DOI: 10.1002/chem.200500758

(*S*)-Selective Kinetic Resolution and Chemoenzymatic Dynamic Kinetic Resolution of Secondary Alcohols

Linnéa Borén, Belén Martín-Matute,* Yongmei Xu, Armando Córdova, and Jan-E. Bäckvall*[a]

Abstract: (*S*)-Selective kinetic resolution was achieved through the use of a commercially available protease, which was activated with a combination of two different surfactants. The kinetic resolution (KR) process was optimized with respect to activation of the protease and to the acyl donor. The KR proved to be compatible with a range of functionalized *sec*-alcohols, giving good to high enantiomeric ratio values (up to >200). The enzymatic resolution was combined with a ruthenium-catalyzed racemization to give an (*S*)-selective dynamic kinetic resolution (DKR) of *sec*-alcohols. The DKR process works under very mild reaction conditions to give the corresponding esters in high yields and with excellent enantioselectivities.

Keywords: enzyme catalysis · kinetic resolution · metal catalysis · proteases · racemization

Introduction

Although catalytic asymmetric synthesis has developed dramatically during the past few decades,[1] the most common way in industry to obtain enantiomerically pure compounds is still by kinetic resolution (KR) and chiral chromatography of racemic mixtures.[2] In this context, dynamic kinetic resolution (DKR) is a powerful tool for the conversion of a racemic substrate into one single enantiomer.[3] For an efficient DKR, it is required that the enantiomers of the racemic starting material can be interconverted (racemized) with a reasonable rate and that the kinetic resolution conditions are compatible with the in situ racemization process. The number of examples of chemoenzymatic DKR that combine an enzymatic KR with an in situ racemization method has increased during the past few years.[4–10] Lipases constitute the most popular class of hydrolases and have been extensively used by organic chemists in enantioselective transesterifications.[11] A powerful approach for DKR of alcohols is the combination of a lipase-catalyzed resolution with a transition-metal-catalyzed racemization.[4–6,9,10] Because naturally occurring lipases are (*R*)-selective for alcohols according to the Kazlauskas rule,[12,13] lipases can only be used to transform the racemic alcohol into the (*R*)-acetate. This is a limitation of the method and there is presently a need for engineering lipases in order to obtain mutants that are (*S*)-selective.

Serine proteases, a sub-class of hydrolases, are known to catalyze transesterifications similar to those catalyzed by lipases, but interestingly, often with reversed enantioselectivity.[14,15] Subtilisin Carlsberg is a commercially available serine protease that has been used for the (*S*)-selective resolution of alcohols with moderate enantioselectivity. Proteases contain a catalytic machinery that is the approximate mirror image of that in lipases (Figure 1).[12] This makes it possible to use subtilisin as an (*S*)-selective resolution catalyst.

Subtilisin Carlsberg is not a thermostable enzyme; after 35 minutes at 70 °C all the activity is lost.[16] The catalyst used for the racemization must therefore be active at ambient temperature. For this reason, Shvo's complex (**1**),[17]

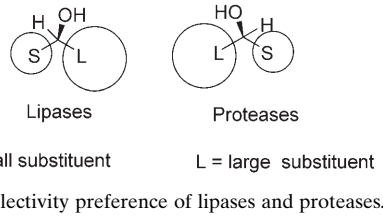
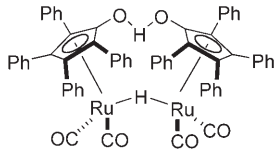


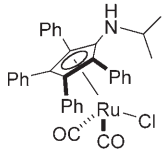
Figure 1. Enantioselectivity preference of lipases and proteases.

[a] L. Borén, Dr. B. Martín-Matute, Dr. Y. Xu, Dr. A. Córdova, Prof. Dr. J.-E. Bäckvall
Department of Organic Chemistry, Arrhenius Laboratory
Stockholm University, 106 91 Stockholm (Sweden)
Fax: (+46) 8-154-908
E-mail: belen@organ.su.se
jeb@organ.su.se

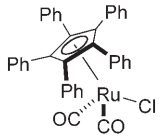
which has been successfully employed for DKR of *sec*-alcohols,[4] could not be used. Recently, Kim and Park et al. reported the use of catalyst **2** for the racemization of secondary alcohols at room temperature in a lipase-based DKR.[6] However, very long reaction times were required for this enzyme–ruthenium combination. The same catalyst was also employed for the (*S*)-selective DKR of alcohols using subtilisin Carlsberg as the enzyme component.[15] Again, very long reaction times (3–4 d) were required due to poor compatibility between the enzyme and the ruthenium catalyst. More recently, we discovered that catalyst **3** racemizes alcohols rapidly at room temperature,[5,18] and more importantly, combination of this catalyst with a lipase gave a highly efficient DKR at room temperature with reaction times down to 3 h.[5]



1

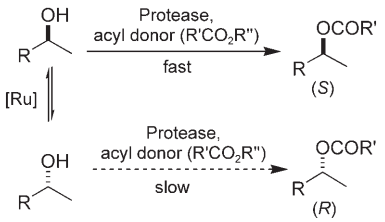


2



3

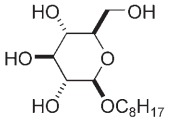
We now report the combination of this catalyst (**3**) with specially treated subtilisin Carlsberg for the (*S*)-selective DKR of secondary alcohols at room temperature. Subtilisin Carlsberg usually shows a low to moderate E value (E = Enantiomeric ratio of the enzyme) towards 1-phenylethanol (E = 4–36).[19] By using two different surfactants and employing isopropenyl valerate as the acyl donor, we have now been able to improve the E value for 1-phenylethanol to 66, and with this system, a range of functionalized *sec*-alcohols gave high E values, some up to >200. The enzymatic resolution under these conditions was highly compatible with the racemization catalyzed by **3**, resulting in a DKR with significantly shorter reaction times than those previously reported.[15] This DKR process yields acylated products with the opposite configuration to those obtained by the use of lipases (Scheme 1).



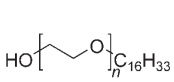
Scheme 1. (*S*)-Selective dynamic kinetic resolution.

Results and Discussion

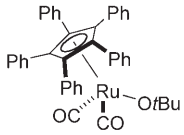
Kinetic resolution and racemization: A primary requirement for a successful DKR is that the KR conditions are compatible with those required for the racemization process. Toluene is an excellent solvent for the racemization catalyzed by complex **3**, however, subtilisin usually exhibits very low activity in organic solvents and an enhancement of the activity is required for an efficient kinetic resolution. Identification of the factors contributing to optimum enzyme activity in organic solvents is very difficult. Differences in the catalytic activity of enzymes in organic solvents and water can be related to changes in the structure and dynamic properties of the protein or changes in solvation.[20] It is known that one of the simplest ways to increase the activity of an enzyme in organic solvents is to coat the enzyme with a lipid or surfactant before lyophilization. Adsorption increases the surface area and also avoids denaturation of the enzyme during lyophilization.[21,22] Furthermore, surfactants help to lock the enzyme in a conformational state more suitable for catalysis.[19] We decided to improve the catalytic activity of subtilisin Carlsberg by physical modification with two surfactants:[23] octyl β-D-glycopyranoside (**4**) and Brij 56[24] (**5**).



4



5 ($n \approx 10$)



7

In a first attempt, the KR of 1-phenylethanol (**6a**) was performed in toluene at room temperature. Isopropenyl acetate (1.5 equiv) was used as the acyl donor because it is known to be compatible with the racemization reaction catalyzed by **3**.[5] The enzyme was treated with either surfactant **4** or **5**. However, we obtained not only very low enantioselectivity (E = 2–3), but also a very low yield of acetate (<2%) after 5 days. The use of a mixture of both surfactants did not show any improvement in the outcome of the reaction. Therefore, we next turned our attention to the use of THF as the solvent, as KRs catalyzed by subtilisin Carlsberg in THF have been reported previously.[12,14b] First, we tested whether the racemization catalyzed by complex **3** has a similar reaction rate in THF to that in toluene. We have previously reported the efficient activation of **3** by KO*t*Bu, and we identified the formation of the ruthenium alkoxide complex **7** as a key intermediate.[5] Therefore, **3** was activated by using a slight excess of KO*t*Bu in toluene or in THF at room temperature before adding (*S*)-1-phenylethanol ((*S*)-**6a**). As shown in Figure 2, the racemization takes place with very similar rates in the two solvents. We next turned our attention to the optimization of the subtilisin-catalyzed KR of 1-phenylethanol (**6a**) in THF.

In order to improve the enantioselectivity of the reaction, we studied the effect of different acyl donors on the outcome of the KR. An acyl donor that is commonly used in combination with subtilisin is 2,2,2-trihaloethyl butyrate.[25] When 2,2,2-trifluoroethyl butyrate (**8**) is used as an acyl

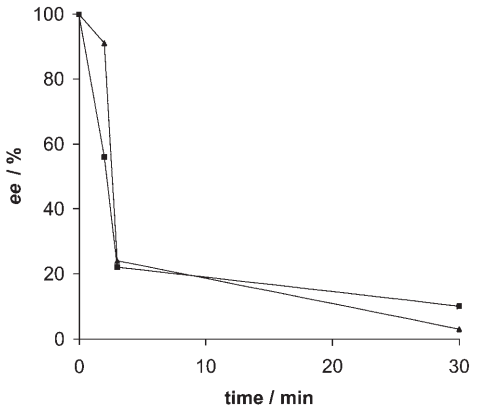
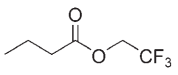
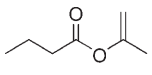


Figure 2. Racemization of (*S*)-1-phenylethanol ((*S*)-**6a**) (0.5 M) catalyzed by complex **3** after treatment with KO*t*Bu in toluene (▲) and in THF (■). Catalyst concentration: 0.0025 M (0.5 mol %).

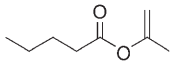
donor it yields trifluoroethanol as a byproduct. On the other hand, isopropenyl butyrate (**9**) has the same butyrate group as **8** but it yields only acetone as a byproduct. These byproducts can also influence the KR. To investigate how a longer carbon chain would affect the enantioselectivity of the reaction, isopropenyl valerate (**10**) and *p*-chlorophenyl octanoate (**11**) were tried. The surfactant used can also influence the enantioselectivity and the activity of the reaction. Table 1 shows the results obtained when varying the acyl donor, the surfactants, and the enzyme/surfactant ratio.



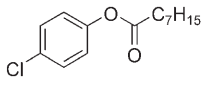
8



9



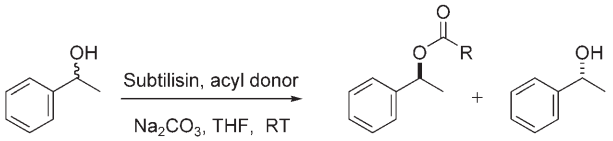
10



11

The acylation of 1-phenylethanol (**6a**) to yield the corresponding ester (**12a′**) catalyzed by subtilisin immobilized in Brij 56 (**5**) (entries 1, 2, 4, and 8) resulted in a slow but in general selective KR. On the other hand, the reaction catalyzed by subtilisin immobilized in octyl β-D-glycopyranoside (**4**) resulted in a fast but slightly less selective KR (entries 3 and 5–7). When the enzyme/surfactant ratio was increased, the difference between the reactions with the enzyme activated with either **4** or **5** decreased (entry 8 vs 9). Fortunately, the combination of both surfactants gave a fast and selective kinetic resolution. Thus, an enzyme/**4**/**5** ratio of 4:1:1 gave a similar E value, but the reaction took place faster (entry 10). When the acyl donors were varied, we observed that the KR becomes slower but more selective as the carbon chain of the acyl part becomes longer (cf. acyl donors **9** and **10** in entries 10 and 11, respectively). However, when this carbon chain is too long (acyl donor **11**), the reaction rate decreases considerably, and does not increase when double the amount of the supported enzyme is used (compare entries 12 and 13). Isopropenyl esters gave higher enantioselectivity than trifluoroethyl esters (compare entries 1 and 2). The best results were obtained by using isopropenyl valerate as the acyl donor, and with the enzyme treated with both surfactants in a subtilisin/**4**/**5** ratio of 4:1:1 (entry 11).

Table 1. Kinetic resolution of 1-phenylethanol (**6a**).[a]



rac-**6a** (*S*)-**12a'** (*R*)-**6a**

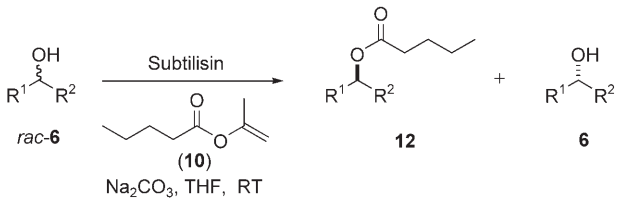
Entry	Immobilized enzyme [mg]	Enzyme/**4**/**5**	Acyl donor	Yield of **12a′** [%][b]	*ee* of **12a′** [%][c]	E[d]
1	15	1:0:1	**9**	14	96	57
2	15	1:0:1	**8**	30	93	40
3	15	1:1:0	**9**	42	88	30
4	11.3	2:0:1	**9**	18	96	60
5	6	2:1:0	**9**	40	89	31
6	11.3	2:1:0	**9**	40	90	34
7	22.5	2:1:0	**9**	51	85	36
8	9.4	4:0:1	**9**	32	92	36
9	9.4	4:1:0	**9**	30	91	31
10	11.3	4:1:1	**9**	52	84	35
11[e]	11.3	4:1:1	**10**	46	93	66
12	11.3	4:1:1	**11**	22	95	50
13	22.5	4:1:1	**11**	24	95	52

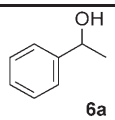
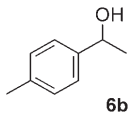
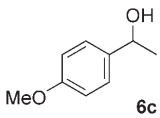
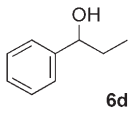
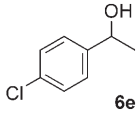
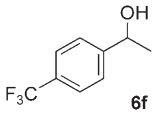
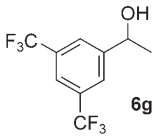
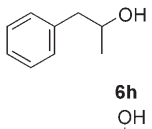
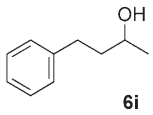
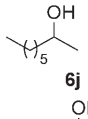
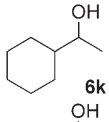
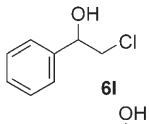
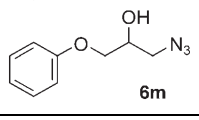
[a] Unless otherwise stated, all reactions were performed on a 1.0 mmol scale employing 1.5 equiv of acyl donor, and 1 mmol Na_2CO_3 in 2 mL of THF at room temperature for 17 h. [b] Determined by applying the formula conversion $= ee_s/(ee_s+ee_p)$, in which the subscripts s and p represent starting materials and products, respectively. [c] Determined by chiral GC by using a chiral CP-Chirasil-Dex CB column and employing racemic compounds as references. [d] Enantiomeric ratio (see ref. [3c]). [e] Reaction time 16 h.

The KR of a variety of alcohols was performed by using the reaction conditions shown in Table 1, entry 11 (Table 2). Similarly to 1-phenylethanol (**6a**) (Table 2, entry 1), the *p*-methyl-substituted alcohol (**6b**) gave the corresponding valerate (**12b**) with similar selectivity (E = 52; Table 2, entry 2). A slightly lower enantioselectivity was obtained for the *p*-methoxy-substituted alcohol **6c** (entry 3). The KR of ethyl carbinol **6d** proceeded with a rather low reaction rate (entry 4). Surprisingly, substrates bearing electron-withdrawing groups on the ring (**6e**–**g**) gave the corresponding esters in a highly enantioselective reaction (entries 5–7). Non-benzylic alcohols **6h**–**k** also gave high E values. However, in some cases (**6i** and **6k**), the reaction rate was rather low. Also, some functionalized alcohols (**6l**–**n**) were subjected to KR (entries 12–14), but unfortunately the enzyme showed low enantioselectivity for these substrates.

Dynamic kinetic resolution: The combination of a metal-catalyzed racemization with an enzyme-catalyzed KR is not always straightforward. The metal may interfere with the enzyme to give poor resolution, or the enzyme may slow down or inhibit the racemization by the metal catalyst. In

Table 2. Kinetic resolution of secondary alcohols **6**.[a]

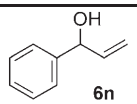


Entry	Substrate	*t* [h]	Yield of **12** [%][b]	*ee* of **12** [%][c]	E[d]
1[e]	 **6a**	16	46	93 (*S*)	66
2	 **6b**	17	47	91 (*S*)	52
3	 **6c**	18	45	91 (*S*)	47
4	 **6d**	17	34	95 (*S*)	63
5	 **6e**	15	46	97 (*S*)	170
6	 **6f**	17	41	97 (*S*)	133
7	 **6g**	16	40	>99 (*S*)	>200
8	 **6h**	16	40	96 (*S*)	95
9	 **6i**	17	25	97 (*S*)	90
10	 **6j**	15	45[f]	>99 (*S*)	>200
11	 **6k**	26	26[f]	97 (*S*)	91
12	 **6l**	24	27	88 (*R*)[g]	21
13	 **6m**	22	19	54 (*S*)	4

addition, the acyl donor, the by-products produced upon the acylation, or the surfactants can also interfere with the racemization or with the KR. We employed acyl donor **8** in the early experiments. Because it was not known how the activated enzyme would affect catalyst **3**, the DKR of 1-phenylethanol (**6a**) was optimized with respect to the combination of enzyme and surfactants (Table 3). The combination of surfactants **4** and **5** gave a better result than when just **4** was used for activation of the enzyme (entries 1–3 vs 4). When the amount of activated enzyme was decreased, the acylation rate decreased (entry 4 vs 5). As expected from Table 1, the best DKR result was obtained by using isopropenyl valerate (**10**) as the acyl donor and employing 18 mg of surfactant-treated subtilisin Carlsberg in an enzyme/**4**/**5** ratio of 4:1:1. These conditions resulted in 96% yield and 95% *ee* of **12a** after a reaction time of 18 h (entry 6).

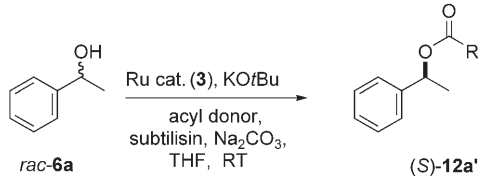
The DKR using subtilisin Carlsberg activated by **4** and **5** was extended to a range of secondary alcohols (Table 4). The catalyst (**3**) was activated by a catalytic amount of KO*t*Bu in the presence of the enzyme and Na_2CO_3.[5] The amount of base required depends on the amount of enzyme used and on the substrate. Therefore, the amount of base was optimized for each entry. The amount of enzyme used depends on each substrate too. For a successful DKR, the resolution rate should not exceed the racemization rate too much to avoid depletion of the resolved enantiomer; this could result in a decrease in *ee* of the ester product. Similar to 1-phenylethanol (**6a**), alcohol **6b** gave the corresponding ester in excellent yield and enantiomeric excess. The DKR of alcohol **6c**, bear-

Table 2. (Continued)

Entry	Substrate	*t* [h]	Yield of **12** [%][b]	*ee* of **12** [%][c]	E[d]
14	 **6n**	21	51	83 (*S*)	30

[a] Unless otherwise stated, all reactions were performed on a 1.0 mmol scale with 1.5 equiv of isopropenyl valerate (**10**), 18 mg of the enzyme surfactant mixture (enzyme/**4**/**5** = 4:1:1), 1 mmol Na_2CO_3, in 2 mL of THF at room temperature. [b] Determined by applying the formula: conversion = $ee_s/(ee_s+ee_p)$. [c] Determined by GC using a CP-Chirasil-Dex CB column using racemic compounds as references. [d] Enantiomeric ratio of the enzyme (see ref. [3c]). [e] 11.3 g of immobilized enzyme was employed. [f] Determined by ^{1}H NMR spectroscopy. [g] The configuration descriptor changes from *S* to *R* because of group priority changes as defined by the Cahn–Ingold–Prelog system.

Table 3. Dynamic kinetic resolution of 1-phenylethanol (**6a**).[a]



rac-**6a** (*S*)-**12a'**

Entry	Immobilized enzyme [mg]	Enzyme/**4**/**5**	*t* [h]	Yield of **12a'** [%][b]	*ee* of **12a'** [%][b]
1	30	1:1:0	19	>80	86
2	22.5	2:1:0	13	70	87
3[c]	22.5	2:1:0	13	70	90
4	11.25	4:1:1	16	93	91
5	7.5	4:1:1	17	67	92
6[d,e]	18	4:1:1	18	96	95

[a] Unless otherwise stated, all reactions were performed on a 1.0 mmol scale with 1.5 equiv of **8** as an acyl donor, 1 mmol Na_2CO_3, and 5 mol% **3**, 6 mol% KO*t*Bu, in 2 mL of THF at room temperature. [b] Determined by GC using a CP-Chirasil-Dex CB column using racemic compounds as references. [c] 7 mol% of KO*t*Bu. [d] 6 mol% **3**. [e] **10** was used as the acyl donor.

ing a methoxy group at the *para* position, was more sluggish, but the ester was obtained in 80% yield and 87% *ee* after 38 h. The ethyl-substituted carbinol (**6d**) also required longer reaction times due to a slow KR (vide supra), but by using 36 mg of enzyme added in two portions, a 70% yield of valerate **12d** with 94% *ee* was obtained. In general, excellent yields and enantioselectivities were obtained for aromatic substrates bearing electron-withdrawing groups (entries 5–7). Non-benzylic alcohols also gave the corresponding esters in good yields and with excellent enantioselectivities (entries 8–11). The latter results are particularly important because the aliphatic alcohols formed after hydrolysis of the esters are not readily accessible by asymmetric reduction. Synthesis of enantiomerically pure aromatic alcohols has been successfully accomplished through stereoselective hydrogenation of ketones and through transfer hydrogenation reactions, but the corresponding aliphatic substrates proceed with poor enantioselectivity.[26–29]

Conclusion

A commercially available protease, subtilisin Carlsberg, was efficiently activated by the use of two different surfactants: octyl β-D-glycopyranoside (**4**) and Brij 56[24] (**5**). Both of them increase the stability of the enzyme in organic solvents. Surfactant **4** enhances the catalytic activity of the enzyme and surfactant **5** improves the enantioselectivity. The combination of these two surfactants led to an optimal enzymatic resolution process, that is, fast reaction and high enantioselectivity. The enantioselectivity was further increased by the use of isopropenyl valerate (**10**) as the acyl donor, which has not previously been used in subtilisin-catalyzed KR. Subtilisin Carlsberg usually shows a low to moderate E value towards 1-phenylethanol (E = 4–36).[19] By using surfactants **4** and **5**, and acyl donor **10**, we have been able to improve the E value to 66. This system proved to be efficient for KR for a range of functionalized *sec*-alcohols, giving E values up to >200. The optimized KR was combined with a racemization catalyzed by ruthenium complex **3**, which rapidly racemizes alcohols at room temperature and thus allows the use of a nonthermostable enzyme. This resulted in an (*S*)-selective[13] DKR of *sec*-alcohols, which proceeded in high yields (up to 97%), with high enantioselectivity (up to 99%), and with short reaction times. The present combination of catalyst **3** and the specially treated subtilisin Carlsberg is about 4–5 times faster than the previously reported (*S*)-selective DKR.[15]

Experimental Section

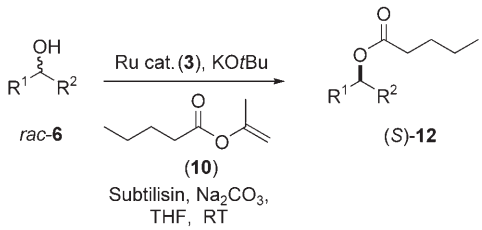
General: All reactions were carried out under a dry argon atmosphere in flame-dried glassware. Solvents were purified according to standard procedures. ^{1}H and ^{13}C NMR spectra were recorded at 400 or 300 MHz and at 100 or 75 MHz, respectively. Chemical shifts (δ) are reported in ppm, using the residual solvent peak in $CDCl_3$ ($\delta_H = 7.26$ and $\delta_C = 77.00$ ppm) as internal standard, and coupling constants (*J*) are given in Hz. Enantiomeric excesses were determined by using analytical gas chromatography with a CP-Chirasil-Dex CB chiral capillary column. Solvents for extraction and chromatography were of technical grade quality and distilled before use. Purification of synthesized material was performed by using column chromatography with Merck silica gel 60 (240–400 mesh).

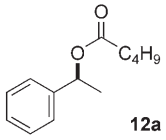
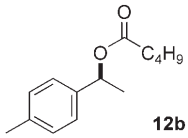
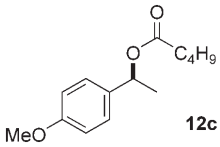
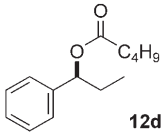
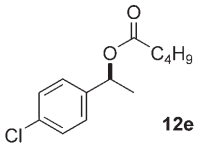
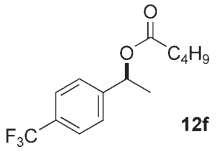
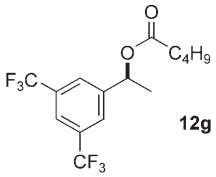
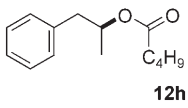
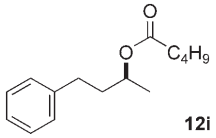
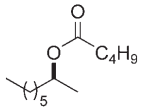
Commercially available alcohols were used without further purification. 2,2,2-Trifluoroethyl butyrate (**8**) was purchased from Sigma Aldrich. Ruthenium catalyst **3** was synthesized according to a literature procedure.[5]

Immobilization of subtilisin Carlsberg: Subtilisin Carlsberg (60 mg) was dissolved in a solution of octyl β-D-glycopyranoside (**4**; 15 mg) and Brij 56 (polyoxyethylene (10) cetyl ether, **5**; 15 mg) in a phosphate buffer (pH 7.2, 6 mL) and the mixture was rapidly frozen in liquid N_2 and lyophilized for 12 h.

Isopropenyl butyrate (9):[30] ^{1}H NMR (400 MHz, $CDCl_3$, 25°C) $\delta = 4.71$–4.58 (m, 2H; CH_2), 2.40–2.28 (m, 2H; CH_2), 1.87 (s, 3H; CH_3), 1.75–1.60

Table 4. Dynamic kinetic resolution of secondary alcohols **6**.[a]



Entry	Substrate	KO*t*Bu [mol%]	*t* [h]	Product	Yield [%][b]	*ee* [%][c]
1	**6a**	6	18	 **12a**	>96 (96)	95
2	**6b**	6	25	 **12b**	92	93
3	**6c**	6	38	 **12c**	80	87
4[d]	**6d**	6	43	 **12d**	70	94
5	**6e**	6	30	 **12e**	86 (84)	97
6	**6f**	7	25	 **12f**	94 (90)	97
7	**6g**	6	24	 **12g**	97 (94)	95
8[e]	**6h**	7	24	 **12h**	83	91
9[f]	**6i**	7	42	 **12i**	97 (94)	95
10	**6j**	6	41	 **12j**	80	99

(m, 2H; CH_2), 0.93 ppm (t, ^3J(H,H) = 7.2 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.56, 152.95, 101.73, 36.08, 19.43, 18.30, 13.43 ppm.

Isopropenyl valerate (10): This acyl donor was prepared according to a literature procedure for preparation of similar isopropenyl esters:[31] ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ = 4.69 (br s, 1H; C=C(*H*)H), 4.66 (br s, 1H; C=C(*H*)H), 2.37 (t, ^3J(H,H) = 7.4 Hz, 2H; CH_2), 1.92 (s, 3H; CH_3), 1.65 (quint, ^3J(H,H) = 7.5 Hz, 2H; CH_2), 1.37 (sext, ^3J(H,H) = 7.4 Hz, 2H; CH_2), 0.93 ppm (t, ^3J(H,H) = 7.4 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.56, 152.95, 101.73, 36.08, 19.43, 18.30, 13.43 ppm.

***p*-Chlorophenyl octanoate (11)**:[32] ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.32 (d, ^3J(H,H) = 8.8 Hz, 2H; 2× CH), 7.01 (d, ^3J(H,H) = 8.8 Hz, 2H; 2× CH), 2.54 (t, ^3J(H,H) = 7.6 Hz, 2H; CH_2), 1.80–1.70 (m, 2H; CH_2), 1.42–1.22 (m, 8H; 4× CH_2), 0.89 ppm (t, ^3J(H,H) = 6.8 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 172.28, 149.47, 131.27, 129.65, 123.18, 34.53, 31.86, 29.26, 29.12, 25.09, 22.81, 14.27 ppm.

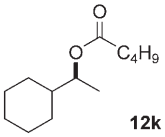
General procedure for the kinetic resolution of *sec*-alcohols

*Kinetic resolution of 1-phenylethanol (**6a**)*: In a typical experiment, surfactant-treated subtilisin Carlsberg (enzyme/**4**/**5** in a mass ratio 4:1:1; 18 mg) and Na_2CO_3 (106 mg, 1.0 mmol) were added to a dry Schlenk tube under argon. Then THF (2 mL), 1-phenylethanol (**6a**; 120 μL, 1.0 mmol), and isopropenyl valerate (**10**; 230 μL, 1.5 mmol) were added subsequently under argon. The mixture was stirred under an argon atmosphere at ambient temperature for 17 h. After filtration, the solvent was evaporated and the residue was analyzed by using chiral GC (see Table 2).

General procedure for the dynamic kinetic resolution of *sec*-alcohols

*Dynamic kinetic resolution of 1-phenylethanol (**6a**)*: A solution of KO*t*Bu (0.5 M in THF; 120 μL, 6 mol%) was added to a mixture of surfactant-treated subtilisin Carlsberg (18 mg), Na_2CO_3 (106 mg, 1.0 mmol), and ruthenium complex **3** (38 mg, 6 mol%) in THF (2 mL) in a 10 mL Schlenk tube under an argon atmosphere. The mixture was stirred and after 6 min, 1-phenylethanol (**6a**; 120 μL, 1.0 mmol) was added. After another 4 min isopropenyl valerate (**10**; 230 μL, 1.5 mmol) was added. After being stirred at ambient temperature for 18 h, the reaction mixture was filtered and concentrated. Purification by column

Table 4. (Continued)

Entry	Substrate	KO*t*Bu [mol%]	*t* [h]	Product	Yield [%][b]	*ee* [%][c]
11[g]	**6k**	8	18	 **12k**	67	95

[a] Unless otherwise stated, all reactions were performed on a 1.0 mmol scale with 1.5 equiv of **10**, 18 mg of the enzyme surfactant mixture (enzyme/**4**/**5** = 4:1:1), 1 mmol Na_2CO_3, 6 mol% of **3**, 6–8 mol% of KO*t*Bu, in 2 mL of THF at room temperature. Isolated yields in parentheses. [b] Determined by GC using a CP-Chirasil-Dex CB column using racemic compounds as references. [c] Enantiomeric excess. [d] The enzyme surfactant mixture was added in 2 portions (18 mg in each portion). The second portion was added after 16 h. [e] Reaction run at 38 °C. The enzyme surfactant mixture was added in 2 portions (12 mg in each portion). The second portion was added after 4.5 h. [f] 28 mg of supported enzyme were employed. After 24 h, a further 9 mg was added. [g] Reaction run at 38 °C and 30 mg of enzyme were employed.

chromatography (silica gel; pentane/diethyl ether 98:1) afforded (*S*)-1-phenylethyl pentanoate (**12a**) as a colorless oil (198 mg, 96%, 95% *ee*). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.25–7.36 (m, 5H; 5×CH), 5.91 (q, ^3J(H,H) = 6.4 Hz, 1H; CH), 2.33 (t, ^3J(H,H) = 7.6 Hz, 2H; CH_2), 1.61–1.66 (m, 2H; CH_2), 1.54 (d, ^3J(H,H) = 6.4 Hz, 3H; CH_3), 1.30–1.40 (m, 2H; CH_2), 0.91 ppm (t, ^3J(H,H) = 7.6 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 173.05, 141.85, 128.43, 127.74, 126.01, 71.97, 34.32, 27.00, 22.23, 22.19, 13.66 ppm.

*(S)-1-(4-Chlorophenyl)ethyl pentanoate (**12e**)*: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.31 (d, ^3J(H,H) = 8.8 Hz, 2H; 2×CH), 7.27 (d, ^3J(H,H) = 8.8 Hz, 2H; 2×CH), 5.84 (q, ^3J(H,H) = 6.4 Hz, 1H; CH), 2.32 (t, ^3J(H,H) = 7.6 Hz, 2H; CH_2), 1.63–1.56 (m, 2H; CH_2), 1.51 (d, ^3J(H,H) = 6.4 Hz, 3H; CH_3), 1.36–1.28 (m, 2H; CH_2), 0.90 ppm (t, ^3J(H,H) = 7.6 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 172.95, 140.38, 133.51, 128.62, 127.45, 71.26, 34.24, 26.97, 22.18, 22.16, 13.65 ppm.

*(S)-1-(4-Trifluoromethylphenyl)ethyl pentanoate (**12f**)*: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.60 (d, ^3J(H,H) = 8.2 Hz, 2H; 2×CH), 7.45 (d, ^3J(H,H) = 8.2 Hz, 2H; 2×CH), 5.91 (q, ^3J(H,H) = 6.9 Hz, 1H; CH), 2.34 (br d, ^3J(H,H) = 7.4 Hz, 2H; CH_2), 1.67–1.52 (m, 2H; CH_2), 1.53 (d, ^3J(H,H) = 6.6 Hz, 3H; CH_3), 1.3 (sext, ^3J(H,H) = 7.2 Hz, 2H; CH_2), 0.90 ppm (t, ^3J(H,H) = 7.4 Hz, 3H; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 172.89, 145.92, 129.94 (q, ^2J(^{13}C,^{19}F) = 32.4 Hz), 126.22, 125.45 (q, ^3J(^{13}C,^{19}F) = 3.7 Hz), 124.04 (q, ^1J(^{13}C,^{19}F) = 272.3 Hz), 71.29, 34.17, 26.95, 22.25, 22.18, 13.61 ppm.

*(S)-1-(3,5-Trifluoromethylphenyl)ethyl pentanoate (**12g**)*: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.78 (s, 3H; 3×CH), 5.95 (q, ^3J(H,H) = 6.6 Hz, 1H; CH), 2.37 (t, ^3J(H,H) = 6.3 Hz, 2H; CH_2), 1.62 (quint, ^3J(H,H) = 7.1 Hz, 2H; CH_2), 1.56 (d, ^3J(H,H) = 6.6 Hz, 3H; CH_3), 1.32 (sext, ^3J(H,H) = 6.8 Hz, 2H; CH_2), 0.90 ppm (t, ^3J(H,H) = 7.1 Hz, 3H; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 172.79, 146.54, 131.90 (q, ^2J(^{13}C,^{19}F) = 33.2 Hz), 126.18 (q, ^3J(^{13}C,^{19}F) = 2.6 Hz), 123.22 (q, ^1J(^{13}C,^{19}F) = 272.5 Hz), 121.73 (q, ^3J(^{13}C,^{19}F) = 3.7 Hz), 70.66, 34.9, 26.96, 22.29, 22.17, 13.57 ppm.

*(S)-1-Methyl-3-phenylpropyl pentanoate (**12i**)*: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.31–7.26 (m, 2H; 2×CH), 7.21–7.19 (m, 3H; 3×CH), 4.97 (sext, ^3J(H,H) = 6.3 Hz, 1H; CH), 2.74–2.57 (m, 2H; CH_2), 2.29 (t, ^3J(H,H) = 7.7 Hz, 2H; CH_2), 2.05–1.75 (m, 2H; CH_2), 1.69–1.58 (m, 2H; CH_2), 1.37 (sext, ^3J(H,H) = 7.5 Hz, 2H; CH_2), 1.25 (d, ^3J(H,H) = 6.1 Hz, 3H; CH_3), 0.94 ppm (t, ^3J(H,H) = 7.4 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 173.42, 141.55, 128.35, 128.26, 125.85, 70.13, 37.63, 34.35, 31.79, 27.11, 22.23, 20.01, 13.68 ppm.

Valerate esters **12b–d**, **12h**, **12j**, and **12k** were transformed to the corresponding alcohols and compared to commercially available pure samples by using NMR spectroscopy.

Acknowledgements

We are grateful for financial support from the Swedish Research Council, the Swedish Foundation for Strategic Research, and the Ministerio de Educación y Ciencia of Spain.

[1] a) J. Halpern, B. M. Trost, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5347; b) *Catalytic Asymmetric Synthesis*, 2nd ed. (Ed.: I. Ojima), Wiley-VCH, New York, **2000**.

[2] M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keβeler, R. Stürmer, T. Zelinski, *Angew. Chem.* **2004**, *116*, 806–843; *Angew. Chem. Int. Ed.* **2004**, *43*, 788–824.

[3] a) R. S. Ward, *Tetrahedron: Asymmetry* **1995**, *6*, 1475–1490; b) U. T. Strauss, U. Felfer, K. Faber, *Tetrahedron: Asymmetry* **1999**, *10*, 107–117; c) H. Stecher, K. Faber, *Synthesis* **1997**, *1*, 1–16.

[4] a) O. Pàmies, J.-E. Bäckvall, *Trends Biotechnol.* **2004**, *22*, 130–135; b) O. Pàmies, J.-E. Bäckvall, *Chem. Rev.* **2003**, *103*, 3247–3261; c) O. Pàmies, J.-E. Bäckvall, *Curr. Opin. Biotechnol.* **2003**, *14*, 407–413; d) M.-J. Kim, Y. Anh, J. Park, *Curr. Opin. Biotechnol.* **2002**, *13*, 578–587, and erratum: M.-J. Kim, Y. Anh, J. Park, *Curr. Opin. Biotechnol.* **2003**, *14*, 131; e) F. F. Huerta, A. B. E. Minidis, J.-E. Bäckvall, *Chem. Soc. Rev.* **2001**, *30*, 321–331.

[5] a) B. Martín-Matute, M. Edin, K. Bogár, J.-E. Bäckvall, *Angew. Chem.* **2004**, *116*, 6697–6701; *Angew. Chem. Int. Ed.* **2004**, *43*, 6535–6539; b) B. Martín-Matute, M. Edin, K. Bogár, F. B. Kaynak, J.-E. Bäckvall, *J. Am. Chem. Soc.* **2005**, *127*, 8817–8825.

[6] 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.

[7] For a DKR employing acid zeolites for racemization of benzylic alcohols, see: S. Wuyts, K. De Temmerman, D. E. De Vos, P. A. Jacobs, *Chem. Eur. J.* **2005**, *11*, 386–397.

[8] For a related DKR of amines, see: M. T. Reetz, K. Schimossek, *Chimia* **1996**, *50*, 668–669.

[9] a) G. K. M. Verzijl, J. G. de Vries, Q. B. Broxterman, *Tetrahedron: Asymmetry* **2005**, *16*, 1603–1610; b) B. A. C. van As, J. van Buijtenen, A. Heise, Q. B. Broxterman, G. K. M. Verzijl, A. R. A. Palmans, E. W. Meijer, *J. Am. Chem. Soc.* **2005**, *127*, 9964–9965.

[10] T. H. Riermeier, P. Gross, A. Monsees, M. Hoff, H. Trauthwein, *Tetrahedron Lett.* **2005**, *46*, 3403–3406.

[11] a) R. D. Schmid, R. Verger, *Angew. Chem.* **1998**, *110*, 1694–1720; *Angew. Chem. Int. Ed.* **1998**, *37*, 1608–1633; b) K. Faber, *Biotransformations in Organic Chemistry*, 4th ed., Springer, Berlin, **2000**.

[12] R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport, L. A. Cuccia, *J. Org. Chem.* **1991**, *56*, 2656–2665.

[13] The terms (*R*)- and (*S*)-selective are used for typical *sec*-alcohols in which the large group (cf. Figure 1) has the higher priority in the sequential rule for determining the configuration (according to the Cahn–Ingold–Prelog system).

[14] a) R. J. Kazlauskas, A. N. E. Weissfloch, *J. Mol. Catal. B* **1997**, *3*, 65–72; b) P. A. Fitzpatrick, A. M. Klibanov, *J. Am. Chem. Soc.* **1991**, *113*, 3166–3171; c) R. J. Kazlauskas, A. N. E. Weissfloch, *J. Org. Chem.* **1991**, *56*, 2656–2665.

[15] For an example of (*S*)-selective DKR, see: M.-J. Kim, Y. Chung, Y. Choi, H. Lee, D. Kim, J. Park, *J. Am. Chem. Soc.* **2003**, *125*, 11494–11495.
[16] Product information from Sigmaaldrich. Sigma. Prod. No. P 5380.
[17] a) Y. Blum, D. Czarkle, Y. Rahamin, Y. Shvo, *Organometallics* **1985**, *4*, 1459–1461; b) Y. Shvo, D. Czarkie, Y. Rahamin, *J. Am. Chem. Soc.* **1986**, *108*, 7400–7402; c) N. Menasche, Y. Shvo, *Organometallics* **1991**, *10*, 3885–3891.
[18] G. Csjernyik, K. Bogár, J.-E. Bäckvall, *Tetrahedron Lett.* **2004**, *45*, 6799–6802.
[19] *Hydrolases in Organic Synthesis* (Eds.: U. T. Bornscheurer, R. J. Kazlauskas), Wiley-VCH, Weinheim, **1999**.
[20] a) J. L. Schmitke, C. R. Wescott, A. M. Klibanov, *J. Am. Chem. Soc.* **1996**, *118*, 3360–3365; b) A. M. Klibanov, *Trends Biotechnol.* **1997**, *15*, 97–101.
[21] K. Dabulis, A. M. Klibanov, *Biotechnol. Bioeng.* **1993**, *31*, 41–53.
[22] a) A. M. Santos, M. Vidal, Y. Pacheco, J. Frontera, C. Báez, O. Ornellas, G. Barletta, K. Griebenow, *Biotechnol. Bioeng.* **2001**, *74*, 295–308; b) K. Griebenow, M. Vidal, C. Báez, A. M. Santos, G. Barletta, *J. Am. Chem. Soc.* **2001**, *123*, 5380–5381; c) A. M. Santos, M. González, Y. Pacheco, K. Griebenow, *Biotechnol. Bioeng.* **2003**, *84*, 324–331.
[23] F. Bordusa, *Chem. Rev.* **2002**, *102* , 4817–4868.
[24] Brij 56: polyoxyethylene(10) cetyl ether, $C_{16}H_{33}(OCH_2CH_2)_nOH$.
[25] a) M. Therisod, A. M. Klibanov, *J. Am. Chem. Soc.* **1986**, *108*, 5638–5640; b) S. Riva, J. Chopineau, A. P. G. Kieboom, A. M. Klibanov, *J. Am. Chem. Soc.* **1988**, *110*, 584–589.
[26] Review on metal-catalyzed hydrogenation of ketones: R. Noyori, T, Ohkuma, *Angew. Chem.* **2001**, *113*, 40–75; *Angew. Chem. Int. Ed.* **2001**, *40*, 40–73.
[27] Reviews on asymmetric transfer hydrogenation of ketones: a) M. J. Palmer, M. Wills, *Tetrahedron: Asymmetry* **1999**, *10*, 2045–2061; b) M. Wills, M. Palmer, A. Smith, J. Kenny, T. Walsgrove, *Molecules* **2000**, *5*, 4–18.
[28] For reduction of 2-alkanones, see: I. Sarvary, F. Almqvist, T. Frejd, *Chem. Eur. J.* **2001**, *7*, 2158–2166.
[29] Reduction of 2-hexanone using catalytic hydrogenation gives 96% yield of the alcohol in 75% *ee*, and reduction of 2-nonanone gives the alcohol in 100% yield, 1% *ee*. See ref. [26].
[30] Y.-F. Wang, J. J. Lalonde, M. Momongan, D. E. Bergbreiter, C.-H. Wong, *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.
[31] L. J. Goossen, J. Paetzold, D. Koley, *Chem. Commun.* **2003**, 706–707.
[32] D. Direktor, R. Effenberger, *J. Chem. Technol. Biotechnol. Chem. Technol.* **1985**, *35A*, 281–284.

Received: June 30, 2005
Published online: November 3, 2005

