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Enantioselective Cascade Biocatalysis via Epoxide Hydrolysis and Alcohol Oxidation: One-Pot Synthesis of (*R*)-*α*-Hydroxy Ketones from *Meso*- or Racemic Epoxides

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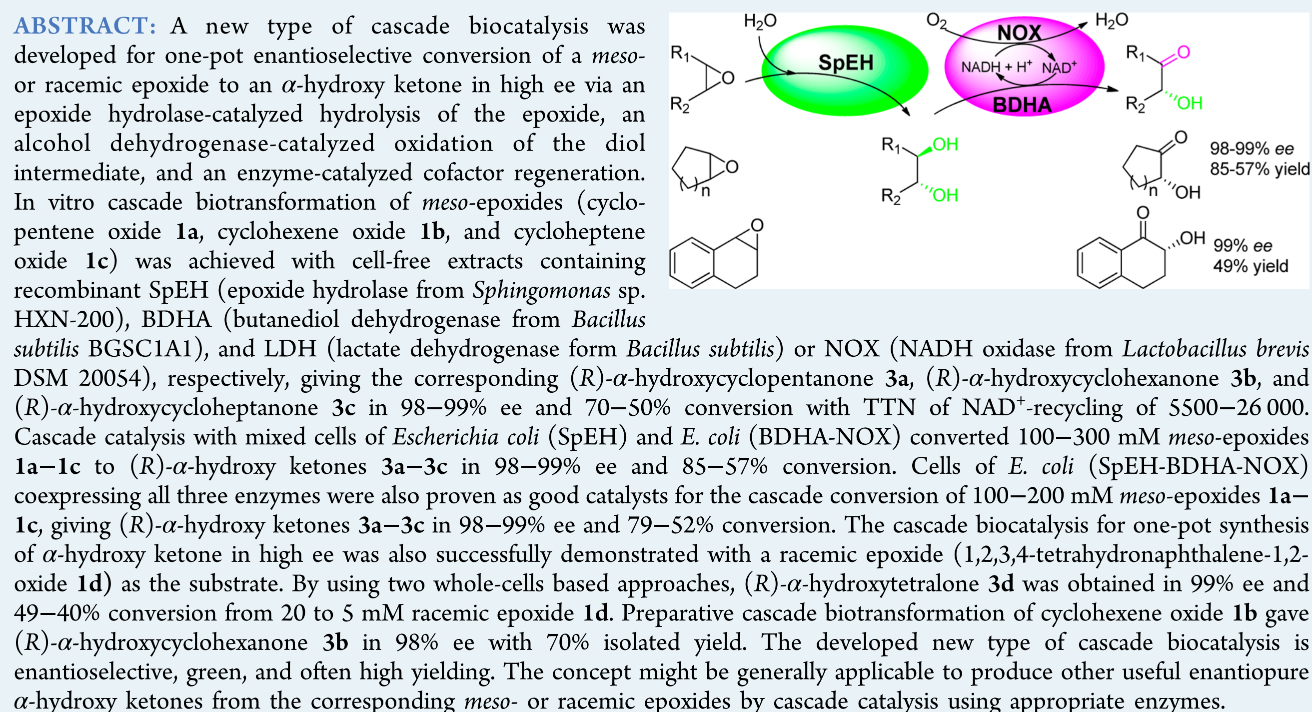
Supporting Information



ABSTRACT: A new type of cascade biocatalysis was developed for one-pot enantioselective conversion of a *meso*- or racemic epoxide to an *α*-hydroxy ketone in high ee via an epoxide hydrolase-catalyzed hydrolysis of the epoxide, an alcohol dehydrogenase-catalyzed oxidation of the diol intermediate, and an enzyme-catalyzed cofactor regeneration. In vitro cascade biotransformation of *meso*-epoxides (cyclopentene oxide **1a**, cyclohexene oxide **1b**, and cycloheptene oxide **1c**) was achieved with cell-free extracts containing recombinant SpEH (epoxide hydrolase from *Sphingomonas* sp. HXN-200), BDHA (butanediol dehydrogenase from *Bacillus subtilis* BGSC1A1), and LDH (lactate dehydrogenase form *Bacillus subtilis*) or NOX (NADH oxidase from *Lactobacillus brevis* DSM 20054), respectively, giving the corresponding (*R*)-*α*-hydroxycyclopentanone **3a**, (*R*)-*α*-hydroxycyclohexanone **3b**, and (*R*)-*α*-hydroxycycloheptanone **3c** in 98−99% ee and 70−50% conversion with TTN of NAD^+-recycling of 5500−26 000. Cascade catalysis with mixed cells of *Escherichia coli* (SpEH) and *E. coli* (BDHA-NOX) converted 100−300 mM *meso*-epoxides **1a**−**1c** to (*R*)-*α*-hydroxy ketones **3a**−**3c** in 98−99% ee and 85−57% conversion. Cells of *E. coli* (SpEH-BDHA-NOX) coexpressing all three enzymes were also proven as good catalysts for the cascade conversion of 100−200 mM *meso*-epoxides **1a**−**1c**, giving (*R*)-*α*-hydroxy ketones **3a**−**3c** in 98−99% ee and 79−52% conversion. The cascade biocatalysis for one-pot synthesis of *α*-hydroxy ketone in high ee was also successfully demonstrated with a racemic epoxide (1,2,3,4-tetrahydronaphthalene-1,2-oxide **1d**) as the substrate. By using two whole-cells based approaches, (*R*)-*α*-hydroxytetralone **3d** was obtained in 99% ee and 49−40% conversion from 20 to 5 mM racemic epoxide **1d**. Preparative cascade biotransformation of cyclohexene oxide **1b** gave (*R*)-*α*-hydroxycyclohexanone **3b** in 98% ee with 70% isolated yield. The developed new type of cascade biocatalysis is enantioselective, green, and often high yielding. The concept might be generally applicable to produce other useful enantiopure *α*-hydroxy ketones from the corresponding *meso*- or racemic epoxides by cascade catalysis using appropriate enzymes.

KEYWORDS: *biocatalysis, cascade catalysis, enantioselective synthesis, epoxide hydrolase, alcohol dehydrogenase, α-hydroxy ketone*

■ INTRODUCTION

Cascade biocatalysis enables multistep reactions in one pot without the time-consuming, waste-generating, energy-intensive, yield-reducing, and cost-increasing isolation of intermediates, thus being a green and useful tool for sustainable chemical synthesis.[1−7] Many types of cascade biocatalysis have been recently reported for organic synthesis,[8−14] and enantioselective cascade biocatalysis has also been developed for chiral synthesis, such as the deracemization of secondary alcohols,[15,16] asymmetric dihydroxylation of aryl olefins to diols,[17−19] conversion of unsaturated ketones to chiral lactones or ketones,[20,21] transformation of carvenol to chiral lactones,[22] conversion of ethyl 4-oxo-pent-2-enoates to chiral *γ*-butyrolactones,[23] amination of *sec*-alcohols to chiral amines,[24] transformation of benzaldehyde and pyruvate to nor(pseudo)-ephedrine,[25] and conversion of asymmetric diketone to chiral substituted pyrrolidines.[26] Despite of the big achievement in this field, it is still necessary to develop new types of cascade biocatalysis for many different synthetic applications.

We have been interested in developing novel cascade biocatalysis for enantioselective synthesis of *α*-hydroxy ketones. Many enantiopure *α*-hydroxy ketones are useful templates in asymmetric reactions and important building blocks for the synthesis of chiral chemicals and pharmaceuticals.[27,28] For instance, (*R*)-*α*-hydroxycyclopentanone **3a** and (*R*)-*α*-hydroxycyclohexanone **3b** are useful and valuable intermediates for chiral synthesis.[29] (*R*)-*α*-Hydroxytetralone **3d** is useful for the synthesis of 1-amino-1, 2, 3, 4-tetrahydronaphthalen-2-ol, a chiral auxiliary and ligand in asymmetric transformations.[30−32] Chiral *α*-hydroxy ketones can be prepared by using chemical

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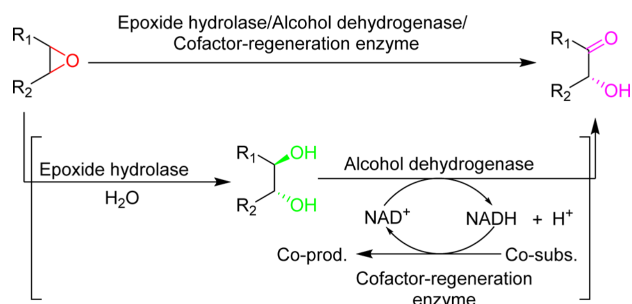
methods,[33−35] which often suffers from one or more drawbacks such as the use of toxic catalyst and reagent, unsatisfied product ee, and low catalytic turnover. Chiral α-hydroxy ketones can also be prepared by using enzymatic methods via resolution, which often encounters problem of unsatisfied enantioselectivity: acetylation-based kinetic resolution produced (*S*)-**3d** with an enantiomeric ratio (*E*) of 16−35;[36] reductive kinetic resolution gave (*R*)-**3a** with an *E* of 10;[37] and oxidative kinetic resolution of *trans*-cyclohexane-1,2-diol **2b** with diacetyl reductases afforded (*S*)-**3a** or (*R*)-**3a** in high ee but with low yield (<20%).[38,39] We recently reported the oxidation of racemic *trans*-vicinal diols with butanediol dehydrogenase (BDHA) from *Bacillus subtilis* BGSC1A1 to give (*R*)-α-hydroxy ketones **3a−d** in high ee and 49−50% yield with excellent enantioselectivity.[40] Nevertheless, the maximum yield for kinetic resolution is only 50%. Lyase-catalyzed aldehyde−ketone cross-coupling via carboligation was also known for the synthesis of chiral α-hydroxy ketones;[41] however, excellent enantioselectivity was observed only in special cases such as the reaction between pyruvate and benzaldehyde.[25,42]

Here we report a new type of cascade biocatalysis for efficient one-pot synthesis of enantiopure α-hydroxy ketone from the corresponding easily available *meso*- or racemic epoxide via enantioselective hydrolysis of the epoxide and enantioselective oxidation of the major enantiomer of the diol intermediate (Scheme 1). The novel concept and synthetic methodology are demonstrated with the syntheses of (*R*)-α-hydroxyketones **3a−d** as the model reactions by the combination of an epoxide hydrolase, an alcohol dehydrogenase, and a cofactor regeneration enzyme as the efficient biocatalysts.

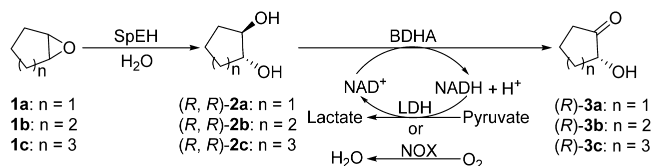
Scheme 1. One-Pot Synthesis of Enantiopure α-Hydroxy Ketones from *meso*- or Racemic Epoxides via Cascade Biocatalysis



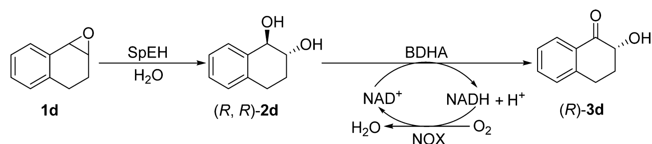
RESULTS AND DISCUSSION

Selection of the Enzymes and Design of the Biocatalytic Systems for the One-Pot Cascade Catalysis To Convert *meso*- and Racemic Epoxides 1a−d to Enantiopure α-Hydroxy Ketones 3a−d. We recently discovered a highly active epoxide hydrolase (SpEH) from *Sphingomonas* sp. HXN-200 as a unique bacterial EH for enantioselective hydrolysis of *meso*-epoxides such as cyclopentene oxide **1a** and cyclohexene oxide **1b** to give the corresponding (1*R*, 2*R*)-vicinal diols such as *trans*-cyclopentane-1,2-diol **2a** and *trans*-cyclohexane-1,2-diol **2b** in 86−88% ee and 99% yield.[43,44] The EH catalyzed also the highly *R*-enantioselective hydrolysis of racemic epoxides such as styrene oxides to afford the corresponding (*S*)-epoxides in >98% ee and 47−36% yield.[44−47] SpEH was thus selected as the appropriate enzyme for the hydrolysis step in the designed cascade biotransformation of *meso*-epoxides **1a−c** and racemic epoxide 1,2,3,4-tetrahydronaphthalene-1,2-oxide **1d** to the corresponding enantiopure α-hydroxy ketones **3a−d**, respectively (Schemes 2 and 3). Previously, we also discovered BDHA for

Scheme 2. One-Pot Synthesis of (*R*)-α-Hydroxy Ketones 3a−c from *meso*-Epoxides 1a−c via Cascade Biocatalysis Using SpEH, BDHA, and LDH or using SpEH, BDHA, and NOX



Scheme 3. One-Pot Synthesis of (*R*)-α-Hydroxy Ketone 3d from Racemic Epoxide 1d via Cascade Biocatalysis Using SpEH, BDHA, and NOX



the highly *R*-enantioselective oxidation of diols **2a−d** to give the corresponding (*R*)-α-hydroxy ketones **3a−d** in >99% ee at 49% conversion.[40] Accordingly, BDHA was chosen as the oxidation enzyme in the cascade biocatalysis to convert the major enantiomers of the diol intermediates to the corresponding (*R*)-α-hydroxy ketones **3a−d**. Because BDHA-catalyzed oxidation depends on NAD^+, the well-known lactate dehydrogenase (LDH) from *Bacillus subtilis* was selected for the regeneration of the cofactor NAD^+ to achieve high productivity in the oxidation step.[40] While LDH-catalyzed regeneration of NAD^+ requires the addition of pyruvate as the cosubstrate, it could be more economical and greener to use NADH oxidase (NOX) for NAD^+ regeneration, which requires only molecular oxygen as the cosubstrate. The NOX from *Lactobacillus brevis* DSM 20054[48] was thus also explored as the cofactor-regeneration enzyme in the three-enzyme cascade.

To demonstrate the concept of using the three enzymes for the new type of cascade biocatalysis, in vitro biocatalysis with the cell-free extracts containing the three necessary enzymes was first explored. To reduce the cost and enhance the productivity of the new cascade biocatalysis, cells of the mixed strains expressing separately the three enzymes and cells of a single strain expressing all three enzymes were designed as the catalytic system, respectively, and compared for the target reactions.

Genetic Engineering of *E. coli* Strains Expressing the Individual Enzyme and *E. coli* Strains Coexpressing the Necessary Multiple Enzymes. To produce the enzymes and microbial cells for the designed cascade reactions, *E. coli* (BDHA),[40] *E. coli* (LDH),[40] *E. coli* (SpEH),[44] *E. coli* (NOX), *E. coli* (BDHA-NOX), and *E. coli* (SpEH-BDHA-NOX) expressing the necessary enzymes were constructed. *E. coli* strains were grown at 37 °C in TB medium containing appropriate antibiotics for 2 h to reach an OD_{600} of 0.6−0.8 and then induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 0.5 mM. The cell growth was continued at 22 °C for 10−14 h. The specific activity of the

Table 1. One-Pot Enantioselective Conversion of *meso*-Epoxides 1a−c to (*R*)-α-Hydroxy Ketones 3a−c via Cascade Biocatalysis in Vitro with the Mixtures of SpEH, BDHA, and LDH or with the Mixtures of SpEH, BDHA, and NOX

entry[a]	sub.	sub. concn (mM)	SpEH[b] (g pro. /L)	BDHA[c] (g pro. /L)	LDH[d] (g pro. /L)	NOX[e] (g pro. /L)	NADH (mM)	pyruvate (mM)	time (h)	conv. to 3 (%)[f]	ee of (*R*)-3 (%)[g]	TTN of NADH
1	**1a**	50	5	10	−	−	0.002	−	12	4	98	−
2	**1a**	50	5	10	5	−	0.002	200	9	65	98	15 000
3	**1b**	25	3	10	5	−	0.002	200	12	50	99	5500
4	**1c**	100	5	10	5	−	0.002	200	12	55	99	26 000
5	**1a**	50	5	12	−	5	0.004	−	18	69	98	8100
6	**1b**	20	3	10	−	5	0.002	−	18	70	99	6200
7	**1c**	100	5	12	−	5	0.004	−	12	58	99	14 000

[a]Reactions were carried out in 10 mL of Tris buffer (100 mM, pH 7.5) at 30 °C and 250 rpm. [b]Lyophilized cell-free extract of *E. coli* (SpEH). [c]Lyophilized cell-free extract of *E. coli* (BDHA). [d]Lyophilized cell-free extract of *E. coli* (LDH). [e]Lyophilized cell-free extract of *E. coli* (NOX). [f]Conversion was determined by GC analysis, error limit: <2% of the state values. [g]ee was determined by chiral GC analysis.

cells of *E. coli* (NOX) harvested at 12 h was determined to be 5.2 U/mg protein by using the cell-free extract for NADH oxidation with UV detection. *E. coli* (BDHA-NOX) cells grown for 6 to 14 h reached high specific activity (116−129 U/g cdw) for the oxidation of racemic *trans*-cyclohexane-1,2-diol **2b** to α-hydroxycyclohexanone **3b** (Figure S2A), which is 3-fold higher than the activity of *E. coli* (BDHA).[40] *E. coli* (SpEH-BDHA-NOX) cells showed a specific activity of 34−42 U/g cdw for the conversion of cyclohexene oxide **1b** to α-hydroxycyclohexanone **3b** after growth for 6−14 h (Figure S2B).

The SDS-PAGE of the cell-free extracts of the *E. coli* cells harvested at 12 h (after 10 h induction) was shown in Figure S2C. The protein bands of the coexpressed SpEH, BDHA, and NOX in *E. coli* (SpEH-BDHA-NOX) (Lane 1), the coexpressed BDHA and NOX in *E. coli* (BDHA-NOX) (Lane 2), the expressed NOX in *E. coli* (NOX) (lane 3), the expressed SpEH in *E. coli* (SpEH) (lane 4), and the expressed BDHA in *E. coli* (BDHA) (lane 5) were clearly visible.

Cascade Biocatalysis with SpEH, BDHA, and LDH or with SpEH, BDHA, and NOX in vitro for One-Pot Conversion of *meso*-Epoxide 1a−c to (*R*)-α-Hydroxyketones 3a−c. Cell-free extracts of *E. coli* (SpEH), *E. coli* (BDHA), *E. coli* (LDH), and *E. coli* (NOX) were prepared from cells harvested at late exponential growing phase at 12−14 h, respectively, and they were used for in vitro cascade biocatalysis to demonstrate the concept (Scheme 2). To facilitate the adjustment of the ratios among different enzymes for accomplishing the cascade reaction, the cell-free extracts of the *E. coli* strains were lyophilized to give the corresponding enzyme powders, respectively. SpEH and BDHA were used at a ratio of 1:2 in the presence of 0.002 mM NADH for biotransformation of cyclopentene oxide **1a**, and only 4% conversion to α-hydroxycyclopentanone **3a** was obtained at 12 h (Table 1, entry 1). The conversion was greatly improved by the addition of LDH as a cofactor-regeneration enzyme in the catalytic system. Combining SpEH, BDHA, and LDH at 3−5:10:5 in the presence of 0.002−0.004 mM NADH and 200 mM pyruvate gave rise to the conversion of *meso*-epoxides **1a−c** (25−100 mM) to (*R*)-hydroxy ketones (*R*)-**3a−c** in excellent ee (98−99%) and good conversion (50−65%) after 9−12 h reactions (Table 1, entries 2−4). TTN of 5500−26 000 for NAD^+ regeneration was achieved, which is high enough for economical application of NAD^+. However, the regeneration of NAD^+ requires the excessive of pyruvate as cosubstrate, thus generating additional cost and waste. The use of NOX as the cofactor regeneration enzyme was then examined. By using a catalytic system containing SpEH, BDHA, and NOX at 3−5:10−12:5 in the presence of 0.002−0.004 mM NADH, (*R*)-hydroxy ketones **3a−c** were obtained in excellent ee (98−99%) and good conversion (58−70%) from *meso*-epoxides **1a−c** (20−100 mM) after 12−18 h reactions (Table 1, entries 5−7). In such a system, the regeneration of NAD^+ requires only molecular oxygen as cosubstrate and gave only water as byproduct, and the TTN for the regeneration of NAD^+ reached 6200−14000 which is also high enough for practical application of the cofactor. Considering the higher atom efficiency and less waste generation, NOX-catalyzed regeneration of NAD^+ is chosen for further development of the cascade biocatalysis.

The in vitro biotransformations results successfully demonstrated the new type of cascade biocatalysis and novel methodology for one-pot production of (*R*)-hydroxy ketones from the corresponding *meso*-epoxides via enantioselective epoxide hydrolysis and enantioselective oxidation of the diol intermediate. In the first step, SpEH catalyzed the enantioselective hydrolysis of *meso*-epoxides **1a−b** to give the corresponding (*R*, *R*)-diols **2a−b** in 85−86% ee. In the second step, BDHA catalyzed the highly enantioselective oxidation of the major enantiomer (*R*, *R*)-**2a−b** to afford the corresponding (*R*)-hydroxy ketones **3a−b** in 98−99% ee. In the case of conversion of cycloheptene oxide **1c** to (*R*)-hydroxycycloheptanone **3c**, hydrolysis with SpEH gave (*R*, *R*)-*trans*-cycloheptane-1,2-diol **2c** in only 19% ee. Nevertheless, the highly selective oxidation of (*R*, *R*)-**2c** with BDHA gave rise to the formation of (*R*)-hydroxycycloheptanone **3c** still in excellent ee (99%). In comparison, chemical conversion of epoxides to α-hydroxy ketones with strong acids gave racemic product together with several byproducts.[49−51]

Cascade Biocatalysis with the Mixtures of Resting Cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) for One-Pot Conversion of *meso*-Epoxide 1a−c to (*R*)-α-Hydroxyketones 3a−c. The use of whole-cell biocatalysts is more economic for cofactor dependent biotransformation than the use of free enzymes, because the cells can be easily produced in large amount and at low cost, the intact cells can help to maintain enzyme stability, and the cofactor NAD^+/NADH in cells can be used for target reaction with cofactor recycling. To develop whole-cell system for the cascade biocatalysis, the resting cells of *E. coli* (BDHA-NOX) were first examined for the oxidation reaction in the second step. The cells showed a specific activity of 115, 150, 143 U/g cdw for the oxidation of racemic *trans*-cyclic diols **2a−c** to (*R*)-α-hydroxyketones **3a−b**, respectively, being 3-fold higher than the cells of *E. coli* (BDHA).[40] The enhanced activity is possibly due to the regeneration of intracelluer cofactor NAD^+. Bio-

Table 2. One-Pot Enantioselective Conversion of *meso*-Epoxides 1a−c to (*R*)-*α*-Hydroxy Ketones 3a−c via Cascade Biocatalysis with the Mixtures of Resting Cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) or with the Resting Cells of *E. coli* (SpEH-BDHA-NOX)

entry[a]	sub.	sub. concn (mM)	*E. coli* (SpEH) (g cdw/L)	*E. coli* (BDHA-NOX) (g cdw/L)	*E. coli* (SpEH-BDHA-NOX) (g cdw/L)	time (h)	conv. to 3 (%)[b]	ee of (*R*)-3 (%)[c]
1	**1a**	100	4	10	–	6	84	98
2	**1b**	100	4	12	–	9	85	98
3	**1b**	200	4	16	–	12	78	98
4	**1b**	300	4	16	–	12	70	98
5	**1c**	200	4	13	–	13	57	99
6	**1a**	100	–	–	12	6	79	98
7	**1b**	100	–	–	12	9	71	98
8	**1b**	200	–	–	16	16	60	98
9	**1c**	100	–	–	12	9	52	99

[a]Reactions were carried out in a two-liquid phase system containing 10 mL of Tris buffer (100 mM, pH 7.5) and 10 mL hexadecane at 30 °C and 250 rpm. [b]Conversion was determined by GC analysis, error limit: <2% of the state values. [c]ee was determined by chiral GC analysis.

oxidation of racemic *trans*-cyclic diol **2a−c** (100 mM) with resting cells of *E. coli* (BDHA-NOX) gave (*R*)-*α*-hydroxyketones **3a−c** in 97−99% ee and 49−50% conversion, being also more productive than that with resting cells of *E. coli* (BDHA).[40] On the other hand, the resting cells of *E. coli* (SpEH) were also highly active for the hydrolysis reaction in the first step of the cascade, converting *meso*-epoxides **1a−c** (100 mM) to the corresponding diols in 99% within only 1−2 h. As same as the free SpEH, the whole-cells gave the hydrolysis products (*R, R*)-**2a−b** in 85−86% ee and (*R, R*)-**2c** in 19% ee, respectively.

On the basis of the different specific activities of the two bacterial cells, the resting cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) were mixed at a ratio of 4:10−16 to achieve efficient cascade biocatalysis. To solve the solubility and toxicity problem of the epoxides, an aqueous−organic two-phase system consisting of Tris-buffer and hexadecane at 1:1 (v/v) was used as the reaction medium in which the hydrophobic organic phase acts as a reservoir for the toxic epoxides. As shown in Table 2, combining *E. coli* (SpEH) cells at 4 g cdw/L and *E. coli* (BDHA-NOX) cells at 12 g cdw/L was able to produce (*R*)-*α*-hydroxycyclohexanone **3b** in 98.0% ee with 85% conversion from 100 mM cyclohexene oxide **1b** (Table 2, entry 2). The time course of this reaction is shown in Figure 1. At 1 h, epoxide **1b** was totally converted to *trans*-cyclohexane-1,2-diol **2b** due to the high activity of SpEH, 72 mM diol **2b** remained in the reaction mixture, and the rest of diol **2b** was converted to 26 mM (*R*)-*α*-hydroxycyclohexanone **3b** in 99% ee by BDHA coupled with NOX. From 1 to 6 h, the concentration of (*R*)-**3b** increased linearly with the linear decrease of the concentration of **2b**. At 9 h, 85 mM (*R*)-**3b** was produced in 98% ee, and 13 mM **2b** remained in the reaction mixture. To achieve even higher product concentration, 200−300 mM cyclohexene oxide **1b** was used for the cascade catalysis with the cell mixtures of *E. coli* (SpEH) at 4 g cdw/L and *E. coli* (BDHA-NOX) at 16 g cdw/L as catalysts. These gave (*R*)-*α*-hydroxy-cyclohexanone **3b** in 98% ee with 78−70% conversion.

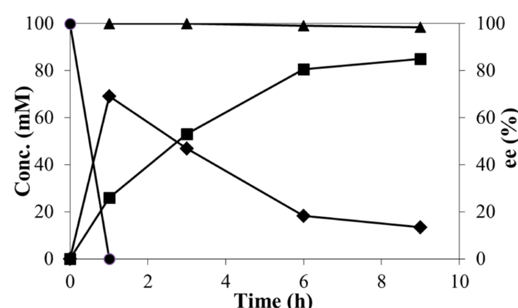


Figure 1. Time course of one-pot enantioselective conversion of cyclohexene oxide **1b** (100 mM) to (*R*)-*α*-hydroxycyclohexanone **3b** via cascade biocatalysis with the mixtures of resting cells of *E. coli* (SpEH) (4 g cdw/L) and *E. coli* (BDHA-NOX) (12 g cdw/L) in a two-liquid phase system consisting of Tris-buffer (100 mM, pH 7.5) and hexadecane (1:1). ●, concentration of **1b**; ♦ concentration of **2b**; ■, concentration of (*R*)-**3b**; ▲, ee of (*R*)- **3b**.

The generality of using the mixtures of the cells containing three necessary enzymes for performing the cascade reaction was demonstrated by using other *meso*-epoxides **1a** and **1c** as the substrates. As shown in Table 2 (entries 1 and 5), conversion of cyclopentene oxide **1a** (100 mM) and cycloheptene oxide **1c** (200 mM) with a mixture of 4 g cdw/L of *E. coli* (SpEH) cells and 10−13 g cdw/L of *E. coli* (BDHA-NOX) cells gave (*R*)-*α*-hydroxycyclopentanone **3a** in 98% ee with 84% conversion and (*R*)-*α*-hydroxycycloheptanone **3c** in 99% ee with 57% conversion, respectively. In comparison with the cascade biocatalysis using free enzymes, the use of cell mixtures afforded high product concentration and cost-effectiveness (considering the cost and availability of catalyst and cofactor) with the potential for industrial application.

Cascade Biocatalysis with Resting Cells of *E. coli* (SpEH-BDHA-NOX) for One-Pot Conversion of *meso*-Epoxide 1a−c to (*R*)-*α*-Hydroxyketones 3a−c. The use of single recombinant strain coexpressing all necessary enzymes for the cascade biocatalysis could avoid the cell cultivation of multiple strains and reduce the total cell density for the cascade biocatalysis. Resting cells of *E. coli* (SpEH-BDHA-NOX) were thus used for the one-pot conversion of *meso*-epoxide **1a−c** to (*R*)-hydroxy ketones **3a−c** (Scheme 2). As shown in Table 2 (entries 6−9), reactions of 100−200 mM epoxide **1b** with *E. coli* (SpEH-BDHA-NOX) at 12−16 g cdw/L afforded (*R*)-*α*-hydroxycyclohexanone **3b** in 98% ee and 71−60% conversion. Reactions of epoxides **1a** and **1c** (100 mM) with the same catalysts at 12 g cdw/L gave (*R*)-*α*-hydroxycyclopentanone **3a** in 98% ee and 79% conversion and (*R*)-*α*-hydroxycycloheptanone **3c** in 99% ee and 52% conversion, respectively. These results proved that the use of cells of a single microorganism coexpressing three necessary enzymes as catalysts worked well for the desired cascade biocatalysis. In comparison with the use

Table 3. One-Pot Enantioselective Conversion of Racemic Epoxide 1d to (*R*)-α-Hydroxy Ketone 3d via Cascade Biocatalysis with the Mixtures of Resting Cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) or with the Resting Cells of *E. coli* (SpEH-BDHA-NOX)

entry[a]	sub.	sub. concn (mM)	*E. coli* (SpEH) (g cdw/L)	*E. coli* (BDHA-NOX) (g cdw/L)	*E. coli* (SpEH-BDHA-NOX) (g cdw/L)	time (h)	conv. to **3d** (%)[b]	ee of (*R*)-**3d** (%)[c]
1	**1d**	5	4	16	–	18	49	99
2	**1d**	20	4	16	–	24	45	99
3	**1d**	10	–	–	16	24	40	99

[a]Reactions were carried out in a two-liquid phase system containing 10 mL of Tris buffer (100 mM, pH 7.5) and 10 mL of hexadecane at 30 °C and 250 rpm. [b]Conversion was determined by HPLC analysis, error limit: <2% of the state values. [c]ee was determined by chiral HPLC analysis.

of the cell mixtures of *E. coli* (SpEH) and *E. coli* (BDHA-NOX), similar product yields were achieved at lower cell density with *E. coli* (SpEH-BDHA-NOX) as catalysts. On the other hand, the use of mixed microorganisms is of advantages regarding the easy adjustment of appropriate ratio of different enzymes.

Cascade Biocatalysis for One-Pot Conversion of Racemic Epoxide 1d to (*R*)-α-Hydroxyketones 3d with the Mixtures of Resting Cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) or with Resting Cells of *E. coli* (SpEH-BDHA-NOX). To further prove the generality of the new cascade biocatalysis, racemic 1,2,3,4-tetrahydronaphthalene-1,2-oxide **1d** was selected as a substrate for one-pot synthesis of α-hydroxyketone with resting cells of *E. coli* (SpEH). This reaction was not very enantioselective, but it could quickly reach 100% conversion to produce racemic *trans*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene **2d**. On the other hand, the oxidation of racemic *trans*-diol **2d** with resting cells of *E. coli* (BDHA-NOX) was highly enantioselective, providing (*R*)-α-hydroxytetralone **3d** in >99% ee at 49% conversion. The combining of cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) for the cascade conversion of 5−20 mM racemic epoxide **1d** gave (*R*)-**3d** in 99% ee with 49−44% conversion (Table 3, entries 1 and 2). Alternatively, the use of resting cells of *E. coli* (SpEH-BDHA-NOX) alone converted 10 mM racemic **1d** to (*R*)-α-hydroxytetralone **3d** in 99% ee and 40% conversion (Table 3, entry 3). All these results demonstrated the generality of using the cascade biocatalysis for one-pot synthesis of enantiopure α-hydroxyketones from the corresponding epoxides, either *meso*-epoxides or racemic epoxides. The low yield in the cascade conversion of a racemic epoxide to an enantiopure α-hydroxyketone might be improved by using an enantioconvergent EH[52,53] as the first enzyme in the cascade reaction to convert a racemic epoxide into an enantio-enriched or pure diol with high conversion.

Preparation of (*R*)-α-Hydroxycyclohexanone 3b from Cyclohexene Oxide 1b by One-Pot Cascade Biocatalysis with the Mixtures of the Resting Cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX). The applicability of this new type of cascade biocatalysis for preparative biotransformation was examined on a 100 mL-scale biotransformation. Reaction of cyclohexene oxide **1b** (100 mM, 500 mg) was carried out at 30 °C for 9 h in a two-liquid phase system consisting of 50 mL of hexadecane and 50 mL of aqueous buffer containing the resting cells of *E. coli* (SpEH) (4 g cdw/L) and *E. coli* (BDHA-NOX) (12 g cdw/L). Although some *trans*-cyclohexanene-1,2-diol **2b** still remained in the reaction mixtures, simple workup and subsequent purification by flash chromatography gave pure (*R*)-α-hydroxycyclohexanone **3b** in 98% ee with 70% isolated yield (350 mg). This example demonstrated the potential of using the new type of cascade biocatalysis for facile, green, and high-yielding synthesis of enantiopure α-hydroxycyclohexanone from the corresponding easily available epoxide.

CONCLUSION

A new type of cascade biocatalysis was successfully developed for one-pot synthesis of useful and valuable α-hydroxy ketones in high ee from the corresponding easily available *meso*- or racemic epoxides via enantioselective epoxide hydrolysis and enantioselective oxidation of the diol intermediate. Efficient cascade biocatalysis was achieved by the combination of an epoxide hydrolase, an alcohol dehydrogenase, and a cofactor regeneration enzyme as the catalysts. The novel concept and synthetic methodology were successfully demonstrated by in vitro biocatalysis with the mixtures of recombinant enzymes SpEH, BDHA, and LDH or NOX, in the presence of trace amount of NADH, to convert several cyclic *meso*-epoxides **1a**−**c** to the corresponding (*R*)-α-hydroxy ketones **3a**−**c** in 98−99% ee and 70−50% conversion with a TTN of 5500−26 000 for NAD^+ recycling. In comparison with LDH, NOX catalyzed the regeneration of NAD^+ by using molecular oxygen as cosubstrate with water as coproduct, thus being more economic and sustainable. Whole-cell based cascade biocatalysis was achieved by using the cell mixtures of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) at the optimized ratio in a two-liquid phase system to convert 100−300 mM *meso*-epoxide **1a**−**c** to (*R*)-α-hydroxy ketones **3a**−**c** in 98−99% ee and 85−57% conversion. Moreover, the cells of *E. coli* (SpEH-BDHA-NOX) coexpressing all three enzymes were also good catalysts for the cascade conversion, converting 79−52% of 100−200 mM *meso*-epoxide **1a**−**c** to (*R*)-α-hydroxy ketones **3a**−**c** in 98−99% ee. The generality of the cascade biocatalysis was further demonstrated with these whole-cell approaches to convert 5−20 mM racemic epoxide **1d** to (*R*)-α-hydroxytetralone **3d** in 99% ee with 49−40% conversion. Preparative biotransformation was demonstrated on a 100 mL-scale with mixed cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) to give (*R*)-α-hydroxycyclohexanone **3b** in 98% ee and 70% isolated yield from cyclohexene oxide **1b** (100 mM). The new type of cascade biocatalysis might be generally applicable to prepare other useful enantiopure α-hydroxyketones from the corresponding *meso*- or racemic epoxides by selecting and combining the appropriate enzymes in the similar way.

EXPERIMENTAL SECTION

Engineering of *E. coli* (BDHA), *E. coli* (LDH), *E. coli* (SpEH), *E. coli* (NOX), *E. coli* (BDHA-NOX), and *E. coli* (SpEH-BDHA-NOX). *E. coli* (BDHA) and *E. coli* (LDH) were constructed in our previous research.[40]

E. coli (SpEH). Epoxide hydrolase (SpEH) gene[44] was amplified via PCR with primers of SpEH-F: CGC GGATCC G ATG ATG AAC GTC GAA CAT ATC CG and SpEH-R: CCC

AAGCTT TCA AAG ATC CAT CTG TGC AAA GGC by using genome DNA from *Sphingomonas* sp. HXN-200 as the template. The PCR amplifications were performed with Pfu DNA polymerase (New England Biolabs Inc.). The resulting 1146 bp fragment was digested with *Bam*HI and *Hind*III and then ligated into pETduet-1, which was then digested with the same restriction enzymes to generate the construct pETduet-SpEH. The resulting construct was then transformed into *E. coli* T7 competent cells and plated on LB plates containing ampicillin (100 μg/mL). The transformed strain was abbreviated as *E. coli* (SpEH).

E. coli (NOX). NADH oxidase (NOX) gene from *Lactobacillus brevis* DSM 20054[48] was synthesized by Genscript Corp (Piscataway, NJ). The gene was PCR amplified further using forward primer NOX-F: GGA AGATCT C ATG AAA GTC ACA GTT GTT GGT TGT AC and reverse primer NOX-R: CCG CTCGAG TTA AGC GTT AAC TGA TTG GGC AAC T. The amplified gene was digested with *Bgl*II and *Xho*I and ligated into pETduet vector. The resulting construct was then transformed into *E. coli* T7 competent cells and plated on LB plates containing 100 μg/mL ampicillin. The transformed strain was abbreviated as *E. coli* (NOX).

E. coli (BDHA-NOX). The constructed plasmids pET28a-BDHA[40] and pETduet-NOX with the same origin of replication and different antibiotic selection were transformed into *E. coli* T7 competent cells simultaneously and plated on LB plates containing ampicillin (100 μg/mL) and kanamycin (50 μg/mL). The transformed strain coexpressing BDHA and NOX was abbreviated as *E. coli* (BDHA-NOX).

E. coli (SpEH-BDHA-NOX). pETduet-SpEH was first digested with *Bgl*II and *Xho*I, and the NOX gene (digested with *Bgl*II and *Xho*I) was ligated into pETduet-SpEH vector, generating the construct pETduet-SpEH-NOX. The constructed plasmids pET28a-BDHA[40] and pETduet-SpEH-NOX with the same origin of replication and different antibiotic selection were transformed into *E. coli* T7 competent cells simultaneously and plated on LB plates containing ampicillin (100 μg/mL) and kanamycin (50 μg/mL). The transformed strain coexpressing BDHA, SpEH, and NOX was abbreviated as *E. coli* (SpEH-BDHA-NOX).

Cell Growth and Activity of *E. coli* (SpEH), *E. coli* (BDHA), *E. coli* (LDH), *E. coli* (NOX), *E. coli* (BDHA-NOX), and *E. coli* (SpEH-BDHA-NOX). The recombinant *E. coli* strains were grown, respectively, overnight at 37 °C in LB medium (3 mL in 20 mL tube) containing appropriate antibiotics (100 μg/mL ampicillin, or 50 μg/mL kanamycin, or both) as indicated above. One milliliter of seed culture was transferred to 50 mL of TB medium containing appropriate antibiotics in a 250 mL flask. The cells were grown for 2 h to reach an OD_{600} of 0.6−0.8 and then induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) (final concentration of 0.5 mM). Cell growth was continued at 22 °C and 250 rpm for 10−12 h. Cells were harvested by centrifugation at 8,500 × g and 4 °C for 10 min, washed twice with Tris buffer (100 mM, pH 7.5), and resuspended in the same buffer as resting cells for activity test and biotransformation.

The activity tests for *E. coli* (SpEH), *E. coli* (BDHA), and *E. coli* (LDH) were performed as described before.[44,40] The specific activity of *E. coli* (BDHA-NOX) was determined by performing the biotransformation of racemic cyclohexane-diol **2b** (50 mM) to (*R*)-α-hydroxycyclohexanone **3b** with the resting cells (2 g cdw/L) in 10 mL of Tris buffer (pH 7.5, 100 mM) at 30 °C and 250 rpm for 10 min and quantifying the product concentration by GC analysis. The specific activity of *E. coli* (SpEH-BDHA-NOX) was determined by performing the biotransformation of cyclohexene oxide **1b** (50 mM) to (*R*)-α-hydroxycyclohexanone **3b** with the resting cells (2 g cdw/L) in 10 mL of Tris buffer (pH 7.5, 100 mM) at 30 °C and 250 rpm for 10 min, and quantifying the product concentration by GC analysis. Analytic samples for these assays were prepared by removing the cells by centrifugation, saturating with NaCl, extracting with ethyl acetate containing 5 mM phenylacetone as an internal standard (1:1), and drying over Na_2SO_4. The special activities were expressed in U/g cdw, and all experiments were performed in duplicate.

The specific activity of *E. coli* (NOX) was performed by using the cell-free extract. The harvested cell pellets were resuspended in 20 mL of Tris buffer (pH 7.5, 100 mM) to an OD_{600} of 25 and passed through a homogenizer (one time, at 21 bar) to break the cells. The cell lysate was centrifuged at 15 000*g* and 4 °C for 30 min, and the supernatant (cell-free extract) was used to determine the activity of NOX. The protein concentration in the cell-free extracts was determined by the Bradford method. The activity assay was performed with a mixture of 989 μL of Tris buffer (pH 7.5, 100 mM), 10 μL of NADH (20 mM) in distilled water, and 1 μL of enzyme solution (5 g protein/L) in a cuvette. The UV absorbance at 340 nm was followed to monitor the decrease of NADH concentration by the oxidation with NOX. One unit of enzyme activity was defined as 1 μmol NADH decreased per minute. The special activity was expressed in U/g protein, and all experiments were performed in duplicate.

General Procedure for Enantioselective Cascade Conversions of *meso*-Epoxides 1a−c to (*R*)-α-Hydroxy Ketones 3a−c with the Mixture of Lyophilized Cell-Free Extract of *E. coli* (SpEH), *E. coli* (BDHA), and *E. coli* (LDH) or *E. coli* (NOX). The freshly prepared cells of *E. coli* (SpEH), *E. coli* (BDHA), *E. coli* (LDH), and *E. coli* (NOX) were resuspended in deionized (DI) water to a cell density of 20 g cdw/L, respectively. The cell suspension was passed through a Constant Cell Disruption System at 20 KPSi to break the cells, and the mixture was ultracentrifuged at 15 000*g* at 4 °C for 30 min to remove the cell debris. The protein concentration of the cell-free extracts was determined by Bradford method. The cell-free extracts were frozen at −80 °C overnight, followed by lyophilization for 48 h to get the lyophilized enzyme powders.

The lyophilized powders of the cell free-extracts of *E. coli* (SpEH), *E. coli* (BDHA), and *E. coli* (LDH) or *E. coli* (NOX) were mixed at different protein concentration (3−5 g/L, 10−12 g/L, and 5 g/L, respectively) in 10 mL of 100 mM Tris buffer (pH 7.5), followed by the addition of *meso*-epoxides **1a−c** (20−100 mM), NADH (0.002−0.004 mM), and cosolvent DMSO (5% v/v). In the case of using lyophilized power of the cell free-extracts of *E. coli* (LDH), pyruvate (200 mM) was also added as the cosubstrate. The mixtures were shaken at 250 rpm and 30 °C. Aliquots (300 μL) were taken out at different time points, saturated with NaCl, and extracted with ethyl acetate containing 5 mM phenylacetone as an internal standard (1:1). The organic phase was collected and dried over Na_2SO_4. After filtration, the samples were used for GC analysis to determine the yield and ee of (*R*)-α-hydroxy ketones **3a−c**.

General Procedure for Enantioselective Oxidation of Racemic Diols 2a−d to (*R*)-α-Hydroxy Ketones 3a−d with Resting Cells of *E. coli* (BDHA-NOX). The freshly prepared *E. coli* (BDHA-NOX) cells were resuspended in 10 mL of 100 mM Tris buffer (pH 7.5) to a cell density of 10−16

g cdw/L, racemic diols **2a−d** (0.2−2.0 mmol) was added, and the mixtures were shaken at 250 rpm and 30 °C. For the biotransformation of **2a−c**, 300 μL aliquots were taken out from the reaction mixtures at different time points for GC analysis of the ee and concentration of diols **2a−c** and α-hydroxy ketones **3a−c**. Analytic samples were prepared by removing the cells by centrifugation, saturating with NaCl, extracting with ethyl acetate containing 5 mM phenylacetone as an internal standard (1:1), and drying over Na_2SO_4. For the biotransformation of **2d**, 100 μL aliquots were taken out from the reaction mixtures at different time points, mixed with 400 μL of ACN containing 2 mM benzylacetone as an internal standard, and used for HPLC analysis to determine the concentration of diol **2d** and α-hydroxy ketone **3d**. For analyzing the ee of **2d** and **3d**, 200 μL aliquots were taken out and centrifuged to remove the cells, followed by extraction with 200 μL of chloroform, evaporation, and dissolving the residues in 200 μL of isopropyl alcohol for chiral HPLC analysis.

General Procedure for Enantioselective Cascade Conversions of Epoxides 1a−d to (*R*)-α-Hydroxy Ketones 3a−d with the Mixtures of Resting Cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX). The freshly prepared cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) were mixed in 10 mL of 100 mM Tris buffer (pH 7.5) to a cell density of 4 g cdw/L and 10−16 g cdw/L, respectively. Ten milliliters of hexadecane containing epoxides **1a−d** (0.1−2.0 mmol) was added. The mixture was incubated at 250 rpm and 30 °C. For **1a−c**, 300 μL aqueous phases were taken out at different time points for analysis. Analytic samples were prepared by removal of the cells via centrifugation, saturation with NaCl, extraction with ethyl acetate (1:1) containing 5 mM phenylacetone as an internal standard, and drying the organic phase over Na_2SO_4. The samples were analyzed by GC to quantify the ee and concentration of diols **2a−c** and α-hydroxy ketones **3a−c**. For **1d**, 10 μL of organic and 100 μL of aqueous phases were taken out separately at different time points and diluted with 490 and 400 μL of ACN containing 2 mM benzylacetone as an internal standard, respectively. The samples were analyzed by HPLC to determine the concentration of diol **2d** and α-hydroxy ketone **3d**. The concentration of each compound was calculated as the total concentration in aqueous and organic phase. For analyzing the ee of (*R*)-**3d**, 200 μL aliquots were taken out from the reaction mixture, the cells were removed by centrifugation, the product was extracted with 200 μL chloroform, the solvent was evaporated, and the residue was redissolved in 200 μL of isopropyl alcohol for chiral HPLC analysis.

General Procedure for Enantioselective Cascade Conversions of Epoxides 1a−d to (*R*)-α-Hydroxy Ketones 3a−d with the Resting Cells of *E. coli* (SpEH-BDHA-NOX). The freshly prepared *E. coli* (SpEH-BDHA-NOX) cells were resuspended in 10 mL of 100 mM Tris buffer (pH 7.5) to a cell density of 12−16 g cdw/L. Ten milliliters of hexadecane containing epoxides **1a−d** (0.1−2.0 mmol) was added. The mixture was incubated at 250 rpm and 30 °C. Samples were taken out at different time points to prepare analytic samples for the determination of the product concentration and ee, as described above.

Preparation of (*R*)-α-Hydroxycyclohexanone 3b from Cyclohexene Oxide 1b by Cascade Biocatalysis with the Mixtures of Resting Cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX). The freshly prepared cells of *E. coli* (SpEH) and *E. coli* (BDHA-NOX) were resuspended in 50 mL 100 mM Tris buffer (pH 7.5) to a cell density of 4 g cdw/L and 12 g cdw/L, respectively. Fifty milliliters of hexadecane containing cyclohexene oxide **1b** (100 mM, 500 mg) was added. The mixtures were shaken at 250 rpm and 30 °C for 9 h. The reaction mixture was centrifuged to remove the cells. Aqueous phase was separated, saturated with NaCl, and extracted with ethyl acetate for three times (3 × 50 mL). All the organic phases were combined and then dried over Na_2SO_4. After filtration, the organic solvent was removed by evaporation at reduced pressure. The crude products were purified by flash chromatography on a silica gel column with *n*-hexane/ethyl acetate of 50:50 (R_f = 0.3) to give (*R*)-**3b** as a colorless oil in 70.0% yield (350 mg) and 98% ee. $[\alpha]_D^{25}$ +20.6 (c 1.0, $CHCl_3$); lit. (ref 14) $[\alpha]_D^{20}$ +20.8 (c 0.65, $CHCl_3$, >99% ee). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44−1.78 (m, 3H), 1.85−1.93 (m, 1H), 2.08−2.15 (m, 1H), 2.32−2.41 (m, 1 H), 2.43−2.50 (m, 1 H), 2.54−2.60 (m, 1H), 3.64 (d, J = 3.2 Hz, 1H), 4.10−4.15 (m, 1H).

■ ASSOCIATED CONTENT

Supporting Information

The following file is available free of charge on the ACS Publications website at DOI: 10.1021/cs5016113.

Chemicals, biochemicals, and strains; analytical methods; specific activity of NOX for the cell-free extract; specific activity of SpEH, BDHA, LDH, and NOX for the lyophilized cell-free extracts; procedure for the hydrolysis of epoxides with *E. coli* (SpEH); data for the oxidation of diols with *E. coli* (BDHA) and *E. coli* (BDHA-NOX); chiral GC and HPLC chromatograms; and ^{1}H NMR spectrum (PDF)

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Notes

The authors declare no competing financial interest.

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