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Synthesis of alkyl (*R*)-lactates and alkyl (*S*,*S*)-*O*-lactyllactates by alcoholysis of *rac*-lactide using Novozym 435

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Abstract—Enzymatic alcoholysis of *rac*-lactide for kinetic resolution was carried out in organic solvents. Effects of organic solvent, reaction temperature, and alcohol as a nucleophile were also investigated in Novozym 435-catalyzed alcoholysis of *rac*-lactide. Both alkyl (*R*)-lactate and alkyl (*S*,*S*)-*O*-lactyllactate were simultaneously obtained in high yields (>45%) and high enantiopurities (>97% ee) through Novozym 435-catalyzed ring-opening of *rac*-lactide and subsequent enantioselective alcoholysis of the resultant alkyl *O*-lactyllactate.

Lactic acid (2-hydroxypropanoic acid) is one of the simplest chiral compounds, and enantiopure (*R*)- and (*S*)-lactic acid derivatives are important synthons for chiral drugs.[1] (*S*)-Lactic acid is commercially produced through microbial fermentation, while (*R*)-lactic acid is relatively difficult to obtain. In addition, ethyl (*S*,*S*)-*O*-lactyllactate, which is a linear dimer of (*S*)-ethyl lactate, was reported to be used as a starting material for the alkyl lactate oligomers, which have anticancer activity.[2]

Lipases have been extensively used to obtain chiral alcohols and carboxylic acids, due to their excellent chiral recognition.[3] Among the various lipases, Novozym 435, which is an immobilized form of lipase B from *Candida antarctica* (CALB), possesses the high enantioselectivity for a broad range of substrates.[4] Previously, we reported the enantioselective acylations of racemic alkyl lactates with vinyl alkanoates using Novozym 435.[5] We have succeeded in obtaining both butyl (*R*)-*O*-butanoyllactate and butyl (*S*)-lactate in excellent yields (48%) and enantioselectivities (>99.5% ee) on a large scale.

In the present work, Novozym 435 was applied to obtain enantiopure alkyl (*R*)-lactate and alkyl (*S*,*S*)-*O*-lactyllactate from *rac*-lactide, which is a cyclic dimer produced from the dehydration of lactic acid. Lipase-catalyzed ring-opening polymerizations of lactide into poly(lactic acid)s have been reported.[6] However, to the best of our knowledge, no previous report has been issued on enzymatic production of chiral compounds from *rac*-lactide.

In preliminary experiments, commercially available lipases[7] were screened for alcoholysis of *rac*-lactide (*rac*-**1**) with *n*-butanol (**4d**) in hexane/THF (90:10).[8] Among the lipases tested, a few lipases including Novozym 435 functioned in the formation of butyl *O*-lactyllactate (**2d**), the corresponding ring-opened product. However, their enantioselectivities were low so that *SS*-**1** was alcoholyzed slightly more than *RR*-**1**. Nevertheless, *SS*-**2d** was successfully obtained in high ee when Novozym 435 was used. It turned out that this is because Novozym 435 catalyzed enantioselective alcoholysis of the ring-opened *RR*-**2d** with an excess of **4d** to generate a butyl (*R*)-lactate (*R*-**3d**). It is noteworthy that Novozym 435 has quite different enantioselectivity toward cyclic **1** and its ring-opened **2**. This finding prompted us to examine a novel route for enantioselective synthesis of *SS*-**2** and *R*-**3** from *rac*-**1** by Novozym 435 (Scheme 1). It is known that CALB demonstrates (*R*)-stereoselectivity toward secondary alcohols.[3] Accordingly, *R*-**3** was produced by the alcoholysis of ring-opened **2** using Novozym 435 as shown in this experiment. The reason for

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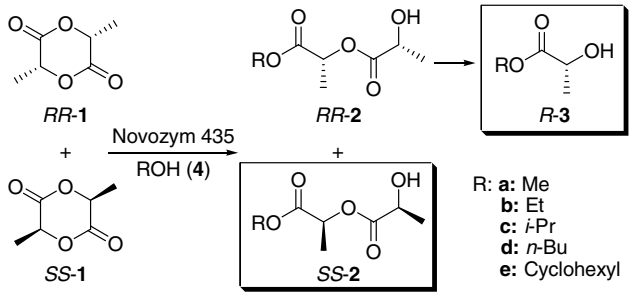

Scheme 1. Enzymatic alcoholysis of *rac*-lactide by Novozym 435.

(*S*)-preference of Novozym 435 toward cyclic **1** was not clear. However, when CALB was employed for the ring-opening reaction of racemic cyclic esters such as δ- and ε-lactone derivatives, it has been reported to show (*S*)-preference of alcohol moiety although its enantioselectivity was low.[9] Novozym 435 is expected to show the same enantioselectivity toward cyclic **1**, which has a structure similar to that of lactones.

The enzymatic alcoholyses of *rac*-**1** with **4d** by Novozym 435 were conducted in various conditions (Table 1).[10] Although in hexane/THF solvent both *R*-**3d** and *SS*-**2d** were predominantly produced by alcoholysis, in THF itself only *SS*-**2d** was formed as a major product with poor enantioselectivities (entries 1 and 2). When acetonitrile or toluene was used as a solvent, *SS*-**2d** was mainly obtained (entries 3 and 4). In ethers such as *i*-Pr_2O, Et_2O, and *t*-BuOMe except for THF, the two successive alcoholyses of *rac*-**1** produced *R*-**3d** in good yields (entries 5–7). Interestingly, CH_2Cl_2 afforded *RR*-**1** and *SS*-**2d** with high ee without the formation of *R*-**3d**, which could be applied to kinetic resolution of *rac*-**1** to produce *RR*-**1** and *SS*-**2d** (entry 8). Novozym 435 is known to be very heat-tolerant and can be used even at 70–80 °C.[4a] Novozym 435-catalyzed alcoholyses were also examined at various temperatures in hexane/THF. The higher the reaction temperature was, the faster the reaction was without deterioration of enantioselectivities (entries 9–12). The alcoholyzed products, *SS*-**2d** and *R*-**3d**, were obtained in high yields with high ee within 5 h at 60 °C.

Lipase-catalyzed alcoholyses of *rac*-**1** with various alcohols were also conducted at 60 °C in hexane/THF to afford *SS*-**2** and *R*-**3** with excellent yields (>45%) and enantioselectivities (>97% ee) as shown in Table 2.[11] Compared to Table 1, the times required to reach 50% conversion were prolonged because the ratio of Novozym 435 to *rac*-**1** was reduced for large-scaled reactions from 2 mg/0.3 mmol to 40 mg/20 mmol. *n*-Butanol (**4d**) showed the highest reactivity among primary alcohols used (entries 1, 2, and 4). The reaction of **4d** was completed within 20 h, giving a *SS*-**2d** and a *R*-**3d** in 48% and 48% of isolated yields, respectively. Isopropyl alcohol (**4c**) exhibited reactivity comparable to **4d** (entry 3). Some secondary alcohols such as isopropyl alcohol were reported to be efficient substrates for CALB.[4a] But cyclohexanol (**4e**) had low reactivity to achieve a 50% conversion in 260 h due to the bulkiness of **4e** (entry 5). When *tert*-butanol (**4f**) or phenol (**4g**) was used, *rac*-**1** remained even after 260 h without production of *SS*-**2** or *R*-**3**.

When Novozym 435 was recycled for the alcoholysis of *rac*-**1** with **4d**, its activity was decreased slowly while its enantioselectivity was maintained (Table 3). The time required to reach a 50% conversion increased by an hour per cycle. As a result, in the fourth cycle the 50% conversion was attained in 9 h.

Typical procedure for the syntheses of the alkyl (*S*,*S*)-*O*-lactyllactates (*SS*-**2a**–**e**) and alkyl (*R*)-lactates (*R*-**3a**–**e**) from the *rac*-lactide (*rac*-**1**) is as follows: To a solution of *rac*-lactide[12] (*rac*-**1**, 20 mmol) and alcohol (**4**, 60 mmol) in hexane/THF (10 mL, 90:10) was added Novozym 435 (40 mg). The solution was shaken at 200 rpm at 60 °C. The progress of the reaction was monitored by GC analyses on the chiral column (Cyclosil-B®, Agilent). After completion of alcoholysis, Novozym 435 was removed by filtration and the solvent and the

Table 1. Optimization of alcoholysis of *rac*-lactide (**1**) with *n*-butanol (**4d**) by Novozym 435[a]

Entry	Solvent	Temp (°C)	Time (h)	GC ratio (%)[b]		
				RR-**1**/*SS*-**1**	*RR*-**2**/*SS*-**2**	*R*-**3**/*S*-**3**
1	Hexane/THF[c]	30	8	3/0	9/>49	37/0
2	THF	30	8	35/12	10/37	4/0
3	Toluene	30	8	37/15	12/34	1/0
4	Acetonitrile	30	8	35/14	6/35	8/0
5	*i*-Pr_2O	30	8	5/0	8/>49	36/0
6	*t*-BuOMe	30	8	15/1	6/48	28/0
7	Et_2O	30	8	0/0	17/>49	32/0
8	CH_2Cl_2	30	8	41/2	9/47	0/0
9	Hexane/THF[c]	30	14[d]	0/0	0/>49	>49/0
10	Hexane/THF[c]	40	9[d]	0/0	0/>49	>49/0
11	Hexane/THF[c]	50	7[d]	0/0	0/>49	>49/0
12	Hexane/THF[c]	60	5[d]	0/0	0/>49	>49/0

[a] Reaction conditions: *rac*-**1** (0.3 mmol), **4d** (0.9 mmol), and Novozym 435 (2 mg) in solvent (3 mL) were shaken for 8 h.
[b] Determined by GC (Cyclosil-B®).
[c] Hexane:THF = 90:10.
[d] Monitored by GC till 50% conversions.

Table 2. Alcoholysis of *rac*-lactide (**1**) with various alcohols by Novozym 435[a]

Entry	Alcohol	Time[c] (h)	*SS*-**2**[b]		*R*-**3**[b]	
			GC Yield (%)	% ee	GC Yield (%)	% ee
1	MeOH (**4a**)	160	49	98	49	>99
2	EtOH (**4b**)	66	48	97	49	>99
3	*i*-PrOH (**4c**)	24	49	97	47	>99
4	*n*-BuOH (**4d**)	20	48 (48)[d]	>99	49 (48)[d]	>99
5	CyOH (**4e**)	260	45	99	46	>99
6	*t*-BuOH (**4f**)	>260		No rxn		
7	PhOH (**4g**)	>260		No rxn		

[a] Reaction conditions: *rac*-**1** (20 mmol), **4** (60 mmol), and Novozym 435 (40 mg) in hexane/THF (90:10, 10 mL) were shaken at 60 °C.
[b] Determined by GC (Cyclosil-B®).
[c] Monitored by GC till 50% conversions.
[d] Isolated yields.

Table 3. Recycling of Novozym 435 for the alcoholysis of *rac*-lactide (**1**)[a]

Recycle	Time[c] (h)	*SS*-**2d**[b]		*R*-**3d**[b]	
		GC Yield (%)	% ee	GC Yield (%)	% ee
1	5	49	>99	49	>99
2	6	49	>99	49	>99
3	7	49	>99	49	>99
4	9	49	>99	49	>99

[a] Reaction conditions: *rac*-**1** (0.3 mmol), **4d** (0.9 mmol), and Novozym 435 (2 mg) in *n*-Hexane/THF (90:10, 10 mL) were shaken.
[b] Monitored by GC till 50% conversions.
[c] Determined by GC (Cyclosil-B®).

remaining alcohol were evaporated under reduced pressure. The corresponding products, alkyl (*R*)-lactates and alkyl (*S*,*S*)-*O*-lactyllactates, were obtained by vacuum distillation. The absolute configurations of the *SS*-**2a**–**e** were identified by comparison with authentic compounds prepared by enzymatic alcoholyses of commercially available *SS*-**1**.[13]

In conclusion, we found that *C. antarctica* lipase B (Novozym 435) was not very stereospecific to lactide, but highly enantioselective to alkyl *O*-lactyllactate in hexane/THF. A new synthetic route for alkyl (*R*)-lactate and alkyl (*S*,*S*)-*O*-lactyllactates from *rac*-lactide was developed. Alcoholysis of *rac*-lactide catalyzed by Novozym 435 produced both alkyl (*R*)-lactates and alkyl (*S*,*S*)-*O*-lactyllactates at the same time in high yields (>45%) and high enantiomeric purities (>97% ee).

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7. The lipases screened are as follows: *Candida antarctica* lipase B (Novozym 435, Novozymes), *Candida rugosa* lipase (Sigma), porcine pancreatic lipase (Sigma), *Rhizomucor miehei* lipase (Lipozyme IM, Novozymes), *Candida rugosa* lipase (AY, Amano), *Pseudomonas fluorescence* (AK, Amano), *Burkholderia cepacia* (PS, Amano), *Aspergillus niger* (A, Amano), *Rhizopus oryzae* (F-AP 15, Amano), *Penicillium camembertii* (G, Amano), *Mucor javanicus* (M, Amano).
8. THF (10%) was added to hexane to increase the solubility of *rac*-**1**.
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10. The concentration effect of *rac*-**1** was not significant (data not shown).
11. A trace of trimer of alkyl lactate was observed after prolonged reaction time.
12. *rac*-Lactide was purchased from Aldrich, USA.
13. *SS*-**2a**: bp 76–78 °C/2 mmHg; $[\alpha]_D^{25}$ −35.2 (*c* 0.015, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50 (d, 3H, $J = 7.2$ Hz), 1.53 (d, 3H, $J = 6.9$ Hz) 2.79 (s, 1H), 3.76 (s, 3H), 4.36 (q, 1H, $J = 6.9$ Hz), 5.20 (q, 1H, $J = 6.9$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.9, 20.5, 52.5, 66.8, 69.3, 170.7, 175.1.
SS-**2b**: bp 78–80 °C/2 mmHg; $[\alpha]_D^{25}$ −32.4 (*c* 0.015, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, 3H, $J = 7.2$ Hz), 1.43–1.54 (m, 6H), 2.76 (br, 1H), 4.21 (q, 2H, $J = 7.2$ Hz), 4.36–4.39 (m, 1H), 5.18 (q, 1H, $J = 6.9$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1, 16.9, 20.5, 61.6, 66.8, 69.5, 170.2, 175.2; MS (*m*/*z*) 191 (MH^+).
SS-**2c**: bp 76–78 °C/5 mmHg; $[\alpha]_D^{25}$ −31.8 (*c* 0.014, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.24 (s, 3H, $J = 6.6$ Hz), 1.27 (d, 3H, $J = 6.6$ Hz), 1.46–1.69 (m, 6H),

2.76 (d, 1H, $J = 5.7$ Hz), 4.32–4.39 (m, 1H), 5.01–5.07 (m, 1H), 5.09–5.16 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.8, 20.6, 21.7, 66.8, 69.3, 69.6, 169.7, 175.2.

SS-**2d**: bp 131–138 °C/2 mmHg; $[\alpha]_D^{25}$ −32.7 (*c* 0.014, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (t, 3H, $J = 7.4$ Hz), 1.34–1.65 (m, 10H), 2.76 (d, 1H, $J = 6.0$ Hz), 4.12–4.18 (m, 2H), 4.33–4.38 (m, 1H), 5.18 (q, 1H, $J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.6, 16.9, 19.0, 20.5, 30.5, 65.4, 66.7, 69.5, 170.2, 175.2; MS (*m*/*z*) 219 (MH^+).

SS-**2e**: bp 126–130 °C/2 mmHg; $[\alpha]_D^{25}$ −29.1 (*c* 0.022, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25–1.85 (m, 16H), 2.72 (br, 1H), 4.35 (q, 1H, $J = 6.8$ Hz), 4.77–4.84 (m, 1H), 5.14 (q, 1H, $J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ16.9, 20.6, 23.6, 25.3, 31.4, 66.7, 69.7, 74.1, 169.6, 175.2; MS (*m*/*z*) 245 (MH^+).