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The Use of Microorganisms in Organic Synthesis. I. Microbiological Asymmetric Reduction of 2-Methyl-3-oxobutyrates

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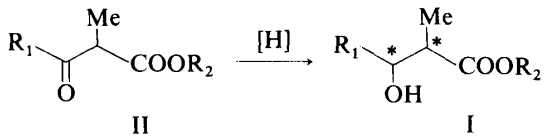
The synthesis of optically active α-methyl β-hydroxy esters I by means of microbiological reduction of the corresponding β-keto esters II was carried out. Benzyl 2-methyl-3-oxobutyrate **1** was found to be reduced by a variety of yeasts to the α-methyl β-hydroxy esters with (2*R*, 3*S*)- and (2*S*, 3*S*)-configurations (**2** and **3**, respectively), and by carrying out screening experiments, yeasts which give each product with high optical purity were isolated. Moreover, the absolute configuration and the optical purity of the reduction products were found to be determinable from the 400 MHz nuclear magnetic resonance spectra of the (+)-α-methoxy-α-trifluoromethylphenylacetyl esters of the alcohols produced.

Keywords——α-methyl β-keto ester; α-methyl β-hydroxy ester; asymmetric reduction; microbiological reduction; yeast

The use of optically active building blocks has become increasingly important in the synthesis of polyoxomacrolide antibiotics and related polyfunctional natural products.[1] The present paper deals with the synthesis of optically active α-methyl β-hydroxy esters I by means of microbiological reduction of the corresponding β-keto esters II. The reason why the β-hydroxy esters I were selected as target compounds is that the 3-hydroxy-2-methylpropionic ester moiety frequently appears in a variety of natural products such as erythromycins,[2] oudemansin,[3] and other polyoxoantibiotics.[4]



II I

It is well known that the reduction of β-keto esters by actively fermenting baker's yeast (*Saccharomyces cerevisiae*) affords optically active β-hydroxy esters.[5] We first examined[6] whether the 2-methyl derivatives II could be reduced by yeast in the same way as in the cases of simple β-keto esters and we then tried to find a facile and practical method for the determination of the absolute configuration and the optical purity of the reduction products. Benzyl 2-methyl-3-oxobutyrate (**1**)[7] was initially chosen as a substrate and extensive microbiological reduction studies were undertaken.

Reduction of **1** with fermenting baker's yeast produced an inseparable mixture of α-methyl β-hydroxy esters (**2** and **3**) in 44% yield, the ratio being 67:33 in favor of **2**. The mixture was converted to the silyl ethers (**4** and **5**), which could then be separated by means of high pressure liquid chromatography to the less polar fraction **4** ($[\alpha]_D$ +10.25° (c=5.05, $CHCl_3$)) and the more polar fraction **5** ($[\alpha]_D$ +28.48° (c=5, $CHCl_3$)) by elution with *n*-hexane–AcOEt (200:1). Both silyl ethers (**4** and **5**) were treated with tetrabutylammonium

fluoride ($Bu_4N^+F^-$) to afford the optically active α-methyl β-hydroxy esters (**2**; $[\alpha]_D$ +1.82° (c=4.9, $CHCl_3$), and **3**; $[\alpha]_D$ +18.41° (c=3.65, $CHCl_3$)), as shown in Chart 1.

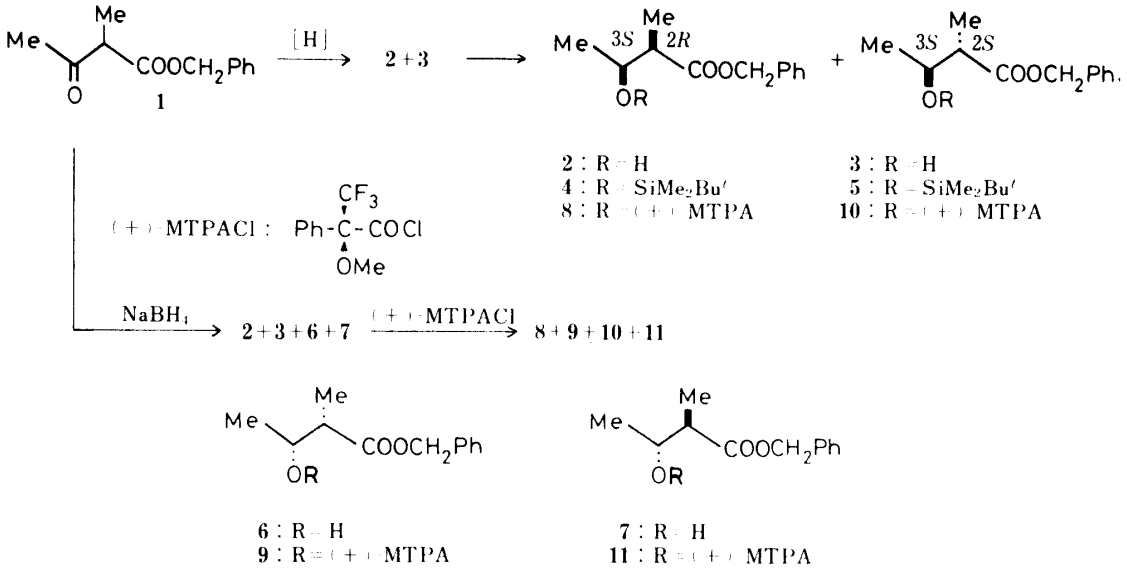


Chart 1

For the determination of the absolute structure and the optical purity of **2** and **3**, the four possible stereoisomers (**2**, **3**, **6**, and **7**) were prepared by $NaBH_4$ reduction of **1**. The four alcohols were converted into the corresponding (+)-MTPA esters (**8**—**11**) by treatment with (+)-MTPACl ((+)-α-methoxy-α-trifluoromethylphenylacetyl chloride.[8] In the 400 MHz nuclear magnetic resonance (NMR) spectra of the above mixtures of the (+)-MTPA esters, eight distinctly separated doublets due to eight types of secondary methyl groups were observed clearly in the absence of shift reagents as shown in Fig. 1.

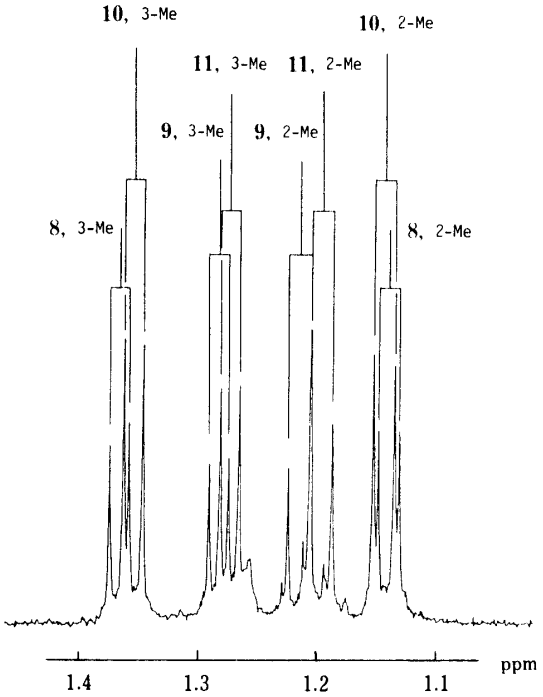
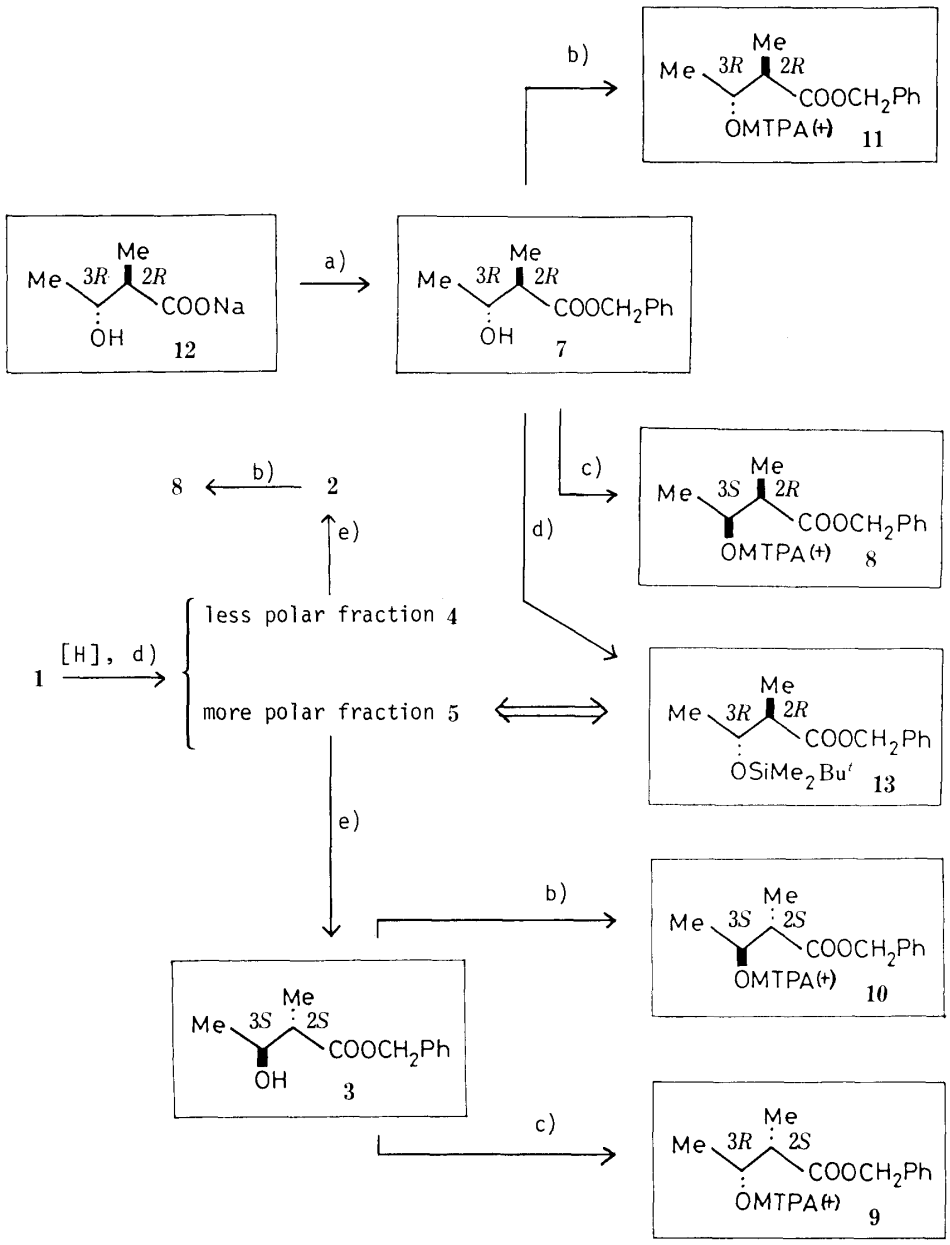


Fig. 1

In order to clarify the relationship between chemical shift and stereostructure, authentic samples (**8**—**11**) were prepared as shown in Chart 2.



a) $PhOP(O)Cl_2$, $PHCH_2OH$; b) (+)-MTPACl, pyridine;
c) EtOOC–N=N–COOEt, Ph_3, (+)-MTPA;
d) $^tBuMe_2SiCl$, imidazole; e) $Bu_4N^+F^-$.

Chart 2

Esterification of the known sodium (2*R*, 3*R*)-β-hydroxy carboxylate **12**[9] with benzyl alcohol in the presence of phenyl dichlorophosphate ($PhOP(O)Cl_2$)[10] gave the (2*R*, 3*R*)-benzyl ester **7**, which was treated with (+)-MTPACl to give the (2*R*, 3*R*)-(+)-MTPA ester **11**. Here, no indication of epimerization of C_2–Me was observed in the NMR spectrum. On the other hand, when **7** was treated with (+)-MTPA ((+)-α-methoxy-α-trifluoromethylphenylacetic acid)[8] in the presence of diethyl azodicarboxylate (EtOOC–N=N–COOEt)

Table I

Compound	2-Me	3-Me
8 (2*R*, 3*S*)	1.137, d, J=7.1 Hz	1.363, d, J=6.4 Hz
9 (2*S*, 3*R*)	1.214, d, J=7.1 Hz	1.280, d, J=6.4 Hz
10 (2*S*, 3*S*)	1.142, d, J=7.1 Hz	1.352, d, J=6.4 Hz
11 (2*R*, 3*R*)	1.195, d, J=7.1 Hz	1.272, d, J=6.4 Hz

and triphenylphosphine (Ph_3P) (Mitsunobu's method[11]), the (2*R*, 3*S*)-(+)-MTPA ester **8** was obtained. Silylation of **7** with *tert*-butyldimethylchlorosilane ($Me_2{}^tBuSiCl$) gave the corresponding (2*R*, 3*R*)-silyl ether **13** ($[\alpha]_D$ −30.79° (c=4.28, $CHCl_3$)). The NMR spectrum of **13** was identical with that of the above mentioned polar fraction **5**, but the sign of $[\alpha]_D$ was opposite to that of **5**. Consequently, the isomer **3** derived from **5** was confirmed to have (2*S*, 3*S*)-absolute configuration. Treatment of **3** with (+)-MTPACl gave the (2*S*, 3*S*)-(+)-MTPA ester **10**, while treatment of **3** with (+)-MTPA after conversion of the configuration of the C_3-hydroxyl group by Mitsunobu's method afforded the (2*S*, 3*R*)-(+)-MTPA ester **9**. The 400 MHz NMR spectra of the four standard (+)-MTPA esters (**8**—**11**) thus obtained were measured and the signals due to the secondary methyl groups were assigned as shown in Table I and Fig. 1.

Using these data, the absolute configuration of the reduction product **8** and hence that of **2** was determined as follows. Treatment of the isomer **2** derived from the less polar fraction **4** with (+)-MTPACl gave the (+)-MTPA ester **8**, whose chemical shifts due to the two methyl groups were identical with those of the authentic (2*R*, 3*S*)-(+)-MTPA ester **8**. Therefore, the isomer **2** was found to have (2*R*, 3*S*)-absolute configuration. Then, the optical purity of the microbiological reduction products was calculated on the basis of the intensity of C_2- or C_3-secondary methyl groups in the 400 MHz NMR spectra. The *syn*[12]-(+)-MTPA ester **8** derived from **4** was found to be almost optically pure (more than 99% *e.e.*) since no signals due to the antipodal isomer **9** were detected. On the other hand, the optical purity of the *anti*[12]-(+)-MTPA ester **10** derived from the more polar fraction **5** was shown to be 84% *e.e.*

Next, *Candida albicans* was used for reduction. The products (**2** and **3**) were found to have the same absolute configuration (**2**, (2*R*, 3*S*), optical purity 97% *e.e.*; **3**, (2*S*, 3*S*), optical purity 95% *e.e.*) as obtained by fermenting baker's yeast reduction. It should be emphasized here that the optical purity of **3** was significantly improved by the use of this microorganism.

Since a relatively simple method for the determination of the absolute structure and the optical purity of the reduction products was thus established, screening experiments were carried out using about 50 mg of **1** as a substrate for 16 kinds of yeasts.[13] The progress of the reduction was monitored by thin-layer chromatography (TLC). After completion of the reduction, the products were directly treated with (+)-MTPACl in pyridine and the resulting crude (+)-MTPA esters were separately purified by means of preparative thin-layer chromatography. The 400 MHz NMR spectra of these (+)-MTPA esters were measured and the absolute configuration, the optical purity (% *e.e.*) and the ratio of *syn*/*anti* isomers were determined. Selected data are shown in Table II.

In conclusion, α-methyl β-keto esters were found to be reduced by a variety of yeasts to the α-methyl β-hydroxy esters with (2*R*, 3*S*)- and (2*S*, 3*S*)-configurations and by carrying out screening experiments, yeasts which give products with very high optical purity were isolated. Moreover, the absolute configuration and the optical purity of the reduction products were found to be determinable from the 400 MHz NMR spectra of the (+)-MTPA esters of the alcohols produced. The present method was successfully applied for the determination of the stereostructure of the reduction products in other related systems, as will be described in the

Table II

Entry	Yeast	Chemical yield (%) (as (+)MTPA ester)	*syn*/*anti*	Optical yield (% *e.e.*)
1	*Endomycopsis fibligera*	68	31/69	*syn* 91 *anti* 94
2	*Hansenula anomala*	56	36/64	*syn* 92 *anti* 91
3	*Hansenula anomala* NI-7572	57	55/45	*syn* 97 *anti* 96
4	*Lipomyces starkeyi*	57	50/50	*syn* 92 *anti* 88
5	*Pichia farinosa*	64	50/50	*syn* >99 *anti* >99
6	*Pichia membranaefaciens*	57	67/33	*syn* 98 *anti* 94
7	*Rhodotorula glutinis*	22	94/6	*syn* 97 *anti* 87
8	*Saccharomyces acidifaciens*	63	42/58	*syn* 92 *anti* 90
9	*Candida albicans*	92 (as **2**+**3**)	26/74 (as **4**/**5**)	*syn* 97 *anti* 95
10	*Saccharomyces cerevisiae* (Baker's yeast)	44 (as **2**+**3**)	67/33 (as **4**/**5**)	*syn* >99 *anti* 84

subsequent paper.

Experimental

Melting points were measured with a Kofler micro melting point apparatus and are uncorrected. Infrared (IR) spectra (CCl_4) were measured on a JASCO A-3 spectrophotometer. NMR spectra were measured either on a JEOL FX-60 spectrometer or a JEOL FX-400 instrument. Spectra were taken as 5—10% w/v solutions in $CDCl_3$ with Me_4Si as an internal reference. Gas chromatography-mass spectroscopy (GC-MS) was carried out on a Hitachi RMU-6M mass spectrometer and high-resolution mass spectra were taken with a Hitachi M-80 GC-MS spectrometer. High pressure liquid chromatography (HPLC) was carried out on a JASCO TRI ROTAR (Senshu Pack N 50-5 column (Nucleosil, 5 mμ, 8 i.d. × 300 mm)). $[\alpha]_D$ was measured on a Perkin-Elmer model 241 MC polarimeter.

Asymmetric Reduction of 1 with Baker's Yeast (*Saccharomyces cerevisiae*)——i) A suspension of baker's yeast (available from Oriental Yeast Co., Ltd., 20 g), sucrose (20 g) and substrate **1** (1 g) in H_2O (100 ml) was shaken at 30 °C for 5 h. The reaction mixture was filtered with the aid of celite and the filtrate was extracted with ether. The ether extract was washed with sat. NaCl aq., then dried over $MgSO_4$. Removal of the solvent gave an oily product, which was chromatographed on silica gel (40 g) to give the reduction products (**2**+**3**; 441 mg, 44% yield) from the *n*-hexane–ethyl acetate (12 : 1—10 : 1) eluate.

ii) Imidazole (157 mg) and *tert*-butyldimethylchlorosilane (350 mg) were added to a solution of the reduction products (**2**+**3**; 400 mg) in dimethylformamide (DMF, 3 ml), and the mixture was stirred for 68 h at room temperature. After the addition of H_2O, the mixture was extracted with ether. The ether extract was washed with sat. NaCl aq., then dried over $MgSO_4$ and concentrated to give an oil, which was chromatographed on silica gel (50 g). Elution with *n*-hexane–ethyl acetate (100 : 1) afforded 446 mg (72% yield) of silyl ethers (**4**+**5**). This mixture was subjected to HPLC with *n*-hexane–ethyl acetate (200 : 1) as a solvent system (flow rate; 3 ml/min), yielding the less polar fraction **4** (119 mg) and the more polar fraction **5** (78 mg). In this case, the ratio of **4**/**5** was found to be 67/33. **4**: *Anal.* High-resolution MS. Calcd for $C_{18}H_{30}O_3Si$ ($M^+ + 1$; *m*/*e*): 323.204. Found: 323.209. $[\alpha]_D^{26}$ +10.25° (*c* = 5.05, $CHCl_3$). IR ν_{max} cm^{-1}: 1732. 400 MHz NMR δ: 0.012, 0.039 (each 3H, s; $SiMe_2$), 0.858 (9H, s; *tert*-Bu), 1.135 (3H, d, J = 6.1 Hz; 3-Me), 1.174 (3H, d, J = 7 Hz; 2-Me), 2.468 (1H, qq, J = 6.1, 7 Hz; 2-H), 4.093 (1H, qq, J = 6.1, 6.1 Hz; 3-H), 5.107 (2H, q, J = 12.5 Hz; $\underline{CH_2}Ph$) **5**: *Anal.* High-resolution MS. Calcd for $C_{18}H_{30}O_3Si$ ($M^+ + 1$; *m*/*e*): 323.204.

Found: 323.202. $[\alpha]_D^{26}$ +28.48° (c=5, $CHCl_3$). IR ν_{max} cm^{-1}: 1732. 400 MHz NMR δ: 0.022, 0.049 (each 3H, s; $SiMe_2$), 0.853 (9H, s; *tert*-Bu), 1.104 (3H, d, J=7.1 Hz; 2-Me), 1.121 (3H, d, J=6.3 Hz; 3-Me), 2.556 (1H, qq, J=7.1, 7.1 Hz; 2-H), 4.049 (1H, qq, J=6.3, 7.1 Hz; 3-H), 5.103 (2H, s; CH_2Ph).

iii) A mixture of **4** (100 mg) and $Bu_4N^+F^-\cdot 3H_2O$ (300 mg) in tetrahydrofuran (THF, 5 ml) was stirred for 3 h at room temperature and the reaction mixture was washed with sat. NaCl aq., then dried over $MgSO_4$ and concentrated to give an oil, which was chromatographed on silica gel (10 g) to afford *syn*-**2** (57 mg; 88% yield) as a homogeneous oil from the *n*-hexane–ethyl acetate (10 : 1) eluate. *Anal.* High-resolution MS. Calcd for $C_{12}H_{16}O_3$ (M^+, *m/e*): 208.110. Found: 208.108. $[\alpha]_D^{27}$ +1.82° (c=4.9, $CHCl_3$). IR ν_{max} cm^{-1}: 3550, 1720. 400 MHz NMR δ 1.162 (3H, d, J=6.4 Hz; 3-Me), 1.206 (3H, d, J=7.1 Hz; 2-Me), 2.555 (1H, qq, J=7.1, 3.9 Hz; 2-H), 4.088 (1H, qq, J=6.4, 3.9 Hz; 3-H), 5.145 (2H, s; CH_2Ph), 2.515 (1H, br s; OH). A mixture of **5** (78 mg) and $Bu_4N^+F^-\cdot 3H_2O$ (240 mg) in THF (3 ml) was stirred for 1.5 h at room temperature and the reaction mixture was worked up in the same way as in the case of **4** to provide *anti*-**3** (36.5 mg; 72% yield) as a homogeneous oil from *n*-hexane–ethyl acetate (10 : 1) eluate. *Anal.* High-resolution MS. Calcd for $C_{12}H_{16}O_3$ (M^+, *m/e*): 208.110. Found: 208.110. $[\alpha]_D^{26}$ +18.41° (c=3.65, $CHCl_3$). IR ν_{max} cm^{-1}: 3530, 1720. 400 MHz NMR δ 1.196 (3H, d, J=7.3 Hz; 2-Me), 1.206 (3H, d, J=6.4 Hz; 3-Me), 2.508 (1H, qq, J=7.3, 6.9 Hz; 2-H), 3.903 (1H, qq, J=6.4, 6.9 Hz; 3-H), 5.151 (2H, s; CH_2Ph), 2.652 (1H, br s; OH).

Preparation of Four Alcohols (2, 3, 6 and 7) and Their (+)-MTPA Esters (8—11)——i) A mixture of **1** (265 mg) and $NaBH_4$ (200 mg) in EtOH (3 ml) was stirred for 1 h at 0 °C and the reaction mixture was extracted with ether after the addition of H_2O. The ether extract was washed with sat. NaCl aq., dried over $MgSO_4$ and concentrated to give a mixture of four alcohols (**2**+**3**+**6**+**7**; 254 mg, 95% yield). A part of the mixture (50 mg) was converted to the corresponding 3,5-dinitrobenzoates, which were recrystallized from MeOH to give pale yellow prisms, mp 59.5—60 °C. *Anal.* Calcd for $C_{19}H_{18}N_2O_8$: C, 56.71; H, 4.51; N, 6.96. Found: C, 56.60; H, 4.41; N, 6.93. The ratio of *syn*-(**2**+**6**)/*anti*-(**3**+**7**) was found to be 2 : 3 by a comparison of the integral intensity of methylene group signals (δ 5.145 and 5.151 for **2**+**6** and **3**+**7**, respectively in the 400 MHz NMR spectra.

ii) Pyridine (0.5 ml) was added to a mixture of the four alcohols (**2**+**3**+**6**+**7**; 53 mg) and (+)-MTPACl (96 mg), and the reaction mixture was stirred for 96 h at room temperature. After the addition of H_2O, the reaction mixture was extracted with ether. The ether extract was washed with sat. NaCl aq., dried over $MgSO_4$ and concentrated to give an oil, which was chromatographed on silica gel (20 g) to afford the (+)-MTPA esters (**8**+**9**+**10**+**11**; 106 mg, 98% yield) as a homogeneous oil from the *n*-hexane–ethyl acetate (9 : 1) eluate. **8**+**9**: 400 MHz NMR δ 1.137 (3H, d, J=7.1 Hz; 2-Me), 1.214 (3H, d, J=7.1 Hz; 2-Me), 1.363 (3H, d, J=6.4 Hz; 3-Me), 1.280 (3H, d, J=6.4 Hz; 3-Me). **10**+**11**: 400 MHz NMR δ 1.142 (3H, d, J=7.1 Hz; 2-Me), 1.195 (3H, d, J=7.1 Hz; 2-Me), 1.352 (3H, d, J=6.4 Hz; 3-Me), 1.272 (3H, d, J=6.4 Hz; 3-Me).

Synthesis of Authentic (2*R*, 3*R*)-7 and Its Silyl Ether 13 from the Known Sodium Carboxylate 12——i) Pyridine (96 μl), phenyl dichlorophosphate (89 μl) and then benzyl alcohol (83 μl) were added to a solution of **12** (56 mg) in dimethoxyethane (2 ml) under an argon atmosphere at 0 °C. The reaction mixture was stirred for 17 h at room temperature then extracted with chloroform after acidification with aq. 1 N HCl. The chloroform extract was washed with sat. NaCl aq., dried over $MgSO_4$ and concentrated to give an oil, which was chromatographed on silica gel (8 g) to provide a mixture (92 mg) of **7** and a trace of $PhCH_2OH$ from the *n*-hexane–ethyl acetate (10 : 1) eluate. The NMR spectrum of **7** was identical with that of *anti*-**3** derived from **5**.

ii) Imidazole (57 mg) and *tert*-butyldimethylchlorosilane (100 mg) were added to a solution of the above mixture (92 mg) in dimethylformamide (DMF) (1 ml), and the reaction mixture was stirred for 20 h at room temperature. After the addition of H_2O, the reaction mixture was extracted with ether. The ether extract was washed with sat. NaCl aq., dried over $MgSO_4$ and concentrated to give an oil, which was subjected to preparative thin layer chromatography (silica gel, 20 cm × 20 cm; solvent, *n*-hexane–ethyl acetate (10 : 1)) to provide (2*R*, 3*R*)-**13** (43 mg, 33% yield from **12**). The NMR spectrum of **13** was identical with that of the more polar fraction **5** derived from baker's yeast reduction of **1**. $[\alpha]_D^{26}$ −30.79° (c=4.28, $CHCl_3$).

Preparation of Authentic (2*R*, 3*R*)-(+)-MTPA Ester 11——Pyridine (0.5 ml) was added to a mixture of **7** (26 mg, prepared from **12**) and (+)-MTPACl (47 mg) and the reaction mixture was stirred for 17 h at room temperature, then worked up in the same way as described for the preparation of the four (+)-MTPA esters (**8**+**9**+**10**+**11**) to give an oil, which was subjected to preparative TLC to afford **11** (19 mg, 35% yield) as a homogeneous oil. MS *m/e*: 424 (M^+). 400 MHz NMR δ: 1.195 (3H, d, J=7.1 Hz; 2-Me), 1.272 (3H, d, J=6.4 Hz; 3-Me), 2.797 (1H, qq, J=7.1, 2-H), 3.458 (3H, d, J=1.2 Hz; OMe), 5.06 (2H, q, J=12.5 Hz; CH_2Ph), 5.398 (1H, qq, J=6.4 Hz; 3-H).

Preparation of Authentic (2*R*, 3*S*)-(+)-MTPA Ester 8——A mixture of **7** (42 mg, prepared from **12**), (+)-MTPA (71 mg), EtOOC–N=N–COOEt (106 mg) and Ph_3P (158 mg) in dry benzene (3 ml) was stirred under an argon atmosphere for 137 h at room temperature. After the addition of H_2O, the reaction mixture was extracted with ether. The ether extract was washed with sat. NaCl aq., dried over $MgSO_4$ and concentrated to give an oil, which was subjected to preparative TLC to afford **8** (28 mg, 33% yield) as a homogeneous oil. MS *m/e*: 424 (M^+). 400 MHz NMR δ: 1.137 (3H, d, J=7.1 Hz; 2-Me), 1.363 (3H, d, J=6.4 Hz; 3-Me), 2.691 (1H, qq, J=7.1 Hz; 2-H), 3.509 (3H, d, J=1 Hz; OMe), 5.058 (1H, q, J=12.5 Hz; CH_2Ph), 5.459 (1H, qq, J=6.4 Hz; 3-H).

(+)-MTPA Esterification of Baker's Yeast Reduction Product (2*S*, 3*S*)-3——Pyridine (0.5 ml) was added to a

mixture of **3** (21 mg) and (+)-MTPACl (39 mg), and the reaction mixture was stirred for 17 h at room temperature. The reaction mixture was worked up and purified in the same way as in the case of **8** to provide **10** (13 mg, 30% yield) as a homogeneous oil. *Anal.* High-resolution MS. Calcd for $C_{22}H_{23}F_3O_3$ (M^+, *m/e*): 424.150. Found: 424.152. 400 MHz NMR δ: 1.142 (3H, d, J=7.1 Hz; 2-Me), 1.352 (3H, d, J=6.4 Hz; 3-Me), 2.791 (1H, qq, J=7.1 Hz; 2-H), 3.502 (3H, d, J=1 Hz; OMe), 4.952 (2H, q, J=12.5 Hz; $\underline{CH_2}$Ph), 5.407 (1H, qq, J=6.4 Hz; 3-H). The optical purity of **10** and hence that of **3** was found to be 84% *e.e.*

Preparation of Authentic (2*S*, 3*R*)-(+)-MTPA Ester 9 from 3——A mixture of **3** (42 mg), (+)-MTPA (71 mg), EtOOC–N=N–COOEt (53 mg) and Ph_3P (79 mg) in dry benzene (3 ml) was stirred under an argon atmosphere for 120 h at room temperature. The reaction mixture was worked up and purified in the same way as in the case of **8** to afford **9** (35 mg, 41% yield) as a homogeneous oil. MS *m/e*: 424 (M^+). 400 MHz NMR δ: 1.214 (3H, d, J=7.1 Hz; 2-Me), 1.280 (3H, d, J=6.4 Hz; 3-Me), 2.718 (1H, qq, J=7.1 Hz; 2-H), 3.475 (3H, d, J=1 Hz; OMe), 5.102 (2H, q, J=12.2 Hz; $\underline{CH_2}$Ph), 5.486 (1H, qq, J=6.4 Hz; 3-H).

(+)-MTPA Esterification of Baker's Yeast Reduction Product 2——Pyridine (0.5 ml) was added to a mixture of **2** (20 mg) and (+)-MTPACl (36 mg), and the reaction mixture was stirred for 17 h at room temperature. The reaction mixture was worked up and purified in the same way as in the case of the preparation of authentic **8** to provide (+)-MTPA ester **8** (13 mg, 33% yield) as a homogeneous oil. *Anal.* High-resolution MS. Calcd for $C_{22}H_{23}F_3O_3$ (M^+, *m/e*): 424.150. Found: 424.148. The 400 MHz NMR spectrum of **8** was identical with that of authentic (+)-MTPA ester **8**. The optical purity of **8** and hence that of **2** was found to be more than 99% *e.e.*

Asymmetric Reduction of 1 with *Candida albicans*——i) Asymmetric reduction of **1** with *Candida albicans* was carried out according to our reported fermentation procedure.[13] A test tube (25 mm × 200 mm) containing 10 ml of culture medium comprising 5% glucose, 0.1% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.05% urea, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% $CaCl_2 \cdot 2H_2O$, 0.1% yeast extract, a trace of mineral solution (0.1% $FeSO_4 \cdot 7H_2O$, 0.1% $MnCl_2 \cdot 4H_2O$, 0.1% $ZnSO_4 \cdot 7H_2O$; 0.2 ml per 100 ml of culture medium) and tap water (pH 7.0) was inoculated with *Candida albicans* and the yeast was cultured at 30 °C for 2 d with continuous shaking. Then 1 ml of the above seed culture was transferred to 200 ml of the same medium. After cultivation for 3 d, 100 mg of the substrate **1** was added and cultivation was continued for a further 3 d under the same conditions. This asymmetric reduction of **1** (finally 1.272 g of **1**) was carried out twelve times on the same scale.

ii) The reaction mixture was worked up and purified in the same way as in the case of baker's yeast reduction of **1** to provide the reduction products (**2**+**3**; 1.181 g, 92% yield). Then imidazole (733 mg) and *tert*-butyldimethylchlorosilane (1.284 g) were added to a solution of the reduction products (**2**+**3**; 1.181 g) in DMF (3 ml) and the reaction mixture was stirred for 97 h at room temperature. The reaction mixture was worked up and purified in the same way as in the case of silylation of the baker's yeast reduction product to give silyl ethers (**4**+**5**; 1.894 g). A part of the silyl ethers was subjected to the same HPLC separation as applied to the silyl ethers (**4**+**5**) derived from the baker's yeast reduction product and separated to the less polar fraction **4** (92 mg) and the more polar fraction **5** (223 mg). In this case, the ratio of **4**/**5** was found to be 26/74. **4**: $[\alpha]_D^{26}$ +11.21° (c=6.2, $CHCl_3$). **5**: $[\alpha]_D^{26}$ +35.42° (c=4.96, $CHCl_3$). Both the less polar fraction **4** and the more polar fraction **5** were identical with previous samples of **4** and **5** derived from the baker's yeast reduction products with respect to NMR and IR spectra.

iii) Each silyl ether (**4**; 92 mg and **5**; 110 mg) thus obtained was separately treated with $Bu_4N^+F^- \cdot 3H_2O$ (276 mg in the case of **4** and 330 mg in the case of **5**) in THF (3 ml) for 1.5 h at room temperature to give the desilylated product (**2**, 46 mg, 77% yield; **3**, 65 mg, 91% yield) by the same work-up and purification procedures as in the case of baker's yeast reduction. Both desilylated products **2** and **3** were identical with previous samples **2** and **3** prepared by baker's yeast reduction of **1** with respect to NMR (60 MHz) and IR spectra **2**: $[\alpha]_D^{22}$ +2.84° (c=4.58, $CHCl_3$). **3**: $[\alpha]_D^{22}$ +21.85° (c=6.48, $CHCl_3$).

(+)-MTPA Esterification of *Candida albicans* Reduction Products (2 and 3)——i) Pyridine (0.5 ml) was added to a mixture of **2** (46 mg) and (+)-MTPACl (68 mg), and the reaction mixture was stirred for 17 h at room temperature, then worked up and purified in the same way as in the case of the preparation of authentic **8** to provide (+)-MTPA ester **8** (69 mg, 74% yield) as a homogeneous oil. The optical purity of **8** and hence that of **2** was found to be 97% *e.e.*

ii) Pyridine (0.5 ml) was added to a mixture of **3** (36 mg) and (+)-MTPACl (66 mg), and the reaction mixture was stirred for 17 h at room temperature, then worked up and purified in the same way as in the case of **8** to afford the (+)-MTPA ester **10** (61 mg, 84% yield) as a homogeneous oil. The optical purity of **10** and hence that of **3** was calculated as 95% *e.e.*

Screening of Various Yeasts——Microorganisms previously described[13] by us were used to reduce the starting material **1**. Erlenmeyer flasks (500 ml) containing 100 ml of the same culture medium as in the case of *Candida albicans* cultivation were inoculated with microorganisms, and culture was carried out at 30 °C for 4 d with continuous shaking. Then the substrate (*ca.* 50 mg of compound **1**) was added, and culture was continued for a further 3 d under the conditions. Each reaction mixture was separately worked up in the same way as in the case of baker's yeast reduction to give the crude reduction product. Pyridine (0.5 ml) was added to a mixture of each reduction product and (+)-MTPACl (*ca.* 80 mg), and the reaction mixture was stirred for 48 h at room temperature, then worked up and purified in the same way as in the case of **8** to afford the (+)-MTPA ester as shown in Table II.

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