Highly Enantioselective Reduction of Carbonyl Compounds Using a Reductase Purified from Bakers' Yeast

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An NADPH-dependent reductase that shows reducing activity for 1-chloro-2-hexanone has been purified from bakers' yeast. SDS−PAGE and gel filtration suggested that the purified reductase is a monomeric enzyme with a molecular weight of ca. 37 kDa. Asymmetric reduction of several carbonyl compounds using the purified reductase has been carried out. 1-Chloro-2-hexanone, 1-acetoxy-2-heptanone, methyl acetoacetate, ethyl pyruvate, 1-chloro-2,4-pentanedione, and 2,4-hexanedione were reduced to the corresponding alcohols with high enantiomeric purities (>98% ee). The reductase showed high specificity constants ($k_{cat}/K_m = 10^3-10^5$ s^{-1} M^{-1}) and relatively low Michaelis constants ($K_m = 10^{-4}-10^{-3}$ M) for all the substrates examined.

Introduction

Asymmetric synthesis with biocatalysts is becoming more and more important because of its great potential in environmentally benign organic syntheses. Among many kinds of transformations with biocatalysts,[1] asymmetric reduction of carbonyl compounds with microorganisms such as bakers' yeast (BY, *Saccharomyces cerevisiae*)[2] is a traditional and useful method for the preparation of optically active alcohols.[3] Although the BY whole-cell reduction is inexpensive and convenient, stereoselectivity is often incomplete due to several oxidoreductases contained in the BY cells. On the other hand, the enzymatic reduction using a purified reductase usually shows high stereoselectivity.[4−7] Complete stereoselectivity, as frequently observed in enzymatic reactions, is one of the requirements for modern asymmetric synthesis. The problem that the purification of an enzyme is laborious can be eliminated, once the method for producing the enzyme on a large scale using the cloning technique has been established.[8] Since many enzymes whose substrate specificity is not strict are currently known,[1] it is an important task of synthetic chemists to develop the potential ability of a purified enzyme by applying a variety of multifunctional substrates. In this paper, we report the highly enantio- and regioselective reduction of several carbonyl compounds using an NADPH-dependent reductase purified from BY.

Results

Purification of the Reductase. We decided to purify the enzyme that shows reducing activity toward 1-chloro-2-hexanone (**1**), because the enantioselectivities in the BY whole-cell reduction of a series of 1-chloro-2-alkanones were only moderate.[9] The enzyme assay for the cell-free extract (CFE) of BY using UV spectroscopy revealed that the reductase requires NADPH as a coenzyme. The reductase in the CFE was successfully purified to homogeneity by hydrophobic chromatography and ion-exchange chromatography. The detailed procedure is described in the Experimental Section. After the final chromatographic separation, only a single band (37 kDa) was detected by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and by gel filtration, suggesting that the reductase is a monomeric enzyme.[10]

Asymmetric Reduction with the Purified Reductase. We carried out the enzymatic reduction of carbonyl

(1) (a) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (b) Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695. (c) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114. (d) *Preparative Biotransformations: Whole Cell and Isolated Enzymes in Organic Synthesis*; Roberts, S. M., Wiggins, K., Casy, G., Eds.; John Wiley & Sons: Chichester, 1993. (e) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994. (f) *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; VCH: New York, 1994; Vol. 1. (g) Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Berlin, 1995.

(2) Abbreviations used in this paper: BSA, bovine serum albumin; BY, bakers' yeast; CFE, cell-free extract; GL6P, glucono-δ-lactone-6-phosphate; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; MES, 2-(*N*-morpholino)ethanesulfonic acid; $NADP^+$, β-nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of $NADP^+$; SDS−PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TTN, total turnover number.

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(7) Ishihara, K.; Nakajima, N.; Tsuboi, S.; Utaka, M. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 3314.

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(9) Sakai, T.; Wada, K.; Murakami, T.; Kohra, K.; Imajo, N.; Ooga, Y.; Tsuboi, S.; Takeda, A.; Utaka, M. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 631.

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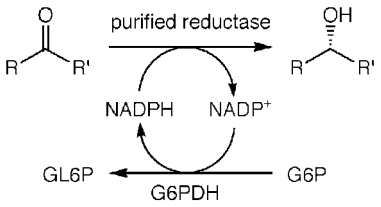
Scheme 1[a]



[a] For abbreviations, see ref 2.

compounds **1**−**7** using the purified reductase. To regenerate a catalytic amount of NADPH in situ, the glucose-6-phosphate (G6P)/glucose-6-phosphate dehydrogenase (G6PDH) system[11] was employed (Scheme 1). It was confirmed by UV spectrophotometric analysis that G6PDH has little or no reducing activity for **1**−**7** in the presence of NADPH. The results of the enzymatic reduction of **1**−**7** are summarized in Table 1, where the stereochemistry of the products is drawn in such a way that the β (front) face of the carbonyl group in the substrates is attacked by NADPH. The results of the whole-cell reduction of **1**−**7** are also shown in Table 1 for comparison. The obtained alcohols are useful for organic synthesis. For example, alcohols **9** and **13** are chiral building blocks for prostaglandins[12] and nonactin,[13] respectively.

The reductase successfully reduced ketones **1**−**4** to the corresponding alcohols **8**−**11** with high enantiomeric purities (>98% ee). In the cases of 1-substituted-2,4-pentanediones **5** and **6**, the more hindered but more activated carbonyl group was regioselectively reduced, in contrast to 2,4-hexanedione (**7**) undergoing the reduction at the less hindered carbonyl (acetyl) group. The absolute configuration of alcohols **8** and **12** was found to be *R*, which is opposite to the *S*-configuration of other alcohols.

Kinetic Parameters. We determined the kinetic parameters of the reductase for **1**−**7** in order to evaluate the capacity of the reductase as a catalyst from a physicochemical viewpoint. The number of the kinetic parameters so far determined and evaluated for enantioselective enzymatic reactions toward nonnatural substrates is limited.[5−7] The initial rates v_0 at different substrate concentrations $[S]_0$ were measured by monitoring the decrease in the absorbance of NADPH at 340 nm. The plot of v_0 versus $[S]_0$ afforded a typical saturation curve. The apparent k_{cat} and K_m values were calculated using the nonlinear least-squares method assuming the Michaelis−Menten equation. The results are listed in Table 2.

Discussion

The reductase presented in this paper showed excellent stereoselectivity toward several nonnatural substrates. The enzymatic reduction showed higher stereoselectivity than the whole-cell reduction. In particular, the high enantioselectivity in the enzymatic reduction of **1** encouraged us, because any attempt to improve the enantioselectivity in the BY whole-cell reduction of **1** has been unsuccessful so far.[9,14] The reductase exhibited several interesting features as shown below.

Table 1 suggests that the purified reductase is one of the enzymes contributing to Prelog's rule, the empirical rule proposed for the microbial reductions of carbonyl compounds;[15] the right-hand moiety of each structure is bulkier than the left-hand moiety (see Table 1), and the β face of the carbonyl group (the Si face in the cases of **1** and **5**, and the Re face in the cases of the other substrates) is attacked by NADPH. We suppose that the right-hand moiety of the substrates is either accommodated in a binding pocket of the reductase by attractive interactions or disfavored by some steric repulsion. In the case of the polyfunctional ketone **6**, the steric bulkiness and the polarity of the acetoxy group (the right-hand moiety) are very similar to those of the acetyl group (the left-hand moiety). Therefore, it is conceivable that **6** has two types of binding modes, with the Re or Si face of the carbonyl group directed toward NADPH in some ratio. Such ambiguous recognition of the enantioface may be responsible for the exceptionally low enantioselectivity for **6**. Ketones **2**−**4**, **6**, and **7** were reduced to the corresponding (*S*)-alcohols, whereas chlorine-containing substrates **1** and **5** were reduced to the corresponding (*R*)-alcohols. This difference in the absolute configuration may be convenient for organic synthesis, because derivatives of both enantiomers of 1,2-diol can be prepared by the choice of the α substituent (Cl or OAc).[3c]

Another interesting observation is the regioselectivity for β-diketones **5**−**7**. It is known that β-diketones undergo the BY reduction selectively at the less hindered carbonyl group.[16] The purified reductase has the ability to completely recognize the steric difference between methyl and ethyl groups of **7**. This regiospecificity is impressive when it is compared, for example, to the result that the reduction of **7** with $NaBH_4$ in water showed little regioselectivity even at 0 °C. Furthermore, the regioselectivity of the reductase was changed when 1-substituted-2,4-pentanediones **5** and **6** were used (Table 1), indicating that in this case the chemical reactivity of the functional groups dominates over the steric effect.[17]

In the enzymatic reduction of **1**, the isolated yield of **8** was initially very low under our standard reaction conditions. By adding a small amount of bovine serum albumin (BSA) to the reaction mixture according to the report of Nakamura et al.,[6a] the yield was significantly improved. The amino acid residues at the active sites of the enzymes may be susceptible to functionalization by the potential alkylating agent **1**, which could have been suppressed by BSA. In the enzymatic reduction of **5**, a large amount (51%) of furanone **15** was obtained, although the enantiomeric purity of alcohol **12** was high. Control experiments revealed that both the reductase and

(10) The facts that the purified reductase showed high activity for 1-acetoxy-2-alkanones and that its molecular weight determined by SDS−PAGE and gel filtration is ca. 37 kDa strongly suggest that it is identical with the α-acetoxy ketone reductase (AcKR) previously reported by us.[7] We have suggested that AcKR is identical with L-enzyme-2,[7] which is one of the four β-keto ester reductases isolated by Nakamura et al.[6a]

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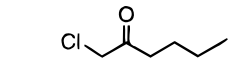
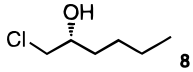
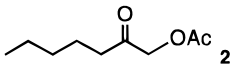
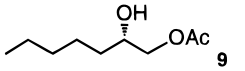
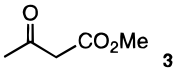
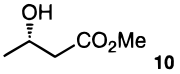
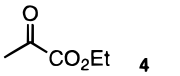
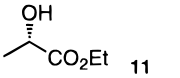
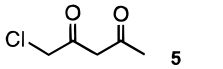
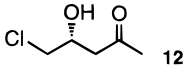
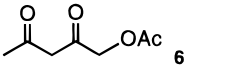
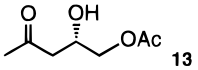
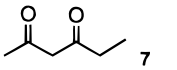
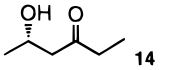
Table 1. Asymmetric Reduction of Carbonyl Compounds Using the Purified Reductase and BY Whole Cells

reduction		purified reductase[a]			whole cells[b]		
substrate	product	time h	% yield[c] (% ee)	*R/S*	time h	% yield[c] (% ee)	*R/S*
 **1**	 **8**	8	64 (>99)[d]	*R*	4	74 (80)[e]	*R*
 **2**	 **9**	4	80 (98)	*S*	4	52 (96)[f]	*S*
 **3**	 **10**	5	64 (>99)	*S*	5	49 (97)	*S*
 **4**	 **11**	6	41 (>99)	*S*	1	65 (93)[g]	*S*
 **5**	 **12**	3	19 (>99)[h]	*R*	2	34 (54)[i]	*S*
 **6**	 **13**	15	65 (68)	*S*	4	33 (65)[j]	*S*
 **7**	 **14**	16	69 (>99)	*S*	8	35 (98)[k]	*S*

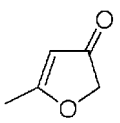
[a] Conditions: substrate (0.25 mmol), purified reductase (1.5 U with respect to **1**), $NADP^+$ (2.5 μmol), G6PDH (22 U), G6P (0.29 mmol), 10 mM phophate buffer (pH 7.0, 25 mL), 30 °C. [b] Conditions: substrate (1.0 mmol), pressed bakers' yeast (6 g), glucose (2 g), water (60 mL), 30 °C. [c] Isolated yield. [d] BSA (2 mg) was added as an additive. [e] The values reported in ref 9 (with immobilized BY) are 69 (80).[f] The values reported in ref 7 (with immobilized BY) are 51 (99). [g] The values reported in ref 28 are 47 (91). [h] BSA (2 mg) was added as an additive. A large amount (51%) of furanone **15** was obtained. [i] The values reported in ref 17 (with dry BY) are 53 (29, *S*). [j] The values reported in ref 24 (with dry BY) are 42 (82). [k] The values reported in ref 16a are 48 (97).

Table 2. Kinetic Parameters of the Purified Reductase[a]

substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m
1	6.9 ± 0.5	$(2.1 \pm 0.5) \times 10^{-3}$	3.3×10^3
2	$(4.1 \pm 0.1) \times 10$	$(3.2 \pm 0.2) \times 10^{-4}$	1.3×10^5
3	$(1.0 \pm 0.04) \times 10$	$(8.0 \pm 0.9) \times 10^{-4}$	1.3×10^4
4	1.2 ± 0.09	$(3.3 \pm 0.9) \times 10^{-4}$	3.6×10^3
5	3.4 ± 0.2	$(3.2 \pm 0.4) \times 10^{-3}$	1.1×10^3
6	$(1.5 \pm 0.06) \times 10$	$(4.0 \pm 0.5) \times 10^{-3}$	3.8×10^3
7	$(1.0 \pm 0.1) \times 10$	$(7.1 \pm 1.4) \times 10^{-3}$	1.4×10^3

[a] Measurement conditions: reductase (2.8×10^{-8} or 6.3×10^{-8} M), NADPH (3.0×10^{-4} or 3.4×10^{-4} M), substrate concentration range (substrate) = 0.7−14 mM (**1**), 0.008−0.8 mM (**2**), 1.5−15 mM (**3**), 0.07−6 mM (**4**), 0.7−14 mM (**5**), 0.4−16 mM (**6**), 1.8−12 mM (**7**), 10 mM phosphate buffer (pH 7.0), 30 °C. The kinetic parameters were calculated by means of the nonlinear least-squares method applied to the Michaelis−Menten equation: $v_0 = k_{cat}[E]_0[S]_0/(K_m + [S]_0)$.

the buffer are responsible for the cyclization of **5**.[18]



15

The total turnover number (TTN)[1e] for NADPH in the enzymatic reduction is ca. 100 at 100% conversion, and the TTN roughly estimated for the enzyme is ca. 29 000.[19] The former value is lower than that reported for membrane−reactor systems (TTN = 10^3−10^5).[20] The latter value is also lower than that observed for other enzymatic reductions toward natural substrates (TTN = 10^6−10^7)[11] and is higher than that for the lipase-catalyzed enantioselective transesterifications of secondary alcohols in an organic solvent (TTN = ca. 5000).[21] The specificity constants, k_{cat}/K_m, of the reductase (Table 2) amount to the order of 10^3−10^5 s^{-1} M^{-1}. Despite the reactions toward nonnatural substrates, the specificity constants are high and are comparable to those of other enzymes for natural substrates; recall that the rate constant for the diffusion-controlled bimolecular association in water is 10^9 s^{-1} M^{-1} and that the specificity constants of most of the enzymatic reactions for natural substrates are in the range of 10^4−10^8 s^{-1} M^{-1}.[22] The results that the reductase showed relatively low K_m values for all the substrates examined may suggest broad substrate specificity of the reductase, which is advantageous for organic synthesis. The apparent binding energies approximately calculated from the equation $\Delta G° = RT \ln K_m$ range from −3.0 to −4.8 kcal/mol. The ample binding energy of the reductase for **1**−**7** may be used to recognize the enantioface of the carbonyl group, because the differential activation energy required for 98% ee can be calculated to be −2.8 kcal/mol.[23] These considerations confirm that the reductase is an excellent catalyst for asymmetric reduction.

(18) Control experiments were done without NADP(H), G6PDH, and G6P in 10 mM phosphate buffer (pH 7.0). Conversions of **5** to **15** in the presence and absence of the reductase (1.5 U with respect to **1**) after 4 h were 82% and 54%, respectively.

(19) The weight of the reductase used in the preparative reduction was ca. 0.32 mg as determined by the method of Bradford. Because the molecular weight of the reductase is 37 000, the amount of reductase used is 8.6 nmol.

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(22) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman: New York, 1985.

Experimental Section

General. ^{1}H NMR spectra were measured in $CDCl_3$ at 500 or 200 MHz. Silica gel column chromatography was carried out using Fuji Silysia BW-127 ZH (100−270 mesh), and TLC was performed on Merck silica gel 60 F_{254}. Butyl-Toyopearl 650M, Phenyl-Toyopearl 650M, and DEAE-Toyopearl 650M were purchased from Tosoh Co. The ultrafiltration membrane (10-kDa cutoff) and the seamless cellulose tubing for dialysis were purchased from Advantec and Viskase Co., respectively. G6PDH (from BY, lyophilized powder) and the molecular weight markers for SDS−PAGE (lysozyme (14 kDa), β-lactoglobulin (18 kDa), trypsinogen (24 kDa), ovalbumin (45 kDa), albumin (66 kDa)) were purchased from Sigma. The pressed BY, NADPH, $NADP^+$, and G6P were purchased from Oriental Yeast Co., Ltd. The substrates **1**,[9] **2**,[7] **5**,[17] **6**,[24] and **7**[25] were prepared according to the literature. The reduction products **8**,[9] **9**,[26] **10**,[27] **11**,[28] **12**,[17] **13**,[24] and **14**[16a] were characterized according to the literature.

Enzyme Assay. A chromatographic fraction (300 μL) was added to a solution (2.4 mL) of 1-chloro-2-hexanone (**1**) (4.8 mM) and NADPH (0.33 mM) in 10 mM MES buffer (pH 6.0) in a UV cuvette thermostated at 25 °C. After the solution was quickly shaken, the reaction rate was measured by following the decrease in the absorbance of NADPH at 340 nm as a function of time. In this paper, 1.0 U of the enzymatic activity is defined as the amount of enzyme that oxidizes 1.0 μmol of NADPH/min at 25 °C under the reaction conditions indicated above. The amount of proteins was determined by the method of Bradford using BSA as the standard.[29]

Kinetic Parameters. An aliquot (200 or 500 μL) of the purified reductase (0.37 μM) in 10 mM phosphate buffer (pH 7.0) was added to a solution (2.4 mL) of substrate and NADPH (0.37 mM) in the phosphate buffer in a UV cuvette; the substrate concentration range (substrate) = 0.7−14 mM (**1**), 0.008−0.8 mM (**2**), 1.5−15 mM (**3**), 0.07−6 mM (**4**), 0.7−14 mM (**5**), 0.4−16 mM (**6**), and 1.8−12 mM (**7**). After the solution was magnetically stirred for 30 s in a UV cell compartment thermostated at 30 °C, the initial rate v_0 was measured by monitoring the decrease in the absorption at 340 nm as a function of time. The initial rates v_0 were measured at different substrate concentrations $[S]_0$. Plot of v_0 versus $[S]_0$ afforded the saturation curve. The apparent k_{cat} and K_m values were calculated using the nonlinear least-squares method assuming the Michaelis−Menten equation: $v_0 = k_{cat}[E]_0[S]_0/(K_m + [S]_0)$.

Purification of the Reductase. Raw pressed BY (40 g) suspended in 0.1 M MES buffer (pH 6.0, 40 mL) was homogenized by glass beads (0.5 mm in diameter, 60 mL) with a cell mill (Vibrogen Co.) chilled with ice. This procedure was repeated eight times (BY totally 320 g). The homogenate was centrifuged at 12 000 rpm for 30 min at 4 °C. To the supernatant CFE (360 mL) obtained by decantation was added $(NH_4)_2SO_4$ (52 g, 30%). After the solution was allowed to cool in an ice bath for 45 min, the solution was centrifuged at 12 000 rpm for 30 min at 4 °C. All the purifications by column chromatography that are described below were carried out at 4 °C. The supernatant solution was applied on a Butyl-Toyopearl column (ϕ 4 × 12 cm) equilibrated with 10 mM MES buffer (pH 6.0) containing 1 M $(NH_4)_2SO_4$. The proteins were eluted with the MES buffer using a stepwise gradient of $(NH_4)_2SO_4$ (from 1 to 0.6 M, 150 mL each). The elution pattern of the proteins was examined by the absorbance at 280 nm. The active fractions were detected by the enzyme assay method described above. The active fractions (170 mL) eluted at 0.9−0.8 M $(NH_4)_2SO_4$ were then combined. After the concentration of $(NH_4)_2SO_4$ in the enzyme solution was adjusted to ca. 1.2 M by adding $(NH_4)_2SO_4$, the enzyme solution was applied on a Phenyl-Toyopearl column (ϕ 3 × 8 cm) equilibrated with 10 mM MES buffer (pH 6.0) containing 1.2 M $(NH_4)_2SO_4$. The proteins were eluted with the MES buffer using a stepwise gradient of $(NH_4)_2SO_4$ (from 1.2 to 0.8 M, 125 mL each). The active fractions (160 mL) eluted at 0.9 M $(NH_4)_2SO_4$ were then combined. After the concentration of $(NH_4)_2SO_4$ in the enzyme solution was adjusted to ca. 1.0 M by adding $(NH_4)_2SO_4$, the enzyme solution was applied on a Butyl-Toyopearl column (ϕ 2.5 × 8 cm) equilibrated with 10 mM MES buffer (pH 6.0) containing 1 M $(NH_4)_2SO_4$. The proteins were eluted with the MES buffer using a stepwise gradient of $(NH_4)_2SO_4$ (from 1 to 0.6 M, 125 mL each). The active fractions (75 mL) eluted at 0.8 M $(NH_4)_2SO_4$ were combined and concentrated to 20 mL by ultrafiltration. After the solution was dialyzed against 10 mM phosphate buffer (pH 7.0, 250 mL × 4, 1000 mL × 2), the solution was applied on a DEAE-Toyopearl column (ϕ 2.5 × 5 cm) equilibrated with the phosphate buffer. The proteins were eluted with the phosphate buffer using a stepwise gradient of NaCl (from 0 to 0.05 M, 100 mL each). The active fractions (32 mL) eluted at 0.04 M NaCl were combined. Only a single band was detected by SDS−PAGE (12.5%) around ca. 37 kDa as estimated by using the molecular weight markers. These results are summarized in Table 3. Gel filtration using a TSK-GEL G3000 SW column (Tosoh Co.) (0.1 M phosphate buffer (pH 7.0, 0.1 M Na_2SO_4), flow rate 0.5 mL/min, detection at 280 nm) showed a single peak at ca. 37 kDa as estimated by using the molecular weight markers. The reductase thus purified was used for the enzymatic reduction of carbonyl compounds **1**−**7** and for the determination of the kinetic parameters.

Table 3. Purification of the Reductase

purification step	total activity[a] (U)	total proteins[b] (mg)	specific activity (U/mg)	yield (%)
CFE	35.6	3800	0.0094	100
butyl	23.2	567	0.041	65
phenyl	15.3	85.6	0.18	43
butyl	12.5	13.8	0.91	35
DEAE	3.47	1.18	2.9	10

[a] Determined by the spectrophotometric enzyme assay method described in the Experimental Section. One unit of enzymatic activity is defined as the amount of enzyme that oxidized 1.0 μmol of NADPH/min at 25 °C. [b] Determined by the method of Bradford.[29]

General Procedure for Enzymatic Reduction. To a solution of the purified reductase (1.5 U with respect to **1**), $NADP^+$ (2.1 mg, 2.5 μmol), G6PDH (100 μg, 22 U), and G6P (100 mg, 0.29 mmol) in 10 mM phosphate buffer (pH 7.0, 25 mL) was added the substrate (0.25 mmol). The mixture was stirred with a magnetic stirrer in a water bath thermostated at 30 °C for the appropriate reaction time. The progress of the reaction was monitored by TLC. After the solution was saturated with NaCl, the crude product was extracted with ethyl acetate or ether (15 mL × 4). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography or distillation under reduced pressure to give the product.

General Procedure for BY Whole-Cell Reduction. To bakers' yeast (6.0 g) suspended in water (60 mL) was added glucose (2.0 g). After the mixture was stirred with a magnetic stirrer at 30 °C for 30 min, the substrate (1.0 mmol) was added to the mixture. The reaction mixture was stirred at 30 °C for the appropriate reaction time. After Celite (13 g) was added to the reaction mixture, the mixture was filtered through Celite. The Celite was washed with acetone (6 mL) and then with ethyl acetate or ether (20 mL × 3). The filtrate was

(23) The ratio of the rate constants for parallel reactions is equal to the ratio of the amounts of two products: $E = (k_{cat}/K_m)^S/(k_{cat}/K_m)^R = S/R$. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294. The above equation can be transformed to the equation $\Delta\Delta G^{\ddagger} = -RT \ln E = -RT \ln [(1 + ee(P))/(1 - ee(P))]$.

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saturated with NaCl. After the organic phase was separated, the crude product was extracted with ethyl acetate or ether (40 mL × 3). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography or distillation under reduced pressure to give the product.

Determination of Enantiomeric Purity and Absolute Configuration. The enantiomeric purities of alcohols **9** and **13** were determined by ^{1}H NMR spectroscopy after the alcohols were converted to the MTPA esters.[30] The enantiomeric purities of alcohols **8**, **10** (acetate derivative), **11**, **12**, and **14** (acetate derivative) were determined by gas chromatography with a CP-cyclodextrin-β-2,3,6-M-19 capillary column (Chrompack Co.). The absolute configurations of alcohols **8**−**14** were determined by comparison with the signs of the reported specific rotations or with the retention time of authentic samples by gas chromatography.

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Supporting Information Available: Spectroscopic data for the optically active alcohols **8**−**14**, copies of ^{1}H NMR spectra for **8**−**14**, $[S]_0-v_0$ plots for **1**−**7**, and results of SDS−PAGE and gel filtration (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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