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Enzymatic oxidation of *para*-substituted arenes: access to new non-racemic chiral metabolites for synthesis

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ABSTRACT

A series of *para*-substituted benzene derivatives were subjected to whole-cell fermentation with *Escherichia coli* JM109 (pDTG601), an organism expressing toluene dioxygenase (TDO). Several compounds proved to be excellent substrates for TDO, including 4-bromo-phenylacetylene, 4-bromobenzaldehyde, 4-bromobenzyl alcohol and 4-bromo-allylbenzene. Some of the first *para*-functionalized diene diols produced using TDO, are useful substrates for further synthetic manipulations, including their use in the potential synthesis of complex natural products.

1. Introduction

Chiral cyclohexadiene-*cis*-diols derived from aromatic precursors through chemoenzymatic oxidation by mutants of the soil bacterium *Pseudomonas putida* and recombinant clones of *Escherichia coli* have proven to be very useful additions to the chiral pool.[1–6] In wild-type organisms, this family of enzymes allow the use of benzene, naphthalene and biphenyl compounds to be metabolized as an energy source. Gibson et al. reported the first bio-oxidative degradation of benzene and its simple derivatives in 1968[7,8] and then in 1970 reported the first example of a mutant, Pp39D, capable of blocking the further degradation of diols of type **1** (Scheme 1).[9] This result was first exploited by Ley who used *cis*-1,2-dihydroxycyclohexa-3,5-diene for the synthesis of pinitol.[10] Since this report, the bio-oxidation of arenes has provided access to many useful chiral building blocks with an accompanying interest regarding the substrate-tolerance of the enzyme. Known metabolites were comprehensively reviewed up to 2004 by Johnson et al.,[6,11–17] while recent reviews describe the range of synthetic targets that have been attained from *cis*-diene-diols.[1–6,18]

The published library of compatible mono-substituted and *ortho*-substituted substrates is considerable, presumably because their enzymatic dihydroxylation is almost completely enantioselective.[11,12] However, there have been very few *para*-substituted substrates published to date, and the majority of the metabolites do not contain useful functional groups at the 1- and 4-positions that can be easily manipulated. Notable exceptions include several dihalogenated metabolites, which have been used to access the *ent*-series of diene diols after downstream reduction of an iodine atom, as reported by Boyd et al. (Fig. 1).[19,20] These compounds have not been exploited for synthetic purposes, possibly because of low enantiotopic differentiation by the enzyme. One exception is the use of **4** by Hudlicky et al. in order to access the *ent*-series for 7-deoxypancratastatin synthesis;[21] however, the 1,4-substitution arising from the original functionality of the arene derivative was not conserved in the final product. Consequently, there remains a need to investigate the enzymatic tolerance for *para*-substituted benzenes. Herein we report the isolation of several new metabolites derived from their bio-oxidation.

2. Results and discussion

Diene diol metabolites provide ideal chiral starting materials for an approach towards tetrodotoxin (Fig. 2), a marine toxin first isolated in 1950,[22] which has gathered significant synthetic attention[23–34] since its structural elucidation in 1965.[35] We envisioned that 1,4-functionalized cyclohexadiene diols will allow access to the target in a very efficient manner. The greatest challenge in this approach towards the desired alkaloid was thought to be the installation of the two *syn*-carbon chains at opposite poles of the core cyclohexane (Fig. 2). If both carbon chains could be introduced prior to the enzymatic dihydroxylation, the target molecule could be readily accessed through simple elaboration of the two dienes. To this end, a series of 1,4-difunctionalized substrates were examined as potential substrates for the toluene dioxygenase enzyme expressed by *E. coli* JM109 (pDTG601A) a recombinant organism developed by Gibson (Table 1).[36]

Initial results supported the hypothesis that *para*-substituted benzene derivatives were poorer substrates than their better-studied *ortho*- or mono-substituted counterparts. The majority of the compounds were not converted by the endogenous enzymes into the desired diene diols. All three carboxylic acid derivatives **6**, **7** and **8** failed to provide the desired compound when fed to the cells

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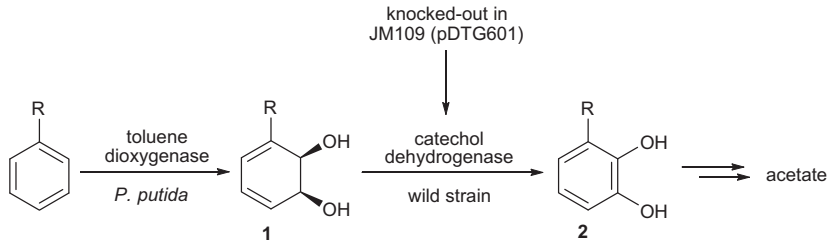


Scheme 1. Comparison of the degradation pathways of aromatics by wild type *P. putida* and the bioengineered strain.

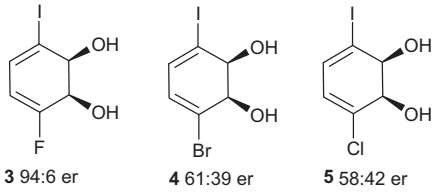


Figure 1. Previously generated 1,4-'difunctionalized' diene diols.

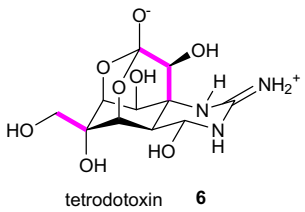


Figure 2. Tetrodotoxin with *syn* 1,4 carbon chains highlighted.

as either the free acid (dissolved in DMSO prior to addition to broth) or as the sodium salt; in all cases the substrates were recovered in high yields. This was somewhat surprising since **6** had been previously reported by Ribbons et al. in 1987 using *P. putida* JT107 to be an excellent substrate, with complete enantiotopic discrimination.[37,38] Even simpler structures provided unsatisfactory results. Benzyl bromide derivative **9** proved not to be a substrate and was recovered unchanged. Similar results were obtained for dioxolane **10** and acetate **11**. These substrates were chosen to serve as protected derivatives of the benzyl alcohol (a known toxin to the bacteria) and benzaldehyde derivatives, respectively, but neither provided any diene diol products; interestingly, dithiane **14** was completely digested in the fermentation, presumably through oxidation of the sulfur atoms as this is a generally recognized phenomenon.[39] However, when benzaldehyde derivative **12** or benzyl alcohol derivative **13** was examined, both provided the same diol, **27**, with similar yields, approximately 700 mg/L. This result was particularly satisfying as the protected derivatives, hypothesized to be more stable to the enzymatic conditions, failed to provide the product; the aldehyde was presumably reduced to the alcohol during the reaction through another process. However, as the enantiomeric ratios of the **27** differ based on whether the source material was the aldehyde **12** (60:40) or the benzyl alcohol **13** (78:22), it appears that this competing reduction occurs on the same time-scale as the dihydroxylation.

As a control of the fermentation methodology and bacterial strain, 4-bromo-iodobenzene **4** and 1,4-dibromo benzene **15** were treated under the fermentation conditions. Both provided their diene diols, 730 mg/L (65% yield, 60:40 er) and 920 mg/L (80% yield, *meso*), respectively. It is important to note that these were prepared only on a 1-L scale with 1 g of substrate; consequently these are excellent yields and conversions. The enantiomeric ratio determined for **4** was identical to that previously reported by Boyd.[19] This, however, is the first reported isolation of *meso* diene diol **28**. The diol had been previously prepared by Hudlicky et al. in 2006 in low yield during the study dealing with the dihydroxylation of all three isomeric dibromobenzenes and the synthesis of (−)-conduritol E and consequently was not fully characterized at the time.[40]

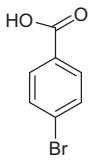
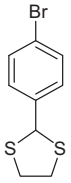
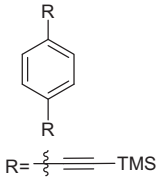
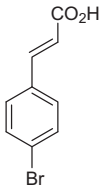
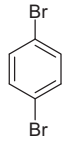
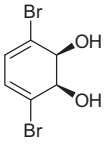
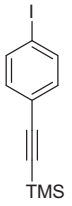
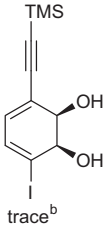
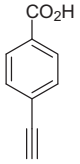
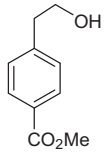
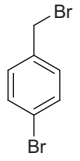
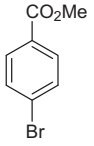
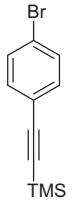
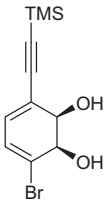
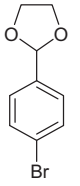
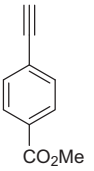
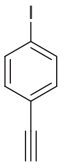
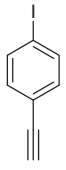
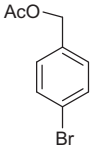
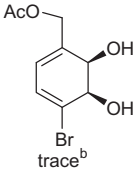
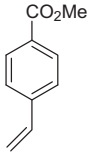
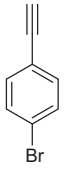
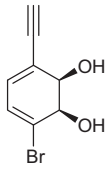
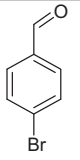
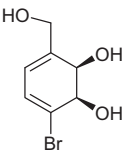
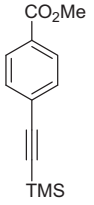
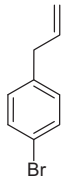
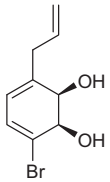
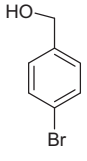
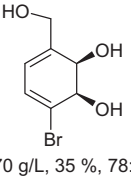
The ideal substrate for our current purposes, **16**, was investigated along with a series of related methyl esters, **17**, **18**, **19** and **20**. All of these esters failed to provide any detectable metabolites, and the starting material was recovered from the fermentation broth. Esters have proven to be less than ideal substrates in the past. Previous reports from our laboratory have examined the substrate scope of this enzyme using a variety of esters. Methyl benzoate provides its analogous diene diol in 1.3 g/L (on 9-L scale), considerably less than the 20–22 g/L routinely achieved with bromobenzene. As the steric bulk of the ester increases, the yield rapidly decreases; thus butyl benzoate is not a suitable substrate for this enzyme.[41] The introduction of an *ortho* halogen group has been shown to be slightly tolerated: 2-bromomethyl benzoate provides the diene diol in 200 mg/L;[42] however it appears that the introduction of a *para* substituent completely destroys the enzyme's tolerance of the substrate, as opposed to the better tolerated *ortho* substitution. Consequently, it appears that adding the steric concerns of *para* substitution to the poor enzyme tolerance of esters produces a compound incompatible with the TDO enzymatic pocket. To circumvent this problem, a series of *para*-bromo- and iodophenylacetylenes were prepared, as small alkyl substituents are well precedented as good substrates. Symmetrical *bis*-trimethylsilylprotected diacetylene **21** was not metabolized and hence was quantitatively recovered from the fermentation. However, both the iodo-TMS acetylene derivative **22** and the bromo analogue **23** produced small amounts of metabolites along with the recovered starting material. *para*-Iodophenyl acetylene **24**, provided only trace amounts of the product, while bromo-substituted **25** furnished a respectable 640 g/L (30% yield) of diene diol **29** (in a 95:5 enantiomeric ratio) after extraction of the fermentation mixture. Due to the successful conversion of the acetylene compound, the allyl derivative **26** was also prepared. It furnished, upon fermentation, diene diol **30** in 850 g/L (40% yield, 82:18 enantiomeric ratio).

In all cases, the enantiomeric ratios were determined through chiral HPLC of the diene diols. Their conversion to known compounds demonstrated their absolute stereochemistry. The derivatizations of the diene diols were carried out for the determination of the absolute stereochemistry and are shown in Scheme 2.

Alkynyl diol **29** was protected as acetonide **31** and then reduced with Adam's catalyst to the fully saturated cyclohexane **32**. Although this compound has been previously reported by Gibson et al.,[43] they provided no characterization data for it at the time; consequently, enantiopure acetonide diol **33**, derived from ethyl benzene, was treated in a similar fashion to provide an enantiopure sample of **32**.[44,45]

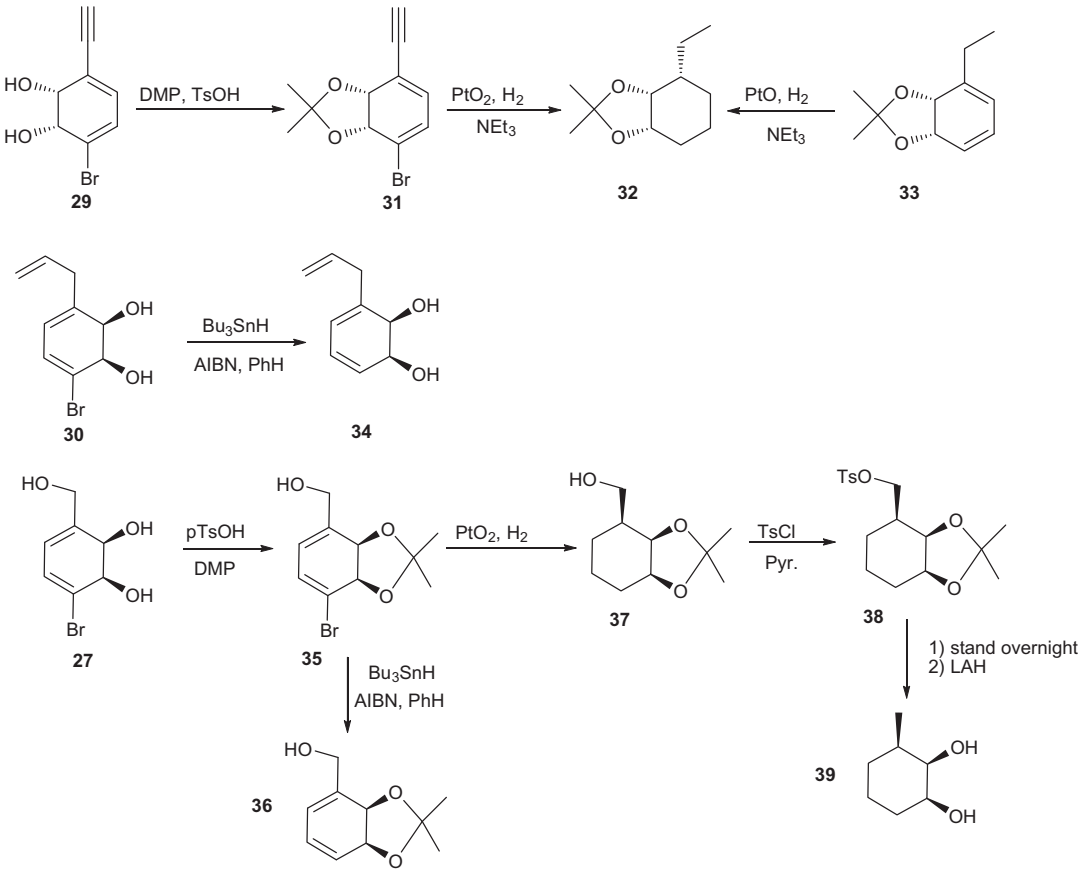
Allyl diene diol **30** was dehalogenated under standard radical conditions to provide diol **34**, whose properties were compared

Table 1
Results of the whole-cell fermentation of potential metabolites using *E. coli* JM 109 (pDTG601)

Substrate	Product (g/L; % yield, er)	Substrate	Product (g/L; % yield, er)	Substrate	Product (g/L; % yield, er)
6 	—[a,c]	**14** 	—[d]	**21** 	—[a]
7 	—[a,c]	**15** 	**28**  1.5 g/L, 80 %, *meso*	**22** 	 trace[b]
8 	—[a,c]	**16** 	—[a]		
9 	—[a]	**17** 	—[a]	**23** 	 trace[b]
10 	—[a]	**18** 	—[a]	**24** 	 trace[b]
11 	 trace[b]	**19** 	—[a]	**25** 	**29**  0.64 g/L, 30 %, 95:5
12 	**27**  0.68 g/L, 34 %, 60:40	**20** 	—[a]	**26** 	**30**  0.85 g/L, 40 %, 81.5:18.5
13 	**27**  0.70 g/L, 35 %, 78:22				

[a] Unreacted arene recovered.
[b] Trace diene diol observed by crude NMR of extract, deemed insufficient for isolation. Stereochemistry assigned by analogy to similar compound.
[c] Substrate tested as both carboxylic acid and sodium salt.
[d] No unreacted arene or other product observed in crude NMR of extract.

to those of a previously published standard.[45] Diene diol **27** derived from benzyl alcohol could not be successfully dehalogenated but its acetonide derivative was successfully dehalogenated in low yield to provide **36**, whose enantiomer is known.[46] Alternatively, Adams' reduction provided cyclohexane **37**, which was tosylated to the activated **38,** which could be chromatographed. This



Scheme 2. Derivatization of metabolites to known compounds for determination of absolute stereochemistry.

compound decomposed readily (in under 12 h) when left under argon at 4 °C, presumably through elimination of the tosylate and then acid-catalysed loss of the acetonide. Treatment of the decomposition residue with lithium aluminum hydride provided the known diol **39** as the major product.[47]

None of the three new diols obtained in good yield could be isolated with complete selectivity, although alkynyl derivative **29** was obtained in a 95:5 enantiomeric ratio, which is consistent with the size difference between the larger alkyne and smaller bromine atom as predicted by Boyd.[19] Allyl derivative **30** is not generated with higher selectivity, although the allyl group is still considered 'larger' by the enzyme than the bromine substituent, and the enantiomeric ratio drops to 82:18. This discrepancy could be related to the differences in the geometry of the linear alkyne and the more flexible allyl chain. As aforementioned, benzyl alcohol has not been reported on as a substrate, possibly because of its inherent toxicity towards *E. coli* (when benzyl alcohol was subjected to fermentation conditions, only trace conversion was noted).[48,49] However, the theoretical product of this fermentation, diene-diol **36**, can be readily generated from either *para*-bromobenzyl alcohol or *para*-bromobenzaldehyde in acceptable yields. Both substrates are better tolerated by the enzyme than methyl benzoate, an alternative source of **36**. The enantioselectivity of the two processes varies greatly. The benzyl alcohol derivative provides sufficient bias to allow for the formation of the product in a 78:22 enantiomeric ratio. However, the benzaldehyde derivative provides almost no enantiotopic bias, as diol **27** is generated in a near-racemic 60:40 enantiomeric ratio. The size difference between the two substrates is not large, the difference in hydrogen-bonding properties is maybe responsible for the observed difference in enantioselectivity.

3. Conclusion

We have identified several *para*-substituted arenes suitable as substrates for toluene dioxygenase. The flexibility in the choice of the substituents carries the potential for further elaboration to readily incorporate carbon chains at the 1- and 4-positions of the resulting cyclohexadiene ring. Although the enantioselectivity of these transformations has been found to not be extremely high in most cases, it could be significantly improved upon by investigating substrates containing different halogen atoms, as based on recent precedents.[19,42] Studies to this effect are currently underway. Consequently, second-generation substrates of this family could provide useful chiral starting materials to access complex natural products containing this chiral *cis*-diol motif, such as tetrodotoxin, (+)-tutin,[50] forskolin D,[51] picrotoxin and picrotoxinin[52] and nimbin,[53] among many others. The formal synthesis of tetrodotoxin based on this technology will be reported in due course.

4. Experimental

4.1. General

All non-hydrogenation reactions were carried out under an argon atmosphere, hydrogenation reactions were carried out under a hydrogen balloon. Glassware used for moisture-sensitive reactions was flame-dried under vacuum and subsequently purged with argon. THF, DME and toluene were distilled from potassium/benzophenone. Methylene chloride and acetonitrile were distilled from calcium hydride. Flash column chromatography was performed using Kieselgel 60 (230–400 mesh) pre-neutralized with 2% triethylamine. Analytical thin-layer chromatography was

performed using silica gel 60-F_{254} plates. Melting points are reported uncorrected. IR spectra were recorded as neat samples or in KBr pellets. ^{1}H and ^{13}C NMR spectra were obtained on either a 300 or 600 MHz instrument. Data are reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants(s) in Hz, integration. Specific rotation measurements are given in deg cm^3 g^{-1} dm^{-1}. Mass spectra and high resolution mass spectra were performed by the analytical division at Brock University. Chiral HPLC was carried out on an Agilent 1100 series instrument equipped with a UV detector monitoring at 254 nm and an ODH chiral column. HPLC flow-rate was 0.5 mL/min using a gradient from 95:5 hexane/*iso*-propanol to 5:95 hexane/*iso*-propanol over 20 min (Condition A); or 90:10 hexane:*iso*-propanol to 60:40 hexanes/*iso*-propanol over 20 min (Condition B).

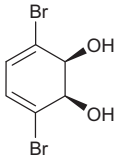
4.2. Small scale fermentation

In two 1 L fermentation cultures of grown *E. coli* JM109 (pDTG601) cells was added 500 mg of substrate [for the detailed procedure see the literature[42,54]]. The fermentation cultures were shaken at 37 °C for the reaction period. After this time, the cells were separated from the broth by centrifugation at 7000 rpm for 20 min. The cell-free broth was extracted three times with a total of 2.4 L of base-washed ethyl acetate. Evaporation of the ethyl acetate afforded the diene diol that could be further purified using column chromatography.

4.3. Large scale fermentation

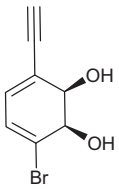
Protocol carried out as previously described in a 9 L fermenter.[42,54]

4.3.1. 3,6-Dibromocyclohexa-3,5-diene-1,2-diol 28



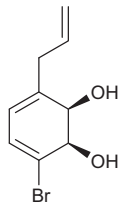
This compound was previously prepared by Hudlicky et al. in 2006.[40] 1,4-Dibromobenzene was shaken overnight with mature cells of *E. coli* JM109 (pDTG601A) under the general protocol (small scale) described above. The crude white solid was recrystallized from ether to provide 1.6 g of white needles (1.6 g/L). White needles. R_f = 0.3 (4:1 ethyl acetate:hexanes); mp = 131–133 °C ($CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/MeOD): δ_{ppm} 6.12 (s, 2H), 4.30 (s, 2H), 3.52 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$/MeOD): δ_{ppm} 129.2, 125.7, 72.6; IR ν_{max}: (KBr) cm^{-1}. HRMS (EI) Calcd for $C_{15}H_{20}O_2$ (M^+): 232.1463. Found 232.1465.

4.3.2. (1*R*,2*R*)-3-Bromo-6-ethynylcyclohexa-3,5-diene-1,2-diol 29



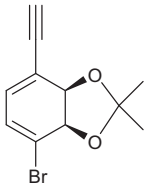
p-Bromoethynylbenzene[55] was fermented according to standard procedures in an 18 L fermenter. Following standard work-up and recovery of the unreacted material, the supernatant was extracted three times with ethyl acetate. The combined organics were then washed with brine and concentrated to provide 15.5 g of crude material which was determined to be 75% pure diol. The crude was purified by column chromatography (3:1 hexanes:ethyl acetate) to provide 5.75 g of pure product as an off-white solid (0.64 g/L). Off-white solid. R_f = 0.31 (3:1 hexanes:ethyl acetate); mp = 118–120 °C (MeOH/Et_2O); $[\alpha]_D^{20} = +34.6$ (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 6.28 (d, *J* = 6.2 Hz, 1H), 6.05 (d, *J* = 6.1 Hz, 1H), 4.22 (d, *J* = 5.9 Hz, 1H), 4.15 (d, *J* = 6.0 Hz, 1H), 3.24 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_{ppm} 129.8, 127.8, 126.4, 122.1, 82.7, 81.6, 71.8, 66.8; IR ν_{max}: (KBr) 3734, 3288, 3200, 2839, 1557, 1414, 1304, 1102, 1085, 1014, 844 cm^{-1}; HRMS (EI) Calcd for $C_8H_7BrO_2$ (M^+): 213.9629. Found: 213.9627; MS (EI) 216 (49.6), 214 (53.9), 198 (42.9), 196 (43.9), 145 (17.8), 143 (18.4), 135 (52.2), 117 (22.6), 118 (16.7), 89 (83.9), 77 (100); HPLC Condition A as described above (minor enantiomer rt = 5.4 min, major enantiomer rt = 10.2 min).

4.3.3. (1*R*,2*R*)-3-Allyl-6-bromocyclohexa-3,5-diene-1,2-diol 30



p-Bromoallylbenzene was fermented according to standard procedures in an 18 L fermenter. Following standard work-up the supernatant was extracted three times with ethyl acetate and concentrated and purified by column chromatography (3:1 hexanes:ethyl acetate) to provide 7.66 g of pure product as an off-white solid (0.85 g/L). Off-white amorphous powder. R_f = 0.37 (1:1 hexanes:ethyl acetate); mp = 128–130 °C (MeOH/Et_2O); $[\alpha]_D^{20} = +13.6$ (*c* 0.86, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 6.33 (d, *J* = 6.07 Hz, 1H), 5.85 (tdd, *J* = 16.89, 10.10, 6.71, 6.71 Hz, 1H), 5.61 (td, *J* = 5.97, 1.39, 1.39 Hz, 1H), 5.18–5.09 (m, 2H), 4.32 (br s, 2H), 2.97 (ddd, *J* = 6.47, 2.62, 1.49 Hz, 2H), 2.51 (s, 1H), 2.31 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_{ppm} 140.0, 135.0, 127.1, 123.9, 119.7, 117.3, 72.8, 71.0, 37.3. IR ν_{max}: (MeOH) 3429, 2095, 1642, 1424, 1286, 1097, 1079, 1049, 1018, 913, 838 cm^{-1}. HRMS (EI) Calcd for $C_9H_{11}BrO_2$ (M^+): 229.9942. Found: 229.9945. HPLC Condition A as described above (minor enantiomer rt = 4.1 min, major enantiomer rt = 7.0 min).

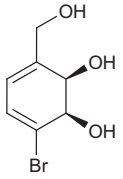
4.3.4. (1*R*,2*R*)-3-Bromo-6-ethynyl-[1,2]-isopropylidenedioxycyclohexa-3,5-diene 31



Diol **29** (300 mg, 1.4 mmol) was dissolved in acetone (10 mL) and 2,2-dimethoxypropane (1 mL) along with catalytic *p*TsOH (10 mg). The reaction mixture was stirred for 4 h, neutralized with triethylamine (1 mL) and concentrated under reduced pressure. The crude mixture was directly purified by flash chromatography (4:1 hexanes:ethyl acetate, 0.5% triethylamine) to provide 278 mg of the acetonide as a white solid. White powder. R_f = 0.32 (9:1 hexanes:ethyl acetate); mp = 78–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 6.39 (d, *J* = 6.5 Hz, 1H), 6.21 (d, *J* = 6.5 Hz, 1H), 4.77 (d, *J* = 8.4 Hz, 1H), 4.67 (d, *J* = 8.4 Hz, 1H), 3.28 (s, 1H), 1.45 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_{ppm} 135.8, 125.5,

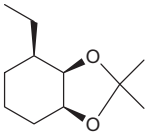
122.9, 119.0, 106.6, 76.1, 72.9, 63.2, 26.5, 24.9; IR ν_{max}: ($CHCl_3$) 3288, 2934, 1626, 1557, 1415, 1305, 1085, 1014, 896, 844, 778 cm^{-1}. HRMS (EI) Calcd for $C_{11}H_{11}BrO_2$ (M^+): 253.9942. Found: 253.9938.

4.3.5. (1*R*,2*R*)-3-Bromo-6-(hydroxymethyl)cyclohexa-3,5-diene-1,2-diol 27



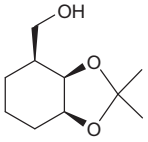
p-Bromobenzyl alcohol was fermented according to standard procedures in an 18 L fermenter. Following standard work-up the supernatant was extracted three times with ethyl acetate and concentrated and purified by recrystallization from diethyl ether to provide 6.12 g of pure product as a white solid (0.68 g/L). White powder. $[\alpha]_D^{20} = -8.3$ (*c* 1.0, MeOH, 7:3 er); ^1H NMR (300 MHz, $CDCl_3$): δ_{ppm} 6.38 (d, *J* = 6.0 Hz, 1H), 5.80 (ddd, *J* = 6.1, 3.0, 1.6 Hz, 1H), 4.38 (dd, *J* = 6.0, 0.8 Hz, 1H), 4.24–4.14 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_{ppm} 142.3, 127.9, 126.5, 119.2, 74.1, 71.2, 63.0; IR ν_{max}: ($CHCl_3$) 3337, 2920, 2861, 1648, 1579, 1388, 1333, 1096, 1014, 842 cm^{-1}; HRMS (EI) Calcd for $C_7H_9BrO_3$ (M^+): 219.9735. Found: 219.9731; HPLC Condition B as described above (minor enantiomer rt = 11.4 min, major enantiomer rt = 10.0 min).

4.3.6. (1*S*,2*R*,3*R*)-3-Ethyl-[1,2]-isopropylidenedioxycyclohexane 32



Crude (1*S*,2*R*)-3-ethyl-[1,2]-isopropylidenedioxycyclohexa-3,5-diene (98 mg, 0.54 mmol) was dissolved in 2 mL of methanol and 500 μL of triethylamine and the solvent was degassed. Adams' catalyst (10 mg) was then added, and the flask was flushed with hydrogen gas; a balloon was charged with hydrogen and the reaction mixture was stirred for 16 h under the hydrogen atmosphere. The reaction mixture was then filtered through a Celite pad along with diethyl ether and the solvent was removed under reduced pressure. The crude mixture was then purified through flash chromatography (9:1 hexanes:ethyl acetate) to provide 46 mg of a clear oil (46% yield). Clear oil. R_f = 0.44 (9:1 hexanes:ethyl acetate); bp = 221–223 °C (760 mm Hg); $[\alpha]_D^{20} = +10.9$ (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ_{ppm} 4.13 (dd, *J* = 5.0, 1.9 Hz, 1H), 4.02 (td, *J* = 8.9, 5.5, 5.5 Hz, 1H), 1.80–1.58 (m, 2H), 1.47 (s, 6H), 1.54–1.38 (m, 1H), 0.94, 1.25–1.08 (m, 3H), (t, *J* = 7.1, 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_{ppm} 107.3, 75.1, 74.6, 39.0, 28.9, 28.0, 25.8, 25.5, 25.3, 21.0, 11.4; IR ν_{max}: ($CHCl_3$) 3752, 3155, 293, 2254, 1816, 1793, 1717, 1643, 1465, 1381, 1096, 905, 717 cm^{-1}; HRMS (EI) Calcd for $C_{11}H_{20}O_2$ (M^+-CH_3): 169.1229. Found 169.1232.

4.3.7. (1*S*,2*R*,3*S*)-3-Hydroxymethyl-[1,2]-isopropylidenedioxy-cyclohexane 37



To a degassed solution of acetonide-protected diene diol **35** (800 mg, 3.07 mmol) in methanol (16.0 mL) and triethylamine (3.2 mL) was added Adams' catalyst (PtO_2, 80 mg) and the atmosphere was evacuated and replaced with hydrogen. The reaction mixture was stirred for 12 h at ambient temperature under a hydrogen atmosphere, and then filtered through Celite along with methanol. The solvent was removed under reduced pressure and the residue was purified by flash chromatography to provide 320 mg of the product as a clear oil in 57% yield. Clear oil. R_f = 0.48 (1:1 hexanes:ethyl acetate); $[\alpha]_D^{20} = -6.1$ (*c* 2.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ_{ppm} 4.33 (dd, *J* = 5.3, 3.5 Hz, 1H), 4.16 (td, *J* = 9.0, 5.5, 5.5 Hz, 1H), 3.83 (dd, *J* = 11.0, 4.3 Hz, 1H), 3.73 (dd, *J* = 11.0, 5.8 Hz, 1H), 1.89–1.72 (m, 3H), 1.54 (s, 3H), 1.58–1.45 (m, 3H), 1.39 (s, 3H) 1.35–1.27 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_{ppm} 108.0, 74.7, 74.5, 60.2, 39.3, 28.4, 27.9, 25.9, 21.5, 20.2; IR ν_{max}: ($CHCl_3$) 3691, 3626, 3011, 2940, 2870, 1732, 1451, 1382, 1372, 1235, 1039, 863 cm^{-1}; HRMS (EI) Calcd for $C_9H_{15}O_3$ (M^+-CH_3): 171.1021. Found: 171.1036.

Acknowledgments

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