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

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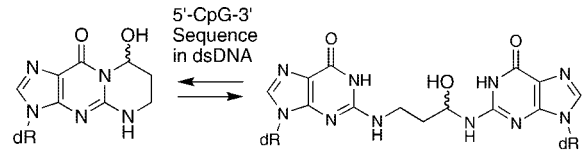
CONSPECTUS

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 smoke[5] 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

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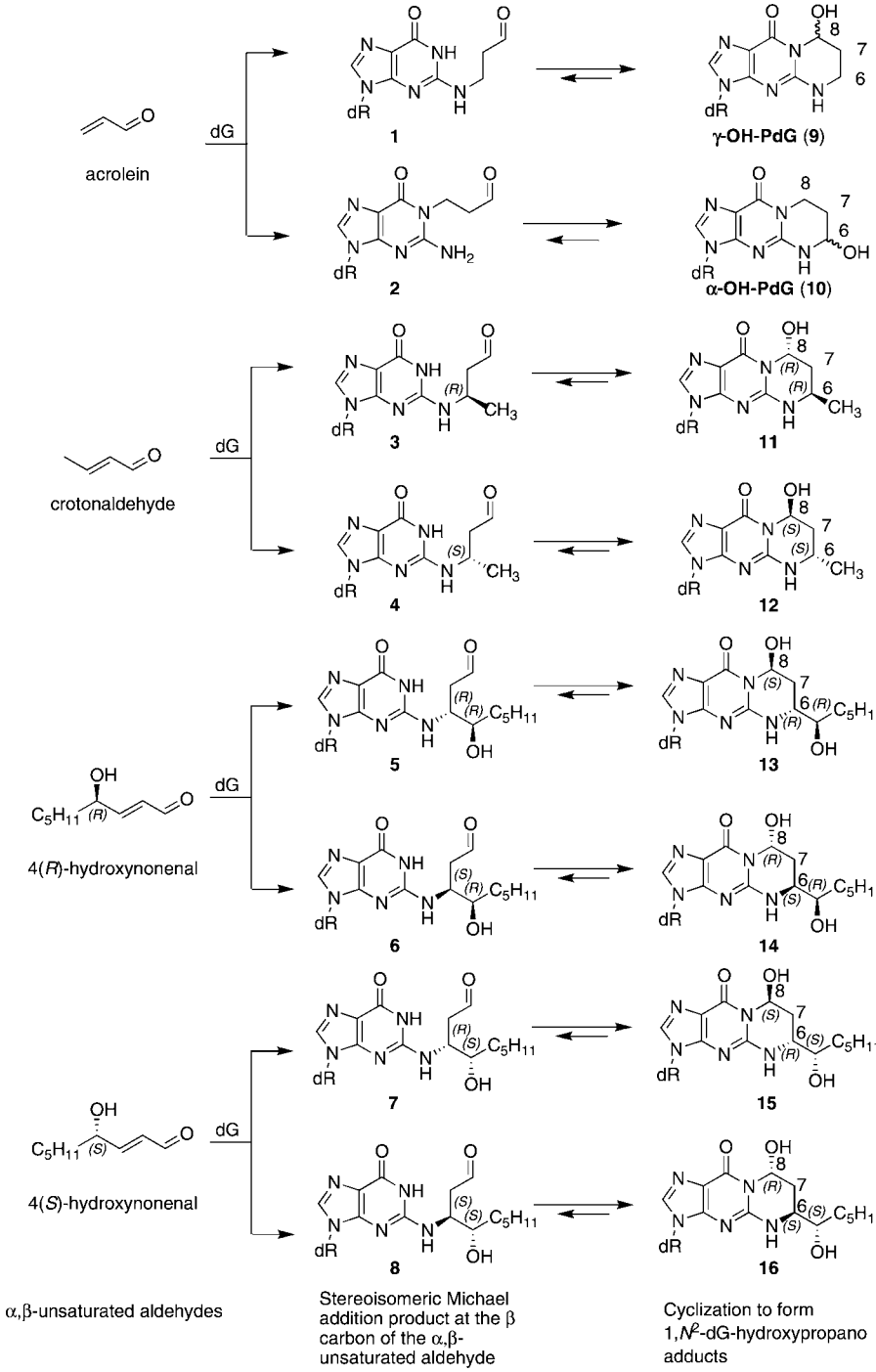
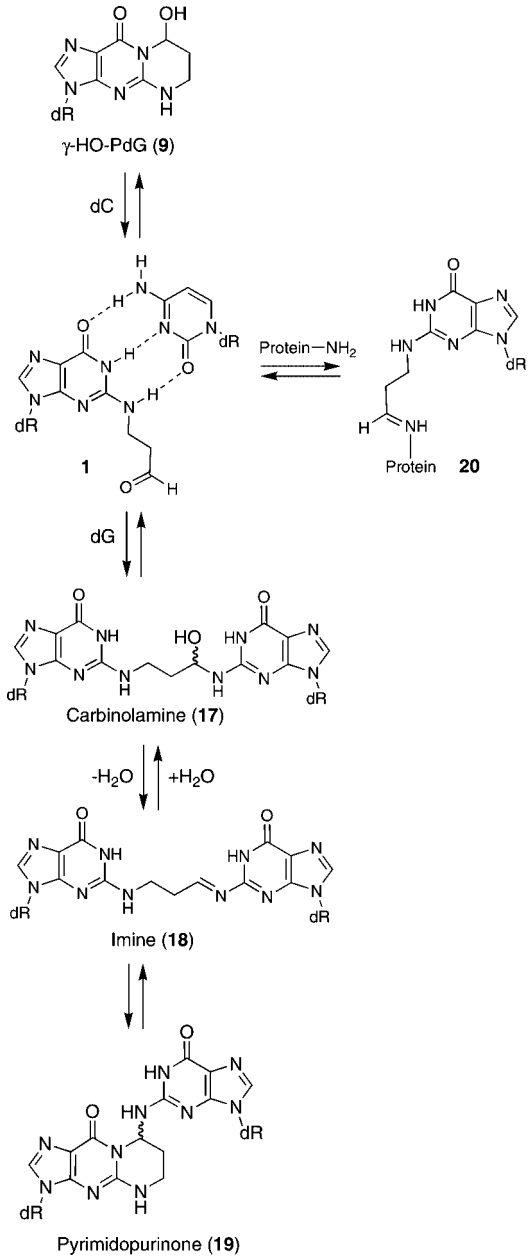
SCHEME 1. 1,N^2-dG Cyclic Adducts Arising from Michael Addition of Enals to dG



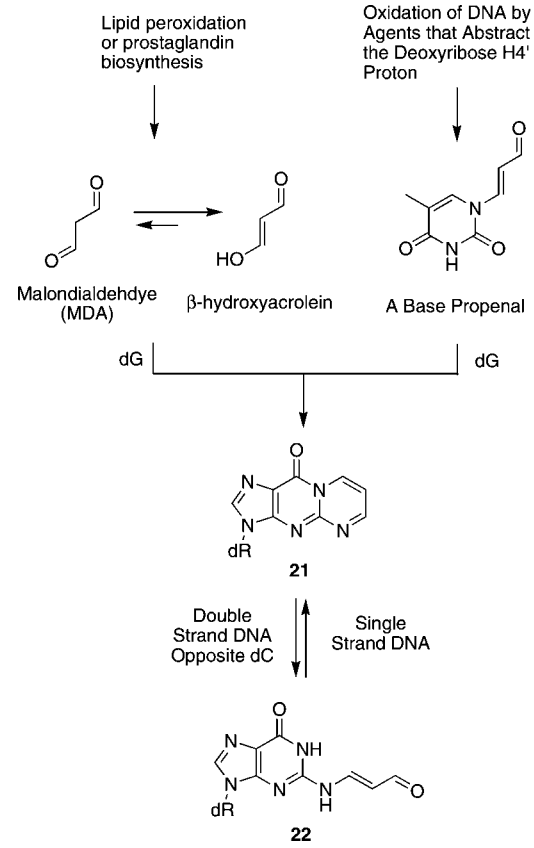
bis-electrophiles.[8,9] Galliana and Pantarotto characterized the 8-hydroxypyrimido[1,2-α]purin-10(3*H*)-one (γ-OH-PdG, **9**) adduct from the reaction of acrolein with dG.[10] Chung and Hecht concurrently reported the crotonaldehyde adduct of dG (**11**, **12**)[11] and explored the reactivity of enals 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-*a*]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



SCHEME 3. Formation and Chemistry of the Malondialdehyde-Derived M_1dG Adduct



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 is carcinogenic in rats.[27] Crotonaldehyde is genotoxic and mutagenic in human lymphoblasts[28] 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]

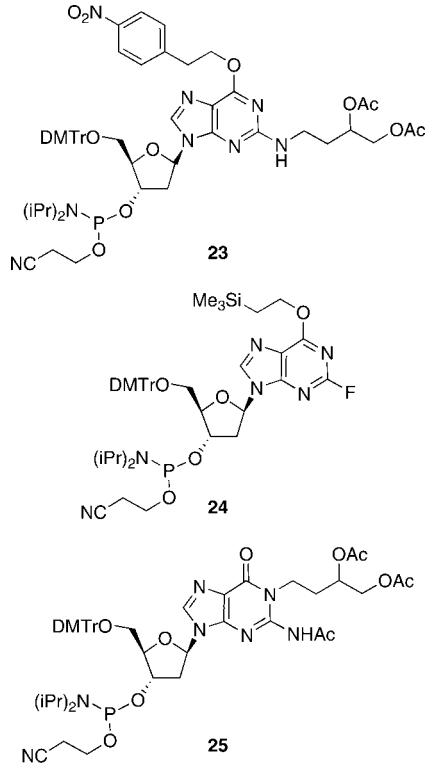


FIGURE 1. Phosphoramidite reagents for the site-specific synthesis of oligodeoxynucleotides containing 1,N^2-dG enal adducts.

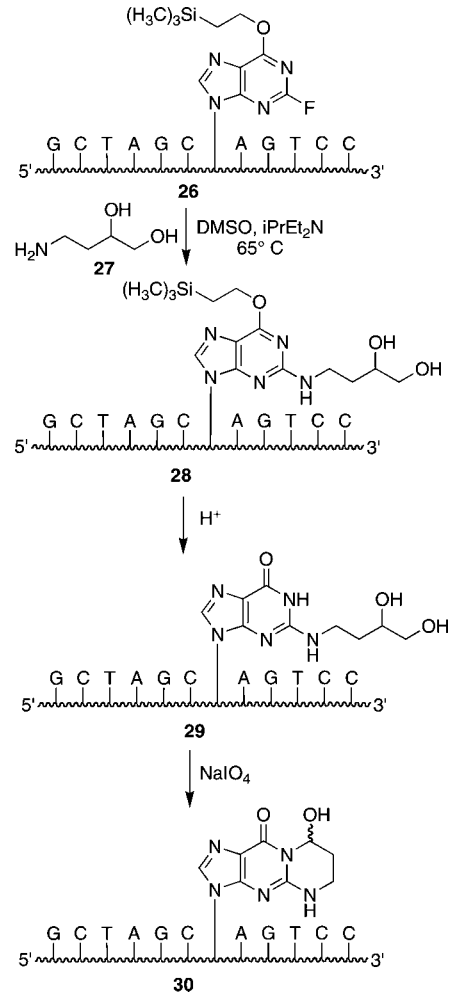
We further hypothesized that these aldehydes react with other nucleobases in the cDNA strand, forming interstrand cross-links, which exist as equilibrium mixtures of carbinolamine (**17**), imine (**18**), or pyrimidopurinone (**19**) species. The aldehyde in structure **1** also yields peptide− and 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 pyrimidopurinone 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. synthesized γ-OH-PdG (**9**) by condensation of 4-amino-1,2-butanediol 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 strategy[48] allowed preparation of various dG enal adducts from a single modified phosphoramidite. A challenge was the prepara-

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



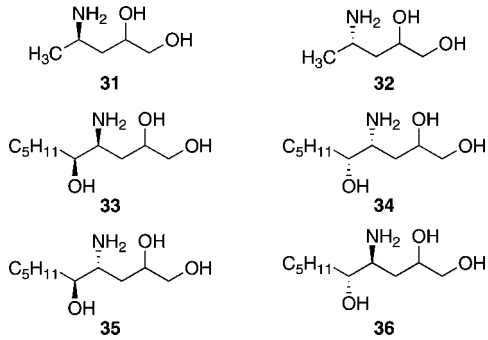


FIGURE 2. Amino alcohols for synthesis of crotonaldehyde- and 4-HNE-modified oligodeoxynucleotides.

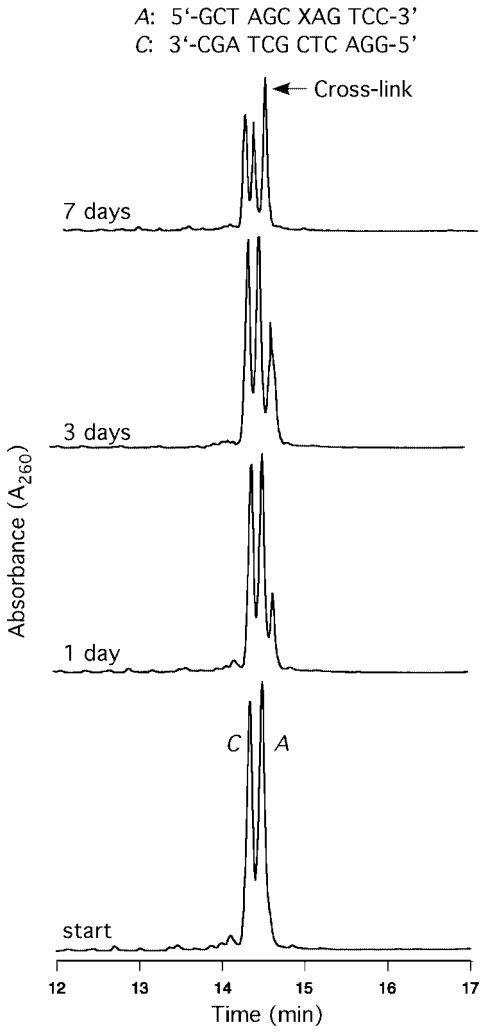


FIGURE 3. Cross-linking of γ-OH-PdG adducts in the 5′-CpG-3′ sequence, monitored by CGE. The adducted and complementary strands are identified by the letters *A* and *C*, respectively; the arrows indicate interstrand cross-links.

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]

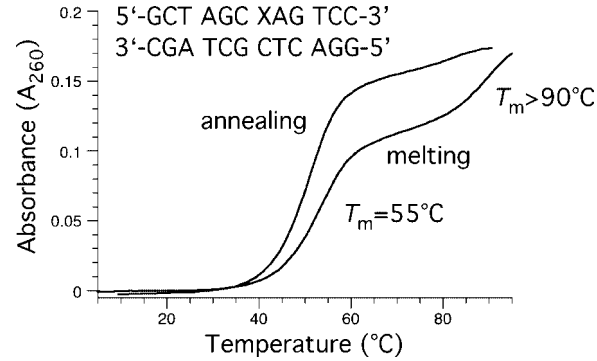


FIGURE 4. Thermal melting analysis of an acrolein-modified oligonucleotide in a CpG sequence after incubation for 5 days. The higher melting transition is assigned to the interstrand cross-link.

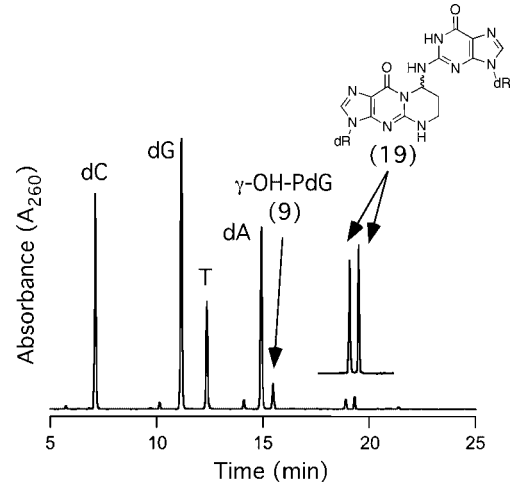


FIGURE 5. Digestion of the cross-linked γ-OH-PdG-adducted duplex. The pyrimidopurinone bis-nucleosides were identified by comparison with authentic standards.

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 ∼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, ∼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

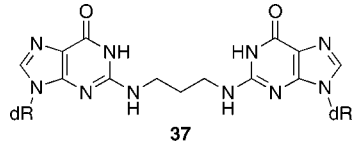


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FIGURE 6. N^2-dG:N^2-dG trimethylene cross-link derived from the reduction of **18**.

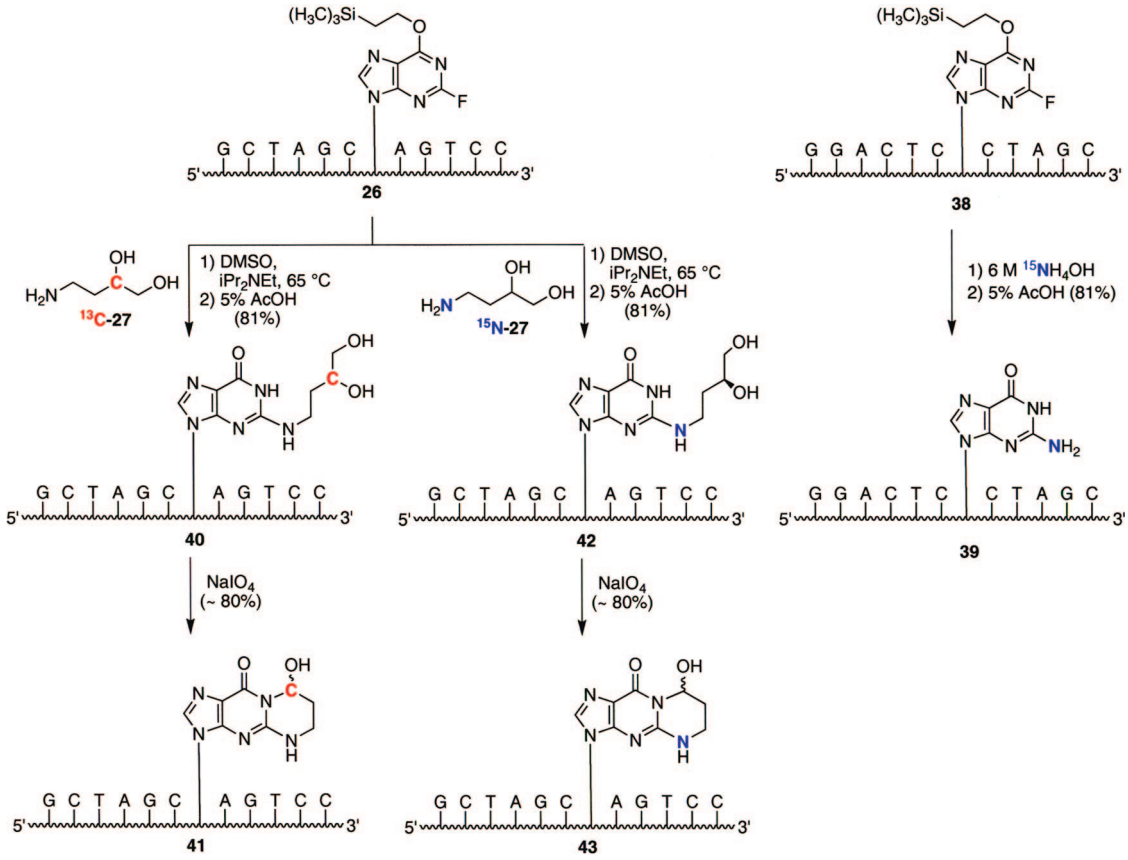
to those arising from acetaldehyde-treated DNA.[19] Reduction of **19** afforded N^2-dG:N^2-dG bis-nucleosides tethered by a trimethylene chain (**37**, Figure 6).[56] If the cross-linked duplex was reduced with $NaB(CN)H_3$ prior to its digestion, N^2-(3- hydroxypropyl)-dG from the reduction of γ-OH-PdG (**9**) and cross-link **37** were observed.

Although the cross-link could be reductively trapped, ^{13}C NMR experiments utilizing X = γ-^{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-$^{15}N^2$-PdG was annealed with its complementary strand. An ^{15}N-HSQC filtered spectrum revealed the NOE between X^7 $^{15}N^2H$ and the imino proton X^7 N1H (Figure 8), precluding the pyrimidopurinone structure (**19**). A triple resonance $^1H^{13}C^{15}N$ experiment conducted subsequent to annealing γ-^{13}C-modified oligodeoxynucleotide **41** with $^{15}N^2$-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(AGGCXCCT)$_2$; 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**)



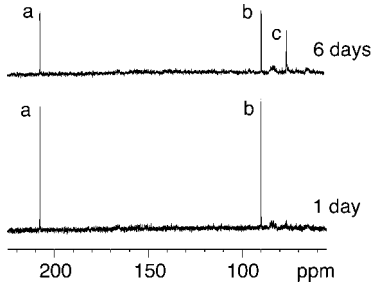


FIGURE 7. Data from an isotopically enriched sample containing ^{13}C-γ-OH-PdG. The imine linkage remained below the level of NMR detection. The top spectrum shows a ^{13}C resonance assigned as the diastereomeric carbinolamine forms of the cross-link. Assignments of resonances: (a) aldehyde **1**; (b) 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 $^{15}N^2$-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 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(TCCXCGGA)$_2$, its structure was distorted, and its T_m was reduced.[59,63]

Interstrand Cross-Linking of (6*R*) and (6*S*) 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, ~40% cross-link formation occurred for the 6*R* diastereomer **11** (Figure 10), whereas <5% cross-link was observed for the 6*S* diasteromer **12**.[55] Digestion of the cross-link yielded the bis-nucleoside pyrimidopurinone,[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 predominetly an imine or pyrimidopurinone with some of the carbinolamine linkage present.[20]

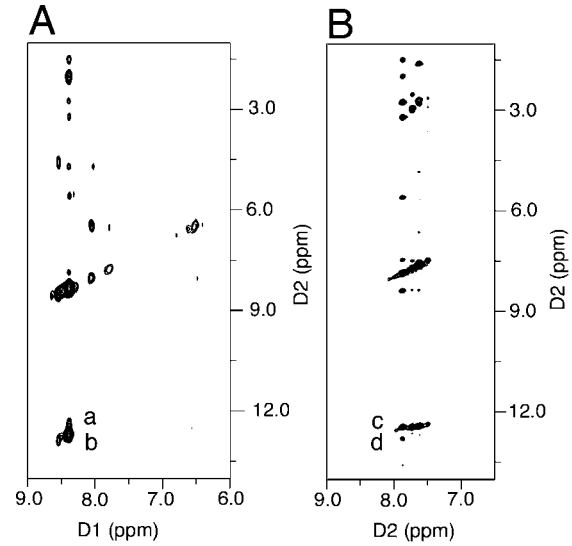


FIGURE 8. Isotope-edited NMR identified the carbinolamine linkage for the γ-OH-PdG cross-link: (A) ^{15}N-HSQC NOESY spectrum for oligodeoxynucleotide **39** annealed with oligodeoxynucleotide **31**. Nucleotides are numbered 5′-d(G^{1}C^{2}T^{3}A^{4}G^{5}C^{6}X^{7}A^{8}G^{9}T^{10}C^{11}C^{12})-3′·5′-d(G^{13}G^{14}A^{15}C^{16}T^{17}C^{18}Y^{19}C^{20}T^{21}A^{22}G^{23}C^{24})-3′, X^7 = γ-OH PdG; Y^{19} = ^{15}N^{2}dG. Crosspeaks (a) Y^{19} $^{15}N^2$H→X^7 N1H (weak); (b) Y^{19} $^{15}N^2$H→Y^{19} N1H (strong). (B) ^{15}N-HSQC NOESY spectrum for γ-OH-$^{15}N^2$-PdG-labeled oligodeoxynucleotide **43** annealed with its complement. Crosspeaks (c) X^7 $^{15}N^2$H→X^7 N1H (strong); (d) X^7 $^{15}N^2$H→G^{19} 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 6*R* 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 pyrimidopurinone 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 6*R*- and 6*S* cross-links indicated that both retained Watson−Crick hydrogen bonds at the cross-linked base pairs (Figure 11).[64] However, the 6*S* diastereomer showed lower stability. Whereas for the 6*R* diastereomer, the CH_3 group was positioned in the center of the minor groove, for the 6*S* 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 pyrimidopurinone cross-link arising from the 6*R* stereochemistry exhibited a more favorable orientation of the C6 CH_3 group.[20]

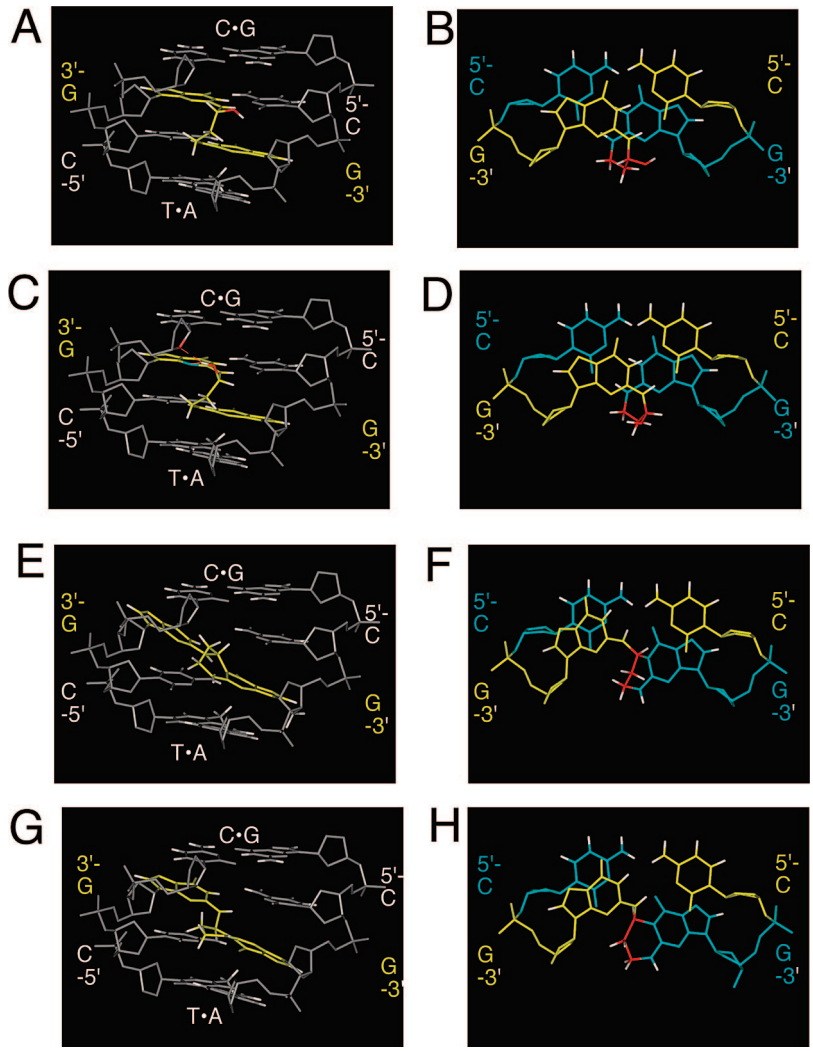


FIGURE 9. Modeling the 8*R* and 8*S* epimers of the 5′-CpG-3′ acrolein-induced cross-links. A C·G pair is 5′ and a T·A pair is 3′ to the 5′-CpG-3′ sequence. (A) 8*R*-diastereomer of carbinolamine cross-link **17**, minor groove view. (B) 8*R*-diastereomer of cross-link **17**, base-stacking. (C) 8*S*-diastereomer of cross-link **17**, minor groove view. (D) 8*S*-diastereomer of cross-link **17**, base-stacking. (E) 8*R*-diastereomer of pyrimidopurinone 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 (G^{19}), 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 pyrimidopurinone 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′

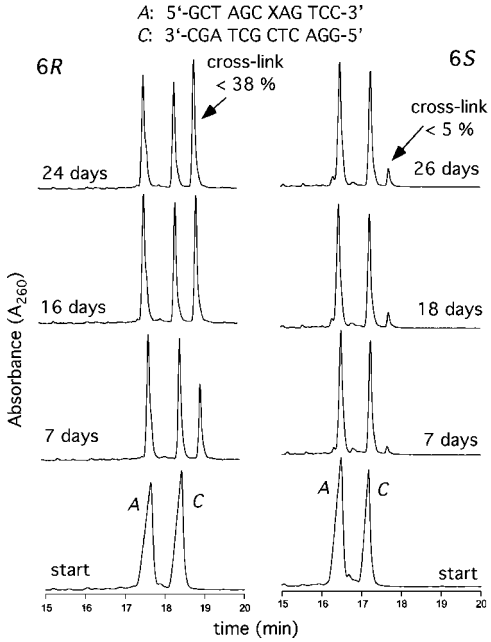


FIGURE 10. Cross-linking reactions of the 6*R* and 6*S* crotonaldehyde-modified duplexes in the 5′-CpG-3′ sequence monitored by CGE. The adducted and complementary strands are identified by the letters *A* and *C*, respectively; the arrows indicate the interstrand cross-links.

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]

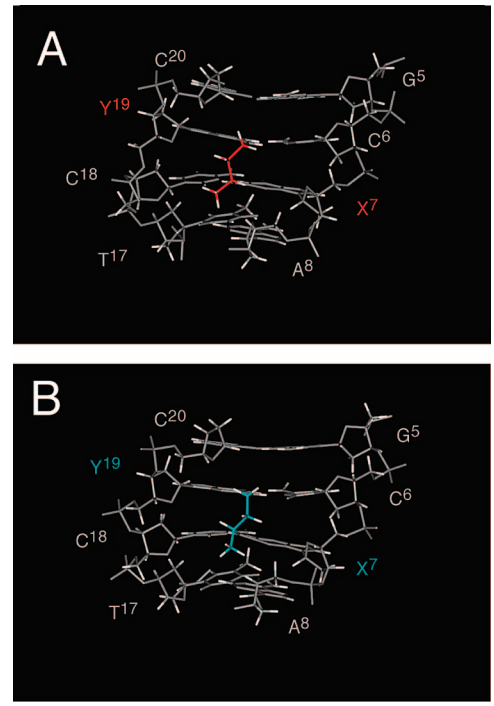


FIGURE 11. Structures of reduced cross-links arising from crotonaldehyde: (A) the 6*R* cross-link (red) oriented in the center of the minor groove; (B) The 6*S* cross-link (blue) interfered sterically with the DNA and exhibited lower stability. Nucleotides are numbered 5′-d($G^1C^2T^3A^4G^5C^6X^7A^8G^9T^{10}C^{11}C^{12}$)-3′·5′-d($G^{13}G^{14}A^{15}C^{16}T^{17}C^{18}Y^{19}C^{20}T^{21}A^{22}G^{23}C^{24}$)-3′, X^7= 6*R* or 6*S*-crotonaldehyde-adducted dG in the 5′-CpG-3′ sequence; Y^{19} = cross-linked dG in the complementary strand. Reproduced from ref 64. Copyright 2007 American Chemical Society.

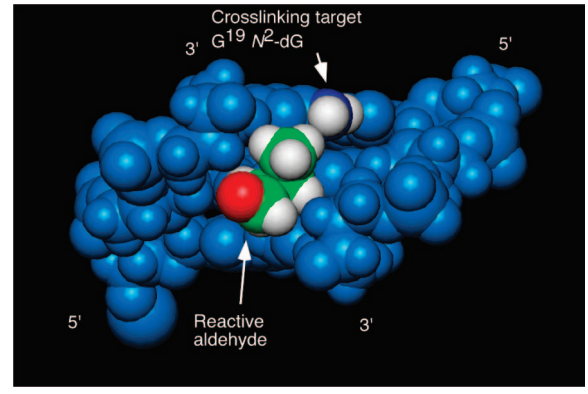


FIGURE 12. Base pairs $C^6 \cdot G^{19}$, $X^7 \cdot C^{18}$, and $A^8 \cdot T^{17}$ in the oligodeoxynucleotide containing the N^2-(3-oxo-1*S*-methyl-propyl)-dG adduct **12**. The orientation of the aldehyde does not favor cross-linking to the target G^{19} N^2-dG. Nucleotides are numbered 5′-d($G^1C^2T^3A^4G^5C^6X^7A^8G^9T^{10}C^{11}C^{12}$)-3′·5′-d($G^{13}G^{14}A^{15}C^{16}T^{17}C^{18}Y^{19}C^{20}T^{21}A^{22}G^{23}C^{24}$)-3′, X^7 = N^2-(3-oxo-1*S*-methyl-propyl)-dG adduct **12**. Reproduced from ref 65. Copyright 2006 American Chemical Society.

Summary

The 1,N^2-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-

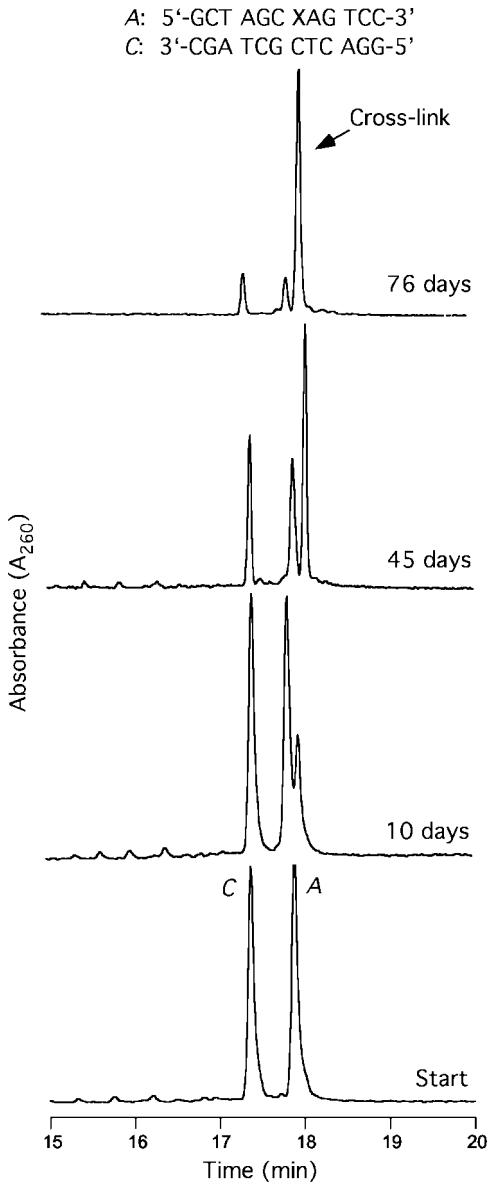


FIGURE 13. Cross-linking of the (6*S*,8*R*,11*S*)-4-HNE-containing oligodeoxynucleotide.

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 6*R* configuration for crotonaldehyde and the stereochemically equivalent 6*S* configuration for 4-HNE.

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FOOTNOTES

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