Synthesis of an Oligodeoxynucleotide Containing the Alkaline Labile Malondialdehyde−Deoxyguanosine Adduct Pyrimido[1,2-*a*]purin-10(3*H*)-one

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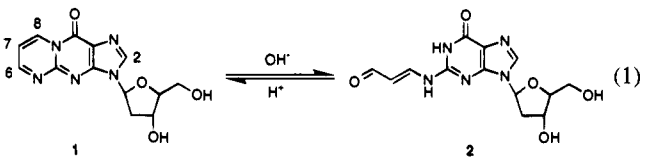
Received January 18, 1995

Studies of the chemistry and biology of DNA damage have focused mainly on DNA adducts derived from exogenous chemicals and ultraviolet radiation.[1] However, recent studies indicate that DNA adducts are also produced from endogenous chemicals that arise during normal metabolism, oxidative stress, etc., and that these adducts may contribute to the etiology of genetic diseases such as cancer.[2] Malondialdehyde is a major mutagenic and carcinogenic product generated during peroxidation of membrane lipids that reacts with deoxynucleosides to produce a variety of adducts.[3,4] The major adduct at physiological pH is 3-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-pyrimido[1,2-*a*]purin-10(3*H*)-one (**1**). This adduct is also formed by reaction of malondialdehyde with single- and double-stranded DNA and recently has been identified as a constituent of liver DNA from healthy human beings.[2a,5] The levels of **1** in human liver represent ~5500 adducts/cell, which suggests that 1 may contribute significantly to the development of human genetic disease.

The existence of high levels of **1** in normal DNA places a high priority on studies of its mutagenic potential and repair. The most direct approach to such studies is the construction of viral genomes or shuttle vectors containing **1** at defined positions.[6] These genomes can be introduced into recipient cells and the fate of the adduct and its mutagenic potency evaluated. Unfortunately, the instability of **1** under the alkaline conditions typically used to deprotect synthetic oligodeoxynucleotides has prevented its site-specific incorporation into appropriate vectors.[7] This prompted us to explore alternate chemical strategies for the incorporation of **1** into oligodeoxynucleotides that avoid the use of aqueous ammonia or strong organic bases for deprotection. We report herein the synthesis of oligodeoxynucleotides containing **1**, utilizing 2-(acetoxymethyl)benzoyl (AMB)-protected nucleosides as monomers and anhydrous potassium carbonate/methanol as deprotecting reagent.

AMB protecting groups have been shown previously to be removable from nucleosides by treatment with K_2CO_3.[8] Treatment of **1** with 50 mM K_2CO_3 in anhydrous methanol for 10 h followed by neutralization led to quantitative recovery of **1** as judged by HPLC analysis. This indicated that **1** is stable under the conditions used for removal of the AMB protecting groups. However, an aqueous wash is necessary to remove the deprotected oligodeoxynucleotides from the solid phase resin and treatment of **1** with aqueous K_2CO_3 quantitatively converted it to a compound with an ultraviolet absorbance maximum at 320 nm. The similarity of this spectrum to those of previously characterized malondialdehyde adducts with deoxyadenosine and deoxycytidine suggested that the new compound was an oxopropenyl derivative formed by ring-opening of **1** (eq 1).[4a,b,e]



1 2 (1)

The product was isolated by reversed phase HPLC, and the ^1H and ^{13}C NMR spectra verified the presence of the oxopropenyl group.[9] The electrospray ionization mass spectrum (positive ion mode) exhibited peaks at *m/z* (relative intensity) 322 (23), 304 (58), 206 (75), and 188 (100), consistent with a ring-opened structure. The isomeric composition was verified by monitoring the ring-opening of [8-^{2}H]**1** by NMR. A single product was formed that did not exhibit an aldehyde resonance.

Compound **2** slowly converted to **1** on standing in neutral aqueous solution, but exposure of **2** to a trace of acid led to rapid and quantitative recyclization. The reversible conversion of **1** and **2** provided an opportunity to incorporate **1** into oligodeoxynucleotides by carrying out deprotection, removal from the resin, and acid treatment to regenerate **1** in the polymers.

AMB-protected deoxynucleosides were synthesized and converted to 5′-*O*-(dimethoxytrityl)-3′-(2-cyanoethyl)phosphoramidite derivatives.[8] Deoxynucleoside **1** was synthesized by reaction of malondialdehyde with guanine followed by enzymatic transribosylation with thymidine.[10] 5′-*O*-(Dimethoxytrityl)-3′-(2-cyanoethyl)phosphoramidite **3** was synthesized utilizing standard procedures.[11] The DMT•AMB derivative of deoxyguanosine was attached to long-chain alkylamine-controlled pore glass beads via a succinyl linker. The nucleoside loading was 35 μmol/g, as determined by the dimethoxytrityl cation assay.

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(9) ^1H NMR (D_2O, 200 MHz): δ 2.35 (m, 1H, H-2′$_\alpha$), 2.75 (m, 1H, H-2′$_\beta$), 3.64 (m, 2H, H-5′,5″), 3.96 (m, 1H, H-4′), 4.50 (m, 1H, H-3′), 5.65 (dd, 1H, *J* = 9.0, 13.2 Hz, H-7), 6.24 (t, 1H, *J* = 7.0 Hz, H-1′), 7.85 (s, 1H, H-2), 8.27 (d, 1H, *J* = 13.2 Hz, H-6), 8.97 (d, 1H, *J* = 9.0 Hz, H-8). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 190.

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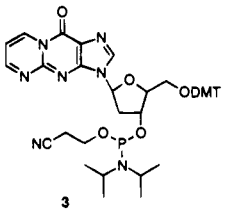

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The first sequence for incorporation of **1** was 5′-CGCXCG-GCATG-3′ (**4**). This sequence represents a mutable hotspot of an M13 vector that we have developed for site-specific mutagenesis experiments.[12] The modified oligodeoxynucleotide was synthesized on an automated synthesizer using the standard 1 μmol protocol. The coupling yields were >97% at each step as judged by the amount of DMT cation released. The fluorescence of **1** was evident on the resin at all steps following its incorporation. The solid support was treated with 2 mL of anhydrous 50 mM K_2CO_3/MeOH for 12 h at room temperature, and then the deprotecting solution was carefully neutralized with acetic acid and evaporated to a residue. Following workup, the oligodeoxynucleotide was purified to homogeneity by reversed phase and anion exchange HPLC (Figure 1). The UV spectrum of **4** contained the absorption bands of **1** (Figure 1, inset). Its purity was estimated to be >99.5% by electrophoresis on a 20% denaturing polyacrylamide gel of ^{33}P-labeled material. An unmodified 11-mer, synthesized using AMB protecting groups, comigrated with **4** between 10-mer and 12-mer markers. Electrospray ionization mass spectrometry of **4** revealed the presence of ions at *m/z* 1130.1, 847.5, and 677.9, corresponding to 3^-, 4^-, and 5^- charged ions, respectively. The theoretical molecular weight of **4** is 3394.16, and the observed molecular weight calculated from the above ions was 3393.93. The presence of **1** was further verified by digesting **4** with snake venom phosphodiesterase and alkaline phosphatase followed by HPLC analysis.

A second oligodeoxynucleotide representing a mutable hotspot of M13, 5′-GGTXTCCG-3′ (**5**), was synthesized and removed from the resin with K_2CO_3/MeOH. The basic aqueous solution, containing DMT·**5**, was purified by C_{18} reversed phase HPLC. Removal of the DMT group and simultaneous ring closure of **2** to **1** in the oligodeoxynucleotide were effected by treatment with aqueous acetic acid. The detritylated oligodeoxynucleotide **5** was purified by reversed phase and anion exchange HPLC. The NMR spectrum of **5** is shown in Figure 2. The proton signals of the exocyclic ring of **1** are evident at δ 7.35, 9.05, and 9.30. No signals are detectable in the region δ 5.1−5.8 corresponding to the ring-opened residue **2**. Thus, the pyrimidopurinone residue in oligodeoxynucleotides is stable in neutral aqueous medium with respect to ring-opening.

The successful incorporation of the alkaline labile malondialdehyde−deoxyguanosine adduct into oligodeoxynucleotides **4** and **5** will enable investigation of its structural and functional effects. These studies will provide a better understanding of the pathways by which a product of endogenous DNA damage may lead to cancer and other genetic diseases.

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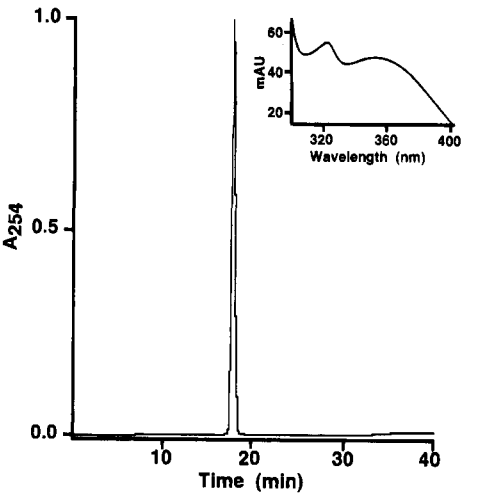

Figure 1. HPLC profile of purified **4**. The column was eluted with a gradient of 5−25% acetonitrile (0−20 min), 25−100% acetonitrile (30 min) in 50 mM triethylamınonium acetate (TEAA) buffer, pH 6.0. Inset: UV spectrum of **4**. The absorbance between 300 and 400 nm is due to the presence of **1**.

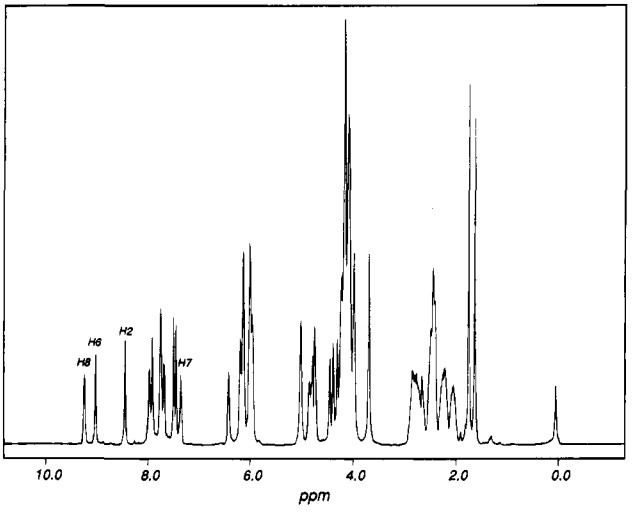

Figure 2. NMR spectrum (400 MHz) of the nonexchangeable protons of 5′-GGTXTCCG-3′ (X = **1**) in D_2O.

Acknowledgment. This work was supported by a research grant (CA 47479) from the National Institutes of Health. We are grateful to G. Sundaruvan, A. Chaudhary, and J. Weisenseel for technical assistance.

JA9501676