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# Enantioselective trans-Dihydroxylation of Aryl Olefins by Cascade Biocatalysis with Recombinant *Escherichia coli* Coexpressing Monooxygenase and Epoxide Hydrolase

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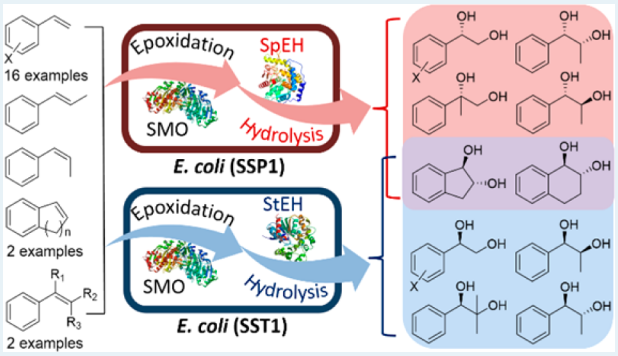
Supporting Information

**ABSTRACT:** Cascade biocatalysis via intracellular epoxidation and hydrolysis was developed as a green and efficient method for enantioselective dihydroxylation of aryl olefins to prepare chiral vicinal diols in high ee and high yield. *Escherichia coli* (SSP1) coexpressing styrene monooxygenase (SMO) and epoxide hydrolase SpEH was developed as a simple and efficient biocatalyst for *S*-enantioselective dihydroxylation of terminal aryl olefins **1a**−**15a** to give (*S*)-vicinal diols **1c**−**15c** in high ee (97.5−98.6% for 10 diols; 92.2−93.9% for 3 diols) and high yield (91−99% for 6 diols; 86−88% for 2 diols; 67% for 3 diols). Combining SMO and epoxide hydrolase StEH showing complementary regioselectivity to SpEH as a biocatalyst for the cascade biocatalysis gave rise to *R*-enantioselective dihydroxylation of aryl olefins, being the first example of this kind of reversing the overall enantioselectivity of cascade biocatalysis. *E. coli* (SST1) coexpressing SMO and StEH was also engineered as a green and efficient biocatalyst for *R*-dihydroxylation of terminal aryl olefins **1a**−**15a** to give (*R*)-vicinal diols **1c**−**15c** in high ee (94.2−98.2% for 7 diols; 84.2−89.9% for 6 diols) and high yield (90−99% for 6 diols; 85−89% for 5 diols; 65% for 1 diol). *E. coli* (SSP1) and *E. coli* (SST1) catalyzed the trans-dihydroxylation of *trans*-aryl olefin **16a** and *cis*-aryl olefin **17a** with excellent and complementary stereoselectivity, giving each of the four stereoisomers of 1-phenyl-1,2-propanediol **16c** in high ee and de, respectively. Both strains catalyzed the trans-dihydroxylation of aryl cyclic olefins **18a** and **19a** to afford the same *trans*-cyclic diols (1*R*,2*R*)-**18c** and (1*R*,2*R*)-**19c**, respectively, in excellent ee and de. This type of cascade biocatalysis provides a tool that is complementary to Sharpless dihydroxylation, accepting *cis*-alkene and offering enantioselective trans-dihydroxylation.




**KEYWORDS:** *biocatalysis, biotransformations, cascade catalysis, dihydroxylation, enantioselectivity, epoxide hydrolase, monooxygenase, vicinal diol*

## INTRODUCTION

One-pot cascade catalysis is an important tool for sustainable chemical manufacturing, since it could avoid the energy- and time-consuming, yield-decreasing, waste-producing, and labor-demanding separation and purification of the intermediates in traditional multistep synthesis.[1−5] Compared with cascade chemical catalysis, cascade biocatalysis has several distinctive features: it can be easily combined in one pot because of the similar reaction conditions of enzyme catalysis; it is nontoxic; and it is often highly chemo-, regio-, and enantioselective.[6−10] Recently, enantioselective cascade biocatalysis has received increasing attention for the synthesis of enantiopure compounds for chiral pharmaceutical and fine chemical manufacturing. Although cascade biocatalysis exists naturally in the metabolic pathways in cells, it is necessary to develop nonnatural cascade biocatalysis for the synthesis of nonnatural chemical compounds. Over the years, several types of enantioselective cascade biocatalysis have been reported.[11−27] Nevertheless, it is still very important to develop a new type of enantioselective cascade biocatalysis for practical synthesis.

We recently developed a novel type of enantioselective cascade biocatalysis for trans-dihydroxylation of olefins to prepare enantiopure vicinal diols via one-pot cascade epoxidation and hydrolysis, a formal dihydroxylation that is different from dioxygenase-catalyzed dihydroxylation. Cells of *Sphingomonas* sp. HXN-200 containing a P450 monooxygenase[28−31] and epoxide hydrolase (EH)[32−34] were used to catalyze the enantioselective trans-dihydroxylation of several cyclic olefins.[35] A tandem biocatalysts system consisting of the *Escherichia coli* cells expressing styrene monooxygenase

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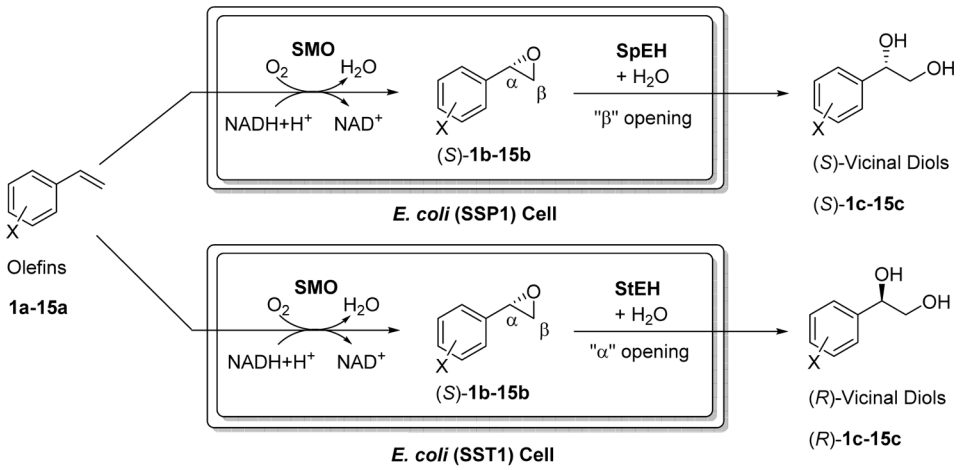
**Scheme 1. Enantioselective Dihydroxylation of Aryl Olefins 1a−15a with *E. coli* (SSP1) Coexpressing Styrene Monooxygenase (SMO) and Epoxide Hydrolase from *Sphingomonas* sp. HXN-200 (SpEH) To Produce (*S*)-Vicinal Diols (*S*)-1c−15c and with *E. coli* (SST1) Coexpressing SMO and Epoxide Hydrolase from *Solanum tuberosum* (StEH) To Produce (*R*)-Vicinal Diols (*R*)-1c−15c, Respectively**




**1a-c**: X = H **4a-c**: X = *p*-Br **7a-c**: X = *p*-$CF_3$ **10a-c**: X = *m*-Br **13a-c**: X = *o*-F
**2a-c**: X = *p*-F **5a-c**: X = *p*-Me **8a-c**: X = *m*-F **11a-c**: X = *m*-Me **14a-c**: X = *o*-Cl
**3a-c**: X = *p*-Cl **6a-c**: X = *p*-OMe **9a-c**: X = *m*-Cl **12a-c**: X = *m*-OMe **15a-c**: X = *o*-Me

(SMO)[36−42] and the cell-free extract containing the EH (SpEH)[32−34] of *Sphingomonas* sp. HXN-200 was engineered for one-pot simultaneous *S*-enantioselective dihydroxylation of aryl olefins;[43] lipase-mediated epoxidation, and EH-catalyzed epoxide hydrolysis were combined for the enantioselective trans-dihydroxylation of cyclic olefins via one-pot sequential epoxidation and hydrolysis.[44] Although asymmetric cis-dihydroxylation of olefins can be achieved in one step by Sharpless dihydroxylation using a heavy metal oxide catalyst with chiral ligands,[45] chemical trans-dihydroxylation of olefins requires two reaction steps: epoxidation of olefins[46] and subsequent hydrolysis of epoxides,[47,48] which needs the separation of toxic and unstable epoxide intermediates and also utilizes toxic metals (e.g., osmium, cobalt). Thus, one-pot cascade biocatalysis for trans-dihydroxylation of alkenes provide a greener and more efficient synthetic method for the preparation of enantiopure vicinal diols than the corresponding two-step chemical catalysis. It is also a complementary tool to Sharpless dihydroxylation.

We are interested in further developing this type of cascade biocatalysis as a practical method for the preparation of enantiopure vicinal diols that are useful and valuable synthetic intermediates for many pharmaceuticals, bioactive compounds, and chiral reagents.[49−58] A group of enantiopure vicinal diols are selected as target compounds (Scheme 1−4). For example, (*S*)-1-phenyl-1,2-ethanediol, **1c**, is a crucial synthetic precursor for pharmaceutical (*R*)-fluoxetine,[50] chiral phosphoramidite ligand,[51] and an auxiliary for stereoselective glycosylation;[52] (*R*)-1-(4-fluorophenyl)-1,2-ethanediol, **2c**, is an intermediate for preparing the cholesterol-lowering medicine Ezetimibe;[53] (*R*)-1-(3-chlorophenyl)-1,2-ethanediol, **9c**, is a key chiral synthon for $\beta_3$-adrenergic agonists;[54] (*S*)-1-(3-methoxyphenyl)-1,2-ethanediol, **12c**, is a chiral ligand for chromium complex catalysts;[55] (1*R*,2*S*)-phenylpropanediol, **16c**, and (1*S*,2*S*)-**16c** are useful for the synthesis of muscle relaxant phenylcarbanate[56] and selegiline,[57] respectively; and (1*R*,2*R*)-indanediol, **18c**, can be easily converted to (1*S*)-amino-(2*R*)-indanol for the synthesis of the anti-HIV drug Indinavir.[58] Thus far, the reported cascade biocatalysis system for the dihydroxylation of olefins is relatively complicated, and its efficiency needs to be further improved for practical application, the substrate scope is not fully explored, and the dihydroxylation is only *S*-selective.

Here, we report the development of *E. coli* (SSP1) cells coexpressing SMO and SpEH as a simpler and more efficient biocatalyst for *S*-enantioselective dihydroxylation of 15 terminal aryl olefins, **1a−15a**, to produce the corresponding (*S*)-vicinal diols **1c−15c** in high ee and good yield. We also report the development of *E. coli* (SST1) cells coexpressing SMO and another EH (StEH) with the complementary regioselectivity of SpEH as a simple and efficient biocatalyst for *R*-enantioselective dihydroxylation of 15 terminal aryl olefins, **1a−15a**, to produce the corresponding (*R*)-vicinal diols **1c−15c** in high ee and good yield. *E. coli* (SSP1) and *E. coli* (SST1) cells are further explored for the highly enantioselective trans-dihydroxylation of nonterminal aryl olefins **16a−17a** to synthesize all four enantiomers of 1-phenyl-1,2-propanediol **16c** and of aryl cyclic olefins **18a−19a** to prepare (1*R*,2*R*)-*trans*-diols **18c−19c**.

## RESULTS AND DISCUSSION

**Genetic Engineering of *E. coli* Coexpressing SMO and SpEH for *S*-Selective Dihydroxylation of Styrenes.** SMO was chosen as the enzyme for epoxidation of olefins in the first step of the cascade because it is a well-known enzyme for the epoxidation of styrenes to give (*S*)-epoxides in high ee.[36−42] In addition, the epoxidation of styrenes with recombinant *E. coli* expressing SMO has been developed as one of the most productive biocatalytic oxyfunctionalization processes.[41,42] EH from *Sphingomonas* sp. HXN-200 (SpEH) was used as the enzyme for the hydrolysis of the epoxides in the second step of the cascade because this EH is known to hydrolyze (*S*)-styrene oxides at the "*β*" position to give (*S*)-diols in high ee with the retention of configuration.[32−34] The *E. coli* strain expressing SpEH was also developed as an efficient catalyst for the preparation of enantiopure epoxides by kinetic resolution.[34]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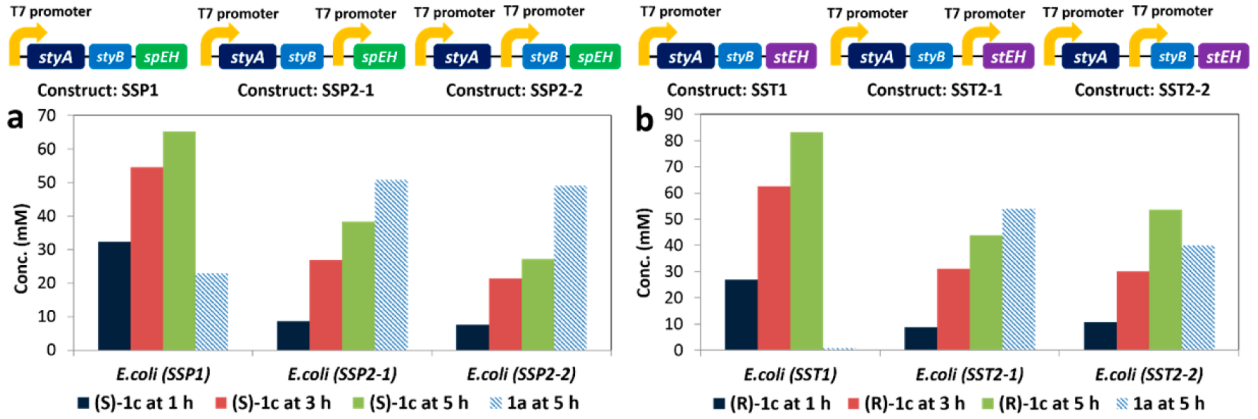


**Figure 1.** (a) Genetic constructions of and dihydroxylation with three different *E. coli* strains coexpressing SMO (StyA and StyB) and SpEH; (b) genetic constructions of and dihydroxylation with three different *E. coli* strains coexpressing SMO (StyA and StyB) and StEH. The dihydroxylation of 100 mM styrene, **1a**, to diol, **1c**, was performed with resting cells (10 g cdw/L) of the corresponding *E. coli* strain at 30 °C in a two-liquid-phase system (*n*-hexadecane/KP buffer = 1:1).

To develop recombinant *E. coli* containing both SMO and SpEH as a simple and efficient catalyst system for the dihydroxylation of olefins, the two coding sequences of SMO (*styA* and *styB*) were amplified from pSPZ10 plasmid,[37] and the gene of SpEH was amplified from the genome of the *Sphingomonas* sp. HXN-200.[34] To optimize the expression level of the three genes, we engineered three different expression cassettes on the commercially available plasmid pRSFduet (Figure 1a): SSP1, an artificial operon of *styA*, *styB*, and *spEH* controlled by one T7 promoter; and SSP2-1 and SSP2-2, where *styA*, *styB*, and *spEH* are under the control of two T7 promoters. These plasmids were transformed into *E. coli* T7 expression cells to give *E. coli* (SSP1), *E. coli* (SSP2-1), and *E. coli* (SSP2-2), respectively. These strains were grown in TB medium, and IPTG was added to induce protein expression. As a result, all three strains were able to coexpress SMO (StyA and StyB) and SpEH, but at different levels. The strains were examined for biotransformation of 100 mM styrene, **1a**, in a two-liquid-phase system consisting of potassium phosphate (KP) buffer and *n*-hexadecane (1:1). *E. coli* (SSP1) showed the best results, producing 65 mM (*S*)-1-phenylethane-1,2-diol, **1c**, in 99% ee at 5 h (Figure 1a). The other two strains also gave (*S*)-**1c**, but in lower concentrations. In comparison with other two strains, *E. coli* (SSP1) has a higher ratio of SMO/SpEH, which is desirable for the cascade dihydroxylation because the catalytic efficiency of SpEH ($k_{cat}/K_m$ = 6.0 $mM^{-1}$ $s^{-1}$)[34] is higher than that of SMO ($k_{cat}/K_m$ = 4.2 $mM^{-1}$ $s^{-1}$).[59] The superiority of *E. coli* (SSP1) is also probably due to the more homogeneous expression of several genes in one operon.[60,61] *E. coli* (SSP1) was selected for further development.

**Genetic Engineering of *E. coli* Coexpressing SMO and StEH for *R*-Selective Dihydroxylation of Styrenes.** To engineer a cascade biocatalysis for *R*-selective dihydroxylation of styrenes, the EH from *Solanum tuberosum* (StEH) was selected as the enzyme for the hydrolysis step because StEH is known to hydrolyze (*S*)-styrene oxides to offer (*R*)-diols by opening at the $\alpha$ position (inversion of configuration).[62−64] The regioselectivity for the hydrolysis with StEH is complementary to that with SpEH. The concept was successfully proven by using a tandem biocatalysts system consisting of resting cells of *E. coli* (pSPZ10) expressing SMO (5 g cdw/L) and *Pichia pastoris* CBS 7435_MutS_PotHis expressing StEH (5 g cdw/L) for the dihydroxylation of 100 mM styrene, **1a**, in the same two-liquid-phase system as described above. (*R*)-1-Phenyl-1,2-ethanediol, **1c**, was formed in 95% ee and 74% yield. To avoid the difficulty in cultivation of yeast cells and the preparation of cells of two microorganisms, we started to engineer an *E. coli* strain coexpressing SMO and StEH as a simple and efficient catalyst for *R*-selective dihydroxylation of styrenes. *E. coli* (pSPZ10_pMS470Δ8) was constructed by electronically transforming pMS470Δ8 (containing *StEH*) to competent cells of *E. coli* (pSPZ10) (containing *styA* and *styB*). During cell growth, SMO was induced by adding 0.1% (v/v) DCPK, and StEH was induced by adding 0.05 mM IPTG. The harvested cells were used at 20 g cdw/L for the dihydroxylation of 100 mM styrene **1a** in the two-liquid-phase system for 8 h to produce (*R*)-1-phenyl-1,2-ethanediol, **1c**, in 93% ee and 90% yield. The specific activity was ∼25 U/g cdw, which is lower than that of *E. coli* (SSP1) (50 U/g cdw).

To engineer a more active *E. coli* strain coexpressing SMO and StEH for *R*-selective dihydroxylation of styrenes, the gene of StEH was synthesized according to the reported potato cDNA sequence[65] with codon optimization for the expression in *E. coli*. Similar to the engineering of SMO and SpEH, three different expression cassettes of SMO and StEH were constructed, and three strains *E. coli* (SST1), *E. coli* (SST2-1), and *E. coli* (SST2-2) were obtained and evaluated for the dihydroxylation (Figure 1b). *E. coli* (SST1) gave a specific activity of 40 U/g cdw and produced 82 mM (*R*)-1-phenylethane-1,2-diol, **1c**, in 96% ee at 5 h in the dihydroxylation of 100 mM styrene **1a** at a cell density of 10 g cdw/L in the two-liquid-phase system. It is the best among the three strains. It also showed higher activity than *E. coli* (pSPZ10_pMS470Δ8). Thus, *E. coli* (SST1) was chosen for further development as a powerful catalyst for *R*-enantioselective dihydroxylation of styrenes, being complementary to *E. coli* (SSP1) for the cascade biocatalysis.

**Cell Growth and Dihydroxylation Activity of *E. coli* (SSP1) and *E. coli* (SST1).** *E. coli* (SSP1) strain was grown easily in M9 medium, with glucose as the carbon source, in a shaking flask, and SMO and SpEH were coexpressed by adding IPTG as the inducer. The growth and activity of the cells were monitored by taking samples at different time points for measuring the optical density and dihydroxylation activity. As


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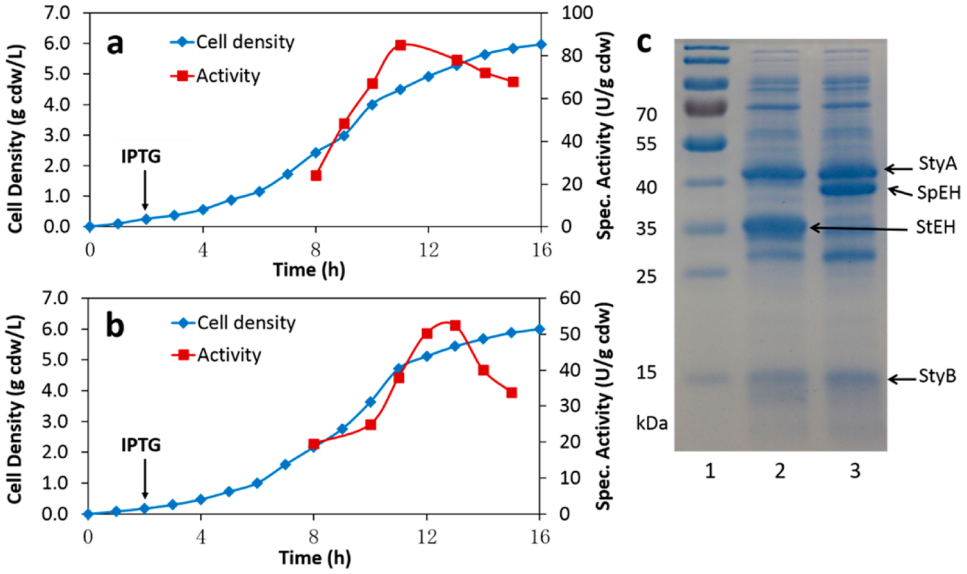


**Figure 2.** (a, b) Cell growth and specific activity for dihydroxylation of the recombinant strains: (a) *E. coli* (SSP1), (b) *E. coli* (SST1). Cells were initially cultured at 37 °C, induced at 2 h by the addition of IPTG (0.5 mM), and then grown at 25 °C. The activities were based on 30 min dihydroxylation of 10 mM styrene **1a** with resting cells (1 g cdw/L) in aqueous buffer. (c) SDS−PAGE. Lane 1, protein marker; lane 2, cell-free extract of *E. coli* (SST1) coexpressing SMO and StEH taken at 12 h; lane 3, cell-free extract of *E. coli* (SSP1) coexpressing SMO and SpEH taken at 12 h.

shown in Figure 2a, the cells reached a high density (5−6 g cdw/L; cdw: cell dry weight) at 13−16 h. High specific activities (70−80 U/g cdw) toward *S*-dihydroxylation of styrene **1a** were achieved at the late exponential growing phase (11−13 h). In the SDS−PAGE of the cell free extract of the *E. coli* (SSP1) taken at 12 h (Figure 2c, lane 3), StyA, StyB, and SpEH are clearly visible.

*E. coli* (SST1) showed a similar cell growth curve and reached high cell density (5−6 g cdw/L) at 13−16 h (Figure 2b). Good specific activities (40−50 U/g cdw) for *R*-dihydroxylation of styrene **1a** were also achieved at the late exponential growing phase (12−14 h). The SDS−PAGE of the cell free extract of the *E. coli* (SST1) taken at 12 h (Figure 2c, lane 2) also clearly demonstrated the expressing of StyA, StyB, and StEH in *E. coli* (SST1).

**Asymmetric Dihydroxylation of Terminal Aryl Olefins 1a−15a to (*S*)-Vicinal Diols 1c−15c with Resting Cells of *E. coli* (SSP1).** To explore the synthetic potential of *E. coli* (SSP1), the resting cells of the strain were employed for the dihydroxylation of 20 mM styrene **1a** and substituted styrenes **2a−15a** (Scheme 1) in a two-liquid-phase system containing KP buffer and *n*-hexadecane (1:1). The *n*-hexadecane phase acts as a reservoir of the substrate and possible epoxide intermediate to reduce their inhibition effect on the enzymes. As listed in Table 1, (*S*)-vicinal diols **1c−15c** were produced in high ee from **1a−15a** by the one-pot cascade epoxidation and hydrolysis with resting cells of *E. coli* (SSP1). Importantly, many vicinal diols, such as (*S*)-**1c−4c**, (*S*)-**7c−10c**, (*S*)-**12c**, and (*S*)-**13c**, were produced in excellent ee (≥97.5%). Other vicinal diols, including (*S*)-**5c**, (*S*)-**11c**, and (*S*)-**14c**, were obtained in high ee (92.2−93.9%). The configurations of **1c−15c** were established by comparing bioproducts with the standard diols that were either commercially available or prepared via Sharpless asymmetric dihydroxylation (Figures S2−S16, Supporting Information). The high *S*-enantioselectivity of dihydroxylation is due to the high *S*-enantioselectivity of SMO-catalyzed epoxidation of styrenes and the high regioselectivity of SpEH-catalyzed hydrolysis of (*S*)-epoxides at the *β* position. Only two diols, (*S*)-**6c** and (*S*)-**15c**, were produced in moderate ee (83.2 and 65.7%), possibly because of the autohydrolysis of unstable epoxide **6b** or the reduced regioselectivity of SpEH in the hydrolysis of epoxide **15b**.

Most of the dihydroxylations gave high conversion and high yield. (*S*)-Vicinal diols **1c**, **8c**, **9c**, **11c**, **12c**, and **13c** were obtained in 91−>99% yields; (*S*)-**2c** and (*S*)-**5c** were formed in 86−88% yields; and (*S*)-**3c**, (*S*)-**6c**, and (*S*)-**10c** were produced in 67% yields. This confirmed that SMO and SpEH coexpressed in the *E. coli* cells are very active for the cascade biocatalysis. The specific activity for these *S*-dihydroxylations is 11−55 U/g cdw, with an exception for the *S*-dihydroxylation of **10a** (8 U/g cdw). In the previously reported tandem biocatalysts system, (*S*)-epoxide **1b** accumulated as the intermediate in the early stage of the biotransformation.[43] Because of the high activity of SpEH in *E. coli* (SSP1), there was no epoxide accumulated during the cascade biocatalysis with resting cells of *E. coli* (SSP1). The time curve of the dihydroxylation of **9a** was a representative example (Figure S1, Supporting Information), which clearly evidences this point. In comparison with the previously reported tandem biocatalysts (20 mM **1a** was converted in 21 h with 2.5 g cdw/L of *E. coli* cells expressing SMO and 20 g of protein/L of cell free extract containing SpEH),[43] the use of resting cells of *E. coli* (SSP1) coexpressing SMO and SpEH provides a much simpler catalyst and much higher catalytic efficiency: 20 mM **1a** was converted to (*S*)-**1c** in only 2 h with 10 g cdw/L of *E. coli* (SSP1) cells.

The *S*-dihydroxylations of **4a**, **7a**, **14a**, and **15a** with resting cells of *E. coli* (SSP1) gave lower yields (25−34%). This is mainly due to the low epoxidation activity of SMO toward these substrates, which have either a strong electron-withdrawing group or an ortho substitution.[38−40]

**Asymmetric Dihydroxylation of Terminal Aryl Olefins 1a−15a to (*R*)-Vicinal Diols 1c−15c with Resting Cells of *E. coli* (SST1).** To explore the synthetic potential of another strain, *E. coli* (SST1), the resting cells of the strain were used


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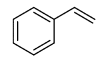
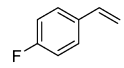
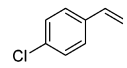
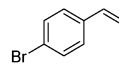
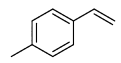
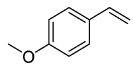
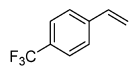
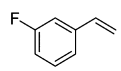
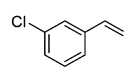
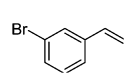
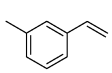
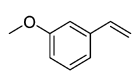
**Table 1. Enantioselective Dihydroxylation of Aryl Olefins 1a−15a with Resting Cells of *E. coli* (SSP1) and *E. coli* (SST1), Respectively**

| | Substrate[a] | Catalyst | Act. (U/g cdw)[b] | Conv. (%)[c] | Prod. | Yield (%)[d] | *ee* (%)[e] |
|---|---|---|---|---|---|---|---|
| **1a** |  | *E. coli* (SSP1) | 46 | >99 | (*S*)-**1c** | 92 | 98.1 |
| | | *E. coli* (SST1) | 39 | >99 | (*R*)-**1c** | 93 | 95.5 |
| **2a** |  | *E. coli* (SSP1) | 33 | >99 | (*S*)-**2c** | 88 | 97.9 |
| | | *E. coli* (SST1) | 41 | >99 | (*R*)-**2c** | 90 | 95.2 |
| **3a** |  | *E. coli* (SSP1) | 20 | 67 | (*S*)-**3c** | 67 | 97.8 |
| | | *E. coli* (SST1) | 22 | 90 | (*R*)-**3c** | 89 | 95.6 |
| **4a** |  | *E. coli* (SSP1) | 7 | 40 | (*S*)-**4c** | 34 | 97.7 |
| | | *E. coli* (SST1) | 7 | 92 | (*R*)-**4c** | 86 | 94.4 |
| **5a** |  | *E. coli* (SSP1) | 12 | 98 | (*S*)-**5c** | 86 | 93.9 |
| | | *E. coli* (SST1) | 15 | >99 | (*R*)-**5c** | 85 | 87.7 |
| **6a** |  | *E. coli* (SSP1) | 26 | >99 | (*S*)-**6c** | 67 | 83.2 |
| | | *E. coli* (SST1) | 20 | >99 | (*R*)-**6c** | 65 | 85.4 |
| **7a** |  | *E. coli* (SSP1) | 2 | 31 | (*S*)-**7c** | 25 | 97.5 |
| | | *E. coli* (SST1) | 2 | 18 | (*R*)-**7c** | 19[f] | 87.7 |
| **8a** |  | *E. coli* (SSP1) | 41 | >99 | (*S*)-**8c** | >99 | 98.4 |
| | | *E. coli* (SST1) | 43 | >99 | (*R*)-**8c** | >99 | 94.2 |
| **9a** |  | *E. coli* (SSP1) | 22 | 96 | (*S*)-**9c** | 95 | 97.5 |
| | | *E. coli* (SST1) | 15 | 97 | (*R*)-**9c** | 95 | 95.8 |
| **10a** |  | *E. coli* (SSP1) | 8 | 80 | (*S*)-**10c** | 67 | 97.5 |
| | | *E. coli* (SST1) | 6 | 98 | (*R*)-**10c** | 86 | 84.2 |
| **11a** |  | *E. coli* (SSP1) | 11 | 97 | (*S*)-**11c** | 91 | 93.1 |
| | | *E. coli* (SST1) | 9 | 98 | (*R*)-**11c** | 92 | 98.2 |
| **12a** |  | *E. coli* (SSP1) | 55 | >99 | (*S*)-**12c** | 96 | 97.6 |
| | | *E. coli* (SST1) | 40 | >99 | (*R*)-**12c** | >99 | 87.3 |
| **13a** |  | *E. coli* (SSP1) | 11 | 94 | (*S*)-**13c** | 94 | 98.6 |
| | | *E. coli* (SST1) | 17 | 98 | (*R*)-**13c** | 89 | 68.1 |
| **14a** |  | *E. coli* (SSP1) | 4 | 31 | (*S*)-**14c** | 34[f] | 92.2 |
| | | *E. coli* (SST1) | 2 | 26 | (*R*)-**14c** | 10 | 36.9 |
| **15a** |  | *E. coli* (SSP1) | 5 | 36 | (*S*)-**15c** | 34 | 65.7 |
| | | *E. coli* (SST1) | 5 | 34 | (*R*)-**15c** | 15 | 89.9 |

[a]The reactions were performed with substrates **1a−15a** (20 mM in organic phase) and resting cells (10 g cdw/L) in a two-liquid-phase system consisting of KP buffer (200 mM, pH 8.0, 2% glucose) and *n*-hexadecane (1:1) at 30 °C for 8 h. [b]Activity is the specific activity determined for the initial 30 min. [c]Conversion is the consumption of starting substrate, determined by normal phase HPLC analysis of the remaining substrate in the *n*-hexadecane phase. Error limit: 3% of the state values. [d]Yield is the analytical yield of the formation of diol product, determined by reversed phase HPLC analysis of the product in the aqueous phase. Error limit: 3% of the state values. [e]The ee value was determined by chiral HPLC analysis. Error limit: 0.2% of the state values. [f]Yield is slightly higher than conversion due to the error limit in the measurement of yield and conversion.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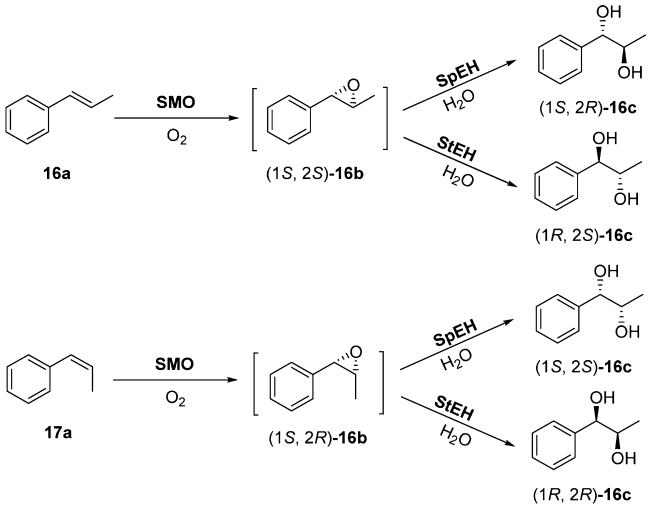
for the *R*-dihydroxylation of 20 mM olefins **1a**−**15a** (Scheme 1) in the same two-liquid-phase system as described above. The results are listed in Table 1, and (*R*)-vicinal diols (*R*)-**1c**−**15c** were produced from **1a**−**15a** by the one-pot cascade epoxidation and hydrolysis with resting cells of *E. coli* (SST1). Many (*R*)-vicinal diols, such as (*R*)-**1c**−**4c**, (*R*)-**8c**, (*R*)-**9c**, and (*R*)-**11c**, were produced in excellent ee (94.2−98.2%). The diols (*R*)-**5c**−**7c**, (*R*)-**10c**, (*R*)-**12c**, and (*R*)-**15c** were also formed in high ee (84.2−89.9%). The high *R*-enantioselectivity of dihydroxylation is the combined result of high *S*-enantioselectivity of SMO-catalyzed epoxidation of styrenes and the high regioselectivity of StEH-catalyzed hydrolysis of (*S*)-epoxides at the $\alpha$ position. Only (*R*)-**13c** and (*R*)-**14c** were obtained in low ee (68.1 and 36.9%). The low enantioselectivity was probably caused by the hindrance of the ortho-substitution in epoxides **13b**−**14b** for the hydrolysis at the $\alpha$ position with StEH.

Most of the *R*-dihydroxylations with the resting cells of *E. coli* (SST1) gave also high conversion and high yield. (*R*)-Vicinal diols **1c**, **2c**, **8c**, **9c**, **11c**, and **12c** were obtained in 90−>99% yields; (*R*)-**3c**, (*R*)-**4c**, (*R*)-**5c**, (*R*)-**10c**, and (*R*)-**13c** were formed in 85−89% yields; and (*R*)-**6c** was produced in 65% yield. This also confirmed that SMO and StEH coexpressed in the *E. coli* cells are very active for the cascade biocatalysis. The specific activity for these *R*-dihydroxylations is 15−43 U/g cdw, with an exception for the *R*-dihydroxylations of **4a**, **10a**, and **11a** (6−9 U/g cdw). Because of the high activity of StEH in *E. coli* (SST1), no epoxide was accumulated in the dihydroxylations of **1a**−**12a**. Accumulation of expoxide was observed only in the dihydroxylation of ortho-substituted olefins **13a**−**15a**. Here, the resting cells of *E. coli* (SST1) coexpressing SMO and StEH were developed as a simple and efficient catalyst for the synthesis of these (*R*)-vicinal diols via dihydroxylation, being the first biocatalytic system for the *R*-enantioselective dihydroxylations of styrenes. Clearly, *E. coli* (SSP1) and *E. coli* (SST1) are excellent cascade biocatalysts with complementary enantioselectivity for the dihydroxylation of styrene and its derivatives. It is the first example to achieve the reversal of overall enantioselectivity of cascade biocatalysis by changing the regioselectivity in an individual reaction step. The concept can be applied to solve the problem of lacking mirror-image enzymes in biocatalysis, in addition to the discovery and development of enantiocomplementary enzymes.[66]

The *R*-dihydroxylations of **7a**, **14a**, and **15a** with resting cells of *E. coli* (SST1) gave lower yields (10−19%). This is similar to the cases with *E. coli* (SSP1) and due to the low activity of SMO.

**Asymmetric Trans Dihydroxylation of Nonterminal Aryl Olefins 16a and 17a with Resting Cells of *E. coli* (SSP1) and *E. coli* (SST1).** To prepare vicinal diols with two chiral centers and evidently distinguish our trans-dihydroxylation with Sharpless cis-dihydroxylation, we tested the dihydroxylation of nonterminal olefin substrates **16a** and **17a** with our catalysts (Scheme 2). The trans-dihydroxylation was also performed in a two-phase system with resting cells as catalysts. As shown in Table 2, trans-dihydroxylation of **16a** and **17a** with *E. coli* (SST1) gave (1*R*,2*S*)-**16c** and (1*R*,2*R*)-**16c** in excellent ee (>98%) and de (≥98%), respectively. The configuration of **16c** was established by comparing bioproducts with the standard diols that were prepared via Sharpless asymmetric dihydroxylation (Figures S17, S18, Supporting Information). The yields (96 and 89%) and the specific activities (15 and 20 U/g cdw) were also high. On the other hand, trans-dihydroxylation of $\beta$-methyl styrenes **16a** and **17a** with *E. coli* (SSP1) afforded (1*S*,2*R*)-**16c** in 94.2% ee and 91.8% de and (1*S*, 2*S*)-**16c** in 85.6% ee and >99% de, respectively. The regioselectivity and the yield for the hydrolysis were decreased, possibly as a result of the steric hindrance of a $\beta$-methyl group in the epoxide intermediate to the hydrolysis at the $\beta$ position with SpEH. Nevertheless, the product ee and de were still quite high. The great achievement here was the production of all four stereoisomers of 1-phenyl-1,2-propanediol, **16c**, in high ee and de by the trans-dihydroxylation of *trans*-alkene **16a** and *cis*-alkene **17a** with the two complementary biocatalysts, respectively. In comparison, the elegant Sharpless dihydroxylation has difficulty in transforming *cis*-alkene, such as **17a**, with high selectivity.[45]

**Scheme 2. Enantioselective Trans-Dihydroxylation of Nonterminal Aryl Olefins 16a and 17a with *E. coli* (SSP1) (expressing SMO and SpEH) To Produce Vicinal Diols (1*S*,2*R*)-16c and (1*S*,2*S*)-16c, and with *E. coli* (SST1) (expressing SMO and StEH) To Produce Vicinal Diols (1*R*,2*S*)-16c and (1*R*,2*R*)-16c, Respectively**




**Asymmetric Trans Dihydroxylation of Aryl Cyclic Olefins 18a and 19a with Resting Cells of *E. coli* (SSP1) and *E. coli* (SST1).** The trans-dihydroxylation of cyclic aryl olefins **18a** and **19a** (Scheme 3) was performed with resting cells of *E. coli* (SSP1) and *E. coli* (SST1). As shown in Table 2, *E. coli* (SSP1) gave (1*R*,2*R*)-**18c** and (1*R*,2*R*)-**19c** in very high ee (>96%), de (>98%), and yield (73−80%) from **18a** and **19a**, respectively. The configurations of **18c**−**19c** were established by comparing bioproducts with the standard diols that were commercially available (Figures S19, S20, Supporting Information). The unexpected change in the enantioselectivity of the dihydroxylation was possibly caused by the change in the regioselectivity of SpEH in *E. coli* (SSP1) to the cyclic epoxides **18b** and **19b**. On the other hand, *E. coli* (SST1) also produced (1*R*,2*R*)-**18c** and (1*R*,2*R*)-**19c** in very high ee (>96%), de (>98%), and yield (67−71%) from **18a** and **19a**, respectively. These results demonstrated once again the unique potential of *E. coli* (SSP1) and *E. coli* (SST1) in the asymmetric trans-dihydroxylation of *cis*-alkenes. In comparison, Sharpless dihydroxylation has difficulty with *cis*-alkenes as starting materials and could not produce *trans*-vicinal diols (1*R*,2*R*)-**18c** and (1*R*,2*R*)-**19c** from *cis*-alkenes **18a** and **19a**.[45]

**Asymmetric Dihydroxylation of Other Aryl Olefins 20a−22a with Resting Cells of *E. coli* (SSP1) and *E. coli***


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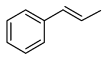
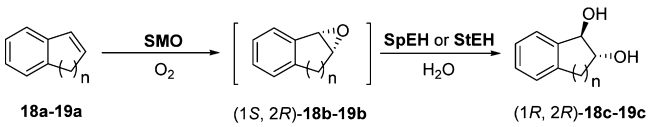
**Table 2. Enantioselective Trans-Dihydroxylation of Nonterminal Aryl Olefins 16a, 17a and Aryl Cyclic Olefins 18a, 19a with Resting Cells of *E. coli* (SSP1) and *E. coli* (SST1), Respectively**

| Substrate[a] | | Catalyst | Act. (U/g cdw)[b] | Conv. (%)[c] | Prod. | Yield (%)[d] | *ee* (%)[e] | *de* (%)[f] |
|---|---|---|---|---|---|---|---|---|
| **16a** |  | *E. coli* (SSP1) | 23 | 80 | (1*S*, 2*R*)-**16c** | 22 | 94[g] | 91.8 |
| | | *E. coli* (SST1) | 15 | >99 | (1*R*, 2*S*)-**16c** | 96 | >98[g] | 98.0 |
| **17a** |  | *E. coli* (SSP1) | 19 | 94 | (1*S*, 2*S*)-**16c** | 75 | 85.6 | >99 |
| | | *E. coli* (SST1) | 20 | 97 | (1*R*, 2*R*)-**16c** | 89 | 98.8 | >99 |
| **18a** |  | *E. coli* (SSP1) | 28 | 97 | (1*R*, 2*R*)-**18c** | 80 | 98.0 | 98.8 |
| | | *E. coli* (SST1) | 20 | 98 | (1*R*, 2*R*)-**18c** | 71 | 96.1 | 98.1 |
| **19a** |  | *E. coli* (SSP1) | 4 | 75 | (1*R*, 2*R*)-**19c** | 73 | 96.8 | >99 |
| | | *E. coli* (SST1) | 4 | 69 | (1*R*, 2*R*)-**19c** | 67 | 99.6 | >99 |

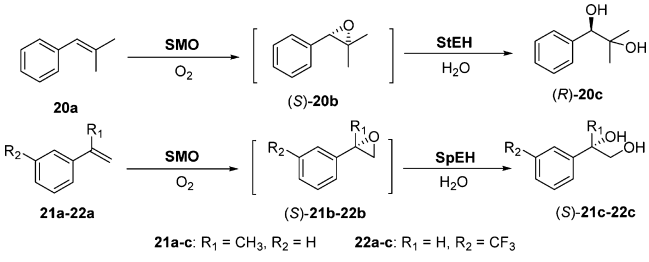
[a]The reactions were performed with substrates **16a**−**19a** (20 mM in organic phase) and resting cells (10 g cdw/L) in a two-liquid-phase system consisting of KP buffer (200 mM, pH 8.0, 2% glucose) and *n*-hexadecane (1:1) at 30 °C for 8 h. [b]Activity is the specific activity determined for initial 30 min. [c]Conversion is the consumption of starting substrate, determined by normal phase HPLC analysis of the remaining substrate in the *n*-hexadecane phase. Error limit: 3% of the state values. [d]Yield is the analytical yield of the formation of diol product, determined by reversed phase HPLC analysis of the product in the aqueous phase. Error limit: 3% of the state values. [e]The ee value was determined by chiral HPLC analysis. Error limit: 0.2% of the state values. [f]The de value was determined by chiral HPLC analysis. Error limit: 0.2% of the state values. [g]Error limit: 0.5% of the state values.

**Scheme 3. Enantioselective Trans-Dihydroxylation of Aryl Cyclic Olefins 18a and 19a with *E. coli* (SSP1) (expressing SMO and SpEH) or *E. coli* (SST1) (expressing SMO and StEH) To Produce Vicinal Diols (1*R*,2*R*)-18c and (1*R*,2*R*)-19c**




**(SST1).** We further tested *E. coli* (SSP1) and *E. coli* (SST1) for the dihydroxylation of substituted olefins (**20a**−**22a**) (Scheme 4, Table 3) in a two-liquid-phase system with the resting cells as catalysts. With two methyl groups on the $\beta$ carbon, **20a** could not be converted to (*S*)-**20c** in high ee by *E. coli* (SSP1) because of the huge steric hindrance at the $\beta$ position for hydrolysis with SpEH. On the other hand, dihydroxylation of **20a** with *E. coli* (SST1) gave (*R*)-**20c** in 98.2% ee and 83% yields. In contrast, dihydroxylation of **21a** and **22a** with *E. coli* (SSP1) afforded (*S*)-**21c** in 94.5% ee and (*S*)-**22c** in 97.6% ee, respectively, but dihydroxylation of **21a** and **22a** with *E. coli* (SST1) failed to produce (*R*)-**21c** and (*R*)-**22c**, which is probably due to the poor $\alpha$-regioselectivity of StEH to the corresponding epoxide intermediates. The configurations of **20c**−**22c** were established by comparing bioproducts with the standard diols that were prepared via Sharpless asymmetric dihydroxylation (Figure S21−23, Supporting Information).

**Scheme 4. Enantioselective Dihydroxylation of Aryl Olefins 20a−22a with *E. coli* (SSP1) (expressing SMO and SpEH) or *E. coli* (SST1) (expressing SMO and StEH) To Produce Vicinal Diols (*R*)-20c, (*S*)-21c, and (*S*)-22c**




**Preparation of Vicinal Diols (*S*)-1c, (*S*)-2c, (*S*)-5c, (*S*)-9c, (*S*)-12c, (*R*)-1c, (*R*)-2c, (*R*)-9c, (1*R*,2*S*)-16c, and (1*R*,2*R*)-16c by Asymmetric Dihydroxylation of Aryl Olefins with Resting Cells of *E. coli* (SSP1) or *E. coli* (SST1).** To further demonstrate the synthetic potential of trans-dihydroxylation via cascade biocatalysis, we carried out the preparation of 10 valuable vicinal diols from 7 aryl olefins—**1a**, **2a**, **5a**, **9a**, **12a**, **16a**, **17a**—on a 50 mL scale with the resting cells of *E. coli* (SSP1) or *E. coli* (SST1). The syntheses were performed at a substrate concentration of 50 mM (based on total reaction volume) in a modified two-phase system containing 45 mL of aqueous KP buffer and 5 mL of *n*-hexadecane. The reactions were monitored by TLC by checking the disappearance of the substrates. After 5−8 h, the reactions were stopped, and the products were separated and purified by flash chromatography. All 10 useful and valuable vicinal diols—(*S*)-**1c**, (*S*)-**2c**, (*S*)-**5c**, (*S*)-**9c**, (*S*)-**12c**, (*R*)-**1c**, (*R*)-**2c**, (*R*)-**9c**, (1*R*,2*S*)-**16c**, and (1*R*,2*R*)-**16c**—were obtained in high ee (92.4−98.6%), de (de ≥ 98%, if applicable), and good isolated yield (70.6−85.5%) (Table 4). The dihydroxylation via cascade biocatalysis gave around 0.3 g of product/g of cells. In the representative procedure of Sharpless asymmetric dihydroxylation,[45] 1.4 g of AD-mix-$\alpha$ was used for the dihydroxylation of 1 mmol of olefin, corresponding to ∼0.1 g of product/g of catalyst. In our cascade biocatalysis for trans-dihydroxylation of olefins, whole cells are used as a less expensive and greener catalyst, molecular oxygen is used as the less expensive and green oxidant, and


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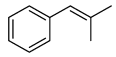
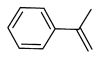
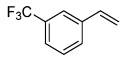
**Table 3. Enantioselective Dihydroxylation of Aryl Olefins 20a−22a with Resting Cells of *E. coli* (SSP1) and *E. coli* (SST1), Respectively**

| Substrate[a] | | Catalyst | Act. (U/g cdw)[b] | Conv. (%)[c] | Prod. | Yield (%)[d] | *ee* (%)[e] |
|---|---|---|---|---|---|---|---|
| **20a** |  | *E. coli* (SSP1) | 16 | 76 | (*S*)-**20c** | 11 | 3.4 |
| | | *E. coli* (SST1) | 17 | 80 | (*R*)-**20c** | 83[f] | 98.2 |
| **21a** |  | *E. coli* (SSP1) | 8 | 62 | (*S*)-**21c** | 56 | 94.5 |
| | | *E. coli* (SST1) | 11 | 68 | (*S*)-**21c** | 24 | 46.0 |
| **22a** |  | *E. coli* (SSP1) | 2 | 41 | (*S*)-**22c** | 46[f] | 97.6 |
| | | *E. coli* (SST1) | 3 | 22 | (*S*)-**22c** | 13 | 74.0 |

[a]The reactions were performed with substrates **20a−22a** (20 mM in organic phase) and resting cells (10 g cdw/L) in a two-liquid-phase system consisting of KP buffer (200 mM, pH 8.0, 2% glucose) and *n*-hexadecane (1:1) at 30 °C for 8 h. [b]Activity is the specific activity determined for the initial 30 min. [c]Conversion is the consumption of starting substrate, determined by normal phase HPLC analysis of the remaining substrate in the *n*-hexadecane phase. Error limit: 3% of the state values. [d]Yield is the analytical yield of the formation of diol product, determined by reversed phase HPLC analysis of the product in the aqueous phase. Error limit: 3% of the state values. [e]The ee value was determined by chiral HPLC analysis. Error limit: 0.2% of the state values. [f]Yield is slightly higher than conversion because of the error limit in the measurement of yield and conversion.

**Table 4. Preparation of (*R*)- or (*S*)-Vicinal Diols in High ee by Enantioselective Dihydroxylation of Aryl Alkenes with Resting Cells of *E. coli* (SSP1) or *E. coli* (SST1)**

| | | | | isolated yield | | | | |
|---|---|---|---|---|---|---|---|---|
| sub[a] | catalyst | time (h) | prod | g | % | ee (%)[b] | de (%)[c] | prod./cells (g/g cdw) |
| **1a** | *E. coli* (SSP1) | 5 | (*S*)-**1c** | 0.295 | 85.5 | 96.3 | n.a.[d] | 0.30 |
| **1a** | *E. coli* (SST1) | 5 | (*R*)-**1c** | 0.289 | 83.8 | 95.8 | n.a. | 0.29 |
| **2a** | *E. coli* (SSP1) | 6 | (*S*)-**2c** | 0.299 | 76.7 | 96.7 | n.a. | 0.30 |
| **2a** | *E. coli* (SST1) | 5 | (*R*)-**2c** | 0.325 | 80.7 | 96.7 | n.a. | 0.33 |
| **5a** | *E. coli* (SSP1) | 8 | (*S*)-**5c** | 0.279 | 73.4 | 92.4 | n.a. | 0.28 |
| **9a** | *E. coli* (SSP1) | 8 | (*S*)-**9c** | 0.326 | 75.6 | 96.5 | n.a. | 0.33 |
| **9a** | *E. coli* (SST1) | 8 | (*R*)-**9c** | 0.304 | 70.6 | 96.3 | n.a. | 0.30 |
| **12a** | *E. coli* (SSP1) | 6 | (*S*)-**12c** | 0.358 | 85.3 | 96.8 | n.a. | 0.36 |
| **16a** | *E. coli* (SST1) | 7 | (1*R*,2*S*)-**16c** | 0.313 | 82.3 | >98[e] | 98.2 | 0.31 |
| **17a** | *E. coli* (SST1) | 8 | (1*R*,2*R*)-**16c** | 0.300 | 78.8 | 98.6 | >99 | 0.30 |

[a]The reactions were performed with substrates (50 mM based on total volume) and resting cells (20 g cdw/L) in a two-liquid-phase system (50 mL) consisting of KP buffer (200 mM, pH 8.0, 2% glucose) and *n*-hexadecane (9:1) at 30 °C. [b]The ee value was determined by chiral HPLC analysis. Error limit: 0.2% of the state values. [c]The de value was determined by chiral HPLC analysis. Error limit: 0.2% of the state values. [d]n.a.: not applicable. [e]Error limit: 0.5% of the state values.

water is used for the epoxide hydrolysis. The green and efficient cascade biocatalysis provides practical syntheses of the useful and valuable vicinal aryl diols in high ee and high yield.

**Dihydroxylation of Styrene 1a with Growing Cells in Fermentor.** We further explored the potential of using growing cells of the recombinant *E. coli* strain for the dihydroxylation of aryl olefins. Dihydroxylation of styrene **1a** with *E. coli* (SST1) was chosen as a model reaction. To avoid the possible environmental concerns and reduce the additional cost, an organic phase (*n*-hexadecane) was not applied in the growing cell experiment. Instead, styrene **1a** was fed directly and slowly into the reaction mixture to alleviate the toxicity of styrene.

*E. coli* (SST1) was grown in a fermentor overnight to a cell density of 7 g cdw/L, glucose was fed, and IPTG was added to induce the enzyme expression. After 5 h of growth, the cell density reached 20 g cdw/L, and styrene **1a** was fed to start the dihydroxylation. After 5 h of biotransformation with the growing cells, 120 mM (16.6 g/L) (*R*)-1-phenyl-1,2-ethanediol **1c** was produced in 96.2% ee with an average volumetric productivity of 3.3 g/L/h for the reaction period. The enantioselectivity of the dihydroxylation with growing cells was the same as that with resting cells. Thus, the cascade biocatalysis for enantioselective dihydroxylation can be performed with either growing cells or resting cells as catalysts, it can also be carried out in either aqueous phase or a two-phase system, and it can be easily scaled up by using a fermentor. The use of growing cells as the catalyst may further improve the product titer and volumetric productivity. Further optimization of the process could make the cascade biocatalysis even more practical for the enantioselective trans-dihydroxylation of aryl olefins.

## CONCLUSION

*Escherichia coli* (SSP1) cells coexpressing styrene monooxygenase (SMO) and epoxide hydrolase SpEH were developed as a green and efficient biocatalyst for *S*-enantioselective dihydroxylation of aryl olefins via intracellular cascade epoxidation and hydrolysis. The *S*-enantioselectivity was generated by SMO-catalyzed *S*-selective epoxidation and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SpEH-catalyzed regioselective hydrolytic opening of the (*S*)-epoxide at the $\beta$ position. Dihydroxylation of terminal aryl olefins **1a**−**15a** with resting cells of *E. coli* (SSP1) offered (*S*)-vicinal diols **1c**−**15c** in high ee (97.5−98.6% for 10 diols; 92.2−93.9% for 3 diols) and high yield (91−99% for 6 diols; 86−88% for 2 diols; 67% for 3 diols).

Combining SMO and epoxide hydrolase StEH showing an $\alpha$ opening of aryl epoxides as the catalyst for the cascade biocatalysis gave rise to *R*-enantioselective dihydroxylation of aryl olefins. *E. coli* (SST1) coexpressing SMO and StEH was also engineered as a green and efficient biocatalyst for *R*-dihydroxylation of aryl olefins, being complementary to *E. coli* (SSP1). Dihydroxylation of terminal aryl olefins **1a**−**15a** with resting cells of *E. coli* (SST1) afforded (*R*)-vicinal diols **1c**−**15c** in high ee (94.2−98.2% for 7 diols; 84.2−89.9% for 6 diols) and high yield (90−99% for 6 diols; 85−89% for 5 diols; 65% for 1 diol). To the best of our knowledge, it is the first report of reversing the overall enantioselectivity of cascade biocatalysis by changing the regioselectivity in an individual reaction step, which will help to solve the problem of lacking mirror-image enzymes in biocatalysis.

*E. coli* (SSP1) and *E. coli* (SST1) catalyzed the trans-dihydroxylation of either *trans*-aryl olefin **16a** or *cis*-aryl olefin **17a** with excellent and complementary stereoselectivity, giving each of the four stereoisomers of 1-phenyl-1,2-propanediol **16c** in high ee and de, respectively. Both strains catalyzed the trans-dihydroxylation of aryl cyclic olefins **18a** and **19a** to afford the same *trans*-cyclic diols, (1*R*,2*R*)-**18c** and (1*R*,2*R*)-**19c**, respectively, in excellent ee and de. This type of cascade biocatalysis provides a complementary tool to Sharpless dihydroxylation, accepting *cis*-alkene and offering enantioselective trans-dihydroxylation.

Preparative dihydroxylations with the resting cells of *E. coli* (SSP1) or *E. coli* (SST1) were successfully demonstrated to prepare five (1*S*)-vicinal diols and five (1*R*)-vincinal diols in high ee (92.4−98.6%) with high isolated yield (70.6−85.5%). Growing cells of *E. coli* (SST1) were also proven to be a good catalyst for the enantioselective dihydroxylation of styrene **1a** to produce 120 mM (16.6 g/L) (*R*)-1-phenyl-1,2-ethanediol, **1c**, in 96.2% ee.

The cascade biocatalysis for dihydroxylation of olefins reported here utilizes molecular oxygen as an inexpensive and green oxidant and water as a simple reagent, thus being sustainable. The developed catalysts show a relatively broad substrate range for aryl olefins, high and complementary enantioselectivity, and high activity and yield, thus being useful for the production of several useful and valuable enantiopure vicinal diols and deserving further development for potential industrial application. The reported concept and methodology on engineering efficient, enantioselective, and enantiocomplementary catalysts could be extended to the development of new biocatalysts for enantioselective trans-dihydroxylation of other types of olefins by combining other monooxygenases and EHs.

## EXPERIMENTAL SECTION

**Genetic Engineering of *E. coli* Strains Coexpressing Styrene Monooxygenase (SMO) and Epoxide Hydrolase from *Sphingomonas* sp. HXN-200 (SpEH).** The genes of SMO (*styA* and *styB*) and SpEH were amplified by PCR (Phusion DNA polymerase) from previously constructed pSPZ10[37] and the genome of *Sphingomonas* sp. HXN-200[34] by using appropriate primers (Supporting Information). The PCR products were subjected to double digestion with appropriate restriction enzymes (New England Biolabs). The *styA* fragment was first ligated to the pRSFduet plasmid (Novagen); the *styB* fragment was then ligated to the resulting plasmid; and finally, *spEH* was added to the resulting construct to give a recombinant plasmid containing three genes (*styA*, *styB*, and *spEH*). By using different cloning sites on the plasmid, three different expression cassettes, SSP1, SSP2-1, and SSP2-2, were constructed on the pRSFduet (Figure 1a). They were transformed into T7 Express Competent *E. coli* cells (New England Biolabs) to yield *E. coli* (SSP1), *E. coli* (SSP2-1), and *E. coli* (SSP2-2), respectively.

**Genetic Engineering of *E. coli* Strains Coexpressing Styrene Monooxygenase (SMO) and Epoxide Hydrolase from *Solanum tuberosum* (StEH).** The *stEH* gene was synthesized with codon optimization for *E. coli* from Genscript according to the sequence Genbank U02497.[65] Similar to the engineering of SMO with SpEH, the genes *styA*, *styB*, and *stEH* were amplified by PCR and then cloned to the pRSFduet to construct three expression cassettes: SST1, SST2-1, and SST2-2, respectively (Figure 1b). The transformation of these plasmids gave three strains (*E. coli* (SST1), *E. coli* (SST2-1), and *E. coli* (SST2-2)), respectively, coexpressing SMO and StEH.

**Cell Growth and Dihydroxylation Activity of *E. coli* (SSP1) or *E. coli* (SST1).** *E. coli* strain *E. coli* (SSP1) or *E. coli* (SST1) was cultured in LB medium (2 mL) containing kanamycin (50 mg/L) at 37 °C for 7−10 h and then inoculated into 50 mL M9 medium containing glucose (30 g/L), yeast extract (5 g/L), and kanamycin (50 mg/L). The cells were grown at 37 °C for 2 h to reach an $OD_{600}$ of 0.6, and then IPTG (0.5 mM) was added to induce the expression of enzymes. The cells continued to grow for 10−12 h at 25 °C to reach a cell density of 5−6 g cdw/L. The cells were harvested by centrifugation (5000*g*, 5 min), and the cell pallets were used as catalysts for the activity test or biotransformation.

Activity test: freshly prepared *E. coli* (SSP1) or *E. coli* (SST1) cells were suspended to a cell density of 1.0 g cdw/L in KP (potassium phosphate) buffer (200 mM, pH 8.0) containing glucose (2%, w/v) and 40 $\mu$L of styrene **1a** stock solution (0.5 M in ethanol) to a 2 mL system. The reaction mixture was shaken at 250 rpm and 30 °C for 30 min. One milliliter aliquots were taken out and mixed with 1 mL of acetonitrile to stop the reaction. After centrifugation, the supernatant was used for HPLC analysis of the diol product.

**General Procedure for Enantioselective Dihydroxylation of Aryl Olefins 1a−22a with Resting Cells of *E. coli* (SSP1) or *E. coli* (SST1).** Freshly prepared *E. coli* (SSP1) or *E. coli* (SST1) cells were resuspended to a cell density of 10 g cdw/L in KP buffer (200 mM, pH 8.0) containing glucose (2%, w/v) to a 2 mL system in a shaking flask (100 mL). Two milliliters of *n*-hexadecane containing 20 mM aryl olefins **1a**−**22a** was added to the reaction system to form a second phase. The reaction mixture was shaken at 250 rpm and 30 °C for 8 h, then 200 $\mu$L aliquots of each phase were taken out at 0, 0.5, 2, and 8 h to follow the reaction. For the organic phase, *n*-hexadecane (100 $\mu$L) was separated after centrifugation, diluted with 900 $\mu$L of *n*-hexane (containing 2 mM benzyl alcohol as an internal standard), and subjected to normal phase HPLC analysis for quantifying the olefin substrates **1a**−**22a** and possible epoxide intermediate. For the aqueous phase, supernatants (100 $\mu$L) were separated after centrifugation, diluted with 400 $\mu$L of water and 500 $\mu$L of acetonitrile (containing 2 mM benzyl alcohol as internal standard), and then used for


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reversed phase HPLC analysis of the diol products **1c**−**22c**. The remaining aqueous phase (about 1 mL), after 8 h in the flask, was subjected to centrifugation to remove the cells, followed by extraction with ethyl acetate and drying over $Na_2SO_4$. After evaporation, the residue was dissolved in 2 mL of solvent (hexane/IPA = 9:1) for chiral HPLC analysis of the ee and de of the diol products **1c**−**22c**.

**General Procedure for Preparation of (1*S*)-Vicinal Diols (*S*)-1c, 2c, 5c, 9c, 12c by Enantioselective Dihydroxylation of Aryl Olefins 1a, 2a, 5a, 9a, 12a with Resting Cells of *E. coli* (SSP1).** Freshly prepared *E. coli* (SSP1) cells were resuspended to a cell density of 20 g cdw/L in KP buffer (200 mM, pH 8.0) containing glucose (2%, w/v) to a 45 mL system in a shaking flask (250 mL with tribaffle). Five milliliters of *n*-hexadecane containing 2.50 mmol substrate (0.260 g of **1a**, 0.305 g of **2a**, 0.295 g of **5a**, 0.346 g of **9a**, and 0.335 g of **12a**) was added to the reaction system to form a second phase (50 mM substrate concentration based on total reaction volume). The reaction mixture was shaken at 250 rpm and 30 °C, and the reaction was monitored by TLC. After 5−8 h, the substrate disappeared totally, and the reaction mixture was then saturated with NaCl. After centrifugation, the aqueous phase was collected and washed with 10 mL of *n*-hexane. The aqueous phase was then extracted with ethyl acetate three times (3 × 50 mL), and all the organic phases were combined. After drying over $Na_2SO_4$, the solvents were removed by evaporation. The crude diol products were purified by flash chromatography on a silica gel column with *n*-hexane/ethyl acetate (2−1:1) as eluent ($R_f \approx 0.3$ for all diol products).

(*S*)-**1-Phenyl-1,2-ethanediol** (*S*)-**1c.** White solid; 0.295 g; yield: 85.5%; ee: 96.3%.

(*S*)-**1-(4-Fluorophenyl)-1,2-ethanediol** (*S*)-**2c.** White solid; 0.299 g; yield, 76.7%; ee, 96.7%.

(*S*)-**1-(4-Methylphenyl)-1,2-ethanediol** (*S*)-**5c.** White solid; 0.279 g; yield, 73.4%; ee, 92.4%.

(*S*)-**1-(3-Chlorophenyl)-1,2-ethanediol** (*S*)-**9c.** Colorless syrup; 0.326 g; yield, 75.6%; ee, 96.5%.

(*S*)-**1-(3-Methoxyphenyl)-1,2-ethanediol** (*S*)-**12c.** Colorless syrup; 0.358 g; yield, 85.3%; ee, 96.8%.

**General Procedure for Preparation of (1*R*)-Vicinal Diols (*R*)-1c, 2c, 9c, (1*R*,2*S*)-16c, (1*R*,2*R*)-16c by Enantioselective Dihydroxylation of Aryl Olefins 1a, 2a, 9a, 16a, 17a with Resting Cells of *E. coli* (SST1).** Freshly prepared *E. coli* (SST1) cells were resuspended to a cell density of 20 g cdw/L in KP buffer (200 mM, pH 8.0) containing glucose (2%, w/v) to a 45 mL system in a shaking flask (250 mL with tribaffle). Five milliliters of *n*-hexadecane containing 2.50 mmol of substrate (0.260 g of **1a**, 0.305 g of **2a**, 0.346 g of **9a**, 0.295 g of **16a**, and 0.295 g of **17a**) was added to the reaction system in the flask. The reaction mixture was shaken at 250 rpm and 30 °C. The reaction was monitored by TLC. After 5−8 h, the substrate disappeared totally. The reaction mixture was then saturated with NaCl. After centrifugation, the aqueous phase was collected and then washed with 10 mL of *n*-hexane. The aqueous phase was extracted with ethyl acetate three times (3 × 50 mL), and all the organic phases were combined. After drying over $Na_2SO_4$, the solvents were removed by evaporation. The crude diol products were then purified by flash chromatography on a silica gel column with *n*-hexane/ethyl acetate (2−1:1) ($R_f \approx 0.3$ for all diol products).

(*R*)-**1-Phenyl-1,2-ethanediol** (*R*)-**1c.** White solid; 0.289 g; yield, 83.8%; ee, 95.8%.

(*R*)-**1-(4-Fluorophenyl)-1,2-ethanediol** (*R*)-**2c.** White solid; 0.325 g; yield, 80.7%; ee, 96.7%.

(*R*)-**1-(3-Chlorophenyl)-1,2-ethanediol** (*R*)-**9c.** Colorless syrup; 0.304 g; yield, 70.6%; ee, 96.3%.

(1*R*,2*S*)-**1-Phenyl-1,2-propanediol** (1*R*,2*S*)-**16c.** Colorless syrup; 0.313 g; yield, 82.3%; ee, >98%; de, 98.2%.

(1*R*,2*R*)-**1-Phenyl-1,2-propanediol** (1*R*,2*R*)-**16c.** Colorless syrup; 0.300 g; yield, 78.8%; ee, 98.6%; de, >99%.

**Enantioselective Dihydroxylation of Styrene 1a with Growing Cells of *E. coli* (SST1).** *E. coli* (SST1) was cultured in LB medium (2 mL) containing kanamycin (50 mg/L) at 37 °C for 7−10 h, and then inoculated into 100 mL of M9 medium containing glucose (30 g/L), yeast extract (5 g/L), and kanamycin (50 mg/L). The cells were grown at 30 °C for 12 h to reach an $OD_{600}$ of 15. All culture was transferred into 900 mL of sterilized modified Riesenberg medium with 15 g/L glucose as the carbon source in a 3 L fermentor (Sartorius). The cells were grown in the fermentor at 30 °C for 12 h to reach an $OD_{600}$ of 15−18. During the batch growth, the pH value was maintained at 7.0 by adding 30% phosphoric acid or 25% ammonia solution, based on pH sensing, and the stirring rate was kept constant at 1000 rpm and aeration rate was kept constant at 1 L/min. At the end of the batch growth (12 h), the $P_{O_2}$ started to increase, indicating glucose depletion. Fed-batch growth was started by feeding a solution containing 730 g/L glucose and 19.6 g/L $MgSO_4 \cdot 7H_2O$. The feeding rate was stepwise increased: 6.5 mL/h for 1 h, 8 mL/h for 1 h, 10 mL/h for 1h, 13 mL/h for 1 h, then kept at 16 mL/h until the end of the reaction. The stirring rate was stepwise increased: 1200 rpm for 2 h and 1500 rpm for 2 h, then kept at 2000 rpm until the end of the reaction. The aeration rate was stepwise increased: 1.2 L/min for 2 h, 1.5 L/min for 2h, then kept at 2.0 L/min until the end of the reaction. Antifoam PEG2000 (Fluka) was added when necessary. After fed-batch growth for 2 h, IPTG (0.5 mM) was added to induce the expression of protein. After fed-batch growth for 5 h, the cell density reached 20 g cdw/L, and the biotransformation started by dropwise adding styrene **1a** at the rate of 6 mL/h for 4 h and then 3 mL/h for additional 1 h. The reaction was monitored by taking a sample every hour for analyzing the formation of diol **1c** by reversed phase HPLC. After 5 h of reaction, 120 mM (*R*)-1-phenyl-1,2-ethanediol was produced in 96.2% ee.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Chemicals, chemical synthesis, analytical methods, strains and biochemicals, culture media, genetic engineering of recombinant strains, NMR data and specific rotations of bioproducts, procedures of other related biotransformations, time course of representative dihydroxylation, chiral HPLC chromatograms, and $^1$H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

(1) Bruggink, A.; Schoevaart, R.; Kieboom, T. *Org. Process Res. Dev.* **2003**, *7*, 622−640.
(2) Fogg, D. E.; dos Santos, E. N. *Coord. Chem. Rev.* **2004**, *248*, 2365−2379.
(3) Wasilke, J. C.; Obrey, S. J.; Baker, R. T.; Bazan, G. C. *Chem. Rev.* **2005**, *105*, 1001−1020.
(4) Kolarovic, A.; Schnürch, M.; Mihovilovic, M. D. *J. Org. Chem.* **2010**, *76*, 2613−2618.
(5) Denard, C. A.; Hartwig, J. F.; Zhao, H. *ACS Catal.* **2013**, *3*, 2856−2864.
(6) Breuer, M.; Ditrich, K.; Habicher, T.; Hauer, B.; Kesseler, M.; Stürmer, R.; Zelinski, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 788−824.
(7) Panke, S.; Held, M.; Wubbolts, M. *Curr. Opin. Biotechnol.* **2004**, *15*, 272−279.
(8) Patel, R. N. *ACS Catal.* **2011**, *1*, 1056−1074.
(9) *Biocatalysis for green chemistry and chemical process development*; Tao, J. A., Kazlauskas, R. J., Eds.; Wiley: New York, 2011.
(10) Clouthier, C. M.; Pelletier, J. N. *Chem. Soc. Rev.* **2012**, *41*, 1585−1605.
(11) Santacoloma, P. A.; Sin, G.; Gernaey, K. V.; Woodley, J. M. *Org. Process Res. Dev.* **2011**, *15*, 203−212.
(12) Ricca, E.; Brucher, B.; Schrittwieser, J. H. *Adv. Synth. Catal.* **2011**, *353*, 2239−2262.
(13) Schrittwieser, J. H.; Sattler, J.; Resch, V.; Mutti, F. G.; Kroutil, W. *Curr. Opin. Chem. Biol.* **2011**, *15*, 249−256.
(14) Mateo, C.; Chmura, A.; Rustler, S.; van Rantwijk, F.; Stolz, A.; Sheldon, R. A. *Tetrahedron: Asymmetry* **2006**, *17*, 320−323.
(15) Seisser, B.; Lavandera, I.; Faber, K.; Spelberg, J. H. L.; Kroutil, W. *Adv. Synth. Catal.* **2007**, *349*, 1399−1404.
(16) Voss, C. V.; Gruber, C. C.; Faber, K.; Knaus, T.; Macheroux, P.; Kroutil, W. *J. Am. Chem. Soc.* **2008**, *130*, 13969−13972.
(17) Voss, C. V.; Gruber, C. C.; Kroutil, W. *Angew. Chem., Int. Ed.* **2008**, *47*, 741−745.
(18) Cabirol, F. L.; Lim, A. E.; Hanefeld, U.; Sheldon, R. A. *Org. Process Res. Dev.* **2010**, *14*, 114−118.
(19) Kalaitzakis, D.; Smonou, I. *J. Org. Chem.* **2010**, *75*, 8658−8661.
(20) Tauber, K.; Fuchs, M.; Sattler, J. H.; Pitzer, J.; Pressnitz, D.; Koszelewski, D.; Faber, K.; Pfeffer, J.; Haas, T.; Kroutil, W. *Chem.—Eur. J.* **2013**, *19*, 4030−4035.
(21) Liu, J.; Li, Z. *ACS Catal.* **2013**, *3*, 908−911.
(22) Siirola, E.; Mutti, F. G.; Grischek, B.; Hoefler, S. F.; Fabian, W. M.; Grogan, G.; Kroutil, W. *Adv. Synth. Catal.* **2013**, *355*, 1703−1708.
(23) Oberleitner, N.; Peters, C.; Muschiol, J.; Kadow, M.; Saß, S.; Bayer, T.; Schaaf, P.; Iqbal, N.; Rudroff, F.; Mihovilovic, M. D.; Bornscheuer, U. T. *ChemCatChem* **2013**, *5*, 3524−3528.
(24) Chmura, A.; Rustler, S.; Paravidino, M.; van Rantwijk, F.; Stolz, A.; Sheldon, R. A. *Tetrahedron: Asymmetry* **2013**, *24*, 1225−1232.
(25) Staudt, S.; Burda, E.; Giese, C.; Müller, C. A.; Marienhagen, J.; Schwaneberg, U.; Hummel, W.; Drauz, K.; Gröger, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 2359−2363.
(26) Müller, C. A.; Akkapurathu, B.; Winkler, T.; Staudt, S.; Hummel, W.; Gröger, H.; Schwaneberg, U. *Adv. Synth. Catal.* **2013**, *355*, 1787−1798.
(27) Staudt, S.; Bornscheuer, U. T.; Menyes, U.; Hummel, W.; Gröger, H. *Enzyme Microb. Technol.* **2013**, *53*, 288−292.
(28) Zhang, W.; Tang, W. L.; Wang, Z.; Li, Z. *Adv. Synth. Catal.* **2010**, *352*, 3380−3390.
(29) Tang, W. L.; Li, Z.; Zhao, H. *Chem. Commun.* **2010**, *46*, 5461−5463.
(30) Pham, S. Q.; Pompidor, G.; Liu, J.; Li, X. D.; Li, Z. *Chem. Commun.* **2012**, *48*, 4618−4620.
(31) Li, A.; Liu, J.; Pham, S. Q.; Li, Z. *Chem. Commun.* **2013**, *49*, 11572−11574.
(32) Liu, Z.; Michel, J.; Wang, Z.; Witholt, B.; Li, Z. *Tetrahedron: Asymmetry* **2006**, *17*, 47−52.
(33) Jia, X.; Wang, Z.; Li, Z. *Tetrahedron: Asymmetry* **2008**, *19*, 407−415.
(34) Wu, S.; Li, A.; Chin, Y. S.; Li, Z. *ACS Catal.* **2013**, *3*, 752−759.
(35) Chang, D.; Heringa, M. F.; Witholt, B.; Li, Z. *J. Org. Chem.* **2003**, *68*, 8599−8606.
(36) Panke, S.; Witholt, B.; Schmid, A.; Wubbolts, M. G. *Appl. Environ. Microbiol.* **1998**, *64*, 2032−2043.
(37) Panke, S.; Wubbolts, M. G.; Schmid, A.; Witholt, B. *Biotechnol. Bioeng.* **2000**, *69*, 91−100.
(38) Schmid, A.; Hofstetter, K.; Feiten, H.-J.; Hollmann, F.; Witholt, B. *Adv. Synth. Catal.* **2001**, *343*, 732−737.
(39) Hollmann, F.; Lin, P.-C.; Witholt, B.; Schmid, A. *J. Am. Chem. Soc.* **2003**, *125*, 8209−8217.
(40) Bernasconi, S.; Orsini, F.; Sello, G.; Di Gennaro, P. *Tetrahedron: Asymmetry* **2004**, *15*, 1603−1606.
(41) Panke, S.; Held, M.; Wubbolts, M. G.; Witholt, B.; Schmid, A. *Biotechnol. Bioeng.* **2002**, *80*, 33−41.
(42) Kuhn, D.; Kholiq, M. A.; Heinzle, E.; Bühler, B.; Schmid, A. *Green Chem.* **2010**, *12*, 815−827.
(43) Xu, Y.; Jia, X.; Panke, S.; Li, Z. *Chem. Commun.* **2009**, 1481−1483.
(44) Xu, Y.; Li, A.; Jia, X.; Li, Z. *Green Chem.* **2011**, *13*, 2452−2458.
(45) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.
(46) Zhang, W.; Loebach, J. L.; Wilson, S. R.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1990**, *112*, 2801−2803.
(47) Tokunaga, M.; Larrow, J. F.; Kakiuchi, F.; Jacobsen, E. N. *Science* **1997**, *277*, 936−938.
(48) Albrecht, Ł.; Jiang, H.; Dickmeiss, G.; Gschwend, B.; Hansen, S. G.; Jørgensen, K. A. *J. Am. Chem. Soc.* **2010**, *132*, 9188−9196.
(49) Nicolaou, K. C.; Huang, X.; Snyder, S. A.; Rao, P. B.; Bella, M.; Reddy, M. V. *Angew. Chem., Int. Ed.* **2002**, *41*, 834−838.
(50) Pandey, R. K.; Fernandes, R. A.; Kumar, P. *Tetrahedron Lett.* **2002**, *43*, 4425−4426.
(51) Gavrilov, K. N.; Zheglov, S. V.; Gavrilova, M. N.; Chuchelkin, I. V.; Groshkin, N. N.; Rastorguev, E. A.; Davankov, V. A. *Tetrahedron Lett.* **2011**, *52*, 5706−5710.
(52) Kim, J.-H.; Yang, H.; Park, J.; Boons, G.-J. *J. Am. Chem. Soc.* **2005**, *127*, 12090−12097.
(53) Zhao, D.; Song, S.; Li, Y.; Xiong, X.; Sun, L.; Miao, J. Shenyang Pharmaceutical University; CN Patent 102477008A, 2012; SciFinder Scholar, 2012, 782417.
(54) Hattori, K.; Nagano, M.; Kato, T.; Nakanishi, I.; Imai, K.; Kinoshita, T.; Sakane, K. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2821−2824.
(55) Jones, G. B.; Guzel, M. S.; Heaton, B. *Tetrahedron: Asymmetry* **2000**, *11*, 4303−4320.
(56) Choi, Y. M.; Lee, F. Bio-Pharm Solutions Co., Inc., KR; U.S. Patent 20130005801A1, 2013; SciFinder Scholar, 2013, 12968.
(57) Sayyed, I. A.; Sudalai, A. *Tetrahedron: Asymmetry* **2004**, *15*, 3111−3116.
(58) Chartrain, M.; Jackey, B. A.; Heimbuch, B.; Taylor, C. S. Merck & Co., Inc., USA; U.S. Patent 5871981A, 1999; SciFinder Scholar, 1999:119785.
(59) Otto, K.; Hofstetter, K.; Röthlisberger, M.; Witholt, B.; Schmid, A. *J. Bacteriol.* **2004**, *186*, 5292−5302.
(60) Keasling, J. D. *ACS Chem. Biol.* **2008**, *3*, 64−76.
(61) Ajikumar, P. K.; Xiao, W.-H.; Tyo, K. E.; Wang, Y.; Simeon, F.; Leonard, E.; Mucha, O.; Phon, T. H.; Pfeifer, B.; Stephanopoulos, G. *Science* **2010**, *330*, 70−74.
(62) Monterde, M. I.; Lombard, M.; Archelas, A.; Cronin, A.; Arand, M.; Furstoss, R. *Tetrahedron: Asymmetry* **2004**, *15*, 2801−2805.
(63) Lindberg, D.; Gogoll, A.; Widersten, M. *FEBS J.* **2008**, *275*, 6309−6320.

(64) Wang, W.; Wang, D. I. C.; Li, Z. *Chem. Commun.* **2011**, *47*, 8115−8117.
(65) Morisseau, C.; Beetham, J. K.; Pinot, F.; Debernard, S.; Newman, J. W.; Hammock, B. D. *Arch. Biochem. Biophys.* **2000**, *378*, 321−332.
(66) Mugford, P. F.; Wagner, U. G.; Jiang, Y.; Faber, K.; Kazlauskas, R. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 8782−8793.