NMR Characterization of a DNA Duplex Containing the Major Acrolein-derived Deoxyguanosine Adduct γ-OH-1, N²-Propano-2'-deoxyguanosine*

Carlos de los Santos‡, Tanya Zaliznyak, and Francis Johnson
From the Department of Pharmacological Sciences, State University of New York at Stony Brook, New York 11794-8651

The environmental and endogenous mutagen acrolein reacts with cellular DNA to produce several isomeric 1,N²-propanodeoxyguanosine adducts. High resolution NMR spectroscopy was used to establish the structural features of the major acrolein-derived adduct, γ-OH-1,N²-propano-2'-deoxyguanosine. In aqueous solution, this adduct was shown to assume a ring-closed form. In contrast, when γ-OH-1,N²-propano-2'-deoxyguanosine adducts with dC at the center of an 11-mer oligodeoxynucleotide duplex, the exocyclic ring opens, enabling the modified base to participate in a standard Watson-Crick base pairing alignment. Analysis of the duplex spectra reveals a regular right-handed helical structure with all residues adopting an anti orientation around the glycosidic torsion angle and Watson-Crick alignments for all base pairs. We conclude from this study that formation of duplex DNA triggers the hydrolytic conversion of γ-OH-1,N²-propano-2'-deoxyguanosine to an open chain form, a structure that facilitates pairing with dC during DNA replication and accounts for the surprising lack of mutagenicity associated with this DNA adduct.

Acrolein is a ubiquitous environmental pollutant formed by incomplete combustion of organic materials, including wood, food, tobacco, and fuels. This α,β-unsaturated aldehyde reacts to form hydroxylated 1,N²-propano-2'-deoxyguanosine adducts in DNA (1–3). Acrolein also is formed endogenously during the metabolic oxidation of polyamines (4) and is an end product of lipid peroxidation (5–7). Acrolein also is formed endogenously during the metabolic oxidation of polyamines (4) and is an end product of lipid peroxidation (5–7). Acrolein also is formed endogenously during the metabolic oxidation of polyamines (4) and is an end product of lipid peroxidation (5–7).

References

Published, JBC Papers in Press, October 27, 2000, DOI 10.1074/jbc.M009028200

Received for publication, October 3, 2000

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

* This work was supported by National Institutes of Health Grants CA47995 and CA77094. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed: Dept. of Pharmacological Sciences, Basic Science Tower, 7th Fl., Rm. 147, State University of New York, Stony Brook, NY 11794-8651. Tel.: 631-444-3649; Fax: 631-444-3218; E-mail: cds@pharm.sunysb.edu.
§ The abbreviations used are: γ-OH-PdG, γ-OH-1,N²-propano-2'-deoxyguanosine; TSP, (2,2,3,3-d₄)sodium 3-trimethylsilyl-propionate; NOESY, nuclear Overhauser effect spectroscopy; COSY, correlation spectroscopy; TOCSY, total correlation spectroscopy; NOE, nuclear Overhauser effect; dC, 2'-deoxycytidine; dA, 2'-deoxyadenosine; dG, 2'-deoxyguanosine; T, thymidine; acr, acrolein.

Acrolein-derived Deoxyguanosine Adduct
No base line correction was applied to the transformed spectra. A three-dimensional model of the acr-dG-dC duplex was built using INSIGHTII (Biosym Technologies, Inc.) by replacing the nonhydrogen-bonded amino proton of a deoxyguanosine residue at the sixth position of a B-form 11-mer duplex for the γ-OH-propyl moiety. Using the conjugate gradient method, this model was energy-minimized to ensure that distances between Ha/Hs’ and Hb/Hb’ protons of γ-OH-PdG and the H1’ protons of C7 and G18 residues were within the observable NOE range (see text). Energy minimization was performed on Silicon Graphics computers using the program X-PLOR 3.851 (33).

RESULTS

NMR Characterization of the acr-dG-dC Duplex: Nonexchangeable Protons—At pH values over 6.5, the one-dimensional proton spectrum of the acr-dG-dC duplex displays a main set of sharp signals manageable for NMR characterization. Below this pH value, a second conformation of the duplex in solution is evident by the presence of minor resonances that become stronger as the pH is reduced (see Fig. 5 below). Therefore, assignment of the proton signals follows the examination of NOESY and COSY spectra collected at pH 6.5 using standard analysis procedures (34, 35). Fig. 2 shows an expanded region of a NOESY spectrum (300-ms mixing time) recorded in 100% D2O buffer at 30 °C, depicting interactions between the base and the H1’ proton regions. Indicative of a right-handed helix, each base proton (purine H8 or pyrimidine H6) shows NOE cross-peaks to the H1’ proton of the ipso and 5’-flanking sugar residues. At the center of the duplex these NOE interactions can be traced without interruption, suggesting that the presence of γ-OH-PdG does not cause large perturbations of the double-helix structure. In addition, the intensity of intra-residue base-H1’ NOE peaks is much weaker than that of the H5-H6 cross-peaks of cytosine residues suggesting an anti-conformation around the glycosidic torsion angle for all residues of the acr-dG-dC duplex (35). Additional evidence of a regular right-handed helix is the observation of NOE peaks between each cytosine (H5) and the base proton of its 5’-side neighbor (Fig. 2, peaks A–F). Analogous directionality of NOE interactions is present between the base and sugar H3’, H2’, H2’’ protons in other regions of the same spectrum (regions not shown). Similarly, nonexchangeable protons of the central (A4 C5 acr-dG C7 A8) (T15 G16 C17 G18 T19) segment have chemical shift values almost identical to those of the corresponding unmodified control duplex, indicating only a minor deviation from the canonical DNA conformation. Chemical shifts of the nonexchangeable protons of the acr-dG-dC duplex measured at 30 °C are listed in Table 1.

Identification of the proton signals of the propyl bridge follows from the analysis of COSY, TOCSY, and NOESY spectra collected in 100% D2O buffer solutions. In the 300-ms mixing time NOESY spectrum, a proton signal at 4.93 ppm, assigned to H7, displays NOE cross-peaks to the overlapping Hb/Hb’ protons as well as the Ha/Hs’ protons within the propyl moiety (Fig. 3A, peaks A and B, respectively). Accordingly, in a TOCSY (120-ms mixing time) spectrum recorded under identical temperature and pH conditions, cross-peaks are present between these same proton signals (Fig. 3B, peaks A and B, respectively), and among the Ha, Ho’, Hs’, and Hb/Hb’ protons of γ-OH-PdG (region not shown). An intriguing observation is the simultaneous presence of NOE peaks between the Hb/Hb’ of the aduct and the H1’ protons of G18 and C7 residues located in opposite strands of the duplex (Fig. 3A, peaks E and C, respectively). Besides this, the presence of a sharp nonexchangeable proton signal is evident at 9.58 ppm, at 30 °C, and slightly upfield at 5 °C (see Fig. 5 below), in a region of the spectrum that is normally devoid of proton signals associated with the duplex. This minor signal shows no cross-peak to any exchangeable or nonexchangeable proton of the duplex and,
based on its chemical shift, is assigned to a small percentage of the aldehydic open form of γ-OH-PdG (see Fig. 6 below).

Exchangeable Protons—In the sample dissolved in 10% D2O buffer, the 1D proton spectrum shows 11 imino proton signals resonating between 12.0 and 14.0 ppm, in the Watson-Crick region (see Fig. 5 below). Sequence-specific assignment of the exchangeable proton signals results from the analysis of a NOESY (220-ms mixing time) spectrum collected at 2 °C (pH 6.5). Fig. 4 shows expanded contour plots depicting NOE interactions between the imino and the amino/base proton regions of this spectrum. Each thymine imino proton displays a strong NOE interaction to the H2 proton of the corresponding adenine partner, thus establishing the formation of Watson-Crick alignments for all A-T base pairs of the duplex (Fig. 4, peaks A–D). Similarly, the presence of NOE cross-peaks between the guanine imino and the amino protons of the cytosine partner indicates the formation of Watson-Crick alignments in all non-lesion-containing G-C base pairs of the duplex (Fig. 4, peaks E, E', I, and T'). Surprisingly, a remaining imino proton signal at 12.64 ppm, which is originated at the acr-dGz dC pair of the duplex, displays strong NOE cross-peaks with three different amino proton signals. Based on interactions to the previously assigned C17(H5) proton and their strong NOE connectivity, which is only observed in 10% D2O buffer, two of these signals are readily assigned to the amino protons of the lesion-partner C17 residue. Thus, peaks J and J' in Fig. 4 originate from NOE interactions between the imino and the amino/base proton regions of this spectrum. Each thymine imino proton displays a strong NOE interaction to the H2 proton of the corresponding adenine partner, thus establishing the formation of Watson-Crick alignments for all A-T base pairs of the duplex (Fig. 4, peaks A–D). Similarly, the presence of NOE cross-peaks between the guanine imino and the amino protons of the cytosine partner indicates the formation of Watson-Crick alignments in all non-lesion-containing G-C base pairs of the duplex (Fig. 4, peaks E, E', I, and T'). Surprisingly, a remaining imino proton signal at 12.64 ppm, which is originated at the acr-dGz dC pair of the duplex, displays strong NOE cross-peaks with three different amino proton signals. Based on interactions to the previously assigned C17(H5) proton and their strong NOE connectivity, which is only observed in 10% D2O buffer, two of these signals are readily assigned to the amino protons of the lesion-partner C17 residue. Thus, peaks J and J' in Fig. 4 originate from NOE interactions between the imino and the amino/base proton regions of this spectrum. Each thymine imino proton displays a strong NOE interaction to the H2 proton of the corresponding adenine partner, thus establishing the formation of Watson-Crick alignments for all A-T base pairs of the duplex (Fig. 4, peaks A–D). Similarly, the presence of NOE cross-peaks between the guanine imino and the amino protons of the cytosine partner indicates the formation of Watson-Crick alignments in all non-lesion-containing G-C base pairs of the duplex (Fig. 4, peaks E, E', I, and T'). Surprisingly, a remaining imino proton signal at 12.64 ppm, which is originated at the acr-dGz dC pair of the duplex, displays strong NOE cross-peaks with three different amino proton signals. Based on interactions to the previously assigned C17(H5) proton and their strong NOE connectivity, which is only observed in 10% D2O buffer, two of these signals are readily assigned to the amino protons of the lesion-partner C17 residue. Thus, peaks J and J' in Fig. 4 originate from NOE interactions between the imino and the amino/base proton regions of this spectrum. Each thymine imino proton displays a strong NOE interaction to the H2 proton of the corresponding adenine partner, thus establishing the formation of Watson-Crick alignments for all A-T base pairs of the duplex (Fig. 4, peaks A–D). Similarly, the presence of NOE cross-peaks between the guanine imino and the amino protons of the cytosine partner indicates the formation of Watson-Crick alignments in all non-lesion-containing G-C base pairs of the duplex (Fig. 4, peaks E, E', I, and T'). Surprisingly, a remaining imino proton signal at 12.64 ppm, which is originated at the acr-dGz dC pair of the duplex, displays strong NOE cross-peaks with three different amino proton signals. Based on interactions to the previously assigned C17(H5) proton and their strong NOE connectivity, which is only observed in 10% D2O buffer, two of these signals are readily assigned to the amino protons of the lesion-partner C17 residue. Thus, peaks J and J' in Fig. 4 originate from NOE interactions between the imino and the amino/base proton regions of this spectrum. Each thymine imino proton displays a strong NOE interaction to the H2 proton of the corresponding adenine partner, thus establishing the formation of Watson-Crick alignments for all A-T base pairs of the duplex (Fig. 4, peaks A–D). Similarly, the presence of NOE cross-peaks between the guanine imino and the amino protons of the cytosine partner indicates the formation of Watson-Crick alignments in all non-lesion-containing G-C base pairs of the duplex (Fig. 4, peaks E, E', I, and T'). Surprisingly, a remaining imino proton signal at 12.64 ppm, which is originated at the acr-dGz dC pair of the duplex, displays strong NOE cross-peaks with three different amino proton signals. Based on interactions to the previously assigned C17(H5) proton and their strong NOE connectivity, which is only observed in 10% D2O buffer, two of these signals are readily assigned to the amino protons of the lesion-partner C17 residue. Thus, peaks J and J' in Fig. 4 originate from NOE interactions between the imino and the amino/base proton regions of this spectrum. Each thymine imino proton displays a strong NOE interaction to the H2 proton of the corresponding adenine partner, thus establishing the formation of Watson-Crick alignments for all A-T base pairs of the duplex (Fig. 4, peaks A–D). Similarly, the presence of NOE cross-peaks between the guanine imino and the amino protons of the cytosine partner indicates the formation of Watson-Crick alignments in all non-lesion-containing G-C base pairs of the duplex (Fig. 4, peaks E, E', I, and T'). Surprisingly, a remaining imino proton signal at 12.64 ppm, which is originated at the acr-dGz dC pair of the duplex, displays strong NOE cross-peaks with three different amino proton signals. Based on interactions to the previously assigned C17(H5) proton and their strong NOE connectivity, which is only observed in 10% D2O buffer, two of these signals are readily assigned to the amino protons of the lesion-partner C17 residue. Thus, peaks J and J' in Fig. 4 originate from NOE
interactions between acr-dG(N1H) imino and C17(N4H2) protons. The third NOE cross-peak originates from the interaction between acr-dG(N1H) and acr-dG(N2H2) protons of the adduct (Fig. 4, peak K). These connectivities are only possible when the adduct exists in a ring-opened state so that the lesion-containing base pair adopts the standard Watson-Crick alignment. Consistent with these assignments and supporting the open form of γ-OH-PdG, N1H and N2H display NOE cross-peaks to the Ho/Hα' and Hά/Hβ' protons of the propyl chain (Fig. 4, peaks Q–T).

Evidence of base stacking is seen in the connectivities between the adenine H2 protons and the imino protons of the flanking base pairs (Fig. 4, peaks M–O) and those among the imino protons of the duplex (region not shown). Likewise, the strong NOE peak between the amino proton of the adduct and G16(N1H) at the 3′-flanking base pairs indicates proper stacking of γ-OH-PdG inside the duplex (Fig. 4, peak L). Chemical shifts of the exchangeable protons of the acr-dGdc duplex measured at 2 °C are listed in Table I.

Proton Spectra of the γ-OH-PdG Nucleoside—The unexpected observation that γ-OH-PdG exists in an open form in the duplex prompted us to investigate its state at the nucleoside level. In contrast to observations made with the duplex sample, no proton signals are observed around 9.60 and 4.90 ppm (Fig. 5B). The analysis of a TOCSY spectrum of the nucleoside dissolved in 100% D2O buffer, pH 6.5, 30 °C, reveals that the Ho/Hα', Hά/Hβ', and Hγ protons resonate at 3.52/3.48, 2.22/1.92, and 6.36 ppm, respectively (data not shown). These chemical shift values are slightly downfield from those previously reported for the adduct dissolved in dimethyl sulfoxide (30, 36) and suggest a prevalent closed state for the γ-OH-PdG nucleoside in water. In addition, the exocyclic form of the adduct is insensitive to pH changes and only the ring-closed state is observed under a wide range of values (Fig. 5B).

Upon duplex formation, the chemical shifts of protons on the propyl chain move significantly upfield, especially Hγ, that changes from 3.63 ppm in the nucleoside to 4.93 ppm in the duplex. This chemical shift value, which is inconsistent with the aldehyde proton of the lesion that resonates at 9.58 ppm, is ascribed to the Hγ proton of the propyl chain in which the carbonyl group is present in the hydrated form (dihydroryl) of the adduct (Fig. 6). The relative population of these two forms is dependent on the pH of the sample, the aldehyde form being favored by basic conditions. Apart from these states of γ(OH)-PdG, an alternative conformation of the acr-dGdc duplex, which may involve protonated cytosine residues, becomes evident at pH 6.4 and lower values (Fig. 5A). Proton chemical shifts of the γ(OH)-1, N2-PdG nucleoside are listed in Table II.

**DISCUSSION**

**Solution Conformations of the acr-dGdc Duplex—**Early in the course of these studies it became evident that the acr-dGdc duplex adopts a single conformation only at neutral or basic pH (Fig. 5). However, adduct-containing sequences are unstable to the basic conditions used during sample purification, which promote oligomer polymerization (data not shown). Therefore,
we chose to conduct our studies at pH 6.5 where the acr-dG-dC duplex is in the conformation present at basic pH. The directionality of sequential NOE interactions indicates that this conformation is a double-stranded helix with residues adopting an anti orientation around the glycosidic bond (Fig. 2). The pattern of NOE peaks observed for the exchangeable imino protons establish that all base pairs of the acr-dG-dC duplex have a Watson-Crick alignment (Fig. 4). At the lesion-containing base pair, this becomes possible only if γ-OH-PdG adduct exists as an open form with the N²-propyl chain pointing away from the helix and toward the solvent. In this conformation, the Hα/Hα' and Hβ/Hβ' protons of γ-OH-PdG are found in the minor groove of the helix, close to H1' protons of residues in both strands of the duplex (Fig. 3A, peaks B, C, and D), and its Watson-Crick edge remains accessible forming a fully hydrogen-bonded acr-dG-dC base pair (Fig. 4, peaks J, J', and K). These structural characteristics are readily fulfilled within a regular B-form helix, as shown by the energy-minimized model of the acr-dG-dC duplex (Fig. 7).

Duplex DNA Induces Ring Opening—Spectroscopic data of the γ-OH-PdG nucleoside in aqueous solutions establish a pH-independent 1,N²-closed conformation for the adduct (Fig. 5B), suggesting that duplex formation catalyzes the rearrangement of the propyl bridge to an open form. An analogous transformation was described recently for DNA duplexes in which the deoxyguanosine-malondialdehyde adduct M1G is positioned opposite dC (37). However, a “canonical” Watson-Crick base pair forms only in the case of γ-OH-PdG. This difference may explain in part why M1G is mutagenic in bacteria (38), whereas γ-OH-PdG is not (see accompanying articles (39, 40)). The role of the partner base in promoting ring opening of γ-OH-PdG adducts will be the subject of future investigations.

Comparison with Duplexes Containing PdG—An unsubstituted 1,N²-propano-2-deoxyguanosine adduct has been used extensively in biological (17–22) and structural (23–27) studies as a model for natural acrolein lesions. PdG tends to adopt the syn conformation when the adduct is positioned opposite dG at neutral pH and when dA or dC residues in the complementary strand are protonated under acidic conditions (23–25). The syn

![FIG. 6. Chemical rearrangement exerted by γ-(OH)-PdG. The exocyclic form present on the free nucleoside can add a water molecule to afford the hydrated open conformation observed in the acr-dG-dC duplex. Alternatively, chemical rearrangement of γ-OH-PdG produces the N²-(γ-exopropyl) configuration of the adduct. The two open forms of the adduct reach equilibrium with the hydrated structure favored at neutral basic solutions.](image)

**TABLE II**

| Proton chemical shifts of γ-(HO)-PdG |
|-------------------------------------|
| Values are given in ppm relative to TSP. Chemical shifts were recorded in phosphate buffer (10 mM), pH 6.5, containing 50 mM NaCl. Nonexchangeable protons are at 30 °C; exchangeable protons are at 5 °C. |
| Free nucleoside | Duplex DNA |
|-----------------|-----------|
| H8              | 7.88      |
| Hα/Hα'          | 3.52/3.48 |
| Hβ/Hβ'          | 2.22/1.92 |
| Hγ              | 6.36      |
| N1H             | 12.64     |
| N2H             | 7.38      |
| H1'             | 6.22      |
| H2'             | 2.45      |
| H2''            | 2.73      |
| H3'             | 4.57      |
| H4'             | 4.06      |
| H5'/H5''        | 3.77/3.75 |
| NA              |           |

* Value on the aldehydic form.
* NA, not assigned.

![FIG. 7. Left, central segment of the energy-minimized model of the acr-dG-dC duplex having the adduct in the hydrated configuration. The adduct stays in the minor groove of the helix pointing the dihydroxypropyl moiety directly toward the solvent. The model fulfills short distances detected as NOE cross-peaks and predicts the formation of a strong hydrogen bond between acr-dG(OγH) and C17(O4') (yellow dotted lines). Right, view from the top of the helical axis showing Watson-Crick alignment at the acr-dG-dC base pair.](image)
conformation permits formation of hydrogen-bonded base pairs through the Hoogsteen edge of the adduct while stacking with flanking residues. Results of the present study establish a fundamental difference between γ-OH-PdG and PdG in that, under appropriate conditions, the former can undergo a chemical rearrangement in aqueous solution to assume an open chain form. Thus, when γ-OH-PdG is in an anti conformation, a fully hydrogen-bonded acr-dGdc base pair exists at neutral/basic pH values, which does not perturb the duplex structure (Fig. 7). However, at acidic pH, the spectra of the acr-dGdc duplex show exchangeable proton signals that appear to originate from the amino group of a $C^+$ residue (Fig. 5A). Considering the strong tendency of PdG to adopt a syn conformation, it is likely that, at acidic pH, the duplex contains a syn γ-OH-PdG adduct paired to a protonated cytosine residue forming an alignment similar to the one described for PdGdc (25). The structural characteristics of this conformation in the acr-dGdc duplex is currently under investigation.

**Biological Implications**—Two laboratories have performed primer extension and site-specific mutagenesis studies in bacteria using DNA containing γ-OH-PdG. Synthesis past the lesion is reduced indicating that γ-OH-PdG blocks DNA synthesis and, when translesional synthesis occurs, dCMP is incorporated opposite the lesion almost exclusively (see accompanying articles (39, 40)). The present study provides structural grounds for understanding this behavior. At the replication fork γ-OH-PdG would adopt the closed $1,N^2$-exocyclic form described for the free nucleoside in solution. As with PdG, this conformation of the adduct is expected to hinder incorporation of DAMP, dGMP, and TMP, resulting in the inhibition of DNA synthesis. However, incorporation of dCMP opposite γ-OH-PdG would trigger the chemical rearrangement from the exocyclic closed form of the adduct to an opened conformation. The subsequent formation of a replication structure stabilized by Watson-Crick hydrogen bonds would facilitate rapid extension of the γ-OH-PdGdc pair resulting in error-free translesional DNA synthesis. Thus, chemical rearrangement of γ-OH-PdG to an open form during DNA synthesis would account for the lack of mutagenicity observed with the major acrolein-derived 2'-deoxyguanosine adduct in bacteria.

**Acknowledgments**—We thank Cecilia Torres for the synthesis and purification of modified oligodeoxynucleotides and Arthur P. Grollman for critical reading of this manuscript.

**REFERENCES**

1. World Health Organization Publications (1992) The WHO Environmental Health Criteria Series, Vol. 127

2. Galliani, G., and Pantarotto, C. (1983) *Tetrahedron Lett.* 24, 4491–4492

3. Chung, F. L., Young, R., and Hecht, S. S. (1984) *Cancer Res.* 44, 990–995

4. Lee, Y., and Sayre, L. M. (1998) *J. Biol. Chem.* 273, 19490–19494

5. Esterbauer, H., Schaur, R. J., and Zoller, H. (1991) *Free Radic. Biol. Med.* 11, 81–128

6. Wu, H.-Y., and Lin, Y.-L. (1995) *Anal. Chem.* 76, 1603–1612

7. Chung, F. L., Nath, R. G., Nagao, M., Nishikawa, A., Zhou, G. D., and Rand-erath, K. (1999) *Mutat. Res.* 423, 71–81

8. Nath, R. G., and Chung, F.-L. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 7491–7495

9. Nath, R. G., Ocando, J. E., and Chung, F.-L. (1996) *Cancer Res.* 56, 452–456

10. Chung, F.-L., Zhang, L., Ocando, J. E., and Nath, R. G. (1999) *JARC Sci. Publ.* 150, 45–54

11. Alarcon, R. A. (1976) *Cancer Treat. Rep.* 60, 327–335

12. McDermid, M. A., Iype, P. T., Kolodner, K., Jacobson-Kram, D., and Strickland, P. T. (1991) *Mutat. Res.* 248, 93–99

13. Marnett, L. J., Hurd, H. K., Hollstein, M. C., Levin, D. E., Esterbauer, H., and Ames, B. N. (1985) *Mutat. Res.* 148, 25–34

14. Curren, R. D., Yang, L. L., Cooklin, P. M., Grafostron, R. C., and Harris, C. C. (1988) *Mutat. Res.* 209, 17–22

15. Smith, R. A., Cohen, S. M., and Lawson, T. A. (1990) *Carcinogenesis* 11, 497–498

16. Kawasaki, M., Matsuda, T., Nakayama, A., Takebe, H., Matsui, S., and Yagi, T. (1998) *Mutat. Res.* 417, 63–75

17. Shihbutsi, S., and Grollman, A. P. (1993) *J. Biol. Chem.* 268, 11703–11710

18. Hashim, M. F., and Marnett, L. J. (1996) *J. Biol. Chem.* 271, 9160–9165

19. Hashim, M. F., Schnez-Boutaud, N., and Marnett, L. J. (1997) *J. Biol. Chem.* 272, 20205–20212

20. Benamira, M., Singh, U., and Marnett, L. J. (1992) *J. Biol. Chem.* 267, 22392–22400

21. Moriya, M., Zhang, W., Johnson, F., and Grollman, A. P. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 11899–11903

22. Burcham, P. C., and Marnett, L. J. (1994) *J. Biol. Chem.* 269, 28844–28850

23. Kouchakdjan, M., Marinnelli, E., Gao, X. L., Johnson, F., Grollman, A., and Patel, D. (1989) *Biochemistry* 28, 5647–5657

24. Huang, P., and Eisenberg, M. (1992) *Biochemistry* 31, 6518–6532

25. Singh, U. S., Moe, J. G., Reddy, G. R., Weisenseel, J. P., Marnett, L. J., and Stone, M. P. (1993) *Chem. Res. Toxicol.* 6, 825–836

26. Kouchakdjan, M., Eisenberg, M., Liv, D., Marinnelli, E., Grollman, A. P., and Patel, D. J. (1990) *Biochemistry* 29, 4456–4465

27. Huang, P., Patel, D. J., and Eisenberg, M. (1993) *Biochemistry* 32, 3852–3866

28. Plum, G. E., Grollman, A. P., Johnson, F., and Breslauer, K. J. (1992) *Biochemistry* 31, 12096–12102

29. Khullar, S., Varaprasad, C. V., and Johnson, F. (1999) *J. Med. Chem.* 42, 947–950

30. Neebe, L. V., Harris, C. M., and Harris, T. M. (2000) *Chem. Res. Toxicol.* 13, 421–429

31. States, D. J., Habekorn, R. A., and Ruben, D. J. (1982) *J. Magn. Res.* 48, 286–292

32. Plateau, P., and Gueron, M. (1982) *J. Am. Chem. Soc.* 104, 7310–7311

33. Brunger, A. (1993) *X-FLOR, Version 3.1: A system for X-Ray Crystallography and NMR*, Yale University Press, New Haven, CT

34. van de Ven, J. M., and Hillebre, C. W. (1988) *Eur. J. Biochem.* 176, 1–18

35. de los Santos, C. (1999) in *Comprehensive Natural Products Chemistry*, vol 7: DNA and Aspects of Molecular Biology (Barton, D., Nakanishi, K., and Meth-Cohn, O., eds) pp. 55–80, Elsevier Science Ltd., Oxford, UK

36. Boerth, D. W., Eder, E., Hussain, S., and Hoffman, C. (1999) *Chem. Res. Toxicol.* 11, 284–294

37. Mao, H., Schnetz-Boutaud, N. C., Weisenseel, J. P., Marnett, L. J., and Stone, M. P. (1998) *Mutat. Res.* 409, 261–265

38. Bouchard, B. P., and Marnett, L. J. (2001) *J. Med. Chem.* 44, 1609–1612

39. Chung, F. L., Nath, R. G., Nagao, M., Nishikawa, A., Zhou, G. D., and Rand-erath, K. (1999) *Mutat. Res.* 423, 71–81

40. VanderVeen, L. A., Hashim, M. F., Nechev, L. V., Harris, T. M., Harris, C. M., and Marnett, L. J. (2001) *J. Biol. Chem.* 276, 9066–9070