Tetrahedron: *Asymmetry* 22 (2011) 315–322

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Lipases A and B from *Candida antarctica* in the enantioselective acylation of ethyl 3-heteroaryl-3-hydroxypropanoates: aspects on the preparation and enantiopreference

Jürgen Brem [a,b], Arto Liljeblad [a], Csaba Paizs [b], Monica Ioana Toşa [b], Florin-Dan Irimie [b], Liisa T. Kanerva [a,*]

[a] *Institute of Biomedicine, Department of Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry and Department of Chemistry, University of Turku, FIN-20014, Finland*

[b] *Department of Biochemistry and Biochemical Engineering, Babeş-Bolyai University of Cluj-Napoca, Ro-400028 Cluj-Napoca, Arany János 11, Romania*

ARTICLE INFO

Article history:
Received 21 December 2010
Accepted 20 January 2011
Available online 14 March 2011

ABSTRACT

The preparative scale kinetic resolution of racemic ethyl 2- and 3-furyl- and 2- and 3-thienyl-3-hydroxypropanoates has been performed by *Candida antarctica* lipases A and B with vinyl esters. A study based on the present work together with the literature has been carried out in terms of lipase enantiopreference and substrate structure. We also discuss the excellent behavior of the lipase A in O-acylations of secondary alcohols with respect to enantiopreference.

1. Introduction

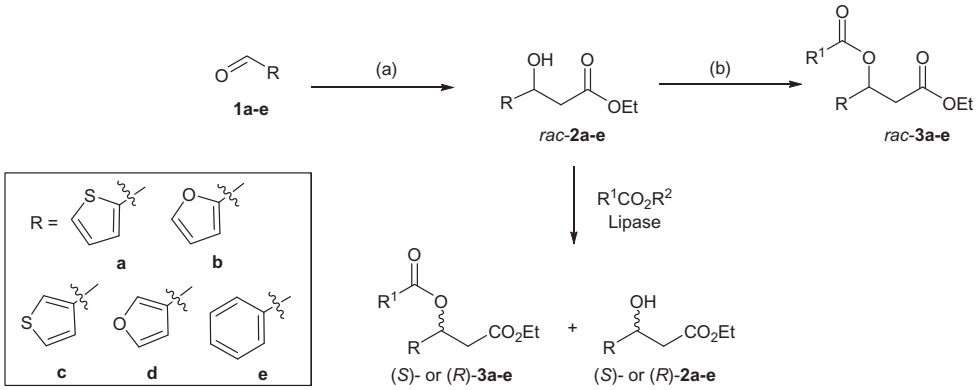
β-Hydroxy esters are versatile chiral intermediates in the synthesis of several pharmaceuticals and fine chemicals. For instance, (*R*)-3-hydroxybutanoate esters serve as starting materials for β-lactam antibiotics, including carbapenems and penems with broad antimicrobial spectra, and for dorzolamide, a topically active human carbonic anhydrase II inhibitor.[1–7] Amongst aryl-substituted β-hydroxy esters, ethyl (*S*)-3-hydroxy-3-(thiophen-2-yl)-propanoate (*S*)-**2a** is a precursor for the synthesis of duloxetine (a serotonin-norepinephrine re-uptake inhibitor) and ethyl (*R*)-3-hydroxy-3-phenylpropanoate (*R*)-**2e** for that of atomoxetine (a norepinephrine re-uptake inhibitor).[8–10] These examples indicate that an active drug molecule may have either an (*R*)- or (*S*)-configuration. More generally, the preparation of both enantiomers of a drug and its key intermediates is necessary to recognize the physiological properties of these molecules.

With regards to green chemistry, biocatalysts have been increasingly included and used by modern synthetic chemists in addition to expensive and often complicated chemical asymmetric catalysts and natural products used in traditional resolution. The enzymatic kinetic resolution of a racemate with a suitable hydrolytic enzyme is one of the most common biocatalytic methods used today. In the best case, both enantiomers (one as the less reactive enantiomer and the other as a new product) can be obtained in one-pot in a single reaction. Kinetic resolution can be further transformed into a dynamic kinetic resolution (DKR) by combining an enzyme as an enantioselective catalyst with a suitable racemization catalyst in one-pot.[11,12] The choice of an enzyme is determined by the reactive functional groups of the racemate. High stability, enantioselectivity, and good commercial availability in free and immobilized forms have made lipases (EC 3.1.1.3) especially attractive kinetic resolution catalysts via the acylation of a nucleophilic (such as alcohol) functionality and through the deacylation (hydrolysis, alcoholysis or interesterification) of an ester functionality in the molecule.[13] Thus, β-hydroxy esters enable a variety of possibilities as both alcohols and esters for kinetic resolutions with lipases.

Lipase-catalyzed methods have previously been used for the kinetic resolution of racemic ethyl 3-benzofuranyl-, 3-benzo[*b*]-thiophenyl-, and 3-(10-alkyl-10*H*-phenothiazin-3-yl)-3-hydroxypropanoates,[14,15] and for the DKR of *rac*-**2e**.[16] Herein our aim was to study the chemoenzymatic possibility of preparing the enantiomers of ethyl 3-heteroaryl-3-hydroxypropanoates via chemical synthesis of *rac*-**2a**–**e** followed by enantioselective lipase-catalyzed O-acylation of racemic mixtures in organic solvents (Scheme 1). Compound **2e**, although not heteroaromatic, was included in the initial screens for comparison. We also wanted to study the enantiopreferences of lipases A (CAL-A) and B (CAL-B) from *Candida antarctica*; the two lipases were thoroughly studied in the kinetic resolution of various β-amino esters.[17] Some other commercial lipases were also screened for the sake of comparison. Many lipases have been shown to produce the (*R*)-ester in the O-acylation of secondary alcohols. For such (*R*)-enantioselectivity, the large group at the stereogenic center must owe CIP priority over the medium size group as with (*R*)-1-phenylethanol.[18] Interestingly, an opposite enantiopreference in O- and N-acylations was sometimes detected with the title enzymes CAL-A and CAL-B (Fig. 1), and we have illustrated this phenomenon with *rac*-**2a**–**e**. The value of the

* Corresponding author. Tel.: +358 2 3336773; fax: +358 2 3337955.
E-mail address: lkanerva@utu.fi (L.T. Kanerva).

0957-4166/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2011.01.027



Scheme 1. Chemoenzymatic route to the enantiomers of 3-heteroaryl-3-hydroxypropanoates. Reagents and conditions: (a) Zn, $BrCH_2CO_2Et$, THF, reflux; (b) R′COCl, Et_3N, DMAP, CH_2Cl_2, rt or $(R'CO)_2O$, $CoCl_2$, CH_3CN, reflux.

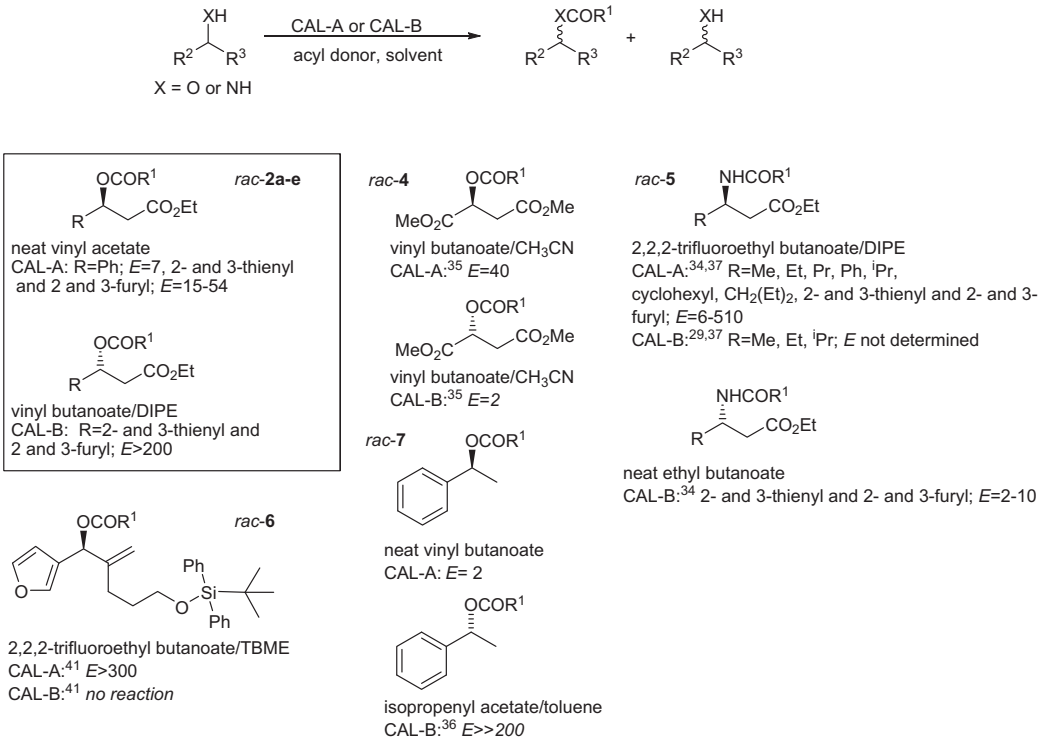


Figure 1. Enantiodiscrimination in O- and N-acylations by CAL-A (on Celite) and CAL-B (Novozym 435). O-Acylated product enantiomers are presented by drawing the aromatic ring always on the left side of the stereogenic center.

enantiopreference mapping resides in finding lipases with an opposite enantiopreference for DKR purposes.

Since the 1990s, CAL-B used as Novozym 435 preparation (CAL-B adsorbed on a divinylbenzene-crosslinked, hydrophobic macroporous polymer based on methyl and butyl methacrylic esters) has become the most common lipase used in synthetic organic chemistry due to its ability to effectively catalyze various highly enantioselective acyl transfers in polar as well as in non-polar solvents. In our previous studies, CAL-B indicated low chemoselectivity for the N-acylation of various β-amino esters leading to competitive interesterification with an acyl donor.[17] On the other hand, excellent chemoselectivity was reported for CAL-A in the N-acylation of the same β-amino esters.[17] The high chemoselectivity has been explained by recent studies which proved the highly restricted acyl binding pocket of CAL-A.[19–21] Moreover, the ability of CAL-A to catalyze the acylation of bulky nucleophilic substrates is noteworthy. This has been explained by the presence of a GGGX-motif at the active site, whereas most lipases consist of a GX-motif.[22] The excellent properties of CAL-A with regards to its ability to hydrolyze amides have also been reported.[23] Along with its peculiar catalytic behavior, the use of CAL-A is now rapidly increasing. Summaries for CAL-A[17,24–26] exist but the catalytic properties of CAL-A toward secondary alcohols are still relatively scattered in the literature. Herein we integrate the obtained results for *rac*-**2a**–**e** to the existing knowledge of CAL-A related to secondary alcohols.

2. Results and discussion

2.1. Synthesis of the racemic starting materials

In principle, various chemical methods exist for the preparation of racemic ethyl 3-hydroxy-3-arylpropanoates. Amongst them, the

Reformatsky reaction is a straightforward method which was previously successful in the preparation of 3-hydroxy-3-heteroarylpropanoates from the corresponding aldehydes.[14,15] Accordingly, the method was used to transform aldehydes **1a**–**e** into *rac*-**2a**–**e** with ethyl bromoacetate in tetrahydrofuran in the presence of zinc dust (Scheme 1, route a). Compounds *rac*-**2a** and *rac*-**2e** were obtained with acceptable yields (92% and 72%, respectively). With *rac*-**2b** the yield was improved from 5% to 28% by performing the reaction without solvent (THF). With *rac*-**2b** and *rac*-**2d**, the low yields (28% and 17%, respectively) obtained were possibly due to the instability of the furan ring under acidic conditions and to the interference of the zinc with the ring oxygen. Efforts to synthesize the hydroxy esters through the corresponding β-keto esters which were first prepared by condensation of aldehydes **1b**–**d** with ethyl diazoacetate proved to be impractical as low keto ester yields (35–40%) discouraged us from continuing with reduction into *rac*-**2b**–**d**.[27]

To obtain racemic resolution products *rac*-**3a**–**e** (R^1 = Me, Et or Pr) for analytical identification, the racemates *rac*-**2a**–**e** were esterified (route b) with the corresponding acyl chloride in dichloromethane in the presence of triethylamine and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine, or with the corresponding acid anhydride in the presence of anhydrous cobalt chloride in acetonitrile, leading to good yields as described in the Section 4.

2.2. Enzymatic kinetic resolution

Extensive screening with various lipase preparations for the O-acylation of *rac*-**2a** and *rac*-**2e** as model compounds in neat vinyl acetate started our analytical scale studies (Scheme 1). The formation of an acyl-enzyme intermediate, an ester intermediate between the serine hydroxyl at the active site of the lipase and an acyl donor, is pivotal for lipase catalysis. This means that a β-hydroxy ester as an added alcohol nucleophile binds enantioselectively in the nucleophile binding site before reacting with the intermediate, whereas as an acyl donor, it enantioselectively binds in the acyl binding site and forms the intermediate by itself. Accordingly, care is needed when choosing an achiral acyl donor for enzymatic O-acylation due to the dual nature of the substrates present. For CAL-B catalysis competitive interesterification may be an especially serious side reaction[28,29] as it might also be exploitable in various applications.[30,31] To prevent interesterification as a side reaction with CAL-B, vinyl acetate was chosen as a commonly used and irreversible acyl donor. Vinyl acetate is more reactive an acyl donor than the substrate ester *rac*-**2a** or *rac*-**2e** and also prevents another possible side reaction, the enzymatic dimerization of the substrate. With CAL-A, interesterification and dimerization are less likely due to the restricted acyl binding pocket as already mentioned.

The results with the model substrates show two interesting features. Firstly, the *S*-enantiomers reacted with CAL-A and *Rhizopus oryzae* lipase (Table 1, entries 1–4 and 20–22), whereas the other lipases indicated (*R*)-enantioselectivity (entries 5–19). Secondly, enantioselectivity in terms of enantiomer ratio (*E*) was clearly different with the phenyl (*rac*-**2e**) and heteroaryl (*rac*-**2a**) substrates. Thus, lipases from *Burkholderia cepacia* and *Pseudomonas fluorescens* were favorable with *rac*-**2e** (entries 7–16), whereas the other lipases favored *rac*-**2a** (entries 1–6 and 17–22). The performance of lipase *Burkholderia cepacia* preparations with *rac*-**2e** is also in accordance with the excellent enantioselectivity observed in the DKR of *rac*-**2e** in the presence of (pentaphenylcyclopentadienyl)ruthenium complex.[16] Clearly, CAL-B (entries 5 and 6) along with various CAL-A preparations, especially when adsorbed on Celite in the presence of sucrose (entries 2 and 3),[32] gave fast and enantioselective reactions with *rac*-**2a** but not with *rac*-**2e**. In addition to the lipases shown in Table 1, lipases from porcine pancreas, *Mucor miehei*, *Rhizopus arrhizus*, *Mucor javanicus*, *Aspergillus niger*, *Candida lipolytica*, and *Penicillium camemberti* were practically inactive under the screening conditions.

As shown in Table 1, CRL and *Rhizopus oryzae* lipase seem to have potential for the kinetic resolution of *rac*-**2a** with *E* values of 71 and 124, respectively (entries 17 and 20). However, CRL gave considerable ester hydrolysis as a side reaction and was not studied further. *Rhizopus oryzae* lipase kept low activity even after tedious optimization studies. Accordingly, attention was paid to CAL-A and CAL-B, two fascinating lipases, which have been previously exploited, for instance in the N-acylation of β-amino esters.[28–30,33–37] The acylation of *rac*-**2a** in neat vinyl acetate, propanoate, and butanoate (used as acyl donors and solvents) revealed the opposite effects of CAL-A and CAL-B on the *E*-values (Table 2). With CAL-A, $E = 54$ (entry 1) was the highest in neat vinyl acetate, whereas the reaction with CAL-B in vinyl butanoate gave excellent enantioselectivity ($E > 200$, entry 3). Solvent effects were studied further for the acylation of *rac*-**2a** with CAL-A using vinyl acetate

Table 1
Acylation of *rac*-**2a** and *rac*-**2e** (0.050 M) with enzymes (50 mg/mL) in neat $CH_3CO_2CH{=}CH_2$ at room temperature

Entry	Substrate	Lipase	Time (h)	Conv. (%)	Reactive enantiomer	*E*
1	*rac*-**2a**	CAL-A (lyo.)	1	25	(*S*)	7
2	*rac*-**2a**	CAL-A on Celite	1	50	(*S*)	54
3	*rac*-**2e**	CAL-A on Celite	1	10	(*S*)	7
4	*rac*-**2a**	CAL-A CLEA	1	38	(*S*)	10
5	*rac*-**2a**	CAL-B (Novozym 435)	20	7	(*R*)	40
6	*rac*-**2e**	CAL-B (Novozym 435)	20	48	(*R*)	5
7	*rac*-**2a**	*Burkholderia cepacia* (lyo.)	20	4	(*R*)	2
8	*rac*-**2e**	*Burkholderia cepacia* (lyo.)	20	21	(*R*)	34
9	*rac*-**2a**	*Burkholderia cepacia*-C II	20	70	(*R*)	5
10	*rac*-**2e**	*Burkholderia cepacia*-C II	20	41	(*R*)	104
11	*rac*-**2a**	*Burkholderia cepacia*-D	20	55	(*R*)	10
12	*rac*-**2e**	*Burkholderia cepacia*-D	20	35	(*R*)	276
13	*rac*-**2a**	*Pseudomonas fluorescens* (lyo.)	20	25	(*R*)	12
14	*rac*-**2e**	*Pseudomonas fluorescens* (lyo.)	20	31	(*R*)	33
15	*rac*-**2a**	*Pseudomonas fluorescens* on Celite	20	20	(*R*)	7
16	*rac*-**2e**	*Pseudomonas fluorescens* on Celite	20	8	(*R*)	37
17	*rac*-**2a**	CRL	20	35	(*R*)	71
18	*rac*-**2e**	CRL	20	40	(*R*)	12
19	*rac*-**2a**	CRL sol-gel	20	29	(*R*)	50
20	*rac*-**2a**	*Rhizopus oryzae* lipase	20	7	(*S*)	124
21	*rac*-**2e**	*Rhizopus oryzae* lipase	20	2	(*S*)	39
22	*rac*-**2a**	*Rhizopus oryzae* lipase IMMARO-T2-150	20	6	(*S*)	2

Table 2
CAL-A- and CAL-B-catalyzed (50 mg/mL) acylations of *rac*-**2a** (0.25 M) in neat $R^1CO_2CH{=}CH_2$ at room temperature

Entry	R^1	CAL-A on Celite[a]		Novozym 435	
		Conv. (%)	*E*	Conv. (%)	*E*
1	CH_3	50	54	5	6
2	CH_3CH_2	62	19	15	74
3	$CH_3CH_2CH_2$	51	13	45	>200

Reaction time 1 h (CAL-A) and 60 h (CAL-B).
[a] 20% (w/w) of CAL-A and 12% (w/w) of sucrose on Celite.[32]

and with CAL-B using vinyl butanoate as an acyl donor in organic solvents (Table 3). With both lipases the reactions in the neat vinyl ester were acceptable (entry 12). In addition, reactivity with CAL-B in ethers and in hydrophobic solvents was good and in most cases the reactions proceeded with excellent enantioselectivities (*E* >200) (entries 1–6 and 9). As vinyl butanoate is relatively expensive and its boiling point is high, the work with CAL-B was continued using vinyl butanoate in *tert*-butyl methyl ether (MTBE) as a low-boiling solvent.

Table 3
CAL-A- and CAL-B-catalyzed (50 mg/mL) acylations of *rac*-**2a** (0.25 M) with $R^1CO_2CH{=}CH_2$ (R^1 = Me) and $R^1CO_2CH{=}CH_2$ (R^1 = Pr) as acyl donors (5 equiv), respectively, in different solvents at room temperature

Entry	Solvent	CAL-A on Celite[a]		Novozym 435	
		Conv. (%)	*E*	Conv. (%)	*E*
1	*n*-Octane	21	8	44	94
2	*n*-Hexane	20	8	46	>200
3	Toluene	14	7	49	>200
4	Benzene	12	10	46	>200
5	MTBE	19	10	45	>200
6	Diethyl ether	31	9	50	>200
7	THF	9	15	8	>200
8	1,4-Dioxane	1	3	3	>200
9	Acetonitrile	21	16	8	27
10	Chloroform	3	5	6	>200
11	Dichloromethane	7	15	9	>200
12	Neat acyl donor	50	54	45	>200

Reaction time 1 h (CAL-A) and 60 h (CAL-B).
[a] 20% (w/w) of CAL-A and 12% (w/w) of sucrose on Celite.[32]

Finally, the acylation of *rac*-**2a**–**d** was tested with CAL-A in neat vinyl acetate and with CAL-B with vinyl butanoate in MTBE (Scheme 1, Table 4). The results clearly indicated excellent (*R*)-enantioselectivity in the CAL-B-catalyzed O-acylations (*E* >200), whereas CAL-A displayed moderate (*S*)-selectivity (*E* = 14–54). Finally, the preparative scale kinetic resolutions of *rac*-**2a**–**d** were performed successfully using CAL-B in MTBE as described in Section 4, allowing the preparation of the (*R*)-esters **3a**–**d** (R^1 = Pr) and the unreacted (*S*)-**2a**–**d** in highly enantiomerically enriched forms with ee values between 95% and 99% (Table 5). In spite of low enantioselectivities (*E* 14–54), the preparative scale kinetic resolutions were also performed with CAL-A in neat vinyl acetate. In accordance with the opposite enantiodiscriminations of CAL-A and CAL-B, the signs of the specific rotations $[\alpha]_D$ of the starting materials **2** changed, as did those of products **3**. The absolute configurations of the present resolution products are based on the $[\alpha]_D$ values in Table 5 and on the literature values +19 (*c* 1.7, $CHCl_3$, ee = 84%)[38] for (*R*)-**2a**, +18 (*c* 1.6, $CHCl_3$, ee = 54)[38] and +15 (*c* 0.9, $CHCl_3$, ee = 75%)[39] for (*R*)-**2b** and −94.2 (*c* 1.3, $CHCl_3$, ee = 90%)[39] for (*R*)-**3b** (R^1 = Pr).

2.3. Enantiopreference of CAL-A and CAL-B

Integration of the results obtained for the O-acylation of *rac*-**2a**–**e** (Tables 4 and 5) with the previously published O- and

Table 4
Acylations of *rac*-**2a**–**d** (0.25 M) with CAL-A (50 mg/mL) in neat $R^1CO_2CH{=}CH_2$ (R^1 = Me), and with CAL-B (50 mg/mL) and $R^1CO_2CH{=}CH_2$ (R^1 = Pr, 5 equiv) in MTBE

Entry	Substrate	CAL-A on Celite[a]		Novozym 435	
		Conv. (%)	*E*	Conv. (%)	*E*
1	*rac*-**2a**	50	54	50	>200
2	*rac*-**2b**	49	33	51	>200
3	*rac*-**2c**	50	14	50	>>200
4	*rac*-**2d**	46	22	48	>>200

Reaction time 1 h (CAL-A) and 60 h (CAL-B).
[a] 20% (w/w) of CAL-A and 12% (w/w) of sucrose on Celite.[32]

N-acylations (Figs. 1 and 2)[5,14,15,29,34–37,40–49] reveals some interesting facts about the enantiodiscrimination of CAL-A and fundamental differences of lipases CAL-A and CAL-B. Lipase CAL-B catalyzes the O-acylation of a series of simple secondary alcohols with excellent enantioselectivity. Examples include the O-acylation of 1-phenylethanol *rac*-**7**, a frequently used model compound, and 3-hydroxybutanoate *rac*-**8** (*E* >200).[5,36] This is based on a so-called stereospecificity pocket delimited by Thr42, Ser47, and Trp104 at the active site of CAL-B. The pocket is able to accommodate groups smaller than propyl.[50–52] Also the enantiopreference of the reactions is predictable. Thus, with *rac*-**7**, *rac*-**8**, and *rac*-**16** the methyl group (the medium-sized group) at the asymmetric center fits into the stereospecificity pocket, leading to the excellent *R*-enantiopreference with CAL-B. With all other substrates showing reaction with CAL-B, the faster reacting enantiomer has the same stereostructure around the asymmetric center with the aforementioned substrates, although the medium-sized group exceeds the size of propyl *rac*-**4**, -**5**, -**12**, -**14**, and -**15** or the group is electron rich such as CN (*rac*-**9**–**11**). With these compounds, CAL-B considerably lost its enantioselective character. The present substrates *rac*-**2a**–**d** with CH_2CO_2Et and substrate *rac*-**15** (X = S, *E* = 108) with CH_2CN as medium-sized groups are exceptions with excellent enantioselectivity. However, either of the groups, heteroaryl or CH_2CO_2Et/CH_2CN, evidently does not fit into the stereospecificity pocket. Common to these substrates, a five-membered heteroaryl ring and a group with a lone-pair of electrons are located next to the asymmetric center. The interactions of these groups with CAL-B stay currently unclear.

Lipase CAL-A, on the other hand, has a wide nucleophile binding site,[19] and the O-acylation of *rac*-**7** proceed with low enantioselectivity (*E* = 2), even though the reaction is otherwise very fast. There are a lot of data concerning the kinetic resolution of 1-phenylethanol but studies with CAL-A seem to be not published. The results given in Figure 1 were performed using vinyl butanoate as an acyl donor. Based on the crystal structure of CAL-A and molecular modeling, it has been proposed that upon substrate binding, CAL-A undergoes a conformational change whereby the active-site 'flap' (Gly426–Gly440) moves around the active site, widening the available space essentially infinitely.[19,20] This can explain why CAL-A is active and shows enantioselectivity in the O-acylation of highly sterically hindered secondary alcohols, such as *rac*-**6**, *rac*-**10**–**14**, *rac*-**17,** and *rac*-**18**, whereas CAL-B, or some other lipase, hardly catalyzes the reactions. As an interesting feature, amines *rac*-**5** (*E* = 75–510)[34,37] display much higher enantioselectivity when compared to the corresponding alcohols *rac*-**2a**–**e** (*E* = 7–54).

As can be seen in Figures 1 and 2, CAL-A may change the enantiopreference in O- (and N-)acylation according to the substrate structure, whereas CAL-B always prefers the same stereostructure with the substrates considered. Thus, when the bulkiness of the heteroaromatic rings increases from thienyl and furyl [*rac*-**2a**–**d**, (*S*)-enantiopreference] to benzothiophenyl and benzofuranyl (*rac*-**12** and *rac*-**14**), or the ring is substituted with another aromatic substituent (*rac*-**9**, *rac*-**11**, and *rac*-**15**), the enantiopreference of

Table 5
Kinetic resolution of *rac*-**2a–d**, characterization of the isolated products

Novozym 435				CAL-A on Celite			
Product	Yield (%)/ee (%)/$[\alpha]_D$[a]	Product (R^1 = Pr)	Yield/ee /$[\alpha]_D$[a]	Product	Yield (%)/ee (%)/$[\alpha]_D$[a]	Product (R^1 = Me)	Yield/ee/$[\alpha]_D$[a]
(*S*)-**2a**	46/99/−17	(*R*)-**3a**	48/96/+88	(*R*)-**2a**	45/68/+11	(*S*)-**3a**	44/94/−63
(*S*)-**2b**	47/99/−16	(*R*)-**3b**	48/97/+85	(*R*)-**2b**	47/63/+10	(*S*)-**3b**	45/90/−60
(*S*)-**2c**	46/99/−44	(*R*)-**3c**	48/99/+49	(*R*)-**2c**	47/51/+21	(*S*)-**3c**	40/81/−47
(*S*)-**2d**	46/97/−29	(*R*)-**3d**	47/99/+52	(*R*)-**2d**	46/60/+16	(*S*)-**3d**	41/88/−58

[a] 10^{-1} deg cm^2 g^{-1}; *c* 1.0, $CHCl_3$, *T* = 25 °C. Isolated yields based on the maximum theoretical recovery from the racemic starting material.

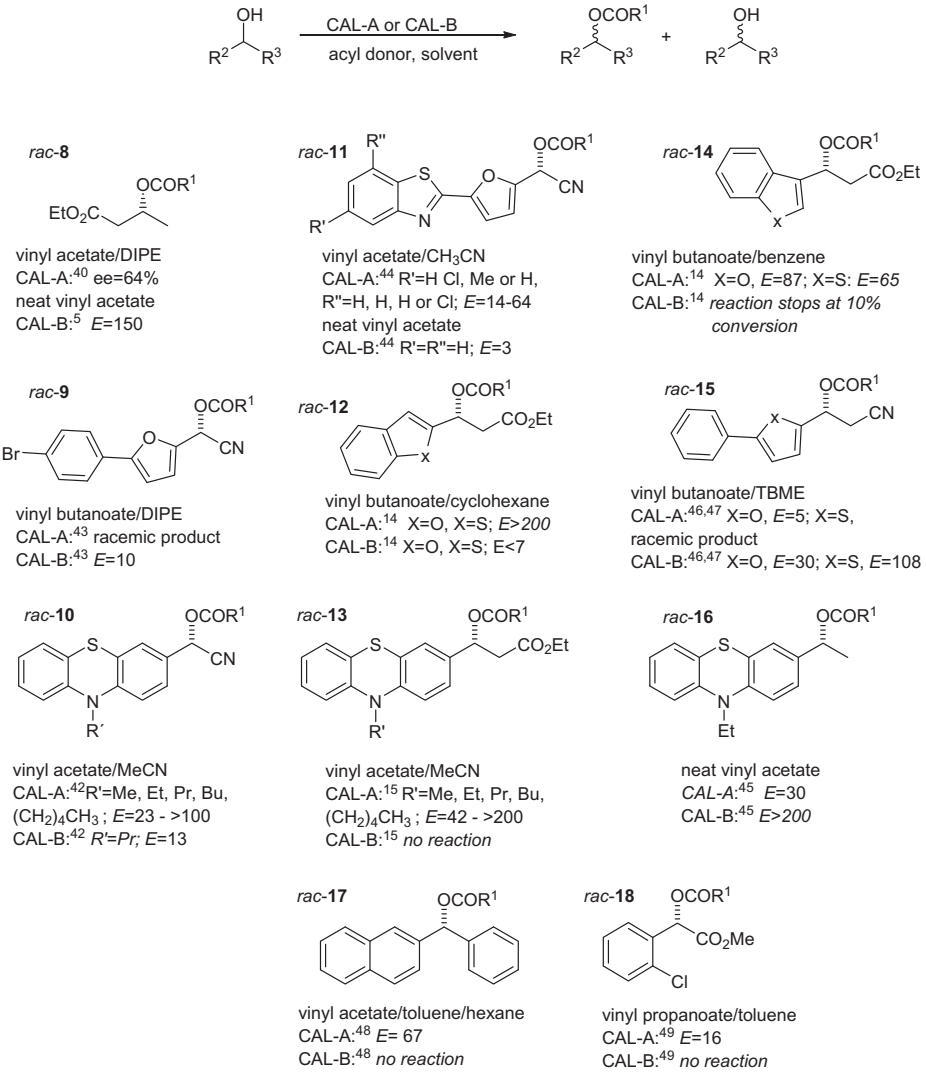


Figure 2. Enantiodiscrimination in O-acylations by CAL-A (on Celite) and CAL-B (Novozym 435). O-Acylated product enantiomers are presented by drawing the aromatic ring always on the left side of the stereogenic center.

CAL-A reverses. Reversal from an (*S*)- to an (*R*)-enantiopreference is also seen when the phenyl in *rac*-**2e** becomes more bulky (in *rac*-**13**) and in compounds *rac*-**10** and *rac*-**16**–**18**. The (*S*)-enantiopreference is also evident with *rac*-**4**, both the α- and β-hydroxy diesters. It is possible that the heteroaryl ring is connected to enantiopreference and high enantioselectivity in the O-acylation of heteroaryl-substituted secondary alcohols. These observations are rather difficult to explain without modeling. It is also worth remembering that all the given results were obtained with commercially available CAL-A, which not only contains contaminant enzymes, but also decomposed (evidently the active-site flap was lost), yet functional, CAL-A.[23] The presence of two catalytically active forms of CAL-A may have an effect not only on the enantioselectivity but also on the enantiopreference of the reactions.

3. Conclusions

An enantioselective O-acylation method has been developed to resolve ethyl 3-hydroxy-3-(thiophenyl/furanyl)propanoates *rac*-**2a–d** with vinyl butanoate in MTBE by using CAL-B (Novozym 435) as an enantioselective catalyst. The highly enantioselective

(E >200) reactions allowed the separation of the enantiomers in almost theoretical yields. Interestingly, CAL-A (on Celite) showed an opposite enantiopreference to that obtained with CAL-B. Although the enantioselectivities with CAL-A were more or less moderate (E = 14–54) the resolution products (*R*)-**2a**–**d** and (*S*)-**3a**–**d** (R^1 = Me) were separated, and the specific rotations were determined to witness the opposite specific rotations to the (*S*)-**2a**–**d** and the butanoate esters (*R*)-**3a**–**d** (R^1 = Pr) obtained with CAL-B catalysis.

We have shown that with regards to the enantiodiscrimination, the behavior of CAL-A and CAL-B depends on the substrate structure. Using literature data in addition to the present results it is clear that in CAL-B catalysis the more reactive enantiomer in the O-acylation of secondary alcohols has always similar predictable stereostructure around the stereogenic center. With CAL-A heteroaryl analogs seems to be favored and enantiopreference is changed by changing the size and substitution of an aromatic ring.

4. Experimental

4.1. Analytical methods

^{1}H and ^{13}C NMR spectra were recorded on Bruker spectrometers operating at 500 or 300 MHz with tetramethylsilane (TMS) as an internal standard. Analytical data for compounds **2** and **3a**,**b**,**e** correspond to the literature data.[14,15] HRMS were measured in ESI$^+$ mode with a Bruker Avance microOTOF-Q quadrupole-TOF spectrometer. The optical rotations were measured with a PerkinElmer 341 or a Bellingham-Stanley ADP 220 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm g^{-1}. 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 Å (63–200 μm). The determination of the E-value was based on the equation $E = \ln[(1 - c)(1 - ee_S)]/\ln[(1 - c)(1 + ee_S)]$.[53]

The enantiomeric separations of **2c**,**e** and **3c**,**e** were performed by GC (HP1090 gas chromatograph) equipped with Cyclosil-B (30 × 0.32 mm × 0.25 μm) column and those of **2a**,**d** and **3a**,**d** Chrompack CP-Chirasil-Dex CB column (25 × 0.25 mm). The following oven temperatures were used: **2a** and **3a** 125 °C, **2b**,**c** and **3b**,**c** 140 °C, **2d** and **3d** 110 °C, and **2e** and **3e** 125 °C. The retention times for the enantiomers are shown in Table 6.

4.2. Reagents and solvents

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. Lipases from *Aspergillus niger*, *Pseudomonas fluorescens*, *Burkholderia cepacia*, *Rhizopus oryzae*, *Rhizopus arrhizus*, *Penicillium camemberti*, and *Mucor javanicus* were products of Amano. Immobilized *Rhizopus oryzae* lipase (IMMARO-T2-150) was from ChiralVision. Lipases from *Candida rugosa* (CRL), *Candida lipolytica*, *Mucor miehei*, and porcine pancreas (PPL) were purchased from Fluka. Lipase A from *C. antarctica* (CAL-A) was the product from Roche (Chirazyme L-5, lyo.), and it (5 g) was adsorbed on Celite (17 g) in the presence of sucrose (3 g).[32] CAL-A CLEA was from Fluka. Lipase B from *C. antarctica* (CAL-B, Novozym 435) and lipase from *Thermomyces lanuginosus* (Lipozyme TL IM) were purchased from Novozymes, Denmark. Lipase from *Candida rugosa* (CRL) immobilized on sol-gel was a gift from Professor László Poppe, Budapest University of Technology and Economics, Hungary.

4.3. Synthesis of racemic compounds

4.3.1. Synthesis of racemic β-hydroxy esters rac-2a–e

A solution of ethyl bromoacetate (84 g, 56 mL, 0.50 mol) in tetrahydrofuran (100 mL) was added in small portions under slight warming into a mixture of zinc powder (40 g, 0.61 mol) and one of the aldehydes **1a**–**e** (0.60 mol) in tetrahydrofuran (10 mL) which was previously refluxed for 30 min. After completion of the reaction (approx. 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) under vigorous stirring. After separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was washed with water (50 mL), then dried over anhydrous magnesium sulfate, and evaporated in vacuo. The crude product was purified by preparative vacuum-chromatography using hexane/EtOAc (3:1, v/v) as an eluent. Spectroscopic data are in accordance with the literature data.[38,39]

4.3.1.1. Ethyl 3-hydroxy-3-(thiophen-2-yl)propanoate *rac*-2a. Yield: 92%; ^{1}H NMR: (300 MHz, $CDCl_3$): δ = 1.27 (t, J = 7.2 Hz, 3H); 2.86 (ddd, J = 5.4, J = 8.7 Hz, J = 15.6 Hz, 2H); 4.19 (q, J = 7.2 Hz, 2H); 5.37 (dd, J = 5.5 Hz, J = 8.5 Hz, 1H); 6.95–7.01 (m, 2H); 7.27–7.31 (m, 1H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 14.1; 43.2; 61.0; 66.5; 123.6; 124.8; 126.7; 146.3; 171.9. HRMS: calcd for $C_9H_{12}NaO_3S$ [M+Na]$^+$ 233.0399, found 233.0394.

4.3.1.2. Ethyl 3-hydroxy-3-(furan-2-yl)propanoate *rac*-2b. Yield: 28%; ^{1}H NMR: (300 MHz, $CDCl_3$): δ = 1.25 (t, J = 7.2 Hz, 3H); 2.84 (ddd, J = 4.5 Hz, J = 8.4 Hz, J = 16.2 Hz, 2H); 4.17 (q, J = 7.2 Hz, 2H); 5.12 (dd, J = 4.5 Hz, J = 8.4 Hz, 1H); 6.25–6.35 (m, 2H); 7.35–7.39 (m, 1H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 14.0; 40.0; 60.9; 64.0; 106.1; 110.2; 142.1; 154.9; 171.7. HRMS: calcd for $C_9H_{12}NaO_4$ [M+Na]$^+$ 207.0627, found 207.0617.

4.3.1.3. Ethyl 3-hydroxy-3-(thiophen-3-yl)propanoate *rac*-2c. Yield: 27%; ^{1}H NMR: (500 MHz, $CDCl_3$): δ = 1.24 (t, J = 7.5 Hz, 3H); 2.75 (ddd, J = 5.5 Hz, J = 8.5 Hz, J = 15.5 Hz, 2H); 4.15 (q, J = 7.5 Hz, 2H); 5.18 (dd, J = 5.5 Hz, J = 8.5 Hz, 1H); 7.08–7.07 (m, 1H); 7.20–7.22 (m, 1H); 7.27–7.30 (m, 1H); ^{13}C NMR: (126 MHz, $CDCl_3$): δ = 14.1; 42.6; 60.8; 66.7; 120.9; 125.5; 126.2; 144.0; 172.2. HRMS: calcd for $C_9H_{12}NaO_3S$ [M+Na]$^+$ 233.0399, found 233.0392.

4.3.1.4. Ethyl 3-hydroxy-3-(furan-3-yl)propanoate *rac*-2d. Yield: 17%; ^{1}H NMR: (500 MHz, $CDCl_3$): 1.26 (t, J = 7.5 Hz, 3H); 2.73 (ddd, J = 5.5 Hz, J = 8.5 Hz, J = 15.5 Hz, 2H); 4.17 (q, J = 7.5 Hz,

Table 6
Retention times [(*S*)/(*R*)] of the enantiomers of **2** and **3** (R^1 = Me and R^1 = Pr)

Compound	t_r (min)	Compound (R^1 = Pr)	t_r (min)	Compound (R^1 = Me)	t_r (min)
2a	78.8/80.8	**3a**	127.4/124.4	**3a**	50.9/48.8
2b	6.3/14.3	**3b**	4.2/4.5	**3b**	5.2/6.2
2c	32.9/34.2	**3c**	69.3/65.5	**3c**	31.3/29.9
2d	60.3/57.2	**3d**	118.8/114.0	**3d**	47.4/45.8
2e	50.1/52.8	**3e**	72.3/68.7	**3e**	46.2/44.9

2H); 5.09 (dd, J = 5.5 Hz, J = 8.5 Hz, 1H); 6.38–6.43 (m, 1H); 7.36–7.42 (m, 2H); ^{13}C NMR: (126 MHz, $CDCl_3$): δ = 14.1; 42.1; 60.9; 63.4; 108.4; 127.4; 139.0; 143.4; 172.2. HRMS: calcd for $C_9H_{12}NaO_4$ $[M+Na]^+$ 207.0627, found 207.0620.

4.3.1.5. Ethyl 3-hydroxy-3-phenylpropanoate *rac*-2e. Yield: 72%; ^{1}H NMR: (300 MHz, $CDCl_3$): 1.26 (t, J = 7.2 Hz, 3H); 2.72 (ddd, J = 4.2 Hz, J = 8.7 Hz, J = 16.2 Hz, 2H); 4.18 (q, J = 7.2 Hz, 2H); 5.13 (dd, J = 4.2 Hz, J = 8.7 Hz, 1H); 7.25–7.41 (m, 5H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 14.1; 43.4; 60.8; 70.3; 125.7; 127.7; 128.5; 142.6; 172.3. HRMS: calcd for $C_{11}H_{14}NaO_3$ $[M+Na]^+$ 217.0835, found 217.0841.

4.3.2. Synthesis of racemic diesters *rac*-3a–e

Method A. At first, Et_3N (6.17 mmol, 624 mg, 860 μL), acetyl chloride (6.17 mmol, 485 mg, 440 μL), and DMAP (0.16 mmol, 20 mg) were added into a solution of racemic **2a–e** (5.61 mmol) in dry CH_2Cl_2 (15 mL). The mixture was stirred at room temperature for 2–3 h and then quenched with water (15 mL). The isolated organic layer was dried over anhydrous sodium sulfate and the solvent was distilled off by rotatory evaporation. The crude product was purified by vacuum-chromatography on silica gel using hexane–EtOAc (3:1, v/v) as eluent to give the product as a semisolid with 60–70% yield.

Method B. A mixture of racemic **2a–e** (50 mmol), acid anhydride (60 mmol, 9.5 g, 9.55 ml), and anhydrous cobalt (II) chloride (32.4 g, 250 mmol) in acetonitrile (100 mL) was refluxed overnight. After cooling at room temperature, the cobalt (II) chloride was filtered off. The filtrate was evaporated in vacuo and the crude product was purified by preparative vacuum-chromatography on silica gel using hexane/EtOAc (3:1, v/v) as an eluent to give the product as a semisolid with 80–90% yield.

4.3.2.1. Ethyl 3-acetoxy-3-(thiophen-2-yl)propanoate *rac*-3a (R^1 = Me). Yield: 91%; ^{1}H NMR: (300 MHz, $CDCl_3$): δ = 1.23 (t, J = 7.2 Hz, 3H); 2.03 (s, 3H); 2.96 (ddd, J = 5.4 Hz, J = 8.7 Hz, J = 15.6 Hz, 2H); 4.15 (q, J = 6.9 Hz, 2H); 6.47 (dd, J = 5.4 Hz, J = 8.7 Hz, 1H); 6.94–6.97 (m, 1H); 7.09 (d, J = 3,3 Hz, 1H); 7.27 (d, J = 5,1 Hz, 1H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 14.1; 21.0; 41.4; 60.9; 67.3; 125.8; 126.2; 126.7; 141.8; 169.3; 169.7. HRMS: calcd for $C_{11}H_{14}NaO_5S$ $[M+Na]^+$ 265.0505, found 265.0501.

4.3.2.2. 3-Ethoxy-3-oxo-1-(thiophen-2-yl)propyl butyrate *rac*-3a (R^1 = Pr). Yield: 87%; semisolid; ^{1}H NMR: (300 MHz, $CDCl_3$): δ = 0.91 (t, J = 7.5 Hz, 3H); 1.24 (t, J = 7.0 Hz, 3H); 1.63 (overlapped tq, $J_1 \sim J_2 \sim$ 7.5 Hz, 2H); 2.28 (t, J = 7.5 Hz, 2H); 2.97 (ddd, J = 5.4 Hz, J = 9 Hz, J = 16.2 Hz, 2H); 4.14 (q, J = 7.2 Hz, 2H); 6.49 (dd, J = 5.4 Hz, J = 9 Hz, 1H); 6.94–6.97 (m, 1H); 7.08–7.11 (m, 1H); 7.26–7.29 (m, 1H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 13.5; 14.1; 18.3; 36.1; 41.4; 60.8; 67.1; 125.6; 126.0; 126.6; 141.9; 169.3; 172.3. HRMS: calcd for $C_{13}H_{18}NaO_5S$ $[M+Na]^+$ 293.0818, found 293.0818.

4.3.2.3. Ethyl 3-acetoxy-3-(furan-2-yl)propanoate *rac*-3b (R^1 = Me). Yield: 81%; ^{1}H NMR: (300 MHz, $CDCl_3$): δ = 1.24 (t, J = 7.2 Hz, 3H); 2.05 (s, 3H); 3.00 (ddd, J = 6 Hz, J = 8.1 Hz, J = 15.6 Hz, 2H); 4.15 (q, J = 7.2 Hz, 2H); 6.25–6.38 (m, 3H); 7.39–7.42 (m, 1H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 14.1; 20.9; 37.6; 60.8; 64.7; 109.0; 110.3; 142.7; 151.1; 169.4; 169.8. HRMS: calcd for $C_{11}H_{14}NaO_5$ $[M+Na]^+$ 249.0733, found 249.0730.

4.3.2.4. 3-Ethoxy-1-(furan-2-yl)-3-oxopropyl butyrate *rac*-3b (R^1 = Pr). Yield: 85%; ^{1}H NMR: (300 MHz, $CDCl_3$): δ = 0.91 (t, J = 7.5 Hz, 3H); 1.23 (t, J = 7.2 Hz, 3H); 1.63 (overlapped tq, $J_1 \sim J_2 \sim$ 7.5 Hz, 2H); 2.27 (t, J = 7 Hz, 2H); 2.98 (ddd, J = 6 Hz, J = 9 Hz, J = 15.9 Hz, 2H); 4.14 (q, J = 7.2 Hz, 2H); 6.26–6.38 (m, 3H); 7.38–7.42 (m, 1H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 13.5; 14.1; 18.3; 36.0; 37.7; 60.8; 64.6; 108.8; 110.3; 142.7; 151.3; 169.4; 172.4. HRMS: calcd for $C_{13}H_{18}NaO_5$ $[M+Na]^+$ 277.1046, found 277.1095.

4.3.2.5. Ethyl 3-acetoxy-3-(thiophen-3-yl)propanoate *rac*-3c (R^1 = Me). Yield: 90%; ^{1}H NMR: (500 MHz, $CDCl_3$): δ = 1.22 (t, J = 7.5 Hz, 3H); 2.04 (s, 3H); 2.88 (ddd, J = 5.5 Hz, J = 8.5 Hz, J = 15.5 Hz, 2H); 4.13 (q, J = 7.5 Hz, 2H); 6.30 (dd, J = 5.5 Hz, J = 8.5 Hz, 1H); 7.08–7.09 (m, 1H); 7.28–7.30 (m, 2H); ^{13}C NMR: (126 MHz, $CDCl_3$): δ = 14.1; 21.0; 40.7; 60.7; 67.8; 122.9; 125.9; 126.2; 140.0; 169.6; 169.8. HRMS: calcd for $C_{11}H_{14}NaO_5S$ $[M+Na]^+$ 265.0505, found 265.0511.

4.3.2.6. 3-Ethoxy-3-oxo-1-(thiophen-3-yl)propyl butyrate *rac*-3c (R^1 = Pr). Yield: 86%; ^{1}H NMR: (500 MHz, $CDCl_3$): δ = 0.90 (t, J = 7.5 Hz, 3H); 1.22 (t, J = 7.0 Hz, 3H); 1.63 (overlapped tq, $J_1 \sim J_2 \sim$ 7.5 Hz, 2H); 2.27 (t, J = 7.5 Hz, 2H); 2.88 (ddd, J = 5.5 Hz, J = 8.5 Hz, J = 15.5 Hz, 2H); 4.12 (q, J = 7.5 Hz, 2H); 6.31 (dd, J = 5.5 Hz, J = 8.5 Hz, 1H); 7.07–7.09 (m, 1H); 7.27–7.31 (m, 2H); ^{13}C NMR: (126 MHz, $CDCl_3$): δ = 13.5; 14.1; 18.4; 36.2; 40.8; 60.7; 67.6; 122.8; 125.9; 126.2; 140.2; 169.9; 172.4. HRMS: calcd for $C_{13}H_{18}NaO_5S$ $[M+Na]^+$ 293.0818, found 293.0818.

4.3.2.7. Ethyl 3-acetoxy-3-(furan-3-yl)propanoate *rac*-3d (R^1 = Me). Yield: 81%; ^{1}H NMR: (500 MHz, $CDCl_3$): 1.23 (t, J = 7.5 Hz, 3H); 2.03 (s, 3H); 2.85 (ddd, J = 5.5 Hz, J = 8.5 Hz, J = 15.5 Hz, 2H); 4.14 (q, J = 7.5 Hz, 2H); 6.20 (dd, J = 5.5 Hz, J = 8.5 Hz, 1H); 6.41–6.42 (m, 1H); 7.36–7.38 (m, 1H); 7.46–7.48 (m, 2H); ^{13}C NMR: (126 MHz, $CDCl_3$): δ = 14.1; 21.0; 40.0; 60.7; 64.7; 108.7; 123.8; 140.5; 143.3; 169.5; 169.8. HRMS: calcd for $C_{11}H_{14}NaO_5$ $[M+Na]^+$ 249.0733, found 249.0726.

4.3.2.8. 3-ethoxy-1-(furan-3-yl)-3-oxopropyl butyrate *rac*-3d (R^1 = Pr). Yield: 85%; ^{1}H NMR: (500 MHz, $CDCl_3$): δ = 0.91 (t, J = 7.5 Hz, 3H); 1.23 (t, J = 7.0 Hz, 3H); 1.62 (overlapped tq, $J_1 \sim J_2 \sim$ 7.5 Hz, 2H); 2.26 (t, J = 7.5 Hz, 2H); 2.85 (ddd, J = 5.5 Hz, J = 8.5 Hz, J = 15.5 Hz, 2H); 4.13 (q, J = 7.5 Hz, 2H); 6.21 (dd, J = 5.5 Hz, J = 8.5 Hz, 1H); 6.40–6.42 (m, 1H); 7.36–7.38 (m, 1H); 7.45–7.47 (m, 2H); ^{13}C NMR: (126 MHz, $CDCl_3$): δ = 13.5; 14.1; 18.4; 36.2; 40.1; 60.8; 64.5; 108.8; 124.0; 140.5; 143.3; 169.6; 172.5. HRMS: calcd for $C_{13}H_{18}NaO_5$ $[M+Na]^+$ 277.1046, found 277.1058.

4.3.2.9. Ethyl 3-acetoxy-3-phenylpropanoate *rac*-3e (R^1 = Me). Yield: 88%; ^{1}H NMR: (300 MHz, $CDCl_3$): 1.23 (t, J = 7.2 Hz, 3H); 2.03 (s, 3H); 2.88 (ddd, J = 5.0 Hz, J = 9 Hz, J = 15.6 Hz, 2H); 4.14 (q, J = 7.2 Hz, 2H); 6.21 (dd, J = 5.1 Hz, J = 9 Hz, 1H); 7.29–7.41 (m, 5H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ = 14.1; 21.0; 41.6; 60.7; 71.8; 126.4; 128.3; 128.5; 139.4; 169.6; 169.8. HRMS: calcd for $C_{13}H_{16}NaO_4$ $[M+Na]^+$ 259.0941, found 259.1030.

4.4. Enzymatic acylations

4.4.1. Analytical scale kinetic resolution of *rac*-2a and *rac*-2e

One of the lipases (50 mg/mL) and an acyl donor (vinyl acetate or butanoate, 5 equiv) were added into a solution of substrate (*rac*-**2a** or *rac*-**2e**, 0.25 M) in an organic solvent (1 mL) or in neat vinyl acetate. The reaction mixture was shaken (300 rpm) at room temperature (23–24 °C). For HPLC or GC analysis, the samples taken from the reaction mixture (10 μL) were diluted to 500 μL with 2-propanol or TBME and filtered before injection

4.4.2. Preparative scale kinetic resolution of *rac*-2a–d

4.4.2.1. General procedure with CAL-A. At first, CAL-A on Celite (250 mg) was added into a solution of one of the substrates

rac-**2a**–**d** (1.25 mmol) in vinyl acetate (5 mL). The reaction mixtures were shaken (300 rpm) for 1 h at room temperature to reach the conversion and enantiomeric composition of the products shown in Table 4. The enzyme was removed by filtration and the excess vinyl acetate by distillation. The crude products were purified by column chromatography on silica gel using hexane/EtOAc (3:1, v/v) as an eluent to give the enantiomerically enriched products as semisolids with the isolated yields shown in Table 5.

NMR and MS spectra of the resolution products were indistinguishable from those of the racemates.

4.4.2.2. General procedure with CAL-B. Vinyl butanoate (5 equiv) and CAL-B (250 mg) were added into a solution of one of the substrates *rac*-**2a**–**d** (approx. 250 mg, 1.25 mmol) in MTBE (5 mL). The reaction mixture was shaken (300 rpm) for 60 h at room temperature to reach the conversions and enantiomeric compositions shown in Table 4. Work-up as above gave the products shown in Table 5.

Acknowledgments

J.B. thanks The Sectoral operational program human resources development, Contract POSDRU 6/1.5/S/3—'Doctoral studies: through science toward society' and the grant from the Center of International Mobility (CIMO) in Finland for financial support.

References

1. Bush, K.; Macielag, M.; Weidner-Wells, M. *Curr. Opin. Microbiol.* **2004**, *7*, 466–476.
2. Kobayashi, R.; Konomi, M.; Hasegawa, K.; Morozumi, M.; Sunakawa, K.; Ubukata, K. *Antimicrob. Agents Chemother.* **2005**, *49*, 889–894.
3. Dalhoff, A.; Janjic, N.; Echols, R. *Biochem. Pharmacol.* **2006**, *71*, 1085–1095.
4. Berks, A. H. *Tetrahedron* **1996**, *52*, 331–375.
5. Turcu, M. C.; Kiljunen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2007**, *18*, 1682–1687.
6. Hung, H.; Pan, X.; Tan, N.; Zeng, G.; Ji, C. *Eur. J. Med. Chem.* **2007**, *42*, 365–372.
7. Turcu, M. C.; Rantapaju, M.; Kanerva, L. T. *Eur. J. Org. Chem.* **2009**, 5594–5600.
8. Zhang, S.; Norrlöw, O.; Wawrzynczyk, J.; Dey, E. S. *Appl. Environ. Microbiol.* **2004**, *70*, 6776–6782.
9. Collins, A. N.; Sheldrake, G. N.; Crosby, J. *Chirality in Industry II*; John Wiley & Sons, 1997.
10. Ratovelomanana-Vidal, V.; Girard, C.; Touati, R.; Tranchier, J. P.; Ben Hassine, B.; Genêt, J. P. *Adv. Synth. Catal.* **2003**, *345*, 261–274.
11. Turner, N. J. *Curr. Opin. Chem. Biol.* **2010**, *14*, 115–121.
12. Lee, J. H.; Han, K.; Kim, M.-J.; Park, J. *Eur. J. Org. Chem.* **2010**, 999–1015.
13. Kanerva, L. T.; Liljeblad, A. Transesterification—Biological, Wiley Online Library, Encyclopedia of Catalysis 2010, doi:10.1002/0471227617.eoc197.
14. Brem, J.; Paizs, C.; Toşa, M. I.; Vass, E.; Irimie, F. D. *Tetrahedron: Asymmetry* **2009**, *20*, 489–496.
15. Brem, J.; Toşa, M. I.; Paizs, C.; Vass, E.; Irimie, F. D. *Tetrahedron: Asymmetry* **2010**, *21*, 365–373.
16. Huerta, F. F.; Bäckvall, J.-E. *Org. Lett.* **2001**, *3*, 1209–1212.
17. Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831–5854.
18. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665.
19. Ericsson, D. J.; Kasrayan, A.; Johansson, P.; Bergfors, T.; Sandström, A. G.; Bäckvall, J.-E.; Mowbray, S. L. *J. Mol. Biol.* **2008**, *376*, 109–119.
20. Engström, K.; Nyhlén, J.; Sandström, A. G.; Bäckvall, J.-E. *J. Am. Chem. Soc.* **2010**, *132*, 7038–7042.
21. Naik, S.; Basu, A.; Saikia, R.; Madan, B.; Paul, P.; Chaterjee, R.; Brask, J.; Svendsen, A. *J. Mol. Catal. B: Enzym.* **2010**, *65*, 18–23.
22. Henke, E.; Pleiss, J.; Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 3211–3213.
23. Liljeblad, A.; Kallio, P.; Vainio, M.; Niemi, J.; Kanerva, L. T. *Org. Biomol. Chem.* **2010**, *8*, 886–895.
24. Domínguez de María, P.; Carboni-Oerlemans, C.; Tuin, B.; Bargeman, G.; Van der Meer, A.; Van Gemert, R. *J. Mol. Catal. B: Enzym.* **2005**, *37*, 36–46.
25. Nielsen, T. B.; Ishii, M.; Kirk, O. Lipases A and B From the Yeast *Candida Antarctica*. In *Biotechnological Application of Cold-Adapted Organisms*; Margesin, R., Schinner, S., Eds.; Springer: Berlin-Heidelberg, Texas, 1999; pp 49–61.
26. Kirk, O.; Christensen, M. W. *Org. Process. Res. Dev.* **2002**, *6*, 446–451.
27. Balaji, B. S.; Chanda, B. M. *Tetrahedron* **1998**, *54*, 13237–13252.
28. Gedey, S.; Liljeblad, A.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 2573–2581.
29. Gedey, S.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Can. J. Chem.* **2002**, *80*, 565–570.
30. Li, X.-G.; Kanerva, L. T. *Org. Lett.* **2006**, *8*, 5593–5596.
31. Liljeblad, A.; Kavenius, H.-M.; Tähtinen, P.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2007**, *18*, 181–191.
32. Kanerva, L. T.; Sundholm, O. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407–2410.
33. Solymár, M.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2002**, *13*, 1923–1928.
34. Solymár, M.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2002**, *13*, 2383–2388.
35. Liljeblad, A.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 4405–4415.
36. Mavrynsky, D.; Päiviö, M.; Lundell, K.; Sillanpää, R.; Kanerva, L. T.; Leino, R. *Eur. J. Org. Chem.* **2009**, 1317–1320.
37. Gedey, S.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2001**, *12*, 105–110.
38. Fernández-Ibáñez, Á. M.; Maciá, B.; Minnaard, A. J.; Feringa, B. L. *Angew. Chem., Int. Ed.* **2008**, *47*, 1317–1319.
39. Xu, C.; Yuan, C. *Tetrahedron* **2005**, *61*, 2169–2186.
40. Fishman, A.; Eroshov, M.; Dee-Noor, S. S.; van Mil, J.; Cogan, U.; Effenberger, R. *Biotechnol. Bioeng.* **2001**, *74*, 256–263.
41. Pihko, A. J.; Lundell, K.; Kanerva, L.; Koskinen, A. M. P. *Tetrahedron: Asymmetry* **2004**, *15*, 1637–1643.
42. Paizs, C.; Tähtinen, P.; Toşa, M.; Majdik, C.; Irimie, F.-D.; Kanerva, L. T. *Tetrahedron* **2004**, *60*, 10533–10540.
43. Paizs, C.; Tähtinen, P.; Lundell, K.; Poppe, L.; Irimie, F.-D.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2003**, *14*, 1895–1904.
44. Paizs, C.; Toşa, M.; Majdik, C.; Tähtinen, P.; Irimie, F.-D.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2003**, *14*, 619–627.
45. Brem, J.; Toşa, M. I.; Paizs, C.; Munceanu, A.; Matkovíc-Čalogović, D.; Irimie, F.-D. *Tetrahedron: Asymmetry* **2010**, *21*, 1993–1998.
46. Turcu, M. C.; Perkiö, P.; Kanerva, L. T. *Arkivoc* **2009**, *iii*, 251–263.
47. Turcu, M. C.; Perkiö, P.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2010**, *21*, 739–745.
48. Tjosås, F.; Anthonsen, T.; Jacobsen, E. E. *Arkivoc* **2008**, *vi*, 81–90.
49. Uhm, K.-N.; Lee, S.-J.; Kim, H.-K.; Kang, H.-Y.; Lee, Y. *J. Mol. Catal. B: Enzym.* **2007**, *45*, 34–38.
50. Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jones, T. A. *Structure* **1994**, *2*, 293–308.
51. Rotticci, D.; Hæffner, F.; Orrenius, C.; Norin, T.; Hult, K. *J. Mol. Catal. B: Enzym.* **1998**, *5*, 267–272.
52. Uppenberg, J.; Öhrner, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T. A. *Biochemistry* **1995**, *34*, 16838–16851.
53. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.