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'Easy-on, easy-off' resolution of chiral 1-phenylethylamine catalyzed by *Candida antarctica* lipase B

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Abstract—An 'easy-on, easy-off' process for the effective resolution of (±)-1-phenylethylamine was designed using the lipase B of *Candida antarctica*. This two step lipase-catalyzed process for the resolution of a chiral arylalkylamine involves a high-conversion enantioselective condensation of (*R*)-(+)-1-phenylethylamine with capric acid (conversion 99%, <24 h), followed by the hydrolysis of the corresponding synthesized (*R*)-(+)-amide (conversion 98%, 48 h). As a result, this efficient enzymatic process yields both (*R*)- and (*S*)-enantiomers of 1-phenylethylamine in high enantiomeric purity.

1. Introduction

Enantiopure compounds are used in the fine-chemical, pharmaceutical, and agrochemical industries for applications as resolving agents, chiral auxiliaries, and chiral synthetic building blocks.[1] Consequently, a current research goal is to develop robust and cost-effective catalytic techniques for the production of enantiopure compounds in high enantiomeric excess.[2] Particular attention has been given to the effective resolution of chiral amines such as (±)-1-phenylethylamine (PhEA), broadly used as simple and yet powerful intermediate in industrial asymmetric synthesis or as chiral adjuvant.[3,4] A variety of chemical and enzymatic methods have been reported to obtain both enantiomers of chiral 2-alkylamines in very high enantiomeric excess (99% minimum, preferably >99.5%).[2] However, most of these strategies involve multi-step transformations[5] and low effective fractional recrystallizations.[6]

Although lipase-catalyzed acyl transfer reactions have been demonstrated to be a very useful alternative for the resolution of chiral amines, in general these procedures do not meet the criteria of high yield and high enantiomeric excess for both enantiomers. In fact, the main drawback of enzymatic procedures has been attributed to the null or very low amidase activity of lipases,[7–9] restricting part of the biocatalytic process with the resolution of only one of the enantiomers (the unreactive chiral amine); indeed, the harsh conditions associated to the hydrolysis of the acylated pure enantiomers (concentrated acid media, 16–48 h, $T > 100$ °C)[2,10,11] make the isolation of the pure enantiomer from the resolved amide difficult. Recently, a full enzymatic process, coined as 'easy-on, easy-off', was proposed as a practical tool for the effective resolution of *rac*-PhEA. This process consists of a sequential amide synthesis–hydrolysis process catalyzed by penicillin acylase from *Alcaligenes faecalis* in an aqueous medium, yielding highly pure PhEA enantiomers.[12] Herein, we report a contribution to the effective resolution of PhEA and to the 'easy-on, easy-off' technology by the use of lipase B from *Candida antarctica* (CaL-B) as the enantioselective agent for the effective resolution of *rac*-PhEA. CaL-B, contrary to proteases, amidases, or esterases, has a broad specificity, and its stability in organic solvents allows the extension of the 'easy-on, easy-off' technology to a wide variety of molecules.

2. Results and discussion

The general strategy for this process consists of a first step of enantioselective acylation of *rac*-PhEA *rac*-**1** with capric acid **2** carried out in 2-methyl-2-butanol (2M2B) (Scheme 1) and a second step in which the hydrolysis of

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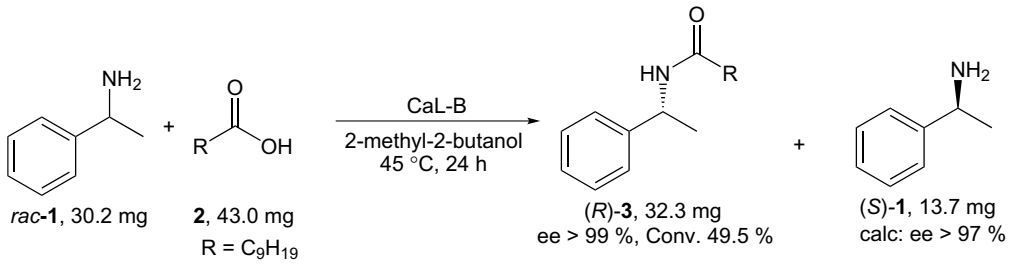
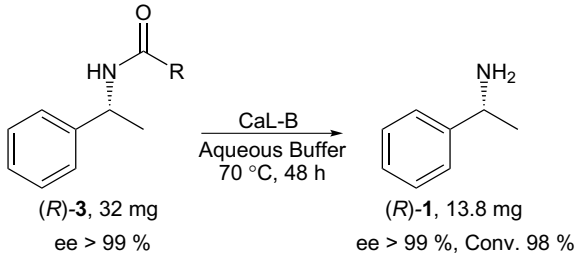


Scheme 1. Lipase-catalyzed preparation of enantiomerically pure (*S*)-**1**.

the acylated (*R*)-(+)-PhEA-enantiomer (*R*)-**3** is carried out at pH 7 in phosphate buffer (Scheme 2). Other acids can be used instead of capric acid either for the synthesis or hydrolysis, although as previously reported for amide hydrolysis, higher reaction rates were observed for the C_{10} acyl residue.[21]



Scheme 2. Lipase-catalyzed preparation of enantiomerically pure (*R*)-**1**.

For the enantioselective acylation of *rac*-PhEA, a reaction mixture containing 12.1 mg (0.01 mmol) of *rac*-**1**, 17.2 mg (0.01 mmol) of **2**, 50 mg of CaL-B, 50 mg of molecular sieves and 5 ml of 2M2B in a 7 ml vessels was agitated at 45 °C for 24 h, to reach a conversion of 49.5% of *rac*-**1** as determined by HPLC.[13] At the end of the acylation reaction, the mixture was filtered to remove the enzyme and molecular sieves, and 2M2B was evaporated; the unreacted PhEA and amide **3** were purified from the crude reaction by flash chromatography. The enantiomerically pure (*R*)-**3** amide was obtained as a white powder (12.8 mg, yield 95%) and the enantiomerically pure (*S*)-**1** amine as a colorless liquid (5.5 mg, yield 90%, calcd ee = 97%). The enantiomeric excess (ee) of (*R*)-**3** was determined by chiral HPLC (ee = 99%). These results confirm the high enantioselectivity of CaL-B and the feasibility of amide synthesis in 2M2B.[13]

In fact, when the lipase-catalyzed amidation of PhEA is carried out in hydrophobic solvents such as hexane, ethyl or di-isopropyl ether, or in solvent free media, yields are considerably lower.[2,10,14] Recently, the high conversion of **1** (97%) in ionic liquids was reported using dodecanoic acid as the acyl donor.[15] It is interesting to note that high yields on amide synthesis obtained in ionic liquids and 2M2B are the consequence of a high solubility of amides in polar solvents,[16] which favor the accumulation of more polar products at equilibrium.[16,17]

Up until now, the enantioselective hydrolysis of amides has been limited to proteases or amidases. However, these enzymes have a narrow specificity and a low stability in organic solvents. On the other hand, although it has been reported that lipases have a restricted activity toward amide hydrolysis, various strategies have been proposed to cope with this limitation. One alternative is the use of activated substrates such as methoxyacetate, to enhance lipase activity.[18,19]

Recently, we have reported that, contrary to the generalized belief that lipases do not hydrolize amides, CaL-B possess an amidase activity which is strongly dependent on the specific structural features of the substrates; indeed, amides with non-substituted aryl residues and medium acyl chain are readily hydrolyzed by CaL-B.[20,21]

Based on these reports, a small scale hydrolysis reaction was carried out with 2.06 mg (0.0075 mmol) of (*R*)-**3**, obtained in the first step, in 5 ml of phosphate buffer incubated at 70 °C with 30 mg of CaL-B (6 mg/ml). After 48 h of reaction 71% yield was obtained as determined by HPLC. The unreacted amide (*R*)-**3** and the enzyme were filtered from the reaction medium, and the filtrate pH adjusted to 11 with NaOH. Finally, the enantiomerically pure (*R*)-**1** amine was recovered completely by ethyl acetate extractions (80% yield). In agreement with our previous work, an efficient hydrolysis was observed for a C_{10} acyl derivative and a non-substituted aryl ring.[21] Indeed, it is well known that the catalytic mechanism of lipases acting on an acylated substrate is undertaken through the formation of an acyl-enzyme intermediate that facilitates nucleophilic attack; this intermediate is particularly favored with substrate structures, such as amides of medium- or long-chain acyl residues.

To evaluate the whole process on a larger scale, an amide synthesis (easy-on) was carried out in 2.5 ml of 2M2B with 30.2 mg of *rac*-**1**, 43.0 mg of **2** (100 mM of both substrates) and 25 mg of CaL-B. After 24 h of reaction, a 49.5% of *rac*-**1** was converted and 32.3 mg of (*R*)-**3** and 13.7 mg of (*S*)-**1** were recovered by flash chromatography (Scheme 1).

In the first attempt to hydrolyze (easy-off) the (*R*)-**3** amide (32 mg) in volumes lower than 20 ml, severe stirring and diffusional problems were encountered. Therefore, to scale-up the process under constant hydrodynamic conditions, we maintained equivalent concentration ratios as those used for the hydrolysis on a low scale: 32 mg of (*R*)-**3** amide and 495 mg of CaL-B (6 mg/ml) resuspended in 82 ml of phosphate buffer, incubated at 70 °C. With

these scaled conditions, better yields than those obtained in the small scale experiment for the enantiomerically pure (*R*)-**1** amine were observed (98%) in 48 h (Scheme 2).[22] The enzymatic hydrolysis herein described results in better yields than those already reported for the selective hydrolysis of arylamides.[23] Indeed, conversion yields of 98% were obtained in the hydrolysis of (*R*)-**3** amide in 48 h, while in general 7–10 days are required to obtain similar yields in lipase-catalyzed hydrolysis of amides.[1]

3. Conclusion

In conclusion, the whole lipase-catalyzed 'easy-on, easy-off' strategy described here considers an integrated synthesis–hydrolysis process where both enantiomerically pure (*R*)-**1** and (*S*)-**1** amines are obtained in good yields and high enantiomeric excess. This strategy involves not only a single biocatalyst in an effective two step resolution process, but also it is successfully applied to the resolution of PhEA, one of the most powerful intermediates used in industrial asymmetric synthesis as a chiral adjuvant and as a ligand in asymmetric catalysis.[3] We are currently working on an extension of this effective enzymatic resolution process for other amines, alcohols, and amino-alcohols.

4. Experimental

4.1. Materials

Methanol (99%) and 2-methyl-2-butanol (2M2B, 99.7%) were purchased from J.T. Baker (Edo. De México, México). Tetrahydrofuran (>99%), (±)-1-phenylethylamine (*rac*-PhEA, 99%), (*S*)-(−)-1-phenylethylamine (⩾98%), capric acid (99%) and molecular sieves (8–12 mesh) were purchased from Aldrich (WI, USA). The lipase B from *C. antarctica*, Novozym 435 (CaL-B) was obtained from Novozymes-México A/C (México).

4.2. HPLC analysis

Previous to HPLC analysis, enzyme and molecular sieves were separated from all samples by centrifugation at 1700*g* for 3 min. The reactions were quantified by HPLC using a Waters Spherisorb 80-5 ODS-2 column (4.6 × 250) mm and a Waters 600E system controller (Waters Corp. Milford, MA, USA) with a flow rate of 1 ml min^{-1}. The effluent was a methanol–phosphate buffer (20 mM, pH 3.8) 70:30 (v/v) solution. The production or consumption of amine in the hydrolysis and synthesis, respectively, was quantified with a water 996 photodiode array detector at 206 nm.

4.3. Chiral HPLC analysis

Enantiomeric excesses of the amide were quantified by chiral HPLC with a CHIRALCEL OJ-H column (4.6 × 250 mm) (Chiral Technologies Inc., West Chester PA, USA). The effluent was *n*-heptane/ethanol 97:3 (v/v) with a flow rate of 1 ml min^{-1}. The retention time for the *N*-[(*S*)-(−)-1-phenylethyl]decanamide is 8.7 min and 7.5 min for the *N*-[(*R*)-(+)-1-phenylethyl]decanamide. To identify each enantiomer, a standard of *N*-[(*S*)-(−)-1-phenylethyl]decanamide enantiomer was chemically synthesized.

4.4. Structure determination

^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Ultra Shield spectrometer run at 300 MHz and 75 MHz, respectively. Elemental analysis was obtained in a Thermofinnigan EA 112 instrument. High resolution mass spectrometry analyses (FAB+) were performed on a Jeol JMS-SX 102A mass spectrometer. Xenon was used as the bombarding gas with an energy of 10 kV using PEG 600 as internal standard and 3-nitrobenzyl alcohol as the matrix. Optical rotations were determined in a 10 cm, 1 ml cell, Perkin–Elmer-341 polarimeter. All melting points were determined in an Electrothermal MEL-TEMP apparatus and are uncorrected.

4.4.1. Chemical synthesis of *N*-[(*S*)-(−)-1-phenylethyl]decanamide (*S*)-3. A stirred solution of capric acid **2** (1.0 g, 1.1 ml, 5.8 mmol) in 100 ml of CH_2Cl_2 was treated with 1.29 g (6.3 mmol) of DCC, 0.04 g (0.4 mmol) of DMAP, and 0.81 ml (0.76 g, 6.3 mmol) of (*S*)-(−)-1-phenylethylamine (*S*)-**1**. The mixture was stirred overnight and then filtered and concentrated in vacuo. Flash Chromatography was packed with silica gel 230–400 mesh (Macherey-Nagel, Düren, Germany) and eluted with 90:10 hexane/ethanol, afforded the pure *N*-[(*S*)-(−)-1-phenylethyl]decanamide enantiomer [(*S*)-**3**] as a white powder. Yield after isolation 81%, white powder, mp = 50–52 °C, $[\alpha]_D^{24} = -64.7$ (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 0.89$ (t, 3H, $J = 6.93$), 1.27 (s, 10H), 1.49 (d, 3H, $J = 6.9$), 1.63 (m, 2H), 2.17 (t, 2H, $J = 7.9$), 5.15 (m, 1H), 5.77, (d, 1H, $J = 7.2$), 7.27–7.36 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.46 MHz) $\delta = 15.4$, 21.9, 22.86, 25.95, 29.45, 29.46, 29.54, 29.64, 32.05, 37.10, 48.71, 126.37, 127.5, 128.83, 143.49, 172.4. Elemental Anal. Calcd for $C_{18}H_{29}NO$: C, 78.49; H, 10.61; N, 5.09. Found: C, 78.35; H, 10.99; N, 4.68.

4.5. Enzymatic reactions

4.5.1. Synthesis of *N*-[(*R*)-(+)-1-phenylethyl]decanamide (*R*)-3. In a general procedure, the reaction was carried out at 45 °C in magnetically agitated 7 ml sealed vessels containing 12.1 mg (0.01 mmol) of *rac*-PhEA *rac*-**1**, 17.2 mg (0.01 mmol) of capric acid **2**, 50 mg of molecular sieves and 5 ml of 2M2B previously dehydrated with molecular sieves. The reactions were started by the addition of 50 mg of CaL-B. After 24 h, the *N*-[(*R*)-(+)-1-phenylethyl]decanamide enantiomer (*R*)-**3** and unreacted 1-phenylethylamine were recovered in a flash chromatography system. Column was packed with silica gel 230–400 mesh (Macherey-Nagel, Düren, Germany) and eluted with 90:10 hexane/ethanol.

Yield after isolation 95%, white powder, melting point 45–47 °C, $[\alpha]_D^{24} = +67.5$ (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) $\delta = 0.89$ (t, 3H, $J = 6.93$), 1.27 (s, 10H), 1.49 (d, 3H, $J = 6.9$), 1.63 (m, 2H), 2.17 (t, 2H, $J = 7.9$), 5.15 (m, 1H), 5.77, (d, 1H, $J = 7.2$), 7.27–7.36 (m, 5H). ^{13}C

NMR ($CDCl_3$, 75.46 MHz) $\delta = 15.4$, 21.9, 22.86, 25.95, 29.45, 29.46, 29.54, 29.64, 32.05, 37.10, 48.71, 126.37, 127.5, 128.83, 143.49, 172.4. Elemental Anal. Calcd for $C_{18}H_{29}NO$: C, 78.49; H, 10.61; N, 5.09. Found: C, 77.51; H, 11.14; N, 4.72.

4.5.2. Hydrolysis of *N*-[(*R*)-(+)-1-phenylethyl]decanamide (*R*)-3. In a general procedure, the reaction was carried out with 2.06 mg (0.0075 mmol) of *N*-[(*R*)-(+)-1-phenylethyl]decanamide enantiomer (*R*)-**3** obtained in the enzymatic synthesis, in 5ml of phosphate buffer incubated at 70 °C with 30 mg of CaL-B (6 mg/ml). After 48 h, the yield of liberated (*R*)-(+)-PhEA enantiomer was determined by HPLC.

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