Received: 6 June 2017 | Revised: 19 July 2017 | Accepted: 24 July 2017

DOI: 10.1002/chir.22764

SPECIAL ISSUE ARTICLE

WILEY

Enantiopurity and absolute configuration determination of arene *cis*-dihydrodiol metabolites and derivatives using chiral boronic acids

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Abstract

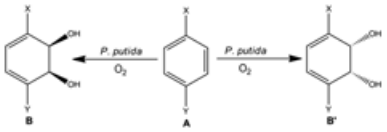
The relative merits of the methods employed to determine enantiomeric excess (*ee*) values and absolute configurations of chiral arene and alkene *cis*-1,2-diol metabolites, including boronate formation, using racemic or enantiopure (+) and (−)-2-(1-methoxyethyl)phenylboronic acid (MEPBA), are discussed. Further applications of: 1) MEPBA derived boronates of chiral mono- and poly-cyclic arene *cis*-dihydrodiol, cyclohex-2-en-1-one *cis*-diol, heteroarene *cis*/*trans*-2,3-diol, and catechol metabolites in estimating their *ee* values, and 2) new chiral phenylboronic acids, 2-[1-methoxy-2,2-dimethylpropyl]phenyl boronic acid (MDPBA) and 2-[1-methoxy-1-phenylmethyl]phenyl boronic acid (MPPBA) and their advantages over MEPBA, as reagents for stereochemical analysis of arene and alkene *cis*-diol metabolites, are presented.

KEYWORDS

asymmetric synthesis, bacterial metabolism, boronate diastereomers, boronic acid enantiomers, *cis*-1,2-diol bioproducts, enantiomeric excess values

The first examples of chiral arene *cis*-dihydrodiol bacterial metabolites **B** (X ≠ Y) or **B′** (X ≠ Y) (Scheme 1) were found by Gibson and co-workers almost 50 years ago.[1] Among the early substrates used were toluene **A** (X = Me, Y = H),[2] *p*-chlorotoluene **A** (X = Me, Y = Cl)[1] and naphthalene.[3] The main focus of Gibson's group was on the structural/stereochemical identification of new *cis*-dihydrodiol metabolites, from mono- and di-substituted benzene and polycyclic aromatic hydrocarbon substrates, using different dioxygenase enzymes.[1-10] The instability of arene *cis*-dihydrodiol metabolites, and their unavailability in sufficient quantities, were early barriers to their utilization in synthetic chemistry.[1-8] In 1983, the development of a proprietary constituent mutant strain of *P. putida* (UV4) by ICI, expressing toluene dioxygenase (TDO), led to the commercial production of arene *cis*-dihydrodiols, *e.g.*, **B** (X = Y = H and X = Me, Y = H), and the first applications of this strain in synthesis.[11-13]

The availability of other mutant (*Pseudomonas* and *Sphingomonas*) and recombinant (*Escherichia coli*) strains, expressing TDO, naphthalene dioxygenase (NDO), biphenyl dioxygenase (BPDO), and benzoate dioxygenase for biotransformation resulted in a marked increase in worldwide interest in this type of bacterial metabolite. To date, more than 400 new *cis*-dihydrodiols have been isolated and are being used in chemoenzymatic syntheses.[1-19]



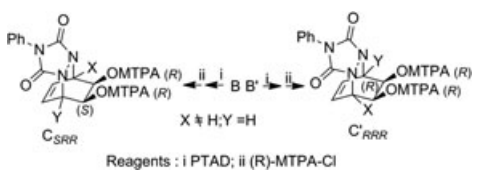
SCHEME 1 Formation of *cis*-dihydrodiols (**B** and **B**′) from substituted benzene substances (**A**)

1 | MATERIALS AND METHODS

1.1 | Methods for determining the enantiopurities and absolute configurations of arene cis-dihydrodiols

With the marked increase in the numbers and uses of chiral *cis*-dihydrodiols, reliable methods were required for the determination of both enantiomeric excess (*ee*) values and absolute configurations (ACs). During early reports of *cis*-dihydrodiol formation from mono- and poly-cyclic arene substrates,[3-10] stereochemical correlation methods, e.g., catalytic hydrogenation and oxidative ring cleavage reactions, were commonly used to assign *ee* values and ACs. Due to the instability of most *cis*-dihydrodiols, early efforts, to form di[2-methoxy-2-(trifluoromethyl) phenylacetate] (diMTPA) diastereomers for *ee* determination[20] were unsuccessful and X-ray crystallographic analysis was rarely employed for AC assignments. Thus, alternative methods for the determination of *ee* values and ACs were developed.

DiMTPA derivatives ($\mathbf{C}_{SRR}$ and $\mathbf{C}'_{RRR}$) of stable Diels-Alder cycloadducts, obtained by cycloaddition of *cis*-dihydrodiols **B** (Y = H and X = F, Me, CF_3) with 4-phenyl-1,2,4-triazoline-3,5-dione, were often crystalline. X-ray crystallographic analysis then provided an unequivocal indirect assignment method for ACs and *ee* values of several *cis*-dihydrodiols **B** (Scheme 2).[21,22] Later studies, using low-temperature X-ray crystallographic facilities, showed that ACs of unstable *cis*-dihydrodiols **B**, derived from monosubstituted benzene **A** (X = F, Y = H; X = Br, Y = H),[23] and 1,4-disubstituted benzene substrates **A** (X = CF_3, Y = F; X = CN, Y = F; X = CN, Y = Me), [24] could be determined directly with the anomalous dispersion method.



SCHEME 2 Formation of diMTPA diastereomers (C_{SRR} and C'_{RRR}) derived from 4-phenyl-1,2,4-triazoline-3,5-dione adducts of *cis*-dihydrodiol enantiomers (B and B′, X not H, Y = H)

Following the earlier isolation of two non-enantiopure arene *cis*-dihydrodiol metabolites **B**/**B**′ (X = Me, Y = Cl; X = Me, Y = Br),[4] further TDO-catalyzed biotransformation studies of these and other 1,4-disubstituted benzene substrates were conducted[24,25]; the *ee* values of isolated metabolites were determined by the formation of diMTPA diastereomers of their stable cycloadducts and ^{1}H-(or ^{19}F-)-NMR analysis of the distinguishable OMe (or CF_3) signals of the diastereomers ($\mathbf{C}_{SRR}$ and $\mathbf{C}'_{RRR}$, Scheme 2).[21,22] Formation of diMTPA esters ($\mathbf{C}_{SRR}$ and $\mathbf{C}'_{RRR}$), from 1,4-disubstituted benzene substrates **A** (X ≠ Y), confirmed that most *cis*-dihydrodiols were not single enantiomers and that *cis*-diols **B**/**B**′, metabolites from *P. putida* UV4 (X = Me, Y = Cl, 15% *ee* and X = Me, Y = Br, 37% *ee*), were not racemic.

The partial hydrogenation of unstable *cis*-dihydrodiol metabolites of polycyclic arenes (naphthalene, phenanthrene) and azaarenes (quinoline and isoquinoline) substrates, to yield the corresponding stable tetrahydro-*cis*-diols, followed by diMTPA ester formation, using both (*R*)- and (*S*)-MTPA chloride, showed the metabolites to be single enantiomers (>98% *ee*).[26] A combination of experimental, and Density Functional Theory (DFT)-calculated electronic circular dichroism (ECD) spectra, was later applied successfully for the direct determination of ACs of *cis*-dihydrodiol metabolites derived from monosubstituted, 1,2-, 1,3-, and 1,4- disubstituted benzenes,[23,27-29] bicyclic arenes,[30] and azaarenes.[31]

Enantiomeric mixtures of *cis*-dihydrodiols were available from biotransformations of substituted benzenes, with TDO,[24,25,32,33] chlorobenzene dioxygenase,[34,35] and from both mono- and poly-cyclic arenes, using site-directed mutants of NDO.[36] Application of chiral stationary phase (CSP) high-performance liquid chromatography (HPLC) methods (Chiralcel OJ and AD columns) separated individual *cis*-dihydrodiol enantiomers and also gave *ee* values directly.[24,36] Separation of the *n*-butylboronate derivatives of *cis*-dihydrodiol enantiomers was reported, using chiral gas chromatography (CGC) with a cyclodextrin column. The availability of suitable CSP HPLC (or CGC) columns thus provided a very useful method for the determination of *ee* values of alkene and arene *cis*-dihydrodiol metabolites. Since arene *cis*-dihydrodiol metabolites are often found to be enantiopure, and as the chiral columns are generally available in only one enantiomeric configuration, the observation of a single peak by CSP HPLC (or CGC) analysis could be due to the presence of a single enantiomer or an unresolved mixture of enantiomers.

While X-ray crystallography[23,24] and ECD spectroscopy[23,27-31] have provided rigorous methods for AC assignments of arene *cis*-dihydrodiols, better methods for determination of *ee* values, than reported earlier,[21,22,26]

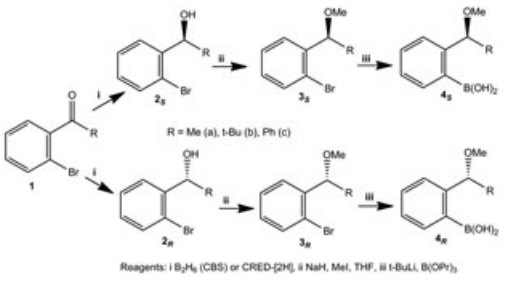
were required. A major objective of our work was to synthesize and utilize already available and new chiral boronic acids for the determination of *ee* values and ACs of a wide range of *cis*-diol metabolites, by ^{1}H–NMR analysis of the corresponding boronate derivatives.

^{1}H–NMR spectra were generally obtained using a Bruker (Billerica, MA) Avance DRX-500 instrument and $CDCl_3$ as solvent. X-ray diffraction intensities were measured on a Siemens (Erlangen, Germany) P3 diffractometer. All chiral vicinal diol and catechol metabolites used in the study, isolated as single enantiomers in most cases (>98% *ee*), were available from earlier dioxygenase enzyme-catalyzed dihydroxylations of the corresponding alkene, arene, and heteroarene substrates with mutant bacterial strains. Optical rotations and ACs of arene *cis*-diol metabolites were reported in earlier reviews[14-16] and later references in Tables 1–6. The synthesis of racemic and enantiopure [2-(1-methoxyethyl)phenyl]boronic acid (MEPBA) was conducted according to the literature procedures.[37,38] Diastereomeric boronates were obtained by reacting the *cis*-diol with a slight excess of MEPBA reagent in $CDCl_3$ solution and the reaction was followed to completion by ^{1}H–NMR spectroscopy. Baseline resolutions of OMe singlets (δ_{OMe}), were generally observed when $\Delta\delta_{OMe}$ values were >7 ppb for boronate diastereomers formed, either using: 1) single enantiomer *cis*-diols and racemic MEPBA or 2) enantiomeric mixtures of *cis*-diols with (1*R*)- or (1*S*)-MEPBA.

2 | RESULTS AND DISCUSSION

The method used for the determination of enantiopurity of arene *cis*-dihydrodiols in the study entailed formation of chiral boronate derivatives using (±)-(MEPBA) $\mathbf{4a_S}$/$\mathbf{4a_R}$, and (−)-(*S*)-MEPBA $\mathbf{4a_S}$ or (+)-(*R*)-MEPBA $\mathbf{4a_R}$, followed by their ^{1}H–NMR analysis (Scheme 3).

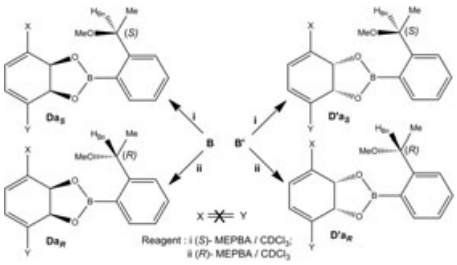
The initial step, reported in the literature for the synthesis of MEPBA reagent $\mathbf{4a_S}$ involved an asymmetric reduction of *o*-bromoacetophenone **1a** with diborane in the presence of a chiral oxazaborolidine catalyst (CBS method, Scheme 3).[37] Recrystallization of the resulting chiral alcohol $\mathbf{2a_S}$ (85% *ee*) yielded a single enantiomer (>98% *ee*), which was methylated (MeI/NaH). Treatment of methyl ether $\mathbf{3a_S}$, with *tert*-butyl lithium and triisopropylborate, followed by hydrolysis yielded enantiopure (+)-(*R*)-MEPBA $\mathbf{4a_R}$.[37]



SCHEME 3 Formation of chiral boronic acid enantiomers $\mathbf{4_S}$ and $\mathbf{4_R}$ from ketone 1 (OMe changed to OH)

Asymmetric reduction of ketone **1a**, catalyzed by a carbonyl reductase (CRED) expressed in baker's yeast cells was later used to produce (−) (*S*)-alcohol $\mathbf{2a_S}$ (>98% *ee*) as the first step in the synthesis of enantiopure (−)-(*S*)-boronic acid $\mathbf{4a_S}$ (Scheme 3).[38] This chemoenzymatic route to alcohol $\mathbf{2a_S}$, followed by a Mitsunobu inversion step, yielded the (+)-(*R*) enantiomer $\mathbf{2a_R}$ and finally boronic acid (+)-(*R*)-MEPBA $\mathbf{4a_R}$.[38] During the study a SelectAZyme screening kit of CREDs was also used with substrate *o*-bromoacetophenone **1a**. In common with bakers' yeast, most of the available CREDs favored formation of (−)-(*S*) alcohol $\mathbf{2a_S}$ with many giving *ee* values of >90%. One member of the kit (CRED A161) produced mainly the (+)-(*R*) enantiomer $\mathbf{2a_R}$ (87% *ee*), which can yield a single enantiomer on careful recrystallization. Thus, while both boronic acids (−)-(*S*)-$\mathbf{4a_S}$ and (+)-(*R*)-$\mathbf{4a_R}$ can be produced by a chemoenzymatic method, the more readily available (−)-(*S*) enantiomer $\mathbf{4a_S}$ was used preferentially along with (±)-MEPBA $\mathbf{4a_S}$ /$\mathbf{4a_R}$ in the study.

The first applications of (+)-(*R*)-boronic acid $\mathbf{4a_R}$ for the determination of *ee* values were reported by Burgess and Porte. It was applied to six acyclic 1,2-diols, two acyclic 1,3-diols, and an acyclic *β*-amino alcohol, all as racemates.[37] Resnick et al. utilized both (−)-(*S*)-$\mathbf{4a_S}$ and (+)-(*R*)-$\mathbf{4a_R}$ boronic acid enantiomers to determine *ee* values and ACs of *cis*-dihydrodiol metabolites, e.g., **B** or **B′** (Y = H and X = F, Cl, I, Me, CF_3, Ph), derived from the corresponding monosubstituted benzenes, and several polycyclic arene substrates.[38-40] *cis*-Dihydrodiol metabolites **B** (Y = H and X = Cl, I, Me, CF_3, Ph), previously shown to be single enantiomers (>98% *ee*),[21,22] were reacted in situ with boronic acids $\mathbf{4a_S}$ and $\mathbf{4a_R}$.[38] The resulting corresponding single boronate diastereomers, $\mathbf{Da_S}$ and $\mathbf{Da_R}$, each exhibiting distinguishable ^{1}H–NMR signals associated with the exocyclic chiral center (Scheme 4). The methoxy group (OMe) appeared as a singlet (δ_{OMe}), the other methyl group (CMe) as a doublet (δ_{Me}), and the benzylic proton H_{Bn} (δ_{Bn}) as a quartet. Identification of δ_{OMe} (or δ_{Me}) signals in the ^{1}H–NMR spectrum of $\mathbf{Da_S}$ diastereomer was possible by the reaction of boronic acid enantiomer $\mathbf{4a_S}$ with metabolites **B** (Y = H and X = Cl, I, Me, CF_3, Ph). *cis*-Dihydrodiol metabolite **B** (Y = H and X = F) was found to be of lower enantiopurity (80% *ee*),[21,22] as shown by its reaction with boronic acid $\mathbf{4a_S}$, which yielded a mixture of boronate diastereomers $\mathbf{Da_S}$ and $\mathbf{D'a_S}$.



SCHEME 4 Formation of boronates $\mathbf{Da_S}$, $\mathbf{Da_R}$, $\mathbf{D'a_S}$ and $\mathbf{D'a_R}$ from the reactions of *cis*-dihydrodiol enantiomers B and B′ with (−) -(*S*)-MEPBA $\mathbf{4a_S}$ and (+)-(*R*)-MEPBA $\mathbf{4a_R}$

The diastereomeric boronate method was also applied to confirm *ee* values (>98%) for *cis*-diol metabolites from polycyclic arenes (naphthalene, anthracene),[38] heteroarenes (dibenzofuran, dibenzothiophene),[39] and dihydroarenes (1,2-dihydronaphthalene, 9,10-dihydroanthracene and 9,10-dihydrophenanthrene).[38,40] The relative trends observed, for ^{1}H-NMR directional chemical shift values of the MeO (δ_{OMe}) and Me (δ_{Me}) groups of boronate diastereomers $\mathbf{Da_S}$ and $\mathbf{D'a_S}$ with boronic acid (−)-(*S*)-$\mathbf{4a_S}$, or $\mathbf{Da_R}$ and $\mathbf{D'a_R}$ with boronic acid (+)-(*R*)-$\mathbf{4a_R}$, were also used to assign ACs of *cis*-dihydrodiol metabolites **B** and **B′**.[38-40] Thus, a consistent upfield shift of δ_{OMe} signals of boronates, formed using (−)-(*S*)-$\mathbf{4a_S}$, was observed with monocyclic arene *cis*-dihydrodiols **B** (Y = H and X = Me, CF_3 and Ph), relative to the δ_{OMe} value found using (+)-(*R*)-$\mathbf{4a_R}$. While stereodifferentiation of *cis*-diol enantiomers was shown by the separation of MeO singlets ($\Delta\delta_{OMe}$) and Me doublets ($\Delta\delta_{Me}$) of boronate diastereomers in $CDCl_3$ solvent, in some cases better signal separations were observed using C_6D_6 solvent.[38-40]

Following the earlier reports of the successful application of MEPBA reagent in the racemic ($\mathbf{4a_S/4a_R}$) and enantiopure ($\mathbf{4a_S}$ or $\mathbf{4a_R}$) forms, for the determination of both *ee* values and ACs of *cis*-dihydrodiol metabolites isolated from six monocyclic and four polycyclic arenes,[38-40] this valuable reagent has since been used extensively in our laboratories. The new results presented herein include: 1) the applicability of the MEPBA reagent for determining the *ee* values and ACs of a range of other arene *cis*-diol metabolites (*e.g.*, from carbocyclic and heterocyclic arenes and *meta* phenols) and of *cis*/*trans*-diol metabolites (from heterocyclic arenes), 2) the synthesis of two new chiral boronic acids 2-[1-methoxy-2,2-dimethylpropyl]phenyl boronic acid (MDPBA) and 2-[1-methoxy-1-phenylmethyl]phenyl boronic acid (MPPBA) and preliminary comparative results showing their great potential for the stereochemical analysis of *cis*-diol metabolites of arenes or alkenes and chiral catechol metabolites derived from benzylic alcohols.

2.1 | MEPBA-derived boronates of *cis*-dihydrodiols 5–32 formed from the corresponding substituted benzene substrates

The relevant diagnostic ^{1}H–NMR signals (δ_{OMe} and δ_{Me}) from MEPBA derivatives of *cis*-dihydrodiols, formed by TDO-catalyzed *cis*-dihydroxylation of the corresponding monosubstituted (**5–23**) and disubstituted benzene substrates (**24–32**), are shown in Table 1.[14,16,33,41-43]

Only one MeO singlet (δ_{OMe}) was observed, for boronate derivatives $\mathbf{Da_S}$ (Y = H) of monocyclic arene *cis*-diol metabolites (**5–31**), formed using (−)-(*S*)-MEPBA $\mathbf{4a_S}$ (Scheme 4). With (±)-MEPBA $\mathbf{4a_R/4a_S}$, two MeO singlets ($\Delta\delta_{OMe}$) of equal peak area were obtained for boronates $\mathbf{Da_R}$ and $\mathbf{Da_S}$ (Y = H), consistent with metabolites (**5–31**) being single enantiomers (>98% *ee*). The presence of a doublet for Me (δ_{Me}) in the spectrum of boronate $\mathbf{Da_S}$ (Y = H), with (*S*)-MEPBA $\mathbf{4a_S}$ and two separated doublets ($\Delta\delta_{Me}$) in the spectrum of boronates $\mathbf{Da_R}$ and $\mathbf{Da_S}$ (Y = H) with (±)-MEPBA $\mathbf{4a_R/4a_S}$) was also employed in some cases to establish enantiopurity. A wide variation in the separation of OMe singlets ($\Delta\delta_{OMe}$ ppb) was found with boronates $\mathbf{Da_R}$ and $\mathbf{Da_S}$ (Y = H) formed with (±)-MEPBA $\mathbf{4a_R/4a_S}$ (Diagram 1, Table 1).

The smallest $\Delta\delta_{OMe}$ values for boronate derivatives of *cis*-dihydrodiols ($\Delta\delta_{OMe}$ 0–3) were associated with vinyl halides,[38] e.g., metabolite **5** ($\Delta\delta_{OMe}$, 0). Those bearing alkyl (**15**), substituted alkyl (**9**, **10**, **16,** and **17)** and vinyl substituents (**11–14**) showed slightly larger values (5-18). The largest values were associated with electron-withdrawing groups (**6–8**, $\Delta\delta_{OMe}$ 11–27) and particularly aryl or azaaryl groups (**18–23**, $\Delta\delta_{OMe}$ 56–89). The (±)-MEPBA $\mathbf{4a_R/4a_S}$ reagent was also effective in forming boronates from *cis*-dihydrodiol metabolites of *ortho*-, *meta*-, and *para*-disubstituted benzenes (**24–30**, **32**, $\Delta\delta_{OMe}$ 10–24). The 1,4-disubstituted benzene *cis*-dihydrodiol **32** was found to be a mixture of enantiomers (15% *ee*, using MEPBA $\mathbf{4a_S}$) and *cis*-diol metabolites (**6–31**) were confirmed as single enantiomers (Table 1, >98% *ee*, using MEPBA $\mathbf{4a_S}$ and (±)-MEPBA $\mathbf{4a_R/4a_S}$).

The consistently smaller upfield δ_{OMe} chemical shifts and negative $\Delta\delta_{OMe}$ values recorded earlier for boronate derivatives of monocyclic *cis*-dihydrodiols **B** (Y = H and X = Me, CF_3 and Ph), using MEPBA $\mathbf{4a_S}$[38] (relative to δ_{OMe} values using MEPBA $\mathbf{4a_R}$), were again observed for boronates of *cis*-dihydrodiols (**6–32**). As most of the ACs of metabolites (**6–32**, Table 1) had already been established by other methods, the advantage of this approach for the determination of ACs of monocyclic *cis*-dihydrodiols by formation of boronates using MEPBA $\mathbf{4a_S}$ and (±)-MEPBA $\mathbf{4a_R/4a_S}$, is clearly demonstrated.

TABLE 1 $[\alpha]_D$, AC and % *ee* values of *cis*-diols (**5**-**31**), δ and $\Delta\delta$ values of boronates

Cis- diol	$[\alpha]_D$[a]	AC	δ_{OMe}[b] (−)-(*S*)	$\Delta\delta_{OMe}$[c] (ppb)	δ_{Me}[d] (−)-(*S*)	$\Delta\delta_{Me}$[e] (ppb)	% *ee*	Lit.[f]
5	+41	1*S*,2*S*	3.257	0	1.441	+1	>98	14
6	+201	1*S*,2*R*	3.234	−11	1.435	+74	>98	14
7	+98	1*S*,2*R*	3.206	−25	1.410	+31	>98	14
8	+59[g]	1*S*,2*R*	3.209	−27	1.415	+24	>98	14
9	+69[g]	1*S*,2*R*	3.152	−6			>98	41
10	+73[g]	1*S*,2*R*	3.151	−7			>98	41
11	+115[g]	1*S*,2*R*	3.212	−18	1.416	+55	>98	14
12	+128	1*S*,2*R*	3.200	−10	1.428	+42	>98	14
13	+78	1*S*,2*R*	3.218	−8	1.418	+26	>98	14
14	+87	1*S*,2*R*	3.218	−14	1.418	+29	>98	14
15	+80	1*S*,2*R*	3.225	−11	1.420	+21	>98	16
16	+70[h]	1*S*,2*R*	3.222	−18	1.410	+34	>98	14
17	+33[g]	1*S*,2*R*	3.349	−5			>98	41
18	+144[h]	1*S*,2*R*	3.176	−56	1.421	+102	>98	42
19	+140[h]	1*S*,2*R*	3.179	−56	1.415	+111	>98	42
20	+252	1*S*,2*R*	3.123	−89	1.562	+128	>98	14
21	+129[h]	1*S*,2*R*	3.135	−77	1.393	+136	>98	42
22	+249[h]	1*S*,2*R*	3.143	−70	1.400	+150	>98	42
23	+181[h]	1*S*,2*R*	3.151	−71	1.418	+149	>98	42
24	+110	1*S*,2*S*	3.227	−12			>98	33
25	+103	1*S*,2*S*	3.229	−10			>98	33
26	+123	1*S*,2*S*	3.228	−12			>98	33
27	+139	1*S*,2*S*	3.229	−13			>98	33
28	+64	1*S*,2*R*	3.215	−24	1.420	+18	>98	14
29	+28	1*S*,2*R*	3.238	−19	1.426	+37	>98	43
30	+56	1*S*,2*R*	3.217	−23	1.421	+43	>98	43
31	+60	1*S*,2*R*	3.216	−26	1.421	+38	>98	43
32	+5	1*R*,2*R*	3.219	−16	1.431	+41	15	43

[a]MeOH;

[b]upfield δ value using $\mathbf{4a}_S$;

[c]difference in δ_{MeO} values using $\mathbf{4a}_S$ and $\mathbf{4a}_R$;

[d]downfield δ value $\mathbf{4a}_S$;

[e]difference in δ_{Me} values of $\mathbf{4a}_S$ and $\mathbf{4a}_R$;

[f]Literature reference;

[g]$CHCl_3$;

[h]THF.

2.2 | MEPBA derivatives of cyclohex-2-en-1-one cis-diols 33–41 derived from the corresponding meta-phenols

TDO-catalyzed *cis*-dihydroxylation of *meta* phenols resulted in the initial formation of the corresponding monocyclic *cis*-dihydrodiols, which preferred to exist as cyclohex-2-en-1-one *cis*-diol tautomers $\mathbf{E}_S$ (Scheme 5).[41-44] Reacting MEPBA reagents $\mathbf{4a}_S$ and (±) $\mathbf{4a}_R$ /$\mathbf{4a}_S$ with tautomers $\mathbf{E}_S$, the corresponding boronate diastereomers $\mathbf{F}_{SS}$ and $\mathbf{F}_{SR}$ were formed. Their $\Delta\delta_{OMe}$ values indicated that *cis*-diol metabolites (**33–41**) were enantiopure (>98% *ee*, Table 2).[41-44] Boronate derivatives of *cis*-diol metabolites having an aryl substituent (**18–23**, Table 1) resulted in

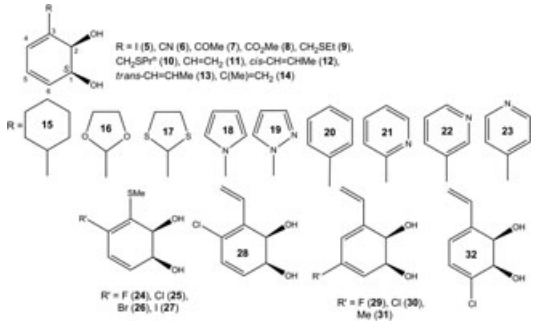
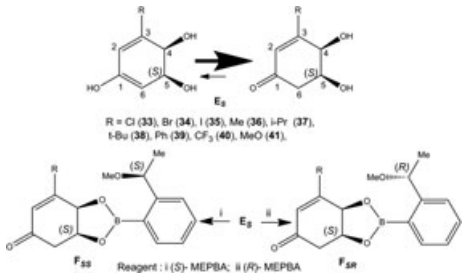


DIAGRAM 1 Structures **5–32**



SCHEME 5 Boronates $\mathbf{F_{SS}}$ and $\mathbf{F_{SR}}$ from (*S*)- or (*R*)-MEPBA and *cis*-dihydrodiols $\mathbf{E_S}$ (**33–41**)

larger $\Delta\delta_{OMe}$ values (56–89). A much larger value was observed for the boronate of *cis*-diol **39** ($\Delta\delta_{OMe}$ 136), which could be the result of the anisotropic effect of the phenyl substituent and closer proximity of the OMe group compared with the corresponding boronate of metabolite **20** ($\Delta\delta_{OMe}$ 89, Table 1). As the number of cyclohex-2-en-1-one *cis*-diols studied was relatively small, other trends in the magnitude of $\Delta\delta_{OMe}$ values were not apparent.

The OMe signals (δ_{OMe}) recorded for MEPBA $\mathbf{4a_S}$ derivatives of *cis*-diols (**6–32**) were located upfield in all cases (Table 1). Conversely, OMe signals for *cis*-diols (**33–38** and **41**) were found downfield, except for *cis*-diols (**39** and **40),** which had upfield δ_{OMe} signals (Table 2).[44-47] These results demonstrate the value of the MEPBA reagents for *ee* determination and also their limitations as a reliable method for the determination of ACs of cyclohex-2-en-1-one *cis*-diol metabolites of phenol substrates.

2.3 | MEPBA derivatives of *cis*-dihydrodiols 42–67 derived from the corresponding polycyclic arenes and heteroarenes

Relevant MeO singlet values (δ_{OMe}) and their separations ($\Delta\delta_{OMe}$), for boronate derivatives, of bi-, tri-, and tetracyclic arene *cis*-diol metabolites (**42–67**) (Diagram 2), formed using (*S*)-MEPBA $\mathbf{4a_S}$, and (±) MEPBA $\mathbf{4a_R/4a_S}$, are presented in Table 3.[9,10,14,15,36,42,48-50] As the MeO signal separation values ($\Delta\delta_{OMe}$) were generally higher (30–160) compared to those reported (0–89, Table 1), δ_{Me} and $\Delta\delta_{Me}$ values for Me doublets were not recorded. Polycyclic *cis*-dihydrodiol metabolites from carbocyclic arenes (**42–44, 55, 59, 61, 64**) and heterocyclic arenes (**45–54, 56–58, 60, 62, 63, 65–67**) were single enantiomers (>98% *ee*), based on $\Delta\delta_{OMe}$ values of boronates (Table 3).

The enhanced effect of aromatic substituents on $\Delta\delta_{OMe}$ values of boronates, of monocyclic *cis*-dihydrodiols (**18–23**, $\Delta\delta_{OMe}$ 56–71, Table 1), and a cyclohex-2-en-1-one *cis*-diol (**39**, $\Delta\delta_{OMe}$ 136, Table 2) was also evident in Table 3, where fused aromatic rings had a similar effect.

TABLE 2 $[\alpha]_D$, AC and % *ee values* of *cis*-diols (**33-41**), δ_{OMe} *and* $\Delta\delta_{OMe}$ values of boronates

Cis-diol	$[\alpha]_D$[a]	AC	δ_{OMe}[b] (−)-(*S*)	δ_{OMe}[c] (+)-(*R*)	$\Delta\delta_{OMe}$[d] (ppb)	% *ee*	Lit.[e]
33	−52	4*S*,5*S*	3.249	3.228	+21	>98	44
34	−45	4*S*,5*S*	3.245	3.225	+20	>98	44
35	−38	4*S*,5*S*	3.261	3.235	+26	>98	45,46
36	−151	4*R*,5*S*	3.222	3.202	+20	>98	45, 46
37	−79	4*R*,5*S*	3.215	3.204	+9	>98	47
38	−9	4*R*,5*S*	3.231	3.224	+7	>98	47
39	−20	4*R*,5*S*	3.017	3.153	−136	>98	45
40	−65	4*R*,5*S*	3.214	3.221	−7	>98	45
41	−120	4*S*,5*S*	3.213	3.195	+18	>98	46

[a]MeOH;

[b]OMe δ value using $\mathbf{4a_S}$;

[c]OMe δ value using $\mathbf{4a_R}$;

[d]difference (ppb) in δ_{OMe} values using$\mathbf{4a_S}$ and $\mathbf{4a_R/4a_S}$;

[e]Literature reference.

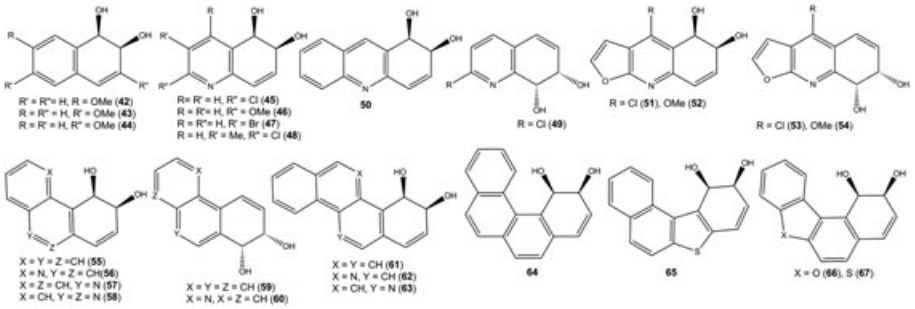


DIAGRAM 2 Structures **42–67**

TABLE 3 $[\alpha]_D$, AC and % *ee* values of *cis*-diols (42–70) and $\Delta\delta_{OMe}$ values of boronates

Cis-diol	$[\alpha]_D$[a]	Ab. Config.	δ_{OMe} [b](−)-(*S*)	δ_{OMe} [c](+)-(*R*)	$\Delta\delta_{OMe}$	% *ee*	Lit.[d]
42	+236	1*R*,2*S*	3.164	3.208	−44	>98%	14
43	+202	1*R*,2*S*	3.174	3.212	−36	>98%	14
44	−188	1*S*,2*R*	3.139	3.207	−68	>98%	14
45	+140	5*R*,6*S*	3.095	3.129	−34	>98%	14
46	+80	5*R*,6*S*	3.170	3.212	−42	>98%	48
47	+220	5*R*,6*S*	3.102	3.132	−30	>98%	48
48	+172	5*R*,6*S*	3.174	3.203	−34	>98%	49
49	+148	8*R*,7*S*	3.117	3.157	−40	>98%	48
50	+102	1*R*,2*S*	3.115	3.179	−64	>98%	48
51	+214	5*R*,6*S*	3.19	3.25	−60	>98%	50
52	−110	5*R*,6*S*	3.19	3.25	−60	>98%	50
53	+138	8*R*,7*S*	3.19	3.24	−50	>98%	50
54	+94	8*R*,7*S*	3.198	3.230	−32	>98%	50
55	+35	4*R*,3*S*	3.115	3.241	−126	>98%	15
56	+167	10*R*,9*S*	3.12	3.25	−130	>98%	42
57	+82	10*R*,9S	3.09	3.22	−130	>98%	42
58	−280	10*R*,9*S*	3.08	3.19	−110	>98%	42
59	+ve[f]	1*R*,2*S*	3.148	3.220	−72	>98%	36
60	+46	7*R*,8S	3.16	3.21	−50	>98%	42
61	+112	4*R*,3S	2.97	3.11	−140	>98%	9
62	+114	4*R*,3S	3.15	3.29	−140	>98%	9
63	+94	10*R*,9S	3.08	3.23	−150	>98%	9
64	−222	1*R*,2*S*	3.25	3.32	−70	>98%	10
65	+21	11*R*,10*S*	3.14	3.23	−91	>98%	10
66	+42	11*R*,10*S*	3.01	3.17	−160	>98%	10
67	+18	1*R*,2*S*	3.15	3.21	−60	>98%	10

[a]MeOH;

[b]upfield δ value using $\mathbf{4a}_{S}$;

[c]downfield δ value using $\mathbf{4a}_{R}$;

[d]Literature reference;

[e]$CHCl_3$;

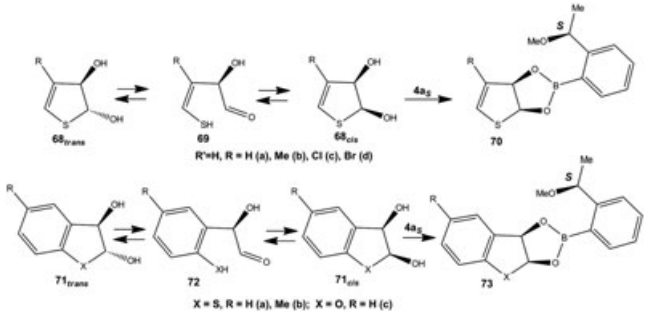
[f]only positive sign of rotation recorded.

Larger values ($\Delta\delta_{OMe}$ 30–68) were similarly observed for boronates of *cis*-dihydrodiol metabolites of bicyclic (**42–49**) and linear tricyclic arenes (**50–54**). The effect was further magnified for boronates in which the *cis*-diol bonds were located within the bay regions of tricyclic arenes (**55–58**, $\Delta\delta_{OMe}$ 110–130) and tetracylic arenes (**61–63**, $\Delta\delta_{OMe}$ 140–150), compared to those in the non-bay region positions of tricyclic arenes (**59** and **60**, $\Delta\delta_{OMe}$ 50–72). Relatively large $\Delta\delta_{OMe}$ values were also observed for boronates of tetracyclic arene *cis*-dihydrodiols located within the fjord (**64**, $\Delta\delta_{OMe}$ 70) or pseudo-fjord regions (**65–67**, $\Delta\delta_{OMe}$ 60–160). In common with the results shown in Table 1, a consistent upfield shift of the OMe singlet (negative $\Delta\delta_{OMe}$) was also noted when using (−)-(*S*)-MEPBA **4a*S*** (Table 3). The results of boronates of more than 50 *cis*-dihydrodiol metabolites, presented in Tables 1 and 3, demonstrate strong support to the earlier proposal[37]; that AC assignments for mono- and polycyclic arene and heteroarene *cis*-dihydrodiols can be made, based on the direction of chemical shifts of the boronate OMe signals (δ_{OMe}), formed using (−)-(*S*)-MEPBA **4a*S*** and (±)-MEPBA **4a*R*/4a*S*** reagents.

2.4 | Application of MEPBA reagents for the determination of *ee* values of chiral *cis/trans* dihydrodiols 68a–d and 71a–d derived from the corresponding heteroarenes

Stereochemical characterization of *cis*-diol metabolites, initially formed by TDO-catalyzed dihydroxylation of thiophene (**68a$_{cis}$-d$_{cis}$**, **71a$_{cis}$**, and **71b$_{cis}$**) and furan (**71c$_{cis}$**) rings (Scheme 6), proved to be difficult, due to their equilibration (epimerization) with the corresponding *trans* isomers (**68a$_{trans}$-d$_{trans}$** and **71a$_{trans}$-c$_{trans}$**) at ambient temperature.[51-54]

The epimerization involved a spontaneous ring opening process via acyclic aldehyde intermediates (**69a–d** and **72a–c**), with the equilibrium favoring *trans* isomers in hydroxylic solvents (80–100% *trans* in CD_3OD or D_2O) and *cis* isomers in less polar solvents (60–80% *cis* in $CDCl_3$).[51-54] Reaction with (−)-(*S*)-MEPBA **4a*S*** simplified the problem of stereochemical analysis, presented by *cis*/*trans* isomerization of the heterocyclic diols, as the corresponding boronates (**70a–d** and **73a–c**) were only formed from *cis* isomers.



SCHEME 6 Boronates (**70a–d and 73a–c**) formed from the reaction of (*S*)-MEPBA **4a*S*** with heteroarene *cis/trans*-dihydrodiols (**68–d and 71a–c**)

Separation of the isomeric mixtures of diol metabolites (**68a$_{cis}$-d$_{cis}$/68a$_{trans}$-d$_{trans}$** and **71c$_{cis}$ /71c$_{trans}$**) was unsuccessful, due to their rapid epimerization; therefore, individual $[\alpha]_D$ values could not be recorded. Recrystallization of mixtures of diols (**71a$_{cis}$ /71a$_{trans}$**) and (**71b$_{cis}$ / 71b$_{trans}$**) from $CHCl_3$ led to the isolation of enantiopure samples of *cis* isomers (**71a$_{cis}$** and **71b$_{cis}$**), and single enantiomers of *trans* isomers (**71a$_{trans}$** and **71b$_{trans}$**) were crystallized from MeOH. The maximum $[\alpha]_D$ values of isomers (**71a$_{cis}$**, **71b$_{cis}$**, **71a$_{trans}$**, and **71b$_{trans}$**) were recorded immediately after dissolution in order to minimize epimerization.

The enantiomeric mixtures of diols (**68a$_{cis}$-d$_{cis}$** and **71a$_{cis}$-c$_{cis}$**), on reaction with (−)-(*S*)-MEPBA **4a$_S$**, formed the major boronate diastereomers (**70a–d** and **73a–c**). ^{1}H–NMR analyses of the boronates showed the upfield OMe signals between (δ_{OMe} 3.06–3.27) and provided a range of $\Delta\delta_{OMe}$ values (15–120); the *ee* values were found in the range 43–63% (Table 4).[53,54] As recorded earlier for boronates of carbocyclic *cis*-dihydrodiols, from polycyclic arenes, larger $\Delta\delta_{OMe}$ values were again observed for metabolites (**71a$_{cis}$-c$_{cis}$**, $\Delta\delta_{OMe}$ 90–120 Diagram 4) compared with the much smaller $\Delta\delta_{OMe}$ values from the monocyclic *cis* diols (**68a$_{cis}$-d$_{cis}$**, $\Delta\delta_{OMe}$ 15–25). The upfield shift of δ values noted using MEPBA **4a$_S$** (Table 5) is consistent with similar ACs for the major enantiomer of heterocyclic *cis*-diols (**68a$_{cis}$-d$_{cis}$** and **71a$_{cis}$-c$_{cis}$**) and carbocyclic *cis*-dihydrodiols (**5–32** and **42–67**) shown in Tables 1 and 3. The *ee* values of *cis*/*trans* metabolites (**68a–d** and **71a–d**), found following reaction with MEPBA **4a$_S$**, were confirmed by the formation of corresponding diMTPA derivatives, and the ACs were established in some cases by X-ray crystallography.

2.5 | Synthesis of the new chiral boronic acids MDPBA and MPPBA

The advantages and limitations of (−)-(*S*)-MEPBA **4a*S*** and (±)-MEPBA **4a$_R$/4a$_S$**, as reagents for determination of *ee* values and ACs of arene *cis*-dihydrodiol (**5–32** and **46–67**), and cyclohex-2-en-1-one *cis*-diol metabolites (**33–41**), is clearly evident from the results (Tables 1–3). In some cases, the use of MEPBA resulted in: 1) very low $\Delta\delta_{OMe}$ values precluding base-line separation of diagnostic OMe singlets in the ^{1}H–NMR spectra of boronate diastereomers, formed from monocyclic *cis*-dihydrodiols, e.g., **B** or **B′** (Y = H and X = F, Cl, I; $\Delta\delta_{OMe}$ 0–3),[38]

TABLE 4 $[\alpha]_D$, AC, % *ee* values and $\Delta\delta_{OMe}$ values from boronates of diols ($68a_{cis}$-d_{cis} / $68a_{trans}$-d_{trans}) and ($71a_{cis}$-c_{cis} / $71a_{trans}$-c_{trans})

Cis-diol	$[\alpha]_D$[a]	AC[b,c]	$\Delta\delta_{OMe}$[d]	% *ee*	Lit.[e]
68a$_{cis}$ /68a$_{trans}$	-83[c]	2*S*,3*R* (60) 2*R*,3*R* (40)	−20	43	53
68b$_{cis}$ /68b$_{trans}$	+19[c]	2*S*,3*R* (60) 2*R*,3*R* (40)	−20	48	53
68c$_{cis}$ /68c$_{trans}$	-55[c]	2*S*,3*S* (65) 2*R*,3*S* (35)	−15	49	53
68d$_{cis}$ /68d$_{trans}$	-36[c]	2*S*,3*S* (63) 2*R*,3*S* (37)	−25	44	53
71a$_{cis}$ /71a$_{trans}$(80/20)[c]	+117$_{cis}$[f]-97$_{trans}$[g]	2*S*,3*R* 2*R*,3*R*	−90	63 → >98[d] 63 → >98[e]	54
71b$_{cis}$ /71b$_{trans}$ (78/22)[c]	+136$_{cis}$[f]-80$_{trans}$[g]	2*S*,3*R* 2*R*,3*R*	−90	>98[d] >98[d]	54
71c$_{cis}$ /71c$_{trans}$	-14[c]	2*S*,3*R* (60) 2*R*,3*R* (40)	−120	55	54

[a]$CDCl_3$;

[b]major isomer;

[c]*cis*/*trans* equilibrium mixture in $CDCl_3$;

[d]difference (ppb) in δ_{OMe} values using **4a$_S$**;

[e]Literature reference;

[f]isomers **71a$_{cis}$** and **71b$_{cis}$** recrystallized from $CHCl_3$ (>98% *ee*);

[g]isomers **71a$_{trans}$** and **71b$_{trans}$** recrystallized from MeOH (>98% *ee*).

TABLE 5 $[\alpha]_D$, AC and % *ee* values of *cis*-diols (5, 76–79) and $\Delta\delta_{MeO}$, $\Delta\delta_{Me}$, $\Delta\delta_{Bn}$ and $\Delta\delta_{t\text{-}Bu}$ values of boronates (5′a-c) and (76′-79′a-c) using racemic MEPBA 4a$_R$ /4a$_S$, MDPBA 4b$_R$ /4b$_S$ and MPPBA 4c$_R$ /4c$_S$

Cis-diol	$[\alpha]_D$[a]	AC	$\Delta\delta_{OMe}$, $\Delta\delta_{Me}$ 4a$_R$ /4a$_S$	$\Delta\delta_{OMe}$, $\Delta\delta_{Bn}$, $\Delta\delta_{t\text{-}Bu}$ 4b$_R$ /4b$_S$	$\Delta\delta_{OMe}$, $\Delta\delta_{Bn}$ 4c$_R$ /4c$_R$	% *ee*	Lit.[b]
5	+41	1*S*,2*S*	0_{OMe}, 1_{Me}	29_{OMe}, 30_{Bn}, 12_{t-Bu}	5_{OMe}, 60_{Bn}	>98	15, 21
76	+39	1*S*,2*R*	115_{OMe}, 61_{Me}	97_{OMe}, 22_{Bn}, 189_{t-Bu}	129_{OMe}, 32_{Bn}	>98	59
77	+21	1*R*,2*S*	7_{OMe}, 5_{Me}	3_{OMe}, 5_{Bn}, 5_{t-Bu}	6_{OMe}, **76**$_{Bn}$	>98	43
78	+103	1*R*,2*S*	9_{OMe}, 3_{Me}	24_{OMe}, 13_{Bn}, 58_{t-Bu}	24_{OMe}, 64_{Bn}	>98	43
79	−60	1*R*	5_{OMe}, 0_{Me}	8_{OMe}, 2_{Bn}, 4_{t-Bu}	7_{OMe}, 56_{Bn}	88→ > 98	43

[a]$CHCl_3$;

[b]Literature reference.

and 2) signal multiplicity, and possible overlap of ^{1}H–NMR signals associated with the exocyclic chiral group of boronates **Da$_S$** or **Da$_R$**, including methyl doublets (Me) and benzylic quartets (Bn). To address these limitations, the syntheses of two new racemic phenylboronic acids, (±)-MDPBA **4b$_S$/4b$_R$** and (±)-MPPBA **4c$_S$ /4c$_R$**, were carried out (Scheme 3). The ^{1}H–NMR spectra of their boronate derivatives would have several diagnostic singlets, e.g., using (±)-MDPBA **4b$_S$/4b$_R$** (OMe, t-Bu and Bn) and (±)-MPPBA **4c$_S$ /4c$_R$** (OMe and Bn). Thus, reducing the possibility of signal overlap and the probability of increasing signal resolution, i.e., larger $\Delta\delta_{OMe}$, $\Delta\delta_{t\text{-}Bu}$, $\Delta\delta_{Bn}$ values.

The synthetic sequence used for the new boronic acids MDPBA and MPPBA, was identical to that reported earlier for MEPBA (Scheme 3).[37,38] Ketones **1b**, **1c** and racemic alcohols **2b$_R$/2b$_S$** and **2c$_R$/2c$_S$** were prepared using literature methods. The alcohols were obtained in good yield (91–95%) by reduction ($NaBH_4$/MeOH) of the corresponding ketones **1b** and **1c** (see Supporting Information). The synthesis of enantiopure alcohols **2b** and **2c** via asymmetric reduction of ketones **1b** and **1c** and their AC assignments had been reported.[55,56]

Asymmetric reduction methods, reported earlier for ketones (**1a–c**), were: 1) chemocatalysis with chiral oxazaborolidines, to give alcohols **2a$_S$** or **2a$_R$** and **2b$_S$** or **2b$_R$**,[37,55,57] 2) chiral Ru-BINAP/diamine complexes, to form alcohols **2c$_S$** or **2c$_R$**[56] and 3) biocatalysis using CREDs, to give alcohol **2a$_S$**.[38] Enantiopure (−)-(1*S*,2*R*)-*cis*-1-amino-2-indanol **74$_{1S,2R}$** and its (+)-(1*R*,2*S*)

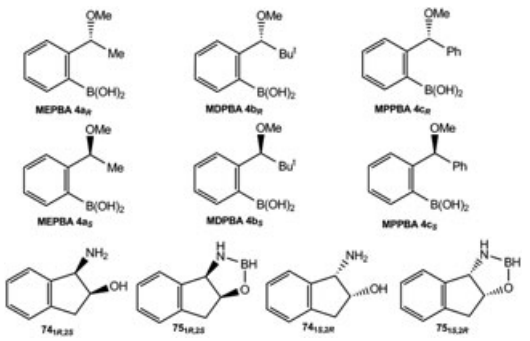


DIAGRAM 3 Structures $\mathbf{4a}_R$–$\mathbf{c}_R$, $\mathbf{4a}_S$–$\mathbf{c}_S$, $\mathbf{74}_{1R2S}$, $\mathbf{74}_{1S2R}$, $\mathbf{75}_{1R2S}$, $\mathbf{75}_{1S2R}$

enantiomer $\mathbf{74}_{1R,2S}$, available commercially and by chemoenzymatic synthesis,[58] were reacted ($BH_3.SMe_2$/THF), to form the corresponding oxazaborolidines ($\mathbf{75}_{1S,2R}$ and $\mathbf{75}_{1R,2S}$, Diagram 3). These chiral hydride transfer reagents were used in the asymmetric reduction of a range of ketones, to give the corresponding alcohols (79–95% *ee*).[58] This literature method and oxazaborolidines ($\mathbf{75}_{1S,2R}$ and $\mathbf{75}_{1R,2S}$, Diagram 3), were applied to the asymmetric reduction of ketones **1b** and **1c**, to give alcohols **2b** (77% yield, 94% *ee*) and **2c** (84% yield, 98% *ee*). Reduction of ketone **1a**, under similar conditions, yielded alcohol **2a** (83% yield, 81% *ee*); Ru-complex with aminoalcohol $\mathbf{74}_{1S,2R}$ (or $\mathbf{74}_{1R,2S}$) gave better results (89% yield, 98% *ee*).

Methylation of racemic and enantioenriched alcohols (**2a**–**c**, 94–98% *ee*), conducted according to the literature method (NaH/MeI/THF),[37,38] yielded the corresponding methyl ethers **3a** (95% yield), **3b** (92% yield), and **3c** (98% yield). Transmetallation, followed by crystallization from hexane,[38,39] yielded both racemic and enantiopure forms of the chiral boronic acids **4a** (59% yield), **4b** (64% yield) and **4c** (59% yield) **(**Scheme 3). Recrystallization of the new boronic acids (**4b** and **4c**), derived from the alcohols **2b** (94% *ee*) and **2c** (98% *ee*), provided single enantiomers, whose structures, ACs and *ee* values (>98%) were confirmed by X-ray crystallography.

The monomeric structures of the (±)-boronic acids $\mathbf{4b}_R/\mathbf{4b}_S$ and (+)-$\mathbf{4c}_R$, shown in Figure 1, contained dihedral angles of 26° and 60°, respectively, between the disubstituted aryl rings and the attached planar $B(OH)_2$ groups. Only intermolecular hydrogen bonding was found in crystalline (+)-boronic acid $\mathbf{4c}_R$ enantiomer, the molecules being linked in infinite chains via hydrogen bonding engaging both hydroxyl groups. Surprisingly, racemic $\mathbf{4b}_{R/S}$ showed a strong intramolecular hydrogen bond between an OH group and a proximate OMe group (B-O-H - - - O-Me), thus rendering the OMe oxygen atom chiral in the solid state. There is also intermolecular hydrogen bonding between OH groups, and the molecules existing as hydrogen-bonded dimers in the solid state. While the OMe oxygen atom is pyramidal, with respect to the attached covalently-bonded and hydrogen-bonded atoms, the OH oxygen atoms in both (±)-$\mathbf{4b}_R$ /$\mathbf{4b}_S$ and (+)-$\mathbf{4c}_R$ are trigonal planar. This implies sp^2 hybridization, with the filled vertical *p* orbitals on oxygen interacting with the empty *p* orbital on boron.

Boronic acid monomers are known to dehydrate, to form the corresponding cyclotrimeric anhydrides (boroxines), and both readily interconvert in solution at ambient temperature. Although the racemic boronic acid $\mathbf{4b}_R$ /$\mathbf{4b}_S$ was found to be a monomer in the crystalline state, during the removal of solvent (CH_2Cl_2) under vacuum, the (+)-(*R*) enantiomer $\mathbf{4b}_R$ crystallized out preferentially as boroxine $\mathbf{4b'}_R$; the molecule lies on a crystallographic three-fold axis of symmetry. The boroxine ring, being isoelectronic with benzene, is planar with the aryl substituents adopting a propeller conformation and an interplanar angle of 35.6° between the boroxine and aryl rings. The AC and *ee* value (>98%) of (+)-boronic acid monomer $\mathbf{4b}_R$ was established from the corresponding boroxine X-ray crystal structure $\mathbf{4b'}_R$ (Figure 1). A solution of the enantiopure crystalline (+)-boroxine $\mathbf{4b'}_R$ was found to react spontaneously with *cis*-diols in a similar manner to (±)-monomer $\mathbf{4b}_R$ /$\mathbf{4b}_S$.

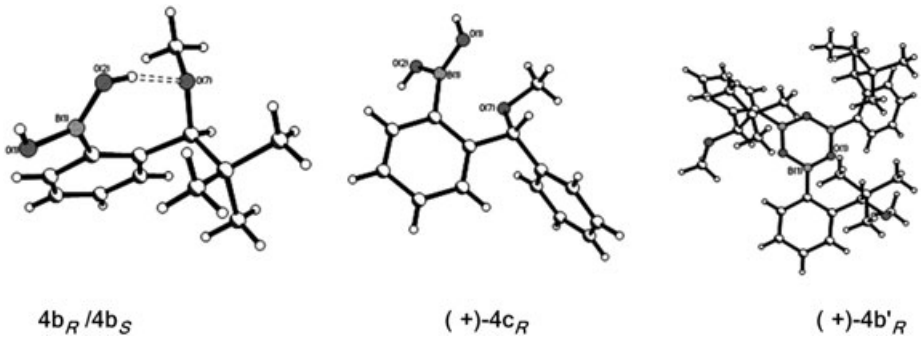


FIGURE 1 X-ray crystal structures of (±)-boronic acids $4b_R/4b_S$, (+)-$4c_R$ and (+)-boroxine $4b'_R$

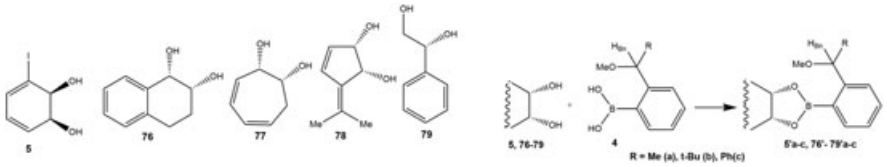


DIAGRAM 4 Structures **5**, **76**–**79**, **5′a**–**c**,**76′a**–**c**, **77′a**–**c**, **78′a**–**c**, **79′a**–**c**

2.6 | Determination of % *ee* values of *cis*-diols (5, 76–79) using MEPBA $4a_R$ /$4a_S$, MDPBA $4b_R$ /$4b_S$, and MPPBA $4c_R$ /$4c_S$

The cyclic *cis*-diols metabolites (**5**, **76–78**) were isolated as single enantiomers (>98% *ee*), but an enantiopure sample of acyclic diol metabolite **79** was only obtained by recrystallization of the enantioenriched metabolite (88% *ee*). ^{1}H–NMR separations of diagnostic signals from MeO ($\Delta\delta_{OMe}$), benzylic H ($\Delta\delta_{Bn}$) and *tert*-butyl ($\Delta\delta_{t\text{-}Bu}$) groups of boronate diastereomers formed from vicinal *cis*-diol metabolites (**5**, **76–79**, Diagram 4), using MEPBA $\mathbf{4a_R}$ / $\mathbf{4a_S}$, MDPBA $\mathbf{4b_R}$ /$\mathbf{4b_S}$, and MPPBA $\mathbf{4c_R}$ /$\mathbf{4c_R}$ are presented in Table 5.[15,21,43,59]

A comparison of $\Delta\delta_{MeO}$ values of boronates of *cis*-diol **76**, having a fused aromatic ring, formed with (±)-boronic acids $\mathbf{4a_R}$ /$\mathbf{4a_S}$, $\mathbf{4b_R}$ /$\mathbf{4b_S}$, and $\mathbf{4c_R}$ /$\mathbf{4c_R}$, showed that they were consistently larger (97–129) compared to *cis*-diols **5** (0–29), **77** (3-7), **78** (9-24), and **79** (5-8). The $\Delta\delta_{Me}$ values for boronates found, using these *cis*-diols and boronic acid $\mathbf{4a_R}$ /$\mathbf{4a_S}$, were smaller than their $\Delta\delta_{OMe}$ values. Boronates formed using MDPBA $\mathbf{4b_R}$ /$\mathbf{4b_S}$ and diols (**5**, **76–79**) showed a wide range of values, e.g., $\Delta\delta_{t\text{-}Bu}$ (4–189) and $\Delta\delta_{Bn}$ (2-30), while boronates of MPPBA $\mathbf{4c_R}$ /$\mathbf{4c_R}$, that recorded larger $\Delta\delta_{Bn}$ values (32–76), proved to be the most consistently reliable chiral boronic acid.

2.7 | Application of (+)-MPPBA reagent $\mathbf{4c_R}$ for the determination of *ee* values of chiral catechols

The TDO-catalyzed *cis*-dihydroxylation of substituted benzyl alcohol enantiomers yielded triol diastereomers (**80a–e** and **81a–e**, Diagram 5).[60] These triols, in turn, proved to be good substrates for a naphthalene diol

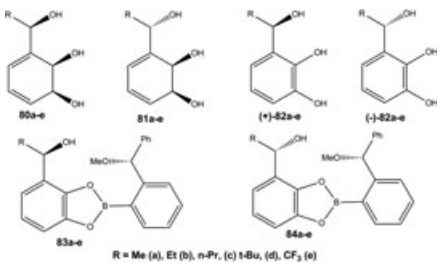


DIAGRAM 5 Structures **80a–e, 81a–e, (+)–82a–e, (−)–82a–e, 83a–e, 84a–e**

TABLE 6 $[\alpha]_D$, AC and % *ee* values of catechols (82a–e) and $\Delta\delta_{OMe}$ values of boronates (83a–e and 84a–e)

Catechol	$[\alpha]_D$[a]	AC	$\Delta\delta_{OMe}$[b](−)-(*S*)	% *ee*	Lit.[c]
82a	+18[c]	*R*	20	>98	61
82b	+20[c]	*R*	30	>98	61
82c	+16[c]	*R*	30	>98	61
82d	+23	*R*	120	>98	63
82e	+13	*S*	90	>98	63

[a]$CDCl_3$;

[b]difference in δ_{OMe} values of boronates (**83a–e** and **84a–e**) formed using MEPBA $\mathbf{4a_S}$;

[c]Literature reference.

dehydrogenase enzyme, which catalyzed their biotransformations, to yield individual (+)- and (−)-catechol enantiomers (**82a–e**).[61-63] As separations ($\Delta\delta_{OMe}$) of diagnostic ^{1}H–NMR signals of the boronates, formed using MEPBA $\mathbf{4a_S}$ with (+) and (−)-catechols (**82a–e**), were relatively small (δ_{OMe} 3.15–3.28), it was considered appropriate to test the new boronic acid (+)-MPPBA $\mathbf{4c_R}$ (Table 6).[61,63] The magnitude of the observed values ($\Delta\delta_{OMe}$ 20–120), for boronates from enantiomers of catechols (**82a–e**) with (+)-MPPBA $\mathbf{4c_R}$, confirmed that the reagent can be successfully applied, to determine the *ee* values of chiral catechol metabolites derived from benzylic alcohols, and possibly also to catechols derived from alkylaryl sulfoxides.[54]

3 | CONCLUSION

The versatility of racemic MEPBA $\mathbf{4a_R}$ /$\mathbf{4a_S}$ and enantiopure MEPBA ($\mathbf{4a_R}$ and $\mathbf{4a_S}$) for the determination of *ee* values was demonstrated by the formation of boronate diastereoisomers of chiral *cis*-diol metabolites of alkenes, mono- and poly-cyclic arenes, and heteroarenes, phenols, and catechol metabolites. The MEPBA reagents ($\mathbf{4a_R}$ /$\mathbf{4a_S}$, $\mathbf{4a_R}$, and $\mathbf{4a_S}$) were also utilized in stereochemical analysis of 1) *cis* β-amino alcohols,[64] synthesized in five steps from polycyclic *cis*-dihydrodiol metabolites, 2) 1,3-diols,[37] and 3) 2-hydroxyacids.[37] Preliminary results on the synthesis and applications of racemic and enantiopure forms of the new chiral boronic acids MDPBA **4b** and MPPBA **4c**

suggest that they may offer significant advantages over the MEPBA reagents **4a**. Although the boronates of chiral boronic acids (**4b** and **4c**) in this study were only used to determine *ee* values of catechols and *cis*-diols of alkenes and arenes, they should equally be applicable to *cis* β-amino alcohols,1,3-diols, and 2-hydroxyacids.

The consistent upfield shift ($CDCl_3$) in the direction of OMe signals (δ_{OMe}), associated with (−)-(*S*)-MEPBA boronate derivatives, originally observed for a relatively small number of arene *cis*-dihydrodiols,[38-40] was also recorded in the large number of examples shown in Tables 1, 3, and 5. These examples lend further support to the boronate method for the assignment of absolute configurations to this type of metabolite. It should be noted, however, that an inconsistent trend in the OMe signal direction of shift (δ_{OMe}) was observed with boronate derivatives of cyclohex-2-en-1-one *cis*-diol metabolites with (−)-MEPBA (Table 2). Thus, where possible, more rigorous alternative methods, e.g., X-ray crystallography and ECD spectroscopy, should also be used for AC determination.

ACKNOWLEDGMENTS

We thank Dr. C-T Yeung (Hong Kong Polytechnic University) for providing helpful chiroptical information about compound **2b**$_R$ and Prof. T. Moody (Almac Sciences Ltd) for preliminary results obtained using SelectAZyme CREDs. Funding was provided for CAST Awards by the Department of Education, Northern Ireland (GMMcC, PAG) and for a Postgraduate Studentship by the European Social Fund (JSH).

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How to cite this article: Boyd DR, Sharma ND, Goodrich PA, et al. Enantiopurity and absolute configuration determination of arene *cis*-dihydrodiol metabolites and derivatives using chiral boronic acids. *Chirality*. 2017;1–14. https://doi.org/10.1002/chir.22764