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PAPER

Asymmetric *trans*-dihydroxylation of cyclic olefins by enzymatic or chemo-enzymatic sequential epoxidation and hydrolysis in one-pot†

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Novel and efficient one-pot enzymatic and chemo-enzymatic synthetic methods are developed for the asymmetric *trans*-dihydroxylations of cyclic olefins **1a** and **1b** *via* sequential epoxidation and hydrolysis. The Novozym 435®-mediated epoxidation of cyclohexene **1a** and subsequent hydrolysis of the intermediate cyclohexene oxide **2a** with resting cells of *Sphingomonas* sp. HXN-200 in one-pot gave (1*R*,2*R*)-cyclohexane diol **3a** in 84% ee and 95% conversion. *trans*-Dihydroxylation of *N*-benzyloxycarbonyl 3-pyrroline **1b** with the same enzymatic system gave the corresponding (3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine **3b** in 93% ee and 94% conversion. In the one-pot chemo-enzymatic system, epoxidation of *N*-benzyloxycarbonyl 3-pyrroline **1b** by *m*-CPBA and subsequent hydrolysis of epoxide intermediate **2b** with resting cells of *Sphingomonas* sp. HXN-200 gave the *trans*-diol (3*R*,4*R*)-**3b** in 92% ee and 94–97% conversion. While the *trans*-dihydroxylation of cyclohexene **1a** to (1*R*,2*R*)-cyclohexane diol **3a** is reported for the first time, the *trans*-dihydroxylation of *N*-benzyloxycarbonyl 3-pyrroline **1b** to (3*R*,4*R*)-**3b** with such an enzymatic or chemo-enzymatic system afforded a much higher product concentration than the same reaction with the system using a microorganism containing the two necessary enzymes. The developed one-pot enzymatic and chemo-enzymatic systems for the asymmetric *trans*-dihydroxylation of olefins are new, easy to prepare, adjust and operate, are high yielding, complementary to Sharpless asymmetric dihydroxylation and particularly useful for the asymmetric synthesis of cyclic *trans*-diols.

Introduction

The preparation of fine chemicals and pharmaceuticals often involves complex multi-step syntheses, which require expensive isolation and purification of intermediates, and generate a large amount of waste.[1] A potential economic and sustainable solution is to perform multi-step reactions in one-pot. A key criterion for such a process is the compatibility of the individual reactions. Chemical reactions are often carried out under different conditions, thus being difficult to be performed in one-pot. On the other hand, enzymatic transformations, which are non-toxic and often highly selective, have similar reaction conditions and therefore are preferred for one-pot reactions. Multi-step enzymatic reactions are common in microbial cells during the metabolism of natural compounds by living microorganisms. However, when non-natural compounds are used as substrates for chemical synthesis, a microorganism often does not have the appropriate, active and selective enzymes for the desired multi-step reaction. The appropriate enzymes for individual reaction steps may be selected and combined to enable the multi-step biotransformation of non-natural substrates in one-pot. Up to now, there have been only a few such examples of enzymatic multi-step syntheses from non-natural substrates in one-pot.[2–3]

We have been interested in developing the asymmetric *trans*-dihydroxylation of olefins *via* one-pot sequential epoxidation and hydrolysis for the preparation of optically active vicinal diols, which are useful and valuable fine chemicals and pharmaceutical intermediates. This type of transformation is unique and different from asymmetric *cis*-dihydroxylation with Sharpless' catalyst[4] or a dioxygenase.[5] Rapid dihydroxylation of alkenes was achieved by using a lipase and hydrogen peroxide under microwave conditions,[6] which was elegant but not enantioselective. Previously, we discovered a bacterial strain *Sphingomonas* sp. HXN-200 containing the necessary monooxygenase and epoxide hydrolase for the *trans*-dihydroxylation of specific olefins with high enantioselectivity.[7] However, the efficiency and generality of using a wild-type microorganism for this type of transformation may be limited by the availability of the appropriate monooxygenase and epoxide hydrolase at the desired ratio within the cells. Recently, we developed a

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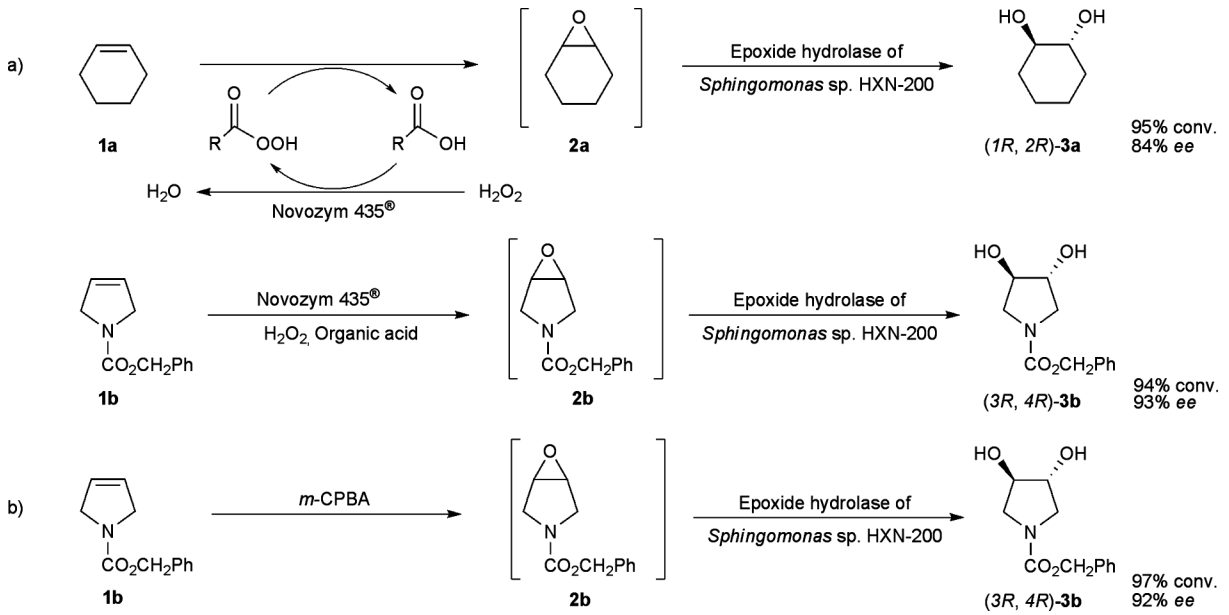

Scheme 1 The asymmetric *trans*-dihydroxylation of cyclohexene **1a** and *N*-benzyloxycarbonyl 3-pyrroline **1b** *via* a one-pot sequential epoxidation and hydrolysis: (a) enzymatic system and (b) chemo-enzymatic system.

novel and more general approach for the asymmetric dihydroxylation of styrenes to prepare the corresponding (*S*)-vicinal diols in high ee and good yield by using a tandem biocatalyst system combining styrene monooxygenase and epoxide hydrolase from different microorganisms.[8] Here, we report our recent success on the development of asymmetric *trans*-dihydroxylations of cyclic olefins *via* sequential epoxidation and hydrolysis in one-pot by two new systems: an enzymatic one, with lipase-mediated epoxidation and epoxide hydrolase-catalyzed hydrolysis, and a chemo-enzymatic one, with epoxidation by *m*-CPBA and hydrolysis *via* epoxide hydrolase.

Results and discussions

Cyclohexene **1a** and *N*-benzyloxycarbonyl 3-pyrroline **1b** were selected as model substrates for *trans*-dihydroxylations (Scheme 1) since they represent two different types of cyclic alkene and the corresponding *trans*-diols, (1*R*,2*R*)-**3a** and (3*R*,4*R*)-**3b**, are useful pharmaceutical intermediates that are difficult to make. As starting cyclic alkenes **1a** and **1b** are symmetric, Sharpless dihydroxylations of these compounds would give achiral *cis*-diols. On the other hand, the *trans*-dihydroxylation of **1a** and **1b** *via* epoxidation and hydrolysis affords *trans*-vicinal diols **3a** and **3b**, respectively. Since the epoxidation step does not involve any enantioselectivity, efficient but less enantioselective oxidation systems such as lipase-mediated oxidation[9] and chemical oxidation with *m*-CPBA were selected to produce achiral *meso*-epoxides **2a** and **2b**. The ee of the final products **3a** and **3b** was determined by the enantioselectivity of the hydrolysis step, thus the enantioselective epoxide hydrolase of *Sphingomonas* sp. HXN-200 was selected for the catalytic hydrolysis.[7,10] The epoxidation and hydrolysis were firstly investigated separately and then coupled in one-pot for the desired *trans*-dihydroxylation of alkenes **1a** and **1b**.

Lipase-mediated epoxidation of cyclohexene 1a

A high yield of cyclohexene oxide **2a** can be achieved by the lipase-mediated epoxidation of cyclohexene **1a** in toluene, chloroform or dichloromethane.[11a] However, these solvents are very toxic to microbial cells and thus are not suitable for the subsequent enzymatic hydrolysis step. The lipase-mediated epoxidation of **1a** was then investigated in acetonitrile[11b] since it has some compatibility with the enzymatic system. Lauric acid (LA), which showed a high reaction rate in the formation of peroxy acid,[11c] was selected as the perhydrolysis substrate. The easily available Novozym 435® (*Candida Antarctica* lipase B immobilized on macroporous acrylic resin) was chosen as the enzyme. The epoxidation of cyclohexene **1a** was thus performed by using a different amount of cyclohexene **1a**, H_2O_2, Novozym 435® and LA in acetonitrile for 24 h (Table 1). Excellent yields of cyclohexene oxide **2a** were obtained in all cases (Table 1, entries 1–5). The epoxidation of 0.5 M of **1a** with 0.25 M LA and 1.25 M H_2O_2 in the presence of 25 g L^{-1} Novozym 435® gave nearly a 100% yield of cyclohexene oxide **2a** (Table 1, entry 5).

Lipase-mediated epoxidation of *N*-benzyloxycarbonyl 3-pyrroline 1b

The lipase-mediated epoxidation of N-containing olefins is much more difficult, possibly due to the existence of basic nitrogen groups.[12] Epoxidation of *N*-benzyloxycarbonyl 3-pyrroline **1b** using the same conditions optimized for the epoxidation of cyclohexene **1a** afforded only 37% of **2b** after 48 h, and increasing the reaction time to 96 h only improved the conversion to 55% (Table 1, entries 6 and 7). Further increasing the acid concentration would increase the conversion. However, it was impossible to do so with lauric acid due to its limited solubility in acetonitrile. Other organic acids such as 6-hydroxy caporic acid (HCA), octanoic acid (OA) and phenyl acetic acid (PAA) were thus used at 1.0 M, respectively. While the first two acids did not show any significant improvement (Table 1, entries 8–11),

Table 1 Lipase-mediated epoxidation of cyclohexene **1a** and *N*-benzyloxycarbonyl 3-pyrroline **1b**

Entry	Sub.[a]	Sub. conc./M	H_2O_2/M	Novozym 435®/g L^{-1}	Organic acid[b]	Acid conc./M	Time/h	Prod.	Conv. (%)
1	**1a**	0.25	0.625	100	LA	0.125	24	**2a**	97
2	**1a**	0.25	0.625	100	LA	0.25	24	**2a**	99
3	**1a**	0.50	1.25	100	LA	0.25	24	**2a**	99
4	**1a**	0.50	1.25	50	LA	0.25	24	**2a**	99
5	**1a**	0.50	1.25	25	LA	0.25	24	**2a**	99
6	**1b**	0.50	1.25	25	LA	0.25	48	**2b**	37
7	**1b**	0.50	1.25	25	LA	0.25	96	**2b**	55
8	**1b**	0.50	1.25	25	HCA	1.0	48	**2b**	29
9	**1b**	0.50	1.25	25	HCA	1.0	96	**2b**	46
10	**1b**	0.50	1.25	25	OA	1.0	72	**2b**	50
11	**1b**	0.50	1.25	25	OA	1.0	144	**2b**	69
12	**1b**	0.50	1.25	25	PAA	1.0	72	**2b**	70
13	**1b**	0.50	1.25	25	PAA	1.0	144	**2b**	82
14	**1b**	0.50	1.25	25	PAA	1.5	72	**2b**	81
15	**1b**	0.50	1.25	25	PAA	1.5	144	**2b**	88
16	**1b**	0.50	1.25 + 0.50[c]	25 + 10[c]	PAA	1.5	144	**2b**	96

[a] A mixture of cyclic olefin **1a** or **1b**, H_2O_2 (50% aqueous solution), organic acid and Novozym 435® in acetonitrile (1 mL) was shaken at 300 rpm and 30 °C. [b] LA: lauric acid; HCA: 6-hydroxy caproic acid; OA: octanoic acid; PAA: phenyl acetic acid. [c] Another portion of H_2O_2 and Novozym 435® was added after 72 h.

Table 2 Epoxidation of *N*-benzyloxycarbonyl 3-pyrroline **1b** by *m*-CPBA

Entry	Sub.[a]	Sub. conc./M	*m*-CPBA/M	Reaction medium	Time/h	Prod.	Conv. (%)
1	**1b**	0.10	0.20	Acetonitrile	4.0	**2b**	46
2	**1b**	0.10	0.20	Acetonitrile	72	**2b**	99
3	**1b**	0.10	0.20	Hexane	2.5	**2b**	92
4	**1b**	0.10	0.20	Hexane	4.0	**2b**	98
5	**1b**	0.10	0.15	KPB[b]	4.0	**2b**	99
6	**1b**	0.20	0.30	KPB	4.0	**2b**	99
7	**1b**	0.04	0.06	KPB	6.0	**2b**	99

[a] A mixture of cyclic olefin **1b** and *m*-CPBA in a 1 mL reaction medium was shaken at 300 rpm and 30 °C. [b] KPB: potassium phosphate buffer (50 mM, pH 8.0).

the last one demonstrated a higher conversion (Table 1, entries 12 and 13). Increasing the PAA concentration to 1.5 M afforded epoxide **2b** in 82% conversion after 72 h and 88% after 144 h (Table 1, entries 14 and 15). Under the same initial conditions, the conversion was further increased to 96% after 144 h by the addition of a second portion of H_2O_2 and enzyme at 72 h (Table 1, entry 16). To the best of our knowledge, this is the first example of a lipase-mediated epoxidation of an N-containing olefin.

Epoxidation of *N*-benzyloxycarbonyl 3-pyrroline 1b by *m*-CPBA

Chemical epoxidation with *m*-CPBA as the oxidant is usually carried out in dichloromethane.[13] However, dichloromethane is very toxic to the second step enzymatic hydrolysis. Thus, the epoxidation of **1b** with *m*-CPBA was examined at different ratios in other reaction media, including hexane, acetonitrile and an aqueous buffer. As listed in Table 2, the oxidations with *m*-CPBA were faster than the lipase-mediated examples in all cases. Hexane and aqueous buffer were much better solvents for the oxidation. Complete conversion of 0.2 M **1b** was achieved with 0.3 M *m*-CPBA in 50 mM aqueous potassium phosphate buffer (pH = 8.0) in only 4 h.

Enantioselective hydrolysis of alicyclic *meso*-epoxides 2a/b with the epoxide hydrolase of *Sphingomonas* sp. HXN-200

Hydrolytic reactions[7,10] were investigated with the view of coupling them with the first epoxidation step. Since acetonitrile and excess hydrogen peroxide were used in the enzymatic epoxidation of **1a** and **1b**, the effects of acetonitrile and hydrogen peroxide on the hydrolysis of the corresponding *meso*-epoxides **2a** and **2b** with the epoxide hydrolase of *Sphingomonas* sp. HXN-200 were studied. When the hydrolysis of **2a** was carried out with resting cells of *Sphingomonas* sp. HXN-200 in the presence of 50 mM of H_2O_2, no inhibitory effect on enzyme activity by H_2O_2 was observed (Table 3, entries 1, 2, 4 and 5). Later on, we found that whole cells of *Sphingomonas* sp. HXN-200 could decompose some of the H_2O_2, indicating the possible existence of catalase in the cells. While no inhibition of 2% acetonitrile on the enzymatic hydrolysis was observed (Table 3, entries 3 and 1), the presence of 5% acetonitrile resulted in some inhibition of the enzymatic hydrolysis of **2a** and nearly no inhibition of the enzymatic hydrolysis of **2b** (Table 3, entries 4 and 8). A big inhibition was observed with 10% acetonitrile (Table 3, entries 6 and 9)

Asymmetric *trans*-dihydroxylation of cyclohexene 1a and *N*-benzyloxycarbonyl 3-pyrroline 1b by a one-pot enzymatic sequential epoxidation and hydrolysis

To perform the sequential epoxidation and hydrolysis of cyclohexene **1a** in one-pot, the epoxidation of **1a** was first carried out by lipase-mediated epoxidation under the established conditions (Table 1, entry 5). A mixture of cyclohexene **1a** (0.5 mmol), H_2O_2 (50% aqueous solution, 1.25 mmol), LA (0.25 mmol) and

Table 3 Enantioselective hydrolysis of cyclic *meso*-epoxides **2a** and **2b** with resting cells of *Sphingomonas* sp. HXN-200

Entry	Sub.[a]	Sub. conc./mM	HXN-200/g cdw L^{-1}	ACN (%)	H_2O_2/mM	Time/h	Product	Conv. (%)	ee (%)
1	**2a**	10	10	0	0	48	(1*R*,2*R*)-**3a**	99	85
2	**2a**	10	10	0	50	48	(1*R*,2*R*)-**3a**	99	85
3	**2a**	10	10	2	0	48	(1*R*,2*R*)-**3a**	99	85
4	**2a**	10	10	5	0	48	(1*R*,2*R*)-**3a**	75	85
5	**2a**	10	10	5	50	48	(1*R*,2*R*)-**3a**	76	85
6	**2a**	10	10	10	0	48	(1*R*,2*R*)-**3a**	26	85
7	**2b**	10	15	2	0	28	(3*R*,4*R*)-**3b**	99	93
8	**2b**	10	15	5	0	28	(3*R*,4*R*)-**3b**	97	93
9	**2b**	10	15	10	0	28	(3*R*,4*R*)-**3b**	2.2	93

[a] A mixture of cyclic *meso*-epoxide **2a** or **2b** and resting cells of *Sphingomonas* sp. HXN-200 in 10 mL potassium phosphate buffer (100 mM, pH 8.0) was shaken at 300 rpm and 25 °C for **2a**, and 30 °C for **2b**, respectively.

Table 4 The asymmetric *trans*-dihydroxylation of cyclohexene **1a** and *N*-benzyloxycarbonyl 3-pyrroline **1b** by one-pot enzymatic or chemo-enzymatic sequential epoxidation and hydrolysis

Entry	Sys.[a]	Sub.	Conc./M	Sol.	Vol./mL	H_2O_2/Novozym 435®/acid (M/g L^{-1}/M)	*m*-CPBA/M	Time/h	HXN-200 cells[b]/g L^{-1}	Sol.	Total vol./mL	Time/h	Prod.	Conc./mM	Conv. (%)	ee (%)
1	EE	**1a**	0.50	ACN	1.0	1.25/25/0.25		24	24	KPB[c]	50	48	**3a**	9.5	95	84
2	EE	**1b**	0.50	ACN	1.0	1.75/35/1.5		144	15	KPB	50	24	**3b**	9.4	94	93
3	CE	**1b**	0.10	KPB	1.0		0.20	4	15	KPB	10	24	**3b**	9.6	96	92
4	CE	**1b**	0.20	KPB	1.0		0.30	4	25	KPB	10	45	**3b**	18.8	94	92
5	CE	**1b**	0.02	KPB	5.0		0.03	4	21	KPB	10	16	**3b**	9.4	94	92
6	CE	**1b**	0.04	KPB	5.0		0.06	6	30	KPB	10	20	**3b**	19.4	97	92

[a] EE: enzymatic system; CE: chemo-enzymatic system. [b] Cell suspension was added after the finishing of the first reaction, resulting in an increase in the total reaction volume. For CE, sodium sulfite was added before the addition of the cell suspension. [c] KPB: potassium phosphate buffer (50 mM, pH 8.0).

Novozym 435® (25 mg) in acetonitrile (1 mL) was shaken at 300 rpm and 30 °C for 24 h. The conversion of cyclohexene oxide **2a** was over 99%. Then, a cell suspension of *Sphingomonas* sp. HXN-200 (49 mL, 24 g cdw L^{-1}) was added, and the mixture shaken at 300 rpm and 25 °C. After 30 h of reaction, the concentration of (1*R*,2*R*)-cyclohexane 1,2-diol **3a** reached 9.5 mM, corresponding to an overall conversion of 95% based on the cyclohexene **1a** initially added (Table 4, entry 1). The ee value of (1*R*,2*R*)-**3a** was 84%, which was nearly the same as that (85% ee) obtained in the single step hydrolysis of cyclohexene oxide **2a** with resting cells of *Sphingomonas* sp. HXN-200. The ee of **3a** might be further improved by using other epoxide hydrolases with higher enantioselectivities.[14]

Similarly, the *trans*-dihydroxylation of *N*-benzyloxycarbonyl 3-pyrroline **1b** in one-pot was performed by the epoxidation of **1b** (0.5 M) using Novozym 435® (15 + 10 g L^{-1}), H_2O_2 (1.25 + 0.50 M) and PAA (1.5 M) for 144 h, and the subsequent addition of a cell suspension of *Sphingomonas* sp. HXN-200 (15 g cdw L^{-1}) for a 24 h hydrolysis reaction (Table 4, entry 2). (3*R*,4*R*)-*N*-Benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine **3b** was obtained in 9.4 mM, corresponding to a 94% total conversion and in 93% ee, the same ee value as that achieved by the enzymatic enantioselective hydrolysis of *meso*-epoxide **2b** with resting cells of *Sphingomonas* sp. HXN-200. Simple purification by chromatography on a short silica gel column gave 82 mg (3*R*,4*R*)-**3b** in 98% purity with a 71% isolated yield.

Recycling of biocatalysts in the asymmetric *trans*-dihydroxylation of cyclohexene 1a by a one-pot enzymatic sequential epoxidation and hydrolysis

To reduce the cost of the immobilized enzyme (Novozym 435®) and to explore the reuse of whole cells, catalyst recycling was performed in the asymmetric *trans*-dihydroxylation of cyclohexene **1a**. The oxidation of cyclohexene (0.5 mmol) with 50% hydrogen peroxide (1.25 mmol), lauric acid (0.25 mm) and Novozym 435® (25 mg) in acetonitrile (1 mL) was performed at 300 rpm and 30 °C for 12 h, followed by the addition of a cell suspension of *Sphingomonas* sp. HXN-200 (49 mL, 24 g cdw L^{-1}) for the hydrolysis reaction for another 48 h. After this time, Novozym 435® was separated by removal of the cell suspension, and washed with de-ionized water and then cold acetone. After drying, the recovered Novozym 435® was used for the next reaction cycle. From the cell suspension, the cells were separated from the supernatant after centrifugation, washed with de-ionized water and re-used in the next reaction cycle. As shown in Fig. 1a, the recovered Novozym 435® gave nearly the same high conversion for the epoxidation of cyclohexene **1a** to cyclohexene oxide **2a** in each cycle and remained at 98% of the original productivity after four cycles of *trans*-dihydroxylation experiments. The results demonstrate that the immobilized enzyme is stable in the current one-pot reaction system, and that such an efficient recycling and effective reuse can significantly reduce its cost. The results also increase the greenness of the

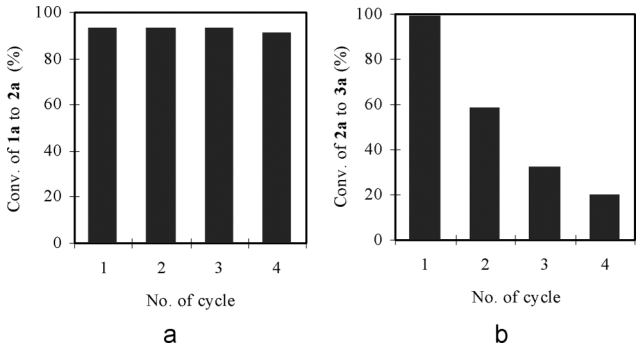

Fig. 1 Recycling and reuse of biocatalysts in the asymmetric *trans*-dihydroxylation of cyclohexene **1a** to **3a**: (a) Novozym 435® and (b) cells of *Sphingomonas* sp. HXN-200.

current method. On the other hand, the recovered cells of *Sphingomonas* sp. HXN-200 could also be recycled and reused to some extent. As shown in Fig. 1b, the cells showed 60% of the productivity achieved in the previous cycle for the hydrolysis of cyclohexene oxide **2a** to the corresponding diol **3a**. The decrease of activity is understandable, since cells are generally less stable than immobilized biocatalysts. To achieve full conversion, some new cells could be added. As cells are easily available and cheap, the use of cells as biocatalysts remains practical. Of course, it would be interesting to further develop the immobilized epoxide hydrolase as a second biocatalyst for one-pot asymmetric *trans*-dihydroxylations.

Asymmetric *trans*-dihydroxylation of *N*-benzyloxycarbonyl 3-pyrroline 1b by a one-pot chemo-enzymatic sequential epoxidation and hydrolysis

There are only limited reports on chemo-enzymatic reactions in one-pot,[15] possibly due to the compatibility problem. Simply coupling of the two reactions by the epoxidation of 0.10 M *N*-benzyloxycarbonyl 3-pyrroline **1b** with 0.20 M *m*-CPBA in 1 mL KP buffer for 4 h, followed by hydrolysis with the addition of a 9 mL cell suspension (15 g L^{-1}) of *Sphingomonas* sp. HXN-200 for 24 h, did not give any diol product **3b**. The enzymatic activity was possibly destroyed by the unreacted *m*-CPBA remaining in the reaction system. To solve this problem, Na_2SO_3 was added to remove the unreacted *m*-CPBA after completion of the epoxidation reaction, and cells of *Sphingomonas* sp. HXN-200 were then added to start the second hydrolysis reaction. By following this procedure, the *trans*-diol (3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine **3b** was obtained as the only product in 96% conversion and 92% ee (Table 4, entry 3). When the initial concentration of **1b** was doubled (Table 4, entry 4), (3*R*,4*R*)-**3b** was obtained in 92% ee and 94% conversion, corresponding to a final product concentration of 20 mM, which is 10 times higher than that previously reported with a bacterial strain containing the two necessary enzymes.[7] When the volumetric ratio of the two sequential reactions was changed from 1 : 10 to 1 : 1 (Table 4, entries 5 and 6), similar results were obtained: (3*R*,4*R*)-**3b** was obtained in 94–97% conversion and 92% ee.

Conclusions

Asymmetric *trans*-dihydroxylations of cyclic olefins **1a** and **1b** were successfully achieved by a one-pot enzymatic or chemo-enzymatic sequential epoxidation and hydrolysis. The Novozym 435®-mediated epoxidation of cyclohexene **1a** and subsequent hydrolysis of the intermediate cyclohexene oxide **2a** with resting cells of *Sphingomonas* sp. HXN-200 in one-pot gave (1*R*,2*R*)-cyclohexane diol **3a** in 85% ee and 95% conversion. The *trans*-dihydroxylation of *N*-benzyloxycarbonyl 3-pyrroline **1b** with the same enzymatic system gave (3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine **3b** in 93% ee and 94% conversion. The compatibility problem caused by the organic solvent used for the oxidation was successfully solved by the efficient epoxidation of the substrate at a high concentration in a small amount of solvent. Novozym 435® was efficiently recycled and effectively reused, with no significant decrease of productivity after 4 cycles. On the other hand, cells of *Sphingomonas* sp. HXN-200 were also recycled and reused, retaining 60% of their productivity from the previous cycle. In the one-pot chemo-enzymatic system, the epoxidation of *N*-benzyloxycarbonyl 3-pyrroline **1b** by *m*-CPBA and subsequent hydrolysis of epoxide **2b** with resting cells of *Sphingomonas* sp. HXN-200 gave (3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine **3b** in 92% ee and 94–97% conversion. Both reactions were carried out in the same aqueous buffer system, and the compatibility problem caused by the unreacted *m*-CPBA was easily solved by the addition of Na_2SO_3. While the *trans*-dihydroxylation of cyclohexene **1a** to (1*R*,2*R*)-cyclohexane 1,2-diol **3a** is reported for the first time, the *trans*-dihydroxylation of *N*-benzyloxycarbonyl 3-pyrroline **1a** to (3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine **3b** with either an enzymatic or chemo-enzymatic system afforded much higher product concentrations than the same reaction with a bacterial strain containing the two necessary enzymes. Compared with the two-step synthesis procedure, the one-pot approach avoids the isolation of the epoxide intermediate.

The developed one-pot enzymatic and chemo-enzymatic systems are novel for the asymmetric *trans*-dihydroxylation of olefins. They are better than the only other known system comprising a bacterial strain containing the two necessary enzymes, with easy selection and combination of the appropriate systems for the desired individual reactions, and easy adjustment of the individual systems for complete conversion. The asymmetric *trans*-dihydroxylation methods reported here are complementary to the Sharpless asymmetric dihydroxylation, being particularly useful for the asymmetric synthesis of cyclic *trans*-diols. The principles and knowledge obtained here could be generally useful for the development of asymmetric *trans*-dihydroxylations of other types of olefins by the selection and combination of appropriate tandem enzymes or chemo-enzymatic systems. While oxidations with Novozym-435® or *m*-CPBA work with a variety of olefins, the substrate scope for the enantioselective hydrolysis could be broadened by using different epoxide hydrolases. In addition to the existing ones, new and appropriate epoxide hydrolases could be obtained by enzyme engineering. It would also be interesting to develop the immobilized epoxide hydrolase as the second biocatalyst for one-pot asymmetric *trans*-dihydroxylations.

Experimental

Materials

Novozym 435® (*Candida Antarctica* lipase B immobilized on macroporous acrylic resin with a specific activity of 10 000 U g^{-1} catalyst for the synthesis of propyl laurate from lauric acid and 1-propanol) was purchased from Novozymes. Ethyl acetate (>99%) was purchased from Merck. Cyclohexene (>99.5%), cyclohexene oxide (98%), *N*-benzyloxycarbonyl 3-pyrroline (90%), lauric acid (98%), phenylacetic acid (>99%) and octanoic acid (98%) were purchased from Sigma-Aldrich. H_2O_2 (50% w/w) was obtained from Analar. 3-Chloroperoxybenzoic acid (*m*-CPBA) (*ca.* 70%) was purchased from Fluka. 6-Hydroxycaproic acid (95%) was bought from Alfa Aesar. Acetonitrile (>99%) was purchased from Fisher. Hexane (95%) was purchased from Tedia. Sodium sulfite (98%) was obtained from Acros.

Analytical methods

GC analysis was performed by using an Agilent 7890A gas chromatograph with an HP-5 column (30 m × 0.32 mm × 0.25 μm). Temperature program: 40 °C for 1 min, then to 140 °C at 12 °C min^{-1} and finally to 280 °C at 50 °C min^{-1}. Retention times: 3.6 min for **1a**, 5.6 min for **2a**, 8.2 min for **3a** and 9.3 min for n-dodecane (internal standard). Temperature program: 150 °C for 1 min, then to 280 °C at 10 °C min^{-1}. Retention times: 2.8 min for n-dodecane (internal standard), 7.0 min for **1b**, 8.6 min for **2b** and 11.0 min for **3b**.

HPLC analysis was carried out by using a Shimadzu™ Prominence HPLC with a Hypersil BDS-C18 column (4.0 × 125 mm, 5 μm) and UV detection at 210 nm. Mobile phase: 5% ACN/95% water from 0 to 5 min, changed to 55% ACN/45% water from 5 to 10 min and remaining at 55% ACN/45% water from 10 to 16 min; flow rate, 1.0 mL min^{-1}. Retention time: 11.3 min for benzyl alcohol (internal standard), 12.4 min for **3b**, 13.7 for **2b** and 14.9 min for **1b**.

The ee of **3a** was determined by GC analysis with a chiral column (Supelco, β-DEX™ 120, 30 m × 0.25 mm × 0.25 μm). Temperature program: from 100 °C to 157 °C at 3 °C min^{-1}, then to 200 °C at 30 °C min^{-1}. Retention time: 17.4 min for (1*S*,2*S*)-**3a** and 17.7 min for (1*R*,2*R*)-**3a**.

The ee of **3b** was determined by HPLC analysis with a chiral column (Chiralpak AS, 250 mm × 4.6 mm). Mobile phase: n-hexane–isopropanol (95 : 5), flow rate: 1.0 mL min^{-1},UV detection at 210 nm. Retention time: 53.2 min for (3*R*,4*R*)-**3b** and 66.4 min for (3*S*,4*S*)-**3b**.

Bacterial strain and growth medium

The strain *Sphingomonas* sp. HXN-200 was cultivated in E2 media using n-octane as the carbon source according to a published procedure.[16]

General procedure for the lipase-mediated epoxidation of cyclohexene 1a and *N*-benzyloxycarbonyl 3-pyrroline 1b

A mixture of olefin **1a** or **1b** (0.5 mmol), H_2O_2 (50% aqueous solution, 1.25 mmol), organic acid (0.125–1.5 mmol) and Novozym 435® (10–250 mg) in different organic solvents (1 mL) was shaken at 300 rpm and 30 °C. 2 μL samples were taken at different time intervals and mixed with 198 μL ethyl acetate containing 1 mM dodecane. After centrifugation at 15000 rpm for 10 min, the supernatant was analyzed by GC to quantify the conversion of **1a** to **2a** and of **1b** to **2b**, respectively.

General procedure for the enantioselective hydrolysis of cyclohexene oxide 2a and *N*-benzyloxycarbonyl 3,4-epoxy pyrrolidine 2b with resting cells of *Sphingomonas* sp. HXN-200

The hydrolysis of epoxide **2a** or **2b** (10 mM) was performed with frozen/thawed cells (10–15 g cdw L^{-1}) of *Sphingomonas* sp. HXN-200 in 10 mL 100 mM KP buffer (pH 8.0) at 300 rpm. The reaction temperature was 25 °C for **2a** and 30 °C for **2b**, respectively. For GC analysis of the conversions of **2a** to **3a**, as well as the ee of **3a**, 200 μL aliquots were taken at predetermined time points, followed by removal of the cells *via* centrifugation and extraction with ethyl acetate (1 : 1) containing 2 mM n-dodecane as an internal standard. For HPLC analysis of the conversion of **2b** to **3b**, an analytic sample was prepared by taking 100 μL aliquots, removing cells by centrifugation and mixing 20 μL of the supernatant with 180 μL ethanol containing 1 mM benzyl alcohol as an internal standard. For chiral HPLC analysis of the ee of **3b**, the sample was prepared by taking 200 μL aliquots, removing the cells *via* centrifugation, adding sodium chloride and extracting with ethyl acetate (1 : 1).

The asymmetric *trans*-dihydroxylation of cyclohexene 1a and *N*-benzyloxycarbonyl 3-pyrroline 1b by enzymatic sequential epoxidation and hydrolysis in one-pot

The asymmetric *trans*-dihydroxylation of cyclohexene 1a to prepare (1*R*,2*R*)-cyclohexane 1,2-diol 3a. A mixture of cyclohexene **1a** (0.5 mmol), H_2O_2 (50% aqueous solution, 1.25 mmol), lauric acid (0.25 mmol) and Novozym 435® (25 mg) in acetonitrile (1 mL) was shaken at 300 rpm and 30 °C for 24 h. A 49 mL suspension of frozen/thawed cells of *Sphingomonas* sp. HXN-200 (24 g cdw L^{-1}) in 100 mM KP buffer (pH 8.0) was added to the reaction mixture. The reaction was incubated at 300 rpm and 25 °C for 48 h. (1*R*,2*R*)-Cyclohexane 1,2-diol **3a** was formed in 84% ee, as determined by GC analysis with a chiral column, and in 95% overall conversion, as measured by GC analysis.

The asymmetric *trans*-dihydroxylation of *N*-benzyloxycarbonyl 3-pyrroline 1b to prepare (3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine 3b. A mixture of *N*-benzyloxycarbonyl 3-pyrroline **1b** (0.5 mmol), H_2O_2 (50% aqueous solution, 1.25 mmol), phenyl acetic acid (1.5 mmol) and Novozym 435® (25 mg) in acetonitrile (1 mL) was shaken at 300 rpm and 30 °C for 72 h. Another portion of H_2O_2 (50% aqueous solution, 0.5 mmol) and Novozym 435® (10 mg) was added to the reaction mixture, and the reaction continued for another 72 h. After adjusting the pH to 8 by adding an Na_2CO_3 aqueous solution (1 M), a 49 mL suspension of frozen/thawed cells of *Sphingomonas* sp. HXN-200 (15 g cdw L^{-1}) in 50 mM KP buffer (pH 8.0) was added to the reaction mixture. The reaction was performed at 300 rpm and 30 °C for 24 h, which gave (3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine **3b** in 93% ee and 94% overall conversion, as analysed by HPLC with a chiral column and a C18 column, respectively.

To purify the product, cells were removed from the biotransformation mixtures *via* centrifugation, and the product extracted into ethyl acetate. The organic phase was separated, dried over $MgSO_4$ and the solvent removed by evaporation. Purification by column chromatography on silica gel (R_f = 0.27, ethyl acetate) afforded 81.8 mg (70.7%) of (3*R*,4*R*)-**3b** in 93% ee and 98% purity.

The asymmetric *trans*-dihydroxylation of *N*-benzyloxycarbonyl 3-pyrroline 1b by chemo-enzymatic sequential epoxidation and hydrolysis in one-pot

A mixture of *N*-benzyloxycarbonyl 3-pyrroline **1b** (0.2 mmol) and *m*-CPBA (0.3 mmol, 1.5 equiv.) in KP buffer (pH 8.0, 1 mL) was shaken at 300 rpm and 30 °C for 4 h. After adding 1 M Na_2SO_3 aqueous solution (1 equiv.), the mixture was stirred for 20 min to remove the unreacted *m*-CPBA. 9 mL suspension of frozen-thawed cells of *Sphingomonas* sp. HXN-200 (25 g cdw L^{-1}) in 50 mM KP buffer (pH 8.0) was then added. The mixture was incubated at 300 rpm and 30 °C for 45 h. This gave (3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-pyrrolidine **3b** in 92% ee, analyzed by HPLC with a chiral column, and at 18.8 mM with 94% overall conversion, analyzed by reversed phase HPLC with a C18 column.

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