Structure Elucidation of the Adducts Formed by Fjord Region Dibenzo[a,l]pyrene-11,12-dihydriodol 13,14-Epoxides with Deoxyguanosine

Kai-Ming Li,† Mathai George,§ Michael L. Gross,§ Cheng-Huang Lin,** Ryszard Jankowiak,‡ Gerald J. Small,†,‡ Albrecht Seidel,‡ Heiko Kroth,† Eleanor G. Rogan,† and Ercole L. Cavalieri*‡,†

Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, Nebraska 68198-6805, Department of Chemistry, Washington University, One Brookings Drive, St. Louis, Missouri 63130-4899, Department of Chemistry and Ames Laboratory-U.S. Department of Energy, Iowa State University, Ames, Iowa 50011, and Institute of Toxicology, University of Mainz, Mainz, Germany

Received October 21, 1998

(±)-anti-Dibenzo[a,l]pyrene-11,12-dihydriodol 13,14-epoxide ((±)-anti-DB[a,l]PDE) was reacted with deoxyguanosine (dG) in dimethylformamide at 100 °C for 30 min, and two sets of adducts were isolated: a mixture of (±)-anti-cis- & -trans-N2dG (43%) and a mixture of (±)-anti-cis- & -trans-N7Gua (45%). Both are mixtures of four stereoisomers that cannot be separated by HPLC. Similarly, (±)-syn-DB[a,l]PDE was reacted with dG under the same conditions, and (±)-syn-cis- & -trans-N2dG (38%) and (±)-syn-cis- & -trans-N7Gua (59%) were obtained. The structures of the adducts were determined by a combination of NMR and fast atom bombardment mass spectrometry. By reacting (−)-anti-DB[a,l]PDE or (+)-syn-DB[a,l]PDE with dG under the same conditions, however, optically pure N2dG and N7Gua isomers were obtained: (−)-anti-cis-N2dG (12%), (−)-anti-trans-N2dG (17%), (−)-anti-trans-N7Gua (43%), (+)-syn-cis-N2dG (7%), (+)-syn-trans-N2dG (3%), (+)-syn-cis-N7Gua (36%), and (+)-syn-trans-N7Gua (22%). The structures of the optically pure adducts were assigned by NMR. syn- and anti-DB[a,l]PDE–N2dG adducts can be distinguished by fluorescence line-narrowing spectroscopy (FLNS). Moreover, distinction between cis- and trans-stereochemistry of the adducts is also straightforward by FLNS, because the FLN spectra for the four DB[a,l]PDE–N2dG adducts, anti-cis, anti-trans, syn-cis, and syn-trans, are spectroscopically unique.

Introduction

Polycyclic aromatic hydrocarbons (PAH)† are activated by two major pathways, one-electron oxidation with formation of radical cations and monoxygenation with formation of diol epoxides, or, more frequently, a combination of both pathways (1, 2). In DNA, the Ade and Gua bases are the most frequent targets of metabolically activated PAH. Electrophilic attack by PAH radical cations and diol epoxides at the N-3 and N-7 of Ade or the N-7 and sometimes C-8 of Gua leads to formation of depurinating adducts that are lost from DNA by cleavage of the glycosidic bond (2). When reaction occurs at the exocyclic amino group of deoxyguanosine (dG) or deoxyadenosine (dA), the adducts obtained are stable and remain in DNA.

Investigating the postulated mechanism of metabolic activation of dibenzo[a,l]pyrene (DB[a,l]P) requires reference adducts formed by DB[a,l]P radical cation and by fjord region DB[a,l]P-11,12-dihydriodol 13,14-epoxides (DB[a,l]PDE). Synthesis of DB[a,l]P adducts by one-electron oxidation was already reported (3, 4). In addition, adducts formed by DB[a,l]PDE with dA were recently synthesized (5). In this article, we report the synthesis and structure elucidation of the adducts formed by reaction of DB[a,l]PDE with dG.

Stable and depurinating adducts of DB[a,l]P were obtained by microsomal activation of this compound in the presence of DNA (6). Furthermore, stable dA and dG adducts of DB[a,l]PDE have been identified in cell culture, indicating that DB[a,l]P is stereoselectively converted to the (+)-syn- and (−)-anti-DB[a,l]PDE with 11S,12R,13S,14R- and 11R,12S,13S,14R-configurations, respectively (7, 8).

Fluorescence line-narrowing spectroscopy (FLNS) can be used for fingerprint identification of various PAH–DNA adducts and PAH metabolites (9–12), and the combination of FLNS and non-line-narrowing (NLN) fluorescence spectroscopy provides conformational information (13–16). Spectral characterization of DB[a,l]PDE–N2dG and DB[a,l]PDE–N4dA standard adducts is
essential for identification of adducts derived from syn-and anti-DB[a]PDE and provides the necessary reference information for studying DB[a]PDE–DNA adducts formed in vitro and in vivo. In this article, we also report the characterization of anti-trans-, anti-cis-, syn-trans-, and syn-cis-DB[a]PDE–N2dG adducts by FLNS. The results obtained for the corresponding DB[a]PDE–N6-dA adducts will be published elsewhere (17). Differences in the FLN spectra for the four N2dG adducts provide a means for selective, unambiguous identification of the four adducts after their formation in biological systems.

**Experimental Section**

**Caution.** DB[a]PDE are hazardous chemicals and were handled according to NIH guidelines (18).

**General Procedures.** All of the procedures for UV, NMR, fast atom bombardment mass spectrometry (FAB MS), and HPLC were the same as previously described (5). The spectra of the (±)-anti-cis- & trans- and (±)-syn-cis- & -trans adducts were recorded in Me2SO-d6 at 25 °C, whereas the spectra of the (−)-anti and (+)-syn adducts were recorded in Me6SO-d2/D2O at 25 °C. The procedure for FLNS analysis of DB[a]PDE–N2-dG adducts was the same as that described in previous publications (16, 17, 19).

**Chemicals.** (±)-anti-DB[a]PDE and (±)-syn-DB[a]PDE were obtained from ChemSyn Science Laboratories (Lenexa, KS). (−)-anti-DB[a]PDE and (+)-syn-DB[a]PDE were synthesized as previously described (20, 21). The dG was purchased from Aldrich (Milwaukee, WI) and was desiccated over P2O5 under vacuum at 110 °C for 48 h prior to use. Commercially available dimethylformamide (DMF; Aldrich) was purified by refluxing over CaH2, followed by distillation under vacuum and was stored over 4 Å molecular sieves under argon.

**Chemical Synthesis of DB[a]PDE Adducts.** The reaction of (±)-anti-DB[a]PDE is described here as an example. The same methods were used for reaction of the other DB[a]PDE. (±)-anti-DB[a]PDE (5 mg, 0.0142 mmol) was dissolved in 1 mL of dry DMF at room temperature under argon. The dG (35 mg, 0.133 mmol) was added to the above solution, and the reaction was carried out at 100 °C for 30 min. DMF was then removed under vacuum; the residue was dissolved in Me6SO-de at 110 °C for 48 h prior to use. Commercially available dimethylformamide (DMF; Aldrich) was purified by refluxing over CaH2, followed by distillation under vacuum and was stored over 4 Å molecular sieves under argon.

**Table 1. Adducts Obtained by Reaction of (±)-anti-DB[a]PDE with dG in DMF at 100 °C for 30 min**

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<th>Product</th>
<th>Yield (%)</th>
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</table>

* Only trace amounts of (−)-anti-cis-N7Gua were obtained.
exchanged with D2O). J = 6.20 (bs, 1H, 2-H), 7.29 (bs, 1H, 7-H, J = 7.5 Hz), 8.23 (1H, 4-H), 8.38 (1H, 4-H, J = 3.4 Hz = 8.0 Hz). 8.88 (d, 1H, 4-H, J = 7.5 Hz).

(11,12,13,14-Trihydroxy-14-N7Gua-11,12,13,14-tetrahydro-DB[a]JP [(--)-anti-cis & -trans-N7Gua]: UV λmax 243, 278 (sh), 286, 295 (sh), 328, 342 nm; 1H NMR δ 3.95 (d, 1H, 12-H), 3.98 (m, 1H, 11-H, J = 10.0 Hz), 4.91 (d, 1H, 11a-H, J 11,a,11 = 10.0 Hz). 8.02 (1H, 7-H, J = 7.5 Hz), 8.04 (1H, 6-H, J = 7.5 Hz), 8.07 (2H, 8-H, J = 8.5 Hz). 8.23 (1H, 2-H, J = 8.0 Hz), 8.38 (1H, 4-H). 8.48 (d, 1H, 4-H, 3.4 Hz = 8.0 Hz). 8.94 (d, 1H, 4-H, J = 7.5 Hz), 8.98 (d, 1H, 4-H, J = 7.5 Hz).

(11,12,13,14-Trihydroxy-14-N7dG-11,12,13,14-tetrahydro-DB[a]JP [(--)-syn-cis & -trans-N7dG]: UV λmax 242, 286 (sh), 290, 297 (sh), 321, 344 nm; 1H NMR δ 3.75 (d, 1H, 12-H), 3.78 (bs, 1H, 11-H, J = 10.0 Hz), 4.05 (sh, 1H, 11a-H, J = 10.0 Hz). 8.02 (1H, 7-H, J = 7.5 Hz), 8.06 (1H, 6-H, J = 7.5 Hz), 8.16 (1H, 5-H, J = 6.20 Hz).

(11,12,13,14-Trihydroxy-14-N7dG-11,12,13,14-tetrahydro-DB[a]JP [(--)-syn-cis-N7dG]: UV λmax 242, 286, 290, 297 (sh), 321, 344 nm; 1H NMR δ 3.85 (d, 1H, 12-H), 3.95 (m, 1H, 11-H, J = 10.0 Hz), 4.05 (sh, 1H, 11a-H, J = 10.0 Hz). 8.02 (1H, 7-H, J = 7.5 Hz), 8.06 (1H, 6-H, J = 7.5 Hz), 8.16 (1H, 5-H, J = 6.20 Hz).

Results and Discussion

Synthesis and Isolation of Adducts. When both racemic and optically pure DB[a]IPD were reacted with dG at 100 °C for 30 min, two types of adducts were isolated in high yield (Table 1): one formed by reaction of DB[a]IPD at the benzylc C14-position with the NH2 group of dG (N7dG adducts) and the second formed by...
Only one N7Gua adduct was isolated, and its yield was much greater than those of the N2dG adducts. The structures of all adducts were determined by NMR spectroscopy (COSY), and D2O treatment of exchangeable hydrogens. Furthermore, the signal of the H-14 proton of the DB[a, l]PDE moiety at 7.48 ppm (Table 2) is shifted significantly downfield compared to the signals of the other methine protons of the cyclohexenyl ring, indicating that the adduct has been formed between the C-14 of DB[a, l]PDE and the 2-NH2 of dG. The signals of the remaining protons were assigned by a combination of 1H NMR, two-dimensional chemical shift correlation spectroscopy (COSY), and D2O treatment of exchangeable protons.

The NMR spectrum of these adducts (Figure 3B), the two proton signals at 6.96 ppm, exchangeable with D2O, show a chemical shift close to that of the amino group in dG (6.48 ppm, not shown). This indicates that the amino group is not involved in the formation of the adduct. The lack of a deoxyribose moiety in the adduct suggests that the bond between the dihydrodiol epoxide and dG occurs at N-7, with destabilization of the glycosidic bond and loss of the deoxyribose moiety. The chemical shifts of the remaining protons were assigned by 1H NMR, COSY, and D2O exchange. The substantial broadening of the signals of all protons is attributable to the almost identical NMR spectra of the four stereoisomers, which could not be separated by HPLC.

The N2dG adducts can be easily separated from the N7Gua adducts (Figure 1). However, efforts to further separate diastereomeric mixtures of cis- and trans-N2dG adducts, or the four individual stereoisomers of optically active N2dG adducts obtained from racemic DB[a, l]PDE (Scheme 1) and syn-DB[a, l]PDE (Scheme 2), were not successful under isocratic or gradient HPLC conditions. By using (±)-syn- and (±)-anti-DB[a, l]PDE, however, pure (±)-anti-cis-N2dG, (±)-anti-trans-N2dG, (±)-anti-trans-N7Gua (Scheme 3, Figure 2A), (±)-syn-cis-N2dG, (±)-syn-trans-N2dG, (±)-syn-cis-N7Gua, and (±)-syn-trans-N7Gua (Scheme 4, Figure 2B) were obtained. The structures of all adducts were determined by NMR (Figures 3, 4, 7). The formulae of all the racemic N7Gua and stereoisomeric N2dG adducts were confirmed by FAB MS, and more confirmation was achieved by tandem MS, while the optically pure adducts were investigated by FLNS.

Structure Elucidation of Racemic Adducts. (1) (±)-anti-11,12,13-Trihydroxy-14-N2dG-11,12,13,14-tetrahydro-DB[a, l]P [(±)-anti-cis- & -trans-N2dG]. The NMR spectrum (Figure 3A) of this mixture of four stereoisomers does not show different sets of chemical shifts, suggesting that the isomers exhibit almost identical NMR spectra. The absence of the NH2 signal for dG around 7 ppm indicates that this group participates in the bond of dG to the dihydrodiol epoxide. The characteristic proton signals of the deoxyribose moiety further substantiate formation of the adduct at the exocyclic amino group. Furthermore, the signal of the H-14 proton of the DB[a, l]PDE moiety at 7.48 ppm (Table 2) is shifted significantly downfield compared to the signals of the other methine protons of the cyclohexenyl ring, indicating that the adduct has been formed between the C-14 of DB[a, l]PDE and the 2-NH2 of dG. The signals of the remaining protons were assigned by a combination of 1H NMR, two-dimensional chemical shift correlation spectroscopy (COSY), and D2O treatment of exchangeable protons.

(2) (±)-anti-11,12,13-Trihydroxy-14-N7Gua-11,12,13,14-tetrahydro-DB[a, l]P [(±)-anti-cis- & -trans-N7Gua]. In the NMR spectrum of these adducts (Figure 3B), the two proton signals at 6.96 ppm, exchangeable with D2O, show a chemical shift close to that of the amino group in dG (6.48 ppm, not shown). This indicates that the amino group is not involved in the formation of the adduct. The lack of a deoxyribose moiety in the adduct suggests that the bond between the dihydrodiol epoxide and dG occurs at N-7, with destabilization of the glycosidic bond and loss of the deoxyribose moiety. The chemical shifts of the remaining protons were assigned by 1H NMR, COSY, and D2O exchange. The substantial line broadening of the signals of all protons is attributable to the almost identical NMR spectra of the four stereoisomers, which could not be separated by HPLC.

(3) (±)-syn-11,12,13-Trihydroxy-14-N2dG-11,12,13,14-tetrahydro-DB[a, l]P [(±)-syn-cis- & -trans-N2dG]. The NMR spectrum of the title compounds (Figure 4A) shows two sets of proton signals, suggesting that the cis and trans stereoisomers can be identified. Assignment of the structure of these adducts is based on the lack of the NH2 signal of dG (around 7 ppm) and the shift downfield of the H-14 proton, compared to the chemical shifts of the other methine protons of the cyclohexenyl ring. Furthermore, the proton signals of the deoxyribose are present. These data suggest that the adduct is formed by a bond between C-14 in the dihydrodiol epoxide moiety and the 2-NH2 group of dG. Determination of the cis- and trans-opened adducts from the two sets of proton signals is based on the coupling constant J13a,14a, which is larger for the trans- than cis-opened adducts (ref 5 and see below). It was found that syn isomers preferentially produce cis-opened adducts and anti isomers preferentially yield trans-opened adducts (5). Thus, in the two sets of signals, the major one is designated as deriving from the cis-opened adduct. The ratio of the cis- to trans-opened adducts is 70:30, as determined from peak areas.

(4) (±)-syn-11,12,13-Trihydroxy-14-N7Gua-11,12,13,14-tetrahydro-DB[a, l]P [(±)-syn-cis- & -trans-N7Gua]. The NMR spectrum of these adducts (Figure 4B) shows two sets of proton signals that are analogous to those of (±)-syn-cis- & -trans-N2dG (Figure 4A). The lack of deoxyribose signals and the presence of the 2-NH2 proton signals indicate that these two diastereomers are the syn-cis- and syn-trans-N7Gua adducts. The smaller coupling constant J13a,14a = 3.5 Hz (Table 2) is...
designated as deriving from the cis-opened adduct and the larger \( J_{13b,14b} = 4.5 \) Hz (Table 2) from the trans-opened adduct. Integrating the peaks gives a ratio of syn-cis versus syn-trans adducts of 65:35.

Mass Spectra of the Racemic Adducts. FAB MS produces [M + H]$^+$ and [M + Na]$^+$ ions for the dihydrodiol epoxide adducts. Sufficient signal intensity was produced to enable exact-mass measurements of either the protonated or sodiated molecules of the N2dG and N7Gua adducts (see Experimental Section). The elemental compositions were confirmed, as the measured masses were within 2.5–2.7 ppm of the theoretical masses for the four adducts.

In an effort to obtain structural information, the protonated or sodiated adducts were submitted to tandem mass spectrometry, and their collisionally activated decomposition (CAD) spectra were recorded. The dihy-
drodiol epoxides do not desorb well, probably owing to the hydrophilicity of the three hydroxyl groups. Because of the poor sensitivity, we were able to obtain the CAD spectra only by using the array detector of the four-sector instrument. We present four spectra here to demonstrate that they are consistent with the proposed structures, but like the similar CAD spectra of dA adducts (5), the spectra are compound-class-specific, but not isomer-specific.

For the (±)-anti-cis- & -trans-N2dG adducts, the CAD spectrum of the [M + H]+ caused fragmentation of the molecules into their constituent parts (Figure 5A). Losses occur of the deoxyribose as C5H8O3 and neutral Gua to give the ions of m/z 504 and 469, respectively. The latter is unexpected and may be due to migration of the PAH triol moiety from Gua to deoxyribose in the MS experiment. We also see formation of deoxyribose ion and protonated Gua at m/z 117 and 152, respectively. The triol moiety is expelled as an ion of m/z 353. There is a signature pattern for the triol moiety that consists of the fragment ions of m/z 353, 335, 317, 307, 289, 276/7, and 263. These ions are connected by losses of CO, H2O, and portions of the six-membered ring containing the triol. An identical set of ions is formed in the fragmentation of DB[a,l]PDE-dA and -dC adducts, although their relative abundances are different (5). Benzo[a]pyrene-7,8-dihydrodiol 9,10-epoxide adducts also give an analogous pattern of m/z 303, 285, 267, 257, 239, and 226/7 fragment ions (22), with m/z values 50 units (C4H2) lower than those of DB[a,l]P adducts.

The CAD or product-ion spectrum of the [M + H]+ of (±)-anti-cis- & -trans-N7Gua (Figure 5B) shows most of the signature pattern for the triol (some ions are missing because there is a small gap in the center of the array detector). The presence of Gua is indicated by the fragment of protonated Gua at m/z 152. To obtain CAD spectra of the (±)-syn-cis- & -trans-N2-dG and (±)-syn-cis- & -trans-N7Gua adducts, we had to turn to [M + Na]+ ions because the sample contained sufficient Na+ to render the [M + H]+ species poorly abundant. The spectrum (Figure 6A) of (±)-syn-cis- & -trans-N2-dG shows again that the dominant fragmentation is to disassemble the adduct into its constituent parts: [Gua + Na]+ shifted to m/z 174 from m/z 152 for [Gua + H]+ and ions formed by losses of deoxyribose (at m/z 526) and neutral Gua (at m/z 491). The latter ion suggests that N2dG adducts may be susceptible to rearrangement of the PAH-triol moiety because the loss of the base is not seen for N6dA adducts of this type (5). The pattern of peaks in the region of m/z 289, when amplified, is similar in ion membership, but not abundance, to the triol signature (m/z 335, 317, 307...), indicating that the Na+ is bound preferentially with the deoxyribose/Gua portion of the molecule and is lost as part of the neutral moiety.

The CAD spectrum (Figure 6B) of the [M + Na]+ of (±)-syn-cis- & -trans-N7Gua illustrates more clearly the pattern of fragment ions in the m/z 289 region, and another version of this spectrum was published previously (23). One sees clearly that many of the fragment ions constitute the signature pattern of the PAH-triol and do not bear the Na+, indicating again a preference of Na+ for the deoxyribose or base. A notable exception is the...
abundant ion at m/z 375, which is the PAH-triol bound to Na⁺.

Structure Elucidation of (−)-anti-[DB[a,l]PDE-dG and (+)-syn-[DB[a,l]PDE-dG Adducts. The small amounts of the two dihydrodiol epoxides (−)-anti-[DB[a,l]PDE and (+)-syn-[DB[a,l]PDE available for synthesizing the dG adducts rendered challenging the elucidation of their structures. The NMR spectra were recorded in Me₂SO-d₆/D₂O to sharpen the broad signals corresponding to the hydroxy and amino protons and, at the same time, to sharpen the signals corresponding to the C-H protons.

Of particular importance in elucidating the structures of the optically pure stereoisomers is an empirical rule that we developed by studying similar adducts obtained from the reaction of [DB[a,l]PDE with dA: the anti-[DB[a,l]PDE tend to produce more trans-opened adducts, whereas the syn-[DB[a,l]PDE yield more cis-opened adducts. More important for establishing the structure of these adducts is the coupling constant J₁₃,₁₄, which is relatively low for the cis-opened adducts and larger for the trans-opened adducts (5).

In the reaction of (−)-anti-[DB[a,l]PDE with dG, three adducts were isolated and identified: namely the (−)-anti-trans-N²dG (17% yield), (−)-anti-cis-N²dG (12%), and (−)-anti-trans-N⁷Gua (43%; Table 1). The NMR spectra of these three adducts are shown in Figure 7. Assignment of the various proton signals was determined by ¹H NMR and COSY, and the rationale for distinguishing the N²-dG from the N⁷Gua adduct is based on the presence of the signals for the deoxyribose moiety in the former and their absence in the latter. The J₁₃,₁₄ = 3.5 Hz observed in spectrum 7A (Table 2) is small compared to J₁₃,₁₄ = 8.0 Hz in spectrum 7B. Therefore, the adduct with the smaller coupling constant is designated as the one that has 13-H and 14-H cis to each other, and thus, the absolute stereochemistry of the adduct must be 11R,12S,13R,14R. The adduct in Figure 7B, with J₁₃,₁₄ = 8.0 Hz, has the 13-H and 14-H trans to each other, and its absolute stereochemistry must be 11R,12S,13R,14S. Of the N⁷Gua adduct (Figure 7C), only one optically pure isomer was isolated; the other was obtained in trace amount (Figure 2A). The isolated adduct is designated...
small dG and two N7Gua adducts were obtained. From the relatively high yield of the isomer designated as (−)-anti-trans-N7Gua because the yield was high, and the value for the coupling constant J_{13,14} = 6.0 Hz is also relatively high. Therefore, this adduct is designated with 11R,12S,13R,14S-absolute stereochemistry. As (−)-anti-trans-N7Gua because the yield was high, and the value for the coupling constant J_{13,14} = 6.0 Hz is also relatively high. Therefore, this adduct is designated with 11R,12S,13R,14S-absolute stereochemistry.

From reaction of (−)-syn-DB[a,l]PDE with dG, two N2-dG and two N7Gua adducts were obtained. From the small J_{13,14} = 3.0 Hz and the higher yield obtained for the isomer shown in Figure 8A, this adduct is designated as the (−)-syn-cis-N2-dG, with 11S,12R,13R,14R-absolute stereochemistry. Thus, the adduct shown in Figure 8B must be the (−)-syn-trans-N2-dG. Elucidation of the structure of the two N7Gua adducts obtained relies on

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<td></td>
<td></td>
</tr>
<tr>
<td>(±)-syn-trans-N7Gua</td>
<td>4.94</td>
<td>3.70</td>
<td>4.00</td>
<td>6.78</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subscripts a and b refer to cis-opened adducts and trans-opened adducts, respectively.

Figure 5. Portion of the CAD mass spectra of (A) [M + H]^+ ions of m/z 620 from (±)-anti-cis- & trans-N2-dG and (B) [M + H]^+ ions of m/z 504 from (±)-anti-cis- & trans-N7Gua.

Figure 6. Portion of the CAD mass spectra of (A) [M + Na]^+ ions of m/z 642 from (±)-syn-cis- & trans-N2-dG and (B) [M + Na]^+ ions of m/z 526 from (±)-syn-cis- & trans-N7Gua.

the higher yield of the isomer designated as (±)-syn-cis-N7Gua (36%) with a relatively small J_{13,14} = 4.0 Hz (Figure 9A) versus the assigned (±)-syn-trans-N7Gua (22%) with J_{13,14} = 6.5 Hz.


FLN multiplet origin structures for anti-trans- and anti-cis-N2-dG adducts are shown in Figure 10; the FLN...
spectra in Figure 10A,B were acquired for two different excitation wavelengths: 379.0 and 376.0 nm, respectively. Spectra a and c were obtained for the trans isomer in glycerol/water at 4.2 K and spectra b and d for the cis isomer. The FLN peaks are labeled with their S_1 vibrational frequencies in cm\(^{-1}\). For \(\lambda_{\text{ex}} = 376.0\) nm (Figure 10B), the FLN spectra of anti-trans- and anti-cis-N\(^2\)dG adducts are fairly similar; however, for \(\lambda_{\text{ex}} = 379.0\) nm (Figure 10A), there are significant differences in the vibrational frequencies and intensities that enable spectral distinction of these two adducts. There are characteristic modes at 532, 545, 625, 635, and 698 cm\(^{-1}\) for the anti-trans-N\(^2\)dG adduct, whereas the anti-cis-N\(^2\)dG adduct has modes at 536, 551, and 627 cm\(^{-1}\). In addition, the relative intensities of the FLN peaks are different for these two adducts, with the higher frequency modes of anti-trans-N\(^2\)dG being more intense than the corresponding modes for the anti-cis-N\(^2\)dG adduct.

The same excitation wavelengths were used to generate FLN spectra for the syn-trans- and syn-cis-N\(^2\)dG adducts, shown in Figure 11A,B, corresponding to different excitation wavelengths (\(\lambda_{\text{ex}} = 379.0\) and 376.0 nm, respectively) that expose different regions of the vibronic spectrum. In Figure 11 the FLN peaks in the \(387-390\) nm region correspond to excited-state vibrational modes of the major conformer II of these adducts. Spectra a and c are FLN spectra for syn-trans-N\(^2\)dG in the glycerol/water glass at 4.2 K and spectra b and d for syn-cis-N\(^2\)dG. Comparison of the spectra for these two adducts at the two excitation wavelengths reveals that syn-cis can be distinguished from its syn-trans stereoisomer on the basis of its characteristic vibrational modes at 543, 730, 865, 891, and 917 cm\(^{-1}\); moreover, the modes at 690 and 882 cm\(^{-1}\) are only observed for the syn-trans isomer. Because the FLN spectra for syn-trans- and syn-cis-N\(^2\)dG in Figure 11 are significantly different from the spectra for the anti-trans- and anti-cis-N\(^2\)dG adducts shown in Figure 10, differentiation of these four dG stereoisomeric adducts by FLNS is straightforward.

Conclusions
The reaction of (±)-anti-DB[a,l]PDE with dG gave two sets of adducts in good yield: (±)-anti-cis- & -trans-N\(^2\)dG and (±)-anti-cis- & -trans-N7Gua; both sets were mixtures of four stereoisomers that could not be sepa-
The FLN peaks are labeled with their excited-state vibrational frequencies in cm$^{-1}$.

The products from (a) $\text{syn}$-$\text{DB}[\text{a},\text{l}]\text{PDE}$ with dG afforded optically pure N$^2$-$\text{dG}$ and N$^7$-Gua adducts. The $\text{(−)}$-$\text{anti}$-$\text{DB}[\text{a},\text{l}]\text{PDE}$ yielded more adducts trans-opened at the benzylic C-14, whereas $\text{(−)}$-$\text{syn}$-$\text{DB}[\text{a},\text{l}]\text{PDE}$ afforded mainly cis-opened adducts. It was shown that FLNS possesses the necessary selectivity to distinguish the four stereoisomeric $\text{DB}[\text{a},\text{l}]\text{PDE}$-N$^2$-$\text{dG}$ adducts. With this technique, adducts from anti-$\text{DB}[\text{a},\text{l}]\text{PDE}$ are easily distinguished from syn-$\text{DB}[\text{a},\text{l}]\text{PDE}$ adducts, and the spectra of trans-opened adducts are different from those of cis-opened adducts. The FLN spectra acquired for these four stereoisomeric N$^2$-$\text{dG}$ adducts will serve future research projects as standards for positive identification of $\text{DB}[\text{a},\text{l}]\text{PDE}$-DNA stable adducts formed in biological systems.

**Acknowledgment.** This research was supported by Grants R01 CA49917 and P01 CA49210 from the National Cancer Institute. Core support at the Epilepsy Institute is provided by Grant P30-CA36727 from the National Cancer Institute and at the Washington University MS Research Resource by Grant P41-RR00954 from the National Center for Research Resources. Ames Laboratory is operated for the U.S. Department of Energy by Iowa State University under Contract No. W-7405-Eng-82. This research was also supported by the Deutsche Forschungsgemeinschaft (SFB 302).

**References**


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