Babeş-Bolyai University
Faculty of Chemistry and Chemical Engineering
Department of Biochemistry and Biochemical Engineering

Biocatalytic approach towards enantiomerically enriched heteroaryl-1,2-ethanediols

Ph.D. Thesis Abstract

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-Cluj Napoca-
2011

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Defense: 24 March 2011

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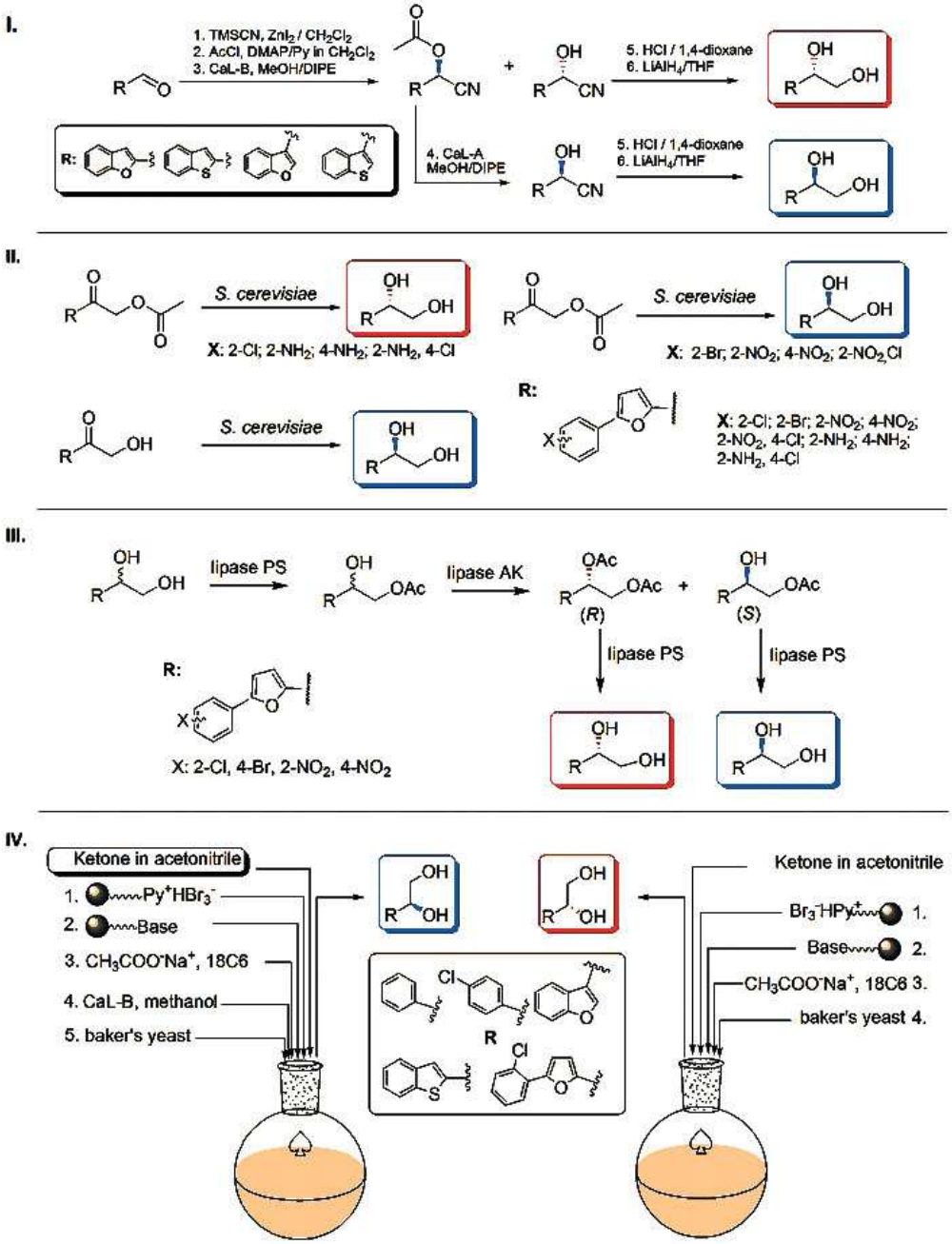
Keywords: baker's yeast, biocatalysis, biotransformation, (hetero)aryl-1,2-ethanediols, kinetic resolution, lipase, one-pot, stereoselective synthesis, substituent effect

Graphical abstract:



1. Introduction

The progress of human civilization is directly linked to the progress of chemistry. The need for comfort, health maintenance and restoration are untouchable goals in absence of chemistry.

The new materials made by the chemical processes found applications in the field of medicine, food, cleansing agents, industries (e.g. oil, construction industry), cosmetics and etc. The pharmaceutical industry is one of the world's most emerging industries designed to provide drugs, used as medicines to prevent, treat and heal the main diseases of the humanity. The structural complexity of the novel validated drugs is continuously increasing and, unsurprisingly, given that the human body functions using chiral catalyst, the trend for new chiral pharmaceutical reagents continuosly increased in the last decades. Nowadays these chiral compounds are usually manufactured in single enantiomeric form.

This thesis belongs to the field of biocatalysis and biotransformation, providing novel highly selective and greener processes, as well as novel potential chiral synthons for the pharmaceutical and fine chemical industry. The biocatalysis, the chemical synthesis mediated by a biocatalyst (isolated enzyme, whole cell systems) became a key component in the pharmaceutical sector. The exquisite chemo-, regio-, and stereoselective properties of biocatalysts enabled difficult synthesis to be circumvented, especially in the synthesis of 'single enantiomeric form'- products.

The chemical industry also faces pressure in developing new, process integrated and "greener" technologies, with the aim to decrease the environmental impact. Biocatalysis has the potential to be used as integrated clean technology, due to the mild reaction conditions (pH, temperature, pressure), the environmentally friendly and compatible biocatalysts, which allows to enable sequential reactions.

The recent advances in the field of biocatalysts enabled the biocatalytic processes to compete successfully with the conventional chemical processing, significantly increasing the industrial applications of biocatalysis. The recent advances in protein design and engineering, reaction engineering, and further disciplines resulted in improvements of existing enzymatic processes and developed alternative or completely novel ones. Thus the increasement of the share of biotechnology in organic fine chemical synthesis and the replacement of traditional synthetic manufacturing procedures is precognized.

This thesis limits to the discussion of the stereoselective synthesis of enantiopure (hetero)aryl-1,2-ethanediols, important chiral precursors and intermediates in the

pharmaceutical industry. Besides obtaining various novel compounds with potential pharmaceutical applicability, novel chemoenzymatic synthetic procedures were also succesfully developed.

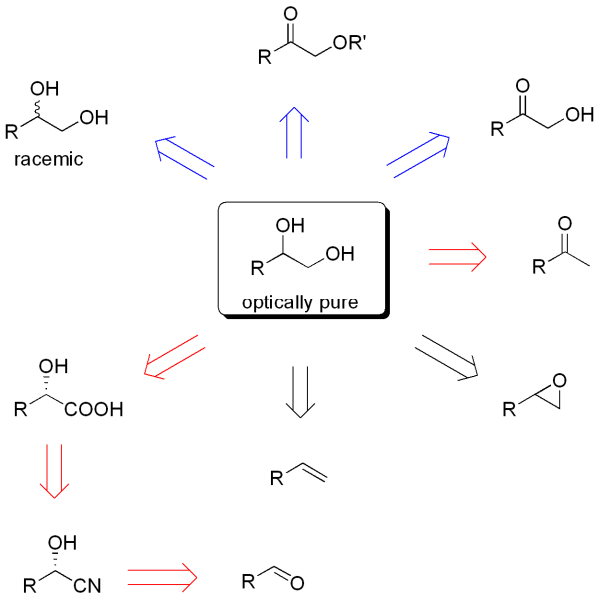
2. T**heoretical part** *(literature data*)

3. Aims of the study

In the present doctoral thesis, devoted to the stereoselective synthesis of heterocyclic optically pure 1,2-ethanediols with potential applicability in the pharmaceutical industry, the following aims were proposed:

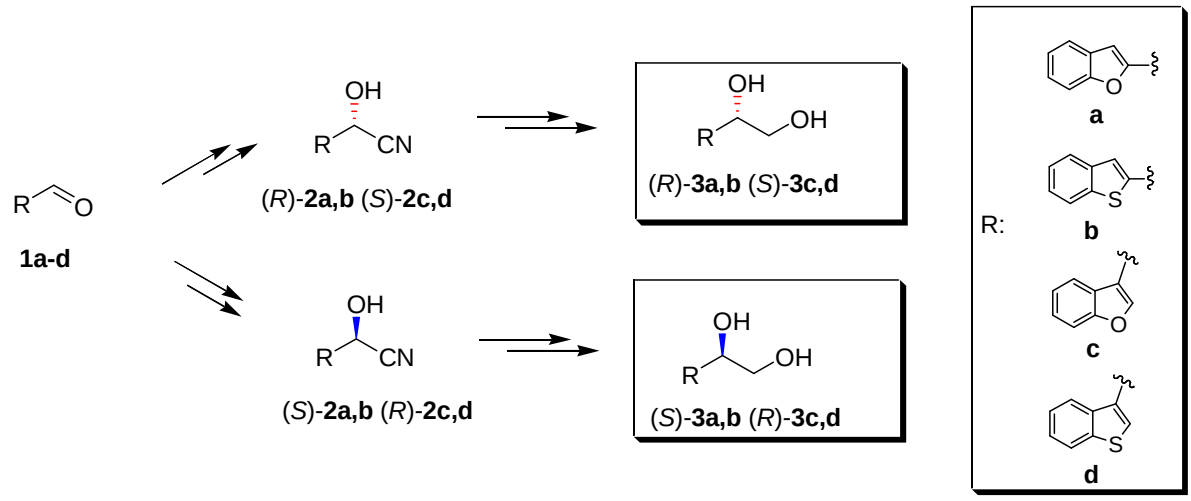
1. Enhancement of the enantiopurity of the previously obtained benzofuran and benzo[*b*]thiophene 1,2-ethanediols, by developing a novel chemoenzymatic synthetic procedure.

In case of these previously prepared (*R*)- and (*S*)-1,2-ethanediols **3a-d** the global yield and the enantiopurity of the products were not satisfactory in all cases (see chapter 2.3.4).[63] To overcome these drawbacks, by further retrosynthetic analysis (Scheme 1 red lines on left), we proposed a new alternative synthetic route based on novel optically pure cyanohydrins **2a-d**, well-known versatile chiral synthons[87], which could be easily prepared from the corresponding, more available aldehydes **1a-d**.



Scheme 1. *Retrosynthetic pathways for the optically pure 1,2-ethanediols (red trace – new retrosynthetic pathways proposed by us, blue trace – previous retrosynthetic routes employed for the synthesis of novel heteroaryl-1,2-ethanediols, black trace- known routes, not employed in the thesis)*

A widely used approach for synthesis of pure enantiomers of cyanohydrins is based on the kinetic (KR)[88] or dynamic kinetic resolutions (DKR)[89] of the racemic cyanohydrins or their esters with different hydrolases, especially lipases[90.] The chemical[91] and enzymatic hydrolysis[92] of the nitrile group of the enantiomerically enriched cyanohydrins, into the corresponding hydroxy acids and amides is already described and thus, by the subsequent chemical reduction both enantiomers of the desired 1,2-ethanediols (*R*)- and (*S*)-**3a-d** can be obtained. The studied reaction sequence is presented in Scheme 2.



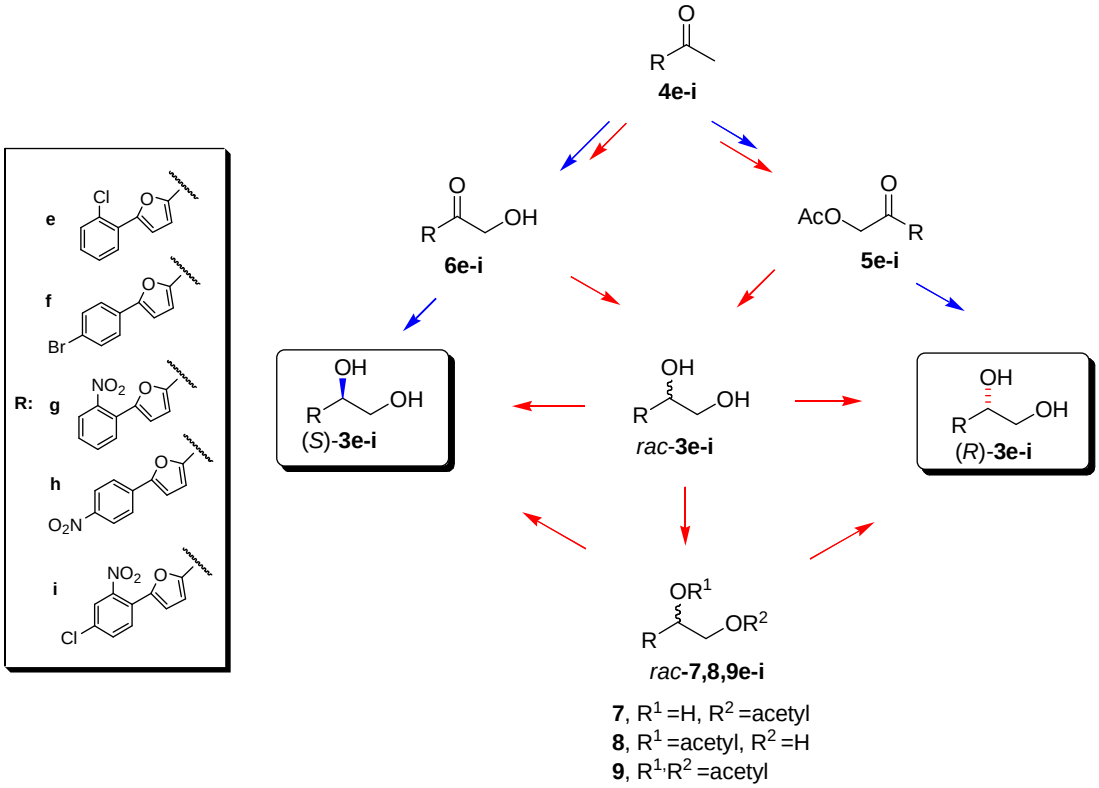
Scheme 2. *The synthesis of both (R)- and (S)-benzofuranyl- and benzo[b]thiophenyl-1,2-ethanediols **3a-d** via optically pure cyanohydrins **2a-d***

2. Synthesis of novel optically pure phenylfuran-2-yl-ethanediols **3e-i** by two different biocatalytic procedures: the baker's yeast mediated biotransformation of α-hydroxymethyl ketones **6e-i** and α-acetoxymethyl ketones **5e-i** (Scheme 3, blue lines) and the lipase mediated kinetic resolution of the corresponding racemic 1,2-ethanediols *rac*-**3e-i** and their acylated derivatives *rac*-**7,8,9e-i** (Scheme 3, red lines). In both cases the synthetic procedures use the corresponding heteroaryl ethanones **4e-i** as starting materials.

The baker's yeast mediated chemoenzymatic procedure is an environmentally friendly and efficient method for the synthesis of both enantiomers of several heteroaryl-1,2-ethanediols (Chapter 2.3.4.), thus its employment is worthily justified.

The lipase mediated biotransformation of aryl-1,2-ethanediols have also been successfully employed for the synthesis of various aryl-1,2-ethanediols (see Chapter 2.3.3.3.). In this case we tested all the possible kinetic resolution processes of the differently substituted

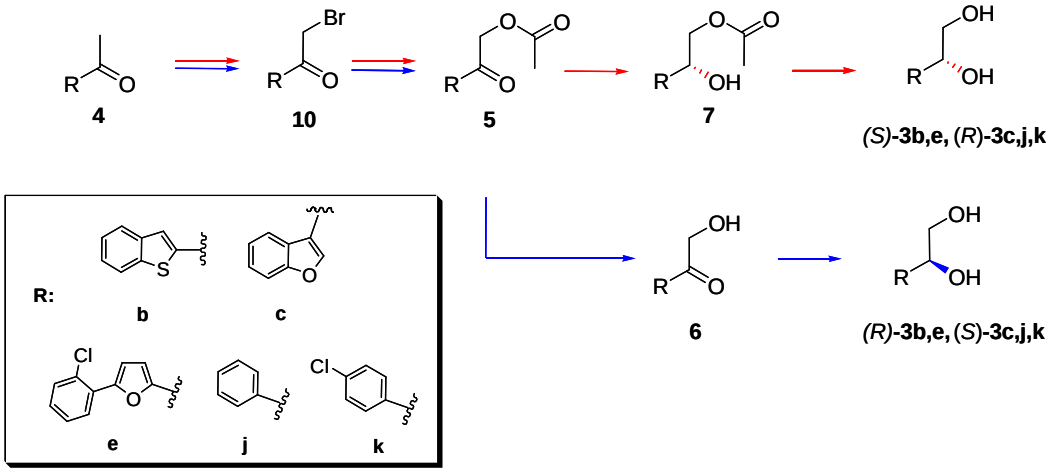
phenylfuran-2-yl-ethane-1,2-diols *rac*-**3e-i** and their primary **7e-i** or secondary monoacylated **8e-i** or diacylated **9e-i** derivatives, in order to develop an efficient chemoenzymatic method, based upon the most efficient kinetic resolution process.



Scheme 3. *Synthesis of both enantiomers of the optically active novel phenylfurane-1,2-ethanediols **3e-i** by biotransformations with baker's yeast (blue line) or by lipase mediated kinetic resolution (red lines)*

3. Development of a general, one-pot, highly efficient methodology for the synthesis of enantiomerically enriched aryl-1,2-ethanediols starting from a cheep, achiral compound.

An enantioselective enzymatic synthesis by a simple and efficient one-pot method for the synthesis of aryl-1,2-ethanediols starting from the achiral and cheep ethanones is still an awarding goal. Thus the previously developed chemoenzymatic multistep synthesis[63] of both (*R*)- and (*S*)-1-aryl-1,2-ethanediols, based on the enantiotope selective baker's yeast mediated reduction, by further process optimization was transformed into a chemoenzymatic one-pot procedure using as starting materials the corresponding ketones **4** (Scheme 4).



Scheme 4. *Blue lines- the one-pot process involving the biotransformation of α-hydroxy ketones **6**; red lines – the one-pot process involving the biotransformation of α-acetoxymethyl ketones **5***

4. Results and discussion

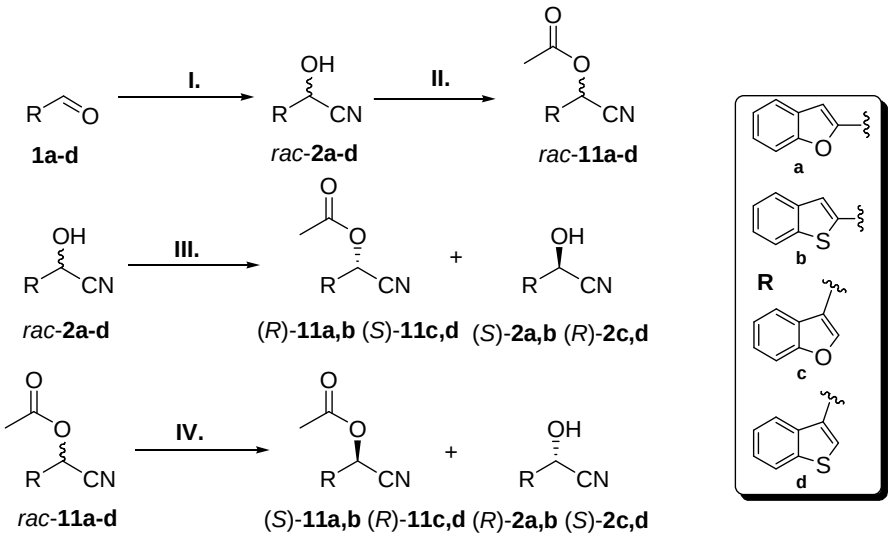
4.1. Synthesis of enantiomerically enriched (*R*)- and (*S*)-benzofuranyl- and benzo[*b*]thiophenyl-1,2-ethanediols 3a-d *via* enantiopure cyanohydrins 2a-d as intermediates

4.1.1. Synthesis of the racemic cyanohydrins 2a-d and their acylated derivatives 11a-d.

The synthesis of the racemic cyanohydrins *rac*-**2a-d** from the corresponding aldehydes **1a-d** was performed using trimethyl sylil cyanide in the presence of a catalytic amount of anhydrous ZnI_2 in dichloromethane. Further the racemic cyanohydrins *rac*-**2a-d** were acylated with acetyl chloride in presence of Py/DMAP in dichloromethane, yielding the corresponding racemic cyanohydrin acetates *rac*-**11a-d** (Scheme 5)**.**

4.1.2. Analytical scale enzymatic transformations

To investigate the stereoselectivity of the enzymatic reactions and the activity of the enzymes, first the chromatographic enantiomeric separation of the racemates *rac*-**2,11a-d** was established. In order to obtain highly enantiomerically enriched (*R*)- and (*S*)-heteroaryl cyanohydrins, potential useful lipases were screened in various organic solvents for the enantiomer selective acylation with vinyl acetate (5 eq.) of the racemic cyanohydrin *rac*-**2a-d** and alcoholysis (methanol, ethanol, propanol and butanol, 8 eq.) of the racemic cyanohydrin-acetates *rac*-**11a-d** respectively (Scheme 5).



I. $(CH_3)_3SiCN$, ZnI_2 in CH_2Cl_2, rt.; **II.** CH_3COCl, DMAP/Py in CH_2Cl_2, rt.; **III.** vinyl acetate, L-AK / organic solvent; **IV.** CH_3OH, CaL-B / DIPE.

Scheme 5. *The synthesis and the enantiomer selective biotransformation of the racemic heteroaryl cyanohydrins and cyanohydrin acetates.*

The behavior of lipases greatly differs for both types of the enzymatic reactions. For the analytical scale enzymatic acylation of *rac*-**2a-d,** most of the lipases, including lipase PS, previously shown to be one of the most useful in many cases[87], were catalytically inactive. Lipase A from *Candida antarctica* immobilized on Celite (CaL-A), reticulated with glutaraldehyde, CaL-A (CLEA), or covalently immobilized (IMMCal-A T2-150), catalyzed with slow rate and poor selectivity the acylation of *rac*-**2a-d** in all tested solvents. Surprisingly, lipase B from *Candida Antarctica* (CaL-B) was also found to be an inefficient catalyst. The reaction rate of the enzymatic acylation was around 5 % after 7 days, however the enantiopurity of the acylated products were high (ee > 98 %).

For the enantioselective acylation of the racemic cyanohydrins *rac*-**2a-d,** only lipase from *Pseudomonas fluorescens* (L-AK) was highly active and selective. This result was in good accordance with the previous observations, when L-AK was found as the optimal catalyst for the enantiomer selective acylation of benzofuranyl- and benzo[*b*]thiophenyl-ethanols.[93]

As expected, the stereoselectivity of the reaction was influenced by the nature of the solvent. While lipase AK mediated acylation with vinyl acetate (5 eq.) of *rac*-**2a-c** showed the highest selectivity in dichloromethane, using the same enzyme DIPE was found as optimal

solvent for the biotransformation of *rac*-**2d** (Table 1, entries 4-8, data shown only for the enzymatic acylation of *rac*-**2a**).

To reach the maximal enantiomeric excess for the resolution products, the influence of the nature and the amount of the acyl donor was also tested. While the use of vinyl butanoate as acyl donor (Table 1, entry 5) did not improve the enantioselectivity of reaction in dichloromethane, testing the stereoselectivity with different amount of vinyl acetate (Table 1, entries 4, 6-8) it was found that 4 eq. of vinyl acetate is the optimal quantity for the highest ee of the resolution products (Table 1, entry 7).

Table 1. The influence of the type of solvent and acyl donor upon the selectivity for the lipase AK mediated acylation of *rac*-**2a**.

Entry	Solvent	Acylating agent	Time (h)	c (%)	ee_P	ee_S	E
1	Toluene	Vinyl acetate (5 eq.)	36	41	97	68	134
2	DIPE	Vinyl acetate (5 eq.)	21	40	96	64	95
3	*t*-BME	Vinyl acetate (5 eq.)	21	48	97	90	~200
4		Vinyl acetate (5 eq.)	21	51	97	> 99.5	»200
5		Vinyl butanoate (5 eq.)	27	48	98	96	»200
6	Dichloromethane	Vinyl acetate (8 eq.)	16	50	97	96	>200
7		Vinyl acetate (4 eq.)	16	50	98	98	»200
8		Vinyl acetate (2 eq.)	16	50	96	98	>200
9	*n*-hexane	Vinyl acetate (8 eq.)	21	45	91	75	48

The results of the analytical scale LAK mediated acetylation of *rac*-**2a-d** with 4 eq. of vinyl acetate are shown in Table 3, entries 1-4. It is important to note that the stereoselectivity and the rate of the enzymatic reaction were influenced by the structure of the heteroaryl cyanohydrins. While the enzymatic kinetic resolution of benzofuran-2-yl- and benzo[*b*]thiophen-2-yl-cyanohydrins (*rac*-**2a,b**) undergoes with high enantioselectivity (Table 3, entries 1-2), when benzofuran-3-yl- and benzo[*b*]thiophen-3-yl-cyanohydrins (*rac*-**2c,d**) were used as substrates, the transformation occurred slowly (46 % conversion after 18 h and 41% conversion after 23 h respectively), affording the resolution products with unsatisfactory enantiopurity (Table 3, entries 3 and 4).

Further the analytical scale enantiomer selective alcoholysis of the racemic heteroaryl cyanohydrin acetates *rac*-**11a-d** was investigated. Using an optimization procedure in order to select the proper enzyme, solvent, type and amount of nucleophile, it was found that diisopropyl ether as solvent and methanol (2 eq.) as nucleophile are the most appropiate for the highly enantioselective CaL-B mediated alcoholysis of *rac*-**11a-d**. CaL-A, CaL-A(CLEA) and IMMCal-A T2-150 catalyzed rapidly with poor selectivity the alcoholysis of *rac*-**11a-d**. In Table 2, a selection of data for the enantiomer selective alcoholysis of *rac*-**11a** in various conditions is presented. Thus, both cyanohydrins and cyanohydrin acetates were obtained after 2 hours at around 50% conversion with high ee (Table 2, entry 9). Higher amounts of methanol decreased the reaction rate without significantly altering the stereoselectivity of the CaL-B mediated methanolysis of *rac*-**11a-d** (Table 2, entries 7,8). CaL-A, CaL-A(CLEA) and IMMCal-A T2-150 catalyzed rapidly with poor selectivity the alcoholysis of *rac*-**11a-d**.

Table 2. The influence of the nature of solvent and nucleophile upon the selectivity of the CaL-B mediated transesterification of *rac*-**11a**

Entry	Solvent	Reactant	Time (h)	c (%)	ee_p	ee_s	E
1	Acetonitrile	methanol (8 eq.)	3	26	> 99.5	35	»200
2	*n*-hexane	methanol (8 eq.)	3	49	97	92	~200
3		methanol (8 eq.)	1.5	48	98	90	>200
4		ethanol (8 eq.)	1.5	39	99	64	>200
5		1-propanol (8 eq.)	1.5	43	99	76	»200
6	DIPE	1-butanol (8 eq.)	1.5	41	99	68	»200
7		methanol (6 eq.)	2	48	99	93	»200
8		methanol (4 eq.)	2	49	99	95	»200
9		methanol (2 eq.)	2	49.5	99	97	»200
10	Dichloromethane	methanol (8 eq.)	3	15	> 99.5	17	»200
11	Toluene	methanol (8 eq.)	3	39	> 99.5	64	»200
12	*t*-BME	methanol (8 eq.)	3	49	96	91	156

The results for the optimal analytical scale CaL-B mediated methanolysis of *rac*-**11a-d** in DIPE are presented in Table 3, entries 5-8. The same dependence of the reaction rate and stereoselectivity on the structure of the heteroaryl cyanohydrin acetates *rac*-**11a-d** was observed as found for the enzymatic acylation of *rac*-**2a-d**. While the enzymatic kinetic resolution of benzofuran-2-yl- and benzo-[*b*]thiophen-2-yl-cyanohydrin acetates (*rac*-**11a,b**) undergoes with high enantioselectivity (Table 3, entries 5,6), the reaction rate and the ee

values of the resolution products were lower when benzofuran-3-yl- and benzo[*b*]thiophen-3-yl-cyanohydrin acetates (*rac*-**11c,d**) were used as substrates (Table 3, entries 7,8).

Table 3. The optimal conditions for the enantioselective resolution of racemic cyanohydrins *rac*-**2a-d** and cyanohydrin acetates *rac*-**11a-d**

Entry	Substrate	Enzyme	Solvent	Time (h)	c (%)	ee_P (%)	ee_S (%)
1	*rac*-**2a**	Lipase AK	dichloromethane	15	50	98	98
2	*rac*-**2b**	Lipase AK	dichloromethane	13	50	97	97
3	*rac*-**2c**	Lipase AK	dichloromethane	18	46	92	79
4	*rac*-**2d**	Lipase AK	DIPE	23	41	82	57
5	*rac*-**11a**	CaL-B	DIPE	2	50	> 99.5	>99.5
6	*rac*-**11b**	CaL-B	DIPE	1	50	> 99.5	> 99.5
7	*rac*-**11c**	CaL-B	DIPE	13	50	98	98
8	*rac*-**11d**	CaL-B	DIPE	21	49	97	93

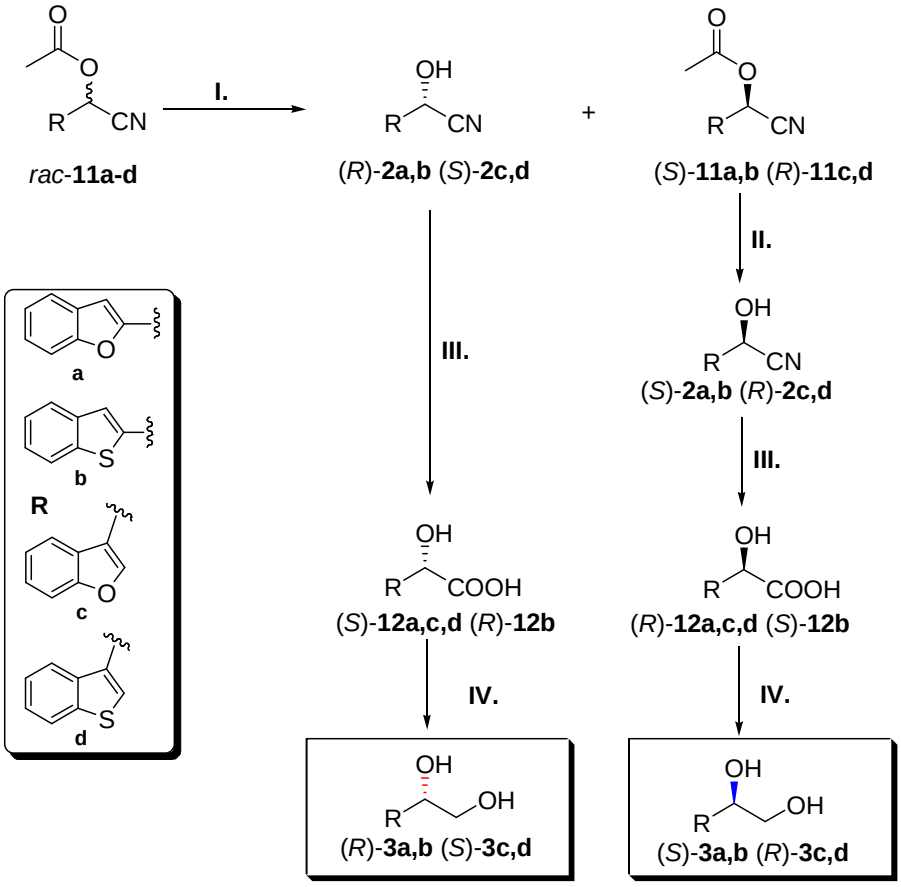
4.1.3. The preparative synthesis of (*R*)- and (*S*)- heteroaryl-1,2-ethanediols 3a-d

Using the procedure depicted in Scheme 6, the preparative scale synthesis of both (*R*)- and (*S*)-heteroaryl-1,2-ethanediols starting from the racemic heteroaryl cyanohydrins was also performed. Because the lipase catalyzed methanolysis of *rac*-**11a-d** undergoes with higher stereoselectivity than the enzymatic acylation of *rac*-**2a-d**, further using the same reagents, enzyme and solvent as in the case of the analytical scale reactions, the preparative scale resolution of *rac*-**11a-d** was performed (Table 4A). All dilutions, substrate–biocatalyst *ratio* and reaction conditions were the same as in the case of the analytical scale reactions. The reactions were monitored by HPLC and TLC and were stopped at an approx. 50% conversion, removing the enzyme by filtration.

As already mentioned, all three lipases A from *Candida antarctica* used in the analytical scale enzymatic methanolysis of *rac*-**11a-d** were found to be highly active, but non-selective enzymes. Therefore, the isolated enantiomerically enriched heteroaryl cyanohydrin acetates (*S*)-**11a,b**, (*R*)-**11c,d,** the products of the enantiomer selective enzymatic alcoholysis of *rac*-**11a-d**, were almost quantitatively transformed into the corresponding cyanohydrins (*S*)-**2a,b**, (*R*)-**2c,d** by CaL-A(CLEA) mediated methanolysis in DIPE (Table 4B, Scheme 6). Data on yields, enantiomeric excess and optical rotation of the obtained enantiomers are presented in Table 4.

Further, both enantiomers of the heteroaryl cyanohydrins (*R*)- and (*S*)-**2a-d** were chemically hydrolyzed into the corresponding α-hydroxy acids (*R*)- and (*S*)-**12a-d**. After isolation, the latest compounds were reduced with $LiAlH_4$ affording with good yields and

with small decreasing of the enantiopurity the corresponding heteroaryl-1,2-ethanediols (*R*)- and (*S*)-**3a-d** as shown in Table 5**.**



I.CH_3OH, CaL-B / DIPE; **II.**CH_3OH, CaL-A (CLEA) / DIPE ;
III. 6N aqueos HCl / dioxane, reflux; **IV.** $LiAlH_4$ / THF, rt.

Scheme 6. *Preparative scale synthesis of both (R)- and (S)-heteroaryl cyanohydrins* ***2a-d*** *and their transformation into the corresponding heteroaryl-1,2-ethanediols* ***3a-d****.*

Table 4. Yields, ee and optically rotatory power for the CaL-B mediated kinetic resolution products (A) and for the CLEA mediated methanolysis of the enantiopure cyanohydrin acetates.

	A							B			
	Yield*	ee	$[\alpha]_D^{25}$		Yield*	ee	$[\alpha]_D^{25}$		Yield**	ee	$[\alpha]_D^{25}$
(*R*)-**2a**	48	98	-53.9	(*S*)-**11a**	48	98	-54.9	(*S*)-**2a**	96	99	+55.7
(*R*)-**2b**	48	98	-26.3	(*S*)-**11b**	48	98	-30.8	(*S*)-**2b**	93	>99.5	+27.9
(*S*)-**2c**	49	96	-37.4	(*R*)-**11c**	46	98	+11.9	(*R*)-**2c**	95	99	+40.5
(*S*)-**2d**	48	96	-47.8	(*R*)-**11d**	46	97	+24.8	(*R*)-**2d**	96	>99.5	+51.5

based on *rac*-**11a-d**; ** based on (*S*)-**11a,b** and (*R*)-**11c,d**

Table 5. The preparative-scale synthesis of both enantiomerically enriched enantiomers of the heteroaryl-1,2-ethanediols **3a-d**

3	(*S*)-**3a-d**			(*R*)-**3a-d**		
	Yield	ee	$[\alpha]_D^{20}$	Yield	ee	$[\alpha]_D^{20}$
a	46	97	-28.2	46	97	+28.2
b	46	96	-13.1	47	96	+13.1
c	42	91	-24.6	48	95	+25.7
d	31	94	-44.8	47	93	+44.3

The sense of the optical rotation and the chromatographic retention times of enantiomers of the heteroaryl-1,2-ethanediols **3a-d** were compared with those described in literature[63] in order to establish the stereochemical course of the selective enzymatic reactions of the benzofuranyl- and benzo[*b*]thiophenyl-cyanohydrins and their acetates since their absolute configurations were unknown.

4.1.4. Determination of the absolute configuration by VCD measurements

The absolute configuration of optically active cyanohydrin acetates **11a-d** obtained by the enantioselective methanolysis of *rac*-**11a-d** was also determined using (VCD) measurements combined with quantum chemical calculations. VCD spectra in $CDCl_3$ of (-)-**11a,** (-)-**11b,** (+)-**11c** and (+)-**11d**, with unknown absolute configuration, obtained by the enantioselective methanolysis of *rac*-**11a-d**, are shown in Figure 1a. All the four spectra are dominated by the negative $\nu_{C=O}$ band of the ester carbonyl group at ~1750 cm^{-1}, and they have a more or less similar pattern in the fingerprint region (1600-1100 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 does not influence the position and sign of the ester $\nu_{C=O}$ VCD band and has only moderate 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.[94]

The determination of the absolute configuration was based on the comparison of the computed and measured spectra for compound (-)-**11a** (see Figure 1b).

The calculations were performed for the (*S*)-**11a** enantiomer and the three lowest-energy conformers, shown in Figure 2, with a total estimated population of 99% considered for the simulation of the theoretical VCD spectrum. The agreement between the calculated

and measured VCD spectra is reasonably good, both in terms of wave number values and the signs of the VCD bands (the matching pairs are labeled with corresponding numbers in Figure 1b) which permits to unambiguously assign the absolute configuration to *S*.

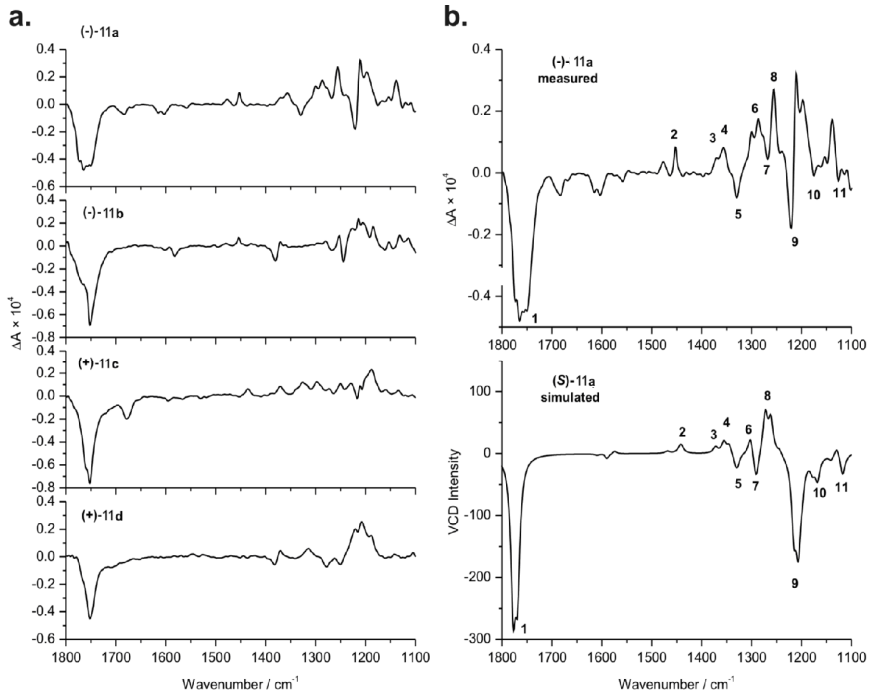


Figure 1.

a. *VCD spectra of compounds (-)-**11a**, (-)-**11b**, (+)-**11c** and (+)-**11d** measured in $CDCl_3$;* ***b.*** *VCD spectrum of (-)-**11a** measured in $CDCl_3$ solution (top) in comparison with the simulated VCD spectrum of (S)-**11a** (bottom), obtained as a population-weighted sum of the calculated spectra of individual conformers. Corresponding bands are labeled with identical numbers*

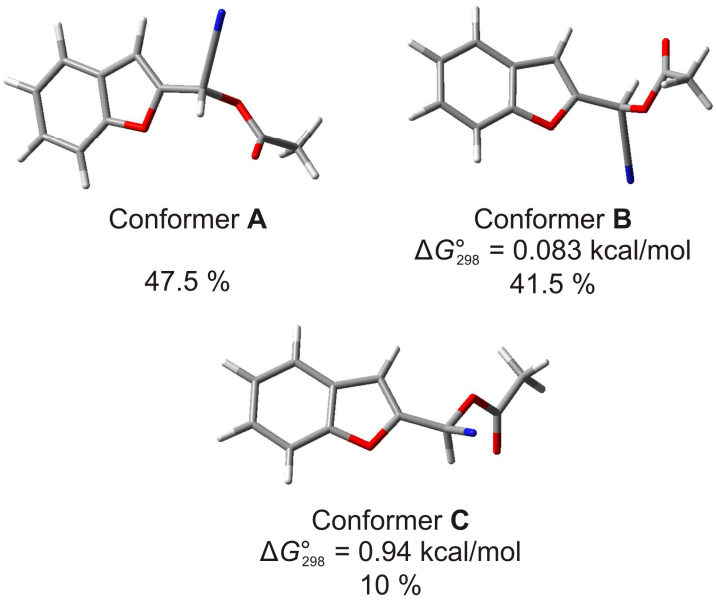


Figure 2.

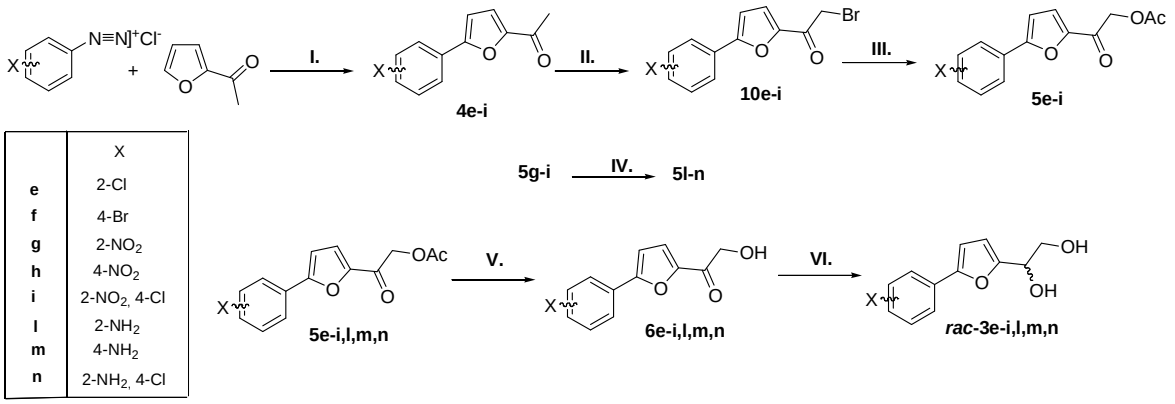
*Computed structures of the three most abundant eq.uilibrium conformers of (S)-**11a,** with the indication of their relative Gibbs free energies and estimated populations*

4.2 Baker's yeast-mediated biotransformation of α-hydroxy- and α-acetoxymethyl- 5-phenylfuran-2-yl-ethanones 5,6e-i,l,m,n

4.2.1 Synthesis of the substrates 5,6e-i,l,m,n

The synthesis of the substrates was performed in accordance with the earlier reported chemoenzymatic method.[63] The heteroaryl-ethanones **4e-i** used as starting materials, were prepared by the Meerwein method[95] from the corresponding diazonium salts of various anilines and 2-acetyl-furan. Further the obtained ketones **4e-i** were α-brominated, followed by their transformation into α-acetoxymethylketones **5e-i** using sodium acetate as reactant, dioxane as solvent and 18C6 crown ether as phase transfer catalyst. The α-acetoxymethylketones **5l-n** were prepared from the α-acetoxymethylketones **5g-i** by the selective reduction of the nitro group with $SnCl_2$ in ethanol. Further by the enzymatic ethanolysis of **5e-i,l,m,n** the α-hydroxymethylketones **6e-i,l,m,n** were obtained with excellent yields. Finally the latest compounds were reduced with sodium borohydride to yield the *rac*-heteroaryl-1,2-ethanediols *rac*-**3e-i,l,m,n** (Scheme 7).

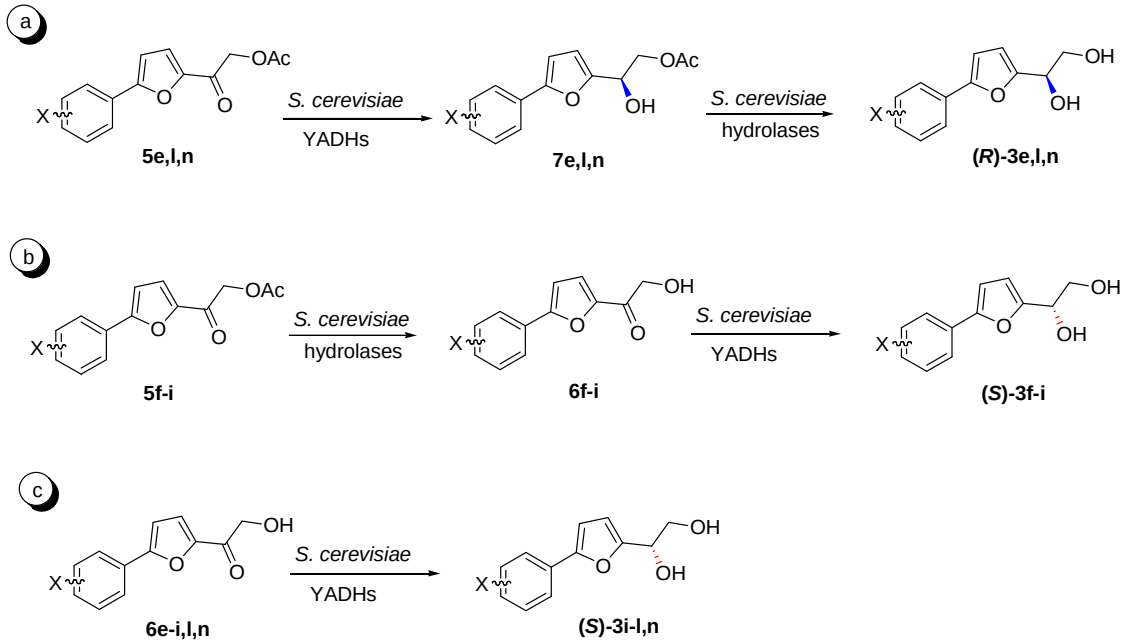
After that, the chromatographic separation of the enantiomers of *rac*-**3e-i,l,m,n** was developed, which allows us to investigate the stereochemical outcome of the baker's yeast mediated biotransformations (Scheme 8).



Scheme 7. *Synthesis of the prochiral ketones* ***5,6e-i,l,m,n*** *and racemic 1,2-heteroaryl-ethane diols* ***3e-i,l,m,n****. I.* $CuCl_2/H_2O$*, acetone; II. pyridinium tribromide/*CH_3COOH*, 80* ^0C*; III.* $CH_3COO^-Na^+$*, 18C6/1,4-dioxane, reflux; IV.* $SnCl_2$*/EtOH, ultrasound; V. Novozyme 435/EtOH; VI.* $NaBH_4$*/MeOH*

4.2.2. Baker's yeast mediated cellular transformations

In our first approach, the baker's yeast mediated transformation of **5,6e-i** under fermenting and non-fermenting conditions was performed (Table 6). Further, in order to increase the enantiopurity of the products, the reactions were performed in the conditions with higher stereoselectivity (fermenting or nonfermenting system) using various additives (Table 7) which can influence the stereoselectivity of the cellular transformation as described in Chapter 2.3.5.4. and other previous reports.[96,97]



Scheme 8. *Baker's yeast mediated stereoselective biotransformation of ketones* ***5,6e-i,l,n***

The influence of various additives upon the stereoselectivity of the reactions differs for each substrate. As example, while the bioreduction of **5e** showed the highest selectivity in the presence of allyl alcohol and ethyl-bromoacetate (Table 7, entries 3 and 6), in case of **6i** these additives decreased the selectivity of the bioreduction (Table 7, entries 3 and 6) and the highest selectivity appeared in the presence of Mg^{2+} ions (Table 7, entry 7). For the biotransformations of **5g,h** the best results were obtained using $MgCl_2$ and dimethyl-sulfoxide (DMSO) respectively as additive (Table 8, entries 4 and 5). In case of **5f,i** and **6e** the use of different additives didn't increased the selectivity of the processes, for **6e** the nonfermenting system (Table 6, entry 6), while in case of **5f,i** the fermenting system (Table 6, entry 2,5) showed the highest selectivity.

Table 6. The fermentative and nonfermentative biotransformation of **5,6e-i**

Entry	Substrate	Product	ee (%)		Yield [c] (%)	
			a	b	a	b
1	**5e**	(*R*)-**3e**	67	60	81	85
2	**5f**	(*S*)-**3f**	58	46	58	49
3	**5g**	(*S*)-**3g**	80	73	85	79
4	**5h**	(*S*)-**3h**	87	69	90	60
5	**5i**	(*S*)-**3i**	39	37	80	75
6	**6e**	(*S*)-**3e**	90	97	89	90
7	**6f**	(*S*)-**3f**	41	36	65	61
8	**6g**	(*S*)-**3g**	83	79	70	59
9	**6h**	(*S*)-**3h**	60	52	75	72
10	**6i**	(*S*)-**3i**	75	52	61	60

a. Fermenting system; b. Nonfermenting system; [c.] After 3 days

Table 7. The influence of various additives upon the stereoselectivity of bioreduction of ketones **5e** and **6i**

Entry	Additives	ee (%)		Yield (%)		Time (h)
		(*S*)-**3i**	(*R*)-**3e**	(*S*)-**3i**	(*R*)-**3e**	
1	A[a]	75	67	58	85	48
2	B[a]	52	60	52	81	48
3	Allyl alcohol[b]	40	89	91	93	48
4	*n*-hexane[b]	71	51	49	87	48
5	L-Cysteine[b]	77	66.7	58	85	48
6	Ethyl bromoacetate[b]	20	93	47	91	48
7	$MgCl_2$[b]	87	71.9	55	89	48

A. Fermenting system; B. Nonfermenting system;
[a] without additives; [b] in fermenting system

Generally, the cellular transformation of the α-acetoxymethylketones involves two concurring processes: the reduction of the carbonyl group catalyzed by the yeast alcohol dehydrogenases (YADHs) and the enzymatic hydrolysis of the α-acetoxy group respectively (Scheme 8a.). In previous works it was demonstrated that the reduction is faster than the hydrolysis.[63]

As we expected, the biotransformation of α-acetoxymethyl ketones **5e** and the respective α-hydroxyethanones **6e** takes place with opposite enantiopreference and with good selectivity (Table 6, entries 1 and 6). However, in contrast to the most of the earlier reported results[63,75c,98] the biotransformation of α-acetoxymethyl ketones **5g-i** (Scheme 8b, Table 6, entries 2-5) and the corresponding α-hydroxyethanones **6g-i** (Scheme 8c, Table 6, entries 7-10) showed the same stereochemical outcome and the selectivity of the processes were also lower than in case of the cellular transformation of **5,6e**.

The same stereochemical preference for the transformation of **5f** and **6f** (Table 6, entries 2 and 7) and the lower enantioselectivity for the reduction of **5,6f** could be explained by the fact that baker's yeast possesses several alcohol dehydrogenases, both (*R*)- and (*S*)-specific enzymes, which could display nearly equal activities towards this substrate, or due to the high sterical demand of the bromine group only one enzyme acts, but with low stereoselectivity. It is important to note that during the biotransformation of **5f** the presence of **6f** could not be detected proving that the rate of the reduction is considerably higher that of the hydrolysis of the esteric group.

The same stereochemical outcome for the biotransformation of α-acetoxymethyl ketones **5g-i** (Table 6, entries 3-5) and α-hydroxymethyl ketones **6g-i** (Table 6, entries 8-10) respectively could be explained by the higher activity of the hydrolases than those of the YADHs present in baker's yeast cells, towards α-acetoxymethyl ketones **5g-i**. By monitoring in time the biotransformation of **5g-i** the appearance of the hydroxyethanones **6g-i** was observed, proving that in these cases the hydrolysis is faster than the reduction (Scheme 8b).

We supposed that the strong electron withdrawing electronic effects of the nitro group reduce the electron density on the esteric carbon, enhancing its reactivity, thus the enzymatic hydrolytic process being favored.

To prove our supposition, further, by the selective chemical reduction of the nitro group, prochiral ketones **5g-i** were transformed into **5l-n** (Scheme 7, step IV) followed by their enzymatic alcoholysis into **6l-n**. As it was expected, the baker's yeast mediated biotransformations of α-acetoxyketones **5l,n** and α-hydroxyketones **6l,n**, all of them bearing an amino group as substituent, takes place with opposite enantiopreference and no trace of α-hydroxyethanones **6l,n** could be detected during the biotransformations of **5l,n**.

However, in both fermenting and nonfermenting system the enantiopurity of the products was low (Table 8, entries 7-10) and it could not be enhanced considerably using additives. Moreover, the produced (*R*)- and (*S*)-1-(5-aminophenyl-furan-2-yl)ethane-1,2-diols

3l-n were very sensitive, instable compounds. The produced diol **3m** decomposed completely *in situ* during the reaction.

Table 8. Baker's yeast mediated preparative scale biotransformation of heteroaryl-ketones **5e-i,l,n** and **6e-i,l,n**

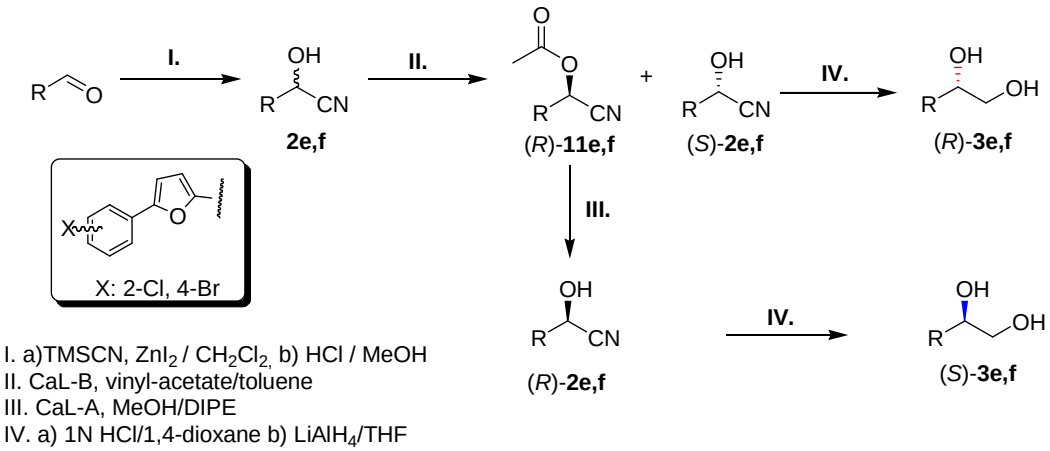
Entry	Substrate	Product	ee (%)	Reaction time (days)	Yield (%)	α_D^{25}
1	**5e**	(*R*)-**3e** [a]	94	2	88	+ 45
2	**6e**	(*S*)-**3e** [b]	97	2	90	−48.1
3	**5f**	(*S*)-**3f** [c]	50	2	60	− 17.1
4	**6g**	(*S*)-**3g** [d]	91	2	75	− 61
5	**5h**	(*S*)-**3h**[e]	88	3	79	− 41.7
6	**6i**	(*S*)-**3i** [d]	87	3	82	− 49
7	**5l**	(*R*)-**3l** [c]	9	2	65	+ 4.1
8	**6l**	(*S*)-**3l** [c]	80	2	72	− 28.1
9	**5n**	(*R*)-**3n** [c]	29	2	56	+ 9.8
10	**6n**	(*S*)-**3n** [c]	41	2	78	− 16.1

[a] Fermenting system with ethyl-bromoacetate as additive
[b] Nonfermenting system (without sucrose)
[c] Fermenting system
[d] Fermenting system with Mg^{2+} as additive
[e] Fermenting system with dimethylsulfoxide as additive

4.2.3. The absolute configuration of the synthesized diols

Because the absolute configuration of the produced (+)- and (−)-diols were unknown, the both enantiomers of **3e,f** were prepared from the corresponding (*R*)- and (*S*)-cyanohydrins[90a] **2e,f** as shown in Scheme 9.

The absolute configuration of the products was established comparing the chromatographic retention times and the sense of the optically rotatory power of the diols obtained by the two different methods. The same signs of the optically rotatory power of (*R*)-**3e,l,n** and other (*R*)- 1-(5-phenylfuran-2-yl)ethane-1,2-diols[99,100] was also in accordance with the assignment of the absolute configuration for the (+)-enantiomers described herein.



Scheme 9. *Retrosynthetic pathway to determinate the absolute configuration*

4.3. Lipase mediated kinetic resolutions of 5-phenylfuran-2-yl-ethane-1,2- diols

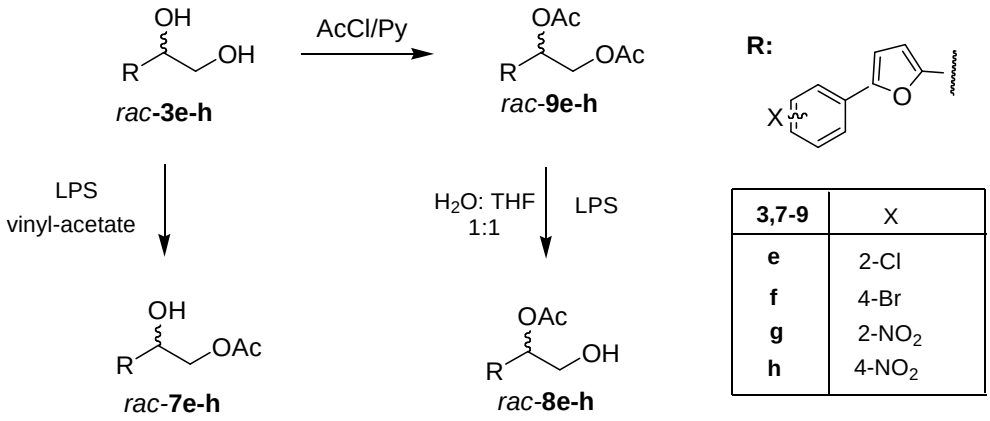
The previous presented work devoted to the chemoenzymatic synthesis of both enantiomeric forms of phenylfuran-2-yl-ethane-1,2-diols by the baker's yeast mediated biotransformation of α-acetoxymethyl-5-phenylfurane-2-yl-ethanones **5e-i** and of the α-hydroxymethyl-5-phenylfuran-2-yl-ethanones **5e-i** do not give the expected results. Due to the observed strong substituent effect, in some cases no biotransformation, or low enantiomeric excess values were obtained, the method proving to be unsatisfying for the general synthesis of both enantiomers of the purposed 1,2-ethanediols **3e-i**.

Therefore our interest turned towards the employment of the lipase mediated kinetic resolution for the synthesis of the highly enantiomerically enriched forms of phenylfuran-2-yl-ethane-1,2-diols.

4.3.1. Preparation of the racemic substrates *rac*-3,7,8,9e-h

The racemic 1-(5-phenylfuran-2-yl)ethane-1,2-diols *rac*-**3e-h** were prepared by the chemo-enzymatic method described earlier (Scheme 7). These were transformed by chemical acylation into racemic diacetates *rac*-**9e-h** (Scheme 10). In order to avoid the drawbacks of the protective groups employment for the regioselective acylation of *rac*-**3e-h** and because the chemical synthesis of racemic 2-hydroxy-1-(5-phenylfuran-2-yl)ethyl acetates *rac*-**7e-h** and 2-hydroxy-2-(5-phenylfuran-2-yl)ethyl acetates *rac*-**8a-d** by the previously described methods[56] failed, our attention turned towards highly regioselective but non stereoselective enzymatic methods. In accordance with earlier results[47-50,52,54] in case of enzymatic acylation of the racemic 1,2-ethanediols *rac*-**3e-h** it was observed that LPS acts in a highly regioselective manner, yielding only *rac*-**7e-h** (Scheme 10). Even after long reaction time no

trace of *rac*-**9e-h** and/or *rac*-**8e-h** was found in the reaction mixture. However, this result is in contrast with previous reports[47-50,52,54], where a second LPS catalyzed enantiomer selective acylation step also occurs, yielding finally the opposite enantiomeric forms of the optically active diacetylated and the monoacetylated 1,2-diols.



Scheme 10. *Chemoenzymatic synthesis of rac-**7,8,9e-h***

LPS was found as the proper biocatalyst also for the synthesis of racemic 2-hydroxy-2-(5-phenylfuran-2-yl)ethyl acetates *rac*-**8e-h**. Thus, by the LPS mediated hydrolysis of the racemic diacetate *rac*-**9e-h** in THF-water mixture (1:1, v/v) *rac*-**8e-h** occurs quantitatively (Scheme 10). Also in this case no trace of the possible byproducts was detected in the reaction mixture.

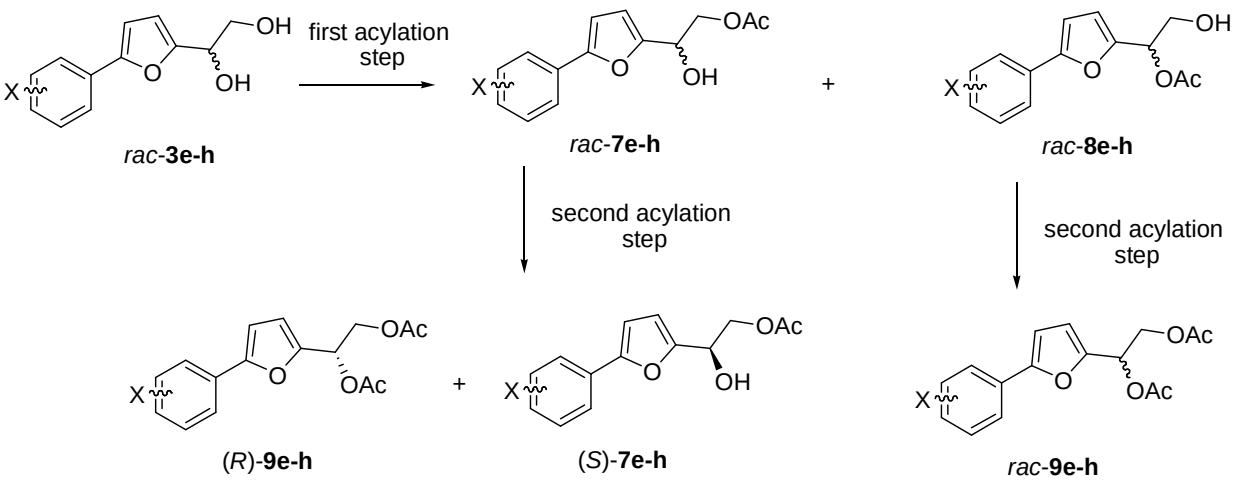
It is important to note that the same gram-scale procedures undergoes in similar way, the isolation and the purification of the target compounds was easy and simple providing an accessible synthetic route to both monoacetylated phenylfuran-2-yl-ethane-1,2-diols *rac*-**7,8e-h**. In this way the use of difficult chemical methods[51,53], requiring special reagent or experimental conditions, were avoided.

In order to synthesize optically pure 5-phenylfuran-2-yl 1,2-ethanediols further the enzymatic kinetic resolution of authentic racemic compounds *rac*-**3,7,8,9e-h** was investigated.

4.3.2. The enzymatic acylation of racemic diols *rac*-3e-h

In our first approach we focused on the enzymatic acylation of the racemic 1,2-ethanediols *rac*-**3e-h**. Using various solvents as reaction media and vinyl acetate as acyl donor most of the tested lipases like CaL-B (Novozyme 435, lipase from B *Candida antarctica*), LAK, CrL (lipase from *Candida rugosa*), lipase from *Mucor javanicus* showed

no or small activity. Only the highly active LPS and PPL (porcine pancreatic lipase) with moderate activity catalyzed regioselectively the acylation of the *rac*-**3e-h**, yielding the racemic 2-hydroxy-2-(5-phenylfuran-2-yl)ethyl acetates *rac*-**7e-h**. CaL-A (lipase A from *Candida antarctica*) catalyzed in a first step with poor regioselectivity the acylation of *rac*-**3e-h** yielding both *rac*-**7e-h** and *rac*-**8e-h** with an approximate 4:1 *ratio*. In the second step the formed monoacylated compounds were further acylated by CaL-A. As it is described in the next paragraph, while the acetylation of *rac*-**7e-h** undergoes stereoselectively, *rac*-**8e-h** was transformed in a non-stereoselective manner into the racemic diacetate *rac*-**9e-h** lowering the ee of (*R*)-**9e-h** produced in the reaction mixture as shown in Scheme 11. Thus the use of the racemic heteroaryl-ethane-1,2-diols *rac*-**3e-h** as substrates for an efficient enzymatic enantiomer selective resolution is ruled out.

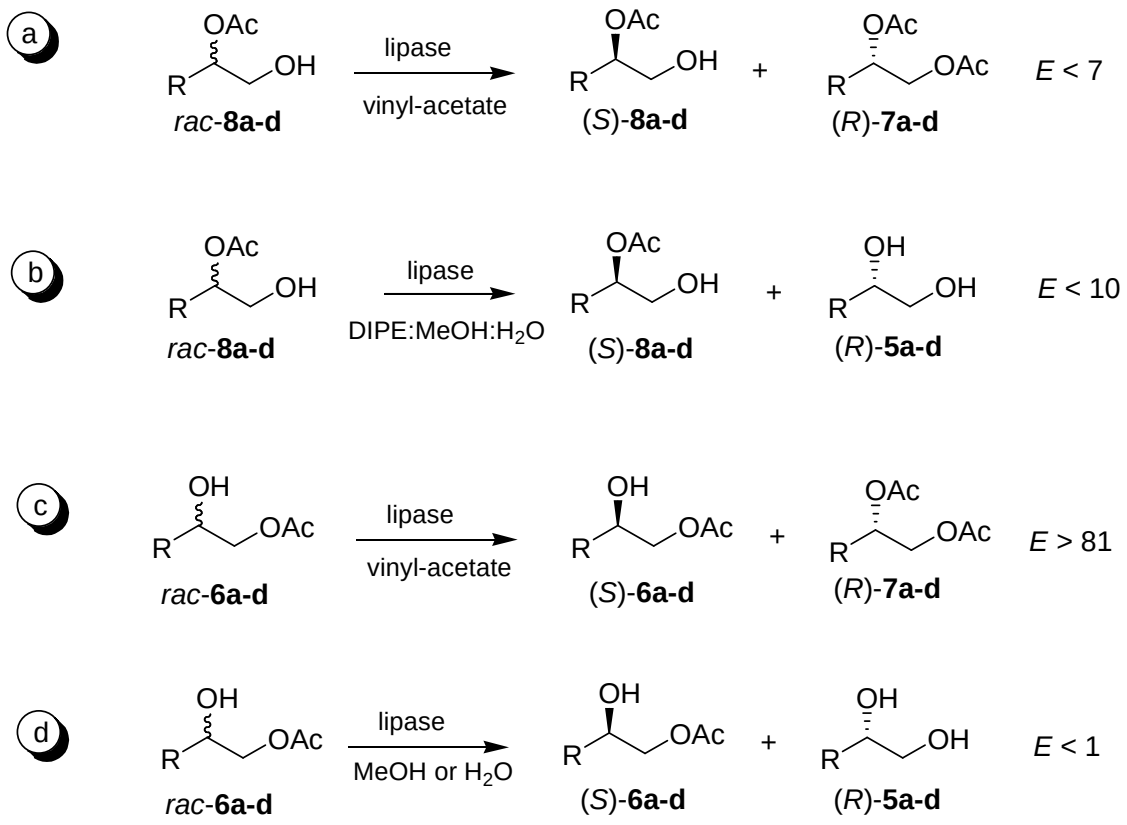


Scheme 11. *CaL-A mediated acylation of rac-**3e-h***

4.3.3. The enzymatic acylation, alcoholysis and hydrolysis of secondary and primary monoacetates *rac*-7,8e-h

It is known that lipases catalyze in their biological function the 1,3-regioselective hydrolysis of triacylglycerols at water-lipid interfaces. Consequently, we decided to investigate first the enzymatic kinetic resolution of racemic 2-hydroxy-2-(5-phenylfuran-2-yl)ethyl acetates *rac*-**8e-h**, since the enzymatic acylation of the primary hydroxyl group should undergo easily.

Using racemic 1-(5-(4-bromophenyl)furan-2-yl)2-hydroxyethyl acetate *rac*-**8f** as model compound, potentially useful lipases were screened for the enantiomer selective acylation with vinyl acetate (8 eq.) in various organic solvents. Most of the lipases showed high activity, in all tested solvents, only lipase from *Mucor javanicus* and PPL were catalytically inactive. The stereoselectivity of the enzymatic reaction was influenced by the nature of the used solvent as reaction media. Best results were obtained in diisopropyl ether (DIPE), however the obtained results were unsatisfactory (E<7.4, Scheme 12a) as shown in Table 9. Moreover, in all tests LAK was a highly active and totally non stereoselective enzyme. These results can be explained with the fact that the reacting hydroxyl group is near to the asymmetric center, thus the sterical recognition between the catalytic center of the enzyme and the enantiomers of the substrate is poor. Interestingly CaL-B showed opposite enantiopreference than all the other lipases, producing the (*S*)-diacetate and the (*R*)-secondary monoacetate both with moderate enantiomeric excess (Table 9, entry 3).



Scheme 12. *Enzymatic kinetic resolution of the racemic monoacylated diols*

Table 9. Enzymatic acylation with vinyl acetate of *rac*-**8f** in DIPE.

Entry	Enzyme	Time	c (%)	ee_P (%)	ee_S (%)	E
1	CaL-A	2h	30	31	13	2
2	LPS	2h	61	52	81	7
3	CaL-B[a]	2h	61	46	72	6
4	CrL	16 h	54	49	58	5

[a] anti-Kazlauskas type

The enzymatic alcoholysis or hydrolysis of the racemic secondary monoacetate *rac*-**8f** also gave unsatisfactory results. Most of the lipases, and other hydrolases like PLE, Acylase I and esterase from *Rhizopus oryzae* were catalytically inactive in neat alcohols like methanol, ethanol, propanol and butanol as well as in various organic solvent with a content of 8 eq. of the previously mentioned nucleophiles. The enzymatic hydrolysis performed in THF-water (1:1, v/v)mixture also failed. Interestingly a mixed enzymatic alcoholysis-hydrolysis process, with moderate enantioselectivity (E<10), yielding the (*R*)-**3f** (ee: 65%) and the (*S*)-**8f** (ee: 35%), occurred when the reaction was performed in a DIPE: MeOH : water (1:1:2, v/v) mixture in presence of LPS (Scheme 12b). Similar results were found also for the LPS mediated mixed alcoholysis-hydrolysis for the rest of the substrates *rac*-**8e,g,h**.

Further the enzymatic acylation of primary monoacetate derivatives *rac*-**7e-h** was performed (Scheme 12c). 2-hydroxy-2-(5-(2-nitrophenyl)furan-2-yl)ethyl acetate *rac*-**7g** was used as model compound for the screening of the potential useful lipases. All screening procedures were performed in DIPE in presence of vinyl acetate (8 eq.) as reactant.

Table 10. Lipase catalyzed acylation of the primary monoacetate *rac*-**7g**

Entry	Enzyme	Time (h)	c (%)	ee_P (%)	ee_S (%)	E
1	CaL-A	6	47	91	76	49
2	CaL-A (CLEA)	4	40	85	56	22
3	IMMCalA T2–150	4	34	73	37	9
4	LAK	24	43	94	71	69
5	CaL-B	16	3	50	2	3
6	CrL	16	21	53	14	4

Among the tested lipases, only CaL-A (Table 10, entry 1-3), and LAK (Table 10, entry 4) proved to be efficient catalysts, while lipase from *Mucor javanicus* and LAK were catalytically inactive. CaL-A showed higher activity than LAK, but the latest enzyme displays a higher selectivity. Beside CaL-A, the celite supported form of enzyme (Table 10, entry 1), other immobilized lipase A from *Candida antarctica* like CLEA (enzyme reticulated with

glutaraldehyde, Table 10, entry 2) or IMMCalA T2–150 (covalently immobilized enzyme, Table 10, entry 3) were tested and it was found that in term of stereoselectivity they are inefficient biocatalysts.

These behavior of CaL-A is in accordance with the *sn*-2 preference showed by the enzyme towards triacylglycerols. However CaL-A was rarely used in enantioselective reactions, usually being reported to be highly active in a non-selective manner[101], high E-values have been obtained with substrates having a bulky group vicinal to the stereogenic centre[90b,c,102,103].

More surprising is the contrary behavior of the LAK compared to previously reported observations, when it was found as a highly selective biocatalyst for the acylation of the secondary monoacetate of several 1,2-ethanediols, while in case of acylation of the primary monoacetate showed significantly lower activity and selectivity[51].

Further, using the most efficient enzymes (CaL-A, LAK) solvents effects on the selectivity of the enzymatic acylation was tested. A strong solvent influence upon the reaction rate and selectivity was observed. Beside various organic solvents, ionic liquids, previously found as proper media for the LPS-catalyzed enzymatic acylation of different substituted phenyl-1,2-ethanediols,[54] were also used (Table 11, entry 7,14) with unsatisfactory results in the present case (Table 11, entry 7,14). Best results for the LAK mediated reaction were obtained in DIPE (Table 11, entry 9).

Table 11. The influence of the nature of solvent upon the CaL-A and lipase AK mediated acylation of *rac*-**7g**

Entry	Enzyme	Solvent	Time (h)	c (%)	ee_P (%)	ee_S (%)	E
1	CaL-A	DIPE	6	50	89	91	54
2	CaL-A	*t*BME	6	41	81	56	16
3	CaL-A	CH_2Cl_2	4	6	85	5	13
4	CaL-A	Acetonitrile	4	16	70	13	6
5	CaL-A	Toluene	4	34	88	44	23
6	CaL-A	Vinyl acetate	6	50	89	92	56
7	CaL-A	[bmim]PF_6	6	30	88	37	22
8	LAK	DIPE	24	43	95	71	83
9	LAK	*t*BME	24	35	90	48	31
10	LAK	Toluene	24	34	93	47	44
11	LAK	Vinyl acetate	24	16	89	17	20
12	LAK	CH_2Cl_2	24	17	93	19	33
13	LAK	Acetonitrile	24	12	91	13	24
14	LAK	[bmim]PF_6	24	20	91	23	27

The same screening procedure was performed for the rest of the substrates and in all cases the optimal transformation was identical with that found in case of *rac*-**7g** (Table 12, entry 2,4,6,8). It is important to note that the CaL-A mediated transformation of *rac*-**7e-h** in neat vinyl acetate was also satisfactory in most of cases (Table 12, entry 1,3,5). Excepting the enzymatic transformation of *rac*-**7h** (*E*=5, Table 12, entry 7), the CaL-A mediated acylations underwent with good enantioselectivities (*E*= 56-133).

Table 12. Comparison of the CaL-A and LAK mediated acylation of *rac*-**7e-h**

Entry	Substrate	Enzyme	Solvent	Time (h)	c (%)	ee_p (%)	ee_s (%)	E
1	*rac*-**7e**	CaL-A	Vinyl acetate	6	48	95	83	133
2	*rac*-**7e**	LAK	DIPE	13	50	97	97	>200
3	*rac*-**7f**	CaL-A	Vinyl acetate	9	50	92	91	76
4	*rac*-**7f**	LAK	DIPE	9	50	97	96	>200
5	*rac*-**7g**	CaL-A	Vinyl acetate	6	50	89	92	56
6	*rac*-**7g**	LAK	DIPE	30	50	93	95	102
7	*rac*-**7h**	CaL-A	Vinyl acetate	12	55	46	56	5
8	*rac*-**7h**	LAK	DIPE	22	50	92	93	81

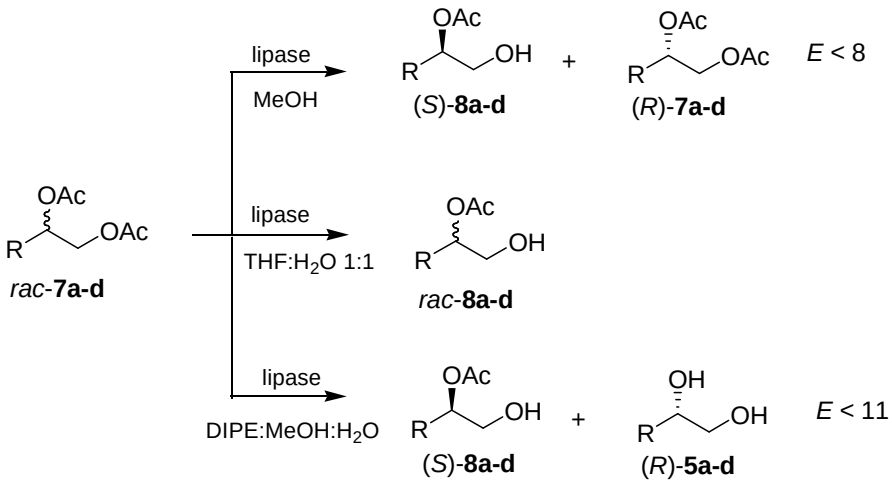
The enzymatic hydrolysis or alcoholysis of the *rac*-**7e-h** was also tested (Scheme 12d). Using the same methodology previously described for *rac*-**8e-h** it was found that most of the tested lipases (CaL-A, LAK, LPS, PLE, CrL, lipase from *Mucor javanicus*) were useless. Only LPS and CaL-B transformed in a totally nonselective manner all the substrates into the corresponding racemic diols *rac*-**3e-h**, when the reactions were performed in a mixture of diisopropyl ether-methanol-water (1:1:2, v/v).

4.3.4. Kinetic resolution of the racemic diacetylated derivatives *rac*-9e-h

Finally the same hydrolases were tested as potential catalysts for the methanolysis of the racemic diacetylated derivatives *rac*-**9e-h**. The reactions were performed in various solvents containing 8 eq. of nucleophile as well as in neat methanol. While most of the lipases were catalytically inactive, as it was expected[53] CaL-B was able to transform *rac*-**9e-h** in an *anti*-Kazlauskas manner[36a] into (*R*)-diacetate and the (*S*)-secondary monoacetate. However, the ee of the reaction products were only moderate (ex. ee 82% for (*R*)-**9h** and ee 51% for (*S*)-**8h**) and at higher conversions also the presence of slight amount of the diol (5-10%) was observed.

When the reactions were performed in the mixture of THF:H_2O (1:1, v/v), CaL-B and LPS were able to hydrolyze regioselectively *rac*-**9e-h** into *rac*-**8e-h**. Using a mixture of DIPE:

MeOH: H_2O as reaction media LPS catalyzed rapidly the transformation of *rac*-**9e-h** into *rac*-**8e-h**, followed by the stereoselective lysis of the latest racemates yielding (*R*)-**3e-h** the (*S*)-**8e-h** with moderate enantioselectivity (ee 65-71% for (*R*)-**3e-h** and 59-67 % for (*S*)-**8e-h**). The possible routes are depicted in Scheme 13.



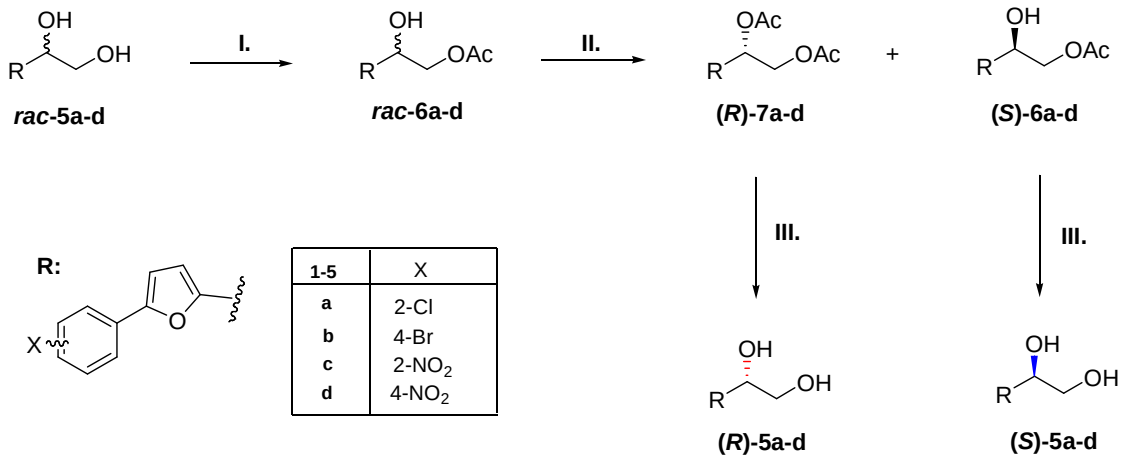
Scheme 13. *Lipase catalyzed reactions of the racemic diacylated diols*

4.3.5. Preparative scale synthesis of optically pure (*R*)- and (*S*)-3e-h

The preparative scale synthesis of both enantiomeric forms of the phenylfuran-2-yl-ethane-1,2-diols was based on the results of the analytical scale enzymatic reactions described in the previous paragraph (Scheme 14).

Using racemic 1,2-diols *rac*-**3e-h** as starting materials first their regioselective LPS-mediated enzymatic acylation was performed yielding quantitatively *rac*-**7e-h**. Further the LAK mediated enantioselective enzymatic acylation of *rac*-**7e-h** was performed obtaining (*S*)-**7e-h** and (*R*)-**9e-h** in highly enantiomerically enriched forms by stopping the reactions at an approx. 50% conversion (monitored with HPLC) removing the enzyme by filtration. All dilutions, substrate-biocatalyst ratio and reaction conditions were the same as in the case of the analytical scale reactions. Data on yields, enantiomeric excess and the specific rotation of the obtained enantiomers are presented in Table 13. The isolated (*S*)-**7e-h** and (*R*)-**9e-h** were quantitatively transformed into the corresponding diols (*S*)- and (*R*)-**3e-h** without any loss of enantiopurities, by the LPS catalyzed mixed alcoholysis-hydrolysis procedure. The chemical hydrolysis[104] and alcoholysis[53,54] of (*S*)-**7e-h** and (*R*)-**9e-h** were also tested, but due to the structural instability of diols in acidic or basic media, in most of the cases the appearance of several by-products and partial racemization of the produced diols was observed.

The absolute configurations of the produced optically enriched 1,2-diols was established comparing the chromatographic retention times of the enantiomers and the signs of the specific rotations for the produced enantiomerically enriched diols with earlier reported data[105].



I. LPS / vinyl acetate; **II.** LAK, vinyl acetate / DIPE; **III.** LPS / MeOH:DIPE:H_2O (1:1:2, v/v)

Scheme 14. *Enzyme catalyzed preparative scale synthesis of (R)- and (S)-* ***3e-h***

Table 13. Yields, ee and optically rotatory power for products of the preparative scale procedure

Entry	Product	Yield[a] (%)	ee (%)	$[\alpha]_D^{25}$ [b]	Product	Yield[a] (%)	ee (%)	$[\alpha]_D^{25}$ [b]
1	(*S*)-**7e**	49	97	-6.7	(*R*)-**9e**	49	97	+ 33.1
2	(*S*)-**7f**	48	96	-10.4	(*R*)-**9f**	49	97	+ 42.7
3	(*S*)-**7g**	49	95	-23.8	(*R*)-**9g**	47	93	+82.3
4	(*S*)-**7h**	48	93	- 18.7	(*R*)-**9h**	47	92	+65.5
5	(*S*)-**3e**	47	97	− 24.5	(*R*)-**3e**	47	97	+ 24.3
6	(*S*)-**3f**	46[b]	96	− 21.2	(*R*)-**3f**	48	96	+ 21.9
7	(*S*)-**3g**	48	95	− 33.9	(*R*)-**3g**	46	93	+ 31.4
8	(*S*)-**3h**	46	93	− 25.8	(*R*)-**3h**	46	92	+25.1

[a] calculated *rac*-**3e-h**
[b] c 0.5

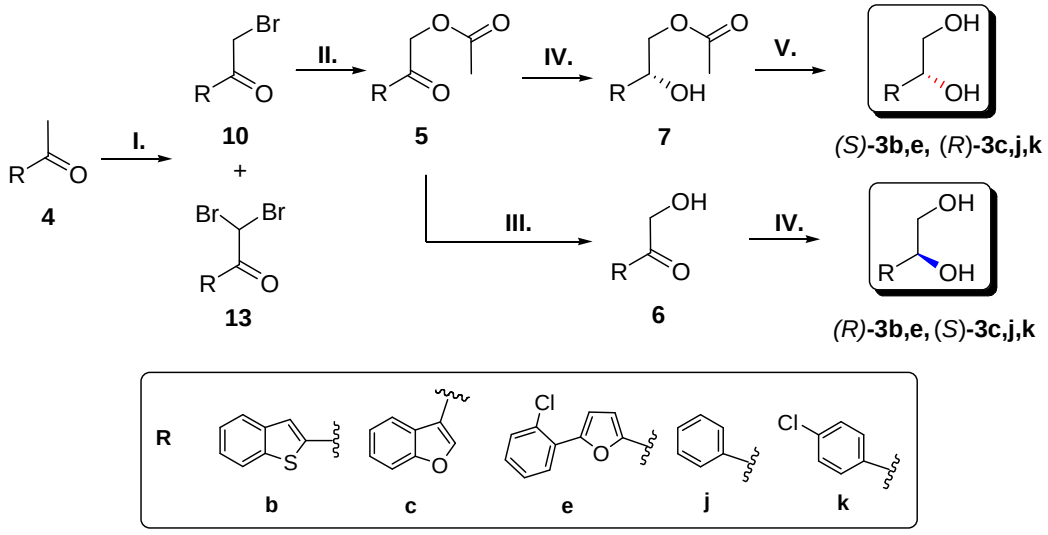
4.4. Chemoenzymatic One-Pot Synthesis of both (*R*)- and (*S*)-aryl-1,2-ethanediols 3b,c,e,j,k

The previously developed chemoenzymatic synthesis of both (*R*)- and (*S*)-1-aryl-1,2-ethanediols using arylketones as starting materials is a multistep procedure.[62,63] In order to remove side products or reagents that would impair the next steps, the purification of the

intermediates is usually necessary. To avoid such problems, practically quantitative conversions and non-disturbing reagents and solvents are required. In this case a one-pot procedure can be achieved without purification of intermediates as shown in Scheme 15. For this purpose, using polymer bound pyridinium tribromide in acetonitrile, the α-bromination of arylketones **4** occurs in quantitative yields. The subsequent complete conversion of α-bromo-arylketones **10** into α-acetoxymethyl arylketones **5** undergoes with sodium acetate in presence of phase-transfer catalyst 18C6. Further addition of lipase B from *Candida antarctica* immobilized on Celite (Novozyme 435) and methanol afforded quantitatively the α-hydroxy-arylketones **6**. By pouring water suspended baker's yeast cells into the same pot containing α-hydroxy- or α-acetoxymethyl arylketones, the enantiomerically enriched (*R*)- or (*S*)-1-aryl-1,2-ethanediols **3** can be obtained.

Using various arylketones **4b,c,e,j,k** as starting materials, we demonstrated that this is a reliable one-pot procedure for the synthesis of both (*R*)- and (*S*)-1-aryl-1,2-ethanediols with high yields and ee values.

The reaction sequences are shown in Scheme 15. To determine the conditions for quantitative conversions, the reactions were monitored after each step by HPLC and/or by TLC. After the completion of a reaction, the conditions (dilution, temperature, pH, etc.) were adjusted to optimal for the next step.

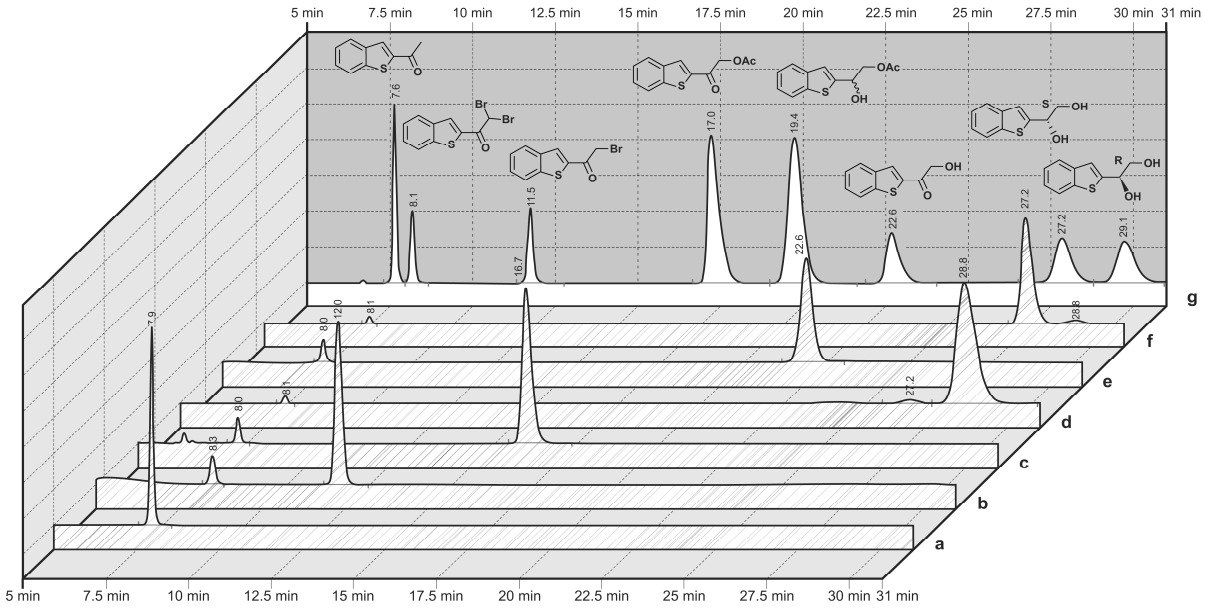


I. Polymer bound pyridinium tribromide / acetonitrile, reflux; polymer bound 4-(*N*-benzyl-*N*-methylamino)pyridine, rt. **II.** $CH_3COO^-Na^+$, 18C6 / acetonitrile, reflux. **III.** Novozyme 435, methanol / acetonitrile, rt. **IV.** Oxidoreductases from baker's yeast. **V.** Hydrolases from baker's ysest for **b,c,e** and PLE for **j,k**.

Scheme 15. *The one-pot synthesis of (R)- and (S)-1-aryl-1,2-ethanediols*

As example, the HPLC elution diagrams corresponding to all steps of the one-pot transformation of 1-(benzo[*b*]thiophene-2-yl)ethanone **4b** into the desired products are presented in Figure 3.

Figure 3. *Elution diagrams from a chiral column corresponding to each reaction step for the one-pot synthesis of (S)- and (R)-**3b***



Under the used conditions, the retention times for authentic 1-(benzo[*b*]thiophene-2-yl)ethanone **4b**, 1-(benzo[*b*]thiophene-2-yl)-2-bromoethanone **10b**, 1-(benzo[*b*]-thiophene-2-yl)-2,2-dibromoethanone **13b**, 2-(benzo[*b*]thiophene-2-yl)-2-oxoethyl acetate **5b**, 2-(benzo[*b*]thiophene-2-yl)-2-hydroxyethyl acetate **7b**, 1-(benzo[*b*]thiophene-2-yl)-2-hydroxy-ethanone **6b**, (*S*)- and (*R*)-1-(benzo[*b*]thiophene-2-yl)ethane-1,2-diol **3b** were approximately 7.9, 11.5, 8.1, 17.0, 19.4, 22.6, 27.2 and 29.1 minutes, respectively (Figure 3, trace g).

In the first stage the starting ketone **4b** (Figure 3, trace a) was totally transformed into the brominated products (Figure 3, trace b). In the second stage the accumulated HBr, which would compromise the further reactions, was removed from the reaction mixture using polymer bond 4-(*N*-benzyl- *N*-methylamino)-pyridine. The use of polymer bond reactants in the first step is crucial. The free pyridinium hydrobromide and 4-(*N*,*N*-dimethylamino)pyridine hydrobromide in the reaction mixture strongly decreased the activity and selectivity of the further enzymatic reactions and also the appearance of several undesired

side products has been detected. It is important to note that 5-8% of dibrominated ketone **13b** was also formed (Figure 3, trace b, 8.2 min.), however this compound was inert in the further reactions, its presence was detected after each step of the one-pot procedure (Figure 3, traces c-g). The 18C6 phase-transfer catalyst mediated acetoxylation of the α-bromo-arylketone **10b** (Figure 3, trace c) and the subsequent Novozyme 435 catalyzed transformation of α-acetoxymethyl-arylketone **5b** into α-hydroxymethyl-arylketone **6b** (Figure 3, trace e) underwent with complete conversions, the formation of undesired side-products was not observed. Finally the baker's yeast mediated biotransformation of the α-acetoxymethyl- and α-hydroxymethyl-arylketone yielded the opposite enantiomeric forms (Figure 3, trace d respectively f) of the desired 1-aryl-1,2-ethanediol **3b** and they were easily isolated by extraction with ethyl acetate. It is important to note, that during the biotransformation of 2-(benzo[*b*]thiophene-2-yl)-2-oxoethyl acetate **5b** no trace of 2-(benzo[*b*]thiophene-2-yl)-2-hydroxy-ethyl acetate **7b** was detected, indicating that the reduction product is a good substrate for hydrolases also present in baker's yeast cells.

The procedure for the other 1-aryl-1,2-ethanediols **3c,e,j,k** was similar and the yields of the isolated products are shown in Table 14. In case of biotransformation of **5j,k** besides the desired (*S*)-**3j,k** unhydrolysed (*S*)-**7j,k** was also detected. In these cases, after completion of the bioreduction, the addition of esterase from porcine liver (PLE) rapidly converted (*S*)-**7j,k** into the corresponding diols (*S*)-**3j,k**.

Table 14. Yields, reaction times and conditions for the one-pot synthesis of (*R*)- and (*S*)-1-aryl-1,2-ethane diols

Entry	Product	Yield (%)	ee (%)	Conditions	T (h)
1	(*S*)-**3b**[b)]	79	96	fermenting[c)]	110
2	(*R*)-**3b**[a)]	82	95	fermenting	126
3	(*S*)-**3c**[a)]	80	95	fermenting	38
4	(*R*)-**3c**[b)]	82	98	fermenting	48
5	(*S*)-**3e**[b)]	81	97	fermenting[d)]	48
6	(*R*)-**3e**[a)]	79	94	non-fermenting	72
7	(*S*)-**3j**[a)]	75	77	fermenting	36
8	(*R*)-**3j**[b)]	73	95	fermenting	48
9	(*S*)-**3k**[a)]	68	82	fermenting	34
10	(*R*)-**3k**[b)]	75	95	fermenting	48

[a)] reaction product involving the cellular transformation of **5b,c,e,j,k**. [b)] reaction product involving the cellular transformation of **6b,c,e,j,k**. [c)] with L-cysteine as additive. [d)] with ethyl bromoacetate as additive.

5. Conclusions

In the previously developed synthesis of the (*R*)- and (*S*)-benzofuranyl- and benzo[*b*]thiophenyl-1,2-ethanediols, the global yield and the enantiopurity of the products were not satisfactory in all cases. Thus a novel, highly efficient chemoenzymatic procedure was developed for the synthesis of both (*R*)- and (*S*)-benzofuranyl- and benzo[*b*]thiophenyl-1,2-ethanediols, based on the well known lipase catalyzed synthesis of novel optically active cyanohydrins followed by their chemical hydrolysis into the corresponding α-hydroxy acids and the subsequent reduction with $LiAlH_4$ of the latest compounds.

For the synthesis of both enantiomeric forms of novel differently substituted 5-phenylfuran-2-yl-ethane-1,2-diols one of the previously reported methods, the baker's yeast mediated biotransformation of the α-acetoxymethyl ketones and of the α-hydroxymethyl ketones was employed. Several phenylfuran-2-yl-ethane-1,2-diols had been obtained in high enantiomeric excesses and with high yield values. However, due to the observed strong substituent effect, in some cases no biotransformation, or low enantiomeric excess values were obtained.

By employment of the lipase mediated kinetic resolution of racemic 1,2-ethanediols, the enantiomeric excesses obtained for the novel differently substituted 5-phenylfuran-2-yl-ethane-1,2-diols were superior than those obtained by the baker's yeast mediated biotransformation of the corresponding α-acetoxymethyl ketones and of the α-hydroxy ketones. Thus, exploiting the regioselectivity of lipase PS and the enantioselectivity of lipase AK, a highly efficient, and general enzymatic method for the synthesis of both enantiomeric forms of novel differently substituted 5-phenylfuran-2-yl-ethane-1,2-diols was successfully developed.

Besides the well established methods mentioned above, an enantioselective, simple and efficient one-pot method, starting from the achiral and cheep ethanones was developed for the synthesis of enantiomerically enriched (*R*)- and (*S*)-aryl-1,2-ethanediols. A wide range of aryl groups, phenyl, 4-chlorophenyl, benzo[*b*]thiophene-3-yl, benzofuran-2-yl, 2-chlorophenylfuran-2-yl were used, and owing to the large number of existing arylethanones and the broad substrate specifity of baker's yeast, the presented method proved to be convenient for the synthesis of (*R*)- and (*S*)-1,2-ethanediols.

Aknowledgements

The experimental work was carried out at the Department of Biochemistry, Faculty of Chemistry and Chemical Engineering, University of Babes-Bolyai.

First of all I would like to express my deepest gratitude and warmest thanks towards my supervisor Professor Florin-Dan Irimie for giving me the chance to enter into the beautiful world of biocatalysis, for supervising me in the whole process of this thesis work, for giving always useful, supportive advices and opportunities to develop myself.

Special thanks to Dr. Paizs Csaba, Dr. Monica Ioana Toşa and for all other members of the research group of the department: Dr. Györfi Zoltán Attila, Dr. Paula Podea Moldovan, for sharing me their knowledge, for creating an efficient and warm working environment and for giving useful advices.

I am also grateful to all my present and former collegues: Brem Jürgen, Laura Pop, Maria Trif, Laura Chiş, Dr. Monica Hrişcu, Augustin Cătălin Moţ, Florina Violeta Deac, Cristina Bişchin, Pöllnitz Alpár, Mara Naghi, Roxana Redis, Mihaela Stevar, Kállay András, Kalló Noémi-Hajnalka, Dima Nórbert, Gálfi Katalin, Sándor Mónika, Pócs Géza, Gui Mara-Oana, Klaus Dobrezberger for their friendly, helpful attitude, for the nice discussions related to chemistry, science or anyother theme.

The reviewers of this thesis, Professor Mircea Dărăbanţu, Professor Poppe László and Professor Dan Caşcaval are acknowledged for their valuable comments during the reviewing process.

Professor János Rétey from the Institute of Organic Chemistry, Karlsruhe Institute of Technology is acknowledged for HRMS analysis and for supervising the scientific publications. Special thanks for Dr. Vass Elemér from the Eötvös Lóránd Uviversity for the determination of the absolute configuration of the novel optically pure cyanohydrins by VCD measurements, and for showing me this technique. Professor Poppe László is also gratefully acknowledged for his generous gifts of enzymes, and for his heplful advices. Professor Novák Lajos and Dr. Hornyánszky Gábor from the Budapest University of Technology and Economics are acknowledged for introducing me in the field of the scientific research and for their helpful attitude during my doctoral studies.

The financial support from the Romanian National Council of Scientific Research of Higher Education, from the Hungarian Academy of Science (Hungarian Science Abroad Scholarship Programe and Domus Hungarica Fellowship), and from the Eurotrans foundation is gratefully acknowledged.

Last but not least I sincerely thanks to my family: my parents, my brother, my sister and to all my friends. I am glad having all of you in my life, helping to remind the reasons and happiness of my existance.

My highest admiration and special thanks to my fiancee, Bálint Jutka M.D. for understanding, supporting, helping and loving me during all these years.

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