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Microbial asymmetric reduction of α-hydroxyketones in the anti-Prelog selectivity

Toshikuni Tsujigami, Takeshi Sugai and Hiromichi Ohta*

Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

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Abstract—*Yamadazyma farinosa* IFO 10896 was found to reduce α-hydroxyketones bearing a phenyl ring to give optically active diols with anti-Prelog selectivity. The distance between the carbonyl group and the phenyl ring was shown to have an interesting effect on the reactivity and selectivity of the enzyme system.

1. Introduction

The most commonly used biocatalyst in asymmetric reduction is baker's yeast. This microorganism is commercially available, easy to handle, and able to be used in the asymmetric reduction of wide range of carbonyl functionalities and carbon–carbon double bonds.[1] Recently, the scope of biotransformation seems to be expanding increasingly wider, as the whole cell biocatalysts have been shown to be used in solvents other than aqueous medium. For example Nakamura et al. showed that baker's yeast can be used in organic solvents[2] and resting cells of *Geotricum* were demonstrated to work well in supercritical carbon dioxide by Matuda et al.[3] Moreover, microbial reduction can be performed on a large scale so that it can be applied in an industrial process by utilizing an engineered microorganism[4] and designing the reaction conditions.[5] In this way, it is desirable to find many kinds of microorganisms which have their own characteristics in their substrate specificity and selectivity to fill the variety of demands from asymmetric synthesis. Although baker's yeast has established to have plural reducing enzymes,[6,7] it generally gives the (*S*)-alcohol on reduction of ketones,[1] known as Prelog's rule.[8]

We have screened for a microorganism which reduces carbonyl compounds in an anti-Prelog manner and found that *Yamadazyma farinosa* is a useful strain which is registered to the Institute for Fermentation, Osaka with the registry number IFO 10896. This biocatalyst promotes the reduction of various ketones and exhibits enantioselectivity to those which have a π-electron system or heteroatom moiety. Typical products from the corresponding ketones are shown in Fig. 1.[9]

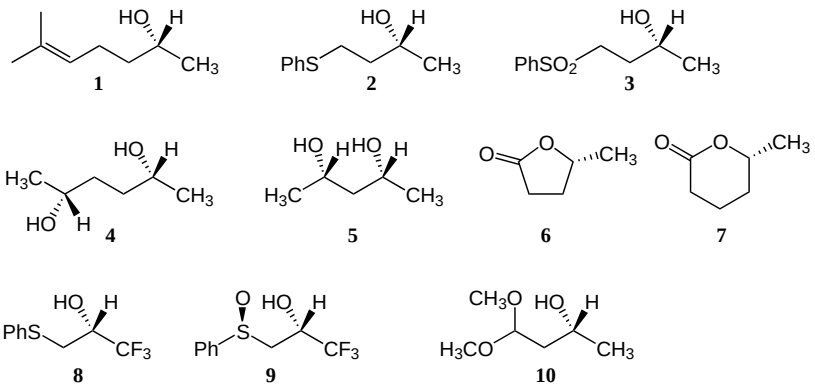

Figure 1. Optically active alcohols obtained via the *Y. farinosa*-mediated reduction of the corresponding ketones. See Refs. 9a for **1**, 9b and 9f for **2** and **3**, 9c for **4** and **5**, 9d for **6** and **7**, 9f for **8** and **9**, and 9g for **10**.

* Corresponding author. Tel./fax: +81-(0)45-566-1703; e-mail: hohta@chem.keio.ac.jp

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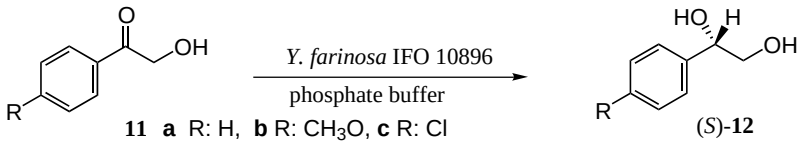
Table 1. Asymmetric reduction of α-hydroxyacetophenone derivatives



11 **a** R: H, **b** R: CH_3O, **c** R: Cl (*S*)-**12** (1)

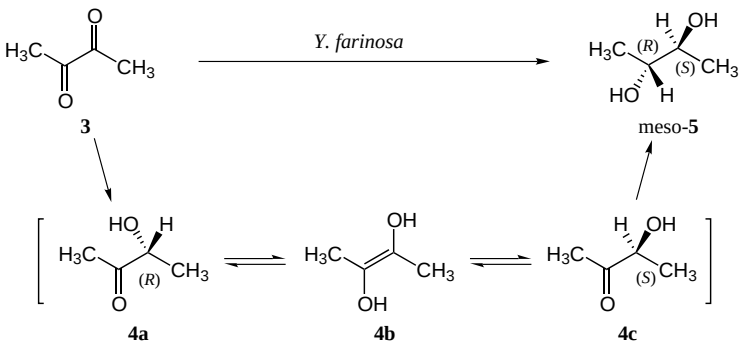
Entry	R	Atmosphere	Conc. (% w/v)	Time (h)	Yield (%)	Config.	E.e. (%)
1	H	Ar	0.5	24	91	*S*	>99
2	MeO	Ar	0.5	48	95	*S*	>99
3	Cl	Ar	0.3	48	85	*S*	>99
4	H	Air	0.5	24	86	*S*	>99

As seen from these compounds, the substrates so far subjected to the reduction system of *Y. farinosa* were limited to methyl or trifluoromethyl ketones. Aiming to widen the applicability of this unique biocatalyst, we tried the reaction using substrates which are expected to give chiral products for which C–C bond elongation on both sides of the stereogenic center is possible.

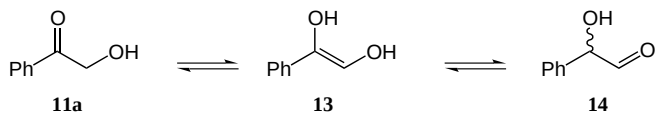
2. Results and discussion

Y. farinosa was grown on a nutrient medium and harvested by centrifugation. The reduction was performed using resting cells in a phosphate buffer under an atmosphere of argon. As shown in Table 1 (entries 1–3), α-hydroxyacetophenone and *p*-substituted derivatives gave the corresponding diol in high yields and high e.e., regardless to the electronic properties of the substituents (Eq. (1)). Redley et al. have reported that baker's yeast reduces **11** to give (*R*)-styrene glycol **12**.[10] Thus, the enantioselectivity of the present biotransformation is opposite to that obtained by using intact cells of baker's yeast. Some years ago, we demonstrated that this yeast reduces 1,3- and 1,4-diones to the corresponding (*R*,*R*)-diols with high e.e. However, in contrast to these, in the case of butan-2,3-diones **3**, the yeast gave the *meso*-isomer **5** as the major product.[9c] The mechanism of formation of *meso*-isomer was supposed to be due to the racemization of intermediary formed ketol **4a** via tautomerism prior to the second-step reduction (Eq. (2)). If the same type of equilibrium is present for hydroxyacetophenone derivatives **11**, they might possibly isomerize to hydroxy aldehydes **14** as shown in Eq. (3), and if it were the real substrates of microbial reduction, then the e.e. of the final product would not be necessarily high. The sharp enantioselectivity actually observed indicates that biotransformation of hydroxyacetophenone **11** is the direct reduction of the ketone moiety of the added substrates. Another interesting feature of the present reduction is that the presence of air has no effect on the enantioselectivity in contrast to the reduction of other compounds so far investigated, where the presence of air lowered the e.e. of the products.

Although it is possible to transform a phenyl ring to a carbonyl group, which can be used for C–C bond formation, it would be more convenient if the substrate has some protected functional groups, which will be converted more easily to the active forms. Thus, we tried the reaction with mono-protected dihydroxyacetones **15a** and **15b** (Table 2 and Eq. (4)). In both cases, the microbial reduction proceeded, although the rates of the reaction was very slow for benzoyl derivatives **15b**. The spatial arrangement of the ligands around the stereogenic center of mono-benzylated triol **16a** was the same as that in the case of reduction of hydroxyacet-

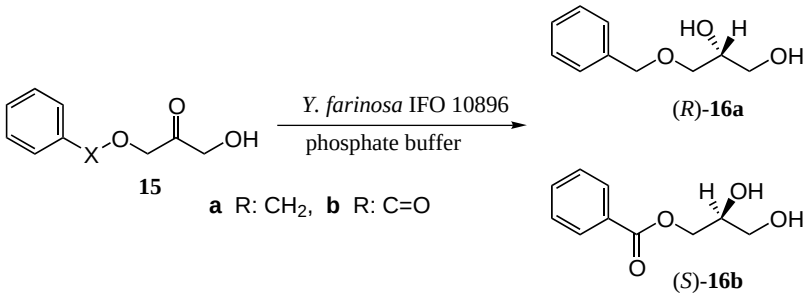


(2)



(3)

Table 2. Asymmetric reduction of mono-protected dihydroxyacetone



(4)

Entry	X	Conc. (% w/v)	Time (h)	Yield (%)	Config.	E.e. (%)
1	CH_2	0.5	48	98	*R*	73
2	CO	0.5	168	65	*S*	80

ophenone, although the absolute configuration was opposite because of the priority rule. However, the mode of reaction of benzoyl derivative **15b** was entirely different from those mentioned so far. The configuration of the resulting mono-protected glycerol was shown to be (*S*) on the basis of the sign of the optical rotation and the order of elution in HPLC. The structural difference between the substrate **15a** and **15b** is that **15b** has a carbonyl group instead of a methylene of **15a**. The difference of the polarity the facility in the formation of hydrogen bonding, and other electronic and steric effects are supposed to be enough for the inversion of the binding mode. In fact, the free energy required to reverse the two binding modes from the ratio of 95/5 to 5/95 can be calculated to be only 3.5 kcal/mol at room temperature. The selectivity of the reaction is lower compared to the reaction of **11**. We supposed that this may be attributed to the long distance between the carbonyl carbon and the phenyl ring. In the case of compound **11**, the aromatic ring is directly attached to the carbonyl carbon, whilst in the case of dihydroxyacetone derivative **15**, there are three atoms (C–O–C) between the carbonyl group and the phenyl ring.

To examine the effect of carbon numbers, two kinds of ketones which have two atoms between the phenyl ring and the reaction center were designed and subjected to the reaction. The results are summarized in Table 3. The reaction proceeded smoothly. For both compounds **17a** and **17b** (Eq. (5)), the e.e. of the products were better than those of **16**, as expected. Thus, it can be concluded that the distance between the phenyl ring and the reaction center is important for the binding mode of the substrates to the active site of the enzyme.

3. Conclusion

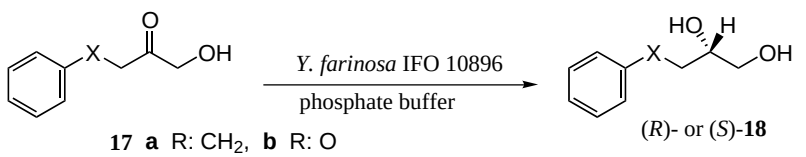
Various types of α-hydroxyketones bearing a phenyl ring on the other side of the carbonyl group were reduced with good to high enantioselectivities by the aid of the enzyme system of *Y. farinosa*. The enantioselectivity was anti-Prelog in most cases as expected. The numbers of atoms between the carbonyl group and the phenyl ring had a clear effect on the enantioselectivity of the reaction, the nearer the phenyl ring to the carbonyl group, the higher the e.e. of the products.

4. Experimental

4.1. General

All melting points were uncorrected. Optical rotation values were recorded on a Jasco DIP 360 polarimeter. IR spectra were measured as films for oils and as KBr discs for solids with a Jasco FT/IR-410 instrument. ^1H NMR spectra were recorded at 270 MHz on a JEOL JNM-EX 270 unless otherwise stated. Column chro-

Table 3. Asymmetric reduction of α-hydroxyketones with a two-atom spacer between the carbonyl group and the phenyl ring



(5)

Entry	X	Conc. (% w/v)	Time (h)	Yield (%)	Config.	E.e. (%)
1	CH_2	0.5	48	94	*S*	94
2	O	0.5	48	63	*R*	88

matography was carried out with Katayama 60 K070-WH 70–230 mesh silica gel.

4.2. Synthesis of substrates

4.2.1. *p*-Methoxy-α-hydroxyacetophenone 11b (general procedure for the selective oxidation of secondary hydroxyl group of 1,2-diol). This compound was prepared via oxidation of 1-(*p*-methoxyphenyl)-1,2-ethanediol. To a stirred suspension of *p*-methoxystyrene (4.0 g, 23.2 mmol) in water (130 mL), powdered *m*-CPBA (2.0 g, 19.2 mmol) was added in small portions over a period of 5–10 min at 0°C. The mixture was stirred at rt for 2 h. Then 10% aqueous sulfuric acid (3.3 mL) was added and the mixture was further stirred for 2 h. The progress of the reaction was monitored by TLC. Solid NaOH was added until the solution became homogeneous. The reaction mixture was extracted with ethyl acetate (5×10 mL). The combined organic extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel column chromatography. Elution with hexane/ethyl acetate (1/1) afforded 1-*p*-methoxyphenyl-1,2-ethanediol **12** (474 mg, 15%); ^{1}H NMR (270 MHz): δ 3.72 (m, 2H), 3.83 (s, 3H), 4.79 (m, 1H), 6.91 (d, J=8.73, 2H), 7.34 (d, J=8.73, 2H).

To a stirred solution of 1-*p*-methoxyphenyl-1,2-ethanediol **12** (500 mg, 2.97 mmol) and 4-dimethylaminopyridine (14.5 mg, 0.12 mmol) in dichloromethane (15 mL) was added triethylamine (331 mg, 3.27 mmol) at rt. *tert*-Butyldimethylsilyl chloride (493 mg, 3.27 mol) was added dropwise over 20 min and the stirring was continued overnight at rt. The progress of the reaction was monitored by TLC. Removal of the solvent afforded a residue, which was purified by silica-gel column chromatography. Elution with hexane/ethyl acetate (5/1) afforded *p*-methoxyphenyl)-2-*tert*-butyldimethylsilyloxyethanol (800 mg, 95%). Solid tetrapropylammonium perruthenate (TPAP, 46.7 mg, 0.13 mmol) was added in one portion to a stirred solution of 1 - (*p* - methoxyphenyl) - 2 - *tert* - butyldimethylsilyloxyethanol (750 mg, 2.66 mmol) and *N*-methylmorpholine-*N*-oxide (MMO, 467 mg, 398 mmol) in dichloromethane at rt. The stirring was continued for 3 h at the same temperature. Then the reaction mixture was filtered through a pad of silica, eluting with ethyl acetate. The filtrate was evaporated to afford the residue, which was stirred in a 50% aqueous methanolic solution of Oxone (1.8 g, 2.93 mmol) at rt for 3 h. The progress of the reaction was monitored by TLC. On completion, the methanol was removed under reduced pressure and the residue was extracted five times with 10 mL portions of ethyl acetate. The combined organic extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel column chromatography. Elution with hexane/ethyl acetate (2/1) afforded *p*-methoxy-α-hydroxyacetophenone **11b** as colorless crystals; yield 386 mg (three steps, 83%); mp 105.0–105.5°C (from ethanol), lit.[11] 105–106°C; IR ν_{max}: 3418, 1681, 1673, 1604, 1233, 836 cm^{-1}; ^{1}H NMR (270 MHz): δ 3.87 (s, 3H), 4.80 (s, 2H), 6.96 (d, J=9.07, 2H), 7.87 (d, J=9.07, 2H).

4.2.2. *p*-Chloro-α-hydroxyacetophenone 11c. To a stirred solution of *m*-CPBA (1.27 g, 7.36 mmol) in chloroform (12 mL), *p*-chlorostyrene (1.03 g, 7.43 mmol) was added in small portions over a period of 5–10 min at 0°C and the mixture was stirred overnight at rt. Then 1% (w/v) aqueous solution of sulfuric acid (36 mL) was added and the mixture was stirred at rt for 1 h. 10% NaOH (30 mL) was added and the aqueous solution was extracted with ethyl acetate (5×10 mL). The combined organic extract was washed with brine and dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel column chromatography. Elution with hexane/ethyl acetate (1/1) afforded 1-(*p*-chlorophenyl)-1,2-ethanediol (304 mg, 23%).

According to the general procedure, **11c** was prepared from 1-(*p*-chlorophenyl)-1,2-ethanediol (500 mg, 2.88 mmol); yield 183 mg (37%); mp 116.0–118.0°C (from ethanol), lit.[12] 114–115°C; IR ν_{max}: 3424, 3382, 1680, 1592, 1232, 1093, 827 cm^{-1}; ^{1}H NMR (270 MHz): δ 4.83 (s, 2H), 7.46 (d, J=8.57 Hz, 2H), 7.85 (d, J=8.57 Hz, 2H).

4.2.3. 1-Benzyloxy-3-hydroxy-2-propanone 15a. To a stirred mixture of epichlorohydrin (2.0 g, 21.6 mmol), benzyl alcohol (2.12 g, 19.6 mmol), and tetrabutylammonium hydrogen sulfate (0.29 g, 0.86 mmol) was added dropwise 50% aqueous sodium hydroxide (10 mL) at 0°C. Stirring was continued for 30 min at the same temperature and then for 21 h at rt. The mixture was extracted with ether (5×10 mL). The combined organic extract was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel column chromatography. Elution with hexane/ether (6/1) afforded 1-benzyloxy-2,3-epoxypropane; yield 1.84 g (57%); oil; ^{1}H NMR (270 MHz): δ 2.54 (dd, J=4.94, 2.80, 1H), 2.72 (dd, J=4.61, 4.45, 1H), 3.11 (m, 1H), 3.35 (dd, J=11.33, 5.77, 1H), 3.67 (dd, J=11.38, 2.96, 1H), 4.45 (d, J=2.03, 1H), 4.53 (d, J=2.03, 1H), 7.22 (m, 5H).

A mixture of 1-benzyloxy-2,3-epoxypropane (1.0 g, 6.09 mmol) and 1% aqueous solution of sulfuric acid (30 mL) was stirred for 5 h at rt. The aqueous solution was extracted with ethyl acetate (10×10 mL). The combined organic extract was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give 1-benzyloxy-2,3-propanediol **16a**; yield 1.06 g (96%); oil; ^{1}H NMR (270 MHz): δ 3.59 (m, 4H), 3.83 (m, 1H), 4.49 (s, 2H), 7.27 (m, 5H).

According to the general procedure, **15a** was prepared from 1-benzyloxy-2,3-propanediol (**16a**) (500 mg, 2.74 mmol); yield 84 mg (17%); mp 77.0–78.0°C; IR ν_{max}: 3361, 3300, 2939, 2878, 1731, 1498, 1127, 1094 cm^{-1}; ^{1}H NMR (270 MHz): δ 4.20 (s, 2H), 4.50 (s, 2H), 4.62 (s, 2H), 7.37 (m, 5H).

4.2.4. 1-Benzoyloxy-3-hydroxypropanone 15b. To a stirred mixture of dihydroxyacetone dimer (180 mg, 2.00 mmol) and benzoic anhydride (693 mg, 3.02 mmol) in 1,4-dioxane (20 mL), lipase PS (15 g) was added, and the mixture was stirred for 48 h at 30°C. After the removal of the enzyme by filtration with Celite, the filtrate was concentrated under reduced pressure. The residue was purified by silica-gel column chromatography. Elution with hexane/ethyl acetate (1/1) afforded **15b**; yield 178.0 mg (46%); mp 94.0–95.0°C (from ethanol), lit.[13] 92–93°C; IR ν_{max}: 3430, 3382, 1730, 1714, 1273, 1110, 713 cm^{-1}; ^1H NMR (270 MHz): δ 4.49 (s, 2H), 5.00 (s, 2H), 7.52 (m, 3H), 8.07 (m, 2H).

4.2.5. 1-Hydroxy-4-phenyl-2-butanone 17a. According to the procedure for the preparation of **12**, diol **18a** was prepared from 4-phenyl-1-butene (3 g, 23 mmol); yield 3 g (80%); IR ν_{max}: 3400–3100, 2930, 2862, 2361, 2339, 1496, 1455, 1097, 1069, 1038, 699, 519 cm^{-1}; ^1H NMR (270 MHz): δ 1.69–1.82 (m, 2H), 2.65–2.87 (m, 2H), 3.47 (m, 1H), 3.71 (m, 2H), 7.17–7.32 (m, 5H).

According to the general procedure, **17a** was prepared from (±)-4-phenyl-1,2-butanediol (**18a**, 967 mg, 5.82 mmol); yield 216 mg (23%); oil; IR ν_{max}: 3500–3400, 3027, 2927, 2361, 2339, 1721, 1604, 1496, 1454, 1064, 1496, 1454, 1064, 993, 749, 701 cm^{-1}; ^1H NMR (270 MHz): δ 2.72 (d, $J=7.75$, 2H), 2.96 (d, $J=7.75$, 2H), 4.17 (s 2H), 7.14–7.27 (m, 5H).

4.2.6. 1-Hydroxy-3-phenoxypropanone 17b. According to the procedure of the preparation of styrene glycol derivatives **12**, 1-phenoxy-2,3-propanediol **18b** was prepared from 1,2-epoxy-3-phenoxypropane (3 g, 20 mmol); yield 2.66 g (79%); mp 56.5–57.0 (from ethanol); IR ν_{max}: 3450–3300, 2916, 2871, 2360, 2338, 1600, 1497, 1252, 1046, 748 cm^{-1}; ^1H NMR (270 MHz): δ 4.02–4.11 (m, 3H), 6.88–6.99 (m, 3H), 7.23–7.31 (m, 2H).

According to the general procedure, **17b** was prepared from (±)-**18b** (1 g, 5.95 mmol); yield 309 mg (31%); mp 147.5–148.0°C (from ethanol); IR ν_{max}: 3375, 1749, 1738, 1706, 1601, 1587, 1499, 1249, 1076, 1053 cm^{-1}; ^1H NMR (270 MHz, acetone-d_6): δ 3.68 (d, $J=11.54$, 2H), 3.94 (d, $J=11.71$, 1H), 3.98 (d, $J=11.71$, 1H), 4.26 (d, $J=11.54$, 1H), 6.90–6.97 (m, 3H), 7.25–7.31 (m, 2H).

4.3. General procedure for the microbial reductions

The substrates were added to the suspension of grown cells of *Yamadazyma farinosa* IFO 10896 and incubated at 30°C.

4.3.1. (*S*)-(+)-1-Phenyl-1,2-ethanediol (*S*)-12a. *Y. farinosa* IFO 10896 was incubated in a glucose medium [containing glucose (2.0 g), peptone (0.7 g), yeast extract (0.5 g), K_2HPO_4 (0.2 g), KH_2PO_4 (0.3 g), pH 6.5, total volume 100 mL] for 2 days at 30°C. The wet cells (ca. 8 g from 100 mL of the broth) were harvested by centrifugation (3000 rpm) and washed with phosphate buffer (0.1 M, pH 6.5). The wet cells (4 g) were re-suspended in 20 mL of a phosphate buffer solution (pH 6.5, 0.1 M) in a 500 mL shaking culture (Sakaguchi) flask with the substrate **11a** [103 mg, 0.76 mmol, 0.5% w/v]. After glucose (1.0 g) was added, the flask was purged with argon, equipped with a balloon filled with argon and shaken on a reciprocal shaker for 2 days at 30°C. The cell mass was removed by filtration using Celite. The filtrate was extracted with ethyl acetate (10×10 mL). The combined organic extract was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo and the residue was purified by silica-gel column chromatography. Elution with hexane/ethyl acetate (1/1) afforded (*S*)-1-phenyl-1,2-ethanediol **12a** (95 mg, 91%); IR ν_{max}: 3300–3200, 3030, 2933, 2871, 1448, 1101, 1054, 700 cm^{-1}; ^1H NMR (270 MHz): δ 3.61 (m, 2H), 4.72 (m, 1H), 7.20 (m, 5H); MS m/e (%): 31 (110), 77 (80), 79 (75), 107 (20), 138 (M^+, 2); $[\alpha]_D^{22}$ +71.9 (*c* 1.04, $CHCl_3$), lit.[14] $[\alpha]_D^{18}$ +60 (*c* 1.0, $CHCl_3$); mp 64.5–65.0°C (from ethanol, lit.[15] 62–63°C. Its e.e. was determined by HPLC analysis (Chiralcel OD of Daicel Chemical Industries Ltd., 4.6×250 mm); eluent: hexane-2-propanol=9:1; flow rate 0.5 mL/min, retention time: (*R*)-form; 22.3 min (minor), (*S*)-form; 23.8 min (major).

4.3.2. (*S*)-(+)-1-(*p*-Methoxyphenyl)-1,2-ethanediol (*S*)-12b. The cultivation of the *Y. farinosa* and the following reaction were carried out according to the procedure described for the preparation of (*S*)-**12a**. 1-Hydroxy-2-(4-methoxyphenyl)ethanone **11b** (103 mg, 0.62 mmol) was used as the substrate; yield 99 mg (95%); IR ν_{max}: 3400–3300, 2936, 1612, 1515, 1245, 1086, 1025, 834 cm^{-1}; ^1H NMR (270 MHz) δ 3.72 (m, 2H), 3.83 (s, 3H), 4.79 (m, 1H), 6.91 (d, $J=8.73$, 2H), 7.34 (d, $J=8.73$, 2H); MS m/e (%): 31 (46), 77 (71), 94 (73), 109 (85), 137 (100), 168 (M^+, 18); $[\alpha]_D^{23}$ +64.5 (*c* 1.02, $CHCl_3$); mp 78.0–79.0°C (from ethanol), lit.[16] 75–76°C. Its e.e. was determined by HPLC analysis (Chiralcel OD of Daicel Chemical Industries Ltd., 4.6×250 mm; eluent: hexane-2-propanol=9:1; flow rate 0.5 mL/min, retention time: (*R*)-form; 29.8 min (minor), (*S*)-form; 32.2 min (major).

4.3.3. (*S*)-(+)-1-(4-Chlorophenyl)-1,2-ethanediol (*S*)-12c. The cultivation of the *Y. farinosa* and the following reaction were carried out according to the procedure described for the preparation of (*S*)-**12a**. 2-(4-Chlorophenyl)-1-hydroxyethanone (**11c**, 61 mg, 0.36 mmol) was used as the substrate; yield 52 mg (85%); IR ν_{max}: 3400–3300, 2933, 2895, 1492, 1087, 1037, 900, 829 cm^{-1}; ^1H NMR (270 MHz): δ 3.60 (dd, $J=11.21$, 8.08 Hz, 1H), 3.74 (dd, $J=11.21$, 3.46 Hz, 1H), 4.79, (dd, $J=8.08$, 3.46 Hz, 1H), 7.30 (m, 4H); MS m/e (%): 31 (40), 77 (100), 141 (45), 143 (15), 172 (M^+, 2.6), 174 (0.9); $[\alpha]_D^{22}$ +59.5 (*c* 1.07, $CHCl_3$) [lit.[17] $[\alpha]_D^{20}$ −31.4 for the (*R*) enantiomer (*c* 0.97, EtOH)]; mp 80.0–80.5°C (lit.[17] 83–84°C). Its e.e. was determined by HPLC analysis (Chiralcel OD of Daicel Chemical Industries Ltd., 4.6×250 mm; eluent: hexane-2-propanol=9:1; flow rate 0.5 mL/min, retention time: (*R*)-form; 36.6 min (minor), (*S*)-form; 39.6 min (major).

4.3.4. (*R*)-1-Benzyloxy-2,3-propanediol (*R*)-16a. The cultivation of the *Y. farinosa* and the following reaction were carried out according to the procedure described for the preparation of (*S*)-**12a**. 1-Benzyloxy-3-hydroxypropan-2-one **15a** (99 mg, 0.55 mmol) was used as the substrate; yield 98 mg (98%); IR ν_{max}: 3450–3350, 2929, 2869, 1736, 1107, 1045, 699 cm^{-1}; ^1H NMR (270 MHz) δ 3.59 (m, 4H), 3.83 (m, 1H), 4.49 (s, 2H), 7.27 (m, 5H); MS m/e (%): 31 (25), 77 (232), 91 (100), 105 (49), 182 (M^+, 4.4); $[\alpha]_D^{21}$ −0.98 (*c* 1.02, $CHCl_3$) lit.[18] $[\alpha]_D^{20}$ −1.9 (*c* 0.84, $CHCl_3$) for the (*R*) enantiomer. Its e.e. was determined by HPLC analysis (Chiralcel OD of Daicel Chemical Industries Ltd., 4.6×250 mm; eluent: hexane-2-propanol = 9:1; flow rate 1.0 mL/min, retention time: (*R*)-form; 18.5 min (major), (*S*)-form; 22.9 min (minor).

4.3.5. (*S*)-1-Benzoyloxy-2,3-propanediol (*S*)-16b. The cultivation of the *Y. farinosa* and the following reaction were carried out according to the procedure described for the preparation of (*S*)-**12a**. 1-Benzoyloxy-3-hydroxypropanone (**15b**, 103 mg, 0.53 mmol) was used as the substrate; yield 68 mg (65%); IR ν_{max}: 3450–3350, 2951, 2888, 1719, 1602, 1452, 1316, 1278, 1107, 1070, 712 cm^{-1}; ^1H NMR (270 MHz): δ 3.71 (dd, J = 11.46, 5.69, 1H), 3.81 (dd, J = 11.46, 3.87, 1H), 4.09 (m, 1H), 4.43 (dd, J = 11.54, 5.77, 1H), 4.49 (dd, J = 11.54, 4.95, 1H), 7.45–8.09 (m, 5H); MS m/e (%): 31 (7), 39 (64), 51 (32), 77 (80), 105 (100), 197 (M^++1, 0.26); $[\alpha]_D^{20}$ +4.08 (*c* 0.94, EtOH), lit.[19] $[\alpha]_D^{23}$ −19.0 (*c* 1.0, EtOH) for the (*R*) enantiomer. Its e.e. was determined by HPLC analysis (Chiralcel OD of Daicel Chemical Industries Ltd., 4.6× 250 mm; eluent: hexane-2-propanol = 9:1; flow rate 1.0 mL/min, retention time: (*R*)-form; 14.3 min (minor), (*R*)-form; 16.5 min (major).

4.3.6. (*S*)-4-Phenyl-1,2-butanediol (*S*)-18a. The cultivation of the *Y. farinosa* and the following reaction were carried out according to the procedure described for the preparation of (*S*)-**12**. 1-Hydroxy-4-phenyl-2-butanone **17a** (99 mg, 0.60 mmol) was used as the substrate; yield 94 mg (94%); IR ν_{max}: 3500–3400, 3027, 2927, 2361, 2339, 1721, 1604, 1496, 1454, 1064, 1496, 1454, 1064, 993, 749, 701 cm^{-1}; ^1H NMR (270 MHz): δ 2.72 (d, J = 7.75, 2H), 2.96 (d, J = 7.75, 2H), 4.17 (s 2H), 7.14–7.27 (m, 5H); MS m/e (%): 39 (34), 57 (19), 65 (66), 78 (8), 91 (100), 117 (45), 135 (6), 148 (49), 167 (M^++1, 40); $[\alpha]_D^{20}$ −32.3 (*c* 1.02, EtOH), lit.[20] $[\alpha]_D^{20}$ +27.1 (*c* 1.0, EtOH) for the (*R*)-isomer. Its e.e. was determined by HPLC analysis (Chiralcel OD of Daicel Chemical Industries Ltd., 4.6×250 mm; eluent: hexane-2-propanol = 9:1; flow rate 2.0 mL/min, retention time: (*S*)-form; 11.0 min (major), (*R*)-form; 24.6 min (minor).

4.3.7. (*R*)-1-Phenoxy-2,3-propanediol (*R*)-18b. The cultivation of the *Y. farinosa* and the following reaction were carried out according to the procedure described for the preparation of (*S*)-**12a**. 1-Hydroxy-3-phenoxypropanone **17b** (102 mg, 0.61 mmol) was used as the substrate; yield 65 mg (63%); IR ν_{max}: 3450–3300, 2916, 2871, 2360, 2338, 1600, 1497, 1252, 1046, 748 cm^{-1}; ^1H NMR (270 MHz): δ 4.02–4.11 (m, 3H), 6.88–6.99 (m, 3H), 7.23–7.31 (m, 2H); MS m/e (%): 31 (100), 39 (64), 51 (54), 65 (66), 77 (26), 95 (21), 168 (M^+, 40); $[\alpha]_D^{20}$ −1.61 (*c* 1.02, $CHCl_3$), lit.[21] $[\alpha]_D^{20}$ −10.8 (*c* 1.0 EtOH) for the (*R*) isomer; mp 56.5–57.0°C (from ethanol), lit.[22] 53.5–55°C. Its e.e. was determined by HPLC analysis (Chiralcel OD of Daicel Chemical Industries Ltd., 4.6× 250 mm; eluent: hexane-2-propanol = 9:1; flow rate 2.0 mL/min, retention time: (*R*)-form; 9.7 min (major), (*S*)-form; 21.6 min (minor).

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