Pergamon

Tetrahedron: Asymmetry Vol. 5, No. 10, pp. 1935-1944, 1994
Elsevier Science Ltd
Printed in Great Britain
0957-4166/94 $7.00+0.00

0957-4166(94)00275-4

Application of Microbial Enantiofacially Selective Hydrolysis in Natural Product Synthesis

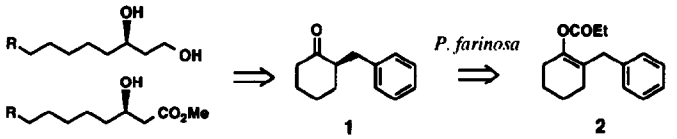
Osamu Katoh, Takeshi Sugai, and Hiromichi Ohta*

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

Abstract: *Pichia farinosa* IAM 4682 mediated enantiofacially selective hydrolysis worked efficiently (65-70% yield) on the interface-bioreactor in a reproducible manner, which established the product, (*R*)-2-benzylcyclohexanone (84-87%*e.e.*), to be the starting material for the synthesis of optically active natural products. Methyl (*R*)-3-hydroxy-12-methyltridecanoate, a constituent of lipopolysaccharide, and (*R*)-1,3-nonanediol, a secretion of cucumber fly, were synthesized *via* this common intermediate, of which the optically active secondary alcohol moiety was derived from the above chiral ketone by Baeyer-Villiger oxidation. Final products were enantiomerically enriched to 94-95% *e.e.*, by the lipase-mediated enantioselective transesterification, which could remove the minor enantiomer as the corresponding acetate.

Introduction

Optically active α-substituted ketones are important starting matrials for natural product synthesis.[1] Among a number of synthetic methods for the preparation of such compounds, the enantiofacially selective protonation of the corresponding enolate[2] has attracted much attention, because the starting material, the enolates, are readily available from the racemic α-substituted ketones *via* deprotonation. We were the first to develop the hydrolysis of enol esters by yeast or lipases which causes the enzymatic enatiofacially selective protonation, and that was particularly successful from the synthetic standpoint .[2a-g] Those results prompted us to apply this protocol to the synthesis of natural products, which possess 1,3-bifunctional groups.



1 **2**

Scheme I

Interface-Bioreactor Mediated Microbial Hydrolysis

In the present work, the starting material is (*R*)-2-benzylcyclohexanone (**1**). The hydrolysis of the corresponding enol propionate by whole cells of *Pichia farinosa* IAM 4682 has already been shown to give the desired product.[2b] Through this study, however, it turned out that a large amount of yeast cells was required to get both high yield (75%) and enantiomeric excess (*e.e.*, 84%) of (*R*)-**1**. On occasion, such a large amount of cell mass (15 g of wet cell / 80 mg of enol ester **2**, in 40 mL of buffer) causes a severe emulsion during the extraction procedure. The demand for a large-scale preparation prompted us to establish another procedure improved in both of incubation and workup.

At first, the best way to avoid the tedious extraction procedure seemed to be the use of immobilized biocatalysts in organic solvents.[3] The immobilized yeast was prepared by using conventional supports, such as sodium alginate,[3c] κ-carageenan,[4] and photo-sensitive polymers.[5] To our disappointment, however, the hydrolysis with immobilized cells resulted in only very poor conversions (0–5%) even after prolonged incubation. Apparently, the immobilization of *P. farinosa* caused damage to the hydrolytic enzyme.

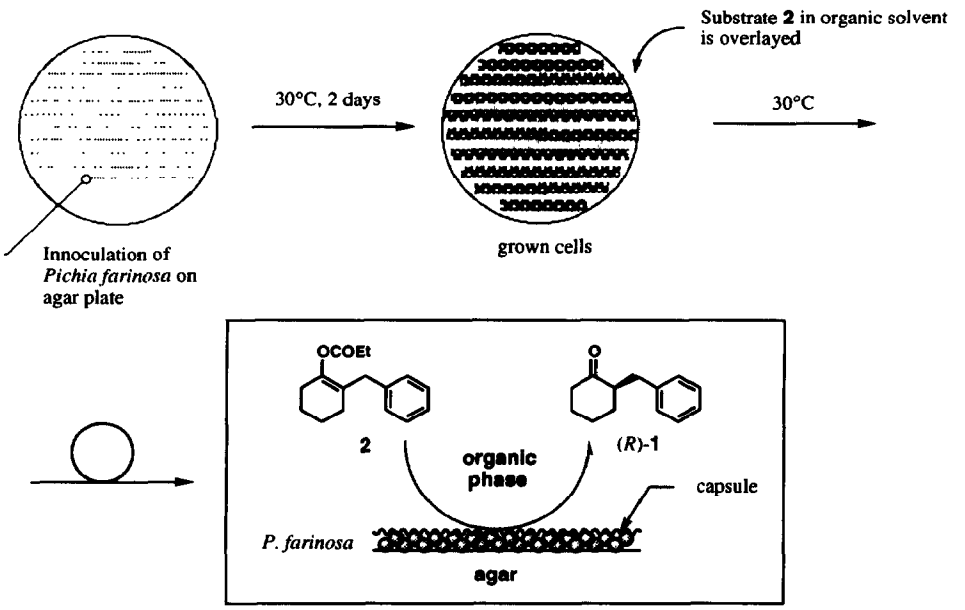
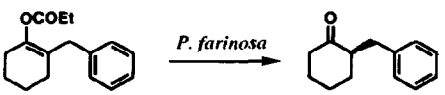


Figure I

The problem was successfully solved by the use of interface-bioreactor,[6] which has recently been developed as an alternative to immobilized microorganisms in organic solvents. The interface-bioreactor is set up and works as illustrated in Fig. I (see above). The microorganism is grown on a hydrophilic carrier (agar plate), and subsequently a solution of substrate **2** in hydrophobic solvent is overlaid. After the reaction has proceeded in cells on the solvent-carrier interface, the product **1** is recovered form the organic layer. Two advantages in this system has been advocated: 1) a hydrophilic polysaccharide layer (capsule) is developed on the interface, and this layer protects the microorganism against toxic compounds; 2) freshly grown microorganism can be used directly in organic solvents, without any lowering the activity of enzyme during the process of immobilization.

Table I. Effect of the solvent on the interface-bioreactor mediated hydrolysis of **2**[a]

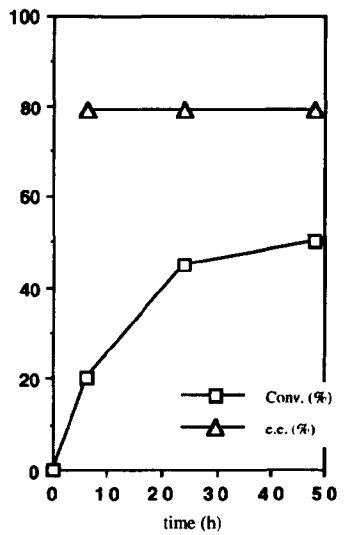


solvent	recovery of **2** (%)	yield of **1** (%)	*e.e.* of **1** (%)[b]
hexane	61	trace	–
diisopropyl ether	50	trace	–
toluene	70	trace	–
isooctane	23	42	67
liquid paraffin	51	17	74

a) **2** (80 mg) / solvent (40 mL) on *Pichia farinosa* grown on agar plate (56 cm^2); 30 °C, 1 day.
b) determined by HPLC analysis (Daicel, Chiralcel OJ).

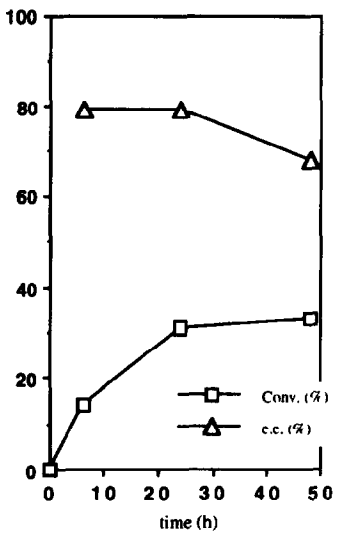
To begin with the use of interface-bioreactor, the effect of solvent which is overlaid on *P. farinosa* was examined. Among the hydrophobic solvents tested,[cf.6] it turned out that isooctane (2,2,4-trimethylpentane) was the best choice (Table I). Isooctane has an appropriate medium boiling point (99 °C), low viscosity, and especially, low toxicity which is characteristic for branched chain hydrocarbons.

Next, the optimization of the incubation conditions was investigated. The conversion, productivity and *e.e.* of the product depend on the two variables, 1) the ratio of [amount of cell mass / the amount of enol ester **2**]; 2) the incubation time. First, 80 mg of the substrate **2** was charged on grown cells of *P. farinosa* (on glucose-agar plate, 56 cm^2). A smooth hydrolysis proceeded to give **1**, the conversion reaching to 50% after 48 h incubation. At intervals, the *e.e.* of the product was checked and it showed a constant value (*ca.* 80%, Fig. II). Encouraged by this result, the feeding amount of **2** was raised 5 times (400 mg on the same plate), expecting that the productivity would increase. The result, however, turned out to be disappointing. The incubation at a higher charge of substrate has a really malignant effect on the *e.e.* of the product, which dropped to as low as 69% (Fig. III), especially after prolonged incubation.



2 (80 mg) / isooctane (40 mL)
on *Pichia farinosa* grown on agar plate (56 cm^2)

Fig. II



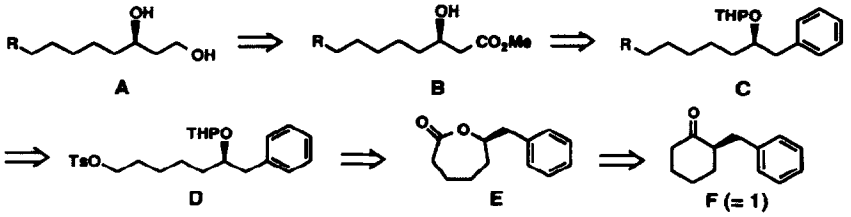
2 (400 mg) / isooctane (40 mL)
on *Pichia farinosa* grown on agar plate (56 cm^2)

Fig. III

Based on the information obtained so far, the interface-bioreactor was set up and the reaction was carried out as follows. *P. farinosa* was grown on an agar plate (638 cm^2 in an aluminum rectangle vessel, 21 g of wet cells) and 500 mg of **2** in 50 mL of isooctane was overlaid. The hydrolysis proceeded to 98% conversion within 1 day, in a highly reproducible manner. The desired product was easily recovered only by distillation of organic materials in 65-70% with 84-87% *e.e.* In this way, the microbial enantiofacially selective hydrolysis mediated in an interface-bioreactor established (*R*)-2-benzylcyclohexanone (**1**) as starting material for the synthesis of optically active natural products.

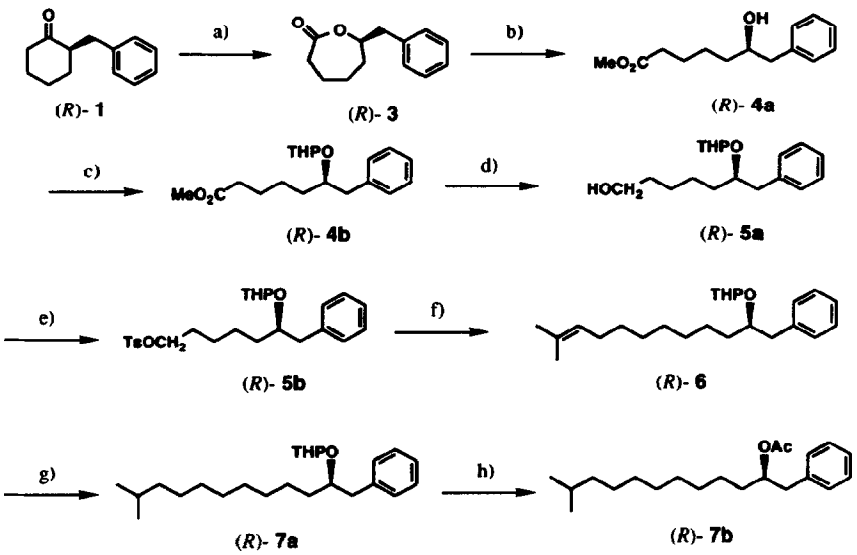
Synthesis of Methyl (*R*)-3-Hydroxy-12-methyltridecanoate

The synthetic plan to optically active 3-hydroxy acids and1,3-diols is shown in Scheme II. The primary hydroxyl group in **A** and ester group in **B** would be derived from the oxidation of aromatic group in **C**. The alkyl (R) chain extension of tosylate **D** will give **C**. Optically active secondary alcohol moiety in **C** can be derived from the Baeyer-Villiger oxidation of (*R*)-2-benzylcyclohexanone (**F** = **1**). This scheme enables the synthesis of 3-hydroxy acids or 1,3-diols *via* **D**, of which absolute configuration is unambiguously established to be (*R*).



Scheme II

Our primary target molecule is methyl (*R*)-3-hydroxy-12-methyltridecanoate, an *iso*-type branched long chain β-hydroxy acid ester, found in a rickettsia, *Coxiella burnetii*.[7] Long chain β-hydroxy acid derivatives are widespread in nature, and work as the architecture of cell walls of microorganisms, especially in lipopolysaccharides and are responsible for endotoxicity, antitumor activity and adjuvanticity. The lipopolysaccharide of *Coxiella burnetii* consist of mainly the *iso*- and *anteiso*-type β-hydroxy acids, whose (*R*)-configurations have been suggested based on the GLC analysis of the diastereomeric derivatives.[7] 3-Hydroxy-12-methyltridecanoate has also been found in liposidomycin B, a microbial metabolite which inhibits bacterial peptidoglycan synthesis.[8]

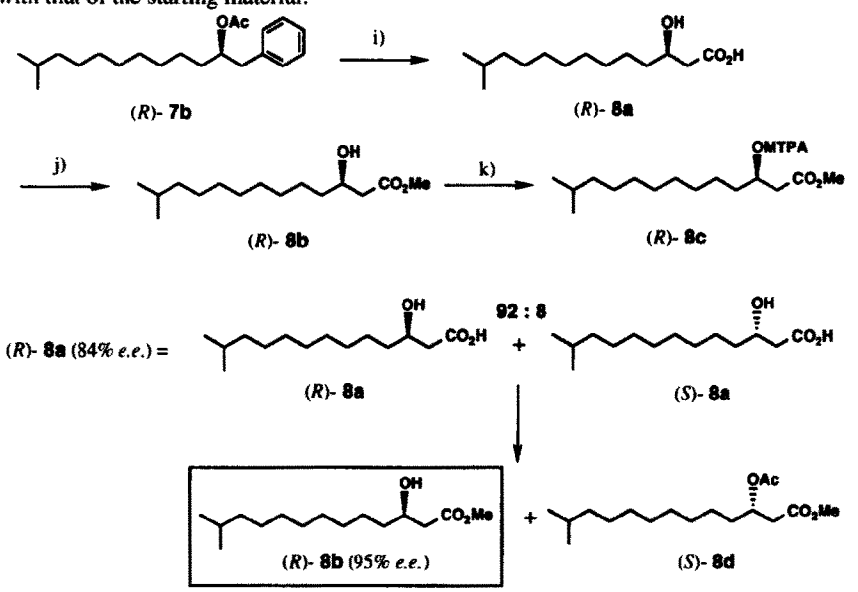


a) mCPBA / CH_2Cl_2; b) NaOH-H_2O-EtOH, H^+, then CH_2N_2; c) DHP, PPTS / CH_2Cl_2; d) $LiAlH_4$ / Et_2O; e) *p*-TsCl / C_5H_5N; f) R_2LiCu / Et_2O; g) H_2, Pd-C / EtOH; h) PPTS / EtOH, then Ac_2O / C_5H_5N.

Scheme III

Toward this end, Baeyer-Villiger oxidation of (*R*)-**1** afforded lactone (*R*)-**3** (99%). It is well known that Baeyer-Villiger oxidation proceeds with retention of configuration, and the *e.e.* of (*R*)-**3** (84%*e.e.*) was in good accordance with that of the starting material. The preparation of the key-intermediate (*R*)-**5b** (83%, 5 steps) was carried out in a sequence of conventional steps. The alkylation of tosylate **5b** was realized only by the use of lithium dialkylcuprate in ether at low temperature[9] to give (*R*)-**6** (87%). For the sake of further transformation, the THP protective group was replaced by acetyl group to afford (*R*)-**7b** (90%).

The remaining step is the conversion of the aromatic ring to the desired carboxylate moiety. We carefully examined this transformation by using the combination of catalytic amounts of ruthenium oxide and sodium metarperiodate.[cf.10] It turned out that prolonged reaction brought about the further degradation of the desired product. Thus the reaction was stopped before all the starting material disappeared on TLC. After methylation, (*R*)-**8b** was obtained (85%, based on the consumed starting material) along with the recovery of **7b** (15%). Based on the ^{1}H NMR measurement of the corresponding α-methoxy-α-trifluoromethylphenylacetate (MTPA[11] ester) **8c**, the *e.e.* of **8b** was determined to be 84%, which was consistent with that of the starting material.



i) $RuCl_3$, $NaIO_4$; j) CH_2N_2; k) MTPA-Cl, C_5H_5N; l) *Pseudomonas* lipase, vinyl acetate, BHT, THF.

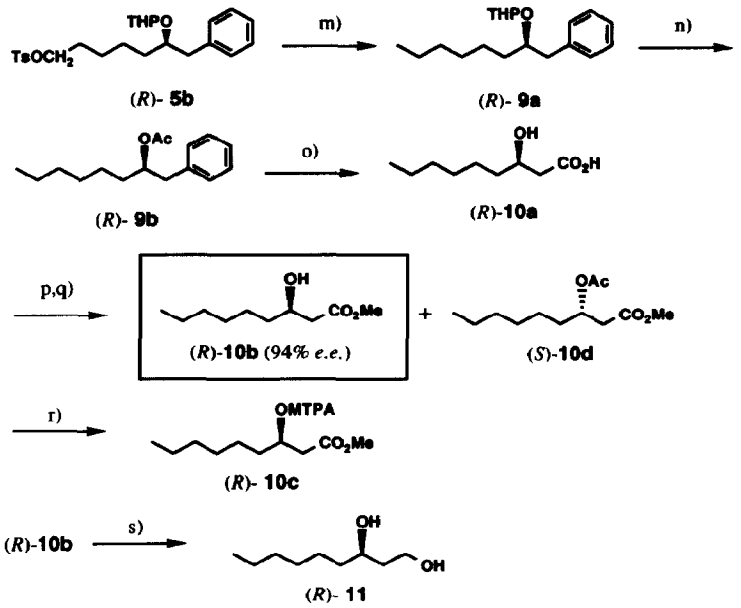
Scheme IV

For the enhancement of *e.e.* of the final product, lipase-catalyzed enantioselective transesterification was utilized.[12] Thus, at the stage of β-hydroxy acid **8a**, the contaminated (*S*)-**8a** (*ca.* 8%) could be removed as its corresponding acetate, by the treatment of *Pseudomonas* lipase (Amano PS) and vinyl acetate. Subsequent methylation afforded enantiomerically enriched (*R*)-**8b** (82% yield, 95%*e.e.*) together with (*S*)-**8d** (4% yield, 54%*e.e.*). In this way, starting from (*R*)-2-benzylcyclohexanone (**1**), methyl (*R*)-3-hydroxy-12-methyltridecanoate of high *e.e.* was synthesized, whose (*R*)-absolute configuration is unambiguously defined.

Synthesis of (*R*)-1,3-Nonanediol

The synthetic scheme was then extended to (*R*)-1,3-nonanediol, a component of rectal gland secretion of male cucumber fly, *Dacus cucumis*.[13] The tosylate **5b** was treated with lithium dimethylcuprate to afford the

product **9a** which had a desired carbon skeleton. According to the procedure as described above, this was converted into a β-hydroxy ester (*R*)-**10b** with high *e.e.* (94%). Finally, $LiAlH_4$ treatment afforded (*R*)-1,3-nonanediol (**11**), $[\alpha]_D^{18}$ –6.8 (EtOH) [lit.[13b] $[\alpha]_D^{20}$ –5.4 (EtOH)].



m) Me_2LiCu / Et_2O; n) PPTS / EtOH, then Ac_2O / C_5H_5N; o) $RuCl_3$, $NaIO_4$;
p) *Pseudomonas* lipase, vinyl acetate, BHT, THF; q) CH_2N_2; r) MTPA-Cl, C_5H_5N; s) $LiAlH_4$ / Et_2O

Scheme V

Conclusion

Interface-bioreactor mediated enantiofacially selective microbial hydrolysis of enol esters established the preparation of (*R*)-2-benzylcyclohexanone (**1**). The product was shown to be a useful starting material for the synthesis of optically active natural products, which possess 3-hydroxy acid or 1,3-diol moieties.

EXPERIMENTAL

All b.ps were uncorrected. IR spectra were measured as films on a Jasco IRA-202 spectrometer. ^1H NMR spectra were measured in $CDCl_3$ with TMS as the internal standard at 90 MHz on a JEOL JNM FX-90 spectrometer unless otherwise stated. Hitachi 163 Gas chromatograph and Shimadzu LC-5A liquid chromatograph were used for GLC and HPLC analyses, respectively. Optical rotations were recorded on a Jasco DIP 360 polarimeter. Freshly distilled THF and Et_2O from sodium-benzophenone ketyl for anhydrous reaction. Wako Gel B-5F and silica gel 60 K070-WH (70-230 mesh) of Katayama Chemical Co. were used for prep TLC and column chromatography, respectively.

(R)-2-benzylcyclohexanone **1**. The interface-bioreactor was set up as follows. A glucose-agar medium [containing glucose (1.0%), peptone (0.7%), yeast extract (0.5%), K_2HPO_4 (0.5%), agar (2.0%), pH 7.5, total volume 500 mL] was poured in an aluminum rectangle vessel [29 cm x 22 cm (=638 cm^2)] and solidified. On that plate, loopfuls of *P. farinosa* was streaked aseptically (see Fig. I) and grown at 30 °C for 2 days. Then a soln of **2** (500 mg, 2.5 mmol) in isooctane (50 mL) was overlaid, and the vessel was sealed with a rid and left to stand at 25 °C. After 24 h, GLC analysis showed the reaction proceeded to 98%; GLC (column, OV-1, 2 m, 100 °C + 2 °C/min; N_2, 0.6 kg/cm^2) t_R 2.3 min (98%), 5.7 min (2%). Then the organic layer was recovered and the cell mass was stirred vigorously with EtOAc. The combined organic solution was washed with brine and concentrated *in*

vacuo. The residue was dissolved in hexane/EtOAc (20/1) and the soln was passed through a pad of SiO_2 (10 g). Subsequent distillation afforded **1** (250 mg, 66%), b.p. 170-175 °C/1.5 Torr (bulb-to-bulb distillation); $[\alpha]_D^{20}$ +35.2 (c=1.16, MeOH) [lit.[2b] $[\alpha]_D^{26}$ +40.0 (c=1.01, MeOH)]; HPLC analysis: column, Daicel Chiralcel OJ 25 cm x 4.6 mm; solvent, hexane-*i*-PrOH (180:1); flow rate: 1.0 mL/min, detected at 254 nm: t_R 15.7 min (92.7%) 18.4 min (7.3%). IR vmax 3045, 2945, 2850, 1715, 1600, 1500, 1450, 1130, 735, 705 cm^{-1}; ^1H NMR δ 1.10-2.25 (6H, m), 2.26-2.90 (3H, m), 3.05-3.45 (2H, m), 7.09-7.41 (5H, m). Its IR and NMR spectra were identical with those reported previously.[2b]

(R)-7-benzyloxacycloheptan-2-one **3**. To a stirred soln of **1** $[[\alpha]_D^{22}$ +36.5 (c=1.87, MeOH), 0.96 g, 5.1 mmol] in CH_2Cl_2 (40 mL) was added mCPBA (3.0 g, 70% purity, 12 mmol) with ice-cooling and the mixture was stirred overnight at room temp. The mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with 10% $Na_2S_2O_3$ aq. soln, 10% K_2CO_3 aq. soln and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (25 g). Elution with hexane/EtOAc (4/1) and subsequent distillation afforded **3** (1.1 g, 99%), b.p. 170-180 °C/1.0 Torr (bulb-to-bulb distillation); $[\alpha]_D^{19}$ –40.2 (c=1.22, $CHCl_3$); HPLC analysis: column, Daicel Chiralcel OJ 25 cm x 4.6 mm; solvent, hexane-*i*-PrOH (9:1); flow rate: 1.0 mL/min, detected at 254 nm: t_R 31.5 min (8.3%) 40.2 min (91.7%). IR vmax 3025, 2945, 2855, 1725, 1600, 1500, 1450, 1350, 1330, 1280, 1255, 1170, 1130, 1050, 755, 705 cm^{-1}; ^1H NMR δ 1.20-2.15 (6H, m), 2.48-2.68 (2H, m), 2.78 (1H, dd, J = 7.0, 15.0 Hz), 3.18 (1H, dd, J = 7.0, 15.0 Hz), 4.20-4.60 (1H, m), 7.19-7.40 (5H, m) (Found: C, 76.22; H, 8.12. Calc. for $C_{13}H_{16}O_2$: C, 76.44; H, 7.89%.)

Methyl (R)-6-hydroxy-7-phenylheptanoate **4a**. To a stirred soln of **3** (1.0 g, 4.9 mmol) in EtOH (40 mL) and water (10 mL) was added 10 N NaOH aq. soln (2.7 mL, 27 mmol) and the mixture was stirred overnight. EtOH was evaporated *in vacuo* and the resulting mixture was diluted with water. The pH of the solution was adjusted to 1.0 by the addition of 6 N HCl aq. soln and the mixture was extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was treated with diazomethane solution, and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (20 g). Elution with hexane/EtOAc (5/1) and subsequent distillation afforded **4a** (1.0 g, 91%), b.p. 175-185 °C/0.7 Torr (bulb-to-bulb distillation); $[\alpha]_D^{20}$ –6.7 (c=1.59, $CHCl_3$); IR vmax 3450, 3025, 2945, 2855, 1735, 1600, 1500, 1450, 1435, 1200, 1175, 1090, 755, 705 cm^{-1}; ^1H NMR δ 1.30-1.85 (7H, m), 2.33 (2H, t, J = 6.3 Hz), 2.55 (1H, dd, J = 7.0, 12.5 Hz), 2.85 (1H, dd, J = 7.0, 12.5 Hz), 3.67 (3H, s), 3.70-4.00 (1H, broad), 7.11-7.48 (5H, m). (Found: C, 70.76; H, 8.68. Calc. for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53%.)

Methyl (6R)-6-tetrahydropyranyloxy-7-phenylheptanoate **4b**. A mixture containing **4a** (0.94 g, 4.0 mmol), dihydropyran (0.54 g, 6.4 mmol), pyridinium *p*-toluenesulfonate (100 mg, 0.4 mmol) and CH_2Cl_2 (6.0 mL) was stirred at room temp overnight. The mixture was diluted with ether (20 mL), washed with brine, and dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (30 g). Elution with hexane/EtOAc (20/1) afforded **4b** as a diastereomeric mixture (1.3 g, quant.); IR vmax 3250, 2945, 2855, 1735, 1600, 1500, 1450, 1435, 1350, 1200, 1175, 1130, 1075, 1025, 865, 815, 745, 705 cm^{-1}; ^1H NMR δ 1.05-1.90 (12H, m), 2.29 (2H, t, J = 7.0 Hz), 2.69 (1H, dd, J = 7.0, 12.5 Hz), 2.98 (1H, dd, J = 7.0, 12.5 Hz), 3.25-3.55 (2H, m), 3.67 (3H, s), 3.72-4.02 (1H, m), 4.32-4.48, 4.62-4.78 (total 1H, m), 7.11-7.48 (5H, m). This was employed for next step without further purification.

(6R)-6-Tetrahydropyranyloxy-7-phenyl-1-heptanol **5a**. A soln of **4b** (1.3 g, 4.0 mmol) in ether (6 mL) was added to a stirred and ice-cooled suspension of $LiAlH_4$ (130 mg) in ether (10 mL). The mixture was stirred for 4 h at room temp. Then excess $LiAlH_4$ was destroyed by the addition of water (0.13 mL), 10% NaOH aq soln (0.13 mL) and water (0.39 mL) to the stirred and ice-cooled mixture. The stirring was continued for 1 h. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (20 g). Elution with hexane/EtOAc (9/1) afforded **5a** as a diastereomeric mixture (1.1 g, quant.); IR vmax 3045, 3025, 2945, 2855, 1600, 1500, 1450, 1350, 1200, 1130, 1115, 1075, 1020, 865, 815, 745, 705 cm^{-1}; ^1H NMR δ 1.05-1.90 (15H, m), 2.69 (1H, dd, J = 7.0, 12.5 Hz), 2.98 (1H, dd, J = 7.0, 12.5 Hz), 3.20-3.50 (2H, m), 3.61 (2H, t, J = 7.0 Hz), 3.72-4.09 (1H, m), 4.32-4.48, 4.62-4.78 (total 1H, m), 7.11-7.48 (5H, m). This was employed for next step without further purification.

(6R)-6-Tetrahydropyranyloxy-7-phenylheptyl tosylate **5b**. *p*-Toluenesulfonyl chloride (1.5 g) was added to a soln of **5a** (1.1 g, 3.9 mmol) in pyridine (13 mL) with stirring and ice-cooling. The mixture was stirred for 5 h. Then it was poured into ice-water and extracted with ether. The ether soln was washed with water, sat. $CuSO_4$ aq. soln, $NaHCO_3$ aq. soln and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (25 g). Elution with hexane/EtOAc (9/1) afforded **5b** as a diastereomeric mixture (1.5 g, 91%); IR vmax 3025, 2945, 2855, 1600, 1500, 1450, 1360, 1175, 1130, 1115, 1025, 865, 820, 745, 705 cm^{-1}; ^1H NMR δ 1.05-1.90 (14H, m), 2.46 (3H, s), 2.72 (1H, dd, J = 7, 12.5 Hz), 2.95 (1H, dd, J = 7, 12.5 Hz), 3.20-3.50 (2H, m), 3.65-3.90 (1H, m), 3.98 (2H, t, J = 7.0 Hz), 4.32-4.48, 4.62-4.78 (total 1H, m), 7.11-7.32 (6H, m), 7.37 (1H, s?), 7.69-7.89 (2H, m). This was employed for next step without further purification.

(11R)-2-Methyl-12-phenyl-11-tetrahydropyranyloxy-2-dodecene **6**. A 0.2 M soln of (4-methyl-3-pentenyl)lithium in ether (25 mL) was prepared at –20 to –15 °C under Ar from 1-bromo-4-methyl-3-pentene (2.5 g) and lithium wire (0.46 g). The resulting soln

(20 mL) was added to a cooled and stirred suspension of powdered CuI (I) (0.38 g) in ether (3.5 mL) at –40 to –30 °C under Ar. The mixture was stirred for 30 min at –30 °C and then cooled to –78 °C. A soln of **5b** (520 mg, 1.16 mmol) in ether (4 mL) was added dropwise to the cuprate soln at –78 °C. Stirring is continued for 2 h at –70 °C and the reaction was quenched at that temp by the addition of sat. NH_4Cl aq. soln. After warming to the room temp, the mixture was poured onto ice/sat. NH_4Cl aq. soln. The organic layer was separated and the aqueous layer was extracted with ether several times. The combined ether soln was washed with sat. NH_4Cl aq. soln, $NaHCO_3$ aq. soln and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (50 g). Elution with hexane/EtOAc (20/1) afforded **6** as a diastereomeric mixture (360 mg, 87%); IR vmax 3025, 2945, 2855, 1600, 1500, 1450, 1375, 1350, 1245, 1200, 1130, 1115, 1075, 1015, 865, 820, 745, 705 cm^{-1}; ^1H NMR δ 0.90-1.40 (10H, m), 1.40-1.80 (7H, m), 1.60 (3H, s), 1.70 (3H, d, J = 2.0 Hz), 1.80-2.16 (3H, m), 2.78 (1H, dd, J = 7.0, 12.5 Hz), 2.95 (1H, dd, J = 7.0, 12.5 Hz), 3.20-3.60 (2H, m), 3.65-4.05 (1H, m), 4.32-4.48, 4.62-4.78 (total 1H, m), 5.12 (1H, tq, J = 7.0, 2.0 Hz), 7.11-7.48 (5H, m). This was employed for next step without further purification.

(2R)-11-Methyl-1-phenyl-2-tetrahydropyranyloxydodecane **7a**. A mixture of **6** (360 mg, 1.0 mmol) and Pd-C (10%, 50 mg) in EtOH (18.0 mL) was vigorously stirred under H_2 for 30 min. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (15 g). Elution with hexane/EtOAc (40/1) afforded **7a** as a diastereomeric mixture (340 mg, 93%); IR vmax 3025, 2945, 2855, 1600, 1500, 1465, 1455, 1385, 1365, 1355, 1260, 1200, 1130, 1115, 1075, 1025, 870, 820, 750, 705 cm^{-1}; ^1H NMR δ 0.85 (6H, d, J = 8.0 Hz), 1.10-1.90 (23H, m), 2.75 (1H, dd, J = 7.0 Hz, 12.5 Hz), 2.95 (1H, dd, J = 7.0, 12.5 Hz), 3.20-3.60 (2H, m), 3.65-4.05 (1H, m), 4.32-4.48, 4.62-4.78 (1H, m), 7.11-7.40 (5H, m). This was employed for next step without further purification.

(R)-10-Methyl-(1-benzyl)undecyl acetate **7b**. A soln of **7a** (360 mg, 1.0 mmol), pyridinium *p*-toluenesulfonate (30 mg, 0.12 mmol) in EtOH (8 mL) was stirred at 55 °C for 3 h. After cooling to room temp, the solvent was evaporated in vacuo. The residue was diluted with water and extracted with EtOAc. The extract was washed with water, sat. $NaHCO_3$ aq. soln and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (15 g). Elution with hexane/EtOAc (30/1) afforded an alcohol (240 mg). This was dissolved in pyridine (2.5 mL) and added acetic anhydride (2.5 mL) to it, and the mixture was stirred overnight at room temp. The mixture was poured into ice-water and extracted with ether. The extract was washed with water, dilute HCl aq soln, $NaHCO_3$ aq. soln and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (10 g). Elution with hexane/EtOAc (30/1) and subsequent distillation afforded **7b** (270 mg, 90% from **7a**), b.p. 180-185 °C/0.7 Torr (bulb-to-bulb distillation); $[\alpha]_D^{20}$ –0.62 (c=0.96, $CHCl_3$); IR vmax 3025, 2945, 2855, 1735, 1600, 1500, 1465, 1450, 1375, 1240, 1125, 750, 705 cm^{-1}; ^1H NMR δ 0.85 (6H, d, J = 8.0 Hz), 1.22 (14H, br), 1.40-1.70 (3H, m), 2.00 (3H, s), 2.84 (2H, d, J = 7.0 Hz), 5.06 (1H, m), 7.10-7.48 (5H, m). (Found: C, 78.82; H, 11.58. Calc. for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76%.)

Methyl (R)-3-hydroxy-12-methyltridecanoate **8b**. To a mixture of **7b** (180 mg, 0.56 mmol), $NaIO_4$ (2.2 g, 10 mmol), CH_3CN (2.0 mL), CCl_4 (2.0 mL) and water (3.0 mL), $RuCl_3$ (6.2 mg) was added and the resulting mixture was vigorously stirred at room temp. After 8 h, the mixture was diluted with water and extracted with ether. The extract was washed with brine and concentrated *in vacuo*. The residue was diluted with EtOH (7 mL) and 10N NaOH aq soln (0.55 mL) was added to it at 0 °C. The mixture was stirred for 30 min at room temp and concentrated *in vacuo*. The residue was dissolved in hot water and treated with activated carbon. The activated carbon was filtered, and the pH of the filtrate was adjusted to 3 by the addition of 2N HCl aq soln. The mixture was extracted with $CHCl_3$. The extract was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* to give the crude product (130 mg). A portion (4.0 mg) was treated with TMS-diazomethane, and was purified by preparative SiO_2 TLC [developed with hexane/EtOAc (4/1)] to afford the corresponding alcohol of **7b** (2.0 mg, 15%) and **8b** (2.0 mg, 50% from **7b**, 85% based on the consumed **7b**). The *e.e* of **8b** was determined to be 84% by ^1H NMR measurement of the corresponding (*R*)-MTPA ester **8c**: ^1H NMR (JEOL JNM-GX-400, 400 MHz, $CDCl_3$) δ 3.46 [3H, br.s, $C(CF_3)OCH_3$, 8.0%], 3.49 [3H, br.s, $C(CF_3)OCH_3$, 92.0%], 3.52 (3H, s, CO_2CH_3, 8.0%), 3.59 (3H, s, CO_2CH_3, 92.0%).

Enhancement of the e.e. To a solution of crude **8a** (130 mg, containing also the corresponding alcohol of **7b**) and BHT (1 mg) in a mixture of vinyl acetate (2 mL) and THF (dried over molecular sieves 4A, 1 mL) was added lipase PS-30 (68 mg), and the mixture was stirred with heating at 65 °C for 48 h. After cooling, the mixture was filtered and the filtrate was concentrated *in vacuo* and the residue was treated with ethereal soln of diazomethane. The residue was chromatographed on silica gel (5 g). Elution with hexane/EtOAc (9/1) afforded a mixture of **7b** and (*S*)-**8d**. Further elution with hexane/EtOAc (4/1) gave (*R*)-**8b** (55.0 mg, 82.0%), $[\alpha]^{18}_D$ -13.8 (c=0.97, $CHCl_3$); IR vmax 3460, 2945, 2855, 1735, 1460, 1435, 1365, 1200, 1170 cm^{-1}; ^1H NMR (JEOL JNM-GX-400, 400 MHz, $CDCl_3$) δ 0.86 (6H, d, J = 8.0 Hz), 1.10-1.20 (2H, m), 1.20-1.38 (9H, m), 1.40-1.50 (1H, m), 1.50-1.59 (2H, m), 1.60-1.64 (3H, m), 2.41 (1H, dd, J = 6.0, 10.8 Hz), 2.52 (1H, dd, J = 2.0, 10.8 Hz), 2.81-2.89 (1H, m), 3.71 (3H, s), 3.97-4.05 (1H, br). HRMS Found: 258.2188. Calc. for $C_{15}H_{30}O_3$: 258.2192. The *e.e* of **8b** was determined to be 95% by ^1H NMR measurement of the corresponding (*R*)-MTPA ester **8c**:

(3H, s), 2.52 (2H, d, J = 7.6 Hz), 3.69 (3H, s), 5.20 (1H, m). Its *e.e.* was determined to be 54% by the comparison of its specific rotation with that of (*R*)-**8d** which was obtained by the acetylation of (*R*)-**8b**; $[\alpha]^{18}_D$ +0.7 (c=1.34, $CHCl_3$).

(2R)-1-Phenyl-2-tetrahydropyranyloxyoctane **9a**. A 1.8 M soln of methyllithium in ether (1.6 mL) was added to a cooled and stirred suspension of powdered CuI (I) (0.24 g) in ether (1 mL) at temperatures of –15 to 0 °C under Ar. The mixture was stirred for 30 min at –10 °C. A soln of **5b** (250 mg, 0.56 mmol) in ether (2.5 mL) was added dropwise to the cuprate soln at 0 °C. Stirring is continued for 2 h at 0 °C and the reaction was quenched at that temp by the addition of sat. NH_4Cl aq. soln. The work-up was carried out by the similar procedure for **6**, and the product was purified by preparative SiO_2 TLC [developed with hexane/EtOAc (7/1)] to afford **9a** as a diastereomeric mixture (140 mg, 87%); IR νmax 3025, 2925, 2855, 1600, 1500, 1460, 1375, 1350, 1255, 1200, 1130, 1115, 1075, 1020, 865, 815, 750, 705 cm^{-1}; ^1H NMR δ 0.79 (3H, t, J = 5.5 Hz), 1.00-1.90 (16H, m), 2.78 (1H, dd, J = 7.0, 12.5 Hz), 2.90 (1H, dd, J = 7.0, 12.5 Hz), 3.15-3.50 (2H, m), 3.60-4.00 (1H, m), 4.26-4.45, 4.58-4.78 (total 1H, m), 7.05-7.32 (5H, m). This was employed for next step without further purification.

(R)-(1-Benzyl)heptyl acetate **9b**. In the same manner as described for **7b**, (*R*)-1-phenyl-2-octanol was obtained from **9a** (270 mg, 0.93 mmol). B.p. 180-185 °C/1.5 Torr (bulb-to-bulb distillation); $[\alpha]_D^{20}$ –10.9 (c=0.85, $CHCl_3$); IR νmax 3370, 3025, 2925, 2855, 1600, 1500, 1125, 1075, 1030, 750, 705 cm^{-1}. This was further acetylated to give (*R*)-**9b** (180 mg, 87% from **9a**), b.p. 165-170 °C/1.0 Torr (bulb-to-bulb distillation); $[\alpha]_D^{19}$ –0.82 (c=1.56, $CHCl_3$); IR νmax 3025, 2925, 2855, 1735, 1600, 1450, 1375, 1240, 1015, 750, 705 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J = 6.3 Hz), 1.05-1.40 (7H, br. s), 1.40-1.62 (3H, m), 2.00 (3H, s), 2.84 (2H, d, J = 7.0 Hz), 5.07 (1H, m), 7.10-7.45 (5H, m). (Found: C, 77.37; H, 9.60 Calc. for $C_{16}H_{24}O_2$: C, 77.38; H, 9.74%.)

Methyl (R)-3-hydroxynonanoate **10b**. In the same manner as described for **8b**, **9b** (170 mg, 0.68 mmol) was oxidized in the presence of RuO_4 to give the crude product (110 mg). A portion (4.0 mg) was treated with TMS-diazomethane, and was purified by preparative SiO_2 TLC [developed with hexane/EtOAc (4/1)] to afford the corresponding alcohol of **9b** (1.3 mg, 43%) and **10b** (2.0 mg, 35% from **9b**, 57% based on the consumed **9b**). The *e.e* of **10b** was determined to be 84% by ^1H NMR measurement of the corresponding (*R*)-MTPA ester **10c**: ^1H NMR (JEOL JNM-GX-400, 400 MHz, $CDCl_3$) δ 3.45 [3H, br.s, $C(CF_3)OCH_3$, 8.0%], 3.46 [3H, br.s, $C(CF_3)OCH_3$, 92.0%], 3.52 (3H, s, CO_2CH_3, 8.0%), 3.59 (3H, s, CO_2CH_3, 92.0%).

Enhancement of the *e.e.* was carried out in the same manner as above. Starting from crude **10a** (100 mg, containing also the corresponding alcohol of **9b**), (*R*)-**10b** (30 mg, 23%) was obtained, $[\alpha]^{16}_D$ -16.4 (c=0.83, $CHCl_3$); IR νmax 3450, 2925, 2855, 1735, 1435, 1200, 1165 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J = 6.3 Hz), 1.10-1.80 (10H, m), 2.48 (2H, t, J = 5.0 Hz), 2.75-3.05 (1H, br.), 3.74 (3H, s), 3.85-4.30 (1H, m). (Found: C, 63.77; H, 10.53. Calc. for $C_{10}H_{20}O_3$: C, 63.80; H, 10.71%.) The *e.e* of **10b** was determined to be 94% by ^1H NMR measurement of the corresponding (*R*)-MTPA ester **10c**.

(*R*)-**10d** (13 mg, 8%) was also obtained, $[\alpha]^{21}_D$ +1.3 (c=0.65, $CHCl_3$); IR νmax 2925, 2855, 1745, 1465, 1440, 1365, 1235, 1200, 1170, 1025 cm^{-1}. Its *e.e.* was determined to be 61% by the comparison of its optical rotation with that of (*R*)-**10d** which was obtained by the acetylation of (*R*)-**10b**; $[\alpha]^{21}_D$ +2.0 (c=0.70, $CHCl_3$).

(R)-1,3-nonanediol **11**. A soln of **10d** (15.0 mg, 0.08 mmol) in ether (1 mL) was added to a stirred and ice-cooled suspension of $LiAlH_4$ (5 mg) in ether (0.5 mL). The mixture was stirred for 4 h at room temp. Then excess $LiAlH_4$ was destroyed by the addition of water (0.05 mL), 10% NaOH aq soln (0.05 mL) and water (0.15 mL) to the stirred and ice-cooled mixture. The stirring was continued for 1 h. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by SiO_2 preparative TLC and subsequent distillation to afford **11** (10.0 mg, 80%), b.p. 140-150 °C/0.9 Torr (bulb-to-bulb distillation); $[\alpha]^{18}_D$ –6.8 (c=0.95, EtOH) [lit.[13b] $[\alpha]^{20}_D$ –5.4 (*c* 1.40 EtOH)]; IR νmax 3350, 2945, 2870, 1465, 1060 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J = 6.3 Hz), 1.20-1.80 (12H, m), 2.22-2.55 (2H, br.), 3.70-4.02 (3H, m). (Found: C, 67.21; H, 13.58. Calc. for $C_9H_{20}O_2$: C, 67.45; H, 12.58%.)

Acknowledgments: The authors thank Mr. Shinobu Oda, Research Institute of Kansai Painting Co. for the discussion on interface-bioreactor, Amano Pharmaceutical Co. for generous gift of lipase. We are grateful to Dr. Makoto Ubukata of RIKEN for the information of 3-hydroxy-12-methyltridecanoate. This research was supported by the Grant-in-Aid for Scientific Research on Priority Areas (No. 0527424) from the Ministry of Education, Science and Culture, Japan. Financial support from Iwaki Foundation is also acknowledged with thanks.

References

1. Review: (a) Evans, D. A. *Asymmetric Synthesis*, Morison, J. D. *ed.*; Academic Press: New York **1984**; Vol. 3, Chapter 2, pp. 2-110. Recent examples: (b) Murakata, M.; Nakajima, M.; Koga, K. *J. Chem. Soc., Chem. Commun.* **1990**, 1657. (c) Tomooka, K.; Matsumoto, K.; Suzuki, K.; Tsuchihashi, G. *Synlett.* **1992**, 129. (d) Nishida, M.; Nakaoka, K.; Ono, S.; Yonemitsu, O.; Nishida, A.; Kawahara, N.; Takayanagi, H. *J. Org. Chem.* **1993**, *58*, 5870. (e) Mori, K.; Takayama, S.; Yoshimura, S. *Liebigs Ann. Chem.* **1993**, 91.

Yonemitsu, O.; Nishida, A.; Kawahara, N.; Takayanagi, H. *J. Org. Chem.* **1993**, *58*, 5870. (e) Mori, K.; Takayama, S., Yoshimura, S. *Liebigs Ann. Chem.* **1993**, 91.

2. (a) Ohta, H.; Matsumoto, K.; Tsutsumi, S.; Ihori, T. *J. Chem. Soc. Chem. Commun.*, **1989**, 485. (b) Ohta, H.: Matsumoto, K.; Tsutsumi, S.; Ihori, T. *J. Am. Chem. Soc.*, **1990**, *112*, 9614. (c) Matsumoto, K.; Ohta, H.; *Chem. Lett.* **1989**, 1589. (d) Sugai, T.; Kakeya, H.; Ohta, H.; Morooka, M.; Ohba, S. *Tetrahedron* **1989**, *45*, 6135. (e) Matsumoto, K.; Suzuki, N.; Ohta, H. *Tetrahedron Lett.* **1990**, *31*, 7163. (f) Kume, Y.; Ohta, H. *Tetrahedron Lett.* **1992**, *42*, 6367. (g) Matsumoto, K.; Ohta, H.; *Tetrahedron Lett.*, **1991**, *32*, 4729. (h) Fujii, I., Lerner, R. A.; Janda, K. D. *J. Am. Chem. Soc.*, **1991**, *113*, 8528. (i) Fuji, K.; Tanaka, K.; Miyamoto, H. *Tetrahedron: Asymmetry* **1993**, *4*, 247. (j) Duhamel, P.; Yebga, A.; Cahard, D.; Renouf, P.; Poirier, J.-M. *Tetrahedron: Asymmetry* **1993**, *4*, 2447.

3. Review: (a) Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, *91*, 49. (b) Tanaka, A. *Bio-Industry* **1992**, *9*, 563. Recent examples: (c) Naoshima, Y.; Maeda, J.; Munakata, Y. *J. Chem. Soc., Perkin I*, **1992**, 659. (d) Nakamura, K.; Takano, S.; Terada, K.; Ohno, A. *Chem. Lett.* **1992**, 951.

4. Kierstan, M.; Bucke, C. *Biotechnol. Bioeng.* **1977**, *19*, 387.

5. Fukui, S.; Tanaka, A.; Iida, T.; Hasegawa, E. *FEBS Lett.* **1976**, *66*, 179.

6. Oda, S.; Ohta, H. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 1515. Oda, S.; Ohta, H. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 2041.

7. Wollenweber, H.-W.; Schramek, S.; Moll, H.; Rietschel, E. T. *Arch. Microbiol.* **1985**, *142*, 6.

8. Ubukata, M.; Isono, K.; Kimura, K.; Nelson, C. C.; McCloskey, J. A. *J. Am. Chem. Soc.* **1988**, *110*, 4416.

9. Mori, K.; Sugai, T. *Synthesis* **1982**, 752.

10. Mori, K.; Bernotas, R. *Tetrahedron: Asmmetry* **1990**, *1*, 87.

11. Dale, J. A.; Mosher, H. S. *J. Am. Chem*, Soc. **1973**, *95*, 512.

12. Sugai, T.; Ritzén, H.; Wong, C.-H. *Tetrahedron: Asymmetry* **1993**, *4*, 1051.

13. (a) Kitching, W.; Lewis, J. A.; Fletcher, M. T.; Drew, R. A. I.; Moore, C. J.; W. A.; Francke, W. *J. Chem. Soc., Chem. Commun.* **1986**, 853. (b) Kitching, W.; Lewis, J. A.; Perkins, M. V.; Drew, R.; Moore, C. J.; Schurig, V.; König, W. A., Francke, W. *J. Org. Chem.* **1989**, *54*, 3893.

(*Received in Japan* 30 *June* 1994; *accepted* 10 *August* 1994)