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Processing of cyclopropylarenes by toluene dioxygenase: isolation and absolute configuration of metabolites

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Abstract—Several arenes possessing a cyclopropyl substituent were subjected to enzymatic oxidation with toluene dioxygenase. The absolute configuration of metabolites was established by chemical means.

1. Introduction

Enzymatic oxidation of aromatics with recombinant organisms expressing toluene dioxygenase (TDO) provides valuable synthons for asymmetric synthesis. Several hundreds of cyclohexadiene *cis*-diols are already known[1] and many have found application in total synthesis of natural products.[1c,2] Reliable procedures have been published for the whole-cell fermentation of arenes using either a recombinant organism[3] (*Escherichia coli* JM 109 pDTG601) or a blocked mutant[4] (*Pseudamonas putida* 39D), both of which express toluene dioxygenase. To extend the functional content of such metabolites, we wished to examine a series of cyclopropylarenes that would provide diene diols of type **1**, ideally suited for many further reactivity options and cascade processes, as illustrated in Figure 1.

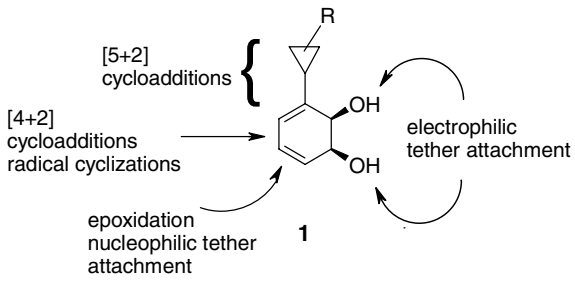

Figure 1. Reactivity options of homochiral dienylcyclopropanes obtained from enzymatic oxidation of the corresponding cyclopropylarenes.

2. Results and discussion

Cyclopropylbenzene, previously investigated in connection with the mechanism of the enzymatic oxidation,[5] has proven to be an excellent substrate for the enzyme. We prepared the cyclopropylarenes[6] listed in Table 1 and subjected them to the whole-cell fermentation protocol.

The products of fermentation were isolated by extraction of the fermentation broth with base-washed ethyl acetate, then characterized and subjected to the proof of absolute configuration, as outlined in Schemes 1–3. In all cases, the absolute configuration was proven by comparison with synthetic material derived from diol **15**, whose absolute stereochemistry has already been firmly established,[7] with the exception of metabolite **8**, whose match was made with the known diol **23**.[8]

Oxidation of racemic *trans*-cyclopropylstilbene gave diene diols **12a** and **12b**, which was reduced with potassium azodicarboxylate/acetic acid and the diol protected as an acetonide,[5] to provide **20a** and **20b** (Scheme 1). ^{1}H and ^{13}C NMR spectroscopic data for compounds **20a** and **20b** revealed an approximate 1:1 ratio of diastereoisomers. The absolute configuration was proven by conversion of phenylacetylene **17** to the corresponding cyclopropylboronic acid,[9] which was subjected to a Suzuki coupling protocol with vinyl bromide **21**, prepared from homochiral bromodiene diol **15**.[10]

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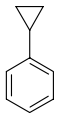
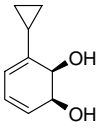
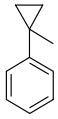
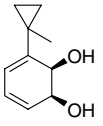
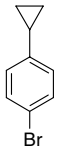
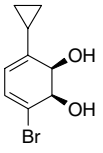
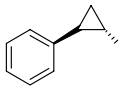
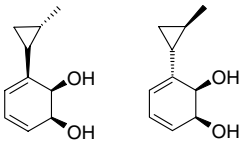
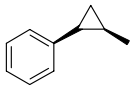
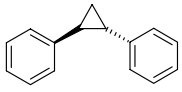
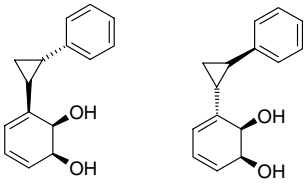
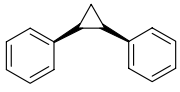
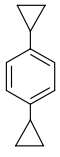
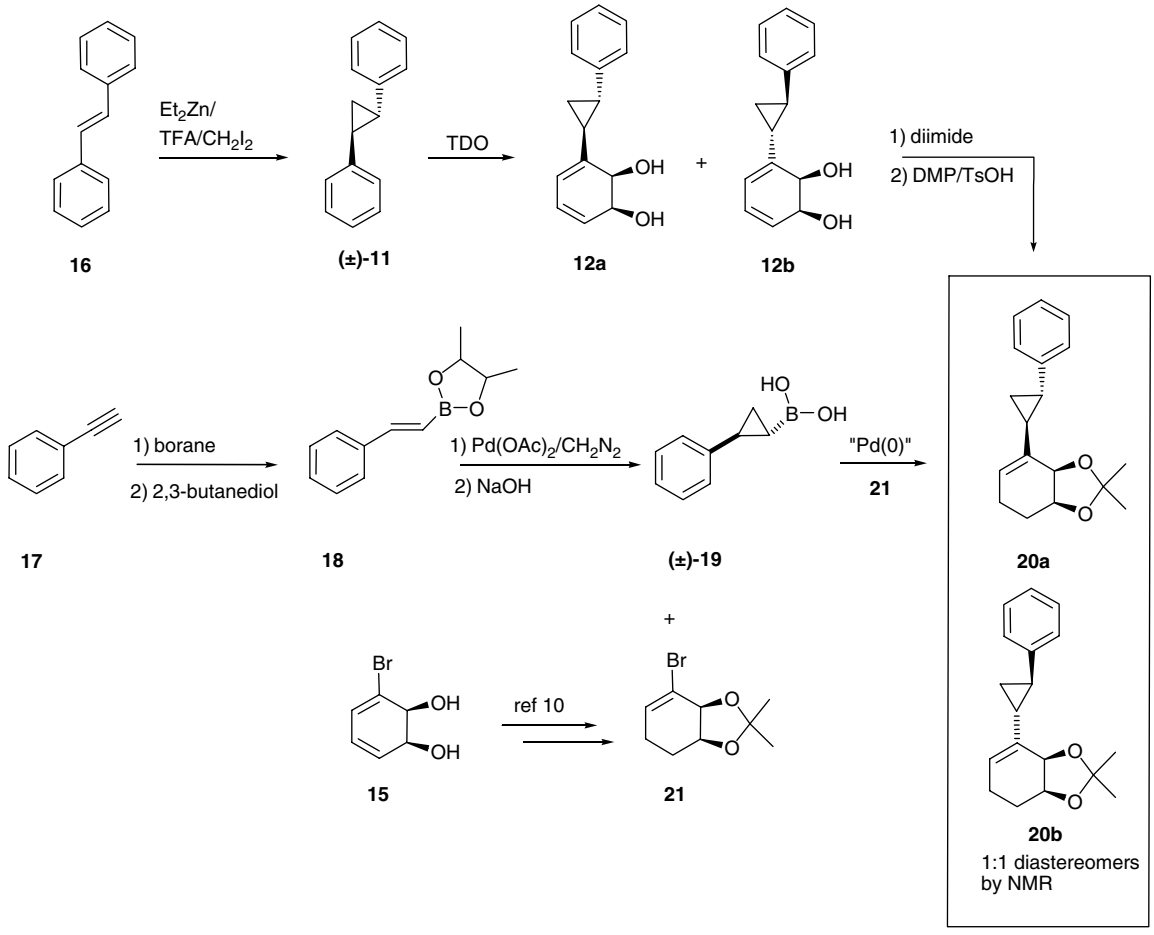
Table 1. Results of enzymatic oxidation of cyclopropylarenes by *E. coli* JM 109 (pDTG601)

Entry	Substrate	Product	Yield (mg/L)
1[a]	 **2**	 **3**	2500
2	 **4**	 **5**	56
3	 **6**	 **7**	32
4	 **(±)-8**	 **9a (1:1) 9b**	90
5[b]	 **(±)-10**	—	—
6	 **(±)- 11**	 **12a (1:1) 12b**	140
7[b]	 **13**	—	—
8[c]	 **14**	—	—

[a] Biotransformation of substrate **2** was performed in a 15-L fermentor.
[b] ^{1}H NMR spectra of the crude residue from biooxidation of *cis*-configured cyclopropanes revealed the presence of only trace amounts of the corresponding diene diols.
[c] Only starting material was recovered.

Spectroscopic data and the specific rotations of **20a** and **20b**, synthesized from compound **15**, provided the proof of the absolute configuration of the *trans*-isomer **12a** and **12b**.

The proof of the absolute configuration for diols **9a** and **9b** was initially attempted by oxidative cleavage and reduction of phenylcyclopropyl metabolite **12** according to the literature methods.[11] When this approach failed, the methyl derivative was prepared from a known compound, triene **23**,[8] as shown in Scheme 2. Fermentation of *trans*-β-methylstyrene **22**, initially reported by Boyd et al., gave the corresponding chiral triene diol **23**, which was reduced with diimide to the more stable diene. The diol functionality was protected as its acetonide and the less-substituted olefin converted to



Scheme 1.

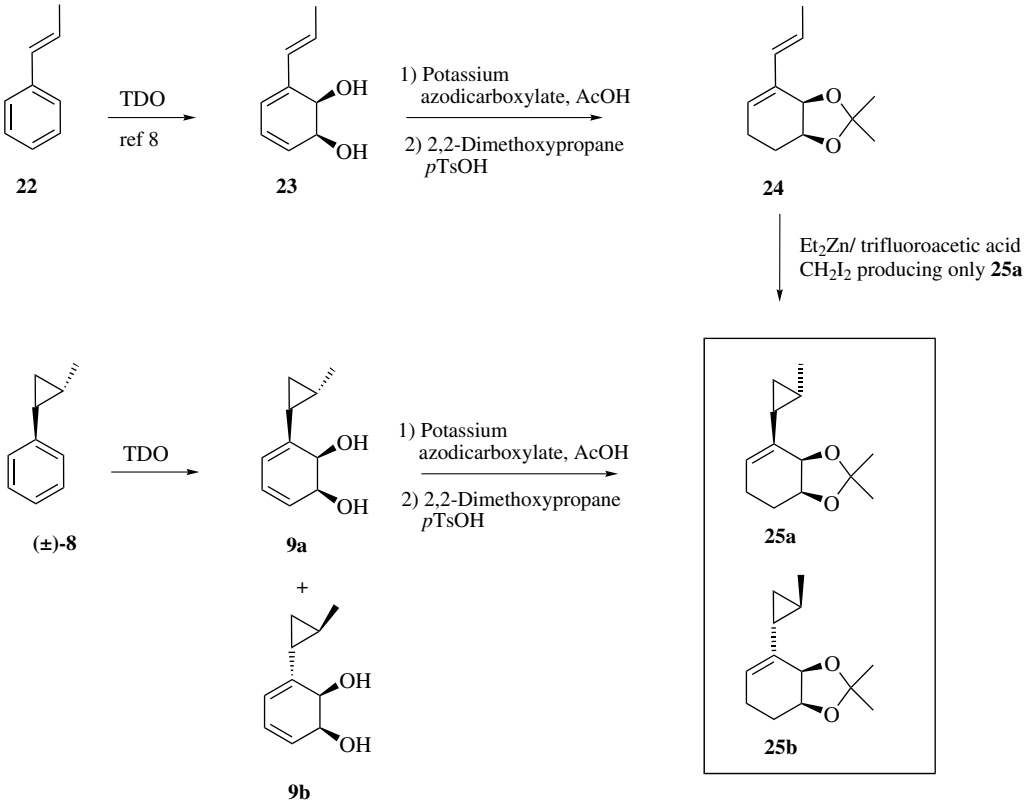
its corresponding cyclopropane[6] **25a**, formed as a single diastereoisomer whose specific rotation sign matched that of compounds **25a** and **25b** prepared by fermentation of cyclopropyl benzene **8**.[12] The enantiomeric excesses of the inseparable diastereomeric mixtures of **20a** and **20b** and **25a** and **25b** cannot be determined accurately. We base the estimate on the fact over 200 diols derived from mono-substituted arenes are produced in >95% ee.

Proof of the absolute configuration of metabolite **7** was accomplished by conversion to vinylcyclopropane **26** by hydrogenation with Adams' catalyst in triethylamine. The configuration of diol **26** was correlated to diol **3**, as shown in Scheme 3. The absolute configuration of diol **3** had previously been matched to diol **15**.[5] It has been shown that oxidation of cyclopropylbenzene proceeds to give essentially enantiomerically pure diene diol, whereas enzymatic oxidation of *p*-bromocyclopropylbenzene provides the product with only 27% enantiomeric excess[13] in the absolute configuration shown in Scheme 3.

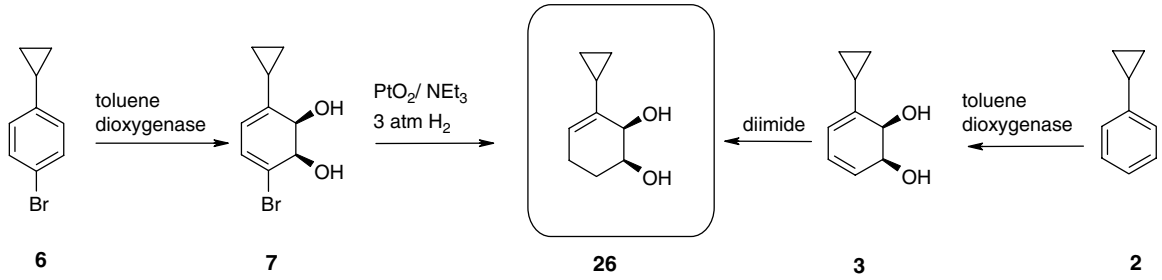
3. Conclusion

In summary, new metabolites derived from cyclopropylarenes have been identified for future use in asymmetric synthesis. It is interesting to note that the enzyme did not differentiate between the enantiomers of racemic substrates **8** and **11**, producing a 1:1 mixture of diastereomers. This observation is consistent with previous results obtained from the fermentation of various substrates containing stereogenic centers proximal to the aromatic ring.[10b] It appears that the substituents containing the remote stereogenic centers lie outside the active site and therefore no enantiomeric discrimination takes place during the oxidation; this rationale is consistent with the model of enzymatic oxidations proposed by Boyd.[1a]

It should also be noted that the *cis*-isomers **10** and **13** proved not to be substrates at all. This is surprising, especially in case of **13**, whose molecular volume resembles phenanthrene, an excellent substrate for both toluene dioxygenase and the closely related enzyme naphthalene dioxygenase.[14] Equally surprising is the fact that *p*-dicyclopropylbenzene was not oxidized by toluene dioxygenase, especially since *p*-bromocyclopropylbenzene is a substrate. Further studies on the enzymatic oxidation of substrates containing remote stereogenic centers will be conducted. The currently available cyclopropylarene metabolites will be employed in various cycloaddition and cascade processes as outlined in Figure 1. The results of these endeavors will be reported in due course.



Scheme 2.



Scheme 3.

4. Experimental

4.1. General biotransformation procedure

4.1.1. Shake-flask fermentation using *E. coli* JM 109 (pDTG601).

4.1.1.1. 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 (50 mg L^{-1}). *E. coli* JM 109 pDTG601 cells were streaked onto a plate and incubated at 35 °C for 18 h. Only single bacterial colonies were selected for preculture preparations.

4.1.1.2. 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 (50 mg L^{-1}). A sterile plastic culture tube containing 3 mL LB medium was inoculated with a single colony of *E. coli* JM 109 (pDTG601) and the preculture grown at 35 °C on an orbital shaker (180 rpm) for 6 h.

4.1.1.3. 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 (50 mg L^{-1}). A 2-L sterile fernbach flask containing 500 mL of LB medium was inoculated with 1 mL of *E. coli* JM 109 (pDTG601) preculture and the resulting culture grown at 35 °C on an orbital shaker (180 rpm) for 5 h. A chemical inducer of protein synthesis, β-isopropylthiogalactopyranoside (IPTG) (10 mg L^{-1}), was added via a sterile filter, and the cells grown for an additional 7 h at 35 °C on an orbital shaker (180 rpm).

4.1.1.4. Substrate addition. The supernatant was separated from the cells by centrifugation at 7000 rpm for 15 min. The supernatant was decanted and the cell pellet resuspended 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 (250 mg L^{-1}) was added as a solution in isopropyl alcohol (for solid substrates) or as a neat liquid (for oils). Product formation was monitored by thin-layer chromatography (silica gel, hexane–ethyl acetate, 1:1).

4.1.1.5. 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 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 with a saturated solution of Na_2CO_3. The extract was dried over $MgSO_4$, filtered, and the solvent 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 diene diols.

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

4.1.2. (1*S*,2*R*)-3-(1-Methyl-cyclopropyl)-cyclohexa-3,5-diene-1,2-diol 5. The diol was isolated from a crude mixture from the biotransformation of the corresponding cyclopropylbenzene by *E. coli* JM 109 (pDTG601) according to the general procedure for shake-flask fermentation. After purification by flash silica gel chromatography (2:1 Hex/EA, 10% deactivated silica), the *cis*-diene diol was recrystallized from ethyl acetate/pentane to afford the *cis*-dihydroarene diol as a white crystalline solid, 56 mg/L; mp 81–81.5 °C; $[\alpha]_D^{26} = +65.5$ (*c* 1.0, MeOH); R_f 0.30 (hexanes–ethyl acetate, 1:1); IR (film) *v* 3290, 1639, 1582, 1453 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 5.90 (d, $J = 5.6$ Hz, 1H), 5.85 (d, $J = 6.0$ Hz, 1H), 5.63 (d, $J = 9.0$ Hz, 1H), 4.32 (s, 1H), 4.10 (dd, $J = 14$, 10 Hz, 1H), 3.72 (d, $J = 5.0$ Hz, 1H), 1.23 (s, 3H), 0.92 (m, 2H), 0.56 (m, 2H); HRMS (EI) calcd for $C_{10}H_{14}O_2$, 166.0994; found, 166.0995.

4.1.3. (1*R*,2*R*)-3-Bromo-6-cyclopropyl-cyclohexa-3,5-diene-1,2-diol 7. The diol was isolated from a crude mixture from the biotransformation of the corresponding cyclopropylbenzene by *E. coli* JM 109 (pDTG601) according to the general procedure for shake-flask fermentation. Purification by flash silica gel chromatography (3:2 Hex/EA, 10% deactivated silica) furnished the *cis*-dihydroarene diol as a white crystalline solid, 32 mg/L; $[\alpha]_D^{26} = -17.5$ (*c* 0.65, $CHCl_3$); R_f 0.76 (hexanes–ethyl acetate, 1:1); IR (film) *v* 3290, 1631, 1550 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ: 6.27 (d, $J = 6.3$ Hz, 1H), 5.47 (d, $J = 6.3$ Hz, 1H), 5.63 (d, $J = 9$ Hz, 1H), 4.23 (d, $J = 2.4$ Hz, 2H), 4.18 (m, 1H), 3.92 (d, $J = 7.5$ Hz, 1H), 2.88 (s, 1H), 1.65 (m, 1H), 0.76 (m, 2H), 0.60 (m, 2H); ^{13}C NMR (100 MHz, acetone-d_6) δ: 144.2, 126.6, 124.2, 115.6, 72.6, 70.3, 13.2, 6.4, 6.0.

4.1.4. (1*S*,2*R*)-3-(2-Methyl-1-cyclopropyl)-cyclohexa-3,5-diene-1,2-diols 9a and 9b 1:1 mixture of diastereoisomers. The crude mixture of diols was isolated from fermentation with *E. coli* JM 109 (pDTG601) according to the general procedure for shake-flask fermentation. The product was purified by flash column chromatography on 10% deactivated silica gel (hexanes–ethyl acetate, 1:1) to provide the crystalline diols **9a** and **9b** as an inseparable mixture of diastereoisomers (90 mg/L). Mp 42–46 °C; $[\alpha]_D^{22} = +71.3$ (*c* 0.5, $CHCl_3$); R_f 0.3 (hexanes–ethyl acetate, 1:1); IR (film) *v* 3350, 2951, 1585, 1384, 1074 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 5.83 (m, 1H), 5.62 (d, $J = 9.8$ Hz, 1H), 5.55 (m, 1H), 4.25 (s, 1H), 3.78 (s, 1H), 2.30 (br s, 1H), 1.87 (br s, 1H), 1.20 (m, 1H), 1.05 (d, $J = 2.8$ Hz, 3H), 0.97 (m, 1H), 0.65 (m, 1H), 0.45 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 143.5, 128.0, 127.9, 124.9, 124.8, 120.7, 117.4, 117.3, 70.3, 70.2, 69.6, 69.5, 23.4, 23.3, 19.2, 16.4, 16.0, 15.2, 14.6, 14.5; HRMS (EI) calcd for $C_{10}H_{14}O_2$ (M^+), 166.0993; found, 166.0994.

4.1.5. (1*S*,2*R*)-3-(2-Phenyl-cyclopropyl)-cyclohexa-3,5-diene-1,2-diols 12a and 12b 1:1 mixture of diastereoisomers. The crude mixture of diols was isolated from fermentation with *E. coli* JM 109 (pDTG601) according to the general procedure for shake-flask fermentation. The product was purified by flash column chromatography on 10% deactivated silica gel (hexanes–ethyl acetate, 1:1) to provide the title compound as a clear oil, which was an inseparable mixture of diastereoisomers (140 mg/L): $[\alpha]_D^{22} = +38.6$ (*c* 0.89, $CHCl_3$); R_f 0.28 (hexanes–ethyl acetate, 1:1); IR (film) *v* 3346, 2922, 1603, 1498, 1403 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.24–7.19 (m, 2H), 7.13–7.01 (m, 3H), 5.86 (m, 1H), 5.67–5.60 (m, 2H), 4.27 (s, 1H), 3.87 (d, $J = 5.8$ Hz, 1H), 2.58–2.41 (br s, 1H), 2.07 (m, 1H), 1.81–1.71 (m, 1H), 1.33 (m, 1H), 1.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 142.4, 142.3, 142.1, 142.0, 128.9, 128.8, 128.6, 128.5, 128.2, 126.4, 126.3, 126.2, 126.2, 124.8, 124.7, 120.9, 118.5, 118.3, 70.3, 70.1, 69.4, 27.1, 26.9, 26.5, 25.5, 25.3, 16.9, 16.2, 15.7; HRMS (EI) calcd for $C_{15}H_{16}O_2$ (M^+): 228.1150; found, 228.1139.

4.1.6. (3a*R*,7a*S*)-2,2-Dimethyl-4-(2-phenylcyclopropyl)-3a,4,5,7a-tetrahydrobenzo[1,3]dioxole 20 1:1 mixture of diastereoisomers. Diene diols **12a** and **12b** (140 mg, 0.61 mmol, 1 equiv) were dissolved in 5 mL methanol and the solution then transferred to a 25-mL round-bottomed flask equipped with magnetic stirring bar and addition funnel. The flask was cooled externally to 0 °C at which time a PAD reagent (potassium azodicarboxylate, 331 mg, 1.71 mmol, 2.8 equiv) was added to the reaction flask. The yellow slurry was stirred for 10 min, then the addition funnel charged with acetic acid (236 μL, 3.99 mmol, 6.5 equiv) dissolved in 5 mL methanol. The acetic acid solution was added dropwise over a 1 h period and the yellow slurry allowed to warm to rt overnight. A saturated solution of sodium bicarbonate was used to adjust the pH to approximately 8.5 and the reaction mixture diluted with 20 mL ethyl acetate.

The layers were separated and the aqueous layer extracted with portions of ethyl acetate. The combined organic layers were washed with brine, dried ($MgSO_4$), and the drying agent removed by filtration. The solvent was removed under reduced pressure to provide 125 mg crude product, which was used directly in the subsequent reaction without further purification.

The crude product from diimide reduction was dissolved in the minimum amount of acetone and transferred to a 25 mL round-bottomed flask equipped with magnetic stirring bar. 2,2-Dimethoxypropane (471 μL, 3.9 mmol, 6.4 equiv) was added as a neat solution followed by several crystals of *p*-toluenesulfonic acid. The reaction mixture was stirred for 1.5 h at which time DMP was removed under vacuum. The residue was purified by flash column chromatography (hexanes–ethyl acetate, 3:1), affording the title compound, which was an inseparable mixture of diastereoisomers, as a clear and colorless oil (66 mg, 40% yield over two-step sequence); $[\alpha]_D^{22} = +30.2$ (*c* 1.0, $CHCl_3$); R_f 0.55 (hexanes–ethyl acetate, 5:1); IR (film) *v* 2984, 2931, 1604, 1498, 1455, 1367, 1241 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.16 (m, 2H), 7.05 (m, 3H), 5.72/5.50 (t, $J = 3.8$ Hz, 1H), 4.35 (m, 1H), 4.25 (m, 1H), 2.20–2.05 (m, 1H), 2.05 (m, 1H), 1.90–1.75 (m, 2H), 1.70–1.60 (m, 1H), 1.35 (d, $J = 3.5$ Hz, 3H), 1.30 (d, $J = 3.2$ Hz, 3H), 1.22 (m, 1H), 1.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 142.0, 141.9, 135.3, 135.1, 127.5, 127.2, 124.9, 124.7, 124.4, 124.4, 123.0, 122.0, 119.4, 107.3, 73.3, 72.9, 72.4, 27.0, 26.9, 25.7, 25.7, 25.5, 24.5, 24.1, 22.5, 19.6, 19.5, 14.2, 14.0; HRMS (EI) calcd for $C_{18}H_{22}O_2$ (M^+): 270.1619; found, 270.1603; Anal. Calcd for $C_{18}H_{22}O_2$: C, 79.56; H, 8.20. Found, C, 79.23; H, 8.15.

4.1.7. (1*S*,2*R*)-3-Propenyl-cyclohex-3-ene-1,2-diol. To a solution of diene diol **23** (1.35 g, 8.8 mmol, 1.0 equiv) in 20 mL MeOH at 0 °C was added portionwise potassium azodicarboxylate (5.16 g, 26.6 mmol, 3 equiv) over 10 min. The yellow slurry was stirred for 10 min before the addition of AcOH (3.55 mL, 61.6 mmol, 7 equiv) in 20 mL MeOH over a 1 h period. The reaction mixture was stirred overnight, warming slowly to rt over 12 h. The reaction mixture was quenched by addition of 12 mL saturated solution of sodium carbonate. Methanol was removed under vacuum and replaced with ethyl acetate. The layers were separated and the aqueous portion extracted three times with 15 mL ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Filtration of the drying agent and removal of the solvent under reduced pressure provided a solid, which was recrystallized to give 828 mg of the title compound as a tan solid (61%). Mp 107–108 °C (from methylene chloride-pentane); $[\alpha]_D^{24} = -133$ (*c* 0.5, $CHCl_3$); R_f 0.21 (hexanes–ethyl acetate, 1:1); IR (film) *v* 3260, 2945, 2871, 1633 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 5.93 (d, $J = 16$ Hz, 1H), 5.84 (m, 1H), 5.63 (t, $J = 3.9$ Hz, 1H), 4.30 (d, $J = 3.6$ Hz, 1H), 3.63 (m, 1H), 2.23 (br s, 2H), 2.15 (m, 2H), 1.72 (d, $J = 5.9$ Hz, 3H), 1.66 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.9, 131.5, 128.9, 123.7, 69.7, 65.9, 25.3, 25.1, 18.3; HRMS (EI) calcd for $C_9H_{14}O_2$ (M^+): 154.0993; found, 154.0994.

4.1.8. (3a*S*,7a*R*)-2,2-Dimethyl-4-propenyl-3a,4,5,7a-tetrahydro-benzo[1,3]dioxole 24. The diol derived from diimide reduction of diene diol **23** (828 mg, 5.37 mmol, 1 equiv) was transferred to a 50-mL round-bottomed flask and dissolved in 5 mL acetone. 2,2-Dimethoxy propane (1.243 mL, 10.1 mmol, 1.88 equiv) was added followed by 1 spatula tip of *p*-toluenesulfonic acid. The solution was stirred at rt for 2.5 h before the reaction was quenched with 10 mL of saturated sodium carbonate solution and the acetone removed under reduced pressure. The cloudy mixture was diluted with 15 mL ethyl acetate and 2 mL of distilled water. The layers were separated and the aqueous layer back-extracted with fresh 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 tan oil, which was purified by flash column chromatography (98:2 hexanes–ethyl acetate) to afford the title compound as a clear and colorless oil, 900 mg (86%): $[\alpha]_D^{24} = +68.1$ (*c* 0.72, $CHCl_3$); R_f 0.56 (10% ethyl acetate in hexanes); IR (neat) *v* 2984, 2932, 1451, 1367 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 5.96 (d, $J = 15.9$ Hz, 1H), 5.83 (m, 1H), 5.73 (t, $J = 4.2$ Hz, 1H), 4.58 (d, $J = 6.0$ Hz, 1H), 4.22 (m, 1H), 2.15 (m, 1H), 1.96 (m, 1H), 1.71 (d, $J = 6.3$ Hz, 3H), 1.69 (m, 2H), 1.42 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 134.3, 131.9, 129.3, 124.5, 108.3, 73.6, 71.4, 28.1, 26.3, 26.1, 21.6, 18.4; HRMS (EI) Calcd for $C_{12}H_{18}O_2$, 194.1306; found, 194.1304.

4.1.9. (3a*R*,7a*S*)-2,2-Dimethyl-4-(2-methylcyclopropyl)-3a,4,5,7a-tetrahydro-benzo[1,3]dioxole 25. To a solution of diethyl zinc (16.5 mL of 1.0 M solution in hexanes, 16.5 mmol, 4 equiv) in 16 mL anhydrous methylene chloride at 0 °C was added freshly distilled trifluoroacetic acid (0.65 mL, 8.25 mmol, 2 equiv) in 8 mL methylene chloride *very slowly* (ca. 20 min). The thick, white slurry was stirred at 0 °C for 20 min at which time diiodomethane (0.66 mL, 8.25 mmol, 2 equiv) in 8 mL was introduced to the reaction flask by cannulation. The resulting gray slurry was stirred for 20 min before addition of propenyl protected diol **24** (800 mg, 4.06 mmol, 1 equiv) dissolved in 8 mL methylene chloride. The reaction flask was removed from the ice bath and the slurry allowed to warm to rt over 30 min. Progress of the reaction was monitored by TLC (10% ethyl acetate in hexanes, $KMnO_4$ stain). When deemed complete, the reaction was quenched by the addition of 20 mL saturated solution of NH_4Cl and the layers separated. The aqueous layer was extracted with two portions of methylene chloride and the combined organic layers dried over anhydrous $MgSO_4$. Evaporation of the solvent under reduced pressure provided an oil, which was purified by flash column chromatography (10% ethyl acetate in hexanes) to afford the corresponding cyclopropane as a clear and colorless oil (567 mg, 67%). $[\alpha]_D^{24} = +64.3$ (*c* 0.4, $CHCl_3$); R_f 0.44 (hexanes–ethyl acetate, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ: 5.32 (m, 1H), 4.17 (m, 1H), 4.15 (m, 1H), 1.98 (s, 1H), 1.73 (m, 2H), 1.27 (m, 6H), 0.94 (m, 4H), 0.85 (2H), 0.42 (m, 1H), 0.21 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.1, 122.4, 108.2, 74.2, 73.6, 28.0, 26.5, 25.7, 23.1, 20.7, 18.9, 14.2, 13.4 ppm (single diastereomer).

4.1.10. (3a*R*,7a*S*)-2,2-Dimethyl-7-(2-methyl-cyclopropyl)-3a,4,5,7a-tetrahydrobenzo[1,3]dioxole 25 1:1 mixture of diastereoisomers. Diols **9a** and **9b** (40 mg, 0.24 mmol, 1 equiv) were transferred to a 10-mL round-bottomed flask and dissolved in 2 mL acetone. 2,2-Dimethoxy propane (250 μL, 2.4 mmol, 10 equiv) was added followed by 1 crystal *p*-toluenesulfonic acid. The solution was stirred at rt for 24 h before the reaction was quenched with 1 mL of saturated sodium bicarbonate solution. The cloudy mixture was diluted with 15 mL ethyl acetate and 2 mL of distilled water. The layers were separated and the aqueous layer back-extracted with fresh 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 tan oil (24 mg), which was purified by flash column chromatography (10% ethyl acetate in hexanes) to afford the title compound as a clear oil and inseparable mixture of diastereoisomers, 16 mg (23%): $[\alpha]_D^{22} = +47.5$ (*c* 0.4, $CHCl_3$); R_f 0.44 (10% ethyl acetate in hexanes); IR (film) *v* 3353, 2984, 2930, 1430, 1366 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ*: 5.43/5.37 (t, $J = 3.8$ Hz, 1H), 4.47 (m, 1H), 4.17 (m, 1H), 2.15–2.00 (m, 1H), 1.85–1.70 (m, 2H), 1.65–1.50 (m, 2H), 1.34 (d, $J = 6$ Hz, 1H), 1.03 (d, $J = 5.8$ Hz, 2H), 0.9–0.75 (m, 1H), 0.68 (m, 1H), 0.51 (dd, $J = 8.9$, 4.8 Hz, 1H), 0.30 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 137.5, 122.8, 122.5, 108.5, 74.8, 74.6, 73.9, 28.3, 26.9, 26.1, 23.5, 23.4, 21.1, 21.0, 19.6, 19.2, 15.2, 14.5, 13.8, 13.6; HRMS (EI) calcd for $C_{13}H_{20}O_2$, 208.1463; found, 208.1468.

4.1.11. (1*S*,2*R*)-3-Cyclopropyl-cyclohexa-3-ene-1,2-diol 26. From (1*R*,2*R*)-3-bromo-6-cyclopropyl-cyclohexa-3,5-diene-1,2-diol: Diol **7** (100 mg, 0.43 mmol, 1 equiv) was dissolved in 4 mL of MeOH and transferred to a thick-walled hydrogenation flask. Distilled triethylamine (62 μL, 0.43 mmol, 1 equiv) was added followed by PtO_2 (Adams catalyst, 19 mg, 0.087 mmol, 20 mol %). The flask was placed on a Parr hydrogenation apparatus, evacuated, and the headspace replaced with hydrogen gas (3 atm). The flask was allowed to shake at rt for a period of 1.5 h. The reaction mixture was filtered through Celite and washed with several portions of methanol, concentrated, and purified by flash column chromatography (hexanes–ethyl acetate, 3:2) and the solvent removed under reduced pressure to provide the title compound as an oily solid (27 mg, 41%). Spectral data are consistent with that of the same compound prepared by enzymatic oxidation from cyclopropyl benzene and subsequent diimide reduction.[5] R_f 0.27 (hexanes–ethyl acetate, 1:1); $[\alpha]_D^{26} = -33$ (*c* 1.0, MeOH), lit. $[\alpha]_D^{23} = -126$ (*c* 1.0, MeOH). 26% enantiomeric excess.

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