Kinetic Resolution of Optically Pure 3-Alkanol by *Pseudomonas cepacia* Lipase-Catalyzed Transesterification with Vinyl Acetate in Organic Solvent

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Abstract: Kinetic resolution of 3-alkanol by *Pseudomonas cepacia* lipase-catalyzed transesterification has been studied using vinyl acetate as an acetylating reagent, five alcohols and different organic solvents. The optical resolution data did not fit the previously reported theory: the enantioselectivity (*E*: the enantiomeric ratio for the homocompetitive reaction) showed the maxima against the extent of conversion (*c*) in all systems studied. The optical resolution of (*S*)-3-alkanol with above 95% of enantiomeric excess (*ee*) was attained by controlling *c*, but only a moderate *ee* was observed in the resolution of (*R*) isomer from the racemate, owing to low or moderate enantioselectivity. The resolution of enantiomers with high optical purity was carried out using enantiomerically enriched alcohol: the *ee* values of the resolved alcohols showed an excellent agreement with those determined by the previously reported equations using the optical resolution data. Based on these observations, optically pure (*R*)- and (*S*)-3-alkanols (*ee* ≥ 99%) were resolved from the racemates in a preparative scale by repeating the transesterification.

Key words: *Pseudomonas cepacia* lipase (PCL), transesterification, vinyl acetate, kinetic resolution, optically pure 3-alkanol

1 Introduction

Vinyl acetate (VA) is a powerful acetylating reagent for the resolution of enantiomers from chiral compounds by lipase (triacylglycerol hydrolase, EC 3.1.1.3) catalyzed transesterification in organic solvent (1-3): VA irreversibly reacts with the active serine residue in lipase to give the acetyl-enzyme intermediate [Enz-Ac] because vinyl alcohol produced is immediately convert-

ed to acetaldehyde by keto-enol tautomerization and this irreversibility serves to acceleration in the reaction rate (1-4). Such a reaction is called "irreversible transesterification" and used for kinetic resolution of various secondary alcohols (3, 5-8).

Some information is available on the quantitative analyses of optical resolution data in lipase catalyzed transesterification between VA and chiral compounds with hydroxy group at C-2 position (2-alkanol and its

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analogue). It has been reported that the reaction is reversible based on the fact that the enantioselectivity [E : the enantiomeric ratio for the homocompetitive reaction, *i.e.*, as the reaction is irreversible] at low conversion is greater than that at high conversion ($>50\%$) in *Pseudomonas fluorescens* lipase (PFL)-catalyzed transesterification with racemic β -methyl-(2-thiophene)propanol in chloroform (9). Furthermore it has been reported that the optical resolution data did not fit the previously resolution format (5, 10-12) based on the observation: the E value increases with c in the range of below 0.5 in *Pseudomonas cepacia* lipase (PCL)-catalyzed transesterification with racemic sulcatol, ($\pm$)-6-methyl-5-hepten-2-ol, in chlorinated solvents (13). In the previous papers on PCL-catalyzed transesterification with 2-alkanol, we reported that the E value showed the maxima at around 0.5 of c in all systems studied (14) and that the reaction of enantiomerically enriched substrate was a powerful strategy for kinetic resolution of chiral compounds with high optical purity (15). So far, no information is available about quantitative analyses of the optical resolution data in kinetic resolution of chiral compounds with OH group at C-3.

Thus, we selected PCL catalyzed transesterification between VA and 3-alkanol (**1**) in organic solvent (Scheme 1) and investigated the quantitative analyses of optical resolution data and the resolution of optically pure (R)- and (S)-(**1**) ($ee \geq 99\%$). In this paper, individual 3-alkanol was abbreviated to X3-OH, where X is carbon number of the alcohol and 3- shows the position of hydroxy group, and its acetate was also abbreviated to X3-OAc.

Enz : *Pseudomonas cepacia* Lipase (PCL)

3-Alkanol (Abbreviation)

R = C₄H₉ (73-OH) C₅H₁₁ (83-OH) C₆H₁₃ (93-OH)
C₇H₁₅ (103-OH) C₈H₁₇ (113-OH)

Scheme 1

2 Experimental

2.1 Materials

Vinyl acetate (VA) (GR, Wako Pure Chemical, Osaka) was dried over molecular sieves (3A 1/16, Wako Pure Chemical, Osaka). 3-Alkanol (**1**) [3-heptanol (73-OH), 3-octanol (83-OH), 3-nonanol (93-OH), 3-decanol (103-OH) and 3-undecanol (113-OH)] and (R)-1-(1-naphthyl)ethyl isocyanate (optical purity $ee > 99\%$) were purchased from Tokyo Chemical Industry, Tokyo and used without purification. Silica gel (Silica gel 60, 70-230 mesh, Katayama Chemicals, Osaka) was used without treatment or after heating at 200°C for 2 h when the activity was not sufficient for separation of the product (**2**) and the unreacted substrate (**1**). Organic solvents were purified by the ordinary method (16). The reaction solvents were dried over molecular sieves before use. *Pseudomonas cepacia* lipase (PCL) [Lipase PS, 43 U/mg for hydrolysis and Lipase PS-D (adsorbed on celite), 0.8 U/mg for the transesterification between VA and 1-phenylethanol] was obtained from Amano Enzyme Inc., Nagoya.

2.2 Transesterification

To a stirred solution (33 mL) containing VA (1.63 M) (1 M=1 mol dm⁻³) and racemic 3-alkanol (RS)-(**1**) (0.42-0.63 M) at 30°C was added PCL (Lipase PS, 100 mg/mL or Lipase PS-D, 50 mg/mL) and the stirring was continued. The reaction was followed by GC in an appropriate interval and the reaction mixture was filtered at the desired conversion. The enzyme was washed with the solvent (20 mL) twice. In the case of dioxane, the combined filtrates were poured into water (300 mL) and the compounds were extracted with hexane (100 mL) three times. The combined extracts were washed with water (100 mL) three times, dried over anhydrous sodium sulfate and evaporated. The residue, which contained the product and the unreacted alcohol, was dissolved with hexane (30 mL) and loaded on the silica gel column (Silica gel 100 g, ϕ 30×600 mm). The compounds were eluted with ethyl acetate-hexane (5:95 vol/vol, 600 mL) and ethanol-hexane (20:80 vol/vol, 400 mL). In the cases of other solvents, the filtrates were combined and evaporated. The resulting residue was treated by the same manner as described above.

The fractions (400-550 mL) containing the ester were combined and evaporated, giving (R)-1-ethylalkyl

acetate [(*R*)-(2)]. Yield : 80-95%. An alkaline solution (1 M KOH dissolved in ethanol containing 10 vol% of water) (10 mL/g-ester) was added to the obtained ester and the mixture was heated at 56°C for 40-60 min and cooled to room temperature or left at room temperature for 12-16 h. A 100 mL of saturated sodium chloride solution (sat. NaCl) was added to the reaction mixture and the compounds were extracted with hexane (30 mL) three times. The combined extracts were washed with sat. NaCl (30 mL) three times, dried over anhydrous sodium sulfate and evaporated, giving (*R*)-3-alkanol [(*R*)-(1)]. Yield: 70-90% [based on (*R*)-(2)].

The fractions (700-1000 mL) were combined and concentrated until crystals of silica gel appeared. A 100 mL of sat. NaCl and 5 mL of 1M KOH were successively added to the residue and the compounds were taken up by a similar procedure to that for purification of the ester fractions, giving (*S*)-3-alkanol [(*S*)-(1)]. Yield: 60-85%.

The resolution from enantiomerically enriched 3-alkanols, (*R*)-enriched-(1) (initial optical purity: $ee_0=0.52-0.91$) and (*S*)-enriched-(1) ($ee_0=0.53-0.85$), was carried out in a similar manner as described above. Yields were 85-96, 75-99 and 65-93% for the ester, its alcohol (based on the ester) and the unreacted alcohol for (*R*)-enriched-(1), and 70-92, 55-90 and 68-99% for (*S*)-enriched-(1), respectively.

2·3 Resolution of Optically Pure 3-Alkanol in a Preparative Scale

2·3·1 Optically pure (*R*)-(1)

1st Reaction — To a stirred mixture of racemic 3-octanol [(*RS*)-83-OH, 0.62 mol], VA (1.08 mol) and 1,2-dichloroethane (500 mL) at 30°C was added PCL (Lipase PS-D, 35 g) and the stirring was continued. After 2 h (49% of conversion), the reaction mixture was filtered and the enzyme was washed with 1,2-dichloroethane (200 mL) three times. The filtrates were combined and evaporated. The residue was dissolved in hexane (500 mL), left at room temperature for 1-3 h, filtered and evaporated. The hexane treatment was carried out twice for removing the crystalline impurities. The refined residue (100g) was divided into 5.5-5.6 g portions. Each portion was dissolved with hexane (30 mL) and loaded on a silicagel column (Silica gel 100g, ϕ 30×600 mm) and purified by a similar method as described above (2·2). As a result, we obtained enantiomerically enriched (*R*)-83-OH ($ee=80\%$, yield: 84%)

and (*S*)-83-OH ($ee=76\%$, yield: 82%), which were used as the starting materials in the following reactions.

2nd Reaction — A mixture of (*R*)-83-OH ($ee=80\%$, 0.25 mol), VA (0.43 mol), 1,2-dichloroethane (200 mL) and PCL (Lipase PS-D, 14 g) was stirred at 30°C for 2 h (83% of conversion). The reaction mixture was treated by a similar method as described above (the 1st reaction and 2·2), giving highly enantiomerically enriched (*R*)-83-OH ($ee=97\%$) in a yield of 82%.

3rd Reaction — A mixture of (*R*)-83-OH ($ee=97\%$, 0.18 mol), VA (0.32 mol), 1,2-dichloroethane (150mL) and PCL (Lipase PS-D, 10.5 g) was stirred at 30°C for 100 min (79% of conversion). The reaction mixture was treated by a similar method as described above. Finally we obtained optically pure (*R*)-3-octanol with above 99% of ee in a yield of 80%

Similarly optically pure (*R*)-(1) was resolved from (*RS*)-(1) in a preparative scale by repeating the transesterification three times. The results were summarized in **Table 1**. PCL(Lipase PS) and PCL(Lipase PS-D) were used for the reactions in acetonitrile and 1,2-dichloroethane, respectively.

2·3·2 Optically pure (*S*)-(1)

1st Reaction was identical to the 1st reaction for (*R*)-83-OH (2·3·1).

2nd Reaction — A mixture of (*S*)-83-OH ($ee=76\%$, 0.25 mol), VA (0.43 mol), 1,2-dichloroethane (200 mL) and PCL (Lipase PS-D, 14 g) was stirred at 30°C for 3 h (30% of conversion). The reaction mixture was treated by a similar method as described above. The obtained unreacted substrate was colored in yellow or brown and contaminated with a small amount of impurities which was determined by GC. We therefore purified the unreacted substrate as follows: a mixture of the obtained alcohol (0.20 mol), pyridine (130 mL) and acetic anhydride (65 mL) was left at room temperature. After 16 h, the reaction mixture was poured into a stirring sat. NaCl (1200 mL) cooled in an ice bath. The resulting solution was acidified with 150 mL of conc. hydrochloric acid and stirred at 0°C for 1 h. The compounds were extracted with hexane (500 mL) five times. The combined filtrates were washed with sat. NaCl (500 mL) five times, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed and hydrolyzed with 1M KOH soln. by a similar method as described above, giving colorless optically pure (*S*)-83-OH with above 99% of ee in a yield of 85%

Table 1 Kinetic Resolution of Optically Pure (*R*)-(1) from (*RS*)-(1) in a Preparative Scale by PCL-Catalyzed Transesterification with VA in Organic Solvent at 30°C.

PCL ^{a)}	Solv. ^{b)}	(<i>R</i>)-(1)	1st Reaction ^{c)}			2nd Reaction			3rd Reaction		
			<i>c</i> / %	Y / %	<i>ee</i> _P / %	<i>c</i> / %	Y / %	<i>ee</i> _P / %	<i>c</i> / %	Y / %	<i>ee</i> _P / %
PS	MCN	73-OH	52	63	78	75	60	97	85	58	>99
		83-OH	54	81	78	77	78	97	88	81	>99
		93-OH	54	87	73	80	81	95	85	84	>99
		103-OH	53	92	73	75	89	95	80	85	>99
		113-OH	55	94	73	75	92	95	82	88	>99
PS-D	DCE	73-OH	46	65	75	67	66	97	87	64	>99
		83-OH	49	84	80	83	82	97	79	80	>99
		93-OH	49	92	74	65	83	97	86	83	>99
		103-OH	50	97	78	69	90	98	85	86	>99
		113-OH	52	99	71	65	94	97	84	92	>99

a) PS: Lipase PS, PS-D: Lipase PS-D. b) MCN: acetonitrile, DCE: 1,2-dichloroethane.

c) VA=1.08 mol, 73-OH=0.69 mol, 83-OH=0.62mol, 93-OH=0.56 mol, 103-OH=0.51 mol, 113-OH=0.47 mol

Lipase PS=70.0g, Lipase PS-D=35g. Similar reaction conditions were used in the 2nd and 3rd reactions.

Y : Yield of (*R*)-(1).

Similarly optically pure (*S*)-(1) was resolved from (*RS*)-(1) in a preparative scale. The results were summarized in **Table 2**. PCL(Lipase PS) and PCL(Lipase PS-D) were also used for the reactions in acetonitrile and 1,2-dichloroethane, respectively.

2.4 Analytical Methods

GC analysis was done with a Shimadzu 14B

Gaschromatograph equipped with a FID detector. Column: G-100 column, ϕ 1.2 mm $\times$ 20 m, film thickness 1 μ m (purchased from GL Sciences, Tokyo); Injection and detector temperature : 250°C; Carrier (N₂) pressure : 40 kPa at 80°C. Column temperature: 70 $\rightarrow$ 120°C, 80 $\rightarrow$ 135°C, 95 $\rightarrow$ 150°C, 110 $\rightarrow$ 165°C, and 125 $\rightarrow$ 180°C programmed with an increase of 10°C/min for the reaction mixtures of 73-OH, 83-OH, 93-OH, 103-

Table 2 Kinetic Resolution of Optically Pure (*S*)-(1) from (*RS*)-(1) in a Preparative Scale by PCL-Catalyzed Transesterification with VA in Organic Solvent at 30°C.

PCL ^{a)}	Solv. ^{b)}	(<i>S</i>)-(1)	1st Reaction ^{c)}			2nd Reaction		
			<i>c</i> / %	Y / %	<i>ee</i> _S / %	<i>c</i> / %	Y / %	<i>ee</i> _S / %
PS	MCN	73-OH	52	58	84	25	65	>99
		83-OH	54	73	91	20	80	>99
		93-OH	54	78	86	31	78	>99
		103-OH	53	83	84	33	79	>99
		113-OH	55	88	89	21	83	>99
PS-D	DCE	73-OH	46	64	64	43	68	>99
		83-OH	49	82	76	30	85	>99
		93-OH	49	89	72	35	91	>99
		103-OH	50	90	77	31	90	>99
		113-OH	52	99	76	35	93	>99

a) PS: Lipase PS, PS-D: Lipase PS-D. b) MCN: acetonitrile, DCE: 1,2-dichloroethane.

c) The reaction conditions were described in **Table 1**. Y : Yield of (*S*)-(1).

OH and 113-OH, respectively.

HPLC analysis was carried out with a JASCO (Japan Spectroscopic, Co., Tokyo) Intelligent HPLC equipped with a wavelength variable UV detector. Column: combined two columns of a Finepak SIL (JASCO, particle size: 5 μm , ϕ 4.6 $\times$ 250 mm) and an Inertsil SIL (GL Sciences, particle size: 5 μm , ϕ 4.6 $\times$ 250 mm); Mobile phase: dichloromethane containing 0.05 vol% of methanol; Flow rate: 1.0 mL/min; Detection wavelength: 254 nm. The enantiomeric excess (*ee*) of enantiomerically enriched and optically pure 3-alkanols, (*R*)- and (*S*)-(**1**), were determined as the corresponding carbamates (diastereomers) after derivatization with (*R*)-1-(1-naphthyl)ethyl isocyanate (17).

Optical rotations were measured by a JASCO DIP-370 Polarimeter using mercury 405-577 lines at 20°C in chloroform. The specific rotations of optically pure (*R*)- and (*S*)-(**1**) were listed in **Table 3**.

3 Results and Discussion

3.1 Solvent Screening

According to the optical resolution theory (5, 10-12), the enantioselectivity (*E*: the enantiomeric ratio) is expressed as Eq. (1) when the enzymatic reaction is reversible:

$$E = \frac{\ln[1 - (1 + K)c(1 + ee_p)]}{\ln[1 - (1 + K)c(1 - ee_p)]} = \frac{\ln[1 - (1 + K)(c + ee_s\{1 - c\})]}{\ln[1 - (1 + K)(c + ee_s\{1 + c\})]} \quad (1)$$

where *K* is the equilibrium constant, *c* is the extent of

conversion, and *ee_p* and *ee_s* are the optical purities in enantiomeric excess of the product and the unreacted substrate, respectively. When the reaction is irreversible or the reverse reaction is negligible (*K*=0), Eq. (1) is reduced to Eq. (2).

$$E = \frac{\ln[1 - c(1 + ee_p)]}{\ln[1 - c(1 - ee_p)]} = \frac{\ln[(1 - c)(1 - ee_s)]}{\ln[(1 - c)(1 + ee_s)]} \quad (2)$$

If *E* calculated by Eq. (2) for the reversible reaction, it essentially decreases as *c* increases (18). However, it has been reported that *E* can be determined by Eq. (2) in the range of *c* where the reverse reaction is negligible even though the reaction is reversible (5, 11, 12).

It has been reported that *E* is affected by a combination of the structures of solvent and substrate and the solvent hydrophobicity (log *P*) in PCL-catalyzed transesterification with tributylglycerol (TB) (19-21). We therefore screened more than a dozen solvents for PCL-catalyzed transesterification between VA and (*RS*)-83-OH at 30°C. The results were summarized in **Table 4**. The *E* values were determined by Eq. (2). **Table 4** shows that 1,2-dichloroethane, acetonitrile and acetone gave higher *E* than the other solvents. A similar tendency has been reported in PCL-catalyzed transesterification between TB and secondary alcohol (18-21) and also in the reaction between VA and 2-alkanol (14). Thus, we selected 1,2-dichloroethane and acetonitrile as the reaction solvents and also used hexane as a standard solvent in the following studies.

Table 3 Specific Rotation Values ($[\alpha]^\circ$) of Optically Pure (*R*)-(**1**) and (*S*)-(**1**)^a.

Substrate	λ/nm^b							
	(<i>R</i>)-(1) ^c				(<i>S</i>)-(1) ^d			
	577	546	435	405	577	546	435	405
73-OH	-9.2	-10.4	-17.2	-20.4	9.2	10.4	17.2	20.4
83-OH	-9.6	-10.8	-17.9	-21.2	9.5	10.8	17.8	21.2
93-OH	-8.9	-10.0	-16.6	-19.6	8.9	10.1	16.6	19.7
103-OH	-8.0	-8.9	-14.7	-17.5	8.0	8.8	14.7	17.5
113-OH	-7.5	-8.5	-14.0	-16.6	7.5	8.5	14.0	16.6

a) *C*=7-8 (g/100 mL) in chloroform at 20°C.

b) Mercury lamp.

c) The optical purity was listed in **Table 1**.

d) The optical purity was listed in **Table 2**.

Table 4 Solvent Effect on the Enantioselectivity (E) of Optical Resolution by PCL-Catalyzed Transesterification between VA and (RS)-83-OH at 30°C^a.

Solvent	(R)-83-OH				(S)-83-OH			
	$t / \text{min}^{\text{b}}$	$c / \%$	$ee_{\text{P}} / \%$	$E / ^{\circ}$ ^c	t / h^{b}	$c / \%$	$ee_{\text{P}} / \%$	$E / ^{\circ}$ ^c
1,2-Dichloroethane	130	33	87	23	24	63	99	18
Acetonitrile	160	37	86	22	16	61	>99	23
Acetone	140	35	86	21	24	65	>99	18
Tetrahydrofuran	160	33	85	18	24	65	>99	18
Diisopropyl ether	120	35	84	18	24	72	98	9
Hexane	80	33	85	18	24	65	97	12
Octane	80	32	85	18	24	65	97	12
Toluene	105	31	84	17	24	69	>99	13
Heptane	80	32	84	17	24	65	97	12
Benzene	100	33	84	16	24	69	99	12
Isooctane ^d	70	31	83	16	24	65	98	13
Dioxane	130	33	82	15	24	67	>99	15
Cyclohexane	70	30	78	11	24	69	99	11
Carbon tetrachloride	75	33	74	10	24	70	99	12

a) [VA]=1.53 M, [83-OH]=0.88 M and PCL(Lipase PS)=100 mg/mL. b) t : the reaction time.

c) Determined by Eq. (2). d) 2,2,4-Trimethylheptane.

3.2 Quantitative Analyses of Optical Resolution Data

The optical resolution data (ee_{P} and ee_{S} as functions of c) were measured for PCL-catalyzed transesterification between VA and (RS)-83-OH in 1,2-dichloroethane at 30°C. The E values were determined by Eq. (2) and the dependence of E on c was investigated. The results

were illustrated in Fig. 1. As shown in Fig. 1B, E increased with c in the range of below 0.5. This demonstrates that the transesterification did not obey the previously reported optical resolution format (5, 9-11), neither Eq. (1) nor Eq. (2). The E value sharply decreased with c in the range of above 0.55 (Fig. 1B) due to the reverse reaction. A similar tendency has been reported

Fig. 1 Plots of ee vs. c and E vs. c for PCL-Catalyzed Transesterification between VA and (RS)-83-OH in 1,2-Dichloroethane at 30°C.A: $\circ$ ee_{P} vs. c , $\square$ ee_{S} vs. c . [VA]=1.53 M, [83-OH]=0.88 M and PCL(Lipase PS)=100 mg/mL.B: E vs. c . The E values were determined by Eq. (2) using the data in Fig. 1A.

in PCL-catalyzed transesterification between VA and 2-alkanol in various organic solvents (14). In contrast, we observed that the optical resolution data fit Eq. (1) with $E=23$ and $K=0.02$ in the transesterification between triacetyl glycerol (TA) and (*RS*)-83-OH in 1,2-dichloroethane at 30°C as for the reversible reaction of TA with (*RS*)-2-octanol in 1,2-dichloroethane ($E=18$ and $K=0.01$ at 30°C)(14).

In the previous paper, we reported that the extent of conversion of enantiomers in the racemate was calculated by the following equations:

$$c_R = c(1+ee_p) = (1-c)(1-ee_S) \quad (3)$$

and

$$c_S = c(1-ee_p) = (1-c)(1+ee_S) \quad (4)$$

where c_R and c_S are the extents of conversion of (*R*) and (*S*) isomers in the racemate (14). Then we investigated the analysis of optical resolution data by Eqs. (3) and (4) using the data of the transesterification with (*RS*)-83-OH in **Fig. 1A**. The results were illustrated in **Fig. 2**. As shown in **Fig. 2A**, time courses show that both c_R and c_S increased with the reaction time (t). This suggests that enantiomers in (*RS*)-83-OH competitively react with the acetyl-enzyme intermediate ([Enz-Ac] in

Scheme 1) to give the corresponding products. As shown in **Fig. 2B**, the pseudo first order plot [$\ln(1-c)$ vs. t] gave a linearity for the reaction of (*R*)-83-OH in (*RS*)-83-OH, but curvature for (*S*)-83-OH. This suggests that acetylation of (*S*)-83-OH with the acetyl-enzyme intermediate is suppressed during the reaction. This suppression caused an increase of E in the range of $c < 0.5$ (**Fig. 1B**). A similar tendency has been reported in the transesterification with 2-alkanol (14).

3.3 Optical Resolution

Figure 1A demonstrates that the resolution of (*S*)-(1) with high ee is possible by controlling c of PCL catalyzed transesterification between VA and (*RS*)-(1). As shown in **Table 5**, we succeeded in the resolution of (*S*)-(1) with $ee > 95\%$ as expected.

In contrast, (*R*)-(1) with $ee > 95\%$ could not be resolved from (*RS*)-(1) in all cases because of the moderate enantioselectivity (**Fig. 1A** and **Table 5**). A similar resolution has been reported in PCL-catalyzed transesterification between VA and racemic 2-alkanol in organic solvent (15).

Fig. 2 Time Courses and the First Order Plots of Enantiomers in (*RS*)-83-OH for PCL-Catalyzed Transesterification with VA and (*RS*)-83-OH in 1,2-Dichloroethane at 30°C.

A : Time courses. [VA]=1.53 M, [83-OH]=0.88 M and PCL(Lipase PS)=100 mg/mL. These curves were generated from Eqs. (3) and (4) using the data in **Fig. 1A** and the time course of the reaction with (*RS*)-83-OH.

B : The first order plots. These curves were generated from the data in **Fig. 2A**.

Table 5 Resolution of (*R*)- and (*S*)-(**1**) from (*RS*)-(**1**) by PCL-Catalyzed Transesterification with VA in Organic Solvent at 30°C.

(R)-(1)	Hexane ^{a)}			1,2-Dichloroethane ^{a)}			Acetonitrile ^{b)}		
	<i>c</i> / %	<i>ee</i> _P / %	<i>E</i> / - ^{c)}	<i>c</i> / %	<i>ee</i> _P / %	<i>E</i> / - ^{c)}	<i>c</i> / %	<i>ee</i> _P / %	<i>E</i> / - ^{c)}
73-OH	44	78	15	46	76	14	41	84	21
83-OH	43	83	19	40	85	22	43	84	22
93-OH	42	71	10	43	76	13	44	80	17
103-OH	43	72	11	43	82	19	45	80	18
113-OH	43	63	7	41	77	13	45	80	18
(S)-(1)	<i>c</i> / %	<i>ee</i> _S / %	<i>E</i> / - ^{c)}	<i>c</i> / %	<i>ee</i> _S / %	<i>E</i> / - ^{c)}	<i>c</i> / %	<i>ee</i> _S / %	<i>E</i> / - ^{c)}
73-OH	64	98	15	63	97	14	59	98	21
83-OH	63	99	18	62	99	19	61	>99	23
93-OH	73	98	9	63	98	16	62	99	21
103-OH	64	95	11	62	98	17	63	>99	23
113-OH	74	95	7	65	97	12	64	>99	21

a) [VA]=1.63 M, [73-OH]=0.63 M, [83-OH]=0.56 M, [93-OH]=0.51 M, [103-OH]=0.46 M, [113-OH]=0.42 M, PCL(Lipase PS-D)=50 mg/mL.

b) [VA]=1.53 M, [73-OH]=0.99 M, [83-OH]=0.88 M, [93-OH]=0.79 M, [103-OH]=0.72 M, [113-OH]=0.66 M, PCL(Lipase PS)=100 mg/mL.

c) Determined by Eq. (2).

3·4 Kinetic Resolution from Enantiomerically Enriched Substrate

Kinetic resolution of chiral compounds with *ee* > 95% is considered to be practical in a synthetic or industrial sense. For satisfying it, *E* should be above 150 for the optical resolution at *c*=50% even though the reaction is irreversible [Eq. (2)]. If the reaction is reversible, for example *K*=0.02 in Eq. (1), above 210 of *E* is required at *c*=50%. Such cases are not necessarily obtained for the common enzymatic resolution of certain chiral compound because available enzymes and their enantioselectivity are restricted. Thus, kinetic resolution from enantiomerically enriched substrate becomes a powerful strategy for the preparation of optically pure or highly enantiomerically enriched compounds.

When enzymatic reaction with racemate obey Eq. (2), optical purity (*ee*'_P) of the fast-reacting enantiomer resolved from the reaction of enantiomerically enriched substrate (initial optical purity: *ee*₀) is predicted by

$$\left[1 - c' \left(\frac{1 + ee'_P}{1 + ee_0} \right)\right] = \left[1 - c' \left(\frac{1 - ee'_P}{1 - ee_0} \right)\right]^E \quad (5)$$

where *c*' is the extent of conversion based on the starting substrate (10). This equation, however, requires that

E is independent of *c* and *K*=0. Then, we examined the dependence of *E* on *c* for PCL-catalyzed transesterification between VA and (*RS*)-(**1**). The plots of log *E* vs. *c* were shown in Fig. 3, which demonstrates that the *E* value increased at first, maximized at around 0.5-0.6 of *c*, and then decreases against *c* in all systems studied, i.e., *E* is clearly dependent on *c*. We therefore abandoned the use of Eq. (5) and adopted the previously reported equations using the optical resolution data for predicting optical purities of enantiomers resolved from enantiomerically enriched substrate (15).

3·4·1 Resolution from (*R*)-enriched-(**1**)

The following equations have been reported for predicting the optical purities of enantiomers resolved by the reaction with fast reacting enantiomer enriched substrate with initial optical purity of *ee*₀:

$$ee'_P = \frac{ee_0 + ee_P}{1 + ee_0 ee_P} \quad (6)$$

$$ee'_S = \frac{ee_0 - ee_S}{1 - ee_0 ee_S} \quad (7)$$

ee'_S > 0 : (*R*) configuration

ee'_S < 0 : (*S*) configuration

and

Fig. 3 Plots of $\log E$ vs. c for PCL-Catalyzed Transesterification with VA and (*R*)- (1) in Organic Solvent at 30°C.

For the reaction in hexane and 1,2-dichloroethane:
[VA]=1.63 M, [73-OH]=0.63 M, [83-OH]=0.56 M, [93-OH]=0.51 M, [103-OH]=0.46 M, [113-OH]=0.42 M and PCL(Lipase PS-D)=50 mg/mL.

For the reactions in acetonitrile:
[VA]=1.53 M, [73-OH]=0.99 M, [83-OH]=0.88 M, [93-OH]=0.79 M, [103-OH]=0.72 M, [113-OH]=0.66 M and PCL(Lipase PS)=100 mg/mL.

The E values were determined by Eq. (2).

$$c' = \frac{ee_s(1 + ee_0 ee_p)}{ee_s + ee_p} \quad (8)$$

where ee'_p and ee'_s are optical purities of the product and the unreacted substrate, respectively, and c' is the extent of conversion based on the enantiomerically enriched substrate (15).

In order to ensure Eqs. (6)–(8) for the prediction, we compared the plots of $ee'(\text{calc})$ vs. $c'(\text{calc})$ determined by these equations using the resolution data in **Fig. 3** with those [$ee'(\text{meas})$ vs. $c'(\text{meas})$] experimentally obtained by the resolution from (*R*)-enriched 93-OH ($ee_0=0.880, 0.749$ and 0.552). The results were illustrated in **Fig. 4**, which shows an excellent agreement between the calculated and measured plots. **Figure 4** also suggests that (*R*)-93-OH with $ee'_p > 95\%$ can be resolved in a good yield by controlling c of the reaction when ee_0 is sufficiently high for the resolution. Similar-

Fig. 4 Plots of ee' vs. c' for PCL-Catalyzed Transesterification between VA and (*R*)-Enriched 83-OH in Organic Solvent at 30°C.

Solvent — DCE : 1,2-Dichloroethane, MCN : Acetonitrile.

ee_0 : (a) 0.880, (b) 0.749 and (c) 0.522.

A, B : [VA]=1.63 M, [93-OH]=0.51 M and PCL(Lipase PS-D)=50 mg/mL.

C : [VA]=1.53 M, [93-OH]=0.79 M and PCL(Lipase PS)=100 mg/mL.

○, △, □ : $ee'(\text{calc})$ vs. $c'(\text{calc})$.

●, ▲, ■ : $ee'(\text{meas})$ vs. $c'(\text{meas})$.

ly highly enantiomerically enriched (*R*)- (1) ($ee'_p > 95\%$) was obtained from (*R*)-enriched-(1) as shown in **Table 6**. This table shows that the values of $ee'_p(\text{meas})$ were in an excellent accordance with those of $ee'_p(\text{calc})$. The values of $ee'_s(\text{meas})$ were also in agreement with those of $ee'_s(\text{calc})$.

3·4·2 Resolution from (*S*)-enriched-(1)

As shown in **Table 5**, (*S*)- (1) with $ee > 95\%$ could be resolved from (*RS*)- (1) by controlling c of the reaction, but in the expense of low chemical yield because poorly enantiomerically enriched (*R*)- (1) was obtained in a higher yield than (*S*)- (1). The use of enantiomerically enriched substrate may avoid this problem. In the previous paper (15), we reported the following equations for

Table 6 Kinetic Resolution of Highly Enantiomerically Enriched (*R*)-(**1**) from (*R*)-enriched-(**1**) by PCL-Catalyzed Transesterification with VA in Organic Solvent at 30°C.

(R)-(1)	<i>ee</i> ₀ / %	Hexane ^{a)}			1,2-Dichloroethane ^{a)}			Acetonitrile ^{b)}		
		<i>c</i> ' / %	<i>ee</i> ' _P / %		<i>c</i> ' / %	<i>ee</i> ' _P / %		<i>c</i> ' / %	<i>ee</i> ' _P / %	
			meas	calc ^{c)}		meas	calc ^{c)}		meas	calc ^{c)}
73-OH	73	70	96	96	69	96	96	76	96	96
	85	80	98	98	83	98	97	87	98	98
73-OH	67	69	96	96	65	95	96	75	96	96
	80	80	97	98	82	98	97	84	97	97
93-OH ^{d)}	52	50	90	90	58	92	92	56	94	94
	75	60	95	95	73	96	96	79	96	96
	88	86	97	97	91	97	97	82	99	99
103-OH	73	67	94	95	65	97	97	68	96	97
	83	82	96	97	82	98	97	85	97	97
113-OH	71	72	92	92	75	95	96	75	96	96
	81	75	95	95	78	96	96	84	97	97
	91	90	97	97	90	98	98	91	98	99

a) [VA]=1.63 M, [73-OH]=0.63 M, [83-OH]=0.56 M, [93-OH]=0.51 M, [103-OH]=0.46 M, [113-OH]=0.42 M, PCL(Lipase PS-D)=50 mg/mL.

b) [VA]=1.53 M, [73-OH]=0.99 M, [83-OH]=0.88 M, [93-OH]=0.79 M, [103-OH]=0.72 M, [113-OH]=0.66 M, PCL(Lipase PS)=100 mg/mL.

c) Determined by Eq. (6).

d) From the data in **Fig. 3**.

kinetic resolution from the slow reacting enantiomer enriched substrate (initial optical purity: *ee*₀):

$$ee''_s = \frac{ee_0 + ee_s}{1 + ee_0 ee_s} \quad (9)$$

$$ee''_p = \frac{ee_0 - ee_p}{1 - ee_0 ee_p} \quad (10)$$

ee'_P > 0 : (*S*) configuration

ee'_P < 0 : (*R*) configuration

and

$$c'' = \frac{ee_s(1 - ee_0 ee_p)}{ee_s + ee_p} \quad (11)$$

where *ee*'_s and *ee*'_p are optical purities of the product and the unreacted substrate, and *c*' the extent of conversion based on the enantiomerically enriched substrate.

In order to ensure Eqs. (9)-(11) for the prediction, we investigated the plots of *ee*'(meas) vs. *c*'(meas) in PCL catalyzed transesterification with (*S*)-enriched 93-OH (*ee*₀=0.853, 0.718 and 0.530) and compared them with the plots of *ee*'(calc) vs. *c*'(calc) determined by these equations using the data in **Fig. 3**. The results were

illustrated in **Fig. 5**. This shows a good agreement between the calculated and measured plots. The higher *c*' was required for the resolution of optically pure (*S*)-93-OH by the reaction with (*S*)-enriched 93-OH with lower *ee*₀. **Figure 5** also suggests that *ee*'_s > 99% can be attained in a good chemical yield by controlling *c* of the reaction and *ee*₀ of the substrate, which is easily predicted by the *ee*'_s(calc) vs. *c*'(calc) plots.

3.5 Resolution of Optically Pure 3-Alkanols in a Preparative Scale

Based on the above observations, we attempted the kinetic resolution of optically pure (*R*)- and (*S*)-(**1**) (*ee* ≥ 99%) from (*RS*)-(**1**) in a preparative scale by the transesterification in 1,2-dichloroethane and acetonitrile at 30°C. The results were summarized in **Tables 1** and **2**. Optically pure (*R*)-(**1**) was obtained by repeating the reaction three times and twice for optically pure (*S*)-(**1**). The *ee* values of the resolved alcohols agreed with those determined by Eqs. (6) and (9). This procedure will be a practical method for the resolution of optically pure or highly enantiomerically enriched substrates by enzymatic reactions with a moderate enantioselectivity.

Fig. 5 Plots of ee'' vs. c'' for PCL-Catalyzed Transesterification between VA and (S)-Enriched 83-OH in Organic Solvent at 30°C.

Solvent — DCE: 1,2-Dichloroethane, MCN: Acetonitrile.

ee_0 : (a) 0.853, (b) 0.718 and (c) 0.530.

A,B: [VA]=1.63 M, [93-OH]=0.51 M and PCL(Lipase PS-D)=50 mg/mL.

C: [VA]=1.53 M, [93-OH]=0.79 M and PCL(Lipase PS)=100 mg/mL.

○, △, □: ee'' (calc) vs. c'' (calc).

●, ▲, ■: ee'' (meas) vs. c'' (meas).

3·6 Specific Rotations

Finally we measured the specific rotations of optically pure (R)- and (S)-(1). As shown in **Table 3**, the $[\alpha]$ value of (R)-(1) was negative and positive for (S)-(1). A similar tendency was already reported for 2-alkanol (15). Next, we investigated the dependence of $[\alpha]=[\alpha](S)=-[\alpha](R)$ on carbon number (CN) of the alcohol and wavelength (λ /nm) and compared them with those of 2-alkanol (**Figs. 6 and 7**). As shown in **Fig. 6B**, the $[\alpha]$ value of 2-alkanol decreased with CN of the alcohol. In contrast, the $[\alpha]$ value of 3-alkanol maximized at CN=8 (3-octanol) (**Fig. 6A**). As shown in **Fig. 7**, the $[\alpha]$ value increased as λ decreased for both

Fig. 6 Plots of $[\alpha]$ vs. Carbon Number of Secondary Alcohol.

$[\alpha]=[\alpha](S)=-[\alpha](R)$.

A: These curves were generated from the data in **Table 3**.

B: These curves were generated from the data in Ref. (15) and the related experiments.

Fig. 7 Plots of $[\alpha]$ vs. Wavelength (λ /nm).

$[\alpha]=[\alpha](S)=-[\alpha](R)$

Left: These curves were generated from the data in **Table 3**.

Right: These curves were generated from the data in Ref. (15) and the related experiments.

alcohols. This recommends the measurement of $[\alpha]$ at shorter wavelength for the determination of optical purity of secondary alcohols by optical rotation.

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