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Enantioselective hydrolysis of various substituted styrene oxides with *Aspergillus Niger* CGMCC 0496†

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Enantioselective biohydrolysis of various substituted styrene oxides using whole fungus cells of *Aspergillus niger* **CGMCC** 0496 are described. The results show not only *para*- but also some *ortho*- substituted styrene oxides can achieve high enantioselectivity during the hydrolysis.

Introduction

Enantiomerically pure forms of substituted phenylethanediols and substituted styrene oxides are important intermediates for the synthesis of various biologically active molecules. Recently, a number of chemical and biochemical methods have been developed to obtain these optically pure building blocks.[1] One of the most useful bioapproaches is enantioselective hydrolysis of racemic epoxides by epoxide hydrolases.

Epoxide hydrolases (EHs) are very important enzymes which have been detected in many organisms such as mammals,[2] plants,[3] insects,[4] and microorganisms.[5] Because of the widespread distribution in metabolism of various xenobiotics, mammalian epoxide hydrolases have been extensively studied during the past two decades.[6] However, due to their low availability, the potential of mammalian enzymes as chiral catalysts is seriously limited in the light of the preparative scale.

Recently detailed searches for EHs from microbial sources have been undertaken by several groups. A lot of microbial origin EHs have been identified,[7] and some show extremely good enantioselectivity when hydrolyzing *para*-substituted styrene oxides.[8] However, there have been few results on the substrates of *ortho*-[9] and *meta*-substituted styrene oxides. More recently, we screened EHs among several strains of *Aspergillus niger* which were isolated from soil. Several of them were found to have extremely highly active and enantioselective EHs,[10] especially *A. niger* **CGMCC** 0496.[9]

In this paper, we investigated the biocatalytic hydrolysis of various substituted styrene oxides using whole cells of *A. niger* **CGMCC** 0496.

Results and discussion

As we know, most epoxides are unstable in acidic and basic conditions. Hence it was important to minimize the chemical hydrolysis of the epoxide by selecting appropriate substrates for the study of the enantioselectivity of this strain. During the research, the substituted styrene oxides, especially nitrostyrene oxides, were found to be stable and resistant to a non-enzymatic spontaneous degradation. In order to get reliable results on the enantioselectivity and specificity of the strain, *p*-nitrostyrene oxides were used as the model substrates.

Temperature is an important factor in a biocatalytic system. It has been reported that the temperature greatly affected not only the performance of the reaction but also the enantioselectivity of the kinetic resolution.[11] As a result, in this case, the most favorable temperature for the biocatalytic hydrolysis reaction was determined first. (Table 1)

From Table 1 it clearly appears that the best temperature for the whole cell biocatalytic system was 25 °C. After 109 min, (*S*)-epoxide was recovered in 42% yield with 94% ee and the corresponding (*R*)-diol was obtained in 45% yield with 95% ee. When the reaction was carried out at 30 °C and the product diol reached 93% ee, the residue epoxide was only recovered with 78% ee. When the reaction was carried out at 20 °C, no observable hydrolysis could be found even with a prolonged reaction time.

The stereochemical pathways for the biohydrolysis of 1,2-epoxides using epoxide hydrolases are complex.[12] Two enantiomers of diols may be found when hydrolyzing epoxides using a single enantiomer. When the enzyme attacks the terminal carbon of the epoxide, the configuration of the product can be retained. Otherwise, the enzyme attacks the secondary carbon of the epoxide, *via* a S_N2 mechanism and the configuration of the product is reversed.

In order to obtain detailed information about the bioprocess, (*S*)-*p*-nitrostyrene oxide (>99% ee) and (*R*)-*p*-nitrostyrene oxide (>98% ee) were selected as microbial substrates for hydrolysis. (*S*)-*p*-Nitrostyrene oxide was obtained by biohydrolysis of its racemic form. (*R*)-*p*-Nitrostyrene oxide was prepared from its corresponding (*R*)-diol (Scheme 1). The (*R*)-diol was also obtained by hydrolysis and could easily be recrystallized from $CHCl_3$ leading to enantiopure material.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/ob/b3/b312469j/

Table 1 Biohydrolysis of *p*-nitrostyrene oxide by *A. niger* **CGMCC** 0496[a] under different temperatures

Temperature/°C	Reaction time/min	Yield of epoxide (%)	Ee of the epoxide (%) (abs. config.[b])	Yield of diol (%)	Ee of the diol (%) (abs. config.[b])
30	83	45	78 (*S*)	39	93 (*R*)
25	109	42	94 (*S*)	45	95 (*R*)
20	120	Biohydrolysis was not detected by TLC			

[a] The reactions were performed with 10 g cells : 200 mg substrate. [b] The absolute configurations were confirmed by comparing the optical rotations reported in the literature.[8]

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Table 2 Biohydrolysis of (*S*)- and (*R*)-*p*-nitrostyrene oxide with *A. niger* **CGMCC** 0496[a]

Substrate	Reaction time/min	Yield of diol (%)	Ee of diol (%) (abs. config.[b])
(*S*)-**1**	720	8	89(*S*)
(*R*)-**1**	32	>95	97(*R*)

[a] The reactions were carried out at 25 °C with 10 g cells : 0.1 g substrate. [b] The absolute configurations were established by comparisons with the elution order of the standard sample, (*S*)-*p*-nitrostyrene oxide, on a Chiralpak AD column.

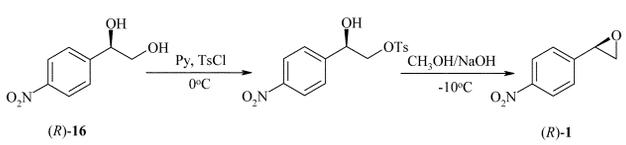
Table 3 Different rate constants for the production of the (*S*)- and (*R*)-diols from the corresponding chiral epoxides by *A. niger* **CGMCC** 0496

Substrate	Rate constants of production (*S*)-diol k/min^{-1}	Rate constants of production (*R*)-diol k/min^{-1}
(*R*)-**1**	$>1.4 \times 10^{-3}$	$>9.22 \times 10^{-2}$
(*S*)-**1**	1.09×10^{-4}	6.4×10^{-5}

Table 4 Biohydrolysis of various substituted nitrostyrene oxides by *A. niger* **CGMCC** 0496

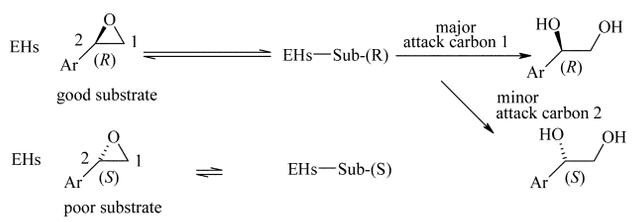
Entry	Substitution of nitro group	Reaction time/min	Yield of epoxide[c] (%)	Ee of epoxide (%) (abs. conf.)	Yield of diol[c]	Ee of diol (%) (abs. conf.)[d]
1	*p*-[a]	109	42	94(*S*)[d]	45	95(*R*)
2	*p*-[b]	23	47	97(*S*)	46	93(*R*)
3	*m*-[a]	140	45	55(*S*)[e]	48	66(*R*)
4	*m*-[b]	39	40	57(*S*)	33	79(*R*)
5	*o*-[a]	170	52	58(*S*)[e]	42	96(*R*)
6	*o*-[b]	68	34	98(*S*)	38	>99(*R*)
7	*o*-[f]	68	42	84(*S*)	—	—

[a] 200 mg substrate with 10 g cells. [b] 100 mg substrate with 20 g cells. [c] Isolated yield. [d] By comparing the optical rotation reported in the literature.[8,13] [e] The absolute configuration was obtained by checking the optical rotation of the epoxides which were prepared from corresponding optical diols with a chemical correlation method.[14] [f] Same conditions as the entry **6**, except adding 7 mg of (*R*)-diol.[9]



(*R*)-**16** (*R*)-**1**

Scheme 1 Preparation of (*R*)-*p*-nitrostyrene oxide from (*R*)-(4-nitrophenyl)-1,2-ethanediol.



Scheme 2 Biohydrolysis process of substituted styrene oxide with EHs in *A. niger* strain.

The biohydrolysis of (*S*)- and (*R*)-*p*-nitrostyrene oxide were carried out respectively under the same conditions (Table 2).

No hydrolysis of (*S*)-*p*-nitrostyrene oxide could be detected by TLC after 60 min. Even after an additional 660 min, the yield of the product diol only reached 8.0% with the (*S*)-configuration in 89% ee. This was not the result of chemical hydrolysis because only a yield of 1.2% of the diol was found by HPLC in a blank test after 720 min in the same buffer. When (*R*)-*p*-nitrostyrene oxide was tested, the rate of the reaction was much faster than that of the (*S*)-form. No residue epoxide could be detected by TLC after 32 min. The (*R*)-configuration product was obtained with a 97% ee.

Through the methods of Michaelis–Menten we checked the rate constants of the forming (*S*)- and (*R*)-diols from the corresponding chiral epoxides (Table 3). The results showed that the stereochemical pathway of the biohydrolysis of the EHs is more complex.

From the results in Table 3, we found the following profiles of the bioprocess: (1) The rate constants for the production of (*R*)-diols from the corresponding (*R*)-epoxides were approximately 1.44×10^3 times faster than the one which formed from (*S*)-epoxide. (2) The rate constants for the production of (*S*)-diols from the corresponding (*R*)-epoxides were approximately 12 times than that formed from (*S*)-epoxide. It was suggested that the EHs in *A. niger* favorably attack the (*R*)-configuration enantiomer, especially at the first carbon atom of the 1,2-epoxide. (Scheme 2)

In order to obtain further reaction data on the effect of the substitution pattern, various nitrostyrene oxides were investigated at 25 °C (Table 4).

Excellent results were found when the strain hydrolyzed *p*- and *o*-substituted nitrostyrene oxides. The high ratio of cells to substrate reduced the time of the complete resolutions. When *o*-nitrostyrene oxide was used as the substrate, a high ratio of cells to substrate was required for complete resolution. We thought it probably due to the product inhibition. In order to get further evidence to support our surmise, a carefully designed reaction was carried out with the same conditions as entry **6** except adding 7 mg of (*R*)-diol to the substrate epoxide. The residue epoxide was recovered in 42% yield and 84% ee when the reaction was stopped within 68 min. This confirmed our hypothesis.[9]

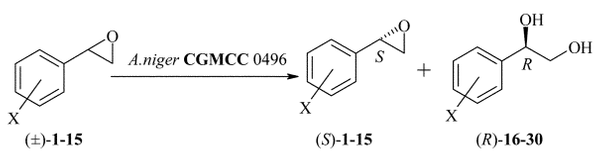
After investigating the substitute site specificities of the strain, a range of substituted styrene oxides were investigated in order to broaden the applicability of this fungus (Scheme 3 and Table 5).

The strain was found to have high affinity for a wide range of substituted styrene oxides. The enantioselectivity of the reactions strongly depended on the position of the substituent group. Remarkable results were obtained in most cases when hydrolyzing *para*- and *ortho*-substituted styrene oxides. Product inhibitions were also found during the hydrolysis of other *ortho*-substituted substrates, such as (±)-**7**, when using the low ratio of cells to substrate. To our surprise, unexpectedly low

Table 5 Different rate constants for the production of (*S*)- and (*R*)- diols from the corresponding chiral epoxides by *A. niger* **CGMCC** 0496

Substrate[a] Entry	Time/min	Residue epoxide			Product of diol		
		Entry[b]	Yield (%)	Ee (%)	Entry[c]	Yield (%)	Ee (%)
(±)-**1**	109	(*S*)-**1**	42	94	(*R*)-**16**	45	95
(±)-**2**	140	(*S*)-**2**	45	55	(*R*)-**17**	48	66
(±)-**3***	68	(*S*)-**3**	34	98	(*R*)-**18**	38	>99
(±)-**4**	32	(*S*)-**4**	24	41	(*R*)-**19**	33	74
(±)-**5**	60	(*S*)-**5**	32	95	(*R*)-**20**	46	85
(±)-**6**	24	(*S*)-**6**	47	28	(*R*)-**21**	40	74
(±)-**7**	45	(*S*)-**7**	17	47	(*R*)-**22**	37	84
(±)-**7***	44	(*S*)-**7**	22	94	(*R*)-**22**	30	84
(±)-**8**	40	(*S*)-**8**	30	>99	(*R*)-**23**	43	86
(±)-**9**	38	(*S*)-**9**	43	35	(*R*)-**24**	43	70
(±)-**10**	65	(*S*)-**10**	22	>99	(*R*)-**25**	46	80
(±)-**11**	52	(*S*)-**11**	25	97	(*R*)-**26**	39	72
(±)-**12**	43	(*S*)-**12**	45	70	(*R*)-**27**	43	63
(±)-**13**	41	(*S*)-**13**	29	63	(*R*)-**28**	41	70
(±)-**14**	215	(*S*)-**14**	15	75	(*R*)-**29**	36	52
(±)-**15***	126	(*S*)-**15**	34	98	(*R*)-**30**	31	82

[a] The reactions were carried out at 25 °C with 200 mg substrate and 10 g cells except *, which were carried out with 100 mg substrate and 20 g cells. [b] The absolute configurations of (*S*)-**4**, (*S*)-**5**, (*S*)-**6**, (*S*)-**7**, (*S*)-**8**, (*S*)-**12**, (*S*)-**14** were established by comparisons with the optical rotation reported in the literatures.[8,15,16] The absolute configurations of (*S*)-**11**, (*S*)-**13** were defined by analogy with the common spectroscopic behavior of (*S*)-*para*-substituted styrene oxide. The absolute configurations of (*S*)-**9**, and (*S*)-**10** were obtained by checking the optical rotations of the epoxides which were prepared from the corresponding optical diols with a chemical correlation method.[14] The absolute configuration of (*S*)-**15** was deduced by common behavior of *A. niger* hydrolyzing substituted styrene oxide. [c] The absolute configurations of (*R*)-**19**, (*R*)-**20**, (*R*)-**21**, (*R*)-**22**, (*R*)-**23**, (*R*)-**25**, (*R*)-**27**, (*R*)-**29** were established by comparison of the optical rotations reported in the literature.[8,13,17] The absolute configurations of (*R*)-**24**, (*R*)-**26**, (*R*)-**28**, (*R*)-**30** were deduced by common behavior of *A. niger* hydrolyzing substituted styrene oxide.



epoxides:
1. X= *p*-NO_2; **2**. X= *m*-NO_2; **3**. X= *o*-NO_2; **4**. X= *p*-F; **5**. X= *p*-Cl;
6. X= *m*-Cl; **7**. X= *o*-Cl; **8**. X=*p*-Br; **9**. X= *m*-Br; **10**. X=*o*-Br;
11. X= *p*-I; **12**. X= *p*-CH_3; **13**. X= *p*-CH_3CH_2; **14**. X= H; **15**. X= *o*-CF_3
diols:
16. X= *p*-NO_2; **17**. X= *m*-NO_2; **18**. X= *o*-NO_2; **19**. X= *p*-F; **20**. X= *p*-Cl;
21. X= *m*-Cl; **22**. X= *o*-Cl; **23**. X=*p*-Br; **24**. X= *m*-Br; **25**. X=*o*-Br;
26. X= *p*-I; **27**. X= *p*-CH_3; **28**. X= *p*-CH_3CH_2; **29**. X= H; **30**. X= *o*-CF_3

Scheme 3

enantioselectivities were found in the hydrolysis of (±)-**4**, (±)-**12**, (±)-**13**. The fair results of the reaction of (±)-**4** were probably due to the small size of the fluorine atom, which makes the enantioselectivity of the reaction much more like the case of (+)-**14**. On the other hand, the poor results of (±)-**12**, (±)-**13** may be attributable to the electron donating properties of the methyl and ethyl groups which decrease the essential stability of the epoxides and increase the non-enzymatic, concomitant hydrolysis during the attempted resolution.

The results reported in Table 5 also showed that more time was needed for complete resolution when there was no substituted group in the aromatic ring [case (±)-**14**], and the substituent group in the benzene ring accelerated the rate of the biohydrolysis.

Conclusion

In summary, the *A. niger* **CGMCC** 0496 strain in asymmetric hydrolysis of various substituted styrene oxides is a useful methodology for preparing chiral styrene oxides and its corresponding diols, especially for some *para*- and *ortho*-substituted styrene oxides.

Experimental

1 General

The strain of *A. niger* used in this work is registered at the China General Microbiological Culture Collection Center, Institute of Microbiology, the Chinese Academy of Sciences (Beijing, China) under no. **CGMCC** 0496. All melting points of the compounds are uncorrected. IR spectra were recorded on a Shimadzu IR-440 spectrometer. EI mass spectra (MS) were run on a HP-5989A mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker AMX-300 spectrometer with tetramethylsilane as the internal standard. Chemical shifts are reported in ppm and *J* are in Hz. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. HPLC was carried out using Chiralcel OD or OJ, and Chiralpak AD or AS (0.46 cm ϕ × 25 cm) detected at UV 254 nm. GC was carried out using a Rt-βDEXcst™column (Resteck) or Chiraldex™ G-PN column (Astec); TLC was carried out using HSG F_{254} silica gel plates and silica gel (200–400 mesh) was used for chromatography.

2 General procedure for the culture of microorganisms

The strain of *A. niger* **CGMCC** 0496 was maintained on agar medium which contained K_2HPO_4 (1.0 g L^{-1}), KCl (0.5 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), $FeSO_4 \cdot 7H_2O$ (0.0 g L^{-1}), fructose (10 g L^{-1}), corn steep liquor (15 g L^{-1}), and agar (3 g L^{-1}) pH 6.8–7.0. The cells were grown on medium[19] of K_2HPO_4 (1.0 g L^{-1}), KCl (0.5 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), $FeSO_4 \cdot 7H_2O$ (0.01 g L^{-1}), fructose (10 g L^{-1}) and corn steep liquor (15 g L^{-1}) in tap water. For large-scale cultures, a two-stage process was used. 100 mL of medium (in a 500 mL flask) was first inoculated and then cultured at 30 °C on a reciprocating shaker set at 100 cpm for 20 h. In the second stage, 1000 mL of medium (in a 5 L flask) was inoculated under the same conditions with 5% of the first stage culture. The second stage lasted for 52 h. The cells were harvested by centrifugation (HITACHI, CR20B2) at 7000 rpm and 5 °C for 30 min, and were then washed twice with 100 mL of 0.1 mol L^{-1} (pH 8.0) phosphate buffer. Fresh wet mycelium was used for further biotransformation studies.

3 General procedure for the biotransformation of racemic epoxide [(±)-1]-[(±)-15] with *A. niger* CGMCC 0496

Wet mycelium 10 g or 20 g was suspended in 100 mL of sodium phosphate buffer 0.1 M (pH = 8.0) and maintained at 25 °C. A portion of 100 or 200 mg of the epoxide as a solution in DMF (3 mL) was added to the cells suspension, and the medium was stirred by a mechanical stirrer at 600 rpm. The course of

bioconversion was monitored by the residual epoxide and the formation of the corresponding diol by TLC. When an appropriate degree of conversion was reached, the reaction was stopped by adding 50 mL of ethyl acetate. The mycelium was filtered off. The fungal cake was washed twice in 20 mL ethyl acetate. The aqueous phase was saturated with sodium chloride, and then extracted three times with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Flash chromatography (petroleum ether–EtOAc, gradient from 8 : 1 to 1 : 1) of the residue gave the epoxides and corresponding diols. The yields, the enantiomeric purity and the absolute configuration of the reside epoxides and corresponding diols are summarized in Tables 1–4.

3.1 (*S*)-(+)-2-(4-Nitrophenyl) oxirane (S)-1. Following the general procedure (10 g cells to 200 mg substrate), epoxide (*S*)-**1** was obtained in the yield as indicated in Table 5. Light yellow solid; mp 73–75 °C; 94% ee; $[\alpha]_D^{25}$ +35.0 (*c* 1.20, $CHCl_3$), {Lit.[8] $[\alpha]_D^{20}$ +37.6 (*c* 1.99, $CHCl_3$) for >98% ee, (*S*)}; MS *m*/*z* (rel. intensity %): 166([M + 1]$^+$, 2), 165(M$^+$, 3), 164(6), 148(47), 118(68), 91(30), 89(100), 77(12), 65(21), 63(47), 51(21); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.78 (dd, 1H, J_1 = 5.5 Hz, J_2 = 2.5 Hz), 3.23 (dd, 1H, J_1 = 5.5 Hz, J_2 = 4.1 Hz), 3.96 (dd, 1H, J_1 = 4.1 Hz, J_2 = 2.5 Hz), 8.22, 7.46 (AB, 4H, J = 8.7 Hz); IR (KBr): ν_{max} 1610, 1530, 1410, 1350, 855 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.2 (*S*)-(+)-2-(3-Nitrophenyl)-oxirane (*S*)-2. Following the general procedure (10 g cells to 200 mg substrate); epoxide (*S*)-**2** was obtained in the yield as indicated in Table 5. Yellow oil; 57% ee; $[\alpha]_D^{18}$ +2.5 (*c* 2.8, $CHCl_3$); MS *m*/*z* (rel. intensity %): 165(M$^+$, 18), 150(32), 136(68), 120(25), 105(17), 90(100), 77(22), 74(12), 65(52), 63(59); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.80 (dd, 1H, J_1 = 4.8 Hz, J_2 = 2.4 Hz), 3.22 (dd, 1H, J_1 = 4.2 Hz, J_2 = 0.9 Hz), 3.97 (dd, 1H, J_1 = 3.9 Hz, J_2 = 1.2 Hz), 7.40–7.75 (m, 2H), 8.02–8.24 (m, 2H); IR (film): ν_{max} 3113, 2995, 1517, 1343, 1301, 1042, 983, 887, 789, 741 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.3 (*S*)-(−)-2-(2-Nitrophenyl)-oxirane (*S*)-3. Following the general procedure (20 g cells to 100 mg substrate), epoxide (*S*)-**3** was obtained in the yield as indicated in Table 5. Light yellow solid; mp 51–52 °C; 98% ee; $[\alpha]_D^{19.5}$ −107.2 (*c* = 1.7; $CHCl_3$); MS *m*/*z* (rel. intensity %): 165(M$^+$, 0.3), 149(2), 135(21), 105(10), 104(10), 91(79), 89(21), 79(71), 77(100); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.67 (dd, 1H, J_1 = 5.4 Hz, J_2 = 2.4 Hz), 3.30 (dd, 1H, J_1 = 5.4 Hz, J_2 = 4.2 Hz), 4.48 (dd, 1H, J_1 = 4.5 Hz, J_2 = 3.0 Hz), 7.40–7.55 (m, 1H), 7.58–7.75 (m, 2H), 8.15 (dd, 1H, J_1 = 8.1 Hz, J_2 = 1.2 Hz); IR (KBr): ν_{max} 3150, 2997, 1532, 1353, 1254, 899, 859, 809, 737, 684 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.4 (*S*)-(+)-2-(4-Fluorophenyl)-oxirane (*S*)-4. Following the general procedure (10 g cells to 200 mg substrate), epoxide (*S*)-**4** was obtained in the yield as indicated in Table 5. Oil; 41% ee; $[\alpha]_D^{27}$ +7.0 (*c* 1.9, $CHCl_3$), {lit.[8] $[\alpha]_D^{20}$ +15.6 (*c* 0.97, $CHCl_3$) for 98% ee, (*S*)}; MS *m*/*z* (rel. intensity %): 139 (M + 1, 0.92), 138(M$^+$, 13), 110(13), 109(100), 107(11), 83(22), 81(4); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.75 (dd, 1H, J_1 = 5.4 Hz, J_2 = 2.5 Hz), 3.13 (dd, 1H, J_1 = 5.3 Hz, J_2 = 4.1 Hz), 3.84 (dd, 1H, J_1 = 3.9 Hz, J_2 = 2.7 Hz), 6.90–7.12 (m, 2H), 7.14–7.35 (m, 2H); IR (film): ν_{max} 3055, 2995, 2926, 1608, 1514, 1479, 1383, 1222, 1157, 1015, 988, 882, 836, 820 cm^{-1}. The enantiomeric excess was measured by capillary GC analysis using Rt-βDEXcst™(Resteck).

3.5 (*S*)-(+)-2-(4-Chlorophenyl)-oxirane (*S*)-5. Following the general procedure (10 g cells to 200 mg substrate), epoxide (*S*)-**5** was obtained in the yield as indicated in Table 5. Light oil; 95% ee; $[\alpha]_D^{27}$ +18.7 (*c* 1.8, $CHCl_3$), {lit.[8] $[\alpha]_D^{20}$ +19.3 (*c* 1.16, $CHCl_3$) for >98% ee, (*S*)}; MS *m*/*z* (rel. intensity %): 156, 154 (M + 1, 2, 8), 155, 153 (M$^+$, 3, 7), 138(3), 125(40), 119(39), 91(29), 89(100), 63(34), 50(17); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.74 (dd, 1H, J_1 = 5.4 Hz, J_2 = 2.4 Hz), 3.13 (dd, 1H, J_1 = 5.4 Hz, J_2 = 4.2 Hz), 3.82 (dd, 1H, J_1 = 4.2 Hz, J_2 = 2.4 Hz), 7.10–7.21 (m, 2H), 7.26–7.35 (m, 2H); IR (film): ν_{max} 3054, 2992, 2920, 1602, 1496, 1478, 1417, 1381, 1199, 1090, 1015, 987, 879, 831, 769 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OJ column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.6 (*S*)-(+)-2-(3-Chlorophenyl) oxirane (*S*)-6. Following the general procedure (10 g cells to 200 mg substrate), epoxide (*S*)-**6** was obtained in the yield as indicated in Table 5. Light oil; 28% ee; $[\alpha]_D^{27}$ +3.6 (*c* 1.0, $CHCl_3$), {lit.[15] $[\alpha]_D^{22}$ −11.15 (*c* 1.56, $CHCl_3$) for >99% ee, (*R*)}; MS *m*/*z* (rel. intensity %): 157, 155(M + 1, 0.8, 1.4), 156, 154(M$^+$, 10, 24), 141(15), 139(14), 125(50), 111(11), 91(56), 89(100); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.79 (dd, 1H, J_1 = 5.3 Hz, J_2 = 2.4 Hz), 3.17 (dd, 1H, J_1 = J_2 = 4.8 Hz), 3.86 (dd, 1H, J_1 = J_2 = 2.8 Hz), 7.16–7.62 (m, 4H); IR (film): ν_{max} 3059, 2993, 1602, 1575, 1481, 1435, 1386, 1079, 999, 880, 823, 692 cm^{-1}. Anal. calcd. For C_8H_7ClO: C, 62.15; H, 4.56; Cl, 22.93. Found: C, 62.11; H, 4.58; Cl, 23.35. The enantiomeric excess was measured by capillary GC analysis using Chiraldex™ G-PN column (Astec).

3.7 (*S*)-(+)-2-(2-Chlorophenyl) oxirane (*S*)-7. Following the general procedure (20 g cells to 100 mg substrate), epoxide (*S*)-**7** was obtained in the yield as indicated in Table 5. Oil; 94% ee; $[\alpha]_D^{23}$ +48.3 (*c* 1.2, $CHCl_3$) {lit.[16] $[\alpha]_D^{25}$ +32.2 (*c* 1.19, $CHCl_3$) for 99% ee, (*S*)}; MS *m*/*z* (rel. intensity %): 156, 154(M$^+$, 4, 16), 155, 153(M$^+$ − 1, 10, 23), 134(9), 124(28), 119(75), 91(33), 89(100), 63(17); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.65 (dd, 1H, J_1 = 5.9 Hz, J_2 = 3.6 Hz), 3.19 (dd, 1H, J_1 = 5.3 Hz, J_2 = 4.2 Hz), 4.21 (dd, 1H, J_1 = J_2 = 3.3 Hz), 7.10–7.50 (m, 4H); IR (film): ν_{max} 3061, 2993, 1699, 1593, 1482, 1442, 1383, 1249, 1121, 1053, 1035, 880, 755 cm^{-1}. The enantiomeric excess was measured by capillary GC analysis using Chiraldex™ G-PN column (Astec).

3.8 (*S*)-(+)-2-(4-Bromophenyl) oxirane (*S*)-8. Following the general procedure (20 g cells to 100 mg substrate); epoxide (*S*)-**8** was obtained in the yield as indicated in Table 5. Oil; >99% ee; $[\alpha]_D^{23}$ +13.9 (*c* 1.2, $CHCl_3$) {lit.[8] $[\alpha]_D^{20}$ +13.6 (*c* 1.46, $CHCl_3$) for >98% ee, (*S*)}; MS: *m*/*z* (rel. intensity %): 200, 198(M$^+$, 4), 199, 197(M − 1, 3), 169(14), 119(41), 89(100), 63(36); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.73 (dd, 1H, J_1 = 5.4 Hz, J_2 = 2.7 Hz), 3.13 (dd, 1H, J_1 = 5.1 Hz, J_2 = 3.6 Hz), 3.81 (dd, 1H, J_1 = 3.9 Hz, J_2 = 2.4 Hz), 7.05–7.18 (m, 2H), 7.40–7.48 (m, 2H); IR (film): ν_{max} 3051, 2991, 2919, 1595, 1490, 1415, 1378, 1101, 1073, 1011, 987, 878, 828 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralcel OJ column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 100 : 1).

3.9 (*S*)-(+)-2-(3-Bromophenyl) oxirane (*S*)-9. Following the general procedure (10 g cells 200 mg substrate), epoxide (*S*)-**9** was obtained in the yield as indicated in Table 5. Oil; 35% ee; $[\alpha]_D^{27}$ +4.0 (*c* 1.1, $CHCl_3$); MS *m*/*z* (rel. intensity %): 200, 198(M$^+$, 23, 23), 199, 197(M$^+$ − 1, 32, 32), 169(27), 141(16), 119(67), 91(60), 89(100); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.77 (dd, 1H, J_1 = 5.4 Hz, J_2 = 2.5 Hz), 3.15 (dd, 1H, J_1 = 5.4 Hz, J_2 = 4.1 Hz), 3.81 (dd, 1H, J_1 = 4.0 Hz, J_2 = 2.5 Hz),

7.10–7.50 (m, 4H); IR (film): ν_{max} 3057, 2992, 1600, 1571, 1478, 1431, 1385, 1369, 1201, 1070, 997, 877, 786, 691 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AS column (eluent at $V = 0.8$ mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.10 (*S*)-(+)-2-(2-Bromophenyl) oxirane (*S*)-10. Following the general procedure (20 g cells to 100 mg substrate), epoxide (*S*)-**10** was obtained in the yield as indicated in Table 5. Oil; >99% ee; $[\alpha]_D^{18}$ +68.7 (*c* 1.1, $CHCl_3$); MS *m*/*z* (rel. intensity %): 200, 198(M^+, 17, 18), 199, 197($M^+ - 1$, 16, 15), 185(1), 171(9), 169(10), 141(1), 120(7), 119(84), 91(63), 90(41), 89(100); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ 2.64 (dd, 1H, J_1 = 5.9 Hz, J_2 = 2.6 Hz), 3.18 (dd, 1H, J_1 = 5.9 Hz, J_2 = 4.1 Hz), 4.15 (dd, 1H, J_1 = 4.1 Hz, J_2 = 2.6 Hz), 7.08–7.38 (m, 3H), 7.54 (dd, 1H, J_1 = 7.9 Hz, J_2 = 1.1 Hz); IR (film): ν_{max} 3055, 2991, 2916, 1569, 1472, 1440, 1381, 1248, 1045, 1026, 879, 753 cm^{-1}. The enantiomeric excess was measured by capillary GC analysis using Chiraldex™ G-PN column (Astec).

3.11 (*S*)-(+)-2-(4-Iodophenyl)oxirane (*S*)-11. Following the general procedure (20 g cells to 100 mg substrate), epoxide (*S*)-**11** was obtained in the yield as indicated in Table 5. Yellow oil; 97% ee; $[\alpha]_D^{26}$ +25.1 (*c* 1.0, $CHCl_3$); MS *m*/*z* (rel. intensity %): 247($M + 1$, 10), 246(M^+, 53), 245(36), 233(19), 230(10), 217(53), 127(6), 119(75), 91(69), 90(54), 89(75); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.74 (dd, 1H, J_1 = 5.4 Hz, J_2 = 2.6 Hz), 3.13 (dd, 1H, J_1 = 5.3 Hz, J_2 = 2.6 Hz), 2.80 (dd, 1H, J_1 = 3.8 Hz, J_2 = 2.5 Hz), 6.90–7.05 (m, 2H), 7.60–7.78 (m, 2H); IR (film): ν_{max} 3053, 2990, 1589, 1474, 1413, 1377, 1056, 1006, 876, 825, 792cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AS column (eluent at $V = 0.8$ mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.12 (*S*)-(+)-2-(4-Methylphenyl)oxirane (*S*)-12. Following the general procedure (10 g cells to 200 mg substrate), epoxide (*S*)-**12** was obtained in the yield as indicated in Table 5. Oil; 70% ee; $[\alpha]_D^{16}$ +19.5 (*c* 1.2, $CHCl_3$); MS: *m*/*z* (rel. intensity %): 235($M + 1$, 3), 134(M^+, 2), 121(100), 91(42), 77(24), 65(12), 51(6); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.34 (s, 3H), 2.80 (dd, 1H, J_1 = 5.4 Hz, J_2 = 2.7 Hz), 3.13 (dd, 1H, J_1 = 5.7 Hz, J_2 = 4.2 Hz), 3.83 (dd, 1H, J_1 = 3.9 Hz, J_2 = 2.4 Hz), 7.10–7.20 (m, 4H); IR (film): ν_{max} 3051, 2988, 2922, 1519, 1477, 1386, 1131, 1255, 1199, 1109, 986, 881, 818 cm^{-1}. The enantiomeric excess was measured by capillary GC analysis using Rt-βDEXcst™ column (Resteck).

3.13 (*S*)-(+)-2-(4-Ethylphenyl)oxirane (*S*)-13. Following the general procedure (10 g cells to 200 mg substrate), epoxide (*S*)-**13** was obtained in the yield as indicated in Table 5. Oil; 63% ee; $[\alpha]_D^{16}$ +18.4 (*c* 1.4, $CHCl_3$); MS *m*/*z* (rel. intensity %): 149($M + 1$, 4), 148(M^+, 14), 131(5), 119(62), 117(11), 115(5), 104(9), 91(21), 77(7); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.23 (t, 3H, J = 7.6 Hz), 5.30 (q, 2H, J = 7.6 Hz), 2.80 (dd, 1H, J_1 = 5.5 Hz, J_2 = 2.6 Hz), 3.13 (dd, 1H, $J_1 = J_2$ = 4.7 Hz), 3.83 (dd, 1H, J_1 = 4.0 Hz, J_2 = 2.5 Hz) 7.02–7.25 (m, 4H); IR (film): ν_{max} 3057, 2967, 2874, 1908, 1617, 1519, 1384, 1255, 1128, 987, 881, 834 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AS column (eluent at $V = 0.8$ mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.14 (*S*)-(−)-2-Phenyloxirane (*S*)-14. Following the general procedure (10 g cells to 200 mg substrate), epoxide (*S*)-**14** was obtained in the yield as indicated in Table 5. Oil; 75% ee; MS *m*/*z* (rel. intensity %): 122($M + 2$, 15), 121($M + 1$, 43), 120(M^+, 20), 105(100), 91(68), 77(88); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.67 (dd, 1H, J_1 = 5.7 Hz, J_2 = 2.7 Hz), 3.15 (dd, 1H, J_1 = 5.4 Hz, J_2 = 3.9 Hz), 3.86 (dd, 1H, J_1 = 3.9 Hz, J_2 = 2.7 Hz) 7.15–7.45 (m, 4H); IR (film): ν_{max} 3040, 2991, 2913, 1608, 1497, 1477, 1453, 1390, 1254, 1202, 985, 877, 759, 699 cm^{-1}. The enantiomeric excess was measured by capillary GC analysis using Chiraldex™ G-PN column (Astec).

3.15 (*S*)-(+)-2-((2-Trifluoromethyl)phenyl)oxirane (*S*)-15. Following the general procedure (10 g cells to 200 mg substrate), epoxide (*S*)-**15** was obtained in the yield as indicated in Table 5. Oil; 98% ee; $[\alpha]_D^{21}$ +35.4 (*c* 0.2, $CHCl_3$) MS *m*/*z* (rel. intensity %): 188(M^+, 16), 187(51), 174(33), 173(75), 159(39), 157(30), 138(27), 119(100), 108(25), 95(11); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.63 (dd, 1H, J_1 = 6.0 Hz, J_2 = 2.7 Hz), 3.18 (dd, 1H, J_1 = 5.1 Hz, J_2 = 4.5 Hz), 3.83 (dd, 1H, $J_1 = J_2$ = 1.8 Hz), 7.20–7.85 (m, 4H); IR (film): ν_{max} 1655, 1606, 1458, 1316, 1168, 1121, 1060, 1035, 887, 769 cm^{-1}. The enantiomeric excess was measured by capillary GC analysis using Chiraldex™-G-PN column (Astec).

3.16 (*R*)-(−)-1-(4-Nitrophenyl)-1,2-ethanediol (*R*)-16. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**16** was obtained in the yield as indicated in Table 5. Yellow solid; mp 89–90 °C; 94% ee; $[\alpha]_D^{25}$ −19.5 (*c* 1.0, EtOH) {lit.[13] $[\alpha]_D^{20}$ −20.0 (*c* 1.15, MeOH) for 96% ee, (*R*)}; MS *m*/*z* (rel. intensity %): 184([M + 1]$^+$, 100), 182 (2) 166 (17), 152 (63), 136 (26), 122 (16), 106 (43), 94 (35), 91 (11), 78 (44); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 3.45 (br s, 2H), 3.58 (dd, 1H, J = 11.1, 6.7 Hz), 3.66 (dd, 1H, J = 11.1, 4.9 Hz), 4.85 (dd, 1H, J = 6.7, 4.9 Hz), 8.18, 7.69 (AB, 4H, J = 8.7 Hz); IR (KBr): ν_{max} 3250, 1600, 1510, 1410, 1350, 1100, 1060, 850, 800, 720 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at $V = 0.8$ mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.17 (*R*)-(−)-1-(3-Nitrophenyl)-1,2-ethanediol (*R*)-17. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**17** was obtained in the yield as indicated in Table 5. Yellow solid; mp 76–77 °C; 79% ee, $[\alpha]_D^{25}$ −13.9 (*c* 1.6, EtOH) {lit.[13] $[\alpha]_D^{20}$ +26 (*c* 1.5, MeOH) for 92% ee, (*S*)}; MS *m*/*z* (rel. intensity %): 184([M + 1]$^+$, 5.1), 166 (70), 152 (100), 136 (34.4), 105 (60), 91 (16), 77 (52); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 3.40 (br s, 2H), 3.70–3.50 (m, 2H), 4.87 (dd, 1H, $J_1 = J_2$ = 5.7 Hz), 7.61 (t, 1H, $J_1 = J_2$ = 8.2 Hz), 7.83 (d, 1H, J = 7.5 Hz), 8.10 (d, 1H, J = 8.2 Hz), 8.28 (s, 1H); IR (KBr): ν_{max} 3300, 1520, 1340, 1070, 1030, 860, 800, 720 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at $V = 0.8$ mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.18 (*R*)-(+)-1-(2-Nitrophenyl)-1,2-ethanediol (*R*)-18. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**18** was obtained in the yield as indicated in Table 5. Light yellow solid; mp 108–109 °C; >99% ee, $[\alpha]_D^{19.5}$ +53.4 (*c* = 1.1; EtOH) {lit.[13] $[\alpha]_D^{20}$ +9 (*c* 0.8, MeOH) for 47% ee, (*R*)}; MS *m*/*z* (rel. intensity %): 184([M + 1]$^+$, 1.1), 166 (21), 152 (88), 135 (52), 104 (100), 91 (54), 79 (69), 77 (88); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 3.45 (br s, 2H), 3.60 (dd, 1H, J_1 = 11.1Hz, J_2 = 7.0 Hz), 3.74 (dd, 1H, J_1 = 11.1 Hz, J_2 = 4.0 Hz), 5.28 (dd, 1H, J_1 = 7.0 Hz, J_2 = 4.0 Hz), 7.53 (dd, 1H, $J_1 = J_2$ = 7.8 Hz), 7.73 (dd, 1H, $J_1 = J_2$ = 7.8 Hz), 7.91–7.86 (m, 2H); IR (KBr): ν_{max} 3250, 1610, 1530, 1065, 825, 795, 750, 700 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel O*J* column (eluent at $V = 0.8$ mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.19 (*R*)-(−)-1-(4-Fluorophenyl)-1,2-ethanediol (*R*)-19. Following the general procedure (20 g cells to 100 mg substrate), diol (*R*)-**19** was obtained in the yield as indicated in Table 5. White solid; mp 55–56 °C; 74% ee; $[\alpha]_D^{24}$ −23.5 (*c* 1.5, EtOH) {lit.[8] $[\alpha]_D^{20}$ −49 (*c* 1.07, $CHCl_3$) for 81% ee, (*R*)}; MS *m*/*z* (rel. intensity %): 156(M^+, 2), 140(3), 139(47), 126(6), 125(100), 123(15), 121(5), 119(4), 109(9), 107(4), 95(26), 91(3), 77(38), 70(4); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ 3.29(s, 2H),

3.45–3.78 (m, 2H), 4.70–4.83 (m, 1H), 7.02–7.20 (m, 2H), 7.22–7.54 (m, 2H); IR (KBr): ν_{max} 3207, 2934, 2878, 1608, 1512, 1470, 1245, 1234, 1105, 1086, 1033, 891, 831 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AS column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.20 (*R*)-(+)-1-(4-Chlorophenyl)-1,2-ethanediol (*R*)-20. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**20** was obtained in the yield as indicated in Table 5. White solid; mp 83–84 °C; 95% ee; $[a]_D^{27}$ +18.7 (*c* 1.8, $CHCl_3$) {lit.[13] $[a]_D^{20}$ +26 (*c* 2.1, EtOH) for 83% ee, (*R*)}; MS: *m/z* (rel. intensity %): 172(M^+, 2), 155(11), 141(86), 113(26), 89(8), 77(100), 51(21); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ 3.13 (br s, 2H), 3.52 (dd, 1H, J_1 = 10.9 Hz, J_2 = 7.4 Hz), 3.60 (dd, 1H, J_1 = 11.0Hz, J_2 = 4.6 Hz), 4.71 (dd, 1H, J_1 = 11.9 Hz, J_2 = 4.5 Hz), 7.30–7.40 (m, 2H), 7.40–7.50 (m, 2H); IR (KBr): ν_{max} 3310, 2977, 2939, 2879, 1596, 1490, 1457, 1341, 1110, 1085, 1031, 1015, 890, 823 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 95 : 5).

3.21 (*R*)-(−)-1-(3-Chlorophenyl)-1,2-ethanediol (*R*)-21. Following the general procedure (20 g cells to 100 mg substrate), diol (*R*)-**21** was obtained in the yield as indicated in Table 5. Oil; 74% ee; $[a]_D^{24}$ −15.8 (*c* 1.1, EtOH) {lit.[13] $[a]_D^{20}$ +17 (*c* 1.4, EtOH) for 55% ee, (*S*)}; MS *m/z* (rel. intensity %): 175, 173(M + 1, 0.38, 1.09), 174, 172(M^+, 3.65, 11.65), 143(25), 141(80), 139(4), 125(2), 115(10), 113(33), 105(4), 89(4), 77(100); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ 2.92 (s, 2H), 3.59 (dd, 1H, J_1 = 11.2 Hz, J_2 = 8.1 Hz), 3.73 (dd, 1H, J_1 = 11.2 Hz, J_2 = 2.6 Hz), 4.76 (dd, 1H, J_1 = 7.8 Hz, J_2 = 2.8 Hz) 7.01–7.28 (m, 3H), 7.35 (s, 1H); IR (film): ν_{max} 3369, 2926, 2878, 1599, 1575, 1479, 1431, 1197, 1102, 1077, 1029, 786, 693 cm^{-1}. Anal. calcd. for $C_8H_9ClO_2$: C, 55.67; H, 5.26; Cl, 20.54. Found: C, 55.69; H, 5.22; Cl, 20.53%. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.22 (*R*)-(−)-1-(2-Chlorophenyl)-1,2-ethanediol (*R*)-22. Following the general procedure (20 g cells to 100 mg substrate), diol (*R*)-**22** was obtained in the yield as indicated in Table 5. White solid; mp 99–100 °C; 84% ee; $[a]_D^{23}$ −50.4 (*c* 1.7, EtOH) {lit.[13] $[a]_D^{25}$ −50 (*c* 2.0, EtOH) for 66% ee, (*R*)}; MS *m/z* (rel. intensity %): 172(M^+, 1.8), 143(38), 141(100), 113(24), 77(70);[1] H NMR (300 MHz, CD_3COCD_3, TMS): δ 3.39 (dd, 1H, J_1 = 11.2 Hz, J_2 = 7.8 Hz), 3.46 (br s, 2H), 3.69 (dd, 1H, J_1 = 11.2 Hz, J_2 = 3.0 Hz), 5.12 (dd, 1H, J_1 = 7.8 Hz, J_2 = 3.0 Hz), 7.61–7.29 (m, 4H); IR (KBr): ν_{max} 3300, 1635, 1440, 1070, 1040, 760 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 8 : 2).

3.23 (*R*)-(−)-1-(4-Bromophenyl)--1,2-ethanediol (*R*)-23. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**23** was obtained in the yield as indicated in Table 5. White solid; mp 104–105 °C; 86% ee; $[a]_D^{23}$ −41.4 (*c* 1.2, EtOH) {lit.[8] $[a]_D^{20}$ −37.2 (*c* 1.03, $CHCl_3$) for 79% ee, (*R*)}; MS *m/z* (rel. intensity %): 218,216(M^+, 3,3), 187(71), 185(83), 159(16), 157(19), 77(100), 51(18); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS) δ 3.26 (br s, 2H), 3.53 (dd, 1H, J_1 = 11.4 Hz, J_2 = 7.5 Hz), 3.61 (dd, 1H, J_1 = 11.1 Hz, J_2 = 4.8 Hz), 4.70 (dd, 1H, J_1 = 7.5 Hz, J_2 = 4.8 Hz), 7.20–7.38 (m, 2H), 7.40–7.60 (m, 2H); IR (KBr): ν_{max} 3051, 2991, 2919, 1595, 1490, 1073, 1011, 828 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AS column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 8 : 2).

3.24 (*R*)-(−)-1-(3-Bromophenyl)-1,2-ethanediol (*R*)-24. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**24** was obtained in the yield as indicated in Table 5. Oil; 70% ee; $[a]_D^{23}$ −8.5 (*c* 1.2, EtOH); MS: *m/z* (rel. intensity %): 218, 216(M^+, 8), 191(22), 187(70), 185(74), 157(34), 117(7), 105(12), 81(21), 77(100); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 3.50–2.80(br s, 2H), 3.58–3.85 (m, 2H), 4.74 (dd, 1H, J_1 = 8.2Hz, J_2 = 3.3 Hz), 7.12–7.52 (m, 3H), 7.55 (s,1H); IR (film): ν_{max} 3260, 2918, 1656, 1594, 1569, 1415, 1193, 1111, 1068, 1057, 1022, 995, 906, 779, 695 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 95 : 5).

3.25 (*R*)-(−)-1-(2-Bromophenyl)--1,2-ethanediol (*R*)-25. Following the general procedure (10 g diol to 200 mg substrate), diol (*R*)-**25** was obtained in the yield as indicated in Table 5. White solid; mp 118–119 °C; 80% ee; $[a]_D^{18}$ −24.8 (*c* 1.3, EtOH) {lit.[17] $[a]_D^{20}$ −7.5 (*c* 0.99, $CHCl_3$) for (*R*)}; MS *m/z* (rel. intensity %): 219,217(M + 1, 0.3, 0.2), 218,216(M^+, 2, 2), 201(2), 199(2), 188(7), 187(72), 185(76), 159(15), 157(19), 155(3), 137(3), 119(3), 107(2), 105(11), 91(5), 89(6), 78(44), 77(100); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ 3.29 (s, 2H), 3.42 (dd, 1H, J_1 = 11.2 Hz, J_2 = 7.9 Hz), 3.74 (dd, 1H, J_1 = 11.2 Hz, J_2 = 3.1 Hz), 5.07 (dd, 1H, J_1 = 7.9 Hz, J_2 = 3.1 Hz), 7.20 (ddd, 1H, J_1 = J_2 = 7.7 Hz, J_3 = 1.8 Hz), 7.39 (ddd, 1H, J_1 = J_2 = 7.4 Hz, J_3 = 1.2 Hz), 7.54 (ddd, 1H, J_1 = J_2 = 8.0 Hz, J_3 = 1.2 Hz), 7.66 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.6 Hz); IR (KBr): ν_{max} 3276, 2923, 1589, 1568, 1467, 1431, 1363, 1193, 1127, 1093, 1069, 1023, 953, 898, 836, 756 cm^{-1}. Anal. calcd. for C_8H_7BrO: C, 44.27; H, 4.18; Br, 36.81. Found: C, 44.32; H, 4.27; Br, 36.90%. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.26 (*R*)-(−)-1-(4-Iodophenyl)-1,2-ethanediol (*R*)-26. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**26** was obtained in the yield as indicated in Table 5. White solid; mp 120–121 °C; 72% ee; $[a]_D^{27}$ −17.7 (*c* 1.1, EtOH); MS *m/z* (rel. intensity): 265(M + 1, 3), 264(M^+, 30), 234(13), 233(100), 205(3), 141(11), 127(3), 107(25), 105(21), 79(25), 78(99); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ 3.25 (s, 2H), 3.52 (dd, 1H, J_1 = 11.0 Hz, J_2 = 7.6 Hz), 3.61 (dd, 1H, J_1 = 11.2 Hz, J_2 = 4.2 Hz), 4.70 (dd, 1H, J_1 = 7.6 Hz, J_2 = 4.3 Hz), 7.20, 7.66 (AB, 4H, J = 8.1 Hz); IR (KBr): ν_{max} 3369, 2923, 1586, 1484, 1396, 1092, 1066, 1034, 1023, 1006, 895, 832, 821, 523 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AS column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.27 (*R*)-(−)-1-(4-Methylphenyl)-1,2-ethanediol (*R*)-27. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**27** was obtained in the yield as indicated in Table 5. White solid; mp 68–69 °C; 63% ee; $[a]_D^{27}$ −48.1 (*c* 1.1, EtOH) {lit.[8] $[a]_D^{25}$ −44.7 (*c* 0.75, $CHCl_3$) for 66% ee, (*R*)}; MS *m/z* (rel. intensity %): 152(M^+, 2.4), 135(11), 121(100), 105(5), 93(45), 77(25), 65(6); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ 2.32 (s, 3H), 3.18 (br s, 2H), 3.50 (dd, 1H, J_1 = 11.1 Hz, J_2 = 8.1 Hz), 3.60 (dd, 1H, J_1 = 11.1 Hz, J_2 = 4.2 Hz), 4.68 (dd, 1H, J_1 = 7.8 Hz, J_2 = 4.2 Hz), 7.14, 7.28 (AB, 4H, J = 7.8 Hz); IR (KBr): ν_{max} 3264, 2921, 2863, 1515, 1347, 1097, 1070, 1033, 900, 849, 819 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AS column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 95 : 5).

3.28 (*R*)-(−)-1-(4-Ethylphenyl)-1,2-ethanediol (*R*)-28. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**28** was obtained in the yield as indicated in Table 5. White solid; mp 64–65 °C; 70% ee; $[a]_D^{23}$ −23.8 (*c* 1.2, EtOH); MS *m/z* (rel. intensity %): 166(M^+, 0.16), 165(2), 155(1), 149(100), 136(3), 135(51), 133(7), 131(64), 120(4), 105(6), 91(20), 79(39); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ 1.23 (t, 3H, J = 5.2 Hz), 2.65 (q, 2H, J = 7.6 Hz) 2.74–3.15 (br s, 2H),

3.60–3.78 (m, 2H), 4.78 (dd, 1H, J_1 = 8.0 Hz, J_2 = 3.5 Hz), 7.20, 7.26 (AB, 4H, J = 8.1 Hz); IR (KBr): ν_{max} 3259, 2959, 2921, 2867, 1513, 1456, 1358, 1097, 1072, 1050, 1031, 900, 849, 831 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using a Chiralpak AS column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

3.29 (*R*)-1-(−)-Phenyl-1,2-ethanediol (*R*)-29. Following the general procedure (10 g cells to 200 mg substrate), diol (*R*)-**29** was obtained in the yield as indicated in Table 5. white solid; mp 66–67 °C; 52% ee; MS: *m*/*z* (rel. intensity %): 138(M^+, 3), 121(11), 107(100), 79(85), 77(65), 63(4), 51(14); ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 2.92 (br s, 2H), 3.56–3.81 (m, 2H), 4.82 (dd, 1H, J_1 = 8.1 Hz, J_2 = 3.3 Hz), 7.26–7.50 (m, 5H); IR (KBr): ν_{max} 3203, 3061, 3030, 2934, 1469, 1448, 1343, 1228, 1101, 1089, 1054, 887, 833, 760, 748, 699 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 95 : 5).

3.30 (*R*)-(−)-1-((2-Trifluoromethyl)phenyl)-1,2-ethanediol (*R*)-30. Following the general procedure (20 g cells to 100 mg substrate), diol (*R*)-**30** was obtained in the yield as indicated in Table 5. White solid; mp 66–67 °C; 82% ee; $[\alpha]_D^{21}$ −38.4 (*c* 0.5, EtOH); MS: *m*/*z* (rel. intensity %): 206(M^+, 4), 175(44), 173(25), 155(100), 145(18), 127(55); ^{1}H NMR (300 MHz, CD_3COCD_3, TMS): δ): δ 3.18(br s, 2H), 3.48 (dd, 1H, J_1 = 11.4 Hz, J_2 = 8.1 Hz), 3.54 (dd, 1H, J_1 = 11.1 Hz, J_2 = 3.0 Hz), 5.10 (dd, 1H, J_1 = 8.1Hz, J_2 = 1.5 Hz), 7.40–7.56 (m, 1H), 7.58–7.78 (m, 2H), 7.82–7.98 (m, 1H); IR (KBr): ν_{max} 3323, 2939, 2877, 1610, 1586, 1456, 1316, 1164, 1110, 1025, 770, 755, 667 cm^{-1}. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (eluent at V = 0.8 mL min^{-1}, hexane : 2-propanol = 9 : 1).

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