Babeş-Bolyai University

Cluj-Napoca

Faculty of Chemistry and Chemical Engineering

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PhD Thesis Abstract

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Cluj-Napoca

2015

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Jury

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Defense: July 2015

Table of contents

Keywords: lipase, biocatalysis, hydroxy acids, kinetic resolution, organic synthesis

1. Introduction

Chirality is certainly one of the most intriguing phenomena in nature. It generally leads to specific activities and properties that differentiate stereoisomers. As a result, there is a high demand for chiral building blocks, and the search for new and efficient methods for the synthesis of enantiomerically pure compounds has been a major area of research in chemistry.

The structural complexity of novel drugs is continuously increasing and considering that the human body functions using chiral catalysts, the trend for new chiral pharmaceutical reagents is also increasing, therefore, nowadays these chiral compounds are usually obtained in single enantiomeric form.

Biocatalysis is used for the synthesis of optically pure compounds due to the high enantio- and stereospecificity of modern enzymatic catalysts. It has emerged as a modern field, meeting the demands of green chemistry and sustainable development. The main advantages of using biocatalysts (an isolated enzyme or whole-cell) are reducing energy cost (due to the mild reaction conditions: pH, temperature, pressure) as well as minimizing production and disposal of waste. Enzymes are environmentally friendly and compatible to each other, enabling sequential reactions.

Chiral carboxylic acids are important building blocks for the synthesis of many pharmaceutical drugs and natural compounds, where substances of high enantiomeric excess are often needed. These compounds are used as chiral intermediates because they have at least two functional groups (hydroxyl and carboxyl groups) which can be chemically transformed into various other functional groups.

Kinetic resolution has the advantage of providing both enantiomers of a racemate with excellent enantiopurity in the presence of a highly enantioselective enzyme. The main drawbacks of traditional kinetic resolution are maximum 50% theoretical yield and the decrease of the enantiomeric excess (*ee*) with the increase of conversion above 50%.

This thesis addresses the field of lipase-based catalysis, in organic solvents, useful in developing highly selective and ecofriendly processes for obtaining enantiopure hydroxy acids.

1.1. Optically pure hydroxy acids and their derivatives

Optically pure hydroxycarboxylic acids can be widely used as chiral precursors for several reasons: they contain at least two functional groups: a hydroxy group and a carboxy group; the functional groups can easily be modified chemically; and a second chiral center can be introduced.

1.1.1. α-Hydroxy acids

Chiral carboxylic acids are important building blocks for the synthesis of many pharmaceutical drugs[1] and natural compounds such as pheromones[2] and pesticides,[3] in which substances of very high enantiomeric excess (>99% *ee*) are often needed. Particularly, pure isomers of (*R*)- and (*S*)-α-hydroxy-phenylacetic acid (mandelic acid) and their esters are very useful in organic synthesis. (*R*)-Mandelic acid is used for the synthesis of interesting cephalosporin antibiotics such as Cephamandole and Cephonicid[4], penicillins, anti-tumor agents and anti-obesity agents[5]. Enantiomerically pure acids are also used in the resolution of racemates by selective precipitation.[6] Optically active α-hydroxy acids and α-ketoacids bearing a substituent with an aryl group, such as phenyllactic acid, phenylpyruvic acid, mandelic acid, benzoylformic acid and their derivatives, play significant roles as synthons in the synthesis of natural and biologically active compounds[7]. (*R*)-2-Hydroxy-2-(2'-chlorophenyl)acetic acid is the key chiral intermediate in the synthesis of (*S*)-clopidogrel, a platelet aggregation inhibitor, used for heart attack and stroke treatment.

1.1.2. β-Hydroxy acids

Optically active β-hydroxy-β-aryl-propanoic acids and their derivatives are highly functionalized chiral synthons, of which the chiral β-hydroxy-β-aryl propionates are precursors for the synthesis of enantiopure pharmaceuticals covering a plethora of actions. Optically active β-hydroxycarboxylic acids and their derivatives bearing various aromatic moieties have been used as starting materials for the preparation of enantiopure bioactive compounds, such as vitamins, antibiotics, pheromones and flavor compounds.[8]

β-Hydroxy esters are versatile chiral intermediates in the synthesis of several pharmaceuticals and fine chemicals. For instance, (*R*)-3-hydroxybutanoate esters serve as starting materials for β-lactam antibiotics, including carbapenems and penems with broad antimicrobial spectra, and for dorzolamide, a topically active human carbonic anhydrase II inhibitor.[9]

Amongst aryl-substituted β-hydroxy esters, ethyl (*S*)-3-hydroxy-3-(thiophen-2-yl)-propanoate is a precursor for the synthesis of duloxetine (a serotonin-norepinephrine re-uptake inhibitor) and ethyl-(*R*)-3-hydroxy-3-phenylpropanoate for that of atomoxetine (a norepinephrine re-uptake inhibitor).[10]

1.2. Kinetic resolution using lipases

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are perhaps the most frequently used enzymes in organic chemistry because they couple a broad range of substrate specificity with high regio- and enantioselectivity, and thus, they have been employed as catalysts for kinetic resolutions of racemates (e.g. racemic secondary alcohols, racemic carboxylic acids, etc).[11] From a chemical point of view, lipases can be considered as mild and selective reagents that are able to activate a generic carboxylate and transfer it to a large number of nucleophiles in different organic solvents. Amongst the very large collection of enzymes, lipases have emerged as one of the most suitable enzymes for kinetic resolution processes in asymmetric synthesis. Most lipases can accept a broad range of unnatural substrates and are thus very versatile for applications in organic synthesis. Microbial lipases represent the most important class of biocatalysts used for a wealth of applications in organic synthesis. Some of the advantages of using lipases are that, they do not require cofactors, many lipases are available in free and immobilized form, and can be produced in large quantities.

Industrial application is often hampered by a lack of long-term operational stability along with difficult recovery and reuse of the enzyme. These drawbacks can often be overcome by immobilization of the enzyme. Among the advantages of immobilization, there is the easier handling of the enzyme, the facile separation from the product, thereby minimizing protein contamination of the product, it also facilitates the efficient recovery and reuse of costly enzymes, for economic viability, and enables their use in continuous, fixed-bed operation. A further benefit is enhanced stability,[12] and operational conditions, towards denaturation by heat or organic solvents or by autolysis.

Enzymatic reactions are known to be highly sensitive to the solvent selection, especially in hydrophilic solvent systems. Generally, comparable to the hydrophobic counterparts, the hydrophilic solvents could more easily strip the“essential water” bound to the lipase, which is necessary to preserve the flexibility of the enzyme conformation; this phenomenon deactivates the lipase.[13] The hydrophobicity of the solvent, defined as log *P* (the logarithm of the partition coefficient of a compound between *n*-octanol and water), is the physical property of a solvent which best describes the solvent effect on enzyme activity.[14]

The acyl donor may influence the equilibrium position and the rate of acylation/deacylation. Many investigators hypothesized that increasing the amount of acyl donor could aid in forming the acyl-enzyme intermediate and accelerate the reaction rate[15].

Regarding the lipase-catalyzed transesterification of vinyl esters and alcohols, it is known to result in the formation of acetaldehyde which could deactivate microbial lipases,

presumably by structural changes caused by initial Schiff-base formation at solvent accessible lysine residues.

Three main routes towards enantiopure compounds are available: making use of chiral pool, resolution (separation) of racemates and asymmetric synthesis.The strategies for the generation of single enantiomers from racemates include kinetic resolution, dynamic kinetic resolution, stereoinversion (via nonchiral intermediate to yield its mirror-image counterpart as the sole product) and enantioconvergent process (transformation of substrate enantiomers through opposite stereochemical pathways forming only one enantiomer product).[16]

Simple kinetic resolution[17] relies on a difference in reaction rate between enantiomers in order to obtain enantioenriched material. Enzymatic kinetic resolution is based on the difference between the reaction rates of the enantiomers of a racemate in the presence of an enzyme as a chiral catalyst. In the optimal case of a kinetic resolution, the transformation of one of the enantiomers into the product takes place while the other enantiomer stays unreacted. There are advantages for these processes such as higher reaction rates up to 10^{12} times (compared to chemical methods), improved efficiency, higher chemo-, regio- and enantioselectivity. The selectivity factor is defined as the *ratio* of the rates of the two reactions. In the absence of selectivity, in a simple kinetic resolution, a racemic mixture of products would be formed. Ideally, a large rate difference would exist and one could obtain enantioenriched product in a 50% yield, as well as recover enantioenriched starting material in 50% yield. A disadvantage of this strategy is that in order to obtain high yields of each compound (~50%) with excellent enantioenrichment (≥95% *ee*), high selectivity factors (»200), which are difficult to achieve, are required.[18]

Empirical rules have been formulated to predict the fast reacting enantiomer for lipases. The rule proposed by Kazlauskas[19] to predict the fast reacting enantiomer of a racemic secondary alcohol during acylation in the presence of *Burkholderia cepacia* lipase also proved to be valid for many other lipases. According to this rule, the discrimination of the enantiomers is based on the size of the substituents (medium-size substituent, large-size substituent) attached to the asymmetric center, which bind to different hydrophobic pockets at the active site of the enzyme.

In this thesis, the efforts to increase the enantioselectivity are oriented towards conditions of the lipase-catalyzed kinetic resolution which are easy to be adjusted in a synthetic chemistry laboratory.

1.3. Absolute configuration

Absolute configuration refers to the spatial arrangement of the atoms of a physically identified chiral molecular entity (or group) and its stereochemical description (e.g., (*R*) or (*S*), (P) or (M), D or L, etc). The determination of absolute configuration in chiral molecules is a problem of significant importance to molecular stereochemistry, therefore many methods have been developed over the years to address this problem. Among them: X-ray crystallography, followed by chiroptical methods (e.g., circular dichroism (CD), optical rotatory dispersion (ORD), or specific optical rotation).

The optical activity is a very useful way of characterizing the bulk compound, unfortunately, polarimeters require a higher concentration than that obtained by dissolving the single crystal used for diffraction measurements, as it would be more helpful to characterize the optical activity in solution of only this single crystal.[20]As the measurement of specific rotation is a single-wavelength technique, the presence and effect of impurities can go without detection, also optical rotation can only provide a measure of enantiomeric excess if the specific rotation of the enantiomerically pure compound is sufficiently strong and has been determined previously.

However, one of the most widely used experimental techniques is without a doubt nuclear magnetic resonance (NMR). This technique is appealing because of its advantages, which include the following: the instrument is available in most laboratories, an in-depth understanding of the fundamentals of the method is not necessary to apply the method, a small amount of sample is needed which can be recovered and it is applicable to both solid and liquid samples because the analysis is conducted in solution. The analysis of the derivatives prepared from the substrate and the two enantiomers from a chiral derivatizing agent (CDA) is the most widely used approach to determine absolute configurations as the covalent bonding of the substrate and the auxiliary reagent produces species with a greater conformational rigidity, which in turn produces greater differences in the NMR spectra. For the derivatization of the substrate, there are two options: (a) the preparation of two derivatives from the two enantiomers of the chiral derivatizing agent and the substrate (double derivatization), and (b) the preparation of a single derivative from the substrate and one enantiomer of the chiral derivatizing agent (single derivatization). Among the designed auxiliary agents, there are: α-methoxyphenylacetic acid (MPA), α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA), 9-anthrylmethoxyacetic acid (9-AMA,) Boc-phenylglycine (BPG), etc.[21]

2. Synthesis of optically pure hydroxy acids and their derivatives

2.1. Chemical synthesis

2.1.1. α-Hydroxy acids

Although the chemical method is the most popular way to prepare chiral α-hydroxy acids and α-ketoacids in industry, it does not always satisfactorily work in terms of their enantioselectivity or yield. Utilization of chemical catalysts has also made the green production of α-hydroxy acids and α-ketoacids difficult to accomplish. Among the methods developed, there are the asymmetric Friedel-Crafts alkylation of aromatic compounds with glyoxylic acid ethyl ester in presence of (*S*)-6,6'-dibromo-1,1'-binaphthalene-2,2'-diol[22], the enantioselective scandium-catalyzed vinylsilane additions as a new approach to the synthesis of enantiopure *β,γ*-unsaturated α-hydroxy acid derivatives[23], the enantioselective synthesis of α-hydroxy esters by ruthenium catalyzed 1,2-addition of arylboronic acids to *t*-butyl glyoxylate[24] or organocatalyst mediated reduction[25] of keto-esters.

2.1.2. β-Hydroxy acids

Extensive effort in obtaining optically active β-hydroxy acids or their derivatives has resulted in fruitful synthetic methods for their synthesis. The aldol reaction is one of the most important methods for the stereoselective construction of complex acyclic molecules. Enantioselective aldol reaction of *tert*-butyl acetate using titanium-carbohydrate complexes was achieved in high optical purity by Duthaler, R. O.*et al*[26]. The chiral auxilary trans-2-phenylcyclohexanol provided a good level of asymmetric induction as a chiral Reformatsky reagent for the enantioselective synthesis of β-hydroxy acids[27]. Regioselective carbomethoxylation of chiral epoxides proved to be a good method for obtaining enantiomerically pure β-hydroxy esters[28]. Also, converting β-oxocarboxylic acids to chiral β-hydroxy carboxylic acids by employing DIP-Cl™ (B-Chlorodiisopinocampheylborane) as the reducing agent, resulted in high enantioselectivity[29].

2.2. Enzymatic synthesis

2.2.1. α-Hydroxy acids

Biocatalysis has emerged as a powerful strategy for the production of optically active α-hydroxy acids due to its remarkable stereoselectivity and high yield[30]. Various biocatalytic approaches to the synthesis of enantiopure α-hydroxy acids have been developed,[31] including reductase-catalyzed asymmetric reduction of the corresponding keto ester,[32] nitrilase-catalyzed enantioselective hydrolysis of mandelonitrile and its derivatives[33] and esterase or

lipase catalyzed resolution of the *O*-acetates or esters of hydroxy acids[34]. By using dynamic kinetic resolution,[35] deracemization[36] of the racemic substrates or bioreduction[37] it is possible to produce one of the enantiomers with theoretically 100% yield. In the case of kinetic resolution, it is possible to use biocatalysts, especially lipases,[38,39,40] for selective acylation[41] or hydrolysis[42] in order to obtain the desired substances and both of the enantiomers can be obtained with good optical purity at a theoretical yield of 50%.[43].

Biocatalytic oxidation could be an environmentally benign procedure as a substitute for traditional chemical reactions[44] and furthermore, enantioselective oxidation of racemic α-hydroxy acids by α-hydroxy acid dehydrogenases (α-HADHs) could produce the corresponding α-ketoacids and optically active α-hydroxy acids with high enantiomeric excess (*ee*) as remaining substrates[45].

Among these pathways, the enzymatic resolution of *O*-acetylated hydroxy acids by esterase or lipase shows great potential for application due to their practical use in chemical industry,[46] therefore it has been extensively studied.[47]

2.2.2. β-Hydroxy acids

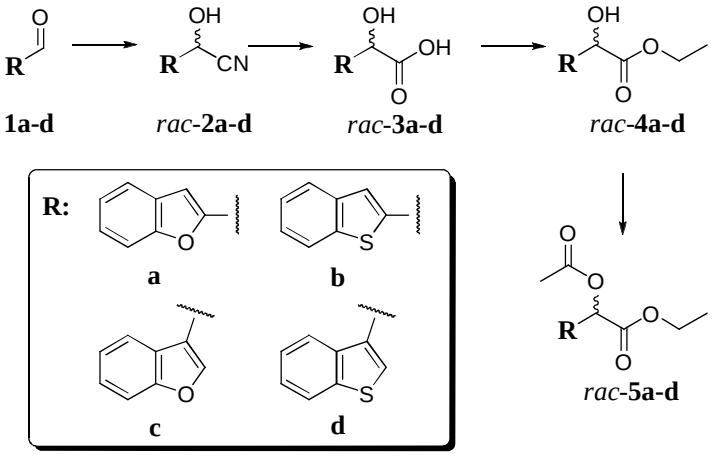
A large variety of chemo- and biocatalytic procedures used for the enantioselective preparation of 3-hydroxypropanoic acids and their derivatives were reported.[48]

Several chiral organometallic or enzymatic procedures are available: enantioselective reduction of the corresponding β-ketoesters; kinetic or dynamic kinetic resolution (KR or DKR) of the racemic β-hydroxycarboxylic acids and their derivatives; deracemization of β-hydroxy esters[49]. The enzymatic kinetic resolution of various chiral substrates with fatty acids or their derivatives as acyl donors or the stereoselective enzymatic hydrolysis of long chain fatty acid esters have already been investigated by our group[50].

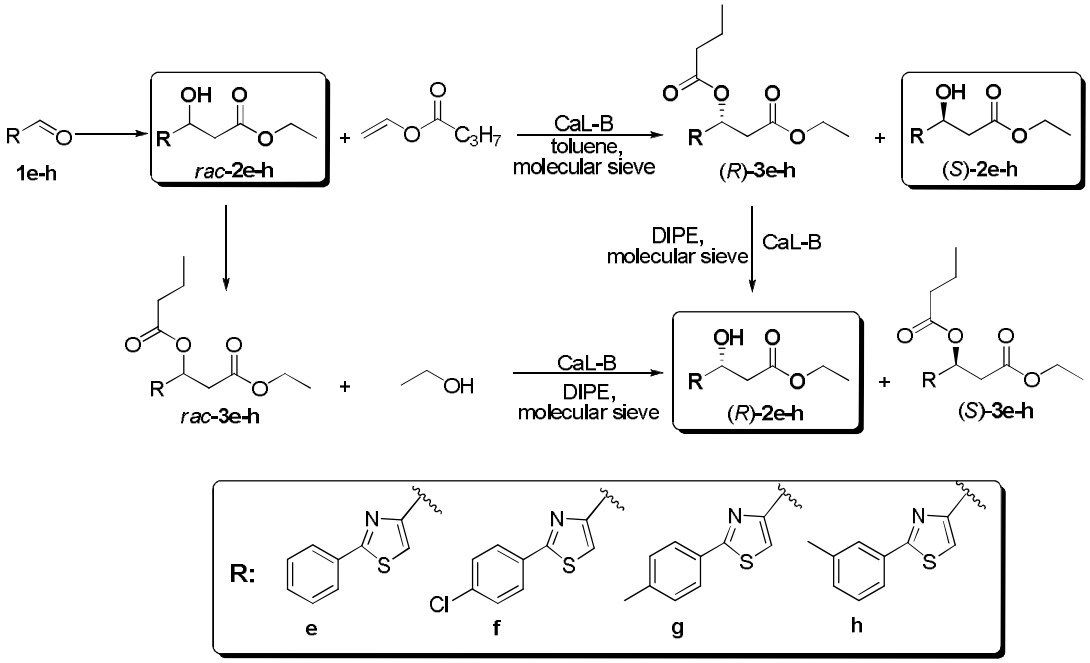
3. Aims of this study

Lipases are frequently used in enzymatic kinetic resolutions in organic chemistry because they accept a broad range of substrates and present high enantioselectivity. Moreover, from the environmental context, biocatalytic processes are greener, less hazardous and least polluting. Therefore, this thesis has been devoted to the synthesis of highly enantiopure α- and β-hydroxy acid derivatives by means of lipase-mediated kinetic resolution. For this purpose, the following objectives were proposed:

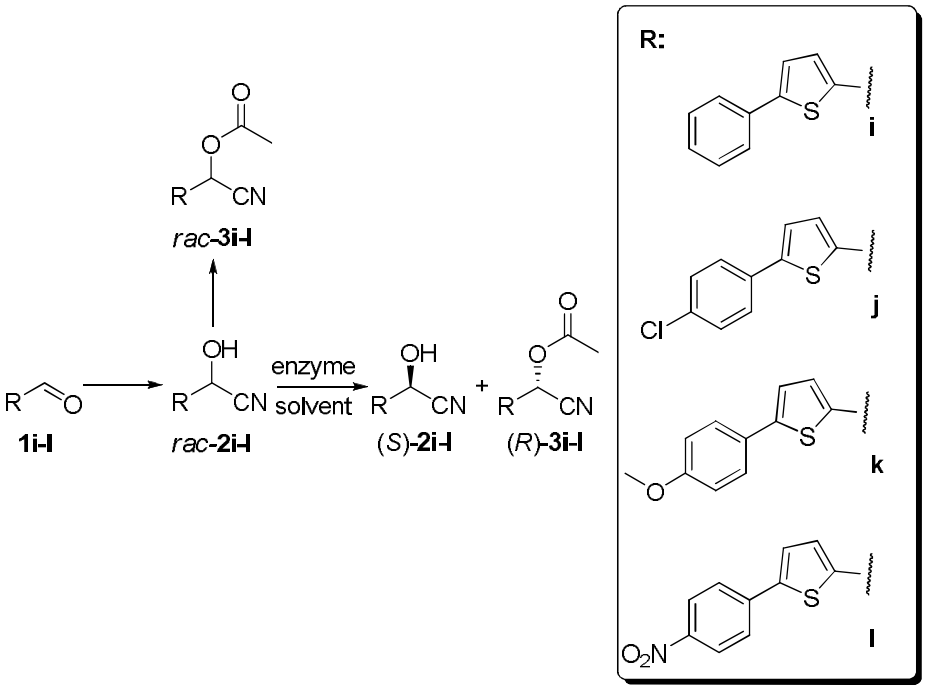
1. The synthesis of four different heteroaromatic α-hydroxy esters and their *O*-acylated counterparts, followed by their lipase-mediated resolution, in order to obtain the corresponding enantiomerically enriched α-hydroxy acids.



2. The synthesis of four different ethyl-3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates and their *O*-acylated counterparts, followed by the lipase mediated synthesis of optically pure (*R*)- and (*S*)-ethyl-3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates.



3. The synthesis of four different 2-hydroxy-2-(5-phenylthiophene-2-yl)acetonitrile derivatives and their *O*-acylated counterparts, followed by their lipase mediated kinetic resolution in order to obtain the corresponding enantiomerically enriched α-cyanohydrins.

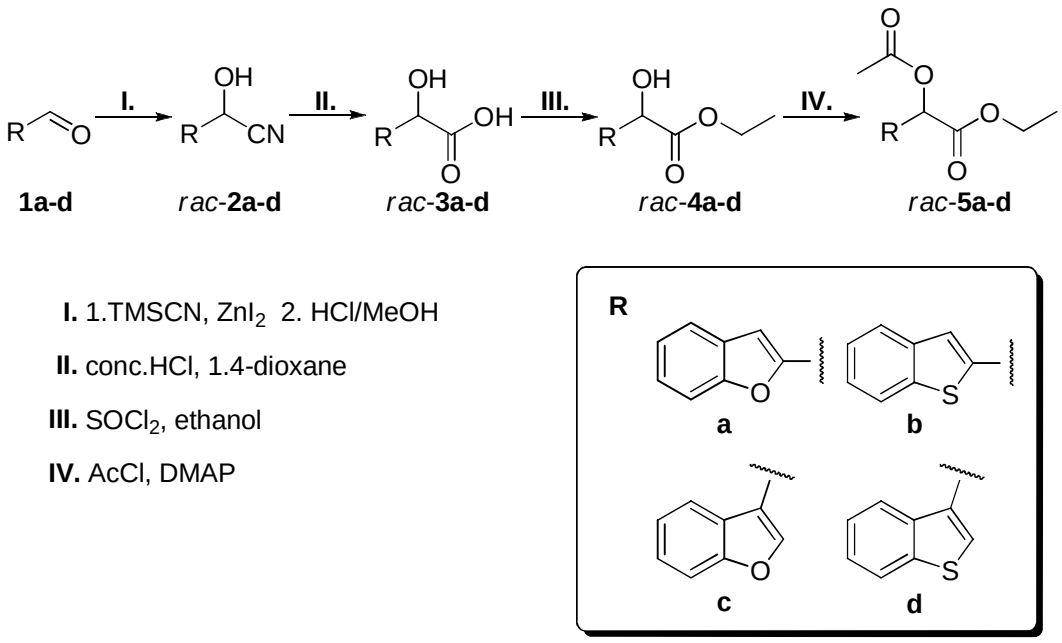


4. Results and discussion

4.1. Chemical synthesis of the racemic compounds

4.1.1. Chemical synthesis of the α-hydroxy acids derivatives

As illustrated in **Scheme 1**, the synthesis of the racemic cyanohydrins *rac*-**2a-d** from the corresponding heteroaromatic aldehydes **1a-d** was performed using trimethyl silyl cyanide in the presence of a catalytic amount of ZnI_2 in dichloromethane. The racemic cyanohydrins *rac*-**2a-d** were further hydrolyzed using conc. HCl in 1,4-dioxane and reflux, yielding the corresponding racemic 2-heteroaryl-hydroxyacetic acids *rac*-**3a-d**, which in ethanol and a catalytic amount of $SOCl_2$ were transformed in the racemic ethyl-2-heteroaryl-hydroxyacetates *rac*-**4a-d**. Finally, *rac*-**4a-d** were chemically acylated into racemic ethyl 2-acetoxy-heteroaryl-2-acetates *rac*-**5a-d** using acetyl chloride in dichloromethane and a catalytic amount of 4-*N*,*N*-dimethylamino-pyridine 1% in pyridine.

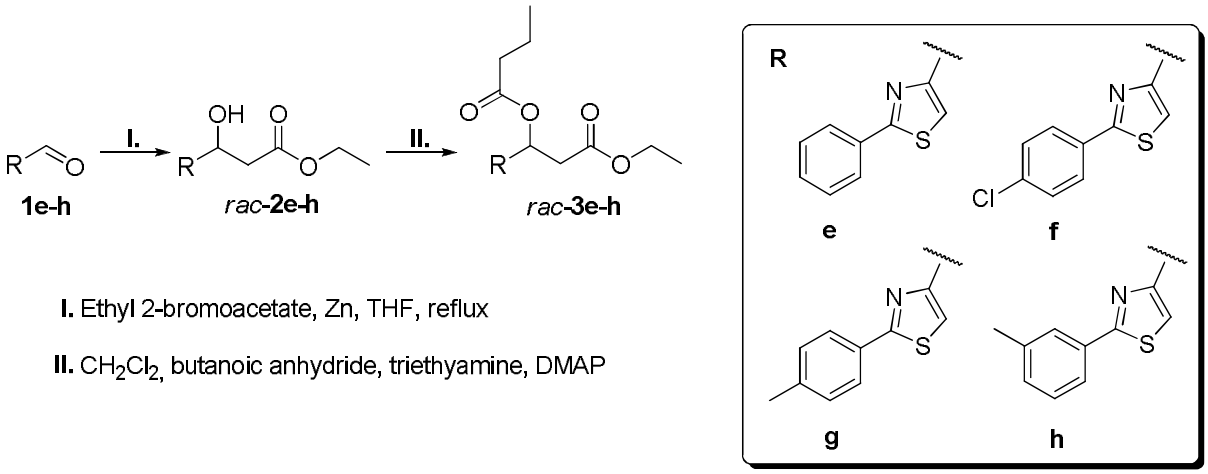


Scheme 1. Chemical synthesis of the racemic *rac*-**5a-d**

4.1.2. Chemical synthesis of the β-hydroxy esters and their corresponding diesters

Racemic β-hydroxy acids can be obtained by several methods: through the Reformatsky reaction of the corresponding aldehyde with β-bromo esters, or if the bromo ester is not available, by using the dianion of carboxylic acids; using ethyl diazoacetate and different Lewis acids as catalysts, followed by the reduction to the corresponding hydroxy esters. The β-hydroxy esters can be subsequently hydrolized to the corresponding β-hydroxy acids.

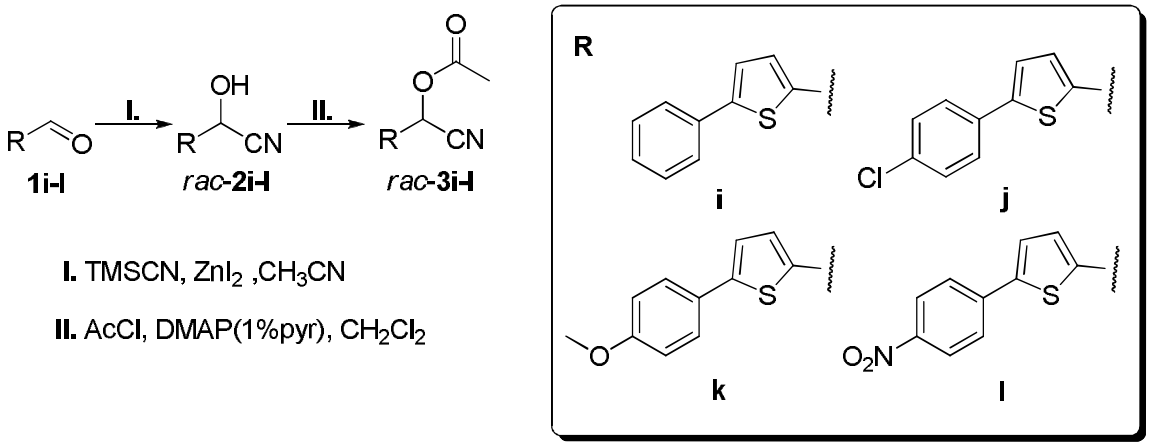
Racemic ethyl 3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates (*rac*-**2e-h**) were prepared by the Reformatsky reaction starting from the corresponding 2-aryl-thiazol-4-carbaldehydes **1e-h**. By the chemical acylation of *rac*-**2e-h** using butanoic anhydride in presence of triethylamine and a catalytic amount of 4-*N*,*N*-dimethylamino-pyridine (DMAP) in dichloromethane, the preparation of the racemic diesters *rac*-**3e-h** was also performed, as shown in **Scheme 2**.



Scheme2. Chemical synthesis of the *rac*-**2e-h** and *rac*-**3e-h**

4.1.3. Chemical synthesis of the α-cyanohydrins

As illustrated in **Scheme 3**, the synthesis of the racemic cyanohydrins *rac*-**2i–l** from the corresponding aldehydes **1i–l** was performed using trimethyl silyl cyanide in the presence of a catalytic amount of anhydrous ZnI_2 in acetonitrile or dichloromethane. The racemic cyanohydrins *rac*-**2i-l** were further acylated with acetyl chloride in the presence of Py/DMAP in dichloromethane, yielding the corresponding racemic cyanohydrin acetates *rac*-**3i–l**.



Scheme 3. Chemical synthesis of *rac*-**2i-l** cyanohydrins and their corresponding *O*-acylated esters *rac*-**3i-l**

4.2. Enzyme catalyzed synthesis of the enantiomerically enriched compounds

4.2.1. Enzyme-mediated kinetic resolution of the α-hydroxyesters and of the *O*-acylated α-hydroxyesters

In order to investigate the stereoselectivity of the enzymatic kinetic resolution and the activity of the enzymes, the chromatographic separation of the enantiomers was first established. Using various HPLC chiral columns, the baseline separation of all the enantiomers of *rac*-**3,4,5a-d** was performed.

Analytical scale enzymatic acylation of *rac*-4a-d

In order to obtain highly enantiomerically enriched (*R*)-ethyl 2-acetoxy-heteroaryl-2-acetates, comercially available free or immobilized lipases were screened in various organic solvents for enantioselective acylation with vinyl acetate as irreversible acyl donor of the racemic ethyl-2-heteroaryl-hydroxyacetates *rac*-**4a-d**.

First, the analytical scale enantiomer selective enzyme catalyzed acylation of racemic ethyl-2-heteroaryl-hydroxyacetates *rac*-**4a-d** was studied, using *rac*-**4a** as model compound. Thus, the acetylation of *rac*-**4a** in the presence of various lipases using vinyl acetate as acylation reagent was performed. Most of the tested enzymes like CaL-B, lipase F, lipase AK, *Pseudomonas cepacia* lipase (LPS), and *Mucor miehei* lipase were catalytically inactive, lipase from *Candida rugosa also* showed moderate enantioselectivity and low activity (ee_p = 46% for (*R*)-**5a**, *c* = 4 % after 24h). Only immobilized lipase A from *Candida antarctica* (CaL-A) proved to be a highly active and selective catalyst, however the catalytic performance of this enzyme was strongly influenced by the type of the immobilization. Reactions performed with CaL-A reticulated with glutaraldehyde (CaL-A CLEA) or covalently attached to dry acrylic beads (IMMCALA) showed poor enantiomeric excess for the reaction product even at low conversion. Using lipase A from *Candida antarctica* adsorbed on Celite (CaL-A), the reaction underwent faster and the selectivity was considerably improved (*ee* >90% for both reaction products at ~50% conversion). Investigating the influence of substrate:enzyme *ratio* upon the enantioselectivity and conversion, we found that an 1:2 (w:w) substrate:enzyme *ratio* is most appropriate. The nature of the solvent and the nucleophile can also significantly influence the activity and selectivity of the enantiomer selective enzymatic acylation. Thus, the CaL-A mediated acylation of *rac*-**4a** with vinyl acetate in several organic solvents was tested (**Table 1**). DIPE and MTBE (**Table 1**, entries **1** and **2**) proved to be the most appropriate solvents for the acetylation of *rac*-**4a** (*E* = 70 at 51 % conversion after 2 hours). In CH_2Cl_2 and acetonitrile the

selectivity was moderate (**Table 1**, entry **3** and **4**), while in chloroform and THF the catalyst was inefficient. The CaL-A mediated acylation of *rac*-**4a** with vinyl butanoate and isopropenyl acetate in DIPE was also studied and no considerable changes for the selectivity of reaction were found as compared to those obtained by using vinyl acetate (*E* = 21 at *c* = 40% for the vinyl butanoate, *E* = 19 at *c* = 30% for the isopropenyl acetate and *E* = 39 at *c* = 45% for vinyl acetate, after 2h). In accordance with the optimal conditions found for the biotransformation of *rac*-**4a**, the CaL-A mediated analytical scale acetylation with vinyl acetate of *rac*-**4-b,c.d** in DIPE was also performed (**Table 1**, entries **5**, **6**, **7**) with good reactivity and selectivity.

Table 1. The enantioselective acylation of *rac*-**4a-d** with vinyl acetate and CaL-A in different solvents

Entry	Substrate	Solvent	Time (h)	*c* (%)	ee_S(%)	ee_P(%)	*E*
1	*rac*-**4a**	DIPE	2	51	95	90	70
2	*rac*-**4a**	*t*-BME	2	23	23	74	8
3	*rac*-**4a**	CH_2Cl_2	23	34	44	85	19
4	*rac*-**4a**	CH_3CN	23	42	62	84	21
5	*rac*-**4b**	DIPE	2	50	93	90	65
6	*rac*-**4c**	DIPE	4	49	95	97	>200
7	*rac*-**4d**	DIPE	3	49	98	99	»200

Analytical scale enzymatic alcoholysis of racemic compounds *rac*-5a-d

Lipases usually retain their enantiomer preference in hydrolysis or alcoholysis.[1-3.6] Consequently, such reactions should result in opposite enantiomeric forms of the enantiomerically enriched ethyl 2-heteroaryl-2-hydroxyacetates **4a-d** and ethyl 2-acetoxy-2-heteroaryl-acetates **5a-d** as those found in the kinetic resolution by acylation. Therefore, the study of alcoholysis and hydrolysis of the corresponding racemic ethyl 2-acetoxy-2-heteroaryl-acetates *rac*-**5a-d** has been also considered.

Due to their low water solubility, first the enzymatic alcoholysis of *rac*-**5a-d** was tested. The same wide selection of commercial hydrolases used also for the enzymatic acylation were screened for the analytical scale alcoholysis of the racemic diesters *rac*-**5a-d**. First, the experiments were carried out in neat anhydrous methanol, ethanol, propanol and

butanol, followed by experiments using the same enzymes in all the solvents checked for the enzymatic esterification, adding 5 equivalents of alcohol into the reaction mixture. Because in all cases the enzymatic alcoholysis proved to be totally inefficient (yields < 5% after 2 days with PLE, LPS and esterase from *Rhyzopus oryzae*, substrate:enzyme *ratio* 1:2), further on the enzymatic hydrolysis of *rac*-**5a-d** was studied.

Analytical scale enzymatic hydrolysis of compounds *rac*-5a-d

Experiments were performed in several organic solvents saturated with water in order to increase the rate of hydrolysis. As DIPE was also used for the enzymatic acylation and we observed that it has good results with the hydrolysis reaction, further on we conducted the experiments using a substrate:enzyme *ratio* 1:2 (w/w) and a mixture of DIPE:water 18:1 (v:v).

Next, an enzyme screening was performed separately for both benzofuranyl-2-yl and benzofuranyl-3-yl, using lipases, esterases and acylases and after several hours, results show only LPS, CaL-A, CrL and PLE, while CaL-B and Acylase I shows no results for *rac*-**5a,c**. All the enzymes tested presented (*R*) selectivity, except CrL which proved to be (*S*) selective. The enzymatic hydrolysis of *rac*-**5a,c** as substrate showed the presence of the corresponding hydroxyester, hydroxy acid and the *O*-acylated hydroxy acid. These results can be explained by the different regioselectivities of the employed hydrolases**.** Based on the results showed in **Table 2**, we further used LPS for the hydrolysis of *rac*-**5b** and CaL-A for the hydrolysis of *rac*-**5d**, the results obtained being presented in **Table 3**. Therefore, by the employment of the enzymatic acylation of *rac*-**4a-d** followed by the LPS or CaL-A mediated hydrolysis of (*R*)-**5a-d** the highly enantiomerically enriched hydroxyesters (*R*)-**4a-d** can be obtained.

Table 2. The enzymatic hydrolysis of *rac*-**5a,c** in DIPE:water 18:1 (v:v), monitored by TLC and HPLC :

Entry	Substrate	Enzyme	Config[a]	Products		
				Hydroxyester	*O*-acetylated hydroxyacid	Hydroxyacid
1	*rac*-**5a**	CaL-A	(*R*)	+ +	-[b]	-[b]
2	*rac*-**5a**	LPS	(*R*)	+ + +	-[b]	-[b]
3	*rac*-**5a**	CrL	(*S*)	+ +	-[b]	+
4	*rac*-**5a**	PLE	(*R*)	+	+	+ +
5	*rac*-**5c**	CaL-A	(*R*)	+ + +	-[b]	-[b]
6	*rac*-**5c**	LPS	(*R*)	-[b]	-[b]	+
7	*rac*-**5c**	CrL	(*S*)	+ +	-[b]	+
8	*rac*-**5c**	PLE	(*R*)	-[b]	+	+ +

[a] the absolute configuration of the faster reactive enantiomer determined for the **5a,c** as substrate
[b] - not detected (c< 2%)
The presence of this products is marked with 1 to 3 plus (+), according to their concentration and their absence with minus(-).

Table 3. The enzymatic hydrolysis of *rac*-**5a-d** in DIPE:water 18:1 (v:v):

Entry	Substrate	Enzyme	Time (h)	c (%)	ee_S (%)	ee_P(%)	E
1	*rac*-**5a**	LPS	22	50	95	94	120
2	*rac*-**5b**	LPS	22	49	94	97	>200
3	*rac*-**5c**	CaL-A	12	45	78	99	100
4	*rac*-**5d**	CaL-A	12	48	80	92	>200

Analytical scale enzymatic hydrolysis of compounds *rac*-4a-d

For the enzymatic hydrolysis of the hydroxy esters *rac*-**4a-d**, we used *rac*-**4a** as our model compound and we performed an enzyme screening in a water:DIPE 1:1 (v/v) mixture using PLE, *Pseudomonas cepacia* lipase (LPS), PPL, CaL-A, CaL-B, CrL, L-AK, lipase F, BUTE lipase, *Mucor miehei,* Acylase I from Porcine kidney and from *Aspergillus,* esterase F from *Rhyzopus oryzae* as biocatalysts. CaL-B showed the highest activity and selectivity values, while PLE, Esterase F from *Rhyzopus oryzae* and Acylase I showed lower activity and

enantioselectivity values. Interestingly enough, only Esterase F showed enantiopreference for the (*R*) enantiomer of the substrate. Thus, the resolution of *rac*-**4b,c,d** was performed by the CaL-B mediated hydrolysis obtaining from moderate to excellent enantioselectivity values (**Table 4**).

To investigate the stereoselectivity of the enzymatic kinetic resolution and the activity of the enzymes, the products (*R*)/(*S*)-**3a-d** were derivatized with *t*-amyl alcohol and the chromatographic separation of the enantiomers was first established.

Table 4. The enzymatic hydrolysis of *rac*-**4a-d** DIPE:water 1:1 (v:v):

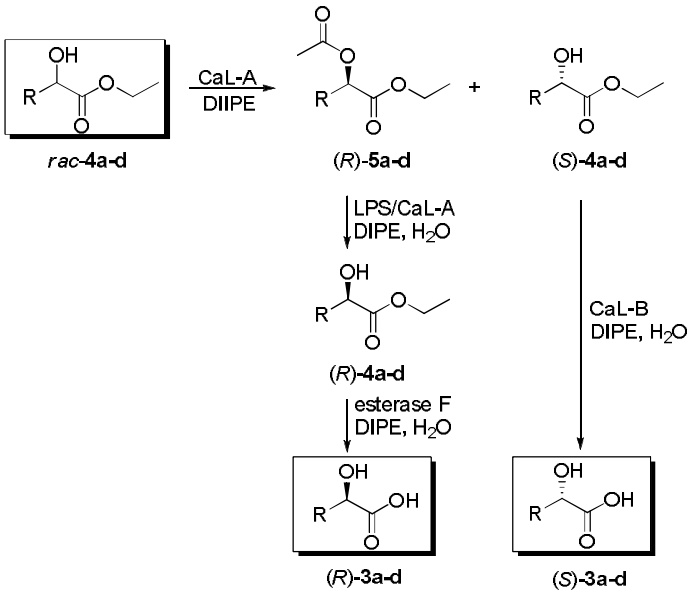
Entry	Substrate	Enzyme	Config[a]	Time (h)	*c* (%)	ee_S(%)	ee_P(%)[b]	*E*
1	*rac*-**4a**	PLE	(*S*)	40	42	52	72	10
2	*rac*-**4a**	Esterase F	(*R*)	40	11	10	75	11
3	*rac*-**4a**	Acylase I	(*S*)	40	2	2	63	101
4	*rac*-**4a**	CaL-B	(*S*)	17	42	70	97	124
5	*rac*-**4b**	CaL-B	(*S*)	15	45	80	93	>200
6	*rac*-**4c**	CaL-B	(*S*)	14	50	98	96	»200
7	*rac*-**4d**	CaL-B	(*S*)	40	51	98	95	153

[a] the absolute configuration of the faster reactive enantiomer
[b]enantiomeric exces for optically pure acid **3a-d** was determined after derivatization of the corresponding acid with 2,2-dimethyl-1-propanol to the corresponding 2-hydroxy neopentyl ester

Preparative scale synthesis

In **Scheme 4**, the preparative scale synthesis of both (*R*)- and (*S*)- **3a-d** was set up. All the dilutions, substrate:enzyme *ratio* and reaction conditions were the same as in the case of the analytical scale reactions.

Starting from the *rac*-**4a-d,** which we enzymatically acylate in the presence of CaL-A in DIPE, we obtain the (*R*)-**5a-d** and the (*S*)-**4a-d**. The (*S*)-**4a-d** is enzymatically hydrolyzed with CaL-B, which showed (*S*) selectivity, in a DIPE:water mixture, to the corresponding (*S*)-**3a-d**, while (*R*)-**5a-d** is first hydrolyzed with LPS or CaL-A to the corresponding hydroxyester (*R*)-**4a-d** and then, using esterase F, which showed (*R*) selectivity, to the (*R*)-**3a-d**. In this way we can obtain both optically pure α-hydroxy acids.



Scheme 4. Enzymatic route to obtaining optically pure heteroaryl α-hydroxy acids

Table 5. Enantiomeric excesses and specific rotations for the isolated enantiomerically enriched **3,4,5a-d :**

Compound	Yield(%)	ee [%]	$[\alpha]_D$[a]
(*R*)-**3a**	90	99	+26
(*S*)-**3a**	89	99	-27
(*R*)-**3b**	92	99	-26.1
(*S*)-**3b**	94	99	+25
(*R*)-**3c**	91	99	+3
(*S*)-**3c**	90	99	-2.7
(*R*)-**3d**	90	99	+46
(*S*)-**3d**	88	98	-48.3
(*R*)-**4a**	90	95	-38.5
(*S*)-**4b**	96	82	-37.1
(*R*)-**4c**	92	75	-68.5
(*R*)-**4d**	95	98	-103.7
(*S*)-**5a**	86	92	+131.4

(*R*)-**5b**	92	83	+81.3
(*S*)-**5c**	89	93	+80.3
(*S*)-**5d**	93	99	+100.8

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

Conclusions

The synthesis of highly enantiomerically enriched (*ee* up to 99%) (*R*)- and (*S*)-2-benzofuranyl- and 2-benzo[b]thiophenyl-2-hydroxyacetic acids was accomplished by the CaL-A mediated kinetic resolution of the corresponding racemic α-hydroxyesters, followed by CaL-B mediated hydrolysis of the untransformed enantiomer of the substrate (*S*)-**4a-d** to yield the (*S*)-**3a-d** and a two step enzymatic hydrolysis first of the optically pure diacetate (*R*)-**5a-d** with LPS or CaL-A to the corresponding hydroxyester (*R*)-**4a-d** and then, using esterase F, which showed (*R*) selectivity, to obtain the optically pure α-hydroxy acid (*R*)-**3a-d**.

4.2.2. Lipase-mediated synthesis of optically pure β-hydroxyesters and *O*-acylated β-hydroxyesters

In order to investigate the stereoselectivity of the reactions involving chiral 3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates and their diesters, the chromatographic separation of their enantiomers was first established.

Analytical scale enzymatic acylation of *rac*-2e-h

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

promote hydrolytic reactions, thus yielding undesired by-products or causes a decrease in the enantiopurity of the products.

The nature of the solvent and the nucleophile can significantly influence the activity and selectivity of the enantiomer selective enzymatic acylation. Thus, the solvents used were selected and tested as they are commonly accepted by lipases. Among the enzymes tested for the *O*-acylation of our model compound *rac*-**2e** using vinyl acetate and vinyl butanoate, lipase AK on Celite and *Pseudomonas cepacia* lipase (L-PS free) were catalytically inactive in all the tested solvents, while CrL, depending on the acylation agent and the solvent used, showed higher or lower selectivity and activity. For the CrL mediated acylation using vinyl acetate in MTBE, DIPE, hexane and toluene both, reaction rate and selectivity, were low (**Table 6**, entries **10-13**), while by using vinyl butanoate, the reaction undergoes with low selectivity in MTBE, DIPE and hexane (**Table 7**, entries **6-8**). By performing the CaL-A on Celite mediated *O*-acylation of *rac*-**2e** using vinyl acetate in various ethers like MTBE and DIPE or nonpolar solvents like hexane, toluene and acetonitrile, the reactions showed moderate enantioselectivities (*E* =5-14, **Table 6**, entries **1-5**). When, in the same experiments, vinyl butanoate was used as acyl donor, a considerably decreased enzyme activity was detected in MTBE (**Table 7**, entry **1**), while in the rest of the tested solvents CaL-A on Celite was inactive. In terms of activity and selectivity (*E* = 3-9, **Table 6**, entries **6-9**) for the CaL-B catalyzed *O*-acylation using vinyl acetate in all tested solvents, we obtained similar results as those found for CaL-A on Celite. However, a major improvement in selectivity was obtained for the CaL-B catalyzed *O*-acylation with vinyl butanoate using *n*-hexane and toluene as solvents (**Table 7**, entries **4-5**), yielding highly enantiomerically enriched resolution products at approx. 50% conversion. Interestingly, CaL-B-SWCNT proved to be completely inactive for the present purpose, however it showed high activity and selectivity for the acylation of *rac*-1-phenylethanol.

Table 6. Lipase and solvent screenings for the selective *O*-acylation of the racemic substrate *rac*-**2e** using vinyl acetate, after 17 hours

Entry	Enzyme	Solvent	*c* (%)	$ee_{(R)\text{-}3e}$	$ee_{(S)\text{-}2e}$	*E*
1	CaL-A on Celite	MTBE	35.3	64	35	6
2	CaL-A on Celite	DIPE	78.3	26	94	5
3	CaL-A on Celite	CH_3CN	68	45	96	9
4	CaL-A on Celite	Hexane	72.4	38	>99	14

5	CaL-A on Celite	Toluene	29.6	64	27	6
6	CaL-B	MTBE	35.6	47	26	3.5
7	CaL-B	DIPE	32.3	65	31	6.3
8	CaL-B	Hexane	53.7	62	72	8.9
9	CaL-B	Toluene	24.5	74	24	8.4
10	CrL	MTBE	5.2	36	2	2
11	CrL	DIPE	27.2	40	15	2.7
12	CrL	Hexane	33.8	47	24	3.5
13	CrL	Toluene	12.8	68	10	5.8

Table 7. Lipase and solvent screenings for the selective *O*-acylation of the racemic substrate *rac*-**2e** using vinyl butanoate, after 17 hours

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

Analytical scale enzymatic alcoholysis of *rac*-3e-h

Lipases usually retain their enantiomer preference found in the stereoselective acylation of chiral alcohols including the case of hydrolysis or alcoholysis of the esteric counterparts. Consequently, such reactions should result in opposite enantiomeric forms of the enantiomerically enriched ethyl 3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates **2e-h** and their

diesters **3e-h** as those found in the enzymatic acylation of *rac*-**2e-h**. Further, the enzymatic alcoholysis of *rac*-**3e**, used as model compound, was investigated. The experiments were carried out using the same enzymes in all of the tested solvents checked for the enzymatic acylation, by adding 5 equivalents of ethanol or 1-butanol into the reaction mixture. Ethanolysis mediated by CaL-B showed the highest selectivity and activity when MTBE (**Table 8**, entry **5**) and DIPE (**Table 8**, entry **6**) were used as reaction media. Due to the higher activity of the enzyme in DIPE, this solvent was used in further experiments.

Table 8. Alcohol, lipase and solvent screening for the selective alcoholysis of the racemic diester *rac*-**3e**, after 14 hours

Entry	Alcohol	Enzyme	Solvent	*c* (%)	$ee_{(R)\text{-}2e}$	$ee_{(S)\text{-}3e}$	*E*
1	EtOH	CaL-A	MTBE	11.1	64	8	4.9
2			DIPE	32.2	59	28	5
3			CH_3CN	8.3	77	7	8.2
4			Hexane	19.3	71	17	7
5		CaL-B	MTBE	49.7	97	96	>200
6			DIPE	48.4	>99	93	»200
7			Toluene	6.6	>99	7	>200
8		CRL	Hexane	9.8	55	6	3.6
9	BuOH	CaL-A	CH_3CN	3	>99	3	>200
10		CaL-B	DIPE	83	19	94	4
11			Hexane	39.3	71	46	9
12			Toluene	16.6	85	17	14
13		CrL	DIPE	13.1	53	8	3.5
14			Hexane	14.5	47	8	3

Preparative scale enzymatic acylation of *rac*-2e-h and ethanolysis of *rac*-3e-h

Using the optimal conditions found for the analytical scale biotransformations of both *rac*-**2e** and *rac*-**3e**, the CaL-B mediated preparative scale acylation of *rac*-**2e-h** with vinyl butanoate in toluene (**Table 9**, entries **1-4**) and ethanolysis of *rac*-**3e-h** in DIPE (**Table 9**, entries **5-8**) was next performed with good reactivity and selectivity (*E* »200, 99% ee_s for

most of the resolution products at approx. 50% conversion). All the dilutions, substrate:enzyme *ratio* and reaction conditions were the same as in the case of the analytical scale reactions. The yields of the isolated and chromatographically purified resolution products were in the range of 45-48%, calculated related to the starting racemic substrates and are given in **Table 9**, together with the optically rotatory power of the enantiopure products.

Table 9. Preparative scale enzymatic acylation of *rac*-**2e-h** and ethanolysis of *rac*-**3e-h**

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

Conclusion

An efficient enzymatic procedure on the synthesis of enantiomerically pure ethyl 3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates has been described (*ee* 99%). By using enzymatic kinetic resolution, both optically pure enantiomers of four ethyl 3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates **2e-h** and four butanoates **3e-h** were synthesized with high yields. CaL-B proved to be the optimal biocatalyst for both acylation of *rac*-**2e-h** with vinyl butanoate in toluene and the ethanolysis of *rac*-**3e-h** in DIPE. CaL-B also showed to be efficiently reusable in ten cycles, since its activity and stereoselectivity remain unaltered.

4.2.3. Lipase-mediated kinetic resolution of α-cyanohydrins and *O*-acylated α-cyanohydrins

In order to investigate the stereoselectivity of the reactions involving chiral derivatives of 2-hydroxy-2-(5-phenylthiophen-2-yl)acetonitrile and their esters, the chromatographic separation of the enantiomers was first established. The baseline separation of the enantiomers of all *rac*-**2,3i-l** was performed using HPLC columns and different mixtures of *n*-hexane and 2-propanol (v/v) as eluent.

Analytical scale enzymatic acylation of *rac*-2i-l

In order to obtain highly enantiomerically enriched (*R*)-cyano(5-heteroaryl-2-yl)methyl acetate, commercially available immobilized lipases were screened in various organic solvents for the enantioselective acylation of the racemic 2-heteroaryl-2-hydroxyacetonitrile *rac*-**2i-l** with irreversible acyl donors. First, the analytical scale enantiomer selective enzyme catalyzed acylation of racemic 2-heteroaryl-2-hydroxyacetonitrile *rac*-**2i-l** was studied using *rac*-**2i** and vinyl acetate in the presence of different solvents and various lipases. Most of the enzymes tested such as immobilized lipase B from *Candida antarctica* (CaL-B, Novozym 435), lipase from *Pseudomonas fluorescens* (AK) on sol-gel and lipase from *Candida rugosa* (CrL) were catalytically inactive after 3 hours. The lipase from *Pseudomonas fluorescens* (AK) immobilized by adsorbtion on Celite showed good enantioselectivity and activity (ee_p = 98% and ee_S=72% at c = 42% after 3 h, in MTBE). By using CaL-A from *Candida antarctica* immobilized on Celite, the reaction was faster and the selectivity was improved (*ee*>83%) for both reaction products at 48% conversion.

Investigating the influence of the substrate:enzyme *ratio* upon the enantioselectivity and conversion, a 1:5 (w/w) substrate:enzyme *ratio* was found to be the most appropriate. The nature of the solvent and the nucleophile can also significantly influence the activity and selectivity of an enantioselective enzymatic acylation. Thus, the CaL-A mediated acetylation of *rac*-**2i** with vinyl acetate in several organic solvents was tested (**Table 10**). Acetonitrile (**Table 10**, entry **1**) proved to be the most appropriate solvent (E = 50 at 47% conversion after 3 h). In CH_2Cl_2 and ethyl acetate the selectivity and the activity were moderate after 3 hours (**Table 10**, entries **2** and **3**), while in MTBE and methyl-THF the biocatalyst was inefficient. The CaL-A mediated acylation of *rac*-**2i** with isopropenyl acetate in acetonitrile was also studied and no significant changes for the activity and selectivity of the reaction were found compared to those obtained by using vinyl acetate (ee_p=81% at c=20% after 42h), also ethyl-

metoxyacetate, ethyl-etoxyacetate and vinyl pivaloate were tested, but they proved to be inactive.

Table 10. Enantioselective acylation mediated by different enzymes, of *rac*-**2i** with vinyl acetate in different solvents

Entry	Enzyme	Solvent	Time (h)	*c* (%)	ee_S(%)	ee_P (%)	*E*
1	CaL-A	CH_3CN	3	46	85	99	>200
2		Ethyl acetate	3	22.7	21.5	73.1	7.9
3		MTBE	3	28.8	18.8	46.3	3.2
4		CH_2Cl_2	3	15.8	16.5	87.4	17.6
5		Methyl-THF	17	34	22.8	44.3	3.2
6	L-AK	MTBE	3	42	72	98	>200
7		Methyl-THF	3	12.6	14	98	121
8		CH_2Cl_2	17	44.6	80	99	>200
9	CaL-B	CH_2Cl_2	17	23	28	91	29
10	L-AK solgel	MTBE	17	9.5	10	98	109

In the case of 2-hydroxy-2-(5-(4-methoxyphenyl)thiophen-2-yl)acetonitrile *rac*-**2j-l**, the screening for the best enzymatic kinetic resolution conditions revealed different results (**Table 11** entry **3**, **Table 12**, entry **9**; **Table 13** entry **2**). The screening for the optimal conditions for the KR of *rac*-**2k** included the use of vinyl acetate, vinyl butanoate and vinyl decanoate as irreversible acylation agents, but the best activity and selectivity of the lipases was achieved when using vinyl acetate (**Table 12**).

Table 11. Lipase and solvent screening for for the kinetic resolution of *rac*-**2j** using vinyl acetate as an acylating agent

Entry	Enzyme	Solvent	*Time* (h)	c (%)	ee_S(%)	ee_P(%)	*E*
1	CaL-A	MTBE	3	2	6	22	1.6
2	CaL-A	CH_3CN	3	41	62	89	32
3	L-AK	CH_2Cl_2	3	50	99	>99	»200
4	L-PS	MTBE	3	14.5	17	99	>200

Table 12. Lipase and solvent screening for the kinetic resolution of *rac*-**2k** using vinyl acetate as an acylating agent

Entry	Enzyme	Solvent	Time (h)	*c* (%)	ee_S(%)	ee_P (%)	*E*
1	CaL-A	CH_3CN	3	47	74.4	82	23.3
2		CH_2Cl_2	3	38.6	38.6	90	27.8
3		MTBE	3	67	67	66	9.5
4		Methyl-THF	17	14	14	86	15.4
5		DIPE	3	14.9	14.9	21	1.8
6	CaL-B	CH_2Cl_2	3	63	93	54	10.6
7		DIPE	17	54.7	84	69	14.8
8	L-AK	CH_3CN	3	4	4	96.5	58.8
9		CH_2Cl_2	17	49	99	99	>200
10		MTBE	3	48.5	83	88	41.7
11		Methyl-THF	3	44.6	78.7	97	>200
12		DIPE	3	52	83.6	77	20.2
13	L-PS	CH_2Cl_2	17	49.6	93	94	110
14		DIPE	3	0.1	94	0.1	52

Table 13. Lipase and solvent screening for the kinetic resolution of *rac*-**2l** using vinyl acetate as an acylating agent

Entry	Enzyme	Solvent	Time (h)	*c* (%)	ee_S(%)	ee_P(%)	*E*
1	L-AK	CH_3CN	17	53	99	85	189
2	L-AK	CH_2Cl_2	17	50	99	99	»200
3	L-AK	MTBE	3	52	99	90	>200

Considering that for substituted heteroaryl cyanohydrins, L-AK showed good activity and selectivity in CH_2Cl_2, it was employed for the preparative scale enzymatic acetylation of all three tested substrates *rac*-**2j-l**, while for *rac*-**2i** the enzymatic kinetic resolution was performed using vinyl acetate and CaL-A, in acetonitrile (**Table 14**).

Table 14. Preparative scale enzymatic acylation of *rac*-**2i-l** using the best conditions found on analytical scale and specific rotations of the isolated enantiomerically enriched **2,3i-l**

Entry	Substrate	Time (h)	Products						*E*
			$ee_{(R)\text{-}3}$	Yield (%)	$[\alpha]_D$[a]	$ee_{(S)\text{-}2}$	Yield(%)	$[\alpha]_D$[a]	
1	*rac*-**2i**	17	99	47	+62.1	80	45	+18.5	>200
2	*rac*-**2j**	17	99	45	+16.4	99	47	+156.1	»200
3	*rac*-**2k**	17	99	45	+2.6	99	45	+81.46	»200
4	*rac*-**2l**	17	99	46	+23.7	99	45	+59.8	»200

[a] 10^{-1} deg cm^2 g^{-1}; c 1.0, $CHCl_3$, CH_2Cl_2 or MeOH, T = 25 °C. Isolated yields based on the maximum theoretical recovery from the racemic starting material.

Conclusion

A new lipase-mediated kinetic resolution starting from racemic α-cyanohydrins was developed in order to obtain the corresponding *O*-acylated α-cyanohydrins with high enantiopurity. While for the substituted heteroaromatic α-cyanohydrins *rac*-**2j-l**, L-AK showed good activity and selectivity in CH_2Cl_2, for the enzymatic kinetic resolution of the unsubstituted heteroaromatic α-cyanohydrin *rac*-**2i**, CaL-A and acetonitrile proved efficient.

5. General conclusions

This thesis describes the potential of lipases as highly applicable chiral catalysts for the preparation of enantiopure α- and β-hydroxy acids and their derivatives.

By exploiting the different enantioselectivity of several lipases, a new sequential kinetic resolution procedure was established for the synthesis of highly enantiomerically enriched (*ee* up to 99%) (*R*)- and (*S*)-2-benzofuranyl- and 2-benzo[b]thiophenyl-2-hydroxyacetic acids.

A new method for the lipase mediated kinetic resolution of a series of differently substituted heteroaromatic β-hydroxy esters was reported, providing high enantiomeric excesses of the formed products (*ee* 99%). CaL-B proved to be the optimal biocatalyst for both the *O*-acylation and the ethanolysis reactions, in different reaction medium.

A series of four new differently substituted phenylthiophen-based (*S*)-α-cyanohydrins and their (*R*)-*O*-acetylated counterparts were successfully obtained in high enantiomeric excesses by CaL-A and L-AK mediated kinetic resolution of the racemic α-cyanohydrins.

7. Acknowledgements

The experimental work was carried out at the laboratory of the Biochemistry and Biochemical Engineering Research Group leaded by Prof. Florin Dan Irimie, within the Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University.

I would first like to express my deepest gratitude to my scientific supervisor Prof. Florin Dan Irimie for introducing me to the fascinating field of biocatalysis, for supervising and advising me during my thesis research work and for encouraging me to embrace the challenges which allowed me to improve my research skills.

Special thanks to Assoc. Prof. Eng. Habil. Paizs Csaba, Assoc. Prof. Eng. Monica Ioana Toşa, Assoc. Prof. Gabriel Katana, Assist. Prof. Paula Podea, Assist. Lect. Csaba László Bencze, Dr. Brem Jürgen, for sharing their knowledge with me, for creating an efficient working environment and for their helpful attitude during my doctoral studies.

I want to express my gratitude to our collaborators, Professor László Poppe from the Eötvös Lóránd University and Professor Valentin Zaharia from the Iuliu Haţieganu University of Medicine and Pharmacy for sharing their expertise and reagents. Professor Liisa Kanerva from the University of Turku is also gratefully acknowledged for her generous gift of enzymes, and for her heplful advices. I also warmly thank the reviewers of this thesis for their helpful comments and advice.

I've had the privilege to work in a great research group, with passionate people who contributed to my professional development and to whom I am grateful for their help with the lab experiments, for their friendship and for the moments at the coffee breaks with the interesting discussions which were always welcome and refreshing interruptions in the working days: Alexandra Mantu, Andrea Varga, Hajnal Vari-Bartha, Botond Nagy, Norbert Dima, Mădălina Elena Moisă, Denisa Hathazi, Alina Filip, Annamaria Varga, Denisa Hapău, Dr. Melinda Füstös, Dr. Laura Pop, Dr. Laura Chiş, Dr. Györfi Zoltán Attila, Dr. Maria Trif, Dr. Monica Hriscu, Dr. Augustin Cătălin Moţ, Dr. Florina Violeta Deac, Dr. Cristina Bischin, Kállo Noemi-Hajnalka.

I thank my colleagues in the other research groups of the department for their help with the scientific issues I encountered. Assist. Lect. Emese Gál is acknowledged for MS analyses. Dr. Dan Porumb and Dr. Alpár Pöllnitz are acknowledged for introducing me to the NMR spectra measurements.

The financial support from the Sectoral Operational Programme for Human Resources Development 2007-2013, co-financed by the European Social Fund, under the project POSDRU/159/1.5/S/132400 and financial support from the Romanian Ministry of Education and Research (PN II ID PCE 3-0775/2011) are gratefully acknowledged.

Last, but not least, I have the deepest regard towards my family, for their loving, confident, encouraging and patient attitude throughout my doctoral studies.

Sincere thanks to all my friends, for being there for me whenever I needed, whether it was a happy or a sad moment in my life, I really appreciate it.

8. List of publications

This thesis is based on the following publicatons, which are reffered in the text by roman numerals I and III. Unpublished data is also included.

Scientific publications:

I. Varga A., Naghi M. A., Füstös M., Katona G., Zaharia V.: CaL-B mediated synthesis of optically pure (*R*)- and (*S*)- ethyl 3-hydroxy-3-(2-aryl-thiazol-4-yl)propanoates, *Tetrahedron: Asymmetry* **2014**, *25*, 298-304.

II. Toma A., Hapau D., Naghi M., Vlase L., Mogoşan C., Zaharia V.: Synthesis and antiinflammatory activity of new polyheterocyclic Schiff bases and Mannich bases, *Studia Universitatis Babes-Bolyai Chemia* **2013**, 2, 93-104.

III. Naghi M. A., Bencze L.C., Brem J., Paizs C., Irimie F. D., Tosa M. I.: Sequential enzymatic procedure for the preparation of enantiomerically pure 2-heteroaryl-2-hydroxyacetic acids, *Tetrahedron: Asymmetry* **2012**, 23, 181–187.

IV. Brem J., Naghi M., Tosa M. I., Boros Z., Poppe L., Irimie F. D., Paizs C.: Lipase mediated sequential resolution of aromatic b-hydroxy esters using fatty acid derivatives, *Tetrahedron: Asymmetry* **2011**, 22, 1672–1679.

V. Trif M., Kallo N. H., Naghi M. A., Bencze L. C.: Stereoselective bioreduction of 1-(5-phenylfuran-2-yl)-ethanones mediated by baker's yeast, *Biocatalysis and Biotransformation* **2011**, 30(2), 177-183.

Conference publications:

I. Naghi M.A., Tosa M.I., Paizs C., Irimie F.D.: Lipase-mediated kinetic resolution towards enantiomerically enriched α- and β-hydroxy acids and derivatives, Biotransformations for Pharmaceutical and Cosmetic Industry, Warsaw, **2014** (oral presentation).

II. Naghi M. A., Vari J.H., Tosa M.I., Paizs C., Irimie F.D.: CaL-A mediated kinetic resolution of racemic 2-hydroxy-2-(5-phenylthiophen-3-yl)acetonitrile and its derivatives, 13th Symposium and Summer School on Bioanalysis (13th ISSSB), Debrecen, Hungary, **2013** (poster).

III. Naghi M.A., Paizs C., Irimie F.D., Tosa M.I.: A sequential enzymatic methodology as a new approach for the synthesis of optically pure heteroaryl-α-hydroxy acids, ‘’Centenary of Education in Chemical Engineering’’ International Conference, Iasi, Romania, **2012** (poster).

IV. Naghi M. A., Lăcătus M., Nagy B., Brem J., Irimie F. D.: Biocatalytic synthesis of various optically active (hetero)aryl-β-hydroxy-propanoic acid derivatives, 7th International Conference "Students for students", Cluj-Napoca, Romania, **2011** (poster).

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