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Complete List of Authors:	Boyd, Derek; Queens University Belfast, Chemistry and Chemical Engineering Sharma, narain; Queens University Belfast, Chemistry and Chemical Engineering Berberian, Victoria; Queens University Belfast, Chemistry and Chemical Engineering Cleij, Marcel; Queens University Belfast, Chemistry and Chemical Engineering Hardacre, Christopher; Queen's University, Chemistry and Chemical Engineering Ljubez, Vera; Queens University Belfast, Chemistry and Chemical Engineering McConville, Gareth; Queens University Belfast, Chemistry and Chemical Engineering Stevenson, Paul; Queens University, School of Chemistry and Chemical Engineering Kulakov, Leonid; Queens University, School of Biological Sciences Allen, C; Queens University, School of Biological Sciences
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Arene *cis*-Diol Dehydrogenase-Catalysed Regio- and Stereo-Selective Oxidation of Arene-, Cycloalkane- and Cycloalkene-*cis*-Diols to Yield Catechols and Chiral α-Ketols

Derek R. Boyd,[a,b*] Narain D. Sharma,[a,b] M. Victoria Berberian,[a,b] Marcel Cleij,[b] Christopher Hardacre,[a,b] Vera Ljubez,[b] Gareth M[c] Conville,[b] Paul J. Stevenson,[b] Leonid A. Kulakov and [c] Christopher C. R. Allen[c*]

[a]CentTACat, [b]School of Chemistry and Chemical Engineering, Queen's University of Belfast, Belfast BT9 5AG, UK [c] School of Biological Sciences, Queen's University of Belfast, Belfast, BT9 5AG, UK

FAX: (+44)-28-9097-4687; e-mail: dr.boyd@qub.ac.uk

FAX: (+44)-28-9097-5877; e-mail:c.allen@qub.ac.uk

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Dedicated to the memory of Professor David T. Gibson, a good friend and excellent scientist.

Abstract: Benzene *cis*-diol dehydrogenase and naphthalene *cis*-diol dehydrogenase enzymes, expressed in *Pseudomonas putida* wild-type and *Escherichia coli* recombinant strains, were used to investigate regioselectivity and stereoselectivity during dehydrogenations of arene, cyclic alkane and cyclic alkene vicinal *cis*-diols. The dehydrogenase-catalysed production of enantiopure *cis*-diols, α- ketols and catechols, using benzene *cis*-diol dehydrogenase and naphthalene *cis*-diol dehydrogenase, involved both kinetic resolution and asymmetric synthesis methods. The chemoenzymatic production and applications of catechol bioproducts in synthesis were investigated.

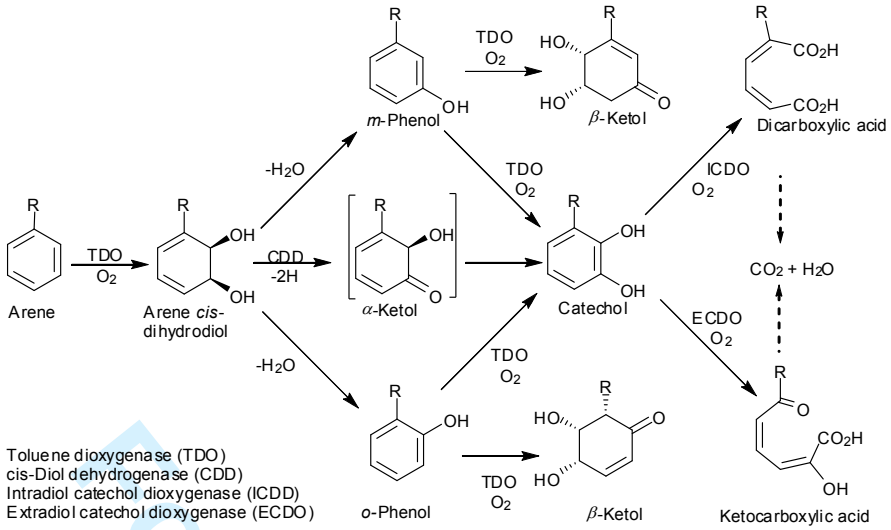
Keywords: Alkene/arene *cis*-1,2-diols; catechols; *cis*-diol dehydrogenases; α-ketols; stereoselectivity

Introduction

Arene *cis*-dihydrodiols, resulting from aromatic ring hydroxylating dioxygenase-catalysed oxidation, are initial metabolites formed during bacterial biodegradation of the corresponding monocyclic arenes and are valuable synthetic precursors.[1a-o] Other arene derivatives and metabolites include α-ketols/catechols,[2a-e] *o*-/*m*-phenols,[3a-d] β-ketols[4a-f] and carboxylic acids.[5a,5b] (Scheme 1). The vast majority of chiral *cis*-dihydrodiols, formed from monosubstituted benzene substrates, *e.g.* **1c-h**, with toluene dioxygenase (TDO) as biocatalyst, are single enantiomer *cis*-dihydrodiols, resulting from dihydroxylation at the arene 2,3-bond and having an [*S*] configuration at C-1 (*e.g.* **2c$_S$-h$_S$**, Scheme 2).[6a,6b]

cis-Dihydrodiol enantiomers of type **2$_R$** and regioisomers of types **3$_S$** or **3$_R$** are rarely produced as arene metabolites. [6a-f] This may account for the regio- and stereo-selectivity aspects of *cis*-diol dehydrogenase-catalysed formation of catechols having received relatively little attention.[7a-h] The recent development of chemoenzymatic routes to 2,3-*cis*-dihydrodiol enantiomers **2$_R$**,[8a,8b] *cis*-dihydrodiol diastereoisomers **2$_S$**[8c,8d] and 3,4-*cis*-dihydrodiol regioisomers **3$_S$** [8e] provided the opportunity for further studies of regio- and stereo-selectivity of benzene *cis*-diol dehydrogenase (BCDD) and naphthalene *cis*-diol dehydrogenase (NCDD) enzymes, during formation of catechols **4** or **5** (Scheme 2).

The structure, biochemistry and mechanism of *cis*-diol dehydrogenases from *Pseudomonas* species, belong to the type II short chain alcohol dehydrogenase / reductase family (SDR). They have been investigated, using a range of methods including X-ray crystallography and molecular modelling. These methods have recently been utilized for studies of the biphenyl *cis*-diol dehydrogenase (BphB) that catalyses the



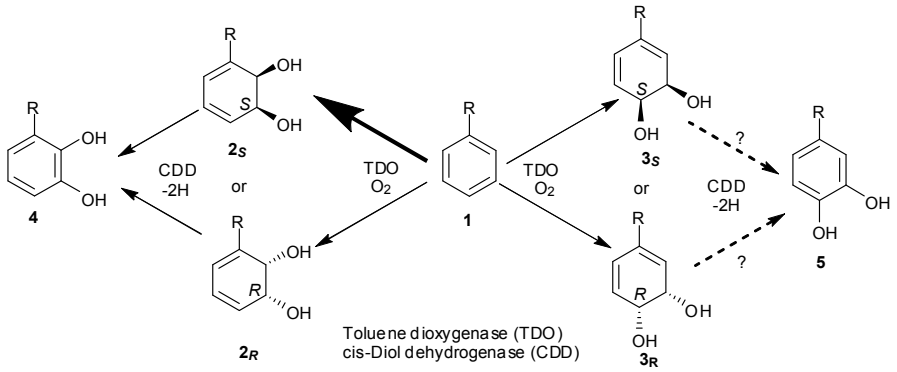
Scheme 1. Enzyme-catalysed oxidation of arenes in bacteria to yield *cis*-dihydrodiols, of phenols to give α- and β- ketols and/or catechols leading to carboxylic acids.

dehydrogenation of 2,3-*cis*-dihydrodiol metabolite of biphenyl.[9a,9b] The mechanism involving abstraction of a proton from the more accessible OH group in the *cis*-dihydrodiol substrate (and structural pattern adopted by BphB are similar to those associated with most other SDR enzymes (Scheme 1).[2e]

cis-Dihydrodiol metabolites are not normally isolated from biotransformations of monocyclic arenes, when using wild-type strains of *P. putida*, *e.g.* NCIMB 11767 and NCIMB 8859, but *P. putida* NCIMB 12910 (ML2) proved to be an exception.[10a] The different *cis*-diol dehydrogenase enzymes, expressed in relevant strains, *e.g.* toluene *cis*-diol dehydrogenase (TCDD) in *P. putida* 11767, NCDD in *P. putida* 8859 and BCDD in *P. putida* ML2, all catalysed the dehydrogenation of monocyclic *cis*-dihydrodiols **2**, to yield catechols **4**. Since these catechols undergo further metabolism they were not found as bioproducts. The development of a *P. putida* double mutant[7a] and *E. coli*[2b, 7b-e] recombinant strains, containing both dioxygenase (*e.g.* TDO) and *cis*-diol dehydrogenase (*e.g.* TCDD) enzymes, allowed a range of catechols **4** to be formed directly from the corresponding monosubstituted arenes **1**. The yields of catechols **4**, obtained from the biotransformation of monocyclic arenes **1**, by the combination of two biocatalysts, were limited by the catechol toxicity and feed-back control, inhibiting the TDO enzyme activity required to produce the *cis*-dihydrodiol intermediates **2** (Scheme 2).[7a,7f]

An alternative approach, involving biotransformation of monosubstituted benzene substrates, *e.g.* **1b**, using a combination of a biocatalyst (TDO) with a chemocatalyst (Pd/C) was also found to yield the corresponding catechol, *e.g.* **4b**.[7g] However, this tandem effect of two catalysts resulted in a disproportionation reaction, producing equal quantities of catechol and the corresponding *cis*-tetrahydrodiol. With *cis*-dihydrodiol substrates **2b*s*-h*s*** available from earlier large-scale biotransformations of arene substrates **1b-h**, using the constitutive mutant strain *P. putida* UV4, and with access to a new recombinant strain, *E. coli narB*, containing the *narB* gene and expressing NCDD*narB*,[7h,7i] it was possible to produce multigram quantities of some of the corresponding catechols **4b-h**.[7g]

The names attributed to dioxygenase enzymes are based on their natural arene substrates *e.g.* benzene, toluene, naphthalene and biphenyl. The accommodating capacities of these dioxygenase active sites, being dependent on the substrate dimensions, are thus expected to increase in the sequence: benzene dioxygenase (BDO) < toluene dioxygenase (TDO) < naphthalene dioxygenase (NDO) < biphenyl dioxygenase (BPDO). As arene dioxygenase genes are closely linked with *cis*-diol dehydrogenase genes, in wild-type operons of *P. putida*, a similar correlation between size of substrate and capacity of active site,



Scheme 2. Dioxygenase (TDO)-catalysed formation of *cis*-2,3-dihydrodiols $\mathbf{2}_{S}$ or $\mathbf{2}_{R}$ or *cis*-3,4-dihydrodiols $\mathbf{3}_{S}$ or $\mathbf{3}_{R}$ from monosubstituted benzene substrates **1** and *cis*-diol dehydrogenase (CDD)-catalysed desaturation to yield catechols **4** or **5**.

would be expected for both types of enzyme. This trend was observed in earlier studies, where optimal activity was associated with BDO-catalysed *cis*-dihydroxylation of the smallest monocyclic parent arene **1a**, to yield arene *cis*-dihydrodiol **2a**. Optimal *cis*-diol dehydrogenase activity was then found when using BCDD with the smallest arene *cis*-diol **2a** as substrate to yield catechol **4a**.[10a-c] Similarly, the activity of NCDD was found to be optimal, when using larger *cis*-diol metabolites, derived from naphthalene or benzene substrates with bulky substituents *e.g.* **2** (R = Ph) and **6** (R = CF_3, *t*-Bu; Scheme 3) and using NDO.[7g,10b] The difference in the relative capacities of active sites of BCDD and NCDD enzymes should play an important role in the regio- and stereo-selective catalysed dehydrogenation reactions of vicinal *cis*-diols by these enzymes.

When employing *P. putida* ML2 strain for the current study, it was considered important to establish the isoenzyme responsible for the stereoselectivity, since two different *cis*-diol dehydrogenase enzymes (BCDD*bedD* or BCDD*BedD*) had been isolated and identified earlier.[10b,10c] Although the BCDD*bedD* enzyme, encoded by the *bedD* gene ($BCDD_{bedD}$), showed good activity with 1,2 propane diol and *cis*-1,2-cyclohexane diol, the only acceptable arene *cis*-dihydrodiol substrate was found to be the achiral parent compound **2a**.[10b] The BCDD*BedD* enzyme, encoded by the *BedD* gene ($BCDD_{BedD}$), showed a similar activity with these three substrates. However, while the chiral *cis*-dihydrodiol metabolites $\mathbf{2b}_{S}$, $\mathbf{2c}_{S}$ and $\mathbf{2f}_{S}$, derived from fluorobenzene, chlorobenzene and toluene, were acceptable substrates, the largest member of the group, $\mathbf{2h}_{S}$, was not.[10b] During the earlier biotransformations of the unnatural *cis*-dihydrodiol enantiomers $\mathbf{2b}_{R}$, $\mathbf{2c}_{R}$ and $\mathbf{2d}_{R}$ to catechols, using *P. putida* ML2 cells,[10a] it appeared that the BCDD*bedD* isoenzyme was mainly responsible for the preference shown for the $\mathbf{2}_{R}$ enantiomers. Without further investigation, an unequivocal confirmation of this preference could not be provided.

In our earlier studies, with different wild-type strains of *P. putida* and enantiomeric mixtures of monocyclic *cis*-dihydrodiol substrates, *e.g.* $\mathbf{2b}_{S}$ / $\mathbf{2b}_{R}$, enantiocomplementarity was observed. Thus, *P. putida* ML2 whole cells had an exclusive preference for the relatively rare $\mathbf{2}_{R}$ enantiomers,[10a] and *P. putida* 8859 for the more common $\mathbf{2}_{S}$ enantiomers (Scheme 2).[8a,8d] In this study, using a mixture of *cis*-dihydrodiol enantiomers $\mathbf{2b}_{R}$ / $\mathbf{2b}_{S}$, enantioselectivity of TCDD, expressed in the *P. putida* NCIMB 11767 wild-type strain, was found to be rather poor. Hence we focused our investigation on the relative activities of *P. putida* strains expressing BCDD and NCDD. The earlier kinetic resolution studies, using *P. putida* ML2[10a] and 8859 wild-type strains,[8a] provided a kinetic resolution route to either *cis*-dihydrodiol enantiomer but the catechol bioproducts were not recovered, due to mineralization *via* water-soluble and relatively unstable carboxylic acid intermediates (Scheme 1). A similar enantioselectivity trend was observed using partially purified BCDD and NCDD enzymes where *cis*-dihydrodiol enantiomers **2a-h** were converted to catechols **4a-h** albeit on a very small scale.[10a]

A recent report convincingly demonstrated the value of bacterial *trans*-diol dehydrogenase enzymes, as biocatalysts for the stereoselective

dehydrogenation of cyclic vicinal *trans*-diols and formation of chiral α-ketols.[10e] A complementary study of stereoselectivity, observed during *cis*-diol dehydrogenase-catalysed dehydrogenation of cyclic vicinal *cis*-diols and formation of chiral α-ketols, is presented herein. Major objectives of this programme include: (i) a study of the regio- and stereo-selectivity of BCDD and NCDD enzymes expressed in wild-type *P. putida* strains and *E. coli* recombinant strains, (ii) an evaluation of BCDD and NCDD as biocatalysts for production of chiral ketols *via* asymmetric synthesis and kinetic resolution and (iii) the application of catechol metabolites in synthesis.

Results and Discussion

Regio- and stereo-selectivity of BCDD- and NCDD-catalysed dehydrogenation of cyclic *cis*-dihydrodiols during the formation of catechols and ketols

(i) Enantioselectivity of BCDD and NCDD enzymes during the dehydrogenation of *cis*-dihydrodiols 2 to yield catechols

Practical procedures for the TDO-catalysed synthesis of *cis*-dihydrodiols from the corresponding arenes have been widely reported using both *P. putida* mutant, *e.g.* 39/D or UV4, and *E. coli* recombinant strains, *e.g.* JM109(pDTG601).[1a-o] These biotransformations can readily be scaled up and high isolated yields are often obtained. *cis* Diols **2c$_S$-e$_S$** have all been obtained using *P. putida* UV4 with yields > 80% and each has been scaled up (>100 g). *cis*-Dihydrodiol **2a** was produced in kilogram quantities (>1 kg) from a single biotransformation of benzene **1a,** using a 150 litre fermentor. *cis*-Diol dehydrogenase-catalysed reactions are easier to conduct than arene dioxygenase-catalysed reactions, due to the increased solubility of *cis*-diols, tolerance of higher substrate concentrations (>25 g/L) and no special requirement for oxygenation.

The construction of a new *E. coli* clone, containing the benzene *cis*-diol dehydrogenase gene (BCDD$_{bedD}$), allowed further aspects of enantioselectivity to be investigated. The *bedD* gene was cloned into the pBAD expression vector system, using arabinose as a relatively inexpensive inducer for the new *E. Coli bedD* recombinant strain, to optimise and achieve the highest possible levels of enzyme expression. The enantioselectivity of the BCDD*bedD* enzyme, expressed in *E. coli bedD*, was tested using enantiomeric mixtures of the *cis*-dihydrodiols **2b$_S$** / **2b$_R$**. Addition of enantioenriched substrate **2b$_R$** (32% *ee*) yielded catechol **4b** (40%), with the residual **2b$_S$** enantiomer being recovered in similar yield (40%) and >98% *ee (*Scheme 2). The experiment was repeated, with further enantioenriched substrate **2b$_R$** of 80% *ee*, and catechol **4b** was isolated in higher yield (60%), along with the remaining enantiopure *cis*-dihydrodiol **2b$_S$** (10% yield, >98% *ee*). This result provided strong evidence that BCDD*bedD* was the enzyme mainly responsible for dehydrogenation of *cis*-dihydrodiol **2$_R$** enantiomers, when using *P. putida* ML2.

(ii) Regio- and stereo-selectivity of NCDD during the dehydrogenation of *cis*-dihydrodiol 3d$_S$ to yield catechol 5d

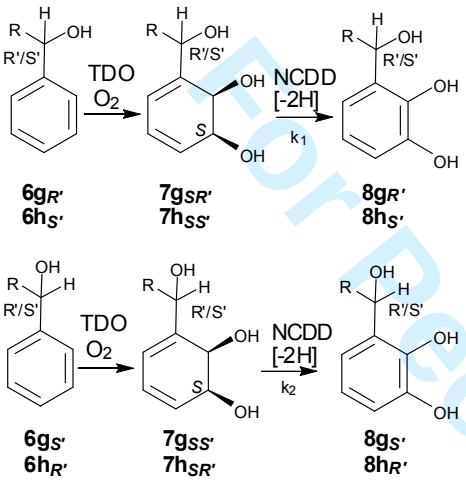
Although the NCDD enzyme was found to catalyse the preferential dehydrogenation of **2$_S$** enantiomers of 2,3-*cis*-dihydrodiols, its ability to accept the isomeric 3,4-*cis*-dihydrodiols **3$_S$**, as substrates, had not been investigated in the past, due to their unavailability (Scheme 2). Several 3,4-*cis*-dihydrodiols **3$_S$** have been reported in recent years, as very minor metabolites,[6c,6e,6f] and others have been obtained by a four-step chemoenzymatic synthesis, from the corresponding 2,3-*cis*-dihydrodiols **2$_S$**.[8d] A sample of 3,4-*cis*-dihydrodiol **3d$_S$**, available from the latter study, when used as a substrate for *E.coli narB* yielded bromocatechol **5d** in excellent yield (84%). Thus, demonstrating that NCDD*narB* activity, expressed in *E.coli narB,* was not restricted to 1,2-*cis*-dihydrodiols **2$_S$**. Based on this observation, it is expected that enantiomers **3$_S$** of: (i) 3,4- *cis*-dihydrodiol metabolites, *e.g.* **3$_S$** / **3$_R$** (R = Ph, 2-Py), resulting from NDO-catalysed *cis*-dihydroxylation of the corresponding arene substrates **1**[6c,6e,6f] and (ii) other chemoenzymatically produced 3,4-regioisomers, *e.g.* **3c$_S$**, **3e$_S$**, **3f$_S$**,[8e] would also be utilized as substrates for NCDD.

(iii) Regio- and stereo-selectivity of NCDD, present in *E. coli narB*, during the dehydrogenation of triol diastereoisomers 7g$_{SR'}$, 7h$_{SS'}$, 7g$_{SS'}$, and 7h$_{SR'}$ to yield the corresponding catechols 8g$_{R'}$, 8h$_{S'}$, 8g$_{S'}$ and 8h$_{R'}$

Triols **7$_{SR'}$** (R = Me, Et, Pr, *t*-Bu) had been produced earlier, from *cis*-dihydroxylation of the corresponding alkyl benzenes **1**, using *P. putida* UV4 cultures, without any evidence of the alternative diastereoisomers **7$_{SS'}$** being formed.[8b] The individual benzylic alcohol enantiomers **6$_{R'}$** and **6$_{S'}$** (R = Me, Et and Pr) as substrates, under

similar conditions, yielded the corresponding triol diastereoisomers $\mathbf{7}_{SR'}$ and $\mathbf{7}_{SS'}$. When these diastereoisomeric triols were used as substrates for NCDD, the resulting crude catechols $\mathbf{8}_{R'}$ and $\mathbf{8}_{S'}$ (R = Me, Et and Pr) were found to be unstable,[10d] and decomposed into unidentified products, during attempted chromatographic purification; these catechols were therefore characterized as dimethyl ether derivatives.

The study of regio- and diastereo-selectivity of NCDD*narB*, using *E.coli narB* for biotransformation of triols **7**, required the resulting catechols **8** to be stable, unlike catechols $\mathbf{8}_{R'}$ and $\mathbf{8}_{S'}$



Scheme 3. TDO-catalysed formation of triols $\mathbf{7g}_{SR'}$, $\mathbf{7h}_{SS'}$, $\mathbf{7g}_{SS'}$, $\mathbf{7h}_{SR'}$ from benzylic alcohols $\mathbf{6g}_{R'}$, $\mathbf{6h}_{S'}$, $\mathbf{6g}_{S'}$, $\mathbf{6h}_{R'}$ and their application as substrates for NCDD-catalysed formation of chiral catechols $\mathbf{8g}_{R'}$, $\mathbf{8h}_{S'}$, $\mathbf{8g}_{S'}$, $\mathbf{8h}_{R'}$.

(R = Me, Et and Pr).[10d] Triol $\mathbf{7h}_{SR'}$ had been isolated, as a major metabolite, from biotransformation of 2,2,2-trifluoroacetophenone using *P. Putida* UV4, after PLC separation from the minor diastereoisomer $\mathbf{7h}_{SS'}$.[8d] The racemic benzylic alcohol $\mathbf{6g}_{R'}$ / $\mathbf{6g}_{S'}$, as substrate, with *P. putida* UV4 cells, under similar conditions, gave separable triol diastereoisomers $\mathbf{7g}_{SR'}$ and $\mathbf{7g}_{SS'}$ (Scheme 3). Triols $\mathbf{7g}_{SR'}$, $\mathbf{7h}_{SR'}$, $\mathbf{7h}_{SS'}$, and $\mathbf{7g}_{SS'}$, each containing three allylic alcohol groups, proved to be very good substrates for *E. coli narB* and suitable models to investigate diastereoselectivity of NCDD*narB*, during formation of stable catechols $\mathbf{8g}_{R'}$, $\mathbf{8h}_{R'}$, $\mathbf{8g}_{S'}$, and $\mathbf{8h}_{S'}$ (Scheme 3).

Alcohol dehydrogenation at the C-1 position, to form α-ketol intermediates, had been observed earlier (Scheme 1), when using TCDD and BphB enzymes, to produce catechols from *cis*-dihydrodiols of toluene[2d] and biphenyl[2e, 9a, 9b] respectively. Dehydrogenation of the exocyclic allylic alcohol group, present in each of the triol diastereoisomers $\mathbf{7g}_{SR'}$ / $\mathbf{7g}_{SS'}$ and $\mathbf{7h}_{SR'}$ / $\mathbf{7h}_{SS'}$, offered NCDD*narB* enzyme with a potential alternative metabolic pathway. However, this pathway was not adopted, and the regioselectivity of NCDD*narB* was again directed, exclusively, towards the oxidation of an endocyclic allylic alcohol group (Scheme 3). The resulting α-ketol intermediates, presumably formed *via* oxidation of the less sterically hindered C-1 hydroxyl group, preferred to exist as their catechol tautomers $\mathbf{8g}_{R'}$, $\mathbf{8h}_{R'}$, $\mathbf{8g}_{S'}$, and $\mathbf{8h}_{S'}$. In the absence of the opposite triol enantiomers, *e.g.* $\mathbf{7g}_{RR'}$ and $\mathbf{7g}_{RS'}$, the exclusive enantioselectivity observed for NCDD*narB*-catalysed dehydrogenation of *cis*-dihydrodiols $\mathbf{2}_S$ using *E. coli narB*,[10a] was also assumed to apply to triols $\mathbf{7g}_{SR'}$, $\mathbf{7h}_{SR'}$, $\mathbf{7h}_{SS'}$, and $\mathbf{7g}_{SS'}$. Furthermore, as no significant difference was observed in the relative rates of formation of catechols $\mathbf{8g}_{R'}$, or $\mathbf{8g}_{S'}$, and $\mathbf{8h}_{R'}$,or $\mathbf{8h}_{S'}$ ($k_1 = k_2$), during NCDD*narB*-catalysed oxidation of each diastereoisomeric member of the triol pairs $\mathbf{7g}_{SR'}$ /$\mathbf{7g}_{SS'}$ and $\mathbf{7h}_{SR'}$/ $\mathbf{7h}_{SS'}$, no evidence of diastereoselectivity was found (Scheme 3).

(iv) Regio- and stereo-selectivity of BCDD- and NCDD-catalysed dehydrogenation of meso monocyclic alkane *cis*-diols 9 and 12 to yield α-ketols 10 and 13

The purified BCDD*bedD* enzyme, isolated from *P. putida* ML2, displayed exclusive enantioselectivity, but the activity was confined to the relatively rare *cis*-dihydrodiol $\mathbf{2}_R$ enantiomers, *e.g.* $\mathbf{2b}_R$.[10a] A limited range of acyclic alkane *vic*-diols was also found to be acceptable substrates for *P. putida* ML2, however their metabolites were not isolated or detected. In this context, investigation of the regio- and stereo-chemistry associated with BCDD-catalysed oxidation of cycloalkane *cis*-diol substrates **9** and **12** was undertaken, using *P. putida* ML2 and the new *E. coli bedD* clone for biotranformations, to give the corresponding α-ketol metabolites **10** and **13** (Scheme 4). The absolute configurations of the alkene *cis*-diols **15**, **17**, **19**, **21**, *trans*-diols **11**, **14** and α-ketols **10**, **13**, **16**, **18**, **20** and **22** involved in this study, had been established earlier (Schemes 4 and 5).

The enantiopurities of the residual *cis*-diol and α-ketol metabolites were determined by a combination of methods including: (i) comparison of specific optical rotations ($[\alpha]_D$) with the reported values, (ii) chiral stationary phase GC-MS

Table 1. Yields (%), *ee* values (%) and absolute configurations of α-ketols **10**, **13**, **16**, **18**, **20**, **22**, residual *cis*-diols **15**, **17**, **19**, **21** and *trans*-diol **14**, obtained using *cis*-diol substrates and BDD or NDD as biocatalysts.

Entry	Enz[a-d]	*cis*-Diol substrate	Diol %Yield	Diol % *ee*[d]	Diol Config.	Ketol	Ketol %Yield	Ketol % *ee*[d]	KetolConfig.
1	BDD[a]	**9**				**10**$_S$	63	> 98[e]	2*S*
2	BDD[b]	**9**				**10**$_S$	39	> 98[e]	2*S*
3	BDD[a]	**12**	**14**$_{SS}$,74	97[e]	1*S*,2*S*	**13**$_S$	11	> 98[e]	2*S*
4	BDD[b]	**12**	**14**$_{SS}$,12	> 98[e]	1*S*,2*S*	**13**$_S$	9	> 98[e]	2*S*
5	BDD[a]	**15**$_{1R,2S}$	22	> 98[e]	1*R*,2*S*	**16**$_S$	36	> 98[e]	5*S*
6	BDD[b]	**15**$_{1R,2S}$	42	> 98[e]	1*R*,2*S*	**16**$_S$	23	> 98[e]	5*S*
7	BDD[a]	**17**$_{1R,2S}$	16	> 98[e]	1*R*,2*S*	**18**$_S$	22	> 98[e]	6*S*
8	BDD[b]	**17**$_{1R,2S}$	20	96[e]	1*R*,2*S*	**18**$_S$	43	> 98[e]	6*S*
9	NDD[c]	**19**$_{1S,2R}$	35	> 98[f]	1*S*,2*R*	**20**$_S$	12	> 98[f]	2*S*
10	NDD[c]	**21**$_{1S,2R}$	28	> 98[f]	1*S*,2*R*	**22**$_S$	12	> 98[f]	2*S*

[a] *P. putida* ML2; [b] *E. coli. bedD*; [c] *P. putida* 8859; [d] Maximum *ee* value observed; [e] CSPGC-MS; [f] CSPHPLC.

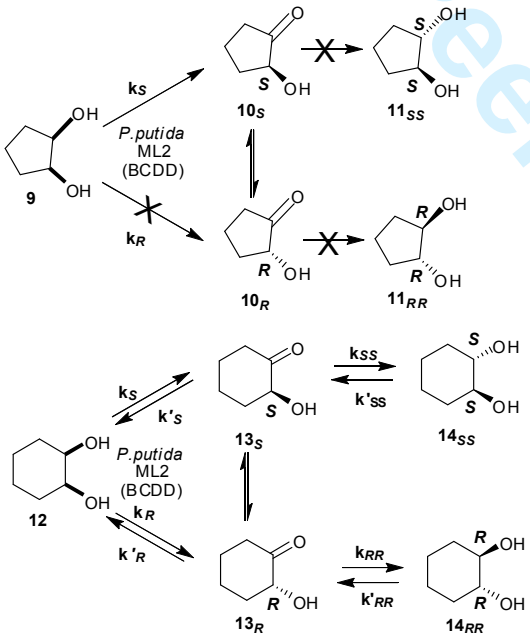
(CSPGC-MS), (iii) chiral stationary phase HPLC (CSPHPLC), (iv) formation of diMTPA using CSPGC-MS or CSPHPLC, during the early stages of biotransformations, prior to the isolation and purification of metabolites and before significant racemization. Enantiopurity values, based on optical rotations of isolated metabolites, were lower in some cases, due to their partial racemization occurring during the biotransformation, workup and purification process, but allowed absolute configurations to be established. Time course experiments, using CSPGC-MS and CSPHPLC analysis, allowed the metabolic profile and maximum *ee* values of metabolites to be determined.

Initial biotransformation studies of *cis*-diol substrates **9** and **12** were conducted, using *P. putida* ML2 whole cells as a source of BCDD (Scheme 4). The possibility of dehydrogenation occurring, during oxidation of the meso substrates **9** and **12**, was investigated, using relatively high cell densities (OD_{600} = 30, *e.g.* 22 g/L dcw) and substrate concentrations (2-25 g/L). A small-scale time course study, involving extraction (EtOAc) and CSPGC-MS analysis of the extract, showed that the substrate **9** was totally consumed after 36h and the α-ketol product **10** was formed in quantitative yield, essentially, as a single enantiomer (98 % *ee*). This proved to be a very efficient asymmetric synthesis (k_S >>> k_R). Under esters of *cis*- and *trans*-diols and analysis by NMR spectroscopy. The percentage *ee* values (Table 1, entries 1-4) were the optimal values observed, these conditions, no evidence of racemization of α-ketol **10** or its enzyme-catalysed reduction to *trans*-diol **11** was observed. This biotransformation of substrate **9** (1.0 g) was repeated for a shorter period (5 h). Extraction and column chromatography of the crude product yielded (+)-(2*S*)-α-ketol **10**$_S$ (63% yield, 95% *ee*), after separation from the residual achiral *cis*-diol **9** (Table 1, entry 1). The enantiopurity of α-ketol **10**$_S$ was found to be optimal at higher concentrations of substrate *cis*-diol **9**, *i.e.* 98% *ee* at 8 g/L and 95% *ee* at 25 g/L (Figure S1). The enantiopurity of α-ketol **10**$_S$ was found to be slightly lower (98% → 95% *ee*), when using a higher concentration of substrate *cis*-diol **9** (Figure S1). Employing a high cell density (OD_{600} = 24) of *E. coli bedD*, as source of BCDD*bedD*, and a lower concentration (2 g/L) of achiral substrate *cis*-diol **9**, again formed (2*S*)-α-ketol **10**$_S$ as the sole metabolite (Table 1, entry 2).

Time course studies of the biotransformation of meso *cis*-diol **12**, using *P. putida* ML2 and conducted over 24 h, involved sequential extraction and analysis of metabolites by CSPGC-MS. The final yield of α-ketol **13**$_S$ was found to vary between 11% at a lower substrate concentration (10 g/L, Figure S2) and 80% at a higher concentration (25 g/L, Figure S3); the metabolic path was more complex than that found using *cis*-diol **9**. The

BCDD-catalysed dehydrogenation of *cis*-diol **12**, initially, resulted in a marked preference (k_S >>> k_R) for the formation of (*S*)-α-ketol enantiomer $\mathbf{13}_S$ (98% *ee*, Table 1, entry 3). The *ee* value of α-ketol $\mathbf{13}_S$ decreased with time (98% → 45% *ee* after 24 h) and showed a concomitant increase in the proportion of *trans*-diol $\mathbf{14}_{SS}$. The crude bioproduct mixture was worked up after 24 h, and separated by column chromatography, to give α-ketol $\mathbf{13}_S$ (11%, yield, 63% *ee*) and *trans*-diol $\mathbf{14}_{SS}$ in higher yield (74%) and enantiopurity (97% *ee*).

The metabolic pathway for *cis*-diol **12**, using *P. putida* ML2, was more complex than that found for *cis*-diol **9**. This was mainly due to the formation of *trans*-diol $\mathbf{14}_{SS}$ as the major bioproduct and the unexpected racemization of α-ketol **13**. As this required further investigation, a time course biotransformation of racemic α-ketol **13** was conducted, over a 2 h period, and monitored by CSGC-MS analysis of the EtOAc extracts of aliquots (Figure S4).



Scheme 4. BCDD*bedD*-catalysed asymmetric synthesis of α-ketols $\mathbf{10}_S$ and $\mathbf{13}_S$, from *cis*-diols **9** and **12** and formation of *trans*-diols $\mathbf{14}_{SS}$ and $\mathbf{14}_{RR}$.

These results indicated that (*S*)-α-ketol enantiomer $\mathbf{13}_S$ was totally reduced, within 2 h, to yield a mixture of meso *cis*-diol **12** (93%) and *trans*-diol $\mathbf{14}_{SS}$ (7%), *i.e.* k'_S >> k_{SS} (Scheme 4) Most of the (*R*)-α-ketol enantiomer $\mathbf{13}_R$ (83%) was unused, with biotransformation occurring at a much slower rate, to form *trans*-diol $\mathbf{14}_{RR}$ (16%) and *cis*-diol **12** as a very minor metabolite (1%), *i.e.* k_{RR} > k'_R. As the residual α-ketol $\mathbf{13}_R$ (90% *ee*), present after 2 h, had the opposite configuration to α-ketol $\mathbf{13}_S$ (98% *ee*), obtained from biotransformation of *cis*-diol **12** under similar conditions, it provided a rationale for this unexpected enantiocomplementarity and the unexpected racemization of α-ketol $\mathbf{13}_S$.

A time course biotransformation of a racemic sample of *trans*-diol $\mathbf{14}_{RR}$ / $\mathbf{14}_{SS}$, over a 24 h period, revealed that very little of enantiomer $\mathbf{14}_{SS}$ was being utilized (k'_{RR} >> k'_{SS}, Figure S5). *trans*-Diol $\mathbf{14}_{RR}$ was bioconverted to α-ketol $\mathbf{13}_R$ (22% relative yield, 80% *ee*), leaving a slight enantiomeric excess of the residual *trans*-diol $\mathbf{14}_{SS}$ (78% relative yield, 12% *ee*). It was evident, from these results, that alcohol dehydrogenation and the reverse reduction process were both in operation, during the biotransformation of *cis*-diol **12**, ketol **13** and *trans*-diol **14**. To decrease the formation of *trans*-diol $\mathbf{14}_{SS}$, and increase the yield and enantiopurity of ketol $\mathbf{13}_S$, during the metabolism of *cis*-diol **12** by *P. putida* ML2, acetone was added as a competitive inhibitor. It proved to be very effective with the proportion of *trans*-diol **14** decreasing from 82% to 1%, after the addition of acetone (10% vol.), and with the amount of ketol $\mathbf{13}_S$ increasing from 18% to 99% and *ee* from 40% to 98% (Table 2). The asymmetric synthesis of ketols $\mathbf{10}_S$ and $\mathbf{13}_S$, as single enantiomers, established the value of *P. putida* ML2, and possibly BCDD*bedD*, in the deracemization of meso *cis*-diols **9** and **12**.

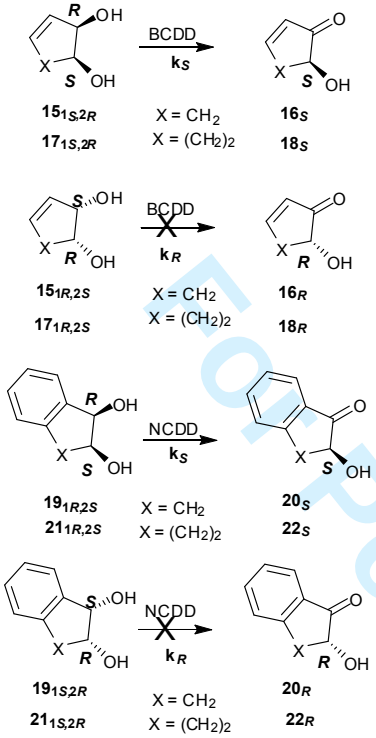
Since the activities of either of the two alcohol dehydrogenase enzymes, (BCDD*bedD* and BCDD*BedD*), present in *P. putida* ML2 cells, could have been responsible for the reversible formation of *cis*-diol **12**, *trans*-diol **14** and α-ketol **13**, the biotransformation of *cis*-diol **12** was repeated with *E. coli bedD*. Under conditions, similar to those used earlier with *cis*-diol **9**, a (1:4) mixture of α-ketol $\mathbf{13}_S$ (98% *ee*) and *trans*-diol $\mathbf{14}_{SS}$ (>98% *ee*) was obtained, in a lower yield compared to the earlier *P. putida* ML2 biotransformation (Table 1, entry 4). This observation is consistent with the BCDD*bedD* enzyme being responsible for the dehydrogenation of *cis*-diol **12** to yield α-ketol $\mathbf{13}_S$ and also its reduction to *trans*-diol $\mathbf{14}_{SS}$.

(v) Regio- and stereo-selectivity of BCDD-catalysed dehydrogenation of racemic monocyclic *cis*-diols and NCDD-catalysed dehydrogenation of racemic bicyclic *cis*-diols to yield the corresponding α-ketols

Preliminary studies, of the potential of BCDD*bedD* in the kinetic resolution of racemic alkene *cis*-diols **15** and **17** and production of α-

ketol metabolites **16** and **18**, were conducted with *P. putida* ML2 (Scheme 5).

The biotransformation of substrate $\mathbf{15}_{1R,2S}$/ $\mathbf{15}_{1S,2R}$, followed by extraction and sequential CSPGC analysis, showed that a kinetic resolution was taking place. When the biotransformation was terminated after 5 h, formation of diMTPA ester



Scheme 5. BCDD- or NCDD-catalysed synthesis of α-ketols $\mathbf{16}_S$, $\mathbf{18}_S$, $\mathbf{20}_S$ and $\mathbf{22}_S$, and kinetic resolution of racemic *cis*-diols **15**, **17**, **19** and **21**, to yield residual *cis*-diols $\mathbf{15}_{1R,2S}$, $\mathbf{17}_{1R,2S}$, $\mathbf{19}_{1S,2R}$ and $\mathbf{21}_{1S,2R}$.

and NMR analysis indicated that an enantiopure sample (>98% *ee*) of residual *cis*-diol $\mathbf{15}_{1R,2S}$ was obtained in 22% recovered yield (Table 1, entry 5). It had been reported[11a-c] that an enantiomerically enriched form of α-ketols **16** and **18** could not be isolated due to racemization and that *ee* values and absolute configurations were only determined from their acetate derivatives. These reports[11a-c] suggested that the task of isolating, recording reproducible optical rotation measurements, and obtaining reliable enantiopurity values, based on comparison with literature data for α-ketols $\mathbf{16}_S$/$\mathbf{16}_R$ and $\mathbf{18}_S$/$\mathbf{18}_R$, would be particularly challenging.

Furthermore, enantiomeric separation of these α-ketol enantiomers, by CSPGC-MS, could also be difficult. To resolve these problems, each small aliquot was reduced ($NaBH_4$ / MeOH) to the corresponding *trans*-diols, which were configurationally stable and readily separable by CSPGC-MS.

Evidence of racemization of α-ketol $\mathbf{16}_S$ was found, during the bioconversion of *cis*-diol **15**, using *P. putida* ML2; it was enantiopure (>98% *ee*) after 1 h (k_S >>> k_R), but after 5 h, the value had decreased to 69% *ee* (Figure S6). This biotransformation was then repeated using *E. coli bedD*. After 12 h, the residual *cis*-diol $\mathbf{15}_{1R,2S}$ was recovered as a single enantiomer (>98% *ee*, 42% yield), but the enantiopurity of α-ketol $\mathbf{16}_S$ was lower (>98% → 33% *ee*, 23% yield, Table 1, entry 6). Throughout the course of biotransformation of *cis*-diol **15**, racemization of initially formed enantiopure α-ketol $\mathbf{16}_S$ was occurring, and presumably also during the isolation of metabolite $\mathbf{16}_S$. Thus, while the negative sign of optical rotation values for metabolite $\mathbf{16}_S$ was consistent, the magnitude varied, *e.g.* using *P. putida* ML2 ($[\alpha]_D$ = -52) and *E. coli bedD* ($[\alpha]_D$ = -23). Evidence of a facile chemical racemization process for α-ketols **16** and **18**, *via* an enol tautomer, was observed earlier.[11a] A bacterial strain of *P. putida* (ATCC 12633) was reported[11b] to express racemase activity and catalyse the racemization of a range of α-hydroxy ketones, including compounds **20** and **22**. While no direct evidence of racemaze activity in our *P. putida* strains (ML2, 8859) was found, the possibility of a contribution from an enzyme-catalysed racemization process occurring cannot be excluded.

Biotransformation of *cis*-diol **17**, with *P. putida* ML2, was monitored, in a manner similar to *cis*-diol **15** (CSPGC-MS analysis), over a 4 h period (Figure S7). The residual *cis*-diol $\mathbf{17}_{1R,2S}$ had an *ee* value of 96% at 4 h and the metabolite α-ketol $\mathbf{18}_S$, formed after 1 h, was a single enantiomer (k_S >>> k_R), which had partially racemized (>98 → 87% *ee*) after 4 h (Table 1, entry 7). Using *E. coli bedD* for the metabolism of *cis*-diol **17**, a similar result was obtained, to give *cis*-diol $\mathbf{17}_{1R,2S}$ (>98% *ee*) and α-ketol $\mathbf{18}_S$ with a lower *ee* value (> 98% → 43%, Table 1, entry 8). It is noteworthy that optimal regio- and stereo-selectivity were observed with BCDD*bedD* as biocatalyst, when the allylic hydroxyl group of *cis*-diols **15** and **17** was, exclusively oxidized, to give α–ketols $\mathbf{16}_S$ and $\mathbf{18}_S$. Although cycloalkene metabolites, α-ketol $\mathbf{16}_S$ and $\mathbf{18}_S$, were initially formed as single enantiomers, they appeared to racemize more readily than the corresponding cycloalkane metabolites α-ketol $\mathbf{10}_S$ and $\mathbf{13}_S$; this was assumed to be due to keto-enol tautomerization, facilitated by conjugation.

In conjunction with the study of *cis*-diol dehydrogenase-catalysed dehydrogenation of

Table 2. Relative composition and % *ee* of α-ketol **13** and *trans*-diol **14** metabolites obtained using *cis*-diol substrate **12** and acetone as co-substrate with *P. putida* ML2.

Acetone (% vol.)	*cis*-Diol **12** (%)	α-Ketol **13** (%)	*trans*-Diol **14** (%)	α-Ketol $\mathbf{13}_S$ (% *ee*)	*trans*-Diol $\mathbf{13}_{S,S}$ (% *ee*)
0	0	18	82	40	96
1	0	20	80	44	97
3	0	41	59	66	97
5	0	78	22	91	98
10	0	99	1	98	98

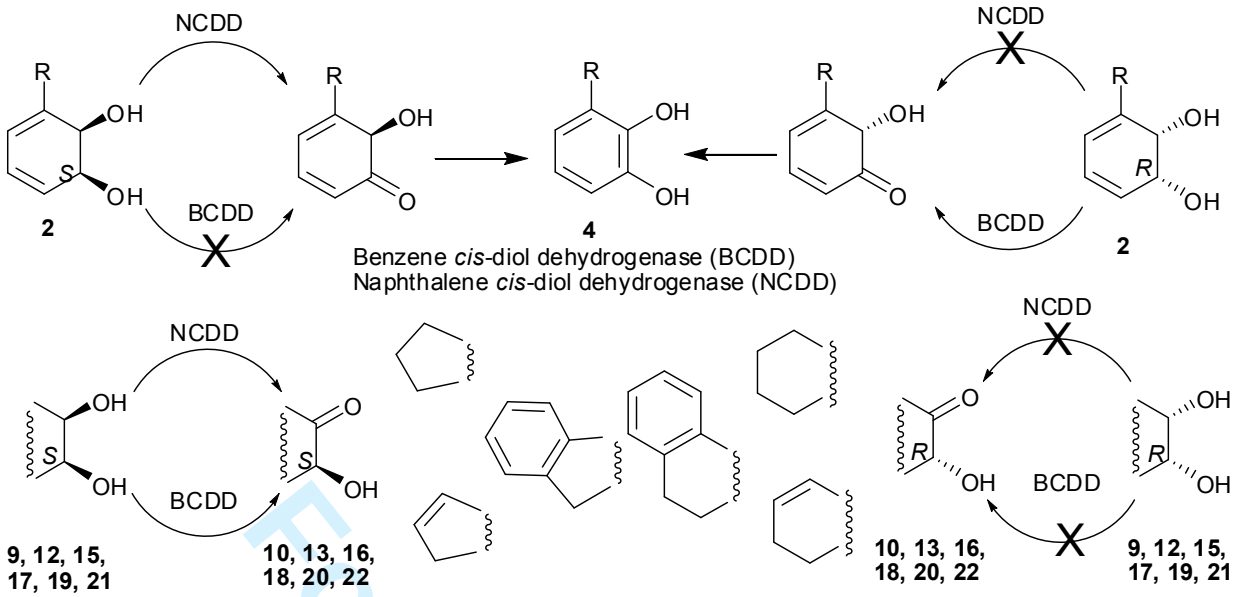
monocyclic *cis*-diols, a brief study of the biotransformation of larger racemic substrates, *e.g.* *cis*-diol **19** and **21** was also undertaken. Using NCDD, expressed in whole cells of a wild type strain, *P. putida* 8859, for racemic *cis*-diol **19** and analysis of biotransformed material by CSPHPLC, regioselectivity was observed, with exclusive oxidation of the benzylic hydroxyl group yielding ketol $\mathbf{20}_S$. The residual *cis*-diol $\mathbf{19}_{1S,2R}$ (35% yield, 98% *ee*) and ketol metabolite $\mathbf{20}_S$ (12% yield, 88% *ee*) were separated by PLC .

In order to determine if partial racemization of metabolite $\mathbf{20}_S$ had occurred, Enantiopure samples of both enantiomers of *cis*-diol **19**, available from earlier biotransformation studies, were separately added as substrates. After 24 h, the (1*R*,2*S*) enantiomer was completely bioconverted to α-ketol $\mathbf{20}_S$ (80% *ee*), while the (1*S*,2*R*) enantiomer remained unmetabolized. Similar results were obtained with racemic and enantiopure samples of *cis*-diol **21** as substrates with *P. putida* 8859. Thus, racemic substrate *cis*-diol **21** gave metabolite ketol $\mathbf{22}_S$ (12% yield, 86% *ee*) and the residual *cis*-diol $\mathbf{21}_{1S,2R}$ (28% yield, > 98% *ee*).

An enantiopure sample of *cis*-diol $\mathbf{21}_{1R,2S}$ was bioconverted to α-ketol $\mathbf{22}_S$ (88% *ee*). The opposite enantiomer $\mathbf{21}_{1S,2R}$ was not biotransformed. These results demonstrate that the relative rates of dehydrogenation of the *cis*-diol enantiomers $\mathbf{15}_{1S,2R}$, $\mathbf{17}_{1S,2R}$, $\mathbf{19}_{1R,2S}$ and $\mathbf{21}_{1R,2S}$, involving dehydrogenation at allylic (*R*) or benzylic (*R*) chiral centres and formation of (*S*)-α-ketols, were consistently much higher ($k_S >>> k_R$) with either BCDD or NCDD as biocatalysts. Since biotransformation of enantiopure (>98% *ee*) *cis*-diol enantiomer substrates $\mathbf{19}_{1R,2S}$ and $\mathbf{21}_{1R,2S}$, with *P. putida* 8859, yielded partially racemized α-ketols $\mathbf{20}_S$ (80% *ee*) and $\mathbf{22}_S$ (88% *ee*), it is probable that metabolites $\mathbf{20}_S$ and $\mathbf{22}_S$, isolated from the biotransformation of racemic *cis*-diol substrates **19** and **21**, were also formed initially as enantiopure metabolites (> 98% *ee*, Table 1. Entries 9 and 10).

It was anticipated that the relatively large bicyclic *cis*-diols **19** and **21** would not be acceptable substrates for BCDD. However, based on a preliminary CSPHPLC study, using *P. putida* ML2 without isolation of bioproducts, a racemic sample of *cis*-diol **19** was biotransformed into enantiopure α-ketol $\mathbf{20}_S$, leaving the residual *cis*-diol $\mathbf{19}_{1S,2R}$ as a single enantiomer. A relative nine fold decrease in reaction rate was observed, *i.e.* substrate consumed per minute of biomass, when bicyclic substrate **21** was used, presumably, due to a smaller active site in BCDD.

The results, presented in Table 1, indicate that the residual chiral *cis*-diols $\mathbf{15}_{1R,2S}$, $\mathbf{17}_{1R,2S}$, $\mathbf{19}_{1S,2R}$ and $\mathbf{21}_{1S,2R}$ were single enantiomers, resulting from a *cis*-diol dehydrogenase-catalysed kinetic resolution process. Similarly, the ketol metabolites $\mathbf{10}_S$, $\mathbf{13}_S$, $\mathbf{16}_S$, $\mathbf{18}_S$, $\mathbf{20}_S$ and $\mathbf{22}_S$ also appeared to be initially formed as single enantiomers. Evidence was found of the variable degree of racemization occurring during the α-ketol biotransformations. It is noteworthy that during biotransformations of: arene *cis*-dihydrodiols, BCDD accepted only (1*R*)-enantiomers, *e.g.* $\mathbf{2b}_R$ and NCDD accepted only (1*S*)-enantiomers, *e.g.* $\mathbf{2b}_S$, as substrates to yield catechols, *e.g.* **4b**. However, in the case of alkene *cis*-diols, both BCDD and NCDD accepted meso *cis*-diols **9** and **12** and racemic *cis*-diols **15**, **17**, **19**, and **21** as substrates, to yield only (*S*)-α-ketols $\mathbf{10}_S$, $\mathbf{13}_S$, $\mathbf{16}_S$, $\mathbf{18}_S$, $\mathbf{20}_S$, $\mathbf{22}_S$ (Scheme 6).



Scheme 6. Complementary enantioselectivity of BCDD for arene *cis*-dihydrodiols $\mathbf{2}_{R}$ and NCDD for arene *cis*-dihydrodiols $\mathbf{2}_{S}$, to yield catechols **4** and similar enantioselectivity of BCDD and NCDD for meso *cis*-diols **9**, **12** and racemic *cis*-diols **15**, **17**, **19** and **21** to yield (*S*)-α-ketols $\mathbf{10}_{S}$, $\mathbf{13}_{S}$, $\mathbf{16}_{S}$, $\mathbf{18}_{S}$, $\mathbf{20}_{S}$ and $\mathbf{22}_{S}$.

(vi) Applications of catechols 4b, 4e, $\mathbf{8h}_{R'}$ and $\mathbf{8h}_{S'}$ obtained by NCDD*narB*-catalysed dehydrogenation of monosubstituted benzene *cis*-dihydrodiols

We have reported[8d] that the dehydration of triol $\mathbf{7h}_{SR'}$, under acidic conditions, gave a mixture (1:2) of the corresponding *ortho*-phenol $\mathbf{23h}_{R}$ and *meta*-phenol $\mathbf{24h}_{R}$. As part of a wider study, to synthesise and evaluate the potential of chiral compounds, obtained by enzymatic hydroxylation of arenes and their derivatives, as ligands and reagents,[12a,12b] monomethyl ethers of phenols $\mathbf{23h}_{R}$ and $\mathbf{24h}_{R}$ were synthesised. The methyl ethers proved to be very useful resolving and chiral solvating agents (^{1}H and ^{19}F NMR analysis) for racemic or enantioenriched carboxylic acids and sulfoxides. The range of chiral reagents was extended by treating catechols $\mathbf{8h}_{R'}$ and $\mathbf{8h}_{S'}$ with an excess of diazomethane, which yielded separable mixtures of mono- and di-methylated products $\mathbf{25h}_{S}$ / $\mathbf{26h}_{S}$ and $\mathbf{25h}_{R}$ / $\mathbf{26h}_{R}$. (Scheme 3). It was surprising to find that the more sterically hindered OH groups at C-2 were methylated, in preference to the more accessible OH groups at C-1. The strong electron withdrawing effect of the CF_3 substituent may be responsible for the preference, by rendering the phenolic OH group at C-2 more acidic. The potential of chiral reagents $\mathbf{25h}_{S}$, $\mathbf{26h}_{S}$, $\mathbf{25h}_{R}$ and $\mathbf{26h}_{R}$, and related derivatives with exocylic MeO groups, is currently under investigation.

The enzyme-catalysed hydroxylation of phenols to yield catechols has been reported, using both arene dioxygenases (TDO, NDO)[4a-f] and monooxygenases, including toluene monooxygenases (T3MO, T4MO).[13a-c]] It is difficult to make a direct comparison between results found using T3MO-, T4MO- or TDO/CDD-catalysed methods for making catechols since other metabolites are also formed in each case. TDO-catalysed monohydroxylation of phenols can thus yield both cyclohexenone *cis*-diols and/or catechols, depending on the substituent present. [4a-f] Toluene monooxygenases can yield both dihydroxylated (catechols, hydroquinones) and trihydroxylated benzene metabolites, from monophenols. While process development studies have been carried out, using 2-hydroxybiphenyl 3-monooxygenase (HbpA) to yield catechols, [13d] optimization work remains to be carried out using BCDD- and NCDD-catalysed dehydrogenations of *cis*-diol substrates used in this study. One of the potential benefits of the method discussed herein is that of the >400 examples of arene *cis*-dihydrodiol metabolite reported, to date, it is likely that many more will be biotransformed into the corresponding catechols, using the appropriate *cis*-diol dehydrogenase.

The relative activities of NCDD*narB* during biotransformations of *cis*-dihydrodiols **2a-h**, using *E. Coli narB*, to yield catechols **4a-h** and **4i**, was reported earlier (Scheme 2).[7f] It showed

that from NCDD-catalyzed dehydrogenation of a

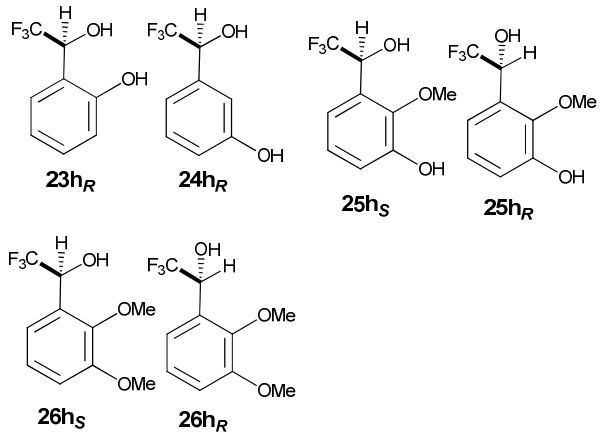
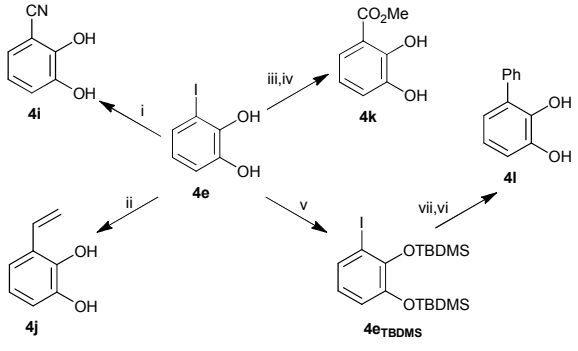


Figure 1. Enantiopure benzylic alcohols obtained from the triols **7h**$_{SR'}$ or **7h**$_{SS'}$ and catechol **8h**$_{R'}$ or **8h**$_{S'}$ metabolites.

range of *cis*-dihydrodiols, bioconversion of the iodo-substituted compound **2e** was the most efficient and the resulting catechol **4e** showed very useful synthetic potential. Catechol **4e** was obtained earlier, by the metabolism of iodobenzene, using a recombinant strain, *E. coli* JM109 (pDTG602), expressing both TDO and TCDD;[7c] it was also recently reported as an uncharacterized intermediate, formed by chemical synthesis.[7j] In the present unoptimized study, biotransformation of *cis*-dihydrodiol **2e**, using *E. Coli narB* as a source of NCDD*narB*, provided multigram quantities (> 10 g) of catechol **4e**, which allowed an evaluation of its reactions, including substitution of the iodine atom, to produce of a wider range of catechols **4** (Scheme 7).



Reagents: i Bu_3SnCN, $(Ph_3P)_4Pd$, THF; ii $Bu_3SnCH{=}CH_2$, $(Ph_3P)_4Pd$, THF; iii $Pd(OAc)_2$, NaOAc, CO, MeOH: iv HCl, MeOH; v TBDMS-triflate, Et_3N, CH_2Cl_2; vi PhMgBr, $Ni(acac)_2$, Et_2O; vii Bu_4NF, THF

Scheme 7. Substitution reactions of catechol **4e** to yield catechols **4i-l**.

Palladium-catalysed (Stille) cross-coupling reactions of catechol **4e** with tributyltin cyanide and vinyl tributyl tin reagents, yielded the corresponding catechols **4i** (24%) and **4j** (32%). Palladium-catalysed carbonylation of catechol **4e** resulted in the substitution of the iodine atom to give catechol **4k** in better yield (60%). Cross-coupling of a phenyl Grignard reagent with the protected catechol derivative **4e**$_{TBDMS}$, in the presence of nickel acetylacetonide, resulted in formation of phenyl-substituted catechol **4l** in excellent yield (90%) after deprotection. This approach should also be applicable to synthesis of a wider range of catechols bearing different alkyl and aryl groups.

Conclusions

Regioselectivity, enantioselectivity and diastereoselectivity were investigated, using both wild type and recombinant bacterial strains for BCDD*bedD*- and NCDD*narB*-catalysed biotransformations of monosubstituted benzene 2,3- and 3,4-*cis*-dihydrodiols, to yield catechols. Alcohol dehydrogenations, catalysed by BCDD*bedD* and NCDD*narB*, resulted in the desymmetrization of meso cycloalkane 1,2-*cis*-diols and kinetic resolution of racemic cycloalkene 1,2-*cis*-diols, during the formation of chiral α–ketol metabolites. Among the advantages of the current approach are: (i) availability of very wide range of *cis*-diol substrates by a single enzymatic or chemical step (ii) the increased water-solubility of substituted benzene *cis*-dihydrodiol, cycloalkane *cis*-diol and cycloalkene *cis*-diol substrates, in some cases, allowing relatively high concentrations of substrates (>20 g/L) to be used (iii) acceptable isolated yields of enantiopure vicinal *cis*-diols, catechols and α-ketols (generally 60-80%) and (iv) synthetic applications of achiral and chiral catechols, produced from *cis*-dihydrodiol substrates by NCDD*narB* catalysis. Some α-ketols were, however, obtained with evidence of partial racemization occurring *via* alternative pathways.

Experimental Section

General Methods

NMR analysis was carried out using Bruker Avance DPX-300 (300 MHz) and DRX-500 (500 MHz) instruments. All NMR samples were run in $CDCl_3$ solvent, using tetramethylsilane as reference, unless stated otherwise. Coupling constants were expressed in Hz. Mass spectra were recorded at 70 eV, on an AE1-MS902 spectrometer updated by V.G. Autospec instruments, using a heated inlet system. Accurate molecular weights were obtained by the peak matching method, using perfluorokerosene as standard reference and were accurate to within ± 6 x 10^{-6} p.p.m.

Optical rotation values were determined on a Perkin-Elmer automatic precision polarimeter Model 241. Concentrations were measured in g/100 cm^3 using the specified solvent at a wavelength of 589 nm (sodium D-line) and at ambient temperature.

GC/MS analyses were carried out, using a Hewlett Packard 6890 gas chromatograph directly linked to a Hewlett Packard 5973 Mass Selective Detector (MSD). The gas chromatographs were fitted with fused silica capillary columns including 30 m Supelco-β-Dex 120, 30 m Supelco-β-Dex 225, 30 m Supelco-α-Dex 120 and 10 m Chrompak CP-Chirasil-DEX CB columns. Quantification of analytes was performed, using gas chromatography, with flame ionisation detection (GC/FID). Chiral stationary phase HPLC (CSPHPLC) analysis was conducted, using a Perkin Elmer Series 3B model coupled to a Perkin Elmer LC-75 spectrophotometric detector, and LC1-100 computing integrator. Chromatographic separations were achieved using a Chiralcel OB and OJ columns.

Chemicals

All meso cycloalkane *cis*-diols, racemic cycloalkane *trans*-diols and cycloalkene *cis*-diols, used as substrates for *cis*-diol dehydrogenase enzymes, were either available commerically (**9**, **11**, **14**) or by chemical synthesis through literature methods (**16**, **18**, **20**). *cis*-Diols **2b**$_S$, **2e**$_S$, **3d**$_S$, **19**$_{1S,2R}$, **19**$_{1R,2S}$, **21**$_{1S,2R}$, **21**$_{1R,2S}$ and triols **7h**$_{SR'}$, **7h**$_{SS'}$ were available from earlier biotransformation studies with *P. putida* UV4.[6b, 8c, 8d] The triol diastereoisomers **7g**$_{SR'}$ and **7g**$_{SS'}$ were also obtained under similar conditions. The catechol **4e**, **4i**, **4j**, **4k**, **4l**, **5d** and α-ketol metabolites **10**$_S$, **13**$_S$, **16**$_S$, **18**$_S$, **20**$_S$ and **22**$_S$ were all known compounds, which were identified by comparison with authentic samples and literature data.

Bacterial Strains

The *P. putida* wild-type strains were obtained from Prof. J. R. Mason, King's College, University of London, UK (*P. putida* ML2) and from the National Collections of Industrial and Marine Bacteria, Ltd., Aberdeen, UK (*P. putida* NCIMB 8859). Typical biotransformation conditions, used with these strains, were similar to those reported earlier.[14a,14b] The proprietary constituent mutant strain, *P. putida* UV4, was obtained from Avecia Pharmaceuticals, Billingham, Cleveland, UK and was used for the *cis*-dihydroxylation of both alkenes and arenes as reported.[14b] The *E.coli narB*,[7h,7i] and new *E.coli bedD* recombinant strains, were developed at Queen's University, Belfast, and were utilized to produce 3-substituted catechols,[7f] in the current study.

Construction of a new *E.coli bedD* recombinant strain

A derivative of the arabinose P_{BAD} promoter vector pBAD24 was used. This vector, pBADET9, obtained from Dr. V. Ksenzenko, has a translation start codon within a unique NdeI restriction site. Primers were designed for cloning of the bedD gene, from *Pseudomonas putida*ML2 (Accession number for the DNA sequence used is U08463, AF148496). Forward primer (7005-7023 nt):5'-GTT CAT ATG GAC CGC GCC ATT CAA TCA C-3'. Reverse primer (8182-8211 nt): 5'-GCA GAA TTC CGG CGG AGC CCA GGC CAG AGA -3'. The NdeI restriction site was introduced into the 5'-part of the forward primer and EcoRI site into reverse primer (sites are underlined). The DNA fragment, amplified with these primers, included the complete bedD gene sequence. The fragment containing the complete bedD gene was amplified, using PfuTurbo polymerase obtained from Stratagene. Reaction conditions were as follows: 95 °C for 3 min and then 32 cycles of 95° C (30 s), 55° C (30 s) and 72 °C (4.5 min). The fragment was purified, using PCR DNA and a Gel Band Purification Kit (Amersham), and digested with the corresponding enzymes.
PBAD-ET DNA was digested with both NdeI and EcoRI restriction enzymes and then used for the cloning. Ligated DNA was transformed into *E. coli* TOP 10 cells (Invitrogen).Transformation mixtures were plated onto 2YT media containing ampicillin (100 μg ml^{-1}) and arabinose (0.02%). Colonies were analysed after incubation at 30 °C for 24-30 h. This involved spraying with 0.1 M benzene *cis*-dihydrodiol; those developing a dark colour, indicating catechol formation, were selected. The resulting plasmid that was used in transformation experiments was designated pCL-bd. The cloned gene was placed under tight regulation by P_{BAD} promotor, which allowed efficient modulation of its expression by addition of an inducer (arabinose), to cultivation media. *E. coli* TOP 10 cells, containing this plasmid, were used in biotransformation experiments and designated pCL-bd.

Biotransformation of racemic benzylic alcohols 6g$_{R'}$ / 6g$_{S'}$ to yield triols 7g$_{SR'}$ and 7g$_{SS'}$ using *P.putida* UV4

A biotransformation of benzyl alcohol **(±)**-2,2-dimethyl-1-phenyl-1-propanol **6g**$_{R'}$ / **6g**$_{S'}$ (5.0 g, 30 mmol) was carried out, with *P. putida* UV4 in a 10 L fermenter for 18 h, under reported conditions.[8d] Ethyl acetate extraction of the centrifuged culture medium yielded a diastereoisomeric mixture of triols **7g**$_{SR'}$ and **7g**$_{SS'}$. The diastereoisomers were separated by multiple elution PLC (5% MeOH/$CHCl_3$), to give the minor isomer, (+)-*cis*-(1*S*,2*R*)-3-[(1'*R*)-1'-hydroxy-2',2'-dimethylpropyl]-3,5-cyclohexadiene-1,2-diol **7g**$_{SR'}$ (860 mg, 14%), and the major isomer, **(+)**-*cis*-(1*S*,2*R*)-3-[(1'*S*)-1'-hydroxy-2',2'-dimethylpropyl]-3,5-cyclohexadiene-1,2-diol **7g**$_{SS'}$ (1.74 g, 29%).

(+)-*cis*-(1*S*,2*R*)-3-[(1'*R*)-1'-Hydroxy-2',2'-dimethylpropyl]-3,5-cyclohexadiene-1,2-diol 7g$_{SR'}$

A gum; R_f 0.22 (7% MeOH / $CHCl_3$); $[\alpha]_D$ +71 (*c* 0.41, MeOH); (Found: M^+, 198.1265; $C_{11}H_{18}O_3$ requires 198.1256); δ_H (500 MHz, $CDCl_3$) 0.97 (9 H, s, CH^tBu), 4.00 (1 H, d, $J_{2,1}$ 5.7, 2-H), 4.03 (1 H, s, CH^tBu), 4.38 (1 H, m, 1-H), 5.81 (1 H, dd, $J_{6,5}$ 9.2, $J_{6,1}$ 2.2, 6-H), 6.02 (1 H, ddd, $J_{5,6}$ 9.3, $J_{5,4}$ 5.5, $J_{5,1}$ 2.1, 5-H), 6.06 (1 H, d, $J_{4,5}$ 4.9, 4-H); δ_C (125 MHz, $CDCl_3$) 25.99, 36.06, 69.55, 70.08 (x2), 79.78, 121.93, 123.89 (x2), 129.79, 124.71; *m/z* (EI): 198 (M^+, 2%), 196 (27), 181 (7), 180 (24), 178 (44), 163 (52), 147 (31), 139 (91), 124 (90), 123 (88), 111 (49), 107 (93), 106 (91), 105 (88), 95 (88), 93 (98), 91 (61), 69 (73), 65 (91), 55 (100), 51 (91), 45 (57), 41 (90), 39 (88), 31 (38), 29 (83), 27 (90). electronic CD (MeOH): 282 nm $\Delta\varepsilon$ +0.566, 255 nm $\Delta\varepsilon$ +0.326, 226 nm $\Delta\varepsilon$ -1.885.

(+)-*cis*-(1*S*,2*R*)-3-[(1'*S*)-1'-Hydroxy-2',2'-dimethylpropyl]-3,5-cyclohexadiene-1,2-diol 7g$_{SS'}$

White crystalline solid; mp 74-78 ºC (from ether/hexane); R_f 0.32 (7% MeOH/$CHCl_3$); $[\alpha]_D$ +123 (*c* 1.44, MeOH); (Found: M^+-H_2O,180.1150; $C_{11}H_{16}O_2$, requires 180.1150); δ_H (500 MHz, $CDCl_3$) 0.95 (9 H, s, CH^tBu), 4.02 (1 H, s, CH^tBu), 4.33 (1 H, d, $J_{2,1}$ 5.7, 2-H), 4.38 (1 H, m, 1-H), 5.77 (1 H, d, $J_{4,5}$ 5.4, 4-H), 5.83 (1 H, dd, $J_{6,5}$ 9.6, $J_{6,1}$ 2.8, 6-H), 5.97 (1 H, ddd, $J_{5,6}$ 9.6, $J_{5,4}$ 5.4, $J_{5,1}$ 2.1, 5-H); δ_C (125 MHz, $CDCl_3$) 26.19, 35.78, 67.67, 69.41, 83.17, 123.65, 123.74 (x2), 130.45, 138.37; m/z (EI): 180 (21%), 165 (5), 141 (14), 139 (8), 124 (72), 123 (91), 121 (19), 107 (34), 106 (67), 105 (67), 95 (100), 77 (72), 67 (54), 65 (41), 57 (87), 43 (51), 41 (81), 39 (64), 29 (86), 27 (40); electronic CD (MeOH): 282 nm $\Delta\varepsilon$ +1.204, 258 nm $\Delta\varepsilon$ +0.981, 226 nm $\Delta\varepsilon$ -1.174

Biotransformations of triols 7g$_{SR'}$, 7g$_{SS'}$, 7h$_{SR'}$, 7h$_{SS'}$ with *E. coli narB (NCDDnarB)*

Typical biotransformation procedure using *E. coli narB*

E. coli narB was grown at 37 ºC in Luria broth (LB) medium. Antibiotic ampicillin (0.1 mg cm^{-3}) was added to the culture medium, to maintain plasmid-harbouring cells. *iso*-Propyl-β-D-thiogalactopyranoside (IPTG), an enzyme-inducer, was added (0.05 mg/cm^3) before inoculation of the liquid LB-media for the cell-growth. Cells were harvested, in late exponential growth phase, by centrifugation, washed and re-suspended (shake flasks; OD_{600} = 5-10) in potassium phosphate buffer (50 mM, pH 7.2), prior to the biotransformation at 30 °C. Substrates (0.5-2 mg/cm^3) were added separately and the reaction terminated after 18 h. Separate biotransformations of triols **7g**$_{SR'}$ (170 mg, 1.04 mmol), **7g**$_{SS'}$ (320 mg, 1.95 mmol),

7h$_{SR'}$, (2.24 g, 10.67 mmol) and **7h$_{SS'}$** (70 mg, 0.33 mmol) were carried out with *E. coli narB* for 18 h. Ethyl acetate extractions of the centrifuged culture media yielded the corresponding single catechols **8g$_{R'}$**, **8g$_{S'}$**, **8h$_{R'}$** and **8h$_{S'}$**, which were purified by PLC (5% MeOH/$CHCl_3$).

3-[(1'*R*)-1'-Hydroxy-2',2'-dimethylpropyl]-1,2-benzenediol 8g$_{R'}$

Viscous oil (100 mg, 49%); R_f 0.37 (5% MeOH/$CHCl_3$); $[\alpha]_D$ +23 (*c* 0.6, MeOH); (Found: M^+, 196.1099; $C_{11}H_{16}O_3$ requires 196.1099); δ_H (500 MHz, $CDCl_3$) 0.99 (9 H, s, CH ^tBu), 2.71 (1 H, br s, OH), 4.55 (1 H, s, C*H* ^{t}Bu), 5.62 (1 H, br s, OH), 6.44 (1 H, dd, $J_{4,5}$ 7.7, $J_{4,6}$ 1.4, 4-H), 6.70 (1 H, dd, $J_{5,4}$ =$J_{5,6}$ 7.8, 5-H), 6.83 (1 H, dd, $J_{6,5}$ 7.9, $J_{6,4}$ 1.5, 6-H), 8.45 (1 H, br s, OH); δ_C (125 MHz, $CDCl_3$) 26.00, 37.27, 85.06, 113.69, 119.03, 120.75, 123.86, 143.14, 145.41 (x2); *m/z* (EI): 196 (M^+, 15%), 178 (30), 163 (24), 145 (6), 140 (7), 139 (100), 137 (9), 123 (9), 122 (6), 111 (12), 93 (41), 84 (8), 77 (5), 65 (30), 63 (5), 57 (25), 53 (8), 49 (12), 43 (24), 41 (26), 39 (23), 29 (22), 27 (12); electronic CD (MeOH): 204 nm $\Delta\varepsilon$ -1.976.

(-)-3-[(1'*S*)-1'-Hydroxy-2',2'-dimethylpropyl]-1,2-benzenediol 8g$_{S'}$

Viscous oil (280 mg, 73%); $[\alpha]_D$ -25 (*c* 0.48, MeOH); CD (MeOH): 204 nm $\Delta\varepsilon$ +1.852. The NMR and MS data were identical to that found for the **8g$_{R'}$** enantiomer.

(-)-3-[(1'*R*)-2',2',2'-Trifluoro-1'-hydroxyethyl]-1,2-benzenediol 8h$_{R'}$

Viscous oil (1.33 g, 60%); R_f 0.24 (7% MeOH/$CHCl_3$); $[\alpha]_D$ -15 (*c* 1.1, MeOH); (Found: M^+, 208.0341; $C_8H_7O_3F_3$ requires 208.0347); δ_H (500 MHz, $CDCl_3$) 3.95 (1 H, br s, OH), 5.18 (1 H, q, $J_{H',F}$ 7.1, C*H*CF_3), 5.70 (1 H, br s, -OH), 6.71 (1 H, dd, $J_{6,5}$ 7.8, $J_{6,4}$ 1.1, 6-H), 6.79 (1 H, dd, $J_{5,4}$ =$J_{5,6}$ 7.9, 5-H), 6.92 (1 H, dd, $J_{4,5}$ 7.9, $J_{4,6}$ 1.5, 4-H), 7.21 (1 H, br s, OH); δ_C (125 MHz, $CDCl_3$) 72.95 (q, *J* 33.12), 116.03, 117.72, 120.42, 120.59 (x2), 124.34 (q, *J* 282.75), 143.50, 144.71 (x2); *m/z* (EI): 208 (M^+, 100%), 190 (88), 162 (5), 161 (8), 143 (10), 142 (75), 139 (15), 137 (17), 133 (10), 114 (50), 93 (36), 81 (10), 69 (17), 65 (40); electronic CD (MeOH): 277 nm $\Delta\varepsilon$ -0.197, 206 nm $\Delta\varepsilon$ +3.922, 201 nm $\Delta\varepsilon$ -1.281.

(+)-3-[(1'*S*)-2',2',2'-Trifluoro-1'-hydroxyethyl]-1,2-benzenediol 8h$_{S'}$

Viscous oil (50 mg, 72%); $[\alpha]_D$ +13 (*c* 0.74, MeOH). The NMR and MS data were identical to that found for the 8h$_{R'}$ enantiomer.

Methylation of 3-[(1'*R*)-2',2',2'-trifluoro-1'-hydroxyethyl]-1,2-benzenediol 8h$_{R'}$

Methylation of 3-[(1'*R*)-2',2',2'-trifluoro-1'-hydroxyethyl]-1,2-benzenediol **8h$_{R'}$** (600 mg, 0.29 mmol) with an excess of diazomethane, at ambient temperature, yielded a crude mixture of (-)-2-methoxy-3-[(1'*R*)-2',2',2'-trifluoro-1'-hydroxyethyl]phenol **25h$_{R'}$** and (+)-(1*R*)-1-(2',3'-dimethoxyphenyl)-2,2,2-trifluoroethan-1-ol **26h$_{R'}$**, which were separated by PLC (20% diethyl ether/hexane).

2-Methoxy-3-[(1'*R*)-2',2',2'-trifluoro-1'-hydroxyethyl]phenol 25h$_{R'}$

White crystalline solid (30 mg, 46%); mp 114-117 ºC (from $CHCl_3$); R_f 0.26 (10% diethyl ether/hexane); $[\alpha]_D$ -2.0 (*c* 0.62, $CHCl_3$); (Found: M^+, 222.0503; $C_9H_9O_3F_3$ requires 222.0504); δ_H (500 MHz, $CDCl_3$) 3.08 (1 H, d, $J_{OH,H}$ 6.7, CHO*H*), 3.89 (3 H, s, O*Me*), 5.32 (1 H, qd, $J_{H,F}$ = $J_{H,OH}$ 6.9, C*H*CF_3), 5.36 (1 H, br s, ArO*H*), 7.00 (1 H, dd, $J_{6,5}$ 7.7, $J_{6,4}$ 1.9, 6-H) 7.05 (1 H, d, $J_{4,5}$ 7.5, $J_{4,6}$ 1.9, 4-H), 7.09 (1 H, dd, $J_{5,4}$ = $J_{5,6}$ 7.8, 5-H); δ_C (125 MHz, $CDCl_3$) 62.37, 68.56 (q, *J* 32.7), 118.04, 120.46, 124.77 (q, *J* 282.3), 125.37, 127.91, 146.34, 149.21; *m/z* (EI): 222 (M^+, 100%), 204 (10), 184 (10), 161 (10), 153 (81), 151 (7), 138 (23), 137 (28), 133 (31), 125 (13), 123 (30), 110 (12), 107 (8), 95 (8), 93 (37), 81 (10), 77 (11), 65 (18), 63 (9).

2-Methoxy-3-[(1'*S*)-2',2',2'-trifluoro-1' hydroxyethyl]phenol 25h$_{R'}$

White crystalline solid; mp 114 ºC; $[\alpha]_D$ +2.0 (*c* 0.58, $CHCl_3$).

(1*R*)-1-(2',3'-Dimethoxyphenyl)-2,2,2-trifluoroethan-1-ol 26h$_{R'}$

Light yellow viscous oil (30 mg, 44%); R_f 0.44 (10% diethyl ether/hexane); $[\alpha]_D$ +6.0 (*c* 2.38, $CHCl_3$); (Found: M^+, 236.0660; $C_{10}H_{11}O_3F_3$ requires 236.0669); δ_H (500 MHz, $CDCl_3$) 3.81 (1 H, d, $J_{OH,H}$ 7.7, CHO*H*), 3.88 (3 H, s, O*Me*), 3.95 (3 H, s, O*Me*), 5.26 (1 H, qd, $J_{H,F}$ =$J_{H,OH}$ 7.3, C*H*CF_3), 6.95 (1 H, dd, $J_{4',5'}$ 8.1, $J_{4',6'}$ 1.4, 4'-H), 6.98 (1 H, dd, $J_{6',5'}$ 7.6, $J_{6',4'}$ 1.0, 6'-H), 7.09 (1 H, dd, $J_{5',4'}$ = $J_{5',6'}$ 8.0, 5'-H); δ_C (125 MHz, $CDCl_3$) 55.85, 61.21(x 2), 69.97 (q, *J* 32.7), 113.53, 120.65, 124.23, 124.48 (q, *J* 282.8), 127.16, 147.69, 152.53; *m/z* (EI): 236 (M^+, 100%), 167 (60), 152 (12), 151 (7), 139 (27), 137 (18), 124 (12), 123 (5), 109 (15), 106 (14), 81 (10), 77 (12), 69 (17), 65 (8), 63 (8).

(1*S*)-1-(2',3'-Dimethoxyphenyl)-2,2,2-trifluoroethan-1-ol 26h$_{R'}$

Light yellow viscous oil;$[\alpha]_D$ -5.0 (*c* 1.43, $CHCl_3$).

Biotransformation of 4-bromo-3,5-cyclohexadiene-1,2-diol 3d$_S$ with *E. coli narB*

A small-scale biotransformation of (+)-(1*S*,2*R*)-4-bromo-3,5-cyclohexadiene-1,2-diol **3d$_S$** (10 mg, 0.05 mmol) was carried out with *E. coli narB* for 18 h. Ethyl acetate extraction of the centrifuged culture medium yielded 4-bromo-1,2-benzenediol **5d** as the only metabolite. Catechol **5d** was purified by PLC (50% ether/hexane) and identified by NMR spectroscopy.

4-Bromo-1,2-benzenediol 5d[15]

White solid (8.3 mg, 84%); R_f 0.27 (50% diethyl ether/hexane); δ_H (500 MHz, $CDCl_3$) 6.74 (1 H, d, $J_{6,5}$ 8.5, 6-H), 6.92 (1 H, dd, $J_{5,6}$ 8.4, $J_{5,3}$ 2.3, 5-H), 7.02 (1 H, d, $J_{3,5}$ 2.3, 3-H).

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