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Toluene dioxygenase-mediated oxidation of dibromobenzenes. Absolute stereochemistry of new metabolites and synthesis of (−)-conduritol E

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Abstract—Dibromobenzenes (*o*-, *m*-, and *p*-isomers) were converted to the corresponding *cis*-cyclohexadiene diols by whole-cell fermentation with *Escherichia coli* JM 109 (pDTG601A), an organism over-expressing the enzyme toluene dioxygenase (TDO). Absolute stereochemistry of new metabolites was determined, and (−)-conduritol was synthesized.

1. Introduction

Oxidation of aromatic compounds to the corresponding *cis*-cyclohexadiene diols with toluene dioxygenase represents a reaction that has, as yet, no equivalent in synthetic methodology. To date over 400 metabolites have been isolated[1] and many served as optically pure material in total synthesis of natural products.[2,3] The whole-cell fermentation of aromatic compounds with the recombinant organism *Escherichia coli* JM 109 (pDTG601)[4] produces moderate to excellent yields of the corresponding diols, which are easily extracted from the fermentation broth using base-washed ethyl acetate.[3a]

The mechanism of the enzymatic oxidation remains unknown although some predictive models have been proposed regarding the expected regio- and stereochemistry of oxidation in single-ring, disubstituted aromatic compounds.[5] For several such compounds the fate of oxidation is known for all three regioisomers: *ortho*, *meta*, and *para*. The metabolites of these are shown in Table 1. A large number of diene diols is known for at least two of the three possible isomers, and their metabolites have been listed in several recent compilations.[1,2] Many diol metabolites have been observed in variously substituted biphenyls but were not rigorously characterized. A total of nearly 100 such compounds has been noted.[1] This manuscript reports the results of oxidation of a series of dibromobenzenes as well as the determination of absolute stereochemistry for a new diol derived from *o*-dibromobenzene by chemical conversion to (−)-conduritol E.

2. Results and discussion

Of the three isomers of dibromobenzene, only one, *m*-dibromobenzene (**28**), had previously been subjected to

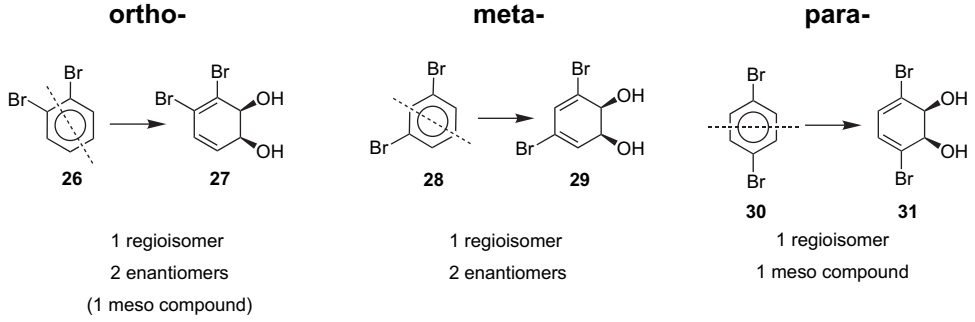

Figure 1. Possible modes of oxidation for the series of dibromobenzenes.

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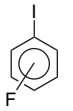
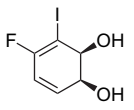
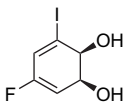
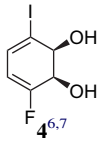
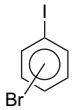
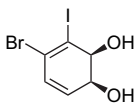
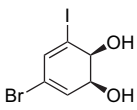
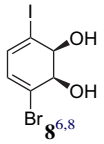
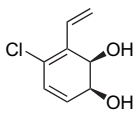
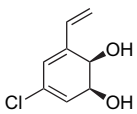
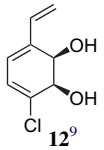
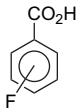
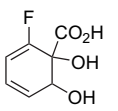
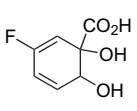
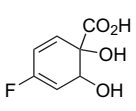
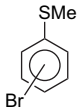
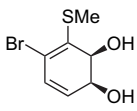
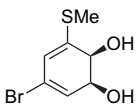
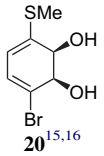
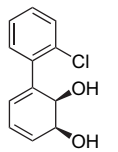
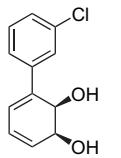
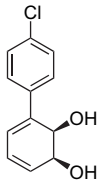
Table 1. Oxidation of isomeric series of disubstituted arenes

Substrate	Products (references in superscript)		
 **1**	 **2**[6]	 **3**[6]	 **4**[6,7]
 **5**	 **6**[6]	 **7**[6]	 **8**[6,8]
 **9**	 **10**[9,10]	 **11**[9]	 **12**[9]
 **13**	 [a]**14**[11,12,13]	 [a]**15**[14]	 [a]**16**[11,12,14]
 **17**	 **18**[15,16]	 **19**[15,16]	 **20**[15,16]
 **21**	 **22**[17,18]	 **23**[17]	 **24**[18]

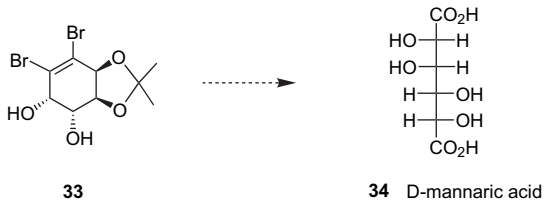
[a] Absolute stereochemistry not determined.

toluene dioxygenase-mediated oxidation. A single isomer, **29**, was produced in a yield of 4 g L^{-1} [3a] and served as a convenient starting material for a short synthesis of the amaryllidaceae constituent narciclasine.[19]

Because of the symmetry of dibromobenzenes, only one regioisomer of a diol is possible, as shown in Figure 1. In principle, *o*-dibromobenzene could also form a *meso* compound if the oxidation took place with a 3,4-regiochemistry, however, TDO oxidations of disubstituted arenes yield exclusively 1,2-regioisomers with respect to the directing group. Whole-cell oxidation of *m*-dibromobenzene with *E. coli* JM 109 (pDTG601) produced a single enantiomer (>99% ee). Similarly, the *o*-isomer provided diol **27** in a yield of 4.1 g L^{-1}, and the *p*-isomer furnished the *meso*-diol **31** in 55 mg L^{-1}.[20] Though this particular metabolite is *meso*, it may find application in asymmetric synthesis through further desymmetrization.[21] Procedures such as lipase resolution have been used to enrich enantiomeric excesses of those diols that are produced as scalemic mixtures. For example, *p*-bromoiodobenzene provides the corresponding diol with only 20% ee and may be enriched by either subsequent lipase resolution of acetyl derivatives or by resubmission of the scalemic diol to further fermentation with a wild *Pseudomonas* strain in which one of the enantiomer is consumed. Such procedures have been applied in the preparation of *ent*-diene diols[22] and *ent*-7-deoxypancratistatin.[8] The symmetrical nature of the molecule may be exploited through the use of double radical or Heck-type cyclizations from the vinyl bromides to appropriately tethered functionalities.

The absolute configuration of **27** was established by its conversion to conduritol E,[23] as shown in Scheme 1, and its enantiomeric excess was conveniently determined by ^{19}F NMR evaluation of the Mosher ester derived from monoprotected diol **38**, (Scheme 2). The Mosher ester **39** was previously prepared from homochiral and racemic alcohols **38** in connection with a study on the oxidation of a series of methylsulfanyl bromobenzenes.[15,16] Analysis of the crude ^{19}F NMR spectrum indicated a single peak corresponding to an enantioselectivity of greater than 95% in the enzymatic oxidation of *o*-dibromobenzene.

Enantiomerically pure diol **33** can easily be converted into D-mannaric acid by ozonolysis according to established procedures for the conversion of vinyl bromides of this type into hexoses.[24] The provision of isomeric sugars D-glucaric acid and D-altaric acid is also possible, as all four diastereoisomers at C-4 and C-5 are accessible by directed hydroxylation or epoxidation procedures. Diol **27** would appear to be suitable for synthesis of such acids (Fig. 2).



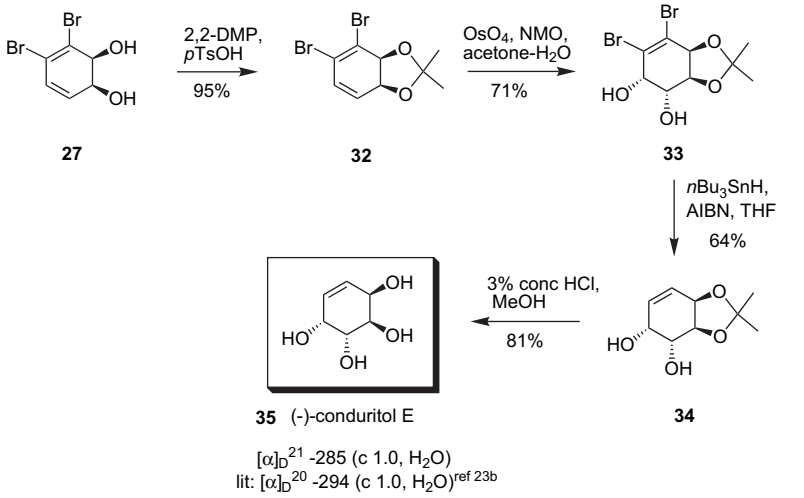
33 **34** D-mannaric acid

Figure 2.

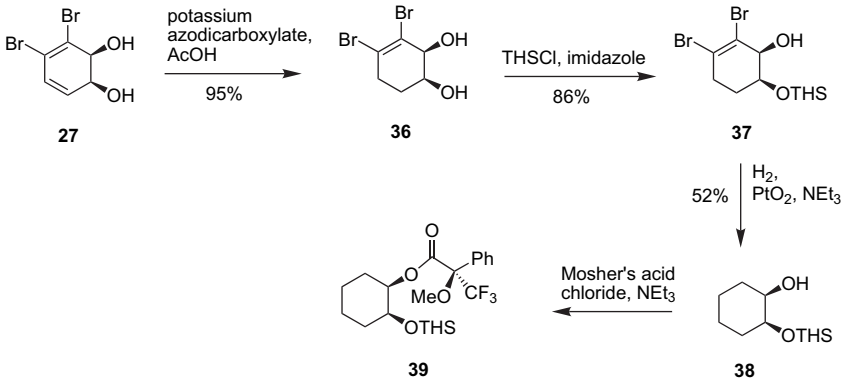
3. Experimental

3.1. General

All non-hydrolytic reactions were carried out under an argon atmosphere. Glassware used for moisture-sensitive reactions was flame-dried under vacuum and subsequently purged with argon. THF was distilled from potassium/benzophenone. Methylene chloride was distilled from calcium hydride. Flash column chromatography was performed using Kieselgel 60 (230–400 mesh). Analytical thin-layer chromatography was performed using silica gel 60-F_{254} plates. Melting points are reported uncorrected. IR spectra were recorded as a film, unless otherwise specified. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker instrument at 300 and 75 MHz, respectively. Specific rotation measurements are given in deg cm^3 g^{-1} dm^{-1}. Ultraviolet spectroscopy was performed using a diode array spectrophotometer. Large-scale fermentation was performed in a 15-L B. Braun Biostat C-15 Fermentor. All biological media was purchased through Fisher Canada. Combustion analyses



Scheme 1. Correlation of absolute configuration by conversion to (−)-conduritol E.



Scheme 2. Synthesis of Mosher's ester **39**.

were performed by Atlantic Microlabs, Norcross, Georgia, USA.

3.2. General biotransformation procedure

3.2.1. Small-scale fermentation with *E. coli* JM 109 (pDTG601).

3.2.1.1. Growth of colonies. Agar plates consisted of bactotryptone (10 g L^{-1}), yeast extract (5 g L^{-1}), NaCl (5 g L^{-1}), agar (30 g L^{-1}), and ampicillin (100 mg L^{-1}). *E. coli* JM 109 (pDTG601) cells were streaked onto a plate and were incubated at 35 °C for 12–24 h. A single bacterial colony was selected for preculture preparations as described in the following section.

3.2.1.2. Preparation of preculture. Luria Bertani (LB) liquid medium consisted of bactotryptone (10 g L^{-1}), yeast extract (5 g L^{-1}), NaCl (5 g L^{-1}), and ampicillin (100 mg L^{-1}). The preculture medium (3 mL) was inoculated with a single colony of *E. coli* JM 109 (pDTG601) and the resulting inoculum was grown at 35 °C on an orbital shaker (200 rpm) for 6 h.

3.2.1.3. Fernbach flask preparation. LB liquid medium consisted of bactotryptone (10 g L^{-1}), yeast extract (5 g L^{-1}), NaCl (5 g L^{-1}), glucose (5 g L^{-1}), and ampicillin (100 mg L^{-1}). LB medium (500 mL) was inoculated with 1 mL of *E. coli* JM 109 (pDTG601) of the preculture medium. This inoculum was grown at 35 °C on an orbital shaker (180 rpm) for 5 h. A chemical inducer, isopropyl-1-thio-β-D-galactopyranoside (IPTG) (10 mg L^{-1}), was added via sterile filter and the cells were grown for an additional 7 h at 35 °C on an orbital shaker (200 rpm).

3.2.1.4. Substrate addition. The supernatant was separated from the cells by centrifugation at 7000 rpm for 15 min. The cell pellet was re-suspended in 500 mL of 0.1 M phosphate buffer consisting of KH_2PO_4 (6.8 g L^{-1}), K_2HPO_4 (8.7 g L^{-1}), and glucose (2 g L^{-1}). The aromatic substrate (400 mg L^{-1}) was added as a solution in isopropyl alcohol. Product formation was monitored by thin-layer chromatography (silica gel, hexane/ethyl acetate, 1:1).

3.2.1.5. Product isolation. After 5 h of incubation with substrate the pH of the culture medium was adjusted with 6 M NaOH to 8.5, and a cell pellet was obtained by centrifugation at 7000 rpm and 4 °C for 20 min. The supernatant liquid was extracted with acid-free ethyl acetate, prepared by stirring the organic solvent with a saturated solution of Na_2CO_3 and separation of the organic layer from the aqueous layer. The extract was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed under reduced pressure.

The crude material was purified by crystallization or flash column chromatography (silica gel deactivated with 10% distilled water) immediately after concentration of the solvent in order to minimize decomposition of the unstable dienediols.

3.2.2. Large-scale fermentations. Large-scale fermentations were carried out in a 15-L (8-L working volume) B. Braun Fermentor according to a published procedure.[3]

3.2.3. Extraction of products. Dienediols obtained from large-scale (8-L fermentation) were extracted from the aqueous fermentation broth into ethyl acetate either by standard manual extraction or by continuous extraction. Large-scale manual extraction requires up to 20 L of ethyl acetate, whereas the use of a rotary-evaporator-driven continuous extractor facilitates the extraction of up to 9 L of aqueous broth using as little as 3 L of ethyl acetate. The diene diols derived from small-scale fermentations (1-L) were extracted manually. Progress of either manual or continuous extraction was monitored by thin-layer chromatographic analysis of the aqueous layer.

3.2.3.1. (1*S*,2*S*)-3,4-Dibromo-cyclohexa-3,5-diene-1,2-diol (27). The biooxidation of *o*-dibromobenzene was performed according to the general procedure for large-scale fermentation.[3] *o*-Dibromobenzene **26** (60 g) was added dropwise over 45 min to a 15-L fermentor containing a growing culture of *E. coli* JM 109 (pDTG601). After stirring the media for an additional 1 h, the cell broth was separated from the cells by centrifugation. The broth was extracted using a rotary-evaporator-driven continuous extractor over a three-days period with 3 L of ethyl acetate. The combined organic layers were washed twice with approximately 10% w/v sodium carbonate solution to remove any phenolic residue. The organic extracts of the fermentation broth were concentrated in vacuo and the dienediol precipitated by addition of pentane. Recrystallization from ethyl acetate/pentane provided the title compound as a white solid (32.8 g, 4.1 g L^{-1}). Mp 144–146 °C (from ethyl acetate/pentane); $[\alpha]_D^{22}$ +104 (*c* 0.15, diethyl ether); R_f 0.36 (hexanes/ethyl acetate, 1:1); IR (film) ν 3175, 1621 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 6.03 (dd, *J*=9.9, 2.1 Hz, 1H), 6.00 (dd, *J*=6.6, 2.4 Hz, 1H), 4.62 (d, *J*=6.9 Hz, 1H), 4.55 (m, 1H), 4.28 (m, 2H); ^{13}C NMR (75 MHz, acetone-d_6) δ 133.4, 126.5, 126.2, 120.6, 74.2, 69.0; HRMS (EI) Calcd for $C_6H_6Br_2O_2$ (M^+), 267.8731; Found, 267.8733; Anal. Calcd for $C_6H_6Br_2O_2$: C, 26.70; H, 2.24. Found: C, 27.04; H, 2.32.

3.2.3.2. (3a*S*,7a*S*)-2,2-Dimethyl-4,5-dibromo-4,6-benzo[1,3]dioxole (32). Diene diol **27** (3.0 g, 11.1 mmol, 1 equiv) was transferred to a 100-mL round-bottomed flask and suspended in 5 mL acetone and 15 mL of 2,2-dimethoxypropane. A few crystals of *p*-toluenesulfonic acid were added, and the reaction mixture was stirred at rt for 3 h. The reaction was quenched with 12 mL of 10% aq sodium hydroxide solution, and the acetone was removed under reduced pressure. The residue was diluted with ethyl acetate, and the layers were separated. The aqueous layer was then extracted with several portions of ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. The solvent was removed under vacuum to provide a light oil (3.21 g, 95%). An analytical sample was obtained after chromatography over 10% deactivated silica gel to afford the title compound as a clear oil. $[\alpha]_D^{22}$ +101 (*c* 0.75, $CHCl_3$); R_f 0.50 (50% ethyl acetate in hexanes); IR (film) ν 2988, 2933, 2896, 1634 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.14 (d, *J*=9.9 Hz, 1H), 5.93 (dd, *J*=9.9, 3.6 Hz, 1H), 4.82–4.72 (m, 2H), 1.45 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.7, 125.6, 123.8, 120.3, 106.9, 77.3, 71.3, 26.6, 25.0; HRMS (EI) Calcd for $C_9H_{10}Br_2O_2$, 307.9047; Found, 307.9037.

3.2.3.3. (3a*S*,4*R*,5*S*,7a*S*)-2,2-Dimethyl-6,7-dibromo-4,5-dihydroxybenzo[1,3]dioxole (33). The protected dienediol **32** (3.1 g, 10.0 mmol, 1 equiv) was suspended in 60 mL of 8:1 (by volume) mixture of acetone/water. *N*-Methylmorpholine-*N*-oxide (2.34 g, 20.0 mmol, 2 equiv) was added followed by the addition of four crystals of osmium tetraoxide. The reaction mixture darkened slightly and was stirred for 18 h until consumption of starting material was complete. The reaction mixture was quenched by addition of 5 mL satd aq sodium bisulfite and 2 g solid sodium bisulfite, and the pH of the mixture was adjusted by addition of concd HCl. The mixture was stirred for 15 min, and the acetone was removed under reduced pressure. The aqueous portion was extracted repeatedly with ethyl acetate, and the combined organic extracts were washed with 1 N HCl, 20% aq solution of KOH, and brine before being dried over anhydrous magnesium sulfate. The extracts were filtered through a short column of silica gel and the solvent evaporated to furnish 2.4 g of a white crystalline solid, 71% yield, which required no further purification for the subsequent reaction. An analytically pure sample was obtained by recrystallization from ethyl acetate/pentane. Mp 155–156 °C; $[\alpha]_D^{22}$ +5.47 (*c* 0.75, MeOH); R_f 0.3 (50% ethyl acetate in hexanes); IR (KBr) ν 3435, 3360, 2994, 2905, 1606 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.77 (dd, *J*=1.2, 5.4 Hz, 1H), 4.50–4.45 (m, 2H), 4.34 (m, 1H), 2.68 (br s, 2H), 1.43 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 127.6, 125.6, 77.6, 75.3, 70.9, 69.1, 27.6, 26.1; HRMS (EI) Calcd for $C_8H_9O_4Br$, 326.8867; Found, 326.8863; Anal. Calcd for $C_9H_{12}Br_2O_4$: C, 31.42; H, 3.52. Found: C, 31.59; H, 3.53.

3.2.3.4. (−)-Conduritol E (35). Dibromide **33** (0.50 g, 1.4 mmol, 1 equiv) was dissolved in 50 mL distilled THF and transferred to a flame-dried 100-mL round-bottomed flask equipped with a reflux condenser. The solution was degassed in an ultrasound bath and under positive argon pressure for 10 min. Azoisobutyronitrile (23 mg, 0.14 mmol, 0.1 equiv) was added, and the solution was heated to steady reflux (83 °C external temp). At this time, tributyl tin hydride (1.0 mL, 3.36 mmol, 2 equiv) was added in portion. Reflux was maintained for 1.5 h until complete consumption of starting material was witnessed by TLC analysis. The reaction mixture was cooled, and potassium fluoride (2 g) was added. The resulting precipitate was filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography using 4:1 hexanes/ethyl acetate to 100% ethyl acetate to provide 170 mg (64%) of the de-brominated material. The solid was dissolved in 5 mL of methanol and to this solution was added 2 mL of a 3% (by volume) solution of concd HCl in methanol and the resulting solution was stirred for 40 h after which time the solvent was removed under reduced pressure to

provide a crude white solid. The solid was purified by flash column chromatography (4:1 chloroform/methanol) to give conduritol E as a white crystalline solid (78 mg, 81%). Mp 194–195 °C (lit.[23b] mp 193 °C); $[\alpha]_D^{20}$ −285 (*c* 1.0, H_2O), lit.[23b]: $[\alpha]_D^{20}$ −294 (*c* 1.0, H_2O); R_f 0.18 (chloroform/methanol, 4:1); IR (film) ν 3434, 1634 cm^{-1}; ^1H NMR (300 MHz, MeOD) δ 5.79 (d, *J*=2.1 Hz, 2H), 4.27 (s, 2H), 3.93 (d, *J*=0.9 Hz, 2H); ^{13}C NMR (75 MHz, MeOD) δ 130.7, 70.9, 67.6; HRMS (EI) Calcd for $C_6H_8O_3$ (M^+-H_2O), 128.0473; Found, 128.0455.

3.2.3.5. (1*S*,2*S*)-3,4-Dibromo-cyclohexa-3-ene-1,2-diol (36). Diol **27** (0.38 g, 1.47 mmol, 1 equiv) was dissolved in 6 mL MeOH, and the round-bottomed flask containing the solution was subsequently placed into an ice/NaCl bath. Potassium azodicarboxylate (0.93 g, 4.27 mmol, 3 equiv) was added in two portions to the methanolic solution. Acetic acid (0.85 mL, 12.78 mmol, 9 equiv) in 2 mL MeOH was added dropwise over 40 min. The reaction flask was allowed to warm to room temperature overnight (15 h). The reaction was quenched by adding 2 mL saturated Na_2CO_3 solution and stirring for 20 min. Methanol was removed under reduced pressure and the residue diluted with 10 mL EtOAc. The layers were separated and the aqueous phase was extracted with 3×10 mL EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and treated with activated charcoal. Filtration and concentration of the filtrate under reduced pressure afforded **36** as a white crystalline solid (0.367 g, 95%). Mp 175–176 °C; $[\alpha]_D^{21}$ −50.4 (*c* 0.75, MeOH); R_f 0.23 (Hex/EtOAc, 1:1); IR (KBr pellet) ν 3246, 1626 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 4.61 (d, *J*=6 Hz, 1H), 4.26 (s, 1H), 3.96–3.84 (m, 2H), 2.79–2.51 (m, 2H), 2.05–1.92 (m, 1H), 1.85–1.73 (m, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 127.1, 124.9, 73.5, 68.25, 35.2, 26.9; HRMS (EI) Calcd for $C_6H_8Br_2O_2$, 271.8872; Found, 271.8871; Anal. Calcd for $C_6H_8Br_2O_2$: C, 26.50; H, 2.97. Found: C, 27.34; H, 3.16.

3.2.3.6. (1*S*,2*S*)-1-[(Hexyldimethylsilyl)oxy]-3,4-dibromocyclohexa-3-ene-2-ol (37). A 5-mL round-bottom flask was charged with diol **36** (200 mg, 0.74 mmol, 1 equiv), imidazole (65 mg, 0.96 mmol, 1.3 equiv), and 1 mL anhydrous dimethylformamide. The flask was cooled externally to −30 °C, then hexyldimethylsilyl chloride (0.15 mL, 0.78 mmol, 1.05 equiv) was added. The mixture was stirred at −30 °C for 1 h and then the reaction flask was placed in a freezer (−18 °C) for 21 h. The mixture was allowed to warm to room temperature and diluted with 50 mL ether, washed with 10×1 mL distilled H_2O, brine, and dried over $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure. The crude silyl ether was purified by flash column chromatography (pentane/Et_2O, 10:1) to give **37** as a clear and colorless oil (0.26 g, 86%). $[\alpha]_D^{21}$ −41.1 (*c* 0.75, MeOH); R_f 0.23 (pentane/Et_2O, 10:1); IR (film) ν 3547, 2958, 2868, 1628 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.26–4.17 (m, 1H), 4.03–3.93 (m, 1H), 2.84 (d, *J*=4 Hz, 1H), 2.77–2.64 (m, 1H), 2.63–2.48 (m, 1H), 2.10–1.9 (m, 1H), 1.77–1.57 (m, 2H), 0.95–0.84 (m, 13H), 0.17 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 130.8, 127.8, 122.9, 74.1, 73.6, 69.6, 35.1, 34.1, 27.2, 24.8, 20.2, 20.0, 18.5, 18.4; HRMS (EI) Calcd for $C_{14}H_{26}Br_2O_2Si$, 328.9032; Found, 328.9026; Anal. Calcd for $C_{14}H_{26}Br_2O_2Si$: C, 40.59; H, 6.33. Found: C, 40.96; H, 6.36.

3.2.3.7. (1*R*,2*S*)-2-[(Hexyldimethylsilyl)oxy]cyclohexan-1-ol (38). A flask containing a magnetic stirring bar was charged with dibromide **37** (0.219 g, 0.53 mmol, 1 equiv), triethylamine (0.5 mL, 3.56 mmol, 7 equiv), platinum oxide (Adam's catalyst, 24 mg, 0.11 mmol, 0.2 equiv), and 0.5 mL MeOH. The reaction flask was evacuated, flushed with hydrogen via a balloon (1 atm), and stirred until total consumption of starting material as was observed by TLC control (6 h). The crude mixture was filtered through a short plug of Celite, and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (pentane/Et_2O, 10:1) to give the title compound as a clear and colorless oil (71 mg, 52%) with spectral data matching that of previously reported compound **38**. $[\alpha]_D^{23}$ +3.4 (*c* 1.0, $CHCl_3$), lit.[16]: $[\alpha]_D^{23}$ +3.3 (*c* 1.0, $CHCl_3$).

3.3. General procedure for the formation of Mosher ester derivative 39

Alcohol **38** (20 mg, 0.076 mmol, 1 equiv) was transferred to a flame-dried round-bottomed flask containing magnetic stirring bar under an argon atmosphere. Anhydrous triethylamine (17 μL) was added followed by 4-dimethylaminopyridine (4.8 mg, 0.038 mmol, 0.5 equiv). (*R*)-(−)-α-Methoxy-α-trifluoromethyl-phenylacetic acid chloride (23 μL, 0.11 mmol, 1.5 equiv) was added dropwise. Within minutes a white precipitate was observed. The reaction was stirred overnight. The reaction mixture was then diluted with 5 mL methylene chloride, transferred to a separatory funnel, and washed with 5 mL saturated solution of sodium bicarbonate. The layers were separated, the organic layer dried ($MgSO_4$), and the solvent evaporated to provide the ester as a crude oil. The compound was purified by flash column chromatography (pentane/Et_2O, 10:1) to afford the ester as a clear and colorless oil (20 mg, 57%). Physical and spectral data matched that for the compound previously reported.[16]

R_f 0.46 (pentane/Et_2O, 10:1); IR (film) ν 2948, 2867, 1745, 1463, 1450, 1379, 1263, 1169 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.60 (m, 2H), 7.40 (m, 3H), 5.12 (m, 1H), 3.80 (m, 1H), 3.55 (m, 3H), 1.75–1.55 (m, 7H), 1.5–1.2 (m, 2H), 0.88 (dd, *J*=6.8, 5.2 Hz, 6H), 0.81 (d, *J*=4.1 Hz, 6H), 0 (s, 3H), −0.10 (s, 3H); ^{19}F NMR (188 MHz, $CDCl_3$) δ −72.4 ppm. Lit.[15]: ^{19}F NMR (188 MHz, $CDCl_3$) δ −72.8 ppm.

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