

Interstrand DNA Cross-Links Induced by α,β -Unsaturated Aldehydes Derived from Lipid Peroxidation and Environmental Sources

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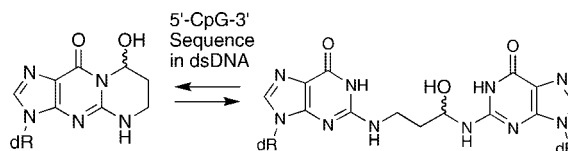
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CON SPECTUS

Significant levels of the 1,*N*²- γ -hydroxypropano-dG adducts of the α,β -unsaturated aldehydes acrolein, crotonaldehyde, and 4-hydroxy-2E-nonenal (HNE) have been identified in human DNA, arising from both exogenous and endogenous exposures. They yield interstrand DNA cross-links between guanines in the neighboring C·G and G·C base pairs located in 5'-CpG-3' sequences, as a result

of opening of the 1,*N*²- γ -hydroxypropano-dG adducts to form reactive aldehydes that are positioned within the minor groove of duplex DNA. Using a combination of chemical, spectroscopic, and computational methods, we have elucidated the chemistry of cross-link formation in duplex DNA. NMR spectroscopy revealed that, at equilibrium, the acrolein and crotonaldehyde cross-links consist primarily of interstrand carbinolamine linkages between the exocyclic amines of the two guanines located in the neighboring C·G and G·C base pairs located in 5'-CpG-3' sequences, that maintain the Watson–Crick hydrogen bonding of the cross-linked base pairs. The ability of crotonaldehyde and HNE to form interstrand cross-links depends upon their common relative stereochemistry at the C6 position of the 1,*N*²- γ -hydroxypropano-dG adduct. The stereochemistry at this center modulates the orientation of the reactive aldehyde within the minor groove of the double-stranded DNA, either facilitating or hindering the cross-linking reactions; it also affects the stabilities of the resulting diastereoisomeric cross-links. The presence of these cross-links *in vivo* is anticipated to interfere with DNA replication and transcription, thereby contributing to the etiology of human disease. Reduced derivatives of these cross-links are useful tools for studying their biological processing.

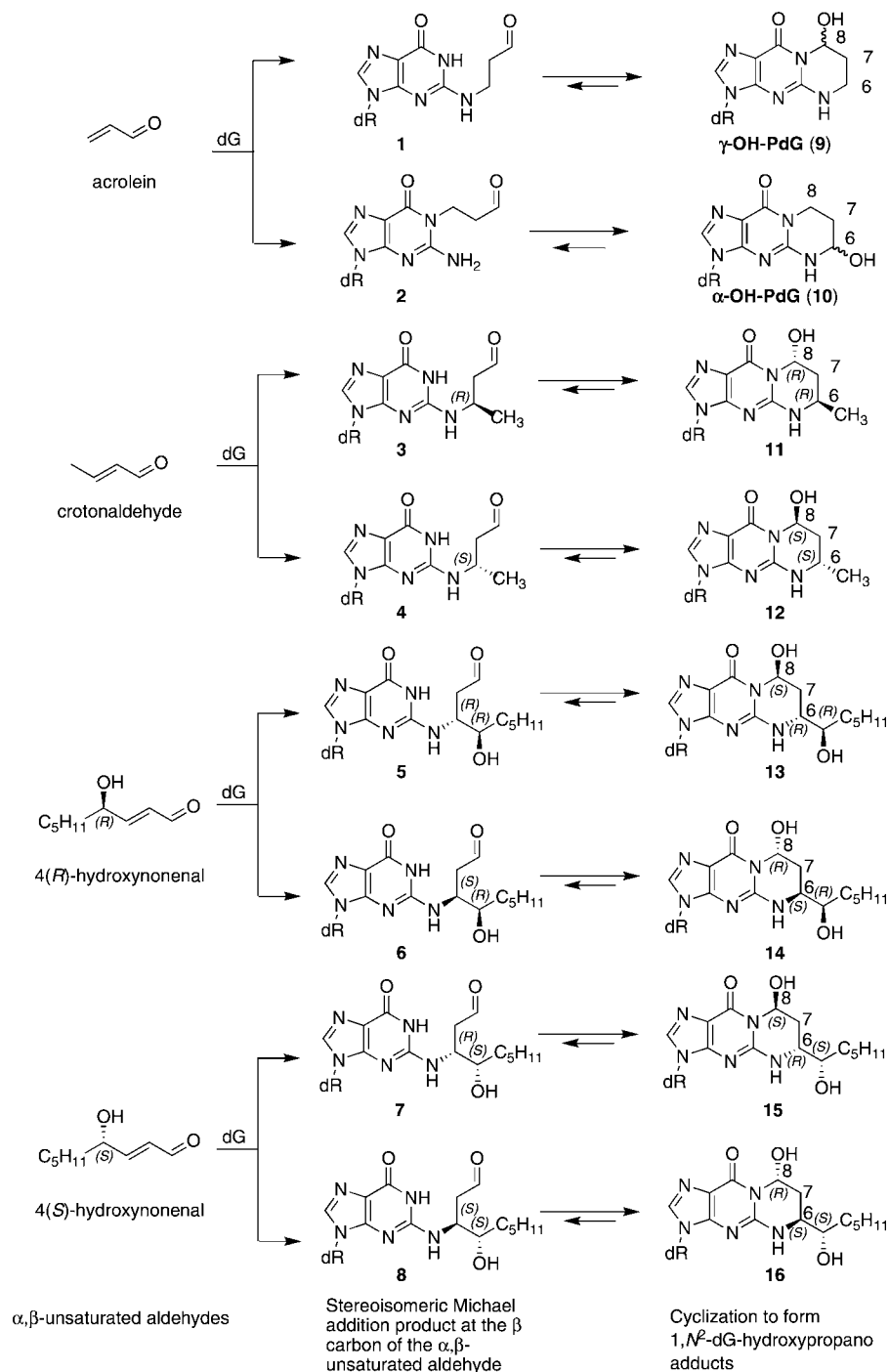


Interstrand DNA Cross-link Formation by γ -HO-PdG-type Adducts

Introduction

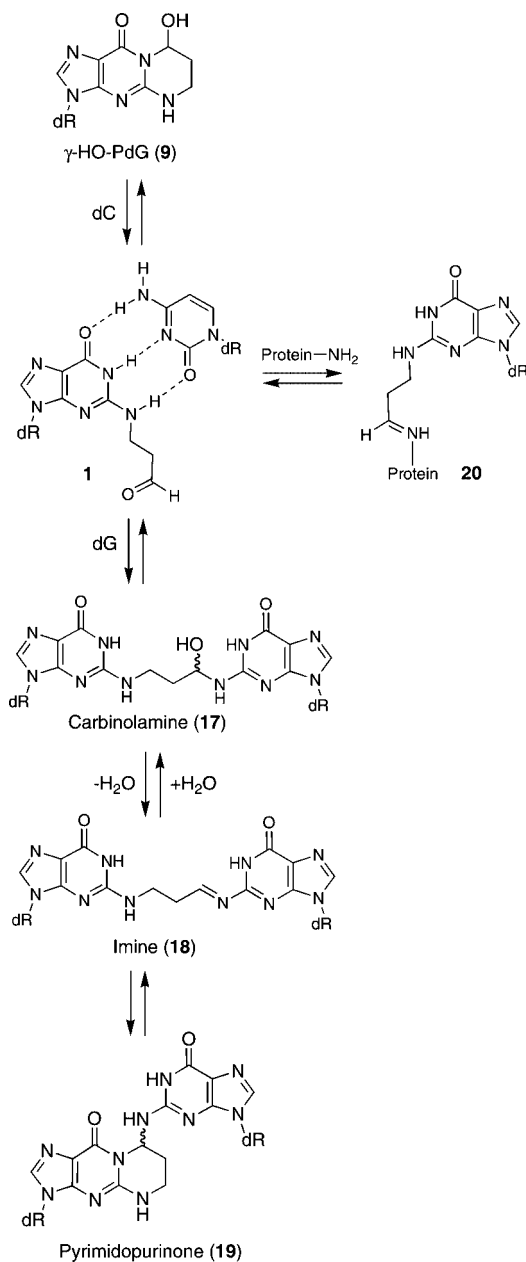
The α,β -unsaturated aldehydes (enals) acrolein, crotonaldehyde, and 4-hydroxynonenal (4-HNE) (Scheme 1) are endogenous byproducts of lipid peroxidation, arising as a consequence of oxidative stress.^{1–4} Acrolein and crotonaldehyde exposures also occur from exogenous sources, for example, cigarette smoke⁵ and automobile exhaust.⁶ Enals react with DNA nucleobases to

give exocyclic adducts; they also react with proteins.⁷ Addition of enals to dG involves Michael addition of the *N*²-amine to give *N*²-(3-oxopropyl)-dG adducts (**1**, **3–8**), followed by cyclization of N1 with the aldehyde, yielding the corresponding 1,*N*²- γ -hydroxypropano-dG products (**9**, **11–16**). Early work is traced to Shapiro and Leonard, who independently examined the reactions of nucleosides with glyoxal, malondialdehyde, chloroacetaldehyde, and related

SCHEME 1. 1, N^2 -dG Cyclic Adducts Arising from Michael Addition of Enals to dG

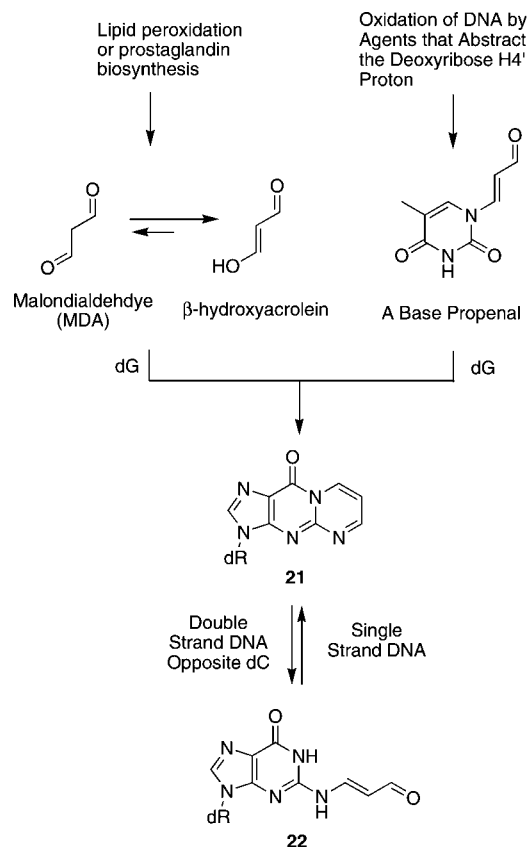
bis-electrophiles.^{8,9} Galliana and Pantarotto characterized the 8-hydroxypyrimido[1,2- α]purin-10(3*H*)-one (γ -OH-PdG, **9**) adduct from the reaction of acrolein with dG.¹⁰ Chung and Hecht concurrently reported the crotonaldehyde adduct of dG (**11**, **12**)¹¹ and explored the reactivity of enals and enones with dG.^{12,13} The lipid peroxidation product 4-HNE afforded related dG-adducts (**13**–**16**).¹⁴ Identification of acrolein adducts of other nucleosides followed.^{15,16}

The principal acrolein adduct is γ -OH-PdG (**9**),^{10,12} although the regioisomeric 6-hydroxypyrimido[1,2- α]purin-10(3*H*)-one (α -OH-PdG, **10**) has also been observed.^{12,17} The γ -OH-PdG adduct (**9**) exists as a mixture of C8-OH epimers. With crotonaldehyde, addition at N^2 -dG creates a stereocenter at C6. Of four possible products, the two with the *trans* relative stereochemistry at C6 and C8 (**11**, **12**) are observed.^{12,18} These are also formed through the reaction of dG with 2 equiv of

SCHEME 2. Enal-dG Adducts Mediate DNA Interstrand Cross-Link Formation

acetaldehyde.^{5,19,20} The corresponding 4-HNE-derived 1, N^2 -dG adducts possess an additional stereocenter on the C6 side chain, resulting in four observable diastereomers (**13–16**).

The 1, N^2 -dG exocyclic adducts from acrolein (**9, 10**), crotonaldehyde (**11, 12**), and 4-HNE (**13–16**) exist in human and rodent DNA.^{2–4,17,21} The binding pattern of acrolein–DNA adducts is similar to the p53 mutational pattern in human lung cancer, implicating acrolein as a major cigarette-related lung cancer inducing agent.²² Acrolein is mutagenic in bacterial and mammalian cells,^{23,24} including human cells,^{25,26} and

SCHEME 3. Formation and Chemistry of the Malondialdehyde-Derived M₁dG Adduct

is carcinogenic in rats.²⁷ Crotonaldehyde is genotoxic and mutagenic in human lymphoblasts²⁸ and induces liver tumors in rodents.²⁹ 4-HNE induces a DNA damage response in *Salmonella typhimurium*^{30,31} but is inactive in bacterial mutagenesis assays.²³ However, it causes mutations in V79 CHO cells, and DNA from liver specimens from individuals suffering from Wilson's disease and hemochromatosis contain mutations attributed to 4-HNE–dG adducts.³² Site-specific mutagenesis reveals that these 1, N^2 - γ -hydroxypropano-dG adducts induce predominantly G→T transversions in COS-7 cells.^{33–35}

The hypothesis explored in this Account posits that in duplex DNA, 1, N^2 -dG enal adducts (**9, 11–16**) open, unmasking a reactive aldehyde (**1, 3–8**) in the minor groove, as shown for γ -OH-PdG (**9**) in Scheme 2. This hypothesis was, in part, developed from the observation that the malondialdehyde-derived adduct **21** opens to a related aldehyde **22** when placed opposite dC in DNA (Scheme 3).^{36–38} Enal adducts are lower oxidation state homologues of **21** and the notion that acrolein, crotonaldehyde, and 4-HNE undergo similar chemistry was confirmed by the observation that γ -OH-PdG (**9**) ring-opens to the N^2 -(3-oxopropyl)-dG aldehyde (**1**) when placed opposite dC.³⁹

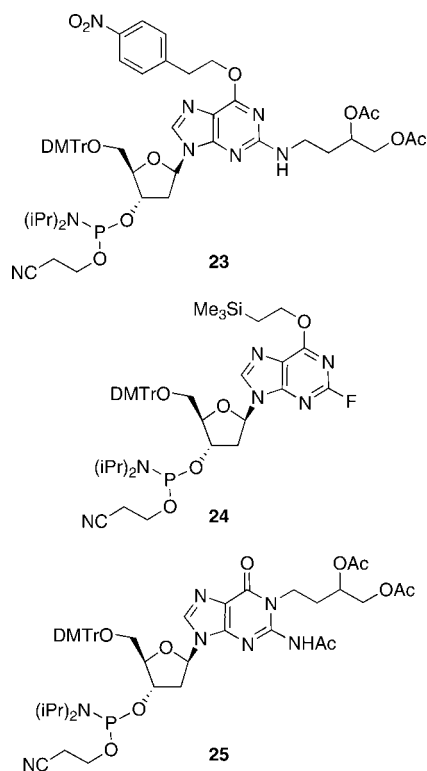


FIGURE 1. Phosphoramidite reagents for the site-specific synthesis of oligodeoxynucleotides containing $1,N^2$ -dG enal adducts.

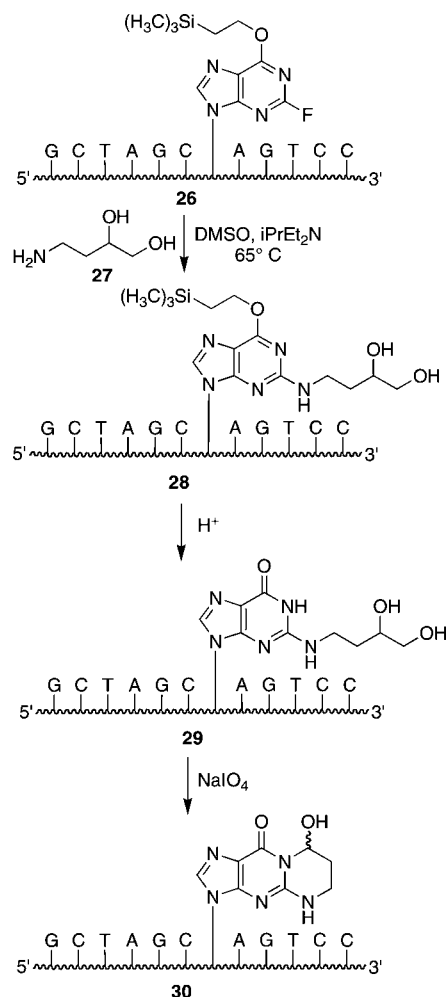
We further hypothesized that these aldehydes react with other nucleobases in the cDNA strand, forming interstrand cross-links, which exist as equilibrium mixtures of carbinolamine (**17**), imine (**18**), or pyrimidopyrimidinone (**19**) species. The aldehyde in structure **1** also yields peptide– and protein–DNA conjugates (**20**);^{40,41} however, analysis of this literature is beyond the scope of this Account. Interstrand DNA cross-linking was proposed based upon analysis of acrolein-treated DNA.²⁶ Few site-specific interstrand cross-links are chemically characterized.^{42,43} Hecht characterized a pyrimidopyrimidinone bis-nucleoside cross-link analogous to **19** from acetaldehyde-treated calf thymus DNA;¹⁹ the cross-link was formally derived from crotonaldehyde, the aldol condensation product of acetaldehyde.

In this Account, we discuss the chemistry of interstrand cross-links that are likely to be generated in DNA as secondary dG adducts of acrolein, crotonaldehyde, and 4-HNE. These and their reduced derivatives provide tools to study the processing of interstrand cross-links and to define their roles in the etiology of human disease.

Synthesis of Oligodeoxynucleotides Containing $1,N^2$ -dG Enal Adducts

Aldehyde groups have been introduced into DNA through periodate cleavage of vicinal diols.⁴⁴ Khullar et al. synthe-

SCHEME 4. Synthesis of $1,N^2$ - γ -OH-PdG in Oligodeoxynucleotides by the Postsynthetic Modification Strategy



sized γ -OH-PdG (**9**) by condensation of 4-amino-1,2-butane-diol with 3',5'-*O*-bis-*tert*-butyldimethylsilyloxy-*O*⁶-*p*-nitrophenylethyl-protected 2-fluoroinosine deoxynucleoside.⁴⁵ The N^2 -(3,4-dihydroxybutyl) moiety was oxidized to yield N^2 -(3-oxopropyl)-dG (**1**), which cyclized to γ -OH-PdG (**9**).⁴⁵ Preparation of phosphoramidite **23** (Figure 1) allowed for incorporation of γ -OH-PdG (**9**) into oligodeoxynucleotides; oxidative cleavage of the diol to **9** was achieved after oligodeoxynucleotide assembly and deprotection.⁴⁵ Our approach introduced the N^2 -(3,4-dihydroxybutyl) group *after* oligodeoxynucleotide synthesis (Scheme 4).⁴⁶ We prepared oligodeoxynucleotide **26** containing *O*⁶-[(2-trimethylsilyl)ethyl]-2-fluorohypoxanthine from phosphoramidite **24**; nucleophilic aromatic substitution with amino diol **27** provided **28**. Removal of the *O*⁶ protecting group under acidic conditions yielded **29**, which was oxidized to oligodeoxynucleotide **30**.⁴⁷ Our postsynthetic modification strategy⁴⁸ allowed preparation of various dG enal adducts from a single modified phosphoramidite. A challenge was the prepara-

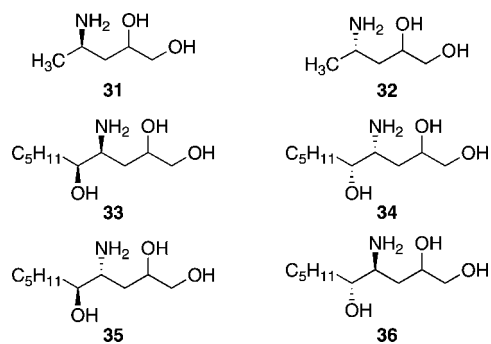


FIGURE 2. Amino alcohols for synthesis of crotonaldehyde- and 4-HNE-modified oligodeoxynucleotides.

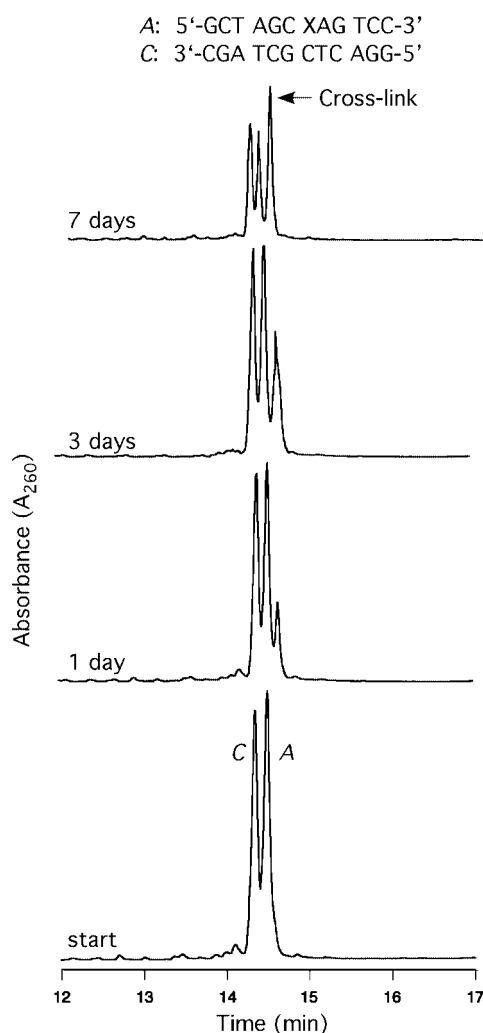


FIGURE 3. Cross-linking of γ -OH-PdG adducts in the 5'-CpG-3' sequence, monitored by CGE. The adducted and complementary strands are identified by the letters A and C, respectively; the arrows indicate interstrand cross-links.

tion of stereochemically defined amino diols of the crotonaldehyde (**31**, **32**) and 4-HNE adducts (**33**–**36**) (Figure 2).^{49–52} This strategy could not be applied to oligodeoxynucleotides containing α -OH-PdG (**10**), which were prepared using the modified phosphoramidite **25**.^{53,54}

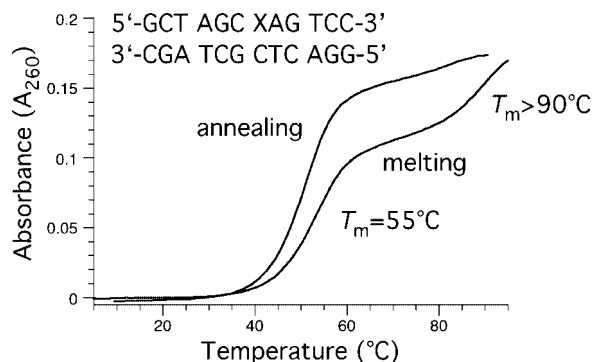


FIGURE 4. Thermal melting analysis of an acrolein-modified oligonucleotide in a CpG sequence after incubation for 5 days. The higher melting transition is assigned to the interstrand cross-link.

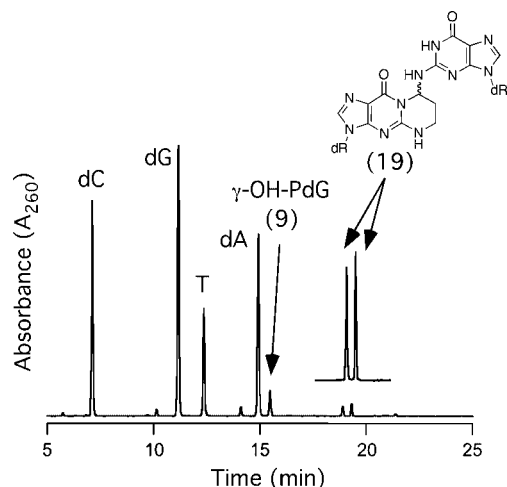


FIGURE 5. Digestion of the cross-linked γ -OH-PdG-adducted duplex. The pyrimidopurinone bis-nucleosides were identified by comparison with authentic standards.

Interstrand Cross-Linking by γ -OH-PdG

Oligodeoxynucleotide **30**, containing γ -OH-PdG adduct **9**, was annealed to its complement, and the formation of an interstrand cross-link was monitored by capillary gel electrophoresis (CGE; Figure 3).^{55,56} A new species formed and reached a level of $\sim 50\%$ yield after 7 days at 25 °C. Mass spectrometric analysis suggested that the chemical nature of the cross-link was a carbinolamine linkage (**17**), in equilibrium with either or both the imine (**18**) or pyrimidopurinone (**19**) forms.⁵⁶ This cross-link exhibited a reversible melting transition (T_m) at >90 °C,^{51,55} which was assigned as the interstrand cross-link (Figure 4); the T_m of the un-cross-linked duplex containing **9** was 55 °C, 10 °C lower than the unmodified duplex. In duplex DNA, $\sim 20\%$ of the cross-linked form reverted to the un-cross-linked form over 16 h at pH 7, whereas reversion occurred within 1 h under conditions that disrupted the duplex.⁵⁶ Enzymatic digestion yielded diastereomeric pyrimidopurinone bis-nucleoside cross-links **19** (Figure 5), which are structurally related

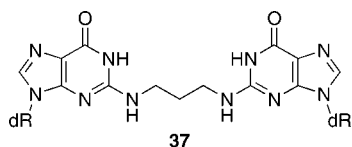


FIGURE 6. N^2 -dG: N^2 -dG trimethylene cross-link derived from the reduction of **18**.

to those arising from acetaldehyde-treated DNA.¹⁹ Reduction of **19** afforded N^2 -dG: N^2 -dG bis-nucleosides tethered by a trimethylene chain (**37**, Figure 6).⁵⁶ If the cross-linked duplex was reduced with $\text{NaB}(\text{CN})\text{H}_3$ prior to its digestion, N^2 -(3-hydroxypropyl)-dG from the reduction of γ -OH-PdG (**9**) and cross-link **37** were observed.

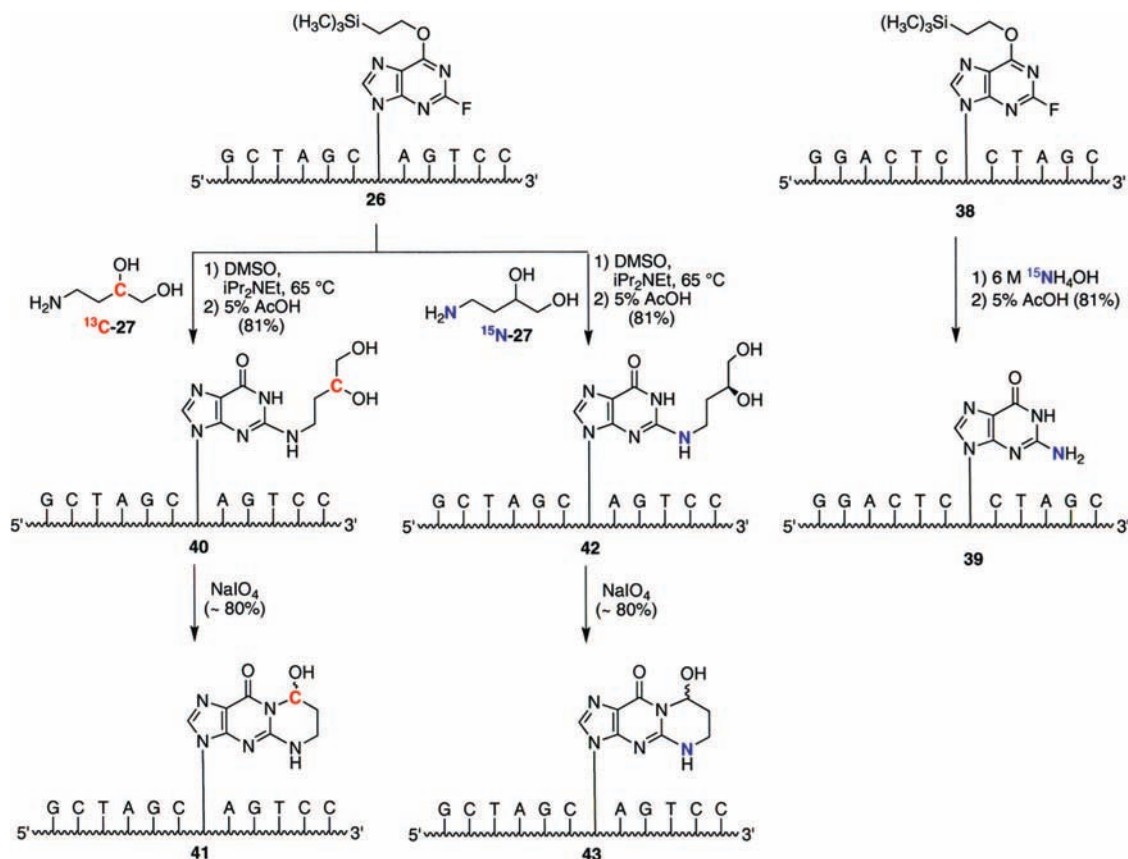
Although the cross-link could be reductively trapped, ^{13}C NMR experiments utilizing $X = \gamma$ - ^{13}C - γ -OH-PdG-adducted oligodeoxynucleotide **41** (Scheme 5) failed to detect the imine linkage in duplex DNA (Figure 7).⁵⁷ The identification of the cross-link in duplex DNA as the carbinolamine (**17**) and not the pyrimidopurinone (**19**)⁵⁶ was accomplished by isotope-edited NMR experiments in which oligodeoxynucleotide **43** containing γ -OH- $^{15}\text{N}^2$ -PdG was annealed with its complementary strand. An ^{15}N -HSQC filtered spectrum revealed the NOE between X^7 $^{15}\text{N}^2\text{H}$ and the imino proton X^7 N1H (Figure 8),

precluding the pyrimidopurinone structure (**19**). A triple resonance $^1\text{H}^{13}\text{C}^{15}\text{N}$ experiment conducted subsequent to annealing γ - ^{13}C -modified oligodeoxynucleotide **41** with $^{15}\text{N}^2$ -dG-modified oligodeoxynucleotide **39** revealed correlation between the γ - ^{13}C carbinol and the ^{15}N amine.⁵⁸

The clue as to why the cross-link preferred the carbinolamine (**17**)^{57,58} and not the pyrimidopurinone cross-link (**19**)⁵⁶ was provided by an experiment in which an N^2 -dG: N^2 -dG trimethylene linkage **37**, a surrogate for the carbinolamine cross-link (**17**), was constructed in 5'-d(AGGCXCT)₂; X represents the linked guanines.⁵⁹ The saturated linkage caused minimal distortion.⁵⁹ Additionally, modeling suggested that the carbinolamine linkage maintained Watson–Crick hydrogen bonding at both of the cross-linked C·G pairs (Figure 9).⁵⁸ Dehydration of carbinolamine **17** to an imine (**18**) or cyclization of the latter to pyrimidopurinone linkage (**19**) was predicted to disrupt Watson–Crick bonding at one or both of the cross-linked base pairs.

Interstrand cross-linking by γ -OH-PdG was specific to the 5'-CpG-3' sequence (Figure 9). When γ -OH-PdG (**9**) was engineered into 5'-d(CGTACXCATGC)-3', containing both the 5'-CpG-3' or 5'-GpC-3' sequences,⁵⁵ and the complement

SCHEME 5. Preparation of Oligodeoxynucleotides Containing ^{13}C (Red) and ^{15}N (Blue) Isotopes in the γ -OH-PdG Adduct (**41**, **43**), and an ^{15}N Isotope (Blue) in the Complementary Strand (**39**)



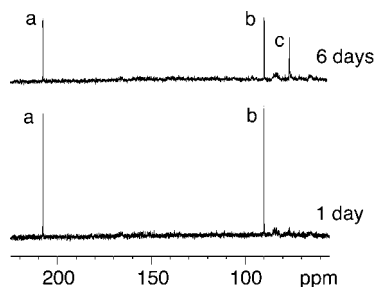


FIGURE 7. Data from an isotopically enriched sample containing ^{13}C - γ -OH-PdG. The imine linkage remained below the level of NMR detection. The top spectrum shows a ^{13}C resonance assigned as the diastereomeric carbinolamine forms of the cross-link. Assignments of resonances: (a) aldehyde **1**; (b) hydrated-aldehyde; (c) diastereomeric carbinolamines **17**. An imine resonance would be expected at ~ 130 ppm. Reproduced from ref 58. Copyright 2005 American Chemical Society.

5'-(GCATGCGTACG)-3' was labeled with $^{15}\text{N}^2$ -dG (the underlined residue corresponds to the potential 5'-CpG-3' cross-link), only ^{15}N -labeled bis-nucleoside cross-link **19** was observed after enzymatic digestion and analysis by LC-ESI-MS, establishing the 5'-CpG-3' sequence dependence for cross-linking. Other 5'-CpG-3' interstrand cross-links are known, for example, arising from mitomycin $\text{C}^{60,61}$ and nitrous acid. 62 When the N^2 -dG: N^2 -dG trimethylene linkage (**37**), a surrogate for the nonobserved cross-link in the 5'-GpC-3' sequence, was constructed in $d(\text{TCCXCGGA})_2$, its structure was distorted, and its T_m was reduced. 59,63

Interstrand Cross-Linking of (6R) and (6S) Crotonaldehyde 1, N^2 - γ -Hydroxypropano-dG Adducts

Ring opening of the diastereomeric 1, N^2 -dG adducts **11** and **12** was incomplete at pH 7 when placed opposite dG in duplex DNA. The incomplete ring opening was attributed to the positioning of the CH_3 groups to avoid steric clash with N3 of guanine, which becomes significant in the N^2 -(1-methyl-3-oxopropyl)-dG aldehydes (**3**, **4**). The abilities of these adducts to form interstrand cross-links in the 5'-CpG-3' sequence as oligodeoxynucleotide **30** depended upon stereochemistry at the C6 carbon. After >20 days, $\sim 40\%$ cross-link formation occurred for the 6R diastereomer **11** (Figure 10), whereas $<5\%$ cross-link was observed for the 6S diastereomer **12**. 55 Digestion of the cross-link yielded the bis-nucleoside pyrimidopurine, 55 identical to that isolated from acetaldehyde-treated DNA. 19 The presence of the imine linkage was inferred since the cross-link was reductively trapped. 55 Lao and Hecht concluded that the cross-link was predominantly an imine or pyrimidopurine with some of the carbinolamine linkage present. 20

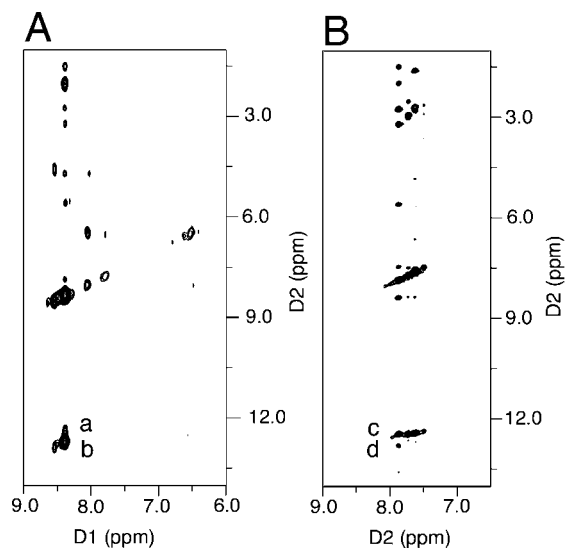


FIGURE 8. Isotope-edited NMR identified the carbinolamine linkage for the γ -OH-PdG cross-link: (A) ^{15}N -HSQC NOESY spectrum for oligodeoxynucleotide **39** annealed with oligodeoxynucleotide **31**. Nucleotides are numbered 5'-d(G¹C²T³A⁴G⁵C⁶X⁷A⁸G⁹T¹⁰C¹¹C¹²)-3'. 5'-d(G¹³G¹⁴A¹⁵C¹⁶T¹⁷C¹⁸Y¹⁹C²⁰T²¹A²²G²³C²⁴)-3', X⁷ = γ -OH PdG; Y¹⁹ = $^{15}\text{N}^2$ -dG. Crosspeaks (a) Y¹⁹ $^{15}\text{N}^2\text{H}$ -X⁷ N1H (weak); (b) Y¹⁹ $^{15}\text{N}^2\text{H}$ -Y¹⁹ N1H (strong). (B) ^{15}N -HSQC NOESY spectrum for γ -OH- $^{15}\text{N}^2$ -PdG-labeled oligodeoxynucleotide **43** annealed with its complement. Crosspeaks (c) X⁷ $^{15}\text{N}^2\text{H}$ -X⁷ N1H (strong); (d) X⁷ $^{15}\text{N}^2\text{H}$ -G¹⁹ N1H (weak). Reproduced from ref 58. Copyright 2005 American Chemical Society.

Although the cross-link could be reduced, NMR failed to detect the imine linkage in duplex DNA (Figure 8). 57 Using isotopically labeled adducts, 50 it was established that the carbinolamine form of the 6R cross-link was the only detectable cross-link species present in duplex DNA. As for the γ -OH-PdG adduct, modeling revealed that the carbinolamine linkage maintained Watson-Crick pairing at the cross-linked base pairs. Dehydration of the carbinolamine to the imine or cyclization of the latter to the pyrimidopurine cross-link would disrupt Watson-Crick pairing at one or both of the cross-linked C-G base pairs, providing a rationalization for why the carbinolamine is preferred.

Structural studies utilizing saturated analogs of the 6R- and 6S cross-links indicated that both retained Watson-Crick hydrogen bonds at the cross-linked base pairs (Figure 11). 64 However, the 6S diastereomer showed lower stability. Whereas for the 6R diastereomer, the CH_3 group was positioned in the center of the minor groove, for the 6S diastereomer, it was positioned in the 3' direction, interfering sterically with the DNA duplex structure. 64 These results were consistent with modeling of the native cross-links. 50 Lao and Hecht also concluded that the pyrimidopurine cross-link arising from the 6R stereochemistry exhibited a more favorable orientation of the C6 CH_3 group. 20

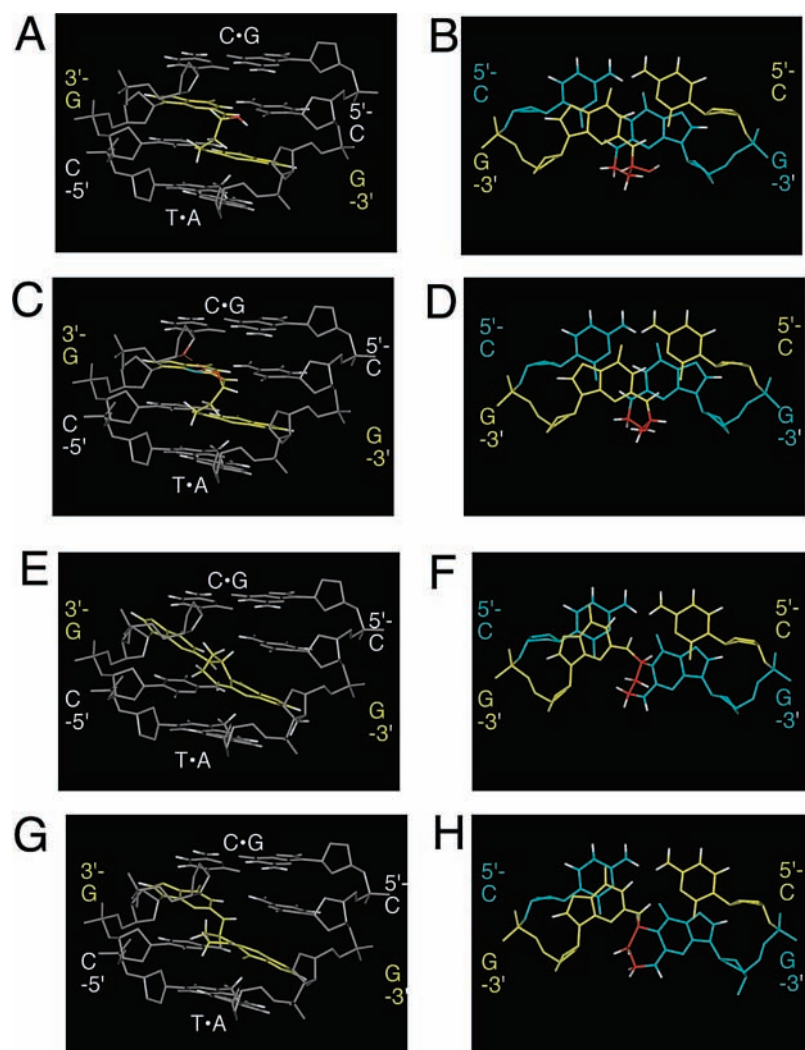


FIGURE 9. Modeling the 8*R* and 8*S* epimers of the 5'-CpG-3' acrolein-induced cross-links. A C·G pair is 5' and a T·A pair is 3' to the 5'-CpG-3' sequence. (A) 8*R*-diastereomer of carbinolamine cross-link **17**, minor groove view. (B) 8*R*-diastereomer of cross-link **17**, base-stacking. (C) 8*S*-diastereomer of cross-link **17**, minor groove view. (D) 8*S*-diastereomer of cross-link **17**, base-stacking. (E) 8*R*-diastereomer of pyrimidopurine cross-link **19**, minor groove view. (F) 8*R*-diastereomer of cross-link **19**, base-stacking. (G) 8*S*-diastereomer of cross-link **19**, minor groove view. (H) 8*S*-diastereomer of cross-link **19**, base-stacking. Reproduced from ref 58. Copyright 2005 American Chemical Society.

Additional studies of the 6*S* diastereomer **12** were performed at pH 9.3. This pH favors the ring-opened aldehyde adducts. The aldehyde group of the ring-opened 6*S* adduct is oriented in the 3'-direction within the minor groove (Figure 12).⁶⁵ Consequently, the aldehyde was distant from the exocyclic amine of the guanine involved in cross-linking (G¹⁹), explaining why this diastereomer generated interstrand cross-links less efficiently than the 6*R* diastereomer.⁶⁵ These observations also corroborated modeling studies.⁵⁰

Interstrand Cross-Linking by *trans*-4-HNE Adducts

Only the HNE adducts with the (6*S*,8*R*,11*S*) configuration (**16**) formed an interstrand cross-link in the 5'-CpG-3' sequence (Figure 13). This configuration possessed the same relative

stereochemistry at C6 as did the 6*R* configuration of the crotonaldehyde adduct (**11**), further supporting the role of stereochemistry at C6 in modulating interstrand cross-linking. Cross-linking proceeded slowly. However, after 2 months, the yield was a remarkable ~85%.⁵¹ Digestion of the DNA yielded the pyrimidopurine bis-nucleoside cross-link.⁵¹ Spectroscopic studies to delineate the cross-linking chemistry of the HNE adducts are continuing.

Potential Biological Significance

One major goal of continuing research is to demonstrate that these acrolein, crotonaldehyde, and HNE-derived interstrand cross-links are present *in vivo*, utilizing mass spectrometric-based analysis.^{17,66–68} Since they equilibrate with non-cross-linked species, and require the presence of the 5'-CpG-3'

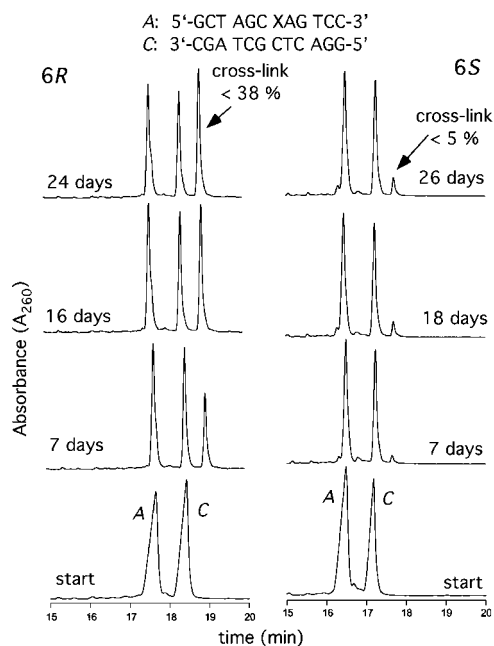


FIGURE 10. Cross-linking reactions of the 6R and 6S crotonaldehyde-modified duplexes in the 5'-CpG-3' sequence monitored by CGE. The adducted and complementary strands are identified by the letters A and C, respectively; the arrows indicate the interstrand cross-links.

sequence, they may be present at low levels in tissue samples. Nevertheless, it has been reported that acrolein preferentially binds at 5'-CpG-3' sites, a consequence of cytosine methylation at these sequences.²²

The potential presence of these cross-links *in vivo* is anticipated to interfere with DNA replication and transcription. Moreover, in humans, interstrand cross-link repair requires the cooperation of multiple proteins belonging to different biological pathways, including but not limited to nucleotide excision repair, homologous recombination, translesion DNA synthesis, double-strand break repair, and the Fanconi anemia pathway.^{43,69–73} Current models suggest that interstrand cross-link repair is initiated by dual incisions around the cross-link in one of the two affected strands. This “unhooking” depends on the endonucleolytic activity of the XPF/ERCC1 complex, a component of NER. The result is a gap that may be filled by pairing of the 3' end of the preincised strand with the homologous sequence, followed by DNA synthesis. Alternatively, the complementary strand with the cross-link attached may be used as a template for translesion DNA synthesis. Once the integrity of one DNA strand is restored, the second strand may be repaired by conventional NER. When repair is concomitant with replication, a DNA double-strand break could be formed; thus, additional biological processing would be required to tolerate interstrand cross-links.^{43,70}

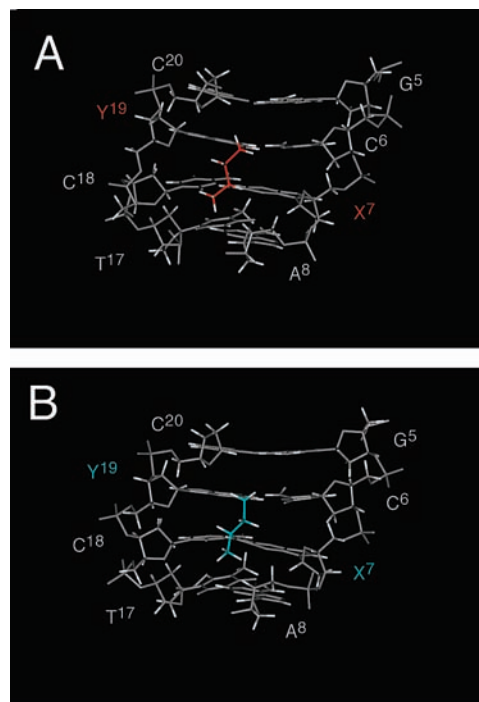


FIGURE 11. Structures of reduced cross-links arising from crotonaldehyde: (A) the 6R cross-link (red) oriented in the center of the minor groove; (B) The 6S cross-link (blue) interfered sterically with the DNA and exhibited lower stability. Nucleotides are numbered 5'-d(G¹C²T³A⁴G⁵C⁶X⁷A⁸G⁹T¹⁰C¹¹C¹²)-3' · 5'-d(G¹³G¹⁴A¹⁵C¹⁶T¹⁷C¹⁸Y¹⁹C²⁰T²¹A²²G²³C²⁴)-3', X⁷ = 6R or 6S-crotonaldehyde-adducted dG in the 5'-CpG-3' sequence; Y¹⁹ = cross-linked dG in the complementary strand. Reproduced from ref 64. Copyright 2007 American Chemical Society.

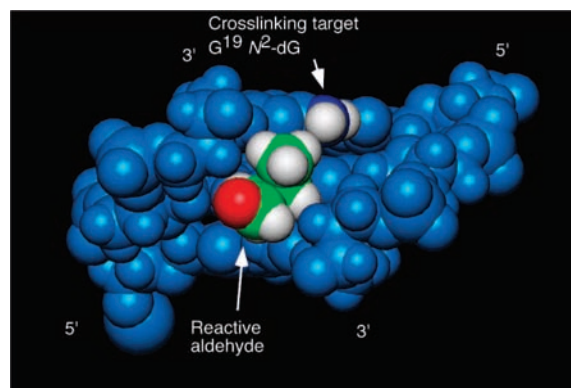


FIGURE 12. Base pairs C⁶·G¹⁹, X⁷·C¹⁸, and A⁸·T¹⁷ in the oligodeoxynucleotide containing the N²-(3-oxo-1S-methyl-propyl)-dG adduct **12**. The orientation of the aldehyde does not favor cross-linking to the target G¹⁹ N²-dG. Nucleotides are numbered 5'-d(G¹C²T³A⁴G⁵C⁶X⁷A⁸G⁹T¹⁰C¹¹C¹²)-3' · 5'-d(G¹³G¹⁴A¹⁵C¹⁶T¹⁷C¹⁸Y¹⁹C²⁰T²¹A²²G²³C²⁴)-3', X⁷ = N²-(3-oxo-1S-methyl-propyl)-dG adduct **12**. Reproduced from ref 65. Copyright 2006 American Chemical Society.

Summary

The 1,N²-dG adducts of acrolein, crotonaldehyde, and 4-HNE yield interstrand cross-links in the 5'-CpG-3' sequence. These arise via opening of the 8-hydroxypropano ring to the corre-

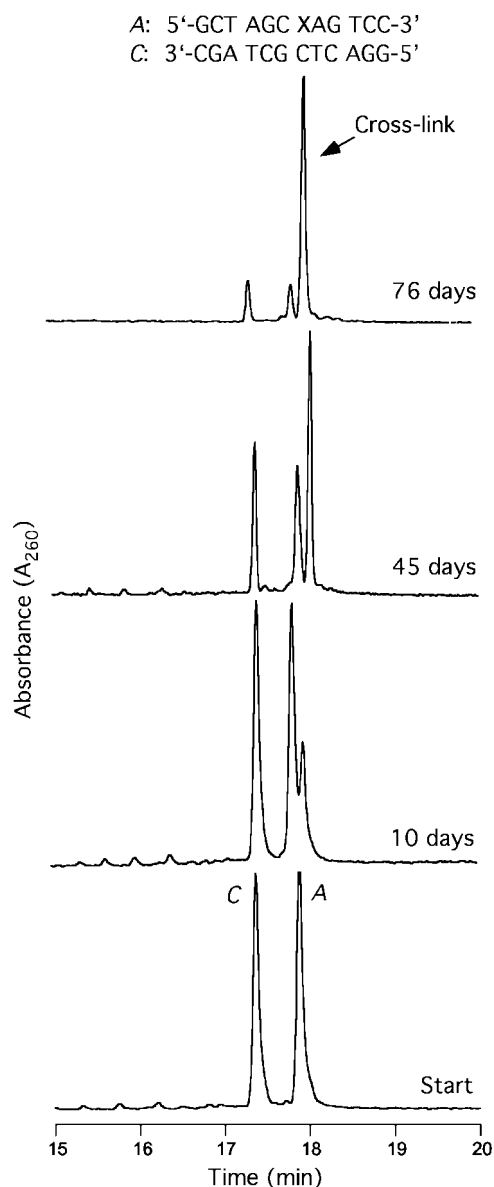


FIGURE 13. Cross-linking of the (6S,8R,11S)-4-HNE-containing oligodeoxynucleotide.

sponding aldehydes, which undergo attack by the N^2 -amino group of the cross-strand dG in the 5'-CpG-3' sequence. The cross-links arising from acrolein and crotonaldehyde exist in duplex DNA as carbinolamine linkages, which enable the cross-linked C·G base pairs to maintain Watson–Crick hydrogen bonding with minimal distortion of the duplex. The cross-linking chemistry depends upon the stereochemistry of the C6 carbon, which favorably orients the reactive aldehyde within the minor groove in the 5'-CpG-3' sequence, favoring the 6R configuration for crotonaldehyde and the stereochemically equivalent 6S configuration for 4-HNE.

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