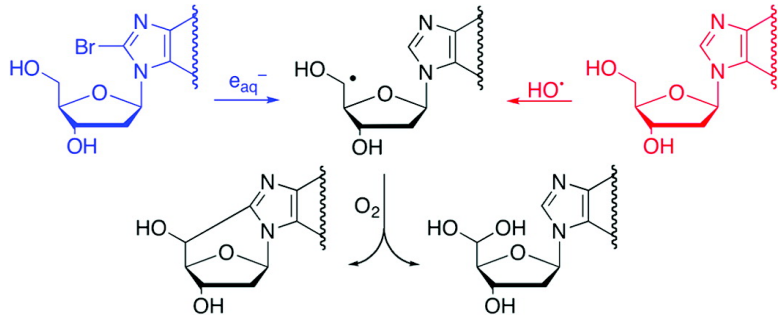
Article

The Fate of C5# Radicals of Purine Nucleosides under Oxidative Conditions

Fabien Boussicault, Panagiotis Kaloudis, Clara Caminal, Quinto G. Mulazzani, and Chryssostomos Chatgilialoglu

J. Am. Chem. Soc., **2008**, 130 (26), 8377-8385 • DOI: 10.1021/ja800763j • Publication Date (Web): 04 June 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES

Published on Web 06/04/2008

The Fate of C5′ Radicals of Purine Nucleosides under Oxidative Conditions

Fabien Boussicault, Panagiotis Kaloudis, Clara Caminal, Quinto G. Mulazzani, and Chryssostomos Chatgilialoglu*

ISOF, Consiglio Nazionale delle Ricerche, Via P. Gobetti 101, 40129 Bologna, Italy

Received January 30, 2008; E-mail: chrys@isof.cnr.it

Abstract: The factors that influence the reactivity of C5′ radicals in purine moieties under aerobic conditions are unknown not only in DNA, but also in simple nucleosides. 5′,8-Cyclopurine lesions are the result of a rapid C5′ radical attack to the purine moieties before the reaction with oxygen. These well-known lesions among the DNA modifications were suppressed by the presence of molecular oxygen in solution. Here we elucidate the chemistry of three purine-substituted C5′ radicals (i.e., 2′-deoxyadenosin-5′-yl, 2′-deoxyinosin-5′-yl, and 2′-deoxyguanosin-5′-yl) under oxidative conditions using γ-radiolysis coupled with product studies. 2′-Deoxyadenosin-5′-yl and 2′-deoxyinosin-5′-yl radicals were selectively generated by the reaction of hydrated electrons (e_{aq}^-) with 8-bromo-2′-deoxyadenosine and 8-bromo-2′-deoxyinosine followed by a rapid radical translocation from the C8 to the C5′ position. Trapping these two C5′ radicals with $Fe(CN)_6^{3-}$ gave corresponding hydrated 5′-aldehydes in good yields that were isolated and fully characterized. When an oxygen concentration in the range of 13−266 μM (typical oxygenated tissues) is used, the hydrated 5′-aldehyde is accompanied by the 5′,8-cyclopurine nucleoside. The formation of 5′,8-cyclopurines is relevant in all experiments, and the yields increased with decreasing O_2 concentration. The reaction of HO$^\bullet$ radicals with 2′-deoxyadenosine and 2′-deoxyguanosine under normoxic conditions was also investigated. The minor path of C5′ radicals formation was found to be ca. 10% by quantifying the hydrated 5′-aldehyde in both experiments. Rate constants for the reactions of the 2′-deoxyadenosin-5′-yl with cysteine and glutathione in water were determined by pulse radiolysis to be $(2.1 \pm 0.5) \times 10^7$ and $(4.9 \pm 0.6) \times 10^7$ M^{-1} s^{-1} at 22 °C, respectively.

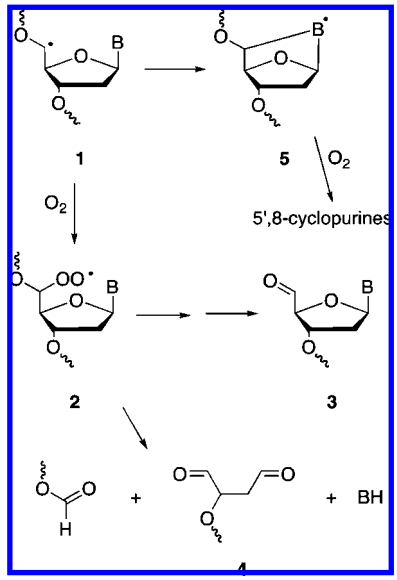
Introduction

Hydroxyl radicals (HO$^\bullet$) are known to react with DNA either by hydrogen abstraction from the 2-deoxyribose units or by addition to the base moieties causing deleterious chemical modifications.[1] The order of reactivity of HO$^\bullet$ radicals toward the various hydrogen atoms of the 2-deoxyribose moiety is under debate.[2] The proposed order that also parallels the exposure to solvent of the 2-deoxyribose hydrogen atoms (i.e., H5′ > H4′ > H3′ ≈ H2′ ≈ H1′)[3,4] was recently challenged by a few publications.[5,6] Computational data in a simple nucleotide indicated that the most probable sites of H-atom abstraction by radical species are the H5′ and H4′ positions.[7] Under biologically relevant conditions (i.e., aerobic), the corresponding carbon-centered radicals resulted in the formation of oxidized abasic sites, 5′,8-cyclopurine nucleotides, strand breaks terminated with a variety of sugar residues, and freely diffusible degradation products.[8–13] There is growing evidence that the oxidation of 2-deoxyribose in DNA plays a critical role in the genetic toxicology of oxidative stress and inflammation.[2]

The chemistry of 2-deoxyribose oxidation in DNA and model nucleosides or nucleotides has been the subject of periodic reviews.[1,2,8–10,12,13] Several mechanistic aspects such as the 5′-oxidation pathways are not well understood. Under aerobic conditions, the forming C5′ radical **1** reacts with oxygen to generate the peroxyl radical **2**, which partitions between two reaction channels. One path yields the 5′-aldehyde terminus **3**, while the other gives the 5′-(2-phosphoryl-1,4-dioxobutane) residue **4** with the concomitant loss of a base (Scheme 1).[14–16]

(1) (a) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis: Philadelphia, PA, 1987. (b) von Sonntag, C. *Free-Radical-Induced DNA Damage and Its Repair: A Chemical Perspective*; Springer-Verlag: Berlin, 2006.
(2) Dedon, P. C. *Chem. Res. Toxicol.* **2008**, *21*, 206–219.
(3) Balasubramanian, B.; Pogozelski, W. K.; Tullius, T. D. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9738–9743.
(4) Sy, D.; Savoye, C.; Begusova, M.; Michalik, V.; Charlier, M.; Spotheim-Maurizot, M. *Int. J. Radiat. Biol.* **1997**, *72*, 147–155.
(5) Xue, L.; Greenberg, M. M. *Angew. Chem., Int. Ed. Engl.* **2007**, *46*, 561–564.
(6) Roginskaya, M.; Bernhard, W. A.; Marion, R. T.; Razskazovskiy, Y. *Radiat. Res.* **2005**, *163*, 85–89.
(7) Toure, P.; Villena, F.; Melikyan, G. G. *Org. Lett.* **2002**, *4*, 3989–3992.
(8) (a) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191–198. (b) Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 312–332.
(9) Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746–769.
(10) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089–1107.
(11) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109–1147.
(12) Chatgilialoglu, C.; O'Neill, P. *Exp. Gerontol.* **2001**, *36*, 1459–1471.
(13) Greenberg, M. M. *Org. Biomol. Chem.* **2007**, *5*, 18–30.
(14) (a) Kawabata, H.; Takeshita, K.; Fujiwara, T.; Sugiyama, H.; Matsura, T.; Saito, I. *Tetrahedron Lett.* **1989**, *30*, 4263. (b) Angeloff, A.; Dubey, I.; Pratviel, G.; Bernadou, J.; Meunier, B. *Chem. Res. Toxicol.* **2001**, *14*, 1413–1420.
(15) (a) Chen, B.; Bohnert, T.; Zhou, X.; Dedon, P. C. *Chem. Res. Toxicol.* **2004**, *17*, 1406–1413. (b) Montevecchi, P. C.; Manetto, A.; Navacchia, M. L.; Chatgilialoglu, C. *Tetrahedron* **2004**, *60*, 4303–4308.

Scheme 1. Fate of C5′ Radical under Aerobic Conditions

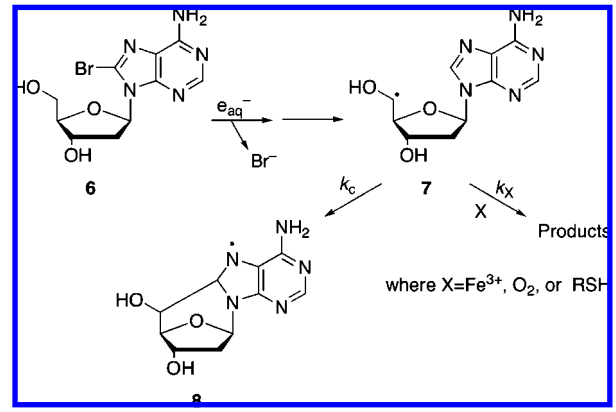


The 5′,8-cyclo-2′-deoxyadenosine and 5′,8-cyclo-2′-deoxyguanosine moieties, also denominated as 5′,8-cyclopurines, are the result of a rapid C5′ radical attack to the purine moiety to give **5** before the reaction with oxygen (Scheme 1). These lesions are observed among the DNA modifications.[17,18] They have also been identified in mammalian cellular DNA in vivo, where their levels are enhanced by conditions of oxidative stress.[19–21] It was also reported that the formation of 5′,8-cyclopurines was suppressed by the presence of molecular oxygen in solution.[22]

Interestingly, these lesions do give neither strand breaks nor abasic sites, and therefore they have not been considered in the overall reactivity of HO$^{\bullet}$ radicals toward the various hydrogen atoms of the 2-deoxyribose moieties. The factors that influence the reactivity of C5′ radicals in purine moieties under aerobic conditions are unknown not only in DNA and related systems, but also in simple nucleosides.

There are only a few methods available for site-specific generation of C5′ radicals. We have recently demonstrated that photolysis of 5′-*tert*-butyl ketone derivatives of thymine and 2′-deoxyguanosine leads to formation of thymidin-5′-yl and 2′-deoxyguanosin-5′-yl radicals, respectively.[23] We have also previously demonstrated by using pulse radiolysis techniques that the reaction of hydrated electrons (e_{aq}^-) with 8-bromo-2′-deoxyadenosine (**6**) produces bromide anion and the corresponding C8 radical, which abstracts intramolecularly a hydrogen atom from the C5′ position, affording selectively the 2′-deoxyadenosin-5′-yl radical **7** (Scheme 2).[24] Radical **7** undergoes cyclization with a rate constant of $k_c = 1.6 \times 10^5$ s^{-1} to give the heteroaromatic radical **8**. Furthermore, the reactivity of C5′ radical **7**toward $K_3Fe(CN)_6$ and O_2 was examined in competition with the cyclization process.[24] Similar reactions with almost identical rate constants have been observed with 8-bromo-2′-deoxyinosine (**10**), which affords the 2′-deoxyinosin-5′-yl radical.[25] In this article, we extended the latter approach for site-specific generation of C5′ radical to include the reaction of **7** with biologically relevant thiols and, more importantly, to study the fate of purine-substituted C5′ radical under aerobic conditions or in the presence of oxidants. Furthermore, we reinvestigated the reaction of radiation-generated HO$^{\bullet}$ radicals with 2′-deoxyadenosine and 2′-deoxyguanosine under aerobic conditions.

Scheme 2. Chemical Studies of Hydrated Electron (e_{aq}^-) with 8-Bromo-2′-deoxyadenosine (**6**)



Results and Discussion

Radiolytic Production[26,27] of Transients. Radiolysis of neutral water leads to the species e_{aq}^-, HO$^{\bullet}$, and H$^{\bullet}$ as shown in eq 1, where the values in parentheses represent the chemical radiation yields (G) expressed in μmol J^{-1}. The reactions of e_{aq}^- with substrates were studied by irradiating solutions containing 0.25 M *t*-BuOH. Hydroxyl radicals were scavenged efficiently by *t*-BuOH, (eq 2, $k_2 = 6.0 \times 10^8$ M^{-1} s^{-1}), whereas hydrogen atoms reacted only slowly (eq 2, $k_2 = 1.7 \times 10^5$ M^{-1} s^{-1}). The presence of N_2O transforms efficiently e_{aq}^- into the O$^{\bullet-}$ species (eq 3, $k_3 = 9.1 \times 10^9$ M^{-1} s^{-1}). The HO$^{\bullet}$ radical [pK_a(HO$^{\bullet}$) = 11.9] is in equilibrium with its conjugated base O$^{\bullet-}$ (eq 4/−4, $k_4 = 1.2 \times 10^{10}$ M^{-1} s^{-1}, $k_{-4} = 1 \times 10^8$ s^{-1}).

$$H_2O \rightsquigarrow e_{aq}^- (0.27), \quad HO^{\bullet} (0.28), \quad H^{\bullet} (0.062) \qquad (1)$$

(16) (a) Recent results on DNA adducts derived from electrophilic products of 5′-oxidation of deoxyribose in DNA and on oligonucleotides containing 5′-aldehyde at one terminus have been also reported. See: Chen, B.; Vu, C. C.; Byrns, M. C.; Dedon, P. C.; Peterson, L. A. *Chem. Res. Toxicol.* **2006**, *19*, 982–985. (b) Kodama, T.; Greenberg, M. M. *J. Org. Chem.* **2005**, *70*, 8122–8129.
(17) Dizdaroglu, M.; Jaruga, P.; Rodriguez, H. *Free Radical Biol. Med.* **2001**, *30*, 774–784.
(18) Jaruga, P.; Birincioglu, M.; Rodriguez, H.; Dizdaroglu, M. *Biochemistry* **2002**, *41*, 3703–3711.
(19) Randerath, K.; Zhou, G. D.; Somers, R. L.; Robbins, J. H.; Brooks, P. J. *J. Biol. Chem.* **2001**, *276*, 36051–36057.
(20) D'Errico, M.; Parlanti, E.; Teson, M.; Bernardes de Jesus, B. M.; Degan, P.; Calcagnile, A.; Jaruga, P.; Bjørås, M.; Crescenzi, M.; Pedrini, A. M.; Egly, J.-M.; Zambruno, G.; Stefanini, M.; Dizdaroglu, M.; Dogliotti, E. *EMBO J.* **2006**, *25*, 4305–4315.
(21) These lesions represent a unique class of oxidative DNA lesions in that they are repaired by the nucleotide excision repair pathway but not by base excision repair or direct repair. For a review, see: Brooks, P. J. *Neuroscience* **2007**, *145*, 1407–1417.
(22) Dizdaroglu, M. *Biochem. J.* **1986**, *238*, 247–254.
(23) Manetto, A.; Georganakis, D.; Gimisis, T.; Leondiadis, L.; Mayer, P.; Carell, T.; Chatgilialoglu, C. *J. Org. Chem.* **2007**, *72*, 3659–3666.
(24) (a) Chatgilialoglu, C.; Guerra, M.; Mulazzani, Q. G. *J. Am. Chem. Soc.* **2003**, *125*, 3839–3848. (b) Flyunt, R.; Bazzanini, R.; Chatgilialoglu, C.; Mulazzani, Q. G. *J. Am. Chem. Soc.* **2000**, *122*, 4225–4226.
(25) Russo, M.; Jimenez, L. B.; Mulazzani, Q. G.; D'Angelantonio, M.; Guerra, M.; Miranda, M. A.; Chatgilialoglu, C. *Chem.−Eur. J.* **2006**, *12*, 7684–7693.
(26) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513–886.
(27) Ross, A. B.; Mallard, W. G.; Helman, W. P.; Buxton, G. V.; Huie, R. E.; Neta, P. NDRL-NIST Solution Kinetics Database, ver. 3; National Institute of Standards and Technology: Gaithersburg, MD, 1998.

$$HO^{\bullet}/H^{\bullet} + t\text{-BuOH} \rightarrow (CH_3)_2C(OH)CH_2^{\bullet} + H_2O/H_2 \quad (2)$$

$$e_{aq}^{-} + N_2O \rightarrow O^{\bullet-} + N_2 \quad (3)$$

$$HO^{\bullet} + HO^{-} \rightleftarrows O^{\bullet-} + H_2O \quad (4)$$

Reactivity of 2′-Deoxyadenosin-5′-yl Radical toward Thiols. The reactivity of radical **7** toward cysteine (CySH) and glutathione (GSH) was investigated by pulsing Ar-purged solutions of 1 mM **6** containing 0.25 M *t*-BuOH and different concentrations of thiol (0−5 mM) at natural pH.[28] The reactions were monitored at 360 nm, where the radical **8** has a λ_{max} with ϵ = 9600 M^{-1} cm^{-1} (Scheme 2).[24] The absorption at 360 nm was found to decrease by increasing the concentration of thiol (see Figure 1 for CySH and Supporting Information for GSH). This decrease was almost identical to the one expected from the partition of e_{aq}^{-} between **6** ($k = 1.6 \times 10^{10}$ M^{-1} s^{-1})[24] and CySH ($k = 8.5 \times 10^{9}$ M^{-1} s^{-1}).[27] On the other hand, the first-order growth (k_{obs}) increased linearly with increasing CySH concentration; that is, $k_{obs} = k_c + k_x$[CySH] (insets a and b). From the slopes of k_{obs} versus [RSH], the bimolecular rate constants were found to be $(2.1 \pm 0.5) \times 10^7$ and $(4.9 \pm 0.6) \times 10^7$ M^{-1} s^{-1} for CySH and GSH, respectively.

Table 1 summarizes the results obtained for the trapping of C5′ radical **7**, together with the analogous reactions of the C1′ radical (2′-deoxyuridin-1′-yl).[29] It is worth emphasizing that the rate constants for the reaction of C5′ radical with thiols are 1 order of magnitude higher than that of the anologous reaction of C1′ radical (presumably both enthalpic and entropic factors play a role) and that GSH is two times faster than CySH for both radicals. The oxygen trapping is quite fast and similar for the two species, whereas the oxidation by Fe^{3+} is ca. 40 times faster for C5′ radical.

Oxidation of 2′-Deoxyadenosin-5′-yl and 2′-Deoxyinosin-5′-yl Radicals. C5′ radicals such as 2′-deoxyadenosin-5′-yl (**7**) and 2′-deoxyinosin-5′-yl were found to react with $K_3Fe(CN)_6$ with rate constants higher than 10^9 M^{-1} s^{-1} (cf. Table 1), presumably affording the corresponding cation because the electron transfer is thermodynamically quite favorable; that is, $E°[Fe(CN)_6^{3-}/Fe(CN)_6^{4-}] = 0.36$ V[30a,b] and $E°[CH_3CHO, H^+/CH_3CH(^{\bullet})OH] = -1.25$ V (cf. Scheme 2).[30c] We investigated these reactions by detailed product studies. On the basis of the above-mentioned kinetic data, it is expected that in the presence of a few millimolar concentration of $K_3Fe(CN)_6$ the reaction follows this path. Since e_{aq}^{-} reacts with $K_3Fe(CN)_6$ ($k = 3.1 \times 10^9$ M^{-1} s^{-1})[27] only 5 times slower than 8-bromo-2′-deoxyadenosine or 8-bromo-2′-deoxyinosine ($k = 1.6 \times 10^{10}$ M^{-1} s^{-1}),[24,25] the amount of $K_3Fe(CN)_6$ should be added preferably in portions to keep a steady-state concentration ≤1 mM.[31]

Ar-purged aqueous phosphate-buffered solutions (pH 7.1) of 1.52 mM **6** (or 1.04 mM **10**) containing 0.25 M *t*-BuOH and 1 mM $K_3Fe(CN)_6$ were initially γ-irradiated with a dose of 1.5 kGy. Subsequently, amounts of $K_3Fe(CN)_6$ (1 mM) were added at 1.5 kGy dose intervals to keep the concentration of oxidant in the desired range. Both sets of reactions were monitored by HPLC after passage of the reaction mixtures through an ion-exchange resin to eliminate the iron salts. The HPLC analyses are shown in Figure 2A,B for the two 8-bromopurine nucleosides. In both sets of experiments, there is the consumption of starting bromide (blue peaks) and the formation of a main product (red peaks). After workup, the hydrated 5′-aldehydes **9** and **11** were isolated as compounds of quite good stablility[32] and fully characterized (Scheme 3).

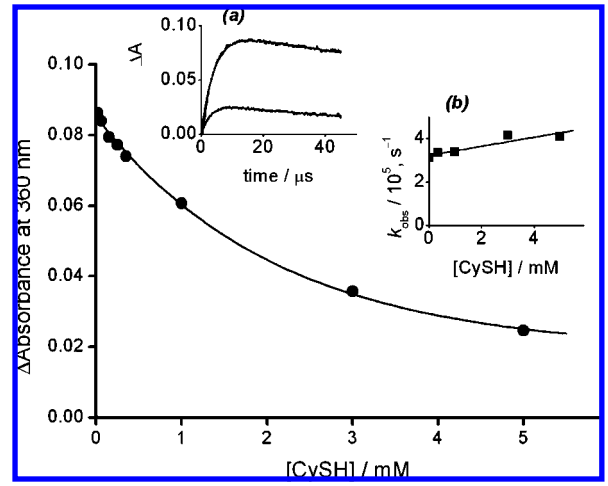


Figure 1. Dependence of ΔAbsorbance at 360 nm on [CySH] obtained from the pulse radiolysis of Ar-purged, unbuffered solutions containing 1 mM **6** and 0.25 M *t*-BuOH; optical path = 2.0 cm, dose = 20 Gy. The solid line represents an empirical fit to the data. Insets: (a) Time dependence of ΔAbsorbance at 360 nm in the absence (upper curve) and in the presence (lower curve) of 5 mM CySH. (b) Dependence of k_{obs} for buildup at 360 nm on [CySH].

Table 1. Rate Constants for Reaction of 2′-Deoxyadenosin-5′-yl and 2′-Deoxyuridin-1′-yl[a]

	rate constant (M^{-1} s^{-1})	
trapping agent	2′-deoxyadenosin-5′-yl	2′-deoxyuridin-1′-yl[b]
CySH	$(2.1 \pm 0.5) \times 10^7$	$(2.9 \pm 0.4) \times 10^6$
GSH	$(4.9 \pm 0.6) \times 10^7$	$(4.4 \pm 0.3) \times 10^6$
O_2	$(1.8 \pm 1) \times 10^{9c}$	2×10^9
Fe^{3+d}	$(4.2 \pm 0.4) \times 10^{9c}$	ca. 1×10^8

[a] Reactions in neutral water at 22 ± 2 °C. [b] Data from ref.[29] [c] Data from ref.[24] [d] $Fe(CN)_6^{3-}$ with 2′-deoxyadenosin-5′-yl and $FeCl_3$ with 2′-deoxyuridin-1′-yl.

The insets of Figure 2A,B show the change in concentration of the bromide (blue) and the hydrated 5′-aldehyde (red). On the basis of the consumption of starting material, the yields of hydrated 5′-aldehydes **9** and **10** are 85 and 87%, respectively. Two minor products eluted before the red peaks in all chromatograms and were assigned to the corresponding free base and (5′*R*)-5′,8-cyclopurine nucleoside (in order of appearance) in comparison with the authentic samples (vide infra). Their yields are estimated to be in the range of 6−8% each.[33]

Aldehydic protons usually exhibit a peak at 9−10 ppm at ^{1}H NMR, while the corresponding carbon atoms usually absorb downfield from 160 ppm at ^{13}C NMR. On the contrary, compounds **9** and **11** exhibited a peak at ∼5.2 ppm at ^{1}H NMR

(28) The pH of the solutions decreases from 7.5 to 5.5 when the amount of CySH (0−5 mM) is increased.

(29) Chatgilialoglu, C.; Ferreri, C.; Bazzanini, R.; Guerra, M.; Choi, S.-Y.; Emanuel, C. J.; Horner, J. H.; Newcomb, M. *J. Am. Chem. Soc.* **2000**, *122*, 9525–9533.

(30) (a) All redox potentials are vs NHE. (b) Hausler, K. E.; Lorenz, W. J. In *Standard Potentials in Aqueous Solution*; Bard, A. J., Parsons, R., Jordan, J. Eds.; Marcel Dekker: New York, 1985; p 408. (c) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637–1755.

(31) $K_3Fe(CN)_6$ can also trap $H^{\bullet}$ atoms ($k = 6.3 \times 10^9$ M^{-1} s^{-1})[27] and $Me_2C(OH)CH_2^{\bullet}$ radicals ($k \approx 3 \times 10^6$ M^{-1} s^{-1}). See: Candeias, L. P.; Wolf, P.; O'Neill, P.; Steenken, S. *J. Phys. Chem.* **1992**, *96*, 10302–10307.

(32) An aqueous solution of **9** or **11** (1 mM) left at room temperature for 24 h and additionally heated at 40 °C for 12 h did not result in any decomposition.

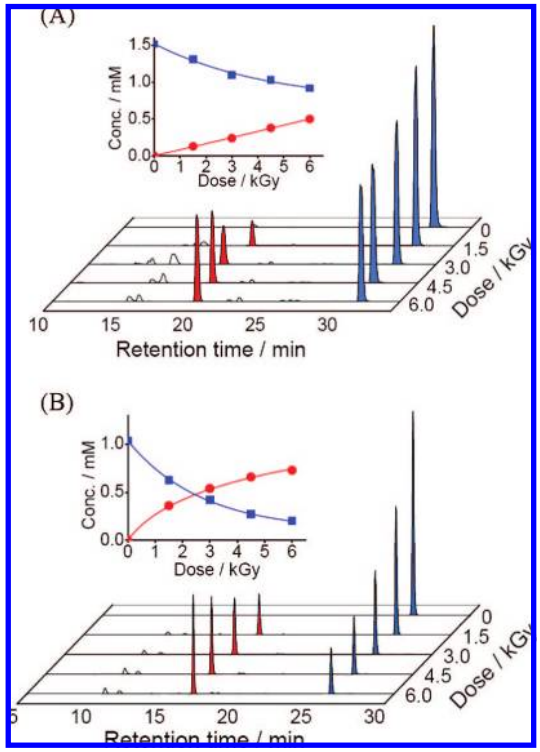
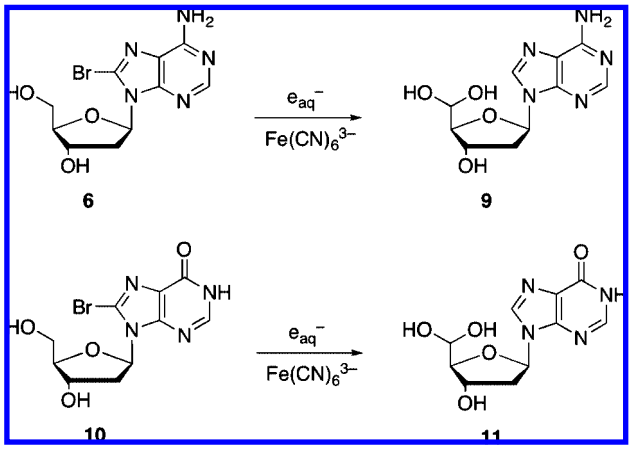


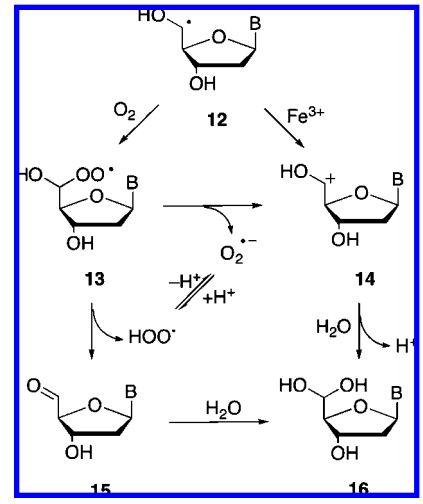
Figure 2. (A) HPLC runs of γ-irradiation of **6** (1.52 mM) in Ar-purged phosphate-buffered aqueous solutions (pH = 7.1) containing *t*-BuOH (0.25 M) and 1 mM $K_3Fe(CN)_6$ at a dose rate of 8.2 Gy min^{-1}. Extra amounts of $K_3Fe(CN)_6$ (1 mM) were added at 1.5 kGy dose intervals. The HPLC peaks of **6** are highlighted in blue, while the peaks of the product **9** are highlighted in red. The inset shows the concentration of **6** (blue) and **9** (red) as a function of the irradiation dose. (B) HPLC runs of γ-irradiation of **10** (1.04 mM) in Ar-purged phosphate-buffered aqueous solution (pH = 7.1) containing *t*-BuOH (0.25 M) and 1 mM $K_3Fe(CN)_6$ at a dose rate of 8.7 Gy min^{-1}. Extra amounts of $K_3Fe(CN)_6$ (1 mM) were added at 1.5 kGy dose intervals. The HPLC peaks of **10** are highlighted in blue, while the peaks of the product **11** are highlighted in red. The inset shows the concentration of **10** (blue) and **11** (red) as a function of the irradiation dose.

Scheme 3. One-Pot Synthesis of Hydrated 5′-Aldehydes **9** and **11** from the Reaction of Corresponding 8-Bromopurine Nucleoside with Hydrated Electrons in the Presence of $Fe(CN)_6^{3-}$



and ~90 ppm at ^{13}C NMR, a clear indication that only the hydrated form is present in aqueous solutions. In the ribo series, adenosine 5′-aldehyde has been previously synthesized and shown also by NMR to exist in the hydrated form in aqueous solution.[34] Therefore, the radiolytic conditions allowed to develop a synthetically useful one-pot procedure by an unconventional radical-polar crossover reaction,[35] for the conversion of 8-bromo derivatives **6** and **10** to hydrated 5′-aldehyde **9** and **11** in good yields. Scheme 4 shows the mechanism that involves oxidation of C5′ radical **12** by Fe^{3+} to the carbocation **14** and the subsequent addition of water to form the hydrated 5′-aldehyde **16**.

Scheme 4. Proposed Mechanisms for the Fate of C5′ Radical and Formation of Hydrated 5′-Aldehyde under Oxidative Conditions (the pK_a of $HOO^{\bullet}$ Is 4.8)



Effect of Oxygen Concentration on the Fate of 2′-Deoxyadenosin-5′-yl and 2′-Deoxyinosin-5′-yl Radicals. Our method of selective generation of C5′ radicals in purine nucleosides is also suitable for investigating these reactions under aerobic conditions. Air-saturated aqueous phosphate-buffered solution (pH 7.1) of 1.58 mM **6** containing 0.25 M *t*-BuOH was γ-irradiated at a dose rate of 8.2 Gy min^{-1}. During γ-radiolysis, continuous bubbling of air through the sample ensured the constant concentration of O_2. The reaction was monitored by HPLC at 1.5 kGy dose intervals up to 6 kGy as shown in Figure 3A. An analogous experiment was performed with 0.95 mM **10**, where the reaction was monitored at 1 kGy dose intervals up to 7 kGy. Figure 3B shows the HPLC runs at 0, 2, 5, and 7 kGy. The consumption of the starting material (blue peaks) was accompanied by the formation of a main product (red peaks), which is assigned to the corresponding hydrated aldehyde (**9** or **11**) in comparison with the authentic sample. The insets of Figure 3A,B show the change in concentration of the starting bromide and the main product. On the basis of the consumption of starting material, the yields of hydrated aldehydes **9** and **11** are 42 and 76%, respectively.

The mechanism we envisaged for the formation of hydrated 5′-aldehydes under these conditions is also outlined in Scheme 4. Reaction of C5′ radical **12** with oxygen gives the peroxyl radical **13**. The rate constant for the oxygen trapping of

(33) Analogous experiments of bromide **6** replacing $K_3Fe(CN)_6$ with $FeCl_3$, CuCl, or $CuCl_2$ were also carried out. In all cases, the yields of **9** were much poorer because of (i) the lower conversion of starting material, the rate constants of e_{aq}^- with $FeCl_3$, CuCl, or $CuCl_2$ being 10−20 times faster than that of $K_3Fe(CN)_6$,[27] and (ii) the formation of similar amounts of 5′,8-cyclo-2′-deoxyadenosine, indicating a slower quenching of C5′ radical by the oxidants $FeCl_3$, CuCl, or $CuCl_2$.

(34) (a) Liu, S.; Wnuk, S. F.; Yuan, C.; Robins, M. J.; Borchardt, R. T. *J. Med. Chem.* **1993**, *36*, 883–887. (b) Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins, M. J. *J. Med. Chem.* **1997**, *40*, 1608–1618.

(35) Murphy, J. A. In *Radicals in Organic Synthesis*; Renaud, P., Sibi, M. P. Eds.; Wiley-VCH: Weinheim, Germany, 2001; pp 298−315.

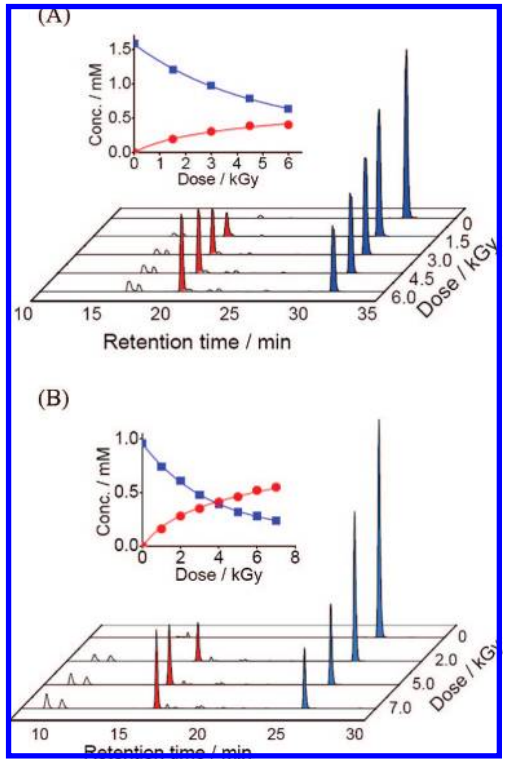
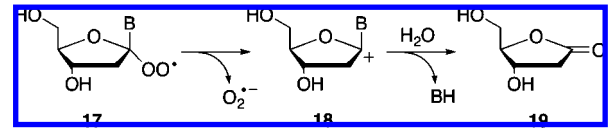


Figure 3. (A) HPLC runs of γ-irradiation of **6** (1.58 mM) in air-saturated phosphate-buffered aqueous solution (pH = 7.1) containing *t*-BuOH (0.25 M) at a dose rate of 8.2 Gy min^{-1}. The HPLC peaks of **6** are highlighted in blue, while the peaks of the product **9** are highlighted in red. The inset shows the concentration of **6** (blue) and **9** (red) as a function of the irradiation dose. (B) HPLC runs of γ-irradiation of **10** (0.95 mM) in air-saturated phosphate-buffered aqueous solution (pH = 7.1) containing *t*-BuOH (0.25 M) at a dose rate of 8.1 Gy min^{-1}. The HPLC peaks of **10** are highlighted in blue, while the peaks of the product **11** are highlighted in red. The inset shows the concentration of **10** (blue) and **11** (red) as a function of the irradiation dose.

2′-deoxyadenosin-5′-yl or 2′-deoxyinosin-5′-yl is 2×10^9 M^{-1} s^{-1}.[24,25] The peroxyl radical **13** could decay either via a cyclic transition state leading to HOO$^\bullet$ radical and aldehyde **15** or via heterolytic cleavage to generate carbocation **14** and superoxide radical anion. The two pathways are formally identical.[36,37] Although the analogous α-hydroxyethylperoxyl radical is reported to eliminate HOO$^\bullet$ with formation of acetaldehyde (k = 50 s^{-1} at 20 °C),[39] the heterolytic cleavage has an important precedent in nucleosides. Indeed, the C1′ peroxyl radical **17** has been demonstrated to split to **18** and superoxide with a rate

Scheme 5. Fate of C1′ Peroxyl Radicals[40]



constant of 1.4×10^4 s^{-1}, followed by hydrolysis with formation of the ribonolactone **19** (Scheme 5).[40]

In the above-mentioned experiments, a consistent percentage of e_{aq}^- and H$^\bullet$ produces $O_2^{\bullet-}$,[41] whereas additional $O_2^{\bullet-}$ is produced in the reaction of C5′ radicals with oxygen. However, no evidence of further reaction between $O_2^{\bullet-}$ with the starting 8-bromopurine nucleosides is found. To investigate deeply this issue, O_2-saturated phosphate-buffered solutions of lower concentrations of 8-bromopurine derivatives (0.14 mM) containing 0.25 M *t*-BuOH were γ-irradiated as usual. Under these conditions, where O_2 quenched nearly all the primary reducing species forming $O_2^{\bullet-}$, the consumption of the starting nucleoside was not observed.[37b]

Two minor products eluted before the red peaks in all chromatograms of Figure 3A,B and were assigned (in order of appearance) to the corresponding free base and (5′*R*)-5′,8-cyclopurine nucleoside in comparison with the authentic samples.[24,25] To understand better the relation of these two minor products with the hydrated 5′-aldehyde, we performed analogous experiments by varying the oxygen concentration. The solubility of molecular oxygen in H_2O is 1.33×10^{-3} M at 22 °C and 1 atm partial pressure.[42] The air-saturated solution corresponds to 2.66×10^{-4} M of O_2, which is ca. 6 times higher than typical well-oxygenated tissues; that is, $[O_2] \cong 4 \times 10^{-5}$ M (the oxygen concentration is even lower in the nucleus).[43] We performed additional experiments using 5 or 1% O_2, which corresponds to 6.65×10^{-5} and 1.33×10^{-5} M, respectively, where the cyclization should be favored. Indeed, by decreasing the O_2 concentration the yields of hydrated 5′-aldehyde and (5′*R*)-5′,8-cyclopurine nucleoside decreased and increased, respectively. The yields of free base also increased.

To quantify these changes, analysis of the data was performed in terms of radiation chemical yield. The disappearance of the starting material (mol kg^{-1}) divided by the absorbed dose (1Gy = 1 J kg^{-1}) gives the radiation chemical yield (G). Figure 4 shows examples of the G of disappearance of starting bromide **6** and the G of formation of products vs dose for 2.66×10^{-4} and 1.33×10^{-5} M of oxygen, respectively.[37b]

In Tables 2 and 3, all the data are collected for the two series, showing that the sum of the yields of the three products, on the basis of the converted starting bromide, are ca. 70% for **6** and ca. 90% for **10**. Taking into account that $G(e_{aq}^-) + G(H^\bullet)$ = 0.33 μmol J^{-1} (eq 1), the $G(-\mathbf{6}) = 0.27$ and $G(-\mathbf{10}) = 0.23$ μmol J^{-1} in air-saturated experiments correspond to 82 and 70% of the reaction of e_{aq}^- and H$^\bullet$ with each derivative. These percentages reflect fairly well the competition existing between the reduction of starting bromide ($k = 1.6 \times 10^{10}$ M^{-1} s^{-1})[24,25] and molecular oxygen ($k = 1.9 \times 10^{10}$ M^{-1} s^{-1})[26,27] by solvated

(36) The pK_a of HOO$^\bullet$ is 4.8. See: Bielski, B. H. T.; Cabelli, D. E.; Arudi, R. L. *J. Phys. Chem. Ref. Data* **1985**, *14*, 1041–1100.
(37) (a) Hydrogen peroxide was also found to be a major reaction product. Air-saturated aqueous phosphate-buffered solution (pH 7.5) of 2.2 mM **6** containing 0.25 M *t*-BuOH was γ-irradiated at a dose rate of 7.7 Gy min^{-1}. During γ-radiolysis, continuous bubbling of air through the sample ensured the constant concentration of O_2. Hydrogen peroxide was determined iodometrically at 0, 1, and 2 kGy.[38] A $G(H_2O_2) \approx 0.18$ μmol J^{-1} was found, considerably higher than the $G(H_2O_2) = 0.07$ produced from the water radiolysis. (b) See Supporting Information for more details.
(38) Allen, A. O.; Hochanadel, C. J.; Ghormley, J. A.; Davis, T. W. *J. Phys. Chem.* **1952**, *56*, 575–586.
(39) (a) Bothe, E.; Behrens, G.; Schulte-Frohlinde, D. *Z. Naturforsch., B: Chem. Sci.* **1977**, *32*, 886–889. (b) Bothe, E.; Schuchmann, M. N.; Schulte-Frohlinde, D.; von Sonntag, C. *Z. Naturforsch., B: Chem. Sci.* **1983**, *38*, 212–219.
(40) (a) Chatgilialoglu, C.; Gimisis, T. *Chem. Commun.* **1998**, 1249–1250. (b) Emanuel, C. J.; Newcomb, M.; Ferreri, C.; Chatgilialoglu, C. *J. Am. Chem. Soc.* **1999**, *121*, 2927–2928.
(41) The rate constants for the reaction of e_{aq}^- and H$^\bullet$ with O_2 to produce $O_2^{\bullet-}$ are 1.9×10^{10} and 1.2×10^{10} M^{-1} s^{-1}, respectively.[27]
(42) Battino, R.; Rettich, T. R.; Tominaga, T. *J. Phys. Chem. Ref. Data* **1983**, *12*, 163–178.
(43) (a) Zander, R. *Z. Naturforsch., C: Biosci.* **1976**, *31*, 339–352. (b) Zander, R. *Adv. Exp. Med. Biol.* **1976**, *75*, 463–467.

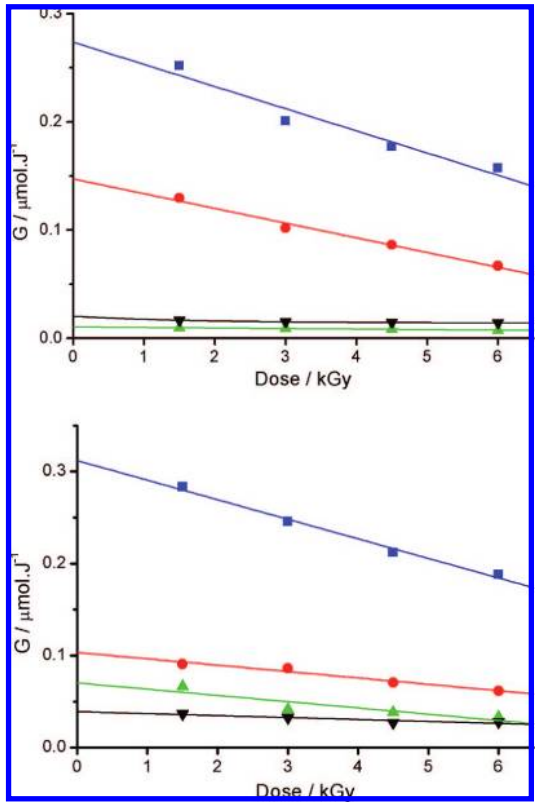


Figure 4. Irradiation of 8-bromo-2′-deoxyadenosine **6** in phosphate-buffered aqueous solutions (pH = 7.1) containing *t*-BuOH (0.25 M) under aerobic conditions. Upper: 1.58 mM of **6** and $[O_2] = 2.66 \times 10^{-4}$ M. Lower: 1.77 mM of **6** and $[O_2] = 1.33 \times 10^{-5}$ M. The chemical irradiation yields $G(-\mathbf{6})$ (■), $G(\mathbf{9})$ (●), G(adenine) (▼) and $G(\mathbf{20})$ (▲), as a function of the irradiation dose. The line extrapolation to a zero dose leads to the G values given in Table 2.

Table 2. Radiation Chemical Yield (G) Extrapolated to Zero Dose from the Irradiation of 8-Bromo-2′-deoxyadenosine **6** at Various Oxygen Concentrations[a]

6, mM	O_2, M	$G(-\mathbf{6})$	$G(\mathbf{9})$	G(adenine)	$G(\mathbf{20})$
1.77	1.33×10^{-5}	0.31	0.10	0.04	0.07
2.22	6.65×10^{-5}	0.31	0.12	0.04	0.05
1.58	2.66×10^{-4}	0.27	0.15	0.02	0.01

[a] Units of G in μmol J^{-1}.

Table 3. Radiation Chemical Yield (G) Extrapolated to Zero Dose from the Irradiation of 8-Bromo-2′-deoxyadenosine **10** at Various Oxygen Concentrations[a]

10, mM	O_2, M	$G(-\mathbf{10})$	$G(\mathbf{11})$	G(Hy)[b]	$G(\mathbf{22})$
1.17	1.33×10^{-5}	0.29	0.10	0.07	0.09
0.94	6.65×10^{-5}	0.28	0.12	0.05	0.07
0.95	2.66×10^{-4}	0.23	0.16	0.03	0.04

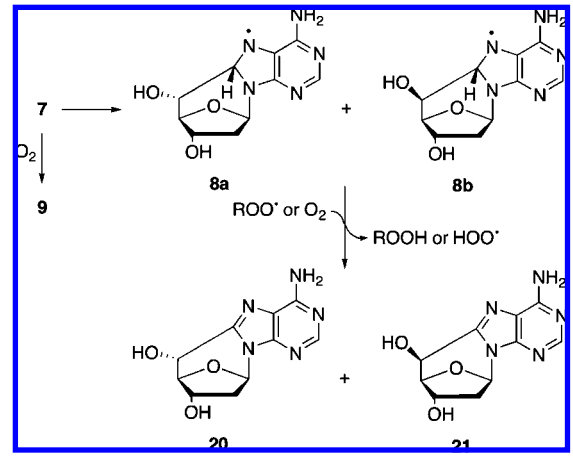
[a] Units of G in μmol J^{-1}. [b] Hy = Hypoxanthine.

electrons, taking into consideration the initial concentration of nucleoside (1.58 and 0.95 mM for **6** and **10**, respectively) and O_2 (2.66×10^{-4} M). By decreasing the $[O_2]$, the G of disappearance increased accordingly.

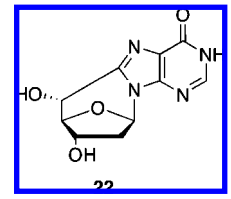
The 5′,8-cyclo-2′-deoxyadenosine is formed in two diastereomeric forms depending on the configuration at C5′ position; that is, (5′*R*)- and (5′*S*)-isomers (**20** and **21**, respectively).[24,44] The (5′*R*)/(5′*S*) ratio varies substantially with experimental

(44) Jimenez, L. B.; Encinas, S.; Miranda, M. A.; Navacchia, M. L.; Chatgilialoglu, C. *Photochem. Photobiol. Sci.* **2004**, *3*, 1042–1046.

Scheme 6. Fate of 2′-Deoxyadenosin-5′-yl radical and the Formation of (5′*R*)-5′,8-Cyclo-2′-deoxyadenosine (**20**) and (5′*S*)-5′,8-Cyclo-2′-deoxyadenosine (**21**) under Aerobic Conditions



conditions and the presence of protecting groups.[44,45] A (5′*R*)/(5′*S*) = 6:1 ratio was found in γ-irradiated aqueous solutions.[24] In the present study, a (5′*R*)/(5′*S*) ≈ 6:1 ratio is also found in all experiments.[37b] The cyclization of C5′ radical **7** occurs through its *pro*-(5′*R*) and *pro*-(5′*S*) to give **8a** and **8b** radicals, respectively, which are in the chair conformation (Scheme 6). It was suggested that the **8a**/**8b** ratio depends on the energies of the two chair transition states, where *pro*-(5′*R*) and *pro*-(5′*S*) conformations have the 5′OH in axial and equatorial arrangement, respectively, and only the *pro*-(5′*R*) conformer can be stabilized by hydrogen bonding, involving either the oxygen of the sugar ring or the N3 of the base.[24,45] Therefore, such hydrogen bonding is most likely the origin of the observed diastereoselective ratio. Oxidative rearomatization of **8a** and **8b** completes the reaction mechanism, affording the diastereomers **20** and **21** (Scheme 6).[46] Similar results were also reported for the (5′*R*)- and (5′*S*)-isomers of 5′,8-cyclo-2′-deoxyinosine,[25] and in the present study the observed diastereomeric ratio of ca. 5:1 is in favor of (5′*R*)-isomer **22**.[37b] Our findings clearly indicate that the formation of 5′,8-cyclopurines in the range of 13−266 μM of oxygen is relevant and, in agreement with Scheme 6, the yield increases with decreasing O_2 concentration.



The formation of free base (adenine or hypoxanthine) accounts also substantially on the reaction products. Interestingly, the yield decreased by increasing O_2 concentration. The base release mechanism probably involves several minor paths of the sugar radicals C1′, C3′, and C4′.[1] For example, the C1′ radical path would follow the mechanism reported in Scheme 5. We suggest that the primary alkyl radicals derived from the reaction of $HO^•/H^•$ with *t*-BuOH (eq 2) are able to abstract hydrogen unselectively from the sugar moiety. By increasing the oxygen concentration, the primary alkyl radical is trans-

formed to peroxyl radical whose reactivity toward hydrogen abstraction is much more bland.[47]

Reaction of Hydroxyl Radicals with 2′-Deoxyadenosine and 2′-Deoxyguanosine in the Presence of Oxygen. The above-described approach of selectively generated C5′ radicals by reaction of e_{aq}^- with 8-bromopurine derivatives does not operate with 8-bromo-2′-deoxyguanosine because the electron adduct undergoes a fast protonation at C8 to afford the one-electron-oxidized 2′-deoxyguanosine.[48,49] However, the two diastereomeric forms (5′*S*) and (5′*R*) of 5′,8-cyclo-2′-deoxyguanosine were identified in γ-irradiation of N_2O-saturated aqueous solution of 2′-deoxyguanosine in a (5′*R*)/(5′*S*) ratio of 8.3:1.[22,50] It is well-known that the reaction of $HO^•$ radicals with 2′-deoxyguanosine (**23**) occurs mainly at the base moiety by addition (ca. 85%), and to a minor extent at the deoxyribose unit by hydrogen abstraction (ca. 15%).[51] Our recent results have shown that the hydrogen abstraction from the C5′ position is an important component of the sugar moiety.[50] Therefore, it is reasonable to expect substantial amounts of hydrated 5′-aldehydes **24** in the reaction of $HO^•$ radicals with **23** under oxic conditions (Scheme 7).[52]

An aqueous phosphate-buffered solution (pH 7.1) of 1.44 mM **23** was saturated with $N_2O(80\%)/O_2(20\%)$ before irradiation. During γ-radiolysis (dose rate = 8.0 Gy min^{-1}), continuous bubbling of the gas mixture in the sample ensured the constant concentration of O_2 throughout the reaction time. The reaction was monitored by HPLC at 0.5 kGy dose intervals up to 2 kGy (Figure 5). The consumption of **23** (32% after 2 kGy) was accompanied by the formation of two main products, identified as guanine and the hydrated 5′-aldehyde **24**. In an analogous experiment after 6 kGy, the irradiated mixture was concentrated to ca. 2 mL and passed through a column packed with RP-18 silica eluted with 0−2% acetonitrile/water. The hydrated 5′-aldehyde **24** was isolated and fully characterized.[52,53]

Under our normoxic conditions, 93% of the e_{aq}^- was converted to give further $HO^•$ (cf. eqs 3 and 4) for a total $G(HO^•) = 0.53$ μmol J^{-1}, whereas the remaining e_{aq}^- and $H^•$ atoms were mainly converted to superoxide radical anion.[41] The $HO^•$ radicals are partitioned between guanine and sugar moieties of **23** with an overall rate constant of $\sim 5 \times 10^9$ M^{-1} s^{-1}.[27] Furthermore, the oxygen concentration would be high enough to trap most of the alkyl radicals derived from the sugar moiety on nucleoside, in analogy also with the reactions described in the previous section.

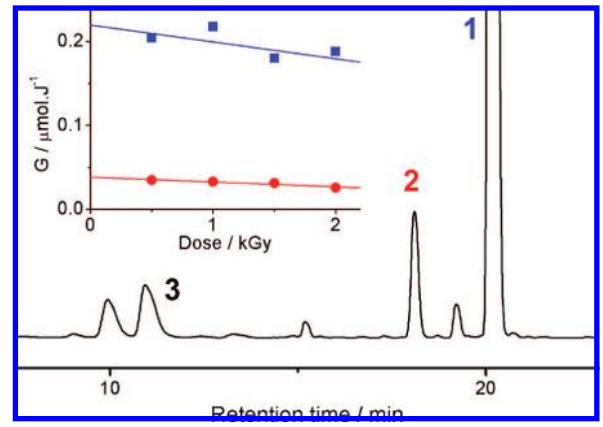
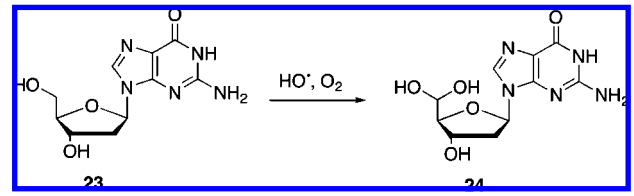


Figure 5. HPLC run of γ-irradiation of **23** (1.44 mM) in N_2O (80%)/O_2 (20%)-purged phosphate-buffered aqueous solution (pH = 7.1) with a dose of 1.0 kGy (dose rate = 8.0 Gy min^{-1}). The peaks **1**, **2**, and **3** are the starting materials (**23**), hydrated 5′-aldehyde (**24**), and guanine, respectively. Inset: Radiation chemical yield $G(-\mathbf{23})$ (■) and $G(\mathbf{24})$ (●) as a function of the irradiation dose.

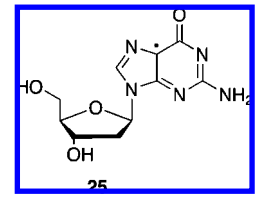
Scheme 7. Reaction of Hydroxyl Radicals with 2′-Deoxyguanosine in the Presence of Oxygen



The inset of Figure 5 shows the G of disappearance of 2′-deoxyguanosine (**23**) and the G of formation of hydrated 5′-aldehyde **24** vs dose. By extrapolating the radiation chemical yields to zero dose, the values $G(-\mathbf{23}) = 0.22$ and $G(\mathbf{24}) = 0.04$ μmol J^{-1} were found. Two important facts can be deduced:

(i) Considering $G(HO^•) = 0.53$ vs $G(-\mathbf{23}) = 0.22$, it can be suggested that ca. 60% of the attack of $HO^•$ radicals on **23** is not productive, affording **23** back. Interestingly, ca. 65% of $HO^•$ radicals attack the guanine moiety to afford the deprotonated one-electron-oxidized 2′-deoxyguanosine (**25**) that does not react with oxygen.[51] We suggest that under our experimental condition the intermediate **25** is converted back to the 2′-deoxyguanosine.[54,55]

(ii) The ratio $G(HO^•)/G(\mathbf{24})$ approximately reflects the percentage (ca. 8%) of $HO^•$ radicals attacking the H5′ of the 2′-deoxyguanosine.[56]



On the basis of the above-described findings, it is reasonable to expect substantial amounts of hydrated 5′-aldehydes **9** in the reaction of $HO^•$ radicals with 2′-deoxyadenosine (**26**) under

(45) Navacchia, M. L.; Chatgilialoglu, C.; Montevecchi, P. C. *J. Org. Chem.* **2006**, *71*, 4445–4452.
(46) Chatgilialoglu, C. *Chem.−Eur. J.* **2008**, *14*, 2310–2320.
(47) We cannot exclude minor paths of radical translocation in the intermediate **7** to positions other than the C5′ of the sugar radical. Theoretical calculation at the B3LYP/6-31G* level suggested that radical translocations to the C5′ and C3′ positions have activation energies of 3.2 and 11. 1 kcal mol^{-1}, respectively. See: Chatgilialoglu, C.; Duca, M.; Ferreri, C.; Guerra, M.; Ioele, M.; Mulazzani, Q. G.; Strittmatter, H.; Giese, B. *Chem.−Eur. J.* **2004**, *10*, 1249–1255.
(48) Chatgilialoglu, C.; Caminal, C.; Guerra, M.; Mulazzani, Q. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 6030–6032.
(49) Chatgilialoglu, C.; Caminal, C.; Altieri, A.; Vougioukalakis, G. C.; Mulazzani, Q. G.; Gimisis, T.; Guerra, M. *J. Am. Chem. Soc.* **2006**, *128*, 13796–13805.
(50) Chatgilialoglu, C.; Bazzanini, R.; Jimenez, L. B.; Miranda, M. A. *Chem. Res. Toxicol.* **2007**, *20*, 1820–1824.
(51) Candeias, L. P.; Steenken, S. *Chem.−Eur. J.* **2000**, *6*, 475–484.
(52) For an analogous experiment, see: Langfinger, D.; von Sonntag, C. *Z. Naturforsch., C: Biosci.* **1985**, *40*, 446–448.
(53) The formation of 5′-carboxaldehyde has been reported in the heavy ion-mediated decomposition products of 2′-deoxyguanosine. See: Gromova, M.; Nardin, R.; Cadet, J. *J. Chem. Soc., Perkin Trans.* **1998**, *2*, 1365–1374.
(54) Candeias and Steenken also found that in the reaction of $HO^•$ radicals with 9-methylguanine the depletion of the starting purine is incomplete.[51]
(55) A similar phenomenon has been also observed in the radiolytic degradation of 2′-deoxyguanosine in aerated aqueous solution. See: Douki, T.; Spinelli, S.; Ravanat, J.-L.; Cadet, J. *J. Chem. Soc., Perkin Trans.* **1999**, *2*, 1875–1880.

normoxic conditions. Evidence exists that some H-abstraction occurs from the deoxyribose unit and in particular from C5′ position.[57] Aqueous phosphate-buffered solution (pH 7.1) of 1.17 mM **26** was saturated with $N_2O(80\%)/O_2(20\%)$ before irradiation. During γ-radiolysis (dose rate = 8.0 Gy min^{-1}), continuous bubbling of the gas mixture in the sample ensured the constant concentration of O_2 during the experiment. The reaction was monitored by HPLC at 0.5 kGy dose intervals up to 2 kGy.[37b] The consumption of the starting material (48% after 2 kGy) was accompanied by the formation of two main products which elute at shorter retention times and are assigned to adenine and the hydrated 5′-aldehyde **9**. In terms of radiation chemical yields, the values $G(-\mathbf{26}) = 0.37$ and $G(\mathbf{9}) = 0.06$ μmol J^{-1} were found. Following the above-mentioned analysis, we suggest that under our experimental condition ca. 30% of the attack of $HO^\bullet$ radicals on **26** affords adenine-derived radicals that are converted back to starting nucleoside.[58] On the other hand, on the basis of the ratio $G(HO^\bullet)/G(\mathbf{9})$, we suggest that ca. 11% of $HO^\bullet$ radical attack occurs at the H5′ of the 2′-deoxyadenosine.[56]

Conclusions

The site-specific generation of C5′ radicals under various oxygen concentrations has been achieved by the reaction of hydrated electrons (e_{aq}^-) with 8-bromo-2′-deoxyadenosine or 8-bromo-2′-deoxyinosine. In the range of 13−266 μM of oxygen (typical oxygenated tissues), the C5′ radicals are partitioned between two reaction channels (i.e., the reaction with the O_2 and an unimolecular rearrangement) providing the hydrated 5′-aldehyde and the 5′,8-cyclopurine nucleoside, respectively. The formation of 5′,8-cyclopurines is relevant in all experiments, and the yield increased by decreasing O_2 concentration. Under aerobic conditions (266 μM of O_2), the 2′-deoxyadenosin-5′-yl radical affords a 15:1 ratio in favor of the hydrated 5′-aldehyde. In the reaction of $HO^\bullet$ radicals with 2′-deoxyadenosine or 2′-deoxyguanosine, under analogous conditions, the 5′,8-cyclopurines could not be quantified because the formation of C5′ radicals account only by ca. 10%. Without doubt, the most relevant site of H-atom abstraction by $HO^\bullet$ radical from 2-deoxyribose moiety of these two nucleosides results to be the H5′ position,[56] in agreement with the computational data.[7] The rate constant for the reaction of C5′ radical with GSH, the so-called "repair reaction", is found to be 5×10^7 M^{-1} s^{-1}. Since the intracellular level of GSH in mammalian cells is in the 0.5−10 mM range,[59] the repair reaction, the trapping by O_2, and the cyclization process should be in competition. However, it is not appropriate to extend the present findings to the behavior of the double-stranded DNA. Local conformations due to the supramolecular organization should influence considerably (either accelerating or reducing) the cyclization rate, and therefore it is necessary to provide measurements of 5′,8-cyclopurine lesions in different environments. Their identification in mammalian cellular DNA in vivo, where levels can be enhanced by conditions of oxidative stress, deserves further attention.[19−21]

Experimental Section

Materials. 8-Bromo-2′-deoxyadenosine, 2′-deoxyinosine, 2′-deoxyguanosine, and 2′-deoxyadenosine were purchased from Berry & Associates and used without any further purification. All other chemicals and solvents were purchased from Sigma-Aldrich and used as received. Solutions were freshly prepared using water purified with a Millipore (Milli-Q) system; their pH was buffered with potassium phosphates (Merck). 8-Bromo-2′-deoxyinosine was synthesized by bromination of 2′-deoxyinosine.[25] The solutions of nucleosides were freshly prepared immediately before each experiment.

Pulse Radiolysis. Pulse radiolysis with optical absorption detection was performed as previously reported.[24]

Continuous Radiolysis. The experiments were performed at room temperature (22 ± 2 °C) on 5-mL samples using ^{60}Co-Gammacells, with a dose rate of ca. 8 Gy min^{-1}. The absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking $G(Fe^{3+}) = 1.61$ μmol J^{-1}.[60] Reaction mixtures were analyzed with a Zorbax SB-C18 column (4.6 × 150 mm) and eluted in triethylammonium acetate buffer (20 mM, pH 7) with a 0−20% acetonitrile linear gradient over 35 min with a flow rate of 1.0 mL/min (detection at 254 nm).

2′-Deoxyadenosine Hydrated 5′-Carboxaldehyde. *Method A.* An Ar-purged aqueous phosphate-buffered solution (10 mM, pH = 7.1) of 8-bromo-2′-deoxyadenosine (50 mg, 0.15 mmol, 1.5 mM) containing 0.25 M *t*-BuOH and 1 mM $K_3Fe(CN)_6$ was initially γ-irradiated with a dose of 1.5 kGy. Subsequently, amounts of $K_3Fe(CN)_6$ (1 mM) were added at 1.5 kGy dose intervals to keep the oxidant concentration in the desired range. The solution was irradiated with a total dose of 6 kGy. Under these conditions, there is ca. 40% conversion of the starting material and an 85% yield of the aldehyde among the products. After passage of reaction crude through an ion-exchange resin (Amberlite IRA-400) to eliminate the iron salts, the volume of the mixture was reduced to ∼2 mL by lyophilization and chromatographed on RP-18 silica using 0−4% acetonitrile/water as the eluent. The fractions containing the hydrated 5′-aldehyde were lyophilized to afford 13.6 mg of the pure product as a white powder. *Method B.* An aqueous phosphate-buffered solution (10 mM, pH = 7.1) of 8-bromo-2′-deoxyadenosine (50 mg, 0.15 mmol, 2 mM) was γ-irradiated under air bubbling with a dose of 10 kGy. Under these conditions, there is a 70% conversion of the starting material and a 50% yield of the aldehyde among the products. The volume of the reaction crude was reduced to ∼5 mL by lyophilization and chromatographed on RP-18 silica using 0−4% acetonitrile/water as the eluent. The fractions containing the hydrated 5′-aldehyde were lyophilized to afford 18.7 mg of the pure product as a white powder. ^{1}NMR (400 MHz, D_2O) δ 2.51 (m, 1H, H-2″), 2.78 (m, 1H, H-2″), 4.08 (dd, 1H, $J_{H3'} = 1.9$, $J_{H5'} = 3.7$ Hz, H-4′), 4.67 (m, 1H, H-3′), 5.16 (d, 1H, $J_{H4'} = 3.7$ Hz, H-5′), 6.41 (dd, 1H, $J_{H2'} = 6.1$, $J_{H2''} = 8.2$ Hz, H-1′), 8.06 (s, 1H, H-2), 8.21 ppm (s, 1H, H-8). ^{13}C NMR (100 MHz, D_2O) δ 38.9 (C-2′), 71.5 (C-3′), 85.4 (C-1′), 89.2 (C-4′), 89.4 (C-5′), 118.8 (C-5), 140.5 (C-8), 148.1 (C-4), 152.3 (C-2), 155.4 (C-6). (+)-ESI-MS: 267.8 [100 $(M + H)^+$], (+)-ESI-MS/MS: 249.8, 135.9 UV (H_2O, nm): 213.5, 257.0 (max), 228.5 (min). The assignment of the NMR resonances was carried out by COSY and HSQC experiments.

2′-Deoxyinosine Hydrated 5′-Carboxaldehyde. The above-described Methods A and B were used for 8-bromo-2′-deoxyinosine (50 mg). *Method A.* The hydrated 5′-aldehyde was isolated in 60%

(56) It is possible to estimate roughly the total percentage of $HO^\bullet$ radical attacks on the deoxyribose unit by assuming that the free base can result from attacks other than the one to the H5′ position.[1,2] The values of G(guanine) = 0.03 and G(adenine) = 0.06 μmol J^{-1} were found in the experiments of $HO^\bullet$ radical with **23** and **26**, respectively. We calculated that ca. 14 and 22% of $HO^\bullet$ radicals attack the deoxyribose unit of 2′-deoxyguanosine and 2′-deoxyadenosine, respectively, which is in excellent agreement with the estimated values obtained from the reducing properties of sugar-derived radicals by pulse radiolysis.[51,58]

(57) Mariaggi, N.; Cadet, J.; Teoule, R. *Tetrahedron* **1976**, *32*, 2385–2387.

(58) Vieira, A. J. S. C.; Steenken, S. *J. Am. Chem. Soc.* **1990**, *112*, 6986–6994.

(59) Meister, A.; Anderson, M. E. *Annu. Rev. Biochem.* **1983**, *52*, 711–760.

(60) Spinks, J. W. T.; Woods, R. J. *An Introduction to Radiation Chemistry*, 3rd ed.; Wiley: New York, 1990; p 100.

yield (22.8 mg, 0.084 mmol). *Method B.* The hydrated 5′-aldehyde was isolated in 25% yield (10 mg, 0.037 mmol). ^{1}NMR (400 MHz, D_2O) δ 2.57 (m, 1H, H-2′), 2.87 (m, 1H, H-2″), 4.08 (m, 1H, H-4′), 4.72 (m, 1H, H-3′), 5.17 (d, 1H, $J_{H4'} = 3.7$ Hz, H-5′), 6.51 (dd, 1H, $J_{H2'} = 6.7$, $J_{H2''} = 7.4$ Hz, H-1′), 8.20 (s, 1H, H-2), 8.26 ppm (s, 1H, H-8). ^{13}C NMR (100 MHz, D_2O) δ 39.1 (C-2′), 71.5 (C-3′), 85.5 (C-1′), 89.2 (C-4′), 89.4 (C-5′), 124.2 (C-5), 140.0 (C-8), 148.2 (C-2), 148.7 (C-4), 157.4 (C-6). *m*/*z* (ESI) 268.7 $[M + H]^+$, *m*/*z* (ESI-MS/MS): 136.9 UV (H_2O, nm): 248.7 (max), 217.9 (min). The assignment of the NMR resonances was carried out by COSY and HSQC experiments.

2′-Deoxyguanosine Hydrated 5′-Carboxaldehyde. An aqueous phosphate-buffered solution (10 mM, pH = 7) of 2′-deoxguanosine (40 mg, 2 mM) was γ-irradiated with a dose of 5 kGy under continuous bubbling of a mixture of N_2O (80%) and O_2 (20%). The reaction crude was concentrated to ~2 mL and passed through a column packed with RP-18 silica eluted with water. The samples containing the hydrated 5′-aldehyde were lyophilized to afford 2 mg of the pure product as a white powder. ^{1}NMR (400 MHz, D_2O) δ 2.47 (m, 1H, H-2′), 2.82 (m, 1H, H-2″), 4.04 (m, 1H, H-4′), 4.42 (m, 1H, H-3′), 5.16 (d, 1H, $J_{H4'} = 4.0$ Hz, H-5′), 6.35 (dd, 1H, $J_{H2'} = 6.1$, $J_{H2''} = 8.6$ Hz, H-1′), 7.94 ppm (s, 1H, H-8). ^{13}C NMR (100 MHz, D_2O) δ 38.6 (C-2′), 71.3 (C-3′), 85.6 (C-1′), 89.0 (C-4′), 89.5 (C-5′), 117.2 (C-5), 139.1 (C-8), 151.4 (C-4), 153.8 (C-2), 158.4 (C-6). *m*/*z* (ESI) 283.7 $[M + H]^+$, 265.8 $[M + H - H_2O]^+$ *m*/*z* (ESI-MS/MS): 151.8 UV (H_2O, nm): 252.3, 273.8 (max), 220.2 (min). The assignment of the NMR resonances was carried out by COSY and HSQC experiments.

Acknowledgment. This work was supported in part by the European Community's Marie Curie Research Training Network under Contract MRTN-CT-2003-505086 [CLUSTOXDNA]. The support and sponsorship concerned by COST Action CM0603 on "Free Radicals in Chemical Biology (CHEMBIORADICAL)" are kindly acknowledged. We thank M. Lavalle, A. Monti, and A. Martelli for assistance with the pulse radiolysis experiments.

Supporting Information Available: Product studies and pulse radiolysis experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

JA800763J