DOI: 10.1002/ejoc.200700251

Identification of Adducts Formed in the Reactions of 2′-Deoxyguanosine and Calf Thymus DNA with Glutaraldehyde

Raymond Olsen,[a,b] Josefin Backman,[c] Paal Molander,[a,b] Karel D. Klika,*[d] and Leif Kronberg*[c]

Keywords: DNA alkylation / Nucleobases / Glutaraldehyde / NMR spectroscopy

Glutaraldehyde (1,5-pentanedial) is a widely used industrial chemical that has been found to be mutagenic in bacteria and mammalian cells. It has been claimed that glutaraldehyde forms adducts with 2′-deoxyguanosine, 2′-deoxyadenosine and 2′-deoxycytidine, but until recently none of the adducts had been identified. In a very recent contribution from our laboratories, the structures were determined of three glutaraldehyde adducts formed in the reaction with 2′-deoxyadenosine, and it was shown that one of the adducts is formed in calf thymus DNA. In the current work, we have studied the reaction of glutaraldehyde with 2′-deoxyguanosine and calf thymus DNA in aqueous buffered solutions. Among the numerous adducts formed in the reaction with 2′-deoxyguanosine, six compounds were found to be stable and could be isolated by semi-preparative liquid chromatography and identified by UV absorbance and ^{1}H and ^{13}C NMR spectroscopic and mass spectrometric studies. Adduct **dG350b**, comprising two diastereomers, was found to be derived from one unit of glutaraldehyde which has been transformed to a tetrahydropyran ring so that C-2 and C-6 of the ring are attached to N-1 and N^2 of the purine unit resulting in a bridged structure. The adducts **dG349a** and **b** are analogous to **dG350b**, but the oxygen atom in the bridgehead position has been replaced by a nitrogen atom resulting in a piperidine ring. Adducts **dG349a** and **b** bear a diastereomeric relationship to one another and were not only separable, but were configurationally assigned. Adduct **dG599**, also comprising two diastereomers, is similar to **dG349a** and **b**, but the nitrogen atom in the piperidine ring in this case is the exocyclic amino nitrogen atom of another 2′-deoxyguanosine unit. It was found that adducts **dG350b** and **dG349a** and **b** were formed in calf thymus DNA. Plausible mechanisms for the formation of these adducts are presented.

Introduction

Glutaraldehyde (1,5-pentanedial) is an aliphatic dialdehyde with a wide variety of uses, most of which depend on the protein binding and biocidal properties of the compound. Glutaraldehyde has many industrial applications, including use as a crosslinking and tanning agent, a hardener in some X-ray development fluids, a biocide in metalworking fluids and in oil and gas pipelines, an antimicrobial in water treatment systems, a slimicide in paper manufacturing, a preservative in cosmetics, toiletries and chemical products such as fabric softeners, a fixative for biological specimens, a stabilizing agent of collagen-based bioimplantable materials, and in the production of carbonless paper.[1,2] Furthermore, glutaraldehyde is also used as a cold sterilizer in medical applications, a disinfectant in animal housing and air ducts and for the treatment of some dermatological disorders.[1,2] Consequently, occupational exposure for glutaraldehyde may occur in a number of industries.

Glutaraldehyde has been reported to be a skin and mucous membrane irritant,[3] it is also a skin allergen and may cause respiratory allergic reactions,[4–7] and histopathological effects in the nose have been demonstrated[8,9] in rats and mice. Glutaraldehyde has been found to be genotoxic in vitro and induces mutations in both bacterial and mammalian cells.[9–12] It also produces sister chromatid exchanges and chromosomal aberration in mammalian cells in vitro.[9] However, published data to assess the carcinogenic potential of glutaraldehyde are only limited[1] though two studies on the long-term exposure of rats and mice to glutaraldehyde by inhalation or oral routes have shown no evidence of carcinogenic activity.[13,14]

[a] National Institute of Occupational Health,
P. O. Box 8149 Dep., 0033 Oslo, Norway
[b] Department of Chemistry, University of Oslo,
P. O. Box 1033 Blindern, 0315 Oslo, Norway
[c] Laboratory of Organic Chemistry Åbo Akademi University,
Biskopsgatan 8, 20500 Turku/Åbo, Finland
Fax: +358-2-21-54-866
E-mail: leif.kronberg@abo.fi
[d] Department of Chemistry, University of Turku,
Vatselankatu 2, 20014 Turku, Finland
Fax: +358-2-33-36-700
E-mail: klikakd@yahoo.co.uk
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

Glutaraldehyde has been reported to form adducts with 2′-deoxyguanosine, 2′-deoxyadenosine and 2′-deoxycytidine, but not with thymidine.[15] The 2′-deoxyguanosine-glutaraldehyde adducts were reported to be relatively stable, whilst the 2′-deoxyadenosine-glutaraldehyde and 2′-deoxycytidine-glutaraldehyde adducts were reported to be comparatively labile.[15] However, the chemical structures of the adducts were not determined. Recently, we studied the reaction of glutaraldehyde with 2′-deoxyadenosine and determined the structures of three adducts.[16] Information on the chemical structures of adducts is important for understanding the genotoxic potential of the chemical compounds and thus the danger that glutaraldehyde poses to human health.

Liquid chromatography (LC) coupled to ultraviolet (UV) and mass spectrometric (MS) detection for the analyses of reaction mixtures, in combination with semi-preparative LC and nuclear magnetic resonance spectroscopic analyses of the isolated adducts, have proven to be an expedient method for the isolation and structural characterization of nucleoside adducts.[17–25] The aim of this study was to determine the structures of adducts formed in the reaction of glutaraldehyde with 2′-deoxyguanosine and calf thymus DNA in aqueous solutions.

Results and Discussion

Reactions of Glutaraldehyde with 2′-Deoxyguanosine

Analyses of the reaction mixtures by LC electrospray ionization tandem quadrupole MS (LC-ESI-MS/MS) (extracted ion chromatograms presented in the Supporting Information) and LC-UV [diode array detector (DAD) chromatogram presented in Figure 1] revealed that the most favorable reaction conditions for adduct formation were a pH of 8.8 and a reaction time of 3 d. However, the adducts could also be observed in reactions performed at pH = 7.4. The LC-ESI-MS/MS analyses of the reaction mixture provided pseudomolecular ions giving rise to peaks at *m*/*z* = 349 (**dG349a** and **dG349b**), 350 (**dG350a** and **dG350b**), 368 (**dG368a** and **dG368b**), 450 (**dG450a**–**d**) and 599 (**dG599**). The compounds **dG599**, **dG350b**, **dG349a** and **dG349b** were sufficiently stable to be isolated by semi-preparative LC. The number of glutaraldehyde units that were incorporated and the number of dG moieties assembled together in the compounds were clearly evident by MS analysis. However, following isolation, only by ^{13}C NMR was the number of diastereomers present in each sample revealed. The refined structures and conformational features were then elucidated by comprehensive NMR studies using a standard set of experiments and methodology[22,25–27] including the acquisition of ^{15}N NMR data as this nucleus has been demonstrated to be of great utility in determining the structures of nucleobase adducts.[22–24,26,28,29] The structures of the identified adducts are presented in Scheme 1.

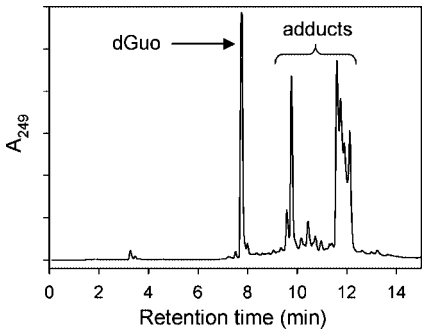
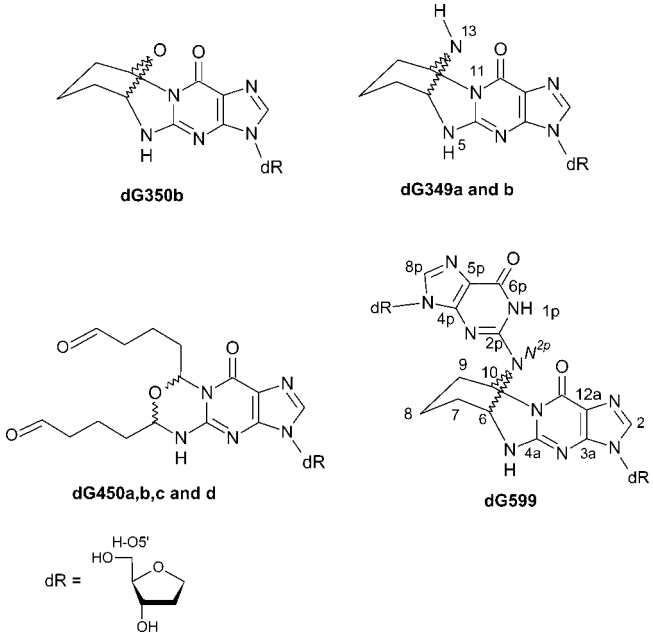


Figure 1. C18 analytical column LC-UV chromatogram recorded at 249 nm of the reaction mixture of glutaraldehyde with 2′-deoxyguanosine in 0.5 M phosphate buffer (pH = 8.8) kept at 37 °C for 3 d.



Scheme 1. Structures of the glutaraldehyde-derived nucleoside adducts identified in this study.

Adduct **dG350b** was ascertained to be a mixture of diastereomers. Their gross structures were readily determined following the $J_{H,H}$ coupling pathway along the alkyl chain starting from H-5. The sp^3-hybridized states of C-6 and C-10, together with heteronuclear correlations across the ethereal bridge, devolved the final structure, although the signals as sets could not be allotted to single diastereomers and the sets assigned.

The mass spectra of **dG349a** and **b** provided unexpected values of 348 amu (by $ESI^{+,-}$ and FAB^+) for the nominal masses of the compounds. Even values imply that a nitrogen atom has either been gained or lost from the guanosine substrate. The former was indicated by the presence of guanine in the product ions and proven to be the case by accurate mass measurements of the pseudomolecular ions and the indirect NMR observation of all six nitrogen atoms. To confirm the number of labile hydrogen atoms, silylation of

a sample was performed to provide the tetrasilylated product with an accurate mass also consistent with the presence of six nitrogen atoms. The introduction of a sixth nitrogen atom raises the immediate question of its origin, and its precise source was not substantiated. An artefact arising from a source other than the reactants (e.g. ammonium acetate used in the LC solvents) was the initial concern addressed, but removal of ammonium acetate from the LC eluent still provided the same result. Consideration was then given to guanosine being the nitrogen source, a notion supported by the observation of a structure (**dG599**) incorporating two guanosine units and one glutaraldehyde segment (vide infra). However, it was unequivocally proven that the introduced nitrogen atom did not come from dG since in a test reaction using fully labeled $^{15}N_5$-dG, the **dG349** adducts that formed contained only five labeled nitrogen atoms, not six as would be the case if the bridgehead nitrogen atom had originated from dG.

The similarity of the chemical shifts of **dG349a** and **b** to each other (each was composed of only one set of signals) implied a diastereomeric relationship between the two. Indeed, their similarity to the chemical shifts of **dG350b** rendered their structural elucidation facile after realizing the presence of the N^{2p}-H group and the couplings of H-6 and H-10 to the N^{2p}-H proton and the cross-bridging correlations. Diastereomeric adducts due solely to the presence of a single asymmetric center in the adduct segment have previously been successfully separated by LC.[23,24]

One aspect complicating the structural analysis[23–25,30–33] was the mobility of the nitrogen atoms, in particular N-5 and N-13 with respect to the orientation of N5-H and N-11. Thus, looking across the plane of the base unit, for either enantiomeric moiety {aza nitrogen atom (N-13) up (β-aza diastereomer) or aza nitrogen atom down (α-aza diastereomer)} N5-H could conceivable be orientated towards or away from the sugar unit. Furthermore, it was not possible to discern a direct interaction between the N5-H or N13-H groups and H-O5′. The supposition is therefore that a solvent molecule can be conveniently placed between the adduct vicinity and the sugar body and be held in place by attractive forces from both entities and therefore be long-term retained relative to other molecules comprising the solvent sheath – an arrangement only functional for the β-aza diastereomer. Such an assemblage would provide a much more polar, compact entity and would therefore elute first, and thus the β-aza stereochemistry is assigned to the first eluting diastereomer **dG349a**. Furthermore, the NOEs and coupling constants together indicate that the HO-5′ group is in the main directed down and away from the base unit for both **dG349a** and **b**, thus leaving the O-5′ lone pairs available for complexation in the case of **dG349a**. Because the vicinal couplings $J_{H5',HO5'}$, $J_{H5'',HO5'}$, $J_{H5',H4'}$, and $J_{H5'',H4'}$ are virtually identical for both diastereomers, there is little difference between the two in terms of the population-weighted average conformations for this portion of the molecule. However, the NOEs, expressed[34] as r_{NOE} to convey the dependency of the sixth power, indicate some proximity of the HO-5′ group in the α-aza diastereomer **dG349b** to both N5-H and H-2, consistent with the group being able to free-wheel into such positioning unhindered by the retention of a bound solvent molecule. Neither of these NOEs were observed for the β-aza diastereomer **dG349a**. A final indication is the chemical shift of N-4 in **dG349a**, considerably deshielded (–45.2 ppm) in comparison to its counterpart in **dG349b** (–64.5 ppm). Presumably N-4 in **dG349a** also participates in complexation, or is at least deshielded by the anisotropy of the bound solvent molecule. It should be noted that the chemical shifts of all other observed nuclei for the two diastereomers are exceedingly similar in value.

For **dG349a** and **b**, the piperidine ring resulting from adduct formation can adopt either a boat or a chair conformation. A boat conformation would provide two large couplings (or one large and one medium coupling) for both methine hydrogen atoms H-6 and H-10 (in addition to couplings to H-5 and N^{2p}-H), whilst a chair conformation would only provide two small/medium couplings for these two hydrogen atoms. The extracted $J_{H,H}$ coupling constants from spin simulation[35] clearly indicate that chair conformations are adopted by the piperidine rings in both diastereomers.

The chemical shifts of **dG599**, comprised of two diastereomers, bear remarkable analogies to **dG349a** and **b**, and elucidation of their gross structure followed compactly from the presumption that the nitrogen atom in the piperidine ring in this case is the exocyclic amino nitrogen atom of another 2′-deoxyguanosine unit. An analogous precedent to this structure has been reported.[16] The placement of a large group on N-1p (the bridging atom) is clearly evident by the steric compression effects on H-6 and H-10, both deshielded by 1.3 ppm relative to **dG349a** and **b**. Again, the signals as sets could not be allotted to single diastereomers and the sets assigned.

Dynamic effects for **dG599** were clearly apparent at 25 °C in both the ^{1}H (especially H-6, H-10 and one pair of the H-2/H-8p signals) and ^{13}C NMR spectra (e.g. C-6, C-10, and all the carbon atoms of one guanosine base unit). Specifically, the dynamic effects were clearly stronger on one guanosine unit than the other. Raising the temperature to 65 °C did not alleviate matters and in fact it seemed to exacerbate the situation for some spins, though as has been observed[22] previously for DMSO solutions, the exchange rate actually varied with time. However, it can safely be concluded that multiple species participate in the exchange system and the potential conformers are due to restricted rotation about the N^{2p}–"oxopurinyl" bond. It is the spins of this guanosine moiety (labeled "p" in Scheme 1) that exhibit the observed stronger dynamic effects. The two dG units were differentiated by correlations from the sharp H-2 signals to the tertiary N-11 at –285 ppm rather than the N(H)-1p signal resonating at –226 ppm.

Compound **dG350a** exhibited a UV absorption maximum at 272 nm, an indication of adduct formation at the exocyclic amino group.[15] Unfortunately, following isolation, it decomposed back to glutaraldehyde and dG before it could be characterized.

The adducts **dG450a** and **dG450b** were separated form the reaction mixture, but subsequent NMR analyses revealed that the samples contained mainly impurities, and distinct signals attributable to **dG450a** and **dG450b** could not be allotted. The pseudomolecular ion at *m*/*z* = 450 shows that the adducts **dG450a**–**d** are all evidently derived from two units of glutaraldehyde, but during the condensation with dG only one unit of water is lost. A possible structure to account for these adducts consists of a 1,3,5-oxadiazinane ring substituted with two butanal chains whereby four diastereomers in all are possible. The mechanism for the formation of such a ring would be the same as that for the formation of tetrahydropyrans when glutaraldehyde reacts with anilines.[36]

Adducts **dG368a** and **b** were also not amenable to isolation but are of interest as one or both could be the hydrated precursors to **dG350b**, i.e. the tetrahydropyran ring is opened to provide two diol groups. With two asymmetric carbon atoms at C-6 and C-10, four diastereomers in total are possible.

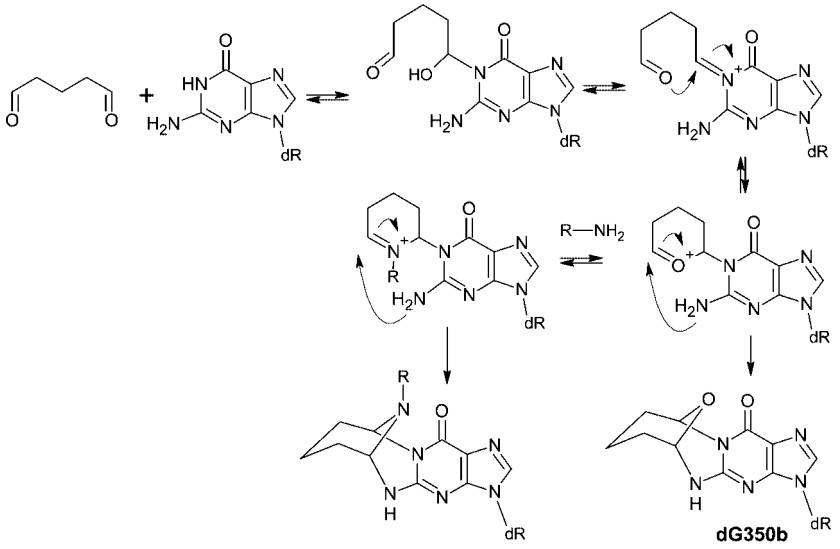
Adduct Formation

The compounds **dG350b**, **dG349a**, **dG349b** and **dG599** were formed faster and in higher yields in reactions performed at pH = 8.8 than at pH = 7.4. This indicates that the initial reaction takes place at the N-1 position, which is the most acidic position in dG (pK_a = 9.4). The initially formed carbinolamine is in equilibrium with a charged tetrahydropyran intermediate which undergoes an intramolecular ring closure by attack from the exocyclic amino group to yield **dG350b** (Scheme 2). However, the charged tetrahydropyran intermediate is susceptible to attack from any amino function and will consequently react with the exocyclic amino group of dG or with ammonia yielding, in these cases, charged tetrahydropyridine derivatives which subsequently undergo ring closure to yield **dG599** and **dG349a**–**b**, respectively.

Reaction of Glutaraldehyde with DNA

The reactions of glutaraldehyde with single- and double-stranded calf thymus DNA were performed at 37 °C and pH = 7.4 or 8.8. The modified DNA was enzymatically hydrolyzed to deoxynucleosides. As a control sample served calf thymus DNA that was not incubated with glutaraldehyde, but was otherwise treated as the incubated sample. The DNA hydrolysates were analyzed with LC-ESI-MS/MS in the multiple reaction monitoring (MRM) mode, and the adducts were identified by their selective MRM transitions and retention time equability with the adduct standards. The ion peaks monitored were the protonated molecular ions and the fragment peaks arising from cleavage of the sugar moiety (*m*/z = 116) from the parent ions.

The adducts **dG349a** and **dG349b** were observed in all DNA hydrolysates and the level of the adduct in dsDNA following 2 d of reaction at pH = 7.4 corresponded to 98 pmol/mg DNA (304 adducts/10^7 nucleotides) and 116 pmol/mg DNA (357 adducts/10^7 nucleotides), respectively (Figure 2). The adduct **dG350b** was observed in dsDNA only after 7 d of reaction at pH = 7.4, and the level of the adduct corresponded to 3 pmol/mg DNA (11 adducts/10^7 nucleotides) (Figure 2). In ssDNA the adducts were formed in about 10 times higher yields, and especially the reactions performed at pH = 8.8 favored the formation of adducts in ssDNA.



Scheme 2. Mechanism of formation of adducts **dG349a** and **b**, **dG350b** and **dG599**.

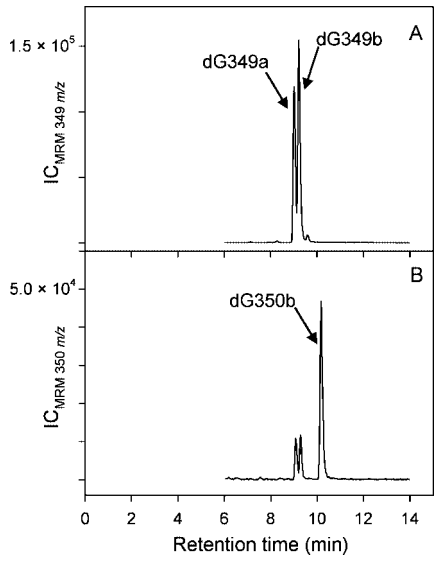


Figure 2. A: MRM chromatogram of the adducts **dG349a** and **dG349b** (the transition m/z = 349 to 233 was monitored and the collision voltage was set to 12 V) obtained by the analysis of the hydrolysate of ssDNA incubated with glutaraldehyde at pH = 7.4 for 7 d. B: MRM chromatogram of the adduct dG350b (the transition m/z = 350 to 234 was monitored and the collision voltage was set to 15 V) obtained by the analysis of the hydrolysate of ssDNA incubated with glutaraldehyde at pH = 8.8 for 3 d.

Conclusions

Previously it has been argued that glutaraldehyde forms adducts with deoxyguanosine, but the adducts were reported to be unstable and their identity remained unknown. In this work, we have successfully isolated and carried out structural identification of six adducts formed in the reaction of glutaraldehyde with deoxyguanosine. In the adducts the glutaraldehyde unit had been transformed to either a tetrahydropyran or a piperidine ring. The heteroatoms in the rings made up a one-atom bridge, and the bridgehead carbon atoms are fused to N-1 and N^2 of deoxyguanosine. The tetrahydropyrane adduct **dG350b** and the piperidine adduct **dG599** were found to both be compromised by two diastereomers which differ from each other with respect to the orientation of the bridge in respect to the plane of the base unit. The piperidine adducts **dG349a** and **dG349b** were isolated as pure compounds and found to have a diastereomeric relationship. The nitrogen atom in the piperidine ring of adduct **dG599** was found to be derived from the exocyclic amino group of deoxyguanosine and thus **dG599** consisted of two deoxyguanosine units.

In addition, LC-ESI-MS/MS analyses of the reaction mixture revealed the presence of four peaks giving rise to a pseudomolecular ion peak at m/z = 450. These adducts, designated **dG450a–d**, were considered as four diastereomers of a structure containing a 1,3,5-oxadiazine ring substituted with two butanal chains. The LC-ESI-MS/MS analyses showed also formation of adducts giving rise to a pseudomolecular ion peak at m/z = 368 which may represent compounds where the tetrahydropyrane ring in **dG350b** is opened by hydrolysis providing two diol groups.

In the hydrolysates of calf thymus DNA incubated with glutaraldehyde at pH = 7.4 and 8.8, the adducts **dG350b**, and **dG349a** and **b** could be detected. It is noteworthy that adducts **dG349a** and **b** maintain their relevancy from a biological point of view, given the ubiquitous presence of ammonia in biological systems, as well as other liberal nitrogen sources, i.e. their creation from DNA in vivo is assured. The miscoding properties of these adducts are not known, but it is obvious that the adducts will affect the formation of hydrogen bonds to the adenine base unit in the complementary DNA strand.

Experimental Section

Chemicals: Calf thymus DNA (Type I: sodium salt, highly polymerized), 2′-deoxyguanosine, acid phosphatase (wheat germ Type I), alkaline phosphatase (*E. coli* Type III), nuclease P1 from *Penicillum citrinum*, bis-tris buffer [bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane hydrochloride], and [D_6]DMSO were obtained from Sigma–Aldrich (St. Louis, MO, USA). Guanosine (u-$^{15}N_5$, 96–98%) was provided by Cambridge Isotope Laboratories (Andover, MA, USA). A 24% aqueous glutaraldehyde solution was purchased from NMD (Oslo, Norway). HPLC grade acetonitrile (ACN) and methanol (MeOH) were obtained from Rathburn Chemicals (Walkerburn, UK). Ethanol (EtOH, 96%) was purchased from Arcus (Oslo, Norway). Water was obtained from a Milli-Q® Academic water purification system from Millipore (Billerica, MA, USA). Analytical grade ammonium acetate (NH_4Ac), zinc chloride ($ZnCl_2$) and magnesium chloride hexahydrate ($MgCl_2 \times 6H_2O$) were provided by Merck (Darmstadt, Germany). Nitrogen, used as nebulizer and desolvation gas in the mass spectrometer, was produced by a Whatman nitrogen generator (Whatman International, Haverhill, MA, USA).

Chromatographic System: LC analyses were performed with a Waters CapLC™ System (Waters, Milford, MA, USA) consisting of a binary capillary gradient pump, an autosampler, and a DAD. The reaction mixtures were separated with a (3.5 μm, 1.0 mm i.d. × 150 mm) Kromasil C18 analytical column (G&T Septech, Kolbotn, Norway). The analytical chromatographic system was operated at 20 °C. The LC system was operated isocratically for 3 min with ACN and 10 mM NH_4Ac (2:98, v/v), prior to a gradient from 2–50% ACN over the course of 12 min at a flow rate of 40 μL/min. Semi-preparative isolation of the products was performed with a (5 μm, 10 mm i.d. × 250 mm) Thermo Hypersil-Keystone BDS Hypersil C18 column (Thermo Electron Corporation, Waltham, MA, USA). A Waters XTerra RP18 guard column (3.5 μm, 3.9 mm i.d. × 20 mm) was coupled to the semi-preparative column. The column was coupled to an Agilent 1100 series LC system (Agilent Technologies, Palo Alto, CA, USA) consisting of a quaternary pump, a micro degasser, a thermostatted column compartment, a DAD and a thermostatted analytical scale fraction collector. Injections were performed by a Waters 717 autosampler. The chromatographic system was operated at 30 °C using a flow rate of either 2.0 or 2.5 mL/min.

Spectroscopic and Spectrometric Methods: NMR spectra of adducts were recorded with NMR spectrometers equipped with a z-axis field-gradient 5 mm inverse broadband probe operating at 600 (and 500) MHz for ^1H, 150 (and 125) MHz for ^{13}C and 60 MHz for ^{15}N. All spectra were recorded in [D_6]DMSO at 25 °C, except the spectra of **dG599** which were recorded at 65 °C. The solvent signals were used as internal standards (δ = 2.500 ppm for ^1H and δ =

39.99 ppm for ^{13}C) except for ^{15}N spectra which were referenced externally to 90% nitromethane in CD_3NO_2 (δ = 0.0 ppm). ^{15}N signals were only observed indirectly whereby the *f*1 dimension taken from a ^{1}H-^{15}N HSQC or HMBC experiment was forward linear predicted to 11 Hz pt^{-1} (acquisition resolution 90 Hz pt^{-1}). For ^{1}H-^{15}N/^{13}C HMBC experiments, $^{n}J_{H,N/C}$ was optimized for 8 Hz and 4 Hz. Spin analysis was performed using Perch[35] iteration software for the extraction of ^{1}H chemical shifts and $J_{H,H}$ coupling constants. For ^{1}H, chemical shifts extracted from Perch are reported to three decimal places, whilst manual measurements are reported to two decimal places (two and one decimal places, respectively, for *J*); for ^{13}C, chemical shifts are reported to two decimal places except for broad signals which are reported to one decimal place; for ^{15}N, chemical shifts are reported to one decimal place except for samples containing diastereomers wherein signals are reported to the nearest whole number due to uncertainty arising from overlap. Full experimental details for all NMR experiments are given in refs.[22,26,27] The MS analyses of the column effluent were provided by a QuattroLC™ tandem quadrupole MS equipped with a Z-spray atmospheric pressure ionization source prepared for electrospray ionization (Micromass Manchester, UK). ESI was performed in positive mode with applied voltages: capillary voltage: 4000 V; sample cone voltage: 15 V and extraction cone voltage: 2 V. The nebulizer gas flow was 90 L/h, the desolvation gas flow was 360 L/h, the desolvation temperature was 450 °C and the source temperature was 110 °C. When the instrument was operated in MRM mode, argon (99.999%, Yara Industrial, Oslo, Norway) was used as the collision gas wherein the collision cell pressure was set to 2.8×10^{-3} mbar and the collision and RF lens voltages set to 15 V and 0.4 V, respectively. MRM experiments were performed using dwell times of 300 ms for each channel. The CapLC™ system and the tandem quadrupole MS instrument were controlled and data were acquired using MassLynx v4.1 software. Exact mass determination was performed by direct infusion of the nucleoside adducts with a Micromass Q-TOF 2™ quadrupole time-of-flight (Q-TOF) mass spectrometer equipped with a Z-spray atmospheric pressure ionization source prepared for ESI. The Q-TOF mass spectrometer was operated at a resolution of 13700. Elemental composition and accurate mass for the silylated **dG349b** adduct was determined with a Fisons ZAB-Spec high-resolution MS instrument. The ionization mode was electron impact (70eV) and the resolution was 7000.

Small-Scale Reactions of Glutaraldehyde with 2′-Deoxyguanosine: Glutaraldehyde (112 mg, 1.12 mmol) was treated with 2′-deoxyguanosine (15 mg, 0.056 mmol) in 8 mL of 0.5 M phosphate buffer solutions at 37 °C and pH = 7.4 and 8.8. The progress of the reactions was monitored by LC-ESI-MS/MS analysis of aliquots taken from the reaction mixtures.

Preparation of the 2′-Deoxyguanosine-Glutaraldehyde Adducts: To a solution of 2′-deoxyguanosine (300 mg, 1.12 mmol) in 0.5 M phosphate buffer (pH = 8.8, 150 mL) was added 24% aqueous glutaraldehyde solution (9.37 mL, 22.5 mmol). The reaction mixture was stirred at 37 °C for 3 d and monitored by LC-ESI-MS/MS analyses. After 3 d, the reaction was complete and the mixture was filtered and concentrated by rotary evaporation at 37 °C to about one-tenth of the initial volume. Precipitated phosphate buffer was removed by filtration. The adducts were purified using the semi-preparative LC system which was operated isocratically for 5 min with ACN and 10 mM NH_4Ac (2:98, v/v), prior to a gradient from 2 to 20% ACN over the course of 10 min, and finally maintained at 20% ACN for 9 min. The flow rate was 2.5 mL/min. The collected fractions containing the adduct were combined and rotary-evaporated at 37 °C to about one-tenth of the initial volume. Further purification was done by re-injection of the concentrated solution on the semi-preparative LC system using the same gradient elution. Once more, the collected fractions containing the adducts were combined and rotary-evaporated at 37 °C to about one-tenth of the initial volume. Finally, the sample was desalted using the semi-preparative LC system run with a gradient from 20 to 40% MeOH over the course of 10 min and then maintained at 40% MeOH for a further 7 min. The flow rate was 2.0 mL/min. The desalted pure adducts were rotary-evaporated at 37 °C to dryness, further dried under vacuum overnight and finally dissolved in [D_6]DMSO.

Adduct dG349a: Retention time: 9.50 min. UV (EtOH/ water, 80:20, v/v): λ_{max} (ε/dm^3 mol^{-1} cm^{-1} 9000) = 206, 259 and 270 (sh) nm; λ_{min} = 228 nm. MS (ESI): *m*/*z* (%) = 349 (100) [M + H$^+$], 233 (71) [MH$^+$ – deoxyribosyl + H], product ion scan of 349: 233 (100) [MH$^+$ – deoxyribosyl + H], 152 (55) [MH$^+$ – $C_{10}H_{16}NO_3$ + H]. HRMS: calcd. for [$C_{15}H_{20}N_6O_4$ + H$^+$] 349.1624; found 349.1643. ^{1}H NMR ([D_6]DMSO): δ = 8.108 (d, J_{H6} = 3.65 Hz, H-5), 7.892 (s, H-2), 6.113 (dd, $J_{H2'\beta}$ = 7.97, $J_{H2'\alpha}$ = 5.98 Hz, H-1′), 5.563 (m, $J_{H9\beta}$ = 4.25, J_{H1p} = 3.20, $J_{H9\alpha}$ = 2.17 Hz, H-10), 5.262 (d, $J_{H3'}$ = 3.92 Hz, HO-3′), 4.952 (t, $J_{H5'proR}$ = 5.70, $J_{H5'proS}$ = 5.46 Hz HO-5′), 4.484 (m, $J_{H7\alpha}$ = 4.08, J_{H5} = 3.65, J_{H1p} = 2.64, $J_{H7\beta}$ = 2.46 Hz, H-6), 4.339 (m, $J_{H2'\beta}$ = 5.64, $J_{HO3'}$ = 3.92, $J_{H2'\alpha}$ = 2.91, $J_{H4'}$ = 2.57 Hz, H-3′), 3.810 (m, $J_{H5'proS}$ = 4.85, $J_{H5'proR}$ = 4.51, $J_{H3'}$ = 2.57 Hz, H-4′), 3.554 (m, $J_{H5'proR}$ = –11.67, $J_{HO5'}$ = 5.46, $J_{H4'}$ = 4.85 Hz, H-5′*proS*), 3.525 (br., J_{H10} = 3.20, J_{H6} = 2.64 Hz, N_{13}-H), 3.492 (m, $J_{H5'proS}$ = –11.67, $J_{HO5'}$ = 5.70, $J_{H4'}$ = 4.51 Hz, H-5′*proR*), 2.55 (overlapped, $J_{H2'\alpha}$ = –13.10, $J_{H1'}$ = 7.97, $J_{H3'}$ = 5.64 Hz, H-2′β), 2.178 (ddd, $J_{H2'\beta}$ = –13.10, $J_{H1'}$ = 5.98, $J_{H3'}$ = 2.91 Hz, H-2′α), 1.774 (overlapped m, $J_{H8\alpha}$ = 14.66, $J_{H9\alpha}$ = –12.13, J_{H10} = 4.25, $J_{H8\beta}$ = 3.09 Hz, H-9β), 1.771 (overlapped m, $J_{H9\beta}$ = –12.13, $J_{H8\beta}$ = 3.92, $J_{H8\alpha}$ = 2.80, J_{H10} = 2.17 Hz, H-9α), 1.745 (overlapped m, $J_{H8\alpha}$ = 13.33, $J_{H7\alpha}$ = –12.59, $J_{H8\beta}$ = 4.67, J_{H6} = 2.46 Hz, H-7β), 1.583 (overlapped m, $J_{H8\alpha}$ = –14.27, $J_{H7\beta}$ = 4.67, $J_{H9\alpha}$ = 3.92, $J_{H9\beta}$ = 3.09, $J_{H7\alpha}$ = 2.26 Hz, H-8β), 1.581 (overlapped m, $J_{H7\beta}$ = –12.59, J_{H6} = 4.08, $J_{H8\alpha}$ = 4.06, $J_{H8\beta}$ = 2.26 Hz, H-7α), 1.415 (m, $J_{H8\beta}$ = –14.27, $J_{H9\beta}$ = 14.66, $J_{H7\beta}$ = 13.33, $J_{H7\alpha}$ = 4.06, $J_{H9\alpha}$ = 2.80 Hz, H-8α) ppm. ^{13}C NMR ([D_6]-DMSO): δ = 155.80 (C-12), 151.98 (C-4a), 149.91 (C-3a), 135.71 (C-2), 116.26 (C-12a), 88.04 (C-4′), 82.98 (C-1′), 71.28 (C-3′), 62.25 (C-5′), 61.86 (C-10), 59.90 (C-6), 39.76 (C-2′), 31.80 (C-7), 29.57 (C-9), 15.30 (C-8) ppm. ^{15}N NMR ([D_6]DMSO): δ = –45.2 (N-4), –131.4 (N-1), –209.0 (N-3), –283.0 (N-5), –286.3 (N-11), –336.8 (N-13) ppm.

Adduct dG349b: Retention time: 9.69 min. UV (EtOH/water, 80:20, v/v): λ_{max} (ε/dm^3 mol^{-1} cm^{-1}) = 206, 259 and 270 (sh, 9300) nm; λ_{min} = 228 nm. MS (ESI): *m*/*z* (%) = 349 (100) [M + H$^+$], 233 (71) [MH$^+$ – deoxyribosyl + H], product ion scan of 349: 233 (100) [MH$^+$ – deoxyribosyl + H], 152 (55) [MH$^+$ – $C_{10}H_{16}NO_3$ + H]. HRMS: calcd. for [$C_{15}H_{20}N_6O_4$ + H$^+$] 349.1624; found 349.1638. ^{1}H NMR ([D_6]DMSO): δ = 8.116 (d, J_{H6} = 3.71 Hz, H-5), 7.896 (s, H-2), 6.117 (dd, $J_{H2'\beta}$ = 7.96, $J_{H2'\alpha}$ = 5.98 Hz, H-1′), 5.563 (m, $J_{H9\alpha}$ = 4.39, J_{H1p} = 2.77, $J_{H9\beta}$ = 2.13 Hz, H-10), 5.260 (d, $J_{H3'}$ = 3.93 Hz HO-3′), 4.956 (t, $J_{H5'proR}$ = 5.61, $J_{H5'proS}$ = 5.48 Hz HO-5′), 4.485 (m, $J_{H7\beta}$ = 4.16, J_{H5} = 3.71, $J_{H7\alpha}$ = 2.38, J_{H1p} = 2.32 Hz, H-6), 4.334 (m, $J_{H2'\beta}$ = 5.56, $J_{HO3'}$ = 3.93, $J_{H2'\alpha}$ = 2.92, $J_{H4'}$ = 2.58 Hz, H-3′), 3.808 (m, $J_{H5'proS}$ = 4.82, $J_{H5'proR}$ = 4.52, $J_{H3'}$ = 2.58 Hz, H-4′), 3.553 (m, $J_{H5'proR}$ = –11.70, $J_{HO5'}$ = 5.48, $J_{H4'}$ = 4.82 Hz, H-5′*proS*), 3.533 (br., J_{H10} = 2.77, J_{H6} = 2.32 Hz, N_{13}-H), 3.501 (m, $J_{H5'proS}$ = –11.70, $J_{HO5'}$ = 5.61, $J_{H4'}$ = 4.52 Hz, H-5′*proR*), 2.5 (overlapped, $J_{H2'\alpha}$ = –13.11, $J_{H1'}$ = 7.96, $J_{H3'}$ = 5.56 Hz, H-2′β), 2.188 (ddd, $J_{H2'\beta}$ = –13.11, $J_{H1'}$ = 5.98, $J_{H3'}$ = 2.92 Hz, H-2′α), 1.772 (overlapped m, $J_{H8\beta}$ = 14.25, $J_{H9\beta}$ = –12.08, J_{H10} = 4.39, $J_{H8\alpha}$ = 3.27 Hz, H-9α), 1.763 (overlapped m, $J_{H9\alpha}$ =

–12.08, $J_{H8\beta}$ = 3.77, $J_{H8\alpha}$ = 3.56, J_{H10} = 2.13 Hz, H-9β), 1.744 (overlapped m, $J_{H8\beta}$ = 13.48, $J_{H7\beta}$ = –12.46, $J_{H8\alpha}$ = 4.60, J_{H6} = 2.38 Hz, H-7α), 1.575 (overlapped m, $J_{H8\beta}$ = –14.14, $J_{H9\beta}$ = 3.56, $J_{H7\beta}$ = 2.55, $J_{H7\alpha}$ = 4.60, $J_{H9\alpha}$ = 3.27 Hz, H-8α), 1.572 (overlapped m, $J_{H7\alpha}$ = –12.46, $J_{H8\alpha}$ = 2.55, $J_{H8\beta}$ = 4.20, J_{H6} = 4.16 Hz, H-7β), 1.408 (m, $J_{H8\alpha}$ = –14.14, $J_{H9\alpha}$ = 14.25, $J_{H7\alpha}$ = 13.48, $J_{H7\beta}$ = 4.20, $J_{H9\beta}$ = 3.77 Hz, H-8β) ppm. ^{13}C NMR ([D$_6$]-DMSO): δ = 155.83 (C-12), 152.01 (C-4a), 149.92 (C-3a), 135.59 (C-2), 116.12 (C-12a), 88.00 (C-4′), 82.84 (C-1′), 71.28 (C-3′), 62.24 (C-5′), 61.88 (C-10), 59.89 (C-6), 39.93 (C-2′), 31.81 (C-7), 29.56 (C-9), 15.30, (C-8) ppm. ^{15}N NMR ([D$_6$]DMSO): δ = –64.5 (N-4), –131.3 (N-1), –209.0 (N-3), –283.1 (N-5), –286.5 (N-11), –336.7 (N-13) ppm.

Silylated Adduct dG349b: High-resolution EIMS: calcd. for $C_{27}H_{52}N_6O_4Si_4$ 636.3127; found 636.3116.

Adduct dG368a: Retention time: 9.71 min. UV (LC eluent, 10 mM NH_4Ac and ACN): λ_{max} = 208, 255 and 268 (sh) nm; λ_{min} = 224 nm. MS (ESI): *m/z* (%) = 368 (100) [M + H$^+$], 252 (6) [MH$^+$ – deoxyribosyl + H], product ion scan of 368: 252 (36) [MH$^+$ – deoxyribosyl + H], 234 (100) [MH$^+$ – deoxyribosyl – H_2O + H], 152 (5) [MH$^+$ – $C_{10}H_{17}O_5$ + H].

Adduct dG368b: Retention time: 9.92 min. UV (LC eluent, 10 mM NH_4Ac and ACN): λ_{max} = 208, 255 and 268 (sh) nm; λ_{min} = 224 nm. MS (ESI): *m/z* (%) = 368 (100) [M + H$^+$], 252 (6) [MH$^+$ – deoxyribosyl + H], product ion scan of 368:252 (36) [MH$^+$ – deoxyribosyl + H], 234 (100) [MH$^+$ – deoxyribosyl – H_2O + H], 152 (5) [MH$^+$ – $C_{10}H_{17}O_5$ + H].

Adduct dG599: Retention time: 9.87 min. UV (EtOH/water, 80:20, v/v): λ_{max} (ε/dm^{3}mol^{-1}cm^{-1}) = 208 and 257 (10300) nm; λ_{min} = 228 nm. MS (ESI): *m/z* (%) = 599 (100) [M + H$^+$], 483 (59) [MH$^+$ – deoxyribosyl + H], 367 (84) [M + H$^+$ – 2 deoxyribosyl + 2 H], product ion scan of 599:483 (100) [MH$^+$ – deoxyribosyl + H], 367 (49) [M + H$^+$ – 2 deoxyribosyl + 2 H], 332 (52) [MH$^+$ – dG + H], 268 (10) [MH$^+$ – $C_{15}H_{18}N_5O_4$ + H], 216 (12) [MH$^+$ – dG – deoxyribosyl + H], 152 (4) [MH$^+$ – $C_{20}H_{26}N_5O_7$ + H]. HRMS: calcd. for [$C_{25}H_{30}N_{10}O_8$ + H$^+$] 599.2326; found 599.2312. ^{1}H NMR ([D$_6$]DMSO at 65 °C): δ = 8.27 and 8.24 (2×d, J_{H6} = 2.3 and 2.4 Hz, H-5), 7.99 (br. s, H-8p), 7.874 and 7.872 (s, H-2), 6.96 and 6.95 (br. m, H-10), 6.14–6.06 (m, H-1′), 5.84 (br. m, H-6), 5.04 (br., HO-3′), 4.69 and 4.61 (br., HO-5′), 4.39–4.29 (m, H-3′), 3.84–3.79 (m, H-4′), 3.61–3.38 (m, H-5′ and H-5′′), 2.64–2.45 (overlapped m, H-2′β), 2.24–2.13 (overlapped m, H-2′α), 2.06–2.00 (overlapped m, H-9eq), 2.00–1.92 (overlapped m, H-7ax and H-9ax), 1.87–1.82 (overlapped m, H-7eq), 1.76–1.71 (overlapped m, H-8eq), 1.61–1.50 (overlapped m, H-8ax) ppm; N_{1p}-H not observed. ^{13}C NMR ([D$_6$] DMSO): δ = 158.5 (C-6p), 155.26 and 155.23 (C-12), 152.4 (C-2p), 151.03 and 150.95 (C-4a), 149.53 and 149.48 (C-3a), 149.3 (C-4p), 137.9 and 137.6 (C-8p), 135.71 and 135.65 (C-2), 119.4 (C-5p), 116.15 and 116.08 (C-12a), 87.99 and 87.86 and 87.85 (C-4′), 83.69 and 83.38 and 82.84 and 82.74 (C-1′), 70.99 and 70.94 and 70.84 and 70.67 (C-3′), 62.03 and 61.97 and 61.87 (C-5′), 61.28 and 60.99 (C-6), 61.04 and 60.91 (C-10), 39.99 and 39.97 and 39.73 and 39.55 (C-2′), 31.65 (C-7), 29.34 and 29.33 (C-9), 14.24 (C-8) ppm. ^{15}N NMR ([D$_6$]DMSO): δ = –131 (N-1), –204 (N-9p), –207 (N-3), –226 (N-1p), –285 (N-12), –288 (N-5), –296 (N^{2p}) ppm.

Adduct dG350a: Retention time: 10.28 min. UV (LC eluent, 10 mM NH_4Ac and ACN): λ_{max} = 204, 215 (sh), 272 and 282 (sh) nm; λ_{min} = 224 nm. MS (ESI): *m/z* (%) = 350 (100) [M + H$^+$], product ion scan of 350: 332 (3) [MH$^+$ – H_2O], 234 (96) [MH$^+$ – deoxyribosyl + H], 216 (100) [MH$^+$ – deoxyribosyl – H_2O + H].

Adduct dG350b: Retention time: 10.57 min. UV (EtOH/water, 80:20, v/v): λ_{max} (ε/dm^{3}mol^{-1}cm^{-1}) = 206 and 258 (9100) nm; λ_{min} = 227 nm. MS (ESI): *m/z* (%) = 350 (100) [M + H$^+$], 234 (79) [MH$^+$ – deoxyribosyl + H], product ion scan of 350: 234 (100) [MH$^+$ – deoxyribosyl + H], 216 (10) [MH$^+$ – deoxyribosyl – H_2O + H]. HRMS: calcd. for [$C_{15}H_{19}N_5O_5$ + H$^+$] 350.1464; found 350.1460. ^{1}H NMR ([D$_6$]DMSO): δ = 8.62 and 8.61 (d, J_{H6} = 3.7 Hz, H-5), 7.98 and 7.97 (s, H-2), 6.131 and 6.128 (t, $J_{H2'}$ = 7.0 Hz, H-1′), 6.124 (m, H-10), 5.27 (d, $J_{H3'}$ = 3.9 Hz, HO-3′), 5.26 (m, H-6), 4.93 and 4.94 (t, $J_{H5'}$ = 5.4 Hz, HO-5′), 4.34 (m, H-3′), 3.82 (m, H-4′), 3.55 (m, H-5′), 3.50 (m, H-5′′), 2.52 (overlapped m, H-2′), 2.21 (ddd, $J_{H2'}$ = –13.1, $J_{H1'}$ = 6.0, $J_{H3'}$ = 3.1 Hz, H-2′′), 1.96 (overlapped m, H-9b), 1.92 (overlapped m, H-7b), 1.82 (m, H-9a), 1.63 (overlapped m, H-8b), 1.61 (overlapped m, H-7a), 1.54 (overlapped m, H-8a) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 155.05 and 155.03 (C-12), 150.25 and 150.22 (C-4a), 150.02 (C-3a), 136.3 and 136.2 (C-2), 116.2 and 116.1 (C-12a), 88.1 (C-4′), 82.98 and 82.92 (C-1′), 77.61 and 77.59 (C-10), 77.04 and 77.03 (C-6), 71.2 (C-3′), 62.17 and 62.15 (C-5′), 39.9 (C-2′), 31.4 (C-7), 29.1 (C-9), 13.1 (C-8) ppm.

Adduct dG450a: Retention time: 10.88 min. UV (EtOH/water, 80:20, v/v): λ_{max} (ε/dm^{3}mol^{-1}cm^{-1}) = 208, 256 and 270 (sh, 7500) nm; λ_{min} = 226 nm. MS (ESI): *m/z* (%) = 450 (100) [M + H$^+$], 334 (34) [MH$^+$ – deoxyribosyl + H], product ion scan of 450: 432 (5) [MH$^+$ – H_2O], 334 (86) [MH$^+$ – deoxyribosyl + H], 316 (100) [MH+ – deoxyribosyl – H_2O + H], 152 (5) [MH$^+$ – $C_{15}H_{23}O_6$ + H]. HRMS: calcd. for [$C_{20}H_{27}N_5O_7$ + H$^+$] 450.1989; found 450.1966].

Adduct dG450b: Retention time: 11.09 min. UV (EtOH/water, 80:20, v/v): λ_{max} (ε/dm^{3}mol^{-1}cm^{-1}) = 208, 256 and 270 (sh, 9600) nm; λ_{min} = 226 nm. MS (ESI): *m/z* (%) = 450 (100) [M + H$^+$], 334 (12) [MH$^+$ – deoxyribosyl + H]; product ion scan of 450: 432 (5) [MH$^+$ – H_2O], 334 (86) [MH$^+$ – deoxyribosyl + H], 316 (100) [MH+ – deoxyribosyl – H_2O + H], 152 (5) [MH$^+$ – $C_{15}H_{23}O_6$ + H].

Adduct dG450c: Retention time: 11.90 min. UV (LC eluent, 10 mM NH_4Ac and ACN): λ_{max} = 208, 256 and 270 (sh) nm; λ_{min} = 226 nm. MS (ESI): *m/z* (%) = 450 (100) [M + H$^+$], 334 (12) [MH$^+$ – deoxyribosyl + H]; product ion scan of 450: 432 (5) [MH$^+$ – H_2O], 334 (86) [MH$^+$ – deoxyribosyl + H], 316 (100) [MH+ – deoxyribosyl – H_2O + H], 152 (5) [MH$^+$ – $C_{15}H_{23}O_6$ + H].

Adduct dG450d: Retention time: 11.95 min. UV (LC eluent, 10 mM NH_4Ac and ACN): λ_{max} = 208, 256 and 270 (sh) nm; λ_{min} = 226 nm. MS (ESI): *m/z* (%) = 450 (100) [M + H$^+$], 334 (12) [MH$^+$ – deoxyribosyl + H], product ion scan of 450: 432 (5) [MH$^+$ – H_2O], 334 (86) [MH$^+$ – deoxyribosyl + H], 316 (100) [MH$^+$ – deoxyribosyl – H_2O + H], 152 (5) [MH$^+$ – $C_{15}H_{23}O_6$ + H].

Determination of Nucleoside Adduct Yields: Subsequent to the NMR analyses, the NMR samples were concentrted to dryness under vacuum for 48 h, weighed and re-dissolved in EtOH/water (80:20, v/v). These solutions served as stock solutions, from which calibration solutions were prepared. The adducts in the reaction mixtures were quantitatively determined by LC-ESI-MS/MS using MRM mode. The molar yields were calculated from the original amount of nucleoside in the reaction mixture.

Reactions of Glutaraldehyde with Calf Thymus DNA: Single-stranded (ss) DNA was prepared from the double-stranded (ds) DNA by heating the DNA to 100 °C for 5 min and rapidly cooling the solution with ice. Glutaraldehyde (0.105 mL, 0.25 mmol) was treated with ssDNA and dsDNA (2.5 mg) in 2.5 mL of 0.1 M phosphate buffer (pH = 7.4 and 8.8) at 37 °C. The mixtures were stirred for 2 d (pH = 7.4), 3 d (pH = 8.8) and 7 d (pH = 7.4), respectively, and then the mixtures were pipetted to a 50-mL NUNC™ (Nalge Nunc Int., Rochester, NY, USA) centrifuge tube. The DNA was

precipitated by the addition of 5 M NaCl (0.5 mL) and cold EtOH (8 mL) followed by cooling to –20 °C. The mixture was centrifuged (10 min at 3000 rpm), and the supernatant was removed. The recovered DNA was washed with cold 70% EtOH (3 mL), cold EtOH (3 mL), and dissolved in water (3 mL). The DNA was re-precipitated from the solution by the addition of cold EtOH (8 mL) and cooling to –20 °C. The DNA was recovered by centrifuging and dried in vacuo for 5 h. Then the DNA was dissolved in 4 mL of 100 mM bis-tris buffer (pH = 6.5) containing 2 mM $MgCl_2$ overnight. The DNA was enzymatically hydrolyzed by the addition of Nuclease P1 solution (1 mg/mL in 1 mM $ZnCl_2$) until a final concentration of 50 units/mL was attained. The mixture was stirred and incubated at 37 °C for 4 h. Finally, bacterial alkaline phosphatase and wheat germ acid phosphatase (the latter dissolved in bis-tris-$MgCl_2$ buffer at a concentration of 10 mg/mL) were added to realize a final concentration of 6 units/mL and 0.4 units/mL, respectively. The mixture was incubated at 37 °C for 18 h. The enzyme digest mixture was loaded onto a pre-rinsed (water) Centricon YM-3 filter from Millipore and centrifuged with a Sorvall (Kendro Laboratory Products, Asheville, NC, USA) Superspeed fixed-angle rotor (SS-34) for 150 min (10 °C) at 7000 rpm. The ultrafiltrate was recovered, dried under vacuum and reconstituted in 1000 μL of water. The resulting solution was analyzed with LC-ESI-MS/MS operating in the MRM mode.

Supporting Information (see footnote on the first page of this article): Extracted ion chromatograms of the adducts in the reaction mixture, UV spectra for all compounds, and ^1H and ^{13}C NMR spectra for the compounds **dG349a**, **dG349b**, **dG350b**, and **dG599**.

Acknowledgments

Financial support from The Research Council of Norway is acknowledged, whilst the Center for Scientific Computing (CSC) is thanked for computational support.

[1] American Conference of Industrial Hygienists (ACGIH). *Glutaraldehyde*, *TLV® Chemical Substances*, 7th ed. documentation, **2001**, p. 1–10.
[2] B. Beije, P. Lundberg, *Arbete och Hälsa* **1997**, *20*, 1–30.
[3] P. S. Burge, *Br. Med. J.* **1989**, 299–342.
[4] O. J. Corrado, J. Osman, R. J. Davies, *Human Toxicol.* **1986**, *5*, 325–327.
[5] P. F. G. Gannon, P. Bright, M. Campbell, S. P. O'Hickey, P. S. Burge, *Thorax* **1995**, *50*, 156–159.
[6] S. C. Stenton, J. R. Beach, J. H. Dennis, N. P. Keaney, D. J. Hendrick, *Occup. Med.* **1994**, *44*, 95–98.
[7] M. Chan-Yeung, T. McMurren, F. Catonio-Begley, S. Lam, *J. Allergy Clin. Immunol.* **1992**, *91*, 974–978.
[8] M. G. B. St Clair, E. A. Gross, K. T. Morgan, *Toxicol. Pathol.* **1990**, *18*, 353–361.
[9] U. S. National Toxicology Program, *Toxicity studies of glutaraldehyde administrated by inhalation to F344/N rats and B6C3F1 mice*, **1993**, NTP Technical Report 25; DHHS (NIH) Pub. No. 93-3348.
[10] D. E. Levin, M. Hollstein, M. F. Christman, E. A. Schwiers, B. N. Ames, *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 7445–7449.
[11] S. Haworth, T. Lawlor, K. Mortelsmans, W. Speck, E. Zeiger, *Environ. Mutagen. Suppl.* **1983**, *1*, 3–142.
[12] L. J. Marnett, H. K. Hurd, M. C. Hollstein, D. E. Levin, H. Esterbauer, B. N. Ames, *Mutat. Res.* **1985**, *148*, 25–34.
[13] U. S. National Toxicology Program, *Toxicology and carcinogenesis studies of glutaraldehyde in F344/N rats and B6C3F1 mice*, **1998**, NTP Technical Report 490; DHHS (NIH) Pub. No. 98-3980.
[14] B. Ballantyne, *Toxicology of Glutaraldehyde: Review of Studies and Human Health Effects*, Union Carbide Corporation, Bound Brook, NJ, **1995**, p. 47–54.
[15] K. Hemminki, R. Suni, *Arch. Toxicol.* **1984**, *55*, 186–190.
[16] R. Olsen, J. Backman, P. Molander, S. Øvrebø, S. Thorud, E. Lundanes, T. Greibrokk, L. Kronberg, *Chem. Res. Toxicol.*; DOI: 10.1021/tx7000477.
[17] L. V. Nechev, I. D. Kozekov, A. K. Brock, C. J. Rizzo, T. M. Harris, *Chem. Res. Toxicol.* **2002**, *15*, 607–613.
[18] T. Munter, L. Cottrell, S. Hill, L. Kronberg, W. P. Watson, B. T. Golding, *Chem. Res. Toxicol.* **2002**, *15*, 1549–1560.
[19] J. Backman, R. Sjöholm, L. Kronberg, *Chem. Res. Toxicol.* **2004**, *17*, 1652–1658.
[20] F. A. Beland, M. I. Churchwell, L. S. Von Tungeln, S. Chen, P. P. Fu, S. J. Culp, B. Schoket, E. Gyorffy, J. Minárovits, M. C. Poirier, E. D. Bowman, A. Weston, D. R. Doerge, *Chem. Res. Toxicol.* **2005**, *18*, 1306–1315.
[21] A. J. Pawłowicz, T. Munter, Y. Zhao, L. Kronberg, *Chem. Res. Toxicol.* **2006**, *19*, 571–576.
[22] J. Mäki, P. Tähtinen, L. Kronberg, K. D. Klika, *J. Phys. Org. Chem.* **2005**, *18*, 240–249.
[23] K. D. Klika, J. Mäki, R. Sjöholm, L. Kronberg, *ARKIVOC* **2006**, *(xii)*, 65–74.
[24] A. J. Pawłowicz, K. D. Klika, L. Kronberg, *Eur. J. Org. Chem.* **2007**, *9*, 1429–1437.
[25] A. J. Pawłowicz, T. Munter, K. D. Klika, L. Kronberg, *Bioorg. Chem.* **2006**, *34*, 39–48.
[26] P. Virta, A. Koch, M. U. Roslund, P. Mattjus, E. Kleinpeter, L. Kronberg, R. Sjöholm, K. D. Klika, *Org. Biomol. Chem.* **2005**, *3*, 2924–2929.
[27] E. Balentová, J. Imrich, J. Bernát, L. Suchá, M. Vilková, N. Prónayová, P. Kristian, K. Pihlaja, K. D. Klika, *J. Heterocycl. Chem.* **2006**, *43*, 645–656.
[28] J. Mäki, K. D. Klika, R. Sjöholm, L. Kronberg, *J. Chem. Soc. Perkin Trans. 1* **2001**, 1216–1219.
[29] M. U. Roslund, P. Virta, K. D. Klika, *Org. Lett.* **2004**, *6*, 2673–2676.
[30] A. Rosling, K. Klika, F. Fülöp, R. Sillanpää, J. Mattinen, *Heterocycles* **1999**, *51*, 2575–2588.
[31] A. Rosling, K. D. Klika, F. Fülöp, R. Sillanpää, J. Mattinen, *Acta Chem. Scand.* **1999**, *53*, 103–113.
[32] A. Rosling, M. Hotokka, K. D. Klika, F. Fülöp, R. Sillanpää, J. Mattinen, *Acta Chem. Scand.* **1999**, *53*, 213–221.
[33] P. Tähtinen, A. Bagno, K. D. Klika, K. Pihlaja, *J. Am. Chem. Soc.* **2003**, *125*, 4609–4618.
[34] K. D. Klika, J. Imrich, I. Danihel, S. Böhm, P. Kristian, S. Hamuľaková, K. Pihlaja, A. Koch, E. Kleinpeter, *Magn. Reson. Chem.* **2005**, *43*, 380–388.
[35] See for example: R. Laatikainen, M. Niemitz, U. Weber, J. Sundelin, T. Hassinen, J. Vepsäläinen, *J. Magn. Reson. Ser. A* **1996**, *120*, 1–10; see also: *Peak Research NMR Software*, Perch Solutions Ltd., Kuopio, Finland, **2003** (http://www.perchsolutions.com).
[36] A. P. Henderson, C. Bleasdale, W. Clegg, B. T. Golding, *Chem. Res. Toxicol.* **2004**, *17*, 378–382.

Received: March 21, 2007
Published Online: June 28, 2007

