Thiocrown Ether Additive Effects on Diastereoselectivity of the Lipase-Catalyzed Reaction: Preparation of Optically Active 3-Hydroxy-2-methylalkanenitriles through a Double Enzymatic Reaction Strategy

Koichi Mitsukura,† Hiroko Choraku, San Thi Da, and Toshiyuki Itoh*

Department of Chemistry, Faculty of Education, Okayama University, Okayama 700-8530

†Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530

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The additive effect on diastereoselectivity towards the lipase-catalyzed hydrolysis of acetates of 3-hydroxy-2-methyl- or 3-hydroxy-2-ethylalkanenitriles has been investigated. Diastereoselectivity was not influenced by thiocrown ether additives, although a significant modification of enantioselectivity was observed. Origin of the diastereoselectivity of the lipase-catalyzed reaction was thus evidently different from that of enantioselectivity. Based on these results, an easy preparation of optically active 3-hydroxy-2-methylpentanenitrile and 3-hydroxy-2-methylbutanenitrile have been demonstrated through lipase-catalyzed reaction by a double enzymatic reaction strategy.

The value of an enzymatic reaction in organic synthesis is extensively increased by its environmentally friendly aspect.[1] Since only a limited number of lipase-catalyzed reactions is applicable for practical optical resolution, development of an efficient strategy to improve their reaction performance is desirable.[2] We have reported that thiacrown ethers, especially 1,4,8,11-tetrathiacyclotetradecane, have the potential to enhance both the enantioselectivity and reaction rate in the lipase-catalyzed reaction (Fig. 1).[3]

Extensive studies have been made to disclose the factors deciding the stereoselectivity of lipases.[4,5] Among these, we have been especially fascinated by "the transition-state model" proposed by Ema et al.[5] in which the authors rationalize the enantioselectivity of lipases towards secondary alcohols. They showed the usefulness of their theory to realize successful kinetic resolution of a large secondary alcohol,[6] and in fact, we previously demonstrated that a lipase-catalyzed reaction was applicable even for the reaction of extremely bulky compounds such as α-(tributylstannyl)-alkanols.[7] Our finding of thiocrown ether modification of the lipase-catalyzed reaction could not be explained by the traditional "binding step model" in which the enantioselectivity originated from the fitness of the substrate towards the active site of the enzyme. We therefore decided to investigate the detail of stereoselectivity on diastereoselectivity using 3-hydroxy-2-methyl or 3-hydroxy-2-methylalkanenitriles as model compounds from the standpoint of the regulating effects of thiocrown ethers on stereoselectivity of the lipase-catalyzed reaction.

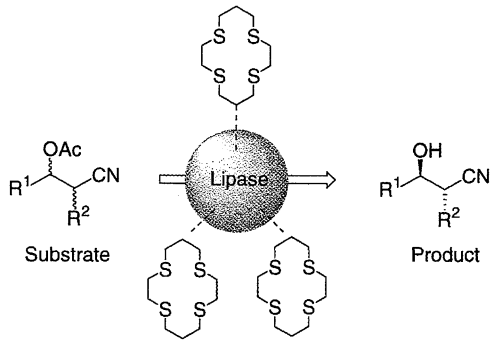


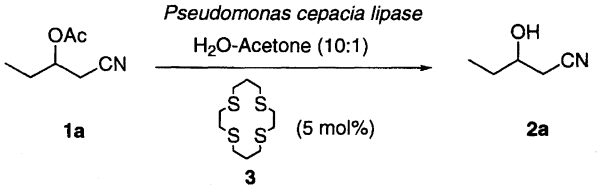
Fig. 1. Thiocrown ether modification of lipase-catalyzed reaction.

In this paper we wish to report the results of the thiocrown ether effect on diastereoselectivity of the lipase-catalyzed hydrolysis of acetate of 3-hydroxy-2-alkylalkanenitriles, and discuss the differences of the origin between diastereoselectivity and enantioselectivity in the lipase-catalyzed reaction. Based on these results on diastereoselectivity and enantioselectivity of the lipase-catalyzed reaction, a double enzymatic reaction method to isolate one enantiomer from four enatiomeric isomers of 3-hydroxy-2-alkylalkanenitriles has been demonstrated.

Results and Discussion

Kinetic Aspect of the Thiocrown Ether Modified Lipase-Catalyzed Reaction. Before our study of thiocrown ether effect on diastereoselectivity using lipase-catalyzed reaction of 3-hydroxy-2-alkylalkanenitriles as model compounds, we measured the kinetic parameters of

Pseudomonas cepacia lipase (PCL)-catalyzed hydrolysis of 1-cyanobutan-2-yl acetate (**1a**) because the most drastic modification was recorded when various types of acetates of 2-hydroxyalkanenitriles were submitted to the lipase-catalyzed reaction in the presence of thiocrown ether (Eq. 1).[3e]



(1)

Enantiomerically pure acetates (*R*)-**1a**[3e] and (*S*)-**1a**[3e] were reacted with the lipase-catalysis, and the progress of the reaction was monitored by gas chromatography to obtain the initial rate (v_0). The hydrolysis of **1a** was done in a non-buffered aqueous solution to exclude any effect of complexation between the thiocrown ether **4** and metal cation (Eq. 1). The reaction was typically done as follows: 12.5 mL of an aqueous lipase solution was added to a solution of acetate **1a**, together with a crown ether **4** additive (5 mol% toward the substrate) in 1.25 mL of acetone. The resulting mixture was stirred at 35 °C. The concentration of the acetate [S_0] was systematically changed, and the plot of v_0 against [S_0] afforded a typical saturation curve. The apparent V_{max} and K_m were obtained by the nonlinear-squares method applied to the Michaelis–Menten type of equation (Table 1).[8]

The Table 1 clearly shows that the thiocrown ether modification on enantioselectivity in the lipase-catalyzed reaction originated from the differences of V_{max} and not from K_m. Thiocrown ether significantly accelerated the reaction using both enantiomers, and the most important fact is that *the difference in V_{max} values of (R)-1a between that of (S)-1a drastically enlarged by 20-fold* when the reactions were done in the presence of 5 mol% of thiocrown ether **4** (Entries 2 and 4), while it was 10-fold in the absence of thiocrown ether (Entries 1 and 3). No significant difference in K_m was observed between (*R*)-**1a** and (*S*)-**1a** (Entries 1 and 3) in the absence of thiocrown ether, and the difference was expanded only slightly when the reactions done in the presence of 5 mol% of thiocrown ether **4** (Entries 2 and 4). It is well known that K_m of the lipase-catalyzed reaction are generally large, indicating that the binding of lipases to the substrates is weak.[9] A very interesting point is that K_m were increased by addition of thiocrown ether; the additive compound weakened the binding force of the enzyme to the substrate. These experimental results strongly support the transition-state model of Ema et al.[5] in that the ability of lipases to discriminate between the enantiomers at the transition-state is high, while the ability to recognize the chirality in the binding step is poor. Thiocrown ether may modify the transition state and expand the difference of V_{max} between the enantiomers, increasing the enantioselectivity, though there still remains another possibility to explain the origin of the thiacrown ether modification. The value of the catalytic efficiency (V_{max}/K_m) on (*S*)-**1a** was slightly reduced by addition of thiocrown ether (Entry 4), while no difference was observed for (*R*)-**1a** (Entries 1 and 2), so that increased enantioselectivity may be produced by the enantioselective inhibition of (*S*)-**1b** in the presence of the thiocrown ether near the active site.

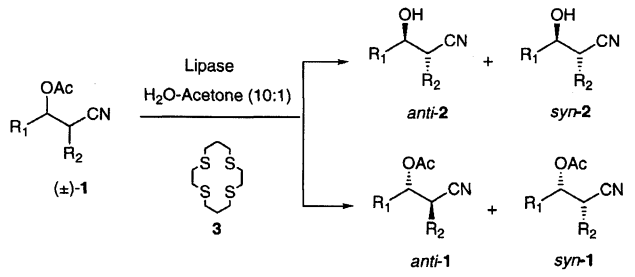
Thiocrown Ether Effect on Diastereoselectivity of the Lipase-Catalyzed Reaction. The origin of stereoselectivity of lipase-catalyzed reaction seems to be explained by the transition-state model as described above, however, another question remains concerning the origin of stereoselectivity for diastereomers, whether a neighborhood functional group of the hydrolysis point does or does not affect the stereochemistry of an enzymatic reaction. We investigated this point using 3-hydroxy-2-methyl or 3-hydroxy-2-ethylalkanenitriles, **1b** (R^1 = Et, R^2 = Me), **1c** (R^1, R^2 = Et), and **1d** (R^1, R^2 = Me), as model substrates from the standpoint of modifying the property of the thiocrown ether towards diastereoselectivity (Eq. 2). Racemic 3-hydroxy-2-methyl- or 3-hydroxy-2-ethylalkanenitriles, (±)-**2b-2d**, were prepared through nucleophilic addition of lithiopropionitrile or lithiobutyronitrile to aldehydes.[3e] The alcohols obtained were converted to the corresponding acetates, **1b—1d**, in almost quantitative yields by treatment with acetyl chloride in the presence of pyridine as base. The hydrolysis was done using two types of lipases, lipase AL from *Achromobacter* sp (Meito)[10] and PCL (Amano). Because the starting acetate was a mixture of four enantiomers, the reaction was stopped when the hydrolysis ratio reached about 25% conversion. The initial rate was calculated from the percentage conversion per reaction time. Because this reaction is a kinetic resolution of a racemic substrate, the optical purity of

Table 1. Kinetic Parameters for the PCL-Catalyzed Hydrolysis of 1-Cyanobutan-2-yl Acetate **1a** in the Presence of Thiocrown Ether **3**

Entry	Substrate	**3**	V_{max}	K_m	V_{max}/K_m
		mol. amount	mM min^{-1} mg (lipase)$^{-1}$	M	min^{-1} mg^{-1}
1	(*R*)-**1a**	0	$(5.6\pm2.0)\times10^{-4}$	$(5.7\pm2.5)\times10^{-2}$	1.0×10^{-5}
2	(*R*)-**1a**	0.05	$(2.2\pm0.84)\times10^{-3}$	$(2.2\pm0.91)\times10^{-1}$	1.0×10^{-5}
3	(*S*)-**1a**	0	$(4.7\pm2.6)\times10^{-5}$	$(3.7\pm2.1)\times10^{-2}$	1.3×10^{-6}
4	(*S*)-**1a**	.0.05	$(9.9\pm7.2)\times10^{-5}$	$(9.5\pm7.0)\times10^{-2}$	1.0×10^{-6}

a) Conditions: lipase (typically, 3—10 mg and 150—300 mg for (*R*)- and (*S*)-enantiomers, respectively), optically pure (> 99%ee) acetate (typically, ca. 0.05—0.4 M) and deionized water (5.0 mL) and acetone (0.5 mL), 35 °C. Because of the heterogenous reaction, the nonlinear least-squares method was applied to the Michaelis–Menten type of equations: $v_0 = V_{max}$ (E)mg [S_0]/(K_m + [S_0]), where V_{max} is normalized by the weight of lipase (E)mg.

the producing alcohol and remaining acetate depends on the reaction conversion. The effect of this thiocrown ether on enantioselectivity was evaluated by comparison of E values, which were calculated by the equation proposed by Sih et al.[11] The alcohol **2** produced and remaining ester **1** were extracted with ethyl acetate and separated by silica-gel flash column chromatography (hexane / ethyl acetate = 5 : 1) and the diastereomeric excess (de) of alcohol **2** produced was measured by capillary GC analysis. The enantiomeric excess of alcohol *anti*-**2b**, *syn*-**2b**, *anti*-**2c**, and *syn*-**2c** was measured by capillary GC analysis using the chiral stationary phase (Chiraldex G-Ta) of the corresponding acetate. The enantiomeric excess (ee) of the remaining acetate *anti*-**1b**, *syn*-**1b**, *anti*-**1c**, and *syn*-**1c** was also measured by GC analysis. Unfortunately, we could not measured enantioselectivity of **1d** by GC analysis at an early stage of the reaction because signals responsible for the four enantiomers are too close to calculate the ee of each isomer with sufficient reliability. The results are summarized in Table 2.



(2)

There were significant differences on the additive effect on the reaction rate for lipase AL-catalyzed reaction of **1b** and **1c** with slightly increased diastereoselectivity (Entries 4 and 6), while no acceleration was observed for **1d** (Entry 10). With both substrates **1b** and **1c**, the enantioselectivity towards *anti*-isomers was slightly lowered by addition of thiocrown ether, while that of *syn* isomers was increased (Entries 2 and 4). On the other hand, thiocrown ether slightly accelerated the reaction rate of PCL-catalyzed reaction of **1d**, but slightly reduced the diastereoselectivity (Entry 8). Although we tried to measure kinetic parameters for these compounds, unfortunately, this was unsuccessful because separation of the isomers **1b** and **1c** was impossible by preparative means of silica-gel thin-layer chromatography or column chromatography.

A great increase of enantioselecivity was caused by thiocrown ether for acetate of 3-hydroxypentanenitrile **1a** as described earlier (Table 1), while no marked increase in diastereoselectivity was found for any of the acetates used with addition of the thiocrown ether. These results obviously suggest that diastereoselectivity and enantioselectivity are decided by different origins. It may suggest that the thiocrown ether modified conformational change of lipase protein is so small that it does not reflect diastereo-favoritism, though it is high enough to cause modification of the transition state.

Preparation of Optically Active 3-Hydroxy-2-methylalkanenitriles through Lipase-Catalyzed Reaction by a Double Enzymatic Reaction Strategy. The high enantioselectivity and broad substrate specificity is quite an important property of the lipase catalyzed reaction because these factors are usually difficult to make compatible with each other in other enzymatic reaction systems. It was found, however, that diastereoselectivity and enantioselectivity of lipase-catalyzed reaction have different origins. This makes it desirable to form a strategy to obtain optically pure α-alkyl-β-hydroxyalkanenitriles by lipase-catalyzed optical resolution of racemic diastereomixture. We chose 3-hydroxy-2-methylpentanenitrile **2a** and 3-hydroxy-2-methylbutanenitrile **2b**[12] as model substrates to demonstrate such strategy of preparing both diastereo- and enantiomeric pure compounds by a lipase-catalyzed reaction, because they can be used as building blocks for the synthesis of sex attractant pheromones.[13,14]

We hypothesized that a double enzymatic reaction system provides a useful means to resolve 3-hydroxy-2-methylalkanenitriles; the first stage is evaluation of an enzyme that has high diastereoselectivity, and the second is to iden-

Table 2. Thiocrown Ether Additive Effect on the Lipase-Catalyzed Reaction

Entry	R^1	R^2	Lipase	**3** mol. amount	Time h	Rate [a]	%conv. [a] (*syn*+*anti*)	%de [b] of **2**	%ee [c] of *anti*-**2**	*E* value [d] for *anti*	*E* value [d] for *syn*
1	Et	Me	PCL	0	5	5.2	26	72	>99	>243	>214
2	Et	Me	PCL	0.05	5	5.1	25	72	>99	>285	>222
3	Et	Me	AL	0	24	0.9	21	86	>99	>501	>500
4	Et	Me	AL	0.05	10	2.6	26	94	>99	>412	>700
5	Et	Et	AL	0	48	0.4	21	86	96	74	212
6	Et	Et	AL	0.05	24	0.9	21	90	95	55	222
7	Me	Me	PCL	0	24	0.7	16	10	—[e]	—	—
8	Me	Me	PCL	0.05	24	1.3	31	5	—[e]	—	—
9	Me	Me	AL	0	72	0.3	19	60	—[e]	—	—
10	Me	Me	AL	0.05	72	0.3	21	61	—[e]	—	—

a) Rate shows % conv. per reaction time (h) and was determined by capillary GC (MS) analysis. b) *anti*-**2** was obtained as a major isomer in all reactions tested. c) Determined by capillary GC analysis using chiral stationary phase (Chiraldex G-Ta). Optical purity of the product shows >99%ee when no isomer was detected by GC analysis. d) E[11] $= \ln[1-c(1+\mathrm{ee2})]/\ln[1-c(1-\mathrm{ee2})]$, $c = \mathrm{ee1}/\mathrm{ee2}+\mathrm{ee1}$. e) It was impossible to determine %ee of **2** by GC analysis because the four signals responsible for each enantiomer were too close to allow calclulation of the enantiomeric excess with reliability.

tify an enzyme that reacts specifically with one enantiomer. Synthesis of *anti*-alkanenitrile **1b** (R^1 = Et, R^2 = Me) was accomplished through this reaction protocol as shown in Scheme 1. Lipase AL-catalyzed hydrolysis of (±)-**1b** gave *anti*-**2b** in 26% yield with 86% de, and this was then treated with PCL in the presence of vinyl acetate as acyl donor in diidopropyl ether to afford (2*S*,3*R*)-**1b** in optically pure form (>99%ee) with 96% de (Scheme 1, upper equation). It was difficult, however, to find an enzyme that hydrolyze acetate **1d** (R^1, R^2 = Me) with sufficient *anti* diastereoselectivity; lipase AL-catalyzed hydrolysis of (±)-**1d** (R^1, R^2 = Me) gave *anti*-**2d** with the highest diastereoselectivity (60% de). Using the same strategy, (2*S*,3*R*)-**1d** was obtained in optically pure form (>99%ee), though diastereoselectivity was insufficient (65%de) (Scheme 1, middle equation). Incidentally, another enzyme combination was required to prepare (2*R*, 3*S*)-**1d** because the hydrolysis reaction stopped at about 30% conversion when lipase AL was used. The best lipase providing (2*R*,3*S*)-**1d** was lipase OF; acetate *anti*-**1d** was obtained with 58%de and this was further treated with Hemi-cellulase (from *Aspergillus* sp.) to afford (2*R*,3*S*)-**1d** with >99%ee with 86%de (Scheme 1, lower equation). Based on the GC analysis of the reaction course, it was found that (2*S*,3*R*)-**1d**(*anti*) was initially hydrolyzed by lipase AL or OF, (2*R*, 3*R*)-**1d**(*syn*) was next consumed, the third isomer hydrolyzed was (2*S*,3*S*)-**1d**(*syn*), and (2*R*,3*S*)-**1d**(*anti*) was the last isomer hydrolyzed by the lipase.

Conclusions

Kinetic study of lipase-catalyzed hydrolysis of acetate 3-hydrokyalkanenitriles in the presence or absence of thiocrown ether suggested that diastereoselectivity and enantioselectivity should be viewed from different mechanistic aspects. A double lipase catalyzed reaction was therefore useful for resolving 3-hydroxy-2-methylalkanenitriles by the lipase-catalyzed reaction. The reactions are particularly beneficial even for large scale preparative organic synthesis, because the key step of this synthesis is an environmentally friendly enzymatic reaction, and particularly of note is that all lipases used are commercially available and not expensive. Hence this protocol can undoubtedly allow us to evolve a smarter and more convenient synthesis of optically active building blocks for organic synthesis.

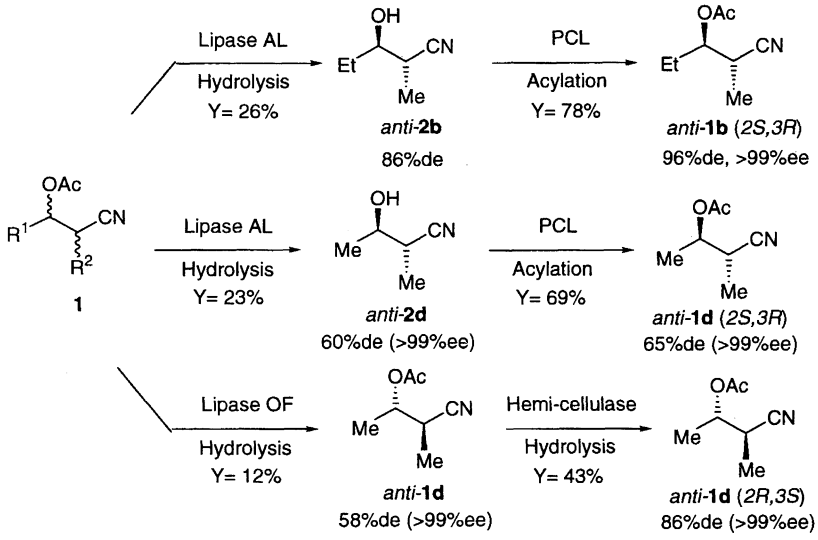
Experimental

Materials. Crown ethers were purchased from Aldrich and used without further purification. Lipases were provided by Amano Pharmaceutical Co., Ltd. and Meito Sangyo Ltd. (Japan).

Kinetic Measurement. A typical example is as follows: An aqueous solution of lipase PS (5.25 mL, which includes 0.0037 g of the enzyme)*, optically pure acetate **1a**[3e] (typically, about 0.01—1.0 M) (1 M = 1 mol dm^{-3}) in acetone (0.50 mL), and thiacrown ether **4** (0.0074 g) was stirred at 200 rpm in a test tube with a rubber stopper in a water bath at 35 °C. At an appropriate time, samples (50—100 μL) were withdrawn and filtered through a cellulose acetate membrane filter, and eluted with ethyl acetate. The filtrate was analyzed by gas chromatography ("Quadrex" bonded fused silica methyl silicone, ϕ0.25 mm×25 m, N_2) to obtain the conversion; $v = 2.44\times10^{-3}$ (mmol min^{-1})/0.0037 g (E) = 6.6×10^{-4} mol dm^{-3} mg(E)$^{-1}$ min^{-1}. Five data points were routinely collected to measure the initial rate (v_0) at each substrate concentration [S_0]. Plot of v_0 against [S_0] afforded a saturation curve, and the apparent V_{max} and K_m values were obtained by the nonlinear least-squares method applied to the Michaelis–Menten type of equation as follows: $v_0 = V_{max}(E)mg[S_0]/(K_m+[S_0])$, where V_{max} is normalized by the weight of lipase (E)mg.

***Preparation of the Lipase Solution:** A typical example is as follows: A Boltex shaking suspension of PCL (0.0518 g) in deionized water (7.35 mL) was centrifuged at 3000 rpm for 5 min at room temperature, then 5.25 mL of the supernatant was immediately used as the enzyme solution. Because the enzyme content in PCL used here was about 10% by weight and the remainder was mostly amorphous inorganic compounds (celite),[3b] the content of the enzyme was thus estimated as 3.7 mg; this (3.7 mg/ g PCL) was used as the enzyme amount when the kinetic parameters were calculated because we used PCL having the same lot number in all experiments. (*R*)-**1a** (>99%ee):[3e] $[\alpha]_D^{25}$ +79.2 (*c* 1.77, $CHCl_3$), (*S*)-**1a** (>99%ee):[3e] $[\alpha]_D^{25}$ −80.0 (*c* 1.05, $CHCl_3$)

(±)-3-Hydroxy-2-methylpentanenitrile (2b). To a solution



Scheme 1. Preparation of optically active 3-hydroxy-2-methylalkanenitriles using a double-enzymatic reaction method.

of diisopropyl amine (3.04 g, 30.0 mmol) in THF (30.0 mL) under argon atmosphere at −10 °C was added a solution of *n*-BuLi in hexane (1.62 M, 17.0 mL, 27.5 mmol). The mixture was stirred at 10 °C for 1 h and cooled to −78 °C and then a solution of propionitrile (1.23 g, 30.0 mmol) in THF (15.0 mL) was added. The mixture was stirred at −78 °C for 1 h, then a solution of propionaldehyde (1.40 g, 25.0 mmol) in THF (15.0 mL) was added, and the mixture was again stirred at −78 °C for 3 h. The reaction was quenched with saturated aqueous NH_4Cl and treated with aqueous 2 M HCl. The mixture was extracted with Et_2O, and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The obtained yellow oily residue was purified by flash column chromatography (gradient elution hexane/ethyl acetate = 8 : 1 to 4 : 1) to give an alcohol **2b** as a colorless oil (2.23 g, 23.0 mmol) in 92% yield: R_f 0.29 (hexane/ ethyl acetate = 2 : 1); bp 95 °C/ 533 Pa (Kugelrohr); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.99 (3H, t, J = 7.2 Hz), 1.36 (3H, d, J = 7.2 Hz), 1.83 (2H, dq, J = 7.4, 7.0 Hz), 2.17 (1H, s), 2.71 (1H, dq, J = 7.2, 4.3 Hz), 3.52 (1H, ddd, J = 7.2, 5.7, 4.3 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 9.85, 14.59, 20.08, 32.50, 120.92; IR (neat) 3459, 2973, 2246, 1143, 977 cm^{-1}. Found: C, 63.43; H, 9.88; N, 12.41%. Calcd for $C_7H_{11}NO$: C, 63.69; H, 9.80; N, 12.38%. Using the same procedure, hydoxyalkanenitriles, **2c**—**2d**, were also prepared from the corresponding aldehydes.

(±)-3-Hydroxy-2-ethylpentanenitrile (2c). R_f 0.40 (hexane/ ethyl acetate = 2 : 1); bp 102 °C/ 400 Pa (Kugelrohr); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.01 (3H, t, J = 7.5 Hz), 1.11 (3H, t, J = 7.5 Hz), 1.25 (1H, s), 1.59—1.90 (4H, m), 2.54 (1H, ddd, J = 9.6, 5.7, 3.9 Hz) 3.61 (1H, dt, J = 6.5, 3.9 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 9.95, 11.94, 22.57, 28.57, 40.86, and 119.96; IR (neat) 3420, 2925, 2240, 1465, 1410, 1120, 1020 cm^{-1}. Found: C, 66.21; H, 10.35; N, 11.08%. Calcd for $C_7H_{13}NO$: C, 66.11; H, 10.30; N, 11.01%.

(±)-3-Hydroxy-2-methylbutanenitrile (2d).[12] R_f 0.24 (hexane/ ethyl acetate = 2 : 1); bp 90 °C/ 667 Pa (Kugelrohr); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.94 (6H, t, J = 6.5, 6.3 Hz), 1.79 (1H, dq, J = 13.3, 6.7 Hz), 2.50 (1H, d, J = 3.1 Hz), 2.53 (1H, d, J = 1.4 Hz), 2.63—2.77 (1H, brs, OH), 3.63—3.71 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 17.24, 18.40, 23.52, 33.16, 72.42, 118.28; IR (neat) 3460, 2970, 2250, 1470, 1420, 1130, 1060 cm^{-1}.

(±)-2-Cyanpopentan-3-yl Acetate (1b). To 2-methyl-3-hydroxypentanenitrile (**2b**) solution (0.963 g, 9.92 mmol) in dichloromethane (CH_2Cl_2) (20.0 mL) and pyridine (1.0 mL) was added a CH_2Cl_2 solution (20 mL) of acetylchloride (1.56 g, 19.9 mmol) at 0 °C and the solution was stirred at room temperature for 3 h. The reaction was quenched by the addition of crushed ice, extracted with CH_2Cl_2, dried over $MgSO_4$ and evaporated. The crude product was purified by flash column chromatography (hexane/ ethyl acetate = 10 : 1) to provide acetate **1b** (1.24 g, 8.91 mmol) in 90% yield: R_f 0.55 (hexane/ ethyl acetate = 2 : 1); bp 100 °C/ 4933 Pa (Kugelrohr); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.92 (3H, t, J = 7.4 Hz), 1.29 (3H, d, J = 7.0 Hz), 1.73 (2H, dq, J = 9.7, 7.5 Hz), 2.12 (3H, s), 2.88 (1H, dq, J = 7.27, 4.13 Hz), 4.86 (1H, ddd, J = 7.4, 5.9, 4.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 9.52, 14.47, 20.74, 25.40, 29.85, 73.94, 119.80, 170.40; IR (neat) 2977, 2247, 1743, 1458, 1387, 1235 cm^{-1}. Found: C, 61.73; H, 8.39; N, 9.00%. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03%. Using the same procedure, acetates **1b**, **1c**, and **1d** were also prepared from the corresponding hydoxyalkanenitriles.

(±)-4-Cyanohexan-3-yl Acetate (1c). R_f 0.68 (hexane/ ethyl acetate = 2 : 1); bp 113 °C/ 800 Pa (Kugelrohr); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.93 (3H, t, J = 7.6 Hz), 1.09 (3H, t, J = 7.4 Hz), 1.57—1.83 (4H, m), 2.12 (3H, s), 2.68 (1H, ddd, J = 8.8, 6.4, 3.9 Hz), 4.94 (1H, ddd, J = 7.5, 5.9, 3.9 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 9.59, 11.81, 20.81, 22.48, 25.79, 37.98, 72.82, 119.01, 170.39; IR (neat) 2972, 2243, 1744, 1463, 1374, 1231, 1022 cm^{-1}. Found: C, 63.50; H, 8.95; N, 8.32%. Calcd for $C_9H_{15}NO_2$: C, 63.88; H, 8.93; N, 8.28%.

(±)-3-Cyanobutan-2-yl Acetate (1d).[12] R_f 0.55 (hexane/ ethyl acetate = 2 : 1); bp 95 °C/ 800 Pa (Kugelrohr); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.32 (3H, d, J = 7.2 Hz), 1.37 (3H, d, J = 6.4 Hz), 2.11 (3H, s), 2.82 (1H, dq, J = 7.3, 4.7 Hz), 4.97 (1H, dq, J = 6.4, 4.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 14.16, 17.90, 20.85, 31.46, 69.61, 119.79, 170.06; IR (neat) 2988, 2246, 1742, 1453, 1378, 1238, 1038 cm^{-1}.

Lipase-Catalyzed Hydrolysis for the Experiment in Table 2. A typical example is described below: To an acetone solution (10.0 mL) of ester (±)-**1b** (1.41 g, 10.0 mmol) and thiacrown ether **4** (0.134 g, 0.50 mmol), lipase PS aqueous solution (100 mL) was added and the resulting mixture was incubated at 35 °C. The progress of the reaction was monitored by GLC analysis using Quadrex MS (ϕ0.25 mm×25 m). The reaction was stopped by the addition of small pieces of ice, then the mixture was extracted with ethyl acetate, dried over $MgSO_4$ and evaporated to dryness. The product **2b** and remaining substrate (−)-**1b** were separated by silica-gel flash column chromatography (hexane /ethyl acetate = 10 : 1 to 2 : 1). The lipase solution was prepared in the following way: A Boltex shaking suspension of PCL (0.705 g) in deionized water (110 mL) was centrifuged at 3000 rpm for 5 min at room temperature, then 100 mL of the supernatant was immediately used as the enzyme solution. GLC analysis for measurement of % ee of (±)-**1b** was done using a capillary column on chiral phase; Chiraldex G-TA, ϕ0.25 mm×20 m; Carrier gas: He 40 mL min^{-1}; Temp (°C); 100 or 130, Inlet pressure; 1.35 kg cm^{-2}; Amount 400 ng; Detection; FID. The results of GC analyses of racemic **1b** and **1c** are summarized as follows. *anti*-**1b**: t_R = 7.04 min (2*S*,3*R*) and 12.86 min (2*R*, 3*S*), *syn*-**1b**: t_R = 8.62 min and 9.86 min ; *anti*-**1c**: t_R = 9.20 min (2*S*,3*R*) and 12.88 min (2*R*,3*S*), *syn*-**1c**: t_R = 10.35 min and 10.72 min; *anti*-**1d**: t_R = 5.00 min (2*S*,3*R*) and 7.89 min (2*R*,3*S*), *syn*-**1d**: t_R = 5.25. Two signals due to *syn*-isomers ((2*R*,3*R*)-**1d** and (2*S*,3*S*)-**1d**) completely overlapped. A slight overlap of the large signal (t_R = 5.25) corresponded to two *syn*-isomers with the signal of (2*S*,3*R*)-**1d** (t_R = 5.20) was occurred, so that it was difficult to calculate the %ee of *anti*-**1d** precisely. Fortunately, we succeeded in measuring the %ee of *anti*-**1d** after the lipase-catalyzed reaction because the process reduced the content of the *syn*-isomers included in the product at a level that did not affect any result for calculating the %ee of *anti*-**1d**.

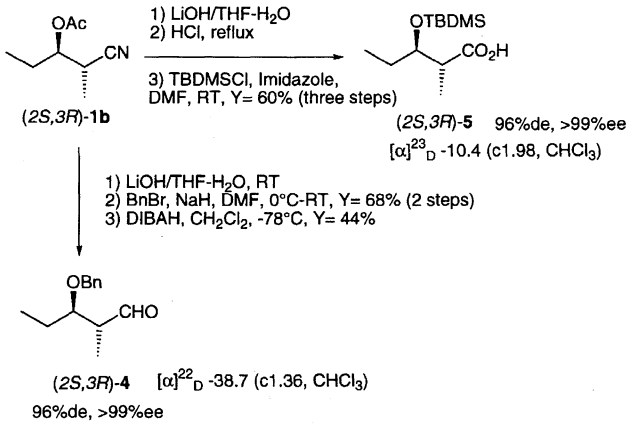
Preparation of Optically Active 3-Hydroxy-2-methylalkanenitriles through Lipase-Catalyzed Reaction by a Double Enzymatic Reaction Strategy.

(2*S*,3*R*)-2-Cyanopentan-3-yl Acetate (1b). To a suspension of lipase AL (4.04 g) in deionized water (260 mL) was added an acetone (26 mL) solution of racemic acetate **1b** (8.08 g, 52.1 mmol) and this was stirred at 35 °C for 160 h. The reaction was monitored by GC analysis and stopped by crushed ice and NaCl when reaction conversion reached about 25%. The mixture was extracted with ethyl acetate, dried over $MgSO_4$ and evaporated to dryness. Silica-gel flash column chromatography (hexane/ethyl acetate 10 : 1) gave the product **2b** (1.49 g, 13.2 mmol, 25%) and the unreacted acetate **1b** (4.05 g, 26.1 mmol, 50%). Capillary GC analysis showed that a diastereomeric ratio of **2b** was 86% *anti* selectivity. A mixture of **2b** (1.06 g, 9.40 mmol, 86%de), lipase PS (0.530 g), and vinyl acetate (1.23 g, 14.3 mmol) in 25.0 mL of diisopropyl ether was stirred at r.t. for 41 h. The mixture was filtered through a glass

sintered filter with a celite pad to remove the lipase and washed with CH_2Cl_2. The filtrate was evaporated and chromatographed on silica-gel (hexane/ ethyl acetate = 7 : 1) and gave acetate **1b** (0.864 g, 5.57 mmol, 59%) and alcohol **2b** (0.430 g, 3.80 mmol, 41%). Capillary GC analysis using chiral column (G-Ta) showed that the enantiomeric excess of **1b** :$[\alpha]_D^{25}$ +49.5 (*c* 1.01, $CHCl_3$) obtained was >99%ee with 96%de (*anti*).

The stereochemistry of **2b** produced by the lipase AL-catalyzed reaction was analyzed as reference compounds as follows (Scheme 2): To a DMF (13.0 mL) solution of NaH (0.239 g, 5.99 mmol) was added a DMF (2.0 mL) solution of **2b**: $[\alpha]_D^{25}$ +0.5 (*c* 0.88, $CHCl_3$) (0.452 g, 3.99 mmol) and benzylbromide (0.819 g, 4.79 mmol) at 0 °C and the mixture was stirred for 0.5 h at r.t. Silica gel flash column chromatography of the product gave 548 mg (2.70 mmol) of 3-benzyloxy-2-methylpentanenitrile in 68% yield. The nitrile (0.460 g, 2.26 mmol) was dissolved in CH_2Cl_2 (10 mL) and then treated with DIBAH (3.45 mmol in 1.5 M toluene) at −78 °C for 1 h. The reaction was quenched by NH_4Cl aqueous solution and acidified by 2 M HCl, and the mixture was extracted with CH_2Cl_2. The combined organic layers were evaporated and silica gel flash column chromatography (hexane/ ethyl acetate = 50 : 1 to 20 : 1) gave 0.204 g (0.994 mmol) of 3-benzyloxy-2-methylpentanal (**4**): $[\alpha]_D^{22}$ −38.7 (*c* 1.36, $CHCl_3$) in 44% yield. Comparing the spectrum data of ^{1}H- and ^{13}C NMR analysis of **4** with those of reference,[13] aldehyde **4** was confirmed to have *anti* configuration.

The absolute configuration of **1b** obtained by the lipase-catalyzed reaction was identified as follows: Acetate **1b** was treated with 1.0 equiv of lithium hydroxide (LiOH·H_2O) in a mixed solvent (THF–MeOH–H_2O = 3 : 1 : 1) at r.t. for 18 h to provide alcohol **2b**: $[\alpha]_D^{25}$ +0.5 (*c* 0.88, $CHCl_3$) which was treated with 6 M HCl under reflux conditions for 72 h to give 3-hydroxy-2-methylpentanoic acid:



Scheme 2.

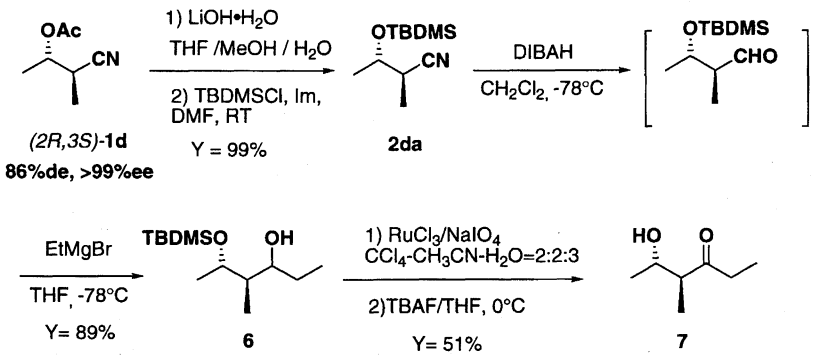
[α]$_D^{23}$ −7.0 (*c* 1.59, $CHCl_3$). This acid was then reacted with *t*-butyldimethylsilylchloride in the presence of imidazole as base in DMF at r.t. for 6 h to give the *t*-butyldimethylsilyl ester which was then treated with LiOH·H_2O in a mixed solvent of THF–MeOH–H_2O (3 : 1 : 1) at r.t. and afforded 3-(*t*-butyldimethylsilyloxy)-2-methylpentanoic acid (**5**): $[\alpha]_D^{23}$ −10.4 (*c* 1.98, $CHCl_3$) in 27% yield (two steps). Comparing the value of the optical rotation with the authentic sample (2*R*,3*S*)-**5**: $[\alpha]_D^{23}$ +10.0 (*c* 1.11, $CHCl_3$),[14] the configuration of the 2-position and 3-position of the starting nitrile **1b** was assigned as 2*S*,3*R*.

(2*S*,3*R*)-3-Benzyloxy-2-methylpentanal (4).[13] ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.96 (3H, t, *J* = 7.4 Hz), 1.08 (3H, d, *J* = 7.1 Hz), 1.55—1.75 (2H, m) 2.68 (1H, dq, *J* = 6.9, 2.2 Hz), 3.67—3.71 (1H, m) 4.48 (1H, d, AB, *J* = 11.5 Hz) 4.60 (1H, d, AB, *J* = 11.5 Hz) 7.26—7.36 (5H, m), 9.76 (1H, d, *J* = 2.1 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 8.83, 10.12, 23.41, 48.94, 71.53, 80.42, 127.71, 127.84, 128.38, 138.18, 204.67; IR (neat) 2969, 2873, 1724, 1458, 1070, 743 cm^{-1}. These spectra were identical with those in reference.[13]

(2*S*,3*R*)-3-(*t*-Butyldimethylsilyloxy)-2-methylpentanoic Acid (5).[14] ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.08 (3H, s), 0.09 (3H, s) 0.85 (9H, s) 0.91 (3H, t, *J* = 5.4 Hz) 1.18 (3H, d, *J* = 7.0 Hz) 1.49—1.64 (2H, m) 2.67 (1H, dq, *J* = 7.2, 5.4 Hz) 3.78—3.85 (1H, m) 11.15 (1H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 8.90, 13.51, 15.23, 25.73, 26.98, 44.30, 75.09, 178.66; IR (neat) 3104, 2954, 1710, 1465, 1255, 1119, 1013, 839 cm^{-1}.

(2*S*,3*R*)-3-Cyanobutan-2-yl Acetate (1d). Using the same protocol described above, (2*S*,3*R*)-**1d** was obtained. $[\alpha]_D^{23}$ +10.5 (*c* 1.30, $CHCl_3$). 65%de (*anti*), >99%ee. Preparation of (2*R*, 3*S*)-**1d** was accomplished using two different types of enzymes as described in Scheme 1.

(2*R*,3*S*)-3-Hydroxy-2-methylbutanenitrile (2d). To a suspension of lipase OF (4.34 g) in de-ionized water (180 mL) was added an acetone (15 mL) solution of acetate (±)-**1d**[12] (7.24 g, 51.3 mmol) and thiocrown ether **3** (0.040 g, 0.15 mmol, 0.5 mol%) and the mixture was stirred at 35 °C for 36 h. The reaction mixture was extracted with CH_2Cl_2 and evaporated to dryness. Silica gel flash column chromatography (hexane/ ethyl acetate = 7 : 1 to 2 : 1) gave alcohol **2d** (3.90 g, 35.1 mmol, 68%) and acetate **1d** (0.869 g, 6.16 mmol, 12%). Diastereomeric excess of **1d** was measured by capillary GC analysis as 58%de with >99%ee. Acetate **1d** (1.24 g, 8.78 mmol) was then hydrolyzed by hemicellulase (Amano) (0.744 g) in 15 mL of 0.1 M phosphate buffer (pH 7.2) at 35 °C for 137 h. Silica gel flash column chromatography of the extract gave alcohol **2d** (0.456 g, 4.60 mmol, 52%) hydrolyzed and acetate **1d** (0.534 g, 3.78 mmol, 43%) unreacted. Capillary GC analysis using chiral column (G-Ta) showed that the enantiomeric excess of **1d** was >99%ee with 86%de (*anti*). $[\alpha]_D^{25}$ −11.8 (*c* 1.27, $CHCl_3$).



Scheme 3.

Measurement of the Diastereo-Favoritism of the Lipase-Catalyzed Reaction. Acetate **2d** produced by the lipase-catalyzed reaction was converted to the authentic sample, i.e. 5-hydroxy-4-methylhexan-3-on (**7**)[14] as shown in Scheme 3.

To *N*,*N*-dimethylformamide (DMF) (26.0 mL) solution of **2d** (1.35 g, 13.6 mmol, 87%de) and TBDMSCl (2.47 g, 16.4 mmol) was added a DMF (4.0 mL) solution of imidazole (1.39 g, 20.4 mmol) at 0 °C and the mixture was stirred at r.t. for 6 h. The reaction mixture was extracted with ether and the combined organic layer was dried over $MgSO_4$ and evaporated to dryness. Silica gel flash column chromatography (hexane/ ethyl acetate = 100 : 1) gave TBDMS ether **2da** (2.88 g, 13.8 mmol) in 99% yield. A dichloromethane (8.0 mL) solution of **2da** (0.274 g, 1.28 mmol) was added to 1.50 mmol of diisobutylaluminum hydride (DIBAH, 1.5 M in toluene) dropwise at −78 °C and the reaction mixture was stirred at the same temperature for 1.5 h. The reaction was quenched by addition of 2 M HCl and the mixture was extracted with ether. The combined organic layer was dried over $MgSO_4$ and evaporated to give a crude oil which was used in the next reaction without purification. The crude oil was dissolved in 6.0 mL of THF and was treated with ethylmagnesium bromide (2.6 mmol, 0.65 M in THF) at −78 °C for 1 h. The reaction was quenched by addition of NH_4Cl saturated aqueous solution and acidified by 2 M HCl. The mixture was extracted with ether, and the combined organic layer was dried and evaporated. Silica-gel flash column chromatography (hexane /ethyl acetate = 20 : 1) gave diol **6** (0.280 g, 1.14 mmol) in 89% yield. To a suspension of solution of $RuCl_3 \cdot H_2O$ (0.0068 g, 0.032 mmol) and $NaIO_4$ (0.103 g, 0.481 mmol) in 5 mL of mixed solvent ($CH_3CN : H_2O = 2 : 3$) was added a CCl_4 (2.0 mL) solution of **6** (0.0810 g, 0.329 mmol) at r.t. and the mixture was stirred for 24 h at r.t.[15] The reaction was quenched by addition of 2-propanol and was extracted with CH_2Cl_2. The combined organic layer was washed with brine, $NaHCO_3$ saturated aqueous solution, and finally with water. The organic layer was dried ($MgSO_4$) and evaporated to dryness to give an oily product. This was dissolved in THF (2.0 mL) and treated with tetrabutylammonium fluoride (TBAF) (1.0 M in THF) at 0 °C for 3 h. The mixture was extracted with ether, dried ($MgSO_4$), and evaporated to dryness. Silica gel flash column chromatography (hexane/ ethyl acetate = 10 : 1) gave 5-hydroxy-4-methylhexan-3-one (**7**)[14] (0.022 g, 0.169 mmol) in 51% yield (two steps from **6**). This hydroxy ketone was composed of two isomers. ^{1}H NMR analysis of **7** showed that the major isomer was the *anti* isomer because the coupling constant (*J* value) of H_4–H_5 was 7.22 Hz, while that of the minor isomer was 3.25 Hz.

(4*SR*,5*RS*)-5-Hydroxy-4-methylhexan-3-one (7)[14] (85%de). ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.06 (3H, t, J = 7.3 Hz), 1.11 (3H, d, J = 7.4 Hz), 1.20 (3H, d, J = 6.3 Hz), 1.25 (1H, s), 2.55 (2H, d, J = 7.3 Hz), 2.50—2.62 (1H, m), 3.91 (1H, m)*. * The spin decoupling test showed that the coupling constant of C-5 proton (J_{H4-H5}) of the major isomer was 7.22 Hz, while that of the minor one is 3.25 Hz.; ^{13}C NMR (50 MHz, $CDCl_3$) δ = 7.52, 14.15, 20.90, 35.76, 52.85, 69.62, 216.55; IR (neat) 3315, 2929, 2858, 1726, 1471, 1257, 1007, 837 cm^{-1}.

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