

Stereocontrolled Syntheses of All Four Stereoisomeric 1,*N*²-Deoxyguanosine Adducts of the Lipid Peroxidation Product *trans*-4-Hydroxynonenal

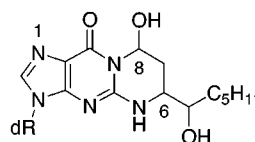
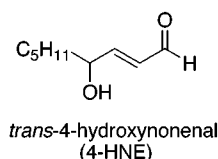
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ABSTRACT



trans-4-Hydroxynonenal (4-HNE) is a unique product from the peroxidation of ω -6 polyunsaturated fatty acids. The major reaction of racemic 4-HNE with DNA is with deoxyguanosine to give four stereoisomeric exocyclic propano adducts. The stereospecific syntheses of these four adducts has been achieved at the nucleoside level. The synthetic approach is amenable to the synthesis of structurally defined oligonucleotides containing these endogenous genotoxins.

Reactive oxygen species such as hydroxyl radical are produced by cellular respiration. These species abstract hydrogen atoms from polyunsaturated fatty acids to generate carbon-centered radicals, which in turn react with molecular oxygen to generate the corresponding hydroperoxy radicals.¹ Subsequent fragmentation of the lipid hydroperoxides generates complex arrays of lipid peroxidation products. A number of these products have been identified as relatively simple aliphatic α,β -unsaturated aldehydes (enals) such as acrolein, crotonaldehyde, 4-hydroxynonenal, and 4-oxononenal.² Acrolein and crotonaldehyde are also ubiquitous environmental pollutants and are found in cigarette smoke. These substances react with proteins and are toxic. The simpler enals acrolein and crotonaldehyde induce revertants in the Ames *Salmonella* tester strains TA100 and TA104;³ thus the lipid peroxidation products represent a class of endogenous mutagens.⁴ 4-Hydroxynonenal (4-HNE), the major lipid peroxidation product

derived from ω -6 polyunsaturated fatty acids, was reported to be inactive in these *Salmonella* tester strains, while its lower homologue 4-hydroxypentenal was shown to be mutagenic. It is likely that the toxicity of 4-HNE masked its genotoxicity in these assays. Consistent with this hypothesis is that 4-HNE induced mutations in V79 Chinese hamster ovary cells and induced an SOS response in *Salmonella typhimurium*.^{5,6}

The reaction of enals with nucleobases produces exocyclic propano adducts. For deoxyguanosine, this involves initial Michael addition of the exocyclic amino group followed by ring closure of N1 onto the aldehyde group. The reaction of deoxyguanosine with 4-HNE gives three new stereogenic centers. The relative stereochemistry of the C8-hydroxy group and the C6-hydroxyalkyl side chain has been determined to be *trans*.⁷ Thus, four diastereomeric adducts, **1–4** (Figure 1), are possible, which may have different properties when incorporated into oligonucleotides.⁸ We report here the stereocontrolled syntheses of all four stereoisomers of the

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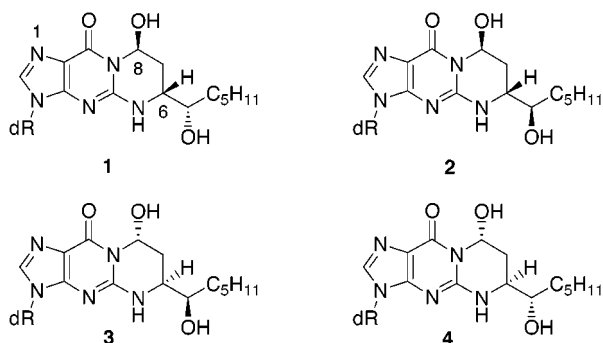


Figure 1. 1,*N*²-Deoxyguanosine adducts of 4-HNE.

1,*N*²-deoxyguanosine adduct of 4-HNE. The synthesis is amenable to site-specific syntheses of oligonucleotides containing stereochemically defined adducts of 4-HNE.

The site-specific syntheses of oligonucleotides containing 1,*N*²-deoxyguanosine adducts of acrolein (**7**) have been reported by Johnson and by Harris.^{9,10} In both cases, a vicinal diol unit was used as a surrogate for the aldehyde; the vicinal diol was cleaved with sodium periodate after assembly of the oligonucleotides. The three-carbon acrolein unit was introduced via a nucleophilic aromatic substitution reaction of amino alcohol **6** with an O⁶-protected 2-fluoroinosine derivative **5** (Scheme 1).

We envisioned a similar strategy for the synthesis of 1,*N*²-deoxyguanosine adducts of 4-HNE. Enantiomeric amino alcohols **8** and **9**, possessing the anti stereochemistry, are required for the synthesis of adducted nucleosides **1** and **2**, respectively (Figure 2), whereas syn amino alcohols **10** and **11** would give **3** and **4**. Enantioselective syntheses of amino alcohols **9** and **11** are shown in Schemes 2 and 3.

The synthesis of **9** began with a Sharpless asymmetric epoxidation of (*E*)-oct-2-en-1-ol using (–)-diethyl tartrate as the ligand, to give the epoxy alcohol **13**; a minor stereoisomer could not be detected by high field NMR analysis of the corresponding Mosher's ester of **13**.¹¹ Reaction of **13** with benzyl isocyanate afforded a separable mixture of two cyclic *N*-benzyl urethanes **14** and **15**; the minor isomer **14** could be equilibrated by treatment with sodium hydride.¹² At this point, a two-carbon extension of the aliphatic chain was accomplished by converting the primary alcohol of **15** to the corresponding iodide followed by displacement with vinylmagnesium bromide to give **16**.

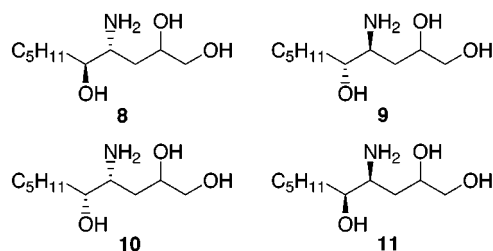
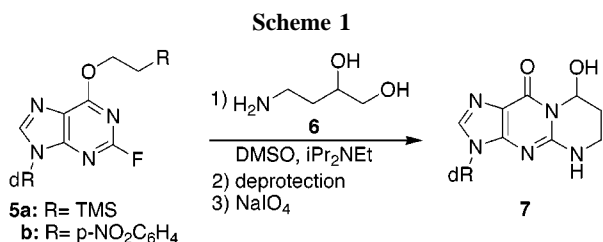
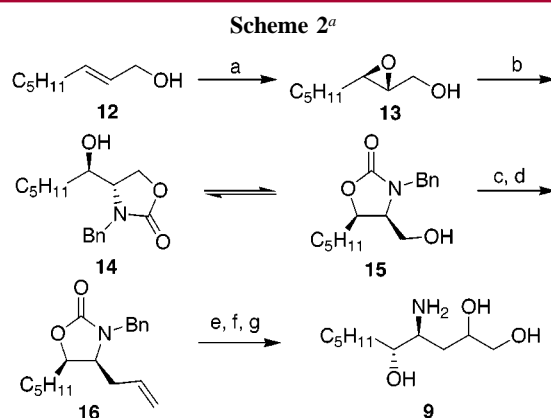


Figure 2. Amino alcohols for the synthesis of **1–4**.

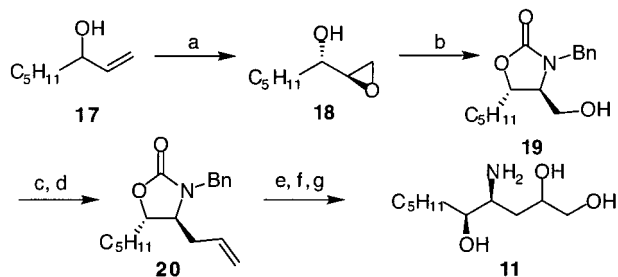
Dihydroxylation gave a 1:1 mixture of vicinal diols; since the vicinal diol is eventually oxidatively cleaved to the corresponding aldehyde by periodate, the mixture of stereoisomers at this center is of no consequence. Deprotection gave the desired anti amino alcohol (**9**) in enantiomerically pure form. The enantiomeric amino alcohol **8** was prepared using (+)-diethyl tartrate as the ligand for the Sharpless epoxidation.

Using a similar synthetic strategy, we have synthesized the syn amino alcohol **11** (Scheme 3). The stereochemistry is established through a Sharpless kinetic resolution of racemic 3-hydroxy-1-octene using (+)-diisopropyl tartrate as the chiral ligand for the titanium catalyst. We estimate that the epoxy alcohol (**18**) was produced in 92% ee on the basis of high field NMR analysis of the corresponding Mosher's ester.^{11b} Treatment of **18** with benzyl isocyanate gave a single cyclic *N*-benzyl urethane **19**. The remainder of the synthesis proceeded by an identical sequence of reactions as for the anti amino alcohol discussed above to give **11**. Once again, the enantiomeric syn amino alcohol **10** was synthesized by simply changing to the antipodal tartrate ligand during the Sharpless kinetic resolution.

Amino alcohols **8–11** were individually condensed with 2'-deoxy-*O*⁶-(2-trimethylsilylethyl)-2-fluoroinosine (**5a**) to

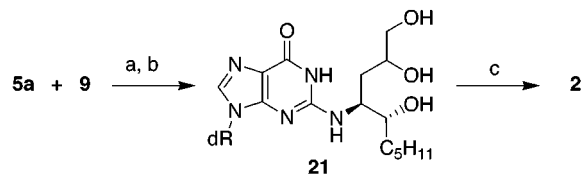


^a Reagents: (a) Ti(OiPr)₄, *t*BuOOH, (–)-DET, 4 Å MS, CH₂Cl₂, –20 °C, 85%; (b) BnNCO, NaH, THF, reflux, 50%; (c) Ph₃P, I₂, imidazole, 84%; (d) H₂C=CHMgBr, CuI, HMPA, THF, 67%; (e) OsO₄, NMO, THF, *t*BuOH, H₂O, 72%; (f) KOH, EtOH, reflux, 68%; (g) H₂, Pd(OH)₂, MeOH, 100%

Scheme 3^a

^a Reagents: (a) $\text{Ti}(\text{OiPr})_4$, $t\text{BuOOH}$, (+)-DIPT, CH_2Cl_2 , -20°C , 40%, 91% ee; (b) BnNCO , NaH , THF, reflux, 58%; (c) Ph_3P , I_2 , imidazole, 82%; (d) $\text{H}_2\text{C}=\text{CHMgBr}$, CuI , HMPA, THF, 69%; (e) OsO_4 , NMO, THF, $t\text{BuOH}$, H_2O , 69%; (f) KOH , EtOH, reflux, 70%; (g) H_2 , $\text{Pd}(\text{OH})_2$, MeOH, 100%

give the corresponding N^2 -dG derivative (i.e., **21** from **9**) in 57–64% yield after deprotection of the O^6 -(2-trimethylsilyl-ethyl) group (Scheme 4). In the case of the syn amino

Scheme 4^a

^a Reagents: (a) $i\text{Pr}_2\text{NEt}$, DMSO, 70°C ; (b) 5% AcOH, 64% from **5a**; (c) NaIO_4 , H_2O , 87%

alcohols, the minor stereoisomer from the Sharpless kinetic resolution could be separated at this stage by HPLC. Oxidative cleavage of the vicinal diol with sodium periodate gave the $1,N^2$ -propano adducts **1–4** in 80–87% yield.

Methods have been developed for the detection of DNA adducts of 4-HNE and related species.¹³ This involves digestion of the oligonucleotide and analysis by HPLC. The four stereoisomers of the 4-HNE-dG adduct at the nucleoside

or the corresponding 3',5'-bisphosphate can be partially separated by reverse phase HPLC. It was observed that two stereoisomers coeluted while the two others were cleanly resolved; no definitive stereochemical assignments were made. With stereospecific syntheses of **1–4**, we are now in a position to assign the absolute stereochemistry of the 4-HNE adducts.

Using the published HPLC conditions we were able to reproduce the reported elution profile. On the basis of the HPLC retention times and published ^1H NMR data, we have established that the two resolved isomers are **3** and **4**, derived from the syn amino alcohols. This is consistent with the previous observation that acid deglycosylation of these isomers gave a single, presumably racemic guanine derivative.^{13a} Interestingly, the reaction of racemic 4-HNE with calf thymus DNA followed by digestion and analysis by HPLC with ^{32}P postlabeling detection has been reported to give only two of the stereoisomers.^{13c} On the basis of a comparison of the reported HPLC retention time with our own analysis, we tentatively assign the major adduct as **4** and the minor adduct as **3**. It is worth noting that all four stereoisomers have been detected from animal tissue using the ^{32}P postlabeling method, although not always in equal concentrations.^{13c}

Harris has shown that oligonucleotides containing an O^6 -protected 2-fluorinosine residue can undergo nucleophilic aromatic substitution with aliphatic and aromatic amines to give site-specifically adducted oligonucleotides.¹⁰ The O^6 -protected 2-fluorinosine was incorporated into oligonucleotides using standard phosphoramidite chemistry. Reaction of amino alcohols **8–11** with oligonucleotides containing 2-fluorinosine followed by deprotection and periodate cleavage should be a viable route to structurally and stereochemically defined oligonucleotides containing 4-HNE adducts. These modified oligonucleotides will be essential for mutagenesis, repair, and structural studies on this endogenous genotoxin.

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Supporting Information Available: Copies of the ^1H and ^{13}C NMR spectra of all new compounds and CD spectra of **1–4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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