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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 exposure. 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.

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, e.g., 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

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cyclization of N1 with the aldehyde, yielding the corresponding 1,N^2-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 bis-electrophiles.[8,9] Galliana and Pantarotto characterized the 8-hydroxypyrimido[1,2-α]purin-10(*3H*)-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(*3H*)-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 configurations at C6 and C8 (**11,12**) are observed.[12,18] These are also formed through the reaction of dG with two equivalents of acetaldehyde.[5,19,20] The corresponding 4-HNE-derived 1,N^2-dG adducts possess an additional stereocenter on the C6-sidechain, 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 agent.[22] Acrolein is mutagenic in bacterial and mammalian cells,[23,24] including human,[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-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]

We further hypothesized that these aldehydes react with other nucleobases in the complementary DNA 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.

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.

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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*-nitro-phenylethyl-protected 2-fluoroinosine deoxynucleoside.[45] The N^2-(3,4-dihydroxy-butyl) 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 ofγ-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 post-synthetic modification strategy[48] allowed preparation of various dG enal adducts from a single modified phosphoramidite. A challenge was the preparation of stereochemically-defined amino diols for 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]

Interstrand Cross-linking by γ-OH-PdG

Oligodeoxynucleotide **30**, containingγ-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 seven days at 25 °C. Mass spectrometric analysis suggested 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 uncross-linked duplex containing **9** was 55° C, 10° lower than the unmodified duplex. In duplex DNA, ~20% of the cross-link reverted to uncross-linked over 16 hr at pH 7, whereas reversion occurred within 1 hr under conditions that disrupted the duplex.[56] Enzymatic digestion yielded diastereomeric pyrimidopurinone bis-nucleoside cross-links **19** (Figure 5), which are structurally related 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 crosslink **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** containingγ-OH-$^{15}N^2$-PdG was annealed with its complementary strand. A ^{15}N-HSQC filtered spectrum revealed the NOE between X^7 $^{15}N^2$H 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-PdG-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 a 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

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distortion.[59] Additionally, modeling suggested that the carbinolamine linkage maintained Watson-Crick 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**), would have disrupted 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(CGTAC<u>X</u>CATGC)-3′, containing both the 5′-CpG-3′ or 5′-GpC-3′ sequences,[55] and the complement 5′-(GCAT<u>G</u>CGTACG)-3′ was labeled with $^{15}N^2$-dG (the underlined 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, e.g., 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 non-observed cross-link in the 5′-GpC-3′ sequence, was constructed in d(TCC<u>X</u>CGGA)$_2$, 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}-PdG 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 the cross-link was predominetly an imine or pyrimidopurinone with some of the carbinolamine linkage present.[20]

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 bonding at the cross-linked base pairs. Dehydration of the carbinolamine to the imine, or cyclization of the latter to form the pyrimidopurinone cross-link, would disrupt Watson-Crick bonding 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 12).[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]

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 to the exocyclic amine of the guanine involved in cross-linking (G^{19}), explaining why

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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 posessed 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 two 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′ 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 pre-incised strand with the homologous sequence, followed by DNA synthesis. Alternatively, the complementary strand with the crosslink 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 is formed; thus, additional biological processing would be required to tolerate interstrand cross-links.[43,70]

Summary

The 1,N^2-dG adducts of acrolein, crotonaldehyde, and 4-HNE yield interstrand cross-links in the5′-CpG-3′ sequence. These arise via opening of the 8-hydroxypropano ring to the corresponding 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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Biographies

Michael P. Stone received his B.S. in Biochemistry at the University of California, Davis, and the Ph.D. in Chemistry at the University of California, Irvine, with Philip N. Borer. After postdoctoral training at the University of Rochester with Thomas R. Krugh, he joined the faculty at Vanderbilt University, where he is Professor of Chemistry and Biochemistry. His research interests include the structural consequences of DNA damage.

Carmelo J. Rizzo received his B.A in Chemistry from Temple University and the Ph. D. in Chemistry from the University of Pennsylvania under Amos B. Smith. After an NIH postdoctoral fellowship at Columbia University with Ronald Breslow, he joined the faculty at Vanderbilt University where he is currently Professor of Chemistry and Biochemistry and Co-Deputy Director of the Vanderbilt Center in Molecular Toxicology. He is interested in the chemical aspects of DNA damage.

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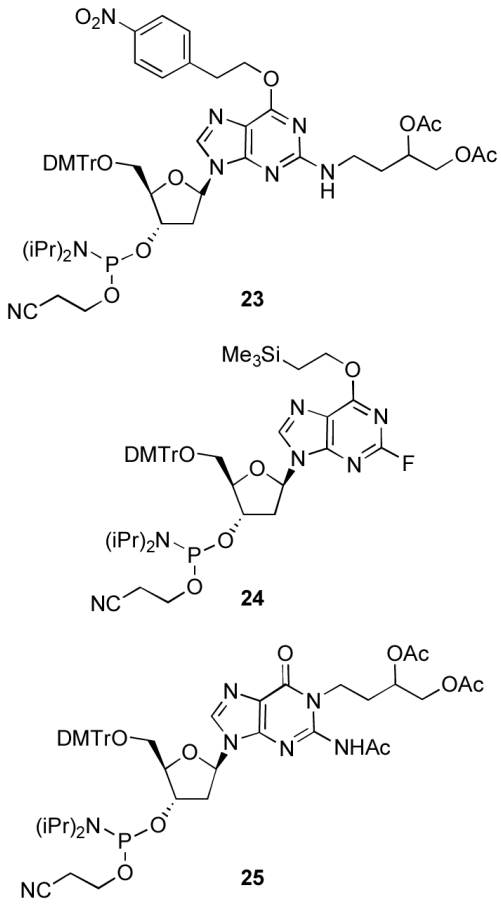

Figure 1.
Phosphoramidite reagents for the site-specific synthesis of oligodeoxynucleotides containing 1,N^2-dG enal adducts.

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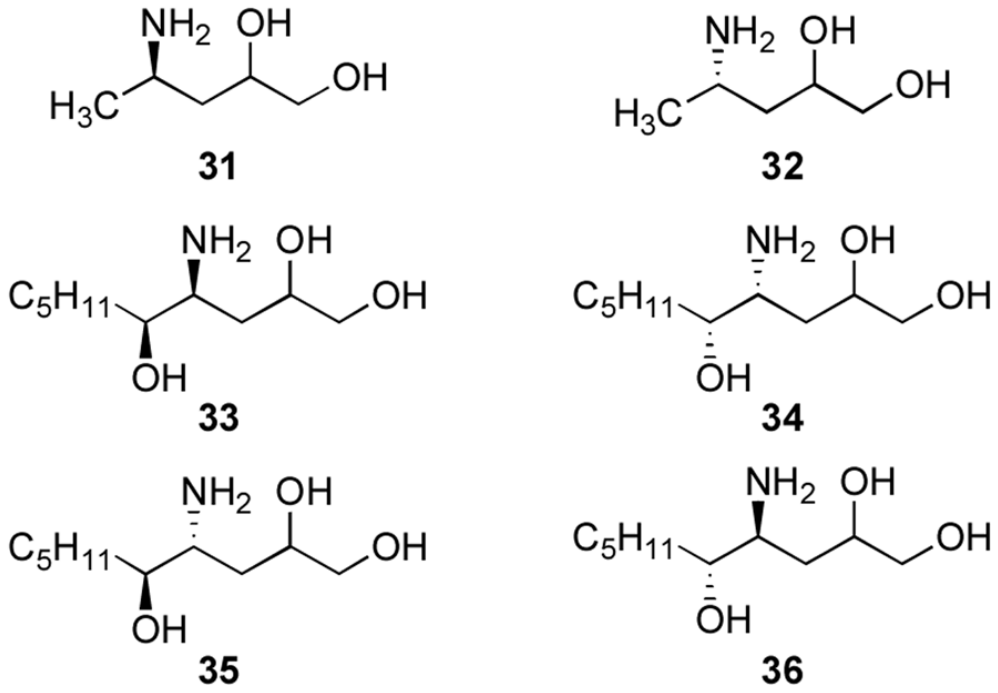

Figure 2.
Amino alcohols for synthesis of crotonaldehyde and 4-HNE-modified oligodeoxynucleotides.

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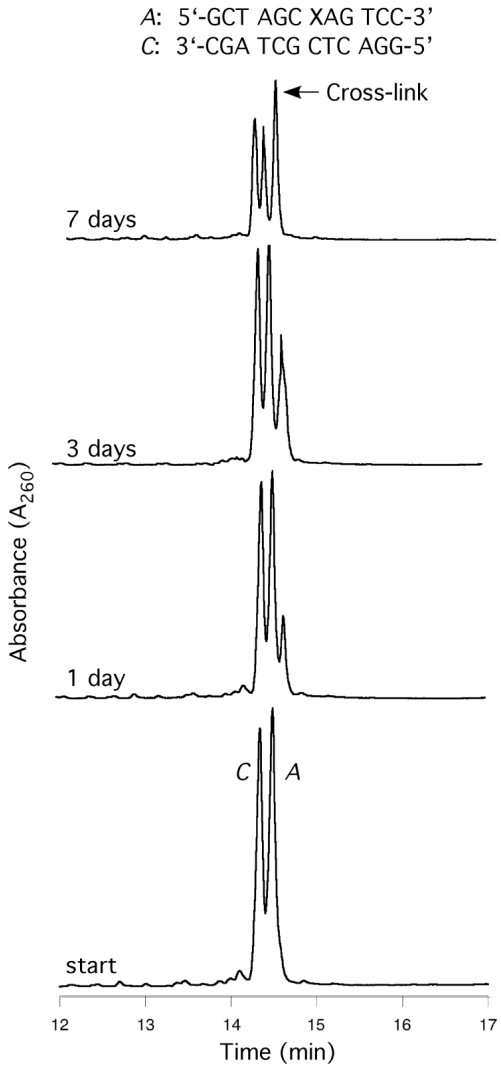

Figure 3.

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.

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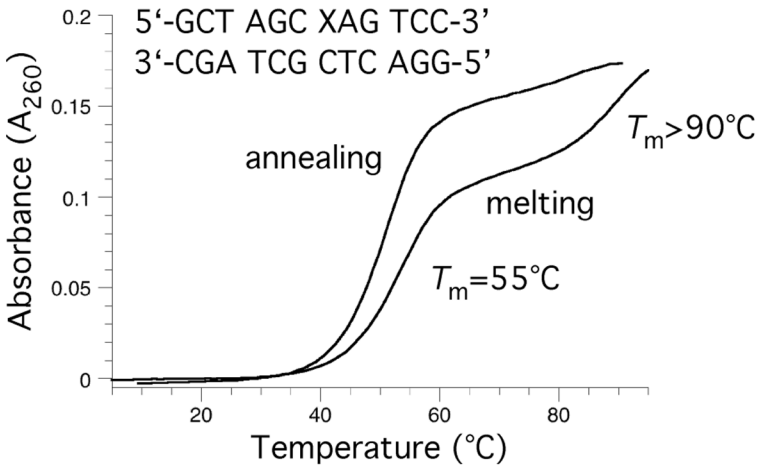

Figure 4.
Thermal melting analysis of and acrolein modified oligonucleotide in a CpG sequence after incubation for five day. The higher melting transition is assigned to the interstrand cross-link.

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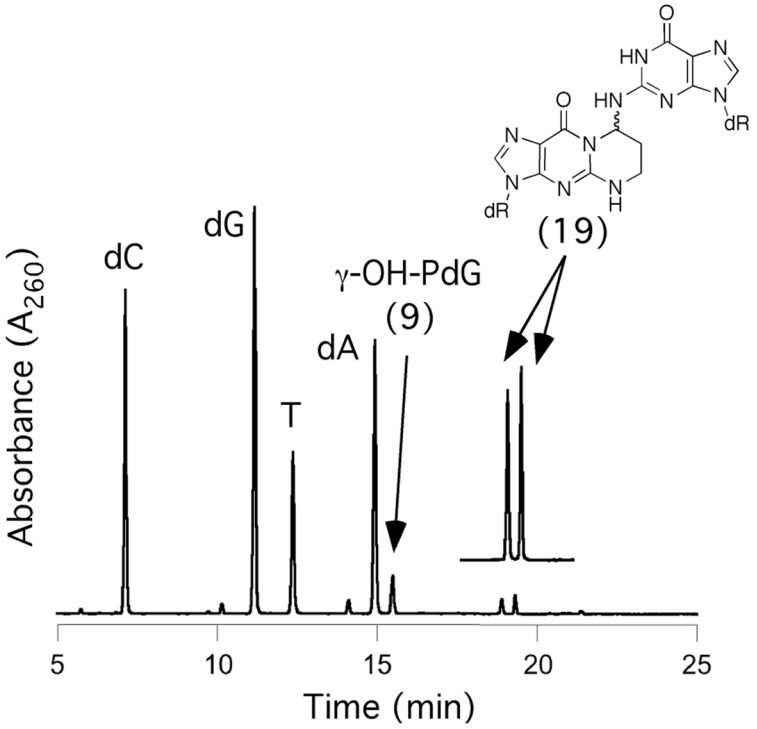

Figure 5.
Digestion of the cross-linkedγ-OH-PdG-adducted duplex. The pyrimidopurinone bis-nucleosides were identified by comparison with authentic standards.

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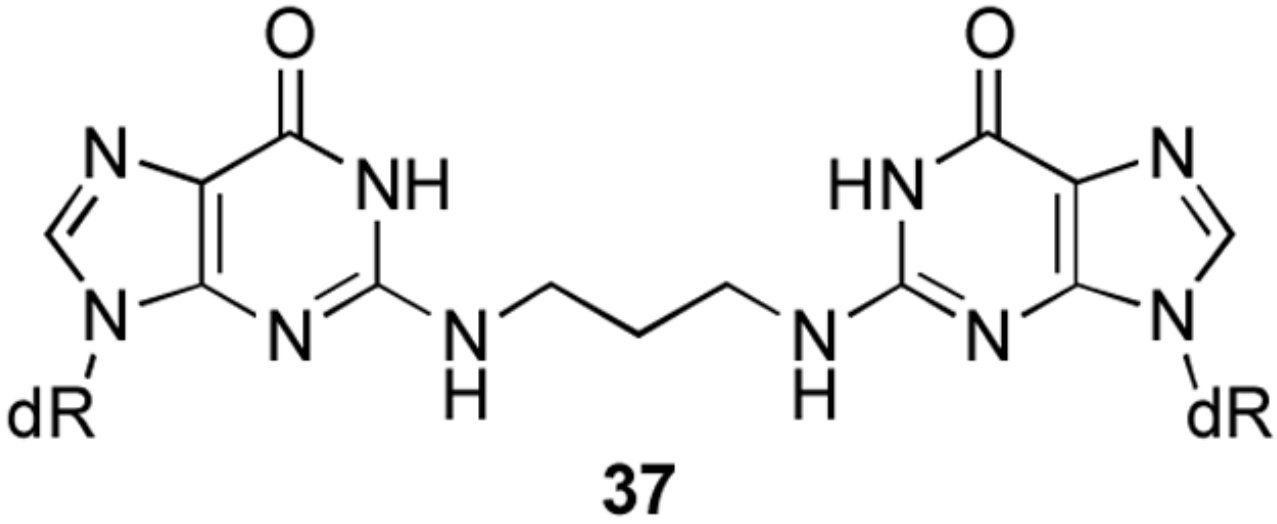

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Figure 6.
N^2-dG:N^2-dG trimethylene cross-link derived from the reduction of **18**.

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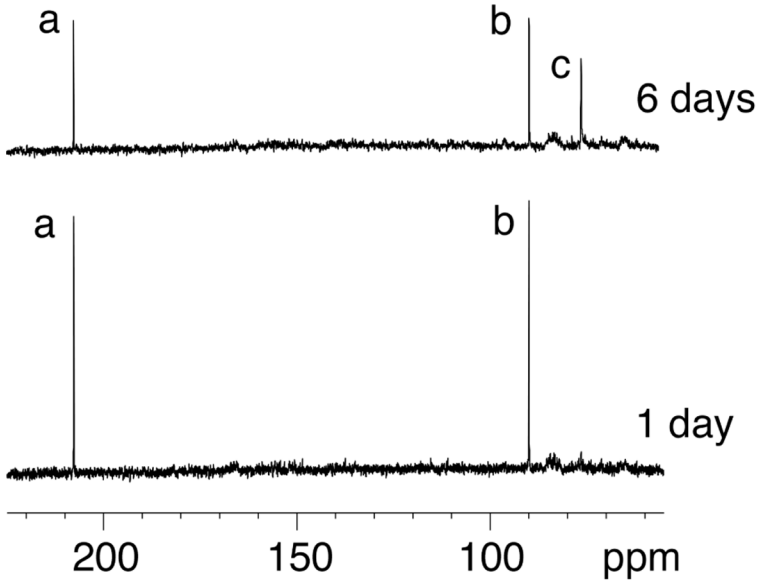

Figure 7.
Data from an isotopically enriched sample containing ^{13}C-γ-OH-PdG. The imine linkage remained below the level of NMR detection. The top spectrum shows a ^{13}C resonance assigned as the diastereomeric carbinolamine forms of the cross-link. Assignments of resonances: **a**, aldehyde **1;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

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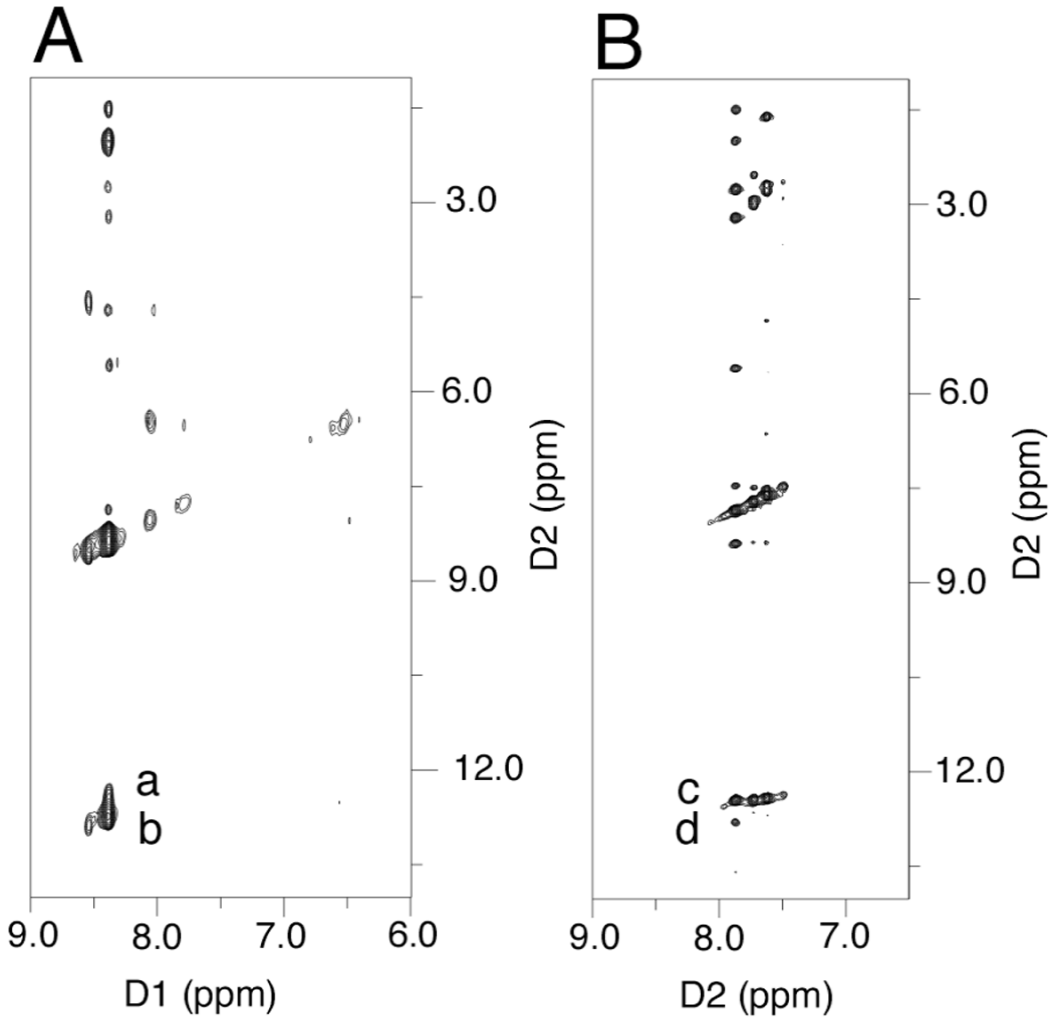

Figure 8.

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} $^{15}N^{2}H\rightarrow X^{7}$ N1H (weak); b, Y^{19} $^{15}N^{2}H\rightarrow Y^{19}$ N1H (strong). **B.** ^{15}N-HSQC NOESY spectrum for γ-OH-$^{15}N^{2}$-PdG labeled oligodeoxy-nucleotide **43** annealed with its complement. Crosspeaks c, X^{7} $^{15}N^{2}H\rightarrow X^{7}$ N1H (strong); d, X^{7} $^{15}N^{2}H\rightarrow G^{19}$ N1H (weak). Copyright American Chemical Society 2005.

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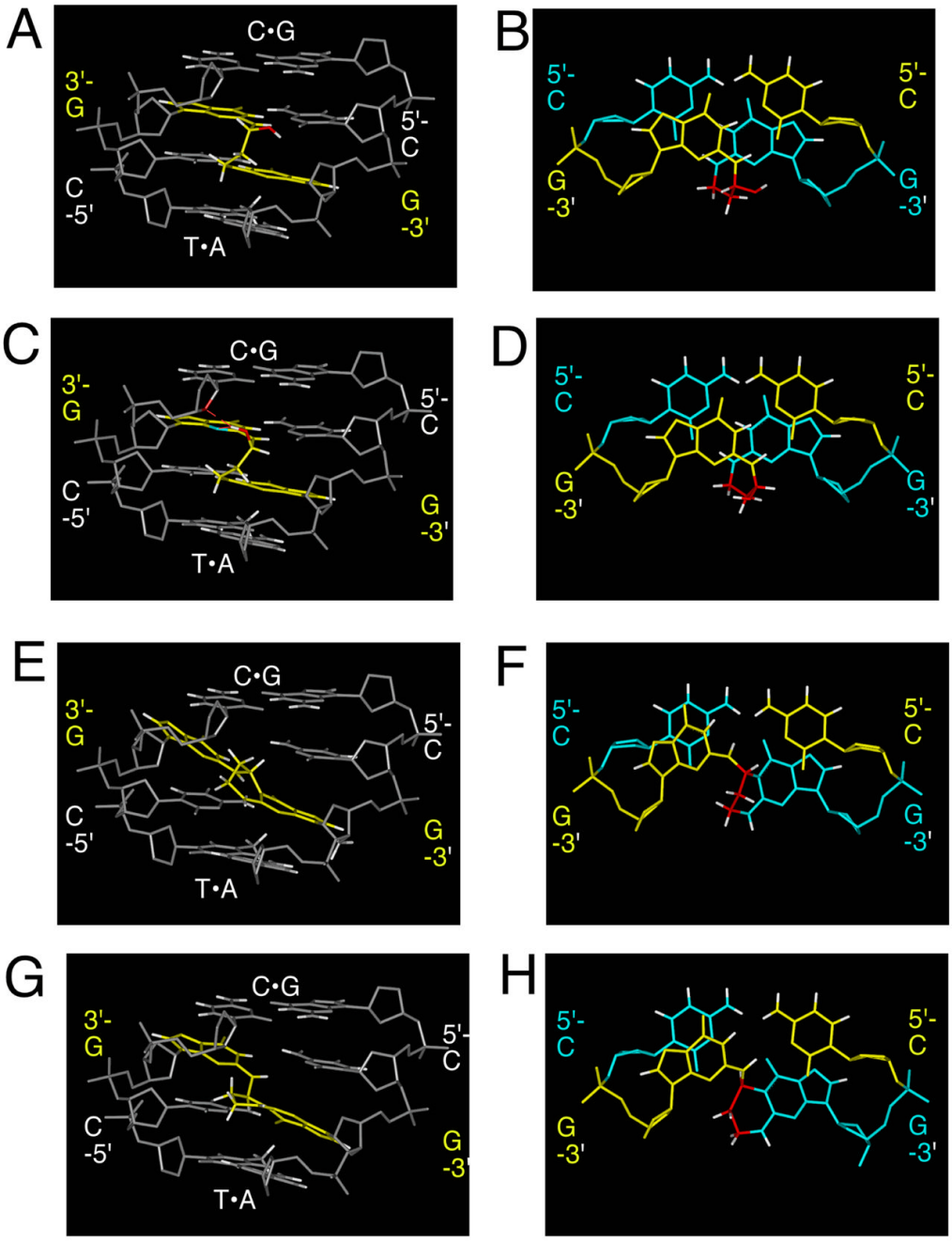

Figure 9.

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. Copyright American Chemical Society 2005.

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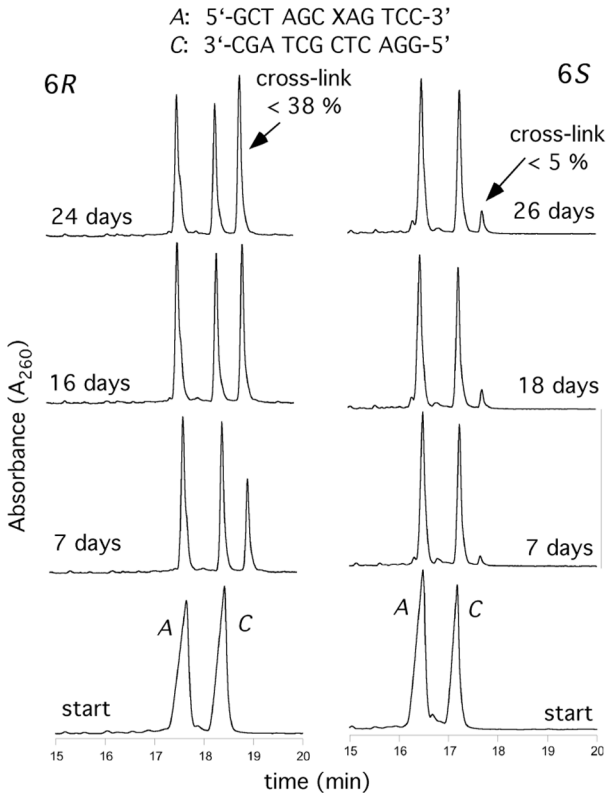

Figure 10.

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.

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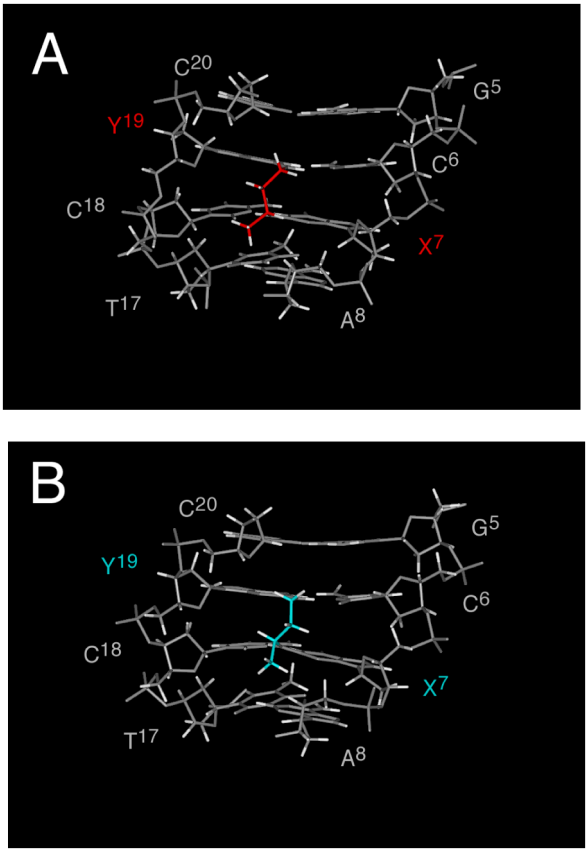

Figure 11.
Structures of reduced cross-links arising from crotonaldehyde. **A.** The 6*R* cross-link (red) oriented in the center of the minor groove. **B.** The 6*S* cross-link (blue) interfered sterically with the DNA and exhibited lower stability. Nucleotides are numbered 5′-d ($G^{1}C^{2}T^{3}A^{4}G^{5}C^{6}X^{7}A^{8}G^{9}T^{10}C^{11}C^{12}$)-3′•5′-d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

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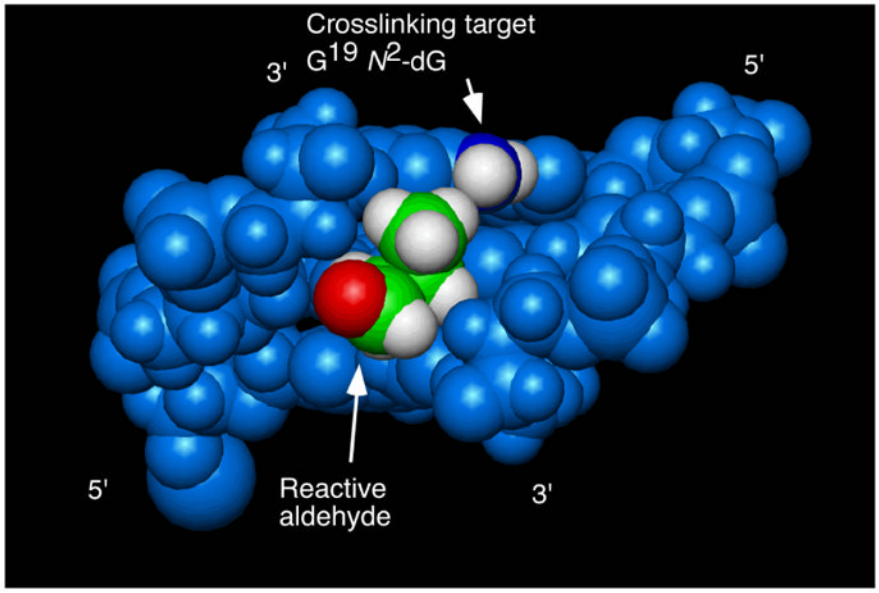

Figure 12.

Base pairs C^{6}•G^{19}, X^{7}•C^{18}, and A^{8}•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 ($G^{1}C^{2}T^{3}A^{4}G^{5}C^{6}X^{7}A^{8}G^{9}T^{10}C^{11}C^{12}$)-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}= N^{2}-(3-oxo-1*S*-methyl-propyl)-dG adduct **12**. Copyright American Chemical Society 2006.

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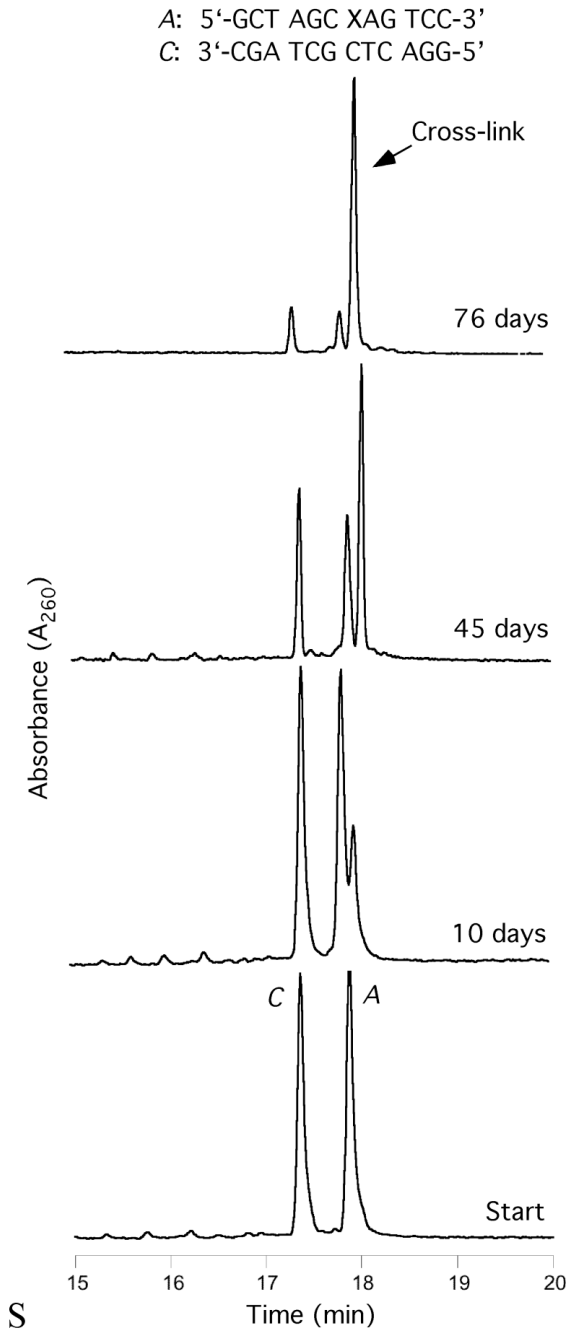

Figure 13.
Cross-linking of the (*6S,8R,11S*)-4-HNE-containing oligodeoxynucleotide.

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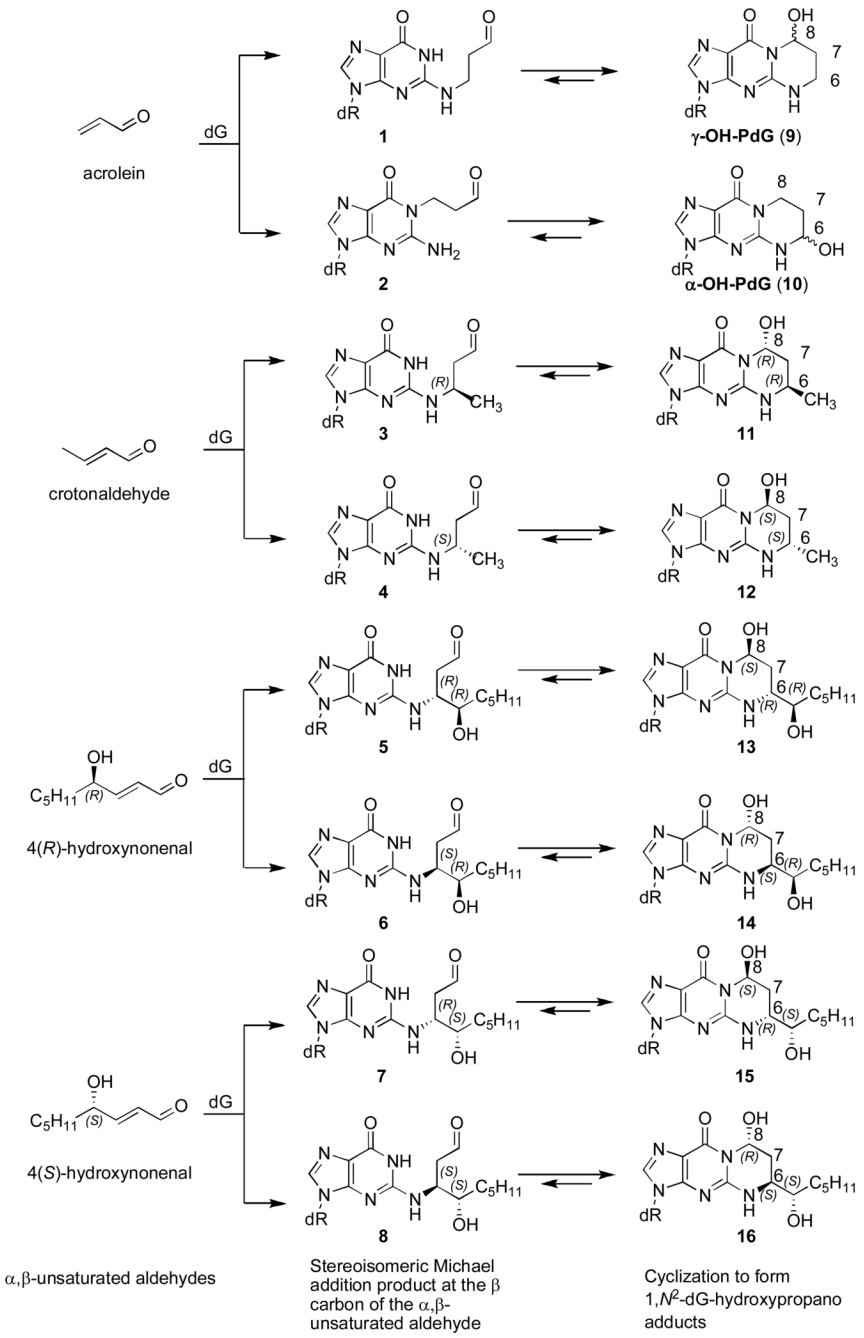

Scheme 1.
1,N^2-dG cyclic adducts arising from Michael addition of enals to dG.

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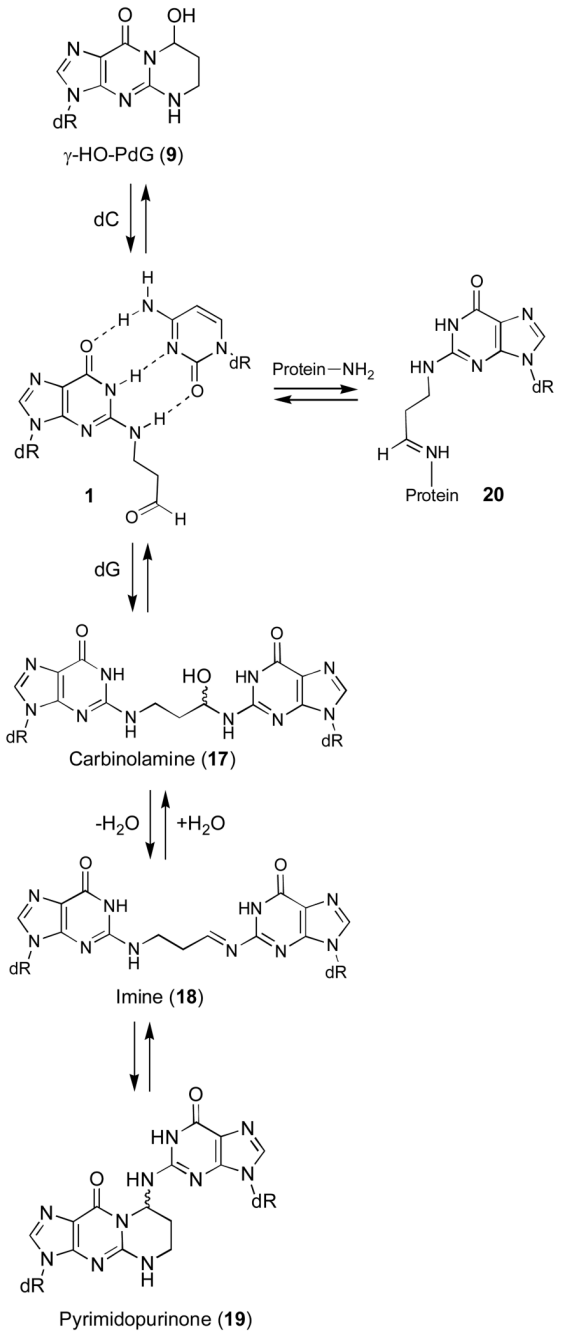

Scheme 2.
Enal-dG adducts mediate DNA interstrand cross-link formation.

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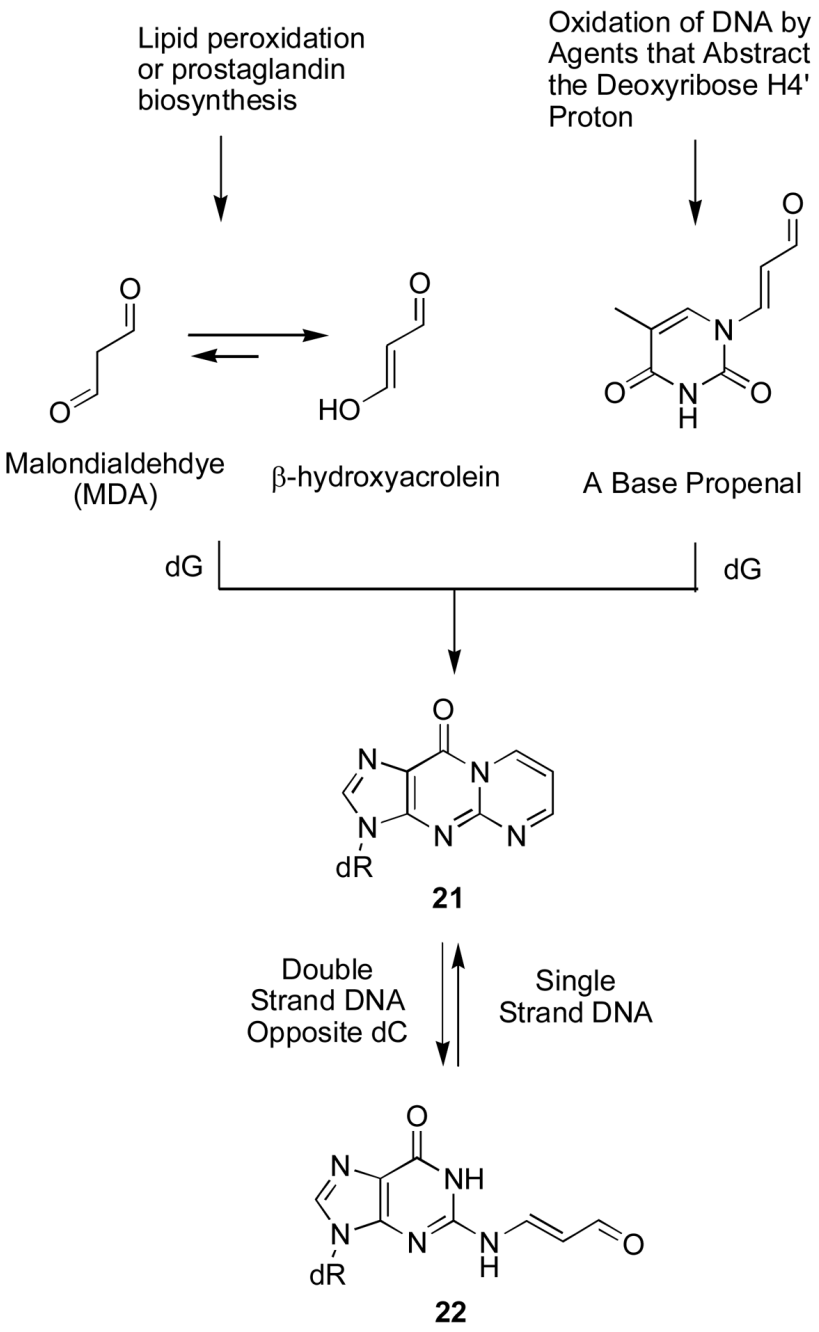

Scheme 3.
Formation and Chemistry of the Malondialdehyde-derived M_1dG Adduct.

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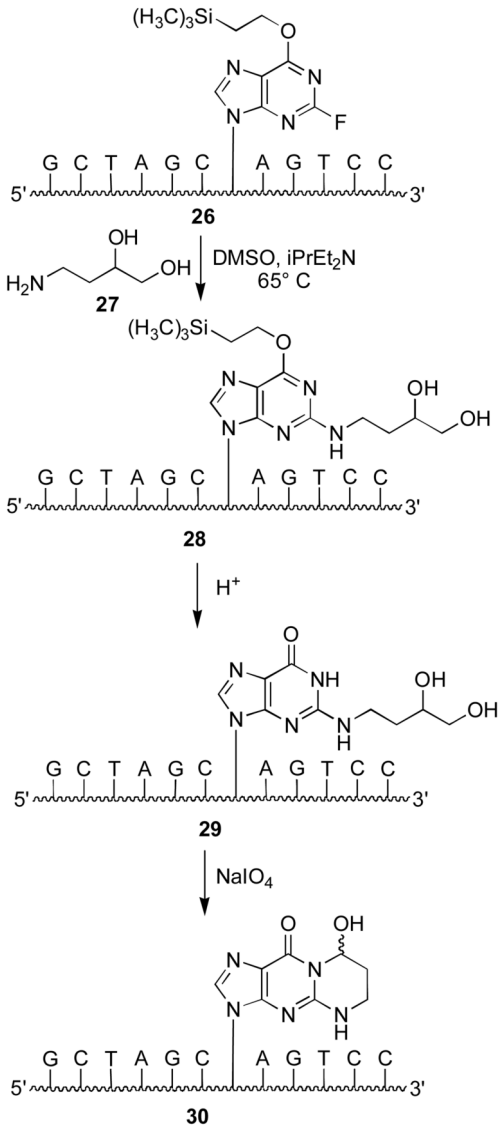
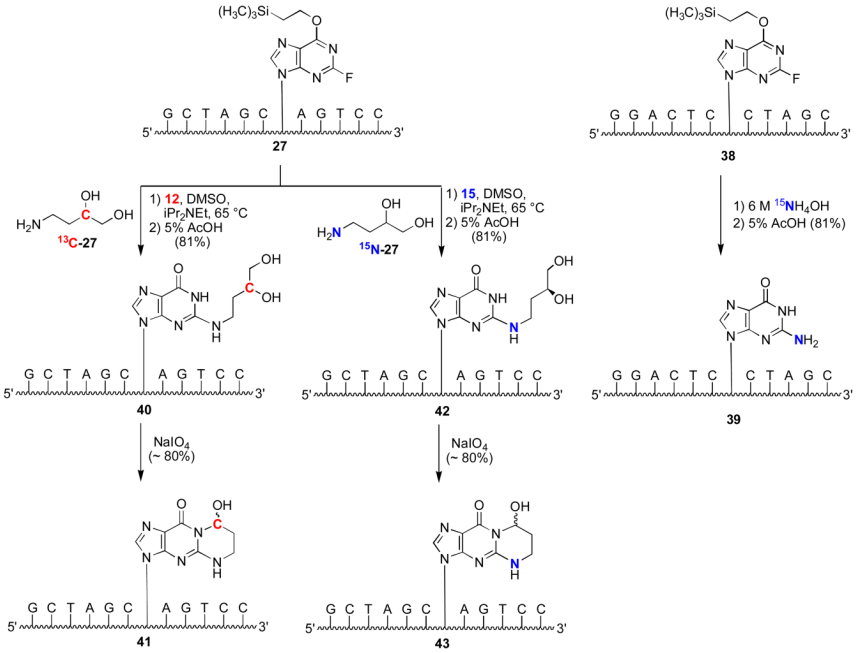


Scheme 4.

Synthesis of 1,N^2-γ-OH-PdG in oligodeoxynucleotides by the post-synthetic modification strategy.



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**).

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