Mechanism of 1,N^2-Etheno-2′-deoxyguanosine Formation from Epoxyaldehydes†

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Background levels of etheno adducts have been attributed to the reaction of DNA with 2,3-epoxyaldehydes, a proposed product of lipid peroxidation. We have examined the reaction of (2*R*,3*S*)-epoxyhexanal with dGuo to give 7-(1*S*-hydroxybutyl)-1,N^2-etheno-dGuo. We observed that the stereochemistry of the side chain scrambled over time. This process provided insight into the mechanism for the formation of 1,N^2-etheno-dGuo from 4,5-epoxy-2-decenal [Lee, S. H., et al. (2002) *Chem. Res. Toxicol. 15*, 300–304]. The mechanistic proposal predicts that 2-octenal is a by-product of the reaction. The reaction of 4,5-epoxy-2-decenal was reinvestigated, and the 2-octenal adduct of dGuo was identified as a product of this reaction in support of the mechanistic proposal. Also observed are products that appear to be derived from 2,3-epoxyoctanal, which can be formed through Schiff base formation of 4,5-epoxy-2-decenal with the dGuo followed by hydration of the double bond and retro-aldol reaction.

Introduction

Leonard and coworkers initially investigated the reaction of chloroacetaldehyde with dAdo, Cyt, and dGuo to form the corresponding etheno (ϵ) adducts in which an ethylene unit bridges two nucleophilic sites of the base (**1–3**; Figure 1) (*1, 2*). Some of these modified bases have found utility as fluorescent probes to examine the structure, function, and dynamics of DNA. It was subsequently found that chlorooxirane (**5**), formed by the in vivo epoxidation of vinyl chloride by a cytochrome P450, is also a source of etheno adducts upon reaction with DNA (*3, 4*). Exposure to vinyl chloride, which is produced in large quantities in the plastics industry, has been correlated to a unique tumor, hepatic angiosarcoma. Etheno adducts are miscoding and have been attributed at least in part to the carcinogenicity of vinyl chloride and related vinyl monomers (*3, 5–9*). There has been significant interest in the chemistry and biology of etheno adducts, which are prototypes for so-called exocyclic DNA adducts.

Of particular interest, background levels of etheno adducts have been found in unexposed populations (*10–13*). The endogenous C_2 donors were hypothesized to be bis-electrophiles such as 2,3-epoxyaldehydes (**6**), derived from free radical degradation to polyunsaturated fatty acids initated by reactive oxygen species (*14–16*). The mechanism of formation of 2,3-epoxyaldehydes from lipid peroxidation has not been fully elucidated. The epoxidation of the corresponding α,β-unsaturated aldehyde, which is produced in abundance through lipid peroxidation (*17, 18*), is a possible source, although the cellular oxidant for this reaction has not been identified (*19, 20*). Chung postulated that lipid hydroperoxides, a key intermediate in lipid peroxidation, could act as the epoxidation agent for the conversion of α,β-unsaturated aldehydes to the corresponding 2,3-epoxyaldehyde (*21, 22*). Model studies showed that hydrogen peroxide, *t*-butylhydroperoxide, or lipid hydroperoxides react with *trans*-4-hydroxynonenal (HNE)[1] at neutral pH to give 2,3-epoxy-4-hydroxynonanal (EHN) in modest yield (*16, 23, 24*). Blair and co-workers demonstrated that 4,5-epoxy-2-decenal is a product of linoleic acid peroxidation, suggesting that epoxyaldehydes may be direct products of lipid peroxidation rather than secondary products (*25*).

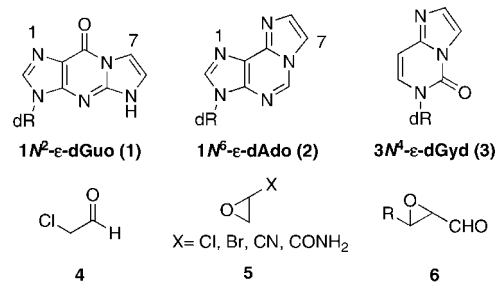

Figure 1. Etheno adducts of DNA bases and two-carbon bis-electrophiles.

The reaction of EHN with dGuo results in a variety of products as shown in Figure 2 (*16*). These include etheno adducts with a C7-hydroxyalkyl side chain (**7**) and the parent 1,N^2-etheno-2′-deoxyguanosine (1,N^2-ϵ-dGuo; **1**). Products from the reaction of dGuo with glycidaldehyde (*26, 27*), 2,3-epoxybutanal (*14*), 4,5-epoxydec-2-enal (*28*), and 2,3:4,5-diepoxydecanal (*29*) have also been characterized (*21*). The mechanism of formation of etheno adducts from 2,3-epoxyaldehydes is shown in Scheme 1. Although an alternative mechanism has been proposed (*10, 15*), we favor that outlined

† This manuscript is dedicated to Professor Lawrence J. Marnett in celebration of his 60th birthday.

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[1] Abbreviations: 1,N^2-ϵ-dGuo, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4-dihydro-9*H*-imidazo[1,2-*a*]purin-9-one or 1,N^2-etheno-2′-deoxyguanosine; ϵ-dAdo, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[2,1-*i*]purine or 1,N^6-etheno-2′-deoxyadenosine; ϵ-dCyd, 6-(2-deoxy-β-D-*erythro*-pentofuranosyl)imidazo[1,2-*c*]pyrimidin-5(6*H*)one or 3,N^4-etheno-2′-deoxycytidine; HNE, 4-hydroxy-2-nonenal; EHN, 2,3-epoxy-4-hydroxynonanal; ONE, 4-oxo-2-nonenal; EDE, 4,5-epoxy-2-decenal; HPNE, 4-hydroperoxy-2-nonenal; DET, diethyl tartrate; TEMPO, 2,2,4,4-tetramethylpiperidine-*N*-oxide; UPLC, ultraperformance liquid chromatography; SRM, selected reaction monitoring.

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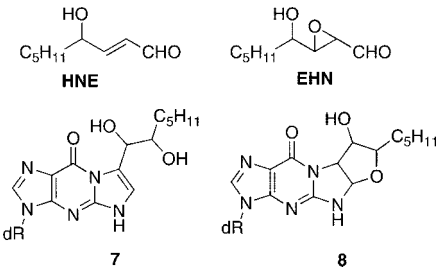
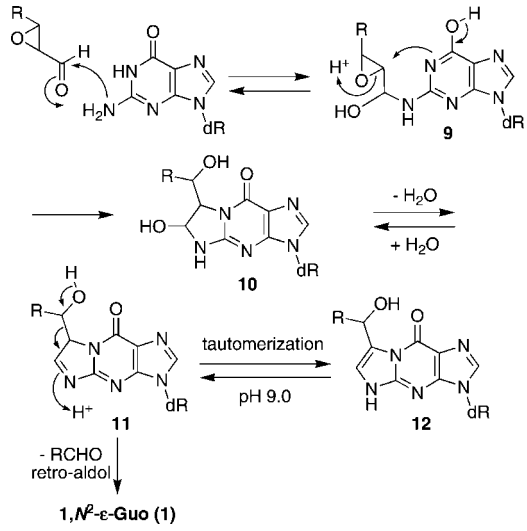


Figure 2. Products from the reaction of 2,3-epoxy-4-hydroxynonanal (EHN) with dGuo.

Scheme 1



by Golding, who studied the reaction of dGuo with glycidaldehyde (*26, 27*).

Condensation of the exocyclic amino group of dGuo with the aldehyde initially gives carbinol amine **9**. Epoxide ring opening by N1 gives the cyclized intermediate **10**, which can undergo reversible dehydration to imine **11**. Tautomerization of the imine provides the C7-(1-hydroxyalkyl)-1,N^2-ϵ-dGuo (**12**). Alternatively, imine **11** can lose the C7 side chain as the corresponding aldehyde via a retro-aldol reaction, resulting in the formation of the unsubstituted parent 1,N^2-ϵ-dGuo (**1**). Exposure of the C7-hydroxyalkyl-substituted etheno adduct (**12**) to alkaline conditions results in quantitative loss of the C7 side chain, presumably through the imine intermediate **11** (*16*). The bicyclic adduct **8**, which is uniquely derived from the reaction of dGuo with EHN, arises by trapping of the cyclic imine (**11**) by the side chain hydroxyl group. A similar mechanism can be envisioned for the formation of the 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3*H*-imidazo[2,1-*i*]purine (ϵ-dAdo) adducts from the reaction with 2,3-epoxyaldehydes, but to our knowledge, bicyclic dAdo adducts analogous to **8** have not been observed.

EHN was shown to be more tumorgenic than HNE itself (*30*). The 1,N^6-ϵ-dAdo and 1,N^2-ϵ-dGuo adducts with intact C7 side chains have been observed from calf thymus DNA and the DNA isolated from intact cells that were treated with EHN (*24, 31*). Etheno adducts with intact side chains are likely to be present in cellular DNA and may have differential biology than parent etheno adducts. In addition, the side chain stereochemistry of these adducts may play a significant role in the structure and mutagenicity of the modified nucleobase. We report here the diastereospecific synthesis of 7-(1*S*-hydroxybutyl)-1,N^2-ϵ-dGuo from the reaction of dGuo with (2*R*,3*S*)-epoxyhexanal and examination of its side chain reactivity. We find that the side chain stereochemistry scrambles over time. The scrambling reaction provides insight in the mechanism of 1,N^2-ϵ-dGuo formation from the lipid peroxidation product 4,5-epoxy-2-decenal (EDE) (*28*).

Experimental Procedures

All solvents were distilled before use according to standard procedures. Moisture and air sensitive reactions were conducted under a nitrogen atmosphere in oven-dried glassware. Thin-layer chromatography was performed on silica gel glass plates (Merck, Silica Gel 60 F_{254}; layer thickness, 250 μm) and visualized under UV light or by staining with anisaldehyde followed by charring. ^{1}H NMR spectra were recorded at 600, 400, or 300 MHz, and ^{13}C NMR spectra were recorded at 150.9 MHz in $CDCl_3$ or in DMSO-d_6.

(2*S*,3*S*)-Epoxy-1-hexanol (14). In a flame-dried flask under an argon atmosphere were added activated, 4 Å molecular sieves (0.65 g) and anhydrous dichloromethane (75 mL). In a separate flask, (+)-diethyl tartrate [(+)-DET, 1.23 g, 6 mmol] was stirred in anhydrous dichloromethane over activated 4 Å molecular sieves (~0.2 g) for 15 min and then transferred to the reaction flask via syringe. In a separate flask, Ti(O^iPr)$_4$ (1.42 g, 5 mmol) and cumene hydroperoxide (9.55 g, 62.5 mmol) were stirred over activated 4 Å molecular sieves (~0.2 g) in dichloromethane (10 mL) for 15 min and then transferred to the reaction flask via syringe. The reaction mixture was stirred for 40 min at −40 °C. A solution of *trans*-2-hexen-1-ol (2.50 g, 25 mmol) in dichloromethane (10 mL) was stirred over activated 4 Å molecular sieves (~0.2 g) for 15 min and then added to the reaction flask dropwise via syringe. The reaction mixture was stirred at −25 °C for 4 h and then quenched by the addition of ferrous sulfate (15.0 g) in 10% aqueous tartaric acid (50 mL) to the cooled reaction mixture. The mixture was stirred for 1 h after which time the organic phase was separated, washed with water (2 × 100 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was diluted with ether (200 mL) and stirred with 30% NaOH in saturated brine (25 mL) at 0 °C for 30 min. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered, and evaporated. Purification by flash chromatography on silica, eluting with 20% ethyl acetate in hexanes, afforded **14** (1.66 g, 57%). $[\alpha]_D^{24.7} = -43.7$ (*c* 1.0, $CHCl_3$), [lit. $[\alpha]_D = -46.6°$ (*c* 1.0, $CHCl_3$), 94% ee] (*32*). ^{1}H NMR ($CDCl_3$): δ 3.88–3.92 (m, 1H, C1-H′), 3.62–3.64 (m, 1H, C1-H″), 2.90–2.96 (m, 2H, C2-H, C3-H), 2.13 (br s, 1H, –OH), 1.44–1.56 (m, 4H, 2 CH_2), 0.96 (t, J = 7.2 Hz, 3H, CH_3).

(2*R*,3*S*)-Epoxyhexanal (15). To a solution of **14** (0.58 g, 5 mmol) in dichloromethane (5 mL) was added 2,2,6,6-tetramethylpiperidine-*N*-oxide (TEMPO, 0.078 g, 0.5 mmol) followed by bisacetoxyiodobenzene (1.77 g, 5.5 mmol). The reaction mixture was stirred at room temperature for 3 h and then diluted with dichloromethane (25 mL). The mixture was washed with a saturated aqueous solution of $Na_2S_2O_3$ (15 mL), and the aqueous layer was extracted with dichloromethane (3 × 25 mL). The phases were separated, and the aqueous layer was extracted with dichloromethane (3 × 5 mL). The combined organic phases were successively washed with a saturated $NaHCO_3$ solution (25 mL) and saturated brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography using 10% ethyl acetate in pentane as the eluant afforded **15** (0.346 g, 60%). $[\alpha]_D^{24.8}$ +99.42° (*c* 0.35, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 9.02 (d, J = 6.3 Hz, 1H, C1-H′), 3.16 (m, 1H, C3-H), 3.06 (dd, J_1 = 6.3 Hz, J_2 = 1.8 Hz, 1H, C2-H), 1.56 (m, 4H, 2 × C4-H, 2 × C5-H), 0.91 (t, J = 7.2 Hz, 3H, 3 × C6-H).

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-7-(1*S*-hydroxybutyl)-3,4-dihydro-9*H*-imidazo[1,2-*a*]purin-9-one (16). To a suspension of dGuo·H_2O (50 mg, 0.18 mmol) in DMF (1 mL) was added K_2CO_3 (57 mg, 0.41 mmol), and the mixture was stirred for 15 min. A solution of (2*R*,3*S*)-epoxyhexanal (61 mg, 0.54 mmol) in DMF (1 mL) was added to the suspension, and the reaction was stirred for 12 h. The reaction was monitored by HPLC (gradient I). The reaction mixture was neutralized to pH 7 with 5% acetic acid and purified by HPLC (gradient II). The fractions collected

from HPLC were immediately cooled to −78 °C and lyophilized to afford (*S*)-**16** (36 mg, 55%). Scrambling of the side chain stereochemistry was observed if the HPLC fractions were left for longer periods at room temperature. ^{1}H NMR (DMSO-d_6): δ 8.13 (s, 1H, H-2), 7.21 (s, 1H, H-6), 6.22 (dd, $J_1 = 7.68$, $J_2 = 6.28$ Hz, 1H, H-1′), 5.28 (d, $J = 4.12$ Hz, 1H, 3′-OH), 5.21 (d, $J = 6.04$ Hz, 1H, C9-OH), 5.18 (m, 1H, C10-H), 4.94 (t, $J = 5.6$ Hz, 1H, 5′-OH), 4.36 (m, 1H, H-3′), 3.83 (m, 1H, H-4′), 3.55 (m, 2H, H-5′, H-5″), 2.57 (m, 1H, H-2″), 2.24 (m, 1H, H-2′), 1.8 (m, 1H, C11-H′), 1.65 (m, 1H, C11-H″), 1.5–1.33 (m, 2H, C12-H′, H″), 0.89 (t, $J = 8$ Hz, 3H, C13-H′, H″,H‴). ^{13}C NMR (DMSO-d_6): δ 153.8, 149.5, 146.9, 137.8, 128.3, 116.4, 113.3, 87.7, 83.1, 70.8, 64.6, 61.8, 39.4, 38.5, 18.7, 13.7. LC-ESI-MS *m*/*z* calcd for $C_{16}H_{21}N_5O_5$ [M + H], 364.15; found, 364.10.

7,7′-Butylidene-bis[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4-dihydro-9*H*-imidazo[1,2-*a*]purin-9-one (18a). (*S*)-**16** (3.6 mg, 0.01 mmol) was stirred in pH 9 buffer (boric acid–KCl–NaOH, 0.1 M, 1 mL) for 72 h. The reaction mixture was neutralized to pH 7 with 5% acetic acid and purified by HPLC (gradient II) to afford **18a** (1.9 mg, 59 %). ^{1}H NMR (DMSO-d_6): δ 8.02 (d, 2H, 2 × H-2), 6.89 (d, 2H, 2 × H-6), 6.28 (t, 1H, C10-H, $J = 6.8$ Hz), 6.19 (t, 2H, 2 × H-1′, $J = 6.8$ Hz), 5.27 (d, 2H, 2 × 3′-OH, $J = 4.0$ Hz), 4.97 (d, 1H, 2 × 5′-OH, $J = 5.6$ Hz), 4.35 (m, 2H, 2 × H-3′), 3.82 (m, 2H, 2 × H-4′), 3.55 (m, 4H, 2 × H-5′, 2 × H-5″), 2.57 (m, 2H, 2 × H-2″), 2.22 (m, 2H, 2 × H-2′), 1.98 (q, 2H, C11-H′, H″, $J = 7.2$ Hz), 1.5 (m, 2H, C12-H′, H′), 0.93 (t, 3H, C13-H′, H″,H‴, $J = 7.2$ Hz). LC-ESI-MS *m*/*z* calcd for $C_{28}H_{32}N_{10}O_8$ [M + H], 637.25; found, 637.21.

General Procedure for Epimerization. (*S*)-**16** (1 mg, 0.0028 mM) was stirred in pH 5.5, 7.4, and 9.0 buffer (potassium biphthalate–NaOH, 0.05 M, 1 mL) for up to 7 days at room temperature. The scrambling reaction was monitored by HPLC. In addition to the epimerization of (*S*)-**16**, etheno adduct **1** and dimer **18a** were observed.

EDE. EDE was prepared in two steps starting from 2-octenal as previously described (*28, 33*). The final product and intermediate were purified by flash chromatography. The EDE was analyzed by GC-MS and judged free of any starting 2-octenal.[2]

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4,6,7,8-tetrahydro-8-hydroxy-6-pentyl-pyrimido[1,2-a]purin-10(3H)-one (26). A solution of *trans*-2-octenal (2.52 mg, 20 μmol) in 100 μL of degassed acetonitrile was added to a suspension of dGuo·H_2O (2.85 mg, 10 μmol) and L-arginine (3.48 mg, 20 μmol) in bicine buffer (100 mM, pH 8.0, 450 μL). The reaction mixture was heated at 60 °C for 60 h. Additional *trans*-2-octenal (1.89 mg, 15 μmol) in acetonitrile (100 μL) and L-arginine (2.61 mg, 15 μmol) in bicine buffer (100 μL) was added to the reaction mixture every 15 h (three additions). The total amount of 2-octenal and L-arginine added was 65 μmol. The reaction was monitored by HPLC gradient II. The reaction mixture was extracted with hexanes and acidified to pH 5.0 with 1 N HCl, and the resulting solution was purified by HPLC using gradient system I (flow rate, 5.0 mL/min) to give **26** (1.85 mg, 47% yield) as a white solid. The purity of the product was judged to be >99% by HPLC (gradient II). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.88 (s, 1H, H2), 7.49 (s, 1H, N^2-H), 6.58 (br s, 1H, C8-OH), 6.20 (s, 1H, H8), 6.1 (t, $J = 6.6$ Hz,1H, H-1′), 5.23 (br s, 1H, C3′-OH), 4.89 (br s, 1H, C5′-OH), 4.32 (s, 1H, H3′), 3.78 (s, 1H, H4′), 3.57 (m, 1H, H6), 3.52 (m, 2H, H5′, H5″), 2.5 (m, 1H, H2′), 2.16 (m, 1H, H2″), 2.05 (m, 1H, H7), 1.36 (m, 1H, H7), 1.31 (m, 8H, 4CH_2), 0.88 (t, $J = 6.6$ Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 156.0, 151.3, 150.3, 135.7, 115.9, 87.9, 82.6, 71.1, 69.7, 62.1, 44.8, 34.5, 32.9, 31.7, 24.4, 22.5, 14.3. UV λ_{max} (ϵ) 260 nm (14020). Positive ESI-MS *m*/*z* 394 $[M + H]^+$. MS/MS of *m*/*z* 394 (collision energy, 30 eV): *m*/*z* (relative intensity) 278 $[BH]^+$ (52), 260 $[BH - H_2O]^+$ (30), 234 $[BH - CH_3CHO]^+$ (100), 190 $[BH - H_2O - C_5H_{11}]^+$ (20), 152 $[Gua + H]^+$ (20).

The same procedure was also used starting from [$^{15}N_5$]dGuo to prepare [$^{15}N_5$]-**26** (32% yield). [$^{15}N_5$]-**26** was quantified by its UV absorbance at 260 nm.

3-(2-Deoxy-β-D-*erythro*)pentofuranosyl)-7-(1-hydroxyhexyl)-3,4-dihydro-9*H*-imidazo[1,2-a]purin-9-one (30). To a suspension of dGuo·H_2O (8.0 mg, 28 μmol) in DMF (0.5 mL) was added K_2CO_3 (9.0 mg, 65 μmol), and the mixture was stirred for 15 min. A solution of 2,3-epoxyoctanal (12.0 mg, 84.5 μmol, in 1.0 mL of DMF) was added to the suspension, and the reaction was stirred for 14 h. The reaction was monitored by HPLC for the formation of the desired product using gradient system II. The reaction mixture was diluted with water (3.0 mL) and neutralized to pH 7.0 with 1% HCl, and the resulting solution was purified by HPLC using gradient system I (flow rate, 5.0 mL/min) to give **30** (5.6 mg, 51% yield) as a white solid. The purity of the product was judged to be >99% by HPLC (gradient II). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.10 (s, 1H, H-2), 7.26 (s, 1H, H-6), 6.22 (t, 1H, $J = 6.8$ Hz, H-1′), 5.28 (d, 1H, $J = 3.6$ Hz, 3′-OH), 5.24 (d, 1H, $J = 6$ Hz, C10-OH), 5.15 (m, 1H, H-9), 4.97 (t, 1H, 5′-OH), 4.36 (m, 1H, H-3′), 3.83 (q, 1H, $J = 2.8$ Hz, H-4′), 3.52 (m, 2H, H-5′, H-5″), 2.59 (m, 1H, H-2′), 2.24 (m, 1H, H-2″), 1.81 (m, 1H, H′-11), 1.66 (m, 1H, H″-11), 1.46 (m, 1H, H′-12), 1.27 (m, 5H, 2CH_2, H″-12), 0.85 (t, 3H, CH_3). ^{13}C NMR: δ 154.3, 150.2, 147.4, 137.7, 128.8, 116.4, 113.8, 88.0, 83.4, 71.4, 66.5, 65.3, 61.5, 39.8, 36.8, 31.4, 25.5, 22.4, 14.4. Positive ESI-MS *m*/*z* 392 $[M + H]^+$. MS/MS of *m*/*z* 392 (collision energy, 33 eV): *m*/*z* (relative intensity) 276 $[BH]^+$ (28), 258 $[BH - H_2O]^+$ (100), 188 (4), 176 $[BH - CHOH - C_5H_{11}]^+$ (20). Following the procedure described above, [$^{15}N_5$]-**30** was obtained from [$^{15}N_5$]dGuo in 29% yield.

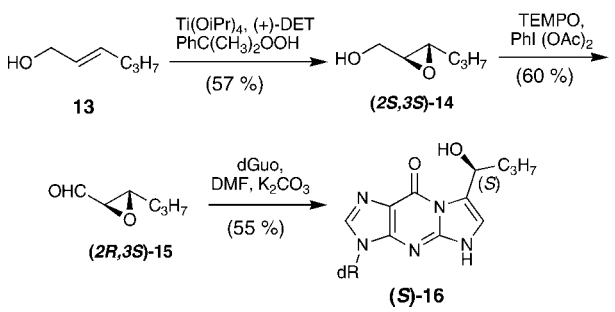
Reaction of EDE with dGuo. The solution of *trans*-EDE (1680 μg, 10 μmol) in ethanol (25 μL) was added to dGuo·H_2O (1335 μg, 4.68 μmol) dissolved in MOPS buffer (100 mM, pH 7.5, 611 μL) containing 150 mM NaCl. The reaction mixture was sonicated for 15 min at room temperature and then incubated at 37 °C with continuous shaking for 60 h. The pH of the reaction was adjusted to 8.0 with 1 N NaOH, and then, L-arginine (1740 μg, 10 μmol) and EDE (840 μg, 5 μmol) were added. A second portion of L-arginine (870 μg, 5 μmol) and EDE (840 μg, 5 μmol) was added after 24 h, and the reaction mixture was incubated at 37 °C for 24 h (total time for the incubation with arginine was 48 h). At the end of the incubation, the sample was filtered through a 0.45 μm Millipore cartridge, lyophilized, and then subjected to UPLC-ESI-MS analysis.

HPLC. The purification of nucleosides and the analysis of reaction mixtures were performed on a Beckman HPLC gradient system with a diode array UV detector monitoring at 260 nm using YMC ODS-AQ columns (250 mm × 4.6 mm i.d.) at a flow rate of 1.5 mL/min for analysis and (250 mm × 10 mm i.d.) at a flow rate of 5 mL/min for purification with H_2O and CH_3CN. HPLC gradient I was as follows: initially 90% H_2O, a 25 min linear gradient to 20% H_2O, followed by a 5 min linear gradient to the initial conditions. HPLC gradient II was as follows: initially 99% H_2O, a 15 min linear gradient to 90% H_2O, a 5 min linear gradient to 80% H_2O, isocratic at 80% H_2O for 5 min, 10 min linear gradient to 20% H_2O, isocratic at 20% H_2O for 5 min, followed by a 5 min linear gradient to the initial conditions.

UPLC-ESI-MS Analysis. UPLC-ESI-MS analysis was performed with a Waters (Milford, MA) Acquity Ultra Performance LC system equipped with a 100 mm × 1.0 mm, 1.7 μm particle size C_{18} column (ACQUITY UPLC BEH C_{18}) and coupled to Finigan LTQ (Thermo Electron, San Jose, CA) linear ion trap mass spectrometer. The mass spectrometer was equipped with an Ion Max electrospray ionization source operated in positive ionization mode. The Xalibur software package (version 2.0, Thermo Electron) was used for the system operation and data manipulation. The UPLC flow rate was set to 0.15 mL/min at 50 °C using the following solvents: (A) 1:99 CH_3CN in 10 mM ammonium acetate buffer and (B) 90:10 CH_3CN in 10 mM ammonium acetate buffer. Elution

[2] The purity of EDE was examined by GC-SIM-MS. 2-Octenal could not be detected in the sample (<0.05%). A trace amount of 2,3-epoxyoctanal was observed (<0.16%). The reaction of 2,3-epoxyoctenal with dGuo was examined under the same reaction conditions as the reaction with EDE. We found that the yield of 7-(1-hydroxyhexyl)-1,N^2-ϵ-dGuo (**30**) was <10%. When this reactivity is taken into consideration, the observed amount of **30** from the reaction of EDE and dGuo is at least twice that from the 2,3-epoxyoctanal initially present.

Scheme 2



programs were as follows. UPLC gradient III: starting at 10% B and isocratic for the first 2 min, followed by a 5 min linear gradient to 100% B, isocratic at 100% B for 0.5 min, then a 0.5 min linear gradient back to starting conditions (10% B), followed by a 3 min isocratic period to allow the column to re-equilibrate to the starting conditions (11 min). UPLC gradient IV: starting at 1% B, then a 2 min linear gradient to 10% B, a 3.5 min linear gradient to 50% B, a 2.5 min linear gradient to 100% B, then isocratic at 100% B for 0.5 min, then a 0.5 min linear gradient back to the starting conditions (1% B), followed by a 3 min isocratic period to allow the column to re-equilibrate to the starting conditions (12 min). Individual MS instrument parameters were optimized by infusing the adduct **26** with the syringe pump into the MS source through a mixing tee at a flow rate of 0.025 mL/min. The LC solvent (1:1 A/B) flowed at 0.15 mL/min.

LTQ MS Parameters. Samlpes (10 μL) were injected through an 10 μL injection loop into a six-port switching valve injector that diverted the column eluent to waste for the first 2.0 min of UPLC gradient III. The ESI source was set up in the positive ion mode as follows: capillary temperature, 400 °C; source spray voltage, 3.7 kV; source current, 6.5 μA; sheath, 60 units; and auxiliary gas, 10 units. The adducts were analyzed by MS/MS using selection reaction monitoring (SRM). The ion transition was *m/z* 394 → *m/z* 278 (adduct **26**) and *m/z* 399 → *m/z* 283 ([$^{15}N_5$]-**26**) with a collision energy of 21 eV. Other MS parameters were optimized to achieve maximum signal intensity.

Quantitation of 26 and 1. Aliquots of the reaction of dGuo with EDE were diluted by four-fold (v/v). A portion of this sample (10 μL) was mixed with [$^{15}N_5$]-**26** (0.2 ng, 10 μL). A portion of this mixture (10 μL) was then subjected to UPLC-ESI-MS/MS-SRM analysis using UPLC gradient III by monitoring ion transitions of *m/z* 394 → 278 for **26** and *m/z* 399 → 283 for [$^{15}N_5$]-**26**.

For a determination of the quantity of 1,N^2-ϵ-dG adduct (**1**), aliquots of the reaction mixture were diluted by 20-fold, and then, a portion of this sample (10 μL) was mixed with [$^{15}N_5$]-**26** (0.2 ng, 10 μL). A portion of this mixture (10 μL) was analyzed by UPLC-ESI-MS/MS-SRM using UPLC gradient IV by monitoring ion transitions of *m/z* 292 → 176 for **1** and *m/z* 399 → 283 for [$^{15}N_5$]-**26**.

Results and Discussion

Reaction of dGuo with 2,3-Epoxyhexanal and the Side Chain Chemistry of 7-(1-Hydroxybutyl)-1,*N*^{2}-ϵ-dGuo. The reaction of dGuo with epoxyaldehydes to give 1,*N*^{2}-ϵ-dGuo and their derivatives has been reported in the literature under a variety of conditions; these conditions generally employ aqueous buffers (pH 7.1–11.0), and in some cases, an organic cosolvent (tetrahydrofuran or acetonitrile) was used to solubilize the epoxyaldehyde (*14, 16, 22, 26, 28, 29, 31, 34–38*). Mixtures of products were often observed that result from various stereoisomers as well as loss of the C7 side chain to give the parent 1,*N*^{2}-ϵ-dGuo adduct. We prepared the 7-(1*S*-hydroxybutyl)-1,*N*^{2}-ϵ-dGuo (**16**) in a stereospecific manner by the reaction of dGuo and enantiomerically enriched (2*R*,3*S*)-epoxyhexanal (**15**), which was prepared via Sharpless asymmetric epoxidation of *trans*-hex-2-en-1-ol (**13**) followed by oxidation (Scheme 2) (*32, 39*). The (2*S*,3*S*)-**14** was judged to be approximately 90% ee based on optical rotation (*32*). Reaction of the (2*R*,3*S*)-**15** with dGuo in the presence of K_2CO_3 and DMF as the solvent gave the desired product (*S*)-**16** in 55% yield with few side products.

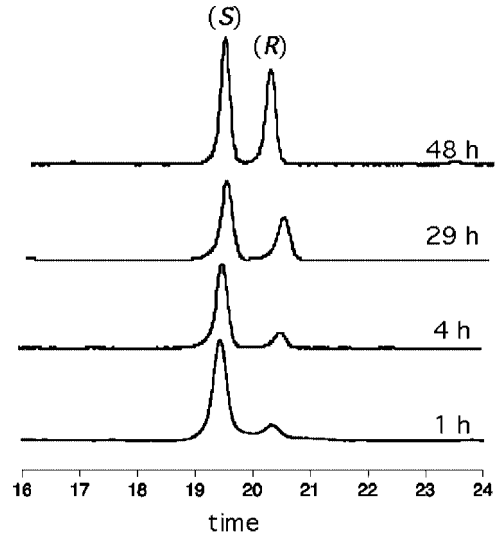
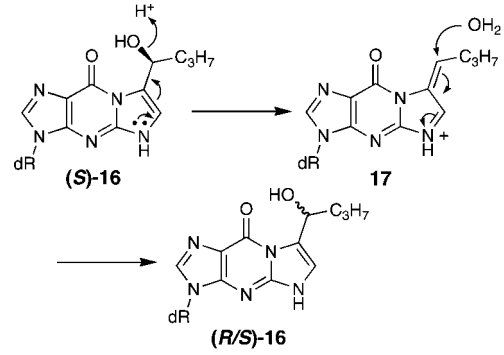
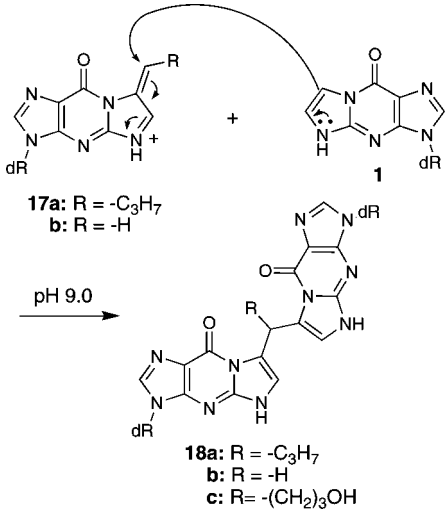


Figure 3. Scrambling of the (*S*)-**16** stereochemistry at pH 5.5 as monitored by RP-HPLC.

Scheme 3

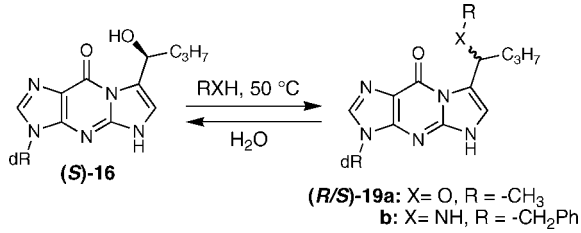


Scheme 4

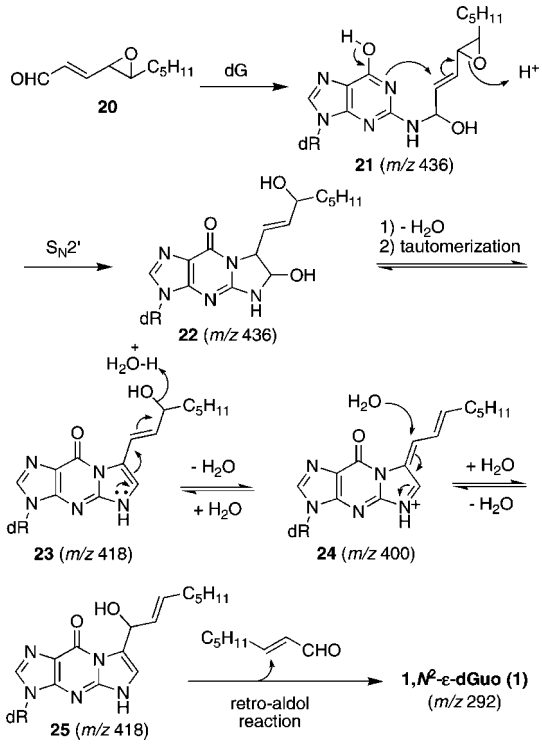


We found that (*S*)-**16** was relatively stable at neutral pH. Under acidic conditions (pH 5.5), the side chain stereochemistry scrambled over the course of ~48 h at room temperature to give a 1:1 mixture of stereoisomers as observed by HPLC analysis (Figure 3). The diastereomer with the *R* side chain

Scheme 5



Scheme 6

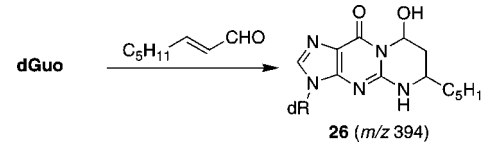


stereochemistry was synthesized as an authentic standard according to Scheme 2, except that the (–)-DET ligand was used for the Sharpless asymmetric epoxidation to give the (2*R*,3*R*)-epoxyalcohol. The loss of stereochemical integrity can be explained by the mechanism shown in Scheme 3.

As higher pH, the scrambling of the side chain stereochemistry competes with loss of the side chain to give 1,N^2-ϵ-dGuo (**1**). At pH 9.0, a third product was observed, which was identified as the dimer **18a** on the basis of mass spectrometry and two-dimensional NMR data. This product arises from the reaction of **16** with 1,N^2-ϵ-dG (**1**) as proposed in Scheme 4. Golding observed an analogous product (**18b**) from the reaction of dGuo and glycidaldehyde at pH 11 presumably from the reaction of **17b** with **1** (*26, 40*). A related dimer (**18c**) was characterized from the reaction of dGuo with α,β-unsaturated aldehydes and 2-hydroperoxytetrahydrofuran and probably arises from the reaction of 1,N^2-ϵ-dGuo and 4-hydroxybutanal, a product from the ring opening of 2-hydroxytetrahydrofuran (*40*). Consistent with the mechanism shown in Scheme 4 is the observation that incubation of **16** at lower concentrations gave less of the dimer **18a** and more **1**. The potential nucleophilicity of the C7-position of 1,N^2-ϵ-dGuo has been reported (*41, 42*).

We observed that intermediate **17a** could be trapped by other nucleophiles. If (*S*)-**16** was stirred in methanol at 50 °C, two new products were observed by HPLC. These products had identical masses as observed by LC-ESI-MS and are consistent with the structure possessing a side chain methoxy group (**19a**,

Scheme 7



Scheme 5). However, all attempts to isolate and further characterize this product failed. Attempted purification by HPLC gave (*R*/*S*)-**16** instead. The side chain methoxy group apparently solvolyzes back to **17a** during purification, which is subsequently trapped by water in the HPLC mobile phase. We could also trap **17a** with benzylamine, but once again, the product could not be isolated and was only characterized by LC-ESI-MS. These observations suggest that 7-hydroxyalkyl-1,N^2-ϵ-dGuo adducts have the potential to form cross-links with other nucleophilic groups in DNA or with proteins. However, our inability to isolate products **19** indicates that any such cross-links would be readily reversible.

Reaction of dGuo with EDE. Blair and co-workers identified 1,N^2-ϵ-dGuo (**1**) as a product of the reaction between EDE (**20**) and dGuo (*28*). A proposed mechanism for this reaction is outlined in Scheme 6 and is an extension of our observation that the side chain stereochemistry of **16** scrambles. The formation of the initial etheno adduct with an intact side chain would follow a slightly different course. In this case, epoxide opening by N1 would proceed by an S_N2' mechanism to give 7-(3-hydroxy-1-octenyl)-1,N^2-ϵ-dGuo adduct **23** after dehydration and tautomerization. Solvolysis of the side chain hydroxyl group of **23** is facilitated through the extended π-systems. Rehydration of the solvolysis product (**24**) could now give 7-(1-hydroxy-2-octenyl)-1,N^2-ϵ-dGuo **25**, which would subsequently lose its side chain by a retro-aldol reaction to give 1,N^2-ϵ-dGuo (**1**) in an analogous manner to Scheme 1.

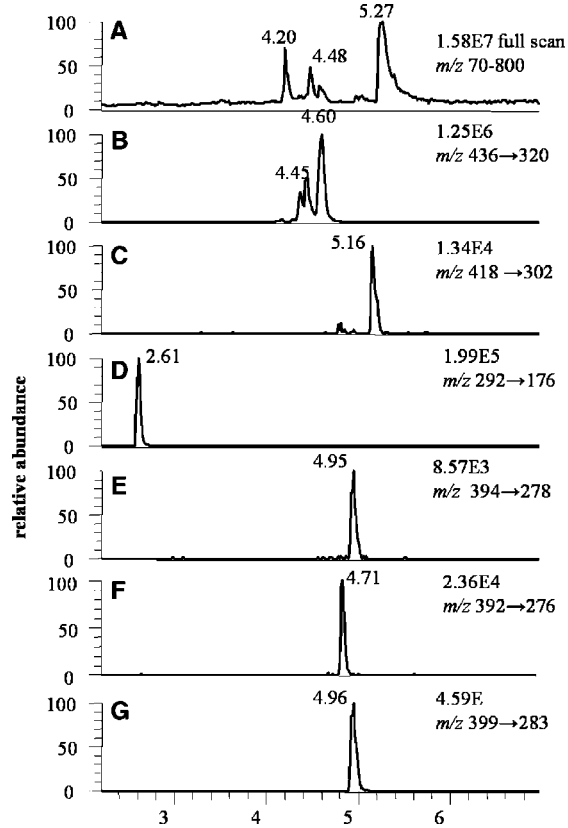


Figure 4. UPLC-ESI-MS-SRM (–116 Da) profile for the reaction of dGuo with EDE. The peaks at retention times 4.20 and 5.27 are unidentified, low molecular weight, trace contaminants.

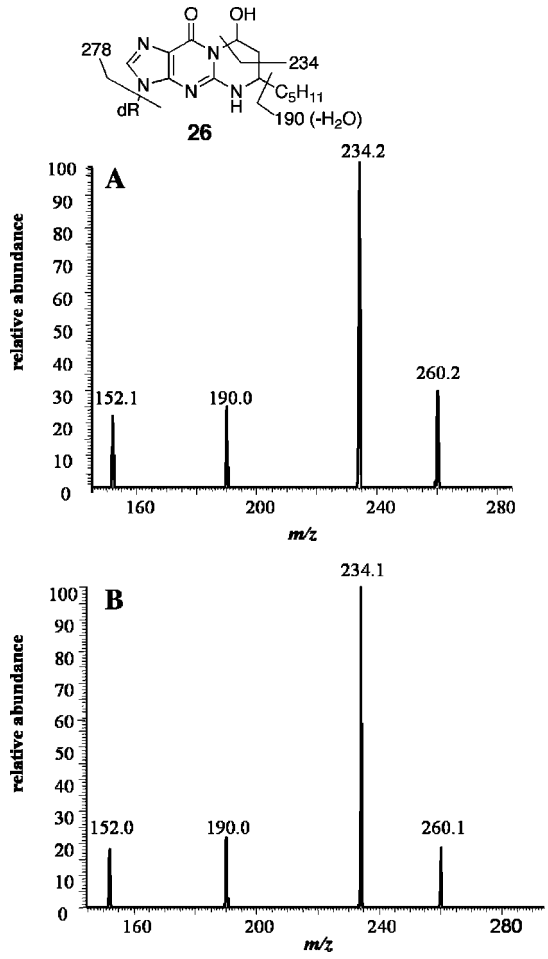


Figure 5. MS^3 spectrum and fragmentation of **26**. (A) From the reaction of dGuo with EDE and (B) an authentic standard.

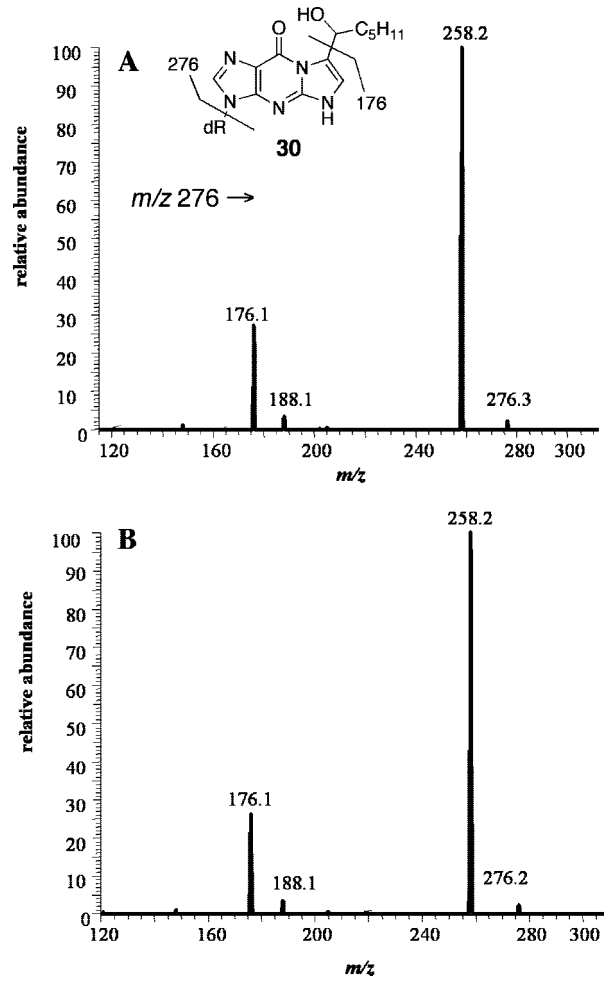


Figure 6. MS^3 spectrum of 7-(1-hydroxyhexyl)-1,N^2-ϵ-dGuo (**30**). (A) From the reaction of dGuo with EDE and (B) an authentic standard.

According to our mechanistic proposal, a by-product of 1,N^2-ϵ-dGuo formation is 2-octenal, which is also capable of forming stable adducts with dGuo (Scheme 7). We re-examined the reaction of dGuo with excess EDE (**20**) with the plan of using UPLC-ESI-MS to observe the 2-octenal adduct of dGuo (**26**), thereby providing support for the mechanism in Scheme 6. An authentic standard of the 2-octenal adduct of dGuo was prepared according to the method of Sako, who showed that the reaction of dGuo with crotonaldehyde could be accelerated by the addition of arginine (*43*).

The reaction of dGuo with EDE proceeded in low overall conversion and gave a complex mixture of products; as such, UPLC-ESI-MS with selected reaction monitoring is an ideal method for the analysis of the reaction mixture. Arginine was added to the reaction to accelerate the formation of the octenal adduct (*43*). Modified dGuo nucleosides were identified by the neutral loss of the deoxyribose group (–116 Da) from the parent ion. The UPLC-ESI-MS analysis of the reaction between dGuo and EDE is shown in Figure 4. A large cross-section of products possessed [M + H] ions at *m/z* 436 and 418 Da (Figure 4B,C), suggesting their identities to be intermediates **21**–**23** and **25** (Scheme 6); each of these intermediates is predicted to exist as multiple stereoisomers. A component of this mixture possessed a mass of *m/z* 394 [M + H] (Figure 4E), which corresponds to the 2-octenal adduct of dGuo (**26**). After loss of deoxyribose (*m/z* 394 → 278), the major product ion (*m/z* 278 → 234) corresponds to the loss of –CH(OH)CH_2– (Figure 5). In addition, dehydration of the octenal adduct (*m/z* 278 → 260) and loss of the pentyl side chain (*m/z* 260 → 190) was also observed. The mass spectrum was identical to that of the authentic standard (Figure 5B).

To simplify the analysis, we used an isotopically labeled standard of the octenal adduct **26**, prepared from labeled [$^{15}N_5$]dGuo (Figure 4G), to quantitate the formation of both **26** and **1**. In principle, **26** and **1** should be formed in equimolar amounts. However, the formation of enal adducts with dGuo is usually slow, and we, therefore, expect the relative amount of **1** to be higher than **26**. Calibration curves were determined by plotting the concentration of **1** (12.5–375 pg)/[$^{15}N_5$]-**26** (100 pg) or **26** (0.5–507 pg)/[$^{15}N_5$]-**26** (100 pg) vs the integrated ratio of **1** (*m/z* 292 → 176)/[$^{15}N_5$]-**26** (*m/z* 399 → 283) or **26** (*m/z* 394 → 278) [$^{15}N_5$]-**26** (*m/z* 399 → 283) (see Figures S14–S16 of the Supporting Information). We find that 1,N^2-ϵ-dGuo (**1**) was produced in at least eight-fold excess to **26**.

A product of *m/z* 392 was also observed in the reaction, which showed a neutral loss of 116 Da to *m/z* 276, indicating that it was a dGuo nucleoside adduct (Figure 4F). We speculated that this product is 7-(1-hydroxyhexyl)-1,N^2-ϵ-dGuo (**30**), which would arise from the reaction of dGuo with 2,3-epoxyoctanal. The 7-(1-hydroxyhexyl)-1,N^6-ϵ-dAdo adducts have been observed from the reaction of dAdo with 2,4-decadienal in the presence of hydrogen peroxide (*36, 44*). It was proposed that 2,3-epoxyoctanal arose from the hydration of the 2,3-double bond followed by retro-aldol reaction to 2-octenal and acetaldehyde; 2-octenal was then epoxidized by hydrogen peroxide. Similarly, EDE can undergo hydration and retro-aldol reaction

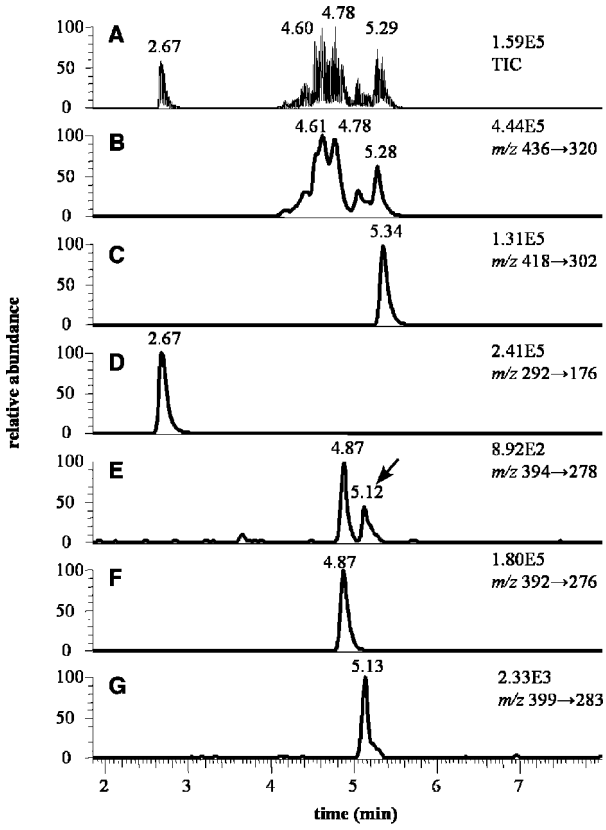
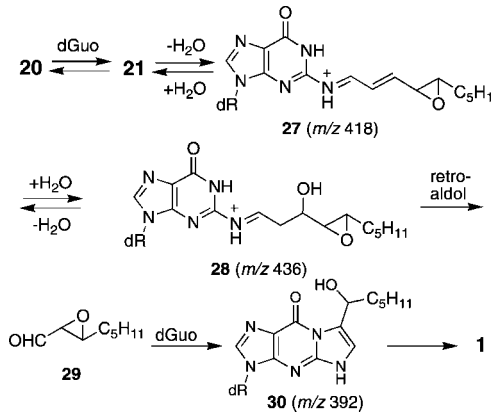


Figure 7. UPLC-ESI-MS-SRM profile for the reaction of EDE with dGuo without the addition of arginine.

to give acetaldehyde and 2,3-epoxyoctanal. Mass spectrometric analysis of **30** is shown in Figure 6. After neutral loss of deoxyribose (*m/z* 392 → 276), product ions were observed that corresponded to dehydration (*m/z* 276 → 258) and loss of the hydroxyhexyl side chain (*m/z* 276 → 176). The mass spectrum was identical to that of an authentic standard prepared by reaction dGuo with 2,3-epoxyoctanal. Because **30** can lose its side chain as hexanal, this represents an alternative mechanism for the formation of 1,N^2-ϵ-dGuo (**1**) from EDE. This alternative pathway is not predicted to produced 2-octenal as a by-product and, therefore, contributes to the observation that **1** was produced in higher concentration than **26**.

In the original study by Blair and co-workers, no 2,3-epoxyoctanal was detected after incubation of EDE at 37 °C for up to 72 h in 100 mM, pH 7.4, MOPS buffer (*28*).[2] This suggests that treatment of the reaction with arginine to promote the reaction of 2-octenal with dGuo also facilitated the hydration and retro-aldol reaction of EDE. The later reaction presumably involves initial Schiff base formation between arginine and EDE. Interestingly, the product of *m/z* 392 was still observed when arginine was omitted from the reaction (Figure 7F). We propose that Schiff base formation between the EDE and the N^2-amino group of dGuo also facilitates the hydration and retro-aldol reactions yielding 2,3-epoxyoctanal (Scheme 8). Schiff base formation has been shown to accelerate a variety of organic reactions including aldol reactions and Michael additions (*43, 45, 46*). The Schiff base intermediate (**27**) and its hydrate (**28**) possess an *m/z* of 418 and 436, respectively; these masses were observed in the analysis of the reaction and can also be attributed to intermediates **21**–**23** and **25** (Scheme 6). The mechanism in Scheme 8 would yield the N^2-dGuo adduct of acetaldehyde, which can readily hydrolyze (*47, 48*). Unfortunately, attempts to trap the N^2-acetaldehyde adduct by reduction

Scheme 8



with $NaBH_4$ or $NaB(CN)H_3$ were unsuccessful. The 1,N^2-octenal adduct of dGuo (**26**) was also observed in the absence of arginine (Figure 7E; R, 5.12); however, its concentration was considerably lower. The peak eluting at retention time 4.87 min also shows a 394 → 278 transition and is attributed to the [M + 2] isotope distribution of 7-(1-hydroxyhexyl)-1,N^2-ϵ-dGuo (**30**), which is calculated to be 1.8% of the molecular ion. It has been noted that dGuo reacts more readily with 2,3-epoxyaldehydes than enal; thus, the relative amount of **1** and **26** cannot be used to gauge the relative contributions of the two pathways.

Conclusions

7-(1*S*-Hydroxybutyl)-1,N^2-ϵ-dGuo was prepared from the reaction of (2*R*,3*S*)-epoxyhexanal and dGuo. The C7 side chain stereochemistry was found to be labile with the 1,N^2-ϵ-dGuo ring system stabilizing the cation as a result of solvolysis of the side chain hydroxyl group (Scheme 3). The observation was extended to the mechanism of 1,N^2-ϵ-dGuo formation from the reaction of EDE with dGuo, which was predicted to result in the formation of 2-octenal as a by-product (Scheme 6). The 1,N^2-octenal adduct of dGuo was observed as a product of the reaction thereby supporting the proposed mechanism. In addition, 7-(hydroxyhexyl)-1,N^2-ϵ-dGuo, which is derived from the reaction of dGuo with 2,3-epoxyoctenal, was also observed. The later process is also a source of 1,N^2-ϵ-dGuo. It is proposed that the Schiff base formation between EDE and dGuo results in 2,3-epoxyoctanal by a hydration (Michael addition of water) and retro-aldol reaction (Scheme 8). This process can potentially be facilitated by amino acids, peptides, and proteins, thereby making it of interest as it provides a viable mechanism for the formation of 2,3-epoxyaldehydes from EDE, a product from the peroxdiation of linoleic acid (*25*).

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Supporting Information Available: Spectroscopic data for compounds (*S*)-**16**, **18a**, **26**, and **30** and calibration curves for **1** and **26** vs [$^{15}N_5$]-**26** and **30** vs [$^{15}N_5$]-**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Leonard, N. J. (1984) Etheno-substituted nucleotides and coenzymes: fluorescence and biological activity. *CRC Crit. Rev. Biochem. 15*, 125–199.

(2) Leonard, N. J. (1992) Etheno-bridged nucleotides in structural diagnosis and carcinogenesis. *Chemtracts: Biochem. Mol. Biol. 3*, 273–297.

(3) Barbin, A. (2000) Etheno-adduct-forming chemicals: from mutagenicity testing to tumor mutation spectra. *Mutat. Res. 462*, 55–69.
(4) Bartsch, H., Barbin, A., Marion, M.-J., Nair, J., and Guichard, Y. (1994) Formation, detection, and role in carcinogenesis of ethenobases in DNA. *Drug Metab. Rev. 26*, 349–371.
(5) Bolt, H. M. (2005) Vinyl chloride—A classical industrial toxicant of new interest. *Crit. Rev. Toxicol. 35*, 307–323.
(6) Choi, J.-Y., Zang, H., Angel, K. C., Kozekov, I. D., Goodenough, A. K., Rizzo, C. J., and Guengerich, F. P. (2006) Translesion synthesis across 1,N^2-ethenoguanine by human DNA polymerases. *Chem. Res. Toxicol. 19*, 879–886.
(7) Hang, B., Chenna, A., Guliaev, A. B., and Singer, B. (2003) Miscoding properties of 1,N^6-ethanoadenine, a DNA adduct derived from reaction with the antitumor agent 1,3-bis(2-chloroethyl)-1-nitrosourea. *Mutat. Res. 531*, 191–203.
(8) Zhang, W., Johnson, F., Grollman, A. P., and Shibutani, S. (1995) Miscoding by the exocyclic and related DNA adducts 3,N^4-etheno-2′-deoxycytidine, 3,N^4-ethano-2′-deoxycytidine, and 3-(2-hydroxyethyl)-2′-deoxyuridine. *Chem. Res. Toxicol. 8*, 157–163.
(9) Pandya, G. A., and Moriya, M. (1996) 1,N^6-Ethenodeoxyadenosine, a DNA adduct highly mutagenic in mammalian cells. *Biochemistry 35*, 11487–11492.
(10) Nair, J., Barbin, A., Guichard, Y., and Bartsch, H. (1995) 1,N^6-Ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine in liver DNA from humans and untreated rodents detected by immunoaffinity/^{32}P-postlabelling. *Carcinogenesis 16*, 613–617.
(11) Swenberg, J. A., La, D. K., Scheller, N. A., and Wu, K.-y. (1995) Dose-response relationships for carcinogens. *Toxicol. Lett. 82–83*, 751–756.
(12) Barbin, A., Ohgaki, H., Nakamura, J., Kurrer, M., Kleihues, P., and Swenberg, J. A. (2003) Endogenous deoxyribonucleic acid (DNA) damage in human tissues: a comparison of ethenobases with aldehydic DNA lesions. *Cancer Epidemiol. Biomarkers Prev. 12*, 1241–1247.
(13) El Ghissassi, F., Barbin, A., Nair, J., and Bartsch, H. (1995) Formation of 1,N^6-ethenoadenine and 3,N^4-ethenocytosine by lipid peroxidation products and nucleic acid bases. *Chem. Res. Toxicol. 8*, 278–283.
(14) Nair, V., and Offerman, R. J. (1985) Ring-extended products from the reaction of epoxy carbonyl compounds with nucleic acid bases. *J. Org. Chem 50*, 5627–5631.
(15) Sodum, R. S., and Chung, F.-L. (1988) 1,N^2-Ethenodeoxyguanosine as a potential marker for DNA adduct formation by trans-4-hydroxy-2-nonenal. *Cancer Res. 48*, 320–323.
(16) Sodum, R. S., and Chung, F.-L. (1989) Structural characterization of adducts formed in the reaction of 2,3-epoxy-4-hydroxynonanal with deoxygunaosine. *Chem. Res. Toxicol. 2*, 23–28.
(17) Esterbauer, H., Schaur, R. J., and Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radical Biol. Med. 11*, 81–128.
(18) Pan, J., and Chung, F.-L. (2002) Formation of cyclic deoxyguanosine adducts from ω-3 and ω-6 polyunsaturated fatty acids under oxidative conditions. *Chem. Res. Toxicol. 15*, 367–372.
(19) Hartley, D. P., Ruth, J. A., and Petersen, D. R. (1995) The hepatocellular metabolism of 4-hydroxynonenal by alcohol dehydrogenase, aldehyde dehydrogenase, and glutathione S-transferase. *Arch. Biochem. Biophys. 316*, 197–205.
(20) Ullrich, O., Grune, T., Henke, W., Esterbauer, H., and Siems, W. G. (1994) Identification of metabolic pathways of the lipid peroxidation product 4-hydroxynonenal by mitochondria isolated from rat kidney cortex. *FEBS Lett. 352*, 84–86.
(21) Chung, F.-L., Chen, H.-J. C., and Nath, R. G. (1996) Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts. *Carcinogenesis 17*, 2105–2111.
(22) Chen, H.-J. C., and Chung, F.-L. (1994) Formation of etheno adducts in reactions of enals via autoxidation. *Chem. Res. Toxicol. 7*, 857–860.
(23) Chen, H.-J. C., and Chung, F.-L. (1996) Epoxidation of *trans*-4-hydroxy-2-nonenal by fatty acid hydroperoxides and hydrogen peroxide. *Chem. Res. Toxicol. 9*, 306–312.
(24) Douki, T., Odin, F., Caillat, S., Favier, A., and Cadet, J. (2004) Predominance of the 1,N^2-propano 2′-deoxyguanosine adduct among 4-hydroxy-2-nonenal-induced DNA lesions. *Free Radical Biol. Med. 37*, 62–70.
(25) Lee, S. H., Oe, T., and Blair, I. A. (2001) Vitamin C-induced decomposition of lipid hydroperoxides to endogenous genotoxins. *Science 292*, 2083–2086.
(26) Golding, B. T., Slaich, P. K., Kennedy, G., Bleasdale, C., and Watson, W. P. (1996) Mechanism of formation of adducts from the reactions of glycidaldehyde with 2′-deoxyguanosine and/or guanosine. *Chem. Res. Toxicol. 9*, 147–157.
(27) Golding, B. T., Slaich, P. K., and Watson, W. P. (1986) Reaction of guanosine with glycidaldehyde. *IARC Sci. Publ. 70*, 227–231.
(28) Lee, S. H., Oe, T., and Blair, I. A. (2002) 4,5-Epoxy-2(*E*)-decenal-induced formation of 1N^6-etheno-2′-deoxyadenosine and 1,N^2-etheno-2′-deoxyguanosine adducts. *Chem. Res. Toxicol. 15*, 300–304.
(29) Loureiro, A. P. M., Di Mascio, P., Gomes, O. F., and Medeiros, M. H. G. (2000) *trans*,*trans*-2,4-Decadienal-induced 1,N^2-etheno-2′-deoxyguanosine adduct formation. *Chem. Res. Toxicol. 13*, 601–609.
(30) Chung, F. L., Chen, H.-J. C., Guttenplan, J. B., Nishikawa, A., and Hard, G. C. (1993) 2,3-Epoxy-4-hydroxynonanal as a potential tumor-initiating agent of lipid peroxidation. *Carcinogenesis 14*, 2073–2077.
(31) Chen, H.-J. C., Zhang, L., Cox, J., Cunningham, J. A., and Chung, F.-L. (1998) DNA Adducts of 2,3-epoxy-4-hydroxynonanal: Detection of 7-(1′,2′-dihydroxyheptyl)-3*H*-imidazo[2,1-*i*]purine and 1,N^6-ethenoadenine by gas chromatography/negative ion chemical ionization/mass spectrometry. *Chem. Res. Toxicol. 11*, 1474–1480.
(32) Gao, Y., Klunder, J. M., Hanson, R. M., Masamune, H., Ko, S. Y., and Sharpless, K. B. (1987) Catalytic asymmetric epoxidation and kinetic resolution: Modified procedures including in situ derivatization. *J. Am. Chem. Soc 109*, 5765–5780.
(33) Lin, J., Fay, L. B., Welti, D. H., and Blank, I. (1999) Synthesis of *trans*-4,5-epoxy-(E)-2-decenal and its deuterated analog for the development of a sensitive and selective quantification method based on isotope dilution assay with negative chemical ionization. *Lipids 34*, 1117–1126.
(34) Sodum, R. A., and Chung, F.-L. (1991) Stereoselective formation of *in vitro* nucleic acid adducts of 2,3-epoxy-4-hydroxynonanal. *Cancer Res. 51*, 137–143.
(35) Chen, H.-J. C., Gonzalez, F. J., Shou, M., and Chung, F.-L. (1998) 2,3-Epoxy-4-hydroxynonanal, a potential lipid peroxidation product for etheno adduct formation, is not a substrate of human epoxide hydrolase. *Carcinogenesis 19*, 939–943.
(36) Carvalho, V. M., Asahara, F., Di Mascio, P., de Arruda Campos, I. P., Cadet, J., and Medeiros, M. H. (2000) Novel 1,N^6-etheno-2′-deoxyadenosine adducts from lipid peroxidation products. *Chem. Res. Toxicol. 13*, 397–405.
(37) Carvalho, V. M., Asahara, F., Di Mascio, P., Campos, I. P., Cadet, J., and Medeiros, M. H G. (2001) 1,N^6-etheno-2′-deoxyadenosine adducts from trans,trans-2,4-decadienal and trans-2-octenal. *Adv. Exp. Med. Biol. 500*, 229–232.
(38) Loureiro, A. P. M., de Arruda Campos, I. P., Gomes, O. F., Di Mascio, P., and Medeiros, M. H. G. (2004) Structural characterization of diastereoisomeric ethano adducts derived from the reaction of 2′-deoxyguanosine with *trans*,*trans*-2,4-decadienal. *Chem. Res. Toxicol. 17*, 641–649.
(39) De Mico, A., Margarita, R., Parlanti, L., Vescovi, A., and Piancatelli, G. (1997) A versatile and highly selective hypervalent iodine (III)/2,2,6,6-tetramethyl-1-piperidinyloxyl-mediated oxidation of alcohols to carbonyl compounds. *J. Org. Chem 62*, 6974–6977.
(40) Loureiro, A. P. M., de Arruda Campos, I. P., Gomes, O. F., Possari, E. P. M., Di Mascio, P., and Medeiros, M. H. G. (2005) Structural characterization of an etheno-2′-deoxyguanosine adduct modified by tetrahydrofuran. *Chem. Res. Toxicol. 18*, 290–299.
(41) Goodenough, A. K., Kozekov, I. D., Zang, H., Choi, J.-Y., Guengerich, F. P., Harris, T. M., and Rizzo, C. J. (2005) Site-specific synthesis and polymerase bypass of oligonucleotides containing a 6-hydroxy-3,5,6,7-tetrahydro-9H-imidazo[1,2-*a*]purin-9-one base, an intermediate in the formation of 1,N^2-etheno-2′-deoxyguanosine. *Chem. Res. Toxicol. 18*, 1701–1714.
(42) Guengerich, F. P., Persmark, M., and Humphreys, W. G. (1993) Formation of 1,N^2- and N^2,3-ethenoguanine from 2-halooxiranes: Isotopic labeling studies and isolation of a hemiaminal derivative of N^2-(2-oxoethyl)guanine. *Chem. Res. Toxicol. 6*, 635–648.
(43) Sako, M., and Yaekura, I. (2002) A convenient preparative method for the 1N^2-cyclic adducts of guanine nucleosides and nucleotides with crotonaldehyde. *Tetrahedron 58*, 8413–8416.
(44) Carvalho, V. M., Gasparutto, D., Di Mascio, P., Medeiros, M. H. G., and Cadet, J. (2003) Site-specific incorporation of the 1-hexanol-1,N^6-etheno-2′-deoxyadenosine adduct into oligodeoxyribonucleotides. *Bioorg. Med. Chem. 11*, 2445–2452.
(45) Lelais, G., and MacMillan, D. W. C. (2006) Modern strategies in organic catalysis: The advent and development of iminium activation. *Aldrichim. Acta 39*, 79–87.
(46) Dalko, P. I., and Moisan, L. (2004) In the golden age of organocatalysis. *Angew. Chem. Int. Ed. 43*, 5138–5175.
(47) Wang, M., McIntee, E. J., Cheng, G., Shi, Y., Villalta, P. W., and Hecht, S. S. (2001) A Schiff base is a major DNA adduct of crotonaldehyde. *Chem. Res. Toxicol. 14*, 423–430.
(48) Wang, M., McIntee, E. J., Cheng, G., Shi, Y., Villalta, P. W., and Hecht, S. S. (2000) Identification of DNA adducts of acetaldehyde. *Chem. Res. Toxicol. 13*, 1149–1157.

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