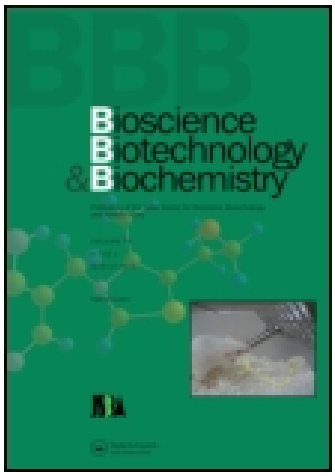

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Biochemical Reduction of 3-Oxoalkanoic Esters by a Bottom-fermentation Yeast, *Saccharomyces cerevisiae* IFO 0565

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Received March 30, 1994

The scope and limitation of a bottom-fermentation yeast (*Saccharomyces cerevisiae* IFO 0565) toward the reduction of 3-oxoalkanoic esters were examined. The substrate specificity of this microorganism for various kinds of 3-oxoalkanoic esters was studied. This microorganism was distinct from converntional bakers' yeast in terms of its selectivity in the reduction and its high expression of a hydrolytic enzyme. 3-Oxoalkanoic ester with an aromatic substituent, a halogen substituted 3-oxoalkanoic ester, an aliphatic longer-chain 3-oxoalkanoic ester and its α,α-difluoro analog were also accepted by this microorganism. The products are useful intermediates in the synthesis of physiologically active compounds.

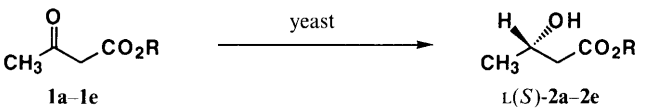
Optically active 3-hydroxyalkanoic esters are an important starting material for the synthesis of natural products, medicines, ferroelectric liquid crystals, and other industrially useful materials.[1] Their asymmetric syntheses have been extensively studied, resulting in great progress in both chemical[2] and chemo-enzymatic[3] approaches. Among them, the whole cell-mediated reduction of the corresponding 3-oxoalkanoic ester is particularly attractive,[4] because the reaction can be simply carried out only by mixing the substrate and yeast cells in water at room temperature. Therefore, many workers have studied the substrate specificity and stereoselectivity.

In this context, we became interested in the bottom-fermentation yeasts that are widely used in the brewing industry, expecting both unique selectivity and reactivity. The notion came from the demand for nutrition and the metabolic pathway of bottom-fermentation yeasts being considerably different from those of top-fermentation yeasts (*e.g.*, bakers' yeast), which implicates, in turn, a difference in the type and amount of enzymes that are expressed with these two classes of microorganisms. In this study, we chose *Saccharomyces cerevisiae* IFO 0565, which is one of the typical bottom-fermentation yeasts. This strain has previously been classified as *S. carlsbergensis*.

Substrate specificity and stereoselectivity

At first, the reduction of ethyl 3-oxobutanoate (acetoacetate) and related simple alkyl 3-oxoalkanoates was investigated, analogous studies having been made in the field of bacteria[5] and fungi.[6] The length of the alcohol part was changed, while the substituent attached to the carbonyl group was fixed as methyl. For comparison, the same substrates were reduced by both bakers' yeast and bottom-fermentation brewers' yeast (*Saccharomyces* sp., Asahi Breweries Ltd.). Table I summarizes the results. The results obtained by the use of bakers' yeast are in good accordance with the reported data,[7] in terms of a uniformly high enantiomeric excess (*e.e.*, 85–97%) and L(*S*)-configuration of the newly created chiral center. The *e.e.* values increased as the number of carbons in the ester group increased. In contrast, brewers' yeast and *S. cerevisiae* IFO 0565 reduced lower homologues (**1a** and **1b**) to give products with moderate *e.e.* (*ca.* 50%).[cf.8] Moreover, steric hindrance of the alcohol moiety (larger than butyl: **1c–1e**) totally retarded the reduction. Thus, in brewers' yeast and *S. cerevisiae* IFO 0565, the relative activity of the enzyme that produces the D(*R*)-form of the hydroxy ester ("D-

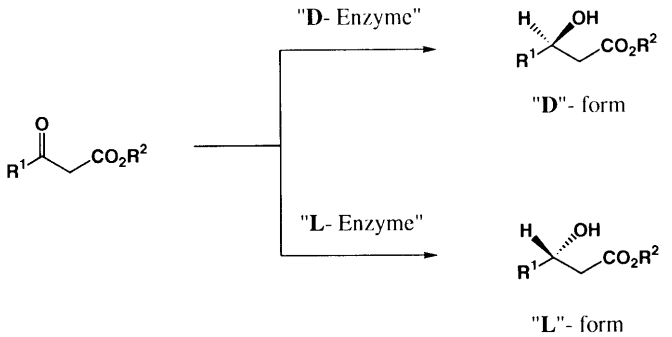
Table I. Reduction of Alkyl 3-Oxobutanoate with Yeasts[a]



1a–1e L(*S*)-**2a–2e**

Substrate	Bakers' yeast % yield	Bakers' yeast % *e.e.*[b]	Brewers' yeast % yield	Brewers' yeast % *e.e.*[b]	*S. cerevisiae* IFO 0565 % yield	*S. cerevisiae* IFO 0565 % *e.e.*[b]
1a R = Et	40.0	83.6	47.3	46.7	33.6	48.6
1b R = *n*-Pr	66.6	85.5	58.3	50.4	45.2	51.2
1c R = *n*-Bu	41.2	88.8	0	—	0	—
1d R = *n*-C_5H_{11}	24.2	96.6	0	—	0	—
1e R = *n*-C_6H_{13}	14.0	96.7	0	—	0	—

[a] See the Experimental section for the reaction conditions.
[b] Determined by GLC analysis of the corresponding (*R*)-MTPA esters.



Scheme 1. Stereochemical Course for Yeast-mediated Reduction of the Carbonyl Group.

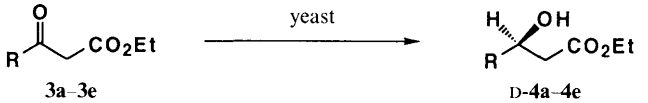
† Address correspondence to this author.

enzyme," Scheme 1)[9] is larger in comparison with that in bakers' yeast. Alkyl 3-oxobutanoates, especially higher homologues, are poor substrates for the present "D-enzyme." In bakers' yeast, the "L-enzyme" is expressed in a large quantity and works very well with these substrates.

These conclusions were further supported by observations from a series of experiments on changing the substituent attached to the carbonyl group, the alcohol moiety being fixed as ethyl. The results are summarized in Table II, which shows that "D-enzymes" were predominantly expressed in brewers' yeast and *S. cerevisiae* IFO 0565.

Table II includes other examples. A branched-chain substrate (**3c**) was accepted to give a D(*S*)-product in high *e.e.* (>99%). The substrate with an aromatic substituent (**3d**) and a halogenated 3-oxoalkanoic ester (**3e**) underwent the reduction in a highly enantioselective manner (80–90% *e.e.*), which is in contrast to bakers' yeast reduction that led to a lower *e.e.* (60–66%). Products **4d** and **4e** serve as the optically active starting material for synthetic medicines such as (*S*)-1-benzyl-3-hydroxypyrrolidine[10] and fluoxetine,[11] respectively.

Table II. Reduction of Ethyl 3-Substituted 3-Oxobutanoate with Yeasts[a]



3a–3e D-4a–4e

Substrate	Bakers' yeast % yield	Bakers' yeast % *e.e.*[b]	Brewers' yeast % yield	Brewers' yeast % *e.e.*[b]	*S. cerevisiae* IFO 0565 % yield	*S. cerevisiae* IFO 0565 % *e.e.*[b]
3a R = Et	40.3	43.1	64.5	86.7	69.8	95.0
3b R = *n*-Pr	63.9	95.6	73.4	95.0	81.0	98.7
3c R = *i*-Pr	29.0	>99	47.5	>99	29.2	>99
3d R = Ph	20.7	60.7	26.9	75.7	36.2	79.1
3e R = $-CH_2Cl$	55.2	65.5	51.2	84.3	41.6	89.6

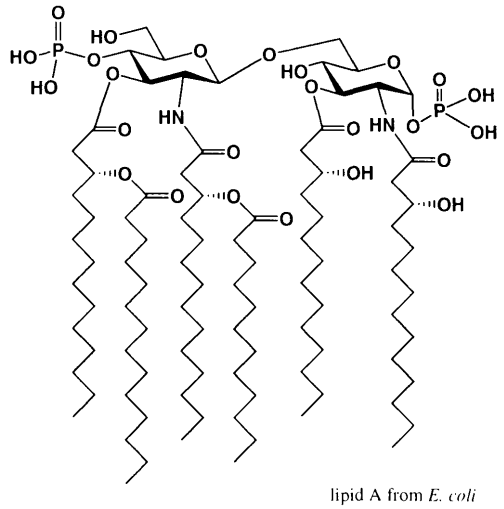
[a] See the Experimental section for the reaction conditions.
[b] The *e.e.* values were determined by HPLC analysis of the corresponding (*R*)-MTPA esters, except for **4c**, which was determined by a comparison of optical rotation values.

Application to the longer-chain 3-oxoalkanoic ester

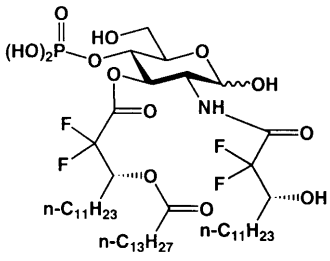
We next turned our attention to applying this procedure for the preparation of longer-chain 3-hydroxyalkanoic acids related to lipid A and its analog.

Lipid A constitutes the lipophilic component in lipopolysaccharide (LPS) of Gram-negative bacteria (Scheme 2). This particular structure is responsible for the endotoxicity, antitumorial activity and adjuvanticity of LPS,[12] which has created recent interest in the synthesis community.[12c,13] Lipid A itself is a hybrid of hydrophilic oligosaccharide and hydrophobic longer-chain fatty acids, the chain length as well as the absolute configuration of a fatty acid having an important effect on the physiological activity of this class of compound.[14] As part of our recent study on the synthesis of glycolipid and related compounds,[15] we tried a preparative-scale synthesis of (*R*)-3-hydroxytetradecanoic acid, a key synthetic intermediate of the lipophilic side chain. While a number of procedures for the preparation of optically active forms have been reported so far,[16] the practice still depends on the classical preferential crystallization of the diastereomeric ammonium salt.[16b] Very recently, the optical resolution of (±)-3-hydroxytetradecanoic acid mediated by lipase-catalyzed transesterification has been reported,[17] although strong dependence of the reactivity on the substrate structure severely limited the scope of this approach. For example, the 2,2-difluoro analog is a very poor substrate and makes kinetic resolution impossible. The present yeast-mediated reduction system, which is rich in the "D-enzyme," seems to provide a potent method for preparing (*R*)-3-hydroxytetradecanoic acid and its 2,2-disubstituted analogs.

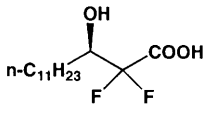
When the substrate **5** was treated with the whole cell of this yeast, it was rapidly consumed. A hydroxyacid as the primary product was isolated as methyl ester **6** in a 20% yield by treating with diazomethane (Scheme 3). The *e.e.* of **6** was 97%, which is nearly the same as the result obtained by the bakers' yeast-mediated reduction of 3-oxotetradecanoic acid.[16e] These observations strongly suggest



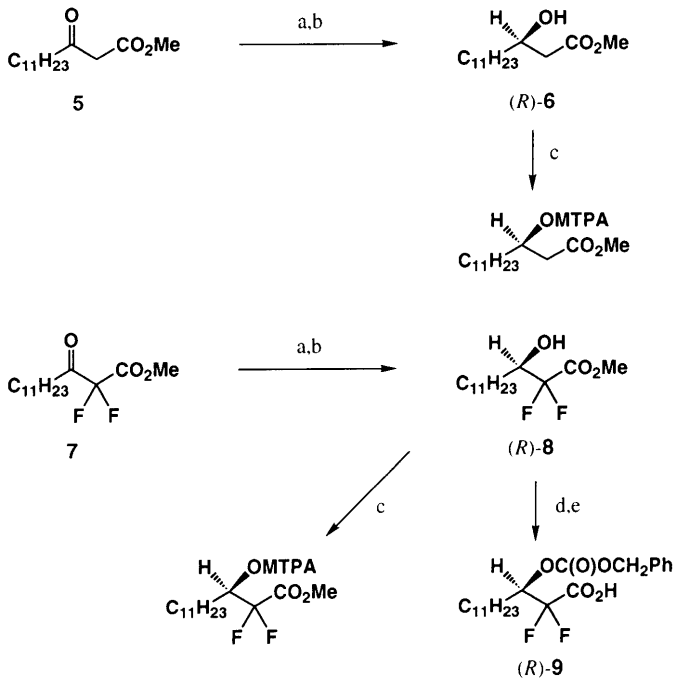
lipid A from *E. coli*



fluoro analog of GLA-60

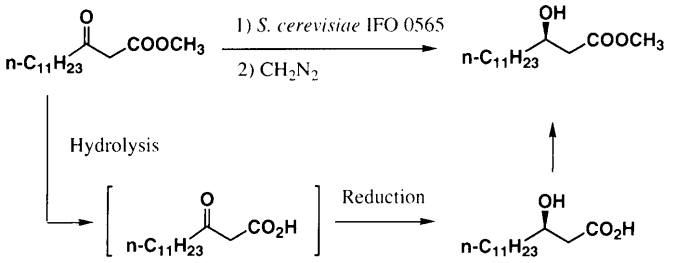


Scheme 2. Lipid A and Its Analog.



Scheme 3. Reduction of Methyl 3-Oxotetradecanoate and Its α,α-Difluoro Analog.

Reagents: (a) *Saccharomyces cerevisiae* IFO 0565; (b) CH_2N_2; (c) MTPA-Cl/pyridine; (d) $PhCH_2OCOCl$/pyridine; (e) NaOH, H_2O–EtOH.



Scheme 4. Proposed Reaction Pathway for Methyl 3-Oxotetradecanoate.

that hydrolysis of the methyl ester would occur prior to reduction of the carbonyl group,[cf.18] as depicted in Scheme 4. Bakers' yeast reportedly gave only a 5% yield of the product when methyl 3-oxotetradecanoate was subjected to the reaction, while a 40% yield of the product was obtained by starting from the corresponding oxoacid.[16e,19] These observations suggest that expression of the hydrolytic enzyme in bakers' yeast is far less when compared with *S. cerevisiae* IFO 0565.

We used an optimization study to establish the reaction conditions that would improve the yield; that is, (1) aerobic conditions, (2) the use of maltose as the carbon source, and (3) a higher pH value (7.6) with higher concentration of the buffer solution (200 mM). Under these conditions, we were able to obtain **6** in a yield of 56%. The present method has the extra advantage that 3-oxoalkanoic ester, and not the corresponding acid, can be used as the substrate, since the internal hydrolytic enzyme gives an improved yield. In contrast, the substrate for bakers' yeast needs to be 3-oxoalkanoic acid, which is susceptible to non-enzymatic degradation, leading to a reportedly lower yield that does not exceed 40%.[16e]

Finally, this whole cell system was applied to prepare the 2,2-difluoro analog of 3-hydroxytetradecanoic acid. This compound, designed as the synthetic intermediate of the new analog of GLA-60[20] (Scheme 2), has so far been synthesized only *via* a multistep approach from a carbohydrate precursor.[21]

The optimized reaction conditions were applied to oxoester **7** to give **8** in a 54% yield after methylation. The (*R*) configuration of the newly created chiral center of the hydroxy ester was determined by converting **8** to synthetic intermediate (*R*)-**9**,[21] whose absolute configuration has already been established. The present synthesis is highly efficient in terms of its simplicity (5 steps) and high overall yield (10% from undecanal), in comparison with the reported procedure (14 steps and 0.4% from methyl α-D-galactoside[21]).

Experimental

All boiling point (bp) and melting point (mp) data are uncorrected. IR spectra were measured as films for oils and as KBr discs for solids with a Jasco IRA-202 spectrometer or a Shimadzu 8100M spectrometer. ^{1}H-NMR spectra were measured in chloroform-d with tetramethylsilane as the internal standard at 270 MHz with a JEOL JNM EX-270 spectrometer, or at 400 MHz with a JEOL JNM GX-400 spectrometer, unless otherwise stated. Mass spectra were obtained at 70 eV with a Hitachi M-80B spectrometer. Optical rotation values were recorded by a Jasco DIP 360 polarimeter with chloroform as the solvent. A Hewlett 5890 gas chromatography was used for GLC analysis, and HPLC analyses were performed on a Shimadzu LC-10 chromatograph. Wako gel B-5F and silica gel 60 K070-WH (70–230 mesh) from Katayama Chemical Co. were used for preparative TLC and column chromatography, respectively.

General procedure for the incubation of S. cerevisiae IFO 0565 and the whole cell-mediated reduction of 3-oxoesters. The yeast (*S. cerevisiae* IFO 0565) was pre-incubated in a medium (200 ml) containing malt extract (4 g), peptone (0.2 g), and glucose (4 g) for 2 days at 30°C with shaking. This was added to 1800 ml of the same medium and the incubation was continued for 2 days at 30°C on gyratory shaker. The yeast cells (20 g in wet weight) were collected by centrifugation (3000 rpm for 10 min), resuspended in a mixture of deionized water (150 ml) and glucose (15 g), and stirred at 25°C for 30 min. The substrate (**1a–e** and **3a–e**, 10 mmol) was added as an ethanol solution (2 ml), and the mixture was stirred at 25°C. After 48 h, the mixture was filtered through a pad of Celite, saturated with sodium chloride and extracted with ether. The ethereal solution was washed with brine, dried over sodium sulfate and concentrated *in vacuo* to give a crude product, which was further purified by column chromatography (silica gel, 20 g). Elution with hexane–ethyl acetate (3:1) and subsequent distillation afforded the purified product (**2a–b** and **4a–e**).

Ethyl (S)-3-hydroxybutanoate (**2a**). 528 mg (40.0% yield) from 1.3 g of **1a**, bp 47°C at 2.5 mmHg, $[\alpha]_D^{24}$ +12.3° (*c* 1.26) [lit.[7c] $[\alpha]_D^{25}$ +37.2° (*c* 1.3), lit.[8] $[\alpha]_D^{22}$ +43.9° (*c* 0.998), lit.[22a] $[\alpha]_D^{20}$ +38.5°, lit.[22b] $[\alpha]_D^{23}$ +41.6° (*c* 1.0)]; IR ν_{max} cm^{-1}: 3445, 2980, 1730, 1250, 1180, 1120, 1088, 1030, 950; ^{1}H-NMR (270 MHz) δ: 1.23 (3H, d, *J*=6.3 Hz), 1.28 (3H, t, *J*=7.1 Hz), 2.41 (1H, dd, *J*=16.5, 8.3 Hz), 2.50 (1H, dd, *J*=16.5, 4.0 Hz), 3.01 (1H, br.), 4.18 (2H, q, *J*=7.2 Hz), 4.12–4.23 (1H, m). *Anal.* Found: C, 54.49; H, 9.22%. Calcd. for $C_6H_{12}O_3$: C, 54.53; H, 9.15%. Its IR and NMR spectra were identical with those reported previously.[8]

GLC analysis of its MTPA ester: (DB-WAX column, 30 m, 170°C; He, 150 Kpa) t_R 17.5 min (25.6%), 17.8 min (73.4%).

Propyl (S)-3-hydroxybutanoate (**2b**). 669 mg (45.2%) from 1.46 g of **1b**: bp 46°C at 1.0 mmHg, $[\alpha]_D^{28}$ +21.9° (*c* 0.95); IR ν_{max} cm^{-1}: 3470, 2970, 1732, 1184, 1122, 1088, 1030, 949; ^{1}H-NMR (270 MHz) δ: 0.95 (3H, t, *J*=7.4 Hz), 1.23 (3H, d, *J*=6.3 Hz), 1.66 (2H, tq, *J*=7.4, 6.8 Hz), 2.41 (1H, dd, *J*=16.5, 7.9 Hz), 2.50 (1H, dd, *J*=16.5, 4.0 Hz), 3.06 (1H, br.), 4.08 (2H, t, *J*=6.8 Hz), 4.10–4.26 (1H, m). *Anal.* Found: C, 57.50; H, 9.76%. Calcd. for $C_7H_{14}O_3$: C, 57.51; H, 9.65%.

GLC analysis of its MTPA ester was carried out under the same conditions as the used for **2a**: t_R 20.2 min (24.4%), 20.7 min (75.6%). The absolute configuration of (+)-**2b** was concluded to be (*S*) for the following

reasons: 1) All of (*S*)-**2a**, (*S*)-**2c**[7c] and the present sample showed a consistent (+) sign of rotation; 2) the GLC analysis of the MTPA ester showed that the slow-moving diastereomer was predominant. Indeed, $[\alpha]_D^{29}$ +31.8° (*c* 0.91) for (*S*)-(+)-**2c** was obtained by bakers' yeast reduction of **1c** and conversion to the corresponding MTPA ester. GLC analysis: (DB-WAX column, 30 m, 170°C; He, 100 Kpa) t_R 24.2 min (5.6%), 24.9 min (94.4%).

Ethyl (R)-3-hydroxypentanoate (**4a**). 1.03 g (69.8% yield) from 1.46 g of **3a**, bp 75°C at 2.5 mmHg, $[\alpha]_D^{29}$ −31.2° (*c* 0.93) [lit.[6b] $[\alpha]_D^{20}$ −32.3° (*c* 2.25), lit.[23a] $[\alpha]_D^{22}$ −13.44° (*c* 1.2), lit.[23b] $[\alpha]_D^{22}$ +20.64° [for (*S*), *c* 3.85]]; IR ν_{max} cm^{-1}: 3450, 2970, 1736, 1261, 1176, 1118, 1026; ^{1}H-NMR (270 MHz) δ: 0.96 (3H, t, *J*=7.4 Hz), 1.28 (3H, t, *J*=7.3 Hz), 1.43–1.61 (2H, m), 2.39 (1H, dd, *J*=16.5, 8.9 Hz), 2.52 (1H, dd, *J*=16.5, 3.3 Hz), 2.92 (1H, br.), 3.88–3.98 (1H, m), 4.18 (2H, q, *J*=7.3 Hz). *Anal.* Found: C, 57.51; H, 9.72%. Calcd. for $C_7H_{14}O_3$: C, 57.51; H, 9.65%.

Its NMR spectrum was in good accordance with that reported previously.[6b]

HPLC analysis of its MTPA ester: (Merck Lichrospher Si60 column, 25 cm × 4 mm; solvent, hexane–THF–MeOH (6000 : 100 : 1); flow rate: 2.0 ml/min; detection at 217 nm) t_R 51.7 min (97.5%), 60.5 min (2.5%).

Ethyl (R)-3-hydroxyhexanoate (**4b**). 1.30 g (81.0% yield) from 1.58 g of **3b**, bp 134°C at 2.0 mmHg, $[\alpha]_D^{28}$ −26.1° (*c* 1.23) [lit.[6b] $[\alpha]_D^{20}$ −23.6° (*c* 1.5), lit.[23b] $[\alpha]_D^{22}$ −11.91° (*c* 4.24), lit.[24] $[\alpha]_D^{20}$ −22.1° (*c* 1.04)]; IR ν_{max} cm^{-1}: 3457, 2970, 1732, 1265, 1176, 1122, 1026, 972; ^{1}H-NMR (270 MHz) δ: 0.93 (3H, t, *J*=6.9 Hz), 1.27 (3H, t, *J*=7.3 Hz), 1.32–1.56 (4H, m), 2.39 (1H, dd, *J*=16.5, 8.6 Hz), 2.50 (1H, dd, *J*=16.5, 3.3 Hz), 2.90 (1H, br.), 3.98–4.08 (1H, m), 4.17 (2H, q, *J*=7.2 Hz). *Anal.* Found: C, 60.11; H, 10.27%. Calcd. for $C_8H_{16}O_3$: C, 59.98; H, 10.07%.

Its NMR spectrum was in good accordance with that reported previously.[6b]

HPLC analysis of its MTPA ester was carried out under the same conditions as those used for **4a**: t_R 41.3 min (99.4%), 47.5 min (0.6%).

Ethyl (S)-3-hydroxy-4-methylbutanoate (**4c**). 467 mg (29.2% yield) from 1.58 g of **3c**: bp 62°C at 3 mmHg, $[\alpha]_D^{26}$ −37.3° (*c* 1.16) [lit.[6b] $[\alpha]_D^{24}$ −33° (*c* 1.6)]; IR ν_{max} cm^{-1}: 3503, 2970, 1732, 1280, 1180, 1107, 1030; ^{1}H-NMR (270 MHz) δ: 0.93 (6H, d, *J*=6.8 Hz), 1.27 (3H, d, *J*=7.2 Hz), 1.64–1.70 (1H, m), 2.39 (1H, dd, *J*=16.1, 8.9 Hz), 2.50 (1H, dd, *J*=16.1, 3.3 Hz), 3.11 (1H, br.d, *J*=3.6 Hz), 3.72–3.82 (1H, m), 4.17 (2H, q, *J*=7.2 Hz). *Anal.* Found: C, 59.88; H, 10.21%. Calcd. for $C_8H_{16}O_3$: C, 59.98; H, 10.07%.

Its NMR spectrum was in good accordance with that reported previously.[6b]

Ethyl (S)-3-hydroxy-3-phenylpropanoate (**4d**). 710 mg (36.2% yield) from 1.94 g of **3d**, bp 116°C at 3.0 mmHg, $[\alpha]_D^{28}$ −41.4° (*c* 0.76) [lit.[11c] $[\alpha]_D^{20}$ +44.0° [for (*R*), *c* 1.015], lit.[11d] $[\alpha]_D^{20}$ −51.0° (*c* 1.5), lit.[22a] $[\alpha]_D^{20}$ −25.8°]; IR ν_{max} cm^{-1}: 3450, 2975, 1732, 1495, 1196, 1161, 1034, 760, 698; ^{1}H-NMR (270 MHz) δ: 1.26 (3H, t, *J*=7.3 Hz), 2.68 (1H, dd, *J*=16.5, 3.5 Hz), 2.76 (1H, dd, *J*=16.5, 8.3 Hz), 3.32 (1H, br.d, *J*=3.6 Hz), 4.17 (2H, q, *J*=7.3 Hz), 5.09–5.15 (1H, m), 7.25–7.39 (5H, m). *Anal.* Found: C, 67.92; H, 7.45%. Calcd. for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27%.

Its IR and NMR spectra were identical with those reported previously.[11d]

HPLC analysis of its MTPA ester was carried out under the same conditions as those used for **4a**: t_R 69.7 min (89.6%), 81.7 min (10.4%).

Ethyl (S)-4-chloro-3-hydroxybutanoate (**4e**). 685 mg (41.6% yield) from 1.65 g of **3e**, bp 80°C at 2.5 mmHg, $[\alpha]_D^{29}$ −17.6° (*c* 0.93) [lit.[7a] $[\alpha]_D^{23}$ −11.7° (*c* 5.75), lit.[23b] $[\alpha]_D^{22}$ +5.22° [for (*R*) isomer, *c* 6.12]]; IR ν_{max} cm^{-1}: 3456, 2950, 1732, 1192, 1157, 1091, 1057, 1034; ^{1}H-NMR (270 MHz) δ: 1.28 (3H, t, *J*=7.3 Hz), 2.59 (1H, dd, *J*=16.5, 7.6 Hz), 2.67 (1H, dd, *J*=16.5, 4.9 Hz), 3.59 (1H, dd, *J*=10.6, 6.3 Hz), 3.63 (1H, dd, *J*=10.6, 4.6 Hz), 4.19 (2H, q, *J*=7.3 Hz), 4.20–4.31 (1H, m). *Anal.* Found: C, 43.40; H, 6.71%. Calcd. for $C_6H_{11}O_3Cl$: C, 43.26; H, 6.65%.

HPLC analysis of its MTPA esteer was carried out under the same conditions as those used for **4a**: t_R 87.6 min (94.8%), 96.9 min (5.2%).

Reduction with compressed bakers' yeast (Oriental) and with brewers' yeast from a production source was carried out in a similar manner, results were summarized in Tables I and II.

Methyl (R)-3-hydroxytetradecanoate (**6**). Cells of *S. cerevisiae* IFO 0565 (5 g, wet weight) were re-suspended in a phosphate buffer (0.2 M, pH 7.6, 37.5 ml), and maltose (3.75 g) was added to the mixture while stirring at 30°C. After 30 min, a substrate (**5**, 101 mg, 0.39 mmol) was added as an ethanol solution (1 ml), and the mixture was stirred at 30°C for 24 h. After acidification, the mixture was centrifuged (3000 rpm), and the supernatant was saturated with sodium chloride and extracted with ether. The precipitated cells were treated by ultrasound sonication in acetone, and then filtered. The filtrate was concentrated *in vacuo*, and the residue was extracted with ether. The solid material on the filter was further extracted with ether by applying sonication. The organic extracts were combined and successively washed with water and brine, dried over sodium sulfate and concentrated *in vacuo*. After being treated with an ethereal solution of diazomethane, the residue was purified by SiO_2 flash column chromatography (10 g). Elution with hexane–ethyl acetate (5 : 1) afforded **6** (56 mg, 55.6% yield), bp 145°C at 1.0 mmHg, $[\alpha]_D^{20}$ −15.3° (*c* 1.19) [lit.[16e] $[\alpha]_D^{23}$ −18.5° (*c* 1.05), lit.[17] $[\alpha]_D^{26}$ −11.1° (*c* 2.53)]; IR ν_{max} cm^{-1}: 3460, 2945, 2855, 1735, 1460, 1435, 1365, 1200, 1170; ^{1}H-NMR (270 MHz) δ: 0.88 (3H, t, *J*=6.6 Hz), 1.20–1.35 (18H, m), 1.40–1.58 (2H, m), 2.41 (1H, dd, *J*=16.4, 8.6 Hz), 2.52 (1H, dd, *J*=16.4, 3.5 Hz), 2.83 (1H, d, *J*=4.0 Hz), 3.71 (3H, s), 4.02 (1H, m). *Anal.* Found: C, 69.77; H, 11.95%. Calcd. for $C_{15}H_{30}O_3$: C, 69.72; H, 11.70%.

Its NMR spectrum was identical with that reported previously.[17]

^{1}H-NMR analysis of its MTPA ester (400 MHz) δ: 3.53 (0.045H, br. s, MTPA–OCH_3), 3.55 (2.955H, br. s, MTPA–OCH_3), 3.59 (0.045H, s, –$COOCH_3$), 3.66 (2.955H, s, –$COOCH_3$).

Methyl (R)-3-hydroxy-2,2-difluorotetradecanoate (**8**). Substrate **7** was obtained by methylation and subsequent pyridinium chlorochromate oxidation of the known 3-hydroxy-2,2-difluorotetradecanoic acid[17,25] in a 98% yield; IR ν_{max} cm^{-1}: 2940, 2860, 1790, 1755, 1460, 1440, 1320, 1200, 1140, 980, 820. This was employed in the next step without further purification. The reduction of **7** was carried out as already described. (*R*)-**8** (57 mg, 53.7% yield from 105 mg of **7**) was recrystallized from hexane to afford an analytical sample, mp 33–34°C, $[\alpha]_D^{21}$ −7.6° (*c* 0.83); IR ν_{max} cm^{-1}: 3402, 2940, 2900, 1763, 1469, 1338, 1312, 1288, 1261, 1215, 1099, 1072, 1026, 833, 790, 729, 671; ^{1}H-NMR (270 MHz) δ: 0.88 (3H, t, *J*=6.6 Hz), 1.20–1.47 (18H, m), 1.50–1.72 (2H, m), 1.97 (1H, d, *J*=7.2 Hz), 3.91 (3H, s), 3.95–4.10 (1H, m). *Anal.* Found: C, 61.24; H, 9.73%. Calcd. for $C_{15}H_{28}O_3F_2$: C, 61.20; H, 9.59%.

^{1}H-NMR analysis of MTPA ester of (±)-**8** δ: 3.51 (1.5H, br. s, MTPA–OCH_3), 3.58 (1.5H, br. s, MTPA–OCH_3), 3.74 (1.5H, s, –$COOCH_3$), 3.84 (1.5H, s, –$COOCH_3$). In the NMR spectrum of the MTPA ester of (*R*)-**8**, the δ 3.58 and 3.84 signals were not detected.

(R)-3-[(Benzyloxycarbonyl)oxy]-2,2-(difluoro)tetradecanoic acid (**9**). A solution of **8** (160 mg, 0.54 mmol), and carbobenzoxy chloride (185.6 mg, 1.09 mmol) and DMAP (132 mg) in dichloromethane (5 ml) was stirred for 10 min at 0°C, and then for 3 h at room temperature. The reaction mixture was diluted with ethyl acetate, and the solution was successively washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo* to give an oily substance. This was chromatographed on SiO_2 (10 g). Elution with hexane–ethyl acetate (20 : 1) gave an oil (173 mg, 77% yield), ^{1}H-NMR (400 MHz) δ: 0.88 (3H, t, *J*=6.0 Hz), 1.19–1.51 (18H, m), 1.70–1.75 (2H, m), 3.77 (3H, s), 5.10–5.25 (3H, m), 7.27–7.42 (5H, m). This was employed in the next step without further purification. The product (52 mg) was dissolved in ethanol (0.24 ml), a 2 N sodium hydroxide solution (0.084 ml) was added at 0°C, and the mixture was stirred for 2 h. After acidification, the reaction mixture was diluted with water and extracted with ethyl acetate. The extract was successively washed with water and brine, dried over sodium sulfate, and concentrated *in vacuo* to give an oily material, which solidified after standing in a refrigerator. This was recrystallized from hexane to afford **9** (34 mg, 68% yield from **8**), mp 36–38°C [lit.[21] mp 43–44°C], $[\alpha]_D^{20}$ +12.8° (*c* 0.81) [lit.[21] $[\alpha]_D^{24}$ +11.5° (*c* 0.9)]; IR ν_{max} cm^{-1}: 3456, 2037, 1732, 1643, 1466, 1396, 1350, 1296, 1280, 1207, 1145, 1088, 1026, 972, 941, 906, 825, 790, 744, 698; ^{1}H-NMR (400 MHz) δ: 0.87 (3H, t, *J*=6.5 Hz), 1.26 (18H, m), 1.70–1.80 (2H, m), 5.15–5.25 (2H, m), 7.27–7.38 (5H, m). Its IR and NMR spectra were identical with those reported previously.[21] HRMS: found, 415.2314; calcd. for $C_{22}H_{33}O_5F_2$ (M^++H), 415.2294.

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