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Synthesis, Absolute Configuration, and Bacterial Mutagenicity of the 8 Stereoisomeric Vicinal Diol Epoxides at the Terminal Benzo Ring of Carcinogenic Dibenz[*a,h*]anthracene

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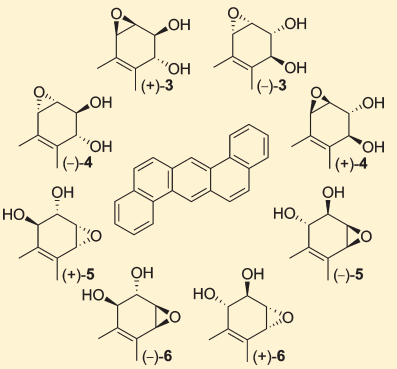
Supporting Information

ABSTRACT: The synthesis of the 8 possible stereoisomeric diol epoxides (DEs) at the terminal benzo ring of carcinogenic dibenz[*a,h*]anthracene (DBA) is reported. *trans*-3,4-Dihydroxy-3,4-dihydro-DBA (**1**) afforded the 4 bay region DEs: the enantiomeric pairs of the *anti* diastereomers (+)-**3**/(−)-**3** and of the *syn* diastereomers (−)-**4**/(+)-**4**, respectively. *trans*-1,2-Dihydroxy-1,2-dihydro-DBA (**2**) served as precursor of the 4 reverse DEs: the enantiomeric pairs of the *anti* diastereomers (+)-**5**/(−)-**5** and of the *syn* diastereomers (−)-**6**/(+)-**6**, respectively. The transformation of the olefinic double bond in the enantiomeric *trans*-dihydrodiols to epoxides was achieved by either (i) oxidation with *m*-chloroperoxybenzoic acid or (ii) formation of a bromohydrin with *N*-bromoacetamide/H_2O followed by dehydrobromination with an anion exchange resin. Because of the pseudodiequatorial conformation of the hydroxyl groups in **1**, both reactions proceeded highly stereoselectively, while the stereoselectivity was impaired by the pseudodiaxial conformation of the hydroxyl groups in **2**. Diastereomers and racemic compounds were efficiently separated without derivatization by HPLC on achiral or chiral stationary phases, respectively. The absolute configurations of the DEs were deduced from the absolute configuration of **1** and **2** considering the regio- and stereoselectivity of the subsequent reactions and resulted in (+)-(1*R*,2*S*,3*S*,4*R*)-**3**/(−)-(1*S*,2*R*,3*R*,4*S*)-**3**, (−)-(1*S*,2*R*,3*S*,4*R*)-**4**/(+)-(1*R*,2*S*,3*R*,4*S*)-**4**, (+)-(1*R*,2*S*,3*S*,4*R*)-**5**/(−)-(1*S*,2*R*,3*R*,4*S*)-**5**, and (−)-(1*R*,2*S*,3*R*,4*S*)-**6**/(+)-(1*S*,2*R*,3*S*,4*R*)-**6**. The bacterial mutagenicity of the 8 stereoisomeric DEs was determined in histidine-dependent strains TA98 and TA100 of *Salmonella typhimurium* in the absence of a metabolizing system. In general, the bay region DEs of DBA were stronger mutagens than the reverse DEs. In strain TA98, the *syn* diastereomers of bay region DEs were stronger mutagens than their *anti* isomers, while in the case of reverse DEs the *anti* diastereomers were more potent than their *syn* isomers. In strain TA100, all *syn* diastereomers surpassed the bacterial mutagenicity of their *anti* isomers. Concerning the bay region DEs of DBA, this corresponds to the situation described for benzo[*a*]pyrene: of the 4 enantiomeric bay region DEs of DBA and benzo[*a*]pyrene, the *syn* diastereomer with [(*R,S*)-diol (*R,S*)-epoxide] absolute configuration is the most potent mutagen in both bacterial strains, while the *anti* isomer with [(*S,R*)-diol (*R,S*)-epoxide] configuration is the weakest mutagen.



INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) play an important role as environmental contaminants due to their chemical stability and because many of them exhibit strong genotoxicity.[1] Like most chemical carcinogens, PAHs have to be transformed enzymatically to chemically reactive metabolites in order to exert their mutagenic and carcinogenic potential.[2] Vicinal diol epoxides with the oxirane ring occupying a sterically hindered bay region have been considered as important genotoxic metabolites of PAHs since the mid-1970s.[3,4] These electrophilic metabolites are prone to nucleophilic attack by water and consequently hydrolyze in the predominantly aqueous environment of biological systems to tetrahydroxy derivatives,[5] which therefore indicate the metabolic formation of vicinal diol epoxides.

The carcinogenic PAH dibenz[*a,h*]anthracene (DBA) is enzymatically converted at its terminal benzo ring to *trans*-dihydrodiols.[6−10] The *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,h*]anthracene (3,4-dihydrodiol, **1**) plays an important role in the genotoxicity of the parent hydrocarbon because of its substantial metabolic formation[6,8,9] and its strong mutagenic[11,12] and carcinogenic activity.[13,14] The occurrence of several 1,2,3,4-tetrahydroxy derivatives of DBA during the biotransformation of 3,4-dihydrodiol **1**[8,15,16] as well as of *trans*-1,2-dihydroxy-1,2-dihydrodibenz[*a,h*]anthracene (1,2-dihydrodiol, **2**)[9,17] indicates the formation of vicinal diol epoxides as metabolic precursors. Chromatographic evidence exists for the formation of *anti*- and *syn*-diol epoxides (**3** and **4**, Scheme 1) from 3,4-dihydrodiol **1**.[16] The biotransformation of 1,2-dihydrodiol **2** to its *anti*- and *syn*-diol epoxides (**5** and **6**, Scheme 1) can only be established indirectly from the occurrence of *r*-1,*t*-2,*t*-3,*c*-4- and *r*-1,*t*-2,*c*-3,*t*-4-tetrahydroxy-1,2,3,4-tetrahydro-DBA as microsomal metabolites of **2**.[9] In order to account for the genotoxic properties of

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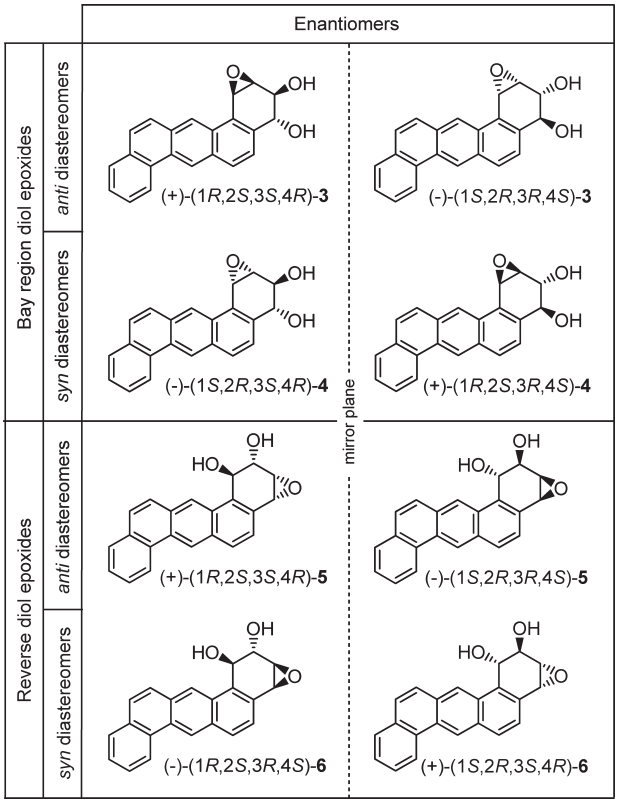
Scheme 1. Chemical Structures of the 8 Stereoisomers of Vicinal Diol Epoxides at the 1,2,3,4-Positions of Dibenz[*a,h*]-anthracene



these reactive metabolites of DBA, their synthetic preparation was required. Since not only the formation of the *trans*-dihydrodiols of DBA[8−10,12] but also the mutagenicity of the enantiomers[12] is highly stereoselective, differences in the genotoxicity of their metabolites, the enantiomeric diol epoxides, could be expected. Therefore, the 8 possible stereoisomers of the vicinal diol epoxides at the terminal benzo ring of DBA (Scheme 1) were synthesized. The 3,4-dihydrodiol **1** afforded the 4 bay region diol epoxides: the enantiomeric pair of the *anti* diastereomers (+)-**3**/(−)-**3** and the enantiomeric pair of the *syn* diastereomers (−)-**4**/(+)-**4** (Scheme 1). The 1,2-dihydrodiol **2** served as precursor of the 4 reverse diol epoxides: the enantiomeric pair of the *anti* diastereomers (+)-**5**/(−)-**5** and the enantiomeric pair of the *syn* diastereomers (−)-**6**/(+)-**6** (Scheme 1).

The absolute configuration of the 8 stereoisomeric diol epoxides was deduced from the absolute configuration of the starting *trans*-dihydrodiols **1** and **2**[18] considering the regio- and stereoselectivity of the subsequent reactions. Finally, as a standardized indicator of genotoxic activity the bacterial mutagenicity in *Salmonella typhimurium* of the 8 stereoisomeric diol epoxides was determined.

EXPERIMENTAL PROCEDURES

Caution: *PAHs and their derivatives are potentially hazardous and should be handled in accordance with NIH Guidelines for the Laboratory Use of Chemical Carcinogens.*

Chemicals. (±)-*trans*-3,4-Bis(benzoyloxy)-1,2,3,4-tetrahydrodibenz[*a,h*]anthracene was prepared from 1,2-dihydrodibenz[*a,h*]anthracene-4(3*H*)-one[19] by the method described by McCaustland and Engel[20] for the synthesis of the corresponding derivative of benzo[*a*]pyrene (BaP). The 1,2-dihydrodiol (±)-**2** was obtained as reported elsewhere.[21] The chiral stationary phases based on silica-bound (−)-2-(2,4,5,7-tetranitrofluoren-9-ylideneaminooxy)propionic acid ((−)-TAPA) or (−)- and (+)-2-(2,5,9,11-tetranitro-7*H*-benzo[*c*]fluoren-7-ylideneaminooxy)propionic acid ((−)-/(+)-TBFPA) were prepared as previously described.[22,23] Pure *m*-chloroperoxybenzoic acid (MCPBA) used for the epoxidations was obtained by extensive washing of commercial MCPBA (77%; Fluka, Neu-Ulm, Germany) with phosphate buffer (1 M, pH 7.5)[24] and drying. *N*-Bromoacetamide (NBA), *N*-bromosuccinimide (NBS), the anion exchange resin Amberlite IRA-400(Cl), and deuterated solvents for NMR spectroscopy were provided by Aldrich, Steinheim, Germany. LiCl and Li_2CO_3 were delivered by Fluka, Neu-Ulm, Germany. All other chemicals and the achiral stationary phases for HPLC were supplied by Merck, Darmstadt, Germany. All chemicals used were of analytical or HPLC grade.

Analytical Methods. Preparative HPLC was conducted with system 830 (Du Pont, Bad Homburg, Germany), analytical HPLC with model 3500 B (Spectra Physics, Darmstadt, Germany), monitoring the UV absorbance at 254 nm. Melting point determinations were carried out in unsealed capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on system AM 400 (Bruker, Karlsruhe, Germany) at 400 MHz or with a Bruker WH 90 at 90 MHz. ^{1}H chemical shifts (ppm) are reported relative to tetramethylsilane. In the case of racemic compounds and their two enantiomers, a ^{1}H NMR spectrum is displayed just once since the spectra of the three compounds are identical. UV/vis absorbance spectra were measured in ethanol with a MPS-2000 spectrophotometer (Shimadzu, Düsseldorf, Germany). Specific rotations were determined in THF at 589 nm (sodium D-line) and 20 °C with a 241 LC polarimeter (Perkin-Elmer, Frankfurt, Germany). Mass spectra were run on a MAT CH 711 (Varian, Darmstadt, Germany) using the electron impact ionization method (EI).

*(±)-trans-3,4-Bis(benzoyloxy)-3,4-dihydrodibenz[a,h]anthracene [(±)-**7**].* To a solution of (±)-*trans*-3,4-bis(benzoyloxy)-1,2,3,4-tetrahydrodibenz[*a,h*]anthracene (1.2 g, 2.3 mmol) in CCl_4 at 60 °C, NBS (0.6 g, 3.0 mmol) and benzoyl peroxide (5 mg) were added. The mixture was irradiated with a UV lamp (300 W; Ultra Vitalux, Osram, München, Germany) for 20 min and heated at reflux for 40 min. After evaporation, the residue was dissolved in hexamethylphosphoric triamide (HMPT; 150 mL), LiCl (5.0 g, 118 mmol), and Li_2CO_3 (7.5 g, 102 mmol) were added, and the suspension was heated to 100 °C under an argon atmosphere for 2.5 h. The mixture was cooled to room temperature, poured into 500 mL ice water, and extracted with ethyl acetate (2 × 250 mL). The organic layer was washed successively with water, 5% aqueous acetic acid, water, 10% aqueous $NaHCO_3$ solution and water, dried with $MgSO_4$, and concentrated under reduced pressure. Purification by chromatography on silica (chloroform/*n*-hexane 8:2, v/v) and final trituration with boiling methanol yielded (±)-**7** (0.88 g, 74%) as a pale yellow solid: mp 233 °C (239−240 °C).[24] ^{1}H NMR (400 MHz; Me_2SO-d_6/D_2O) δ 9.49 (s, 1H, H_7), 9.06 (s, 1H, H_{14}), 8.99 (d, 1H, H_8, $J_{8,9}$ = 7.9 Hz), 8.27−7.47 (m, 17H, H_1, $H_{aromat.}$), 6.78 (d, 1H, H_4, $J_{3,4}$ = 7.2 Hz), 6.52 (dd, 1H, H_2, $J_{1,2}$ = 9.1 Hz, $J_{2,3}$ = 3.3 Hz), 6.14 (dd, 1H, H_3, $J_{3,4}$ = 7.2 Hz, $J_{2,3}$ = 3.3 Hz); EI-MS *m/z* (rel intensity, %) 520 (8) $[M]^+$, 398 (43) $[MH - C_6H_5CO - H_2O]^+$, 105 (100) $[C_6H_5CO]^+$.

*(±)-trans-3,4-Dihydroxy-3,4-dihydrodibenz[a,h]anthracene [(±)-**1**].* The methanolysis of (±)-**7** (0.6 g, 1.15 mmol) was carried out according to ref 24 and afforded (±)-**1** (0.34 g, 94%) as a pale yellow solid: mp 265 °C (278−280 °C).[24] ^{1}H NMR (400 MHz; acetone-d_6/Me_2SO-d_6 [3:2, v/v]/D_2O) δ 9.33 (s, 1H, H_7), 8.92 (d, 1H, H_8, $J_{8,9}$ = 7.9 Hz), 8.79 (s, 1H, H_{14}), 8.14 (d, 1H, H_{12}, $J_{12,13}$ = 8.6 Hz), 7.92−7.59 (m, 6H, $H_{5,6,9-11,13}$), 7.42 (dd, 1H, H_1, $J_{1,2}$ = 10.1 Hz, $J_{1,3}$ = 2.4 Hz), 6.22 (dd, 1H, H_2, $J_{1,2}$ = 10.1 Hz, $J_{2,3}$ = 2.4 Hz), 4.84 (d, 1H, H_4, $J_{3,4}$ = 11.5 Hz),

4.47−4.43 (dt, 1H, H_3, $J_{3,4}$ = 11.5 Hz, $J_{2,3}$ = $J_{1,3}$ = 2.4 Hz). UV/vis: λ_{max} (nm) (ε [M^{-1} cm^{-1}]): 225 (24 400), 233 (22 800), 247 (16 900), 277 (46 300), 286 (63 500), 295 (55 400), 337 (1 400), 348 (3 100), 369 (4 200), 387 (2 000); EI-MS *m/z* (rel intensity, %) 312 (100) $[M]^+$, 294 (91) $[M - H_2O]^+$, 266 (87) $[M - H_2O - CO]^+$, 265 (78) $[M - H_2O - CHO]^+$.

*(+)-(3S,4S)-3,4-Dihydroxy-3,4-dihydrodibenz[a,h]anthracene [**(+)-1**] and (−)-(3R,4R)-3,4-Dihydroxy-3,4-dihydrodibenz[a,h]anthracene [**(−)-1**].* The separation of (±)-**1** (238 mg, 0.73 mmol) into the enantiomers was performed by preparative HPLC (dichloromethane/methanol 4:1, v/v; 21 mL/min) in 20 mg portions using a chiral stationary phase based on silica-bound (−)-TBFPA (5 μm; 16 × 250 mm) as previously described.[23] The first eluted fraction contained (−)-**1** as the major product and the second fraction (+)-**1**. The latter was purified by 3-fold rechromatography under the conditions described above and final chromatography on Florisil (50 g; chloroform/diethyl ether 9:1, v/v) to yield a pale yellow solid: (+)-**1** (42.7 mg, 38%), $[\alpha]_D^{20}$ = +202° (*c* = 0.61 mg/mL). Because of the tailing of (−)-**1** during chromatographic separation, the first fraction had to be enantiomerically purified by application of a chiral stationary phase based on silica-bound (+)-TBFPA (5 μm; 16 × 250 mm; dichloromethane/methanol 4:1, v/v; 18 mL/min), which improved the separation by inversion of the elution order of the enantiomers. 2-Fold rechromatography and final purification with Florisil as described above afforded a pale yellow solid: (−)-**1** (49.7 mg, 44%), $[\alpha]_D^{20}$ = −193° (*c* = 0.56 mg/mL).

*(±)-c-3,t-4-Dihydroxy-r-1,2-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(±)-3**].* Epoxidation of (±)-**1** (114 mg, 0.36 mmol) with MCPBA carried out as described[24] yielded (±)-**3** (67.1 mg, 56%) as a white solid: mp 264 °C (dec) (196−197 °C).[24] ^{1}H NMR (400 MHz; acetone-d_6/Me_2SO-d_6 [3:2, v/v]/D_2O) δ 9.37 (s, 1H, H_7), 9.04 (s, 1H, H_{14}), 8.92 (d, 1H, H_8, $J_{8,9}$ = 8.1 Hz), 8.22 (d, 1H, H_{13}, $J_{12,13}$ = 8.8 Hz), 7.94−7.88 (m, 3H, $H_{5,11,12}$), 7.70 (d, 1H, H_6, $J_{5,6}$ = 9.1 Hz), 7.68−7.60 (m, 2H, $H_{9,10}$), 5.08 (d, 1H, H_1, $J_{1,2}$ = 4.5 Hz), 4.59 (d, 1H, H_4, $J_{3,4}$ = 8.6 Hz), 3.92 (d, 1H, H_3, $J_{3,4}$ = 8.6 Hz), 3.83 (d, 1H, H_2, $J_{1,2}$ = 4.5 Hz). UV/vis: λ_{max} (nm) (ε [M^{-1} cm^{-1}]): 203 (21 100), 222 (32 000), 236 (24 000), 262 (31 500), 271 (38 700), 281 (69 700), 292 (87 000), 337 (5 800), 353 (6 300), 369 (4 200), 389 (1 200); EI-MS *m/z* (rel intensity, %) 310 (100) $[M - H_2O]^+$, 282 (8) $[M - H_2O - CO]^+$, 281 (16) $[M - H_2O - CHO]^+$, 254 (6) $[M - H_2O - 2CO]^+$, 252 (16) $[M - H_2O - 2CHO]^+$.

*(+)-(1R,2S,3S,4R)-3,4-Dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrodibenz-[a,h]anthracene [(+)-3] and (−)-(1S,2R,3R,4S)-3,4-Dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-3**].* Epoxidation of (−)-**1** (20 mg, 0.064 mmol) as described above afforded (+)-**3** (17.2 mg, 82%): $[\alpha]_D^{20}$ = +43° (*c* = 0.58 mg/mL). By an analogous reaction of (+)-**1** (19.2 mg, 0.061 mmol), (−)-**3** (18.2 mg, 90%) was obtained: $[\alpha]_D^{20}$ = −37° (*c* = 0.48 mg/mL).

*(±)-t-2-Bromo-r-1,t-3,c-4-trihydroxy-1,2,3,4-tetrahydrodibenz[a,h]-anthracene [**(±)-8**].* According to the procedure described by Yagi et al.,[25] (±)-**1** (100 mg, 0.32 mmol) was transformed with NBA (50 mg, 0.36 mmol) to the bromotriol (±)-**8** (105 mg, 80%), which was obtained as a white solid: mp 163 °C (dec). ^{1}H NMR (90 MHz; acetone-d_6/D_2O) δ 9.34 (s, 1H, H_7), 8.99−8.91 (m, 1H, H_8), 8.82 (s, 1H, H_{14}), 8.23 (d, 1H, H_{13}, $J_{12,13}$ = 8.8 Hz), 8.00−7.65 (m, 6H, $H_{5,6,9-12}$), 5.92 (d, 1H, H_1, $J_{1,2}$ = 2.6 Hz), 4.96−4.79 (m, 2H, $H_{3,4}$), 4.42−4.30 (m, 1H, H_2); EI-MS *m/z* (rel intensity, %) 374 (92) $[M (^{81}Br) - 2 \times H_2O]^+$, 372 (100) $[M (^{79}Br) - 2 \times H_2O]^+$, 263 (51) $[M - 2H_2O - HBr - CHO]^+$.

*(+)-(1S,2R,3S,4R)-2-Bromo-1,3,4-trihydroxy-1,2,3,4-tetrahydrodibenz [a,h]anthracene [**(+)-8**] and (−)-(1R,2S,3R,4S)-2-Bromo-1,3,4-trihydroxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-8**].* Separation of the racemic bromotriol (±)-**8** (78 mg, 0.19 mmol) was performed by preparative HPLC (dichloromethane/methanol 3:2, v/v; 24 mL/min) in 26 mg portions using a chiral stationary phase based on silica-bound (−)-TAPA (5 μm; 16 × 250 mm). The first eluted fractions gave (−)-**8** (28.3 mg, 73%), $[\alpha]_D^{20}$ = −17° (*c* = 0.18 mg/mL), the second fractions (+)-**8** (35.2 mg, 90%), $[\alpha]_D^{20}$ = +24° (*c* = 0.49 mg/mL), as white solids.

*(±)-t-3,c-4-Dihydroxy-r-1,2-epoxy-1,2,3,4-tetrahydrodibenz[a,h]-anthracene [**(±)-4**].* Dehydrobromination of (±)-**8** (92 mg, 0.22 mmol) with the anion exchange resin Amberlite IRA-400 according to the method of Yagi et al.[25] yielded (±)-**4** (37.0 mg, 50%) as a white solid: mp 214−216 °C (dec). ^{1}H NMR (400 MHz; acetone-d_6/Me_2SO-d_6 [3:2, v/v]/D_2O) δ 9.39 (s, 1H, H_7), 8.95 (s, 1H, H_{14}), 8.93 (d, 1H, H_8, $J_{8,9}$ = 7.7 Hz), 8.24 (d, 1H, H_{13}, $J_{12,13}$ = 8.7 Hz), 7.96−7.89 (m, 2H, $H_{11,12}$), 7.73 (d, 1H, H_5, $J_{5,6}$ = 9.3 Hz), 7.70−7.61 (m, 3H, $H_{6,9,10}$), 4.84 (d, 1H, H_1, $J_{1,2}$ = 4.2 Hz), 4.73 (d, 1H, H_4, $J_{3,4}$ = 5.9 Hz), 4.00 (d, 1H, H_3, $J_{3,4}$ = 5.9 Hz), 3.84−3.83 (m, 1H, H_2). UV/vis: λ_{max} (nm) (ε [M^{-1} cm^{-1}]): 223 (41 900), 235 (33 100), 262 (47 600), 270 (60 500), 280 (103 600), 291 (126 000), 336 (7 600), 351 (8 000), 363 (5 700), 389 (1 500); EI-MS *m/z* (rel intensity, %) 328 (10) $[M]^+$, 310 (70) $[M - H_2O]^+$, 282 (20) $[M - H_2O - CO]^+$, 281 (45) $[M - H_2O - CHO]^+$, 254 (22) $[M - H_2O - 2CO]^+$, 253 (41) $[M - H_2O - CO - CHO]^+$, 252 (52) $[M - H_2O - 2CHO]^+$.

*(+)-(1R,2S,3R,4S)-3,4-Dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrodibenz [a,h]anthracene [**(+)-4**] and (−)-(1S,2R,3S,4R)-3,4-Dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-4**].* Dehydrobromination of (−)-**8** (25 mg, 0.061 mmol) as described above yielded (+)-**4** (15.7 mg, 78%) as a white solid: $[\alpha]_D^{20}$ = +103° (*c* = 0.48 mg/mL). Similarly, the reaction of (+)-**8** (32.4 mg, 0.079 mmol) afforded (−)-**4** (21.7 mg, 84%): $[\alpha]_D^{20}$ = −77° (*c* = 0.64 mg/mL).

*(−)-(1R,2R)-1,2-Dihydroxy-1,2-dihydrodibenz[a,h]anthracene [**(−)-2**] and (+)-(1S,2S)-1,2-Dihydroxy-1,2-dihydrodibenz[a,h]anthracene [**(+)-2**].* Compound (±)-**2** (107 mg, 0.34 mmol) was separated in 21 mg portions by preparative HPLC (dichloromethane/methanol 7:3, v/v; 33 mL/min) using a chiral stationary phase based on silica-bound (−)-TAPA (5 μm; 16 × 250 mm) as previously described.[23] The first eluted fraction afforded (−)-**2** (52.6 mg), the second fraction (+)-**2** (50.6 mg), as crude products. Final purification of the first fraction was carried out by chromatography on Florisil (50 g; chloroform/diethyl ether 9:1, v/v) to yield pure (−)-**2** (39.7 mg, 74%) as a pale yellow solid: $[\alpha]_D^{20}$ = −468° (*c* = 2.33 mg/mL), mp 241 °C (257−259 °C).[24] ^{1}H NMR (400 MHz; acetone-d_6/D_2O) δ 9.31 (s, 1H, H_7), 8.92 (d, 1H, H_8, $J_{8,9}$ = 8.0 Hz), 8.87 (s, 1H, H_{14}), 8.15 (d, 1H, H_{12}, $J_{12,13}$ = 8.5 Hz), 7.94−7.60 (m, 5H, $H_{5,6,9-11}$), 7.44 (d, 1H, H_{13}, $J_{12,13}$ = 8.5 Hz), 6.75 (d, 1H, H_4, $J_{3,4}$ = 9.5 Hz), 6.30−6.26 (m, 1H, H_3, $J_{2,3}$ = 5.3 Hz, $J_{1,3}$ = 0.8 Hz, $J_{3,4}$ = 9.5 Hz), 5.56 (s, 1H, H_1, $J_{1,2}$ = 0 Hz), 4.52−4.43 (dd, 1H, H_2, $J_{2,3}$ = 5.3 Hz, $J_{2,4}$ = 1.7 Hz). UV/vis: λ_{max} (nm) (ε [M^{-1} cm^{-1}]): 258 (36 700), 267 (32 000), 280 (46 200), 293 (83 700), 307 (110 400), 329 (6 300), 345 (7 000), 363 (6 000), 383 (2 500); EI-MS *m/z* (rel intensity, %) 312 (2.2) $[M]^+$, 294 (100) $[M - H_2O]^+$, 265 (39) $[M - CHO]^+$.

Pure (+)-**2** (41.4 mg, 77%) $[\alpha]_D^{20}$ = +406° (*c* = 2.17 mg/mL) was obtained by similar treatment of the second fraction.

*(−)-(1R,2S,3R,4S)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz [a,h]anthracene [**(−)-6**] and (+)-(1R,2S,3S,4R)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(+)-5**].* Epoxidation of (−)-**2** (100 mg, 0.32 mmol) with MCPBA by the procedure employed for the preparation of (±)-**3** gave a mixture (73 mg) of *syn* and *anti* diastereomers.[24] Separation was accomplished in 10 mg portions of the crude product by HPLC (LiChrosorb Si 60, 5 μm, 16 × 250 mm; THF/diethyl ether/*n*-heptane 3:3:2, v/v/v; 4.8 mL/min). The first eluted fraction afforded the *syn* diastereomer (−)-**6** (4.81 mg, 4.6%), $[\alpha]_D^{20}$ = −171° (*c* = 0.14 mg/mL), and the second fraction yielded the *anti* diastereomer (+)-**5** (10.2 mg, 9.7%), $[\alpha]_D^{20}$ = +200° (*c* = 0.20 mg/mL), as white powders. (+)-**5**: ^{1}H NMR (400 MHz; acetone-d_6/Me_2SO-d_6 [3:2, v/v]/D_2O) δ 9.52 (s, 1H, H_7), 9.33 (s, 1H, H_{14}), 8.91 (d, 1H, H_8, $J_{8,9}$ = 8.2 Hz), 8.16 (d, 1H, H_{13}, $J_{12,13}$ = 8.6 Hz), 7.93−7.87 (dd, 1H, H_{11}, $J_{10,11}$ = 7.2 Hz, $J_{9,11}$ = 1.2 Hz), 7.82 (d, 1H, H_6, $J_{5,6}$ = 9.0 Hz), 7.70−7.59 (m, 4H, $H_{5,9,10,12}$), 5.07 (d, 1H, H_1, $J_{1,2}$ = 7.1 Hz), 4.16 (d, 1H, H_4, $J_{3,4}$ = 4.4 Hz), 4.15−4.13 (dd, 1H, H_2, $J_{1,2}$ = 7.1 Hz,

$J_{2,3}$= 1.8 Hz), 3.84−3.82 (dd, 1H, H_3, $J_{3,4}$ = 4.4 Hz, $J_{2,3}$= 1.8 Hz). UV/vis: λ_{max} (nm) (ε [M^{-1} cm^{-1}]) 222 (70 400), 239 (62 200), 262 (86 000), 272 (107 900), 282 (192 400), 293 (245 000), 337 (13 300), 354 (12 900), 391 (2 700). EI-MS *m*/*z* (rel intensity, %) 310 (100) [M − H_2O]$^+$, 281 (7) [M − H_2O − CHO]$^+$, 252 (10) [M − H_2O − 2 × CHO]$^+$.

*(+)-(1S,2R,3S,4R)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(+)-6**] and (−)-(1S,2R,3R,4S)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-5**].* Epoxidation of (+)-**2** (100 mg, 0.32 mmol) as described above yielded a mixture (69 mg) of *syn* and *anti* diastereomers. HPLC separation on LiChrosorb Si60 as outlined above afforded as the first eluted fraction the *syn* diastereomer (+)-**6** (5.44 mg, 5.2%), $[\alpha]_D^{20}$ = +142° (*c* = 0.15 mg/mL), and as the second fraction the *anti* diastereomer (−)-**5** (8.00 mg, 7.6%), $[\alpha]_D^{20}$ = −102° (*c* = 0.23 mg/mL). Both diastereomers were obtained as white powders.

*(±)-t-3-Bromo-r-1,t-2,c-4-trihydroxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(±)-9**)] and (±)-c-3-Bromo-r-1,t-2,t-4-trihydroxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(±)-10**].* According to the procedure described by Yagi et al.,[25] (±)-**2** (250 mg, 0.80 mmol) was transformed with NBA (125 mg, 0.91 mmol) to a mixture (297 mg) of the diastereomeric bromotriols (±)-**9** and (±)-**10**. Separation was performed by reversed-phase HPLC (LiChrosorb RP-18, 10 μm, 16 × 250 mm; methanol/water 3:2, v/v; 17 mL/min). The first eluted fraction contained (±)-**9** (81.2 mg, 25%) and the second fraction (±)-**10** (83.5 mg, 26%). Both diastereomers were obtained as white solids:

(±)-**9**: mp 223 °C (dec). ^{1}H NMR (90 MHz; Me_2SO-d_6/D_2O) δ 9.33 (s, 1H, H_7), 9.00−8.89 (m, 1H, H_8), 8.73 (s, 1H, H_{14}), 8.20 (d, 1H, H_{13}, $J_{12,13}$ = 8.8 Hz), 7.98−7.67 (m, 6H, $H_{5,6,9-12}$), 5.40 (d, 1H, H_1, $J_{1,2}$ = 3.6 Hz), 4.98 (d, 1H, H_4, $J_{3,4}$ = 8.6 Hz), 4.82−4.69 (dd, 1H, H_3, $J_{3,4}$ = 8.6 Hz, $J_{2,3}$ = 2.0 Hz), 4.40−4.33 (m, 1H, H_2); EI-MS *m*/*z* (rel intensity, %) 374 (72) [M (^{81}Br) − 2 × H_2O]$^+$, 372 (71) [M (^{79}Br) − 2 × H_2O]$^+$, 265 (30) [M − 2 × H_2O − Br − CO]$^+$, 263 (34) [M − 2 × H_2O − HBr − CHO]$^+$.

(±)-**10**: mp 134 °C (dec). ^{1}H NMR (90 MHz; Me_2SO-d_6/D_2O) δ 9.35 (s, 1H, H_7), 8.93 (s, 1H, H_{14}), 9.00−8.87 (m, 1H, H_8), 8.22 (d, 1H, H_{13}, $J_{12,13}$ = 9.1 Hz), 8.00−7.67 (m, 6H, $H_{5,6,9-12}$), 5.35 (d, 1H, H_1, $J_{1,2}$ = 3.8 Hz), 4.96 (d, 1H, H_4, $J_{3,4}$ = 5.0 Hz), 4.11−4.02 (m, 2H, $H_{2,3}$); EI-MS *m*/*z* (rel intensity, %) 374 (19) [M (^{81}Br) − 2 × H_2O]$^+$, 372 (19) [M (^{79}Br) − 2 × H_2O]$^+$, 265 (11) [M − 2 × H_2O − Br − CO]$^+$, 263 (13) [M − 2 × H_2O − HBr − CHO]$^+$.

*(+)-(1S,2R,3S,4R)-3-Bromo-1,2,4-trihydroxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(+)-9**] and (−)-(1R,2S,3R,4S)-3-Bromo-1,2,4-trihydroxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-9**].* Bromotriol (±)-**9** (80 mg, 0.20 mmol) was separated into the enantiomers in 10 mg portions by preparative HPLC (dichloromethane/methanol 3:2, v/v; 16 mL/min) using a chiral stationary phase based on silica-bound (−)-TAPA (5 μm; 16 × 250 mm). In the first fraction, (−)-**9** (29.2 mg, 73%), $[\alpha]_D^{20}$ = −16° (*c* = 0.48 mg/mL), was collected, and in the second fraction, (+)-**9** (35.7 mg, 89%), $[\alpha]_D^{20}$ = +17° (*c* = 0.76 mg/mL), was collected.

*(−)-(1R,2S,3R,4S)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-6**].* Dehydrobromination of the enantiomeric bromotriol (−)-**9** (24.8 mg, 0.061 mmol) with the anion exchange resin Amberlite IRA-400 performed according to Yagi et al.,[25] and final purification by HPLC (LiChrosorb Si 60, 5 μm, 16 × 250 mm; THF/diethyl ether/*n*-heptane 3:3:2, v/v/v; 5.3 mL/min) afforded (−)-**6** (5.37 mg, 27%) as a white solid: $[\alpha]_D^{20}$ = −187° (*c* = 0.23 mg/mL). ^{1}H NMR (400 MHz; acetone-d_6/Me_2SO-d_6 [3:2, v/v]/D_2O) δ 9.44 (s, 1H, H_7), 8.97 (d, 1H, H_8, $J_{8,9}$ = 8.0 Hz), 8.81 (s, 1H, H_{14}), 8.26 (d, 1H, H_{13}, $J_{12,13}$ = 8.5 Hz), 7.97 (d, 1H, H_6, $J_{5,6}$ = 9.1 Hz), 7.93 (d, 1H, H_{11}, $J_{10,11}$ = 7.7 Hz), 7.79 (d, 1H, H_5, $J_{5,6}$ = 9.1 Hz), 7.75 (d, 1H, H_{12}, $J_{12,13}$ = 8.5 Hz), 7.72−7.63 (m, 2H, $H_{9,10}$), 5.47 (t, 1H, H_1), 4.61 (t, 1H, H_2, $J_{1,2}$ = 2.3 Hz, $J_{2,3}$ = 1.2 Hz), 4.25 (d, 1H, H_4, $J_{3,4}$ = 3.9 Hz), 3.96−3.94 (m, 1H, H_3). UV/vis: λ_{max} (nm) (ε [M^{-1} cm^{-1}]): 222 (56 000), 240 (50 700), 262 (74 700), 271 (94 600), 282 (170 000), 293 (221 000), 336 (11 100), 352 (11 400), 390 (2 500); EI-MS *m*/*z* (rel intensity, %) 310 (100) [M − H_2O]$^+$, 281 (7) [M − H_2O − CHO]$^+$, 252 (10) [M − H_2O − 2 × CHO]$^+$.

*(+)-(1S,2R,3S,4R)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(+)-6**].* Dehydrobromination of (+)-**9** (29.9 mg, 0.073 mmol) and purification as described above afforded (+)-**6** (9.36 mg, 39%) as a white solid: $[\alpha]_D^{20}$ = +167° (*c* = 0.21 mg/mL).

*(+)-(1R,2S,3S,4R)-3-Bromo-1,2,4-trihydroxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(+)-10**] and (−)-(1S,2R,3R,4S)-3-Bromo-1,2,4-trihydroxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-10**].* Bromotriol (±)-**10** (80 mg, 0.20 mmol) was separated by preparative HPLC under the conditions described for (±)-**9**. The first eluted fraction afforded (+)-**10** (24.7 mg, 62%), $[\alpha]_D^{20}$ = +119° (*c* = 0.97 mg/mL), and the second fraction afforded (−)-**10** (31.4 mg, 79%), $[\alpha]_D^{20}$ = −119° (*c* = 0.56 mg/mL).

*(+)-(1R,2S,3R,4R)-1,4-Dihydroxy-2,3-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(+)-11**] and (+)-(1R,2S,3S,4R)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(+)-5**].* Dehydrobromination of (+)-**10** (22.8 mg, 0.056 mmol) with the anion exchange resin Amberlite IRA-400 according to Yagi et al.[25] yielded a mixture of the regioisomeric epoxides (+)-**11** and (+)-**5**. HPLC separation (LiChrosorb Si 60, 5 μm, 16 × 250 mm; THF/diethyl ether/*n*-heptane 3:3:2, v/v/v; 5.0 mL/min) afforded as the first eluted fraction (+)-**11** (3.66 mg, 20%): $[\alpha]_D^{20}$ = +120° (*c* = 0.10 mg/mL). ^{1}H NMR (400 MHz; acetone-d_6/Me_2SO-d_6 [3:2, v/v]/D_2O) δ 9.39 (s, 1H, H_7), 9.04 (s, 1H, H_{14}), 8.93 (d, 1H, H_8, $J_{8,9}$ = 8.0 Hz), 8.23 (d, 1H, H_{13}, $J_{12,13}$ = 8.7 Hz), 7.95 (d, 1H, H_6, $J_{5,6}$ = 9.0 Hz), 7.91−7.88 (m, 2H, $H_{5,11}$), 7.72−7.60 (m, 3H, $H_{9,10,12}$), 5.02 (d, 1H, H_2, $J_{2,3}$ = 4.0 Hz), 4.65 (d, 1H, H_1, $J_{1,2}$ = 4.2 Hz), 4.49 (t, 1H, H_4, $J_{3,4}$ = 3.4 Hz), 3.81 (t, 1H, H_3, $J_{3,4}$ = 3.4 Hz). As the second fraction, (+)-**5** (1.18 mg, 6.5%), $[\alpha]_D^{20}$ = +189° (*c* = 0.10 mg/mL), was obtained.

*(−)-(1S,2R,3S,4S)-1,4-Dihydroxy-2,3-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-11**] and (−)-(1S,2R,3R,4S)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz[a,h]anthracene [**(−)-5**].* Dehydrobromination of (−)-**10** (28.7 mg, 0.070 mmol) affording a mixture of the regioisomeric epoxides (−)-**11** and (−)-**5** and their separation were performed as described above. The first fraction yielded (−)-**11** (6.40 mg, 28%), $[\alpha]_D^{20}$ = −43° (*c* = 0.31 mg/mL), and the second fraction yielded (−)-**5** (2.82 mg, 12%), $[\alpha]_D^{20}$ = −114° (*c* = 0.14 mg/mL).

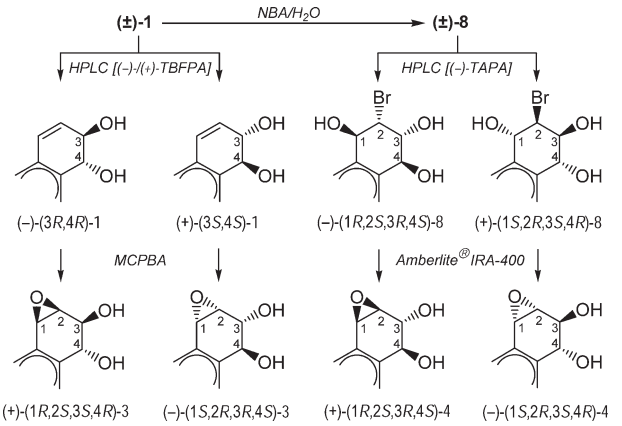
Mutagenicity Studies. The bacterial mutagenicity was determined by employing histidine-dependent strains TA98 and TA100 of *Salmonella typhimurium* (generously provided by Dr. B. N. Ames, University of California, Berkeley, CA) as described by Maron and Ames[26] with only minor modifications[16] omitting an exogenous metabolizing system. The mutagenicity test was performed according to the plate incorporation protocol.[26] The test compounds were dissolved in 30 μL/plate *N*,*N*-dimethylformamide immediately before use. The specific mutagenicity was calculated from the slope of the initial linear part of the dose−response curve.

RESULTS AND DISCUSSION

Synthesis. The diol epoxides **3**, **4**, **5**, and **6** (Scheme 1) were prepared from the 3,4-dihydrodiol **1** or the 1,2-dihydrodiol **2** by employing two reactions: either epoxidation with MCPBA or dehydrobromination of suitable bromotriols as shown in Schemes 2 and 3.

The 3,4-dihydrodiol (±)-**1** was obtained by the classical stepwise construction of the DBA nucleus[19,27] via *trans*-3,4-bis(benzoyloxy)-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene,[24] its allylic bromination, and dehydrobromination to the dihydrodibenzoate (±)-**7** followed by methanolysis. The overall yield (70%) for these three steps, a considerable improvement over the published yield (36%),[24] was achieved by using LiCl/Li_2CO_3 in HMPT[28] as previously reported for dehydrobromination of

Scheme 2. Synthesis of the Stereoisomers of *trans*-3,4-Dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrodibenz[*a,h*]anthracene (Bay Region Diol Epoxides)[a]



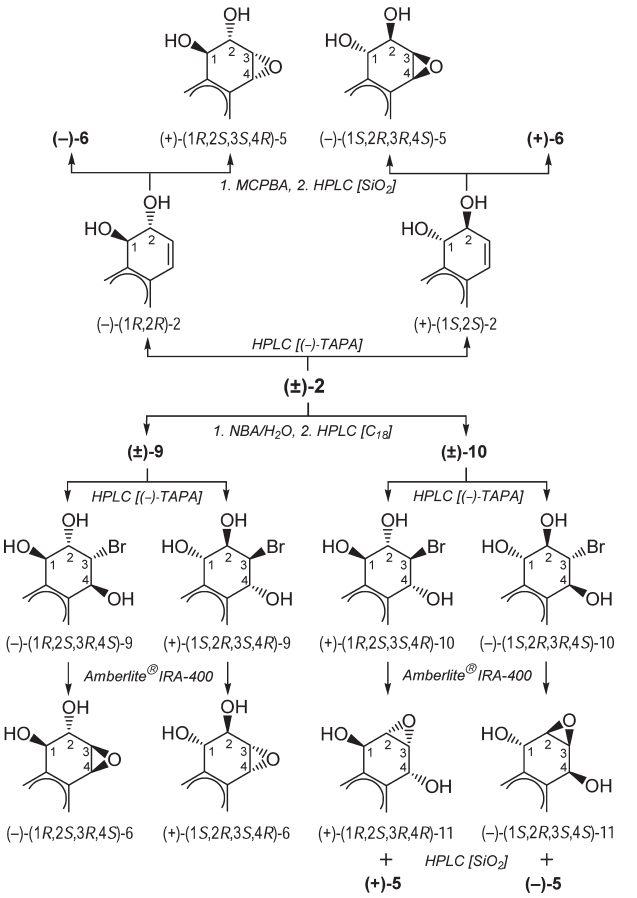
[a] Chemical structures show only the terminal benzo ring of dibenz[*a,h*]anthracene. Reagents and/or chromatographic methods are indicated in italics: *MCPBA*, *m*-chloroperoxybenzoic acid; *NBA*, *N*-bromoacetamide; (−)-*TAPA*, silica-bound (−)-2-(2,4,5,7-tetranitrofluoren-9-ylideneaminooxy)propionic acid; (−)-*TBFPA*, silica-bound (−)-2-(2,5,9,11-tetranitro-7*H*-benzo[*c*]fluoren-7-ylideneaminooxy)propionic acid.

1,2-diacetoxy-4,5-dibromocyclohexane in the synthesis of benzene dihydrodiol.[29] The racemic 3,4-dihydrodiol (±)-**1** was separated into its enantiomers by chiral HPLC using silica-bound (−)-TBFPA as stationary phase.[23] Because of massive tailing, the first eluting (−)-**1** was contaminated with the later eluting enantiomer (+)-**1**. Therefore, (−)-**1** was purified using silica-bound (+)-TBFPA, i.e., the chiral stationary phase of opposite absolute configuration.

Epoxidation of the 1,2-double bond in the enantiomerically pure 3,4-dihydrodiols (−)-**1** and (+)-**1** with MCPBA[20,24,30] afforded highly stereoselective *anti*-diol epoxides (+)-**3** and (−)-**3**, respectively (Scheme 2) as a result of the hydrogen bonding in the transition state between the allylic hydroxyl group and the peroxy acid.[31] Since the hydroxyl groups in **1** exist predominantly in the pseudodiequatorial conformation,[24] the more distant benzylic hydroxyl group exerts no cis-directing effect on the attack of the peroxy acid.

For the synthesis of the *syn*-diol epoxides (+)-**4** and (−)-**4**, the 3,4-dihydrodiol (±)-**1** was reacted with NBA in water leading to bromotriol (±)-**8** followed by dehydrobromination with the anion exchange resin Amberlite IRA-400 according to the method of Yagi et al.[25,30] (Scheme 2). The diastereoselective formation of the *syn*-diol epoxide from the *trans*-dihydrodiol is the result of several regio- and stereoselective reactions: First, the electrophilic Br^+ formed from protonated NBA adds stereoselectively to the 1,2-double bond in a fashion similar to that of the peroxy acid by the cis-directing effect of the allylic hydroxyl group;[32] the subsequent attack of water is regiospecific with respect to the benzylic position of the hydroxyl group and stereospecific with respect to the trans-opening of the cyclic bromonium ion; finally, the intramolecular nucleophilic substitution of bromine can only take place by the adjacent alkoxide in trans-position, i.e., by the benzylic hydroxyl group. Thus, the bromohydrin is converted into the epoxide with retention of configuration at the hydroxyl-bearing carbon and inversion at the bromine-carrying one.[32] The dehydrobromination leading to

Scheme 3. Synthesis of the Stereoisomers of *trans*-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrodibenz[*a,h*]anthracene (Reverse Diol Epoxides[a]



[a] Chemical structures show only the terminal benzo ring of dibenz[*a,h*]anthracene. Reagents and/or chromatographic methods are indicated in italics: *MCPBA*, *m*-chloroperoxybenzoic acid; *NBA*, *N*-bromoacetamide; (−)-*TAPA*, silica-bound (−)-2-(2,4,5,7-tetranitrofluoren-9-ylideneaminooxy)propionic acid.

enantiomerically pure *syn*-diol epoxides (+)-**4** and (−)-**4** required the bromotriols (−)-**8** and (+)-**8**, which were obtained in high yields by preparative HPLC of (±)-**8** on a chiral stationary phase based on silica-bound (−)-TAPA.[22,23]

For the synthesis of enantiomerically pure *anti*- and *syn*-diol epoxides of 1,2-dihydrodiol, (±)-**2** was very efficiently separated by preparative HPLC on a chiral stationary phase based on silica-bound (−)-TAPA into (−)-**2** and (+)-**2**.[23] Epoxidation of the 3,4-double bond in 1,2-dihydrodiol **2** with MCPBA[24] afforded a mixture of the diastereomeric *anti*- and *syn*-diol epoxides **5** and **6** (Scheme 3). This is the result of the pseudodiaxial conformation of the hydroxyl groups in bay region *trans*-dihydrodiols[33] such as the 1,2-dihydrodiol **2**.[24] Here, not only the cis-directing effect of the allylic but also that of the benzylic hydroxyl group leads to the entry of the oxiranyl oxygen in the *anti*- and *syn*-position with respect to the benzylic hydroxyl group. The mixture of *anti*- and *syn*-diol epoxides could be separated into the diastereomers by preparative HPLC on silica gel as described,[24] however, only when a modified mobile phase was employed. The *anti*- and *syn*-diol epoxides **5** and **6** were obtained in a ratio of about 2:1 (3:1).[24]

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Table 1. Vicinal Coupling Constants *J* of the Protons at the Hydroxyl Group-Bearing Carbon Atoms in *trans*-3,4- and -1,2-Dihydrodiols of Dibenz[*a*,*h*]anthracene and in Their *anti*- and *syn*-Diol Epoxides

	vicinal coupling constants *J* of the protons at the hydroxyl group-bearing carbon atoms		
position of the hydroxyl groups	*trans*-dihydrodiols	*anti*-diol epoxides	*syn*-diol epoxides
3,4	**1**	**3**	**4**
	$J_{3,4}$ = 11.5 Hz (11 Hz)[24]	$J_{3,4}$ = 8.6 Hz (9 Hz)[24]	$J_{3,4}$ = 5.9 Hz
1,2	**2**	**5**	**6**
	$J_{1,2}$ = 0 Hz (2 Hz)[24]	$J_{1,2}$ = 7.1 Hz (0 Hz)[24]	$J_{1,2}$ = 2.3 Hz

Since the *syn*-diol epoxide **6** was obtained in a rather low yield by peroxy acid epoxidation, its synthesis from 1,2-dihydrodiol via the bromotriol was attempted (Scheme 3). As in the case of epoxidation of the 1,2-dihydrodiol with peroxy acid, the cis-directing effect of the allylic and of the benzylic hydroxyl group on the entry of the bromine atom was observed leading to a 1:1 mixture of bromotriols **9** and **10** after reaction of **2** with NBA in the presence of water[25,30] (Scheme 3). This mixture was separated by preparative reversed-phase HPLC into the diastereomeric racemic bromotriols (±)-**9** and (±)-**10**. Very efficient separation of the racemates into the enantiomers was then achieved by preparative HPLC on a chiral stationary phase based on silica-bound (−)-TAPA (Scheme 3).

Dehydrobromination of bromotriols (−)-**9** and (+)-**9** afforded stereospecifically the *syn* diastereomers of the 3,4-epoxides (−)-**6** and (+)-**6** (Scheme 3). Since in bromotriol **10** the hydroxyl groups in 2- and 4-position are trans to the bromine atom, nucleophilic displacement of bromine in 3-position by both adjacent alkoxides resulted in a mixture of the 2,3-epoxide **11** and the *anti* diastereomers of the 3,4-epoxide **5** (Scheme 3) in a ratio of about 5:2. The predominant formation of the 2,3-epoxide is in agreement with an observation in the case of BaP where the 8,9-epoxide was the main product of dehydrobromination of an analogous bromotriol.[34]

Both synthetic pathways to the *anti* and *syn* diastereomers of the diol epoxides of the 1,2-dihydrodiol (Scheme 3) complement each other. Starting with racemic 1,2-dihydrodiol **2**, peroxy acid epoxidation yielded the *anti* diastereomer **5** with an overall yield of 13% compared to 7% of the *syn* diastereomer **6**, whereas with the bromotriol method, the *syn* diastereomer **6** was obtained with 14% overall yield in contrast to 3% of the *anti* diastereomer **5**.

Conformation. The preferred conformation of the diol epoxides can be deduced from the magnitude of the vicinal coupling constants (*J*) for the carbinol hydrogens obtained by ^{1}H NMR.[35] As in the case of *trans*-dihydrodiols of a series of PAHs,[33] large values of *J*, i.e., 8−9 Hz, indicate a pseudodiequatorial conformation and low values, i.e., 0−3 Hz, a pseudodiaxial conformation of the vicinal hydroxyl groups. *anti*-Diol epoxides have a relatively strong preference for the conformation in which the hydroxyl groups adopt pseudodiequatorial positions.[5,36] This is demonstrated by the bay region *anti*-diol epoxide **3** with $J_{3,4}$ = 8.6 Hz (Table 1; Supporting Information). For the reverse *anti*-diol epoxide **5**, the diagnostic coupling constant $J_{1,2}$ was reported as 0 Hz,[24] characteristic for a pseudodiaxial conformation of the vicinal hydroxyl groups. However, on reinvestigation we found $J_{1,2}$ = 7.1 Hz for **5** (Table 1; Supporting Information), which indicates a preference for the pseudodiequatorial conformation. Although in both ^{1}H NMR experiments different solvents were used, acetone-d_6 in ref 24 and acetone-d_6/Me_2SO-d_6 [3:2, v/v]/D_2O in this study, a considerable alteration of the coupling constant by this change in solvent composition[37] seems to be unlikely. For $J_{1,2}$ in **5**, the value of 7.1 Hz is more plausible since $J_{1,2}$ of the reverse *anti*-diol epoxide of benz[*a*]anthracene with a terminal benzo ring structurally identical to that of **5**, determined in acetone-d_6, was reported as 6.8 Hz.[5] In *syn*-diol epoxides, the benzylic hydroxyl group and the oxirane oxygen are on the same side of the ring and can thus undergo intramolecular hydrogen bonding,[35] resulting in a preference for the conformation in which the hydroxyl groups are pseudodiaxial.[5,36] This is illustrated by the reverse *syn*-diol epoxide **6** with $J_{1,2}$ = 2.3 Hz (Table 1; Supporting Information). However, when the oxirane is part of a highly hindered bay or fjord region the expected preferred pseudodiaxial conformation of the hydroxyl groups in *syn*-diol epoxides is shifted to the pseudodiequatorial conformation. Reported examples are the *syn*-diol epoxides of BaP ($J_{7,8}$ = 6.0 Hz),[38] benz[*a*]anthracene ($J_{3,4}$ = 7.0 Hz),[39] benzo[*c*]phenanthrene ($J_{3,4}$ = 9.0 Hz),[36] and now *syn*-diol epoxide **4** with a coupling constant $J_{3,4}$ = 5.9 Hz (Table 1; Supporting Information).

Absolute Configuration. The absolute configuration of the 8 enantiomeric and hence optically active diol epoxides (Scheme 1) was deduced from the absolute configuration of their synthetic precursors, the 3,4- and 1,2-dihydrodiols **1** and **2**, considering the regio- and stereoselectivity of the subsequent reactions discussed above. The (*R*,*R*) configuration has been established for the enantiomers of both *trans*-dihydrodiols with negative optical rotation, i.e., (−)-**1** and (−)-**2**, by two spectroscopic methods;[18] consequently, (+)-**1** and (+)-**2** are assigned the (*S*,*S*) configuration.

Therefore, the bay region *anti*-diol epoxide (+)-**3** prepared from (−)-**1** has the configuration (1*R*,2*S*,3*S*,4*R*) and its enantiomer (−)-**3** (1*S*,2*R*,3*R*,4*S*) (Scheme 2). The use of racemic bromotriol (±)-**8** for the preparation of the enantiomeric *syn*-diol epoxides of **1** (Scheme 2) required an additional experiment: (−)-(3*R*,4*R*)-**1** was stereospecifically transformed with NBA in aqueous THF as described above, yielding bromotriol **8** with positive optical rotation and hence (1*S*,2*R*,3*S*,4*R*) configuration (Scheme 2); the same absolute configuration has the *syn*-diol epoxide (−)-**4**, while its enantiomer (+)-**4** is assigned the (1*R*,2*S*,3*R*,4*S*) configuration.

Analogous considerations apply to the absolute configuration of the reverse diol epoxides from 1,2-dihydrodiol **2**. Thus, the *anti*-diol epoxide with positive optical rotation, (+)-**5**, obtained by peroxy acid epoxidation of (−)-(1*R*,2*R*)-**2**, is associated with (1*R*,2*S*,3*S*,4*R*) absolute configuration, while the accompanying *syn*-diol epoxide with negative optical rotation, (−)-**6**, is assigned the (1*R*,2*S*,3*R*,4*S*) configuration (Scheme 3). Finally, the enantiomers of these diol epoxides, (−)-**5** and (+)-**6** possess (1*S*,2*R*,3*R*,4*S*) and (1*S*,2*R*,3*S*,4*R*) absolute configurations, respectively.

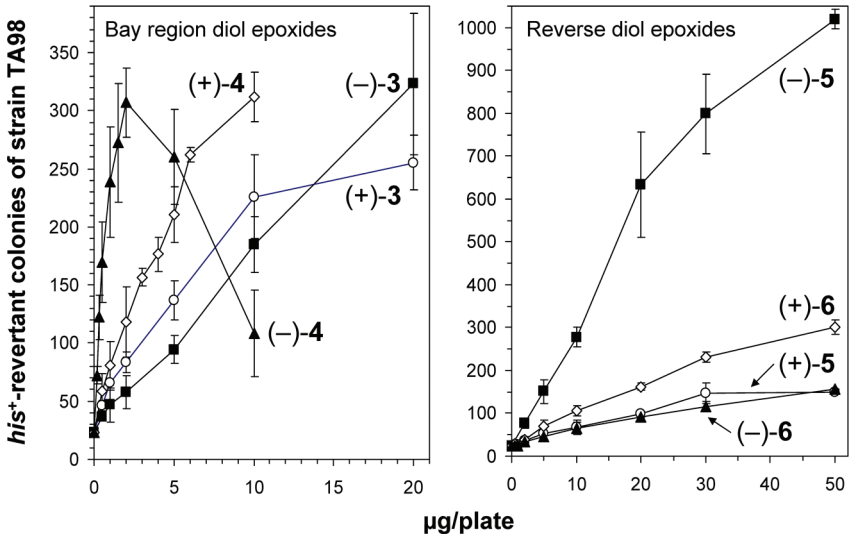

Figure 1. Bacterial mutagenicity in strain TA98 of *Salmonella typhimurium* of the 8 stereoisomers of vicinal diol epoxides at the 1,2,3,4-positions of dibenz[*a*,*h*]anthracene. Data are presented as the mean values ± SD from three independent experiments, each conducted in triplicate.

A perfect correspondence exists between the absolute configuration and the signs of optical rotation of the enantiomeric bay region diol epoxides of DBA (this study) and those of BaP,[40] benz[*a*]anthracene,[41] and chrysene.[41]

Mutagenicity. The genotoxicity of the 8 stereoisomeric diol epoxides was determined as bacterial mutagenicity by the reversion of histidine-dependent strains TA98 and TA100 of *Salmonella typhimurium* to histidine prototrophy[42] in the absence of a metabolizing system employing the plate incorporation protocol.[26] Strain TA98 is reverted by frameshift mutagens, while TA100 detects mutagens causing base-pair substitutions.[26] As reported for BaP,[34,43,44] benz[*a*]anthracene,[45] chrysene,[46−48] and phenanthrene,[46] the bacterial mutagenicity of bay region diol epoxides of DBA was in all cases stronger in strain TA100 than in TA98 (Figure 1−4). Remarkably, reverse diol epoxides of BaP[34,44] and chrysene[48] are stronger mutagens in strain TA98 than in TA100. In the case of DBA, this difference was only observed for the reverse *anti*-diol epoxide (−)-**5** (Figure 1−4). The decline in the number of his^+-revertant colonies at higher concentrations of (−)-**4** (cf. Figures 1 and 2) is a result of the toxicity of the *syn*-diol epoxide as indicated by a thinning of the his^- background lawn.[26]

The purity of the stereoisomeric diol epoxides prepared in this study requires some comments. The ^{1}H NMR spectra indicate a chemical purity of >95%, though the optical purity, especially of (−)-**4** and (−)-**5**, seems to be lower as demonstrated by the values of optical rotation. Obviously, diol epoxides (−)-**4** and (−)-**5** are somewhat contaminated with (+)-**4** and (+)-**5**, respectively. Since (−)-**4** and (−)-**5** are much stronger mutagens than (+)-**4** and (+)-**5** (cf. Figure 3), a higher purity of (−)-**4** and (−)-**5** had not changed this difference but only enhanced its extent.

With the exception of (−)-**5**, the bay region diol epoxides of DBA turned out to be stronger mutagens than the reverse diol epoxides (Figures 3 and 4). This was also observed in the case of the diol epoxides of BaP[34,44] and chrysene[48] and is obviously a consequence of the stronger electrophilic reactivity of the oxirane moiety in bay region vs reverse diol epoxides. As proposed by Jerina et al.,[4] the quantum chemical parameter $\Delta E_{deloc}/\beta$ represents the ease of formation of a resonance stabilized carbocation upon heterolytic cleavage of the benzylic C−O bond of the oxirane ring. It could indeed be verified that a quantitative relationship exists between mutagenic activity and the calculated value of $\Delta E_{deloc}/\beta$: when this quantum chemical parameter was plotted against the logarithm of relative bacterial mutagenicity of tetrahydroepoxides[49] or diol epoxides[50] of various PAHs, a satisfying linear correlation was obtained. As anticipated, the $\Delta E_{deloc}/\beta$ value of 0.738[4] calculated for the bay region diol epoxides of DBA is high in contrast to the lower value of 0.593[51] for the reverse diol epoxides.

The 8 stereoisomeric diol epoxides of DBA constitute 4 diastereomeric pairs of *anti* and *syn* isomers (Scheme 1). In strain TA98, the *syn* diastereomers of bay region diol epoxides are stronger mutagens than their *anti* isomers: (−)-**4** (*R*,*S*,*R*,*S*) > (+)-**3** (*R*,*S*,*S*,*R*), and (+)-**4** (*S*,*R*,*S*,*R*) > (−)-**3** (*S*,*R*,*R*,*S*) (Figure 3), while in the case of reverse diol epoxides, the *anti* diastereomers are more potent than their *syn* isomers, (+)-**5** (*R*,*S*,*S*,*R*) > (−)-**6** (*R*,*S*,*R*,*S*), and (−)-**5** (*S*,*R*,*R*,*S*) > (+)-**6** (*S*,*R*,*S*,*R*) (Figure 3). When the absolute configurations of diol epoxides of different polycyclic aromatic hydrocarbons are compared, the configuration is given beginning with the absolute configuration of the benzylic carbon bearing a hydroxyl group while omitting the numbering of the carbon atoms, e.g., (*R*,*S*,*S*,*R*) stands for [(*R*,*S*)-diol (*S*,*R*)-epoxide]. In strain TA100, all *syn* diastereomers surpass the bacterial mutagenicity of their *anti* isomers (Figure 4). Concerning the bay region diol epoxides, this corresponds to the situation described for BaP.[52] Of the 4 enantiomeric bay region diol epoxides of DBA and BaP, the *syn* diastereomer with (*R*,*S*,*R*,*S*) absolute configuration is the most potent mutagen in both strains of *Salmonella typhimurium*, while the *anti* isomer with (*S*,*R*,*R*,*S*) configuration is the weakest mutagen. In contrast to DBA and BaP, the *anti* diastereomers of the bay region diol epoxides of chrysene[47] and benz[*a*]anthracene[45] with (*R*,*S*,*S*,*R*) absolute configuration were by far the strongest mutagens for strain TA100. Finally, among the 4 stereoisomers of the structurally closely related fjord region diol epoxide of benzo[*c*]phenanthrene, again the *anti* diastereomer, in this case with (*S*,*R*,*R*,*S*) absolute configuration, was the strongest bacterial mutagen in strains TA98 and TA100.[53] A more pronounced correlation between the absolute

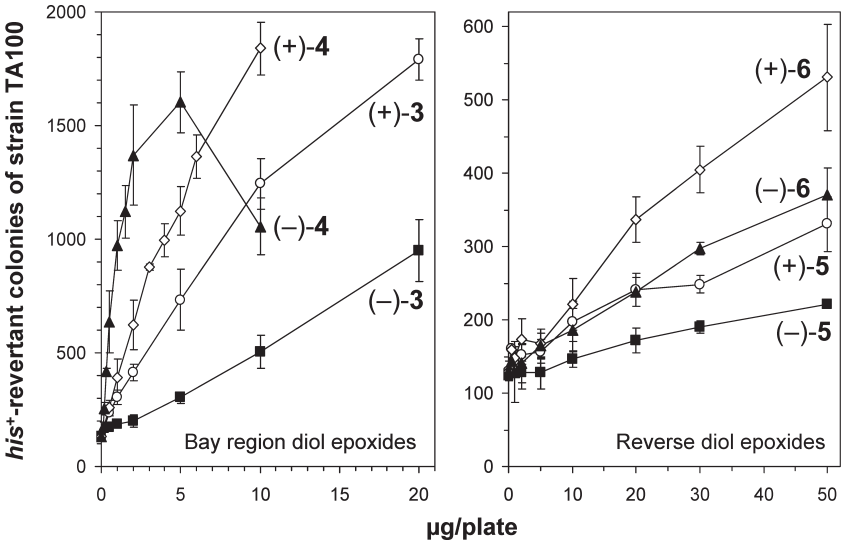

Figure 2. Bacterial mutagenicity in strain TA100 of *Salmonella typhimurium* of the 8 stereoisomers of vicinal diol epoxides at the 1,2,3,4-positions of dibenz[*a*,*h*]anthracene. Data are presented as the mean values ± SD from three independent experiments, each conducted in triplicate.

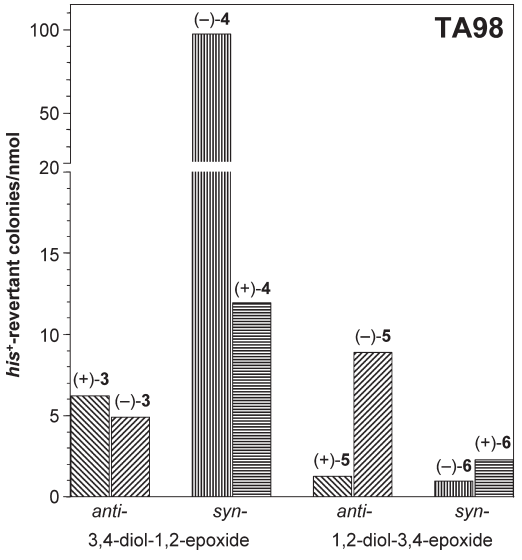

Figure 3. Specific mutagenicity in strain TA98 of *Salmonella typhimurium* of the 8 stereoisomers of vicinal diol epoxides at the 1,2,3,4-positions of dibenz[*a*,*h*]anthracene.

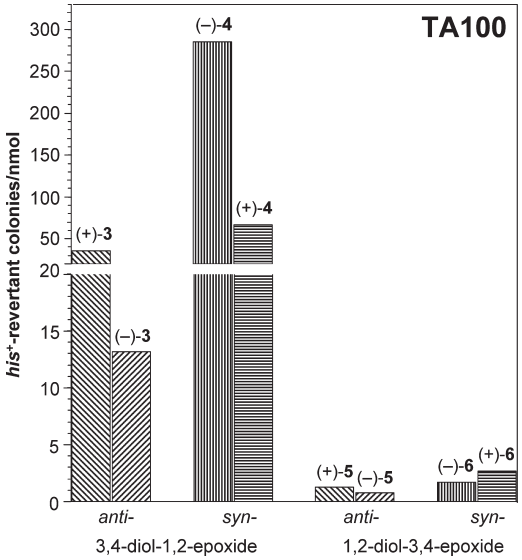

Figure 4. Specific mutagenicity in strain TA100 of *Salmonella typhimurium* of the 8 stereoisomers of vicinal diol epoxides at the 1,2,3,4-positions of dibenz[*a*,*h*]anthracene.

configuration of bay (and fjord) region diol epoxides and genotoxicity was observed for their carcinogenic activity: of the 16 configurational isomers studied from BaP,[54,55] chrysene,[56] benz[*a*]anthracene,[57] and benzo[*c*]phenanthrene,[58] the one with the highest activity in two murine tumor models was the *anti* diastereomer with (*R*,*S*,*S*,*R*) absolute configuration.

As in the case of other PAHs,[59] the microsomal biotransformation at the terminal benzo ring of DBA to *trans*-dihydrodiols, the precursors of vicinal diol epoxides, proceeds highly regio- and stereoselectively:[8−10,12] (−)-(3*R*,4*R*)-**1** was obtained with 11% of total metabolic conversion succeeded by its enantiomer (+)-(3*S*,4*S*)-**1** with 5%, (−)-(1*R*,2*R*)-**2** with 4% and its enantiomer (+)-(1*S*,2*S*)-**2** with 1%.[12] The bacterial mutagenicity of the enantiomeric 1,2- and 3,4-dihydrodiols of DBA follows nearly the same order: (−)-(3*R*,4*R*)-**1** > (+)-(3*S*,4*S*)-**1** > (+)-(1*S*,2*S*)-**2** > (−)-(1*R*,2*R*)-**2**.[12] Assuming that diol epoxides are responsible for the genotoxicity of the *trans*-dihydrodiols of DBA, the high bacterial mutagenicity of the bay region diol epoxides and the low mutagenicity of the reverse diol epoxides of DBA determined in this study could have been predicted from the results of previous work.[12]

Both DBA and BaP belong to the carcinogenic PAHs, and DBA like BaP[60] is metabolized predominantly to the most genotoxic enantiomer of the 8 possible stereoisomeric diol epoxides. However, in contrast to BaP, DBA was demonstrated to be transformed additionally to bisdiol epoxides[16] that obviously play a more important role than simple diol epoxides in the covalent binding of this PAH to DNA in vivo.[61]

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■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR spectra of (±)-**3**, (±)-**4**, (+)-**5**, and (−)-**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ DEDICATION

This work is dedicated to the memory of Donald M. Jerina (1940−2011), the founder of the bay region theory of polycyclic aromatic hydrocarbon carcinogenesis.

■ ABBREVIATIONS

BaP, benzo[*a*]pyrene; DBA, dibenz[*a,h*]anthracene; 1,2-dihydrodiol, *trans*-1,2-dihydroxy-1,2-dihydrodibenz[*a,h*]anthracene; 3,4-dihydrodiol, *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,h*]anthracene; HMPT, hexamethylphosphoric triamide; MCPBA, *m*-chloroperoxybenzoic acid; NBA, *N*-bromoacetamide; NBS, *N*-bromosuccinimide; PAH, polycyclic aromatic hydrocarbon; (−)-TAPA, (−)-2-(2,4,5,7-tetranitrofluoren-9-ylideneaminooxy)propionic acid; (−)-TBFPA, (−)-2-(2,5,9,11-tetranitro-7*H*-benzo[*c*]fluoren-7-ylideneaminooxy)-propionic acid

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