Enantioselective toluene dioxygenase catalysed di- and tri-hydroxylation of monosubstituted benzenes

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Asymmetric *cis*-dihydroxylation to yield diols **2A**–**2G** and sequential benzylic monohydroxylation–*cis*-dihydroxylation to yield triols **4A**–**4G** (trihydroxylation), occurred during biotransformation of a series of monosubstituted alkylbenzene substrates **1A**–**1G** using toluene dioxygenase, a biocatalyst present in *Pseudomonas putida* UV4. Dioxygenase-catalysed *cis*-dihydroxylation of the *R* and *S* benzylic alcohol enantiomers **3B**–**3D**, **3B′**–**3D′** gave the corresponding enantiopure triols **4B**–**4D**, **4B′**–**4D′**. Biotransformation of substrates **1J**–**1L** yielded *cis*-diols **2J**–**2L** and a minor triol metabolite **4A**. Benzylic alcohols **3J**–**3L** were postulated as unstable intermediates yielding triol **4A** *via* benzaldehyde **5** and benzyl alcohol **3A** intermediates. *cis*-Dihydroxylation of monosubstituted benzylic substrates containing bulky groups (**1H**, **1I**) or 1,4-dialkyl-substituted benzene substrates (**10A**–**10C**) gave the corresponding *cis*-dihydrodiol metabolites (**2H**, **2I**, **11A**–**11C**) exclusively. The *cis*-diols **2A**–**2L**, **11A**–**11C** and triols **4A**–**4F**, **4B′**–**4D′** were stereochemically assigned as single enantiomers of 1*S*,2*R*-configuration based on NMR and CD spectroscopy. The absolute configurations of the exocylic chiral centres in the triol bioproducts **4A**–**4F**, **4B′**–**4D′** were established by stereochemical correlation and aromatisation/hydrogenation to yield the corresponding enantiopure phenolic benzylic alcohols having similar CD spectra.

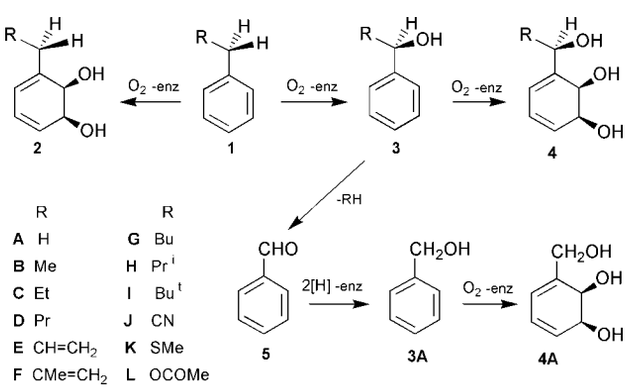
Introduction

Bacterial dioxygenase enzymes (Rieske oxygenases)[1] have been widely reported to catalyse the introduction of either two oxygen atoms (*cis*-dihydrodiol formation)[2–7] or a single oxygen atom (benzylic hydroxylation[8–12] or sulfoxidation[13–15]) to substrates containing a benzene ring. The formation of *cis*-dihydrodiol metabolites of arenes in particular has been studied using mutant strains of bacteria (mainly *Pseudomonads*) and to date in excess of three hundred examples have been reported.[6,7]

Recent studies have shown that dioxygenase-catalysed benzylic monohydroxylation is relatively common in bicyclic substrates; more than thirty examples have been reported.[5,12] Although dioxygenase-catalysed trihydroxylation of bicyclic substrates, *e.g.* benzocycloalkanes[12,16] and dihydroarenes,[17] has been reported or postulated, very few examples of benzylic monohydroxylation of monosubstituted benzene substrates or sequential mono-hydroxylation followed by *cis*-dihydroxylation (trihydroxylation) have been found.[6,18,19] This study provides evidence of benzylic monohydroxylation, ring dihydroxylation, and trihydroxylation occurring in substituted benzene substrates to yield a new range of potentially valuable enantiopure bioproducts containing two or three chiral centres. The factors determining the dioxygenase-catalysed regioselectivity and stereoselectivity during oxidation of alkyl-substituted benzene substrates, which may allow the metabolic sequence to be predicted, are also discussed.

Results and discussion

Initial studies of monosubstituted alkylbenzenes, containing at least two benzylic hydrogen atoms as substrates, were carried out using a mutant strain (UV4) of *Pseudomonas putida* which contains toluene dioxygenase (TDO) but lacks the *cis*-dihydrodiol dehydrogenase enzyme responsible for the catalytic conversion of *cis*-dihydrodiols to catechols. Substrates **1A**–**1I** were found to yield the expected *cis*-dihydrodiols **2A**–**2I** in variable yields (9–60%) as the less polar metabolites (R_f 0.3–0.4, 5% MeOH in $CHCl_3$) (Scheme 1). The structures of metabolites



Scheme 1

2A–**2L** were deduced from ^{1}H NMR, MS, CD and microanalytical data. The enantiopurity values of *cis*-dihydrodiols **2A**–**2L** were mainly determined by the formation of the (*R*)- and (*S*)-bis-[α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA)] esters of the 4-phenyl-1,2,4-triazoline-3,5-dione cycloadducts[20] (method A) or the diastereoisomeric boronate esters using (*R*)- and (*S*)-2-(1-methoxyethyl)benzeneboronic acid (MEBBA, method B)[21] having the structures shown in Scheme 2. *cis*-Dihydrodiols **2A**, **2B** and **2D** were found to be of >98% enantiomeric excess (ee) and of the (1*S*,2*R*) configuration.[20] *cis*-Dihydrodiols **2A**–**2L** all proved to be single enantiomers having the same (1*S*,2*R*) configuration from chiral stationary-phase HPLC analysis, comparison of their CD spectra, and from the NMR spectra of their MTPA cycloadducts and MEBBA derivatives as reported in the Experimental section.

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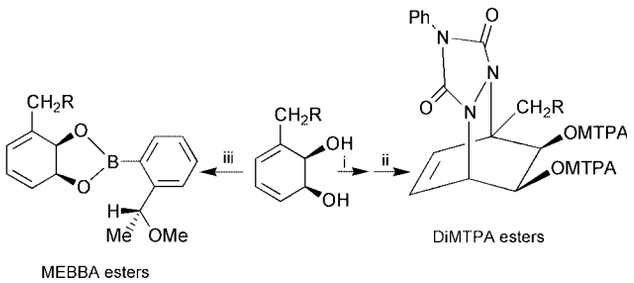
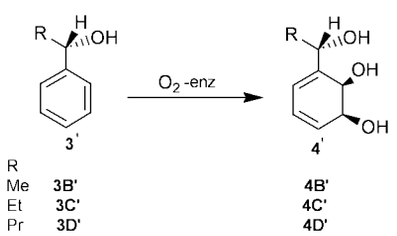


Scheme 2 *Reagents*: i 4-Phenyl-1,2,4-triazoline-3,5-dione; ii (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride; iii (*R*)- or (*S*)-2-(1-methoxyethyl)benzeneboronic acid.

The anticipated *cis*-dihydrodiol metabolites **2A**–**2F** were accompanied by more polar metabolites (R_f 0.09–0.12, 5% MeOH in $CHCl_3$) which were identified as the corresponding triols **4A**–**4F** from ^{1}H NMR, MS, and CD data (see Experimental section). The triol metabolites were isolated in variable yields, *i.e.* **4A** (≈4%) and **4B** (≈5%), **4C** (26%), **4D** (9%), **4E** (15%), **4F** (18%) and triol **4G** only being detected by GC/MS analysis (≪1%). Careful examination of the ^{1}H NMR spectra of triols **4B**–**4F** indicated that all were diastereoisomerically homogeneous except for triol **4F** where a minor proportion of the other diastereoisomer **4F′** (≈5%) was separated by multiple elution preparative TLC (PLC) on silica gel (5% MeOH in CH_2Cl_2). Triols **4C** and **4F/4F′** were found to be more abundant than the corresponding *cis*-diol metabolites **2C** and **2F**. Except for triol **4B**, which was reported as a minor metabolite (≈5% of total metabolites isolated) from ethylbenzene **1B**,[18] none of the other triol metabolites, to our knowledge, has been previously observed.

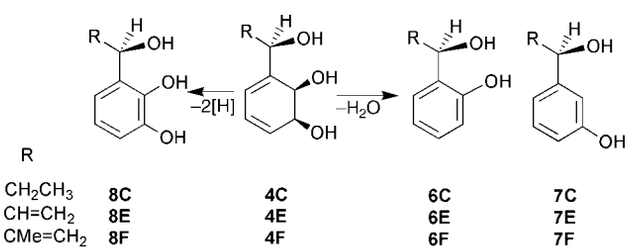
The formation of triols **4B**–**4E** as single diastereoisomers of >98% ee (methods A and B) is consistent with a biotransformation sequence involving the initial formation of benzylic alcohols **3B**–**3E** mainly as the *R* enantiomers followed by *cis*-dihydroxylation. In order to test this metabolic pathway and to establish the absolute configurations at the exocyclic chiral centres of the triols, the individual benzylic alcohol enantiomers (*R*)-(+)-**3B**, (*S*)-(−)-**3B′**, (*R*)-(+)-**3C**, (*S*)-(−)-**3C′**, (*R*)-(+)-**3D** and (*S*)-(−)-**3D′** were added as substrates (Scheme 3).



Scheme 3

The latter benzylic alcohols proved to be either equally reactive or better substrates than the corresponding alkylbenzenes; yields of the triol metabolites depended on the nature of the substituent on the benzene ring. The samples of triols **4B**, **4C** and **4D**, obtained from biotransformation of either the alkylbenzene **1B**, **1C** and **1D** or the benzyl alcohol **3B**, **3C** and **3D**, were found to be structurally and stereochemically indistinguishable. A similar result was reported by Gibson *et al.*[18] when benzylic alcohols **3B** and **3B′** were added as substrates to *P. putida* 39/D and triols **4B** and **4B′** were isolated. Although the benzylic alcohols **3A**–**3F** were generally not detected as intermediate metabolites from the corresponding alkylbenzene substrates **1A**–**1F**, it was assumed that stereoselective benzylic hydroxylation had occurred to yield mainly alcohols **3B**–**3F** with an excess of the *R* configuration. Support for this assumption was obtained during biotransformation of ethylbenzene **1B**; the benzyl alcohol intermediate **3B** was isolated as a minor metabolite with an 80% excess of the *R* configuration. Since the corresponding triol metabolite **4B** was enantiopure, some degree of kinetic resolution appears to have occurred during the subsequent *cis*-dihydroxylation process. This was supported by the observation that when individual enantiomers of the benzylic alcohol **3B** were added as substrates the yield of the resulting triol **4B** obtained from the *R* enantiomer was found to be higher than for **4B′**. Additional evidence for the metabolic sequence: alkylbenzene **1** ⟶ benzyl alcohol **3** ⟶ triol **4**, rather than the sequence alkylbenzene **1** ⟶ *cis*-dihydrodiol **2** ⟶ triol **4**, was obtained when the *cis*-dihydrodiols **2A**–**2E** were added as potential substrates and no triols were detected. Since the CD spectra of the triols **4B**–**4D**, formed either by biotransformation of the corresponding alkylbenzenes or by oxidation of the benzylic alcohols **3B**, **3B′**, **3C**, **3C′**, **3D**, **3D′**, were very similar to those of the *cis*-diols **2A**–**2I**, it was concluded that the absolute configurations at the endocyclic chiral centres were identical (1*S*,2*R*).

The formation of triols **4B**, **4B′**, **4C**, **4C′**, **4D** and **4D′**, from the corresponding alcohol precursors **3B**, **3B′**, **3C**, **3C′**, **3D** and **3D′** of known configuration, allowed the absolute stereochemistry at all three chiral centres to be unequivocally established. Since the allylic alcohol single enantiomers **3E** and **3F** were unavailable as substrates, it was not possible to determine the configuration of the benzylic chiral centre in the corresponding triols **4E** and **4F/4F′** by this method. Aromatisation of the triols **4C**, **4E** and **4F/4F′** by acid-catalysed dehydration (based on earlier work with *cis*-dihydrodiols[22]) gave the corresponding phenols **6C/7C**, **6E/7E**, **6F/7F** and *via* *cis*-diol dehydrogenase-catalysed dehydrogenation yielded the corresponding catechols **8C**, **8E** and **8F** (Scheme 4). Phenols **6E/7E**,



Scheme 4

6F/7F, catechols **8E**, **8F** and their derivatives, obtained after hydrogenation of the vinyl group, were found to have CD spectra very similar to those of phenols **6C/7C** and catechol **8C** of *R* configurations. Further studies on the formation of phenols from a wider range of *cis*-dihydrodiols and -triols including those mentioned above are in progress and the results will be presented elsewhere.

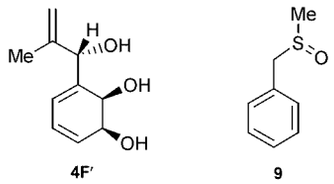
The benzylic hydroxylation of a propyl group in preference to a methyl, ethyl, or butyl group among substrates **1A**–**1D** may result from an optimal fit at the active site of the TDO since propylbenzene **1C** can adopt a conformation very similar in shape to that of indane. Mono- and bis-benzylic hydroxylation of indane and 2-substituted indanes were found to be particularly favoured biotransformation pathways using *P. putida* UV4.[12] Benzylic hydroxylation of alkylbenzene substrates containing larger groups, *e.g.* isobutyl **1H** and neopentyl **1I**, did not proceed, possibly due to steric inhibition.

Monohydroxylation of the allylbenzene substrates **1E** and **1F**, followed by *cis*-dihydroxylation, appears to be a result of the double activation (benzylic and allylic) of the methylene group. The presence of electron-withdrawing groups or heteroatoms adjacent to the benzylic position, *e.g.* the nitrile group in benzyl cyanide **1J**, the sulfur atom in methyl benzyl sulfide **1K** and the oxygen atom in phenyl acetate **1L**, was also expected to produce a similar combined activating effect facilitating benzylic hydroxylation, *i.e.* formation of alcohol intermediates **3J**, **3K** and **3L**.

Addition of benzyl cyanide, **1J**, as substrate to *P. putida* UV4 yielded the expected (1*S*,2*R*)-*cis*-dihydrodiol **2J** as the major metabolite. However, a time–course study of this biotransformation revealed that the *cis*-diol **2J** was also accompanied by a more polar metabolite which appeared to be a triol. Structural and stereochemical analysis showed that this triol was the (1*S*,2*R*)-enantiomer of compound **4A** and was indistinguishable from the product obtained when benzyl alcohol **3A** was used as substrate. The remote possibility that biotransformation involved the direct substitution of the nitrile group with a hydroxy group was eliminated by a series of further biotransformations. A metabolic sequence which could account for the formation of triol **4A** is shown in Scheme 1. Dioxygenase-catalysed benzylic hydroxylation to yield mandelonitrile **3J** as the initial step, spontaneous loss of hydrogen cyanide to yield benzaldehyde **5**, enzyme-catalysed reduction to form benzyl alcohol **3A**, and finally dioxygenase-catalysed *cis*-hydroxylation is postulated as the preferred pathway for the formation of triol **4A**. Support for this metabolic scheme was obtained when racemic mandelonitrile **3J** was separately added as a substrate to *P. putida* UV4 in a time–course experiment and both benzyl alcohol **3A** and triol **4A** were isolated as metabolites. The decomposition of mandelonitrile **3J** appeared to be spontaneous rather than enzyme-catalysed, since residual mandelonitrile analysed, by the MTPA method during the biotransformation, was found to be racemic. Furthermore, mandelonitrile **3J** was totally converted to benzaldehyde **5** after being shaken at ambient temperature (16 h) in a flask containing *P. putida* UV4 which had earlier been autoclaved to destroy enzyme activity. Similarly, when benzaldehyde **5** and benzyl alcohol **3A** were added separately to *P. putida* UV4 both yielded the triol **4A** which was structurally and stereochemically indistinguishable from that obtained using benzyl cyanide **1J** and mandelonitrile **3J** as substrates. The presence of an alcohol dehydrogenase in *P. putida* UV4 has previously been inferred from its ability to catalyse the reduction of an aldehyde intermediate formed from *cis*-dihydroxylation of a furan ring.[23] A 60 : 40 ratio of the *cis*-diol **2J** and triol **3A** was formed when benzonitrile **1J** was used as substrate. When this biotransformation was repeated using [α,α-D_2]benzyl cyanide as substrate, the ratio of *cis*-diol to triol increased markedly to 97 : 3. This results is entirely consistent with an initial dioxygenase-catalysed benzylic hydroxylation step which is inhibited by a primary kinetic isotope effect. Although the putative benzylic alcohol intermediate mandelonitrile **3J** could not be detected during the time–course studies, it is postulated that, like the other benzyl alcohol metabolites **3B**–**3F**, it was also formed with a strong preference for the *R* configuration.

The benzyl methyl sulfide substrate **1K** yielded three bioproducts when added to cultures of *P. putida* UV4. The major product, (1*S*,2*R*)-*cis*-dihydrodiol **2K** (90% relative yield), was accompanied by sulfoxide **9** (8%) and triol **4A** (2%). The isolation of racemic dialkyl sulfoxide **9** is noteworthy since alkyl aryl or diaryl sulfoxides, previously obtained[15] using *P. putida* UV4, were often enantiopure. The isolation of triol **4A** in low yield was assumed to be the result of competition between the ring-dihydroxylation and heteroatom-oxidation pathways. Benzylic hydroxylation of sulfide **1K** to an unstable hemithioacetal intermediate **3K** followed by spontaneous decomposition to benzaldehyde **5**, enzyme-catalysed reduction to benzyl alcohol **3A**, and dioxygenase-catalysed *cis*-dihydroxylation to yield triol **4A** is shown in Scheme 1. The O-demethylation process is similar to that found during monooxygenase-catalysed (P-450) dealkylation which occurs widely during mammalian drug metabolism.

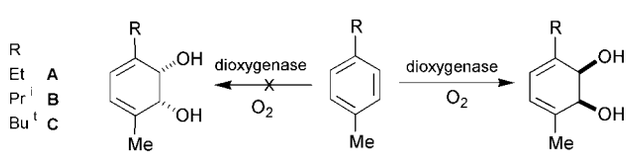
The biotransformation of benzyl acetate **1L**, using *P. putida* UV4, produced the expected *cis*-diol **2L** as the major metabolite (≈ 80% relative yield) accompanied by benzyl alcohol **3A** (≈20% yield) and triol **4A** (≈1% yield). The formation of triol **4A** as a minor metabolite could be due to benzylic hydroxylation



4F′ **9**

forming the transient benzylic alcohol **3L**, decomposition to benzaldehyde **5**, followed by reduction and dihydroxylation as proposed for substrates **1J** and **1K** (Scheme 1). However, the presence of a significant proportion of benzyl alcohol **3A** suggests that esterase-catalysed hydrolysis of the ester **1L** may be the major pathway and that a large proportion of triol **4A** had resulted from *cis*-dihydroxylation of the hydrolysis product. Further evidence was obtained by using two recombinant strains (*E. coli* pKST7 and *E. coli* pKST11) each containing the same TDO as was present in *P. putida* UV4 and benzyl acetate **1L** as substrate. In neither case could the ester hydrolysis product **3A**, or the derived triol **4A**, be observed and *cis*-dihydrodiol **2L** was isolated as the sole metabolite. The availability of triol **4A** from substrates **1J**–**1L** is of potential interest in the context of synthesis of single-enantiomer pseudosugars.

It is surprising that despite evidence of benzylic oxidation occurring in substrates **1A**–**1F** the initially formed benzyl alcohol metabolites **3A**–**3F** were generally not intercepted as bioproducts. This indicates that in general the benzyl alcohols are much better substrates than the parent arenes **1A**–**1F**. In an attempt to intercept the benzyl alcohol intermediates, and to reduce the possibility of triol formation, the *para*-alkyl-substituted toluene substrates **10A**–**10C** were examined. Earlier work had demonstrated that disubstituted benzene substrates gave lower yields of *cis*-dihydrodiols[24] and might thus facilitate benzylic hydroxylation. 1,4-Disubstituted benzenes **10A**–**10C**, however, showed no evidence of benzylic hydroxylation and only the corresponding *cis*-diols **11A**–**11C** were isolated as metabolites. This observation suggests that, as shown with compounds **1H** and **1I**, the size of substrate is an important factor in determining whether TDO-catalysed benzylic monohydroxylation of *cis*-dihydroxylation occurs. The yields of *cis*-diol products obtained decreased sharply with increasing size of *para*-substituent on the substrates **10A**–**10C** (Et > ^{i}Pr > ^{t}Bu). *cis*-Dihydrodiols **11A**–**11C** containing two weakly electron-donating alkyl groups proved to be rather unstable during attempted purification. However *cis*-diol **11A** was obtained in sufficient quantity to form the corresponding MEBBA derivatives which showed that it was enantiopure (>98% ee) and was of the (1*S*,2*R*) configuration. A model has been devised for the TDO system based on extensive studies of 1,4-disubstituted benzene substrates which allows the preferred absolute configuration of the *cis*-dihydrodiol metabolites to be predicted.[15] Thus a strong preference for the (1*S*,2*R*) configuration was predicted and later confirmed where the R group was larger than the substituent at the *para* position (R > Me, Scheme 5).[24]



Scheme 5

Based upon this model it was assumed that the *cis*-diols **11A**–**11C** were enantiopure and had (1*S*,2*R*) configuration. Recent unpublished results from these laboratories on a wider range of substrates has confirmed that an ethyl group is more dominant than a methyl group in directing both the regioselectivity and stereoselectivity of asymmetric dihydroxylation in disubstituted benzene substrates and thus supports the prediction that the isolated products **11A**–**11C** were of the (1*S*,2*R*) configuration.

Conclusions

Dioxygenase-catalysed oxidation of benzene rings containing a methylene group can result in either benzylic monohydroxylation or *cis*-dihydroxylation of the ring in a predictable manner according to the size and nature of the benzylic substituents. In general, benzylic alcohols are better substrates than the corresponding benzyl precursors and thus undergo rapid *cis*-dihydroxylation to yield a range of enantiopure triols having synthetic potential. The formation of transient benzylic alcohol metabolites, followed by spontaneous decomposition yielding benzaldehyde and ultimately the *cis*-dihydrodiol derivative of benzyl alcohol, has been observed. Benzylic alcohol formation occurs with a strong preference to the (*R*) configuration while the *cis*-diol and triol bioproducts were exclusively of the (1*S*,2*R*) and (1*S*,2*R*,1′*R*) configuration, respectively.

Experimental

^{1}H NMR spectra were recorded at 300 MHz (Bruker Avance DPX-500) and at 500 MHz (Bruker Avance DRX-500) in $CDCl_3$ solvent unless stated otherwise. Chemical shifts (δ) are reported in ppm relative to $SiMe_4$ and coupling constants (J) are given in Hz. Mass spectra were recorded at 70 eV on a VG Autospec Mass Spectrometer, using a heated inlet system. Accurate relative molecular masses were determined by the peak-matching method with perfluorokerosene as standard. Elemental microanalyses were obtained on a Perkin-Elmer 2400 CHN microanalyser. CD spectra were recorded on a JASCO J-720 instrument for samples in acetonitrile solvent. CSP/HPLC was carried out using a Shimadzu LC-6A liquid chromatograph connected to Hewlett Packard diode array detector and the specified Daicel CSP column. Optical rotations were measured on a Perkin-Elmer Model 214 polarimeter, and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}.

Shake flask (<0.5 g) and fermenter (>0.5 g) biotransformations were carried out using *P. putida* UV4 under reported conditions.[20]

The diol and triol bioproducts, obtained after bioconversion of the corresponding substrates (**1A**–**1L**, **10A**–**10C**), were separated and purified by PLC (silica gel; 50% EtOAc in hexane). The diastereoisomeric mixture of triols **4F** and **4F′** was also separated by PLC (silica gel; 5% MeOH in $CHCl_3$). The ^{1}H NMR spectra data from diol and triol metabolites are shown in Table 1. 4-Phenyl-1,2,4-triazoline-3,5-dione cycloadduct MTPA esters (Method A) and MEBBA esters (Method B) were prepared by the methods reported earlier.[20,21]

cis-(1*S*,2*R*)-3-Methylcyclohexa-3,5-diene-1,2-diol 2A

From substrate **1A** (60%), mp 56–58 °C (from ethyl acetate–hexane) (lit.,[25] 59 °C); $[\alpha]_D$ +26 (*c* 1.76, MeOH) (lit.,[25] $[\alpha]_D$ +25); >98% ee (Method A).

cis-(1*S*,2*R*)-3-Ethylcyclohexa-3,5-diene-1,2-diol 2B

From substrate **1B** (60%), mp 37–38 °C (from hexane) (lit.,[18] 38 °C); $[\alpha]_D$ +40 (*c* 1.3, MeOH) (lit.,[18] $[\alpha]_D$ +42); >98% ee (Method A).

cis-(1*S*,2*R*)-3-Propylcyclohexa-3,5-diene-1,2-diol 2C

From substrate **1C** (15%), mp 48–51 °C (from hexane); $[\alpha]_D$ +77 (*c* 0.76, MeOH) (Found: C, 70.3; H, 9.1. $C_9H_{14}O_2$ requires C, 70.1; H, 9.25%); *m*/*z* 154 (M^+, 1%), 136 (50), 107 (100); CD λ 273 nm ($\Delta\varepsilon$ 1.193), 215 nm ($\Delta\varepsilon$ −3.767); >98% ee (Method B).

cis-(1*S*,2*R*)-3-Butylcyclohexa-3,5-diene-1,2-diol 2D

From substrate **1D** (33%), mp 56–58 °C (from hexane); $[\alpha]_D$ + 87 (*c* 0.66, MeOH) (Found: C, 71.6; H, 9.3. $C_{10}H_{16}O_2$ requires C, 71.4; H, 9.6%); *m*/*z* 168 (M^+, 20%), 150 (27), 107 (100); CD λ 272 nm ($\Delta\varepsilon$ 0.830), 213 nm ($\Delta\varepsilon$ −3.231); >98% ee (Method B).

cis-(1*S*,2*R*)-3-Allylcyclohexa-3,5-diene-1,2-diol 2E

From substrate **1E** (18%), mp 38 °C (from hexane–CH_2Cl_2); $[\alpha]_D$ +21 (*c* 0.73, MeOH) (Found: M^+, 152.0834. $C_9H_{12}O_2$ requires *M*, 152.0837); *m*/*z* 152 (M^+, 8%), 134 (100), 91 (72); CD λ 268 nm ($\Delta\varepsilon$ 0.295), 212 nm ($\Delta\varepsilon$ −0.794); >98% ee (Method B).

cis-(1*S*,2*R*)-3-(2′-Methylallyl)cyclohexa-3,5-diene-1,2-diol 2F

From substrate **1F** (9%), mp 75–76 °C (from hexane); $[\alpha]_D$ +10 (*c* 0.7, $CHCl_3$) (Found: C, 72.3; H, 8.4. $C_{10}H_{14}O_2$ requires C, 72.3; H, 8.5%); *m*/*z* 166 (M^+, 5%), 148 (85), 133 (100); CD λ 270 nm ($\Delta\varepsilon$ 1.20) 218 nm ($\Delta\varepsilon$ −3.404); >98% ee (Method B).

cis-(1*S*,2*R*)-3-Pentylcyclohexa-3,5-diene-1,2-diol 2G

From substrate **1G** (17%), mp 65–67 °C (from hexane); $[\alpha]_D$ +113 (*c* 0.43, MeOH) (Found: C, 72.1; H, 9.9. $C_{11}H_{18}O_2$ requires C, 72.5; H, 10.0%); *m*/*z* 182 (M^+, 24%), 136 (41), 179 (100); CD λ 276 nm ($\Delta\varepsilon$ 2.271), 219 nm ($\Delta\varepsilon$ −3.06).

cis-(1*S*,2*R*)-3-Isobutylcyclohexa-3,5-diene-1,2-diol 2H

From substrate **1H** (27%), mp 74–76 °C (from hexane); $[\alpha]_D$ +94 (*c* 0.68, MeOH) (Found: M^+, 168.1149. $C_{10}H_{16}O_2$ requires *M*, 168.1150); *m*/*z* 168 (M^+, 15%), 107 (100), 91 (72); CD λ 273 nm ($\Delta\varepsilon$ 2.335), 218 nm ($\Delta\varepsilon$ −3.122).

cis-(1*S*,2*R*)-3-Neopentylcyclohexa-3,5-diene-1,2-diol 2I

From substrate **1I** (22%), mp 61–63 °C (from hexane); $[\alpha]_D$ +152 (*c* 0.44, MeOH) (Found: M^+, 182.1311. $C_{11}H_{18}O_2$ requires *M*, 182.1307); *m*/*z* 182 (M^+, 30%), 164 (21), 108 (100); CD λ 270 nm ($\Delta\varepsilon$ 6.453), 218 nm ($\Delta\varepsilon$ −5.016).

cis-(1*S*,2*R*)-3-(Cyanomethyl)cyclohexa-3,5-diene-1,2-diol 2J

From substrate **1J** (20%), oil; $[\alpha_D$ +13 (*c* 0.4, MeOH) (Found: C, 63.9; H, 6.5; N, 9.4. $C_8H_9NO_2$ requires C, 63.6; H, 6.0; N, 9.3%); *m*/*z* 151 (M^+, 40%), 133 (45), 122 (55), 105 (100); CD λ 264 nm ($\Delta\varepsilon$ 1.770), 205 nm ($\Delta\varepsilon$ −1.781); >98% ee (Method B).

cis-(1*S*,2*R*)-3-(Methylthiomethyl)cyclohexa-3,5-diene-1,2-diol 2K

From substrate **1K** (50%), mp 68–69 °C (from hexane–diethyl ether); $[\alpha]_D$ + 82 (*c* 0.48, $CHCl_3$) (Found: C, 56.0; H, 7.4. $C_8H_{12}O_2S$ requires C, 55.8; H, 7.0%); *m*/*z* 172 (M^+, 30%), 154 (37), 124 (100); CD λ 274 nm ($\Delta\varepsilon$ 4.638), 230 nm ($\Delta\varepsilon$ 1.851), λ 208 nm ($\Delta\varepsilon$ −1.083).

cis-(1*S*,2*R*)-3-(Acetoxymethyl)cyclohexa-3,5-diene-1,2-diol 2L

From substrate **1L**, mp 67–68 °C (from chloroform–hexane); $[\alpha]_D$ +104 (*c* 0.7, MeOH) (Found: C, 58.5; H, 6.2. $C_9H_{12}O_4$ requires C, 58.7; H, 6.5%); *m*/*z* 184 (M^+, 3%), 166 (1), 124 (100); >98% ee (Method A).

cis-(1*S*,2*R*)-3-Ethyl-6-methylcyclohexa-3,5-diene-1,2-diol 11A

From substrate **10A** (16%), mp 78–79 °C (from hexane–CH_2Cl_2); $[\alpha_D$ +7 (*c* 0.4, MeOH) (Found: M^+, 154.0989. $C_9H_{14}O_2$ requires *M*, 154.0994); *m*/*z* 154 (M^+, 2%), 136 (100); CD λ 270 nm ($\Delta\varepsilon$ −0.013), 227 nm ($\Delta\varepsilon$ 0.016), λ 211 nm ($\Delta\varepsilon$ −0.018); >98% ee (Method B).

cis-(1*S*,2*R*)-3-Isopropyl-6-methylcyclohexa-3,5-diene-1,2-diol 11B

From substrate **10B** (15%), *unstable solid*; mp 45–49 °C (decomp.); $[\alpha]_D$ +43 (*c* 0.4, MeOH) (Found: M^+, 168.1155. $C_{10}H_{16}O_2$ requires *M*, 168.1150); *m*/*z* 168 (M^+, 30%), 122 (100).

Table 1 ^{1}H NMR spectral data for the *cis*-diol and triol metabolites

Compound	H-1	H-2	H-4	H-5	H-6	Me	Other exocyclic protons		
2A	4.29m	4.03d, *J* 6.0	5.72d, *J* 4.7	5.91m	5.79dd, *J* 3.4, 9.5	1.93s			
2B	4.31m	4.00d, *J* 5.9	5.73m	5.93m	5.73m	1.09m	2.29m (CH_2)		
2C	4.31m	4.01, *J* 5.9	5.71d, *J* 5.2	5.71m	5.71dd, *J* 3.4, 9.5	0.94t, *J* 7.4	2.23m (CH_2)	1.35m (CH_2)	
2D	4.38m	3.96d, *J* 5.7	5.73d, *J* 5.7	6.02m	5.81dd, *J* 3.4, 9.4	0.92t, *J* 6.6	2.42m (CH_2)	1.57m (CH_2)	1.23m (CH_2)
2E	4.32m	4.06d, *J* 6.0	5.74d, *J* 5.5	5.90m	5.90m		3.00d, *J* 6.7 (CH_2)	5.85m (2 × =CH)	5.14dd, *J* 17.7, 1.7 (=CH)
2F	4.33m	4.04d, *J* 6.0	5.76m	5.95m	5.82m	1.74s	2.88d, *J* 16.0 (CH_AH_B)	2.98d, *J* 16.0 (CH_AH_B)	4.82s, 4.86s (2 × =CH)
2G	4.33m	3.98d, *J* 5.8	5.69d, *J* 5.4	5.93m	5.75dd, *J* 3.1, 9.5	0.83t, *J* 6.5	2.26m (CH_2)	1.41m (CH_2)	1.26m $(CH_2)_2$
2H	4.31m	3.98d, *J* 5.7	5.69d, *J* 4.9	5.93m	5.72dd, *J* 2.8, 9.8	0.88t, *J* 6.8	2.24m (CH_2)	0.80d, *J* 6.8 (Me)	1.78m ($CHMe_2$)
2I	4.38m	3.97d, *J* 5.6	5.72d, *J* 5.5	5.95m	5.76dd, *J* 3.4, 9.8	0.94s $(Me)_3$	2.05d, *J* 13.0 (CH_AH_B)	2.18d, *J* 13.0 (CH_AH_B)	
2J	4.27m	4.25dd, *J* 5.1, 1.3	6.10dd, *J* 5.1, 9.4	6.06dd, *J* 5.1, 9.4	6.01dd, *J* 3.9, 9.4		3.31d, *J* 19.7 (CH_AH_B)	3.39d, *J* 19.7 (CH_AH_B)	
2K	4.26m	4.18d, *J* 6.2	5.83m	5.83m	5.83m	1.98s	3.21d, *J* 13.6 (CH_AH_B)	3.34d, *J* 13.6 (CH_AH_B)	
2L	4.35m	4.19d, *J* 6.3	5.98m	5.98m	5.98m	2.1s	4.69d, *J* 13.5 (CH_AH_B)	4.80d, *J* 13.5 (CH_AH_B)	
11A	4.06d, *J* 5.6	4.09d, *J* 5.6	5.63d, *J* 5.6	5.67d, *J* 5.6		1.88s	2.22q, *J* 7.4 (CH_2)	1.07t, *J* 7.4 (Me)	
11B	4.15d, *J* 5.3	4.06d, *J* 5.3	5.64d, *J* 5.7	5.68d, *J* 5.7		1.95s	2.51m (CH)	1.09d, *J* 3.5 $(Me)_2$	
11C	4.24d, *J* 5.5	4.11d, *J*, 5.5	5.74d, *J* 5.7	5.66, *J* 5.7		1.95s		1.25s $(Me)_3$	
4A	4.28m	4.38d, *J* 6.2	5.98m	5.98m	5.98m		4.33s (C*H*OH)		
4B	4.27m	4.20d, *J* 6.1	5.97m	5.97m	5.97m	1.37d, *J* 6.4	4.50q, *J* 6.4 (C*H*OH)		
4C	4.29m	4.19d, *J* 5.1	5.93d, *J* 5.3	6.00m	5.85dd, *J* 3.1, 9.6	0.97t, *J* 7.4	4.23m (C*H*OH)	1.73 (CH_2)	
4D	4.30m	4.22d, *J* 6.1	5.93d, *J* 5.3	5.97m	5.85dd, *J* 3.1, 9.8	0.94t, *J* 9.7	4.32m (C*H*OH)	1.72m (CH_2)	1.37m (CH_2)
4E	4.29m	4.21d, *J* 6.2	5.88m	6.00m	6.00m		4.83d, *J* 6.2 (C*H*OH)	4.83m (2 × =CH)	5.22m, 5.36m ($=CH_AH_B$)
4F	4.31m	4.14d, *J* 6.1	5.93m	6.02m	5.85dd, *J* 3.1, 8.2	1.72s	4.77s (C*H*OH)	5.00d, *J* 1.3 ($=CH_AH_B$)	5.14d, *J* 1.3 ($=CH_AH_B$)
4B′	4.29m	4.39d, *J* 6.1	5.86d, *J* 5.3	5.97m	5.89m	1.41d, *J* 6.4	4.50q, *J* 6.4 (C*H*OH)		
4C′	4.28m	4.35d, *J* 6.1	5.81d, *J* 5.3	5.93m	5.83dd, *J* 3.1, 9.2	0.91t, *J* 9.7	4.19 (C*H*OH)	1.75m (CH_2)	
4D′	4.26m	4.38d, *J* 5.7	5.84d, *J* 5.2	5.99m	5.91m	0.95t, *J* 7.9	4.26m (C*H*OH)	1.69m (CH_2)	1.35m (CH_2)

***cis*-(1*S*,2*R*)-3-*tert*-Butyl-6-methylcyclohexa-3,5-diene-1,2-diol 11C**

From substrate **10C** (5%), unstable solid; mp 56–60 °C (decomp.); $[\alpha]_D$ +94 (*c* 0.4, MeOH) (Found: M^+, 182.1304. $C_{11}H_{18}O_2$ requires *M*, 182.1307); *m*/*z* 182 (M^+, 10%), 122 (100).

***cis*-(1*S*,2*R*)-3-(Hydroxymethyl)cyclohexa-3,5-diene-1,2-diol 4A**

From substrates **1A** (4%), **1J** (15%), **3J** (18%) and **5** (8%), oil; $[\alpha]_D$ +35 (*c* 0.1, MeOH) (Found: $M^+ - 18$, 124.0525. $C_7H_8O_2$ requires *m*/*z*, 124.0524); *m*/*z* 142 (M^+, <1%), 124 (10); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*R*)-1′-Hydroxyethyl]cyclohexa-3,5-diene-1,2-diol 4B**

From substrate **1B** (5%), (*R*)-**3B** (20%), oil; $[\alpha]_D$ +55 (*c* 0.57, MeOH) (lit.,[18] $[\alpha]_D$ +55); *m*/*z* 154 (M^+, <1%), 95 (100); CD λ 282 nm ($\Delta\varepsilon$ 0.421), 222 nm ($\Delta\varepsilon$ −4.233); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*S*)-1′-Hydroxyethyl]cyclohexa-3,5-diene-1,2-diol 4B′**

From substrate (*S*)-**3B** (8%), unstable oil; $[\alpha]_D$ +5 (*c* 0.81, MeOH) (lit.,[18] $[\alpha]_D$ + 6); *m*/*z* 154 (M^+, <1%), 43 (100); CD λ 285 nm ($\Delta\varepsilon$ 0.3206), 222 nm ($\Delta\varepsilon$ −4.030); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*R*)-1′-Hydroxypropyl]cyclohexa-3,5-diene-1,2-diol 4C**

From substrate **1C** (26%) and (*R*)-**3C** (8%), oil; $[\alpha]_D$ +52 (*c* 0.95, MeOH) (Found: M^+, 170.0941. $C_9H_{14}O_3$ requires *M*, 170.0943); *m*/*z* 170 (M^+, 10%), 152 (29), 95 (100); CD λ 282 nm ($\Delta\varepsilon$ 0.421), 222 nm ($\Delta\varepsilon$ −4.233); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*S*)-1′-Hydroxypropyl]cyclohexa-3,5-diene-1,2-diol 4C′**

From substrate (*S*)-**3C** (53%), mp 98–104 °C (decomp.) (from $CHCl_3$–Et_2O); $[\alpha]_D$ + 16 (*c* 0.41, MeOH) (Found: M^+, 170.0944); *m*/*z* 170 (M^+, 10%), 152 (29), 95 (100); CD λ 284 nm ($\Delta\varepsilon$ 0.321), 222 nm ($\Delta\varepsilon$ −4.030); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*R*)-1′-Hydroxybutyl]cyclohexa-3,5-diene-1,2-diol 4D**

From substrate **1D** (9%) and (*R*)-**3D** (31%), oil; $[\alpha]_D$ + 58 (*c* 0.49, MeOH) (Found: M^+, 184.2343. $C_{10}H_{16}O_3$ requires *M*, 184.2346); *m*/*z* 184 (M^+, 15%), 134 (47), 107 (100); CD λ 280 nm ($\Delta\varepsilon$ 0.407), 222 nm ($\Delta\varepsilon$ −4.462); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*S*)-1′-Hydroxybutyl]cyclohexa-3,5-diene-1,2-diol 4D′**

From substrate (*S*)-**3D** (23%), mp 59–63 °C (from $CHCl_3$–Et_2O); $[\alpha]_D$ +3 (*c* 0.42, MeOH) (Found: M^+, 184.2349; *m*/*z* 184 (M^+, 15%), 134 (47%), 107 (100); CD λ 283 nm ($\Delta\varepsilon$ 0.333), 222 nm ($\Delta\varepsilon$ −4.393); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*R*)-(1′-Hydroxyallyl]cyclohexa-3,5-diene-1,2-diol 4E**

From substrate **1E** (15%), oil; $[\alpha]_D$ −9 (*c* 1.6, MeOH) (Found: M^+, 168.0787. $C_9H_{12}O_3$ requires *M*, 168.0786); *m*/*z* 168 (M^+, 13%), 150 (57), 133 (74), 94 (100); CD λ 292 nm ($\Delta\varepsilon$ 0.1839), 256 nm ($\Delta\varepsilon$ −0.7936), 216 nm ($\Delta\varepsilon$ −6.452); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*R*)-(1′-Hydroxy-2′-methylallyl]cyclohexa-3,5-diene-1,2-diol 4F**

From substrate **1F** (18%), oil; $[\alpha]_D$ −13 (*c* 0.6, $CHCl_3$) (Found: M^+, 182.0940. $C_{10}H_{14}O_3$ requires *M*, 182.0943); *m*/*z* 182 (M^+, 1%), 164 (41), 146 (28), 49 (100); CD λ 286 nm ($\Delta\varepsilon$ 0.439), 208 nm ($\Delta\varepsilon$ −3.791); >98% ee (Method B).

***cis*-(1*S*,2*R*)-3-[(*S*)-(1′-Hydroxy-2′-methylallyl]cyclohexa-3,5-diene-1,2-diol 4F′**

From substrate **1F** (5%), oil; $[\alpha]_D$ −8 (*c* 0.3, MeOH); MS and CD data were virtually identical with those found for diastereoisomer **4F**; >98% ee (Method B).

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