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Highly Efficient Synthesis of Optically Pure (*S*)-1-phenyl-1,2-ethanediol by a Self-Sufficient Whole Cell Biocatalyst

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Terminal vicinal diols are important chiral building blocks and intermediates in organic synthesis. Reduction of α-hydroxy ketones provides a straightforward approach to access these important compounds. In this study, it has been found that asymmetric reduction of a series of α-hydroxy aromatic ketones and 1-hydroxy-2-pentanone, catalyzed by *Candida magnolia* carbonyl reductase (CMCR) with glucose dehydrogenase (GDH) from *Bacillus subtilis* for cofactor regeneration, afforded 1-aryl-1,2-ethanediols and pentane-1,2-diol, respectively, in up to 99% *ee*. In order to evaluate the efficiency of the bioreduction, lyophilized recombinant *Escherichia coli* whole cells coexpressing CMCR and GDH genes were used as the biocatalyst and α-hydroxy acetophenone as the model substrate, and the reaction conditions, such as pH, cosolvent, the amount of biocatalyst and the presences of a cofactor (i.e., $NADP^+$), were optimized. Under the optimized conditions (pH 6, 16 h), the bioreduction proceeded smoothly at 1.0 M substrate concentration without the external addition of cofactor, and the product (*S*)-1-phenyl-1,2-ethanediol was isolated with 90% yield and 99% *ee*. This offers a practical biocatalytic method for the preparation of these important vicinal diols.

Introduction

Terminal vicinal diols are important chiral building blocks and intermediates for the synthesis of natural products, agrochemicals and pharmaceuticals.[1] Therefore, many synthetic methods have been developed for the preparation of optically pure vicinal diols. For example, organometallic complexes have been used to catalyze the asymmetric hydrogenation of α-hydroxy acetophenone to give optically active 1-phenyl-1,2-ethanediol,[1b,2] although the chemical reduction needed high temperature, high pressure of hydrogen, oxygen-free operation and alkaline reaction conditions under which the substrate was less stable.[3]

Several biocatalytic methods for the preparation of 1,2-diols have also been developed. Stereospecific dihydroxylation of styrene catalyzed by naphthalene dioxygenase afforded (*R*)-1-phenyl-1,2-ethanediol with 78.6% *ee*.[4] Racemic 1-phenyl-1,2-ethanediol was enantioselectively oxidized by glycerol dehydrogenase, resulting in its resolution, but the oxidation product α-hydroxy acetophenone strongly inhibited the enzyme activity.[5] With whole-cell *Brevibacterium lutescens* CCZU12-1 as the catalyst, (*R*)-1-phenyl-1,2-ethanediol was oxidized to (*R*)-mandelic acid leaving (*S*)-1-phenyl-1,2-ethanediol intact with 99% *ee* at a substrate concentration of 50 mM.[6] Lipase-catalyzed transesterification of 1-phenyl-1,2-ethanediol has also been conducted; under the reported conditions, a mixture of monoester, diester and unreacted 1-phenyl-1,2-ethanediol with low *ee* was obtained.[7] Enantioselective conversion of racemic 1-phenyl-1,2-ethanediol to (*S*)-1-phenyl-1,2-ethanediol by *Candida parapsilosis* has been shown to give high yield and *ee*, but the reaction was performed at low substrate concentration.[8]

Enantioselective carbonyl reductases have been successfully used in the reduction of terminal α-hydroxy alkyl carbonyl compounds.[9] However, the bioreduction has been usually carried out at low substrate concentration with a large amount of cofactor. In an effort to develop a practical method for the synthesis of optically pure vicinal diols, we screened the reductases available in our laboratory; *Candida magnolia* carbonyl reductase (CMCR), which belongs to the short-chain dehydrogenase family,[10] was found to show excellent reactivity and stereoselectivity toward the reduction of α-hydroxy ketones. Furthermore, we demonstrated the efficient asymmetric synthesis of (*S*)-1-phenyl-1,2-ethanediol as the model compound at high concentration without addition of an external cofactor.

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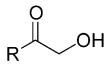
Table 1. Screening of carbonyl reductases toward the reduction of α-hydroxy acetophenone.

Enzyme[a]	Specific activity[b] U mg^{-1}	Conversion [%]	*ee*[c] [%]
CMCR	3.50	99	99 *S*
SSCR	0.05	45	92 *S*
GCY1	0.01	8	99 *R*
Ymr226c	0.92	86	83 *R*
PFADH	0.03	12	32 *R*
Gre3	–	–	–

[a] For details, see the general comments in the Experimental Section. [b] Ketone (6.25 mM), NADPH (0.40 mM) and 10% *v/v* DMSO in sodium phosphate buffer (100 mM, 190 μL). The reaction was initiated by addition of carbonyl reductase (10 μL solution containing 0.5–20 μg of enzyme); 1 U is defined as the enzyme converting 1 μmol of NADPH to NADP$^+$ per minute with α-hydroxyacetophenone as the substrate. [c] The configuration was determined by comparison of the HPLC retention times with those of standard samples; chiral HPLC analyses were performed on a Chiralcel OD-H column.

Results and Discussion

When the reductases available in our laboratory were screened by using α-hydroxy acetophenone as the substrate, CMCR showed high activity and enantioselectivity (Table 1). The reactivity and enantioselectivity of CMCR for the reduction of phenyl-substituted α-hydroxy acetophenones and 1-hydroxy-2-pentanone were then studied (Figure 1).



a: R = C_6H_5, b: R = 4'-Me-C_6H_4, c: R = 4'-CN-C_6H_4,
d: R = 4'-F-C_6H_4, e: R = 4'-Cl-C_6H_4, f: R = 4'-Br-C_6H_4,
g: R = 4'-OMe-C_6H_4, h: R = 2'-Cl-C_6H_4, i: R = 3'-Cl-C_6H_4,
j: R = $CH_3CH_2CH_2$

Figure 1. α-Hydroxy ketones evaluated in this study.

The results are presented in Table 2. It can be seen that CMCR effectively catalyzed the reduction of various substituted α-hydroxy acetophenones. The substituent at the phenyl ring exerted some effect on the specific activity. Electron-donating substituents such as CH_3 or OCH_3 decreased the specific activity, while electron-withdrawing substituents increased the specific activity compared with unsubstituted substrate. Among the tested substrates, 4′-bromo α-hydroxy acetophenone showed the highest specific activity. This pattern is consistent with the observed activity tendency of CMCR toward the reduction of acetophenones with 4′-substituent at the phenyl group.[11] The position of the substituent at the phenyl ring also affected the enzyme activity, with activity increasing in the order of 2′ < 3′ < 4′ for the chloro group. The 2′-chloro substituent not only affected the enzyme specific activity, but also the *ee* value of the product. The *Candida parapsilosis* carbonyl reductases SCR1 and SCR3,[9e] which were used for the reduction of α-hydroxy acetophenones, showed detectable activity toward the reduction of 2′-chloro substituted substrate, but no

Table 2. Reduction of α-hydroxy ketones.

Substrate	Specific activity[a] U mg^{-1}	Conversion[b] [%]	Isolated yield[b] [%]	*ee*[c] [%]
a	3.50	99	95	99 *S*
b	0.78	99	94	99 *S*
c	3.20	99	96	99 *S*
d	3.91	99	95	99 *S*
e	3.88	99	94	99 *S*
f	4.86	99	96	99 *S*
g	0.91	99	94	99 *S*
h	< 0.01	90[d]	–	94 *S*
i	1.83	99	95	99 *S*
j	1.08	99	62	99 *S*[e]

[a] Ketone (6.25 mM), NADPH (0.40 mM) and 10% *v/v* DMSO in sodium phosphate buffer (100 mM, 190 μL). The reaction was initiated by addition of *Candida magnolia* carbonyl reductase (CMCR) (10 μL solution containing 3.5 U CMCR; 1 U is defined as the enzyme converting 1 μmol of NADPH to NADP$^+$ per minute with α-hydroxyacetophenone as the substrate). [b] For the procedure for enzymatic reduction of α-hydroxy ketones, see the Experiment Section. [c] The configuration was determined by comparison the HPLC retention times with those of standard samples and reported in the literature;[2a,13] chiral HPLC analyses were performed on a Chiralcel OD-H column. [d] Sodium phosphate buffer (1 mL, 100 mM, pH 6.5) containing 10% *v/v* DMSO, 5 mM substrate, 10 mM glucose, 1 mM NADP$^+$, 7 U CMCR and 4 U GDH (1 U is defined as the enzyme converting 1 μmol of NADP$^+$ to NADPH per minute with D-glucose as the substrate) was shaken for 24 h. The reaction mixture was extracted with 1 mL EtOAc, and the extract was analyzed by chiral HPLC. [e] The configuration was determined by the sign of the optical rotation.

detectable activity when the substituent was OCH_3 in the 4′-position. CMCR also exhibited high activity toward the α-hydroxy aliphatic ketone (**j**). 1-Hydroxy-2-pentanone was reduced to (*S*)-pentane-1,2-diol, which is the key intermediate of triazole fungicide propiconazole.[12]

The substituent at the α-position of acetophenone also dramatically affected the specific activity. When the α-position was substituted by hydroxy group, CMCR showed nearly 100-fold higher (3.50 U mg^{-1}) than the unsubstituted acetophenone (0.04 U mg^{-1}). When the α-substituent was Cl (0.78 U mg^{-1}), Br (0.19 U mg^{-1}) or CN (0.11 U mg^{-1}), the substituent showed positive effect but less than an OH group. The *ee* values of the products all reached 99%.[11,14]

Considering the low solubility of the aromatic substrates in aqueous solution, methanol, ethanol, isopropanol, tetrahydrofuran (THF), 1,4-dioxane, dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), ethyl acetate, butyl acetate, acetone, toluene were screened as cosolvent for the reduction of α-hydroxy acetophenone by CMCR. The results showed that in the reaction media with DMSO or butyl acetate as the cosolvent, α-hydroxy acetophenone was completely reduced in 6 h, while the conversions with other cosolvents were not finished as detected by TLC analysis. As such, DMSO was chosen as the cosolvent for the reductions of various phenyl-substituted α-hydroxy acetophenones on a 50 mL scale with a substrate concentration of 50 mM (except 1-hydroxy-2-pentanone with a concentration of 100 mM). The glucose dehydrogenase (GDH) from *Bacillus subtilis* was used for the regeneration of NADPH. As shown in Table 2, the (*S*)-enantiomers of the corre-

sponding vicinal diol products were isolated in greater than 94% yield and 99% *ee* after reaction for 12 h. DMSO was also used as cosolvent for the effective reduction of ethyl 2-oxo-4-phenylbutyrate.[15]

Using *Escherichia coli* cells coexpressing both carbonyl reductase and GDH has been proven to be a practical method for effective cofactor regeneration,[16] even in unconventional media and multistep reaction systems.[17] This method could decrease or avoid the external addition of cofactor and simplify the process. In order to demonstrate the applicability of the asymmetric bioreduction of α-hydroxy acetophenones for the synthesis of optically pure (*S*)-configured vicinal diols, CMCR and GDH genes were coexpressed in *E. coli* strain BL21(DE3). The functional expression of both CMCR and GDH genes was confirmed by measuring their activities in cell-free extract, which were 1160 U (1 U was defined as the enzyme converting 1 μmol of NADPH to $NADP^+$ per minute with α-hydroxyacetophenone as the substrate) and 540 U (1 U was defined as the enzyme converting 1 μmol of $NADP^+$ to NADPH per minute with D-glucose as the substrate) per gram of lyophilized cells, respectively. The lyophilized cells as the catalyst were reported,[16c,d,f] and in our experiment, the lyophilized cells were stable for months at 4 °C without loss of activity and used as the biocatalyst.

By monitoring the conversion at different pH by using TLC, it was found that when the reaction mixture was maintained at pH 6.0, the substrate could be completely reduced in 6 h, while at other pH the substrate was still present in the reaction mixture after 6 h. As such, pH 6.0 was chosen for subsequent reactions. The results encouraged us to optimize the substrate concentration. The reduction of α-hydroxy acetophenone was conducted at a substrate concentration of 0.5, 0.75 and 1.0 M. For the reduction of α-hydroxy acetophenone at 0.5 M by 16 mg mL^{-1} of lyophilized whole cells coexpressing CMCR (93 U) and GDH (43 U), the reaction was completed in 6 h with addition of 1 mM $NADP^+$. Without addition of the external cofactor, the reaction was not finished until 24 h. When the quantity of lyophilized cells was increased to 32 mg mL^{-1} (196 U CMCR, 86 U GDH), the reaction was completed in 12 h without the external addition of cofactor. When the substrate concentration was enhanced to 0.75 M, the reaction was completed in 16 h. Further increase of the substrate concentration to 1.0 M resulted in incomplete reduction of α-hydroxy acetophenone with the reaction time prolonging to 24 h. However, the complete reduction of 1.0 M α-hydroxy acetophenone was achieved by using 50 mg mL^{-1} lyophilized whole cells (290 U CMCR, 135 U GDH) within 16 h without external addition of the cofactor, optically pure (*S*)-1-phenyl-1,2-ethanediol was isolated in 90% yield. The time courses for the reduction of α-hydroxy acetophenone at substrate concentrations of 0.75 M and 1.0 M with and without addition of 1 mM $NADP^+$ are presented in Figure 2. The results show that the addition of the external cofactor dramatically accelerates the initial bioreduction. In the reaction with addition of 1 mM $NADP^+$, the conversion reached 70% in the first 2 h. The biotransformation without addition of external cofactor became faster in the following 8 h. The optical purity of the product was 99% *ee* in all cases. To the best of our knowledge, this is the first example of the bioreduction of α-hydroxy acetophenone without addition of external cofactor at such high substrate concentration (136 g L^{-1}), much higher than the previous reports with substrate concentration of 1.0 g L^{-1}[9a] or 5.0 g L^{-1}.[9f] Without the external addition of the cofactor, the reaction was conducted in 50 mL, the concentration of the substrate was 1.0 M, and the reaction was could still be completed in 16 h; (*S*)-1-phenyl-1,2-ethanediol was obtained with 90% yield and 99% *ee*.

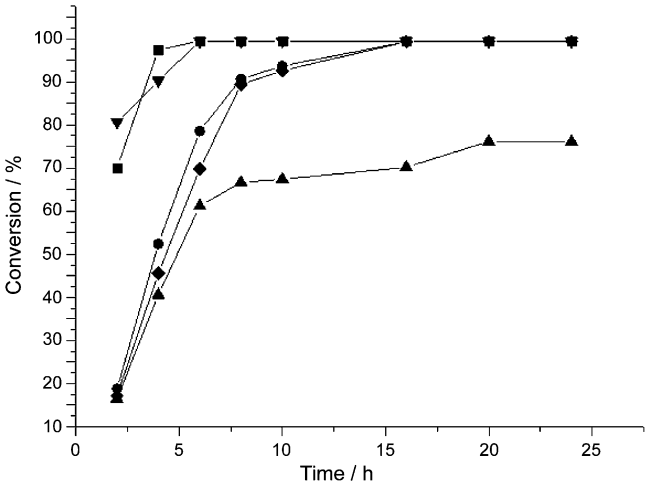


Figure 2. Reduction of α-hydroxy acetophenone at a concentration of 0.75 M (102 g L^{-1}) and 1.0 M (136 g L^{-1}) by *Escherichia coli* cells harboring pRSFDuet-1-GDH-CMCR with the addition of 0 and 1.0 mM $NADP^+$. (■) 0.75 M substrate, 1.0 mM $NADP^+$ and 32 mg mL^{-1} lyophilized cells; (●) 0.75 M substrate, 0 mM $NADP^+$ and 32 mg mL^{-1} lyophilized cells; (▲) 1.0 M substrate 0 mM $NADP^+$ and 32 mg mL^{-1} lyophilized cells; (▼) 1.0 M substrate 1.0 mM $NADP^+$ and 50 mg mL^{-1} lyophilized cells; (◆) 1.0 M substrate, 0 mM $NADP^+$ and 50 mg mL^{-1} lyophilized cells.

Conclusion

CMCR showed excellent activity and enantioselectivity toward phenyl-substituted α-hydroxy acetophenones. The electronic effect and steric factor of the substituents on the aromatic ring affected the enzyme activity, but exerted minimal effect on the enantioselectivity. As such, CMCR seemed to be a good choice of enzyme for the synthesis of optically pure (*S*)-1-phenyl-1,2-ethanediol and its analogues via the reduction of the corresponding α-hydroxy acetophenones.

To avoid addition of expensive NADPH into the reaction system, CMCR and GDH genes were coexpressed in *E. coli*. Using lyophilized recombinant *E. coli* whole cells as the biocatalyst, α-hydroxy acetophenone was reduced in 16 h at a substrate concentration of 1.0 M to give (*S*)-1-phenyl-1,2-ethanediol with 90% yield and 99% *ee*. This demonstrated for the first time that the bioreduction of α-hydroxy acetophenones can proceed smoothly at such high substrate concentration without the addition of an external cofactor, offering new opportunity for developing scalable biocatalytic processes for the synthesis of optically pure vicinal diols, a class of important chemicals for pharmaceutical, agrichemical and material industries.

Experimental Section

General: Carbonyl reductase from *Sporobolomyces salmonicolor* AKU4429 (SSCR),[18] GCY1, Ymr226c and Gre3 from *Saccharomyces cerevisiae*,[19] PFADH from *Pyrococcus furiosus*,[20] carbonyl reductase from *Candida magnolia* (CMCR) and D-glucose dehydrogenase (GDH)[21] were prepared as described previously. Substituted α-hydroxy acetophenones and 1-hydroxy-2-pentanone were prepared and purified according to the literature.[22] (*S*)-1-Phenyl-1,2-ethanediol, (*R*)-1-phenyl-1,2-ethanediol and all other ketones were purchased from commercial sources, and the cofactors were obtained from F. Hoffmann-La Roche AG. The racemic alcohol standard samples used in HPLC analysis were prepared by reduction of the corresponding ketones with $NaBH_4$. $NaBH_4$ (2 mmol) was added into the ketone (1 mmol) solution in MeOH (10 mL). The reaction mixture was stirred for 30 min at rt. After the solvent was removed, saturated NH_4Cl solution (10 mL) was added, and the aqueous phase was extracted with ethyl acetate to give the solution of the product. The substrate conversion and product *ee* values were determined by gas chromatography (GC) analysis using a CP-Chirasil-DEX CB (Varian, USA) after trimethylsilyl (TMS) derivatization (carried out by addition of anhydrous EtOAc (700 μL) and bis(trimethylsilyl)trifluoroacetamide (90 μL) + trimethyl chlorosilane (10 μL) at 60 °C for 1 h, or HPLC analysis using a Chiralcel OD-H column (4.6×250 mm; Daicel Co., Japan). Enzyme activities toward the reduction of ketones were assayed using a SpectraMax M2 microplate reader (Molecular Devices). The ^{1}H NMR spectra were measured on a Brucker Avance 600 spectrophotometer using $CDCl_3$ as the solvent. When the reaction was conducted, $NADP^+$ not NADPH was added, because NADPH could be regenerated by GDH and D-glucose.

Activity assay of carbonyl reductase for the reduction of α-hydroxy acetophenone: The specific activity of purified carbonyl reductase SSCR, GCY1, Ymr226c, Gre3, PFADH, CMCR toward the reduction of α-hydroxy acetophenone in Table 1 and Table 2 were determined by spectrophotometrically measuring the oxidation of NADPH at 340 nm ($\varepsilon = 6.22\ mM^{-1}cm^{-1}$) in the presence of an excess amount of ketone. The activity was measured at room temperature in a 96-well plate, in which each well contained ketone (6.25 mM), NADPH (0.40 mM) and 10% *v/v* DMSO in sodium phosphate buffer (100 mM, 190 μL). The reaction was initiated by the addition of the carbonyl reductase (10 μL solution containing 0.5–20 μg of enzyme). The specific activity ($U\,mg^{-1}$) was defined as the number of micromoles of NADPH converted in 1 min by 1 mg of enzyme ($\mu mol \cdot min^{-1}\,mg^{-1}$).

Screening of the cosolvent: Sodium phosphate buffer (1 mL, 100 mM, pH 6.5) containing 10% *v/v* organic solvent (methanol, ethanol, isopropanol, THF, 1,4-dioxane, DMSO, DMF, EtOAc, butyl acetate, acetone, toluene), substrate (50 mM), glucose (100 mM), $NADP^+$ (1.0 mM), CMCR (3.5 U, 1 U was defined as the enzyme converting 1 μmol of NADPH to $NADP^+$ per min with α-hydroxyacetophenone as the substrate) and GDH (4 U, 1 U was defined as the enzyme converting 1 μmol of $NADP^+$ to NADPH per min with D-glucose as the substrate) was shaken at rt. The process of the reaction was monitor by TLC every 2 h.

Enzymatic reduction of α-hydroxy ketones: A typical procedure for the enzymatic reduction of α-hydroxy ketones was as follows: a solution of α-hydroxy acetophenone in DMSO (5 mL, 0.5 M) was added to sodium phosphate buffer (100 mM, pH 6.5, 45 mL) containing CMCR (70 U), GDH (80 U), $NADP^+$ (5 mg), and D-glucose (1.0 g). The reaction mixture was stirred at 30 °C with TLC monitoring from time to time. All the reactions were finished within 6 h. After complete consumption of the substrate, the reaction mixture was saturated with solid NaCl, extracted with EtOAc (3×40 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford the optically pure product. The yields and *ee* values are presented in Table 2.

(*S*)-1-Phenyl-1,2-ethanediol [(*S*)-2 a]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.61 (dd, *J* = 8.4, 11.4.0 Hz, 1 H), 3.71 (dd, *J* = 3.0, 11.4 Hz, 1 H), 4.78 (dd, *J* = 3.6, 8.4 Hz, 1 H), 7.26–7.47 ppm (m, 5 H); Analytical HPLC: *n*-hexane/2-propanol = 97:3, flow rate = 1.0 mL min^{-1}, *T* = 30 °C, UV detection: 230 nm; t_R: 33.6 min for (*R*)-**2 a** and 36.4 min (*S*)-**2 a**.

(*S*)-1-(4-Methylphenyl)-1,2-ethanediol [(*S*)-2 b]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.34 (s, 3 H), 3.61 (dd, *J* = 5.4, 10.2 Hz, 1 H), 3.71 (dd, *J* = 6.6, 9.0 Hz, 1 H), 4.75 (dd, *J* = 5.4, 11.4 Hz, 1 H), 7.16–7.27 ppm (m, 4 H); Analytical HPLC: *n*-hexane/2-propanol = 96:4, flow rate = 0.9 mL min^{-1}, *T* = 30 °C, UV detection: 230 nm; t_R: 25.3 min for (*R*)-**2 b** and 28.4 min for (*S*)-**2 b**.

(*S*)-1-(4-Cyanophenyl)-1,2-ethanediol [(*S*)-2 c]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.61 (dd, *J* = 3.6, 9.6 Hz, 1 H), 3.74 (dd, *J* = 4.2, 7.2 Hz, 1 H), 4.75 (dd, *J* = 6.6, 9.0 Hz, 1 H), 7.16–7.27 ppm (m, 4 H); Analytical HPLC: *n*-hexane/2-propanol = 92:8, flow rate = 0.9 mL min^{-1}, *T* = 30 °C, UV detection: 254 nm; t_R: 31.9 min for (*R*)-**2 c** and 35.9 min for (*S*)-**2 c**.

(*S*)-1-(4-Fluorophenyl)-1,2-ethanediol [(*S*)-2 d]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.57 (dd, *J* = 8.4, 11.4 Hz, 1 H), 3.69 (dd, *J* = 3.6, 12.0 Hz, 1 H), 4.76 (dd, *J* = 3.6, 8.4 Hz, 1 H), 7.01–7.32 ppm (m, 4 H); Analytical HPLC: *n*-hexane/2-propanol = 96:4, flow rate = 0.9 mL min^{-1}, *T* = 30 °C, UV detection: 230 nm; t_R: 27.4 min for (*R*)-**2 d** and 30.3 min for (*S*)-**2 d**.

(*S*)-1-(4-Chlorophenyl)-1,2-ethanediol [(*S*)-2 e]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.57 (dd, *J* = 8.4, 11.4 Hz, 1 H), 3.70 (dd, *J* = 3.0, 11.4 Hz, 1 H), 4.76 (dd, *J* = 3.6, 8.4 Hz, 1 H), 7.26–7.32 ppm (m, 4 H); Analytical HPLC: *n*-hexane/2-propanol = 97:3, flow rate = 1.0 mL min^{-1}, *T* = 30 °C, UV detection: 230 nm; t_R: 34.8 min for (*R*)-**2 e** and 38.5 min for (*S*)-**2 e**.

(*S*)-1-(4-Bromophenyl)-1,2-ethanediol [(*S*)-2 f]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.58 (dd, *J* = 8.4, 11.4 Hz, 1 H), 3.72 (dd, *J* = 3.0, 10.8 Hz, 1 H), 4.77 (dd, *J* = 3.6, 7.8 Hz, 1 H), 7.01–7.32 ppm (m, 4 H); Analytical HPLC: *n*-hexane/2-propanol = 97:3, flow rate = 1.0 mL min^{-1}, *T* = 30 °C, UV detection: 230 nm; t_R: 38.6 min for (*R*)-**2 f** and 42.1 min for (*S*)-**2 f**.

(*S*)-1-(4-Methoxyphenyl)-1,2-ethanediol [(*S*)-2 g]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.61 (dd, *J* = 7.8, 10.8 Hz, 1 H), 3.69 (dd, *J* = 3.6, 11.4 Hz, 1 H), 3.80 (s, 3 H), 4.74 (dd, *J* = 3.6, 8.4 Hz, 1 H), 6.88–7.32 ppm (m, 4 H); Analytical HPLC: *n*-hexane/2-propanol = 95:5, flow rate = 0.8 mL min^{-1}, *T* = 30 °C, UV detection: 230 nm; t_R: 39.8 min for (*R*)-**2 g** and 42.2 min for (*S*)-**2 g**.

(*S*)-1-(2-Chlorophenyl)-1,2-ethanediol [(*S*)-2 h]: Analytical HPLC: *n*-hexane/2-propanol = 95:5, flow rate = 0.8 mL min^{-1}, *T* = 30 °C, UV detection: 230 nm; t_R: 18.0 min for (*S*)-**2 h** and 20.5 min for (*R*)-**2 h**.

(*S*)-1-(3-Chlorophenyl)-1,2-ethanediol [(*S*)-2 i]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.61 (dd, *J* = 7.8, 11.4 Hz, 1 H), 3.75 (dd, *J* = 3.6, 11.4 Hz, 1 H), 4.79 (dd, *J* = 3.6, 7.8 Hz, 1 H), 7.23–7.39 ppm (m, 4 H); Analytical HPLC: *n*-hexane/2-propanol = 95:5, flow rate = 0.8 mL min^{-1}, *T* = 30 °C, UV detection: 230 nm; t_R: 23.5 min for (*R*)-**2 i** and 27.2 min for (*S*)-**2 i**.

(*S*)-Pentane-1,2-diol [(*S*)-2 j]: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.94 (t, *J* = 7.2 Hz, 3 H), 1.35–1.51 (m, 4 H), 3.42 (dd, *J* = 7.8, 10.8 Hz, 1 H), 3.64 (dd, *J* = 3.0, 10.8 Hz, 1 H), 3.71—3.75 (m, 1 H); Analytical GC:

50 °C for 15 min, then 2 °C min^{-1} to 90 °C, then hold at 90 °C for 10 min; t_R: 36.1 min for (*S*)-**2j** and 36.5 min for (*R*)-**2j**; $[\alpha]_D^{25} = -13.4$ ($c = 0.4$ MeOH), $[\alpha]_D^{25} = -17.3$ ($c = 1$ MeOH).[8b] The *ee* value was determined after TMS derivation.

Coexpression of CMCR and GDH genes: The GDH gene was amplified using primers GDH-*Nco*I-F/GDH-*Hin*dIII-R and pET15b-*GDH* as template. The plasmid pRSFDuet-1-*GDH* was constructed by inserting GDH between the *Nco*I and *Hin*dIII sites of pRSFDuet-1 (Novagen). The CMCR gene was amplified using forward primer CMCR-*Nde*I-F and reverse primer CMCR-*Xho*I-R and pET21b-*CMCR* as template and then ligated into pRSFDuet-1-GDH, to generate the recombinant plasmid pRSFDuet-1-GDH-CMCR (Figure 3).The recombinant plasmid pRSFDuet-1-GDH-CMCR was transformed into *E. coli* BL21(DE3). The recombinant plasmid pRSFDuet-1-GDH-CMCR was transformed into the *E. coli* BL21(DE3) for coexpression of the GDH and CMCR. Single colony was picked into 4 mL Luria–Bertani (LB) medium supplemented with 50 μg mL^{-1} kanamycin at 37 °C and 200 rpm for 10–12 h. Then 1.0% seed was inoculated into 2 L flasks containing 800 mL of LB medium, and grown at 37 °C, 200 rpm until the OD_{600} reached 0.8–1.0, 1.0 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce GDH and CMCR expression at 25 °C, 200 rpm for 12 h. Cells were harvested by centrifugation at 4 °C and 5000 rpm for 15 min.

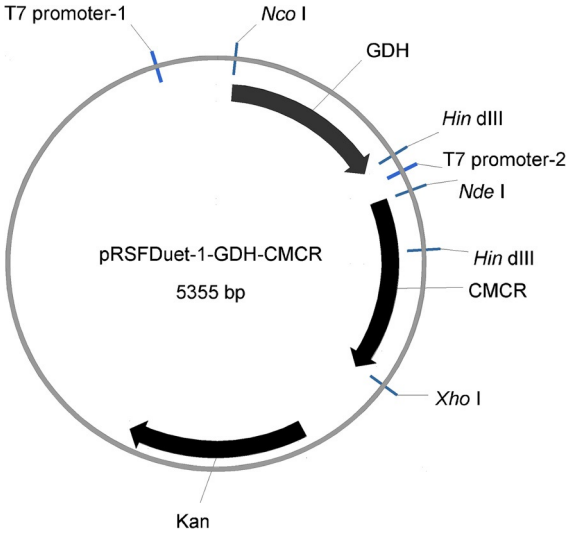


Figure 3. Schematic representation of the recombinant plasmid pRSFDuet-1-GDH-CMCR used in this study.

Effect of pH on the reduction of α-hydroxy acetophenone by lyophilized whole cells: A solution of α-hydroxy acetophenone in DMSO (0.5 mL, 5 M) was added to sodium phosphate buffer (100 mM, 4.5 mL) containing lyophilized whole cells coexpressing CMCR (93 U) and GDH (43 U), $NADP^+$ (1.0 mM), and D-glucose (1.1 M). The reaction mixture was stirred at 30 °C, and the pH of the reaction was maintained at the appropriate level (6.0, 6.5, 7.0 or 7.5) by automatically adding 4 M aq NaOH. The reaction was monitored by TLC analysis every 2 h.

Reduction of α-hydroxy acetophenone to (*S*)-1-phenyl-1,2-ethanediol by lyophilized cells: A solution of α-hydroxy acetophenone in DMSO (0.5 mL, 5 M, 7.5 M or 10 M) was added to sodium phosphate buffer (100 mM, 4.5 mL) containing the desired amount of lyophilized whole cells coexpressing CMCR and GDH, $NADP^+$ (0 mM or 1.0 mM), and D-glucose (1.1 M, 1.65 M or 2.22 M). The reaction mixture was stirred at 30 °C, and the pH of the reaction was maintained at 6.0 by automatically adding 4 M aq NaOH. The reaction was monitored by HPLC analysis. The results are summarized in Figure 2.

Preparation of (*S*)-1-phenyl-1,2-ethanediol by lyophilized cells: A solution of α-hydroxy acetophenone in DMSO (5 mL, 10 M) was added to sodium phosphate buffer (100 mM, 45 mL) containing the desired amount of lyophilized whole cells coexpressing CMCR and GDH (2900 U CMCR, 1350 U GDH), and D-glucose (2.22 M). The reaction mixture was stirred at 30 °C, and the pH of the reaction was maintained at 6.0 by automatically adding 4 M aq NaOH. The substrate was completely consumed after 16 h, and the reaction mixture was then centrifuged (5000 *g* for 5 min at rt). The solid was washed with EtOAc (2×20 mL), and the supernatant was saturated with solid NaCl and extracted with EtOAc (3×40 mL). The combined organic extracts were washed with saturated aqueous NaCl solution (40 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford optically pure (*S*)-1-phenyl-1,2-ethanediol (6.2 g, 90% yield).

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Keywords: asymmetric synthesis · biocatalysis · bioreductions · carbonyl reductases · stereochemistry · vicinal diols

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