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Communications to the Editor

Postsynthetic Generation of a Major Acrolein Adduct of 2′-Deoxyguanosine in Oligomeric DNA†

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Acrolein, the simplest member of the class of α,β-unsaturated carbonyl compounds, is a ubiquitous substance in the environment.[2] It arises from a wide range of sources, from the incomplete combustion of organic matter to its occurrence as a secondary metabolite of some anticancer drugs.[3] Several research groups have shown that a number of α,β-unsaturated aldehydes form adducts with DNA that are mutagenic in bacteria.[4] The adducts[5] of acrolein with DNA bases have been characterized,[5,6] and of these the cyclic derivative **1** (Figure 1), formed from 2′-deoxyguanosine (dG) residue, is one of the more dominant. However, little is known about the precise mutagenic behavior of this adduct at the level of DNA replication.

For such biological studies, homogeneous segments of DNA in which adduct **1** is located site-specifically are required. Unfortunately the base lability of adduct **1** precludes its direct incorporation into DNA by the standard synthetic protocol whereas the direct treatment of DNA with acrolein leads to attack at multiple sites.

Because of these problems our group[7] and others[8] have pioneered the use of the base-stable propano-dG (**3**) as a model to study the mutagenic spectrum of these adducts. The direct treatment of dG with acrolein results in an initial Michael addition at the C2 amino group, which is followed by cyclization at N-1 to give adduct **1**. To introduce this lesion into DNA we reasoned that a viable approach would be to generate the aldehyde function in **1a** after the synthesis of the DNA was complete. Although this might be accomplished using an acetal, the conditions for unmasking this group would undoubtedly compromise the glycosidic linkages.

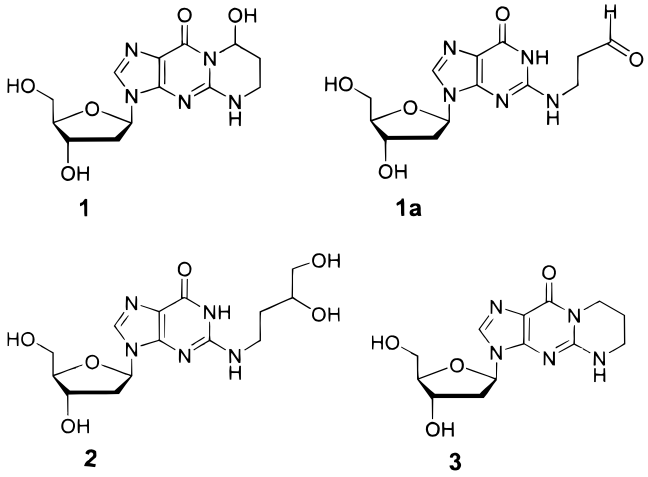

Figure 1.

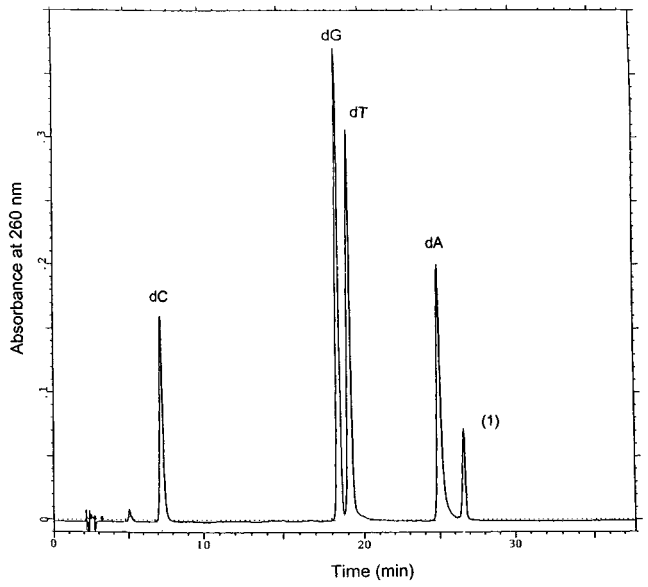

Figure 2. HPLC spectrum at 260 nm of the hydrolysate after enzymatic degradation of the 24-mer by phosphodiesterase and alkaline phosphatase. The peaks represent normal DNA bases and adduct **1**.

Therefore we elected to introduce the N^2-dihydroxybutyl derivative **2** of dG into DNA, as an alkali-stable precur-

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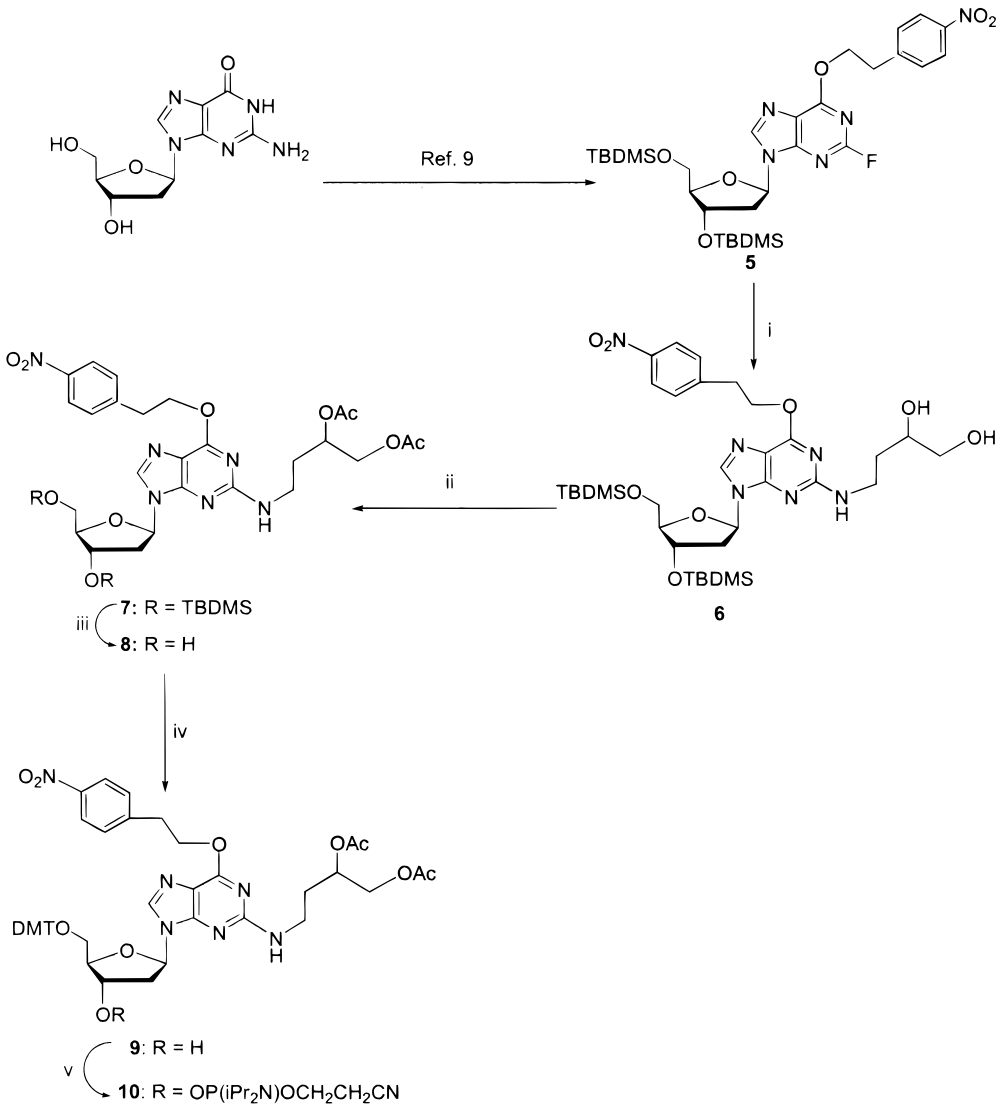
Scheme 1[a]



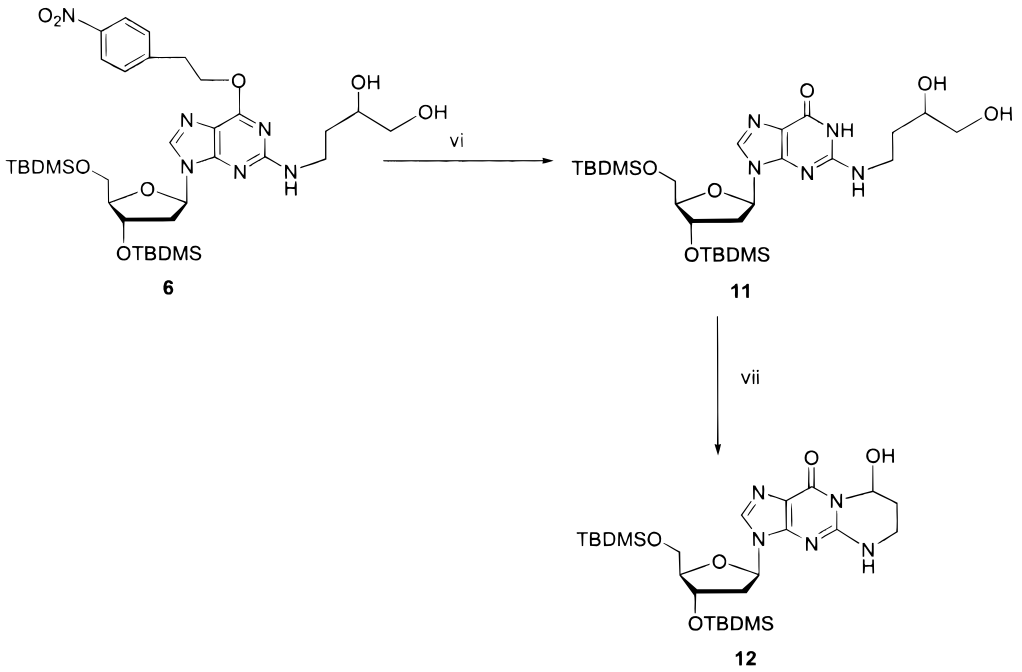
[a] Reagents and conditions: (i) amino diol **4**, MeOH, $CaCl_2$, 58 °C, 16 h; (ii) Ac_2O, Py, rt, 16 h; (iii) HF, Py, rt, 4 h; (iv) DMT-Cl, Py, rt, 1 h; (v) $ClP(OCH_2CH_2CN)N^iPr_2$, NEt_3, CH_2Cl_2, rt, 2 h.

sor to adduct **1**. This has the advantages of ease of protection and a ready fission by periodate to the desired aldehyde **1a**, postsynthetically. The latter could be expected to cyclize spontaneously to **1** given that this is thought to be part of the mechanism for the formation of these adducts (Figure 1).

Thus the fluoro-inosine **5** (Scheme 1), which can be obtained from 2′-deoxyguanosine in three steps according to a literature procedure,[9] was treated with the previously known[10] amino diol **4** to obtain[11] the dihydroxy-dG derivative **6**. Acetylation of diol **6** in the presence of acetic anhydride in pyridine then afforded the diacetate **7**. The cleavage of the silyl ethers in compound **7** by HF in pyridine resulted in diol **8** which was smoothly transformed in two steps into the required DMT-phosphoramidite **10** under standard conditions via the DMT intermediate **9**.

To confirm the formation of adduct **1** at the monomer level, the *p*-nitrophenethyl group in compound **6** was removed using DBU in pyridine[12] to obtain the diol **11** (Scheme 2). This was then subjected to periodate cleavage in aqueous MeOH which led to adduct **12**, having a protected sugar moiety. Analytical data (^{1}H NMR and ^{13}C NMR spectra) indicate that **12** may be a single isomer.[13] Having confirmed that the cyclic adduct can be formed under these conditions, we directed attention to the generation of lesion **1** in DNA. This was accomplished by employing the DMT-phosphoramidite **10** in a standard DNA synthesis protocol[14] (Scheme 1). Normal HPLC methods were used for the purification of the oligomers. By way of examples, oligomers 5′- CGT ACX CAT GC-3′ and 5′-GCC ATG CAT GTT XTG CTA GAT TGC-3′ (where X = compound **2**) were purified twice by reversed-phase HPLC {eluents: triethylammonium acetate (0.1 M, pH 7.1) and CH_3CN; a linear gradient of 16−36% and 0−20% CH_3CN over 30 min was used for DMT-on DNA and DMT-off DNA, respectively}. When these purified oligomers (DMT-off) were treated with excess of an aqueous solution of sodium periodate (0.1 M, room temperature) until no starting material remained (2−6 h, monitored by HPLC), they afforded single-stranded DNA containing the desired adduct **1** at position X. These again were purified by reversed-phase HPLC to give essentially pure material. Both oligomers were characterized by electrospray mass spectroscopy (11-mer *m*/*z*: obsvd, 3372.5; calcd, 3373.6

Scheme 2[a]



[a] Reagents and conditions: (vi) DBU, MeOH, rt, 16 h; (vii) $NaIO_4$, H_2O−MeOH, rt, 2 h.

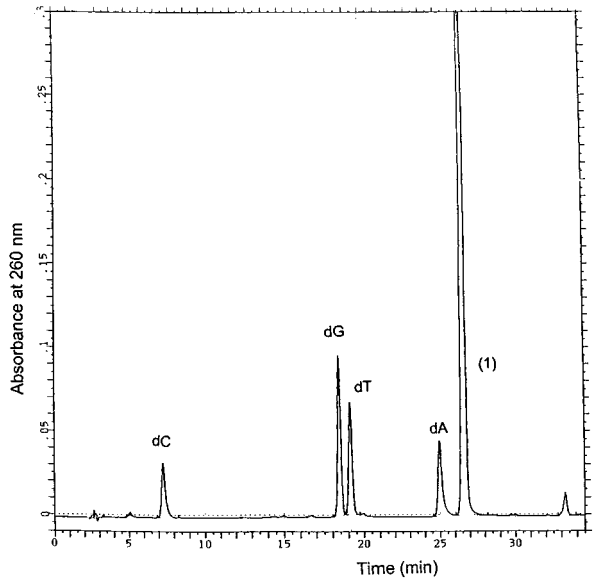
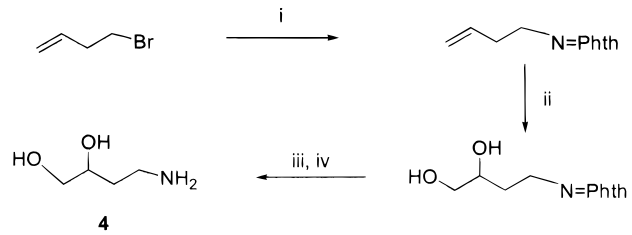


Figure 3. HPLC spectrum of the hydrolysate as shown in Figure 2, augmented by the addition of an authentic sample of adduct **1**.

Da) and (24-mer *m*/*z*: obsvd, 7428.4; calcd, 7430.8 Da). The 24-mer was also subjected to enzymatic degradation by phosphodiesterase and alkaline phosphatase.[15] HPLC of the hydrolysate clearly showed the presence of only 2′-deoxycytidine, 2′-deoxyguanosine, 2′-deoxythymidine, 2′-deoxyadenosine, and the adduct **1**—elution times being 7.27, 18.45, 19.13, 24.96, 26.62 min, respectively (Figure 2)**.** The HPLC peak associated with the acrolein adduct of dG was identified by the addition of an authentic sample to the hydrolysate (Figure 3). Work is now in progress to determine the mutational spectrum of adduct **1** in both bacterial and mammalian cell systems, and to determine the effect of this lesion on DNA structure using NMR methods.

Scheme 3[a]



[a] Reagents and conditions: (i) potassium phthalimide (Phth), DMF, rt, 16 h; (ii) OsO_4, *N*-methylmorpholine *N*-oxide, THF−H_2O, rt, 16 h; (iii) H_2N−NH_2, MeOH, rt, 16 h; (iv) acetic acid, $NaHCO_3$.

In conclusion, we have developed a synthetic pathway that allows the generation in DNA of the alkali-sensitive acrolein adduct **1**. We believe that this methodology has the general potential to allow the synthesis of DNA containing other alkali-sensitive lesions. Further work in this direction is being pursued.

Acknowledgment. This work was supported in part by a grant awarded by the National Institutes of Health, National Cancer Institute (CA799508), and the National Institute of Environmental Sciences (ES0406812). We thank Dr. Charles R. Iden and especially Mr. Robert Rieger for the mass spectra of the nucleosides and DNA containing the adducts, and we also thank Mrs. Cecilia Torres for the HPLC analytical data.

Supporting Information Available: Synthetic procedures and spectral data for compounds **6**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) This paper was presented at the 216th National ACS Meeting in Boston, MA (August 23−27, 1998). Division of Medicinal Chemistry abstract no. 188.
(2) Chung F−L.; Roy, K. R.; Hecht, S. S. A Study of Reactions of α,β-Unsaturated Carbonyl Compounds with Deoxyguanosine. *J. Org. Chem.* **1988**, *53*, 14−17.

(3) Nath, R. G.; Ocando, J. E.; Chung, F.-L. Detection of 1,N^2-Propanodeoxyguanosine Adducts as Potential Endogenous DNA Lesions in Rodent and Human Tissues. *Cancer Res.* **1996**, *56*, 452−456.
(4) Lutz, D.; Eder, E.; Neudecker, T.; Henschler, D. Structure-mutagenicity relationships in α,β-unsaturated carbonylic compounds and their corresponding allylic alcohols. *Mutat. Res.* **1982**, *93*, 305−315.
(5) Chung, F.-L.; Young, R.; Hecht, S. S. Formation of Cyclic 1,N^2-Propanodeoxyguanosine Adducts in DNA upon Reaction with Acrolein or Crotonaldehyde. *Cancer Res.* **1984**, *44*, 990−995.
(6) (a) Chenna, A.; Rieger, R. A.; Iden, C. R. Characterization of thymidine adducts formed by acrolein and 2-bromoacrolein. *Carcinogenesis* **1992**, *13*, 2361−2365. (b) Douki, T.; Ames, B. N. An HPLC-EC Assay for 1,N^2-Propano Adducts of 2′-Deoxyguanosine with 4-Hydroxynonenal and Other α,β-Unsaturated Aldehydes. *Chem. Res. Toxicol.* **1994**, *7*, 511−518.
(7) (a) Marinelli, E. R.; Johnson, F.; Iden, C. R.; Yu, P.-L. Synthesis of 1, N^2-(1,3-Propano)-2′-deoxyguanosine and Incorporation into Oligodeoxynucleotides: A Model for Exocyclic Acrolein DNA Adducts. *Chem. Res. Toxicol.* **1990**, *3*, 49−58. (b) Moriya, M.; Zhang, W.; Johnson, F.; Grollman, A. P. Mutagenic potency of exocyclic DNA adducts: Marked differences between *Escherichia coli* and simian kidney cells. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11899−11903.
(8) (a) Basu, A. K.; O'Hare, S. M.; Valladier, P.; Stone, K. Mols, O.; Marnett, L. J. Identification of Adducts Formed by Reaction Of Guanine Nucleosides with Malondialdehyde and Structurally Related Aldehydes. *Chem. Res. Toxicol.* **1988**, *1*, 53−59. (b) Benamira, M.; Singh, U. S.; Marnett, L. J. Site Specific Frameshift Mutagenesis by a Propanodeoxyguanosine Adduct Positioned in the $(CpG)_4$ Hot-spot of *Salmonella typhimurium his D3052* carried on an M13 Vector. *J. Biol. Chem.* **1992,** *31*, 22392−22400.
(9) Adib, A.; Potier, P. F.; Doronina, S.; Huc, I.; Behr, J. P. A High-Yield Synthesis of Deoxy-2-Fluoroinosine and its Incorporation into Oligonucleotides. *Tetrahedron Lett.* **1997**, *38*, 2989−2992.
(10) Knapp, S.; Hale, J.; Bastos, M.; Molina, A.; Cheng, K Y. Synthesis of Hypusine and Other Polyamines Using Dibenzyltriazones for Amino Protection. *J. Org. Chem.* **1992**, *57*, 6239−6256. However, we prepared the racemic amino diol **4** according to Scheme 3.
(11) Harris, T. M.; Harris, C. M.; Horton, P.; Cooper. M. D.; Kuchimanchi, S.; Tsarouhtsis, D.; DeCorte, B. L. Improved Strategies for Postoligomerization Synthesis of Oligodeoxynucleotides Bearing Structurally Defined Adducts at the N^2 Position of Deoxyguanosine. *Chem. Res. Toxicol.* **1996**, *9*, 630−637.
(12) Himmelsbach, F.; Schulz, B. S.; Trichtinger, T.; Charubala, R.; Pleiderer, W. The *p*-Nitrophenylethyl (NPE) Group: A Versatile New Blocking Group for Phosphate and Aglycone Protection in Nucleosides and Nucleotides. *Tetrahedron* **1984**, *40*, 59−72.
(13) The formation of two hydroxyl isomers might have been expected here as was found by Guengerich and his associates (ref 16) for the corresponding lower homologue the 1,N^2-hydroxyethano-2′-deoxyguanosine. Nevertheless, the HPLC data and the NMR spectra indicate that this is probably a single compound. Further work to characterize this material is in progress.
(14) Beaucage, S. L. Protocols for Oligonucleotides and Analogues. in *Methods in Molecular Biology*; Agrawal, S., Ed.; Humana Press Inc.: Totowa, NJ, 1993.
(15) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory: Plainview, NY, 1989.
(16) Langouët, S.; Müller, M.; Guengerich, F. P. Misincorporation of dNTPs Opposite 1,N^2-Ethenoguanine and 5,6,7,9-tetrahydro-7-hydroxy-9-oxoimidazo[1,2-a]purine in Oligonucleotides by *Escherichia coli* Polymerase I exo$^-$ and II exo$^-$, T7 Polymerase exo$^-$, Human Immunodeficiency Virus−1 Reverse Transcriptase, and Rat Polymerase β. *Biochemistry* **1997**, *36*, 6069−6079.

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