PERKIN 1

Stereoselective benzylic hydroxylation of 2-substituted indanes using toluene dioxygenase as biocatalyst

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Received (in Cambridge) 22nd February 1999, Accepted 16th April 1999

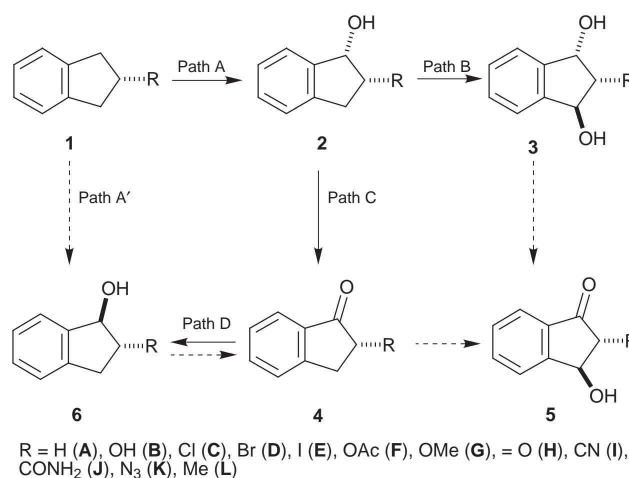
Indane, **1A**, and a series of 2-substituted indane substrates, **1B**–**1D**, **1G**, **1I**–**1L**, were found to undergo benzylic monohydroxylation catalysed by toluene dioxygenase, present in the intact cells of *Pseudomonas putida* UV 4, to yield enantiopure *cis*-indan-1-ols, **2A**–**2D**, **2G**, **2I**–**2L** of the same absolute configuration at C-1 as major bioproducts. Enantiopure *trans*-indan-1-ols **6B**, **6C**, and **6G** were also obtained as minor metabolites. Evidence of further sequential benzylic hydroxylation (*bis*-hydroxylation) was found only with substrates **2A**, **1C**, **1D** and **1L** to yield the corresponding enantiopure *trans*-1,3-diols, **3A**, **3C**, **3D** and **3L**. Minor enzyme-catalysed processes also observed include benzylic alcohol oxidation to ketones (**4A**, **5A**, **4B**, **4L**, **5L**), ketone reduction to benzylic alcohol **6A**, ester hydrolysis to indan-2-ol **1B**, and *cis*-dihydroxylation of indan-1-ol **6A** to triol **7**. The enantiopurities and absolute configurations of bioproducts have been determined using MTPA ester formation, circular dichroism spectroscopy and stereochemical correlation methods.

The contribution of asymmetric oxidation and kinetic resolution to the production of bioproducts of high ee (>98%), and the metabolic sequence involved in their biotransformation by *P. putida* UV4 is discussed. Enantiocomplementarity was found during the benzylic hydroxylation of indan-2-ol **1B**, using toluene dioxygenase and naphthalene dioxygenase, when both single enantiomers of the metabolites **2B**, **4B** and **6B** of opposite configurations were obtained.

Introduction

Dioxygenases have been widely regarded as enzymes which *catalyse reactions in which both of the oxygen atoms of O_2 are incorporated into the substrate molecule*.[1] This description of the catalytic role of the dioxygenase system has however recently been shown to be inaccurate. Thus, in addition to catalysing the *vicinal* dihydroxylation of arenes (*cis*-dihydrodiol formation) and cyclic or acyclic alkenes (*vicinal* diol formation), dioxygenases have also been found to catalyse the monooxygenation of a wide range of substrates. Dioxygenase-catalysed monohydroxylations of methyl and methylene groups activated by neighbouring aryl rings (benzylic hydroxylation), vinyl groups (allylic hydroxylation), carbonyl groups (keto alcohol formation), oxygen atoms (O-dealkylation), sulfur atoms (S-dealkylation) and nitrogen atoms (N-dealkylation) have all been reported in recent reviews of dioxygenase activity.[2–4] Although dioxygenation and monooxygenation reactions have been observed using the same bacterial dioxygenase enzymes, the main bioproducts found in this study have been the result of benzylic hydroxylation.

A preliminary report of this work focused on the role of toluene dioxygenase (TDO, present in cultures of the soil bacterium *Pseudomonas putida* UV4) in catalysing benzylic hydroxylation of indane, **1A**,[5] and 2-hydroxyindane, **1B**.[6] Since the major bioproduct in each case proved to be a single benzylic alcohol enantiomer, (1*R*)-**2A**[5] and (1*S*,2*R*)-**2B**,[6] the potential of this enzyme-catalysed route to yield a series of enantiopure indan-1-ols (**2A**–**2L**) has been examined further in this study. The relative contributions of dioxygenase-catalysed asymmetric synthesis (alkane monohydroxylation) and kinetic resolution (hydroxylation of a benzylic monol to a *trans*-1,3-diol or oxidation of a benzylic alcohol to a ketone) to the production of an enantiopure indan-1-ol were also of interest in this programme.



Scheme 1

The major objective of this study was, however, to examine the potential of the enzyme-catalysed hydroxylation route to chiral benzylic alcohols of synthetic value.

Results and discussion

All the indane substrates, with the exceptions of compounds **1A**, **1B** and **1H** (available commercially) were synthesised using standard literature procedures. Intact cells of the constitutive mutant strain (UV4), derived from the wild type bacterium *Pseudomonas putida* NCIMB 11767, provided the main source of TDO used in the present study. The UV4 strain was deficient in the *cis*-dihydrodiol dehydrogenase enzyme and thus *cis*-dihydrodiol metabolites of arene substrates accumulated in the

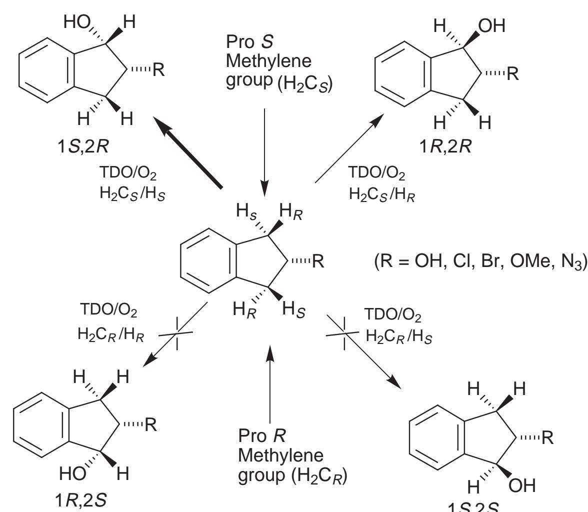
Table 1 Formation of bioproducts **2**–**6** from the biotransformation of indane substrates **1** using *Pseudomonas putida* UV4

Compound number	Substrate **1** (R)	Bioproduct **2**	Bioproduct **3**	Bioproduct **4**	Bioproduct **5**	Bioproduct **6**
A	H	+	+ [a,b]	+ [b]	+ [b]	
B	OH	+		+ [b]		+
C	Cl	+	+			+
D	Br	+	+			
E	I	+ [c]	+ [c]			
F	OAc	+ [d]		+ [d]		
G	OMe	+				+
H	=O	+ [e]				
I	CN	+				
J	$CONH_2$	+				
K	N_3	+				
L	Me	+	+	+	+	

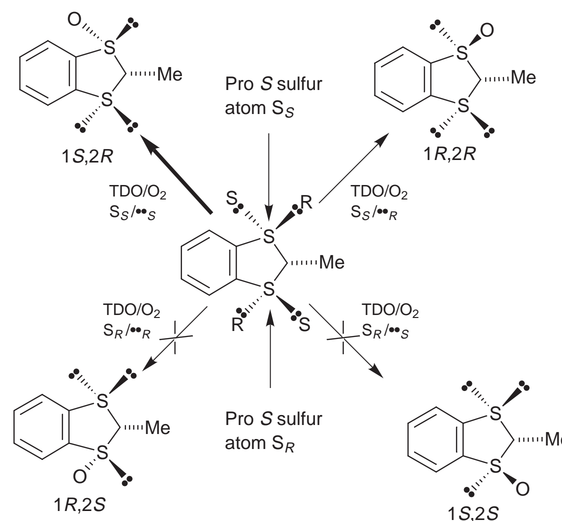
[a] Only isolated when (*R*)-indan-1-ol was used as substrate. [b] Only isolated when (*S*)-indan-1-ol was used as substrate. [c] Trace quantities only detected. Substrate mainly dehydrohalogenated to yield indene **8** and the derived metabolites *cis*-diol **2B** and inden-1-ol **9**. [d] Substrate hydrolysed to yield indan-2-ol, **1B** before hydroxylation. [e] Substrate reduced to yield indan-2-ol, **1B** before hydroxylation.

bioextracts. When the indane substrates **1A**–**1L** were added to intact cells of *P. putida* UV4, benzylic hydroxylation was generally the initial step observed in the biotransformation process. In Table 1 are listed the range of *cis*-indan-1-ols **2A**–**2E**, **2G**, **2I**–**2L** and other metabolites isolated from the bioextracts. The *cis*-geometry of the latter bioproducts was in each case established by spectral methods and comparison with reported[7–9] *cis*- and *trans*-2-substituted indan-1-ols (*e.g.* **2B**, **6B**, **6C**, **6D**, **2K**, **6K**, **2L** and **6L**). ^{1}H NMR spectral analyses of *cis*/*trans*-2-substituted indan-1-ols had earlier suggested[7–9] that reliable stereochemical assignments could be made based on a larger difference between δ values for H_3 and $H_{3'}$ in the *trans*-isomer as compared to the *cis*-isomer. This differential in δ values for the methylene protons allowed the major indan-1-ol bioproducts **2B**, **2C**, **2D**, **2G**, **2I**–**2L** to be assigned the *cis*-geometry and the minor metabolites **6B**, **6C**, and **6G** the *trans* configuration. *Vicinal* coupling constant values in five-membered rings are not generally regarded as reliable indicators of *cis*/*trans* geometry.[7] However, in this case the *cis*-isomers were found to have slightly smaller *vicinal* ($J_{1,2}$) coupling constants. The indan-1-ol bioproducts **2A**–**2D**, **2G**, **2I**–**2L** were determined to be enantiopure by comparison with reported $[a]_D$ values,[8–10] and/or by formation and ^{1}H NMR analyses of their 2-methoxy-2-phenyl-2-trifluoromethylacetate (MTPA) ester derivatives, and/or by chiral stationary phase high pressure liquid chromatography (CSPHPLC). The absolute configurations of the metabolites **2A**, **2B**, **2K** and **2L** are already known.[8–11] Additional methods used for absolute configuration assignment include stereochemical correlation between indan-1-ols **2A** (with **2B** and **2D**), **2D** (with **2K**), **2I** (with **2J**), and a comparison of circular dichroism spectra (**2A**–**2D**, **2G**, and **2L**), which were dominated by a strong absorption in the region λ 195 to 198 nm ($\Delta\varepsilon$ −8 to −15 $dm^3\ mol^{-1}\ cm^{-1}$).

It is noteworthy that the absolute configuration at C-1 of the single enantiomer indan-1-ol bioproducts **2A**–**2D**, **2G**, **2I**–**2L** is identical in all cases (Scheme 1) but, due to changes in substituent priorities on application of the Sequence Rules, this configuration is assigned as 1*R* for metabolites **2A**, **2I**, **2J** and **2L** and as 1*S* for metabolites **2B**–**2D**, **2G** and **2K**. The formation of the 2-substituted indan-1-ol bioproducts **2B**–**2D**, **2G**, **2I**–**2L**, as essentially single enantiomers (>98% ee) having an identical absolute configuration at C-1 (Scheme 1, Path A), is assumed to result mainly from asymmetric oxidation (benzylic hydroxylation) where the dioxygenase enzyme is able to stereodifferentiate between hydroxylation of the prochiral methylene groups at C-1 and C-3 each bearing two prochiral hydrogen atoms (Scheme 2). A similar stereoselective asymmetric synthesis has been postulated[12,13] to account for the formation of *cis*-2-methyl-1,3-benzodithiole 1-oxide during dioxygenase-catalysed oxidation (sulfoxidation) of prochiral sulfur atoms at positions



Scheme 2



Scheme 3

1 and 3 each bearing prochiral lone pairs (Scheme 3). The preferred stereoselective substitution of a hydrogen atom by a hydroxy group at one of the two prochiral benzylic groups and one of the two prochiral benzylic hydrogen atoms (*pro-S*, R = Cl, Br, OH, OMe, N_3, and *pro-R*, R = H, Me, CN, $CONH_2$, Scheme 2), during the TDO-catalysed benzylic hydroxylation, occurred in a configurationally similar manner to the TDO-

catalysed sulfoxidation process in 2-methyl-1,3-benzodithiole (Scheme 3).

The similarity in shape of the latter thioacetal to the 2-substituted indane substrates **1A**–**1E**, **1G** and **1I**–**1L** suggests that they are all accommodated in a similar manner at the active site of TDO. Unfortunately neither the crystal structure of the TDO enzyme, nor the mechanism of monooxygenation (benzylic hydroxylation or sulfoxidation) has yet been determined. The results are consistent with a similar mechanism for both TDO-catalysed benzylic hydroxylation and alkylaryl sulfoxidation reactions. Evidence that TDO was responsible for biocatalysis of these types of monooxygenation processes was obtained by using a recombinant strain, *Escherichia coli* pKST 11. This strain expresses the toluene dioxygenase gene found in the wild type strain of *P. putida* (11767) on plasmid pKST 11. The inducible mutant strain (UV4) was originally derived from the 11767 strain. In a typical example when *E. coli* pKST 11 was used with substrate **1L**, *cis*-diol **2L** (40%), of high enantiopurity (>90%), and of identical absolute configuration to that obtained using *P. putida* UV4, was formed. When the parent *E. coli* strain was used as a control the bioproduct **2L** was not observed. Similar results were found when *E. coli* pKST 11 was used with the sulfide substrates.[14]

In our earlier work[12,13] the sulfoxidation of 2-methyl-1,3-benzodithiole using oxygen and *P. putida* UV4 (TDO) was found (Scheme 3) to yield the *cis* isomer of 2-methyl-1,3-benzodithiole 1-oxide as the major metabolite (96%, 1*S*,2*R*, >98% ee) and the *trans* isomer as a very minor bioproduct (4%, 1*S*,2*S*, >98% ee). *trans* Isomers **6B**, **6C**, **6G** (*ca.* 10% relative yield, >98% ee, 1*R*,2*R*) were also found as minor benzylic hydroxylation metabolites of indan-2-ol **1B**, 2-chloroindane **1C**, and 2-methoxyindane **1G** respectively (Scheme 1, Path A′). Based upon stereochemical correlations with compounds of established absolute configuration it was found that these minor *trans* bioproducts **6B**, **6C** and **6G** had the opposite (1*R*) configuration at the benzylic chiral centre compared with the corresponding (1*S*) *cis*-bioproducts **2B**, **2C** and **2G**. The reversal of absolute configuration at the benzylic chiral centre in the *cis* and *trans*-indan-1-ol metabolites was unexpected since it was assumed that both hydroxylations (Paths A and A′) were catalysed by the same dioxygenase.

When indan-1-one **4A** was added to *P. putida* UV4 it was reduced to indan-1-ol **2A** having a high enantiomeric preference (77%) for the same absolute configuration at C-1 as was found in the *trans* indan-1-ol metabolites, **6B**, **6C**, and **6G**. Although the definitive experiments using the individual enantiomers of the ketones **4B**, **4C** and **4G** as substrates with *P. putida* UV4 have not yet been carried out, it is probable that they were formed *via* the metabolic sequence Path A ⟶ Path C ⟶ Path D. The oxidation of *cis*-indan-1-ols **2B**, **2C** and **2G** to yield the ketones **4B**, **4C** and **4G**, followed by reduction to the corresponding *trans*-indan-1-ols **6B**, **6C**, and **6G** using intact cells of *P. putida* UV4 (and other strains of *P. putida*[15]) are well precedented and are assumed to involve both dioxygenase and dehydrogenase enzymes. If the formation of the enantiopure bioproducts **2B**–**2D**, **2G**, **2I**–**2L** results from asymmetric benzylic hydroxylation alone then the pattern of stereoselectivity is consistent with exclusive attack (>98%) at one prochiral methylene group and at one prochiral hydrogen atom in substrates **1B**–**1D**, **1G**, **1I**–**1L** (Scheme 2).

The weight of currently available evidence would appear to favour a biotransformation process involving a marked degree of stereoselectivity during asymmetric hydroxylation (Scheme 1, path A). The formation of enantiopure indan-1-ol bioproducts **2A**–**2D**, **2G**, **2I**–**2L** could also arise from an enzyme-catalysed kinetic resolution process (Scheme 1, path B and/or path C) or by TDO-catalysed benzylic hydroxylation to yield a mixture of enantiomers followed by a kinetic resolution process (Scheme 1, paths A, B and C). Among observed enzyme-catalysed reactions of indan-1-ols, which might result in asymmetric destruction of one enantiomer, are further oxidations including benzylic hydroxylation to yield *trans*-1,3-diols (**3A**, **3C**, **3D** and **3L**, Scheme, path B) and benzylic alcohol oxidation to yield cyclic ketones (**4A**, **4B**, **4L** and **5L**, Scheme 1, path C).

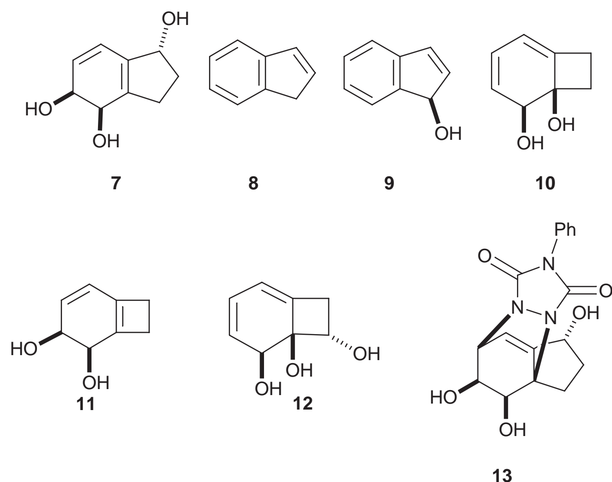
The possible role of kinetic resolution, in the production of enantiopure indan-1-ol metabolites, was also investigated. The benzylic alcohol derivative **2A,** of the parent substrate indane **1A**, which was readily available commercially in both enantiomeric forms, was used as substrate. (−)-(*R*)-Indan-1-ol **2A** (66%, >98% ee) and indan-1-one (**4A**, 34%) were previously reported as the major metabolites of indane **1A** in cultures of *P. putida* UV4; *trans*-1,3-dihydroxyindane **3A** was not however isolated as a metabolite.[5] When enantiopure (−)-(*R*)-indan-1-ol **2A** was examined under similar conditions it proved to be a relatively poor substrate (72% recovery of substrate) and (−)-*trans*-(1*R*,3*R*)-1,3-dihydroxyindane **3A** was isolated as the major metabolite (13%, >98% ee by CSPHPLC analysis). By contrast, enantiopure (+)-(*S*)-indan-1-ol **2A**, was found to be a much better substrate (6% recovery) yielding indan-1-one **4A** as the major (82%) with (+)-*trans*-(1*S*,3*S*)-diol (9%) and (3*R*)-3-hydroxyindane-1-one **5A** (4%) as the minor metabolites. From the aforesaid observations, using *P. putida* UV4 or similar results from *P. putida* F39/D,[15,16] both of which are sources of TDO, it is clear that the biotransformation of the (1*S*)-enantiomer of indan-1-ol is faster than the (1*R*)-enantiomer. The ee value of (*R*)-indan-1-ol **2A** (>98% ee) may therefore be the combined result of asymmetric synthesis, yielding an excess of (*R*)-indan-1-ol **2A**, followed by a kinetic resolution process favouring the oxidation of (*S*)-indan-1-ol **2A** largely to indan-1-one **4A**.

The 1,3-diols **3A**, **3C**, **3D, 3E** and **3L**, isolated as bioproducts from the corresponding indane **1A** or 2-substituted indanes (**1C**, **1D**, **1E** and **1L**), were all found to be optically active and thus must have a 1,3-*trans* configuration and C_2 symmetry. A time-course experiment on the biotransformation of 2-bromoindane **1D**, to yield *cis*-2-bromoindan-1-ol **2D** and *trans*-1,3-diol **3D**, was carried out by HPLC analysis of the bioextracts; the formation of *trans*-diol **3D** was found to coincide with the disappearance of *cis*-2-bromoindan-1-ol **2D**. The initially formed enantiopure *cis*-2-bromoindan-1-ol **2D**, when used as substrate to *P. putida* UV4 confirmed the formation of a single enantiomer of the *trans*-diol **3D** as the sole metabolite through the metabolic sequence shown in Scheme 1 (**1**→**2**→**3**, path B). The samples of diol **3D** obtained from biotransformation of 1-bromoindane **1D** or the indan-1-ol derivative **2D** were indistinguishable. Based upon the established formation of the enantiopure *trans*-diols **3A** and **3D** from the corresponding indan-1-ol enantiomers (1*R*)-**2A** and (1*S*,2*R*)-**2D** respectively**,** it is assumed that the other *trans* diols, **3C** (>98% ee by MTPA analysis) and **3E** (>98% ee by CSPHPLC analysis), were also formed from the corresponding indan-1-ol enantiomers, (1*S*,2*R*)-**2C** and (1*S*,2*R*)-**2E**. Since the absolute configurations of the indan-1-ol precursors (**2A**, **2C**, **2D** and **2L**) were known, the derived *trans* 1,3-diols (**3A**, **3C**, **3D** and **3L**) must have the configurations shown in Scheme 1. This was also evident from their similar CD spectra (λ 192 to 200 nm, $\Delta\varepsilon$ −2.4 to −14 dm^3 mol^{-1} cm^{-1}). While no attempts were made to optimise the yields of indan-1-ols **2A**–**2D**, **2G**, **2I**–**2L**, the time-course study carried out on the metabolism of 2-bromoindane **1D** suggested that the proportion of monohydroxylation and dihydroxylation products could be controlled. Thus longer biotransformation times led to the isolation of *trans*-1,3-diols **3A**, **3C**, **3D** and **3L** as major or exclusive metabolites. The importance of controlling biotransformation time and other parameters was evident from the variable proportions of metabolites formed when small scale (shake flask) and large scale (fermenter, where the biotransformation conditions are different) procedures were used for the metabolism of substrates **1B** and **1C**.

Ketone metabolites from indane substrates were generally

found to be less common than mono- or *bis*- benzylic hydroxylation products. Indan-1-one **4A** was thus isolated from indane **1A** in low yield (7%). Indan-1-one **4A** (80%) and (*R*)-3-hydroxyindane-1-one **5A** (4%; 82% ee) were however obtained as metabolites from (*S*)-indan-1-ol **2A**. Similarly (2*R*)-2-hydroxyindan-1-one (**4B**, 0 to 2%; 26% ee) was derived from indan-2-ol **1B**. The only other ketone bioproducts, obtained in significant quantities, were from 2-methylindane **1L**. Compound **1L** was an excellent substrate yielding in addition to the benzylic hydroxylation products **2L** and **3L,** the ketones **4L** and **5L**. 2-Methylindan-1-one **4L** was estimated to have a small excess (7% ee) of the 2*R* enantiomer from a comparison with the literature $[a]_D$ value.[11] The ketoalcohol bioproduct from 2-methylindane **1L** consisted mainly of the *cis* isomer of **5L** (66% ee by CSPHPLC analysis) with a minor proportion of the *trans* isomer **5L**. At present no unequivocal explanation can be provided for the isolation of chiral ketone bioproducts **5A** (82% ee), **4B** (26% ee) **4L** (7% ee) and **5L** (66% ee) with lower ee values compared to the benzylic alcohol bioproducts **2A**–**2D**, **2G**, **2I**–**2L**, **3A**, **3C**, **3D** and **3L** (>98% ee). The possibility of racemisation of chiral centres adjacent to a keto group (*e.g.* **4B** and **4L**) cannot at present be excluded. The formation of both benzylic alcohol enantiomers followed by stereoselective dehydrogenase enzyme-catalysed oxidation to yield ketones (**2**→**4**, **6**→**4**, **3**→**5**) or other metabolic steps involving achiral intermediates may also contribute to the lower ee values observed. The nature of the enzyme in *P. putida* UV4 responsible for ketone formation has not been established. It is likely that an alcohol dehydrogenase enzyme is involved since Gibson *et al.* have shown[15] that a toluene inducible indan-1-ol dehydrogenase enzyme was present in *P. putida* F39/D and was responsible for the formation of indan-1-one **4A** from indane **1A**.

The formation of monohydroxylation products from indane (**2A**–**2D**, **2G**, **2I**–**2L**) and indan-1-ol (**3A**, **3C**, **3D** and **3L**) substrates is typical of the biotransformation of benzocycloalkenes by TDO present in *P. putida* UV4. Dioxygenase-catalysed *cis*-dihydroxylation in the benzene ring of benzocycloalkenes is rather unusual but has been found using *P. putida* UV4 and benzocyclobutene as substrate. *cis*-Diols **10** and **11** and a triol **12** were formed from benzocyclobutene.[5,17] During the present study of indane substrates, a minor metabolite of (*R*)-indan-1-ol **2A** was isolated and identified as 1,4,5-trihydroxy-4,5-dihydroindane **7** (4%). Triol **7** was found to partially decompose to a phenol product during separation and purification procedures. Despite its instability, a sufficient sample of triol **7** was purified to allow its complete characterisation by ^{1}H (NOE and COSY) and ^{13}C NMR, MS, and CD analyses and from formation of the cycloadduct **13** with *N*-phenyl-1,2,4-triazoline-3,5-dione. Since triol **7**, a metabolite of enantiopure (*R*)-indan-1-ol, had a very similar CD spectrum to that of (2*R*,3*S*)-*cis*-diol **11**, it was concluded that it was a single enantiomer having the (1*R*,4*R*,5*S*) absolute configuration.



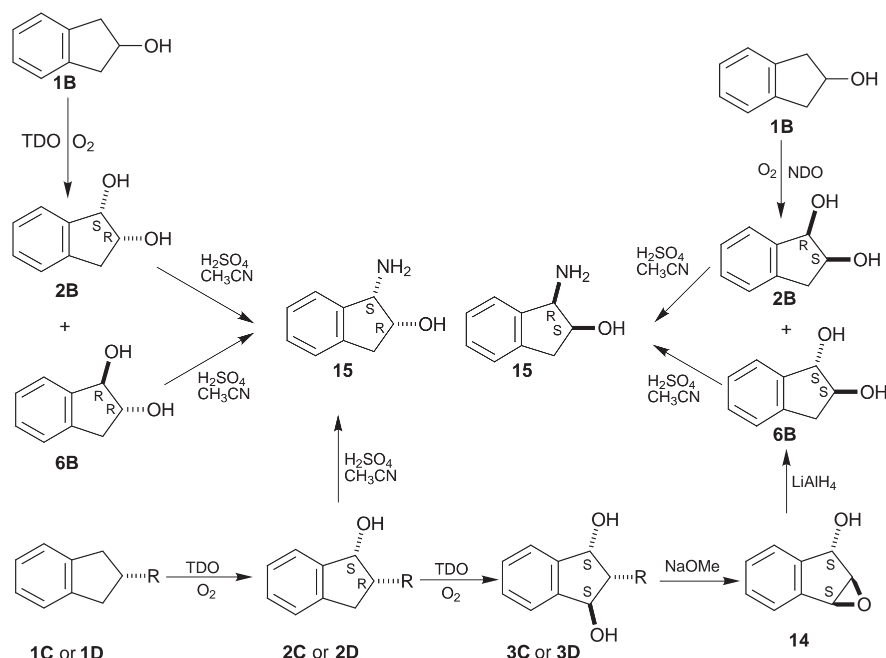
Biotransformation of 2-substituted indanes **1E**, **1F**, and **1H** did not provide any significant quantities of the corresponding indan-1-ol metabolites (**2E, 2F,** and **2H**) possibly due to competition from other dioxygenase-catalysed pathways or alternative enzyme reactions, *e.g.* dehydrohalogenation of 2-iodoindane **1E** gave indene **8** and the derived bioproducts **9** and **2B**. The dehydroiodination of 2-iodoindane is consistent with the involvement of a benzylic radical intermediate. It is evident that (i) the carbon–iodine bond in substrate **1E** is weaker than any of the equivalent (C–R) bonds in substrates **1A**–**1D**, and **1F**–**1L**, and (ii) an iodine atom β to a radical centre will undergo rapid homolytic cleavage.[18,19] The formation of indene **8** and the derived bioproducts **2B** and **9** may be used as evidence of radical involvement. Hydrolysis of the ester **1F** and reduction of ketone **1H** was in each case found to yield indan-2-ol **1B**, and the derived diols **2B** and **6B**.

The concept of enantiocomplementarity, *i.e*. synthesis of either enantiomer, is of particular value in the context of enzyme-catalysed reactions since it is often assumed that enzyme systems produce an excess of only one enantiomer. Recent studies have shown that toluene dioxygenase (TDO) and naphthalene dioxygenase (NDO) enzymes can yield sulfoxides of opposite chirality, from the parent sulfides.[3,20,21] Similarly when these dioxygenase enzyme systems were used in the benzylic hydroxylation of indane **1A**, enantiocomplementarity was again observed with the (*R*) configuration being preferred by TDO (84 to >98% ee) and the (*S*) configuration by NDO (58 to 92% ee). The best example to date of enantiocomplementarity, occurring during dioxygenase-catalysed benzylic hydroxylation, was provided by the biotransformation of 2-substituted indanes, when TDO (from *P. putida* UV4) and NDO (from *P. putida* NCIMB 8859) were used with indan-2-ol **1B** as substrate (Scheme 4). In each case three metabolites **2B**, **4B**, and **6B** were isolated. While the relative proportions of each metabolite differed, all were found to be enantiopure (>98% ee) and of opposite absolute configuration (Table 2). Further work is in progress to establish if enantiocomplementarity also occurs during benzylic hydroxylation of other 2-substituted indane substrates shown in Table 1.

An ultimate objective of this study was to examine the feasibility of using the biotransformation route to enantiopure chiral compounds for use in synthesis. While no attempt has been made to optimise the yields of bioproducts the results indicate that (i) it is possible to obtain isolated yields in excess of 75%, (ii) generally the preferred bioproduct can be obtained in higher yield by carefully monitoring the progress of the biotransformation (HPLC) and stopping the process at the appropriate stage, (iii) benzylic hydroxylation has been demonstrated successfully on a 10 g scale and should be feasible on a much larger scale providing that the fermenter capacity is available. *cis*-and *trans*-1,2-Dihydroxyindanes (**2B** and **6B**), obtainable as single enantiomers from the benzylic hydroxylation of indan-2-ol **2B** using TDO and NDO, have been used as precursors for *cis*-1-amino-2-hydroxyindane **15**.[6,22] Individual enantiomers of the amino alcohol **15** have been synthesised from the *cis*- and *trans*-diol metabolites (**2B** and **6B**), using the Ritter reaction (Scheme 4).[6] The Ritter reaction conditions, applied to synthesis of the *cis*-amino alcohol **15** from *trans*-1-hydroxy-2- bromoindane **6D**,[22] have also been found to apply to the enantiopure *cis*-halohydrin metabolites **2C** and **2D** yielding the corresponding amino alcohol enantiomers **15**.[6] Conversion of the derived *trans*-1,3-diol derivatives **3C** and **3D** to *trans*-diol **6B** *via* the epoxyalcohol **14** allowed the synthesis of amino alcohols of opposite configuration to that obtained from the halohydrin metabolites **2C** and **2D**. This provides a further demonstration of enantiocomplementarity. Synthetic applications of the enantiomers of *cis*-1-amino-2-hydroxyindane, derived from indan-1-ol metabolites **2B**, **4B**, **2C** and **2D**, and other types of enantiopure diol metabolites, will be discussed elsewhere.

Table 2 Yields, ee values, and absolute configurations of metabolites using TDO and NDO enzyme systems and indan-2-ol **1B** as substrate

	Isolated yield (%)		Ee (%)		Absolute configuration	
Metabolite	TDO	NDO	TDO	NDO	TDO	NDO
2B	35	3	>98	>98	1*S*,2*R*	1*R*,2*S*
4B	1	35	>98	>98	2*R*	2*S*
6B	4	10	>98	>98	1*R*,2*R*	1*S*,2*S*



Scheme 4

Experimental

^{1}H NMR spectra were recorded at 300 MHz (General Electric QE 300 and Bruker Avance DPX-500) and at 500 MHz (General Electric GN 500 and Bruker Avance DRX-500) in $CDCl_3$ solvent unless stated otherwise. Chemical shifts (δ) are reported in ppm relative to $SiMe_4$ and coupling constants (J) are given in Hz. ^{13}C NMR spectra were recorded at 125 MHz using the General Electric GN 500 and Bruker Avance DRX-500 instruments. Mass spectra were recorded at 70 eV on an AEI-MS902 instrument updated by VG Autospec Instruments, using a heated inlet system. Accurate molecular weights were determined by the peak matching method using perfluorokerosene as standard. Elemental microanalyses were obtained on a Perkin-Elmer 2400 CHN microanalyser. Circular dichroism spectra were recorded on a Jasco J-720 instrument in acetonitrile solvent; ε values are given in dm^3 mol^{-1} cm^{-1}. CSPHPLC was carried out using a Shimadzu LC-6A liquid chromatograph connected to Hewlett Packard diode array detector and the specified Daicel CSP column.

Shake flask (small, <0.5 g) and fermenter (large, >0.5 g) biotransformations were carried out using *P. putida* UV4 and *P. putida* NCIMB 8859 under reported conditions.[20]

Synthesis of substrates

2-Chloroindane 1C. Thionyl chloride (55 g, 0.46 mol) was added, in small portions over 1 h with stirring at 0 °C, to a solution of indan-2-ol (30 g, 225 mmol) in anhydrous pyridine (20 cm^3) and chloroform (250 cm^3). The mixture was refluxed for 3 h, cooled and poured onto crushed ice. The organic layer was separated, dried (Na_2SO_4) and solvent was distilled off. The crude product obtained was purified by flash column chromatography (silica gel, pentane), to yield 2-chloroindane **1C**, (21 g, 61% yield) as a colourless oil, bp 120 °C/5 mmHg, (lit.,[23] 90–91 °C/3 mmHg); δ_H (500 MHz) 3.21 (2H, dd, $J_{1,1}$ 16.7, $J_{1,2}$ 3.9, 1-H), 3.44 (2H, dd, $J_{1,1'}$ 16.7, $J_{1',2}$ 6.4, 1′-H), 4.72 (1H, tt $J_{2,1}$ 3.9, $J_{2,1'}$ 6.4, 2-H), 7.19–7.25 (4H, m, Ar-H); *m*/*z* 152 (M$^+$, 50%), 117 (100).

2-Bromoindane 1D. Phosphorus tribromide (55 g, 0.20 mol) was added in small portions during 1 h with stirring at 0 °C to a solution of indan-2-ol (30 g, 225 mmol) in anhydrous pyridine (20 cm^3) and chloroform (250 cm^3). The reaction mixture when worked up and purified by flash column chromatography as above yielded 2-bromoindane (19 g; 43%); bp 83–87 °C/5 mmHg, (lit.,[24] 83–85 °C/4 mmHg); δ_H (500 MHz) 3.34 (2H, dd, $J_{1,1}$ 17.1, $J_{1,2}$ 3.9, 1-H), 3.51 (2H, dd, $J_{1,1}$ 17.1, $J_{1',2}$ 6.3, 1′-H), 4.76 (1H, tt, $J_{2,1}$ 3.9, $J_{2,1'}$ 6.3, 2-H), 7.19–7.29 (4H, m, Ar-H); *m*/*z* 198 (M^+, ^{81}Br, 30%), 196 (M^+, ^{79}Br, 37%), 117 (100).

2-Iodoindane 1E. To a solution of 2-(*p*-tolylsulfonyl)indane (3.6 g, 12.5 mmol) in dry acetone (100 cm^3) was added anhydrous sodium iodide (5 g, 36.2 mmol). The reaction mixture was stirred at 55 °C for 18 hours, cooled and diluted with water (100 cm^3). Most of the acetone was removed under reduced pressure and the aqueous reaction mixture extracted with ether (3 × 75 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the crude product by flash chromatography (hexane ⟶ 25% Et_2O–hexane) gave 2-iodoindane **1E** (2.4 g, 80%); mp 51–52 °C (lit.,[25] 51–52 °C); δ_H (500 MHz) 3.39 (2H, dd, $J_{1,1'}$ 17.0, $J_{1,2}$ 5.0, 1-H), 3.48 (2H, dd, $J_{1',1}$ 17.0, $J_{1',2}$ 6.2, 1′-H), 4.35 (1H, m, 2-H), 7.20–7.26 (2H, m, Ar-H), 7.27–7.28 (2H, m, Ar-H).

2-Acetoxyindane 1F. A solution of indan-2-ol **1B** (5 g, 37 mmol), in anhydrous pyridine (2 cm^3), was treated with excess of acetic anhydride (5 cm^3) and the mixture stirred at room temperature overnight. Pyridine and the excess of acetic

anhydride were removed by distillation with toluene under reduced pressure. Purification of the crude product by distillation yielded 2-acetoxyindane **1F** (6.5 g, 99%) as a clear oil, bp 110 °C/12 mmHg (Found: M^+, 176.0845. $C_{11}H_{12}O_2$ requires M^+, 176.0837); δ_H (500 MHz) 2.02 (3H, s, OAc), 3.01 (2H, dd, $J_{1,1'}$ 16.9, $J_{1,2}$ 2.9, 1-H), 3.31 (2H, dd, $J_{1',1}$ 16.9, $J_{1',2}$ 6.4, 1′-H), 5.51 (1H, m, 2-H), 7.17–7.25 (4H, m, Ar-H); *m/z* 176 (M^+, 1%), 117 (15), 116 (100).

2-Methoxyindane 1G. To a stirring mixture of indan-2-ol **1B** (2.68 g, 20 mmol) and powdered KOH (2.2 g, 40 mmol) in DMSO (20 cm^3) was added dropwise methyl iodide (2.8 g, 20 mmol) at room temperature and the stirring continued for 2 h. Water (50 cm^3) was added and the product extracted into ether (2 × 50 cm^3). The ether extract was washed with water (2 × 25 cm^3), dried ($MgSO_4$) and concentrated. The crude product obtained was purified by distillation, to yield 2-methoxyindane **1G** (2.96 g, 88%) as a colourless oil, bp 76–80 °C/12 mmHg (Found: M^+, 148.0892. $C_{10}H_{12}O$ requires M^+, 148.0888); δ_H (500 MHz) 3.07 (2H, dd, $J_{1,1'}$ 16.09, $J_{1,2}$ 4.24, 1-H), 3.24 (2H, dd, $J_{1',1}$ 16.14, $J_{1',2}$ 6.39, 1′-H), 3.46 (3H, s, OMe), 4.32 (1H, m, 2-H), 7.22–7.32 (4H, m, Ar-H); *m/z* 148 (M^+, 60%), 117 (30), 116 (100).

2-Cyanoindane 1I. A solution of 2-carbamoylindane **1J** (0.1 g, 0.62 mmol) in thionyl chloride (2.0 cm^3) was heated under reflux for 15 h. Excess of thionyl chloride was carefully distilled off and water added to the cooled reaction mixture. The crude 2-cyanoindane **1J** was extracted into ether and purified by distillation to yield an oil (0.060 g, 73%), bp 90 °C/3 mmHg (lit.,[26] 79–81 °C/0.05 mmHg); δ_H (500 MHz) 3.04 (2H, dd, $J_{1,1}$ 16.9, $J_{1,2}$ 3.9, 1-H), 3.21 (2H, dd, $J_{1,1}$ 16.9, $J_{1',2}$ 6.5, 1′-H), 3.76 (1H, tt, $J_{2,1}$ 3.9, $J_{2,1}$ 6.5, 2-H), 7.19–7.29 (4H, m, Ar-H); *m/z* 133 (M^+, 26%), 117 (37), 77 (100).

2-Carbamoylindane 1J. To a mixture of indan-2-ol **1B** (1.0 g, 7.4 mmol), sodium cyanide (0.73 g, 15 mmol) and sodium iodide (0.005 g) in acetonitrile (10 cm^3) and DMF (10 cm^3) was added dropwise trimethylsilyl chloride (1.02 g, 15 mmol) and the reaction mixture refluxed at 60 °C for 5 h. Water (20 cm^3) followed by a saturated solution of sodium hypochlorite (25 cm^3) was added to the reaction mixture, the crude product extracted with ether (2 × 50 cm^3) and the ethereal layer dried. Purification by column chromatography (ether–hexane; 1:9) yielded compound **1J** as a colourless oil (0.7 g, 73%), bp 83–87 °C/5 mmHg (Found: M^+, 161.0843, $C_{10}H_{11}NO$ requires M^+, 161.0841); δ_H (500 MHz) 2.95 (2H, dd, $J_{1,1}$ 17.1, $J_{1,2}$ 3.9, 1-H), 3.23 (2H, dd, $J_{1,1}$ 17.1, $J_{1',2}$ 6.3, 1′-H), 5.57 (1H, tt, $J_{2,1}$ 3.9, $J_{2,1}$ 6.3, 2-H), 7.19–7.29 (4H, m, Ar-H); *m/z* 161 (M^+, 20%), 117 (37), 77 (100).

2-Azidoindane 1K. A mixture of 2-(*p*-tolylsulfonyl)indane (Lancaster) (0.46 g, 1.6 mmol) and sodium azide (0.15 g, 2.3 mmol) in anhydrous DMF (6 cm^3) was stirred for 5 h at 70 °C. The cooled reaction mixture was extracted with ether (2 × 30 cm^3) after quenching with water (25 cm^3). The ether extract was washed with water (15 cm^3), dried ($MgSO_4$) and concentrated under pressure to yield product **1K** as a colourless oil (0.21 g, 83%); bp 55–57 °C/8 mmHg (Found: M^+, 159.0801, $C_9H_9N_3$ requires M^+, 159.0796); δ_H (500 MHz) 3.01 (2H, dd, $J_{1,1}$ 16.2, $J_{1,2}$ 4.4, 1-H), 3.23 (2H, dd, $J_{1,1}$ 16.2, $J_{1',2}$ 6.6, 1′-H), 4.35 (1H, tt, $J_{2,1}$ 4.4, $J_{2,1}$ 6.6, 2-H), 7.17–7.25 (4H, m, Ar-H); *m/z* 159 (M^+, 35%), 131 (4), 117 (5).

2-Methylindane 1L. Following reaction conditions reported in the literature,[27] magnesium turnings (0.96 g, 41 mmol), methyl iodide (4.15 g, 39 mmol) and indan-2-one (5 g, 37 mmol) were reacted together. After the usual workup the crude product was purified by column chromatography (ethyl acetate–hexane; 3:7) to yield 2-methylindan-2-ol as a colourless oil (4.1 g, 73%); δ_H (300 MHz) 1.43 (3H, s, Me), 3.95 (4H, s, 2 × CH_2), 7.10–7.38 (4H, m, Ar-H). A solution of 2-methylindan-2-ol (3.1 g, 4 mmol) in dry benzene (40 cm^3) containing toluene-*p*-sulfonic acid (0.02 g) was refluxed using a Dean–Stark apparatus until the dehydration reaction was complete (4 h, TLC analysis). The organic layer was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the crude product by flash chromatography (hexane) gave 2-methylindene as a colourless oil (2.5 g, 69%), bp 43–45 °C/3 mmHg (lit.,[28] 79 °C, 10 mmHg); δ_H (300 MHz) 2.18 (3H, s, CH_3), 3.28 (2H, s, 1-H), 6.50 (1H, s, 3-H), 7.05–7.38 (4H, m, Ar-H); *m/z* 130 (M^+, 100%), 115 (69). The ^1H NMR spectrum of the sample was similar to that reported.[27]

A solution of 2-methylindene (1.0 g, 7.7 mmol) in hexane (5 cm^3) was stirred at 1 atm under hydrogen for 5 h in the presence of 10% Pd/C. The catalyst was removed by filtration and the hexane under reduced pressure. The hydrogenated crude product was distilled under reduced pressure to yield pure 2-methylindane **1L** (0.910 g, 91%); bp 42–46 °C/3 mmHg (lit.,[27] bp 70 °C/10 mmHg); δ_H (300 MHz) 1.13 (3H, d, $J_{Me,H}$ 6.2, Me), 2.54 (3H, m, 2-H and 3-H), 3.05 (2H, m, 1-H), 7.09–7.20 (4H, m, Ar-H). ^1H NMR spectrum of the sample was similar to that reported.[29]

Biotransformation of indane and 2-substituted indanes by *P. putida* UV4

Biotransformations were carried out with *P. putida* UV4 for *ca.* 24 h and were worked up using the standard conditions.

Biotransformation of indane 1A. Biotransformation of indane **1A** (1.93 g, 16.4 mmol) yielded the following two products on separation by PLC (ethyl acetate–hexane; 1:4).

(−)-(R)-Indan-1-ol ***2A***. (0.67 g, 34%) mp 76 °C (chloroform) (lit.,[8] 72 °C); $[a]_D$ −32 (*c* 1.0, $CHCl_3$) (lit.,[8] −30.8, $CHCl_3$); δ_H (300 MHz) 1.89–2.01 (1H, m, 2-H), 2.44 (1H, m, 2′-H), 2.77–2.87 (1H, m, 3-H), 3.02 (1H, m, 3′-H), 5.25 (1H, t, $J_{1,2}$ 5.8, $J_{1,2'}$ 6.1, 1-H), 7.21–7.25 (3H, m, Ar-H), 7.41–7.45 (1H, m, Ar-H); ee >98% (MTPA derivative).

Indan-1-one ***4A***. (0.150 g, 7%) was spectrally indistinguishable from an authentic sample.

Biotransformation of (−)-(*R*)-indan-1-ol 2A. Biotransformation of (−)-(*R*)-indan-1-ol **2A** (0.5 g, 3.73 mmol) gave three compounds on separation by PLC (MeOH–$CHCl_3$; 1:9) including recovered (−)-(*R*)-indan-1-ol **2A** (0.36 g, 72%).

(−)-trans-(1R,3R)-1,3-Dihydroxyindane ***3A***. (0.07 g, 13%); R_f 0.4, mp 106–107 °C (from MeOH–$CHCl_3$); $[a]_D$ −43 (*c* 0.6, MeOH) (Found: M^+, 150.0677. $C_9H_{10}O_2$ requires M^+, 150.0681); δ_H (500 MHz) 2.35 (2H, m, 2-H), 5.42 (2H, m, 1-H, 3-H), 7.28–7.46 (4H, m, Ar-H); *m/z* 150 (M^+, 37%), 132 (100); CD: λ 221.5 nm $\Delta\varepsilon$ 1.84, λ 195.40 nm $\Delta\varepsilon$ −1.47; >98% ee (CSPHPLC, Chiralpak AD, propan-2-ol–hexane; 1:9).

(−)-(1R,4R,5S)-1,4,5-Trihydroxy-4,5-dihydroindane ***7***. (0.03 g, 5%), R_f 0.2, unstable solid; mp 102–105 °C (decomp.) (from MeOH–$CHCl_3$); $[a]_D$ −36 (*c* 0.3, MeOH); δ_H (500 MHz) 1.83 (1H, m, 2′-H), 2.33 (2H, m, 3-H), 2.58 (1H, m, 2-H), 4.31 (1H, m, 5-H), 4.38 (1H, d, $J_{4,5}$ 6.6, 4-H), 5.01 (1H, m,1-H), 5.96 (1-H, dd, $J_{6,7}$ 9.5, $J_{6,5}$ 3.3, 6-H), 6.02 (1-H, dd, $J_{6,7}$ 9.5, $J_{7,5}$ 1.5, 7H); δ_C (125 MHz) 30.89, 33.16, 45.70, 68.14, 68.74, 123.85, 131.85, 136.15, 138.65; *m/z* 168 (M^+, 1%), 151 (100); CD: λ 213.9 nm $\Delta\varepsilon$ +1.39. To a suspension of triol **7** (0.01 g) in CH_2Cl_2 (3 cm^3) was added 4-phenyl-1,2,4-triazoline-3,5-dione (0.012 g). After stirring the reaction mixture at ambient temperature for 2 h, the solvent was removed and the residue purified by PLC (ethyl acetate–methanol, 9:1) to yield cycloadduct **13** (0.015 g, 75%), R_f 0.4, solid which slowly decomposes on standing: mp 213–216 °C (decomp.); $[a]_D$ −48 (*c* 0.45, MeOH); δ_H (500 MHz) 1.97 (1H, ddd, $J_{3,3'}$ 13.6, $J_{3,2}$ 7.6, $J_{3,2'}$ 11.0, 3-H), 2.24 (1H, ddd, $J_{3,3'}$ 13.6, $J_{3',2'}$ 7.8, $J_{3',2}$ 2.7, 3′-H), 2.44 (1H, ddd, $J_{2',2'}$ 16.8, $J_{2,3'}$

2.7, $J_{2,3}$ 7.6, 2-H), 2.84 (1H, ddd, $J_{2',2'}$ 16.8, $J_{2',3'}$ 7.8, $J_{3,2'}$ 11.0, 2′-H), 3.69 (1H, d, $J_{4,5}$ 8.1, 5-H), 3.94 (1H, dd, $J_{4,5}$ 8.1, $J_{4,6}$ 2.6, 4-H), 4.68 (1-H, br s, OH), 4.98 (1H, dd, $J_{6,7}$ 6.2, $J_{6,4}$ 2.6, 6-H), 6.16 (1-H, m, 7-H), 7.39–7.49 (5H, m, Ar-H); *m*/*z* 348 ($M^+ - 1$, 1%), 329 (14), 259 (76), 185 (100).

Biotransformation of (+)-(*S*)-indan-1-ol 2A. Biotransformation of (+)-(*S*)-indan-1-ol **2A** (0.5 g, 3.73 mmol) yielded four compounds by PLC purification (Et_2O–pentane, 1 : 1) including recovered substrate **2A** (0.03 g, 6%) and the achiral compound indan-1-one **4A** (0.2 g, 41%), R_f 0.7.

(+)-trans-(1S,3S)-1,3-Dihydroxyindane 3A. (0.05 g, 9%), R_f 0.2, mp 105–106 °C (from MeOH–$CHCl_3$); $[a]_D$ +42 (*c* 0.5, MeOH) was spectrally indistinguishable from the (1*R*,3*R*) enantiomer derived from (−)-(*R*)-indan-1-ol **2A**. Both (1*S*,3*S*) and (1*R*,3*R*)-enantiomers of compound **3A** were found to be >98% ee by CSPHPLC analysis (Chiralpak AD, propan-2-ol–hexane, 1 : 9).

(−)-(3R)-3-Hydroxyindan-1-one 5A. (0.02 g, 4%), R_f 0.6, bp 128–132 °C, 4 mmHg, $[a]_D$ −101 (*c* 2.97, $CHCl_3$); ν_{max}/cm^{-1} 1700 (C=O) (Found: M^+, 148.0523. $C_9H_8O_2$ requires M^+, 148.0524); δ_H (500 MHz) 2.63 (1H, dd, $J_{2',2}$ 18.7, $J_{3,2'}$ 2.9, 2′-H), 3.14 (1H, dd, $J_{2',2}$ 18.7, $J_{2,3}$ 6.8, 2-H), 5.44 (1H, dd, $J_{2',3}$ 2.9, $J_{2,3}$ 6.8, 3-H), 7.27–7.76 (4H, m, Ar-H); *m*/*z* 148 (M^+, 100%), 130 (7); CD λ 199.7 nm Δε −1.31, λ 213.1 nm Δε +6.29, λ 289.6 nm Δε +4.76, λ 337.8 nm Δε −3.69; the ee value was estimated to be 82% by CSPHPLC (Chiralcel OB, propan-2-ol–hexane, 1 : 9).

Biotransformation of indan-2-ol 1B. Biotransformation of indan-2-ol **1B** (0.260 g, 1.94 mmol) yielded four compounds on separation by PLC (solvent A: ether–hexane, 2 : 3; solvent B: EtOAc–hexane, 3 : 1) including the recovered substrate **1B** (0.15 g).

(−)-cis-(1S,2R)-1,2-Dihydroxyindane 2B. (0.10 g, 35%), R_f 0.5 (solvent B), mp 95–96 °C (from chloroform–hexane) (lit.,[8] 99–100 °C); $[a]_D$ −48 (*c* 0.5, $CHCl_3$) (lit.,[8] −52); δ_H (300 MHz) 2.95 (1H, $J_{3,2}$ 3.66, $J_{3,3'}$ 16.22, 3-H), 3.11 (1H, dd, $J_{3',2}$ 5.7, $J_{3',3}$ 16.4, 3′-H), 4.49 (1H, m, 2-H), 4.99 (1-H, d, $J_{1,2}$ 4.9, 1-H), 7.22–7.29 (3H, m, Ar-H) and 7.41–7.44 (4H, m, Ar-H); *m*/*z* 150 (M^+, 21%), 132 (100); CD: λ 195.4 nm Δε −8.9; ee >98% (MTPA).

(−)-trans-(1R,2R)-1,2-Dihydroxyindane 6B. (0.011 g, 4%) R_f 0.4 (solvent B), $[a]_D$ −26 (*c* 0.5, EtOH) (lit.,[8] −29); δ_H (300 MHz) 2.83 (1H, dd, $J_{3,2}$ 5.0, $J_{3,3'}$ 15.9, 3-H), 3.29 (1H, dd, $J_{3',2}$ 7.7, $J_{3',3}$ 16.1, 3′-H), 4.39 (1H, m, 2-H), 5.02 (1H, d, $J_{1,2}$ 5.5, 1-H), 7.2–7.4 (4H, m, Ar-H); *m*/*z* 150 (M^+, 12%), 132 (100); ee >98% (MTPA derivative).

(−)-(2R)-2-Hydroxyindan-1-one 4B. (0.003 g, 1%), R_f 0.5 (solvent A), $[a]_D$ −16 (*c* 0.2, $CHCl_3$) (lit.,[30] −62, $CHCl_3$); ν_{max}/cm^{-1} 1721 (C=O); δ_H (300 MHz) 3.04 (1H, dd, $J_{3,2}$ 4.9, $J_{3,3'}$ 16.6, 3-H), 3.59 (1H, dd, $J_{3',2}$ 7.9, $J_{3',3}$ 16.6, 3′-H), 4.59 (1H, dd, $J_{2,3'}$ 7.8, $J_{2,3}$ 5.2, 2-H), 7.38–7.79 (4H, m, Ar-H); *m*/*z* 148 (M^+, 100%), 131 (21), 91 (94).

Biotransformation of indan-2-ol 1B using a fermenter. Biotransformation of indan-2-ol **1B** (10.0 g, 74.6 mmol) by *P. putida* UV4 in a 10 l fermenter and subsequent ethyl acetate extraction of the centrifuged culture medium yielded two products, which were separated by fractional crystallisation (from $CHCl_3$). The metabolites, identified as (−)-*cis*-(1*S*,2*R*)-1,2-dihydroxyindane **2B** (4.53 g, 40%) and (−)-*trans*-(1*R*,2*R*)-1,2-dihydroxyindane **6B** (0.51 g, 5%), were found to be spectrally and stereochemically indistinguishable from the metabolites isolated from the smaller scale biotransformation of substrate **1B**.

Biotransformation of 2-chloroindane 1C. Biotransformation of 2-chloroindane **1C** (0.25 g, 1.6 mmol) yielded two products on separation by reverse phase PLC (MeOH–H_2O, 4 : 1).

(−)-cis-(1S,2R)-2-Chloroindan-1-ol 2C. (0.18 g, 65%,) R_f 0.4; mp 109 °C (from CH_2Cl_2–pentane); $[a]_D$ −52 (*c* 0.6, $CHCl_3$) (Found: C, 64.6; H, 5.6. C_9H_9OCl requires C, 64.1; H, 5.4%); δ_H (500 MHz) 2.50 (1H, br s, OH), 3.26 (1H, dd, $J_{3,2}$ 3.5, $J_{3,3'}$ 16.7, 3-H), 3.34 (1H, dd, $J_{3',2}$ 5.4, $J_{3',3}$ 16.7, 3′-H), 4.79 (1H, m, 2-H), 5.16 (1-H, d, $J_{1,2}$ 5.0, 1-H), 7.25–7.32 (3H, m, Ar-H), 7.44–7.47 (1H, m, Ar-H); *m*/*z* 168 (M^+, 40%), 133 (100); CD: λ 196 nm Δε −7.47; ee >98% (MTPA derivative).

(+)-trans-(1R,2R)-2-Chloroindan-1-ol 6C. (0.020 g, 7%); R_f 0.6; mp 89–90 °C; $[a]_D$ +10 (*c* 0.1, $CHCl_3$) (Found: M^+, 168.0342. $C_9H_9O^{35}Cl$ requires M^+, 168.0342; δ_H (500 MHz) 2.46 (1H, br s, OH), 3.08 (1H, dd, $J_{3,2}$ 7.3, $J_{3,3'}$ 16.2, 3-H), 3.50 (1H, dd, $J_{3',2}$ 7.3, $J_{3',3}$ 16.2, 3′-H), 4.28 (1H, dt, $J_{2,3}$ 7.3, $J_{2,1}$ 5.8, 2-H), 5.19 (1-H, d, $J_{1,2}$ 5.8, 1-H), 7.21-7.23 (3H, m, Ar-H), 7.35–7.44 (1H, m, Ar-H); *m*/*z* 168 (M^+, 45%), 133 (100); ee >98% by CSPHPLC (Chiralcel OD, propan-2-ol–hexane, 1 : 9) of the derived indane-1,2-dioxide.

Biotransformation of 2-chloroindane 1C using a fermenter. Biotransformation of 2-chloroindane **1C** (3.0 g, 19.7 mmol) by *P. putida* UV4 and subsequent ethyl acetate extraction of the centrifuged bioextract gave only (−)-*trans*-(1*S*,3*S*)-1,3-dihydroxy-2-chloroindane **3C** (1.4 g, 38%) mp 154–156 °C (from $CHCl_3$–hexane); $[a]_D$ −92 (*c* 0.6, MeOH) (Found: C, 59.0; H, 5.2. $C_9H_9O_2Cl$ requires C, 58.6; H, 4.9%); δ_H (300 MHz) 4.35 (1H, dd, $J_{2,1}$ 4.8, $J_{2,3}$ 5.3, 2-H), 5.22 (1H, d, $J_{1,2}$ 4.8, 1-H), 5.29 (1H, d, $J_{3,2}$ 5.3, 3-H), 7.28–7.48 (4H, m, Ar-H); *m*/*z* 184 (M^+, 1%), 148 (100), 131 (30); CD: λ 311.90 nm Δε −1.058, λ 219.60 nm Δε −9.207, λ 194.80 nm Δε −2.476; ee >98% (MTPA derivative).

Biotransformation of 2-bromoindane 1D. Biotransformation of 2-bromoindane **1D** (5 g, 25.4 mmol) yielded two products on separation by flash chromatography (1-5% MeOH in $CHCl_3$).

(−)-cis-(1S,2R)-2-Bromoindan-1-ol 2D. (1.9 g, 35%); mp 110–111 °C (from CH_2Cl_2–pentane); $[a]_D$ −61 (*c* 0.62 in $CHCl_3$) (Found: C, 50.4; H, 4.0. C_9H_9OBr requires C, 50.7; H, 4.3%); δ_H (500 MHz) 2.44 (1H, d, $J_{OH,1}$ 9.42, OH), 3.38 (1H, dd, $J_{3,2}$ 3.2, $J_{3,3'}$ 16.7, 3-H), 3.45 (1H, dd, $J_{3',2}$ 5.3, $J_{3',3}$ 16.9, 3′-H), 4.92 (1H, m, 2-H), 4.98 (1-H, d, $J_{1,2}$ 4.3, 1-H), 7.26–7.46 (4H, m, Ar-H); *m*/*z* 214 (M^+, ^{81}Br, 55%), 212 (M^+, ^{79}Br, 53), 133 (100); CD: λ 269.90 nm Δε +0.2967, λ 217.10 nm Δε −1.509, λ 195.20 nm Δε −4.483; ee >98% (MTPA derivative).

(+)-trans-(1S,3S)-1,3-Dihydroxy-2-bromoindane 3D. (1.5 g, 26%); colourless semisolid; $[a]_D$ +6 (*c* 0.40 in $CHCl_3$) (Found: C, 47.4, H, 4.1. $C_9H_9O_2Br$ requires C, 47.2; H, 4.0%); δ_H (500 MHz) 4.42 (1H, dd, $J_{2,1}$ 2.7, $J_{2,3}$ 5.3, 2-H), 5.17 (1H, d, $J_{1,2}$ 2.7, 1-H), 5.41 (1H, d, $J_{3,2}$ 5.4, 3-H), 7.25–7.48 (4H, m, Ar-H); CD: λ 200.80 nm Δε −2.34, λ 221.60 nm Δε +1.128.

Biotransformation of 2-iodoindane 1E. Biotransformation of 2-iodoindane **1E** (0.33 g, 1.35 mmol) by *P. putida* UV4 gave three major products, on separation by PLC: (−)-*cis*-(1*S*,2*R*)-1,2-Dihydroxyindane **2B** (0.042 g, 20%), (−)-(1*R*)-1-hydroxyindene **9** (0.023 g, 13%) and (+)-(1*S*,3*S*)-1,3-dihydroxy-2-iodoindane ***3E*** (0.002 g, 0.5%) Compounds **2B** and **9** were spectrally and stereochemically identical to the metabolites obtained from indene **8** with *P. putida* UV4.[31] (1*S*,2*S*)-2-Iodoindan-1-ol **2E** was detected only in trace amounts by GC/MS.

(+)-(1S,3S)-1,3-Dihydroxy-2-iodoindane 3E. (0.002 g, 0.5%); semisolid; $[a]_D$ +4 (*c* 0.2, $CHCl_3$) (Found: (M^+ − HI), 148.0528. $C_9H_8O_2$ requires 148.0524); δ_H (500 MHz) 4.18 (1H, dd, $J_{2,1}$ 5.1, $J_{2,3}$ 5.3, 2-H), 5.08 (1H, d, $J_{1,2}$ 5.1, 1-H), 5.17 (1H, d, $J_{3,2}$ 5.3, 3-H), 7.24–7.45 (4H, m, Ar-H); *m*/*z* 149 (M^+ − I, 54%), 148 (100).

Biotransformation of 2-acetoxyindane 1F. Biotransformation of 2-acetoxyindane **1F** (0.1 g, 0.56 mmol) yielded indan-2-ol **1B** (0.039 g, 51%), (−)-*cis*-(1*S*,2*R*)-1,2-dihydroxyindane **2B** (0.021 g, 25%), (−)-*trans*-(1*R*, 2*R*)-1,2-dihydroxyindane **6B** (0.003 g, 3%) and (−)-(2*R*)-2-hydroxyindan-1-one **4B** (0.002 g, 2%).

These products were spectrally and stereochemically identical to those obtained with indan-2-ol **1B** as substrate.

Biotransformation of 2-methoxyindane 1G. Biotransformation of 2-methoxyindane **1G** (0.6 g, 4.05 mmol) by *P. putida* UV4 gave two products on separation by reverse phase PLC ($MeOH–H_2O$, 4 : 1).

(−)-cis-(1S,2R)-2-Methoxyindan-1-ol ***2G***. (0.06 g, 9%); R_f 0.3; colourless semisolid; $[a]_D$ −59 (*c* 0.3, $CHCl_3$) (Found: M^+, 164.0837. $C_{10}H_{12}O_2$ requires 164.0837); δ_H (500 MHz) 3.01 (1H, dd, $J_{3,2}$ 5.5, $J_{3,3'}$ 15.9, 3-H), 3.06 (1H, dd, $J_{3',2}$ 4.1, $J_{3',3}$ 16.2, 3′-H), 3.49 (3H, s, OMe), 4.10 (1H, m, 2-H), 5.1 (1-H, d, $J_{1,2}$ 4.8, 1-H), 7.17–7.47 (4H, m, Ar-H); *m*/*z* 164 (M^+, 20%), 132 (100), 131 (35); CD: λ 194.60 nm $\Delta\varepsilon$ −9.452; ee >98% by CSPHPLC (Chiralcel AD, propan-2-ol–hexane, 1 : 9) of the 1,2-dimethoxy derivative.

(−)-trans-(1R,2R)-2-Methoxyindan-1-ol ***6G***. (0.006 g, 1%); R_f 0.35; semisolid; $[a]_D$ −12 (*c* 0.3, $CHCl_3$); δ_H (500 MHz) 2.80 (1H, dd, $J_{3,2}$ 5.1, $J_{3,3'}$ 16.2, 3-H), 3.24 (1H, dd, $J_{3',2}$ 7.4, $J_{3',3}$ 16.2, 3′-H), 3.51 (3H, s, OMe), 4.02 (1H, m, 2-H), 5.15 (1-H, d, $J_{1,2}$ 5.51, 1-H), 7.16–7.48 (4H, m, Ar-H); *m*/*z* 164 (M^+, 20%), 132 (100), 131 (35); ee >98% by CSPHPLC (Chiralcel AD, propan-2-ol–hexane, 1 : 9) of the 1,2-dimethoxy derivative.

Biotransformation of indan-2-one 1H. Biotransformation of indan-2-one **1H** (0.1 g, 0.75 mmol) by *P. putida* UV4 yielded indan-2-ol **1B** (0.012 g, 12%), (−)-*cis*-(1*S*,2*R*)-1,2-dihydroxyindane **2B** (0.032 g, 28%) and (−)-*trans*-(1*R*,2*R*)-1,2-dihydroxyindane **6B** (0.004 g, 3%). The metabolites were spectrally and stereochemically identical to those obtained from indan-2-ol **1B**.

Biotransformation of 2-cyanoindane 1I. Biotransformation of 2-cyanoindane **1I** (0.12 g, 0.84 mmol) gave only (−)-*cis*-(1*R*,2*S*)-2-cyanoindan-1-ol **2I** (0.05 g, 37%); mp 129–130 °C (from chloroform–hexane); $[a]_D$ −81 (*c* 0.5, $CHCl_3$) (Found: C, 75.4; H, 5.7; N, 8.8. $C_{10}H_9NO$ requires C, 75.5; H, 5.7; N, 8.8%); ν_{max}/cm^{-1} 1712 (CN); δ_H (500 MHz) 3.01(1H, dd, $J_{3,2}$ 4.5, $J_{3,3'}$ 16.4, 3-H), 3.19 (1H, dd, $J_{3,2}$ 4.5, $J_{3',3}$ 16.3, 3′-H), 4.31 (1H, m, 2-H), 4.98 (1H, d, $J_{1,2}$ 5.3, 1-H), 7.22–7.46 (4H, m, Ar-H); *m*/*z* 175 (M^+, 1%), 146 (15); CD: λ 222.0 nm $\Delta\varepsilon$ −0.730, λ 203.6 nm $\Delta\varepsilon$ −1.014.

Biotransformation of 2-carbamoylindane 1J. Biotransformation of 2-carbomoylindane **1J** (0.2 g, 1.24 mmol) yielded one product, (−)-*cis*-(1*R*,2*R*)-2-carbamoylindan-1-ol **2J** (0.08 g, 36%), mp 149–150 °C (from chloroform–hexane); $[a]_D$ −56 (*c* 1.0, $CHCl_3$) (Found: C, 67.9; H, 6.3; N, 7.8. $C_{10}H_{11}NO_2$ requires C, 67.8; H, 6.3; N, 7.9%); ν_{max}/cm^{-1} 1684 (C=O); δ_H (500 MHz) 3.06 (1H, dd, $J_{3,2}$ 4.7, $J_{3,3'}$ 15.9, 3-H), 3.16 (1H, dd, $J_{3,2}$ 4.7, $J_{3',3}$ 15.9, 3′-H), 4.5 (1H, m, 2-H), 5.01 (1H, d, $J_{1,2}$ 5.2, 1-H), 7.27–7.44 (4H, m, Ar-H); *m*/*z* 177 (M^+, 19%), 159 (15); CD: λ 250.0 nm $\Delta\varepsilon$ +1.630, λ 207.80 nm $\Delta\varepsilon$ −2.573.

Biotransformation of 2-azidoindane 1K. Biotransformation of 2-azidoindane **1K** (0.6 g, 3.77 mmol) gave (−)-*cis*-(1*S*,2*R*)-2-azidoindan-1-ol **2K** (0.4 g, 61%); mp 149–150 °C (from hexane–chloroform); $[a]_D$ −111 (*c* 0.6, $CHCl_3$) (lit.,[10] −111); δ_H (500 MHz) 3.11 (1H, dd, $J_{3,2}$ 4.1, $J_{3,3'}$ 16.3, 3-H), 3.16 (1H, dd, $J_{3',2}$ 4.7, $J_{3',3}$ 16.3, 3′-H), 4.35 (1H, m, 2-H), 5.2 (1-H, d, $J_{1,2}$ 5.1, 1-H), 7.27–7.44 (4H, m, Ar-H); *m*/*z* 175 (M^+, 0.1%), 146 (15%); CD: λ 218.50 nm $\Delta\varepsilon$ +1.474.

Biotransformation of 2-methylindane 1L. Biotransformation of 2-methylindane **1L** (1.2 g, 9.1 mmol) yielded four products on separation by PLC (ethyl acetate–pentane, 2 : 3).

(−)-trans-(1R,3R)-1,3-Dihydroxy-2-methylindane ***3L***. (0.5 g; 34%) R_f 0.1; mp 103–105 °C (from $CHCl_3$–hexane); $[a]_D$ −18.6 (*c* 0.21, MeOH) (Found: C, 73.1; H, 7.5. $C_{10}H_{12}O_2$ requires C, 73.2; H, 7.4%), δ_H (300 MHz) 1.13 (3H, d, $J_{Me,H}$ 7.1, Me), 2.09 (1H, m, 2-H), 4.75 (1H, d, $J_{1,2}$ 6.6, 1-H), 4.93 (1H, d, $J_{3,2}$ 5.8, 3-H), 7.25–7.34 (4H, m, Ar-H); *m*/*z* 164 (M^+, 49%), 146 (52), 131 (100); CD: λ 220.6 nm $\Delta\varepsilon$ + 3.53, λ 196.8 nm $\Delta\varepsilon$ − 1.55.

(−)-cis-(2S,3R)-3-Hydroxy-2-methylindan-1-one ***5L***. (0.25 g; 17%); R_f 0.2; semisolid; $[a]_D$ −105 (*c* 0.28, $CHCl_3$) (Found: M^+, 162.0685. $C_{10}H_{10}O_2$ requires M^+, 162.0681); δ_H (300 MHz) 1.40 (3H, d, $J_{Me,2}$ 7.4, Me), 2.60 (1H, dd, $J_{2,Me}$ 7.4, $J_{2,3}$ 3.8, 2-H), 4.94 (1H, d, $J_{3,2}$ 3.8, 3-H), 7.48–7.75 (4H, m, Ar-H); *m*/*z* 162 (M^+, 70%), 144 (26), 147 (100); CD: λ 336.3 nm $\Delta\varepsilon$ −2.95, λ 288.5 nm $\Delta\varepsilon$ +3.49, λ 220.8 nm $\Delta\varepsilon$ +4.74, λ 200.6 nm $\Delta\varepsilon$ −1.42.

(−)-cis-(1R,2R)-1-Hydroxy-2-methylindane ***2L***. (0.27 g; 20%) R_f 0.5; mp 51–52 °C (from ether–pentane) (lit.,[11] 45 °C); $[a]_D$ −38 (*c* 0.25 in $CHCl_3$) (lit.,[11] $[a]_D$ +30); δ_H (300 MHz) 1.13 (3H, d, $J_{Me,H}$ 7.0, Me), 2.53 (1H, m, 2-H), 2.67 (1H, dd, $J_{3',3}$ 15.6, $J_{3',2}$ 7.1, 3′-H), 2.94 (1H, dd, $J_{2,3}$ 7.3, $J_{3,3'}$ 15.6, 3-H), 4.98 (1H, d, $J_{1,2}$ 7.3, 1-H), 7.20–7.41 (4H, m, Ar-H); *m*/*z* 148 (M^+, 88%), 147 (100); CD: λ 196.6 nm $\Delta\varepsilon$ −15.64.

(−)-(2R)-2-Methylindan-1-one ***4L***. (0.24 g; 18%); R_f 0.6; $[a]_D$ −2.9 (*c* 1.22, dioxane) (lit.,[11] $[a]_D$ −42) (Found: M^+ 146.0733. $C_{10}H_{10}O$ requires M^+ 146.0732); δ_H (300 MHz) 1.30 (3H, d, $J_{Me,H}$ 7.3, Me), 2.60–2.72 (1H, m, 3-H), 3.36 (1H, m, 2-H), 7.37–7.80 (4H, m, Ar-H); *m*/*z* 146 (M^+, 75%), 131 (100); CD: λ 221.6 nm $\Delta\varepsilon$ −8.64.

Biotransformation of indan-2-ol 1B by *P. putida* NCIMB 8859. Biotransformation of indan-2-ol **1B** (0.260 g, 1.94 mmol) by *P. putida* NCIMB 8859 and ethyl acetate extraction of the bioextract yielded (+)-(2*S*)-2-hydroxyindan-1-one **4B** (0.010 g; 35%, 26% ee by comparison of $[a]_D$ values), (+)-*trans*-(1*S*,2*S*)-1,2-dihydroxyindane **6B** (0.030 g; 10%, >98% ee; MTPA derivative) and (−)-*cis*-(1*R*,2*S*)-1,2-dihydroxyindane **2B** (0.01 g; 3%, >98% ee; MTPA derivative) on separation by PLC as before.

Conversion of (−)-*trans*-(1*S*,3*S*)-1, 3-dihydroxy-2-chloroindanol 3C to (+)-*trans*-(1*S*,2*S*)-1,2-dihydroxyindane 6B. A solution of (−)-*trans*-1,3-dihydroxy-2-chloroindanol **3C** (0.10 g, 0.54 mmol) in diethyl ether (20 cm^3) was stirred (3 h, room temperature) with sodium methoxide (0.10 g, 1.85 mmol). The salts were filtered off from the reaction mixture and the ether solution washed with water, dried ($MgSO_4$), and concentrated to yield a crude sample of (−)-(1*S*,2*R*, 3*S*)-1,2-epoxy-3-hydroxyindane **14**, (0.076 g, 95%), $[a]_D$ −63 (*c* 2.3, $CHCl_3$); δ_H (300 MHz) 4.13 (1H, d, $J_{3,2}$, 2-H), 4.30 (1H, dd, $J_{3,2}$ 2.6, $J_{2,1}$ 3.8, 3-H), 5.03 (1H, d, $J_{1,2}$ 3.8, 3-H), 7.26–7.41 (2H, m, Ar-H), 7.55–7.60 (2H, m, Ar-H). A solution of crude epoxide **14** (0.075 g) in methanol (5 cm^3) was stirred under hydrogen (1 atm, ambient temperature, 4 h) in the presence of Pd/C (10%) catalyst. Workup of the reaction mixture yielded (+)-*trans*-(1*S*,2*S*)-1,2-dihydroxyindane **6B** (0.063 g, 82%); >98% ee (MTPA derivative). (+)-*trans*-(1*S*,3*S*)-1,3-Dihydroxy-2-bromoindane **3D** was similarly converted to the (+)-*trans*-(1*S*,2*S*) enantiomer; >98% ee (MTPA derivative).

Acknowledgements

We thank the BBSRC (N. D. S. and P. G.), DENI (Distinction Award to N. I. B. and a CAST Award to P. A. G.) and the TBNI (M. R. G.) for support of this programme.

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