Supplementary Information

Asymmetric dihydroxylation of aryl olefins by sequential enantioselective epoxidation and regioselective hydrolysis with tandem biocatalysts

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Materials and methods

1) Materials

Styrene (**1a**), 4-, 3-, 2-chlorostyrenes (**1b-d**), styrene oxide (**2a**), racemic 1-phenyl-1, 2-ethanediol (**3a**), AD-MIX-α, kanamycin and dicyclopropyl ketone (DCPK) were purchased from Sigma-Aldrich. Racemic 4-chlorophenyl-1,2-ethanediol (**3b**) and 3-chlorophenyl-1,2-ethanediol (**3c**) were bought from Spectra group limited Inc. (USA). Hexadecane was purchased from Merck. All chemicals were in analytical grade and used without further purification.

4-, 3- and 2-chlorostyrene oxides (**2b-d**) were prepared by oxidation corresponding olefins using m-CPBA as oxidant (Botes, A.L. *et al.*, PCT. Int. Appl., WO 2005100569, 2005). Racemic 2-chlorophenyl-1,2-ethanediol (**3d**) was prepared by acid catalyzed hydrolysis of corresponding epoxide. The (*S*)-diols (**3a-d**) were prepared from corresponding aryl olefins using AD-MIX-α as catalyst (F. J. Moreno-Dorado *et al*., *Tetrahedron: Asymmetry* 2003, **14**, 503). The purity of the product was tested by GC and HPLC.

2) Bacterial strains and growth media for enantioselective epoxidation

The organism used in this study was *Escherichia coli* JM101 harboring the plasmid pSPZ10 containing the styAB genes of *Pseudomonas* sp. strain VLB120. The strain was cultivated in M9* media according to the reference (Panke *et al.*, *Biotechnol. Bioeng.* 2000, **69**, 91). The only difference is the cultivation temperature was 25 ^{o}C (it was 30 ^{o}C in the reference) after adding of 0.05% (vol/vol) DCPK. When the resulting culture was grown to the stationary phase (usually after 12-14 h), the cells were collected by centrifugation and stored at -80 ^{o}C. The enzyme activity was ~95 U/g cdw under standard activity test conditions.

3) Bacterial strain and growth media for regioselective hydrolysis of epoxide

The strain *Sphingomonas* sp. HXN-200 was cultivated in E2 media using *n*-Octane as carbon source (In detail, please see the supplying materials of the paper published on *J. Org.Chem.*, 2001, **66**, 8424).

4) Preparation of cell-free extract and lyophilized cell free extract powder from *Sphingomonas* sp. HXN-200.

Cell suspension (25 g cdw/L) of *Sphingomonas* sp. HXN-200 in KP buffer (pH 7.5, 100 mM) was broken at 30 KPSi by Constant Cell Disruption System. The mixture was ultracentrifuged at 245,000 g and 4 ^{o}C for 30 min to give the supernatant free of cell debris. The protein amount of cell free extract was determined by Bradford method. For the preparation of lyophilized powder, the cell-free extract was frozen at -80 ^{o}C overnight, and then it was lyophilized for 48 h to get the lyophilized powder.

5) Determination of the specific epoxidation activity of *E. coli* JM101 (pSPZ10)

Aliquots of 0.5-1 ml cell suspension were centrifuged and re-suspended with 0.475 ml KPB (pH 7.5, 0.1 M, containing 1% glucose) to a cell density of 1.0-2.5 g/L cdw (Depends on the enzyme activity). The cell suspension was incubated horizontally on a shaker (1000 rpm, 30 ^{o}C) for 2 min. 25 μL of a 30 mM styrene's solution in ethanol was added to a final concentration of 1.5 mM. The reaction continued for 5 min in the shaker and was finally stopped by adding 1 ml of cold ethyl acetate containing 0.5 mM dodecane as an internal standard. The water phase was extracted by vigorous shaking for 20 s and the phases were separated by centrifugation. The organic phase was dried over anhydrous magnesium sulfate and analyzed by GC. One unit (U) is defined as the activity that produces 1 μmol of a styrene oxide in 1 min at 30 ^{o}C.

6) General procedure for dihydroxylation of olefins (**1a-d**)

Dihydroxylation of styrene (**1a**): Lyophilized cell free extract (200 mg) was added to a 10 mL suspension of frozen/thawed cells of *E. coli* JM 101 pSPZ10 (2.5 g cdw/L) in 100 mM KP buffer (pH 7.5) containing glucose (28 mM), then hexadecane containing 20 mM of styrene was added. The mixture was incubated at 200 rpm and 30 ^{o}C. Aliquots were taken at different time points, centrifuged, and separated into aqueous and organic phase. 100 μL organic and 100 μL aqueous phases were diluted with 900 μL ethanol containing 1 mM benzylalcohol as the internal standard, respectively. All the samples were analyzed by HPLC for determination of concentration of olefins, epoxides and diols. The final concentration of each compound was the total concentration in both aqueous and organic phase. For determination of the ee value of

produced diols, 100 μL aqueous phases was saturated with NaCl and extracted by 200 μL ethyl acetate, after centrifugation, the organic phase was separated, dried over Na_2SO_4 and analyzed by chiral HPLC. After 35 h, (*S*)-**3a** was obtained in 95% yield and 99.4% ee.

Similar reactions were conducted for chlorostyrenes (**1b-d)** with different biocatalysts amounts shown in table 1.

7) Analytical methods

(a) GC analysis of styrene and styrene oxide.

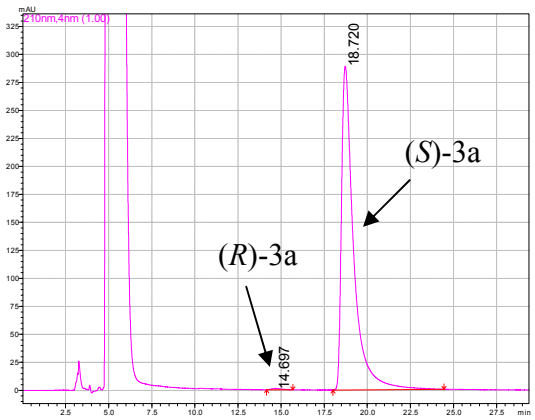
The concentration of styrene and styrene oxide were determined using HP-5 column (Agilent, 30m*0.32mm*0.25μm) connected to a Agilent 6890 gas chromatograph (Agilent, USA) with splitless injection and a temperature profile from 100 to 170°C at 7 °C min^{-1} with helium as the carrier gas. Compounds were detected by a flame ionization detector. The retention time for styrene (**1a**) and styrene oxide (**2a**) is 2.6 and 3.7 min, respectively.

(b) Reverse HPLC analysis

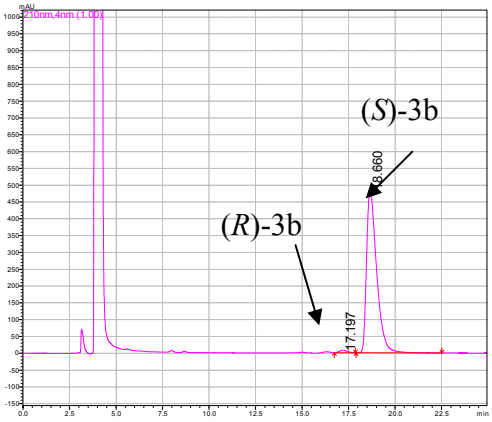
Styrene, 4-, 3-, 2-chlorostyrene (**1a-d**), corresponding epoxides (**2a-d**) and diols (**3a-d**) were analyzed by using HPLC with Hypersil BDS-C18 column (Agilent, 4.0 × 125 mm, 5um) at 210 nm. For **1a**, **2a** and **3a**, the retention time is 14.2, 9.5 and 2.7 min, respectively, Mobile phase: 10% ACN/ 90% Water at beginning changed to 40% ACN / 60% Water at 5 min and lasted for 17 min. Then the mobile phase was changed back to the beginning composition; flow rate, 1.5 mL/min; For **1b**, **2b**, **3b** and **1c**, **2c**, **3c**, Mobile phase: 20% ACN/ 80% Water at beginning changed to 45% ACN / 55% Water at 5 min and lasted for 17 min. Then the mobile phase was changed back to the beginning composition; flow rate, 1.0 mL/min. The retention time for **1b**, **2b**, **3b** is 4.8, 14.9 and 20.0 min, respectively. The retention time for **1c**, **2c**, **3c** is 4.6, 15.0 and 20.1 min, respectively; for **1d**, **2d** and **3d**, the retention time is 15.5, 12.3 and 9.9 min, respectively, Mobile phase: 10% ACN/ 90% Water at beginning changed to 55% ACN / 45% Water at 5 min and lasted for 15 min. Then the mobile phase was changed back to the beginning composition; flow rate, 1.0 mL/min;

(c) Chiral HPLC analysis of diols (3a-d) ee value.

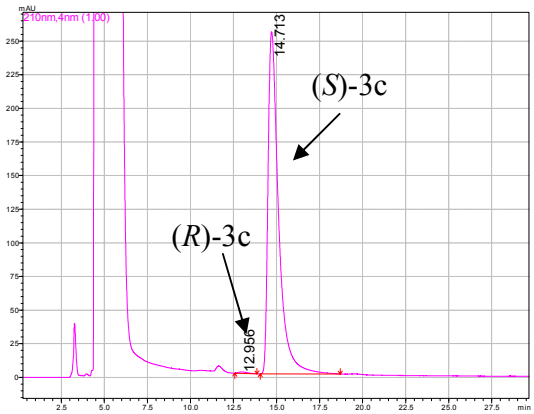
Chiralcel OD-H or OB-H (250 mm × 4.6 mm) column were used for the determination the ee value of diols; UV detection at 210 nm; a mixture of *n*-hexane and 2-propanol (95:5) was used as mobile phase with a flow rate of 1.0 mL/min. For 1-phenyl-1,2-ethanediol (**3a**) and 3-chloro-1-phenyl-1, 2-ethanediol (**3c**), Chiralcel OB-H column was used. For 4-chloro-1-phenyl-1, 2-ethanediol (**3b**) and 2-chloro-1-phenyl-1,2-ethanediol (**3d**), Chiralcel OD-H column was used. The retention times of (*R)*- and (*S*)-**3a** is 14.7 and 18.7 min, respectively; (*R)*- and (*S*)-**3b** is 17.2 and 18.7 min, respectively; (*R)*- and (*S*)-**3c** is 12.9 and 14.7 min, respectively; (*R)*- and (*S*)-**3d** is 12.9 and 16.9 min, respectively. The following figures are the chiral HPLC chromatograms of (*S*)-diols (**3a-d**) prepared by dihydroxylation listed in table 1.



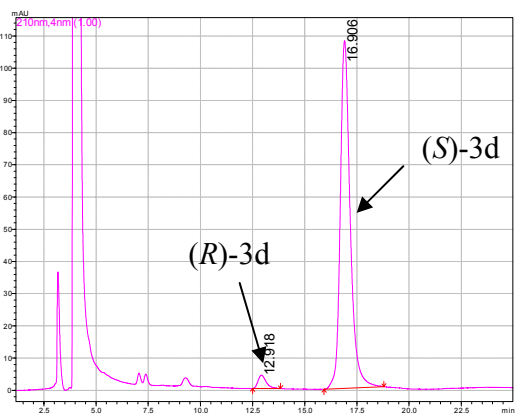
Phenyl-1,2-ethanediol
(Table 1, entry 2)



4-chlorophenyl-1,2-ethanediol
(Table 1, entry 5)



3-chlorophenyl-1,2-ethanediol
(Table 1, entry 6)



2-chlorophenyl-1,2-ethanediol
(Table 1, entry 7)