Synthesis of Oligonucleotides Containing the Alkali-Labile Pyrimidopurinone Adduct, M_1G

Nathalie C. Schnetz-Boutaud,[†] Hui Mao,[‡] Michael P. Stone,[‡] and Lawrence J. Marnett*[,†,‡]

Departments of Biochemistry and Chemistry, Center in Molecular Toxicology, and Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee 37235

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An improved method for the synthesis of oligodeoxyribonucleotides containing the endogenous adduct, pyrimido[1,2-*a*]purin-10(3*H*)-one (M_1G), is reported. The key features of the methodology include improved synthesis of the deoxynucleoside of M_1G by transribosylation with deoxycytidine catalyzed by nucleoside 2′-deoxyribosyltransferase and the use of commercially available 4-*tert*-butylphenoxyacetyl protecting groups for normal nucleotides. Facile deprotection and removal of the M_1G-containing oligomers from the solid support were achieved by treatment with a solution of potassium carbonate in methanol. NMR studies were performed to determine the stability of the oligonucleotides at different pHs.

Introduction

The synthesis of oligonucleotides containing structurally defined adducts of carcinogens, drugs, and other xenobiotics is critical to an evaluation of the structural and genetic effects of DNA damage. Most synthetic approaches to them include direct reactions of electrophiles with oligonucleotides (*1*, *2*) or assembly of oligonucleotides from adduct-modified nucleosides (*3*−*6*). Our laboratory is interested in the chemistry and biology of DNA damage by the endogenous mutagen malondialdehyde (MDA)[1] (*7*−*9*). MDA is formed enzymatically as a byproduct of arachidonic acid metabolism, as well as nonenzymatically during lipid peroxidation, and it reacts with deoxynucleosides to produce a variety of adducts (*10*−*14*). The most abundant adduct at physiological pH is 3-(2′-deoxy-*β*-D-*erythro*-pentofuranosyl)pyrimido[1,2-*a*]-purin-10(3*H*)-one (M_1G-dR, **1**). The existence of high levels of M_1G adducts in human tissues places a high priority on studies of its mutagenic potential and repair (*15*−*18*).

The instability of M_1G-dR under the strongly alkaline conditions typically used to deprotect synthetic oligodeoxynucleotides was a major hurdle to its site-specific incorporation into appropriate vectors (see Scheme 1). Previous studies in our laboratory resulted in the first successful synthesis of an oligonucleotide containing M_1G by using the 2-(acetoxymethyl)benzoyl (AMB) protecting group on the free amino moieties of dG, dC, and dA (*6*). AMB protecting groups are removable from nucleosides by treatment with potassium carbonate (*19*), and M_1G-dR proved to be stable under these conditions (*6*). However, AMB protecting groups are cumbersome to incorporate into monomers and are not stable to extensive storage (*19*). Therefore, we adapted commercially available protecting groups to the synthesis of M_1G-containing oligonucleotides. In the course of this work, we also developed a significantly improved synthesis of the deoxynucleoside of M_1G.

Materials and Methods

Reagents and Enzymes. HPLC grade solvents were obtained from Fisher (Pittsburgh, PA). Reagent grade chemicals and deuterated solvents were obtained from Aldrich (Milwaukee, WI) and were used as received. Nucleoside 2-deoxyribosyltransferase (EC 2.4.2.6, transferase) was a generous gift from S. Short (Glaxo-Wellcome Inc., Research Triangle Park, NC).

Instrumental Analysis. HPLC analyses were performed with a Varian model 9010 solvent delivery system and a Hewlett-Packard model 1040A diode array detector interfaced with a Hewlett-Packard model 9000, Series 300 Chemstation. Samples were injected onto an Ultrasphere ODS reversed phase column (10 mm × 250 mm) from Beckman (Fullerton, CA) and eluted at 2.5 mL/min. A gradient from 100 mM ammonium formate (pH 6.4) to 50% acetonitrile was used as follows: from 0 to 5 min, linear gradient to 10% acetonitrile; from 5 to 20 min, linear gradient to 15% acetonitrile; from 25 to 35 min, linear gradient to 25% acetonitrile; from 35 to 40 min, linear gradient to 35% acetonitrile; and from 40 to 45 min, linear gradient to 50% acetonitrile. The eluant was monitored at 260 and 320 nm. UV spectra were recorded using a Hewlett-Packard UV/VIS model 89500 spectrometer. Mass spectra were recorded on a Finnigan TSQ 7000 triple-quadrupole spectrometer.

Synthesis of M_1G-dR. To a solution of guanine hydrochloride (1 g, 5.3 mmol) in 1 N HCl (100 mL) at 70 °C was added dropwise a solution of tetraethoxypropane (1.3 mL, 5.86 mmol) in methanol (1.25 mL). After addition was complete, the reaction mixture was further stirred for 0.5 h at 70 °C. The reaction mixture was allowed to cool to 0 °C and neutralized with K_2CO_3, and the unreacted guanine was filtered. 2-(*N*-Morpholino)-ethanesulfonic acid (MES) was added to obtain a final concentration of 0.5 M, followed by the addition of deoxycytidine (1.2 g, 5.3 mmol) to the solution. The pH of the reaction mixture was adjusted to 6.0, and the transferase (230 *μ*g) was added (*20*, *21*). The solution was incubated for 2 h at 37 °C with gentle

* To whom correspondence should be addressed: Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232-0146. Phone: (615) 343-7329. Fax: (615) 343-7534. E-mail: marnett@toxicology.mc.vanderbilt.edu.

[†] Department of Biochemistry.

[‡] Department of Chemistry.

[1] Abbreviations: MDA, malondialdehyde; transferase, nucleoside 2-deoxyribosyltransferase; M_1G, pyrimido[1,2-*a*]purin-10(3*H*)-one; M_1G-dR, M_1G-deoxyribose; AMB, 2-(acetoxymethyl)benzoyl; MES, 2-(*N*-morpholino)ethanesulfonic acid; t-BPAC, *tert*-butylphenoxyacetyl.

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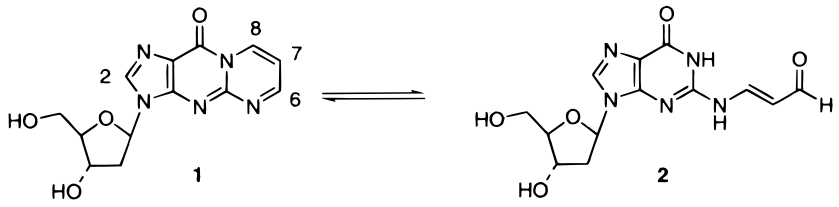
Scheme 1



agitation, after which complete conversion to M_1G-dR was observed. The crude product was purified by chromatography on silica gel (95:5 CH_2Cl_2/MeOH). Yield: 220 mg, 13%.

5′-*O*-(4,4′-Dimethoxytrityl)M_1G-dR. The synthesis of the dimethoxytrityl derivative was achieved by slight modification of the previously reported procedure (*22*). M_1G-dR (0.1 g, 0.33 mmol) was treated with anhydrous pyridine (10 mL), and the pyridine was evaporated. This procedure was repeated twice, and the residue was placed under vacuum overnight. Anhydrous pyridine (10 mL) and diisopropylethylamine (0.34 mL, 1.98 mmol) were added to the flask containing M_1G-dR, and the mixture was stirred under argon. The solution was cooled to 0 °C, and 4,4′-dimethoxytrityl chloride (0.678 g, 1.32 mmol) was added. The reaction mixture was allowed to warm slowly to room temperature and was stirred for 18 h. The reaction was quenched by the addition of methanol (100 μL) and the mixture stirred for 10 min. The reaction mixture was concentrated under vacuum, dissolved in CH_2Cl_2 (50 mL), and washed with 10% K_2CO_3 (2 × 50 mL). The organic solution was dried ($MgSO_4$), filtered, and evaporated under vacuum. Chromatography over silica gel (99.5:0.5 CH_2Cl_2/Et_3N) yielded the desired product (0.16 g, 80%). ^{1}H NMR ($CDCl_3$): δ 9.43 (dd, $J_{6,8}$ = 2.3 Hz and $J_{7,8}$ = 7.2 Hz, 1H, H_8), 8.91 (dd, $J_{6,8}$ = 2.3 Hz and $J_{6,7}$ = 3.8 Hz, 1H, H_6), 8.12 (s, 1H, H_2), 7.41−7.37 (m, 2H, aromatic), 7.30−7.15 (m, 7H, aromatic), 7.08 (dd, $J_{6,7}$ = 3.8 Hz and $J_{7,8}$ = 7.2 Hz, 1H, H_7), 6.80−6.74 (m, 4H, aromatic), 6.64 (t, $J_{1',2'}$ = 6.3 Hz, 1H, $H_{1'}$), 4.65−4.62 (m, 1H, $H_{3'}$), 4.20−4.18 (m, 1H, $H_{4'}$), 3.75 [s, 6H, $(OCH_3)_2$], 3.51−3.34 (m, 2H, $H_{5'}$ and $H_{5''}$), 2.71−2.48 (m, 2H, $H_{2'}$ and $H_{2''}$).

3′-*O*-[(*N*,*N*-Diisopropylamino)(2-cyanoethyl)phosphinyl]-5′-*O*-(dimethoxytrityl)M_1G-dR. The phosphoramidite reagent was synthesized according to the procedure described previously (*22*). 5′-*O*-(Dimethoxytrityl)M_1G-dR (100 mg, 0.2 mmol) was treated with anhydrous pyridine (10 mL), and the pyridine was evaporated. This procedure was repeated twice, and then the compound was placed under vacuum overnight. To a solution of anhydrous 1*H*-tetrazole (20 mg, 0.26 mmol) was added a solution of the tritylated compound (100 mg, 0.2 mmol) in dry CH_2Cl_2 (3 mL), followed by 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphoramidite (95 μL, 0.3 mmol). The reaction mixture was stirred under argon at room temperature for 3 h. The mixture was treated with $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layer was dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by chromatography on silica gel (99.5:0.5 CH_2Cl_2/Et_3N) to afford pure 3′-*O*-[(*N*,*N*-diisopropylamino)(2-cyanoethyl)-phosphinyl]-5′-*O*-(dimethoxytrityl)M_1G-dR (120 mg, 76%). ^{31}P NMR ($CDCl_3$): δ 149.42, 149.45.

Oligonucleotides. Oligonucleotides were synthesized on an Expedite model 8909 Nucleic Acid Synthesis System (PerSeptive Biosystems, Framingham, MA) on a 1 μmol scale using the manufacturer's standard protocol. The beads from four 1 μmol cassettes were suspended in a 50 mM solution of K_2CO_3 in dry methanol (4 mL) under argon and stirred slowly for 18 h. The beads were allowed to settle; the supernatant was removed, and the beads were washed with H_2O (2 × 1 mL). The combined aqueous fractions were neutralized cautiously with 1 M acetic acid to pH 6 and filtered. The oligonucleotide was purified using the HPLC system described above.

Chromatography Gel Electrophoresis. Oligonucleotide purity was evaluated on a Beckman P/ACE 2000 instrument (Beckman) using "ssDNA 100" gel capillary and "TRIS-borate-urea buffer" from the manufacturer. Samples were applied at −10 kV and run at −15 kV (30 °C).

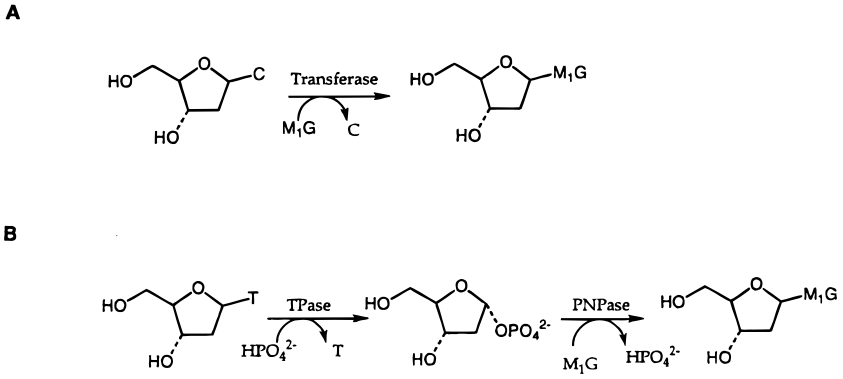
NMR Measurements. The purified oligonucleotides were desalted using a G25 column (Sigma, St. Louis, MO). The collected fraction was lyophilized and redissolved in 0.5 mL of buffer solution containing 0.1 M NaCl, 10 mM NaH_2PO_4, and 50 μM Na_2EDTA at pH 6.8. The solutions were lyophilized and exchanged three times with 99.96% D_2O. The final sample was dissolved in 0.5 mL of 99.996% D_2O. The oligonucleotide concentration was estimated to be 0.5 mM from an extinction coefficient of 9.31 × 10^4 M^{-1} cm^{-1} calculated at 260 nm. Experiments were performed on a Bruker DMX500 spectrometer at a ^{1}H frequency of 500.13 MHz at a temperature of 35 °C. One-dimensional spectra were recorded with a sweep width of 6000 Hz and 32K data points. Two-dimensional spectra were recorded with 512 real data points in the d_1 dimension and 2048 real data points in the d_2 dimension for COSY experiments. Phase-sensitive TOCSY experiments were performed using a mixing time of 80 ms and MLEV17 spin lock pulse with TPPI phase cycling. Spectra were processed using FELIX (Biosym Technologies) on Silicon Graphics Indigo^2 workstations. The data in the d_1 dimension were zero-filled to give a matrix of 2K × 2K real points. A sine-bell apodization function with a 90° phase shift was used in both dimensions.

Interconversion of M_1G-dR and N^2-(3-Oxo-1-propenyl)-dG. The process of the interconversion between M_1G and N^2-(3-oxo-1-propenyl)dG was investigated by recording the NMR spectra of the sample as a function of pH and time. The pH of the sample was adjusted by titration with a 10% (by volume) NaOD or DCl solution. A 3 mm (diameter) micro pH probe (Ingold, Inc.) was used to measure the pH in the NMR tube. The spectra at different pHs were recorded using the same acquisition parameters.

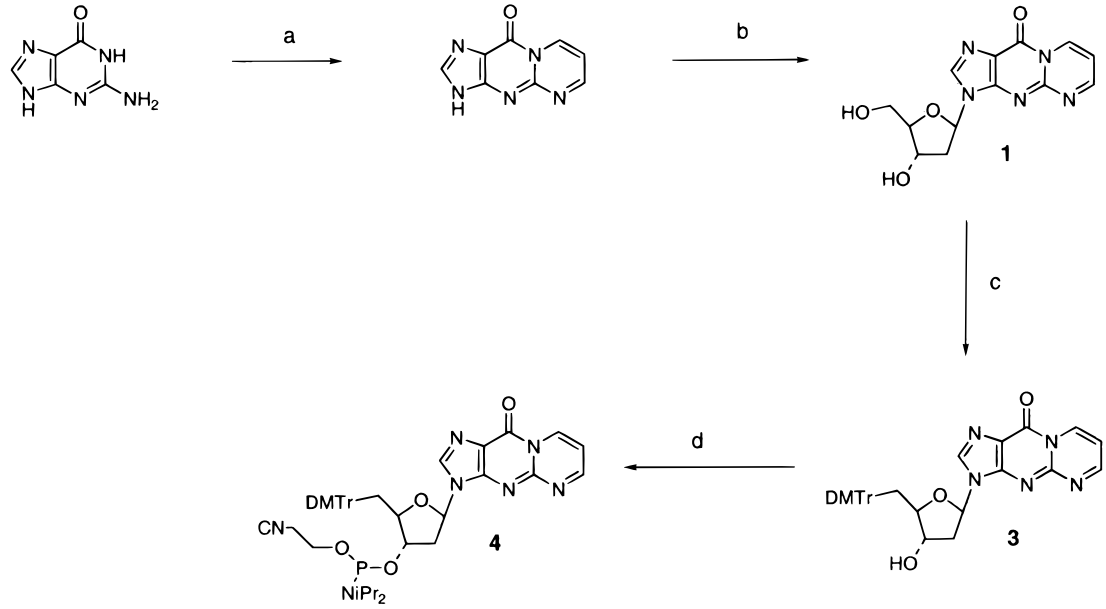
Results and Discussion

Our previous synthesis of M_1G-dR utilized a two-step enzymatic transribosylation of M_1G with the thymine base of dT (*23*). The yields in this procedure were limited by the requirement to purify the M_1G base after its synthesis from dG and tetraethoxypropane. Attempts to convert the overall procedure to a one-pot reaction were unsuccessful because of contamination by side products. However, we found that a one-pot reaction could be developed by using nucleoside 2′-deoxyribosyltransferase for the transribosylation. This enzyme exchanges bases for the cytosine of deoxycytidine, generating the deoxyriboside directly (Scheme 2). This enzyme was reported to form only the N9 isomer of 9-β-D-2′-deoxyribofuranosyl-1-deazapurine by Betbeder et al., and Muller et al. used a partially purified enzyme to add deoxyribose to guanine and modified DNA bases, including M_1G, and found that after 15 h, the coupling yield was 97% and the ratio of

Scheme 2



Scheme 3[a]



[a] (a) Tetraethoxypropane, 1 N HCl; (b) MES, transferase, dC; (c) DMtr chloride, pyridine; (d) 1*H*-tetrazole, 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphoramidite, CH_2Cl_2.

N9 to N7 was 85:15 (*24*, *25*). We used pure nucleoside 2′-deoxyribosyltransferase and observed complete conversion from the M_1G base to M_1G-dR, with no N7 isomer formed. Purification by silica gel chromatography (two times) and reversed phase medium-pressure liquid chromatography yielded pure M_1G-dR. The modification not only increased the yield of the synthesis but also decreased the time necessary for the synthesis and purification.

Synthesis of M_1G-Containing Oligonucleotides. Cyanoethyl phosphoramidite **4** (Scheme 3) was prepared by alkylation of the 5′-position of M_1G-dR with 4,4′-dimethoxytrityl chloride, followed by phosphitylation with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphophoramidite, as described by De Corte et al. (*22*). The phosphoramidite **4** was then used to prepare oligonucleotides by solid phase synthesis. The method used for the synthesis of the oligodeoxyribonucleotide varied from the one previously described by the use of the *tert*-butyl PAC group instead of the AMB protecting group for unmodified nucleosides. PAC and alkyl-PAC protecting groups have previously been used by Sinha et al. and Fujimoto et al., who showed that they could be removed with K_2CO_3/methanol (*26*, *27*).

An 8-mer, d(GGTXTCCG) where X = M_1G, was prepared and was deprotected with 50 mM K_2CO_3 in dry methanol. To complete the cleavage of the oligonucleotide, the beads were washed with water. The same deprotection procedure was used for the M_1G-containing oligonucleotides synthesized with the AMB protecting group (*6*). The fully deprotected oligonucleotide was analyzed and purified by HPLC injection on a reversed phase column as described in Materials and Methods. The purity was confirmed by capillary gel electrophoresis (Figure 1).

NMR Characterization of d(GGTXTCCG) (X = M_1G). The spectrum of the M_1G oligomer had no characteristic resonance from the t-BPAC protecting group, indicating complete deprotection. Two resonances at 1.58 and 1.71 ppm integrated for three protons each and were assigned to the methyl groups of the two thymines adjacent to M_1G. A total of 11 resonances were observed in the downfield region between 7 and 10 ppm that arise from the base aromatic protons of each nucleotide, in addition to three exocyclic ring protons of M_1G. In a two-dimensional TOCSY spectrum, two H5−H6 cross-peaks from two cytosine residues, two CH_3−H6 cross-peaks from two thymine residues, and a total of eight sets of H1′−H2′ and H1′−H2″ cross-peaks were observed in their corresponding regions. Although a complete assignment was not determined, the observed aromatic protons were assigned as the H6 protons of the two thymines at

Table 1. Assignments of the Exocyclic Ring Protons of M_1G

proton	M_1G-dR nucleotide[a](ppm)	N^2-(3-oxo-1-propenyl)dG nucleotide[a] (ppm)	M_1G oligomer (ppm)	N^2-(3-oxo-1-propenyl)dG oligomer (ppm)	$\Delta\delta$[b] (ppm)
H2[c]	8.22	7.80	8.42	7.99	0.43
H6	8.92	8.21	8.98	8.28	0.70
H7	7.20	5.60	7.24	5.71	1.53
H8	9.20	8.95	9.23	8.99	0.24

[a] Obtained from ref *28*. Chemical shifts were measured in DMSO-d_6. [b] $\Delta\delta$ values are not calibrated for the pH change. [c] This proton was numbered as H8 for deoxyguanosine. For the unmodified oligonucleotide, $\delta_{H8} = 8.01$ ppm.

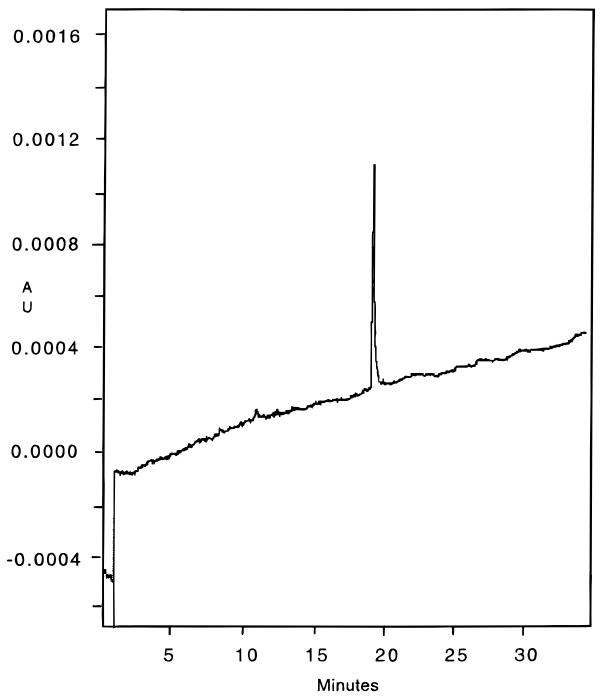


Figure 1. CGE profile of 5′-GGTXTCCG-3′.

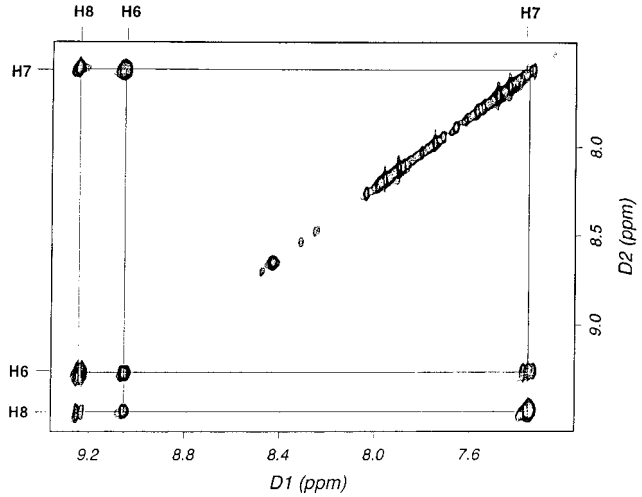


Figure 2. Expanded plot of a TOCSY spectrum showing the scalar coupling correlation of exocyclic protons of M_1G in 5′-GGTXTCCG-3′ at pH 6.8 and 35 °C.

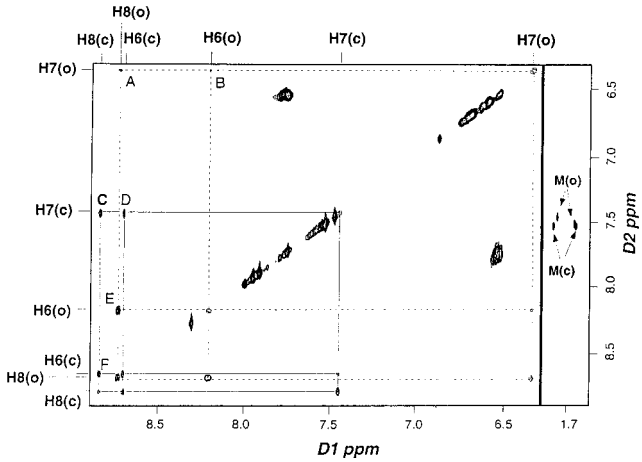


Figure 3. Expanded plot of a TOCSY spectrum showing the scalar coupling correlation of exocyclic protons of M_1G in 5′-GGTXTCCG-3′ at pH 10 and 35 °C. Both ring-closed and ring-opened forms of M_1G are apparent.

7.42 and 7.47 ppm, the H6 of two cytosines showing as a doublet at 7.66 and 7.72 ppm, and the H8 of the three guanines at 7.87, 7.92, and 7.94 ppm, respectively. A resonance at 8.40 ppm was assigned as H2 of M_1G, corresponding to H8 in guanine. These resonances demonstrated the correct nucleotide composition of the oligonucleotide.

The assignments of exocyclic ring protons of M_1G were determined by examining the scalar coupling correlation of these protons in a series of COSY experiments. Figure 2 shows the expanded plot of a TOCSY spectrum. The cross-peaks arising from the three M_1G exocyclic ring protons are labeled, and the assignments are in agreement with a previous report (*28*). A TOCSY experiment was also performed at pH 10 in order to assign the protons of the N^2 propenyl group for N^2-(3-oxo-1-propenyl)dG, an exocyclic ring-opened form of M_1G (Figure 3). Examining the scalar coupling pattern gave the assignments of 5.71, 8.28, and 7.99 ppm for H7, H6, and H8, respectively. The chemical shifts of the methyls (1.59 and 1.72 ppm) of the two thymidines adjacent to the modified base were slightly shifted, and the H6s of both thymines merged into one resonance upon formation of N^2-(3-oxo-1-propenyl)dG. The chemical shifts of the exocyclic ring protons are listed in Table 1.

Interconversion of M_1G and N^2-(3-Oxo-1-propenyl)dG. Increasing the pH resulted in M_1G converting to a ring-opened form, N^2-(3-oxo-1-propenyl)dG (*6*). After the sample was titrated with NaOD to bring the pH from 6.5 to 10, the magnitudes of the signals of the three exocyclic ring protons, H6−H8, of the closed form (7.4, 8.9, and 9.4 ppm, respectively) decreased as the magnitudes of the three resonances corresponding to H6−H8 (5.71, 8.28, and 7.99 ppm, respectively) of the ring-opened N^2-(3-oxo-1-propenyl)dG increased. H2 (8.40 ppm) of M_1G disappeared, and a new resonance arose at 7.99 ppm, which was assigned as H2 of the N^2-(3-oxo-1-propenyl)-dG oligomer. Figure 4 represents a set of spectra recorded as a function of time after the pH was adjusted to 10. The conversion of the M_1G oligomer to the N^2-(3-oxo-1-propenyl)dG oligomer was slow; only about 50% of the M_1G oligomer converted to the N^2-(3-oxo-1-propenyl)dG oligomer after 30 min. About 90% of the M_1G oligomer converted after 5 h. However, the resonances from the

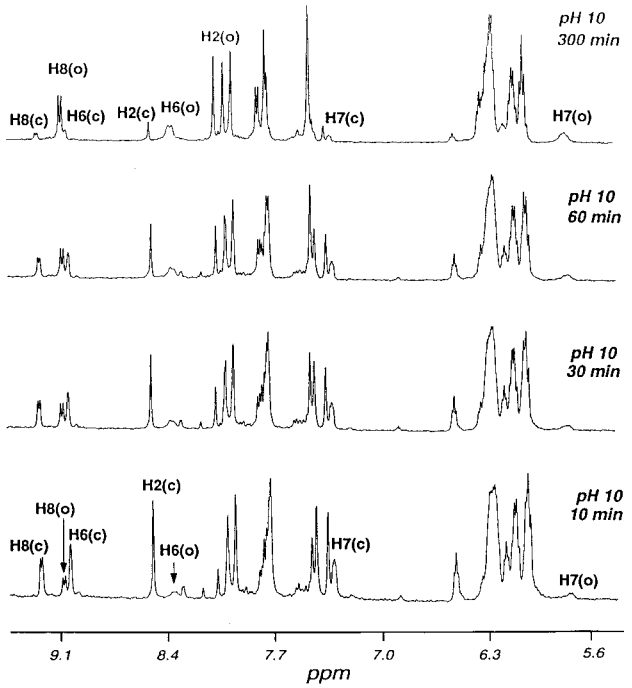


Figure 4. Expanded plots of spectra showing the conversion of M_1G and N^2-(3-oxo-1-propenyl)dG as a function of time at pH 10.

ring-closed M_1G oligomer were still detectable 24 h after the pH was adjusted to 10. It is interesting to note that the ring opening of the M_1G moiety in an oligonucleotide, as a function of pH, is slow compared with the ring opening of a duplex M_1G oligomer in which dC is opposite the adduct and catalyzes the opening of M_1G very rapidly (*29*).

Further evidence for the proposed structure of the M_1G-containing oligodeoxynucleotide was obtained by LC/MS with electrospray ionization. The oligonucleotide was analyzed by direct loop injection. The peaks corresponding to *m/z* 823.4 and 1234.8 were observed and represent 3^- and 2^- charged ions, respectively. The theoretical molecular weight of this 8-mer is 2470.6, and the molecular weight calculated from the ions described above was 2469.9.

This report describes a straightforward procedure for the generation of M_1G-containing oligonucleotides from readily available commercial materials. This provides a convenient method for the generation of oligonucleotides for a range of structural, biochemical, and genetic studies. The 8-mer sequence described herein has been used in in vivo mutagenesis and structural studies (*29*, *30*), but we also have synthesized 19-mers and 31-mers for in vitro replication and repair experiments. Thus, the synthetic operations described can be readily adapted for different oligonucleotide lengths and amounts.

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References

(1) Cosman, M., Ibanez, V., Geacintov, N. E., and Harvey, R. G. (1990) Preparation and isolation of adducts in high yeild derived from the binding of two benzo[*a*]pyrene-7,8-dihydroxy-9,10-oxide stereoisomers to the oligonucleotide d(ATATGTATA). *Carcinogenesis* **11**, 1667−1672.

(2) Baertschi, S. W., Raney, K. D., Stone, M. P., and Harris, T. M. (1988) Preparation of the 8,9-epoxide of the mycotoxin aflatoxin B1: The ultimate carcinogenic species. *J. Am. Chem. Soc.* **110**, 7929−7931.

(3) Lee, H., Hinz, M., Stezowski, J. J., and Harvey, R. G. (1990) Syntheses of polycyclic aromatic hydrocarbon-nucleoside and oligonucleotide adducts specifically alkylated on the amino functions of deoxyguanosine and deoxyadenosine. *Tetrahedron Lett.* **31**, 6773−6776.

(4) Zhou, Y., and Romano, L. J. (1993) Solid-phase synthesis of oligonucleotides containing site-specific N-(2′-deoxyguanosine-8-yl)-2-(acetylamino)fluorene adducts using 9-fluorenylmethoxycarbonyl as the base-protecting group. *Biochemistry* **32**, 14043−14052.

(5) Laksham, M. K., Sayer, J. M., Yagi, H., and Jerina, D. M. (1992) Synthesis and duplex-forming properties of a nonanucleotide containing an N6-deoxyadenosine adduct of a bay-region diol epoxide. *J. Org. Chem.* **57**, 4585−4590.

(6) Reddy, G. R., and Marnett, L. J. (1995) Synthesis of an oligodeoxynucleotide containing the alkaline labile malondialdehyde-deoxyguanosine adduct pyrimido[1,2-*a*]purin-10(3H)-one. *J. Am. Chem. Soc.* **117**, 5007−5008.

(7) Marnett, L. J., Hurd, H. K., Hollstein, M. C., Levin, D. E., Esterbauer, H., and Ames, B. N. (1985) Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. *Mutat. Res.* **148**, 25−34.

(8) Yau, T. M. (1979) Mutagenicity and cytotoxicity of malondialdehyde in mammalian cells. *Mech. Ageing Dev.* **11**, 137−144.

(9) Basu, A. K., and Marnett, L. J. (1983) Unequivocal demonstration that malondialdehyde is a mutagen. *Carcinogenesis* **4**, 331−333.

(10) Seto, H., Akiyama, K., Okuda, T., Hashimoto, T., Takesue, T., and Ikemura, T. (1981) Structure of a new modified nucleoside formed by guanosine-malonaldehyde reaction. *Chem. Lett.*, 707−708.

(11) Nair, V., Turner, G. A., and Offerman, R. J. (1984) Novel adducts from the modification of nucleic acid bases by malondialdehyde. *J. Am. Chem. Soc.* **106**, 3370−3371.

(12) Marnett, L. J., Basu, A. K., O'Hara, S. M., Weller, P. E., Rahman, A. F. M. M., and Oliver, J. P. (1986) Reaction of malondialdehyde with guanine nucleosides: formation of adducts containing oxadiazabicyclononene residues in the base-pairing region. *J. Am. Chem. Soc.* **108**, 1348−1350.

(13) Stone, K., Ksebati, M., and Marnett, L. J. (1990) Investigation of the adducts formed by reaction of malondialdehyde with adenosine. *Chem. Res. Toxicol.* **3**, 33−38.

(14) Stone, K., Uzieblo, A., and Marnett, L. J. (1990) Studies of the reaction of malondialdehyde with cytosine nucleosides. *Chem. Res. Toxicol.* **3**, 467−472.

(15) Chaudhary, A. K., Nokubo, M., Reddy, G. R., Yeola, S. N., Morrow, J. D., Blair, I. A., and Marnett, L. J. (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. *Science* **265**, 1580−1582.

(16) Rouzer, C. A., Chaudhary, A. K., Nokubo, M., Ferguson, D. M., Reddy, G. R., Blair, I. A., and Marnett, L. J. (1997) Analysis of the malondialdehyde-2′-deoxyguanosine adduct, pyrimidopurinone, in human leukocyte DNA by gas chromatography/electron capture negative chemical ionization/mass spectrometry. *Chem. Res. Toxicol.* **10**, 181−188.

(17) Leuratti, C., Singh, R., Lagneau, C., Farmer, P. B., Plastaras, J. P., Marnett, L. J., and Shuker, D. E. (1998) Determination of malondialdehyde-induced DNA damage in human tissues using an immunoslot blot assay. *Carcinogenesis* **19**, 1919−1924.

(18) Vaca, C. E., Fang, J.-L., Mutanen, M., and Valstar, L. (1995) ^{32}P-Postlabelling determination of DNA adducts of malonaldehyde in humans: total white blood cells and breast tissue. *Carcinogenesis* **16**, 1847−1851.

(19) Kuijpers, W. H., Kuyl-Yeheskiely, E., van Boom, J. H., and van Boeckel, C. A. (1993) The application of the AMB protective group in the solid-phase synthesis of methylphosphonate DNA analogues. *Nucleic Acids Res.* **21**, 3493−3500.

(20) Armstrong, S. R., Cook, W. J., Short, S. A., and Ealick, S. E. (1996) Crystal structures of nucleoside 2-deoxyribosyltransferase in native and ligand-bound forms reveal architecture of the active site. *Structure* **4**, 97−107.

(21) Boutaud, N. S., and Marnett, L. J. (2000) Condensation by Enzymatic Coupling. In *Current Protocols in Toxicology* (Jones, R., Ed.) John Wiley and Sons, New York (in press).
(22) Decorte, B. L., Tsarouhtis, D., Kuchimanchi, S., Cooper, M. C., Horton, P., Harris, C. M., and Harris, T. M. (1996) Improved strategies for postoligomerization synthesis of oligodeoxynucleotides bearing structurally defined adducts at the N2 position of deoxyguanosine. *Chem. Res. Toxicol.* **9**, 630−637.
(23) Chapeau, M. C., and Marnett, L. J. (1991) Enzymatic synthesis of purine deoxynucleoside adducts. *Chem. Res. Toxicol.* **4**, 636−638.
(24) Betbeder, D., Hutchinson, D. W., and Richards, A. O. (1989) The stereoselective enzymatic synthesis of 9-β-D-2′-deoxyribofuranosyl 1-deazapurine. *Nucleic Acids Res.* **17**, 4217−4222.
(25) Muller, M., Hutchinson, L. K., and Guengerich, F. P. (1996) Addition of deoxyribose to guanine and modified DNA bases by *Lactobacillus helveticus trans-N*-deoxyribosylase. *Chem. Res. Toxicol.* **9**, 1140−1144.
(26) Sinha, N. D., Davis, P., Perez, J., Hodge, R., Kremsky, J., and Casale, R. (1993) Labile exocyclic amine protection of nucleosides in DNA, RNA and oligonucleotide analog synthesis facilitating N-deacylation, minimizing depurination and chain degradation. *Biochimie* **75**, 13−23.
(27) Fujimoto, J., Tran, L., and Sowers, L. C. (1997) Synthesis and cleavage of oligodeoxynucleotides containing a 5-hydroxyuracil residue at a defined site. *Chem. Res. Toxicol.* **10**, 1254−1258.
(28) Niedernhofer, L. J., Riley, M., Schnetz-Boutaud, N., Sanduwaran, G., Chaudhary, A. K., Reddy, G. R., and Marnett, L. J. (1997) Temperature-dependent formation of a conjugate between tris-(hydroxymethyl)aminomethane buffer and the malondialdehyde-DNA adduct pyrimidopurinone. *Chem. Res. Toxicol.* **10**, 556−561.
(29) Mao, H., Schnetz-Boutaud, N. C., Weisenseel, J. P., Marnett, L. J., and Stone, M. P. (1999) Duplex DNA catalyzes the chemical rearrangement of a malondialdehyde deoxyguanosine adduct. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6615−6620.
(30) Fink, S. P., Reddy, G. R., and Marnett, L. J. (1997) Mutagenicity in *Escherichia coli* of the major DNA adduct derived from the endogenus mutagen, malondialdehyde. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8652−8657.

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