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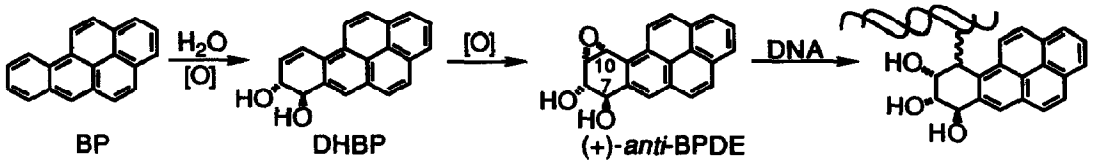
Improved Formal Preparation of Enantiomerically Pure *anti*-Benzo[*a*]pyrene Diol Epoxide.

George R. Negrete[1] and Thomas Meehan*

Departments of Pharmacy and Pharmaceutical Chemistry
University of California, San Francisco, CA 94143-0446

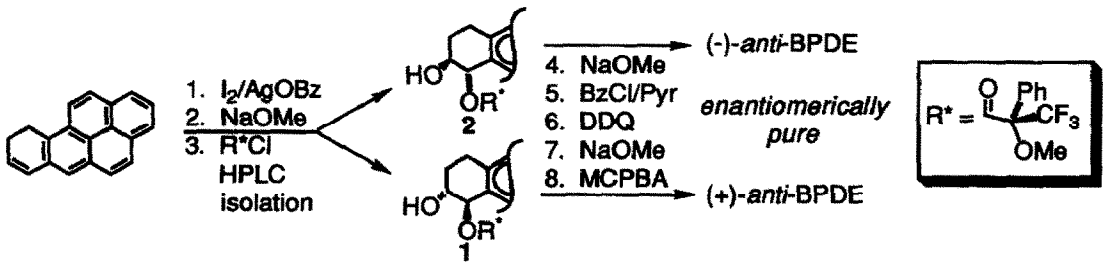
Abstract: We report an improved, economical formal route to enantiomerically pure *anti*-benzo[*a*]pyrene diol epoxide (BPDE). A trimethylaluminum catalyzed regio- and stereoselective opening of racemic 7,8-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene with Mosher's acid delivers benzylic esters exclusively. This step is a significant improvement over the lack of regioselectivity of standard procedures. We also show that modification of the subsequent chemical steps further shortens the preparation of enantiomerically pure *anti*-BPDE.

Benzo[*a*]pyrene (BP), a widespread environmental contaminant, is metabolically activated to the highly carcinogenic diol epoxide, (+)-*anti*-benzo[*a*]pyrene diol epoxide (BPDE) and its much less carcinogenic enantiomer.[2] Covalent binding of (+)-*anti*-BPDE at nucleic acid exocyclic amino groups produces an array of regio- and stereochemically related products that are believed to initiate the molecular events leading to tumorigenesis. The details of these processes, including the correlation between the individual adducts (and their stereoisomers) and tumorigenesis, remain to be elucidated.[3] Our continuing study of BP-DNA interactions necessitates the use of gram quantities of enantiomerically pure *anti*-BPDE. The prohibitive cost[4] and inefficient preparation of this compound prompted our search for an alternative route. We report here the development of a significantly improved, efficient, and relatively inexpensive preparation of resolved *anti*-BPDE enantiomers.



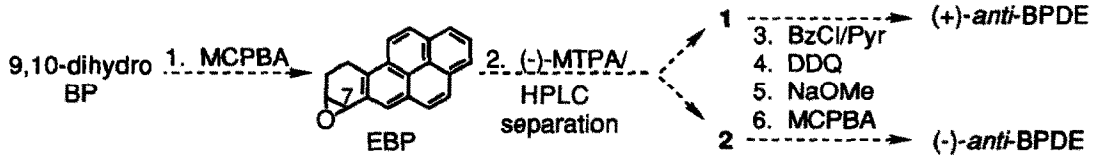
Scheme 1. BP Metabolism and Covalent Binding to DNA.

We have prepared enantiomerically pure *anti*-BPDE following the procedure of Jerina[5] (Scheme 2). Silver benzoate/iodine oxidation of 9,10-dihydro BP gives the racemic trans dibenzoate. Resolution requires methanolysis of the dibenzoates, Mosher's acid ((-)-α-methoxy-α-(trifluoromethyl)phenylacetic acid; MTPA) monoesterification of the C7 hydroxy to give separable diastereomers (1 and 2), and subsequent saponification. The concurrent formation of poorly separable C8 mono and C7,8-diesters (the ratio C7-MTPA:C8-MTPA:diesters is approximately 0.6:1.0:0.1) severely reduces the efficiency of this resolution. Benzoylation and DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) treatment delivers 7,8-dibenzoyl-7,8-dihydro BP. Saponification yields 7,8-dihydroxy-7,8-dihydro BP (DHBP, Scheme 1) and enantiomerically pure *anti*-BPDE is obtained after *m*-chloroperoxybenzoic acid (MCPBA) epoxidation.



Scheme 2. Usual Preparation of Enantiomerically Pure *anti*-BPDE.

We envisaged a modified approach to enantiomerically pure DHBP based on the treatment of a 7,8-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (EBP) with Mosher's acid to selectively produce the common intermediate diastereomers **1** and **2** (Scheme 3; hashed arrows represent proposed transformations). The proposed regioselectivity of this addition follows from benzylic activation of the C7 center for which there is well documented precedent.[6] After the usual diastereomer separation, we bypass MTPA saponification and proceed directly to benzoylation. Subsequent DDQ oxidation of each diastereomer and methanolysis produces enantiomerically pure DHBP (the immediate *anti*-BPDE precursor). Such a route remedies the poor selectivity encountered in the usual monoesterification of diols described above and shortens the route from 9,10-dihydro BP to *anti*-BPDE by two steps. We have successfully implemented this improved protocol to make enantiomerically pure DHBP.



Scheme 3. Proposed route to enantiomerically pure *anti*-BPDE.

Our synthesis began with the preparation of racemic EBP via MCPBA epoxidation[7] of 9,10-dihydro BP[5a] in 81% recrystallized yield.[8] Opening of epoxides with poor nucleophiles, such as our proposed addition of MTPA, often requires the assistance of Lewis acid catalysts. In preliminary studies, we investigated the use of several catalysts that have been reported to enhance epoxide additions including $AlMe_3$,[9] Al_2O_3,[10] $ZnBr_2$,[11] $LiClO_4$,[10] $(NH_4)_2Ce(NO_3)_6$,[12] and $Pd[P(Ph_3)]_4$.[13] In addition, Cs_2CO_3 was tested based on reports that this reagent accelerates the nucleophilic additions of carboxylic acids.[14] These preparations were performed at room temperature, in the reported solvents (3 mL), by addition of catalyst to solutions of epoxide (3 mg) and *S*-(-)-MTPA (5 mg). The reactions were monitored by TLC (silica; 20% THF/hexanes) at 1 and 2 hr and by HPLC at 72 hr and compared with authentic samples prepared by the method of Jerina.[5a]

At 1 hr, there appeared to be no reaction progress except in Entry 2 ($AlMe_3$). For this sample, vigorous boiling had been observed upon addition of the catalyst to the reaction milieu and complete conversion of substrate to product was indicated by TLC. HPLC (silica gel; 20% THF/hexanes; 345 nm abs) of the Entry 2 products showed that trans adducts strongly predominated though trace quantities of more mobile products were detected (these may be either cis products or C8-MTPA regioisomers). At 72 hr many of the samples showed significant conversion to products (see Table). The only exceptions were $ZnBr_2$ and Cs_2CO_3. Notably, moderate diastereoselectivity was observed in several preparations, though, this was not investigated further.

Based on these initial results, more carefully controlled conditions for epoxide opening with ethereal $AlMe_3$ were examined. In these preparations, ethereal solutions of epoxide (10 mg) and MTPA (10 mg; 1 eq) were

cooled to the indicated temperature, treated with catalyst (3 μL), and allowed to stir for 3 hr. An aliquot was then removed, quenched with saturated sodium bicarbonate, and assayed by HPLC. Use of several temperatures (-100, -78, -46, -23, 0 °C) showed conversion to product occurred at -46 °C and above (the respective conversions are: 0, 0, 7, 89, 100%). To determine diastereoselectivity, the preparations were repeated with 0.3 eq MTPA. HPLC again demonstrated that the products were obtained as 1:1 mixtures at each temperature investigated.

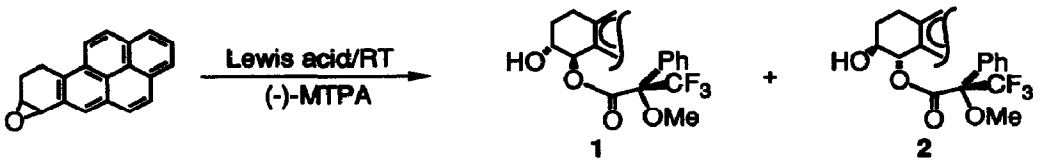


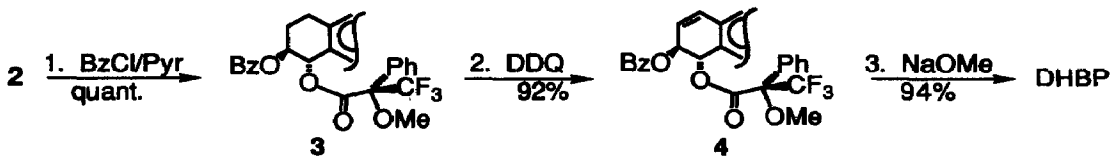
Table: MTPA Additions to EBP.

entry	catalyst (quantity)	solvent	time (hr)	% conversion† (1 + 2)	ratio **2:1**
1	none	Et_2O	72	10	2.8:1
2	$AlMe_3$ (2M in toluene; 60 μL)	Et_2O	1	97	1.0:1
3	Al_2O_3 (3 mg)	THF	72	42	4.5:1
4	$ZnBr_2$ (3 mg)	acetonitrile	72	7	2.0:1
5	$LiClO_4$ (3 mg)	acetonitrile	72	33	2.2:1
6	Cs_2CO_3 (3 mg)	Et_2O	72	2	--*
7	$(NH_4)_2Ce(NO_3)_6$ (3 mg)	Et_2O	72	65	1.9:1
8	$Pd[P(Ph_3)]_4$ (3 mg)	Et_2O	72	37	1.7:1

† ratio of HPLC signal areas: (1 + 2)/(1 + 2 + EBP)
* unable to determine due to an overlapping contaminant

To confirm the preparative utility of this reaction, we successfully converted racemic EBP (100 mg) to the separable diastereomers **1** and **2**. Treatment of a solution of epoxide and (-)-MTPA with trimethylaluminum (-78 to 0 °C, 4 hr) gave a diastereomeric mixture of products in good yield (77%). Proton NMR of the crude product showed no evidence of stereochemical (C7-C8 cis isomers) or regiochemical (C8-MTPA) contaminants.[15] These results demonstrate that this is an excellent alternative for producing these advanced intermediates for the preparation of enantiomerically pure *anti*-BPDE.

To show the feasibility of the shortened route from the 7-MTPA esters to DHBP, we successfully converted **2** to the corresponding enantiomerically pure DHBP. This diastereomer was benzoylated (BzCl/pyridine; room temperature; 18 hr) in quantitative yield.[16] Subsequent DDQ treatment gave the corresponding unsaturated product (two steps, 92% yield).[17] HPLC and NMR (^1H and ^{13}C) showed signals for a single product at each step. Simultaneous methanolysis of the benzoyl and MTPA esters occurred in 95% yield under standard conditions to deliver the desired enantiomerically pure DHBP. The overall yield of this sequence



is 86% from **2**. We are currently applying this procedure to the preparation of (+)-*anti*-BPDE and several related polycyclic aromatic hydrocarbon analogs and we will shortly report full details of our results with these important metabolites.

CONCLUSION. We developed a modified route to enantiomerically pure *anti*-BPDE that results in significantly improved yields and a shorter synthetic sequence. This is accomplished by: (i) the trimethylaluminum catalyzed, regioselective ring opening of EBP by MTPA, and (ii) direct benzoylation and DDQ oxidation of the resulting 7-MTPA esters to yield DHBP. The improved MTPA ester preparation and shorter overall route result in yields that are twice that reported via the previously used route. This protocol will assist investigators in BP carcinogenesis studies and may find application in the preparation of other important PAH metabolites.

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16. **(7*R*,8*S*)-7-[(*S*)-(α-methoxy-α-(trifluoromethyl)phenylacetoxy)]-8-benzoyl-7,8,9,10-tetrahydrobenzo[*a*]pyrene (3).** ^{1}H NMR: (300.6 MHz; $CDCl_3$): δ 2.26 (m, 2H, *H9*), 2.65 (m, 2H *H10*), 3.53 (s, 3H, OCH_3), (m, 1H, *H8*), 6.94 (d, J = 16.5 Hz, 1H, *H7*), 7.3-8.2 (m, 18H, H_{aryl}); ^{13}C NMR (75.59 MHz; $CDCl_3$): δ 24.1, 25.9, 29.7, 55.6, 72.9, 74.3, 122.4, 123.5, 124.0, 125.2, 125.2, 126.1, 127.1, 127.2, 127.7, 128.5, 128.8, 129.4, 129.5, 129.7, 129.7, 130.5, 130.7, 131.1, 132.2, 133.4, 134.5, 165.8, 166.7.
17. **(7*R*,8*S*)-7-[(*S*)-(α-methoxy-α-(trifluoromethyl)phenylacetoxy)]-8-benzoyl-9,10-dihydrobenzo[*a*]pyrene (4).** ^{1}H NMR: δ 3.46 (s, 3H, OCH_3), 6.06 (d, J = 9.9 Hz, 1H, *H8*), 6.22 (dd, J = 2.7, 9.9 Hz, 1H, *H9*), 7.04 (d, J = 9.3 Hz, 1H, *H10*), 7.09 (d, J = 7.2 Hz, 1H, *H9*), 7.0-8.2 (m, 20 H, H_{aryl}); ^{13}C NMR: δ 54.7, 71.7, 73.7, 120.9, 121.4, 124.2, 124.4, 124.7, 124.8, 125.4, 126.0, 126.1, 126.3, 126.6, 127.0, 127.2, 127.4, 127.5, 127.6, 127.8, 128.0, 128.3, 128.7, 128.9, 164.9, 165.3.

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