Tetrahedron: *Asymmetry* 19 (2008) 1844–1852

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

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

Lipase-catalyzed kinetic resolution of racemic 1-heteroarylethanols—experimental and QM/MM study

Monica Toşa [a], Sarolta Pilbák [b], Paula Moldovan [a], Csaba Paizs [a], Gábor Szatzker [b], György Szakács [c], Lajos Novák [b], Florin-Dan Irimie [a,*], László Poppe [b,*]

[a] *Department of Biochemistry and Biochemical Engineering, Babeş-Bolyai University of Cluj-Napoca, Ro-400028 Cluj-Napoca, Arany János 11, Romania*
[b] *Department of Organic Chemistry and Technology, and Research Group for Alkaloid Chemistry of the Hungarian Academy of Sciences, Budapest University of Technology and Economics, H-1111 Budapest, Műegyetem rkp. 3, Hungary*
[c] *Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, H-1111 Budapest, Műegyetem rkp. 3, Hungary*

ARTICLE INFO

Article history:
Received 13 June 2008
Accepted 2 July 2008
Available online 30 July 2008

ABSTRACT

The kinetic resolution of racemic 1-(benzothiazol-2-yl)ethanol *rac*-**2a**, 1-(benzo[*b*]thiophen-2-yl)ethanol *rac*-**2b**, 1-(benzo[*b*]furan-3-yl)ethanol *rac*-**2c** and 1-(benzo[*b*]thiophen-3-yl)ethanol *rac*-**2d** was studied by enantiomer selective acylation catalyzed by a selection of commercially available and in house produced lipases. Alcoholysis of the corresponding racemic acetates *rac*-**3a**–**d** catalyzed by *Candida antarctica* lipase B (CaLB) was also investigated. Two racemic 1-heteroarylethanols *rac*-**2a**,**b** were prepared by addition of the corresponding lithiated heteroarylic compounds **1a**,**b** to acetaldehyde, whereas two others, *rac*-**2c**,**d** were synthesized by the addition of MeMgI onto the corresponding heteroaryl-carbaldehydes **1c**,**d**. The high enantiomer selectivities of CaLB in the acylation of racemic 1-heteroarylethanols *rac*-**2a**–**d** allowed the determination of the enantiomeric preference of these enzymatic acetylation reactions by QM/MM [pm3/uff or hf(3-21+g**)/uff] calculations. For acetylation of each of the racemic alcohols *rac*-**2a**–**d**, four possible tetrahedral intermediate states were compared and analyzed.

1. Introduction

The need for the production of optically active compounds as single enantiomers is growing rapidly. Catalytic enantioselective synthesis is highly preferred in the preparation of enantiopure compounds, although classical resolution via diastereoisomeric salts is still widely employed. Due to their chiral nature, biocatalysts are predominantly suited for the production of enantiopure stereoisomers. Thus, biocatalysis has developed from a research technology to a widely used manufacturing method in the pharmaceutical and fine chemicals industry.[1–3] Biotransformations are already being used by industry to manufacture various products, such as drugs, agricultural chemicals, fine chemicals and plastics.[4,5] Hydrolases—especially lipases—proved to be versatile biocatalysts for synthetic biotransformations.[6] Lipases, in many cases, show remarkable chemoselectivity, regioselectivity and enantioselectivity towards a broad range of substrates. Lipases can retain their remarkably high stereoselectivity in organic media.[6] The stereoselectivity of lipases has been utilized to perform kinetic resolutions,[6,7] deracemizations and dynamic kinetic resolutions.[8] The low cost of many lipases make them very useful catalysts for the kinetic resolution of secondary alcohols including those containing heteroaromatic group. For example, the optically active 1-(benzofuran-2-yl)ethanol was prepared by enantiotopic selective bioreduction[9] or by lipase-catalyzed enantiomer selective reactions.[10]

As part of our interest in the development of stereoselective methods for the preparation of optically active heteroaromatic compounds, we wanted to follow the study of lipase-catalyzed kinetic resolutions of further racemic 1-heteroarylethanols **2a**–**d** by lipase-catalyzed enantiomer selective acylation (Fig. 1). Lipases usually retain their enantiomeric preference in hydrolysis or alcoholysis.[1–3,6] Consequently, such reactions should result in opposite enantiomeric forms of the enantiomerically enriched 1-heteroarylethanols **2a**–**d** and 1-heteroarylethyl acetates **3a**–**d** as the kinetic resolution by acetylation. Therefore, the study of hydrolysis or alcoholysis of the corresponding racemic 1-heteroarylethyl acetates **3a**–**d** has been also considered.

Stereoselective chemical methods for the 1-heteroarylethanols **2b**–**d** have already been studied. The enantioselective reduction of the corresponding ketone with (*S*)-2-methyl-CBS-oxazaborolidine gave the optically active 1-(benzo[*b*]thiophen-2-yl)ethanol (*R*)-**2b**.[11] The optically active 1-(benzo[*b*]thiophen-3-yl)ethanol (*R*)-**2d** was obtained by addition of $(CH_3)_2Zn$ to the corresponding aldehyde **1d** catalyzed by a commercially available ClCr(Salen).[12] The (*S*)-enantiomer of 1-(benzo[*b*]furan-3-yl)ethanol (*S*)-**2c** was obtained by the reduction of (*S*)-benzo[*b*]furan-3-yl)oxirane with $LiAlH_4$ or by the stereoselective reduction of 3-acetylbenzofuran

* Corresponding authors. Tel.: +36 1 4632229; fax: +36 1 4633297 (L.P.).
E-mail address: poppe@mail.bme.hu (L. Poppe).

0957-4166/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2008.07.004

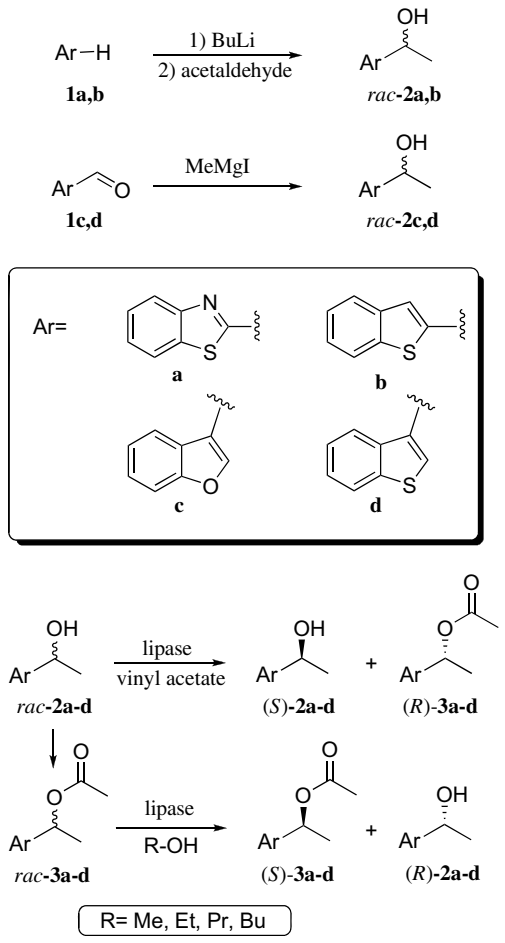

Figure 1. Preparation and enzymatic kinetic resolution of racemic 1-heteroarylethanols *rac*-**2a**–**d**.

with (−)-DIP-Cl.[13] Although enzymatic kinetic resolution of quite similar structural derivatives (benzimidazolyl ethanols) is already described,[14] no enzymatic methods for the enantioselective preparation of optically active 1-heteroarylethanols **2a**–**d** have been found.

Although the absolute configuration of the enantiomers of 1-(benzo[*b*]thiophen-2-yl)ethanol (*R*)-**2b**,[11] 1-(benzo[*b*]furan-3-yl)-ethanol (*S*)-**2c**[13] and (*R*)-**2c** and 1-(benzo[*b*]thiophen-3-yl)ethanol (*R*)-**2d**[12] was assigned, in many cases the absolute configurations were assumed only on the basis of the known stereopreference of the reagent or catalyst. As there is no data for the optically active forms of 1-(benzothiazol-2-yl)ethanol **2a**, the absolute configuration of its enantiomers cannot be assigned by specific rotation.

Traditionally, proteins and other large biological molecules have been out of the reach of electronic structure methods. The QM/MM computational technique allows the study of large molecules by defining two or three layers within the structure that are treated at different levels of accuracy. In a typical two-layer approach, the active site is treated using an electronic structure method, whilst the rest of the system is modelled with molecular mechanics. Calibration studies have demonstrated that the resulting predictions are essentially equivalent to those that would be produced by the high accuracy method. For example, in a study, the excited states of bacteriorhodopsin were calculated using QM/MM (ONIOM) methods.[15]

Well resolved X-ray crystal structures are available for several lipases, such as *Candida antarctica* lipase B (CaLB),[16] or lipase of *Burkholderia cepacia* (formerly *Pseudomonas cepacia*).[17] Moreover, the excellent reactivity and selectivity of CaLB with chiral alcohols were explained by several modelling[18,19] and molecular dynamics[20,21] studies. Very recently, QM/MM calculations within the lipase of *P. cepacia* revealed that such calculations performed on the tetrahedral intermediate (THI) states of the reaction can explain better the reactivity of the lipase towards the enantiomers of the racemic alcohol substrates than the X-ray structure co-crystallized with the (*S*)- or (*R*)-phosphonate inhibitors mimicking the ester forms of the acetylated products.[17] As systematic conformational analysis performed within the CaLB active site at MM level has already been applied to absolute configuration assignments,[22] we proposed to extend this method using the experimentally determined enantiomer selectivities of CaLB towards the enantiomers of 1-heteroarylethanols **2a**–**d** combined with QM/MM calculations within the CaLB structure for assignment of their absolute configuration.

2. Results and discussion

The preparation of racemic 1-heteroarylethanols *rac*-**2a**–**d** was straightforward (Fig. 1). The 1-(benzothiazol-2-yl)ethanol *rac*-**2a** and 1-(benzo[*b*]thiophen-2-yl)ethanol *rac*-**2b** were prepared by the addition of the corresponding lithiated heteroarylic compounds **1a**,**b** to acetaldehyde, whereas 1-(benzo[*b*]furan-3-yl)ethanol *rac*-**2c** and 1-(benzo[*b*]thiophen-3-yl)ethanol *rac*-**2d** were synthesized by the addition of MeMgI onto the corresponding heteroaryl-carbaldehydes **1c**,**d**.

With the racemic 1-heteroarylethanols *rac*-**2a**–**d** and with a wide selection of commercial and in house prepared lipases[23,24] in hand, the enantiomer selectivity of the enzyme-catalyzed acylations of the racemic alcohols *rac*-**2a**–**d** was screened first (Fig. 1, Tables 1–4). As a result of the screen, a number of biocatalysts exhibiting high enantiomer selectivity towards the racemic alcohols *rac*-**2a**–**d** were found (Tables 1–4). As expected, several commercial lipases such as lipase B from *C. antarctica* (CaLB, formulated as Novozym 435), and lipase AK exhibited high enantiomer selectivity (E >100) towards all 1-heteroarylethanols *rac*-**2a**–**d** (Tables 1–4). Within the series of the four racemic alcohols *rac*-**2a**–**d**, the lipases usually retained their degree of enantiomer selectivity. Lipase AY, lipases from *Candida rugosa/cyclindracae* and porcine pancreas (PPL) exhibited only moderate enantiomer selectivities towards the racemic 1-heteroarylethanols *rac*-**2a**–**d**

Table 1
Enantiomer selective enzymatic acylation of racemic 1-(benzo[*d*]thiazol-2-yl)ethanol (rac-**2a**)

Enzyme	Time [h]	*c* [%]	ee (*S*)-**2a** [%]	ee (*R*)-**3a** [%]	*E*[a]
Lipase AK	6	46.1	97.9	98.0	>200
Novozym 435	72	50.4	98.8	99.1	≫200
Lipase AY	24	38.5	59.5	84.2	21
CrL	8	27.9	27.1	87.0	19
Lipozyme TL IM	28	8.7	0.1	68.5	5
CcL	25	41.3	63.5	88.2	31
PPL	26	2.2	16.1	85.7	15
Lipase PS	24	42	78.7	98.9	>200
Lipase 3b	38	48.5	99.4	99.5	≫200
SSF-9	38	46.4	81.2	98.7	>200
SSF-23	38	28.8	32.3	99.2	>200
SSF-97	38	26.4	28.4	99.7	≫200
SSF-101	38	12.4	2.6	99.6	≫200
SSF-117	38	15.4	2.1	85.2	13
Lipase F	26	13.0	42.9[b]	89.4[b]	27

[a] The degree of enantiomer selectivity (E) was determined from $ee_{(S)\text{-}\mathbf{2a}}$ and $ee_{(R)\text{-}\mathbf{3a}}$.[25] Due to sensitivity to experimental errors, E values calculated in the 100–300 range are reported as >100, values in the 300–500 range are reported as >200 and values calculated above 500 are given as ≫200.

[b] Configurations of the major enantiomers are opposite to those given in the headline: (*R*)-**2a** and (*S*)-**3a**.

Table 2
Enantiomer selective enzymatic acylation of racemic 1-(benzo[*b*]thiophen-2-yl)ethanol *rac*-**2b**

Enzyme	Time [h]	*c* [%]	$ee_{(S)\text{-}\mathbf{2b}}$ [%]	$ee_{(R)\text{-}\mathbf{3b}}$ [%]	*E*[a]
Lipase AK	24	49.6	99.2	97.8	≫200
Novozym 435	24	49.8	99.4	99.3	≫200
Lipase AY	46	47.0	60.0	87.9	37
CrL	24	26.5	35.9	82.2	15
Lipozyme TL IM	46	12.5	27.1	99.4	>200
CcL	46	40.8	50.0	88.5	30
PPL	46	10.6	11.1	91.2	24
Lipase PS	46	50.5	99.8	98.9	≫200
Lipase 3b	15	49.8	98.8	99.4	≫200
SSF-9	22	48.2	99.7	99.2	≫200
SSF-23	22	48.8	90.6	98.8	≫200
SSF-97	22	46.2	69.5	97.0	>100
SSF-101	22	31.3	20.0	96.1	77
SSF-117	22	36.3	50.0	96.1	87
Lipase F[b]	24	9.1	37.9[b]	7.7[b]	2

[a] The degree of enantiomer selectivity (*E*) was determined from $ee_{(S)\text{-}\mathbf{2b}}$ and $ee_{(R)\text{-}\mathbf{3b}}$.[25] Due to sensitivity to experimental errors, *E* values calculated in the 100–300 range are reported as >100, values in the 300–500 range are reported as >200 and values calculated above 500 are given as ≫200.
[b] Configurations of the major enantiomers are opposite to those given in the headline: (*R*)-**2b** and (*S*)-**3b**.

Table 3
Enantiomer selective enzymatic acylation of racemic 1-(benzo[*b*]furan-3-yl)ethanol *rac*-**2c**

Enzyme	Time [h]	*c* [%]	$ee_{(S)\text{-}\mathbf{2c}}$ [%]	$ee_{(R)\text{-}\mathbf{3c}}$ [%]	*E*[a]
Lipase AK	16	47.0	97.7	97.5	>200
Novozym 435	16	48.1	96.2	97.6	>200
Lipase AY	40	18.2	22.0	92.6	32
Lipozyme TL IM	40	5.1	8.5	91.1	23
CcL	40	16.6	18.4	90.7	25
PPL	24	5.1	17.5	97.2	75
Lipase PS	48	7.1	11.2	91.5	25
Lipase 3b	21	49.8	99.7	97.7	≫200
SSF-9	21	26.0	42.7	96.0	74
SSF-23	21	15.5	30.6	93.7	41
SSF-97	21	7.5	14.5	85.2	14
SSF-101	21	6.3	10.3	97.1	76
SSF-117	21	19.4	20.8	87.4	18
Lipase F[b]	40	4.7	4.4[b]	74.6[b]	7

[a] The degree of enantiomer selectivity (*E*) was determined from $ee_{(S)\text{-}\mathbf{2c}}$ and $ee_{(R)\text{-}\mathbf{3c}}$.[25] Due to sensitivity to experimental errors, *E* values calculated in the 100–300 range are reported as >100, values in the 300–500 range are reported as >200 and values calculated above 500 are given as ≫200.
[b] Configurations of the major enantiomers are opposite to those given in the headline: (*R*)-**2c** and (*S*)-**3c**.

(*E* between 7 and 75, see Tables 1–4). The enhanced steric bulkiness of 1-(benzo[*b*]thiophen-3-yl)ethanol *rac*-**2d** compared to 1-(benzo[*b*]thiophen-2-yl)ethanol *rac*-**2b** only influenced the reactivity, but not the degree of the selectivity of the lipases (Tables 2 and 4). Only Lipozyme TL IM (immobilized form of lipase from *Thermomyces lanuginosus*) behaved differently. This lipase acted with high enantiomer selectivities on the lipophilic heterocycles (*E* >100 for *rac*-**2b** and *rac*-**2d**; Tables 2 and 4). On the other hand, these high selectivities dropped significantly in the kinetic resolutions of the more polar heterocycles (*E* = 5 and 23 for *rac*-**2a** and *rac*-**2c**, respectively; Tables 1 and 3).

In addition, a number of our novel preparations from thermophilic fungi (lipase 3b)[23] or solid state fermentation of mesophilic fungi (SSF-9, 23, 97, 117),[24] also exhibited excellent enantiomer selectivities (*E* >100, see Tables 1–4).

As expected, almost all lipases in this study followed the so-called Kazlauskas rule,[26] which predicted the enantiomeric preference for lipase-catalyzed kinetic resolutions. The rule is

Table 4
Enantiomer selective enzymatic acylation of racemic 1-(benzo[*b*]thiophen-3-yl)ethanol (*rac*-**2d**)

Enzyme	Time [h]	*c* [%]	$ee_{(S)\text{-}\mathbf{2d}}$ [%]	$ee_{(R)\text{-}\mathbf{3d}}$ [%]	*E*[a]
Lipase AK	24	47.5	99.0	99.4	≫200
Novozym 435	24	42.2	77.3	95.8	>100
Lipase AY	46	24.1	32.6	82.8	15
CrL	24	9.3	12.3	72.7	7
Lipozyme TL IM	46	8.1	10.8	98.4	>100
CcL	46	16.0	19.1	83.5	13
PPL	46	3.0	4.8	94.5	37
Lipase PS	46	20.6	27.7	99.3	>200
Lipase 3b	15	49.8	98.8	99.5	≫200
SSF-9	22	12.8	13.6	96.2	59
SSF-23	22	12.7	12.4	98.6	>100
SSF-97	22	7.1	6.7	99.1	>100
SSF-101	22	4.4	0.5	98.6	>100
SSF-117	22	8.8	9.7	96.0	54
Lipase F[b]	24	6.9	9.6[b]	84.6[b]	13

[a] The degree of enantiomer selectivity (*E*) was determined from $ee_{(S)\text{-}\mathbf{2d}}$ and $ee_{(R)\text{-}\mathbf{3d}}$.[25] Due to sensitivity to experimental errors, *E* values calculated in the 100–300 range are reported as >100, values in the 300–500 range are reported as >200 and values calculated above 500 are given as ≫200.
[b] Configurations of the major enantiomers are opposite to those given in the headline: (*R*)-**2d** and (*S*)-**3d**.

based on the assumption that the binding pockets of the lipases can discriminate between the enantiomers of a secondary alcohol. The sterically different arrangement of the large substituent and the medium substituent in the two enantiomers of secondary alcohols result as in different binding affinity and thus different rates of reaction. Usually, the (*R*)-enantiomer is the fast reacting one. Interestingly, lipase F exhibited an opposite enantiomer preference and resulted in the formation of the (*S*)-acetates (*S*)-**3a–d** (Tables 1–4).

Next, preparative scale kinetic resolutions of the four racemic 1-heteroarylethanols *rac*-**2a–d** were performed (200 mg, each) with the most selective immobilized commercial enzyme, Novozym 435, for full characterization of the optically active products (*R*)-**3a–d** and (*S*)-**2a–d** (Table 5). The high enantiomer selectivity of this lipase allowed the preparation of the alcohols (*S*)-**2a–d** and acetates (*R*)-**3a–d** in high enantiomeric excess (ee 96.5–99.3; Table 5).

The opposite enantiomeric forms of the optically active products [(*S*)-**3a–d** and (*R*)-**2a–d**] can be prepared by alcoholysis of the corresponding racemic acetates *rac*-**3a–d** with the same immobilized lipase (Novozym 435, Table 6). The degree of enantioselectivity in the alcoholysis reactions of the racemic acetates *rac*-**3a–d** was systematically tested in four alcohols (methanol, ethanol, propan-1-ol and butan-1-ol; Table 6). The highest selectivities were achieved in the alcoholyses of racemic 1-acetoxy-1-benzo[*b*]thiophen-2-yl-ethane *rac*-**3b** (*E* >100 in each alcohol). Considering

Table 5
Novozym 435 (CaLB) catalyzed kinetic resolution of the heteroaryl alcohols (*rac*-**2a–d**)

Compound	Yield [%]	ee [%]	*E*[a]	$[\alpha]_D^{24}$[b]
(*S*)-**2a**	44	97.2	>200	−18.4
(*R*)-**3a**	45	98.0		+75.5
(*S*)-**2b**	45	99.1	≫200	−21.2
(*R*)-**3b**	46	99.3		+142.2
(*S*)-**2c**	45	96.7	>100	−18.5
(*R*)-**3c**	44	97.0		+73.2
(*S*)-**2d**	46	96.5	>200	−27.1
(*R*)-**3d**	43	97.8		+78.2

[a] The degree of enantiomer selectivity (*E*) was determined from $ee_{(S)\text{-}\mathbf{2}}$ and $ee_{(R)\text{-}\mathbf{3}}$.[25] Due to sensitivity to experimental errors, *E* values calculated in the 100–300 range are reported as >100, values in the 300–500 range are reported as >200.
[b] (*c* 1.0, $CHCl_3$).

Table 6
Alcoholysis of racemic 1-heteroarylethyl acetates *rac*-**3a–d** with Novozym 435 (CaLB)

Compound	Alcohol	Time [h]	*c* [%]	$ee_{(S)\text{-}3}$ [%]	$ee_{(R)\text{-}2}$ [%]	*E*[a]
rac-**3a**	Methanol	28	19.5	7.3	7.3	1
	Ethanol	28	51.6	99.3	97.5	>100
	Propan-1-ol	28	51.7	87.1	85.7	37
	Butan-1-ol	28	42.2	70.5	94.6	77
rac-**3b**	Methanol	48	10.7	9.4	99.3	>100
	Ethanol	48	49.7	94.6	97.5	>100
	Propan-1-ol	48	42.6	71.3	97.4	>100
	Butan-1-ol	48	37.7	65.0	96.8	>100
rac-**3c**	Methanol	24	1.4	1.6	96.6	58
	Ethanol	24	18.1	21.8	95.6	55
	Propan-1-ol	24	7.1	7.1	97.7	92
	Butan-1-ol	24	7.1	7.1	97.8	98
rac-**3d**	Methanol	48	2.5	5.9	75.1	2
	Ethanol	48	22.1	21.8	99.3	>100
	Propan-1-ol	48	17.3	13.0	96.3	60
	Butan-1-ol	48	9.5	6.8	96.7	65

[a] The degree of enantiomer selectivity (*E*) was determined from $ee_{(R)\text{-}2}$ and $ee_{(S)\text{-}3}$.[25] Due to sensitivity to experimental errors, *E* values calculated in the 100–300 range are reported as >100, values in the 300–500 range are reported as >200.

the productivity and selectivity together, ethanol is the most appropriate solvent for the Novozym 435-catalyzed alcoholyses of the racemic acetates *rac*-**3a–d** (Table 6).

The absolute configuration of the enantiomers of 1-(benzofuran-3-yl)ethanol (*S*)-**2c**[13] and (*R*)-**2c** and 1-(benzothiophene-3-yl)ethanol (*R*)-**2d**[12] was known. Therefore, configurations of all the residual enantiomers in the CaLB-catalyzed reactions **3a–d** were assigned as (*S*) on the basis of the same sign of their specific rotation and also on the basis of the empirical Kazlauskas rule. Consequently, an (*R*)-configuration can be assigned to the products **3a–d** on a similar basis.

There is no absolute configuration assignment based on experimental data for the fast reacting enantiomers (*R*)-**3a** and (*R*)-**3b**. Therefore, it was thought worthy to determine the sense of the enantiomer selectivities of CaLB towards the enantiomers of 1-heteroarylethanols **2a–d** based on the structure of CaLB.[27] The interpretation of the enantiomer selectivity of CaLB with secondary alcohols was investigated by docking the two alcohol enantiomers into the active site.[28] Conformation searches of the covalent tetrahedral intermediates (THI) at molecular mechanics level were already applied for absolute configuration assignments.[22] Here, we extended this method by using more reliable QM/MM calculations to determine the absolute configurations of the fast reacting enantiomers of **3a–d**.

The stereoselectivity towards the enantiomers of racemic 1-heteroarylethanols *rac*-**2a–d** in CaLB was examined by a systematic conformation search (CS) at molecular mechanics level within the rigid enzymic environment followed by QM/MM relaxation. For the calculations, the THI's, formed by the reaction of the (*R*)- or (*S*)-alcohol with the acyl enzyme, were used to model the transition states.

It is known that the mechanism of serine proteases involves the Asp-His-Ser catalytic triad. It is also generally accepted that a tetrahedral intermediate (THI) is formed during the acylation and deacylation step. Within CaLB, the specific regions important for the prediction of enantioselectivity were determined with the aid of molecular dynamics-based methodology.[20] The 'double-proton-transfer' mechanism involves a proton transfer from Ser to His and a proton transfer from His to Asp at the tetrahedral intermediate. Full enzyme empirical valence bond studies concluded that Asp in the catalytic triad stays ionized during the reaction, the 'double-proton-transfer' mechanism.[29,30] The analysis indicates that the most important factor in the catalysis of serine proteases is the stabilization of the negatively charged tetrahedral intermediate (THI) by the main-chain N–H dipoles of the 'oxoanion-hole'. High level calculations on 30 atom models reveal that 'double-proton-transfer' mechanism is comparable in energy to the single proton transfer mechanism, but may not be relevant for real enzymes where other hydrogen bonds can stabilize the Asp and are therefore likely to disfavour the proton transfer from His to Asp.[31] High level calculations revealed that THI's can be good approximations of the transition states.[31] Molecular dynamics and mechanics were used to find low energy conformations and the calculated energy difference of the THI's formed by the interaction of the (*R*)- or (*S*)-substrate with the acyl enzyme.[28,32] A systematic conformational search was applied in case of acyloxypyrrolinones[33] where a very good agreement was found between the models and empirical results.

The reactions of the (*R*)- or (*S*)-enantiomers of a secondary alcohol with the acyl enzyme result in the formation of four possible THI's. To explain the stereoselectivity of the reactions of racemic 1-heteroarylethanols *rac*-**2a–d**, calculations on the four THI's of each alcohol were performed.

First, the THI's were constructed within the CaLB structure and the systematic conformation search was performed for each THI by molecular mechanics within a rigid enzyme environment. Next, the best five structures were calculated by a three-layer ONIOM model (pm3/uff/uff; over 1020/1019 atoms). To determine the stereoselectivity for the secondary alcohols **2a–d**, the lowest energy ONIOM models for each of the four possible THI's were compared (Table 7). The four possible THI structures for racemic 1-(benzothiazol-2-yl)ethanol *rac*-**2a** revealed that the bulky heteroaromatic part of the substrate always occupies the same binding pocket whilst the carbonyl oxygen of the acyl enzyme always points to the so-called oxoanion hole (Fig. 2).

The calculations determined that for each of the 1-heteroarylethanols *rac*-**2a–d**, the (*R*)-**2**-(*S*)-THI structure is the most favoured (Table 7). Accordingly, the fast reacting acetates from all racemic alcohols **2a–d** have an (*R*)-configuration.

Amongst the four calculated racemic alcohols, the THI's of the more bulky 1-(benzo[*b*]furan-3-yl)ethanol *rac*-**2c** and 1-(benzo[*b*]-thiophen-3-yl)ethanol *rac*-**2d** were structurally more diverse (Fig. 3). Here, the bicyclic heteroaromatic substituents were

Table 7
Comparison of the THI states of acylation of 1-heteroarylethanols *rac*-**2a–d** within the active site of CaLB determined by QM/MM (pm3/uff) method

Substrate	(*R*)-**2**-(*R*)-THI[a]		(*R*)-**2**-(*S*)-THI[a]		(*S*)-**2**-(*R*)-THI[a]		(*S*)-**2**-(*S*)-THI[a]	
	Δ*E* (kcal/mol)	$d_{N-OS}/_{N-OE}$ (Å)	Δ*E* (kcal/mol)	$d_{N-OS}/_{N-OE}$ (Å)	Δ*E* (kcal/mol)	$d_{N-OS}/_{N-OE}$ (Å)	Δ*E* (kcal/mol)	$d_{N-OS}/_{N-OE}$ (Å)
rac-**2a**	20.1	2.65/4.06	0.0	2.55/2.68	8.4	2.63/3.17	21.2	2.57/3.93
rac-**2b**	37.5	2.65/4.10	0.0	2.57/2.66	14.8	2.67/3.21	35.3	2.52/3.64
rac-**2c**	16.1	2.57/2.77	0.0	2.54/2.69	10.8	2.64/3.16	10.0	2.53/2.71
rac-**2d**	21.4	2.63/4.20	0.0	2.55/2.68	10.7	2.64/3.15	16.8	2.51/3.62

[a] The relative energies (Δ*E*) and N–O distances between His224-N_τ and THI oxygens (d) for the THI states are shown [d_{N-OS}: His224-N_τ–Ser105–O_γ; d_{N-OE}: His224-N_τ–ester–O (i.e., O of **2**)].

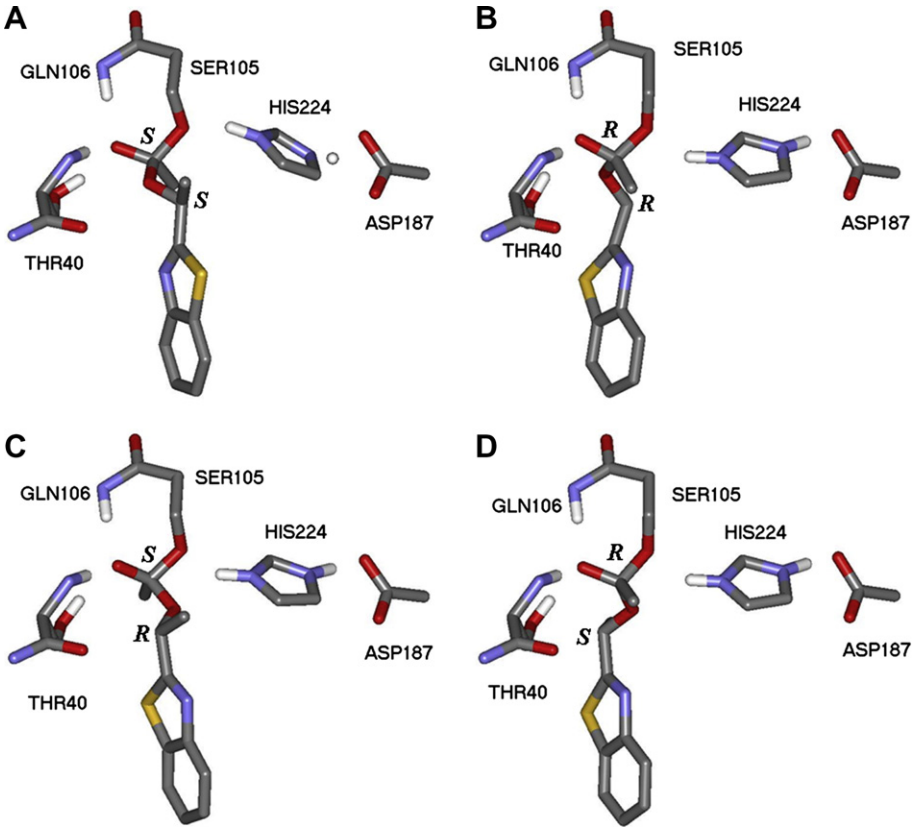

Figure 2. Tetrahedral intermediate (THI) states within the active site of CaLB determined by QM/MM (pm3/uff) calculations for acetylation of racemic 1-(benzothiazol-2-yl)ethanol *rac*-**2a** [A, (*S*)-**2a**-(*S*)-THI; B, (*R*)-**2a**-(*R*)-THI; C, (*R*)-**2a**-(*S*)-THI; D, (*S*)-**2a**-(*R*)-THI].

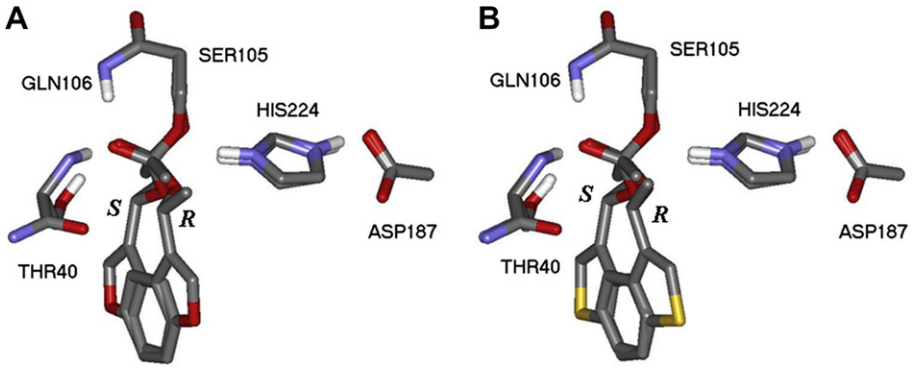

Figure 3. Comparison of the two lower energy THI states for acylation of racemic 1-(benzo[*b*]furan-3-yl)ethanol *rac*-**2c** and 1-(benzo[*b*]thiophen-3-yl)ethanol *rac*-**2d** within the active site of CaLB determined by QM/MM (pm3/uff) calculations [A, (*S*)-**2c**-(*R*)-THI and (*R*)-**2c**-(*S*)-THI; B, (*S*)-**2d**-(*R*)-THI and (*R*)-**2d**-(*S*)-THI].

arranged oppositely but larger six-membered benzo parts occupied almost the same position. In fact, the absolute configurations of these alcohols **2c**,**d**[12,13] unambiguously supported the validity of our calculation method.

At last, to determine more accurately the absolute configurations of the fast reacting enantiomers of racemic 1-(benzothiazol-2-yl)ethanol *rac*-**2a** and 1-(benzo[*b*]thiophen-2-yl)ethanol *rac*-**2b**, a higher level ONIOM method was applied (Table 8). The higher level method [hf(3-21+g**)/uff/uff, Table 8] as well as the lower level calculations (pm3/uff/uff, Table 7) indicated (*R*)-acetates (*R*)-**2a** and (*R*)-**2b** as the fast forming enantiomers. The heteroaromatic substituents of **2a** and **2b** enantiomers occupy the same position (Fig. 4). In addition to the energy differences (8.3 kcal/mol for **2a** and 6.8 kcal/mol for **2b**), the distance data between His224-N_τ and THI oxygens also indicate that the (*R*)-**2**-(*S*)-THI's are more favoured (Table 8). The significantly longer N_τ–O_{ester} distances (3.34 vs 2.59 Å, for **2a**; 3.23 vs 2.56 Å, for **2b**; Table 8) for (*S*)-**2**-(*R*)-THI

Table 8
Comparison of the THI states of acylation of 1-heteroarylethanols *rac*-**2a** and *rac*-**2b** within the active site of CaLB determined by QM/MM [hf(3-21+g**)/uff] method

Substrate	(*R*)-**2**-(*S*)-THI[a]		(*S*)-**2**-(*R*)-THI[a]	
	ΔE (kcal/mol)	$d_{N-OS}/_{N-OE}$ (Å)	ΔE (kcal/mol)	$d_{N-OS}/_{N-OE}$ (Å)
rac-**2a**	0.0	2.75/2.59	8.3	2.55/3.34
rac-**2b**	0.0	2.74/2.56	6.8	2.56/3.23

[a] Relative energies (ΔE) and N–O distances between His224-N_τ and THI oxygens (d) for the THI states are shown [d_{N-OS}: His224-N_τ–Ser105-O_3; d_{N-OE}: His224-N_τ–ester–O (i.e., O of **2**)].

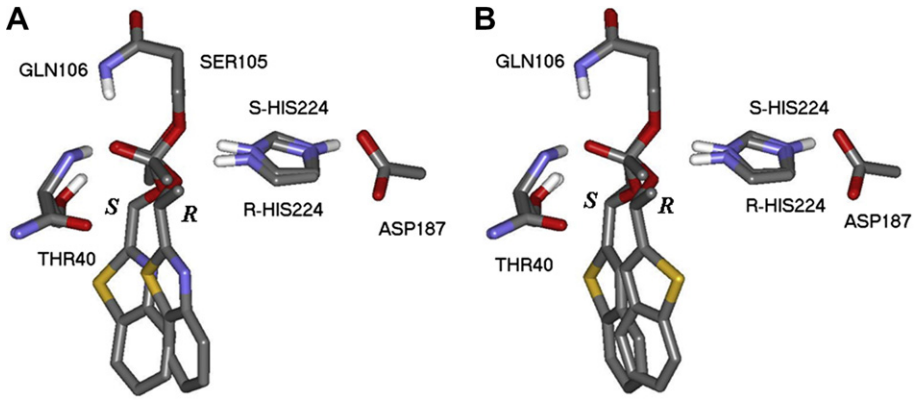

Figure 4. Comparison of the two lower energy THI states for acylation of 1-(benzothiazol-2-yl)ethanol *rac*-**2a** and 1-(benzo[*b*]thiophen-2-yl)ethanol *rac*-**2b** within the active site of CaLB determined by QM/MM [hf(3-21+g**)/uff] calculations [A, (*S*)-**2a**-(*R*)-THI and (*R*)-**2a**-(*S*)-THI; B, (*S*)-**2b**-(*R*)-THI and (*R*)-**2b**-(*S*)-THI].

than the (*R*)-**2**-(*S*)-THI states indicate that the energy differences between the real transition states may be even higher than the ones between the THI states.

3. Conclusion

An efficient synthesis of both enantiomeric forms of four racemic 1-heteroarylethanols *rac*-**2a–d**, and their acetates *rac*-**2a–d** has been achieved by enzymatic kinetic resolution. Among several highly selective commercial and in-house developed lipases, Novozym 435 has been selected as a catalyst for the acetylation of racemic alcohols *rac*-**2a–d** with vinyl acetate. Whereas the enzymatic acetylation afforded the (*R*)-enantiomers of acetates (*R*)-**3a–d** and (*S*) enantiomers of the alcohols (*S*)-**2a–d** in high enantiomeric excess, alcoholysis of the racemic acetates *rac*-**3a–d** yielded the opposite enantiomeric forms [(*R*)-**2a–d** and (*S*)-**3a–d**].

For the determination of the absolute configuration of the fast reacting enantiomers of **2a** and **2b**, a new computational methodology based on QM/MM calculations on the tetrahedral intermediates of the enantiomers within the *C. antarctica* lipase B structure has been applied.

4. Experimental

4.1. Materials and methods

4.1.1. Analytical methods

The ^{1}H 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 with TMS as the internal standard. IR spectra were recorded in KBr or CsBr plates on a Jasco FT-IR spectrometer and the wavenumbers are reported in cm^{-1}. GC analyses were made by an Konik HRGC 4000 B gas chromatograph (carrier gas N_2; head pressure: 60 psi, injector: 250 °C; FID detector: 250 °C) on a Astec-Chiral column (30 m × 0.25 mm, dimethylated-β-cyclodextrin, No. 777023 G0507-30). EI mass spectra were taken on a VG 7070E mass spectrometer operating at 70 eV, and are characterized by *m/z* (relative intensity %) values. Optical rotations were determined on a Bellingham-Stanley ADP 220 polarimeter. Preparative vacuum-chromatography[34] was performed on Merck Kieselgel 60 (0.063–0.200 μm).

4.1.2. Reagents and solvents

Benzothiazol **1a**, benzo[*b*]thiophen **1b**, vinyl acetate and the other commercial chemicals and solvents were products of Aldrich or Fluka. All solvents were purified and dried by standard methods. Benzofuran-3-carbaldehyde **1c**[35] and benzo[*b*]thiophene-3-carbaldehyde **1d**[36] are known compounds.

4.1.3. Biocatalysts

Lipase AK, lipase AY, lipase F and lipase PS were obtained from Aldrich. Lipozyme TL IM and Novozym 435 were products of Novozymes, Denmark. Lipases from *C. rugosa* (CrL) and *Pseudomonas fluorescens* (PfL) were purchased from Fluka. PPL and lipase from *C. cyclindracae* (CcL) were obtained from Sigma. Several extracellular hydrolases were isolated as acetone dried supernatant of shake flask fermentation of thermophilic fungus (Lipase 3b)[23] or air dried preparations from solid state fermentation of mesophilic fungi (SSF-9 SSF-117).[24]

4.2. Synthesis of racemic 1-heteroarylethanols *rac*-2a–d

4.2.1. Racemic 1-(benzothiazol-2-yl)ethanol, *rac*-2a and 1-(benzo[*b*]thiophen-2-yl)ethanol, *rac*-2b

To a stirred solution of the heteroaryl compound **1a** or **1b** (20 mmol) in anhydrous THF (120 mL) under argon, 2.7 M *n*-BuLi in heptane (8.15 mL, 1.1 equiv) was added below −70 °C, and the mixture was stirred for 1 h at −78 °C. Then, anhydrous acetaldehyde (4.38 g, 4.62 mL, 60 mmol) was added below −70 °C, and the solution was allowed to warm to −10 °C in 2 h. The mixture was quenched with saturated NH_4Cl solution (30 mL) and diluted with water (100 mL). After separation of the forming layers, the water layer was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with water (50 mL) and brine (30 mL), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel using CH_2Cl_2 as eluent to yield *rac*-**2a** or *rac*-**2b** as a white solid.

4.2.1.1. Racemic 1-(benzo[*d*]thiazol-2-yl)ethanol *rac*-2a. Yield: 92%; mp: 55–56 °C (lit. 54–56 °C[37]); ^{1}H NMR: 1.73 (d, 3H, *J* = 6.6 Hz), 3.31 (s, 1H), 5.29 (q, 1H, *J* = 6.6 Hz), 7.37–7.52 (m, 2H), 7.89 (d, 1H, *J* = 8.0 Hz), 7.99 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR: 24.0, 68.4, 121.8, 122.7, 125.1, 126.2, 134.7, 152.5, 177.1; IR (KBr): 3212, 2977, 2682, 2364, 1513, 1438, 1365, 1313, 1290, 1241, 1176, 1157, 1101, 1072, 1058, 1012, 890, 757, 730, 505; HRMS: M^+ found (M^+ calculated for C_9H_9NOS): 179.04067 (179.04048); MS: 179(M^+, 27), 164(16), 136(100), 108(35), 91(9), 82(13), 69(28), 63(18), 50(10) 45(35), 39(16).

4.2.1.2. Racemic 1-(benzo[*b*]thiophen-2-yl)ethanol *rac*-2b.[11] Yield: 88% (lit. 84%[11]); mp: 62 °C (lit. 62–63 °C[38]); ^{1}H NMR: 1.66 (d, 3H, *J* = 6.4 Hz), 2.53 (s, 1H), 5.19 (q, 1H, *J* = 6.4 Hz), 7.17 (s, 1H), 7.31–7.40 (m, 2H), 7.73 (d, 1H, *J* = 7.6 Hz), 7.83 (d, 1H,

J = 7.6 Hz); ^{13}C NMR: 25.1, 66.8, 119.5, 122.5, 123.5, 124.1, 124.3, 139.3, 139.5, 150.5; IR (KBr): 3301, 2967, 2923, 2358, 1733, 1455, 1434, 1369, 1315, 1249, 1203, 1143, 1128, 1066, 985, 937, 889, 831, 742, 725, 584; HRMS: M^+ found (M^+ calculated for $C_{10}H_{10}OS$): 178.04573 (178.04524); MS: 178(M^+, 37), 163(29), 135(100), 115(14), 91(52), 69(19), 63(24), 51(16), 43(64), 39(24).

4.2.2. Racemic 1-(benzofuran-3-yl)ethanol *rac*-2c and 1-(benzo[*b*]thiophen-3-yl)ethanol *rac*-2d

To a stirred solution of methylmagnesium iodide, prepared from magnesium (7.4 mmol, 0.177 g) and iodomethane (7.4 mmol, 1.05 g, 0.460 mL) in Et_2O (5 mL), a solution of a heteroaryl-3-carbaldehyde (**1c** or **1d**, 6.17 mmol) dissolved in Et_2O (3 mL) was added slowly at 0 °C under argon. The resulting mixture was stirred at room temperature overnight. After quenching the reaction mixture by the slow addition of saturated ammonium chloride solution (8 mL), the organic layer was separated and the aqueous layer extracted with Et_2O (2 × 10 mL). The combined Et_2O solutions were dried over anhydrous sodium sulfate. After removing the solvent by rotatory evaporation, the solid residue was purified by vacuum-chromatography on silica gel using CH_2Cl_2 as eluent resulting in the racemic alcohol *rac*-**2c** or *rac*-**2d** as a colourless semisolid.

4.2.2.1. Racemic 1-(benzo[*b*]furan-3-yl)ethanol *rac*-2c. Yield: 89%; semisolid; ^{1}H NMR: 1.65 (d, 3H, J = 6.7 Hz), 2.33 (s, 1H), 5.13 (q, 1H, J = 6.7 Hz), 7.24–7.36 (m, 2H), 7.50 (d, 1H, J = 7.4 Hz), 7.55 (s, 1H), 7.71 (d, 1H, J = 7.4 Hz); ^{13}C NMR: 23.4, 63.1, 111.6, 120.5, 122.6, 124.5, 125.1, 126.1, 140.8, 155.7; IR (CsBr): 3359, 2977, 1579, 1452, 1369, 1282, 1074, 1004, 858, 746; HRMS: M^+ found (M^+ calculated for $C_{10}H_{10}O_2$): 162.06838 (162.06808); MS: 162(M^+, 36), 147(59), 115(18), 91(100), 63(26), 51(13), 43(30), 39(22).

4.2.2.2. Racemic 1-(benzo[*b*]thiophen-3-yl)ethanol *rac*-2d. Yield: 85%; mp: 55 °C (lit. 53.5–55 °C[39]); ^{1}H NMR: 1.65 (d, 3H, J = 6.6 Hz), 2.37 (s, 1H), 5.24 (q, 1H, J = 6.6 Hz), 7.43 (s, 1H), 7.36–7.41 (m, 2H), 7.87–7.92 (m, 2H); ^{13}C NMR: 23.4, 65.7, 121.5, 122.3, 122.9, 124.1, 124.4, 137.3, 140.7, 141.0; IR (CsBr): 3359, 2973, 2364, 1456, 1427, 1369, 1255, 1122, 1070, 838; HRMS: M^+ found (M^+ calculated for $C_{10}H_{10}OS$): 178.04552 (178.04524); MS: 178(M^+, 49), 163(67), 161(12), 135(100), 128(7), 115(18), 91(55), 69(14), 63(20), 51(12), 43(43), 39(15).

4.3. Chemical acetylation of racemic 1-heteroarylethanols *rac*-2a–d

To a solution of the racemic 1-heteroarylethanol (*rac*-**2a**–**d**, 5.61 mmol) in 15 mL of dry CH_2Cl_2, Et_3N (6.17 mmol, 0.62 g, 0.85 mL), acetic anhydride (6.17 mmol, 0.63 g, 0.58 mL) and DMAP (20 mg) were added. The resulting mixture was stirred at room temperature overnight and was quenched with water (15 mL). The organic layer was separated and dried over anhydrous sodium sulfate, and the solvent was distilled off by rotatory evaporation. The residue was purified by vacuum-chromatography on silica gel using CH_2Cl_2 as eluent to give acetate *rac*-**3a**–**d** as a colourless semisolid.

4.3.1. Racemic 1-acetoxy-1-benzo[*d*]thiazol-2-yl-ethane *rac*-3a

Yield: 97%; semisolid; ^{1}H NMR: 1.78 (d, 3H, J = 6.6 Hz), 2.20 (s, 3H), 6.26 (q, 1H, J = 6.6 Hz), 7.38–7.53 (m, 2H), 7.89 (d, 1H, J = 8.2 Hz), 8.05 (d, 1H, J = 8.2 Hz); ^{13}C NMR: 20.8, 21.1, 70.2, 121.7, 123.2, 125.3, 126.2, 134.6, 152.9, 169.8, 171.3; IR (CsBr): 2991, 2933, 2358, 1749, 1521, 1438, 1371, 1230, 1060, 937, 759, 730; HRMS: M^+ found (M^+ calculated for $C_{11}H_{11}NO_2S$): 221.05131 (221.05105); MS: 221(M^+, 13), 178(64), 162(15), 136(34), 109(19), 90(4), 69(17), 65(10), 55(5), 43(100), 39(9).

4.3.2. Racemic 1-acetoxy-1-benzo[*b*]thiophen-2-yl-ethane *rac*-3b

Yield: 98%; mp: 30 °C (lit. mp = 30–31 °C[40]); ^{1}H NMR: 1.72 (d, 3H, J = 6.6 Hz); 2.12 (s, 3H); 6.27 (q, 1H, J = 6.6 Hz); 7.30 (s, 1H), 7.32–7.37 (m, 2H), 7.76 (d, 1H, J = 6.6 Hz), 7.83(d, 1H, J = 6.6 Hz); ^{13}C NMR: 21.3, 21.9, 68.2, 121.7, 122.4, 123.7, 124.4, 124.5, 139.2, 139.4, 145.1, 170.2; IR (CsBr): 3058, 2983, 2933, 2355, 1741, 1436, 1371, 1236, 1151, 1049, 1018, 937, 831, 748, 727, 584; HRMS: M^+ found (M^+ calculated for $C_{12}H_{12}O_2S$): 220.05597 (220.0558); MS: 220(M^+, 50), 178(95), 161(85), 135(30), 128(52), 115(41), 89(30), 69(12), 63(17), 51(9), 43(100), 39(12).

4.3.3. Racemic 1-acetoxy-1-benzo[*b*]furan-3-yl-ethane *rac*-3c

Yield: 96%; semisolid; ^{1}H NMR: 1.72 (d, 3H, J = 6.8 Hz), 2.11(s, 3H), 6.23 (q, 1H, J = 6.8 Hz), 7.26–7.37 (m, 2H), 7.52 (d, 1H, J = 6.6 Hz), 7.64 (s, 1H), 7.68 (d, 1H, J = 6.6 Hz); ^{13}C NMR: 20.4, 21.3, 64.9, 111.7, 120.4, 121.2, 122.8, 124.6, 125.9, 142.0, 155.6, 170.4; IR (CsBr) 2983, 2933, 2364, 1735, 1454, 1371, 1238, 1087, 1049, 1016, 941, 856, 746; HRMS: M^+ found (M^+ calculated for $C_{12}H_{12}O_3$): 204.07848 (204.07864); MS: 204(M^+, 23), 162(42), 145(51), 115(57), 89(26), 63(26), 51(11), 43(100), 39(19).

4.3.4. Racemic 1-acetoxy-1-benzo[*b*]thiophen-3-yl-ethane *rac*-3d

Yield: 98%; semisolid; ^{1}H NMR: 1.73 (d, 3H, J = 6.6 Hz), 2.13 (s, 3H), 6.35 (q, 1H, J = 6.6 Hz), 7.38–7.43 (m, 2H), 7.45 (s, 1H), 7.86–7.91 (m, 2H); ^{13}C NMR: 20.6; 21.3; 67.4; 122.1; 122.9; 123.3; 124.2; 124.5; 136.3; 137.1; 140.7; 170.4; IR (CsBr): 2981, 2933, 2358, 1735, 1428, 1369, 1240, 1043, 939, 761, 734; HRMS: M^+ found (M^+ calculated for $C_{12}H_{12}O_2S$): 220.05563 (220.0558); MS: 220(M^+, 48), 178(85), 163(43), 161(91), 135(30), 128(54), 115(45), 89(33), 69(11), 63(16), 51(8), 43(100), 39(10).

4.4. Lipase-catalyzed acylations of racemic 1-heteroarylethanols *rac*-2a–d

4.4.1. Acylations on an analytical scale

To a solution of racemic 1-heteroarylethanol (*rac*-**2a**–**d**, 20 mg) in vinyl acetate (0.5 mL), lipase (20 mg) was added. The reaction mixture was shaken at 1000 rpm at room temperature. For GC analysis, samples were taken directly from the reaction mixture (10 μL, diluted to 200 μL with CH_2Cl_2). Data on reaction time, conversion and enantiomeric composition of the products [(*S*)-**2a**–**d** and (*R*)-**3a**–**d**] are presented in Tables 1–4.

4.4.1.1. GC analysis of 1-(benzo[*d*]thiazol-2-yl)ethanol 2a and 1-(benzo[*d*]thiazol-2-yl)ethyl acetate 3a. GC (110 °C, R_t/min): 44.89/51.82 [(*S*)-/(*R*)-enantiomers of **2a**, respectively], 22.3/23.57 [(*S*)-/(*R*)-enantiomers of **3a**, respectively].

4.4.1.2. GC analysis of 1-(benzo[*b*]thiophen-2-yl)ethanol 2b and 1-(benzo[*b*]thiophen-2-yl)ethyl acetate 3b. GC (140 °C, R_t/min): 12.48/12.92 [(*R*)-/(*S*)-enantiomers of **2b**, respectively], 9.79/10.19 [(*S*)-/(*R*)-enantiomers of **3b**, respectively].

4.4.1.3. GC analysis of 1-(benzo[*b*]furan-3-yl)ethanol 2c and 1-(benzo[*b*]furan-3-yl)ethyl acetate 3c. GC (120 °C, R_t/min): 23.3/24.73 [(*R*)-/(*S*)-enantiomers of **2c**, respectively], 14.41/16.32 [(*S*)-/(*R*)-enantiomers of **3c**, respectively].

4.4.1.4. GC analysis of 1-(benzo[*b*]thiophen-3-yl)ethanol 2d and 1-(benzo[*b*]thiophen-3-yl)ethyl acetate 3d. GC (140 °C, R_t/min): 14.6/15.46 [(*S*)-/(*R*)-enantiomers of **2d**, respectively], 7.94/8.23 [(*S*)-/(*R*)-enantiomers of **3d**, respectively].

4.4.2. Preparative scale acylation of racemic 1-heteroarylethanols *rac*-2a–d

To a solution of racemic 1-heteroarylethanols *rac*-**2a–d** (200 mg, each) in vinyl acetate (6 mL) Novozym 435 (200 mg) was added and the mixture was stirred at room temperature overnight. The enzyme was filtered off and washed with acetone (2 × 5 mL). Solvents were distilled off from the combined filtrates, and the residue was purified by column chromatography (silica gel, CH_2Cl_2) resulting in the optically active alcohol (−)-(*S*)-**2a–d** and acetate (+)-(*R*)-**3a–d** fractions as white semisolids. IR, NMR and MS spectra of the optically active alcohols, (*S*)-**2a–d** and acetates, (*R*)-**3a–d** were indistinguishable from those of their racemates (for spectra of *rac*-**2a–d** and *rac*-**3a–d**, see Sections 4.2 and 4.3). Data on yield, enantiomeric composition and specific rotation of the products [(*S*)-**2a–d** and (*R*)-**3a–d**] are shown in Table 5.

4.5. Lipase-catalyzed alcoholysis of racemic 1-heteroarylethyl acetates *rac*-3a–d

To a mixture of racemic 1-heteroarylethyl acetates *rac*-**3a–d** (20 mg, each) in alcohol (0.5 mL), Novozym 435 (20 mg) was added, and the mixture was shaken at 1000 rpm and room temperature. Samples were analyzed by GC similarly as described in Section 4.4.1. Data on solvent, reaction time, conversion and enantiomeric composition of the products (*R*)-**2a–d** and (*S*)-**3a–d** are presented in Table 6.

4.6. Computational methods

4.6.1. Systematic conformational search within the active site of CaLB at MM level

The calculations were performed within the active site of the crystal structure CaLB containing Tween80 (T80) as substrate mimic (PDB code: 1LBT).[27] From this CaLB structure, a part containing the residues within 10 Å spheres around the Ser105 O_γ, T80 C_{10} and T80 C_{20} atoms was cut off as the active site model for CS and later QM/MM calculations. Next, T80 was removed and by the Hyperchem[41] standard procedure, hydrogens were added to the amino acid residues and water oxygens. The C- and N-termini at cutting were completed to neutral aldehyde and amino moieties. During our calculation His224 was protonated and Asp187 was deprotonated.

The tetrahedral intermediate (THI) states were built in the active site model by constructing a covalent bond between the O_γ atom of Ser105 and the carbonyl carbon of the (*R*)- and (*S*)-enantiomers of **3a–d** acetates. In this way, starting structures were constructed for the four THI states (*R*)-**2**-*R*-THI, (*R*)-**2**-*S*-THI, (*S*)-**2**-*R*-THI, (*S*)-**2**-*S*-THI for acetylation of each of the racemic alcohols *rac*-**2a–d**. The initial THI's including C_α and C_β (with their hydrogens) of Ser105 (33 atoms for **3b–d** and 32 atoms for **3a** acetates) were optimized by the MM+ method of HYPERCHEM[41] within the rigid enzymic environment (including no waters).

The conformational search (CS) within this active site model was performed by the CS module implemented in Hyperchem[41] at molecular mechanics (MM+) level using default settings (MM+ forcefield; gradient: 0.1 kcal/mol; Polak-Ribiere method until 0.05 kcal/mol RMS gradient or maximum 600 cycles; limits: 300 iterations, 150 optimizations, 15 conformations; test options: 'skip if atoms are closer than 0.3 Å'). During CS, five torsion angles (starting from C_α of Ser105) and no rings of the alcohols **2a–d** were chosen to vary in a rigid enzymic environment (including no waters). The three lowest energy conformations of each of the **2a–d** alcohol–acyl enzyme THI systems were investigated further by QM/MM methods.

4.6.2. QM/MM calculations within the active site of CaLB

All QM/MM optimizations were carried out using the ONIOM protocol as implemented in GAUSSIAN 03[42] with the aid of GAUSSVIEW[43] for selecting and preparing the calculation levels.

The best three conformations for each THI's were optimized using a three-layer ONIOM method (pm3/uff/uff) over 1020/1019 atoms. The high layer (HL) included the atoms of the alcohols **2a–d**, the acetate part of THI, the side chains of the catalytic triad (Ser105, His224, Asp187 + one water), the relevant parts of the residues forming the so-called oxoanion hole (Gln106, Thr40). The HL was relaxed with the semiempirical PM3 methods. The medium layer (ML) included Trp104 (+ one water), Asp134, Gln157, Ile189, Leu278, Ala281 and Ala282. The low layer (LL) consisted of the remaining residues which were kept fixed during the optimization. The ML and LL were optimized by molecular mechanic force-field (UFF). In addition to the (pm3/uff/uff) calculations, several THI's of **2a** and **2b** [(*R*)-**2**-S-THI and (*S*)-**2**-R-THI] were optimized with Hartree–Fock methods in the HL [hf(3-21+g**)/uff/uff] as well.

Acknowledgements

L.P. thanks the financial support from Hungarian OTKA Foundation (T-048854). The financial support to C.P from the Romanian Ministry of Education and Research (No. 1480-6/07.04.2006) and Domus Hungarica Foundation is gratefully acknowledged.

References

1. Faber, K. *Biotransformations in Organic Chemistry*, 4th ed.; Springer: Berlin, 2004.
2. Rehm, H.-J.; Reed, G.; Pühler, A.; Stadler, P.; Kelly, D. R., 2nd ed. In *Biotechnology: Biotransformations I and II*; Wiley-VCH: Weinheim, 1998; Vols. 8a and 8b.
3. Poppe, L.; Novák, L. *Selective Biocatalysis: A Synthetic Approach*; VCH: Weinheim-New York, 1992.
4. *Industrial Biotransformations*; Liese, A., Seelbach, K., Wandrey, C., Eds., 2nd ed.; Wiley-VCH: Weinheim-New York, 2006.
5. Panke, S.; Held, M.; Wubbolts, M.; Schmid, A.; Hollmann, F.; Park, J. B.; Bühler, B. *Curr. Opin. Biotechnol.* **2004**, *15*, 272–279.
6. Bornschauer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations*; Wiley-VCH: Weinheim-New York, 2006.
7. Ghanem, A.; Aboul-Enein, H. Y. *Chirality* **2005**, *17*, 1–15.
8. Turner, N. J. *Curr. Opin. Chem. Biol.* **2004**, *8*, 114–119.
9. Paizs, Cs.; Toşa, M.; Majdik, C.; Moldovan, P.; Novák, L.; Kolonits, P.; Marcovici, A.; Irimie, F.-D.; Poppe, L. *Tetrahedron: Asymmetry* **2003**, *14*, 1495–1501.
10. Paizs, Cs.; Toşa, M.; Bódai, V.; Szakács, G.; Kmecz, I.; Simándi, B.; Majdik, C.; Novák, L.; Irimie, F.-D.; Poppe, L. *Tetrahedron: Asymmetry* **2003**, *14*, 1943–1949.
11. Yokoyama, Y.; Shiraishi, H.; Tani, Y.; Yokoyama, Y.; Yamaguchi, Y. *J. Am. Chem. Soc.* **2003**, *125*, 7194–7195.
12. Cozzi, P. G.; Kotrusz, P. *J. Am. Chem. Soc.* **2006**, *128*, 4940–4941.
13. Zaidlewicz, M.; Chechlowska, A.; Prewysz-Kwinto, A.; Wojtczak, A. *Heterocycles* **2001**, *55*, 569–577.
14. Ravi Kumar, C.; Rachna, S.; Palakodety, R. K. *Tetrahedron: Asymmetry* **2008**, *19*, 901–905.
15. Vreven, T.; Morokuma, K. *Theor. Chem. Acc.* **2003**, *109*, 125–132.
16. (a) Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jones, T. A. *Structure* **1994**, *2*, 293–308; (b) Uppenberg, J.; Patkar, S.; Bergfors, T.; Jones, T. A. *J. Mol. Biol.* **1994**, *235*, 790–792; (c) 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.
17. Luic, M.; Stefanic, Z.; Ceilinger, I.; Hodoscek, M.; Janezic, D.; Lenac, T.; Lescic, A.; Sepac, D.; Tomic, S. *J. Phys. Chem. B* **2008**, *112*, 4876–4883.
18. Haeffner, F.; Norin, T.; Hult, K. *Biophys. J.* **1998**, *74*, 1251–1262.
19. Otto, R. T.; Scheib, H.; Bornscheuer, U. T.; Pleiss, J.; Syldatk, C.; Schmid, R. D. *J. Mol. Catal. B* **2000**, *8*, 201–211.
20. Raza, S.; Fransson, L.; Hult, K. *Protein Sci.* **2001**, *10*, 329–338.
21. Ottosson, J.; Fransson, L.; Hult, K. *Protein Sci.* **2002**, *11*, 1462–1471.
22. (a) Szatzker, G.; Pilbák, S.; Tőke, E. R.; Bódai, V.; Poppe, L. *FEBS J.* **2005**, *272*, 114; (b) Tőke, E. R.; Kolonits, P.; Novák, L.; Poppe, L. *Tetrahedron: Asymmetry* **2006**, *17*, 2377–2385.
23. Bódai, V.; Peredi, R.; Bálint, J.; Egri, G.; Novák, L.; Szakács, G.; Poppe, L. *Adv. Synth. Catal.* **2003**, *345*, 811–818.
24. Nagy, V.; Tőke, E. R.; Chee Keong, L.; Szatzker, G.; Ibrahim, D.; Che-Omar, I.; Szakács, G.; Poppe, L. *J. Mol. Catal. B, Enzym.* **2006**, *39*, 141–148.
25. Rakels, J. L. L.; Straathof, A. J. J.; Heijnen, J. J. *Enzyme Microb. Technol.* **1993**, *15*, 1051–1056.

26. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665.
27. Uppenberg, J.; Ohmer, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthomen, T.; Jones, T. A. *Biochemistry* **1995**, *34*, 16838–16851.
28. Cuiper, A. PhD thesis, University of Groningen (NL), 1999.
29. Warshel, A.; Russell, S. *J. Am. Chem. Soc.* **1986**, *108*, 6569–6579.
30. Warshel, A.; Naray-Szabó, G.; Sussman, F.; Hwang, J.-K. *Biochemistry* **1989**, *28*, 3629–3637.
31. Hu, C. H.; Brinck, T.; Hult, K. *Int. J. Quantum Chem.* **1998**, *69*, 89–103.
32. Rotticci, D.; Haeffner, F.; Orrenius, C.; Norin, T.; Hult, K. *J. Mol. Catal. B* **1998**, *5*, 267–272.
33. Orrenius, C.; Haeffner, F.; Rotticci, D.; Öhrner, N.; Norin, T.; Hult, K. *Biocatal. Biotransf.* **1998**, *16*, 1–15.
34. Poppe, L.; Novák, L. *Magy. Kém. Lapja* **1985**, *40*, 366–369.
35. Zaidlewicz, M.; Chechlowska, A.; Prewysz-Kwinto, A.; Wojtczak, A. *Heterocycles* **2001**, *55*, 569–577.
36. King, W. J.; Nord, F. F. *J. Org. Chem* **1948**, *13*, 635–637.
37. Florio, S.; Capriati, V.; Colli, G. *Tetrahedron* **1997**, *53*, 5839–5846.
38. Yokoyama, Y.; Shiraishi, H.; Tani, Y.; Yokoyama, Y. *J. Am. Chem. Soc.* **2003**, *125*, 7194–7195.
39. Noyce, D. S.; Forsyth, D. A. *J. Org. Chem.* **1974**, *19*, 2828–2831.
40. Hill, E. A.; Gross, M. L.; Stasiewicz, M.; Manion, M. *J. Am. Chem. Soc.* **1969**, *91*, 7381–7392.
41. HYPERCHEM 7.5, Hypercube, 2003.
42. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A.; GAUSSIAN 03, Revision B.05, Gaussian, Wallingford, CT, 2003.
43. GAUSSVIEW, Version 3.09, R. Dennington II, T. Keith, J. Millam, K. Eppinnett, W. L. Hovell, R. Gilliland, Semichem, Shawnee Mission, KS, 2003.