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Separation of secondary alcohols via enzymatic kinetic resolution using fatty esters as reusable acylating agents

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ABSTRACT

A two consecutive step procedure for the resolution-separation of secondary alcohols employing ethyl tetradecanoate in the presence of lipase allowed the enzymatic kinetic resolution of two target molecules, 1-phenylethanol and 6-methylhept-5-en-2-ol. (*S*)-1-Phenylethanol was isolated in a yield of 47% with an ee of 94% and (*R*)-1-phenylethanol in a yield of 51% with an ee of 95%. (*S*)-6-Methylhept-5-en-2-ol was isolated in a yield 47% and an ee of 87% and (*R*)-6-methylhept-5-en-2-ol in a yield 49% and an ee of 90%.

1. Introduction

The design of chemical methods that minimize the usage of hazardous or toxic substances and production of waste is a major focus of modern organic synthesis.[1] In recent years this has become a major concern for the chemical industries as they are required to adhere to increasingly stringent guidelines and policies. This has provided an impetus for the development of new sustainable chemical processes.[2,3]

As part of the drive for sustainability, chemists have continued to be attracted to the area of biocatalysis, which has many useful features, particularly for asymmetric synthesis. In recent years, the biocatalysis approach has become widely utilized for the synthesis of complex enantiomerically pure molecules, and there has been particular interest from the industrial sector. A number of important developments have helped to increase the utility of biocatalysis including for example: (1) rapid access to new classes of enzymes through high throughput sequencing; (2) development of robust screening technologies in order to identify more specific, selective and stable enzyme(s); and (3) the availability of a wider range of expression systems required for the large-scale production of biocatalysts in an efficient and cost-effective manner.[4–6]

Another benefit of using biocatalysts in organic synthesis is that they can often be carried out under conditions which allow the minimization of undesirable organic solvents. The development of new solvents in the green chemistry arena is important because it can help to improve the overall efficiency of a reaction and enhance the process, for example, by allowing catalyst recovery.[7] Examples of recent developments include the use of aqueous biphasic, fluorous biphasic,[8–10] alkyl carboxylates,[11] supercritical fluids,[12,13] and ionic liquids,[14,15] and all these have become attractive as alternatives to more traditional approaches.[7,16,17] Most recently there has been considerable interest in the use of fatty esters as reaction media,[18–22] because of their availability in large scale from sustainable sources.[23]

The first reports on lipase-catalyzed reaction on fatty acids were reported by Sym in 1930 on the esterification of oleic acid in the presence of pancreatic lipase.[24] The physical properties of fatty esters make them a good reaction media for performing enzymatic esterification or transesterification reactions and they can allow the continuous removal of volatile compounds under vacuum.[25] Moreover, they can themselves act as reagents with the ester functionality used as an acyl donor for lipases.[18–20]

Several acylating agents have already been described,[26] from which the more used ones are vinyl esters. However, even those have their limitations, such as the formation of side products, such as acetaldehyde, that can deactivate the enzyme or react in the reaction media and form new products.[26] In recent years, new acylating agents have been developed, namely succinic anhydride,[27,28] carbonates[29] and fatty esters.[18,19] Additionally, more elaborated systems have been described as facilitators in the separation process of free alcohol enantiomer and the other enantiomer as an ester,[30,31] such as fluorous solvent,[32] ionic liquids,[33] ionic liquids-scCO$_2$,[34] membrane[35] or PEG.[36]

Recently we described a new approach to enzymatic kinetic resolution for the separation of secondary alcohols in which we used ionic liquids to assist in the enantiomer separation. This allowed us to use a sequence of simple extraction methods to achieve an efficient resolution-separation process.[33] However, this approach is not without some limitations, particularly the requirements for a specific ionic liquid as acylating agent and reaction medium, and the use of an organic solvent for the extraction step.

Herein, we report a more general and effective process for the resolution and isolation of both enantiomers of secondary alcohols

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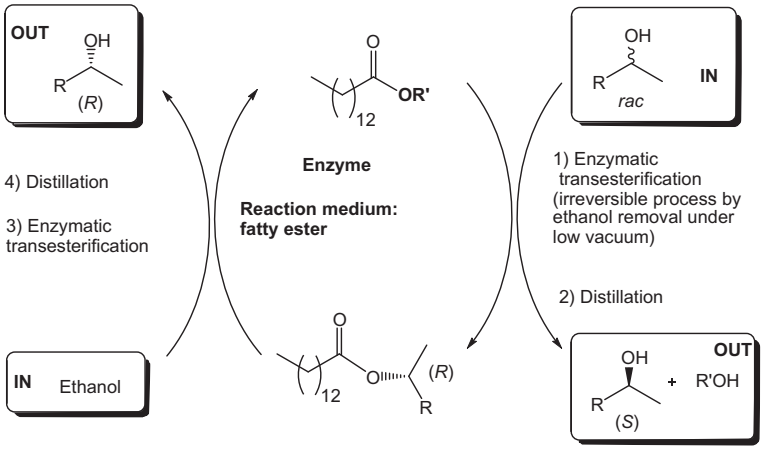


Scheme 1. One-pot resolution of *sec*-alcohols, based in the usage of fatty esters as solvent and acylating agent.

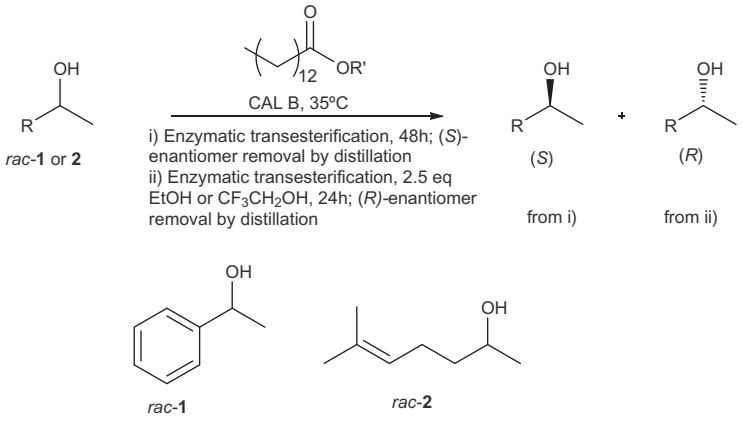
derived from resolution, without the use of organic solvents (Scheme 1). The strategy employs two steps; in the first an enzymatic reaction allows the isolation of the (*S*)-alcohol enantiomer by distillation. In the second step another enzymatic reaction is utilized, in order to release the (*R*)-enantiomer that is also isolated by distillation.

2. Results and discussion

The choice of long chain fatty acid-derived esters as acyl donors for the resolution of secondary alcohols was based on a number of factors including physico-chemical properties and its origin from fatty esters, a sustainable source. These acylating agents were deemed ideal because of their low melting point and high boiling point characteristics, which should allow the reactions to be carried out without any additional solvent and also offering the prospect of product isolation via distillation.

In the event, a two-step resolution-separation method was evaluated using two model substrates, 1-phenylethanol **1** and 6-methylhept-5-en-2-ol (sulcatol) **2**. Using the enzyme *Candida antarctica* lipase B (CAL-B), a variety of fatty acids were evaluated including, ethyl tetradecanoate (ethyl myristate) **3** and 2,2,2-trifluoroethyl tetradecanoate (2,2,2-trifluoroethyl myristate) **4** (Table 1).

Table 1
Effect of the acyl donor on the enzymatic resolution of 1-phenylethanol 1 and 6-methylhept-5-en-2-ol (sulcatol) **2**



Entry[a]	Step (i)			Step (ii)	
	Acylating agent R′	Racemic alcohol	(*S*)-Alcohol yield/ee[b] (%)	Primary alcohol	(*R*)-Alcohol yield/ee[b] (%)
1	Et	**1**	48/86	EtOH[e]	52/90
2	Et	**2**	47/87	EtOH[e]	49/90
3	CH_2CF_3	**1**	45/93	CF_3CH_2OH	0/-
4	CH_2CF_3	**1**	47/95	CF_3CH_2OH[c]	0/-
5	CH_2CF_3	**1**	47/94	CF_3CH_2OH[d]	8/62
6	CH_2CF_3	**1**	49/95	EtOH[e]	31/87
7	CH_2CF_3	**1**	48/96	[f]	46/98
8	CH_2CF_3	**2**	49/94	[f]	47/87

[a] All reactions were carried out with 4.1 mmol alcohol, 4.1 mmol of acylating agent and 160 mg of CAL B, 35 °C, 100 mm Hg. To perform the second step, 2.5 equiv of primary alcohol was added.
[b] Enantiomeric excess determined by GLC.
[c] 2,2,2-Trifluoroethanol added reduced to 1.1 equiv.
[d] 1.1 equiv of 2,2,2-trifluoroethanol added slowly over 6 h.
[e] 2.5 equiv of EtOH.
[f] Basic hydrolysis, KOH/MeOH.

The first enzymatic reaction performed with ethyl tetradecanoate **3** demonstrating that (*S*)-**1** could be obtained in good yield and ee (48%, 86% ee, entry 1). We observed that the second step also proceeded well to give (*R*)-**1** in good yield and ee (52%, 90% ee, entry 1). When we used as an alternative 2,2,2-trifluoroethyl tetradecanoate **4**, (*S*)-**1** was isolated in moderate yield and good ee (45%, 93% ee, entry 3), but no further reaction was observed indicating perhaps that enzyme inhibition was occurring due to a high concentration of 2,2,2-trifluoroethanol.[37]

In an attempt to improve the efficiency of the protocol we decreased the concentration of 2,2,2-trifluoroethanol in solution (entry 4), and modified the addition of the alcohol such that it was slowly added over 6 h (entry 5). However, in both cases we did not observe any improvement in the protocol. We also replaced 2,2,2-trifluoroethanol with ethanol in the second transesterification step (entry 6). Under these conditions, (*R*)-**1** was isolated in a 31% yield and 87% ee. However, these results are lower than might be expected given our assumption that excess 2,2,2-trifluoroethanol was compromising the efficiency of the process.

In order to quantify the yield and enantiomeric excess of the (*R*)-enantiomer, a basic hydrolysis was performed, providing (*R*)-**1** in a very good yield and excellent enantiomeric excess (46%, 98% ee, entry 7).

We then decided to test the scope of the methodology using another substrate. Hence 6-methylhept-5-en-2-ol (sulcatol) **2**[38] was evaluated under the previous optimum conditions. Using ethyl tetradecanoate **3**, (*S*)-**2** was isolated in good yields and enantioselectivities: 47%, 87% ee for (*S*)-**2** and 49%, 90% ee for (*R*)-**2** (entry 2). The use of 2,2,2-trifluoroethyl tetradecanoate **4** confirmed that it was possible to isolate (*S*)-**2** in a good yield and ee (49%, 94% ee), and we isolated (*R*)-**2** after basic hydrolysis, in a 47% yield and 87% ee (entry 8).

It is known that the particular structure of the acylating agent is important for the enzymatic resolution of *sec*-alcohols, by its influence on the equilibrium of reaction. We assumed that the structure of the acylating agent would be particularly important in the present methodology because of its dual role as an acylating agent and solvent. A study was therefore carried out on the fatty ester/substrate ratio and as shown in Table 2, increasing the acylating agent/ substrate ratio from 1 to 10 resulted in a moderate increase in the enantiomeric excess of (*S*)-**1** and (*R*)-**1** obtained (entry 1 vs entry 2). Identical conditions were used on the enzymatic resolution of 2, and similar results were obtained for (*S*)-**2** (entry 3 vs entry 4). On the other hand, we noted that under these conditions (*R*)-**2** presented a slight decrease in ee (48% yield, 86% ee, entry 4). These unexpected results could be attributed to the incomplete distillation of the (*S*)-enantiomer during the first step, and consequent erosion of the enantiomeric excess on the second step.

We also examined the influence of temperature on the overall reaction and although we noted a modest decrease in the enantiomeric purity with an increase in temperature from 35 °C to 40 °C (entry 1 vs entry 5). However, the considerable reduction of reaction time is also noteworthy (entry 1 vs entries 5 and 6).[39]

We examined the ability to reuse the acylating agent and carried out experiments using recycled acylating agent and enzyme. These experiments were carried out at 40 °C, using ethyl tetradecanoate **3** as acylating agent, 1-phenylethanol as substrate in a 1:1 ratio in the presence of *C. antarctica* lipase B (CAL B) (Fig. 1).

As shown in Figure 1, the efficiency of the enzyme was retained for at least five cycles (>43% yield, >80% ee). We note that the loss of 3–7% yield in each step should be noted which is probably due to incomplete distillation, and product loss during this process. Since the overall process of five cycles consisted of ten sequential enzymatic reactions with the same enzyme, fatty ester and ten distillations of each enantiomer (0.6 g scale), these results show the simplicity, feasibility and robustness of the overall process.

Table 2
Study of the reaction conditions for the resolution/separation of 1-phenylethanol **1** and 6-methylhept-5-en-2-ol (sulcatol) **2**

Entry	Acylating agent/racemic alcohol ratio	Racemic alcohol	Temp (°C)	Time (h) 1st/2nd steps	(*S*)-Alcohol yield/ee[c] (%)	(*R*)-Alcohol yield/ee[c] (%)
1[a]	1:1	**1**	35	48/24	48/86	52/90
2[a]	10:1	**1**	35	48/24	47/94	51/95
3[a]	1:1	**2**	35	48/24	47/87	49/90
4[a]	10:1	**2**	35	48/24	46/95	48/86
5[b]	1:1	**1**	40	24/24	45/85	45/86
6[b]	1:1	**1**	40	24/12	49/90	44/80

[a] The reactions at 35 °C were carried out with 4.1 mmol alcohol, and 160 mg of CAL B, 100 mm Hg.
[b] The reactions at 40 °C were carried out with 10.35 mmol alcohol and 500 mg of CAL B. To perform the second step, 2.5 equiv of absolute ethanol was added.
[c] Enantiomeric excess was determined by GLC.

3. Conclusions

In conclusion a simple, effective and practical methodology for efficient kinetic resolution and enantiomer separation of secondary alcohols has been developed. The key features of the approach are (1) the use of fatty esters as acylating agents and reaction solvent medium; (2) a second enzymatic reaction to liberate the alcohol from the ester produced in step 1; and (3) the ability to recycle

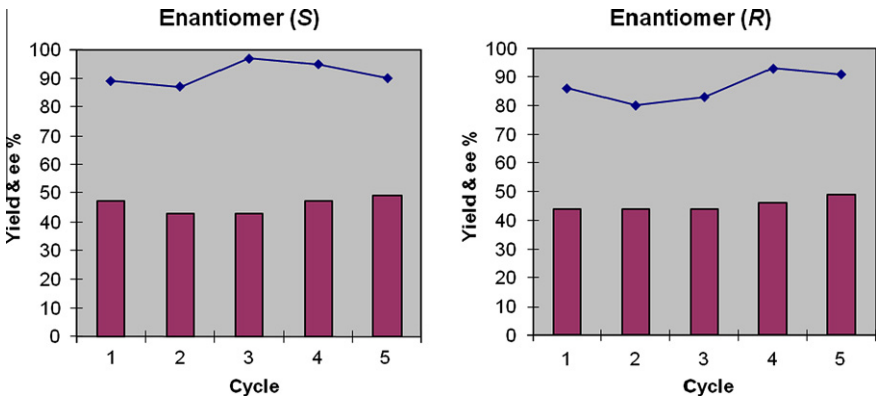


Figure 1. Reuse process for one-pot resolution of **1**: (solid bar) yield, (♦) ee.

both the acylating agent and the enzyme. The simplicity of this process offers potential advantages over other kinetic resolution protocols and we believe that it will find significant utility as a strategy for the large-scale production of enantiomerically pure volatile products.

4. Experimental

4.1. General experimental details

4.1.1. Reagents

The reactions were performed in the presence of *C. antarctica* lipase B (Novozym 435® with 1–2% water w/w and 7000 PLU/g) produced from Novozymes Co. (Denmark). The fatty ester used was prepared from tetradecanoic acid (myristic acid) (from Fluka) or commercially available from SAFC (Ref. W24,450-3-K). The absolute ethanol and 2,2,2-trifluoroethanol were obtain from *Riedel-de* Haën and Sigma–Aldrich, respectively. The secondary alcohols 1-phenylethanol and 6-methylhept-5-en-2-ol (sulcatol) were purchased from Sigma-Aldrich and distilled before use.

4.1.2. Analysis

The enantiomeric excesses (ee) were determined by GC analysis, performed on Trace Focus Unicam, FID detection, using capillary column Astec chiraldex™ G-TA (30 m × 0.25 mm × 0.12 μm) (Ref. 73033AST); injector: 250 °C; detector: 250 °C; split ratio = 6, column flow (H_2): 60 kPas (1.2 mL/min); 1-phenylethanol: oven: 100 °C for 15 min and ramp 8 °C/min to 155 °C), t_R (*S*) = 12.78 min; t_R (*R*) = 13.45 min; t_R ethyl tetradecanoate = 40.12 min; 6-methylhept-5-en-2-ol (sulcatol): oven: 75 °C for 15 min and ramp 8 °C/min to 155 °C); t_R (*S*) = 12.43 min; t_R (*R*) = 12.94 min; t_R ethyl tetradecanoate = 43.72 min; mass spectrometry analysis were performed by the mass spectrometry service of Santiago de Compostela University, Spain. NMR spectra were recorded at room temperature in a Bruker AMX 300, $CDCl_3$ as solvent and $(CH_3)_4Si$ (^1H) as internal standard. Infrared (IR) spectra were recorded with a Jasco FT/IR-430 model as thinly dispersed films on NaCl disks. For the procedures under vacuum was used a diaphragm pump (1–760 mm Hg).

4.2. Preparation of ethyl tetradecanoate (ethyl myristate) 3

A single-necked round-bottomed flask equipped with a magnetically stir bar was filled with tetradecanoic acid (100 g; 0.438 mol), ethanol 96% (V/V) (573.5 mL; 9.75 mol) and sulfuric acid (12.0 mL, 0.225 mol). The reaction mixture was stirred at 80 °C (temperature measured in the silicon bath) for 16 h. After this time, the mixture was cooled at room temperature and the ethanol was removed under vacuum in a rotavapor. The mixture was transferred to a separating funnel (1 L) and washed with water (3 × 300 mL) and a solution of sodium bicarbonate (50% (v/v), 300 mL). The organic phase was dissolved in hexane (300 mL), washed with water (300 mL), dried with anhydrous magnesium sulfate and filtered. The solvents were evaporated under reduced pressure in a rotavapor and the product was isolated by distillation under vacuum (120 °C, 1 mm Hg) in order to obtain ethyl tetradecanoate as a colourless oil (95.38 g, 86%). ^1H NMR δ 4.14–4.07 (2H, q, J = 7.1 Hz), 2.29–2.42 (2H, t, J = 7.5 Hz), 1.62–1.58 (2H, t, J = 7.2 Hz), 1.26–1.21 (23H, m), 0.88–0.84 (3H, t, J = 6.6 Hz); ^{13}C NMR δ 173.9 (*C*O), 60.21 (*C*H_2O), 34.47 (*C*H_2(CO)), 32.01 (*C*H_2), 31.96 (*C*H_2), 29.70 (*C*H_2), 29.56 (*C*H_2), 29.52 (*C*H_2), 29.44 (*C*H_2), 29.37 (*C*H_2), 29.45 (*C*H_2), 25.02 (*C*H_2), 22.78 (*C*H_2(CH_3)), 14.32 (*C*H_3), 14.18 (*C*H_3); FAB-MS (*m/z*): 256.20 (M, 4%), 200 (M+1−C_4H_8, 7.5%), 157 (M−C_7H_{15}, 22%), 129 (M−C_9H_{19}, 22%), 88 (M−$C_{12}H_{24}$, 100%), 73 (M−$C_{13}H_{27}$); IR ν_{max} (film): 2924, 2853, 1739, 1467, 1372, 1349, 1302, 1247, 1179, 1115, 1037, 722 cm^{-1}.

4.3. Preparation of 2,2,2-trifluoroethyl tetradecanoate 4

A double-necked round-bottomed flask equipped with a magnetic stirrer bar was filled with tetradecanoic acid (5.0 g; 0.219 mol) and heated to 40 °C with a few drops of DMF. Thionyl chloride (13.8 g; 0.117 mol) was added slowly and the reaction mixture was heated to 70 °C and stirred for 1 h to produce tetradecanoyl chloride. Volatile compounds were distilled at 120 °C under vacuum (1 mm Hg). Triethylamine (27 mL, 0.195 mol) was added to 2,2,2-trifluoroethanol (11,7 g, 0.117 mol) in CH_2Cl_2 (125 ml) in another round-bottomed flask. After stirring for 30 min, tetradecanoyl chloride was added and the reaction mixture was stirred at room temperature for 3 h. After this time, the reaction was quenched with aqueous HCl 5% (250 mL) and the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was washed with an aqueous solution of saturated sodium bicarbonate (200 mL) and dried with anhydrous magnesium sulfate and filtered. The solvents were evaporated under reduced pressure in a rotavapor and the product was isolated by distillation of the volatile compounds under vacuum (120 °C, 1 mm Hg) yielding the 2,2,2-trifluoroethyl tetradecanoate **4** as a colourless oil (29.99 g, 89%). ^1H NMR δ 4.52–4.43 (2H, q, J = 8.6 Hz), 2.45–2.40 (2H, t, J = 7.5 Hz), 1.69–1.64 (2H, t, J = 6.9 Hz), 1.27 (20H, m), 0.92–0.87 (3H, t, J = 6.6 Hz); ^{13}C NMR δ 172.3 (*C*O), 125.0–121.3 (d, J = 278 Hz, *C*F_3), 61.0–59.5 (q, J = 36.2 Hz, *C*H_2O), 33.79 (*C*H_2(CO)), 32.07 (*C*H_2), 29.81 (*C*H_2), 29.78 (*C*H_2), 29.71 (*C*H_2), 29.55 (*C*H_2), 29.02 (*C*H_2), 29.31 (*C*H_2), 29.11 (*C*H_2), 24.84 (*C*H_2), 22.83 (*C*H_2(CH_3)), 14.25 (*C*H_3).

4.4. Preparation of 1-phenylethyl tetradecanoate 5

A double-necked round-bottomed flask equipped with a magnetic stirrer bar was filled with tetradecanoic acid (5.0 g; 0.022 mol) and heated to 40 °C with a few drops of DMF. Thionyl chloride (2.8 g; 0.023 mol) was added slowly. The reaction mixture was heated to 80 °C and stirred for 1 h to produce tetradecanoate chloride. Volatile compounds were distilled at 120 °C under vacuum (1 mm Hg). In another round-bottomed flask, triethylamine (5.4 mL, 0.039 mol) was added to 1-phenylethanol (2.86 g, 0.023 mol) in CH_2Cl_2 (10 ml). After 30 min stirring, tetradecanoyl chloride obtained previously was added to the reaction mixture, which was stirred at room temperature for 3 h. After this time, the reaction was quenched with aqueous HCl 5% (20 mL) and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was washed with an aqueous solution of saturated sodium bicarbonate (200 mL) and dried with anhydrous magnesium sulfate and filtered. The solvents were evaporated under reduced pressure in a rotavapor and the product was isolated by distillation of the volatile compounds under vacuum (120 °C, 1 mm Hg). 1-Phenylethyl tetradecanoate was obtained as colourless oil (6.33 g, 87%). ^1H NMR δ 7.37–7.30 (5H, m), 5.97–5.90 (1H, q, J = 6.7 Hz), 2.38–2.33 (2H, t, J = 7.5 Hz), 1.68–1.64 (2H, t, J = 6.9 Hz), 1.58–1.56 (3H, d, J = 6.6 Hz), 1.30 (20H, m), 0.95–0.90 (3H, t, J = 6.6 Hz); ^{13}C NMR δ 173.1 (*C*O), 142.0 (Ar), 128.5 (Ar), 127.9 (Ar), 126.1 (Ar) 72.08 (*C*HO), 34.71 (*C*H_2(CO)), 32.04 (*C*H_2), 29.78 (*C*H_2), 29.76 (*C*H_2), 29.70 (*C*H_2), 29.57 (*C*H_2), 29.47 (*C*H_2), 29.37 (*C*H_2), 29.20 (*C*H_2), 25.08 (*C*H_2), 22.80 (*C*H_2), 22.35 (*C*H_2(CH_3)), 14.21 (*C*H_3).

4.5. General procedure for resolution of 1-phenylethanol1

At first, CAL B (Novozym 435®; 160 mg) and racemic 1-phenylethanol (0.502 g, 4.1 mmol) were added to a plastic test tube (10 mL) inside a glass trap attached to a vacuum pump system, where a fatty ester (4.1 mmol) was being stirred. The reaction mixture was stirred under reduced pressure (100 mm Hg) in a thermostatic water bath. The above-mentioned reaction mixture was

filtered and the enzyme was washed with hexane (3 × 10 mL). The solvent was then evaporated and the reaction mixture was distilled under reduced pressure (1 mm Hg, 60 °C) in order to obtain (*S*)-1-phenylethanol. The enzyme was dried under reduced pressure (20 mm Hg) for 2 h. After distillation, the recovered enzyme and the collected reaction medium containing the other enantiomer as an ester, and the ethyl tetradecanoate were transferred to a plastic test tube (10 mL). Alcohol (absolute ethanol or 2,2,2-trifluoroethanol; for specific amount see Table 1) was added and the mixture was stirred in a thermostatic water bath. The reaction mixture was filtered and the enzyme washed with hexane (3 × 10 mL). The solvent was then evaporated and the reaction mixture distilled under reduced pressure (1 mm Hg, 60 °C) to isolate (*R*)-1-phenylethanol. Both compounds obtained by distillation were analyzed by GLC.

4.6. General procedure for resolution of 6-methylhept-5-en-2-ol (sulcatol) 2

At first, CAL B (Novozym 435®; 160 mg) and racemic 6-methylhept-5-en-2-ol (0.531 g, 4.1 mmol) were added to a plastic test tube (10 mL) inside a glass trap attached to a vacuum pump system where the fatty ester (4.1 mmol) was being stirred. The reaction mixture was stirred under reduced pressure (350 mm Hg) in a thermostatic water bath. Then it was filtered and the enzyme washed with hexane (3 × 10 mL). The solvent was evaporated and the reaction mixture distilled under reduced pressure (1 mm Hg, 50 °C) in order to obtain (*S*)-6-methylhept-5-en-2-ol. The enzyme was dried under reduced pressure (20 mm Hg) for 2 h. After distillation, the recovered enzyme and the collected reaction medium containing the other enantiomer as an ester and the ethyl tetradecanoate were transferred to a plastic test tube (10 mL). The alcohol (absolute ethanol or 2,2,2-trifluoroethanol; for specific amount see Table 1) was added and the mixture was stirred in a thermostatic water bath. The reaction mixture was filtered and the enzyme washed with hexane (3 × 10 mL). The solvent was then evaporated and the reaction mixture distilled under reduced pressure (1 mm Hg, 50 °C) to give the (*R*)-6-methylhept-5-en-2-ol. Both compounds obtained by distillation were analyzed by GLC.

4.7. Hydrolysis reaction

After filtering the enzyme, KOH in methanol/water (90:10) (10% w/v) was slowly added to the reaction mixture at 0 °C. Mixture was stirred at 80 °C for 3 h and cooled. 20 mL of water was added and the alcohol was recovered with diethyl ether (3 × 30 mL). Alcohols were analyzed by GLC.

4.8. Procedure for reused resolution experiments (Fig. 1)

At first, CAL B (Novozym 435®; 500 mg) and racemic 1-phenylethanol (1.32 g, 10.35 mmol) were added to a magnetically stirred ethyl tetradecanoate (2.95 g, 10.35 mmol) in a plastic test tube (50 mL) inside a glass trap attached to a vacuum pump system. The reaction mixture was stirred for 24 h under reduced pressure (100 mm Hg) at 40 °C in a thermostatic water bath. The above-mentioned reaction mixture was filtered and the enzyme washed with hexane (3 × 15 mL). The solvent was then evaporated and the reaction mixture distilled under reduced pressure (1 mm Hg, 60 °C) in order to obtain the (*S*)-1-phenylethanol. The enzyme was dried under reduced pressure (20 mm Hg) for 2 h. After distillation, the recovered enzyme and the collected reaction medium containing the other enantiomer as an ester and the ethyl tetradecanoate were transferred to a plastic test tube (50 mL) Absolute ethanol (2.5 equiv) was added and the mixture was stirred for 24 h at 40 °C in a thermostatic water bath. The reaction mixture was filtered and the enzyme washed with hexane (3 × 15 mL). The solvent was then evaporated and the reaction mixture distilled under reduced pressure (1 mm Hg, 60 °C) in order to obtain the (*R*)-sec-alcohol. The regenerated ethyl tetradecanoate and the enzyme were reused for the next cycle. Both compounds obtained by distillation were analyzed by GLC.

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