Selective Generation and Reactivity of 5′-Adenosinyl and 2′-Adenosinyl Radicals

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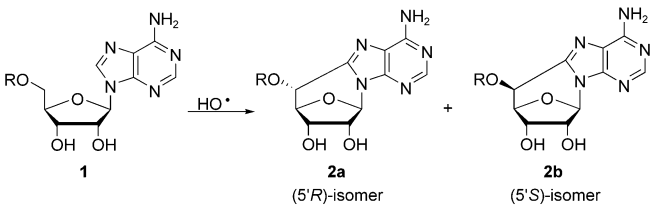
Abstract: The reaction of hydrated electrons (e^-_{aq}) with 8-bromoadenosine **7** has been investigated by radiolytic methods coupled with product studies. Pulse radiolysis revealed that one-electron reductive cleavage of the C–Br bond gives the C8 radical **8** followed by a fast radical translocation 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 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 $1.0 \times 10^4\ s^{-1}$ and gives the aromatic aminyl radical **10**, whereas C2′ radical **11** liberates adenine with a rate constant of $1.1 \times 10^5\ s^{-1}$.

Keywords: cyclization · elimination · nucleosides · pulse radiolysis · radical reactions

Introduction

In 1968 Keck discovered that attack of HO• radicals at adenine-5′-phosphate **1** ($R = PO_3^{2-}$) leads among other products to the 5′,8-cycloadenosides **2a** and **2b**.[1] The effect of pH on the formation of the diastereomers **2a**,**b** has been studied in detail,[2] and it turned out that only the (5′*R*)-isomer **2a** is enzymatically active.[3] Radiation-induced damages to polyadenylic acid, as well as to free adenosine have also been investigated in some details.[4] Depending on the substrate and the experimental conditions, the ratio of the (5′*S*)- and (5′*R*)-isomers changes substantially. γ-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.[4] 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. γ-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.[5,6] Some of us recently investigated the reaction of hydrated electrons (e^-_{aq}) with 8-bromo-2′-deoxyadenosine (**3**) by radiolytic methods.[7] 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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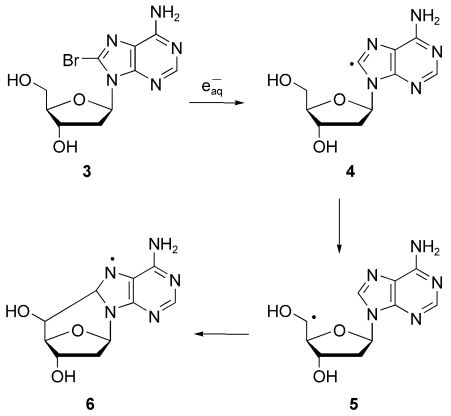
lecularly a hydrogen atom exclusively from the C5′ position affording selectively C5′ radical **5** (Scheme 2). This allowed for the first time to study the fate of the 2′-deoxyadenosin-5′-yl radical **5** properly, and in particular the cyclization step **5**→**6**, which occurs with a rate constant of $1.6\times10^5\ s^{-1}$.



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

We have now found in radiation studies with 8-bromoadenosine **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.[8]

Results and Discussion

Reaction of hydrated electrons (e_{aq}^-) with 8-bromoadenosine 7: Radiolysis of neutral water leads to e_{aq}^-, $HO^\bullet$ and $H^\bullet$ as shown in [Eq. (1)]. The values in parentheses represent the radiation chemical yields (G values) in units of $\mu mol\,J^{-1}$.[9] The reactions of e_{aq}^- with the substrates were studied in O_2-free solutions containing 0.25 M *t*BuOH. With this amount of *t*BuOH, $HO^\bullet$ is scavenged efficiently [Eq. (2), $k_2=6.0\times10^8\ M^{-1}\,s^{-1}$], whereas $H^\bullet$ reacts only slowly [Eq. (3), $k_3=1.7\times10^5\ M^{-1}\,s^{-1}$].[9,10] Therefore, the reactions of $H^\bullet$ may be relevant since they can account for as much as ~20% of the products (see below).

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

$$HO^\bullet + t\text{BuOH} \xrightarrow{k_2} (CH_3)_2C(OH)CH_2^\bullet + H_2O \quad (2)$$

$$H^\bullet + t\text{BuOH} \xrightarrow{k_3} (CH_3)_2C(OH)CH_2^\bullet + H_2 \quad (3)$$

The pseudo first-order rate constant, k_{obs}, for the reaction of e_{aq}^- with a defined amount of 8-bromoadenosine **7** was determined by measuring the rate of the decrease of the optical density of e_{aq}^- at 720 nm ($\varepsilon=1.9\times10^4\ M^{-1}\,cm^{-1}$).[11] From the slope of k_{obs} versus [**7**], the bimolecular rate constant was determined to be $1.1\times10^{10}\ M^{-1}\,s^{-1}$, which is very similar to the analogous reactions with adenosine ($1.0\times10^{10}\ M^{-1}\,s^{-1}$),[12] and with the 8-bromo-2′-deoxyadenosine **3** ($1.6\times10^{10}\ M^{-1}\,s^{-1}$).[7]

The reaction of an aqueous solution of **7** (1 mM) and *t*BuOH (0.25 M) at pH ~7 with e_{aq}^- in the absence of 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 μs (Figure 1). The time profile for the formation of the transient with $\lambda_{max}=350$ nm (Figure 1, inset a) follows a first-order kinetic

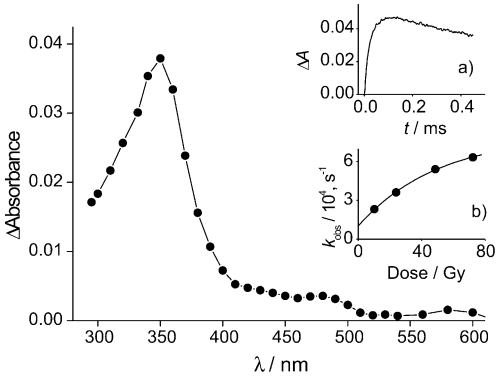


Figure 1. Absorption spectrum obtained from the pulse radiolysis of an Ar-purged solution containing 1 mM **7** and 0.25 M *t*BuOH at pH ~7, taken 100 μs after the pulse; dose = 20 Gy, optical path = 2.0 cm. Insets: a) Time dependence of absorption at 350 nm; dose = 23.8 Gy. b) Dependence of k_{obs}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

with a rate constant (k_{obs}) that is independent of **7** in the concentration range 0.2–1 mM. But k_{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,[13] a rate constant $k_c=(1.0\pm0.2)\times10^4\ s^{-1}$ at 20 °C is obtained. The absorbance at 350 nm is also found to vary substantially with the dose. An apparent molar extinction coefficient (ε_{app}) of $6100\pm100\ M^{-1}\,cm^{-1}$ at 350 nm was calculated by extrapolating to zero dose and assuming a radiation chemical yield $G=0.27\ \mu mol\,J^{-1}$, which is the G of hydrated electrons.[9]

In analogy to the reactions of 8-bromo-2′-deoxyadenosine **3**,[7] 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 μs) and the absorbance at the maximum (350 vs 360 nm) was about two thirds. The cyclization rate constant for reaction **9**→**10** ($k_c=1.0\times10^4\ s^{-1}$) 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. $1\times10^4\ M^{-1}\,cm^{-1}$),[7,14] 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).[15] Pulsing O_2-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 $TMPD^{\bullet+}$ were measured at 565 nm ($\varepsilon = 1.25\times10^4\,M^{-1}cm^{-1}$), which represents one of the absorption maxima of $TMPD^{\bullet+}$ (Figure 2).[16,17] Because under these experimental conditions $HO^{\bullet}$ 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,[18] we concluded that the $TMPD^{\bullet+}$ radical cation is formed from intermediates generated by the reaction of e^-_{aq} with **7**.[19]

The yield of $TMPD^{\bullet+}$ (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^-_{aq}. Figure 2 shows that an induction period is observed for the formation of $TMPD^{\bullet+}$, 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 $k_f = (1.1\pm0.1)\times10^5\,s^{-1}$. The second component depends on the concentration of TMPD and the rate constant $k_{TMPD} = (4.6\pm0.3)\times10^8\,M^{-1}s^{-1}$ (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

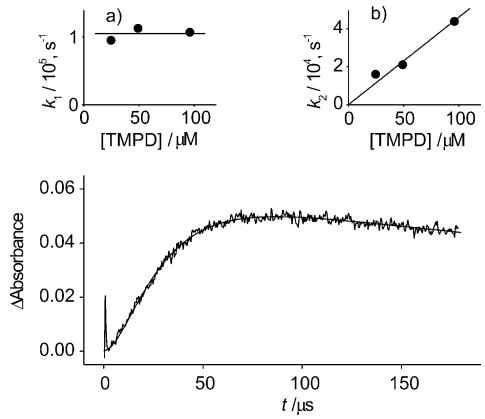
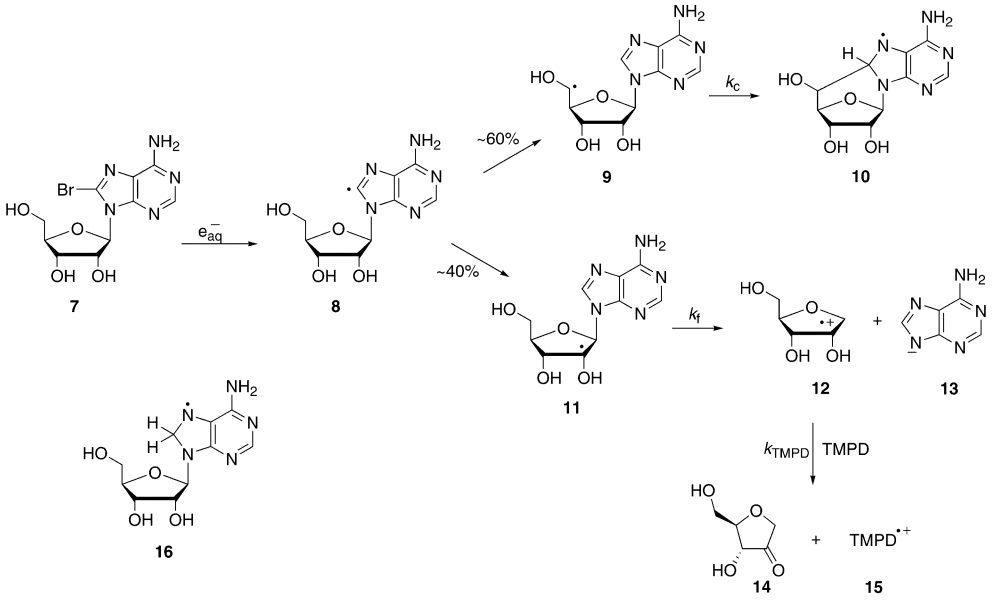


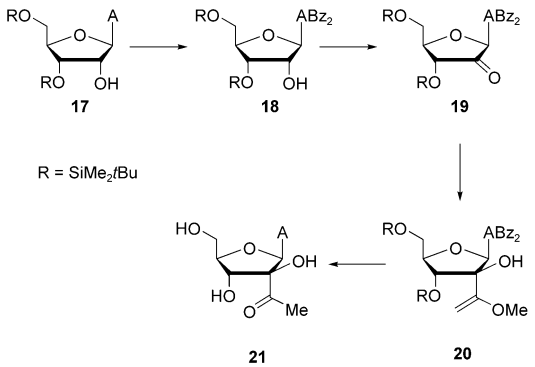
Figure 2. Time dependence of absorption at 565 nm obtained from the pulse radiolysis of an Ar-purged solution containing 1 mM **7**, 0.25 M *t*BuOH and 96 μM TMPD at pH ~7; dose = 22 Gy, optical path = 2.0 cm. Insets: a) Dependence of the first component k_1 from the [TMPD]. b) Dependence of the second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

($k_f = 1.1\times10^5\,s^{-1}$) 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.[20] Radical cation **12** oxidizes TMPD giving presumably compound **14**. It is worth mentioning that in the case of the deoxyribosides the build up of $TMPD^{\bullet+}$ was not observed.[7] 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-



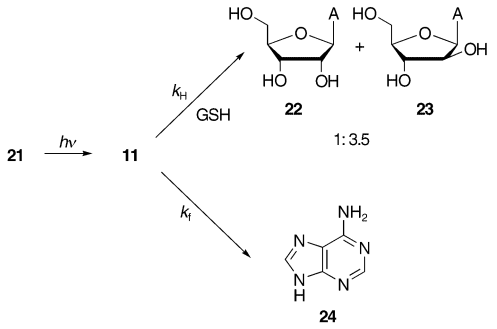
Scheme 3. Proposed mechanism for the fate of radical **5** based on pulse radiolysis studies.



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

tected adenosine **17**, which was benzoylated at the NH_2 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)].

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

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

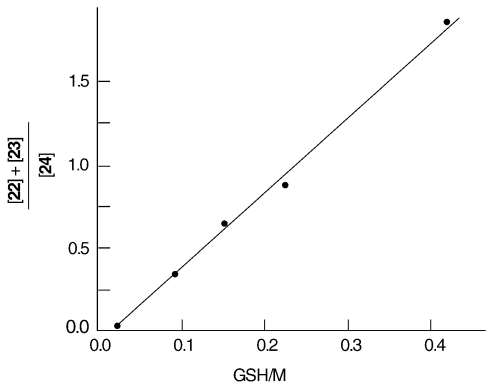


Figure 3. Plot obtained from the competition between fragmentation and hydrogen abstraction from GSH by radical **11** (Scheme 5) according to Equation (4).

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 $10^6\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$,[21] which suggests a rate constant k_f of about $2\times 10^5\,\mathrm{s}^{-1}$ 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[23,24] 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.[25] All three reactions are calculated to be strongly exothermic as expected on the thermochemical grounds.[26] Radical translocation to the C5′ position has the lowest activation energy ($3.1\,\mathrm{kcal\,mol}^{-1}$), which is similar to that of the 2′-deoxyribose analogue ($3.2\,\mathrm{kcal\,mol}^{-1}$).[7] Hydrogen abstraction from the C2′ position occurs with a barrier of $9.6\,\mathrm{kcal\,mol}^{-1}$, whereas the analogous reaction in the deoxyribonucleoside has a much higher activation energy ($16.2\,\mathrm{kcal\,mol}^{-1}$). This difference of $6.6\,\mathrm{kcal\,mol}^{-1}$ 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 ($11.1\,\mathrm{kcal\,mol}^{-1}$) 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 $6.5\,\mathrm{kcal\,mol}^{-1}$, 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 Å) to the hydrogen at C5′ than to that at C2′ (2.70 Å). In order to reach the transition state for the H-abstraction from C5′, one looses 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′.

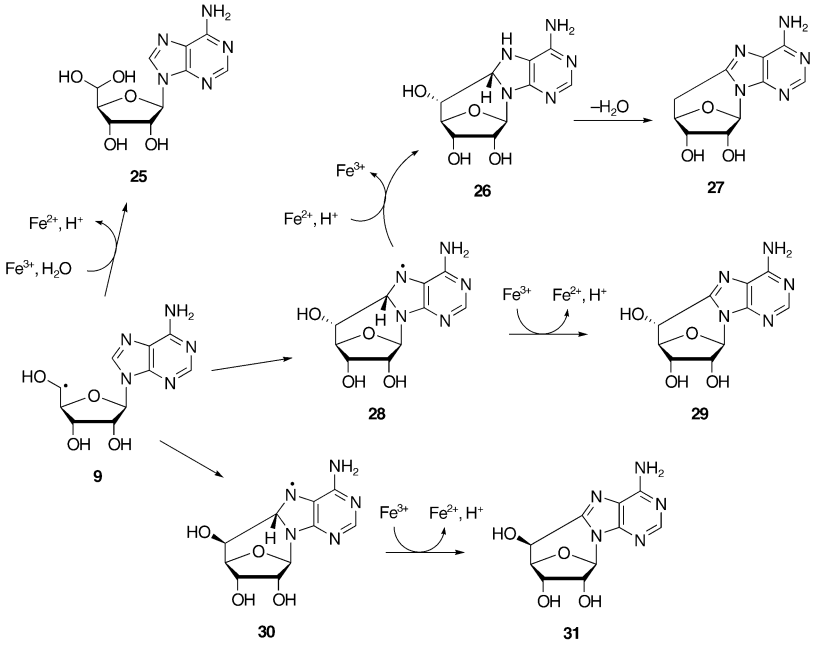
Product studies from continuous radiolysis of 8-bromoadenosine 7: In order to further strengthen our mechanistic sug-

gestions derived from the pulse radiolysis of **7**, we also carried out product studies from the γ-radiolysis experiments. Samples containing 3 mL of aqueous solution of **7** (ca. 1.5 mM) and *t*BuOH (0.25 M) at pH ~7, were irradiated under stationary-state conditions with a total dose up to 1.5 kGy at a dose rate of about 20 Gy min^{-1}, 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**,[7] 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 $Fe(CN)_6^{3-}$, the rate constants being 4.2×10^9 and 8.3×10^8 M^{-1} s^{-1} respectively, whereas radical **6** can also be reduced by strong reductants.[7] It was suggested that under γ-irradiation of aqueous solutions of 4 mM $K_4Fe(CN)_6$ (reductant), a continuous generation of micromolar levels of the oxidant $Fe(CN)_6^{3-}$ could be obtained. Such a low concentration of oxidant should allow radical **9** before being oxidized to cyclize to radical **10**.

A deareated aqueous solution (1 L) containing 1.5 mM of **7** (520 mg), 0.25 M *t*BuOH and 4 mM $K_4Fe(CN)_6$ at pH ~7 was γ-irradiated with a total dose up to 3 kGy at a dose rate of 18 Gy min^{-1}. 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 μM levels of $Fe(CN)_6^{3-}$ generated in situ. Regarding the formation of products that derived from C5′ radical chemistry, Scheme 6 shows our mechanistic proposal.[27] 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 μM level of $Fe(CN)_6^{3-}$ 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 $Fe(CN)_6^{4-}$, since its concentration is several orders higher than that of $Fe(CN)_6^{3-}$. 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_{aq}^- at pH ~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 1.0×10^4 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 1.1×10^5 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 HO•, H•, and e_{aq}^- are generated with 1–20 μM concentrations. 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 cut-off filters. The bandwith 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 N_2O-saturated solution containing 0.1 M HCO_2^- and 0.5 mM methyl viologen, using $G\varepsilon = 9.66\times10^{-4}\ m^2\,J^{-1}$ at 602 nm.[28] $G(X)$ 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°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 $NaHCO_3$ (1000 mL). The solution was extracted with dichloromethane (4×100 mL), the extracts dried over $MgSO_4$ 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°C. After 2 h, the reaction was quenched with $NaHCO_3$ (1 g, 11.9 mmol) and the ethanol removed under reduced pressure. The residue was solved in water (100 mL), extracted with dichloromethane (4× 100 mL), dried over $MgSO_4$ 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}H NMR (300 MHz, $CDCl_3$): δ = 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, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 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 $[M+H]^+$; HR-MS (FAB): calcd for $C_{36}H_{49}N_5O_6Si_2$: 705.0163; found: 705.0161 $[M+H]^+$.

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°C to nucleoside **18** (5.70 g, 8.10 mmol) in dichloromethane (35 mL). After 23 h the solution was quenched at 0°C with sodium thiosulfate (25.0 g, 100 mmol) and a saturated solution of $NaHCO_3$ (200 mL). The mixture was extracted with dichloromethane, dried with $MgSO_4$ 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}H NMR (300 MHz, $CDCl_3$): δ = 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_b-5′), 3.92 (dd, J = 12.4, 3.6 Hz, 1H, H_a-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), −012 (s, 3H, MeSi); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 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)-β-D-arabinofuranosyl]-adenine (20): At −78°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°C for 3 min and then cooled down again to −78°C. During this procedure the yellow solution became pale yellow-green. After 5 min the reaction mixture was added to a cooled (0°C) solution of ketone **19** (213 mg, 0.3 mmol) in tetrahydrofuran (5 mL). The mixture was quenched after 10 min with a saturated NH_4Cl solution (50 mL), water was added (50 mL) and extracted with dichloromethane (3×50 mL). From the dried solution ($MgSO_4$) 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}H NMR (300 MHz, $CDCl_3$): δ = 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, C($CH_\alpha H_\beta$)OCH_3), 4.19 (s, 1H, H-4′), 4.13 (d, J = 3.1 Hz, 1H, C($CH_\alpha H_\beta$)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, 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}C NMR (75.5 MHz, $CDCl_3$): δ = 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-β-D-arabinofuranosyl)-adenine (21): Trimethylsilyl chloride (33.5 mg, 0.31 mmol) was added at 20°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 NH_4Cl (60 mL) and the mixture extracted with dichloromethane (3×60 mL). After drying over $MgSO_4$ 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 (180μL 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):CH_3CN 98:2 (2 min)→ 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}H NMR (300 MHz), D_2O): δ = 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_b-5′), 3.90 (dd, J = 12.8, 4.4 Hz, 1H, H_a-5′), 2.39 (s, 3H, $CO\mathit{CH_3}$); ^{13}C NMR (75.5 MHz, D_2O): δ = 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 $[M+H]^+$; HR-MS (FAB): calcd for $C_{12}H_{15}N_5O_5$: 310.1151; found: 310.1151 $[M+H]^+$.

Kinetic experiments: The modified adenosine **21** (0.62 mg, 0.20 μmol) and a large excess of glutathione (20 μmol to 420 μmol) were solved in aqueous tetraethylammonium chloride (TEAA, 200 μL of a 100 mM solution, pH 7.0) in a polymethyl methacrylate cuvette. The solution was deoxygenated with argon (30 min), thermostated at 20°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:CH_3CN 98:2 to 84:16 within 13 min). The compounds **22**:**23** and **24** were quantified using known material. A plot of [**22**]+[**23**]/[**24**] against the glutathione concentration gave a straight line with a slope of 4.3 M^{-1} (r = 0.998).

Continuous radiolysis: Continuous radiolyses were performed at room temperature (22±2°C) on 3 mL or 1 L samples using a ^{60}Co-Gammacell, with dose rates between 18 −20 Gy min^{-1}. The absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking $G(Fe^{3+})$ = 1.61 μmol J^{-1}.[29] The reactions of **7** (purchased from Sigma) with e^-_{aq} and H$^\bullet$ were investigated using deareated aqueous solutions containing 1.5 mM substrate and 0.25 M *t*BuOH in the presence or absence of 4 mM $K_4Fe(CN)_6$ at pH ~7. The 1 L solution in the presence of 4 mM $K_4Fe(CN)_6$ was γ-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**[30] (R_f = 0.91; 11 mg, 0.043 mmol; 5%), **29**[31] (R_f = 0.83; 31 mg; 0.12 mmol; 14%), adenosine (R_f = 0.62; 23 mg; 0.086 mmol; 10%), **27**[32] (R_f = 0.56; 6.4 mg; 0.026 mmol; 3%), **31**[31] (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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[1] K. Keck, *Z. Naturforsch. B* **1968**, *23*, 1034–1043.

[2] J. A. Raleigh, W. Kremers, R. Whitehouse, *Radiat. Res.* **1976**, *65*, 414–422; J. A. Raleigh, A. F. Fuciarelli, *Radiat. Res.* **1985**, *102*, 165–175.

[3] A. Hampton, P. J. Harper, J. Sasaki, *Biochemistry* **1972**, *11*, 4965–4969; T. P. Haromy, J. Raleigh, M. Sundaralingam, *Biochemistry* **1980**, *19*, 1718–1722.

[4] A. F. Fuciarelli, F. Y. Shum, J. A. Raleigh, *Radiat. Res.* **1987**, *110*, 35–44; A. J. Alexander, P. Kebarle, A. F. Fuciarelli, J. A. Raleigh, *Anal. Chem.* **1987**, *59*, 2484–2491.

[5] M. Dizdaroglu, P. Jaruga, H. Rodriguez, *Free Radical Biol. Med.* **2001**, *30*, 774–784; for early work, see: N. Marriagi, J. Cadet, R. Teoule, *Tetrahedron* **1976**, *32*, 2385–2387.

[6] M.-L. Dirksen, W. F. Brakely, E. Holwitt, M. Dizdaroglu, *Int. J. Radiat. Biol.* **1988**, *54*, 195–204; M. Dizdaroglu, M.-L. Dirksen, H. Jiang, J. H. Robbins, *Biochem. J.* **1987**, *241*, 929–932.

[7] C. Chatgilialoglu, M. Guerra, Q. G. Mulazzani, *J. Am. Chem. Soc.* **2003**, *125*, 3839–3848; R. Flyunt, R. Bazzanini, C. Chatgilialoglu, Q. G. Mulazzani, *J. Am. Chem. Soc.* **2000**, *122*, 4225–4226.

[8] It is worth mentioning that the analogous reaction of hydrated electrons (e^-_{aq}) with 8-bromoguanosine behaves in a completely different manner. See: M. Ioele, R. Bazzanini, C. Chatgilialoglu, Q. G. Mulazzani, *J. Am. Chem. Soc.* **2000**, *122*, 1900–1907.

[9] G. V. Buxton, C. L. Greenstock, W. P. Helman, A. B. Ross, *J. Phys. Chem. Ref. Data* **1988**, *17*, 513–886, and references therein. A. B. Ross, W. G. Mallard, W. P. Helman, G. V. Buxton, R. E. Huie, P. Neta, *NDRL-NIST Solution Kinetic Database, Ver. 3*, Notre Dame Radiation Laboratory, Notre Dame, IN and NIST Standard Reference Data, Gaithersburg, MD, **1998** and references therein.

[10] The rate constant of HO$^\bullet$ radical with **7** was found to be $3.8\times10^9\,\text{M}^{-1}\text{s}^{-1}$ (unpublished work). Therefore, more than 97% of HO$^\bullet$ radicals are scavenged by *t*BuOH when $[\mathbf{7}]=1\leq10^{-3}\,\text{M}$.

[11] G. L. Hug, *Natl. Stand. Ref. Data Ser. (US Natl. Bur. Stand.)* **1981**, *69*, 1–159.

[12] K. J. Visscher, M. P. de Haas, H. Loman, B. Vojnovic, J. M. Warman, *Int. J. Radiat. Biol.* **1987**, *52*, 745–753.

[13] For example, see: G. V. Buxton, Q. G. Mulazzani, A. B. Ross, *J. Phys. Chem. Ref. Data* **1995**, *24*, 1055–1349.

[14] L. P. Candeias, S. Steenken, *J. Phys. Chem.* **1992**, *96*, 937–944.

[15] TMPD, $E°(\text{TMPD}^{\bullet+}/\text{TMPD})=0.27$ V. See: P. Wardman, *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637–1755, and references therein.

[16] S. Fujita, S. Steenken, *J. Am. Chem. Soc.* **1981**, *103*, 2540–2545; S. Steenken, A. J. S. C. Vieira, *Angew. Chem.* **2001**, *113*, 581; *Angew. Chem. Int. Ed.* **2001**, *40*, 571–573.

[17] P. S. Rao, E. Hayon, *J. Phys. Chem.* **1975**, *79*, 1063–1066.

[18] Generally electrophilic-type alkyl radicals like α-acyl derivatives react with TMPD with a rate constant of ca. $10^8\,\text{M}^{-1}\,\text{s}^{-1}$. The rate constant of H$^\bullet$ atom with TMPD is reported to be $2.2\times10^8\,\text{M}^{-1}\,\text{s}^{-1}$ at pH 1 where TMPD is doubly protonated.[9]

[19] Under the conditions employed: i) the competition between **7** and TMPD for e^-_{aq} is well in favor of **7** since $k(e^-_{aq}+\text{TMPD})=9.1\times10^7\,\text{M}^{-1}\text{s}^{-1}$,[17] and $[\mathbf{7}]\geq10\times[\text{TMPD}]$, and ii) although the rate constant of HO$^\bullet$ with TMPD is $1.0\times10^{10}\,\text{M}^{-1}\text{s}^{-1}$, (ref. [16]) the HO$^\bullet$ radicals are still scavenged efficiently by *t*BuOH (ref. [10]).

[20] For example, see: A. Gugger, R. Batra, P. Rzadek, G. Rist, B. Giese, *J. Am. Chem. Soc.* **1997**, *119*, 8740–8741; H. Strittmatter, A. Dussy, U. Schwitter, B. Giese, *Angew. Chem.* **1999**, *111*, 238; *Angew. Chem. Int. Ed.* **1999**, *38*, 135–137.

[21] C. Tronche, F. N. Martinez, J. H. Horner, M. Newcomb, M. Senn, B. Giese, *Tetrahedron Lett.* **1996**, *37*, 5845–5848.

[22] An absolute rate constant of $4.4\times10^6\,\text{M}^{-1}\text{s}^{-1}$ for the reaction of 2′-deoxyuridin-1′-yl radical with GSH was directly measured by laser flash photolysis, see: C. Chatgilialoglu, C. Ferreri, R. Bazzanini, M. Guerra, S.-Y. Choi, C. J. Emanuel, J. H. Horner, M. Newcomb, *J. Am. Chem. Soc.* **2000**, *122*, 9525–9533.

[23] DFT calculations were carried out with the GAUSSIAN 98 system of programs[24] employing the B3LYP functional and the valence double-ζ basis set supplemented with polarization *d*-function on heavy atoms (B3LYP6-31G*). Unrestricted wavefunction was used for radical species (UB3LYP6-31G*). The nature of the transition states were verified by frequency calculations (one imaginary frequency) and energy values were corrected for the zero-point vibrational energy (ZPVE).

[24] Gaussian 98, Revision A.7, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery Jr., R. E. Stratmann, B J. C. urant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh, PA, **1998**.

[25] The *anti* conformer **8**, which undergoes radical translocation, is separated from the more stable *syn* conformer ($\Delta E=0.9\,\text{kcal mol}^{-1}$) by a rotational barrier around the N-glycosidic bond of $2.9\,\text{kcal mol}^{-1}$; calculated here at the UB3LYP6-31G* level.

[26] J. Berkowitz, G. B. Ellison, D. Gutman, *J. Phys. Chem.* **1994**, *98*, 2744–2765.

[27] For possible mechanistic paths for the formation of adenosine via the reaction of H$^\bullet$ with **7**, see ref. [7].

[28] C. A. Kelly, E. L. Blinn, N. Camaioni, M. D'Angelantonio, Q. G. Mulazzani, *Inorg. Chem.* **1999**, *38*, 1579–1584 (Corrigenda p. 2756).

[29] J. W. T. Spinks, R. J. Woods, *An Introduction to Radiation Chemistry*, 3rd ed., Wiley, New York, **1990**, p. 100.

[30] S. Liu, S. F. Wnuk, C. Yuan, M. J. Robins, R. T. Borchradt, *J. Med. Chem.* **1993**, *36*, 883–887.

[31] A. Matsuda, M. Tezuka, K. Niizuma, E. Sugiyama, T. Ueda, *Tetrahedron* **1978**, *34*, 2633–2637.

[32] A. Matsuda, K. Muneyama, T. Nishida, T. Sato, T. Ueda, *Nucleic Acids Res.* **1976**, *3*, 3349–3357.

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