New Chiral Synthons from the Microbial Oxidation of Bromonaphthalenes

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Abstract: 1-Bromo- and 2-bromonaphthalene were subjected to bio-oxidation with whole cells of *Pseudomonas putida* NCIB 9816-11. The major metabolites were isolated and spectroscopically characterized as (+)-*cis*-(1R,2S)-Dihydroxy-1,2-dihydro-8-bromonaphthalene 1, (+)-*cis*-(1R,2S)-Dihydroxy-1,2-dihydro-5-bromonaphthalene 2 from 1-bromonaphthalene and (+)-*cis*-(1R,2S)-Dihydroxy-1,2-dihydro-7-bromonaphthalene 8 from 2-bromonaphthalene. The absolute stereochemistry and enantiomeric excess were determined by conversion of each metabolite to the known (-)-*cis*-tetrahydronaphthalene diol 6.

INTRODUCTION

Arene *cis*-dihydrodiols are important starting materials in asymmetric synthesis as evidenced by the increasing number of applications appearing in the literature.[1] The majority of the published material consists of reports of the microbial oxidation of monocyclic aromatic hydrocarbons and the use of such metabolites as enantiomerically pure synthons.

Recently, there has been an increase in interest in the microbial dihydroxylation of bicyclic arenes,[2] and heterocycles.[3,4] Compared with the number of cases in the area of single-ring aromatics, relatively few such oxidations have been reported to date. Among the compounds studied previously were naphthalene,[5a] biphenyl,[5b] 2-methylnaphthalene,[5c] 2-methoxynaphthalene,[2] 1-chloro and 2-chloronaphthalene,[5d,6] 2-methylcarboxynaphthalene,[5d] 2-naphthalenesulfonic acid,[5e] 2-naphthol,[5f] benzofuran,[5g] benzothiophene[3] and quinoline.[4] With additional functionality, such bicyclic arene *cis*-diols would augment their potential as synthons for the synthesis of polycyclic natural products. The bromine atom was envisioned as a versatile group that would render the aromatic moiety amenable to further chemical manipulations following the biocatalytic incorporation of asymmetry.

The initial studies of bacterial degradation of halogenated naphthalene derivatives, conducted in 1955, were concerned with the investigation of the fate of primary metabolites in the presence of soil bacteria. Walker and Wiltshire[6] studied the biodegradation of 1-chloro- and 1-bromonaphthalene by naphthalene-degrading bacteria isolated from soil. They isolated 1,2-dihydroxy-1,2-dihydro-8-chloronaphthalene and 3-chlorosalicylic acid from 1-chloronaphthalene. Similarly, they isolated 3-bromosalicylic acid from 1-bromonaphthalene. Indirect evidence for a dienediol intermediate was obtained by observing

the formation of phenolic degradation products when the broth was heated with acid. In our study, in which metabolically blocked bacterial mutants of such microorganisms were used,[7] the dienediol intermediates Wiltshire and Walker claimed to have observed (but not isolated) were completely characterized.

RESULTS AND DISCUSSION

1-Bromonaphthalene. Naphthalene-dioxygenase-mediated biotransformation of 1-bromonaphthalene using *Pseudomonas putida* NCIB 9816-11 (Pp NCIB 9816-11), as described in the Experimental Section, resulted in the formation of diols **1** and **2** in a ratio of 65:35 and traces of **3** (determined by ^{1}H NMR and HPLC of the crude mixture and in agreement with the values obtained after actual separation) in a combined yield of 250 mg/L of culture. Metabolites **1** and **2** were separated by preparative HPLC. Small amounts of **3** were detected in the fractions containing **2** and flash column chromatography was used for its separation. With the exclusion of traces of acid, the pure diols were reasonably stable at room temperature ($t_{1/2}$ in CDCl$_3$ > 2 months) and indefinitely stable when kept at -78 °C.
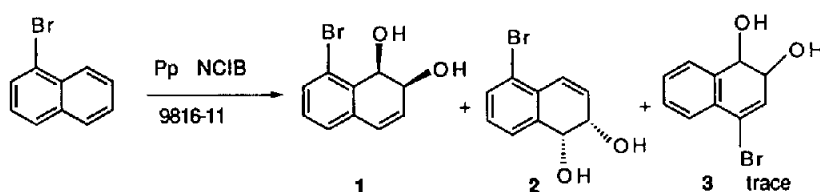

Because of the scarcity of material only ^{1}H NMR and ^{13}C NMR were compiled for **3**. To determine the regiochemistry of the bio-oxidation, **1** and **2** were dehydrated to the corresponding naphthols. As expected,[6,9] one isomer was predominantly formed from each diol thus confirming the regiochemistry of oxidation by comparison with known naphthols.[10]
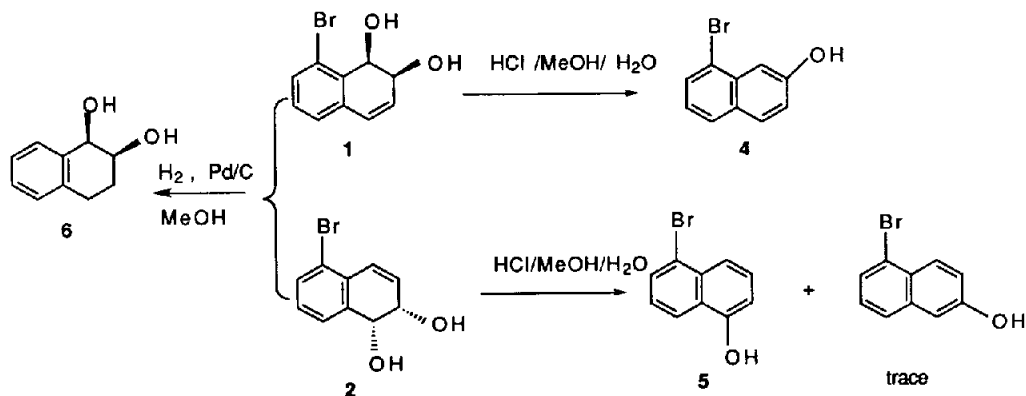
To determine the absolute stereochemistry of the diol metabolites, each diol was converted to the known naphthalene diol **6**.[5a] The enantiopurity of these diols was determined to be >98% based on the comparison of optical rotation. (For **6** from **1**: $[\alpha]_D^{25}$= - 36.0 (c 1.55, CHCl$_3$); For **6** from **2**: $[\alpha]_D^{25}$= - 36. 1

(c 0.8, CHCl₃); lit [α]$_D^{25}$ = - 38 (c 0.87, CHCl₃).[5a,11] Chiral lanthanide shift reagents[12] and Mosher ester method were used to further ascertain the optical purity by ¹H NMR methods.[13]

For the determination of optical purity by the lanthanide-induced shift method, racemic *cis*-tetrahydronaphthalene diol **7a** was prepared as a reference compound via oxidation of commercially available 1,2-dihydronaphthalene and converted to the Mosher diester **7b**.
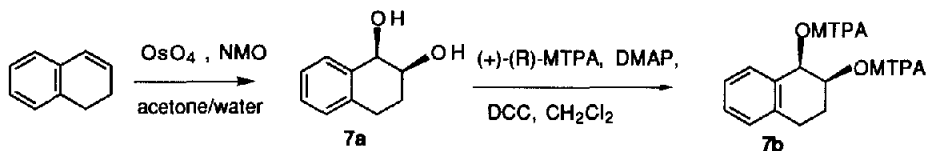
The ¹H NMR spectrum of this racemic mixture in the presence of an equimolar quantity of tris [3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium (III), (Eu(hfc)₃) showed well-resolved signals for benzylic methines (δ $_{α-isomer}$ = 6.85 ppm and δ $_{β-isomer}$ = 6.70 ppm) and was used to assess enantiopurity of **6** derived from the metabolites. An authentic sample of (-)*cis*-tetrahydronaphthalene diol (obtained from hydrogenation of microbial oxidation product of naphthalene), showed a single signal for this proton and each hydrogenated diol obtained from **1** and **2** also showed a single signal for the benzylic proton.

NMR analysis of the corresponding Mosher esters **7b** (see fig. 1) provided further evidence for the enantiomeric purity and further confirmed the assignment of absolute stereochemistry, **7b** itself being a known compound.[13] By a combination of the above methods the enantiomeric excess was proven to be >98% for all hydrogenated diols.

2-Bromonaphthalene. The corresponding biotransformation of 2-bromonaphthalene produced **8** and traces of **9** in a combined yield of 200 mg/L of culture media. None of the third possible diol isomer was observed. Separation of **8** from minute amounts of **9** was accomplished by flash chromatography (silica gel, 2:1 EtOAc/hexanes). Only the ¹H NMR of **9** was obtained to confirm the regiochemistry of oxidation. Diol **8** exhibited comparable stability at room temperature to that of **1** and **2** (t₁/₂ in CDCl₃ > 2 months). Acid-induced dehydration of **8** led exclusively to the formation of bromonaphthol **10**.[6]
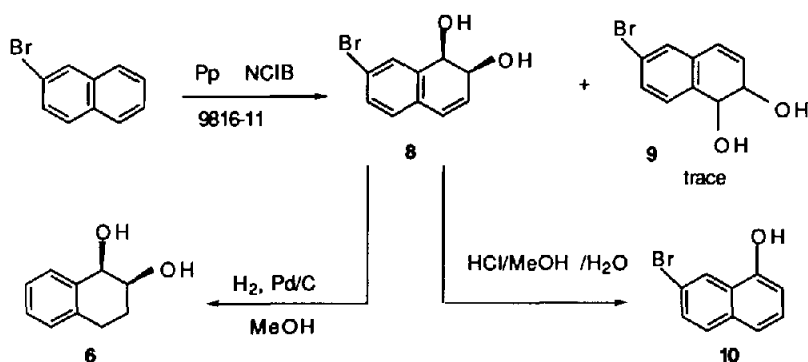
Catalytic hydrogenation with concomitant debromination of **8** resulted in the formation of **6** ($[\alpha]_D^{25}=$ - 35 (c 1.03, CHCl₃). Treatment of a diol **6** derived from **8** with Eu(hfc)₃ showed a single signal for benzylic methine indicating a single enantiomer within the detection limits of the ^{1}H NMR spectrometer.

The Mosher Ester Method

Mosher's acid[14] ([α-methoxy-α-(trifluoromethyl)]phenyl acetic acid) was used to assess enantiomeric excess of chiral alcohols by the formation of diastereomeric esters, which can be differentiated by NMR. Figure 1 summarizes the determination of enantiomeric excess and absolute stereochemistry of the hydrogenated diols from **1, 2,** and **8**.
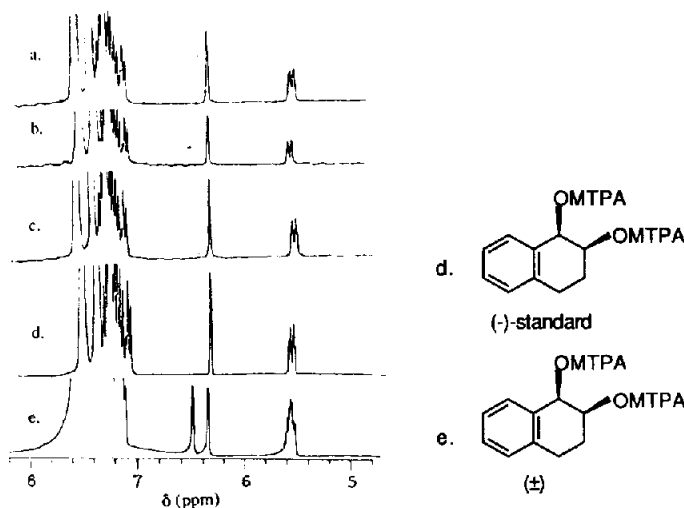

Figure 1. Expanded Region of ^{1}H NMR of Mosher Diesters from Hydrogenated Bromonaphthalene Diol **6**: a) from 2-bromonaphthalene diol **8**; b) from 1-bromonaphthalene diol **2**; c) from 1-bromonaphthalene diol **1**; d) from naphthalene diol, standard homochiral compound and e) from racemic diol **7**.

CONCLUSION

Metabolites **1, 2** and **8** from 1-bromo and 2-bromonaphthalene were obtained in reasonable yields and high enantiopurity. They are attractive synthons for asymmetric syntheses of carbocyclic natural product skeletons and their applications will be reported in due course.

EXPERIMENTAL SECTION

General

The GC instrument used was a HP 5790 A GC. GC traces were recorded on a HP Integrator. NMR spectra were recorded in CDCl₃ (unless otherwise stated) on a Bruker WP-270, GE QE-300 or Varian Unity-400. Coupling constants are given in Hertz, chemical shifts are given in ppm downfield from TMS. [13]C multiplicities were determined by APT experiments. IR spectra were obtained on a Perkin Elmer 283B instrument. HPLC was performed on Microsorb 5 μm C18, 4.6 mm ID x 25 cm L (analytical) and

Microsorb 5 μm C18, 21.4 mm ID x 25 cm L (preparative) columns. Flash column chromatography was performed on Merck silica gel (grade 60, 230-240 mesh). Melting points were determined on a Thomas Hoover Uni-melt apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer model 241 polarimeter. Mass spectra were measured on a VG 7070 E-HF instrument.

Purification of starting materials. Commercial bromonaphthalenes were purified by several recrystallizations from ethanol. The purity (>99%)was assayed by GC. t_r 1-bromo- = 11.7 mins., t_r 2-bromo- = 11.4 mins. (HP-1 50m capillary column, injector T = 300 °C, detector T = 300 °C, isothermal at 150 °C).

Microbial oxidation of bromonaphthalenes. Precultures (4 x 50 mL) in 100-mL Fernbach flasks were prepared by inoculating 50 mL of MSB[8] with 0.5% succinate as carbon source (pH 7.2) with a vial of Pp NCIB 9816-11 cells. The microorganisms were grown for 24 hrs. at 30°C, shaking at 120 rpm. Each preculture was transferred to (4 x 300mL) MSB with 0.5% succinate (pH 7.2) in 2.8-L Fernbach flasks. The culture media were shaken at 150 rpm, 30°C for 6 hrs., after which 125 mg of the substrate in 0.5 ml DMSO was added. A drop of antifoaming agent (Mazu DF 204) was added to each Fernbach flask. Incubation with shaking was continued for the next 24 hrs. and the biotransformation was stopped. The culture medium (1.4 L) was centrifuged at 7000 rpm for 15 mins. at 10 °C. The supernatant solution was extracted with ethyl acetate (4 x 300 mL), was dried with Na_2SO_4 and evaporated to obtain an oily mixture which was filtered through silica gel using ethyl acetate as eluent (to remove residual cell material); evaporation afforded 250-300 mg crude diol mixture. For 1-bromonaphthalene, the crude diol mixture was dissolved in MeOH and purified via preparative HPLC (MeOH:H_2O/60:40), retention times: t_r (1) = 10.07 mins. t_r(2) = 11.42 mins.; 2 was separated from traces of 3 by flash chromatography (silica gel, 2:1 ethyl acetate/ hexanes). In the case of 2-bromonaphthalene, metabolite 8 was purified by flash chromatography (silica gel, 2:1 EtOAc/hexanes).

(+)-*cis*-(1R,2S)-Dihydroxy-1,2-dihydro-8-bromonaphthalene (1). R_f = 0.43 (2:1 EtOAc:hexanes), $[\alpha]_D^{25}$ = + 28 (c 0.44, CHCl₃), mp 120 °C (dec). IR (KBr, cm⁻¹) 3200, 2900, 2500, 1650, 1530, 1440, 1180, 1060, 980, 860, 800, 730. ¹H NMR (270 MHz) δ 7.45 (d, J = 8.0, 1H), 7.17 (2d, J = 8.0, J = 7.5, 1H), 7.07 (d, J = 7.5, 1H), 6.37 (dd, J = 10.0, J = 2.8, 1H), 5.92 (d, J = 10.0, 1H), 5.03 (t, J = 5.0, 1H), 4.62 (m, 1H), 2.81 (exch, 1H), 2.16 (exch, 1H). ¹³C NMR (400 MHz) δ 134.49 (C), 133.42 (C), 132.59 (CH), 132.04 (CH), 130.63 (CH), 126.41 (CH), 126.23 (CH), 125.52 (C), 69.97 (CH), 68.59 (CH). MS m/z (rel. int.) (EI+) 242 (M+2, 10), 240 (M,12), 225 (M+2-H_3O^+, 33), 223 (M-H_3O^+, 35), 196(15), 172 (10), 144 (100), 115, 30). HRMS calculated for $C_{10}H_9O_2Br$ 239.9786 found 239.9786 error 2.1 ppm.

(+)-*cis*-(1R,2S)-Dihydroxy-1,2-dihydro-5-bromonaphthalene (2). R_f = 0.37 (2:1 EtOAc/hexanes), $[\alpha]_D^{25}$ = + 84.4 (c 0.5, MeOH) mp 142-142.5 °C. IR (KBr, cm⁻¹) 3200, 2900, 1650, 1620, 1430, 1160, 1120, 1090, 830, 750. ¹H NMR (270 MHz) δ 7.49 (d, J = 7.8, 2H), 7.14 (t, J = 7.8, 1H), 6.94 (d, J = 10, 1H), 6.18 (dd, J = 10, J = 4.4, 1H), 4.66 (m, 1H), 4.38 (m, 1H), 2.46 (exch, 1H), 1.99 (exch, 1H). ¹³C NMR (270 MHz, acetone-d_6) δ 140.9 (2C), 133.0 (CH), 132.6 (CH), 129.8 (CH), 127.6 (CH), 126.9 (C), (C), 71.4 (CH), 67.7 (CH). MS m/z (rel. int.) (EI+) 223 (M-17, 30), 196 (30), 144 (100), 115 (70).

cis-1,2-Dihydroxy-1,2-dihydro-4-bromonaphthalene (3). ^{1}H NMR (270 MHz) δ 7.63 (m, 1H), 7.52 (m, 1H), 7.36 (m, 2H), 6.51 (d, J = 4.8, 1H), 4.73 (m, 1H), 4.35 (m, 1H), 2.38 (exch, 1H), 2.17 (exch, 1H). ^{13}C NMR (270 MHz) δ 136.2 (C), 131.0 (C), 130.9 (CH), 130.1 (CH), 128.8 (CH), 128.1 (CH), 127.2 (CH), 125.1 (C), 69.9 (CH), 68.4 (CH). The assignment of *cis* relative stereochemistry for this compound was a speculation.

8-Bromo-2-naphthol (4).[10] The diol 1 (100 mg, 0.42 mmol) was dissolved in MeOH (5 mL) and 3N HCl (3 mL) was added. The solution was stirred at room temperature until the disappearance (observed by TLC) of 1 was complete. The reaction mixture was neutralized with solid NaHCO$_3$ and was concentrated by rotary evaporation. The residue was diluted with H$_2$O (5 mL) and extracted with EtOAc (2 x 10 mL), the extract was then dried with MgSO$_4$ and evaporated to dryness to afford a white solid. The white solid (76.9 mg, 83%yield) was recrystallized from hexanes, mp 112 ° C (lit. 113-114 °C). ^{1}H NMR (270 MHz) δ 7.75 (m, 3H), 7.55 (s, 1H), 7.18 (m, 2H), 5.11 (s, 1H).

5-Bromo-1-naphthol (5).[10] The diol 2 (38.5 mg, 0.16 mmol) was dissolved in MeOH (5 ml), 3N HCl (2 mL) was added and the solution stirred at room temperature until aromatization was complete. Solid NaHCO$_3$ was added until effervescence has ceased and the mixture was evaporated to remove MeOH and was diluted with H$_2$O (5 mL). The aqueous solution was extracted with EtOAc (2 x 10 mL) and dried with MgSO$_4$. The concentrated EtOAc extract was chromatographed on silica (2:1 EtOAc /hexanes). Only the major isomer was collected. The eluent was concentrated to afford a white solid (23.7 mg, 67% yield) which was recrystallized from H$_2$O. mp 136.5-137 ° C (lit 137 °C). ^{1}H NMR (270 MHz) δ 8.20 (d, J = 8.5, 1H), 7.80 (d, J = 7.6, 1H), 7.41 (dd, J = 7.6, J = 8.5 , 1H), 7.31 (dd, J = 7.3 , J = 8.4, 1H), 6.87 (d, J = 7.3, 1H), 5.59 (s, 1H).

(-)-cis-(1R,2S)-Dihydroxy-1,2,3,4-tetrahydronaphthalene (6). *Typical procedure.* The diol 8 (42 mg, 0.17 mmol) was weighed out and dissolved in MeOH (5 mL), 5 mg of 10% Pd/C and a drop of triethylamine were added. The round-bottom flask was evacuated and filled with H$_2$ at atmospheric pressure. After the reaction, (observed by TLC), the suspension was filtered through Celite; concentrated in the rotary evaporator and chromatographed on silica using mixture of hexanes/ EtOAc as eluent. The eluent was concentrated to give a white solid (8.7 mg, 31% yield). ^{1}H NMR (270 MHz) δ 7.43 (m, 1H), 7.23 (m, 2H), 7.13 (m, 1H), 4.66 (d, J = 3.5, 1H), 3.97 (dt, J = 3.5, J = 9.7, 1H), 2.95 (m, 1H), 2.78 (m, 1H), 2.60 (exch, 2H), 2.05 (m, 1H), 1.95 (m, 1H).

(±)-cis-1,2-Dihydroxy-1,2,3,4-tetrahydronaphthalene (7a). 1,2-Dihydronaphthalene (130 mg, 1mmol) was dissolved in 10 ml 3:1 acetone/water mixture, NMO (100mg, 0.85 mmol) was added to this followed by 0.30 mL of 0.05 M OsO$_4$ in t-BuOH. At the completion of the reaction, it was quenched 15% aq. NaHSO$_3$ (10 mL), saturated with NaCl and extracted with EtOAc (2 x 15 mL). The combined extracts were dried with Na$_2$SO$_4$ and concentrated under reduced pressure. Chromatography on silica (2:1 EtOAc:hexanes) afforded 107.8 mg (0.66 mmol, 66% yield) of product as white solid.

Chiral Lanthanide-Induced Shift Study. To determine the optimum ratio of the chiral shift reagent Eu(hfc)$_3$ to the hydrogenated diol, the method of incremental increase in concentration of shift reagent was

used.[12,15] Diol 7 (10 mg) was dissolved in 1 mL CHCl$_3$-d. To a half of this solution 100 mg Eu(hfc)$_3$ was added. The ^{1}H NMR of the other half of the diol solution was recorded and served as reference spectrum. The solution containing the lanthanide reagent was incrementally added to this solution to make up 5, 10, 20, 30, 40, 50 mol% and until all the remaining lanthanide reagent was added. It was found that the optimum diastereodifferentiation was realized with a 1:1 molar ratio of the diol and shift reagent.

(+)-cis-($1R,2S$)-Dihydroxy-1,2-dihydro-7-bromonaphthalene (8). R$_f$ = 0.40 (2:1 ethyl acetate/hexane), [α]$_D^{25}$ = +255 (c = 1.0, MeOH), mp = 146 °C (dec). IR (KBr, cm^{-1}) 3200, 2900, 1590, 1560, 1470, 1100, 1040, 1010, 930, 840, 825. ^{1}H NMR (270 MHz) δ 7.72 (d, J = 2.0, 1H), 7.41 (dd, J = 8.1, J = 2.0, 1H), 7.00 (d, J = 8.1, 1H), 6.51 (d, J = 9.9, 1H), 6.12 (dd, J = 9.9, J = 4.7, 1H), 4.69 (m, 1H), 4.33 (m, 1H), 2.4 (exch, 1H), 1.8 (exch, 1H). ^{13}C NMR (270 MHz) δ 140.8 (C), 133.0 (C), 131.1 (CH), 130.8 (CH), 130.5 (CH), 128.9 (CH), 128.2 (CH), 121.6 (C), 70.7 (CH), 67.1 (CH). MS m/z (rel.int.) (EI+) 242 (M+2, 20), 240 (M, 23), 226 (M+2-H$_2$O, 40), 224(M-H$_2$O, 43), 196 (40), 144 (100), 115 (90) HRMS calcd for C$_{10}$H$_9$O$_2$Br 239.97859 found 239.9786 error 1.2 ppm.

cis-1,2-Dihydroxy-1,2-dihydro-6-bromonaphthalene (9). ^{1}H NMR (270 MHz) δ 7.40 (s, 2H), 7.26 (s, 1H), 6.47 (d, J = 10.8 , 1H), 6.10 (dd, J = 1.3, J = 10.8, 1H), 4.66 (m, 1H), 4.36 (m, 1H), 2.45 (exch, 1H), 1.98 (exch, 1H). The assignment of cis relative stereochemistry for this compound was made in analogy to other metabolites and is viewed as an assumption.

7-Bromo-1-naphthol (10). To diol 8 (65 mg, 0.27 mmol) in MeOH (5 mL), 3N HCl (2 mL) was added and the reaction stirred at room temperature until it was complete. The mixture was neutralized with NaHCO$_3$ and extracted with EtOAc after removing MeOH. The white solid (50 mg, 85% yield) had a mp of 104-105 °C (lit 105-106 °C). ^{1}H NMR (270 MHz) δ 8.37 (s, 1H), 7.66 (d, J = 8.8, 1H), 7.55 (d, J = 8.8 , 1H), 7.40 (d, J = 8.2 , 1H), 7.31 (dd, J = 7.4, J = 8.2, 1H), 6.82 (d, J = 7.4, 1H), 5.33 (s, 1H).

General procedure for the preparation of the Mosher diester [16]. To one equivalent of the tetrahydrodiol, dissolved in dichloromethane, was added three equivalents of the (+)-MTPA, three equivalents of DCC and one-tenth equivalent of DMAP. The mixture was stirred at room temperature until the conversion was complete as seen by TLC. The mixture was filtered to remove dicyclohexyl urea, the filtrate was washed with water followed by dilute HOAc solution, and then water again. The dichloromethane extract was dried (MgSO$_4$) and concentrated under reduced pressure. The crude diester product was further purified by flash chromatography (silica gel using EtOAc/hexanes as eluent).

ACKNOWLEDGMENTS

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