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Preparation of Optically Pure α-Alkyl β-Hydroxy Nitriles by the Bakers' Yeast Reduction

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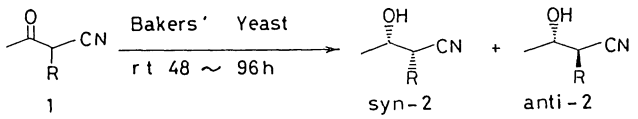
The bakers' yeast reduction of 2-alkyl-3-oxobutyronitrile gave optically pure 2-alkyl-3-hydroxybutyronitriles. Especially 2-phenyl-3-oxobutyronitrile gave only syn diastereomer of 2-phenyl-3-hydroxybutyronitrile in high optical yield.

Nitrogen-functionalyzed optically active secondary alcohol derivatives are sometimes found in biologically active compounds as important moiety for their activity.[1] It is, therefore, required to develop the efficient and convenient methods to supply nitrogen-functionalized alcohol derivatives. Since the bakers' yeast has been recognized as a useful reducing agent for asymmetric reduction of various functionalized ketones to produce optically active secondary alcohols under mild conditions and simple operations.[2] Chemical yield and enantioselectivity in the yeast reduction of various substrates, however, are not always satisfactory i.e. it has sometimes been observed that low enantioselectivity and decomposition of the substrate occured. Although much efforts have been paid for screening microbes which have good ability to reduce various kind of ketones with high enantioselectivity, it is also important to get a knowledge for the functional groups which have a good affinity toward the microbe.[3] We would like to report in this paper that the bakers' yeast reduction of 2-alkyl β-keto nitriles into the corresponding hydroxy nitriles with high enantioselectivity (Scheme 1). Since the cyano group is a functional group widely used as a precursor of both of amino group and carbonyl group,[4] hydroxy nitriles obtained should become wide variety of useful key intermediates in the synthesis of optically active natural products which are involving nitrogen functional group and carbonyl group.



Scheme 1.

Results and Discussion

Several keto nitriles, i.e. 2-methyl- (**1a**), 2-ethyl- (**1b**), 2-butyl- (**1c**), and 2-phenyl-3-oxobutyronitrile (**1d**) were prepared by the reaction of acetyl chloride with α-lithiated nitriles and subjected to the reduction with bakers' yeast. Results of the present microbial reduction of β-keto nitriles are summarized in Table 1.

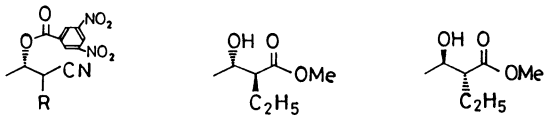
The products **2** consisted of syn and anti isomers, which could be separated into each of pure diastereomers as 3,5-dinitrobenzoates by silica gel flash column chromatography except for the case of nitrile **2c**. Each of benzoates were converted into (+)-α-methoxy-α-(trifluoromethyl)phenylacetates (MTPA esters) of 2-alkyl-3-hydroxybutyronitriles by treatment with lithium aluminum hydride followed by (+)-MTPA chloride for determination of their enantiomeric excess by ^{1}H NMR and ^{19}F NMR analysis.[5] Completely enantioselective reduction was achieved (Entries 2, 3, and 4), except for the reduction of 2-methyl-3-oxobutyronitrile (Entry 1). To determine the configuration, each of diasteromers of **2b** were converted into the correesponding hydroxy esters. According to ^{1}H NMR analysis of the hydroxy ester,[6] the assignment of diastereomers could be achieved. Consequently it was found that the chemical shift of the methine proton at 2-position of *syn*-**2b** was in lower field than the anti one, in analogy with compounds **2a** and **2c**, the isomers which have higher value of chemical shift of the methine proton were presumed to be the syn isomers. Hence the ratio of each of diastereo-

Table 1. Results of the Bakers' Yeast Reduction of 3-Oxobutyronitriles

Entry	Product	R	Yield/%	syn : anti[a]	$[\alpha]_D$ in EtOH, *c* ca. 1	
					syn-**2**	*anti*-**2**
1	**2a**	CH_3	69	56 : 44	+17.3°(82%ee, 3*S*)[b]	−11.9° (74%ee, 3*S*)[b]
2	**2b**	C_2H_5	46	54 : 46	+ 9.5°(>98%ee, 3*S*)[b]	−18.8° (>98%ee, 3*S*)[b]
3	**2c**	*n*-C_4H_9	43	58 : 42	+14.3°(>98%ee, 3*S*)[b]	—[c]
4	**2d**	Ph	61	>99 : <1	−33.3°(>98%ee, 3*S*)[b]	

a) Determined by ^{1}H NMR analysis. b) The enantiomeric excess was determined by ^{1}H and ^{19}F NMR analyses of (+)-MTPA ester.[5] c) Anti isomer could not be isolated in pure state by the flash column chromatography on silica gel.

mers of yeast reduction products of **2a**, **2b**, and **2c** could be determined by ^{1}H NMR analysis. For the determination of the absolute configuration, anti isomer of 2-ethyl-3-hydroxybutyronitrile (**2b**) was converted into the corresponding hydroxy ester **4**. Comparing the specific rotation of methyl (2*S*,3*R*)-2-ethyl-3-hydroxybutanoate (**5**) which was derived from methyl (3*R*)-3-hydroxybutanoate,[7] the absolute configuration of the starting nitrile *anti*-**2b** was assigned to be 2*R*, 3*S*.

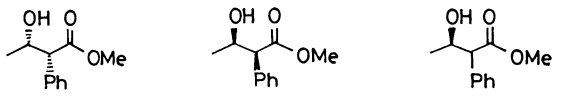


3 4 5

Fig. 1.

Absolute configuration of 3-position of **2a** and **2c** were presumed by the specific rotations and the chemical shift of the methoxyl group of (+)-MTPA esters by comparison with the result in the case of (3*R*)-2-ethyl-3-hydroxybutyronitrile which was assigned by the method above mentioned.

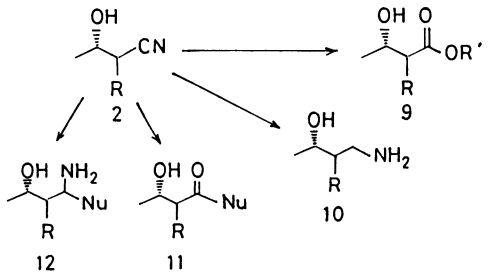
Although the starting material was racemic at C-2 position, only single diastereoisomer was obtained in the case of the reduction of 2-phenyl-3-oxobutyronitrile (**1d**) in optically pure state (Entry 4). To determine the absolute configuration, nitrile **2d** obtained by bakers' yeast reduction was converted into the hydroxy ester **6** by the acid hydrolysis of the cyano group followed by treatment of diazomethane. ^{1}H NMR spectrum of the acetate of **6** agreed completely with that of syn isomer of (±)-**6**.[8] The absolute configuration of nitrile **2d** at C-2 and C-3 position was presumed to be 2*R*, 3*S* on the basis of the sign of the optical rotation of the hydroxy ester **6** with that of (−)-(2*S*, 3*R*)-**7**. This result agreed with a general rule for the enantioselectivity in the bakers' yeast reduction.[9]



6 7 8

Fig. 2.

This microbial reduction of 3-oxobutyronitriles possesses two outstanding characteristics for the preparation of optically active building blocks for organic synthesis. First, the reduction of 2-phenyl-3-oxobutyronitrile gave a single diastereoisomer of the hydroxy nitrile in optically pure state in good yield. We presumed the reason of this extremely high syn diastereoselectivity of the reduction of 2-phenyl-3-oxobutyronitrile was due to the enhanced enolization of the 3-oxo group[10,11] by the phenyl group. Recently, it has been reported that details of the diastereoselectivity of the bakers' yeast reduction of 2-alkyl-3-oxobutanoate.[12] The keto nitrile **1d** is regarded as equivalent in 2-phenyl-3-oxobutanoate. The bakers' yeast reduction of methyl 2-phenyl-3-oxobutanoate, however, gave a corresponding hydroxy ester in very low yield. In addition, only the reduction of **1d**, the high diastereoselectivity was observed. Other ketones of **1a**, **1b**, and **1c**, however, the bakers' yeast reduction provided good enantioselectivity but poor diastereoselectivity. It is of much interest to compare the results of this microbial reduction of 3-oxobutyronitrile **1** with those of 2-alkyl-3-oxobutanoates[12] in considering the factor which define the stereoselectivity in the enzymatic systems of the yeast. Second, the reduction products involve a cyano group in the molecule. Since the cyano group is a precursor of both of amino group and carbonyl group,[4] hydroxy nitriles **2** obtained in this reaction should become useful building blocks for the synthesis of variety of optically active natural products. As described above, 3-hydroxy esters **9** could be converted from the nitrile **2** easily. And the reduction of **2b** at room temperature for 24 h with 10 mol% of PtO_2 under hydrogen atmosphere[13] provided the amino alcohol **10** in 85% yield. Nitriles react with some nucleophilic reagents under presence of acidic catalyst,[4] both of β-hydroxy ketone **11** and γ-amino alcohol **12** may also be derived from **2** (Scheme 2).



Scheme 2.

It should be emphasized here that the most important thing is to use yeast as a reagent in organic synthesis in designing the substrate to supply the microbial reduction and to modify the substrate which could become a good chiral synthon after reduction. In the present reaction, it has been revealed that the bakers' yeast could play an important role to reduce 3-oxobutyronitriles giving 3-hydroxybutyronitriles with high optical purity.

Experimental

Instruments. NMR spectra were recorded on Varian VXR-200, VXR-500, and JEOL MH-100 spectrometers in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. Optical rotations were measured with a JASCO DIP-

4 digital polarimeter. IR spectra were recorded on JASCO A-102 spectrometer on KBr disk.

Materials. Solvents and commercially available starting materials were generally used without additional purification unless otherwise indicated. Flash chromatography was done on a column of silica gel (Wako gel C-300). Tetrahydrofuran (THF) and diethyl ether (Et_2O) were freshly distilled from sodium benzophenone ketyl. Pyridine and dichloromethane were refluxed on calcium hydride for 5 h and distilled and stored under argon in the presence of Molecular Sieves 4A.

Preparation of 2-Alkyl-3-oxobutyronitrile (1). To a solution of lithium diisopropylamide (LDA) in 30 mL of THF was added a solution of a nitrile (30 mmol) in 60 mL of THF at −50 °C under argon atmosphere and the solution was stirred for 3 h to produced a white sluggish solution, then THF (15 mL) solution of acetyl chloride (1.178 g, 15 mmol) was added one portion into the sluggish solution at −78 °C. After the addition was complete the mixture was stirred and allowed to warm to 0 °C. The reaction mixture was acidified with 2 M HCl solution, then extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to give the crude product. Purification by silica gel flash chromatography (ethyl acetate/hexane=1 : 20 to 1 : 5) gave 3-oxobutyronitrile **1** in 51 to 80% yields. Chemical yield (%), boiling point (°C/mmHg; 1 mmHg≈133.322 Pa), R_f value on silica gel TLC, ^{1}H NMR spectral data (100 MHz, δ TMS in $CDCl_3$), and IR spectral data of ketones **1** are summarized below.

1a: 60%; 60/75 mmHg by Kugelrohr; 0.4 (AcOEt/hexane=1 : 2); 1.5 (3H, d, J=7.8 Hz), 2.4 (3H, s), and 3.7 (1H, q, J=8.4 Hz) ; 3000, 2950, 2310 (CN), 1725 (C=O), 1480, 1360, and 1170 cm^{-1} (neat).

1b: 58%; 85/75 mmHg by Kugelrohr; 0.4 (AcOEt/hexane=1 : 2); 1.1 (3H, t, J=6.6 Hz), 1.8—2.1 (2H, m) , 2.3 (3H, s), and 3.5 (1H, t, J=7.0 Hz) 2970, 2920, 2870, 2230 (CN), 1720 (C=O), 1360, 1160, and 1010 cm^{-1} (neat).

1c: 51%; 110/25 mmHg by Kugelrohr; 0.5 (AcOEt/hexane=1 : 5); 1.0 (3H, t , J=6.5 Hz) , 1.2—2.2 (6H, m), 2.5 (3H, s) 3.5—3.9 (1H, t, J=4.0 Hz); 2970, 2930, 2250 (CN), 1720 (C=O) cm^{-1} (neat).

1d: 80%; mp 97 °C; 0.5 (AcOEt/hexane=1 : 1); 2.3 (3H, s), 4.8 (1H, s), and 7.6 (5H, s); 3050, 2950, 2200(CN), 1730(C=O), 1360, and 1140 cm^{-1} on KBr disk.

Reduction of 2-Alkyl-3-oxobutyronitrile with Bakers' Yeast. To a stirred solution of 30 g of D-glucose in 200 mL of water was added 25 g of bakers' yeast (Oriental Yeast Co.) and the suspension was stirred for 30 min, then, 20 ml of an ethanol solution of 2-phenyl-3-oxobutyronitrile (**1d**) (0.815 g, 5.12 mmol) was added. The mixture was stirred at room temperature for 48 h, and then the yeast suspension of 25 g of yeast, 30 g of D-glucose in 200 mL of water was added and stirred for an additional 148 h. The mixture was filtered through a Celite pad. The filtrate was saturated with NaCl, and extracted with ethyl acetate. The extracts were concentrated in vacuo and the residue was subjected to silica gel flash column to give the alcohol **2d** (0.502 g, 3.12 mmol, 65%) as a yellow oil. The chemical yield of another alcohols **2** obtained by the same method as above are described as followed. **2a**=69%, **2b**=46%, and **2c**=43%. The ratio of diastereomers was determined by 200 MHz ^{1}H NMR analysis. In the case of the reduction of **1d**, no diastereomer could not be detected in **2d** produced in the yeast reduction using 500 MHz ^{1}H NMR.[14]

Preparation of 3,5-Dinitrobenzoates (3).[15] To a solution of **2** (1.0 mmol), pyridine (1.0 mmol), and 4-dimethylaminopyridine (DMAP)(0.1 mmol) in 2.5 mL of dichloromethane (CH_2Cl_2) solution was added 1.5 mmol of 3,5-dinitrobenzoyl chloride in 1.5 mL of CH_2Cl_2 under argon at 0 °C and stirred for 3 h at room temperature. Some pieces of crashed ice were then added to the reaction mixture, and this mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give a yellow solid. Separation of diastereomers could be performed by silica gel flash column chromatography or silica gel TLC. Chemical yield (%), melting point (°C), R_f value on silica gel TLC, and IR spectral data(KBr disk) are summarized below.

3a: 95%; 70 (syn), 65 (anti); 0.6 (syn) and 0.5 (anti), AcOEt/hexane=1 : 1 after 5 times of development; 3100, 2960, 2240(CN), 1730(CO) , 1550, 1170, 920, 720 cm^{-1}.

3b: 95%; 70 (syn), 92 (anti); 0.6, (syn), 0.45 (anti), AcOEt/hexane=1 : 5, after 4 times of development; 3100, 2960, 2920, 2240(CN) , 1730(CO) , 1550, 1340, 1270, 1170, 920, 720 cm^{-1}.

3c: 87%; 98 (syn); 0.65 (syn), AcOEt/hexane=1 : 5, after 10 times of development; 3150, 2950, 2850, 2230(CN), 1730(CO), 1550, 920 cm^{-1}.

3d: 61%; 115; 0.25, AcOEt/hexane=1 : 3, after 3 times of development; 3110, 2950, 2850, 2230 (CN), 1780 (CO), 1530, 950 cm^{-1}.

Spectral data, each diastereomers of the esters of **3** of ^{1}H HMR (200 MHz, δ, $CDCl_3$, J/Hz), ^{13}C NMR (50 MHz, δ, $CDCl_3$, ppm) are summarized below.

3a(syn): 1.423 (3H, d, J=7.25), 1 579 (3H, d, J=6.40), 3.172 (1H, dq, J_1=4.99, J_2=7.26), 5.245, (1H, dq, J_1=5.05, J_2=6.38), 9.131 (2H, d, J=2.11), 9.236 (1H, t, J=2.12); 14.146, 16.085, 31.143, 72.740, 122.840(CN), 119.259, 129.405, 133.071, 148.612, 161.555.

3a(anti): 1.420 (3H, d, J=6.35), 1.555 (3H, d, J=6.35), 3.025 (1H, dq, J_1=5.42, J_2=7.23), 5.318 (1H, dq, J_1=5.40, J_2=6.30), 9.141 (2H, d, J=2.06), 9.230 (1H, t, J=2.12); 14.366, 17.925, 31.627, 72.925, 122.711(CN), 199.259, 129.043, 133.043, 148.805, 161.555.

3b(syn): 1.128 (3H, t, J=7.40), 1.523 (3H, d, J=6.24), 1.60—1.76 (2H, m), 2.980 (1H, dq, J_1=5.21, J_2=7.54), 5.253 (1H, dq, J_1=5.61, J_2=5.96), 9.097 (2H, d, J=3.00) 9.187 (1H, t, J=2.20); 11.640, 16.588, 22.284, 39.192, 71.699, 118.426, 122.364(CN), 129.451, 133.142, 148.707, 161.578.

3b(anti): 1.156 (3H, t, J=7.20), 1.569 (3H, d, J=6.30), 1.750 (2H, dq, J_1=7.57, J_2=7.30), 2.834 (1H, dt, J_1=5.08, J_2=7.49), 5.386 (1H, dq, J_1=6.15, J_2=5.22), 9.150 (2H, d, J=2.17), 9.244 (1H, t, J=2.14); 11.701, 18.535, 22.478, 39.835, 71.713, 118.422, 122.809(CN), 129.514, 133.185, 148.762, 161.620.

3c(syn): 0.931 (3H, t, J=6.64), 1.581 (3H, d, J=6.41), 1.27—1.50 (2H, m), 1.50—1.75 (4H, m), 3.00—3.13 (1H, m), 5.27 (1H, dq, J_1=6.35, J_2=4.97), 9.133 (2H, d, J=2.12) 9.239 (1H, t, J=2.14); 13.706, 16.487, 22.078, 28.488, 29.251, 37.455, 72.112, 118.663, 122.816(CN), 129.482, 133.176, 148.734, 161.667.

3c(syn+anti mixture): 0.917 (3H, t, J=7.38), 0.935 (3H, t, J=6.64), 1.30—1.50 (2H, m), 1.570 (3H, d, J=6.35), 1.50—1.80 (4H, m), 2.84—2.94 (1H, m), 3.00—3.13 (1H, m), 5.27 (1H, dq, J_1=6.35, J_2=4.97), 5.373 (1H, dq, J=6.35, J_2=6.40), 9.137 (2H, d, J=2.12), 9.153 (2H, d, J=2.13), 9.243 (1H, t, J=2.17); 13.705, 16.487, 18.517, 22.078, 28.473, 28.586, 29.170, 29.243, 37.442, 38.099, 71.916, 72.094, 118.589, 122.775(CN), 129.490,

133.161, 148.726, 161.598. Underlined signals are presumed to be of anti isomer's.

3d(syn): 1.510 (3H, d, J=6.40), 4.407 (1H, d, J=5.20), 7.30—7.50 (5H, m), 9.121 (2H, d, J=2.20), 9.233 (1H, t, J=2.00); 16.059, 43.077, 73.830, 117.309, 122.830(CN), 128.080, 129.121, 129.403, 129.467, 130.819, 133.049, 148.712, 161.681.

Lithium Aluminum Hydride Reduction of 3. To a suspension of 45 mg (1.16 mmol) of lithium aluminum hydride in 1.8 mL of THF at −78°C under argon was added a solution of 144.1 mg (0.469 mmol) of **3b** in 4 mL of THF, and the solution was stirred for 3.5 h. After quench reaction by the addition of wet ether and aqueous solution saturated with Na_2SO_4, the reaction solution was dried by anhydrous K_2CO_3, filtration through a glass filter and evaporation provided a crude oil. Kugelrohr distillation gave **2b** as a colorless oil (47.4 mg, 0.419 mmol, 89%). By the same method other benzoyl esters **3** could be coneverted into the alcohol **2** in 80 to 99% yield. Specific rotation in EtOH at 23°C, boiling point, IR spectral data and elemental analyses of **2** are summarized below.

2a: (syn) +17.3°, c 1.00, (anti) −11.3°, c 0.890; 80°C/1.5 mmHg (Kugelrohr); 3430, 2930, 2240(CN), 1460, 1120 cm^{-1} (neat); Calcd for C_5H_9ON: C, 60.58; H, 9.15; N, 14.13%. Found: C, 60.62; H, 9.29; N, 14.25%

2b: (syn) +9.5°, c 0.820, (anti) −18.8°, c 0.920; 110°C/12 mmHg (Kugelrohr); 3430, 2970, 2870, 2240(CN), 1460, 950 cm^{-1} (neat); Calcd for $C_6H_{11}ON$: C, 63.69; H, 9.80; N, 12.38%. Found: C, 63.41; H, 9.82; N, 12.47%.

2c: (syn) +14.3°, c 0.650; 135°C/2.0 mmHg (Kugelrohr); 3450, 2950, 2930, 2870, 2240(CN), 1470, 1410, 1120 cm^{-1} (neat); Calcd for $C_8H_{155}ON$: C, 68.05; H, 10.71; N, 9.92%. Found: C, 67.87; H, 10.61; N, 10.04%.

2d: (syn) −33.3°, c 0.650; 150°C/2.0 mmHg (Kugelrohr); 3400, 3050, 2950, 2790, 2230(CN), 1480, 730, 680 cm^{-1} (neat); Calcd for $C_{10}H_{11}ON$: C, 74.51; H, 6.88; N, 8.69%. Found: C,74.48; H, 6.80; N, 8.82%.

^1H NMR (200 MHz, $CDCl_3$, δ, J/Hz) and ^{13}C NMR (50 MHz), $CDCl_3$, δ, ppm) spectral data, and ^{19}F NMR (470 MHz, $CDCl_3$, δ, C_6F_6, ppm) spectral data of (+)-MTPA ester are summarized below.

2a(syn): 1.30 (3H, d, J=7.25), 1.34 (3H, d, J=6.29), 1.85 (1H, broad, s, OH), 2.74 (1H, dq, J_1=5.54, J_2=7.21), 3.88 (1H, dq, J_1=6.11, J_2=5.99); 13.717, 20.069, 33.918, 68.574, 121.054; 90.280(p1), 90.310(p2), p1 : p2=8.94 : 91.04, 82%ee.

2a(anti): 1.33 (3H, d, J=6.27), 1.34 (3H, d, J=7.19), 1.947 (1H, d, J=5.60, OH), 2.632 (1H, dq, J_1=4.75, J_2=7.75), 3.87 (1H, dq, J_1=6.16, J_2=5.68); 14.338, 21.209, 34.245, 68.588, 121.231; 90.123(p1), 90.405 (p2), p1 : p2=13.9 : 86.1, 72%ee.

2b(syn): 1.08 (3H, t, J=7.33), 1.33 (3H, d, J=6.24), 1.50—1.70 (2H, m), 2.28 (1H, s, OH), 2.60 (1H, dt, J_1=9.52, J_2=5.67), 3.89 (1H, dq, J_1=6.40, J_2=6.20); 11.755, 20.428, 21.748, 42.172, 67.310, 120.231; 90.224 (single peak), >98%ee.

2b(anti): 1.06 (3H, t, J=7.38), 1.32 (3H, d, J=6.34), 1.58—1.78 (2H, m), 2.28 (1H, s, OH), 2.46 (1H, dq, J_1=4.56, J_2=6.20), 3.82—3.99 (1H, m); 11.721, 21.460, 22.217, 42.366, 67.053, 120.168; 90.256 (single peak), >98%ee.

2c(syn): 0.90 (3H, t, J=6.80), 1.34 (3H, d, J=6.20), 1.18—1.43 (2H, m), 1.48—1.78 (2H, m), 2.03 (1H, broad s, OH), 2.50 (1H, q, J=4.45), 3.89 (1H, dq, J_1=6.34, J_2=6.23); 13.732, 21.576, 22.179, 28.510, 29.400, 40.671, 67.464, 120.179; 90.268 (single peak), >98%ee.

2c(syn+anti mixture): 0.90 (3H, t, J=6.80), 1.325 (3H, d, J=6.29), 1.333 (3H, d, J=6.20), 1.20—1.45 (2H, m), 1.45—1.80 (2H, m), 2.00 (1H, s, OH), 2.46—2.56 (1H, m), 2.60—2.70 (1H, m), 3.82—3.94 (1H, m); 13.732, 13.746, 20.361, 21.566, 21.576, 22.179, 28.043, 28.056, 28.516, 29.410, 40.478, 40.677, 67.442, 67.625, 120.179, 129.427. Underlined signals are presumed to be of anti isomer's.

2d(syn): 1.330 (3H, d, J=6.18), 2.002 (1H, s, OH), 3.911 (1H, d, J=6.24), 4.09 (1H, dq, J_1=6.21, J_2=6.16), 7.20—7.50 (5H, m); 19.842, 46.254, 70.150, 76.933, 128.217, 128.593, 129.154, 132.354; 90.119(single peak), >98%ee.

Methyl (2*R*,3*S*)-2-Ethyl-3-hydroxybutanoate (4). A solution of *anti*-**2b** (49.3 mg, 0.436 mmol) with 5 mL of concd HCl was refluxed for 8 h. After dilution with 10 mL of ethyl acetate, the organic layer was washed with brine, dried, and evaporated to give a colorless oil. The oil was diluted with ether and treated with diazomethane. Purification by silica gel TLC gave **4** as a colorless oil (21.3 mg, 0.146 mmol, 33%).

$[\alpha]_D^{23}$+8.48° (c 0.825, $CHCl_3$); R_f 0.75, AcOEt/hexane=1 : 2; ^1H NMR (100 MHz, δ, $CDCl_3$), 0.9 (3H, t, J=4.4 Hz), 1.2 (3H, d, J=6.0 Hz), 1.3—1.8 (2H, m), 2.2 (1H, q, J=6.4 Hz), 3.0 (OH, s), 3.7 (3H, s), 3.7—4.1 (1H, m); IR (neat), 3430, 2960, 2860, 1730, 1430, 1260, 1200, 1020, and 795 cm^{-1}.

Methyl (2*S*,3*R*)-2-Ethyl-3-hydroxybutanoate (5).[7] To a solution of 6.0 mmol of LDA in 3 mL of THF was added a THF (2.0 mL) solution of 351.4 mg (2.97 mmol) of methyl (3*R*)-3-hydroxybutanoate, $[\alpha]_D^{23}$+43.0° (neat), under argon at −50°C and the solution was stirred for 2 h. Into the resulting orange solution of dianion was added a solution of 1.404 g (19.00 mmol) of ethyl iodide in THF (1.0 mL) and 1.3 mL of HMPA and the mixture was stirred allowed to warm to −25°C for 8 h. After quenching of the reaction by the addition of 2 M HCl, the mixture was extracted with ethyl acetate. The organic layer was dried and evaporated to give a colorless oil. Purification by silica gel TLC gave **5** as a colorless oil in 58% yield (251.3 mg, 1.72 mmol, anti:syn=98.2:1.8). $[\alpha]_D^{23}$ −9.21° (c 1.086, $CHCl_3$); ^1H NMR (100 MHz, δ, $CDCl_3$) 0.9 (3H, t, J=4.4 Hz), 1.2 (3H, d, J=6.4 Hz), 1.3—1.8 (2H, m), 2.2 (1H, q, J=6.4 Hz), 3.0 (1H, broad s, OH), 3.7 (3H, s) 3.7—4.1 (1H, m).

Methyl (2*R*,3*S*)-2-Phenyl-3-hydroxybutanoate (6). The nitrile **2d** (105.5 mg, 0.657 mmol) was converted into the ester **7** (55.6 mg, 0.554 mmol) by the acid hydrolysis of the cyano group followed by the treatment of diazomethane as described above in 65% yield.

$[\alpha]_D^{23}$ +99.8° (c 1.85, $CHCl_3$); R_f 0.6, AcOEt/hexane=1 : 5, after 10 times of development; ^1H NMR (100 MHz, δ, $CDCl_3$) 1.1 (3H, d, J=13.0 Hz), 2.9 (1H, s, OH), 3.4 (1H, d, J=8.4 Hz), 3.6 (3H, s), 4.0—4.5 (1H, m), and 7.2—7.6 (5H, broad s); IR (neat) 3345, 3000, 1740 (C=O), 1240, 1060, 960, 730, and 700 cm^{-1}.

Methyl (±)-2-Phenyl-3-hydroxybutanoate (8).[8,16] To a solution of 10 mmol of LDA in 10 mL of THF was added 1.502 g (10.0 mmol) of methyl 2-phenylacetate at −78°C under argon and the solution was stirred for 1.5 h and was added a solution of 0.44 g (10 mmol) of acetaldehyde in THF (5 mL) at the same temperature and stirred for 1.0 h. After addition of 2 M HCl, the solution was extracted with ether, and the organic layer was dried, evaporated and purification using silica gel flash column chromatography to give *syn*-**8** (0.590 g, 3.04 mmol, 30%) and *anti*-**8** (0.587 g, 3.01 mmol, 30%) as a colorless oil respectively.

***syn*-8:** ^1H NMR (100 MHz, δ, $CDCl_3$), 1.1 (3H, d, J=13.0

Hz), 2.9 (1H, s, OH), 3.4 (1H, d, J=8.4 Hz), 3.6 (3H, s), 4.0—4.5 (1H, m), 7.2—7.6 (5H, broad s); IR (neat) 3400, 3000, 1740 (C=O), 1240, 960, and 730 cm^{-1}. *anti*-**8**: ^1H NMR (100 MHz, δ, $CDCl_3$) 1.0 (3H, d, J=6.5 Hz), 3.0 (1H, s, OH), 3.5 (1H, d, J=10.9 Hz), 4.1(3H, s), 4.2—4.5 (1H, m), 7.3—7.5 (5H, m).

Acetate of *syn*-8: ^1H NMR (200 MHz, δ, $CDCl_3$), 1.292 (3H, d, J=6.40 Hz), 1.801 (3H, s, OAc), 3.669 (3H, s, OMe), 3.719 (1H, d, J=8.80 Hz), 5.527 (1H, dq, J_1=8.80 Hz, J_2=6.20 Hz), 7.20—7.30 (5H, m); ^{13}C NMR (50 MHz, δ, $CDCl_3$), 18.650 (4C), 20.831 ($\underline{C}H_3CO$), 52.146 (2C), 56.943 ($COO\underline{C}H_3$), 70.622 (3C), 127.590, 128.429, 128.753, 135.240, 169.954(1C), 171.736 ($CH_3\underline{C}OO$). Acetate of *anti*-**8**: ^1H NMR (200 MHz, δ, $CDCl_3$), 1.048 (3H, d, J=6.20 Hz), 2.042 (3H, s, OAc), 3.666 (3H, s), 3.701 (1H, d, J=10.4 Hz), 5.495 (1H, dq, J_1=10.4 Hz, J_2=6.2 Hz), 7.2—7.3 (5H, m); ^{13}C NMR (50 MHz, δ, $CDCl_3$) 17.673(4C), 21.124, 52.079 (2C), 57.427, 71.785 (3C), 128.070, 128.581, 128.876, 134.782, 180.011 (1C), and 171.974.

Methyl (2*S*,3*R*)-2-Phenyl-3-hydroxybutanoate (7). To a suspension of 91.5 mg (0.387 mmol) of *syn* acetate of **8** in 2.0 mL of 0.1 M phosphate buffer (pH 7.2) was added 46 mg of lipase A6 (from *Aspergillus niger* by Amano Co.), and the suspension was stirred at room temperature for 143 h (30% conversion determined by ^1H NMR). Extraction from the reaction mixture with ethyl acetate and purification by silica gel TLC gave the alcohol **7** as a colorless oil (16.9 mg, 0.087 mmol, 22%).

$[\alpha]_D^{26}$ −97.0° (*c* 1.71, $CHCl_3$), 96%ee determined by the 200 MHz ^1H NMR analysis using chiral shift reagent Eu(hfc)$_3$. When 30 mol% of Eu(hfc)$_3$ was added into the $CDCl_3$ solution of **7**, the methoxy peak at δ 3.70 (s) was split up into two peaks at δ 3.97 and 4.21. According to the ratio of the peak intensity of these two peaks (the low field peak: the high field one=92 : 2), the absolute configuration at C-3 position of **7** was presumed as 3*R* because this ratio was just the same tendency of the chemical shift of the ester methyl group of 74%ee of methyl (2*R*,3*R*)-2-phenyl-3-hydroxybutanoate,[16] $[\alpha]_D^{23}$ +57.0° (*c* 1.05, $CHCl_3$) in ^1H NMR analysis in the presence of the same chiral shift reagent of 30 mol% of Eu(hfc)$_3$.

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