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Peracetylated β-cyclodextrin as additive in enzymatic reactions: enhanced reaction rate and enantiomeric ratio in lipase-catalyzed transesterifications in organic solvents

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Abstract—Peracetylated β-cyclodextrin has been employed as a macrocyclic additive to enhance the enantiomeric ratio *E* and reaction rate in *Pseudomonas cepacia* lipase (PSL)-catalyzed enantioselective transesterification of 1-(2-furyl)ethanol in organic solvents. The beneficial action of the cyclodextrin used as a regulator of lipase was tentatively interpreted as increasing the conformational flexibility of the enzyme and undergoing host–guest complexation with the product, thereby preventing product inhibition and leading to an enhancement of the enantiomeric ratio *E* and the reaction rate. The effect of the organic solvent on the present cyclodextrin-mediated enzymatic transesterification has been studied.

1. Introduction

Enzymes, especially lipases, are now well established as valuable catalysts in organic synthesis. These natural catalysts have also been shown to be very useful tools for access to chiral molecules under non-aqueous conditions.[1] Compared to aqueous conditions, the use of an organic reaction medium can have some interesting advantages, including enhanced thermal stability of the undissolved enzyme, easy separation of the suspended enzyme from the reaction medium for recycling, increased solubility of the substrate, favorable equilibrium shift to synthesis over hydrolysis in transesterification reactions, the elimination of undesired side-reactions caused by water and the generation of novel selectivities of certain enzymes.[2]

Many non-aqueous enzymatic reactions are prone to substrate or product inhibition, which deactivate the enzyme at higher substrate or product concentration leading to a decrease in the reaction rate and enantiomeric ratio *E*. In fact, keeping the substrate concentration at a low level through continuous addition can circumvent substrate inhibition, however, product inhibition is still difficult to control even by gradual removal of the product by physical means.[3] The use of enzymes in organic solvents has also the drawback of their decreased catalytic activities, which are generally several orders of magnitude lower than in aqueous solution. Several strategies have been explored to overcome the lower activity of enzymes in organic solvents to make them more appealing to organic chemists. These include the methods of enzyme preparation,[4] directed evolution,[5] chemical modification of the enzyme,[6] control of the pH value,[7] co-lyophilization with lyoprotectants[8] and salts,[9] addition of water-mimicking compounds like formamide, glycol or DMF,[10] immobilization techniques,[11] imprinting with substrates and substrate analogues,[12] and cross-linking crystallization.[13] It has been reported that an additional improvement in the rate and selectivity of the reaction in non-aqueous media can be achieved by using macrocyclic organic additives, which might have a beneficial influence on the microenvironment of the enzymes and hence improve their catalytic activity and selectivity in organic solvents.[14]

Among these supramolecular additives, crown ethers and especially thiacrown ethers have been successfully used to enhance the catalytic activity and the enantiomeric ratio *E* of PSL in the formation and hydrolysis of carboxylic esters.[15–17] In fact, co-lyophilization of various enzymes with crown ether leads to enhancement of the catalytic activity of the enzyme and also increases the enantiomeric ratio *E* in organic solvents. It is conceivable that the crown ethers bind to the

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enzyme in the co-lyophilization process leading to increased solubility of the enzymes in the organic phase, thus accelerating the rate of transesterification.[18] Also, such binding can increase the flexibility of the enzymes in organic solvents leading to enhancement of its activity and the enantiomeric ratio E.[19] The enhancement of the enzyme activity by crown ethers may also be attributable to the capability of the crown ether to facilitate the removal of water molecules from the active site upon substrate binding.[18]

Cyclodextrins (CDs), another class of compound with a macrocyclic structure, have been successfully used to improve enzymes activity and to increase the reaction rate and E in enzyme-catalyzed reactions in organic solvents.[20,21] They are a family of chiral cyclic α-(1-4)-linked D-glucose oligomers with six, seven, or eight glucose units, corresponding to α-, β-, and γ-homologues, possessing toroidal conformation in the solid state and in solution. The internal hydrophobic cavity and the external hydrophilic rim of chemically modified cyclodextrins render them ideal for modelling enzyme-substrate binding,[22] drug delivery,[23] catalysis,[24] host–guest association,[25] chiral separation[26] and molecular recognition in self-assembled monolayers[27] etc.

Previously, permethylated β-cyclodextrin has been used to enhance the reaction rate and enantiomeric ratio, E of the reactions of subtilisin Carlsberg suspended in an organic solvent.[21] In terms of bulkiness and hydrogen-bonding ability, the introduction of an acetyl group to free β-cyclodextrin may drastically alter its role as regulator for enzymes in organic solvents. Therefore, peracetylated β-cyclodextrin (cf. Fig. 1) is expected to exhibit novel behavior in enhancing the reaction rate and E of PSL-catalyzed transesterification reactions under non-aqueous conditions when added prior to the lyophilization of the enzyme in buffer.

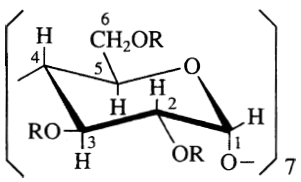


Figure 1. Structure of peracetylated-β-CD (R = -$COCH_3$).

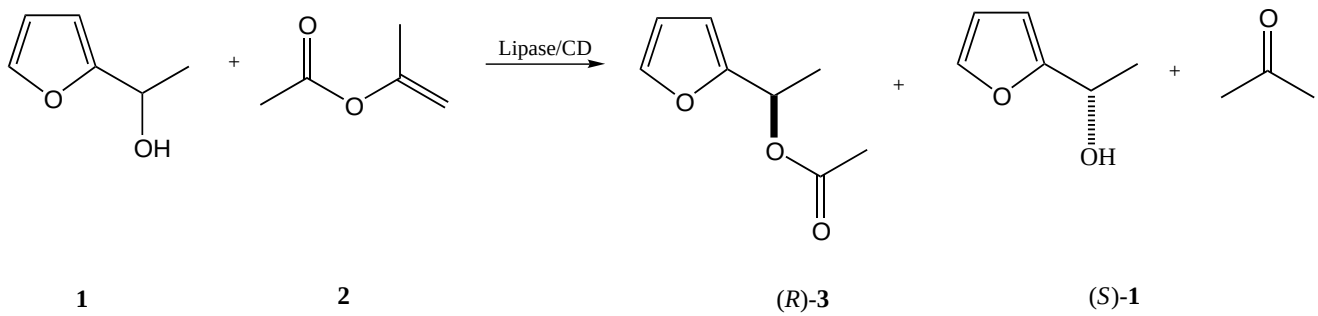
Herein, we report the utility of peracetylated β-cyclodextrin employed as an additive to enhance the reaction rate and enantiomeric ratio E in the *Pseudomonas cepacia* lipase (PSL)-catalyzed enantioselective transesterification of 1-(2-furyl)ethanol in an organic solvent.

2. Results and discussion

Two methods of enzyme preparation have been performed. The first consisted of the lyophilization of *P. cepacia* lipase (PSL), the present model enzyme, from phosphate buffer pH 6.0 without the cyclodextrin additive. The second enzyme preparation was concerned with the co-lyophilization of PSL with peracetylated β-cyclodextrin using the same phosphate buffer. The effect of the cyclodextrin on the reaction rate and E of PSL-catalyzed transesterification in various organic solvents was studied in the model kinetic resolution of lipase-catalyzed transesterification of 1-(2-furyl)ethanol using isopropenyl acetate as acyl donor (cf. Scheme 1).

Compared to the commercially available PSL used as purchased, a slight decrease in the catalytic activity was observed when the enzyme was lyophilized from phosphate buffer pH 6. This finding is accounted for by partial denaturation of PSL during lyophilization. Interestingly, no such decrease in catalytic activity was observed when the enzyme was co-lyophilized with peracetylated β-cyclodextrin using the same phosphate buffer. Since the magnitude of the enzyme activation in organic solvents by macrocyclic compounds depends on the ratio of the enzyme and the additive,[18] three different mixtures were investigated, i.e. 1:1; 1:2; 1:6 (enzyme to cyclodextrin, w/w). When the peracetylated β-cyclodextrin was employed at a 1:6 weight ratio of enzyme to cyclodextrin, a significant activation of PSL has been detected as compared to the data of the lyophilized PSL from buffer alone (cf. Fig. 2). The lyophilized lipase from buffer alone catalyzed kinetic resolution of **1** was characterized by the following data: time = 24 h, e.e.$_s$ = 99%, e.e.$_p$ = 61%, conv. = 60% and E = 28.

The enhancement of the reaction rate and the E of the PSL-catalyzed transesterification of **1** was in the order 1:6>1:2>1:1 (enzyme to peracetylated β-cyclodextrin, w/w) (cf. Fig. 3).



1 **2** (*R*)-**3** (*S*)-**1**

Scheme 1. Co-lyophilized PSL with peracetylated-β-cyclodextrin catalyzed transesterification of (±)-1-(2-furyl)ethanol **1** using isopropenyl acetate **2** in toluene.

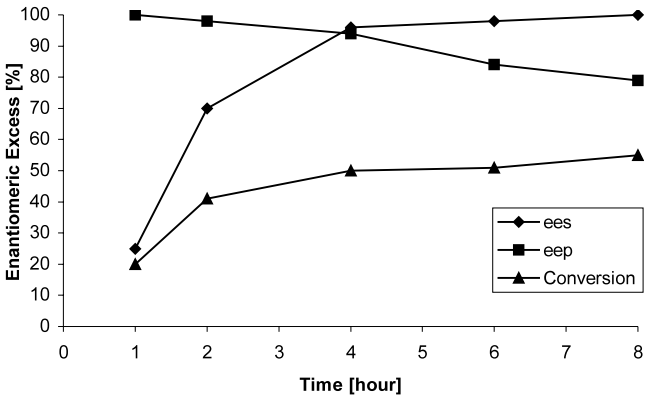


Figure 2. Co-lyophilized lipase with peracetylated β-cyclodextrin (1:6 weight ratio) catalyzed enantioselective transesterification of 1(2-furyl)ethanol in toluene. The reaction was terminated in 8 h with 56% conv., >99% e.e.$_s$, 80% e.e.$_p$, $E=52$.

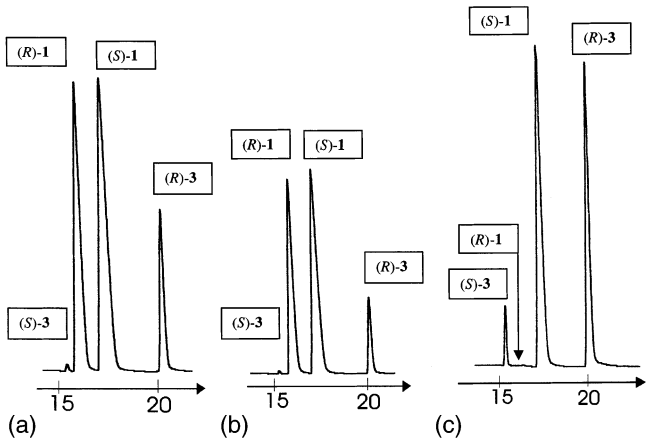


Figure 3. Gas-chromatographic separation of the enantiomer of both substrate **1** and product **3** on heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin of the co-lyophilized PSL with peracetylated β-cyclodextrin-catalyzed transesterification of **1** in toluene at t = 8 h. (a) 1:1 enzyme/Ac β-cyclodextrin weight ratio, e.e.$_s$ = 13.7%, e.e.$_p$ = 94.3%, conv. = 12.7%, $E=39$. (b) 1:2 enzyme/Ac β-cyclodextrin weight ratio, e.e.$_s$ = 14%, e.e.$_p$ = 94%, conv. = 13%, $E=38$. (c) 1:6 enzyme/Ac β-cyclodextrin weight ratio, e.e.$_s$ = 99.9%, e.e.$_p$ = 79.1%, conv. = 56%, $E=88$.

In a control experiment it was found that no reaction took place when peracetylated β-cyclodextrin was employed without any lipase. It is concluded from this finding that peracetylated β-cyclodextrin does not catalyze the enantioselective transesterification of **1** by itself but regulates enzyme activity, probably by changing the conformation of the enzymatic catalytic site or undergoes host–guest complexation with substrate and/or product. In an additional experiment, PSL was first lyophilized from buffer alone, suspended in toluene and only afterwards peracetylated β-cyclodextrin was added to the reaction mixture (at the 1:6 weight ratio as before). A slight enhancement in the reaction rate and E was observed. However, this enhancement was much smaller than that observed when lipase was co-lyophilized with cyclodextrin in phosphate buffer using the same weight ratio. Therefore, the beneficial effect of the cyclodextrin additive is believed to arise mainly from the direct interaction with the enzyme rather than complexation with either substrate or product. Thus, in the co-lyophilization step, the cyclodextrin may interact with the enzyme in a specific but as yet unknown way by changing its conformation and hence influencing its catalytic behaviour as already inferred above.

Since the cyclodextrin is used in excess, host–guest complexation in solution with the substrate and product cannot be excluded. This might explain why the reaction rate was higher when using the 1:6 as compared to the 1:1 ratio (enzyme to cyclodextrin, w/w). In fact, the ability of cyclodextrins to form host–guest complexes may indeed prevent substrate as well as product inhibition in enzymatic reactions.[20] The inclusion of product in the cavity of the cyclodextrin may also shift the equilibrium in the desired direction. This thermodynamic effect, however, would not explain the enhancement of the reaction rate. The ability of 1-(2-furyl)ethanol **1** as well as its ester **3** to undergo inclusion in the peracetylated β-cyclodextrin cavity was inferred by (upfield/downfield) complexation-induced shifts in ^{1}H and ^{13}C NMR spectroscopic studies. Whether enantioselective binding of substrate **1** or product **3** with cyclodextrin, rendering one enantiomer more accessible to the enzyme, contributes to the observed enhancement of enantiomeric ratio is still open to question.

Indeed, when performing the esterification of **1** with acetyl chloride with pyridine as the catalyst instead of lipase, and peracetylated β-cyclodextrin as chiral additive, enantioselective esterification to give **3** was observed. It was found that the inclusion of enantiomers of **1** in the peracetylated β-cyclodextrin cavity occurred in a preferential way allowing the (*R*)-**1** enantiomer to react faster with acetyl chloride to form the enantiomerically biased ester. The following data were measured: time = 2 days, e.e.$_s$ = 5.2% (*S*)-**1**, e.e.$_p$ = 31% (*R*)-**3**, conv. = 15% and $E=2.03$. Also, this effect has been observed when using permethylated β-cyclodextrin as additive. The following data were measured: time = 2 days, e.e.$_s$ = 0.6% (*S*)-**1**, e.e.$_p$ = 7.0% (*R*)-**3**, conv. = 8.0% and $E=1.16$. This supports the assumption that the enhancement of E in the PSL and cyclodextrin co-lyophilized catalytic transesterification of **1** in organic solvent is based on a beneficial effect of the cyclodextrin on the enzyme rather then preferential enantioselective complexation of **1** with the cyclodextrin.

It should be noted that simple esters like peracetylated β-cyclodextrin cannot be used as an acylating agent for **1** in the presence of an activated ester like isopropenyl acetate. In general, acylation with a normal ester as the acyl donor for the transesterification of alcohols in the presence of lipase are often slow and reversible with an equilibrium constant of near to one. This was confirmed by allowing **1** to react with peracetylated β-cyclodextrin in the presence of lipase in toluene under

the same conditions as detailed above, no reaction was observed and the recovered cyclodextrin was found to be the peracetylated β-cyclodextrin. In an additional experiment, perbutyrated β-cyclodextrin was synthesized and used instead of peracetylated β-cyclodextrin as additive and in co-lyophilized form with lipase to catalyze the enantioselective transesterification of **1** in toluene in the presence of isopropenyl acetate as the acyl donor, surprisingly, reaction inhibition was observed. We concluded that the acetyl group is involved in the enhancement of the reaction rate as well as the enantiomeric ratio of lipase-catalyzed transesterification of **1**.

The solubility of lipase in toluene could also play an important role in the enhancement of the reaction rate and enantioselectivity, Reinhoudt et al.[18] reported that crown ethers could solubilize the lipase in organic solvents and accelerate the rate of transesterification. The solubility of lipase as well as co-lyophilized lipase with peracetylated β-cyclodextrin and cyclodextrin alone has been studied in toluene using light scattering techniques. We found that only lipase dissolved in toluene forms aggregates. Co-lyophilized lipase with peracetylated β-cyclodextrin and cyclodextrin alone did not form aggregates. We concluded from this observation that peracetylated β-cyclodextrin increases the solubility of the *P. cepacia* lipase in toluene when they are mixed prior to lyophilization in buffer.

This increase in solubility of the lipase in toluene might contribute to the enhancement of the reaction rate as well as the enantiomeric ratio of co-lyophilized lipase-catalyzed transesterification of **1** in toluene.

2.1. Effect of organic solvents on the PSL and cyclodextrin co-lyophilized catalytic transesterification of 1

The choice of the organic solvent for a lipase-catalyzed reaction is known to be crucial in determining the E values.[30,31] It has been reported that solvents with log P values more than two exhibit high E, whereas solvents with log P of less than two show detrimental effects on the E of the enzyme-catalyzed reactions in organic solvents.[32] P is employed as an index for the solvent hydrophobicity in biocatalytic reactions[33] and is defined as the ratio of the concentration of a substance in two immiscible phases (1-octanol and water) at equilibrium. We tried to correlate the observed E with various solvents in dependence on their log P. However, for the present enzyme preparations (co-lyophilized lipase with peracetylated β-cyclodextrin and lyophilized enzyme only) no straightforward correlation between the enzyme prepared, solvents and E was found (cf. Table 1). For example, as compared with lyophilized lipase only, the enhancement of E using co-lyophilized lipase with peracetylated β-cyclodextrin was 6.7 fold higher in magnitude in isooctane (log $P=4.5$), while the enhancement was 6.2 times higher in magnitude in tetrahydrofuran (log $P=0.49$). Also, in 1,4-dioxane (log $P=-1.1$) the enhancement in enantiomeric ratio E was 5.8 times higher in magnitude, while in toluene (log $P=2.5$) the enhancement was only 1.8 times higher in magnitude. With regard to the E value of the reaction, isooctane was the best solvent employed in the transesterification of **1** using the co-lyophilized lipase with peracetylated β-cyclodextrin. However, with regard to the reaction rate and the enantiomeric excess of both substrate and product, toluene proved to be the best solvent employed.

3. Experimental

3.1. Materials and methods

3.1.1. Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded at 250 and 400 MHz, respectively, on Bruker spectrometers (Karlsruhe, Germany). Chemical shifts (δ) are given in ppm relative to TMS as internal standard. Specific rotations were measured with a Perkin-Elmer 241 polarimeter operating at the sodium D line and at room temperature.

3.1.2. Chemicals and enzymes. All chemicals were purchased from Fluka (Switzerland) and dried over molecular sieves prior to use. Lipase from *P. cepacia* (PSL) was a gift from Amano (Nagoya, Japan). β-Cyclodextrin was purchased from Wacker-Chemie GmbH (Burghausen/Germany). The peracetylated β-cyclodextrin has been synthesized as described later.

Table 1. Enantiomeric ratio E enhancement of co-lyophilized lipase with peracetylated β-cyclodextrin-catalyzed transesterification of **1** in different organic solvents

Solvent	Log P[a]	Lyophilized[b] lipase	Peracetylated β-cyclodextrin co-lyophilisate[c]	Enhancement
Isooctane	4.5	E[d] = 46.2	$E=>300$	6.7
THF	0.49	$E=93.6$	$E=>300$	6.2
1,4-Dioxane	−1.1	$E=27.9$	$E=161.6$	5.8
n-Hexane	3.5	$E=11.3$	$E=40.1$	3.5
Acetonitrile	−0.33	$E=99.7$	$E=>300$	3.4
Toluene	2.5	$E=28.9$	$E=51.4$	1.8

[a] P = partition coefficient; defined as the ratio of the concentration of a substance in two immiscible phases at equilibrium (1-octanol and water).
[b] PSL lyophilized from phosphate buffer alone, pH 6.
[c] Lyophilized from aqueous phosphate buffer containing peracetylated β-cyclodextrin at a 1:6 weight ratio of lipase to cyclodextrin.
[d] E = enantiomeric ratio.[29]

3.1.3. Synthesis and biochemical transformation reactions. The racemic ester **3** was synthesized on an analytical scale to optimize a baseline separation of the enantiomers using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin as a stationary phase in GC. The separation factor $\alpha = 1.32$ and resolution $R_s = 14.3$ for the ester **3** and $\alpha = 1.08$ and $R_s = 2.5$ for the alcohol **1**. Peak integration for the racemic sample showed the unusual elution order (*S*)-**3**, <(*R*)-**1**, <(*S*)-**1**, <(*R*)-**3** which should be noted. The retention times were 14.7, 15.5, 16.8, and 20.2 min, respectively.

3.1.4. Peracetylated β-cyclodextrin. β-Cyclodextrin (5.6 g) was reacted with acetic anhydride (113 mL) in pyridine (225 mL). The reaction was stirred for 24 h at room temperature. Water was added and the extraction was performed using ethyl acetate. The organic layer was washed with 1 M HCl followed by water, dried with anhydrous sodium sulphate, and the excess solvent was removed by rotary evaporator. The residue was purified by column chromatography using CH_2Cl_2/acetone (4:1, v/v). The yellowish product was crystallized with ethanol/diethyl ether (1:1, v/v) to give white crystals; yield 60%; mp 201; $[\alpha]_D^{24}$ 125 (*c* 0.01, $CHCl_3$); MS (positive FAB, 0.1% in methanol+0.1% acetic acid) *m*(nominal mass)/*z* 2017 $[M+H]^+$; anal. calcd for $C_{84}H_{112}O_{56}$: C, 49.97; H, 5.55. Found: C, 49.80; H, 5.20%; ^{1}H NMR (benzene-d_6) δ 5.26 (dd, 1H, H-3), 5.03 (d, 1H, H-1), 4.7 (t, 1H, H-2), 4.52 (d, 1H, H-6a), 4.22 (m, 1H, H-6b), 3.67 (t, 1H, H-4), 2.10 (s, 3H, CH_3); ^{13}C NMR (benzene-d_6) δ 170.44 (CO), 170.31 (CO), 169.24 (CO), 97.48 (C-1), 77.53 (C-4), 71.23 (C-3), 70.69 (C-2), 70.33 (C-5), 63.11 (C-6), 20.62 (CH_3), 20.54 (CH_3), 20.47 (CH_3).

3.1.5. Co-lyophilization of PSL with peracetylated β-cyclodextrin. *P. cepacia* lipase (PSL, 100 mg) was dissolved (2 mg/1 μL) in 20 mM phosphate buffer (pH 6.0) and lyophilized for 48 h (control). The co-lyophilization of lipase with peracetylated β-cyclodextrin was performed by the same method, except that the cyclodextrin was added prior to lyophilization with different ratios of lipase to cyclodextrin (1:1; 1:2; 1:6, w/w). After lyophilization, the enzyme preparation was dried under vacuum and stored at −18°C for further use.

3.1.6. Lipase-catalyzed transesterification of 1-(2-furyl)ethanol. All reactants (alcohol, ester) were stored over activated 4 Å molecular sieves. (±)-1-(2-Furyl)ethanol (56 mg, 0.5 mmol) and isopropenyl acetate (108.8 mg, 1 mmol) were dissolved in organic solvent (3 mL) in a reaction vial (5 mL). The reaction mixture was thermostated in an oil bath to 40°C. Then, a 100 μL sample of the reaction mixture was withdrawn for GC analysis ($t = 0$ of sample). Afterward, 100 mg of lyophilized lipase or lipase co-lyophilized with 100, 200 or 600 mg peracetylated β-cyclodextrin was added. 100 μL samples were taken after several time intervals. The samples were centrifuged to separate lipase. The organic layer was diluted by 100 μL toluene. The reaction progress was monitored qualitatively by thin layer chromatography. An aliquot of the supernatant was used for the GC analysis. When maximum conversion was reached the reaction was terminated by filtration. The enzyme was washed with solvent and then with acetone. Substrate (*S*)-**1** and product (*R*)-**3** were distilled and the recovered cyclodextrin was purified and analyzed.

3.1.7. Enantioselective gas-chromatographic analysis. Enantioselective analysis of 1-(2-furyl)ethanol **1** (substrate) and acetoxy-1-(2-furyl)ethane **3** (product) was performed simultaneously on a gas chromatograph (Hewlett Packard 580, Waldbronn, Germany) equipped with a flame ionization detector (FID). The chiral stationary phase heptakis-(2,3-di-*O*-methyl-6-*O*-tert-butyldimethylsilyl)-β-cyclodextrin, 20% (w/w) was dissolved in PS 86 (Gelest, ABCR GmbH & Co., Karlsruhe, Germany) and coated on a 25 m×0.25 mm fused silica capillary column (0.25 μm film thickness) according to literature.[28] The analytical conditions were: injector temperature, 200°C; FID temperature, 250°C; oven temperature 70°C for the simultaneous separation of enantiomers of **1** and **3**. Hydrogen was used as the carrier gas (40 KPa column head pressure). The retention time of (*S*)-**3**, (*R*)-**1**, (*S*)-**1**, (*R*)-**3**, were 14.7, 15.5, 16.8, 20.2 min, respectively. The substrate **1** and product **3** were identified by using a GC/MSD-system HP 6890/5973 (Hewlett Packard, Waldbronn, Germany) equipped with an HP 7683 autosampler. The enantiomeric excess e.e. of both substrate ($e.e._s$) and product ($e.e._p$) as well as conversion (conv.) and enantiomeric ratio (*E*) were determined by the computer program available on the internet http://www.orgc.TUGraz.at/orgc/programs/selectiv/selectiv.htm, developed by Faber et al.[29]

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References

1. Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Wiley-VCH: Weinheim, Germany, 1999; ISBN 3-527-30104-6.
2. Klibanov, A. M. *Trends Biochem. Sci.* **1989**, *14*, 141.
3. Faber, K. *Biotransformations in Organic Chemistry*, 3rd ed.; Springer Verlag: Heidelberg, Germany, 1997; ISBN 3-540-61688-8.
4. Almarsson, Ö.; Klibanov, A. M. *Biotechnol. Bioeng.* **1996**, *49*, 87–92.
5. Reetz, M. T.; Jaeger, K. E. *Biocatalysis* **1999**, *200*, 31–57.
6. Babonneau, M. T.; Jacquier, R.; Lazaro, R.; Viallefont, P. *Tetrahedron Lett.* **1989**, *30*, 2787.
7. Yang, Z.; Zacherl, D.; Russell, A. J. *J. Am. Chem. Soc.* **1993**, *115*, 12251–12257.

8. Dabulis, K.; Klibanov, A. M. *Biotechnol. Bioeng.* **1993**, *41*, 566–571.
9. Khmelnitsky, Y. L.; Welch, S. H.; Clark, D. E.; Dordick, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 2647–2648.
10. Kitaguchi, H.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 9272–9273.
11. Reslow, M.; Adlercreutz, P.; Mattiasson, B. *Eur. J. Biochem.* **1988**, *172*, 573–578.
12. Rich, J. O.; Dordick, J. S. *J. Am. Chem. Soc.* **1997**, *119*, 3245–3252.
13. Lalonde, J. J.; Govardhan, C.; Khalaf, N.; Martinez, A. G.; Visuri, K.; Margolin, A. L. *J. Am. Chem. Soc.* **1995**, *117*, 6845–6852.
14. Theil, T. *Tetrahedron* **2000**, *56*, 2905–2919.
15. Takagi, Y.; Teramoto, J.; Kihara, H.; Itoh, T.; Tsukube, H. *Tetrahedron Lett.* **1997**, *37*, 4991–4992.
16. Takagi, Y.; Ino, R.; Kihara, H.; Itoh, T.; Tsukube, H. *Chem. Lett.* **1997**, 1247–1248.
17. Itoh, T.; Takagi, Y.; Murakami, T.; Hiyama, Y.; Tsukube, H. *J. Org. Chem.* **1996**, *61*, 2158–2163.
18. Bross, J.; Sakodinskaya, I. K.; Engbersen, J.; Verboon, W.; Reinhoudt, D. N. *J. Chem. Soc., Chem. Commun.* **1995**, 255–256.
19. Bross, J.; Visser, A. J. W. G.; Engbersen, J. F. J.; Verboom, W.; Hoek, A. V.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 12657–12663.
20. Harper, J. B.; Easton, C. J.; Lincoln, S. F. *Curr. Org. Chem.* **2000**, *4*, 429–454.
21. Griebenow, K.; Laureano, Y. D.; Santos, A. M.; Clemente, I. M.; Rodriguez, L.; Vidal, M. W.; Barletta, G. *J. Am. Chem. Soc.* **1999**, *121*, 8157–8163.
22. D'Souza, V. T.; Bender, M. L. *Acc. Chem. Res.* **1987**, *20*, 146–152.
23. Pfannemüller, B.; Burchard, W. *Macromol. Chem.* **1969**, *121*, 1–17.
24. Ye, H.; Tong, W.; D'Souza, V. T. *J. Am. Chem. Soc.* **1992**, *144*, 5470–5472.
25. Andersson, T.; Sundahl, M.; Westman, G.; Wennerstroem, O. *Tetrahedron Lett.* **1994**, *35*, 7103–7106.
26. Schurig, V. *J. Chromatogr. A* **2001**, *906*, 275–299.
27. Rojas, M. T.; Koeniger, R.; Stoddart, J. F.; Kaifer, A. E. *J. Am. Chem. Soc.* **1995**, *117*, 336–343.
28. Dietrich, A.; Maas, B.; Messer, W.; Bruche, G.; Karl, V.; Kaunzinger, A.; Mosandl, A. *High Resolut. Chromatogr.* **1992**, *15*, 590–593.
29. Kroutil, W.; Kleewein, A.; Faber, K. *Tetrahedron: Asymmetry* **1997**, *8*, 3251–3261.
30. Wescott, C. R.; Klibanov, A. M. *J. Am. Chem. Soc.* **1993**, *115*, 1629–1631.
31. Nakamura, K.; Kinoshita, M.; Ohno, A. *Tetrahedron* **1994**, *50*, 4681–4690.
32. Hansch, L. C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525–616.
33. Terradas, F.; Tenston-Henry, M.; Fitzpatrick, P. A. *J. Am. Chem. Soc.* **1993**, *115*, 390–396.