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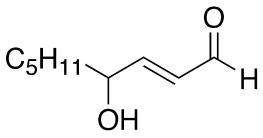
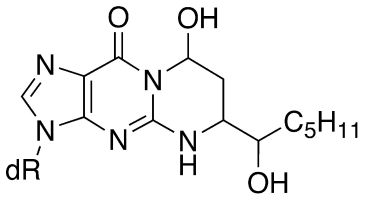


trans-4-hydroxynonenal
(4-HNE)



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Site-Specific Synthesis and Reactivity of Oligonucleotides Containing Stereochemically Defined 1,N^2-Deoxyguanosine Adducts of the Lipid Peroxidation Product *trans*-4-Hydroxynonenal

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Abstract: *trans*-4-Hydroxynonenal (HNE) is a major peroxidation product of ω-6 polyunsaturated fatty acids. The reaction of HNE with DNA gives four diastereomeric 1,N^2-γ-hydroxypropano adducts of deoxyguanosine; background levels of these adducts have been detected in animal tissue. Stereospecific syntheses of these four adducts at the nucleoside level have been accomplished. In addition, a versatile strategy for their site-specific incorporation into oligonucleotides has been developed. These adducts are destabilizing as measured by melting temperature when compared to an unadducted strand. The thermal destablization of the adducted 12-mers ranged from 5 to 16 °C and is dependent on the absolute stereochemistry of the adduct. The HNE adducts were also examined for their ability to form interstrand DNA−DNA cross-links when incorporated into a CpG sequence. We find that only one of the HNE stereoisomers formed interstrand DNA−DNA cross-links.

Introduction

The autoxidation of polyunsaturated fatty acids is a free radical process initiated by reactive oxygen species such as hydroxyl radical. The initial product is a lipid hydroperoxide which can undergo metal ion mediated fragmentation to produce a complex array of products.[1] Among the peroxidation products are α,β-unsaturated aldehydes (enals).[2] Enals react with sulfhydryl and amino groups of proteins and are toxic.[2a] They also react with DNA bases and have mutagenic potential.[3] Thus, the peroxidation of lipids represents an endogenous source of cytotoxins and genotoxins. The simplest enals, acrolein and crotonaldehyde, have been shown to be products of lipid peroxidation although the mechanism of their formation is not obvious.[4,5] Acrolein can also arise by the oxidative degradation of threonine.[6] In addition, acrolein, and crotonaldehyde are widely dispersed environmental pollutants and are constituents of cigarette smoke.[7] Acrolein and crotonaldehyde induce revertants in the Ames *Salmonella typhimurium* tester strains TA100 and TA104.[8]

A major product of the peroxidation of ω-6 polyunsaturated fatty acids is (±)-4-hydroxynonenal (HNE).[9] HNE is highly cytotoxic and induces apoptosis and necrosis in tumor cells.[10] HNE is inactive in Ames assays; however, the lower homologue 4-hydroxypentenal was found to be mutagenic.[8] It is likely that the toxicity of HNE masks its genotoxicity in these assays. Consistent with this hypothesis is the fact that HNE induces mutations in V79 Chinese hamster ovary cells and induces a dose-dependent SOS response in *S. typhimurium*.[11,12] Recently, livers from humans who had suffered from Wilson's disease and hemochromatosis were examined and found to contain mutations at C:G sites in the coding regions of the p53 tumor suppressor gene.[13] Evidence was presented that these mutations may be caused by HNE modification of a deoxyguanosine.

Enals are bis-electrophiles and can potentially undergo tandem reactions with pairs of nucleophilic sites on the DNA bases. Reaction with deoxyguanosine gives the cyclic 1,N^2-hydroxypropano adducts (Figure 1). Two regioisomers are possible. The one having the hydroxyl group at C8, i.e., proximal to the purine ring system, arises by conjugate addition of the exocyclic N^2-

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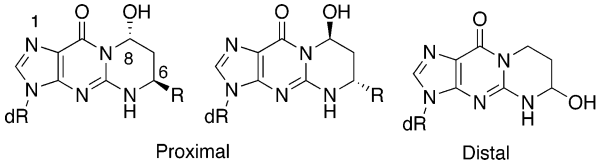

Figure 1. 1,N^2-hydroxypropano adducts of deoxyguanosine.

amino group to the enal followed by ring closure of N1 with the aldehyde. The acrolein adduct has a new stereogenic center at C8. For crotonaldehyde and higher enals, two new stereogenic centers are formed; however, only the stereoisomers with the C8-hydroxyl group trans to the C6-substituent are observed.[14] The second type of adduct, i.e., the 6-hydroxy or distal adduct, results from initial conjugate addition of N1 followed by ring closure of the aldehyde with the N^2-amino group or, alternatively, by formation of the Schiff base with the N^2-amino group followed by ring closure onto N1 and rehydration of the Schiff base. The distal adduct has only been observed for the reaction of deoxyguanosine with acrolein and methyl vinyl ketone.[15] A number of studies have demonstrated the formation of proximal deoxyguanosine adducts in DNA incubated with acrolein and other enals and in DNA samples isolated from enal-exposed and unexposed animal tissue, but distal adducts have not been identified in any of these DNA samples.[16]

Recently, 1,N^2-deoxyguanosine adducts of acrolein and crotonaldehyde have been site-specifically incorporated into oligonucleotides.[17] Site-specific mutagenesis studies of the acrolein adduct revealed this lesion to be essentially nonmutagenic in point mutation assays in *E. coli*, but mutagenic in mammalian cells.[18,19] The synthesis of the distal acrolein deoxyguanosine adduct has recently been reported by Johnson and by our laboratory, and we have also reported the preparation of oligonucleotides containing this lesion.[20,21] Mutagenesis studies for the distal adducts have not yet been reported.

At the nucleoside level, the 1,N^2-deoxyguanosine adducts of enals exist exclusively in the ring-closed form. However, recent NMR structures of an oligonucleotide duplex containing the proximal acrolein and closely related malondialdehyde adducts of deoxyguanosine showed that the ring-opened form predominated.[22,23] The ring-opened structure leaves an electrophilic aldehyde group in the minor groove of DNA, which can react with nucleophilic sites of other DNA bases to form intra- or interstrand DNA−DNA cross-links or with proteins to give DNA−protein cross-links. Recent chemical and spectroscopic studies have demonstrated the ability of the acrolein adduct to

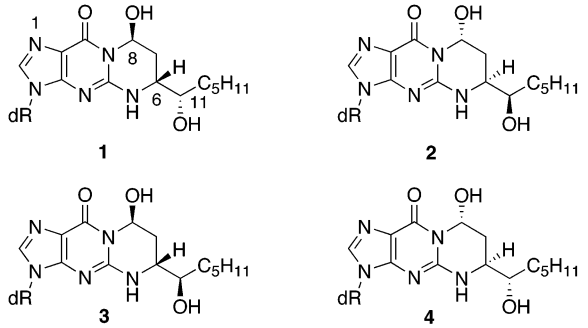

Figure 2. 1,N^2-deoxyguanosine adducts of HNE.

form both interstrand and intrastrand DNA−DNA cross-links.[24−26] Hecht and co-workers have also isolated a formal crotonaldehyde cross-link between two deoxyguanosines from the reaction of calf thymus DNA with acetaldehyde.[27] The mechanism by which that cross-link is formed probably does not involve the reaction of DNA with crotonaldehyde, which is the aldol product of acetaldehyde, but rather the reaction of acetaldehyde with the acetaldehyde−deoxyguanosine Schiff base.[17c,27] The ability of enals to form DNA−DNA cross-links could play a significant role in their biological processing.

The reaction of HNE with deoxyguanosine creates three new stereogenic centers in the nucleoside. One center arises from the HNE itself; the other two are generated by the adduction reaction. As in the proximal crotonaldehyde adducts, the relative stereochemistry between C8 and C6 is trans. Thus, there are four possible stereoisomers of the HNE adducts of deoxyguanosine, **1**−**4** (Figure 2).[28] Oligonucleotides containing the diastereomeric HNE adducts may have different local conformations. Consequently, it is possible that the various stereoisomers of the HNE adduct could elicit different DNA−DNA and DNA-protein cross-linking reactions and different biological responses.

We report here the stereocontrolled syntheses of all four stereoisomers of the proximal 1,N^2-deoxyguanosine adduct of HNE.[29] In addition, oligonucleotides containing the four stereoisomers of the HNE adduct have been prepared in a structurally specific manner. Finally, we have examined the ability of these adducts to form interchain cross-links and found the cross-linking reaction is strongly dependent on adduct stereochemistry and gives a remarkably stable cross-link.

Results

Stereospecific Synthesis of the Deoxyguanosine Adducts of HNE. Syntheses of the proximal 1,N^2-deoxyguanosine adduct of acrolein (**7a**) and of oligonucleotides containing **7a** have been independently reported by Johnson and Harris.[17a,b] Similar syntheses of the two diastereomers of the crotonaldehyde adduct

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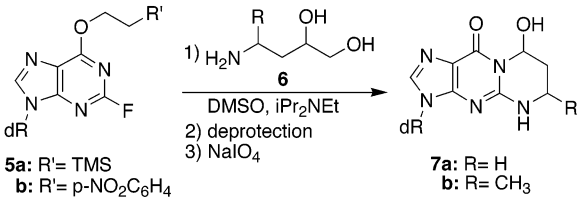
(29) Wang, H.; Rizzo, C. J. *Org. Lett.* **2001**, *3*, 3603. In our initial report, compounds **2** and **3** in Figure 1 were interchanged and should be as shown in Figure 2 of this manuscript. Corrrection: Wang, H.; Rizzo, C. J. *Org. Lett.* **2002**, *4*, 155.

Scheme 1



7b were reported as well as site-specific syntheses of oligonucleotides containing the individual diastereomers.[17c] In each case, a vicinal diol unit was used as a surrogate for the aldehyde group and the vicinal diol was cleaved with sodium periodate to yield an aldehyde which then cyclized (Scheme 1). The three- and four-carbon units for acrolein and crotonaldehyde, respectively, were introduced via a nucleophilic aromatic substitution reaction of amino alcohol **6** with an O^6-protected 2-fluoroinosine derivative **5**. In the oligonucleotide syntheses by Harris et al., these displacements were accomplished on an oligonucleotide containing O^6-protected 2-fluoroinosine nucleotide **5a**.[17b,c]

A similar strategy can be envisioned for the synthesis of 1,N^2-deoxyguanosine adducts of HNE and of oligonucleotides containing these adducts. The stereocontrolled synthesis of the four diastereomeric HNE adducts would require enantioselective syntheses of the amino alcohols shown in Figure 3. The antipodal amino alcohols **8** and **9** possessing the anti relative stereochemistry between the critical amino and hydroxyl groups are required for the synthesis of adducted nucleosides **1** and **2**, respectively, 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 enantioselective synthesis of **9** began with a Sharpless asymmetric epoxidation of *E*-oct-2-en-1-ol (**12**) using (−)-diethyl tartrate as the ligand, to give the epoxy alcohol **13**.[30] Reaction of **13** with benzyl isocyanate afforded a separable mixture of two cyclic *N*-benzyl urethanes **14** and **15**; the undesired isomer **14** could be equilibrated to form additional amounts of **15** by treatment with sodium hydride.[31] A two-carbon extension of the aliphatic chain was accomplished by converting the primary hydroxyl group of **15** to the corresponding iodide (**16**) followed by displacement with vinylmagnesium bromide in the presence of copper iodide to give **17**. Dihydroxylation gave a 1:1 mixture of vicinal diols (**18**). Because 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 high enantiomeric purity at carbons 4 and 5. The enantiomeric amino alcohol **8** was prepared in an identical fashion using (+)-diethyl tartrate as the ligand for the Sharpless epoxidation.

Using a related synthetic strategy, we have synthesized the syn amino alcohol **11** (Scheme 3). The absolute configuration was established through a Sharpless kinetic resolution of racemic 3-hydroxy-1-octene (**20**) using (+)-diisopropyl tartrate as the chiral ligand for the titanium catalyst.[30,32] Treatment of **21** with benzyl isocyanate gave a single cyclic *N*-benzyl urethane **22**.

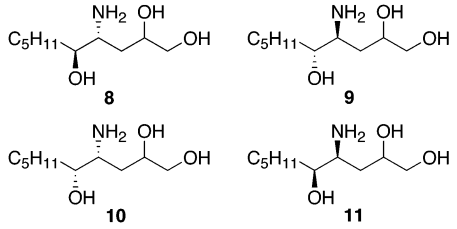
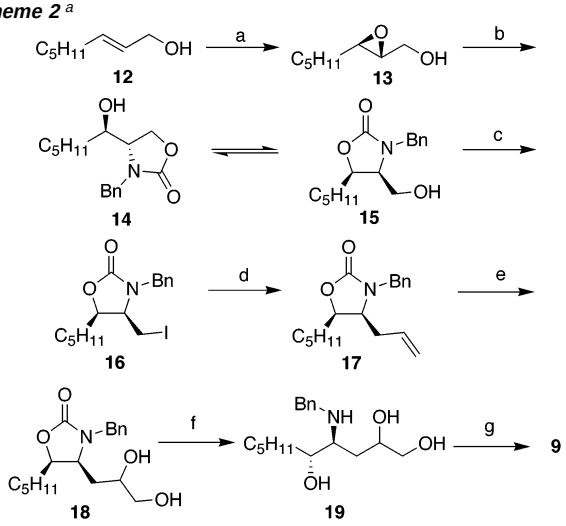


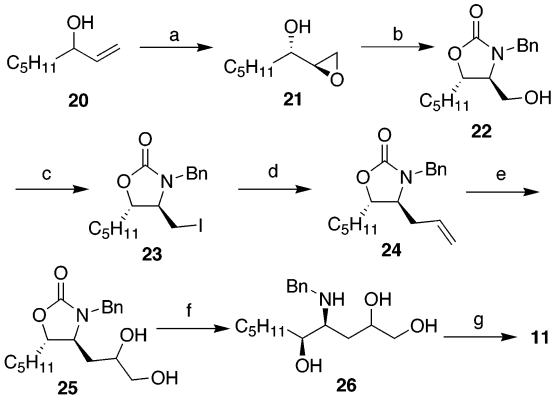
Figure 3. Amino alcohols for the synthesis of **1**−**4**.

Scheme 2 [a]



[a] Reagents: (a) $Ti(OiPr)_4$, tBuOOH, (−)-DET, 4 Å MS, CH_2Cl_2, −20 °C, 85%, (b) BnNCO, NaH, THF, reflux, 50%, (c) Ph_3P, I_2, imidazole, 84%, (d) H_2C═$CHMgBr$, CuI, HMPA, THF, 67%, (e) OsO_4, NMO, THF, tBuOH, H_2O, 72%, (f) KOH, EtOH, reflux, 68%, (g) H_2, $Pd(OH)_2$, MeOH, 100%.

Scheme 3 [a]



[a] Reagents: (a) $Ti(OiPr)_4$, tBuOOH, (+)-DIPT, CH_2Cl_2, −20 °C, 40%, 91% ee, (b) BnNCO, NaH, THF, reflux, 58%, (c) Ph_3P, I_2, imidazole, 82%, (d) H_2C═$CHMgBr$, CuI, HMPA, THF, 69%, (e) OsO_4, NMO, THF, tBuOH, H_2O, 69%, (f) KOH, EtOH, reflux, 70%, (g) H_2, $Pd(OH)_2$, MeOH, 100%.

The remainder of the synthesis to give **11** proceeded by a sequence of reactions identical to those for anti amino alcohols **8** and **9** discussed above. Once again, the enantiomeric syn amino alcohol **10** was synthesized by simply changing to the antipodal tartrate ligand during the Sharpless kinetic resolution.

The optical purity of epoxy alcohols **13** and **21** was established by analysis of the corresponding (−)-Mosher's esters by HPLC and high-field NMR. Richardson and Rychnovsky previously reported that the Sharpless asymmetric epoxidation

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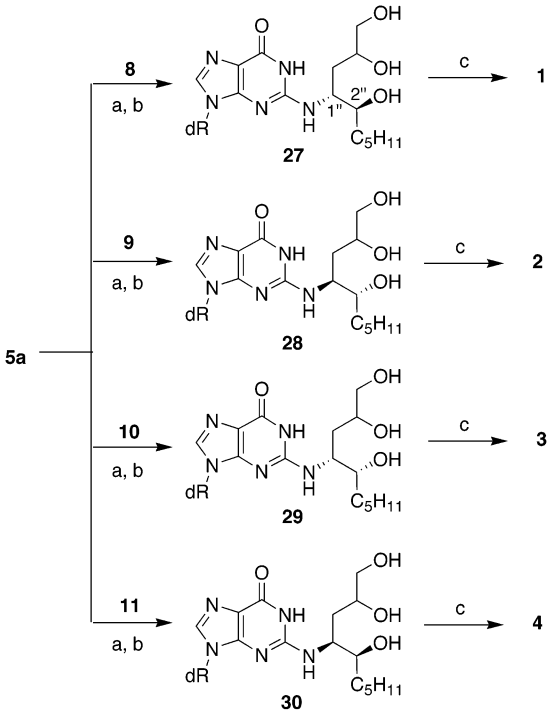
Scheme 4 [a]



[a] Reagents: (a) $^{i}Pr_2NEt$, DMSO, 70 °C, (b) 5% AcOH, 57−64% from **5a**, (c) $NaIO_4$, H_2O, 80−87%.

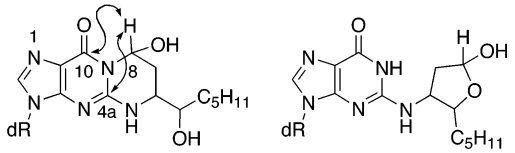


Figure 4. HMBC correlation establishing the structures of **1**−**4**.

of **12** proceeded in greater than 99% ee as determined by chiral GC analysis.[33] We could not detect a minor stereoisomer of the corresponding (−)-Mosher's ester of **13** by HPLC with UV detection or by NMR. From a similar analysis of the corresponding (−)-Mosher's ester of **21**, we estimate that the Sharpless kinetic resolution of **20** proceeded in 92−94% ee.[30b,32]

Amino alcohols **8**−**11** were individually condensed with 2-fluoro-O^6-(2- trimethylsilylethyl)-2′-deoxyinosine (**5a**) to give the corresponding N^2-deoxyguanosine derivatives **27**−**30** in 57−64% yield after deprotection of the O^6-(2-trimethylsilylethyl) group (Scheme 4). In the case of the syn amino 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^2-8-hydroxypropano deoxyguanosine derivatives **1**−**4** in 80−87% yield.

The structures of HNE adducts **1**−**4** were firmly established by $^1H-^{13}C$ long-range heteronuclear correlation NMR spectra (HMBC). For each sample we observed correlation between H8 and the C10 carbonyl carbon as well as C4a (Figure 4). For **4** these resonances were at 6.40 and 157.8 and 152.3 ppm (Figure 5). No such correlations would have been observed in the alternative hemi-acetal structure shown in Figure 4; thus, this structure is ruled out for all four diastereomers.

The ^1H NMR spectra of the four nucleosides **1**−**4** are listed in Table 1. It should be noted that the spectra of **1** and **2** are

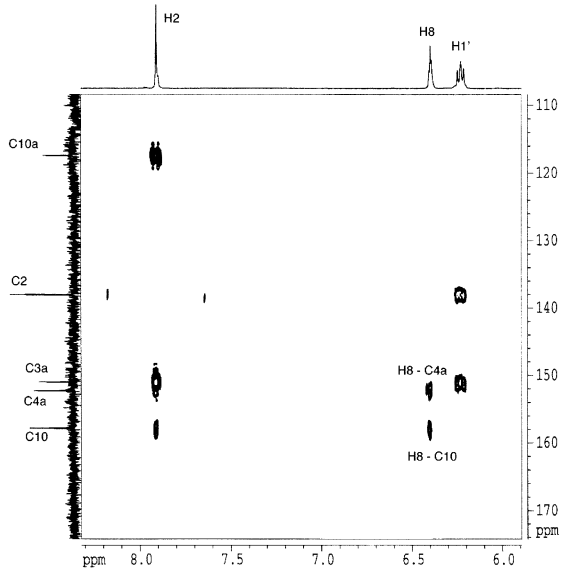


Figure 5. HMBC spectra of **4**.

Table 1. ^1H NMR Spectra of Nucleosides **1**−**4** in Methanol-d_4

assignment	1	2	3	4
2	7.91	7.91	7.91	7.91
6	3.75−3.70[a]	3.75−3.70[a]	3.61	3.61
7a	2.10	2.11	2.18	2.19
7b	1.74	1.74	1.60[c]	1.60[c]
8	6.42	6.42	6.40	6.40
11	3.82−3.75[b]	3.81−3.75[b]	3.47	3.47
12−15	ND[d]	ND[d]	ND[d]	ND[d]
16	0.92	0.93	0.93	0.93
1′	6.23	6.24	6.23	6.23
2′	2.67	2.69	2.68	2.68
2″	2.33	2.33	2.33	2.33
3′	4.51	4.51	4.51	4.51
4′	3.98	3.98	3.98	3.99
5′	3.78	3.78	3.78	3.78
5″	3.72	3.73	3.70	3.71

[a] The peak is overlapped with H-5′ and its chemical shift was estimated from COSY. [b] The peak is overlapped with H-5″ and its chemical shift was estimated from COSY. [c] The peak is hidden under the bigger peaks of protons from H12−H15, and its chemical shift was estimated from COSY. [d] Not determined.

virtually superimposable. Likewise, the spectra of **3** and **4** are essentially identical. This relationship is not unexpected because each pair is enantiomeric with respect to the three stereogenic sites in the base. The deoxyribosyl moiety is too distant from the stereogenic centers of the adducted base to produce more than miniscule perturbations in the chemical shifts.

We have examined the circular dichroism spectra of nucleosides **1**−**4**. In contrast to NMR, each diastereomer gives a unique CD spectrum (Figure 6). Nevertheless, the 280 nm band arises primarily from the nucleobase. Thus, **1** and **3**, which have the (6*R*,8*S*) configuration show negative ellipticity at 280 nm, whereas **2** and **4**, which have mirror image stereochemistry at these sites, show positive ellipticity. A similar result had been observed for the crotonaldehyde adducts which have only a methyl group at C6 (Figure 7).[17c,34] A negative ellipticity was observed for crotonaldehyde adduct **31** having the (6*S*,8*S*) configuration whereas diastereomer **32** gave a positive signal. It should be noted that the designation of absolute configuration at C6 for the crotonaldehyde adducts is opposite that for the

(33) Richardson, T. I.; Rychnovsky, S. D. *Tetrahedron* **1999**, *55*, 8977.

(34) Chung, F.-L.; Hecht, S. S. *Cancer Res.* **1983**, *43*, 1230.

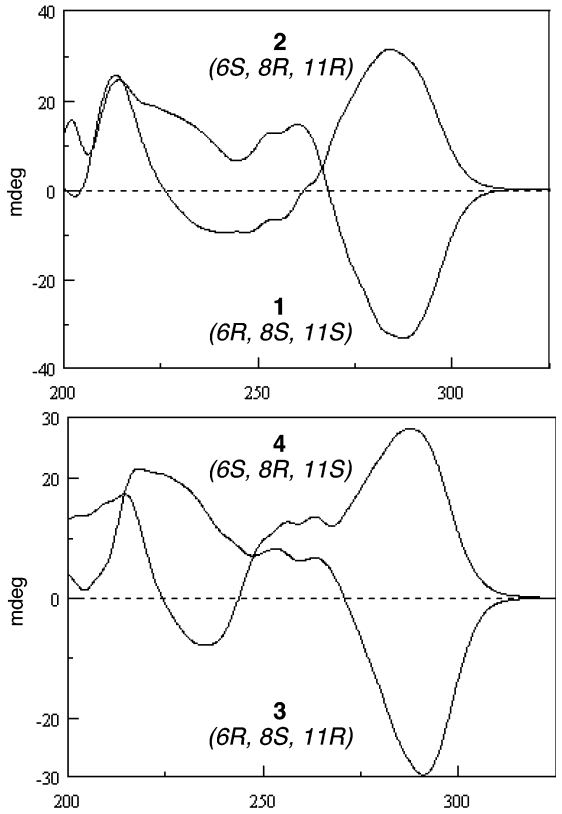
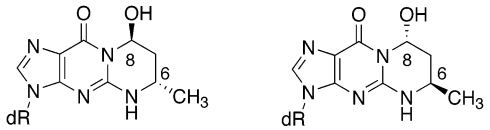


Figure 6. CD Spectra of **1**−**4**. Sample concentration is 1 mg/mL in methanol.



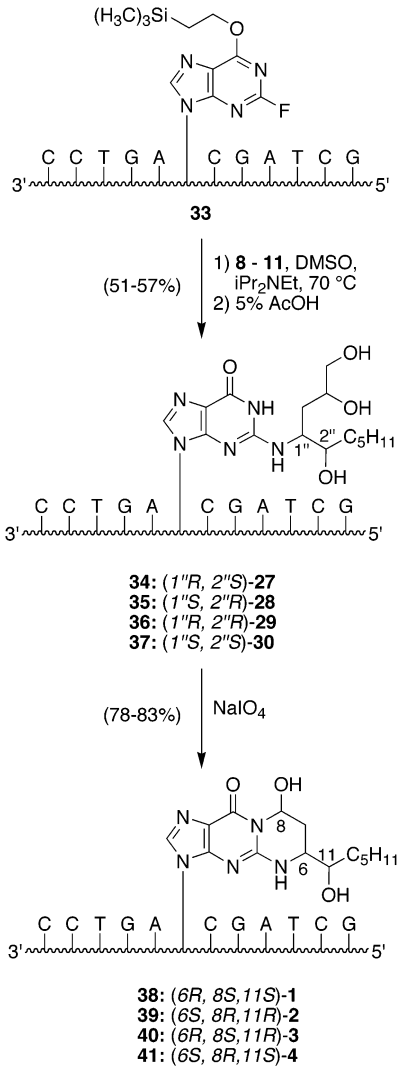
31 (*6S, 8S*) **32** (*6R, 8R*)

Figure 7. 1,N^2-deoxyguanosine adducts of crotonaldehyde.

HNE adducts because of a change of priority for the side-chain (a methyl group in **31** and **32** versus a hydroxyhexyl group for **1**−**4**). The trans relative stereochemistry between H6 and H8 is observed for both types of adducted nucleosides. For the six example, we have examined thus far (**1**−**4**, **31**−**32**), the sign of the CD signal at 280 nm correlates with the absolute configuration at C6 and C8.

Site-Specific Synthesis of Oligonucleotides Containing Stereochemically Defined Adducts of HNE. A post-oligomerization strategy was the method of choice for the synthesis of oligonucleotides containing stereochemically defined HNE adducts. The alternative use of phosphoramidite derivatives of HNE-adducted nucleosides **1**−**4** or of aminotriol-adducted nucleosides **27**−**30** would require cumbersome strategies for orthogonal protection of the side-chain functionality. The phosphoramidite reagent of **5a** has previously been incorporated into oligonucleotides.[35] Many examples have been reported of oligonucleotides containing **5a** undergoing substitution by amine analogues of mutagens to give sequence specific mutagen-linked oligonucleotides at N^2 of deoxyguanosine.[17,36] These syntheses have included the preparation of oligonucleotides bearing 1,N^2-deoxyguanosine adducts of acrolein and crotonaldehyde.[17]

Scheme 5



Stereo- and site-specific syntheses of 12-mer oligodeoxynucleotides containing the four 1,N^2-deoxyguanosine adducts of HNE was accomplished according to Scheme 5. Oligonucleotide **33** was prepared using standard solid-phase methods with phenoxyacetyl-protected phosphoramidite reagents for the unmodified bases. Deprotection of the oligonucleotide without hydrolysis of the O^6-(2-(trimethylsilyl)ethyl)-2-fluoroinosine base was accomplished with 0.1 M NaOH at room temperature as previously described.[17b,c,36] Aminotriols **8**−**11** were individually reacted with oligonucleotide **33** in DMSO at 70 °C in the presence of Hünig's base to give the corresponding site-specifically adducted oligonucleotides **34**−**37** after deprotection of O^6 with dilute acetic acid. Confirmation that the adduction reaction had proceeded as planned was provided by MALDI-TOF mass spectrometric analysis of **34**−**37**. In addition, the oligonucleotides were subjected to enzymatic digestion followed by HPLC analysis to confirm the presence of modified nucleosides **27**−**30** (Figure 7). Periodate cleavage of the vicinal diol unit provided the corresponding stereochemically defined HNE-adducted oligonucleotides **38**−**41**. Aminotriols **10** and **11** had been prepared in 92−94% ee. However, HPLC purification of the oligonucleotides both before and after periodate cleavage

(35) Harris, C. M.; Harris, T. M. *Curr. Prot. Nucleic Acid Chem.* Boyle, A. L., Ed.; J. Wiley & Sons: New York: 1999; p 1.3.1−1.3.15.
(36) DeCorte, B. L.; Tsarouhtsis, D.; Kuchimanchi, S.; Cooper, M. D.; Horton, P.; Harris, C. M.; Harris, T. M. *Chem. Res. Toxicol.* **1996**, *9*, 630.

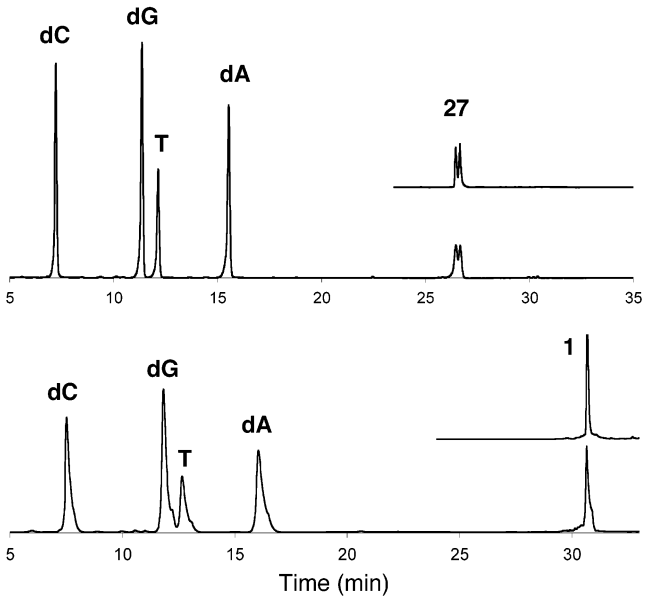
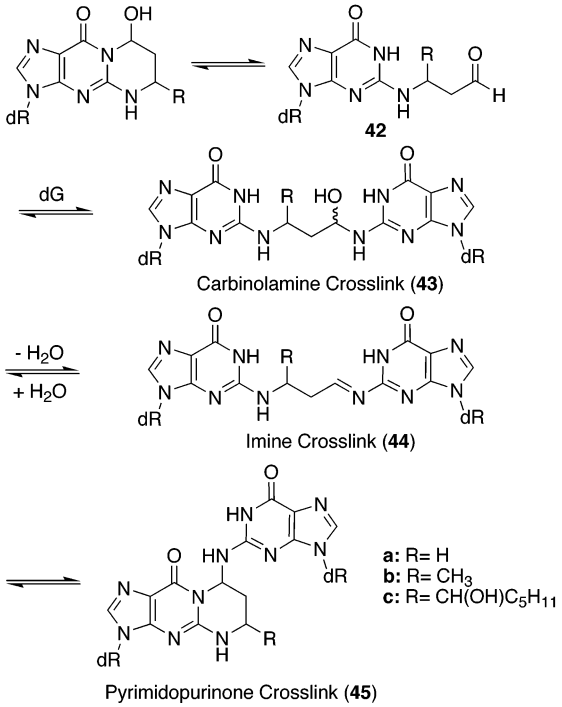


Figure 8. HPLC analysis of the enzymatic digestion of oligonucleotides **34** (top) and **38** (bottom).

Scheme 6



gave purified oligonucleotides that were totally free of diastereomeric contamination. Again, the structures of **38**−**41** were confirmed by MALDI-TOF mass spectrometry and enzymatic digestion, which established the presence of HNE-adducted nucleosides **1**−**4** (Figure 8).

DNA−DNA Cross-Linking Studies of HNE-Adducted Oligonucleotides. We have previously shown that 1,N^2-deoxyguanosine adducts of acrolein are capable of forming interstrand DNA−DNA cross-links in a CpG sequence. In DNA duplexes the hydroxypropano adduct opens to give the free aldehyde (**42**, Scheme 6). Nucleophilic addition by the exocylic amino group gives a carbinolamine interstrand cross-link (**43**). Recent NMR studies of a cross-linked DNA duplex have provided compelling evidence for the carbinolamine cross-link.[25] The carbinolamine

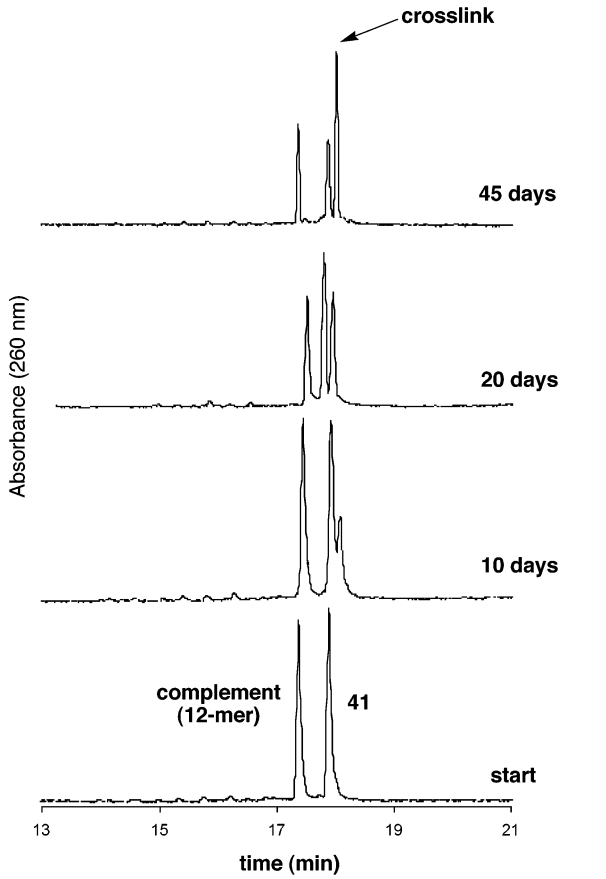


Figure 9. Cross-linking of (*6S,8R,11S*)-HNE adducted oligonucleotide **41** with a complementary 12-mer in 50 mM phosphate buffer, pH 7.0 containing 1 M KCl at 37 °C.

can undergo reversible dehydration to the corresponding imine cross-link (**44**). The imine cross-link has not been detected spectroscopically but its presence, at least in small quantities, has been established by chemical probes.

Interchain cross-linking of oligonucleotides containing the (*6S,8S*)- and (*6R,8R*)-crotonaldehyde adducts (**31** and **32**, respectively, in Figure 7) has also been studied.[37] Interestingly, a strong stereochemical preference for the interstrand cross-linking reaction was observed with the (*6R,8R*)-stereoisomer (**32**) cross-linking efficiently (38%), whereas the (*6S,8S*)-stereoisomer cross-linked only to the extent of about 5%.

Duplexes were formed by hybridization of **38**−**41** with the complementary strand and their ability to form interstrand cross-links examined. As with the crotonaldehyde adducts, we observed a strong stereochemical preference in the cross-linking reaction. Oligonucleotides **38**−**40** showed no evidence of cross-linking with a complementary strand after incubation for 60 days in 50 mM, pH 7.0 phosphate buffer with 1M KCl. Oligonucleotide **41** having the (*6S,8R,11S*) configuration, however, formed a new species over time which proved to be the interstrand cross-link. With the oligonucleotide containing the (*6R,8R*)-crotonaldehyde adduct, the rate of cross-linking had been significantly slower than for the acrolein adduct. The cross-linking reaction with oligonucleotide **41** was slower yet. Figure 9 shows the electrophoretograms obtained when the reaction was monitored for more than eight weeks by CZE.

(37) Kozekov, I. D.; Nechev, L. V.; Moseley, M. S.; Harris, C. M.; Rizzo, C. J.; Stone, M. P.; Harris, T. M. *J. Am. Chem. Soc.* **2003**, *125*, 50.

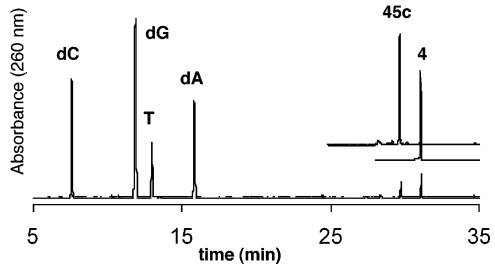


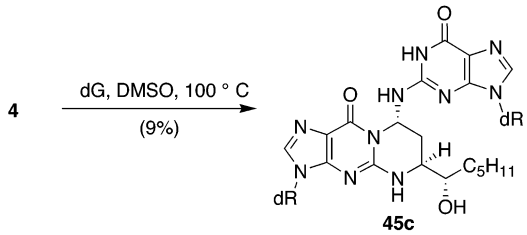
Figure 10. Enzyme digest of the cross-linking reaction of **41** with its complement after 61 days at 37 °C.

The cross-linking reaction with **41**, although slow, gave a remarkably high yield. The duplex containing the acrolein-adducted oligonucleotide had come to equilibrium within 7 days and gave approximately 50% of the cross-link, whereas the (*6R,8R*)-crotonaldehyde-adducted oligonucleotide had come to equilibrium within 24 days and gave 38% of the cross-link. For **41**, the cross-linking reaction did not appear to reach equilibrium, even after 61 days. When the reaction was stopped after this time, we estimated that the yield of cross-link was 85% based on integration of the UV absorbance in the electrophoretogram.

Multiple lines of evidence had been obtained to establish that acrolein could mediate interstrand cross-links in a CpG sequence. MALDI-TOF mass spectrometry of the cross-linked oligonucleotide had supported the presence of carbinolamine and imine species. Enzymatic digestion of the cross-linked oligonucleotides with nucleases gave pyrimidopurinone bis-nucleoside **45a**. When the cross-linked oligonucleotide was treated with sodium cyanoborohydride prior to enzymatic digestion, the N^2-N^2 trimethylene-linked bis-nucleoside was obtained. Authentic samples of the pyrimidopurinone and trimethylene bis-nucleoside standards were prepared by independent routes. The presence of the carbinolamine has been confirmed by NMR spectroscopy. Similar lines of evidence have established the interstrand cross-link in a duplex containing the (*6R,8R*)-adduct of crotonaldehyde.

The presence of an interstrand cross-link in the duplex formed from oligonucleotide **41** was established by enzymatic digestion of the cross-linking reaction with snake venom phosphodiesterase, DNase I, and alkaline phosphatase which gave pyrimidopurinone bis-nucleoside **45c** along with the four unmodified nucleosides in the proper relative amounts for a duplex of **41** with its complement (Figure 10). In addition, some of nucleoside **4** was observed; it arose from un-cross-linked oligonucleotide **41** and also by hydrolysis of the cross-link during the course of the enzymatic digestion. The assignments of nucleosides **4** and **45c** in the digestion reaction were confirmed by HPLC co-injection with authentic samples. Nucleoside **45c** was prepared according to Scheme 7 in low yield. The pyrimidopurine structure **45c** was established by

Scheme 7



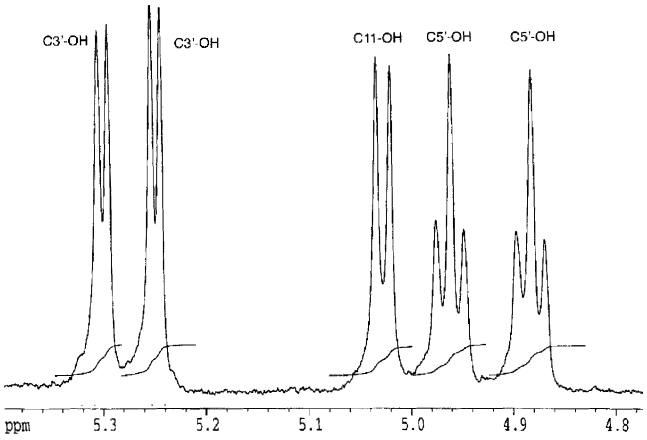


Figure 11. ^{1}H NMR spectrum of **45c** in DMSO-d_6.

Table 2. T_m Studies of Adducted Oligonucleotide Duplexes

5′-d(G-G-A-C-T-C-G-C-T-A-G-C)-3′ 3′-d(C-C-T-G-A-**X**-C-G-A-T-C-G)-5′	
Unadducted (**X** = dG)	65° C
Acrolein[a] (**46**)	55 °C (55 + 91°C)[d]
Crotonaldehyde[b]	
47: **X** = **31** (*6S,8S*)	51°C
48: **X** = **32** (*6R,8R*)	51 °C (51 + 88 °C)[e]
HNE[c]	
38: **X** = **1** (*6R,8S,11S*)	60 °C
39: **X** = **2** (*6S,8R,11R*)	60 °C
40: **X** = **3** (*6R,8S,11R*)	53 °C
41: **X** = **4** (*6S,8R,11S*)	49 °C (49 + >90 °C)[f]

Temperature was raised 1 °C per minute. Conditions: 1M NaCl, 10 mM pH 7 Phosphate, 0.05 mM EDTA, 0.5 AU/mL of each Oligonucleotide). [a] Ref. [b] Ref. [c] This work. [d] After 7 days at 37 °C. [e] After 24 days at 37 °C. [f] After 61 days at 37 °C.

observation of the ^{1}H−^{1}H coupling of the hydroxyl protons in the NMR spectrum taken in DMSO. The proton assignments were made by a COSY spectrum. The C11 hydroxyl proton was observed as a doublet, which is consistent with structure **45c** (Figure 11). The structure was further confirmed by HMBC.

Melting Studies. Modified oligonucleotides **38**−**41** were hybridized with the complementary 12-mer and the influence of the HNE adducts on the thermal stability of the duplexes was assessed via melting studies monitored by the increase in molar absorptivity at 260 nm which occurs when a DNA duplex dissociates. The results of this study are summarized in Table 2. A melting experiment carried out with the unadducted 12-mer duplex gave a T_m value of 65 °C (Figure 12, Panel A). For the modified oligonucleotides, the T_m values were measured immediately after annealing with the complementary strands. The samples were then incubated at 37 °C to allow cross-linking to occur.

The initial T_m values for the duplexes formed from HNE-adducted oligonucleotides **38**−**41** varied with the stereochemistry of the adduct. In general, DNA adducts destabilize duplex structure and lower the T_m of the duplex. Oligonucleotides **38** and **39** containing the (*6R,8S,11S*) and (*6S,8R,11R*) stereochemistry in the HNE adduct, respectively, had T_m values of 60 °C, only 5° lower than the unmodified duplex. Oligonucleotides **40** and **41** were significantly more destabilized and had T_m values of 53 and 49 °C, respectively. With all four adducted duplexes, there was only a small hysteresis effect between the melting and annealing processes, comparable to that observed with the unadducted duplex.

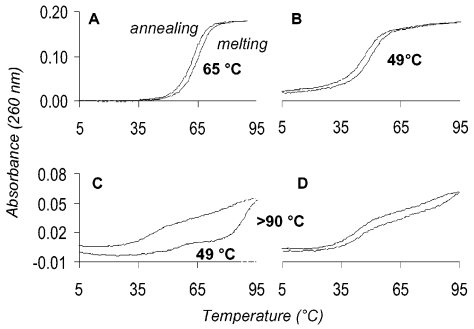


Figure 12. Melting curves for (A) the unadducted 12-mer duplex, (B) the duplex containing the (*6S,8R,11S*) adduct of HNE (**41**) immediately after duplex preparation, (C) the same sample after incubation for 61 days at 37 °C, (D) a second melting-annealing cycle carried out immediately after the measurements shown in Panel C.

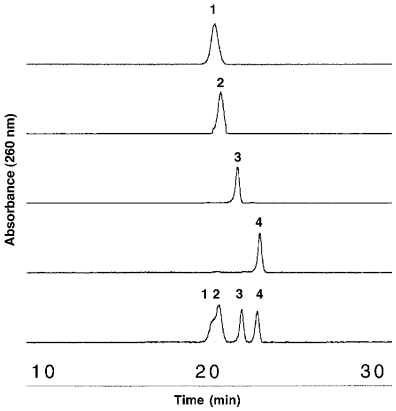


Figure 13. HPLC chromatogram of **1**−**4**.

The oligonucleotides were then incubated at 37 °C for extended periods of time to allow an opportunity for interstrand cross-linking to occur. There was little or no change in the melting curves for the duplexes formed from oligonucleotides **38**−**40**, which had also shown no evidence of cross-link formation by the CZE assay. However, for the duplex containing adducted oligonucleotide **41** with the (*6S,8R,11S*)-adduct configuration, a second melting transition was observed. Panel C of Figure 12 shows a biphasic melting curve observed after the sample had incubated for 61 days. The higher transition occurred at >90 °C. It should be noted that the cooling curve is substantially displaced from the heating curve although clearly not identical with the curve in Panel B. Immediately after the melting-annealing cycle had been completed the cycle was repeated. Two melting transitions can still be seen (Panel D), although the high melting transition is less pronounced than in Panel C.

Discussion

Syntheses of the HNE-adducted nucleosides **1**−**4** and oligonucleotides containing them have been accomplished in a structurally specific manner by taking advantage of the displacement of fluoride from 2-fluorohypoxanthine derivatives by aminotriols. The present syntheses are modeled on previous work from our laboratory for the syntheses of acrolein- and crotonaldehyde-adducted nucleosides and oligonucleotides.[17b,c,37] The present reactions differ in one key aspect from the earlier reports, namely, the condensation reactions using aminotriols **27**−**30** are significantly slower than the corresponding reactions of the aminodiols used to produce the acrolein and crotonaldehyde adducts. The basis for this reduced reactivity may, in part, be steric because of the greater bulk of the α-hydroxyhexyl moiety attached to the aminodiol. However, electronic factors stemming from the vicinal amino alcohol structure are believed to play a greater role. The strong dipole of the hydroxyl group reduces electron density on the amine, thus decreasing its nucleophilicity. Furthermore, the hydroxyl group is able to hydrogen bond with the amine, further decreasing its reactivity with electrophiles. Attenuated reactivity has also been observed in similar reactions of aminotriols derived from PAH diol epoxides, even in cases where steric factors do not play a major role.[38] Despite this problem, the reactivity of aminotriols **27**−**30** was sufficient for the syntheses of adducted nucleosides **1**−**4** and oligonucleotides containing them to be achieved in a reasonably efficient fashion.

The HPLC analysis of 1,N^2-deoxyguanosine adducts of HNE, as well as the corresponding 5′-phosphates, 3′-phosphates, and 3′,5′-bis-phosphates, has been reported in connection with the development of methods to detect these adducts in animal tissue using ^{32}P-postlabeling or electrochemical detection.[39] In all of these reports, the elution profile of the four stereoisomers shows that the first two diastereomers that eluted from reverse phase columns are only partially resolved, whereas the subsequent pair are cleanly resolved from the first pair and from each other. Importantly, Fu showed that the four diastereomers of the nucleosides eluted in the same relative order as the corresponding phosphate derivatives.[39b] It was previously demonstrated that acid-catalyzed deglycosylation of the partially resolved pair of nucleoside adducts gave a single, presumably racemic, guanine derivative.[39b] Deglycosylation of the second pair also gave a single guanine derivative, which had a diastereomeric relationship to that derived from the partially resolved pair of HNE adducts.[39a] This result indicates that the partially resolved pair of adducts have the same relative stereochemistry, but opposite absolute configurations at C6, C8, and C11. Likewise, the second pair have the same relative stereochemistry, but opposite configurations, at C6, C8, and C11. The two pairs are diastereomeric at C11 relative to C8 and C6.

Using our nucleosides **1**−**4**, we are now able to assign the absolute configurations of the HNE adducts. Using the published HPLC conditions, we have reproduced the chromatogram with mixtures of the synthetic HNE-adducted nucleosides (Figure 13). In the order of elution, the unresolved pair of diastereomers are assigned as diastereomers **1** (*6R,8S,11S*) and **2** (*6S,8R,11R*) and the resolved pair as **3** (*6R,8S,11R*) and **4** (*6S,8R,11S*). (Our numbering of **1**−**4** in this paper has been chosen so that the numbers would correspond to the order of HPLC elution.) A comparison of the NMR data (Table 1) for nucleosides characterized by Fu with those of the synthetic samples prepared in the present investigation, particularly protons **7a** and **7b**, fully supports these assignments.[28,39b]

The reaction of racemic HNE with calf thymus DNA followed by enzymatic digestion and detection by ^{32}P-postlabeling has

(38) McNees, A. G.; O'Donnell, M.; Horton, P. H.; Kim, H.-Y.; Kim, S. J.; Harris, C. M.; Harris, T. M.; Lloyd, R. S. *J. Biol. Chem.* **1997**, *272*, 33 211.
(39) (a) Douki, T.; Ames, B. N. *Chem. Res. Toxicol.* **1994**, *7*, 511. (b) Yi, P.; Zhan, D.; Samokyszyn, V. M.; Doerge, D. R.; Fu, P. P. *Chem. Res. Toxicol.* **1997**, *10*, 1259. (c) Chung, F.-L.; Nath, R. G.; Ocando, J.; Nishikawa, A.; Zhang, L. *Cancer Res.* **2000**, *60*, 1507. (d) Wacker, M.; Schuler, D.; Wanek, P.; Eder, E. *Chem. Res. Toxicol.* **2000**, *13*. 1165.

been reported to give only two of the four possible stereoisomers of the deoxyguanosine adducts.[39b] The major adduct can now be assigned as **4** possessing the *6S,8R,11S* stereochemistry and the minor adduct is **3**, possessing the *6R,8S,11R* stereochemistry. However, it should be noted that all four adducts have been detected in animal tissue using the ^{32}P-postlabeling method.[39c]

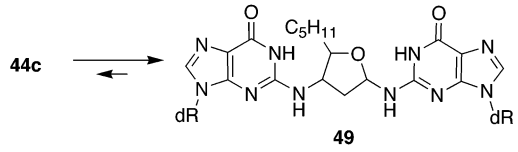
The T_m measurements summarized in Table 2 provide some insight into the structure of DNA duplexes containing the enal adducts. Previous studies have confirmed that the acrolein adducted oligonucleotide (**46**) is present entirely in the ring-opened N^2-(3-oxopropyl) form (**42**, Scheme 6) in the DNA duplexes rather than in the pyrimidopurinone form.[22−25] The ring opening allows for the stabilizing effects of Watson−Crick hybridization to be maintained. We believe that the (*6R,8R*)-crotonaldehyde (**32**) and (*6S,8R,11S*)-HNE (**41**) adducts also exist, at least in part, in the ring-opened form because they are able to form cross-links and ring-opening is a prerequisite for the cross-linking reaction. Adducted oligonucleotides **47** and **38**−**40** form interstrand cross-links inefficiently or not at all and may exist predominantly in the ring-closed pyrimidopurinone form. The adducted duplexes are all destabilized relative to the unmodified duplex.

The reported T_m of the acrolein-modified oligonucleotide was depressed by 10 °C, whereas the slightly bulkier crotonaldehyde adducts caused a 14 °C depression. Duplexes containing HNE-adducted oligonucleotides **40** and **41** have T_m values comparable to those for crotonaldehyde whereas those containing **38** and **39** are less destabilizing than the acrolein adduct. We hypothesize that HNE adducts, in particular those in oligonucleotides **38** and **39** are able to adopt conformations in which the side-chain hydroxyl groups can hydrogen bond with basic sites in the duplex, thus offsetting the inherent destabilization by the hydrophobic hexyl chain.

For the acrolein-, (*6R,8R*)-crotonaldehyde-, and (*6S,8R,11S*)-HNE-adducted oligonucleotides, an additional, higher melting transition had been observed at approximately 90 °C after the duplexes have been allowed to incubate for an extended period of time to permit cross-linking (Table 2). The higher melting transition is assigned to the interstrand cross-link. When the cross-link was present, a large hysteresis effect was seen during the cooling phase of the experiment, indicating that the cross-link was undergoing cleavage after the second melting transition took place. In the melting studies, a second melting-annealing cycle was routinely carried out immediately after completion of the first one. For the oligonucleotides containing the acrolein and (*6R,8R*)-crotonaldehyde adducts, the higher melting species was no longer observed in the second melting cycle. However, the cross-link derived from the (*6S,8R,11S*)-HNE adduct was much more robust; only partial loss of the cross-linked species occurred during the first melting cycle.

Oligonucleotides containing the (*6R,8R*)-crotonaldehyde adduct (**48**) and the (*6S,8R,11S*)-HNE adduct (**41**) have the same relative stereochemistry at C6 and cross-link effectively. Interestingly, the oligonucleotide containing the (*6S,8R,11R*) adduct of HNE (**40**) failed to cross-link even though it has the same relative stereochemistry at C6. In addition, the (*6S,8R,11S*) adduct cross-linked to a greater extent than the (*6R,8R*) adduct of crotonaldehyde or even the acrolein adduct. This suggests that the C11 side-chain hydroxyl group in the (*6S,8R,11S*) adduct of HNE stabilizes the cross-linked product. In support of this

Scheme 8



hypothesis, the resulting cross-link has much higher thermal stability than those derived from the acrolein and crotonaldehyde adducts. A possible explanation for the high yield and thermal stability of the HNE interstrand cross-link, as well as the dependence on the C11 stereochemistry for the HNE adducts is that the side-chain hydroxyl group of **41** can trap the imine form of the cross-link to form tetrahydrofuran **49** (Scheme 8), which we believe would be more stable than either the carbinolamine or imine cross-links. The disposition of the pentyl group in cross-link **49** would be dependent upon the C11 stereochemistry of **44c** and one could easily imagine that the configuration of the carbon bearing the hydroxyl group could greatly influence the stability of tetrahydrofuran **49**.

An alternative explanation for the stereochemical dependence for the cross-linking reaction is that only the (*6R,8R*)-crotonaldehyde and (*6S,8R,11S*)-HNE adducts open to the N^2-3-oxopropyl structure (**42**, Scheme 6) when hybridized to a complementary strand of DNA and the other diastereomers remain in the ring-closed pyrimidopurinone form. It is likely that the modified base would exist in the syn conformation about the glycosidic bond for the ring closed structure as was shown for oligonucleotides containing 1,N^2-propano-2′-deoxyguanosine (PdG).[40] UV melting studies showed that a PdG-containing 13-mer was destabilized by 14 °C when hybridized to a complementary strand under low salt (1 mM NaCl) conditions relative to an unadducted oligonucleotide. This T_m difference is the same as that observed for the crotonaldehyde adducted oligonucleotides. The thermal destabilization of the HNE-adducted oligonucleotides due to a possible syn conformation of the nucleobase could be partially offset by formation of a hydrogen bond by the side chain hydroxyl group. We plan to establish the structure and conformation of both the cross-linked and uncross-linked crotonaldehyde and HNE-adducts by NMR spectroscopy.

Summary

We have developed stereospecific syntheses of nucleosides and oligonucleotides containing 1,N^2-deoxyguanosine adducts of HNE. We have also demonstrated that one of these adducts, i.e., that possessing the (*6S,8R,11S*) stereochemistry, is capable of efficiently forming a very stable interstrand cross-link in a CpG context. The cross-linking adduct shares a common C6 stereochemistry with the crotonaldehyde adduct that also forms interstrand cross-links.

DNA−DNA cross-links represent a severe form of DNA damage that will interfere with DNA replication. We have now shown that simple enals that arise endogenously from lipid peroxidation are able to form interstrand DNA−DNA cross-links. In these in vitro experiments, the rate of cross-link was on the order of days to weeks. However, this rate could be

(40) (a) Weisenseel, J. P.; Reddy, R.; Marnett, L. J.; Stone, M. P. *Chem. Res. Toxicol.* **2002**, *15*, 127. (b) Singh, U. S.; Moe, J. G.; Reddy, G. R.; Weisenseel, J. P.; Marnett, L. J.; Stone, M. P. *Chem. Res. Toxicol.* **1993**, *6*, 825.

dramatically influenced by the local structure of DNA such as those imposed by supercoiling. In addition, the persistence of the DNA lesion is an important issue. In preliminary structural work, the ring-opened form of the acrolein adduct did not perturb the duplex structure. The synthesis of authentic standards of the cross-linked nucleosides will greatly aid in the identification of these cross-links in the DNA of exposed cells and tissue samples. Work along these lines is currently in progress.

Experimental Section

All commercially obtained chemicals were used as received. Methylene chloride was freshly distilled from calcium hydride. Anhydrous THF was freshly distilled from a sodium/benzophenone ketyl. Anhydrous *tert*-butyl hydroperoxide in isooctane was prepared as previously described.[30b] All reactions were performed under an argon atmosphere. Glassware was oven-dried and cooled under argon. Proton NMR data were recorded at 300 and 400 MHz; carbon-13 data was recorded at 75 and 100 MHz. High-resolution FAB mass spectra were obtained from the University of Notre Dame Mass Spectrometry Center using nitrobenzyl alcohol (NBA) as the matrix. MALDI-TOF mass spectra (negative ion) of modified oligonucleotides were obtained on a Voyager Elite DE instrument (Perseptive Biosystems) at the Vanderbilt Mass Spectrometry Facility using a 3-hydroxypicolinic acid (HPA) matrix containing ammonium hydrogen citrate (7 mg/mL) to suppress sodium and potassium adducts. The cross-linked oligonucleotides were unstable in the MALDI matrix, rapidly reverting to the individual strands. Flash column chromatography was performed using silica gel (70−230 mesh). Nucleosides were purified by C-18 reverse phase HPLC in water/acetonitrile with a diode array detector monitoring at 260 nm. Gradient A and B were used for HPLC purification and analysis: gradient A, 99% water initially, 15 min linear gradient to 90% water, 5 min linear gradient to 80% water, and 10 min linear gradient to 0% water followed by 5 min linear gradient to initial conditions; and gradient B, 85% water initially, 40 min linear gradient to 70% water, 5 min linear gradient to 0% water, followed by 5 min linear gradient to initial conditions. Electrophoretic analyses were carried out using a Beckman P/ACE Instrument System 5500 Series monitored at 260 nm on a 27 cm × 100 μm column packed with the manufacturer's 100-R gel (for ss DNA) using a Tris-borate buffer system containing 7 M urea.

(*2R,3R*)-3-Pentyloxirane-2-methanol (13).[30,33] To a 200 mL flame-dried flask were added activated, powdered 4 Å molecular sieves (0.70 g) and anhydrous methylene chloride (80 mL). A solution of diethyl D-(−)-tartrate (0.69 g, 3.37 mmol) in methylene chloride (5 mL) was stirred over activated 4 Å molecular sieves (pellets) for 15 min, then transferred to the reaction flask via cannula. The reaction mixture was cooled to −25 °C then titanium tetraisopropoxide (5.42 mL, 18.2 mmol) and anhydrous *tert*-butyl hydroperoxide (6.0 M in isooctane, 6.30 mL, 37.86 mmol) were successively added dropwise and the mixture stirred at −25 °C for 40 min. A solution of *E*-2-octenol (**12**, 1.80 g, 14.1 mmol) in methylene chloride (10 mL) was stirred over activated 4 Å molecular sieves (pellets) for 15 min, then transferred to the reaction flask dropwise via cannula. The mixture was stirred at −25 °C over 4 h, then quenched by the addition of a solution of ferrous sulfate (15 g) in 10% aqueous tartaric acid (50 mL) to the cooled reaction mixture. The mixture was stirred for 1 h and the organic phase was separated, washed with water, dried over Na_2SO_4, filtered and concentrated. The residue was diluted with ether (100 mL) and stirred with 1 N NaOH (50 mL) at 0 °C for 40 min. The ether solution was separated, washed with brine, dried over $MgSO_4$, filtered and evaporated. Purification by flash chromatography on silica, eluting with 10% ethyl acetate in hexanes gave **13** (1.73 g, 85.0%): $[\alpha]^{20}_D$ +40.0° (*c* 0.56, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.94−3.88 (m, 1H), 3.65−3.60 (m, 1H), 2.97−2.90 (m, 2H), 1.70 (br s, 1H),1.61−1.24 (m, 8H), 0.90 (t, J = 6.7 Hz, 3H); ^{13}C NMR δ61.7, 58.5, 56.0, 31.5, 31.4, 25.6, 22.6,13.9.

(*2S,3S*)-3-Pentyloxirane-2-methanol (*ent*-13). The Sharpless epoxidation of **12** was performed in the same manner as described above for **13** using diethyl L-(+)-tartrate to give ***ent*-13** in 82% yield. $[\alpha]^{20}_D$ −40.4° (*c* 1.08, $CHCl_3$).

(*4S,5R*)-3-Benzyl-4-hydroxymethyl-5-pentyl-2-oxazolidinone (15) and 3-Benzyl-4-(1-hydroxyhexyl)-2-oxazolidinone (14). A suspension of 60% NaH (390 mg, 1.51 mmol) in dry THF (12 mL) was cooled to 0 °C, and a solution of **13** (360 mg, 2.50 mmol) in dry THF (2 mL) was added dropwise over 5 min. The reaction was stirred for an additional 10 min, then freshly distilled benzyl isocyanate (460 μL, 53.8 mmol) was added dropwise over 5 min. The reaction was heated at reflux for 2 h. The mixture was cooled to 0 °C and 60% NaH (85 mg, 2.13 mmol) was added. The reaction was heated at reflux for an additional 1 h after which time the solution was cooled to 0 °C and quenched with saturated NH_4Cl (10 mL). The layers were separated and the aqueous phase was extracted with methylene chloride (3 × 10 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification by flash chromatography on silica, eluting with 5−20% ethyl acetate in hexane gave a mixture of **14** and **15**. The mixture was separated by flash chromatography on silica, eluting with 5−50% methylene chloride in 2:1 ether in hexane to give **15** (258 mg, 37%). The remaining **14** was recycled by the treatment with NaH in THF and the resulting mixture purified as above to afford additional **15** (91 mg, 13%). **14**: ^{1}H NMR ($CDCl_3$) δ 7.60−7.26 (m, 5H), 4.69 (d, J = 15.2 Hz, 1H), 4.34 (dd, J = 8.5, 6.9 Hz, 1H), 4.30 (d, J = 15.2 Hz, 1H), 4.20 (t, J = 8.8 Hz, 1H), 3.81−3.78 (m, 1H), 3.60 (m, 1H), 2.21 (br s, 1H), 1.45−1.05 (m, 8H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR δ 159.2, 136.2, 129.1, 128.2, 128.0, 67.5, 62.0, 59.2, 46.6, 31.7, 31.5, 25.5, 22.4, 13.9. **15**: $[\alpha]^{20}_D$ +13.1° (*c* 0.61, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.39−7.26 (m, 5H), 4.79 (d, J = 15.2 Hz, 1H), 4.47 (ddd, J = 9.9, 7.8, 3.6 Hz, 1H), 2.43 (br s, 1H), 4.25 (d, J = 15.2 Hz, 1H), 3.80−3.67 (m, 2H), 3.55 (ddd, J = 7.8, 3.8, 3.2 Hz, 1H), 1.93−1.83 (m, 1H), 1.69−1.56 (m, 2H),1.42−1.26 (m, 5H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR δ 158.8, 136.5, 128.8, 128.0, 127.8, 77.5, 58.6, 58.3, 46.5, 31.4, 29.0, 25.8, 22.4, 13.9; HRMS (FAB, NBA) *m/z* calcd for $C_{16}H_{24}NO_3$ (M+H) 278.1756, found 278.1757. Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.29; H, 8.36; N, 5.05. Found: C, 69.18; H, 8.47; N, 4.91.

(*4R,5S*)-3-Benzyl-4-hydroxymethyl-5-pentyl-2-oxazolidinone (*ent*-15). Following the procedure described above for **15**, ***ent*-13** was converted to ***ent*-15** in 49% yield: $[\alpha]^{20}_D$ −13.3° (*c* 0.55, $CHCl_3$).

(*4S,5R*)-3-Benzyl-4-iodomethyl-5-pentyl-2-oxazolidinone (16). Triphenylphosphine (706 mg, 2.68 mmol) and imidazole (183 mg, 2.68 mmol) were dissolved in ether (4 mL) and acetonitrile (1 mL). The mixture was cooled to 0 °C, and iodine (680 mg, 2.68 mmol) was added with vigorous stirring. The resulting slurry was warmed to 20 °C, then cooled to 0 °C again, and a solution of **15** (248 mg, 0.89 mmol) in ether/acetonitrile/benzene (3 mL, 1:1:1) was added dropwise over 5 min. The mixture was warmed to 20 °C and stirred for 1 h, then cooled to 0 °C and quenched with 5% sodium bicarbonate (10 mL). The layers were separated and the aqueous phase was extracted with ether (3 × 10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Purification by flash chromatography on silica, eluting with 5−7% ethyl acetate in hexanes afforded **16** (291 mg, 84.0%): $[\alpha]^{20}_D$ + 40.6° (*c* 1.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.41−7.26 (m, 5H), 4.85 (d, J = 15.4 Hz, 1H), 4.50 (ddd, J = 10.4, 7.5, 3.3 Hz, 1H), 4.13 (d, J = 15.4 Hz, 1H), 3.71 (ddd, J = 7.5, 7.2, 3.1 Hz, 1H), 3.21−3.08 (m, 2H), 1.71−1.92 (m, 2H), 1.60−1.63 (m, 1H), 1.26−1.42 (m, 5H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR δ 157.8, 135.7, 129.0, 128.1, 128.0, 77.5, 56.9, 46.4, 31.4, 27.8, 25.6, 22.4, 13.9, −0.9.

(*4R,5S*)-3-Benzyl-4-iodomethyl-5-pentyl-2-oxazolidinone (*ent*-16). Following the procedure described above for **16**, ***ent*-15** was converted to ***ent*-16** in 83% yield. $[\alpha]^{20}_D$ − 40.6 ° (*c* 0.725, $CHCl_3$).

(*4S,5R*)-4-Allyl-3-benzyl-5-pentyl-2-oxazolidinone (17). To a solution of **16** (294 mg, 0.759 mmol), cuprous iodide (15 mg, 0.08 mmol), and HMPA (0.56 mL, 3.04 mmol) in THF (3 mL) was added

vinylmagnesium bromide (1.52 mL, 1.0 M solution in THF) dropwise at −23 °C over 5 min. The resulting mixture was stirred for 40 min at −23 °C, then quenched with saturated NH_4OH (10 mL). The layers were separated and the aqueous phase was extracted with ether (3 × 10 mL). The combined organic extracts were dried with $MgSO_4$, filtered, and concentrated. Purification by flash chromatography on silica, eluting with 5−7% ethyl acetate in hexanes afforded **17** (146 mg, 67%). $[\alpha]^{20}_D$ +23.2 (*c* 1.34 in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.37−7.26 (m, 5H), 5.74−5.67 (m, 1H), 5.17−5.12 (m, 2H), 4.85 (d, J = 15.3 Hz, 1H), 4.44−4.40 (m, 1H), 4.08 (d, J = 15.3 Hz, 1H), 3.63−3.58 (m, 1H), 2.38−2.34 (m, 2H), 1.80−1.72 (m, 1H), 1.67−1.30 (m, 7H), 0.89 (t, J = 6.1 Hz, 3H); ^{13}C NMR δ 158.2, 136.2, 133.0, 128.8, 127.9, 127.8, 118.9, 78.1, 56.5, 46.1, 31.9, 31.4, 29.1, 25.6, 22.4, 13.9; HRMS (FAB, NBA) *m*/*z* calcd for $C_{18}H_{26}NO_2$ (M+H) 288.1964, found 288.1964. Anal. Calcd for $C_{18}H_{25}NO_2$: C, 75.22; H, 8.77; N, 4.87. Found: C, 74.97; H, 8.62; N, 4.87.

(*4R,5S*)-4-Allyl-3-benzyl-5-pentyl-2-oxazolidinone (*ent*-17). Following the procedure described above for **17**, ***ent*-16** was converted to ***ent*-17** in 70% yield. $[\alpha]^{20}_D$ −23.8° (*c* 1.40, $CHCl_3$).

(*4S,5R*)-3-Benzyl-4-(2′,3′-dihydroxypropyl)-5-pentyl-2-oxazolidinone (18). To a stirred solution of **17** (66 mg, 0.23 mmol), *N*-methylmorpholine *N*-oxide (31 mg, 0.253 mmol), THF (0.8 mL), *t*-BuOH (0.32 mL), and water (0.16 mL) was added osmium tetroxide (14 μL, 0.1 M in benzene, 1.4 μmol). The mixture was stirred for 12 h, then a second portion of osmium tetroxide (9 μL, 0.1 M in benzene 0.9 μmol) was added and stirring continued for 8 h. The reaction was quenched with 5% aqueous $NaHSO_3$ (5 mL) with vigorous stirring for 15 min then poured into water (5 mL), transferred to a separatory funnel and extracted with methylene chloride (10 × 3 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. Purification by flash chromatography on silica, eluting with 1−2% methanol in chloroform gave **18** (53 mg, 72%) as an inseparable mixture of diastereomers: ^{1}H NMR ($CDCl_3$, assignment of diastereomeric pairs based on coupling pattern and COSY) δ 7.35−7.22 (m, 5H), 4.79 and 4.67 (diastereomers, d, d, J = 15.4 and 15.4 Hz, 1H), 4.50−4.40 (m, 1H), 4.21 and 4.13 (diastereomers, d, d, J = 15.4 and 15.4 Hz, 1H), 3.87 and 3.76 (diastereomers, m, m, 1H), 3.75 and 3.64 (diastereomers, m, m, 1H), 3.59−3.53 (m, 1H), 3.38−3.32 (m, 1H), 2.99 (d, J = 21.3 Hz, 1H), 2.89 (br s, 1H), 1.74−1.46 (m, 5H), 1.37−1.29 (m, 5H), 0.90−0.86 (m, 3H); ^{13}C NMR ($CDCl_3$, assignment of diastereomeric pairs based on relative intensity and DEPT) δ 159.9 and 158.6, 136.5 and 136.2, 128.8 and 128.7, 127.9, 127.8 and 127.8, 79.0 and 78.2, 69.1 and 68.7, 67.8 and 66.7, 55.2 and 54.7, 46.8 and 45.9, 31.5, 30.6 and 30.1, 29.4 and 29.4, 25.5, 22.5 and 22.4, 13.9; HRMS (FAB, NBA) *m*/*z* calcd for $C_{18}H_{28}NO_4$ (M+H) 322.2018, found 322.2010.

(*4R,5S*)-3-Benzyl-4-(2′,3′-dihydroxypropyl)-5-pentyl-2-oxazolidinone (*ent*-18). Following the procedure described above for **18**, ***ent*-17** was converted to ***ent*-18** in 75% yield.

(*4S,5R*)-4-(Benzylamino)-1,2,5-decanetriol (19). A solution of **18** (32 mg, 0.108 mmol) and 50% aqueous NaOH (0.25 g) in absolute ethanol (1 mL) was heated at reflux overnight. The reaction was cooled to room temperature and poured into water (5 mL). The reaction was transferred to a separatory funnel and extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. Purification by flash chromatography, eluting with 4−6% methanol in hexanes afforded **19** (20 mg, 68%) as an inseparable mixture of diastereomers: ^{1}H NMR (CD_3OD, assignment of diastereomeric pairs based on coupling pattern and COSY) δ 7.35−7.26 (m, 5H), 3.98 and 3.90 (diastereomers, d, d, J = 12.8 and 12.4 Hz, 1H), 3.89−3.76 (m, 2H), 3.77 and 3.74 (diastereomers, d, d, J = 12.7 and 12.7, 1H), 3.47−3.37 (m, 2H), 2.79−2.74 (m, 1H), 1.72−1.31 (m, 10 H), 0.93−0.89 (m, 3H); ^{13}C NMR (assignment of diastereomeric pairs based on relative intensity and DEPT) δ 141.0 and 140.8, 129.6 and 129.5, 129.5 and 129.4, 128.3 and 128.2, 73.6 and 71.9, 71.5 and 71.1, 67.5 and 67.4, 62.1 and 59.4, 52.1 and 51.6, 34.8 and 34.6, 33.0 and 32.9, 31.5, 27.2 and 27.1, 23.7, 14.4; HRMS (FAB, NBA) *m*/*z* calcd for $C_{17}H_{30}NO_3$ (M+H) 296.2226, found 296.2221.

(*4R,5S*)-4-(Benzylamino)-1,2,5-decanetriol (*ent*-19). Following the procedure described above for **19**, ***ent*-18** was converted to ***ent*-19** in 69% yield.

(*4R,5S*)-4-Amino-1,2,5-decanetriol (8). Following the procedure described below for **9**, ***ent*-19** was converted to **8** as an inseparable mixture of diastereomers in 98% yield. The spectra properties were identical to **9**.

(*4S,5R*)-4-Amino-1,2,5-decanetriol (9). A suspension of 20% $Pd(OH)_2$ on carbon (5 mg) in methanol (1 mL) was stirred at room temperature under a hydrogen atmosphere for 30 min. A solution of **19** (18 mg, 0.061 mmol) in methanol (1 mL) was added via cannula, and the reaction mixture was stirred under a hydrogen atmosphere for 5 h. The catalyst was removed by filtration, and the filtrate was concentrated to give **9** (12.5 mg, 100%) as an inseparable mixture of diastereomers: ^{1}H NMR (CD_3OD) 3.82−3.73 (m, 1H), 3.62−3.40 (m, 3H), 2.98−2.88 (m, 1H), 1.88−1.16 (m, 10 H), 0.93−0.89 (m, 3H); ^{13}C NMR (assignment of diastereomeric pair based on relative intensity and DEPT) δ 75.9 and 75.6, 73.0 and 70.4, 67.8 and 67.5, 56.4 and 53.5, 35.7 and 34.6, 33.6, 33.0, 26.9, 23.7, 14.4; HRMS (FAB, NBA) *m*/*z* calcd for $C_{10}H_{24}NO_3$ (M+H) 206.1756, found 206.1759.

(*2R,3S*)-1,2-Epoxy-3-octanol (21).[30b,32] Dry methylene chloride (120 mL) was added to a 500 mL flame-dried flask under argon. A solution of (±)-octen-3-ol (**20**, 2.37 g, 18.2 mmol) and diisopropyl L-(+)-tartrate (5.13 g, 21.8 mmol) in 10 mL of CH_2Cl_2 was stirred with 4 Å molecular sieves (pellets) for 15 min, then transferred to the reaction flask via cannula, and the mixture was cooled to −20 °C. Titanium tetraisopropoxide (5.42 mL, 18.2 mmol) was added dropwise, and the mixture was stirred for 15 min at −20 °C. Anhydrous *tert*-butyl hydroperoxide (6.0 M in isooctane, 6.30 mL, 37.86 mmol) was added dropwise. The mixture was stirred at −20 °C for 5 h, after which time GC−MS analysis showed 45% conversion. To the stirred and cooled solution was added a solution of ferrous sulfate (15 g) in 10% aqueous tartartic acid (50 mL). The mixture was stirred for 1 h and then the organic phase was separated, washed with water, dried over Na_2SO_4, filtered, and evaporated. The residue was diluted with ether (100 mL) and stirred with 1N NaOH (50 mL) at 0 °C for 40 min. The ether phase was separated, washed with brine, dried over $MgSO_4$, filtered and evaporated. Purification by flash chromatography on silica, eluting with 8% ethyl acetate in hexanes gave **21** (1.06 g, 40%): $[\alpha]^{20}_D$ +21.4° (*c* 1.17, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.81−3.80 (m, 1H), 3.02−2.99 (m, 1H), 2.81 (dd, J = 5.0, 2.9 Hz, 1H), 2.73 (dd, J = 5.0, 4.1 Hz, 1H), 2.10 (br s, 1H), 1.31−1.55 (m, 8H), 0.90 (t, J = 6.7 Hz, 3H); ^{13}C NMR δ 68.4, 54.5, 43.4, 33.4, 31.8, 24.9, 22.5, 13.9.

(*2S,3R*)-1,2-Epoxy-3-octanol (*ent*-21). The Sharpless kinetic resolution of **20** was performed in the same manner as described above for **21** using diisopropyl D-(−)-tartrate to give ***ent*-21** in 41% yield: $[\alpha]^{20}_D$ −20.2° (*c* 1.29, $CHCl_3$).

(*4S,5S*)-3-Benzyl-4-hydroxymethyl-5-pentyl-2-oxazolidinone (22). A solution of **21** (450 mg, 3.12 mmol) in dry THF (10 mL) was treated with 60% NaH (269 mg, 6.72 mmol) under argon and stirred for 10 min at room temperature. Freshly distilled benzyl isocyanate (0.50 mL, 4.06 mmol) was added dropwise over 10 min, and the resulting mixture heated at reflux for 1.5 h. The mixture was then cooled to 0 °C and quenched with saturated NH_4Cl (10 mL) and the layers separated. The aqueous layer was extracted with methylene chloride (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica, eluting with 20% ethyl acetate in hexane gave a partially purified product. Further purification was accomplished by flash chromatography on silica, eluting with 1−18% ethyl acetate in hexane to afford **22** (0.502 g, 58%): $[\alpha]^{20}_D$ −72.0 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.36−7.26 (m, 5H), 4.72 (d, J = 15.3 Hz, 1H), 4.39 (ddd, J = 7.0, 5.6, 5.6 Hz, 1H), 4.26 (d, J = 15.3 Hz, 1H), 3.73−3.69 (m,

1H), 3.54−3.48 (m, 1H), 3.25 (ddd, J = 5.6, 3.3, 3.3 Hz, 1H), 2.67 (br s, 1H), 1.67−1.46 (m, 2H), 1.44−1.19 (m, 6H), 0.860 (t, 6.7 Hz, 3H); ^{13}C NMR δ 156.9, 144.9, 135.2, 128.4, 127.7, 127.3, 81.5, 78.4, 45.2, 35.3, 31.3, 23.5, 22.4, 13.9; HRMS (FAB, NBA) m/z calcd for $C_{16}H_{24}NO_3$ (M+H) 278.1756, found 278.1767. Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.29; H, 8.36; N, 5.05. Found: C, 69.06; H, 8.29; N, 5.11.

(*4R,5R*)-3-Benzyl-4-hydroxymethyl-5-pentyl-2-oxazolidinone (*ent*-22). Following the procedure described above for **22**, ***ent*-21** was converted to ***ent*-22** in 56% yield: $[\alpha]^{20}_D$ +67.5 (c 0.830, $CHCl_3$).

(*4S,5S*)-3-Benzyl-4-iodomethyl-5-pentyl-2-oxazolidinone (23). Triphenylphosphine (1.11 g, 4.22 mmol) and imidazole (288 mg, 4.22 mmol) were dissolved in ether (4.8 mL) and acetonitrile (1.2 mL). The mixture was cooled to 0 °C, and iodine (1.07 g, 4.22 mmol) was added with vigorous stirring. The resulting slurry was warmed to 20 °C and then cooled to 0 °C again, and a solution of **22** (390 mg, 1.41 mmol) in ether/acetonitrile/benzene (3 mL, 1:1:1) was added dropwise over 5 min. The mixture was warmed to 20 °C and stirred 1 h, then cooled to 0 °C and quenched with 5% sodium bicarbonate (10 mL). The layers were separated and the aqueous phase extracted with ether (3 × 10 mL). The combined organic extracts were dried with $MgSO_4$, filtered and concentrated. Purification by flash chromatography on silica, eluting with 4% ethyl acetate in hexanes afforded **23** (449 mg, 82%): $[\alpha]^{20}_D$ −10.5° (c 1.22, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.37−7.26 (m, 5H), 4.84 (d, J = 15.3 Hz, 1H), 4.22−4.16 (m, 1H), 4.08 (d, J = 15.3 Hz, 1H), 3.20−3.15 (m, 3H), 1.61−1.59 (m, 2H), 1.45−1.20 (m, 6H), 0.87 (m, 3H); ^{13}C NMR δ 157.4, 135.4, 129.0, 128.2, 128.1, 79.1, 59.0, 46.0, 35.0, 31.3, 23.9, 22.4, 13.9, 6.9.

(*4R,5R*)-3-Benzyl-4-iodomethyl-5-pentyl-2-oxazolidinone (*ent*-23). Following the procedure described above for **23**, ***ent*-22** was converted to ***ent*-23** in 83% yield: $[\alpha]^{20}_D$ + 10.9° (c 0.86, $CHCl_3$).

(*4S,5S*)-4-Allyl-3-benzyl-5-pentyl-2-oxazolidinone (24). To a solution of **23** (530 mg, 1.37 mmol), cuprous iodide (27 mg, 0.14 mmol) and HMPA (1.01 mL, 5.48 mmol) in THF (5 mL) was added vinylmagnesium bromide (2.74 mL, 1.0 M solution in THF) dropwise at −30 °C over 5 min. The resulting mixture was stirred for 1 h at −30 °C then quenched with saturated NH_4OH (10 mL). The layers were separated and the aqueous phase extracted with ether (3 × 10 mL). The combined organic extracts were dried with $MgSO_4$, filtered and concentrated. Purification by flash chromatography on silica, eluting with 3−7% ethyl acetate in hexanes afforded **24** (274 mg, 70%). $[\alpha]^{20}_D$ −52.4° (c 0.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.39−7.26 (m, 5H), 5.62 (m, 1H), 5.16 (m, 2H), 4.84 (d, J = 15.2 Hz, 1H), 4.16 (ddd, J = 7.2, 5.1, 5.1 Hz, 1H), 4.07 (d, J = 15.2 Hz, 1H), 3.21 (ddd, J = 7.2, 5.1, 4.1 Hz, 1H), 2.39−2.22 (m, 2H), 1.67−1.26 (m, 8H), 0.86 (t, J = 6.6 Hz, 3H); ^{13}C NMR δ 157.9, 135.8, 131.4, 128.7, 128.0, 127.8, 119.7, 78.1, 58.4, 45.8, 36.0, 34.8, 31.3, 24.1, 22.3, 13.8; HRMS (FAB, NBA) m/z calcd for $C_{18}H_{26}NO_2$ (M+H) 288.1964, found 288.1973. Anal. Calcd for $C_{18}H_{25}NO_2$: C, 75.22; H, 8.77; N, 4.87. Found: C, 75.12; H, 8.80; N, 4.83.

(*4R,5R*)-4-Allyl-3-benzyl-5-pentyl-2-oxazolidinone (*ent*-24). Following the procedure described above for **24**, ***ent*-23** was converted to ***ent*-24** in 72% yield. $[\alpha]^{20}_D$ + 51.8° (c 0.845, $CHCl_3$).

(*4S,5S*)-3-Benzyl-4-(2′,3′-dihydroxypropyl)-5-pentyl-2-oxazolidinone (25). To a stirred solution of **24** (150 mg, 0.522 mmol), *N*-methylmorpholine *N*-oxide (70 mg, 0.574 mmol), THF (1 mL), t-BuOH (0.4 mL), and water (0.2 mL) was added osmium tetroxide in benzene (32 μL, 0.1 M in benzene, 3.2 μmol). The mixture was stirred for 12 h, then a second portion of osmium tetroxide (20 μL, 0.1 M in benzene, 2.0 μmol) was added and stirring continued for 8 h. The reaction was quenched with 5% aqueous $NaHSO_3$ (10 mL) with vigorously stirring for 15 min. The mixture was poured into water, transferred to a separatory funnel and extracted with methylene chloride (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica, eluting with 1−2% methanol in chloroform gave **25** (116 mg, 69%) as an inseparable mixture of diastereomers: ^{1}H NMR ($CDCl_3$, assignment of diastereomeric pairs based on coupling pattern and COSY) δ 7.37−7.26 (m, 5H), 4.82 and 4.73 (diastereomers, d, d, J = 15.3 and 15.2 Hz, 1H), 4.40−4.20 (m, 1H), 4.12 and 4.10 (diastereomers, d, d, J = 15.3 and 15.2 Hz, 1H), 3.72−3.71 (m, 1H), 3.58−3.51 (m, 1H), 3.48−3.44 and 3.25−3.28 (diastereomers, m, m, 1H), 3.40−3.33 (m, 1H), 2.63 (m, 1H), 2.25 (br s, 1H), 1.81−1.50 (m, 4H), 1.39−1.15 (m, 6H), 0.86 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, assignment of diastereomeric pairs based on relative intensity and DEPT) δ 158.3, 135.8, 128.8, 128.0, 127.9 and 127.9, 80.1 and 79.2, 69.2 and 67.6, 66.9 and 66.8, 57.9 and 57.0, 46.0 and 45.7, 35.0 and 34.9, 34.6 and 34.2, 31.3 and 31.3, 24.0 and 24.0, 22.3, 13.8; HRMS (FAB, NBA) m/z calcd for $C_{18}H_{28}NO_4$ (M+H) 322.2018, found 322.2017.

(*4R,5R*)-3-Benzyl-4-(2′,3′-dihydroxypropyl)-5-pentyl-2-oxazolidinone (*ent*-25). Following the procedure described above for **25**, ***ent*-24** was converted to ***ent*-25** as an inseparable mixture of diastereomers in 71% yield.

(*4S,5S*)-4-(Benzylamino)-1,2,5-decanetriol (26). A solution of **25** (70 mg, 0.218 mmol) and 50% NaOH (0.5 mL) in 95% ethyl alcohol (2 mL) was heated at reflux overnight, cooled to room temperature and poured into water (10 mL). The reaction was transferred to a separatory funnel and extracted with ethyl acetate (4 × 10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Purification by flash chromatography, eluting with 4−6% ethanol in hexanes afforded **26** (45 mg, 70%): ^{1}H NMR ($CDCl_3$, assignment of diastereomeric pairs based on coupling pattern and COSY) δ 7.35−7.26 (m, 5H), 3.98 and 3.93 (diastereomers, d, d, J = 12.8 and 12.4 Hz, 1H), 3.96−3.94 and 3.91−3.87 (diastereomers, m, m, 1H), 3.84 and 3.71) (diastereomers, d, d, J = 12.4 and 12.8, 1H), 3.74−3.67 and 3.68−3.65 (diastereomers, m, m, 1H), 3.61−3.52 (m, 1H), 3.46−3.39 (m, 1H), 3.24 (br s, 4H), 2.90−2.86 and 2.82−2.78 (diastereomers, m, m, 1H), 1.95−1.26 (m, 10H), 0.92−0.87 (m, 3H); ^{13}C NMR ($CDCl_3$, assignment of diastereomeric pairs based on relative intensity and DEPT) δ 139.3 and 139.2, 128.5, 128.3 and 128.2, 127.3 and 127.2, 72.9 and 71.9, 71.5 and 69.9, 67.0 and 66.9, 60.8 and 60.2, 51.8 and 51.5, 33.9 and 33.9, 33.3 and 30.1, 31.8, 25.8 and 25.2, 22.6, 14.0; HRMS (FAB, NBA) m/z calcd for $C_{17}H_{30}NO_3$ (M+H) 296.2226, found 296.2221.

(*4R,5R*)-4-(Benzylamino)-1,2,5-decanetriol (*ent*-26). Following the procedure described above for **26**, ***ent*-25** was converted to ***ent*-26** in 68% yield.

(*4R,5R*)-4-Amino-1,2,5-decanetriol (10). Following the procedure described below for **11**, ***ent*-26** was converted to **10** as an inseparable mixture of diastereomers in 100% yield.

(*4S,5S*)-4-Amino-1,2,5-decanetriol (11). A suspension of 20% $Pd(OH)_2$ on carbon (10 mg) in methanol (1 mL) was stirred at room temperature under a hydrogen atmosphere for 30 min. A solution of **26** (29 mg, 0.098 mmol) in methanol (1 mL) was added via cannula and the reaction mixture stirred under a hydrogen atmosphere for 5 h. The catalyst was removed by filtration and the filtrate concentrated to give **11** (20 mg, 100%) as an inseparable mixture of diastereomers: ^{1}H NMR (CD_3OD, assignment of diastereomeric pairs based on coupling pattern and COSY) δ 3.83−3.76 (m, 1H), 3.53−3.43 (m, 2H), 3.50−3.44 (m, 1H), 2.96−2.93 (m, 1H), 1.81−1.18 (m, 10 H), 0.93−0.89 (m, 3H); ^{13}C NMR (CD_3OD, assignment of diastereomeric pairs based on relative intensity and DEPT) δ 75.9 and 75.0, 72.6 and 70.6, 67.8 and 67.5, 55.4 and 53.4, 38.0 and 37.4, 34.5, 33.1, 26.8 and 26.7, 23.7, 14.4; HRMS (FAB, NBA) m/z calcd for $C_{10}H_{24}NO_3$ (M+H) 206.1756, found 206.1764.

***N*^{2}-[1-(*R*)−(2,3-Dihydroxypropyl)-2-(*S*)-hydroxyheptyl]deoxyguanosine (27).** Following the procedure described below for **28**, the reaction of (*4R,5S*)-**8** (11 mg, 0.053 mmol) with **5a** (13.0 mg, 0.035 mmol) gave **27** (10 mg, 60%) after hydrolysis. ^{1}H NMR (DMSO-d_6) δ 10.21 (m, 1H, N1−H), 7.89 and 7.88 (diastereomers, s, s, 1H, H-8), 6.43−6.34 (m, 1H, N^2-H), 6.13−6.08 (m, 1H, H-1′), 5.25 (d, J = 3.7 Hz, 1H, 3′-OH), 4.86 (t, J = 3.4 Hz, 1H, 5′-OH), 4.87−4.58 (m, 2H,

5*-OH and 2*-OH), 4.58−4.40 (m,1H, 1*-OH), 4.32 (m, 1H, H-3′), 4.013 and 3.99 (diastereomers, m, m, 1H, H-4*), 3.79 (m, 1H, H-4′), 3.63−3.42 (m, 4H, H-5′, H-5″, H-2*, H-5*), 3.30−3.19 (m, 2H, H-1*, H-1**, hidden under DMSO peak but visible in CD_3OD), 2.58 (m, 1H, H-2′), 2.18 (m, 1H, H-2″), 1.78 (m, 1H, H-3*), 1.57−1.14 (m, 9H), 0.85 (t, J = 6.6 Hz, 3H); HRMS (FAB, NBA) *m*/*z* calcd for $C_{20}H_{34}N_5O_7$ (M+H) 456.2458, found 456.2440.

***N*²-[1-(*S*)-(2,3-Dihydroxypropyl)-2-(*R*)-hydroxyheptyl]deoxyguanosine (28).** A solution of (*4S*,*5R*)-**9** (10.0 mg, 0.049 mmol) in anhydrous DMSO (200 μL) was added to a mixture of **5a** (11.9 mg, 0.032 mmol), DMSO (100 μL), and diisopropylethylamine (100 μL). The mixture was stirred at 65−70 °C for 2 days, then cooled, and concentrated under high vacuum using a centrifugal evaporator. The residue was dissolved in 5% acetic acid (1 mL) and stirred at room temperature for 1 h. Purification by HPLC (gradient B) gave **28** (8.3 mg, 57%): ^{1}H NMR (DMSO-d_6) δ 10.41 (m, 1H, N1−H), 7.88 and 7.87 (diastereomers, s, s, 1H, H-8), 6.41−6.33 (m, 1H, N^2-H), 6.12−6.08 (m, 1H, H-1′), 5.25 (d, J = 4.0 Hz, 1H, 3′-OH), 4.87−4.80 (m, 2H, 5′-OH and 5*-OH), 4.73−4.63 (m, 1H, 2*-OH), 4.51 and 4.45 (diastereomers, t, t, J = 5.6 and 5.5 Hz, 1H, 1*-OH), 4.32 (m, 1H, H-3′), 4.02 and 3.91 (diastereomers, m, m, 1H, H-4*), 3.79 (m, 1H, H-4′), 3.57−3.44 (m, 4H, H-5′, H-5″, H-2*, H-5*), 3.26 (m, 2H, H-1*, H-1**, hidden under DMSO peak but visible in CD_3OD), 2.57 (m, 1H, H-2′), 2.17 (m, 1H, H-2″), 1.79 (m, 1H, H-3*), 1.57−1.23 (m, 9H), 0.85 (t, J = 6.6 Hz, 3H); HRMS (FAB, NBA) *m*/*z* calcd for $C_{20}H_{34}N_5O_7$ (M+H) 456.2458, found 456.2450.

***N*²-[1-(*R*)-(2,3-Dihydroxypropyl)-2-(*R*)-hydroxyheptyl]deoxyguanosine (29).** Following the procedure described below for **30**, the reaction of (*4R*,*5R*)-**28** (15.2 mg, 0.074 mmol) with **5a** (13.7 mg, 0.037 mmol) gave **29** (10.7 mg, 63.5%) after hydrolysis of the O^6-trimethylsilylethyl group. ^{1}H NMR (DMSO-d_6) δ 10.35 (m, 1H, N1−H), 7.89 and 7.86 (diastereomers, s, s, 1H, H-8), 6.34 (m, 1H, N^2-H), 6.08 (m, 1H, H-1′), 5.24 (m, 1H, 3′-OH), 4.99 and 4.90 (diastereomers, m, 1H, 5*-OH), 4.88 (m, 1H, 5′-OH), 4.60 and 4.49 (diastereomers, s, s, 1H, 2*-OH), 4.47 (m, 1H, 1*-OH), 4.33 (m, 1H, H-3′), 4.11−4.05 (m, 1H, H-4*), 3.79 (m, 1H, H-4′), 3.60−3.40 (m, 4H, H-5′, H-5″, H-2*, H-5*), 3.30−3.15 (m, 2H, H-1*, H-1**, hidden under water peak but visible in CD_3OD), 2.58 (m, 1H, H-2′), 2.18 (m, 1H, H-2″), 1.72 (m, 1H, H-3*), 1.50−1.10 (m, 9H), 0.81 (t, J = 6.8 Hz, 3H); HRMS (FAB, NBA) *m*/*z* calcd for $C_{20}H_{34}N_5O_7$ (M+H) 456.2458, found 456.2439.

***N*²-[1-(*S*)-(2,3-Dihydroxypropyl)-2-(*S*)-hydroxyheptyl]deoxyguanosine (30).** A solution of (*4S*,*5S*)-**11** (20.1 mg, 0.098 mmol) in anhydrous DMSO (200 μL) was added to a solution of **5a** (18.2 mg, 0.049 mmol), DMSO (100 μL), and diisopropylethylamine (100 μL). The mixture was stirred at 70−75 °C for 26 h, cooled, and concentrated under high vacuum using a centrifugal evaporator. The residue was dissolved in 5% acetic acid (1 mL) and stirred at room temperature for 1 h. Purification by HPLC (gradient A) gave **30** (13.5 mg, 60.5%) as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6) δ 10.37 (br s, 1H, N1−H), 7.88 and 7.86 (diastereomers, s, s, 1H, H-8), 6.39 (m, 1H, N^2-H), 6.10 (m, 1H, H-1′), 5.26 (m, 1H, 3′-OH), 4.99 and 4.90 (diastereomers, d, d, J = 4.2 and 4.2 Hz, 1H, 5*-OH), 4.88 (m, 1H, 5′-OH), 4.58 and 4.45−4.38 (diastereomers, d, d, J = 4.5 and 4.5 Hz, 1H, 2*-OH, the later peak overlapped with 1*-OH), 4.48 (m, 1H, 1*-OH), 4.33 (m, 1H, H-3′), 4.11−4.03 (m, 1H, H-4*), 3.80 (m, 1H, H-4′), 3.60−3.40 (m, 4H, H-5′, H-5″, H-2*, H-5*), 3.30−3.15 (m, 2H, H-1*, H-1**, hidden under water peak but visible in CD_3OD), 2.58 (m, 1H, H-2′), 2.16 (m, 1H, H-2″, 1.72(m, 1H, H-3*), 1.50−1.10 (m, 9H), 0.80 (t, J = 6.7 Hz, 3H); HRMS (FAB, NBA) *m*/*z* calcd for $C_{20}H_{34}N_5O_7$ (M+H) 456.2458, found 456.2471.

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-(*R*)-[1-(*S*)-hydroxyhexyl]pyrimido[1,2-*a*]purin-10(*3H*)-one (1). Following the same procedure described below for **4**, **27** was converted to **1** in 80% yield. ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, H-2), 6.42 (m, 1H, H-8), 6.23 (dd, J = 7.5, 6.5 Hz, 1H, H-1′), 4.51 (m, 1H, H-3′), 3.98 (m, 1H, H-4′), 3.78 (dd, J = 12.0, 3.5 Hz, 1H, H-5′), 3.82−3.75 (m, 1H, H-11, overlapped with H-5′), 3.72 (dd, J = 12.0, 3.9 Hz, 1H, H-5″), 3.75−3.70 (m, 1H, H-6, overlapped with H-5″), 2.67 (m, 1H, H-2′), 2.33 (m, 1H, H-2″), 2.10 (m, 1H, H-7a), 1.74 (m, 1H, H-7b), 1.57−1.27 (m, 8H), 0.923 (t, J = 6.5 Hz, 3H); HRMS (FAB, NBA) *m*/*z* calcd for $C_{19}H_{30}N_5O_6$ (M+H) 424.2196, found 424.2190.

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-(*S*)-[1-(*R*)-hydroxyhexyl]pyrimido[1,2-*a*]purin-10(*3H*)-one (2). Following the procedure described below for **4**, **28** was converted to **2** in 84% yield. ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, H-2), 6.42 (t, J = 2.5, 1H, H-8), 6.24 (dd, J = 7.6, 6.2 Hz, 1H, H-1′), 4.51 (m, 1H, H-3′), 3.98 (m, 1H, H-4′), 3.78 (dd, J = 12.0, 3.4 Hz, 1H, H-5′), 3.81−3.75 (m, 1H, H-11, overlapped with H-5′), 3.73 (dd, 12.0, 3.9 Hz, 1H, H-5″), 3.75−3.70 (m, 1H, H-6, overlapped with H-5″), 2.69 (m, 1H, H-2′), 2.33 (m, 1H, H-2″), 2.11 (m, 1H, H-7a), 1.74 (m, 1H, H-7b), 1.57−1.28 (m, 8H), 0.93 (t, J = 6.7 Hz, 3H); HRMS (FAB, NBA) *m*/*z* calcd for $C_{19}H_{30}N_5O_6$ (M+H) 424.2196, found 424.2177.

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-(*R*)-[1-(*R*)-hydroxyhexyl]pyrimido[1,2-*a*]purin-10(*3H*)-one (3). Following the procedure described below for **4**, **29** was converted to **3** in 83% yield. ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, H-2), 6.40 (t, J = 2.5, 1H, H-8), 6.23 (t, J = 6.9 Hz, 1H, H-1′), 4.51 (m, 1H, H-3′), 3.98 (dt, J = 3.3, 3.0 Hz, 1H, H-4′), 3.78 (dd, J = 12.1, 3.4 Hz,1H, H-5′), 3.70 (dd, J = 12.1, 3.8 Hz, 1H, H-5″), 3.61 (m, 1H, H-6), 3.47 (m, 1H, H-11), 2.68 (m, 1H, H-2′), 2.33 (m, 1H, H-2″), 2.18 (m, 1H, H-7a), 1.64−1.28 (m, 9H), 0.93 (t, J = 6.6 Hz, 3H); HRMS (FAB, NBA) *m*/*z* calcd for $C_{19}H_{30}N_5O_6$ (M+H) 424.2196, found 424.2192.

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-(*S*)-[1-(*S*)-hydroxyhexyl]pyrimido[1,2-*a*]purine-10(*3H*)-one (4). A solution of sodium periodate (505 μL, 0.0101 mmol, 20 mM in water) was added to a solution of **30** (4.6 mg, 0.010 mmol) in 50 mM, pH 7 phosphate buffer (500 μL). The reaction mixture was stirred at room temperature for 10 min and immediately purified by HPLC (gradient A) to give **4** (3.7 mg, 87%). ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, H-2), 6.40 (t, J = 2.5 Hz, 1H, H-8), 6.23 (t, J = 6.9 Hz, 1H, H-1′), 4.51 (m, 1H, H-3′), 3.99 (m, H-4′), 3.78 (dd, J = 12.1, 3.4 Hz, H-5′), 3.71 (dd, J = 12.1, 3.7 Hz, 1H, H-5″), 3.61 (m, 1H, H-6), 3.47 (m, 1H, H-11), 2.68 (m, 1H, H-2′), 2.33 (m, 1H, H-2″), 2.19 (m, 1H, H-7a), 1.64−1.28 (m, 9H), 0.93 (t, J = 6.6 Hz, 3H); HRMS (FAB, NBA) *m*/*z* calcd for $C_{19}H_{30}N_5O_6$ (M+1) 424.2196, found 424.2181.

3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-6-(*S*)-[1-(*S*)-hydroxyhexyl)]-5,6,7,8-tetrahydro-8-(*R*)-(*N*²-deoxyguanosinyl)-pyrimido[1,2-*a*]purin-10(*3H*)-one (45c). In a glass tube was added **4** (5 mg) and 2′-deoxyguanosine monohydrate (10 mg) in DMSO (250 μL). The mixture was stirred in an oil bath at 100 °C for 11 days. The solvent was removed under vacuum. Purification by HPLC (gradient B) gave recovered **4** (3 mg) and **45c** (0.7 mg, 9%): ^{1}H NMR (DMSO-d_6) δ 10.20 (s, 1H), 7.97 (s, 1H), 7.94 (s, 1H), 7.43 (s, 1H), 7.31 (d, J = 6.8 Hz, 1 H), 6.66 (m, 1H), 6.20 (m, 1H), 6.13 (m, 1H), 5.31 (d, J = 4.0 Hz, 1H), 5.25 (d, J = 3.7 Hz), 5.03 (d, J = 5.5 Hz, 1H), 4.96 (t, J = 5.4 Hz), 4.88 (t, J = 5.6 Hz, 1H), 4.36 (m, 1H), 3.90 (m, 1H), 3.52 (m, 6H), 2.62 (m, 1H), 2.44 (m, 1H, hidden under solvent peak, visible in COSY spectrum), 2.20 (m, 3H), 1.65 (m, 1H), 1.38−1.10 (m, 9H), 0.84 (t, J = 6.7 Hz, 1H); MS: LC-ESMS *m*/*z* calcd for $C_{29H_{41}}N_{10}O_9$ (M+1) 673.31, found 673.38.

Synthesis of 5′-d(GCTAGC(27)AGTCC) (34). The oligonucleotide 5′-d(GCTAGC(X)AGTCC) (**33**, X = **5a**; 10 A_{260} units) was mixed in a plastic tube with diisopropylethylamine (50 μL), DMSO (100 μL), and **1** (0.5 mg). The mixture was stirred at 65 °C for 72 h, adding additional portions of **1** (0.5 mg) after 24 and 48 h. HPLC showed the disappearance of **33**. The solvent was evaporated under vacuum and the residue dissolved in 5% acetic acid (500 μL), then stirred for 2 h at room temperature. The reaction mixture was neutralized to pH 7 (pH paper) with 1 M NaOH and purified by HPLC (gradient A) to give oligonucleotide **27** (5.4 A_{260} units, 54%). The modified oligonucleotide (**27**) was characterized by enzyme digestion followed by

HPLC analysis and MALDI-TOF MS. MS: MALDI (HPA) *m*/*z* calcd for (M−H) 3832.9, found 3832.2.

Synthesis of 5′-d(GCTAGC(28)AGTCC) (35). Following the procedure described for **34**, **35** was prepared in 57% yield. MS: MALDI (HPA) *m*/*z* calcd for (M−H) 3832.9, found 3833.0.

Synthesis of 5′-d(GCTAGC(29)AGTCC) (36). Following the procedure described for **34**, **36** was prepared in 53% yield. MS: MALDI (HPA) *m*/*z* calcd for (M−H) 3832.9, found 3833.3.

Synthesis of 5′-d(GCTAGC(30)AGTCC) (37). Following the procedure described for **34**, **37** was prepared in 51% yield. MS: MALDI (HPA) *m*/*z* calcd for (M−H) 3832.9, found 3833.7.

Synthesis of 5′-d(GCTAGC(1)AGTCC) (38). Sodium periodate (100 μL of a 20 mM solution in pH 7 phosphate buffer) was added to a solution of **34** (3.2 A_{260} units) in phosphate buffer (300 μL, pH 7.0), and the reaction stirred at room temperature for 10 min. HPLC purification afforded 5′-d(GCTAGC(**1**)AGTCC) (**38**, 2.5 A_{260} units, 78%). The structure was confirmed by enzyme digestion followed by HPLC analysis using nucleoside **1** as an authentic sample and MALDI-TOF MS. MS: MALDI (HPA) *m*/*z* calcd for (M−H) 3800.8, found 3799.9.

Synthesis of 5′-d(GCTAGC(2)AGTCC) (39). Following the procedure described for **38**, **39** was prepared in 82% yield. MS: MALDI (HPA) *m*/*z* calcd for (M−H) 3800.8, found 3799.3.

Synthesis of 5′-d(GCTAGC(3)AGTCC) (40). Following the procedure described for **38**, **40** was prepared in 83% yield. MS (MALDI) *m*/*z* calcd for (M−H) 3800.8, found 3801.8.

Synthesis of 5′-d(GCTAGC(4)AGTCC) (41). Following the procedure described for **38**, **41** was prepared in 80% yield. MS: MALDI (HPA) *m*/*z* calcd for (M−H) 3800.8, found 3800.8.

Enzymatic Digestion. The oligonucleotide (0.2 A_{260} units) was dissolved in 30 μL of buffer (pH 7.0, 10 mM Tris-HCl, 10 mM $MgCl_2$) and incubated with DNase I, snake venom phosphodiesterase I, and alkaline phosphatase at 37 °C for 90 min. The mixture was then analyzed by reverse phase HPLC using a YMC ODS-AQ column (250 × 4.6 mm i.d., 1.5 mL/min) with UV detection monitoring at 260 nm. The following solvent gradient was used: 1−10% acetonitrile over 15 min, 10−20% acetonitrile over 5 min, hold for 5 min, 20−100% acetonitrile over 10 min, hold for 5 min and then 100−1% acetonitrile over 5 min. The modified nucleosides **1**−**4**, **27**−**30**, and **45c** were identified by comparison with authentic samples based on retention times, co-injection, and UV spectra.

Cross-linking Reactions. Equal molar mixtures of the adducted (1.0 A_{260} units) and complementary (1.0 A_{260} units) oligonucleotides in 60 μL of potassium phosphate buffer (0.05 M, pH 7.0) containing 1.0 M KCl were incubated at 37 °C and 46 °C in plastic Eppendorf tubes. The reactions were analyzed periodically by CZE with UV detetction. The cross-linked species eluted later than the starting oligonucleotides. Relative amounts of remaining starting and cross-linked oligonucleotides were estimated from peak areas. Conversion of peak areas to relative molar amounts was made using molar absorptivities calculated on the basis of sequence using the method of Borer.[41] The molar absorptivity of nucleosides **1**−**4** were assumed to be identical to that of dG and that of bis-nucleoside **45c** to be twice that of dG. The molar absorptivity contributions of the individual nucleosides were assumed to be identical for single-stranded and cross-linked oligonucleotides, i.e., no allowance was made for the hypochromicity of cross-linked duplexes because of uncertainty as to the extent of denaturation under the CZE conditions. The consequence of this assumption is that the extent of cross-linking may be slightly greater than the calculated values. Enzymatic digestion of the cross-linking reaction and analysis by HPLC showed the presence of cross-linked nucleoside **45c**, which was unambiguously identified by comparison with an authentic sample.

Melting Studies. Equal amounts of the modified oligonucleotides and the complementary strands (0.25 A_{260} units each) were dissolved in 0.5 mL of melting buffer (10 mM Na_2HPO_4/NaH_2PO_4, 1.0 M NaCl, 50 μM Na_2EDTA, pH 7.0). UV measurements monitoring the absorbance at 260 nm, were taken at 1 min intervals with a 1 °C/min temperature gradient. The temperature was cycled between 5 and 95 °C. The first derivative of the melting curve was used to establish T_m values.

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Supporting Information Available: Copies of MALDI-TOF spectra of adducted oligonucleotides **34**−**41**. This material is available free of charge via the Internet at http://pubs.acs.org JA0288800

(41) Borer, P. N. *Handbook of Biochemistry and Molecular Biology*, 1st ed.; CRC Press: Cleveland, 1975. Notes: p 359.