Synthesis of *anti*-7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-11-methylbenzo[*a*]pyrene and Its Reaction with DNA

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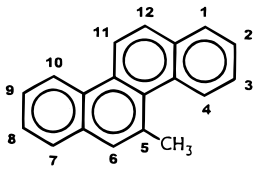
Substitution of a methyl group in the bay region can enhance the tumorigenicity of polycyclic aromatic hydrocarbons such as chrysene, benz[*a*]anthracene, and others. This phenomenon has been related to facile DNA adduct formation of bay region diol epoxides with a methyl group and epoxide ring in the same bay. While *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and its DNA adduct formation have been studied extensively, it is not known whether a methyl substituent in the bay region alters the reactivity of DNA in this system. This is of interest because 11-methylbenzo[*a*]pyrene, which has a bay region methyl group, is more tumorigenic than benzo[*a*]pyrene. To examine the question, we have devised and employed an efficient synthesis based on photochemical cyclization, and prepared *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-11-methylbenzo[*a*]pyrene, the likely ultimate carcinogen of 11-methylbenzo[*a*]pyrene. We have then reacted *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-11-methylbenzo[*a*]pyrene with calf thymus DNA and found that it gives three major adducts. These were identified as having resulted from cis- and trans-ring opening of the (*S*,*R*,*R*,*S*)-enantiomer and from trans-ring opening of the (*R*,*S*,*S*,*R*)-enantiomer. The standard deoxyguanosine adduct markers were prepared, and their structures were tentatively assigned on the basis of their CD and ^{1}H NMR spectra. The adduct distribution of *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-11-methylbenzo[*a*]pyrene is quite different from that observed in the reaction of DNA with the corresponding diol epoxides of benzo[*a*]pyrene or with 5-methylchrysene. The heterogeneity of adducts obtained with *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-11-methylbenzo[*a*]pyrene thus may be related to the enhanced tumorigenicity of 11-methylbenzo[*a*]pyrene.

Introduction

Polycyclic aromatic hydrocarbons (PAHs)[1] with a methyl group in the bay region adjacent to an unsubstituted angular ring are frequently highly tumorigenic, and usually more tumorigenic than other methyl isomers in the same series, or than the parent hydrocarbon (*1*, *2*). We have studied this phenomenon with 5-methylchrysene (5-MeC) as a model carcinogen. Its tumorigenic activity is greater than that of any of the other monomethylchrysenes or of chrysene itself (*1*). The presence of a methyl group and epoxide ring in the same bay region of a chrysene diol epoxide enhances the reactivity with DNA in vitro and in vivo (*1*). These studies provide biochemical support for the generalization that the structural requirements favoring carcinogenicity of methylated PAHs include the presence of a bay region methyl group and a free peri position (e.g., positions 6 and 12 in 5-MeC), both adjacent to an unsubstituted angular ring (*3*).



An extensive database regarding the metabolic activation and DNA binding properties of benzo[*a*]pyrene (BP) is available. Iyer et al. have examined the tumor-initiating activity on mouse skin of all possible monomethylated isomers of BP (*4*). In terms of tumor multiplicity, 11-methylBP (11-MeBP) was the most active compound, its tumor initiating activity being approximately 3 times greater than that of BP. 1-, 3-, 4-, and 12-MeBP had activities approximately equal to that of BP, while the other isomers were less active, or inactive (*4*).

However, no studies have been reported on the metabolic activation or macromolecular binding of 11-MeBP. In this paper, we report our initial investigations on comparative DNA adduct formation by 11-MeBPDE and BPDE. We synthesized *anti*-7,8-dihydroxy-9,10-epoxy-

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[1] Abbreviations: 11-MeBPDE, *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,-10-tetrahydro-11-methylbenzo[*a*]pyrene; BPDE, *anti*-7,8-dihydroxy-9,-10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BP, benzo[*a*]pyrene; 5-MeC, 5-methylchrysene; PAHs, polycyclic aromatic hydrocarbons; 11-MeBP, 11-methylbenzo[*a*]pyrene; 5-MeCDE, *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-5-methylchrysene; dG, 2′-deoxyguanosine; dA, 2′-deoxyadenosine.

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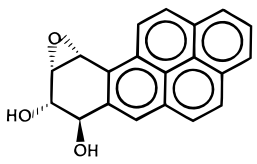
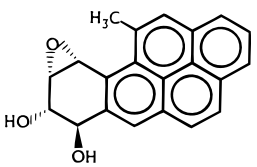
7,8,9,10-tetrahydro-11-methylbenzo[*a*]pyrene (11-MeBPDE) and examined its reaction with calf thymus DNA.



BPDE



11-MeBPDE

Experimental Section

Apparatus. ^{1}H NMR spectra were recorded in $CDCl_3$ using a Bruker model AM 360 WB spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Mass spectra (MS) were recorded on a Hewlett-Packard model 5988A instrument. High-resolution MS were obtained with a Finnigan Mat 95 instrument at the Mass Spectrometry Service (University of Minnesota, Minneapolis, MN). Circular dichroism (CD) spectra were determined in MeOH in an Aviv Associates Inc. (Lakewood, NJ) model 62ADS CD spectrometer. Thin-layer chromatography (TLC) separations were performed on aluminum-supported precoated silica gel plates from EM Industries (Gibbstown, NJ). Starting materials were obtained from Aldrich Chemical Co. (Milwaukee, WI), unless stated otherwise. Ethyl (3-methyl-1-naphthyl)acetate (**1**) was prepared in 50% yield as reported in the literature (*5*).

1-Carbomethoxy-1-(3-methyl-1-naphthyl)-2-(3-methoxyphenyl)ethylene (2). To a solution of **1** (4.28 g, 20 mmol) being stirred in anhydrous tetrahydrofuran (THF) (40 mL) at −78 °C was added dropwise $NaN[(CH_3)_3Si]_2$ (1.0 M, 25 mL, 25 mmol) in anhydrous THF (100 mL). The mixture was stirred at −78 °C for 30 min and the reaction quenched with a solution of *m*-anisaldehyde (2.72 g, 20 mmol) in anhydrous THF (20 mL). After the mixture warmed to room temperature, it was stirred for an additional 16 h, and then poured into a saturated solution of NH_4Cl. After extraction with CH_2Cl_2 (3 × 40 mL), the combined organic layers were dried ($MgSO_4$), filtered, and evaporated to dryness to give crude **2**. The crude compound was purified by chromatography on silica gel with elution by hexane to yield major trans olefin **2** as an oil (3.65 g, 55%): ^{1}H NMR δ 1.20 (t, 3H, $\underline{CH_3}CH_2$), 2.48 (s, 3H, $\underline{CH_3}$), 3.15 (s, 3H, $O\underline{CH_3}$), 3.81 (q, 2H, $C\underline{H_2}$), 6.3 (s, 1H, olefin), 6.65−6.75 (m, 2H), 7.25 (s, 1H), 7.35 (s, 1H), 7.4−7.6 (m, 2H), 7.65−7.95 (m, 3H), 8.05 (s, 1H).

11-Carboethoxy-2-methoxy-5-methylchrysene (3). A solution of olefin **2** (2.7 g, 8.13 mmol) and iodine (5 mg) in anhydrous benzene (1.2 L) was irradiated with a Pyrex-filtered Hanovia 450 W medium-pressure UV lamp, while dry air was bubbled through it. The reaction was stopped after 8 h; the solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel with elution by EtOAc/hexane (1:9) to yield cyclic product **3** (1.0 g, 37%): ^{1}H NMR δ 1.25 (t, 3H, $\underline{CH_3}CH_2$, J = 7.2 Hz), 3.10 (s, 3H, $\underline{CH_3}$), 3.98 (s, 3H, $O\underline{CH_3}$), 4.42 (q, 2H, $CH_3\underline{CH_2}$, J = 7.2 Hz), 7.31 (dd, 1H, H3, $J_{3,4}$ = 9.4 Hz, $J_{1,3}$ = 2.8 Hz), 7.37 (d, 1H, H1, $J_{1,3}$ = 2.8 Hz), 7.48−7.56 (m, 2H, H8 and H9), 7.82 (s, 1H, H6), 7.86 (dd, 1H, H7, $J_{7,8}$ = 7.8 Hz and $J_{7,9}$ = 1.3 Hz), 8.10 (d, 1H, H4 or H10, J = 8.5 Hz), 8.12 (s, 1H, H12), 8.73 (d, 1H, H4 or H10, J = 9.4 Hz).

2-Methoxy-5-methylchrysene-11-carboxaldehyde (4). A solution of **3** (0.90 g, 2.73 mmol) in 6.5 mL of anhydrous THF was added dropwise to a solution of diisobutylaluminum hydride (3 mL, 3 mmol) in 10 mL of anhydrous THF. The mixture was stirred for 16 h at room temperature, poured into H_2O, and extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried over $MgSO_4$. Removal of the solvent gave crude aldehyde **4** which was purified by chromatography on silica gel with elution by hexane/CH_2Cl_2 (45:55) to give **4** (0.44 g, 54%); it was recrystallized from a mixture of hexane and Et_2O: mp 171−173 °C; ^{1}H NMR δ 3.25 (s, 3H, $\underline{CH_3}$), 4.01 (s, 3H, $O\underline{CH_3}$), 7.4 (dd, 1H, H3, $J_{1,3}$ = 2.9 Hz, $J_{3,4}$ = 9.4 Hz), 7.49 (d, 1H, H1, $J_{1,3}$ = 2.9 Hz), 7.60−7.70 (m, 2H, H8 and H9), 7.90−8.00 (m + s, 3H, H7, H10, and H6), 8.40 (s, 1H, H12), 8.78 (m, 1H, H4, $J_{3,4}$ = 9.4 Hz), 10.49 (s, 1H, CHO).

1-(2-Methoxy-5-methylchrysen-11-yl)-2-methoxyethylene (5). Under a N_2 atmosphere, a solution of 1.8 M phenyllithium (in 70:30 cyclohexane/ether, 0.90 mL, 1.60 mmol) was added dropwise to a solution of (methoxymethyl)triphenylphosphonium chloride (0.69 g, 2.0 mmol) in anhydrous Et_2O (15 mL) at −78 °C. The mixture was stirred at −78 °C for 30 min, then warmed to −20 °C for 30 min, and then again cooled to −78 °C. A solution of carboxaldehyde **4** (0.4 g, 1.33 mmol) in anhydrous THF (15 mL) was added. Stirring was continued for 1 h at −78 °C; then the reaction mixture was allowed to warm to room temperature, and stirring was continued for 16 h. After the usual workup, the crude olefin **5** was purified by chromatography on silica gel with elution by hexane/CH_2Cl_2 (80:20) to give **5** (0.27 g, 61%) as a mixture of cis and trans isomers; it was recrystallized from a mixture of hexane and Et_2O: mp 121−123 °C; ^{1}H NMR (acetone-d_6) δ 3.26 (s, 3H, $\underline{CH_3}$), 3.85 (s, 2.25H, CH=CHO$\underline{CH_3}$), 3.95 (s, 0.75H, CH=CHO$\underline{CH_3}$), 4.10 (s, 3H, O$\underline{CH_3}$), 5.90 (d, 0.25H, CH=CH, J = 7.1 Hz), 6.60 (d, 0.75H, CH=CH, J = 12.6 Hz), 6.65 (d, 0.25H, CH=CH, J = 7.1 Hz), 7.25 (dd, 1H, H3, $J_{2,3}$ = 9.4 Hz, $J_{1,3}$ = 2.8 Hz), 7.35 (d, 0.75H, CH=CH, J = 12.7 Hz), 7.40−7.60 (m, 3H, aromatic), 7.80−8.00 (m, 3H, aromatic), 8.75 (m, 1H), 9.05 (m, 1H).

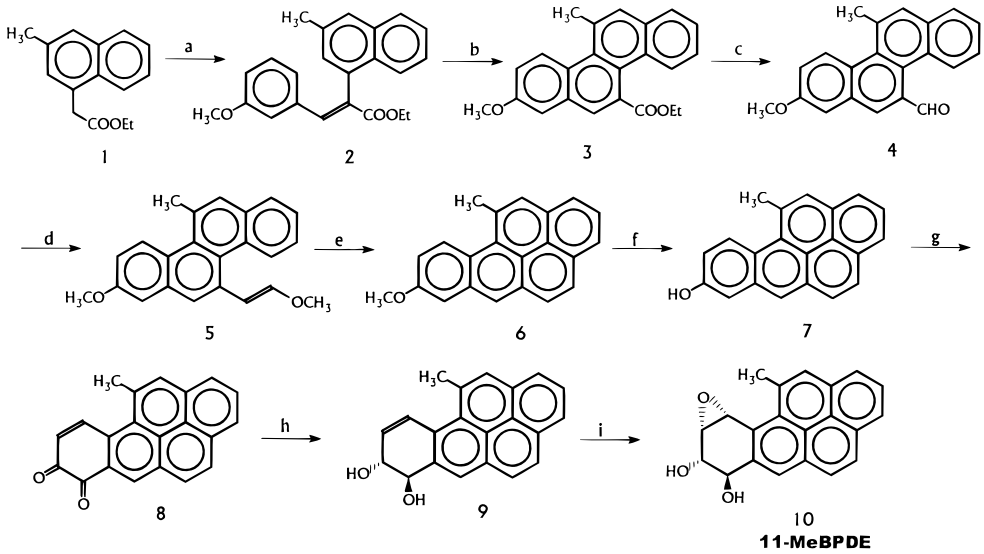
8-Methoxy-11-methylbenzo[*a*]pyrene (6). Under a N_2 atmosphere, a stirred CH_2Cl_2 solution of **5** (0.25 g, 0.76 mmol) was kept at 0 °C, while CH_3SO_3H (5 mL) was added dropwise over the course of 10 min. The reaction mixture was then stirred for an additional 6 h. The usual workup gave a crude product which was purified by chromatography on silica gel with elution by hexane/CH_2Cl_2 (4:1) to give **6** (0.17 g, 77%); it was recrystallized from a mixture of hexane and Et_2O: mp 152−153 °C; ^{1}H NMR δ 3.39 (s, 3H, $\underline{CH_3}$), 4.06 (s, 3H, O$\underline{CH_3}$), 7.41 (dd, 1H, H9, $J_{9,10}$ = 9.7 Hz, $J_{9,7}$ = 2.9 Hz), 7.56 (d, 1H, H7, $J_{7,9}$ = 2.9 Hz), 7.80−8.03 (m, 4H), 8.10−8.40 (m + s, 2H), 8.42 (s, 1H, H6), 9.21 (d, 1H, H10, $J_{9,10}$ = 9.7 Hz).

8-Hydroxy-11-methylbenzo[*a*]pyrene (7). To a stirred solution of BP derivative **6** (0.15 g, 0.51 mmol) in CH_2Cl_2 (150 mL) was added dropwise a solution of BBr_3 in CH_2Cl_2 (0.55 mL, 0.55 mmol) at 0 °C under N_2. The reaction mixture was then stirred at room temperature for 12 h, and the excess reagent was hydrolyzed with ice-cold H_2O. The organic layer was washed with H_2O (3 × 50 mL), dried ($MgSO_4$), filtered, and evaporated to dryness to give crude **7** which was purified by chromatography on silica gel with elution by hexane/CH_2Cl_2 to give **7** (0.12 g, 85%); it was recrystallized from a mixture of hexane and Et_2O: mp 222−224 °C; ^{1}H NMR δ 3.39 (s, 3H, CH_3), 7.45 (dd, 1H, H9, $J_{9,10}$ = 9.6 Hz, $J_{9,7}$ = 2.8 Hz), 7.65 (d, 1H, H7, $J_{7,9}$ = 2.8 Hz), 7.80−8.10 (m, 4H), 8.10−8.45 (m + s, 2H), 8.46 (s, 1H, H6), 9.25 (d, 1H, H10, $J_{9,10}$ = 9.6 Hz); high-resolution MS (positive chemical ionization using ammonia as the reagent gas) calcd for $C_{21}H_{15}O$ 283.1125, found 283.1123.

11-Methylbenzo[*a*]pyrene-7,8-dione (8). Adogen 464 (3 drops) was added to a mixture of **7** (0.1 g, 0.36 mmol), KH_2PO_4 (20 mL, 0.2 M solution), and Fremy's salt (0.19 g, 0.72 mmol) being stirred in CH_2Cl_2/benzene (20 mL, 5:1). Stirring was continued for 3 h at room temperature. The organic layer was collected, and the aqueous solution was extracted with benzene. The benzene extract was washed with H_2O and dried ($MgSO_4$); then the solvent was removed. The residue was purified by chromatography on silica gel with elution by hexane/CH_2Cl_2 (50:50) to give dione **8** (80 mg, 71%); it was recrystallized from a mixture of hexane and Et_2O: mp 218−220 °C; ^{1}H NMR δ 3.10 (s, 3H, $\underline{CH_3}$), 6.50 (d, 1H, H9, $J_{9,10}$ = 10.8 Hz), 7.95−8.35 (m, 6H), 8.81 (d, 1H, H10, $J_{9,10}$ = 10.8 Hz), 8.90 (s, 1H, H6); high-resolution MS calcd for $C_{21}H_{12}O_2$ 296.0837, found 296.0836.

7,8-Dihydroxy-7,8-dihydro-11-methylbenzo[*a*]pyrene (9). To a stirred suspension of dione **8** (60 mg, 0.20 mmol) in ethanol (50 mL) was added $NaBH_4$ (100 mg, 2.64 mmol) over the course of 10 min. The reaction mixture was stirred for 24 h in an open

Scheme 1. Synthesis of 11-MeBPDE[a]



[a] (a) NaN[$(CH_3)_3Si]_2$, *m*-anisaldehyde; (b) *hν*/I_2, PhH; (c) DIBAL; (d) $CH_3OCH_2P^+(Ph)_3Cl^-$, PhLi, THF; (e) CH_3SO_3H; (f) BBr_3; (g) Fremy's salt; (h) $NaBH_4$/MeOH; (i) *m*CPBA.

flask and was then poured into ice-cold H_2O and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with H_2O, dried (Na_2SO_4), and concentrated. The resulting residue was purified by chromatography on Florisil with elution by CH_2Cl_2/EtOAc (50:50) to give diol **9** (50 mg, 82%): ^{1}H NMR (acetone-d_6) δ 3.08 (s, 3H, CH_3), 4.52 (m, 1H, H8), 4.90 (d, 1H, H7, $J_{7,8}$ = 11.3 Hz), 6.22 (dd, 1H, H9, J = 2.0 Hz, $J_{9,10}$ = 10.3 Hz), 7.70 (dd, 1H, H10, $J_{8,9}$ = 2.3 Hz, $J_{9,10}$ = 10.4 Hz), 7.79−7.80 (m, 2H), 8.05−8.18 (m, 4H), 8.51 (s, 1H, H6); high-resolution MS (positive chemical ionization using ammonia as the reagent gas) calcd for $C_{21}H_{17}O_2$ 301.1230, found 301.1228.

***anti*-7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-11-methylbenzo[*a*]pyrene (11-MeBPDE) (10).** A mixture of diol **9** (30 mg, 0.1 mmol), *m*-chloroperbenzoic acid (85 mg, 0.5 mmol), and anhydrous THF (20 mL) was stirred under N_2 for 4 h. Then the reaction mixture was diluted with Et_2O (100 mL), washed with cold 2% NaOH (3 × 20 mL) and H_2O, and dried (K_2CO_3). Removal of the solvent gave a pale yellow powder, which was recrystallized from Et_2O/CH_2Cl_2 to yield diol epoxide **10** (19 mg, 61%): mp 164−165 °C; ^{1}H NMR (DMSO-d_6) δ 3.19 (s, 3H, CH_3), 3.87 (dd, 1H, H9, $J_{9,10}$ = 4.5 Hz, $J_{8,9}$ = 1.6 Hz), 4.04 (d, 1H, H8, $J_{7,8}$ = 8.5 Hz), 4.69 (bs, 1H, OH), 5.00 (d, 1H, H7, $J_{7,8}$ = 8.6 Hz), 5.04 (bs, 1H, OH), 5.20 (d, 1H, H10, $J_{10,9}$ = 4.5 Hz), 8.03 (dd, 1H, H2, J = 7.6 Hz), 8.10 (s, 1H, H12), 8.16−8.19 (m, 3H, H3, H4, and H5), 8.24 (dd, 1H, H1, $J_{1,2}$ = 7.6 Hz, $J_{1,3}$ = 1.1 Hz), 8.62 (s, 1H, H6).

Preparation of DNA Adducts. 11-MeBPDE (500 μg) in 500 μL of THF was added to a solution of calf thymus DNA (5 mg) in 5 mL of 10 mM Tris-HCl buffer (pH 7). This mixture was incubated at 37 °C overnight. Tetraols were removed from the DNA solutions by three extractions with EtOAc (6 mL) and one with Et_2O (6 mL). The DNA was isolated and hydrolyzed enzymatically to deoxyribonucleosides as described previously (*6*). Unmodified deoxyribonucleosides were separated from modified deoxyribonucleosides on Sep-Pak C_{18} cartridges (*7*). The modified deoxyribonucleosides were analyzed by HPLC as described below.

Preparation of Standard 2′-dG or 2′-dA Adducts from 11-MeBPDE. Either 2′-dG-5′-monophosphate (40 mg) or 2′-dA-5′-monophosphate (40 mg) was dissolved in 1 mL of 10 mM Tris-HCl buffer (pH 7.0). To the above solution was added 11-MeBPDE (500 μg) in 0.45 mL of acetone, and the mixture was incubated overnight at 37 °C. The reaction mixture was extracted three times with equal volumes of EtOAc (saturated with H_2O) and once with Et_2O. Finally, the remaining traces of Et_2O were evaporated from the aqueous layer with a gentle stream of N_2, and the modified deoxyribonucleotides were enzymatically hydrolyzed to deoxyribonucleosides as described previously (*6*). A Beckman Ultrasphere ODS 5 μm column (4.6 mm × 250 mm; Beckman Instruments, Fullerton, CA) with the following solvent system was used to separate the modified deoxyribonucleosides: 46% MeOH in H_2O for 34 min, then to 55% MeOH in H_2O (linear gradient) over the course of 10 min, and then 55% MeOH in H_2O for 46 min. The flow rate was 1 mL/min. The three major peaks were collected, and the solvent was evaporated; the resulting residues were redissolved in MeOH, and a CD spectrum of each sample was determined.

Preparation of Peracetates of N^2-dG−11-MeBPDE. The three major N^2-dG−11-MeBPDE adducts were converted to the corresponding peracetates. The solvent was evaporated, and the residue was dissolved in 3 mL of pyridine and mixed with 2 mL of Ac_2O. The resulting mixture was stirred at room temperature overnight. Excess reagent was removed in vacuo. The residue was redissolved in THF and purified by HPLC on a Beckman Ultrasphere ODS 5 μm column (4.6 mm × 250 mm). The following solvent system was used for the separation of modified deoxyribonucleosides at a flow rate of 1 mL/min: 15% MeOH in H_2O to 65% MeOH in H_2O (linear gradient) over the course of 15 min and then to 100% MeOH in 20 min. Each sample was collected and its solvent evaporated, and the sample was then redissolved in acetone-d_6. ^{1}H NMR spectra were recorded at 360 MHz with a 5 mm ^{1}H probe.

Mutagenicity Assays. Diol epoxides were dissolved in DMSO, and *Salmonella typhimurium* strain TA 100 was used for the assay performed as described with preincubation (*8*, *9*). Cytotoxicity was determined by reduction of the number of colonies in nutrient agar plates. 11-MeBPDE was toxic above 1 nmol/plate. Reported mutagenicity values are the means of triplicate assays. Background revertants (145 per plate) have not been subtracted. The number of revertants per nanomole was determined from the slopes of the linear portions of the dose−response curves.

Results and Discussion

Synthesis of 11-MeBPDE. Our synthetic strategy was based on photochemical cyclization of the appropriately substituted ethylene as illustrated in Scheme 1. We have found that the photochemical cyclization provides a very efficient approach to the synthesis of dibenzo[*a*,*l*]-pyrene diol epoxide and various substituted chrysene diol epoxides (*10*, *11*). The precursor for the photocyclization, 1-carbomethoxy-1-(3-methyl-1-naphthyl)-2-(3-methoxy-

Table 1. Chemical Shifts and Coupling Constants for the Methine Protons of BPDE and 11-MeBPDE

	methine protons					
	H_7	H_8		H_9		H_{10}
compound	δ (ppm)	δ (ppm)	coupling constant (Hz)	δ (ppm)	coupling constant (Hz)	δ (ppm)
BPDE[a]	4.71	3.92	$J_{7,8} = 8.8$	3.86	$J_{9,10} = 4.6$	5.21
11-MeBPDE[a]	5.00	4.04	$J_{7,8} = 8.5$	3.87	$J_{9,10} = 4.5$	5.20

[a] NMR spectra were recorded in DMSO-d_6 with a 5 mm ^{1}H probe at 360 MHz on a Bruker AM 360 WB spectrometer.

phenyl)ethylene (**2**), was prepared by condensation of ethyl (3-methyl-1-naphthyl)acetate (**1**) and *m*-methoxybenzaldehyde. Photochemical cyclization of **2** gave 2-methoxy-5-methyl-11-carboethoxychrysene (**3**) in 37% yield. It is interesting to note that the other possible structural isomer, 4-methoxy-5-methyl-11-carboethoxychrysene, was not detected. Conversion of the carboethoxy derivative **3** to aldehyde **4** was achieved in 54% yield with diisobutylaluminum hydride, and the concentration of the corresponding alcohol was determined to be <10% by chromatography. One-carbon chain extension was accomplished by means of the Wittig reaction with (methoxymethyl)-triphenylphosphonium chloride to give **5** in 61% yield. To construct the benzo[*a*]pyrene ring system, standard acid-catalyzed cyclization was employed (*10*). Treatment of **5** with methanesulfonic acid gave 8-methoxy-11-methylBP (**6**) in 77% yield. Demethylation of **6** with boron tribromide afforded **7** in 85% yield. 11-MeBPDE (**10**) was prepared from **7** by application of well-established methods (*12*). The overall yield of 11-MeBPDE (**10**) from **7** was 35%. The presence of a methyl group in the bay region may affect the conformation in the terminal ring. However, ^{1}H NMR shows that chemical shifts and coupling constants of H_7-H_{10} of BPDE and 11-MeBPDE are almost identical (Table 1). These data suggest that 11-MeBPDE and BPDE have similar conformations at the terminal ring.

DNA Adduct Formation. To investigate DNA adduct formation from 11-MeBPDE, the required nucleotide markers were prepared by reacting the diol epoxide with either 2′-dG-5′-monophosphate or 2′-dA-5′-monophosphate (*13*). The modified deoxyribonucleotides were enzymatically hydrolyzed to deoxyribonucleosides which were then analyzed by HPLC (Figure 1). Next, 11-MeBPDE was incubated with calf thymus DNA. The modified DNA was digested enzymatically to give nucleoside adducts that were analyzed by HPLC (Figure 2). Peaks 1−3 were identified as adducts of dG and peaks 4−7 as adducts of dA by comparison of retention times to those of the standard markers. The extent of total calf thymus DNA adduct formation from 11-MeBPDE was 1.7-fold greater than that from BPDE. The extent of formation of dG and dA adducts from 11-MeBPDE was 1.8 and 1.2 times greater than from BPDE, respectively. Similar results were obtained when the *anti*-1,2-diol 3,4-epoxides of 5-MeC and chrysene were compared (*14*). A preliminary mutagenicity assay was conducted with BPDE and 11-MeBPDE in *S. typhimurium* TA 100 (Figure 3). Although 11-MeBPDE is slightly more mutagenic than BPDE at 0.5 and 1 nmol/plate, a more detailed mutagenicity study will be required to fully evaluate the comparative activities of those diol epoxides.

To determine the structure of the major adducts formed by 11-MeBPDE with calf thymus DNA, three deoxyguanosine adducts were collected from HPLC, and their CD and ^{1}H NMR spectra were determined. The CD spectra are illustrated in Figure 4, and the relevant ^{1}H

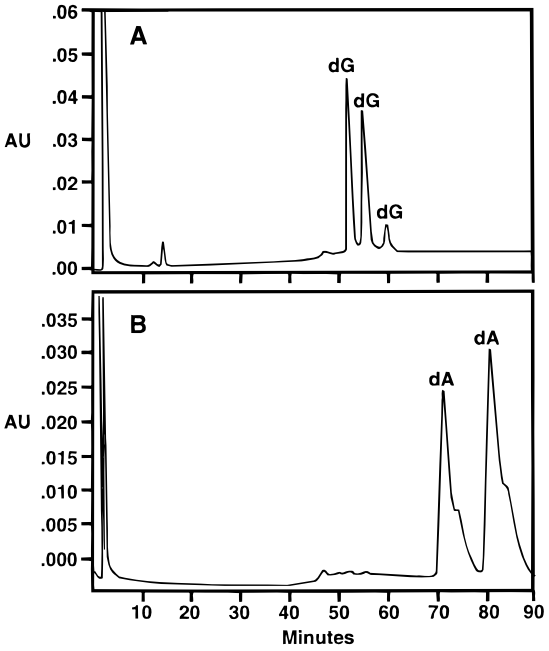


Figure 1. HPLC profile of the products formed upon reaction of *anti*-11-MeBPDE with (A) 2′-dG-5′-monophosphate and (B) 2′-dA-5′-monophosphate.

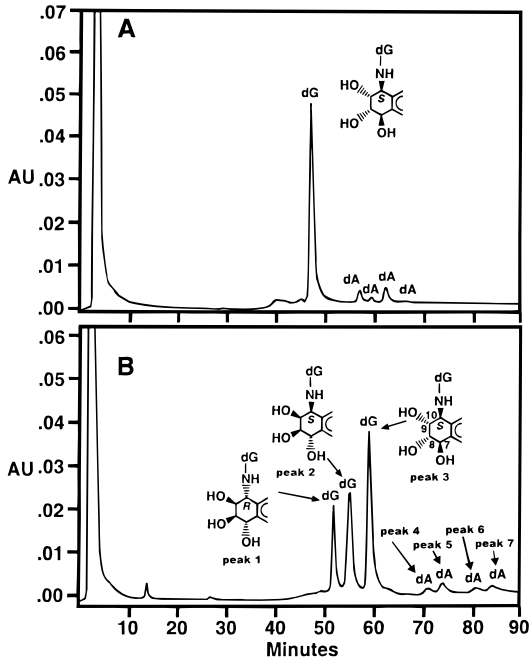


Figure 2. HPLC profiles obtained upon analysis of enzymatic hydrolysates of calf thymus DNA reacted with (A) BPDE and (B) 11-MeBPDE.

NMR data are summarized in Table 2. The most intense bands of the CD spectra of peak 2 and 3 have positive signs; thus, these deoxyguanosine adducts must have the *S* configuration at the point of attachment of the hydrocarbon residue (*15*). The ^{1}H NMR data in Table 2

Table 2. Chemical Shifts and Coupling Constants for the Methine Protons of the Peracetates of Deoxyribonucleoside Adducts of *anti*-11-MeBPDE[a]

	methine protons						
	C_7-H		C_8-H		C_9-H		C_{10}-H
peracetate	δ (ppm)	coupling constant (Hz)	δ (ppm)	coupling constant (Hz)	δ (ppm)	coupling constant (Hz)	δ (ppm)
peak 1, trans[b]	7.00	$J_{7,8} = 9.0$	5.66	$J_{8,9} = 1.9$	6.53	$J_{9,10} = 4.8$	6.93
peak 2, cis[b]	7.00	$J_{7,8} = 8.9$	5.69	$J_{8,9} = 2.0$	6.36	$J_{9,10} = 4.8$	7.11
peak 3, trans	6.83	$J_{7,8} = 9.0$	5.87	$J_{8,9} = 2.1$	6.48	$J_{9,10} = 4.6$	6.73
trans-BP-tetraol	6.80	$J_{7,8} = 9.3$	5.70	$J_{8,9} = 2.4$	5.87	$J_{9,10} = 3.7$	6.95
cis-BP-tetraol	6.62	$J_{7,8} = 4.2$	5.67	$J_{8,9} = 2.6$	5.92	$J_{9,10} = 4.3$	7.33

[a] NMR spectra were recorded in acetone-d_6 with a 5 mm ^{1}H probe at 360 MHz. [b] Trans and cis refer to the relative configuration of the purine and hydroxyl group at the C_{10} and C_9 positions, respectively.

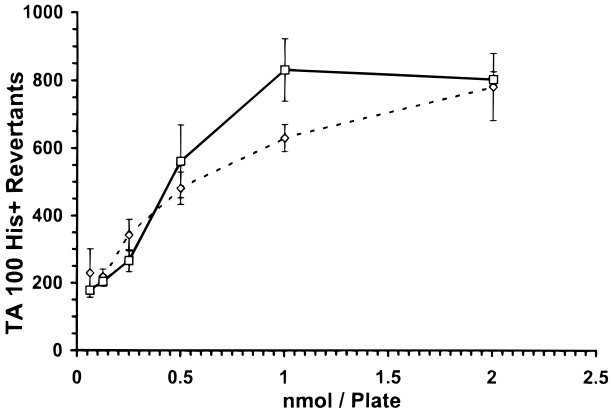


Figure 3. Comparative mutagenicity of BPDE (◇) and 11-MeBPDE (□) in *S. typhimurium* TA 100.

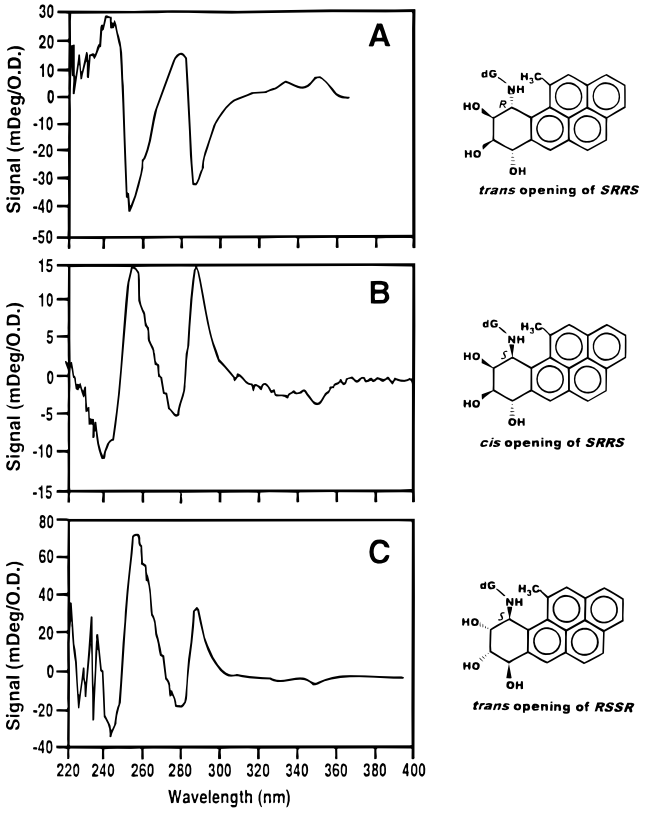


Figure 4. Circular dichroism spectra of (A) 11-MeBPDE−dG adduct peak 1 in MeOH, (B) 11-MeBPDE−dG adduct peak 2 in MeOH, and (C) 11-MeBP−dG adduct peak 3 in MeOH.

demonstrate that peaks 1 and 3 are trans adducts while peak 2 is a cis adduct (*15*). Therefore, peak 1 must be the trans adduct of the (*S*,*R*,*R*,*S*)-enantiomer, peak 2 the cis adduct of the (*S*,*R*,*R*,*S*)-enantiomer, and peak 3 the trans adduct of the (*R*,*S*,*S*,*R*)-enantiomer. These structures are illustrated in Figure 4. Figure 2A shows the HPLC profile obtained after enzymatic hydrolysis of BPDE-modified calf thymus DNA; the major peak has been previously characterized as the trans dG adduct derived from the (*R*,*S*,*S*,*R*)-enantiomer (*13*). Although the formation of the cis adduct derived from the (*R*,*S*,*S*,*R*)-enantiomer of 11-MeBPDE was not detected due to the lack of a reference standard, the possibility that this adduct coeluted with peak 1 or 2 cannot be excluded at this time.

These results demonstrate that introduction of a methyl group into the bay region of BPDE causes a change in the pattern of reaction with DNA. While the reaction of BPDE with DNA gives mainly the trans dG adduct of the (*R*,*S*,*S*,*R*)-enantiomer, reaction of 11-MeBPDE yields substantial amounts of products resulting from both cis and trans addition of dG to the (*S*,*R*,*R*,*S*)-enantiomer, as well as trans addition to the (*R*,*S*,*S*,*R*)-enantiomer. This behavior is also different from that of racemic *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-5-methylchrysene (5-MeCDE), which, in its reaction with DNA, gives the trans adduct of the (*R*,*S*,*S*,*R*)-enantiomer as the major product (*16*). At the present time, the significance of these findings is unclear; however, it is clear that the bay region methyl group affects the reactivity of 11-MeBPDE with DNA, and this may be related to the tumorigenicity of 11-MeBP being higher than that of BP or of the other monomethyl isomers (*4*).

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