Chemoenzymatic Synthesis of Chiral Boronates for the ^{1}H NMR Determination of the Absolute Configuration and Enantiomeric Excess of Bacterial and Synthetic *cis*-Diols

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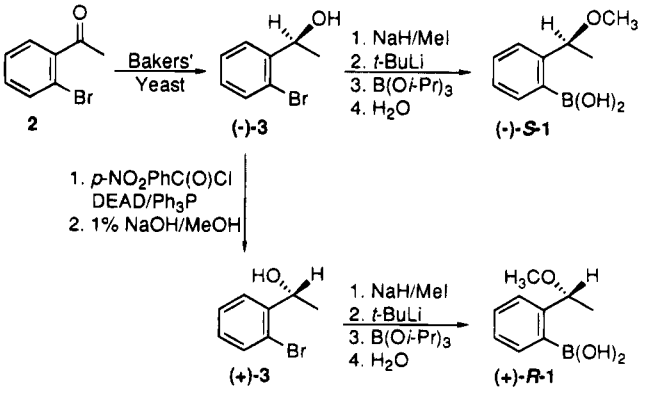
We report on a chemoenzymatic synthesis yielding enantiomerically pure [(+)- and (−)-2-(1-methoxyethyl)-phenyl]boronic acids (**1**) and describe general methods for their use in the ^{1}H NMR determination of the absolute configuration and enantiomeric excess (ee) of *cis*-diols formed by the bacterial dioxygenation of mono- and polycyclic arenes. The procedure is simple, can be applied to small sample amounts (<2 mg), and requires little or no purification of products or intermediates prior to NMR analysis.

The growing demand for single isomer chiral pharmaceuticals[1] accompanied by the desire for the "greening" of organic chemistry[2] has led to an increase in the production of chiral synthons by microbial biocatalysis. These optically active compounds have served as starting points for the synthesis of a wide variety of natural products and biologically active compounds. Some of the most versatile and popular synthons are the homochiral arene-*cis*-diols obtained from the bacterial dioxygenation of mono- and polycyclic arenes.[3] The *cis* dihydroxylation reactions are catalyzed by multicomponent bacterial dioxygenase enzymes which incorporate molecular oxygen into the aromatic nucleus to yield *cis*-diols in high ee.[4–13] The high enantiomeric purity of bacterial *cis*-diols has led to their use in the asymmetric syntheses of a variety of novel acyclic sugars, conduritols, inositols, and complex bioactive natural products, often in better yields and in fewer steps than conventional synthetic methods.[14–17]

As the use of these synthons has increased, so has the need for a simple method for the determination of the absolute configuration and ee of the large number of diols produced during screening for novel metabolites. Traditional methods employed for determining the absolute configuration of monocyclic and polycyclic arene-*cis*-diols have included their chemical transformation to known compounds, conversion to diastereomers, X-ray crystallography, and stereochemical correlation.[5,6,8] Owing to their propensity to dehydrate to the more stable phenols, even under mildly acidic conditions, methodology involving the preparation of bis-Mosher's esters from the acid chlorides has proven difficult. The solution to date has been to initially hydrogenate the double bond to prevent aromatization[18] or to form a stable cycloadduct of the diene prior to its conversion to the diester.[11] Both routes require purification of the final diesters as well as the intermediates prior to esterification. They also require substantial amounts of diol as well as time. However, the recent report of the chiral boronic acid (+)-**1** for the determination of optical purity (ee)[19] appears to have promise in the determination of absolute configuration. We have used a biocatalytic asymmetric reduction coupled with a divergent synthesis to yield both optical isomers of reagent **1**. Use of both (+)- and (−)-forms of chiral boronic acid (**1**), obtained by chemoenzymatic methods, and a series of microbially-produced homochiral arene-*cis*-diols of known absolute stereochemistry (Figure 1) allows us to expand the use of diastereomeric alkyl boronate esters to the determination of absolute configuration.

Results and Discussion

Bakers' yeast reduction of 2′-bromoacetophenone, **2**, yielded the corresponding enantiomerically pure *S*-alcohol, (−)-**3**, in good yield (ca. 70%). Inversion of the alcohol by modified Mitsumobu conditions[20] afforded (+)-**3** (>99% ee) in fair yield (40%). The enantiomerically pure (+)- and (−)-alcohols (**3**) were methylated and converted to their respective chiral *o*-boronic acids, (+)- and (−)-**1**, by previously reported methods.[19] The formation of both



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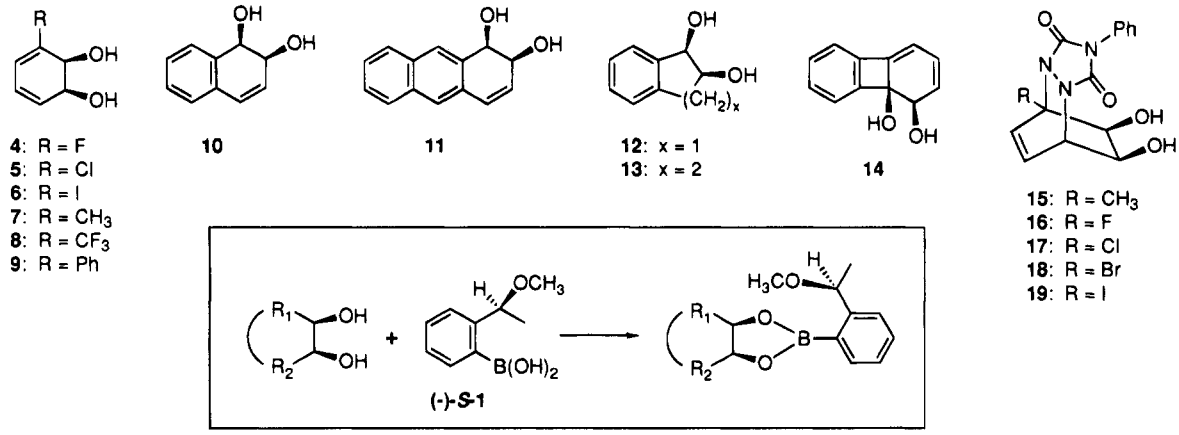


Figure 1. Structures and absolute configuration of the bacterial *cis*-diols used in the present study. Inset depicts the general derivatization scheme for *cis*-diols (**4**–**14**) or their cycloadducts (**15**–**19**) with chiral boronic acids.

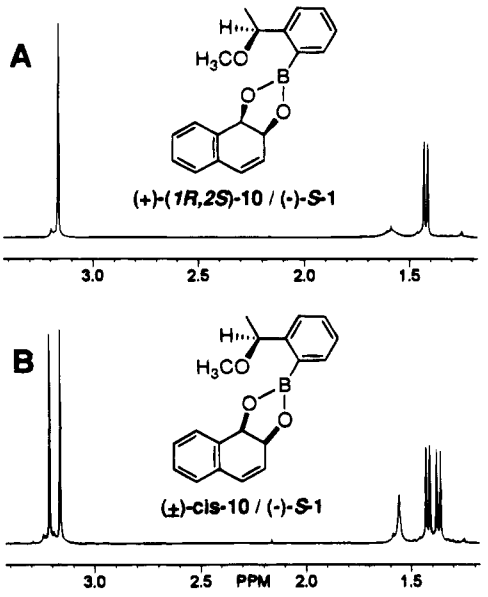


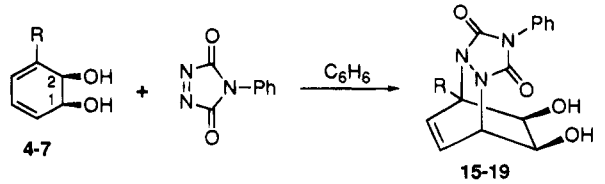
Figure 2. ^{1}H NMR spectra (360 MHz) showing the diastereomeric signals of the boronate esters formed via (−)-*S*-**1** with (A) (+)-*cis*-(*1R,2S*)-dihydroxy-1,2-dihydronaphthalene, **10**, and (B) synthetic (±)-*cis*-1,2-dihydroxy-1,2-dihydronaphthalene.

(+)-*R*- and (−)-*S*-**1** allowed us to form diastereomeric boronate esters with a series of homochiral *cis*-diols and determine if trends in ^{1}H NMR directional shifts could be correlated to the absolute stereochemistry of the diols.

Derivatization of diols **4**–**14** was carried out with a slight excess of (+)- and (−)-boronic acid reagent, **1** (Figure 1). Examination of the resulting boronate esters of diols **7**–**14** by ^{1}H NMR (360 MHz, $CDCl_3$) revealed separation of the diastereomeric OMe and Me signals as illustrated for *cis*-1,2-naphthalene-1,2-dihydrodiol (**10**) in Figure 2. More importantly, a consistent pattern was observed in which the OMe signal for *cis*-diols **7**–**14** derivatized with (−)-**1** was shifted upfield from the OMe signal of the opposite diastereomer formed with (+)-**1** (or the ester formed with (−)-**1** and the opposite enantiomer of the diol) (Table 1). The opposite trend was observed for the Me signal of the esters of diols **7**–**14** formed with (−)-**1**, which were shifted downfield from the Me signal of the esters formed with (+)-**1**. These effects were clearly demonstrated by analyzing the enantiomerically pure *cis*-diols derivatized with (−)- and (+)-**1** and by examining mixtures of the esters (or racemic *cis*-diols derivatized with either (−)- or (+)-**1**). The consistent trend observed in the directional shifts of the diagnostic OMe and Me signals for the boronate esters of **7**–**14** allows for absolute stereochemical determination for novel diols of similar structure and unknown configuration.

The boronate esters of halocyclohexadiene-*cis*-diols **4**–**6** showed little or no resolution in the chemical shifts of the diastereomeric OMe or Me signals in $CDCl_3$. However, when these esters were dissolved in C_6D_6, ^{1}H NMR spectra showed separation of diastereomeric signals and allowed for the use of the reagent's OMe group (**4**–**6**) as a diagnostic signal (Table 1). The esters of **7** and **8** showed little or no resolution of diagnostic signals in C_6D_6. In contrast to the boronate esters of **7**–**14**, the (−)-**1** esters of *cis*-diols **4**–**6** dissolved in C_6D_6 showed a downfield shift for the reagent's OMe signal and an upfield shift for the Me signal (Table 1). The finding that the directional shifts for the boronate esters of **7** and **9** were unchanged in C_6D_6 and $CDCl_3$ suggests that the deviation from the previously observed trend (i.e. esters **7**–**14**) may be related to the electronegativities of the halogens (corresponding decreases in the $\Delta\delta_{OMe}$ are seen with increasing electronegativity of the halogen substituent in the esters of **4**–**6**).

In an attempt to minimize substituent effects and apply the methodology to substituted cyclohexadiene-diols, **4**–**7** were reacted with 4-phenyl-1,2,4-triazoline-3,5-dione and the resulting cycloadducts[11] (**15**–**19**, Figure 1) were derivatized with (+)- and (−)-**1**. Analysis (^{1}H



NMR, 360 MHz) of the boronate esters of the *cis*-diol cycloadducts **15**–**19** showed excellent resolution of dia-

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Table 1. ^{1}H NMR Chemical Shifts[a] of Diastereotopic Signals from *cis*-Diols (4–14) or Cycloadducts (15–19) Derivatized with (−)-*S*-1

no.	compound[e]	*cis*-diol absolute config[b]	% ee	OCH_3 δ (ppm) ($CDCl_3$ or [C_6D_6])	OCH_3 Δδ (ppb)	CH_3 δ (ppm) ($CDCl_3$ or [C_6D_6])	CH_3 Δδ (ppb)
4	fluorobenzene	1*S*,2*S*	74	3.242	0[c]	1.420	0[c]
				[3.158]	[+13]	[1.526]	[−40]
5	chlorobenzene	1*S*,2*S*	>98	3.240	−3[c]	1.425	−5[c]
				[3.175]	[+16]	[1.551]	[−29][c]
6	iodobenzene	1*S*,2*S*	>98	3.255	0[c]	1.440	−15[c]
				[3.207]	[+23]	[1.573]	[−6][c]
7	toluene	1*S*,2*R*	>98	3.225	−9	1.418	+9[c]
				[3.173]	[−2][c]	[1.569]	[+11][c]
8	α,α,α-(trifluoromethyl)benzene	1*S*,2*S*	>98	3.218	−17	1.412	+19[c]
				[3.161]	[0][c]	[1.545]	[0][c]
9	biphenyl	1*S*,2*R*	>98	3.123	−89	1.385	+134
				[3.086]	[−91]	[1.562]	[+128]
10	naphthalene	1*R*,2*S*	>98	3.166	−51	1.424	+50
11	anthracene	1*R*,2*S*	>98	3.165	−66	1.441	+64
12	indene	1*R*,2*S*	68	3.061	−110	1.389	+60
13	1,2-dihydronaphthalene	1*R*,2*S*	>98	3.104	−117	1.418	+74
14	biphenylene	1*R*,1a*S*	>98	3.196	−81	1.441	+53
15	toluene[d]	1*S*,2*R*	>98	3.079	+13	1.324	−1[c]
16	fluorobenzene[d]	1*S*,2*S*	74	3.159	+20	1.352	−7[c]
17	chlorobenzene[d]	1*S*,2*S*	>98	3.145	+28	1.351	−6[c]
18	bromobenzene[d]	1*S*,2*S*	>98	3.139	+29	1.349	−6[c]
19	iodobenzene[d]	1*S*,2*S*	>98	3.132	+27	1.355	−4[c]

[a] Chemical shifts (δ) are reported for the (−)-*S*-1 boronate esters of *cis*-diols of the configuration shown in Figure 1, and the Δδ represents the distance and direction of the shift from the corresponding signal of the boronate ester formed via (+)-*R*-1. A negative Δδ value indicates that the signal of interest is upfield from the corresponding signal of the diastereomer formed with the opposite boronic acid; a positive Δδ means that the signal of interest is downfield from the corresponding signal of the ester formed with the opposite boronic acid. [b] All the *cis*-diols listed have the same relative stereochemistry for the hydroxyl-bearing carbon α to the substituent (**4**–**9**) or to the bridgehead carbon (**10**–**14**); differences in absolute configuration are due to the priorities established by the Cahn–Ingold–Prelog sequence rules. [c] Diastereomeric signals were insufficiently resolved or overlapped, negating their use for diagnostic purposes. [d] Cycloadducts **15**–**19** were analyzed in acetone-d_6 (360 MHz). [e] Refers to the (−)-*S*-1 ester of the *cis*-diol formed by the bacterial dioxygenation of this compound.

stereomeric OMe signals. The directional shifts for the diastereomeric signals of esters of **15**–**19** (Table 1) formed via (−)-**1** were reversed with respect to those of the boronate esters formed from underivatized *cis*-diols **7**–**14**. Thus, for (−)-**1** esters of cycloadducts **15**–**19**, the OMe signal was shifted downfield relative to its diastereomeric counterpart [cycloadducts **15**–**19** derivatized with (+)-**1**] (Table 1). Although the Me signals of these esters (observed as overlapping doublets) were not useful for diagnostic purposes, the absolute stereochemistry of the halocyclohexadiene-*cis*-diols is most suitably analyzed as (+)- and (−)-**1** boronate esters of their cycloadducts (i.e. **16**–**19**) in accordance with the trends observed for the OMe signals (Table 1).

Use of (−)- and (+)-**1** to generate diastereomeric boronate esters of *cis*-diols provides a simple and rapid method for determination of ee[19,21] and, as shown here, can be correlated to the absolute configuration of *cis*-diols (Table 1). The advantages described herin include the biocatalytic asymmetric reduction of **2** to (−)-**3** (>99% ee) by bakers' yeast. The absolute purity of (−)-**3** eliminates the requisite multiple recrystallizations to upgrade the ee of the alcohol obtained via the analogous chemical reduction.[19] In addition, the inversion of the yeast-derived (−)-*S*-alcohol to (+)-**3** allows for the synthesis of both enantiomers of optically pure **1**. The derivatization methodology is efficient and can be performed on as little as 1–2 mg of diol. This should facilitate identification of bacterial dioxygenase systems exhibiting novel enantiospecificity (i.e. diol **14**) and aid in the screening of larger numbers of metabolites. While this examination of *cis*-diols is by no means exhaustive, the trends relating the direction of diastereomeric chemical shifts to absolute stereochemistry are consistent for polycyclic *cis*-dihydrodiols, "angular" *cis*-monohydrodiols, and cycloadducts of substituted cyclohexadiene-*cis*-diols. Analysis of additional classes of diols and other chiral compounds[22] of known configuration should expand the utility of this technique for establishing the absolute configuration and ee of novel compounds which may prove useful as chiral synthons. Such studies are presently underway.

Experimental Section

^{1}H NMR spectra were recorded on a Bruker WM-360 spectrometer. GCMS, chiral stationary phase (CSP) HPLC, $[\alpha]_D$, preparative TLC, and radial dispersion chromatography (RDC) were performed under conditions previously described.[23] Chemicals were commercially available and used without further purification or prepared as described below.

Chemoenzymatic Synthesis of [(−)- and (+)-2-(1-Methoxyethyl)phenyl]boronic Acid (1). Bakers' Yeast Reduction of *o*-Bromoacetophenone (2) to (−)-(*S*)-*o*-bromo-*sec*-phenethyl Alcohol, ((−)-*S*-3). Typical reaction mixture contained 25 g of Fleishman's dry bakers' yeast, 30 g of glucose, and 1.4 mL of **2** (20 mM, added at 30 min) in 500 mL of 0.05 M sodium–potassium phosphate buffer (pH 7.2). Batch reactions (500 mL) were incubated in 1.0 L Erlenmyer flasks at room temperature (23 ● 2 °C) with agitation supplied by 1 in. magnetic stirring bars. Additional yeast and glucose (15 g each) were added to reaction mixtures at 24 h intervals for 4 days. After 5 days, cells were removed by centrifugation (8000*g*, 10

(21) Enantiomeric excesses determined by this method (based on the magnitude of the integrals of diastereomeric signals) were in complete agreement with values determined by CSP-HPLC for synthetic racemic *cis*-diols **10**, **12**, and **13** and for biologically-produced **4** (74% ee).

(22) Other compounds which can yield diastereomeric boronate esters include chiral 1,3-diols, 2-hydroxy acids, and 2-amino alcohols (see ref 19). Although results obtained thus far are compelling and may provide a useful empirical rule, the application of the rule to assign absolute stereochemistry is based on indirect methodology (stereochemical correlation) and, in the absence of known reference compounds, does not constitute proof of stereochemistry.

(23) Resnick, S. M.; Torok, D. S.; Lee, K.; Brand, J. M.; Gibson, D. T. *Appl. Environ. Microbiol.* **1994**, *60*, 3323–3328.

min) and the supernatants were extracted with ethyl acetate, giving (−)-**3**. The crude alcohol (4.1 g) was purified by RDC using 4 mm thick silica plates eluted with a hexane−EtOAc step gradient (0−50% EtOAc, 10% steps over 1 h, flow 8 mL/min) to give (−)-**3** (2.9 g, 70% yield): ^{1}H NMR ($CDCl_3$) δ 7.82 (d, J = 7.82 Hz, 1 H), 7.50 (d, J = 7.92 Hz, 1 H), 7.33 (t, J = 7.05 Hz, 1 H), 7.10 (t, J = 7.82 Hz, 1 H), 5.23 (q, J = 6.4 Hz, 1 H), 2.29 (bs, 1 H), 1.47 (d, J = 6.0 Hz, 3 H) (lit.[19]); ^{13}C NMR ($CDCl_3$) δ 144.6, 132.6, 128.7, 127.8, 126.6, 121.7, 69.2, 23.6; GCMS (EI, 70 eV) m/z (% base peak) 202 (17), 200 (19), 187 (75), 185 (86), 159 (20), 157 (26), 105 (10), 77 (100); $[\alpha]_D$ −58.8 (c 0.17, CH_2Cl_2). The absolute stereochemistry of (−)-**3** was established by stereochemical correlation of the diasteromeric esters, formed with (*R*)-(−)- and (*S*)-(+)-*O*-methylmandelic acid, to those formed with the known (−)-(*S*)-*sec*-phenethyl alcohol.[24] The enantiomeric purity of (−)-*S*-**3** was confirmed to be >99% ee by CSP-HPLC using a Chiralcel OB column [hexane−2-propanol (9:1), 0.5 mL/min]. CSP-HPLC resolved (−)-*S*-**3** at a t_R of 9.6 min; racemic **3**, prepared by $NaBH_4$ reduction of **2**, was resolved as its (−)-*S*- and (+)-*R*-enantiomers at 9.6 and 11.3 min, respectively.

(+)-(*R*)-*o*-bromo-*sec*-phenethyl Alcohol ((+)-*R*-3). Inversion of (−)-*S*-**3** employed modified Mitsunobu conditions.[20] To a solution of (−)-*S*-**3** (502 mg, 2.5 mmol), *p*-nitrobenzoic acid (1.67 g, 10 mmol), and triphenylphosphine (2.62 g, 10 mmol) in dry THF (20 mL) at 0 °C was added diethyl azodicarboxylate (1.58 mL, 10 mmol) dropwise. The resulting solution was stirred for 12 h, concentrated, redissolved in CH_2Cl_2 (40 mL), and dried over anhydrous Na_2SO_4. The solution was filtered through a plug of silica (4 × 4 cm), concentrated, applied to a 2 mm thick silica RDC plate, and eluted with a hexane−EtOAc step gradient (conditions as for (−)-*S*-**3** above) to give 780 mg (89% yield). The resulting ester was dissolved in 1% NaOH−MeOH (25 mL) and stirred for 2 h. The solution was extracted with CH_2Cl_2 (50 mL × 2) and dried over Na_2SO_4. The concentrated material was applied to a 2 mm thick RDC plate and eluted with hexane−EtOAc (as above) to yield pure (+)-*R*-**3** (187 mg, 37%): $[\alpha]_D$ +58.8 (c 0.57, CH_2Cl_2).

Methylation of (−)-*S*-3 and (+)-*R*-3. (−)-*S*-**3** (0.98 g) in THF (15 mL) was treated with NaH (1.5 equiv) for 1 min, followed by MeI (4 equiv). After stirring for 20 h, the reaction mixture was quenched by dropwise addition of water, concentrated to remove the THF, extracted with CH_2Cl_2 (20 mL × 3), dried over Na_2SO_4, and concentrated to give (−)-(*S*)-1-methoxy-1-(2-bromophenyl)ethane as a yellow oil (1.02 g, 97%): GCMS (EI, 70 eV) m/z (% base peak) 216 (6), 214 (6), 202 (8), 201 (98), 199 (100), 185 (11), 183 (12), 104 (24); $[\alpha]_D$ −99.7 (c 1.54, CH_2Cl_2). The methylation of (+)-*R*-**3** yielded the expected (+)-(*R*)-1-methoxy-1-(2-bromophenyl)ethane: $[\alpha]_D$ +100 (c 1.62, CH_2Cl_2). ^{1}H and ^{13}C NMR spectra were consistent with previously reported values,[19] but our data indicate that the specific rotation ($[\alpha]_D$ = −100) of (*R*)-1-methoxy-1-(2-bromophenyl)ethane reported previously[19] should be $[\alpha]_D$ = +100.

[2-(1-Methoxyethyl)phenyl]boronic Acids ((−)-*S*- and (+)-*R*-1). The (*R*)- and (*S*)-1-methoxy-1-(2-bromophenyl)ethanes were converted to their respective *o*-triisopropylborates and then hydrolyzed to the [2-(1-methoxyethyl)phenyl]boronic acids, (+)-*R*- and (−)-*S*-**1**, as previously reported.[19] (−)-*S*-**1**: ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 7.82 Hz, 1 H), 7.35 (m, 2 H), 7.18 (d, J = 7.82 Hz, 1 H), 7.03 (s, 2 H), 4.44 (q, J = 6.62 Hz, 1 H), 3.28 (s, 3 H), 1.55 (d, J = 6.73 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 146.4, 137.6, 130.3, 128.9, 127.7, 104.2, 83.5, 56.3, 22.7; $[\alpha]_D$ −28.7 (c 2.3, CH_2Cl_2). The (+)-*R*-**1** obtained via the inverted alcohol, (+)-*R*-**3**, exhibited the same physiochemical characteristics but had the optical rotation $[\alpha]_D$ = +27.2 (c 4.6, CH_2Cl_2) (lit.[19]).

(24) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.

Bacterial Production of *cis*-Diols. The *cis*-diols shown in Figure 1 were formed by bacterial strains expressing toluene dioxygenase (**4**−**9**),[5,6] naphthalene dioxygenase (**10**−**13**),[7,8] and carbazole dioxygenase (**14**).[25] A number of these and other homochiral *cis*-diols are commercially available from Genencor International, Inc. (South San Francisco, CA).

The general biotransformation procedure for preparing the *cis*-diols[26] was as follows: Cultures (800 mL) of appropriate bacterial strains grown under conditions which induce their hydrocarbon dioxygenases[27] were collected by centrifugation and the cells resuspended in 400 mL of sodium−potassium phoshate buffer (0.05 M, pH 7.2) containing 0.1% hydrocarbon substrate and 0.2% pyruvate. The reaction mixtures were incubated for 24 h at 30 °C with rotary shaking (220 rpm) and centrifuged to remove the cells; the clarified supernatants were extracted (3×) with NaOH-washed EtOAc and the resulting extracts dried over Na_2SO_4 and concentrated under reduced pressure. The *cis*-dienediols were isolated by either PLC or RDC as previously described[23] and analyzed for purity by ^{1}H NMR and GCMS of phenylboronic acid derivatives.[17,27] The specific optical rotation of each purified diol was used to confirm or correlate the absolute stereochemistry (previously reported) for *cis*-diols **4**−**14** in Figure 1. The enantiomeric compositions of the diols **4**−**14** determined by CSP-HPLC (Chiralcel OB or OJ column as previously described[23]) were confirmed (±2%) by the integrals of the diagnostic signals of diastereomeric boronate esters (see Table 1). Diol **14** ($[\alpha]_D$ = −497 (c 1.03, MeOH)) has a structure identical to but a stereochemistry opposite of that previously described.[13] The novel diol enantiomer (**14** , Figure 1) was produced as the sole product (>98% ee) by incubating carbazole-grown cells of *Pseudomonas* sp. strain C250 with biphenylene (0.02%) for 72 h under the biotransformation conditions described above.

General Derivatization Procedures. Derivatization of diols **4**−**14** (typically 2−10 mg) was carried out in $CHCl_3$ (ca. 2 mL), employing a slight excess (1.1 equiv) of (+)- and (−)-**1**. Reaction mixtures were stirred at room temperature for 0.5 h (formation of boronate esters was monitored by TLC) and filtered through a small plug of Na_2SO_4 and silica gel to remove excess acid. The derivatives were then concentrated to dryness under reduced pressure and dissolved in the appropriate solvent for ^{1}H NMR analysis.

A similar methodology was applied to diols **4**−**7** which were first reacted with 4-phenyl-1,2,4-triazoline-3,5-dione (1.1 equiv, C_6H_6, 16 h). The resulting cycloadducts[11] (**15**−**19**, Figure 1) were concentrated to dryness, dissolved in $CHCl_3$, and, without further purification, derivatized with (+)- and (−)-**1** (1.1 equiv, 1 h). The reaction mixtures were concentrated, dissolved in acetone-d_6, and filtered through a small plug of Na_2SO_4 and silica gel directly into NMR tubes.

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(26) Yields of the purified *cis*-diols ranged from 0.2 to 1.0 g/L.
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