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Enzyme-catalyzed synthesis of (*R*)- and (*S*)-3-heteroaryl-3-hydroxy-propanoic acids and their derivatives

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ABSTRACT

Starting from the racemic 3-benzofuranyl- and 3-benzo[*b*]thiophenyl-3-hydroxypropanoic acid ethyl esters as substrates, various multistep enzymatic procedures were developed for the efficient synthesis of the corresponding highly enantiomerically enriched (*R*)- and (*S*)-3-heteroaryl-3-hydroxypropanoic acids.

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1. Introduction

Optically active β-hydroxy-β-arylpropanoic acids and their derivatives are synthetically important and highly functionalized chiral synthons, of which the chiral β-hydroxy-β-arylpropanoates are precursors of enantiopure pharmaceuticals covering a plethora of activity. Taxol is an anticancer agent, fluoxetine is an antidepressant, pravastatin and atorvastatin, with statin moiety, are anticholesterolic drugs, while propranolol and alprenolol are β-blockers.

Recently, the synthesis of fifteen racemic β-hydroxy-β-arylalkanoic acids has been reported, and they were found to be antiproliferative agents.[1] Other acids such as some diastereomeric 3-hydroxy-2-methyl-3-(4-biphenyl)butanoic acids, which are structurally similar to the NSAIDs, were also prepared and evaluated for their anti-inflammatory activity and gastric tolerability by docking calculations.[2]

There are many chemo- and biocatalytic procedures known for the enantioselective preparation of 3-hydroxy-propanoic acids and their derivatives.

One of the chemocatalytic procedures is based on stereoselective reduction of the β-keto-carboxylates. Enantiopure (*S*)-methyl 3-(benzofuran-2-yl)-3-hydroxy-propanoate was prepared by hydrogenation of the corresponding β-keto ester over a chiral heterogenous tartaric acid-modified Raney nickel catalyst.[3] Another enantioselective chemocatalytic method is based on the enantioselective reaction of aldehydes with diketene in the presence of a chiral Schiff's base–titanium alkoxide complex.[4]

Recently, the stereoselective synthesis of (*S*)-methyl 3-(benzo[*b*]thiophen-3-yl)-3-hydroxypropanoate by a Reformatskii reaction in the presence of (−)-*N*,*N*-dimethyl-aminoisoborneol as chiral ligand has also been reported.[5]

However, all these methodologies have noteworthy drawbacks including expensive reagents, low yields, and unsatisfactory enantiomeric purities of the desired products. Thus, many biocatalytic methodologies[6] based on the enantiomer selective kinetic resolution of racemates and enantiotope selective transformation of prochiral substrates have been developed for an efficient, economical, and environmentally friendly synthesis of optically active β-hydroxy-β-propanoic acids and their derivatives.

There are some examples of the bioreduction of β-keto-carboxylates with suspended[7] or with immobilized[8] microorganisms or plant cells, wild type[9] or engineered baker's yeast cells,[10] providing optically active β-hydroxy-carboxylic acids, esters or amides. Based on a biooxidation–reduction process, microbial deracemization of aromatic β-hydroxypropanoates using *Candida parapsilosis* cells yielded highly enantiopure aromatic β-hydroxy-propanoates.[11]

Due to their high regio- and stereoselectivity, enzymes are useful catalysts for the kinetic resolution of racemic 3-substituted 3-hydroxypropionates. *Candida rugosa* lipase (CrL) proved to be a highly enantioselective catalyst for the hydrolysis of various types of racemic aliphatic and aromatic β-hydroxy-β-arylpropanoates or their O-acylated derivatives.[12] Lipase B from *Candida antarctica* (CaL-B) in toluene was successfully used for the stereoselective synthesis of (*S*)-tropic acid (2-phenyl-3-hydroxypropanoic acid) an important building block for some biologically active alkaloids.[13] Since all these enzymatic hydrolyses are carried out in heterogenous systems, the effect of ultrasound was also tested for the enzymatic hydrolysis of ethyl-3-hydroxy-3-phenylpropanoate with PCL, PLE, and CrL.[14] The reaction time was shortened without significant changes in the yield or the enantiomeric excess of the reaction products.

The enantiomer selective acylation of β-hydroxy-β-arylpropanoates was also investigated. Lipases from *Pseudomonas cepacia*, *Chomobacterium viscosum* and porcine pancreas were found as catalysts for the chiral resolutions of racemic aliphatic 3-hydroxy

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esters.[15] The lipase-catalyzed kinetic resolution of different 3-hydroxy esters in organic solvents and supercritical carbon dioxide was also studied. High enantiomeric excesses were found in both cases.[16] Using vinyl acetate as the acyl donor, the effect of supercritical conditions was investigated. The kinetic resolution of 2-substituted 3-hydroxyesters by lipase PS-catalyzed transesterification with vinyl acetate was also reported.[17] It was shown that the volume of the group at the C-3 position, the stereocenter, is crucial for the enantioselectivity of reaction.

A chemoenzymatic route for the synthesis of precursors of β-blockers through the lipase-catalyzed kinetic resolution of *rac*-4-aryloxy-3-hydroxybutanoates by acylation or hydrolysis using lipase from *Burkholderia cepacia* (BCL) was developed.[18]

To the best of our knowledge, no enzymatic kinetic resolution has been described for the synthesis of optically active 3-benzofuranyl- and benzothiophenyl-3-hydroxypropanoates.

As part of our interest in development of enzymatic stereoselective methods for the preparation of optically active heteroaromatic compounds, the enzyme-mediated enantioselective synthesis of various 3-heteroaryl-3-hydroxy-propanoic acids **4a–d** and their derivatives **2**, **3a–d** was developed (Scheme 1).

The absolute configuration of the enantiomerically enriched compound synthesized was assigned by vibrational circular dichroism (VCD) measurements combined with quantum chemical calculations at ab initio (DFT) level of theory, which is a well-established technique for determination of absolute configuration and conformation of small to medium-sized chiral molecules in solution.[19,20]

2. Results and discussion

2.1. Chemical synthesis

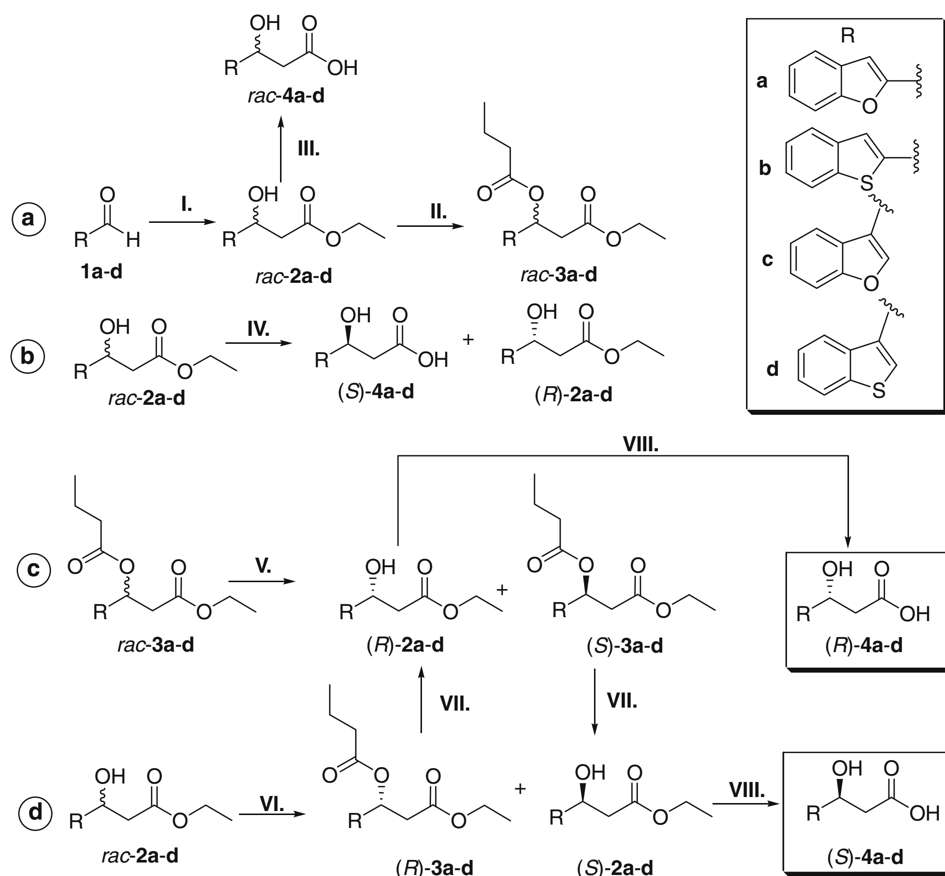
Racemic ethyl 3-heteroaryl-3-hydroxypropanoates *rac*-**2a–d** were prepared by the Reformatskii reaction using the heteroaromatic aldehydes **1a–d** synthesized as previously described by us.[21] *rac*-**2a–d** were hydrolyzed into racemic 3-heteroaryl-3-hydroxypropanoic acid *rac*-**4a–d** in presence of pig liver esterase (PLE) or chemically with refluxing aqueous potassium carbonate. Compounds *rac*-**2a–d** were also used for the preparation of racemic double esters *rac*-**3a–d** by chemical acylation with butanoic anhydride in acetonitrile, in the presence of $CoCl_2$ as catalyst,[22] as shown in Scheme 1a.

2.2. Enzymatic synthesis

To investigate the stereoselectivity of the reaction involving chiral heteroaryl β-hydroxy-β-arylpropanoic acids and their derivatives, the chromatographic separation of the enantiomers was first established (Table 5). Using various HPLC chiral columns, the base-line separation of all the enantiomers of *rac*-**2**–**4a–d** was performed.

2.2.1. Analytical scale biotransformation of *rac*-2,3a–d

2.2.1.1. Analytical scale enzymatic hydrolysis of *rac*-2a–d. A wide selection of commercial hydrolases was screened for the analytical scale selective hydrolysis of racemic esteric compounds



Scheme 1. (a) The synthesis of racemic ethyl 3-heteroaryl-3-hydroxypropanoic acids *rac*-**4a–d** and their derivatives *rac*-**2**,**3a–d**; (b) Enzymatic enantioselective hydrolysis of *rac*-**2a–d**. (c and d) Enzymatic kinetic resolution of *rac*-**2**,**3a–d** followed by the enzymatic transformation of the kinetic resolution products into both (*R*)- and (*S*)-3-heteroaryl-3-hydroxypropanoic acids **4a–d**. Reagents and conditions: (i) Ethyl 2-bromoacetate, Zn, THF, reflux; (ii) $CoCl_2$, $(CH_3–CH_2–CH_2–COO)_2$, CH_3CN, reflux; (iii) PLE in water or K_2CO_3 in water and reflux; (iv) $CH_3–CH_2–CH_2–COO–CH=CH_2$, lipase, solvent; (vii) BCL, H_2O, organic solvent, (viii) PLE, water.

rac-**2a**–**d**. Samples were taken every 30 min and analyzed chromatographically by HPLC. Most of the enzymes tested such as lipase A, AK, AY, F, G, M, R and Lypozyme TL IM were catalytically inactive. Lipases such lipase A and lipase B from *C. antarctica* (CaL-A and CaL-B formulated as Novozym 435), and lipases from *C. rugosa* (CrL) and PLE exhibited high enzymatic activity but low enantiomer selectivity toward all *rac*-**2a**–**d**. The complete hydrolysis of the substrates occurred in 2–4 h.

Only BCL proved to be an efficient catalyst for the synthesis of the highly enantiomerically enriched (*R*)-**2a**–**d** (ee between 97% and 99%), while the enantiomeric composition of the (*S*)-**4a**–**d** formed was found to be low (ee < 50%), when the conversion of the reactions was close to 50% (yields between 45% and 48% for the isolated (*R*)-**2a**–**d** and (*S*)-**4a**–**d** from the analytical scale reaction mixtures (Table 1)). This apparent contradiction could be explained by slow racemization of the 3-heteroaryl-3-hydroxy-propanoic acids in the presence of water. The rate of racemization was even higher when instead of water, different buffers were used in the pH 3–8 range.

Table 1
The enantioselective hydrolysis of *rac*-**2a**–**d** mediated by lipase from *Burkholderia cepacia* (BCL) in distilled water

Substrate	Time (h)	$Yield_{(R)\text{-}2a\text{–}d}$ (%)	$ee_{(R)\text{-}2a\text{–}d}$ (%)	$Yield_{(S)\text{-}4a\text{–}d}$ (%)	$ee_{(S)\text{-}4a\text{–}d}$ (%)
rac-**2a**	24	46	97	47	48
rac-**2b**	24	45	98	47	46
rac-**2c**	32	48	99	49	42
rac-**2d**	32	47	98	48	41

2.2.1.2. Analytical scale enzymatic hydrolysis of *rac*-3a–d. A small-scale biotransformation of the double esteric compounds *rac*-**3a**–**d** was next studied with the same enzymes. Since the enzymatic alcoholysis using several anhydrous alcohols proved to be totally inefficient (yields <5% after 7 days), other enzymatic hydrolyses of *rac*-**3a**–**d** were studied. While most of the enzymes tested were catalytically inactive for this purpose, lipase from *Burkholderia cepacia* (BCL) hydrolyzed *rac*-**3a**–**d** rapidly and regiospecifically but in a non-stereoselective manner into *rac*-**2a**–**d** in cyclohexane–THF (1:1, v:v) media using 10 equiv of water. The presence of *rac*-**4a**–**d** in the reaction mixture was not detected even after 7 days reaction time. Only CrL proved to be a proper biocatalyst for the enantiomer selective hydrolysis of *rac*-**3a**–**d** (Table 2, Scheme 1c). After 24–26 h and approximately 50% conversion, both products were obtained with high enantiomeric excess ($E > 200$) for the resolution of *rac*-**3a**,**c**,**d** but only with moderate enantiomeric excess ($E = 36$) for *rac*-**3b** (Table 2).

Table 2
The enantioselective hydrolysis of *rac*-**3a**–**d** in the presence of CrL

Substrate	Time (h)	*c* (%)	$ee_{(S)\text{-}3a\text{–}d}$	$ee_{(R)\text{-}2a\text{–}d}$	*E*
rac-**3a**	24	49	95	95	>200
rac-**3b**	24	47	79	87	36
rac-**3c**	26	50	95	95	>200
rac-**3d**	26	49	94	94	>200

2.2.1.3. Analytical scale enzymatic acylation of *rac*-2a–d. Another approach for the stereoselective synthesis of 3-heteroaryl-3-hydroxy-propanoic acid derivatives is based on the enantiomer selective acylation of the 3-heteroaryl-3-hydroxypropanoates *rac*-**2a**–**d**. For this, the analytical scale enantiomer selective enzyme-catalyzed acylations in vinyl acetate were tested in the presence of several enzymes.

In the case of *rac*-**2a**,**b**, most of the enzymes tested were catalytically inactive. Lipozyme from *Mucor miehei*, Lipase AK, and Lipozyme TL IM showed little reactivity and moderate selectivity. Only with Novozym 435 after 96 h did the conversion nearly reach 50%. However, the selectivity of the reaction was poor ($E < 7$).

It is known that the nature of the solvent and the nucleophile could significantly influence the selectivity of the enantiomer selective acylation. The acylation of *rac*-**2a** with vinyl acetate in the presence of Novozym 435 in several solvents, such as saturated and aromatic hydrocarbons, cyclic saturated ethers, was tested. Cyclohexane proved to be the most appropriate solvent for the acetylation ($E = 22\%$ and 46% conversion after 4 days). Further acylation of *rac*-**2a** was performed with two other acyl donors (vinyl propanoate and vinyl butanoate) in cyclohexane (Scheme 1c). It was found that the highest selectivity and reactivity for the Novozym 435-catalyzed acylation of *rac*-**2a** was performed with vinyl butanoate ($E > 200$). Similar results were found using the same conditions for the enzymatic resolution of *rac*-**2b** (Table 3).

Table 3
The influence of the type of acyl donor upon the selectivity for the enzymatic acylation of *rac*-**2a**–**d**

Substrate	Reactant	Time (h)	*c* (%)	$ee_{(S)\text{-}2}$	$ee_{(R)\text{-}3}$	*E*
rac-**2a**[a]	Vinyl acetate	106	46	42	49	4
	Vinyl propanoate	160	41	64	94	66
	Vinyl butanoate	60	50	98	98	>200
rac-**2b**[a]	Vinyl acetate	65	17	14	72	7
	Vinyl propanoate	72	32	37	60	12
	Vinyl butanoate	48	50	99	99	>>200
rac-**2c**[b]	Vinyl acetate	16	44	38	49	4
	Vinyl propanoate	16	40	60	90	35
	Vinyl butanoate	16	49	91	93	87
rac-**2d**[b]	Vinyl acetate	24	45	60	72	11
	Vinyl propanoate	24	50	67	67	10
	Vinyl butanoate	24	48	85	92	65

[a] Cyclohexane, Novozym 435.
[b] Benzene, CaL-A.

For the enzymatic acylation of *rac*-**2c**,**d** only CaL-A proved to be catalytically active. After performing the same optimization procedure as described above, it was found that the highest selectivity for the CaL-A-mediated enantiomer selective acylation of *rac*-**2c**,**d** was obtained using benzene as solvent and vinyl butanoate as acyl donor (Scheme 1d).

2.2.1.4. Analytical scale enzymatic synthesis of both (*R*)- and (*S*)-4a–d. Based on our earlier observation that BCL and PLE are highly active and non-stereoselective catalysts for the hydrolysis of *rac*-**3a**–**d** into *rac*-**2a**–**d** and *rac*-**2a**–**d** into *rac*-**4a**–**d**, respectively, these two enzymes were used further for the analytical scale synthesis of (*S*)- and (*R*)-**4a**–**d** without altering the enantiomeric composition of the final isolated products (Scheme 1c and d). First by the BCL-mediated quantitative hydrolysis of (*S*)-**3a**–**d** and (*R*)-**3a**–**d** obtained with the procedures described in Sections 2.2.1.2 and 2.2.1.3, respectively, both (*S*)- and (*R*)-**2a**–**d** were synthesized. Further using a 1:1 substrate–enzyme *ratio*, both (*S*)- and (*R*)-**2a**–**d** were rapidly transformed (15 min) into the corresponding enantiomerically enriched 3-heteroaryl-3-propanoic acids (*S*)- and (*R*)-**4a**–**d**.

2.2.2. Preparative-scale synthesis of both (*R*)- and (*S*)-4a–d

Following the sequence depicted in Scheme 1c and d, the preparative-scale synthesis of both (*R*)- and (*S*)-**4a**–**d** was performed. All the dilutions, substrate–biocatalyst ratio, and reaction conditions were the same as in the case of the analytical scale reactions.

First, the enantiomer selective hydrolysis of *rac*-**3c**,**d** and the enantiomer selective acylation of *rac*-**2a**,**b** were performed. The

Table 4
Yields and specific rotations for the isolated enantiomerically enriched **2**, **3**, **4a–d**

	(*S*)-**2a–d**			(*R*)-**2a–d**			(*R*)-**3a**,**b** and (*S*)-**3c**,**d**			(*S*)-**4a–d**			(*R*)-**4a–d**		
	Yield[a]	*ee*	$[\alpha]_D^{25}$ [b]	Yield[a]	*ee*	$[\alpha]_D^{25}$ [b]	Yield[a]	*ee*	$[\alpha]_D^{25}$ [b]	Yield[a]	*ee*	$[\alpha]_D^{25}$ [b]	Yield[a]	*ee*	$[\alpha]_D^{25}$ [b]
a	49.0	98	−24.3	48.5	98	+24.3	49.0	98	+38.5	48.0	98	−19.6	48.0	98	+19.6
b	48.5	99	−13.5	48.5	99	+13.5	49.0	99	+28.5	47.5	99	−5.8	48.0	98	+5.7
c	49.0	95	−24.5	49.5	95	+24.7	49.5	95	−44.5	48.5	95	−10.0	49.0	94	+9.9
d	48.5	94	−42.5	49.0	94	+42.3	49.5	94	−67.5	48.0	94	−25.1	48.5	94	+25.1

[a] Yields are given for the isolated compounds based on the maximum theoretical recovery from the racemic starting compounds.
[b] Measured in $CHCl_3$, at 10 mg mL^{-1}.

reactions were monitored by HPLC and TLC and were stopped at an approximately 50% conversion, removing the enzyme by filtration. Additionally, by using BCL as catalyst, the full amount of the previously isolated optically active (*S*)- and (*R*)-**3a–d** was quantitatively transformed into (*S*)- and (*R*)-**2a–d**. Finally, via a fast PLE-mediated hydrolysis, both (*R*)- and (*S*)-**2a–d** were transformed into the corresponding hydroxypropanoic acids. The target compounds, (*R*)- and (*S*)-**4a–d**, were isolated with good yields without altering their enantiomeric composition (Table 4).

2.3. The absolute configuration of optically active 2a–d synthesized by the enantiomer selective acylation of *rac*-2a–d

The VCD spectra of (−)-**2a–d** obtained by the enantiomer selective acylation of *rac*-**2a–d** with unknown absolute configuration, recorded in $CDCl_3$ solution, are shown in Figure 1. The spectra, dominated by the negative $\nu_{C=O}$ band of the ester carbonyl group at ~1715 cm^{-1}, have a rather similar pattern between 1800 and 1300 cm^{-1}. This indicates that the nature of the heteroatom (O or S) or the position of the side-chain on the achiral heterocyclic moiety has only a little influence on the overall shape of the VCD spectrum. This can be explained by the fact that molecules with closely related structures typically have regions of similar VCD spectra, particularly those originating from vibrations of structurally identical parts of the molecules which are not strongly coupled with vibrations of the structurally different parts.[19]

The determination of the absolute configuration was based on comparison of the computed and measured spectra for compound (−)-**2b** (see Fig. 1). The calculations were performed for the (*S*)-**2b** enantiomer and the four lowest-energy conformers, as shown in Figure 2, with a total estimated population of 96% were considered for the simulation of the theoretical VCD spectrum. All of these low-energy conformers contain an intramolecular H-bond between the ester carbonyl and the OH group at the β-position, in accordance with the relatively low wavenumber value (~1715 cm^{-1}) of the ester $\nu_{C=O}$ band in the experimental spectrum (a typical, non-H-bonded aliphatic ester would absorb at ~1740 cm^{-1}).

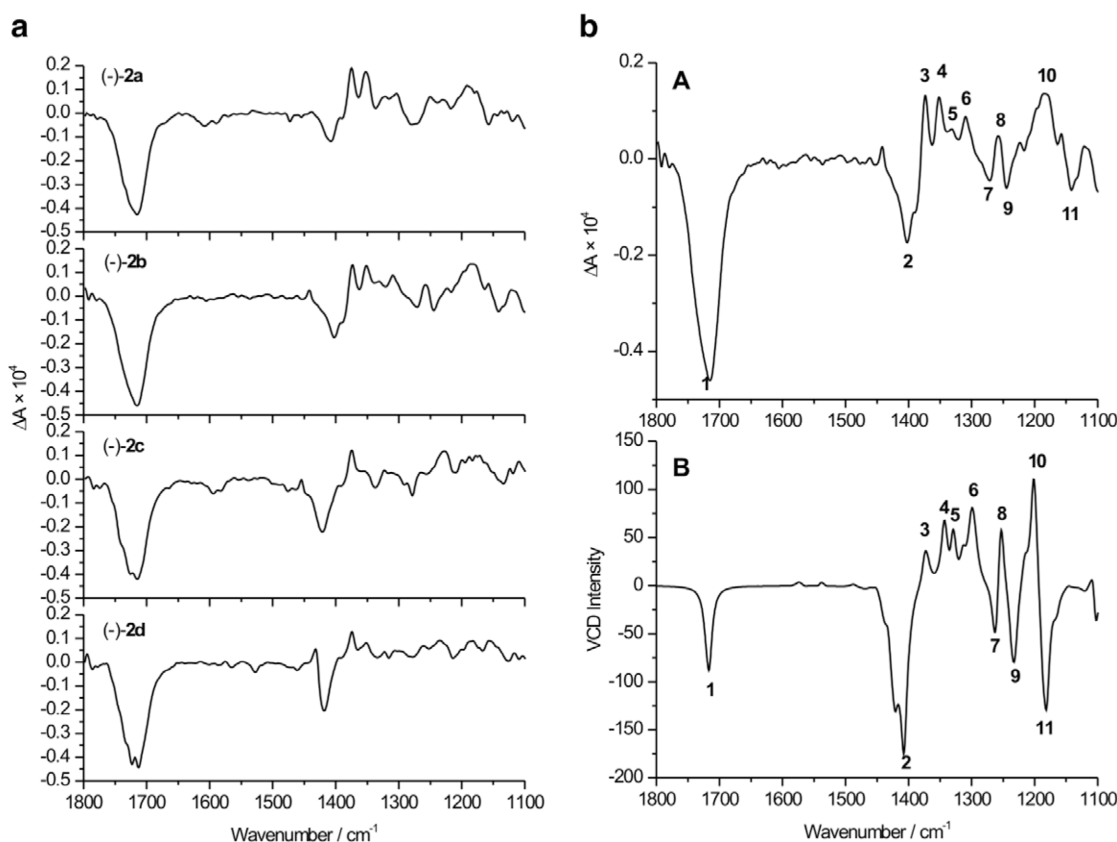


Figure 1. (a) VCD spectra of compounds (−)-**2a–d** measured in $CDCl_3$; (b) VCD spectrum of (−)-**2b** measured in $CDCl_3$ solution (**A**) in comparison with the simulated VCD spectrum of (*S*)-**2b** (**B**), obtained as a population-weighted sum of the calculated spectra of individual conformers. Corresponding bands are labeled with identical numbers.

The agreement between the calculated and measured VCD spectra is reasonably good, both in terms of wavenumber values and the signs of the VCD bands (the matching pairs are labeled with corresponding numbers in Fig. 2) which allows us to unambiguously assign the absolute configuration to be (*S*). Beside the carbonyl vibration (1), bands 3–5 are of particular diagnostic value, being contributed from the coupled bending vibrations of the CH_2, CH, OH, and CH_3 groups. For example, the negative band 2 at 1402 cm^{-1} can be assigned to a $\beta_sCH_2 + \delta CH + \beta OH$ coupled vibration, the positive band 3 at 1374 cm^{-1} is contributed from a $\delta_sCH_3 + \gamma_sCH_2$(ethyl) + $\delta CH + \gamma_sCH_2$ coupled vibration.

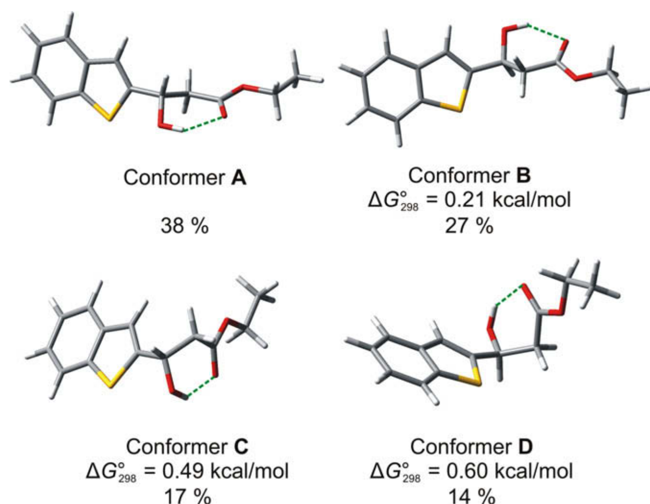


Figure 2. Computed structures of the most abundant equilibrium conformers of (*S*)-**2b** with the indication of their relative Gibbs free energies and estimated populations.

3. Conclusions

The enzyme-catalyzed enantiomer selective hydrolysis of the racemic 3-heteroaryl-3-hydroxypropanoic acid ethyl esters *rac*-**2a**–**d** and 2-(ethoxycarbonyl)-1-heteroaryl-ethyl butyrates *rac*-**3a**–**d**, respectively, and the enantiomer selective acylation of *rac*-**2a**–**d** were studied. After the optimal conditions of the enzymatic kinetic resolutions were found, the preparative-scale multienzymatic synthesis of both highly enantiomerically enriched enantiomers of the 3-heteroaryl-3-hydroxypropanoic acids was performed.

From the high similarity of the experimental VCD spectra of the investigated enantiomeric (−)-**2a**–**d**, especially in the 1800–1300 cm^{-1} spectral region, dominated by vibrations of groups directly attached or close to the stereogenic center and not strongly coupled with vibrations of the heterocyclic moiety, it is reasonable to suppose that all have the same absolute configuration, notably (*S*), as confirmed by quantum chemical calculations in the case of (−)-**2b**.

4. Experimental

4.1. Analytical methods

The ^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometer operating at 300 MHz and 75 MHz, respectively, at 25 °C. Electron impact mass spectra (EI-MS) were taken on a VG 7070E mass spectrometer operating at 70 eV. IR spectra were recorded in KBr pellets on a Jasco 615 FT-IR spectrometer, and the wavenumbers are given in cm^{-1}.

Table 5
The retention times of the enantiomers of *rac*-**2**–**4a**–**d**

Compound	rt (min)
(*S*)-**2a**	28.6
(*R*)-**2a**	27.1
(*S*)-**2b**	35.9
(*R*)-**2b**	38.1
(*S*)-**2c**	10.5
(*R*)-**2c**	11.7
(*S*)-**2d**	11.3
(*R*)-**2d**	13.3
(*S*)-**3a**	15.3
(*R*)-**3a**	14.5
(*S*)-**3b**	21.9
(*R*)-**3b**	27.8
(*S*)-**3c**	7.7
(*R*)-**3c**	8.1
(*S*)-**3d**	8.2
(*R*)-**3d**	8.7
(*S*)-**4a**	9.4
(*R*)-**4a**	8.2
(*S*)-**4b**	10.5
(*R*)-**4b**	9.2
(*S*)-**4c**	9.8
(*R*)-**4c**	8.9
(*S*)-**4d**	10.4
(*R*)-**4d**	10

High performance liquid chromatography (HPLC) analyses were conducted with an Agilent 1200 instrument. Enantiomeric separation of *rac*-**4a**–**d** was performed using the tandem of Astec Chirobiotic-Tag and -R columns (4.6 × 250 mm) and a mixture of methanol and TEAA buffer (pH 4.1), 98:2 (v/v) as eluent. For the enantiomeric separation of *rac*-**2**,**3a**–**b** the tandem of Chiralpak IA and OJ columns (4.6 × 250 mm) and for *rac*-**2**,**3c**–**d** Chiralpak IC column (4.6 × 250 mm) and a mixture of hexane and 2-propanol, 90:10 (v/v) as eluent was used, all at 1 mL/min flow rate (Table 5).

Thin layer chromatography (TLC) was carried out using Merck Kieselgel $60F_{254}$ sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating. Preparative-chromatographic separations were performed using column chromatography on Merck Kieselgel 60 (63–200 μm). Melting points were determined by hot plate method and were uncorrected. Optical rotations were determined on a Bellingham-Stanley ADP 220 polarimeter.

4.2. Reagents and solvents

Commercial chemicals and solvents were purchased from Aldrich or Fluka. All solvents were purified and dried by standard methods as required. Lipases A, AK, AY, F, G, M, R were from Amano, Europe, and England. Lipases from *C. rugosa* (CrL), and Pig liver esterase (PLE) were purchased from Fluka. Lipase B (CAL-B, Novozym 435) and lipase A (CaL-A) from *C. antarctica* and Lypozyme TL IM were purchased from Novozyme, Denmark.

4.3. Synthesis of *rac*-2–4a–d

4.3.1. Synthesis of racemic 3-heteroaryl-3-hydroxy-propanoic acid ethyl esters *rac*-2a–d

A solution of **1a**–**d** (0.6 mol) in tetrahydrofuran (10 mL) was added in small portions with slight warming into the refluxing mixture of zinc powder (40 g, 0.6 mol) and ethyl bromoacetate (83.5 g, 55.5 ml, 0.5 mol) in tetrahydrofuran (100 mL) which was previously heated at reflux for 30 min. After the completion of the reaction (approx. 1 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 redissolved in CH_2Cl_2 (20 mL). The solution was cooled in an ice bath and treated with 10% sulfuric acid (10 mL) under vigorous stirring. After separation, the aqueous phase was extracted with CH_2Cl_2 (3×100 mL). The combined organic layer was washed with saturated $NaHCO_3$ solution (3×50 mL) and water (50 mL), then dried over anhydrous magnesium sulfate, and evaporated in vacuo. The crude product was purified by preparative vacuum-chromatography using dichloromethane–methanol (9:1, v:v) as eluent.

*Ethyl-3-(benzofuran-2-yl)-3-hydroxypropanoate rac-**2a***: Yield: 58%; semisolid; ^1H NMR: (300 MHz, $CDCl_3$): $\delta = 1.29$ (t, 3H); 2.98 (d, 2H); 4.22 (q, 2H); 5.30 (t,1H); 6.69 (s, 1H); 7.21–7.33 (m, 2H); 7.47 (d, 1H); 7.56 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$; 39.8; 61.1; 64.7; 102.9; 111.2; 121.1; 122.8; 124.3; 128.0; 154.8; 157.4; 171.8; IR: IR: $\tilde{\nu} = 3446$, 1734, 1454, 1375, 1253, 1168, 1097; HRMS: M^+ found (M^+ calculated for $C_{13}H_{14}O_4$): 234.0890 (234.08921); MS m/z (%): 235(M+1, 0.8), 234(M^+, 7), 160(2), 147(18), 146(5), 145(8), 144(2), 91(4), 89(2), 58(27), 43(100), 42(6), 39(3).

*Ethyl-3-(benzo[b]thiophen-2-yl)-3-hydroxypropanoate rac-**2b***: Yield: 61%; semisolid; ^1H NMR: (300 MHz, $CDCl_3$): $\delta = 1.29$ (t, 3H); 2.94 (d, 2H); 4.21 (q, 2H); 5.46 (t, 1H); 7.32 (s, 1H); 7.29–7.39 (m, 2H); 7.73 (d, 1H); 7.83 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$; 42.8; 61.1; 67.0; 120.1; 122.4; 123.5; 124.2; 124.3; 139.3; 139.4; 147.0; 171.8; IR: $\tilde{\nu} = 3461$, 1731, 1457, 1373, 1254, 1162, 1052; HRMS: M^+ found (M^+ calculated for $C_{13}H_{14}O_3S$): 250.06636 (250.06637); MS m/z (%): 253(M+3, 4), 251(M+1, 9), 250(M^+, 49), 235(20), 233(20), 211(16), 209(17), 205(10), 203(53), 199(11), 165(18), 163(75), 162(53), 161(41), 157(100), 135(58), 134(26), 115(42), 97(15), 91(19), 89(17), 43(55).

*Ethyl-3-(benzofuran-3-yl)-3-hydroxypropanoate rac-**2c***: Yield: 56%; semisolid; ^1H NMR: (300 MHz, $CDCl_3$): $\delta = 1.29$ (t, 3H); 2.92 (d, 2H); 4.2 (q, 2H); 5.4 (t, 1H); 7.23–7.35 (m, 2H); 7.49 (d, 1H); 7.59 (s, 1H); 7.68 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.2$; 41.4; 61.0; 63.5; 116.6; 120.3; 122.4; 122.7; 124.6; 125.8; 141.4; 155.6; 177.3; IR: $\tilde{\nu} = 3446$, 1734, 1454, 1375, 1253, 1168, 1034; HRMS: M^+ found (M^+ calculated for $C_{13}H_{14}O_4$): 234.08920 (234.08921); MS m/z (%): 235(M+1, 7), 234(M^+, 36), 233(M−1, 36) 157(17), 148(69), 147(100), 146(38), 145(27), 131(36), 119(30), 115(13), 92(14), 91(78), 89(17), 77(12), 65(10), 43(15).

*Ethyl-3-(benzo[b]thiophen-3-yl)-3-hydroxypropanoate rac-**2d***: Yield: 54%; semisolid; ^1H NMR: (300 MHz, $CDCl_3$): $\delta = 1.30$ (t, 3H); 2.93 (d, 2H); 4.25 (q, 2H); 5.54 (t, 1H); 7.37–7.41 (m, 2H); 7.44 (s, 1H); 7.87 (d, 1H); 7.9 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$; 41.6; 61.0; 66.09; 112.09; 122.6; 122.9; 124.19; 124.53; 136.96; 137.4; 140.8; 172.5; IR: $\tilde{\nu} = 3446$, 1732, 1459, 1373, 1279, 1180, 1026; HRMS: M^+ found (M^+ calculated for $C_{13}H_{14}O_3S$): 250.06636 (250.06637); MS m/z (%): 252(M+2, 2), 251(M+1, 5), 250(M^+, 39), 164(7) 163(72), 162(22), 161(17), 136(7), 135(62), 134(19), 115(6), 91(16), 89(12), 82(8), 77(7), 58(24), 43(100), 42(6).

4.3.2. Synthesis of racemic 1-heteroaryl-3-ethoxy-3-oxopropyl butyrates *rac*-3a–d by chemical acylation of the corresponding racemic ethyl 3-heteroaryl-3-hydroxy-propanoates *rac*-2a–d

The mixture of one of the racemic 3-heteroaryl-3-hydroxypropanoic acid ethyl esters *rac*-**2a**–**d** (50 mmol), *n*-butanoic 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 for 8 h. After cooling to room temperature, the cobalt(II) chloride was filtered off. The filtrate was evaporated in vacuo, and the crude solid product was purified by preparative vacuum-chromatography using dichloromethane as eluent.

*2-(Ethoxycarbonyl)-1-(benzofuran-2-yl)-ethyl butyrate rac-**3a***: Yield: 96%; semisolid; ^1H NMR: (300 MHz, $CDCl_3$): $\delta = 0.94$ (t, 3H); 1.25 (t, 3H); 1.66 (m, 2H); 2.33 (t, 2H); 2.99–3.20 (m, 2H); 4.17 (q, 2H); 6.41–6.46 (m, 1H); 6.76 (s, 1H); 7.21–7.34 (m, 2H); 7.48 (d, 1H); 7.57 (d, 1H); ^{13}C NMR(75 MHz, $CDCl_3$): $\delta = 13.5$; 14.1; 18.3; 36.0; 37.8; 60.9; 65.1; 105.4; 111.4; 121.4; 122.9; 124.7; 127.3; 153.7; 154.8; 169.3; 172.3; IR: $\tilde{\nu} = 2967$, 1747, 1456, 1376, 1253, 1168, 1097, 1027; HRMS: M^+ found (M^+ calculated for $C_{17}H_{20}O_5$): 304.13107 (304.13107); MS m/z (%): 305(M+1, 7), 304(M^+, 36), 234(37), 233(100), 216(56), 188(17), 187(22), 171(63), 160(24), 145(77), 131(10), 115(43), 91(6), 89(12), 71(22), 63(5), 43(26).

*2-(Ethoxycarbonyl)-1-(benzo[b]thiophen-2-yl)-ethyl butyrate rac-**3b***: Yield: 95%; semisolid; ^1H NMR: (300 MHz, $CDCl_3$): $\delta = 0.94$ (t, 3H); 1.26 (t, 3H); 1.61–1.75 (m, 2H); 2.33 (t, 2H); 2.93–3.16 (m, 2H); 4.18 (q, 2H); 6.56–6.60 (m, 1H); 7.32 (s, 1H); 7.34–7.39 (m, 2H); 7.75 (d, 1H); 7.81 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 13.6$; 14.1; 18.3; 36.1; 41.2; 61.0; 67.8; 122.4; 122.6; 123.9; 124.4; 124.7; 139.0; 139.4; 142.5; 169.2; 172.3; IR: $\tilde{\nu} = 2966$, 1741, 1458, 1375, 1252, 1173, 1078; HRMS: M^+ found (M^+ calculated for $C_{17}H_{20}O_4S$): 320.10824 (320.10523); MS m/z (%): 321(M+1, 0.7), 320(M^+, 1), 235(12), 232(3), 187(3), 161(4), 157(4), 115(4), 71(3), 60(7), 58(26), 43(100), 42(7), 41(3), 39(4).

*2-(Ethoxycarbonyl)-1-(benzofuran-3-yl)-ethyl butyrate rac-**3c***: Yield: 94%; semisolid; ^1H NMR: (300 MHz, $CDCl_3$): $\delta = 0.91$ (t, 3H); 1.25 (t, 3H); 1.58–1.71 (m, 2H); 2.31 (t, 2H); 2.91–3.22 (m, 2H); 4.16 (q, 2H); 6.50–6.55 (m, 1H); 7.25–7.36 (m, 2H); 7.50 (d, 1H); 7.68 (s, 1H); 7.72 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 13.5$; 14.1; 18.3; 36.1; 39.8; 60.9; 64.6; 111.7; 119.1; 120.3; 122.9; 124.7; 125.5; 142.8; 155.5; 169.6; 172.5; IR: $\tilde{\nu} = 2966$, 1741, 1454, 1371, 1255, 1172, 1107, 1026; HRMS: M^+ found (M^+ calculated for $C_{17}H_{20}O_5$): 304.13107 (304.13107); MS m/z (%): 305(M+1, 7), 304(M^+, 43), 235(12), 234(100), 233(63), 218(13), 217(11), 216(14), 188(45), 175(10), 171(28), 160(12), 148(27), 147(36), 146(46), 145(69), 144(30), 131(27), 115(33), 91(12), 89(10), 71(95), 43(71).

*2-(Ethoxycarbonyl)-1-(benzo[b]thiophen-3-yl)-ethyl butyrate rac-**3d***: Yield: 95%; semisolid; ^1H NMR: (300 MHz, $CDCl_3$): $\delta = 0.93$ (t, 3H); 1.24 (t, 3H); 1.63–1.76 (m, 2H); 2.34 (t, 2H); 2.94–3.18 (m, 2H); 4.16 (q, 2H); 6.63–6.68 (m, 1H); 7.34–7.46 (m, 2H); 7.47 (s, 1H); 7.87 (d, 1H); 7.98 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 13.5$; 14.0; 18.1; 35.9; 40.2; 60.9; 67.3; 122.2; 122.9; 124.33; 124.38; 124.6; 134.1; 136.7; 140.6; 169.8; 172.5; IR: $\tilde{\nu} = 2966$, 1745, 1460, 1371, 1252, 1171, 1097; HRMS: M^+ found (M^+ calculated for $C_{17}H_{20}O_5$): 304.21106 (304.13107); MS m/z (%): 321(M+1, 2), 320(M^+, 11), 250(36), 249(9), 187(10), 163(9), 162(7),161(21), 160(9), 149(22), 135(7),115(12), 71(19), 60(13), 58(23), 43(100).

4.3.3. Synthesis of racemic 3-heteroaryl-3-hydroxy-propanoic acids *rac*-4a–d

4.3.3.1. Chemical hydrolysis. The mixture of *rac*-**2a**–**d** (200 mg) and potassium carbonate (400 mg) in water (50 mL) was heated at reflux for 1 h. The solution was cooled to room temperature, and pH was adjusted to pH 1.5 with concd HCl solution. The product was extracted with ethyl acetate (3×10 mL). The organic layer was dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo, resulting in *rac*-**4a**–**d** as white crystals.

4.3.3.2. Enzymatic hydrolysis. To a mixture of *rac*-**2a**–**d** (200 mg) in water (10 mL), pig liver esterase (100 mg) was added, and the mixture was shaken for 30 min at 1000 rpm at room temperature. After the reaction was completed (checked by TLC), the formed *rac*-**4a**–**d** were extracted with ethyl acetate (3×10 mL). The separated organic layer was dried over anhydrous sodium sulfate, and solvent was removed in vacuo affording the pure product (*rac*-**4a**–**d**) as white crystals.

*3-(Benzofuran-2-yl)-3-hydroxypropanoic acid rac-**4a***: Yield: 98%; mp: 96–97 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ = 2.68–2.88 (m, 2H); 5.01–5.09 (m, 1H); 6.77 (s, 1H); 7.19–7.36 (m, 2H); 7.52–7.61 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 41.4; 64.0; 102.6; 111.4; 121.5; 123.2; 124.4; 128.4; 154.4; 160.3; 172.2; IR: $\tilde{\nu}$ = 3446, 3120, 1681, 1454, 1299, 1253, 1170, 1081; HRMS: M$^+$ found (M$^+$ calculated for $C_{11}H_{10}O_4$): 206.05790 (206.05791); MS *m/z* (%): 207(M+1, 5), 206(M$^+$,47), 188(6), 167(7), 149(17), 147(100), 146(17), 145(7), 118(5), 91(14), 89(5).

*3-(Benzo[b]thiophen-2-yl)-3-hydroxypropanoic acid rac-**4b***: Yield: 98%; mp: 133–135 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ = 2.70–2.86 (m, 2H); 5.31–5.34 (m, 1H); 6.08 (broad s, 1H); 7.30–7.33 (m, 3H); 7.76–7.79 (m, 1H); 7.90–7.94 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 44.7; 66.5; 119.8; 122.8; 123.8; 124.4; 124.6; 138.9; 139.8; 150.8; 172.2; IR: $\tilde{\nu}$ = 3419, 3185, 1668, 1437, 1282, 1087; HRMS: M$^+$ found (M$^+$ calculated for $C_{11}H_{10}O_3S$): 222.03506 (222.03506); MS *m/z* (%): 222(M$^+$, 1), 163(2), 162(1), 135(1), 59(1), 58(10), 57(1), 44(2), 43(100), 42(5), 41(1), 39(3), 38(18).

*3-(Benzofuran-3-yl)-3-hydroxypropanoic acid rac-**4c***: Yield: 97%; mp: 105–106 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ = 2.76–2.84 (m, 2H), 5.22–5.28 (m, 1H); 7.20–7.35 (m, 2H); 7.52–7.55 (m, 1H); 7.75–7.77 (m, 1H); 7.85 (s, 1H); ^{13}C NMR(75 MHz, DMSO-d_6: δ = 43.0; 62.8; 111.8; 121.3; 123.0; 124.5; 124.8; 126.7; 142.2; 155.4; 172.8; IR: $\tilde{\nu}$ = 3442, 3197, 1716, 1454, 1309, 1282, 1176, 1105, 1059; HRMS: M$^+$ found (M$^+$ calculated for $C_{11}H_{10}O_4$): 206.05790 (206.05791); MS *m/z* (%): 207(M+1, 6), 206(M$^+$, 51), 188(6), 188(7), 149(12), 148(9), 147(100), 146(32), 145(14), 131(7), 119(6), 115(8), 92(6), 91(36), 89(8), 63(5), 58(15), 43(45).

*3-(Benzo[b]thiophen-3-yl)-3-hydroxypropanoic acid rac-**4d***: Yield: 97%; mp: 131–132 °C; ^{1}H NMR: (300 MHz, DMSO-d_6): δ = 2.76–2.85 (m, 2H); 5.35–5.41 (m, 1H); 7.33–7.45 (m, 2H); 7.60 (s, 1H); 7.92–8.05 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 43.7; 66.1; 123.4; 123.5; 123.8; 125.0; 125.3; 138.1; 140.6; 141.1; 173.3; IR: $\tilde{\nu}$ = 3521, 2971, 1690, 1429, 1282, 1173, 1093, 1063; HRMS: M$^+$ found (M$^+$ calculated for $C_{11}H_{10}O_3S$): 222.03506 (222.03506); MS *m/z* (%): 222(M$^+$, 2), 218(2), 204(1), 187(1), 177(7), 164(2), 163(17), 162(4), 161(3), 149(2), 135(10), 134(3), 115(3), 43(2).

4.4. Analytical scale enzymatic kinetic resolution of *rac*-2,3a–d

4.4.1. Enzymatic hydrolysis of *rac*-2a–d

A mixture of enzyme (10 mg) and one of *rac*-**2a**–**d** (10 mg) in water (300 μL) was shaken at 1000 rpm and room temperature. Samples (2 μL) were taken at 30 min intervals and diluted with a mixture of hexane–water (1:1, v/v, 400 μL). After phase separation, the aqueous layer was extracted with ethyl acetate (100 μL). Samples (20 μL) from both hexane and ethyl acetate solution were analyzed with HPLC and TLC.

4.4.2. Enzymatic hydrolysis of *rac*-3a–d

A mixture of enzyme (10 mg) and one of *rac*-**3a**–**d** (10 mg) in cyclohexane–THF mixture (1:1, v/v, 300 μL) and water (10 equiv) was shaken at 1000 rpm and room temperature. Samples (2 μL) were taken at 30 min intervals diluted with hexane (400 μL), dried over anhydrous sodium sulfate, filtered, and analyzed with HPLC and TLC.

4.4.3. Enantiomer selective O-acylations of 3-heteroaryl-3-hydroxy-propanoic acid ethyl esters *rac*-2a–d

To a solution of *rac*-**2a**–**d** (20 mg) in organic solvent (200 μL), vinyl ester (20 μL) and enzyme (10 mg) were added, and the mixture was stirred at room temperature. Samples from the reaction mixture (2 μL) were taken at 6-h intervals, diluted with *n*-hexane (400 μL), filtered, and analyzed with HPLC.

4.4.4. Enzymatic hydrolysis of both (*R*)- and (*S*)-3a–d

The reaction and analyses were performed as described in Section 4.4.2.

4.4.5. Enzymatic hydrolysis of both (*R*)- and (*S*)-2a–d

The reaction and analyses were performed as described in Section 4.4.1.

4.5. Preparative-scale enzymatic synthesis of both (*R*)- and (*S*)-4a–d

A mixture of *rac*-**2a**–**b** (200 mg), vinyl butyrate (200 μL) and Novozym 435 (100 mg) in cyclohexane (6 mL) was shaken at room temperature. For the enzymatic resolution of *rac*-**2c**–**d** CrL (200 mg), substrate (200 mg) in cyclohexane–THF mixture (1:1, v/v, 6 mL) and water (10 equiv) were shaken at room temperature. Samples from the reaction mixture (2 μL) were diluted with *n*-hexane (400 μL) and analyzed with HPLC. The reactions were stopped by filtering the enzyme at an approximately 50% conversions. Solvents were removed in vacuo, and the crude product was purified by vacuum chromatography, using a mixture of dichloromethane–methanol (9:1, v:v) as eluent, resulting in both optically active 3-heteroaryl-3-hydroxypropanoates (*S*)-**2a**–**b** and (*R*)-**2c**–**d** and 3-heteroaryl-3-acetoxy-propanoates (*R*)-**3a**–**b** and (*S*)-**3a**–**b** as semisolids. The whole amount of the previously isolated (*R*)-**3a**–**b** and (*S*)-**3c**–**d**, BCL and water (10 equiv) in a cyclohexane–THF mixture (1:1, v/v) were shaken at 1000 rpm until the transformation of the substrate was completed (checked chromatographically). The dilution and substrate–enzyme *ratio* was the same as described in Section 4.4.2. The enzyme was filtered off and solvents were removed in vacuo. The crude product was purified by preparative vacuum-chromatography using dichloromethane–methanol (9:1, v:v) as eluent.

One or both (*R*)- and (*S*)-**2a**–**d** was suspended in water and PLE was added. The dilution and substrate–enzyme *ratio* was the same as described in Section 4.4.2. The reaction mixture was shaken at 1000 rpm. After hydrolysis of the esters was completed (checked by TLC, approx. 15 min), the formed 3-heteroaryl-3-hydroxy-propanoic acids (*R*)- and (*S*)-**4a**–**d** were isolated by extraction with ethyl acetate (3 × 5 mL). The organic layer was dried over anhydrous sodium sulfate, the solvent was removed in vacuo, affording the enantiomerically enriched products (*R*)- and (*S*)-**4a**–**d**.

IR, NMR, and MS spectra of the optically active ethyl 3-heteroaryl-3-hydroxypropanoates (*S*)- and (*R*)-**2a**–**d**, 1-heteroaryl-3-ethoxy-3-oxopropyl butyrates (*R*)-**3a**–**b** and (*S*)-**3c**–**d** and 3-heteroaryl-3-hydroxypropanoic acids (*S*)- and (*R*)-**4a**–**d** were indistinguishable from those of their racemates. Data on yield, enantiomeric composition, and specific rotation of the products (*S*)- and (*R*)-**2**,**4a**–**d** and (*R*)-**3a**–**b** and (*S*)-**3c**–**d** are shown in Table 4.

4.6. VCD spectroscopy and quantum chemical calculations

VCD spectra at a resolution of 4 cm^{-1} were recorded in $CDCl_3$ solution with a Bruker PMA 37 VCD/PM-IRRAS module connected to an Equinox 55 FTIR spectrometer.

The ZnSe photoelastic modulator of the instrument was set to 1300 cm^{-1}, and an optical filter with a transmission range of 1800–800 cm^{-1} was used in order to optimize the sensitivity in the carbonyl and fingerprint regions. The instrument was calibrated for VCD intensity with a CdS multiple-wave plate. A BaF_2 cell with a pathlength of 50 μm and sample concentrations of 100 mg/mL [for compounds (−)-**2a**–**c**] and 125 mg/mL [for compound (−)-**2d**] were used. In order to increase the signal/noise ratio of the low-intensity VCD signals, measurement times of 7 h (corresponding to ∼24,000 accumulated interferograms) were

applied. Baseline correction was achieved by subtracting the spectrum of the solvent obtained under the same conditions.

Geometry optimizations and the computation of vibrational frequencies and VCD rotatory strengths were performed for (*S*)-**2b** at the B3LYP/6–31G(d) DFT level of theory with the GAUSSIAN 03 quantum chemical software package.[23] The vibrational frequencies were scaled by a factor of 0.963.

VCD curves were simulated from the calculated wavenumber and rotatory strength data by using Lorentzian band shape and a half-width at half-height value of 6 cm^{-1}.

Various conformers of compound (*S*)-**2b** were analyzed, out of which four had more than 1% estimated population (the contribution of other, higher-energy conformers could be neglected). The simulated VCD spectrum was obtained as the population-weighted sum of the calculated VCD spectra of the individual conformers.

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