# Reaction of the tamoxifen cation and the bis-(4-methoxyphenyl)methyl cation in aqueous solutions containing 2′-deoxyguanosine

**Robert A. McClelland, Cristina Sanchez, Effiette Sauer, and Sinisa Vukovic**

**Abstract**: The competition between 2′-deoxyguanosine (**dG**) and water has been quantitatively evaluated for the allylic carbocation derived from tamoxifen and for the stabilized diarylmethyl cation (bis-(4-methoxyphenyl)methyl). Both systems were examined by the competition kinetics method, in which the products were quantitatively analyzed after the $S_N1$ solvolysis of the corresponding acetate esters in aqueous solutions containing the nucleoside. The principal product of the reaction of both cations with **dG** is the adduct at the $NH_2$ group, a characteristic of delocalized carbocations. The tamoxifen cation was also examined by laser flash photolysis, with absolute rate constants for the reaction with **dG** and water being obtained and converted into rate constant ratios. The principal result of this study is that there is a three orders of magnitude difference in the reactivity of these cations towards the neutral form of **dG** and its conjugate base. Under acidic conditions where the reaction occurs with neutral **dG**, the guanine–water selectivity is low. Even at relatively high concentrations of **dG**, the majority of the product is alcohol derived from the water reaction. At pH 10 to 11, in contrast, **dG** is present as the anion and this is highly competitive. Yields of adduct as high as 90% can be attained. A consequence of the large difference in reactivities is that at neutral pH the majority of the reaction of the cation with **dG** is actually occurring via the small amount of conjugate base present. A further feature of the results is that the $NH_2$ adduct is the predominant stable product from the anion. To explain the high rate constant for the reaction forming this product, a mechanism is proposed whereby one of the protons of the $NH_2$ group is transferred to N1 as the N2–cation bond is forming.

*Key words*: guanine, DNA adduct, carbocation, tamoxifen.

**Résumé** : On a évalué de façon quantitative la compétition entre la 2′-désoxyguanosine (**dG**) et l'eau pour le carbocation allylique dérivé du tamoxifène et pour le cation diarylméthyle stabilisé, bis-(4-méthoxyphényl)méthyle. On a examiné les deux systèmes par la méthode des cinétiques en compétition dans laquelle les produits sont analysés de façon quantitative après une solvolyse $S_N1$ des esters acétiques correspondants en solutions aqueuses contenant le nucléoside. Le produit principal de chacune des réactions des deux cations avec la **dG** est un adduit au niveau du groupe $NH_2$, une caractéristique des carbocations délocalisés. On a aussi étudié le cation du tamoxifène par photolyses éclairs au laser à partir desquelles on a obtenu des constantes absolues de vitesse pour la réaction avec la **dG** et l'eau, celles-ci étant transformées en rapports de constantes de vitesses. Le principal résultat de cette étude est qu'il y a trois ordres de grandeur de différence dans la réactivité de ces cations vis-à-vis de la forme neutre de la **dG** et de sa forme conjuguée. Dans les conditions acides où la réaction se produit avec la **dG** neutre, la sélectivité guanine–eau est faible. Même à des concentrations élevées de **dG**, la plus grande partie du produit est l'alcool qui provient de la réaction avec l'eau. Par ailleurs, à des pH de 10 à 11, la **dG** est présente sous la forme d'anion qui est fortement compétitif. On peut alors obtenir des rendements d'adduit pouvant atteindre 90%. Une conséquence de la grande différence dans les réactivités est que, au pH neutre, la plus grande partie de la réaction du cation avec la **dG** se produit par le biais de la faible quantité de la base conjuguée qui est présente. Une caractéristique additionnelle des résultats est que l'adduit avec le $NH_2$ est le produit stable prédominant à partir de l'anion. Pour expliquer la constante de vitesse élevée pour la réaction conduisant à la formation de ce produit, on propose un mécanisme dans lequel un des protons du groupe $NH_2$ est transféré vers N1 alors que la liaison N2–cation se forme.

*Mots clés* : guanine, adduit avec l'ADN, carbocation, tamoxifène.

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## Introduction

A common property of chemical carcinogens is the formation of DNA adducts (1). These adducts arise from chemical reactions in which DNA reacts with an electrophile, often an electrophile resulting from metabolism of the parent compound. Tamoxifen **1** (Scheme 1), an antiestrogen used in the treatment and control of breast cancer, is a hepatocarcinogen in the rat (2–4) and causes a small number of endometrial cancers in women (5–7). DNA adducts have been observed in both the animal models (8, 9) and in women treated with the drug (10–14). These are explained by the pathway of Scheme 1 (15–22), whereby an allylic carbocation **3** forms in an $S_N1$ ionization of a sulfate ester **2b**. The latter is derived from metabolic α-hydroxylation of the parent drug to α-hydroxytamoxifen **2a** followed by sulfate transfer. The principle DNA adducts **4** are isomers (see below) where the the α-carbon of the tamoxifen is attached to the $NH_2$ group of guanine residues (20–22). This position of attachment to guanine is characteristic of delocalized carbocations (1).

We have described experiments where the cation **3**, the "tamoxifen carbocation," was observed with laser flash photolysis (LFP) (23). The LFP approach has now been employed to study a number of carbocations (24). These are ground-state cations, identical to ones formed in $S_N1$ solvolyses. The significant advantage of the LFP method is that it is capable of providing direct information on reactivity, both towards the solvent (i.e., water) and to added nucleophiles. A nucleophile of interest because of the relation to carcinogenicity discussed above is guanine or a guanine derivative such as 2′-deoxyguanosine (**dG**). Our previous study (23) showed that the tamoxifen cation is quenched by **dG** in an aqueous solution. However, the selectivity over the reaction with solvent is poor, so that even at the highest concentrations of **dG**, the majority of the cation reacts with solvent. This behavior is typical of carbocations in water (25), but it contrasts dramatically with arylnitrenium ions, the DNA-binding electrophiles derived from carcinogenic amines. Both LFP studies (25, 26) and product analyses (27, 28) show that guanine derivatives are excellent nucleophiles for arylnitrenium ions, even in water.

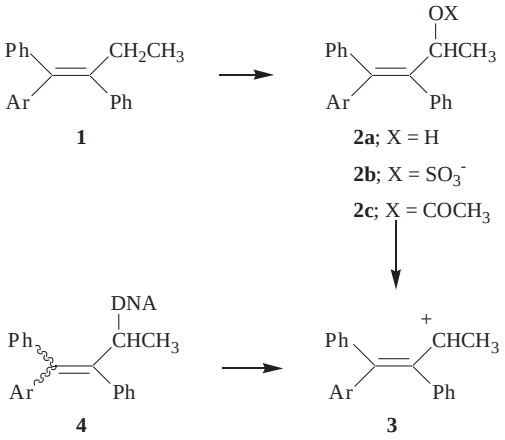
We have now performed a detailed study of the reaction of the tamoxifen cation with **dG**, employing LFP to measure absolute rate constants and product analyses (competition kinetics) for relative rate constants. Competition kinetic experiments have also been carried out with a diarylmethyl cation, the bis-(4-methoxyphenyl)methyl cation, which has a similar lifetime in water to the tamoxifen cation (29). These studies show that there is very pronounced effect of pH on the **dG** reaction. The neutral form of **dG** is quite unreactive as previously found (25). Its conjugate base, however, is far more reactive, so much so that at pH 7 the principal species that is reacting with the cations is the anion. The difference between the two forms of guanine has been recognized (30), but we are not aware of a detailed kinetic study demonstrating the magnitude of the effect, especially for short-lived carbocations.

## Results and kinetic analysis

### Rate pH-profiles in 100% aqueous solutions

In our original paper, the sulfate ester **2b** was employed as the precursor in the LFP experiments (23). A transient with

**Scheme 1.** Ar = $C_6H_4$-4-$OCH_2CH_2N(CH_3)_2$.



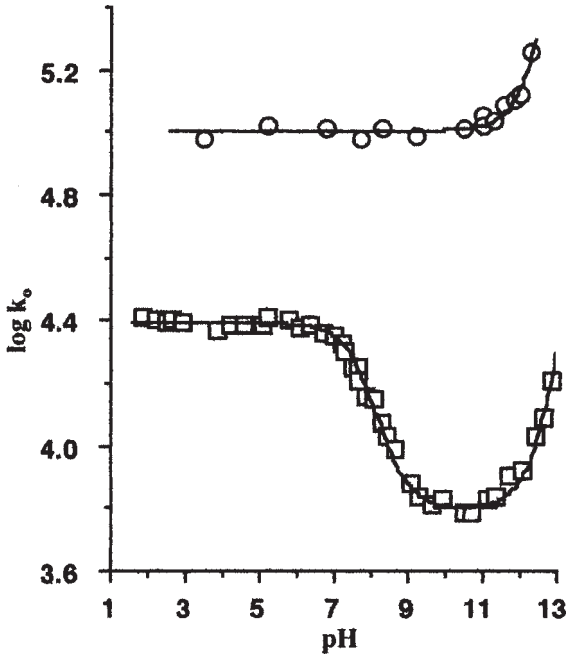

$\lambda_{max}$ at 460 nm was identified as the tamoxifen cation on the basis of several criteria employed to assign carbocation intermediates (24). A key observation was the quenching by azide ion, in particular, the identity of the azide:water rate constant ratio with one obtained by competition kinetics (product analysis) for the ground-state solvolysis of the sulfate in aqueous solutions containing azide ion.

The sulfate ester has a high reactivity towards solvolysis in the ground state. This limits LFP experiments to solutions with considerable organic component (i.e., 40% acetonitrile) where the lower ionizing power slows the $S_N1$ reaction. Since acetate esters have been successfully employed as precursors of carbocations in LFP experiments (24), we investigated the appropriate tamoxifen derivative **2c**. This also produces the tamoxifen cation upon irradiation in aqueous solutions. This was established by comparing LFP results with the acetate and the sulfate in 40% acetonitrile. The two gave the same transient with the same decay kinetics.

The acetate is much more stable towards ground-state solvolysis, with a half-life in 100% water of 2 h. We therefore started by carrying out a detailed kinetic study of the decay of the tamoxifen cation in 100% aqueous solutions. Since the buffers accelerated the decay, experiments were performed with serial buffer dilutions at constant buffer ratio (and constant pH), and the rate constant obtained as the intercept of the plot of $k$ vs. buffer concentration. These rate constants, along with ones obtained in solutions of perchloric acid and sodium hydroxide, are plotted as log $k_o$ vs. pH in Fig. 1. Also plotted in this figure are rate constants obtained in a similar manner for the other cation of this study, the bis-(4-methoxyphenyl)methyl cation.

The profile for the latter cation is straightforward. There is a pH-independent region from acidic pH to a pH of about 11 that corresponds to the reaction of the cation with solvent water molecules. The rate constant is $1.0 \times 10^5$ $s^{-1}$. (Table 1 provides a summary of all rate and equilibrium constants measured in this work, along with their errors provided as one standard deviation.) At pH 11, there is a break to a reaction that is first order in hydroxide ion. This obviously represents the cation–hydroxide combination; the second-order rate constant is $3.8 \times 10^6$ $M^{-1}$ $s^{-1}$. One noteworthy feature is the relatively little difference (a factor of 13) between $k_w$ and $k_{OH}$, typical of short-lived cations in aqueous solutions (31–33).

**Fig. 1.** pH dependence of the logarithm of the rate constants for the decay of the tamoxifen cation (squares) and the bis-(4-methoxyphenyl)methyl cation (circles) in aqueous solutions. Temperature is 20°C and $k_o$ has units of $s^{-1}$. The data for the tamoxifen cation have been obtained at a constant ionic strength of 0.10 M maintained with $NaClO_4$. The data for the bis-(4-methoxyphenyl)methyl cation were obtained in solutions with ionic strength less than 0.01 M. The cations were generated by LFP of acetate precursors with irradiation at 248 nm and detection at 460 nm (tamoxifen cation) and 500 nm (bis-(4-methoxyphenyl)-methyl cation). Rate constants in the region pH 3–11 were obtained in buffer solutions, and are based upon extrapolation to zero buffer concentration. The points are experimental. The line for the tamoxifen cation has been drawn on the basis of eq. [2] and the parameters given in the text.




The profile for the tamoxifen cation can be explained by recognizing that there is a side-chain amine. This means that there are two carbocations, a dication $\mathbf{T}^{2+}$ where the amine is protonated and a monocation $\mathbf{T}^{+}$ where the amine is neutral (Scheme 2). Both, in principle, can react with water and hydroxide, giving rise to eq. [1], where $K_w$ is the autoprotolysis constant of water.

$$[1] \qquad k_o = \frac{k_w^{2+}[H^+] + k_{OH}^{2+}K_w + k_w^{+}K_a(\mathbf{T}) + \dfrac{k_{OH}^{+}K_wK_a(\mathbf{T})}{[H^+]}}{[H^+] + K_a(\mathbf{T})}$$

This takes the form of a four-parameter equation (eq. [2]) where $y = k_o$ and $x = [H^+]$.

$$[2] \qquad y = \frac{ax + b + c/x}{x + d}$$

The experimental data were fit to eq. [2] to provide the four parameters. Parameter $d$ ($1.34 \times 10^{-8}$) is the acidity constant, corresponding to a p$K_a$ of 7.87. This means that the ammonium group in $\mathbf{T}^{2+}$ is one log unit more acidic than in the parent drug. This reflects the electron withdrawing influence of the delocalized positive charge associated with the allylic cation. Parameter $a$ ($2.5 \times 10^4$ $s^{-1}$), is equal to $k_w^{2+}$, the rate constant for water addition to the dication. In terms of the pH profile, this reaction dominates from acidic pH to a pH just before 9; the downward break between pH 7 and 8 is a reflection of the changing acid–base equilibrium. Parameter $c$ ($k_{OH}^{2+}K_wK_a(\mathbf{T}) = 2.2 \times 10^{-17}$) explains the increase in base. This must be due to a reaction of the monocation $\mathbf{T}^{+}$ with hydroxide ion. With $K_a(\mathbf{T})$ known, the rate constant $k_{OH}^{+}$ can be calculated; the value is $1.6 \times 10^5$ $M^{-1}$ $s^{-1}$.

Parameter $b$ ($8.4 \times 10^{-5}$) accounts for the pH-independent region between pH 9 and pH 11. There are two kinetically equivalent processes that could be responsible, the reaction of hydroxide ion with the dication $\mathbf{T}^{2+}$ and the reaction of water with the monocation $\mathbf{T}^{+}$. The former can be ruled out, since it requires a value of $k_{OH}^{2+}$ of $8.4 \times 10^9$ $M^{-1}$ $s^1$, several orders of magnitude greater than expected. As noted above, hydroxide would not react with a cation of the aqueous lifetime of $\mathbf{T}^{2+}$ at anywhere near this rate. Our conclusion, therefore, is that the pH-independent region in weakly basic solution represents water reacting with the monocation $\mathbf{T}^{+}$. The rate constant for this process ($k_w^{+}$) is $6.3 \times 10^3$ $s^{-1}$, fourfold smaller than the rate constant $k_w^{2+}$ for the reaction of water with $\mathbf{T}^{2+}$. This greater reactivity of the dication is not surprising. There is undoubtedly a significant stabilization in both cations by the π-interaction with the oxygen of the dimethylaminoethoxy group. This interaction will be stronger when that group is neutral as opposed to where the amine is protonated. In other words, the $(CH_3)_2NCH_2CH_2O$- group must be a better π donor than the $(CH_3)_2HN^+CH_2CH_2O$- group.

Analysis of the rate constants associated with the buffer revealed that the rate accelerations were due to the basic component of the buffer, the terms in (B) that appear in Scheme 2. As with water and hydroxide, reaction in principle could occur with both $\mathbf{T}^{2+}$ and $\mathbf{T}^{+}$, although of the four buffers employed, only tris resulted in rate constants for both the dication and cation that were statistically significant (Table 1). The actual reaction occurring with the buffer is not known. The most likely scenario is a cation–nucleophile combination. However, a reaction where the buffer base acts as a general base to assist the addition of water to the cation is also possible. Arguments have been made that the water reaction itself is generally base-catalyzed, with a second water molecule acting as the base (29).

### Decay of the tamoxifen cation in the presence of 2′-deoxyguanosine

These experiments were carried out in 20% acetonitrile for solubility reasons. LFP experiments with **dG** as the trapping nucleophile require irradiation, at 308 nm, with our equipment where the **dG** does not absorb. While **2c** does have absorbance at this wavelength, it is weak. Thus, more concentrated solutions of the precursor were required. These concentrations could not be achieved in 100% aqueous solutions, especially solutions of higher pH where the side-chain amine of **2c** is not protonated. The use of 20% acetonitrile also meant that the solubility of **dG** was increased. Solutions with a **dG** concentration of 20 mM were possible, in contrast to 100% aqueous where 8 mM is about the maximum concentration.

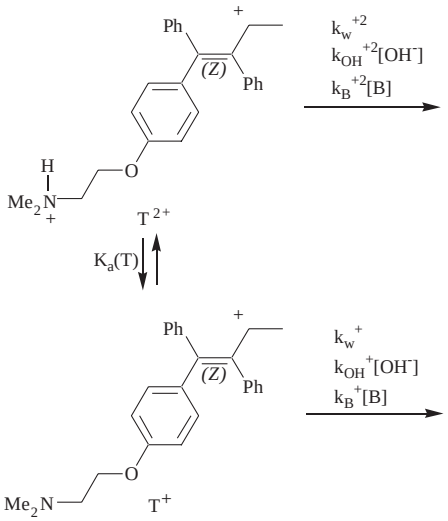
Figure 2 provides the results. The rate constants for the solvent reaction were obtained as described in the previous

**Table 1.** Summary of rate and equilibrium constants for the reactions of tamoxifen dication $\mathbf{T}^{2+}$, tamoxifen monocation $\mathbf{T}^{+}$, and bis-(4-methoxyphenyl)methyl cation ($Ar_2CH^+$).

| Parameter | $\mathbf{T}^{2+}$ | $\mathbf{T}^{+}$ | $Ar_2CH^+$ |
|---|---|---|---|
| $k_w$ ($s^{-1}$) (water) | $(2.5 \pm 0.1) \times 10^4$[a] | $(6.3 \pm 0.2) \times 10^3$[a] | $(1.01 \pm 0.05) \times 10^5$[b] |
| $k_w$ ($s^{-1}$) (20% AN) | $(3.2 \pm 0.1) \times 10^4$[a] | $(8.2 \pm 0.3) \times 10^3$[a] | $(1.3 \times 10^5)$[c] |
| $k_{OH}$ ($M^{-1}$ $s^{-1}$) (water) | —[d] | $(1.6 \pm 0.2) \times 10^5$[a] | $(3.8 \pm 0.8) \times 10^6$[b] |
| p$K_a$(**T**) (water) | 7.87 ± 0.05[a] | | |
| p$K_a$(**T**) (20% AN) | 7.26 ± 0.08[a] | | |
| $k_n$ ($M^{-1}$ $s^{-1}$) (20% AN) | $(1.0 \pm 0.2) \times 10^5$ | ~$(1.5–3) \times 10^4$[e] | |
| $k_a$ ($M^{-1}$ $s^{-1}$) (20% AN) | $(6.4 \pm 0.8) \times 10^7$ | $(1.2 \pm 0.1) \times 10^7$ | |
| p$K_a$(**G**) (20% AN) | 9.27 ± 0.11 (Kinetics) | | 9.44 ± 0.08 (Fig. 6) |
| p$K_a$(**G**) (20% AN) | 9.37 ± 0.05 (Spect.) | | |
| $k_n$:$k_w$ (20% AN) (LFP) | (3.1 ± 0.6) | | |
| $k_n$:$k_w$ (20% AN) (Pdts.) | (2.7 ± 0.3) | —[d] | (1.21 ± 0.07)[f] |
| $k_a$:$k_w$ (20% AN) (LFP) | $(2.0 \pm 0.3) \times 10^3$ | $(1.5 \pm 0.1) \times 10^3$ | |
| $k_a$:$k_w$ (20% AN) (Pdts.) | $(7 \pm 1) \times 10^2$ | $(5.7 \pm 0.6) \times 10^2$ | $(1.52 \pm 0.13) \times 10^3$[f] |
| $k_{AcO}$ ($M^{-1}$ $s^{-1}$) (water) | $(3.4 \pm 0.4) \times 10^5$[a] | —[d] | |
| $k^{2-}_{HPO_4}$ ($M^{-1}$ $s^{-1}$) (water) | $(5.5 \pm 0.5) \times 10^5$[a] | —[d] | |
| $k_{tris}$ ($M^{-1}$ $s^{-1}$) (water) | $(8 \pm 1) \times 10^4$ | $(2 \pm 0.5) \times 10^4$ | |
| $k^{2-}_{CO_3}$ ($M^{-1}$ $s^{-1}$) (water) | —[d] | $(2.1 \pm 0.4) \times 10^5$ | |

[a]Ionic strength = 0.1.
[b]Ionic strength < 0.01.
[c]From ref. 29.
[d]Cannot be measured since not significant in competition with other processes.
[e]Estimated.
[f]Ratio calculated for **DdGa**.

**Scheme 2.**




section, with (where appropriate) serial buffer dilutions providing the rate constants at zero buffer concentration. The rate constants for the reaction with **dG** were obtained using dilute buffers, with 5 to 6 concentrations of **dG** ranging from [**dG**] ~ 0.02 M to [**dG**] = 0 at each pH. The rate constants increased in the presence of **dG**. This acceleration was modest in the solutions with pH 4–6, with only about a 15% increase from the solution with no **dG** to the solution with the highest concentration. As the pH increased, however, the effect of **dG** became much more pronounced, and by pH 9 it was possible to triple the rate constant over that in the absence of **dG**. The observed rate constants were plotted against the concentration of added **dG**. These plots were linear. Their slopes, defined as $k_{dG}$, are plotted as the circles in Fig. 2.

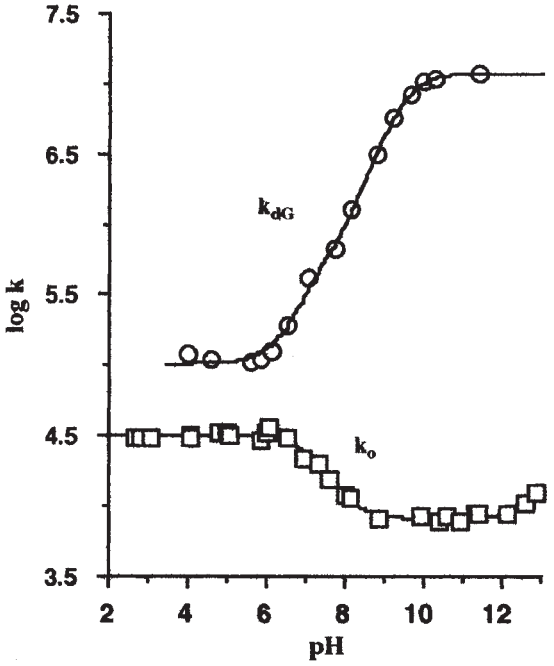
The solvent reaction was analyzed as in the previous section. Values of the parameters so obtained were $K_a(\mathbf{T}) = 5.5 \times 10^{-8}$ (p$K_a(\mathbf{T}) = 7.27$), $k_w^{2+} = 3.2 \times 10^4$ $s^{-1}$, and $k_w^{+} = 8.2 \times 10^3$ $s^{-1}$. The two rate constants are slightly larger than the ones obtained in the study in 100% aqueous solutions. This is a typical observation for carbocation–water reactions as acetonitrile is added (29). There is also a difference in the p$K_a$(**T**) value. This is likely also a solvent effect. Our previous study in 40% acetonitrile resulted in an even lower value of p$K_a$(**T**) (6.6) (23).

An interesting feature of the reaction with **dG** is that at about the point where the curve for the solvent reaction breaks downward, the profile for the **dG** reaction breaks upward. The reasons for this must be different. The downward break in the solvent profile occurs because of the conversion of $\mathbf{T}^{2+}$ to $\mathbf{T}^{+}$. The upward break in the **dG** profile must represent the onset of a new reaction, the reaction of the cation with the conjugate base of **dG**. This leads to the kinetic model of Scheme 3, which shows the nucleoside reaction occurring in four ways. Both the neutral form of **dG** (**dG-H**) and its conjugate base ($\mathbf{dG}^-$) are nucleophiles, and each, in principle, reacts with $\mathbf{T}^{2+}$ and $\mathbf{T}^{+}$.

The kinetic system of Scheme 3 gives rise to eq. [3] for $k_{dG}$.

$$[3] \qquad k_{dG} = \frac{(k_n^{2+}[H^+]^2 + \{k_a^{2+}K_a(\mathbf{G}) + k_n^{+}K_a(\mathbf{T})\}[H^+] + k_a^{+}K_a(\mathbf{G})K_a(\mathbf{T}))}{([H^+] + K_a(\mathbf{T}))([H^+] + K_a(\mathbf{G}))}$$

**Fig. 2.** pH dependence of the logarithm of the rate constants for the decay of the tamoxifen cation in solvent (squares) and the quenching by 2′-deoxyguanosine (**dG**) (circles). Temperature is 20°C, and the solvent is 20:80 (v:v) acetonitrile–water with ionic strength maintained at 0.10 M with sodium perchlorate. Units of $k$ are $s^{-1}$ (solvent reaction) and $M^{-1}$ $s^{-1}$ (**dG** reaction). The cation was generated by excitation of **2c** at 248 nm (solvent data) and 308 nm (**dG** data). The points are experimental. The lines have been drawn on the basis of eq. [2] (solvent reaction) and eq. [4] (**dG** reaction).




This equation contains two acid acidity constants ($K_a$(**T**) and $K_a$(**G**)) referring, respectively, to the ammonium group of the tamoxifen cation and the N1-H proton of **dG**. The rate constants are identified with superscripts "2+" and "+" to differentiate reactions with $\mathbf{T}^{2+}$ and $\mathbf{T}^{+}$ and with subscripts "n" and "a" to differentiate reactions with the neutral and anionic forms of the nucleoside. This equation takes the form of eq. [4], with five adjustable parameters.

$$[4] \qquad y = \frac{ax^2 + bx + c}{(x + d)(x + e)}$$

The experimental data were fit to this equation with the parameter $d = K_a$(**T**) fixed at the value of $5.5 \times 10^{-8}$ obtained from the analysis of the water reaction. The other four were allowed to vary to values providing the best fit.

The parameter $e$ ($5.3 \times 10^{-9}$) is the acidity constant for **dG**, and corresponds to a p$K_a$ value of 9.27. We also measured this value spectroscopically under the same solvent conditions, and obtained a value of 9.37. Within the experimental error (Table 1), the value obtained from the kinetic fit is identical. The parameter $a = k_n^{2+} = 1.0 \times 10^5$ $M^{-1}$ $s^{-1}$ is the rate constant for the reaction of the neutral form of **dG** with the dication $\mathbf{T}^{2+}$. The parameter $c$ ($3.4 \times 10^{-10}$) is equal to $k_a^+ K_a$(**T**)$K_a$(**G**) and represents the reaction of the conjugate base of **dG** with $\mathbf{T}^{+}$. With values of the acidity constants known, $k_a^+$ is calculated as $1.2 \times 10^7$ $M^{-1}$ $s^{-1}$. The parameter $b$ ($3.4 \times 10^{-2}$) represents two kinetically equivalent processes, neutral **dG** reacting with $\mathbf{T}^{+}$ (the $k_n^+$ process) and anionic **dG** reacting with $\mathbf{T}^{2+}$ (the $k_a^{2+}$ process). The latter must be the major contributor. If the former is responsible, $b = k_n^+ K_a$(**T**), which requires $k_n^+$ to be $6.2 \times 10^5$ $M^{-1}$ $s^{-1}$. This is a sixfold greater than $k_n^{2+}$, the rate constant for the reaction of neutral **dG** with the dication. This makes no sense since the dication is the more reactive species. Based upon results for other nucleophiles, $k_n^+$ will lie in the range $1.5–3 \times 10^4$ $M^{-1}$ $s^{-1}$, a factor of three to six times smaller than $k_n^{2+}$. We therefore conclude that the $k_n^+$ process could contribute about 3% to the term in $b$. However, 97% of this term must be due to the process in $k_a^{2+}$. Using this 97% factor, $k_a^{2+}$ is calculated as $6.4 \times 10^7$ $M^{-1}$ $s^{-1}$. It can be seen that this is consistent with the greater reactivity of the dication — $k_a^{2+}$ is fivefold larger than $k_a^+$.

**Scheme 3.**

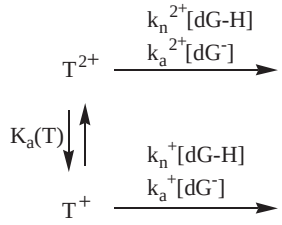


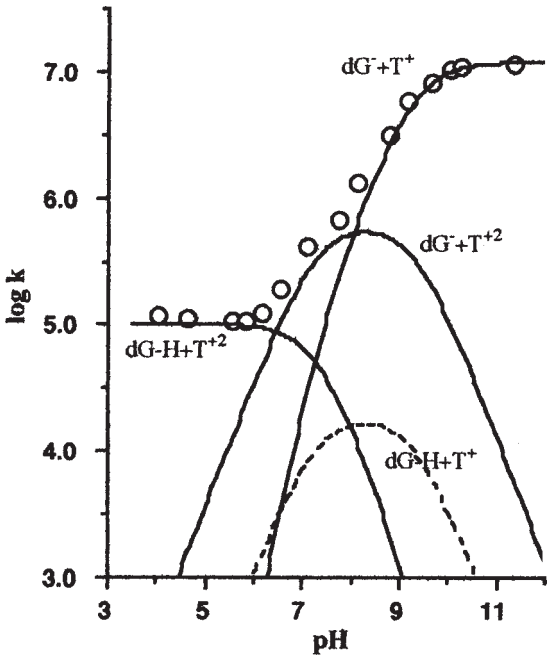

Figure 3 shows how the observed rate constants for the reaction of the tamoxifen cation and **dG** are broken down into the contributions from the four reactions. The curves in this figure were calculated using the rate constants provided in the previous paragraph (with an estimate for $k_n^+$ of $2 \times 10^4$). It can be seen that under acidic conditions the reaction that occurs involves $\mathbf{T}^{2+}$ and the neutral form of **dG**, while under basic conditions, $\mathbf{T}^{+}$ reacts with the anion of **dG**. The principle contributor at pH 7 is the reaction of $\mathbf{T}^{2+}$ with the conjugate base.

### Tamoxifen cation and deoxyguanosine — competition kinetics

The experiments described in this section were performed to determine if the pH effect is also observed in the products. The principle adduct of the tamoxifen cation and **dG** has been isolated and characterized previously as compound **TdG** of Scheme 4 (22). This is formed as four diastereomers, arising from the combinations of the (*R*) and (*S*) forms at the α stereocenter and (*E*) and (*Z*) forms about the C=C. The latter arise because the cation undergoes rapid rotation about this bond before capture by nucleophiles. HPLC analysis of this mixture then provided retention times for the four isomers under our HPLC conditions.

Experiments were then performed in which the acetate **2c** was added to solutions of 20% acetonitrile containing 2′-deoxyguanosine, and the ground-state solvolysis reaction allowed to proceed to completion overnight. The products were analyzed by HPLC. With appropriate correction for relative HPLC sensitivities, this provided the ratio of the sum of the concentrations of the four adducts [**TdG**] to the sum of the concentrations of the (*E*) and (*Z*) alcohols [**TOH**]. This ratio was then converted to a competition-kinetic ratio according to eq. [5], where [**dG**] was the concentration of the nucleoside in the solution.

**Fig. 3.** Breakdown of the reaction of 2′-deoxyguanosine and the tamoxifen cation into components.




[5] $k_{\mathbf{dG}}/k_o = (1/[\mathbf{dG}])([\mathbf{TdG}]/[\mathbf{TOH}])$

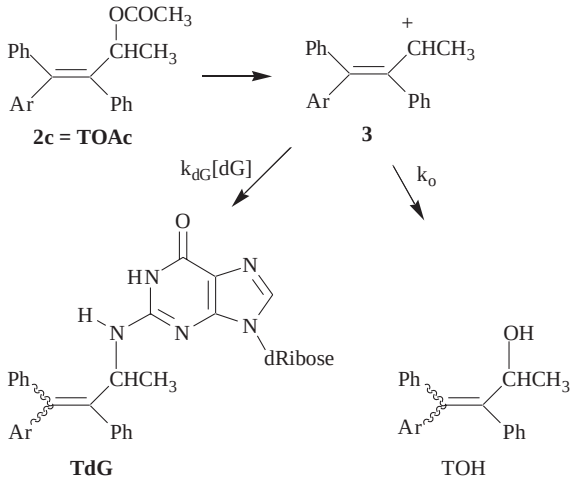
The results are plotted in Fig. 4, and show the same trend as observed in the absolute rate constants, with a significant increase in the selectivity ratio at higher pH. In terms of the analysis of the previous sections, this ratio is given by eq. [6] (see below), obtained by dividing eq. [3] by eq. [1].

The line drawn through the experimental points in Fig. 4 is based on a fit to this equation. Because of the number of parameters involved, the fitting was performed by fixing the values of $K_a(\mathbf{T})$, $K_a(\mathbf{G})$, and $k_w^+:k_w^{2+}$ at the values obtained previously, $5.5 \times 10^{-8}$, $5 \times 10^{-10}$, and 0.256, respectively. This left the three rate constant ratios as variables; values are provided in Table 1. Also given in this table are values of the same ratios calculated from the absolute rate constants measured with LFP. The values of $k_n^{2+}:k_w^{2+}$ obtained by the two methods are not outside of experimental error. The two values of $k_a:k_w$ are, however, statistically different, with the LFP ratio greater than the product ratio in both cases. A possible explanation is provided in the *Discussion*.

### Bis-(4-methoxyphenyl)methyl cation and deoxyguanosine — Competition kinetics

These experiments were performed following the same procedure described in the previous section, with the cation (designated as $\mathbf{D}^+$) being obtained by solvolysis of the acetate ester in solutions of 20% acetonitrile containing **dG**. The HPLC traces showed the peak for the alcohol product **DOH** and three peaks associated with a reaction with **dG**. Since this cation does not generate a new stereocenter (or C=C isomers), each of these peaks must represent a product derived from a different position of attachment to the **dG**.

**Scheme 4.**




This means that there are four reactions occurring in parallel, one forming alcohol and three forming different **dG** adducts (Scheme 5). These adducts are designated as **DdG1**, **DdG2**, and **DdG3** in order of increasing HPLC retention times.

Figure 5 shows how the areas of the peaks for the four products vary with pH for experiments performed with constant concentrations of **dG** and acetate ester. Under acidic conditions (pH 3–5) the areas are constant. The alcohol is the major product, with only small peaks for the three adducts (note the scale in Fig. 5 is logarithmic). As the pH increases, the alcohol peak decreases significantly, the peaks for **DdG1** and **DdG2** grow, and the peak for **DdG3** disappears. Under basic conditions there is another constant region, with the major peak corresponding to **DdG1**.

A sample of **DdG1** was isolated from a scaled-up reaction at high pH. The NMR spectra in DMSO-$d_6$ clearly established that this is the N2-adduct. Features of the NMR leading to this assignment were the down-field proton (10.4 ppm) integrating as 1H characteristic of N1H of guanine derivatives, the proton at C8 in its characteristic position near 7.8 ppm, and a proton at 7.4 ppm integrating only as 1H for N2H. The attachment N2H was most clearly seen by the coupling of this proton and the methine proton of the diarylmethyl group (6.0 ppm). This coupling was verified by a 2D COSY experiment.

With a sample of **DdG1** available, the HPLC area ratio for this peak relative to the alcohol was converted to a concentration ratio, and then, with eq. [7], into a ratio of rate constants.

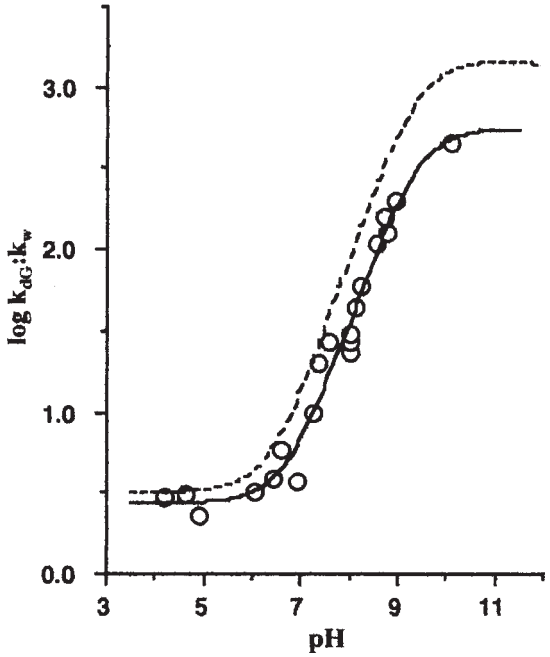
[7] $k_{\mathbf{dG1}}/k_o = (1/[\mathbf{dG}])([\mathbf{DdG1}]/[\mathbf{DOH}])$

The pH dependence of this ratio is shown in Fig. 6. As with the tamoxifen cation the selectivity is modest in acidic solutions, but becomes much more significant at higher pH.

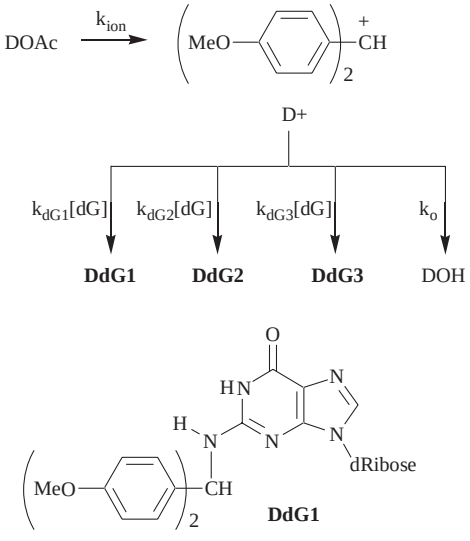
Figure 6 can be explained by a kinetic model where the cation reacts with water in a pH-independent manner to form

[6] $$k_{dG}/k_o = \frac{(k_n^{2+}/k_w^{2+})[H]^{2+} + (k_a^{2+}/k_w^{2+})K_a(\mathbf{G})[H^+] + (k_a^+/k_w^+)(k_w^+/k_w^{2+})K_a(\mathbf{G})K_a(\mathbf{T})}{([H^+] + (k_w^+/k_w^{2+})K_a(\mathbf{T}))([H^+] + K_a(\mathbf{G}))}$$

**Fig. 4.** pH dependence of the logarithm of the ratio of rate constants $k_{dG}$:$k_w$ for the competition for the tamoxifen cation between 2′-deoxyguanosine and water. Temperature is 20°C, and the solvent is 20:80 (v:v) acetonitrile–water with ionic strength maintained at 0.10 M with sodium perchlorate. The circles are the experimental data obtained by analysis of the products of the ground-state solvolysis of the acetate **2c**. The solid line was obtained by fitting the data to eq. [6] as described in the text. The dashed line is the ratio of the absolute rate constants measured by LFP; this line was calculated as the difference between the two lines drawn in Fig. 2.



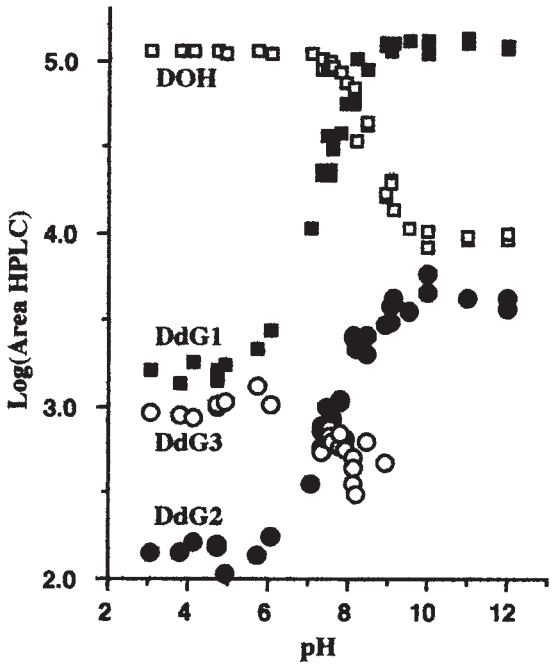

**Scheme 5.**




the alcohol, while adduct **DgD1** is formed from both neutral **dG** and its anionic conjugate base. This leads to eq. [8],

$$[8] \qquad k_{\mathbf{dG1}} / k_o = \frac{(k_n^1/k_w)[H^+] + (k_a^1/k_w)K_a(\mathbf{G})}{[H^+] + K_a(\mathbf{G})}$$

**Fig. 5.** pH dependence of the logarithm of the HPLC areas for the four products observed in the reaction of the bis-(4-methoxyphenyl)methyl cation in 20% acetonitrile in the presence of 2′-deoxyguanosine. The cation was obtained from the solvolysis of bis-(4-methoxyphenyl)methyl acetate. The experiments were performed with a constant concentration of **dG** (0.0096 M) and a constant concentration of the acetate (100 μM).

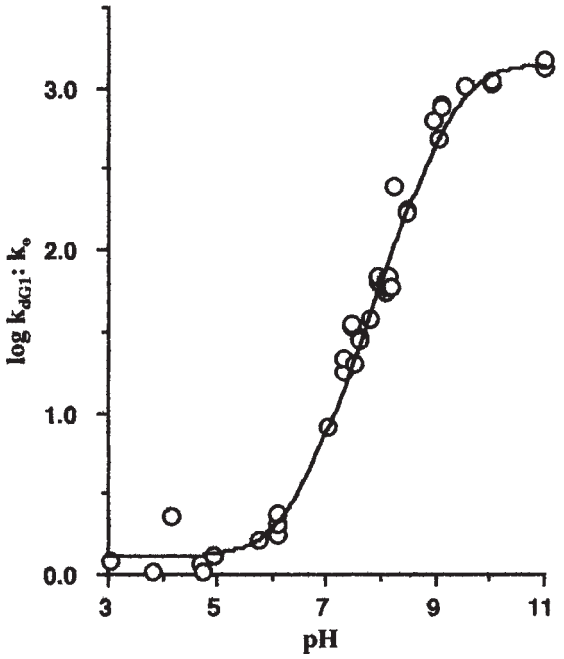



where $K_a(\mathbf{G})$ is the acidity constant of **dG**, $k_w$ is the rate constant for the reaction with water, and $k_n^1$ and $k_a^1$ are the rate constants for reactions forming adduct **DgD1**. As above, the subscripts "n" and "a" represent reactions of the neutral and conjugate base forms of **dG**. The superscript "1" indicates that these are reactions forming **DdG1**. Equation [8] contains three variables, two rate constant ratios, and the acidity constant. The experimental data were fit to this equation with all three parameters as adjustable parameters. The results are provided in Table 1. It can be seen that the value of the acidity constant is within experimental error the same as values obtained by a spectroscopic analysis and a kinetic analysis of the tamoxifen system.

The other two **dG** adducts have not been identified. Under acidic conditions where the neutral form of **dG** is reacting, the area of the peak for **DdG3** is 60% of that for **DdG1**. However, both are minor components of the reaction mixture. With the assumption that the HPLC sensitivity for **DdG3** is the same as that of **DdG1**, the ratio $k_n^3$:$k_w$ is only 0.7 $M^{-1}$. This means that even at the highest concentrations of **dG** attainable (~20 mM), the yield of **DdG3** is only around 1%. The peak for this adduct is not observed in the basic solutions where the anion of **dG** is reacting. Either this adduct is not formed by the reaction with the anion, or its yield is very small in comparison with the other products.

**DdG2** follows the pattern of the major adduct with a significant increase relative to alcohol when the **dG** reacts as the anion. The assumption of the same HPLC sensitivity results in ratios $k_n^2$:$k_w$ = 0.1 $M^{-1}$ and $k_a^2$:$k_w$ = 45 $M^{-1}$. While the latter shows relatively good selectivity over the alcohol,

**Fig. 6.** pH dependence of the logarithm of the ratio of rate constants $k_{dG}$:$k_w$ for the competition for the bis-(4-methoxyphenyl)-methyl cation between 2′-deoxyguanosine and water. Temperature is 20°C, and the solvent is 20:80 (v:v) acetonitrile:water. The circles are the experimental data obtained by analysis of the products of the ground-state solvolysis of the bis-(4-methoxyphenyl)methyl acetate. The line was obtained by fitting the data to eq. [8].




$k_a^2$:$k_a^1$ is only 0.03. Thus the yield of **DdG2** is never greater than 3%.

Finally, we note the evidence that the reaction has proceeded via $S_N1$ solvolyses. This takes the form of experiments where the first-order rate constant for the disappearance of **DOAc** was measured by HPLC. In 20% acetonitrile with the pH at 9.5, rate constants of 0.0018 $s^{-1}$ and 0.0017 $s^{-1}$ were measured for solutions with [**dG**] = 0 and [**dG**] = 0.0096, respectively. The products in the latter are around 90% adducts. The observation that the rate constant is unchanged, within experimental error, shows that the product-determining step has occurred after the rate-determining step; i.e., the reaction is $S_N1$. A similar experiment was not necessary with the tamoxifen system. The observation of C=C isomers in the products shows that there must have been a cationic intermediate.

## Discussion

An attractive approach to model DNA–electrophile interactions is to investigate the reaction with a monomer such as 2′-deoxyguanosine. In this study electrophiles were two delocalized carbocations, the allylic cation derived from the carcinogen tamoxifen, and the bis-(4-methoxyphenyl)methyl cation. Lifetimes of these electrophiles in aqueous solution are 10–200 ms. By carbocation standards, these are relatively long-lived (24). Some relevant comparisons are the benzylic cations derived from ring opening of the diol epoxides of the carcinogen benzo[*a*]pyrene — lifetimes of the order of 50 ns in water (34, 35) — the *p*-methoxybenzyl cation, a benzylic system that has been studied with guanine in the past (36–38) — lifetime of 5 ns (39) — and the diphenylmethyl cation — lifetime of 800 ps (40). The parent benzyl cation is too short-lived to exist in water (41).

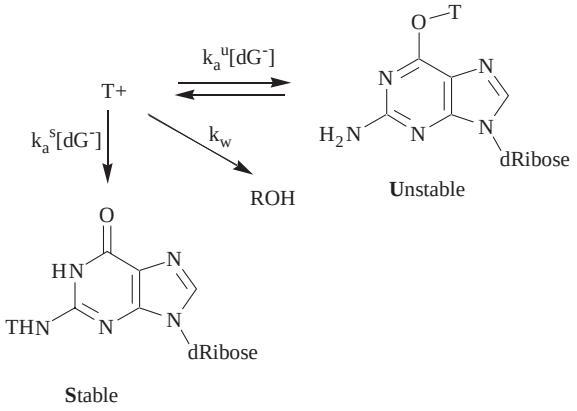
The cations of this study clearly show significant differences in reactivity towards the neutral form of 2′-deoxyguanosine and its conjugate base. This difference is seen in the guanine:water selectivities obtained by analyzing products of solvolysis reactions. It is also seen in the absolute rate constants measured with LFP. A greater reactivity of the conjugate base form of a nucleophile is hardly surprising. Previous studies of guanine–electrophile interactions have commented on this in qualitative terms (30, 36–38). We are not aware of a quantitative study, particularly one where rate constants and rate constant ratios were obtained for intermediate carbocations.

For the cations of this study, the difference between the rate constants for the neutral form of **dG** and the conjugate base is about three orders of magnitude. This results in very different selectivities over the solvent reaction. Under acidic conditions where it is the neutral form of **dG** that reacts, the cations preferentially react with water even at the highest concentrations of **dG**. Arylnitrenium ions, the reactive electrophiles derived from arylamine carcinogens, provide an interesting contrast here. These cations react very efficiently with **dG**, with a high selectivity over the water reaction (25–28). This reaction involves the neutral form of **dG**, since the rate constant is unchanged over the pH range 3.5–8.0 (25). (Studies of arylnitrenium ions with anionic **dG** are currently in progress). The following comparison shows how dramatic the difference is. When the tamoxifen cation (as the $\mathbf{T}^{2+}$ form) reacts in acidic solutions containing 1 mM **dG**, only 0.3% of the products are derived from the nucleoside, 99.7% being the alcohol obtained from capture by solvent. The percentage of adducts is even lower for the bis-(4-methoxyphenyl)methyl cation under the same conditions. The 2-fluorenylnitrenium ion has an aqueous lifetime that is identical to that of $\mathbf{T}^{2+}$. When this cation reacts in 1 mM **dG**, 96% of the products are derived from the nucleoside and only 4% from the solvent (24).

Changing to basic conditions where the **dG** has been converted to its conjugate base results in much higher selectivities towards the nucleoside. For example, the bis-(4-methoxyphenyl)methyl cation reacting at pH 11 in the presence of 1 mM **dG** will form 60% **dG** adduct. This is obviously an attractive method for the preparation of adducts for the purpose of structural characterization. The difference between the two forms of **dG** is such that by pH 7 to 7.5, the majority of the reaction that does occur with the nucleoside is the reaction with the small fraction of anion that is present. This is also reflected in an improved selectivity over the solvent reaction. As one example, the **dG**:water selectivity for the bis-(4-methoxyphenyl)methyl cation at pH 7.3 has increased to 13 from its acidic value of 1.2. This increase occurs since 91% of the reaction with **dG** at pH 7.3 is occurring via the conjugate base.

In the tamoxifen system, a comparison was made between ratios of rate constants for the **dG** and water reactions obtained by product analysis and ratios calculated from the absolute rate constants measured with LFP. The two ratios for

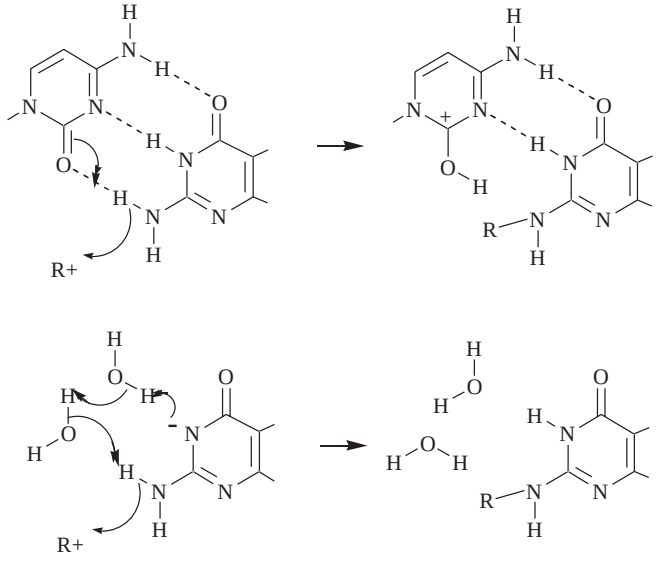
**Scheme 6.**




the competition involving the neutral form of **dG** and water were not outside of experimental error (although this error was quite large). On the other hand, the ratios for the anion of **dG** vs. water were statistically different, with a greater ratio being obtained by the LFP experiments. The difference is almost a factor of three. There is a very large difference in the time scales of the two experiments. The LFP measurements occurred on the microsecond time scale. The product analyses, on the other hand, were carried out after 16–24 h, the time required for complete solvolysis of the acetate. This leads to an explanation whereby the reaction of the cation with **dG** anion proceeds reversibly to an unstable adduct in addition to its irreversible reaction forming the stable product, the N2 adducts (Scheme 6). The quenching by the **dG** anion measured as the second-order rate constant in the LFP experiment would then correspond to the sum of the rate constants for the formation of the unstable and stable products ($k_a^u + k_a^s$). The rate constant in the competition experiment would only refer to the latter ($k_a^s$). Thus, the ratio for the latter would be smaller.

This scenario requires that the unstable adduct be "stable" on the time scale of the LFP experiment, i.e., that it last at least half a millisecond. On the other hand, it must have completely reacted before the products were analyzed. A structure that would satisfy these requirements is the adduct at O6 shown in Scheme 6. Such an adduct is an obvious product of the reaction of a carbocation and the anion of guanine, since it is at O6 that the majority of the negative charge will reside. O6-(*p*-methoxybenzyl)guanosine, the analog derived from the *p*-methoxybenzyl cation, has been shown to be unstable in aqueous solution (36). Its decomposition was pH independent at high pH, and the reaction was proposed to involve ionization to the *p*-methoxybenzyl cation and the guanosine anion. The half-life for this ionization was about 0.5 h. Both the tamoxifen cation and the bis-(4-methoxyphenyl)methyl cation are more stable than *p*-methoxybenzyl, and thus ionizations that result in these cations will be considerably faster. This can be seen in the observation that the acetate esters derived from the two solvolyze at a reasonable rate in water, while *p*-methoxybenzyl acetate is very stable under these conditions. Thus, an O6 adduct formed with the tamoxifen cation (or the bis-(4-methoxyphenyl)methyl cation) is expected to be quite short-lived. It could well survive the time scale of the LFP experiment, but it would not be around after several hours.

**Scheme 7.**




Despite the fact that the negative charge in the **dG** anion is at O6 (and N1), there is still a remarkably large rate constant for the reaction that forms the N2 adduct. This effect is best seen in the competition kinetic experiments with the bis-(4-methoxyphenyl)methyl cation where a single carbocation was involved and the N2 adduct:water product ratio was measured and converted to a rate-constant ratio. The latter changes from 1.2 ($k_n^1$:$k_w$) for neutral **dG** to 1500 ($k_a^1$: $k_w$) for the anion. The rate constant $k_w$ is the same in the two ratios, with a value of $1.3 \times 10^5$ $s^{-1}$ in this solvent (29). Thus, the rate constant $k_n^1$ for the formation of the N2 adduct with neutral **dG** is $1.6 \times 10^5$ $M^{-1}$ $s^{-1}$, and $k_a^1$ for the anion is $2.2 \times 10^8$ $M^{-1}$ $s^{-1}$, over three orders of magnitude greater.

Observations of enhanced formation of N2 adducts in the anion of guanine have been made previously (36–38), although it is not clear if free carbocations were involved in all cases. The N2 adduct is also the principle product of the reaction of DNA with the tamoxifen carbocation (20–22), as well as a number of other electrophiles (1). In DNA one of the $NH_2$ protons is involved in hydrogen bonding to the complementary cytosine. This has led to the recent proposal (42) of a mechanism whereby the proton in the hydrogen bond transfers to the neighboring cytosine as the nitrogen–electrophile bond is forming (top reaction of Scheme 7). In other words, the neighboring cytosine acts as a base to assist the reaction of the $NH_2$ group by removing one of its protons as the reaction proceeds.

A similar mechanism would explain the high reactivity of the $NH_2$ group in the guanine anion. Here the base would be the adjacent partially negatively charged nitrogen at N1. The proton at $NH_2$ could be transferred directly to N1 through a four-center transition state or, perhaps, through the intervention of surrounding water molecules, as shown in the bottom mechanism in Scheme 7. In any case the reaction would lead directly to the product, and there with little or no build-up of positive charge at N2.

## Experimental section

α-Hydroxytamoxifen and α-acetoxytamoxifen ((*E*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-buten-3-ol and its acetate ester) were available from a previous study (43) as were bis-(4-methoxyphenyl)methanol and bis-(4-methoxyphenyl)methyl acetate (44).

*N*2-Bis-(4-methoxyphenyl)methyl-2′-deoxyguanosine was isolated from a scaled-up reaction. 2′-Deoxyguanosine (286 mg, 1 mmol) was added to a mixture of 20 mL acetonitrile and 30 mL of water, and 0.9 mL (0.9 mmol) of sodium hydroxide was added. A solution of bis-(4-methoxyphenyl)methyl acetate (200 mg, 0.7 mmol) in 5 mL of acetonitrile was then slowly added over 8 h, and the mixture left to stand at room temperature, with periodic monitoring by HPLC. When, after 3 days, this indicated that there was no ester remaining, the pH was adjusted to about 5 by the addition of a small amount of 1 M HCl, and the mixture concentrated with a rotary evaporator until only water remained. At this point, a precipitate had formed, which was isolated by filtration. This solid was purified by column chromatography on silica gel with 100% ethyl acetate as the eluting solvent. Fractions were analyzed by HPLC and the fractions containing the adduct were combined and the solvent removed. The solid obtained was purified by dissolving it in a small amount of methanol and then induction of precipitaton by the addition of water. The solid showed only a single peak on HPLC corresponding to the adduct identified as **DdG1**. This was characterized as *N*2-bis-(4-methoxyphenyl)methyl-2′-deoxyguanosine by $^1$H NMR, with coupled protons being identified by gCOSY experiments. $^1$H NMR (500 MHz, DMSO-$d_6$) δ: 2.05–2.10 (m, 1H, H2$_a$′), 2.55–2.6 (m, 1H, H2$_b$′), 3.40–3.45 (m, 1H, H5$_a$′), 3.55–3.60 (m, 1H, H5$_b$′), 3.70 (s, 6H, $OCH_3$), 3.80 (m, 1H, H4′), 4.30 (m, H3′), 4.83 (t, 1H, C5′-OH), 5.22 (d, 1H, C3′-OH), 6.02 (d, 1H, $C_\alpha$-H), 6.08 (t, 1H, H1′), 6.94 (d, 4H, Ar-H), 7.21 (d, 4H, Ar-H), 7.36 (d, 1H, N2-H), 7.84 (s, 1H, H8), 10.35 (s, 1H, N1-H). Mass spectra of this material, even with soft ionization techniques, showed only a very small peak for the molecular ion ($m/z$ = 492).

LFP experiments involved ca. 20 ns pulses at 248 or 308 nm (60–120 mJ per pulse) from a Lumonics excimer laser. A pulsed xenon lamp provided the monitoring light. After passing through a monochromator, the signal from the photomultiplier tube was digitized and sent to a computer for analysis. Stock solutions of the acetates (α-acetoxytamoxifen and bis-(4-methoxyphenyl)methyl acetate) of concentration 20–50 mM were prepared in acetonitrile. Immediately before irradiation a small volume was added to the solution of interest (usually in a 25 mL volumetric flask). Final concentrations of the acetates were 50 μM (α-acetoxytamoxifen in water) and 200–300 μM (α-acetoxytamoxifen in 20% acetonitrile and bis-(4-methoxyphenyl)methyl acetate). The solutions were irradiated with laser light at 248 nm (when studying the reaction with the solvent) and at 308 nm (α-acetoxytamoxifen in the presence of 2′-deoxyguanosine). The signals of the transient carbocations were monitored at 460 nm (tamoxifen cation) and 500 nm (bis-(4-methoxyphenyl)methyl cation). These showed excellent first-order decays. Observed first-order rate constants were obtained by fitting the experimental data of absorbance vs. time to the exponential equation. Rate constants for the solvent reaction were obtained as the average of five to eight kinetic runs. For the decay of the tamoxifen cation in the presence of 2′-deoxyguanosine, 12–16 kinetic runs were averaged. The pH of the solutions was established by the use of perchloric acid (pH < 3), sodium hydroxide (pH > 11) with acetate, phosphate, tris, and carbonate buffers in between. When a buffer was employed, serial dilutions were performed to provide four to five solutions of the same pH (as verified by recording the pH with a pH meter). The observed rate constants were linear in the buffer concentration, and the value of $k_o$, the rate constant in the absence of the buffer, was obtained as the intercept at zero buffer concentration. The experiments with **dG** present were performed with dilute solutions of the buffers (total concentration less than 0.01 M) with four to five concentrations of **dG** ranging up to 20 mM. The rate constants $k_{\mathbf{dG}}$ were obtained by linear regression of the plot of the observed rate constants against the added concentrations of **dG**.

The value of the acid dissociation constant of 2′-deoxyguanosine was measured in 20% acetonitrile, ionic strength = 0.1 M, 20°C by preparing a series of buffer solutions to which had been accurately added the same constant concentration (100 μM) of the nucleoside. Spectra were recorded with a HewlettPackard diode array spectrometer, and titration curves of absorbance vs. pH constructed at several wavelengths. These were fit to the equation $A = (A_{acid}[H^+] + A_{base}K_a)/([H^+] + K_a)$, $A_{acid}$ and $A_{base}$ are the plateau absorbances at low pH and high pH, respectively.

HPLC experiments were performed with a Waters HPLC system comprising a Waters 600E system controller, a Waters 486 tunable absorbance detector set at 238 nm, a Waters 746 data module, and a Waters U6K injector. The column employed was a Waters Symmetry C18 column of 5 μm particle size and dimensions 4.6 mm × 150 mm; the eluting solvent was first passed through a guard column of the same packing material and 4.6 × 25 mm dimensions. Solvents were sparged with helium.

Solutions of α-acetoxytamoxifen and bis-(4-methoxyphenyl)methyl acetate were prepared as described above, by adding the concentrated stock solution in acetonitrile to a solution of 20% acetonitrile. The entire set of experiments with each acetate were performed with the same stock solution to ensure that the final solutions were all of the same concentration, around 100 μM. The solutions were allowed to stand at room temperature for a period of 16–24 h, sufficient time that the signal of the acetate in the HPLC had disappeared.

The products from the α-acetoxytamoxifen were analyzed at 260 nm. Elution at 1 mL min$^{-1}$ involved: (*i*) an initial 0.5 min isocratic run at 25% acetonitrile – 75% acetate buffer (pH 4.5, 0.05 M); (*ii*) a linear gradient to 85% acetonitrile – 15% acetate buffer over 13.5 min; (*iii*) a 3 min isocratic run at 85% acetonitrile – 15% acetate buffer. Peaks were observed at 1 to 2 min (**dG**), 10.5, 10.6 (overlapping (*E*)-**TdG** isomers), 11.8, 11.9 (overlapping (*Z*)-**TdG** isomers), 12.8 ((*E*)-**TOH**)), and 13.5 ((*Z*)-**TOH**). The areas for these peaks were converted into concentration ratios as follows:

$$[9] \qquad [\mathbf{TdG}]/[\mathbf{TOH}] = (1/RF)(\text{Area}(\mathbf{TdG})/\text{Area}(\mathbf{TOH}))$$

where [**TdG**], [**TOH**], Area(**TdG**), and Area(**TOH**) are the sums of the concentrations and HPLC areas of the four **dG** adducts and the two alcohols, respectively, and *RF* is the relative HPLC response factor. The latter was obtained as follows. For a series of solutions of the same total concentration of products, eq. [10] applies:

$$[10] \quad [\mathbf{TdG}] + [\mathbf{TOH}] = \text{Area}(\mathbf{TdG})/F(\mathbf{TdG}) + \text{Area}(\mathbf{TOH})/F(\mathbf{TOH}) = [\mathbf{TOAc}]_o$$

where $F$(**TdG**) and $F$(**TOH**) are the HPLC sensitivities of the adducts and alcohols, respectively, and $[\mathbf{TOAc}]_o$ is the constant total concentration of products determined by the initial concentration of acetate ester. Rearrangement gives eq. [11]:

$$[11] \quad \text{Area}(\mathbf{TdG}) = -(F(\mathbf{TdG})/F(\mathbf{TOH}))\text{Area}(\mathbf{TOH}) + F(\mathbf{TdG})[\mathbf{TOAc}]_o$$

where $F$(**TdG**):$F$(**TOH**) is equal to *RF*. A plot of the total area of the adducts was indeed linear in the sum of the area of the two alcohols. The slope (–2.2) was the negative of *RF*. It should be noted that there are two assumptions in this treatment: **TdG** and **TOH** account for the products and the HPLC sensitivities of the four forms of **TdG** are equivalent, as is also the case for the two forms of **TOH**. The former is true to at least 95%. There were other small peaks in the HPLC chromatogram (including the cyclic indenes (43)) but these never amounted to more than 5% in total. The identical sensitivities of the two alcohols was seen in the previous study. Further confirmation that this *RF* is reasonable is evident from the observation that the sum of the extinction coefficients measured at 260 nm for 2′-deoxyguanosine and α-hydroxytamoxifen divided by the extinction coefficient for α-hydroxytamoxifen is 2.3. The latter is a relative response factor calculated with the assumption that the two chromophores in the adducts are independent and can be modeled by the chromophores in **dG** and the alcohol.

The products from bis-(4-methoxyphenyl)methyl acetate were analyzed at 230 nm. Elution at 2 mL $min^{-1}$ involved: (*i*) an initial 2.0 min isocratic run at 25% acetonitrile – 75% phosphate buffer (pH 7, 0.02 M); (*ii*) a linear gradient to 50% acetonitrile – 50% phosphate buffer over 8 min; (*iii*) a 5 min isocratic run at 50% acetonitrile – 50% acetate buffer. Peaks were observed at 1 min (**dG**), 5.3 min (**DdG1**), 6.2 min (**DdG2**), 6.4 min (**DdG3**), and 9.0 min (DOH). The ratio of the concentration of the $NH_2$ adduct **DdG1** to alcohol was obtained with eq. [12] using a relative response factor of 1.25 obtained by coinjecting authentic samples of the two products.

$$[12] \quad [\mathbf{DdG1}]/[\mathbf{DOH}] = (1/RF)(\text{Area}(\mathbf{DdG1})/\text{Area}(\mathbf{DOH}))$$

First-order rate constants for the solvolysis of bis-(4-methoxyphenyl)methyl acetate were obtained by repeat injections starting immediately after preparation of the solution. The area of the peak for the acetate vs. time was fit to the exponential equation.

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