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PII: S1381-1177(14)00157-X
DOI: http://dx.doi.org/doi:10.1016/j.molcatb.2014.05.007
Reference: MOLCAB 2955

To appear in: *Journal of Molecular Catalysis B: Enzymatic*

Received date: 4-3-2014
Revised date: 30-4-2014
Accepted date: 15-5-2014

Please cite this article as: A. Radu, M.E. Moisă, M.I. Toşa, N. Dima, V. Zaharia, F.D. Irimie, Candida antarctica lipases acting as versatile catalysts for the synthesis of enantiopure (R)- and (S)-1-(2-phenylthiazol-4-yl)ethanamines, *Journal of Molecular Catalysis B: Enzymatic* (2014), http://dx.doi.org/10.1016/j.molcatb.2014.05.007

Graphical Abstract

Candida antarctica lipases acting as versatile catalysts for the synthesis of enantiopure (_R_)- and (_S_)-1-(2-phenylthiazol-4-yl)ethanamines

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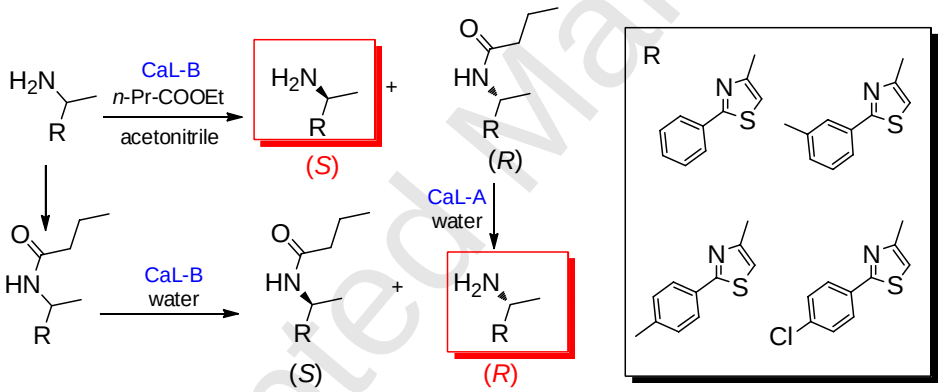

Candida antarctica lipases acting as versatile catalysts for the synthesis of enantiopure (*R*)- and (*S*)-1-(2-phenylthiazol-4-yl)ethanamines

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This paper is dedicated to the anniversary of 95th birthday of Professor Valer Fărcăşanu

Keywords
phenylthiazole, chiral ethanamines, enzymatic kinetic resolution, lipases, amide hydrolysis

Abstract
The synthesis of both enantiomers of four new phenylthiazole-based amines by enantiomer-selective acylation of racemic amines and by hydrolysis of the corresponding racemic amides using lipase B from *Candida antarctica* (Novozyme 435*)* as chiral catalyst was performed with good yields and excellent enantioselectivities. In order to prevent the frequently occurring partial racemization of enantiopure amides during chemical hydrolysis to the corresponding (*R*)-amines, the deprotection of the *N*-acylated (*R*)-enantiomers by mild enzymatic hydrolysis with lipase A from *Candida antarctica* immobilised on Celite was also developed.

1. Introduction

Enantiopure amines and their derivatives are important chiral building blocks and final products especially in the agrochemical, food, pharmaceutical and fine chemical industries. In particular, arylethanamines present valuable industrial applications in the synthesis of pesticides [1].

Heterocyclic ring systems, including thiazole as a well known integral part of all available penicillins, are found as key active component of many drugs, as recently pointed [2]. For instance, 2-phenyl-thiazole derivatives were obtained and a detailed study of their selective antiflaviviral activity was realized [3].

With increasing demand for this type of compounds and interest in environmental aspects, research in this field has led to efforts to establish viable synthethic methods that meet the principles of green chemistry. In this regard, biocatalytic approaches using enzymes as chiral catalysts, especially in organic solvents, are extensively applied.

Several chemocatalytic methods for the preparation of various enantiomerically enriched arylethanamines are described in literature. One of the most common methods for the enantioselective synthesis of amines is the Ru- or Rh-catalysed reductive amination of carbonyl compounds [4]. Another procedure uses a microwave technique to produce the (*S*)-enantiomer of some heteroaryl-ethanamines from the corresponding *N*-protected α-L-amino acids [5]. Rhodium-catalyzed hydrogenation of *N*-sulfonyl ketimine [6] is also described for the asymmetric synthesis of ethanamines. In addition to these, Sigamide-catalyzed enantioselective reduction of ketimines with trichlorosilane also produced selectively the (*S*)-enantiomer of various ethanamines [7].

All these chemical methods require expensive reagents, hard work-up and are time consuming. In contrast, the enzymatic approaches are easier to handle, don't need special reaction conditions and most importantly, they are "*eco-friendly"*. Biocatalytic approaches for obtaining enantiomerically enriched ethanamines commonly use hydrolytic enzymes or transaminases. Lipases were succesfully used in the kinetic resolution of amines through *N*-acylation reactions [1,8]. Hoff and co. reported [9] the synthesis of some chiral *N*-benzyl-*N*-methyl-1-(naphthalen-1-yl)ethanamines and their *in vitro* antifungal activity using in the key step, the enzymatic kinetic resolution of the racemic 1-(naphthalen-1-yl)ethanamine, immobilized lipase B from *Candida antarctica* (commercialized as Novozyme 435) as catalyst, ethyl 2-methoxyacetate as acyl donor and hexane as solvent at 60 °C with excellent yield and enantioselectivity ($E > 200$). A detailed study [10] regarding the kinetic resolution of three racemic ethanamines by acetylation using variously immobilized *Candida antarctica* lipase B indicates that the temperature effect on the selectivity and activity strongly depends on the substrate structure and on the used immobilization method. The CaL-B-catalyzed acylation of various amines and amino-alcohols with myristic acid as acyl donor was also investigated with the aim to explain and control the chemoselectivity of this enzyme, in the specific acylation of bifunctional substrates [11]. The same enzyme was used in the resolution of racemic 1-aryl-allylamines with moderate to excellent enantioselectivity (*ee* 63.5-99.9%) [12a]. Caffeic acid amides in high enantiopuritiy (*ee* 98.5%) were obtained by Novozyme 435-catalyzed aminolysis of racemic ester in isooctane at 70 °C [12b]. Recently, a continuous

flow process for the kinetic resolution of *rac*-1-phenylethylamine using ethyl acetate as acyl donor and Novozyme 435 as catalyst with high enantiomeric *ratios* (E > 200) and short residence time (40 minutes) at 70 °C was reported [13].

A series of primary amine enantiomers were obtained using ω-transaminase from *Vibrio fluvialis* [14], *Arthrobacter sp*. sol-gel immobilized [15a] or with *E. coli* whole cell with ω-transaminase activity [15b].

One of the most frequently used biocatalytic methods is the enzymatic kinetic resolution of a racemate with an adequate hydrolytic enzyme. Enantioselectivity, high stability and commercial availability in both free and immobilized forms have made lipases (EC 3.1.1.3) attractive kinetic resolution catalysts either through the acylation of a nucleophylic functionality (amine or an alcohol) or through the deacylation (hydrolysis, alcoholysis or interesterification) of an amidic or esteric group of the target molecule.

Our aim has been to prepare both enantiomers of four phenylthiazole-based ethanamines in a highly enantiomerically enriched form by a chemoenzymatic pathway (**Scheme 1**). Using the immobilized lipase B from *Candida antarctica* (Novozyme 435) in the enzymatic kinetic resolution of racemic ethanamines *rac*-**4a-d** through *N*-acylation and of racemic ethanamides *rac*-**5a-d** through hydrolysis, a new enzymatic procedure was developed.

2. Experimental Section

2.1. Materials and Methods

All reagents were purchased from Aldrich or Fluka and used as received. Solvents and acyl donors for enzymatic reactions were stored over molecular sieves unless otherwise stated. Lipase from *Pseudomonas fluorescens* (AK) was obtained from Amano, England, while lipase from *Candida rugosa* (CrL) was purchased from Fluka. Lipase A from *Candida antarctica* free or immobilized by adsorption on Celite (CaL-A) was a gift from Professor Liisa T. Kanerva, University of Turku. Immobilized lipase B from *Candida antarctica* (CaL-B, Novozyme 435) was purchased from Novozyme, Denmark and free CaL-B from Chiral Vision, Netherlands. Lipase PS from *Burkholderia cepacia* (*Pseudomonas cepacia*) was purchased from Amano, Aldrich. The enzymatic reactions were performed at 23 °C (room temperature) or at 45 °C.

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker spectrometers operating at 300 or 400 MHz and 75 or 100 MHz, respectively, at 25 ºC. Data for mass spectra analysis (ESI^{+}-

MS) were taken on a VG 7070E mass spectrometer operating at 70 eV. HRMS data were measured in ESI^+ mode with Bruker Avance microOTOF-Qquadrupole-TOF spectrometer.

Thin layer chromatography (TLC) was performed on Merck Kieselgel $60F_{254}$ sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating. Melting points were determined by hot plate method and are uncorrected. The optical rotations were determined with Bellingham-Stanley ADP 220 polarimeter, using chloroform for **5a-d** and methanol for **4a-d** (c = 1mg/mL) as solvents; $[\alpha]_D^{25}$ values are given in units of $10^{-1}deg \times cm^2 \times g^{-1}$.

The enantiomeric separation of *rac*-**4a-d** and *rac*-**5a-d** was performed by High Performance Liquid Chromatography (HPLC) conducted with Agilent 1200 and 1260 systems. Therefore the separation of the racemic ethanamides was accomplished on Chiralcel OJ-H column (4.6 × 250 mm) using *n*-hexane: 2-propanol in different proportions at a flow rate of 1.0 mL/min as mobile phase. The enantiomeric separation of the racemic ethanamines *rac*-**4a,b** was performed on a Chiralpak ZWIX(+) column (4.0 × 250 mm) using acetonitrile: methanol (50 mM formic acid, 25 mM DEA): H_2O (78:28:2, v/v/v) at 1.0 mL/min flow rate as mobile phase, while the separation of *rac*-**4c,d** on Chiralcel OJ-H column was obtained with *n*-hexane: 2-propanol (0.02% DEA) as eluent at 0.9 mL/min flow rate. **Table 1** contains data for the HPLC chromatographic separation of the racemic derivatives**.**

The determination of the *E* value was based on the equation $E = \ln [(1-c)(1-ee_S)] / \ln[(1-c)(1+ee_S)]$, with $c = ee_S/(ee_S+ee_P)$ using linear regression (*E* as the slope of the line $\ln[(1-c)(1-ee_S)]$ versus $\ln[(1-c)(1+ee_S)]$) [16].

2.2. Chemical synthesis

2.2.1. Synthesis of rac-**2a-d**.

One of the aldehydes **1a-d** (1 mmol) dissolved in diethyl ether (5 mL) was added into the ice-cooled solution of the Grignard reagent prepared by treating CH_3I (1.2 equiv.) with magnesium (1.2 equiv.) and I_2 (a crystal) in dry diethyl ether at reflux under argon atmosphere until the magnesium was consumed. The mixture was refluxed overnight. After cooling, saturated ammonium chloride solution was added dropwise under vigorous stirring on an ice bath. The separated organic layer was next extracted with CH_2Cl_2 (3 × 5 mL), dried on anhydrous Na_2SO_4 and evaporated in vacuum. The crude product was purified by column chromatography using CH_2Cl_2: CH_3OH (9:1, v/v) as eluent, affording the pure racemic alcohols *rac*-**2a-d** as yellow semi-solids with 57-70% yields.

*2.2.2. Synthesis of racemic azides (rac-***3a-d***)*

Diphenyl phosphonic azide (1.5 equiv.) was added to a solution of *rac*-**2a-d** (1 mmol) dissolved in dry degassed toluene (2 mL). The mixture was cooled at 0 °C and neat DBU (1.5 equiv.) was added under argon atmosphere. The reaction was stirred for 3 h at 0 °C, then slowly warmed to room temperature and the reaction mixture was stirred for an additional 16 h. The resulting two-phase mixture was washed with water (2 × 20 mL) and 5% HCl solution. (20 mL). The organic layer, dried over anhydrous Na_2SO_4, was further concentrated in vacuum and purified on silica gel with CH_2Cl_2 as eluent providing the desired azides *rac*-**3a-d** as yellow liquids with 65-95% yield.

*2.2.3. Synthesis of racemic amines (rac-***4a-d***)*

The reduction of racemic azides *rac*-**3a-d** to amines *rac*-**4a-d** was performed according to a procedure described in literature [17]. To the solution of azides (1 mmol) and ammonium chloride (2.6 equiv.) in ethyl alcohol (27 mL) and water (9 mL), activated zinc powder (1.3 equiv.) was added. The mixture was stirred vigorously at reflux and after approximately 4 h all the reactions were completed (monitored by TLC). Finally, ethyl acetate and aqueous ammonia (25%) were added and the mixture was filtered to remove the unreacted zinc. The isolated organic layer was dried over anhydrous Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified on silica gel using sequentially two eluents: firstly, CH_2Cl_2: CH_3OH (9:1, v/v) was used to recover the unreacted azide and then CH_2Cl_2: CH_3OH (8:2, v/v) was used to elute the racemic amines. The final products *rac*-**4a-d** were obtained as yellow semi-solids with 90-97% yields.

*2.2.3.1. 1-(2-phenylthiazol-4-yl)ethanamine (rac-***4a***)*. Yield 97%; yellow semi-solid; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.51 (d, *J* = 7 Hz, 3H); 1.91 (broad signal); 4.27 (q, *J* = 7 Hz, 1H); 7.04 (s, 1H); 7.43 (m, 3H); 7.93-7.96 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 23.52; 48.15; 111.90; 125.68; 129.02; 130.05; 133.89; 163.31; 168.31 ppm. HRMS: M^+ found (M^+ calculated for $C_{11}H_{12}N_2S$): 204.0729 (204.0721); ESI$^+$-MS: *m/z* (%): 204 (M, 12); 203 (M-1, 7); 189 (100); 85 (15); 77 (19); 58 (19); 43 (49); 27 (12).

2.2.3.2. 1-(2-m-tolylthiazol-4-yl)ethanamine (*rac-***4b***)*. Yield 94%; yellow semi-solid; ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 1.49 (d, *J* = 7 Hz, 3H); 1.84 (broad signal); 2.38 (s, 3H); 4.37 (q, *J* = 7 Hz, 1H); 7.29-7.42 (m, 2H); 7.62 (s, 1H); 7.73-7.78 (m, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 21.26; 22.41; 47.33; 113.9; 123.40; 126.51; 129.19; 130.94; 133.14; 138.64; 159.02; 166.98 ppm. HRMS: M^+ found (M^+ calculated for

$C_{12}H_{14}N_2S$): 218.0893 (218.0878); ESI^+-MS: *m/z* (%): 219 (M+1, 4); 218 (M, 6); 203 (100); 86 (9); 57 (12); 43 (17); 28 (20).

*2.2.3.3. 1-(2-p-tolylthiazol-4-yl)ethanamine (rac-***4c***).* Yield 93%; yellow semi-solid; ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 1.42 (d, *J* = 7 Hz, 3H); 1.89 (broad signal); 2.35 (s, 3H); 4.23 (q, *J* = 7 Hz, 1H); 7.31 (d, *J* = 9, 2H); 7.48 (s, 1H); 7.83 (d, *J* = 9, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ = 21.17; 22.37; 54.96; 113.73; 126.06; 130.17; 130.55; 140.06; 161.37; 167.00 ppm. HRMS: M^+ found (M^+ calculated for $C_{12}H_{14}N_2S$): 218.0908 (218.0878); ESI^+-MS: *m/z* (%): 218 (M, 19); 203 (100); 176 (17); 159 (17); 118 (21); 101 (16); 85 (24); 57 (20); 43 (47); 27 (7).

*2.2.3.4. 1-(2-(4-chlorophenyl)thiazol-4-yl)ethanamine (rac-***4d***).* Yield 90%; yellow semi-solid; ^1H NMR (400 MHz, CD_3OD, 25 °C): δ = 1.49 (d, *J* = 7 Hz, 3H); 4.22 (q, *J* = 7 Hz, 1H); 7.32 (s, 1H); 7.41 (d, *J* = 6 Hz, 2H); 7.88 (d, *J* = 9 Hz, 2H) ppm. ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 22.76; 48.69; 114.71; 128.86; 130.24; 133.47; 137.04; 163.17; 168.27 ppm. HRMS: M^+ found (M^+ calculated for $C_{11}H_{11}ClN_2S$): 238.0356 (238.0331); ESI^+-MS: *m/z* (%): 239 (M,7); 226 (^{37}Cl, 13); 225 (^{35}Cl, 100); 224 (^{37}Cl, 35); 222 (^{35}Cl, 8); 196 (34); 179 (38); 111 (32); 85 (12); 45 (54); 28 (28).

*2.2.4. Synthesis of racemic amides (rac-***5a-d***)*

DMAP (1%) in pyridine (4 equiv.) and the acylating agent (4 equiv.) were added into the solution of *rac*-**4a-d** (1 mmol) in dry CH_2Cl_2 (15 mL). The mixture was stirred at room temperature for 3 h and then quenched with water (15 mL). The isolated organic layer was dried over anhydrous Na_2SO_4, the solvent evaporated in vacuum and the crude product was purified by column chromatography on silica gel with CH_2Cl_2: CH_3OH (9.5:0.5, v/v) as eluent giving *rac*-**5a-d** as yellow semi-solids with 82-90% yields.

*2.2.4.1. N-(1-(2-phenylthiazol-4-yl)ethyl)butyramide (rac-***5a**). Yield 90%; yellow semi-solid; ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.95 (t, *J* = 7.5 Hz, 3H); 1.55 (d, *J* = 6 Hz, 3H); 1.62-1.75 (m, 2H); 2.19 (t, *J* = 7.5 Hz, 2H); 5.30 (dq, app. quin, *J* = 6 Hz, 6 Hz, 1H); 6.23 (bd, *J* = 6 Hz, 1H); 7.07 (s, 1H); 7.43-7.48 (m, 3H); 7.92-7.95 (d, *J* = 9 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 13.89; 19.29; 22.20; 38.95; 45.78; 113.86; 126.66; 129.08; 130.23; 158.56; 163.44; 169.60; 171.63 ppm. HRMS: M^+ found (M^+ calculated for $C_{15}H_{18}N_2OS$): 274.1136 (274.1140); ESI^+-MS: *m/z* (%): 274 (M, 9); 203 (100); 186 (13); 104 (9); 85 (8); 43 (18); 27 (9).

*2.2.4.2. N-(1-(2-m-tolylthiazol-4-yl)ethyl)butyramide (rac-***5b***).* Yield 87%; yellow semi-solid; ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.94 (t, *J* = 7.5 Hz, 3H); 1.55 (d, *J* = 6 Hz, 3H);

1.62-1.74 (m, 2H); 2.19 (t, J = 7.5 Hz, 2H); 2.42 (s, 3H); 5.27 (dq. app. quin, J = 9 Hz, 6 Hz, 1H); 6.28 (bd, J = 9 Hz, 1H); 7.06 (s, 1H); 7.22-7.32 (m, 2H); 7.70-7.75 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 13.87; 19.26; 21.50; 22.14; 38.90; 45.78; 113.88; 123.88; 127.17; 128.96; 131.03; 133.61; 138.83; 158.57; 168.94; 172.22 ppm. HRMS: M^+ found (M^+ calculated for $C_{16}H_{20}N_2OS$): 288.1322 (288.1296); ESI$^+$-MS: *m/z* (%): 288 (M, 12); 218 (16); 217 (100); 200 (14); 118 (8); 85 (13); 43 (26); 41 (14); 27 (8).

*2.2.4.3. N-(1-(2-p-tolylthiazol-4-yl)ethyl)butyramide (rac-***5c***).* Yield 84%; yellow semi-solid; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.94 (t, J = 7.5Hz, 3H); 1.54 (d, J = 6 Hz, 3H); 1.62-1.74 (m, 2H); 2.19 (t, J = 7.5 Hz, 2H); 2.39 (s, 3H); 5.26 (dq, app. quin, J = 6 Hz, 6 Hz, 1H); 6.25 (bd, J = 6 Hz, 1H); 7.03 (s, 1H); 7.24 (d, J = 6 Hz, 2H); 7.82 (d, J = 6 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 13.88; 19.28; 21.57; 22.19; 38.94; 45.80; 113.34; 126.57; 129.73; 131.10; 140.47; 158.47; 168.31; 172.2 ppm. HRMS: M^+ found (M^+ calculated for $C_{16}H_{20}N_2OS$): 288.1314 (288.1296); ESI$^+$-MS: *m/z* (%): 289 (M+1, 2); 288 (M, 12); 218 (16); 217 (100); 200 (12); 118 (8); 85 (7); 43 (26); 41 (14); 27 (8).

*2.2.4.4. N-(1-(2-(4-chlorophenyl)thiazol-4-yl)ethyl)butyramide (rac-***5d***).* Yield 82%; yellow semi-solid; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.95 (t, J = 7.5 Hz, 3H); 1.55 (d, J = 9Hz, 3H); 1.75-1.65 (m, 2H); 2.19 (t, J = 9 Hz, 2H); 5.27 (dq, app. quin, J = 9 Hz, 6 Hz, 1H); 6.16 (bd, J = 9 Hz, 1H); 7.09 (s, 1H); 7.41 (d, J = 6 Hz, 2H); 7.88 (d, J = 6 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 13.89; 19.28; 22.13; 38.94; 45.73; 114.19; 127.86; 129.30; 132.2; 158.34; 169.98; 177.2 ppm. HRMS: M^+ found (M^+ calculated $C_{15}H_{17}ClN_2OS$), 308.0764 (308.0750); ESI$^+$-MS: *m/z* (%): 308 (M, 9); 239 (^{37}Cl, 40); 237 (^{35}Cl, 100); 222 (^{37}Cl, 13); 220 (^{35}Cl, 12); 85 (^{37}Cl, 7); 83 (^{35}Cl, 5); 43 (^{37}Cl, 15); 41 (^{35}Cl 7); 27 (3).

2.3. Enzymatic synthesis

2.3.1. Analytical scale N-acylation

Novozyme 435 (12.5 mg) and 3-4 pieces of molecular sieves (4 Å) were weighed in a reaction vial before *rac*-**4a-d** (12.5 μmol) in dry organic solvent (0.5 mL) and the acylation agent (50 μmol) were added. Reactions proceeded under shaking (1350 rpm) at 23 °C and were monitored by TLC and HPLC analysis. After approximately 16 h the reactions were considered to be completed.

2.3.2. Analytical scale hydrolysis

10 mg of a CaL-B or CaL-A preparation were weighed in a reaction vial before *rac*-**5a-d** or (*R*)-**5a-d** (7.3 μmol) were added in water (1 mL). The mixtures were shaken at r. t. or at 45 °C. The progress of the reactions was monitored by TLC and HPLC analysis.

2.3.3. Preparative scale N-acylation

The *N*-acylation of racemic amines at preparative scale was performed using substrate:biocatalyst *ratio* and reaction conditions as for the analytical scale.

Therefore, Novozyme 435 (1500 mg) and molecular sieves were weighed in a reaction vial before *rac*-**4a-d** (1.5 mmol) and ethyl *n*-butyrate (6 mmol) were added in ACN (20 mL). The mixtures were shaken at 1350 rpm at 23 °C for approximately 16 h to reach 50% conversion. The enzyme was filtered off, washed with ACN and the combined organic phases were concentrated in vacuum. The crude product was purified by column chromatography on silica gel with CH_2Cl_2: CH_3OH (9.5:0.5, v/v) and CH_2Cl_2: CH_3OH (8:2, v/v) as eluent. The properties of obtained chiral derivatives are presented in **Table 2,** Part A.

2.3.4. Preparative scale hydrolysis

Novozyme 435 or CaL-A on Celite (1000 mg) were weighed in a reaction vial before the aqueous solution of *rac*-**5a-d** or (*R*)-**5a-d** (20 mL, 0.73 mmol) was added. The mixtures were shaken at 45 °C. The progress of the reactions was monitored by TLC and HPLC analysis. When the reactions were completed, the reaction mixtures were freezed and the water was removed by lyophilization. The products were dissolved in CH_2Cl_2 and the enzyme was filtered off. Next, the solution was concentrated in vacuum and the crude product was purified by column chromatography with CH_2Cl_2: CH_3OH as eluent, with a gradient from initial 5% (v/v) to 20% (v/v) to yield (*R*)-amines and (*S*)-amides with excellent enantiomeric excesses at approximately 50% conversion or optically pure (*R*)-**4a-d** as yellow semi-solids data presented in **Table 2,** Part B.

2.4. Absolute configuration

To a solution of *rac*-**2a** (1 mmol) in toluene (10 mL), vinyl acetate (4 mmol), Novozyme 435 (40 mg/mL) and molecular sieves were added. The mixture was shaken at 1350 rpm at room temperature. The process was monitored by TLC and HPLC analysis. The reaction was completed in approximately 6 hours. The enzyme was filtered off, the solvent was removed in vacuum and the crude product was purified by column chromatography with CH_2Cl_2: acetone (25:1, v/v) as eluent. The isolated (*S*)-**2a** was further transformed into the

azide (*R*)-**3a** and next reduced to give (*R*)-**4a** following the procedures described above. Yield: 87%.

3. Results and Discussion

3.1. Chemical synthesis

Based on the methods described in literature, the preparation of amines *rac*-**4a-d** from the corresponding aldehydes **1a-d** was first studied. The racemic ethanols *rac*-**2a-d** obtained by Grignard reaction [18] were transfomed into racemic amines *rac*-**4a-d** *via* azide derivatives *rac*-**3a-d** as shown in **Scheme 1** (route a, steps I-III).

Racemic amides *rac*-**5a-d** were obtained by chemical acylation of *rac*-**4a-d** with butyryl chloride in dichloromethane, in the presence of pyridine and a catalytic amount of DMAP (**Scheme 1,** route a, step IV).

3.2. Enzymatic synthesis

In order to investigate the stereoselectivity of the reactions involving chiral 1-(2-phenylthiazol-4-yl)ethanamines *rac*-**4a–d** and their amides *rac*-**5a-d**, the chromatographic separation of the enantiomers was first established. The base-line separation of all the enantiomers of *rac*-**4,5a-d** was performed using appropriate HPLC chiral columns. The chromatographic enantiomer separation of *rac*-**4c,d** was successfully performed when small amounts of diethylamine (DEA) [19] was added to the mobile phase (**Table 1**).

3.2.3. Analytical scale enzymatic N-acylation of rac-**4a-d**

Common commercial lipase preparations [25 mg/mL; lipases from *Candida rugosa* (CrL), *Pseudomonas fluorescens* (AK), lipase A from *Candida antarctica* immobilized by adsorption on Celite (CaL-A), immobilized lipase B from *Candida antarctica* (CaL-B, Novozyme 435) and Amano lipase PS from *Burkholderia cepacia* (*Pseudomonas cepacia*)] were screened for the *N*-acylation of the model compound, *rac*-**4a**, at room temperature.

In order to find the optimal reaction conditions for the *N*-acylation of *rac*-**4a** the analytical scale enzymatic reactions were conducted in various organic solvents using four different acyl donors like ethyl acetate, isopropyl *n*-butyrate, ethyl *n*-butyrate, ethyl propionate (4 equiv. each). The tested solvents, methyl *tert*-butyl ether (MTBE), acetonitrile (ACN), dichloromethane, toluene and diisopropyl ether (DIPE), were selected as they are frequently used in organic synthesis and industry and are known for their compatibility with lipases. The addition of molecular sieve [4 Å; the *ratio* to the catalyst 1:1 (w/w)] to the reaction medium was necessary since even small traces of water could promote lipase-catalysed hydrolytic reactions. In this case, the hydrolysis of the amidic group could lead to

the formation of an ammonium salt between the amine and the liberated acid, thus lowering the enantioselectivity of the kinetic resolution.

Some lipases (CRL, PS-free and AK on Celite) proved to be catalytically inactive even after 28 hours, while CaL-A showed either no activity or poor selectivity in MTBE with isopropyl *n*-butyrate and ethyl *n*-butyrate.

The highest enantioselectivity (E » 200) and reactivity (c = 50%) for the CaL-B mediated *N*-acylation of *rac*-**4a** were obtained with ethyl *n*-butyrate as acyl donor in dry ACN as solvent after 16 hours (**Table 3**, entry 3).

3.2.4. Preparative scale enzymatic N-acylation of rac-**4a-d**

The developed small-scale kinetic resolution method of *rac*-**4a** was next used for the preparative-scale kinetic resolution of all substrates *rac*-**4a-d** (1.5 mmol) with ethyl *n*-butyrate (6 mmol) and Novozyme 435 (75 mg/mL) in dry ACN (**Scheme 1**, step V). The highly enantioselective *N*-acylations of the reactive (*R*)-**4a-c** (E » 200) were all completed (c ~ 50%) in 16 h. Lower selectivity and activity of the enzyme was observed when the chlorinated *rac*-**4d** was subjected to the same biotransformation (E = 125, c ~ 46% after 16 h).

The resolution products [the unreacted (*S*)-**4a-d** and the obtained (*R*)-**5a-d**] were isolated at close to 50% theoretical yields (93-97% from the theoretical amounts at 50% conversion) in highly enantiopure forms (*ee* 82-99%) (**Table 2**, Part A).

3.2.5. Kinetic resolution and deprotection of (*R*)-**5a-d** *by lipase catalyzed hydrolysis*

Recently, a very important feature that affords amide hydrolysis by serine proteases was attributed to the hydrogen bond formed between the substrate's amide nitrogen and the enzyme or the substrate itself, thus facilitating the nitrogen inversion during the catalytic process [20]. Even though lipases have the same catalytic triad, some of them lack this particular hydrogen bond and, subsequently, the ability to efficiently cleave amides. However, previous results showing that lipases CaL-A [21-22] and CaL-B [1, 22-23] can hydrolyze amides in water, encouraged us to study the hydrolysis of the arylethanamides using lipases as catalysts.

In order to obtain the opposite enantiomeric forms of the enantiomerically enriched 1-(2-phenylthiazol-4-yl)ethanamines [the (*S*)-enantiomers], the lipase-mediated kinetic resolution of *rac*-**5a-d** was investigated (**Scheme 1**, route b). To this extent, several CaL-A and CaL-B preparations were tested for the selective hydrolysis of the model compound *rac*-**5a**.

Analytical scale hydrolytic reactions were performed in water at room temperature and at 45 °C using four enzyme preparations (**Table 4**). It is worth mentioning that the addition of any co-solvent was not necessary. As expected, the results show a greater reaction rate for the reactions performed at 45 °C. All CaL-B-mediated hydrolysis were highly enantioselective, the best reactivity was achieved with Novozyme 435 (**Table 4**, entry 1). CaL-A (free or immobilized on Celite) proved to be a highly active but non-stereoselective catalyst for the same transformations. On terms of reactivity, CaL-A on Celite gave the highest reactivity at 45 °C (**Table 4**, entry 4).

Novozyme 435-catalysed preparative scale hydrolysis of *rac*-**5a-d** proceeded enantioselectively and afforded the (*R*)-amines and (*S*)-amides with excellent enantiomeric excesses at approximately 50% conversion. The enantioselective hydrolytic reactions were completed in 80 hours for *rac*-**5a,b** and 90 hours for *rac*-**5c,d** (**Table 2**, Part B).

Nevertheless (*R*)-**4a-d** amines could be provided also by the deprotection of (*R*)-**5a-d** amides. In our try to avoid the racemization and due to the instability of the heteroaryl ring against traditional acid- and base-catalysed hydrolysis we used the CaL-A mediated hydrolytic deprotection of (*R*)-**5a-d** amides. The CaL-A on Celite mediated preparative scale hydrolytic reactions of (*R*)-**5a-d** were performed at 45 °C yielding quantitatively the corresponding enantiomerically enriched (*R*)-**4a-d** amines in 60 hours (**Table 2**, Part B).

3.3. Determination of the absolute configuration

The absolute configuration of (*R*)-**4a** was determined by first preparing the corresponding alcohol according to the previous method and resolving it with Novozyme 435 in neat vinyl acetate, yielding (*S*)-**2a** with 99% *ee* (**Scheme 1**, route c) [24]. The subsequent transformations of (*S*)-**2a** to (*R*)-**4a** following the procedure described above did not affect significantly the enantiopurity of the involved compounds. The optical rotation measured for (*R*)-**4a** obtained in this way gave $[\alpha]_D^{25}$ = +6.3 (c = 1.0, CH_3OH) at 95% *ee*, which is in accordance with the value $[\alpha]_D^{25}$ = +6.4 (c = 1.0, CH_3OH) at 99% *ee* obtained for the CaL-B-catalyzed hydrolysis of *rac*-**5a** in water (**Table 2**, Part B). It is reasonable to assume that Novozyme 435 manifests the same enantiopreference with the other substrates too in *N*-acylation and hydrolytic reactions. The (*R*)-enantiopreference of Novozyme 435 shown herein has proved to be in accordance with the enantiopreference observed before for the enzyme in *N*-acylations of arylethanamines [8a, 22].

4. Conclusions

An efficient new procedure for the synthesis of enantiomerically enriched (*R*)- and (*S*)-1-(2-phenylthiazol-4-yl)ethanamines has been developed (*ee* 82-99%, *E* » 200 except the chlorinated compound when *E* = 125). It was found that CaL-B (Novozyme 435) is the proper catalyst for both enantioselective *N*-acylation and hydrolysis. The CaL-B-mediated kinetic resolution of *rac*-**4a-d** through *N*-acylation occured best using ethyl *n*-butyrate as acyl donor in acetonitrile as solvent at room temperature. The Novozyme 435-mediated hydrolysis of *rac*-**5a-d** was optimal in water at 45 °C, with no need for additional co-solvents. Deprotection of (*R*)-**5a-d** to the corresponding (*R*)-**4a-d** was achieved faster using CaL-A immobilized on Celite, without affecting the optical purity.

Amide hydrolysis is generally uncommon to lipases which naturally hydrolyze fatty acid glycerol esters but these studies have proven once more the versatility of this type of enzymes. The amidase activity of lipases observed here is of great interest for organic synthesis as it offers an alternative mild method for deprotection of amides or for resolving racemic amides since chemical decomposition and racemization was avoided.

Acknowledgments

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-ID-PCE-2011-3-0775.

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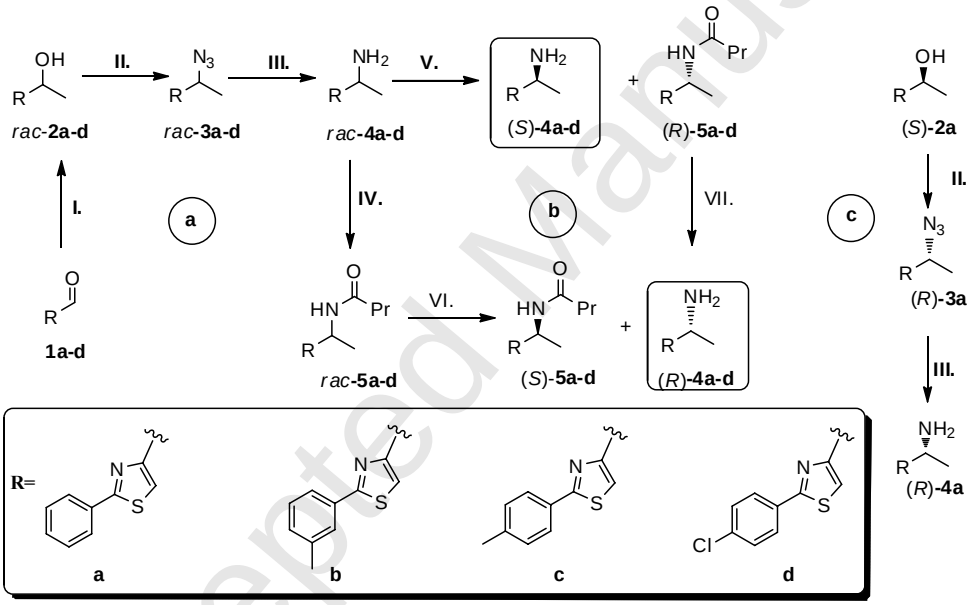
[24] D. Hapău, J. Brem, M. Moisă, M.I. Toşa, F.D. Irimie, V. Zaharia, J. Mol. Catal. B: Enzymatic 94 (2013) 88-94.**Figure legends**



Scheme 1. Synthesis and biotransformations of the studied 1-(2-phenylthiazol-4-yl)ethanamines and ethanacetamides. Reagents and conditions: **I.** CH_3MgI, diethyl ether; **II.** $(PhO)_2PON_3$/toluene; **III.** Zn/NH_4Cl, H_2O/ethanol; **IV.** $CH_3(CH_2)_2COCl$/DMAP/Pyridine/DCM; **V.** CaL-B/ ethyl *n*-butyrate/ACN; **VI.** CaL-B/H_2O; **VII.** CaL-A/H_2O.

Research highlights

- A new procedure for the synthesis of both enantiomerically enriched (*R*)- and (*S*)-1-(2-phenylthiazol-4-yl)ethanamines, based on enzymatic kinetic resolution, was developed.
- The CaL-B-mediated kinetic resolution of racemic amines through *N*-acylation occurred optimal using ethyl *n*-butyrate as acyl donor and acetonitrile as solvent at room temperature.
- The CaL-B-mediated hydrolysis of racemic amides was optimal in water at 45 °C, with no need for additional co-solvents.
- In order to avoid the chemical decomposition and/or racemization during the deprotection of (*R*)-amides to the corresponding (*R*)-amines, CaL-A on Celite, a versatile biocatalyst, was used.

Table legends

Table 1. HPLC chromatographic separation of the racemic derivatives *rac*-**4,5a-d**.
Table 2. Preparative scale enzymatic *N*-acylation of *rac*-**4a-d** (Part A), enzymatic hydrolysis of *rac*-**5a-d** (Part B).
Table 3. *N*-acylation of *rac*-**4a** with CaL-B at 23°C after 16 h.
Table 4. CaL-A and CaL-B-catalyzed analytical scale hydrolysis of *rac*-**5a** in water after 30 h.

Table 1. HPLC chromatographic separation of the racemic derivatives *rac*-**4,5a-d**.

Amine	Condition	Retention time (min)		Amide	Condition	Retention time (min)	
		$t_{r\,(S)}$	$t_{r\,(R)}$			$t_{r\,(S)}$	$t_{r\,(R)}$
4a	70:28:2[a]	25.3	26.7	**5a**	85:15[c]	6.1	7.9
4b	70:28:2[a]	24.4	26.8	**5b**	90:10[c]	6.6	7.9
4c	80:20:2[b]	9.2	10.0	**5c**	85:15[c]	5.8	7.8
4d	90:10:1[b]	10.7	11.2	**5d**	90:10[c]	7.5	8.7

[a] *ratio* of acetonitrile:methanol (FA+DEA):H_2O (Chiralpak ZWIX (+), 1.0 mL/min flow rate)
[b] *ratio* of *n*-hexane:2-propanol:DEA (Chiralcel OJ-H, 0.9 mL/min flow rate)
[c] *ratio* of *n*-hexane:2-propanol (Chiralcel OJ-H, 1.0 mL/min flow rate)

Table 2. Preparative scale enzymatic *N*-acylation of *rac*-**4a-d** (Part A) and enzymatic hydrolysis of *rac*-**5a-d** (Part B).

Part A	**Preparative scale Novozyme 435 mediated *N*-acylation of *rac*-4a-d[b]**					**Part B**	**Preparative scale Novozyme 435 mediated hydrolysis of *rac*-5a-d[c]**			
Compound	**Yield (%)[a]**	***ee* (%)**	**$[\alpha]_D^{25}$**	***mp* (°C)**	***E***	**Compound**	**Yield (%)[a]**	***ee* (%)**	**$[\alpha]_D^{25}$**	***E***
(*S*)-**4a**	97	> 99	-6.2	-	» 200	(*R*)-**4a**	96	> 99	+6.4	» 200
(*R*)-**5a**	97	> 99	+175.9	110±1		(*S*)-**5a**	96	> 99	-176.4	
(*S*)-**4b**	95	> 99	-8.4	-	» 200	(*R*)-**4b**	93	> 99	+8.5	» 200
(*R*)-**5b**	95	> 99	+164.2	113±1		(*S*)-**5b**	93	98	-164.8	
(*S*)-**4c**	93	> 99	-18.3	-	» 200	(*R*)-**4c**	94	> 99	+18.8	» 200
(*R*)-**5c**	96	> 99	+148.8	135±1		(*S*)-**5c**	96	96	- 147.4	
(*S*)-**4d**	93	82	-11.0	-	125	(*R*)-**4d**	91	98	+10.8	> 200
(*R*)-**5d**	93	96	+151.2	130±1		(*S*)-**5d**	95	94	-149.7	

[a] 50% of the racemates taken as 100% theoretical yields; [b] after 16h at 23 °; [c] in water at 45 °C after 80 and 90h respectively.

Table 3. *N*-acylation of *rac*-**4a** with CaL-B at 23 °C after 16 h.

Entry	Acyl donor	Solvent	*c* (%)	*E*
1	isopropyl *n*-butyrate	toluene	40	» 200
2	ethyl *n*-butyrate	toluene	44	» 200
3	ethyl *n*-butyrate	ACN	50	» 200

Table 4. CaL-A and CaL-B-catalyzed analytical scale hydrolysis of *rac*-**5a** in water after 30 h.

Entry	Enzyme	Temperature (°C)	*c* (%)	ee_s (%)	ee_p (%)	*E*
1	CaL-B (Novozyme 435)	23	28	37	> 99	> 200
		45	45	80	> 99	> 200
2	CaL-B (Chiral Vision)	23	39	64	> 99	>200
		45	44	78	> 99	> 200
3	CaL-A free	23	14	3	18	1
		45	31	9	20	2
4	CaL-A on Celite	23	55	5	4	1
		45	75	18	6	1

Research highlights

- A new synthesis of both enantiomers of 1-(2-phenylthiazol-4-yl)ethanamines, by enzymatic kinetic resolution, was developed
- The CaL-B-mediated kinetic resolution of amines occurred optimal using ethyl n-butyrate as acyl donor in acetonitrile
- The CaL-B-mediated hydrolysis of racemic amides was optimal in water at 45 °C, with no need for additional co-solvents.
- To avoid the chemical decomposition and/or racemization during the deprotection of (R)-amides, CaL-A on Celite was used.