Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Published online: 03 Jul 2007.

To cite this article: Kosei Shioji, Yoshimitsu Kurauchi, Keiko Terada, Yasushi Kodera & Kentaro Okuma (2007) Enhancement of Enantioselectivity in the Optical Resolution of Primary Alcohols with Modified Cyclodextrin Colyophilized Lipase, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 37:13, 2163-2170, DOI: 10.1080/00397910701386154

To link to this article: http://dx.doi.org/10.1080/00397910701386154

Synthetic Communications®, 37: 2163–2170, 2007

ISSN 0039-7911 print/1532-2432 online
DOI: 10.1080/00397910701386154

Enhancement of Enantioselectivity in the Optical Resolution of Primary Alcohols with Modified Cyclodextrin Colyophilized Lipase

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Abstract: Colyophilization of lipase was carried out with immobilized β-cyclodextrins (β-CyD) bearing methyl, acetyl, benzoyl, and nicotinoyl substituents. The colyophilizates enhanced stereoselectivity in the acylation of several alcohols. The enantioselectivity in the acylation of ethyl-1-hydroxymethyl-phenylphosphine oxide using colyophilized lipase with nicotinoyl-β-CyD increased approximately threefold (from $E = 34$ to $E = 113$). The amphiphilic character of modified CyDs has been found to influence the enhancement of enantioselectivity.

Keywords: lipase, modified cyclodextrin, optical resolution, primary alcohols

Hydrolases, especially lipase, have been established in organic synthesis as catalysts developed from organisms. These catalysts aid the development of optically active compounds.[1,2] However, the lipase activity in organic solvents is frequently lower than that in aqueous media.[3,4] It is well documented that the lyophilization of enzymes causes a loss of enzyme activity.[5–8] It has been reported that an improvement in the reactivity and selectivity of the enzyme reaction in organic reaction media can be achieved by colyophilization with macrocyclic organic additives, such as cyclodextrins (CyDs). These natural molecular receptors are the subject of

Received September 6, 2006
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considerable interest in many areas of molecular chemistry.[9] Griebenow et al. reported that colyophilization of permethylated β-cyclodextrin increased the activity and enantioselectivity of subtilisin Carlsberg when compared to the lyophilized powder prepared from buffer alone.[10] Ghanem and Schurig reported that peracetylated β-cyclodextrin employed as a macrocyclic additive enhanced the enantiomeric ratio *E* and reaction rate in the *Pseudomonas cepacia* lipase (PCL)–catalyzed transesterification of 1-(2-furyl)ethanol in organic solvent.[11] The action of cyclodextrins used as lipase regulators was interpreted as changing the enzyme conformation, preventing substrate and product inhibition, and/or increasing the lipase solubility in organic solvent. Although it is important to investigate the correlation between hydrophobic cavity size and hydrophilic rim, no modified CyDs were used for the enzyme-catalyzed reaction. Previously, we reported that lipase AK(*pseudomonas fluorescence lipase*)–catalyzed optical resolution afforded optically active alkyl(1-hydroxymethyl)phenylphosphine oxides and the corresponding phosphine boranes.[12,13] However, the optical resolution was not effective for all of the phosphorus compounds investigated. Because enantioselectivity in the enzyme-catalyzed reaction was dominated by the binding between the substrate and the enzyme catalytic site, the optical resolutions of the phosphorus compounds are influenced by the introduction of substituents at the phosphorus atom. The treatment of lipase in the presence of modified CyDs is expected to improve the resolution of those compounds. Herein we report the utility of perbenzoylated and pernicotinoylated β-CyDs as additives to enhance selectivity in the lipase AK–catalyzed enantioselective transesterification of phosphine oxides (Figure 1).

RESULTS AND DISCUSSION

Perbenzoylated β-CyD (**1b**) was prepared according to literature methods,[14] whereas pernicotinoylated β-CyD (**1c**) was prepared by a modified method. The introduction of benzoyl groups to β-CyD is expected to increase the overall hydrophobicity of the molecule. It also appears that the hydrogen

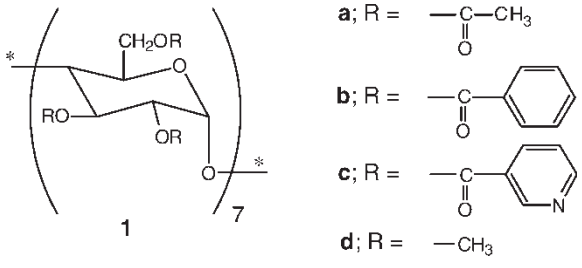
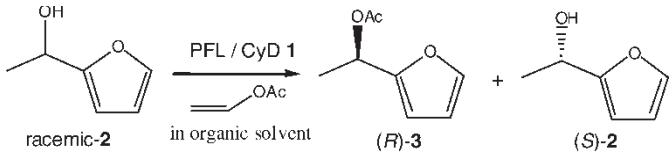


Figure 1. Schematic representation of modified CyD 1.



Scheme 1.

bonding ability of nicotinoylated CyD **1c** is higher than that of **1b**. Colyophilizations of *Pseudomonas fluorescence* (Amano AKG) lipase with modified β-CyDs were carried out by literature methods.[11] Modified CyDs were prepared with a 1:6 weight ratio of enzyme to CyD. The enhancement ability was measured by esterification of the secondary alcohol, 1-(2-furyl) ethanol, with vinyl acetate in cyclohexane using the colyophilized lipase AKG as a catalyst, affording (*R*)-acetate **3** and recovered (*S*)-alcohol **1** (Scheme 1). The enantiopreference of colyophilized lipase AKG was determined by comparison of the retention times in the corresponding gas chromatography (GC) studies. These results are summarized in Table 1. The reaction of **2** with a colyophilizate of **1** was faster than using lyophilized lipase AKG alone.

We applied this method to the transesterification of racemic 1-hydroxyalkylphosphine oxides **4**. The *E*-values using tetrahydrofuran (THF) were higher than those of cyclohexane (1.4–1.6). The esterifications of **4** in THF at 36°C afforded enantiomerically enriched (*R*)-**5** and (*S*)-**4**. These results are summarized in Table 2. The reaction of **4a** using colyophilized lipase with acetyl CyD **1a** resulted in an enhancement of the *E*-values. Moreover, by using colyophilized lipase with nicotinoylated CyD **1c**, the enantioselectivity in the reaction of the ethyl derivative **4a** was fourfold larger than that obtained using lyophilized lipase AKG alone. The isopropyl derivative **4b**, which is bulkier than **4a**, was less reactive than **4a**. The reaction rates of **4** using colyophilizates with **1** were faster than those using AKG alone. The acetylation of *tert*-butyl derivative **4c** was not observed for 2 h. Because of

Table 1. Colyophilized lipase with modified β-CyD **1**-catalyzed acylation of racemic **2** in cyclohexane

1	Time (h)	Conv. (%)	Ee_s (%)[a]	Ee_p (%)[a]	E[b]	Enhancement
—	0.5	30	42	97	102	1.0
—	2	54	98	84	50	—
a	2	50	93	95	118	1.2
b	2	44	74	96	106	1.0
c	2	26	34	99	179	1.8

[a]Determined by GC (cyclodextrin-β-236M-19 (0.25 mm × 25 M).

[b]Calculated by $E = \ln(1 - c)(1 - ee_s)/\ln(1 - c)(1 + ee_p)$, where c is conversion/100.

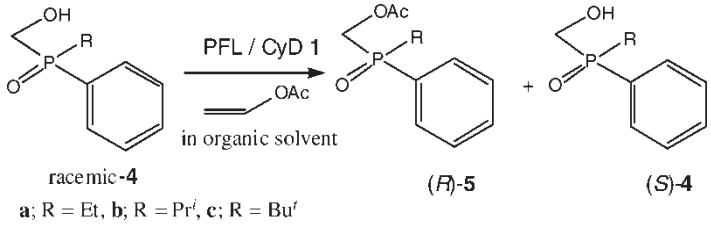
Table 2. Colyophilized lipase AKG with **1** catalyzed acylation of racemic **4** in THF

1	log P	**4**	Time	Solvent	Conv. (%)	Ee_s[a] %	Ee_p[a] (%)	*E*	Enhancement
—		**a**	2	THF	47	76	87	34	1.0
a	0.4				48	88	94	98	2.9
b	1.58				45	74	90	40	1.2
c	−0.04				34	50	97	113	3.4
d	−0.01				30	42	97	94	2.7
—		**b**	17	THF	46	76	91	46	1.0
a					40	60	93	51	1.0
b					40	61	93	47	1.0
c					36	55	97	108	2.3

[a]Determined by HPLC (CHIRALPAK AD with hexane/2-propanol (97:3). (*S*)-**4a**: $[\alpha]_{25}^{D} = -40.9$ ($c = 1.5$, $CHCl_3$, 98% ee). (*R*)-**5a**: $[\alpha]_{25}^{D} = 19.8$ ($c = 1.8$, $CHCl_3$, 53% ee). (*S*)-**4b**: $[\alpha]_{25}^{D} = -41.4$ ($c = 1.6$, $CHCl_3$, 98% ee). (*R*)-**5b**: $[\alpha]_{25}^{D} = 49.6$ ($c = 1.7$, $CHCl_3$, 88% ee).

the steric hindrance of *tert*-butyl group, the reaction was extremely slow (conv. = 2.5% for 48 h) (Scheme 2).

The decrease in the reaction rate of **4** might be caused by host–guest complexation between substrate **4** or product **5** and **1** in solution. The complexation may indeed prevent substrates as well as product inhibition in the enzymatic reaction[15] and improve enantioselectivity by the preferential binding of the (*R*)-enantiomer over the (*S*)-enantiomer. The ability of **4** to undergo complexation with nicotinoyl CyD **1c** was inferred by induced shifts in ^{1}H NMR spectroscopic studies. In the presence of each substrate **2** and **4** (20 equivalents for **1c**), the upfield shift of the aromatic ring protons of **1c** was observed ($\Delta\delta$ 0.025–0.010). However, a specific interaction of (*R*)- or (*S*)-**4** with **1** was not observed. When the mixture of lipase AKG (lyophilized from buffer alone) and lyophilized CyD **1c** at a 1:6 weight ratio as catalyst was carried out, the enhancement in the *E*-value was observed (2.2-fold). This enhancement was smaller than that observed when lipase was colyophilized with **1c** using the same weight ratio. The lipase colyophilized with **1c** was inactivated for 3 days, which indicated that the nicotinoyl



Scheme 2.

group of **1c** interacted with not only the substrate or product, but also the enzyme. Therefore, the clear effect of the modified β-CyD additive is useful in both direct interaction with enzymes and in the complexation with either substrate or product. On the other hand, poor enhancement of the esterification of **4** using benzoylated CyD **1b** was observed. It was reported that the enzyme activity and enantioselectivity of CyDs with varying ring size were both enhanced. These results suggested that the changes in enzyme activity and enantioselectivity were caused by complexation with the phenyl groups of the PCL in the open-lid form.[16,17] Surface hydrophobicity of *Pseudomonas fluorescens* lipase (PFL) was the largest among the lipases.[18] Recently, it was shown that PFL undergoes self-assembly as a result of close interaction between the hydrophobic areas of the lipase surface.[19] The aggregation improves enantioselectivity by the same degree as that afforded by interfacially adsorbed lipase. The partition coefficients (log P) of **1a**, **1b**, **1c**, and **1d** were determined as 0.4, 1.58, −0.04, and −0.1, respectively. Thus, in our case, amphiphilic CyDs are considered to interact with the hydrophobic surfaces of PFL to enhance enantioselectivity.

In summary, enantioselective transesterification of primary-alcohol-type phosphine oxides could be accomplished using colyophilized PFL with modified CyDs. This methodology has potential for practical use, because special acyl moieties or donors are not required.

EXPERIMENTAL

Enzyme and Chemicals

Lipase AKG was obtained as a gift from Amano Pharmaceutical Co., Ltd., and used as a catalyst after lyophilization with modified CyDs. β-CyD was obtained from Wako. Diisopropyl ether was distilled prior to use. Permethyl and peracetyl CyDs were purchased from Wako.

Synthesis

Pernicotinoyl β-CD **1c**

To a solution of β-CyD (1 mmol, 1.1 g) in dry pyridine (50 ml), nicotinoyl chloride (168 mmol, 29.9 g) was added, and the mixture was stirred at 70°C for 7 days. The reaction was quenched by the addition of water and concentrated in vacuo. The pH of the residue was adjusted to 9 by the addition of Na_2CO_3 solution and then extracted with ethyl acetate. The organic layer was washed with water and dried over $MgSO_4$. The filtrate was concentrated to give crude pernicotinoyl β-CyD and purified by recrystallizing from an ethyl acetate–hexane solution in 63% yield. Colorless crystals, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 4.23$ (t, 1H, $J = 9.2$ Hz), 4.66 (d, 1H, $J = 7.6$ Hz), 4.85 (d, 1H,

$J = 10.8$ Hz), 4.94 (dd, 1H, $J = 3.2$ Hz), 5.07 (s, 1H), 5.70 (d, 1H, $J = 3.2$ Hz), 6.06 (t, 1H, $J = 9.2$ Hz), 6.98–705 (m, 2H), 7.55 (s, 1H), 7.63 (d, 1H, $J = 7.2$ Hz), 7.75 (d, 1H, $J = 8.0$ Hz), 8.46 (d, 1H, $J = 8.0$ Hz), 8.53–8.60 (m, 3H), 8.87 (s, 1H), 9.06 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 63.4$, 70.3, 71.7, 72.8, 97.5, 123.3, 123.4, 123.8, 123.9, 124.6, 125.5, 136.8, 137.1, 137.7, 150.5, 150.8, 151.2, 153.9, 154.0, 154.3, 164.0, 164.9, 165.0. MS (MALDI-TOF): 3366.2 $[M + Na]^+$; calc. for $C_{168}H_{133}N_{21}O_{56}$: 3339.8.

Phosphine Oxide **4**

To a solution of ethylphenylphosphinate (30 mmol), in dry THF (30 mL), alkyl magnesium bromide (39 mmol) was added, and the mixture was stirred at 0°C for 2 h. *Tert*-butyllithium (36 mmol) at −78°C and gaseous formaldehyde (150 mmol) were added to the reaction mixture, and the mixture was stirred at −15°C for 2 h. The reaction was quenched with aq. NH_4Cl, and the organic layer was extracted with CH_2Cl_2 (30 mL × 2). The extract was dried over $MgSO_4$ and concentrated in vacuo to give crude phosphine oxide **4**. Purification was carried out by column chromatography (hexane/ethyl acetate) to give **4a** and **4b** in 76% and 68% yields, respectively.

Ethyl(1-hydroxymethyl)phenylphosphine Oxides **4a**

Colorless liquid, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 1.10$–1.18 (m, 3H), 1.85–2.05 (m, 1H), 2.17–2.29 (m, 1H), 4.05–4.15 (m, 2H), 7.46–7.54 (m, 3H), 7.69–7.74 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 5.48$, 5.53, 19.37, 20.05, 60.08, 60.90, 128.74, 128.85, 129.81, 130.71, 130.93, 131.01, 132.16, 132.18. ^{31}P NMR (162 MHz, $CDCl_3$) $\delta = 42.49$. IR (NaCl, $CHCl_3$): 1159, 1169, 3021 cm^{-1}. UV ($CHCl_3$): 255 nm. Found: H, 7.29; C, 57.80, calc.: H, 7.11; C, 57.69.

Isopropyl(1-hydroxymethyl)phenylphosphine Oxides **4b**

Colorless liquid, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 1.90$ (dd, $J = 9.2$ Hz, 3H), 1.33 (dd, $J = 9.2$ Hz, 3H), 2.27–2.35 (m, 1H), 4.60 (dq, $J = 36$ Hz, 2H), 7.50–7.58 (m, 3H), 7.72–7.77 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 15.46$, 15.50, 15.95, 15.98, 26.28, 26.95, 59.25, 60.01, 128.71, 128.82, 129.19, 130.06, 131.13, 131.21, 132.06, 132.09. ^{31}P NMR (162 MHz, $CDCl_3$) $\delta = 45.29$. IR (NaCl, $CHCl_3$): 1149, 1157, 3021 cm^{-1}. UV ($CHCl_3$): 257 nm. Found: H, 7.58; C, 60.76; calc.: H, 7.63; C, 60.60.

Colyophilization of Lipase with CyDs

Modified CyDs (enzyme–CyD = 1:6) were suspended in a solution of lipase AKG (5 mg) in phosphate buffer (20 mM, pH 6.0, 1 mL), sonicated for 30 s, and lyophilized for 48 h.

General Procedure for Esterification of Racemic Alcohols using Colyophilized Lipase

To a solution of racemic alcohols (0.014 mmol) in cyclohexane or THF (0.5 mL), colyophilizates and vinyl acetate (0.042 mmol) were added, and the mixture was stirred at 36°C for an appropriate time. The reaction mixture was filtered, and the solvent was removed under reduced pressure. The reaction was analyzed by HPLC using a Waters 600 dual pump equipped with a tunable absorbance detector (Waters 484) and a CHIRALPAK AD (0.46 × 25 cm) column, and it was eluted with a hexane/2-propanol (97:3) solvent mix including 0.1% trifluoroacetic acid.

Determination of Partition Coefficient

Solutions of modified CyDs were prepared in H_2O at concentrations of 0.02 mM and 0.04 mM, and their concentrations were determined with respect to their optical absorption, obtained using a UV/vis spectrometer. Equal volumes (5 mL) of the aqueous CyD solutions and 1-octanol were stirred at 36°C for 1 h. The H_2O phase was separated and its concentration determined.

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