

# Selective Generation and Reactivity of 5'-Adenosinyl and 2'-Adenosinyl Radicals

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**Abstract:** The reaction of hydrated electrons ( $e_{aq}^-$ ) with 8-bromoadenosine **7** has been investigated by radiolytic methods coupled with product studies. Pulse radiolysis revealed that one-electron reductive cleavage of the C–Br bond gives the C8 radical **8** followed by a fast radical translocation to the sugar moiety. The reaction is partitioned between C5' and C2' positions in a 60:40 ratio leading to 5'-adenosinyl

radical **9** and 2'-adenosinyl radical **11**. This radical translocation from C8 to different sites of the sugar moiety has also been addressed computationally by means of DFT B3LYP calculations. In addition, ketone **21** was prepared

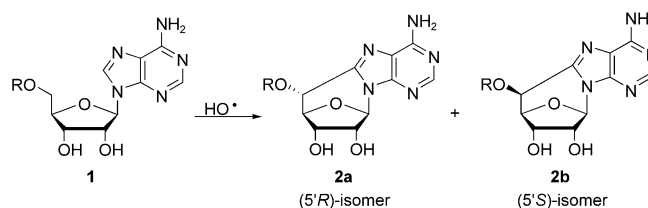
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and photolyzed providing an independent generation of C2' radical **11**. Both C5' and C2' radicals undergo unimolecular reactions. Radical **9** attacks adenine with a rate constant of  $1.0 \times 10^4 \text{ s}^{-1}$  and gives the aromatic aminyl radical **10**, whereas C2' radical **11** liberates adenine with a rate constant of  $1.1 \times 10^5 \text{ s}^{-1}$ .

## Introduction

In 1968 Keck discovered that attack of  $\text{HO}^\bullet$  radicals at adenine-5'-phosphate **1** ( $\text{R} = \text{PO}_3^{2-}$ ) leads among other products to the 5',8-cycloadenosides **2a** and **2b**.<sup>[1]</sup> The effect of pH on the formation of the diastereomers **2a, b** has been studied in detail,<sup>[2]</sup> and it turned out that only the (5'*R*)-isomer **2a** is enzymatically active.<sup>[3]</sup> Radiation-induced damages to polyadenylic acid, as well as to free adenosine have also been investigated in some details.<sup>[4]</sup> Depending on the substrate and the experimental conditions, the ratio of the (5'*S*)- and (5'*R*)-isomers changes substantially.  $\gamma$ -Irradiation of an

aqueous solution of adenosine, adenosine-5'-monophosphate and polyadenylic acid at pH 7 afforded (5'*R*):(5'*S*) ratios of 1.8, 0.4, and 1.6, respectively.<sup>[4]</sup> It has been proposed that a C5' radical might intramolecularly attack the C8,N7 double bond of the adenine moiety to form 5',8-cycloadenosides **2a, b** as final products (Scheme 1). The fact that molecular oxygen inhibits these reactions, was interpreted as trapping of the C5' radical before its attack at the C8 position of adenine.



Scheme 1. The two diastereoisomers of 5',8-cycloadenosine derivatives.

Similar reactions have been observed in the 2'-deoxy-*ribo* series.  $\gamma$ -Irradiation of an aqueous solution of 2'-deoxyadenosine afforded mainly the (5'*R*)-isomer, whereas the (5'*R*):(5'*S*) ratios, using single- and double-stranded DNA, are approximately 2.<sup>[5,6]</sup> Some of us recently investigated the reaction of hydrated electrons ( $e_{aq}^-$ ) with 8-bromo-2'-deoxyadenosine (**3**) by radiolytic methods.<sup>[7]</sup> It was found that **3** captures electrons and rapidly loses the bromide ion to give the corresponding C8 radical **4**. Radical **4** abstracts intramo-

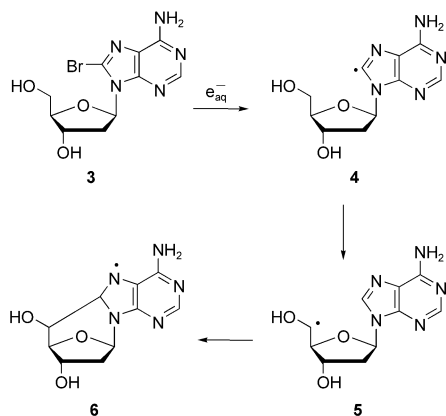
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lecularly a hydrogen atom exclusively from the C5' position affording selectively C5' radical **5** (Scheme 2). This allowed for the first time to study the fate of the 2'-deoxyadenosin-5'-yl radical **5** properly, and in particular the cyclization step **5**→**6**, which occurs with a rate constant of  $1.6 \times 10^5 \text{ s}^{-1}$ .

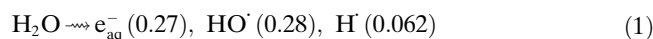


Scheme 2. Chemical studies of hydrated electrons with 8-bromo-2'-deoxyadenosine.

We have now found in radiation studies with 8-bromo-adenosine **7** that in the *ribo* series the intramolecular hydrogen abstraction by the initially formed C8 radical is partitioned between two channels, generating both C5' and C2' radicals with similar rate constants.<sup>[8]</sup>

## Results and Discussion

**Reaction of hydrated electrons ( $e_{\text{aq}}^-$ ) with 8-bromo-adenosine **7**:** Radiolysis of neutral water leads to  $e_{\text{aq}}^-$ ,  $\text{HO}^\cdot$  and  $\text{H}^\cdot$  as shown in [Eq. (1)]. The values in parentheses represent the radiation chemical yields ( $G$  values) in units of  $\mu\text{mol J}^{-1}$ .<sup>[9]</sup> The reactions of  $e_{\text{aq}}^-$  with the substrates were studied in  $\text{O}_2$ -free solutions containing 0.25 M *t*BuOH. With this amount of *t*BuOH,  $\text{HO}^\cdot$  is scavenged efficiently [Eq. (2),  $k_2 = 6.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ ], whereas  $\text{H}^\cdot$  reacts only slowly [Eq. (3),  $k_3 = 1.7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ].<sup>[9,10]</sup> Therefore, the reactions of  $\text{H}^\cdot$  may be relevant since they can account for as much as ~20% of the products (see below).



The pseudo first-order rate constant,  $k_{\text{obs}}$ , for the reaction of  $e_{\text{aq}}^-$  with a defined amount of 8-bromo-adenosine **7** was determined by measuring the rate of the decrease of the optical density of  $e_{\text{aq}}^-$  at 720 nm ( $\epsilon = 1.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ).<sup>[11]</sup> From the slope of  $k_{\text{obs}}$  versus  $[\textbf{7}]$ , the bimolecular rate constant was determined to be  $1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ , which is very similar to the analogous reactions with adenosine ( $1.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ).<sup>[12]</sup>

and with the 8-bromo-2'-deoxyadenosine **3** ( $1.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ).<sup>[7]</sup>

The reaction of an aqueous solution of **7** (1 mM) and *t*BuOH (0.25 M) at pH ~7 with  $e_{\text{aq}}^-$  in the absence of  $\text{O}_2$  was complete within approximately 300 ns. At this time, no significant absorption was detected in the 300–750 nm region. However, a spectrum containing two bands centered at 350 and 480 nm, respectively, developed in 100  $\mu\text{s}$  (Figure 1). The time profile for the formation of the transient with  $\lambda_{\text{max}} = 350 \text{ nm}$  (Figure 1, inset a) follows a first-order kinetic

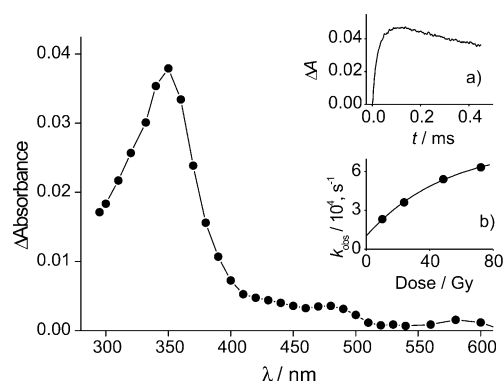


Figure 1. Absorption spectrum obtained from the pulse radiolysis of an Ar-purged solution containing 1 mM **7** and 0.25 M *t*BuOH at pH ~7, taken 100  $\mu\text{s}$  after the pulse; dose = 20 Gy, optical path = 2.0 cm. Insets: a) Time dependence of absorption at 350 nm; dose = 23.8 Gy. b) Dependence of  $k_{\text{obs}}$  (see text) from the radiation dose.

with a rate constant ( $k_{\text{obs}}$ ) that is independent of **7** in the concentration range 0.2–1 mM. But  $k_{\text{obs}}$  increased with the dose/pulse ratio (Figure 1, inset b). This dose dependence is due to the mixing of the first-order growth and the second-order decay of the species. Using an empirical expression for the fitting of the experimental data and extrapolating at zero dose,<sup>[13]</sup> a rate constant  $k_c = (1.0 \pm 0.2) \times 10^4 \text{ s}^{-1}$  at 20 °C is obtained. The absorbance at 350 nm is also found to vary substantially with the dose. An apparent molar extinction coefficient ( $\epsilon_{\text{app}}$ ) of  $6100 \pm 100 \text{ M}^{-1} \text{ cm}^{-1}$  at 350 nm was calculated by extrapolating to zero dose and assuming a radiation chemical yield  $G = 0.27 \mu\text{mol J}^{-1}$ , which is the  $G$  of hydrated electrons.<sup>[9]</sup>

In analogy to the reactions of 8-bromo-2'-deoxyadenosine **3**,<sup>[7]</sup> we assigned the transient in Figure 1 to the conjugated aminyl radical **10**, and the observed rate to the cyclization of radical **9** (Scheme 3). Compared with the 2'-deoxyribosides, in the *ribo* case the spectrum containing the two bands developed more slowly (100 vs 20  $\mu\text{s}$ ) and the absorbance at the maximum (350 vs 360 nm) was about two thirds. The cyclization rate constant for reaction **9**→**10** ( $k_c = 1.0 \times 10^4 \text{ s}^{-1}$ ) is 16 times slower than that of the 2'-deoxy-*ribo* analogue **5**→**6** (Scheme 2). This is probably a consequence of the conformational changes in going from *ribo* to 2'-deoxy-*ribo* derivatives.

Because the extinction coefficients of radicals **6** and **16** are similar (ca.  $1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ).<sup>[7,14]</sup> it is reasonable to

assume a similar extinction coefficient also for radical **10**. This analysis suggests that about 60% of the radicals, produced by reaction of the hydrated electron with **7**, leads to radical **10** (Scheme 3).

Further insight into the reaction was gained by experiments in the presence of *N,N,N,N*-tetramethyl-*p*-phenylenediamine (TMPD).<sup>[15]</sup> Pulsing O<sub>2</sub>-free solutions of 1 mM **7** containing 0.25 M *t*BuOH and different concentrations of TMPD (25–100 μM) at pH ~7 led to the oxidation of TMPD. The rate constants for this oxidation, and the associated formation of TMPD<sup>•+</sup> were measured at 565 nm ( $\epsilon = 1.25 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ), which represents one of the absorption maxima of TMPD<sup>•+</sup> (Figure 2).<sup>[16,17]</sup> Because under these experimental conditions HO• radicals are trapped by *t*BuOH, and the reaction of TMPD with the carbon centered radicals derived from *t*BuOH is unimportant in the time scale of our experiments,<sup>[18]</sup> we concluded that the TMPD<sup>•+</sup> radical cation is formed from intermediates generated by the reaction of e<sub>aq</sub><sup>-</sup> with **7**.<sup>[19]</sup>

The yield of TMPD<sup>•+</sup> (corrected for its decay) increased by increasing TMPD concentration, varying between 15% for [TMPD] = 25 μM to 40% for TMPD = 100 μM relatively to the yield of e<sub>aq</sub><sup>-</sup>. Figure 2 shows that an induction period is observed for the formation of TMPD<sup>•+</sup>, which could be satisfactorily fitted using a two consecutive reactions model. The first component is independent of TMPD concentration (Figure 2, inset a) and occurs with a rate constant of  $k_1 = (1.1 \pm 0.1) \times 10^5 \text{ s}^{-1}$ . The second component depends on the concentration of TMPD and the rate constant  $k_{\text{TMPD}} = (4.6 \pm 0.3) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  (Figure 2, inset b) is assigned to the species reacting with TMPD.

Based on the experimental data obtained from the independent generation of the 2'-adenosinyl radical **11** (see below), we suggest that the initially produced radical **8** affords not only the 5'-radical **9** but also the 2'-radical **11** (Scheme 3). We assigned the observed unimolecular process

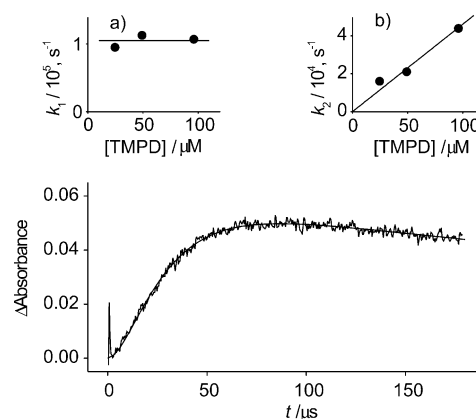
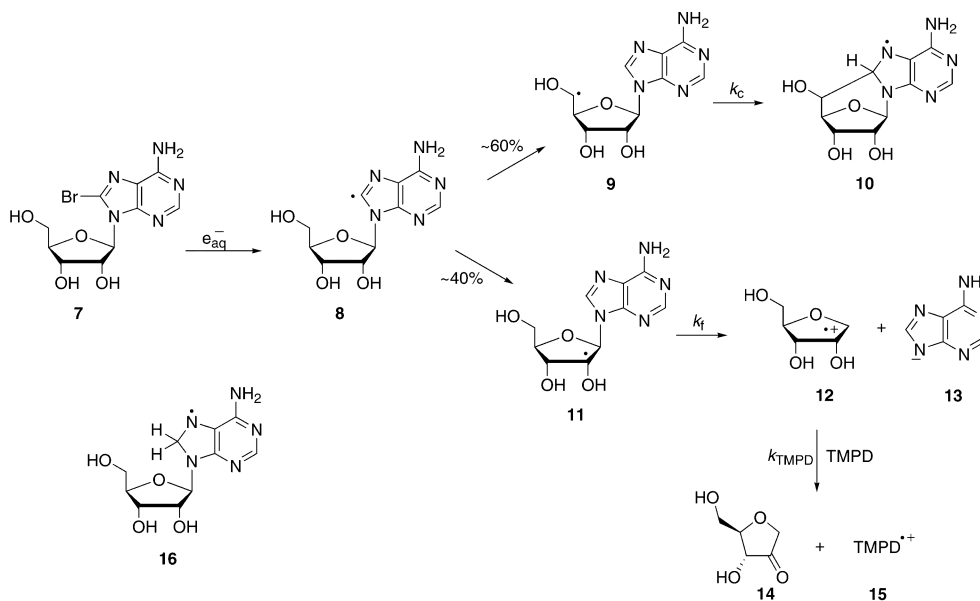


Figure 2. Time dependence of absorption at 565 nm obtained from the pulse radiolysis of an Ar-purged solution containing 1 mM **7**, 0.25 M *t*BuOH and 96 μM TMPD at pH ~7; dose = 22 Gy, optical path = 2.0 cm. Insets: a) Dependence of the first component  $k_1$  from the [TMPD]. b) Dependence of the second component  $k_2$  from the [TMPD].

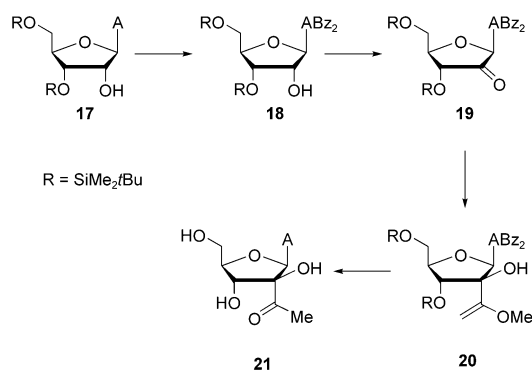
( $k_t = 1.1 \times 10^5 \text{ s}^{-1}$ ) to the heterolysis of the glycosidic bond in radical **11** producing radical cation **12** and the base anion **13**. Analogous heterolytic β-C,O-bond cleavages of tertiary, oxygen-substituted carbon radicals are well known.<sup>[20]</sup> Radical cation **12** oxidizes TMPD giving presumably compound **14**. It is worth mentioning that in the case of the deoxyribosides the build up of TMPD<sup>•+</sup> was not observed.<sup>[7]</sup> This strengthens our mechanistic suggestion because a heterolytic cleavage of β-C,O-bonds in secondary alkyl radicals has never been observed.

#### Independent generation of the 2'-adenosinyl radical (**11**):

The suggestion that radical **11** liberates adenine in a β-bond cleavage was proven by its independent generation in a Norrish photoreaction of 2'-acetylated nucleoside derivative **21** (Scheme 4). The synthesis of **21** started from the partly pro-

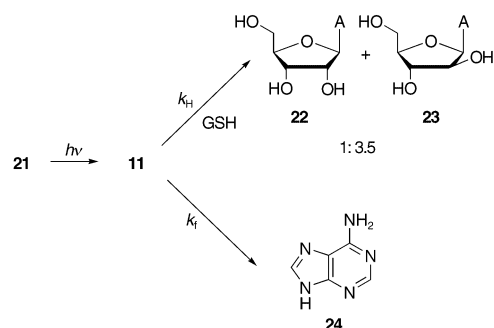


Scheme 3. Proposed mechanism for the fate of radical **5** based on pulse radiolysis studies.

Scheme 4. Synthesis of 2'-acetylated nucleoside **21**.

ected adenosine **17**, which was benzoylated at the NH<sub>2</sub> group of adenine (**17**→**18**), and oxidized at the free 2'-OH function of the ribose (**18**→**19**). Vinylation of the resulting ketone **19** yielded enol ether **20** whose deprotection led to the 2'-acetylated nucleoside derivative **21**. The C,C-bond formation *anti* to the adenine (**19**→**20**) was proven by NOE experiments.

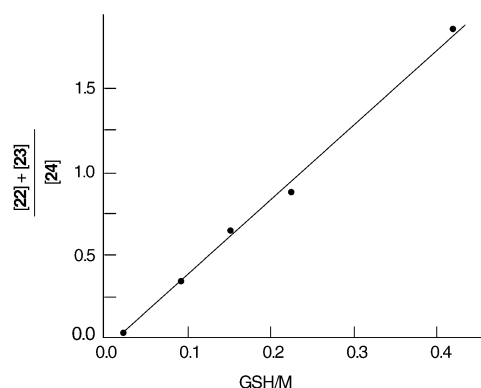
Photolysis of **21** generated the adenosin-2'-yl radical **11**, which was trapped by glutathione (GSH) (Scheme 5). This led to the *ribo*- and *arabino*-products **22** and **23**, respectively, in a 1:3.5 ratio. The preferred attack of the thiol from the α-face of the C2' radical is due to the efficient β-face shielding by the adenine moiety. In addition, adenine **24** was set free. The dependence of the product ratio (**22**+**23**)/**24** upon the concentration of glutathione was determined in competition kinetic experiments (Figure 3).

Scheme 5. Competition kinetics from the selective generation of C2' radical **11**.

Because the measurements were carried out with an at least tenfold excess of glutathione, the H-abstraction could be analyzed by a pseudo first-order kinetic. Thus, the ratio between the rate of the hydrogen abstraction  $k_H$  and the rate of elimination  $k_f$  are described by [Equation (4)].

$$\frac{[\mathbf{22}] + [\mathbf{23}]}{[\mathbf{24}]} = \frac{k_H}{k_f} [\text{GSH}] \quad (4)$$

From the slope of the straight line in Figure 3 a  $k_H/k_f$  ratio of  $4.3 \text{ M}^{-1}$  is obtained. The rates of H-transfer from alkane-thiols to alkyl radicals depend strongly upon the radical substituents.<sup>[21,22]</sup> For example, the α,β-dialkoxyalkyl radical

Figure 3. Plot obtained from the competition between fragmentation and hydrogen abstraction from GSH by radical **11** (Scheme 5) according to Equation (4).

reacts about 10 times slower than the analogous unsubstituted alkyl radical. A reasonable assumption for the reaction of C2' radical **11** with GSH is a rate constant of about  $10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,<sup>[21]</sup> which suggests a rate constant  $k_f$  of about  $2 \times 10^5 \text{ s}^{-1}$  for the β-elimination. This is in good agreement with the rate observed in the radiolysis studies.

**DFT calculations:** Theoretical calculations with the DFT method were performed at the B3LYP6-31G\* level<sup>[23,24]</sup> to determine the factors that affect the activation energies of radical translocation from the adenine to the C5', C3' and C2' positions of the ribose.<sup>[25]</sup> All three reactions are calculated to be strongly exothermic as expected on the thermochemical grounds.<sup>[26]</sup> Radical translocation to the C5' position has the lowest activation energy ( $3.1 \text{ kcal mol}^{-1}$ ), which is similar to that of the 2'-deoxyribose analogue ( $3.2 \text{ kcal mol}^{-1}$ ).<sup>[7]</sup> Hydrogen abstraction from the C2' position occurs with a barrier of  $9.6 \text{ kcal mol}^{-1}$ , whereas the analogous reaction in the deoxyribonucleoside has a much higher activation energy ( $16.2 \text{ kcal mol}^{-1}$ ). This difference of  $6.6 \text{ kcal mol}^{-1}$  can be attributed to the stabilization of the C2' radical by the hydroxyl group in the ribose series. Radical translocation to the C3' positions has the highest activation barrier ( $11.1 \text{ kcal mol}^{-1}$ ) in the ribonucleoside radical **8**.

These calculations show that the difference of the activation energies between H-atom abstraction from C5' (**8**→**9**) and C2' (**8**→**11**) is  $6.5 \text{ kcal mol}^{-1}$ , whereas experimental observations indicate that the processes are competitive. Maybe the activation entropies of these two pathways are different and favor the hydrogen abstraction from C2'. Actually, in the ground state of **8** the distance between the radical site at C8 is considerably longer ( $3.84 \text{ Å}$ ) to the hydrogen at C5' than to that at C2' ( $2.70 \text{ Å}$ ). In order to reach the transition state for the H-abstraction from C5', one loses not only the freedom of rotation around the C4',C5'-bond but one also decreases conformational freedom in the sugar ring. Thus, the activation entropy favors the hydrogen abstraction from C2' compared with the radical translocation to C5'.

**Product studies from continuous radiolysis of 8-bromoadenosine 7:** In order to further strengthen our mechanistic sug-

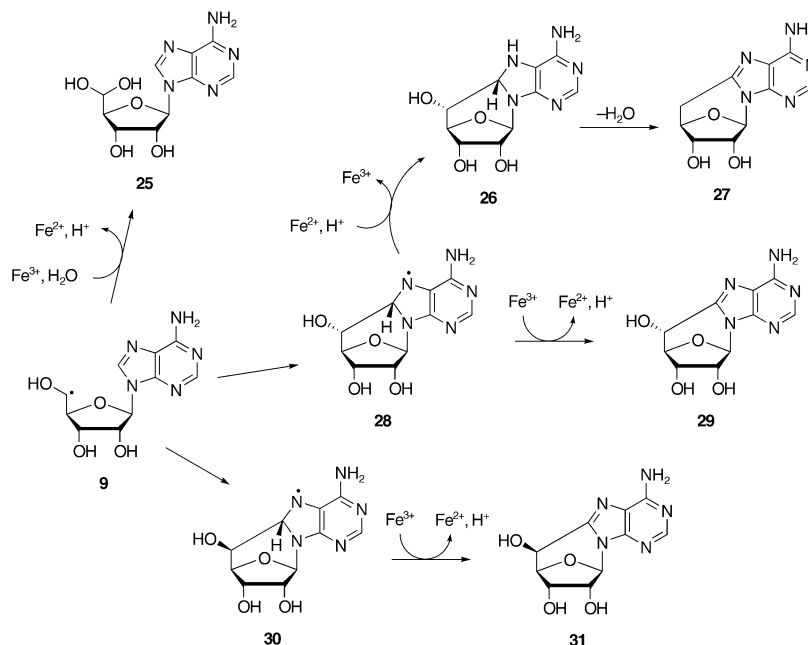
gestions derived from the pulse radiolysis of **7**, we also carried out product studies from the  $\gamma$ -radiolysis experiments. Samples containing 3 mL of aqueous solution of **7** (ca. 1.5 mM) and *t*BuOH (0.25 M) at pH  $\sim$ 7, were irradiated under stationary-state conditions with a total dose up to 1.5 kGy at a dose rate of about 20 Gymin<sup>-1</sup>, followed by HPLC analysis. Although complex chromatograms were obtained, the main reaction product was the free base (adenine) that accounts for 40–45% of the reacted bromide. These results are in excellent agreement with the pulse radiolysis observations reported above. It is worth mentioning that adenine was a minor reaction product in the analogous experiment with **3**,<sup>[7]</sup> which further motivates our conclusion that the free base can derive from the C2' radical chemistry.

In the case of **3**, both radicals **5** and **6** are readily oxidized by  $\text{Fe}(\text{CN})_6^{3-}$ , the rate constants being  $4.2 \times 10^9$  and  $8.3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  respectively, whereas radical **6** can also be reduced by strong reductants.<sup>[7]</sup> It was suggested that under  $\gamma$ -irradiation of aqueous solutions of 4 mM  $\text{K}_4\text{Fe}(\text{CN})_6$  (reductant), a continuous generation of micromolar levels of the oxidant  $\text{Fe}(\text{CN})_6^{3-}$  could be obtained. Such a low concentration of oxidant should allow radical **9** before being oxidized to cyclize to radical **10**.

A deaerated aqueous solution (1 L) containing 1.5 mM of **7** (520 mg), 0.25 M *t*BuOH and 4 mM  $\text{K}_4\text{Fe}(\text{CN})_6$  at pH  $\sim$ 7 was  $\gamma$ -irradiated with a total dose up to 3 kGy at a dose rate of 18 Gymin<sup>-1</sup>. After work-up, the following compounds were eluted in the order (yields in parenthesis): adenosine 5'-carboxyaldehyde **25** (5%), (5'*R*)-5',8-cycloadenosine **29** (14%), adenosine (10%), 5',8-cyclo-5'-deoxyadenosine **27** (3%), (5'*S*)-5',8-cycloadenosine **31** (4%), and adenine (50%).

Also under these conditions, adenine was found once again as the major reaction product (50%) and this further suggests that heterolytic cleavage of the C2' radical is faster than oxidation by  $\mu\text{M}$  levels of  $\text{Fe}(\text{CN})_6^{3-}$  generated in situ. Regarding the formation of products that derived from C5' radical chemistry, Scheme 6 shows our mechanistic proposal.<sup>[27]</sup> Cyclization of C5' radical **9** should afford mainly two aminyl radicals **28** and **30**, which are in the chair conformation. The fate of these radicals strongly depends on the concentration of the iron species. We suggest that the  $\mu\text{M}$  level of  $\text{Fe}(\text{CN})_6^{3-}$  generated in situ can easily oxidize radicals **28** and **30** to give products **29** and **31**, respectively. But radicals **28** and **30** can also be reduced to a minor extent by

$\text{Fe}(\text{CN})_6^{4-}$ , since its concentration is several orders higher than that of  $\text{Fe}(\text{CN})_6^{3-}$ . So that, reduction of radical **28** followed by fast protonation gives compound **26**, which can readily dehydrate (the OH group in the 5' position is *anti* to the H atom in the 8 position), and yields cyclonucleoside **27**. The formation of the hydrated aldehyde **25** (5%) should be due to the oxidation of radical **9**.



Scheme 6. Proposed mechanism for the formation of nucleosides derived from C5' radical **9**.

## Conclusion

The results described herein demonstrate that the reaction of 8-bromoadenosine **7** with  $e_{\text{aq}}^-$  at pH  $\sim$ 7 leads to 5'-adenosinyl radical **9** and 2'-adenosinyl radical **11** in a ratio of about 60:40. In addition, radical **11** was selectively generated by photolyzing the precursor **21**. Using time-resolved spectroscopy and competition kinetic methods we could show that these radicals undergo unimolecular reactions. We found that the C5' radical adds intramolecularly to the C8,N7 double bond of the adenine moiety with a rate constant of  $1.0 \times 10^4 \text{ s}^{-1}$  affording (5'*R*) and (5'*S*)-isomers in a ratio of about 3.5:1, whereas the C2' radical liberates adenine with a rate constant of  $1.1 \times 10^5 \text{ s}^{-1}$  (Scheme 3). Our findings can furnish a molecular basis for forthcoming experiments with RNA radicals, as well as with adenosine derivatives that play crucial roles in biological processes.

## Experimental Section

**Pulse radiolysis:** Pulse radiolysis with optical absorption detection was performed by using the 12 MeV linear accelerator, which delivered 20–200 ns electron pulses with doses between 5 and 50 Gy, by which  $\text{HO}^\bullet$ ,  $\text{H}^\bullet$ , and  $e_{\text{aq}}^-$  are generated with 1–20  $\mu\text{M}$  concentrations. The pulse irradiations

were performed at room temperature ( $22 \pm 2^\circ\text{C}$ ) 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 cut-off 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 a  $\text{N}_2\text{O}$ -saturated solution containing  $0.1\text{ M HCO}_3^-$  and  $0.5\text{ mM}$  methyl viologen, using  $G\epsilon = 9.66 \times 10^{-4}\text{ m}^2\text{J}^{-1}$  at  $602\text{ nm}$ .<sup>[28]</sup>  $G(\text{X})$  represents the number of moles of species X formed or consumed per Joule of energy absorbed by the system.

**6-*N,N*-Dibenzoyl-3',5'-di-*O*-(*tert*-butyldimethylsilyl)-adenosine (18):** Trimethylsilyl chloride (4.70 g, 43.4 mmol) was added at  $20^\circ\text{C}$  to a solution of **17** (10.2 g, 20.6 mmol) in piperidine (80 mL). After 80 min benzoyl chloride (14.4 g, 102 mmol) was added and quenched after 90 min with a saturated solution of  $\text{NaHCO}_3$  (1000 mL). The solution was extracted with dichloromethane ( $4 \times 100\text{ mL}$ ), the extracts dried over  $\text{MgSO}_4$  and the solvent removed under reduced pressure. The residue was solved in ethanol (30 mL) and *p*-toluenesulfonic acid (160 mg, 0.94 mmol) added at  $0^\circ\text{C}$ . After 2 h, the reaction was quenched with  $\text{NaHCO}_3$  (1 g, 11.9 mmol) and the ethanol removed under reduced pressure. The residue was solved in water (100 mL), extracted with dichloromethane ( $4 \times 100\text{ mL}$ ), dried over  $\text{MgSO}_4$  and the solvent removed under reduced pressure. Chromatography over silica gel (dichloromethane/ethyl acetate 10:1) yielded nucleoside **18** as a yellow foam (9.40 g, 13.4 mmol, 67%).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.65, 8.29 (s, H-2 and H-8, 1H each), 7.87–7.84 (m, 4H, Ph), 7.51–7.46 (m, 2H, Ph), 7.38–7.33 (m, 4H, Ph), 6.03 (d,  $J$  = 4.7 Hz, 1H, H-1'), 4.57–4.63 (m, 2H, H-2', H-3'), 4.14 (dd,  $J$  = 3.4, 3.5 Hz, 1H, H-4'), 3.92 (dd,  $J$  = 3.6, 11.5 Hz, 1H, H-5'), 3.77 (dd,  $J$  = 3.2, 11.5 Hz, H-5'), 0.96, 0.88 (s each, 9H each, *t*Bu), 0.18, 0.06, 0.03–0.01 (s each, 3H,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 172.2, 152.8, 152.1, 151.7, 143.5, 134.0, 132.9, 129.4, 128.7, 127.8, 89.0, 85.5, 74.8, 71.5, 62.3, 25.9, 25.7, 18.4, 18.0, –4.6, –4.9, –5.4, –5.6; MS (ESI):  $m/z$ : 705  $[\text{M}+\text{H}]^+$ ; HR-MS (FAB): calcd for  $\text{C}_{30}\text{H}_{49}\text{N}_5\text{O}_6\text{Si}_2$ : 705.0163; found: 705.0161  $[\text{M}+\text{H}]^+$ .

**6-*N,N*-Dibenzoyl-3',5'-di-*O*-(*tert*-butyldimethylsilyl)-2'-keto-adenosine (19):** A solution of Dess–Martin periodinane (6.90 g, 16.3 mmol) and pyridine (6.39 g, 80.8 mmol) in dichloromethane (30 mL) was added at  $20^\circ\text{C}$  to nucleoside **18** (5.70 g, 8.10 mmol) in dichloromethane (35 mL). After 23 h the solution was quenched at  $0^\circ\text{C}$  with sodium thiosulfate (25.0 g, 100 mmol) and a saturated solution of  $\text{NaHCO}_3$  (200 mL). The mixture was extracted with dichloromethane, dried with  $\text{MgSO}_4$  and the solvent removed under reduced pressure. Chromatography on silica gel (dichloromethane/ethyl acetate 10:1) led to **19** as a colorless foam (3.60 g, 5.13 mmol, 63%).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.55, 8.08 (s each, 1H each, H-8 and H-2), 7.86–7.83 (m, 4H, Ph), 7.51–7.46 (m, 2H, Ph), 7.38–7.33 (m, 4H, Ph), 5.84 (s, 1H, H-1'), 5.20 (d,  $J$  = 8.6 Hz, 1H, H-3'), 4.03 (dd,  $J$  = 8.8, 3.8 Hz, 1H, H-4'), 4.03 (d,  $J$  = 12.7 Hz, 1H, H<sub>b</sub>-5'), 3.92 (dd,  $J$  = 12.4, 3.6 Hz, 1H, H<sub>a</sub>-5'), 0.95 (s, 9H, *t*BuSi), 0.77 (s, 9H, *t*BuSi), 0.23 (s, 3H, MeSi), 0.18 (s, 3H, MeSi), 0.01 (s, 3H, MeSi), –0.12 (s, 3H, MeSi);  $^{13}\text{C NMR}$  (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 206.8, 172.0, 153.2, 152.2, 151.9, 144.5, 133.7, 133.6, 133.1, 129.3, 128.6, 126.8, 80.1, 79.9, 71.4, 60.7, 25.5, 18.1, –4.5, –5.3, –5.5, –5.6. Because of the lability of the substance during chromatographic purification, a high resolution mass spectrum could not be performed.

**6-*N,N*-Dibenzoyl-9-[3',5'-di-*O*-(*tert*-butyldimethylsilyl)-2'-(vinyl-1'-methyl enol ether)- $\beta$ -D-arabinofuranosyl]-adenine (20):** At  $-78^\circ\text{C}$  *tert*-butyllithium (0.7 mL of a 1.6 M solution in pentane, 1.12 mmol) was added slowly to a solution of methyl vinyl ether (620 mg, 10.7 mmol) in tetrahydrofuran (1.6 mL). The mixture was warmed up to  $0^\circ\text{C}$  for 3 min and then cooled down again to  $-78^\circ\text{C}$ . During this procedure the yellow solution became pale yellow-green. After 5 min the reaction mixture was added to a cooled ( $0^\circ\text{C}$ ) solution of ketone **19** (213 mg, 0.3 mmol) in tetrahydrofuran (5 mL). The mixture was quenched after 10 min with a saturated  $\text{NH}_4\text{Cl}$  solution (50 mL), water was added (50 mL) and extracted with dichloromethane ( $3 \times 50\text{ mL}$ ). From the dried solution ( $\text{MgSO}_4$ ) the solvent was evaporated under reduced pressure and the residue purified by chromatography over silica gel (pentane/methyl acetate 3:1). This led to enol ether **20** (65.4 mg, 28%).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 8.68 (s, 2H, H-8 and H-2), 7.87–7.84 (m, 4H, Ph), 7.50–7.45 (m, 2H, Ph), 7.37–7.32 (m, 4H, Ph), 6.68 (s, 1H, H-1'), 5.00 (1H, H-3'), 4.44 (d,  $J$  = 3.3 Hz, 1H,  $\text{C}(\text{CH}_3\text{H}_\beta)\text{OCH}_3$ ), 4.19 (s, 1H, H-4'), 4.13 (d,  $J$  = 3.1 Hz, 1H,

$\text{C}(\text{CH}_3\text{H}_\beta)\text{OCH}_3$ ), 4.09 (sb, 1H, HO), 3.97 (d,  $J$  = 11.1, 1H, H-5'), 3.83 (d,  $J$  = 11.1 Hz, 1H, H-5'), 3.48 (s, 3H,  $\text{OCH}_3$ ), 0.95 (s, 9H, *t*BuSi), 0.91 (s, 9H, *t*BuSi), 0.15 (s, 3H, MeSi), 0.14 (s, 3H, MeSi), 0.09 (s, 3H, MeSi), 0.07 (s, 3H, MeSi);  $^{13}\text{C NMR}$  (75.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 172.3, 157.7, 152.0, 153.7, 151.4, 145.6, 134.1, 132.8, 129.4, 128.6, 126.9, 86.0, 85.4, 84.1, 81.7, 79.5, 63.1, 54.8, 25.9, 25.6, 18.5, 17.9, –4.7, –4.9, –5.4, –5.6. Because of the low yield we have directly deprotected the compound.

**(2-Acetyl- $\beta$ -D-arabinofuranosyl)-adenine (21):** Trimethylsilyl chloride (33.5 mg, 0.31 mmol) was added at  $20^\circ\text{C}$  to an acetonitrile solution (3 mL) of enol ether **20** (40.9 mg, 0.54 mmol) and sodium iodide (512 mg, 0.34 mmol). After 1 h the reaction was quenched with a saturated solution of  $\text{NH}_4\text{Cl}$  (60 mL) and the mixture extracted with dichloromethane ( $3 \times 60\text{ mL}$ ). After drying over  $\text{MgSO}_4$  the solvent was removed under reduced pressure. This led to a yellow foam that was treated for 2 h with a 40% methylamine solution (2 mL) and ethanol (0.6 mL). Evaporation under reduced pressure and coevaporation with toluene (two times) afforded a residue that was solved in tetrahydrofuran (2 mL). A tetrahydrofuran solution of tetrabutylammonium fluoride (180  $\mu\text{L}$  of a 1 M solution, 0.18 mmol) was added and, after 1 h, the reaction mixture was quenched with trimethylsilyl chloride (85.9 mg, 0.79 mmol). After addition of water (1 mL), the mixture was co-evaporated twice under reduced pressure with toluene and the residue separated by reversed phase HPLC [tetraethylammonium acetate solution (20 mL, pH 7):  $\text{CH}_3\text{CN}$  98:2 (2 min)  $\rightarrow$  84:16 within 13 min]. This led to the deprotected ketone **21** (9.7 mg, 31.3 mmol, 61.1%) as a colourless powder.  $^1\text{H NMR}$  (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 8.32 and 8.13 (s each, 1H each, H-2 and H-8), 6.57 (s, 1H, H-1'), 4.58 (d,  $J$  = 7.0 Hz, 1H, H-3'), 4.09 (dd,  $J$  = 12.8, 2.0 Hz, 1H, H<sub>b</sub>-5'), 3.90 (dd,  $J$  = 12.8, 4.4 Hz, 1H, H<sub>a</sub>-5'), 2.39 (s, 3H,  $\text{COCH}_3$ );  $^{13}\text{C NMR}$  (75.5 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 212.5, 156.0, 153.2, 149.2, 142.0, 118.7, 87.6, 85.9, 82.8, 77.8, 60.7, 27.9; MS (ESI):  $m/z$ : 310  $[\text{M}+\text{H}]^+$ ; HR-MS (FAB): calcd for  $\text{C}_{12}\text{H}_{15}\text{N}_5\text{O}_5$ : 310.1151; found: 310.1151  $[\text{M}+\text{H}]^+$ .

**Kinetic experiments:** The modified adenosine **21** (0.62 mg, 0.20  $\mu\text{mol}$ ) and a large excess of glutathione (20  $\mu\text{mol}$  to 420  $\mu\text{mol}$ ) were solved in aqueous tetraethylammonium chloride (TEAA, 200  $\mu\text{L}$  of a 100 mM solution, pH 7.0) in a polymethyl methacrylate cuvette. The solution was deoxygenated with argon (30 min), thermostated at  $20^\circ\text{C}$ , and irradiated (500 W, Hg high pressure lamp, 320 nm cut-off filter) for 10 min. Then, water (0.5 mL) was added and the solution analyzed by RP-HPLC (20 mM TEAA:  $\text{CH}_3\text{CN}$  98:2 to 84:16 within 13 min). The compounds **22**–**23** and **24** were quantified using known material. A plot of  $[\text{22}] + [\text{23}]/[\text{24}]$  against the glutathione concentration gave a straight line with a slope of  $4.3\text{ M}^{-1}$  ( $r = 0.998$ ).

**Continuous radiolysis:** Continuous radiolyses were performed at room temperature ( $22 \pm 2^\circ\text{C}$ ) on 3 mL or 1 L samples using a  $^{60}\text{Co}$ -Gammacell, with dose rates between 18–20  $\text{Gy min}^{-1}$ . The absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking  $G(\text{Fe}^{3+}) = 1.61\text{ }\mu\text{mol J}^{-1}$ .<sup>[29]</sup> The reactions of **7** (purchased from Sigma) with  $\text{e}_{\text{aq}}^-$  and  $\text{H}^\cdot$  were investigated using deaerated aqueous solutions containing 1.5 mM substrate and 0.25 M *t*BuOH in the presence or absence of 4 mM  $\text{K}_4\text{Fe}(\text{CN})_6$  at pH  $\sim 7$ . The 1 L solution in the presence of 4 mM  $\text{K}_4\text{Fe}(\text{CN})_6$  was  $\gamma$ -irradiated with a total dose up to 3 kGy. The crude reaction mixture was passed through ion-exchange resin (Amberlite IRA-400) in order to eliminate the iron salts. The reaction crude was lyophilized and then separated by chromatography on RP silica gel (water/acetonitrile 7:3). The following compounds were eluting in the order (yields are based on the recovered starting material): **25**<sup>[30]</sup> ( $R_f = 0.91$ ; 11 mg, 0.043 mmol; 5%), **29**<sup>[31]</sup> ( $R_f = 0.83$ ; 31 mg; 0.12 mmol; 14%), adenosine ( $R_f = 0.62$ ; 23 mg; 0.086 mmol; 10%), **27**<sup>[32]</sup> ( $R_f = 0.56$ ; 6.4 mg; 0.026 mmol; 3%), **31**<sup>[31]</sup> ( $R_f = 0.56$ ; 9.1 mg; 0.034 mmol; 4%), adenine ( $R_f = 0.47$ ; 58 mg; 0.43 mmol; 50%), **7** ( $R_f = 0.34$ ; 221 mg; 0.64 mmol; 43% recovery).

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