Chemical Radiation Studies of 8-Bromoguanosine in Aqueous Solutions

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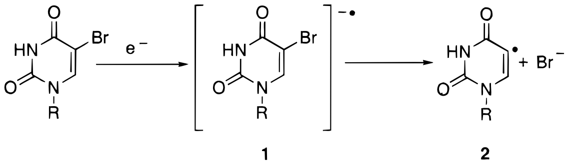
Abstract: Chemical radiolytic methods were used to investigate the reactions of hydrated electrons (e_{aq}^-) with 8-bromoguanosine (8-Br-Guo) as a function of pH. γ-Radiolysis of 8-Br-Guo in aqueous solutions followed by product studies showed the formation of guanosine (Guo) as a single product at various pH. In D_2O solutions the quantitative incorporation of deuterium at the 8-position was also observed. Pulse radiolysis revealed the "instantaneous" formation of a guanosine radical cation ($Guo^{\bullet+}$ or its deprotonated forms) in acid or basic solutions. The same transient species results from the reaction of $H^{\bullet}$ with 8-Br-Guo at pH 3, as well as from the reaction of $(CH_3)_2CO^{\bullet-}$ with 8-Br-Guo at pH 13. In neutral solution, the initial electron adduct was rapidly protonated to give the first observable transient species that decays by first-order kinetics ($k = 5 \times 10^4\ s^{-1}$) to produce the $Guo(-H^+)^{\bullet}$ radical once again. Tailored experiments allowed the reaction mechanism to be defined in some detail.

Introduction

During the last two decades a number of publications have appeared referring to the biological and immunological activities of 8-bromoguanosine (8-Br-Guo) and its 3′,5′-cyclic monophosphate. For example, it has been reported that the metabolic processing of 8-Br-Guo by guanosine specific enzymes is minimal as a consequence of the molecular conformational changes.[1] The presence of a bulky substituent such as the bromine atom at the C8 position of guanosine destabilizes the normal anti orientation of the base, sterically constraining the molecule to the syn conformation.[2] This arrangement, together with the change in the sugar conformation, is reflected in a modification of the ^{13}C and ^{1}H NMR spectra, giving a characteristic upfield shift of the C2′ signal and a downfield shift of the H2′ signal. More recently, 8-bromoguanosine-3′,5′-cyclic monophosphate has been found to be capable of decreasing basal and growth factor-stimulated DNA synthesis in primary aortic smooth muscle cells from newborn rats, displaying an antimitogenic effect similar to some NO-releasing agents.[3]

The interaction of ionizing and ultraviolet radiations with 5-bromo-substituted pyrimidine nucleosides has been intensively investigated.[4−8] It has been shown, for example, that 5-bromo-2′-deoxyuridine, when incorporated into DNA of both mammalian and bacterial cells, makes them more sensitive to the lethal effects of ionizing and ultraviolet radiations.[9] This property of 5-Br-uracil derivatives has been exploited in clinical studies[10] and has been used for mapping protein−nucleic acid interactions.[11] The addition of an electron to 5-Br-uracil derivatives affords the adduct **1** which fast releases a bromide ion with the formation of the vinyl radical **2** (eq 1).[5] Species **1** has also been reported by Saito and co-workers in the photoreaction of duplex



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oligonucleotides containg a 5′-A^{Br}U-3′ sequence.[12] These authors proposed single electron transfer from the adjacent adenine moiety of the 5′-side to the photoexcited 5-Br-uracil residue. In turn, vinyl radical **2** either abstracts a hydrogen (from a hydrogen donor[5,6] and/or from the closest sugar unit of the backbone[12]) or induces cross-linking to amino acid derivatives.[11,13] To our knowledge the analogous electron adducts of 8-bromo purine derivatives have not been reported to date.[14]

The effect of bromination was also considered for macromolecules such as RNA and DNA, where early studies showed the occurrence of mutations and changes in the amino acid composition of an isolated protein.[15] Bromination produces significant biochemical modifications of certain nucleic acids, the most important being the formation of 5-bromopyrimidine and 8-bromopurine nucleotides.[16] In their recent report Sevilla and co-workers studied with ESR spectroscopy the electron transfer occurring within bromine-doped DNA, which contained 5-bromo-6-hydroxy-5,6-dihydrothymine, 5-bromocytosine, and 8-bromoguanine moieties in a 0.2:1:0.23 ratio.[16] The observed paramagnetic species that result from the electron scavenging are assigned to pyrimidine-type adducts. Based on comparison with some available data, they suggested that 8-Br-dGuo is not expected to compete effectively for the electron with the other brominated moieties, although they pointed out the experimental difficulties associated with the determination of 8-Br-dGuo as an electron trap in the system.

Interestingly, von Sonntag and co-workers have shown that the electron adducts (and/or their heteroatom-protonated forms) of thymine, adenine, and cytosine transfer an electron to 5-Br-uridine, whereas the electron adduct of guanine does not behave accordingly.[6] They concluded that guanine can serve as the ultimate sink for the electron which is in antithesis with the general thought of the electron transfer within the DNA molecule, i.e., the electron loss resides at the guanine moieties and the electron gain is at the pyrimidine moieties.[17]

On the basis of these observations, we thought that 8-Br-Guo could be prompted to capture electrons and perhaps it loses the bromine ion rapidly like the 5-Br-uracil derivatives, to give the corresponding radical in the 8-position. This expectation is further strengthened by a very recent report which has shown that debromination of 8-Br-dGuo can be achieved in a quantitative yield by methylene blue and visible light similar to bromoaromatic compounds.[18] Herein we report detailed chemical radiation studies of 8-Br-Guo in aqueous solution where this mechanistic scenario is partially true. In particular, we have investigated the reaction of 8-Br-Guo with the reducing species: e_{aq}^-, $H^\bullet$, $(CH_3)_2CO^{\bullet-}$.

Results and Discussion

Generation of Radicals.[19] Radiolysis of neutral water leads to the species e_{aq}^-, $HO^\bullet$, and $H^\bullet$ as shown in eq 2 where the values in parentheses represent the yields expressed in terms of *G*-values (molecules/100 eV of absorbed radiation). The reactions of e_{aq}^- with the substrates were studied by irradiating deoxygenated solutions containing 0.25 M *t*-BuOH or 0.13 M *i*-PrOH. In the presence of *t*-BuOH, only $HO^\bullet$ radicals are scavenged (eq 3, $k_3 = 6.0 \times 10^8$ M^{-1} s^{-1}), whereas both $HO^\bullet$ and $H^\bullet$ species react with *i*-PrOH (eqs 4 and 5, $k_4 = 1.9 \times 10^9$ M^{-1} s^{-1}, $k_5 = 7.4 \times 10^7$ M^{-1} s^{-1}). In alkaline solution, in the presence of *t*-BuOH, additional e_{aq}^- is produced from the reaction of $H^\bullet$ with HO^- (eq 6, $k_6 = 2.2 \times 10^7$ M^{-1} s^{-1}), whereas at acidic pH (≤ 3) the reaction of e_{aq}^- with H^+ to give $H^\bullet$ (eq 7, $k_7 = 2.3 \times 10^{10}$ M^{-1} s^{-1}) becomes relevant.

$$H_2O \rightsquigarrow e_{aq}^- \ (2.6),\ HO^\bullet\ (2.7),\ H^\bullet\ (0.6) \tag{2}$$

$$HO^\bullet + (CH_3)_3COH \longrightarrow {}^\bullet CH_2C(CH_3)_2OH + H_2O \tag{3}$$

$$HO^\bullet + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2O \tag{4}$$

$$H^\bullet + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2 \tag{5}$$

$$H^\bullet + HO^- \rightleftharpoons e_{aq}^- + H_2O \tag{6}$$

$$e_{aq}^- + H^+ \longrightarrow H^\bullet \tag{7}$$

The $(CH_3)_2CO^{\bullet-}$ species was generated by irradiating N_2O-saturated solutions containing 0.13 M *i*-PrOH at pH 13. The presence of N_2O transforms efficiently e_{aq}^- into the $O^{\bullet-}$ radical (eq 8, $k_8 = 9.1 \times 10^9$ M^{-1} s^{-1}). The $HO^\bullet$ radical [pK_a($HO^\bullet$) = 11.9] is in equilibrium with its conjugated base $O^{\bullet-}$ (eq 9/−9, $k_9 = 1.2 \times 10^{10}$ M^{-1} s^{-1}, $k_{-9} = 1 \times 10^8$ s^{-1}). Hydrogen abstraction from *i*-PrOH by $HO^\bullet$, $H^\bullet$, and $O^{\bullet-}$ produces $(CH_3)_2C(^\bullet)OH$ which deprotonates to give $(CH_3)_2CO^{\bullet-}$ (eq 10, pK_a = 12.03).[20] The radical anion is a better reductant than the neutral radical, ($E°[(CH_3)_2CO,H^+/(CH_3)_2C(^\bullet)OH] = -1.39$ V, $E°[(CH_3)_2CO/(CH_3)_2CO^{\bullet-})] = -2.1$ V).[21,22]

$$e_{aq}^- + N_2O \longrightarrow O^{-\bullet} + N_2 \tag{8}$$

$$HO^\bullet + HO^- \rightleftharpoons O^{-\bullet} + H_2O \tag{9/-9}$$

$$(CH_3)_2\dot{C}OH + HO^- \rightleftharpoons (CH_3)_2CO^{-\bullet} + H_2O \tag{10/-10}$$

The radicals $CO_2^{\bullet-}$ [$E°(CO_2/CO_2^{\bullet-}) = -1.91$ V] and $CH_2O^{\bullet-}$ [the conjugated base of $^\bullet CH_2OH$: pK_a($^\bullet CH_2OH$) = 10.71; $E°(CH_2O/CH_2O^{\bullet-}) = -1.81$ V][21] were generated by irradiating N_2O-saturated solutions at pH 13 containing 0.1 M sodium formate or methanol, respectively. The rate constants for the scavenging of $H^\bullet$ and $HO^\bullet$ radicals by the formate ion are 2.1

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Table 1. Rate Constants (M^{-1} s^{-1})[a] for the Reaction of e_{aq}^-, $H^\bullet$, and $Br_2^{\bullet-}$ with 8-Bromoguanosine (8-Br-Guo) and Guanosine (Guo), Together with pK_a Values of the Two Nucleosides

	8-Br-Guo (pH)[b]	Guo (pH)
pK_a[c]	1.4, 8.4, 12.4	1.9, 9.25, 12.33[d]
$k(e_{aq}^- + S)$	1.1×10^{10} (7)	6×10^9 (7)[e,f]
$k(e_{aq}^- + S^-)$	5.0×10^9 (13)	9×10^7 (12)[e,f]
$k(H^\bullet + S)$	4.5×10^8 (3)	5×10^8 (6.5)[e]
$k(Br_2^{\bullet-} + S)$	4.5×10^7 (7)	3.9×10^7 (7)[g]
$k(Br_2^{\bullet-} + S^-)$	3.0×10^8 (13)	2.5×10^8 (13)[d]

[a] At 22 ± 2 °C; estimated to be accurate to ±10%. [b] This work. [c] p$K_a \cong 12.4$ refers to the deprotonation of the sugar moiety. [d] From ref 23. [e] From ref 26. [f] From ref 6. [g] From ref 27.

$\times 10^8$ and 3.2×10^9 M^{-1} s^{-1}, respectively; the rate constants for the scavenging of these radicals by methanol are 2.6×10^6 and 9.7×10^8 M^{-1} s^{-1}, respectively.

The dibromine radical anion, $Br_2^{\bullet-}$, was generated by irradiating N_2O-saturated solutions containing 0.1 M KBr. Under these conditions, at pH 7, the $HO^\bullet$ radical is converted into the dibromine radical anion through the process shown in eq 11 ($k_{11} = 1.1 \times 10^{10}$ M^{-1} s^{-1}). At pH 13, the $O^{\bullet-}$ species also generates $Br_2^{\bullet-}$ radicals through reaction 12 ($k_{12} = 2.2 \times 10^8$ M^{-1} s^{-1}).

$$Br^- + HO^\bullet \xrightarrow{Br^-} Br_2^{-\bullet} + HO^- \qquad (11)$$

$$Br^- + O^{-\bullet} \xrightarrow{Br^-,\ H_2O} Br_2^{-\bullet} + 2\,HO^- \qquad (12)$$

Acid−Base Properties of 8-Br-Guo. At pH ∼7, the spectrum of 8-Br-Guo shows two bands at 210 and 265 nm with $\epsilon = 2.1 \times 10^4$ and 1.5×10^4 M^{-1} cm^{-1}, respectively. Since the spectrum is pH dependent, the acid−base properties of the species could be determined by following the changes in absorbance at selected wavelengths. From the analysis of the optical density vs pH curves, the pK_a values reported in Table 1 were obtained. For comparison purposes, in Table 1 the pK_a values for Guo are also reported.[23] For 8-Br-Guo the pK_a value of 1.4, representing the protonation of one of the nitrogen sites, is slightly lower than the corresponding value of 1.9 for Guo. The value of pK_a = 8.4 obtained for 8-Br-Guo represents the deprotonation of the NH group and is almost one pK_a unit lower than the corresponding value reported for Guo, i.e., 9.25. The difference probably arises from the presence of the electron-withdrawing bromine atom in the 8-position.[24] The value of pK_a ∼ 12.4 observed for 8-Br-Guo and Guo represents a deprotonation from the sugar moiety, presumably from the 2′ position, where the alkoxy anion formed is stabilized by an intramolecular hydrogen bond with the neighbor OH group.[25]

γ-Radiolysis of 8-Br-Guo. Aqueous solutions of 8-Br-Guo (ca. 1.5 mM) and *t*-BuOH (0.25 M) at pH 3, ∼7, and 13 were prepared. Control experiments showed that 8-Br-Guo is stable at the various pH values used for at least 3 days. The samples containing 10 mL of deoxygenated solution were irradiated under stationary-state conditions with a dose rate of ca. 27 Gy/min followed by HPLC analysis. In all experiments, the formation of Guo was identified by comparison of the retention time with an authentic sample. At pH ∼7 and 13 mass balances obtained by HPLC using 8-Br-Guo and Guo as external standards were close to 100%. At pH ∼7 bromide ion was detected by HPLC and its yield was found to be identical with that of Guo. At pH 3 Guo was the predominant product, although small amounts of a second product which has not yet been identified were also formed. Figure 1 shows the consumption of 8-Br-Guo and the formation of Guo. The concentrations of 8-Br-Guo and Guo decreased and increased, respectively, as the dose increased in the range of 0−1.5 kGy. Furthermore, the efficiency of debromination decreased with decreasing the pH of the medium.

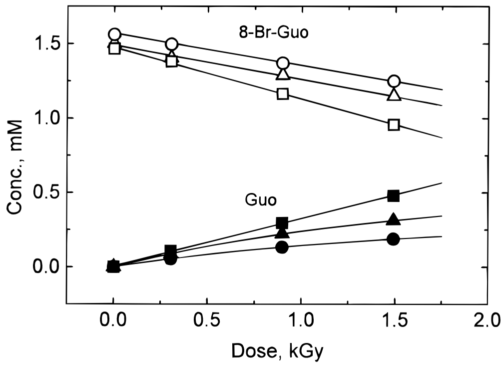

Figure 1. Effect of irradiation dose on the consumption of 8-Br-Guo and the formation of Guo from the continuous irradiation of vacuum degassed solutions containing ca. 1.5 mM 8-Br-Guo and 0.25 M *t*-BuOH at pH 3 (○, ●), ∼7 (△, ▲), and 13 (□, ■).

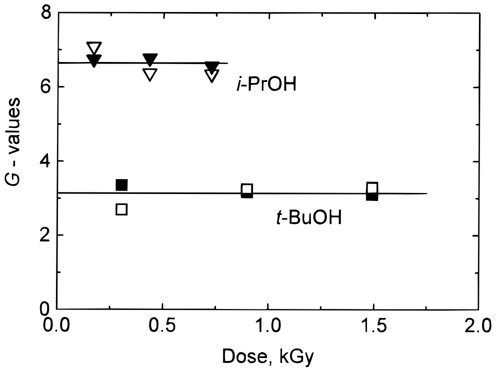

Figure 2. *G*-values as a function of irradiation dose for the consumption of 8-Br-Guo (▽, □) and the formation of Guo (▼, ■) from the continuous irradiation of vacuum degassed solutions containing ca. 1.5 mM 8-Br-Guo and 0.13 M *i*-PrOH or 0.25 M *t*-BuOH at pH 13. The lines are the linear fit to the average data of *G*[−(8-Br-Guo)] and *G*(Guo).

Analysis of the data in terms of *G*-values showed that (i) at pH 13 *G*[−(8-Br-Guo)] and *G*(Guo) are close to 3.2 and independent of the dose (Figure 2), (ii) at pH 7 *G*[−(8-Br-Guo)] and *G*(Guo) are close to 3.2 when the line is extrapolated at zero dose, and (iii) at pH 3 the *G*[−(8-Br-Guo)] and *G*(Guo) are close to 2 when the lines are extrapolated at zero dose (see Supporting Information). Taking into account that $G(e_{aq}^-) + G(H^\bullet) = 3.2$ (eq 2) and that hydrogen atoms are converted into solvated electrons at pH 13 (eq 6) whereas the solvated electrons are partially converted into hydrogen atoms at pH 3 (eq 7), our results lead to the conclusion that all solvated electrons, and to some minor extent the hydrogen atoms, react quantitatively with 8-Br-Guo to yield Guo. In this respect, the reaction could also be a useful synthetic tool.[18] In fact, a sample irradiated for 3 h at pH 13 afforded Guo in 84% yield as determined by HPLC using an external standard (see Supporting Information).

When *i*-PrOH replaced *t*-BuOH at pH 13, the efficiency of the 8-Br-Guo conversion to Guo increased appreciably. Figure 2 shows that the *G*-values in *i*-PrOH are more than twice those

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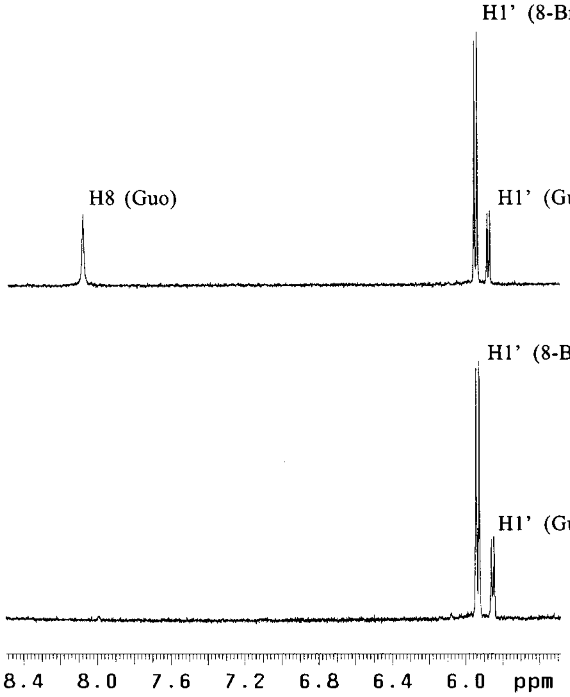
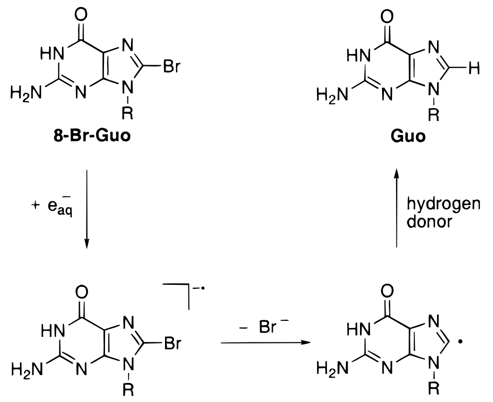


Figure 3. ^{1}H NMR spectra in the range of 5.8 to 8.4 ppm obtained after irradiation of 8-Br-Guo in H_2O (upper spectrum) or in D_2O (lower spectrum) at pH 13.

Scheme 1



in *t*-BuOH. At pH 13 the $(CH_3)_2C(^{\bullet})OH$ radical is mainly in the form of its conjugated base (see eq 10/−10) which is a good reductant. Therefore, we suggest that at pH 13 both e_{aq}^- and $(CH_3)_2CO^{\bullet-}$ are able to reduce 8-Br-Guo.

From a mechanistic point of view, these results suggest that the reaction of e_{aq}^- with 8-Br-Guo produces Guo in a manner similar to the reaction of e_{aq}^- with 5-bromouridine which produces uridine. Thus the addition of an electron to 8-Br-Guo results in the release of a bromide ion and the corresponding radical at the 8-position that reacts with *t*-BuOH or *i*-PrOH to afford Guo (Scheme 1).

To investigate the origin of the hydrogen donor the reactions were also performed in D_2O. The samples containing 8-Br-Guo (ca. 1.5 mM) and *t*-BuOH (0.25 M) in D_2O at different pH were irradiated with a dose of 1.5 kGy followed by ^{1}H NMR analysis. To our surprise, in all cases a deuterium atom was incorporated in the 8-position, i.e., 8-D-Guo. Figure 3 shows for comparison the ^{1}H NMR spectra in the range of 5.5 to 8.5 ppm of the reactions carried out at pH 13 in H_2O and D_2O, respectively. It is well-known from the literature that hydrogen−deuterium exchange at the C8 position of guanosine and related nucleotides is a facile process.[28] Arrhenius expressions are available for these reactions which are governed by pseudo-first-order rate constants. These kinetic data suggest that under our conditions the hydrogen−deuterium exchange is unimportant. In fact, control experiments performed on a solution of Guo under the same experimental conditions showed no such exchange. Therefore, the mechanism reported in Scheme 1 cannot be correct since the hydrogen in the 8-position originated from water.

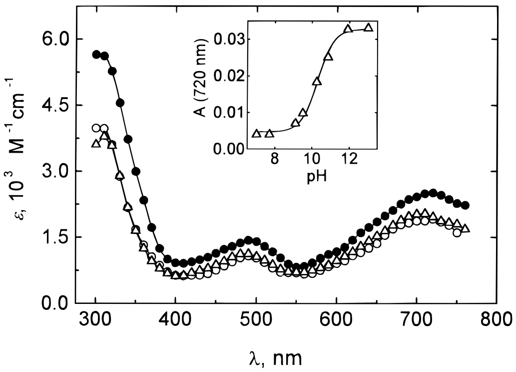


Figure 4. Absorption spectra obtained from the pulse radiolysis at pH 13 of (△) 1 mM 8-Br-Guo, 0.25 M *t*-BuOH, Ar-purged, 2 μs after the pulse; (○) 1 mM Guo, 0.1 M KBr, N_2O-saturated, 30 μs after the pulse; and (●) 1 mM 8-Br-Guo, 0.1 M KBr, N_2O-saturated, 30 μs after the pulse. Inset: pH dependence of absorption at 720 nm obtained from the pulse radiolysis of Ar-purged solutions containing 1 mM 8-Br-Guo and 0.25 M *t*-BuOH; dose = 22 Gy, optical path = 2.0 cm. The solid line represents the pK_a equation fit to the data; the inflection point was obtained at pH 10.4.

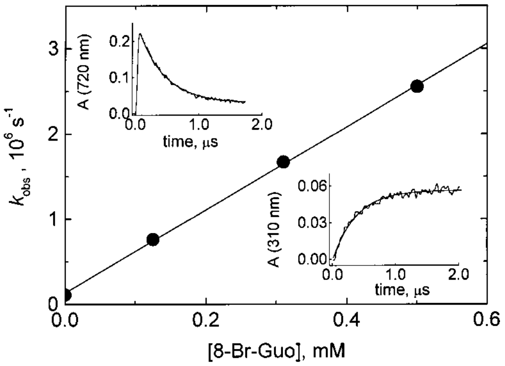


Figure 5. Plot of k_{obs} vs [8-Br-Guo] for the decay of e_{aq}^- (λ_{mon} = 720 nm) and for the buildup of product (λ_{mon}= 310 nm) from the pulse radiolysis of Ar-purged solutions containing 0.25 M BuOH abd 0.1 M NaOH. Insets: Decay observed at 720 nm (upper left) and buildup observed at 310 nm (lower right) in the presence of 0.5 mM 8-Br-Guo; optical path = 2.0 cm, dose per pulse = 22 Gy. The solid lines represent the first-order kinetic fits to the data.

Pulse Radiolysis Studies in Basic Solutions. The optical absorption spectrum obtained after the reaction of e_{aq}^- with 8-Br-Guo at pH 13, taken 2 μs after the pulse, is shown in Figure 4 (open triangles). The spectrum contains three bands centered at 310, 480, and 720 nm, respectively. The ϵ-values were calculated using $G = 3.2$, which represents the sum of $G(e_{aq}^-)$ and $G(H^{\bullet})$ since the $HO^{\bullet}$ radicals are scavenged by the *t*-BuOH (cf. eqs 2 and 3). The time profile of the formation of this transient (Figure 5, right inset) or the disappearance of e_{aq}^-

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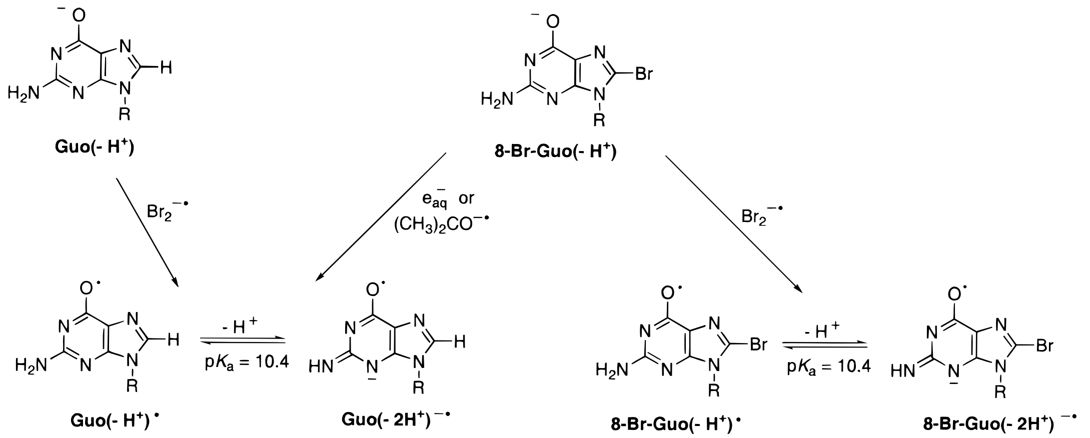
(26) Candeias, L. P.; Wolf, P.; O'Neill, P.; Steenken, S. *J. Phys. Chem.* **1992**, *96*, 10302.

(27) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094.

(28) Lane, M. J.; Thomas, C. J., Jr. *Biochemistry* **1979**, *18*, 3839. Livramento, J.; Thomas, C. J., Jr. *J. Am. Chem. Soc.* **1974**, *96*, 6529. Tomasz, M.; Olson, J.; Mercado, C. M. *Biochemistry* **1972**, *11*, 1235.

Scheme 2



(Figure 5, left inset)[29] leads to a pseudo-first-order rate constant, k_{obs}, and was measured at a number of different 8-Br-Guo concentrations. From the slope of the linear plot, the bimolecular rate constant was found to be 5.0×10^9 M^{-1} s^{-1} (Figure 5). In comparison with the analogous reactions of Guo (see Table 1), the presence of bromine increases the rate constant of the reduction by a factor of 55. The transient decays by second-order kinetics with $2k = 6.8 \times 10^8$ M^{-1} s^{-1}, and its decay does not give rise to absorbing species at $\lambda > 320$ nm.

The above-described spectrum resembled in shape and ϵ-values those obtained by the reaction of Guo with oxidants such as $SO_4^{•-}$ or $Br_2^{•-}$.[27] To closely compare the spectra of oxidized Guo, we also examined the reactions of $Br_2^{•-}$ with Guo and 8-Br-Guo.[30] The reactions were carried out in N_2O-saturated solutions cantaining 0.1 M KBr (vide infra). The optical absorption spectra obtained at pH 13 after the completion of the reactions of Guo and 8-Br-Guo with $Br_2^{•-}$ (30 μs after the pulse) are also shown in Figure 4. The spectra obtained from the oxidation of Guo (open circles) and from the reduction of 8-Br-Guo (open triangles) are identical. Furthermore, the OD change at 720 nm for reduced 8-Br-Guo follows a pK_a curve profile with an inflection point at pH 10.4 (Figure 4, inset and Scheme 2) which is in good agreement with the inflection point at pH 10.7 reported for the oxidized Guo.[27] On the other hand, the spectrum obtained from the oxidation of 8-Br-Guo (solid circles) showed a similar shape although the ϵ-values were higher than those obtained for the oxidized Guo. A similar pK_a value was also measured (Scheme 2). The rate constants for the reactions of $Br_2^{•-}$ with 8-Br-Guo were determined (dose per pulse = 9−23 Gy) by measuring the rate of optical density decrease of $Br_2^{•-}$ as a function of the concentration of 8-Br-Guo.[29] The bimolecular rate constant was found to be 3.0×10^8 M^{-1} s^{-1}, which is similar to that of the analogous reaction of Guo (Table 1). Oxidized 8-Br-Guo and oxidized Guo decay by second-order kinetics with $2k = 1.5 \times 10^8$ and 2×10^8 M^{-1} s^{-1}, respectively.

The reactivity of the $(CH_3)_2CO^{•-}$ radical anion toward 8-Br-Guo at pH 13 was also examined. It was found that $(CH_3)_2CO^{•-}$ reacts with 8-Br-Guo with $k = 8.3 \times 10^7$ M^{-1} s^{-1} giving a transient identical with that obtained after reduction by e_{aq}^-. This transient decays by second-order kinetics with $2k = 1.9 \times 10^8$ M^{-1} s^{-1}. On the other hand, no reaction was observed between the species $CO_2^{•-}$ and $CH_2O^{•-}$ with 8-Br-Guo.

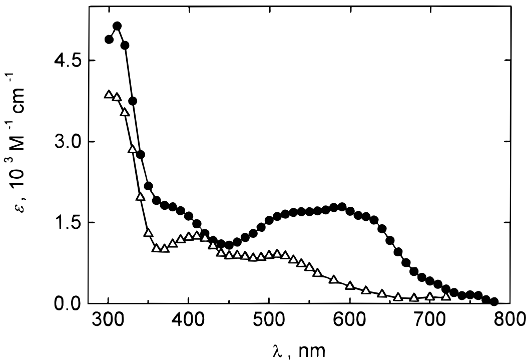


Figure 6. Absorption spectra obtained from the pulse radiolysis of Ar-purged solutions containing 1 mM 8-Br-Guo: (●) at pH ∼7 in the presence of 0.13 M *i*-PrOH, taken 2 μs after the pulse, and (△) at pH 3 in the presence of 0.25 M *t*-BuOH, taken 8 μs after the pulse.

At pH 13, 8-Br-Guo and Guo are present in their deprotonated forms at the base moiety, i.e., 8-Br-Guo(-H^+) and Guo(-H^+), respectively (Table 1 and Scheme 2). There is no doubt that the species obtained from the reaction of 8-Br-Guo(-H^+) with e_{aq}^- or $(CH_3)_2CO^{•-}$ is identical with that obtained from the reaction of Guo(-H^+) with $Br_2^{•-}$. The electron-adduct of 8-Br-Guo(-H^+), upon elimination of Br^-, would give a species equivalent to Guo(-2H^+)$^{•-}$ with a pK_a = 10.4 (Scheme 2). Although the reaction may seem stoichiometrically simple, the γ-radiolysis results show that the mechanism is not. Attempts to detect any intermediate have been unsuccessful. For example, the kinetics at 720 (representing mainly the decay of e_{aq}^-) and 310 nm (representing mainly the formation of Guo(-2H^+)$^{•-}$) were the same even at the highest concentration of the substrate (0.5 mM) used for these experiments. In other words, the formation of Guo(-2H^+)$^{•-}$ is "instantaneous" with a rate constant higher than 10^7 s^{-1}, i.e., the process should be complete in less than 300 ns.

Pulse Radiolysis Studies in Neutral Solutions. A rate constant of 1.1×10^{10} M^{-1} s^{-1} was determined for the reactions of 8-Br-Guo with e_{aq}^- at pH ∼7 by measuring the rate of the optical density decrease of e_{aq}^- as a function of the concentration of the added nucleoside.[29] This value is slightly higher than the analogous reaction of Guo at the same pH (Table 1).[6,26] Figure 6 shows the optical absorption spectrum obtained from the reaction of 8-Br-Guo with e_{aq}^- (solid circles). The ϵ-values were calculated using $G = 2.6$, since the $H^•$ and $HO^•$ species are scavenged by the *i*-PrOH and therefore do not contribute to the reaction.[31] The same spectrum that contains a sharp band with

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[29] For e_{aq}^- at 720 nm, $\epsilon = 1.9 \times 10^4$ $M^{-1}cm^{-1}$; for $Br_2^{•-}$ at 360 nm, $\epsilon = 1 \times 10^4$ $M^{-1}cm^{-1}$: Hug, G. L. *Natl. Stand. Ref. Data Ser., Natl. Bur. Stand.* **1981**, No. 69.

[30] The reaction of $Br_2^{•-}$ with Guo has previously been studied by pulse radiolysis at pH 12−13.[23, 27] However, we were obliged to repeat some of these experiments since some information of interest to us was not given in these papers.

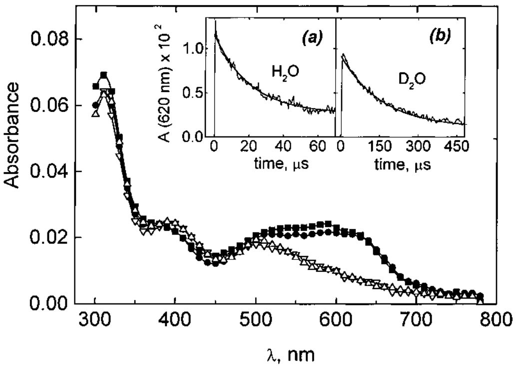

Figure 7. Absorption spectra obtained from the pulse radiolysis (dose = 24 Gy) of Ar-purged solutions containing 1 mM 8-Br-Guo at pH ~7 with 0.25 M *t*-BuOH, taken 2 (■) and 45 μs (△) after the pulse; at pH 7.7 (buffered with phosphates), with 0.25 M *t*-BuOH, taken 2 μs after the pulse (●); and at pH 4 with 0.25 M *t*-BuOH, taken 2 μs after the pulse (▽). Insets: Time dependence of absorption at 620 nm from the pulse radiolysis of Ar-purged solutions containing 1 mM 8-Br-Guo and 0.13 M *i*-PrOH at pH ~7 in H_2O (a) and in D_2O (b); dose per pulse = 9 Gy; optical path = 2.0 cm. The solid lines represent the first-order kinetic fits to the data from which $k(H_2O) = 5.0 \times 10^4$ s^{-1} and $k(D_2O) = 6.2 \times 10^3$ s^{-1} were obtained.

$\lambda_{max} = 310$ nm and a broad band in the 500−650 nm region was obtained by replacing *i*-PrOH with *t*-BuOH (Figure 7, solid squares). The decay of this transient (monitored at 620 nm) follows first-order kinetics.[33] The measured rates were found to increase slightly with increasing doses, which indicates the presence of second-order contributions owing to radical−radical reactions. To minimize this effect, the rate was measured at a low dose (9 Gy), where $k = 5.0 \times 10^4$ s^{-1} was obtained (Figure 7, inset a).[34] When H_2O was replaced by D_2O, the decay at 620 nm became slower, with $k = 6.2 \times 10^3$ s^{-1} (Figure 7, inset b), i.e., the solvent kinetic isotope effect (k_H/k_D) is 8.0. Furthermore, the disappearance of this species gives rise to a new transient the spectrum of which, taken 45 μs after the pulse, is also shown in Figure 7 (open triangles). The last transient is very similar to that obtained after the oxidation of Guo by $Br_2^{\bullet -}$ at pH ~7, taken 100 μs after the pulse to allow the completion of the $Br_2^{\bullet -}$ decay.[35]

It was also found that the rate of decay at 620 nm is influenced by phosphates. The absorption spectra obtained 2 μs after the pulse at pH ~7 in the presence and in the absence of phosphates are almost superimposable (Figure 7). However, the presence of phosphates accelerated the decay at 620 nm, with k_{obs} increasing linearly with $[H_2PO_4^-]$, resulting in $k_{phosp} = 1.4 \times 10^8$ M^{-1} s^{-1}. In addition, the spectra, recorded after completion of the decay of the first transient obtained in the absence or in the presence of phosphates were found to be identical with each other. Furthermore, the rate of decay at 620 nm increases with increasing [H$^+$], so that at pH 4, 2 μs after the pulse, the band at 620 nm has already disappeared (Figure 7, open triangles). From the kinetic analysis of a fast component observed at pH 4 and from computer modeling of the system,[37] the rate constant for the reaction with H$^+$ was estimated to be $k_{H^+} \cong 1 \times 10^{10}$ M^{-1} s^{-1}.

To obtain information about the reactivity of the transient species originating from the reactions of 8-Br-Guo with e_{aq}^- at pH ~7, its redox properties were investigated toward methyl viologen (MV^{2+}) and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD).[38,39] The experiments were performed in Ar-purged solutions containing, in addition to 1 mM 8-Br-Guo and 0.25 M *t*-BuOH, 20−100 μM MV^{2+} or TMPD. The results showed that this radical is unable to reduce MV^{2+} whereas it is able to effect the oxidation of TMPD.[42] Inasmuch as the radical derived from *t*-BuOH is unreactive toward TMPD, we concluded that the TMPD$^{\bullet +}$ radical cation formed in the system originates solely from the species obtained from the reactions of e_{aq}^- and H$^\bullet$ with 8-Br-Guo. The yield of TMPD$^{\bullet +}$ increased with increasing 8-Br-Guo concentration, approaching a plateau that corresponded to 80% (lower limit) of the sum of the yields of e_{aq}^- and H$^\bullet$. A rate constant of $k = 1.7 \times 10^9$ M^{-1} s^{-1} was measured.[44,45]

The mechanism we conceived for the reactions discussed above is illustrated in Scheme 3. The observed initial transient at neutral pH is assigned to radical **4**. That is, the reaction of e_{aq}^- with 8-Br-Guo should afford the radical anion **3** which is expected to be rapidly protonated to yield the neutral radical **4**.[46] This intermediate, which is unreactive toward MV^{2+} and molecular oxygen, efficiently oxidizes TMPD to give the corresponding cation. The decay of **4** follows a first-order kinetics to give Guo(-H$^+$)$^\bullet$ with a rate constant of 5.0×10^4 s^{-1}. By replacing H_2O with D_2O as the reaction medium, a kinetic isotopic effect $k_H/k_D = 8.0$ was determined which

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[31] (a) By examining a N_2O-saturated solution containing 8-Br-Guo and *i*-PrOH we established that the $(CH_3)_2C(^\bullet)OH$ radical does not react with 8-Br-Guo at pH 7. (b) On the other hand, some reactivity of the $(CH_3)_2C(^\bullet)OH$ radical toward 8-Br-Guo was instead observed at pH 3.[32]

[32] This behavior is similar to that previously observed in the case of adenosine which does not react with the 2-propanol radical at pH 7 whereas at pH 2 it does, see: Moorthy, P. N.; Hayon, E. *J. Am. Chem. Soc.* **1975**, *97*, 3345.

[33] The addition of molecular oxygen has no effect on the decay of this transient species.

[34] This rate constant was found to be independent of the nature and concentration of the HO$^\bullet$ scavengers *t*-BuOH or *i*-PrOH (0.1−1.0 M). This observation rules out the possibility that the transient decays by reacting with the alcohol, for example by abstracting from it a hydrogen atom.

[35] The reaction of $Br_2^{\bullet -}$ with Guo is relatively slow (Table 1) so that the disproportionation reaction of $Br_2^{\bullet -}$, $2k = 3.8 \times 10^9$ M^{-1}s^{-1} at the ionic strength = 0.1, had to be taken into account when the ϵ-values of oxidized Guo at pH 7 were calculated. The disproportionation of $Br_2^{\bullet -}$ generates Br$^-$ and Br_3^- which absorbs strongly at 266−270 nm ($\epsilon \sim 4 \times 10^4$ M^{-1}cm^{-1}).[36] The difference between the spectra of oxidized Guo and the spectrum of reduced 8-Br-Guo in the 300 nm region could be due to the effect of the tail of the spectrum of Br_3^- (see Supporting Information).

[36] Rafi, A.; Sutton, H. C. *Trans. Faraday Soc.* **1965**, *61*, 877. Treinin, A.; Hayon, E. *J. Am. Chem. Soc.* **1975**, *97*, 1716. Wong, D.; Di Bartolo, B. *J. Photochem.* **1975**, *4*, 249−268.

[37] Curtis, A. R.; Sweetenham, W. P. FACSIMILE/CHEKMAT Users' Manual; AERE 12805, UKAEA, 1988.

[38] MV^{2+}, $E°(MV^{2+}/MV^{\bullet +}) = -0.45$ V and TMPD, $E°(TMPD^{\bullet +}/TMPD) = 0.27$ V.[39] The rate constants for the reduction of MV^{2+} and/or oxidation of TMPD, and the associated yields of MV$^{\bullet +}$ and/or TMPD$^{\bullet +}$, were determined at 602 and 565 nm, respectively, which represent the absorption maxima of MV$^{\bullet +}$ ($\epsilon = 1.37 \times 10^4$ M^{-1} cm^{-1})[40] and TMPD$^{\bullet +}$ ($\epsilon = 1.25 \times 10^4$ M^{-1} cm^{-1}),[41] respectively.

[39] Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637 and references therein.

[40] Watanabe, T.; Honda, K. *J. Phys. Chem.* **1982**, *86*, 2617.

[41] Fujita, S.; Steenken, S. *J. Am. Chem. Soc.* **1981**, *103*, 2540.

[42] It must be noted that, under the conditions employed, i.e., [8-Br-Guo] ≥ 10 [TMPD], the competition between 8-Br-Guo and TMPD for e_{aq}^- is well in favor of 8-Br-Guo since $k(e_{aq}^- + \text{TMPD}) = 9.1 \times 10^7$ M^{-1} s^{-1}.[43]

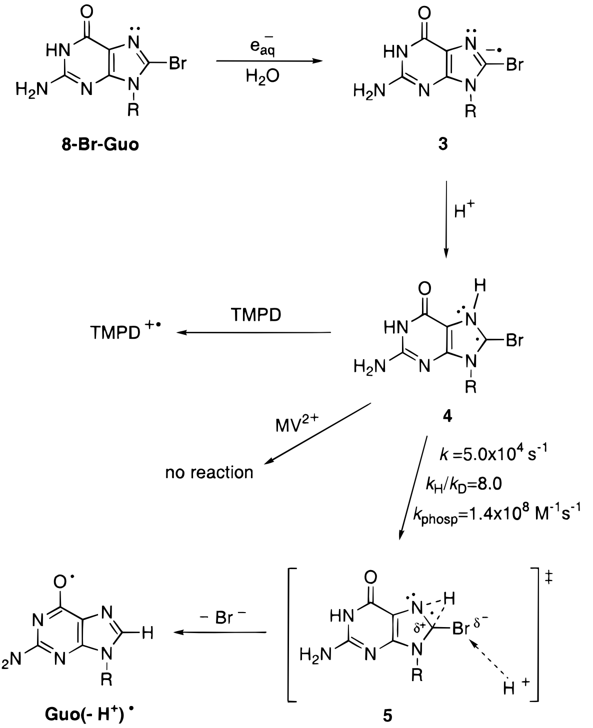
[43] Rao, P. S.; Hayon, E. *J. Phys. Chem.* **1975**, *79*, 1063.

[44] The plot k_{obs} vs [TMPD] shows an intercept corresponding to $k = 2 \times 10^4$ s^{-1}. This suggests a parallel path (possibly related to the second-order contributions observed for the decay at 620 nm) for the decay of the precursor of TMPD$^{\bullet +}$ that would account for about 10% diminution of the yield of TMPD$^{\bullet +}$. Therefore, the reaction of 8-Br-Guo and e_{aq}^-/H$^\bullet$ generates species possessing oxidazing properties in nearly quantitative yiels.

[45] The TMPD$^{\bullet +}$ radical cation is also formed in the presence of 0.01 M of phosphates. Since the formation, yield, and decay of TMPD$^{\bullet +}$ are similar in the absence and presence of phosphates, this suggests that both radical **4** and Guo(-H$^+$)$^\bullet$ (Scheme 3) oxidize TMPD with comparable rate constants. However, at higher concentrations of TMPD, the major path is the reaction of TMPD with radical **4**.

[46] Conductometric detection showed to be the case for the reaction of e_{aq}^- with Guo.[27]

Scheme 3



suggests the involvement of proton transfer or a complex transition state.[47] A transition state such as **5** is expected to be involved since this reaction is accelerated by phosphate and H^+ as found to be the case. It is also worth emphasizing that this mechanistic proposal is consistent with the γ-radiolysis studies which showed the incorporation of H or D atoms at C8 derived from H_2O and D_2O, respectively.[48,50]

Pulse Radiolysis Studies in Acidic Solutions. Figure 6 (open triangles) shows the optical absorption spectrum obtained from the reaction of 8-Br-Guo with e_{aq}^- and $H^\bullet$ at pH 3. The ϵ-values were calculated using $G = 3.2$, which represents the sum of $G(e_{aq}^-)$ and $G(H^\bullet)$.

The spectrum at pH 3 was generated in two steps: the first was complete in less than 1 μs and corresponds to the reaction of 8-Br-Guo with e_{aq}^- (vide infra), the second was complete in about 8 μs and assigned to the reaction of 8-Br-Guo with $H^\bullet$. Figure 8 shows that the two processes generated the same spectrum which contains one band centered at 310 nm and two broad and less intense bands around 400 and 500 nm. This transient spectrum resembles in shape and ϵ-values those obtained by the reactions of Guo with $SO_4^{\bullet-}$ which produced the guanosine radical cation ($Guo^{\bullet+}$).[27] Furthermore, a $pK_a \cong 3.8$ is observed for this transient which matched well with the reported value of 3.9 for $Guo^{\bullet+}$.[27]

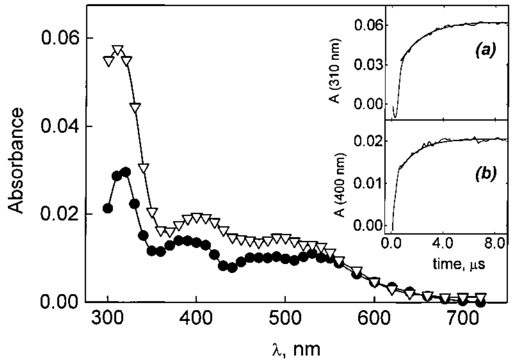
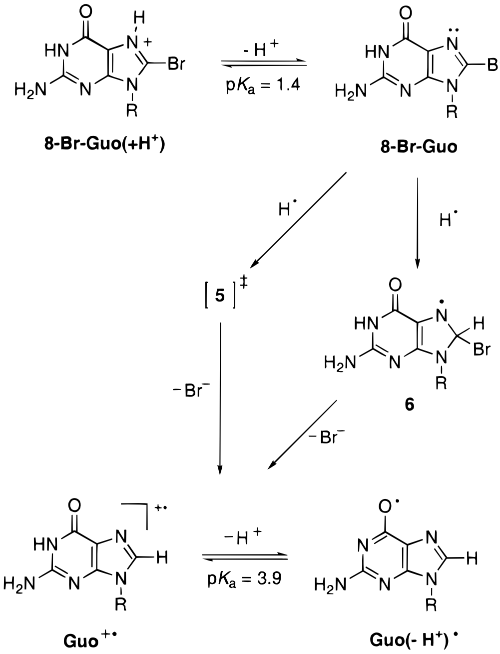


Figure 8. Absorption spectra obtained from the pulse radiolysis of an Ar-purged solution containing 1 mM 8-Br-Guo and 0.25 M *t*-BuOH at pH 3, taken 1 (●) and 8 μs (▽) after the pulse; dose = 24 Gy; optical path = 2.0 cm. Insets: Time profile of the absorptions at 310 (a) and 400 nm (b). The solid lines represent the first-order kinetic fits to the data.

Scheme 4



The rate constant for the reaction of $H^\bullet$ with 8-Br-Guo was obtained at pH 3 (in the presence of 0.25 M *t*-BuOH)[31b] from the linear dependence of the rate of the second buildup at 310 and 400 nm at different 8-Br-Guo concentrations (0.5 and 1.0 mM). Examples of this analysis are shown in Figure 8, insets a and b. A value of 4.5×10^8 M^{-1} s^{-1} was obtained that is similar to that for the reaction of $H^\bullet$ with Guo (Table 1).[26]

The reaction mechanism could involve the addition of $H^\bullet$ to the double bond located between N7 and C8 to give the amino radical **6**, followed by a very fast heterolytic cleavage of the β-CBr bond (Scheme 4).[51] In the transition state, the C−Br bond

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[47] Williams, J. M.; Kreevoy, M. M. In *Advances in Physical Organic Chemistry*; Gold, V., Ed.; Academic Press: London, 1968; Vol. 6, p 63. Saunders, W. H., Jr. In *Techniques of Chemistry*; Lewis, E. S., Ed.; Wiley: New York, 1974; Vol. 6, p 211.

[48] One referee suggested that an alternative mechanism may be present which is the loss of a free bromine atom ($Br^\bullet$) from the radical ion **3**, giving the 8-guanosinyl anion which is directly protonated to the product; the oxidation of TMPD (Scheme 3) could then be attributed to the $Br^\bullet$. Although this proposal could explain some of the observed results,[49] it is easy to verify since it is well-known that in the presence of Br^- the $Br^\bullet$ is converted into the detectable species $Br_2^{\bullet-}$, and therefore easily monitored.[19b] Pulse irradiation of Ar-purged solutions at pH ~7 and 13 containing 2 M *t*-BuOH and 1 mM 8-Br-Guo produced the same results both in the presence and in the absence of 5 mM Br^-. In other words, the presence of 5 mM Br^- did not show detectable amounts of $Br_2^{\bullet-}$ nor did it affect the kinetics for the formation of the spectrum of the oxidized species.

[49] This mechanism has been shown to operate in other systems, e.g., $Br^\bullet$ released from the reaction of e_{aq}^- with *N*-bromosuccinimide, see: Lind, J.; Shen, X.; Eriksen, T. E.; Merenyi, G.; Eberson, L. *J. Am. Chem. Soc.* **1991**, *113*, 4629.

[50] Recent ab initio calculations on the 8-Br-Guo$\bullet^-$ (intermediate **3** in Scheme 3) indicate that the unpaired electron resides at the six-membered ring mainly at the C atom bonded to the three nitrogens. These calculations suggest that the intermediate **3** can release neither a bromide ion nor a bromine atom. (We are grateful to Dr. M. Guerra for sharing these unpublished results with us).

[51] At pH 3 the protonated form of 8-Br-Guo is ca. 5% (Scheme 4). It is expected that e_{aq}^- reacts rapidly also with this form to give $Guo^{\bullet+}$ via intermediate **4** (see Scheme 3).

is expected to be strongly polarized, with the positive charge being delocalized on the neighboring nitrogens, and the interaction of H^+ with Br should facilitate the ejection of the bromide ion. However, a transition state such as **5** could also account for the reaction of the $H^\bullet$ atom with 8-Br-Guo to give $Guo^{+\bullet}$ and ultimately Guo.

Conclusions

The affinity of the solvated electron with 8-Br-Guo is very high. The rate constants of e_{aq}^- with 8-Br-Guo and its deprotonated form is much faster than the corresponding reactions of Guo or the analogous reaction of other nucleosides[19] and comparable to 5-bromouridine.[4,5] The results described demonstrate that the reaction of 8-Br-Guo with e_{aq}^- in neutral and acidic solutions leads quantitatively to Guo. The reaction mechanism is described in Scheme 3 and shows that radical **4** is involved as an intermediate followed by the ejection of a bromide ion to give the deprotonated form of the guanosine radical cation via the proposed transition state **5**. In basic solution the reaction is still more efficient, although the mechanism is not straightforward.[52] Therefore, the mechanistic scenario of the debromination of 8-Br-Guo by the action of e_{aq}^- is different from those determined for the bromoaryl and bromovinyl derivatives.[18] Furthermore, it should be noted that under continuous radiolysis in the whole range of pH we studied, how the $Guo^{+\bullet}$ or its deprotonated forms afford Guo is still unclear.[53,54] Further work on this intriguing mechanism as well as on the analogous reactions of 8-bromopurine derivatives is in progress.

Experimental Section

Materials. 8-Bromoguanosine (Aldrich) and guanosine (Fluka) were used as received. *N*,*N*,*N*′,*N*′-Tetramethyl-*p*-phenylenediamine was purified by sublimation. Sodium formate (Merck), *i*-PrOH (C. Erba for fluorimetry), *t*-BuOH (Fluka, puris.), KBr (Merck, Suprapur), and 1,1′-dimethyl-4,4′-bipyridinium dichloride (methyl viologen, MV^{2+}) (Aldrich or Sigma) were employed. Solutions were freshly prepared using water purified with a Millipore (Milli-Q) system; their pH was the "natural" pH (~7) or was adjusted with $HClO_4$ or NaOH (Merck, Suprapur) or buffered with phosphates (Merck). For the determination of the solvent kinetic isotope effect, D_2O (Aldrich, 99.8 atom % D) was used.

Pulse Radiolysis. Pulse radiolysis with optical absorption detection was performed by using a 12 MeV linear accelerator, which delivered 20−200 ns electron pulses with doses between 5 and 50 Gy, by which $OH^\bullet$, $H^\bullet$, and e_{aq}^- are generated with 1−20 μM concentrations.[55] The pulse irradiations were performed at room temperature (22 ± 2 °C) on samples contained in Spectrosil quartz cells of 2 cm optical path length. Solutions were protected from the analyzing light by means of a shutter and appropriate cutoff filters. The bandwidth used throughout the pulse radiolysis experiments was 5 nm. The radiation dose per pulse was monitored by means of a charge collector placed behind the irradiation cell and calibrated with an N_2O-saturated solution containing 0.1 M HCO_2^- and 0.5 mM methyl viologen, using $G\epsilon = 9.32 \times 10^4$ at 602 nm.[56] G(X) represents the number of molecules of species formed per 100 eV of energy absorbed by the system.

Continuous Radiolyses. Continuous radiolyses were performed at room temperature (22 ± 2 °C) on 10 mL samples using a ^{60}Co-Gammacell with a dose rate of ~27 Gy min^{-1}. The exact absorbed radiation dose was determined with the Fricke chemical dosimeter by taking $G(Fe^{3+}) = 15.5$.[57] The reactions of 8-Br-Guo with e_{aq}^- and $H^\bullet$ were investigated using vacuum degassed solutions containing 1.5 mM substrate and 0.25 M *t*-BuOH that were irradiated with appropriate doses. The solutions irradiated at pH 3 and 13 were neutralized with the required amount of NaOH, 0.1 and HCl 0.1 M, respectively, followed by HPLC or NMR analysis. Qualitative and quantitative HPLC studies were performed on a Waters Associates Unit equipped with a model 600 multisolvent delivery system and controller, a 486 tunable absorbance detector, and a Millenium 2010 data workstation. The aqueous solutions of the samples were loaded after suitable neutralization onto a reverse-phase analytical column (Waters Spherisorb, S5ODS2, C18, 150 × 4.6 mm, 3 mm), eluted in water with a 0−90% methanol gradient over 30 min at a flow rate of 1 mL/min, and detected at 254 nm. Absolute concentrations of 8-Br-Guo and Guo were interpolated from external standard calibration curves. The absolute concentration of the bromide ion released was detected at 210 nm using KBr as external standard. ^{1}H NMR spectra were performed on a Varian Mercury 400 MHz.

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Supporting Information Available: *G*-values dependence, kinetic data, and absorption spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(52) We believe a mechanistic scheme similar to that at neutral solution is the most appropriate one. We suggest that at pH 13 the deprotonated form (at the N1 position) of intermediate **4** could lose a bromide ion rapidly with the concomitant 1,2H shift. The driving force should be the delocalization of the negative charge which favors a C−Br polarized bond.

(53) It has been suggested that the hydration of the guanine radical cation in double-stranded DNA may be responsible for the formation of 8-oxo-dGuo moiety.[54] For example, see: Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692. Cullis, P. M.; Malone, M. E.; Merson-Davies, L. A. *J. Am. Chem. Soc.* **1996**, *118*, 2775. Meggers, E.; Michel-Beyerle, M. E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950.

(54) However, it is well-known that 8-oxo-Guo is not derived from the $Guo^{\bullet+}$ (Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1992**, *114*, 699) which is in agreement with our results.

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