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PAPER

Radical-based alkylation of guanine derivatives in aqueous medium†

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The radical-based alkylation of 8-bromoguanosine (**1a**) and 8-bromo-2′-deoxyguanosine (**1b**) at the C8 position has been investigated in aqueous solutions. Alkyl radicals were generated by scavenging of the primary species of γ-radiolysis by the alcohol substrate. These reactions result in the efficient formation of intermolecular C–C bonds in aqueous media, by using the reactivity of α-hydroxyalkyl radicals derived from alcohols with **1a** and **1b**. A mechanism for the formation of C8 guanine alkylated adducts has been proposed, based on the quantification of radiation chemical yields for the disappearance of starting material and the formation of all products. Two α-hydroxyalkyl radicals are needed to form an alkylated guanine, the first one adding to C8 followed by ejection of Br^- with formation of guanyl adduct and the second one acting as reducing agent of the guanyl adduct.

Introduction

Radical adducts of guanine derivatives at the C8 position are of great importance in DNA damage. The best example is the hydroxyl radical adduct being the precursor of 8-oxoG and FapyG, the most well known lesion of DNA damage caused by oxidative stress.[1–3] Another example is the covalent attachment of the phenoxyl radical to a guanine moiety, which can occur either through oxygen (*O*-bonded adducts) or carbon (C-bonded adducts), due to the delocalization of the unpaired electron in phenoxyl radicals.[4–6] These adducts are biomarkers for phenol exposure and related to phenol-mediated carcinogenesis. Early work was reported on the C8 α-hydroxyalkylation of adenine and guanine moieties in DNA and the reaction responsible for these substitution products was suggested to be the addition of the α-hydroxyalkyl radicals to the C8 position of the purine ring.[7] It was also found that the primary species formed after γ-irradiation in water are trapped by isopropanol to form secondary radicals capable of deactivating DNA.[8]

From a synthetic point of view, generally there are two routes to substituted guanosines at the C8 position. One is by Pd-catalyzed coupling of protected 8-bromoguanosine,[9] as is exemplified by the reaction with terminal alkynes (C–C bond formation)[10] or aromatic amines (C–N bond formation).[11] Alternatively, radical-based approaches have seldom been used, with specific substituents and generally poor yields. Thus, light- and γ-ray-induced reactions of 2′-deoxyguanosine with 2-propanol afforded the C8 α-hydroxylalkyl derivative in 25% and 41% yield, respectively.[12] Photochemical (acetone sensitized) reaction of guanosine with 8-bromoguanosine affords the C8–C8 coupling.[13]

Water is still rarely used as a solvent for radical reactions in organic synthesis,[14–16] mostly due to the lack of solubility for the majority of organic compounds in this medium. In the last decade, we developed several radical reactions in water owing to their relevance for biomimetic chemistry,[17,18] but also to provide an easy access to modified biomolecules. For example, the aqueous procedure is advantageous in the synthesis of modified nucleosides, due to the elimination of protection–deprotection steps owing to the selectivity of the radical approach when compared to the "traditional" methods. Examples of radical reductions,[19] radical oxidation[20] and radical cyclizations[21,22] can be found in the literature. Herein, we report the success of an intermolecular C–C bond formation in water by using the reaction of α-hydroxylalkyl radicals derived from alcohols with 8-bromoguanine derivatives.

Results and discussion

Radiolytic production of transients

Radiolysis of neutral water leads to the reactive species e_{aq}^-, $HO^\bullet$ and $H^\bullet$ together with H^+ and H_2O_2 as shown in eqn (1). The values in parentheses represent the radiation chemical yields (*G*) in units of μmol J^{-1}.[23,24] In a N_2O-saturated solution (~0.02 M of N_2O), e_{aq}^- are transformed into $HO^\bullet$ radical with a rate constant $k_2 = 9.1 \times 10^9$ M^{-1} s^{-1} (eqn (2)), affording a $G(HO^\bullet) = 0.55$ μmol J^{-1}, *i.e.*, $HO^\bullet$ radicals and $H^\bullet$ atoms account for 90% and 10%, respectively, of the reactive species.

$$H_2O \xrightarrow{\gamma} e_{aq}^-(0.27),\ HO^\bullet(0.28), H^\bullet(0.06), H^+(0.27),\ H_2O_2(0.07) \quad (1)$$

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† Dedicated to the memory of Athel Beckwith, a pioneer in radical chemistry.

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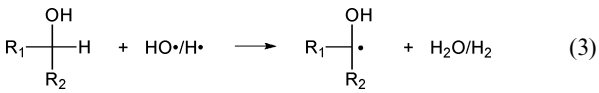
Table 1 Collected rate constants for the reactions of HO• radicals and H• atoms with alcohols and reduction potential of the corresponding α-hydroxylalkyl radicals

Alcohol	k(HO•)[a]/M^{-1} s^{-1}	k(H•)[a]/M^{-1} s^{-1}	E°[b,c]/V
CH_3OH	2.6×10^6	9.7×10^8	−1.18
$MeCH_2OH$	1.7×10^7	1.9×10^9	−1.25
Me_2CHOH	7.4×10^7	1.9×10^9	−1.39
Me_3COH	1.7×10^5	6.0×10^8	

[a] Ref. 23 and 24. [b] E°[$R_1R_2C(O)$, $H^+/R_1R_2C^{\bullet}OH$]. [c] Ref. 25.

$$e_{aq}^- + N_2O + H_2O \rightarrow HO^{\bullet} + N_2 + HO^- \quad (2)$$

When an alcohol is added to the solution, this acts as a scavenger of HO• radicals and H-atoms, to generate α-hydroxyalkyl radicals in an equivalent amount:



(3)

In Table 1, the rate constants for the reactions of various alcohols used in this work with hydroxyl radical and hydrogen atom are shown.[23,24] Together with the rate constants, the reduction potentials for the α-hydroxyalkyl radicals of the corresponding alcohols are also reported.[25] It is worth mentioning that hydroxyl radicals and hydrogen atoms are effectively scavenged by isopropanol and the resulting α-hydroxyalkyl radical is a good reducing radical. *t*-Butanol reacts quite well with hydroxyl radicals but only poorly with hydrogen atoms (Table 1). On the other hand, the rate constants of e_{aq}^- and H• with 8-bromoguanosine are 1.1×10^{10} and 4.5×10^8 M^{-1} s^{-1}, respectively.[26] In order to study the reaction of α-hydroxyalkyl radicals with 8-bromoguanosine (**1a**) and 8-bromo-2′-deoxyguanosine (**1b**), N_2O-saturated buffered solutions (pH 7) of substrate (1.5 mM) in the presence of 0.25 M alcohol were irradiated.

Product studies

Sample solutions (1.5 mM) of **1a** and **1b** in phosphate buffer (the buffer avoids the acidity in the solutions that could lead to hydrolysis of the nucleosides) and in the presence of 0.25 M alcohol were saturated by N_2O prior to irradiation. Irradiation doses were in the 5–7 kGy range and in some cases pushed up to 15 kGy in order to achieve the complete conversion of starting material (*cf*. Table 2). The crude reaction mixture was lyophilised, the residue was taken up in water and purified on reverse-phase silica gel. The main products with methanol, ethanol and isopropanol were the C8-alkylated guanine derivative and in particular α-hydroxylalkyl adducts **3**, **4** and **5**, respectively (Scheme 1), with good to excellent yields (Table 2). In all cases, the replacement of Br with H was also observed as a minor product. The debromination in low conversion was the only reaction observed when *tert*-butanol is used as the alcohol. Table 2 shows that the C8-alkylation products increased in both ribo and deoxyribo series in the order methanol, ethanol, isopropanol. When the alcohol used as a scavenger for hydroxyl radicals is ethanol, the alkylated products **4a** and **4b** were formed in two different diastereoisomeric forms and in a 1/1 ratio.

Reaction mechanism

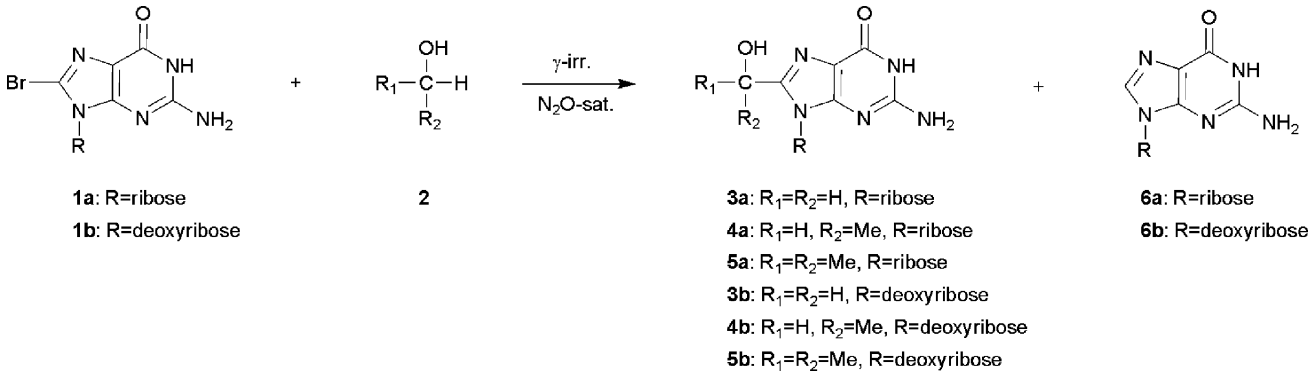
The changes in the concentration of the starting material and products were determined in the initial stages of the reactions (up to 2 kGy) by HPLC using authentic samples as references. From the concentration values, the radiation chemical yields (G) can be calculated by dividing the disappearance of the starting material or the formation of the products (mol kg^{-1}) by the absorbed dose (1 Gy = 1 J kg^{-1}). Analysis of the data in terms of radiation chemical yield (G) can give important information of the reaction mechanism. As an example, Fig. 1 shows the plot of G for the disappearance of 8-bromoguanosine (**1a**) and the formation of products **5a** and **6a** *versus* dose in the experiment with isopropanol. The extrapolation to zero dose gives $G(-\mathbf{1a}) = 0.34$, $G(\mathbf{5a}) = 0.24$, and $G(\mathbf{6a}) = 0.06$ μmol J^{-1}.

In Table 3, the radiation chemical yields (G), extrapolated to zero dose, are given for all the experiments done for both the ribo and deoxyribo series. The estimated percentage error for these values is about 10%. On the basis of the known rate constants for the reactions of e_{aq}^-, HO• and H• reported above, the following observations are underlined: firstly, in all cases the value of $G(-\mathbf{1})$ is similar to the sum of G values of the two products within experimental error, *e.g.*, $G(-\mathbf{1b}) = 0.35$ *vs.* $G(\mathbf{4b}) + G(\mathbf{6b}) = 0.34$ for ethanol; secondly, the $G(-\mathbf{1})$ is approximately half of the calculated G for the α-hydroxylalkyl radicals (eqn (1)–(3)), which suggests that two α-hydroxylalkyl radicals are needed for each molecule of starting material to be alkylated; thirdly, we noted that the G values of α-hydroxylalkyl adducts are *ca.* 0.25, whereas the $G(\mathbf{6})$ values are close to 0.07 μmol J^{-1}. In Scheme 2 the proposed mechanism that is in agreement with the above mentioned observations is depicted and can be explained as follows: the addition of an α-hydroxyalkyl

Table 2 Product studies for the reaction of alcohols with 8-bromoguanosine (**1a**) and 8-bromo-2′-deoxyguanosine (**1b**) under γ-irradiation in N_2O-saturated aqueous solutions

Substrate[a]	Alcohol (**2**)[a]	Dose[b]/kGy	Conv.[c] (%)	**3**[d] (%)	**4**[d,e] (%)	**5**[d] (%)	**6**[d] (%)
1a	CH_3OH	15	100	75			25
	$MeCH_2OH$	10	100		90		10
	Me_2CHOH	7	100			95	5
	Me_3COH	5	9				100
1b	CH_3OH	5	75	65			35
	$MeCH_2OH$	5	95		85		15
	Me_2CHOH	6	100			90	10
	Me_3COH	5	10				100

[a] Conditions: 1.5 mM substrate, 0.25 M alcohol, N_2O-saturated buffered aqueous solution (pH 7). [b] Dose rate: 16 Gy min^{-1}. [c] Conversion of starting material. [d] Yield based on consumed starting material and HPLC analysis using authentic samples as reference for calibration curves. [e] Formation of two diastereoisomers in a 1/1 ratio.



1a: R=ribose
1b: R=deoxyribose

2

3a: R_1=R_2=H, R=ribose
4a: R_1=H, R_2=Me, R=ribose
5a: R_1=R_2=Me, R=ribose
3b: R_1=R_2=H, R=deoxyribose
4b: R_1=H, R_2=Me, R=deoxyribose
5b: R_1=R_2=Me, R=deoxyribose

6a: R=ribose
6b: R=deoxyribose

Scheme 1 The reaction of 8-bromoguanine derivatives **1a** and **1b** with alcohols **2** under γ-irradiation in N_2O-saturated aqueous solutions at pH 7.

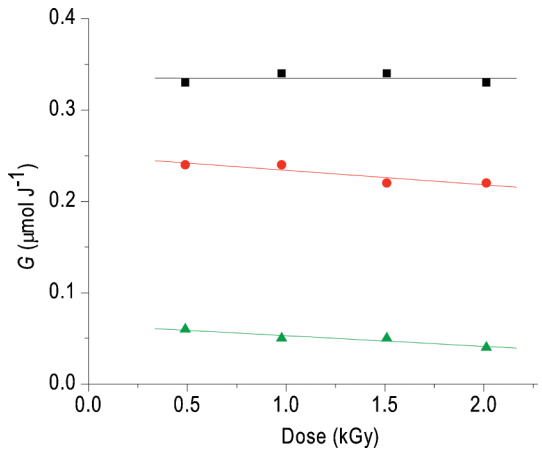


Fig. 1 Radiation chemical yields (G) of 8-bromoguanosine **1a** (■), C8-adduct **5a** (●), and guanosine **6a** (▲) *vs.* dose; G(**–1**) = 0.34, G(**5a**) = 0.24, and G(**6a**) = 0.06 μmol J^{-1} are obtained when the lines are extrapolated to zero dose.

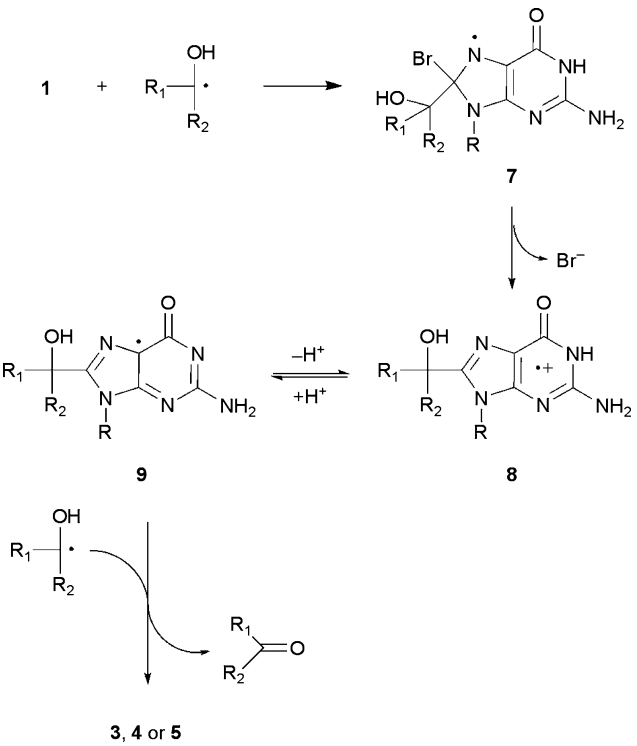
Table 3 Radiation chemical yield (G, μmol J^{-1}) for the reaction of alkyl radicals with 8-bromoguanosine (**1a**) and 8-bromo-2′-deoxyguanosine (**1b**)[a]

Substrate	Alcohol (**2**)	G(–**1**)	G(**3**)	G(**4**)	G(**5**)	G(**6**)
1a	CH_3OH	0.32	0.24			0.11
	$MeCH_2OH$	0.33		0.26		0.09
	Me_2CHOH	0.34			0.25	0.07
	Me_3COH	0.09				0.06
1b	CH_3OH	0.30	0.22			0.08
	$MeCH_2OH$	0.35		0.27		0.07
	Me_2CHOH	0.34			0.28	0.07
	Me_3COH	0.06				0.06

[a] Estimated errors about 10%.

radical to the C8 position leads to radical **7** which, after loss of bromide ion, leads to the radical cation of the alkylguanosine **8**, that is in equilibrium with its deprotonated form, **9** (a pK_a ~ 4 is expected[27]). This neutral radical can be reduced by a second α-hydroxyalkyl radical, to give the product substituted at the 8 position (**3**, **4** or **5**).[28]

In the N_2O-saturated aqueous solution and in the presence of 1.5 mM **1a** or **1b**, solvated electrons are transformed in HO• radicals (90–92%) and 8–10% of them react with **1a** or **1b**. It is well known that the reaction of **1** with e_{aq}^- produces debrominated

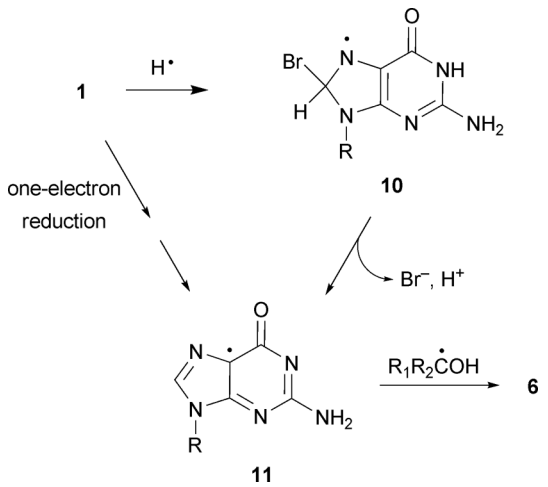


Scheme 2 Reaction mechanism for the α-hydroxylalkyl C8-adduct formation of guanine derivatives.

guanine derivatives **6**.[28,29] Also H-atoms are expected to follow two distinct reactions pathways. In the presence of *tert*-butanol it is expected that the majority of H-atoms will afford the adduct **10** followed by Br^- ejection,[30] whereas in the presence of methanol H-atoms will be equally partitioned between hydrogen abstraction from the alcohol and addition to C8 position of **1**. In ethanol or isopropanol, H-atoms are mainly scavenged by hydrogen abstraction. Therefore, the products **6** arise from all possible reduction processes, as reported in Scheme 3.

Conclusions

The radical-based alkylation of 8-bromoguanosine (**1a**) and 8-bromo-2′-deoxyguanosine (**1b**) at the C8 position has been disclosed. These reactions resulted in the efficient formation of an intermolecular C–C bond in aqueous media, through



Scheme 3 Possible reductive paths of 8-bromoguanine derivatives (**1**).

the intermolecular addition of α-hydroxyalkyl radicals, derived from alcohols, with **1a** or **1b**. Water was confirmed to be a solvent with obvious advantages and provided a convenient media for synthetically useful free radical reactions of biomolecules. Furthermore, a mechanism for the formation of the C8 guanine alkylated adducts has been proposed (Scheme 2), showing that two molecules of α-hydroxyalkyl radicals are needed to form the alkylated guanine, which is in good agreement with the experimental evidence.

Experimental

8-Bromoguanosine **1a** (Aldrich) and 8-bromo-2′-deoxyguanosine **1b** (Berry & Associates) were used as received. Solutions were freshly prepared by using water purified with a Millipore (Milli-Q) system. The pH was buffered with phosphates (pH 7). Continuous radiolysis was performed at room temperature (22 ± 2 °C) on 250 mL samples using a ^{60}Co-Gammacell, with dose rates between 20 and 22 Gy min^{-1}. The absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking $G(Fe^{3+}) = 1.56$ mmol J^{-1}.[31] The reactions of **1a** and **1b** with α-hydroxyalkyl radicals were studied using 1.5 mM buffered solutions (pH 7) of nucleoside and using N_2O-saturated solutions in the presence of 0.25 M alcohol, irradiated with appropriate doses. The crude reaction mixture was lyophilised and the residue was taken up in water and purified on reverse-phase silica gel, eluting with 0 and 3% acetonitrile-containing water. The UV-positive fractions were collected and lyophilised to obtain the desired products as pure materials that were spectroscopically characterised. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Mercury 400 MHz. Qualitative and quantitative HPLC studies were performed on a Waters Associates Unit equipped with a model 600 multisolvent delivery system and controller, a Waters 996 Photodiode Array Detector and a Millenium 21 data workstation. The aqueous solutions of the samples were loaded after dilution onto a reverse-phase analytical column (Waters XTerra MS C_{18} 5 mm 4.6 × 150 mm), eluted in 50 mM ammonium with 0–20% acetonitrile gradient over 25 min at a flow rate of 1 mL min^{-1}, and detected at 254 nm.

8-(Hydroxymethyl)guanosine (3a). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.0 (br s, 1H, disappeared on D_2O shake, NH); 5.81 (d, 1H, J = 6 Hz, H1′); 5.5 (br s, 1H, disappeared on D_2O shake, OH); 5.28 (br s, 2H, disappeared on D_2O shake, 2OH); 5.07 (br s, 1H, disappeared on D_2O shake, OH); 4.74 (t, 1H, J = 6 Hz collapsing to d J = 6 Hz upon irradiation at δ 5.81, H2′); 4.53 (1H, A part of an AB system, J_{AB} = 13 Hz); 4.46 (1H, B part of an AB system, J_{AB} = 13 Hz); 4.1 (1H, dd, J_1 = 6 Hz, J_2 = 3.5 Hz collapsing to d J = 3.5 Hz upon irradiation at δ 4.74, H3′); 3.84 (q, 1H, J = 3.5 Hz, collapsing to t J = 3.5 Hz upon irradiation at δ 4.1, H4′); 3.65 (dd, 1H, J_1 = 12 Hz, J_2 = 3.5 Hz, collapsing to d J = 12 Hz upon irradiation at δ 3.84, H5′); 3.50 (br s, 1H, collapsing to br d J = 12 Hz upon irradiation at δ 3.8 and to dd J_1 = 12 Hz, J_2 = 3.5 Hz on D_2O shake, H5′); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 57.2 (CH_2); 62.6 (CH_2); 71.1 (CH); 72.4 (CH); 86.2 (CH);88.8 (CH); 116.2 (q); 148.1 (q); 152.5 (q); 154.3 (q); 158.0 (q); MS (ES$^-$) m/z 313 (M–1)$^-$, MS(ES$^+$) m/z 336 (M + Na)$^+$

8-(1-Hydroxyethyl)guanosine (4a). Epimer A: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.43 (br s, 2H, disappeared on D_2O shake, NH_2); 5.92 (d, 1H, J = 6 Hz, H1′); 5.8–5.0 (br s, 3H, disappeared on D_2O shake, 3 OH); 4.82 (q, 1H, J = 6 Hz collapsing to s upon irradiation at δ 1.45); 4.73 (t, 1H, J_1 = 6 Hz; collapsing to d J = 6 Hz upon irradiation at δ 5.92, H2′); 4.09 (dd, 1H, J_1 = 6 Hz J_2 = 2.5 Hz collapsing to d J = 2.5 Hz upon irradiation at d 4.73, H3′); 3.85 (m, 1H, H4′); 3.65 (1H, A part of an ABX system J_{AB} = 12 Hz, J_{AX} = 3.5 Hz, H5′); 3.51 (1H, B part of an ABX system, J_{AB} = 12 Hz, J_{BX} = 3 Hz, H5′); 1.45 (d, 3H, J = 6 Hz); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 26.9 (CH_3); 66.8 (CH); 67.6 (CH_2); 76.1 (CH); 77.2 (CH); 91.1 (CH); 93.7 (CH); 120.8 (q); 155.4 (q); 157.1 (q); 159.1 (q); 163.1 (q); MS (ES$^-$) m/z 326 (M–1)$^-$, MS(ES$^+$) m/z 350 (M + Na)$^+$. **Epimer B**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.90 (br s, 1H, disappeared on D_2O shake, NH); 6.37 (br s, 2H, disappeared on D_2O shake, NH_2); 6.0 (d, 1H, J = 5.5 Hz, H1′); 5.5 (br s, 1H, disappeared on D_2O shake, OH); 5.3 (br s, 1H, disappeared on D_2O shake, OH); 5.2 (br s, 1H, disappeared on D_2O shake, OH); 5.0 (br s, 1H, disappeared on D_2O shake, OH); 4.89 (t, 1H, J = 5.5 Hz, H2′); 4.82 (q, 1H, J_1 = 6.5 Hz); 4.20 (dd, 1H, J_1 = 5.5 Hz, J_2 = 4 Hz,H3′); 3.84 (q, 1H, J = 4 Hz H4′); 3.64 (1H, A part of an ABX system J_{AB} = 11.5 Hz, J_{AX} = 4 Hz, H5′); 3.49 (br d, 1H, collapsing to B part of an ABX system, J_{AB} = 11.5 Hz, J_{BX} = 4 Hz, H5′); 1.46 (d, 3H, J = 6.5 Hz); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 22.8 (CH_3); 62.9 (CH_2); 63.3 (CH); 71.4 (CH); 71.9 (CH); 86.1 (CH); 89.0 (CH); 116.3 (q); 151.0 (q); 152.5 (q); 153.9 (q); 157.8 (q); MS (ES$^-$) m/z 326 (M–1)$^-$, MS(ES$^+$) m/z 350 (M + Na)$^+$.

8-(1-Hydroxy-1-methylethyl)guanosine (5a). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.8 (br s, 1H, disappeared on D_2O shake, NH); 6.5 (d, 1H, J = 6 Hz, H1′); 6.27 (br s, 2H, disappeared on D_2O shake, NH_2); 5.5 (br s, 1H, disappeared on D_2O shake, OH); 5.18 (br s, 1H, disappeared on D_2O shake, OH); 5.12 (br s, 1H, disappeared on D_2O shake, OH); 4.89 (t, 1H, J = 6 Hz collapsing to d J = 6 Hz upon irradiation at δ 4.18, H2′); 4.18 (dd, 1H, J_1 = 6 Hz, J_2 = 4 Hz; collapsing to d J = 6 Hz upon irradiation at δ 3.81, H3′); 3.80 (q, 1H, J = 4 Hz collapsing to t J = 4 Hz upon irradiation at δ 4.18, H4′); 3.66 (dd, 1H, J_1 = 12 Hz, J_2 = 4 Hz, collapsing to d J = 12 Hz upon irradiation at δ 3.8, H5′); 3.50 (dd, 1H, J_1 = 12 Hz, J_2 = 4 Hz, collapsing to d J = 12 Hz upon irradiation at δ 3.8, H5′); 3.4 (br s, 1H, disappeared on D_2O shake, OH); 1.54 (s, 3H); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 35.6 (CH_3); 35.9 (CH_3); 67.9 (CH_2); 75.0 (q); 76.2 (CH); 76.6 (CH); 90.7 (CH); 94.6 (CH); 120.5 (q); 157.5 (q); 158.0 (q); 158.1

(q); 162.0 (q); MS (ES^-) m/z 340 $(M-1)^-$, MS(ES^+) m/z 364 $(M + Na)^+$

8-(Hydroxymethyl)-2′-deoxyguanosine (3b). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.0 (br s, 1H, disappeared on D_2O shake, NH); 6.26 (dd, 1H, J_1 = 8 Hz, J_2 = 6 Hz, H1′); 5.52 (br s, 1H, disappeared on D_2O shake, OH); 5.24 (br s, 2H, disappeared on D_2O shake, OH); 4.51 (2H, AB system J_{AB} = 13 Hz; inner line separation 8 Hz); 4.36 (br s, 1H, H3′); 3.82 (m, 1H, H4′); 3.63 (A part of an ABX system, 1H, J_{AB} = 12 Hz, J_{AX} = 5 Hz, H5′); 3.53 (B part of an ABX system, 1H, J_{AB} = 12 Hz, J_{BX} = 5 Hz, H5′); 2.92 (ddd, 1H, J_1 = 14 Hz, J_2 = 8 Hz, J_3 = 6 Hz, H2′); 2.04 (ddd, 1H, J_1 = 14 Hz, J_2 = 6 Hz, J_3 = 2 Hz, H2′); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 41.6 (CH_2); 60.2 (CH_2); 65.5 (CH_2); 74.6 (CH); 87.5 (CH); 91.1 (CH); 119.0 (q); 150.5 (q); 155.2 (q); 157.1 (q); 160.8 (q); MS (ES^-) m/z 296 $(M-1)^-$, MS(ES^+) m/z 320 $(M + Na)^+$

8-(1-Hydroxyethyl)-2′-deoxyguanosine (4b). Epimer A: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.48 (br s, 2H, disappeared on D_2O shake, NH_2);6.43 (dd, 1H, J_1 = 7 Hz, J_2 = 6 Hz, H1′); 5.5(br s, 1H, disappeared on D_2O shake, OH); 5.4 (br s, 1H, disappeared on D_2O shake, OH); 5.20 (br s, 1H, disappeared on D_2O shake, OH); 4.86 (br q, 1H, collapsing to q J = 6.5 Hz on D_2O shake); 4.35 (m, 1H, H3′); 3.80 (m, 1H, H4′); 3.65 (A part of an ABX system, 1H, J_{AB} = 12 Hz, J_{AX} = 4 Hz, H5′); 3.53 (B part of an ABX system, 1H, J_{AB} = 12 Hz, J_{BX} = 3.5 Hz, H5′); 2.91 (ddd, 1H, J_1 = 13 Hz, J_2 = J_3 = 6.5 Hz, H2′); 2.00 (dd, 1H, J_1 = 13 Hz, J_2 = 7 Hz, H2′); 1.45 (d, 3H, J = 6.0 Hz); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 22.5 (CH_3); 38.8 (CH_2); 62.8 (CH_2); 63 (CH); 71.9 (CH); 84.7 (CH); 88.4 (CH); 116.0 (q); 150.1 (q); 152.4 (q); 154.6 (q); 158.7 (q); MS (ES^-) m/z 310 $(M-1)^-$, MS(ES^+) m/z 334 $(M + Na)^+$. **Epimer B**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.80 (br s, 1H, disappeared on D_2O shake, NH); 6.36 (t, 1H, J_1 = 7 Hz, H1′); 6.29 (br s, 2H, disappeared on D_2O shake, NH_2); 5.51 (br d, 1H, J = 6 Hz disappeared on D_2O shake, OH); 5.25 (br s, 1H, disappeared on D_2O shake, OH); 5.21 (br s, 1H, disappeared on D_2O shake, OH); 4.81 (m, 1H, collapsing to d J = 6 Hz on D_2O shake); 4.38 (br s,1H, H3′); 3.81 (br s, 1H, H4′); 3.63 (A part of an ABX system, 1H, J_{AB} = 12 Hz, J_{AX} = 4 Hz, H5′); 3.48 (br d, B part of an ABX system, 1H, J_{AB} = 12 Hz, J_{BX} = 3.5 Hz, H5′); 3.00 (ddd, 1H, J_1 = 13 Hz, J_2 = J_3 = 7 Hz, H2′); 2.02 (dd, 1H, J_1 = 13 Hz, J_2 = 7 Hz, H2′); 1.46 (d, 3H, J = 7.0 Hz); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 21.9 (CH_3); 38.2 (CH_2); 62.3 (CH); 63.0 (CH_2); 72.1 (CH); 85.0 (CH); 88.6 (CH); 116.4 (q); 150.4 (q); 152.3 (q); 153.7 (q); 157.6 (q); MS (ES^-) m/z 310 $(M-1)^-$, MS(ES^+) m/z 334 $(M + Na)^+$

8-(1-Hydroxy-1-methylethyl)-2′-deoxyguanosine (5b). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.0 (br s, 1H, disappeared on D_2O shake; NH); 6.94 (dd, 1H, J_1 = 7 Hz, J_2 = 6 Hz, H1′); 6.28 (br s, 2H, disappeared on D_2O shake, NH_2); 5.61 (br s, 1H, disappeared on D_2O shake, OH); 5.44 (br s, 1H, disappeared on D_2O shake, OH); 5.16 (br s, 1H, disappeared on D_2O shake, OH); 4.40 (m, 1H, H3′); 3.83 (m, 1H, H4′); 3.68 (A part of an ABX system, 1H, J_{AB} = 12 Hz, J_{AX} = 4 Hz, H5′); 3.54 (B part of an ABX system, 1H, J_{AB} = 12 Hz, J_{BX} = 3 Hz, H5′); 2.99 (ddd, 1H, J_1 = 12 Hz, J_2 = 8.5 Hz, J_3 = 7 Hz, H2′); 1.99 (dd, 1H, J_1 = 12 Hz, J_2 = 6 Hz, H2′); 1.53 (s, 3H); 1.51 (s, 3H); ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ 35.2 (CH_3); 35.6 (CH_3); 42.9 (CH_2); 68.0 (CH_2); 74.9 (q); 77.1 (CH); 90.7 (CH); 93.4 (CH); 120.8 (q); 157.2 (q); 157.4 (q); 158.6 (q); 162.9 (q); MS (ES^-) m/z 324 $(M-1)^-$, MS(ES^+) m/z 348 $(M + Na)^+$

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References

1 J. Cadet, T. Douki and J-L. Ravanat, *Acc. Chem. Res.*, 2008, **41**, 1075.
2 G. Pratviel and B. Meunier, *Chem.–Eur. J.*, 2006, **12**, 6018.
3 T. Gimisis and C. Cismas, *Eur. J. Org. Chem.*, 2006, 1351.
4 C. K. McLaughlin, D. R. Lantero and R. A. Manderville, *J. Phys. Chem. A*, 2006, **110**, 6224.
5 K. M. Sun, C. K. McLaughlin, D. R. Lantero and R. A. Manderville, *J. Am. Chem. Soc.*, 2007, **129**, 1894.
6 K. M. Schlitt, K. M. Sun, R. J. Paugh, A. L. Millen, L. Navarro-Whyte, S. D. Wetmore and R. A. Manderville, *J. Org. Chem.*, 2009, **74**, 5793.
7 R. Ben-Ishai, M. Green, E. Graff, D. Elad, H. Steinmaus and J. Salomon, *Photochem. Photobiol.*, 1973, **17**, 155.
8 F. J. Nabben, H. A. van der Stroom and H. Loman, *Int. J. Radiat. Biol.*, 1983, **43**, 495.
9 L. A. Agrofoglio, I. Gillaizeau and Y. Saito, *Chem. Rev.*, 2003, **103**, 1875.
10 G. T. Crisp and J. Gore, *Tetrahedron*, 1997, **53**, 1523.
11 D. W. Old, J. P. Wolfe and S. L. Buchwald, *J. Am. Chem. Soc.*, 1999, **121**, 10453.
12 H. Steinmaus, I. Rosenthal and D. Elad, *J. Org. Chem.*, 1971, **36**, 3594.
13 S. N. Bose, R. J. H. Davies, D. W. Anderson, J. C. Van Niekerk, L. R. Nassimbeni and R. D. Macfarlane, *Nature*, 1978, **271**, 783.
14 C.-J. Li, *Chem. Rev.*, 2005, **105**, 3095.
15 U. M. Lindström, *Chem. Rev.*, 2002, **102**, 2751.
16 *Organic Synthesis in Water*, P. A. Grieco, (ed.), Blackie Academic and Professional, London, 1998.
17 C. Chatgilialoglu and C. Ferreri, *Acc. Chem. Res.*, 2005, **38**, 441.
18 C. Chatgilialoglu and C. Ferreri, in *Biomimetics Learning from Nature*, ed. A. Mukherjee, InTech, Vucovar (Croatia), 2010, pp 117–135, ISBN 978-953-307-025-4, to be found under http://sciyo.com/articles/show/title/biomimetic-chemistry-radical-reactions-in-vesicle-suspensions.
19 A. Postigo, S. Kopsov, C. Ferreri and C. Chatgilialoglu, *Org. Lett.*, 2007, **9**, 5159.
20 F. Boussicault, P. Kaloudis, C. Caminal, Q. G. Mulazzani and C. Chatgilialoglu, *J. Am. Chem. Soc.*, 2008, **130**, 8377.
21 C. Chatgilialoglu, M. Guerra and Q. G. Mulazzani, *J. Am. Chem. Soc.*, 2003, **125**, 3839.
22 C. Chatgilialoglu, R. Bazzanini, L. B. Jimenez and M. A. Miranda, *Chem. Res. Toxicol.*, 2007, **20**, 1820.
23 G. V. Buxton, C. L. Greenstock, W. P. Helman and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1988, **17**, 513.
24 A. B. Ross, W. G. Mallard, W. P. Helman, G. V. Buxton, R. E. Huie and P. Neta, *NDRL-NIST Solution Kinetic Database - Ver. 3*, Notre Dame Radiation Laboratory, Notre Dame, IN and NIST Standard Reference Data, Gaithersburg, MD, 1998.
25 H. A. Schwarz and R. W. Dodson, *J. Phys. Chem.*, 1989, **93**, 409.
26 M. Ioele, R. Bazzanini, C. Chatgilialoglu and Q. G. Mulazzani, *J. Am. Chem. Soc.*, 2000, **122**, 1900.
27 C. von Sonntag, *Free-Radical-Induced DNA Damage and Its Repair: A Chemical Persective*, Springer-Verlag, Berlin, 2006.
28 C. Chatgilialoglu, C. Caminal, A. Altieri, Q. G. Mulazzani, G. C. Vougioukalakis, T. Gimisis and M. Guerra, *J. Am. Chem. Soc.*, 2006, **128**, 13796.
29 C. Chatgilialoglu, C. Caminal, M. Guerra and Q. G. Mulazzani, *Angew. Chem., Int. Ed.*, 2005, **44**, 6030.
30 M. D'Angelantonio, M. Russo, P. Kaloudis, Q. G. Mulazzani, P. Wardman, M. Guerra and C. Chatgilialoglu, *J. Phys. Chem. B*, 2009, **113**, 2170.
31 J. W. T. Spink and R. J. Woods, in *An Introduction to Radiation Chemistry*, Wiley, New York, 3rd edn, 1990, p. 100.

