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Dioxygenase-catalysed oxidation of alkylaryl sulfides: sulfoxidation *versus cis*-dihydrodiol formation

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Toluene- and naphthalene-dioxygenase-catalysed sulfoxidation of nine disubstituted methylphenyl sulfides, using whole cells of *Pseudomonas putida*, consistently gave the corresponding enantioenriched sulfoxides. Using the *P. putida* UV4 mutant strain, and these substrates, differing proportions of the corresponding *cis*-dihydrodiol sulfides were also isolated. Evidence was found for the concomitant dioxygenase-catalysed *cis*-dihydroxylation and sulfoxidation of methyl *para*-tolyl sulfide. A simultaneous stereoselective reductase-catalysed deoxygenation of (*S*)-methyl *para*-tolyl sulfoxide, led to an increase in the proportion of the corresponding *cis*-dihydrodiol sulfide. The enantiopurity values and absolute configurations of the corresponding *cis*-dihydrodiol metabolites from methyl *ortho*- and *para*-substituted phenyl sulfides were determined by different methods, including chemoenzymatic syntheses from the *cis*-dihydrodiol metabolites of *para*-substituted iodobenzenes. Further evidence was provided to support the validity of an empirical model to predict, (i) the stereochemistry of *cis*-dihydroxylation of *para*-substituted benzene substrates, and (ii) the regiochemistry of *cis*-dihydroxylation reactions of *ortho*-substituted benzenes, each using toluene dioxygenase as biocatalyst.

Introduction

The enzyme-catalysed asymmetric oxidation of sulfides, to yield enantiopure sulfoxides, using oxygenases and peroxidases, has been widely reported.[1–25] Ring hydroxylating dioxygenase enzymes from bacterial systems, in particular, have been involved in the oxidative biotransformation of sulfides to yield the corresponding enantiopure sulfoxides.[9–11,19–25] Thus, the soil bacterium *Pseudomonas putida*, and *Escherichia coli* recombinant strains containing the same toluene dioxygenase (TDO) or naphthalene-dioxygenase (NDO) enzymes , have yielded a range of sulfoxides (>30) with high (>90%) enantiomeric excess (*ee*) values. The dioxygenases are particularly efficient at catalysing sulfoxidation of sulfides having an *S*-aryl group (diaryl- or arylalkyl- sulfides) that can be accommodated within a shallow elongated hydrophobic pocket. TDO and NDO enzymes have been often found to yield: (i) sulfoxide enantiomers of either configuration (enantiocomplementarity) as major bioproducts from alkylaryl sulfides and only trace amounts of *cis*-dihydrodiol sulfides[10,19,24] and (ii) *cis*-dihydrodiol sulfides as major metabolites of dialkyl sulfides with the sulfoxides as very minor bioproducts.[10,20]

Recent studies conducted in our laboratories have revealed that, in some cases, further biotransformations of the aryl ring of sulfoxides have occurred to yield *cis*-dihydrodiol sulfoxides. Thus several alkylphenyl sulfides **1** were oxidised, using TDO as biocatalyst (*P. putida* UV 4), to yield the corresponding sulfoxides **2** and *cis*-dihydrodiol sulfoxides **3**, a trioxygenation process (Scheme 1).[21] Methylphenyl sulfide **4** was also found to undergo a tandem TDO-catalysed sulfoxidation/*cis*-dihydroxylation (**4**→**5**→**6**) and a reductase-catalysed *cis*-dihydrodiol sulfoxide deoxygenation process (**6**→**7**, Scheme 2) to yield the corresponding *cis*-dihydrodiol sulfide **7**, using an extended period of biotransformation and a higher cell density of *P. putida* UV4.[25] Although enzyme-catalysed deoxygenation of the *cis*-dihydrodiol sulfoxide **6**, to yield the *cis*-dihydrodiol sulfide **7**, was observed,[25] no direct evidence of the deoxygenation of sulfoxide **5** to yield the parent sulfide **4**, in the presence of the reverse reaction, was obtained (Scheme 2). The possibility of alkylaryl sulfoxide deoxygenation was further investigated using methyl-*p*-tolyl sulfoxide as substrate (**Results and discussion** section).

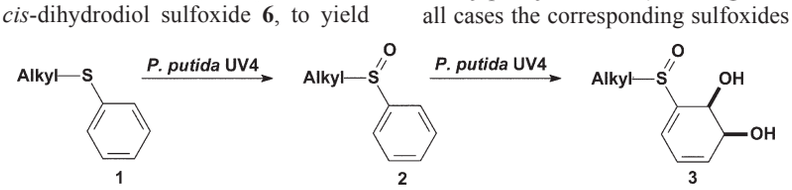
Apart from the formation of metabolite **7**,[19,25,26] few other alkylphenyl sulfide **1** examples of the dioxygenase-catalysed formation of similar *cis*-dihydrodiol sulfides are available and these (*e.g.* Alkyl = Pr^i, Bu^t[10]) were isolated in very low yields.

In this article, the focus is on the dioxygenase-catalysed biotransformation of a range of methyl *para*- (**8**–**12**) and *ortho*-substituted phenyl sulfides (**23**–**26**), where both the corresponding *cis*-dihydrodiol sulfide (**18**–**20**, **22**, **31**–**34**) and sulfoxide metabolites (**13**–**17**, **27**–**30**) were isolated. The *ee* values and absolute configurations of *cis*-dihydrodiols (**18**–**20**,**22**,**31**–**34**) were determined by a combination of chemoenzymatic synthesis, formation of chiral boronate derivatives prior to ^{1}H-NMR analysis, and circular dichroism (CD) spectroscopy. This study also provides an opportunity to evaluate the relative stereodirecting effect of the SMe group in comparison with other atoms and groups, in the context of TDO biocatalysis of *cis*-dihydroxylation of disubstituted benzene substrates. To predict the preferred stereochemical course of TDO-catalysed oxidation of *ortho*- and *para*-substituted thioanisoles, a refined model, based on our preliminary report[27] and reported in subsequent reviews,[28–30] is also presented.

Results and discussion

(a) Asymmetric sulfoxidation of sulfides 8–12, 23–26

An earlier report,[19] on the sulfoxidation of nine *para*-substituted methylphenyl sulfides (including substrates **8**–**12**), showed that in all cases the corresponding sulfoxides were obtained using whole



Scheme 1

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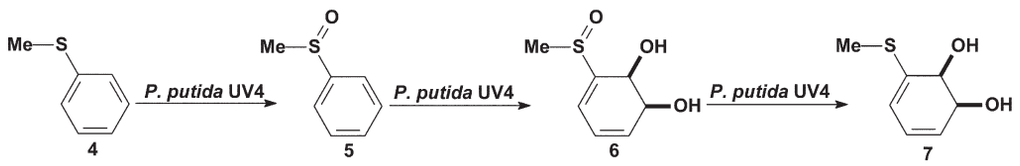


Scheme 2

Table 1 Absolute configurations (Abs. con.) and enantiomeric excess (*ee*) values of sulfoxides **13**–**17** and **27**–**30** from TDO- and NDO-catalysed sulfoxidation

Enzyme	TDO[a]			NDO[b]		
Sulfoxide	% Yield	Abs. con.	*Ee*(%)	% Yield	Abs. con.	*Ee*(%)
13	31[c],50	(+)-*R*	78[c],90	4[c]	(−)-*S*[c]	91[c]
14	2[c]	(−)-*S*	72[c]	5[c]	(−)-*S*[c]	>98[c]
15	2[c],<1	(−)-*S*	73[c]	35[c]	(−)-*S*[c]	>98[c]
16	<1[c]	(−)-*S*	70[c]	3[c]	(−)-*S*[c]	90[c]
17	3,[c] 8	(−)-*S*	18,[c]45	17[c]	(−)-*S*[c]	>98[c]
27	53	(+)-*R*	>98	61	(−)-*S*	>98
28	20	(+)-*R*	41	50	(−)-*S*	69
29	17	(+)-*R*	18	22	(−)-*S*	16
30	5	(−)-*S*	15	7	(−)-*S*	94

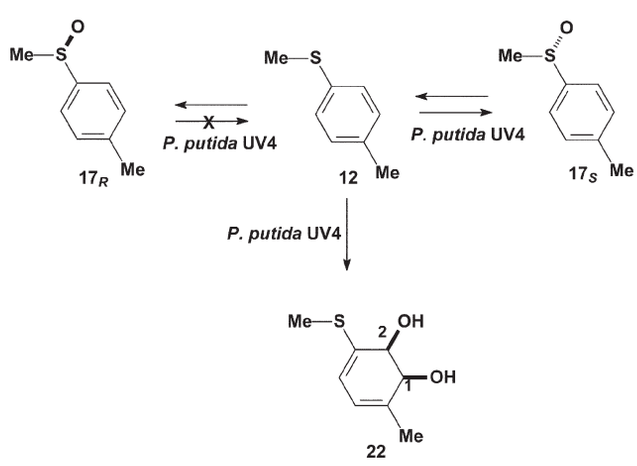
[a] *P. putida* UV4. [b] *P. putida* NCIMB 8859. [c] Reference 19.

cells of either a mutant strain (*P. putida* UV4, a source of TDO) or a wild-type strain (*P. putida* NCIMB 8859, a source of NDO). The earlier yields, absolute configurations and *ee* values of several of these sulfoxides (**13**–**17**)[19] have been combined with additional data and are reproduced in Table 1, to allow comparison with the sulfoxides **27**–**30**, isolated during the current study, using TDO- and NDO-catalysed monooxygenation of *ortho*-substituted methylphenyl sulfides **23**–**26**. As found earlier for sulfoxides **13**–**17**,[19] (≤50% yield), the isolated yields of sulfoxides **27**–**30** were again relatively low (5–53% using TDO, and 7–61% using NDO) and, as expected, generally decreased with increasing size of *ortho* or *para* substituents. The TDO and NDO dioxygenase-catalysed oxidations were found to be stereoselective; seven of the nine sulfoxides (**13**–**17**, **27** and **30**) were obtained with *ee* values of ≥90% (Table 1).

Although most of the absolute configurations of sulfoxides **27**–**30** were known, these assignments, were confirmed by CD spectral comparison, where typical strong positive Cotton effects were found in the region 240–260 nm for the (+)-(*R*) sulfoxide enantiomers **27**–**29** and a negative absorption for (−)-(*S*) sulfoxide **30**.

TDO and NDO biocatalysts and alkylphenyl sulfide substrates have often been found to produce sulfoxide enantiomers of opposite configurations.[10,19,24] This enantiocomplementary trend was not however reflected in the stereochemistry of the isolated methyl *para*-substituted phenyl sulfoxides $\mathbf{14}_S$–$\mathbf{17}_S$, since, with exception of sulfoxide $\mathbf{13}_R$, both TDO and NDO enzymes catalysed the preferential formation of the (*S*)-configuration (Table 1). Similarly, an enzyme-catalysed sulfoxidation of sulfides **9** and **12**, using a wild-type strain of *Pseudomonas frederiksbergensis*, was also recently reported to yield the corresponding (*S*) sulfoxides ($\mathbf{14}_S$ and $\mathbf{17}_S$ respectively) with high ee values (>95%).[31] This preference for the (*S*) enantiomers of sulfoxides **14**–**17**, using dioxygenase bacterial enzymes, contrasts with a strong preference (46→98% *ee*) for the (*R*) enantiomer of sulfoxides **13**–**15** and **17** when the corresponding sulfides (**8**–**10** and **12**) were biotransformed using monooxygenase fungal enzyme(s) found in *Mortierella isabellina*.[32] The isolated methyl *ortho*-substituted phenyl sulfoxides **27**–**29** obtained with *P. putida* UV4 (TDO) and *P. putida* NCIMB 8859 (NDO) did show evidence of enantiocomplementarity except for sulfoxide **30** (Table 1).

The *ee* values for the sulfoxide bioproducts **13**–**17**, derived from the corresponding *para*-substituted methylphenyl sulfide substrates **8**–**12**, and *P. putida* UV4, were generally high (>70%) with the exception of sulfoxide **17** (18%).[19] In order to further investigate these results, the biotransformation of sulfides **8**, **10** and **12** were repeated. The sulfoxides **13** (50% yield), **15** (<1% yield) and **17** (8% yield) were again isolated; *cis*-dihydrodiol sulfides **18**, **20** and **22** were the other bioproducts (see section b). The (*R*) and (*S*) enantiomers of methyl *para*-tolyl sulfoxide **17** (>99% *ee*) were each added as substrates to *P. putida* UV4. While the (*R*) enantiomer, $\mathbf{17}_R$, was recovered unchanged (>99% *ee*), the (*S*) enantiomer, $\mathbf{17}_S$, was partially biotransformed into *cis*-dihydrodiol sulfide **22** and the recovered sulfoxide was found to be a mixture of enantiomers $\mathbf{17}_S$ (96%) and $\mathbf{17}_R$ (4%). This result is consistent with the initial slow formation of both sulfoxide enantiomers $\mathbf{17}_S$/$\mathbf{17}_R$ favouring the (*S*) enantiomer, and a kinetic resolution involving the exclusive deoxygenation of the same enantiomer (Scheme 3). It is thus possible that sulfoxide **17**, in common with sulfoxides **14**–**16**, was initially formed by asymmetric synthesis with a higher proportion of the (*S*) enantiomer. However, the final *ee* value (18–45%) was lower due to a competing kinetic resolution process involving preferential deoxygenation of the (*S*) enantiomer $\mathbf{17}_S$ to form sulfide **12** which was in turn mainly converted to the *cis*-dihydrodiol sulfide **22**. This example provides the first direct evidence of a stereoselective sulfoxide reductase enzyme in *P. putida* UV4 being able to accept an acyclic alkylaryl sulfoxide substrate and supports the view[25] that the whole cells contain a sulfoxide reductase enzyme. This result, allied to earlier evidence from cyclic sulfoxides,[25] suggests that deoxygenation may be a relatively common, but generally hidden, minor metabolic pathway during the formation of enantioenriched sulfoxides (including metabolites **13**–**16** and **27**–**30**).



Scheme 3

Table 2 Yields, absolute configurations (Abs. con.), and enantiomeric excess (*ee*) values of *cis*-dihydrodiol metabolites **18**–**20**, **22**, **31**–**34**, **39**–**42** from the corresponding disubstituted benzene substrates (**8**–**10**, **12**, **23**–**26**, **35**–**38**) obtained using *P. putida* UV4

cis-Dihydrodiol	Yields(%)	Abs. con.	*Ee*(%)
18	2,20	1*R*,2*S*	>98
19	3	1*R*,2*S*	>98
20	8,39	1*R*,2*S*	>98
22	20,40	1*S*,2*S*	>98
31	4	1*S*,2*S*	>98
32	24	1*S*,2*S*	>98
33	16	1*S*,2*S*	>98
34	1	1*S*,2*S*	>98
39	40	1*R*,2*S*	88
40	68	1*R*,2*S*	22
41	83	1*R*,2*S*	15
42	40	1*S*,2*S*	76

(b) Asymmetric *cis*-dihydroxylation of sulfides 8–10, 12, 23–26

The earlier study, using the UV4 and NCIMB 8859 strains of *P. putida* for dioxygenase-catalysed oxidations of alkylaryl sulfides,[19] was focused exclusively on the sulfoxide metabolites. The formation of *cis*-dihydrodiol bioproducts **18**–**20**, **22**, from sulfides **8**–**10**, **12**, was not discussed in this earlier report. This was due to the lack of formation of *cis*-dihydrodiol metabolites from any of the nine substrates using the NCIMB 8859 wild-type strain. Furthermore, four of the *para*-substituted methylphenyl sulfides (including substrate **11** and similar compounds, *e.g.* R = MeO, CN, CF_3, Scheme 4) gave no *cis*-dihydrodiols (*e.g.* compound **21**) using the UV4 mutant strain. Where *cis*-dihydrodiols were formed, *e.g.* diols **18**–**20**, from sulfides **8**–**10** (UV4 mutant strain), these were generally found in low yields (2–8%). *cis*-Dihydrodiol **20** has however recently been isolated from sulfide **10** in good yield (2.3 g L^{-1}) by Hudlicky *et al.* using the recombinant strain *E. coli* JM109(pDTG601).[33] Metabolites **18**–**20**, in common with the unstable *cis*-dihydrodiol **7**,[25,33] contain the electron-donating SMe group which has been found to facilitate spontaneous dehydration to yield the corresponding phenolic products.[34]

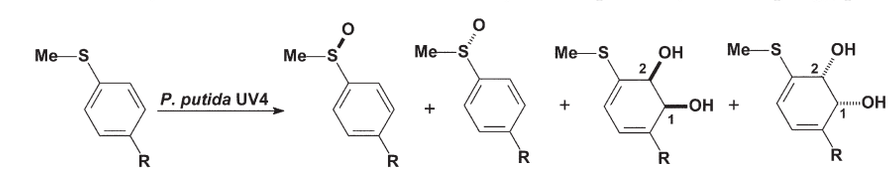
The result of our first biotransformation run, using methyl *para*-tolyl sulfide **12** with *P. putida* UV4, was exceptional; it gave a higher yield (20%) of the corresponding *cis*-dihydrodiol sulfide **22** relative to the sulfoxide **17** (3%, Scheme 3). In light of this increased yield of *cis*-dihydrodiol sulfide **22**, and the recently observed three-step metabolic sequence to yield *cis*-dihydrodiol sulfide **7** from methylphenyl sulfide **4** (Scheme 2),[25] it was considered appropriate to investigate further the formation of the metabolite **22** from methyl *para*-tolyl sulfide **12** and also *cis*-dihydrodiols **18**–**20** isolated earlier. With the help of an improved extraction procedure for harvesting the bioproducts (involving complete removal of water from the culture medium containing the bioproducts prior to ethyl acetate extraction), the yield of *cis*-dihydrodiol sulfide **22** was found to be higher (40%) compared with yields of *cis*-dihydrodiols **18**–**20** (2–8%, Table 2) obtained earlier from this and the other *para*-substituted methylphenyl sulfides, using the standard extraction procedure (ethyl acetate extraction of aqueous solutions saturated with NaCl).

The formation of *cis*-dihydrodiol sulfide **22**, as a major metabolite from the corresponding sulfide substrate **12** in *P. putida* UV4, could have resulted from either a sulfoxidation-*cis*-dihydroxylation-deoxygenation sequence similar to that found for methylphenyl sulfide **4** (Scheme 2) or a direct *cis*-dihydroxylation of the sulfide substrate **4** (Scheme 3). However, when biotransformations of either sulfide **12** or the (*R*) and (*S*) enantiomers of sulfoxide **17** were carried out (section a), no evidence of a *cis*-dihydrodiol sulfoxide metabolite, analogous to compound **6**, was found. Based on the latter observation, it was concluded that *cis*-dihydrodiol sulfide **22** was formed by direct *cis*-dihydroxylation of sulfide **12**. It is noteworthy that when (*R*)-methyl *para*-tolyl sulfoxide **17** was used as substrate, no bioproducts were observed, while the (*S*)-enantiomer yielded the corresponding *cis*-dihydrodiol sulfide **22**. The higher proportion of isolated *cis*-dihydrodiol sulfide **22** (20–40% yield) relative to sulfoxide **17** (3–8%), may be rationalised in terms of partial sulfoxide deoxygenation to yield sulfide **12** which was in turn dihydroxylated to yield *cis*-diol sulfide **22**.

Biotransformations of the *para*-substituted- (**8**, **10**, **12**) and *ortho*-substituted-methyl phenyl sulfides (**23**–**26**) yielded the corresponding *cis*-dihydrodiols (**18**–**20**, **31**–**34**) using *P. putida* UV4 (Schemes 4, 5 and Table 2) in addition to the corresponding sulfoxides (**13**–**15**, **17**, **27**–**30**, Table 2). The isolated yields of *cis*-dihydrodiols **18**–**20**, **31**–**34** were generally low (1–24%), employing the standard extraction procedure. However, repeat biotransformations of sulfides **8**, **10** and **12**, using *P. putida* UV4 and the improved extraction procedure, gave higher yields of *cis*-dihydrodiols **18** (20%), **20** (39%) and **22** (40%), Table 2. It is assumed that *cis*-dihydrodiol sulfides **18**–**20**, **31**–**34** were also formed from a direct asymmetric *cis*-dihydroxylation of the aryl ring of sulfides **8**–**10** and **23**–**26**.

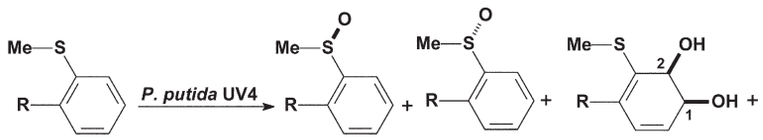
The determination of *ee* values and absolute configurations of the *cis*-dihydrodiols **18**–**20**, **31**–**34** was an important consideration, as TDO-catalysed *cis*-dihydroxylations of *para*-substituted benzene substrates in *P. putida* UV4 have been found to yield enantiomeric mixtures of the corresponding *cis*-dihydrodiol metabolites according to the stereodirecting effects of substituents.[26,27] Furthermore, while *ortho*-substituted benzene substrates were consistently found to yield enantiopure *cis*-dihydrodiols, their regiochemistry was again dependent on the dominant stereodirecting groups.[27–30] In view of the likelihood that both enantiomers of *cis*-dihydrodiol metabolites **18**–**20**, **22**, obtained from methyl *para*-substituted phenyl sulfides **8**–**10**, **12**, (using *P. putida* UV4), would be formed, suitable methods for the determination of *ee* values were developed. One of the methods involved the use of chiral stationary phase HPLC (CSPHPLC) analysis using a Chiralcel OJ column that had been found to separate a different range of *cis*-dihydrodiols isolated from *para*-substituted benzene substrates.[27] Each of the *cis*-dihydrodiol samples **18**–**20**, **22** eluted as a single peak from the CSPHPLC column (Chiralcel OJ) suggesting that these had been formed as single enantiomers; unequivocal confirmation however required both enantiomers to be available for CSPHPLC analysis.

Both enantiomers of the *cis*-dihydrodiols **18**–**20**, and **22** were chemically synthesised from the corresponding *cis*-dihydrodiol metabolites **39**–**42**, available from TDO-catalysed *cis*-dihydroxylation of the appropriate *para*-substituted iodobenzene substrates **35**–**38** (Scheme 6). Palladium-catalysed cross coupling of the *cis*-dihydrodiol metabolites of iodobenzenes with a range of tributyltin compounds (Stille coupling), provides a convenient che-



R = F	8	13_R	13_S	$18_{1R,2S}$	$18_{1S,2R}$
R = Cl	9	14_R	14_S	$19_{1R,2S}$	$19_{1S,2R}$
R = Br	10	15_R	15_S	$20_{1R,2S}$	$20_{1S,2R}$
R = I	11	16_R	16_S	$21_{1R,2S}$	$21_{1S,2R}$
R = Me	12	17_R	17_S	$22_{1S,2S}$	$22_{1R,2R}$

Scheme 4



R = F	23	27_R	27_S	$31_{1S,2S}$
R = Cl	24	28_R	28_S	$32_{1S,2S}$
R = Br	25	29_R	29_S	$33_{1S,2S}$
R = I	26	30_R	30_S	$34_{1S,2S}$

Scheme 5

moenzymatic route to the elusive *cis*-dihydrodiols of alkylphenyl sulfides;[26] this method was used on *cis*-dihydrodiols **39**–**42**. The sulfide *cis*-dihydrodiols, **18**–**22**, after chromatographic purification, were obtained having similar *ee* values to the corresponding substrates (**39**–**42**, 15–88%, Scheme 6, Table 2). With both enantiomers of the *cis*-dihydrodiols **18**–**20** and **22** available, it was confirmed that (i) all enantiomeric pairs were separable by the Chiralcel-OJ column and (ii) the *cis*-dihydrodiol metabolites formed directly from sulfides **8**–**10** and **12** were indeed enantiopure (>98% *ee*). *cis*-Dihydrodiol **20** has also recently been obtained with a high *ee* value using *E. coli* JM109(pDTG601).[33]

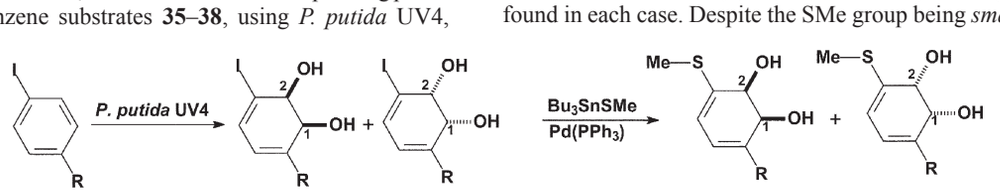
The absolute configurations of the enantioenriched *cis*-dihydrodiols **39**–**42** had been unequivocally established by hydrogenolysis to remove the iodine atom and to give an excess (15–88% *ee*) of the unnatural-configuration *cis*-dihydrodiols of fluorobenzene, chlorobenzene, bromobenzene and toluene of known configurations.[26,35] These unnatural *cis*-dihydrodiols of monosubstituted benzenes have been obtained in enantiopure form by recrystallisation or by kinetic resolution using them as substrates in further biotransformations with wild-type strains of *P. putida*.[28] The isolation of the *para*-substituted *cis*-dihydrodiols **18**–**20** and **22** as single (2*S*) enantiomers, provides a potential method to access the unnatural pure (1*R*) *cis*-dihydrodiols of fluorobenzene, chlorobenzene, bromobenzene and toluene by removal of the SMe group. This route is currently being investigated in our laboratories.

The *ee* values and absolute configurations of *cis*-dihydrodiols **31**–**34**, obtained from methyl *ortho*-substituted phenyl sulfides **23**–**26**, were determined by formation of the corresponding boronate esters (MEPBA) from both the (−)-(*S*)-2-(1-methoxyethyl)phenyl]boronic acid and its racemic form. This method depends upon ^{1}H-NMR spectral analysis of the MeO singlets associated with the diastereoisomeric boronates formed using one *cis*-dihydrodiol enantiomer and the racemic boronic acid or the reverse combination. Utilization of MEPBA derivatives has been successfully applied to a range of *cis*-dihydrodiol metabolites.[20,36,37] Using this method, *cis*-dihydrodiols **31**–**34** were found to be of >98% *ee* and were assigned the (1*S*,2*S*) configuration, in common with all earlier *cis*-dihydrodiols derived from TDO-catalysed dihydroxylation of 1,2-disubstituted benzene substrates.[28–30] The identical (1*S*,2*S*) configurations for *cis*-dihydrodiols **31**–**34** were confirmed by a comparison of their CD spectral data.

(c) Predictive model for the regio- and stereo-directing effects of aryl substituents during TDO-catalysed *cis*-dihydroxylation

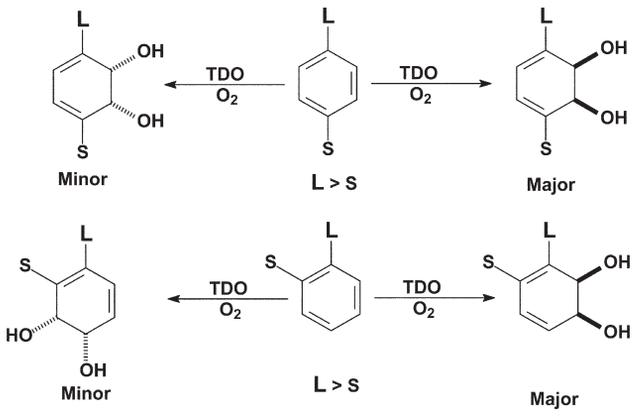
The *cis*-dihydrodiols **39**–**42**, derived from the corresponding *para*-substituted iodobenzene substrates **35**–**38**, using *P. putida* UV4, were mixtures of enantiomers. Comprehensive studies, carried out in our laboratories, on a wider range of 1,4-disubstituted benzene substrates (>25), have shown that in general terms the preferred configuration of the major *cis*-dihydrodiol is largely determined by the difference in size of spherically symmetrical substituents at the 1,4-positions; the dominance decreases in the sequence CF_3 > I > Br > Cl ≥ Me > F > H (Scheme 7).[27–30] Thus the largest substituents (**L**, *e.g.* CF_3 and I) had a dominating stereodirecting effect over the smallest substituents (**S**, *e.g.* F and H). One measure of the size of substituents (**L** and **S**) can be estimated from the Charton steric parameter (ν). However, when the preferred (2*S*)-geometry of the *cis*-dihydrodiol bioproducts **18**–**20** and **22**, obtained from TDO-catalysed dihydroxylation of the methyl *para*-substituted phenyl sulfides **8**–**10** and **12**, is considered, it becomes evident that the SMe group (ν = 0.60), although *smaller* than the Br atom (ν = 0.65), is a more dominant stereodirecting group than the Br, Cl (ν = 0.55) or F atom (ν = 0.27). This suggests that a more appropriate parameter that can take account of the possible non-symmetrical substituent conformations, *e.g.* the Verloop steric parameter, would be more appropriate for the (Scheme 7) predictive model. Our original empirical model for the stereochemistry of *cis*-dihydrodiol metabolites, obtained by TDO-catalysed oxidation of 1,4-disubstituents,[27–30] thus may require slight modification where non-spherically symmetrical substituents such as SMe are present. Recent results have also shown that the non-spherically symmetrical carbomethoxy substituent (CO_2Me, ν = 1.39) is again a dominant stereodirecting group (unpublished data). It seems that while the size of substituents, based on Charton steric parameters, is clearly an important factor, other considerations *e.g.* substituent length and conformation in the vicinity of an active site, should also be taken into consideration.

A stereodirecting effect of the larger group (**L**) during TDO-catalysed dihydroxylation of 1,2-disubstituted benzene substrates was demonstrated by the preferred regiochemistry of the major *cis*-dihydrodiol bioproduct, since each regioisomer has been found to be enantiopure. A predictive model for *ortho*-substituted benzene substrates, similar to that for *para* substituted benzene substrates, had thus been proposed earlier (Scheme 7).[27–30] The effect of the dominant stereodirecting group (**L**) will, in this case, be observed from a preference for *cis*-dihydroxylation at the proximate unsubstituted double bond leading to the formation of the corresponding regioisomer (Scheme 7). In the context of TDO-catalysed *cis*-dihydroxylation of the *ortho*-substituted methylphenyl sulfides **23**–**26**, to yield the corresponding *cis*-dihydrodiols **27**–**30**, the SMe group (ν = 0.60) is clearly dominant over the I atom (ν = 0.78) and the other substituents (F, Cl, Br) since only one regioisomer was found in each case. Despite the SMe group being *smaller* than both



R = F	35	$39_{1R,2S}$	$39_{1S,2R}$	$18_{1R,2S}$	$18_{1S,2R}$
R = Cl	36	$40_{1R,2S}$	$40_{1S,2R}$	$19_{1R,2S}$	$19_{1S,2R}$
R = Br	37	$41_{1R,2S}$	$41_{1S,2R}$	$20_{1R,2S}$	$20_{1S,2R}$
R = Me	38	$42_{1S,2S}$	$42_{1R,2R}$	$22_{1S,2S}$	$22_{1R,2R}$

Scheme 6



Scheme 7

the Br and I atoms (according to the Charton steric parameters), it is evidently a stronger stereodirecting group, in terms of enantioselectivity and regioselectivity. These results demonstrate that while the size of substituent is clearly an important factor for spherically symmetrical groups, other parameters such as preferred conformations or effective lengths of groups can also be equally important in predicting the stereo- and regio-preference during TDO catalysed *cis*-dihydroxylation of substituted benzenes (Scheme 7).

In the absence of X-ray crystallographic data on the TDO enzyme, and an accepted mechanism for dioxygenase-catalysed mono- and di-hydroxylation, it is not possible to be definitive about the preference of this enzyme for sulfoxidation over *cis*-dihydroxylation with alkylphenyl sulfides and the reverse preference with dialkyl sulfides. Based on the reports from recent X-ray crystallographic and mechanistic studies of the NDO enzyme,[38–42] allied to results from this study, a possible stereochemical model for the observed TDO-catalysed sulfoxidation and *cis*-dihydroxylation reactions is presented (Fig. 1).

If it were to be assumed that TDO and NDO have a similar type and shape of binding site, *i.e.* a shallow cavity surrounded by hydrophobic amino acid groups, and that the dioxygen molecule is found bound in a side-on position to Fe(III) (a cyclic peroxide which is readily converted to hydroperoxide by a one electron reduction process),[38–42] then either a single atom or two oxygen atoms can be delivered to the proximate lower face of the substrate. This model could account for formation, regiochemistry and stereochemistry of both monooxygenation of sulfur (sulfoxidation) and carbon atoms (benzylic hydroxylation) attached to a benzene ring, and dioxygenation of the benzene ring (*cis*-dihydroxylation). The oxygen-atom-transfer to methylphenyl sulfide **4** can occur to either the nearby sulfur (sulfoxidation to yield compound **5**) or aryl carbon atoms (*cis*-dihydroxylation to yield **7**), with a strong preference for the former (Fig. 1a). However, the biotransformation of methyl *para*-tolyl sulfide **12** was found to yield mainly the *cis*-dihydrodiol **22** with less of the sulfoxide **17** being formed (Fig. 1b).

Conclusion

Whole cell biotransformations of nine methyl *para*- and *ortho*-substituted phenyl sulfides have been carried out using *P. putida* strains containing both TDO and NDO enzymes to yield the corresponding sulfoxides. Enantioselectivity, during the sulfoxidation, was found to be generally higher (≥90% *ee*) for seven of the nine sulfoxides obtained under NDO biocatalysis. Unequivocal evidence of a kinetic resolution process involving exclusive deoxygenation of (*S*)-methyl *para*-tolyl sulfoxide in *P. putida* UV4 has been discovered and a similar process could be occurring with the other sulfoxides.

Using the UV4 mutant strain of *P. putida*, enantiopure *cis*-dihydrodiol metabolites of both methyl *ortho*- and *para*-substituted phenyl sulfides have been isolated and stereochemically assigned. The *ee* values and absolute configurations of the *cis*-dihydrodiol metabolites, obtained from the methyl *para*-substituted phenyl sulfides, were determined by chemoenzymatic synthesis from the corresponding *cis*-dihydrodiol derivatives from *para*-substituted iodobenzene substrates. The stereochemistry of the *cis*-dihydrodiols, formed from methyl *ortho*-substituted phenyl sulfides was determined by formation of diastereoisomeric chiral boronate esters and CD spectroscopy.

In light of the results reported in this study, the validity of a simple stereochemical model proposed in an earlier communication,[27] was verified and the model updated. This model was used to predict the preferred stereoselectivity and regiochemistry of *cis*-dihydrodiols formed from both *ortho*- and *para*-substituted benzene substrates by TDO-catalysed *cis*-dihydroxylation.

Experimental

^{1}H-NMR spectra of compounds were recorded on Bruker Avance DPX-300 and DPX-500 instruments. Flash column chromatography and PLC were performed on Merck Kieselgel type 60 (250–400 mesh) and $PF_{254/366}$ respectively. Merck Kieselgel $60F_{254}$ analytical plates were used for TLC. Optical rotation ($[\alpha]_D$) measurements were carried out with a Perkin-Elmer 214 polarimeter at ambient temperature (*ca.* 20 °C) and are given in units of 10^{-1} deg cm^2 g^{-1}. Due to the unstable nature and small quantities of *cis*-dihydrodiol metabolites available, the optical rotation values reported may be less than optimal. CD spectra were recorded using a JASCO J-720 instrument and spectroscopic grade methanol as solvent.

The sulfides **8**–**11**, **23**–**26**, and the corresponding racemic sulfoxides **13**–**17**, **27**–**30**, were obtained by the literature methods while sulfide **12**, the corresponding (*R*) and (*S*) sulfoxide enantiomers $\mathbf{17}_S$ and $\mathbf{17}_R$ and substituted iodobenzenes **35**–**38** were available commercially. Sulfide substrates **23**–**26** (0.1–1.0 g) were metabolized, using growing cultures of the mutant strain *Pseudomonas putida* (Strain UV4) or the wild-type strain *Pseudomonas putida* NCIMB *8859* (Strain 8859) according to the method reported earlier for the *para* substituted methylphenyl sulfides **8**–**12**.[19] The bioproducts were generally harvested by repeated solvent extrac-

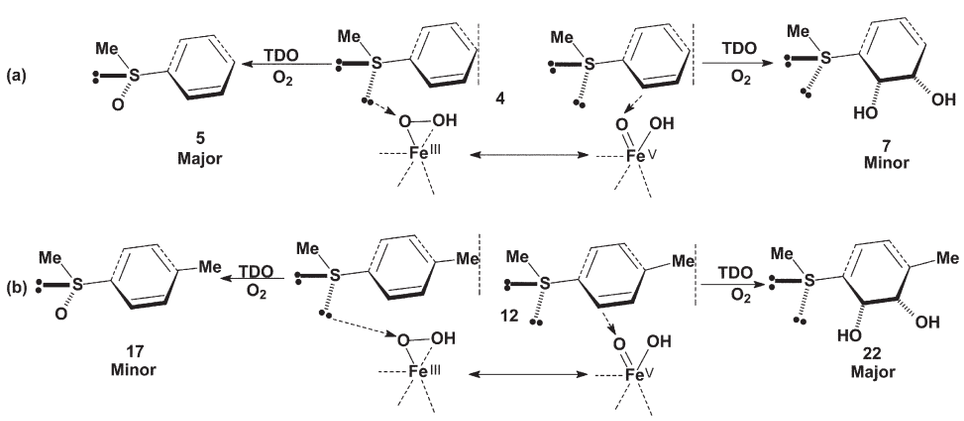


Fig. 1

tion (EtOAc) of the sodium chloride-saturated aqueous solution containing the biotransformed material, and concentration of the combined organic extracts under reduced pressure. In specified cases, an improved extraction procedure, involving removal of water under reduced pressure at 40 °C, prior to ethyl acetate extraction, was used. TLC and ^{1}H NMR spectral analyses, were routinely carried out, before using any purification procedure. The higher R_f sulfoxides were, in each case, separated from the more polar *cis*-dihydrodiol metabolites by PLC ($CHCl_3$: MeOH, 9 : 1).

The yields, *ee* values and absolute configurations of sulfoxide metabolites **13**–**17** of *para*-substituted methylphenyl sulfides **8**–**12**, found using the UV4 and 8859 strains, were reported earlier.[19] The sulfoxidation results obtained, using the same bacterial strains and methyl *ortho*-substituted phenyl sulfides **23**–**26** as substrates, are presented below. The *ee* values of the sulfoxide or *cis*-dihydrodiol bioproducts were determined by ^{1}H-NMR analysis after addition of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (Method A, sulfoxides), CSPHPLC using the specified Chiralcel column (Method B, sulfoxides and *cis*-dihydrodiols), stereochemical correlation, comparison of $[\alpha]_D$ values (Method C, *cis*-dihydrodiols), and addition of (−)-(*S*)- and (+)-(*R*)-2-(1-methoxyethyl)phenyl boronic acid (MEPBA) and ^{1}H-NMR analysis (Method D, *cis*-dihydrodiols).

Sulfoxide metabolites 27–30 formed by biotransformation using *P. putida* strains UV4 and 8859

2-Fluorophenylmethyl sulfoxide 27_R and 27_S from sulfide 23. Strain UV4; an oil, (0.118 g, 53%); bp 116–117 °C/15 mm Hg (Lit.,[43] 103–106 °C/3 mm Hg); (*R*) configuration; $[\alpha]_D$ +163 (*c* 1.6, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 2.84 (3 H, s, Me), 7.13 (1 H, m, Ar–H), 7.43 (1 H, m, Ar–H), 7.51(1 H, m, Ar–H), 7.86 (1 H, m, Ar–H); CD: 242.9 nm Δε 9.57, 216 nm Δε −1.17, 199.7 nm Δε 8.09; *ee* >98% (Method A).

Strain 8859; (0.056 g, 61%); (−)-(*S*) configuration *ee* >98% (Method A).

2-Chlorophenylmethyl sulfoxide 28_R and 28_S from sulfide 24. Strain UV4; an oil; (0.055 g, 20%); (*R*) configuration; $[\alpha]_D$ +116 (*c* 1.2, $CHCl_3$) (Lit.,[44] $[\alpha]_D$ −189, acetone; *S* configuration; 69% *ee*); δ_H (500 MHz, $CDCl_3$) 2.78 (3 H, s, Me), 7.39 (1 H, m, Ar–H), 7.44 (1 H, m, Ar–H), 7.53 (1 H, m, Ar–H), 7.96 (1 H, m, Ar–H); CD: 249.4 nm Δε 6.83, 215.9 nm Δε −6.60, 198.4 nm Δε 8.43; *ee* 41% (Method B, Chiralcel OD, *α* 1.1).

Strain 8859; (0.11 g, 50%); (−)-(*S*) configuration; *ee* 69% (Method B).

2-Bromophenylmethyl sulfoxide 29_R and 29_S from sulfide 25. Strain UV4; an oil; (0.045 g, 17%); (*R*) configuration; $[\alpha]_D$ +35 (*c* 1.8, $CHCl_3$); (Lit.,[45] $[\alpha]_D$ −145, acetone; *S* configuration; 58% *ee*); δ_H (500 MHz, $CDCl_3$) 2.82 (3 H, s, Me), 7.37 (1 H, m, Ar–H), 7.56–7.61 (2 H, m, Ar–H), 7.95 (1 H, m, Ar–H); CD: 250.6 nm Δε 3.19, 212.0 nm Δε −1.76, 201.7 nm Δε 1.54; *ee* 18% (Method B, Chiralcel OD, *α* 1.1).

Strain 8859; (0.048 g, 22%); (−)-(*S*) configuration; *ee* 16% (method B).

2-Iodophenylmethyl sulfoxide 30_S from sulfide 26. Strain UV4; an oil, (0.01 g, 5%); (*S*) configuration; $[\alpha]_D$ −25 (*c* 0.6, $CHCl_3$) (Lit.,[46] +ve $[\alpha]_D$ value; *R* configuration); δ_H (500 MHz, $CDCl_3$) 2.79 (3 H, s, Me), 7.22 (1 H, m, Ar–H), 7.62 (1 H, m, Ar–H), 7.82 (1 H, m, Ar–H), 7.91 (1 H, m, Ar–H); CD: 255.3 nm Δε −1.58, 214.3 nm Δε 1.10, 203.4 nm Δε −1.41; *ee* 15% (Method B, Chiralcel OD, *α* 1.07).

Strain 8859; (0.014 g, 7%); (−)-(*S*) configuration; *ee* 94% (Method B).

cis-Dihydrodiol metabolites 18–20, 22 and 31–34 obtained by biotransformation of disubstituted benzene substrates using *P. putida* UV4 and by chemoenzymatic synthesis

(+)-(1*R*,2*S*)-1,2-Dihydroxy-3-methylthio-6-fluorocyclohexa-3,5-diene $18_{1R,2S}$ from sulfide 8 and from *cis*-dihydrodiol 39. (0.220 g, 20%, from sulfide **8**); mp 120–122 °C (EtOAc/hexane); $[\alpha]_D$ +97 (*c* 0.73, $CHCl_3$); (Found: M^+, 176.0305. $C_7H_9O_2SF$ requires 176.03730); δ_H (300 MHz, $CDCl_3$) 2.28 (3 H, s, Me), 2.37 (1 H, d, $J_{1,OH}$ 3.1, OH), 2.77 (1 H, d, $J_{2,OH}$, OH), 4.35 (1 H, dd, $J_{1,F}$ 11.2 $J_{1,2}$, 5.3, 1-H), 4.47 (1 H, m, H-2), 5.38 (1 H, $J_{4,5}$ 6.0, H-4), 5.68 (1 H, dd, $J_{5,F}$ 10.7, $J_{5,4}$ 6.7, 5-H); *m*/*z* (EI) 176 (M^+, 100%); *ee* >98% (Method B, Chiralcel OJ, *α* 1.5); CD: 299.9 nm Δε 1.37, 223.5 nm Δε 0.58, 200.4 nm Δε −7.87.

Synthesised from cis-dihydrodiol **39**: (0.020 g, 30%); (+)-(1*R*,2*S*) configuration; *ee* 72% (Method C).

(+)-(1*R*,2*S*)-1,2-Dihydroxy-3-methylthio-6-chlorocyclohexa-3,5-diene $19_{1R,2S}$ from sulfide 9 and from *cis*-dihydrodiol 40. (0.004 g, 3%, from sulfide **9**); mp 100–101 °C (EtOAc/hexane); $[\alpha]_D$ +4 (*c* 0.43, $CHCl_3$); (Found: M^+, 192.0002. $C_7H_9O_2SCl$ requires 192.0001) δ_H (300 MHz, $CDCl_3$) 2.28 (3 H, s, Me), 2.45 (2 H, br s, 2 × OH), 4.26 (1 H, d, $J_{1,2}$ 5.5, 1-H), 4.46 (1 H, d, $J_{2,1}$ 5.2, 2-H), 5.42 (1 H, $J_{4,5}$ 6.4, H-4), 6.16 (1 H, d, $J_{5,4}$ 6.5, 5-H); *m*/*z* (EI) 194 (M^+, 20%), 192 (M^+, 57%); *ee* >98% (Method B, Chiralcel OJ, *α* 1.65); CD: 233.5 nm Δε 0.32, 209.2 nm Δε −1.44.

Synthesised from cis-dihydrodiol **40**: (0.065 g, 31%); (+)-(1*R*,2*S*) configuration; *ee* 15% (Method C).

(+)-(1*R*,2*S*)-1,2-Dihydroxy-3-methylthio-6-bromocyclohexa-3,5-diene $20_{1R,2S}$ from sulfide 10 and from *cis*-dihydrodiol 41. (0.365 g, 39%, from sulfide **10**); mp 67–71 °C (Lit.,[33] mp 59–63 °C), $[\alpha]_D$ −15.6 (*c* 0.65, $CHCl_3$), (Lit.,[33] $[\alpha]_D$ −10, $CHCl_3$); (Found: M^+, 237.9494. $C_7H_9O_2SBr$ requires 237.9452); δ_H (300 MHz, $CDCl_3$) 2.27 (3 H, s, Me), 2.45 (1 H, d, $J_{OH,2}$ 8.4, OH), 2.79 (1 H, d, $J_{OH,1}$ 8.4, OH), 4.32 (1 H, d, $J_{1,2}$ 5.5, 1-H), 4.44 (1 H, m, 2-H), 5.35 (1 H, d $J_{4,5}$ 6.2, H-4), 6.38 (1 H, d, $J_{5,4}$ 6.3, 5-H); *m*/*z* (EI) 238 (M^+, 24%), 236 (23%), 142 (100); *ee* >98% (Method B, Chiralcel OJ, *α* 1.51); CD: 329.6 nm Δε −0.130, 291.4 nm Δε 0.23, 276.3 nm Δε 0.23, 234.0 nm Δε 0.79, 211.0 nm Δε −1.17.

Synthesised from cis-dihydrodiol **41** (0.040 g, 53%); (+)-(1*R*,2*S*) configuration; *ee* 22% (Method C).

(+)-(1*S*,2*S*)-1,2-Dihydroxy-3-methylthio-6-methylcyclohexa-3,5-diene $22_{1S,2S}$ from sulfide 12, (*S*)-methyl *para*-tolylsulfoxide 17_S and from *cis*-dihydrodiol 42. White silky needles (0.5 g, 40%, from sulfide **12**); mp 88–94 °C (EtOAc/hexane); $[\alpha]_D$ +31 (*c* 0.95, $CHCl_3$); (Found: M^+, 172.0568. $C_8H_{12}O_2S$ requires 172.0558); δ_H (300 MHz, $CDCl_3$) 1.89 (1 H, d, $J_{OH,2}$ 9.1, OH), 1.92 (3 H, s, Me), 2.27 (3 H, s, SMe), 2.41 (1 H, d, $J_{OH,1}$ 9.1, OH), 4.07 (1 H, dd, $J_{1,OH}$ 9.0 $J_{1,2}$ 5.6, 1-H), 4.24 (1 H, dd,$J_{2,OH}$ 9.8 $J_{1,2}$ 5.6, 2-H), 5.49 (1 H, d $J_{4,5}$ 5.8, H-4), 5.76 (1 H, d, $J_{5,4}$ 5.8, 5-H); *m*/*z* (EI) 172 (M^+, 100%), 154 (63%); *ee* >98% (Method C); CD: 301.8 nm Δε 0.67, 206.5 nm Δε −3.74.

This biotransformation of sulfide **12** also yielded (*S*)-methyl *para*-tolyl sulfoxide 17_S (0.1 g, 17%); *ee* 45%.

From (*S*) *methyl para-tolyl sulfoxide* 17_S (0.1 g, 20%); and recovered substrate 17_S *ee* (0.095 g, 18%); *ee* 92% (Methods A and B, Chiralcel OD–H, *α* 1.2).

Synthesised from cis-dihydrodiol **42**: (0.030 g, 44%); (+)-(1*S*,2*S*) configuration; *ee* 38% (Method C).

cis-Dihydrodiol metabolites 39–42 obtained by biotransformation of disubstituted benzene substrates 35–38 with *P. putida* UV4

(+)-(1*R*,2*S*)-1,2-Dihydroxy-3-iodo-6-fluorocyclohexa-3,5-diene $39_{1R,2S}$. (0.8 g, 40%); mp 126–128 °C (EtOAc/hexane); $[\alpha]_D$ +62 (*c* 0.67, MeOH); (Found: M^+, 255.9405. $C_6H_6O_2FI$ requires 255.9366); δ_H (500 MHz, $CDCl_3$) 4.39 (2 H, m, 1-H, 2-H), 5.43 (1 H, dd, $J_{5,F}$ 9.7, $J_{5,4}$ 6.4, 5-H), 6.56 (1 H, dd, $J_{4,F}$ 5.8, $J_{4,5}$ 6.2, 4-H); *m*/*z* (EI) 256 (M^+, 85%), 238 (29), 129 (78), 83 (100); *ee* 88% (Method B, Chiralcel OJ, *α* 1.2); CD: 266 nm Δε 2.06, 215 nm Δε −1.2.

(+)-(1*R*,2*S*)-1,2-Dihydroxy-3-iodo-6-chlorocyclohexa-3,5-diene $40_{1R,2S}$. (0.39 g, 68%); mp 116–117 °C ($CHCl_3$/hexane); $[\alpha]_D$ +6 (*c* 0.72, MeOH); (Found: C, 26.1; H, 2.0, $C_6H_6O_2ICl$

requires C, 26.4; H, 2.2%); δ_H (500 MHz, $CDCl_3$) 4.38 (1 H, d, $J_{2,1}$ 6.6, 2-H), 4.50 (1 H, dd, $J_{1,2}$ 6.6, $J_{1,5}$ 1.8, 1-H), 5.93 (1 H, d, $J_{5,4}$ 6.3, $J_{5,1}$ 1.8, 5-H), 6.65 (1 H, d, $J_{4,5}$ 6.3, 4-H); *m*/*z* (EI) 272 (M^+, 68%), 254 (6), 237 (2), 109 (100); *ee* 22% (Method B, Chiralcel OJ, α 1.12); CD: 293 nm $\Delta\varepsilon$ 1.77, 243 nm $\Delta\varepsilon$ −6.28, 222 nm $\Delta\varepsilon$ 1.00.

(+)-(1*R*,2*S*)-1,2-Dihydroxy-3-iodo-6-bromocyclohexa-3,5-diene $41_{1R,2S}$. (0.37 g, 83%); mp 125–127 °C ($CHCl_3$/hexane); $[\alpha]_D$ +5 (*c* 0.76, MeOH); (Found: C, 22.6; H, 1.6, $C_6H_6O_2BrI$ requires C, 22.7; H, 1.9%); δ_H (500 MHz, $CDCl_3$) 4.43 (2 H, m, 1-H, 2-H), 6.11 (1 H, d, $J_{5,4}$ 6.3, 5-H), 6.53 (1 H, dd, $J_{4,5}$ 6.3, $J_{4,2}$ 1.1, 4-H); *m*/*z* (EI) 318 (M^+, 16%), 316 (15), 300 (6), 110 (100); *ee* 15% (Method B, Chiralcel OJ, α 1.13); CD: 291 nm $\Delta\varepsilon$ 3.28, 246 nm $\Delta\varepsilon$ −1.17, 221 nm $\Delta\varepsilon$1.40.

(+)-(1*S*,2*S*)-1,2-Dihydroxy-3-methyl-6-iodo-cyclohexa-3,5-diene $42_{1S,2S}$. (0.53 g, 40%); mp 85–87 °C (EtOAc); $[\alpha]_D$ +4.0 (*c* 0.83, MeOH; *ee* 98%); (Found: M^+, 251.9666. $C_7H_9O_2I$ requires 251.9649); δ_H (300 MHz, $CDCl_3$) 1.90 (3 H, s, Me), 4.21 (1 H, d, $J_{1,2}$ 5.7, 1-H), 4.29 (1 H, d, $J_{2,1}$ 5.6, 2-H), 5.49 (1 H, d, $J_{4,5}$ 5.8, 4-H); 6.59 (1 H, d, $J_{5,4}$ 6.0, 1-H); *m*/*z* (EI) 252 (M^+, 23%), 79 (100); *ee* 76% (Method B, Chiralcel OJ, α 1.08); CD: 280 nm $\Delta\varepsilon$ 1.05, 232 nm $\Delta\varepsilon$ −3.71.

(+)-(1*S*,2*S*)-1,2-Dihydroxy-3-methylthio-4-fluorocyclohexa-3,5-diene $31_{1S,2S}$ from sulfide 23. (0.010 g, 4%); R_f 0.36 (10% MeOH/$CHCl_3$); mp 72–73 °C ($CHCl_3$); $[\alpha]_D$ +110 (*c* 0.9, MeOH); (Found: M^+, 176.0350. $C_7H_9O_2SF$ requires 176.0373); δ_H (300 MHz, $CDCl_3$) 2.39 (3 H, s, Me), 3.27–3.52 (2 H, br s, OH), 4.27 (1 H, d, $J_{2,1}$ 5.7, 2-H), 4.50 (1 H, m, 1-H), 5.90 (2 H, m, 5-H, 6-H); *m*/*z* (EI) 176 (M^+, 21%), 158 (100), 143 (65); *ee* >98% (Method D, MEPBA formation); CD: 330.8 nm $\Delta\varepsilon$ 0.27, 292.8 nm $\Delta\varepsilon$ −.1.45, 250.8 nm $\Delta\varepsilon$ 0.75, 232.8 nm $\Delta\varepsilon$ 0.13.

(+)-(1*S*,2*S*)-1,2-Dihydroxy-3-methylthio-4-chlorocyclohexa-3,5-diene $32_{1S,2S}$ from sulfide 24. (0.071 g, 24%); R_f 0.35 (10% MeOH/$CHCl_3$); mp 67–68 °C ($CHCl_3$/MeOH); $[\alpha]_D$ +103 (*c* 1.0, MeOH); (Found: C 43.6, H 4.4; $C_7H_9SO_2Cl$ requires C 43.6, H, 4.7%); δ_H (300 MHz, $CDCl_3$) 2.26 (1 H, br s, OH), 2.44 (3 H, s, Me), 2.71 (1 H, br s, OH), 4.30 (1 H, d, $J_{2,1}$ 5.8, 2-H), 4.53 (1 H, ddd, $J_{1,2}$ 5.7, $J_{1,6}$ 2.5, $J_{1,5}$ 0.9, 1-H), 5.78 (1 H, dd, $J_{5,6}$ 9.8, $J_{5,1}$ 0.9, 5-H), 5.92 (1 H, dd, $J_{6,5}$ 10.0, $J_{6,1}$ 2.3, 6-H); *m*/*z* (EI) 192 (M^+, 24%), 174 (100), 159 (65), 131 (60); *ee* >98% (Method D, MEPBA formation); CD: 334.6 nm $\Delta\varepsilon$ 1.38, 295.6 nm $\Delta\varepsilon$ −.0.73, 265.8 nm $\Delta\varepsilon$ 0.31, 232.0 nm $\Delta\varepsilon$ 4.02, 211.6 nm $\Delta\varepsilon$ −3.51.

(+)-(1*S*,2*S*)-1,2-Dihydroxy-3-methylthio-4-bromocyclohexa-3,5-diene $33_{1S,2S}$ from sulfide 25. (0.045 g, 16%); R_f 0.34 (10% MeOH/$CHCl_3$); mp 57–58 °C ($CHCl_3$); $[\alpha]_D$ +123 (*c* 1.0, MeOH); (Found: C 34.6, H 3.5; $C_7H_9SO_2Br$ requires C 34.5, H, 3.8%); δ_H (300 MHz, $CDCl_3$) 2.45 (3 H, s, Me), 4.27 (1 H, d, $J_{2,1}$ 5.8, 2-H), 4.55 (1 H, dd, $J_{1,2}$ 5.8, $J_{1,6}$ 2.6, 1-H), 5.67 (1 H, dd, $J_{5,6}$ 9.8, $J_{5,1}$ 1.0, 5-H), 6.03 (1 H, dd, $J_{6,5}$ 9.8, $J_{6,1}$ 2.6, 6-H); *m*/*z* (EI) 236 (M^+, 12%), 218 (100), 203 (27), 175 (18); *ee* >98% (Method D, MEPBA formation); CD: 333.2 nm $\Delta\varepsilon$ 1.25, 295.2 nm $\Delta\varepsilon$ −.1.10, 255.2 nm $\Delta\varepsilon$ 1.26, 245.2 nm $\Delta\varepsilon$ 0.96, 231.2 nm $\Delta\varepsilon$ 2.74, 211.2 nm $\Delta\varepsilon$ −5.80.

(+)-(1*S*,2*S*)-1,2-Dihydroxy-3-methylthio-4-iodocyclohexa-3,5-diene $34_{1S,2S}$ from sulfide 26. (0.003 g, 1%); R_f 0.35 (10% MeOH/$CHCl_3$); mp 52–54 °C ($CHCl_3$); $[\alpha]_D$ +139 (*c* 0.3, MeOH); δ_H (500 MHz, $CDCl_3$) 2.46 (3 H, s, Me), 4.21 (1 H, d, $J_{2,1}$ 5.5, 2-H), 4.51 (1 H, dd, $J_{1,2}$ 5.5, $J_{1,6}$ 2.7, 1-H), 5.53 (1 H, d, $J_{5,6}$ 9.9, 5-H), 6.23 (1 H, dd, $J_{6,5}$ 9.8, $J_{6,1}$ 2.6, 6-H); *m*/*z* (EI) 284 (M^+, 24%), 266 (100), 251 (15), 139 (9), 109 (50); *ee* >98% (Method D, MEPBA formation); CD: 336.4 nm $\Delta\varepsilon$ 1.03, 298.4 nm $\Delta\varepsilon$ −.1.17, 232.4 nm $\Delta\varepsilon$ 2.13.

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