Dioxygenase-catalyzed *cis*-dihydroxylation of pyridine-ring systems

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Abstract: Toluene dioxygenase-catalyzed dihydroxylation, in the carbocyclic rings of quinoline, 2-chloroquinoline, 2-methoxyquinoline, and 3-bromoquinoline, was found to yield the corresponding enantiopure *cis*-5,6- and -7,8-dihydrodiol metabolites using whole cells of *Pseudomonas putida* UV4. *cis*-Dihydroxylation at the 3,4-bond of 2-chloroquinoline, 2-methoxyquinoline, and 2-quinolone was also found to yield the heterocyclic *cis*-dihydrodiol metabolite, (+)-*cis*-(3*S*,4*S*)-3,4-dihydroxy-3,4-dihydro-2-quinolone. Heterocyclic *cis*-dihydrodiol metabolites, resulting from dihydroxylation at the 5,6- and 3,4-bonds of 1-methyl 2-pyridone, were isolated from bacteria containing toluene, naphthalene, and biphenyl dioxygenases. The enantiomeric excess (ee) values (>98%) and the absolute configurations of the carbocyclic *cis*-dihydrodiol metabolites of quinoline substrates (benzylic *R*) and of the heterocyclic *cis*-diols from quinoline, 2-quinolone, and 2-pyridone substrates (allylic *S*) were found to be in accord with earlier models for dioxygenase-catalyzed *cis*-dihydroxylation of carbocyclic arenes. Evidence favouring the dioxygenase-catalyzed *cis*-dihydroxylation of pyridine-ring systems is presented.

Key words: dioxygenases; *cis*-dihydroxylation, pyridines, 2-pyridones, absolute configurations.

Résumé : On a trouvé que la dihydroxylation des carbocycles de la quinoléine, de la 2-chloroquinoléine, de la 2-méthoxyquinoléine et de la 3-bromoquinoléine, catalysée par la dioxygénase du toluène en utilisant des cellules complètes de *Pseudomonas putida* UV4, conduit aux métabolites correspondants énantiopurs, les *cis*-5,6- et 7,8-dihydrodiols. On a aussi observé que la *cis*-dihydroxylation de la liaison 3,4 de la 2-chloroquinoléine, de la 2-méthoxyquinoléine et de la quinoléin-2-one conduit au métabolite hétérocyclique *cis*-dihydrodiol (+)-*cis*-(3*S*,4*S*)-3,4-dihydroxy-3,4-dihydroquinoléin-2-one. Lorsqu'on a utilisé des bactéries contenant des dioxygénases du toluène, du naphtalène et du biphényle, on a isolé des métabolites hétérocycliques *cis*-dihydrodiols qui résultent d'une dihydroxylation aux niveaux des liaisons 5,6- et 3,4- de la 1-méthylpyridin-2-one. Les valeurs des excès énantiomériques (ee) (>98%) et les configurations absolues des métabolites des *cis*-dihydrodiols carbocycliques des substrats à base de quinoléine (groupe benzylique étant *R*) et des *cis*-diols hétérocycliques dérivés de substrats à base de quinoléine, de quinoléin-2-one et de pyridin-2-one (groupe allylique étant *S*) sont en accord avec les modèles antérieurs pour les réactions de *cis*-dihydroxylation des arènes carbocycliques catalysées par la dioxygénase. On présente des données favorisant la *cis*-dihydroxylation catalysée par la dioxygénase de systèmes à noyau pyridine.

Mots clés : dioxygénase, *cis*-dihydroxylation, pyridines, pyridin-2-ones, configurations absolues.

[Traduit par la Rédaction]

Introduction

The initial step in the bacterial metabolism of aromatic rings often proceeds via dioxygenase-catalyzed asymmetric dihydroxylation to yield *cis*-dihydrodiol metabolites (1–5). Polycyclic arenes and azaarenes (Scheme 1) are amongst the wide range of substrates (> 400) found to yield carbocyclic *cis*-dihydrodiol metabolites (2, 3).

To date, however, only a small number of *cis*-dihydrodiols has been obtained from enzymatic *cis*-dihydroxylation of

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Dedicated to Professor J. Bryan Jones on the occasion of his 65th birthday.

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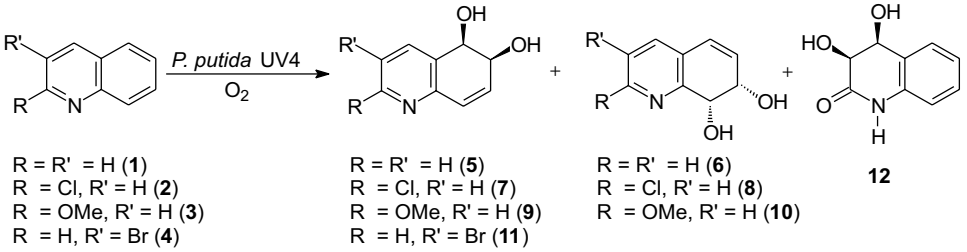
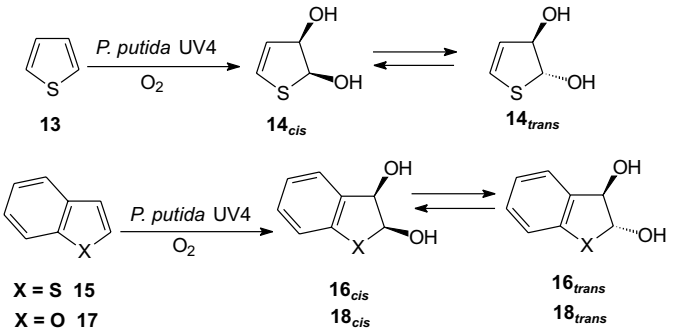
Scheme 1.



Scheme 2.



aromatic heterocyclic rings. These are generally derived from electron-rich five-membered aromatic heterocycles including thiophenes (e.g., **13**), benzothiophenes (e.g., **15**), and benzofurans (e.g., **17**) (Scheme 2).

Heterocyclic *cis*-diols $\mathbf{14}_{cis}$, $\mathbf{16}_{cis}$, and $\mathbf{18}_{cis}$ were found to spontaneously isomerize to the corresponding *trans*-isomers, $\mathbf{14}_{trans}$, $\mathbf{16}_{trans}$, and $\mathbf{18}_{trans}$ (6, 7). No direct evidence of dioxygenase-catalyzed *cis*-dihydroxylation of electron-poor heterocyclic arenes such as pyridine and quinoline has been reported (8–12). Alternative pathways for the bacterial metabolism of pyridine and quinoline rings, including azaarene hydration and monohydroxylation have, however, been discovered (8–12).

cis-Dihydroxylation of the carbocyclic ring and monohydroxylation of the azaarene ring in quinoline (**1**), isoquinoline, quinoxaline, and quinazoline substrates has been reported in *Pseudomonas putida* UV4 (12, 13). Unfortunately, the yields of *cis*-dihydrodiols obtained were generally low. A preliminary study from these laboratories on the biotransformation of quinoline substrates containing a substituent at C-2, such as azaarenes **2** and **3**, has shown that the yield of the corresponding carbocyclic *cis*-dihydrodiols **7**–**10** can be significantly increased (Scheme 1) (14). An unexpected heterocyclic *cis*-diol **12** was also isolated from quinoline substrates **2** and **3**. The possibility of *cis*-dihydroxylation occurring in the heterocyclic ring, either via the corresponding *cis*-dihydrodiols **20** and **21** or via benzo-fused 2-pyridone intermediate **24** as a precursor of metabolite **12**, was also briefly addressed (Scheme 3) (14).

Direct evidence of the stereoselective *cis*-dihydroxylation of a monocyclic 2-pyridone ring (**28**) to yield heterocyclic *cis*-diols **29** and **30** has recently been reported by Modyanova and Azerad (Scheme 4) (15, 16).

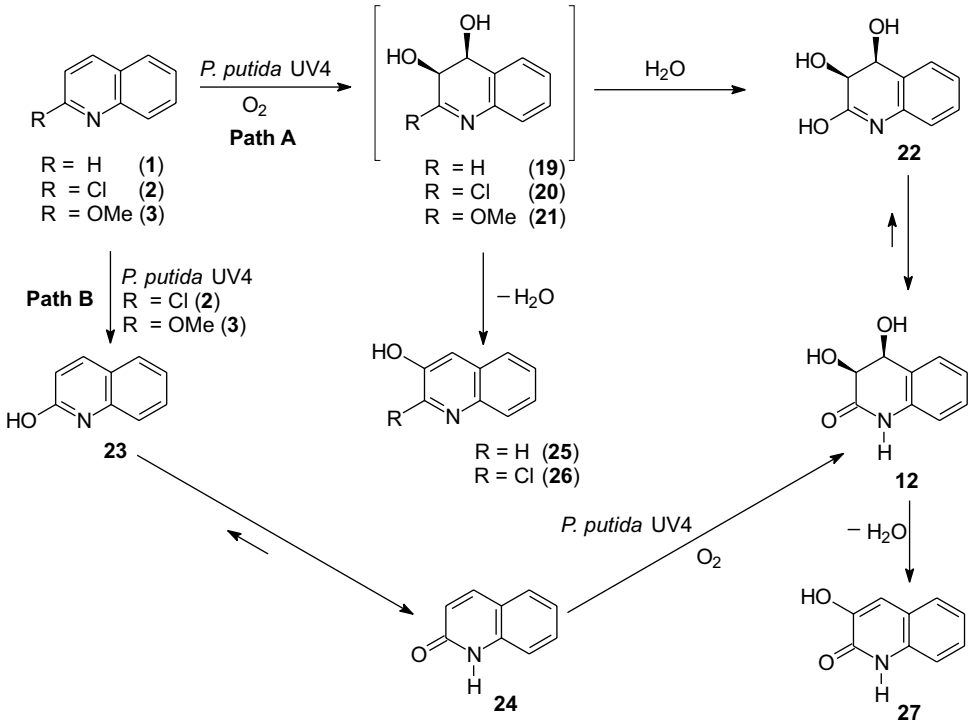
Results and discussion

A major objective of the present study was to conduct a more comprehensive search for evidence of dioxygenase-catalyzed *cis*-dihydroxylation of pyridine-ring systems — a topic that has received little attention in the literature (8–12). In this context, the reported bacterial *cis*-dihydroxylation of the benzo-fused pyridines **2**–**4** (14) and 2-pyridones **24** and **28** (15, 16) has now been re-examined. This extension of earlier work (12–16) involves a wider range of dioxygenases and substrates, with emphasis on the absolute configurations and enantiopurities of metabolites formed by *cis*-dihydroxylation of either the carbocyclic or the heterocyclic ring.

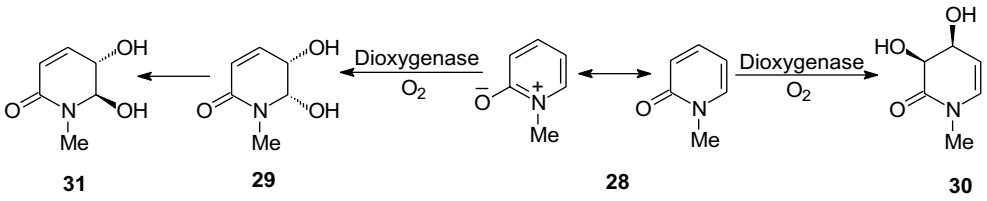
The biotransformation studies were carried out using mutant and recombinant strains of bacteria containing different types of ring-hydroxylating dioxygenase enzymes (Rieske-type nonheme iron oxygenases) (4), devoid of the corresponding *cis*-diol dehydrogenase enzymes, which are responsible for dehydrogenation of *cis*-dihydrodiols to the corresponding catechols. These mutant strains included *Pseudomonas putida* UV4 (a source of toluene dioxygenase, TDO) (17), *P. putida* 9816/11 (a source of naphthalene dioxygenase, NDO) (18), and *Sphingomonas yaniokuyae* B8/36 (a source of biphenyl dioxygenase, BPDO) (19). Recombinant strains used included *Escherichia coli* pKST11 (a source of TDO) (20) and *E. coli* JM 109 (DE3) pDTG141 (a source of NDO) (21) (Table 1).

Earlier studies of the biotransformation of parent quinoline substrate **1** with *P. putida* UV4 showed that diols could be formed by *cis*-dihydroxylation at the 5,6-bond (to yield the major *cis*-diol **5**) and the 7,8-bond (to yield minor *cis*-diol **6**) of the carbocyclic ring (12, 13) in relatively low yields (<10%). Other bioproducts of quinoline (**1**) included 3-hydroxy quinoline (**25**) and anthranilic acid, which could have resulted from *cis*-dihydroxylation at the 3,4-bond in the pyridine ring followed by decompositon of unstable 3,4-*cis*-diol **19** (Scheme 3, path A) (12, 13). When 2-chloroquinoline (**2**) was added as a substrate to the same strain, *cis*-dihydroxylation was found to occur preferentially within the carbocyclic ring at the 7,8-bond to give dihydrodiol **8** (20% yield) and also at the 5,6-bond to yield diol **7** (9% yield). A

Scheme 3.



Scheme 4.



similar trend was found using the TDO-containing recombinant strain (*E. coli* pKST11), where diols **8** (11% yield) and **7** (4% yield) were isolated. The bacterial strains containing NDO showed a preference for *cis*-dihydroxylation at the 7,8-bond to give diol **8** (15% yield using *P. putida* 9816/11 and 5% yield using *E. coli* JM 109). 5,6-Dihydrodiol **7** was also produced using *P. putida* 9816/11 (6% yield). *S. yanoikuyae* B8/36 and *P. fluorescens* Q1 (a wild-type strain grown on quinoline as a carbon source) only yielded *cis*-7,8-dihydrodiol **8** (15% and 1% yields, respectively, Table 1). These results are similar to those reported earlier on the bacterial biotransformation of **1** using *P. putida* 86 (a wild-type strain grown on quinoline as a carbon source) (22). In the latter example, compound **8**, of unspecified ee and absolute configuration, was reported to be the sole *cis*-dihydrodiol metabolite (22).

cis-Diol bioproducts **7** and **8**, obtained from TDO-, NDO-, and BPDO-catalyzed asymmetric dihydroxylation of 2-chloroquinoline (**2**), were all found to be enantiopure (>98% ee) based on the ^{1}H NMR spectral analyses of the corresponding cyclic-boronate derivatives formed by using (–)-(*S*)- and (+)-(*R*)-2-(1-methoxyethyl)benzeneboronic acid (MEBBA). This method has previously been used to determine the absolute configurations of both mono- and polycyclic *cis*-dihydrodiol metabolites (23–25). From a comparison of the characteristic chemical-shift values of the MeO signals in the ^{1}H NMR spectra of the MEBBA esters (23–25) and the circular dichroism (CD) spectral data (compared with parent *cis*-dihydrodiols **5** and **6** of known configurations), metabolites **7** and **8** were assigned the 5*R*,6*S* and 7*S*,8*R* configurations, respectively.

The two major carbocyclic *cis*-dihydrodiols **7** (R_f = 0.3, 8% yield) and **8** (R_f = 0.45, 18% yield), isolated from the biotransformation of **2** using *P. putida* UV4, were accompanied by a more polar metabolite, **12** (R_f = 0.14, 2% yield). X-ray crystallographic analysis, using the anomalous dispersion method, confirmed that bioproduct **12** was an enantiopure sample of (3*S*,4*S*)-3,4-dihydroxy-3,4-dihydro-2-quinolone (**12**) (14). While neither the corresponding MEBBA-boronate derivatives nor the corresponding diMTPA esters could be synthesized, the enantiopurity of *cis*-diol **12** was confirmed as >98% ee (^{1}H NMR analysis) by the formation of a single diastereoisomer of the monocamphanate ester at C-4. It is noteworthy that, with the exception of *P. putida* UV4, no evidence of the more polar *cis*-dihydrodiol metabolite **12** was found with the other five dioxygenase-containing strains. When the biotransformation of **2** was repeated on a larger scale (50 g substrate **2** in 100-L fermenter using *P. putida* UV4) and a more rigorous extraction procedure involving total removal of water prior to extraction was

Table 1. Substrate (arene), bacterial strain (strain), dioxygenase (enzyme), product (diol), % isolated yield (yield), and absolute configuration (Ab. con.) of *cis*-dihydrodiol metabolites (**7**–**12**)[a] obtained from the quinoline substrates **2**–**4** and 2-quinolone (**24**).

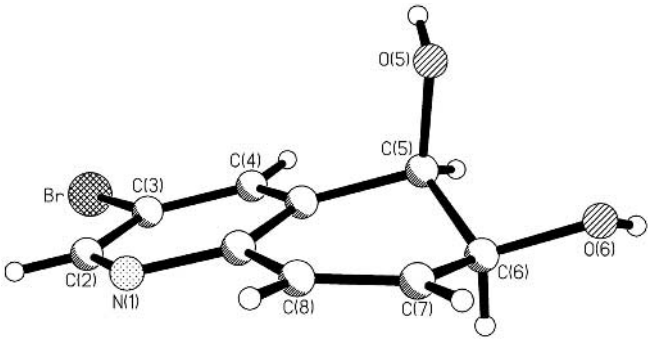
Arene	Strain	Enzyme	Diol	Yield	Ab.con.
2	UV4	TDO	**7**	9	5*R*,6*S*
			8	20	7*S*,8*R*
			12	6	3*S*,4*S*
2	pKST11	TDO	**7**	4	5*R*,6*S*
			8	11	7*S*,8*R*
2	9816/11	NDO	**7**	6	5*R*,6*S*
			8	15	7*S*,8*R*
2	JM109	NDO	**8**	5	7*S*,8*R*
2	B8/36	BPDO	**8**	15	7*S*,8*R*
2	Q1	QDO	**8**	1	7*S*,8*R*
			9	2	5*R*,6*S*
3	UV4	TDO	**10**	7	7*S*,8*R*
			12	13	3*S*,4*S*
3	9816/11	NDO	**10**	54	7*S*,8*R*
3	JM109	NDO	**10**	5	7*S*,8*R*
3	B8/36	BPDO	**10**	47	7*S*,8*R*
4	UV4	TDO	**11**	23	5*R*,6*S*
24	UV4	TDO	**12**	10	3*S*,4*S*

[a]All *cis*-dihydrodiols were enantiopure (>98% ee).

adopted, two additional metabolites were found. Carbocyclic *cis*-dihydrodiols **7** (ca. 5.4 g, 9%) and **8** (ca. 12.0 g, 20%) and the heterocyclic *cis*-diol **12** (ca. 3.1 g, 6%), were again the major bioproducts; the new metabolites were identified as 2-chloro-3-hydroxyquinoline (**26**) (ca. 0.5 g, 1%) and 3-hydroxy-2-quinolone (**27**) (ca. 1.5 g, 3%). It is probable that **27** was obtained by dehydration of the *cis*-diol **12** during the work-up procedure, since its dehydration was observed upon treatment with acid or at elevated temperatures. The presence of the minor phenolic metabolite (**26**) could be accounted for by the spontaneous dehydration of the transient *cis*-dihydrodiol **20**. When the total yields of 2-chloroquinoline metabolites **12**, **26**, and **27** are combined, the dioxygenase-catalyzed oxidation in the heterocyclic ring clearly emerges as a significant metabolic pathway (ca. 10% yield) compared with the total yield (ca. 29%) of carbocyclic ring *cis*-dihydroxylation products **7** and **8** (Scheme 3, path A).

2-Methoxyquinoline (**3**) also proved to be a substrate for *P. putida* UV4; it yielded, from dihydroxylation at the 5,6- and 7,8-positions, the anticipated carbocyclic *cis*-dihydrodiols **9** (R_f = 0.30, 2% yield) and **10** (R_f = 0.40, 7% yield), respectively (Scheme 1). Formation of the corresponding MEBBA derivatives, and CD comparison with *cis*-dihydrodiols **5** and **6**, indicated that metabolites **9** and **10** were enantiopure (>98% ee) and of the 5*R*,6*S* and 7*S*,8*R* configurations, respectively. The major metabolite (R_f = 0.14, 13% yield), formed from 2-methoxyquinoline (**3**) as the substrate and *P. putida* UV4 as a dioxygenase source, was *cis*-diol metabolite **12** of identical configuration (3*S*,4*S*) and enantiopurity (>98% ee) to that found earlier using **2** as substrate. Only 7,8-*cis*-dihydrodiol **10**, of identical ee value and absolute configuration, was obtained using NDO (*P. putida* 9816/11, *E. coli* JM 109) or BPDO (*S. yanoikuyae* B8/36) as the biocatalysts and **3** as the substrate (Table 1).

Fig. 1. X-ray structure of *cis*-diol **11**.

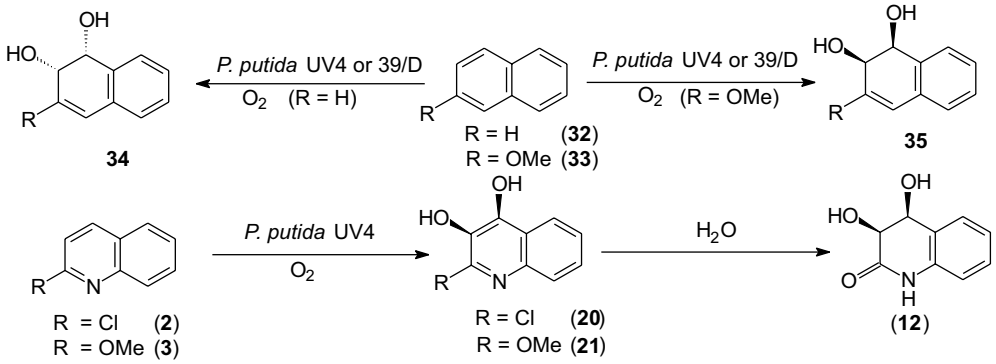


To decrease the possibility of dioxygenase-catalyzed *cis*-dihydroxylation of the heterocyclic ring, 3-bromoquinoline (**4**) was examined as a substrate with TDO (*P. putida* UV4). As anticipated, no hydroxylation occurred at the blocked 3,4-bond in the pyridine ring; the sole metabolite, *cis*-dihydrodiol **11** (23% yield) was enantiopure (>98% ee, (NMR analysis of the MEBBA-boronate derivatives), and resulted from attack at the 5,6-bond. The structure and absolute stereochemistry of *cis*-diol **11** was determined by X-ray crystallography using the anomalous dispersion method (Fig. 1). It again confirmed that 3-bromo-*cis*-5,6-dihydroxy-5,6-dihydroquinoline (**11**) was a single enantiomer and had a 5*R*,6*S* configuration, similar to the other 5,6-*cis*-dihydrodiol metabolites of substituted quinolines (e.g., **7** and **9**). The crystal structure of diol **11** showed no evidence of intramolecular H-bonding, but six intermolecular H-bonding interactions to four neighbouring molecules were observed.

Two feasible mechanisms could account for the formation of heterocyclic *cis*-dihydrodiol metabolite **12** during biotransformation of **2** and **3** in cultures of *P. putida* UV4. The first mechanism assumes that unstable *cis*-dihydrodiols **20** and **21** are the initial products of the direct dihydroxylation of the pyridine rings in the corresponding 2-substituted quinoline substrates, **2** and **3**, respectively (Scheme 3, path A). The postulated intermediates, imidoyl halide **20** and imidate **21**, are likely to spontaneously hydrolyze to yield trihydroxy product **22**, which exists preferentially as 2-quinolone tautomer **12**. Initial metabolites **20** (from **2**) and **19** (from **1**) (12, 13) could also dehydrate yielding the corresponding 3-hydroxyquinolines **26** and **25**, respectively. This evidence, based on the isolation of metabolites **12**, **25**, and **26**, is consistent with the mechanism shown in path A (Scheme 3).

The observation of the "NIH shift" has recently been shown to occur when aromatic hydroxylation proceeds via ei-

Scheme 5.



ther arene oxide isomerization (monooxygenase pathway) or *cis*-dihydrodiol dehydration (dioxygenase pathway) (26). Biotransformation of 3-deuterioquinoline (**1**) using *P. putida* UV4 yielded 3-hydroxyquinoline (**25**), with concomitant migration and retention of the deuterium atom (20% D) at an adjacent carbon atom, i.e., the NIH shift was observed (26). This provides further evidence in support of the formation of unstable *cis*-dihydrodiol **19** derived from the pyridine ring in substrate **1** (Scheme 3, path A).

A comparison of the enantiopurity and absolute configuration of *cis*-dihydrodiol metabolites **34** and **35**, obtained from naphthalene (**32**) and 2-methoxynaphthalene (**33**), respectively (27), with *cis*-dihydrodiol intermediates **20** and **21** from **2** and **3**, respectively, showed striking similarities (Schemes 1 and 5). The major *cis*-dihydrodiol metabolites from **33** were formed at the 5,6- and 7,8- bonds in the unsubstituted ring with TDO (using both *P. putida* 39/D and UV4) as biocatalyst (27); these *cis*-diols, derived from a carbocyclic ring, were of the normal benzylic *R* configuration as found in other bicyclic arenes, such as *cis*-diols **5**–**11** and **34**. Enantiopure *cis*-dihydrodiol **35**, a minor metabolite of **33** (27) was, however, found to have the opposite absolute configuration (benzylic *S*) to that normally found in carbocyclic rings (Schemes 1 and 5).

2-Substituted quinoline substrates **2** and **3** may be considered as isosteres of **33** and, when accepted as substrates by the TDO enzyme, would be expected to form *cis*-diols **20** and **21**, respectively, having the same absolute configuration (benzylic *S*) and enantiopurity as *cis*-diol **35** (>98% ee). As reported earlier, heterocyclic *cis*-diol metabolite **12** was a single enantiomer of identical absolute configuration (benzylic *S*). This observation provides additional indirect evidence favouring dioxygenase-catalyzed *cis*-dihydroxylation of the pyridine ring of substrates **2** and **3** to form the corresponding unstable metabolites **20** and **21**, respectively, as precursors of *cis*-diol **12** (Scheme 3, path A).

Direct evidence of the involvement of *cis*-dihydrodiol intermediates in the initial TDO-catalyzed biotransformation step of electron-rich five-membered aromatic rings was presented earlier (Scheme 2) (6, 7). Indirect evidence of dioxygenase-catalyzed dihydroxylation of the pyrrole ring in indole is also available. Thus, in the manufacture of indigo dye (28, 29), the spontaneous dehydration of the transient *cis*-dihydrodiol metabolite of indole to yield indoxyl (which further undergoes autoxidation) is assumed to occur. Until recently, it was not possible to find direct evidence of *cis*-dihydrodiol formation in the pyridine ring. The electron-poor pyridine-ring system has generally proved to be extremely resistant to dioxygenase-catalyzed *cis*-dihydroxylation where alternative metabolic routes are available. In the case of pyridine-substituted thioethers and arenes, dioxygenase-catalyzed oxidation has been observed only at the sulfur atom (sulfoxidation) (30) or at the carbocyclic ring (*cis*-dihydroxylation) (unpublished data).

In the light of recent direct evidence for *cis*-dihydroxylation of 1-methyl-2-pyridone (**28**) (15, 16), a second mechanism, to account for the formation of *cis*-dihydrodiol **12** from the 2-substituted quinolines **2** and **3**, was considered (Scheme 3, path B). Chemical or enzyme-catalyzed hydrolysis of the quinoline substrates could, in principle, occur in the aqueous-culture medium prior to the *cis*-dihydroxylation process yielding 2-hydroxyquinoline (**23**), which exists mainly as the 2-quinolone tautomeric form (**24**). The halogen and alkoxy substituents at C-2 in the pyridine ring of compounds **2** and **3** are susceptible to hydrolysis via an addition–elimination mechanism normally under acidic conditions. An aqueous–methanolic solution (D_2O–CD_3OD) of **2**, when heated (~45°C) for an extended period, did not undergo hydrolysis, suggesting that it was unlikely that more than a small proportion of substrates **2** and **3** would have hydrolyzed under the relatively mild aqueous conditions that existed during the biotransformation. GC–MS analysis, conducted during the course of the biotransformation of quinoline substrate **2**, showed minute traces of 2-quinolone (**24**) among the bioproducts, which could be due to chemical and (or) enzyme-catalyzed hydrolysis.

To test if **24** was a substrate, it was added to whole cells of *P. putida* UV 4. The corresponding *cis*-dihydrodiol (**12**) was found as the sole metabolite with an identical ee value (>98%) and absolute configuration (3*S*,4*S*) to that isolated from 2-substituted quinoline substrates **2** and **3**, but in a relatively low yield (10%). This observation confirms that metabolite **12** could be derived from **24**. Despite the latter result, the balance of evidence appears to favour path A and involvement of the transient *cis*-diol metabolites **20** and **21** as precursors of heterocyclic *cis*-diol **12**. A recent report of the TDO-catalyzed 3-hydroxylation of 4-picoline (4-methylpyridine) has assumed, without evidence, the initial formation of an unstable *cis*-dihydrodiol metabolite of the pyridine ring (31); the results presented in this study support this conclusion.

The NDO-catalyzed dihydroxylation of the pyridone ring in **28** has recently been found to yield two interesting and

Table 2. Bacterial strains and dioxygenases used in the *cis*-dihydroxylation of 1-methyl 2-pyridone (**28**) and relative (isolated) combined yields of *cis*-diols **29** and **30**.

Strain	Enzyme	% Relative yield of diols **29**:**30**[a]
P. putida 9816/11[b,c]	NDO	93:7 (41)
E. coli JM109(DE3) (pDTG141)[d,e]	NDO	96:4 (52)
E. coli JM109(DE3) (pDTG141-F352V)[d,f]	NDO_{352V}	100:0 (10)
S. yanoikuyae B8/36[b,g]	BPDO	90:10 (2)
P. putida UV4[b,h]	TDO	95:5 (<1)

[a]Relative yield based on the peak ratio obtained after GC–MS analysis.
[b]Without the corresponding *cis*-diol dehydrogenase enzyme activity.
[c]Salicylate inducer.
[d]IPTG inducer, ampicillin resistant.
[e]Recombinant-strain encoding NDO from *P. putida* 9816–4.
[f]Site-directed recombinant NDO with valine at 352.
[g]*m*-Xylene inducer.
[h]Constitutive mutant.

Fig. 2. X-ray structure of *cis*-diol **29**.

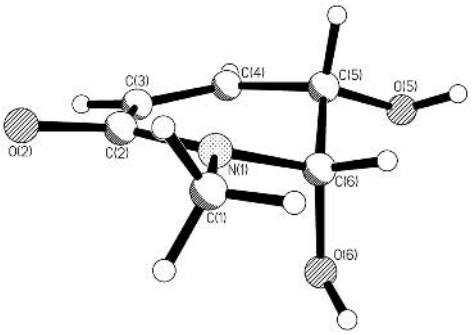
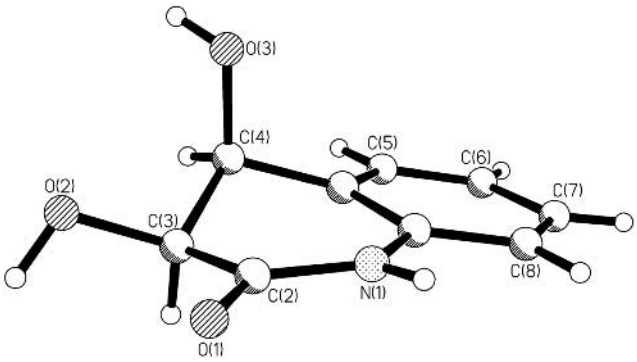


Fig. 3. X-ray structure of *cis*-diol **12**.



relatively stable *cis*-diol metabolites, **29** and **30** (Scheme 4) (16).

In an extension of our earlier work on TDO-catalyzed formation of heterocyclic *cis*-diol **12** from substituted 2-pyridone **24** (14), a programme was undertaken to examine the range of dioxygenases capable of producing *cis*-diols **29** and **30** from pyridone **28**. It was hoped that this would result in higher yields (particularly of the very minor regioisomer **30**), and would allow absolute configurations to be assigned to *cis*-diols **29** and **30**. These *cis*-diol metabolites may be considered as synthetic precursors of polyhydroxylated piperidines, several of which have already been identified as potent inhibitors of glycosidases (32).

An evaluation of the relative merits of the NDO, BPDO, and TDO enzyme systems in the *cis*-dihydroxylation of 1-methyl-2-pyridone (**28**) was carried out by GC–MS analysis. Both of the *cis*-dihydrodiols, **29** and **30**, were found to be detectable and separable as the corresponding bis-trimethylsilyl ethers (*m*/*z* 287). Some evidence of the isomerization of *cis*-diol **29** to *trans*- isomer **31** was found during the analytical process. The yield of the initially formed major diol metabolite **29** from **28** was estimated from the combined yields of *cis*-and *trans*-diols **29** and **31**, respectively.

A range of mutant (*P. putida* 9816/11, *S. yaniokuyae* B8/36, *P. putida* UV 4) and recombinant strains (*E. coli* JM 109 (DE3) pDTG141) (which had previously been used with bicyclic substrates **2** and **3**) and a site-directed mutant (*E. coli* JM 109 (DE3) pDTG141 strain 352V), were applied to the monocyclic substrate **28**; the relative ratios of products are given in Table 2.

Naphthalene dioxygenase, present in *P. putida* 9816/11 and *E. coli* JM 109 (DE3) pDTG141, was found to give a similar ratio of *cis* isomers favouring 5,6-regioisomer **29** (93–96%) and a comparable isolated yield (41–52%). When a site-directed mutant strain was used, where phenylalanine 352 had been replaced by valine (352-V), the isolated yield decreased (10%) but the regioselectivity increased to exclusively yield metabolite **29**. Although the highest proportion of the minor *cis*-diol regioisomer **30** (10% relative yield) was obtained with the BPDO enzyme system, this was offset by a much lower isolated yield (2%). The observation that a monocyclic compound, having a significant degree of aromatic character, was a substrate for both the NDO and BPDO systems was surprising, since only a small number of monocyclic arene derivatives have proved to be substrates for these enzymes. In contrast, more than 200 monocyclic arenes have proved to be acceptable substrates with TDO (2, 3). Surprisingly, when **28** was added as a substrate to *P. putida* UV4, *cis*-diol **29** was detected in very low yield (<1%); the substitution pattern and polarity of substrate **28** may have been contributing factors to the low yield.

The structure and relative stereochemistry of 2-pyridone *cis*-dihydrodiols **29** and **30** were established earlier on the basis of rigorous NMR spectral analysis (16). Similar spectral data was obtained during the current study. In addition, the structure of major *cis*-diol **29** ($[\alpha]_D$ +40, MeOH) was confirmed by X-ray crystallography (Fig. 2); it showed that the C-5 and C-6 hydroxyl groups were in a *cis* relationship with pseudo-equatorial and pseudo-axial conformations, re-

Scheme 6.

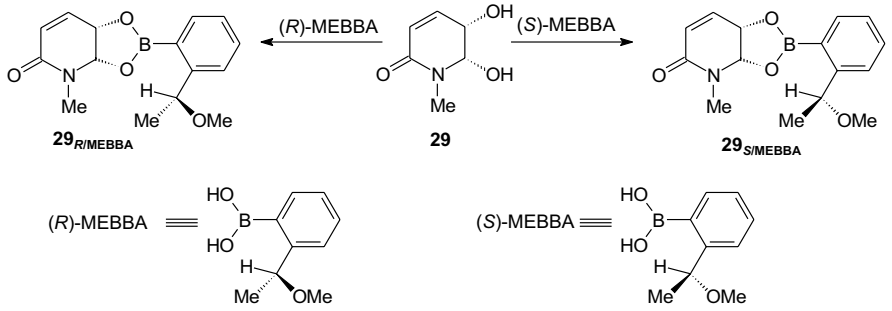
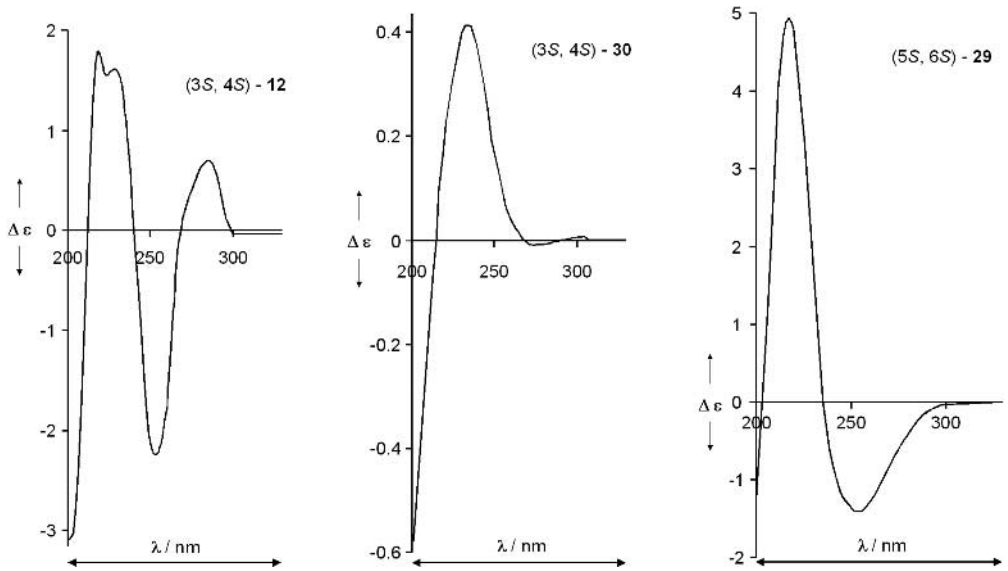


Fig. 4. CD spectra of the *cis*-diols (3*S*,4*S*)-**12**, **(**5*S*,6*S*)-**29** and **(3***S*,4*S*)-**30**.



spectively. The adoption of a pseudo-axial conformation by the OH group at C-6 appears to be the result of steric interaction with the *N*-methyl group. The pseudo-axial conformation of the OH group proximate to a substituent and the formation of a single enantiomer are common features of *cis*-dihydrodiol metabolites of monosubstituted benzene substrates (14, 30). For comparison purposes, the X-ray crystal structure of heterocyclic *cis*-diol **12** is shown in Fig. 3 (14). The OH group at C-4 in **12** and at C-6 in **29** are both pseudo-axial due to steric constraints.

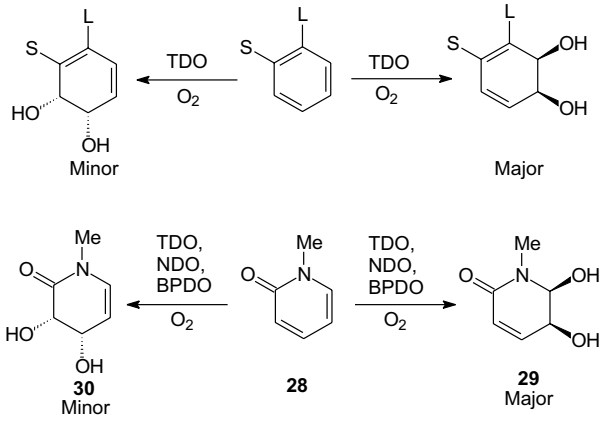
The crystal structure analysis also showed that the sample was enantiopure. There was no evidence of intramolecular H-bonding, but five intermolecular H-bonding interactions to four neighbouring molecules were observed.

From the relative chemical shift positions of methoxyl signals of the cyclic boronate derivatives $\mathbf{29}_{R/\text{MEBBA}}$ (δ_{MeO} 3.24) and $\mathbf{29}_{S/\text{MEBBA}}$ (δ_{MeO} 3.22), a 5*S*,6*S* absolute configuration was tentatively assigned (23–25) to major *cis*-diol **29**; the boronates also confirmed its enantiopurity (>98% ee) (Scheme 6).

In the determination of absolute stereochemistry via analysis of the X-ray crystal structure (using the anomalous dispersion method), the refinement of the Flack (33) absolute-structure parameter (*x*) ideally yields a value of zero for the correct enantiomer and a value of unity for the incorrect enantiomer. In the analysis of *cis*-diol **29**, using the anomalous dispersion method, *x* was refined to a final value of 0.24 (35) for the 5*S*,6*S* enantiomer. This implies that, at a confidence level above 2σ (i.e., >95%), the inverse 5*R*,6*R* structure can be rejected, and hence the true configuration has to be 5*S*,6*S*. Although this indicates an emphatic preference, it fails to meet the commonly accepted level (3σ, 99.8%), which is regarded as equating to statistical certainty. (An independent refinement of the inverse structure (5*R*,6*R*) gave $x = 0.76$ (35), which also independently implies that this configuration can be rejected at better than the 2σ level, albeit not at the preferred 3σ level.)

The circular dichroism (CD) spectra of the bicyclic 3*S*,4*S*-*cis*-diol **12** and monocyclic diols **29** and **30** provided strong supporting evidence for the stereochemical assignments (Fig. 4). Based on a comparison of CD spectra of *cis*-diols **12**, **29**, and **30**, where a strong positive CD absorption was found at lower wavelengths (210–250 nm) and a weaker negative CD absorption at longer wavelengths (235–300 nm), a similar absolute configuration can be assigned to each of the *cis*-diol metabolites. Since the 3*S*,4*S* configuration of (+)-*cis*-diol **12** was rigorously established by X-ray crystallography, monocyclic *cis*-diols **29** and **30** should thus have the 5*S*,6*S* and 3*S*,4*S* configurations, respectively. Since the major *cis*-diol (**29**) was found to be a single enantiomer of 5*S*,6*S* configuration, *trans* isomer (+)-**31** ($[\alpha]_D$ +207, MeOH), obtainable upon gentle heating at ca. 50°C (16) (via

Scheme 7.



reversible ring opening to the corresponding aldehyde) must have a 5*S*,6*R* configuration.

Due to the very low relative yield (<10%) of the minor *cis*-diol (**30**) (Table 2), it was not possible to carry out its complete characterization. The presence of **30** was confirmed by ^{1}H NMR, MS, and GC–MS analysis. Unfortunately neither the corresponding diMTPA esters (from *R*- or *S*-α-methoxy-α-(trifluoromethyl)phenylacetic acid) nor the chiral boronate (MEBBA) derivatives (from *R*- or *S*-2-(1-methoxyethyl)phenylboronic acid) could be formed from *cis*-diols **12** and **30**. In both cases, this may have been due to intramolecular hydrogen bonding between the OH group on C-3 and the adjacent carbonyl group. As stated in the preceding paragraph, however, it was assumed from a comparison of the CD specta that minor *cis*-diol **30** was a single enantiomer of 3*S*,4*S* configuration.

The absolute configurations of *cis*-dihydrodiol metabolites **29** and **30** were found to be in accord with a stereochemical model based on the preferred *cis*-dihydrodiol enantiomers obtained from 1,2-disubstituted benzene substrates with TDO as biocatalyst (Scheme 7) (2). Earlier studies of the TDO-catalyzed *cis*-dihydroxylation of a range of mono-, 1,2-, 1,3-, and 1,4-di-substituted benzene substrates have shown that stereo and regioselectivity can be predicted by considering the substituent size (L = large, S = small) (2). If the *cis*-dihydroxylation of **28** (L = Me, S = O) is considered to occur in a similar manner to 1,2-disubstituted benzene substrates (including polycyclic systems) using several dioxygenase enzymes, then the observed relative yields, ee values, and absolute configurations are in accord with the stereochemical template used earlier (Scheme 7) (2). This premise also supports the view that **28** has a considerable degree of aromatic character and an appreciable resonance energy (34).

The heterocyclic *cis*-dihydrodiols from the quinoline and 2-pyridone substrates have potential as chiral ligands (**5**–**11**) and as precursors for the synthesis of azasugars, alkaloids (**29**, **30**), and enzyme inhibitors (**5**–**11**). Studies are currently in progress to evaluate their potential in these areas.

Conclusion

The biotransformation of quinoline ring systems to yield *cis*-dihydrodiol metabolites appears to be a relatively common metabolic pathway and eight examples (**5**–**12**) have been found. Carbocyclic *cis*-dihydrodiol metabolites **7**–**10**, obtained by TDO-catalyzed dihydroxylation, were single enantiomers of identical configuration (benzylic *R*) to *cis*-diols (e.g., **34**) derived from other polycyclic arenes (e.g., **32**) using TDO, NDO, and BPDO as biocatalyst. A novel enantiopure heterocyclic *cis*-dihydrodiol (**12**) was isolated as a metabolite from 2-chloroquinoline (**2**), 2-methoxyquinoline (**3**), and 2-quinolone (**24**). No direct evidence could be obtained for the formation of *cis*-diol metabolites **20** and **21** from *cis*-dihydroxylation of the pyridine ring of substrates **2** and **3**. Strong indirect evidence has, however, been found for the formation of *cis*-diols **19**–**21** as unstable metabolites of a pyridine ring. The absolute configurations of *cis*-diol metabolites **29** and **30**, derived from 1-methyl-2-pyridone (**28**), are consistent with the dioxygenase enzymes being able to accept substrate **28** as a pyridine derivative having a structure similar to 1,2-disubstituted benzene substrates.

Experimental

^{1}H NMR spectra of compounds were recorded on Bruker Avance DPX-300 and DRX-500 instruments. Mass spectra were recorded using a double-focusing triple-sector VG Autospec instrument. Accurate mass measurements were obtained by the peak-matching method using perfluorokerosene as the standard reference and were accurate to within ±0.006 amu. Flash chromatography and PLC were performed on Merck Kieselgel type 60 (250–400 mesh) and $PF_{254/366}$, respectively. Merck Kieselgel $60F_{254}$ analytical plates were used for TLC. Optical rotation ($[\alpha]_D$) measurements were carried out with a PerkinElmer 214 polarimeter at ambient temperature (ca. 20°C) and are given in units of 10^{-1} deg cm^2 g^{-1}. CD spectra were recorded using a JASCO J-720 instrument and spectroscopic-grade methanol as solvent.

GC–MS analysis of the crude EtOAc extract of *cis*-diol metabolites **29** and **30** was carried out after concentration under a stream of dry N_2 and treatment with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide to yield the corresponding bis-trimethylsilyl ethers. The GC–MS instrument used consisted of a Hewlett Packard 6890 gas chromatograph directly attached to a Hewlett Packard 5973 Mass Selective Detector. The GC oven was equipped with a Hewlett Packard 7683 autosampler and fitted with a wall-coated open-tubular capillary column (12 m × 0.2 mm) with 100%

dimethyl polysiloxane (0.33 μm) as the bonded phase. The injector port was held at 250°C and the oven was programmed at 100°C for 1 min and then ramped to 300°C at 10°C min^{-1}. Helium was employed as carrier gas at a flow rate of 0.8 mL min^{-1} and the sample (1 μL) was injected at a split ratio of 25:1. Under these conditions the *cis*-diols **29** and **30** and the *trans*-diol **31** were eluted at 7.34, 6.96, and 6.00 min, respectively. The mass-selective detector was operated in the full-scan mode measuring ion currents between *m*/*z* 30 and 450 amu. The relative yields of diols were calculated by comparing the relative peak areas of diols (**29**/**31** and **30**).

Substrates **2**, **4**, **24**, and **28** were obtained from commercial sources. Substrate **3** (35) and (–)-(*S*)- and (+)-(*R*)-2-(1-methoxyethyl)benzene boronic acids were synthesized and used according to literature methods (23–25, 36).

Substrates were metabolized using growing cultures of *P. putida* UV4, a constituent mutant strain devoid of *cis*-diol dehydrogenase activity, according to the reported method (30). *P. putida* 9816/11, an inducible mutant, without the corresponding *cis*-diol dehydrogenase enzyme activity, was grown on minimal-salts medium containing 0.05% sodium succinate. The naphthalene dioxygenase (NDO) present was induced by addition of 0.5% sodium salicylate at the late exponential phase of growth. *S. yanoikuyae*, also an inducible mutant without the corresponding *cis*-diol dehydrogenase enzyme activity, was grown on minimal-salts medium with 0.5% sodium pyruvate and 0.05% of yeast extract; the late exponential phase of growth the biphenyl dioxygenase (BPDO) was again induced by the addition of *m*-xylene (0.05 mL L^{-1}) every 1/2 h for 7 h. When the cells were grown in a 100-L fermenter, continuous addition of the volatile inducer (*m*-xylene) was necessary since it was constantly depleted by the air supply. *E. coli* pKST11 biotransformations were carried on cells grown on minimal-salts medium supplemented with glucose (20 mM), thiamin (1 mM), and ampicillin (100 μg mL^{-1}) at 37°C. TDO activity was induced by the addition of 1 mM isopropyl-β-thiogalactopyranoside (IPTG). The NDO in *E. coli* JM109 was similarly induced by the addition of IPTG. *P. putida* Q1 was grown on minimal-salts medium supplemented with quinoline (0.3 mg mL^{-1}) as the carbon source.

Biotransformation of substrates: isolation and identification of metabolites

2-Chloroquinoline 2 with P. putida *UV4*

Biotransformation (10-L fermenter) of 2-chloroquinoline (**2**) (7.5 g) by *P. putida* UV4, repeated extraction of the aqueous biotransformed material with EtOAc, and removal of solvent from the extract under reduced pressure (Procedure 1), yielded a crude mixture of three *cis*-diols **12**, **7**, and **8**; these were separated by a combination of column chromatography ($CHCl_3$ → 10% MeOH–$CHCl_3$) and PLC (7% MeOH–$CHCl_3$). A larger-scale biotransformation (100-L fermenter) of 2-chloroquinoline **2** (50 g) was also carried out. The bioproducts were isolated by removal of water (reduced pressure) from the biotransformed material followed by hot extraction (45°C) of the residual material with 20% MeOH–EtOAc (Procedure 2). Column chromatography and PLC separations yielded five metabolites: **12**, **7**, **8**, **26**, and **27**. The yields obtained from the small and larger scale biotransformations, respectively, are shown together.

(+)-cis-*(3S,4S)-3,4-Dihydroxy-3,4-dihydro-2-quinolone (**12**)*

Colourless crystals (0.164 g, 2.0% and 3.10 g, 5.7%); mp 177–179°C (MeOH–hexane). R_f = 0.14 (7% MeOH–$CHCl_3$). $[\alpha]_D$ +6.0 (*c* 0.72, pyridine). CD λ (nm): 285.20 (Δε = +0.683), 252.40 (Δε = –2.237), 217.50 (Δε = +1.760), 200.40 (Δε = –3.258). IR ν_{max} (cm^{-1}): 3220 (OH), 1674 (CONH). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 4.05 (d, *J* = 3.0 Hz, 1H, OH), 4.13 (d, *J* = 3.0 Hz, 1H, OH), 4.34 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-3), 4.77 (d, $J_{4,3}$ = 3.7 Hz, 1H, H-4), 7.03 (m, 2H, H-6 and H-8), 7.30 (m, 1H, H-7), 7.36 (m, 1H, H-5), 9.36 (br s, 1H, NH): NOE enhancement of H-3 (1%) and H-5 (2%) from irradiation of H-4; NOE enhancement of H-4 (1%) from irradiation of H-3. ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ: 70.0, 71.1, 105.2, 116.1, 123.0, 129.8, 130.1, 137.8, 171.0. MS *m*/*z* (%): 179 (M^+, 77), 161 ([M – H_2O]$^+$, 54), 122 (100). Anal. calcd. for $C_9H_9NO_3$: C 60.3, H 5.1, N 7.8; found: C 59.8, H 4.9, N 7.6.

(+)-cis-*(5R,6S)-2-Chloro-5,6-dihydroquinoline-5,6-diol (**7**)*

Colourless crystals (0.66 g, 8% and 5.4 g, 9%); mp 120–122°C (EtOAc–hexane). R_f = 0.30 (7% MeOH–$CHCl_3$). $[\alpha]_D$ +140 (*c* 0.55, MeOH). CD λ (nm): 294.60 (Δε = –1.891), 254.40 (Δε = +7.648), 226.00 (Δε = +8.017), 195.60 (Δε = –3.821). IR ν_{max} (cm^{-1}): 3402 (OH). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 4.00 (m, 1H, OH), 4.18 (dd, $J_{6,5}$ = 4.9, $J_{6,7}$ = 4.6 Hz, 1H, H-6), 4.24 (m, 1H, OH), 4.55 (d, $J_{5,6}$ = 4.9 Hz, 1H, H-5), 6.27 (dd, $J_{7,6}$ = 4.6, $J_{7,8}$ = 9.9 Hz, 1H, H-7), 6.37 (d, $J_{8,7}$ = 9.9 Hz, 1H, H-8), 7.13 (d, $J_{3,4}$ = 8.1 Hz, 1H, H-3), 7.71 (d, $J_{4,3}$ = 8.1 Hz, 1H, H-4); NOE enhancement of H-4 (1%) from irradiation of H-5. ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ: 67.3, 70.5, 123.6, 130.0, 133.5, 136.8, 138.8, 150.5, 153.5. MS *m*/*z* (%): 197 (M^+(^{35}Cl), 34), 179 ([M – H_2O]$^+$, 8), 168 (100). Anal. calcd. for $C_9H_8ClNO_2$: C 54.7, H 4.1, N 7.1; found: C 54.2, H 3.8, N 6.9.

(+)-cis-*(7S,8R)-2-Chloro-7,8-dihydroquinoline-7,8-diol (**8**)*

Colourless crystals (1.63 g, 18% and 12.0 g, 20%); mp 110–111°C (EtOAc–hexane). R_f = 0.45 (7% MeOH–$CHCl_3$). $[\alpha]_D$ +148° (*c* 0.55 in MeOH). CD λ (nm): 309.80 (Δε = –0.932), 252.20 (Δε = +3.750), 246.80 (Δε = +3.665), 210.00 (Δε = +7.257). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 4.01 (d, *J* = 5.8 Hz, 1H, OH), 4.49 (dd, $J_{7,8}$ = 5.0, $J_{7,6}$ = 4.2 Hz, 1H, H-7), 4.59 (m, 1H, OH), 4.61 (d, $J_{8,7}$ = 5.0 Hz, 1H, H-8), 6.17 (dd, $J_{6,7}$ = 4.2, $J_{6,5}$ = 9.6 Hz, 1H, H-6), 6.57 (d, $J_{5,6}$ = 9.6 Hz, 1H, H-5), 7.34 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-3), 7.61 (d, $J_{4,3}$ = 8.0 Hz, 1H, H-4); NOE enhancement of H-4 (3%) and H-6 (3%) from irradiation of H-5. ^{13}C NMR (125 MHz, $(CD_3)_2CO$) δ: 68.2, 72.4, 124.6, 126.2, 128.3, 133.3, 137.7, 149.6, 158.4. MS *m*/*z* (%): 197 (M^+(^{35}Cl), 37), 179 ([M – H_2O]$^+$, 6), 168 (100). Anal. calcd. for $C_9H_8ClNO_2$: C 54.7, H 4.1, N 7.1; found: C 54.2, H 3.8, N 6.7.

*2-Chloro-3-hydroxyquinoline (**26**)*

White crystalline solid (0.5 g, 1%); mp 311–312°C (EtOAc–hexane) (lit. (37) mp > 210°C). R_f = 0.55 (EtOAc–hexane, 1:1). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 7.41 (ddd, $J_{7,6}$ = 8.3, $J_{7,8}$ = 8.3, $J_{7,5}$ = 1.4 Hz, 1H, H-7), 7.45 (ddd, $J_{6,5}$ = 8.3, $J_{6,7}$ = 8.3, $J_{6,8}$ = 0.6 Hz, 1H, H-6), 7.60 (s, 1H, H-4), 7.68 (dd, $J_{5,6}$ = 8.3, $J_{5,7}$ = 1.4 Hz, 1H, H-5), 7.73 (dd, $J_{8,7}$ = 8.3, $J_{8,6}$ = 0.6 Hz, 1H, H-8). The physical and spectral data

(IR, NMR, MS) of chlorohydroxy compound **26** and its methoxy derivative were similar to the data reported in the literature (37, 38).

*3-Hydroxy-2-quinolone (**27**)*

Colourless crystals (1.5 g, 3%); mp 261–263°C (EtOAc) (lit. (37) mp > 220°C). R_f = 0.2 (EtOAc–hexane, 1:1). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 7.02 (s, 1H, H-4), 7.06 (ddd, $J_{6,5}$ = 7.8, $J_{6,7}$ = 7.0, $J_{6,8}$ = 1.3 Hz, 1H, H-6), 7.23 (ddd, $J_{7,6}$ = 7.0, $J_{7,8}$ = 7.0, $J_{7,5}$ = 1.3 Hz, 1H, H-7), 7.42 (dd, $J_{5,6}$ = 7.8, $J_{5,7}$ = 1.3 Hz, 1H, H-5), 7.45 (dd, $J_{8,7}$ = 7.0, $J_{8,6}$ = 1.3 Hz, 1H, H-8), 8.07 (br s, 1H, NH). Anal. calcd. for $C_9H_7NO_2$: C 67.1, H 4.35, N 8.7; found: C 67.2, H 4.0, N 8.5. The physical and spectral data (IR, NMR, MS) of hydroxyquinolone **27** was similar to the data reported in the literature (37).

2-Chloroquinoline 2 with* E. coli *pKST11

Biotransformation of **2** (0.6 g) with *E. coli* pKST11, extraction (Procedure 1), and purification by PLC (7% MeOH–$CHCl_3$) yielded (+)-*cis*-(5*R*,6*S*)-2-chloro-5,6-dihydroquinoline-5,6-diol (**7**) (0.029 g, 4%) and (+)-*cis*-(7*S*,8*R*)-2-chloro-7,8-dihydroquinoline-7,8-diol (**8**) (0.080 g, 11%).

2-Chloroquinoline 2 with* P. putida *9816/11

Biotransformation of **2** (0.2 g) with *P. putida* 9816/11, extraction (Procedure 1), and separation by PLC (7% MeOH–$CHCl_3$), yielded (+)-*cis*-(5*R*,6*S*)-2-chloro-5,6-dihydroquinoline-5,6-diol (**7**) (0.014 g, 6%) and (+)-*cis*-(7*S*,8*R*)-2-chloro-7,8-dihydroquinoline-7,8-diol (**8**) (0.036 g, 15%).

2-Chloroquinoline 2 with* E. coli *JM109

Biotransformation of **2** (0.05 g) with *E. coli* JM109, extraction (Procedure 1), and PLC purification (7% MeOH–$CHCl_3$), yielded (+)-*cis*-(7*S*,8*R*)-2-chloro-7,8-dihydroquinoline-7,8-diol (**8**) (0.003 g, 5%).

2-Chloroquinoline 2 with* S. yanoikuyae *B8/36

Biotransformation of **2** (0.5 g) with *S. yanoikuyae* B8/36, extraction (Procedure 1), and PLC purification (7% MeOH–$CHCl_3$), gave (+)-*cis*-(7*S*,8*R*)-2-chloro-7,8-dihydroquinoline-7,8-diol (**8**) (0.091 g, 15%).

2-Chloroquinoline 2 with* P. fluorescens *Q1

Biotransformation of **2** (0.5 g) with *P. fluorescens* Q1, extraction (Procedure 1), and PLC purification (7% MeOH–$CHCl_3$) only yielded (+)-*cis*-(7*S*,8*R*)-2-chloro-7,8-dihydroquinoline-7,8-diol (**8**) (0.006 g, 1%).

2-Methoxyquinoline 3 with* P. putida *UV4

Biotransformation (10-L fermenter) of 2-methoxyquinoline (**3**) (7.0 g) with *P. putida* UV4 and extraction (Procedure 1) of bioproducts followed by separation of the crude mixture through a combination of column chromatography (0 → 10% MeOH–$CHCl_3$) and PLC (7% MeOH–$CHCl_3$) gave three compounds. The most abundent component (1.02 g, 13%) was found to be (+)-*cis*-(3*S*,*4S*)-3,4-dihydroxy-1,2,3,4-tetrahydro-2-quinolinone (**12**), which was indistinguishable from the sample obtained from substrate **3**. The other two components were identified as (+)-*cis*-(5*R*,6*S*)-2-methoxy-5,6-dihydroquinoline-5,6-diol (**9**) and (+)-*cis*-(7*R*,8*S*)-2-methoxy-7,8-dihydroquinoline-7,8-diol (**10**).

*(+)-*cis*-(5R,6S)-2-Methoxy-5,6-dihydroquinoline-5,6-diol (**9**)*

White crystalline solid (0.170 g, 2%); mp 182–184°C (MeOH–$CHCl_3$). R_f = 0.30 (7% MeOH–$CHCl_3$). $[\alpha]_D$ +80 (*c* 0.81, MeOH). CD λ (nm): 320.30 (Δε = –0.309), 274.70 (Δε = –0.810), 228.10 (Δε = +3.031), 199.40 (Δε = –4.009). IR ν_{max} (cm^{-1}): 3220 (OH). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 4.00 (m, 1H, OH), 4.18 (dd, $J_{6,5}$ = 4.9, $J_{6,7}$ = 4.6 Hz, 1H, H-6), 4.24 (m, 1H, OH), 4.55 (d, $J_{5,6}$ = 4.9 Hz, 1H, H-5), 6.27 (dd, $J_{7,6}$ = 4.6, $J_{7,8}$ = 9.9 Hz, 1H, H-7), 6.37 (d, $J_{8,7}$ = 9.9 Hz, 1H, H-8), 7.13 (d, $J_{3,4}$ = 8.1 Hz, 1H, H-3), 7.71 (d, $J_{4,3}$ = 8.1 Hz, 1H, H-4); NOE enhancement of H-4 (1%) from irradiation of H-5. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 53.9, 68.2, 70.1, 109.9, 125.4, 130.9, 133.5, 138.5, 149.3, 167.1 MS *m/z* (%): 193 (M^+, 57), 164 (100). Anal. calcd. for $C_{10}H_{11}NO_3$: C 62.2, H 5.7, N 7.3; found: C 62.0, H 5.4, N 6.9.

*(+)-*cis*-(7S,8R)-2-Methoxy-7,8-dihydroquinoline-7,8-diol (**10**)*

White crystalline solid (0.595 g, 7%); mp 80–82°C (MeOH–$CHCl_3$). R_f = 0.40 (7% MeOH–$CHCl_3$). $[\alpha]_D$ +200 (*c* 0.53, MeOH). IR ν_{max} (cm^{-1}): 3367 (OH). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ: 3.55 (d, *J* = 5.0 Hz, 1H, OH), 3.82 (s, 3H, OMe), 4.23 (m, 1H, OH), 4.24 (dd, $J_{7,6}$ = 4.7, $J_{7,8}$ = 5.0 Hz, 1H, H-7), 4.41 (d, $J_{8,7}$ = 5.0 Hz, 1H, H-8), 5.87 (dd, $J_{6,7}$ = 4.7, $J_{6,5}$ = 9.9 Hz, 1H, H-6), 6.37 (d, $J_{5,6}$ = 9.9 Hz, 1H, H-5), 6.52 (d, $J_{3,4}$ = 8.1 Hz, 1H, H-3), 7.47 (d, $J_{4,3}$ = 8.1 Hz, 1H, H-4); NOE enhancement of H-4 (1%) from irradiation of H-5. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 54.2, 64.8, 71.2, 109.7, 120.2, 125.0, 128.9, 137.5, 152.5, 163.9. MS *m/z* (%): 193 (M^+, 96), 164 (100). Anal. calcd. for $C_{10}H_{11}NO_3$: C 62.2, H 5.7, N 7.3; found: C 62.2, H 5.7, N 6.8.

2-Methoxyquinoline 3 with* P. putida *9816/11

Biotransformation of **3** (0.5 g) with *P. putida* 9816/11, extraction (Procedure 1), and PLC purification (7% MeOH–$CHCl_3$), yielded (0.328 g, 54%) (+)-*cis*-(7*S*,8*R*)-2-methoxy-7,8-dihydroquinoline-7,8-diol (**10**).

2-Methoxyquinoline 3 with* S. yanoikuyae *B8/36

Biotransformation of **3** (0.5 g) with *S. yanoikuyae* B8/36, extraction (Procedure 1), and PLC purification (7% MeOH–$CHCl_3$), yielded (0.285 g, 47%) (+)-*cis*-(7*S*,8*R*)-2-methoxy-7,8-dihydroquinoline-7,8-diol (**10**).

2-Methoxyquinoline 3 with* E. coli *JM109

Biotransformation of **3** (0.05 g) with *E. coli* JM109, extraction (Procedure 1), and PLC purification (7% MeOH–$CHCl_3$), yielded (0.003 g, 5%) (+)-*cis*-(7*S*,8*R*)-2-methoxy-7,8-dihydroquinoline-7,8-diol (**10**).

2-Quinolone (24) with* P. putida *UV4

Biotransformation of **24** (0.5 g) with *P. putida* UV4, extraction (Procedure 1), and PLC purification (7% MeOH–$CHCl_3$), yielded (0.062 g, 10%) (+)-*cis*-(3*S*,*4S*)-3,4-dihydroxy-1,2,3,4-tetrahydro-2-quinolinone (**12**).

3-Bromoquinoline (4) with* P. putida *UV4

Biotransformation of **4** (7.5 g) with *P. putida* UV4, extraction (Procedure 1), and purification by column chromatography ($CHCl_3$ and then 10% MeOH–$CHCl_3$) furnished a single compound, which was identified as (+)-*cis*-(5*R*,6*S*)-3-bromo-5,6-dihydroquinoline-5,6-diol (**11**).

(+)-cis-*(5*R*,6*S*)-3-Bromo-5,6-dihydroxy-5,6-dihydroquinoline (**11**)*

Pale yellow crystalline solid (1.7 g, 23%); mp 171°C (MeOH-CH_2Cl_2). R_f = 0.2 (5% MeOH–$CHCl_3$). $[\alpha]_D$ +220 (*c* 0.69, MeOH). CD λ (nm): 318.70 (Δε = –0.016), 260.10 (Δε = +1.439), 214.5 (Δε = +6.222), 197.90 (Δε = –3.931). IR ν_{max} (cm^{-1}): 3392 (OH). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.56 (br s, 2H, OH,), 4.35 (dd, $J_{6,5}$ = 4.8, $J_{6,7}$ = 5.1 Hz, 1H, H-7), 4.77 (d, $J_{5,6}$ = 4.8 Hz, 1H, H-5), 6.46 (dd, $J_{7,8}$ = 9.9, $J_{7,6}$ = 5.1 Hz, 1H, H-7), 6.73 (d, $J_{8,7}$ = 9.9 Hz, 1H, H-8), 8.04 (s, 1H, H-2), 8.48 (s, 1H, H-4); NOE enhancement of H-4 (2%) from irradiation of H-5. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 65.6, 69.6, 120.0, 131.0, 132.2, 133.9, 137.2, 149.1, 149.5. MS *m*/*z* (%): 241 (M^+(^{79}Br), 37), 212 ([M(^{79}Br) – C_2H_5]$^+$, 100). Anal. calcd. for $C_9H_8BrNO_2$: C 44.7, H 3.3, N 5.8; found: C 44.5, H 2.8, N 5.6.

X-ray crystal data for cis-*diol* **11**[3]

$C_9H_8BrNO_2$, M_w = 242.1, orthorhombic, *a* = 4.4973(6), *b* = 11.5223(14), *c* = 17.5491(18) Å, *V* = 909.4(2) Å^3, *T* = 293 K, Cu Kα radiation, λ = 1.54178 Å, space group $P2_12_12_1$, *Z* = 4, D_x = 1.768 g cm^{-3}, 0.50 × 0.50 × 0.25 mm, μ = 5.89 mm^{-1}, *F(000)* = 480, Bruker P3/V2000 diffractometer, ω scan, 9 < 2θ < 110°, measured/independent reflections: 1440/1440, direct methods solution, full matrix least squares refinement on F_0^2, anisotropic displacement parameters for non-hydrogen atoms, hydrogens located in difference Fourier but included at positions calculated from the geometry of the molecule using the riding model, R_1 = 0.037 for 1334 data with $F_0 > 4\sigma(F_0)$, 122 parameters, wR_2 = 0.095 (all data), GoF = 1.13, Flack absolute structure parameter *x* = –0.05(5), $\Delta\rho_{min,max}$ = –0.67/0.31 e Å^{-3}.

*X-ray crystal data for cis-diol **12***

Previously deposited as CCDC 182/770.

1-Methyl-2-pyridone (28) with a range of dioxygenases

Biotransformation of 1-methyl-2-pyridone (**28**) was first carried out using *E. coli* JM109(DE3) pDTG141 an ampicillin-resistant recombinant-strain encoding NDO from *P. putida* 9816–4 and using IPTG as inducer under conditions previously reported (16). This biotransformation was then carried out using the other strains listed in Table 2 employing essentially the same conditions as for substrate **2**. Biotransformation of pyridone **28** with site-directed mutant-strain *E. coli* JM109(DE3) (pDTG141–352V) was carried out as reported (36).

Biotransformations of **28** (0.10 g, dissolved in 1 L of buffer) using strains listed in Table 2, were followed by removal of water by freeze-drying or evaporation under reduced pressure at ~30°C. Extraction of the residue with acetone, yielded a mixture of *cis*-diols **29** and **30**, which were separated by a combination of column chromatography and TLC (MeOH–MeCOMe–$CHCl_3$, 1:1:8). The yields of purified *cis*-diols **29** (major) and **30** (minor) were in the range of 15–20% and 0.7–1.5%, respectively.

(+)-cis-*(5*S*,6*S*)-1-Methyl-5,6-dihydroxy-5,6-dihydro-2-pyridone (**29**)*

Colourless crystals; mp 86–88°C (from CH_2Cl_2). $[\alpha]_D$ +40 (*c* 0.42, MeOH) (lit. (16) $[\alpha]_D$ +41, MeOH). CD λ (nm): 252.0 (Δε = –1.500), 216.2 (Δε = +5.47). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.08 (s, 3H, Me), 4.61 (dd, $J_{5,6}$ = 4.4, $J_{5,4}$ = 2.2, $J_{5,3}$ = 2.2 Hz, 1H, H-5), 4.92 (dd, $J_{6,5}$ = 4.8, $J_{6,4}$ = 1.8 Hz, 1H, H-6), 5.70 (dd, $J_{3,4}$ = 10.0, $J_{3,5}$ = 2.2 Hz, 1H, H-3), 6.37 (dt, $J_{4,3}$ = 10.0, $J_{4,5}$ = 2.2, $J_{4,6}$ = 1.8 Hz, 1H, H-4). Anal. calcd. for C_6H_9NO: 125.04768 ([M – H_2O]$^+$); found: 125.04835.

*X-ray crystal data for cis-diol **29***[3]

$C_6H_9NO_3$, M_w = 143.1, orthorhombic, *a* = 4.627(1), *b* = 10.605(1), *c* = 14.041(2) Å, *V* = 689.0(1) Å^3, *T* = 293 K, Cu Kα radiation, λ = 1.54178 Å, space group $P2_12_12_1$, *Z* = 4, D_x = 1.380 g cm^{-3}, elongated hexagonal prism, 0.40 × 0.12 × 0.12 mm, μ = 0.95 mm^{-1}, Bruker P4 diffractometer, ω scan, 10 < 2θ < 110°, measured/independent reflections: 3381/863, analytical absorption correction, direct methods solution, full matrix least squares refinement on F_0^2, anisotropic displacement parameters for non-hydrogen atoms, hydrogens located in difference Fourier but included at positions calculated from the geometry of the molecule using the riding model, R_1 = 0.027 for 827 data with $F_0 > 4\sigma(F_0)$, 95 parameters, wR_2 = 0.076 (all data), GoF = 1.14, Flack absolute structure parameter *x* = 0.24(35), $\Delta\rho_{min,max}$ = –0.10/0.13 e Å^{-3}.

cis-*(3*S*,4*S*)-1-Methyl-3,4-dihydroxy-3,4-dihydro-2-pyridone (**30**)*

Semi-solid. CD λ (nm): 233.40 (Δε = +0.417). ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ: 2.90 (s, 3H, Me), 3.77 (br s, 1H, OH), 3.92 (br s, 1H, OH), 4.03 (m, 2H, H-3 and H-4), 5.20 (dd, $J_{5,6}$ = 7.8, $J_{5,4}$ = 5.7 Hz, 1H, H-5), 6.17 (d, $J_{6,5}$ = 7.8 Hz, 1H, H-6). Anal. calcd. for C_6H_9NO: 125.04768 ([M – H_2O]$^+$); found: 125.04748.

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[3] Supplementary material may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada. For information on obtaining material electronically go to http://www.nrc.ca/cisti/irm/unpub_e.shtml. Crystallographic information has also been deposited with the Cambridge Crystallographic Data Centre (CCDC Nos. 183535 and 183536). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

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