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# Combination of Novozym 435-catalyzed Enantioselective Hydrolysis and Amidation for the Preparation of Optically Active δ-Hexadecalactone

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**Abstract: A new enzymatic method for synthesis of enantiomerically enriched δ-hexadecalactone (3) based on the enzymatic kinetic resolution of *N*-methyl-5-acetoxyhexadecanamide (1) is described. A combination of lipase-catalyzed hydrolysis and amidation improved enantioselectivity. Lipase-catalyzed amidation was also investigated. Detailed screening of solvents and additive amines was performed. The addition of cyclohexylamine to lipase-catalyzed hydrolysis afforded the best results to give both enantiomers of 3 with more than 90% enantiomeric excess.**

**Key words:** δ-hexadecalactone, lipase-catalyzed resolution, hydrolysis, amidation

## 1 INTRODUCTION

Lactones are present in many plants, usually as components of essential oils[1-4]. They often contribute to the odors of plants, their flowers, and fruits. Lactones also exhibit specific and useful biological activities, and δ-hexadecalactone (**3**) has various useful properties. It is known that δ-hexadecalactone (**3**) was extracted from heads of the queens of the Oriental hornet, *Vespa orientalis*, and that it acts as a sex pheromone[5]. Tanaka *et al*. reported the anti-tumor activity of δ-aklyllactones constituted of 11, 12, 13, 14, and 16 carbons[6]. δ-Hexadecalactone showed the highest anti-tumor activity against EAT cells and anti-invasive activity against human fibrosarcoma HT-1080 cells among these δ-alkyllactones. On the other hand, δ-hexadecalactone is found in bovine fat, ovine fat, and many dairy products[7-12]. It acts as a flavor component in these animal fats. Lehmann *et al*. reported the enantiomer ratio included in the δ-hexadecalactone of dairy products[13]. For instance, the (*R*)- and (*S*)-enantiomer ratios in butter and whipped cream are 90/10 and 88/12, respectively. Generally, lactones have a different odor and threshold among each enantiomer[14, 15]. However, there is no report on the production of δ-hexadecalactone. Therefore, we attempted to synthesize optically active δ-hexadecalactone. Optical resolution using lipase is very useful as a process for the synthesis of optically active substances. Enantioselective hydrolysis and acylation reaction by lipase has been used in recent years as methods of synthesis of drugs such as (*R*)-ibuprofen, (*R*)-stiripentol, and (*S*)-enciprazine have been reported[16-19]. The X-ray structure of CALB was solved in 1994, and a serine-histidine-asparate catalytic triad is responsible for its catalytic activity[20]. This is similar to the active center of serine proteases[21]. Therefore, lipase also catalyzes enantioselective amidation. Methods for synthesis of optically active amides using lipase-catalyzed enantioselective amidation have also been reported[22-26]. However, a method for synthesis of optically active substances by a combination of lipase-catalyzed enantioselective hydrolysis and amidation has not been reported. We investigated the synthesis of optically active δ-hexadecalactone using enantioselective hydrolysis with lipase. Furthermore, we report that simultaneous lipase-catalyzed enantioselective hydrolysis and amidation improved enantioselectivity for the production of optically active δ-hexadecalactone.

## 2 EXPERIMENTAL

### 2.1 General

$^{1}$H and $^{13}$C NMR spectra were recorded on a JNM-ECA-500 spectrometer (JEOL, Tokyo, Japan) at 500 and 126 MHz, respectively, with $CDCl_3$ as a solvent and TMS as the internal standard. Infrared (IR) spectra were recorded on a

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Fourier transform (FT) IR-460-plus spectrometer from JASCO Corp. (Tokyo, Japan) and are reported as wave numbers ($cm^{-1}$). Melting points (mp) were recorded on a MP-500D micro-melting-point apparatus from Yanaco Technical Science Co., Ltd. (Kyoto, Japan) and are uncorrected. Capillary GC was performed using an InertCap CHIRAMIX (30 m × 0.25 mm I.D. 0.25 μm film thickness, GL Science Co., Ltd. Tokyo, Japan) column (Inj. 250℃, Det. 250℃). Optical rotations were obtained on a P-1010 polarimeter from JASCO Corp. (Tokyo, Japan). High-resolution mass spectra (HRMS) were analyzed on an AccuTof GCv 4G (JEOL, Tokyo, Japan). Novozym 435 (immobilized lipase from *Candida antarctica*) was gifts from Novozymes A/S (Paraná, Brazil). Cyclopentyl methyl ether (CPME) was a gift from Zeon Corp. (Tokyo, Japan). Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F254). Flash column chromatography was carried out with silica gel FL60D.

### 2.2 Preparation of racemic *N*-alkyl-5-acetoxyhexadecanamide (*rac*-1a-e)

Racemic *N*-methyl-5-hydroxyhexadecanamide (*rac*-**2a**) was prepared by adding methylamine hydrochloride (1.0 g, 15.0 mmol) and potassium acetate (1.5 g, 15.0 mmol) to a stirred solution of racemic δ-hexadecalactone (*rac*-**3**, 2.5 g, 10.0 mmol) in THF at room temperature. Other *N*-alkyl-5-hydroxyhexadecanamides (*rac*-**2b-e**) were prepared by stirring a solution of δ-hexadecalactone (*rac*-**3**, 2.5 g, 10.0 mmol) and the corresponding amine (20.0 mmol) at room temperature. The aqueous phase was extracted with $CHCl_3$. The combined organic phase was dried over $NaSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography on silica (eluent: AcOEt) to afford the corresponding *N*-alkyl-5-hydroxyhexadecanamides (*rac*-**2a-e**). Racemic *N*-alkyl-5-acetoxyhexadecanamides (*rac*-**1a-e**) were prepared by adding acetic anhydride (0.82 g, 8.0 mmol) and 4-dimethylaminopyridine (0.1 g, 0.8 mmol) to a stirred solution of *rac*-**2a-e** (4.0 mmol) in $CH_2Cl_2$ (25 ml) and stirring the mixture for 24 h. $CH_2Cl_2$ was evaporated, and the mixture was extracted with $CHCl_3$. The combined extracts were washed with brine and dried over $Na_2SO_4$. After filtration, $CHCl_3$ was evaporated to afford the corresponding racemic *N*-alkyl-5-acetoxyhexadecanamides (*rac*-**1a-e**).

2.2.1 *N*-Methyl-5-acetoxyhexadecanamide (*rac*-**1a**)

Yield: 95% (from *rac*-**3**); Colorless solid; mp = 59-60℃; $[\alpha]^{20}_D = -0.98$ [*c* = 1.0, MeOH, 99% e.e. for (*R*)-**1a**]. IR (KBr): $cm^{-1}$ 3277 (N-H), 2954 ($CH_3$), 2919 ($CH_2$), 2870 ($CH_3$), 1732 (O<u>C=O</u>), 1640 (NH<u>C=O</u>), 1241 (C-O). $^1$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3H, -$CH_2$C<u>H</u>$_3$), 1.25-1.32 (m, 18H, -C<u>H</u>$_2$-×9), 1.51-1.58 (m, 4H, -C<u>H</u>$_2$CH(OAc)C<u>H</u>$_2$-), 1.60-1.75 (m, 2H, -C<u>H</u>$_2$-), 2.04 (s, 3H, -OC(=O)C<u>H</u>$_3$), 2.11-2.24 (m, 2H, -NHC(=O)C<u>H</u>$_2$-), 2.80 (d, *J* = 5.0 Hz, 3H, -NHC<u>H</u>$_3$), 4.86 (tt, *J* = 6.0, 6.4 Hz, 1H, -$CH_2$C<u>H</u>(OAc)$CH_2$-), 5.52 (br s, 1H, -N<u>H</u>-). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.1 (-$CH_2$<u>C</u>H$_3$), 21.3, 21.5 (-<u>C</u>H$_2$-×2), 21.5 (-OC(=O)<u>C</u>H$_3$), 22.7, 25.3, 26.2 (-<u>C</u>H$_2$-×3), 26.2 (<u>C</u>H$_3$NHC(=O)-), 29.3, 29.5, 29.6, 31.9, 33.6, 34.0 (-<u>C</u>H$_2$-×6), 36.1 (-NHC=O<u>C</u>H$_2$-), 73.7 (-$CH_2$<u>C</u>H(OAc)$CH_2$-), 171.0 (-O<u>C</u>(=O)$CH_3$), 173.2 (-NH<u>C</u>(=O)-). HRMS (FD) calcd. for $C_{19}H_{37}NO_3$ $(M+H)^+$, 328.2852; found $(M+H)^+$, 328.28018.

2.2.2 *N*-*n*-Propyl-5-acetoxyhexadecanamide (*rac*-**1b**)

Yield: 84% (from *rac*-**3**); Colorless solid; mp = 45-46℃; $[\alpha]^{20}_D = +1.85$ [*c* = 1.0, MeOH, 71% e.e. for (*R*)-**1b**]. IR (KBr): $cm^{-1}$ 3305 (N-H), 2920 ($CH_3$), 2851 ($CH_2$), 1732 (O<u>C=O</u>), 1642 (NH<u>C=O</u>), 1241 (C-O). $^1$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3H, -$CH_2$C<u>H</u>$_3$), 0.92 (t, *J* = 7.3 Hz, 3H, -$CH_2$C<u>H</u>$_3$), 1.17-1.35 (m, 18H, -C<u>H</u>$_2$-×9), 1.47-1.82 (m, 8H, -C<u>H</u>$_2$C<u>H</u>$_2$CH(OAc)C<u>H</u>$_2$-, -NHC<u>H</u>$_2$C<u>H</u>$_2$$CH_3$), 2.04 (s, 3H, -OC(=O)C<u>H</u>$_3$), 2.10-2.26 (m, 2H, -NHC(=O)C<u>H</u>$_2$-), 3.18-3.23 (m, 2H, -NHC<u>H</u>$_2$CH-), 4.87 (tt, *J* = 6.0, 6.0 Hz, 1H, -$CH_2$C<u>H</u>(OAc)$CH_2$-), 5.55 (br s, 1H, -N<u>H</u>-). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 11.3 (-$CH_2$<u>C</u>H$_3$), 14.1 (-$CH_2$<u>C</u>H$_3$), 21.2 (-NHC(=O)$CH_2$<u>C</u>H$_2$-), 21.5 (-OC(=O)<u>C</u>H$_3$), 22.7 (-$CH_2$<u>C</u>H$_2$$CH_3$), 22.9 (-NHC$H_2$<u>C</u>H$_2$$CH_3$), 25.3 (-CH(OAc)$CH_2$<u>C</u>H$_2$-), 29.3, 29.5, 29.6, 31.9 (-<u>C</u>H$_2$-×7), 33.5 (-<u>C</u>H$_2$CH(OAc)$CH_2$-), 34.0 (-$CH_2$CH(OAc)<u>C</u>H$_2$-), 36.2 (-NHC(=O)<u>C</u>H$_2$-), 41.2 (-NH<u>C</u>H$_2$$CH_2$-), 73.7 (-$CH_2$<u>C</u>H(OAc)$CH_2$-), 171.0 (-O<u>C</u>(=O)$CH_3$), 172.5 (-NH<u>C</u>(=O)-). HRMS (FD) calcd. for $C_{21}H_{41}NO_3$ $(M+H)^+$, 356.3165; found $(M+H)^+$, 356.31204.

2.2.3 *N*-*iso*-Propyl-5-acetoxyhexadecanamide (*rac*-**1c**)

Yield: 95% (from *rac*-**3**); Colorless solid; mp = 45-46℃; $[\alpha]^{20}_D = +0.08$ [*c* = 1.0, MeOH, 16% e.e. for (*R*)-**1c**]. IR (KBr): $cm^{-1}$ 3308 (N-H), 2917 ($CH_3$), 2850 ($CH_2$), 1730 (O<u>C=O</u>), 1637 (NH<u>C=O</u>), 1241 (C-O). $^1$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3H, -$CH_2$C<u>H</u>$_3$), 1.14 (dd, *J* = 6.9, 2.9 Hz, 6H, -C(=O)NHCH(C<u>H</u>$_3$)$_2$), 1.25-1.32 (m, 18H, -$CH_2$-×9), 1.51-1.63 (m, 5H, -NHC(=O)C<u>H</u>$_2$$CH_2$C<u>H</u>$_2$CH(OH)C<u>H</u>$_2$-), 1.64-1.74 (m, 1H, -NHC(=O)C<u>H</u>$_2$-), 2.05 (s, 3H, -OC(=O)C<u>H</u>$_3$), 2.07-2.19 (m, 2H, -NHC(=O)C<u>H</u>$_2$-), 4.02-4.13 (m, 1H, -C(=O)NHCH(C<u>H</u>$_3$)$_2$), 4.86 (tt, *J* = 6.3, 6.3 Hz, 1H, -$CH_2$C<u>H</u>(OAc)$CH_2$-), 5.34 (br s, 1H, -N<u>H</u>-). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.1 (-$CH_2$C<u>H</u>$_3$), 21.3 (-NHC(=O)$CH_2$<u>C</u>H$_2$-), 21.5 (-OC(=O)<u>C</u>H$_3$), 22.7 (-<u>C</u>H$_2$$CH_3$), 22.8 (-C(=O)NHCH(<u>C</u>H$_3$)$_2$), 25.3 (-$CH_2$CH(OH)$CH_2$<u>C</u>H$_2$-), 29.3, 29.5, 29.6, (-$CH_2$-×6), 31.9 (-<u>C</u>H$_2$$CH_2$$CH_3$), 33.5 (-NHC(=O)$CH_2$$CH_2$<u>C</u>H$_2$-), 34.0 (-$CH_2$CH(OCOC$H_3$)<u>C</u>H$_2$-), 36.3 (-NHC(=O)<u>C</u>H$_2$-), 41.2 (-C(=O)NH<u>C</u>H(C$H_3$)$_2$), 73.7 (-<u>C</u>HOC(=O)$CH_3$), 171.0 (-O<u>C</u>(=O)$CH_3$), 171.6 (-NH<u>C</u>(=O)-). HRMS (FD) calcd. for $C_{21}H_{41}NO_3$ $(M+H)^+$, 356.3165; found $(M+H)^+$, 356.31238.

2.2.4 *N*-Benzyl-5-acetoxyhexadecanamide (*rac*-**1d**)

Yield: 76% (from *rac*-**3**); Colorless solid; mp = 38-39℃; $[\alpha]^{20}_D = -0.04$ [*c* = 1.0, MeOH, 25% e.e. for (*R*)-**1d**]. IR (KBr): $cm^{-1}$ 3300 (N-H), 3031 (Ar, C-H), 2919 ($CH_3$), 2851 ($CH_2$), 1726 (O<u>C=O</u>), 1637 (NH<u>C=O</u>), 1544, 1456 (Ar, C=

C), 1243 (C-O), 745, 698 (Ar, C-H). $^{1}$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3H, -$CH_2$C$\underline{H}_3$), 1.17-1.32 (m, 18H, -C$\underline{H}_2$-×9), 1.45-1.64 (m, 5H, -C$\underline{H}_2$C$\underline{H}_2$CH(OAc)C$\underline{H}_2$-), 1.66-1.75 (m, 1H, -NHC(=O)$CH_2$C$\underline{H}_2$-), 2.01 (s, 3H, -OC(=O)C$\underline{H}_3$), 2.14-2.26 (m, 2H, -NHC(=O)C$\underline{H}_2$-), 4.41 (d, *J* = 5.7 Hz, 2H, -PhC$\underline{H}_2$NHC(=O)-), 4.85 (tt, *J* = 6.9, 5.7 Hz, 1H, -$CH_2$C$\underline{H}$(OAc)$CH_2$-), 6.04 (br s, 1H, -N$\underline{H}$-), 7.25-7.28 (m, 2H, Ph), 7.31-7.34 (m, 3H, Ph). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.1 (-$CH_2$C$\underline{H}_3$), 21.2 (-NHC(=O)$CH_2$C$\underline{H}_2$-), 21.4 (-OC(=O)C$\underline{H}_3$), 22.6 (-C$\underline{H}_2$$CH_3$), 25.3, 29.3, 29.4, 29.5, 29.6 (-C$\underline{H}_2$-×7), 31.8 (-C$\underline{H}_2$$CH_2$$CH_3$), 33.4 (-NHC(=O)$CH_2$$CH_2$C$\underline{H}_2$-), 34.0 (-NHC(=O)C$\underline{H}_2$-), 36.0 (-$CH_2$CH(OAc)C$\underline{H}_2$-), 43.4 (PhC$\underline{H}_2$NHC(=O)-), 73.6 (-$CH_2$C$\underline{H}$(OAc)$CH_2$-), 127.4, 127.7, 128.6, 138.3 (Ph), 171.0 (-OC$\underline{}$(=O)$CH_3$), 172.4 (-NHC$\underline{}$(=O)-). HRMS (FD) calcd. for $C_{26}H_{43}NO_3$(M)$^+$, 403.3086; found (M)$^+$, 403.30771.

2.2.5 *N*-Cylochexyl-5-acetoxyhexadecanamide (*rac*-**1e**)

Yield: 89% (from *rac*-**3**); Colorless solid; mp = 44-45℃; [α]$^{20}_{D}$ = −0.33 [c = 1.0, MeOH, 64% e.e. for (*R*)-**1e**]. IR (KBr): $cm^{-1}$ 3304 (N-H), 2919 ($CH_3$), 2851 ($CH_2$), 1728 (OC$\underline{}$=O), 1634 (NHC$\underline{}$=O), 1247 (C-O). $^{1}$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3H, -$CH_2$C$\underline{H}_3$), 1.02-1.18 (m, 3H, -C$\underline{H}_2$-×2), 1.19-1.42 (m, 20H, -C$\underline{H}_2$-×10), 1.51-1.65 (m, 6H, -C$\underline{H}_2$C$\underline{H}_2$CH(OAc)C$\underline{H}_2$-), 1.68-1.74 (m, 3H, -C$\underline{H}_2$-×2), 1.88-1.92 (m, 2H, -C$\underline{H}_2$-), 2.04 (s, 3H, -OC(=O)C$\underline{H}_3$), 2.06-2.20 (m, 2H, -NHC(=O)C$\underline{H}_2$-), 3.71-3.80 (m, 1H, -$CH_2$C$\underline{H}$NHC(=O)-), 4.86 (tt, *J* = 6.0, 6.0 Hz, 1H, -$CH_2$C$\underline{H}$(OAc)$CH_2$-), 5.41 (br s, 1H, -N$\underline{H}$-). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.1 (-$CH_2$C$\underline{H}_3$), 21.2 (-NHC(=O)$CH_2$C$\underline{H}_2$-), 21.5 (-OC(=O)C$\underline{H}_3$), 22.6 (-C$\underline{H}_2$$CH_3$), 24.8, 25.3 (-C$\underline{H}_2$-×2), 25.5 (-$CH_2$CH(OAc)$CH_2$C$\underline{H}_2$-), 29.3, 29.4, 29.5, 29.6 (-C$\underline{H}_2$-×6), 31.9 (-C$\underline{H}_2$$CH_2$$CH_3$), 33.2 (-C$\underline{H}_2$CHNHC(=O)-), 33.4 (-C$\underline{H}_2$CH(OAc)$CH_2$-), 34.0 (-$CH_2$CH(OAc)C$\underline{H}_2$-), 36.4 (-NHC(=O)C$\underline{H}_2$-), 48.0 (-$CH_2$C$\underline{H}$NHC(=O)-), 73.7 (-$CH_2$C$\underline{H}$(OAc)$CH_2$-), 171.0 (-OC$\underline{}$(=O)$CH_3$), 171.5 (-NHC$\underline{}$(=O)-). HRMS (FD) calcd. for $C_{24}H_{45}NO_3$(M)$^+$, 395.3399; found (M)$^+$, 395.33767.

### 2.3 General procedure for Novozym 435-catalyzed hydrolysis

Novozym 435 (0.2 g) was added to a solution of *rac*-**1a-e** (0.5 mmol) and MeOH (1.5 mmol) in the corresponding solvent such as cyclohexane, CPME, or the mixed solvents (10 ml), while stirring at 80℃ in an oil bath. After stirring, the mixture was filtered, and the solvent was evaporated. The crude product was purified by flash chromatography on silica and AcOEt to afford the corresponding (*R*)-**1a-e**, (*S*)-**2a-e**, and (*S*)-**3**. Lactonization of (*R*)-**1a-e** and (*S*)-**2a-e** was performed by hydrolysis under alkaline conditions and subsequent methyl esterification in 10% $H_2SO_4$/MeOH, and intraesterification under acidic conditions[27].

2.3.1 (*S*)-*N*-Methyl-5-hydroxyhexadecanamide [(*S*)-**2a**]

Colorless solid; mp = 87-88℃; [α]$^{20}_{D}$ = −0.01 (*c* = 1.0, MeOH, 91% e.e.). IR (KBr): $cm^{-1}$ 3290 (N-H), 2956 ($CH_3$), 2918 ($CH_2$), 2873 ($CH_3$), 2849 ($CH_2$), 1639 (NHC$\underline{}$=O). $^{1}$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3H, -$CH_2$C$\underline{H}_3$), 1.26-1.32 (m, 18H, -C$\underline{H}_2$-×9), 1.38-1.54 (m, 4H, -C$\underline{H}_2$-×2), 1.70-1.81 (m, 2H, -C$\underline{H}_2$-), 2.09 (br s, 1H, -OH), 2.22 (t, *J* = 7.3 Hz, 2H, -NHC(=O)C$\underline{H}_2$-), 2.80 (d, *J* = 5.0 Hz, 3H, -NHC$\underline{H}_3$), 3.58 (m, 1H, -$CH_2$C$\underline{H}$(OH)$CH_2$-), 5.70 (br s, 1H, -NH-). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.1 (-$CH_2$C$\underline{H}_3$), 21.6, 22.7, 25.7, 26.3 (-C$\underline{H}_2$-×4), 26.3 (C$\underline{H}_3$NHC(=O)-), 29.3, 29.6, 29.7, 31.9 (-C$\underline{H}_2$-×4), 36.2 (-NHC(=O)C$\underline{H}_2$-), 36.7, 37.5 (-C$\underline{H}_2$-×2), 71.3 (-$CH_2$C$\underline{H}$(OH)$CH_2$-), 173.8 ($CH_3$NHC$\underline{}$(=O)-). HRMS (FD) calcd. for $C_{17}H_{35}NO_2$(M+H)$^+$, 286.2746; found (M+H)$^+$, 286.27172.

2.3.2 (*S*)-*N*-*n*-Propyl-5-hydroxyhexadecanamide [(*S*)-**2b**]

Colorless solid; mp = 86-87℃; [α]$^{20}_{D}$ = −1.84 (*c* = 1.0, MeOH, 71% e.e.). IR (KBr): $cm^{-1}$ 3285 (O-H, N-H), 2920 ($CH_3$), 2848 ($CH_2$), 1635 (NHC$\underline{}$=O). $^{1}$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3H, -$CH_2$C$\underline{H}_3$), 0.92 (t, *J* = 7.3 Hz, 3H, -$CH_2$C$\underline{H}_3$), 1.17-1.35 (m, 17H, -C$\underline{H}_2$-×9), 1.36-1.56 (m, 7H, -NHC(=O)C$\underline{H}_2$$CH_2$C$\underline{H}_2$CH(OH)C$\underline{H}_2$-, -C$\underline{H}_2$$CH_2$NHC(=O)-), 1.75 (q, *J* = 6.9, 6.9 Hz, 2H, -NHC(=O)$CH_2$C$\underline{H}_2$-), 2.22 (t, *J* = 7.3 Hz, 3H, -NHC(=O)C$\underline{H}_2$-, -$CH_2$CH(OH)$CH_2$C$\underline{H}_2$-), 3.16-3.21 (m, 2H, -C$\underline{H}_2$NHC(=O)-), 3.53-3.64 (m, 1H, -$CH_2$C$\underline{H}$(OH)$CH_2$-), 5.75 (-N$\underline{H}$-). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 11.3 (-$CH_2$C$\underline{H}_3$), 14.1 (-$CH_2$C$\underline{H}_3$), 21.6 (-NHC(=O)$CH_2$C$\underline{H}_2$-), 22.6 (-C$\underline{H}_2$$CH_3$), 22.8 ($CH_3$C$\underline{H}_2$$CH_2$NHC(=O)-), 25.7 (-$CH_2$CH(OH)$CH_2$C$\underline{H}_2$-), 29.3, 29.6, 29.7, (-C$\underline{H}_2$-×4), 31.9 (-C$\underline{H}_2$$CH_2$$CH_3$), 36.3 (-C$\underline{H}_2$CH(OH)$CH_2$-), 36.7 (-NHC(=O)C$\underline{H}_2$-), 37.5 (-$CH_2$CH(OH)C$\underline{H}_2$-), 41.2 (-C$\underline{H}_2$NHC(=O)-), 71.2 (-$CH_2$C$\underline{H}$(OH)$CH_2$-), 173.1 (-NHC$\underline{}$(=O)-). HRMS (FD) calcd. for $C_{19}H_{39}NO_2$(M+H)$^+$, 314.3059; found (M+H)$^+$, 314.30275.

2.3.3 (*S*)-*N*-*iso*-Propyl-5-hydroxyhexadecanamide [(*S*)-**2c**]

Colorless solid; mp = 78-79℃; [α]$^{20}_{D}$ = −0.73 (*c* = 1.0, MeOH, 86% e.e.). IR (KBr): $cm^{-1}$ 3286 (O-H, N-H), 2918 ($CH_3$), 2849 ($CH_2$), 1635 (NHC$\underline{}$=O). $^{1}$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3H, -$CH_2$C$\underline{H}_3$), 1.14 (d, *J* = 6.4 Hz, 6H, -C(=O)NHCH(C$\underline{H}_3$)$_2$), 1.21-1.36 (m, 17H, -C$\underline{H}_2$-×8), 1.38-1.54 (m, 5H, -NHC(=O)C$\underline{H}_2$-, -C$\underline{H}_2$CH(OH)C$\underline{H}_2$-), 1.74 (q, *J* = 7.3 Hz, 2H, -NHC(=O)$CH_2$C$\underline{H}_2$-), 2.12-2.24 (m, 3H, -C$\underline{H}_2$C(=O)NHC$\underline{H}$($CH_3$)$_2$, -C$\underline{H}_2$-), 3.58 (br s, 1H, O$\underline{H}$), 4.02-4.14 (m, 1H, -$CH_2$C$\underline{H}$(OH)$CH_2$-), 5.49 (br s, 1H, N$\underline{H}$). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.1 (-$CH_2$C$\underline{H}_3$), 21.5 (-NHC(=O)$CH_2$C$\underline{H}_2$-), 22.7 (-C$\underline{H}_2$$CH_3$), 22.8 (-C(=O)NHCH(C$\underline{H}_3$)$_2$), 25.7, 29.3, 29.6, 29.7 (-C$\underline{H}_2$-×5), 31.9 (-C$\underline{H}_2$$CH_2$$CH_3$), 36.5 (-C$\underline{H}_2$CH(OH)$CH_2$-), 36.7 (-$CH_2$CH(OH)C$\underline{H}_2$-), 37.5 (-NHC(=O)C$\underline{H}_2$-), 41.2 (-C(=O)NHC$\underline{H}$($CH_3$)$_2$), 71.2 (-$CH_2$C$\underline{H}$(OH)$CH_2$-), 172.2 (-NHC$\underline{}$(=O)-). HRMS (FD) calcd. for $C_{19}H_{39}NO_2$(M+H)$^+$, 314.3059; found (M+H)$^+$, 314.30295.

2.3.4 (*S*)-*N*-Benzyl-5-hydroxyhexadecanamide [(*S*)-**2d**]

Colorless solid; mp = 82-83℃; [α]$^{20}_{D}$ = +0.13 (*c* = 1.0, MeOH, 88% e.e.). IR (KBr): $cm^{-1}$ 3296 (O-H, N-H), 3031 (Ar, C-H), 2918 ($CH_3$), 2849 ($CH_2$), 1639 (NHC$\underline{}$=O), 1557,

1456 (Ar, C=C), 730, 695 (Ar, C-H). $^1$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, $J$ = 6.9 Hz, 3H, -$CH_2$C$\underline{H}_3$), 1.26 (m, 18H, -C$\underline{H}_2$-×9), 1.38-1.55 (m, 4H, -C$\underline{H}_2$CH(OH)C$\underline{H}_2$-), 1.70-1.83 (m, 2H, -NHC(=O)$CH_2$C$\underline{H}_2$-), 2.26 (t, $J$ = 6.9 Hz, 2H, -NHC(=O)C$\underline{H}_2$-), 3.54-3.60 (m, 1H, -$CH_2$C$\underline{H}$(OH)$CH_2$-), 4.43 (d, $J$ = 5.5 Hz, 2H, -C(=O)NHC$\underline{H}_2$Ph), 5.93 (br s, 1H, N$\underline{H}$), 7.26-7.29 (m, 3H, Ph), 7.31-7.35 (m, 2H, Ph). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.1 (-$CH_2$C$\underline{H}_3$), 21.5 (-NHC(=O)$CH_2$C$\underline{H}_2$-), 22.7 (-C$\underline{H}_2$$CH_3$), 25.7 (-$CH_2$CH(OH)$CH_2$C$\underline{H}_2$-), 29.3, 29.6, 29.7, 29.7 (-C$\underline{H}_2$-×4), 31.9 (-C$\underline{H}_2$-), 36.3 (-C$\underline{H}_2$CH(OH)$CH_2$-), 36.7 (-NHC(=O)C$\underline{H}_2$-), 37.6 (-$CH_2$CH(OH)C$\underline{H}_2$-), 43.6 (-C(=O)NHC$\underline{H}_2$Ph), 71.3 (-$CH_2$C$\underline{H}$(OH)$CH_2$-), 127.5, 127.8, 128.7, 138.3 (Ph), 172.9 (-NHC$\underline{}$(=O)-). HRMS (FD) calcd. for $C_{24}H_{41}NO_2$ $(M)^+$, 361.2981; found $(M)^+$, 361.29748.

2.3.5 (*S*)-*N*-Cyclohexyl-5-hydroxyhexadecanamide [(*S*)-**2e**]

Colorless solid; mp = 86-87℃; $[\alpha]^{20}_D$ = +0.45 ($c$ = 1.0, MeOH, 88% e.e.). IR (KBr): $cm^{-1}$ 3300 (O-H, N-H), 2919 ($CH_3$), 2851 ($CH_2$), 1637 (NHC=O). $^1$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, $J$ = 6.9 Hz, 3H, -$CH_2$C$\underline{H}_3$), 1.07-1.20 (m, 4H, -C$\underline{H}_2$-×2), 1.26-1.37 (m, 18H, -C$\underline{H}_2$×9), 1.41-1.53 (m, 4H, -C$\underline{H}_2$CH(OH)C$\underline{H}_2$-), 1.59-1.64 (m, 2H, -NHC(=O)$CH_2$C$\underline{H}_2$-), 1.68-1.78 (m, 4H, -$CH_2$-×2), 1.89-1.91 (m, 2H, -C$\underline{H}_2$-), 2.19 (t, $J$ = 7.3 Hz, 2H, -NHC(=O)C$\underline{H}_2$-), 2.50 (br s, 1H, OH), 3.54-3.60 (m, 1H, -$CH_2$C$\underline{H}$(OH)$CH_2$-), 3.71-3.80 (m, 1H, -C(=O)NHC$\underline{H}$$CH_2$-), 5.69 (br s, 1H, N$\underline{H}$). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.0 (-$CH_2$C$\underline{H}_3$), 21.6 (-NHC(=O)$CH_2$C$\underline{H}_2$-), 22.6 (-C$\underline{H}_2$$CH_3$), 24.8 (-$CH_2$C$\underline{H}_2$$CH_2$-), 25.5 (-$CH_2$CH(OH)$CH_2$C$\underline{H}_2$-), 25.7 (-$CH_2$C$\underline{H}_2$$CH_2$-), 29.3, 29.6, 29.7 (-C$\underline{H}_2$-), 31.8 (-C$\underline{H}_2$$CH_2$$CH_3$), 33.1 (-C(=O)NHCHC$\underline{H}_2$-), 36.4 (-NHC(=O)$CH_2$$CH_2$C$\underline{H}_2$-), 36.6 (-NHC(=O)C$\underline{H}_2$-), 37.5 (-$CH_2$CH(OH)C$\underline{H}_2$-), 48.1 (-C(=O)NHC$\underline{}$$CHCH_2$-), 71.1 (-$CH_2$C$\underline{H}$(OH)$CH_2$-), 172.2 (-NHC$\underline{}$(=O)-). HRMS (FD) calcd. for $C_{22}H_{43}NO_2$ $(M+H)^+$, 354.3372; found $(M+H)^+$, 354.33383.

2.3.6 δ-Hexadecalactone (**3**)

Colorless solid; mp = 33-34℃. Enantiomeric excess determined by GC on an InertCap CHIRAMIX (30 m×0.25 mm I.D. 0.25 μm film thickness) column, temperature: 170℃, flow rate: 2.5 mL/min, $t_R$ = 150.1 min, $t_S$ = 155.4 min; $[\alpha]^{20}_D$ = +42.0 [$c$ = 1.0, THF, (*R*)-**3** with >99% e.e., lit $[\alpha]^{21.5}_D$ = +40.2 ($c$ = 1.76, THF, >99% e.e.)][28], $[\alpha]^{20}_D$ = −39.4 [$c$ = 1.0, THF, (*S*)-**3** with 94% e.e., lit $[\alpha]^{25}_D$ = −40.2 ($c$ = 1.66, THF, >99% e.e.)][28]. IR (KBr): $cm^{-1}$ 2927, 2848 ($CH_3$), 1727 (OC=O), 1242 (C-O). $^1$H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, $J$ = 6.9 Hz, 3H, -$CH_2$C$\underline{H}_3$), 1.26-1.40 (m, 18H, -C$\underline{H}_2$-×9), 1.44-1.62 (m, 2H, -$CH_2$CH(C$\underline{H}_2$-)OC(=O)-), 1.64-1.75, 1.78-1.97 (both m, 4H, -C$\underline{H}_2$C$\underline{H}_2$CH($CH_2$-)OC(=O)-), 2.39-2.49, 2.54-2.63 (both m, 2H, -C(=O)C$\underline{H}_2$$CH_2$-), 4.24-4.31 (m, 1H, -$CH_2$C$\underline{H}$($CH_2$-)OC(=O)-). $^{13}$C NMR (126 MHz, $CDCl_3$): δ = 14.1 (-$CH_2$C$\underline{H}_3$), 18.5, 22.6, 24.9, 27.8, 29.3, 29.4, 29.5, 29.6, 31.9 (-$CH_2$-×9), 35.8 (-$CH_2$CH(C$\underline{H}_2$-)OC(=O)-), 80.6 (-$CH_2$C$\underline{H}$($CH_2$-)OC(=O)-), 171.9 (-OC$\underline{}$(=O)-). HRMS (FD) calcd. for $C_{16}H_{32}O_2$ $(M)^+$, 254.2246; found $(M)^+$, 254.2224.

### 2.4 Novozym 435-catalyzed lactonization of *rac*-1a and *rac*-2a

Novozym 435 (0.2 g) was added to a solution of *rac*-**1a** (0.5 mmol) or *rac*-**2a** (0.5 mmol) in cyclohexane or CPME (10 ml), while stirring at 80℃ in an oil bath. After stirring, Novozym 435 was removed by filtration, and the solvent was evaporated. The crude product was purified by flash chromatography on silica and AcOEt. (*R*)-**1a**, (*S*)-**2a** and (*S*)-**3** were obtained from Novozym 435-catalyzed lactonization of *rac*-**1a**. Racemic **2a** and **3** were obtained from Novozym 435-catalyzed lactonization of *rac*-**2a**.

### 2.5 Novozym 435-catalyzed amidataion of 3

A solution of *rac*-**3** (0.5 mmol), $BnNH_2$ or $cHxNH_2$ (1.0 mmol) and Novozym 435 (0.2 g) in cyclohexane or CPME (10 ml) was stirred for 3 d at 80℃. After stirring, the reaction mixture was filtered to remove Novozym 435, and the solvent was evaporated under vacuum, and $CH_3Cl$ was added to the residue. The resulting solution was then neutralized with aqueous HCl, and extracted with $CH_3Cl$, followed by washing with brine. The combined extracts were dried over anhydrous $MgSO_4$, and the solvent was removed by evaporating under vacuum. The crude product was purified by flash chromatography on silica and AcOEt to give **3** and **2**.

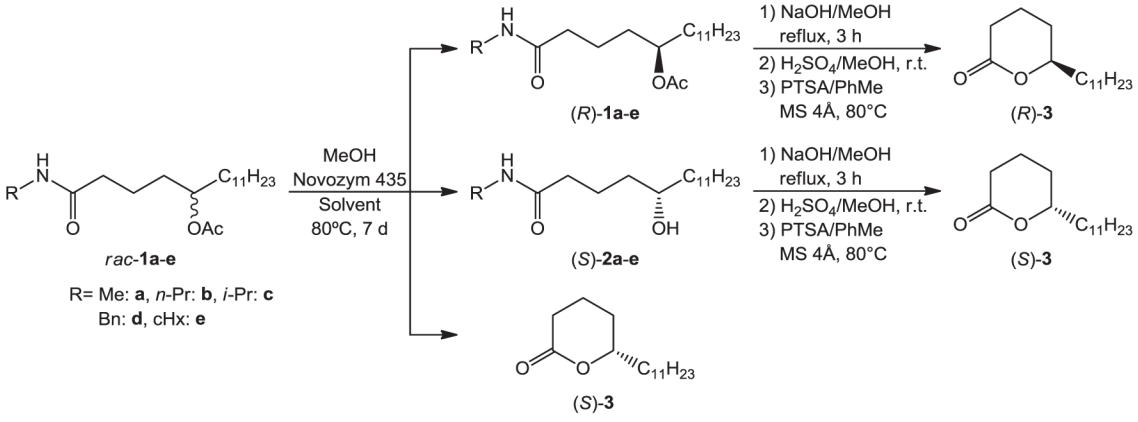
### 2.6 Amine additive Novozym 435-catalyzed hydrolysis of *rac*-1a

A solution of *rac*-**1a** (0.5 mmol), MeOH (1.5 mmol), $BnNH_2$ or $cHxNH_2$ (1.0 mmol), and Novozym 435 (0.2 g) in cyclohexane or CPME (10 ml) was stirred for 7 d at 80℃. After stirring, Novozym 435 was filtered off the reaction mixture, and the solvent was then removed under vacuum. The residue was purified with flash chromatography on silica and AcOEt to afford (*S*)-**2a** and the mixture of (*R*)-**1a** and (*S*)-**2d-e**. $Na_2CO_3$ (2.0 g) was added to a solution of the mixture of (*R*)-**1a** and (*S*)-**2d-e** in MeOH (20 ml), while stirred at 80℃ for 5 hours in an oil bath. After stirring, MeOH was evaporated under vacuum, and $H_2O$ (20 ml) was added to the residue. The resulting solution was then neutralized with aqueous HCl, and extracted with $CH_3Cl$, followed by washing with brine and water. The combined extracts were dried over anhydrous $MgSO_4$, and the solvent was removed by evaporating under vacuum. The crude product was purified by flash chromatography on silica and AcOEt to give (*S*)-**2a** and (*S*)-**2d-e**.

## 3 Results and discussion

### 3.1 Optimization of substrate structure, solvent and temperature

Differences in substrate structure affect the reactivity and enantioselectivity because lipase has substrate specificity. *N*-alkyl-5-acetoxyhexadecanamides (*rac*-**1**) possessing various alkyl groups with different steric hindrances were prepared, and the substrates for lipase-catalyzed hydrolysis using *rac*-**1** were screened (Scheme 1, Table 1). Methyl and *n*-propyl groups as a comparatively small group were selected for the R group in *rac*-**1**. Conversely, *iso*-propyl, benzyl and cyclohexyl groups as a comparatively bulky alkyl group were selected. Various *N*-alkyl-5-hydroxyhexadecanamides (*rac*-**2**) were prepared from racemic δ-hexadecalactone (*rac*-**3**) by a ring-opening reaction using primary amines, and subsequently acetylation of *rac*-**2** using acetic anhydride was performed to afford the corresponding *rac*-**1**. Novozym 435-catalyzed hydrolysis of *rac*-**1a** and **1b** possessing methyl and *n*-propyl group produced (*S*)-**3** besides a deacetylated (*S*)-**2** (Table 1, Entries 1-4). In the case of *rac*-**1c**, **1d**, and **1e** possessing *iso*-propyl, benzyl, and cyclohexyl groups, respectively, (*S*)-**3** did not or scarcely produced them. There was no great difference in enantioselectivity compared with methyl and *n*-propyl groups, but the conversion of *rac*-**1** was low. These showed that R groups of *rac*-**1** did not affect the substrate selectivity of Novozym 435, but the substrate affinity was affected by the difference of R groups. *rac*-**1a** exhibited somewhat higher enantioselectivity than *rac*-**1b**. It was assumed that Novozym 435 shows high substrate specificity for the substrates with a small R group. The enantiomeric excess of (*R*)-**1** was higher than those of (*S*)-**2** and (*S*)-**3** in cyclohexane (Table 1, Entry 1). In contrast, (*S*)-**2** and (*S*)-**3** had higher enantiomeric excesses than (*R*)-**1** in cyclopentyl methyl ether (CPME) (Table 1, Entry 2). Conversion of *rac*-**1** was higher for cyclohexane compared




**Scheme 1** Optical resolution with Novozym 435-catalyzed hydrolysis of *rac*-**1**.

**Table 1** Effect of R group on Novozym 435-catalyzed hydrolysis of *rac*-**1**[1].

| Entry | Substrate | R | Solvent | Yield [%] / Enantiomeric excess [% e.][2] | | |
|---|---|---|---|---|---|---|
| | | | | (*R*)-**1** | (*S*)-**2** | (*S*)-**3** |
| 1 | *rac*-**1a** | Me | Cy-Hexane | 41 / 87 | 18 / 66 | 35 / 77 |
| 2 | | | CPME | 57 / 57 | 14 / 84 | 23 / 88 |
| 3 | *rac*-**1b** | *n*-Pr | Cy-Hexane | 47 / 71 | 17 / 67 | 31 / 76 |
| 4 | | | CPME | 51 / 52 | 26 / 71 | 20 / 80 |
| 5 | *rac*-**1c** | *i*-Pr | Cy-Hexane | 77 / 15 | 13 / 70 | - / - |
| 6 | | | CPME | 76 / 16 | 15 / 86 | - / - |
| 7 | *rac*-**1d** | Bn | Cy-Hexane | 69 / 25 | 22 / 59 | - / - |
| 8 | | | CPME | 80 / 15 | 17 / 88 | - / - |
| 9 | *rac*-**1e** | cHx | Cy-Hexane | 54 / 64 | 38 / 82 | 6 / 83 |
| 10 | | | CPME | 84 / 15 | 14 / 88 | - / - |

1) *rac*-**1**: 0.5 mmol, MeOH: 1.5 mmol, Novozym 435: 0.2 g, Solvent: 10 ml, 80℃, 7 d.
2) Determined by GC using an InertCap CHIRAMIX column. Enantiomeric excesses of (*R*)-**1** and (*S*)-**2** were measured from the corresponding **3**.

to CPME. Several structures of lipases have been elucidated[29]. They all have an α/β hydrolase fold[21, 30, 31], with most of them containing a helical segment called the lid that covers the active site when the enzyme is in the so-called closed formation. Lipases are totally hydrophilic, but the active site of lipase is hydrophobic. Therefore, the active site of lipase is covered by the lid in water. In the presence of lipid aggregates, the lid opens, and the enzyme activity is increased, a phenomenon called interfacial activation[29]. Cyclohexane is a low-polar solvent compared to CPME. It seemed that the active site in cyclohexane was increasingly exposed compared with that in CPME. This state allows the substrate to easily enter the active site. This permitted cyclohexane to show higher conversion than CPME. However, lipase in cyclohexane easily draws the substrate into the active site, which leads to a decrease of enantioselectivity and causes the decrease of enantiomeric excess of (*S*)-**2** and (*S*)-**3** compared with that in CPME. These results showed that the reactivity and enantioselectivity of Novozym 435 were affected by solvents. Next, reaction temperature was considered because it affects reactivity and enantioselectivity greatly for lipase-catalyzed reaction (Table 2). The reaction mixture of *rac*-**1a** was stirred in cyclohexane at 80℃ for 7 days without adding Novozym 435 (Table 2, Entry 1). In the additive-free case, although hydrolysis was progressed in the case where Novozym 435 was added, it did not hydrolyze at all. Only when Novozym 435 carried out a catalyst, hydrolysis of *rac*-**1a** was progressed. In order to confirm the influence of temperature, hydrolysis was performed at 40, 60, and 80℃ for 7 days. The conversion rate became high as temperature became high. When CPME was used as a solvent, in 60 and 80℃, the production of (*S*)-**3** other than (*S*)-**2a** into which the acetyl group was hydrolyzed was observed (Table 2, Entries 6 and 7). In contrast, in 40℃, the reaction was progressed only 10%, and the production of (*S*)-**3** was not observed (Table 2, Entry 5). (*S*)-**2a** and (*S*)-**3** that were produced by Novozym 435-catalyzed hydrolysis showed 89-90% enantiomeric excess in all conditions. From these results, although the reaction temperature affected the reactivity of hydrolysis, the enantioselectivity of Novozym 435 was not influenced greatly. The activity of Novozym 435 is not lost in 80℃. Therefore, 80℃ was the optimal reaction temperature. Additionally, various solvents were

**Table 2** Effect of temperature on Novozym 435-catalyzed hydrolysis of *rac*-**1a**[1].

| Entry | Novozym 435 [g] | Solvent | Temp. [℃] | Yield [%] / Enantiomeric excess [% e.e.][2] | | |
|---|---|---|---|---|---|---|
| | | | | (*R*)-**1** | (*S*)-**2** | (*S*)-**3** |
| 1 | none | Cy-Hexane | 80 | No reaction | | |
| 2 | 0.2 | | 40 | 55 / 46 | 5 / 81 | 15 / 92 |
| 3 | | | 60 | 60 / 48 | 11 / 83 | 16 / 87 |
| 4 | | | 80 | 41 / 87 | 18 / 66 | 35 / 77 |
| 5 | | CPME | 40 | 83 / 13 | 9 / 89 | - / - |
| 6 | | | 60 | 66 / 30 | 13 / 86 | 7 / 91 |
| 7 | | | 80 | 57 / 57 | 14 / 84 | 23 / 88 |

1) *rac*-**1a** 0.5 mmol, MeOH: 1.5 mmol, Novozym 435: 0.2 g, Solvent: 10 ml, 7 d

2) Determined by GC using an InertCap CHIRAMIX column. Enantiomeric excesses of (*R*)-**1** and (*S*)-**2** were measured from the corresponding **3**.

**Table 3** Effect of solvent on Novozym 435-catalyzed hydrolysis[1].

| Entry | Solvent | Temp. [℃] | Yield [%] / Enantiomeric excess [% e.e.][2] | | |
|---|---|---|---|---|---|
| | | | (*R*)-**1** | (*S*)-**2** | (*S*)-**3** |
| 1 | *n*-Hexane | 60 | 38 / 84 | 46 / 72 | 13 / 76 |
| 2 | Cy-Hexane | 80 | 41 / 87 | 18 / 66 | 35 / 77 |
| 3 | CPME | 80 | 57 / 57 | 14 / 84 | 23 / 88 |
| 4 | *i*-$Pr_2O$ | 60 | 67 / 27 | 8 / 89 | 12 / 87 |
| 5 | PhMe | 80 | 74 / 24 | 14 / 84 | 9 / 91 |

1) *rac*-**1**: 0.5 mmol, MeOH: 1.5 mmol, Novozym 435: 0.2 g, Solvent: 10 ml, 80℃, 7 d

2) Determined by GC using an InertCap CHIRAMIX column. Enantiomeric excesses of (*R*)-**1** and (*S*)-**2** were measured from the corresponding **3**.

used in Novozym 435-catalyzed hydrolysis of *rac*-**1a** (Table 3). In the case of toluene, the enantioselectivity was highest, but the conversion was low (Table 3, Entry 5). *n*-Hexane produced results for the enantiomeric excesses of (*R*)-**1** and (*S*)-**3** similar to cyclohexane (Table 3, Entry 1). (*R*)-**1** had higher enantiomeric excess compared with (*S*)-**2** and (*S*)-**3**. In the case of *i*-$Pr_2O$, (*S*)-**2a** and (*S*)-**3a** had a higher enantiomeric excess than (*R*)-**1a** (Table 3, Entry 4). The trend is the same with CPME. It seemed that the use of a mixed cyclohexane/CPME solvent gave both enantiomers with high enantiomeric excess (Table 4). The ratio of cyclohexane/CPME = 80/20 is on the border line of enantioselectivity (Table 4, Entry 5). In this case, all enantiomers were obtained with about 75% enantiomeric excess. By mixing CPME in the range of 5-15% of cyclohexane, the enantioselectivity of (*S*)-**2** increased about 8% compared with the case of using cyclohexane alone without changing most of the enantiomeric excess of (*R*)-**1** (Table 4, Entries 2-4). Similarly, mixtures in the range of 25-75% of cyclohexane for CPME increased the enantioselectivity of (*R*)-**1** more than 10% compared with the case of using CPME alone, which did not change the enantioselectivity of (*S*)-**2** and (*S*)-**3** (Table 4, Entries 6-8). These results indicated that a mixed solvent with an appropriate ratio cyclohexane to CPME gave both enantiomers with high enantiomeric excess compared with the case of using each solvent alone.

### 3.2 Reaction mechanism of lipase in hydrolysis and lactonization

The reaction mechanism of the production of **2a** by lipase-catalyzed hydrolysis of **1a** is shown in Fig. 1. The active site of Novozym 435 is constituted by the three amino acid residues of Ser105, His224, and Asp187, and the reaction proceeds by the mechanism shown in Fig. 1[20]. An oxygen atom of the catalytic serine in the "Free enzyme" attacked the carbonyl carbon of **1a** at the ester group to give a "Tetrahedral intermediate with **1a**". An "Acyl enzyme" was formed by releasing **2a**, and methanol attacked the carbonyl carbon at the acetyl group to afford a "Tetrahedral intermediate with methanol". A catalytic cycle occurs in returning to the "Free enzyme" to release the methyl acetate. The "Free enzyme" preferentially recognized the (*S*)-enantiomer of **1a**, and **2a**, produced by lipase-catalyzed hydrolysis, showed (*S*)-configuration. However, the reaction carried out by changing various conditions for lactone was not produced by this mechanism (Scheme 2, Table 5). Racemic **2a** was stirred with Novozym 435 in cyclohexane or CPME at 80℃ for 7 d (Table 5, Entries 1 and 2). **3** was obtained at yields of 40% and 31% in cyclohexane and CPME, respectively. Enantiomeric excesses of **3** obtained in both solvents were 0%. Figure 2 shows the reaction mechanism of lipase for the production of **3** from **2a**. The oxygen atom of the catalytic serine of the "Free enzyme" becomes the "Tetrahedral intermediate" to attack the carbonyl carbon at the amide group of **2a**. An "Acyl enzyme with a hydroxyl ester form" was formed by releasing methyl amine. Intramolecular esterification proceeds by binding oxygen atoms at hydroxyl groups and carbonyl carbons at ester groups in an "Acyl enzyme with hydroxyl ester form" to form a "Tetrahedral intermediate with lactone form", and release of **3** gives the "Free enzyme". **3** displayed racemic form obtained from **2a** by lipase-catalyzed lactonization (Table 5, Entries 1 and 2). It is assumed that the "Free enzyme" does not recognize the steric configuration in taking **2a** into the active

**Table 4** Effect of mixed solvent on Novozym 435-catalyzed hydrolysis[1].

| Entry | Cy-Hexane / CPME | Yield [%] / Enantiomeric excess [% e.e.][2] | | |
|---|---|---|---|---|
| | | (*R*)-**1** | (*S*)-**2** | (*S*)-**3** |
| 1 | 100 / 0 | 41 / 87 | 18 / 66 | 35 / 77 |
| 2 | 95 / 5 | 42 / 86 | 16 / 74 | 26 / 77 |
| 3 | 90 / 10 | 41 / 85 | 14 / 74 | 33 / 77 |
| 4 | 85 / 15 | 42 / 89 | 20 / 72 | 37 / 79 |
| 5 | 80 / 20 | 43 / 79 | 22 / 74 | 26 / 78 |
| 6 | 75 / 25 | 50 / 71 | 22 / 81 | 21 / 86 |
| 7 | 50 / 50 | 53 / 70 | 22 / 77 | 23 / 86 |
| 8 | 25 / 75 | 45 / 71 | 21 / 81 | 31 / 87 |
| 9 | 0 / 100 | 57 / 57 | 14 / 84 | 23 / 88 |

1) *rac*-**1**: 0.5 mmol, MeOH: 1.5 mmol, Novozym 435: 0.2 g, Solvent: 10 ml, 80℃, 7 d

2) Determined by GC using an InertCap CHIRAMIX column. Enantiomeric excesses of (*R*)-**1** and (*S*)-**2** were measured from the corresponding ***3***.

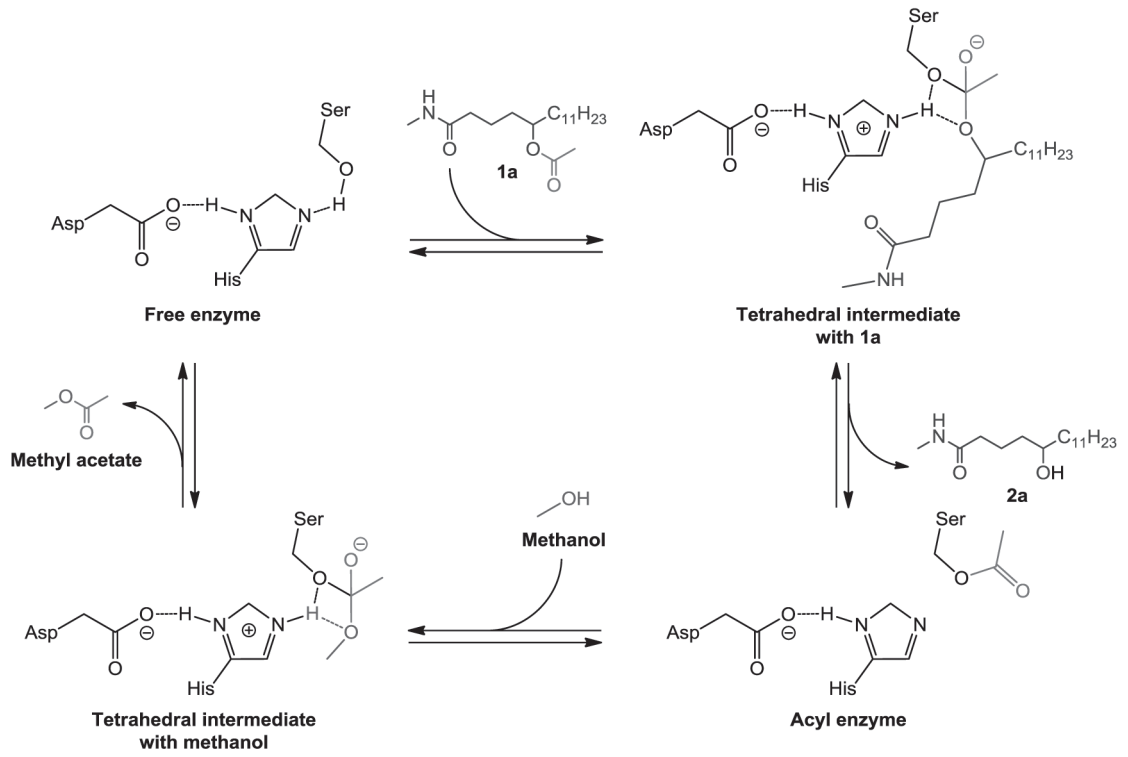
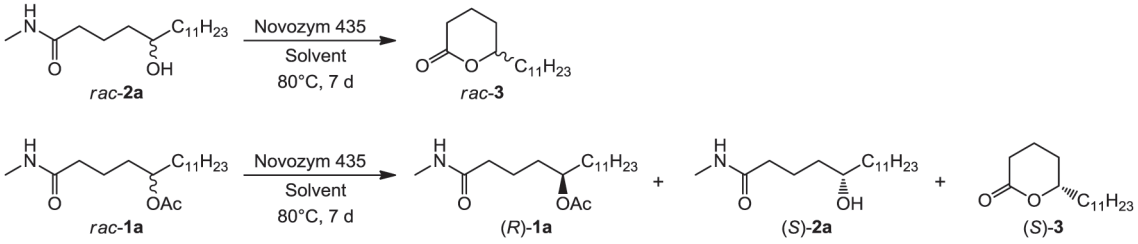



**Fig. 1** Reaction mechanism of lipase for production of **2a** from **1a**.




**Scheme 2** Novozym 435-catalyzed hydrolysis of *rac*-1a and lactonization of *rac*-**2a**.

**Table 5** Reactivity of Novozym 435 toward *rac*-**1a** and *rac*-**2a**[1].

| Entry | Substrate | Solvent | Yield [%] / Enantiomeric excess [% e.e.][2] / Config.[3] | | |
|---|---|---|---|---|---|
| | | | **1** | **2** | **3** |
| 1 | *rac*-**2a** | Cy-Hexane | - / - / - | 54 / *racemic* | 40 / *racemic* |
| 2 | | CPME | - / - / - | 67 / *racemic* | 31 / *racemic* |
| 3 | *rac*-**1a** | Cy-Hexane | 44 / 81 / *R* | 5 / 40 / *S* | 48 / 76 / *S* |
| 4 | | CPME | 67 / 39 / *R* | 9 / 83 / *S* | 20 / 91 / *S* |

1) *rac*-**1a** or *rac*-**2a**: 0.5 mmol, Novozym 435: 0.2 g, Solvent: 10 ml, 80℃, 7 d
2) Determined by GC using an InertCap CHIRAMIX column. Enantiomeric excesses of (*R*)-**1** and (*S*)-**2** were measured from the corresponding **3**.
3) Configuration of **1** and **2** was determined from the corresponding **3** compared with literature data.

site. However, the enantiomeric excess of 3 that was produced when performing Novozym 435-catalyzed hydrolysis of 1a in the presence of methanol was higher than 2a. If 3 produced by the reaction mechanism shown in Fig. 2 in Novozym 435-catalyzed hydrolysis of *rac*-1a, (*S*)-3 should have the same enantiomeric excess as (*S*)-2a. Based on this, it seems that 3 was produced by the different reaction mechanism shown in Fig. 2. Therefore, Novozym 435-cata-

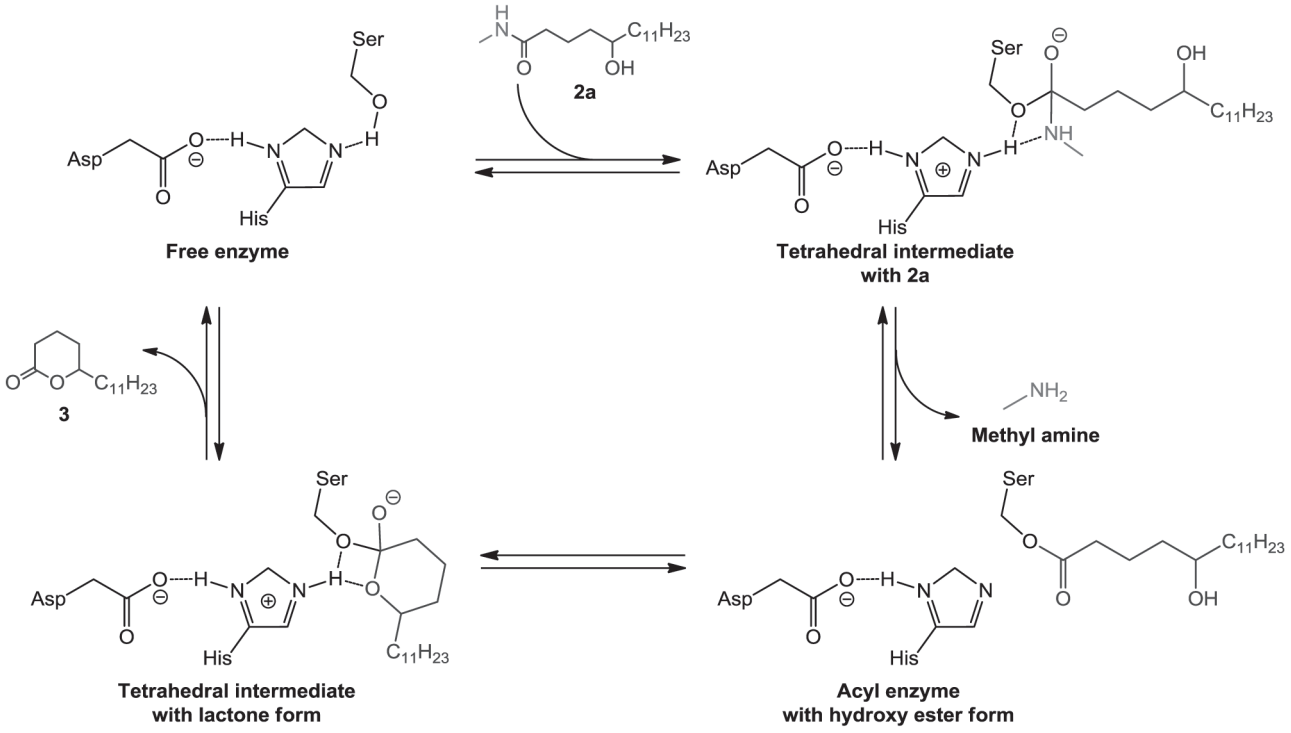



**Fig. 2** Reaction mechanism of lipase for production of **3** from **2a**.

lyzed hydrolysis of *rac*-**1a** was performed in the absence of methanol (Table 5, Entry 3 and 4). (*S*)-**3**, hydrolyzed (*S*)-**2a**, and (*R*)-**1a** were obtained as in the presence of methanol. The enantiomeric excess of (*S*)-**3** obtained with both solvents was higher relative to (*S*)-**2a**. Additionally, as compared with the case in the presence of methanol (Table 1, Entries 1 and 2), the yield of (*S*)-**3** was higher than (*S*)-**2a**. In particular, when cyclohexane was used as a solvent, the amount of **3** was about twice that of **2a** in the presence of methanol, but about 10 times more **3** was produced in the absence of methanol (Table 5, Entry 3). From these facts, it was assumed that mechanism of Novozym 435-catalyzed hydrolysis of *rac*-**1a** and production of (*S*)-**3** is shown as Fig. 3. The oxygen atom of the catalytic serine at the "Free enzyme" is thought to be a "Tetrahedral intermediate with **1a**" and to attack the carbonyl carbon at an ester group of **1a**. The "Free enzyme" selectively takes the (*S*)-enantiomer of **1a**. An "Acyl enzyme" is formed by release of (*S*)-**2a**. Binding an oxygen atom at the hydroxyl group of **2a** with a carbonyl carbon in the "Acyl enzyme" forms the "Tetrahedral intermediate with **2a**". Intramolecular cyclization proceeds and returns to the "Free enzyme" by releasing (*S*)-**3** and *N*-methylacetamide. The "Acyl enzyme" incorporates (*S*)-**2a** selectively into the active site, and the enantiomeric excess of (*S*)-**3** produced by this reaction mechanism is higher than that of (*S*)-**2a**. This indicates that the "Acyl enzyme" has higher affinity to **2a** than the "Free enzyme". On the other hand, methanol has a higher affinity to the "Acyl enzyme" relative to **2a**. Therefore, the "Acyl enzyme" takes methanol preferentially over **2a** in the presence of methanol. It is considered that the yield of (*S*)-**3** is higher in the absence of methanol. The catalytic cycle is slower in the absence of methanol in CPME, and it decreases the conversion of *rac*-**1a**. It is assumed to be the reaction mechanism of the production of **3**, and the reasons the enantiomeric excess of (*S*)-**3** obtained by Novozym 435-catalyzed hydrolysis of *rac*-**1a** is higher than that of (*S*)-**2a** are clearly demonstrated.

### 3.3 Lipase-catalyzed amidation of δ-hexadecalactone

When hydrolyzing *rac*-**1a** using Novozym 435, it seemed that the "Free enzyme" reaction takes *rac*-**1a** to give (*S*)-**2a** (Fig. 1), the "Free enzyme" takes **2a** to give **3** (Fig. 2), and the "Acyl enzyme" takes (*S*)-**2a** to give (*S*)-**3** (Fig. 3), and all occurred at the same time, which decreases the reactivity and enantioselectivity toward *rac*-**1a**. From the results shown in Table 1, *rac*-**1** with a bulky R group such as benzyl and cyclohexyl groups does not produce **3** in Novozym 435-catalyzed hydrolysis. This indicates that **2** with bulky R groups has low affinity for the "Free enzyme" and "Acyl enzyme". In the other words, Novozym 435 has no reactivity toward **1c-e**. Additionally, because lactone produced by Novozym 435 acts as an acyl donor, **3** is considered to have the possibility of inhibiting hydrolysis of *rac*-**1a**. If it is possible to convert (*S*)-**3** into **1c-e** when Novozym 435 showed no reactivity by adding an amine to the hydrolysis reaction, Novozym 435-catalyzed hydrolysis of *rac*-**1a** preferentially proceeds, and it is thought that

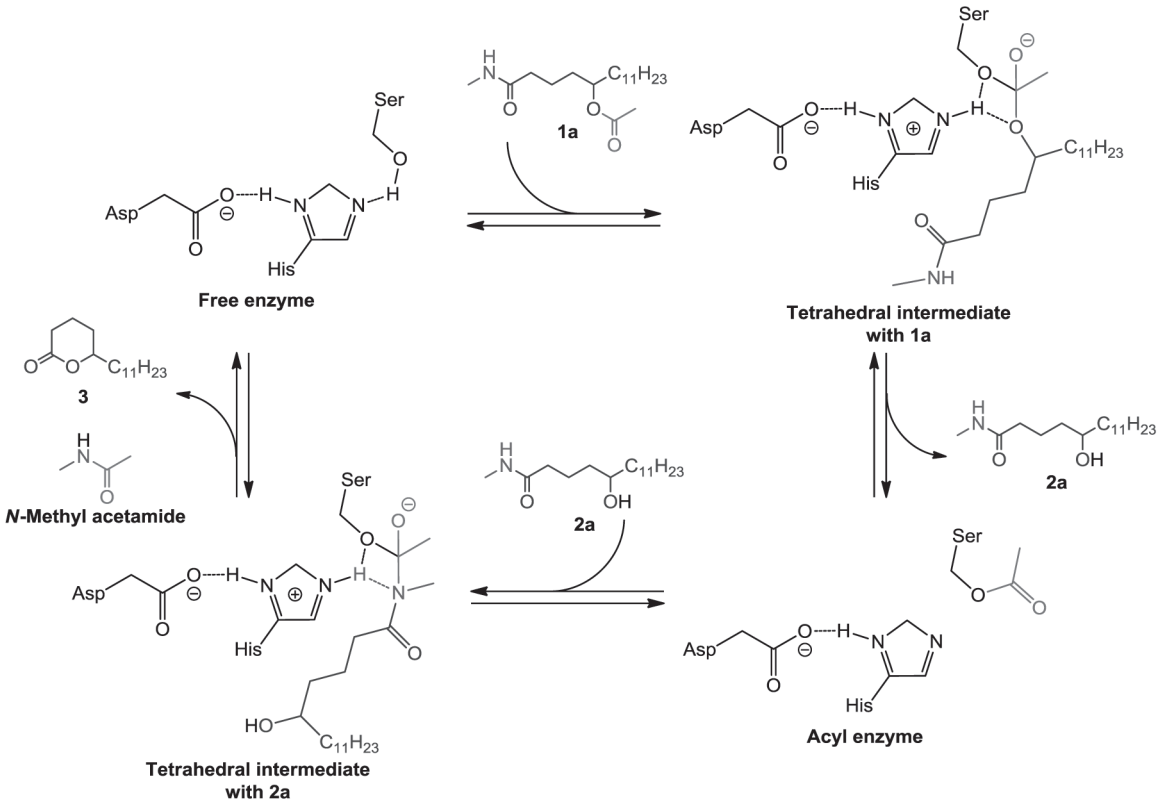
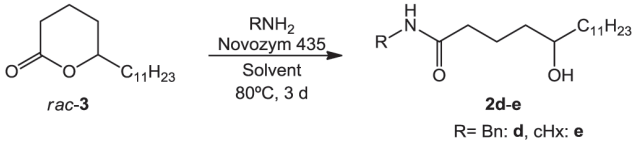



**Fig. 3** Reaction mechanism of lipase for production of **3** from **1a**.




**Scheme 3** Novozym 435-catalyzed amidation of *rac*-**3**.

this might increase the enantioselectivity. However, there is a possibility that the reaction is inhibited by the addition of amine. The amidation of racemic 3 was performed by adding two amine equivalents relative to 3 in the presence or absence of Novozym 435 (Scheme 3, Table 6). From the results shown in Tables 1 and 3, because hydrolysis is carried out at 80℃, benzylamine and cyclohexylamine, which have boiling points higher than 80℃, were selected. In the case of cyclohexane, almost all of 3 were amidated after three days by both amines, but it was not possible to confirm the effects of the presence of lipase (Table 6, Entries 1, 2, 5 and 6). On the other hand, when benzylamine was used in CPME, 2d was obtained at 59% and

**Table 6** Effect of Novozym 435 on amidation of *rac*-**3**[1)].

| Entry | Amine | Novozym 435 [g] | Solvent | Yield [%] | |
|---|---|---|---|---|---|
| | | | | **3** | **2** |
| 1 | $BnNH_2$ | none | Cy-Hexane | - | 92 |
| 2 | | 0.2 | | - | 94 |
| 3 | | none | CPME | 32 | 59 |
| 4 | | 0.2 | | - | 97 |
| 5 | $cHxNH_2$ | none | Cy-Hexane | 6 | 89 |
| 6 | | 0.2 | | 4 | 91 |
| 7 | | none | CPME | 42 | 53 |
| 8 | | 0.2 | | 6 | 89 |

1) *rac*-**3**: 0.5 mmol, amine: 1.0 mmol, Novozym 435: 0.2 g, Solvent: 10 ml, 80℃, 3 d

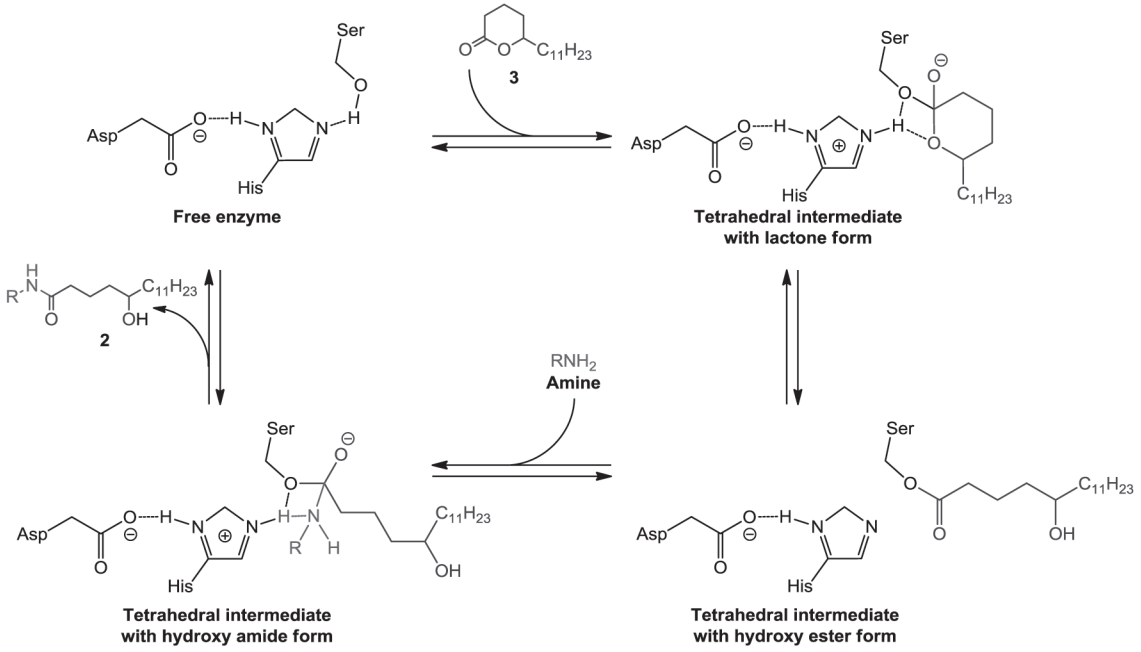
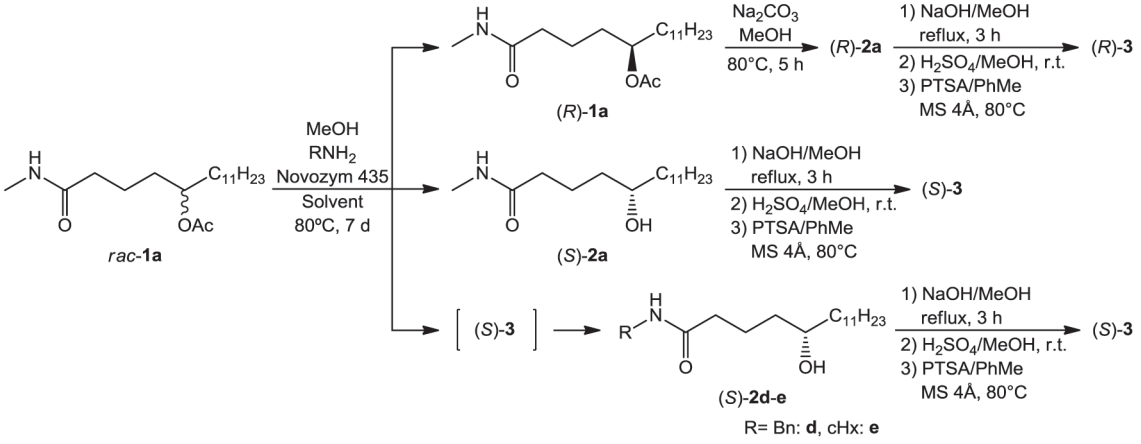



**Fig. 4** Reaction mechanism of lipase for production of **2** from **3**.




**Scheme 4** Amine additive Novozym 435-catalyzed hydrolysis of *rac*-**1a**.

97% in the absence and presence of Novozym 435, respectively (Table 6, Entries 3 and 4). Similarly, in the case of cyclohexylamine in CPME, **2e** was yielded at 53% and 89% in the absence and presence of Novozym 435, respectively (Table 6, Entries 7 and 8). It also shows that the conversion of cyclohexane is higher than that of the CPME as in the above-mentioned results (Tables 1 and 5). These results indicate that the activity of Novozym 435 was not reduced in the presence of amine, but rather that Novozym 435 catalyzed amidation of lactone. The reaction mechanism of amidation catalyzed by Novozym 435 is shown in Fig. 4. An oxygen atom of the catalytic serine in the "Free enzyme" attacks the carbonyl carbon of lactone. After formation of the "Tetrahedral intermediate with lactone", the "Acyl enzyme with hydroxyl ester form" is formed by the ring-opening of lactone. A nitrogen atom of amine attacks carbonyl carbon, and a "Tetrahedral intermediate with hydroxyamide form" is formed. The "Free enzyme" returns by release of **2**. With this mechanism, it is considered that Novozym 435 catalyzed the amidation of **3**.

### 3.4 Optimization of amine and amount added

The type and amount of amine added was optimized. Novozym 435-catalyzed hydrolysis of *rac*-**1a** was performed in cyclohexane or CPME for 7 d adding one, two, or five equal amounts of amine to *rac*-**1a** (Scheme 4, Table 7). Column separation of (*R*)-**1a** and (*S*)-**2d-e** in the reaction mixture was difficult. Therefore, the acetyl group in (*R*)-**1a** was hydrolyzed using $Na_2CO_3$ for the mixture of (*R*)-**1a** and (*S*)-**2d-e**, and the yield was obtained as (*R*)-**2a** and

**Table 7** Optimization of amines and amounts in Novozym 435-catalyzed hydrolysis[1)].

| Entry | Amine / Amount [e.q.] | Solvent | Yield [%] / Enantiomeric excess [% e.e.][2)] | | |
|---|---|---|---|---|---|
| | | | (*R*)-**1a** | (*S*)-**2a** | (*S*)-**2d** or **2e** |
| 1[3)] | None | Cy-Hexane | 41 / 87 | 18 / 66 | 35 / 77 |
| 2 | $BnNH_2$ / 1 | | 38 / 99 | 9 / 40 | 46 / 79 |
| 3 | $BnNH_2$ / 2 | | 40 / 96 | 6 / 58 | 50 /83 |
| 4 | $BnNH_2$ / 5 | | 65 / 52 | 18 / 86 | 14 / 88 |
| 5 | $cHxNH_2$ / 1 | | 35 / 96 | 22 / 41 | 34 / 67 |
| 6 | $cHxNH_2$ / 2 | | 41 / 96 | 25 / 79 | 31 / 82 |
| 7 | $cHxNH_2$ / 5 | | 38 / 86 | 20 / 75 | 39 / 90 |
| 8[3)] | None | CPME | 57 / 57 | 14 / 84 | 23 / 88 |
| 9 | $BnNH_2$ / 1 | | 63 / 34 | 20 / 89 | 13 / 60 |
| 10 | $BnNH_2$ / 2 | | 62 / 41 | 23 / 85 | 7 / 81 |
| 11 | $BnNH_2$ / 5 | | 54 / 39 | 22 / 90 | 23 / 80 |
| 12 | $cHxNH_2$ / 1 | | 53 / 76 | 29 / 87 | 15 / 78 |
| 13 | $cHxNH_2$ / 2 | | 48 / 70 | 32 / 91 | 10 / 94 |
| 14 | $cHxNH_2$ / 5 | | 64 / 28 | 25 / 87 | 5 / 61 |

1) *rac*-**1**: 0.5 mmol, MeOH: 1.5 mmol, Novozym 435: 0.2 g, Solvent: 10 ml, 80℃, 7 d

2) Determined by GC using an InertCap CHIRAMIX column. Enantiomeric excesses of (*R*)-**1** and (*S*)-**2** were measured from the corresponding **3**.

3) Data for (*S*)-**2d** or (*S*)-**2e** were used in Table 1, Entries 1 and 2, respectively.

(*S*)-2d-e. When *rac*-1a was hydrolyzed in cyclohexane using benzylamine as an amine, addition of equal amounts of one and two was compared to when benzylamine additive-free, enantiomeric excesses of (*R*)-1a and (*S*)-2d were improved and that of (*S*)-2a was reduced (Table 7, Entries 2 and 3). The enantiomeric excess of (*S*)-2d was compared to the (*S*)-3 produced by a no amine-added Novozym 435-catalyzed hydrolysis of *rac*-1a (Table 1, Entry 1). On the other hand, when five equal amounts of benzylamine were added, the enantiomeric excesses of (*S*)-2a and (*S*)-2d were improved and that of (*R*)-1a was reduced (Table 7, Entry 4). Adding one and two equal amounts of benzylamine produced no great difference in the yield of (*R*)-1a compared with the benzylamine-free case, but the yield of (*S*)-2a was decreased and that of (*S*)-2d was increased. This is similar to the results obtained by hydrolysis without the addition of methanol to *rac*-1a (Table 5, Entries 3 and 4). It seemed that because benzylamine formed a hydrogen bond with methanol, methanol was hardly incorporated into the active site of lipase. Namely, (*S*)-2a reacted preferentially with the "Acyl enzyme" to give (*S*)-2d. It is considered that the enantiomeric excess of (*S*)-2d improved because the "Acyl enzyme" incorporated (*S*)-2a selectively. Because the lactone with extremely low reactivity was converted to (*S*)-2d for lipase at the same time, which removes the lactone that acts as an acyl donor, the selectivity of (*S*)-1a was improved, and the enantiomeric excess of the unreacted (*R*)-1a was improved. However, when five equal amounts of benzylamine were added, enantiomeric excesses of (*S*)-2a and (*S*)-2d were improved for the above reason, but the conversion of *rac*-1a and enantiomeric excess of (*R*)-1a were reduced because the excess of benzylamine inhibited the progress of the reaction When CPME was used as a solvent, the conversion of *rac*-1a was lower than when no benzylamine was added, and the enantiomeric excesses of (*R*)-1a were decreased (Table 7, Entries 9-11). Because CPME has high polarity compared to cyclohexane, methanol and amines had difficulty in hydrogen bonding. This is because reactivity to the "Acyl enzyme" of benzylamine was increased, and it was preferentially reacted with rather than *rac*-1a. Further, because CPME was easily dissolved in 2a compared to cyclohexane, reactivity to lipase was decreased and the "Acyl enzyme" was hardly incorporated into the active site of (*S*)-2a. This may be because the CPME produced smaller amounts of (*S*)-3 compared to cyclohexane (Table 1, Entries 1 and 2). Thus, (*S*)-2a increased in the reaction system. (*S*)-2a acts both as an acyl receptor (Fig. 2) and as an acyl donor (Fig. 3). Because the increase of (*S*)-2a conceivably competed with the hydrolysis of *rac*-1a, the conversion of *rac*-1a was reduced, and the enantiomeric excess of (*R*)-1a was also reduced. Further, it was considered that because it was difficult to match the substrate specificity of Novozym 435 for 2a dissolved in CPME, the selectivity of Novozym 435

for (*S*)-2a was decreased, and the enantiomeric excess of (*S*)-2d was reduced. When cyclohexylamine was used as an amine in CPME, there was no great difference in the yield of (*R*)-1a compared with the case without cyclohexylamine added, and the enantiomeric excesses of (*R*)-1a were improved about 10% when one and two equal amounts were added (Table 7, Entries 5 and 6). Little difference was observed compared with the yield of (*S*)-2 without an addition, and improved enantiomeric excesses of about 10% were observed when equal amounts of two and five were added (Table 7, Entries 6 and 7). A trend similar to (*S*)-2a was observed in (*S*)-2e. Compared with the case of benzylamine, since the yield of (*S*)-2a was not affected by the addition of cyclohexylamine, cyclohexylamine hardly formed a hydrogen bond with methanol because the cyclohexyl group had a low electron-donating capacity relative to the benzyl group, and it was considered that cyclohexylamine did not affect the incorporation of methanol into the "Acyl enzyme". (*R*)-1a and (*S*)-2e were obtained with more than 90% enantiomeric excess with two and five equal amounts, respectively. However, all enantiomers of (*R*)-1a, (*S*)-2a, and (*S*)-2e were obtained with a higher enantiomeric excess on average with addition of two equal amounts of cyclohexylamine, and this was the optimum for this condition. Even when cyclohexylamine was added to CPME, the yield of (*S*)-2e tended to be comparable to (*S*)-2a with benzylamine (Table 7, Entries 12-14). When five equal amounts of cyclohexylamine were added in order to inhibit the reaction, the yield and enantiomeric excess of (*S*)-2e decreased (Table 7, Entry 14). However, there was no great difference in the yields of (*R*)-1a with one and two equal parts compared to that without cyclohexylamine additive, but enantiomeric excesses of (*R*)-1a were increased more than 10%. When two equal amounts were added, all enantiomeric excesses of (*R*)-1a, (*S*)-2a, and (*S*)-2e were improved, so addition of two equal amounts was considered an appropriate amount (Table 7, Entry 13). From the above, converting 3 produced by lipase to 2d or 2e, which exhibited less reactivity toward lipase by the addition of the corresponding amine, reduced the competitive reaction for hydrolysis of *rac*-1a, and these increased the selectivity of Novozym 435 for 1a. Furthermore, it was found that cyclohexylamine enhanced the selectivity compared to benzylamine, and addition of two equal amounts was optimal.

### 3.5 Lipase-catalyzed hydrolysis of *rac*-1a with two equal amounts of cyclohexylamine in mixed solvent

Cyclohexylamine-added Novozym 435-catalyzed hydrolysis of *rac*-1a was carried out using various cyclohexane/CPME mixed solvents (Table 8). A slight decrease in the enantiomeric excess of (*R*)-1a was observed in 50/50 and 25/75 mixed solvents compared with the case of using cyclohexane alone (Table 8, Entries 7 and 8), but enantiomeric excesses were improved using CPME that had cyclohexane in the range of 5-25% (Table 8, Entries 2-6). In addition, those of (*R*)-1a were more than 20% higher compared with using CPME alone. In the mixed solvents of 95/5, 90/10 and 85/15, enantiomeric excesses of (*S*)-2a were decreased 20-30% compared to cyclohexane (Table 8, Entries 2-4). The yields of (*S*)-2a were more than 10% lower in these mixed solvents relative to cyclohexane alone. Furthermore, the yields of (*S*)-2e were about 10% higher compared to cyclohexane. These were similar to the

**Table 8** Effect of mixed solvent on $cHxNH_2$ additive Novozym 435-catalyzed hydrolysis[1].

| Entry | Cy-Hexane / CPME | Yield [%] / Enantiomeric excess [% e.e.][2] | | |
|---|---|---|---|---|
| | | (*R*)-**1a** | (*S*)-**2a** | (*S*)-**2e** |
| 1 | 100 / 0 | 40 / 96 | 26 / 79 | 31 / 82 |
| 2 | 95 / 5 | 37 / 99 | 13 / 43 | 45 / 87 |
| 3 | 90 / 10 | 36 / 99 | 13 / 60 | 41 / 90 |
| 4 | 85 / 15 | 36 / 99 | 11 / 59 | 40 / 91 |
| 5 | 80 / 20 | 40 / 98 | 17 / 77 | 29 / 92 |
| 6 | 75 / 25 | 37 / 99 | 27 / 76 | 27 / 88 |
| 7 | 50 / 50 | 44 / 95 | 20 / 88 | 20 / 93 |
| 8 | 25 / 75 | 42 / 92 | 27 / 90 | 28 / 79 |
| 9 | 0 / 100 | 48 / 70 | 32 / 91 | 10 / 94 |

1) *rac*-**1a**: 0.5 mmol, MeOH: 1.5 mmol, $cHxNH_2$: 1.0 mmol, Novozym 435: 0.2 g, Solvent: 10 ml, 80℃, 7 d

2) Determined by GC using an InertCap CHIRAMIX column. Enantiomeric excesses of (*R*)-**1** and (*S*)-**2** were measured from the corresponding **3**.

results of the hydrolysis of *rac*-1a in the absence of methanol (Table 5, Entries 3 and 4). Based on these results, (*S*)-2a was preferentially taken into lipase to produce (*S*)-3 because methanol was hardly incorporated into lipase by hydrogen bonded with cyclohexylamine, and the lipase was subsequently converted (*S*)-3 to (*S*)-2e. As above mentioned, the "Acyl enzyme" incorporated (*S*)-2a selectively into the active site to convert (*S*)-3 (Fig. 3). As a result, it was considered that the increase of the ratio of (*R*)-2a in (*S*)-2a caused the decrease of enantiomeric excess of (*S*)-2a. Enantiomeric excesses of (*S*)-2a obtained in 80/20, 75/25, and 50/50 mixed solvents were comparable to those in cyclohexane (Table 8, Entries 4, 6 and 7), and that in 25/75 increased 10% (Table 8, Entry 8). Enantiomeric excesses of (*S*)-2e were observed to decrease slightly in 25/75 mixed solvent (Table 8, Entry 8), but it showed a higher value than cyclohexane alone in all mixed solvents except the ratio of 25/75. Among them, in the cases of 90/10, 85/15, and 80/20, both enantiomers could be efficiently synthesized with more than 90% enantiomeric excess (Table 8, Entries 3-5).

## 4 CONCLUSIONS

*N*-Methyl-5-acetoxyhexandecanamide (*rac*-1a) as a substrate was hydrolyzed using Novozym 435 in the presence of two equal amounts of cyclohexylamine, and both enantiomers of δ-hexadecalactone (3) were synthesized with more than 90% enantiomeric excess. A methyl group was suitable as a substrate for the R group. A moderate mixture of cyclohexane and CPME solvents showed higher enantioselectivity than each solvent alone. Lipase was found to catalyze not only the hydrolysis of *rac*-1a, but also amidation of 3. In general, lipases catalyze the hydrolysis reaction that recognizes the ester groups, but it was confirmed that Novozym 435 recognized the amide groups of 1a and 1b and catalyzed lactonization enantioselectively. It was possible to improve the enantioselectivity of Novozym 435 by the addition of two equal amounts of cyclohexylamine relative to *rac*-1a in the hydrolysis reaction. Furthermore, by using cyclohexane/CPME mixed solvents at ratios of 90/10, 85/15, and 80/20, both enantiomers of 3 were synthesized efficiently with more than 90% enantiomeric excess.

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