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Preparation of *S*-2-Ethylhexyl-*para*-methoxycinnamate by Lipase Catalyzed Sequential Kinetic Resolution

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Abstract: *S*-2-Ethylhexyl-*para*-methoxycinnamate **5-6** is prepared by a sequential biocatalytic resolution. First, either enantioselective acetylation of *rac* 2-ethylhexanol (\pm)-**1** by vinylacetate to *R*-(-)-2-ethylhexylacetate *R*-**2**, or alcoholysis of *rac* 2-ethylhexylbutyrate (\pm)-**3** by *n*-butanol to *S*-(+)-2-ethylhexanol *S*-**1** is completed, then, without isolation of the enantiomerically enriched *S*-alcohol, its enantioselective acylation with the activated *para*-methoxycinnamic acid derivatives **4,5** is performed, both steps being catalyzed by different microbial lipases. The highest amplification of enantioselectivity is obtained by combining acetylation of *rac* 2-ethylhexanol catalyzed by *Penicillium camembertii* lipase or alcoholysis of *rac* 2-ethylhexylbutyrate catalyzed by *Pseudomonas species* lipase in the first step, with acylation of enantiomerically enriched *S*-**1** by vinyl-*para*-methoxycinnamate **4** catalyzed by Lipozyme IM lipase; 84.5% e.e. of **5-6** is achieved in the first, and 88% e.e. in the second approach. Since the *R*-enantiomer of 2-ethylhexanol represents potential source of teratogenic *R*-2-ethylhexanoic acid, **5-6** is regarded as biologically safer UV filter as compared to racemic 2-ethylhexyl-*para*-methoxycinnamate. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In the course of our work on the lipase catalyzed kinetic resolution of some key intermediates in the preparation of biologically active compounds^{1,2}, or of some products which are marketed as the mixtures of stereoisomers^{3,4}, we developed a new model for conformational matching of cyclic vs. acyclic substrates on the lipase active site^{5,6}. For ulterior proof of this concept, we envisaged preparation of model compounds derived from the enantiomerically pure enantiomers of 2-ethylhexanol. With α 2.5 billion tons/year world-wide production, chemical industry offers *rac* 2-ethylhexanol, (\pm)-**1** in the Scheme 1, as one of the cheapest chiral materials, though presently not in the enantiomerically pure form⁷.

Checking toxicological data of potential metabolites of *R*- and *S*-2-ethylhexanol, we noticed that their oxidation products, *R*- and *S*-2-ethylhexanoic acid exhibit notably different biological profiles; the former is highly teratogenic while the latter is not^{8,9}. Oxidation of primary alcohols by both chemical and biochemical agents is well known. It was demonstrated for some primary alcohols that their *in vivo* oxidation is catalyzed by the NAD-dependent oxido-reductases, and it is also known that aldehydes as the intermediate oxidation state can undergo oxido-reduction in the presence of NAD⁺ and some other coenzymes¹⁰. In view of the concern with the adverse possibilities for hydrolytic and subsequent oxidative transformation of the *R*-enantiomer of 2-ethylhexanol in the racemic mixture of Parsol[®]M CX, the commercial product used in sunlight protecting creams¹¹, we regard enantioselective production of *S*-2-ethylhexyl-*para*-methoxycinnamate **5-6** a worthwhile goal for asymmetric synthesis. Differences in biological behaviour displayed by the enantiomers of a plethora of chiral molecules represents the most important reason for developing enantiomerically pure compounds (EPC)¹²⁻¹⁴; **5-6** is a good representative of this class. The need for EPC imposes, however, an extra burden on the task of designing an efficient manufacturing process. With the development of asymmetric synthesis,

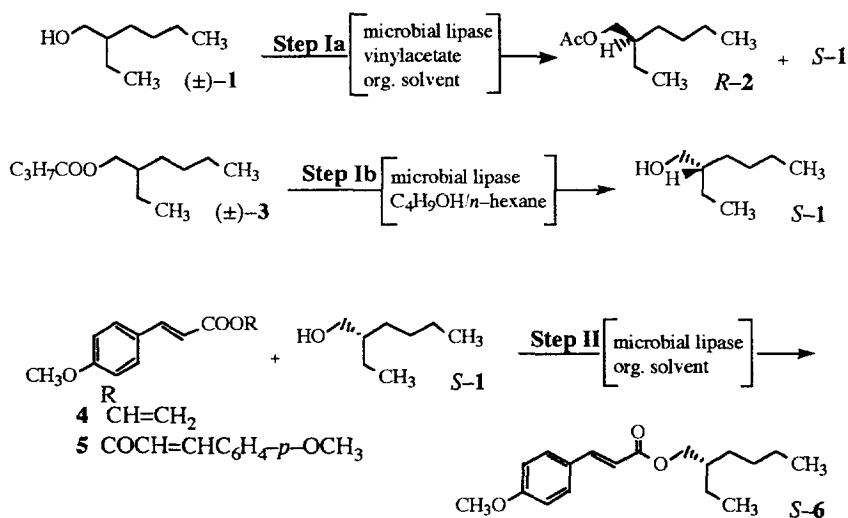
including kinetic resolution of racemates and their scale-up potential complementing the more classical approaches, these difficulties are considerably reduced.

Herein we report on successful application of concept of sequential biocatalytic resolution to enantioselective synthesis of the title target molecule with high enantiomeric purity, Scheme 1. Experimental approach and mathematical analysis of sequential kinetic resolution were developed by Sih and co-workers^{15,16}. Recently reported example confirm the viability of this method in the resolution of a key stereochemical intermediates for the synthesis of (-)-taxol¹⁷.

RESULTS AND DISCUSSION

In order to amplify efficiently the enantioselectivity in biocatalytic preparation of *S*-**6**, Scheme 1, we separately investigated both single steps; lipase catalyzed enantioselective acetylation by vinylacetate of *rac* 2-ethylhexanol (\pm)-**1**, or alcoholysis of *rac* 2-ethylhexylbutyrate ((\pm)-**3**), in the first step, and transesterification of the active *para*-methoxycinnamic acid derivatives **4,5** by racemic 2-ethylhexanol in the second. Initially the lipase catalyzed hydrolysis of racemic **6** in buffers and aqueous-organic media was examined. Only with *Pseudomonas species* lipase in 5% CH₃CN/ buffer very slow conversion (9.2% after 144 h) was observed. In apolar organic solvents, *i*-Pr₂O and cyclohexane, saturated with water, all tested lipases proved ineffective. Finally, alcoholysis with *n*-propanol in *n*-hexane was attempted following the protocol of Bianchi et al¹⁸; none of the tested lipases gave satisfactory results.

Scheme 1



There are few papers reporting kinetic resolution by acylation of *rac* 2-ethylhexanol. Acetylation of racemic **1** with vinylacetate, catalyzed by *Pseudomonas sp.* lipase (PSL) in dichloromethane (69% conversion, 33.7% e.e.)¹⁹, in tetrahydrofuran (20% conversion, 63% e.e.)²⁰, or in benzene (40% conversion, 47% e.e.)¹⁸ has been reported. With oxymacetate or oxymacrylate as irreversible acyl transfer reagents in tetrahydrofuran, and pig pancreatic lipase (PPL) as the catalyst, *S*-acetate was prepared in 60% yield and 75% e.e.²¹. Similar enantioselectivities (55-65%) were obtained in hydrolysis of racemic 2-ethylhexylbutyrate with *Candida*

cylindracea (CC), *Rhizopus arrhizus* (RA) and PS lipase²². By far the highest e.e. is claimed in the patent literature²³; acylation of *rac* 2-ethylhexanol in excess of ethylacetate by PS lipase is claimed to afford *S*-ester in 62% yield and 96% e.e. We repeated this experiment with *Pseudomonas* lipases from different origins and were unable to reproduce the results. At 20.3% conversion the highest e.e. (47%) was obtained.

The results of our screening of microbial lipases in acetylation of *rac* 2-ethylhexanol by vinylacetate are presented in the Table 1 and in the Figs. 1. and 2.

Table 1. Acetylation of *rac*-1 with lipases in organic solvents.

Lipase	Yield of 2 after 24 h (%) ^a				Config. of ester ^b
	<i>n</i> -hexane	<i>i</i> -Pr ₂ O	THF	CH ₃ CN	
<i>Pseudomonas species</i>	97.4	87.1	-	50.0	<i>S</i>
<i>Pseudomonas fluorescens</i>	100	-	-	-	<i>S</i>
<i>Candida cylindracea</i>	100	100	14.4	8.4	<i>rac</i>
<i>Penicillium camembertii</i>	95.3	90.4	18.3	21.4	<i>R</i>
Lipozyme IM	88.2	81.8	10.2	5.0	<i>S</i>
From hog pancreas	93.6	92.6	6.4	42.9	<i>S</i>
<i>Geotrichum candidum</i>	43.8	20.3	0	1.5	-
<i>Humicola lanuginosa</i>	28.6	17.6	0	3.0	-
<i>Penicillium roqueforti</i>	38.8	10.9	0	0	-

^a Determined by GC.

^b Determined in repeated experiments before 100% conversion, based on the data reported in ref. 19.

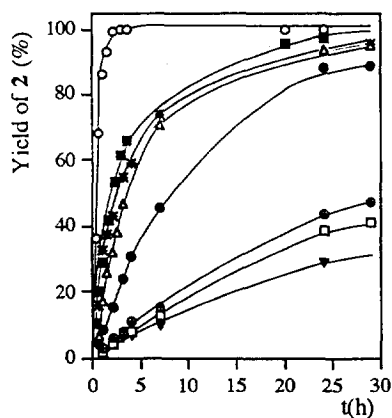


Fig. 1. Progress curves for acetylation of (\pm)-1 with lipases in *n*-hexane.

- *Candida cylindracea* lipase
- *Pseudomonas sp.* lipase
- ▲ *Penicillium camembertii* lipase
- ▲ Lipase from hog pancreas
- Lipozim IM
- *Geotrichum candidum* lipase
- *Penicillium roqueforti* lipase
- ▼ *Humicola lanuginosa* lipase

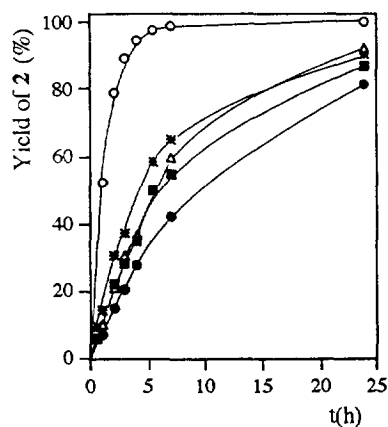


Fig. 2. Progress curves for acetylation of (\pm)-1 with lipases in diisopropylether

- *Candida cylindracea*
- ▲ Lipase from hog pancreas
- ▲ *Penicillium camembertii*
- *Pseudomonas species*
- Lipozim IM

Acceptable conversions after 24 h were obtained in *n*-hexane and in *i*-Pr₂O. In the former solvent progress curves are determined with all tested lipases (Fig. 1), and in the latter for the five most reactive ones (Fig. 2). Most of progress curves approach the saturation at ca 100% conversion without observable inflection at ca 50% conversion, indicating non-selective acetylation of both enantiomers.

Determination of the solvent effect and degree of conversion on the enantiomeric excess of the product has revealed some remarkable features of this reaction, Figs. 3-6.

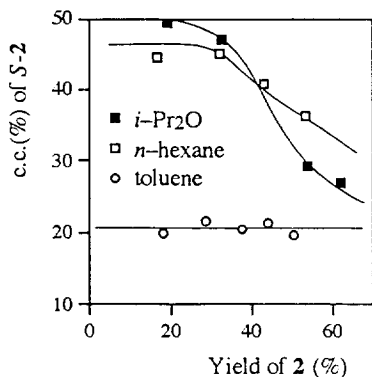


Fig. 3. Effect of solvent on the enantioselectivity of acetylation of (±)-1 by *Pseudomonas sp.* lipase.

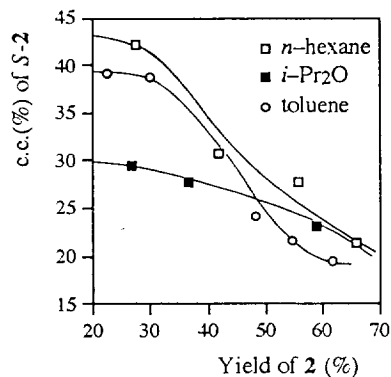


Fig. 4. Effect of solvent on the enantioselectivity of acetylation of (±)-1 by lipase from hog pancreas.

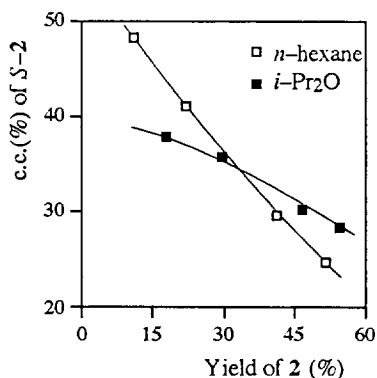


Fig. 5. Effect of solvent on the enantioselectivity of acetylation of (±)-1 by Lipozyme IM lipase.

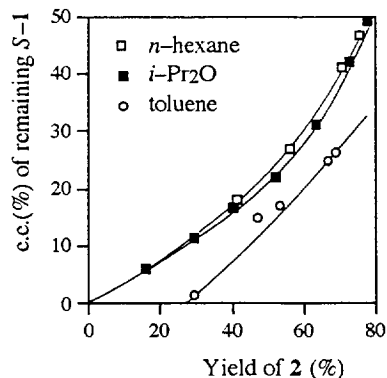


Fig. 6. Effect of solvent on the enantioselectivity of acetylation of (±)-1 by *Penicillium camembertii* lipase.

Most of the tested lipases preferentially acetylate S-enantiomer, but the effect of the solvent and degree of conversion on e.e. vary considerably. *Pseudomonas sp.* lipase exhibit 45-50% e.e. up to ca. 40% conversion in *i*-Pr₂O and *n*-hexane, then enantiomeric purity drops significantly, Fig. 3. In toluene, on the contrary, low but

constant enantioselectivity is observed. Lipase from hog pancreas exhibited also conversion-dependent enantioselectivity in all three solvents, Fig. 4; relative selectivities in toluene and *i*-Pr₂O are reversed as compared to data obtained with PSL. Even stronger conversion-dependent enantioselectivity is observed for Lipozyme IM in both apolar solvents, Fig 5. *Penicillium camembertii* lipase is the only one that preferentially acylates *R*-enantiomer of **1** affording enantiomerically enriched *S*-alcohol. The e.e.'s in all examined solvents rises monotonously with conversion, as depicted in Fig. 6. The accumulated evidence in Figs. 3–6 reveals that the highest e.e.s are in the average achieved in the least polar solvent, *n*-hexane, whereas more polar toluene turned out least effective, in particular for *Pseudomonas* and *Penicillium* lipase. This finding matches the observed reverse correlation between polarity of the solvent, expressed as dipole moment, and enantioselectivity of the lipase catalyzed reactions²⁴. The effect of other characteristic of the solvent are less well correlated^{25,26}, and therefore different variations of e.e. of *S*-**2** with degree of conversion of *rac* **1** cannot be explained; generally, lower e.e.s with higher conversion are determined by exact kinetic parameters^{15,16}. All results reveal low E values (E<5) for the first resolution step, indicating as the necessary strategy to terminate conversion of (±)-**1** at an early stage, and to perform consecutive enantioselective acylation of *S*-**1**.

We therefore next examined the enantioselectivity of the acylation of *rac* 2-ethylhexanol with derivatives of *para*-methoxycinnamic acid. Free acid and its methyl ester prove inactive in lipase catalyzed acyl transfer, whereas vinylcinnamate **4** and cinnamic anhydride **5** were accepted only by *Candida cylindracea* lipase and Lipozyme IM; the exploratory results are presented in Table 2.

Table 2. Transesterification of *rac* -**1** in *n*-hexane.

Acyl donor	Molar ratio (acyl donor:1)	Lipase	Yield of 6 after 24 h (%)	Configuration	e.e. of <i>S</i> - 6 (%)
4 vinyl cinnamate	1:6	CCL	36.0	<i>R</i>	42.3
		Lipozym IM	100	<i>S</i>	60.5
5 cinnamic anhydride	1:6	CCL	100	<i>R</i>	29.3
		Lipozym IM	28.2	<i>S</i>	58.7

Best results were obtained with Lipozyme IM lipase and vinylcinnamate **4** as substrate; progress curves reveal surprising constancy of e.e. (63%) with the degree of conversion, Fig. 7A, B.

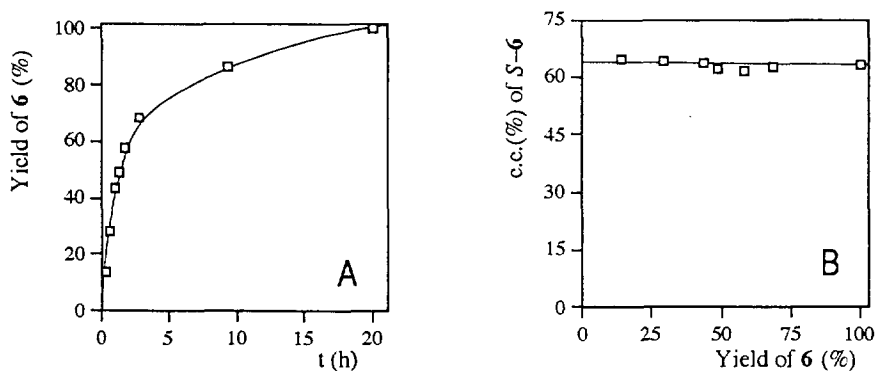


Fig. 7. Conversion (A) and enantioselectivity (B) in transesterification of (±)-**1** by vinylcinnamate **4** in *n*-hexane.

Constancy of e.e. at very high degrees of conversion suggested the use of vinylcinnamate **4** in acylations of enantiomerically enriched *S*-2-ethylhexanol. Therefore the effect of molar ratio vinylcinnamate **4**/*S*-**1** (e.e. 42.5%) on the rate and on the final e.e. of *S*-**6** was examined, Table 3, Fig. 8.

Table 3. Effect of molar ratio **4**:*S*-**1** (e.e. 42.5%) on conversion and e.e. of *S*-**6**.

molar ratio (4 : <i>S</i> - 1)	t (h)	Yield of 6 (%)	e.e. of <i>S</i> - 6 (%)
1:4	48	88.5	81.6
1:6	24	87.0	82
	48	100	
1:12	24	100	82.9

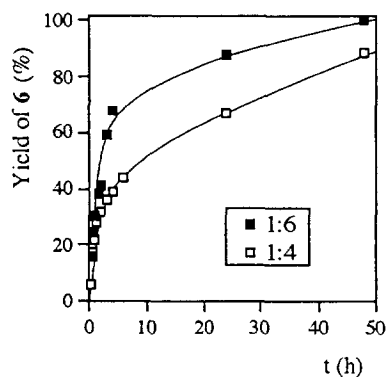


Fig. 8. Progress curves for acylation of *S*-**1** with **4** at different molar ratios **4**:*S*-**1** (e.e. 42.5%) in *n*-hexane.

Transesterification with the enriched *S*-enantiomer afforded *S*-**6** with constant, 82–83% e.e. at very high conversion; the rate of reaction significantly enhances on going from the molar ratio 1:4 to 1:12.

We therefore combined both biocatalytic steps, performing first acetylation of *rac* 2-ethylhexanol with vinylacetate/*Penicillium camembertii* lipase (conversion after 4.5 h 79.8%, e.e. 46.5%, $E=3.0$), and after filtration of the enzyme, evaporation of the solvent and the excess of the reagent, product mixture is dissolved in *n*-hexane and acylation with vinylcinnamate **4** in the presence of Lipozyme IM is continued. Following this protocol, *S*-**6** was obtained with 84.5% e.e., at 100% conversion in the second step.

A silent feature of this process is simply recycling of both lipases after filtration and washing, and reuse of the solvent mixture after distillation of (*n*-hexane)/vinylacetate and adjustment of the concentration of the latter.

In order to enhance the enantioselectivity, hydrolysis of *rac* 2-ethylhexylbutyrate **3** was performed with *n*-butanol in the presence of PSL, step Ib in the Scheme 1. The highest e.e. (60.7%) of *S*-**1** was obtained at ca. 15% conversion, ($E=4.5$), this approach afforded *S*-**6** with highest enantiomeric purity (88%), Table 4.

Table 4. Comparison of various methods in sequential kinetic resolution.

Method	Molar ratio (4:1)	E of the step I	e.e. of S-6 (%)
Acylation of (\pm)-1 by 4	1:6		63
Acylation of (S)-1 (e.e. 46.5%) by 4	1:6	3.0 (I a)	84.5
Acylation of (S)-1 (e.e. 60.7%) by 4	1:6	4.5 (I b)	88

In conclusion, the sequential kinetic resolutions reported herewith resulted in the amplification of enantioselectivity in the preparation of S-6 to the extent which is promising for the large-scale production. Described sequential kinetic resolutions differs in two aspects from the reported examples^{16,17}. First, our racemic starting material is inexpensive, the target chiral molecule contains an achiral carboxylic acid as the more valuable component. Therefore low E values are acceptable in the first step, since high e.e.s of final product S-6 is obtained even at high conversion in the second step. Second, the best results are obtained with two different lipases, whereas previously reported examples made use of the *same enzyme* in the both steps. Finally, easy separation of the solid biocatalysts from the reaction solution, the use of the intermediary S-1 in the second step without separation, and easy isolation of the final optically active ester S-6, make this process amainable to the large scale production.

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EXPERIMENTAL

¹H- and ¹³C NMR spectra were recorded on a Varian XL-GEM 300 spectrometer; shifts are given in ppm downfield from TMS. Optical rotations were measured on Optical Activity LTD automatic polarimeter AA-10.

Acylation of (\pm)-1 by vinylacetate was monitored on a Hawlett Packard instrument Series 1050 with RI detector HP-1047A and HP integrator 3396B using HPLC Econosphere RP C8 5 μ column (250x4.6 mm) under following conditions: methanol-water 5:2 as eluent, flow rate 0.7 ml/min at 170 bar. Optical purity was monitored on a Hawlett Packard GC instrument 5890 Series II, using chiral column, CP-Chirasil-DEX CB (25 mx0.25 mm) at 100°C, detector and injector temperatures: 300°C and 250°C; carrier gas N₂, pressure 50 kPa.

In this reaction following lipases from microbial sources were examined: *Pseudomonas species*, *Humicola lanuginosa*, *Aspergillus niger*, *Geotrichum candidum*, *Rhizopus oryzae*, *Mucor javanicus*, *Candida cylindracea*, *Candida lipolytica*, *Penicillium camembertii*, *Penicillium roqeforti*, *Rhizopus niveus*, *Rhizopus delemar*, are supplied by Amano, lipase from hog pancreas and *Pseudomonas fluorescens* lipase were from Fluka, (activity 3.6 and 31.5 U/mg), *Aspergillus oryzae*, *Aspergillus niger*, *Mucor miehei* and immobilized *Mucor miehei* lipase (Lipozyme IM) are supplied by Novo Nordisk. Lipozyme IM is an immobilized lipase preparation derived from the selected strain of *Mucor miehei*; the gene coding has been transferred to the selected host organism *Aspergillus oryzae*, and belongs to the class of triacylglycerol hydrolases (EC 3.1.1.3)²⁷.

Transesterification of *para*-methoxycinnamic acid derivatives was monitored on Knauer 64 HPLC instrument, with UV detector at 300 nm, and HP integrator 3396A, using chiral HPLC column Chiralpak AS (4.6x250 mm), equipped with precolumn (4.6x50 mm), both from Daicel Co. As the eluent was used *n*-hexane-

2-propanol (90:10), at flow rate 1.0 ml/min and 30 bar. Retention times were 5.9 min for *R*-**6** and 7.3 min for *S*-**6**.

In this reaction following lipases were tested: *Pseudomonas species*, *Candida cylindracea*, *Geotrichum candidum*, *Penicillium roqueforti* (from Amano), *Pseudomonas fluorescens*, lipase from hog pancreas (from Fluka), and immobilized *Mucor mieheii* lipase (Lipozyme IM).

2-Ethylhexylbutyrate 3. 2-Ethylhexanol (2 g, 15.3 mmol) was dissolved in pyridine (30 ml) and butyrylchloride (5.2 g, 48.7 mmol) was slowly added at room temperature. Reaction was terminated after 1.5 h and ethylacetate (50 ml) was added. Reaction mixture was extracted first with water then with diluted HCl solution, water and finally with saturated NaHCO₃ solution. Crude product was purified by distillation on kugelrohr at 0.2 mmHg and 75–100°C, and it was obtained 1.77 g (58%) of pure **3**. ¹H NMR. (CDCl₃) δ: 0.87-1.04 (m, 9H), 1.24-1.40 (m, 9H), 1.59-1.67 (m, 2H), 2.28 (t, *J*=7Hz, 2H), 3.99 (d, *J*=6Hz, 2H). ¹³C NMR. (CDCl₃) δ: 10.81, 13.51, 13.86, 18.34, 22.79, 23.62, 28.74, 30.24, 36.16, 38.57, 66.44, 173.70.

Vinyl-*para*-methoxycinnamate 4. *para*-Methoxycinnamic acid (2.03 g, 11.3 mmol) was added to freshly distilled vinylacetate (9.5 g, 0.11 mol), and stirred until all dissolved. Then 100% H₂SO₄ (0.1 ml) was added and reaction mixture heated under reflux for 1.5 h. To the cooled solution NaOAc (30 g) was added, stirred, filtered and filtrate evaporated. Crude product was purified by chromatography on silicagel (60 g), with CH₂Cl₂/cyclohexane (9:1) as eluent; it was obtained 1.59 g (69%) of pure **4**. ¹H NMR. (CDCl₃) δ: 3.83 (s, 3H), 4.61 (dd, *J*₁=6 Hz, *J*₂=2Hz, 1H), 4.96 (dd, *J*₁=14 Hz, *J*₂=1Hz, 1H), 6.32 (d, *J*₁=16Hz, 1H), 6.91 (dd, *J*₁=9Hz, 2H), 7.42 (dd, *J*₁=14Hz, *J*₂=8Hz, 1H), 7.49 (d, *J*₁=8Hz, 2H), 7.74 (d, *J*₁=16Hz, 1H). ¹³C NMR. (CDCl₃) δ: 55.2, 97.3, 113.8, 114.3, 126.7, 129.9, 141.3, 146.3, 161.7, 164.2.

***para*-Methoxycinnamic acid anhydride 5.** *para*-Methoxycinnamic acid (0.7 g, 3.9 mmol) was added to thionylchloride (4.2 g, 35 mmol) and reaction mixture heated 1 h under reflux. Excess of thionylchloride was evaporated, and crude acid chloride was dissolved in toluene (5 ml). To this solution pyridine (0.45 ml) was added, and then slowly *para*-methoxycinnamic acid (0.45 g, 2.5 mmol). Reaction mixture was heated 1h at 70°C, then cooled, evaporated, and crude anhydride purified on silicagel column (20 g) with CH₂Cl₂ as eluent; it was obtained 0.32 g (38%) of pure **5**. ¹H NMR. (CDCl₃) δ: 3.85 (s, 6H), 6.38 (d, *J*₁=16Hz, 2H), 6.93 (d, *J*₁=8Hz, 4H), 7.52 (d, *J*₁=9Hz, 4H), 7.79 (d, *J*₁=16Hz, 2H). ¹³C NMR. (CDCl₃) δ: 55.2, 114.0, 114.4, 126.4, 130.3, 148.2, 162.1, 163.0.

Screening of microbial lipases in acetylation. Substrate (±)-**1** (ca. 10 mg) and lipase (ca. 10 mg, except in case of *Pseudomonas fluorescens* which was used in amount of 3 mg) were suspended in organic solvent (5 ml) and thermostated at 30°C in an thermostated shaker. Reactions were initiated by addition of vinylacetate (1 ml, 10 mmol), and continued at 30°C and 220 rpm. Samples (0.5 ml) were taken off at the time intervals, filtered through millipore filter, evaporated, dissolved in a mobile phase (100 μl), and analyzed by HPLC on RP C8 column with RI detector.

Determination of optical purity of reaction products 2-ethylhexanol 1 and 2-ethylhexyl-acetate 2. Substrate (±)-**1** (ca. 20 mg) and lipase (ca. 20 mg) were suspended in organic solvent (5 ml) and thermostated at 30°C in a shaker. Reactions were initiated by addition of vinylacetate (1 ml, 10 mmol). Samples (100 μl) were taken off at the time intervals, filtered through millipore filter and analyzed by GC on CP-Chirasil-DEX CB column with FI detector.

Screening of microbial lipases and acyl donors in transacylation. Racemic **1** (40 mg, 0.3 mmol) and lipase (10 mg) were added to *n*-hexane (5 mL) and shaken at 30°C and 220 rpm. Reaction was started by addition of acyl donor **4** or **5** (0.5 mmol). Samples (30 μl) were taken during first 24 hours, diluted by 1 ml of

n-hexane, filtered through millipor filter, evaporated, dissolved in 100 μ l of mobile phase and analyzed on chiral HPLC column Chiralpak AS.

Effect of the molar ration S-1/4 on the rate and optical purity of S-5. Enantiomerically enriched *S*-1 was prepared by acetylation of (\pm)-1 (1.0 g, 7.7 mmol) by vinylacetate (50 ml) in *n*-hexane (250 ml) catalyzed by *Penicillium camembertii* lipase (1.0 g). After 3 h at 30°C conversion was 76.5%, and on separation of acetate *R*-2 by chromatography on silicagel (50 g) with CH₂Cl₂ as eluent it was obtained 225 mg (22.5%) of *S*-1 with e.e. 42.5%. Samples of this material (0.2, 0.3 and 0.6 mmol) were submitted to acylation by vinylcinnamate 4 (10 mg, 0.05 mmol) in *n*-hexane (5 ml) catalyzed by Lipozyme IM (10 mg), at 30°C and 220 rpm. The effect of the variation of molar ratio on the rate and on the e.e.'s of the isolated *S*-6 are presented in the Table 3.

S-(+)-2-Ethylhexyl-*para*-methoxycinnamate, S-6

Method A. Racemic 1 (390 mg, 3.0 mmol) and *Penicillium camembertii* lipase (100 mg) were added to *n*-hexane (20 μ l) at 30°C and 220 rpm. Then vinylcinnamate 4 (102 mg, 0.5 mmol) was added and reaction mixture shaken for 20 h. Enzyme was then separated by filtration, filtrate evaporated, and *S*-6 separated by chromatography on silicagel (20 g) with CH₂Cl₂/cyclohexane (6:4) as eluent, 41 mg of pure *S*-6 were obtained, e.e. 63% (by HPLC), $[\alpha]_D^{25} +2.3$ (c 0.42 in diisopropylether). ¹H NMR. (CDCl₃) δ : 0.86–0.92 (m, 6H), 1.28–1.41 (m, 8H), 1.60–1.64 (m, 1H), 3.77 (s, 3H), 4.09 (dd, $J_1=6$ Hz, $J_2=2$ Hz, 2H), 6.29 (d, $J_1=16$ Hz, 1H), 6.85 (d, $J_1=9$ Hz, 2H), 7.43 (d, $J_1=9$ Hz, 2H), 7.61 (d, $J_1=16$ Hz, 1H). ¹³C NMR. (CDCl₃) δ : 10.6, 13.6, 22.6, 28.6, 30.1, 38.5, 54.9, 66.4, 114.0, 115.5, 127.0, 129.4, 143.9, 161.2, 167.3.

Method B. Racemic 1 (195 mg, 1.5 mmol) and vinylacetate (10 ml) were dissolved in *n*-hexane (50 mL), and at 30°C *Penicillium camembertii* lipase (195 mg) was added. After 4.5 h shaking at 220 rpm conversion was 79.8%, enzyme was filtered off, solvent evaporated and crude reaction mixture, that contained 39.4 mg of *S*-1 with 46.5% e.e., was dissolved in fresh *n*-hexane (5 ml). After addition of vinylcinnamate 4 (10 mg, 0.05 mmol) reaction was initiated by addition of Lipozyme IM (10 mg). After 48 h shaking at 30°C conversion was 100% and e.e. of *S*-6 was 84.5%.

Method C. To the racemic 2-ethylhexylbutyrate 3 (100 mg, 0.5 mmol) and *n*-butanol (60 mg, 0.8 mmol), dissolved in *n*-hexane (5 ml), *Pseudomonas sp.* lipase (100 mg) was added. After 5 days shaking at 35°C conversion was 14.9% and e.e. of *S*-1 was 60.7%. Enzyme was separated by filtration, filtrate evaporated and product mixture dissolved in fresh *n*-hexane (1 ml). Lipozyme IM (2 mg) was added and acylation performed under the same conditions as in the first step. After 48 h conversion was 100% and e.e. of *S*-6 was 88%.

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