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Simplified synthesis of individual stereoisomers of the 4-hydroxynonenal adducts of deoxyguanosine

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Abstract

We previously reported the synthesis of the 1,*N*²-deoxyguanosine adducts of 4-hydroxynonenal, an important product of lipid peroxidation, which involved the nucleophilic aromatic substitution reaction of an *O*⁶-protected-2-fluoroinosine with 4-amino-1,2,5-trihydroxydecanal followed by periodate oxidation of the vicinal diol.³ An improved synthesis of the amino triols has been developed. The *syn* and *anti* diastereomers of a key intermediate, 4-nitro-5-hydroxy-1-decene, were synthesized by a Henry reaction and separated; each diastereomer was further separated into individual enantiomers by chiral supercritical fluid chromatography. Of note, dihydroxylation of the terminal olefin under conventional conditions with catalytic OsO₄ and a tertiary amine oxide as the stoichiometric oxidant led to scrambling of stereochemistry at C4. The scrambling was not observed when *t*-butylhydroperoxide was used as the oxidant.

Keywords

4-hydroxynonenal; DNA adducts; Henry Reaction; dihydroxylation

Introduction

4-Hydroxynonenal (HNE) is a reactive bis-electrophile that is produced endogenously from the free radical oxidation of ω -6 polyunsaturated fatty acids. HNE reacts with proteins and DNA and has cytotoxic and genotoxic potential.^{1,2} The reaction of HNE and DNA occurs predominately with dGuo resulting in four diastereomeric 1,*N*²-cyclic adducts (**1–4**, Scheme 1)^{3–5} We have previously reported the synthesis of the individual stereoisomers of 1,*N*²-HNE-dGuo nucleosides in which *O*⁶-(2-trimethylsilylethyl)-2-fluoro-2-deoxyinosine was reacted with individual stereoisomers of amino triols **5–8**, corresponding to the C6 and C11 positions of the HNE-dGuo adducts (Scheme 2).⁶ A related S_NAr approach was used to synthesize oligonucleotides containing the individual HNE adducts.⁷ In both the nucleoside

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and oligonucleotide syntheses, the vicinal diol was subsequently cleaved with NaIO_4 to unveil the aldehyde group; the aldehyde spontaneously cyclizes with the N1-positions to afford the exocyclic HNE-dGuo adduct with the *trans* relative stereochemistry between C6 and C8. The strategy required the synthesis of four amino triols in which the stereochemistry of the key vicinal amino alcohol unit could be controlled. Each amino triol was synthesized in ~7 steps and the absolute stereochemistry was established by either a Sharpless asymmetric epoxidation (**7** and **8**, ~12 % overall yield) or kinetic resolution (**5** and **6**, ~6% overall yield). Therefore it seemed desirable to develop a more efficient route to the key amino triols (**5–8**) for our ongoing program involving 1, N^2 -HNE-dGuo adducts.^{8,9}

Results and discussion

The new approach is shown in Scheme 3. The fact that the key functional group of the HNE synthon is a vicinal amino alcohol led us to consider a nitroaldol (Henry) reaction between 4-nitro-1-butene and hexanal as the key C-C bonding-forming step. Shibasaki reported pioneering work in asymmetric catalysis for the nitroaldol reaction in 1992;¹⁰ since then considerable progress has been made using a wide variety of chiral ligand-based metal catalysts as well as organocatalysis.^{11–13} However, virtually all of these methods are intended to yield a single stereoisomer of the product in high enantiomeric purity, with some catalysts *syn*-selective and others *anti*-selective. Since our studies require significant amounts of all four stereoisomers, more than one asymmetric strategy would be required. Therefore we decided that it would be more efficient to resolve the individual isomers chromatographically. 4-Nitro-1-butene was prepared from NaNO_2 and 4-bromo-1-butene as previously reported.¹⁴ In our hands, purification by distillation significantly increased the yield of the product. The Henry reaction was carried out under biphasic conditions in 25 mM aqueous NaOH and THF with $\text{Bu}_4\text{N}^+\text{Cl}^-$ as a phase-transfer catalyst in 70–75% yield as a 1:1 mixture of diastereomers.^{15,16}

The *syn* and *anti* nitro alcohols were separated by careful medium pressure chromatography on a Biotage Sp-1 apparatus. The diastereomers were <99% pure as judged by analytical HPLC analysis and mixed fractions could be readily recycled (80% recovery). Each diastereomer was further separated into individual enantiomers using chiral stationary phase chromatography. A CHIRALPAK AD column (Chiral Technologies, INC.) was initially used to separate the enantiomers (2.5% absolute ethanol in hexane); however, superior separation was achieved via stacked injections on a Waters Investigator supercritical fluid chromatography (SFC) using a Phenomenex Lux Cellulose-2 column (Figure 1, 85% recovery).¹⁷ Reinjection of each nitro alcohols on an analytical Agilent 1260 Infinity SFC indicated that they were >98% ee. It is likely that all four isomers could be directly separated with SFC given the resolution.

Dihydroxylation of the olefin was originally accomplished by the Upjohn procedure using catalytic OsO_4 and *N*-methylmorpholine-*N*-oxide as the stoichiometric oxidant.¹⁸ The stereochemistry of the secondary alcohol of the vicinal diol could not be controlled; however, the mixture is of no consequence since this position is fated for oxidation to the aldehyde. Reduction of the nitro group of **13** by catalytic hydrogenation (H_2 , Pd/C) afforded the amino triol **5**.

$\text{S}_\text{N}\text{Ar}$ reaction of **5** with O^6 -protected-2'-fluoroinosine and subsequent periodate oxidation provided 1, N^2 -HNE-dGuo adduct **1**. However, HPLC analysis of this product indicated that it was 70:30 mixture of **1** and **4** (Figure S1A in the Supporting Information). Since these products are isomeric only at C6 suggested that the stereochemistry of the C4-nitro group was scrambled during the dihydroxylation. We reasoned that the *N*-methylmorpholine byproduct was sufficiently basic to deprotonate **9** or the corresponding nitro triol product

(13); indeed, treatment of **9** with *N*-methylmorpholine led to a 3:2 mixture of **9** and **12** (Figure S1B in the Supporting Information). Alternative co-oxidants were examined to allay the scrambling, such as hydrogen peroxide¹⁹, pyridine oxide²⁰, NaClO₃²¹, but these gave unsatisfactory results. Ultimately, we found that *t*-butylhydroperoxide was an effective co-oxidant for the dihydroxylation and did not scramble the stereochemistry of the nitro group (85% yield).^{22,23} After dihydroxylation, the reduction of the nitro group by catalytic hydrogenation (H₂, Pd/C) afforded the amino triols **5–8**. The individual amino triols were converted to specific stereoisomers of the 1,*N*²-HNE-dGuo adducts (**1–4**) as previously described (Scheme 2). The stereochemistry of HNE adducts **1–4** has been unambiguously determined previously^{6,7} and their synthesis served to establish the stereochemistry of nitro alcohols **9–12**.

Summary and conclusion

In conclusion, we have developed a more efficient synthesis of individual stereoisomers of amino triols **5–8**. The sequence required 5 steps and occurred with ~29% overall yield, which also includes two chromatographic separations. Previously, the synthesis of each amino triol required ~7 steps and occurred with ~6% overall yield for **5** and **6**, and with ~12 % overall yield for **7** and **8**.⁷ The key aspect of the new synthesis is the separation of the nitro alcohols from the Henry reaction into individual stereoisomers and the use of *t*BuOOH as the reoxidant for the OsO₄ dihydroxylation reaction to suppress scrambling the nitro group stereochemistry. We have previously shown the utility of the amino triols for the synthesis of stereochemically defined 1,*N*²-HNE-dGuo adducts at the nucleoside and oligonucleotide level.

Experimental Section

Procedures for reactions, NMR data and HPLC chromatograms are provided in the Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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16. 4-Nitro-1-butene (2.0 g, 19.8 mmol) was added to aqueous NaOH (25 mM, 63 mL) followed by addition of THF (5 mL). To this solution, $n\text{Bu}_4\text{N}^+\text{Cl}^-$ (1.27 g, 45.7 mmol) and hexenal (2.0 g, 20.4 mmol) were added, and the mixture was stirred at room temperature for 6 h. Brine was added (60 mL) and the mixture was extracted with diethyl ether (4×100 mL). The combined organic layers were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure to afford the **9–12** as a mixture of stereoisomers. Purification by automated silica chromatography on a Biotage Sp-1 afforded the racemic *syn* (**9** and **10**) and *anti* (**11** and **12**) diastereomers in 70–75% combined yield. Further separation (200 mg) via stacked injections on a Waters Investigator SFC provided the individual enantiomers of **9–12**. See the Supporting Information for spectral data.
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23. Compound **9** (60 mg, 0.30 mmols) was dissolved in acetone/water (9:1, 15 mL), $n\text{Bu}_4\text{N}^+\text{OAc}^-$ (262 mg, 0.87 mmols) was added, and the mixture was stirred at 5 °C for 15 minutes, *t*-Butyl hydroperoxide (5.0 – 6.0 M solution in decane, 90 μL) was added dropwise, followed by addition of OsO_4 (~1 mg). The mixture was allowed to warm to ambient temperature and stirred overnight. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography eluting with methylene chloride/methanol (97:3) to afford **13** (60 mg, 85%). See the Supporting Information for spectra data.

Figure 1.
Chiral stationary phase supercritical fluid chromatography (SFC) of diastereomers **9–12**.

Scheme 1.
Structures of the four 1,*N*²-HNE-dGuo nucleosides.

Scheme 2.

The previous synthesis of the individual stereoisomers of 1,*N*²-HNE-dGuo nucleosides.³

Scheme 3.

Synthesis of the aminotriols **5–8**. a) NaOH , H_2O , THF, $\text{nBu}_4\text{N}^+\text{Cl}^-$ (70–75%); b) medium pressure chromatography (silica gel, 80%); c) Supercritical fluid chromatography (Phenomenex Lux Cellulose-2 column, 85%); 5% isopropanol and 0.1% diethyl amine in CO_2 ; d) OsO_4 , tBuOOH , $\text{n-Bu}_4\text{NOAc}$, acetone/water, (80–85%); e) H_2 , Pd/C , MeOH (90–94%)