Regioselectivity and stereoselectivity of dioxygenase catalysed *cis*-dihydroxylation of mono- and tri-cyclic azaarene substrates†

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cis-Dihydrodiol metabolites were obtained from dioxygenase-catalysed asymmetric dihydroxylations of five monocyclic (azabiphenyl) and four tricyclic (azaphenanthrene) azaarene substrates. Enantiopurity values and absolute configuration assignments were determined using a combination of stereochemical correlation, X-ray crystallography and spectroscopy methods. The degree of regioselectivity found during *cis*-dihydroxylation of monocyclic azaarenes (2,3 bond >> 3,4 bond) and of tricyclic azaarenes (bay region > non-bay region bonds) was dependent on the type of dioxygenase used. The *cis*-dihydrodiol metabolite from an azaarene (3-phenylpyridine) was utilised in the chemoenzymatic synthesis of the corresponding *trans*-dihydrodiol.

Introduction

The asymmetric dihydroxylation of carbocyclic arenes to yield the corresponding *cis*-dihydrodiols, using ring hydroxylating dioxygenase enzymes as biocatalysts, and their applications in synthesis, have been widely reported.[1–11] Most of the known arene *cis*-dihydrodiols have been produced from substituted benzenes (monocyclic arene substrates) and toluene dioxygenase (TDO) as biocatalyst. Bicyclic and tricyclic arenes have been mainly biotransformed to the corresponding *cis*-dihydrodiols, using naphthalene dioxygenase (NDO) and biphenyl dioxygenase (BPDO) enzymes which have larger active sites. Tetracyclic and pentacyclic arenes could only be accommodated by the dioxygenase BPDO having the largest active site; the only acceptable monocyclic arene substrates for NDO and BPDO dioxygenases appeared to be the biaryl type *e.g.* biphenyl **1a**.

The oxidative biotransformations of azaaromatic substrates can, in principle, yield enantiopure bioproducts that are of potential value as chiral building blocks in the synthesis of target molecules.[1–11] Surprisingly, few azaheterocyclic *cis*-dihydrodiols have been isolated, stereochemically assigned and applied as chiral precursors in synthesis. Different types of dioxygenase enzyme (NDO, BPDO and phenanthrene dioxygenase, PDO) were used as biocatalysts in earlier studies for the production of a small number of *cis*-dihydrodiols from di-,[12,13] tri-[14,15] and tetra-cyclic[16] azaarenes.

It was assumed that *cis*-dihydroxylation of an electron-rich pyrrole ring, *e.g.* indole, occurred readily to give the corresponding heterocyclic *cis*-dihydrodiol.[17] Unfortunately the putative indole *cis*-dihydrodiol, being an unstable hemiaminal, spontaneously dehydrated to yield indoxyl which in turn was autoxidised to yield indigo.[18] By contrast, the electron-poor pyridine ring has proved to be much more resistant to dioxygenase-catalysed *cis*-dihydroxylation. Thus, TDO-catalysed oxidation of substituents attached to a pyridine ring, *e.g.* sulfoxidation of SR groups,[19] monohydroxylation of alkyl groups,[20] or *cis*-dihydroxylation of benzofused rings,[12–16] all occur more readily than *cis*-dihydroxylation of a pyridine ring. Indirect evidence for the TDO-catalysed formation of unstable *cis*-dihydrodiols as minor metabolites of a pyridine ring was found when 2-chloro- and 2-methoxy quinolines were both converted to a 2-quinolone *cis*-diol[13] and hydroxypyridines were obtained from the corresponding 2- and 4-alkyl pyridines.[20,21]

As our earlier studies of the TDO-catalysed *cis*-dihydroxylation of azaarenes were mainly focussed on bicyclic substrates, *e.g.* quinoline, isoquinoline, quinoxaline and quinazoline rings,[12,13] the major emphasis of the current programme was on the regio- and stereo-selectivity of dioxygenase-catalysed *cis*-dihydroxylation of monocyclic azaarene analogues of biphenyl **1a** (Scheme 1) and of tricyclic azaarene analogues of phenanthrene **4a** containing a bay region (Scheme 2). These substrates were selected on the basis of similar steric requirements, *i.e.* the coplanar conformations of the monocyclic azaarenes **1b–1d** and the planar tricyclic azaarene analogues of phenanthrene **4a**, *e.g.* benzo[*h*]quinoline **4b**, benzo[*f*]quinoline **4c**, phenanthridine **4d** and benzo[*c*]cinnoline **4e**. It was, therefore, anticipated that all of these azaarenes would be similarly accommodated at the BPDO active site.

Supporting evidence for this presumption is now presented through the biotransformation of the monosubstituted benzene rings bearing pyridine (**1b–1d**), pyrrole (**1e**) or pyrazole (**1f**) ring substituents, to yield the corresponding carbocyclic *cis*-dihydrodiols **2b–2d** as major metabolites and of fused benzene rings in the tricyclic azaarenes **4b–4e** to give mainly the corresponding *cis*-dihydrodiols **5b–5e**.

A further objective of this programme was to examine the comparative effect of different types of dioxygenase enzyme (TDO, NDO, and BPDO) on the regio- and stereo-selectivity

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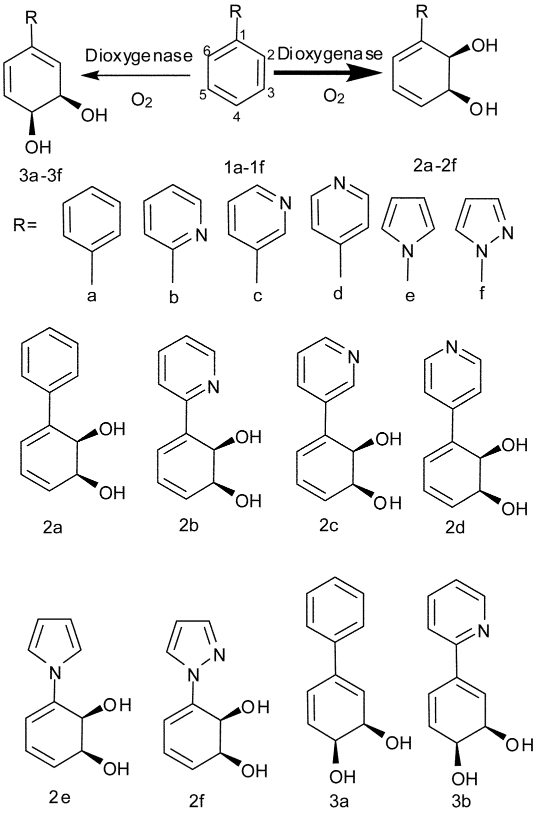
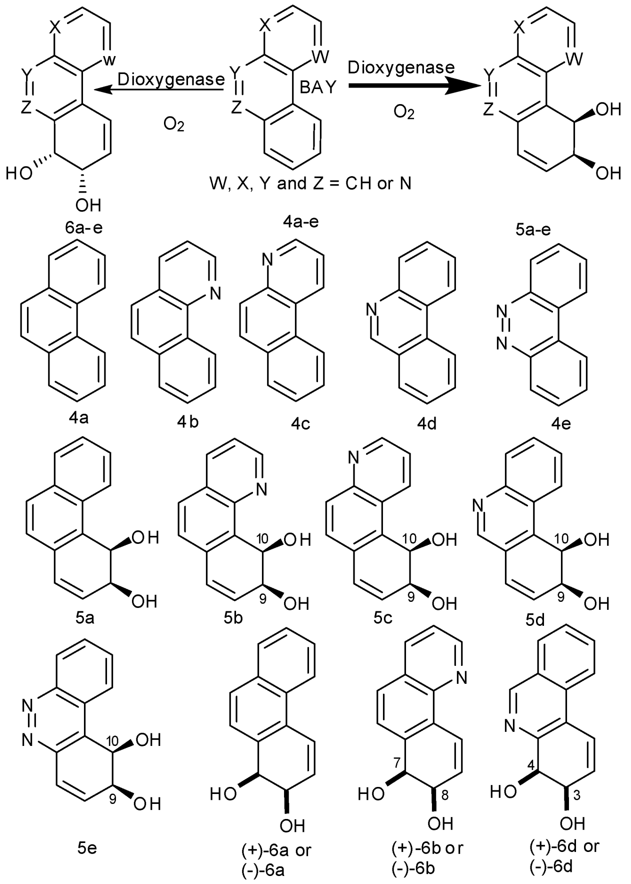


Scheme 1



Scheme 2

of *cis*-dihydroxylation of monosubstituted benzene rings bearing an azarene substituent or of benzene rings fused directly to an azaarene ring. Evidence for the NDO-catalysed *cis*-dihydroxylation of the 3,4-bond of azaarene **1b** to yield the unusual *cis*-dihydrodiol **3b**, as a minor metabolite, has now been obtained. Furthermore, a change in regioselectivity from the preferred bay region has been observed using a site-directed mutant strain of NDO (NDO_{F352V}) and the tricyclic azarene substrates **4b** and **4d** where the corresponding non-bay region *cis*-dihydrodiols **6b** and **6d** have been isolated as major metabolites. The final phase of this study was designed to show how a typical azaarene *cis*-dihydrodiol **2c** could be utilised in a synthesis of the corresponding *trans*-dihydrodiol **17**.

Results and discussion

(a) *Normal* dioxygenase-catalysed *cis*-dihydroxylation at the 2,3 bond of the monosubstituted benzene substrates 1b–1f

At the beginning of this study, biphenyl **1a** was assumed to be unique among monosubstituted benzenes as the only acceptable substrate for all three types of dioxygenases used (TDO, NDO and BPDO). Each of these dioxygenases can catalyse oxidation at the 2,3 bond of biphenyl **1a**, to give enantiopure (1*S*,2*R*)-*cis*-dihydrodiol **2a** with yields increasing in accord with size of the dioxygenase active site, *i.e.* in the sequence TDO < NDO < BPDO.[22,23] The NDO-catalysed biotransformation of biphenyl **1a** was unusual, since *cis*-dihydroxylation occurred not only at the expected 2,3 bond to yield *cis*-dihydrodiol **2a** as the major bioproduct (87%), but also at the 3,4 bond, to yield the abnormal regioisomer **3a** as a minor metabolite (13%, Scheme 1).[22,23]

Addition of the azabiaryls 2-phenyl- (**1b**), 3-phenyl- (**1c**) and 4-phenyl- (**1d**) pyridines to whole cells of a constitutive mutant strain (UV4), of the bacterium *Pseudomonas putida* (expressing TDO) gave the corresponding *cis*-dihydrodiols **2b–2d**, only in low yields (*ca.*: 1%, Scheme 1). The *cis*-dihydrodiol metabolite **2b** of 2-phenylpyridine **1b** had not been found during earlier studies using TDO as biocatalyst.[24] As the phenylpyridine substrates **1b–1d** are of almost identical shape to the parent substrate biphenyl **1a**, it was assumed that they would be more acceptable substrates for BPDO and would thus give higher yields of the corresponding *cis*-dihydrodiols. This premise was confirmed by their biotransformation using whole cells of an inducible mutant strain (B8/36), of *Sphingomomas yanoikuyae* (expressing BPDO). The corresponding *cis*-dihydrodiols **2b–2d**, of identical structure and absolute configuration to those found using TDO, were isolated as the only identified bioproducts with yields in the range of 31–59%.

The *cis*-dihydrodiols **2b** ($[\alpha]_D$ +173, MeOH; 59% yield), **2c** ($[\alpha]_D$ +249, THF; 49% yield) and **2d** ($[\alpha]_D$ +181, THF; 31% yield) were each shown to be single enantiomers (ee >98%) by formation of the corresponding boronate esters using (+)-(*R*) and (–)-(*S*)-2-(1-methoxyethyl)phenylboronic acid (MEBBA). ^{1}H-NMR analysis of the MEBBA derivatives of *cis*-diol metabolites **2b–2d** also allowed their absolute configurations to be tentatively assigned as (1*S*,2*R*), employing methods successfully used for other *cis*-dihydrodiol metabolites.[25,26] The absolute configuration of (+)-*cis*-dihydrodiol **2c** was then rigorously established as (1*S*,2*R*) by hydrogenation of the 5,6-bond to yield the *cis*-tetrahydrodiol **2g** followed by X-ray crystallographic analysis using the anomalous dispersion method (Fig. 1). Compound **2g** in the solid state showed the carbocyclic ring having the half-chair conformation with the hydroxyl group proximate to the pyridine ring being pseudoaxial. The torsion angle along the inter-ring bond is +48°. The (1*S*,2*R*) absolute configurations, assigned to compounds **2b**, **2c** and **2d** by the MEBBA method, were supported by comparison of their very similar circular dichroism (CD) spectra.

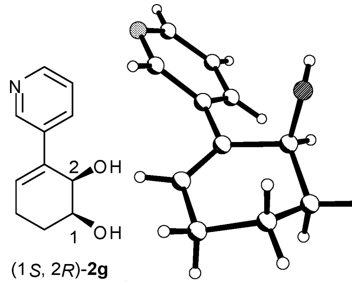


Fig. 1 X-Ray crystal structure of the *cis*-tetrahydrodiol **2g**.

Biotransformations of 1-phenylpyrrole **1e** and 1-phenylpyrazole **1f** were carried out using *P. putida* UV4 to give the corresponding *cis*-dihydrodiols **2e** and **2f** (Scheme 1). These were obtained in higher yield (12%) compared with that of the phenyl pyridine substrates **1b–1d** (*ca.*: 1%) using *P. putida* UV4. This improved *cis*-dihydroxylation of azaarenes **1e** and **1f** with *P. putida* UV4 could be due to their reduced size which allows them to fit more easily into the smaller active site of the TDO enzyme. It is also possible that dihydroxylation of the electron-rich azaarene rings (*cf.*: the *cis*-dihydroxylation of the pyrrole ring of indole)[17] may have occurred with the resultant unstable water-soluble *cis*-diols, or their ring-opened derivatives, not being isolated.

An X-ray crystal structure analysis of *cis*-dihydrodiol **2e** ($[\alpha]_D$ +144, THF. Fig. 2), using the anomalous dispersion method, showed the presence of three crystallographically independent molecules in the solid state. All had the (1*S*,2*R*) configuration and the same conformation for the carbocyclic ring, with a pseudoaxial hydroxyl group adjacent to the pyrrole ring. This preference for the hydroxyl group nearest to the bulky (azaarene) substituent in compound **2e** to adopt a pseudoaxial conformation in the solid state has previously been observed for most other *cis*-dihydrodiol metabolites of monosubstituted[27] and 1,4-disubstituted benzene substrates.[26] Similar preferred pseudoaxial conformations, resulting from reduced steric interactions between substituents R and proximate hydroxyl groups, would be expected to occur in solution. The three independent molecules of **2e** differed only in the conformation of the pyrrole ring, with torsion angles of +14°, +29° and −30°, respectively, along the inter-ring bond. This is most likely due to crystal packing factors as each of the six crystallographically independent hydroxyl groups is involved in intermolecular hydrogen-bonding, as both a hydrogen donor and a hydrogen acceptor. *cis*-Dihydrodiol metabolite **2f** was found to be enantiopure (ee >98%, MEBBA formation) and its CD spectrum was consistent with a (1*S*,2*R*) absolute configuration.

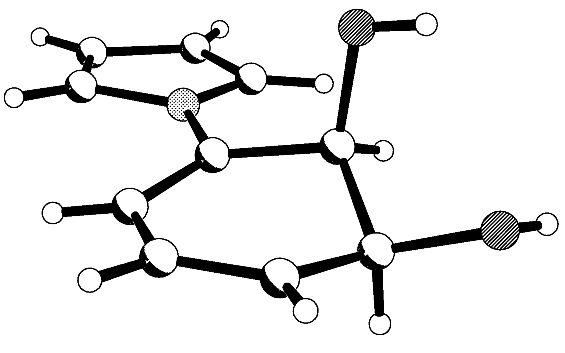


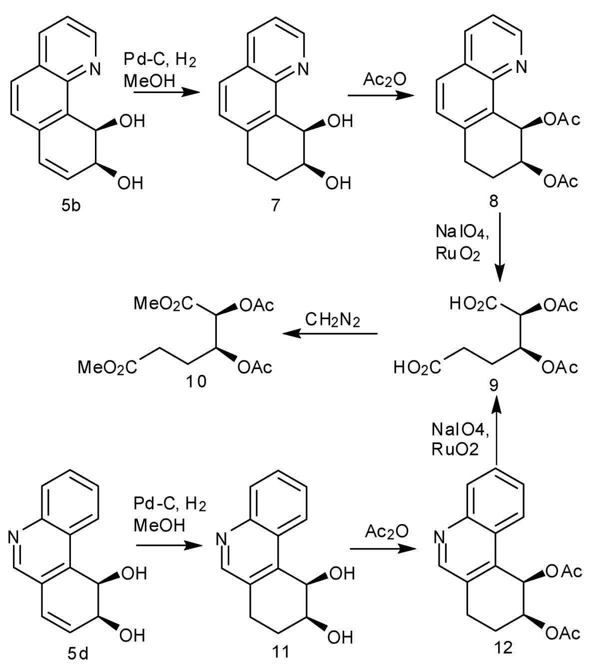
Fig. 2 X-Ray crystal structure view one of the three independent molecules of *cis*-dihydrodiol **2e**.

An earlier study had shown that 2-phenylpyridine **1b**, 1-phenylpyrrole **1e** and 1-phenylpyrazole **1f** were substrates for BPDO.[24] One of the resulting *cis*-dihydrodiols, **2f**, was not isolated as it appeared to spontaneously decompose *via* dehydration to yield the corresponding phenol bioproduct. Although the other *cis*-dihydrodiols **2b** and **2e** were isolated, their optical rotations, ee values and absolute configurations were not reported.[24] While it is probable that metabolites **2b** and **2e** isolated earlier have the same absolute configurations as of those obtained during this study, in the absence of chiroptical data from the previous study,[24] it was not possible to make a direct stereochemical comparison.

(b) *Normal* dioxygenase-catalysed *cis*-dihydroxylation at a bay-region bond of the azaphenanthrene substrates 4b–4e

BPDO-catalysed biotransformation of azaphenanthrenes, benzo[*h*]quinoline **4b**, benzo[*f*]quinoline **4c**, phenanthridine **4d** and benzo[*c*]cinnoline **4e** using *S. yanoikuyae* B8/36, was found in each case, to yield the corresponding bay-region *cis*-dihydrodiol as the only (or major) isolated bioproducts *i.e.* **5b**, ($[\alpha]_D$ +167, MeOH; 50% yield), **5c** ($[\alpha]_D$ +153, MeOH; 67% yield), **5d** ($[\alpha]_D$ +82, MeOH; 72% yield), and **5e** ($[\alpha]_D$ −280, pyridine; 62% yield) (Scheme 2). The biotransformation of phenanthridine **4d**, using an *E. coli* recombinant strain expressing the PDO gene, had earlier been reported to yield **5d** as one of three metabolites.[15] As expected, no evidence of *cis*-dihydroxylation was found in the electron-poor pyridine rings in either this or the earlier study.[15] Using *S. yanoikuyae* B8/36 expressing BPDO as biocatalyst, benzo[*h*]quinoline substrate **4b** was also transformed into the non-bay-region *cis*-diol **6b**, ($[\alpha]_D$ +46, MeOH), as a minor metabolite (13% yield) which was separated from the major metabolite **5b** by PLC. The pattern of a strong preference for BPDO-catalysed *cis*-dihydroxylation at the bay region, observed earlier when phenanthrene **4a** was mainly biotransformed into *cis*-dihydrodiol **5a** (>90%),[28,29] was thus also evident in the azaphenanthrene series. The structures and % ee values (>98%) of the *cis*-dihydrodiols **5b**–**5e** and **6b** were determined by analysis of the ^{1}H-NMR spectra of the diols and their MEBBA derivatives.

In the absence of X-ray crystallographic data, a stereochemical correlation sequence for *cis*-dihydrodiols **5b** and **5d**, involving hydrogenation (**5b** → **7**; **5d** → **11**), diacetylation (**7** → **8**; **11** → **12**), oxidative cleavage (**8** → **9**; **12** → **9**) and methylation (**9** → **10**) to yield dimethyl (2,3-diacetoxy)adipate **10** ($[\alpha]_D$ +153) of established (2*S*,3*S*) configuration, was used (Scheme 3). This provided an unequivocal method for the determination of the (9*S*,10*R*) absolute configuration and enantiopurity (>98% ee) of



Scheme 3

(+)-*cis*-diols **5b** and **5d**. It also confirmed the validity of the method adopted earlier for determination of ee values where analysis of the ^{1}H-NMR spectra of the corresponding MEBBA derivatives was also used in the assignment of absolute configurations to *cis*-diols **5b–5e** (9*S*,10*R*) and **6b** (7*R*,8*S*). A modified Mosher's method was used earlier in assigning the (9*S*,10*R*) configuration to metabolite **5d** but its optical rotation value was not reported.[15]

(c) *Abnormal* dioxygenase-catalysed *cis*-dihydroxylation at the 3,4 bond of the monosubstituted benzene substrate 1b and at non-bay region bonds in azaphenanthrenes 4b and 4d

It is noteworthy that both TDO and BPDO enzymes catalysed the *cis*-dihydroxylation of the biaryls **1a–1f**, exclusively, at the 2,3 bond to yield the corresponding diols **2a–2f** (Scheme 1). Similar results have been reported for all other monosubstituted benzene substrates using TDO as biocatalyst.[1–11] Earlier studies[22,23] showed that *cis*-dihydroxylation of biphenyl **1a** occurred mainly (87%) at the 2,3 bond to yield (1*S*,2*R*)-*cis*-dihydrodiol **2a** (ee >98%) as the major bioproduct with *P. putida* (9816/4, a source of NDO). However, a significant proportion (13%), of (1*S*,2*R*)-dihydrodiol **3a** (ee >98%) was also isolated as a result of *cis*-dihydroxylation occurring at the 3,4 bond (Scheme 1). Regioselectivity for the 3,4 bond became more marked when a site-directed mutant strain of *E. coli* containing a modified form of NDO ($\text{NDO}_{\text{F352V}}$) was employed. Use of this strain, formed by a Phe-352-Val mutation, occurring near the mononuclear non-heme iron atom in the α subunit of the NDO active site, resulted in a remarkable change in both regio- and stereo-selectivity. Thus, *cis*-dihydroxylation occurred almost exclusively at the 3,4 bond (99%), to yield *cis*-dihydrodiol **3a** with an excess of the opposite (1*R*,2*S*) configuration (ee 77%).[23]

Evidence for *ortho*-xylene dioxygenase-catalysed *cis*-dihydroxylation of toluene **1h** occurring at the 2,3 and 3,4 bonds to give *cis*-dihydrodiols **2h** (R = Me, major) and **3h** (R = Me, minor) respectively, based on GC-MS analysis of the corresponding boronate derivatives, was recently obtained (Scheme 1).[30,31] The minor *cis*-dihydrodiol metabolites **3a**[22,23] and **3h**[30,31] are therefore among the relatively few literature examples of the dioxygenase-catalysed *cis*-dihydroxylation of monosubstituted benzene substrates occurring at the 3,4-bond. In view of the difficulty of obtaining these abnormal *cis*-dihydrodiol regioisomers, *e.g.* **3h** (R = Me) and **3i** (R = F), from dioxygenase-catalysed *cis*-dihydroxylation at the 3,4-bonds of monosubstituted benzene substrates **1h** (R = Me) and **1i** (R = F), new chemoenzymatic methods for their synthesis from the normal isomers **2h** (R = Me) and **2i** (R = F) have now been developed.[32]

Recent studies of the large-scale (>100 g) production of the normal *cis*-dihydrodiol metabolite (**2i**, R = F) of fluorobenzene (**1i**, R = F) from our laboratories have produced the first example of a TDO-catalysed oxidation at the 3,4 bond of a monosubstituted benzene (unpublished data). This unusual (1*S*,2*R*)-*cis*-dihydrodiol regioisomer (**3i**, R = F, $[\alpha]_D$ −21, MeOH, ee 20%) (lit.[32] $[\alpha]_D$ −101, *c* 0.5, MeOH, ee >98%) was only present as a minor metabolite (< 3%) that was isolated along with the normal *cis*-dihydrodiol (**2i**, R = F, >97%).

In order to investigate further the unusual regioselective *cis*-dihydroxylation of the 3,4 bond in monosubstituted benzenes, exemplified by the formation of *cis*-dihydrodiol **3a** catalysed by NDO,[22,23] a comparative metabolism study was carried out, using an inducible mutant strain of *P. putida* (9816/11, expressing NDO) and the monocyclic azaarene **1b** as substrate. Although *cis*-dihydroxylation of substrate **1b** using NDO again occurred at the 2,3-bond to give diol **2b** ($[\alpha]_D$ +173, MeOH), the isolated yield (25%) was lower than that found earlier using BPDO (59%). ^{1}H-NMR analysis of the crude product mixture confirmed that *cis*-dihydrodiol **2b** was the dominant bioproduct (≥95% relative yield). Several very weak signals in the relevant baseline section of the ^{1}H-NMR spectrum of the crude extract suggested that a second *cis*-dihydrodiol, could be present as a very minor (≤5%) component.

More reliable evidence of the minor *cis*-dihydrodiol regioisomer of compound **2b** was found by LC/MS analysis, using reverse phase chromatography (aqueous MeOH as eluent) and electrospray ionisation MS of the crude extract; it confirmed the presence of a major peak (≥95%) with an identical retention time (7.1 min) and an accurate mass corresponding to *cis*-dihydrodiol **2b** ($[M + H]^+$ 190.08546). However, a minor peak (≤ 5%) eluting slightly later, at 7.8 min, with an almost identical mass ($[M + H]^+$ 190.08567) and fragmentation pattern as the earlier peak, supported the presence of a regioisomer. MS/MS analysis focussing on the molecular ion at *m*/*z* 190 throughout the analysis period provided further evidence of a regioisomeric *cis*-dihydrodiol. The accurate mass data (172.07503, 144.07786, 78.03393) of the main product ions, from fragmentation of the minor compound, were found to be in accordance with those obtained from the major *cis*-dihydrodiol **2b**. When collision energy of 10 eV was used, the main fragment ion observed for both the unknown minor metabolite and compound **2b**, was at *m*/*z* 172. This facile loss of a molecule of water, in each case, through aromatisation is entirely consistent with the structures of compounds **2b** and a regioisomer, *e.g.* **3b**, where the hydroxyl groups are located on adjacent carbon atoms.

Based on the LC-MS results, the biotransformation was repeated several times to obtain a sufficient quantity of compound **3b** for recording ^{1}H-NMR (2D-COSY), EI MS, CD spectra and an optical rotation. The data collected finally provided unequivocal evidence for the regioisomer structure **3b**, resulting from *cis*-dihydroxylation at the 3,4 bond of azaarene **1b**. *cis*-Dihydrodiol **3b** was found to be enantiopure (ee >98%) by formation and ^{1}H-NMR analysis of the corresponding MEBBA derivatives. The CD spectrum of *cis*-dihydrodiol **3b** confirmed that it had the same (1*S*,2*R*) absolute configuration as for the other *cis*-dihydrodiols **2b**, **2c** and **2d**.

The more sterically demanding tricyclic azaphenanthrenes **4b–4e** were not substrates for the TDO expressed in *P. putida* UV4, but the larger active sites present in NDO and BPDO were able to accommodate them. Thus, when *P. putida* 9816/11 (expressing NDO) was used, the corresponding bay-region *cis*-dihydrodiols **5b–5e** were isolated in low yields (7–17%). Using *S. yanoikuyae* B8/36 (expressing BPDO), these *cis*-dihydrodiols were obtained in higher yields (50–67%), with identical ee values (>98%) and absolute configurations *i.e.* 9*S*,10*R* (**5b–5e**) (Scheme 2, Table 1).

Despite the similar stereoselectivities, observed during NDO- and BPDO-catalysed *cis*-dihydroxylations of azaphenanthrenes **4b–4e** to yield mainly the corresponding *cis*-dihydrodiol metabolites **5b–5e**, some differences in regioselectivity were observed (Scheme 2, Table 1). Benzo[*h*]quinoline **4b** metabolism, using NDO as biocatalyst, gave (9*S*,10*R*)-*cis*-diol **5b** and (7*R*,8*S*) *cis*-diol **6b** (60:40) compared with BPDO (80:20). A marked increase in regioselectivity for NDO-catalysed *cis*-dihydroxylation at the 7,8 bond in compound **4b** was found when the site-directed mutant *E. coli* $_{\text{NDO-F352V}}$ strain was used. It gave the non-bay region *cis*-diol **6b** as the sole metabolite (100%) but in low yield (11%) and of opposite (7*S*,8*R*) configuration (ee >98%) compared with the normal NDO and BPDO enzymes. Similarly, biotransformation of phenanthridine **4d**, using NDO (*P. putida* 9816/11) or BPDO (*S. yanoikuyae* B8/36), yielded the enantiopure (ee >98%) sole metabolite (9*S*,10*R*)-*cis*-diol **5d** as the result of *cis*-dihydroxylation occurring exclusively at the bay-region (Scheme 2, Table 1). When this biotransformation was repeated using the *E. coli* $_{\text{NDO-F352V}}$ recombinant strain, the bay region *cis*-dihydrodiol **5d** was again formed (10% yield) having an identical (9*S*,10*R*) absolute configuration but a much lower enantiopurity (51% ee). *cis*-Dihydrodiol **6d** was the major bioproduct (12% yield) having an excess (84%) of the (3*R*,4*S*) enantiomer.

The dramatic change in regioselectivity, obtained using the site-directed mutant strain *E. coli* $_{\text{NDO-F352V}}$ (source of NDO-352V), is clearly evident compared with a preference for *cis*-dihydroxylation of the bay regions present in phenanthrene **4a** → **5a**, (90%),[22,23] benzo[*h*]quinoline **4b** → **5b**, (60–80%) and phenanthridine **4d** → **5d**, (100%), when using NDO and BPDO (Table 1). This metabolic profile was reversed using $\text{NDO}_{\text{F352V}}$ where a preference for the non-bay regions was found using phenanthrene **4a** → **6a** (83%),[22,23] benzo[*h*]quinoline **4b** → **6b** (100%) and phenanthridine **4d** → **6d** (56%).

cis-Diols **5a**, **6a**, **5b**, **6b**, **5c**, **5d** and **5e**, obtained using NDO and BPDO, were consistently found to be enantiopure (>98% ee) and of allylic (*S*) configuration. By contrast, the enantioselectivity associated with *cis*-diols formed using $\text{NDO}_{\text{F352V}}$, to give bay region *cis*-diols produced of the same (*S*) configuration but with lower % ee values *e.g.* **5a** (95%)[22,23] and **5d** (51%) and non-bay region *cis*-diols having the opposite allylic (*R*) configuration *e.g.* **6a** (91%),[22,23] **6b** (>98%) and **6d** (84%) (Table 1). The structural changes induced by mutation of the Phe-352 amino acid in NDO has recently been shown to result in a different orientation of phenanthrene **4a** and thus in an altered pattern of regio- and enantio-selectivity of *cis*-dihydroxylation.[33] A similar change in orientation could also account for the observed change in regio- and enantio-selectivity associated with $\text{NDO}_{\text{F352V}}$-catalysed dihydroxylation of the azaarenes **4b** and **4d**.

(d) Application of the azarene *cis*-dihydrodiol 2c in the chemoenzymatic synthesis of the corresponding *trans*-dihydrodiol 17

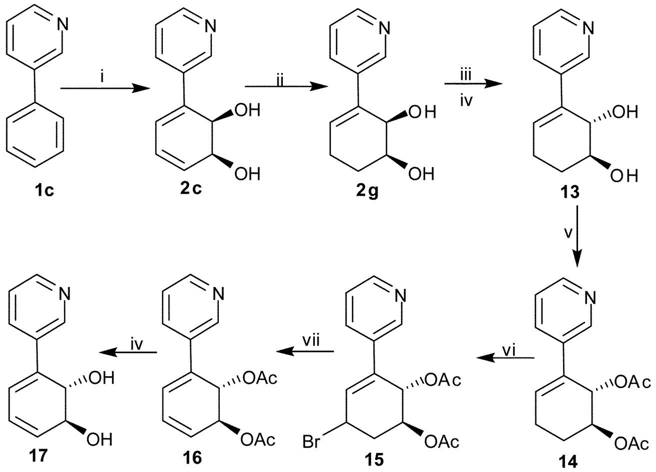
To date, few of the *cis*-dihydrodiol metabolites from azaarene substrates have been employed in synthesis. However *cis*-dihydrodiols from bicyclic azaarenes (*e.g.* quinoline) and tricyclic azaarenes (*e.g.* acridine) have been used in the synthesis of arene oxides and *trans*-dihydrodiols.[11,14] *cis*-Dihydrodiol metabolites of 2-chloroquinolines have been used as precursors of 2,2′-bipyridine ligands in our laboratories (reference 11 and unpublished data). The synthetic potential of the monocyclic azaarene *cis*-dihydrodiol **2c** was demonstrated by its conversion to the corresponding *trans*-dihydrodiol **17**. The six-step synthetic sequence involved (i) partial hydrogenation (**2c** → **2g**), (ii) Mitsunobu inversion (**2g** → **13**),

Table 1 Relative (isolated) yields of *cis*-dihydrodiol products **5b–5e**, **6b** and **6d** enantiomeric excess values (% ee), and absolute configurations (ab.con.)

Substrate	Enzyme	Relative (isolated) product yield, % ee, ab.con.	Relative (isolated) product yield, % ee, ab.con
4b		**5b**	**6b**
	NDO	60 (16) >98, 9*S*,10*R*	40 (10) >98, 7*R*,8*S*
	BPDO	80 (50) >98, 9*S*,10*R*	20 (13) >98, 7*R*,8*S*
	$\text{NDO}_{\text{F352V}}$	0	100 (11)[a] >98, 7*S*,8*R*
4c		**5c**	
	NDO	100 (17) >98, 9*S*,10*R*	0
	BPDO	100 (67) >98, 9*S*,10*R*	0
4d		**5d**	**6d**
	NDO	100 (7) >98, 9*S*,10*R*	0
	BPDO	100 (72)[a] >98, 9*S*,10*R*	0
	$\text{NDO}_{\text{F352V}}$	44 (10) 51, 9*S*,10*R*	56 (12) 84, 3*R*,4*S*
4e		**5e**	
	NDO	100 (16) >98, 9*S*,10*R*	0
	BPDO	100 (62) >98, 9*S*,10*R*	0

[a] ^{1}H-NMR analysis of the crude extract showed traces of a further *cis*-dihydrodiol regioisomer that was neither isolated nor identified.

(iii) diacetylation (**13** → **14**), (iv) allylic bromination (**14** → **15**), (v) dehydrobromination (**15** → **16**) and (vi) hydrolysis (**16** → **17**) (Scheme 4). A similar method had recently been used to synthesise *trans*-dihydrodiols from the corresponding arene *cis*-dihydrodiols.[34]



i *S. yanoikuyae* B8/36, O_2; ii Pd-C, H_2, MeOH; iii DEAD, p-NBA, PPh_3, C_6H_6; iv K_2CO_3, MeOH, H_2O; v Ac_2O, C_5H_5N; vi NBS, CCl_4; vii Li_2CO_3, LiCl, HMPA

Scheme 4

Conclusion

A strong preference for *cis*-dihydroxylation at the 2,3 bond of the carbocyclic ring of substrates **1b–1f** and at a bay region bond of the tricyclic substrates **4b–4e** was observed when NDO and BPDO enzymes were used. Thus, enantiopure *cis*-dihydrodiols **2b–2f** and **5b–5e** were isolated with an allylic (*S*) configuration, based on stereochemical correlation, X-ray crystallography, circular dichroism and NMR spectroscopy methods. The first example of a NDO-catalysed *cis*-dihydroxylation, occurring at the 3,4 bond of a monosubstituted azaarene (**2b**) to yield the corresponding (1*S*,2*R*)-*cis*-dihydrodiol (**3b**), was found. Both regioselectivity and enantioselectivity were found to be reversed using the modified NDO dioxygenase (NDO_{F352V}); the non-bay region *cis*-dihydrodiols **6b** and **6d**, having an excess of the allylic (*R*) enantiomer, were found to be dominant. The general applicability of a chemoenzymatic route, from monocyclic *cis*-dihydrodiols to the corresponding *trans*-dihydrodiols has been demonstrated by the conversion of azaarene *cis*-diol **2c** to *trans*-diol **17**.

Expermental

NMR (^1H and ^{13}C) spectra were recorded on Bruker Avance DPX-300 and DPX-500 instruments and mass spectra were run at 70 eV, on a VG Autospec Mass Spectrometer, using a heated inlet system. Accurate molecular weights were determined by the peak matching method, with perfluorokerosene as the standard. Elemental microanalyses were carried out on a PerkinElmer 2400 CHN microanalyser. For optical rotation ($[\alpha]_D$) measurements (*ca.* 20 °C, 10^{-1} deg cm^2 g^{-1}), a PerkinElmer 341 polarimeter was used. Electronic circular dichroism (ECD) spectra were recorded on a Jasco J-720 instrument in acetonitrile solvent.

Flash column chromatography and PLC were performed on Merck Kieselgel type 60 (250–400 mesh) and $PF_{254/366}$ respectively. Merck Kieselgel type $60F_{254}$ analytical plates were used for TLC. Liquid chromatography/mass spectrometry (LC/MS) analyses were conducted using an Agilent 1100 series HPLC coupled to an Agilent 6510 Q-TOF (Agilent Technologies, USA). Separation was performed using a reverse phase column (Luna C18 (2) 5 μm, 150 × 2.0 mm, Phenomenex, UK) together with the corresponding guard column (C18, 4 × 2.0 mm, Phenomenex, UK). The mobile phase consisted of 95% methanol in channel A, and 5% methanol in channel B. The system was programmed to perform an analysis cycle consisting of 30% A for 1 min, followed by gradient elution from 30% to 95% A over a 11 min period, hold at 95% A for 5 min, return to initial conditions over 5 min and then hold these conditions for a further 5 min. The flow rate was 0.20 ml min^{-1} and the injection volume was 5 μl. MS experiments were carried out using ESI in positive ion mode with the capillary voltage set at 4.0 kV. The desolvation gas was nitrogen set at a flow rate of 11 L min^{-1} and maintained at a temperature of 350 °C. Collision energy values of 10, 20, 30 and 40 eV were employed for MS/MS experiments and data were collected for 100 ms at each value.

The small scale (0.2–5.0 g) shake flask biotransformations and bioproduct isolations were carried out using whole cells of *P. putida* UV4 (TDO), *P. putida* 9816/11 (NDO), *E. coli* $_{F352V}$ (NDO_{F352V}) and *S. yanoikuyae* B8/36 (BPDO) as sources of the dioxygenase enzymes, using methods described earlier.[12,13,16,22,23,34] The bioproducts were isolated by repeated extraction with EtOAc. The extracts were dried (Na_2SO_4), concentrated, and purified using either PLC or flash column chromatography on silica gel. The ee values of bioproducts were determined by analysis of the chemical shift values of methoxyl signals from ^1H-NMR spectra of (–)-(*S*) and (+)-(*R*)-2-(1-methoxyethyl)benzeneboronate esters (MEBBA esters).[25,26]

(+)-(1*S*,2*R*)-1,2-Dihydroxy-3-(2′-pyridyl)cyclohexa-3,5-diene 2b

Substrate **1b**, *S. yanoikuyae* B8/36: colourless oil (0.72 g, 59%); R_f 0.34 (75% ethyl acetate–hexane); $[\alpha]_D$ +173 (*c* 1.2, MeOH) (Found: M$^+$, 189.0798. $C_{11}H_{11}NO_2$ requires 189.0789); ν_{max} (neat) 3353 cm^{-1} (O–H); δ_H (500 MHz, $CDCl_3$) 4.43 (1 H, m, $J_{1,2}$ 6.2, H-1), 4.83 (1 H, d, $J_{2,1}$ 6.2, H-2), 6.07–6.11 (2 H, m, $J_{5,4}$ 5.4, H-5, H-6), 6.58 (1 H, d, $J_{4,5}$ 5.4, H-4), 7.01 (1 H, dd, $J_{4',3'}$ 8.2, $J_{4',5'}$ 3.7, H-4′), 7.54 (1 H, d, $J_{3',4'}$ 8.2, H-3′), 7.61 (1 H, dd, $J_{5',4'}$ 3.7, $J_{5',6'}$ 3.1, H-5′), 8.44 (1 H, d, $J_{6',5'}$ 3.1, H-6′); δ_C (125 MHz, $CDCl_3$) 67.42, 68.99, 119.96, 122.07, 123.64, 124.65, 131.57, 136.91, 136.18, 148.15, 157.16; *m/z* (EI) 189 (M$^+$, 41%), 171 (18), 160 (100), 93 (11); CD: $\Delta\varepsilon$ 5.0 (321 nm) $\Delta\varepsilon$ –2.0 (271 nm), $\Delta\varepsilon$ –8.6 (220 nm); ee >98% (MEBBA).

Substrate **1b**, *P. putida* 9816/11: colourless oil (0.030 g, 25%); >95% relative yield by LC-MS analysis; $[\alpha]_D$ +170 (*c* 1.5, MeOH); ee >98% (MEBBA).

(–)-(1*S*,2*R*)-1,2-Dihydroxy-4-(2′-pyridyl)cyclohexa-3,5-diene 3b

Substrate **1b**, *P. putida* 9816/11: light yellow oil (0.003 g, 2%); R_f 0.20 (5% MeOH–$CHCl_3$); $[\alpha]_D$ –28.0 (*c* 0.32, MeOH) (Found: M$^+$, 189.0790. $C_{11}H_{11}NO_2$ requires 189.0789); δ_H (500 MHz, $CDCl_3$) 4.32 (1 H, m, H-1), 4.48 (1 H, m, H-2), 6.20 (1 H, dd, $J_{6,1}$ 4.3, $J_{6,5}$, 9.7, H-6), 6.56 (1 H, m, $J_{3,2}$ 3.7, H-3), 6.74 (1 H, d, $J_{5,6}$, 9.7, H-5), 7.21 (1 H, m, H-5′), 7.51 (1 H, d, $J_{6',5'}$ 8, H-6′), 7.70 (1 H, m, $J_{5',6'}$ 8, $J_{5',3'}$ 1.8, H-5′), 8.58 (1 H, dd, $J_{3',4'}$ 4.9, $J_{3',5'}$ 1.8, H-3′); CD: $\Delta\varepsilon$ 2.05 (290 nm), $\Delta\varepsilon$ −4.42 (222 nm), $\Delta\varepsilon$ −1.66 (206 nm) and $\Delta\varepsilon$ −1.85 (204 nm); ee >98% (MEBBA).

(+)-(1*S*,2*R*)-1,2-Dihydroxy-3-(3′-pyridyl)cyclohexa-3,5-diene 2c

Substrate **1c**, *S. yanoikuyae* B8/36: light yellow oil (0.718 g, 49%); R_f 0.16 (50% ethyl acetate–hexane); $[\alpha]_D$ +249 (*c* 0.83, THF) (Found: M$^+$, 189.0793. $C_{11}H_{11}NO_2$ requires 189.0789); ν_{max} (neat) 3367 cm^{-1} (O–H); δ_H (500 MHz, $CDCl_3$) 4.50 (1 H, d, $J_{2,1}$ 5.9, H-2), 4.62 (1 H, dd, $J_{1,2}$ 5.9, $J_{1,6}$ 3.1, H-1), 5.99 (1 H, dd, $J_{6,1}$ 3.1, $J_{6,5}$ 9.6, H-6), 6.13 (1 H, m, $J_{5,4}$ 5.5, $J_{5,6}$ 9.6, H-5), 6.40 (1 H, d, $J_{4,5}$ 5.5, H-4), 7.30 (1 H, dd, $J_{5',4'}$ 7.9, $J_{5',6'}$ 4.8, H-5′), 7.87 (1 H, dd, $J_{4',2'}$ 2.0, $J_{4',6'}$ 7.9, H-4′), 8.48 (1 H, d, $J_{6',4'}$ 4.8, H-6′), 8.76 (1 H, d, $J_{2',4'}$ 2.0, H-2′); δ_C (125 MHz, $CDCl_3$) 67.13, 69.20, 122.32, 122.50, 122.65, 131.61, 132.31, 134.03, 134.11, 145.53, 146.84; *m/z*: (EI) 189 (M$^+$, 30%), 171 (91), 160 (100), 93 (9); CD: $\Delta\varepsilon$ 6.0 (312 nm), $\Delta\varepsilon$ −10.3 (229 nm); ee >98% (MEBBA).

(+)-(1*S*,2*R*)-1,2-Dihydroxy-3-(4′-pyridyl)cyclohexa-3,5-diene 2d

Substrate **1d**, *S. yanoikuyae* B8/36: light yellow oil (0.381 g, 31%); R_f 0.11 (50% EtOAc–hexane); $[\alpha]_D$ +181 (*c* 0.85, THF) (Found: M$^+$, 189.0793. $C_{11}H_{11}NO_2$ requires 189.0789); ν_{max} (neat) 3370 cm^{-1} (O–H); δ_H (500 MHz, $CDCl_3$) 4.40 (1 H, dd, $J_{2,1}$ 5.9, $J_{2,4}$ 1.3, H-2), 4.54 (1 H, dd, $J_{1,2}$ 5.9, $J_{1,6}$ 2.3, H-1), 5.94 (1 H, dd, $J_{6,1}$ 2.3, $J_{6,5}$ 9.5, H-6), 6.06 (1 H, dd, $J_{5,4}$ 5.6, $J_{5,6}$ 9.5, H-5), 6.47 (1 H, d, $J_{4,5}$ 5.6, H-4), 7.34 (2 H, d, $J_{3',2'}$ 5.9, $J_{5',4'}$ 5.9, H-3′, H-5′), 8.49 (2 H, d, $J_{2',3'}$ 5.9, $J_{6',5'}$ 5.9, H-2′, H-6′); δ_C (125 MHz, $CDCl_3$) 68.21, 70.50, 119.75 (2 × C), 123.64, 124.82, 133.53, 135.74, 146.07, 150.13 (2 × C); *m/z* (EI) 189 (M$^+$, 25%), 171 (61), 143 (100), 93 (9); CD: $\Delta\varepsilon$ 3.61 (317 nm), $\Delta\varepsilon$ −6.13 (220 nm); ee >98% (MEBBA).

(+)-(1*S*,2*R*)-1,2-Dihydroxy-3-(1′-pyrrolyl)cyclohexa-3,5-diene 2e

Substrate **1e**, *P. putida* UV4: crystalline solid (0.08 g, 12%); mp 102–104 °C (EtOAc–hexane); $[\alpha]_D$ +144 (*c* 0.23, THF) (Found: C, 67.4; H, 6.2, $C_{10}H_{11}NO_2$ requires C, 67.8; H, 6.3%); ν_{max} (KBr) 3347 cm^{-1} (O–H); δ_H (500 MHz, $CDCl_3$) 4.46 (1 H, dd, $J_{1,2}$ 6.2, $J_{1,6}$ 5.9, H-1), 4.64 (1 H, dd, $J_{2,1}$ 6.2, $J_{2,4}$ 2.4, H-2), 5.74 (1 H, d, $J_{5,4}$ 2.6, H-5), 5.93 (1 H, d, $J_{6,1}$ 5.9, H-6), 6.00 (1 H, d, $J_{4,2}$ 2.4, $J_{4,5}$ 2.5, H-4), 6.28 (2 H, m, $J_{2',3'}$ 4.4, H-2′, H-5′), 7.01 (2 H, $J_{3',2'}$ 4.4, m, H-3′, H-4′); δ_C (125 MHz, $CDCl_3$) 68.73, 71.11, 110.04, 110.60 × 2, 118.95 × 2, 123.20, 128.35, 138.52; *m/z* (EI) 177 (M$^+$, 24%), 159 (4), 83 (100); CD: $\Delta\varepsilon$ 4.65 (320 nm), $\Delta\varepsilon$ −9.99 (229 nm); ee >98% (MEBBA).

Crystal data for 2e. $C_{10}H_{11}NO_2$, $M = 177.2$, monoclinic, $a = 13.076(4)$, $b = 4.980(2)$, $c = 21.362(11)$ Å, $\beta = 103.18(3)$, $U = 1354.5(10)$ Å^3, $T = 293(2)$ K, Cu-Kα radiation, $\lambda = 1.5418$ Å, space group $P2_1$ (no. 4), $Z = 6$, $F(000) = 564$, $D_x = 1.303$ g cm^{-3}, $\mu = 0.75$ mm^{-1}, Siemens P3 diffractometer, ω scans, scan range 2°, $4.0° < 2\theta < 110.1°$, measured/independent reflections: 3971/3307, $R_{int} = 0.11$, direct methods solution, full-matrix least squares refinement on F_o^2, anisotropic displacement parameters for non-hydrogen atoms; all hydrogen atoms located in a difference Fourier synthesis but included at positions calculated from the geometry of the molecules using the riding model, with isotropic vibration parameters. $R_1 = 0.074$ for 3210 data with $F_o > 4\sigma(F_o)$, 358 parameters, $\omega R_2 = 0.206$ (all data), GoF = 1.03, Flack × parameter = −0.07(18), $\Delta\rho_{min,max} = -0.25/0.31$ e Å^{-3}. CCDC reference number 691141.

(+)-(1*S*,2*R*)-1,2-Dihydroxy-3-(1′-pyrazolyl)cyclohexa-3,5-diene 2f

Substrate **1f**, *P. putida* UV4: colourless oil (0.028 g, 12%); $[\alpha]_D$ +144 (*c* 0.23, THF) (Found: M$^+$, 178.0742. $C_9H_{10}N_2O_2$ requires 178.0747); ν_{max} (neat) 3356 cm^{-1} (O–H); δ_H (500 MHz, CD_3OD) 4.84 (1 H, ddd, $J_{1,2}$ 6.27, $J_{1,5}$ 2.8, $J_{1,6}$ 2.4, H-1), 4.95 (1 H, d, $J_{2,1}$ 6.3, H-2), 6.07 (1 H, dd, $J_{6,1}$ 2.4, $J_{6,5}$ 7.2, H-6), 6.27 (1 H, ddd, $J_{5,1}$ 2.8, $J_{5,4}$ 5.9, $J_{5,6}$ 7.2, H-5), 6.63 (1 H, d, $J_{4,5}$ 5.9, H-4), 6.68 (1 H, dd, $J_{3',2'}$ 1.9, $J_{3',4'}$ 2.5, H-3′), 7.89 (1 H, d, $J_{2',3'}$ 1.9, H-2′), 8.29 (1 H, d, $J_{4',3'}$ 2.5, H-4′); δ_C (125 MHz, CD_3OD) 70.20, 73.49, 109.53, 114.38, 124.38, 131.16, 132.72, 142.36, 143.97; *m/z* (EI) 178 (M$^+$, 22%), 160 (36), 93 (11); CD: $\Delta\varepsilon$ 1.04 (316 nm), $\Delta\varepsilon$ −3.37 (226 nm); ee >98% (MEBBA).

(+)-(9*S*,10*R*)-9,10-Dihydrobenzo[*h*]quinoline-9,10-diol 5b

Substrate **4b**, *S. yanoikuyae* B8/36: light yellow crystalline solid (3.0 g, 50%); mp 128–129 °C (from $CHCl_3$); R_f 0.25 (7% MeOH/$CHCl_3$); $[\alpha]_D$ +167 (*c* 0.3, MeOH) (Found: M$^+$, 213.0781. $C_{13}H_{11}NO_2$ requires 213.0789); ν_{max} (KBr) 3408 cm^{-1} (O–H); δ_H (500 MHz, $CDCl_3$) 4.46 (1 H, dd, $J_{9,10}$ 5.0 $J_{9,8}$ 5.4, 9-H), 5.57 (1 H, d, $J_{10,9}$ 5.0, 10-H), 6.38 (1 H, dd, $J_{8,7}$ 9.5, $J_{8,9}$ 5.4, 8-H), 6.72 (1 H, d, $J_{7,8}$ 9.6, 7-H), 7.26–7.42 (2 H, m, 5-H, 3-H), 7.73 (1 H, d, $J_{6,5}$ 8.2, 6-H), 8.16 (1 H, dd, $J_{4,3}$ 8.3, $J_{4,2}$ 1.8, 4-H), 8.81 (1H, dd, $J_{2,3}$ 8.2, $J_{2,4}$ 1.8, 2-H); δ_C (125 MHz, $CDCl_3$) 65.25, 71.72, 120.63, 125.89, 126.78, 127.52, 128.26, 129.40, 129.89, 133.10, 137.23, 147.39, 148.31; *m/z* (EI) 213 (M$^+$, 19%), 194 (61), 86 (100), 93 (9); CD: $\Delta\varepsilon$ 2.78 (344 nm), $\Delta\varepsilon$ 3.44 (330 nm), $\Delta\varepsilon$ −0.92 (280 nm); $\Delta\varepsilon$ 5.29 (262 nm); $\Delta\varepsilon$ 6.86 (253 nm); $\Delta\varepsilon$ 1.64 (225 nm); ee >98% (MEBBA).

Substrate **4b**, *P. putida* 9816/11: (0.01 g, 16%) $[\alpha]_D$ +167 (*c* 0.3, MeOH); ee >98% (MEBBA).

(+)-(7*R*,8*S*)-7,8-Dihydrobenzo[*h*]quinoline-7,8-diol 6b

Substrate **4b**, *S. yanoikuyae* B8/36: white crystalline solid (0.76 g, 13%); mp 125–126 °C (from $CHCl_3$); R_f 0.32 (5% MeOH/$CHCl_3$); $[\alpha]_D$ +46 (*c* 0.97, MeOH) (Found: M$^+$, 213.0799. $C_{13}H_{11}NO_2$ requires 213.0789); ν_{max} (KBr) 3319 cm^{-1} (O–H); δ_H (500 MHz, $CDCl_3$) 4.48 (1 H, dd, $J_{8,7} = J_{8,9}$ 4.8, 8-H), 4.89 (1 H, d, $J_{7,8}$ 4.9, 7-H), 6.35 (1 H, dd, $J_{9,10}$ 9.9, $J_{9,8}$ 4.6, 9-H), 7.42 (1 H, dd, $J_{3,4}$ 8.2, $J_{3,2}$ 4.2, 3-H), 7.79 (1 H, d, $J_{5,6}$ 8.3, 5-H), 7.83 (1 H, d, $J_{6,5}$ 8.3, 6-H), 7.93 (1 H, d, $J_{10,9}$ 9.9, 10-H), 8.14 (1 H, dd, $J_{4,3}$ 8.3, $J_{4,2}$ 1.8, 4-H), 8.93 (1 H, dd, $J_{2,3}$ 4.2, $J_{2,4}$ 1.8, 2-H); δ_C (125 MHz, $CDCl_3$) 67.71, 71.45, 121.59, 125.21, 125.98, 128.19, 128.40, 128.99, 129.06, 136.57, 137.10, 144.30, 150.55; *m/z* (EI) 213 (M$^+$, 58%), 194 (60), 167 (100); CD: $\Delta\varepsilon$ +0.17 (273 nm), $\Delta\varepsilon$ −1.05 (247 nm), $\Delta\varepsilon$ +1.59 (224 nm); ee >98% (MEBBA).

Substrate **4b**, *P. putida* 9816/11: (0.006 g, 10%); $[\alpha]_D$ +43 (*c* 0.3, MeOH); ee >98% (MEBBA).

(−)-(7*R*,8*S*)-7,8-Dihydrobenzo[*h*]quinoline-7,8-diol 6b

Substrate **4b**, *E. coli*$_{F352V}$: (0.004 g, 11%); $[\alpha]_D$ −46 (*c* 0.4, MeOH); CD: $\Delta\varepsilon$ −0.09 (273 nm), $\Delta\varepsilon$ +0.66 (247 nm), $\Delta\varepsilon$ −1.043 (224 nm); ee >98%. (MEBBA).

(+)-(9*S*,10*R*)-9,10-Dihydrobenzo[*f*]quinoline-9,10-diol 5c

Substrate **4c**, *S. yanoikuyae* B8/36: light yellow crystalline solid (0.08 g, 67%); R_f 0.25 (7% MeOH–CHCl$_3$); mp 126–128 °C (from CHCl$_3$); $[\alpha]_D$ +153 (*c* 0.3, MeOH) (Found: M$^+$, 213.0791. $C_{13}H_{11}NO_2$ requires 213.0789); ν_{max} (KBr) 3408 cm^{-1} (O–H); δ_H (500 MHz, CDCl$_3$) 4.74 (1 H, ddd, $J_{9,10}$ 5.5, $J_{9,8}$ 2.1, $J_{9,7}$ 2.5, 9-H), 5.26 (1 H, d, $J_{10,9}$ 5.5, 10-H), 6.07 (1 H, dd, $J_{8,7}$ 9.7, $J_{8,9}$ 2.1, 8-H), 6.57 (1 H, dd, $J_{7,8}$ 9.7, $J_{7,9}$ 2.5, 7-H), 7.45 (2 H, m, 5-H, 2-H), 8.01 (1 H, d, $J_{6,5}$ 8.5, 6-H), 8.51 (1 H, dd, $J_{1,2}$ 8.5, $J_{1,3}$ 1.4, 1-H), 8.82 (1H, dd, $J_{3,2}$ 5.6, $J_{3,1}$ 1.4, 3-H); *m*/*z* (EI) 213 (M$^+$, 65%), 194 (33), 86 (100); CD: $\Delta\varepsilon$ –1.74 (311 nm), $\Delta\varepsilon$ +1.13 (257 nm); $\Delta\varepsilon$ –4.82 (223 nm); ee >98% (MEBBA).

Substrate **4c**, *P. putida* 9816/11: (0.02 g, 17%); $[\alpha]_D$ +153 (*c* 0.3, MeOH); ee >98% (MEBBA).

(+)-(9*S*,10*R*)-9,10-Dihydrophenanthridine-9,10-diol 5d

Substrate **4d**, *S. yanoikuyae* B8/36: crystalline solid (0.21 g, 72%); mp 190–192 °C (from EtOAc); R_f 0.15 (5% MeOH–CHCl$_3$); $[\alpha]_D$ +82 (*c* 0.5, MeOH) (Found: C, 72.7; H, 4.7; N, 6.3. $C_{13}H_{11}NO_2$ requires C, 73.2; H, 5.2; N, 6.6%); ν_{max} (KBr) 3390 cm^{-1} (O–H); δ_H (500 MHz, CDCl$_3$) 4.75 (1 H, ddd, $J_{9,10}$ 5.3, $J_{9,8}$ 2.0, $J_{9,7}$ 2.6, 9-H), 5.34 (1 H, d, $J_{10,9}$ 5.4, 10-H), 6.13 (1 H, dd, $J_{8,7}$ 9.8, $J_{8,9}$ 2.0, 8-H), 6.60 (1 H, dd, $J_{7,8}$ 9.8, $J_{7,9}$ 2.6, 7-H), 7.57–7.70 (2 H, m, 2-H, 3-H), 8.05 (1 H, d, $J_{4,3}$ 7.5, 4-H), 8.18 (1H, d, $J_{1,2}$ 8.3, 1-H), 8.67 (1 H, s, 6-H); δ_C (125 MHz, CDCl$_3$) 65.30, 69.73, 120.51, 125.12, 127.78, 127.95, 128.96, 129.40, 129.81, 133.10, 139.07, 146.93, 152.96; *m*/*z* (EI) 213 (M$^+$, 55%), 194 (34), 141 (100); CD: $\Delta\varepsilon$ –0.48 (305 nm), $\Delta\varepsilon$ +1.99 (255 nm), $\Delta\varepsilon$ –0.79 (227 nm); ee >98%. (MEBBA).

Substrate **4d**, *P. putida* 9816/11: (0.004 g, 7%); $[\alpha]_D$ +83 (*c* 0.2, MeOH); ee >98% (MEBBA).

Substrate **4d**, *E. coli*$_{F352V}$: (2.98 g, 10%); $[\alpha]_D$ +42 (*c* 0.4, MeOH); ee 51% (MEBBA).

(–)-(3*R*,4*S*)-3,4-Dihydrophenanthridine-3,4-diol 6d

Substrate **4d**, *E. coli*$_{F352V}$: (3.6 g, 12%); mp 168–170 °C (from EtOAc); R_f 0.20 (5% MeOH–CHCl$_3$); $[\alpha]_D$ –26 (*c* 0.49, MeOH) (Found: M$^+$, 213.0799. $C_{13}H_{11}NO_2$ requires 213.0790); δ_H (500 MHz, CD$_3$OD) 4.62 (1 H, ddd, $J_{3,4}$ 5.2, $J_{3,2}$ 3.2, $J_{3,1}$ 2.0, 3-H), 4.76 (1 H, dd, $J_{4,3}$ 5.2, $J_{4,2}$ 0.7, 4-H), 6.28 (1 H, ddd, $J_{2,1}$ 10.0, $J_{2,3}$ 3.2, $J_{2,4}$ 0.7, 2-H), 7.29 (1 H, dd, $J_{1,2}$ 10.0, $J_{1,3}$ 2.0, 1-H), 7.67 (1 H, ddd, $J_{8,7}$ 8.0, $J_{8,9}$ 7.0, $J_{8,10}$ 1.0, 8-H), 7.82 (1 H, ddd, $J_{9,10}$ 8.4, $J_{9,8}$ 7.0, $J_{9,7}$ 1.4, 9-H), 8.07 (1 H, d, $J_{7,8}$ 8.3, 7-H), 8.23 (1H, d, $J_{10,9}$ 8.6, 10-H), 9.10 (1 H, s, 6-H); δ_C (125 MHz, CD$_3$OD) 68.69, 71.37, 120.53, 121.77, 121.82, 127.10, 127.93, 128.45, 130.88, 132.09, 132.54, 147.66, 150.21; *m*/*z* (EI) 213 (M$^+$, 27%), 196 (9), 195 (29), 184 (48), 166 (29), 156 (15), 149 (22), 112 (38), 105 (64), 97 (45), 83 (50), 77 (33), 71 (55), 57 (100); CD: $\Delta\varepsilon$ –0.29 (269 nm), $\Delta\varepsilon$ –2.14 (236 nm), $\Delta\varepsilon$ –0.24 (220 nm), $\Delta\varepsilon$ –0.48 (212 nm); ee 84% (MEBBA).

(–)-(9*S*,10*R*)-9,10-Dihydrobenzo[*c*]cinnoline-9,10-diol 5e

Substrate **4e**, *S. yanoikuyae* B8/36: (0.08 g, 62%); R_f 0.35 (7% MeOH–CHCl$_3$); mp 132–133 °C (from MeOH); $[\alpha]_D$ –280 (*c* 1.3, pyridine) (Found: M$^+$, 214.0760. $C_{12}H_{10}N_2O_2$ requires 214.0784); ν_{max} (KBr) 3408 cm^{-1} (O–H); δ_H (500 MHz, CDCl$_3$) 4.73 (1 H, ddd, $J_{9,10}$ 5.3, $J_{9,8}$ 2.4, $J_{9,7}$ 2.0, 9-H), 5.38 (1 H, d, $J_{10,9}$ 5.3, 10-H), 6.40 (1 H, dd, $J_{8,7}$ 9.9, $J_{8,9}$ 2.4, 8-H), 7.14 (1 H, dd, $J_{7,8}$ 9.9, $J_{7,9}$ 2.0, 7-H), 7.74 (2 H, m, 3-H, 2-H), 8.34 (2 H, m, 1-H, 4-H); *m*/*z*: 214 (M$^+$, 46%), 195 (29), 87 (100); CD: $\Delta\varepsilon$ –0.26 (287 nm), $\Delta\varepsilon$ –0.75 (263 nm); $\Delta\varepsilon$ +1.26 (257 nm); ee >98% (MEBBA).

Substrate **4e**, *P. putida* 9816/11: (0.02 g, 16%); $[\alpha]_D$ –280 (*c* 0.3, MeOH); ee >98% (MEBBA).

Stereochemical correlation sequence of (+)-(9*S*,10*R*)-9,10-dihydrobenzo[*h*]quinoline-9,10-diol 5b and (+)-(9*S*,10*R*)-9,10-dihydrophenanthridine-9,10-diol 5d with (–)-(2*S*,3*S*)-dimethyl (2,3-diacetoxy)adipate 10

(–)-(9*S*,10*R*)-7,8,9,10-Tetrahydrobenzo[*h*]quinoline-9,10-diol 7. To a solution of enantiopure *cis*-dihydrodiol metabolite **5b** or **5d** (0.2 g, 1.12 mmol) in MeOH (15 cm^3) was added 10% Pd/C (0.010 g), and the mixture stirred (4 h) under an atmosphere of hydrogen at 1 atm pressure. The catalyst was removed by filtration and the filtrate concentrated under reduced pressure to give *cis*-tetrahydrodiol **7** or **11**.

Colourless oil (0.192 g, 80%); $[\alpha]_D$ –43 (*c* 0.5, MeOH) (Found: C, 72.3; H, 6.0; N, 6.3. $C_{13}H_{13}NO_2$ requires C, 72.5; H, 6.1; N, 6.5%); ν_{max} (neat) 3340 cm^{-1} (O–H); δ_H (500 MHz, CDCl$_3$) 1.93–2.01 (1 H, m, 8-H), 2.25–2.35 (1 H, m, 8′-H), 2.77–2.86 (1 H, m, 7-H), 2.26–2.38 (1 H, m, 7′-H), 4.39 (1 H, m, 9-H), 5.48 (1 H, d, $J_{10,9}$ 3.5, 10-H), 7.34 (1 H, d, $J_{6,5}$ 8.4, 6-H), 7.40 (1 H, dd, $J_{3,4}$ 8.2, $J_{3,2}$ 6.1, 3-H), 7.67 (1 H, d, $J_{5,6}$ 8.4, 5-H), 8.16 (1 H, dd, $J_{4,3}$ 8.3, $J_{4,2}$ 1.8, 4-H), 8.83 (1 H, dd, $J_{2,3}$ 6.1, $J_{2,4}$ 1.8, 2-H); δ_C (125 MHz, CDCl$_3$) 25.40, 27.04, 65.18, 70.21, 123.67, 126.49, 127.77, 130.18, 131.66, 133.53, 138.35, 147.37, 148.29; *m*/*z* (EI) 215 (M$^+$, 21%), 196 (14), 186 (69), 171 (43), 143 (100); CD: $\Delta\varepsilon$ +4.84 (235 nm), $\Delta\varepsilon$ –1.58 (218 nm), $\Delta\varepsilon$ –2.22 (201 nm).

(–)-(9*S*,10*R*)-7,8,9,10-Tetrahydrophenanthridine-9,10-diol 11. Colourless oil (0.180 g, 75%); $[\alpha]_D$ –72 (*c* 0.5, MeOH) (Found: C, 72.3; H, 5.9; N, 6.4 $C_{13}H_{13}NO_2$ requires C, 72.5; H, 6.1; N 6.5%); ν_{max} (KBr) 3325 cm^{-1} (O–H); δ_H (500 MHz, CDCl$_3$) 2.03–2.07 (1 H, m, 8-H), 2.09–2.15 (1 H, m, 8′-H), 2.95–2.97 (1 H, m, 7-H), 3.07–3.11 (1 H, m, 7′-H), 4.07 (1 H, m, 9-H), 5.36 (1 H, d, $J_{10,9}$ 4.0, 10-H), 7.51–7.68 (2 H, m, 2-H,3-H), 8.08 (1 H, d, $J_{4,3}$ 8.5, 4-H), 8.23 (1 H, d, $J_{1,2}$ 8.3, 1-H), 8.68 (1 H, s, 6-H); δ_C (125 MHz, CDCl$_3$) 25.42, 26.24, 65.13, 69.72, 123.36, 125.90, 127.33, 128.66, 129.81, 131.27, 139.17, 146.90, 151.74; *m*/*z* (EI) 215 (M$^+$, 44%), 197 (29), 171 (74), 143 (100); CD: $\Delta\varepsilon$ –0.63 (267 nm), $\Delta\varepsilon$ –0.16 (241 nm), $\Delta\varepsilon$ –5.60 (212 nm).

Degradation of *cis*-tetrahydrodiols 7 and 11. *cis*-Tetrahydrodiol **7** (or **11**, 0.1 g, 0.47 mmol) was converted into diacetate **8** (or **11**) by treatment with Ac$_2$O–pyridine. After identification by infrared and ^{1}H NMR spectroscopy, the crude diacetate **8** (or **11**) (0.12 g) was dissolved in a mixture of CCl$_4$ (2 cm^3), MeCN (2 cm^3) and water (3 cm^3). Sodium periodate (3.26 g, 15 mmol) and ruthenium(II) oxide hydrate (0.005 g) were then added to the solution. The reaction mixture was stirred at room temperature for 4 days, a solution of HCl (20 cm^3, 1.5 M) added, and the mixture saturated with NaCl. From the mixture, the product was extracted with EtOAc (3 × 20 cm^3), dried (Na$_2$SO$_4$) and the solvent evaporated. The residue was dissolved in MeOH (1.5 cm^3) and treated with an excess of diazomethane solution in Et$_2$O (4 h, 0 °C). The solvents and excess diazomethane were removed under a stream of nitrogen. Purification of the residue by flash chromatography on silica gel (hexane : Et$_2$O, 90 : 10 → 50 : 50) gave (–)-(2*S*,3*S*)-dimethyl (2,3-diacetoxy)adipate **10**.

(–)-(9*S*,10*R*)-9,10-Diacetoxy-7,8,9,10-tetrahydrobenzo[*h*]quinoline 8. Colourless oil; ν_{max} (neat) 1744 cm^{-1} (C=O); δ_H (500 MHz, CDCl$_3$) 2.04 (3 H, s, Ac), 2.11 (3 H, s, Ac), 2.23–2.46 (2 H, m, 8-H, 8′-H), 3.08–3.19 (2 H, m, 7-H, 7′-H), 5.23 (1 H, m, 9-H), 7.23–7.39 (3 H, m, 3-H, 5-H, 10-H), 7.75 (1 H, d, $J_{6,5}$ 8.2, 6-H), 8.08 (1 H, d, $J_{4,3}$ 8.3, 4-H), 8.89 (1 H, d, $J_{2,3}$ 5.9, 2-H).

(–)-(9*S*,10*R*)-9,10-Diacetoxy-7,8,9,10-tetrahydrophenanthridine-9,10-diol 12. Colourless oil; ν_{max} (neat) 1740 cm^{-1} (C=O); δ_H (500 MHz, CDCl$_3$) 2.10 (3 H, s, Ac), 2.12 (3 H, s, Ac), 2.25–2.38 (2 H, m, 8-H, 8′-H), 3.08–3.29 (2 H, m, 7-H, 7′-H), 5.25 (1 H, m, 9-H), 6.88 (1 H, d, $J_{10,9}$ 2.9, 10-H), 7.58–7.69 (2 H, m, 2-H, 3-H), 7.84 (1 H, d, $J_{4,3}$ 8.0, 4-H), 8.15 (1 H, d, $J_{1,2}$ 8.3, 1-H), 8.79 (1 H, s, 6-H).

(–)-(2*S*,3*S*)-Dimethyl (2,3-diacetoxy)adipate 10. Colourless oil (0.015 g, 13%), $[\alpha]_D$ –14.0 (*c* 1.0, CHCl$_3$) (lit.[35] $[\alpha]_D$ –14.1, CHCl$_3$); ν_{max} (neat) 1736 cm^{-1} (C=O); δ_H (500 MHz, CDCl$_3$) 1.97 (1 H, m, C*H*H), 2.07 (3 H, s, OAc), 2.06–2.15 (1 H, m, CH*H*), 2.18 (3 H, s, OAc), 2.37 (2 H, m, CH$_2$), 3.68 (3 H, s, CO$_2$Me), 3.79 (3 H, s, CO$_2$Me), 5.30 (2 H, m, 2-H, 3-H).

Synthesis of *trans*-(1*S*,2*S*)-1,2-Dihydroxy-3-(3′-pyridyl)-cyclohexa-3,5-diene 17

(i) (1*S*,2*R*)-1,2-Dihydroxy-3-(3′-pyridyl)cyclohex-3-ene 2g. *cis*-Dihydrodiol **2c** (0.1 g, 0.53 mmol, $[\alpha]_D$ +249, THF, ee >98%) in methanol solution (20 cm^3) containing quinoline (50 μl) was catalytically hydrogenated (3% Pd/C) at room temperature and 1 atm pressure. Hydrogen absorption was complete after 4.5 h. The catalyst was removed by filtration and the filtrate evaporated. The hydrogenated product was purified by PLC to give yellow coloured crystalline solid (0.089 g, 88%); mp 86–88 °C (CHCl$_3$–hexane); R_f 0.16 (50% EtOAc–hexane); $[\alpha]_D$ –50 (*c* 0.56, THF) (Found: C, 68.9; H, 7.0; N, 7.1. C$_{11}$H$_{13}$NO$_2$ requires C, 69.1; H, 6.9; N, 7.3%); ν_{max} (KBr) 3344 cm^{-1} (O–H);δ_H (500 MHz, CDCl$_3$) 1.77 (1 H, m, 6-H), 1.86 (1 H, m, 6′-H), 2.24 (1 H, m, 5-H), 2.35 (1 H, m, 5-H′), 3.82 (1 H, m, $J_{1,2}$ 3.7, 1-H), 4.48 (1 H, d, $J_{2,1}$ 3.7, 2-H), 6.18 (1 H, m, 4-H), 7.18 (1 H, dd, $J_{5',4'}$ 3.8, $J_{5',6'}$ 4.9, 5′-H), 7.78 (1 H, dd, $J_{4',2'}$ 2.0, $J_{4',5'}$ 3.8, 4′-H), 8.33 (1 H, d, $J_{6',5'}$ 4.9, 6′-H), 8.61 (1 H, d, $J_{2',4'}$ 2.0, 2′-H); δ_C (125 MHz, CDCl$_3$) 24.96, 25.50, 67.64, 69.94, 123.74, 130.60, 134.10, 135.06, 136.49, 147.28, 147.77; *m*/*z* (EI) 191 (M$^+$, 13%), 173 (56), 147 (100).

Crystal data for 2g. C$_{11}$H$_{13}$NO$_2$, M = 191.2, monoclinic, a = 15.561(6), b = 6.152(2), c = 11.984(6) Å, β = 122.08(3), U = 972.0(7) Å^3, T = 293(2) K, Cu-Kα radiation, λ = 1.5418 Å, space group *C*2 (no. 5), Z = 4, F(000) = 408, D_x = 1.31 g cm^{-3}, μ = 0.73 mm^{-1}, Siemens P3 diffractometer, ω scans, scan range 2°, 4.0° < 2θ < 110.1°, measured/independent reflections: 2214/997, R_{int} = 0.037, direct methods solution, full-matrix least squares refinement on $F_o{}^2$, anisotropic displacement parameters for non-hydrogen atoms; all hydrogen atoms located in a difference Fourier synthesis but included at positions calculated from the geometry of the molecules using the riding model, with isotropic vibration parameters. R_1 = 0.032 for 987 data with $F_o > 4\sigma(F_o)$, 130 parameters, ωR_2 = 0.085 (all data), GoF = 1.14, Flack × parameter = –0.07(17), $\Delta\rho_{min,max}$ = –0.12/0.11 e Å^{-3}. CCDC reference number 691142.

(ii) (1*S*,2*S*)-1,2-Dihydroxy-3-(3′-pyridyl)cyclohex-3-ene 13. To a stirring solution of (1*S*,2*R*)-1,2-dihydroxy-3-(3′-pyridyl)cyclohex-3-ene **7** (1.0 g, 5.24 mmol, $[\alpha]_D$ –50, THF) in anhydrous benzene (20 cm^3) were added dry 3 Å molecular sieves (1 g), triphenyl phosphine (1.51 g, 5.76 mmol) and diethyl azodicarboxylate (1.0 g, 5.76 mmol). The mixture was stirred for 30 min, *p*-nitrobenzoic acid (0.875 g, 5.24 mmol) was then added and the stirring continued for another 30 min at room temperature. The mixture was refluxed at 90 °C until diol **7** had reacted completely (monitored by TLC). The reaction mixture was filtered and the filtrate evaporated under reduced pressure. The residue was dissolved in MeOH (15 cm^3), a 10% aq solution of potassium carbonate (10 cm^3) added to it and the mixture stirred (4 h) at room temperature. On completion of hydrolysis (3 h, monitored by TLC), the solvents were removed *in vacuo* and the crude product taken up in ethyl acetate (40 cm^3). The extract was washed with saturated brine solution (10 cm^3), dried (Na$_2$SO$_4$), and the solvent removed under reduced pressure. Purification of the residue by PLC (R_f 0.19, 50% EtOAc–hexane) yielded *trans*-diol **13** as a yellow crystalline solid (0.62 g, 62%), mp 183 °C (CHCl$_3$–hexane); $[\alpha]_D$ +26 (*c* 1.25, D$_2$O) (Found: M$^+$, 191.0942. C$_{11}$H$_{13}$NO$_2$ requires 191.0946); ν_{max} (KBr) 3355 cm^{-1} (O–H);δ_H (500 MHz, CD$_3$OD): 1.68 (1 H, m, 6-H), 1.88 (1 H, m, 6′-H), 2.18 (1 H, m, $J_{5A,4}$ 4.0, 5-H), 2.29 (1 H, m, 5′-H), 3.83 (1 H, m, $J_{1,2}$ 4.7, 1-H), 4.28 (1 H, d, $J_{2,1}$ 4.7, 2-H), 6.14 (1 H, t, $J_{4,5A}$ 4.0, 4-H), 7.28 (1 H, dd, $J_{5',4'}$ 4.1, $J_{5',6'}$ 4.9, 5′-H), 7.83 (1 H, dd, $J_{4',2'}$ 2.1, $J_{4',5'}$ 4.1, 4′-H), 8.28 (1 H, d, $J_{6',5'}$ 4.9, 6′-H), 8.53 (1 H, d, $J_{2',4'}$ 2.1, 2′-H); δ_C (125 MHz, CD$_3$OD): 21.49, 24.33, 69.20, 70.99, 122.77, 129.27, 133.59, 133.90, 136.44, 145.99, 146.41; *m*/*z* (EI) 191 (M$^+$, 19%), 173 (25), 147 (100).

(iii) *trans*-(1*S*,2*S*)-1,2-Diacetyloxy-3-(3′-pyridyl)cyclohex-3-ene 14. *trans*-(1*S*,2*S*)-1,2-Dihydroxy-3-(3-pyridyl)cyclohex-3-ene (**13**) (0.62 g, 3.25 mmol, $[\alpha]_D$ +26) was acetylated using Ac$_2$O–pyridine. PLC yielded diacetate **14** as a light yellow oil (0.867 g, 97%); R_f 0.01 (30% Et$_2$O–hexane); $[\alpha]_D$ +83 (*c* 1.32, CH$_3$OH) (Found: M$^+$, 275.3011. C$_{15}$H$_{17}$NO$_4$ requires 275.3020); ν_{max} (neat) 1759 cm^{-1} (C=O); δ_H (500 MHz, CDCl$_3$) 1.91 (3 H, s, Ac), 1.99 (2 H, m, 6-H, H-6′-H), 2.01 (3 H, s, Ac), 2.39 (2 H, m, 5-H, 5-H′), 5.15 (1 H, m, $J_{1,2}$ 4.8, $J_{1,6}$ 6.5, 1-H), 5.91 (1 H, dd, $J_{2,1}$ 4.8, $J_{2,4}$ 0.5, 2-H), 6.31 (1 H, dd, $J_{4,2}$ 0.5, $J_{4,5}$ 2.4, 4-H), 7.24 (1 H, dd, $J_{5',4'}$ 7.9, $J_{5',6'}$ 5.1, 5′-H), 7.59 (1 H, dd, $J_{4',2'}$ 1.6, $J_{4',5'}$ 4.8, 4′-H), 8.51 (1 H, d, $J_{6',5'}$ 5.1, 6′-H), 8.59 (1 H, d, $J_{2',4'}$ 1.6, 2′-H); δ_C (125 MHz, CDCl$_3$) 20.74, 21.04, 22.55, 23.53, 68.67, 70.92, 123.02, 131.86, 133.12, 134.47, 147.57, 148.56, 170.13; *m*/*z* (EI) 276 (M + 1, 2%), 275 (6), 215 (25), 155 (100).

(iv) *trans*-(1*S*,2*S*)-1,2-Diacetyloxy-3-(3′-pyridyl)-5-bromocyclohex-3-ene 15. Freshly crystallised *N*-bromosuccinimide (0.591 g, 3.32 mmol) and α,α-azoisobisbutyronitrile (0.01 g) were added to a solution of *trans*-(1*S*,2*S*)-1,2-diacetyloxy-3-(3′-pyridyl)cyclohex-3-ene **14** (0.83 g, 3.02 mmol, $[\alpha]_D$ +83, MeOH) in CCl$_4$ (10 cm^3). The mixture was gently refluxed (~90 °C) using a heat lamp. When the reaction had gone to completion (1 h, ^{1}H-NMR analysis), the mixture was cooled to room temperature, succinimide filtered off, and the solvent removed *in vacuo.* The crude product, obtained as a yellow oil (0.997 g, 93%), was identified as the title compound by ^{1}H-NMR spectroscopy. δ_H (500 MHz, CDCl$_3$) 1.90 (3 H, s, Ac), 2.02 (3 H, s, Ac), 2.50 (1 H, m, 6-H), 2.62 (1 H, m, $J_{6B,1}$ 4.9, 6′-H), 4.93 (1 H, m, 5-H), 5.45 (1 H, m, $J_{1,6B}$ 4.9, $J_{1,2}$ 3.6, 1-H), 6.06 (1 H, d, $J_{2,1}$ 3.6, 2-H), 6.37 (1 H, d, 4-H), 7.27 (1 H, dd, $J_{5',4'}$ 4.9, $J_{5',6'}$ 5.2, 5′-H), 7.61 (1 H, dd, $J_{4',2'}$ 1.6, $J_{4',5'}$ 4.9, 4′-H), 8.55 (1 H, d, $J_{6',5'}$ 5.2, H-6′), 8.60 (1H, s, 2′-H). Due to its unstable nature, it was used without further purification for the next step of the synthesis.

(v) *trans*-(1*S*,2*S*)-1,2-Diacetyloxy-3-(3′-pyridyl)cyclohexa-3,5-diene 16. A mixture of *trans*-(1*S*,2*S*)-1,2-diacetyloxy-3-(3′-pyridyl)-5-bromocyclohex-3-ene **15** (0.97 g, 2.73 mmol), anhydrous lithium chloride (0.325 g, 7.64 mmol) and anhydrous lithium carbonate (0.505 g, 6.83 mmol) in freshly distilled hexamethylphosphoramide (3 cm^3) was heated, with stirring at 95 °C, under nitrogen for 2 h. The ice cooled reaction mixture was diluted with diethyl ether (15 cm^3) and then treated dropwise with stirring with HCl (13.7 cm^3, 1 M solution). The ether layer was separated and the aqueous layer extracted with diethyl ether (2×15 cm^3). The combined ether extract was washed with 2.5% $NaHCO_3$ solution (15 cm^3), dried (Na_2SO_4) and the solvent evaporated. Purification of the residue by PLC (R_f 0.27, Et_2O) yielded *trans*-diacetate **16** as a white crystalline solid (0.708 g, 95%); mp 84 °C (Et_2O–hexane); $[\alpha]_D$ +570 (*c* 1.03, $CHCl_3$) (Found: M^+, 273.2867. $C_{15}H_{15}NO_4$ requires 273.2862); ν_{max} (KBr) 1727 cm^{-1} (C=O); δ_H (500 MHz, $CDCl_3$) 2.01 (3 H, s, Ac), 2.07 (3 H, s, Ac), 5.43 (1 H, dd, 1-H), 6.06–6.10 (2 H, m, 2-H, 6-H), 6.36 (1 H, dd, $J_{5,4}$ 5.9, 5-H), 6.60 (1 H, d, $J_{4,5}$ 5.9, 4-H), 7.28 (1 H, dd, $J_{5',4'}$ 4.2, $J_{5',6'}$ 4.8, 5′-H), 7.68 (1 H, dd, $J_{4',5'}$ 4.2, $J_{4',2'}$ 2.3, 4′-H), 8.53 (1 H, d, $J_{6',5'}$ 4.8, 6′-H), 8.70 (1 H, d, $J_{2',4'}$ 2.3, 2′-H); δ_C (125 MHz, $CDCl_3$) 21.37, 21.46, 68.99, 69.56, 123.76, 124.25, 124.95, 127.45, 131.87, 133.13, 147.60, 149.49, 170.5; *m*/*z* (EI) 273 (10%), 213 (18%), 171 (100).

(vi) *trans*-(1*S*,2*S*)-1,2-Dihydroxy-3-(3′-pyridyl)cyclohexa-3,5-diene 17. To a solution of *trans*-(1*S*,2*S*)-1,2-diacetyloxy-3-(3′-pyridyl)cyclohexa-3,5-diene **16** (0.75 g, 3.97 mmol, $[\alpha]_D$ +570) in MeOH (10 cm^3) were added water and K_2CO_3 (1.24 g, 9 mmol). The mixture was stirred at room temperature. When the deacetylation was complete (3 h, by TLC), the inorganic salts were filtered off and the filtrate concentrated *in vacuo.* The crude product was extracted into EtOAc (25 cm^3) and subsequently purified by PLC to yield the *trans*-dihydrodiol **17** as a light yellow crystalline solid (0.720 g, 96%); mp 157 °C (decomp.) ($CHCl_3$–hexane); R_f 0.2 (50% EtOAc–hexane); $[\alpha]_D$ +189 (*c* 0.51, $CHCl_3$) (Found: M^+, 189.0791. $C_{11}H_{11}NO_2$ requires 189.0790); ν_{max} (KBr) 3816 cm^{-1} (O–H); δ_H (500 MHz, $CDCl_3$) 4.46 (1 H, dd, $J_{1,2}$ 6.2, $J_{1,6}$ 5.3, 1-H), 4.73 (1 H, d, $J_{2,1}$ 6.2, 2-H), 6.12 (1 H, dd, $J_{6,1}$ 5.3, $J_{6,5}$ 4.4, 6-H), 6.22 (1 H, dd, $J_{5,4}$ 5.4, $J_{5,6}$ 4.4, 5-H), 6.36 (1 H, d, $J_{4,5}$ 5.4, 4-H), 7.29 (1 H, dd, $J_{5',4'}$ 4.3, $J_{5',6'}$ 5.5, 5′-H), 7.85 (1 H, d, $J_{4',5'}$ 4.3, 4′-H), 8.50 (1 H, d, $J_{6',5'}$ 5.5, 6′-H), 8.75 (1 H, s, 2′-H); δ_C (125 MHz, $CDCl_3$) 71.08, 71.77, 122.04, 123.62, 124.73, 128.02, 129.05, 133.07, 133.91, 146.98, 148.25; *m*/*z* (EI) 189 (M^+, 11%), 171 (36), 43 (100); CD: $\Delta\varepsilon$ 4.45 (300 nm), $\Delta\varepsilon$ 3.49 (247 nm), $\Delta\varepsilon$ 3.66 (227 nm), $\Delta\varepsilon$ −1.641(203 nm).

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