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

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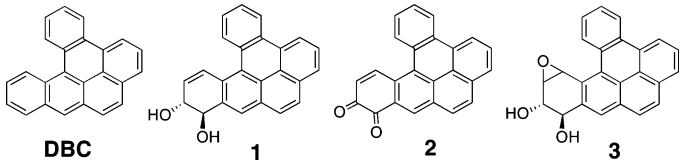
ABSTRACT



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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 $^{13}C_2$-labeled analogues are reported. The $^{13}C_2$-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 condensate[13] 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.

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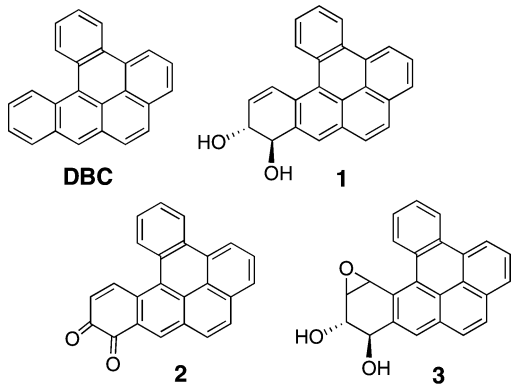


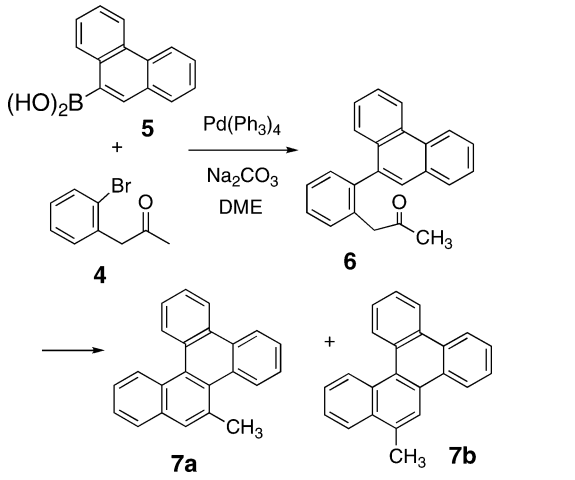
Figure 1. Structures of DBC and its active metabolites.

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 DBC[16] and its active metabolites[17] 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

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% MsOH 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 $TiCl_4$ 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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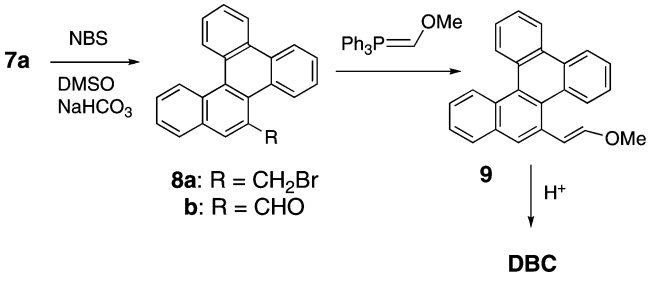
30 h, the yield of **7a** was 72%, and 10% of **6** was recovered. With increasing times, the ratio of **6** continued to decrease, but the ratio of **7a** also began to decrease. At the same time, the ratio of **7b** increased, and by 66 h, it was the sole product. The most practical strategy for synthesis 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

Scheme 2. Synthesis of Dibenzo[*def*,*p*]chrysene

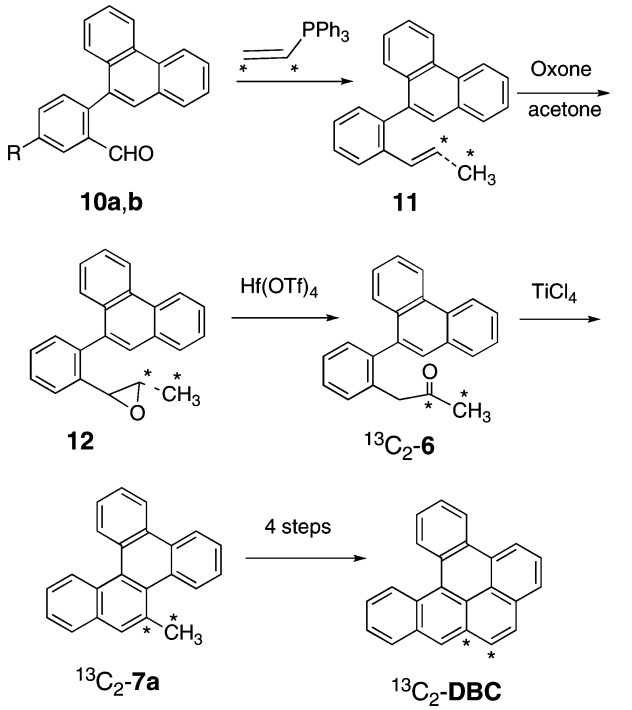


9-bromomethylbenzo[*g*]chrysene (**8a**), which was converted to 9-formylbenzo[*g*]chrysene (**8b**) by treatment with DMSO and $NaHCO_3$.[21] Compound **8a** was somewhat unstable, but satisfactory yields of **8b** were obtainable by use of **8a** directly without purification. Wittig reaction of **8b** 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 $^{13}C_2$−DBC. Because ^{13}C-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 $^{13}C_2$−$EtPPh_3Br$[23] and *t*-BuOK gave **11** as a mixture of *E*- and *Z*-isomers (8:1 by NMR analysis). Epoxidation of **11** with Oxone/acetone[24] gave **12** (87%), and it underwent rearrangement in the presence of $Hf(OTf)_4$ to furnish $^{13}C_2$-2-(9-phenanthryl)phenylacetone ($^{13}C_2$-**6**) (74%). Cyclization of $^{13}C_2$-**6** with $TiCl_4$ by the procedure used for synthesis of **7a** gave $^{13}C_2$-**7a**, and this was converted to $^{13}C_2$-**DBC** by the sequence for **DBC**.

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

Scheme 3. Syntheses of ^{13}C-**DBC**[a]



[a] **a**: R = H; **b**: R = OMe.

Wittig reaction of $^{13}C_2$-**8b** with ^{13}C-labeled $MeOCH{=}PPh_3$ followed by acid-catalyzed cyclization to yield $^{13}C_3$-**DBC**. A similar procedure was employed for introduction of ^{13}C in synthesis of $^{13}C_2$-benzo[*a*]pyrene.[25]

Synthesis of Active Metabolites of DBC (1−3) and their $^{13}C_2$-Labeled Analogues. The active metabolites of DBC were synthesized by modification of the method employed for synthesis of $^{13}C_2$-**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 $EtPPh_3Br$ with *t*-BuOK) provided **13** (89%) as a mixture of *E*- and *Z*- isomers. Epoxidation of **13** with Oxone/acetone[24] 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.

$TiCl_4$-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 stabiization of the reaction intermediate by the electron-donating methoxy group. Bromination of **16** with NBS

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(23) $^{13}C_2$-$EtPPh_3Br$ was prepared by addition of a solution of PPh_3 (4.77 g, 18.2 mmol) in toluene (8 mL) to $^{13}C_2$-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 $^{13}C_2$-$EtPPh_3Br$.

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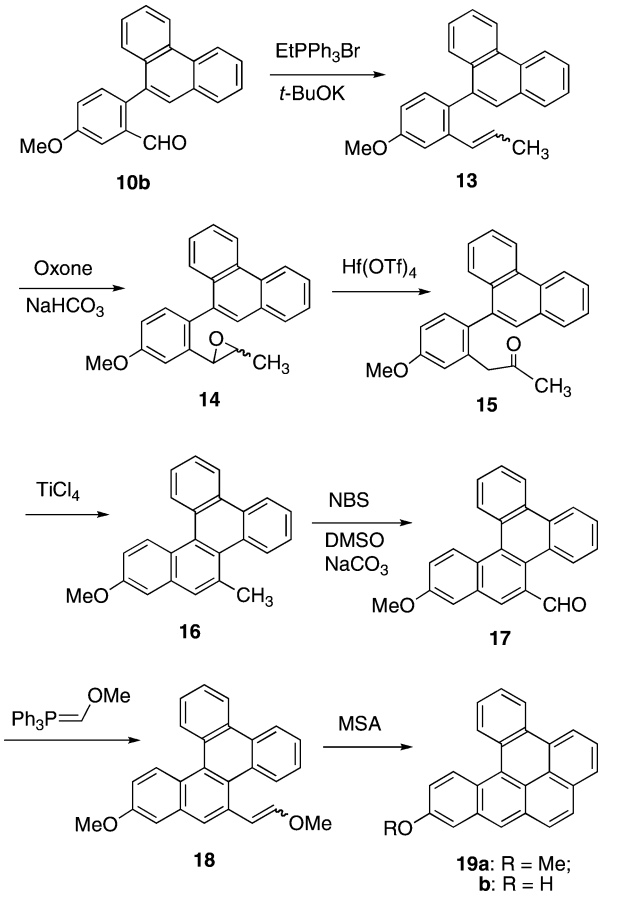
Scheme 4. Synthesis of 12-Hydroxy-DBC (**19b**)



afforded 9-bromomethyl-12-methoxybenzo[*g*]chrysene which was converted to 9-formyl-12-methoxybenzo[*g*]chrysene (**17**) by reaction with DMSO and $NaHCO_3$. 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 BBr_3 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 $NaBH_4$ 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 $^{13}C_2$-labeled analogues of the active carcinogenic metabolites of **DBC** (^{13}C-**1**,^{13}C-**2**, and^{13}C-**3**) were synthesized from$^{13}C_2$-**19b** by procedures analogous to those employed for synthesis of the corresponding unlabeled compounds.$^{13}C_2$-**19b** was prepared from **10b** via Wittig reaction with$^{13}C_2$-$EtPPh_3Br$ 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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