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ARTICLE

Preparation of Passion Fruit-Typical 2-Alkyl Ester Enantiomers via Lipase-Catalyzed Kinetic Resolution

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The preparation of ester enantiomers (acetates, butanoates, hexanoates and octanoates) of the secondary alcohols 2-pentanol, 2-heptanol and 2-nonanol via lipase-catalyzed kinetic resolutions was investigated. Conversion rates and stereochemical courses of esterification and hydrolysis reactions catalyzed by commercially available enzyme preparations were followed for the homologous series of these passion fruit-typical 2-alkyl esters by capillary gas chromatography using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin as chiral stationary phase. An efficient method was developed to prepare the ester enantiomers via lipase-catalyzed esterifications: optically pure (*R*)-2-alkyl esters (ee > 99.9%) were obtained by esterification of the racemic alcohols with enantioselective *Candida antarctica* lipase B (immobilized) as catalyst. The subsequent esterification of the unreacted alcohols using lipase from *Candida cylindracea* yielded the optically enriched (*S*)-esters (ee > 81.4%). The separation of the products via liquid solid chromatography using a mixture of silica gel and aluminum oxide (basic) resulted in high chemical purities and yields (> 40 mol %).

KEYWORDS: Secondary alcohols; 2-alkyl esters; kinetic resolution; *Candida antarctica* lipase B; *Candida cylindracea* lipase

INTRODUCTION

The odd-numbered secondary alcohols 2-pentanol, 2-heptanol and 2-nonanol have been described as volatiles in both purple (*Passiflora edulis* Sims) and yellow (*Passiflora edulis* f. *flavicarpa*) passion fruits (*1*). Short-chain fatty acid esters (acetates, butanoates, hexanoates and octanoates) of these alkan-2-ols were shown to be suitable for a differentiation of the two varieties (*2*). Determinations of the natural enantiomeric ratios showed that nearly optically pure (*R*)-enantiomers of the 2-alkyl esters are characteristic volatiles of the purple variety and not detectable or present only at trace levels in the yellow fruits (*3*).

Enzyme-catalyzed kinetic resolutions of racemic substrates are well established approaches for the preparation of optically pure compounds (*4*). Hydrolytic enzymes, in particular lipases, that catalyze hydrolyses in aqueous medium as well as esterifications and transesterifications in organic solvents are widely employed. These biocatalysts accept a broad range of substrates and exhibit high regio- and enantioselectivity (*5*). Due to their importance in pharmaceutical and pesticide industry the lipase-catalyzed preparations of optically pure secondary alcohols are among the most extensively studied approaches in kinetic resolution (*6*). Based on the observed enantioselectivity of lipases toward secondary alcohols, a rule was proposed to predict which enantiomer reacts faster in lipase-catalyzed reactions (*7*).

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The use of porcine pancreas lipase as biocatalyst for the esterification of odd-numbered racemic secondary alcohols with various short-chain (*8*, *9*) and medium-chain (*10*) fatty acids showed preference for the (*R*)-alcohol, but reaction rate and enantioselectivity were not useful for preparative scale. Suitable esterification rates and high enantioselectivity were only achieved with higher laboratory efforts, such as removal of water by azeotropic distillation and immobilization of the enzyme (*11*). The esterifications of secondary alcohols with *Candida antarctica* lipase B, known as highly enantioselective for a broad range of chiral substrates (*12*), using activated acyl donors, such as succinic anhydride or vinyl acetate, exhibited high reaction rates while leaving the (*S*)-alcohol nearly unreacted (ee > 99%) (*13*). However, the direct application of short-chain fatty acids as substrates and the influence of the chain lengths of homologous secondary alcohols acid have not been pursued.

The objectives of this study were (i) to screen commercially available lipases and esterases regarding their potential to catalyze enantioselective hydrolysis and synthesis, respectively, of secondary alcohols and short-chain fatty acids, (ii) to investigate the influence of the chain lengths of the acyl and alcohol moieties, and (iii) to develop a method to prepare the passion fruit-typical 2-alkyl esters as optically pure compounds in a preparative scale.

MATERIALS AND METHODS

Enzymes. The following enzyme preparations were purchased from Sigma-Aldrich (Taufkirchen, Germany) and Fluka (Taufkirchen, Germany): *Candida antarctica* lipase B (CAL-B, Fluka 62288; 10.9

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U/mg), *Candida antarctica* lipase B immobilized on acrylic resin (CAL-B imm, Sigma-Aldrich L-4777; ≥10 U/mg), *Candida antarctica* lipase (CAL, Fluka 62299; 2.9 U/mg), *Candida cylindracea* lipase (CCL, Fluka 62316; 7.29 U/mg), *Penicillium roqueforti* lipase (PRL, Fluka 62308; 0.65 U/mg), *Aspergillus oryzae* lipase (AOL, Fluka 62285; 2.5 U/mg), porcine pancreas lipase (PPL, Sigma-Aldrich L-3126; 30−90 U/mg), esterase from *Mucor miehei* (MME, Fluka 46059; 1.1 U/mg) and pig liver esterase immobilized on Eupergit C (PLE, Fluka 46064; 0.2 U/mg).

Chemicals. 2-Pentanol (>98%), 2-heptanol (>99%), 2-nonanol (>97%), acetic acid (>99%), butyric acid (>99.5%), hexanoic acid (>98%) and octanoic acid (>98%) were obtained from Fluka (Taufkirchen, Germany). (*S*)-2-Pentanol, (*R*)-2-heptanol and (*S*)-2-nonanol were purchased from Aldrich, Steinheim, Germany. (*S*)-2-Heptanol was obtained from Fluka, Steinheim, Germany. (*R*)-2-Pentanol and (*R*)-2-nonanol were obtained from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. Silica gel 60 (0.063−0.200 mm) was purchased from Merck (Darmstadt, Germany) and aluminum oxide (basic; Brockman activity I) from Fluka (Taufkirchen, Germany).

Esters of the racemic secondary alcohols as well as esters of the enantiopure secondary alcohols were synthesized from the corresponding acyl chlorides using 4-dimethylaminopyridine as catalyst (*3*). Retention indices and mass spectrometric data were in accordance with those previously determined (*3*).

Solvents were distilled prior to use.

Enzyme Screening. In a screw-capped glass vial, 200 μmol of 2-heptanol and 200 μmol of butanoic acid were dissolved in 1 mL of *n*-heptane (dried over molecular sieves 3 Å). The reactions were started by addition of 80 units of enzyme and carried out on a rotary shaker for 24 h at room temperature (20−25 °C). For determination of conversion rate and enantiomeric ratios of substrate and product, aliquots of 20 μL were taken from the solution and diluted in 1000 μL of diethyl ether. The enzyme was removed using a syringe filter. After drying over anhydrous sodium sulfate, the sample was analyzed by capillary gas chromatography (system I).

Enzyme-Catalyzed Esterification. In a screw-capped glass vial, 200 μmol of secondary alcohol, 200 μmol of organic acid and the internal standard (2-heptanone, 22.8 mg) were dissolved in 1 mL of *n*-heptane (dried over molecular sieves 3 Å). After addition of 8 mg of CAL-B or CAL-B imm (80 units), the mixture was continuously stirred on a magnetic stirrer (300 rpm) at room temperature (20−25 °C). For the monitoring of esterification rates, aliquots of 20 μL were taken after defined intervals and treated as described above (enzyme screening). The samples were subjected to GC analysis (system II).

Enzyme-Catalyzed Ester Hydrolysis. In a screw-capped glass vial, 200 μmol of racemic ester was dissolved in 1 mL of potassium phosphate buffer solution (pH 7.4). After addition of 8 mg of CAL-B imm (80 units), the mixture was continuously stirred on a magnetic stirrer (300 rpm) at room temperature (20−25 °C). Aliquots of 20 μL were taken after defined intervals and extracted with 1000 μL of a mixture of *n*-pentane and diethyl ether [1:1, v/v]. The organic layer was dried over anhydrous sodium sulfate and analyzed by GC (system I).

Preparation of Optically Pure Esters. *Kinetic Resolution.* In a 100 mL round-bottom flask, 15 mmol of secondary alcohol and 15 mmol of organic acid were diluted in 75 mL of *n*-pentane (dried over molecular sieves 3 Å). The reaction was started by addition of 300 mg of CAL-B imm (3000 units) and carried out on a rotary shaker for 24 h (2-pentyl- and 2-heptyl esters) and 48 h (2-nonyl esters) at room temperature (20−25 °C). For the monitoring of conversion, aliquots of 5 μL were taken before addition of the enzyme and after termination of the reaction, diluted in 500 μL of diethyl ether, dried with anhydrous sodium sulfate and subjected to GC analysis (system I). After filtration of the enzyme through a round filter and washing of the enzyme, the solution was dried over anhydrous sodium sulfate and concentrated at ~35 °C to a final volume of 5 mL using a Vigreux column (30 cm×2 cm i.d.).

Liquid−Solid Chromatography (*LSC*). The sample was placed in a water-cooled glass column (40×1.6 cm i.d.) filled with a mixture of silica gel and aluminum oxide (basic) [1:1; w/w]. Fractions Ia [pentane/dichloromethane (2:1; v/v); 300 mL] and Ib [pentane/diethyl ether (9:1; v/v); 150 mL] contained the eluted ester. In fraction II (diethyl ether; 350 mL) the nonesterified alcohol was eluted. After removal of the solvent using a Vigreux column and a nitrogen stream the (*R*)-ester was obtained.

Re-esterification. Fraction II containing the nonesterified alcohol was concentrated to a volume of ~3 mL using a Vigreux column. The sample was transferred to a 100 mL round-bottom flask and diluted in 40 mL of *n*-pentane (dried over molecular sieves 3 Å). After addition of an equimolar amount (~8 mmol) of organic acid, the reaction was started with 1.1 g of CCL (~8000 units). For the monitoring of conversion, aliquots of 5 μL were taken before addition of the enzyme and after termination of the reaction, diluted in 500 μL of diethyl ether, dried with anhydrous sodium sulfate and subjected to GC analysis (system I). After filtration of the enzyme through a round filter and washing of the enzyme the solution was dried over anhydrous sodium sulfate and concentrated at ~35 °C to a final volume of 5 mL using a Vigreux column. The (*S*)-ester was obtained by subsequent fractionation and removal of the solvent as described above.

Calculation of Conversion Rates. Three methods were used to determine the conversion rate (*c*):

(i) The amount of produced ester was calculated via determination of the residual alcohol, using 2-heptanone as internal standard and taking into account the following FID response factors experimentally determined for the commercially available 2-alkanols (GC system II): 2-pentanol 1.07; 2-heptanol 1.0; 2-nonanol 0.66; 2-heptanone 1.07.

(ii) The conversion rate starting from a racemic substrate was calculated on the basis of the enantiomeric excesses of the substrate (ee_S) and the product (ee_P) (*14*):

$$c\,(\%) = \frac{ee_S}{ee_S + ee_P} \times 100$$

(iii) The conversion rate starting from an optically enriched substrate was calculated on the basis of the peak areas of the faster reacting substrate (A) and the corresponding product (P) and the slower reacting substrate (B) and the corresponding product (Q) (*14*):

$$c\,(\%) = 1 - \frac{A + B}{(A + P) + (B + Q)} \times 100$$

Determination of Enantioselectivities. The enantioselectivities (*E*) of the reactions were calculated on the basis of the conversion rate (*c*) and the enantiomeric excess of the substrate (ee_S) or the product (ee_P), respectively (*14*):

$$E = \frac{\ln[(1-c) \times (1-ee_S)]}{\ln[(1-c) \times (1+ee_S)]}$$

$$E = \frac{\ln[(1-c) \times (1+ee_P)]}{\ln[(1-c) \times (1-ee_P)]}$$

E was also calculated on the basis of ee_S and ee_P (*15*):

$$E = \frac{\ln[(1-ee_S)/(1+ee_S/ee_P)]}{\ln[(1+ee_S)/(1+ee_S/ee_P)]}$$

Capillary Gas Chromatography (HRGC-FID). *System I.* A Carlo Erba Mega 5160 series gas chromatograph equipped with a flame ionization detector (230 °C) was used. A chiral column was installed with 25% heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in SE54 (30 m×0.32 mm i.d., 0.25 μm film thickness). Synthesis of the cyclodextrin derivative and column preparation were carried out in-house (*16*). The temperature was programmed from 40 °C (2 min hold) to 200 at 2 °C/min. Injection was done in the split mode (220 °C; split ratio 1:15). Carrier gas used was hydrogen at a constant inlet pressure of 110 kPa. Data acquisition was done via Chromcard software (Thermo Fisher Scientific). The order of elution of the enantiomers was assigned by injection of optically pure commercially available (2-alkanols) and chemically synthesized (2-alkyl esters) reference compounds, respectively.

System II. Achiral analyses were performed on a Carlo Erba Mega II 8575 series gas chromatograph equipped with a split/splitless injector (215 °C, split ratio 1:10) and a flame ionization detector operating at 230 °C.

The column used was a 60 m×0.25 mm (i.d.) fused silica capillary column coated with DB-Wax (0.25 μm film thickness; J&W Scientific). The oven temperature was programmed from 40 °C (5 min hold) at 4 °C/min to 230 °C (25 min hold). Carrier gas used was hydrogen at a constant pressure of 105 kPa. Data acquisition was done via Chromcard software (Thermo Fisher Scientific).

Determination of Optical Rotations. Optical rotations were measured on a Polartronic-E polarimeter (Schmidt & Haensch; Berlin, Germany) fitted with a 16.5 mL measuring cell (path length 2 dm) and a sodium lamp (wavelength 589 nm). Samples were diluted in acetone at concentrations of 3 g/100 mL. Specific rotations were calculated as $[\alpha_D] = (\alpha_m \times 100)/(c \times l)$, where α_m is the optical rotation measured, c is the concentration [g/100 mL], and l is the length [dm] of the measuring cell.

RESULTS AND DISCUSSION

Kinetic Resolution via Hydrolysis. Based on the enantioselectivity reported for the hydrolysis of racemic 2-pentyl acetate by lipase B from *Candida antarctica* (CAL-B) (*13*), the potential of this biocatalyst to prepare optically pure short-chain fatty acid esters of 2-alkanols via hydrolysis-based kinetic resolutions was investigated as a first approach. As shown in **Table 1**, the hydrolyses of the 2-alkyl esters proceeded with high enantioselectivity for the (*R*)-esters. The conversion rates and the enantiomeric excesses determined for the reactions of the acetates are in accordance with previously reported data (*13*). However, with increasing chain lengths of the acyl moieties the conversion rates decreased, resulting in significantly reduced enantiomeric excesses of the unreacted ester substrates. Exemplarily, **Figure 1** shows the time-course of the conversion of 2-heptyl esters with immobilized CAL-B as catalyst; for 2-heptyl octanoate the conversion rate leveled off at approximately 20%. Duplicate experiments performed with 2-heptyl hexanoate also resulted in consistently low hydrolysis rates of 27.7% and 25.5%, respectively, after 8 h. Accordingly, this approach is not useful for a preparative scale. Addition of sodium taurocholate, known as a useful emulsifying agent in enzyme-catalyzed processes, e.g. with cholesterol esterases (*17*), did also not result in increased conversion rates.

Table 1. Enantioselectivity of CALB imm toward Esters of Secondary Alcohols (after 8 h Reaction Time)[a]

	conversion[b] (%)	ee_{ester}[c] (%)	$ee_{alcohol}$[d] (%)	E[e]
2-pentyl acetate	48.6	94.6	>99.9	>100
2-pentyl butanoate	42.6	74.3	>99.9	>100
2-pentyl hexanoate	36.8	58.3	>99.9	>100
2-pentyl octanoate	29.8	42.4	>99.9	>100
2-heptyl acetate	48.3	93.4	>99.9	>100
2-heptyl butanoate	39.3	64.7	>99.9	>100
2-heptyl hexanoate	27.7	38.3	>99.9	>100
2-heptyl octanoate	21.4	27.2	>99.9	>100
2-nonyl acetate	49.0	96.2	*f*	*g*
2-nonyl butanoate	37.2	59.4	*f*	*g*
2-nonyl hexanoate	27.6	38.1	*f*	*g*
2-nonyl octanoate	19.0	23.5	*f*	*g*

[a] *E*, enantioselectivity; ee, enantiomeric excess. [b] Calculated according to ref *14*. [c] Enantiomeric excess of the remaining (*S*)-enantiomer. [d] Enantiomeric excess of the formed (*R*)-enantiomer. [e] Calculated on the basis of ee_S according to ref *14*. [f] Enantiomers of 2-nonanol not separated; conversion calculated on the assumption that only (*R*)-2-nonanol is formed. [g] Enantioselectivity not calculated because of the missing separation of the enantiomers of 2-nonanol.

Kinetic Resolution via Esterification. As an alternative approach the enzyme-catalyzed esterification of the secondary alcohols was investigated. Several commercially available lipases and esterases were screened using the kinetic resolution of racemic 2-heptanol via esterification with butanoic acid in heptane as a test system (**Table 2**). Most of the employed enzyme preparations exhibited high enantioselectivity for the (*R*)-configured alcohol substrate. Only the lipase from *Penicillium roqueforti* showed a preference for the (*S*)-alcohol; *Candida cyclindracea* lipase and pig liver esterase did not react enantioselectively. As outlined in **Table 2**, the conversion rates determined after 24 h ranged from 0.1 to 56.6%. The highest yields were obtained with lipase from *Candida cylindracea* and with free and immobilized CAL-B lipase. CAL-B is known as an efficient biocatalyst in organic synthesis (*12*) showing high activity and enantioselectivity toward a wide range of secondary alcohols. All examples reported follow the empirical rule according to ref *7*, which implies that enantioselectivity is proportional to the difference in size between the large (L) and the medium (M) substituents of the substrate. Experiments with a series of varying M and L groups showed that CAL-B is highly enantioselective toward secondary alcohols with an M substituent smaller than *n*-propyl and an L substituent larger than *n*-propyl (*18*). Short chain secondary alcohols, such as the screened substrate 2-heptanol, fulfill the described molecular requirements.

On the other hand, the lipase preparation from *Candida cylindracea* (CCL; in recent times classified as *Candida rugosa*) showed a high conversion rate with only a slight preference for the (*R*)-alcohol. Contradictory results concerning the enantioselectivity

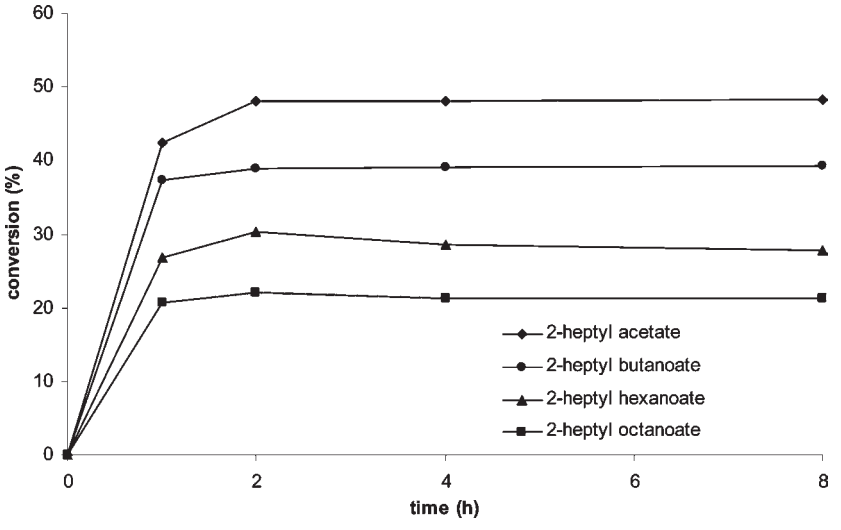

Figure 1. Conversion rates (%) determined for the hydrolysis of 2-heptyl esters catalyzed by CALB imm.

of CCL have been reported in the literature. It has been shown to be a highly stereospecific enzyme (*13*, *19*), but also an unspecific biocatalyst (*20*, *21*). In contrary to CAL-B, the predictive rule according to (*7*) does not apply to the kinetic resolution of acyclic alcohols by CCL. Furthermore, it is well-known that the enantiopreference of CCL is highly dependent on the polarity of the reaction medium (*22*). Because of very low conversion rates the other screened lipases and the esterases from *Mucor miehei* and porcine liver were not considered suitable for an application on preparative scale.

Figure 2 shows the time-course of the esterification of 2-heptanol with a series of homologous short-chain organic acids (C_2−C_8) in heptane using immobilized CAL-B as biocatalyst. For all synthesized 2-heptyl esters maximum esterification rates (46−50%) were achieved after a short reaction time of 2−4 h. Differences in the synthesis rates of the esters could only be seen in the initial stages of the reaction. Duplicate experiments performed exemplarily for 2-heptyl butanoate resulted in consistently high conversion rates of 49.8% and 49.9% after 24 h.

Conversion rates and enantioselectivities determined for the synthesis of the homologous 2-alkyl esters after 24 h are listed in **Table 3**. For the 2-pentyl esters significant differences between the free and the immobilized enzyme were observed. The use of free CAL-B led to higher conversion rates (> 50%) but decreased enantioselectivity toward the secondary alcohol (E < 100). The phenomenon of changing the selectivity of enzymes by immobilization is well-known in biocatalysis (*23*). For CAL-B remarkable differences in enantioselectivity were found depending

Table 2. Screening of Enzymes for Kinetic Resolution of Racemic 2-Heptanol via Esterification with Butanoic Acid in Heptane (after 24 h Reaction Time)[a]

enzymes	conversion[b] (%)	ee_{ester} (%)	$ee_{alcohol}$ (%)	E[c]
lipases				
Candida antarctica lipase B	47.0	>99.8 (*R*)	88.4 (*S*)	>100
Candida antarctica lipase B (imm)	49.1	>99.1 (*R*)	95.5 (*S*)	>100
Candida antarctica lipase	6.0	36.3 (*R*)	2.3 (*S*)	2.2
Candida cylindracea lipase	56.6	6.6 (*R*)	8.6 (*S*)	1.2
Penicillium roqueforti lipase	1.0	57.1 (*S*)	0.6 (*R*)	3.7
Aspergillus oryzae lipase	0.1	>99.9 (*R*)	0.1 (*S*)	>100
porcine pancreas lipase	1.7	85.4 (*R*)	1.5 (*S*)	>12.9
esterases				
Mucor miehei esterase	4.7	>99.9 (*R*)	4.9 (*S*)	>100
pig liver esterase	24.1	2.7 (*S*)	2.7(*R*)	1.2

[a] E, enantioselectivity; ee, enantiomeric excess. [b] Calculated according to ref *14*. [c] Calculated according to ref *15*.

Table 3. Enantioselectivity of CALB and CALB imm toward Secondary Alcohols Using Short-Chain Acids as Acyl Donors (after 24 h Reaction Time)[a]

		conversion[b] (%)	ee_{ester}[c] (%)	$ee_{alcohol}$[d] (%)	E[e]
CALB	2-pentyl acetate	52.1	83.6	85.3	30
	2-pentyl butanoate	53.2	81.4	93.1	33
	2-pentyl hexanoate	52.0	85.3	87.5	36
	2-pentyl octanoate	49.5	93.0	86.4	77
CALB imm	2-pentyl acetate	46.1	99.2	82.0	>100
	2-pentyl butanoate	43.9	99.7	73.6	>100
	2-pentyl hexanoate	45.2	99.1	73.7	>100
	2-pentyl octanoate	47.6	99.1	82.8	>100
CALB	2-heptyl acetate	42.3	99.4	86.5	>100
	2-heptyl butanoate	47.1	99.8	88.4	>100
	2-heptyl hexanoate	46.9	99.3	87.4	>100
	2-heptyl octanoate	47.2	99.5	89.8	>100
CALB imm	2-heptyl acetate	46.5	99.8	83.6	>100
	2-heptyl butanoate	49.8	99.1	95.5	>100
	2-heptyl hexanoate	46.0	99.9	83.4	>100
	2-heptyl octanoate	49.5	99.8	95.3	>100
CALB	2-nonyl acetate	46.8	99.8	*f*	*g*
	2-nonyl butanoate	47.5	99.1	*f*	*g*
	2-nonyl hexanoate	46.2	99.8	*f*	*g*
	2-nonyl octanoate	46.2	99.8	*f*	*g*

[a] E, enantioselectivity; ee, enantiomeric excess. [b] Calculated via determination of the residual alcohol using 2-heptanone as internal standard (see Materials and Methods: Calculation of Conversion Rates). [c] Enantiomeric excess of the (*R*)-enantiomer. [d] Enantiomeric excess of the (*S*)-enantiomer. [e] 2-Pentyl- and 2-heptyl esters: calculated according to ref *15*. 2-Nonyl esters: calculated on the basis of ee_P according to ref *14*. [f] Enantiomers of 2-nonanol not separated. [g] Enantioselectivity not calculated because of the missing separation of the enantiomers of 2-nonanol.

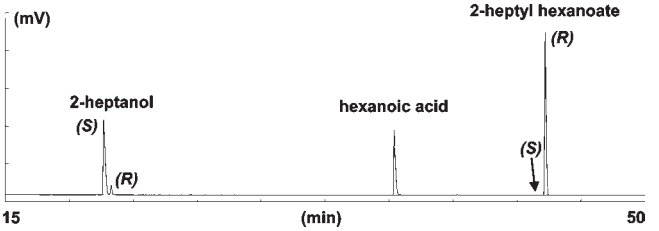

Figure 3. Kinetic resolution of racemic 2-heptanol via esterification with hexanoic acid and CALB imm (after 24 h reaction time; for GC conditions see Materials and Methods).

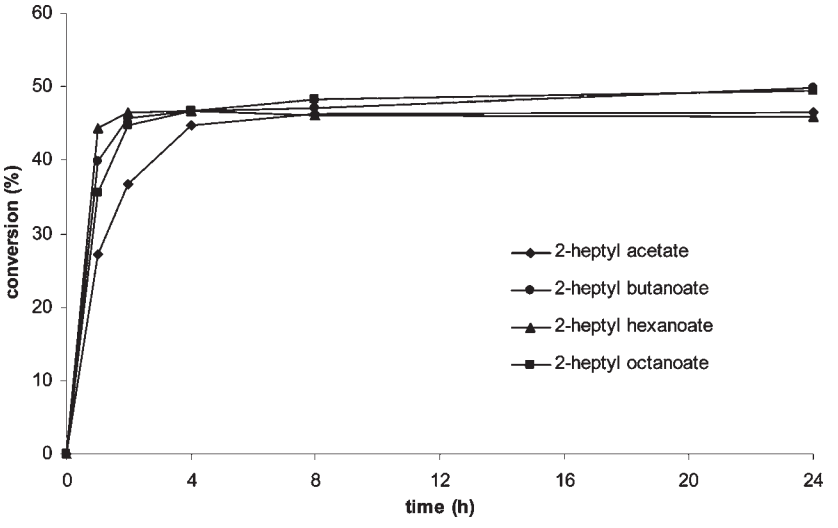

Figure 2. Conversion rates (%) determined for the synthesis of 2-heptyl esters catalyzed by CALB imm.

Table 4. Yields, Chemical Purities, Enantiomeric Excesses (ee), and Optical Rotations [α_D] of the Prepared (*R*)- and (*S*)-Esters

		(*R*)-ester				(*S*)-ester				
product	(*R*,*S*)-alcohol, initial wt [g]	yield [g/mol %]	purity (GC) [%]	opt. purity (ee) [%]	α_D [deg]	yield [g/mol %]	purity (GC) [%]	opt. purity (ee) [%]	α_D [deg]	$\alpha_{D\ calcd}$ [deg]
2-pentyl acetate	1.32	0.81/41.5	99.7	>99.9	−16.01	0.78/40.0	99.6	81.4	+13.08	+13.03
2-pentyl butanoate	1.32	1.02/43.2	99.7	>99.9	−16.61	0.96/40.4	99.5	83.9	+14.02	+13.94
2-pentyl hexanoate	1.32	1.21/43.4	99.3	>99.9	−15.01	1.13/40.3	99.6	83.1	+12.49	+12.47
2-pentyl octanoate	1.32	1.40/43.6	99.0	>99.9	−13.43	1.34/41.7	99.0	83.7	+11.22	+11.24
2-heptyl acetate	1.74	1.04/43.8	99.5	>99.9	−6.32	0.95/40.1	99.0	82.6	+5.29	+5.22
2-heptyl butanoate	1.74	1.27/45.6	99.6	>99.9	−9.40	1.23/43.9	99.3	82.7	+7.81	+7.77
2-heptyl hexanoate	1.74	1.47/45.8	99.7	>99.9	−9.45	1.37/42.6	99.7	86.9	+8.37	+8.22
2-heptyl octanoate	1.74	1.57/43.2	99.2	>99.9	−8.88	1.46/40.1	99.2	82.3	+7.44	+7.31
2-nonyl acetate	2.16	1.16/41.4	99.8	>99.9	−4.51	1.14/40.9	99.8	82.9	+3.67	+3.74
2-nonyl butanoate	2.16	1.29/40.0	99.7	>99.9	−7.27	1.30/40.7	99.9	88.2	+6.51	+6.41
2-nonyl hexanoate	2.16	1.46/40.2	99.7	>99.9	−8.19	1.46/40.1	99.8	84.3	+6.77	+6.90
2-nonyl octanoate	2.16	1.78/43.8	99.1	>99.9	−7.89	1.83/45.1	99.4	84.0	+6.61	+6.63

on the immobilization on different supports (*24*). The enhancement of enantioselectivity of the immobilized enzyme may be explained by the increased stability of the enzyme conformation because of interactions with the polymer (macroporous acrylic resin). Slight shifts in the conformation of the binding pockets of the enzyme provide more space for a docking of the (*S*)-enantiomer, leading to a decrease in enantioselectivity *E* (*18*). Because of the smaller difference in the size of substituents compared to 2-heptanol, this phenomenon was only determined for 2-pentanol.

Using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin as chiral stationary phase, the enantiomers of the substrates and products were separated by means of capillary gas chromatography (except for 2-nonanol) and the stereochemical course of the reactions could be examined. As example, the kinetic resolution of 2-heptanol via esterification with hexanoic acid and immobilized CAL-B as biocatalyst is shown in **Figure 3**. After 24 h of reaction time the (*R*)-ester is produced selectively (ee > 99.9%), resulting in an optical enrichment of the alcohol (91.7% (*S*), 8.3%(*R*)).

The production of optically pure (*S*)-esters via application of "anti-Kazlauskas catalysts" was not pursued, because this type of biocatalyst is rather uncommon or only available by great production efforts (*25*, *26*). Recently, wheat germ lipase has been reported as "anti-Kazlauskas catalysts" in nonaqueous biocatalysis, but low conversion rates and low enantioselectivities were determined for aliphatic secondary alcohols as substrates (*27*).

Preparation of Esters. Optically pure (*R*)-esters and optically enriched (*S*)-esters of secondary alcohols were prepared via lipase-catalyzed kinetic resolution using a two-step procedure. The (*R*)-ester was obtained via esterification of the racemic secondary alcohol (15 mmol) with an equimolar amount of acid using CAL-B imm (300 mg) as biocatalyst. Compared to the microscale experiments, the solvent heptane was replaced by the more volatile pentane in order to facilitate its subsequent removal. After gentle concentration of the reaction mixture using a Vigreux column, the ester was separated from the unreacted alcohol by fractionation. In order to remove the unreacted acid substrate, a mixture of silica gel and aluminum oxide, known as efficient adsorbent for short-chain and aromatic acids (*28*), was used. After total removal of the solvent, the (*R*)-esters were obtained as optically pure compounds (ee >99.9%) with high chemical purities; an overview of the data is presented in **Table 4**. Only minor losses (yields ≥40 mol %) were encountered, demonstrating the effectiveness of the liquid solid chromatography approach employed for purification. The availability of the substances at high chemical and optical purities allowed for the first time the determinatin of the optical rotations $[\alpha]_D$ of the 2-alkyl esters.

Neither the hydrolysis of racemic esters (**Table 1**) nor the application of an "anti-Kazlauskas catalyst" (*27*) seemed suitable for the preparation of optically pure (*S*)-esters. Therefore, the optically enriched (*S*)-alcohols remaining as unreacted substrates after the esterification with CAL-B imm were employed as substrates for further esterification. As shown in **Table 2**, the lipase preparation from *Candida cylindracea* (CCL) had exhibited an acceptable conversion rate in the enzyme screening, while accepting both enantiomers of the alcohol (E = 1.2). After addition of an equimolar amount of acid the remaining alcohol was esterified using this biocatalyst. The (*S*)-esters were obtained by subsequent fractionation and removal of the solvent. The yields, the chemical purities and the enantiomeric excesses (ee) are listed in **Table 4**. The optical purities are lower than those achieved for the (*R*)-esters, because they are determined by the alcohol substrates esterified by a nonspecific lipase. In accordance with the levorotation determined for the (*R*)-esters, the (*S*)-esters were dextrorotatory. The experimentally determined specific rotations $[\alpha]_D$ were in good agreement with the values calculated on the basis of the optical purities determined by GC analysis.

In conclusion, the developed method allows the preparation of optically pure (*R*)- and optically enriched (*S*)-esters of secondary alcohols with moderate experimental efforts. The kinetic resolution-based approach enables the use of the rather inexpensive racemic alcohols as starting compounds. Due to their chemical and optical purities, the substances obtained may serve as materials for subsequent sensory analyses of these esters. Determinations of thresholds via GC/O and sensory evaluations in water are subjects of ongoing investigations.

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