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Heterocycles 35. CaL-B mediated synthesis of enantiomerically pure (*R*)- and (*S*)-ethyl 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates

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ABSTRACT

Herein we present the lipase catalyzed synthesis of four new enantiomerically pure (*R*)- and (*S*)-ethyl 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates and their butanoates by enzymatic enantioselective acylation of the racemic alcohols *rac*-**1a–d** and by ethanolysis of the corresponding racemic esters *rac*-**2a–d** mediated by lipase B from *Candida antarctica* (CaL-B) in organic solvents. In terms of stereoselectivity and activity, both procedures, the acylation and alcoholysis, are successful (50% conversion, $E \gg 200$). The absolute configuration of the resolution products was determined by a detailed ^{1}H NMR study of the Mosher's derivatives of (*S*)-**1a**.

1. Introduction

Thiazoles and their derivatives can be found associated with various antibacterial, antifungal, and anti-inflammatory activities.[1] Some thiazole compounds, such as ritonavir, show anti-inflammatory effects,[2] imidacloprid is used as a pharmaceutical product, while others are agrochemical products.[3]

Enantiopure β-hydroxy esters bearing various aromatic moieties are versatile chiral synthons[4] in organic synthesis and are widely used as starting materials or intermediates for the synthesis of many pharmaceutical products such as L-carnitine[5] or fluoxetine.[6] In addition to chemical methods,[7] milder enzymatic methods have been developed for the synthesis of enantiomerically pure β-hydroxy esters. Biocatalytic approaches for the synthesis of these target molecules broadly involve: (1) enantioselective reduction of the corresponding β-ketoesters; (2) kinetic or dynamic kinetic resolution of β-hydroxy esters; and (3) deracemization of β-hydroxy esters.[8]

The enzymatic kinetic resolution of a racemate with a suitable hydrolytic enzyme is one of the most common biocatalytic methods used nowadays. Enantioselectivity, high stability, and good commercial availability in free and immobilized forms have made lipases (EC 3.1.1.3) attractive kinetic resolution catalysts either through the acylation of a nucleophilic (for example an alcohol) functionality, or through the deacylation (hydrolysis, alcoholysis, or interesterification) of an ester functionality in a molecule.[9] Recently, the lipase catalyzed kinetic resolution of various heteroaryl-β-hydroxy esters such as benzofuranyl- and benzothiophenyl-β-hydroxy esters[10] or phenothiazinyl-β-hydroxy esters[11] has been studied. Moreover, the opposite enantiomer preference of CaL-A and CaL-B in the enantioselective acylation of furan-2-yl- and furan-3-yl-, thiophene-2-yl-, and thiophene-3-yl-β-hydroxy esters was reported.[12] Brem et al. demonstrated that CrL catalyzed esterification with fatty acid vinyl esters can significantly enhance the enantiopurity of the resolution products.[13] The influence of the pretreatment of the lipases, the substrate concentration, and the reaction conditions upon the stereochemical outcome of the enzymatic reaction was also reported on.[14] Furthermore, using adsorption, cross linked enzyme aggregate (CLEA) technology, and sol–gel encapsulation methods, a tailor made enzyme immobilization for the selective O-acylation of the aryl β-hydroxy esters was also successfully realized.[15]

Encouraged by these factors, we have developed a lipase catalyzed kinetic resolution of racemic 1-(2-arylthiazol-4-yl)-β-hydroxypropanoates *rac*-**1a–d** and their corresponding butanoates *rac*-**2a–d** (Scheme 1). The absolute configuration of the novel enantiopure compounds was then determined by a detailed ^{1}H NMR study of the Mosher's derivatives of (*S*)-**1a**.

2. Results and discussion

2.1. Chemical synthesis

Racemic ethyl 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates *rac*-**1a–d** were prepared by the Reformatsky reaction starting from

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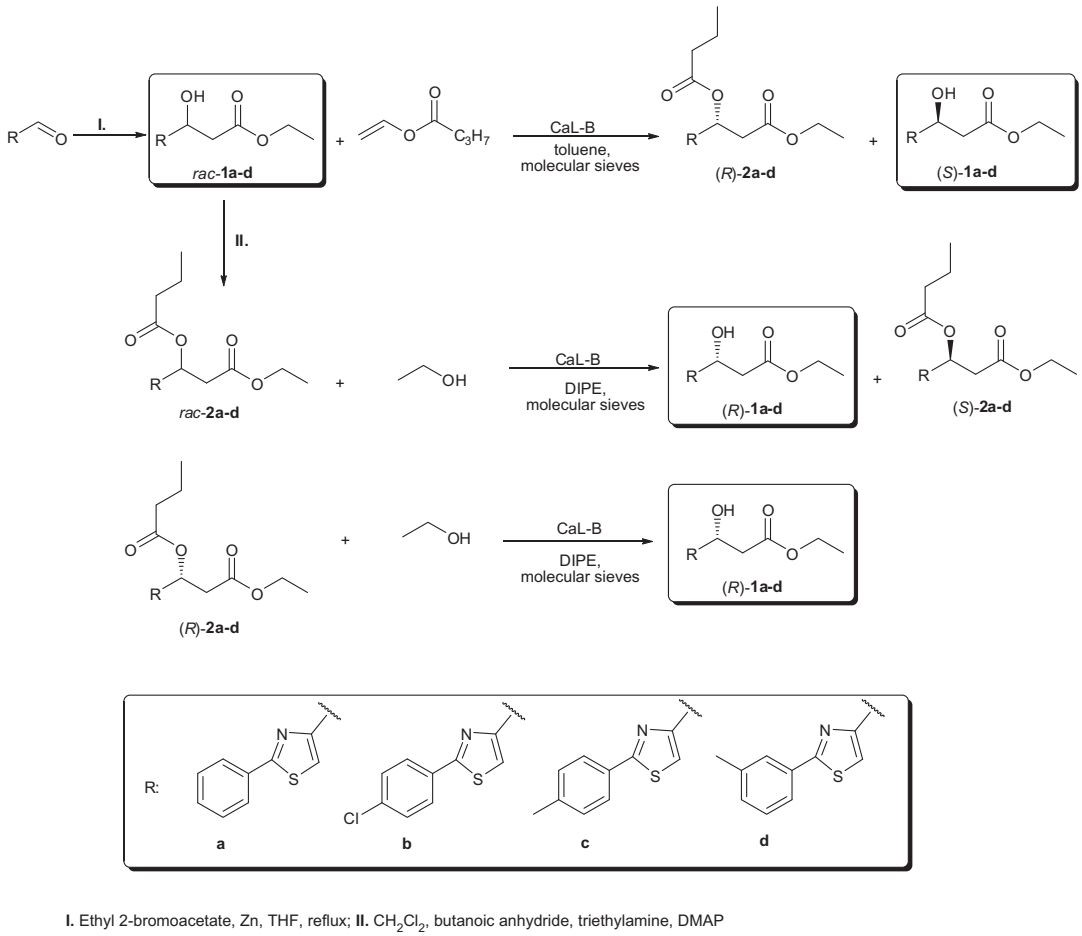


Scheme 1. The chemical synthesis of *rac*-**1a–d** and *rac*-**2a–d** and the enzymatic route for the preparation of enantiomerically pure arylthiazol-β-hydroxy esters and their corresponding diesters.

the corresponding 2-arylthiazol-4-carbaldehydes.[16] By the chemical acylation of *rac*-**1a–d**, with butanoic anhydride in the presence of triethylamine and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine (DMAP) in dichloromethane the preparation of the racemic diesters *rac*-**2a–d** was also performed as shown in Scheme 1, routes I and II.

2.2. Enzymatic synthesis

In order to investigate the stereoselectivity of the reactions involving chiral 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates and their diesters, the chromatographic separation of their enantiomers was first established. The base-line separation of the enantiomers of *rac*-**1, 2a–d** was performed using a Chiralpak IB HPLC column as shown in Section 4.1.

2.2.1. Analytical scale enzymatic acylation of *rac*-1a–d

In an effort to obtain highly enantiomerically enriched resolution products, commercially available free or immobilized lipase preparations (25 mg/ml) were screened in various organic solvents at room temperature, for the enantioselective acylation using vinyl acetate and vinyl butanoate as irreversible acyl donors (0.1 M) of the racemic 3-hydroxy-3-(2-phenylthiazol-5-yl)propanoate *rac*-**1a** (0.025 M), which was used as a model compound. Lipase A from *Candida antarctica* immobilized by adsorption on Celite (CaL-A on Celite), CaL-B (Novozyme 435), lipase B from *Candida antarctica* immobilized by adsorption on single wall carbon nanotube (CaL-B-SWCNT), lipase from *Candida rugosa* (CrL), free *Pseudomonas cepacia* lipase (LPS free) and lipase AK immobilized by adsorption on Celite (AK on Celite) were tested as suitable biocatalysts in dry organic solvents. The experiments were performed in the presence of molecular sieves, since even small traces of water could promote hydrolytic reactions, thus yielding undesired by-products or causing a decrease in the enantiopurity of the products.

The nature of the solvent and the nucleophile can significantly influence the activity and selectivity of the enantioselective enzymatic acylation. Thus, the solvents used were selected and tested as they are commonly accepted by lipases. Moreover, solvents should be chosen from those frequently used in industry, they have to be easy to evaporate and must dissolve both the substrates and products. Among the enzymes tested for the O-acylation of our model compound *rac*-**1a** using vinyl acetate and vinyl butanoate, lipase AK on Celite and *Pseudomonas cepacia* lipase (LPS free) were catalytically inactive in all of the solvents tested, while CrL, depending on the acylation agent and the solvent used, showed higher or lower selectivity and activity. For the CrL mediated acylation using vinyl acetate in methyl *tert*-butyl ether (MTBE), diisopropyl ether (DIPE), hexane and toluene, both the reaction rate and the selectivity were low (Table 1, entries 10–13). When using vinyl butanoate, the reaction gave low selectivity in MTBE, DIPE, and hexane (Table 2, entries 6–8). By performing the CaL-A on Celite mediated O-acylation of *rac*-**1a** using vinyl acetate in various ethers such as MTBE and DIPE or nonpolar solvents such as hexane, toluene, and acetonitrile, the reactions showed moderate enantioselectivities (E = 5–14, Table 1, entries 1–5). When, in the same experiments, vinyl butanoate was used as the acyl donor, a considerably decreased enzyme activity was detected in MTBE (Table 2, entry 1), while for the rest of the solvents tested, CaL-A

Table 1
Lipase and solvent screen for the selective O-acylation of racemic substrate *rac*-**1a** using vinyl acetate, after 17 h

Entry	Lipase	Solvent	*c* (%)	$ee_{(R)\text{-}2a}$	$ee_{(S)\text{-}1a}$	*E*
1	CaL-A on Celite	MTBE	35.3	64	35	6
2	CaL-A on Celite	DIPE	78.3	26	94	5
3	CaL-A on Celite	Acetonitrile	68	45	96	9
4	CaL-A on Celite	Hexane	72.4	38	>99	14
5	CaL-A on Celite	Toluene	29.6	64	27	6
6	CaL-B	MTBE	35.6	47	26	3.5
7	CaL-B	DIPE	32.3	65	31	6.3
8	CaL-B	Hexane	53.7	62	72	8.9
9	CaL-B	Toluene	24.5	74	24	8.4
10	CrL	MTBE	5.2	36	2	2
11	CrL	DIPE	27.2	40	15	2.7
12	CrL	Hexane	33.8	47	24	3.5
13	CrL	Toluene	12.8	68	10	5.8

Table 2
Lipase and solvent screen for the selective O-acylation of racemic substrate *rac*-**1a** using vinyl butanoate, after 17 h

Entry	Lipase	Solvent	*c* (%)	$ee_{(R)\text{-}2a}$	$ee_{(S)\text{-}1a}$	*E*
1	CaL-A on celite	MTBE	81.9	13	59	2
2	CaL-B	MTBE	51.6	91	97	89
3	CaL-B	DIPE	52.6	89	99	89.7
4	CaL-B	Hexane	52.8	89	>99	127.8
5	CaL-B	Toluene	50	>99	>99	≫200
6	CrL	MTBE	20	32	8	2
7	CrL	DIPE	39.6	35	23	2.5
8	CrL	Hexane	54.9	41	50	3.8
9	LPS free	Hexane	47.7	80	73	19.5

Table 3
Alcohol, lipase, and solvent screen for the selective alcoholysis of racemic diester *rac*-**2a**, after 14 h

Entry	Alcohol	Lipase	Solvent	*c* (%)	$ee_{(R)\text{-}1a}$	$ee_{(S)\text{-}2a}$	*E*
1	EtOH	CaL-A	MTBE	11.1	64	8	4.9
2	EtOH	CaL-A	DIPE	32.2	59	28	5
3	EtOH	CaL-A	Acetonitrile	8.3	77	7	8.2
4	EtOH	CaL-A	Hexane	19.3	71	17	7
5	EtOH	CaL-B	MTBE	49.7	97	96	>200
6	EtOH	CaL-B	DIPE	48.4	>99	93	≫200
7	EtOH	CaL-B	Toluene	6.6	>99	7	>200
8	EtOH	CrL	Hexane	9.8	55	6	3.6
9	BuOH	CaL-A	Acetonitrile	3	>99	3	>200
10	BuOH	CaL-B	DIPE	83	19	94	4
11	BuOH	CaL-B	Hexane	39.3	71	46	9
12	BuOH	CaL-B	Toluene	16.6	85	17	14
13	BuOH	CrL	DIPE	13.1	53	8	3.5
14	BuOH	CrL	Hexane	14.5	47	8	3

on Celite was inactive. In terms of activity and selectivity (E = 3–9, Table 1, entries 6–9) for the CaL-B catalyzed O-acylation using vinyl acetate in all solvents tested, similar results were obtained to those found for CaL-A on Celite. However, a major improvement in selectivity was obtained for the CaL-B catalyzed O-acylation with vinyl butanoate using hexane and toluene as solvents (Table 2, entries 4–5), yielding highly enantiomerically enriched resolution products at approximately 50% conversion. CaL-B-SWCNT proved to be completely inactive for the present purpose, although it did show high activity and selectivity for the acylation of *rac*-1-phenylethanol.

2.2.2. Analytical scale enzymatic alcoholysis of *rac*-2a–d

Lipases usually retain the enantiopreference found in the stereoselective acylation of chiral alcohols including the hydrolysis or alcoholysis of the ester counterparts. Consequently, such reactions should result in the opposite enantiomeric forms of the enantiomerically enriched ethyl 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates **1a–d** and their diesters **2a–d** as those found in the enzymatic acylation of *rac*-**1a–d**. The enzymatic alcoholysis of *rac*-**2a**, used as the model compound, was investigated. The experiments were carried out using the same enzymes in all of the solvents tested for the enzymatic acylation by adding 5 equiv of ethanol or 1-butanol into the reaction mixture. Ethanolysis mediated by CaL-B showed the highest selectivity and activity when MTBE (Table 3, entry 5) and DIPE (Table 3, entry 6) were used as reaction media. Due to the higher activity of the enzyme in DIPE, this solvent was used in further experiments.

2.2.3. Preparative scale enzymatic acylation of *rac*-1a–d

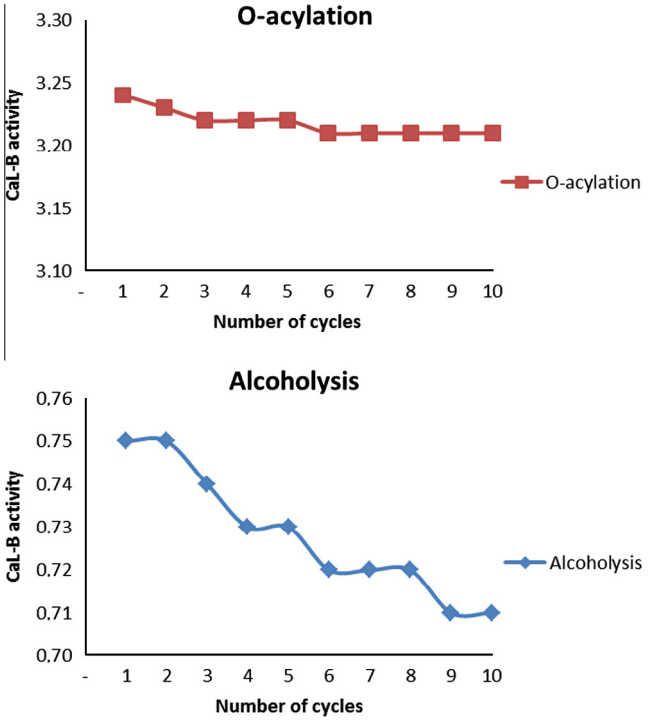
Using the optimal conditions found for the analytical scale biotransformations of both *rac*-**1a** and *rac*-**2a**, the CaL-B mediated preparative scale acylation of *rac*-**1a–d** with vinyl butanoate in toluene (Table 4, entries 1–4) and the ethanolysis of *rac*-**2a–d** in DIPE (Table 4, entries 5–8) was next performed with good reactivity and selectivity ($E \gg 200$, 99% ee_s for the most of the resolution products at approximately 50% conversion). All of the dilutions, substrate/biocatalyst ratios and reaction conditions were the same as in the analytical scale reactions. The yields of the isolated and chromatographically purified resolution products were in the range of 45–48%, calculated related to the starting racemic substrates and are given in Table 4, together with specific rotations of the enantiopure products.

2.2.4. Reuse of the catalysts

An important consideration for the catalytic reaction is the reusability of the catalyst. In order to test the recycling capacity of CaL-B, we used *rac*-**1a** as the substrate for the O-acylation and *rac*-**2a** as the substrate for the alcoholysis. Graphic 1 presents the initial activity of CaL-B as a function of the number of cycles and it can be observed that after 10 cycles, for both the acylation and alcoholysis, the activity of the enzyme decreases only slightly. Every reaction was allowed to proceed up to 50% conversion before the catalyst was subjected to the next cycle. Between cycles, the catalyst was washed with dry solvent and then immediately

Table 4
Preparative scale enzymatic acylation of *rac*-**1a–d** and ethanolysis of *rac*-**2a–d**

Entry	Substrate	Time (h)	Products					*E*
			$ee_{(R)\text{-}2}$	Yield (%)	$[\alpha]_D^a$	$ee_{(S)\text{-}1}$	Yield (%)	$[\alpha]_D^a$	
1	*rac*-**1a**	19	99	47	+78.5	99	46	−56.8	≫200
2	*rac*-**1b**	19	98	45	+75.2	>99	47	−53.5	≫200
3	*rac*-**1c**	19	99	45	+72.8	99	45	−51.3	≫200
4	*rac*-**1d**	17	99	46	+86.2	>99	45	−60.4	≫200
			$ee_{(R)\text{-}1}$	Yield (%)	$[\alpha]_D^a$	$ee_{(S)\text{-}2}$	Yield (%)	$[\alpha]_D^a$	
5	*rac*-**2a**	17	>99	48	+57.2	98	48	−76	≫200
6	*rac*-**2b**	48	98	46	+49.8	>99	45	−74.1	≫200
7	*rac*-**2c**	48	>99	45	+51.9	>99	45	−68.5	≫200
8	*rac*-**2d**	17	>99	45	+59.8	99	46	−83	≫200



Graphic 1. Variation of CaL-B activity in both acylation and alcoholysis reactions during 10 reuse cycles.

reused. During the recycling process, there was a small decrease in reaction rate, but the enantiomeric ratio remained high (E >200).

2.3. Absolute configuration

The absolute configuration of ethyl 3-hydroxy-3-(2-phenylthiazol-4-yl)propanoate (−)-**1a** was determined by ^{1}H NMR using both enantiomers of α-methoxy-α-(trifluoro-methyl)phenylacetic acid (MTPA), of already known absolute configuration.[17] Thus, the enantiomerically pure alcohol (−)-**1a,** recovered during the CaL-B-mediated acylation, was esterified with both (*R*)-MTPA and (*S*)-MTPA. The chemical shifts of the methoxy groups of the two diastereomeric MTPA esters were used as reference signals in order to determine the absolute configuration of (−)-**1a**. The chemical shift difference of the methoxy signals appeared because in one of the diastereomers, the methoxy group and the phenylthiazole ring of the hydroxy ester are on the same side of the MTPA plane, while in the other diastereomer they are opposite to each other. The methoxy signals for the two diastereomers appeared at δ 3.47 for the (*S*)-MTPA-(−)-**1a** ester and δ 3.57 for the (*R*)-MTPA-(−)-**1a** ester (Fig. 1). This proves that the unknown enantiomerically pure (−)-**1a** obtained from the O-acylation reaction of *rac*-**1a** is the (*S*)-enantiomer. The results showed that as expected, in the acylation reaction of the racemic alcohols, all of the enzymes followed Kazlauskas' rule, which predicted the (*R*)-enantiomer preference of the lipases. The configurations of all of the unreacted enantiomers in the CaL-B catalyzed acylations were assigned as (*S*) on the basis of the same sign of their specific rotations.

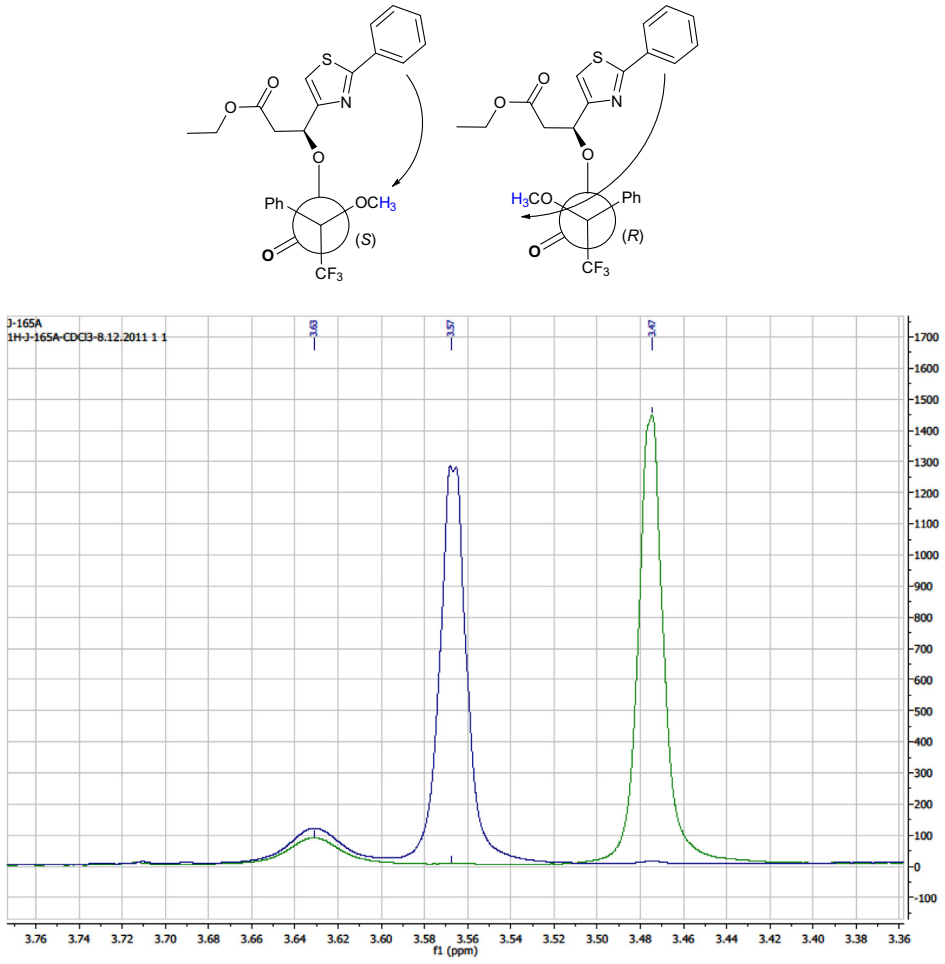


Figure 1. Overlapped H NMR spectra of –OCH_3 in (*S*,*S*) MTPA-**1a** (blue trace) and (*R*,*S*) MTPA-**1a** (green trace) esters.

Table 5
The retention times of the enantiomers of *rac*-**1**–**2a**–**d**

Compound	t_r (min)	Compound	t_r (min)
(*S*)-**1a**	7.6	(*S*)-**2a**	5.2
(*R*)-**1a**	10.3	(*R*)-**2a**	5.6
(*S*)-**1b**	12.4	(*S*)-**2b**	6.5
(*R*)-**1b**	17.5	(*R*)-**2b**	7
(*S*)-**1c**	12.4	(*S*)-**2c**	6.2
(*R*)-**1c**	17	(*R*)-**2c**	6.7
(*S*)-**1d**	10.8	(*S*)-**2d**	5.9
(*R*)-**1d**	15.5	(*R*)-**2d**	6.4

3. Conclusion

An efficient enzymatic procedure for the synthesis of enantiomerically pure ethyl 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates has been described (ee 99%). By using an enzymatic kinetic resolution, both enantiomerically pure stereoisomers of four ethyl 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates **1a**–**d** and four butanoates **2a**–**d** were synthesized with high yields. CaL-B proved to be the optimal biocatalyst for both the acylation of *rac*-**1a**–**d** with vinyl butanoate in toluene and the ethanolysis of *rac*-**2a**–**d** in DIPE. CaL-B has also been shown to be efficiently reusable in up to ten cycles, since its activity and stereoselectivity remain unaltered.

4. Experimental

4.1. Analytical methods

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer operating at 300 MHz and 75 MHz respectively, at 25 °C. Electron impact mass spectra (EI-MS) were taken on a VG 7070E mass spectrometer operating at 70 eV. High Performance Liquid Chromatography (HPLC) analyses were conducted using an Agilent 1200 instrument (Table 5). The enantiomeric separation of *rac*-**1**,**2a** was performed on a Chiralpak IB column (4.6 × 250 mm) and a mixture of *n*-hexane and 2-propanol 90:10 (v/v) as eluent; *rac*-**1**–**2b**,**c**,**d** were separated on Chiralpak IB column with a mixture of *n*-hexane and 2-propanol 95:5 (v/v) as eluent.

Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60F_{254} sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating. Preparative chromatographic separations were performed using column chromatography on Merck Kieselgel 60 (63–200 μm). Melting points were determined by a hot plate method and are uncorrected. Optical rotations were determined on a Bellingham-Stanley ADP 220 polarimeter. The determination of E was based on the equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$, with $c = ee_S/(ee_S + ee_P)$.[18]

4.2. Reagents, solvents, and biocatalysts

All reagents were purchased from Aldrich or Fluka and used as received. Solvents and acyl donors for the enzymatic reactions were stored over molecular sieves unless otherwise stated. Lipases from *Pseudomonas fluorescens* (AK) immobilized by adsorbtion on Celite and *Pseudomonas cepacia* (*Burkholderia cepacia*, LPS) were purchased from Amano England. *Candida rugosa* lipase (CrL) was purchased from Fluka. Lipase B from *Candida antarctica* (CaL-B, Novozyme 435) was purchased from Novozyme, Denmark. Lipase A from *Candida antarctica* immobilized by adsorbtion on Celite (CaL-A) was a gift from Prof. Liisa T. Kanerva, University of Turku, Finland. CaL-B-SWCNT was prepared in our laboratories. Single-walled carbon nanotubes were purchased from Organic Chemicals Co. Ltd. The microcrystalline CaL-B powder used for the preparation of CaL-B-SWCNT was purchased from Chiralvision. The ethyl 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates were synthesized from the corresponding aldehydes using the Reformatsky reaction.[11]

4.3. Synthesis of racemic compounds

4.3.1. Synthesis of the racemic ethyl 3-(2-arylthiazol-4-yl)-3-hydroxypropanoates *rac*-1a–d

At first, β-hydroxy esters *rac*-**1a**–**d** were prepared by adding a solution of ethyl bromoacetate (8.4 g, 5.6 ml, 0.05 mol) in tetrahydrofuran (10 ml) in small portions with slight warming into a mixture of zinc powder (4 g, 0.061 mol) and one of the corresponding aldehydes (0.06 mol) in tetrahydrofuran (10 ml). The aldehyde in THF and zinc powder had been previously refluxed for 30 min. After completion of the reaction (approximately 1.5 h), the mixture was cooled to room temperature and filtered to remove the unreacted zinc. The solvent was evaporated in vacuo and the crude semisolid product was dissolved in CH_2Cl_2 (20 ml). The solution was cooled in an ice bath and treated with saturated ammonium chloride solution (50 ml) with vigorous stirring. After separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 100 ml). The combined organic layers were washed with water (50 ml), dried over anhydrous magnesium sulfate, and evaporated in vacuo. The crude product was purified by preparative vacuum chromatography using hexane:ethyl acetate 3:1 (v/v) as the eluent.

4.3.1.1. *rac*-Ethyl 3-hydroxy-3-(2-phenylthiazol-4-yl)propanoate *rac*-1a. Yield: 92%; yellow solid; mp = 48 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ =1.25 (t, J = 7.5 Hz, 3H), 2.97 (ddd, J = 3 Hz, J = 9 Hz, J = 15 Hz, 2H), 3.86 (s, 1H), 4.18 (q, J = 9 Hz, 2H), 5.3 (dd, J = 3 Hz, J = 9 Hz, 1H), 7.24 (d, J = 3 Hz, 1H), 7.37–7.45 (m, 3H), 7.88–7.94 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.2, 41.5, 60.9, 67.6, 114.0, 126.5, 128.9, 130.1, 133.5, 158.1, 168.4, 172.4; IR: ν = 3019, 2985, 2881, 2826, 2772, 2330, 1777, 1384, 1326, 1275, 1185, 1033, 762, 751; MS: M^+ found (M^+ calculated for $C_{14}H_{15}NO_3S$): 277.0752 (277.0773); MS m/z: 278(M+1, 12), 277(M, 29), 214(13), 204(100), 190(52), 188(17), 162(18), 121(13), 104(18), 77(15), 59(22), 29(16).

4.3.1.2. *rac*-Ethyl 3-(2-(4-chlorophenyl)thiazol-4-yl)-3-hydroxypropanoate *rac*-1b. Yield: 91%; yellow solid; mp = 49 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (t, J = 6 Hz, 3H), 2.94 (ddd, J = 3 Hz, J = 9 Hz, J = 15 Hz, 2H), 3.90 (d, J = 3 Hz, 1H), 4.16 (q, J = 6 Hz, 2H), 5.25–5.30 (m, 1H), 7.25 (s, 1H), 7.35 (d, J = 9 Hz, 2H), 7.81 (d, J = 9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.2, 41.5, 60.9, 67.5, 114.4, 127.7, 129.1, 132.0, 136.0, 159.0, 167.0, 172.3; IR: ν = 3162, 3116, 1792, 1457, 1395, 1275, 1186, 1092, 835, 751; MS: M^+ found (M^+ calculated for $C_{14}H_{14}ClNO_3S$): 311.0407 (311.0383); MS m/z: 314(M+1, ^{37}Cl, 2), 313(M, ^{37}Cl, 10), 312(M+1, ^{35}Cl, 6), 311(M, ^{35}Cl, 30), 240(^{37}Cl, 32), 238(^{35}Cl, 100), 226(^{37}Cl, 20), 224(^{35}Cl, 60), 198(^{37}Cl, 5), 196(^{35}Cl, 15), 157(^{37}Cl, 4), 155(13), 59(25), 43(13), 29(15).

4.3.1.3. *rac*-Ethyl 3-hydroxy-3-(2-*p*-tolylthiazol-4-yl)propanoate *rac*-1c. Yield: 95%; yellow semisolid; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.25 (t, J = 7.5 Hz, 3H), 2.37 (s, 3H), 2.96 (ddd, J = 3 Hz, J = 9 Hz, J = 15 Hz, 2H), 3.84 (d, J = 6 Hz, 1H), 4.17 (q, J = 7.5 Hz, 2H), 5.26-5.32 (m, 1H), 7.21 (d, J = 6 Hz, 3H), 7.79 (d, J = 9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.2, 21.4, 41.6, 60.8, 67.6, 113.5, 126.4, 129.6, 130.9, 140.3, 158.6, 168.6, 172.4; IR: ν = 3371, 3328, 3101, 3037, 2990, 1727, 1517, 1461, 1185, 1034, 756; MS: M^+ found (M^+ calculated for $C_{15}H_{17}NO_3S$): 291.0897(291.0929); MS m/z: 292(M+1, 27), 291(M, 35), 274(11), 219(15), 218(100), 204(52), 202(19), 200(12), 176(16), 135(13), 118(18), 91(12), 29(16).

4.3.1.4. *rac*-Ethyl 3-hydroxy-3-(2-*m*-tolylthiazol-4-yl)propanoate *rac*-1d. Yield: 94%; yellow solid; mp = 40 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.29 (t, J = 6 Hz, 3H), 2.43 (s, 3H), 2.99 (ddd, J = 3 Hz, J = 9 Hz, J = 15 Hz, 2H), 3.69 (d, J = 6 Hz, 1H), 4.21 (q, J = 6 Hz, 2H), 5.35–5.29 (m, 1H), 7.23–7.36 (m, 3H), 7.71–7.78 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.3, 21.4, 41.5, 60.9, 67.7, 113.9, 123.8, 127.1, 128.9, 131.0, 133.5, 138.8, 158.6, 168.7, 172.6; IR: ν= 3571, 1725, 1312, 1286, 1245, 1185, 1029, 798, 752, 733, 687; MS: M^+ found (M^+ calculated for $C_{15}H_{17}NO_3S$): 291.0975 (291.0929); MS *m*/*z*: 292(M+1, 8), 291(M, 29), 219(15), 218(100), 204(46), 202(11), 176(12), 135(8), 118(11), 91(10), 59(11), 43(11), 29(7).

4.3.2. Synthesis of racemic 2-(ethoxycarbonyl)-1-(2-arylthiazol-4-yl)ethyl butanoate *rac*-2a–d

To a solution of the β-hydroxyester *rac*-**1a**–**d** (50 mg) in dichloromethane (1.5 ml), butanoic anhydride (1.1 equiv, 32.16 μl), triethylamine (1.1 equiv, 27.6 μl) and a catalytic amount of 4-*N*,*N*-dimethylamino-pyridine (0.028 equiv, 0.6 mg) were added. After stirring for 3 h at room temperature, the solvent was evaporated and the crude product was separated using column chromatography and a 6:4 mixture of hexane/ethyl acetate as the eluent.

4.3.2.1. *rac*-2-(Ethoxycarbonyl)-1-(2-phenylthiazol-4-yl)ethyl butanoate *rac*-2a. Yield: 92%; yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.93 (t, J = 6 Hz, 3H), 1.23 (t, J = 7.5 Hz, 3H), 1.66 (overlapped tq, $J_1 \sim J_2 \sim$ 7.2 Hz, 2H), 2.33 (t, J = 7.5 Hz, 2H), 3.13 (d, J = 6 Hz, 2H), 4.14 (q, J = 6 Hz, 2H), 6.39 (t, J = 6 Hz, 1H), 7.25 (d, J = 3 Hz, 1H), 7.40–7.45 (m, 3H), 7.90–7.94 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.6, 14.2, 18.5, 36.2, 39.3, 60.8, 68.2, 116.5, 126.6, 128.9, 130.2, 133.5, 154.7, 168.4, 170.0, 172.7; IR: ν = 2975, 1737, 1489, 1286, 1243, 1172, 1089, 1026, 774, 728, 684; MS: M^+ found (M^+ calculated for $C_{18}H_{21}NO_4S$): 347.1182 (347.1191); MS m/z (%): 348(M+1, 3), 347(M, 5), 277(19), 276(100), 231(13), 230(87), 214(18), 190(17), 188(71), 71(15), 58(11), 43(25), 29(16).

4.3.2.2. *rac*-2-(Ethoxycarbonyl)-1-(2-(4-chlorophenyl)thiazol-4-yl)ethyl butanoate *rac*-2b. Yield: 90%; light yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (t, J = 7.5 Hz, 3H), 1.22 (t, J = 7.5 Hz, 3H), 1.60 (overlapped tq,, $J_1 \sim J_2 \sim$ 7.8 Hz, 2H), 2.31 (t, J = 7.6 Hz, 2H), 3.11 (d, J = 9 Hz, 2H), 4.13 (q, J = 6 Hz, 2H), 6.38 (t, J = 6 Hz, 1H), 7.24 (s, 1H), 7.36 (d, J = 8.6 Hz, 2H), 7.8 (d, J = 9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.6, 14.2, 18.4, 36.2, 39.3, 60.7, 68.1, 115.9, 126.5, 129.5, 130.8, 140.4, 154.5, 168.5, 170.0, 172.6; IR: ν = 2975, 1737, 1518, 1462, 1372, 1286, 1243, 1173, 1027, 825; MS: M^+ found (M^+ calculated for $C_{18}H_{20}ClNO_4S$): 381.0793 (381.0802); MS *m*/*z*: 383(M, ^{37}Cl, 2); 381(M, ^{35}Cl, 5); 311(^{37}Cl, 32); 309(^{35}Cl, 100); 265(^{37}Cl, 24); 263(^{35}Cl, 74); 249(^{37}Cl, 8); 247(^{35}Cl, 25); 223(^{37}Cl, 22); 221(^{35}Cl, 69); 71(29); 58(16); 43(59); 29(31).

4.3.2.3. *rac*-2-(Ethoxycarbonyl)-1-(2-*p*-tolylthiazol-4-yl)ethyl butanoate *rac*-2c. Yield: 92%; light yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (t, J = 7.5 Hz, 3H), 1.22 (t, J = 7.5 Hz, 3H), 1.60 (overlapped tq,, $J_1 \sim J_2 \sim$ 7.8 Hz, 2H), 2.27–2.39 (m, 5H), 3.11 (d, J = 9 Hz, 2H), 4.13 (q, J = 6 Hz, 2H), 6.38 (t, J = 6 Hz, 1H), 7.21 (d, J = 6 Hz, 3H), 7.8 (d, J = 9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.6, 14.2, 18.4, 21.4, 36.2, 39.3, 60.7, 68.1, 115.9, 126.5, 129.5, 130.8, 140.4, 154.5, 168.5, 170.0, 172.6; IR: ν = 2975, 1737, 1518, 1462, 1372, 1286, 1243, 1173, 1027, 825; MS: M^+ found (M^+ calculated for $C_{19}H_{23}NO_4S$): 361.1379 (361.1348); MS *m*/*z*: 362(M+1, 20), 361(M, 82), 290(35), 289(100), 273(79), 243(98), 227(45), 203(25), 201(92), 71(28), 43(69), 29(34).

4.3.2.4. *rac*-2-(Ethoxycarbonyl)-1-(2-*m*-tolylthiazol-4-yl)ethyl butanoate *rac*-2d. Yield: 91%; yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.95 (t, J = 7.5 Hz, 3H), 1.26 (t, J = 7.5 Hz, 3H), 1.69 (overlapped tq, $J_1 \sim J_2 \sim$ 7.2 Hz, 2H), 2.36 (t, J = 7.5, 2H), 2.44 (s, 3H), 3.16 (d, J = 6 Hz, 2H), 4.17 (q, J = 6 Hz, 2H), 6.41 (t, J = 7.5 Hz,1H), 7.24–7.36 (m, 3H), 7.73 (d, J = 9 Hz, 1H), 7.79 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.7, 14.2, 18.5, 21.4, 36.3, 39.3, 60.8, 68.2, 116.4, 123.9, 127.2, 128.9, 131.0, 133.4, 138.8, 154.7, 168.7, 170.1, 172.7; IR: ν = 2968, 1738, 1462, 1371, 1286, 1243, 1173, 1033, 798, 751, 706; MS: M^+ found (M^+ calculated for $C_{19}H_{23}NO_4S$): 361.1337 (361.1348); MS *m*/*z*: 362(M+1, 2), 361(M, 4), 290(20), 289(100), 243(71), 227(16), 203(15), 201(70), 71(16), 58(9), 43(31), 29(15).

4.4. Enzyme mediated biotransformation of *rac*-1–2a–d on an analytical scale

4.4.1. Analytical scale enzymatic acylation of *rac*-1a–d

For each reaction, the enzyme (12.5 mg) and molecular sieves (2 pieces) were added into a solution of the substrate *rac*-**1a**–**d** (0.025 M) in 500 μl of solvent, followed by the addition of the acylation agent (0.1 M). The reaction mixture was shaken at 1350 rpm at room temperature for 17 h. The reactions were then verified on TLC after 2 and 17 h, respectively using a hexane/ethyl acetate 6:4 mixture as the eluent. The spots were visualized by treatment with a 5% ethanolic phosphomolybdic acid solution and heating.

4.4.2. Analytical scale enzymatic alcoholysis of *rac*-2a–d

Into a solution of *rac*-**2a**–**d** (5 mg) in each solvent (500 μl), the alcohol (2.4 μl) and the lipase (12.5 mg) were added. The hydrolysis was monitored by TLC and HPLC and was stopped at approximately 50%. For HPLC analysis, samples taken from the reaction mixture (10 μl) were diluted to 500 μl with hexane/2-propanol 90:10 (v/v) and filtered before injection.

4.4.3. Determination of the reusability of CaL-B

For the O-acylation, CaL-B (12.5 mg) and molecular sieves (2 pieces) were added into a solution of the substrate *rac*-**1a**–**d** (0.018 M) in 500 μl of toluene, followed by the addition of vinyl butanoate (0.075 M). The reaction mixture was then shaken at 1350 rpm at room temperature. For HPLC analysis, samples taken from the reaction mixture (10 μl) were diluted to 500 μl with hexane/2-propanol 90:10 (v/v) and injected. When the reaction reached 50% conversion, the enzyme was filtered and thoroughly washed with the solvent and was ready to be reused. This procedure was repeated by using the same CaL-B in order to determine its recyclability. By quantifying the transformed substrate, we were able to determine the enzyme's activity.

For the alcoholysis, into a solution of *rac*-**2a**–**d** (0.015 M) in each solvent (500 μl), the alcohol (0.075 M) and the lipase (12.5 mg) were added. The hydrolysis was monitored by TLC and HPLC and was stopped at approximately 50% conversion. The enzyme was filtered and washed several times with solvent, then reused. For HPLC analysis, samples taken from the reaction mixture (10 μl) were diluted to 500 μl with hexane/2-propanol 90:10 (v/v) and filtered before injection. This procedure was repeated, using the same CaL-B in order to determine its recyclability.

4.5. Preparative scale enzyme mediated biotransformation of *rac*-1–2a–d

The enzyme (750 mg) and molecular sieves (5 pieces) were added into a solution of substrate *rac*-**1a**–**d** (300 mg, 0.025 M) in 30 ml of toluene, followed by the addition of the vinyl butanoate (0.1 M). The reaction mixture was shaken at 1350 rpm at room temperature for 17 h. When the reaction reached 50% conversion,

the enzyme was filtered, the solvent was evaporated, and the crude products were separated by column chromatography using a hexane/ethyl acetate 6:4 mixture as the eluent. Next, into a solution of (*R*)-**2a–d** (120 mg) in DIPE (15 ml), ethanol (58 μl), and CaL-B (375 mg) were added. The hydrolysis was monitored by TLC and after the completion of the reaction, the enzyme was filtered, the solvent was evaporated, and the product was purified using column chromatography and hexane/ethyl acetate 6:4 mixture as the eluent. This way, both stereoisomers of the enantiomerically pure ethyl 3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates were obtained.

4.6. Preparation of the MTPA esters

The enantiomerically pure (*R*)- and (*S*)-MTPA acids (1 equiv) were activated with *N*,*N*′-dicyclohexylcarbodiimide (1.2 equiv) in CH_2Cl_2 (2 ml), dried over molecular sieves, and then (*S*)-**1a** (2 equiv) and a catalytic amount of DMAP were added. The reaction mixture was then stirred overnight at 1350 rpm. Next, the solvent was evaporated and the crude product was purified using column chromatography and CH_2Cl_2 as the eluent. The products were characterized by ^{1}H NMR spectroscopy.

4.7. Preparation of CaL-B-SWCNT

A single-walled carbon nanotube (20 mg) was suspended in a phosphate buffer (50 mM, pH 7.02, 0.5 ml) and was sonicated for 10 min. A solution of *Candida antarctica* Lipase B (400 μg) in phosphate buffer (50 mM, pH 7.02, 0.5 ml) was added into the SWCNT suspension and the mixture was shaken for 3 h at 1500 rpm and 20 °C. Next, the suspension was filtered through a PTFE membrane filter (pore size 0.2 μm) and the precipitate was washed with a phosphate buffer (500 μM, pH 7.02, 3 × 50 μl) in order to remove the unadsorbed enzyme. The global protein content of the combined filtrates was 200 μg, which was determined by using the Bradford method. The obtained CaL-B-SWCNT (protein content ~1%) was freeze-dried and stored under argon.

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