Dioxygenase-catalysed oxidation of disubstituted benzene substrates: benzylic monohydroxylation *versus* aryl *cis*-dihydroxylation and the *meta effect*†

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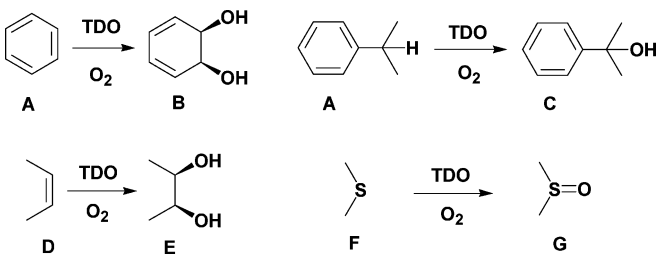
Biotransformations of a series of *ortho*-, *meta*- and *para*-substituted ethylbenzene and propylbenzene substrates have been carried out, using *Pseudomonas putida* UV4, a source of toluene dioxygenase (TDO). The *ortho*- and *para*-substituted alkylbenzene substrates yielded, exclusively, the corresponding enantiopure *cis*-dihydrodiols of the same absolute configuration. However, the *meta* isomers, generally, gave benzylic alcohol bioproducts, in addition to the *cis*-dihydrodiols (the *meta effect*). The benzylic alcohols were of identical (*R*) absolute configuration but enantiomeric excess values were variable. The similar (2*R*) absolute configurations of the *cis*-dihydrodiols are consistent with both the ethyl and propyl groups having dominant stereodirecting effects over the other substituents. The model used earlier, to predict the regio- and stereo-chemistry of *cis*-dihydrodiol bioproducts derived from substituted benzene substrates has been refined, to take account of non-symmetric subsituents like ethyl or propyl groups. The formation of benzylic hydroxylation products, from *meta*-substituted benzene substrates, without further *cis*-dihydroxylation to yield triols provides a further example of the *meta effect* during toluene dioxygenase-catalysed oxidations.

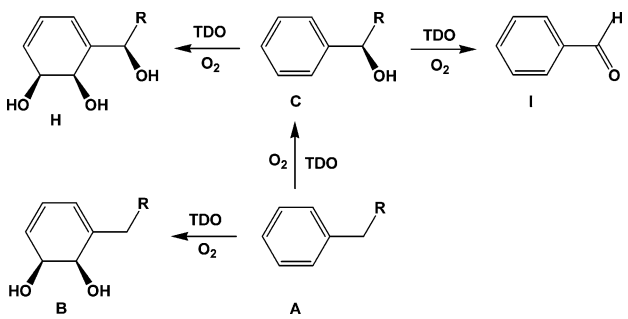
Introduction

Bacterial aryl ring dihydroxylating dioxygenase enzymes (dioxygenases) have been widely recognised for their ability to catalyse the introduction of two oxygen atoms (*cis*-dihydrodiol formation) to substrates containing a benzene ring in a regio- and stereo-selective manner. The formation of *cis*-dihydrodiol metabolites **B**, from arenes **A**, was initially studied using mutant strains of bacteria (mainly *Pseudomonas*) which allow these initial bioproducts and other metabolites to accumulate (Scheme 1). Since the first report of the isolation and identification of a *cis*-dihydrodiol metabolite **B**, *i.e.* from the parent benzene ring **A**,[1] in excess of three hundred examples from substituted benzene substrates have now been reported. The majority of these *cis*-dihydrodiols **B** are enantiopure and are being widely used as synthetic precursors.[2–12]

While dioxygenases, present in whole cells of selected mutants, *e.g. Pseudomonas putida* UV4 (a source of toluene dioxygenase, TDO), can catalyse arene *cis*-dihydroxylations (**A** → **B**, Scheme 1), it has recently been found that they can also catalyse other types of oxidations (*e.g.* **A** → **C**, **D** → **E**, **F** → **G**; Scheme 1 and Scheme 2). In view of the increasing importance of *cis*-dihydrodiols as synthetic precursors it was considered important to rationalise the factors which allow a prediction to be made for the preferred TDO-catalysed oxidation pathway.



Scheme 1



Scheme 2

TDO-catalysed insertion of a single oxygen atom into a C–H bond of an alkyl-substituted benzene ring (benzylic hydroxylation) has also been observed, particularly in benzo-fused bicyclic substrates where the benzylic alcohol products were readily isolated.[13–18]

Although dioxygenase-catalysed benzylic hydroxylation of alkyl-substituted benzene substrates **A** has been observed, prior to the current study, in virtually all cases, isolation of the benzylic alcohols **C** has proved to be elusive due to their being:

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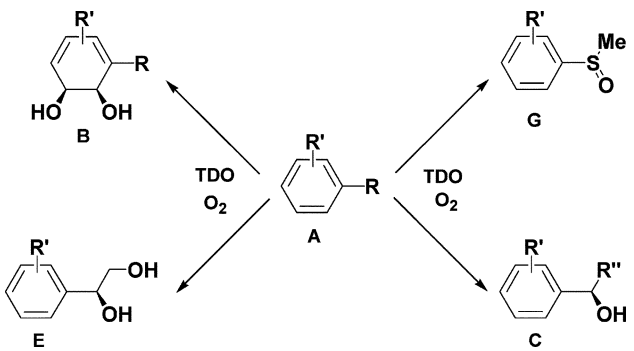
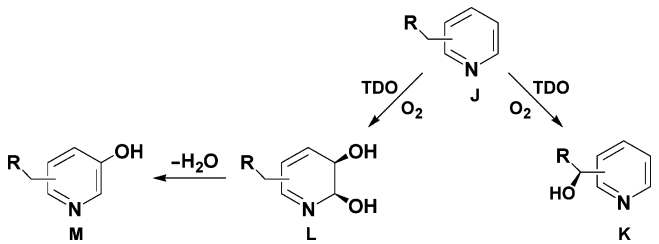
(a) excellent substrates (*e.g.* R = Me, Et, Pr) which are immediately oxidised to the corresponding triols **H** (R = Me, Et, Pr), or (b) unstable intermediates (R = CN, SMe, OAc) that spontaneously decompose to benzaldehyde **I** (Scheme 2).[18] In addition to arene *cis*-dihydroxylation, dioxygenase enzymes have also been found to catalyse the dihydroxylation of acyclic and cyclic conjugated alkenes **D** to give the corresponding alkene diols **E**,[19–32] and monosulfoxidation of alkyl aryl sulfides **F** to give monosulfoxides **G**.[33–43] (Scheme 1).

During the course of earlier studies from these laboratories, using *P. putida* UV4 as a source of TDO, it was observed that *cis*-dihydroxylation of disubstituted benzene substrates **A** (R and R′ ≠ H) was slower, and yields of the corresponding *cis*-dihydrodiols **B** were generally lower (Scheme 3).[32,43,44] This was particularly evident from combinatorial biotransformation studies where the relative rates of *cis*-dihydroxylation of *meta*-disubstituted benzene substrates **A** (R and R′ ≠ H) were lower compared with monosubstituted benzenes **A** (R′ = H) and *ortho*- or *para*-disubstituted benzenes (R and R′ ≠ H, Scheme 3). Similarly, *meta*-substituted styrene substrates (**A**, R = CH=CH$_2$) often yielded more of the exocyclic alkene diol **E** during TDO-catalysed biotransformations (Scheme 3).[32] The structural features of substituted styrenes (**A**, R = CH=CH$_2$), which determine the preference of TDO for dihydroxylation of either the arene ring (to give a *cis*-dihydrodiol **B**) or the alkene group (to give an alkene diol **E**), have also been determined.[32] Furthermore, *meta*-substituted phenyl methyl sulfides (**A**, R = SMe, *meta* R′ = F, Cl) gave sulfoxides (**G**, *meta* R′ = F, Cl) exclusively rather than *cis*-dihydrodiols **B** (R = SMe, *meta* R′ = F, Cl) which were formed when *ortho* and *para* substituents were present (Scheme 3). Thus, the question of selectivity of this enzyme, present in *P. putida* UV4 whole cell systems, for either aryl rings or sulfur atoms has already been addressed;[43] all the results were consistent with the presence of a *meta effect*.



Scheme 3

Recent oxidation studies[45] of alkyl-substituted pyridine substrates **J**, using *P. putida* UV4, also showed evidence of both monohydroxylation of the alkyl group (equivalent to benzylic hydroxylation) to yield alcohol **K** or monohydroxylation of the electron-poor pyridine ring to yield hydroxypyridines **M** (Scheme 4). Aromatic hydroxylation of the pyridine ring presumably occurs *via cis*-dihydroxylation, to give unstable dihydrodiols **L** (or a regioisomer), followed by dehydration. Aromatic monohydroxylation of the aza-arene rings **J** to yield bioproducts **M**, exclusively, when the alkyl group was at C-4 showed a marked



Scheme 4

contrast with aliphatic hydroxylation of C-3 alkyl substrates **J** to yield, exclusively, alcohols **K**. Alkyl substitution at C-2 resulted in both alkyl and aryl hydroxylations, further demonstrating that the regioselectivity of the TDO enzyme, during arene oxidations, is dependent on the substitution pattern.

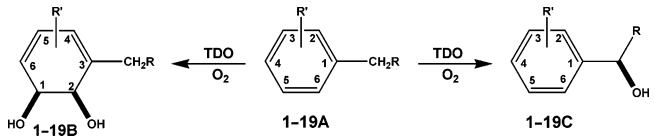
As a consequence of the reactivity of the transient benzylic alcohol intermediates **C** (Scheme 2), very few examples of isolable alcohols have been found from benzylic hydroxylation of alkyl-substituted benzene substrates; the current study is primarily focussed on this objective. In this context, the factors which determine the nature of TDO-catalysed regioselectivity and stereoselectivity, observed during oxidation of disubstituted benzene substrates **A** which contain an alkyl group (R = Et or Pr, Scheme 3 and Table 1), have now been examined to determine: (i) the relative stereodirecting effects of the alkyl groups, (ii) if the arene *cis*-dihydroxylation path to yield *cis*-dihydrodiols **B** is inhibited when a *meta* substituent is present (a *meta effect*) and (iii) the preferred stereochemistry of the benzylic alcohol metabolites **C** (Scheme 3).

Results and discussion

Results obtained from biotransformations of substituted alkylbenzene substrates **A** (R = H, Me, Et and Pr, Scheme 2), using *P. putida* UV4, had shown that the TDO enzyme exhibits a marked preference for oxidation of the arene ring to give the corresponding *cis*-dihydrodiols **B** rather than the methyl or methylene group. Thus, toluene **A** (R = H) gave only a trace of benzyl alcohol **C** (1–2% yield; R = H). When other alkyl groups were present in substrate **A** (R = Me; Et, or Pr), the proportion of attack at the benzylic position could only be estimated from the isolated yields of triols **H** [R = Me (5%), Et (26%), Pr (9%)] formed by rapid oxidation of the corresponding benzyl alcohols **C** which were generally undetected (Scheme 2).

Based on the observations that Et and Pr groups were more likely to undergo benzylic hydroxylation than a Me group, albeit in modest yield, using monosubstituted alkylbenzene substrates, a systematic study of the TDO-catalysed oxidation of a series of substituted ethylbenzene and propylbenzene substrates was undertaken (Table 1). The structures of the bioproducts were confirmed by spectroscopic comparisons with authentic samples, MS and ^{1}H-NMR analyses (chemical shift, coupling constant, NOE), and elemental microanalyses. For consistency of absolute configuration assignment of the bioproducts, the carbon atom bearing the Et or Pr groups was designated as C-1 for the disubstituted benzene substrates **A** and the benzyl alcohol products **C** (Table 1). The 2-substituted alkylbenzene substrates (**2A**–**5A**, **14A**) and 4-substituted benzene substrates (**6A**–**9A**, **15A**–**17A**) were found to give the corresponding *cis*-dihydrodiols (**2B**–**9B**, **14B**–**17B**) exclusively. As expected, the isolated yields were generally

Table 1 Relative yields, ee values and absolute configurations of *cis*-dihydrodiol and benzyl alcohol bioproducts

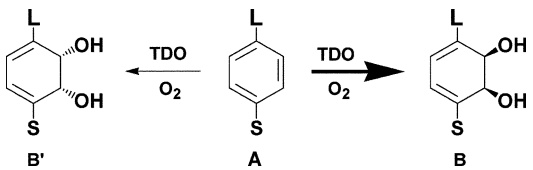


Arene				*cis*-Dihydrodiol				Benzyl alcohol		
R	R′			Yield (%)[a]	Ee	Abs. config.		Yield (%)[a]	Ee	Abs. config.
Me	2-H	**1A**	**1B**	8	>98	1*S*,2*R*[b,c]	**1H**	92	>98	1*S*,2*R*1′*R*[b,c]
Me	2-F	**2A**	**2B**	100	>98	1*S*,2*R*	**2C**	0		
Me	2-Cl	**3A**	**3B**	100	>98	1*S*,2*R*	**3C**	0		
Me	2-Br	**4A**	**4B**	100	>98	1*S*,2*R*	**4C**	0		
Me	2-I	**5A**	**5B**	100	>98	1*S*,2*R*	**5C**	0		
Me	4-F	**6A**	**6B**	100	>98	1*R*,2*R*	**6C**	0		
Me	4-Cl	**7A**	**7B**	100	>98	1*R*,2*R*	**7C**	0		
Me	4-Br	**8A**	**8B**	100	>98	1*R*,2*R*	**8C**	0		
Me	4-I	**9A**	**9B**	100	>98	1*R*,2*R*	**9C**	0		
Me	3-F	**10A**	**10B**	60, 10	>98	1*S*,2*R*	**10C**	40, 90	2	*R*
Me	3-Cl	**11A**	**11B**	14	>98	1*S*,2*R*	**11C**	86	6	*R*
Me	3-Br	**12A**	**12B**	34	>98	1*S*,2*R*	**12C**	66	18	*R*
Et	2-H	**13A**	**13B**	37[b,c]	>98	1*S*,2*R*	**13H**	63[b,c]		1*S*,2*R* 1′*R*
Et	2-F	**14A**	**14B**	100	>98	1*S*,2*R*	**14C**	0		
Et	4-F	**15A**	**15B**	100	>98	1*R*,2*R*	**15C**	0		
Et	4-Cl	**16A**	**16B**	100	>98	1*R*,2*R*	**16C**	0		
Et	4-Br	**17A**	**17B**	100	>98	1*R*,2*R*	**17C**	0		
Et	3-F	**18A**	**18B**	0		1*S*,2*R*	**18C**	100	94	*R*
Et	3Cl	**19A**	**19B**	68	>98	1*S*,2*R*	**19C**	32	46	*R*

[a] Relative yields of bioproducts **B** and **C**. [b] Ref. 18. [c] Isolated as a triol **H**.

low (2–50%) and no evidence of benzylic monohydroxylation or trihydroxylation was observed. These *cis*-dihydrodiol metabolites were all found to be present as single enantiomers (>98% ee), by employing reported methods for enantiopurity determination, *e.g.* ^{1}H-NMR analysis of diastereoisomeric boronate esters formed using (*R*)- and (*S*)-2-(1-methoxyethyl)benzeneboronic acid (MEBBA).[18,29,32,37,43,46] In the ^{1}H-NMR spectra of the boronates, the δ values for the CMe and OMe groups, obtained using perdeuteriochloroform solvent and the (−)-(*S*)-boronic acid, were all shifted downfield (CMe) and upfield (OMe) relative to the signals obtained using (+)-(*R*)-boronic acid. On this basis, all the *cis*-dihydrodiols were tentatively assigned a (2*R*) configuration. This conclusion was supported by circular dichroism (CD) spectroscopy and stereochemical correlation with related *cis*-dihydrodiols of known configurations, *e.g.* those derived from 4-substituted toluenes. With the exception of *cis*-dihydrodiol **2B**, all the *cis*-dihydrodiols showed a positive CD absorption at longer wavelength (268–282 nm), in common with *cis*-dihydrodiol metabolites of monosubstituted alkylbenzene substrates having a (2*R*) configuration.[47] Although *cis*-dihydrodiol **2B** did not show any CD absorption in the longer wavelength region, a very similar CD spectrum was obtained for the corresponding *cis*-dihydrodiol metabolite from 2-fluorotoluene whose absolute configuration was established in an unequivocal manner (X-ray crystallography) to possess a 2*R* configuration. Thus, by analyses of ^{1}H-NMR spectra (MEBBA derivatives) and CD spectroscopy data (*cis*-dihydrodiol), the absolute configuration of each of the *cis*-dihydrodiols **2B**–**12B**, **14B**–**17B** and **19B** was found to be (2*R*) (see ESI†, Table S1, for the ^{1}H-NMR data of these compounds).

The (2*R*) absolute configuration, assigned to each of the *cis*-dihydrodiols **6B**–**9B** and **15B**–**17B** is consistent with the prediction made on the basis of a simple model that had been proposed earlier[44] to account for the preferred absolute configurations of *cis*-dihydrodiol metabolites (TDO enzyme present in *P. putida* UV4) of 1,4-disubstituted benzene substrates (Scheme 5). This model has also been used, recently, to rationalise both the regio- and the stereo-selectivity of TDO-catalysed *cis*-dihydroxylation of 1,2- and 1,3-disubstituted benzene substrates.[7,8,10–12]

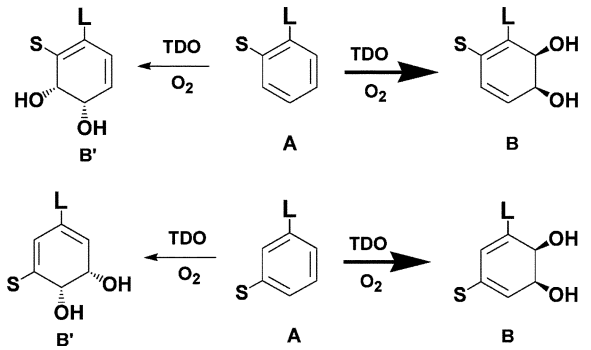


Scheme 5

The model assumes a stereopreference for configuration **B** over the enantiomeric configuration **B′**, based on the relative differences in size between large (L) and small (S) conformationally independent atoms or substituents, using standard steric parameter values, *e.g.* the Charton steric parameter (υ).[48] Thus, the dominant stereodirecting effect of the larger (L) atom or group was found to follow the sequence CF_3 (υ 0.90) > I (υ 0.78) > Br (υ 0.65) > Cl (υ 0.55) ≈ Me (υ 0.52) > F (υ 0.27) > H (υ 0.00) with decreasing enantiopurity values as the size difference between the atoms or groups became smaller. To accomodate substituents whose size will be conformationally dependent, the predictive model has recently been refined. A thiomethoxide group (SMe), having a

smaller υ value (0.60) than a CF_3 group or the halogen atoms (Cl, Br and I), was found to have a stronger stereodirecting effect than the atoms (H, F, Cl, Br, I) or groups (Me, CF_3) studied earlier.[43] The results shown in Table 1 indicate that: (a) the conformationally dependent Et (υ 0.56) and Pr (υ 0.68) groups, present in the 1,4-disubstituted benzene substrates **6A**–**9A** and **15A**–**17A**, exert a stronger stereodirecting effect than the atoms (I, Br, Cl, F) or a Me group (Pr > Et > I > Br > Cl ≈ Me > F > H), (b) the dominant stereodirecting effect of the conformationally dependent tetrahedral groups SMe, Et and Pr is determined by the preferred conformation adopted within the active site rather than their υ values.

The *cis*-dihydrodiol metabolites **2B**–**5B** and **14B** from 1,2-disubstituted benzene substrates **2A**–**5A** and **14A** (Table 1) were all found be enantiopure (>98% ee) and of the same (2*R*) absolute configuration. The dihydroxylation of the arene bond proximate to the dominant stereodirecting group (L) was strongly favoured (no evidence of the alternative regioisomers **B′** was obtained). These results are again consistent with the conclusion that these alkyl groups (L = Et and Pr) are stronger stereodirecting groups than the halogen atoms (S = F, Cl, Br and I; Scheme 6).



Scheme 6

The *cis*-dihydrodiol metabolites **B** of 1,3-disubstituted benzenes, obtained during our current studies, were also formed exclusively (no evidence of **B′**) and found to be enantiopure and of identical absolute configuration (Scheme 6). These *cis*-dihydrodiols were again more difficult to form compared with the corresponding diols from 1,2- or 1,4-disubstituted benzene substrates. Thus, no evidence of *cis*-dihydrodiol **18B** was found from biotransformation of the 1,3-disubstituted benzene **18A**. However, during the biotransformation of 1,3-disubstituted benzenes **10A**–**12A** and **19A**, *cis*-dihydroxylation did occur but exclusively on the arene bond proximate to the Et and Pr groups to yield *cis*-dihydrodiols **10B**–**12B** and **19B** rather than the alternative regioisomers **10B′**–**12B′** and **19B′**. This marked regioselectivity of TDO-catalysed *cis*-dihydroxylation of 1,2- and 1,3-disubstituted benzenes further supports our conclusion that the stereodirecting effects decrease in the sequence Pr > Et > CF_3 > I > Br > Cl ≈ Me > F > H.

The unoptimised isolated yields of *cis*-dihydrodiols **2B**–**12B**, **14B**–**17B** and **19B**, obtained from substituted ethylbenzenes (**2A**–**12A**, 3–55% yields) and propylbenzenes (**14A**–**17A** and **19A**, 2–40% yields), were generally much lower than the corresponding monosubstituted benzene substrates (ethylbenzene **1A**, 65% yield including triol; propylbenzene **13A**, 41% yield including triol). Nevertheless, the determination of the preferred stereo- and regiochemistry of isolated bioproducts has contributed in refining the predictive models (Schemes 5 and 6).

The benzylic alcohol products **10C**–**12C**, **18C** and **19C**, obtained from *meta*-substituted benzene substrates **10A**–**12A**, **18A** and **19A**, were formed in very low yields (1–6%). A wide range of ee values (2–94%) was observed, using chiral stationary phase HPLC analysis (Method B; CSPHPLC, Daicel OB column); in each case an identical (*R*) absolute configuration was found, based on optical rotation measurements and comparison with the literature data. It was assumed that the variable ee values recorded could be the result of asymmetric synthesis and/or kinetic resolution. It is noteworthy that none of the *ortho*- or *para*-substituted alkylbenzene substrates were found to be oxidised *via* benzylic hydroxylation. Furthermore, in contrast with the benzylic alcohol bioproducts **1C** and **13C** (formed initially from ethylbenzene **1A** and propylbenzene **13A** but rapidly converted, *via* TDO-catalysed *cis*-dihydroxylation, to the corresponding triols **1H** and **13H**) none of the benzylic alcohols **10C**–**12C**, **18C** or **19C** were found to be oxidised to the corresponding triols. This observation further supports the view that *meta*-substituted benzene substrates generally show a preference for TDO-catalysed oxidation of a peripheral substituent (benzylic hydroxylation, sulfoxidation and alkene dihydroxylation) rather than an aryl group and that TDO-catalysed *cis*-dihydroxylation is slower for 1,3-disubstituted benzene substrates due to the *meta effect*. From the results presented, it may be assumed that a substituent at a *meta*-postion of a benzene substrate does not allow binding and catalysis of arene *cis*-dihydroxylation at the TDO active site to occur as readily as peripheral oxidation pathways. Our unpublished results on the biotransformation (*P. putida* UV4) of *ortho*-, *meta*- and *para*-xylene substrates show that the corresponding monobenzylic alcohols are the only isolable bioproducts formed (6–13% yields). This may be due to the slower rate of *cis*-dihydroxylation of the disubstituted benzene rings allied to the availability (statistical factor) of six benzylic C–H bonds.

Conclusion

The biotransformation of a series of substituted ethylbenzene and propylbenzene substrates has yielded enantiopure (2*R*) *cis*-dihydrodiols *via* TDO-catalysed dihydroxylation. The stereoselectivity of this oxidation was rationalised, using a refined predictive model where the effective size of conformationally dependent substituents, *e.g.* Et and Pr groups, was found to have a dominant stereodirecting influence. *Meta*-substituted ethylbenzene and propylbenzene substrates were also found to yield benzylic alcohol bioproducts rather than the corresponding triols; this was consistent with earlier evidence of a *meta effect*.

Experimental

^{1}H-NMR spectra were recorded at 300 MHz (Bruker Avance DPX-300) 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 molecular weights were determined by the peak matching method with perfluorokerosene as standard. Elemental microanalyses were

obtained on a Perkin-Elmer 2400 CHN microanalyser. Circular dichroism spectra were recorded on a Jasco J-720 instrument in acetonitrile solvent. CSPHPLC analysis was carried out on a Shimadzu LC-6A liquid chromatograph (Daicel OB column) connected to Hewlett Packard diode array detector. Optical rotation ($[\alpha]_D$) measurements were performed on a Perkin-Elmer polarimeter at ambient temperature temperature (20 °C) and are expressed in units of 10^{-1} deg cm^2 g^{-1}. Ethylbenzene **2A**–**12A** and propylbenzene **14A**–**19A** substrates were prepared from commercially available substituted styrene and alkyl phenyl ketones, using literature methods. The corresponding benzylic alcohol metabolites **10C**–**12C**, **18C**, **19C** were found to have similar ^{1}H-NMR spectral characteristics to those reported in the literature.

Shake flask (<0.5 g) biotransformations were carried out using *P. putida* UV4 under reported conditions.[32] *cis*-Dihydrodiol **2B**–**12B**, **14B**–**17B**, **19B** and benzyl alcohol bioproducts **10C**–**12C**, **18C**, **19C** obtained after bioconversion of the corresponding substrates **2A**–**12A**, **14A**–**19A** were separated and purified by preparative layer chromatography (PLC) (silica gel, 50% EtOAc in hexane). The enantiomeric excess (ee) values of *cis*-dihydrodiols **2B**–**12B**, **14B**–**19B** were determined *via* formation of the corresponding diastereoisomeric boronate esters using *R*- and *S*-2-(1-methoxyethyl)benzeneboronic acids followed by ^{1}H-NMR analysis of the diastereoisomeric composition (Method A).[46]

The absolute configurations were determined by ^{1}H-NMR analysis of the chemical shift values of chiral boronate derivatives, and comparison of CD spectra of *cis*-dihydrodiols. The ee values of benzylic alcohol metabolites **10C**–**13C**, **18C**, **19C** were determined by CSPHPLC analyses using a Daicel OB column (hexane–isopropanol, 95 : 5, Method B), and comparison of the sign of specific optical rotations with the literature values.

cis-Dihydrodiol bioproducts 2B–12B, 14B–17B, 19B

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-3-ethyl-4-fluorocyclohexa-3,5-diene 2B.** From substrate **2A** (0.025 g, 10%); mp 59–61 °C (from hexane); $[\alpha]_D$ −6.2 (*c* 0.45, MeOH); *m*/*z* (EI) (trimethylsilyl derivative from BSTFA) 302 (M$^+$, 90%), 73 (Me$_3$Si, 100); (Found: M$^+$ 158.0745; $C_8H_{11}FO_2$ requires 158.0743); >98% ee (Method A); CD λ 206 nm ($\Delta\varepsilon$ 0.527), 258 nm ($\Delta\varepsilon$ −0.909).

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-4-chloro-3-ethylcyclohexa-3,5-diene 3B.** From substrate **3A** (0.032 g, 7%); mp 57–59 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ +81 (*c* 0.4, MeOH); (Found: C 55.3, H 6.1; $C_8H_{11}ClO_2$ requires C 55.2, H 6.4%); *m*/*z* (EI) 174 (M$^+$, 32%), 156 (26), 93 (100); >98% ee (Method A); CD λ 209 nm ($\Delta\varepsilon$ 2.434), 279 nm ($\Delta\varepsilon$ 0.872).

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-4-bromo-3-ethylcyclohexa-3,5-diene 4B.** From substrate **4A** (0.031 g, 18%); mp 71–72 °C (from hexane); $[\alpha]_D$ +47 (*c* 0.4, MeOH); *m*/*z* (EI) (trimethylsilyl derivative from BSTFA) 364 [(^{81}Br)M$^+$, 20%], 362 [(^{79}Br)M$^+$, 18], 283 (M$^+$ − Br, 30), 73 (Me$_3$Si, 100); (Found: M$^+$ 217.9934; $C_8H_{11}{}^{79}BrO_2$ requires 217.9942); >98% ee (Method A); CD λ 211nm ($\Delta\varepsilon$ 1.36), 276 nm ($\Delta\varepsilon$ 0.701).

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-3-ethyl-4-iodocyclohexa-3,5-diene 5B.** From substrate **5A** (0.132 g, 46%); mp 92–94 °C (from $CHCl_3$); $[\alpha]_D$ +71 (*c* 0.7, MeOH); *m*/*z* (EI) 266 (M$^+$, 15%), 248 (M$^+$ − H_2O, 100), 139 (M$^+$ − I, 17); (Found: M$^+$ 265.9804; $C_8H_{11}IO_2$ requires 265.9804); >98% ee (Method A); CD λ 236 nm ($\Delta\varepsilon$ 2.55), λ 283 nm ($\Delta\varepsilon$ 1.55).

***cis*-(1*R*,2*R*)-1,2-Dihydroxy-3-ethyl-6-fluorocyclohexa-3,5-diene 6B.** From substrate **6A** (0.140 g, 55%); mp 80–81 °C (from hexane); $[\alpha]_D$ +147 (*c* 0.4, MeOH); *m*/*z* (EI) (trimethylsilyl derivative from BSTFA) 302 (M$^+$, 35%), 191 (25), 147 (45), 73 (Me$_3$Si, 100); (Found: M$^+$ 158.0741; $C_8H_{11}FO_2$ requires 158.0743); >98% ee (Method A); CD λ 209 nm ($\Delta\varepsilon$ 0.06), 264 nm ($\Delta\varepsilon$ 3.43).

***cis*-(1*R*,2*R*)-1,2-Dihydroxy-6-chloro-3-ethylcyclohexa-3,5-diene 7B.** From substrate **7A** (0.037 g, 8%); mp 90–91 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ +92 (*c* 0.4, MeOH); (Found: C 55.1, H 6.8; $C_8H_{11}ClO_2$ requires C 55.2, H 6.4%); *m*/*z* (EI) 174 (M$^+$, 46%), 156 (31), 93 (100); >98% ee (Method A); CD λ 217 nm ($\Delta\varepsilon$ 1.451), λ 282 nm ($\Delta\varepsilon$ 0.386).

***cis*-(1*R*,2*R*)-1,2-Dihydroxy-6-bromo-3-ethylcyclohexa-3,5-diene 8B.** From substrate **8A** (0.057 g, 32%); mp 116–118 °C (from $CHCl_3$); $[\alpha]_D$ +27 (*c* 0.4, MeOH); *m*/*z* (EI) (trimethylsilyl derivative from BSTFA) 364 [(^{81}Br)M$^+$, 10%], 362 [(^{79}Br)M$^+$, 10], 283 (M$^+$ − Br, 72), 73 (Me$_3$Si, 100); (Found: M$^+$ 217.9952; $C_8H_{11}{}^{79}BrO_2$ requires 217.9942); >98% ee (Method A); CD λ 218 nm ($\Delta\varepsilon$ 2.87), 283 nm ($\Delta\varepsilon$ 0.253).

***cis*-(1*R*,2*R*)-1,2-Dihydroxy-3-ethyl-6-iodocyclohexa-3,5-diene 9B.** From substrate **9A** (0.145 g, 51%); mp 42–44 °C (from $CHCl_3$); $[\alpha]_D$ +1.7 (*c* 0.7, MeOH); *m*/*z* (EI) 266 (M$^+$, 45%), 248 (M$^+$ − H_2O, 50), 121 (100); (Found: M$^+$ 265.9804; $C_8H_{11}IO_2$ requires 265.9804); >98% ee (Method A).

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-3-ethyl-5-fluorocyclohexa-3,5-diene 10B.** From substrate **10A** (0.029 g, 6%), mp 58–60 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ +99 (*c* 0.4, MeOH); (Found: C 61.1, H 7.2; $C_8H_{11}FO_2$ requires C 60.7, H 7.0%); *m*/*z* (EI) 258 (M$^+$, 64%), 140 (74); >98% ee (Method A); CD λ 212 nm ($\Delta\varepsilon$ −4.222), 279 nm ($\Delta\varepsilon$ 0.872).

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-5-chloro-3-ethylcyclohexa-3,5-diene 11B.** From substrate **11A** (0.023 g, 5%); mp 48–50 °C (from CH_2Cl_2–hexane), $[\alpha]_D$ +13 (*c* 0.8, MeOH); (Found: C 55.0, H 6.0; $C_8H_{11}ClO_2$ requires C 55.2, H 6.4%); *m*/*z* (EI) 174 (M$^+$, 4%), 156 (63), 141 (100); >98% ee (Method A); CD λ 209 nm ($\Delta\varepsilon$ −3.855), 274 nm ($\Delta\varepsilon$ 1.238).

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-5-bromo-3-ethylcyclohexa-3,5-diene 12B.** From substrate **12A** (0.014 g, 3%); mp 54–56 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ −10 (*c* 0.6, MeOH); (Found: C 44.1, H 5.5; $C_8H_{11}BrO_2$ requires C 43.9, H 5.1%); *m*/*z* (EI) 200 (M$^+$, 96%), 185 (100); >98% ee (Method A).

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-4-fluoro-3-propylcyclohexa-3,5-diene 14B.** From substrate **14A** (0.080 g, 21%); mp 75–76 °C (from CH_2Cl_2–hexane), $[\alpha]_D$ +42 (*c* 1.1, MeOH); (Found: M$^+$, 172.2006; $C_9H_{13}FO_2$ requires M$^+$, 172.2060); *m*/*z* (EI) 172 (M$^+$, 61%), 154 (18), 125 (60), 97 (100); >98% ee (Method A); CD λ 206 nm ($\Delta\varepsilon$ 1.028), 288 nm ($\Delta\varepsilon$ 0.147).

***cis*-(1*R*,2*R*)-1,2-Dihydroxy-6-fluoro-3-propylcyclohexa-3,5-diene 15B.** From substrate **15A** (0.055 g, 18%), mp 66–67 °C (from CH_2Cl_2–hexane); $[\alpha]_D$ +120 (*c* 1.1, $CHCl_3$); (Found: C 62.3, H 7.3; $C_9H_{13}FO_2$ requires C 62.8, H 7.6%); *m*/*z* (EI) 172 (M$^+$, 44%),

154 (20), 125 (68), 112 (100); >98% ee (Method A); CD λ 214 nm ($\Delta\varepsilon$ −5.136), 272 nm ($\Delta\varepsilon$ 2.84).

***cis*-(1*R*,2*R*)-1,2-Dihydroxy-6-chloro-3-propylcyclohexa-3,5-diene, 16B.** From substrate **16A** (0.312 g, 32%); mp 91–92 °C (from hexane); $[a]_D$ +45 (*c* 0.8, $CHCl_3$); (Found: C 57.3, H 6.8; $C_9H_{13}ClO_2$ requires C 57.3, H 6.95%); >98% ee (Method A); CD λ 218 nm ($\Delta\varepsilon$ 1.235), 276 nm ($\Delta\varepsilon$ 0.572).

***cis*-(1*R*,2*R*)-1,2-Dihydroxy-6-bromo-3-propylcyclohexa-3,5-diene 17B.** From substrate **17A** (0.281 g, 40%); mp 93 °C (from hexane); $[a]_D$ +68 (*c* 0.6, MeOH); (Found: C 46.2, H 5.5; $C_9H_{13}BrO_2$ requires C 46.4, H 5.6%); *m*/*z* (EI) 234 (M^+, 4%), 232 (4), 216 (39), 214 (40), 187 (98), 185 (100); >98% ee (Method A); CD λ 215 nm ($\Delta\varepsilon$ 2.228), 277 nm ($\Delta\varepsilon$ 0.943).

***cis*-(1*S*,2*R*)-1,2-Dihydroxy-5-chloro-3-propylcyclohexa-3,5-diene 19B.** From substrate **19A** (0.014 g, 2%); mp 61–63 °C (from $CHCl_3$); $[a]_D$ +42 (*c* 1.2, $CHCl_3$); (Found: M^+, 188.0602; $C_9H_{13}ClO_2$ requires M^+, 188.0604); *m*/*z* (EI) 190 (M^+, 20%), 188 (55), 172 (65), 170 (78), 143 (100), 141 (8); >98% ee (Method A); CD λ 211 nm ($\Delta\varepsilon$ −4.458), 268 nm ($\Delta\varepsilon$ 2.021).

Benzylic alcohol bioproducts 10C–12C, 18C, 19C

All benzylic alcohol products were found to be spectroscopically identical with literature data.

(*R*)-1-(3-Fluorophenyl)ethanol 10C. From substrate **10A** (0.017 g, 4%); $[a]_D$ +0.9 (*c* 0.8, MeOH) (lit.[49] $[a]_D$ +38.5, MeOH); 2% ee (Method B).

(*R*)-1-(3-Chlorophenyl)ethanol 11C. From substrate **11A** (0.012 g, 6%); $[a]_D$ +1.1 (*c* 0.8, MeOH) (lit.[50] $[a]_D$ +38.6, MeOH); 6% ee (Method B).

(*R*)-1-(3-Bromophenyl)ethanol 12C. From substrate **12A** (0.012 g, 6%); $[a]_D$ +4.8 (*c* 0.8, MeOH) (lit.[51] $[a]_D$ +29, MeOH); 18% ee (Method B).

(*R*)-1-(3-Fluorophenyl)propanol 18C. From substrate **18A** (0.010 g, 3%); $[a]_D$ +24.8 (*c* 0.36, $CHCl_3$); 94% ee (Method B).

(*R*)-1-(3-Chlorophenyl)propanol 19C (see ref. 52). From substrate **12A** (0.007 g, 1%); $[a]_D$ +7.0 (*c* 0.2, $CHCl_3$) (lit.[52] $[a]_D$ +31, MeOH); 46% ee (Method B).

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