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Enantioselective synthesis of ethyl (*S*)-2-hydroxy-4-phenylbutyrate by recombinant diketoreductase

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ABSTRACT

Recombinant diketoreductase showed excellent stereoselectivity in the double reduction of β,δ-diketo esters. To investigate the substrate specificity and to broaden the applications of this new biocatalyst, a number of ketone substrates were used to evaluate the substrate spectrum and enantioselectivity of this enzyme in the present study. Among the ketone substrates tested, only this enzyme displayed high efficiency and excellent enantioselectivity in the reduction of ethyl 2-oxo-4-phenylbutyrate to ethyl (*S*)-2-hydroxy-4-phenylbutyrate. After optimizing the reaction conditions, the bio-reduction of ethyl 2-oxo-4-phenylbutyrate at a substrate concentration of 0.8 M (164.8 g/L) was achieved by the recombinant diketoreductase in an aqueous-toluene biphasic system coupled with formate dehydrogenase for the regeneration of cofactor, resulting in an overall hydroxyl product yield of 88.7% (99.5% ee). This new enzymatic transformation may offer a practical method for the preparation of this important chiral building block.

1. Introduction

The stereoselective reduction of prochiral ketones is a very useful approach in organic synthesis for producing chiral intermediates that typically are synthetic building blocks for enantiomerically pure pharmaceuticals, food and agrochemicals.[1–3] For instance, enantiopure 2-hydroxy-4-phenylbutanoic acid and its ester have been utilized for the asymmetric synthesis of a wide variety of bioactive molecules, such as angiotensin-converting enzyme (ACE) inhibitors.[4,5] Recently, significant progress has been made in the synthesis of these chiral intermediates by biocatalytic approaches.[6,7] One of the widely used methods is the kinetic resolution of racemic alcohols.[8,9] A lipase from *Pseudomonas cepacia* was employed to enantioselectively hydrolyze racemic ethyl 2-hydroxy-4-phenylbutyrate to give 33% yield of the (*S*)-alcohol.[10] Since only a 50% theoretical yield could be achieved by kinetic resolution, the bio-reduction of prochiral ketones to chiral alcohols exhibits obvious advantages with a 100% theoretical yield after completing the reactions. For example, a dehydrogenase from *Enterobacter* sp. BK2 K was able to reduce 2-oxo-4-phenylbutanoic acid to its *S*-hydroxyl product with a conversion of 96% and ee value of 94%,[11] although the relatively low substrate loading (0.1 M) and moderate enantioselectivity of this reaction do limit its potential as a practical biocatalyst.

Recently, a diketoreductase was cloned from *Acinetobacter baylyi* ATCC 33305, and this novel and unique enzyme was heterogeneously expressed in *Escherichia coli*.[12,13] The recombinant diketoreductase (rDKR) could stereoselectively reduce ethyl-6-(benzyloxy)-3,5-dioxohexanoate to ethyl (3*R*,5*S*)-6-(benzyloxy)-3,5-dihydroxy-hexanoate, a valuable intermediate in the synthesis of statin drugs. Although the bio-reduction of the diketo ester by rDKR has been extensively studied, its substrate specificity towards other ketone substrates, especially mono-keto compounds, remains unclear. Herein, we conducted a survey on the substrate specificity of rDKR against different ketones. Among the ketone substrates examined, rDKR was found to be the only one capable of efficiently converting ethyl 2-oxo-4-phenylbutyrate **1** to ethyl (*S*)-2-hydroxy-4-phenylbutyrate **11a** with high conversion rate and excellent enantioselectivity (Scheme 1). Consequently, an aqueous-toluene biphasic system was established to increase the substrate concentration to a practical level (0.8 M), which provides another useful application of the diketoreductase.

2. Results and discussion

2.1. Reductions of ketone substrates with rDKR

Various ketones with carbonyl group(s) in the side chains (Fig. 1) were examined with rDKR for the conversion and stereoselectivity. The reaction was coupled with formate dehydrogenase (FDH) for NADH regeneration,[14] while reverse phase high perfor-

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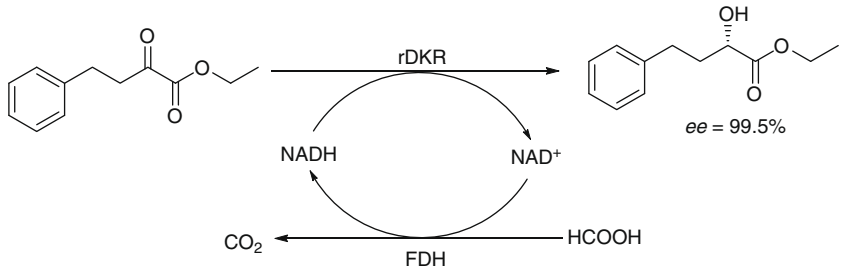

Scheme 1. The reduction of **1** catalyzed by rDKR. Reduction of **1** catalyzed by rDKR gave **11a** with ee value of 99.5%. FDH was used for the regeneration of NADH.

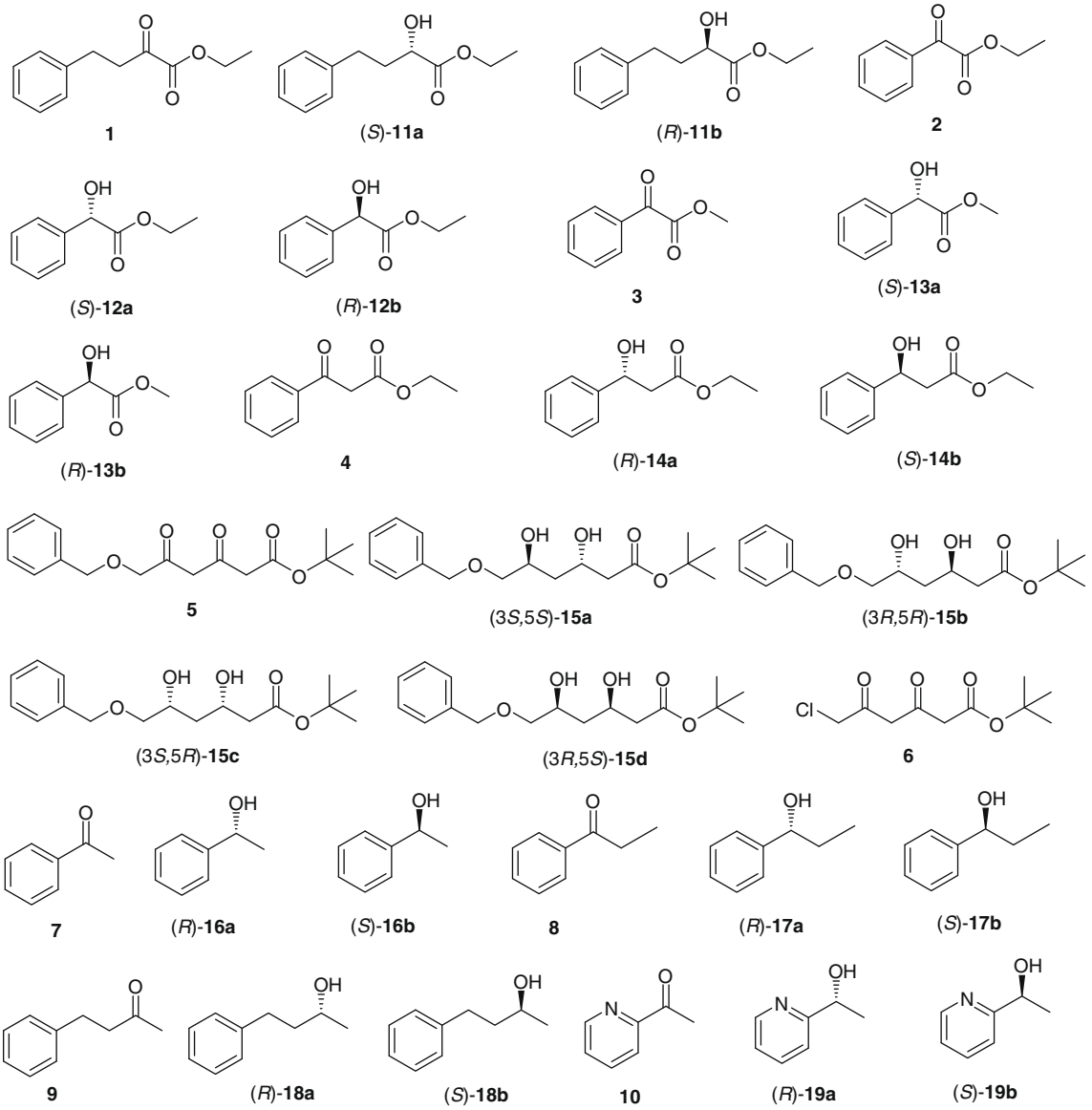

Figure 1. Structures of ketones and their hydroxyl products.

mance liquid chromatography (HPLC) and chiral HPLC were used to assay the conversion and stereoselectivity, respectively.

As shown in Table 1, rDKR showed better conversion in the reduction of α-keto esters than that of β-keto esters. The conversions of **1**, ethyl benzoylformate **2** and methyl benzoylformate **3** were greater than 99%, whereas the conversion of ethyl 3-oxo-3-phenylpropanoate **4 was** only 46.1%. When aryl ketones, such as acetophenone **7**, propiophenone **8** and benzylacetone **9**, were served as substrates, the length of the side chain from the phenyl ring significantly affected the bioconversion by rDKR, in which the conversion rates varied from 10.6% to 88.4%. The bioconversion could be considerably improved by increasing the length of the side chain. Furthermore, the bio-reduction was completely diminished by replacement of the phenyl ring by pyridine in the mono-

Table 1
Reduction of ketones catalyzed by rDKR

Substrate	Conversion[a] (%)	ee[b] (%)	de[b] (%)
1	99.5	99.5 (*S*)	N/A[c]
2	99.1	31.9 (*S*)	N/A
3	99.0	73.3 (*S*)	N/A
4	46.1	99.5 (*S*)	N/A
5	51.7	98.8 (3*R*,5*S*)	86.5 (3*R*,5*S*)
6	0	0	0
7	10.6	30.7 (*R*)	N/A
8	43.8	0.4 (*R*)	N/A
9	88.4	36.7 (*R*)	N/A
10	0	0	N/A

All the reactions coupled with formate dehydrogenase (FDH) for NADH regeneration were performed at room temperature for 18 h.
[a] Conversion of ketones was determined by achiral HPLC.
[b] The ee and de values of hydroxyl products were determined by Chiralcel OD-RH column.
[c] Not applicable.

ketones and by the substitution of benzyloxy group by chlorine in *t*-butyl 3,5-diketo-6-benzyloxyhexanoate **5**, suggesting that the phenyl ring might be essential for the binding between rDKR and substrate. Meanwhile, rDKR generally displayed poor enantioselectivity in the reduction of aryl ketones at a concentration of 2 mg/ml. However, when compounds **1** and **4** were used as substrates, excellent enantioselectivities were observed with different conversion rates (Table 1). The complete conversion (99.5%) and excellent enantioselectivity (99.5% ee) for the transformation of **1** to **11a** prompted us to investigate this enzymatic reaction further as a valuable biocatalytic application.

2.2. Optimization of the reaction media for the reduction of 1

A common obstacle of enzymatic conversion on an industrial scale is the low solubility of hydrophobic substrates in an aqueous media. As a result, enzymatic reactions are usually carried out at a low substrate concentration (5–10 mM), which leads to low efficiency and productivity. One strategy to overcome such problems is to identify an appropriate organic solvent as a co-solvent, in which the hydrophobic substrates can be dissolved without inactivating the enzyme.[15] Therefore, in order to increase the substrate concentration for practical utility, various organic solvents were screened to assess the tolerance of the reaction system consisting of rDKR and FDH in the reduction of **1**. The conversion and enantioselectivity were determined by HPLC.

As shown in Figure 2, both rDKR and FDH could be well tolerated against a variety of organic solvents. In the presence of 20% (v/v) of organic solvents, such as ethanol, DMSO and toluene, the reaction system consisting of rDKR and FDH was stable and performed well. However, the conversion rate decreased dramatically when either ethanol or toluene was used at a higher concentration for the reaction, whereas DMSO could reach values of up to 40% (v/v) in the reaction mixture without a negative impact (Fig. 2). Compared to these solvents, *n*-butanol, tetrahydrofuran, ethyl acetate and *n*-hexane did not show an improvement in the conversion. The enantioselectivity remained unchanged in all cases. Since the practical utility of many enzymes, such as alcohol dehydrogenases from *Pseudomonas fluorescens*[16,17] and from *Rhodococcus erythropolis*,[15] is restricted by their poor tolerance against organic solvents, our results indicated that the complement between rDKR/FDH and organic solvents could be particularly useful for the synthesis of chiral alcohols.

2.3. Reduction of 1 at high substrate concentration

According to the results of organic solvent tolerance, ethanol, DMSO and toluene were selected as co-solvents for further investigation. The reactions were carried out at different concentrations of **1** with 20% of ethanol/toluene or 40% of DMSO. The conversion rates and ee values were examined by HPLC and chiral HPLC. When ethanol was used as a co-solvent, the conversion was only 47.7% at a concentration of 0.05 M of **1** (Fig. 3). Meanwhile, with 20% (v/v) toluene as a co-solvent the conversion was 99.1% at the same substrate concentration. In the case of 40% (v/v) DMSO, the conversion reached 99.6%, and a similar conversion rate was obtained even at 1.2 M of substrate concentration (Fig. 3). Although DMSO showed excellent properties with respect to high substrate concentration, the safety issues and high boiling point of this solvent prohibited its practical use as a co-solvent for industrial applications. Therefore, toluene was ultimately the best choice among the solvents examined. Since toluene is not water miscible, a biphasic system is necessary for the bio-reduction. When whole cells containing rDKR were examined with the same biphasic systems using glucose as a co-substrate for cofactor regeneration, the bio-reduction at a substrate concentration of 10 mg/ml (0.049 M) was only 3.5% after 18 h. On the other hand, evaluation of a biphasic system consisting of an aqueous buffer solution and 20% toluene resulted in 95.2% conversion at substrate concentration of 0.8 M (164.8 g/L) after 9 h (Fig. 4), which shows higher efficiency and productivity than previous reports on (*S*)-alcohol dehydrogenase from *R. ery-*

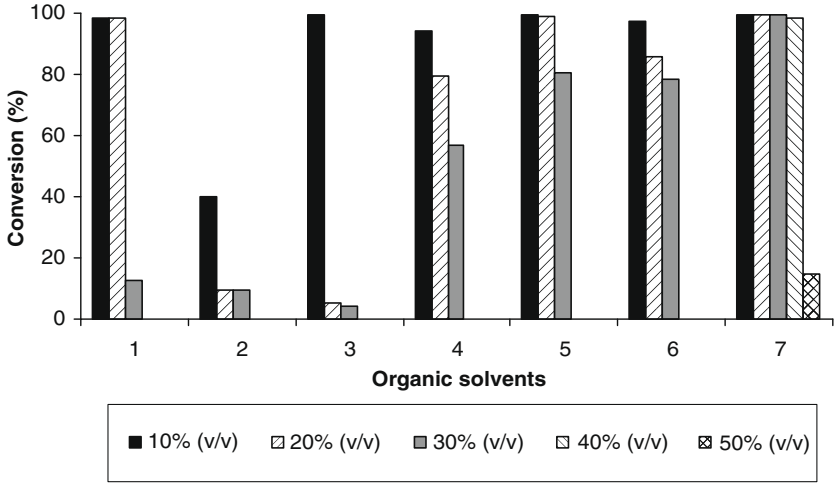

Figure 2. Reduction of **1** in different organic solvents. (1) Ethanol; (2) *n*-butanol; (3) THF; (4) ethyl acetate; (5) toluene; (6) *n*-hexane and (7) DMSO.

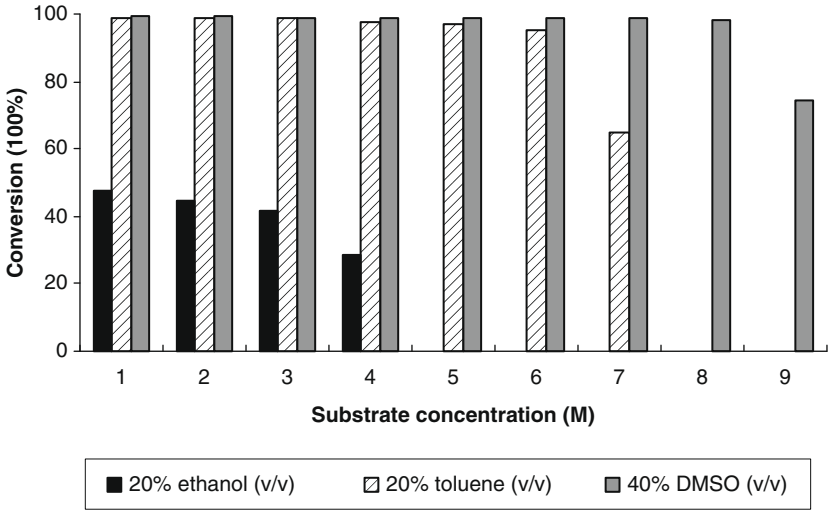

Figure 3. Reduction of **1** at different substrate concentrations. The reduction of **1** at different substrate concentrations was conducted with 20% ethanol, 20% toluene or 40% DMSO as a co-solvent, respectively. Substrate concentrations: (1) 0.05 M; (2) 0.1 M; (3) 0.2 M; (4) 0.4 M; (5) 0.6 M; (6) 0.8 M; (7) 1.0 M; (8) 1.2 M and (9) 1.5 M.

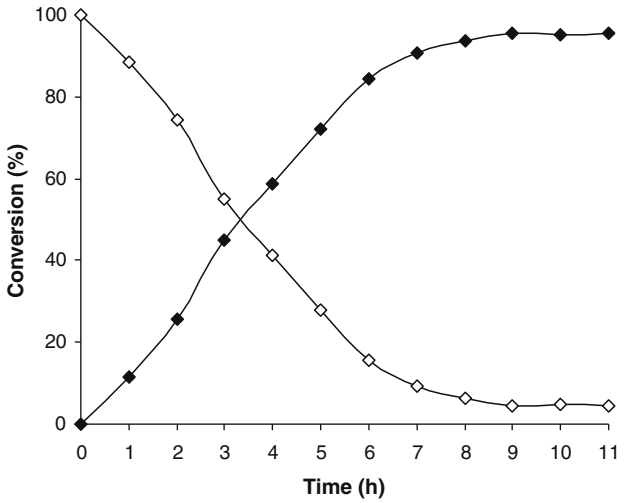

Figure 4. Time courses of the reduction of **1** by rDKR at substrate concentration of 0.8 M. Substrate **1** at 0.8 M (164.8 g/L) was transformed by rDKR coupled with FDH for NADH regeneration. All data represent the averages of three experiments. Open diamond; closed diamond: **11a**.

thropolis for the reduction of *p*-chloroacetophenone (0.2 M) in the aqueous-heptane (4:1) biphasic media.[15,18] Hence, a 50 ml preparative scale with substrate **1** concentration of 0.8 M (164.8 g/L) was performed to confirm this biocatalytic method at this scale. As expected, a conversion rate of 91.8% and 99.5% ee value were obtained after 9 h. After extraction and purification, the desired hydroxyl product **11a** was obtained with an isolated yield of 88.7%. Thus, the aqueous-toluene biphasic system consisting of rDKR and FDH could be used as a practical and efficient method for the preparation of **11a** from **1**.

3. Conclusions

Previous studies have shown that rDKR was able to reduce a diketo ester substrate with excellent stereoselectivity. Herein, the substrate specificity of rDKR on a number of ketones was investigated. An aryl mono-ketone **1** was found to be a suitable substrate for this enzyme. After evaluating different organic solvents, toluene was identified for its usefulness in the biocatalytic reduction of **1**. Coupling with FDH for NADH regeneration, rDKR can efficiently convert **1** to its hydroxyl product **11a** in an aqueous-toluene biphasic system. More importantly, the substrate concentration of **1** in this system was demonstrated to be 0.8 M (164.8 g/L). A practical and simple method for the preparation of **11a** has been developed and optimized, which further broadens the biocatalytic applications of rDKR.

4. Experimental

4.1. Materials and instruments

NAD^+ and IPTG were purchased from Sigma (USA). HPLC-grade acetonitrile was obtained from TEDIA (USA). All ketone substrates and alcohol standards were purchased from Sigma–Aldrich Chemical Co. (USA), ChemPacific (USA) or Nanjing Chemlin Chemical Industry Co., Ltd (China). *t*-Butyl 3,5-diketo-6- benzyloxyhexanoate **5** and *t*-butyl 3,5-dihydroxy-6-benzyloxyhexanoate **15** were prepared according to the procedures described in the literature.[19] The HPLC analyses were performed on a Shimadzu 2010A HT with UV detector. Enantiomeric excess (ee) was determined by chiral HPLC with a Chiralcel OD-RH column (Daicel Instruments, Ltd, USA). FDH was expressed and prepared as described by Shuber et al.[20]

4.2. Expression and enzyme activity of rDKR

rDKR was over-expressed in *E. coli* using LB medium at 15 °C, 220 rpm for 14 h after induction by 50 μM IPTG as previously described.[12,13] After expression, the cells were collected by centrifugation. Next, recombinant *E. coli* cells (5.0 g) were resuspended in 25 ml of 50 mM potassium phosphate buffer (pH 6.0). The cell suspensions were disrupted by passing through a high-pressure cell press at 1000 psi. Cell-free extract was obtained by centrifuging the mixture at 13,500*g* for 30 min. The absorbance change at 340 nm for the oxidation of NADH was used to determine the activity of rDKR with ethyl-6-(benzyloxy)-3, 5-dioxohexanoate as a substrate by spectrophotometric methods at 40 °C. Assay mixture contained 0.15 mM NADH, 0.25 mM ethyl-6-(benzyloxy)-3,5-dioxohexanoate, 5–50 μg DKR and 0.1 M potassium phosphate buffer (pH 6.0) in a final volume of 1.0 ml. For all assays, enzyme activity was defined as one unit representing the oxidation of 1 μmol of NADH per minute per milligram protein. The enzyme

activity of rDKR was approximately 5 μmol/min/mg with a protein concentration of 10.5 mg/ml, which was determined by Bradford method.

4.3. Achiral HPLC

Achiral HPLC methods were performed with various gradients of mobile phase A (0.1% trifluoroacetic acid in water) and mobile phase B (0.1% trifluoroacetic acid in acetonitrile) at 40 °C with UV detection at 220 nm. The analyses were performed on a Waters ODS column (5 μm, 150 × 4.6 mm) with an injection volume of 10 μl and a flow rate of 1 ml/min.

Method 1 was used to determine the conversion of **1** with a gradient from 15% to 80% B in 14 min. The retention times of racemic **11** and **1** were 10.4 min and 12.2 min.

Method 2 was used to determine the conversion of **2** with a gradient from 10% to 80% B in 14 min. The retention times of racemic ethyl mandelate **12** and **2** were 8.7 min and 11.8 min.

Method 3 was used to determine the conversion of **3** with a gradient from 10% to 80% B in 14 min. The retention times of racemic methyl mandelate **13** and **3** were 7.2 min and 10.6 min.

Method 4 was used to determine the conversion of **4** with a gradient from 15% to 85% B in 14 min. The retention times of racemic ethyl-3-hydroxy-3-phenylpropionate **14** and **4** were 8.4 min and 9.8 min.

Method 5 was used to determine the conversion of **5** with a gradient from 30% to 90% B in 14 min. Retention times of racemic *t*-butyl *anti*-3, 5-dihydroxy-6-benzyloxyhexanoate, racemic *t*-butyl *syn*-3, 5-dihydroxy-6-benzyloxyhexanoate and **5** were 7.9 min, 8.3 min and 11.3 min, respectively.

Method 6 was used to determine the conversion of **7** with a gradient from 15% to 65% B in 13 min. The retention times of racemic 1-phenylethanol **16** and **7** were 10.9 min and 12.8 min.

Method 7 was used to determine the conversion of **8** with a gradient from 20% to 80% B in 13 min, and then 80% B was kept for additional 3 min. The retention times of racemic 1-phenyl-1-propanol **17** and **8** were 11.2 min and 13.3 min.

Method 8 was used to determine the conversion of **9** with a gradient from 20% to 65% B in 13 min. Then, the gradient was increased to 90% B and kept for additional 3 min. The retention times of racemic 4-phenyl-2-butanol **18** and **9** were 13.4 min and 14.8 min.

Method 9 was used to determine the conversion of 2-acetylpyridin **10** with a gradient from 5% to 50% B in 14 min. The retention times of racemic 1-(2-pyridyl) ethanol **19** and **10** were 2.9 min and 6.8 min.

4.4. Analysis of the reduction of *t*-butyl 6-chloro-3,5-dioxohexanoate

The HPLC analysis of the reduction of *t*-butyl 6-chloro-3,5-dioxohexanoate **6** was performed on a Waters LC/MS system that consisted of a quaternary pump, an in-line degasser, an autosampler, a column thermostat, and an Micromass Quattro Micro™ series model Ion Trap SL mass spectrometer detector electrospray positive ionization. The chromatographic separation of the reaction mixture of **6** was conducted on a Shimadzu ODS column (5 μm, 250 × 4.6 mm) at 40 °C with an injection volume of 10 μl and a flow rate of 1 ml/min. Elution was achieved with a gradient of 15–90% B (0.1% trifluoroacetic acid in acetonitrile) in 14 min and kept at 90% B for additional 3 min. No hydroxyl products from the reduction of **6** catalyzed by rDKR were detected, except for *m*/*z* 235 of **6** (M+H) at 8.6 min.

4.5. Chiral HPLC

Chiral HPLC methods were performed on a Chiralcel OD-RH column (5 μm, 150 × 4.6 mm) at 25 °C with an injection volume of 5 μl and a flow rate of 0.5 ml/min.

Method 1 was used for the chiral analysis of **11** with a gradient of 30–40% B in 25 min, and then 40% B was kept for additional 5 min. Retention times of **11a** and **11b** were 21.1 min and 23.6 min, respectively.

Method 2 was used for the chiral analysis of **12** with a gradient of 15–30% B in 25 min, and then 30% B was kept for additional 5 min. Retention times of **12a** and **12b** were 23.4 min and 26.7 min, respectively.

Method 3 was used for the chiral analysis of **13** with a gradient of 15–25% B in 25 min, and then 25% B was kept for additional 5 min. Retention times of **13a** and **13b** were 17.5 min and 20.3 min, respectively.

Method 4 was used for the chiral analysis of **14** with a gradient of 25–30% B in 25 min, and then 30% B was kept for additional 5 min. Retention times of **14a** and **14b** were 17.9 min and 20.9 min, respectively.

Method 5 was used for the chiral analysis of the reduction products of **15** with a gradient of 32–40% B in 25 min, and then the gradient of 40% B was kept for additional 5 min. Retention times of dihydroxy ester stereoisomers of **15a**, **15b**, **15c** and **15d** were 18.4 min, 22.6 min, 24.6 min and 27.2 min, respectively.

Method 6 was used for the chiral analysis of **16** with a gradient of 15–30% B in 25 min, and then 30% B was kept for additional 5 min. Retention times of 1**6a** and 1**6b** were 21.1 min and 22.9 min, respectively.

Method 7 was used for the chiral analysis of **17** with a gradient of 32–40% B in 25 min, and then 40% B was kept for additional 5 min. Retention times of **17a** and **17b** were 13.7 min and 15.1 min, respectively.

Method 8 was used for the chiral analysis of **18** with a gradient of 32–40% B in 25 min, and then 40% B was kept for additional 5 min. Retention times of **18a** and **18b** were 17.5 min and 19.2 min, respectively.

4.6. Ketone reduction catalyzed by rDKR

The enzymatic reduction of aryl ketones was performed using rDKR and FDH for NADH regeneration. 1 ml of reaction mixture contained different ketones (2 mg/ml), 5% ethanol (v/v), NAD^+ (0.5 mM), FDH (4 U/ml), sodium formate (0.2 M), rDKR (5 U/ml) and 0.1 M potassium phosphate buffer (pH 6.0). The reaction mixtures were incubated at room temperature, 200 rpm for 18 h. Subsequently, all reactions were terminated by the addition of 1 ml ethanol. After vortexing and centrifugation, the resulting supernatants were subjected to HPLC analysis to determine the conversion and enantioselectivity. The absolute configurations of alcohols were confirmed by chiral HPLC with authentic samples.

4.7. Reduction of 1 in aqueous organic solvent mixtures

The reduction of **1** in an organic solvent was conducted in the presence of various concentrations of organic solvents ranging from 10% to 50% (v/v). Reaction mixture (1 ml) contained **1** (2 mg/ml), NAD^+ (0.5 mM), FDH (4 U/ml), sodium formate (0.2 M), rDKR (5 U/ml) and 0.1 M potassium phosphate buffer (pH 6.0). The reaction mixtures were incubated at room temperature, 200 rpm for 18 h and were terminated by the addition of ethanol (1 ml). The resulting supernatants were subjected to HPLC analyses to determine the conversion and enantioselectivity.

4.8. Reduction of 1 at different substrate concentrations

Substrate concentrations from 0.05 to 1.5 M were used in the reaction media consisting of aqueous buffer solution and 20% ethanol/toluene or 40% DMSO. The reaction mixture (1 ml) contained different concentrations of **1**, sodium formate (2 M), NAD^+ (0.5 mM), FDH (4 U/ml), organic solvent (20% or 40% v/v), rDKR (5 U/ml), 0.1 M potassium phosphate buffer (pH 6.0). The reactions were performed at room temperature, 200 rpm for 18 h. The conversion rate and enantiomeric purity were analyzed by HPLC.

4.9. Reduction of 1 by whole cells containing rDKR

Recombinant *E. coli* cells (0.2 g) containing rDKR were suspended in 1 ml of 0.1 M phosphate buffer (pH 6.0). Glucose (75 mg) was then added along with 10 mg of **1**. The bioconversion of **1** was conducted by shaking at 200 rpm at room temperature for 18 h and was terminated by the addition of 1 ml ethanol. After vortexing and centrifugation, the resulting supernatants were filtered and analyzed by HPLC to determine the conversion and enantioselectivity.

4.10. Time course for the reduction of 1 in the biphasic system

The reduction of **1** were performed at a concentration of 0.8 M in a system containing sodium formate (2 M), NAD^+ (0.5 mM), FDH (4 U/ml), DKR (5 U/ml), toluene (20%, v/v) and 0.1 M potassium phosphate buffer (pH 6.0). The reaction mixtures were incubated at room temperature at 200 rpm. Samples were taken at 1 h intervals for 11 h and were terminated by the addition of ethanol. After centrifugation, the resulting supernatants were subjected to HPLC analyses.

4.11. Reduction of 1 at a preparative scale

A 50 ml reaction mixture contained substrate **1** (8.25 g), $HCOONa{\cdot}2H_2O$ (10.4 g), NAD^+ (16.6 mg), FDH (200 U), toluene (10 ml), DKR (250 U) and 0.1 M potassium phosphate buffer (pH 6.0). The reaction mixture was incubated at room temperature, at 200 rpm for 9 h. Then, the toluene layer was separated from the aqueous phase, and the aqueous phase was extracted with ethyl acetate for three times (3×50 ml). The organic phases were combined and dried over Na_2SO_4. After filtration and evaporation under reduced pressure a yellow oily residue was obtained. Chromatography on silica gel (ethyl acetate/petroleum ether 5:95 v/v) gave 7.3 g **11a** (88.7% isolated yield) as a colorless oil.

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References

1. Patel, R. N. *Curr. Org. Chem.* **2006**, *10*, 1289–1321.
2. Patel, R. N. *Coord. Chem. Rev.* **2008**, *252*, 659–701.
3. Müller, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 362–365.
4. Lin, W. Q.; He, Z.; Jing, Y.; Cui, X.; Liu, H.; Mi, A. Q. *Tetrahedron: Asymmetry* **2001**, *12*, 1583–1587.
5. Dao, D. H.; Kawai, Y.; Hida, K.; Hornes, S.; Nakamura, K.; Ohno, A.; Okamura, M.; Akasaka, T. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 425–432.
6. Chadha, A.; Baskar, B. *Tetrahedron: Asymmetry* **2002**, *13*, 1461–1464.
7. Oda, S.; Inada, Y.; Kobayashi, A.; Ohta, H. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1762–1767.
8. Kalaritis, P.; Regenye, R. W.; Partridge, J. J.; Coffen, D. L. *J. Org. Chem.* **1990**, *55*, 812–815.
9. Huang, S.-H.; Tsai, S.-W. *J. Mol. Catal. B: Enzym.* **2004**, *28*, 65–69.
10. Liese, A.; Kragl, U.; Kierkels, H.; Schulze, B. *Enzyme Microb. Technol.* **2002**, *30*, 673–681.
11. Cha, M.; Kim, E. J.; Yun, H.; Cho, B.-K.; Kim, B.-G. *Biotechnol. Prog.* **2007**, *23*, 606–612.
12. Wu, X. R.; Liu, N.; He, Y. M.; Chen, Y. J. *Nat. Proc.*, **2008**. http://hdl.handle.net/10101/npre.2008.1697.1.
13. Wu, X. R.; Liu, N.; He, Y. M.; Chen, Y. J. *Acta Biochem. Biophys. Sinica* **2009**, *41*, 163–170.
14. Geertman, J. M.; van Dijken, J. P.; Pronk, J. T. *FEMS Yeast Res.* **2006**, *6*, 1193–1203.
15. Gröger, H.; Hummel, W.; Buchholz, S.; Drauz, K.; Nguyen, T.; Rollmann, C.; Hüsken, H.; Abokitse, K. *Org. Lett.* **2003**, *5*, 173–176.
16. Hildebrandt, P.; Riermeier, T.; Altenbuchnerc, J.; Bornscheuera, U. T. *Tetrahedron: Asymmetry* **2001**, *12*, 1207–1210.
17. Zhu, D. M.; Malik, H. T.; Hua, L. *Tetrahedron: Asymmetry* **2006**, *17*, 3010–3014.
18. Gröger, H.; Hummel, W.; Rollmann, C.; Chamouleau, F.; Hüsken, H.; Werner, H.; Wunderlich, C.; Abokitse, K.; Drauz, K.; Buchholz, S. *Tetrahedron* **2004**, *60*, 633–640.
19. Guo, Z. W.; Chen, Y. J.; Goswami, A.; Hanson, R. L.; Patel, R. N. *Tetrahedron: Asymmetry* **2006**, *17*, 1589–1602.
20. Shuber, A. P.; Orr, E. C.; Recny, M. A.; Schendel, P. F.; May, H. D.; Schauer, N. L.; Ferry, J. G. *J. Biol. Chem.* **1986**, *261*, 12942–12947.