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Research highlights

- Kinetic resolution of 1-(2-arylthiazol-4-yl)ethanols and their acetates is described.
- Several lipases were screened as biocatalysts.
- CaL-B efficiently catalyzed both acetylation and methanolysis (ee>90%, E>>200).
- The absolute configuration of the products was determined by the Mosher's method.

Heterocycles 32. Efficient kinetic resolution of 1-(2-arylthiazol-4-yl)ethanols and their acetates using lipase B from *Candida antarctica*

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Keywords

Biocatalysis; kinetic resolution; thiazole; chiral secondary alcohols

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Abstract

In this paper we describe the chemoenzymatic synthesis of new enantiomerically enriched (*R*)- and (*S*)-1-(2-arylthiazol-4-yl)ethanols and their acetates by enzymatic enantioselective acetylation of the racemic alcohols *rac*-**2a-d** and by methanolysis of the corresponding racemic esters *rac*-**3a-d** mediated by lipase B from *Candida antarctica* (CaL-B) in non-aqueous media. In terms of stereoselectivity and activity, both procedures, acylation and alcoholysis, gave similar good results (50% conversion, E>>200). The absolute configuration of the kinetic resolution products was determined by a detailed ^{1}H NMR study of the Mosher's derivatives of (*S*)-**2b**.

1. Introduction

Optically active compounds as single enantiomers have currently a considerable pharmacological importance because chirality causes a high selectivity in interaction with protein receptors and provides specific action to the medication. It is well known that the enantiomers may possess different potency and pharmacoselectivity, due to their different interaction with pharmacological receptors [1]. Consequently, chiral compounds are becoming even more important in therapy and in academic research. In particular, enantiomerically pure secondary alcohols are valuable key synthetic intermediates for various compounds of biological and medicinal interest [2].

Biocatalytic processes have been intensely studied and have become a useful and green alternative in stereoselective synthesis due to their multiple advantages [3]. Among them, lipase mediated kinetic resolutions have found broad applications in the synthesis of various optically active intermediates such as secondary alcohols, amines, amino alcohols, epoxides, amino acids and carboxylic acids [4]. Lipases possess the same enantiomeric preference in both acylation and alcoholysis/hydrolysis process and this constitutes a viable access to the both enantiomeric forms of the chiral products [5]. Furthermore, lipases maintain their stability and selectivity even in organic solvents, because of their oil-water interfacial activation [4].

Heterocyclic ring systems, presenting a diverse array of pharmacophores, can be commonly found in the active components of many medicines. The need for highly enantiopure heterocyclic building blocks and intermediates containing reactive functional groups is a challenge for the modern research.

As a part of our interest in development stereoselective methods for preparation of optically active ethanols bearing a heteroaromatic moiety [6], encouraged by the results concerning the enzymatic kinetic resolution of racemic 1-(2-aryl-4-methyl-thiazol-5-yl)ethanols [7], we turned our attention to the lipase mediated synthesis of enantiopure (*R*)- and (*S*)-1-(2-arylthiazol-4-yl)ethanols (*R*)- and (*S*)--**2a-d** and their (*R*)- and (*S*)-acetates (*R*)- and (*S*)--**3a-d** (Scheme 1).

The absolute configuration of the novel enantiopure compounds was determined by a detailed ^{1}H NMR study of the Mosher's derivatives of (*S*)-**2b**.

2. Experimental

2.1. Analytical methods

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ solution on a Brucker Avance DPX-300 spectrometer operating at 300 and 75 MHz, respectively. Chemical shifts on the δ scale are expressed in ppm values from TMS as internal standard. Mass spectra were recorded on GC-MS Shimadzu QP 2010 Plus spectrometer using direct injection and EI or NCI ionization at 30-70 eV.

High performance liquid chromatography analyses were conducted with an Agilent 1200 instrument using a Chiralpak IB column (4.6 x 250 mm). In all cases, a gradient separation procedure was first established, using a mixture of *n*-hexane and 2-propanol as eluent, all at 1 mL/min flow rate. The gradient separation procedure and the retention times of the enantiomers are showed in Table 1. Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F254 sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates. Preparative chromatographic separations were performed using column chromatography on Merck Kieselgel 60Å (63-200 μm). Optical rotations were determined on a Bellingham-Stanley ADP 220 polarimeter using chloroform as solvent. Melting points were determined on open glass capillaries using an Electrothermal IA 9000 instrument.

2.2. Reagents and solvents

The commercial chemicals and solvents were products of Aldrich or Fluka. All solvents were purified and dried by standard methods as required.

Lipase B from *Candida antarctica* (CaL-B, Novozym 435) was purchased from Novozymes, Denmark. Lipases from *Candida rugosa* (CrL), *Mucor miehei* (MML) and hog pancreas (PPL) were purchased from Fluka. Lipases from *Pseudomonas fluorescens* (AK free), *Burkholderia cepacia* (BcL) were from Amano, Europe, England. Lipase A from *Candida antarctica* covalently immobilized (IMMCal-A T2–150) was purchased from Chiralvision.

2.3. Chemical synthesis of racemic alcohols and their acetates (*rac*-2,3a-d)

The synthesis of racemic secondary alcohols *rac*-**2a-d** and their acetates *rac*-**3a-d** is described in Scheme 1. The 2-arylthiazole-4-carbaldehydes **1a-d** were synthesized as previously described in the literature, using the Hantzsch condensation of various thioamides with 1,3-dichloroacetone, followed by Sommelet reaction [8]. Further, using methyl magnesium iodide as Grignard reagent, the racemic alcohols *rac*-**2a-d** were obtained. Racemic acetates *rac*-**3a-d** were prepared by chemical acetylation of the corresponding alcohols with acetic anhydride.

2.3.1. Synthesis of racemic 1-(2-arylthiazol-4-yl)ethanols (*rac*-2a-d)

To a stirred solution of methyl magnesium iodide, prepared from magnesium (5.6 mmol, 0.134 mg) and iodomethane (5.6 mmol, 0.79 g, 0.348 mL) in diethyl ether (5 mL), under argon, a solution of 2-arylthiazol-4-carbaldehyde (**1a-d**) (3.73 mmol) dissolved in diethyl ether (10 mL) was added slowly. The reaction mixture was stirred at 50°C for 2 hours. After quenching the reaction mixture by the addition of saturated ammonium chloride solution (12 mL) at 0°C, the organic layer was separated and the aqueous layer was extracted with diethyl ether (2 × 10 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotatory evaporation. The crude product was purified by column chromatography using CH_2Cl_2/acetone (25:1 v/v) as eluent.

2.3.1.1. 1-(2-phenylthiazol-4-yl)ethanol (*rac*-2a): Yield: 80%; clear oil; ^{1}H NMR : (300 MHz, $CDCl_3$): δ = 1.60(d, J = 6.1 Hz, 3H); 5.04(q, J = 6.1 Hz, 1H); 7.11(s, 1H); 7.32-7.50(m, 3H); 7.81-8.00(m, 2H); ^{13}C NMR : (75 MHz, $CDCl_3$): δ = 22.9; 66.6; 112.9; 126.6; 128.9; 130.1; 133.4; 161.7; 168.5; HRMS: M^+ found (M^+ calculated for $C_{11}H_{11}NOS$): 205.0565 (205.0561); ESI$^+$-MS: m/z (%): 206 (M+1, 16); 205 (M, 31); 190 (100); 162 (63); 121 (15); 104 (15); 77 (20); 59 (19); 45 (12); 43 (24).

2.3.1.2. 1-(2-m-tolylthiazol-4-yl)ethanol (*rac*-2b): Yield: 82%; clear oil; ^{1}H NMR : (300 MHz, $CDCl_3$): δ = 1.62 (d, J = 6.4 Hz, 3H); 2.41 (s, 3H); 5.04 (q, J = 5.0 Hz, 1H); 7.10 (s, 1H); 7.23 (d, J = 7.5 Hz, 1H); 7.32 (t, J = 7.6 Hz, 1H); 7.72 (d, J = 7.6 Hz, 1H); 7.77 (s, 1H); ^{13}C NMR : (75 MHz, $CDCl_3$): δ = 21.3; 22.9; 66.7; 112.4; 123.7; 127.0; 128.8; 130.9; 133.4; 138.7; 161.5; 168.6; HRMS: M^+ found (M^+ calculated for $C_{12}H_{13}NOS$): 219.0722 (219.0718); ESI$^+$-MS: m/z (%): 220 (M+1, 29); 219 (M, 45); 204 (100); 176 (59); 135 (10); 118 (16); 91 (14); 59 (13); 45 (10); 43 (12).

2.3.1.3. 1-(2-p-tolylthiazol-4-yl)ethanol (*rac*-2c): Yield: 78%; clear oil; ^{1}H NMR : (300 MHz, $CDCl_3$): δ = 1.60 (d, J = 6.5 Hz, 3H); 2.38 (s, 3H); 5.03 (q, J = 6.5 Hz, 1H); 7.1 (s, 1H); 7.22 (d, J = 8.0 Hz, 2H); 7.81 (d, J = 8.1 Hz, 2H); ^{13}C NMR : (75 MHz, $CDCl_3$): δ = 21.4; 22.8; 66.5; 112.1; 126.4; 129.5; 130.8; 140.2; 161.4; 168.5; HRMS: M^+ found (M^+ calculated for $C_{12}H_{13}NOS$): 219.0721 (219.0718); ESI$^+$-MS: m/z (%): 220 (M+1, 20); 219 (M, 46); 204 (100); 176 (61); 135 (10); 118 (20); 91 (15); 59 (16); 45 (12); 43 (16).

2.3.1.4. 1-(2-p-bromophenylthiazol-4-yl)ethanol (*rac*-2d): Yield: 80%; clear oil; ^{1}H NMR : (300 MHz, $CDCl_3$): δ = 1.59 (d, J = 6.5 Hz, 3H); 5.00 (q, J = 6.3 Hz, 1H); 7.13 (s, 1H); 7.53 (d, J = 8.6 Hz, 2H); 7.77 (d, J = 8.6 Hz, 2H); ^{13}C NMR : (75 MHz, $CDCl_3$): δ = 22.9; 66.6; 113.0; 124.2; 127.9; 132.0; 132.3; 161.9; 167.0; HRMS: M^+ found (M^+ calculated for $C_{11}H_{10}BrNOS$): 282.9662 (282.9666); ESI$^+$-MS: m/z (%) = 286 (M+1, ^{81}Br, 17); 285 (M, ^{81}Br, 39); 284 (M+1, ^{79}Br, 21); 283 (M, ^{79}Br, 37); 270 (87); 268 (100); 242 (43); 240 (44); 201 (6); 199 (6); 184 (5); 182 (5); 161 (30); 134 (9); 59 (21); 45 (20); 43 (18).

2.3.2. Synthesis of racemic 1-(2-arylthiazol-4-yl)ethyl acetates (*rac*-3a-d) by chemical acetylation of the corresponding racemic 1-(2-arylthiazol-4-yl)ethanols (*rac*-2a-d)

To a solution of racemic 1-(2-arylthiazol-5-yl)ethanol (*rac*-**2a-d**, 0.6 mmol) in dry dichloromethane (3 mL), triethylamine (0.66 mmol, 66.8 mg, 92.1 µL), acetic anhydride (0.66 mmol, 67.32 mg, 62.2 µL) and 4-dimethylaminopyridine (0.016 mmol, 2 mg) were added. The mixture was stirred at room temperature overnight and then quenched with water (10 mL). The isolated organic layer was drie0d over anhydrous Na_2SO_4 and the solvent was distilled off by rotatory evaporation. The crude product was purified by column chromatography on silica gel (eluent: dichloromethane: acetone = 25:1 (v/v)) to afford *rac*-**3a-d**.

2.3.2.1. 1-(2-phenylthiazol-4-yl)ethyl acetate (***rac*-3a):** Yield: 91%; clear oil; ^{1}H NMR : (300 MHz, $CDCl_3$): δ = 1.68 (d, J = 6.6 Hz, 3H); 2.11 (s, 3H); 6.09 (q, J = 6.6 Hz, 1H); 7.18 (s, 1H); 7.40-7.42 (m, 3H); 7.93-7.96 (m, 2H); ^{13}C NMR : (75 MHz, $CDCl_3$): δ = 20.1; 21.2; 68.7; 114.9; 126.5; 128.7; 130.0; 133.4; 157.0; 168.2; 170.1; HRMS: M^+ found (M^+ calculated for $C_{13}H_{13}NO_2S$): 247.0670 (247.0667); ESI$^+$-MS: m/z (%) = 248 (M+1, 2); 247 (M, 8); 204 (100); 188 (21); 162 (10); 104 (11); 77 (7); 45 (11); 43 (19).

2.3.2.2. 1-(2-m-tolylthiazol-4-yl)ethyl acetate (***rac*-3b):** Yield: 90%; clear oil; ^{1}H NMR : (300 MHz, $CDCl_3$): δ = 1.67 (d, J = 6.6 Hz, 3H); 2.11 (s, 3H); 2.40 (s, 3H); 6.08 (q, J = 6.6 Hz, 1H); 7.17 (s, 1H); 7.22 (d, J = 7.6 Hz, 1H); 7.30 (t, J = 7.6 Hz, 1H); 7.71 (d, J = 7.6; 1H); 7.77 (s, 1H); ^{13}C NMR : (75 MHz, $CDCl_3$): δ = 20.3; 21.3; 68.8; 114.8; 123.8; 127.0; 128.7; 130.8; 133.4; 138.6; 157.0; 168.6; 170.2; HRMS: M^+ found (M^+ calculated for $C_{14}H_{15}NO_2S$): 261.0820 (261.0823); ESI$^+$-MS: m/z (%) = 262 (M+1, 2); 261 (M, 9); 218 (100); 202 (16); 200 (9); 176 (9); 118 (12); 43 (15).

2.3.2.3. 1-(2-p-tolylthiazol-4-yl)ethyl acetate (***rac*-3c):** Yield: 92%; clear oil; ^{1}H NMR : (300 MHz, $CDCl_3$): δ = 1.67 (d, J = 6.6 Hz, 3H); 2.11 (s, 3H); 2.38 (s, 3H); 6.07 (q, J = 6.6 Hz, 1H); 7.15 (s, 1H); 7.22 (d, J = 8.0 Hz, 2H); 7.83 (d, J = 8.2 Hz, 2H); ^{13}C NMR : (75 MHz, $CDCl_3$): δ = 20.3; 21.3; 21.4; 68.8; 114.5; 126.5; 129.5; 130.9; 140.3; 157.0; 168.6; 170.3; HRMS: M^+ found (M^+ calculated for $C_{14}H_{15}NO_2S$): 261.0821 (261.0823);

ESI^+-MS: m/z (%) = 262 (M+1, 2); 261 (M, 10); 218 (100); 202 (17); 200 (9); 176 (10); 118 (13); 43 (14).

2.3.2.4. 1-(2-p-bromophenylthiazol-4-yl)ethyl acetate (*rac*-3d): Yield: 91%; white solid; m.p.: 54-55ºC; ^{1}H NMR : (300 MHz, $CDCl_3$): δ = 1.66 (d, J = 6.6 Hz, 3H); 2.11 (s, 3H); 6.06 (q, J = 6.6 Hz, 1H); 7.20 (s, 1H); 7.54 (d, J = 8.6 Hz, 2H); 7.81 (d, J = 8.6 Hz, 2H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 20.2; 21.3; 68.6; 115.3; 124.2; 128.0; 132.0; 132.4; 157.3; 167.0; 170.2; HRMS: M^+ found (M^+ calculated for $C_{13}H_{12}BrNO_2S$): 324.9769 (324.9772); ESI^+-MS: m/z (%) = 328 (M+1, ^{81}Br, 7); 327 (M, ^{81}Br, 11); 326 (M+1, ^{79}Br, 6); 325 (M, ^{79}Br, 10); 284 (100); 282 (98); 268 (29); 266 (30); 242 (6); 240 (6); 187 (10); 102 (8); 84 (10); 58 (15); 45 (26); 43 (49).

2.4. Analytical scale enzyme mediated biotransformation of 1-(2-arylthiazol-4-yl)ethanols (*rac*-2a-d) and their esters (*rac*-3a-d)

2.4.1. Enzymatic kinetic resolution of racemic 1-(2-phenylthiazol-4-yl)ethanol (*rac*-2a) with vinyl acetate and different lipases

To a solution of racemic 1-(2-phenylthiazol-4-yl)ethanol *rac*-**2a** (20 μmol, 4.1 mg) in vinyl acetate (200 μL) lipase (10 mg) was added. The reaction mixture was shaken at 1300 rpm at room temperature, for 5 hours. For HPLC analysis, samples were taken from the reaction mixture (20 μL), diluted to 2000 μL with a mixture of *n*-hexane and 2-propanol (39:1 v/v) and filtered before injection. Data on conversion and enantiomeric composition of the products for the tested lipases are presented in Table 2.

2.4.2. Enzymatic kinetic resolution of racemic 1-(2-phenylthiazol-4-yl)ethanol (*rac*-2a) with vinyl acetate in different solvents with CaL-B

To a solution of racemic 1-(2-phenylthiazol-4-yl)ethanol *rac*-**2a** (50 μmol, 10.25 mg) in different solvents (500 μL) mentioned in Table 3, vinyl acetate (200 μmol, 18.45 μL) and lipase CaL-B (2.5 mg) were added. The reaction mixtures were shaken at 1300 rpm at room temperature. Samples were analyzed by HPLC similarly as described in Section 2.4.1. Data on conversion and enantiomeric composition of the products for the tested solvents are presented in Table 3. The acylation of racemic alcohols *rac*-**2b-d** was tested

by a similar procedure using the optimal conditions previously found for *rac*-**1a** (Table 4).

2.4.3. Enzymatic alcoholysis of racemic 1-(2-arylthiazol-4-yl)ethyl acetates (*rac*-3a-d)

To a solution of *rac*-**3a** (25 μmol, 6.175 mg) in different solvents (250 μL) mentioned in Table 5, CaL-B (2.5 mg) and methanol (250 μmol, 8 mg, 10 μL) were added. The reaction mixture was shaken at 1300 rpm at room temperature. Samples were analyzed by HPLC similarly as described in Section 2.4.1. Further enzymatic alcoholysis of *rac*-**3b-d** were performed similarly, using the optimal conditions previously obtained for *rac*-**3a**. Data on conversion and enantiomeric composition of the products are presented in Table 5.

2.5. Preparative scale enzyme mediated biotransformation of 1-(2-arylthiazol-4-yl)ethanols (*rac*-2a-d) and their acetates (*rac*-3a-d)

2.5.1. Preparative scale enzymatic acetylation of racemic alcohols *rac*-2a-d

Into the mixture of racemic 1-(2-arylthiazol-4-yl)ethanol (*rac*-**2a-d**, 1 mmol) in toluene (10 mL), vinyl acetate (4 mmol, 344 mg, 369 μL) and lipase CaL-B (50 mg) were added. The reaction mixture was shaken at 1300 rpm at room temperature. The reactions were monitored by HPLC and were stopped at an approx. 50% conversion. The enzyme was removed by filtration. Solvents were removed by rotatory evaporation and the crude product was purified by column chromatography on silicagel using a mixture of dichloromethane: acetone 25:1 v/v as eluent, resulting both optically pure (*S*)-1-(2-arylthiazol-4-yl)ethanols (*S*)-**2a-d** and (*R*)-1-(2-arylthiazol-4-yl)ethyl acetates (*R*)-**3a-d**.

2.5.2. Preparative scale enzymatic methanolysis of *rac*-3a-d

To a solution of *rac*-**3a-d** (0.5 mmol) in methyl *tert*-butyl ether (MTBE, 5 mL), CaL-B (50 mg) and methanol (5 mmol) were added. The reaction mixture was shaken at 1300 rpm at room temperature. The reactions were monitored by HPLC and were stopped at an approx. 50% conversion. The enzyme was removed by filtration. Solvents were removed by rotatory evaporation and the crude product was purified by column chromatography

on silicagel using a mixture of dichloromethane: acetone 25:1 v/v as eluent, resulting both optically pure (*R*)-1-(2-arylthiazol-4-yl)ethanols (*R*)-**2a-d** and (*S*)-1-(2-arylthiazol-4-yl)ethyl acetates (*S*)-**3a-d**.

MS and NMR spectra of the optically active compounds were indistinguishable from those of their racemates.

2.6. Preparation of the Mosher's esters

A solution of (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid ((*S*)-MTPA, 0.064 mmol, 15 mg) and *N,N'*-dicyclohexylcarbodiimide (0.077 mmol, 15.8 mg) in anhydrous dichloromethane (2 mL) was stirred for 30 min followed by addition of (*S*)-**2b** (0.128 mmol, 28.032 mg) and 4-dimethylaminopyridine (1.5 mg). The reaction mixture was stirred overnight at room temperature. After removing the solvent by rotatory evaporation, the crude product was purified by column chromatography on silicagel, using a mixture of dichloromethane: acetone 25:1 (v/v) as eluent. The (*R*)-MTPA-(*S*)-**2b** diastereomer was obtained using a similar procedure.

3. Results and discussion

3.1. Chemical synthesis

As illustrated in Scheme 1, the 2-arylthiazole-4-carbaldehydes **1a-d** were transformed onto the corresponding 1-(2-arylthiazol-4-yl)ethanols (*rac*-**2a-d**) by a Grignard reaction using methyl magnesium iodide as nucleophile. The corresponding acetates *rac*-**3a-d** were obtained by the chemical acetylation of *rac*-**2a-d** with acetic anhydride.

3.2. Enzymatic synthesis

To investigate the stereoselectivity of the kinetic resolution process and the enzymatic activity, first the chromatographic enantiomeric separation of *rac*-**2,3a-d** was established, using various HPLC chiral columns. The retention times of the enantiomers are shown in Table 1.

3.2.1. Analytical scale biotransformation

3.2.1.1. Analytical scale enzymatic acylation of *rac*-2a-d

In order to obtain the highest enantiopurities for the kinetic resolution products, various conditions were screened for the enantiomer selective acylation process. Racemic alcohol *rac*-**2a** was used as the model substrate for the optimization of the enzymatic procedure. First the acetylation of *rac*-**2a** in the presence of various lipases in pure vinyl acetate was tested (Scheme 1, Table 2).

According one reported theory [9], lipase B from *Candida antarctica* (CaL-B) is a useful biocatalyst for the kinetic resolution of secondary alcohols with an substituent smaller than *n*-propyl and the other one much larger. This is due to the steric requirements of the stereoselectivity pocket, a small cavity of the active site involved in the chiral recognition of the substrate [10]. As a confirmation of this theory, in our work CaL-B proved to be the most active and selective enzyme for the kinetic resolution of *rac*-**2a**, a secondary alcohol which possesses the same structural particularities enounced above.

Lipases MML and AK free showed also high enantioselectivity but moderate activity for the acetylation of *rac*-**2a** (Table 2, entries 2, 4).

Several studies have already reported that the nature of the solvent could significantly influence the activity and selectivity of the lipase [11]. The acetylation of *rac*-**2a** mediated by CaL-B with vinyl acetate in several solvents was tested. While the enantioselectivity of the enzyme was found to be high in most of the cases, a strong solvent influence upon the enzyme activity was observed (Table 3). Toluene proved to be the most appropriate solvent for the efficient CaL-B mediated acetylation (E>>200, c ~50% after 5.5 hours) of *rac*-**2a** (Table 3, entry 5). Further a kinetic study was performed for determining the specific activity of CaL-B in the acetylation of *rac*-**2a** at 25 °C. Samples were taken periodically (10 min) and subjected to chiral HPLC separation. Based on the areas under the HPLC traces corrected with the specific molar absorbance of substrate **2a** ($\varepsilon_{\mathbf{2a}}$ = 7548.9 $M^{-1}\times cm^{-1}$) and product **3a** ($\varepsilon_{\mathbf{3a}}$ = 6943.4 $M^{-1}\times cm^{-1}$), the amounts of the substrate (50 μmol), vinyl acetate (100, 150, or 200 μmol) and enzyme (2.5 mg) used in the reaction, the specific enzyme activity was estimated to be 0.1046 $\mu mol\times min^{-1}\times mg_{CaL\text{-}B}^{-1}$ for the enzymatic acylation of *rac*-**2a**. It was found that the concentration of vinyl acetate had no influence upon the enzyme activity demonstrating that the rate-limiting stage of the process is the acyl-enzyme substrate reaction. The same

protocol was successfully used also for the kinetic resolution of alcohols *rac*-**2b-d** (E>>200, c ~50% after 6-9 hours), as shown in Table 4.

3.2.1.2. Analytical scale enzymatic alcoholysis of *rac*-3a-d

In order to obtain the opposite enantiomers of the resolution products, (*R*)-**2a-d** and (*S*)-**3a-d**, the analytical scale enzymatic alcoholysis of racemic 1-(2-arylthiazol-4-yl)ethyl acetate *rac*-**3a** was further investigated. In accordance with the values shown in Table 5, entries 1-3, the best results were achieved in MTBE using CaL-B and methanol (10 equiv) as nucleophile (E >>200). To verify the optimal conditions found for the *rac*-**3a** enzymatic methanolysis, the same procedure was further extended to the other racemic acetates *rac*-**3b-d**. Similar good results were obtained in most cases (Table 5, entries 4-6). Compared to the CaL-B mediated acetylation of *rac*-**2a-d**, the enzyme activity for the methanolysis of *rac*-**3a** was lower (0.018 $\mu mol \times min^{-1} \times mg_{CaL\text{-}B}^{-1}$). In both cases, when brominated *rac*-**2,3d** were used as substrate (Table 4, entry 4; Table 5, entry 6), a decreased CaL-B activity was observed.

3.2.2. Preparative scale synthesis of both (*R*)- and (*S*)-2,3a-d

Using the optimal conditions found for the analytical scale reactions, first the semi-preparative scale enzymatic synthesis of both (*S*)- and (*R*)-**2,3a-d** was performed. Whereas the enzymatic acetylation afforded the (*R*) enantiomers of acetates (*R*)-**3a-d** and (*S*) enantiomers of the alcohols (*S*)-**2a-d**, alcoholysis of the racemic acetates *rac*-**3a-d**, yielded the opposite enantiomeric forms (*R*)-**2a-d** and (*S*)-**3a-d**. No significant differences were found for the reaction rates and for the enantiopurities of the resolution products, compared with those found for the analytical scale reactions (Table 6). Higher amounts of both racemic ethanol *rac*-**2a** and acetate *rac*-**3a** (5 mmols each) were used as starting materials in reliable preparative scale reactions in order to demonstrate the usefulness of the developed procedure for multi-gram scale enzymatic kinetic resolutions. Compared with those found for the analytical or semipreparative scale reactions, no significant differences were observed (ee > 99% for both products, in both enzymatic kinetic resolution processes, see supplementary data).

3.3. The absolute configuration of the kinetic resolution products

The absolute configuration of the novel enantiopure compounds was determined by a detailed ^{1}H NMR study of the Mosher's derivatives of (-)-**2b.** According to one procedure already described in the literature [12], the enantiomerically pure alcohol (-)-**2b** remained untransformed during the CaL-B-mediated acetylation was esterified with both (*R*)-MTPA and (*S*)-MTPA. The resulting diastereomers were differentiated by their ^{1}H NMR spectra (Fig.1-3).

As shown in Figure 1 and 2, in the case of (*S*)-MTPA-(-)-**2b** the strong diamagnetic effect of the phenyl group caused the proximal CH_3 protons to be more shielded, δ = 1.73 ppm, when compared with their analogues in (*R*)-MTPA-(-)-**2b** (δ = 1.79 ppm) where the distance between the phenyl and the methyl group is higher. The methoxy protons of the (*S*)-MTPA-(-)-**2b** were also found to be more shielded, δ =3.56 ppm, in comparison with the methoxy protons of the (*R*)-MTPA-(-)-**2b** (δ =3.61 ppm), due to the diamagnetic effect of the proximal 2-phenylthiazole moiety.

The proton of (*R*)-MTPA-(-)-**2b** located at the 5-position of the thiazole ring was found to be more shielded (δ =6.99 ppm) when compared with the (*S*)-MTPA-(-)-**2b** (δ =7.19 ppm).

Based on these data, the (*S*)-(-) absolute configuration was assigned for (-)-**2b** remained untransformed during the CaL-B mediated acylation.

Configurations of all the enantiomers obtained by the CaL-B catalyzed acetylations were assigned as (*S*) on the basis of the same sign of their specific rotation.

4. Conclusions

An efficient enzymatic kinetic resolution of 1-(2-arylthiazol-4-yl)ethanol derivatives and their acetates has been achieved. For the enantioselective acylation process, the best results were obtained using CaL-B (5 mg/mL), vinyl acetate (4 equiv) and toluene as solvent. CaL-B proved to be an efficient biocatalyst also for the methanolysis (10 equiv) of the corresponding acetates *rac*-**3a-d,** using methyl *tert*-butyl ether as solvent. The rate of the enzymatic reaction was higher for the acylation process, compared to methanolysis.

These results were a valuable prerequisite for the preparative scale production of (*R*)- and (*S*)-**2,3a-d**.

The absolute configuration of (-)-**2b** isolated as unreacted enantiomer during the CaL-B mediated acetylation of *rac*-**2b** was assigned to be (*S*) using a ^{1}H NMR study of its Mosher's derivatives. These results confirmed that in this case the stereochemical outcome of the enzymatic kinetic resolution respect the Kazlauskas empirical rule [13].

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Table legends

Table 1. The retention times of the enantiomers of *rac*-**2,3a-d**, using Chiralpak IB column
Table 2. Lipase screening for the enantioselective acetylation of *rac*-**2a** in vinyl acetate
Table 3. Solvent screening for the CaL-B mediated acetylation of *rac*-**2a** with vinyl acetate
Table 4. CaL-B mediated acetylation of *rac*-**2a-d** with vinyl acetate in toluene
Table 5. CaL-B mediated methanolysis of *rac*-**3a-d** in various solvents
Table 6. Yields and *ee* values for the preparative scale synthesis of (*R*)- and (*S*)-**2,3a-d**

Table 1. The retention times of the enantiomers of *rac*-**2,3a-d**, using Chiralpak IB column

Entry	Compound	t_R (min)	Compound	t_R (min)	Compound	t_R (min)	Compound	t_R (min)	Gradient separation	
									Time (min)	% *i*PrOH
1	(*R*)-**3a**	7.0	(*S*)-**3a**	8.4	(*S*)-**2a**	14.5	(*R*)-**2a**	15.5	0-9 9.1-16	1 20
2	(*R*)-**3b**	6.0	(*S*)-**3b**	6.8	(*S*)-**2b**	12.6	(*R*)-**2b**	14.0	0-7.5 7.6-18	2 20
3	(*R*)-**3c**	6.4	(*S*)-**3c**	7.4	(*S*)-**2c**	13.2	(*R*)-**2c**	14.0	0-7.5 7.6-18	2 20
4	(*R*)-**3d**	6.7	(*S*)-**3d**	7.7	(*S*)-**2d**	13.4	(*R*)-**2d**	14.0	0-7.5 7.6-18	2 15

Table 2. Lipase screening for the enantioselective acetylation of *rac*-**2a** in vinyl acetate

Entry	Enzyme	c[1] (%)	$ee_{(S)\text{-}\mathbf{2a}}$ (%)	$ee_{(R)\text{-}\mathbf{3a}}$ (%)	*E*
1	PPL	5.94	6.29	>99.5	»200
2	MML	21.64	27.48	>99.5	»200
3	CrL	65.96	30.67	15.82	1.78
4	AK free	27.90	38.50	>99.5	»200
5	CaL-A	76.42	99.50	30.70	8.94
6	BcL PS	3.74	3.87	>99.5	»200
7	CaL-B	50.99	>99.5	96.04	»200

[1]after 5 hours

Table 3. Solvent screening for the CaL-B mediated acetylation of *rac*-**2a** with vinyl acetate

Entry	Solvent	c^{1} *(%)*	$ee_{(S)\text{-}\mathbf{2a}}$ (%)	$ee_{(R)\text{-}\mathbf{3a}}$ (%)	*E*
1	*n*-Hexane	37.29	59.16	>99.5	»200
2	*n*-Octane	48.80	94.83	>99.5	»200
3	Cyclohexane	45.95	84.60	>99.5	»200
4	Chloroform	1.66	1.68	>99.5	»200
5	Toluene	49.75	98.50	>99.5	»200
6	DIPE	37.35	59.31	>99.5	»200
7	THF	29.39	40.15	96.48	82.78
8	Acetonitrile	41.08	69.37	>99.5	»200
9	MTBE	38.92	63.31	99.35	»200

[1]after 5 hours 30 min

Table 4. CaL-B mediated acetylation of *rac*-**2a-d** with vinyl acetate in toluene

Entry	Compound	Time (h)	*c* (%)	$ee_{(S)\text{-}\mathbf{2a\text{-}d}}$ (%)	$ee_{(R)\text{-}\mathbf{3a\text{-}d}}$ (%)	*E*
1	**2a**	6	50	>99.5	>99.5	»200
2	**2b**	6	50	>99.5	>99.5	»200
3	**2c**	6	50	>99.5	>99.5	»200
4	**2d**	9	50	>99.5	>99.5	»200

Table 5. CaL-B mediated methanolysis of *rac*-**3a-d** in various solvents

Entry	Compound	Solvent	Time (h)	*c* (%)	$ee_{(R)\text{-}\mathbf{2a\text{-}d}}$ (%)	$ee_{(S)\text{-}\mathbf{3a\text{-}d}}$ (%)	*E*
1	**3a**	Acetonitrile	12	51.5	92.4	98.0	117
2	**3a**	*n*-Hexane	12	51.2	83.0	87.0	30
3	**3a**	MTBE	12	50.3	98.0	99.3	»200
4	**3b**	MTBE	13	42.0	>99.5	72.3	»200
5	**3c**	MTBE	13	41.3	>99.5	70	»200
6	**3d**	MTBE	88	46.9	98.7	87.3	»200

Table 6. Yields and *ee* values for the preparative scale synthesis of (*R*)- and (*S*)-**2,3a-d**

Entry	Product[1]	Yield[3] (%)	*ee* (%)	$[\alpha]_D$[4]	Product[2]	Yield[3] (%)	*ee* (%)	$[\alpha]_D$[4]
1	(*S*)-**2a**	48	>99.5	-8.60	(*R*)-**2a**	48	98	+8.1
2	(*S*)-**2b**	48	98.5	-7.00	(*R*)-**2b**	47	>99.5	+7.2
3	(*S*)-**2c**	49	>99.5	-6.80	(*R*)-**2c**	48	>99.5	+7.0
4	(*S*)-**2d**	48	>99.5	-10.25	(*R*)-**2d**	48	98	+9.8
5	(*R*)-**3a**	48	99	+126.4	(*S*)-**3a**	49	99	-126.0
6	(*R*)-**3b**	47	>99.5	+119	(*S*)-**3b**	47	98	-117.5
7	(*R*)-**3c**	48	99	+112.8	(*S*)-**3c**	46	98	-111.3
8	(*R*)-**3d**	47	99.3	+97	(*S*)-**3d**	49	90	-88.3

[1]from the enantioselective acetylation of *rac*-**2a-d** mediated by CaL-B
[2]from the enantioselective methanolysis of *rac*-**3a-d** mediated by CaL-B
[3]isolated yields based on racemic starting material
[4]c 1.0, $CHCl_3$, t=25ºC

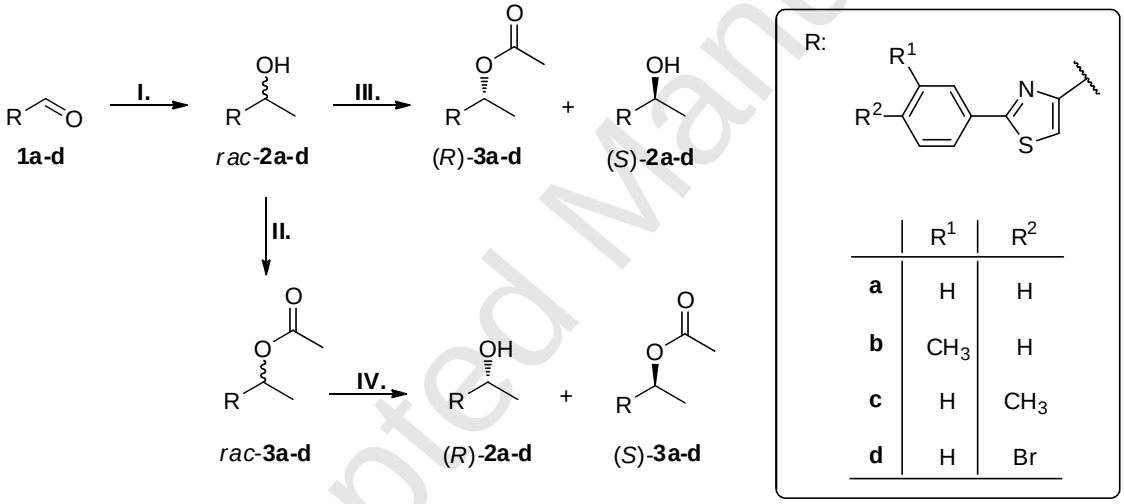
Figure legends

Scheme 1. Synthesis and biotransformation of the 1-(2-phenylthiazol-4-yl)ethanols and their acetates. Reagents and conditions: I. (a) CH_3MgI/diethyl ether; (b) NH_4Cl/H_2O; II. $(CH_3CO)_2O$, DMAP/Et_3N/CH_2Cl_2; III. CaL-B, vinyl acetate/toluene; IV. CaL-B, MeOH/MTBE.

Figure 1. (*S*)- and (*R*)- MTPA derivatives of (*S*)-**2b**.

Figure 2. Signals of the CH_3 and OCH_3 protons from the ^{1}H NMR spectra of (*S*)-MTPA-(*S*)-**2b** and of (*R*)-MTPA-(*S*)-**2b.**

Figure 3. Signals of the proton located at the 5th-position of the thiazole ring from the the ^{1}H NMR spectra of (*S*)-MTPA-(*S*)-**2b** and of (*R*)-MTPA-(*S*)-**2b.**



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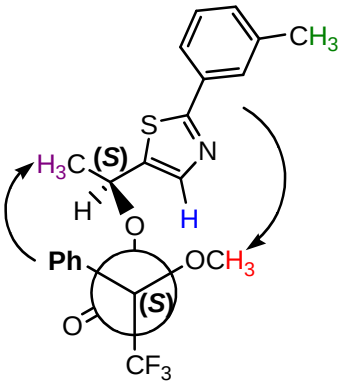
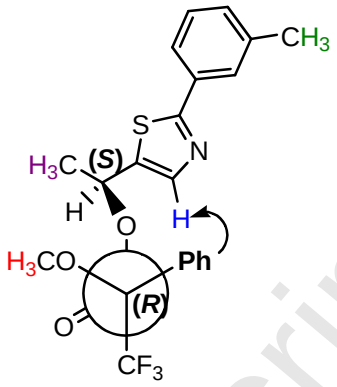


(*S*)-MTPA-(*S*)-**2b**



(*R*)-MTPA-(*S*)-**2b**

Figure 1. (*S*)- and (*R*)- MTPA derivatives of (*S*)-**2b**.

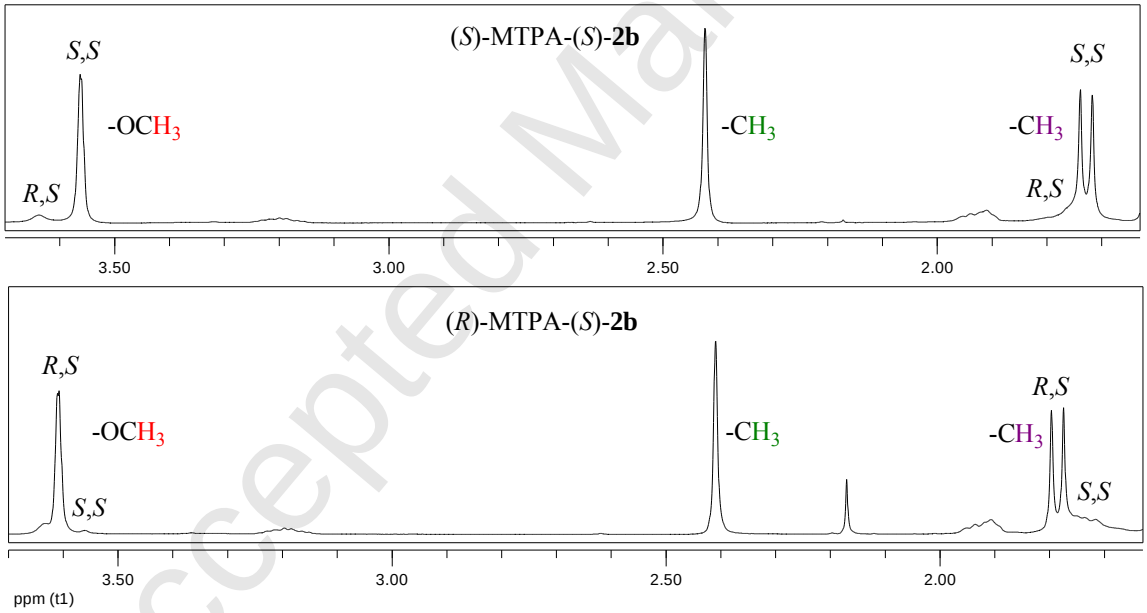


Figure 2. Signals of the CH_3 and OCH_3 protons from the ^1H NMR spectra of (*S*)-MTPA-(*S*)-**2b** and of (*R*)-MTPA-(*S*)-**2b.**

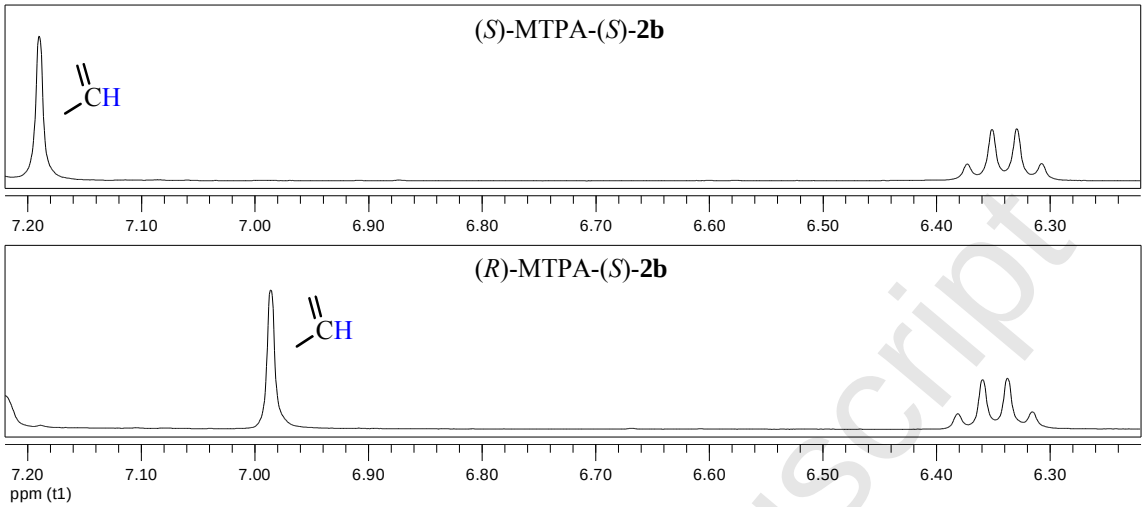


Figure 3. Signals of the proton located at the 5th-position of the thiazole ring from the the ^{1}H NMR spectra of (*S*)-MTPA-(*S*)-**2b** and of (*R*)-MTPA-(*S*)-**2b.**

Graphical Abstract

Heterocycles 32. Efficient kinetic resolution of 1-(2-arylthiazol-4-yl)ethanols and their acetates using lipase B from *Candida antarctica*

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