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Cascade Biotransformations via Enantioselective Reduction, Oxidation, and Hydrolysis: Preparation of (*R*)-*δ*-Lactones from 2-Alkylidenecyclopentanones

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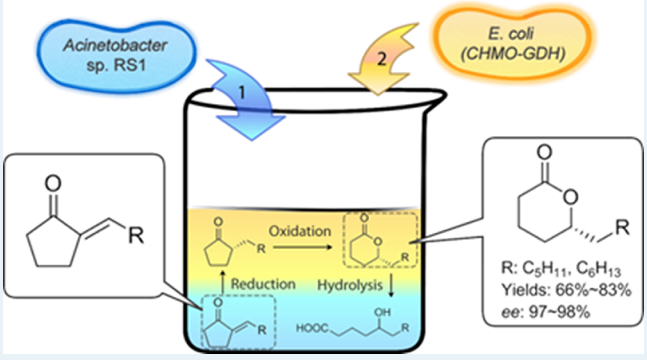
Supporting Information

ABSTRACT: The first cascade biotransformation involving enantioselective reduction of a C=C double bond, Baeyer–Villiger oxidation, and lactone hydrolysis was developed as a green and sustainable tool for synthesizing enantiopure *δ*-lactones. One-pot cascade biotransformations were achieved with *Acinetobacter* sp. RS1 containing a novel enantioselective reductase and an enantioselective lactone hydrolase and *Escherichia coli* coexpressing cyclohexanone monooxygenase and glucose dehydrogenase, converting easily available 2-alkylidenecyclopentanones **1**–**2** into the corresponding valuable flavors and fragrances (*R*)-*δ*-lactones **5**–**6** in high ee. The one-pot synthesis is better than the reported two-step preparation. This concept is useful in developing other redox cascades with the substrates containing C=C double bond.

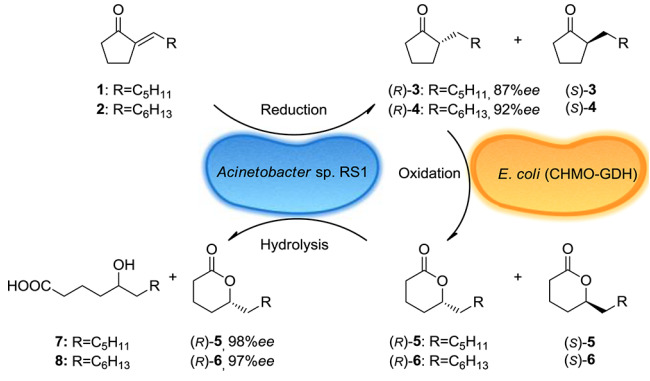


KEYWORDS: *cascade biotransformation, bioreduction, Baeyer–Villiger oxidation, lactone hydrolysis, enantioselective synthesis, δ-lactone*

Conducting cascade reactions in one pot has become a useful tool in sustainable chemical synthesis because it could avoid time-consuming and yield-reducing isolation and purification of intermediates, minimize waste generation and energy consumption, and reduce production cost.[1−4] In comparison with chemical reactions that are often performed at different conditions, enzymatic conversions are more suitable for one-pot cascades because of the similar reaction conditions.[1] Moreover, enzymatic reactions are environmentally friendly and often enantioselective. Therefore, a number of cascade biotransformations have been developed for non-nature synthesis;[5−11] however, there is increasing demand to invent new types of cascade biotransformations to broaden the synthetic application scope.

We have been interested in developing novel one-pot cascade biotransformations for enantioselective syntheses.[9−11] Here, we report the first enantioselective reduction–oxidation–hydrolysis cascade for the synthesis of (*R*)-2-alkyl-*δ*-lactones **5**-**6** from the corresponding 2-alkylidenecyclopentanones **1**–**2** (Scheme 1). *δ*-Lactones **5**–**6** are useful flavor and fragrance materials, and they are produced chemically as a result of the low concentrations in nature.[12,13] It was found that different enantiomers of *δ*-lactones gave different odors.[14] In the only known enantioselective synthesis, optically active **5**–**6** were produced chemically from the easily available starting materials **1**–**2** via catalytic hydrogenation and Baeyer–Villiger (BV) oxidation involving the use of high pressure H_2; a toxic and expensive metal catalyst; and a toxic oxidant, *m*-CPBA.[14]

Scheme 1. One-Pot Reduction–Oxidation–Hydrolysis Cascade for the Preparation of (*R*)-*δ*-Lactones 5–6 from 2-Alkylidenecyclopentanones 1–2



The enantioselective bioreduction of **1**–**2** is the key step in the reduction–oxidation–hydrolysis cascade. Thus far, biocatalytic redox cascades rely mainly on the use of alcohol dehydrogenase as the reducing enzyme.[1] Despite big achievements of biocatalysis in past decades, enantioselective bioreduction of the C=C double bond still remains a

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Table 1. Individual Catalytic Step Involved in the Reduction−Oxidation−Hydrolysis Cascade

sub.	concn (mM)	catalyst[a] (g cdw/L)	prod	spec. act. (U/g cdw)[b]	time (h)	conv (%)	ee_s (%)	ee_p (%)
1	5	12 (cat. A)	(*R*)-**3**	8.8	2	100		87
1	9	12 (cat. A)	(*R*)-**3**	8.5	5	100		80
2	5	12 (cat. A)	(*R*)-**4**	4.7	2	100		92
2	7	12 (cat. A)	(*R*)-**4**	4.5	5	100		88
(±)-**3**	5	2.4 (cat. B)	(*S*)-**5**	28	0.5	39	53	97
(±)-**3**	5	10 (cat. B)	(±)-**5**		1	100		
(±)-**4**	5	2.4 (cat. B)	(*S*)-**6**	30	0.5	42	57	85
(±)-**4**	5	10 (cat. B)	(±)-**6**		1	100		
(±)-**5**	10	4 (cat. A)	**7**	22	1	55	79	
(±)-**6**	10	4 (cat. A)	**8**	22	1	55	73	

[a]Cat. A: *Acinetobacter* sp. RS1; Cat. B: *E. coli* (CHMO−GDH). Biotransformation was performed in 5 mL Tris buffer (50 mM, pH7.5) containing 20 mg/mL glucose at 30 °C, 300 rpm. [b]Specific activity was measured for the first 30 min of the biotransformation.

significant challenge.[15−18] The reductions of 2-methylidenecyclopentanone and 2-propylidenecyclopentanone to the corresponding (*S*)-enantiomers of the products were reported with Baker's yeast and a *Synechococcus* strain, respectively,[17,19] but with long reaction time, low activity and conversion, and unsatisfactory enantioselectivity. No enzyme has been reported for the reduction of **1**−**2** to **3**−**4**, respectively. We initially examined the reduction of **1**−**2** with the well-known old yellow enzymes;[16−18] however, no products could be detected.

Screening of microorganisms was then conducted to discover an appropriate enzyme for the reduction of **1**−**2**. Of 200 alkane-, benzene-, or toluene-degrading strains collected in our lab,[20,21] 9 isolates were identified with the desired activity to reduce **1** and **2**. Among them, *Acinetobacter* sp. RS1 showed good activity, fast cell growth, and high enantioselectivity (Supporting Information). Biotransformation of 5 mM **1** and **2** with the resting cells of the strain gave a specific activity of 8.8 U/g cdw and 4.7 U/g cdw, respectively (1 *U* = 1 μmol product/min). Reactions for 2 h afforded (*R*)-**3** in 87% ee and (*R*)-**4** in 92% ee, both with 100% conversion (Table 1). Obviously, *Acinetobacter* sp. RS1 contains a unique reductase that catalyzes the enantioselective reduction of the C═C double bond. Reduction of 9 mM **1** and 7 mM **2** also gave 100% conversion, with slightly lower product ee. *Acinetobacter* sp. RS1 was, hence, chosen for the reduction step in the designed redox cascades. Its *R* selectivity allows for the access to (*R*)-*δ*-lactones, even with nonselective BV reaction.[22]

Recombinant *Escherichia coli* strains expressing cyclohexanone monooxygenase (CHMO) or cyclopentanone monooxygenase (CPMO), two well-known BVMOs,[22−25] were engineered for the Baeyer−Villiger (BV) oxidation of **3**−**4** to **5**−**6**. Although no activity from *E. coli* (CPMO) was observed, *E. coli* (CHMO) showed the desired oxidation activity with *S* enantioselectivity. *E. coli* strain coexpressing CHMO and glucose dehydrogenase (GDH) from *Bacillus subtilis* was then engineered as a more efficient whole-cell catalyst for the BV oxidations through intracellular cofactor recycling.[26,27] As shown in Table 1, oxidation of 5 mM racemic **3** and **4** with cells (2.4 g cdw/L) of *E. coli* (CHMO−GDH) in the presence of glucose for 0.5 h afforded 39% (*S*)-**5** in 97% ee and 42% (*S*)-**6** in 85% ee. Incubation for 1 h at a cell density of 10 g cdw/L gave 100% conversion in both cases. This indicated a reasonable activity of *E. coli* (CHMO−GDH) for the oxidation of (*R*)-**3** and (*R*)-**4**. Since no *R*-selective BVMOs could be found for the oxidation of **3**−**4**,[22] CHMO was chosen as the second step enzyme in the redox cascade.

Acinetobacter sp. RS1 and *E. coli* (CHMO−GDH) were combined to perform the cascade reduction−oxidation−hydrolysis in one pot. Initial experiments showed that *E. coli* (CHMO−GDH) had also unexpected oxidation activity on substrates **1** and **2** (Supporting Information); thus, the one-pot cascades were carried out in a subsequent manner.[1] Reduction of 6 mM **1** with resting cells of *Acinetobacter* sp. RS1, followed by the oxidation with resting cells of *E. coli* (CHMO−GDH) gave 83% (*R*)-**5** in 98% ee, and the cascade biotransformation of 5.5 mM **2** afforded 66% (*R*)-**6** in 97% ee.

The courses of the cascade biotransformations were investigated in detail. As shown in Figure 1a, substrate **1** was

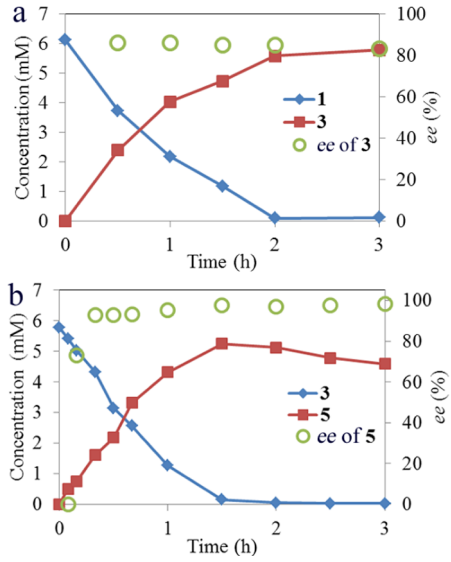

Figure 1. Time course of the subsequent one-pot cascade reduction−oxidation−hydrolysis for the preparation of (*R*)-**5** from **1**. (a) Bioreduction of **1** to **3** by cells of *Acinetobacter* sp. RS1. (b) Simultaneous oxidation of **3** to **5** by cells of *E. coli* (CHMO−GDH) and hydrolysis of **5** by cells of *Acinetobacter* sp. RS1.

rapidly reduced with the resting cells of *Acinetobacter* sp. RS1 within 2 h to give 95% (*R*)-**3** in 83% ee. In the subsequent oxidation with resting cells of *E. coli* (CHMO−GDH), both enantiomers of **3** were converted to **5**. The small amount of (*S*)-**3** generated in the first step was preferentially oxidized to (*S*)-**5** as a result of the *S* selectivity of CHMO, leading to a low ee of (*R*)-**5** at the early stage of the oxidation shown in Figure 1b. Later on, (*R*)-**5** was continuously produced from (*R*)-**3**. At 1.5 h, the intermediate **3** was nearly fully converted, giving 91%

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Table 2. Cascade Reduction, Oxidation, And Hydrolysis for the Preparation of (*R*)-δ-Lactones 5−6 from 2-Alkylidenecyclopentanones 1−2

sub.	concn (mM)	catalyst A[a]	vol (mL)	time (h)	catalyst B[b]	vol (mL)	time (h)	prod	yield (%)	ee (%)
1	6	*Acinetobacter* sp. RS1	5	3	*E. coli* (CHMO−GDH)	15	3	(*R*)-**5**	76	98
1	6	*Acinetobacter* sp. RS1	50	3	*E. coli* (CHMO−GDH)	150	1.5	(*R*)-**5**	83 (56)[c]	98
2	5.5	*Acinetobacter* sp. RS1	5	3	*E. coli* (CHMO−GDH)	15	3	(*R*)-**6**	51	97
2	5.5	*Acinetobacter* sp. RS1	50	3	*E. coli* (CHMO−GDH)	150	1.5	(*R*)-**6**	66 (41)[c]	97

[a]Cell density of 12 g cdw/L was used. [b]Catalyst added after the completion of the first step. Cell density of 10 g cdw/L was used. [c]Numbers in parentheses represent the isolated yield.

(*R*)-**5** in 98% ee. Continuing the reaction to 3 h decreased the yield to 76% while a high ee value was retained. A similar phenomenon was also observed for the cascade transformation of **2** to (*R*)-**6** (Supporting Information).

Because *E. coli* (CHMO−GDH) showed *S* enantioselectivity for the BV oxidation of **3**−**4**, the ee values of the products (*R*)-**5** and (*R*)-**6** in the redox cascade could not exceed those of the intermediates (*R*)-**3** and (*R*)-**4** obtained from the first step reaction. In both cascade cases, however, the ee values of the products (*R*)-**5**−**6** were higher than those of (*R*)-**3**−**4**, indicating the possible enantioselective degradation of the lactones **5**−**6** to improve the ee values. To examine this possibility, racemic **5**−**6** was incubated with resting cells of *Acinetobacter* sp. RS1. *S*-Enantioselective hydrolysis of **5**−**6** was observed, with an *E* of 8−11 (Table 1). The enzymatic degradation products were confirmed to be the hydroxyacids **7**−**8** by LC−MS analysis and by comparison with the products from chemical hydrolysis (Supporting Information). It is also worth mentioning that the enzymatic hydrolysis of δ-lactones is rare,[28−30] and *Acinetobacter* sp. RS1 showed much higher hydrolysis activity (22 U/g cdw) toward the racemic **5**−**6** than the previously reported enzymes.[28]

Preparative cascade biotransformations were carried out (Table 2). The cascade catalysis was started with 50 mL of cell suspension of *Acinetobacter* sp. RS1 in Tris buffer (12 g cdw/L) containing 40 mg **1**−**2** and 20 mg/mL glucose. After 3 h of reaction, 150 mL of Tris buffer containing cells of *E. coli* (CHMO−GDH) (10 g cdw/L) was added to start BV oxidation. After simultaneous oxidation and hydrolysis for 1.5 h, compounds **5**−**6** were produced in 83% and 66%, respectively. Extraction with ethyl acetate and purification by flash chromatography gave (*R*)-**5** in 98% ee and 56% isolated yield and (*R*)-**6** in 97% ee and 41% isolated yield, respectively (Supporting Information).

In summary, the first cascade catalysis involving enantioselective reduction of the C=C double bond and Baeyer−Villiger oxidation is reported. The cascade reaction provides simple and green syntheses of the valuable flavor and fragrance materials, (*R*)-δ-lactones **5**−**6**, from the easily available starting materials **1**−**2**. Such a one-pot synthesis with high product ee and high yield is better than other reported methods, including two-step chemical synthesis. This method allows access to the (*R*)-δ-lactones, which could not be made by kinetic resolution via the oxidation of the racemic ketones **3**−**4** using BVMO, which is commonly *S*-selective. Remarkably, a novel reductase from *Acinetobacter* sp. RS1 was discovered for the enantioselective reduction of C=C double bond with different substrate specificity compared with the well-known old yellow enzymes. Further development of the cascade catalysis includes the improvement of the efficiency by developing a recombinant strain functionally expressing the necessary enzymes and discovering an *R*-selective enzyme for the BV oxidation. The synthetic application of this new type of cascade will also be explored with other substrates. The concept is useful in developing other types of redox cascades with C=C double bond-containing substrates.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures including microbial screening, genetic engineering, single step biotransformation, and one-pot cascade biotransformation; analytic methods; chiral GC, chiral HPLC, and LC−MS chromatograms. This information 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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