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Enzyme-Catalysed Synthesis and Absolute Configuration Assignments of *cis*-Dihydrodiol Metabolites from 1,4-Disubstituted Benzenes

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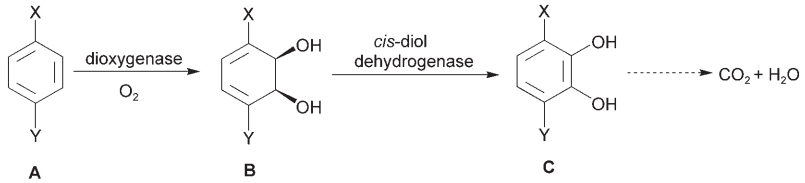
Abstract: A series of ten *cis*-dihydrodiol metabolites has been obtained by bacterial biotransformation of the corresponding 1,4-disubstituted benzene substrates using *Pseudomonas putida* UV4, a source of toluene dioxygenase (TDO). Their enantiomeric excess (*ee*) values have been established using chiral stationary phase HPLC and ^{1}H NMR spectroscopy. Absolute configurations of the majority of *cis*-dihydrodiols have been established using stereochemical correlation and X-ray crystallography and the remainder have been tentatively assigned using NMR spectroscopic methods but finally confirmed by circular dichroism (CD) spectroscopy. These configurational assignments support and extend the validity of an empirical model, previously used to predict the preferred stereochemistry of TDO-catalysed *cis*-dihydroxylation of ten 1,4-disubstituted benzene substrates, to more than twenty-five examples.

Keywords: absolute configuration · conformation analysis · diols · enantiopurity · enzymes

Introduction

The initial step in a major pathway for the bacterial biodegradation of aromatic rings **A** involves dioxygenase-catalysed *cis*-dihydroxylation to yield the corresponding *cis*-dihydrodiols **B**. The wild-type bacterial strains present in the environment contain *cis*-diol dehydrogenases, which can in turn convert the *cis*-dihydrodiols to catechols **C** prior to ring opening and further biodegradation to yield carbon dioxide and water, that is, mineralisation (Scheme 1).



Scheme 1. Bacterial mineralisation of 1,2-disubstituted benzenes.

Toluene dioxygenase is the most widely used dioxygenase enzyme for bacterial biotransformations and has been found to catalyse the *cis*-dihydroxylation of benzene[1] and a large number of mono- and disubstituted benzene substrates in a regio- and stereoselective manner.[2–12] The TDO enzyme present in whole cells of the UV4 constitutive mutant strain of *Pseudomonas putida*, favours the *cis*-dihydroxylation of unsubstituted arene bonds and since the corresponding dehydrogenase enzyme (toluene *cis*-diol dehydrogenase) is blocked, accumulation of the initial *cis*-dihydrodiol metabolites occurs.

Based on preliminary biotransformation results obtained using *P. putida* UV 4 and range of substituted benzene substrates, it was possible to predict the preferred regio-and stereochemistry of the *cis*-dihydrodiol bioproducts using a simple model controlled by the relative steric requirements of substituents X and Y in substrate **A**.[13] Initially the model was developed for the TDO-catalysed *cis*-dihydroxylation of a relatively small number of mono- and 1,4-disubstituted benzene substrates bearing spherically symmetrical substituents, for example, F, Cl, Br, I, Me, and CF_3.[13,14] According to this predictive model, the *cis*-dihydroxylation step will

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occur on the less substituted bond in the benzene ring **A** and the direction of facial attack will favour the absolute configuration shown by enantiomer **B** when the steric requirements of substituent X, are significantly larger than those of Y, for example, using the Charton steric parameter (υ).[15] This model has recently been updated to also allow predictions for substrates bearing substituents which are non-spherically symmetrical and whose size will be conformationally dependent but which have a dominant stereodirecting effect, for example, SMe,[16] CH=CH$_2$,[17] Et,[18] Pr.[18]

With increasing use now being made of disubstituted benzene *cis*-dihydrodiols as chiral precursors in synthesis,[19–27] the development of more convenient and rigorous methods for stereochemical assignment is very important. The simplest literature method used for the direct determination of enantiopurity of benzene *cis*-dihydrodiols is chiral stationary phase HPLC (CSPHPLC) at ambient temperature.[13,16] Chiral stationary phase GC (CSPGC) has also been used but this required the formation of *n*-butyl boronate derivatives and elevated temperatures.[28,29] Comparison of optical rotations, and in some cases X-ray crystallographic analysis,[30,31] of *cis*-dihydrodiols and derivatives, has also been used to confirm their enantiopurity. Other methods that have been developed to confirm *ee* values include NMR analysis after stabilization of the *cis*-dihydrodiol metabolites of monosubstituted benzene substrates by formation of a single diastereoisomeric cycloadduct or regioselective hydrogenation of the unsubstituted alkene bond. This is followed by formation of diastereoisomeric di-MTPA esters using both *R* and *S* forms of the α-methoxy-α-(trifluoromethyl) phenylacetic acid (MTPA).[31,32] The synthesis of diastereoisomeric boronate dervatives using (*R*)- and (*S*)-2-(1-methoxyethyl)phenyl boronic acids followed by ^{1}H NMR analysis has also been used.[15,33]

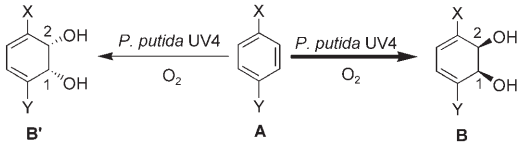
Several of the above methods used for *ee* determination have also been employed for the assignment of absolute configurations of benzene *cis*-dihydrodiols. These include stereochemical correlation,[15] X-ray crystallography,[30,31] and ^{1}H NMR analysis of di-MTPA esters[31,32] or chiral boronates.[16,33] Unfortunately the chromatographic methods (CSPHPLC and CSPGC) did not give information on the absolute configuration and the NMR methods (di-MTPA esters and chiral boronates), and early CD studies[34] provided only tentative assignments.

The present study is focussed on selected *cis*-dihydrodiol metabolites **1a–j**, obtained by biotransformation (*P. putida* UV4) of the corresponding 1,4-disubstituted benzene substrates, containing combinations of the substituents, that is, F, Br, CF$_3$, CN, Me. Several of the previously reported methods (CSPHPLC, di-MTPA ester and chiral boronate formation, stereochemical correlation and X-ray crystallography), allied to experimental and calculated CD spectrocopy,[35] have been used to determine their *ee* values and absolute configurations. This reliable stereochemical information is, in turn, used to verify i) the validity of the preliminary stereochemical model used for prediction of absolute stereochemisty of *cis*-dihydrodiol metabolites formed at the active site of the TDO enzyme,[13] and in particular the stereodirecting effect of the non-spherically symmetrical but rigid nitrile group and ii) the general applicability of confrontation of experimental and calculated CD spectra as a method for determining both the preferred conformations and absolute configurations.[35]

Results and Discussion

Biotransformation of the 1,4-disubstituted benzene substrates **A** using *P. putida* UV4 yielded the corresponding *cis*-dihydrodiols **B** (**1a–j**) and in some cases a minor proportion of the corresponding enantiomers **B′**. The yields were variable for different substrates (5–95% isolated yield) but were generally lower than those obtained from the corresponding monosubstituted benzene substrates (Table 1). The *ee* values (20–98%) were determined using a combination of methods (methods i–v, Schemes 2–6) and are given in Table 1. To maintain uniformity, the numbering system used in Table 1 has also been adopted in the Experimental Section.

Table 1. Isolated yields, optical rotations ($[\alpha]_D$), *ee* values and absolute configurations (ACs) of the major *cis*-dihydrodiol enantiomers **1a–j** (**B**).



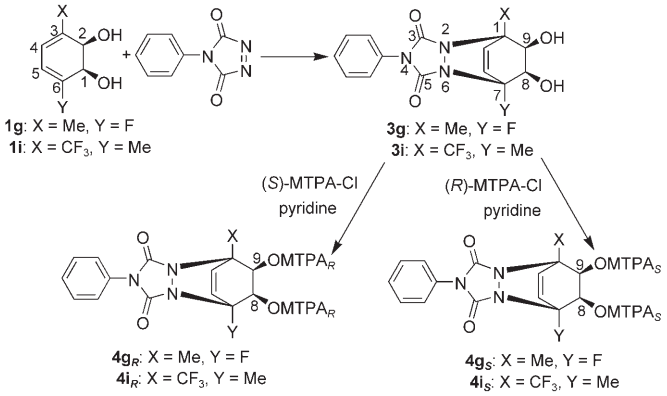
cis-Diol	X	Y	Yield [%]	$[\alpha]_D$[a]	Enantiopurity [% *ee*]	AC
1a	Br	F	95	+52	76[b–d]	**B** (1*R*,2*S*)
1b	CF$_3$	Br	81	−67	>98[b,d,e]	**B** (1*R*,2*R*)
1c	CN	Br	5	−12	>98[b,d,e]	**B** (1*R*,2*R*)
1d	Br	Me	30	−5	20[b–e]	**B** (1*S*,2*S*)
1e	CF$_3$	F	75	−41	>98[b,d,f]	**B** (1*R*,2*R*)
1f	CN	F	50	+68	>98[b,d,e]	**B** (1*R*,2*R*)
1g	Me	F	40	+143	93[b–d]	**B** (1*R*,2*R*)
1h	CF$_3$	CN	80	−32	>98[b,d,e]	**B** (1*S*,2*R*)
1i	CF$_3$	Me	75	−118	>98[b–d]	**B** (1*S*,2*R*)
1j	CN	Me	74	+92	>98[b,d,e]	**B** (1*S*,2*R*)

[a] MeOH. [b] Method i. [c] Method iv. [d] Method v. [e] Method ii. [f] Method iii.

Enantiopurity determination methods for *cis*-dihydrodiols 1a–j: The enantiopurity values for the *cis*-dihydrodiols were measured using CSPHPLC with Chiralcel OJ (**1a**, **c**, **d**, **f** and **g**) or Chiralcel AD (**1j**) columns (method i). Only metabolites **1a**, **d** and **g** appeared to contain both enantiomers after biotransfomation of the parent 1,4-disubstituted arene substrates but three other *cis*-diols (**1c**, **f** and **h**) were obtained as separable enantiomeric mixtures after chemoenzymatic synthesis (Scheme 4). As the other *cis*-diols (**1b**, **e**, **i** and **j**) showed only single peaks on CSPHPLC analysis (OJ column), this could have resulted from poor peak resolution of enantiomers rather than an *ee* value of >98%. Additional methods for *ee* determination were therefore adopted.

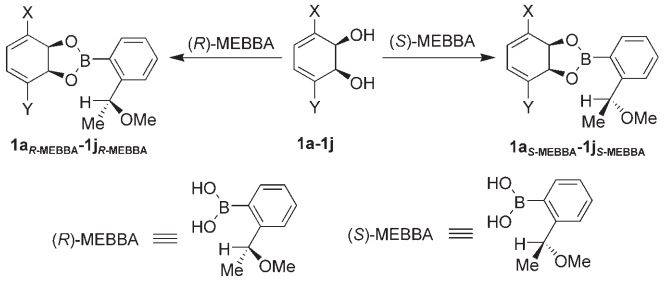
Alternative approaches involved stereochemical correlation (method ii, Schemes 2–4) and comparison of enantiomer ratios from the resulting derivatives by CSPHPLC (method i), optical rotation measurements or X-ray crystallography (method iii) These methods were used to confirm that the *cis*-diols **1b**, **c**, **e**, **f**, **h**, **i** and **j** (Table 1) were enantiopure (>98% *ee*).

^{1}H and ^{19}F NMR analysis of the diastereoisomeric (*R*)- and (*S*)-di-MTPA esters of the corresponding cycloadducts, for example, $\mathbf{4g}_R/\mathbf{4g}_S$ and $\mathbf{4i}_R/\mathbf{4i}_S$ (formed using *cis*-dihydrodiols as dienes and 4-phenyl-1,2,4-triazoline-3,5-dione as dienophile) provided *ee* values (**1a**, **d**, **g**, and **i**, Table 1, method iv, Scheme 2). Unfortunately this method was not generally applicable since formation of the di-MTPA esters of some *cis*-dihydrodiols from 1,4-disubstituted benzenes failed due to reactant unreactivity or product instability.



Scheme 2.

Formation of the corresponding diastereoisomeric boronate esters ($\mathbf{1a}_{(R)\text{-MEBBA}}$ and $\mathbf{1a}_{(S)\text{-MEBBA}}$) using (*R*)- and (*S*)-2-(1-methoxyethyl) benzeneboronic acid (MEBBA), followed by ^{1}H NMR analysis of the diastereoisomeric composition (method v, Scheme 3), proved to be successful for all the *cis*-dihydrodiols **1a**–**j**.[32] The *ee* values shown in Table 1 are an average of those obtained using methods i–v.



Scheme 3.

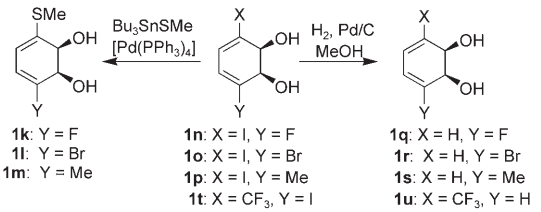
Absolute configuration determination methods for *cis*-dihydrodiols 1a–j: The absolute configurations of *cis*-dihydrodiols **1a**–**j** were determined using several of the methods discussed earlier for the measurement of *ee* values including stereochemical correlation with compounds of established absolute configurations, X-ray crystallography using the anomalous dispersion method and ^{1}H and ^{19}F NMR analyses of diastereoisomeric di-MTPA ester derivatives of cycloadducts.

Stereochemical correlation: The correlation method, used for the determination of absolute configurations in the study, was based on that reported earlier[16] for the synthesis of a series of *cis*-dihydrodiol metabolites of 4-substituted methylphenyl sulfides (**1k**–**m**) where Stille coupling, with organostannanes as the organometallic component, was employed. This earlier stereochemical correlation sequence involved substitution of an iodine atom by an SMe group in the *cis*-dihydrodiols of 4-substituted iodobenzenes (**1n**–**p**) using $[Pd(PPh_3)_4]$-catalysed organotin chemistry to give sulfides (**1k**–**m**) and hydrogenolysis of compounds **1n**–**p** to yield monosubstituted benzene *cis*-dihydrodiols (**1q**–**s**) of known absolute configurations (Scheme 3).

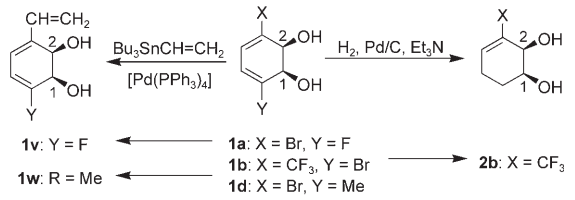
The absolute configurations of seven *cis*-dihydrodiol metabolites (**1a**–**d**, **f**, **h** and **j**) have been determined by rigorous stereochemical correlation methods, during the current programme, as shown in Schemes 4–6. The procedure involved direct substitution of a halogen atom by a vinyl group (**1a**→**1v**, and **1d**→**1w**, Scheme 5) or a nitrile group (**1n**→**1f**, **1o**→**1c**, **1p**→**1j**, **1t**→**1h**, Scheme 6) using Bu_3SnSMe/Bu_3SnCN and $[Pd(PPh_3)_4]$ as catalyst.

Removal of iodine or bromine atoms by catalytic hydrogenolysis (**1n**→**1q**, **1o**→**1r**, **1p**→**1s**, **1t**→**1u**, Scheme 4) or hydrogenolysis/hydrogenation (**1b**→**2b**, Scheme 5) was then used to correlate their absolute configurations to *cis*-diols of known configurations (**1q**, **r**, **s**, **u**, and **2b**). Concomitant catalytic hydrogenolysis and hydrogenation of the substituted bromobenzene *cis*-dihydrodiol **1b** was achieved using a Pd/C catalyst (Scheme 5). The residual alkene bond in compound **2b** proved to be very resistant to further hydrogenation and was of identical 1*S*,2*R* absolute configuration to that obtained from the partial hydrogenation of the *cis*-dihydrodiol metabolite of 1,1,1-trifluoromethylbenzene (**1u**) based on a comparison of optical rotations and CD spectra.

Treatment of the *cis*-dihydrodiol metabolites **1a** (and **1n**) or **1d** (and **1p**) with $Bu_3SnCH{=}CH_2$ and $[Pd(PPh_3)_4]$ catalyst yielded the vinyl *cis*-dihydrodiols **1v** or **1w**, respectively (Scheme 5). *cis*-Diols **1n** and **1p** were stereochemically correlated with the corresponding (1*R*,2*R*)-**1q** and (1*R*,2*S*)-**1s** *cis*-diol enantiomers (Scheme 4). The absolute configura-



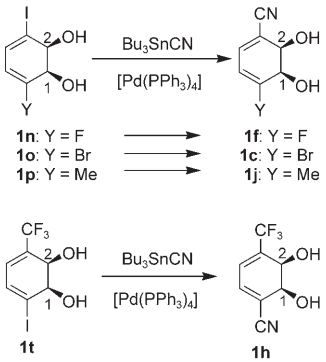
Scheme 4.



Scheme 5.

tions of the metabolites **1a** and **1d** were thus unequivocally established as (1*R*,2*S*)and (1*S*,2*S*), respectively (Table 1).

The 1,4-disubstituted iodobenzene *cis*-dihydrodiol metabolites **1n–p** were all available from an earlier study[16] while compound **1t** was obtained during the current study by a similar TDO-catalysed *cis*-dihydroxylation of the 4-trifluoromethyl substituted iodobenzene substrate under similar conditions. Replacement of the iodine atom in *cis*-dihydrodiols **1n–p** and **t** by a cyano group using Bu_3SnCN in the presence of $[Pd(PPh_3)_4]$ yielded compounds **1f**, **c**, **j** and **h**, respectively (Scheme 6). Hydrogenolysis of *cis*-diols **1n–p** and (H_2, Pd/C) yielded the *ent*-forms of the corresponding *cis*-diol metabolites of fluorobenzene (1*R*,2*R*)-**1q**, bromobenzene (1*R*,2*R*)-**1r** and toluene (1*R*,2*S*)-**1s** (Scheme 4). Conversely the *cis*-dihydrodiol **1u** derived from metabolism of 1,1,1-trifluoromethyl benzene and also from hydrogenolysis of *cis*-diol **1t** both had the natural (1*S*,2*R*) absolute configuration.



Scheme 6.

X-ray crystallography: The *cis*-dihydrodiol **1e** could not be readily assigned an absolute configuration using the unequivocal stereochemical correlation methods shown in Schemes 5 and 6 since neither the hydrogenolysis, hydrogenation nor Stille coupling procedures were applicable and thus X-ray crystallography was used (Figure 1). The hydroxyl group at C1 was pseudoequatorial while that on C2 was pseudoaxial in the solid state. This would also be expected to be the preferred conformation in the solution phase as the steric requirements of the CF_3 group (v 0.90) are much greater than those of a F atom (v 0.27). Thus the OH group proximate to the bulky CF_3 group would prefer to adopt the pseudoaxial conformation. The absolute configuration was

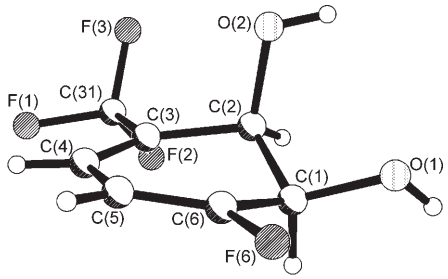


Figure 1. X-ray structure of one of the two independent molecules of (1*R*,2*R*)-**1e**.

tentatively assigned as (1*R*,2*R*) by the Bijvoet method, at about the 90% confidence level. In common with other *cis*-dihydrodiols derived from monosubstituted benzene substrates.[25] compound **1e** was also found to adopt an *M* helical diene conformation.

An X-ray crystal structure analysis of *cis*-dihydrodiol **1j** allowed the absolute configuration to be unequivocally assigned as (1*S*,2*R*), from the anomalous dispersion of the oxygen atoms. This configuration assignment had earlier been determined by the stereochemical correlation method (Scheme 4) and is consistent with the CN group having the dominant stereodirecting effect at the active site of the enzyme. However, the conformation appears to be affected predominantly by the Me group, possibly due to its greater steric bulk (v 0.52). The OH group proximate to the Me was found to be pseudoaxial while the other OH group, closer to the smaller CN group (v 0.40), was pseudoequatorial. In contrast to the earlier X-ray crystal studies of monosubstituted benzene *cis*-dihydrodiols, compound **1e** was found to have a *P* helical conformation (Figure 2).

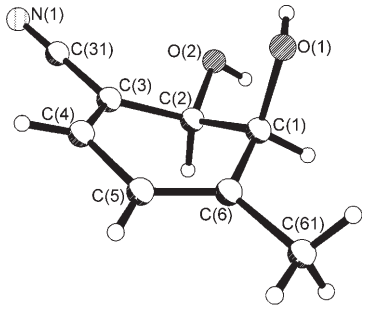


Figure 2. X-ray structure of (1*S*,2*R*)-**1j**.

A crystal structure analysis of *cis*-dihydrodiol **1f** gave the relative configuration and preferred conformations of the OH groups (Figure 3). The absolute configuration was not determined crystallographically but had been unambiguously assigned as (1*R*,2*R*) by stereochemical correlation (Scheme 6). This configuration is consistent with the CN group (v 0.40) having the dominant stereodirecting effect at the active site, as compared with an F atom (v 0.27), but the conformation does not appear to be determined by steric ef-

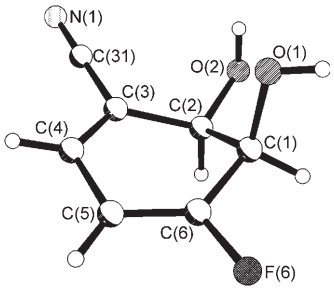


Figure 3. X-ray structure of (1*R*,2*R*)-**1f**.

fects. The OH group proximate to the F was found to be pseudoaxial while the other OH group, closer to the CN group, was pseudoequatorial. Diene **1f** was similar to diene **1j** in again having *P* helicity in the solid state.

***Spectroscopic methods**:* The absolute configurations of most of the *cis*-dihydrodiols in the series **1a–j** were determined by stereochemical correlation or X-ray crystallographic methods with the exception of two compounds (**1g** and **i**). A spectroscopic method used successfully for the determination of enantiopurity values of the *cis*-dihydrodiol metabolites of monosubstituted benzenes, for example, **1q–s** and **u**, involved the initial formation of cycloadducts with 4-phenyl-1,2,4-triazoline-3,5-dione exclusively *syn* to the diol moiety. This was followed by the reaction with the (*S*)- and (*R*)-enantiomers of MTPA chloride in pyridine to yield the corresponding di-MTPA diastereoisomers which were readily distinguishable by ^{1}H and ^{19}F NMR spectrosopy.[31,32]

This approach also provided an empirical assignment of absolute configurations to a range of monosubstituted benzene *cis*-dihydrodiols, based on a consistent trend in ^{1}H NMR signals for MeO groups.[31,32] Thus, for *cis*-dihydrodiols **B** (X = F, Cl, Br, I, Me, CF_3, CN, Y = H) having a (1*S*) configuration, the more downfield ^{1}H NMR signal for the two MeO groups had a larger δ_H value using (*S*)-MTPA chloride (derived from (*R*)-MTPA). The validity of this method for absolute configuration assignment was confirmed by X-ray crystallography of the di-MTPA esters and other methods. The di-MTPA ester method was not as rigorous as methods used earlier. However, in our experience, it was found to be applicable for all *cis*-dihydrodiols **B**, formed from the corresponding monosubstituted benzene rings **A** (Y = H), we had tested.

In the current study, it was anticipated that a similar trend in the ^{1}H NMR spectra would be found for *cis*-dihydrodiols derived from 1,4-disubstituted benzene substrates providing that there was again a significant differential between the group sizes of X and Y (Scheme 5). It was assumed that for *cis*-dihydrodiols **1g** (X = Me, Y = F) and **1i** (X = CF_3, Y = Me) a sufficient difference in the sizes of substituents **X** and **Y** (CF_3 > Me > F) existed, for a similar empirical approach to be valid. Thus, *cis*-dihydrodiols **1g** and **1i** gave the corresponding cycloaddducts (**3g** and **3i**) and di-MTPA esters (**4g**$_R$/**4g**$_S$ and **4i**$_R$/**4i**$_S$). The relevant chemical shifts for the MeO signals (δ_{OMe}) from the di-MTPA esters were then used to make the tentative assignments (1*R*,2*R*)-**1g** and (1*S*,2*R*)-**1i** respectively (Table 2).

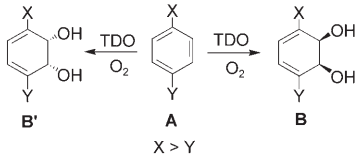
Table 2. Characteristic ^{1}H NMR δ values for the cycloadduct di-MTPA diastereoisomers **4g**$_R$, **4g**$_S$, **4i**$_R$ and **4i**$_S$.

cis-Diol	Cycloadduct	di-MTPA	δ_{OMe}	δ_{OMe}	*cis*-Diol Config.
1g	**3g**[a]	**4g**$_R$	3.07	3.77	**B** (1*R*,2*R*)
		4g$_S$	3.19	3.47	
1i	**3i**	**4i**$_R$	3.41	3.55	**B** (1*S*,2*R*)
		4i$_S$	3.34	3.52	

[a] NMR data from the major enantiomer of *cis*-dihydrodiol **1g**.

In this and earlier studies,[16] *ee* measurements and unequivocal absolute configuration assignments have been made for the 1,4-disubstituted benzene *cis*-dihydrodiols **1a–j** (Table 1 and Scheme 2) and also for a further eighteen examples, based on independent experimental and calculated CD spectroscopy.[35] From this list of 28 *cis*-dihydrodiol derivatives only five (including compounds **1d** and **1g**) have been reported from other laboratories[28,34] but their absolute configurations had not been rigorously established. The stereochemical assignment methods adopted herein and in the other paper,[35] now allow us to confirm and extend the validity of the predictive model for the preferred absolute configuration of bioproducts formed during TDO-catalysed *cis*-dihydroxylation.[7,13,14]

The model shown in Scheme 7 assumes a stereopreference for configuration **B** over the enantiomeric configuration **B′**, based on the relative differences in size between large (X) and small (Y) spherically symmetrical atoms or substituents, using the Charton steric parameter as an indicator or substituent size.[13,14] This has been demonstrated for *cis*-dihydrodiols **1a**, **b**, **d**, **e**, **g** and **i**. Thus, the dominant stereodirecting effect of the larger (X) atom or group was found to follow the sequence CF_3 (υ 0.90) > I (υ 0.78) > Br (υ 0.65) > Cl > (υ 0.53) > Me (υ 0.52) > F (υ 0.27) > H (υ 0.00) and decreased as the size difference between atoms or groups X and Y became smaller.



Scheme 7.

To accommodate substituents of conformationally dependent size, the predictive model has recently been refined. Thus, SMe (υ 0.60)[16] CH=CH_2 (υ 0.60),[16] Et (υ 0.56)[18] and *n*Pr (υ 0.68)[18] groups having smaller υ values were found to have a stronger stereodirecting effect than the substituents having larger υ values (e.g. CF_3, I) studied earlier. The results shown in Table 1 indicate that the conformationally independent size of the CN group, present in the 1,4-disubsti-

tuted benzene substrates **1c**, **f**, **h** and **j**, exerts a stronger stereodirecting effect than the Br and Me substituents despite having a relatively small v value (0.40). CD spectroscopic analysis of the *cis*-dihydrodiol metabolite, derived from 4-chlorobenzonitrile, has also confirmed that the CN group is dominant over the Cl atom during TDO-catalysed *cis*-dihydroxylation.[35] On the basis of the current study, the dominant stereodirecting effect of the X atom or group was found to follow the sequence $CF_3 > CN > Br > Cl \approx Me > F > H$. This is again consistent with the view that non-spherically symmetrical substituents such as CN, SMe, CH=CH_2, Et and Pr have a dominant stereodirecting effect at the active site during the TDO-catalysed *cis*-dihydroxylation of arenes. It also implies that in addition to substituent size (v), the length of the relatively rigid CN group (or of a planar conformation the SMe, CH=CH_2, Et and Pr groups) could also be an important factor during binding and catalysis at the active site of the TDO enzyme.

Conclusion

A series of *cis*-dihydrodiol metabolites **1a–j** has been obtained, using the corresponding 1,4-disubstituted benzenes as substrates and TDO from *P. putida* UV4 as biocatalyst. The enantiopurity values and absolute configurations have been determined employing a range of chemical methods involving halogen substitution, hydrogenolysis, hydrogenation, cycloaddition and esterification reactions. X-ray crystallography, chiral stationary phase HPLC and NMR spectroscopic methods have also been used for stereochemical analysis. Taken in conjunction with the accompanying comprehensive calculated and experimental CD studies on compounds **1a–j** (and 18 other *cis*-dihydrodiol metabolites from 1,4-disubstituted benzene substrates), the absolute configurations can now be assigned in an unequivocal manner.[35] This study has allowed the validity of a stereochemical model, and the dominant stereodirecting effect of the relatively small but inflexible CN group, during TDO-catalysed *cis*-dihydroxylation of substituted benzene substrates, to be further established.

Experimental Section

General information: ^{1}H NMR spectra were recorded at 300 MHz (Bruker Avance DPX-500) and at 500 MHz (Bruker Avance DRX-500) in $CDCl_3$ solvent unless stated otherwise. Chemical shifts (δ) are reported in ppm relative to $SiMe_4$ and coupling constants (J) are given in Hz. Mass spectra were recorded 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 standard. Elemental microanalyses were obtained on a Perkin–Elmer 2400 CHN microanalyser. CSPHPLC was carried out using a Shimadzu LC-6 A liquid chromatograph connected to Hewlett Packard diode array detector. The 1,4-disubstituted benzene substrates and reagents used in the formation of *cis*-dihydrodiol derivatives were obtained commercially.

The enantiomeric excess values of the *cis*-dihydrodiols (**1a–j**) were determined by CSPHPLC using a Daicel OJ or AD column (method i).[13,14] An alternative method (method iv) involved formation of cycloadducts by reaction with Cooksen's reagent (4-phenyl-1,2,4-triazoline-3,5-dione) followed by reaction with the acid chloride derivatives of (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid (MTPA) to yield the corresponding bis-MTPA esters which were analysed by ^{1}H NMR.[31,32] The formation of the corresponding diastereoisomeric boronate esters using (*R*)- and (*S*)-2-(1-methoxyethyl)benzeneboronic acid followed by ^{1}H NMR analysis of the diastereoisomeric composition provided a further approach (method v).[16,32]

The absolute configurations were determined by empirical methods including formation of chiral boronate derivatives, bis-MTPA ester derivatives of Cooksen adducts[31,33] and ^{1}H NMR analysis. Unequivocal methods used include X-ray crystallography and stereochemical correlation.

Shake flask (0.5 g) biotransformations were carried out using *P. putida* UV4 under reported conditions.[31] The *cis*-dihydrodiols (**1a–j**) obtained after bioconversion of the corresponding arene substrates, were separated and purified by PLC (silica gel, 50% EtOAc in hexane).

Physical properties and spectroscopic data for metabolites 1a–j and t

(1*R*,2*S*)-1a (X = Br, Y = F): 0.567 g, 95%; m.p. 90–93 °C (CH_2Cl_2/hexane); $[\alpha]_D = +52$ (c = 0.92, MeOH); 76% *ee*; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.75 (br s, 1H, OH), 2.66 (br s, 1H, OH), 4.49 (m, 2H, 1-H, 2-H), 5.52 (dd, 1H, $J_{5,4}$ = 6.6, $J_{5,F}$ = 10.4 Hz, 5-H), 6.26 ppm (dd, 1H, $J_{4,5}$ = 6.6, $J_{4,F}$ = 5.2 Hz, 4-H); EI MS: m/z (%): 210 (33) $[M]^+$, 190 (2), 83 (100); elemental analysis calcd (%) for $C_6H_6O_2BrF$: C 34.5, H 2.9; found: C 34.7, H 2.8.

(1*R*,2*R*)-1b (X = CF_3, Y = Br): 0.466 g, 81%; m.p. 115–116 °C ($CHCl_3$/hexane); $[\alpha]_D = -67$ (c = 0.82, MeOH); >98% *ee*; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.54 (m, 2H, 1-H, 2-H), 6.42 (m, 1H, 4-H), 6.48 ppm (d, 1H, $J_{4,5}$ = 6.3 Hz, 5-H); EI MS: m/z (%): 260 (39) $[M]^+$, 258 (40), 242 (5), 240(4), 159 (100); elemental analysis calcd (%) for $C_7H_6O_2F_3Br$: C 32.5, H 2.3; found: C 32.4, H 2.4.

(1*R*,2*R*)-1c (X = CN, Y = Br): 0.03 g, 5%; m.p. 137–138 °C ($CHCl_3$/hexane); $[\alpha]_D = -12$ (c = 1.4, MeOH); >98% *ee*; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.42 (d, 1H, $J_{1,2}$ = 6.2 Hz, 1-H), 4.55 (dd, 1H, $J_{2,1}$ = 6.2, $J_{2,4}$ = 1.3 Hz, 2-H), 6.52 (d, 1H, $J_{5,4}$ = 6.3 Hz, 5-H), 6.56 ppm (dd, 1H, $J_{4,5}$ = 6.3, $J_{4,2}$ = 1.3 Hz, 4-H); EI M: m/z (%): 217 (29) $[M]^+$, 215 (31), 199(6), 197 (5), 136 (100), 118 (23); HR MS (EI): calcd for $C_7H_6O_2N^{79}Br$: 214.9582; found: 214.9577 $[M]^+$.

(1*S*,2*S*)-1d (X = Br, Y = Me): 0.180 g, 30%; m.p. 97–98 °C ($CHCl_3$); $[\alpha]_D = -5$ (c = 0.53, MeOH); 20% *ee* (lit.:[13] −9 and 37% *ee*; lit.:[34] 0% *ee*); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.91 (s, 3H, Me), 4.30 (m, 2H, 1-H, 2-H), 5.61 (m, 1H, 5-H), 6.29 ppm (d, 1H, $J_{5,4}$ = 6.0 Hz, 5-H); EI MS: m/z (%): 206 (31) $[M]^+$, 204 (29), 79 (100); HR MS (EI): calcd for $C_7H_9O_2{}^{79}Br$: 203.9786; found: 203.9791 $[M]^+$.

(1*R*,2*R*)-1e (X = CF_3, Y = F): 0.453 g, 75%; m.p. 96–98 °C ($CHCl_3$/hexane); $[\alpha]_D = -41$ (c = 0.86, MeOH); >98% *ee*; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.51 (m, 1H, $J_{1,2}$ = 6.1 Hz, 1-H), 4.57 (d, 1H, $J_{2,1}$ = 6.2 Hz, 2-H), 5.64 (dd, 1H, $J_{5,4}$ = 6.6, $J_{5,F}$ = 9.9 Hz, 5-H), 6.54 ppm (m, 1H, 4-H); EI MS: m/z (%): 198 (90) $[M]^+$, 180 (16), 152 (79), 101 (100); elemental analysis calcd (%) for $C_7H_6O_2F_4$: C 42.4, H 3.1; found: C 42.1, H 3.0.

Crystal data for 1e: $C_7H_6F_4O_2$, M = 198.1, orthorhombic, a = 10.195(5), b = 19.487(8), c = 8.391(4) Å, V = 1667.0(13) Å^3, T = 293(2) K, $Cu_{K\alpha}$ radiation, λ = 1.54178 Å, space group $P2_12_12$ (no. 18), Z = 8, $F(000)$ = 800, ρ_{calcd} = 1.579 g cm^{-3}, μ = 1.552 mm^{-1}, Siemens P3 diffractometer, ω scans, $10.6 < 2\theta < 110°$, measured/independent reflections: 7892/1993, 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 = 0.064 for 1839 data with $F_o > 4\sigma(F_o)$, 243 parameters, wR_2 = 0.148 (all data), GoF = 1.16, Flack parameter x = 0.2(4), $\Delta\rho$kl;$_{min,max}$ = −0.21/0.21 e Å^{-3}. CCDC-631365 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(1*R*,2*R*)-1f (X = CN, Y = F): 0.320 g, 50%; m.p. 106–107 °C (EtOAc/hexane); $[\alpha]_D = +68$ ($c = 0.96$, MeOH); >98% *ee*; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.38$ (dd, 1H, $J_{1,2} = 6.5$ Hz, 2-H), 4.63 (m, 1H, 1-H), 5.77 (dd, 1H, $J_{5,4} = 6.5$, $J_{5,F} = 9.6$ Hz, 5-H), 6.69 ppm (m, 1H, 4-H); EI MS : *m*/*z* (%): 155 (60) $[M]^+$, 138 (21), 126 (100); HR MS (EI): calcd for $C_7H_6O_2NF$: 155.0383; found: 155.0386.

Crystal data for 1f: $C_7H_6FNO_2$, $M = 155.1$, monoclinic, $a = 7.450(2)$, $b = 6.244(1)$, $c = 7.712(2)$ Å, $\beta = 108.06(2)°$, $V = 341.1(1)$ Å^3, $T = 298(2)$ K, $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, space group $P2_1$ (no. 4), $Z = 2$, $F(000) = 160$, $\rho_{calcd} = 1.510$ g cm^{-3}, $\mu = 0.13$ mm^{-1}, Marresearch image plate diffractometer, ω scans, $5.6 < 2\theta < 40.8°$, measured/independent reflections: 1335/649, direct methods solution, full-matrix least squares refinement on F_o^2, anisotropic displacement parameters for non-hydrogen atoms; hydrogen atoms included at positions calculated from the geometry of the molecules using the riding model, with isotropic vibration parameters. $R_1 = 0.069$ for 637 data with $F_o > 4\sigma(F_o)$, 103 parameters, $wR_2 = 0.131$ (all data), GoF = 1.48, $\Delta\rho_{min,max} = -0.55/0.58$ e Å^{-3}. CCDC-631366 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(1*R*,2*S*)-1g (X = Me, Y = F): 0.262 g, 40%; m.p. 120–121 °C ($CHCl_3$); $[\alpha]_D = +143$ ($c = 1.01$, MeOH); 93% *ee* (lit.[13] 90 and 49% *ee*); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.92$ (s, 3H, Me), 4.32 (m, 2H, 1-H, 2-H), 5.54 ppm (m, 2H, 4-H, 5-H); EI MS: *m*/*z* (%): 144 (86) $[M]^+$, 126 (72), 97 (100); HR MS (EI): calcd for $C_7H_9O_2F$: 144.0587; found: 144.0587 $[M]^+$.

(1*R*,2*R*)-1h (X = CF$_3$, Y = CN): 0.480 g, 80%; m.p. 106–108 °C ($CHCl_3$/hexane); $[\alpha]_D = -32$ ($c = 0.96$, MeOH); >98% *ee*; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.44$ (d, 1H, $J_{1,2} = 6.0$ Hz, 1-H), 4.58 (m, 1H, 2-H), 6.66 (d, 1H, $J_{4,5} = 6$ Hz, 4-H), 6.78 ppm (m, 1H, $J_{5,4} = 6$ Hz, 5-H); EI MS: *m*/*z*: 205 (100) $[M]^+$, 187 (14), 126 (100); elemental analysis calcd (%) for $C_8H_6NO_2F_3$: C 46.8, H 2.9, N 6.8; found: C 46.3, H 2.7, N 6.5.

(1*S*,2*R*)-1i (X = CF$_3$, Y = Me): 0.455 g, 75%; m.p. 105–107 °C ($CHCl_3$/hexane); $[\alpha]_D = -118$ ($c = 1.03$, MeOH); >98% *ee*; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.99$ (s, 3H, Me), 4.35 (m, 2H, 1-H, 2-H), 5.82 (d, 1H, $J_{5,4} = 5.0$ Hz, 5-H), 6.51 ppm (dd, 1H, $J_{5,4} = 5.0$, $J_{4,2} = 1.8$ Hz, 4-H); EI MS: *m*/*z*: 194 (14) $[M]^+$, 176 (61), 79 (100); elemental analysis calcd (%) for $C_8H_9O_2F_3$: C 49.5, H 4.6; found: C 49.3, H 4.4.

(1*S*,2*R*)-1j (X = CN, Y = Me): 0.480 g, 74%; m.p. 138–140 °C ($CHCl_3$/hexane); $[\alpha]_D = +92$ ($c = 1.02$, MeOH); >98% *ee*; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.04$ (s, 3H, Me), 4.17 (d, 1H, $J_{1,2} = 6.1$ Hz, 1-H), 4.39 (d, 1H, $J_{2,1} = 6.1$ Hz, 2-H), 5.86 (d, 1H, $J_{5,4} = 5.4$ Hz, 5-H), 6.68 ppm (d, 1H, $J_{4,5} = 5.4$ Hz, 4-H); EI MS: *m*/*z*: 151 (16) $[M]^+$, 133 (57), 86 (100); elemental analysis calcd (%) for $C_8H_9NO_2$: C 63.5, H 6.0, N 9.3; found: C 62.9, H 5.9, N 9.1.

Crystal data for 1j: $C_8H_9NO_2$, $M = 151.2$, orthorhombic, $a = 8.044(2)$, $b = 8.151(2)$, $c = 11.400(2)$ Å, $V = 747.5(3)$ Å^3, $T = 293(2)$ K, $Cu_{K\alpha}$ radiation, $\lambda = 1.54178$ Å, space group $P2_12_12_1$ (no. 19), $Z = 4$, $F(000) = 320$, $\rho_{calcd} = 1.343$ g cm^{-3}, $\mu = 0.81$ mm^{-1}, Siemens P3 diffractometer, ω scans, $13.4 < 2\theta < 114.1°$, measured/independent reflections: 1230/994, 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. $R_1 = 0.041$ for 954 data with $F_o > 4\sigma(F_o)$, 104 parameters, $wR_2 = 0.113$ (all data), GoF = 1.03, Flack parameter $x = -0.5(4)$, $\Delta\rho_{min,max} = -0.16/0.12$ e Å^{-3}. CCDC-631367 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(1*R*,2*R*)-1t (X = CF$_3$, Y = I): 0.300 g, 53%; m.p. 117–118 °C ($CHCl_3$); $[\alpha]_D = -56$ ($c = 0.94$, MeOH); >98% *ee*; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.43$ (m, 2H, 1-H, 2-H), 6.19 (d, 1H, $J_{4,5} = 6.3$ Hz, 4-H), 6.77 ppm (d, 1H, $J_{5,4} = 5.7$ Hz, 5-H); EI MS: *m*/*z*: 306 (100) $[M]^+$, 179 (30); elemental analysis calcd (%) for: $C_7H_6O_2F_3I$: C 27.5, H 1.9; found: C 27.8, H 1.6.

Halogen-substitution reactions of *cis*-dihydrodiols 1a, d, n, o, p and t with tributyltin reagents and [Pd(PPh$_3$)$_4$]

Substitution of a bromine atom by a vinyl group in *cis*-dihydrodiols 1a and 1d to yield *cis*-dihydrodiols 1v and 1w: To a solution of *cis*-diol (**1a** and **1d**, 2 mmol), palladium(II) acetate (10 mol%) and triphenylphosphine (20 mol%) in dry THF (20 mL) was added vinyltributyltin (2.3 mmol). The mixture was stirred at room temperature for 16 h under nitrogen. THF was removed under reduced pressure and the resulting residue purified by flash chromatography followed by PLC (5% MeOH in $CHCl_3$) to give the corresponding vinyl *cis*-dihydrodiols **1v** and **1w** (35–45% yields). Samples of *cis*-dihydrodiols **1v** and **1w** of identical absolute configuration were obtained using the same procedure with the iodo-substituted analogues **1n** and **1p** of known configuration.

(1*R*,2*R*)-1v (X = CH=CH$_2$, Y = F): m.p. 97–99 °C (EtOAc/hexane); $[\alpha]_D = +85$ ($c = 0.59$, MeOH); 76% *ee*; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.54$ (m, 1H, 1-H), 4.62 (m, 1H, 2-H), 5.19 (d, 1H, $J_{cis} = 10.9$ Hz, CH=C*H*$_2$), 5.43 (d, 1H, $J_{trans} = 17.6$ Hz, CH=C*H*$_2$), 5.59 (m, 1H, 5-H), 5.90 (m, 1H, 4-H), 6.38 ppm (dd, 1H, $J_{cis} = 10.9$, $J_{trans} = 17.6$ Hz, C*H*=CH$_2$); EI MS: *m*/*z* (%): 156 (13) $[M]^+$, 138 (90), 109 (100), 83 (21); HR MS (EI): calcd for $C_8H_9O_2F$: 156.0586: found: 156.0669 $[M]^+$.

(1*R*,2*S*)-1w (X = CH=CH$_2$, Y = Me): m.p. 55–57 °C (EtOAc/hexane); $[\alpha]_D = +16$ ($c = 1.13$, MeOH); 30% *ee*; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.94$ (s, 3H, Me), 4.31 (m, 1H, 1-H), 4.42 (m, 1H, 2-H), 5.14 (d, 1H, $J_{cis} = 10.9$ Hz, CH=C*H*$_2$), 5.44 (d, 1H, $J_{trans} = 17.6$ Hz, CH=C*H*$_2$), 5.77 (m, 1H, 5-H), 5.91 (m, 1H, 4-H), 6.39 ppm (dd, 1H, $J_{cis} = 10.9$, $J_{trans} = 17.6$ Hz, C*H*=CH$_2$); EI MS: *m*/*z* (%): 152 (57) $[M]^+$, 134 (45), 106 (42), 91 (100), 79 (62); HR MS (EI): *m*/*z*: calcd for $C_9H_{12}O_2$: 152.0837; found: 152.0836 $[M]^+$.

Substitution of an iodine atom in *cis*-dihydrodiols 1n, o, p and t by a nitrile group to yield *cis*-dihydrodiols 1f, c, j and h: To a solution of *cis*-diol (**1n**, **o**, **p** and **t**, 2 mmol), containing [Pd(PPh$_3$)$_4$] (3 mol%) in dry THF (15 mL) was added tributyltin cyanide (4 mmol). The mixture was stirred under nitrogen at 50 °C for 3 h. The cooled reaction mixture was filtered, the THF removed under reduced pressure and the resulting residue purified by PLC (5% MeOH in $CHCl_3$) to give the corresponding cyano *cis*-dihydrodiols **1f**, **c**, **j** and **h**. The nitrile substituted *cis*-dihydrodiols **1f**, **c**, **j** and **h**, obtained by this method, had identical spectroscopic characteristics and absolute configurations to the same compounds produced as metabolites of the parent 1,4-disubstituted benzene substrates.

Catalytic reduction of *cis*-(1*R*,2*R*)-1b: A solution of *cis*-diol **1b** (2.0 mmol) in MeOH (15 mL) containing Et_3N ($\approx$50 μL) was stirred at room temperature under hydrogen (1 atm) in the presence of 10% Pd/C (0.06 g). When all the starting material had been consumed (5 h, TLC) the resulting residue was purified by flash chromatography (5% MeOH in $CHCl_3$) followed by PLC using the same solvent system.

(1*S*,2*R*)-2b: White crystalline solid: 0.250 g, 69%; m.p. 105–107 °C ($CHCl_3$/hexane), (lit.[36] m.p. 105–107); $[\alpha]_D = -129$ ($c = 0.35$, MeOH), (lit.[36] $[\alpha]_D = -129$).

Cycloadducts 3g and 3i from *cis*-dihydrodiols 1g and 1i: A solution of sublimed 4-phenyl-1,2,4-triazoline-3,5-dione (2 mmol) in CH_2Cl_2 (10 mL) was added, dropwise at room temperature, to a stirring solution of the *cis*-dihydrodiol **1g** or **1i** (1.5 mmol) in CH_2Cl_2 (10 mL), until the pink colour of the reagent persisted. Removal of solvent, after stirring the mixture for 1 h, and purification of the residue by PLC (75% EtOAc in hexane) yielded the respective cycloadduct **3g** or **3i**.

(8*S*,9*R*)-3g: Colourless crystals: 358 mg, 75%; m.p. 209–215 °C (MeOH); $[\alpha]_D = +8$ ($c = 0.92$, MeOH); ^{1}H NMR (300 MHz, CD_3COCD_3): $\delta = 1.96$ (s, 3H, Me), 3.80 (dd, 1H, $J_{8,F} = 1.5$, $J_{8,9} = 8.8$ Hz, 8-H), 4.19 (dd, 1H, $J_{9,F} = 5.8$, $J_{9,8} = 8.8$ Hz, 9-H), 6.40 (dd, 1H, $J_{11,F} = 4.6$, $J_{11,10} = 8.9$ Hz, 11-H), 6.64 (dd, 1H, $J_{10,F} = 9.6$, $J_{10,11} = 8.9$ Hz, 10-H), 7.36–7.51 ppm (m, 5H, Ar-H); EI MS: *m*/*z* (%): 319 (23) $[M]^+$, 119 (100); HR MS (EI): *m*/*z*: calcd for $C_{15}H_{14}FN_3O_4$: 319.0968; found: 319.0973 $[M]^+$.
(8*R*,9*R*)-3i: Colourless crystalline solid: 498 mg, 90%; m.p. 188–190 °C (MeOH); $[\alpha]_D = +8$ ($c = 0.79$, CH_3COCH_3); ^{1}H NMR (300 MHz, CD_3COCD_3): $\delta = 1.98$ (s, 3H, Me), 3.83 (d, 1H, $J_{8,9} = 8.5$ Hz, 8-H), 4.27 (d, 1H, $J_{9,8} = 8.5$ Hz, 9-H), 6.58 (m, 2H, 10-H, 11-H), 7.45–7.47 ppm (m, 5H, Ar-H); EI MS: *m*/*z* (%): 369 (7) $[M]^+$, 119 (100); elemental analysis

calcd (%) for $C_{16}H_{14}O_4N_3F_3$: C 52.0, H 3.8, N 11.4; found: C 51.6, H 3.5, N 11.1.

Bis-(*R*)-(+)- and bis-(*S*)-(−)-MTPA esters $4g_R$/$4g_S$ and **$4is_R$/$4i_S$**: To a solution of the cycloadduct **3g** or **3i** (0.1 mmol) in dry pyridine, containing 4-dimethylaminopyridine (5 mg), was added (*S*)-(+)- or (*R*)-(−)-2-methoxy-2-phenyl-2-trifluoromethylacetyl chloride (0.25 mmol). The mixture was stirred at 60 °C until the esterification reaction had gone to completion (48 h, by ^{1}H NMR analysis). Pyridine was removed under reduced pressure and the residue purified by PLC (2 % MeOH in $CHCl_3$) to yield the respective bis-(*R*)- or bis-(*S*)-MTPA esters (ca. 90 % yield).

(8*R*,9*R*)-$4g_R$: Light yellow semi-solid: 68 mg, 90 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.54 (s, 3H, Me), 3.07 (s, 3H, OMe), 3.77 (s, 3H, OMe), 4.70 (dd, 1H, $J_{8,F}$ = 1.7, $J_{8,9}$ = 9.2 Hz, 8-H), 5.90 (dd, 1H, $J_{9,F}$ = 3.9, $J_{9,8}$ = 9.2 Hz, 9-H), 6.32 (m, 1H, 11-H), 6.73 (dd, 1H, $J_{10,F}$ = 8.3, $J_{10,11}$ = 8.6 Hz, 10-H), 7.29–7.47 ppm (m, 15H, Ar-H); EI MS: *m*/*z* (%): 751 (9) $[M]^+$, 189 (100); HR MS (EI): *m*/*z*: calcd for $C_{35}H_{28}O_8F_7N_3$: 751.1765; found: 751.1782.

(8*R*,9*R*)-$4g_S$: Light yellow semi-solid: 65 mg, 86 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.95 (s, 3H, Me), 3.19 (s, 3H, OMe), 3.47 (s, 3H, OMe), 5.07 (dd, 1H, $J_{8,F}$ = 1.4, $J_{8,9}$ = 9.2 Hz, 8-H), 5.86 (dd, 1H, $J_{9,F}$ = 4.7, $J_{9,8}$ = 9.2 Hz, 9-H), 6.38 (dd, 1H, $J_{11,F}$ = 5.3, $J_{11,10}$ = 8.9 Hz, 11-H), 6.73 (t, 1H, $J_{10,F}$ = 8.5, $J_{10,11}$ = 8.9 Hz, 10-H), 7.31–7.51 (m, 13H, Ar-H), 7.62 ppm (d, 2H, J = 7.4 Hz, Ar-H); EI MS: *m*/*z* (%): 751 (6) $[M]^+$, 189 (100); HR MS (EI): *m*/*z*: calcd for $C_{35}H_{28}O_8F_7N_3$: 751.1765; found: 751.1782.

(8*S*,9*R*)-$4i_R$: White crystalline solid: 64 mg, 80 %; m.p. 86–88 °C (Et_2O/$CHCl_3$); $[\alpha]_D$ = +16 (c = 1.28, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.98 (s, 3H, Me), 3.41 (s, 3H, OMe), 3.55 (s, 3H, OMe), 5.40 (d, 1H, $J_{8,9}$ = 8.9 Hz, 8-H), 5.47 (d, 1H, $J_{9,8}$ = 8.9 Hz, 9-H), 6.54 (d, 1H, $J_{11,10}$ = 8.1 Hz, 11-H), 6.58 (d, 1H, $J_{10,11}$ = 8.1 Hz, 10-H), 7.27–7.54 ppm (m, 15H, Ar-H); EI MS: *m*/*z* (%): 801 (10) $[M]^+$, 568 (9), 189 (100); HR MS (EI): *m*/*z*: calcd for $C_{36}H_{28}O_8F_9N_3$: 801.1733; found: 801.1745 $[M]^+$.

(8*S*,9*R*)-$4i_S$: White crystalline solid: 68 mg, 85 %; m.p. 112–114 °C (Et_2O/$CHCl_3$); $[\alpha]_D$ = +66 (c = 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.53 (s, 3H, Me), 3.34 (s, 3H, OMe), 3.52 (s, 3H, OMe), 5.03 (d, 1H, $J_{8,9}$ = 8.8 Hz, 8-H), 5.76 (d, 1H, $J_{9,8}$ = 8.8 Hz, 9-H), 6.48 (d, 1H, $J_{10,11}$ = 8.4 Hz, 10-H), 6.62 (d, 1H, $J_{11,10}$ = 8.4 Hz, 11-H), 7.37–7.55 ppm (m, 15H, Ar-H); EI MS: *m*/*z*: 801 (M^+, 8 %), 568 (10), 189 (100); HR MS (EI): *m*/*z*: calcd for $C_{36}H_{28}O_8F_9N_3$: 801.1733; found: 801.1746 $[M]^+$.

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