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Bull. Chem. Soc. Jpn., 70, 483—491 (1997)

Preparation of Sulfur-Containing Optically Active Secondary Alcohols Based on *Pichia farinosa*-Catalyzed *anti*-Prelog-Rule Reduction as the Key Step

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(Received October 7, 1996)

A *Pichia farinosa* IAM 4682 mediated reduction of sulfur containing ketones afforded secondary alcohols with (*R*)-absolute configuration. For example, 4-(phenylthio)-2-butanone and 4-(phenylsulfonyl)-2-butanone afforded (*R*)-4-(phenylthio)-2-butanol (91%ee) in 90% yield and (*R*)-4-(phenylsulfonyl)-2-butanol (97%ee) in 94% yield, respectively. In the case that the ee of the product was not satisfactory, any contaminating (*S*)-enantiomer was selectively oxidized by *Rhodococcus rhodochrous* IFO 15564 to leave pure (*R*)-enantiomer. The substrate specificity of *Pichia farinosa*-mediated reduction and *Rhodococcus rhodochrous*-mediated oxidation was further examined.

Preparation by means of yeast-mediated reduction of optically active secondary alcohols with sulfur-containing functionalities, such as sulfinyl and sulfonyl groups, is gaining much attention from many synthetic chemists, because of its simplicity and efficiency.[1] The reduction products are important starting materials for synthesizing natural products, medicines, and other useful materials. So far, due to the stereochemical preference of yeast-mediated reduction[2] expressed as the "Prelog rule", the low availability of the (*R*)-enantiomers of secondary alcohols still remains as an unsolved problem. For example, only (*S*)-enantiomers of 4-(phenylthio)-2-butanol **1a**[3] and 4-(phenylsulfonyl)-2-butanol **2a**[4,5] have been obtained by a conventional bakers' yeast-mediated reduction. Here we report on a biocatalytic approach to these (*R*)-enantiomers of sulfur-containing secondary alcohols **1a**—**7a**, as shown in Fig. 1.

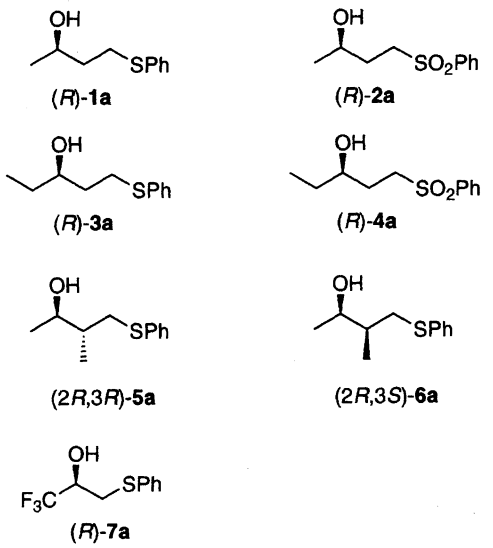


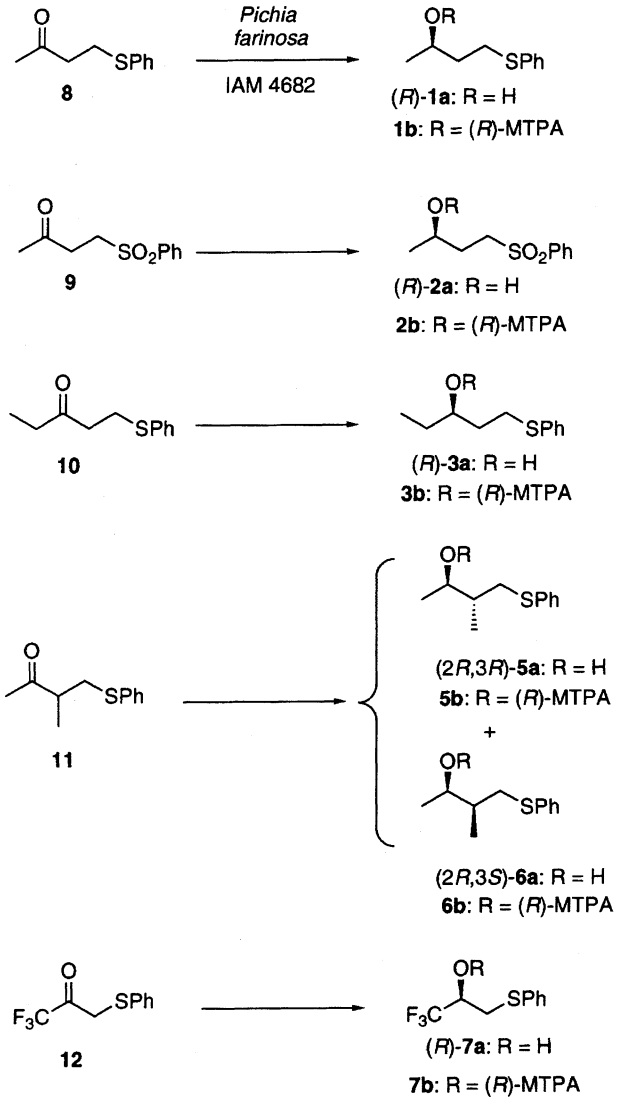
Fig. 1. Synthetically useful sulfur-containing optically active secondary alcohols.

Results and Discussion

***Pichia farinosa*-Mediated Reduction of Sulfur-Containing Ketones.** Among the microorganisms and enzymes whose enantiofacial preference follows an *anti*-"Prelog rule",[6] a yeast, *Pichia farinosa* IAM 4682[7—11] has recently been developed by ourselves as one which provides (*R*)-enantiomers of secondary alcohols. Our first attempt was the reduction of a sulfinyl ketone **8** by using this yeast. The reduction under an anaerobic condition smoothly proceeded to give (*R*)-**1a** of 91%ee in 90% yield (Scheme 1). The reduction of the corresponding sulfonyl ketone **9** also worked well. The higher ee (97%) of the product **2a** was coincided with that the enantioselectivity of the reaction which is enhanced by the presence of neighbouring polar functional groups.[11] Encouraged by the results, we tested some related substrates (Fig. 2); the results are listed in Table 1. A homologous substrate **10**, in which an additional methyl group was attached on the terminal of **8**, was also accepted to give (*R*)-isomer as the product.

Another substrate **11**, whose methyl substituent was placed on the internal position, could be reduced to give a 1 : 1 mixture of the diastereomeric products **5a** and **6a** (Scheme 1). The (*R*)-absolute configurations of the newly created chiral centers were assigned by ^{1}H NMR spectra of the corresponding MTPA esters[8] **5b** and **6b** (see Experimental). The details of the determination of relative and absolute configurations of **5a** and **6a** will be discussed later. There turned out to be no preference with regard to the pre-existing chirality adjacent to the carbonyl group.

As the biocatalyst-mediated synthesis of fluorinated methylcarbinols[12] especially bearing sulfur-containing substituents[13—15] have recently been developed, we next



Scheme 1. Reduction of ketones by *Pichia farinosa*.

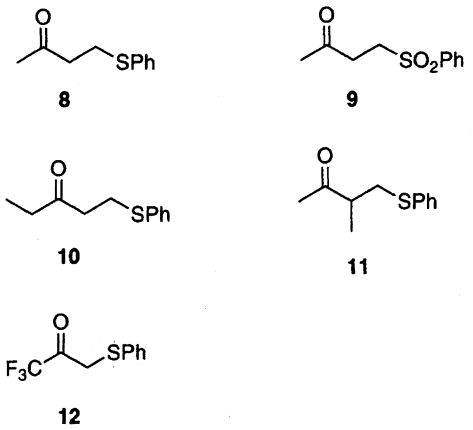


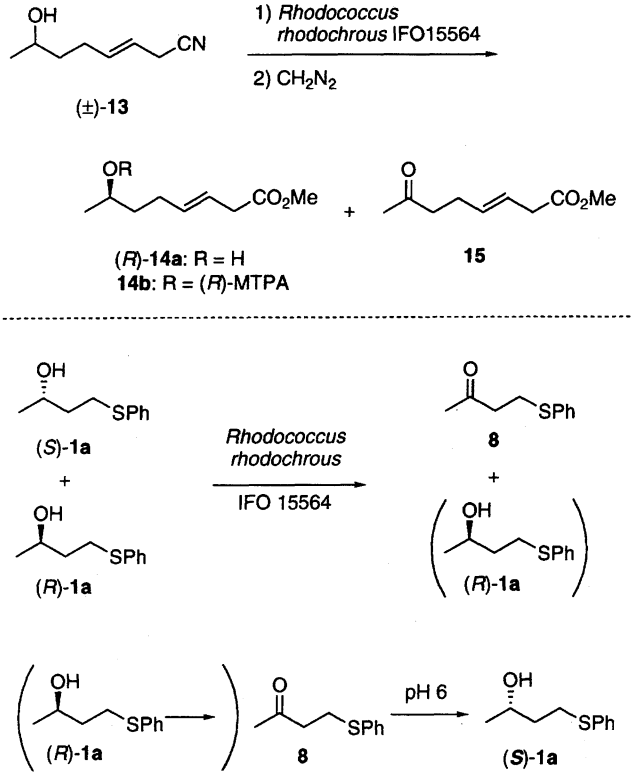
Fig. 2. Substrates for *Pichia farinosa*-mediated reduction.

turned our attention to the reduction of a trifluoromethyl ketone **12**. As expected, the reduction proceeded according to the *anti*-Prelog selectivity[cf.16] and (*R*)[15]-**7a** (88%ee) was obtained in a 72% yield.

Table 1. *Pichia farinosa*-Mediated Reduction of Ketones

Substrate	Product	Yield (%)	%ee
8	**1a**	90	91
9	**2a**	94	97
10	**3a**	93	93
11	**5a+6a**	99	99[a]
12	**7a**	72	88

a) The ee is in regard to the newly created secondary alcohol.



Scheme 2. Enantioselective oxidation of secondary alcohols by *Rhodococcus rhodochrous*.

***Rhodococcus rhodochrous*-Mediated Oxidation of Contaminating (*S*)-Enantiomers.** The ee's of the product, for example, 91% for **1a**, were not always satisfactory for use as optically pure starting materials in synthetic organic chemistry. We turned our attention to the enantioselective oxidation of contaminating (*S*)-isomer (ca. 5%) back to the starting ketone. Toward this end, the possibility of enantioselective microbial oxidation[17] of (*S*)-**1a** was investigated.

The clue to solve this problem was obtained from an unexpected reaction shown below. An enantioselective oxidation of racemic secondary alcohol mediated by *Rhodococcus rhodochrous* IFO 15564 was found in the course of a study of enzymatic hydrolysis of a cyano group.[9] When a racemic nitrile **13** was incubated with this microorganism for a prolonged time, a ketone **15** (28%) was obtained in addition to the desired ester **14a** (48%), after treatment with diazomethane. As the alcohol **14a** was recovered in an enantiomerically enriched form (76%ee) with (*R*)-absolute configuration, a kinetic preference for (*S*)-isomer in enantioselective oxidation of **14a** was suggested. Encouraged by this result,

we attempted the oxidation of (±)-**1a** as a representative of racemic secondary alcohols using this microorganism and the incubation conditions were optimized (Scheme 2).

The oxidation of (±)-**1a** by the resting cells of *R. rhodochrous* proceeded smoothly at pH 8.0 with bubbling of air. The ee of the recovered substrate, (*R*)-**1a**, became as high as 75% after 26 h. The time course of incubation (Fig. 3, solid lines) shows that the oxidation of (*S*)-**1a** is faster (3 : 1) than that of (*R*)-**1a** in the initial phase.

At a lower pH (6.0), the ee of the recovered substrate (37%) was considerably lower than that obtained at pH 8.0. The detailed time-course study (Fig. 3, dotted lines) indicated that the rate of oxidation of (*S*)-isomer at pH 6.0 was slower than that at pH 8.0, while the oxidation of (*R*)-isomer proceeded in a similar rate under either pH. This result suggests that a part of the corresponding ketone **8** was reduced to (*S*)-**1a** under pH 6.0 in the reaction mixture, accompanied with the oxidation of alcohols. An example of pH dependent inversion of oxidation-reduction of a related substrate, 1-(phenylthio)-2-propanol, mediated by *Rhodococcus equi* IFO 3730,[18] has been observed.

The application of the above-mentioned *R. rhodochrous*-mediated oxidation on an enantiomerically enriched (91%ee) (*R*)-**1a** worked well. The contaminating (*S*)-**1a** was effectively removed by this procedure to give (*R*)-**1a** of > 99%ee in 87% yield. It is noteworthy that no oxidation on the sulfur atom was observed; accordingly, the oxidation of the secondary alcohol was supposed to occur by the action of alcohol dehydrogenase.[cf.19] The total yield through the sequential use of two biocatalysts was 78%.

(*R*)-**1a** was converted to (*R*)-**2a** of > 99%ee in 85% yield, by oxidizing its sulfide moiety with hydrogen peroxide in acetic acid (Scheme 3). In this way, enantiomerically pure (*R*)-**1a** and (*R*)-**2a** became available.

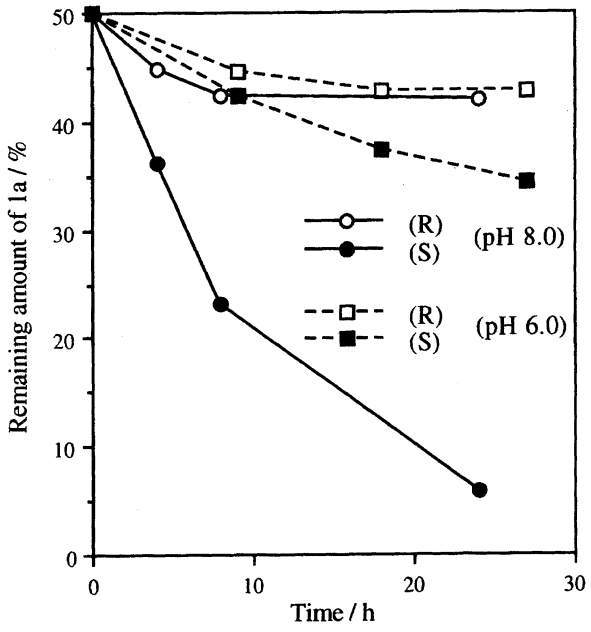
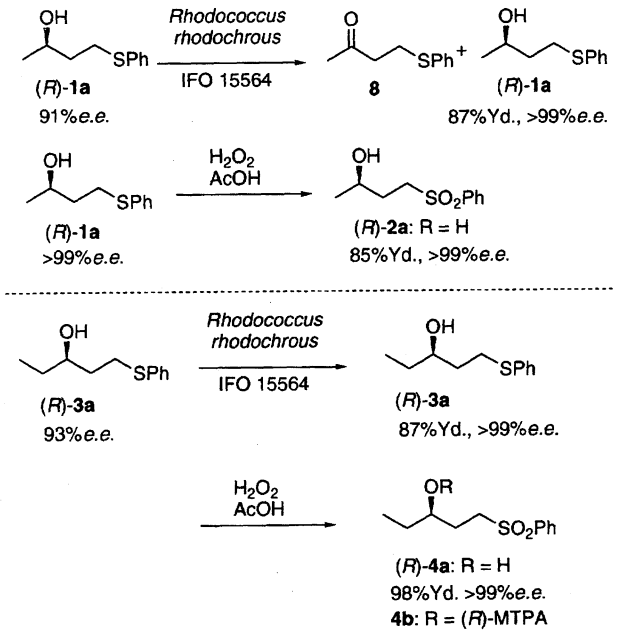


Fig. 3. Time course and pH dependence of *Rhodococcus rhodochrous*-mediated enantioselective oxidation of **1a**.

Next, we embarked upon the investigation on the substrate specificity as well as the enantioselectivity of *Rhodococcus*-mediated dehydrogenation of other secondary alcohols, involving those shown in Fig. 4, the results are listed in Table 2. This method worked well for the enantiomeric enrichment of (*R*)-**3a**. Starting from (*R*)-**3a** of 91%ee, enantiomerically pure (*R*)-**3a** was obtained by *Rhodococcus rhodochrous*-mediated oxidation. The subsequent treatment with hydrogen peroxide afforded (*R*)-**4a** (Scheme 3).

In the case of a diastereomeric mixture of **5a** and **6a** as the substrate, isomers with (*S*)-absolute configuration at the secondary alcohol were oxidized. Thus, the recovered alcohol was a mixture of diastereomers containing (2*R*,3*R*)-**5a** and (2*R*,3*S*)-**6a** as major products. Again, in this case, there was no discrimination on the stereochemistry adjacent to the hydroxyl group. An analogous compound **16**, with no sulfur atom, and an aliphatic secondary alcohol **17** could be



Scheme 3. Preparation of enantiomerically pure alcohols.

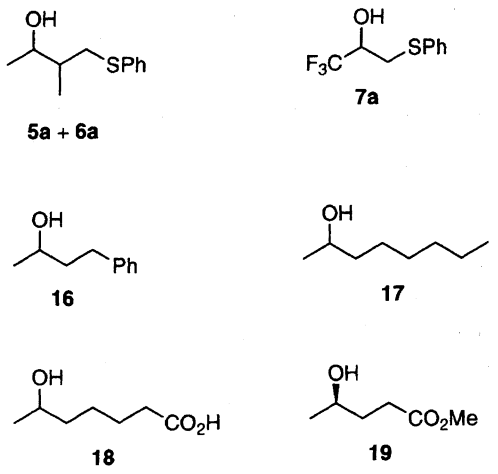
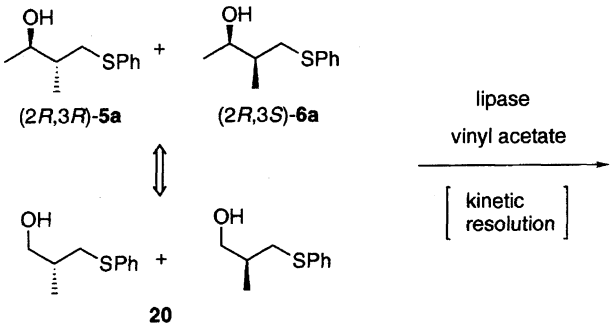


Fig. 4. Substrates and products for *Rhodococcus rhodochrous*-mediated oxidation.

Table 2. *Rhodococcus rhodochrous*-Mediated Oxidation of Alcohols

Substrate	Yield of ketone (%)	Yield of recovered alcohol (%)	%ee of recovered (*R*)-alcohol
(±)-**13**	28	48	76
(±)-**1a**	32	48	75[a]
(±)-**1a**	43	37	37[b]
(*R*)-**1a** (91%ee)	7	87	>99
(*R*)-**3a** (91%ee)	5	87	>99
5a+**6a**[c]	32	41	89[d]
(±)-**7a**	—	40	0
(±)-**16**	27	47	60
(±)-**17**	—	15	94
(±)-**18**	—	26[e]	95[e]

a) The reaction was carried out at pH 8.0. b) At pH 6.0. c) A mixture of four diastereomer was used. d) In regard to the secondary alcohol. e) For (*R*)-**19**.



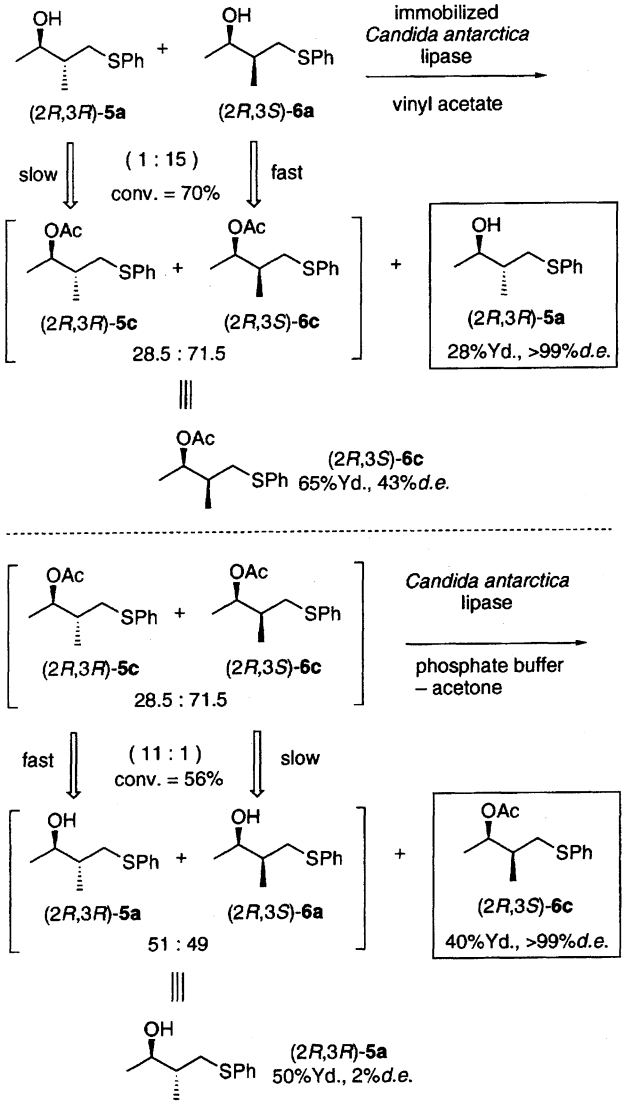
Scheme 4. Resolution of adjacent chiral center.

the substrate. Thus (*S*)-isomers were preferentially oxidized. Although the ee of the recovered alcohol (*R*)-**17** was as high as 94%, the yield was only 15%, due to its volatile nature. In this case, the product 2-octanone was not detected. As the *Rhodococcus*-mediated oxidation proceeds under aerobic condition with vigorous bubbling of air, at any event, it is not suitable for volatile substrates. Another problem was the further metabolism of the product. When hydroxy acid **18** was applied as the substrate, the only isolable product was less homologated (*R*)-**19** after methylation, which was supposingly derived from the primary product, (*R*)-**18**, by a subsequent microbial β-oxidation. Finally, no oxidation was observed on trifluoromethyl carbinol **7a**.

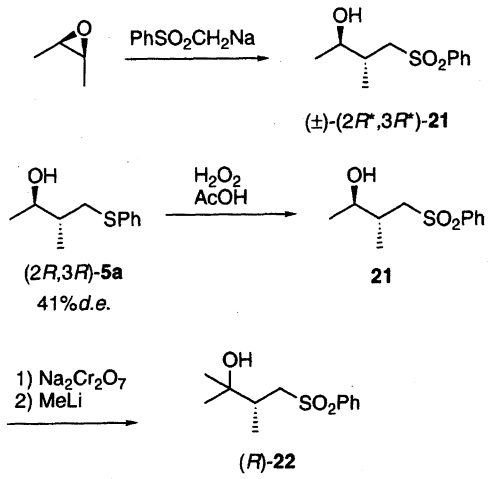
Lipase-Mediated Separation of Diastereomers 5a and 6a and Their Absolute Stereochemistry. Although optically active **5a** and **6a** are expected to be useful intermediates for the synthesis of physiologically active steroids,[20,21] the biocatalyst-mediated kinetic resolution in regard to the stereocenter adjacent to the hydroxyl group was difficult either by reduction or oxidation. Moreover, many attempts for chromatographic separation of these diastereomers resulted in failure. This situation prompted us to provide a new method of separation of those products by a biocatalytic method.

We turned our attention to a lipase-catalyzed kinetic resolution via transesterification. The absolute configuration on the secondary alcohol moiety is commonly (*R*) for **5a** and **6a**, which should be separated. As the preferred absolute configurations of methylcarbinols in many lipase-catalyzed transesterification were (*R*),[22] both (2*R*,3*R*)-**5a** and (2*R*,3*S*)-**6a** can be said to have favorable configurations as secondary alcohols. Accordingly, the situation of the kinetic separation of **5a** and **6a** would be similar to the case of the enantiomers of primary alcohol **20** (Scheme 4). Based on such information for similar types of primary alcohols,[23] the lipases were screened, to obtain a high degree of discrimination of the diastereomers.

Among the lipases tested, *Candida antarctica* lipase (Novo, SP525) showed the highest selectivity. A 1 : 1 mixture of (2*R*,3*R*)-**5a** and (2*R*,3*S*)-**6a** was treated with vinyl acetate in the presence of immobilized[9] *C. antarctica* lipase (Scheme 5). Under this transesterifying condition, the reaction of (2*R*,3*S*)-**6a** was faster than that of (2*R*,3*R*)-**5a**, with an $E(p)$[24] value of 15. At a conversion of 70%, (2*R*,3*R*)-**5a** was recovered in a diastereomerically pure state in 28% yield, while (2*R*,3*S*)-form was obtained as the corresponding



Scheme 5. *Candida antarctica* lipase-catalyzed reactions.



Scheme 6. Determination of relative and absolute configuration of **5a**.

acetate **6c** in 65% yield and 43%d.e.

The remaining task was the preparation of diastereomerically pure (2*R*,3*S*)-**6a**. For this purpose, (2*R*,3*S*)-**6c** [43%d.e.; a mixture of (2*R*,3*S*)-**6c** and (2*R*,3*R*)-**5c** in a ratio of 71.5 : 28.5] was hydrolyzed by the same *Candida antarctica* lipase (Novo, SP525) in aqueous acetone, expecting that (2*R*, 3*S*)-**6c** would react preferentially to give a diastereomerically enriched (2*R*,3*S*)-**6a** while leaving less reactive (2*R*,3*R*)-**5c**. To our surprise, (2*R*,3*S*)-**6a** with lower d.e. (2%) was obtained in 50% yield with the recovery of diastereomerically pure (2*R*,3*S*)-**6c** (40% yield) at a conversion of 56%. The *E*(p) value was 11 for (2*R*,3*R*)-isomer. These results mean that stereochemical preference is reversal between the transesterification in organic solvent (15 : 1) and the hydrolysis in aqueous organic solvent (1 : 11) by the same lipase. A similar reversal of selectivity had been observed by changing the environment of lipase-catalyzed reaction.[25]

As the separation of diastereomers (**5a** and **6a**) could successfully be established by a sequence of lipase-catalyzed reactions, the determination of relative and absolute configurations of the products became possible. The (3*R*) absolute configuration of **5a** was confirmed as follows. The present optically active **5a** (41%d.e.) was treated with hydrogen peroxide in acetic acid to give the corresponding sulfone **21** in 88% yield. Oxidation of secondary alcohol and subsequent nucleophilic attack of methyllithium on carbonyl group afforded a known sulfonyl alcohol (*R*)-**22** $[\alpha]_D^{21}$ −11.6° ($CHCl_3$) [lit,[20] $[\alpha]_D^{25}$ −33.7° ($CHCl_3$)], as shown in Scheme 6. An authentic sample of (±)-(2*R**,3*R**)-**21** was prepared from *cis*-2,3-epoxybutane and phenylsulfonylmethylsodium,[26] and its spectral properties coincided with those of the present optically active **21**. In this way, the absolute configurations of two chiral centers in **5a** and **6a** were unambiguously determined.

Conclusion

In this paper, we have demonstrated a *Pichia farinosa* IAM 4682 mediated reduction of sulfur containing ketones for preparing secondary alcohols with (*R*)-absolute configuration. In case that the ee of the product was not satisfactory, contaminating (*S*)-enantiomer in the resulting product was selectively oxidized by *Rhodococcus rhodochrous* IFO 15564 to leave pure (*R*)-enantiomers. Furthermore, a novel *Candida antarctica* lipase-catalyzed separation of the diastereomeric products were established, even when the stereochemistry of the product was not fully controlled by either of two microbial methods, as mentioned above.

Experimental

IR spectra were measured as films on a JASCO IRA-202 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ with TMS as the internal standard at 270 MHz on a JEOL JNM EX-270 or at 400 MHz on a JEOL JNM GX-400 spectrometer unless otherwise stated. Mass spectra were recorded on Hitachi M-80B spectrometer at 70 eV. Optical rotations were recorded on a JASCO DIP 360 polarimeter. Wako Gel B-5F and silica gel 60 K070-WH (70—230 mesh) of Katayama Chemical Co. were used for preparative TLC and column chromatography, respectively.

(*R*)-4-(phenylthio)-2-butanol (1a) by a *Pichia farinosa* IAM 4682 Mediated Reduction. *Pichia farinosa* IAM 4682 was preincubated according to the reported procedure.[7,8] The wet cells (20 g) were re-suspended in a phosphate buffer solution (pH 6.0, 0.1 M, M = mol dm^{-3}, 100 ml) and Antifoam (Nakalai Tesque Antifoam AF emulsion, 10%, 1 ml) in a 500-ml shaking culture (Sakaguchi) flask together with the substrate **8** [500 mg, 2.77 mmol, 0.5% (w/v)]. After we added glucose (5 g), the air inside the flask was purged with argon, and the flask was equipped with a balloon charged with argon. The flask was shaken at 30 °C on a gyrorotary shaker for 2 d. The cell mass was removed by a filtration with Celite. The filtrate was extracted with ethyl acetate several times and the cell mass was thoroughly washed with ethyl acetate. The combined extracts and washings were washed with brine, dried with sodium sulfate and concentrated in vacuo. The crude residue was purified by silica gel column chromatography (15 g). Elution with hexane/ethyl acetate (8/1) afforded (*R*)-(−)-**1a** (454 mg, 90%, 91%ee). Analytical sample: $[\alpha]_D^{20}$ −25.9° (*c* 0.99, $CHCl_3$). IR ν_{max} 3380, 3060, 2980, 2940, 1585, 1482, 1440, 1380, 1270, 1220, 1130, 1090, 940, 900, 880, 850, 745, 700 cm^{-1}; ^{1}H NMR (270 MHz) δ = 1.28 (d, *J* = 5.9 Hz, 3H), 1.83 (ddd, *J* = 5.9, 7.3, 7.4 Hz, 2H), 3.07 (dt, *J* = 14.8, 7.4 Hz, 1H), 3.14 (dt, *J* = 14.8, 7.3 Hz, 1H), 4.04 (tq, *J* = 5.9, 5.9 Hz, 1H), 7.20—7.28 (m, 1H), 7.31—7.44 (m, 4H). Its IR and NMR spectra were in good accordance with those reported for (*S*)-isomer.[3] Its ee was confirmed by ^{1}H NMR analysis of the corresponding MTPA ester **1b**. ^{1}H NMR (270 MHz) δ = 1.26 (d, *J* = 6.3 Hz, 2.87H), 1.32 (d, *J* = 6.3 Hz, 0.13H), 2.47 (br.s, 2.87H), 3.54 (br.s, 0.13H).

(*R*)-4-(Phenylsulfonyl)-2-butanol (2a). In the same procedure as described for **1a**, ketone **9** (500 mg, 2.36 mmol) was converted to (*R*)-(−)-**2a** (473 mg, 94%, 97%ee) by incubation with *Pichia farinosa* for 2 d. Analytical sample: $[\alpha]_D^{21}$ −21.1° (*c* 0.97, $CHCl_3$) [lit,[5] (*S*)-isomer (>95%ee) $[\alpha]_D^{23}$ +20.7° (*c* 1, $CHCl_3$)]. IR ν_{max} 3520, 3080, 2980, 2940, 1725, 1590, 1450, 1410, 1380, 1310, 1240, 1150, 1090, 1030, 940, 860, 800, 750, 700, 670, 600 cm^{-1}; ^{1}H NMR (270 MHz) δ = 1.20 (d, *J* = 6.1 Hz, 3H), 1.70—2.00 (m, 2H), 3.20 (ddd, *J* = 5.6, 9.9, 14.0 Hz, 1H), 3.30 (ddd, *J* = 5.9, 9.7, 14.0 Hz, 1H), 3.91 (ddq, *J* = 3.8, 12.2, 6.1 Hz, 1H), 7.52—7.70 (m, 3H), 7.88—7.94 (m, 2H). Its IR and NMR spectra were in good accordance with those reported for (*S*)-isomer.[5] Its ee was confirmed by ^{1}H NMR analysis of the corresponding MTPA ester **2b**. ^{1}H NMR (270 MHz) δ = 3.46 (br.s, 2.95H), 3.52 (br.s, 0.05H).

1-(Phenylthio)-3-pentanone (10). The reaction was carried out in a similar manner to the procedure reported for **8**.[3] 1-Penten-3-one (2.16 g, 25.7 mmol) was converted to **10** (4.63 g, 93%) as a colorless oil. Analytical sample: IR ν_{max} 3050, 2980, 2940, 1715, 1680, 1580, 1480, 1455, 1440, 1415, 1375, 1360, 1330, 1280, 1160, 1110, 1090, 1070, 1025, 970, 740, 695 cm^{-1}; ^1H NMR (270 MHz) δ = 1.05 (d, J = 7.3 Hz, 3H), 2.42 (q, J = 7.3 Hz, 2H), 2.73 (t, J = 7.3 Hz, 1H), 3.15 (t, J = 7.3 Hz, 1H), 7.16—7.36 (m, 5H). Its IR and NMR spectra were in good accordance with those reported previously.[27]

(*R*)-1-(Phenylthio)-3-pentanol (3a). In the same procedure as described for **1a**, ketone **10** (500 mg, 2.57 mmol) was converted to (*R*)-(−)-**3a** (505 mg, 93%, 93%ee) by incubation with *Pichia farinosa* for 2 d. Analytical sample: $[\alpha]_D^{23}$ −35.5° (*c* 1.12, $CHCl_3$). Its ee was confirmed by ^1H NMR analysis of the corresponding MTPA ester **3b**. ^1H NMR (270 MHz) δ = 0.74 (t, J = 7.4 Hz, 2.90H), 0.83 (t, J = 7.4 Hz, 0.10H), 3.43 (br.s, 2.90H), 3.49 (br.s, 0.10H).

3-Methyl-4-(phenylthio)-2-butanone (11). The reaction was carried out in a similar manner to the procedure reported for **8**.[3] 3-Methyl-3-buten-2-one (2.11 g, 25.0 mmol) was converted to **11**[28] (4.67 g, 96%) as a colorless oil. Analytical sample: IR ν_{max} 3100, 3000, 2950, 2900, 1720, 1585, 1480, 1460, 1440, 1420, 1360, 1300, 1250, 1160, 1120, 1100, 1075, 1030, 970, 910, 840, 750, 700, 670 cm^{-1}; ^1H NMR (270 MHz) δ = 1.27 (d, J = 7.1 Hz, 3H), 2.21 (s, 3H), 2.81 (ddd, J = 7.1, 13.2, 13.2 Hz, 1H), 2.90 (dd, J = 6.8, 13.2 Hz, 1H), 3.29 (dd, J = 6.8, 13.2 Hz, 1H), 7.22—7.40 (m, 5H).

A Mixture of (2*R*,3*R*)-3-Methyl-4-(phenylthio)-2-butanol (5a) and (2*R*,3*S*)-3-Methyl-4-(phenylthio)-2-butanol (6a). In the same procedure as described for **1a**, **11** (500 mg, 2.57 mmol) was converted to a mixture of diastereomers of alcohols (**5a** : **6a** = 1 : 1, 475 mg, 94%, >99%ee) by incubation with *Pichia farinosa* for 2 d. Its ee and d.e. was confirmed by ^1H NMR analysis of corresponding MTPA esters **5b** and **6b**. ^1H NMR (270 MHz) δ = 1.08 (d, J = 6.8 Hz, 1.50H), 1.09 (d, J = 6.8 Hz, 1.50H), 1.29 (d, J = 5.8 Hz, 1.50H), 1.31 (d, J = 5.9 Hz, 1.50H). No signal due to (2*S*)-isomer could be detected [1.03 (d, J = 6.9 Hz), 1.04 (d, J = 6.8 Hz), 1.36 (d, J = 6.3 Hz), 1.37 (d, J = 6.4 Hz)]. Its relative and absolute configurations were determined as described later.

(*R*)-1,1,1-Trifluoro-3-phenylthiopropan-2-ol (7a). In the same procedure as described for **1a**, ketone **12**[13] (500 mg, 2.27 mmol) was converted to (*R*)-(−)-**7a** (368 mg, 72%, 88%ee) by incubation with *Pichia farinosa* for 4 d. Analytical sample: $[\alpha]_D^{20}$ −62.2° (*c* 0.96, $CHCl_3$), $[\alpha]_D^{20}$ −71.7° (*c* 1.06, CH_3OH) [lit,[15] (*R*)-isomer $[\alpha]_D^{23}$ −67.4° (*c* 0.38, $CHCl_3$), $[\alpha]_D^{23}$ −77.7° (*c* 0.38, CH_3OH)]. IR ν_{max} 3450, 3060, 2930, 1585, 1485, 1440, 1375, 1275, 1170, 1130, 1100, 1030, 1005, 870, 745, 695, 680 cm^{-1}; ^1H NMR (270 MHz) δ = 2.77 (d, J = 4.5 Hz, 1H), 2.87 (dd, J = 10.0, 14.2 Hz, 1H), 3.18 (dd, J = 2.9, 14.2 Hz, 1H), 3.84 (ddd, J = 2.9, 4.5, 10.0 Hz, 1H), 7.11—7.31 (m, 5H). Its IR and NMR spectra were in good accordance with those reported previously.[13] Its ee was confirmed by ^1H NMR analysis of the corresponding MTPA ester **7b**. ^1H NMR (400 MHz) δ = 3.03 (dd, J = 10.4, 14.7 Hz, 0.06H), 3.12 (dd, J = 10.6, 14.7 Hz, 0.94H).

Enantioselective Oxidation of Alcohol 14a. A sterilized medium (pH 7.2, 100 ml) containing glucose (15 g dm^{-3}), KH_2PO_4 (0.4 g dm^{-3}), K_2HPO_4 (1.2 g dm^{-3}), $MgSO_4 \cdot 7H_2O$ (0.5 g dm^{-3}), yeast extract (1 g dm^{-3}), peptone (5 g dm^{-3}) in a 500-ml Erlenmeyer flask with two internal projections was inoculated with a loopful of *R. rhodochrous* IFO 15564. Then the flask was shaken at 30 °C on a gyrorotary shaker for 2 d. The cells were harvested by centrifugation. The wet cells were harvested by centrifugation and washed with 0.1 M phosphate buffer (pH 6.0). The mixture of **13**[9] (*E*/*Z* = 6/1, 570 mg, 4.09 mmol), wet cells (8.0 g) and 0.1 M phosphate buffer (pH 6.0, 57 mL) was stirred at 30 °C for 6 h. After the usual workup and the subsequent methylation with diazomethane and chromatographic purification, a ketone **15** (195 mg, 28%, from **13**) and an alcohol **14a** (338 mg 48% from **13**). **15**; IR ν_{max} 3020, 2970, 2940, 2860, 1740, 1715, 1435, 1415, 1380, 1250, 1195, 1165, 1095, 1015, 970, 885, 850 cm^{-1}; ^1H NMR δ = 2.15 (s, 3H), 2.25—2.40 (m, 2H), 2.52 (t, J = 6.0 Hz, 2H), 3.05 (dd, J = 1.5, 5.6 Hz, 2H), 3.77 (s, 3H), 5.50—5.75 (m, 2H). MTPA ester **14b**; ^1H NMR δ = 1.19 (d, J = 6.2 Hz, major, 88%), 1.21 (d, J = 6.2 Hz, minor, 12%), 1.27 (d, J = 6.2 Hz), 1.29 (d, J = 6.2 Hz); therefore, the major *E* isomer of recovered **14a** was concluded to be 76%ee.

Oxidation of (±)-4-(Phenylthio)-2-butanol (1a) by *Rhodococcus rhodochrous* IFO 15564. In the same manner as described above, *Rhodococcus rhodochrous* was incubated. The wet cells (12 g) were re-suspended in a phosphate buffer solution (pH 8.0, 0.01 M, 100 ml) and the substrate [300 mg, 1.65 mmol, 0.3% (w/v)] was added. After adding Antifoam (Nakalai Tesque Antifoam AF emulsion, 10%, 1 ml), the mixture was stirred at 30 °C with bubbling of air (70 ml min^{-1}). During the incubation, its pH was kept at 8.0 by a pH controller. An analytical sample (1.67 ml) was occasionally withdrawn. To this, chalcone (11.1 mg, as a solution in ethyl acetate) was added as an internal standard and the mixture was extracted with ethyl acetate. The progress of reaction was analyzed by ^1H NMR (270 MHz) δ = 2.07 (s, H-1 of **8**), 2.69 (dd, J = 7.3, 7.3 Hz, H-4 of **8**), 3.91 (ddq, J = 6.3, 6.3, 6.3 Hz, H-2 of **1a**), 7.75 (d, J = 15.8 Hz, H-3 of chalcone). The ee of the recovered **1a** was determined as already described. The results are illustrated in Fig. 3. Other substrates were incubated in a similar manner.

(*R*)- 4- (Phenylthio)- 2- butanol (1a) by a *Rhodococcus rhodochrous* Mediated Oxidation. According to the procedure described above, enantiomerically enriched (*R*)-(−)-**1a** (300 mg, 1.65 mmol, 91%ee) was incubated with *Rhodococcus rhodochrous* for 8 h. The cell mass was removed by a filtration with Celite. The filtrate was extracted with ethyl acetate several times and the cell mass thoroughly washed with ethyl acetate. The combined organic layer was washed with brine, dried with sodium sulfate and concentrated in vacuo. The crude residue was purified by silica gel column chromatography (8 g). Elution with hexane/ethyl acetate (8/1) afforded pure (*R*)-(−)-**1a** (229 mg, >99%ee) in 87% yield. Analytical sample: $[\alpha]_D^{20}$ −27.5° (*c* 0.97, $CHCl_3$) [lit,[3] (*S*)-isomer (96%ee) $[\alpha]_D^{23}$ +26.8° (*c* 1.34, $CHCl_3$)], $[\alpha]_D^{20}$ −35.7° (*c* 1.0, EtOH) [lit,[29] (94%ee) $[\alpha]_D$ −33.0° (*c* 1.1, EtOH)]. Its IR and NMR spectra were identical with those already described. Anal. Found: C, 65.59; H, 8.09%. Calcd for $C_{10}H_{14}OS$: C, 65.89; H, 7.74%.

(*R*)-4-(Phenylsulfonyl)-2-butanol (2a). A solution of hydrogen peroxide (30% in H_2O, 0.3 ml) was added to a solution of (*R*)-(−)-**1a** (100 mg, 0.55 mmol) in acetic acid (1 ml). Then the mixture was stirred under reflux at 80 °C for 2 h. After cooling to room temperature, a 10% $Na_2S_2O_3$ solution (20 ml) was added to the reaction mixture and acetic acid was removed by evaporation in vacuo. The residue was extracted five times with ethyl acetate (10 ml), and the organic layer was sequentially washed with 1 M NaOH solution (5 ml), H_2O (10 ml), saturated NH_4Cl solution (10 ml) and brine (10 ml), then dried with sodium sulfate and concentrated in vacuo. The crude residue was purified by silica gel column chromatography (4 g). Elution with hexane/ethyl acetate (2/1) yielded 100 mg (85%, >99%ee) of (*R*)-(−)-**2a** as a colorless oil. Analytical sample: $[\alpha]_D^{20}$ −21.8° (*c* 1.03, $CHCl_3$) [lit,[4] (*S*)-isomer (>95%ee) $[\alpha]_D^{23}$ +20.7° (*c* 1, $CHCl_3$)]. IR ν_{max} 3520, 3080, 2980, 2940, 1725, 1590, 1450, 1410, 1380, 1310, 1240, 1150,

1090, 1030, 940, 860, 800, 750, 700, 670, 600 cm^{-1}; ^1H NMR (270 MHz) δ = 1.20 (d, J = 6.1 Hz, 3H), 1.70—2.00 (m, 2H), 3.20 (ddd, J = 5.6, 9.9, 14.0 Hz, 1H), 3.30 (ddd, J = 5.9, 9.7, 14.0 Hz, 1H), 3.91 (ddq, J = 3.8, 12.2, 6.1 Hz, 1H), 7.52—7.70 (m, 3H), 7.88—7.94 (m, 2H). Its IR and NMR spectra were in good accordance with those reported for (*S*)-isomer.[4] Anal. Found: C, 56.21; H, 6.82%. Calcd for $C_{10}H_{14}O_3S$: C, 56.05; H, 6.59%. Its ee was confirmed by ^1H NMR analysis of the corresponding MTPA ester **2b**. ^1H NMR (270 MHz) δ = 3.46 (br.s, 3H). No signal due to an (*S*)-isomer could be detected [3.52 (br.s)].

(*R*)-1-(Phenylthio)-3-pentanol (3a). An alcohol (*R*)-(−)-**3a** (57.5 mg, 0.29 mmol, 93%ee) was treated with *Rhodococcus rhodochrous* in a similar manner to that already described for 10 h to afford (*R*)-(−)-**3a** with higher ee (49.8 mg, 87%, >99%ee). Analytical sample: $[\alpha]_D^{20}$ −38.6° (*c* 1.00, $CHCl_3$). IR ν_{max} 3360, 3050, 2960, 2920, 2870, 1580, 1480, 1460, 1440, 1380, 1340, 1270, 1220, 1160, 1120, 1090, 1070, 1020, 980, 920, 900, 870, 740, 690 cm^{-1}; ^1H NMR (270 MHz) δ = 0.91 (t, J = 7.4 Hz, 3H), 1.46 (m, 2H), 1.75 (m, 2H), 2.99 (dt, J = 13, 7.8 Hz, 1H), 3.09 (ddd, J = 8.0, 6.1, 13 Hz, 1H), 3.68 (m, 1H), 7.16—7.36 (m, 5H). Its IR and NMR spectra were in good accordance with those reported for racemate.[30] Anal. Found: C, 67.15; H, 9.06%. Calcd for $C_{11}H_{16}OS$: C, 67.30; H, 8.21%. Its ee was confirmed by ^1H NMR analysis of the corresponding MTPA ester **3b**. ^1H NMR (270 MHz) δ = 0.74 (t, J = 7.4 Hz, 2.91H), 0.83 (t, J = 7.4 Hz, 0.09H), 3.43 (br.s, 2.91H), 3.49 (br.s, 0.09H).

(*R*)-1-(Phenylsulfonyl)-3-pentanol (4a). In the same manner as described for **2a**, (*R*)-(−)-**3a** (156 mg, 0.79 mmol) was converted to 167 mg (98%, >99%ee) of (*R*)-(−)-**4a** as a colorless oil. Analytical sample: $[\alpha]_D^{20}$ −20.8° (*c* 1.00, $CHCl_3$) [lit,[4] (*S*)-isomer (64%ee) $[\alpha]_D^{25}$ +16° (*c* 1, $CHCl_3$)]. IR ν_{max} 3250, 3075, 2960, 2940, 2880, 1590, 1450, 1410, 1310, 1230, 1150, 1090, 1030, 1000, 980, 930, 870, 810, 750, 690, 670 cm^{-1}; ^1H NMR (270 MHz) δ = 0.91 (t, J = 7.4 Hz, 3H), 1.41—1.52 (m, 2H), 1.67—1.81 (br, 1H), 1.74 (dddd, J = 5.3, 9.0, 9.9, 14.0 Hz, 1H), 1.96 (dddd, J = 3.5, 5.9, 9.9, 10.1 Hz, 1H), 3.20 (ddd, J = 5.9, 9.9, 14.1 Hz, 1H), 3.32 (ddd, J = 5.3, 10.1, 14.1 Hz, 1H), 3.61 (m, 2H), 7.53—7.69 (m, 3H), 7.89—7.94 (m, 2H). Its IR and NMR spectra were in good accordance with those reported for (*S*)-isomer.[4] Anal. Found: C, 57.58; H, 7.36%. Calcd for $C_{11}H_{16}O_3S$: C, 57.87; H, 7.06%. Its ee was confirmed by ^1H NMR analysis of corresponding MTPA ester **4b**. ^1H NMR (400 MHz) δ = 3.45 (br.s, 1H). No signal due to an (*S*)-isomer could be detected [3.52 (br.s)].

Oxidation of Other Alcohols: 16 to 18. In the same manner as described for **1a**, alcohols **16**—**18** were incubated with *R. rhodochrous*. The structure of **19** was confirmed by its NMR analysis: ^1H NMR (270 MHz) δ = 1.30 (d, J = 6.3 Hz, 3H), 1.97 (dt, J = 13.9, 7.6 Hz, 2H), 2.36 (t, J = 7.6 Hz, 2H), 3.68 (s, 3H), 4.00 (dt, J = 13.9, 6.3 Hz, 1H). Its NMR spectrum was identical with that of an authentic sample of the corresponding racemate. The ee of these recovered alcohols **16**, **17**, and **19** were determined by ^1H NMR analyses of the corresponding (*R*)-MTPA esters. ^1H NMR (400 MHz) δ = 1.31 (d, J = 6.3 Hz, 2.25H), 1.38 (d, J = 6.3 Hz, 0.75H) for **16**. δ = 3.49 (br.s, 0.09H), 3.55 (br.s, 2.91H) for **17**. δ = 1.29 (d, J = 6.3 Hz, 2.92H), 1.39 (d, J = 6.3 Hz, 0.08H) for **19**.

Lipase-Mediated Separation of (2*R*,3*R*)-3-Methyl-4-(phenylthio)-2-butanol (5a) and (2*R*,3*S*)-3-Methyl-4-(phenylthio)-2-butanol (6a). A mixture of **5a** : **6a** (1 : 1, total 20.0 mg, 0.10 mmol) vinyl acetate (0.5 ml) and *Candida antarctica* lipase (Novo, SP525, 22.0 mg) immobilized with Florisil[9] was stirred for 10 h at 30 °C. The conversion (70%) and diastereomeric excess were estimated by the integration of signals of **5a** (δ = 3.70), **6a** (δ = 3.96), **5c** (δ = 4.88) and **6c** (δ = 5.01). After having been filtered through a pad of Celite, the filtrate was concentrated in vacuo. The crude residue was purified by preparative TLC (hexane/ethyl acetate = 4/1) and afforded 5.5 mg (28%, >99%d.e.) of **5a** and 15.7 mg (65%, 43%d.e.) of **6c**. Analytical sample of **5a**: $[\alpha]_D^{20}$ −49.5° (*c* 0.41, $CHCl_3$). IR ν_{max} 3400, 3050, 2970, 2940, 1580, 1480, 1450, 1440, 1380, 1300, 1270, 1240, 1190, 1140, 1090, 1070, 1025, 1000, 970, 930, 890, 840, 740, 695, 665 cm^{-1}; ^1H NMR (400 MHz) δ = 0.97 (d, J = 6.9 Hz, 3H), 1.12 (d, J = 6.3 Hz, 3H), 1.51 (br.s, 1H), 1.72 (m, 1H), 2.72 (dd, J = 8.1, 12.9 Hz, 1H), 3.13 (dd, J = 4.6, 12.9 Hz, 1H), 3.70 (dq, J = 6.3, 6.3 Hz, 1H), 7.06—7.12 (m, 2H), 7.18—7.30 (m, 3H). Anal. Found: C, 67.15; H, 8.39%. Calcd for $C_{11}H_{16}OS$: C, 67.30; H, 8.22%. Its ee and d.e. were confirmed by ^1H NMR analyses of the corresponding MTPA ester **5b**. ^1H NMR (270 MHz) δ = 1.08 (d, J = 6.8 Hz, 3H), 1.29 (d, J = 5.8 Hz, 3H). No signal due to (2*S*)-isomers [1.03 (d, J = 6.9 Hz), 1.04 (d, J = 6.8 Hz), 1.36 (d, J = 6.3 Hz), 1.37 (d, J = 6.4 Hz)] or (2*R*,3*R*)-isomer [1.09 (d, J = 6.8 Hz), 1.31 (d, J = 5.9 Hz)] could be detected.

Analytical sample of **6c** [43%d.e., i.e. a mixture of **6c** + **5c** (71.5 : 28.5)]: IR ν_{max} 3060, 2980, 2940, 1740, 1585, 1485, 1440, 1375, 1245, 1150, 1135, 1090, 1070, 1040, 1020, 950, 920, 850, 750, 600 cm^{-1}; ^1H NMR (270 MHz): **6c** (71.5% of the total signals) δ = 1.05 (d, J = 6.9 Hz, 3H), 1.18 (d, J = 6.4 Hz, 3H), 1.87 (dddd, J = 3.9, 5.2, 6.9, 8.5 Hz, 1H), 2.02 (s, 3H), 2.68 (dd, J = 8.5, 13.0 Hz, 1H), 3.06 (dd, J = 5.2, 13.0 Hz, 1H), 5.01 (dq, J = 3.9, 6.4 Hz, 1H), 7.07—7.35 (m, 5H); **5c** (28.5% of the total signals) δ = 1.03 (d, J = 6.9 Hz, 3H), 1.17 (d, J = 6.4 Hz, 3H), 1.85 (dddd, J = 4.5, 6.3, 6.9, 8.5 Hz, 1H), 2.01 (s, 3H), 2.64 (dd, J = 8.5, 12.9 Hz, 1H), 3.09 (dd, J = 4.5, 12.9 Hz, 1H), 4.88 (dq, J = 6.3, 6.4 Hz, 1H), 7.07—7.34 (m, 5H).

A mixture of the acetate **6c** (25.1 mg, 43%d.e., >99%ee), *Candida antarctica* lipase (Novo, SP525), acetone and pH 7.0 phosphate buffer (2 : 3, 0.5 ml) was stirred for 11 h at room temperature. The conversion (56%) was determined in the same manner as stated for the lipase-catalyzed acylation. The mixture was extracted five times with ethyl acetate (5 ml), and the organic layer was washed with brine, dried with sodium sulfate and concentrated in vacuo. The crude residue was purified by a preparative TLC (hexane/ethyl acetate = 4/1) to afford 10.1 mg (40%, >99%d.e.) of **6c** and 10.3 mg of **5a** (50%, 2%d.e.). The acetate **6c** was treated overnight with sodium methoxide in methanol at room temperature. Then, the mixture was concentrated in vacuo, extracted three times with ethyl acetate (5 ml). The combined ethyl acetate solution was washed with brine, dried with sodium sulfate and concentrated in vacuo. The crude product was purified by preparative TLC (hexane/ethyl acetate = 4/1) to afford 8.4 mg (quant, >99%d.e., >99%ee) of **6a** as a colorless oil. Analytical sample of **6a**: $[\alpha]_D^{20}$ +17.9° (*c* 1.00, $CHCl_3$). IR ν_{max} 3400, 3050, 2970, 2940, 1580, 1480, 1450, 1440, 1380, 1300, 1270, 1240, 1190, 1140, 1090, 1070, 1025, 1000, 970, 930, 890, 840, 740, 695, 665 cm^{-1}; ^1H NMR (270 MHz) δ = 1.00 (d, J = 6.8 Hz, 3H), 1.16 (d, J = 6.4 Hz, 3H), 1.42 (br.s, 1H), 1.75 (m, 1H), 2.77 (dd, J = 7.8, 13.2 Hz, 1H), 3.10 (dd, J = 6.4, 13.2 Hz, 1H), 3.96 (dq, J = 3.4, 6.4 Hz, 1H), 7.06—7.12 (m, 2H), 7.18—7.30 (m, 3H). Anal. Found: C, 67.15; H, 8.39%. Calcd for $C_{11}H_{16}OS$: C, 67.30; H, 8.22%. Its ee was confirmed to be >99% by the NMR spectrum of **6b**.

(2*R*,3*R*)-3-Methyl-4-(phenylsulfonyl)butan-2-ol (21). In the same manner as described for **2a** (2*R*,3*R*)-**5a** (31.0 mg, 0.16 mmol, 41%d.e.) was converted to **21** (31.7 mg, 88%, 41%d.e.) as a colorless oil. Analytical sample: IR ν_{max} 3500, 3060, 2970, 2930, 2320, 1445, 1400, 1380, 1300, 1240, 1145, 1080, 1020, 1000, 930,

895, 845, 780, 750, 720, 690 cm^{-1}; IR ^1H NMR (400 MHz) of *anti*-isomer $\delta = 1.11$ (d, $J = 6.8$ Hz, 3H), 1.14 (d, $J = 6.4$ Hz, 3H), 2.04 (dddq, $J = 2.9$, 6.4, 8.3, 6.8 Hz, 1H), 2.91 (dd, $J = 8.3$, 14.2 Hz, 1H), 3.44 (dd, $J = 2.9$, 14.2 Hz, 1H), 3.61 (dq, $J = 6.4$, 6.4 Hz, 1H), 7.53—7.92 (m, 5H); ^1H NMR (400 MHz) of *syn*-isomer $\delta = 1.02$ (d, $J = 6.8$ Hz, 3H), 1.09 (d, $J = 6.4$ Hz, 3H), dddq, $J = 3.4$, 4.9, 8.3, 6.8 Hz, 1H), 2.91 (dd, $J = 8.3$, 14.2 Hz, 1H), 3.37 (dd, $J = 4.9$, 14.2 Hz, 1H), 3.95 (dq, $J = 3.4$, 6.4 Hz, 1H), 7.53—7.92 (m, 5H). This was employed in the next step without further purification.

(*R*)-2,3-Dimethyl-4-(phenylsulfonyl)-2-butanol (22). A solution of **21** (30.0 mg, 0.13 mmol, 41%d.e.) in Et_2O (2 ml) was cooled in an ice-water bath. To this solution, Brown's chromic acid solution[30] (1.3 ml) was added over 2 min. The mixture was stirred for 1 h at room temperature. After the reaction had been quenched by adding 2-propanol, the mixture was concentrated in vacuo to remove an excess 2-propanol and acetone. The residue was extracted four times with ethyl acetate (10 ml) and washed with saturated $NaHCO_3$ solution, brine, dried over sodium sulfate, and concentrated in vacuo. The crude residue was purified by preparative TLC (hexane/ethyl acetate = 1/1) to give 29.4 mg (99%) of the desired product as a colorless oil. Analytical sample: IR ν_{max} 3060, 2980, 2930, 2850, 2320, 1720, 1580, 1445, 1400, 1360, 1305, 1240, 1145, 1115, 1085, 1020, 1000, 920, 870, 850, 795, 750, 690 cm^{-1}; ^1H NMR (400 MHz) $\delta = 1.26$ (d, $J = 7.1$ Hz, 3H), 2.19 (s, 3H), 2.97 (dd, $J = 5.1$, 14.2 Hz, 1H), 3.22 (ddt, $J = 5.1$, 7.1, 7.1 Hz, 1H), 3.74 (dd, $J = 7.1$, 14.2 Hz, 1H), 7.54—7.67 (m, 3H), 7.87—7.89 (m, 2H). This was employed in the next step without further purification.

To a solution of this ketone (28.0 mg, 0.12 mmol) in dry THF (1 ml) was added MeLi (1.06 M in Et_2O, 0.2 ml) at 0 °C under argon. Stirring of the solution was continued at the same temperature for 10 min. Then, a saturated NH_4Cl solution (3 ml) was added. The organic layer was separated, and the aqueous layer was extracted four times with ethyl acetate (10 ml). The combined organic layer was dried with sodium sulfate and concentrated in vacuo. The crude residue was purified by preparative TLC (hexane/ethyl acetate = 1/1) to give 14.4 mg (48%) of **22** as a colorless oil. Analytical sample: $[\alpha]_D^{21}$ −11.6° (*c* 0.72, $CHCl_3$) [lit,[20] $[\alpha]_D^{25}$ −33.7° (*c* 1.26, $CHCl_3$)]. IR ν_{max} 3530, 3080, 2990, 2950, 2350, 1585, 1450, 1380, 1250, 1150, 1105, 1090, 1025, 1000, 955, 920, 875, 845, 800, 750, 695, 600 cm^{-1}; ^1H NMR (400 MHz) $\delta = 1.03$ (s, 3H), 1.12 (d, $J = 7.1$ Hz, 3H), 1.22 (s, 3H), 2.14 (ddt, $J = 2.1$, 9.1, 7.1 Hz, 1H), 2.81 (dd, $J = 9.1$, 14.1 Hz, 1H), 3.59 (dd, $J = 2.1$, 14.1 Hz, 1H), 7.54—7.67 (m, 3H), 7.91—7.94 (m, 2H). Its IR and NMR spectra were in good accordance with those reported previously.[20] Anal. Found: C, 59.20; H, 7.79%. Calcd for $C_{12}H_{18}O_3S$: C, 59.48; H, 7.49%.

Conversion of *cis*-2,3-Epoxybutane to (±)-(2*R,3*R**)-21.** According to the reported procedure,[26] *cis*-2,3-epoxybutane (Aldrich, 97%, 50.0 mg, 0.69 mmol) was treated with α-sulfonyl carbanion of phenyl methyl sulfone (132.6 mg, 1.07 mmol) to give 76.5 mg (48%) of (2*R**,3*R**)-**21**. Its IR and NMR spectra were in good accordance with an optically active (2*R*,3*R*)-**21** as mentioned previously.

The authors deeply thank Professors Tamotsu Fujisawa and Makoto Shimizu of Mie University for the discussion on the absolute configuration of fluorine-containing product. We are grateful to Novo Nordisk Co. and Amano Pharmaceutical Co. for the supply of lipases. This work was supported by a Grant-in-Aid for Scientific Research No. 08660140 from the Ministry of Education, Science, Sports and Culture, which is acknowledged with thanks.

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