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# Asymmetric Hydrolysis of Racemic 2-Oxazolidinone Esters with Lipases†

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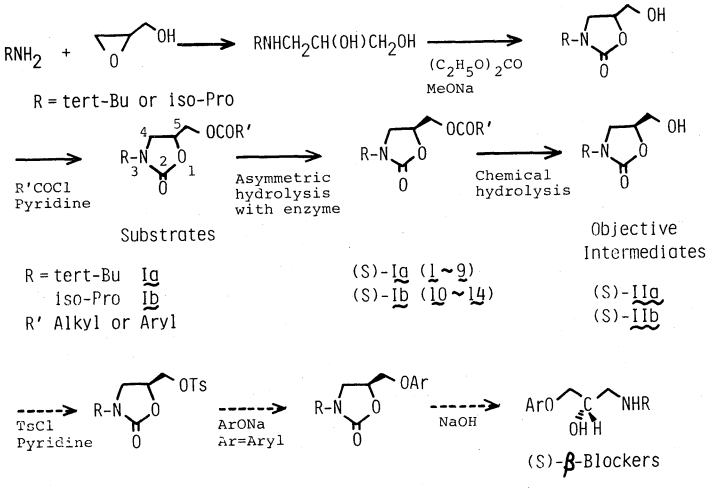
The enzymatic hydrolysis of (*R*, *S*)-5-acyloxymethyl-3-alkyl-oxazolidin-2-one **I** and the behavior of (*S*)-**I** for extraction with an organic solvent were examined so as to extend the biological resolution to racemates, and to learn about more appropriate combinations of substrates with lipases on the asymmetric hydrolysis. The combination of (*R*, *S*)-5-hexanoyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **4** with lipoprotein lipase Amano 3 (L. P. L. Amano 3, origin; *Pseudomonas aeruginosa*) and that of (*R*, *S*)-5-octanoyloxymethyl-3-isopropyl-oxazolidin-2-one **14** with L. P. L. Amano 3 efficiently gave (*S*)-5-hydroxymethyl-3-*tert*-butyl-oxazolidin-2-one (*S*)-**11a** (99% *e.e.*) and (*S*)-5-hydroxymethyl-3-isopropyl-oxazolidin-2-one (*S*)-**IIb** (99% *e.e.*), respectively. (*S*)-**IIa** and (*S*)-**IIb** could be considered to be favorable intermediates for preparing optically active β-blockers.



The authors have reported a new route to produce (*S*)-5-hydroxymethyl-3-*tert*-butyl-oxazolidin-2-one, which was the available intermediate for preparing an optically active β-blocker, by the asymmetric hydrolysis of (*R*,*S*)-5-acetoxymethyl-3-*tert*-butyl-oxazolidin-2-one with lipases or microorganisms (Scheme).[1] Lipoprotein lipase Amano 3 (L.P.L. Amano 3, origin; *Pseudomonas aeruginosa*)[2] and lipase PL 266 (origin; *Alcaligenes* species)[3] were favorable enzymes, judging from the hydrolysis rate and enantioselectivity. In this paper, so as to extend the biological resolution to various racemates of 5-acyloxymethyl-3-alkyl-oxazolidin-2-one **I** and to learn about more appropriate combinations




† Biological Resolution of Racemic 2-Oxazolidinones. Part II. For Part I, see ref. 1.

TABLE I. SUBSTRATE: 5-ACYLOXYMETHYL-3-*tert*-BUTYL-OXAZOLIDIN-2-ONE **Ia**

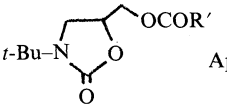
| No. of compound |  R′ | Appearance | $^1$H NMR (90 MHz, $CDCl_3$) |
|---|---|---|---|
| **1** | $CH_3$ | Syrup | 1.4 (9H, s, $(CH_3)_3C$–), 2.2 (3H, s, $CH_3CO$–), 3.35∼3.85 (2H, m, $-CH_2N$–), 4.1∼4.25 (2H, m, $-CH_2O$–), 4.45∼4.75 (1H, m, $-CH_2CH(O-)CH_2$–) |
| **2** | $(CH_3)_2CH$ | Syrup | 1.25∼1.4 (6H, d, $(CH_3)_2CH$–), 1.55 (9H, s, $(CH_3)_3C$–), 2.55∼2.9 (1H, m, $(CH_3)_2CH$–), 3.4∼3.95 (2H, m, $-CH_2N$–), 4.1∼4.4 (2H, m, $-CH_2O$–), 4.55∼4.85 (1H, m, $-CH_2CH(O-)CH_2$–) |
| **3** | $n$-$C_3H_7$ | Syrup | 0.85∼1.1 (3H, d, $CH_3$–), 1.4 (9H, s, $(CH_3)_3C$–), 1.55∼1.85 (2H, m, $CH_3CH_2$–), 2.25∼2.45 (2H, m, $CH_3CH_2CH_2$–), 3.35∼3.9 (2H, m, $CH_2N$–), 4.05∼4.4 (2H, m, $-CH_2O$–), 4.5∼4.8 (1H, m, $-CH_2CH(O-)CH_2$–) |
| **4** | $n$-$C_5H_{11}$ | Syrup | 0.8∼2.6 (20H, m, $C_5H_{11}$–, $(CH_3)_3C$–), 3.3∼3.9 (2H, m, $-CH_2N$–), 4.05∼4.4 (2H, m, $-CH_2O$–), 4.45∼4.75 (1H, m, $-CH_2CH(O-)CH_2$–) |
| **5** | $n$-$C_7H_{15}$ | Syrup | 0.7∼2.5 (24H, m, $C_7H_{15}$–, $(CH_3)_3C$–), 3.3∼3.85 (2H, m, $-CH_2N$–), 4.15∼4.3 (2H, m, $-CH_2O$–), 4.45∼4.75 (1H, m, $-CH_2CH(O-)CH_2$–) |
| **6** | $n$-$C_9H_{19}$ | Syrup | 0.75∼2.55 (28H, m, $C_9H_{19}$–, $(CH_3)_3C$–), 3.3∼3.85 (2H, m, $-CH_2N$–), 4.15∼4.3 (2H, m, $-CH_2O$–), 4.45∼4.8 (1H, m, $-CH_2CH(O-)CH_2$–) |
| **7** | $C_6H_5$ | White powder | 1.4 (9H, s, $(CH_3)_3C$–), 3.4∼3.95 (2H, m, $-CH_2N$–), 4.4∼4.6 (2H, m, $-CH_2O$–), 4.6∼4.9 (1H, m, $-CH_2CH(O-)CH_2$–), 7.25∼8.25 (5H, m, $C_6H_5$–) |
| **8** | $CH_2CH_2COOH$ | White powder | 1.4 (9H, s, $(CH_3)_3C$–), 2.65 (4H, s, $-CH_2CH_2$–), 3.25∼3.85 (2H, m, $-CH_2N$–), 4.15∼4.35 (2H, m, $-CH_2O$–), 4.4∼4.75 (1H, m, $-CH_2CH(O-)CH_2$–), 7.85 (1H, b, –COOH) |
| **9** | $o$-$C_6H_4COOH$ | White powder | 1.35 (9H, s, $(CH_3)_3C$–), 3.4∼3.9 (2H, m, $-CH_2N$–), 4.4∼4.55 (2H, m, $-CH_2O$), 4.6∼4.9 (1H, m, $-CH_2CH(O-)CH_2$–), 7.5∼8.0 (4H, m, $C_6H_4$=), 8.95 (1H, b, –COOH) |

TABLE II. SUBSTRATE: 5-ACYLOXYMETHYL-3-ISOPROPYL-OXAZOLIDIN-2-ONE **Ib**

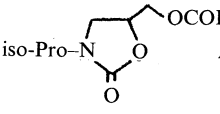
| No. of compound |  R′ | Appearance | $^1$H NMR (90 MHz, $CDCl_3$) |
|---|---|---|---|
| **10** | $CH_3$ | Syrup | 1.15∼1.3 (6H, d, $(CH_3)_2CH$–), 2.1 (3H, s, $CH_3CO$–), 3.2∼3.75 (2H, m, $-CH_2N$–), 3.95∼4.3 (3H, m, $-CH_2O$–, $(CH_3)_2CH$–), 4.6∼4.9 (1H, m, $-CH_2CH(O-)CH_2$–) |
| **11** | $(CH_3)_2CH$ | Syrup | 1.1∼1.3 (12H, m, $(CH_3)_2CHN$–, $(CH_3)_2CHCOO$–), 2.4∼2.9 (1H, m, $(CH_3)_2CHCOO$–), 3.2∼4.9 (6H, m, $-CH_2N$–, $-CH_2O$–, $(CH_3)_2CHN$–, $-CH_2CH(O-)CH_2$–) |
| **12** | $n$-$C_3H_7$ | Syrup | 0.75∼2.5 (13H, m, $C_3H_7$–, $(CH_3)_2CH$–), 3.2∼4.85 (6H, m, $-CH_2N$–, $-CH_2O$–, $(CH_3)_2CH$–, $-CH_2CH(O-)CH_2$–) |
| **13** | $n$-$C_5H_{11}$ | Syrup | 0.75∼2.5 (17H, m, $C_5H_{11}$–, $(CH_3)_2CH$–), 3.2∼3.75 (2H, m, $-CH_2N$–), 3.94∼4.45 (3H, m, $-CH_2O$–, $(CH_3)_2CH$–), 4.45∼4.85 (1H, m, $-CH_2CH(O-)CH_2O$–) |
| **14** | $n$-$C_7H_{15}$ | Syrup | 0.75∼2.5 (21H, m, $C_7H_{15}$–, $(CH_3)_2CH$–), 3.2∼3.75 (2H, m, $-CH_2N$–), 3.95∼4.4 (3H, m, $-CH_2O$–, $(CH_3)_2CH$–), 4.55∼4.85 (1H, m, $-CH_2CH(O-)CH_2$–) |

TABLE III. RELATIVE HYDROLYSIS RATE OF (*R*, *S*)-2-OXAZOLIDINONES WITH LIPASES

| No. of expt. | Enzyme | | Substrate | | Method[a] | Volume of phosphate buffer (ml) | Relative hydrolysis rate |
|---|---|---|---|---|---|---|---|
| | Name | (g) | No. of compound | (g) | | | |
| 1 | L.P.L. Amano 3 | 0.005 | **1** | 5.0 | A | 45 | 0.14 |
| 2 | | | **2** | 5.0 | A | 45 | 0.05 |
| 3 | | | **3** | 5.0 | A | 45 | 1.00 |
| 4 | | | **4** | 5.0 | A | 45 | 0.88 |
| 5 | | | **5** | 5.0 | A | 45 | 0.67 |
| 6 | | | **6** | 5.0 | A | 45 | 0.04 |
| 7 | | 1.0 | **7** | 3.0 | B | 30 | 0.003 |
| 8[b] | | | **7** | 3.0 | B′ | 30 | 0.003 |
| 9[b] | | | **7** | 3.0 | B′ | 30 | 0.003 |
| 10 | | 1.0 | **8** | 3.0 | C | 30 | 0 |
| 11 | | | **9** | 3.0 | C | 30 | 0 |
| 12 | Lipase PL 266 | 0.005 | **1** | 5.0 | A | 45 | 0.11 |
| 13 | | | **2** | 5.0 | A | 45 | 0.03 |
| 14 | | | **3** | 5.0 | A | 45 | 0.57 |
| 15 | | | **4** | 5.0 | A | 45 | 0.21 |
| 16 | | | **5** | 5.0 | A | 45 | 0.11 |
| 17 | | | **6** | 5.0 | A | 45 | 0.001 |
| 18 | | 1.0 | **7** | 3.0 | B | 30 | 0.0004 |
| 19 | L.P.L. Amano 3 | 0.005 | **10** | 5.0 | A | 45 | 0.02 |
| 20 | | | **11** | 5.0 | A | 45 | 0.01 |
| 21 | | | **12** | 5.0 | A | 45 | 0.23 |
| 22 | | | **13** | 5.0 | A | 45 | 0.47 |
| 23 | | | **14** | 5.0 | A | 45 | 0.46 |

[a] See EXPERIMENTAL for methods.
[b] **7** was dissolved in toluene (Expt. 8) or acetone (Expt. 9).



of substrates with enzymes, many substrates are synthesized. The hydrolysis rates, enantioselectivities and separabilities of the desired (*S*)-**I** by extraction with an organic solvent are then examined and discussed.

As shown in Tables I and II, fourteen racemates of **I** were synthesized as illustrated in the Scheme. At first, the hydrolysis rates of the substrates with L.P.L. Amano 3 and lipase PL 266 were examined. The hydrolysis rate was calculated from the sodium hydroxide consumed in 5 min, after starting incubation. The fastest hydrolysis rate was obtained with the combination of (*R*,*S*)-5-butyryloxymethyl-3-*tert*-butyl-oxazolidin-2-one **3** and L.P.L. Amano 3, and this was set as the standard (Expt. 3). The relative hydrolysis rates of other substrates with enzymes were calculated as shown in Table III. Among racemic 5-acyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **Ia**, the relative hydrolysis rates of (*R*,*S*)-5-hexanoyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **4** or (*R*,*S*)-5-octanoyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **5** with L.P.L. Amano 3 were 0.88 and 0.67, respectively, and were particularly high (Expts. 4 and 5). However, those of (*R*,*S*)-5-acetoxymethyl-3-*tert*-butyl-oxazolidin-2-one **1**, (*R*,*S*)-isobutyryloxymethyl-3-*tert*-butyl-oxazolidin-2-one **2**, (*R*,*S*)-5-decanoyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **6**, and (*R*,*S*)-5-benzoyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **7** were low at 0.14, 0.05, 0.04 and 0.003, respectively (Expts. 1, 2, 6 and 7). Since **7** was an insoluble solid in water, it was dissolved in an organic solvent and added to the reaction

TABLE IV. ENANTIOSELECTIVITY AND SEPARABILITY OF (*S*)-**I** ON ASYMMETRIC HYDROLYSIS WITH LIPASES

| No. of expt. | Enzyme | | Substrate[a] no. of compound | Incubation period (hr) | (*S*)-**IIa, b** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Name | (g) | | | Yield[b] (%) | Method[c] | Observed[d] $[\alpha]_D$ | Optical purity[e] (% *e.e.*) | Separability[f] |
| 24 | L.P.L. Amano 3 | 0.1 | **1** | 6 | 30 | D | +44.4° | | − |
| 25 | L.P.L. Amano 3 | 0.1 | **2** | 18 | 17 | D | +44.9° | 98 | − |
| 26 | L.P.L. Amano 3 | 0.01 | **3** | 6 | 32 | D | +45.5° | | − |
| 27 | L.P.L. Amano 3 | 0.01 | **4** | 6 | 35 | E | +44.5° | 99 | ++ |
| 28 | L.P.L. Amano 3 | 0.01 | **5** | 6 | 35 | E | +44.7° | 99 | ++ |
| 29 | Lipase PL 266 | 0.1 | **1** | 10 | 29 | D | +45.5° | | − |
| 30 | Lipase LP 266 | 0.01 | **4** | 24 | 30 | E | +44.4° | | ++ |
| 31 | L.P.L. Amano 3 | 0.01 | **11** | 27 | 25 | D | +52.0° | 98 | − |
| 32 | L.P.L. Amano 3 | 0.01 | **12** | 8 | 30 | D | +48.5° | | − |
| 33 | L.P.L. Amano 3 | 0.01 | **13** | 8 | 35 | E | +55.1° | 99 | + |
| 34 | L.P.L. Amano 3 | 0.01 | **14** | 8 | 36 | E | +53.2° | 99 | ++ |

[a] Substrate (10.0 g)/0.02 M potassium phosphate buffer (pH 7.0, 90 ml).
[b] Yield from **Ia, b**.
[c] See EXPERIMENTAL for methods.
[d] Expts. 24 ~ 30: $[\alpha]_D^{16}$, lit.,[5] $[\alpha]_D^{16}$ +47.8° ($c$ = 1.0, chloroform).
Expts. 31 ~ 34: $[\alpha]_D^{21}$, lit.,[5] $[\alpha]_D^{21}$ +57.12° ($c$ = 1.17, chloroform).
[e] Optical purity was determined from the ratio of the content of each corresponding (*S*)-MTPA diastereomer by GLC analysis.
[f] Separability was judged from the following standpoints and classified: ++, (*S*)-**I** could be efficiently separated by extraction with hexane; +, (*S*)-**I** could be separated by repeated extraction with hexane; −, (*S*)-**I** could be separated by extraction with any organic solvent.

mixture, an emulsive effect not being observed (Expts. 8 and 9). The compounds of (*R,S*)-5-succinyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **8** and (*R,S*)-5-phthaloyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **9** could be easily dissolved in water, but the hydrolysis did not progress (Expts. 10 and 11). The relative hydrolysis rates of **1**～**7** with lipase PL 266 were found to be in the range of 0.0004～0.57 and were all lower than those with L.P.L. Amano 3 (Expts. 12～18). On the other hand, the relative hydrolysis rates of racemic 5-acyloxymethyl-3-isopropyl-oxazolidin-2-one **Ib** such as (*R,S*)-5-acetoxymethyl-3-isopropyl-oxazolidin-2-one **10**, (*R, S*)-5-isobutyryloxymethyl-3-isopropyl-oxazolidin-2-one **11**, (*R, S*)-5-butyryloxymethyl-3-isopropyl-oxazolidin-2-one **12**, (*R,S*)-5-hexanoyloxymethyl-3-isopropyl-oxazolidin-2-one **13** and (*R,S*)-5-octanoyloxymethyl-3-isopropyl-oxazolidin-2-one **14** with L.P.L. Amano 3 were found to be in the range of 0.01～0.47. The relative hydrolysis rates of 3-isopropyl-substituted compounds with L.P.L. Amano 3 were all lower than those of the corresponding 3-*tert*-butyl-substituted ones with L.P.L. Amano 3 (Expts. 19～23). According to the results, it was ascertained that the hydrolysis rates were governed by the groups substituted at the 3 and/or 5 position of **I**. Secondly, the enantioselectivities on the enzymatic hydrolysis of the nine substrates with the lipases, and the separabilities of the desired compounds (*S*)-**I** by extraction with an organic solvent were examined. The enantioselectivity was evaluated by the following method: An obtained compound of (*S*)-**IIa** or (*S*)-**IIb** was converted to the (*S*)-α-methoxy-α-trifluoromethylphenylacetic (MTPA) diastereomer according to the reported method[4] and the optical purity was determined from the ratio of the content of each corresponding diastereomer by GLC analysis.

As shown in Table IV, the variable specific optical rotation values were observed in (*S*)-**IIa**, $[\alpha]_D^{16}$ +44.4～+45.5° (*c*=1.0, chloroform), and in (*S*)-**IIb**, $[\alpha]_D^{21}$ +48.5～+55.1° (*c*=1.0, chloroform), and the specific rotation values were estimated slightly low in comparison with the reported values (lit.,[5] (*S*)-**IIa**: $[\alpha]_D^{16}$ +47.8° (*c*=1.0, chloroform), (*S*)-**IIb**: $[\alpha]_D^{21}$ +57.12° (*c*=1.17, chloroform)). However, the optical purities estimated as (*S*)-MTPA diastereomers on GLC analysis were all found to be 98～99% *e.e.* We can conclude from the above results that the groups substituted at the 3 and/or 5 position of **I** are not particularly effective for the enantioselectivity on the hydrolysis of **I** with L.P.L. Amano 3 or lipase PL 266. Next, the separability was improved in proportion to the length of the carbon chain of the ester part as shown in Table IV. Namely (*S*)-**4**, (*S*)-**5** and (*S*)-**14** could be efficiently separated by extraction with hexane (Expts. 27, 28, 30 and 34). (*S*)-**13** was impractically less soluble in hexane and could be separated by repeating 10 times' its extraction with hexane (Expt. 33). Other organic solvents, *e.g.*, dichloromethane, toluene or ethyl acetate were examined to improve the extraction procedure, but no suitable solvent for separating (*S*)-**13** from (*R*)-**IIb** was found. (*S*)-**1**, (*S*)-**2**, (*S*)-**3**, (*S*)-**11** and (*S*)-**12** could not be separated without silica gel column chromatography (Expts. 24～26, 29 and 31～32).

In conclusion, judging from the hydrolysis rate, the enantioselectivity and the separability among racemic 5-acyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **Ia**, the combination of (*R,S*)-5-hexanoyloxymethyl-3-*tert*-butyl-oxazolidin-2-one **4** with L.P.L. Amano 3, was the best and (*S*)-5-hydroxymethyl-3-*tert*-butyl-oxazolidin-2-one (*S*)-**IIa** was obtained in high enantiomeric excess (99% *e.e.*). On the other hand, among racemic 5-acyloxymethyl-3-isopropyl-oxazolidin-2-one **Ib**, the combination of (*R, S*)-5-octanoyloxymethyl-3-isopropyl-oxazolidin-2-one **14** with L.P.L. Amano 3 was the best and (*S*)-5-hydroxymethyl-3-isopropyl-oxazolidin-2-one (*S*)-**IIb** was obtained in high enantiomeric excess (99% *e.e.*). Optically active β-blockers will be prepared from **4** and **14** with L.P.L. Amano 3, respectively, and the results will be reported in our following paper.

## EXPERIMENTAL

Optical rotations were measured with a PM-101 automatic digital polarimeter (Union Giken Co.). Gas chromatographic analyses were performed on a Hitachi 163 with a column (Silicone OV-17, 100 cm × 0.3 cm, 50 ml/min flow rate of $N_2$) at 220~240°C for the compounds **1**~**14**, and with a column (10% Silicone DC QF-1 on Chromosorb AW DMCS, 60~80 mesh, 500 cm × 0.3 cm, 30 ml/min flow rate of $N_2$) at 225°C to determine the optical purity of **IIa** and **IIb** by analyzing the corresponding (*S*)-MTPA diastereomer mixtures. Melting points were determined in open capillary tubes with a Yamato melting point apparatus, Model 21, and are uncorrected. A Varian EM-390 spectrometer was used to obtain the $^1H$ NMR spectra (90 MHz). Chemical shifts are reported relative to tetramethylsilane as an internal standard. Mass spectra were taken with a Hewlett Packard 5985B System. Thin layer chromatography was performed on Merck silica gel $60F_{254}$ plates developed with a mixed solvent (hexane–acetone = 1 : 1, v/v) and spots were detected with iodine vapor or UV light (254 nm). Silica gel column chromatography was performed on Wako gel C-100 (Wako Pure Chemical Ind. Co., Ltd.) using a mixed solvent (hexane–acetone = 5 : 1, v/v) as the eluent.

Commercial enzymes purchased were as follows: lipoprotein lipase Amano 3 from *Pseudomonas aeruginosa* (L.P.L. Amano 3; Amano Pharmaceutical Co.) and lipase PL 266 from *Alcaligenes* species (Meito Sangyo Co., Ltd.).

Determination of the optical purity of **IIa** or **IIb** was as follows:

A mixture of (*S*)-**IIa** (24 mg, 0.14 mmol), (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (58 mg, 0.23 mmol) and pyridine (18 mg, 0.23 mmol) in dichloromethane (2 ml) was stirred for 1 hr at room temperature. The resulting mixture was injected to a GLC column and the optical purity of (*S*)-**IIa** was determined from the ratio of the content of each corresponding diastereomer. The retention times of the corresponding diastereomers were as follows: (*S*)-**IIa**-(*S*)-MTPA, 104.4 min and (*R*)-**IIa**-(*S*)-MTPA, 96.6 min. The optical purity of (*S*)-**IIb** was also determined in the same manner as in the case of (*S*)-**IIa**. The retention times of the corresponding diastereomers were as follows: (*S*)-**IIb**-(*S*)-MTPA, 112.2 min and (*R*)-**IIb**-(*S*)-MTPA, 102.8 min.

*Syntheses of substrates*

(1) *Syntheses of* (*R,S*)*-5-acyloxymethyl-3-tert-butyl-oxazolidin-2-ones* **Ia**. The various esters of **Ia** (*ca.* 50 g each) were prepared from (*R,S*)-3-*tert*-butylamino-1,2-propanediol according to the reported method.[1] The analytical data of (*R,S*)-3-*tert*-butylamino-1,2-propanediol were as follows: White powder, mp 69~70°C (lit.,[6] mp 81~83°C), $^1H$ NMR ($CDCl_3$) $\delta$: 1.1 (9H, s, $(CH_3)_3C$–), 2.5~2.8 (2H, m, $-CH_2N$–), 2.95~3.4 (3H, b, $-CH_2CH(OH)CH_2OH$, NH), 3.45~3.85 (3H, m, $-NCH_2CH(OH)CH_2$–). MS: *m/z* 147.1 ($M^+$). The NMR data of substrates **Ia** are shown in Table I.

(2) *Syntheses of* (*R, S*)*-5-acyloxymethyl-3-isopropyl-oxazolidin-2-ones* **Ib**. The various esters of **Ib** (*ca.* 50 g each) were also prepared from (*R, S*)-3-isopropylamino-1,2-propanediol according to the reported method.[1] The analytical data of (*R, S*)-3-isopropyl-1,2-propanediol were as follows: Viscous syrup, bp 106~110°C (3 mmHg), $^1H$ NMR ($CDCl_3$) $\delta$: 1.1~1.25 (6H, d, $(CH_3)_2CH$–), 2.45~3.05 (3H, m, $-CH_2N$–. $(CH_3)_2CH$–). 3.45~4.05 (6H, m, $-NHCH_2CH(OH)CH_2OH$). MS: *m/z* 133.1 ($M^+$). The NMR data of substrates **Ib** are shown in Table II.

*Relative hydrolysis rate of racemic 5-acyloxymethyl-3-alkyl-oxazolidin-2-one* **I** *with lipases.*

*Expt. 1* (Method A). The hydrolysis was initiated by adding L.P.L. Amano 3 (5.0 mg) to a solution of the substrate **1** (5.0 g, 23.2 mmol), in 0.02 M potassium phosphate buffer (pH 7.0, 45 ml) with stirring at 30°C, and the pH kept at 7.0 by controlled addition of 1 N sodium hydroxide solution. The hydrolysis rate of **1** with L.P.L. Amano 3 was calculated from the sodium hydroxide consumed in 5 min after incubation had begun.

*Expt. 7* (Method B). The mixture of **7** (3.0 g, 10.8 mmol) and 0.02 M potassium phosphate buffer (pH 7.0, 30 ml) was vigorously stirred for 30 min at 30°C in order to make the suspension homogeneous, **7** being an insoluble solid in water. L.P.L. Amano 3 (1.0 g) was then added to the suspension to initiate the hydrolysis and the pH kept at 7.0 by controlled addition of 1 N sodium hydroxide solution.

*Expt. 8* (Method B′). A solution of the substrate **7** (3.0 g, 10.8 mmol) in toluene (3 ml) was added to the 0.02 M potassium phosphate buffer (pH 7.0. 30 ml) to examine the effect of emulsification of the reaction mixture and to learn the difference from Expt. 7. L.P.L. Amano 3 (1.0 g) was then added to the emulsive mixture and the pH kept at 7.0 by controlled addition of 1 N sodium hydroxide solution.

*Expt. 10* (Method C). The mixture of **8** (3.0 g, 11.0 mmol) and 0.02 M potassium phosphate buffer (pH 7.0, 30 ml) was vigorously stirred for 30 min at 30°C and the pH adjusted to 7.0 by 1 N sodium hydroxide solution. L. P. L. Amano 3 (1.0 g) was then added to the solution, but hydrolysis did not proceed at all.

*Enantioselectivity on the hydrolysis of racemic 5-acyloxymethyl-3-alkyl-oxazolidin-2-one* **I** *with the lipases and the separability of* (*S*)-**I** *by extraction with an organic solvent*

*Expt. 25* (Method D). L.P.L. Amano 3 (100 mg) and the substrate **2** (10.0 g, 41.2 mmol) were incubated in 0.02 M potassium phosphate buffer (pH 7.0, 90 ml) with stirring at 30°C and the pH kept at 7.0 by controlled addition of 5 N sodium hydroxide solution. The hydrolysis was monitored by the addition of 5 N sodium hydroxide solution and reached approximately 50% in 8 hr, the

incubation being continued for more than 10 hr. The reaction mixture was treated with ethyl acetate (200 ml × 3), the separated organic layer being dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residual concentrate containing major (*S*)-**2** and minor (*R*)-**IIa** (approx. 96:4 molar ratio, calculated from the area of the GLC peaks) was chromatographed on a column of silica gel (L/D = 50 cm × 2.4 cm), and the fractions containing the desired (*S*)-**2** were gathered. Removal of the solvent under reduced pressure afforded (*S*)-**2** (2.9 g, 11.9 mmol. yield 29%), $[\alpha]_D^{16}$ +32.1° (*c* = 1.0, chloroform). A mixture of the obtained (*S*)-**2** and sodium hydroxide (1.2 g, 30 mmol) in water (50 ml) was stirred for 4 hr at room temperature. After completing the chemical hydrolysis, the products were extracted with ethyl acetate (100 ml × 3). The organic layer was dried over anhydrous sodium sulfate and evaporated. Hexane was slowly added to the concentrate, and the solution was left to allow the amount of colorless crystals precipitated to increase. Recrystallization from ethyl acetate–hexane (5 ml–5 ml) gave colorless leaflets of pure (*S*)-**IIa** (1.2 g, 6.9 mmol, yield 17%), mp 82.5~83.0°C, $[\alpha]_D^{16}$ +44.9° (*c* = 1.0, chloroform) (lit.,[5] mp 83~84°C, $[\alpha]_D^{16}$ +47.8° (*c* = 1.0, chloroform); lit.,[7] mp 82~83°C, $[\alpha]_D^{20}$ +33.7° (*c* = 1.0, methanol)). $^1$H NMR ($CDCl_3$) $\delta$: 1.4 (9H, s, $(CH_3)_3C$–), 3.4~3.95 (5H, m, $-CH_2N-$, $-CH_2O-$, OH), 4.3~4.6 (1H, m, $-CH_2CH(O-)CH_2-$). MS: *m/z* 173.2 ($M^+$). 98% *e.e.*

*Expt. 27* (Method E). L.P.L. Amano 3 (10 mg) and the substrate **4** (10.0 g, 36.9 mmol) were incubated in 0.02 M potassium phosphate buffer (pH 7.0, 90 ml) with stirring at 30°C and the pH kept at 7.0 by controlled addition of 5 N sodium hydroxide solution. The hydrolysis was monitored by the addition of 5 N sodium hydroxide solution and by GLC analysis. After completing the enzymatic hydrolysis in 6 hr, the resulting mixture was treated with hexane (200 ml × 3). The separated organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residual concentrate (5.2 g) containing major (*S*)-**4** and minor (*R*)-**IIa** (approx. 99.5:0.5 molar ratio, calculated from the area of GLC peaks) was reacted with sodim hydroxide (1.2 g, 30 mmol) in water (50 ml) for 4 hr at room temperature. The reaction mixture was then worked up in the same manner as described in Expt. 25 to give colorless leaflets of pure (*S*)-**IIa** (2.2 g, 12.9 mmol, yield 35%), $[\alpha]_D^{16}$ +44.5° (*c* = 1.0, chloroform), 99% *e.e.*

*Expt. 34.* Colorless needles of pure (*S*)-**IIb** (2.1 g, 13.2 mmol, yield 36%) were prepared according to the Method E, and the analytical data were as follows: mp 56.5~57.5°C, $[\alpha]_D^{21}$ +53.2° (*c* = 1.0, chloroform) (lit.,[5] mp 55~56°C, $[\alpha]_D^{21}$ +57.12° (*c* = 1.17, chloroform); lit.,[7] mp 56~59°C, $[\alpha]_D^{20}$ +49.1° (*c* = 1.0, methanol)). $^1$H NMR ($CDCl_3$) $\delta$: 1.1~1.3 (6H, d, $(C\underline{H}_3)_2CH-$), 3.3~4.25 (6H, m, $-CH_2N-$, $-CH_2O-$, OH, $(CH_3)_2C\underline{H}-$), 4.3~4.75 (1H, m, $-CH_2C\underline{H}(O-)CH_2-$). MS: *m/z* 159.1 ($M^+$). 99% *e.e.*

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