Dynamic Kinetic Resolution

DOI: 10.1002/anie.201104141

Ionic-Surfactant-Coated *Burkholderia cepacia* Lipase as a Highly Active and Enantioselective Catalyst for the Dynamic Kinetic Resolution of Secondary Alcohols**

Hyunjin Kim, Yoon Kyung Choi, Jusuk Lee, Eungyeong Lee, Jaiwook Park, and Mahn-Joo Kim**

Dedicated to Professor Eun Lee on the occasion of his retirement

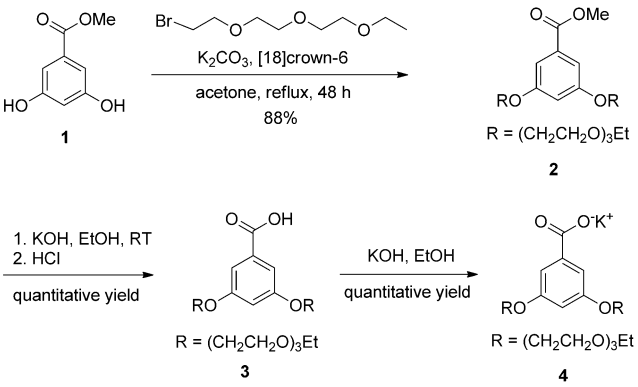
Dynamic kinetic resolution (DKR) is among the most practical methods for the transformation of racemates to single enantiomers.[1] DKR can provide high yields and excellent enantiopurities, both approaching 100%, which are difficult to achieve with classical kinetic resolution (KR).[2] In the past decade, enzymatic resolution coupled with metal-catalyzed racemization has been explored as a new strategy for DKR. Now several procedures are available for the DKR of racemic alcohols[3] and amines.[4] The wider applications of these DKR procedures in organic synthesis, however, can be hampered by the narrow specificity, moderate enantioselectivity, and low activity of the enzyme employed. Therefore, it is of great importance to develop a highly active enzyme having high enantioselectivity toward a wide range of substrates for DKR. We herein report that ionic-surfactant-coated *Burkholderia cepacia* lipase (ISCBCL) has great potential as such an excellent enzyme.

Commercially available *Burkholderia cepacia* lipase (BCL; brand names: Lipase PS and Lipase PS-C) displays significantly lower activity than *Candida antarctica* lipase B (CALB; brand name: Novozym 435) (Table 1, entries 1–3); the latter has been most frequently employed in the metalloenzymatic DKR. Fortunately, we have developed a practical approach for enhancing its activity dramatically. In a typical procedure, buffer-free soluble BCL (24% protein),[5] prepared from its crude preparation (Lipase PS), was lyophilized in the presence of an ionic surfactant **4** (Scheme 1) to yield ISCBCL. The activity of ISCBCL was dependent on the amount of **4** added and the type of solvent system used for lyophilization (Table 1, entries 4–6). The most active ISCBCL (18% protein), lyophilized in the presence of 0.30 mass equiv (23 wt%) of **4** in 1:1 (v/v) water–dioxane cosolvent system, displayed three orders of magnitude higher activity[6] than its crude counterpart (Table 1, entry 6). It was more active than Novozym 435 (20% protein).

Table 1: Activities of lipases.[a]

Entry	Lipase	Amount of **4** [wt%]	Activity[b] [mM h^{-1}]	Activation
1	Lipase PS[c]	–	0.24	
2	Lipase PS-C[d]	–	1.1×10	
3	Novozym 435	–	1.2×10^2	
4	ISCBCL[e]	13	6.4×10	2.7×10^2
5	ISCBCL[f]	13	1.4×10^2	6.0×10^2
6	ISCBCL[f]	23	2.5×10^2	1.0×10^3

[a] The activities were measured with solutions containing 1-phenylethanol (0.50 M), enzyme (1.0 mg), and isopropenyl acetate (1.5 equiv) in toluene at 25 °C. Rate measurements were done twice for each enzyme preparation. [b] Initial rate per mg of enzyme preparation. [c] Crude enzyme. [d] Ceramic-supported enzyme. [e] Lyophilized in 1:1 (v/v) water–THF. [f] Lyophilized in 1:1 (v/v) water–dioxane.



Scheme 1. Preparation of ionic surfactant.

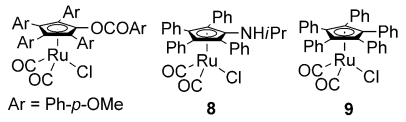
The ISCBCL showed excellent performance in the DKRs of 1-phenylethanol with three ruthenium complexes (**7**–**9**) as the racemization catalysts (Table 2). The ISCBCL-catalyzed DKR with complex **7**[7] proceeded most rapidly and reached completion at room temperature within 1 hour (Table 2, entry 1). The corresponding DKR with complexes **8**[8] and **9**[9] also proceeded well but the reactions were complete in 2 h (Table 2, entries 2 and 3, respectively). Previously, the fastest DKR of 1-phenylethanol took 3 h and was performed with Novozym 435 and **7** or **9**.[7,9] It has been known that Novozym 435 impedes the ruthenium-catalyzed racemiza-

[*] H. Kim, Y. K. Choi, J. Lee, E. Lee, J. Park, Prof. M.-J. Kim
Department of Chemistry
Pohang University of Science and Technology (POSTECH)
San-31 Hyojadong, Pohang 790-784 (Republic of Korea)
E-mail: mjkim@postech.ac.kr

[**] This work was supported by the National Research Foundation of Korea (KRF-2007-511-C00031 and 2009-0074357).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201104141.

Table 2: DKR of 1-phenylethanol with ISCBCL.[a]



Entry	Ru cat.	*t* [h]	Yield [%][b]	*ee* [%][c]
1	**7**	1	95	99
2	**8**	1 (2)	75 (95)	99
3	**9**	1 (2)	85 (97)	99

[a] Performed with solutions containing substrate (1.0 mmol), ISCBCL (23 wt% **4**, 10 mg), Ru catalyst (4.0 mol%), base (1 equiv), and isopropenyl acetate (IPA, 1.5 equiv) in toluene (2.0 mL) at RT. [b] Yield of isolated product. [c] Determined by HPLC using a chiral stationary phase.

tion. In contrast, no significant decrease in racemization rate was observed with ISCBCL. All the results indicate that ISCBCL is more efficient than Novozym 435 as the enzyme for DKR.

To see the scope of ISCBCL-catalyzed DKR, 24 different secondary alcohols were chosen as the substrates (**10a–x**, Figure 1). They carry an aliphatic chain and an aromatic ring at the hydroxymethine center (RCH(OH)Ar). The length of

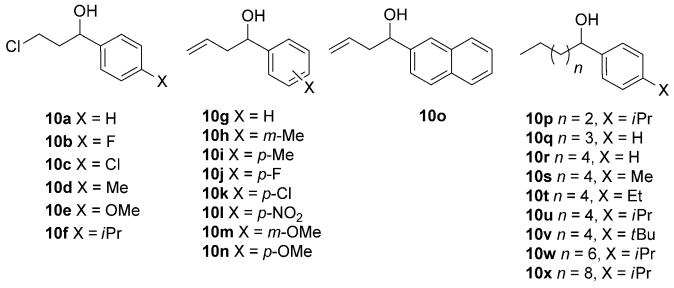
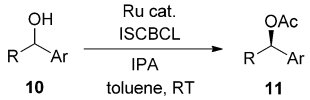


Figure 1. Substrates for DKR.

aliphatic chain ranges widely from three-carbon units such as chloromethyl (**10a–f**) and allyl (**10g–o**) to ten-carbon unit such as *n*-decyl (**10x**). To the best of our knowledge, their DKRs have not been studied previously. It is noted that Novozym 435 is practically inapplicable to their DKRs owing to its narrow specificity.[10]

The DKR reactions of **10a–x** were carried out with solutions containing substrate (0.1–0.3 mmol), ISCBCL (10–30 mg mmol^{-1} substrate), ruthenium complex (**7** or **8**, 4.0–5.0 mol%), K_2CO_3 or Na_2CO_3 (1 equiv), and isopropenyl acetate (IPA, 1.5 equiv) in toluene at room temperature (Table 3). Most of the DKR reactions were complete within 24 h except those of **10e**, **10f**, and **10v**, which took 48 h. In most cases excellent yields and high enantiopurities were obtained (Table 3).[11] These results indicate that all the

Table 3: DKR of secondary alcohols with ISCBCL.



Entry	Substr.	Ru cat.	Prod.	Yield [%][a]	*ee* [%][b]
1	**10a**	**7**	**11a**	96	98
2	**10b**	**7**	**11b**	98	95
3	**10c**	**7**	**11c**	94	98
4	**10d**	**7**	**11d**	90	97
5	**10e**	**7**	**11e**	98	96
6	**10f**	**7**	**11f**	95	99
7	**10g**	**8**[c]	**11g**	96	98
8	**10h**	**8**[c]	**11h**	96	95
9	**10i**	**8**[c]	**11i**	97	97
10	**10j**	**8**[c]	**11j**	97	96
11	**10k**	**8**[c]	**11k**	96	98
12	**10l**	**8**[c]	**11l**	93	96
13	**10m**	**8**[c]	**11m**	95	98
14	**10n**	**8**[c]	**11n**	96	97
15	**10o**	**8**[c]	**11o**	97	97
16	**10p**	**8**[c]	**11p**	94	>99
17	**10q**	**7**	**11q**	97	82
18	**10r**	**7**	**11r**	96	82
19	**10s**	**7**	**11s**	97	81
20	**10t**	**7**	**11t**	97	96
21	**10u**	**7**	**11u**	96	99
22	**10v**	**7**	**11v**	93	99
23	**10w**	**7**	**11w**	96	99
24	**10x**	**7**	**11x**	91	99

[a] Yield of isolated product. [b] Determined by HPLC using a chiral stationary phase. [c] The use of **7** resulted in the formation of a significant amount of byproducts.

substrates tested are accepted by ISCBCL at synthetically useful rates and most of them are transformed with high enantioselectivity. Relatively lower enantiopurities (81–82% *ee*) were observed for the DKRs of substrates carrying a longer aliphatic chain (Table 3, entries 17–19). In contrast to this, the DKRs of substrates with *i*Pr or *t*Bu at the *para* position of phenyl ring gave the highest enantiopurities (99% *ee* or greater) regardless of the length of the aliphatic chain (Table 3, entries 6, 16, and 21–24). In general the enantioselectivity of ISCBCL decreased with the length of the aliphatic chain (compare entries 1, 7, 17, and 18) and increased with increasing bulk of the *para* substituent on the phenyl ring (compare entries 18–22).

All the substrates shown in Figure 1, which have a linear aliphatic side chain and an aromatic ring at the hydroxymethine center, are transformed with the same *R* enantioselectivity[12] in DKR. It would be nice if the enantioselectivity could be switched for the preparation of the opposite enantiomers. We hypothesized that the enantioselectivity should be changed if the aliphatic side chain is sterically enlarged relative to the aromatic ring. To test this hypothesis, α-phenylpropargyl alcohol (**12a**) and its trimethylsilyl (TMS) derivative **13a** were chosen as model substrates (Figure 2). It was found that the DKR of **13a**[13] followed by desilylation with tetrabutylammonium fluoride (TBAF) yielded (*S*)-**14a**, the enantiomer of (*R*)-**14a** produced by the enzymatic acylation of **12a** (Scheme 2). These results prove that the

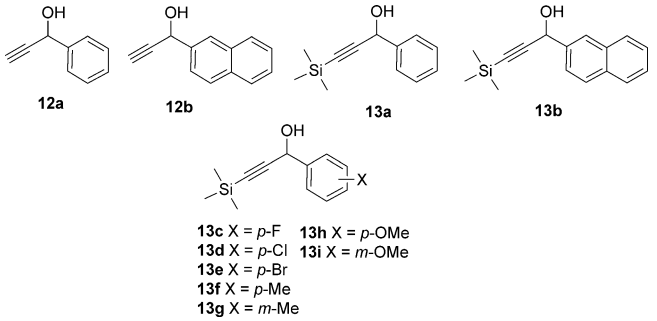
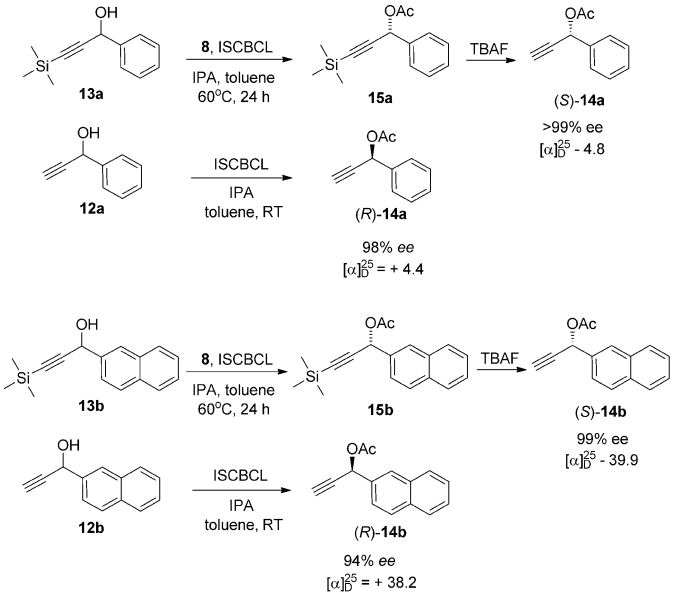


Figure 2. α-Arylpropargyl alcohols.



Scheme 2. KR and DKR of α-arylpropargyl alcohols with ISCBCL.

enantioselectivity was switched between **12a** and **13a**.[14] The switchable enantioselectivity was also confirmed between **12b** and **13b** (Scheme 2). The DKRs of TMS-protected α-arylpropargyl alcohols (**13a–i**) provided excellent enantiopurities approaching 100%[15] with high yields (Table 4), indicating that the switching of enantioselectivity is nearly perfect. The results also imply that sterically demanding substrates can be accepted by ISCBCL with high enantioselectivity at synthetically useful rates.

Table 4: DKR of TMS-protected propargyl alcohols with ISCBCL.[a]

Entry	Substr.	Prod.	Yield [%][b]	*ee* [%][c]
1	**13a**	**15a**	94	>99
2	**13b**	**15b**	89	98
3	**13c**	**15c**	91	96
4	**13d**	**15d**	90	96
5	**13e**	**15e**	91	96
6	**13f**	**15f**	94	>99
7	**13g**	**15g**	92	>99
8	**13h**	**15h**	90	98
9	**13i**	**15i**	91	98

[a] See the Supporting Information for a representative procedure. [b] Yield of isolated product. [c] Determined by HPLC using a chiral stationary phase.

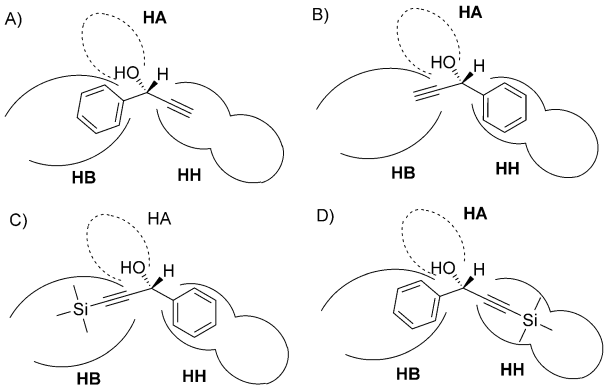


Figure 3. Active-site model of ISCBCL. A,C) Binding modes of more reactive enantiomers. B,D) Binding modes of less reactive enantiomers.

The switchable enantioselectivity of ISCBCL can be explained using a model of the active site based on the X-ray structure of BCL[16] (Figure 3). There are three binding pockets (HA, HB, and HH) at the active site of BCL for anchoring three substituents at the stereocenter of substrate. Among them, the HH binding pocket seems to play an essential role in determining which enantiomer can be accepted more favorably. It has two rooms, a hydrophilic trench, and its entrance (a space of 4.5 Å in diameter), which are separated by a contraction.[16a] Small aliphatic groups such as methyl and ethynyl can be nicely fitted into the pocket (Figure 3A). The binding of flat aromatic rings such as benzene and naphthalene into the pocket is possible (Figure 3B,C) but with some difficulty because their increased sizes should give rise to some repulsive effects around the contraction. The binding of branched and bulky aliphatic groups such as TMS-ethynyl into the same pocket is expected to be even more difficult owing to severe steric repulsion around the contraction (Figure 3D). Therefore, the enantiomers shown in Figure 3A and C react much faster than their respective antipodes in Figure 3B and D. The perfect enantioselectivity of ISCBCL for *para*-isopropyl- and *para*-*tert*-butylphenylalkanols (**10f**, **10p**, **10u–x**) also can be rationalized using the active-site model. The linear aliphatic groups can be fitted into the HH pocket without difficulty while the branched alkyl-substituted aromatic rings are

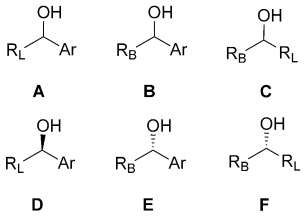


Figure 4. Three different types of secondary alcohols (**A**–**C**) and their enantiomers (**D**–**F**) reacting more rapidly in the ISCBCL-catalyzed DKRs than the others (R = aliphatic, Ar = aromatic, L = linear, and B = branched and bulky).

unable to enter the pocket owing to severe steric repulsion near the contraction.

All the substrates studied in this work can be classified into two types, **A** and **B**, according to the shape of the aliphatic substituent (Figure 4). The results from their DKRs and the active-site model in Figure 3 indicate that the corresponding enantiomers **D** and **E** should react more rapidly than their antipodes in the ISCBCL-catalyzed DKR. For the third type of substrates **C** with two aliphatic substituents at the hydroxymethine center, which have not been studied in this work, **F** should be the more reactive enantiomer. The structural features of **D**, **E**, and **F** can serve as the rules for predicting the enantioselectivity in the ISCBCL-catalyzed DKRs of other secondary alcohols. They also provide guidelines for the rational design of substrates for highly enantioselective DKR.

In conclusion, we have developed a highly active enzyme by coating *Burkholderia cepacia* lipase with the ionic surfactant **4** for use in DKR. With this enzyme (ISCBCL), we were able to achieve the fastest DKR of 1-phenylethanol, the highly enantioselective DKR of a wide range of secondary alcohols (RCH(OH)Ar, $R = C_3$–C_{10}) previously unexplored, and, for the first time, the switching of lipase enantioselectivity in DKR. The switchable enantioselectivity of ISCBCL depending on the shape of aliphatic chain (R) makes it possible to prepare selectively either *R* or *S* enantiomers by DKR. We have also demonstrated that the ISCBCL-catalyzed DKRs provide routes to esters of enantioenriched γ-chlorohydrins and homoallyl and propargyl alcohols, which are useful as versatile building blocks in asymmetric synthesis.[17] Overall, ISCBCL appears to be highly promising as the catalyst for the DKR of secondary alcohols.

Experimental Section

Preparation of ISCBCL: A suspension of crude lipase (6.0 g, Lipase PS from Amano) in a 0.1 M phosphate buffer (50 mL, pH 7.8) was stirred for 10 min at room temperature. Insoluble materials were removed by centrifugation using a COMBI-514R centrifuge at 4 °C. The aqueous solution was then dialyzed using a Spectra/Por Membrane (MWCO: 10 K) against water for 2 days and then freeze-dried to give a white enzyme powder ($\approx$100 mg). For the preparation of ISCBCL, the enzyme powder (50 mg) was dissolved in deionized water (15 mL) and then mixed with **4** (7.5–15 mg, 0.15–0.30 mass equivalent) dissolved in THF or dioxane (15 mL). The resulting solution was freeze-dried to yield ISCBCL, which was then used for kinetic and dynamic kinetic resolution.

Received: June 16, 2011
Published online: September 27, 2011

Keywords: alcohols · catalysts · dynamic kinetic resolution · lipases · ruthenium

[1] a) J. H. Lee, K. Han, M.-J. Kim, J. Park, *Eur. J. Org. Chem.* **2010**, 999–1015; b) Y. Kim, J. Park, M.-J. Kim, *ChemCatChem* **2011**, *3*, 271–277; c) H. Pellissier, *Tetrahedron* **2008**, *64*, 1563–1601; H. Pellissier, *Tetrahedron* **2011**, *67*, 3769–3802; H. Pellissier, *Adv. Synth. Catal.* **2011**, *353*, 659–676.

[2] a) *Hydrolases in Organic Synthesis*, 2nd ed. (Eds.: U. T. Bornscheuer, R. J. Kazlauskas), Wiley-VCH, Weinheim, **2006**; b) *Asymmetric Organic Synthesis with Enzymes* (Eds.: V. Gotor, I. Alfonso, E. Garcia-Urdiales), Wiley-VCH, Weinheim, **2008**.

[3] For DKR of alcohols, see: a) S.-B. Ko, B. Baburaj, M.-J. Kim, J. Park, *J. Org. Chem.* **2007**, *72*, 6860–6864; b) M.-J. Kim, Y. K. Choi, S. Kim, D. Kim, K. Han, S.-B. Ko, J. Park, *Org. Lett.* **2008**, *10*, 1295–1298; c) R. M. Haak, F. Berthiol, T. Jerphagnon, J. A. Gayet, C. Tababiono, C. P. Postema, V. Ritleng, M. Pfeffer, D. B. Janssen, A. J. Minnaard, B. L. Feringa, J. G. De Vries, *J. Am. Chem. Soc.* **2008**, *130*, 13508–13509; d) Q. Chen, C. Yuan, *Chem. Commun.* **2008**, 5333–5335; e) Y. Do, I. C. Hwang, M.-J. Kim, J. Park, *J. Org. Chem.* **2010**, *75*, 5740–5742; f) S. Akai, R. Hanada, N. Fujiwara, Y. Kita, M. Egi, *Org. Lett.* **2010**, *12*, 4900–4903; g) L. K. Thalén, A. Sumic, K. Bogar, J. Norinder, A. K. A. Persson, J.-E. Bäckvall, *J. Org. Chem.* **2010**, *75*, 6842–6847.

[4] For DKR of amines, see: a) M. A. J. Veld, K. Hult, A. R. A. Palmans, E. W. Meijer, *Eur. J. Org. Chem.* **2007**, 5416–5421; b) A. N. Parvulescu, P. A. Jacobs, D. E. De Vos, *Chem. Eur. J.* **2007**, *13*, 2034–2043; c) M.-J. Kim, W.-H. Kim, K. Han, Y. K. Choi, J. Park, *Org. Lett.* **2007**, *9*, 1157–1159; d) A. N. Parvulescu, P. A. Jacobs, D. E. De Vos, *Adv. Synth. Catal.* **2008**, *350*, 113–121; e) K. Han, J. Park, M.-J. Kim, *J. Org. Chem.* **2008**, *73*, 4302–4304; f) L. H. Andrade, A. V. Silva, E. C. Pedrozo, *Tetrahedron Lett.* **2009**, *50*, 4331–4334; g) K. Han, Y. Kim, M.-J. Kim, *Tetrahedron Lett.* **2010**, *51*, 3536–3537; h) Y. Kim, M.-J. Kim, *Tetrahedron Lett.* **2010**, *51*, 5581–5584.

[5] The protein content was determined using the Bradford method. M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–254.

[6] It is assumed that the high activity of ISCBCL may result from two effects of the coating: the protection of enzymes against deactivation during lyophilization and the better dispersion of enzymes in organic solvent. It may be also speculated that the coating could influence the enzymatic activity by changing the pH and water content of enzyme preparation. It was observed that the changes of the pH and water content by coating were not significant. The pH of BCL preparation increased slightly from 6.4 before coating to 6.8 after coating while the water content, determined by the Karl-Fisher titration, decreased from 11–12% to 7–10%.

[7] J. H. Lee, N. Kim, M.-J. Kim, J. Park, *ChemCatChem* **2011**, *3*, 354–359.

[8] a) J. H. Choi, Y. H. Kim, S. H. Nam, S. T. Shin, M.-J. Kim, J. Park, *Angew. Chem.* **2002**, *114*, 2479–2482; *Angew. Chem. Int. Ed.* **2002**, *41*, 2373–2376; b) J. H. Choi, Y. K. Choi, Y. H. Kim, E. S. Park, E. J. Kim, M.-J. Kim, J. Park, *J. Org. Chem.* **2004**, *69*, 1972–1977.

[9] B. Martín-Matute, M. Edin, K. Bogár, F. B. Kaynak, J.-E. Bäckvall, *J. Am. Chem. Soc.* **2005**, *127*, 8817–8825.

[10] Novozym 435 accepts a limited range of secondary alcohols carrying a small and a significantly larger substituent at the hydroxymethine center with high enantioselectivity. In addition, the small substituent of secondary alcohol should be smaller than three-carbon unit for transformation at synthetically useful rate.

[11] It was observed from the NMR spectra of product mixtures that some of ionic surfactant in ISCBCL went into solution during the reaction.

[12] a) R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport, L. A. Cuccia, *J. Org. Chem.* **1991**, *56*, 2656–2665; b) Q. Jing, R. J. Kazlauskas, *Chirality* **2008**, *20*, 724–735.

[13] Here, we used ISCBCL lyophilized in the presence of 13 wt% **4** in 1:1 (v/v) water–THF because more active ISCBCLs were developed later.

[14] This can be also observed with coating-free BCL, but the reactions are expected to be very slow. In a previous study, the resolution of **3a** with Lipase PS took 320 h to reach 51%

conversion at 25 °C (P. Allevi, P. Ciuffreda, M. Anastasia, *Tetrahedron: Asymmetry* **1997**, *8*, 93 – 99).
[15] This is compared to a low enantioselectivity ($E = 16$) observed in the Lipase PS-catalyzed resolution described in Ref. [14].
[16] a) T. Schulz, J. Pleiss, R. D. Schmid, *Protein Sci.* **2000**, *9*, 1053 – 1062; b) D. Guieysse, C. Salagnad, P. Monsan, M. Remaud-Simeon, V. Tran, *Tetrahedron: Asymmetry* **2003**, *14*, 1807 – 1817.
[17] a) S. Denmark, J. Fu, *Chem. Rev.* **2003**, *103*, 2763 – 2793; b) L. Tietze, T. Kinzel, C. C. Brazel, *Acc. Chem. Res.* **2009**, *42*, 367 – 378; c) B. M. Trost, A. H. Weiss, *Adv. Synth. Catal.* **2009**, *351*, 963 – 983.

