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Site-specific synthesis of oligonucleotides containing malondialdehyde adducts of deoxyguanosine and deoxyadenosine via a post-synthetic modification strategy

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Abstract

Malondialdehyde (MDA) and its reactive equivalent, base propenal, are products of oxidative damage to lipids and DNA, respectively; they are mutagenic in bacterial and mammalian systems and MDA is carcinogenic in rats. MDA adducts of deoxyguanosine (M_1dG), deoxyadenosine (OPdA) and deoxycytidine (OPdC) have been characterized. We have developed site-specific syntheses of M_1dG and OPdA adducted oligonucleotides that rely on a post-synthetic modification strategy. This work provides an alternative route to the M_1dG adducted oligonucleotide and to date, the only viable strategy for the site-specific synthesis of OPdA modified oligonucleotides. The stability of the modified oligonucleotides was examined by UV thermal melting studies (T_m). In contrast to the M_1dG adduct, OPdA caused very little change in the T_m.

Introduction

Malondialdehyde (MDA, Figure 1) or reactive equivalents of MDA such as base propenals are produced endogenously through free radical degradation of polyunsaturated fatty acids and DNA or as a by-product of prostaglandin biosynthesis (1–3). MDA and base propenal react with DNA to form adducts of dGuo (M_1dG), dAdo (OPdA) and dCyd (OPdC). Recent studies in *E. coli* indicate that base propenal is likely to be the major endogenous source of M_1dG adducts (4–6). MDA and base propenal are bis-electrophiles and can undergo two reactions with nucleophilic groups of DNA or proteins. Such reactivity can lead to cyclic DNA adducts, DNA-DNA cross-links or DNA-protein cross-links. Recent results have shown that the dGuo adduct of acrolein, a lower oxidation state homologue of MDA, can mediate interstrand DNA-DNA and DNA-peptide cross-links (7–10).

MDA and base propenal are mutagenic in bacteria and mammalian cells and MDA is carcinogenic in rats (11–16). The miscoding properties of M_1dG have been evaluated with site-

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[1]Abbreviations: MDA, malondialdehyde; M_1dG, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrimido[1,2-*a*]purin-10(3*H*)-one; OPdG, N^2-(3-oxo-1-propenyl)-2′-deoxyguanosine; OPdA, N^6-(3-oxo-1-propenyl)-2′-deoxyadenosine; OPdC, N^4-(3-oxo-1-propenyl)-2′-deoxycytidine; PdG, 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4,6,7,8-tetrahydro-pyrimido[1,2-*a*]purin-10(3*H*)-one, THF, tetrahydrofuran; AcOH, acetic acid; DMSO, dimethylsulfoxide; iPr_2NEt, diisopropylethylamine CGE, capillary gel electrophoresis.

specifically adducted oligonucleotides (17). The major base-pair substitution mutation by M_1dG after replication in COS-7 cells and *E. coli* were G → T transversions (17,18). Of particular interest, M_1dG was shown to induce -1 and -2 frameshift mutations when incorporated in a reiterated $(CG)_4$ sequence (18).

The mutagenic potential of the other MDA adducts, namely OPdA and OPdC have not been evaluated by site-specific mutagenesis. Several lines of evidence indicate that these adducts contribute substantially to the overall mutagenic spectrum of MDA. For instance, in random mutagenesis experiments in SOS-induced *E. coli*, a significant proportion of A→G and C→T transitions were observed in addition to G→T transversions (19). Additionally, replication of an MDA-treated double-strand vector in human kidney cells gave 63% insertion or deletion mutations; the remaining base-pair substitution mutations were at CG base pairs (14). Although M_1dG has been shown to be a substrate for nucleotide excision repair (NER), the mutational frequency did not increase when NER deficient cells were used, suggesting that the deletions and insertions were caused by DNA lesions other than M_1dG.

MDA adducts of dAdo and dCyd are base-labile. In order for these adducts to be site-specifically incorporated into oligonucleotides, a strategy must be devised that avoids the strong alkaline conditions generally used for the deprotection step in solid-phase oligonucleotide synthesis. Building on previous synthetic efforts in our laboratory (20), we report here an alternative approach to the synthesis of M_1dG and OPdA-adducted oligonucleotides in which the modification is site-specifically incorporated after oligonucleotide assembly and deprotection, a synthetic approach that has been termed as a post-oligomerization or post-synthetic modification strategy (21–31).

Experimental Procedures

All commercially obtained chemicals were used as received. 4-Amino-3-phenylselanyl-1,2-butanediol (**1**) was synthesized as previous described (20). The reactions of oligonucleotides were carried out in plastic vials. MALDI-TOF mass spectra (negative ion) of modified oligonucleotides were obtained on a Voyager Elite DE instrument (Perseptive Biosystems) at the Vanderbilt Mass Spectrometry Resource Center using a 3-hydroxypicolinic acid (HPA) matrix containing ammonium hydrogen citrate (7 mg/mL) to suppress sodium and potassium adducts.

Synthesis of 5′-d(GCTAGC-(4)-AGTCC)-3′ (7)

Oligonucleotide **6** (10 A_{260} units) was mixed in a plastic test tube with diisopropylethylamine (50 μL), DMSO (100 μL), and **1** (1 mg, 3.8 mmol). The reaction mixture was stirred at 55 °C for 1 day. HPLC analysis showed complete conversion of the starting material (**6**). The solvents were removed *in vacuo* with a centrifugal evaporator. The residue was then dissolved in 5% acetic acid (500 μL) and the mixture stirred for 2 h at room temperature. The mixture was neutralized with 1 M NaOH and purified by reversed-phase HPLC (gradient A) to give modified oligonucleotide **7** (6.5 A_{260} units, ~65%). MS (MALDI-TOF) *m*/*z* calcd for 3888.5, found 3888.7. The presence of the modified base was confirmed by enzymatic hydrolysis of **7** and HPLC analysis of the resulting nucleosides using **4** as an authentic sample (20).

Synthesis of 5′-d(GCTAGC-(M_1dG)-AGTCC)-3′ (9)

A solution of $NaIO_4$ (20 mM) was added to a solution of oligonucleotide **7** (5.0 A_{260} units) in 0.5 % AcOH solution (300 μL), and the reaction mixture was stirred at room temperature for 7 h. The reaction resulted in a single product as observed by reversed phase HPLC (gradient A). Purification gave by reversed-phase HPLC (gradient A) gave M_1dG-modified oligonucleotide **9** (2.75 A_{260} units, ~55%). MS (MALDI-TOF) *m*/*z* calcd for 3680.6, found

3681.4. The presence of the modified base was confirmed by enzymatic hydrolysis of **9** and HPLC analysis of the resulting nucleosides using M_1dG as an authentic standard (20).

Synthesis of 5′-d(TCGTT-(5)-TTGCT)-3′ (11)

The oligonucleotide **10** (35 A_{260} units) was mixed in a plastic test tube with diisopropylethylamine (50 μL), DMSO (100 μL), and **1** (1 mg, 3.8 mmol). The reaction mixture was stirred at 65 °C for 1 day. HPLC analysis (gradient A) showed complete disappearance of **10**. The solution was directly purified by reversed-phase HPLC (gradient A) to give modified oligonucleotide **11** (29 A_{260} units, ~83%). MS (MALDI) *m*/*z* calcd for 3554.5, found 3553.6. The presence of the modified base was confirmed by enzymatic hydrolysis of **11** and HPLC analysis of the resulting nucleosides using **5** as an authentic standard.

Synthesis of 5′-d(TCGTT-(OPdA)-TTGCT)-3′ (12)

A solution of $NaIO_4$ (40 mM) was added to a solution of oligonucleotide **11** (29 A_{260} units) in 0.2 % acetic acid solution (400 μL), and the reaction mixture was stirred at room temperature over 10 h. The reaction mixture was purified directly by reversed-phase HPLC (Gradient A) to give M_1A-modified oligonucleotide **12** (9.4 A_{260} units, ~32%). MS (MALDI-TOF) *m*/*z* calcd for 3365.6, found 3366.8. The presence of the OPdA modification was confirmed by enzymatic digestion of **12** and HPLC analysis of the nucleosides with detection at both 254 and 320 nm and comparison to an authentic standard of OPdA (20).

Synthesis of 5′-d(CAGTC-OPdA-CTAGA)-3′ (13)

Oligonucleotide **13** (5.5 A_{260} units, ~24 %) was prepared from the corresponding oligonucleotide containing the 6-chloropurine base (23 A_{260} units) following the procedure described for the synthesis of oligonucleotides **11** and **12** with the following modification: the initial adduction reaction with **1** was conducted at 55 °C for 10 h. MS (MALDI-TOF) *m*/*z* calcd for 3377.6, found 3377.5.

Synthesis of 5′-d(CAGTG-OPdA-GTACA)-3′ (14)

Oligonucleotide **14** (6.3 A_{260} units, ~18 %) was prepared from the corresponding oligonucleotide containing the 6-chloropurine base (35 A_{260} units) following the procedure described for the synthesis of oligonucleotides **11** and **12** with the following modification: the initial adduction reaction with **1** was conducted at 55 °C for 10 h. MS (MALDI-TOF) *m*/*z* calcd for 3417.7, found 3418.6.

Synthesis of 5′-d(GCAAAAA-OPdA-AAAACATGG)-3′ (15)

The corresponding oligonucleotide containing the 6-chloropurine base (21.4 A_{260} units) was mixed in a plastic test tube with diisopropylethylamine (80 μL), DMSO (150 μL), and **1** (2 mg, 7.6 mmol). The reaction mixture was stirred at 55 °C for 24 h. An additional portion of **1** (2 mg, in 50 μL DMSO) was added and the reaction stirred at 55 °C for an additional 24 h. The solvents were removed *in vacuo* with a centrifugal evaporator and the residue dissolved in 500 μL of water. Purification by reversed-phase HPLC (gradient B) gave the corresponding N^6-(2-phenylselanyl-3,4-butanediol)-dAdo adducted oligonucleotide (11.5 A_{260} units, ~53.7%). MS (MALDI-TOF) *m*/*z* calcd for $[M - H]^-$ 5494.08, found 5493.5

An aqueous solution of $NaIO_4$ (20 mM) was added to a solution of modified oligonucleotide (11.5 A_{260} units) in 0.05 M, pH 7.0 phosphate buffer, (500 μL) and the reaction mixture was stirred at room temperature for 10 min. The mixture was purified directly by HPLC (gradient C) to give oligonucleotide **15** (2.7 A_{260} units, 23.5%). MS (MALDI-TOF) *m*/*z* calcd for $[M - H]^-$ 5304.9, found 5306.4. The presence of the modified base was confirmed by enzymatic

hydrolysis of **15** and HPLC analysis of the resulting nucleosides using OPdA as an authentic standard (20).

Oligonucleotide Purification

The purification of oligonucleotides were performed on a Beckman HPLC system (32 Karat software version 3.1, pump module 125) with a diode array UV detector (module 168) monitoring at 260 nm using Phenomenex Luna 5μ C8 column (250 mm × 10 mm i.d., 3 mL/min for purification) with 0.1 M aqueous ammonium formate and acetonitrile. *HPLC gradients*:

A. initially 1% acetonitrile, 15 min linear gradient to 10 % acetonitrile, 5 min linear gradient to 20 % acetonitrile, 5 min isocratic at 20% acetontrile, 3 min linear gradient to 100 % acetonitrile, 4 min isocratic in 10 0% acetonitrile followed by 3 min linear gradient to initial conditions.

B. initially 1% acetonitrile, 5 min linear gradient to 5 % acetonitrile, 25 min linear gradient to % acetonitrile, 3 min linear gradient to 99% acetonitrile, isocratic at 99 % acetonitrile for 6 min, and 3 min linear gradient to the initial conditions, isocratic at 1 % acetonitrile for 3 min.

C. initially 1% acetonitrile, 5 min linear gradient to 5 % acetonitrile, 20 min linear gradient to 5.7 % acetonitrile, 3 min linear gradient to 99% acetonitrile, isocratic at 99% acetonitrile for 5 min, and then a 3 min linear gradient to the initial conditions, isocratic at 1% acetonitrile for 2 min.

Melting temperature determinations (T_m)

Melting temperatures of modified and unmodified duplexes were determined as previously described (32).

Enzyme digestion

Enzyme digestions were preformed as previously described (33). Reversed phase HPLC analysis of the enzyme digestion reactions were performed using gradient A. Modified oligonucleotides were identified by comparison of the HPLC retention time and diode array UV spectrum with those of authentic standards, which were synthesized as previously described (20).

Sequencing of oligonucleotide 15

The oligonucleotide (0.03 OD) in 20 μL of ammonium citrate buffer (40 mM, pH 9.4) containing 20 mM $MgSO_4$ was incubated at 37 °C with 2 milliunits of phosphodiesterase I. Aliquots (4 μL) were removed after 0, 10, 20, 30 and 40 min and frozen at −20 °C. The aliquots were combined and desalted using a Millipore C_{18} ZipTip. The solution is then used for MALDI-TOF analysis. An identical analysis was performed using phosphodiesterase II.

Crosslinking reaction of oligonucleotide 9

The ability of M_1dG-modified oligonucleotide **9** to form an interstrand crosslink was evaluated using a previously established protocol (8,9,33).

Results and Discussion

Synthesis of M_1dG and OPdA adducted oligonucleotides

We previously described syntheses of MDA adducts M_1dG, OPdA, and OPdC (Figure 1) at the nucleoside level (20). The synthetic approach contained the elements required for the

synthesis of site-specifically adducted oligonucleotides via a post-oligomerization strategy. Although less common than the adducted-phosphoramidite strategy, the post-oligomerization strategy is ideally suited for alkaline-labile adducts that would not survive the strongly basic conditions used for oligonucleotide deprotection (9). The "activated" nucleosides **2** and **3** required for the synthesis of the dGuo and dAdo adducts (Scheme 1), respectively, were reacted with an MDA synthon (**1**) via a nucleophilic aromatic substitution reaction to give the modified nucleosides **4** and **5**. Since **1** was synthesized as a mixture of stereoisomers, the products of this reaction were a mixture of four diastereomers. Oxidation with sodium periodate cleaved the diol to give the corresponding aldehyde. Periodate also oxidized the phenyl selenide to the corresponding selenoxide, which spontaneously eliminated to give the MDA-adducted nucleosides M_1dG and OPdA. In the case of the dGuo adduct, the N^2-3-oxopropenyl group undergoes dehydrative cyclization to give the M_1dG nucleoside. NMR and UV analysis showed that the dAdo adduct of MDA existed in the open-chain N^6-3-oxopropenyl form.

Phosphoramidite reagents containing the required "activated" O^6-(2-(trimethylsilylethyl)-2-fluorohypoxanthine (**2**) and 6-chloropurine (**3**) bases have been previously synthesized and incorporated in oligonucleotides via standard solid-phase methods (25,34–36). The site-specific synthesis of an M_1dG-adducted oligonucleotide is shown in Scheme 2. Reaction of amine **1** with oligonucleotide **6** containing activated base **2** gave the modified oligonucleotide **7**. Upon treatment of **7** with excess sodium periodate, we initially observed two products (**8** and **9**) with one being converted to the other over time (Panel B, Figure 2). The UV spectrum of the transient intermediate, as observed via a diode array detector during HPLC analysis, possessed a long wavelength absorbance suggesting its identity to be the open chain N^2-(3-oxopropenyl) derivative of deoxyguanosine (Panel B, Figure 2). It has been demonstrated that the open chain form undergoes isomerization of the trans-double bond, ring closure and dehydration to M_1dG; the dehydration step occurs under general acid catalysis (37,38). When the periodate oxidation of **7** was carried out in 5 % acetic acid, conversion of **8** to **9** was faster. Oligonucleotides **7** and **9** were characterized by MALDI-TOF mass spectrometry (Table 1) and enzymatic digestion (Figure 3). The modified nucleosides were observed upon HPLC analysis of the enzymatic digestion reaction.

The synthesis of an oligonucleotide site-specifically modified with a dAdo adduct of MDA (OPdA) was achieved according to Scheme 3 starting from oligonucleotide **10** containing activated base **3**. Nucleophilic aromatic substitution of **10** with amine **1** gave oligonucleotide **11**. For the synthesis of the OPdA nucleoside we found that excess periodate gave unidentified by-products and the best yields were obtained with stoichiometric oxidant in the presence of trace acetic acid, although the role of the acetic acid was unclear. However, the oxidation of oligonucleotide **11** was very inefficient under these conditions. We therefore used excess $NaIO_4$ for the conversion of **11** to **12**; although by-products were observed for this reaction, they appeared to be less problematic than at the nucleoside level. MDA-adducted oligonucleotide **12** possessed a strong UV absorbance at ~320 nm, which is characteristic of the open-chain OPdA moiety (39). This oligonucleotide was further characterized by MALDI-TOF mass spectrometry (see supporting information) and enzymatic digestion (Figure 3, panel B), which showed the presence of the desired modified nucleoside.

Three other oligonucleotides that were site-specifically modified with OPdA (**13–15**) were synthesized by an identical strategy and are listed in Table 3 along with their thermal melting temperatures (T_m). Of note is oligonucleotide **15**, which possesses an OPdA adduct in a run of ten dAdo's. Reiterated sequences are prone to frameshift mutations, which are important contributors to human cancer, particularly among individuals with inherited defects in mismatch repair genes (40). There are numerous genes with long runs of dAdo's ($\geq dAdo_8$) in their coding regions that are mutated in individuals with compromised mismatch repair. For instance, the coding regions for *TGFβRII*, *AIM2*, *Caspase-5*, and *SEC63* all contain runs of ten

dAdo's. It has also been demonstrated that oxidative stress can stimulate frameshift mutations in reiterated sequences (41,42). This opens the possibility that DNA modification by MDA and related bis-electrophiles could lead to frameshift mutations.

Oligonucleotide **15** was initially characterized by MALDI-TOF mass spectrometry and enzymatic digestion (see supporting information). To confirm that OPdA modification was in the desired location, **15** was sequenced via controlled enzymatic digestion and MALDI-TOF mass spectrometry (43). The oligonucleotide was separately digested with phosphodiesterase I (snake venom phosphodiesterase) and II (bovine spleen phosphodiesterase). Aliquots were taken at various time points and initially frozen; they were then combined for MALDI-TOF analysis, which provided a mass ladder corresponding to the sequential loss of nucleotides from either the 3′- or 5′-end. Since each nucleotide has a unique mass, the sequence of the adducted oligonucleotide could be readily determined. Figure 5 shows the MALDI-TOF analysis from controlled digestion of **15** (*m/z* 5305.97 Da) with phosphodiesterase I, which possesses 3′→5′ exonuclease activity. A complementary analysis with phosphodiesterase II allowed for sequencing of the oligonucleotide from the 5′-direction and thus provided the sequence of the entire oligonucleotide (see supporting information).

Melting (T_m) studies of M_1dG and OPdA adducted oligonucleotides

The thermal melting temperatures of the MDA-modified oligonucleotides are given in Tables 2 and 3. The M_1dG-containing oligonucleotide **9** is significantly destabilized and its T_m is nineteen degrees lower than that of the unadducted oligonucleotide (Table 2). We have previously examined a number of related dGuo adducts in this sequence, including both regioisomers of the acrolein adduct (**18** and **19**) and the 1,N^2-etheno adduct (**20**) (32). When this sequence contained the major acrolein adduct of dGuo, 8-hydroxy-3,5,6,7-tetrahydro-9*H*-imidazo[1,2-*a*]purine-9-one (**18**), the T_m was twelve degrees lower than unadducted. NMR studies have demonstrated that both M_1dG and the major acrolein adduct exist in the ring-opened N^2-3-oxopropenyl (**21**, OPdG) and N^2-3-oxopropyl (**22**) forms (Scheme 4), respectively when paired with a complementary dCyd (44–46). The difference of seven degrees in T_m's between these adducted oligonucleotides is somewhat surprising given the structural similarities of the open chain forms. One explanation is that ring closure of the OPdG (**21**) is more facile than for **22** and ring closure to M_1dG promotes denaturation of the duplex (38). The 1,N^2-propano-dGuo (PdG, **16**) adduct is a saturated analog of M_1dG but cannot undergo the ring-opening chemistry. NMR studies have shown the PdG base to be in a syn comformation and involved in Hoogsteen pairing with a complementary Cyt (47–49). The T_m for the PdG adduct was identical to M_1dG (Table 2). An alternative explanation for the large destabilization caused by M_1dG is that it actually exists in the ring-closed form with a syn geometry, similar to the PdG-adduct. However, the nineteen degree destabilization for the duplex of **9** is in the same range as previously reported for an M_1dG-modified oligonucleotide containing the *hisD3052* gene sequence, which melted fourteen degree lower than the unmodified oligonucleotide (50,51). In this case, NMR analysis showed that the adduct was in the open N^2-3-oxopropenyl form. The *hisD3052* oligonucleotide duplex containing the PdG adduct melted eleven degrees lower than the corresponding M_1dG-containing oligonucleotide (50,51).

The regioisomeric acrolein adduct, 6-hydroxy-3,5,6,7-tetrahydro-9*H*-imidazo[1,2-*a*]purine-9-one (**19**), also caused the T_m to be lower by 19° compared to unadducted and is the same as for the M_1G adduct. There is little driving force for ring opening of this regioisomer since this would yield an N1-3-oxopropyl-Gua base, which would also severely interfere with Watson-Crick base pairing. NMR studies have shown this regioisomer exists in the ring-closed form in duplex DNA with a syn conformation about the glycosidic bond like that of the PdG adduct (52). The 1,N^2-etheno-dGuo adduct (**20**) and partially reduced M_1dG adduct **17** show similar

T_m values as the M_1dG adduct and also are unable to undergo ring opening. Structural studies of oligonucleotides containing **20** and **17** have not been reported to date.

We observed that the OPdA adduct had little effect on the thermal stability of the oligonucleotide and actually caused a modest stabilization in most cases, a significant contrast to the M_1dG adduct (Table 3). The OPdA-containing oligonucleotide **15** was also hybridized to complements in which one or two dThd's across from the adduct were deleted to mimic potential one- and two-base deletion products (Table 3). These duplexes were less stable than those with a full-length complement by 4° and 7° C, respectively, but were slightly more stable than when the corresponding unadducted oligonucleotide was hybridized to a complement containing a one- or two-base deletion. The modest destabilization due to the N^6-(3-oxopropenyl) group can be attributed to its relatively small size and that it can adopt a conformation that would not interfere with Watson-Crick hydrogen bonding to a complimentary dThd (Figure 6). The effect of the N^6-(2,3,4-trihydroxybutyl)-dAdo adduct derived from butadiene diepoxide, on the thermal stability of an oligonucleotide duplex has also been reported to be modest; the T_m's were 5–8° C lower than unmodified depending on the stereochemistry of the adduct (53,54). The modest destabilization as measured by T_m might suggest that the OPdA adduct causes minimal local distortion of the double helix and therefore may be repaired less efficiently than M_1dG.

Interstrand cross-linking of the M_1dG adducted oligonucleotides

M_1dG has been shown to undergo a ring-opening reaction to OPdG in duplex DNA and the mechanism of this process has been studied in detail (16,37,44,51). It is likely that the ring-opening chemistry is important in defining the biological processing of this lesion. As such, M_1dG can be viewed as a reactive intermediate within DNA. It had been hypothesized that OPdG could form DNA cross-links and such cross-links contribute to the genotoxicity of MDA (14,55). We have previously examined the ability of the 1,N^2-dGuo adduct of acrolein (**18**) as well as the higher congeners crotonaldehyde and 4-hydroxynonenal, to form interstrand DNA cross-links in the identical sequence context as oligonucleotide **9** (8,9,33). Like M_1dG, the acrolein adduct undergoes a ring-opening reaction in duplex DNA when paired opposite a dCyd (45,46). The acrolein adduct is a lower oxidation state homologue of the MDA adduct and the structural similarities between the two can be most readily seen in the ring-opened form (Scheme 4). The interstrand DNA cross-linking reaction for the acrolein, crotonaldhyde and HNE adducts were followed and quantified by capillary gel electrophoresis (CGE) with UV detection, and the acrolein and crotonaldehyde DNA cross-links were recently been characterized by NMR (56,57). When the OPdG-modified oligonucleotide **9** was subjected to our cross-linking reaction conditions, we observed no cross-link formation by our previously established assay. It should be noted that the acrolein, crotonaldehyde and HNE cross-linked oligonucleotides had high melting temperatures (> 90° C), which certainly aided in their detection by CGE. Therefore it is possible that **9** formed a transient interstrand cross-links that was labile under the denaturing conditions of the CGE analysis. Alternatively, these results may indicate that the MDA adduct of dGuo is not involved in DNA-DNA crosslink formation and suggests that the OPdA and OPdC adducts are the relevant crosslinking lesions.

Summary

We have developed site-specific syntheses of oligonucleotides that contain the MDA adducts M_1dG and OPdA using a post-synthetic modification strategy. This involved the reaction of MDA synthon **1** with oligonucleotides containing either the O^6-(2-trimethylsilylethyl)-2-fluorohypoxanthine or 6-chloropurine activated base followed by periodate oxidation (Schemes 2 and 3). Thermal melting (T_m) studies indicated that M_1dG caused significant destabilization of the DNA duplex, whereas the OPdA had little effect on the thermal stability. M_1dG-modified oligonucleotides were previously synthesized using the adducted

phosphoramidite approach; however, the synthesis of the M_1dG nucleoside suffers from low overall yield and difficult purification. The synthesis outlined in this work is likely to improve the availability of M_1dG-adducted oligonucleotides and is to date the only viable route to OPdA-adducted oligonucleotides. This will allow the assessment of the relative contribution of the OPdA adduct to the genotoxicity of MDA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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positioned in a $(CpG)_3$ frameshift hotspot of the *Salmonella typhimurium* hisD3052 gene. Biochemistry 1999;38:13491–13501. [PubMed: 10521256]

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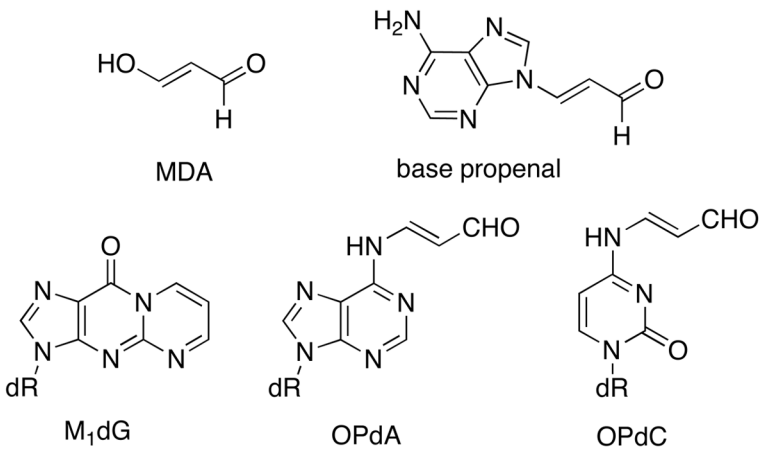


Figure 1.
Structures of NMD, base propenal and their nucleoside adducts

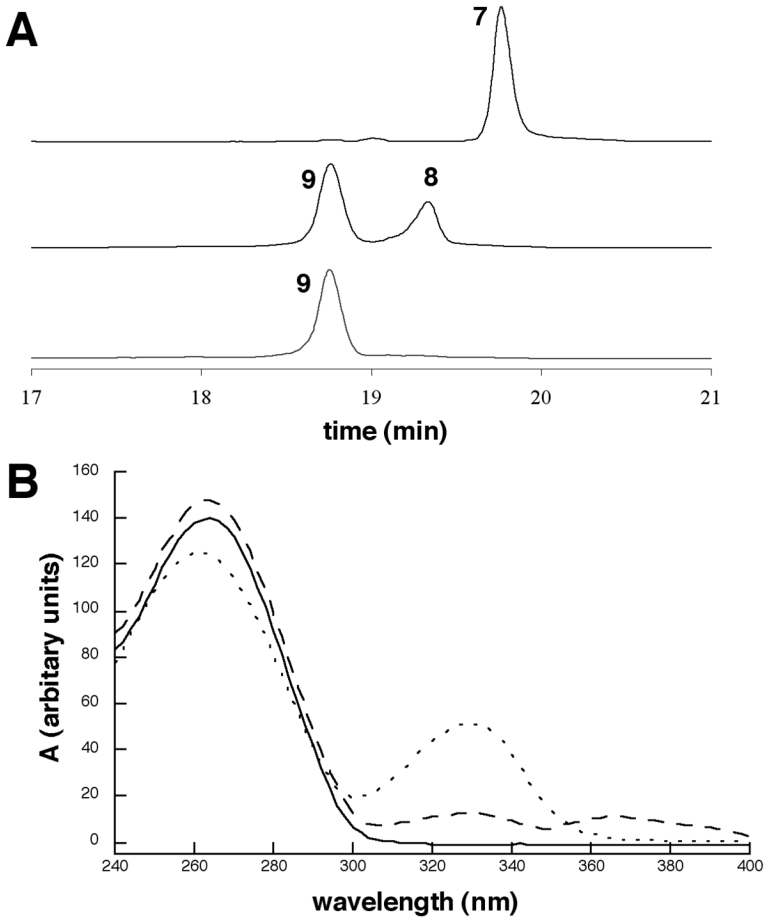


Figure 2.

A. HPLC trace of the conversion of oligonucleotide **7** to **9** upon periodate treatment. The middle trace is the periodate cleavage reaction of **7** after 8 h. The bottom trace is the periodate cleavage of **7** in 5% acetic acid solution after 7 h. **B**. UV spectra of oligonucleotides **7** (——), **8**, (- - - -), and **9** (— — —) as obtained by a diode array detector during HPLC analysis.

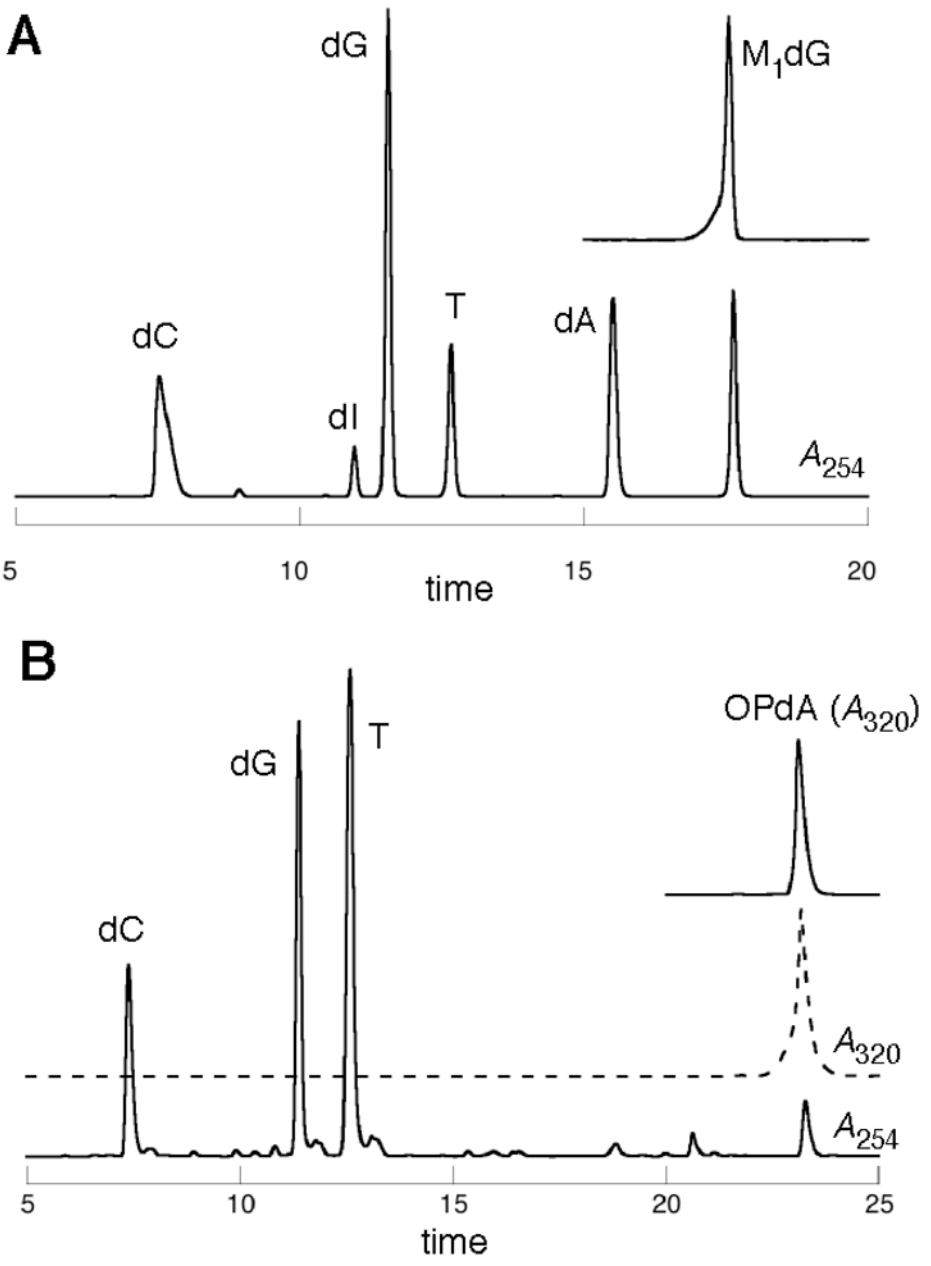


Figure 3.
HPLC analysis of the enzymatic digestion of modified oligonucleotides. **A**. oligonucleotide **9** with detection at 254 (——) and 320 (- - - -) nM and a with a M_1dG standard; B. oligonucleotide **12** OPdA standard (320 nM).

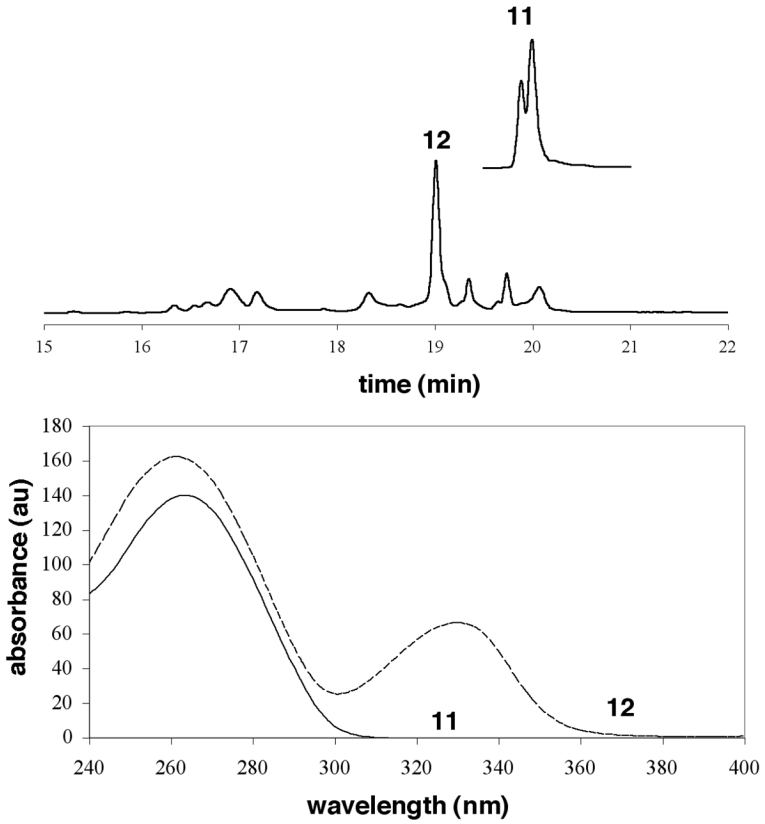


Figure 4.
Panel A: HPLC trace for the conversion of oligonucleotide **11** to **12** upon periodate treatment.
Panel B: Comparison of the UV spectra of oligonucleotides **11** and **12**.

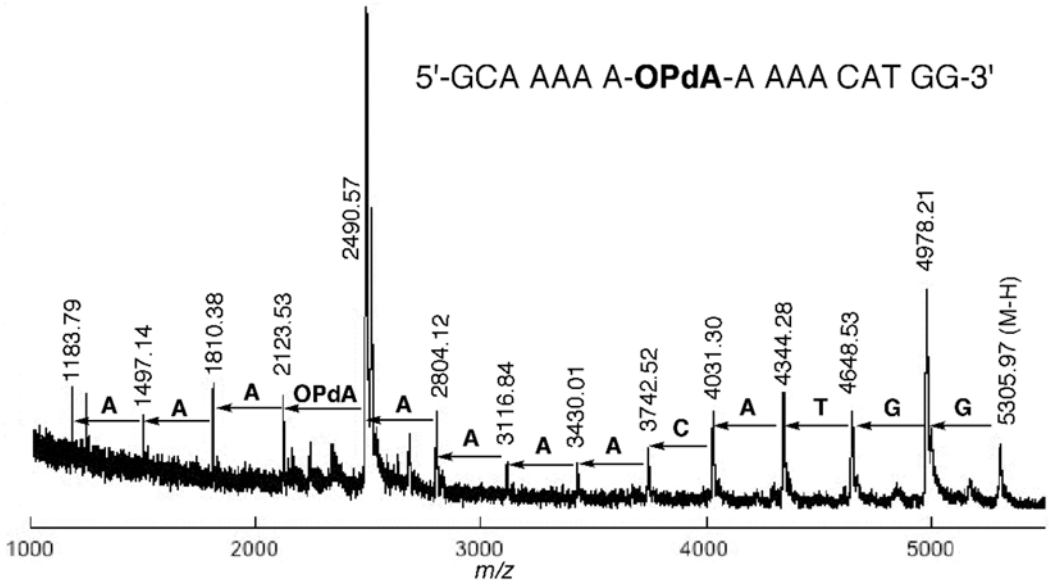


Figure 5.
Sequencing of oligonucleotide **15** by partial digestion with phosphodiesterase I (snake venom phosphodiesterase) and MALDI-TOF mass spectrometry.

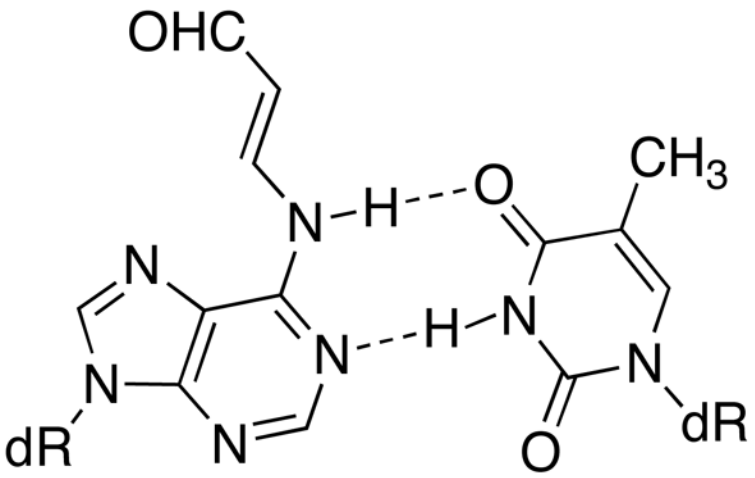
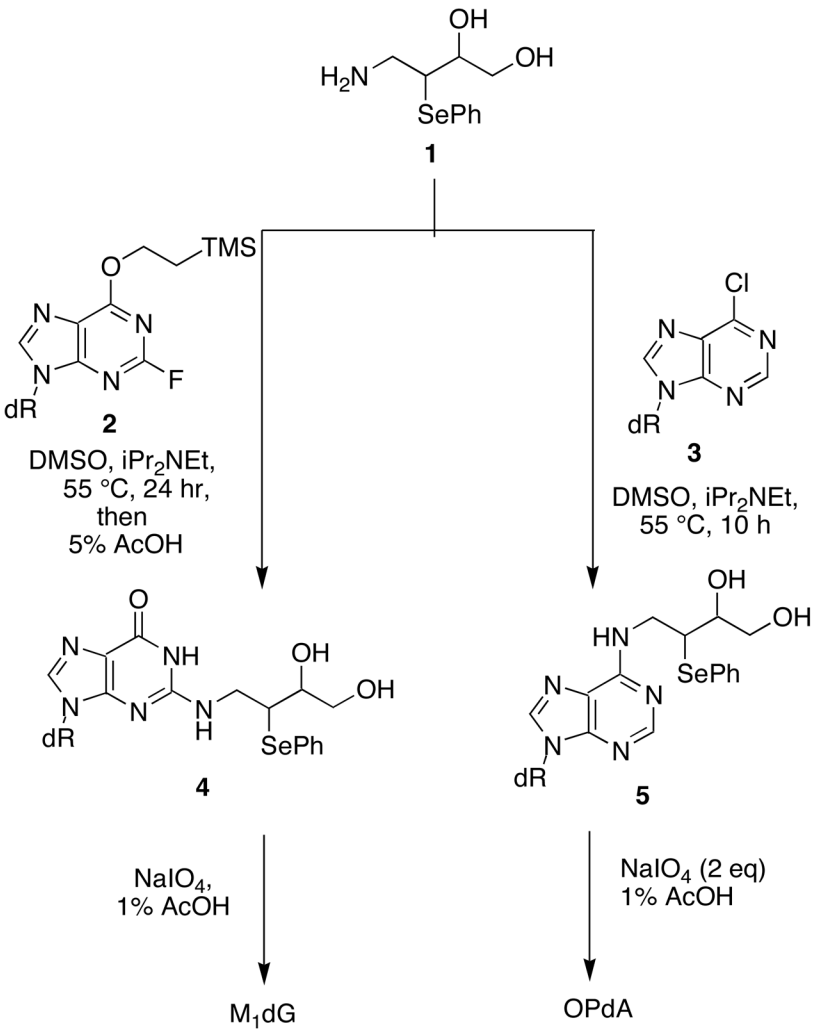
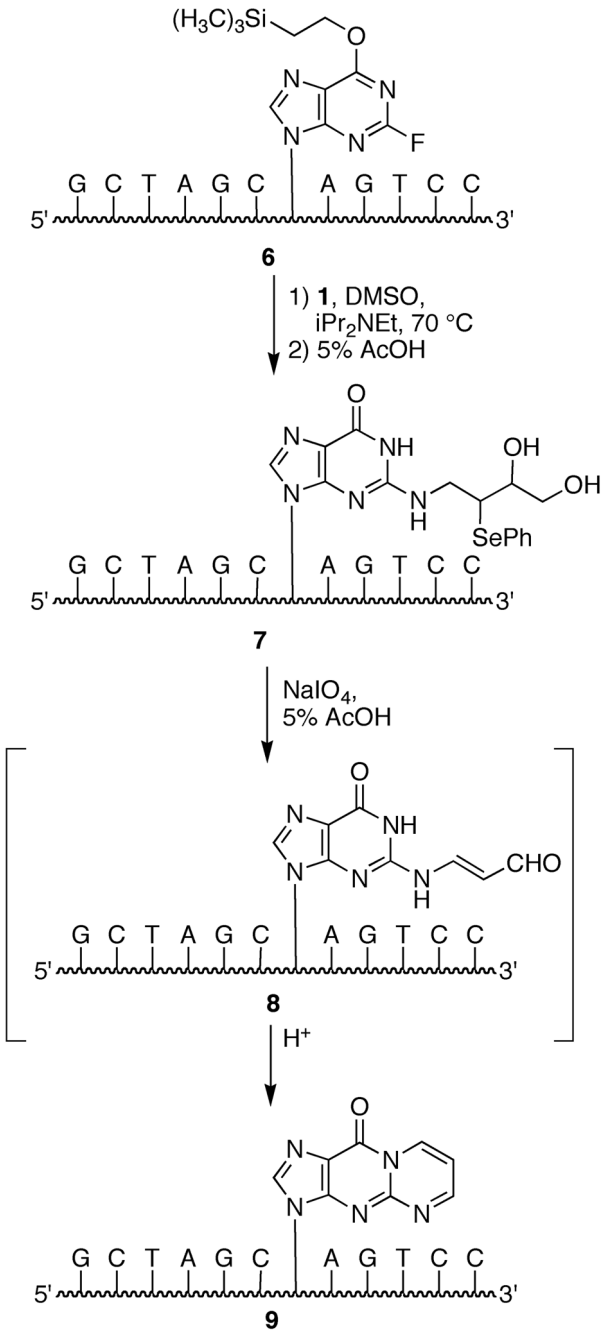


Figure 6.
Conformation of OPdA that does not interfere with Watson-Crick hydrogen bonding.

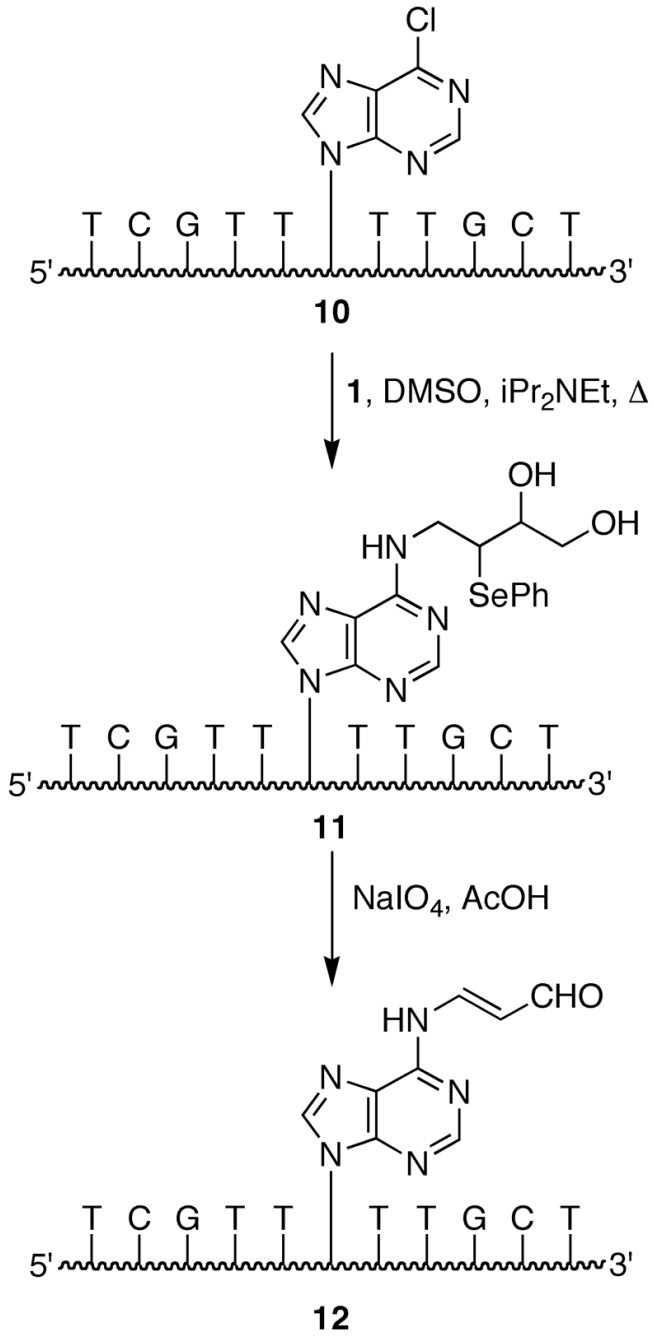


Scheme 1.
Non-biomimetic synthesis of M_1dG and OPdA.

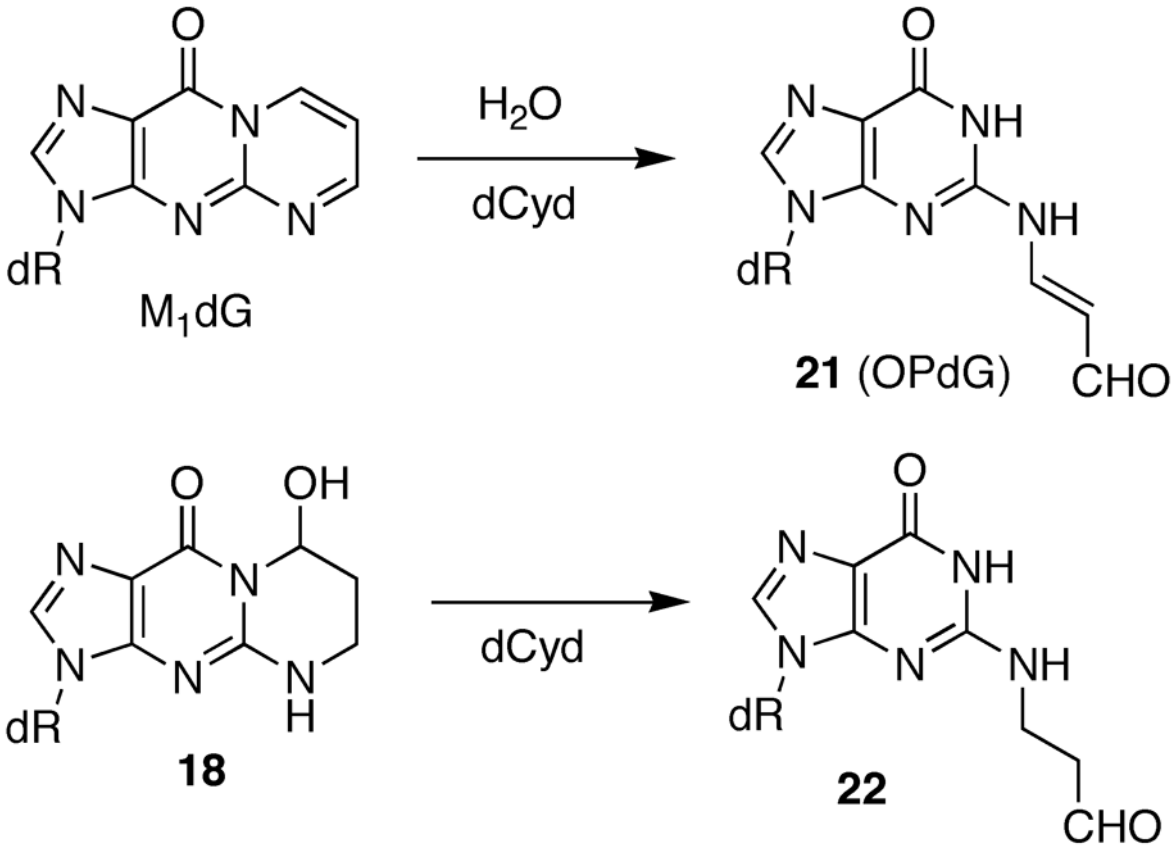


Scheme 2.

Synthesis of an M_1dG-containing oligonucleotide using a post-synthetic modification strategy.



Scheme 3.
Synthesis of an OPdA-containing oligonucleotide using a post-synthetic modification strategy.



Scheme 4.
Structural similarities between the MDA and acrolein adducts of deoxyguanosine.

Table 1

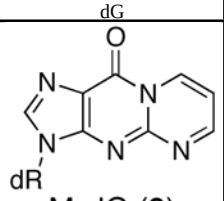
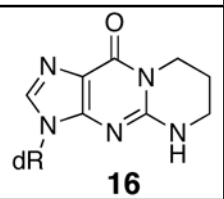
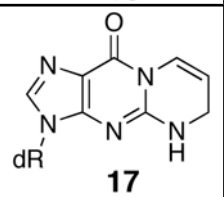
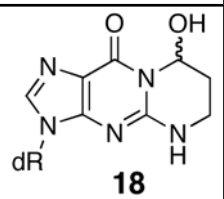
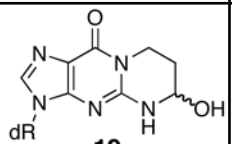
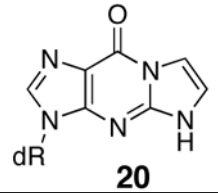
M_1dG- and OPdA-modified oligonucleotides synthesized in this studied and their mass spectral characterization.

Oligonucleotide	*m/z* (da)	
	calcd	observed
5′-GCTAGC-**M_1dG**-AGTCC-3′ (**9**)	3680.6	3681.4
5′-TCGTT-**OPdA**-TTGCT-3′ (**12**)	3365.6	3366.8
5′-CAGTC-**OPdA**-CTAGA-3′ (**13**)	3377.6	3377.5
5′-CAGTG-**OPdA**-GTAGA-3′ (**14**)	3417.7	3418.6
5′-GCAAAAA-**OPdA**-AAAACATGG-3′ (**15**)	5304.9	5306.4

Table 2

T

m of M_1dG and structurally related dGuo adducts in duplex 5′-GCT AGC **X**AG TCC-3′ • 5′-GGACTCGCTACG-3′[a]

X=	T_m	ΔT_m
dG	59 °C	
 M_1dG (**9**)	40 °C[b]	−19 °C
 **16**	40 °C[b]	−19 °C
 **17**	43° C[b]	−17 °C
 **18**	47 °C[c]	−12 °C
 **19**	40 °C[c]	−19 °C
 **20**	41 °C[c]	−18 °C

[a] Conditions for T_m: 100 mM NaCl, 10 mM pH 7 sodium phosphate, 50 μM EDTA, 0.5 A_{260}/mL of each oligonucleotide. The temperature was raised 1° C per minute

[b] This work

[c] reference (32)

Table 3

Thermal melting temperatures (T_m) of OPdA-modified oligonucleotides[a]

Oligonucleotide	T_m (unmodified)	ΔT_m
5′-TCGTT-OPdA-TTGCT-3′ (12) 5′-AGCAA——T——AACGA-5′	46° C (43° C)	3° C
5′-CAGTC-OpdA-CTAGA-3′ (13) 3′-GTCAG T——GATCT-5′	44° C (44° C)	0° C
5′-CAGTG-OPdA-GTAGA-3′ (14) 3′-GTCAC T——CATCT-5′	46° C (45° C)	1° C
5′-GCAAAAA-OPdA-AAAACATGG-3′ (15) 3′-CGTTTTT——T——TTTTGTACC-5′	55° C (56° C)	−1° C
5′-GCAAAAA-OPdA-AAAACATGG-3′ (15) 3′-CGTTTTT————TTTTGTACC-5′	51° C (50° C)	1° C
5′-GCAAAAA-OPdA-AAAACATGG-3′ (15) 3′-CGTTTTT————TTTGTACC-5′	48° C (47° C)	1° C

[a]Conditions: 100 mM NaCl, 10 mM, pH 7 sodium phosphate, 50 μM EDTA, 0.5 A_{260}/mL of each oligonucleotide. The temperature was raised 1° C/min.