

Synthesis of Dibenzo[def,p]chrysene, Its Active Metabolites, and Their ^{13}C -Labeled Analogues

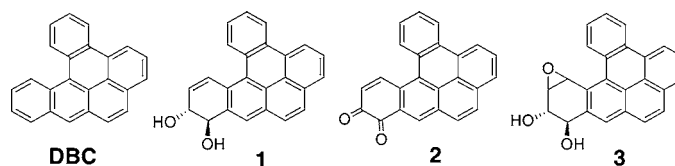
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ABSTRACT



Dibenzo[def,p]chrysene (DBC) is a highly carcinogenic polycyclic aromatic hydrocarbon suspected to be involved in initiation of lung cancer in smokers. Efficient new syntheses of DBC, its active metabolites [DBC diol (1), DBC dione (2), DBC diol epoxide (3)], and their previously unknown $^{13}\text{C}_2$ -labeled analogues are reported. The $^{13}\text{C}_2$ -labeled analogues are required as standards for sensitive methods of analysis of their DNA adducts in human cells using stable isotope dilution liquid chromatography/tandem mass spectrometry.

Polycyclic aromatic hydrocarbons (PAHs) have recently been designated as human carcinogens by the WHO.¹ They are the most potent class of carcinogens commonly present in urban environments.^{2–4} PAHs are formed in combustion of organic matter,^{2–4} and significant levels are present in tobacco smoke,^{5–7} auto and diesel engine emissions,⁴ and charbroiled, smoked, and fried foods.⁴ Current evidence

indicates that PAHs are activated metabolically to reactive forms that attack DNA leading to mutations and cancer.

Three activation pathways have been proposed: the *diol epoxide path* (mediated by cytochrome P-450 enzymes),⁸ the *radical-cation path* (mediated by peroxidase),⁹ and the *quinone path* (mediated by aldo-keto reductase).¹⁰

Dibenzo[def,p]chrysene (DBC)¹¹ (Figure 1) is the most potent PAH carcinogen currently known.¹² It is present in cigarette smoke condensate¹³ and vehicle exhaust condensate.¹³ In connection with studies aimed at elucidation of

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(11) The name dibenzo[def,p]chrysene accords with IUPAC nomenclature rules and Chemical Abstracts, but the older name dibenzo[a,l]pyrene is still in use. See ref 3 for a condensed version of the rules of PAH nomenclature.

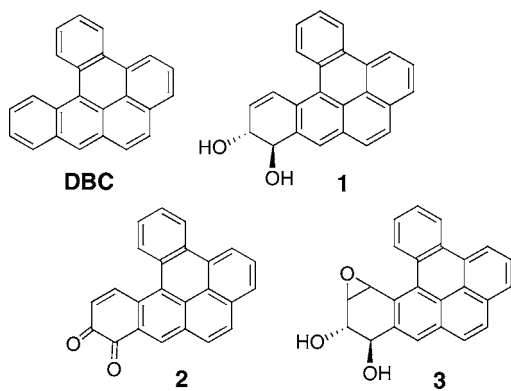


Figure 1. Structures of DBC and its active metabolites.

the role of DBC in human cancer, we required ^{13}C -labeled analogues of DBC and its active metabolites. These compounds were needed as standards for sensitive methods of stable isotope dilution liquid chromatography/tandem mass spectrometric analysis of the metabolites and DNA adducts of DBC.^{14,15}

Although several syntheses of DBC¹⁶ and its active metabolites¹⁷ have been described, they involve multistep procedures that are not adaptable to synthesis of the ^{13}C -labeled analogues. We now report convenient new syntheses of DBC and its active carcinogenic metabolites [DBC *trans*-11,12-dihydrodiol (**1**), DBC 11,12-dione (**2**), and the DBC *anti*- and *syn*-11,12-diol-13,14-epoxides (**3**)]. Also reported is synthesis of ^{13}C -labeled DBC (^{13}C -DBC) and the ^{13}C -labeled analogues of **1–3** (^{13}C -**1**, ^{13}C -**2**, and ^{13}C -**3**) by modification of this synthetic approach.

Synthesis of DBC. Pd-catalyzed Suzuki coupling of 2-bromophenylacetone (**4**) with 9-phenanthrylboronic acid (**5**) took place smoothly in the presence of Na_2CO_3 in DME to provide 2-(9-phenanthryl)phenylacetone (**6**) (82%) (Scheme 1). Attempted cyclodehydration of **6** to 9-methyl-benzo[*g*]chrysene (**7a**) failed to take place under the usual conditions for this type of reaction (20% MSA in CH_2Cl_2 at room temperature for 2 days).¹⁸ However, reaction took place under more vigorous conditions (50% MSA at 85–90 °C) to

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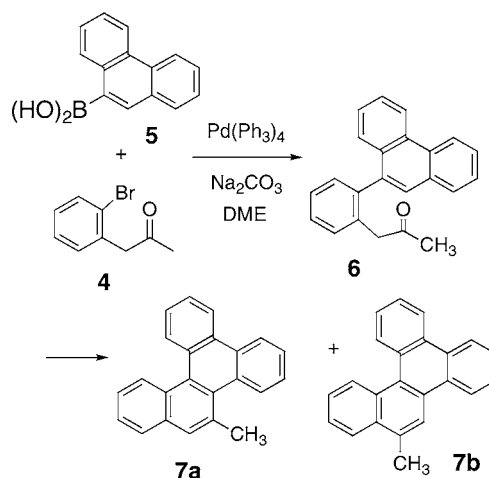
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Scheme 1. Synthesis of 9- and 10-Methylbenzo[*g*]chrysene



furnish a product whose ^1H NMR spectrum was more consistent with the structure of the 10-methyl isomer (**7b**) rather than **7a**. Particularly revealing was the appearance of the methyl proton signal at δ 2.82, not shifted to higher field as expected for a methyl group in a crowded bay region. Also, the ^1H NMR spectrum of **7b** matched closely that reported for this compound.¹⁹ Evidently, migration of the methyl group occurred during or subsequent to cyclodehydration. Methyl migration in acid-catalyzed reactions of PAHs is well known.²⁰

A brief study of this reaction was undertaken to optimize conditions for synthesis of **7a**. Reaction of **6** with 20% MSA at 90 °C for 3 days (Table 1) furnished equal amounts of **7a**

Table 1. Cyclodehydration of **6**

time (h)	temp (°C)	catalyst ^a	7a (%)	7b (%)
48	rt	20% MSA	0	0
72	90	20%MSA	20	20
2	90	50% MSA	0	70
48	90	Hf(OTf) ₄	0	10
20	rt	TiCl ₄	45	0
24	rt	TiCl ₄	60	0
30	rt	TiCl ₄	72	0
40	rt	TiCl ₄	6	76
66	rt	TiCl ₄	0	80

^a MSA = methanesulfonic acid

and **7b** (40%). Similar reaction of **6** with Hf(OTf)₄ as catalyst gave **7b** (10%) as the sole product. Reaction of **6** in the presence of TiCl₄ at room temp for times up to 30 h afforded mixtures of **7a** and unreacted **6** with no detectable **7b**. At

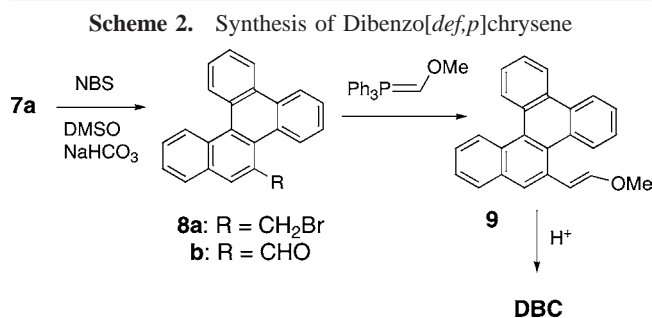
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30 h, the yield of **7a** was 72%, and 10% of **6** was recovered. With increasing times, the ratio of **6** continued to decrease, but the ratio of **7a** also began to decrease. At the same time, the ratio of **7b** increased, and by 66 h, it was the sole product. The most practical strategy for synthesis of **7a** was to quench reaction while a small amount of **6** remained and **7b** was not yet detectable (~30 h), taking advantage of the fact that separation of **6** from **7a** is easy, while separation of **7a** from **7b** is difficult.

Transformation of **7a** to DBC was carried out via the sequence in Scheme 2. Bromination of **7a** with NBS gave



9-bromomethylbenzo[g]chrysene (**8a**), which was converted to 9-formylbenzo[g]chrysene (**8b**) by treatment with DMSO and NaHCO₃.²¹ Compound **8a** was somewhat unstable, but satisfactory yields of **8b** were obtainable by use of **8a** directly without purification. Wittig reaction of **8b** with methoxymethylenetriphenylphosphine afforded 9-(2-methoxy-vinyl)-benzo[g]chrysene (**9**) as a mixture of the *E*- and *Z*-isomers (1:1 by NMR) (90%). Acid-catalyzed cyclization of **9**²² furnished **DBC** (75%).

Synthesis of ¹³C₂-DBC. Because ¹³C-labeled 2-bromophenylacetone (**4**) was unavailable, this synthesis was carried out by a modified procedure (Scheme 3). The starting compound, 2-(9-phenanthryl)benzaldehyde (**10a**), was prepared by Pd-catalyzed coupling of 2-formylphenyl-boronic acid with 9-bromophenanthrene. Wittig reaction of **10a** with ¹³C₂-EtPPh₃Br²³ and *t*-BuOK gave **11** as a mixture of *E*- and *Z*-isomers (8:1 by NMR analysis). Epoxidation of **11** with Oxone/acetone²⁴ gave **12** (87%), and it underwent rearrangement in the presence of Hf(OTf)₄ to furnish ¹³C₂-2-(9-phenanthryl)phenylacetone (¹³C₂-**6**) (74%). Cyclization of ¹³C₂-**6** with TiCl₄ by the procedure used for synthesis of **7a** gave ¹³C₂-**7a**, and this was converted to ¹³C₂-**DBC** by the sequence for **DBC**.

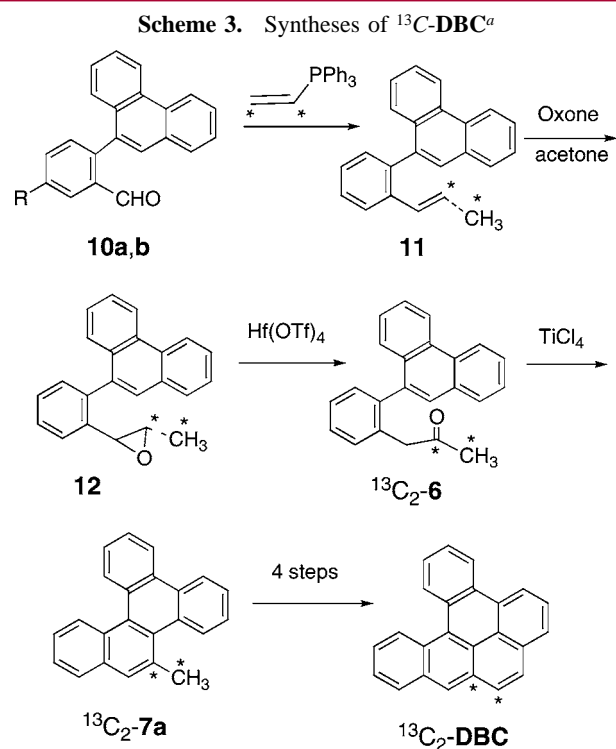
Should a higher level of ¹³C-labeling be desired, an additional ¹³C-atom may be incorporated into ¹³C-DBC via

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(23) ¹³C₂-EtPPh₃Br was prepared by addition of a solution of PPh₃ (4.77 g, 18.2 mmol) in toluene (8 mL) to ¹³C₂-bromoethane (1 g, 9.1 mmol). The mixture was stirred at 120 °C for 2 days and then cooled to room temperature. The precipitate was filtered, washed with cold benzene (2 × 10 mL), and dried under vacuum to afford ¹³C₂-EtPPh₃Br.

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^a **a:** R = H; **b:** R = OMe.

Wittig reaction of ¹³C₂-**8b** with ¹³C-labeled MeOCH=PPh₃ followed by acid-catalyzed cyclization to yield ¹³C₃-**DBC**. A similar procedure was employed for introduction of ¹³C in synthesis of ¹³C₂-benzo[*a*]pyrene.²⁵

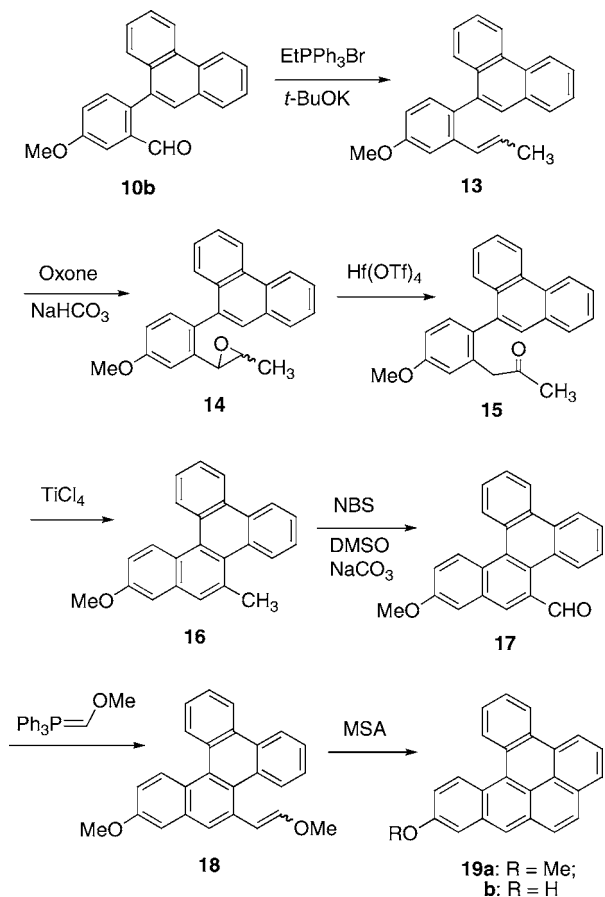
Synthesis of Active Metabolites of DBC (1–3) and their ¹³C₂-Labeled Analogues. The active metabolites of DBC were synthesized by modification of the method employed for synthesis of ¹³C₂-**DBC** (Scheme 4). The initial synthetic target was 10-hydroxy-**DBC** (**19b**), previously shown to be a synthetic precursor of all three metabolites (**1–3**).^{16,17}

Cross-coupling of 2-bromo-5-methoxybenzaldehyde with phenanthrene-9-boronic acid (**5**) provided 2-(9-phenanthryl)-5-methoxybenzaldehyde (**10b**) (62%). Wittig reaction of **10b** with ethylenetriphenylphosphine (generated from reaction of EtPPh₃Br with *t*-BuOK) provided **13** (89%) as a mixture of *E*- and *Z*-isomers. Epoxidation of **13** with Oxone/acetone²⁴ furnished **14** (89%), which was converted to 2-(9-phenanthryl)-5-methoxyphenylacetone (**15**) (70%) on stirring with Hf(OTf)₄ for 15 min at ambient temp.

TiCl₄-catalyzed cyclodehydration of **15** was monitored by TLC to determine the optimum time for minimization of rearrangement of 9-methyl-12-methoxybenzo[*g*]chrysene (**16**) to the 10-methyl isomer. The optimum time was only ~3 h (compared to ~30 h for analogous reaction of **6**). The increased rate of cyclization is likely a consequence of stabilization of the reaction intermediate by the electron-donating methoxy group. Bromination of **16** with NBS

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Scheme 4. Synthesis of 12-Hydroxy-DBC (**19b**)



afforded 9-bromomethyl-12-methoxybenzo[*g*]chrysene which was converted to 9-formyl-12-methoxybenzo[*g*]chrysene (**17**)

by reaction with DMSO and NaHCO₃. Wittig reaction of **17** with methoxymethylenetriphenylphosphine provided **18** as a mixture of *E*- and *Z*-isomers that were used directly in the next step. Cyclization of **18** took place at 0 °C in the presence of MSA to furnish 12-methoxy-DBC (**19a**). Demethylation of **19a** by treatment with BBr₃ provided 12-hydroxy-DBC (**19b**) in good overall yield.

Syntheses of the active carcinogenic metabolites of DBC (**1–3**) from **19b** were carried out by the procedures described previously.^{16,17} Thus, oxidation of **19b** with Fremy's reagent provided DBC 11,12-dione (**2**), and reduction of **2** with NaBH₄ afforded DBC *trans*-11,12-dihydrodiol (**1**). The latter was, in turn, converted to the corresponding *anti*- and *syn*-11,12-diol-13,14-epoxide isomers (**3**) by the established procedures.¹⁷

The ¹³C₂-labeled analogues of the active carcinogenic metabolites of DBC (¹³C-**1**, ¹³C-**2**, and ¹³C-**3**) were synthesized from ¹³C₂-**19b** by procedures analogous to those employed for synthesis of the corresponding unlabeled compounds. ¹³C₂-**19b** was prepared from **10b** via Wittig reaction with ¹³C₂-EtPPh₃Br and subsequent steps analogous to those described for synthesis of unlabeled **19b** from **10b** (Scheme 4).

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Supporting Information Available: Experimental details and spectroscopic data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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