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PAPER

Synthesis of the four stereoisomers of 2,3-epoxy-4-hydroxynonanal and their reactivity with deoxyguanosine†

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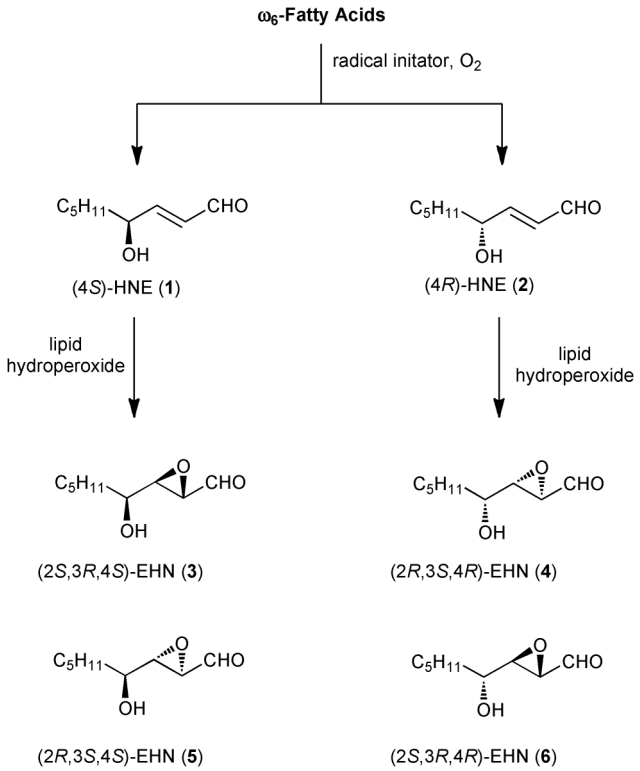
2,3-Epoxy-4-hydroxynonanal (EHN) is a potential product of lipid peroxidation that gives rise to genotoxic etheno adducts. We have synthesized all four stereoisomers of EHN and individually reacted them with 2′-deoxyguanosine. In addition to 1,N^2-etheno-2′-deoxyguanosine, 12 stereoisomeric products were isolated and characterized by ^{1}H NMR and circular dichroism spectroscopy. The stereochemical assignments were consistent with selective NOE spectra, vicinal coupling constants, and molecular mechanics calculations. Reversed-phase HPLC conditions were developed that could separate most of the adduct mixture.

Introduction

Reactive oxygen and nitrogen species formed during normal cellular respiration can initiate oxidative damage to biological membranes through an oxygen-dependent, free radical chain mechanism.[1] The peroxidation of lipids gives a complex array of products including simple enals (acrolein, crotonaldehyde and higher congeners), more oxygenated enals (4-hydroxy-2-nonenal, 4-oxo-2-nonenal and malondialdehyde), and epoxyaldehydes (4,5-epoxy-2-decenal) among other products.[2] Lipid peroxidation has been implicated as a contributing factor of human diseases such as diabetes, cardiovascular injury, neurodegenerative diseases, and cancer.[3–7]

4-Hydroxy-2-nonenal (HNE) is a major lipid peroxidation product from ω-6 polyunsaturated fatty acids.[2] HNE is highly cytotoxic and was shown to induce apoptosis in tumor cell lines.[8,9] HNE reacts predominantly with Gua bases in DNA and has mutagenic potential. Since HNE is produced as a racemate (**1** and **2**, Scheme 1), its reaction with 2′-deoxyguanosine (dGuo) leads to four diastereomeric adducts.[10–12] We have previously reported the synthesis of the oligonucleotides containing stereochemically defined HNE-dGuo adducts and found that the adduct stereochemistry can play a critical role in the chemistry, properties, and mutagenicity of HNE-dGuo adducts.[13,14]

Model studies have shown that HNE can undergo epoxidation with *t*-butylhydroperoxide to yield 2,3-epoxy-4-hydroxynonanal (EHN),[15–18] which is a more potent mutagen.[19] It has been proposed that lipid hydroperoxides may serve as the *in vivo* epoxidizing agents, although the reaction of HNE with *t*-butylhydroperoxide proceeds in modest yield.[17,20] Blair has shown that 4,5-epoxy-2-decenal is produced from the peroxidation of linolenic acid suggesting that epoxyaldehydes can be direct products of lipid peroxidation rather than a secondary product.[21] Each enantiomer



Scheme 1 Stereoisomers of 4-Hydroxynonenal (HNE) and 2,3-epoxy-4-hydroxynonanal (EHN).

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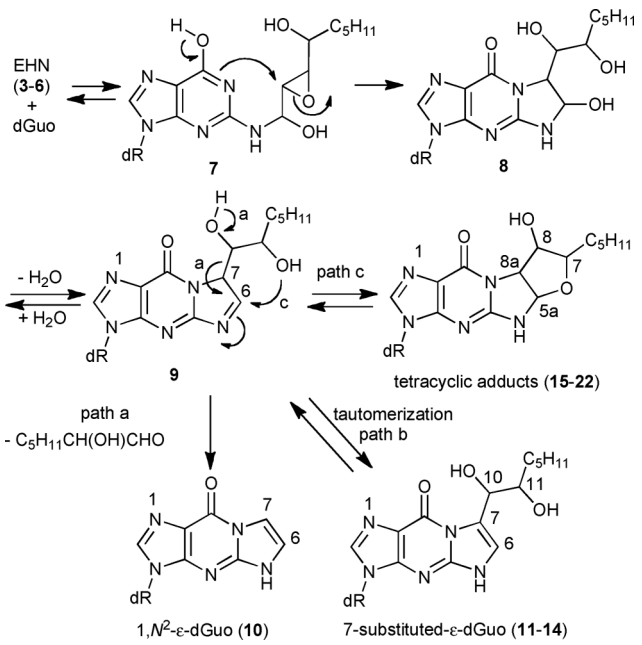
† Electronic supplementary information (ESI) available: Experimental procedures for the synthesis of (4*S*)-HNE, selective ^{1}H NOE spectra (**17–22**), ^{1}H homonuclear decoupling spectra (**15**, **16**, **21**, **22**), CD spectra (**11–14**, **16**, **18–22**), ^{1}H (**3**, **5**, **11–28**) and ^{13}C NMR spectra (**3**, **5**, **11–14,23–28**), GC chromatograms (**3**, **5**, **23–28**), and HPLC chromatograms (**11–22**). See DOI: 10.1039/c0ob00546k

of HNE can lead to a pair of diastereomeric epoxides; thus, four stereoisomers of EHN are possible (**3–6**, Scheme 1).

Scheme 2 outlines the mechanism by which the EHN can react with dGuo to give three types of DNA adducts, which are the parent unsubstituted 1,N^2-ε-dGuo, C7-substituted ε-dGuo adducts, and tetracyclic adducts. EHN is a bis-electrophile, which can react with dGuo at two separate nucleophilic sites to give the 1,N^2-cyclic carbinolamine adduct **8**. The opposite sense of the epoxide-opening, *i.e.*, to give the 1,N^2-dihydroxypropano-dGuo adduct, has been observed as a minor product for the reaction of dGuo with glycidaldehyde.[22] However, products from this competing pathway have not been observed for C3-substituted 2,3-epoxyaldehyde. The carbinolamine **8** can undergo reversible dehydration to give imine **9**, which is a common intermediate to the three observed product types. Although an alternative pathway for the loss of the side chain has been proposed previously,[23] we favor the mechanism outlined by Golding involving loss of the C7-sidechain as the aldehyde *via* a retro-aldol reaction (path a) to give the unsubstituted 1,N^2-etheno-dGuo (ε-dGuo) adduct **10**.[22,24] Imine **9** can also undergo tautomerization to yield four diastereomeric etheno adducts (**11–14**) possessing an intact C7-sidechain (path b). Alternatively, imine **9** can be trapped by the sidechain hydroxyl group to afford eight diastereomeric tetracyclic adducts (**15–22**, path c). Thus, the reaction of racemic EHN with DNA is expected to give a complex mixture of related DNA adducts. Treatment of the C7-substituted ε-dGuo or tetracyclic adducts with base leads to a rapid conversion to the unsubstituted 1,N^2-ε-dGuo adduct **10**. Related etheno adducts have also been characterized from the reaction of EHN and dAdo.[16,25] These adducts have been observed from the treatment of DNA and cells with a combination of HNE and *t*-butylhydroperoxide.[17,25]



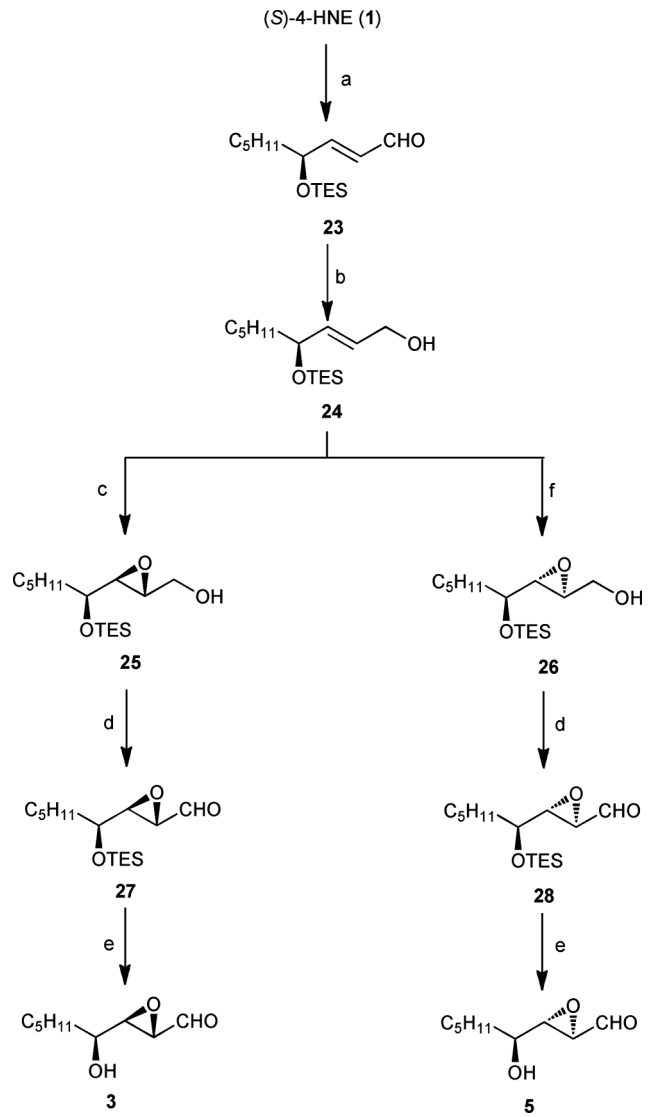
Scheme 2 Mechanism for the formation of the EHN adducts of 2′-deoxyguanosine.

We report here the synthesis of all four stereoisomers of EHN. The individual isomers were then reacted with dGuo and all four C7-substituted ε-dGuo and eight tetracyclic adducts were characterized. In addition, reversed-phase HPLC conditions have been developed that can separate most of these adducts.

Results and discussion

Asymmetric synthesis of all four stereoisomers of EHN

We have previously reported the synthesis of (4*S*)- and (4*R*)-*trans*-HNE (**1** and **2**).[9] The synthesis was based on the work of Yu and Wang and proceeded in four steps from commercially available 2*E*-octenal.[9,26] The absolute stereochemistry was established by a Sharpless asymmetric epoxidation of 2*E*-octen-1-ol.[13,26,27] The HNE enantiomers were estimated to be >90% ee based on optical rotation.[26,28,29] Starting from (4*S*)- and (4*R*)-HNE, all four diastereomers of 2,3-epoxy-4-hydroxynonanal (**3–6**) were synthesized according to Scheme 3.



Scheme 3 Synthesis of EHN from HNE. Reagents: a) TES-Cl, DMAP, Et_3N (67–68%); b) $NaBH_4$, THF (58–62%); c) $Ti(OiPr)_4$, (+)-L-DET, $C_6H_5C(CH_3)_2OOH$, CH_2Cl_2, –25 °C (73–77%); d) $PhI(OAc)_2$, TEMPO (64–76%); e) $nBu_4N^+F^-$, THF (72–92%); f) $Ti(OiPr)_4$, (–)-D-DET, $C_6H_5C(CH_3)_2OOH$, CH_2Cl_2, –25 °C (74–75%).

The hydroxyl group of (4*S*)-HNE (**1**) was protected as a triethylsilyl (TES) ether, then the aldehyde was reduced with $NaBH_4$ in THF to give allyl alcohol **24** in ~40% overall yield. Sharpless asymmetric epoxidation of **24** using the (+)-diethyltartrate ligand gave the epoxyalcohol **25** with the *syn* relative stereochemistry between the epoxide and the protected C4-hydroxyl group. The primary alcohol was oxidized to aldehyde **27**;[30] NMR analysis of **27** showed two aldehyde resonances in a ~93 : 7 ratio, reflective of the diastereoselectivity of the Sharpless epoxidation. Removal of the silyl ether protecting group with fluoride gave (2*S*,3*R*,4*S*)-EHN (**3**). When the (–)-diethyl tartrate ligand was used for the Sharpless epoxidation of **24**, the epoxyalcohol **26** possessing the *anti* relative stereochemistry was obtained. Oxidation and deprotection afforded (2*R*,3*S*,4*S*)-EHN (**5**). The diastereoselectivity of the Sharpless epoxidation of **26** was ~92 : 8 based on NMR analysis of aldehyde **28**. The remaining two stereoisomers of EHN, **4** and **6**, were synthesized in an identical fashion starting from (4*R*)-HNE (**2**). The sequence required five steps from (4*R*)- or (4*S*)-HNE.

Reaction of EHN with dGuo

The reaction of EHN with dGuo was reported to proceed slowly and in low yield.[15] The reactivity can be increased at higher pH; however, a greater proportion of the 1,N^2-ε-dGuo was observed under such conditions. We found that the individual stereoisomers of EHN (**3–6**) react with dGuo in DMF and K_2CO_3 (12–17 h) to give a single stereoisomer of the 7-substituted etheno adducts (**11–14**) in good yield (62–71%) and only a small amount of 1,N^2-ε-dGuo (**10**) was observed (Scheme 4).[31] Interestingly, the tetracyclic adducts (**15–22**) were not observed under these conditions. It should be noted that the stereochemistry of the allylic hydroxyl group of the C7-sidechain (C10) of **11–14** can slowly scramble over time.[31]

Formation of the tetracyclic adducts

Each of the 7-substitued-ε-dGuo adducts **11–14** were individually incubated in 25 mM, pH 6.8 phosphate buffer. Each was consumed over 2–4 days to give two new products, which were identified as the tetracyclic adducts (**15–22**, Scheme 4). The cyclization reaction was performed under high dilution conditions to prevent dimerization of the 7-substituted-ε-dGuo. The dimers were not characterized, but this processes has been reported for other 7-(1-hydroxyalkyl)-ε-dGuo adducts.[22,31,32] The mechanism of the cyclization involves tautomerization back to the imine (**9**) followed by cyclization (Scheme 2). The tautomerization can generate two diastereomers at C7, which corresponds to the C8a-position of the tetracyclic product. Bicyclo[3.3.0]octane systems have a strong preference for a *cis*-ring fusion;[33,34] thus, each diastereomer of the imine is predicted to cyclize to a specific tetracyclic adduct. In the case of **13** and **14**, where the C7-sidechain vicinal diols possess an *anti* relative stereochemistry, the cyclization proceeds to afford near equal amounts of the two tetracyclic adducts. The cyclization was more selective in the case of the *syn vic*-diol (**11** and **12**), affording a major and minor product in ~7 : 1 ratio. The relative stereochemistry of the tetracyclic adducts (**15–22**) explains the observed product ratio. Cyclization of the *syn* diols (**11** and **12**) is predicted to give a product in which the C7-pentyl and C8-hydroxyl groups are either on the convex (**15** and **17**) or concave (**16** and **18**) face of the bicyclo[3.3.0]-ring system. Adducts **16** and **18**, in which both substituents are on the more congested concave face, are expected to be less favored than **15** and **17**, which place the substituents on the convex face. The products from the cyclization of the *anti* diols (**13** and **14**) have one of these substituents on the concave face and the other on the convex face (**19–22**). The pure tetracyclic adducts would undergo ring opening to the corresponding 7-substituted-ε-dGuo adduct and loss of the sidechain to 1,N^2-ε-dGuo over time; **16** and **18** were particularly prone to this process, which complicated the ^{1}H NMR characterization as can be seen in the spectrum in Fig. 1 (right).

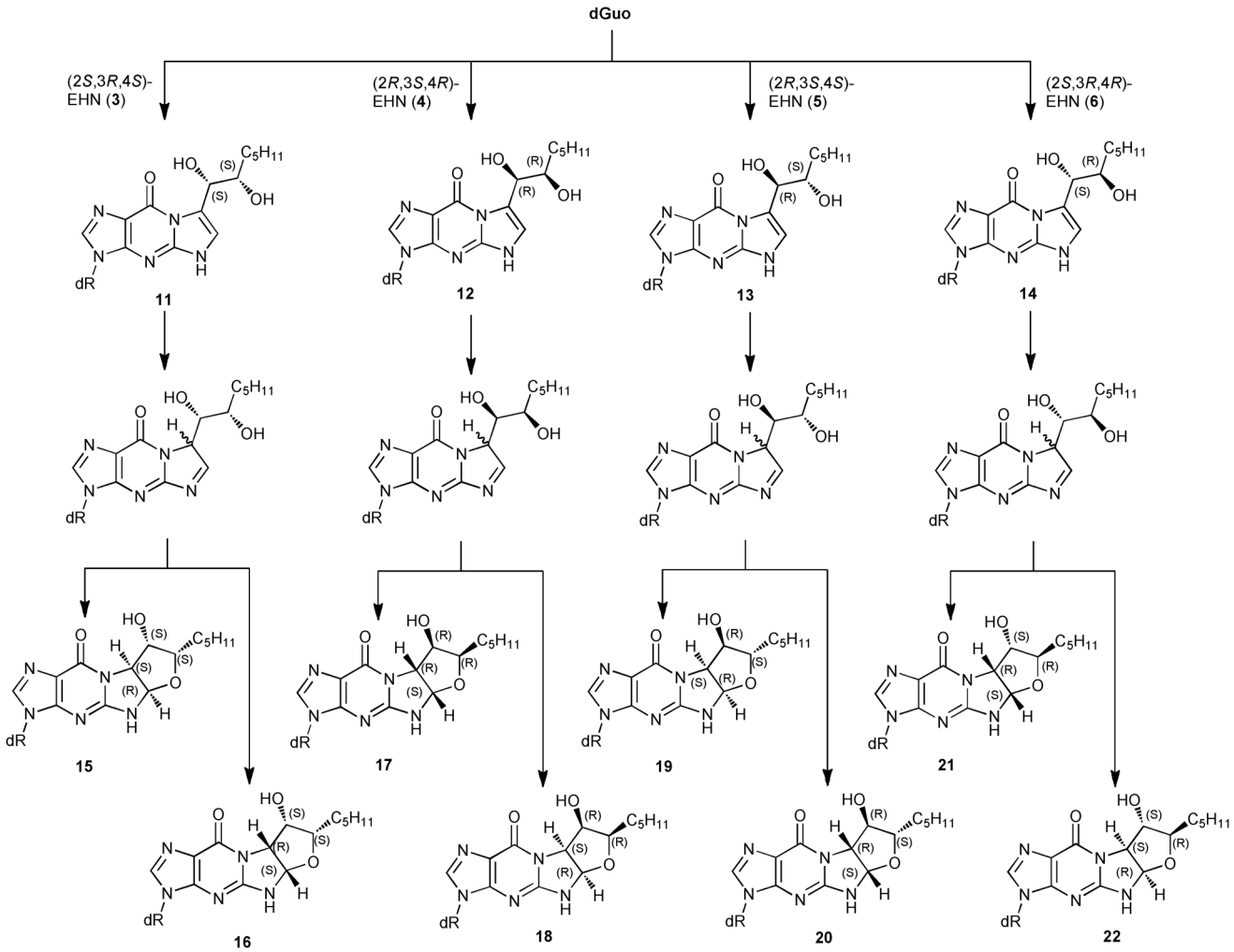
NMR analysis of the tetracyclic adducts 15–22

Comparison of the selective NOE spectra (600 MHz) when protons H5a, H7, H8, and H8a were individually saturated for all eight tetracyclic adducts **15–22** revealed patterns consistent with the relative stereochemical assignments. The ^{1}H NMR assignments were made based on their COSY spectra and are consistent with previous assignments.[15,16] NOE enhancements for vicinal protons (H5a–H8a, H7–H8, H8–H8a) about the furan ring were in the range of 1.5–2.2% when *syn* and ~0.6–0.9% when *anti*. The NOE enhancements for H5a–H7, H5a–H8, and H7–H8a were between 0.1–0.7% when *syn* and <0.1% when *anti*. Although these enhancements were small, they were reproducible and consistent between samples. The NOE enhancements are summarized in Table 1 and an example of the selective NOE spectra of adducts **15** and **16** are shown in Fig. 1.

The conformations of the four relative stereoisomers of the tetracyclic adducts were determined by molecular mechanics using the MMX force field as implemented in PC Model (v. 9.2, Serena Software). The modified Gua bases of **15/17**, **16/18**, **19/21**, and **20/22** have the same relative stereochemistry. Methyl and ethyl groups were used in place of the deoxyribose unit and C7-pentyl chain, respectively, to simply the analysis. Molecular mechanics were performed on adducts **15′**, **16′**, **19′**, and **20′**, where the prime (′) notes the simplified model compound. The predicted vicinal H5a–H8a, H7–H8, and H8–H8a coupling constants were computed from the dihedral angles and compared to experimental values (Table 2) obtained from a series of 1D ^{1}H homonuclear decoupling experiments (Fig. S7–S10, ESI†).[35] The rigidity of the bicyclo[3.3.0]octane fused to the Gua base makes this system ideal for this analysis and there is reasonable agreement between the predicted and observed values for three of the four adducts. The bridgehead H5a and H8a protons are *syn* for all the compounds and the dihedral angle between them is predicted to be small

Table 1 Percentage NOE enhancement when the first proton listed for each pair is saturated (NOE enhancement when the second proton is saturated)

	H5a–H7	H5a–H8	H5a–H8a	H7–H8	H7–H8a	H8–H8a
15	0.1 (0.1)	0.1 (0.1)	1.8 (1.8)	1.8 (1.8)	0.1 (0.1)	0.9 (0.8)
16	0.7 (0.7)	0 (0)	1.9 (2.0)	1.8 (1.8)	0.6 (0.6)	2.2 (2.0)
17	0.1 (0.2)	0.1 (0.1)	1.8 (1.8)	1.7 (1.7)	0.1 (0.1)	0.8 (0.8)
18	0.7 (0.7)	0 (0)	1.5 (1.9)	1.7 (1.8)	0.6 (0.6)	2.1 (2.0)
19	0 (0)	0.1 (0.1)	1.7 (1.9)	0.6 (0.6)	0 (0)	1.9 (1.9)
20	0.4 (0.4)	0.1 (0.1)	1.7 (1.7)	0.7 (0.8)	0.2 (0.2)	0.8 (0.7)
21	0.1 (0.1)	0.3 (0.2)	2.0 (2.0)	0.6 (0.8)	0.1 (0.1)	1.9 (2.0)
22	0.4 (0.4)	0.1 (0.1)	1.6 (1.5)	0.7 (0.7)	0.1 (0.1)	0.7 (0.7)



Scheme 4 2′-Deoxyguanosine adducts of EHN.

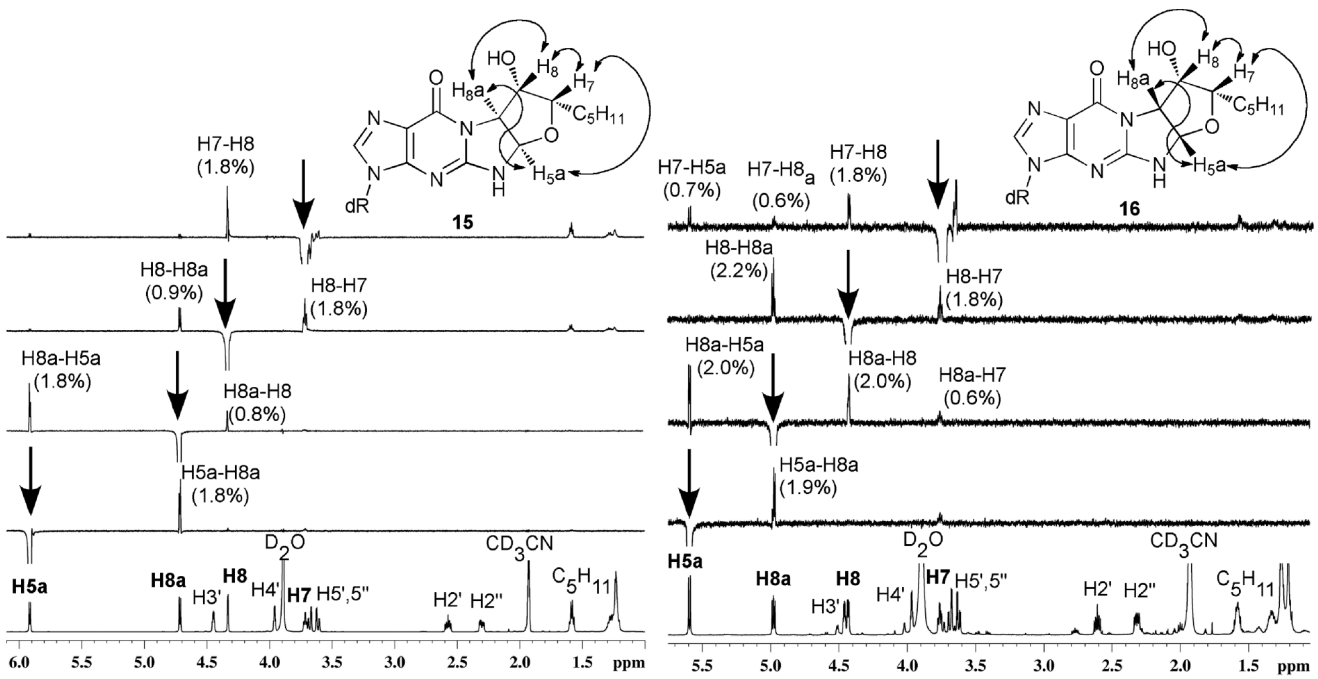


Fig. 1 Selective NOE spectra of tetracyclic adducts **15** and **16**. The NOE spectra for the remaining adducts can be found in Fig. S1–S6, ESI.†

Table 2 The observed vicinal ^1H–^1H coupling constants for tetracyclic adducts **15–22** and the calculated values from MMX minimized structures

	Observed			Calculated (dihedral angle)		
	J_{5a-8a}	J_{7-8}	J_{8-8a}	J_{5a-8a}	J_{7-8}	J_{8-8a}
15	6.6	2.7	0.3	7.2 (1.8°)	1.8 (47°)	0.7 (89°)
16	7.7	2.6	5.8	7.2 (8.1°)	2.2 (44°)	7.5 (19°)
17	6.6	2.7	0.3			
18	7.7	2.6	5.8			
19	6.9	8.0	7.1	7.5 (4.1°)	8.2 (161°)	8.1 (22°)
20	6.7	2.6	1.2	7.3 (6.5°)	8.3 (163°)	5.4 (140°)
21	6.9	8.0	7.1			
22	6.7	2.5	1.2			

(1.8°–4.0°) with *J* values between 7.2 and 7.6 Hz; the observed values were between 6.6 and 7.7 Hz. The terminal furan is predicted to adopt an envelope conformation for all four stereoisomers in which C5a, O6, C8, and C8a were nearly coplanar. The C7 atom is predicted to occupy an *endo* position relative to the other rings for **15′** and **19′**, whereas the C7 atom is predicted to be *exo* for **16′** and **20′**; this designation can be seen for the MMX minimized structures in Fig. 2.

The H7 and H8 protons are *syn* for **15′** and **16′** and are predicted to have dihedral angles of 47° and 44°, corresponding to small coupling constants of 1.8 and 2.2 Hz, respectively. These vicinal coupling constants are in good agreement with the observed values of 2.7 and 2.6 Hz. The relative configuration of H8 and the bridgehead H8a protons is *anti* for **15′** and *syn* for **16′**. The H8–H8a dihedral angles are calculated to be 89° and 19° with predicted coupling constants of 0.7 and 7.5 Hz, respectively. Once again these values are in reasonable agreement with the observed values of 0.3 and 5.8 Hz. A correlation was not observed between H8 and H8a in COSY spectra of **15/17**, indicating that the dihedral angle was ~90° for these protons and is consistent with the calculated value.

The relative stereochemistry of the H7 and H8 is *anti* in **19′** and **20′**. The H7 and H8 protons of **19′** are situated on the *exo* and *endo* faces of the ring system, respectively. The calculated H7–H8 dihedral angle is 161° leading to a predicted vicinal coupling constant of 8.2 Hz; this value is in excellent agreement with the observed value of 8.0 Hz. The relative stereochemistry of H8 and H8a protons is *syn* and predicted to have a dihedral angle of 22°. The calculated coupling constant for this angle is 8.1 Hz, which is again in good agreement with the observed value of 7.1 Hz.

The H7 and H8 protons of the MMX minimized conformation of **20′** occupy pseudo diaxial dispositions off the furan ring, similar to **19′**, resulting in a large predicted dihedral angle of 163°. The H8 and H8a protons are *anti* with a dihedral angle of 140°. The calculated H7–H8 and H8–H8a vicinal coupling constants are 8.3 and 5.4 Hz, respectively, which is in poor agreement with the observed values of 2.6 and 1.2 Hz. A conformational search (GMMX), in which the bonds of the furan ring were varied, was performed as implemented in PC Model using the default parameters. A conformation 5.9 kJ mol^{-1} (1.4 kcals mol^{-1}) higher in energy was found that better matched the observed coupling constants. The main difference between the two conformations is the disposition of C7. The C7 atom is *exo* in the lowest energy conformation (**20′**) and *endo* in the higher energy conformation (**20″**). The C7-*endo* conformation (**20″**) places H7 and H8 in pseudo equatorial positions with a dihedral angle of 89°, while the H8–H8a dihedral angle is predicted to be 95°. These dihedral angles are predicted to give rise to small coupling constants (0.9 and 0.6 Hz, respectively), which are in better agreement with the observed values.

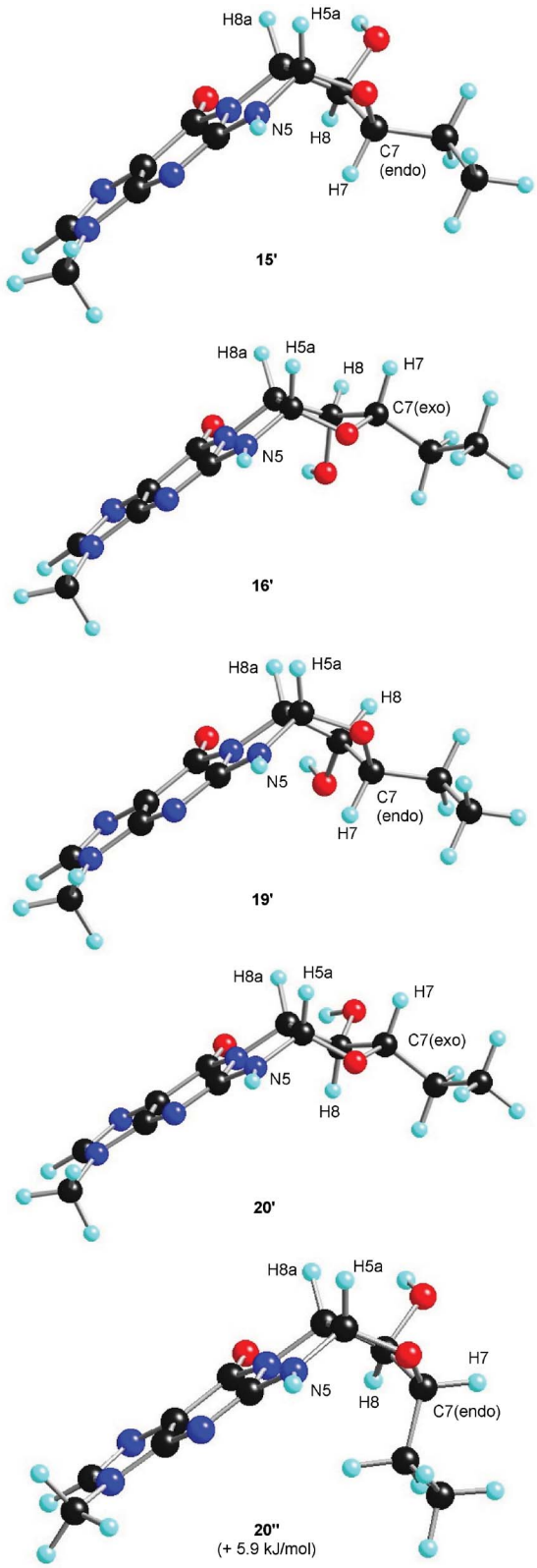


Fig. 2 MMX minimized conformation of the four relative stereoisomers of the tetracyclic adducts.

The C7-*endo* conformation of **20″** also agrees with the observed NOE enhancements as compared to the other tetracyclic adduct. The H5a–H7 and H8a–H7 distances when H7 is in the pseudo-axial position for the C7-*exo* conformation of **20′** are 2.7 and 3.0 Å, respectively. These values are nearly identical to those of **16′** (2.7 and 3.1 Å) in which H7 is also predicted to be pseudo-axial. The H5a–H7 and H8a–H7 NOE enhancements for **16** were 0.7 and 0.6%, respectively, as compared to 0.4 and 0.2% for **20**. The H5a–H7 and H8a–H7 distances predicted when H7 is pseudo-equatorial as for the C7-*endo* conformation of **20″** are 3.8 and 4.1 Å; these longer distances are more consistent with the lower NOE enhancements as compared to **16**. Taken together, the vicinal coupling constants and NOE data are more consistent with the C7-*endo* conformation for adducts **20**/**22** over the energetically more favorable C7-*exo* conformation. The reason for this preference is not entirely clear. The NMR experiments were performed in 1 : 1 CD_3CN/D_2O; hydrophobic considerations would favor the more compact C7-*endo* conformation, which is likely to have a smaller surface area.

Circular dichroism spectra

The eight tetracyclic adducts **15–22** are diastereomers. However, the modified guanine base of adducts **15** and **17**, **16** and **18**, **19** and **21**, and **20** and **22** have enantiomeric relationships, which is reflected in the sign of their CD spectra.[13,15,36,37] An example is shown in Fig. 3 for **15** and **17**. The sign of the CD absorbance at ~275 nm was correlated to the stereochemistry at the bridgehead positions, C5a and C8a. A positive ellipticity was observed for the (5a*R*,8a*S*)-stereochemistry (**15**, **18**, **19**, and **22**) and a negative ellipticity was observed for the (5a*S*,8a*R*)-stereochemistry (**16**, **17**, **20**, and **21**). CD spectra for the remaining adducts can be found in Fig. S11–S13, ESI.† The same enantiomeric relationship was observed in the CD spectra of 7-substituted etheno adduct **11** and **12**, as well as **13** and **14**. In the case of adducts **11–14**, the sign of the CD absorbance at ~295 nm was correlated to the C1-stereochemistry of the C7-(1,2-dihydroxyheptyl) sidechain (see Fig. S14 and S15, ESI†). The 1*S*-stereochemistry resulted in a positive ellipticity (**11** and **14**), while the 1*R*-stereochemistry resulted in a negative ellipticity (**12** and **13**).

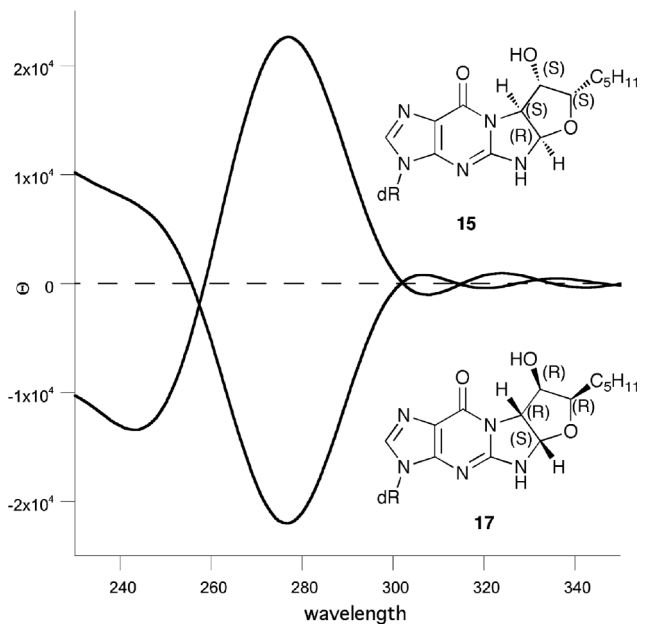


Fig. 3 CD spectra of adducts **15** and **17**. The enantiomeric relationship of the modified Gua bases gives rise to CD signals of opposite signs at ~275 nm.

HPLC separation of the EHN-dGuo adducts

The reaction of EHN with dGuo afforded at least 13 products, including 1,N^2-ε-dGuo (**10**), four diastereoisomers of 7-(1,2-dihydroxyheptyl)-ε-dGuo (**11–14**), and eight diastereomeric tetracyclic adducts (**15–22**). Unfortunately, we were unable to develop HPLC conditions that could resolve all 13 products. Optimal conditions were developed in which 11 peaks could be observed and their identities were verified by comparison of the retention time to the authentic compound (Fig. 4). C7-(1,2-Dihydroxyheptyl)-ε-dGuo adducts **11** and **12** coeluted as did the C7-substituted ε-dGuo adduct **13** and tetracyclic adduct **20**; in addition, tetracyclic adducts **16** and **18** were not well resolved, but distinguishable.

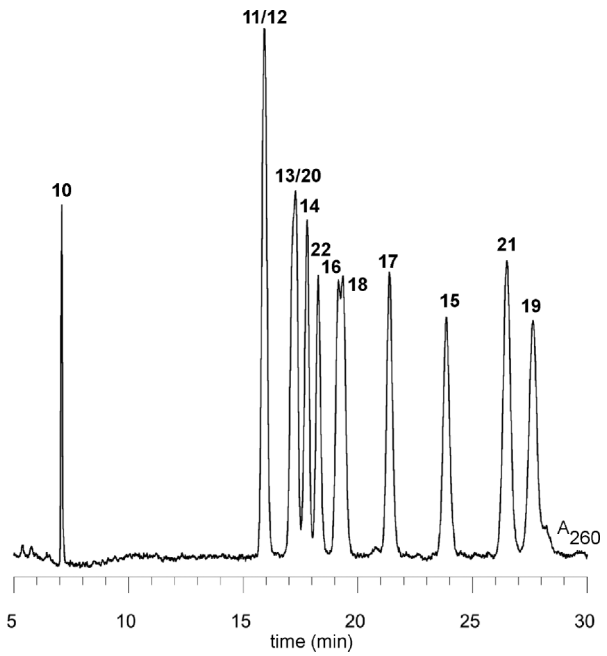


Fig. 4 Reversed-phase HPLC analysis of a mixture of all dGuo adducts of EHN (**10–22**).

Conclusions

The potential role of EHN as an endogenous toxicant was hypothesized by Sodum and Chung who observed EHN-dGuo adducts when dGuo was reacted with HNE using THF as a co-solvent;[18] it was proposed that the hydroperoxide of THF epoxidized HNE. Other hydroperoxides such as H_2O_2, *t*-butylhydroperoxide, and lipid hydroperoxides can also carry out the epoxidation reaction. Subsequently, the reaction of partially separated, racemic *syn* and *anti* diastereomers of EHN with dAdo and dGuo was examined.[15,16] Six products were characterized from the reaction

with dGuo, including 1,N^2-ε-dGuo, a 7-(1,2-dihydroxyheptyl)-ε-dGuo, and four of the tetracyclic adducts, although the absolute stereochemistry was not determined. These adducts were also observed when calf thymus DNA was treated with EHN.[16,17,25] The tetracyclic adducts were subject to acid deglycosylation and the corresponding Gua bases had opposite CD spectra, suggesting enantiomeric relationships of the modified bases. The 7-(1,2-dihydroxyheptyl)-ε-dGuo, and four tetracyclic adducts were rapidly converted to 1,N^2-ε-dGuo under basic conditions. This work established a possible mechanism for the endogenous formation of promutagenic etheno lesions. Other lipid peroxidation products have also been shown to give rise to 1,N^2-ε-dGuo.[31,38–42]

In the present work, we synthesized the four stereoisomers of EHN starting from (4*R*)- and (4*S*)-HNE. The individual EHN stereoisomers were reacted with dGuo to afford a single diastereomer of 7-(1,2-dihydroxyheptyl)-ε-dGuo with minimal formation of 1,N^2-ε-dGuo. Each 7-(1,2-dihydroxyheptyl)-ε-dGuo isomer was then incubated in phosphate buffer to give two tetracyclic adducts. The absolute stereochemistries of all adducts were established. The relative stereochemistry of the tetracyclic adducts was consistent with selective NOE enhancements and the vicinial coupling constants were consistent with predicted values that were calculated from dihedral angles as determined by molecular mechanics.

Experimental section

General

All commercially obtained chemicals were used as received except (methoxymethyl)triphenylphosphonium chloride, which was used after drying overnight in an Abderhalden apparatus under vacuum at 78 °C. CH_2Cl_2 was freshly distilled from calcium hydride. Anhydrous THF was freshly distilled from a sodium/benzophenone ketyl. All reactions were performed under an Ar atmosphere. Glassware was flame-dried and cooled under Ar. Molecular sieves (4 Å) were activated with a microwave oven for at least 7 min and cooled in a desiccator. Flash column chromatography was performed using silica gel (32–63 μm; 230 × 450 mesh). Analytical thin layer chromatography was performed on silica gel glass plates (Merck, silica gel 60 F_{254} layer thickness 250 μm) and visualized by staining with *p*-anisaldehyde followed by charring. High-resolution FAB mass spectra for compounds **11–15**, **17** were obtained from the University of Notre Dame Mass Spectrometry Center using nitrobenzyl alcohol (NBA) as a matrix. High-resolution mass spectra for compounds **16**, **18–22** were obtained from the Vanderbilt University Mass Spectrometry Resource Center and recorded on a Waters Synapt hybrid quadrupole oa-TOF high resolution mass spectrometer operated in "w" mode, posESI. A post-acquisition gain correction factor was applied using sodium formate as lock mass. Qualitative GC-MS analyses were performed on a Varian Saturn 2100T mass spectrometer using the following conditions: electron-impact ionization (EI) mass spectra were generated at 70 eV; ion source temperature was 200 °C and electron multiplier voltage was 1400 V. Scanning was performed from *m*/*z* 40–450. Samples were introduced *via* Varian 3900 GC equipped with CP 8410 autoinjector using the following conditions: split mode injection (ratio 100 : 50) at 180 °C on high resolution gas chromatography column Agilent HP-5 MS (30 m, 0.25 mm ID, 0.25 μm). Helium was used as a carrier gas with 1 mL min^{-1} flow rate. The temperature program was follows: 40 °C (6 min), 7 °C min^{-1} to 70 °C and then 10 °C min^{-1} to 230 °C (hold 13 min). Optical rotations were measured on an Autopol IV polarimeter, using a 1.0 dm cell and $CHCl_3$ as a solvent. All ^1H and ^{13}C NMR spectra for the isomers of EHN and their precursors were acquired on either a Bruker DRX-300 or AV-400 spectrometer in $CDCl_3$ and CD_2Cl_2 which also served as an internal chemical shift standard. Data are reported as follows: chemical shifts, multiplicity, integration, coupling constant (Hz). ^{13}C chemical shifts are reported in ppm with CD_2Cl_2 serving as an internal reference at 53.31 ppm. ECD spectra were recorded on Jasco J720 Spectropolarimeter. A Beckman gradient HPLC system (32 Karat software version 7.0, pump module 125) with a diode array UV detector (module 168) monitoring at 260 nm was used for separations with YMC ODS-AQ columns (Waters Corp).

(4*S*)-4-Hydroxy-2*E*-nonenal (1). (4*S*)-HNE (**1**) was prepared as previously described.[9,26] $[\alpha]_D^{24.5}$ +44.7 (*c* 0.36, $CHCl_3$) (lit:[29] $[\alpha]_D^{20}$ = +48° (*c* 0.69, $CHCl_3$)); δ_H (400 MHz, CD_2Cl_2) 0.91 (t, 3H, *J* = 6.9 Hz, CH_3), 1.29–1.51 (m, 6H, $3CH_2$), 1.54–1.69 (m, 2H, CH_2), 1.86 (br.s., 1H, OH), 4.43 (m, 1H, *J* = 5.2 Hz, H-4), 6.27 (ddd, 1H, *J* = 1.6, 7.9, 15.7 Hz, H-2), 6.84 (dd, 1H, *J* = 4.6, 15.7 Hz, H-3), 9.57 (d 1H, *J* = 7.9 Hz, CHO); *m*/*z* (GC-EI) 157 (100, M^++1), 139 (25), 109 (40), 81 (30).

(4*R*)-Hydroxy-2*E*-nonenal (2). (4*R*)-HNE (**2**) was prepared in the same manner as **1**. $[\alpha]_D^{25}$ –46.7 (*c* 0.73, $CHCl_3$) (lit:[29] $[\alpha]_D^{25}$ –46 (*c* 0.45, $CHCl_3$)).

(4*S*)-4-Triethylsilyloxy-2*E*-nonen-1-al (23). A solution of (4*S*)-HNE (**1**, 0.52 g, 3.36 mmol), 4-(dimethylamino)pyridine (0.049 g, 0.4 mmol) and triethylamine (1.02 g, 10 mmol) in dry CH_2Cl_2 (25 mL) was cooled in an ice-bath. Chlorotriethylsilane (0.60 g, 3.98 mmol) was added dropwise to the stirred solution; the reaction mixture was stirred at 0° C for 10 min, then the ice bath was removed and mixture stirred at room temperature until the starting material was consumed (2 h). Water (25 mL) was added with stirring. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica, eluting with 5% ether in hexanes, afforded **23** (0.62 g, 68%). $[\alpha]_D^{24}$ +17.8 (*c* 0.43, $CHCl_3$). δ_H (400 MHz, CD_2Cl_2) 0.62 (m, 6H, $3CH_2Si$), 0.88 (t, 3H, *J* = 6.8 Hz, CH_3), 0.95 (m, 9H, $3CH_3CH_2Si$), 1.28–1.37 (m, 6H, $3CH_2$), 1.54–1.62 (m, 2H, CH_2), 4.43 (tdd, 1H, *J* = 1.5, 4.6, 6.1 Hz, H4), 6.23 (ddd, 1H, *J* = 1.5, 8.0, 15.5 Hz, H2), 6.82 (dd, 1H, *J* = 4.8, 15.5 Hz, H3), 9.56 (d, 1H, *J* = 8.0 Hz, CHO); δ_C (100 MHz, CD_2Cl_2) 193.9, 160.5, 130.9, 72.0, 37.7, 32.2, 24.9, 23.0, 14.2, 7.0 (3), 5.2 (3); *m*/*z* (GC-EI) 271 (7, M^++1), 253 (100), 241 (50), 157 (28), 139 (12), 81(8).

(4*R*)-4-Triethylsilyloxy-2*E*-nonenal (*ent*-23). *ent*-**23** was prepared from (4*R*)-HNE (**2**) in 67% yield following the procedure described above for **23**. $[\alpha]_D^{24.2}$ –16.0 (*c* 0.45, $CHCl_3$).

(4*S*)-4-Triethylsilyloxy-2*E*-nonen-1-ol (24). Sodium borohydride (0.11 g, 2.82 mmol) was added in portions to a stirred solution of **23** (0.61 g, 2.25 mmol) in dry THF (10 mL) under Ar. The reaction mixture was stirred for 30 min at room temperature, and then deionized water (25 mL) was added. The resulting

mixture was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic layers were washed with water, dried over $MgSO_4$, filtrated, and evaporated. Purification by flash chromatography on silica, eluting with 10% ether in hexanes, afforded **24** (0.38 g, 62%). $[\alpha]_D^{24.2}$ −2.6 (*c* 0.38, $CHCl_3$); δ_H (400 MHz, CD_2Cl_2) 0.55–0.62 (m, 6H, $3CH_2Si$), 0.89–0.99 (m, 12H, $3CH_3CH_2Si$, $-CH_3$), 1.24–1.4 (m, 6H, $3CH_2$), 1.54–1.55 (m, 2H, CH_2), 1.59 (br d, 1H, OH), 4.09–4.13 (m, 3H, CH_2-1, H4), 5.62–5.77 (m, 2H, H2, H3); δ_C (100 MHz, CD_2Cl_2) 134.9, 128.6, 72.7, 62.9, 38.2, 31.8, 24.8, 22.6, 13.7, 6.5 (3), 4.8 (3); *m/z* (GC-EI) 271 (2), 255 (100), 243 (25), 201 (25), 123 (15).

(4*R*)-4-Triethylsilyloxy-2*E*-nonen-1-ol (*ent*-24). *ent*-**24** was prepared in 58% yield from ***ent*-23** following the procedure described above for **24**. $[\alpha]_D^{24.7}$ +2.5 (*c* 0.32, $CHCl_3$).

(2*R*,3*S*,4*S*)-2,3-Epoxy-4-triethylsilyloxy-2*E*-nonan-1-ol (25). A stirred suspension of activated powdered 4 Å molecular sieves (0.075 g) and anhydrous CH_2Cl_2 (15 mL) was cooled under Ar to −40° C. In a separate flask, diethyl L-(+)-tartrate (0.027 g, 0.13 mmol) was stirred in anhydrous CH_2Cl_2 (3 mL) over activated 4 Å molecular sieves (about 0.1 g) for 15 min, then transferred to the reaction flask *via* syringe. In separate flasks, titanium tetraisopropoxide (0.031 g, 0.11 mmol) and cumene hydroperoxide (88%, 0.21 g, 1.38 mmol) were stirred in CH_2Cl_2 (5 mL) over activated 4 Å molecular sieves (about 0.2 g) for 15 min, then consecutively added dropwise to the reaction mixture *via* syringe. The reaction mixture was stirred for 40 min at −40° C under Ar. A solution of **24** (0.15 g, 0.55 mmol) in CH_2Cl_2 (15 mL) was stirred over activated 4 Å molecular sieves (about 0.1 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 (until **24** was no longer detectable TLC); the reaction was quenched by the addition of a solution of ferrous sulfate (15.0 g) in 10% aqueous tartaric acid (3 mL) to the reaction mixture at −25° C. The mixture was stirred for 1h, and then the organic phase was separated, washed with water (2 × 10 mL), dried over $MgSO_4$, filtrated, and concentrated. The residue was diluted with ether (75 mL) and stirred with 30% NaOH in saturated brine (2 mL) at 0° C for 20 min. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtrated and evaporated. Purification by flash chromatography on silica, eluting with 5% ethyl acetate in hexanes afforded **25** (0.115 g, 73%). $[\alpha]_D^{25}$ −15.7 (*c* 0.23, $CHCl_3$); δ_H (300 MHz, CD_2Cl_2) 0.55–0.64 (m, 6H, $3CH_2Si$), 0.90 (t, 3H, *J* = 6.8 Hz, CH_3), 0.93–0.99 (m, 9H, $3CH_3CH_2Si$), 1.29–1.40 (m, 6H, $3CH_2$), 1.55–1.58 (m, 3H, OH, CH_2), 2.93 (dd, 1H, *J* = 2.2, 4.7 Hz, H3), 3.13 (quintet, 1H, *J* = 2.2 Hz, H2), 3.61–3.66 (m, 2H, H1b, H4), 3.95 (dd, 1H, *J* = 2.0, 12.7, H1a); δ_C (75 MHz, CD_2Cl_2) 71.1, 62.0, 58.3, 56.7, 35.6, 32.3, 24.8, 23.0, 14.2, 6.9 (3), 5.2 (3); *m/z* (GC-EI) 289 (0.25, M^++1), 241 (100), 215 (65), 157 (50), 139 (80), 103 (75).

(2*S*,3*R*,4*R*)-2,3-Epoxy-4-triethylsilyloxy-2*E*-nonan-1-ol (*ent*-25). The Sharpless epoxidation of ***ent*-24** was performed in the same manner as described above for **25** using diethyl D-(−)-tartrate to afford ***ent*-25** in 75% yield. $[\alpha]_D^{23.6}$ +16.8 (*c* 0.45, $CHCl_3$).

(2*S*,3*R*,4*S*)-2,3-Epoxy-4-triethylsilyloxy-2*E*-nonan-1-ol (26). The Sharpless epoxidation of **24** was performed in the same manner as described above for **25** using diethyl D-(−)-tartrate to afford **26** in 74% yield. $[\alpha]_D^{24.6}$ +11.3 (*c* 0.47, $CHCl_3$); δ_H (300 MHz, CD_2Cl_2) 0.57–0.65 (m, 6H, $3CH_2Si$), 0.88 (t, 3H, *J* = 6.7 Hz, CH_3), 0.92–0.98 (m, 9H, $3CH_3CH_2Si$), 1.28–1.32 (m, 6H, $3CH_2$), 1.47–1.54 (m, 2H, CH_2), 1.81 (br s, 1H, OH), 2.89 (dd, 1H, *J* = 2.3, 6.6 Hz, H3), 2.97 (quintet, 1H, *J* = 2.4 Hz, H2), 3.37 (q, 1H, *J* = 6.5 Hz, H4), 3.56 (d, 1H, *J* = 4.6, 12.8 Hz, H1b), 3.91 (d, 1H, *J* = 2.3, 12.6 Hz, H1a); δ_C (75 MHz, CD_2Cl_2) 74.0, 61.9, 59.6, 56.8, 35.3, 32.3, 24.3, 22.9, 14.1, 6.9 (3), 5.2 (3); *m/z* (GC-EI) 289 (0.5, M^++1), 241 (100), 215 (60), 157 (40), 139 (80), 103 (60).

(2*R*,3*S*,4*R*)-2,3-Epoxy-4-triethylsilyloxy-2*E*-nonan-1-ol (*ent*-26). The Sharpless epoxidation of ***ent*-24** was performed in the same manner as described above for **25** using diethyl L-(+)-tartrate to afford ***ent*-26** in 77% yield. $[\alpha]_D^{23.9}$ −8.9 (*c* 0.27, $CHCl_3$).

(2*S*,3*S*,4*S*)-2,3-Epoxy-4-triethylsilyloxy-2*E*-nonan-1-al (27). (Diacetoxyiodo)benzene (0.135 g, 0.42 mmol) was added to a solution of **25** (0.11 g, 0.38 mmol) and 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO, 0.006 g, 0.038 mmol) in 10 mL dry CH_2Cl_2. The reaction mixture was stirred at room temperature until **25** was no longer detectable by TLC, and then diluted with CH_2Cl_2 (20 mL). The mixture was washed with a saturated aqueous solution of $Na_2S_2O_3$ (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with 5% $NaHCO_3$ (10 mL) and brine, dried over $MgSO_4$, filtrated, and evaporated. Purification by flash chromatography on silica, eluting with 3% ethyl acetate in hexanes afforded **27** (0.07 g, 64%); δ_H (300 MHz, CD_2Cl_2); 0.55–0.63 (m, 6H, $3CH_2Si$), 0.87–0.97 (m, 12H, CH_3, $3CH_3CH_2Si$), 1.27–1.43 (m, 6H, $3CH_2$), 1.50–1.58 (m, 2H, CH_2), 3.20 (dd, 1H, *J* = 1.9, 3.7 Hz, H3), 3.38 (dd, 1H, *J* = 1.8, 6.3 Hz, H2), 3.79–3.84 (m, 1H, H4), 9.08 (d, 1H, *J* = 6.3 Hz, CHO); δ_C (75 MHz, CD_2Cl_2) 198.7, 69.7, 59.4, 56.7, 35.4, 32.2, 24.7, 22.9, 14.1, 6.8 (3), 5.1 (3); *m/z* (GC-EI) 287 (7.5, M^++1), 269 (75), 241 (100), 227 (50) 215 (30), 157 (65), 109 (30), 81 (20).

(2*R*,3*R*,4*R*)-2,3-Epoxy-4-triethylsilyloxy-2*E*-nonan-1-al (*ent*-27). *ent*-**27** was prepared from ***ent*-25** in 76% yield following the procedure described above for **27**. $[\alpha]_D^{23.6}$ −33.5 (*c* 0.46, $CHCl_3$).

(2*R*,3*R*,4*S*)-2,3-Epoxy-4-triethylsilyloxy-2*E*-nonan-1-al (28). **28** was prepared from **26** in 76% yield following the procedure described above for **27**. $[\alpha]_D^{24.7}$ −59.6 (*c* 0.46, $CHCl_3$); δ_H NMR (300 MHz, $CDCl_3$); 0.58–0.68 (m, 6H, $3CH_2Si$), 0.89 (t, 3H, *J* = 6.8 Hz, CH_3), 0.94–1.0 (m, 9H, $3CH_3CH_2Si$), 1.22–1.42 (m, 6H, $3CH_2$), 1.50–1.60 (m, 2H, CH_2), 3.22–3.26 (m, 2H, H3, H4), 3.44 (q, 1H, *J* = 6.2 Hz, H2), 9.02 (d, 1H, *J* = 6.3 Hz, CHO); δ_C (75 MHz, CD_2Cl_2) 198.2, 73.0, 60.2, 57.6, 35.1, 32.1, 25.1, 22.8, 14.1, 6.9 (3), 5.1 (3); *m/z* (GC-EI) 287 (5, M^++1), 269 (65), 241 (100), 227 (50) 215 (28), 157 (100), 109 (25), 81 (15).

(2*S*,3*S*,4*R*)-2,3-Epoxy-4-triethylsilyloxy-2*E*-nonan-1-al (*ent*-28). *ent*-**28** was prepared from ***ent*-26** in 76% yield following the procedure described above for **28**.

(2*S*,3*R*,4*S*)-2,3-Epoxy-4-hydroxy-2*E*-nonan-1-al (3). Tetrabutylammonium fluoride (0.25 mL, 1.0 M in THF) was added to a stirred solution of **27** (0.06 g, 0.21 mmol) in THF (2 mL) and the reaction was stirred for 1 h at ambient temperature. The solvent was then removed under reduced pressure. Purification by flash chromatography on silica, eluting with 2% methanol in CH_2Cl_2 afforded **3** (0.03 g, 83%). $[\alpha]_D^{23.1}$ +4.8

(c 0.84, CH_2Cl_2); δ_H (400 MHz, CD_2Cl_2); 0.91 (t, 3H, J = 6.8 Hz, CH_3), 1.28–1.62 (m, 8H, $4CH_2$), 1.77 (br s, 1H, OH), 3.31 (dd, 1H, J = 2.1, 2.8 Hz, H3), 3.45 (dd, 1H, J = 1.8, 6.0 Hz, H2), 3.96–3.99 (m, 1H, J = 3.3 Hz, H4), 9.14 (d, 1H, J = 6.1 Hz, CHO); δ_C (75 MHz, CD_2Cl_2) 198.5, 68.3, 59.5, 55.9, 33.7, 32.1, 25.1, 22.9, 14.1; m/z (GC-EI) 173 (0.5, M^++1), 83 (95), 71 (100), 55 (90).

(2*R*,3*S*,4*R*)-2,3-Epoxy-4-hydroxy-2*E*-nonan-1-al (4). 4 was prepared from ***ent*-27** in 72% yield following the procedure described above for **3**. $[\alpha]_D^{24.3}$ –2.1 (c 0.52, $CHCl_3$).

(2*R*,3*S*,4*S*)-2,3-Epoxy-4-hydroxy-2*E*-nonan-1-al (5). 5 was prepared from **28** in 89% yield following the procedure described above for **3**. $[\alpha]_D^{24.3}$ –40.2 (c 0.61, $CHCl_3$). δ_H (400 MHz, CD_2Cl_2); 0.91 (t, 3H, J = 6.8 Hz, CH_3), 1.26–1.36 (m, 6H, $3CH_2$), 1.59–1.65 (m, 2H, CH_2), 1.76 (d, 1H, J = 6.7 Hz, OH), 3.28 (dd, 1H, J = 2.0, 4.4 Hz, H3), 3.38 (dd, 1H, J = 2.0, 6.1 Hz, H2), 3. 62 (ddd, 1H, J = 1.6, 4.7, 6.6 Hz, H4), 9.08 (d, 1H, J = 6.1 Hz, CHO); δ_C NMR (75 MHz, CD_2Cl_2) 198.2, 70.5, 59.8, 57.2, 34.7, 32.0, 25.3, 22.9, 14.1; m/z (GC-EI) 173 (0.2, M^++1), 83 (65), 71 (80), 55 (100).

(2*S*,3*R*,4*R*)-2,3-Epoxy-4-hydroxy-2*E*-nonan-1-al (6). 6 was prepared from ***ent*-28** in 92% yield following the procedure described above for **3**. $[\alpha]_D^{24}$ +38.5 (c 0.52, $CHCl_3$).

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-7-(1*S*,2*S*-dihydroxyheptyl)-3,4-dihydro-9*H*-imidazo[1,2-*a*]purin-9-one (11). K_2CO_3 (8.10 mg, 58.7 μmol) was added to a solution of dGuo·H_2O (7.38 mg, 25.9 μmol) in DMF (500 μL) and the mixture was stirred for 15 min. A solution of (2*S*,3*R*,4*S*)-EHN (**3**, 13.40 mg, 77.9 μmol) in DMF (800 μL) was added to the suspension. The reaction mixture was stirred at room temperature for 12 h; the formation of the desired product was monitored by HPLC using gradient systems I (flow rate 1.5 mL min^{-1}). Water was added (4 mL) and reaction mixture was neutralized to pH 7.0 with 1% HCl (aq). The resulting solution was filtered through a 0.45 μm filter cartridge prior for purification by semi-preparative HPLC using gradient system I (flow rate 5.0 mL min^{-1}). The solution from the reaction was kept frozen during the purification. The fractions collected from the HPLC were immediately cooled to −78° C to prevent epimerization of the product. Lyophilization afforded the modified nucleoside **11** (6.55 mg, 60% yield) as a white solid. The purity of the product was judged to be >99% by analytical HPLC (gradient system II, flow rate 1.5 mL min^{-1}). δ_H NMR (600 MHz, D_2O/CD_3CN); 0.73 (t, 3H, J = 6.8 Hz, CH_3), 1.10–1.30 (m, 5H, $H13_b$, H14, $H15_)$, 1.32–1.41 (m, 3H, $H13_a$, H12), 2.34–2.38 (m, 1H, H2″), 2.65–2.70 (m, 1H, H2′), 3.63 (dd, 1H, J = 4.1, 12.3 Hz, H5″), 3.70 (dd, 1H, J = 3.5, 12.3 Hz, H5′), 3.87–3.91 (m, 1H, H11), 3.97–3.99 (m, 1H, H4′), 4.48–4.51 (m, 1H, H3′), 5.02 (d, 1H, J = 5.5 Hz, H10), 6.22 (t, 1H, J = 6.9 Hz, H1′), 7.16 (s, 1H, H6), 7.95 (s, 1H, H2); δ_C (150.9 MHz, D_2O/CD_3CN) 155.4, 150.3, 147.4, 139.7, 125.6, 117.1, 116.5, 88.3, 85.5, 74.1, 72.3, 70.3, 62.8, 39.8, 33.7, 32.2, 25.8, 23.0, 14.3; m/z (FAB) 422.2020 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040).

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-7-(1*R*,2*R*-dihydroxyheptyl)-3,4-dihydro-9*H*-imidazo[1,2-a]purin-9-one (12). Nucleoside **12** was obtained from (2*R*,3*S*,4*R*)-EHN (**4**) as a white solid in 67% yield and >99% purity by analytical HPLC (gradient system II) following the procedure described above for **11**. δ_H (600 MHz, D_2O/CD_3CN) 0.74 (t, 3H, J = 6.9 Hz, CH_3), 1.13–1.20 (m, 5H, $H13_b$, H14, $H15_)$, 1.27–1.37 (m, 3H, $H13_a$,H12), 2.34–2.38 (m, 1H, H2″), 2.66–2.71 (m, 1H, H2′), 3.63 (dd, 1H, J = 4.2, 12.3 Hz, H5″), 3.70 (dd, 1H, J = 3.6, 12.3 Hz, H5′), 3.86–3.90 (m, 1H, H11), 3.98–4.0 (m, 1H, H4′), 4.48–4.51 (m, 1H, H3′), 5.01 (d, 1H, J = 5.6 Hz, H10), 6.30 (dd, 1H, J = 6.3, 7.4 Hz, H1′), 7.17 (s, 1H, H6), 7.97 (s, 1H, H2); δ_C (150.9 MHz, D_2O/CD_3CN) 155.6, 150.4, 147.6, 139.8, 125.4, 117.3, 116.7, 88.3, 85.6, 74.2, 72.3, 70.4, 62.9, 39.8, 33.7, 32.2, 25.8, 23.0, 14.3: m/z (FAB) 422.2050 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040).

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-7-(1*R*,2*S*-dihydroxyheptyl)-3,4-dihydro-9*H*-imidazo[1,2-a]purin-9-one (13). Nucleoside **13** was obtained as a white solid in 62% yield and >99% purity by analytical HPLC (gradient system *II*) from (2*R*,*3S*,4*S*)-EHN (**5**) following the procedure described above for **11**. δ_H (600 MHz, DMSO-d_6) 0.84 (t, 3H, J = 6.9 Hz, CH_3), 1.21–1.32 (m, 6H, $H12_b$, $H13_b$, 2CH2), 1.45–1.50 (m, 1H, $H13_a$), 1.68–1.73 (m, 1H, $H12_a$), 2.23–2.26 (m, 1H, H2″), 2.58–2.61 (m, 1H, H2′), 3.49–3.53 (m, 1H, H5″), 3.56–3.60 (m, 1H, H5′), 3.67 (q, 1H, J = 6.7 Hz, H11), 3.85 (q, 1H, J = 3.8 Hz, H4′), 4.37–4.39 (m, 2H, H3′, 11-OH), 4.77 (t, 1H, J = 7.9 Hz, H10), 4.94 (t, 1H, J = 5.4 Hz, 5′-OH), 5.28 (d, 1H, J = 4.0 Hz, 3′-OH), 5.35 (d, 1H, J = 8.5 Hz, 10-OH), 6.23 (dd, 1H, J = 6.4, 7.4 Hz, H1′), 7.23 (s, 1H, H6), 8.12 (s, 1H, H2); δ_C (150.9 MHz, DMSO-d_6) 154.0, 149.7, 146.7, 137.5, 125.4, 116.1, 115.3, 87.7, 83.0, 72.5, 70.7, 69.8, 61.7, 40.0, 32.8, 31.5, 24.9, 22.1, 13.9. m/z (FAB) 422.2020 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040)

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-7-(1*S*,2*R*-dihydroxyheptyl)-3,4-dihydro-9*H*-imidazo[1,2-a]purin-9-one (14). Nucleoside **14** was prepared from (2*S*,3*R*,4*R*)-2,3-epoxy-4-hydroxynonanal (**6**) in 71% yield as a white solid and >99% purity by analytical HPLC (gradient system II) following the procedure described above for **11**. δ_H (600 MHz, D_2O/CD_3CN); δ 0.79 (t, 3H, J = 6.9 Hz, CH_3), 1.16–1.32 (m, 6H, $H13_b$, $H12_b$, $2CH_2$), 1.40–1.45 (m, 1H, $H13_a$), 1.68–1.75 (m, 1H, $H12_a$), 2.23–2.30 (m, 1H, H2′), 2.61–2.73 (m, 1H, H2′), 3.65 (dd, 1H, J = 4.4, 12.4 Hz, H5″), 3.70 (dd, 1H, J = 3.4, 12.4 Hz, H5′), 3.92–3.89 (m, 1H, H11), 3.99 (q, 1H, J = 3.6, H4′), 4.37–4.49 (quintet, 1H, J = 3.0 Hz, H3′), 4.91 (d, 1H, J = 7.0 Hz, H10), 6.23 (t, 1H, J = 7.0 Hz, H1′), 7.15 (s, 1H, H6), 7.93 (s, 1H, H2); δ_C (150.9 MHz, D_2O/CD_3CN) 155.6, 150.3, 147.5, 139.8, 125.3, 117.3, 117.0, 88.3, 85.6, 74.0, 72.3, 70.9, 62.9, 39.9, 33.0, 32.3, 25.8, 23.2, 14.4; m/z (FAB) 422.2018 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040).

General procedure for synthesis of the tetracyclic adducts: (5a*R*,7*S*,8*S*,8a*S*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,5a,7,8,8a-hexahydro-8-hydroxy-7-pentyl-10*H*-furo[2′,3′:4,5]imidazo[1,2-*a*]purin-10-one (15) and (5a*S*,7*S*,8*S*,8a*R*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,5a,7,8,8a-hexahydro-8-hydroxy-7-pentyl-10*H*-furo[2′,3′:4,5]imidazo[1,2-*a*]purin-10-one (16). Nucleoside **11** (1.9 mg, 4.5 μmol) was dissolved in phosphate buffer (19 mL, 0.025 M, pH 6.8) and sonicated for 10 min. The reaction mixture was stirred at room temperature for 68 h; during this time, the reaction was monitored by analytical HPLC (every ~24 h) for the formation of desired products using gradient system III. The product mixture was filtrated through a 0.45 μm filter cartridge, rinsed with water (3 × 2 mL), and lyophilized. The resulting solid residue was dissolved in water (3 mL) and purified by semi-preparative HPLC using gradient systems IV (flow rate 5.0 mL min^{-1}). The fractions were

immediately frozen after collection to prevent the epimerization of the products and lyophilized to afford nucleosides **15** (1.0 mg, 51% isolated yield; 69% based on recovered starting material) and **16** (0.2 mg, 9% isolated yield; 12% based on recovered starting material) as white solids. The purity of the obtained adducts were judged to be >99% by analytical HPLC (gradient systems I or II, flow rate 1.5 mL min^{-1}). **15**: δ_H (600 MHz, CD_3CN/D_2O) 0.80 (t, 3H, J = 6.9 Hz, CH_3), 1.18–1.31 (m, 6H, $3CH_2$), 1.59 (q, 2H, J = 7.3 Hz, CH_2 H11), 2.29–2.32 (m, 1H, H2′), 2.56–2.58 (m, 1H, H2″), 3.62 (dd, 1H, J = 3.7, 12.4 Hz, H5″), 3.67 (dd, 1H, J = 3.4, 12.4 Hz, H5′), 3.71 (td, 1H, J = 2.7, 6.9 Hz, H7), 3.96 (q, 1H, J = 3.2 Hz, H4′), 4.33 (d, 1H, J = 2.7 Hz, H8), 4.45 (quintet, 1H, J = 2.8 Hz, H3′), 4.72 (d, 1H, J = 6.6 Hz, H8a), 5.92 (d, 1H, J = 6.6 Hz, H5a), 6.14 (dd, 1H, J = 6.4, 7.7 Hz, H1′), 7.82 (s, 1H, H2); m/z (FAB) 422.2036 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040).

16: δ_H (600 MHz, CD_3CN/D_2O) 0.83 (t, 3H, J = 6.9 Hz, CH_3), 1.23–1.36 (m, 6H, $3CH_2$), 1.54–1.62 (m, 2H, CH_2 H11), 2.29–2.33 (m, 1H, H2′), 2.59–2.63 (m, 1H, H2″), 3.62 (dd, 1H, J = 3.9, 12.4 Hz, H5″), 3.68 (dd, 1H, J = 3.5, 12.4 Hz, H5′), 3.76 (td, 1H, J = 2.7, 6.9 Hz, H7), 3.96 (q, 1H, J = 3.3, H4′), 4.43 (dd, 1H, J = 2.7, 5.8 Hz, H8), 4.45 (quintet, 1H, J = 2.9 Hz, H3′), 4.97 (dd, 1H, J = 5.8, 7.7 Hz, H8a), 5.59 (d, 1H, J = 7.7 Hz, H5a), 6.15 (dd, 1H, J = 6.4, 7.6 Hz, H1′), 7.83 (s, 1H, H2); m/z (FAB) 422.2052 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040).

(5a*S*,7*R*,8*R*,8a*R*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,5a,7,8,8a-hexahydro-8-hydroxy-7-pentyl-10*H*-furo[2′,3′:4,5]-imidazo[1,2-*a*]purin-10-one (17) and (5a*R*,7*R*,8*R*,8a*S*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,5a,7,8,8a-hexahydro-8-hydroxy-7-pentyl-10*H*-furo[2′,3′:4,5]imidazo[1,2-*a*]purin-10-one (18)

Following the general procedure as described above, **12** (6.9 mg, 16.8 μmol) was incubated in phosphate buffer (69 mL, 0.025 M, pH 6.8) for 96 h to afford **17** (4.1 mg, 58% isolated yield; 68% based on recovered starting material) and **18** (0.65 mg, 9% isolated yield; 10% based on recovered starting material) were obtained as white solids. The purity of the products was judged to be >99.9% by analytical HPLC (gradient system *I* or *II*).

17: UV λ_{max} (5 : 1 H_2O/CH_3CN) nm 250 (ε 12,908); δ_H (600 MHz, CD_3CN/D_2O) 0.78 (t, 3H, J = 6.8 Hz, CH_3), 1.18–1.26 (m, 6H, $3CH_2$), 1.58 (q, 2H, J = 7.3 Hz, CH_2 H11), 2.29–2.34 (m, 1H, H2′), 2.56–2.60 (m, 1H, H2″), 3.61 (dd, 1H, J = 4.1 Hz, J = 12.4 Hz, H5″), 3.67 (dd, 1H, J = 3.6 Hz, J = 12.4 Hz, H5′), 3.71 (td, 1H, J = 2.6 Hz, J = 6.9 Hz, H7), 3.95 (q, 1H, J = 3.5 Hz, H4′), 4.33 (d, 1H, J = 2.6 Hz, H8), 4.45 (quintet, 1H, J = 2.9 Hz, H3′), 4.72 (d, 1H, J = 6.6 Hz, H8a), 5.91 (d, 1H, J = 6.6 Hz, H5a), 6.16 (dd, 1H, J = 6.3 Hz, J = 7.7 Hz, H1′), 7.83 (s, 1H, H2); m/z (FAB) 422.2063 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040).

18: δ_H (600 MHz, CD_3CN/D_2O) 0.82 (t, 3H, J = 6.9 Hz, CH_3), 1.24–1.35 (m, 6H, $3CH_2$), 1.55–1.60 (m, 2H, J = 6.9 Hz, CH_2, H11), 2.30–2.35 (m, 1H, H2′), 2.57–2.62 (m, 1H, H2″), 3.62 (dd, 1H, J = 4.0, 12.4 Hz, H5″), 3.68 (dd, 1H, J = 3.5, 12.4 Hz, H5′), 3.76 (td, 1H, J = 2.6, 6.9 Hz, H7), 3.96 (q, 1H, J = 3.1 Hz, H4′), 4.43 (dd, 1H, J = 2.5 Hz, J = 5.8 Hz, H8), 4.45 (quintet, 1H, J = 2.9 Hz, H3′), 4.98 (dd, 1H, J = 5.8, 7.6 Hz, H8a), 5.59 (d, 1H, J = 7.7 Hz, H5a), 6.15 (dd, 1H, J = 6.5, 7.4 Hz, H1′), 7.84 (s, 1H, H2); m/z (ESI-TOF) 422.2056 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040).

(5a*R*,7*S*,8*R*,8a*S*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,5a,7,8,8a-hexahydro-8-hydroxy-7-pentyl-10*H*-furo[2′,3′:4,5]-imidazo[1,2-*a*]purin-10-one (19) and (5a*S*,7*S*,8*R*,8a*R*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,5a,7,8,8a-hexahydro-8-hydroxy-7-pentyl-10*H*-furo[2′,3′:4,5]imidazo[1,2-*a*]purin-10-one (20). Following the general procedure described above, **13** was (2.9 mg, 6.8 μmol) was incubated in phosphate buffer (29 mL, 0.025 M, pH 6.8) for 114 h. Purification by semi-preparative HPLC using gradient system V (flow rate 5.0 mL min^{-1} afforded **19** (0.7 mg, 24% isolated yield; 30% based on recovered starting material) and **20** (1.1 mg, 38% isolated yield; 49% based on recovered starting material) as white solids. The purity of the products was judged to be >99% by analytical HPLC (gradient system *I* or *II*).

19: δ_H (600 MHz, CD_3CN/D_2O) 0.82 (t, 3H, J = 6.1 Hz, CH_3), 1.20–1.25 (m, 4H, $2CH_2$), 1.32–1.40 (m, 2H, $H12_a$, $H12_b$), 1.45–1.52 (m, 1H, $H11_b$), 1.60–1.66 (m, 1H, $H11_a$), 2.30–2.34 (m, 1H, H2″), 2.56–2.60 (m, 1H, H2′), 3.58 (dt, 1H, J = 4.3, 8.0 Hz, H7), 3.61 (dd, 1H, J = 4.0, 12.4 Hz, H5″), 3.71 (dd, 1H, J = 3.5, 12.3 Hz, H5′), 3.97 (m, 1H, H4′), 4.07 (dd, 1H, J = 7.1, 7.9 Hz, H8), 4.45 (q, 1H, J = 2.9 Hz, H3′), 5.0 (t, 1H, J = 7.0 Hz, H8a), 5.89 (d, 1H, J = 6.9 Hz, H5a), 6.14 (dd, 1H, J = 6.4, 7.5 Hz, H1′), 7.83 (s, 1H, H2); m/z (ESI-TOF) 422.2032 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040).

20: δ_H (600 MHz, CD_3CN/D_2O) 0.75 (t, 3H, J = 7.0 Hz, CH_3), 1.12–1.16 (m, 4H, $2CH_2$), 1.20–1.23 (m, 2H, $H11_b$, $H12_b$), 1.30–1.33 (m, 2H, $H11_a$, $H12_a$), 2.28–2.33 (m, 1H, H2′), 2.56–2.60 (m, 1H, H2″), 3.62 (dd, 1H, J = 4.0, 12.3 Hz, H5″), 3.67 (dd, 1H, J = 3.6, 12.4 Hz, H5′), 3.90 (ddd, 1H, J = 2.6, 5.4, 8.1 Hz, H7), 3.95 (q, 1H, J = 3.4 Hz, H4′), 4.33 (dd, 1H, J = 1.2, 2.6 Hz, H8), 4.45 (q, 1H, J = 2.9 Hz, H3′), 4.73 (dd, 1H, J = 1.2, 6.7 Hz, H8a), 5.86 (d, 1H, J = 6.7 Hz, H5a), 6.14 (dd, 1H, J = 6.6, 7.5 Hz, H1′), 7.83 (s, 1H, H2); m/z (ESI-TOF) 422.2037 (MH^+, $C_{19}H_{28}N_5O_6$ requires 422.2040), 444.1859 (M+Na, $C_{19}H_{27}N_5O_6Na$ requires 444.1859).

(5a*S*,7*R*,8*S*,8a*R*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,5a,7,8,8a-hexahydro-8-hydroxy-7-pentyl-10*H*-furo[2′,3′:4,5]-imidazo[1,2-*a*]purin-10-one (21) and (5a*R*,7*R*,8*S*,8a*S*)-[3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,4,5a,7,8,8a-hexahydro-8-hydroxy-7-pentyl-10*H*-furo[2′,3′:4,5]imidazo[1,2-*a*]purin-10-one (22). Following the general procedure described above, **14** (2.5 mg, 5.9 μmol) was incubated in phosphate buffer (24 mL, 0.025 M, pH 6.8) for 72 h. Purification by semi-preparative HPLC using gradient system V (flow rate 5.0 mL min^{-1}) afforded **21** (0.5 mg, 19% isolated yield; 23% based on recovered starting material) and **22** (0.75 mg, 29% isolated yield; 36% based on recovered starting material) as white solids. The purity of the products was judged to be >99.5% by analytical HPLC (gradient system *I* or *II*).

21: δ_H (600 MHz, CD_3CN/D_2O) 0.85 (t, 3H, J = 6.2 Hz, CH_3), 1.27–1.29 (m, 4H, $2CH_2$), 1.37–1.43 (m, 2H, $H12_a$, $H12_b$), 1.49–1.55 (m, 1H, $H11_b$), 1.63–1.69 (m, 1H, $H11_a$), 2.33–2.37 (m, 1H, H2″), 2.62–2.67 (m, 1H, H2′), 3.59 (dt, 1H, J = 4.3, 8.0, H7), 3.64 (dd, 1H, J = 4.0, 12.4 Hz, H5″), 3.72 (dd, 1H, J = 3.5, 12.4 Hz, H5′), 4.05 (m, 1H, H4′), 4.10 (dd, 1H, J = 7.1, 8.0 Hz, H8), 4.49 (quintet, 1H, J = 2.9 Hz, H3′), 5.04 (t, 1H, J = 7.0 Hz, H8a), 5.92 (d, 1H, J = 6.9 Hz, H5a), 6.17 (dd, 1H, J = 6.4, 7.6 Hz, H1′), 7.88 (s, 1H, H2); m/z (ESI-TOF) 444.1855 (M+Na, $C_{19}H_{27}N_5O_6Na$ requires 444.1859.

22: δ_H (600 MHz, CD_3CN/D_2O) 0.78 (t, 3H, J = 7.1 Hz, CH_3), 1.16–1.19 (m, 4H, $2CH_2$), 1.23–1.26 (m, 2H, $H11_b$, $H12_b$), 1.33–1.36 (m, 2H, $H11_a$, $H12_a$), 2.32–2.36 (m, 1H, H2′), 2.58–2.63 (m,

1H, H2″), 3.63 (dd, 1H, J = 3.9, 12.4 Hz, H5″), 3.70 (dd, 1H, J = 3.5, 12.4 Hz, H5′), 3.92 (ddd, 1H, J = 2.6, 5.3, 7.9 Hz, H7), 3.98–3.99 (m, 1H, H4′), 4.32 (dd, 1H, J = 1.2, 2.6 Hz, H8), 4.48 (q, 1H, J = 2.9 Hz, H3′), 4.72 (dd, 1H, J = 1.2, 6.7 Hz, H8a), 5.89 (d, 1H, J = 6.7 Hz, H5a), 6.17 (dd, 1H, J = 6.4, 7.6 Hz, H1′), 7.87 (s, 1H, H2); m/z (ESI-TOF) 444.1858 (M+Na, $C_{19}H_{27}N_5O_6Na$ requires 444.1859).

HPLC. The analysis of reaction mixtures and the purification of nucleosides were conducted on a gradient HPLC system with a diode array UV detector monitoring at 260 nm using C18 reversed-phase columns (250 × 4.6 mm i.d., flow rate of 1.5 mL min^{-1} for analysis and 250 × 10 mm i.d, flow rate 5 mL min^{-1} for purification). The mobile phase consisted of H_2O and CH_3CN using the following gradients: *Gradient I*: 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. *Gradient II*: initially 90% H_2O; a 25 min linear gradient to 20% H_2O, followed by a 5 min linear gradient to the initial conditions. *Gradient III*: initially 90% H_2O; a 5 min linear gradient to 75% H_2O; isocratic at 75% H_2O for 20 min, 4 min linear gradient to 20% H_2O, followed by a 4 min linear gradient to the initial conditions. *Gradient IV*: initially 90% H_2O; a 4 min linear gradient to 75% H_2O; isocratic at 75% H_2O for 20 min, 4 min linear gradient to 20% H_2O; isocratic at 80% H_2O for 2 min, followed by a 4 min linear gradient to the initial conditions. *Gradient V*: initially 90% H_2O; a 3 min linear gradient to 78% H_2O; isocratic at 78% H_2O for 25 min, 4 min linear gradient to 20% H_2O; isocratic at 80% H_2O for 3 min, followed by a 4 min linear gradient to the initial conditions.

Separation of adducts 10–22. A solution containing adducts **11–22** was prepared from standard solutions of the pure compounds (1.0 μg μL^{-1} in 10 : 1 H_2O/CH_3CN). The mixture was analyzed by reversed-phase HPLC (250 mm × 4.6 mm, flow rate 1.0 mL min^{-1}). The mobile phase consisted of H_2O and CH_3CN using the following gradients: initially 90% H_2O; a 3 min linear gradient to 78% H_2O; isocratic at 78% H_2O for 25 min, 4 min linear gradient to 20% H_2O, followed by a 4 min linear gradient to 90% H_2O and remained for 1 min at the initial conditions.

Electronic Circular Dichroism (ECD) spectroscopy. All ECD spectra were recorded at 25 °C in a 1.0 cm circular quartz cell and are an average of three individual scans. The instrument was calibrated with ammonium *d*-camphor-10-sulfonate. The sample concentrations were 0.025–0.05 μg μL^{-1} in 5 : 1 (v/v) H_2O/CH_3CN. The spectra were recorded at a scan speed of 100 nm min^{-1} and a time constant of 1s. The parameters bandwidth of 1 nm, resolution of 1 nm, and sensitivity of 100 mdeg were fixed before recording the spectra. The corresponding solvent spectrum was subtracted from the ECD spectrum of nucleoside solution. The resulting spectra were further processed for smoothening when needed, using the manufacturer's software. The ECD intensities are presented in units of molar ellipticity (Θ) *vs.* wavelength.

NMR experiments for the nucleosides. NMR experiments were recorded using a 14.0 T Bruker magnet equipped with Bruker AV-III console operated at 600.13 MHz. All spectra were acquired in 3 mm NMR tubes using a Bruker 5 mm TCI cryogenically cooled NMR probe. Chemical shifts were referenced internally to CD_3CN (1.93 ppm) or DMSO-d_6 (2.49 ppm), which also served as the ^2H lock solvents. Typical experimental conditions for 1D ^1H NMR spectra included 32 K data points, 13 ppm sweep width, a recycle delay of 1.5 s and 32–512 scans depending on sample concentration. Experimental conditions for 2D ^1H–^1H COSY analysis included 2048 Í 512 data matrix, 13 ppm sweep width, recycle delay of 1.5 s and 4 scans per increment. The data was processed using squared sinebell window function, symmetrized, and displayed in magnitude mode.

Double Pulse Field Gradient Spin Echo (DPFGSE)–NOE and homonuclear decoupling NMR experiments. Selective 1D NOE spectra were recorded using the DPFGSE technique.[43–45] Experimental parameters for this experiment were similar to those for the standard 1D ^1H experiment with the addition of 600 ms mixing time. A standard Gaussian shaped pulse was used for the excitation pulse; the operating software automatically calculated the length and power of the pulse based on the peak integration area of the signal that was irradiated. All samples were dissolved in D_2O/CD_3CN (1 : 1) and degassed by carefully bubbling high purity He gas through the solution in the NMR tube for ~10 min. A fine capillary was used to slowly introduce the He gas to avoid blowing the sample out of the tube. The 1D ^1H homonuclear decoupling experiments were run using the standard pulse sequence included in the manufacturer's software; the software automatically calculated the irradiation frequencies.

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