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LIPASE-CATALYZED APPROACHES TOWARDS SECONDARY ALCOHOLS: INTERMEDIATES FOR ENANTIOPURE DRUGS

by

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Cover: *Burkholderia cepacia* lipase (1OIL – PDB code, see reference 28)

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Abstract

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Lipase-catalyzed Approaches towards Secondary Alcohols: Intermediates for Enantiopure Drugs

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The use of enantiopure intermediates for drug synthesis is a trend in pharmaceutical industry. Different physiological effects are associated with the enantiomers of chiral molecules. Thus, the safety profile of a drug based on an enantiopure active pharmaceutical ingredient is more reliable. Biocatalysis is an important tool to access enantiopure molecules. In biocatalysis, the advantage of selectivity (chemo-, regio- and stereoselectivity) is combined with the benefits of a green synthesis strategy. Chemoenzymatic syntheses of drug molecules, obtained by combining biocatalysis with modern chemical synthesis steps usually consists of fewer reaction steps, reduced waste production and improved overall synthetic efficiency both in yields and enantio- and/or diastereoselectivities compared with classical chemical synthesis.

The experimental work together with the literature review clearly indicates that lipase catalysis is highly applicable in the synthesis of enantiopure intermediates of drug molecules as the basis to infer the correct stereochemistry. By lipase catalysis, enantiopure secondary alcohols used as intermediates in the synthesis of Dorzolamide, an antiglaucoma drug, were obtained. Enantiopure β-hydroxy nitriles as potential intermediates for the synthesis of antidepressant drugs with 1-aryl-3-methylaminopropan-1-ol structure were also obtained with lipases. Kinetic resolution of racemates was the main biocatalytic approach applied. *Candida antarctica* lipase B, *Burkholderia cepacia* lipase and *Thermomyces lanuginosus* lipase were applied for the acylation of alcohols and the alcoholysis of their esters in organic solvents, such as in diisopropyl ether and *tert*-butyl methyl ether. *Candida antarctica* lipase B was used under solvent free conditions for the acylation of ethyl 3-hydroxybutanoate.

Keywords: antidepressant drugs, 1-aryl-3-methylaminopropan-1-ol, Dorzolamide, β-hydroxy nitrile, kinetic resolution, lipase, secondary alcohol.

Tiivistelmä

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Lipaasin katalysoimia lähestymistapoja sekundääristen alkoholien valmistamiseksi: intermediaatteja enantiopuhtaille lääkeaineille

Farmakologia, lääkekehitys ja lääkehoito/ Synteettisen lääkekemian laboratorio, Turun Yliopisto

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Farmaseuttisessa teollisuudessa yleinen suuntaus on enantiopuhtaiden intermediaattien käyttö lääkeainesynteesissä. Kiraalisten molekyylien enantiomeereihin liittyy erilaisia fysiologisia vaikutuksia. Niinpä lääkeaineen turvallisuusprofiili, joka perustuu enantiopuhtaan farmaseuttisesti aktiivisen aineksen käyttöön, on luotettavampaa kuin rasemaattien käyttö. Biokatalyysi on yksi luotettavimmista työkaluista enantiopuhtaiden molekyylien valmistamiseksi. Biokatalyysissä selektiivisyys (kemo-, regio- ja stereoselektiivisyys) yhdistyy vihreän synteesistrategian tuomiin etuihin. Lääkemolekyylien kemoentsymaattinen synteesi, jossa biokatalyysi liitetään moderneihin kemiallisiin synteesivaiheisiin, tarkoittaa yleensä synteesivaiheiden vähentymistä, alentunutta jätteentuottoa sekä saantojen ja enantio- ja/tai diastereoselektiivisyyksien suhteen parantunutta synteesitehokkuutta.

Kokeellinen työ yhdessä kirjallisuuskatsauksen kanssa osoittaa selvästi, että lipaasikatalyysi toimii hyvin käyttökelpoisena perustana oikean stereokemian saamiseksi lääkemolekyylien enantiopuhtaiden intermediaattien synteesissä. Työssä valmistettiin lipaasikatalyysillä enantiopuhtaita sekundäärisiä alkoholeja intermediaateiksi glaukoomalääkkeen, dorzolamidin, synteesiin. Työssä käytettiin lipaasikatalyysiä myös enantiopuhtaiden β-hydroksinitriilien valmistamiseksi. Yhdisteet ovat potentiaalisia intermediaatteja 1-aryyli-3-metyyliaminopropan-1-olirakenteen sisältävien masennuslääkkeiden synteesissä. Raseemisten seosten kineettistä resoluutiota käytettiin pääasiallisena biokatalyyttisenä menetelmänä, joissa CAL-B (*Candida antarctica*n lipaasi B), lipaasi PS (*Burkholderia cepacia*n lipaasi) ja IMMTLL-T1-1500 (*Thermomyces lanuginosu*ksen lipaasi) katalysoivat alkoholien asyloitumista ja vastaavien estereiden alkoholyysiä orgaanisissa liuottimissa kuten di-isopropyyli- ja *tert*-butyylimetyylieettereissä. Etyyli-3-hydroksibutanoaatin asylointiin *Candida antarctica*n lipaasi B soveltui käytettäväksi liuotinvapaissa olosuhteissa.

Avainsanat: 1-aryyli-3-metyyliaminopropan-1-oli, Dorzolamidi, β-hydroksinitriili, kineettinen resoluutio, lipaasi, masennuslääkkeet, sekundäärinen alkoholi.

Rezumat

Metode catalizate de lipaze pentru sinteza alcoolilor secundari: Intermediari în sinteza medicamentelor enantiomeric pure

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O tendință în industria farmaceutică este utilizarea de intermediari enantiomeric puri în sinteza medicamentelor. Moleculele chirale pot prezenta enantiomeri cu efecte fiziologice diferite. În acest context, profilul de siguranță al unui medicament care conține un ingredient activ în formă enantiomeric pură este mult mai fiabil. Biocataliza este o cale valoroasă de obținere a compuşilor enantiomeric puri. În cazul biocatalizei, avantajele unei sinteze selective (chemo-, regio- şi stereoselective) sunt asociate cu beneficiile aduse de strategia prietenoasă față de mediu. În comparație cu metodele de sinteză chimică clasică, metodele de sinteză chemoenzimatice ale substanțelor medicamentoase, obținute prin asocierea reacțiilor enzimatice cu reacții chimice moderne, constau de obicei în mai puțini pași de reacție, conduc la o cantitate mai mică de deşeuri si prezintă o eficiență mărită atât ca şi randament cât şi în ceea ce priveşte enantio şi/sau diastereoselectivitatea

Partea experimentală împreună cu partea de documentare ştiințifică a acestei teze dovedesc clar aplicabilitatea catalizei cu lipaze în sinteza unor intermediari enantiomeric puri, care la rândul lor pot conduce la molecule de substanțe medicamentoase cu stereochimie exactă. În lucrarea de față, cataliza cu lipaze a fost utilizată pentru sinteza de alcooli secundari, intermediari în sinteza Dorzolamidei, medicament folosit pentru tratamentul glaucomului ocular. De asemenea, lipazele s-au folosit în sinteza de β-hidroxi nitrili in formă enantiomeric pură, posibili intermediari în sinteza unor noi medicamente antidepresive cu structură de tipul 1-aril-3-metilaminopropan-1-ol. Rezoluția cinetică a substraturilor racemice a fost principala metodă biocatalitică aplicată. Lipaza B din *Candida antarctica*, lipaza din *Burkholderia cepacia* şi cea din *Thermomyces lanuginosus* s-au utilizat pentru acilarea alcoolilor şi respectiv pentru alcooliza esterilor lor în solvenți organici cum ar fi diizopropil eter şi terț-butilmetil eter. Lipaza B din *Candida antarctica* s-a folosit pentru reacția de acilare a esterului etilic al acidului 3-hidroxibutanoic în absența solventului organic.

Cuvinte cheie: alcool secundar, 1-aril-3-metilaminopropan-1-ol, Dorzolamida, β-hidroxi nitrili, lipaza, medicamente antidepresive, rezoluție cinetică.

TABLE OF CONTENTS

ABBREVIATIONS

Ac	acetyl
Ala (A)	alanine
API	active pharmaceutical ingredient
Asp (D)	aspartic acid
Bn	benzyl
BOC	*tert*-butoxycarbonyl
Bu	butyl
Bz	benzoyl
c	conversion
CAL-A	*Candida antarctica* lipase A
CAL-B	*Candida antarctica* lipase B
COSY	correlation spectroscopy
CRL	*Candida rugosa* lipase
CVL	*Chromobacterium viscosum* lipase
DCM	dichloromethane
de	diastereomeric excess
DEAD	diethyl azodicarboxylate
DKR	dynamic kinetic resolution
DIPE	diisopropyl ether
DMAP	*N*,*N*-dimethyl-4-aminopyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
ee	enantiomeric excess
E	enantiomeric ratio
EC	Enzyme Commission
Et	ethyl
GC	gas chromatography
Gln (Q)	glutamine
Gly (G)	glycine
h	hour
HPLC	high performance liquid chromatography
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum correlation
His (H)	histidine
IA	isopropenyl acetate
IMMCALB	*Candida antarctica* lipase B (ChiralVision preparation)
IMMCALBY	*Candida antarctica* lipase B (ChiralVision preparation)
IMMTLL	*Thermomyces lanuginosus* lipase (ChiralVision preparation)
k	rate constant
KR	kinetic resolution
L	large group
LAH	lithium aluminium hydride
Lipase AK	*Pseudomonas fluorescens* lipase
Lipase PS	*Burkholderia cepacia* lipase
Lipase PS-C II	*Burkholderia cepacia* lipase immobilized on ceramics
Lipase PS-D	*Burkholderia cepacia* lipase immobilized on Celite
log *P*	the logarithm for the partition coefficient of a solvent between 1-octanol and water

M	medium-sized group
Me	methyl
MOM	methoxy methyl
Ms	methane sulfonyl
MS	mass spectroscopy
NOESY	nuclear overhauser effect spectroscopy
NMR	nuclear magnetic resonance
PFL	*Pseudomonas fluorescens* lipase
Ph	phenyl
PPL	porcine pancreatic lipase
Pr	propyl
Py	pyridine
rac-	racemate
rt	room temperature
RL IM	*Rhizomucor miehei* lipase
Ser (S)	serine
TBAB	tetrabutylammonium bromide
TBDPS	*tert*-butyldiphenylsilyl
TBME	*tert*-butyl methyl ether
TEA	triethylamine
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
Thr (T)	threonine
TL IL	*Thermomyces lanuginosus* lipase
TMS	trimethylsilyl
Trp (W)	tryptophan
Ts	*p*-toluenesulfonyl
VA	vinyl acetate
Val (V)	valine

LIST OF ORIGINAL PAPERS

The present thesis is based on the following papers referred by Roman numerals (I-IV) in the text

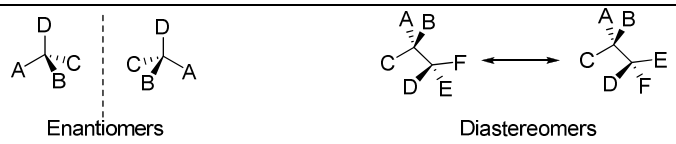
I. Turcu, M. C.; Kiljunen, E.; Kanerva, L. T. Transformation of racemic ethyl 3-hydroxybutanoate into the (*R*)-enantiomer exploiting lipase catalysis and inversion of configuration
Tetrahedron: Asymm. **2007**, *18*, 1682-1687.

II. Turcu, M. C.; Rantapaju, M.; Kanerva, L. T. Applying lipase catalysis to access the enantiomers of dorzolamide intermediates
Eur. J. Org. Chem. **2009**, *32*, 5594-5600.

III. Turcu, M. C.; Perkiö, P.; Kanerva, L. T. Chemoenzymatic method to enantiopure sulphur heterocyclic β-hydroxy nitriles
Arkivoc **2009**, (*iii*), 251-263.

IV. Turcu, M. C.; Perkiö, P.; Kanerva, L. T. Chemoenzymatic method for the preparation of γ-amino alcohols from phenylfuran-based aldehydes; lipase-catalyzed kinetic resolution of β-hydroxy nitriles
Tetrahedron: Asymm. **2010**, *21*, 739-745.

DEFINITIONS



Stereochemistry:	An area of chemistry that studies the relative spatial arrangement of atoms within molecules.
Stereoisomers:	Isomers that possess identical constitution, but which differ in the arrangement of their atoms in space.[1]
Chirality:	The geometric property of a rigid object (or spatial arrangement of points or atoms) of being non-superposable on its mirror image; such an object has no symmetry elements of the second kind (mirror plane, centre of inversion or rotation-reflection axis). If the object is superposable on its mirror image the object is described as being **achiral**.[1]
Enantiomer:	One of a pair of molecular entities which are mirror images of each other and non-superposable.[1]
Diastereomers:	Stereoisomers which are not related as mirror images. Diastereomers are characterized by differences in physical properties and by some differences in chemical behavior towards achiral as well as chiral reagents.[1]
Racemate:	An equimolar mixture of a pair of enantiomers.[1]
Biocatalysis:	Chemical conversion of a substance into a desired product with the aid of a free or immobilized enzyme.[2]
Biotransformation:	Chemical conversion of a substance into a desired product with the aid of a (usually) living whole cell, containing the necessary enzymes.[2]

1. Introduction

The importance of synthetic chemistry on everyday life in modern society is immense, starting from materials with different properties used for clothing, constructions and continuing to complex drugs designed to fight against the main diseases of the century. The complexity of the synthesized molecules is continuously increasing particularly in pharmaceutical industry: there are more different functional groups in molecules and more asymmetric centers. In this context, chemo-, regio- and stereoselective methods are highly demanded in chemical synthesis. The industry faces pressure for sustainable development – '*to produce goods and services in such a manner as to meet the needs and aspirations of the present without compromising the ability of future generations to meet their own needs*' (The World Commission on Environment and Development). In consequence, chemistry needs to become 'greener' by developing environmentally friendly processes, by reducing waste and the use of hazardous reagents and by preferring less energy consuming processes.

This thesis addresses the field of enzyme-based biocatalysis, useful in developing highly selective and 'greener' processes for pharmaceutical and fine chemical industry. The maximum advantage of biocatalysis in large-scale synthesis is wonderfully reached by combining bio- and chemocatalytic steps. A biocatalyst – an isolated enzyme or whole-cell – enables the transformation of unnatural substrates with high degrees of chemo-, regio- and enantioselectivity. In addition, the reactions conditions are mild (pH range 5-8, temperature range 20-40 ^{o}C, normal pressure) and enzymes are environmentally friendly and compatible to each other to enable sequential reactions.

Industrial applications of biocatalysis have steadily increased. Advances in genomics, directed evolution and metagenomics sustain the development of enzymes with the exact properties required by a process conditions. The speed of development in enzymology may favor the vision of Emil Fischer in his Nobel Lecture in 1902 to come true *'… I can foresee a time in which physiological chemistry will not only make greater use of natural enzymes but will actually resort to creating synthetic ones.'*

This thesis will limit the discussion to applications of lipases in organic solvents for the preparation of enantiopure secondary alcohols useful as potential intermediates in chemoenzymatic synthesis of drug molecules. As will be described, lipases are robust and highly active catalysts in organic media.

2. Literature review

2.1. Chirality - Impact on Biological Activity

The phenomenon of chirality, common in Nature, has a great impact on our lives. The result of chirality for a chemical compound is the occurrence of enantiomers. Most often the source of chirality is an asymmetric center, although restricted rotation around axis or planes can be the source of chirality as well. A molecule with one asymmetric center has one pair of enantiomers which are in mirror-image relationship. Most often the asymmetric center is a carbon atom with four different substituents, although for instance phosphorus, sulphur, boron, silicon and more seldom nitrogen might be the asymmetric center. If a molecule contains more than one asymmetric center, the number of pairs of enantiomers is increased. The enantiomers from one pair have identical chemical and physical properties except the ability to rotate the polarized light in opposite directions and to interact differently with other chiral environments. All living organisms, microorganisms, plants, animals and humans are composed of chiral biomolecules, such as amino acids, sugars, proteins and nucleic acids. Moreover, the natural macromolecules are homochiral, *i.e.*, they contain asymmetric centers with the same configurations. Proteins consist of L-amino acids encoded by genomic DNA, whereas carbohydrates are D-saccharides. Thus, physiological processes often involve only one enantiomer of all possible stereoisomers, the other enantiomer and stereoisomers possessing no activity or exhibiting toxicity as the worst scenario. The chiral discrimination of enantiomers affects all bioactive substances, such as pharmaceuticals, insecticides, herbicides, flavors, fragrances and food additives.

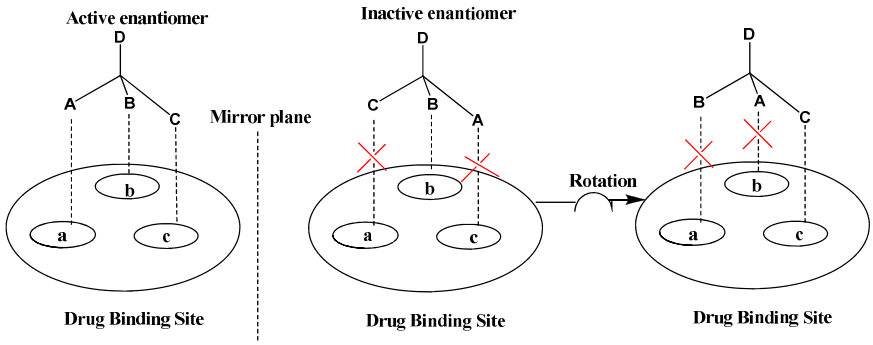


Figure 1. A simplified model for the interaction between the enantiomers of a chiral drug and its binding site[4,5]

Generally the biological activity is achieved by the interaction of specific molecules with biological targets (receptors, enzymes, transporters). Biological receptors usually have a complex structural organization of helices and sheets which displays handedness. The interaction of a biologically active molecule with a target is usually highly stereoselective. A simplified model was developed by Easson and Stedman[3] in 1933 to describe the interaction of a drug molecule with the receptor site based on three-point interactions. Jones has used the three-point contact model to illustrate and to define the enantiomeric and prochiral discrimination of enzymes.[4] In Figure 1 is shown the interaction between the enantiomers of a chiral drug and its binding site based on an illustration proposed by McConathy and Owens.[5] The both enantiomers have the same substituents A, B, C, D at the asymmetric center but with different spatial distribution. The three substituents A, B and C of the active enantiomer of a drug must interact with the corresponding regions of the binding site at the receptor labeled as a, b and c to exert its pharmacological effect. The attachment

of the drug to the target is analogous to 'putting the hand into a glove'. Thus, the 3-dimensional structure of the active enantiomer allows the alignment Aa, Bb and Cc required to achieve the desired biological effect. Even though the inactive or less active enantiomer possesses the same groups A, B, C and D, it cannot bind to the receptor similarly to the active enantiomer, because its 3-dimensional structure does not allow the adequate interactions.

2.2. Enzymes as Synthetic Tools

The synthesis of organic compounds with exact stereochemistry at the asymmetric center represents a real challenge for modern chemistry. If there is more than one asymmetric center in a molecule the complexity of the task increases considerably. Three main routes towards enantiopure compounds are available: making use of chiral pool, resolution (separation) of racemates and asymmetric synthesis. Chiral pool refers to the use of asymmetric centers naturally occurring in enantiopure molecules like carbohydrates, α-amino acids, terpenes, hydroxy acids and alkaloids. Resolution of racemates may be achieved by diastereomer crystallization, by chemical as well as by enzymatic kinetic methods or by chromatographic procedures. Asymmetric synthesis converts achiral molecules to stereoisomers by using chiral chemical catalysts or biocatalysts.

Table 1. Classification of enzymes[6]

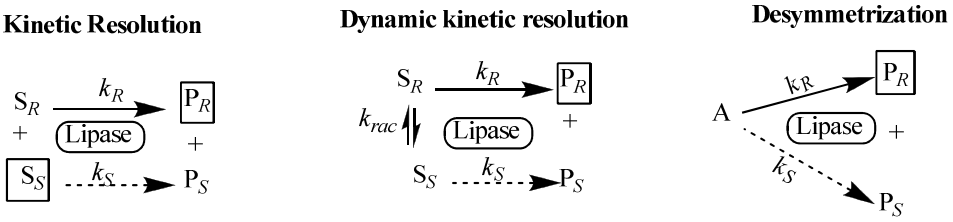
Enzyme class	Reaction type
1. Oxidoreductases	Oxidation/Reduction of C-H, C-C, C=C bonds
2. Transferases	Transfer of groups: aldehydic, ketonic, acyl, sugar, phosphoryl or methyl
3. Hydrolases	Hydrolysis/Formation of esters, amides, lactones, lactams, epoxides, nitriles, anhydrides, glycosides
4. Lyases	Addition/elimination of small molecules on C=C, C=N, C=O bonds
5. Isomerases	Isomerizations such as racemizations, epimerization
6. Ligases	Formation/Cleavage of C-O, C-S, C-N, C-C bonds with concomitant triphosphate cleavage

Biocatalysts - whole-cells or isolated enzymes - may be used to resolve racemates or to create one or more stereogenic centers under mild reaction conditions. Enzymes are the catalysts evolved by Nature to sustain biochemical reactions in a living cell. Considering the chemical structure of enzymes, they all are proteins, except few ribozymes (ribonucleic acids). The enzymes as catalysts follow the chemical definition: they speed up the reaction rate to reach thermodynamic equilibrium without affecting the position of the equilibrium and consuming in the reaction. On the other hand, the thermodynamic equilibrium is not necessarily reached without special arrangements due to product inhibition. The rate acceleration of enzyme-mediated processes in aqueous environments compared with non-enzymatic reactions is by a factor of 10^8-10^{10}. Metabolic pathways and cell growth require highly diverse reactions such as the formation and breaking of carbon-carbon bonds, peptide and ester bonds, saturation/desaturation of carbon-carbon bonds, and oxidation, for instance, by oxygen. As a consequence, enzymes evolve as highly specialized catalysts for different types of chemical reactions. The enzymes are classified in six different classes by the Enzyme Commission from the International Union of Biochemistry and Molecular Biology according to the type of a chemical reaction they catalyze (Table 1). Lipases, the enzymes used in this thesis, belong to the class 3 of hydrolases, and they catalyze the hydrolysis of triglycerides in Nature (see 2.3., Scheme 2).

In 1897 Buchner´s work proved that enzymes do not require the environment of a living cell to be active. This discovery led to the applications of enzymes in various fields, one being the production of chemicals by biotransformations. In early 1980´s the scope of biocatalysis was

expanded for new applications in synthetic chemistry by the observation that enzymes are active in organic solvents containing little or no water.[7,8]

Lipase-catalyzed methods available for the preparation of enantiopure compounds are kinetic resolution (KR), dynamic kinetic resolution (DKR) and desymmetrization (Scheme 1).



S_R, S_S enantiomers of racemic substrate
P_R, P_S enantiomers of the product
A prochiral or *meso*- substrate

$k_R >> k_S$, k_R, k_S - rate constants
k_{rac} - rate constant for racemization

Scheme 1. Lipase-catalyzed methods for the preparation of enantiopure compounds

Enzymatic kinetic resolution is based on the difference between the reaction rates (k_R, k_S) of the enantiomers (S_R, S_S) 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. Kinetic resolution then has an advantage to provide the both enantiomers of a racemate with excellent enantiopurity in the presence of a highly enantioselective enzyme. This is considered as an advantage, especially for drug development where strict regulations require the characterization of biological activity for all possible stereoisomers of a certain drug. When only one enantiomer of a racemate is needed, kinetic resolution offers a relatively low yield, the maximum yield being 50% for one enantiomer.

Enantiomeric ratio (E) is a key parameter to describe the efficiency of a kinetic resolution process. It describes the enantioselectivity of an enzyme for one enantiomer of a racemate. E is a kinetic parameter, defined as a relative proportion of the rate constants of the two enantiomers at the initial stage of the reaction. The mathematical equations for the evaluation of enantioselectivity, based on the conversion and enantiomeric excess of the product (ee_P) and the substrate (ee_S) enantiomers, are shown in section 4.3. of this thesis. E-values lower than 15 obtained for a kinetic resolution can be considered unsuitable for practical purposes. The applicability of a kinetic resolution process with E values 15-30 is moderate, good when E values are in the range 50-100 and excellent when E values are higher than 200. These definitions vary from a scientist to another. To obtain both the product and the remaining substrate in high enantiomeric excess in one reaction step, the E-value needs to be 100 or higher (for instance E=100, ee_S=95% and ee_P=93% should be obtained at 50% conversion). Figure 2 represents graphically the dependence of enantiopurities for the substrate (ee_S) and product (ee_P) versus conversion for three kinetic resolutions with different values for enantiomer ratio (E=5, 50, and 200). According to Figure 2, the substrate is obtained with high enantiopurity (ee_P 99%) when the conversion is higher than 55% for a kinetic resolution with E=50, while for a kinetic resolution with E=200 high enantiopurity of the product (ee_P 99%) is obtained at 51% conversion. When a kinetic resolution has E=5, the product is obtained with ee lower than 70%.

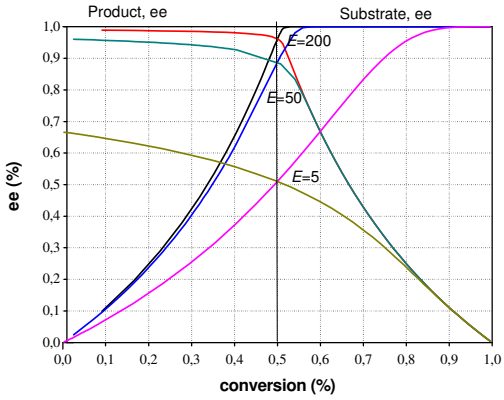


Figure 2. Theoretical plots for enantiomeric excesses of the product and the substrate vs. conversion for KR with *E* values 5, 50 and 200

Dynamic kinetic resolution combines kinetic resolution with the *in-situ* racemization of the unreacted enantiomer. Racemization can take place chemically or enzymatically. DKR was thoroughly reviewed by Pellisier.[9] The racemization of the unreacted enantiomers in DKR is more often performed chemically, because the group of racemases is relatively small. The need for racemization in Nature, governed by stereospecific interactions, is rare. Schnell *et al*[10] have reviewed few racemases applicable for biotransformations

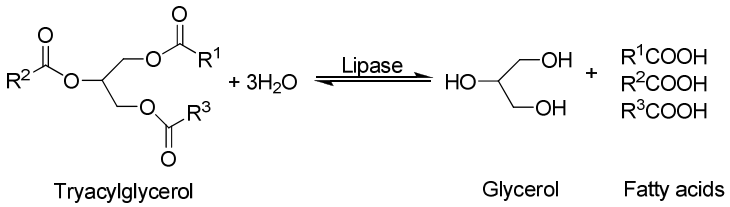
Desymmetrization of *meso* and prochiral compounds has the potential to fulfill theoretical yields of 100%. This method is not that often used as enzymatic kinetic resolution because the substrate *meso* or prochiral has to be available.

All three lipase-catalyzed methods (KR, DKR and desymmetrization) described above may be applied for the preparation of enantiopure alcohols which can be important intermediates for pharmaceutical products, fine chemicals and agrochemicals. Thus, numerous chemical and biocatalytic methods to access enantiopure alcohols exist. Chemical asymmetric synthesis of enantiopure alcohols employs methods like catalytic asymmetric hydrogenation,[11] enantioselective hydride addition to prochiral ketones,[12] asymmetric dialkyl zinc addition to aldehydes[13] and asymmetric aldol reactions.[14] Except lipase catalysis, other biocatalytic methods may use oxidoreductases (EC 1), hydrolases (EC 3) like proteases, esterases, sulphatases, phosphatases in addition to lipases, and lyases (EC 4) like aldolases and hydroxynitrile lyases to access enantiopure alcohols. Biocatalytic methods have advantages over the chemical asymmetric synthesis especially when high requests are imposed for the enantiopurity of products and for reactions to take place under mild green chemistry conditions. Moreover, by using enzymes – natural and non-hazardous catalysts – product contamination detected in the case of asymmetric synthesis involving metal-based chiral catalysts is avoided. For pharmaceutical industry, the typical target for enantiopurity might be as high as 99.5%.[15]

2.3. Lipase Catalysis

Lipases (EC 3.1.1.3) are ubiquitous enzymes found in all types of living organisms from fungi and bacteria to plants and animals. They have essential roles in the digestion, transport and processing of dietary lipids (triglycerides, fats, oils). Lipases exert their natural function on the hydrolysis/synthesis of carboxyl ester bonds of long-chain triacylglycerols (Scheme 2). Applications of lipases are numerous. Beside their role in the lipid metabolism lipases are widely

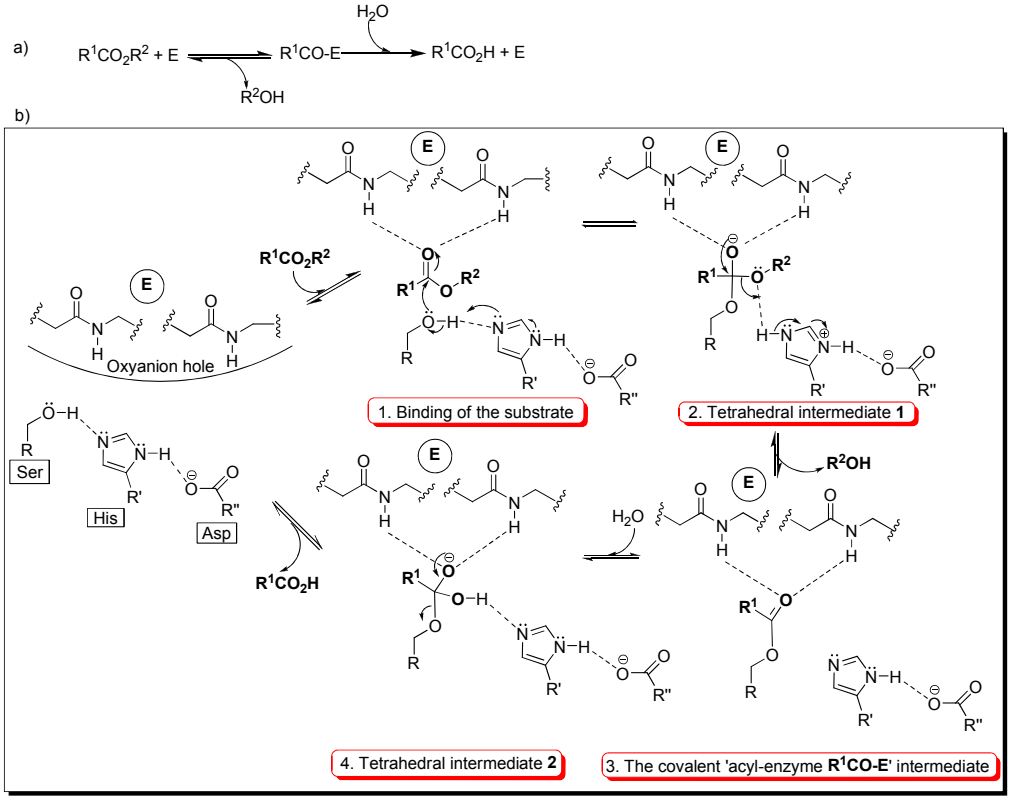
used in biotechnology as additives in detergents, to manufacture food ingredients, in pitch control in the pulp and paper industry, in biopolymers and biodiesel production.[16] Properties of lipases such as stability in organic solvents, catalytic activity without cofactors, broad substrate specificity and high enantioselectivity make them the most widely used class of enzymes in organic synthetic chemistry. A maturing understanding of lipase catalysis and commercial availability of lipases allow their use for industrial-scale biotransformations.



Scheme 2. Natural catalytic function of lipases

2.3.1. Structure and Mechanism of Lipases

The three-dimensional structure of many lipases has been well elucidated, and detailed characterization is available.[17,18,19,20] Considering the three-dimensional structure, most lipases share the same structural architecture, namely the α/β hydrolase fold.[21] Lipases show a high degree of variability at the level of their primary structure. The canonical α/β hydrolase fold is based on the central, mostly parallel β-sheet of 8 strands except the β2 strand which is antiparallel. Strands β3 to β8 are connected by α-helices packed on the both sides of the β-sheet. The number of β-strands and the architecture of the substrate binding subdomains may differ for many lipases from canonical fold. The active site contains a catalytic triad specific to serine-hydrolases, comprising of serine, histidine, and aspartate or glutamate residues. The nucleophile serine of the catalytic triad is located towards the C-terminal end of the β-strand. The nucleophile is encoded by a consensus pentapeptide sequence shared by almost all lipases, Gly-X-Ser-X-Gly located in a 'nucleophile elbow'. The three-dimensional structure of lipases offers explanations for the phenomenon of 'interfacial activation' used long time to distinguish a lipase from other lipolytic enzymes (esterases, phospholipases). 'Interfacial activation' was used to describe the enhanced catalytic activity of lipases on aggregated substrates compared to monomeric substrates. The three-dimensional structure of lipases revealed the presence of an amphiphilic peptidic lid which covers the active site. The X-ray structure of cocrystals of porcine pancreatic lipase-substrate analogs indicates that at lipid/water interface the lid undergoes conformational changes which render the active site accessible for the substrate.[22] However, not all lipases show the phenomenon of interfacial activation. Lipase B from *Candida antarctica* displays no interfacial activation due to the absence of a lid. A short helix (α5) from CAL-B structure was assumed to act as a lid due to its flexibility. Finally, it was concluded that the proposed helix is not involved in conformational changes which control the access to the active site.[23] The overall structure of selected lipases used in the thesis is given in Figure 3.



Scheme 3. The ping-pong bi-bi mechanism of lipases: a) general equation for lipase-catalyzed ester hydrolysis; b) the catalytic cycle of lipase-catalyzed hydrolysis of an ester bond

The catalytic mechanism of lipase-catalyzed ester hydrolysis is represented in Scheme 3.[24] In the first mechanistic step, the nucleophilic attack of the serine residue on the carbonyl carbon of the susceptible lipid ester bond (**$R^1CO_2R^2$**) takes place in the enzyme-substrate complex. The nucleophilicity of the hydroxyl group of the serine residue is enhanced by the hydrogen bond from histidine assisted by the aspartate or glutamate residue. The tetrahedral intermediate **1** is formed where the O atom of the original carbonyl group possesses a negative charge stabilized by hydrogen bonds with at least two amide type NH groups from the main chain at the so-called 'oxyanion hole'. Next the alcohol part (**R^2OH**) is liberated from the intermediate while the acid component of the substrate remains covalently attached to the serine residue in the acyl-enzyme intermediate (**R^1CO-E**). In the next step water, activated by the catalytic triad histidine, acts as a nucleophile hydrolyzing the acyl-enzyme intermediate. The enzyme is regenerated and the carboxylic acid product (**R^1CO_2H**) is obtained.

2.3.2. Characterization of Lipases

Candida antarctica lipase B

The yeast *Candida antarctica*, originally isolated from Lake Vanda in Antarctica, produces two different lipases, A and B (CAL-A and CAL-B). Both lipases have been purified, characterized and are available in immobilized forms. CAL-B was established as a real workhorse of biocatalysis[25] long time before CAL-A. CAL-B is a protein with a molecular mass of 33 kDa and pI of 6.0. The structure is shown in Figure 3a. The enzyme is stable in aqueous media over the pH range 3.5-9.5. The denaturation temperature is between 50-60 ^{o}C. The amino acid sequence of the protein consists of 317 amino acids. The three-dimensional structure of CAL-B was resolved by Uppenberg *et al.* in 1994.[26,27] CAL-B belongs to α/β hydrolase fold, and the catalytic triad consists of Ser105, Asp187 and His224. The highly conserved consensus region in lipases found around catalytic serine, GXSXG (see 2.3.1), is not found in CAL-B, and it is replaced by the sequence TWSQG.[21] Particular for CAL-B is the absence of a functional lid, so interfacial activation is not observed for this lipase. The active site is accessible to external solvent through a narrow channel with hydrophobic walls. The most often used CAL-B preparation is Novozyme 435 which contains the enzyme immobilized on macroporous acrylic polymer resin based on an undisclosed protocol. Recently ChiralVision started the commercialization of different enzymatic preparations containing CAL-B. Some preparations (IMMCALB-T1-1500, IMMCALB-T2-150, IMMCALB-T2-350, IMMCALB-T3-150) contain the enzyme acquired from Novozymes but immobilized with their own methods, whereas other preparations (IMMCALBY-T1-1500, IMMCALBY-T2-150, IMMCALBY-T2-350, IMMCALBY-T3-150) contain the enzyme produced by c-LEcta company and immobilized by ChiralVision. Immobilization methods are either adsorption on dry polypropylene beads or covalent attachment to dry or wet acrylic beads. The size of the beads may vary (150-300 μm, 350-700 μm, <1500 μm).

Burkholderia cepacia lipase

Burkholderia cepacia lipase (previously named *Pseudomonas cepacia*) has a bacterial origin. Its three-dimensional structure was elucidated in 1996 by different groups[28,29] and the structure is shown in Figure 3b. *Burkholderia cepacia* lipase is a protein consisting of 320 amino acids and with a molecular mass of 33 kDa. This enzyme contains one large (C domain) and two smaller domains (U1 and U2). The C domain has the topology specific to canonical α/β hydrolase fold. It contains a central β-sheet formed from six parallel strands, flanked by two α helices on one side and four α helices on the other side. The U1 domain contains three α helices and the U2 domain contains two antiparallel β strands and two α helices. The catalytic triad consists of Ser87, His286, and Asp264 and is located at the C-terminal edge of the central β-sheet. This lipase contains a Ca^{2+} ion, which is coordinated by four oxygen atoms from the protein (carbonyl oxygen atoms of Gln292 and Val296 and the side chain oxygen atoms of Asp242 and Asp288) and by two buried water molecules. This lipase is available in our laboratory from Amano in free form (lipase SL and lipase AH), immobilized by adsorption on diatomaceous earth (PS-D), or immobilized by strong adsorption forces on ceramics Toyonite 200 (PS-C II).

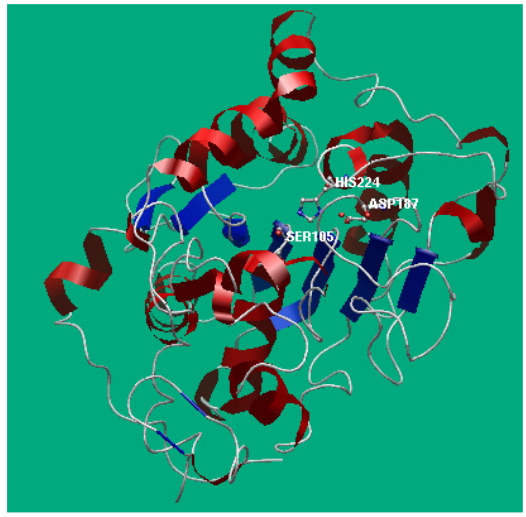
Thermomyces lanuginosus lipase

Thermomyces lanuginosus lipase (previously named *Humicola lanuginosa*) has a fungal origin. This protein has a molecular mass of 30 kDa and 269 amino acids. The optimum pH is 11-12 and thermostability is kept until 55-60 ^{o}C. The three-dimensional structure was reported with 1.85 Å resolution by Derewenda *et al.* in 1994,[30] and the structure is shown in Figure 3c. There are also previous structural data at different resolutions. The structure contains a single roughly spherical

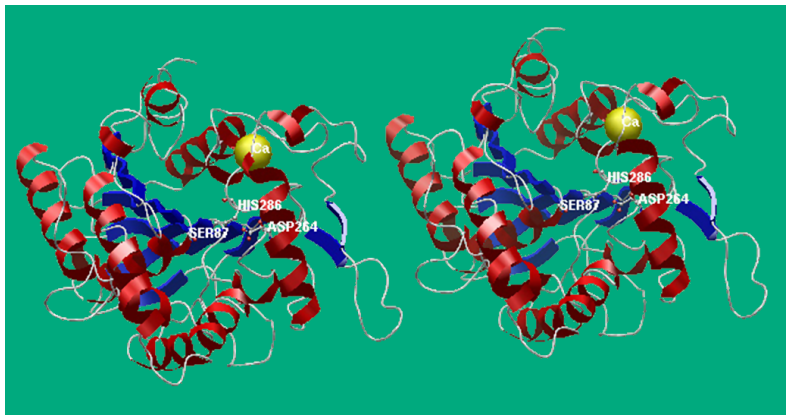
domain with eight predominantly parallel β sheets and five interconnecting α helices.[31] The catalytic triad consisting of Ser146, His258, and Asp201, is located in a hydrophobic groove. The access to the active site is blocked in the inactive conformation by a lid which contains two hinge regions and a strongly amphipatic helix (85-93 residues).[32] The conformational changes take place in the presence of the substrate analogues and the lid makes the active site available for catalysis. *Thermomyces lanuginosus* lipase is the active component of the commercial preparation Lipolase® marketed by Novozymes. In this thesis three different lipase preparations from *Thermomyces Lanuginosus* were screened; Lipozyme TL IM (lipase immobilized on silica) from Novozymes and IMMTLL-T1-1500 (lipase immobilized by adsorption on polypropylene) and IMMTLL-T2-150 (lipase immobilized covalently on polyacrylic beads) from ChiralVision.

Figure 3. The overall structure of important lipases used in this work. The structures are visualized with Bodil[33]: **a)** *Candida antarctica* lipase B (1TCA[27]-PDB code) - the catalytic triad is outlined; **b)** *Burkholderia cepacia* lipase (1OIL[28]-PDB code) - the catalytic triad and Ca^{2+} ion are outlined (the asymmetric unit of the crystal contains two lipase molecules); **c)** *Thermomyces lanuginosus* lipase (1TIB[30]-PDB code) - the catalytic triad and the lid (yellow color) are outlined.

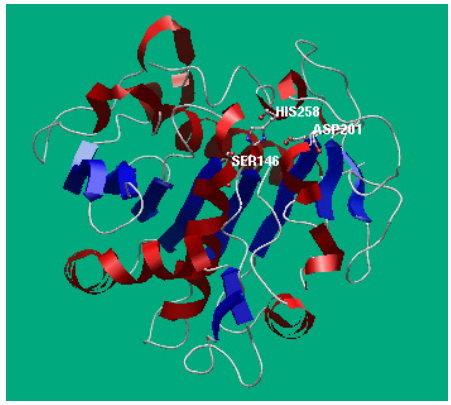
a)

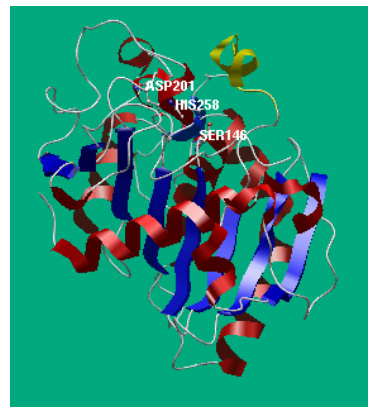

HIS224
ASP187
SER105

b)


Ca
HIS286
SER87
ASP264
Ca
HIS286
SER87
ASP264

c)


HIS258
ASP201
SER146


ASP201
HIS258
SER146

2.3.3. Sources and Forms of Lipases Used in Organic Synthetic Chemistry

Applications of lipases in organic chemistry are favored by the increasing number of lipases commercially available in free or immobilized forms. Lipases used in synthetic reactions are generally derived from microorganisms (Table 2). In addition, one mammalian lipase, PPL, has proved to be particularly useful. The change of the name of certain microorganisms is one of the intriguing aspects for a chemist (see Table 2). The microorganisms are renamed and reclassified when new information about them becomes available. Lipases from microorganisms are generally more stable than those from plants and animals, possess a high variety and are prone to easy genetic manipulation.[34] Industrial production of lipases is based on expression of the gene of interest in recombinant bacteria and yeasts. In organic solvents lipases are used in dried forms obtained by lyophilization and increasingly by immobilization.

Table 2. Origin of commercially available lipases used in organic synthesis

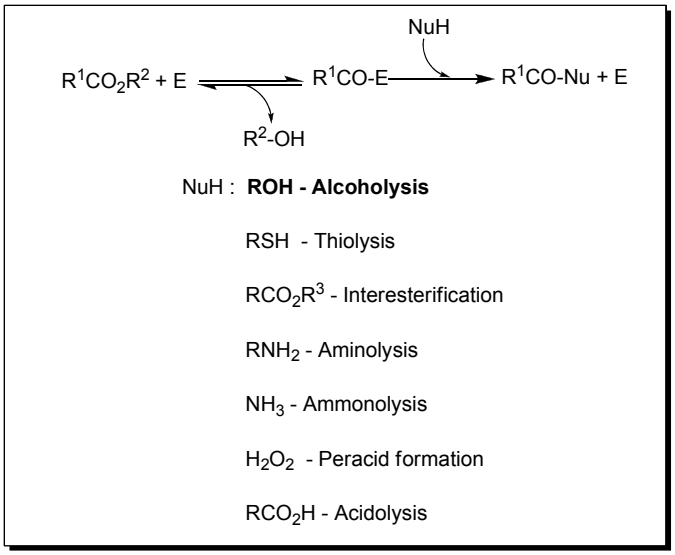
Origin of Lipase	Previous name	Abbreviation
Mammalian origin		
Porcine pancreas		PPL
Fungal lipases		
Candida rugosa	*Candida cylindracea*	CRL
Candida antarctica A		CAL-A
Candida antarctica B		CAL-B
Thermomyces lanuginosus	*Humicola lanuginosa*	TL IL
Rhizomucor miehei	*Mucor miehei*	RL IM
Bacterial lipases		
Pseudomonas fluorescens		AK, PFL
Burkholderia cepacia	*Pseudomonas cepacia*	PS
Chromobacterium viscosum	*Pseudomonas glumae*	CVL

The process of immobilization and various immobilization techniques are thoroughly described in literature.[35] The immediate advantages of using immobilized lipases are those derived from heterogeneous catalysis, such as easy recovery, recyclability, and possibility to develop continuous processes. Immobilization is used to increase the stability in organic solvents. Moreover, activity, substrate specificity and enantioselectivity may be improved by immobilization. The methods available for immobilization are adsorption on a carrier and encapsulation or covalent attachment to a carrier. Special attention is paid to the carriers which may affect the water content of the system, especially when enzymes are exploited in organic solvents. Cross-linking of enzyme is a particular case of immobilization based on the formation of covalent bonds without using a carrier.

2.3.4. Potential of Lipases in Organic Synthetic Chemistry

The potential of lipases in organic chemistry is fully exploited by replacing the aqueous medium with an organic one. The first lipase-catalyzed reaction performed in organic medium was reported in 1984 by Zaks and Klibanov.[36] From the mechanistic point of view, in organic solvents the acyl-enzyme intermediate (R^1CO-E) typical to lipase catalysis (Scheme 3) may be subjected to a nucleophilic attack by different nucleophiles (NuH), such as by alcohols (ROH), thiols (RSH), amines (RNH_2), ammonia (NH_3), hydrogen peroxide (H_2O_2), and carboxylic acids (RCO_2H). Thus, reactions like alcoholysis, thiolysis, aminolysis, ammonolysis, peracid formation and acidolysis are enabled (Scheme 4). Interesterification with another ester (RCO_2R^3) is a special type of alcoholysis in which the nucleophile (R^3OH) is generated *in-situ*. Alcoholysis, thiolysis and interesterification are actually transesterifications, consisting of the formation of a new ester (R^1CONu) from a

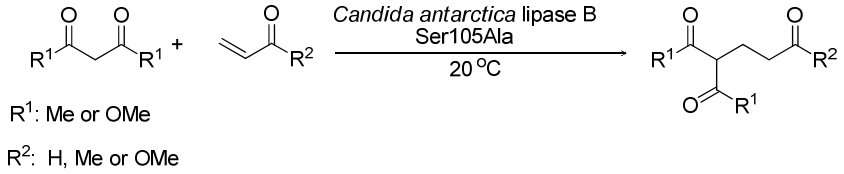
substrate ester ($R^1CO_2R^2$). Exhaustive reviews are available concerning practical applications of lipase-catalyzed transesterification.[37,38,39] Lipase-catalyzed transesterification is applied in this thesis for the preparation of enantiopure secondary alcohols.



Scheme 4. Synthetic potential of lipases in organic solvents

The synthetic applications of lipases exploit their chemo-, regio- and stereoselectivity. Lipases catalyze regio- and chemoselective reactions of polyfunctional compounds which include protective and deprotective techniques. For instance, *Candida antarctica* lipase A was used for the chemoselective *O*-acylation of 2- and 3-hydroxymethylpiperidines.[40] Lipases were also used for regioselective modification of complex natural glycosides.[41] Stereoselectivity of lipases helps them to recognize the chirality in various molecules which may be located on the substrate ester ($R^1CO_2R^2$), on the nucleophile (NuH) or on both of them.

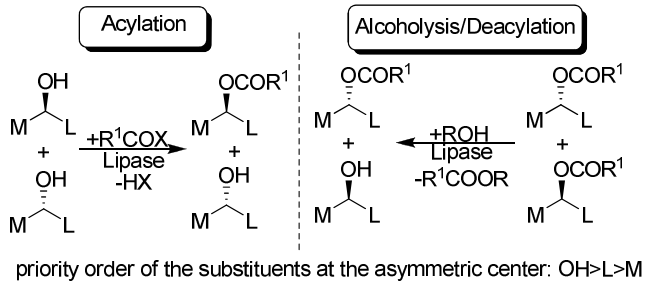
Recently, considerable attention has been paid to catalytic promiscuity of enzymes, as their ability to catalyze other reactions than those already established.[42,43] The catalytic promiscuity of lipases was exploited to enable non-conventional reactions such as aldol and the Michael-type additions. It is expected that protein engineering will enhance the applicability of catalytic promiscuity of lipases and that of enzymes in general. For instance, the mutant Ser105Ala of *Candida antarctica* lipase B catalyzed carbon-carbon bond formation between 1,3-dicarbonyls and α,β-unsaturated carbonyl compounds faster than the wild type (Scheme 5).[44]



Scheme 5. Catalytic promiscuity of lipase CAL-B Ser105Ala mutant for the Michael addition reaction[44]

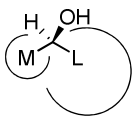
2.4. Towards Enantiopure Secondary Alcohols by Lipase-catalyzed Kinetic Resolution

In this thesis the stereoselectivity of lipase-catalyzed transesterifications ($R^1CO_2R^2$ + ROH= R^1CO_2R + R^2OH) is of interest for the preparation of enantiopure alcohols by the kinetic resolution of the corresponding racemates. Lipases have a broad substrate specificity allowing the synthesis of enantiopure primary, secondary or tertiary alcohols with aliphatic, aromatic and allylic structures.[45,46] Moreover, other functionalities are accepted in the alcohol structure, although lipase catalysis is maintained for the hydroxyl functionality. Compounds like amino alcohols, β-hydroxy nitriles, and hydroxy acids have been successfully resolved by lipase catalysis. The alcohols subjected to lipase catalysis in this thesis are secondary alcohols. The enantioselectivity obtained with secondary alcohols is often high compared to what is observed with primary or tertiary alcohols. Lipase catalysis for secondary alcohols is orientated to the hydroxyl function directly attached to the asymmetric center. Low enantioselectivity with primary alcohols is usually explained by the remote position of the asymmetric center from the site of enzymatic action.[47] Kinetic resolution of tertiary alcohols in the presence of lipases is limited by their steric hindrance and lower reactivity.



Scheme 6. Lipase-catalyzed preparation of enantiopure secondary alcohols

The kinetic resolution of secondary alcohols and esters is performed in organic solvents by lipase-catalyzed acylation and alcoholysis, respectively (Scheme 6). It leads to the formation of one enantiomer as an alcohol and the other enantiomer as an ester. The maximum theoretical yield for each enantiomer is 50%. Empirical rules have been formulated to predict the fast reacting enantiomer for lipases. The rule proposed by Kazlauskas[48] to predict the fast reacting enantiomer of a racemic secondary alcohol during acylation in the presence of *Burkholderia cepacia* lipase proved to be valid also for many other lipases. According to this rule, the discrimination of the enantiomers is based on the size of the substituents (M – medium-size substituent, L – large-size substituent, Figure 4) attached to the asymmetric center, which bind to different hydrophobic pockets at the active site of the enzyme.



Secondary alcohol

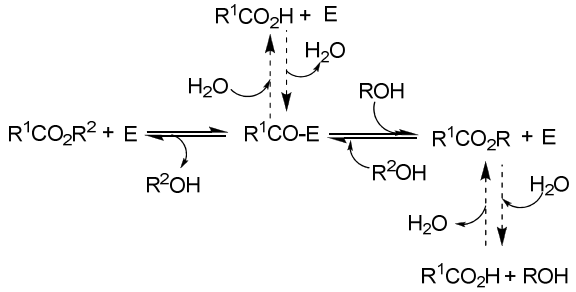
Figure 4. The fast reacting secondary alcohol enantiomer with lipases according to the Kazlauskas rule

2.4.1. Optimization of Kinetic Resolution

Lipases have broad substrate tolerance for unnatural compounds in various environments, although their enantioselectivity often needs to be improved to develop a practical kinetic resolution method. Improvements in selectivity have been achieved by studying solvent effects, water activity of the solvent, temperature effects and the effects of substrate concentrations, acyl donors and different additives added to the reaction mixture and also by engineering the enzyme.[49,50,51] Protein engineering methods like rational protein design and directed evolution have gained importance especially in the last years. Karl Hult´s group succeeded to transform the natural (*R*)-stereopreference for secondary alcohols of wild-type CaL-B into (*S*)-stereopreference by rational design of the mutant Trp104Ala of CaL-B.[52] In this thesis the efforts to increase the enantioselectivity are orientated towards conditions of the lipase-catalyzed kinetic resolution which are easy to be adjusted in a synthetic chemistry laboratory.

2.4.1.1. Reaction Medium in Kinetic Resolution

Reaction conditions for kinetic resolution need to be rigorously controlled to achieve the desired yield and enantiopurity. The sensitivity of the system arises from the reversible nature of transesterification reactions.[53] The alcohol component (R^2OH) of the acyl donor ($R^1CO_2R^2$) can enzymatically react with the new ester (R^1CO_2R) formed leading back to the starting ester ($R^1CO_2R^2$) and cause low enantiopurities for the resolution products. The design of acyl donors to render the reaction irreversible is considered later (See 2.4.1.2). Moreover, water as the natural nucleophile for lipases represents a competing nucleophile for the alcohol (ROH) and may react with the acyl-enzyme intermediate (R^1CO-E) in organic solvents, leading to the undesired hydrolysis of the acyl donor ($R^1CO_2R^2$), of the ester product (R^1CO_2R) or of both of them (Scheme 7). Thus, carefully dried organic solvents are often used for acylations. The solvent itself is not the only source of water in the acylation system: the support material of immobilized lipase, the lipase itself, the acyl donor and side-reactions producing water may contribute to the overall water content of the system. Peter Halling has proposed the use of thermodynamic water activity (a_w) as a parameter to describe efficiently the overall water effect in enzymatic reactions.[54]



Scheme 7. Transesterification – insight into background reactions

The possibility to influence enzyme properties by changing the nature of a solvent was remarked and carefully first studied by Klibanov and co-workers especially for proteases like subtilisin and α-chymotrypsin.[55] The solvent affects also the activity and enantioselectivity of lipase-catalyzed transesterifications. The mechanism is rather complex and not understood at the level which allows the development of a quantitative relationship between certain physical properties of solvents and lipase activity and selectivity. The best solvent for a lipase-catalyzed reaction is usually found by a screening strategy. Ethers (TBME, DIPE, diethyl ether) are solvents

generally preferred in lipase-catalyzed transesterifications.[37] 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. Usually, the highest reaction rates and activity are recorded for transesterifications performed in hydrophobic solvents (log *P*>1.5). Reaction rates are often considerably reduced in hydrophilic solvents. The water solubility in hydrophilic solvents may cause deactivation of the enzyme when the solvent stripes off the 'essential water' necessary to maintain the active conformation and the catalytic activity of the enzyme in an organic solvent.[56]

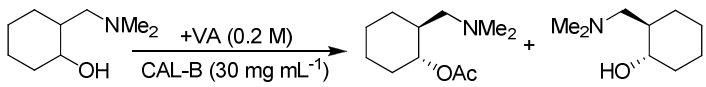
Table 3. Log *P* of commonly used organic solvents[57] in enzymatic reactions

Entry	Solvent	log *P*	Entry	Solvent	log *P*
1	Dimethylsulfoxide	-1.3	9.	Diethylether	0.85
2	*N*,*N*-dimethylformamide	-1.0	10.	TBME[a]	1.5
3	Methanol	-0.76	11.	DIPE[a]	2.03
4	Ethanol	-0.24	12.	Toluene	2.5
5	Acetone	-0.23	13.	Cyclohexane	3.2
6	Methyl acetate	0.16	14.	Hexane	3.5
7	Ethyl acetate	0.68	15.	Heptane	4.0
8	Butanol	0.80			

[a]Calculated with sparc on-line (http://sparc.chem.uga.edu/sparc/)

The work of Forró *et al*.[58] on the acylation of 2-dialkylaminomethylcyclohexanols in the presence of CAL-B clearly shows that solvent effects are not straightforward (Table 4). The lipase was inactive in hydrophilic solvents such as in acetone (log *P*, -0.23) and in tetrahydrofuran (log *P*, 0.49) (entries 1 and 2). The best enantioselectivity was obtained in etheral solutions (entries 3 and 5). Reactivity was highest in diethyl ether which was selected as the most favorable solvent for further studies in the work.

Table 4. Solvent screening for the acylation of *rac-trans*-**1** in the presence of CAL-B[58]



rac-trans-**1** (1*R*,2*S*)-**2** (1*S*,2*R*)-**1**

Entry	Solvent	log *P*	Conversion (%)[a]	*E*
1	Acetone	-0.23	no reaction	-
2	Tetrahydrofuran	0.49	no reaction	-
3	Diethyl ether	0.85	41	>200
4	*tert*-Amyl alcohol	1.40	21	62
5	DIPE	2.03	25	>200
6	Toluene	2.50	20	61

[a]samples taken after 84 h

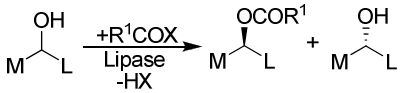
The issue of sustainability cannot be neglected in the selection of a solvent for lipase catalysis. According to the guidelines of green chemistry, a good organic solvent is relatively non-toxic, non-hazardous, not inflammable and not corrosive.[59] Many chlorinated hydrocarbon solvents have already been or are likely to be banned from use in the near future. Whenever possible the

trend is for the use of esters, lower alcohols, toluene and some hydrocarbons, and ethers as solvents. Triacetin (glycerol triacetate), approved by FDA with the label 'Generally Recognized as Safe' human food ingredient, has received particular attention as a potential green solvent. It was used as a solvent and an acyl donor for instance for the kinetic resolution of 2-heptanol in the presence of both free and immobilized CAL-B. Full conversion of 50% and 99% ee was obtained for the formed ester with immobilized CAL-B.[60] Alternative reaction media for lipase-catalysis, such as supercritical fluids[61], ionic liquids,[62] and hydrofluorocarbon solvents[63] are also usable. Reduction of environmental impact is often predicted for these solvents, but this effect is not fully assessed yet in most of the cases. Although each reaction media used for lipase catalysis brings benefits, the great step forward in practical synthetic applications of lipases was the shift to organic solvents.

Especially from the industrial point of view, solvent-free reactions are attractive because a major part of the waste generated in a process consists of solvents. Enzymatic catalysis is often performed with low substrate concentrations to prevent enzyme inhibition. However, lipases act as robust catalysts which often accept relatively high substrate concentrations and even solvent-free reaction conditions. Methyl (*R*)-2-chloromandelate is a chiral building block for the industrial synthesis of (*S*)-Clopidogrel, an anti-thrombotic agent. Enantiopure methyl (*R*)-2-chloromandelate (ee >99%) was obtained with 41% yield by acylation of the racemate with lipase CAL-A under solvent-free conditions.[64] In paper I of this dissertation a solvent-free process was developed for the acylation of ethyl 3-hydroxybutanoate in the presence of CAL-B.

2.4.1.2. Achiral Acyl Donors in Kinetic Resolution

The full potential of lipase catalysis is exploited only when the thermodynamic equilibrium is shifted to the product side. The acyl donor may influence the equilibrium position and the rate of acylation/deacylation. Three main groups of achiral acyl donors are used for kinetic resolution: reversible, quasi-irreversible and irreversible ones (Figure 5).



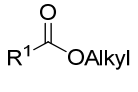
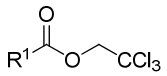
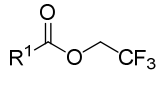
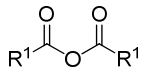
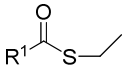
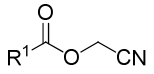
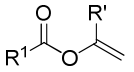
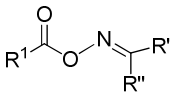
Reversible acyl donor R^1COX	Quasi-irreversible acyl donors R^1COX	Irreversible acyl donors R^1COX
 **Alkyl esters**	  **2,2,2-Trihaloethyl esters**	 **Anhydrides**
 **Thioesters**	 **Cyanomethyl esters**	 **Enol esters**
	 **Oxime esters**	R´: H - **Vinyl esters** CH_3 - **Isopropenyl esters** OEt – **Ethoxy vinyl esters**

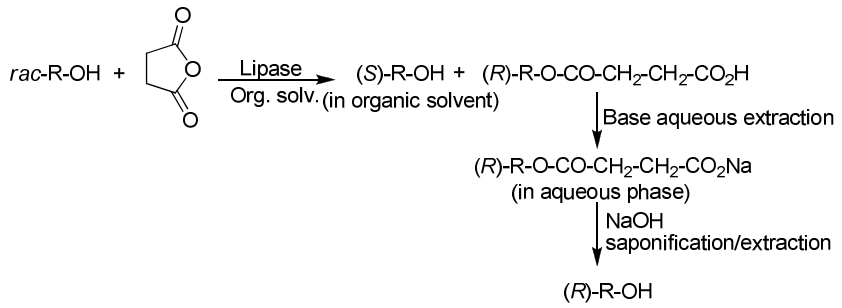
Figure 5. Achiral acyl donors

Hanefeld[65] has thoroughly reviewed the irreversible acyl donors applied in hydrolase-catalyzed acylations, giving details about their synthesis. Quasi-irreversible and irreversible acyl donors belong to activated acyl donors and they contribute to the increase of the rates of enzymatic reactions. When reversible acyl donors (alkyl esters and thioesters) are used for the acylation, thermodynamic equilibrium can be shifted towards the product formation by using an excess of an acyl donor or by removal of a product formed in the reaction mixture. Screening of the acyl donors in the optimization of kinetic resolution in this thesis included mainly 2,2,2-trifluoroethyl esters, enol esters (vinyl and isopropenyl esters) and cyclic anhydrides.

In the case of 2,2,2-trihaloethyl esters as quasi-irreversible acyl donors an alcohol with low nucleophilicity is liberated (CF_3CH_2OH or CCl_3CH_2OH) from the acyl donor which is accordingly expected to be unreactive as a nucleophile. Reduced nucleophilicity is obtained by introducing electron-withdrawing groups in the alkyl part of the acyl donor. Cyanomethyl esters have been seldom used in enzymatic reactions possibly because the hydrogen cyanide is formed in the reaction mixture.[66] Oximes have been used as acylating agents mainly for regioselective protection of sugars and nucleosides, but the work-up of the reaction encountered difficulties in removing the remaining oxime.[67]

Enol esters commonly used as irreversible acyl donors[68] are vinyl esters (R´=H), isopropenyl esters (R´=CH_3) and ethoxy vinyl esters (R´=OEt). The leaving group of the irreversible acyl donors is an enol that immediately tautomerises to the keto form (CH_3CHO, CH_3COCH_3, EtOAc), respectively and, accordingly, no nucleophile is available for backward reaction. However, acetaldehyde released in the case of vinyl esters may cause enzyme deactivation by participation to a Millard type reaction with the lysine residues of the enzyme. Sensitivity of *Candida rugosa* and *Geotrichium candicum* lipases against acetaldehyde was explained by the presence of several lysine residues with high pK_a values (>12) in the lid region which is highly exposed to the solvent.[69]

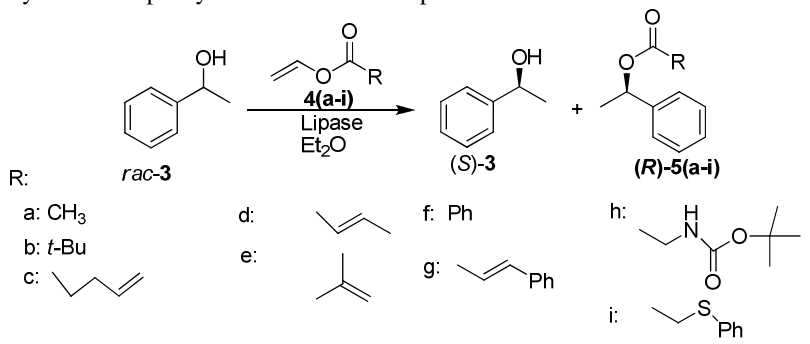
Anhydrides used as acyl donors for transesterification generate carboxylic acids in acylation. The equilibrium is completely shifted towards the product side, because the reverse reaction is thermodynamically unfavored.[70] The applications of anhydrides as acyl donors in enzyme-mediated transesterification of alcohols are not numerous. Possible reasons are the high acylation power of anhydrides which may acylate the enzyme or may lead to chemical background acylation of alcohol racemates. Moreover, the acid product in the reaction medium may change the pH of the enzymatic microenvironment.[65] Promising applications were found for cyclic anhydrides such as succinic anhydride. When succinic anhydride is used as an acyl donor for the kinetic resolution of alcohols, unreacted alcohol may be separated from the product monoester by aqueous-organic extraction (Scheme 8).[71] This procedure is attractive especially for large-scale applications where column chromatography, often used for the purification of resolution mixtures in laboratories, is not suitable.



Scheme 8. Lipase-catalyzed acylation with succinic anhydride as an acyl donor

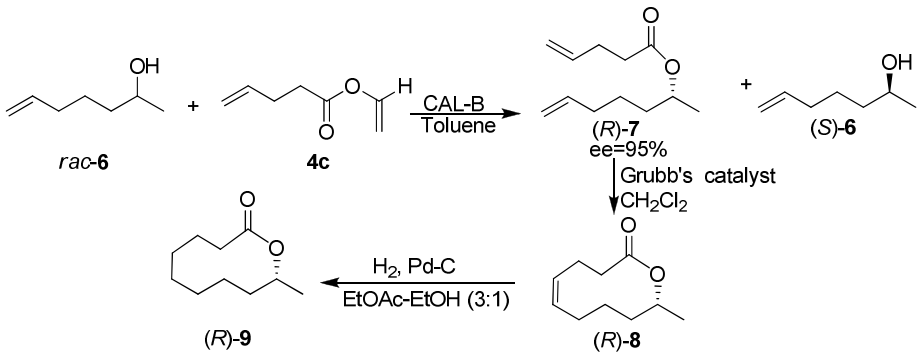
The aim of synthetic chemists is to include lipase-catalyzed reactions in the chemoenzymatic multi-steps synthesis of useful molecules. In the case of acylation with vinyl or isopropenyl acetate, usually the acetyl group introduced in the enzymatic step is removed and replaced by other groups. Chênevert *et al.*[72] have investigated the tolerance of *Candida antarctica* lipase B, *Candida rugosa* lipase, and *Burkholderia cepacia* lipase for the acylation of 1-phenylethanol *rac*-**3** with functionalized vinyl esters (Table 5).

Table 5. Acylation of 1-phenylethanol *rac*-**3** in the presence of CAL-B[72]



Entry	Vinyl ester	c (%)	Time (h)	(*R*)-5(a-i)		(*S*)-3		*E*
				Yield (%)	ee (%)	Yield (%)	ee (%)	
1	**Acetate 4a**	45	24	44	>98	48	>98	>200
2	**Pivalate 4b**	52	90	44	92	42	86	66
3	**4-Pentenoate 4c**	24	144	20	95	62	67	>200
4	**Crotonate 4d**	50	30	50	>98	50	91	>200
5	**Methacrylate 4e**	48	34	45	>98	52	91	>200
6	**Benzoate 4f**	47	43	40	92	54	84	60
7	**Cinnamate 4g**	50	144	43	>98	50	>98	>200
8	**N-Boc Glycinate 4h**	49	140	36	>98	42	>98	>200
9	**Phenyl(thio)acetate 4i**	50	4	48	94	49	>98	>200

The most tolerant lipase was CAL-B which catalyzed the acylation of *rac*-**3** with excellent enantioselectivity (*E*>200) with almost all the acyl donors used. Only in two cases (entry 2 and 6) moderate enantioselectivity was achieved. Pentenoate **4c** was applied in the synthesis of natural product phoracantholide (*R*)-**9**. Phoracantholides are components of odoriferous defense secretion of the Australian longicorn beetle *Phoracantha synonyma*. The combination of acylation in the presence of CAL-B and ring-closing metathesis of the resulted diene ester (*R*)-**7** in the presence of second-generation Grubbs catalyst resulted in the synthesis of (*R*)-**9** (Scheme 9).



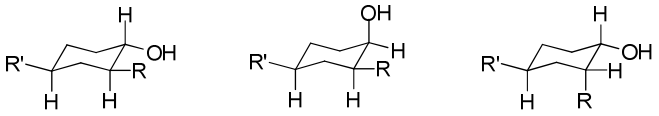
Scheme 9. Synthesis of phoracantholide, (*R*)-**9**[72]

2.4.1.3. Effect of the Structure of Secondary Alcohols in Kinetic Resolution

The structure of secondary alcohols is important for substrate binding during lipase catalysis. Numerous secondary alcohols have been subjected to lipase-catalyzed kinetic resolution, and structural effects vary from one lipase to another. It is also possible to affect them for instance by solvents. Due to huge number of data, I concentrate here only on substituted cyclohexanols and aromatic secondary alcohols, considered relevant for the experimental work of the thesis.

Substituted cyclohexanols with two asymmetric centers exist as racemic *cis*/*trans*-diastereomeric mixtures. Conformational effects on lipase PS-catalyzed acylation with vinyl acetate of 2-substituted[73] and 3-substituted cyclohexanols[74] have been investigated (Tables 6, 7). The ring may adopt different conformations, which has been shown to influence the enantioselectivity and the reactivity of the lipase-catalyzed acylation.

Table 6. Lipase-catalyzed acylation[a] of 2-mono or 2,4-di-substituted cyclohexanols[73]



10,12 **11,13** **14**

10, 12: R'=H
11, **13, 14**: R'=*t*-Bu

a) R=-CH_2-CH_3; b) R=-CH=CH_2; c) R= −C≡CH
d) R=-CH_2-CH_2-CH_2-CH_3; e) R=—C≡C−C(CH_3)=CH_2

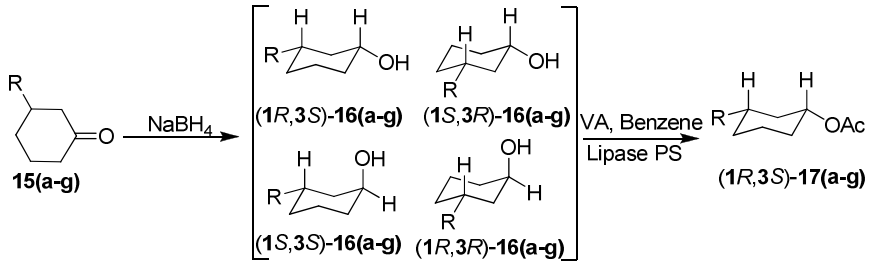
***trans*-2-cyclohexanols**				***cis*-2-cyclohexanols**			
Compound	***E***	**c (%)**	**t (h)**	**Compound**	***E***	**c (%)**	**t (h)**
10a	>200	43	7	**11a**	111	43	46
10b	>200	42	9	**11b**	>200	44	22
10c	>200	42	12	**11c**	>200	42	6
10d	>200	40	8	**11d**	>200	43	51
10e	>200	44	13	**11e**	141	42	104
12b	11	14	48	**13b**	-	0	600
				14b	50	33	102

[a]Lipase PS, vinyl acetate, molecular sieves 4Å, toluene, 30 °C

Table 6 contains data for the acylation of racemic 2-substituted cyclohexanols **10-14** with different R substituents (cases **a-e**). In addition, compounds **11-14** have *t*-Bu group in the position 4. The HO group in *trans*-cyclohexanols **10(a-e)** is orientated favourably for acylation in equatorial position, leading to enhanced reactivity compared to *cis*-cyclohexanols **11(a-e)** with the axial HO groups. On the other hand, the large *t*-Bu group in the position 4 tends to be equatorial, controlling the preferred conformations in **11-14**. Accordingly, *cis*-**13b** with an axial hydroxyl group did not react in 600h. The *cis*-**14b** with an equatorial hydroxyl reacted, although with reduced enantioselectivity.

Table 7 contains similar data for the lipase PS-catalyzed acylation of 3-substituted cyclohexanols with vinyl acetate.[74] Cyclohexanols were used as mixtures of four stereoisomers. The main product obtained with high enantiomeric excess was (1*R*,3*S*)-**17(a-g)**. The separate acylation of *trans*-**16(a-g)** did not proceed efficiently, the HO group being now orientated in an axial position unfavorable for acylation.

Table 7. Preparation of (**1***R*,**3***S*)-**17(a-g)**[74]



(1*R*,3*S*)-17	R	ax/eq[a]	time[b] (h)	yield (%)	ee (%)
a	CH_2NO_2	20/80	4.5	37	86
b	CMe_2NO_2	22/78	25.0	31	>97
c	SPh	12/88	5.0	42	>97
d	SO_2Ph	12/88	48.0	32	>97
e	*n*-Bu	22/78	4.5	38	65
f	Ph	21/79	7.5	36	>97
g	Bn	22/78	6.5	35	>97

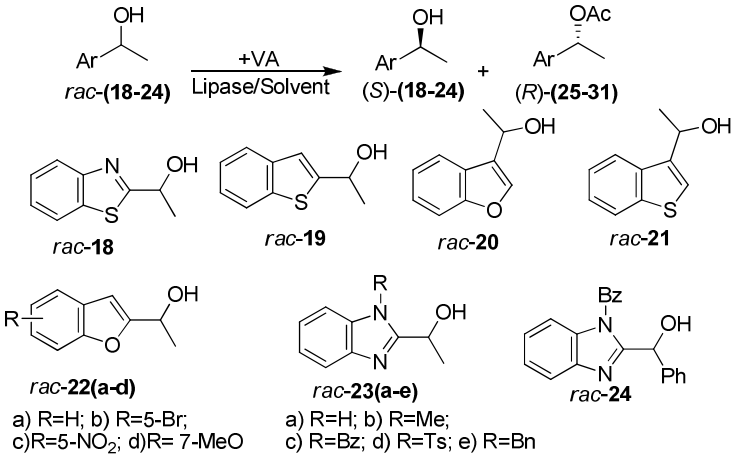
[a] Axial (1*R*,3*R*)/equatorial (1*R*,3*S*) ratio of the alcohol **17** obtained by reduction of **15**
[b] Reaction time for lipase-mediated acylation

Molecular modeling has been applied to study the conformational effects for the lipase CAL-B-catalyzed acylation of *cis*- and *trans*-2-substituted cyclohexanols.[75] The importance of the HO group orientation in an axial position was once more emphasized. In paper II the enzymatic acylation of 3-methyl-substituted tetrahydro-2*H*-thiopyran-4-ol was studied.

Various 1-heteroaryl ethanols containing benzothiazol, benzothiophen, benzofuran and benzimidazol are good examples for the enantioselective acylation of aromatic secondary alcohols. The alcohols have been resolved by lipase-catalyzed acylation (Table 8). CAL-B was used for the preparative-scale acylation of 1-heteroaryl ethanols **18-21** with vinyl acetate used both as a solvent and as an acyl donor.[76] The kinetic resolution of 1-(benzofuran-2-yl)ethanols *rac*-**22(a-d)** was performed in the presence of commercial lipase AK. The enantioselectivity was from good to excellent (*E*>>100) for all alcohols. The effect of the substituent was noticed in the reactivity. The acylation of the unsubstituted alcohol *rac*-**22a** reached 50% conversion in 14 h while the same 50% conversion was achieved in 72 h for compound **22c**, bearing the electon withdrawing NO_2 group in

the position 5.[77] Deacylation of (*R*)-1-acetoxy-1-(benzofuran-2-yl)-ethanes was performed by lipase-catalyzed ethanolysis. Lipase CAL-B (Novozyme 435) was used to resolve benzimidazolyl ethanols *rac*-**23(a-e)** and *rac*-**24** by transesterification with vinyl acetate in toluene. *N*-protection with methyl (*rac*-**23b**) or benzyl (*rac*-**23c**) groups enhanced enantioselectivity compared to the unsubstituted benzimidazolyl ethanol *rac*-**23a** (*E*=14). When electron-withdrawing substituents such as benzoyl or *p*-toluenesulfonyl were the protective groups, lower enantioselectivity was obtained. *Rac*-**24** has two large groups at the asymmetric center and only moderate enantioselectivity (*E*=22) was obtained during acylation.[78]

Table 8. Preparative acylation of 1-heteroaryl ethanols[76,77,78]

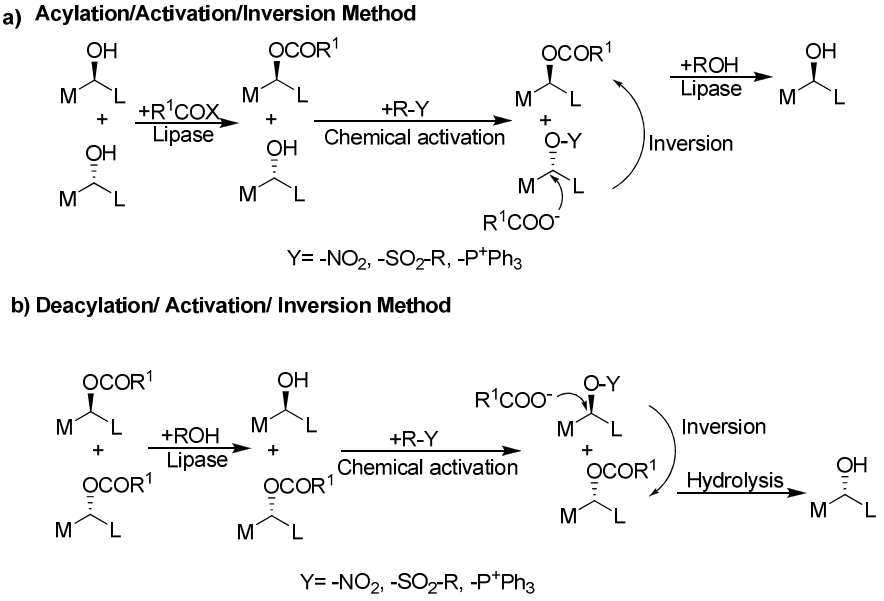


Alcohol	Lipase	Solvent	(*S*)-alcohol		(*R*)-acetate		*E*
			ee (%)	yield (%)	ee (%)	yield (%)	
18	CAL-B	VA[1]	97	44	98	45	>200
19	CAL-B	VA[1]	99	45	99	46	>>200
20	CAL-B	VA[1]	96	45	97	44	>100
21	CAL-B	VA[1]	96	46	97	43	>200
22a	lipase AK	VA[1]	98	49	99	48	>>100
22b	lipase AK	VA[1]	97	48	98	47	>>100
22c	lipase AK	VA[1]	81	51	99	44	>>100
22d	lipase AK	VA[1]	81	51	99	43	>>100
23a	CAL-B	Toluene	90	33	80	38	14
23b	CAL-B	Toluene	99	40	99	42	225
23c	CAL-B	Toluene	88	33	85	35	25
23d	CAL-B	Toluene	86	37	82	39	19
23e	CAL-B	Toluene	97	32	95	34	165
24	CAL-B	Toluene	87	43	84	41	22

[1]VA was used both as a solvent and as an acyl donor

2.5. Kinetic Resolution-Based Methods with Enhanced Yields

2.5.1. Kinetic Resolution/Inversion of Configuration Method

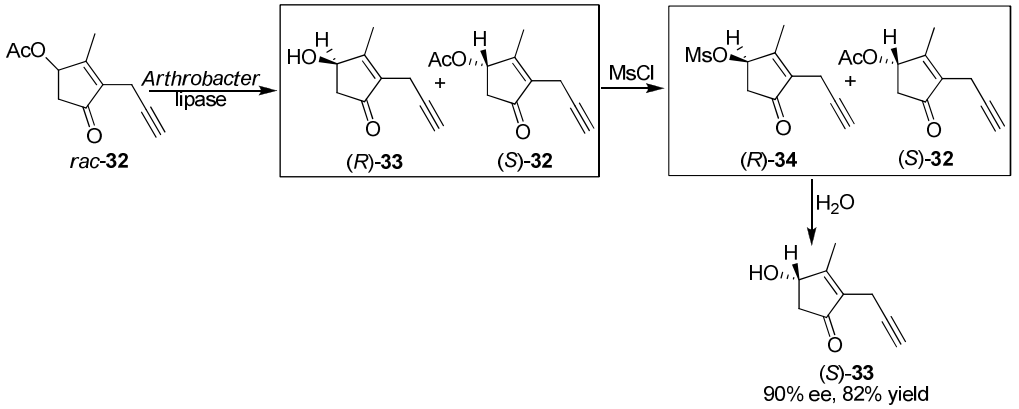


Scheme 10. Kinetic resolution combined with inversion of configuration

The limiting yield of 50% for normal kinetic resolution can be overcome by submitting the resolution mixture obtained by lipase-catalyzed acylation or alcoholysis to the inversion of configuration of the alcohol enantiomer in the mixture (Scheme 10a, b). The inversion of configuration of the hydroxyl group (after conversion into a good leaving group) is based on S_N2 substitution. As shown in Scheme 10a, when the resolution mixture is obtained by the acylation of a racemic alcohol with an *R*-enantioselective lipase (L>M), it contains the *R*-ester and the unreacted *S*-alcohol. When the inversion of configuration of the *S*-alcohol takes place with a carboxylate identical with the acyl part of the acyl donor used in the enzymatic acylation, the unreacted alcohol is converted to the same *R*-ester obtained by the kinetic resolution. Moreover, if the enantioselectivity is not excellent for the kinetic resolution or the inversion step proceeds with a mixed S_N2 and S_N1 reaction, a second resolution step by lipase-catalyzed alcoholysis of the obtained *R*-ester increases the enantiopurity of the *R*-alcohol obtained. In the similar manner the mixture obtained by alcoholysis of an ester of a secondary alcohol may be converted to the *S*-ester which after chemical or enzymatic deprotection gives the *S*-alcohol enantiomer (Scheme 10b).

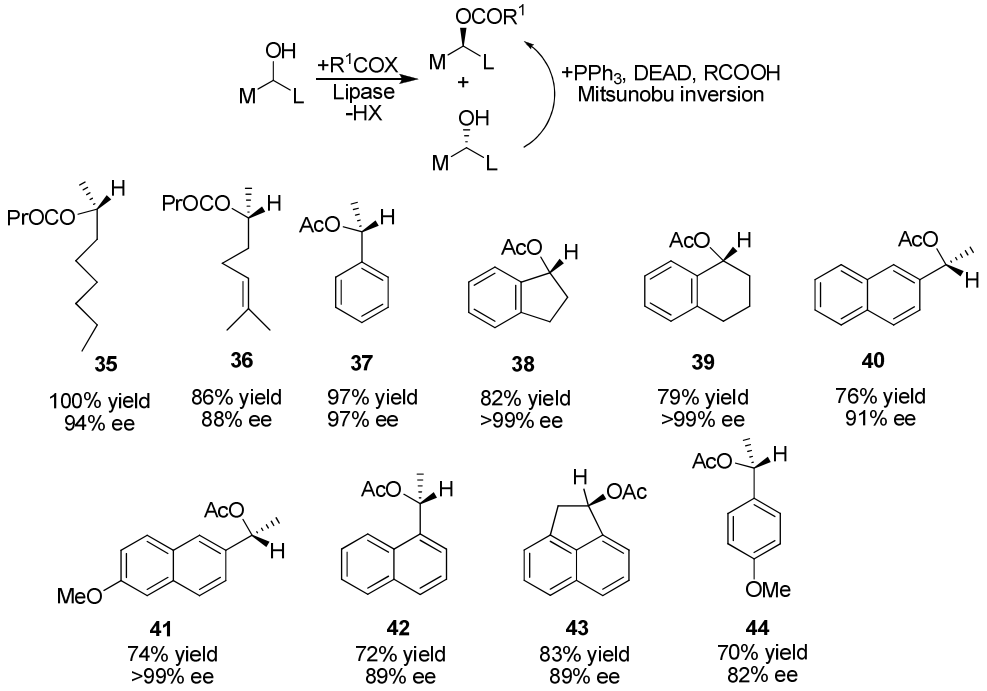
The hydroxyl group of an alcohol may be activated before inversion as a nitrate or sulphonate ester (usually mesylate).[79,80,81,82] As an example, the enantiopure alcohol (*S*)-**33**, intermediate for the synthesis of pyrethroid insecticides was prepared from the corresponding racemic acetate *rac*-**32** by combining hydrolysis with lipase *Arthrobacter* followed by inversion of configuration of the unreacted alcohol by mesylation and hydrolysis with $CaCO_3$ (0.2 equiv.) to give (*S*)-**33** with 90% ee and 82% isolated yield (Scheme 11).[79] In Paper I of this thesis, the CAL-B-catalyzed acylation of racemic ethyl 3-hydroxybutanoate in a solvent-free system was combined with the inversion of configuration to convert the *S*-enantiomer first to the mesylate followed by

acylation with cesium acetate in DMF as a new method to obtain ethyl 3-(*R*)-acetoxybutanoate (ee 92%, yield over 85%) from the racemic mixture.



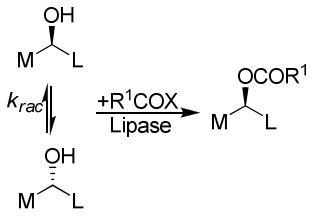
Scheme 11. Lipase-catalyzed hydrolysis combined with inversion of configuration for the synthesis of (*S*)-**33**[79]

The Mitsunobu reaction is another possibility which allows the inversion of configuration of an alcohol enantiomer.[80,83,84,85] The Mitsunobu reaction is an esterification of an alcohol in the presence of diethyl azodicarboxylate (DEAD), triphenylphosphine (PPh_3) and a carboxylic acid. The Mitsunobu reaction may be combined with a mixture obtained from a lipase-catalyzed acylation or deacylation containing an *R*-ester and an *S*-alcohol. When the carboxylic acid used in the Mitsunobu reaction is the same as the acid part of the ester in the resolved mixture, the initially racemic mixture can be again transformed into the same ester enantiomer. Thus, when lipase-catalyzed kinetic resolution by acylation was combined with the Mitsunubu inversion, *rac*-**(35-44)** were transformed into enantiopure esters (ee 82-99%) in good yields (70-99%) (Scheme 12). PPL[85] was used for the synthesis of the products **35** and **36**, lipase PS[85] for the synthesis of the ester **37** and CAL-B[84] for the synthesis of the esters **38**-**44**. In the case of compounds **35** and **36** the Mitsunobu reaction was achieved with butanoic acid, whereas in all other cases acetic acid was used.



Scheme 12. Isolated yields and ee values for esters obtained from racemic alcohols by combined kinetic resolution and Mitsunobu inversion[84,85]

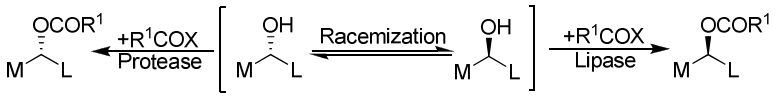
2.5.2. Dynamic Kinetic Resolution



Scheme 13. DKR

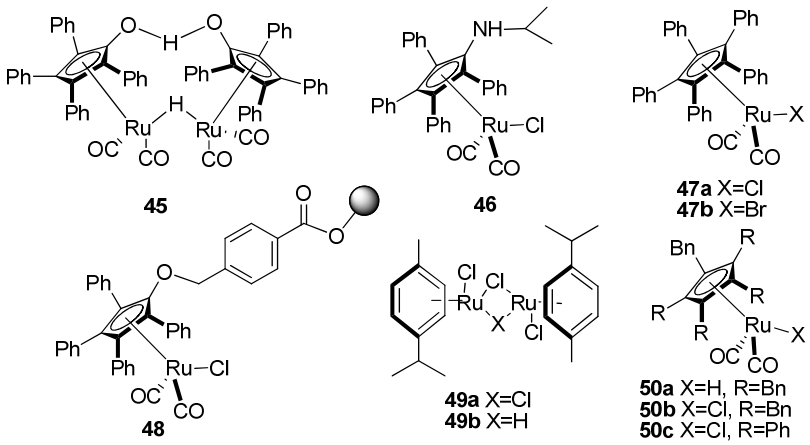
The lipase-catalyzed DKR of alcohols is used to transform kinetic resolution in more profitable processes, affording theoretical yields up to 100% for the fast reacting enantiomer by continuous racemization of the slower reacting enantiomer. In this case only one enantiomer is obtained based on the stereoselectivity of the lipase used. As a drawback, generally lipases manifest the same enantiopreference with secondary alcohols. When the other enantiomer is required an alternative synthetic route or another enzyme in place of a lipase has to be found. Kim and Park[86] and later the Bäckvall group[87] have performed DKR in the presence of proteases to transform a racemate into the opposite enantiomer to the one yielded by a lipase by transesterification (Scheme 14). However, the activity of proteases in organic solvents is often lower than that of lipases and the

substrate tolerance is not that broad. Thus, the scope of DKR with proteases is narrower than with lipases.



Scheme 14. Complementarity of proteases and lipases in DKR of alcohols

The DKR of alcohols obtained by combining lipase catalysis and transition-metal racemization has been under intense study during the last ten years and the area is thoroughly reviewed.[9,88,89] Different metal catalysts, including rhodium, iridium, ruthenium and aluminium are efficient for racemization of secondary alcohols as proved by Wiliams et al.[90] Racemization by transition metals involves a hydrogen transfer mechanism[91] with metal hydrides or dihydrides as key intermediates. Among various metal complexes available for rapid racemization[92,93] only few metal complexes, generally Ru-based complexes, have proved to be compatible with enzymatic resolution (Scheme 15).[94] A transition metal catalyst and an enzyme are very different in structure and origin. They have also different preferences concerning the operating conditions. Thus, it is a real challenge to make them to work efficiently under the same conditions, in the same reaction vessel. Transition metal complexes are generally air and moisture sensitive, and some of them require either temperature or a base to switch to activated forms. The reported formation of the corresponding ketones as side products may justify the low yields obtained in some cases by DKRs. As a consequence, a lot of attention has been paid to improve the DKRs by optimizing the transition metal complex and the kinetic resolution process.



Scheme 15. Transition metal catalysts used in tandem with enzymes for DKR of alcohols

Catalyst **45**, known as Shvo's catalyst,[95] was the first practical ruthenium catalyst applied for the DKR of alcohols by Bäckvall *et al.*[96] It requires thermal activation (70 °C). Thus, this catalyst was used to perform DKRs in combination with thermostable enzymes such as CAL-B and lipase PS. When Shvo´s catalyst was used in the presence of conventional acyl donors such as vinyl acetate or isopropenyl acetate, the products were isolated only with moderate yields (50% and 75%, respectively).[96] The low yield was caused by the oxidation of the substrate based on acetaldehyde

formed from vinyl acetate and on acetone formed from isopropenyl acetate during the enzymatic acylation. When the DKR was performed with *p*-chlorophenyl acetate, the yield of the transformation was increased because *p*-chlorophenol released from the acyl donor is not able to tautomerize like enols do. This acyl donor lades the purification process. The DKR of 1-phenylethanol using *p*-chlorophenyl acetate gave high yield (almost 100%). The scope of the Shvo's catalyst was successfully extended to functionalized alcohols such as diols,[97] hydroxy esters,[98] β-halo alcohols,[99] β-azido alcohols,[100] β-hydroxyalkanephosphonates[101] and β-hydroxy nitriles.[102,103]

Racemization catalysts **46**, **47a** and **47b** perform the racemization at RT but require base activation usually with potassium *tert*-butoxide. Polymer-supported catalyst **48** was developed by Kim and Park[104] to favor recycle and reuse. Catalysts **49a** and **49b** were efficiently employed for the racemization of allylic alcohols even at RT. DKR assisted by these catalysts was developed for aliphatic and aromatic alcohols in the presence of lipase PS.[105] The most recent catalysts are Ru-complexes **50(a-c)**.[106] Their benefits are easy and green preparation and higher air stability when compared to the Bäckval´s catalysts. These catalysts were used to perform the DKR of 1-phenylethanol and 1-(furan-2-yl)ethanol in the presence of CAL-B. The corresponding *R*-acetates were obtained with enantiopurities higher than 97% at 90-95% yields.[107]

2.6. Chemoenzymatic Synthesis of Active Pharmaceutical Intermediates

Chirality in drug synthesis receives special attention due to the implications of different biological activities exhibited by the enantiomers of chiral molecules (see 2.1.). A survey done in 2006 for the syntheses of 128 drug candidate molecules under development in the Process Chemistry R&D departments of GlaxoSmithKline, AstraZeneca and Pfizer revealed that 69 drug molecules contained at least one asymmetric center. Only two chiral drug molecules out of 69 were developed as racemates.[15] In the case of racemic drugs available already on the market, the switch to single-isomer drugs offers the possibility to extend the patent lifetime.

Already in 1984 Ariëns underlined that the neglect of stereochemistry in clinical pharmacology leads to the performance of expensive 'highly sophisticated scientific nonsense.'[108] In 1992, the US Food and Drug Administration (FDA) published a policy statement for the development of new stereoisomeric drugs,[109] followed by similar European guidelines in 1993.[110] These statements made clear that approval of drugs with more stereoisomers is possible only if pharmacokinetic and pharmacodynamic properties of each stereoisomer are fully assessed. Racemic drugs may still be approved if the choice of a racemate versus a single-enantiomer formulation is justified. The advantage of a single stereoisomer drug is a superior therapy which could be accomplished by reduction in dose, reduced variability in metabolism and response and improved tolerability.[111]

As already stated biocatalysis is regarded as an attractive tool for pharmaceutical sector in the synthesis of new drug molecules. The complexity of small APIs is increasing continuously regarding the number of different functional groups and number of asymmetric centers. The properties of biocatalysts such as regio- and stereoselectivity are useful to develop synthetic routes under mild conditions and with reduced number of steps when protection/deprotection steps are avoided. Academic research has already proved that many intermediates for drug synthesis may be accessed by enzymatic routes with high selectivity. The challenge is to transfer the knowledge from the laboratory bench to the production plant and to integrate it into the overall chemical synthesis of a drug molecule. The scale and methods involved are completely different.

The development of a new drug is a costly process and limited by time due to the necessity to perform clinical testing. From the synthetic point of view development of a new drug includes three stages.[112] In the drug discovery stage the intermediates are required in quantities ranging from 1 mg to 100 g. The volumetric productivity and costs are not considered at this stage. The only requirement is to be fast. In this stage enzyme catalysis may be easily included especially when

libraries of enzymes are commercially available for intensive screening. The both enantiomers for a chiral drug candidate are required now for testing and lipase-catalyzed kinetic resolution is a useful method which produces the both enantiomers. In the process development stage quantities between 1-100 kg of a drug preparation are required while in the manufacturing stage ton scale preparation may be required. The synthesis costs dominate the last two stages, so that high productivity is desirable. In the scale-up phase pharmaceutical industry faces the challenge to develop 'greener processes', by reducing synthetic steps, by increasing the degree of use of raw materials and by reducing the energy-intensive processes and operations harmful for the environment. Enzymatic catalysis may contribute to achieve this task.

Excellent reviews are available for applications of biocatalysis for the synthesis of chiral pharmaceutical intermediates.[113,114,115] The examples which follow below are selected to illustrated the potential of the lipase-catalyzed kinetic resolution of secondary alcohols through acylation and that of the corresponding esters through alcoholysis as an enantiopurity producing step of chemoenzymatic synthesis. The kinetic resolution step is shown in the boxes of Schemes 16-24.

<u>Case 1</u> – Paclitaxel (Taxol)

Paclitaxel (**51**) is one of the few drugs successfully used in cancer chemotherapy. It is a drug with a long history and many controversial discussions are associated with its name. This drug was approved by FDA for the treatment of ovarian and breast cancer. Paclitaxel was first isolated in 1967 from the Pacific Yew Tree (*Taxus brevifolia*) and named 'taxol'. Bristol-Myers Squibb has obtained the rights to produce commercially taxol with the same trade name but with the generic name of paclitaxel. The limiting amount of the active component found in yew tree promoted the efforts to synthetize taxol. Skilful chemists have achieved the total syntheses of paclitaxel, but the syntheses developed have only theoretical relevance. The compound 10-deacetyl baccatin-III (**52**, Figure 6) isolated from the fresh leaves of European Yew (*Taxus baccata*) in relatively high concentrations (approx. 1g/kg) proved to be a good candidate for the semi-synthesis of taxol. Paclitaxel is about 1000 times more active than Baccatin III. The structural difference between the two molecules consists of the C-13 side chain moiety existing in Paclitaxel. The side chain of taxol, (2*R*,3*S*)-3-phenylisoserine, is a hydroxy-β-amino acid which challenged the skills of the chemists in the search of a suitable synthetic method.

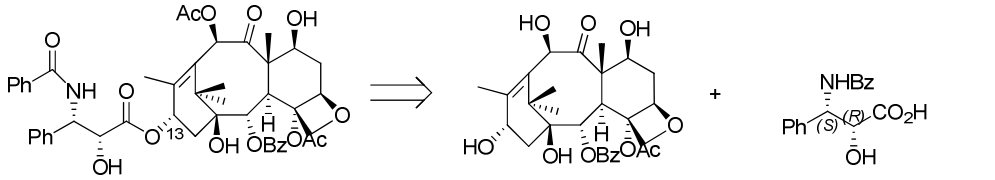


Figure 6. Retrosynthesis of Paclitaxel

Borah *et. al.*[116] have reviewed the synthetic routes towards the C-13 side-chain of taxol. Chemical methods such as asymmetric epoxidation, asymmetric dihydroxylation, inverse electron demand Diels-Alder reaction, enol-imine condensation, chiral auxiliary based strategies and asymmetric catalyst-based methods have been applied. Numerous enzymatic routes towards the C-13 side chain of taxol were also developed, and among them lipase-catalyzed reactions hold an important place. The substrates available for lipase catalysis are *trans*-phenylglycidic esters **54**, 3-hydroxy-4-phenyl-β-lactam derivatives **55(a-c)** and **56(a-c)**, 3-chloro-2-hydroxy-3-

phenylpropanoate **57**, 3-(benzyloxy)-1-nitro-1-phenylpropan-2-ol **58** and 2,3-dihydroxy-3-phenylpropionic acid methyl ester **59** (Figure 7).

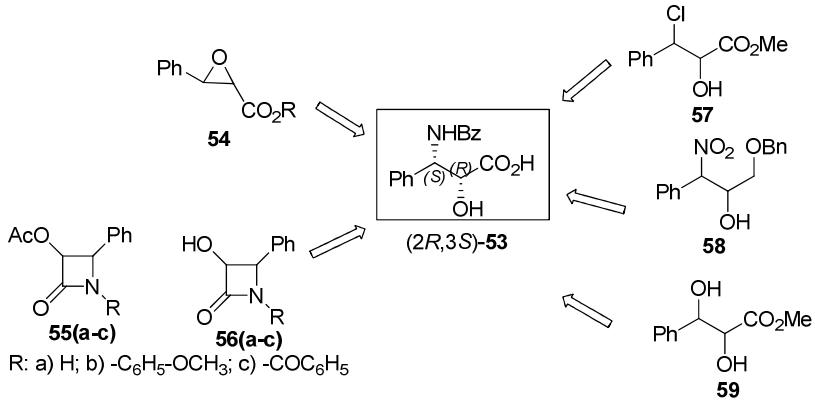
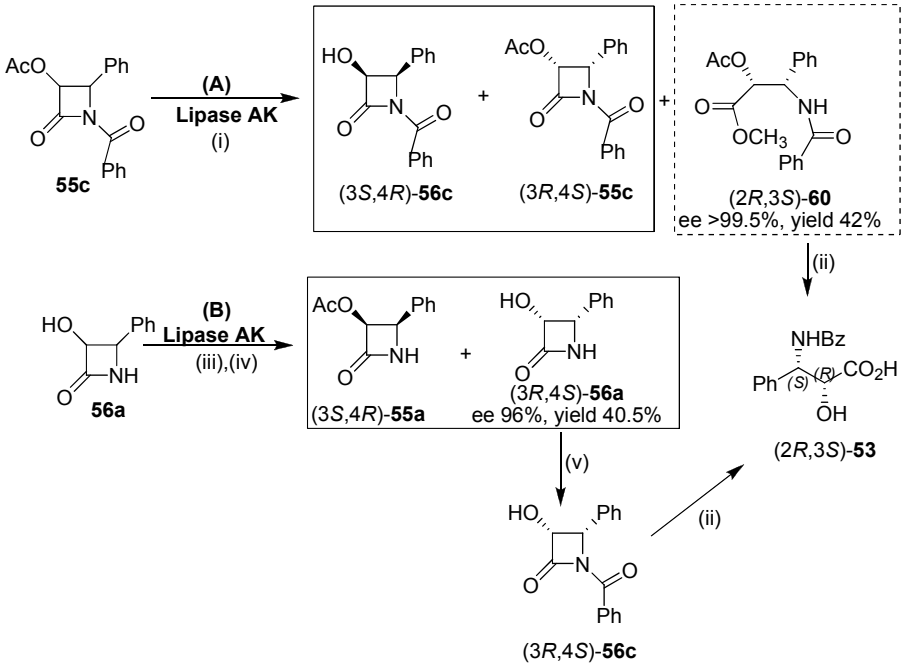


Figure 7. Substrates for synthesis of (2*R*,3*S*)-3-phenylisoserine **53**

Brieva *et al.*[117] have developed methods towards enantiopure 3-hydroxy-4-phenyl β-lactam derivatives **55(a-c)** and **56(a-c)** based on lipase catalysis. Lipase AK was an enantioselective catalyst for the alcoholysis of **55c** (Scheme 16, route A) and also for the acylation of **56a** (Scheme 16, route B). In the case of alcoholysis, lipase AK catalyzed also the cleavage of the β-lactam ring with a high degree of enantioselectivity to yield (2*R*,3*S*)-**60**, which after the hydrolysis of the methyl ester gave (2*R*,3*S*)-**53**. In the case of the lipase AK-catalyzed acylation of **56a**, the desired intermediate (3*R*,4*S*)-**56a** was obtained with 96% ee and 40.5% yield by crystallization from $CHCl_3$. (3*R*,4*S*)-**56a** was converted to (2*R*,3*S*)-3-phenylisoserine after benzoylation and basic hydrolysis.



Reagents and conditions: (i) 5 equiv. CH_3OH, 50 ^oC, lipase AK, TBME; (ii) NaOH; (iii) VA, TBME lipase AK; (iv) crystalization; (v) PhCOCl.

Scheme 16. Lipase catalysis of β-lactam derivatives in the synthesis of the C-13 taxol side-chain[117]

Lipase catalysis was recently applied also to resolve different substituted β-lactam derivatives **61-63** (Figure 8) as an attempt to develope new generation anlogues of Taxol with increased efficiency.[118]

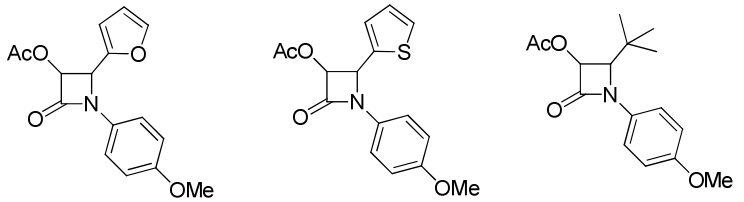
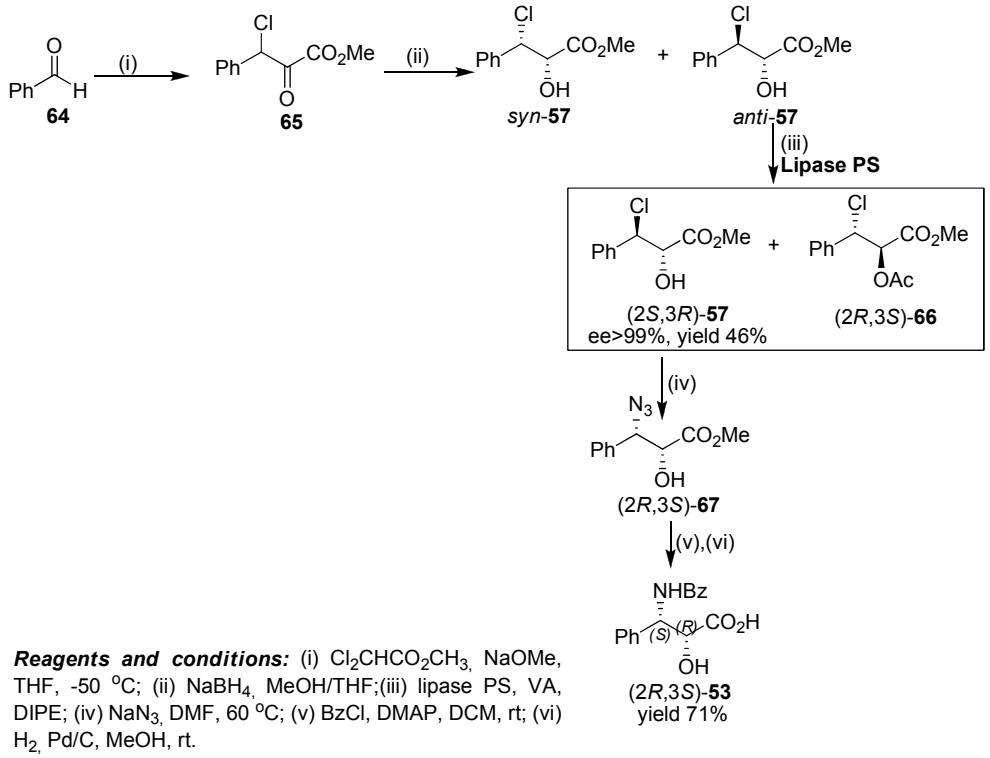


Figure 8. β-lactam derivatives useful for synthesis of new generation analogues of Taxol[118]

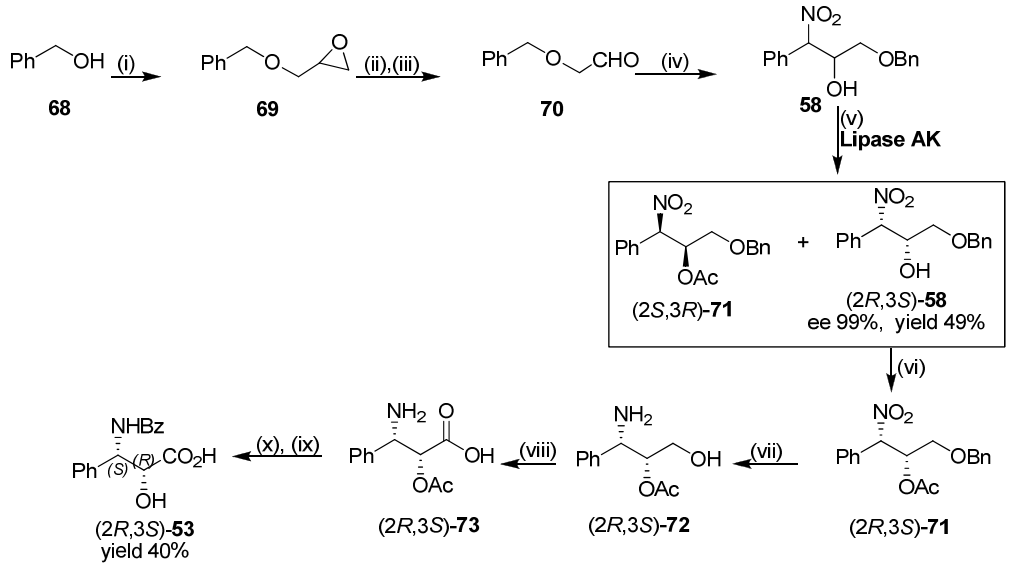
Hamamoto *et. al.*[119] have used 3-chloro-2-hydroxy-3-phenyl propanoate *anti*-**57** for the synthesis of the C-13 taxol side-chain. The intermediate **57** was than obtained from benzaldehyde via Darzen condensation with dichloroacetate followed by reduction with $NaBH_4$. The alcohols of s*yn*-**57** and *anti*-**57** obtained by reduction were separated by column chromatography. In Scheme 17, (2*S*,3*R*)-**57** obtained as the unreacted alcohol enantiomer in the lipase PS-catalyzed acylation of *anti*-**57** with vinyl acetate is used for the synthesis of taxol side-chain. The azide displacement of

(2*S*,3*R*)-**57** achieved the inversion of configuration at C-3 and finally benzoylation, followed by hydrogenation gave the taxol side-chain with 71% yield. Lipase PS can be also used for the acylation of *syn*-**57** with *E*>100 and (2*R*,3*R*)-**66** obtained by acylation can be also converted into (2*R*,3*S*)-**53.**



Scheme 17. Lipase catalysis of 3-chloro-2-hydroxy-3-phenylpropionate for the synthesis of the C-13 taxol side-chain[119]

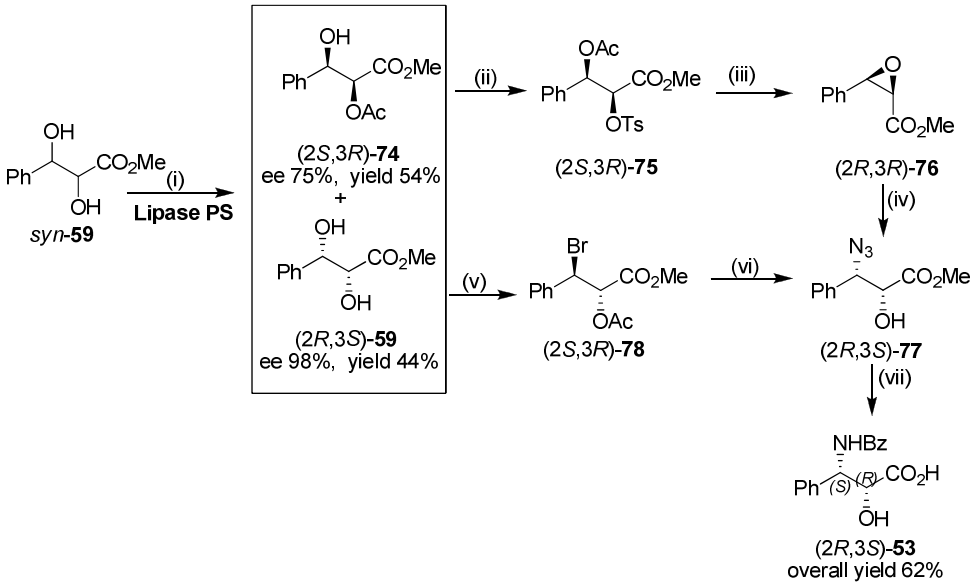
Gogoi *et. al.*[120] have applied lipase-catalyzed acylation of 3-(benzyloxy)-1-nitro-1-phenylpropan-2-ol **58** for the synthesis of the taxol side-chain (Scheme 18). The lipase AK-catalyzed kinetic resolution of **58** with vinyl acetate in hexane gave the product (2*S*,3*R*)-**71** (ee 98.5% and yield 44%) and the unreacted substrate (2*R*,3*S*)-**58** (ee 99% and yield 49%). (2*R*,3*S*)-**58** was converted to the C-13 taxol side chain with an overall yield of 40%.



Reagents and conditions: epichlorohydrin, 50% aq. NaOH, TBAB, <25 °C; (ii) 30% $HClO_4$, Et_2O, rt; (iii) $Pb(OAc)_4$, DCM, 0 °C; (iv) $C_6H_5CH_2NO_2$, THF, -50 °C; (v) lipase AK, VA, hexane; (vi) Ac_2O/Py; (vii) 10% Pd/C, Et_2O, H_2, rt; (viii) CrO_3, CH_3COOH glacial , H_2O, 5 °C; (ix) BzCl, Et_3N, 0 °C; (x) Et_2NMe-H_2O.

Scheme 18. Lipase catalysis of 3-(benzyloxy)-1-nitro-1-phenylpropan-2-ol for the synthesis of the C-13 taxol side-chain[120]

Lee and Kim[121] have used lipase-catalyzed acylation of *syn*-2,3-dihydroxy-3-phenyl-propanoic acid methyl ester, *syn*-**59**, to obtain enantiopure intermediates in the synthesis of the taxol side chain (Scheme 19). The advantage of this method is that the both enantiomers obtained by lipase acylation are used in the synthesis of the taxol side chain, contributing in this way to a higher overall yield. The lipase PS-catalyzed acylation of *syn*-**59** with vinyl acetate gave the monoacetylated product (2*S*,3*R*)-**74** with 75% ee and 54% yield and the unreacted enantiomer (2*R*,3*S*)-**59** with 98% ee and 44% yield. The lower enantiopurity obtained for (2*S*,3*R*)-**74** was increased after tosylation by single crystallization.



Reagents and conditions: (i) lipase PS, VA; (ii) TsCl, Et_3N; (iii) *p*-TsOH, MeOH, K_2CO_3; (iv) NaN_3; (v) HBr-AcOH; (vi) a)NaN_3, b) AcONa; (vii)a) BzCl, Et_3N, b) H_2/Pd-C.

Scheme 19. Lipase catalysis of *syn*-1,2-diols for the synthesis of the C-13 taxol side-chain[121]

Case 2 – 'Bisfuran Alcohol'

'Bisfuran Alcohol**' -** (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-b]furan-3-ol - is an important building block for the synthesis of HIV protease inhibitor candidates (Figure 9). Gilead Sciences have developed HIV protease inhibitor GS-9005. In this project, the synthesis of the enantiopure bisfuran alcohol was achieved by the aid of lipase catalysis (Scheme 20).[122] Different methods have been previously applied for the synthesis of bisfuran alcohol **79,** including methods based on radical cyclization and ozonolysis as well as methods based on a photochemical step which unfortunately is difficult to scale-up.

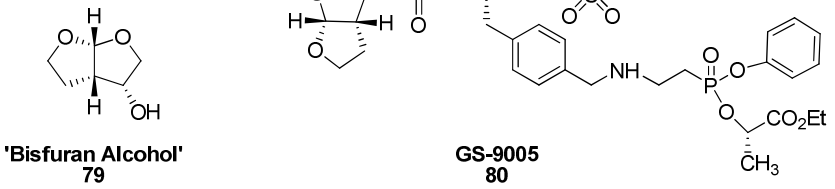
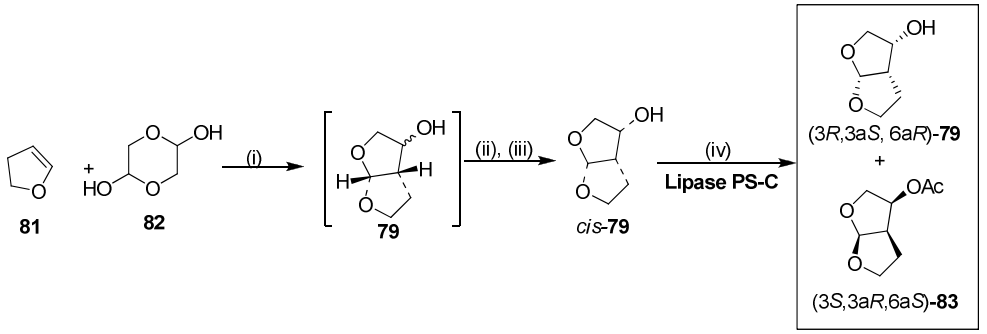
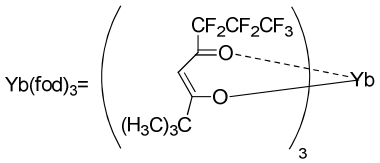


Figure 9. Bisfuran Alcohol and GS-9005

Yu *et al.*[122] have synthesized racemic bisfuran alcohol by condensation of 2,3-dihydrofuran with commercially available glycolaldehyde dimmer in the presence of a lanthanide catalyst $Yb(fod)_3$. The drawback of ytterbium catalyst is a weak control of diastereoselectivity, yielding the diastereomeric *cis*/*trans* ratio 65:35. The control of diastereoselectivity was achieved by performing successive oxidation/reduction of *rac*-**79** which gave the diastereomeric ratio of 95:5 in the favor of *cis*-**79**. The oxidation of **79** was performed with sodium hypochloride and TEMPO, and the reduction was done with sodium borohydride in ethanolic solution. The enzymatic acylation of *cis*-**79** was done with acetic anhydride in 1,2-dimethoxyethane (DME) in the presence of lipase PS-C. The desired enantiomer for the synthesis of HIV inhibitor candidates is the unreacted enantiomer of the resolution mixture, which may be isolated with 99% ee and with 28-35% overall yield at laboratory scale. Lipase-catalyzed kinetic resolution performed poorly in the pilot plant (ee 70% for unreacted enantiomer at 50% conversion). Finally, the kinetic resolution of *cis*-**79** was performed at 4 kg scale (ee 97.2 %, yield 33% for the desired enantiomer) using a loop reactor and controlling the water uptake in the highly hygroscopic DME.



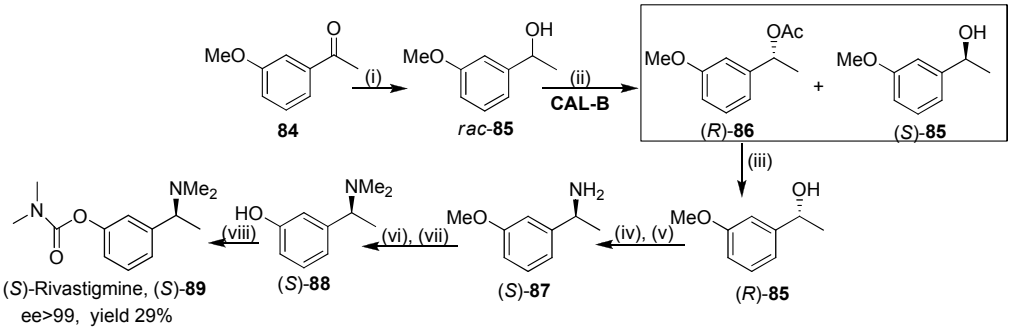
Reagents and conditions: (i) $Yb(fod)_3$ (cat.), rt to 50 ºC; (ii) NaClO, TEMPO; (iii) $NaBH_4$, EtOH; (iv) lipase PS-C, Ac_2O, DME.



Scheme 20. Synthesis of 'bisfuran alcohol'[122]

Case 3 – Rivastigmine

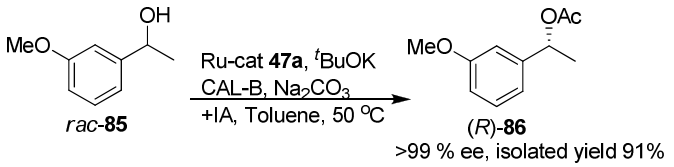
Rivastigmine is a drug used in the treatment of mild to moderate dementia of the Alzheimer´s type and is marketed as a tartrate salt under the name of Exelon. The activity of this drug is based on the (*S*)-enantiomer. Rivastigmine was first synthesized by resolution of *rac*-**89** with (+)-di-*O*,*O*´-*p*-toluoyl tartaric acid monohydrate. A chemoenzymatic synthesis strategy, developed at laboratory scale, was recently proposed for the preparation of (*S*)-rivastigmine using a racemic secondary alcohol, *rac*-1-(3-methoxyphenyl)ethanol, *rac*-**85** as a key intermediate to introduce the enantiopurity into the molecule (Scheme 21).[123]



Reagents and conditions: (i) $NaBH_4$/MeOH; (ii) CAL-B, VA, TBME; (iii) K_2CO_3, MeOH-H_2O, rt; (iv) Phthalimide, PPh_3, DEAD, THF, rt; (v) $N_2H_4 \cdot H_2O$, THF-EtOH, 66 °C; (vi) (a) HCHO, HCO_2H, 100 °C or (b) HCHO, $NaBH(OAc)_3$, Na_2SO_4, rt; (vii) HBr 48% aq., 100 °C; (viii) NaH, ClCON(Et)Me, CH_2Cl_2, 0 °C to rt.

Scheme 21. Chemoenzymatic synthesis of (*S*)-Rivastigmine[123]

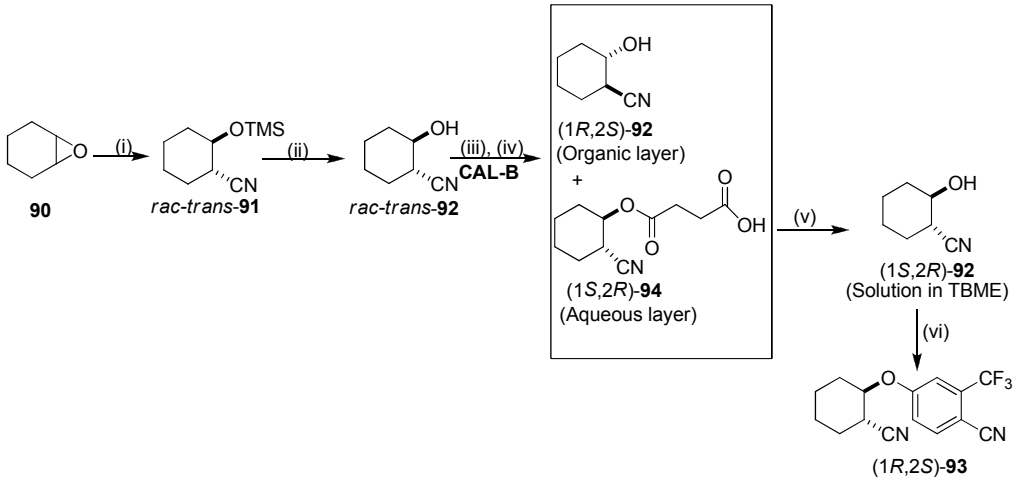
For the acylation of *rac*-**85** with vinyl acetate in TBME, CAL-B showed *E*>200. The CAL-B-catalyzed acylation of *rac*-**85** was combined with Ru catalyst **47a** for *in-situ* racemization of the unreacted enantiomer to develop a DKR process (Scheme 22). DKR afforded (*R*)-**86** with high enantiopurity (>99%) and isolated yield (91%). The synthesis of the amine (*S*)-**87** involves deprotection of (*R*)-**86** and inversion of configuration by the reaction with phthalimide, triphenylphosphine and diethyl azodicarboxylate (DEAD) in dry THF. Enzymatic kinetic resolution of racemic amine *rac*-**87** was also considered but the resolution suffered of low reaction rates compared to the acylation of *rac*-**85**.



Scheme 22. DKR for *rac*-**85**

Case 4 – Androgen receptor antagonist

A chemoenzymatic synthesis was developed as a second-generation process for the synthesis of the androgen receptor antagonist (1*R*,2*S*)-**93** used for the treatment of both alopecia and excess sebum of oily skin (Scheme 23).[124] The first-generation synthesis involved chiral column chromatography purification, which is not an optimal step for large-scale synthesis. An enzymatic kinetic resolution approach was considered as an attractive way to obtain enantiopure *trans*-2-cyanocyclohexanol (**92**)**.** Lipase PS was previously applied for the acylation with vinyl acetate in DIPE of *trans*- and *cis*-2-cyanocyclopentanol and 2-cyclohexanol.[125]



Reagents and conditions: (i) TMSCN, $LaCl_3$; (ii) AcOH; (iii) succinic anhydride, CAL-B, TBME, 40-50 °C; (iv) TBME/K_2HPO_4 extractions; (v) NaOH, TBME/HCl (dil.) extractions; (vi) 4-fluoro-2-(trifluoromethyl)benzonitrile, LiOH, DMF.

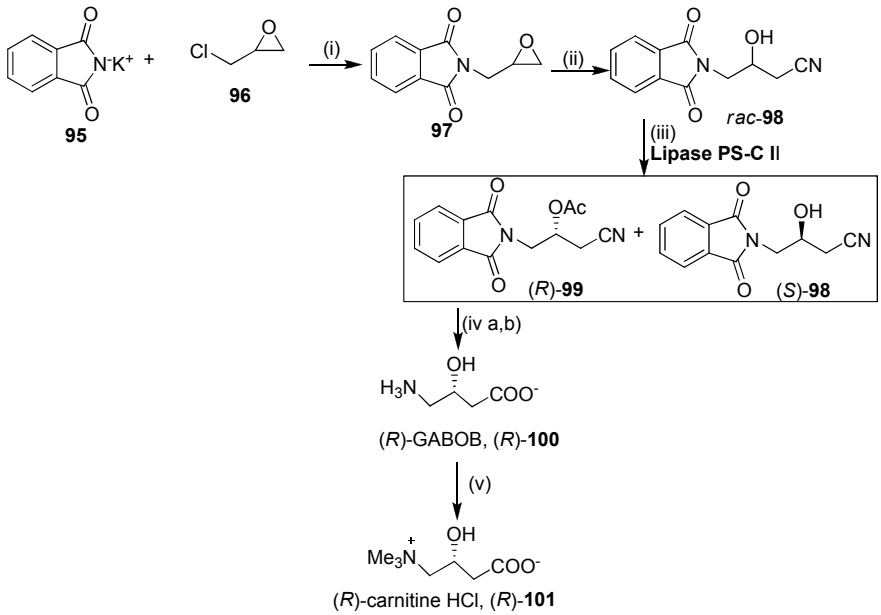
Scheme 23. Chemoenzymatic synthesis of the androgen receptor antagonist, (1*R*,2*S*)-**93**[124]

In the chemoenzymatic synthesis of (1*R*,2*S*)-**93,** succinic anhydride was preferred as an acyl donor for the kinetic resolution of *rac-trans*-**92**. As already stated (page 27) the use of succinic anhydride has the benefit to produce the corresponding monoester that can be separated from the resolution mixture by basic extraction. In this way, column chromatography separation of the resolution products is avoided. The lipase selected after enzyme screening was CAL-B as a commercial preparation Novozym 435. Under optimized conditions, the enzymatic acylation in TBME at 40 °C furnished the product with 94-95% ee and with conversion 47-49% within 30 h.

The second-generation chemoenzymatic synthesis of (1*R*,2*S*)-**93** was scaled up in a pilot plant to produce over 10 kg of API. In addition to the enzymatic step, the second generation manufacturing process for (1*R*,2*S*)-**93** included arylation step performed with LiOH/DMF instead of LiH for which exist safety concerns for the use in large scale synthesis. The isolated yield for the androgen receptor antagonist obtained was ca. 50% and enantiopurity higher than 99.9%.

Case 5 – (*R*)-carnitine

Lipase PS-C II-catalyzed acylation of *rac*-**98** was integrated in the chemoenzymatic synthesis of γ-amino-β-hydroxybutyric acid (GABOB) which acts as neuromodulator in the mammalian nervous system and is a precursor of (*R*)-carnitine (Scheme 24).[126] (*R*)-Carnitine has an important role in fatty acid metabolism for converting stored body fat into energy. Moreover, (*R*)-carnitine has other clinical applications, like in the treatment of myocardial ischaemia and seizure.[127] The reported approaches towards carnitine include asymmetric synthesis and chemical and enzymatic resolution. The method described here applies lipase catalysis to racemic β-hydroxy nitrile **98** as an enantioselective step. *Rac*-**98** was obtained by the treatment of the epoxide **97** with NaCN in ethanol. Lipase PS-C II-catalyzed kinetic resolution of *rac*-**98** in toluene with vinyl acetate produced the alcohol (*S*)-**98** with ee over 99% and the acetate (*R*)-**99** also with ee higher than 99% at 50% conversion. The acetate ester obtained by lipase catalysis was hydrolyzed first to the corresponding alcohol (*R*)-**98** and than to (*R*)-GABOB which was converted by methylation to (*R*)-carnitine (*R*)-**101**.



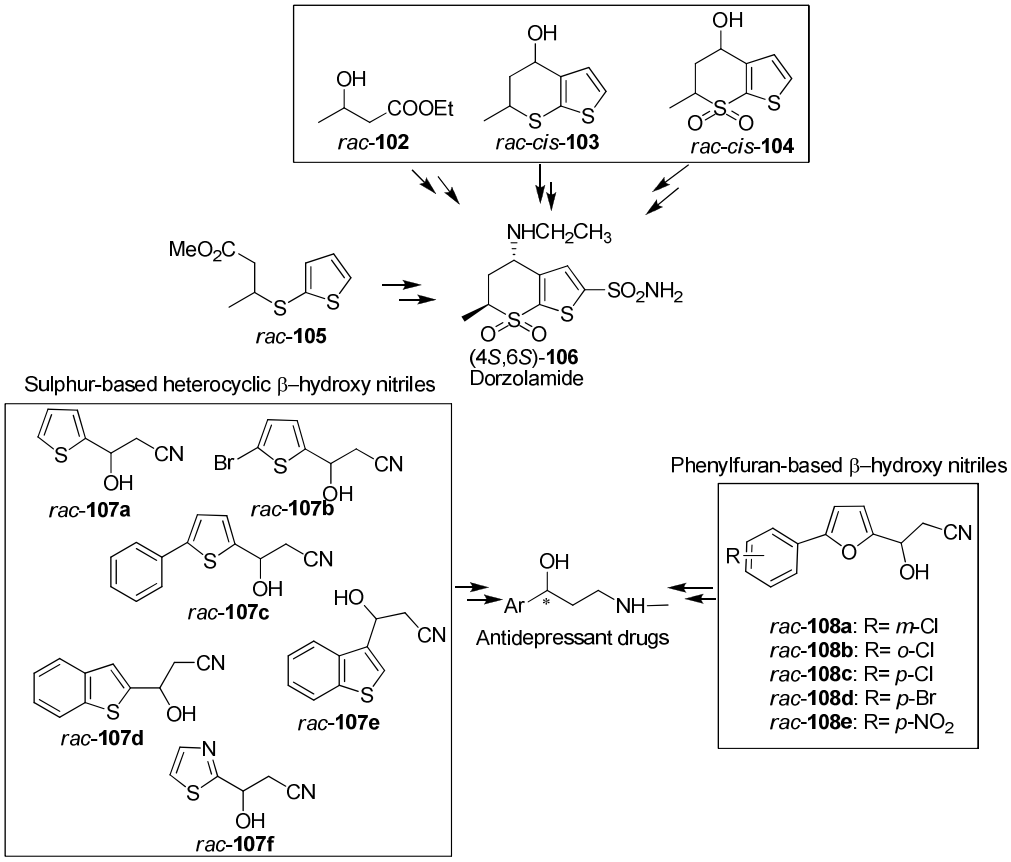
***Reagents and conditions*:** (i) DMF, reflux; (ii) NaCN, EtOH - H2O; (iii) Lipase PS-C II, VA, toluene/chloroform (8:2); (iv)(a) aqueous NH_3 (25%), MeOH, 0 ^oC to rt; (b) concentrated HCl, reflux 10 h; (v) MeI.

Scheme 24. Chemoenzymatic synthesis of (*R*)-carnitine[126]

3. Aim of the Study

The target drugs in the experimental work of this thesis have been Dorzolamide (4*S*,6*S*)-**106** and drugs with 1-aryl-3-methylaminopropan-1-ol structure (Scheme 25). Dorzolamide is a drug used for the treatment of glaucoma, a disease of the eye. Drugs containing 1-aryl-3-methylaminopropan-1-ol structure are efficient in the treatment of depression. The aim was to introduce the enantiopurity into the intermediates or potential intermediates in the synthetic pathways of these drugs by lipase-catalyzed kinetic resolution.

1) Alcohols *rac*-**102-104** and an ester *rac*-**105** were studied as intermediates for the synthesis of Dorzolamide. The purpose was to prepare all four stereoisomers of *rac*-**103** and the enantiomers of *rac*-**102** and *rac-cis*-**104** using lipase-catalyzed kinetic resolution. Lipase catalysis was used to obtain the both enantiomers of the racemates. The work is described in papers I and II.
2) Heterocyclic β-hydroxy nitriles *rac*-**107(a-f)** and *rac*-**108(a-e)** were studied as potential novel intermediates for the synthesis of antidepressant drugs with 3-methylamino-1-arylpropan-1-ol structure. The reduction of the nitrile group in the case of compounds **108(a-e)** with $NaBH_4$ was studied in order to transform the nitriles into γ-amino alcohols. The work is described in papers III and IV.
3) To develop chromatographic methods to detect enantiopurity of the compounds involved has been a challenge in the works.

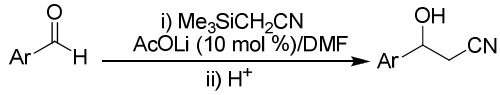


Scheme 25. Molecules under study

4. Materials and Methods

4.1. Chemical Synthesis and Characterization of Racemates

Substrate *rac*-**102** was commercially available from Sigma-Aldrich, while substrates *rac*-**103-105** were generous gifts from PCAS Finland Oy. Racemic β-hydroxy nitriles were synthesized from commercially available aldehydes by cyanomethylation in the presence of lithium acetate as a Lewis base (Scheme 26). The exact procedure is described in the original papers III & IV. The progress of synthesis was monitored by analytical thin layer chromatography (TLC) on Merck Kieselgel $60F_{254}$ sheets. Preparative chromatographic purifications of the synthetic products were performed using column chromatography on Merck Kieselgel 60 (0.063-0.200 μm).



Scheme 26. Synthesis of racemic β-hydroxy nitriles

The characterization of new molecules produced was done by NMR Spectroscopy and MS Spectrometry. HRMS spectra were recorded on a ZabSpec-oaTof instrument. The solution state NMR spectra were recorded on a Brucker Avance 500 spectrometer equipped with BBO-5mm-Zgrad probe operating at 500.13 and 125.77 MHz, respectively. Tetramethylsilane (0.00 ppm) was a reference for both ^{1}H and ^{13}C spectra. All NMR spectra were recorded at +25 ^{o}C. The correct assignment of chemical shifts was confirmed when necessary by application of two-dimensional correlation measurements as ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, NOESY or by one dimensional 1D-NOESY measurements. Melting points were recorded with Gallenkamp apparatus.

4.2. Analytical Methods for Monitoring Enzymatic Reactions

The development of chiral chromatographic technologies sustained the progress achieved in chemical and enzymatic asymmetric syntheses. The description of analytical methods in original publications is rather short, limited to few sentences in the Experimental Sections. In most cases the development of good analytical methods has been a time-consuming procedure.

High performance liquid chromatography (HPLC) and gas-chromatography (GC) with chiral columns were employed for direct separation of the enantiomers and quantification of enantiomeric excesses. Information about the chiral columns used in this thesis is presented in Table 9. GC analyses were performed with an Agilent 6890 Series II Chromatograph equipped with a flame ionization detector (FID) and with autosampler for automatic injection of the samples. The carrier gas used was nitrogen. HPLC analyses were performed with an Agilent HP 1090 Chromatograph equipped with diode-array UV detector and autosampler. Normal phase HPLC analyses were performed varying the content of isopropyl alcohol in hexane as an eluent according to the compound under analysis.

In Figure 10, GC chromatograms for the analysis of *rac*-**107a** and its lipase-catalyzed acylation products are presented. The analysis was made by a GC equipped with CycloSil-B column operated at 160 ^{o}C. In Figure 10a the separated enantiomers of *rac*-**107a** are seen as two peaks with equal areas and at the retention times t_{r1}=32.01 min and t_{r2}=35.13 min. Base-line separation was achieved also for the enantiomers of acetate ester of *rac*-**107a**, the rention times for the peaks being t_{r1}=13.83 min and t_{r2}=14.27 min (Figure 10b). Figure 10c shows the chromatogram for a sample taken from enzymatic kinetic resolution of *rac*-**107a** with vinyl acetate.

Table 9. Chiral columns used in the experimental work

Chiral HPLC columns	Stationary Phase	Compounds analyzed
Daicel Chiralcel OD (250 x 46 mm ID)	Cellulose tris (3,5-dimethylphenylcarbamate), coated on 10 μm silica gel	105
Daicel Chiracel OD-H (250 x 46 mm ID)	Cellulose tris (3,5-dimethylphenylcarbamate), coated on 5 μm silica gel	103, 104, 107(b-f), 108(a-e)
Chiral GC columns	**Stationary Phase**	**Compounds analyzed**
Supelco Beta Dex™ 120 (30 m x 0.25 mm x 0.25μm)	20% permethylated β-cyclodextrin in SPB-35 poly(35%phenyl/65% dimethylsiloxane)	103
CP Chirasil-DEX CB (25 m x 0.25 mm)	Permethylated β-cyclodextrin directly bonded to dimethylpolysiloxane	102, 104
CycloSil-B (30 m x 0.32 mm x 0.25μm)	30% heptakis (2,3-di-O-methyl-6-O-*t*-butyl dimethylsilyl)-β-cyclodextrine in DB-1701	107a

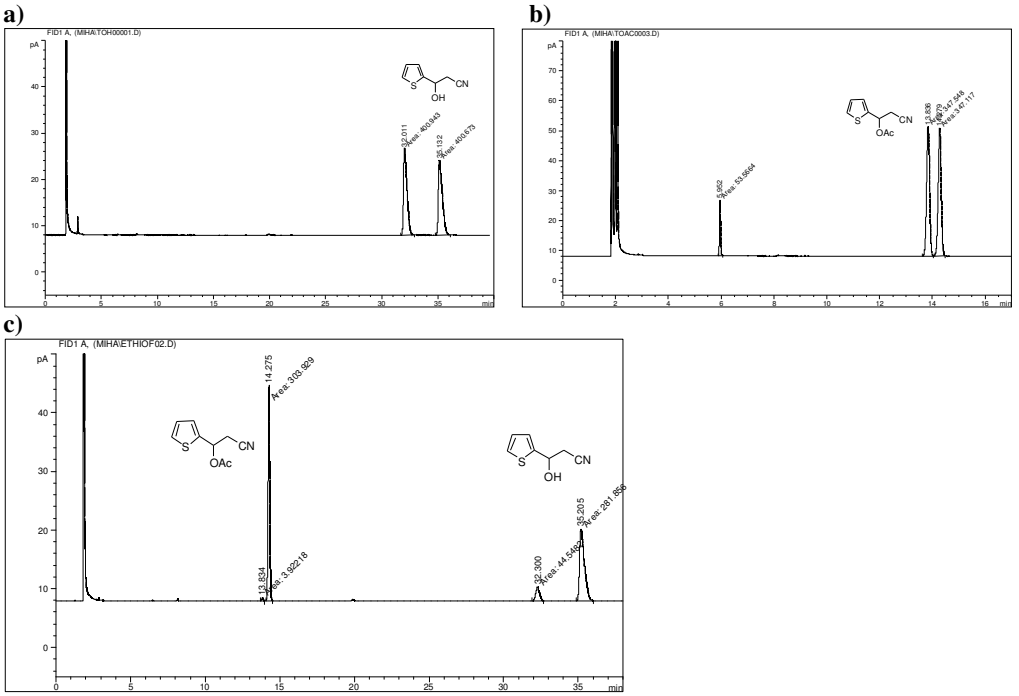


Figure 10. GC Chromatograms obtained for: **a)** *rac*-**107a**; **b)** the acetate of *rac*-**107a**; **c)** the sample taken from enzymatic acylation of *rac*-**107a**

4.3. Mathematical Equations Used in Kinetic Resolution

Conversion (c) and enantiomeric ratio (*E*) for a kinetic resolution were calculated using ee_S and ee_P obtained from GC or HPLC chromatograms and equations (1)-(4).[128]

$$c = \frac{ee_S}{ee_S + ee_P} \quad (1)$$

$$E = \frac{\ln \frac{(1 - ee_S)}{(1 + ee_S / ee_P)}}{\ln \frac{(1 + ee_S)}{(1 + ee_S / ee_P)}} \quad (2) \qquad E = \frac{\ln((1-c)(1-ee_S))}{\ln((1-c)(1+ee_S))} \quad (3) \qquad E = \frac{\ln(1-c(1+ee_P))}{\ln(1-c(1-ee_P))} \quad (4)$$

Equations (1) and (2) are usable when the enantiopurities of the substrate (ee_S) and product (ee_P) are determined simultaneously from the same sample withdrawn from an enzymatic reaction. Equations (1)-(4) are not valid in the case of equilibrium reactions or when side reactions occur. Mathematical equations to describe reversible resolutions include the apparent equilibrium constant of the process. The case is not considered here due to the fact that equilibrium reactions will lead to low enantiopurities for the resolved products.

4.4. Optimization of Enzymatic Reactions

Optimizations for kinetic resolutions were performed in 1-2 ml scale using 0.05 M or 0.1 M racemates. In a typical screening procedure for enzymatic acylation, a racemate (0.05 M or 0.1 M) was dissolved in an organic solvent and the lipase preparation was added. The reaction was started by adding the acyl donor (1-2 equiv. to the racemate). The reactions proceeded by shaking at 170 rpm usually at room temperature (23-24 ^{o}C) if not otherwise stated. The progress of the reactions was followed by removing samples (100 μL) at intervals, filtering off the enzyme, and analyzing the samples by chiral GC or HPLC. In some cases derivatization of the free alcohol enantiomer in the sample was needed in order to get the enantiomers separated in a chromatogram. Derivatization was usually performed with acetic, propionic or butanoic anhydride in the presence of catalytic amount of pyridine containing DMAP (1%) which gave an acylated product other than the one produced by enzymatic acylation.

4.5. Preparative-scale Enzymatic Reactions

Preparative-scale enzymatic reactions were performed using 0.5-5 g of a racemic substrate. The reactions were performed under optimized conditions selected based on the results obtained by screenings. The resolution products (one enantiomer as an ester and the other as an alcohol) were usually separated from the reaction mixture by column purification with Merck Kieselgel 60 (0.063-0.200 μm) and chemically characterized (MS and NMR).

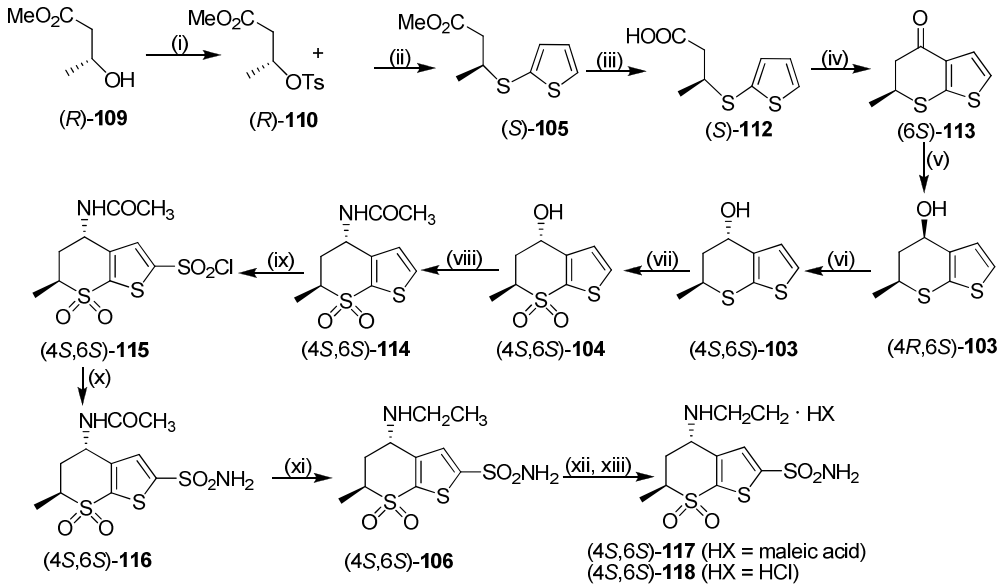
Optical rotations of the prepared enantiomers were measured using Perkin Elmer 341 Polarimeter against the sodium D line, the values for $[\alpha]_D^{25}$ being in units of 10^{-1} deg cm^{-2} g^{-1}.

5. Results and Discussion

In this section a summary of the results obtained experimentally is presented. More detailed discussions are found in the original publications (Papers I-IV). As stated before, the main target is to obtain enantiopure secondary alcohols by lipase catalysis. The secondary alcohols subjected to lipase catalysis include an aliphatic secondary alcohol *rac*-**102**, biciclyc secondary alcohols *rac-cis*-**103** and *rac-cis*-**104** with the HO function attached at the thiopyran ring and heterocyclic bifunctional secondary alcohols, *rac*-**107**(**a-f**) and *rac*-**108**(**a-e**), thus an enantioselective lipase has to be found in every case studied. The substrates alcohols are intermediates or have potential as intermediates for drug synthesis. The method used for the preparation of the both enantiomers of the alcohols is kinetic resolution. The optimization of the kinetic resolution in the quest of high enantiopurity is made according to the description from Literature Review.

5.1. Lipase Catalysis Applied to Intermediates of Dorzolamide (Papers I & II)

Dorzolamide hydrochloride is the drug commercialized by Merck laboratories under the name of Trusopt. It acts as a carbonic anhydrase inhibitor, thus lowering intraocular pressure in open-angle glaucoma and ocular hypertension. Untreated glaucoma can become a serious disease of the eye causing irreversible damage to the optic nerve, eventually leading to blindness. Synthesis of dorzolamide hydrochloride, (4*S*,6*S*)-2-(aminosulfonyl)-4-(ethylamino)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-*b*] thiopyran 7,7-dioxide hydrochloride, (4*S*,6*S*)-**118**, was published by Merck laboratories (Scheme 27).[129] Dorzolamide contains two asymmetric centers with defined absolute stereochemistry, (4*S*,6*S*), achieved in the published protocol by chemical asymmetric synthesis. The first asymmetric center created was C(6) by the tosylation of methyl (*R*)-3-hydroxy butanoate (*R*)-**109** followed by the S_N2 inversion of configuration through displacement with 2-(lithiomercapto)thiophene for the formation of (*S*)-**105**. Diastereomeric control in the reduction of the keto sulphide (6*S*)-**113** gave the alcohol (4*R*,6*S*)-**103** and the acid-catalyzed epimerization at C(4) of the alcohol resulted in (4*S*,6*S*)-**103,** with a ratio of 4*S*:4*R* of only 76:24. The ratio 4*S*:4*R* was improved to 89:11 by Ritter reaction used to introduce the acetamide function. The Ritter reaction of predominantly (4*S*,6*S*)-**104** proceeds with retention of configuration.



Reagents and conditions: (i) TsCl, Et_3N; (ii) C_4H_3S-LiS (2-(lithiomercapto)thiophene), $HCONH_2$, 20 °C; (iii) 12 N HCl, reflux; (iv) TFAA, toluene; (v) LAH, toluene; (vi) 1 N H_2SO_4, 0-5 °C; (vii) H_2O_2, Na_2WO_4; (viii) CH_3CN, H_2SO_4; (ix) HSO_3Cl, $SOCl_2$; (x) NH_4OH, THF, 0-5 °C; (xi) $BH_3 \cdot Me_2S$, THF; (xii) maleic acid, acetone; (xiii) EtOAc, HCl.

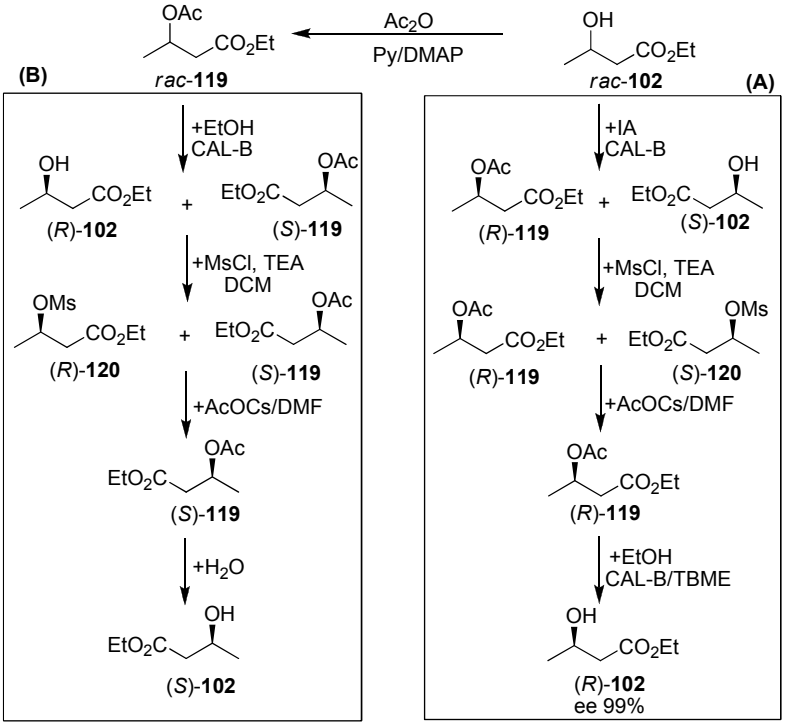
Scheme 27. Synthesis of Dorzolamide hydrochloride[129]

Zeneca has proposed a whole-cell biotransformation for the asymmetric reduction by the fungus *Neurospora crassa* of (6*S*)-ketosulphone in the synthesis of (4*S*,6*S*)-**104**.[130] The advantage of this microorganism is its ability to grow at low pH, which avoids the problem of ketosulphone racemisation. In this process, the reduction of (6*S*)-ketosulphone was accomplished at pilot scale with isolated yield higher than 80% and 98% ee for (4*S*,6S)-**104** (less than 0.5% of (6*R*)-diastereoisomer). The fungus identified by Zeneca works efficiently for the reduction of ketosulphone but difficulties are associated with its growth under controlled fermentation. Moreover, as a whole-cell microorganism *N. crassa* contains more then one enzyme systems which may interfere later in the process development.

It occurred to us to use lipase catalysis to introduce the correct stereochemistry in Dorzolamide. The benefit of lipase catalysis over the whole-cell catalysis is expected particularly in terms of productivity because lipases may tolerate higher substrate concentrations. Moreover, lipases are easy to handle, they do not need cofactors and possess a broad substrate tolerance and they are commercially available in immobilized form. Thus, the substrates suitable for lipase catalysis in the synthesis of Dorzolamide picked from Scheme 27 are ethyl 3-hydroxybutanoate *rac*-**102** commercially available used as a derivative of **109**, *rac-cis*-**103**, *rac-cis*-**104**, and *rac*-**105**. Two substrates, *rac*-**102** and *rac*-**105,** may be used to fix the stereochemistry at C(6) of Dorzolamide, while the lipase catalysis with the other two substrates, *rac-cis*-**103** and *rac-cis*-**104** introduces the stereochemistry at C(4).

5.1.1. Access to the Enantiomers of *rac*-**102**

Ethyl (*R*)-3-hydroxybutanoate is the first possible enantiomer needed for the synthesis of Dorzolamide (Scheme 27). A synthetic protocol consisting of lipase-catalyzed acylation/inversion /lipase-catalyzed alcoholysis was developed to transform racemic commercially available ethyl 3-hydroxybutanoate, *rac*-**102** to the *R*-enantiomer with 99% ee and 85% overall yield (Scheme 28, route A). A similar protocol consisting of lipase-catalyzed alcoholysis/inversion /hydrolysis, although not performed experimentally, may transform *rac*-**119** into the other enantiomer, (*S*)-**102** (Scheme 28, route B).



Scheme 28. Synthetic protocol to access the both enantiomers of ethyl 3-hydroxybutanoate

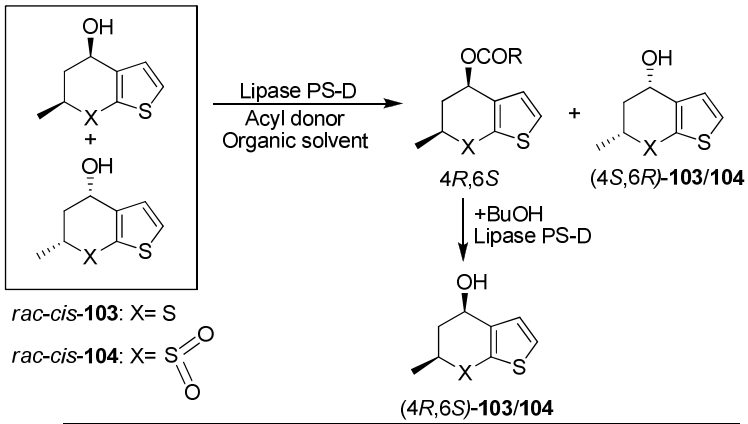
In order to find a lipase that enantioselectively acylates *rac*-**102**, screening with commercial lipases (Apendix 1) was first performed with vinyl butanoate as an acyl donor in TBME. CAL-B was the most enantioselective biocatalyst (E=133), as previously reported.[131] Screening of the acyl donors, included reversible acyl donors used also as reaction media ($PrCO_2Et$ and $MeCO_2Et$), a quasi-irreversible acyl donor ($PrCO_2CH_2CF_3$) and irreversible acyl donors ($PrCO_2CH{=}CH_2$, $MeCO_2CH{=}CH_2$, $MeCO_2C(CH_3){=}CH_2$). The results are given in Table 1 (Paper I). Isopropenyl acetate was the best acyl donor (E=150±4, 48% conversion in 0.5 h with 5 mg mL^{-1} CAL-B). Screening of concentration effects on the acylation of *rac*-**102** with isopropenyl acetate and CAL-B proved that the acylation can be performed in a solvent-free system, where the acyl donor was used just in slight excess over the reactive alcohol enantiomer in the resolution mixture. Thus, the preparative-scale kinetic resolution of *rac*-**102** (5 M) and isopropenyl acetate (3 M) in the presence of CAL-B (30 mg mL^{-1}) was performed to obtain (*S*)-**102** (ee 95%, isolated yield 98% from the

theoretical 50% conversion) and (*R*)-**119** (ee 92%, isolated yield 84% from the theoretical 50% conversion). CAL-B (30 mg mL^{-1}) catalyzed also the alcoholysis of *rac*-**119** (0.1 M) with ethanol (0.2 M) with excellent enantioselectivity (*E*>>200). Ethanol was used for the alcoholysis and no interfering products were formed by possible transesterification at the ethyl carboxylic ester group of **119**.

The inversion of configuration of the less reactive enantiomer (*S*)-**102** was previously reported by the reaction with mesyl chloride followed by hydrolysis under neutral conditions with aqueous $CaCO_3$ to achieve the S_N2 inversion.[132] Inversion of configuration of (*S*)-**102** simultaneously with the hydrolysis of (*R*)-**119** was tested with aqueous $CaCO_3$, but only a minor part of (*R*)-**119** was hydrolyzed. Thus, this was not a method to achieve our goal. Cesium carboxylates were previously employed in S_N2 displacements of mesylates.[133] When the reaction mixture containing (*S*)-**102** and (*R*)-**119** was treated with cesium acetate in DMF also the former compound was transformed into (*R*)-**119** (ee 92%). Ethanolysis of the ester was a mild method for deprotection, increasing at the same time the enantiopurity of the resulting *R*-alcohol (ee 99%). (*R*)-**102** can be used for the incorporation of the chiral methyl group at C-6 in (*S*)-**105** by tosylation and S_N2 displacement with 2-(lithiomercapto)thiophene, as described in Merck protocol. Water elimination from (*R*)-**102** to give ethyl crotanoate favors the formation of *rac*-**105** by Michael addition, rather than (*S*)-**105**.

5.1.2. Access to the Enantiomers of *rac-cis*-103 and *rac-cis*-104

Table 10. Results for lipase-catalyzed large-scale reactions of *rac-cis*-**103** and *rac-cis*-**104**



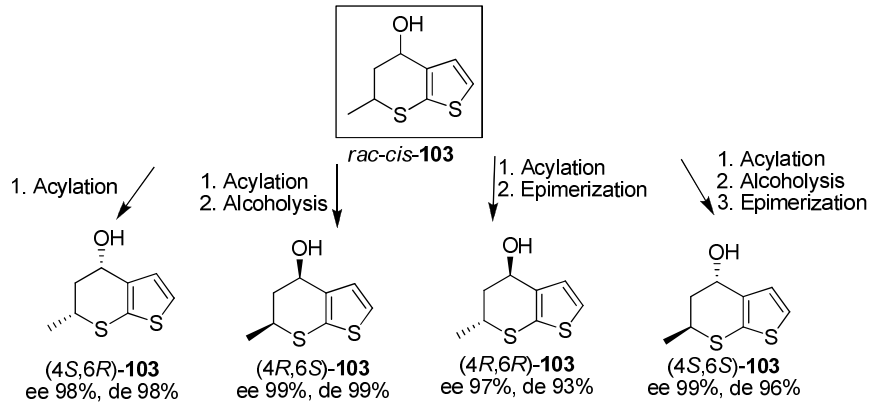
Stereoisomer	ee (%)	de (%)	yield (%)	Specific rotation
(4*S*,6*R*)-**103**	98	98	89	+213.4
(4*R*,6*S*)-**103**	99	99	84	-214.4
(4*S*,6*R*)-**104**	96	99	89	+69.6
(4*R*,6*S*)-**104**	99	99	83	-75.4

For *rac-cis*-**103** (de 91%) and *rac-cis*-**104** (de 97%) the development of a lipase-catalyzed method to introduce the exact stereochemistry at C(4) was targeted in order to overcome a major challenge in the Merck synthesis of Dorzolamide. Moreover, the substrates *rac-cis*-**103**, as derivative of substituted tetrahydro-2*H*-thiopyran-4-ol afforded the study of conformational effects of the ring system on lipase-catalyzed acylation.

The acylation of *rac-cis*-**103**, and that of the corresponding 7,7-dioxide, *rac-cis*-**104,** were highly enantioselective in the presence of lipases. Lipase PS-D was the best enzyme for the

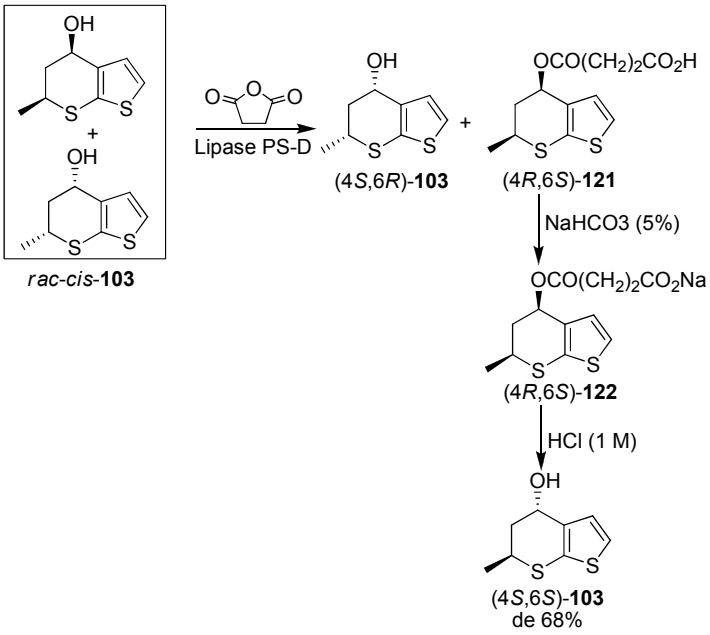
acylation of *rac-cis*-**103** with vinyl butanoate as an acyl donor in TBME with *E*>200 (Table 1, Paper II). *Rac-cis*-**104** was also a good substrate for lipase PS-D but the acyl donor used was vinyl acetate due to the difficulties met in the development of an analytical method for the separation of the enantiomers of the butanoate ester of **104**. *Rac-cis*-**104** was not readily soluble in TBME, thus the reaction in toluene/acetone (9:1) mixture was preferred. Free alcohols (4*R*,6*S*)-**103** and (4*R*,6*S*)-**104** were obtained using the lipase PS-D-catalyzed alcoholysis with 1-butanol of the (4*R*,6*S*)-esters obtained by acylation. The results presented in Table 10 clearly indicate that the preparation of the both enantiomers of *rac-cis*-**103** and *rac-cis*-**104** with high ee and de is possible by lipase catalysis. The yields are given from maximum of the 50% conversion.

The impact of conformation on the lipase PS- and CAL-B-catalyzed acylations was already outlined for substituted cyclohexanols (see 2.4.1.3.). *Rac*-**103** as a methyl-substituted tetrahydro-2*H*-thiopyran-4-ol can adopt different conformations. The acylation of *rac-cis*-**103** (0.1 M) with vinyl butanoate in the presence of lipase PS-D (25 mg mL^{-1}) was highly enantioselective (*E* >200), and 51% conversion was achieved in 0.5 h while the acylation under the same conditions of *rac-trans*-**103** (de 59%; 0.1 M) obtained by acid-catalyzed epimerization of *rac-cis*-**103** proceeded only with *E*=1.4. Thus, *rac-cis*-**103** probably contains HO group in a conformation favorable for acylation while in the case of *rac-trans*-**103** the HO group is orientated in an unfavourable conformation. Although *rac-trans*-**103** was not a suitable substrate to be resolved by lipase-catalyzed kinetic resolution, by combining lipase-catalyzed acylation and alcoholysis steps with acid-catalyzed epimerization, the preparation of all stereoisomers of **103** became possible (Scheme 29).



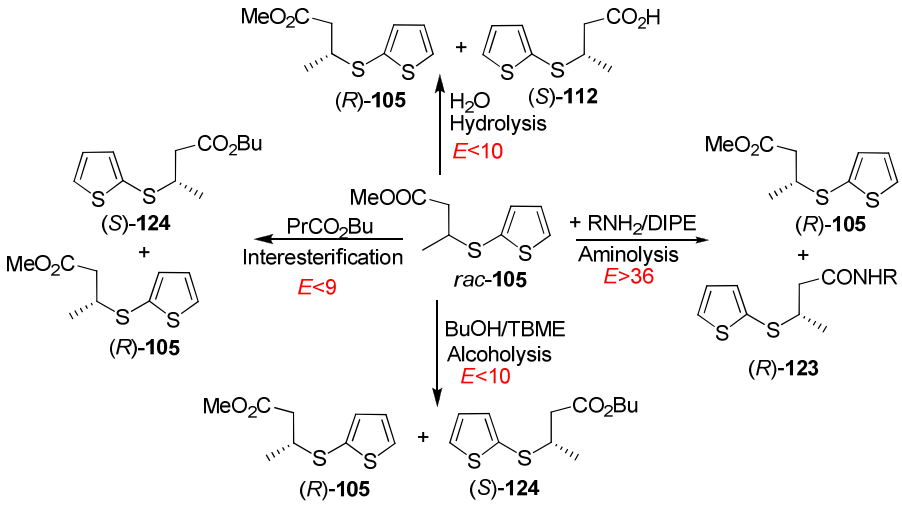
Scheme 29. Stereoisomers of **103**

The acid-catalyzed epimerization of **103** was an impediment for the development of a lipase PS-D-catalyzed kinetic resolution with cyclic anhydrides as acyl donors, and accordingly for improving the separation of the resolved products by the aqueous-organic phase extraction. The screening of acyl donors for the lipase PS-D-catalyzed acylation of *rac-cis*-**103** revealed that cyclic anhydrides, such as succinic and glutaric anhydrides gave good enantioselectivity (*E*=134±2 for succinic anhydride and *E*=80±6 for glutaric anhydride, Table II, Paper II). However, the acylation of *rac-cis*-**103** with succinic anhydride gave the half-ester (4*R*,6*S*)-**121** (Scheme 30). The susceptible epimerization of (4*R*,6*S*)-**103** in the acidic conditions of aqueous extraction used for its isolation conducted to the formation of (4*S*,6*S*)-**103** with 68% de. Thus, succinic anhydride could not be selected as the acyl donor for large-scale acylation of *rac-cis*-**103**.



Scheme 30. Acylation of *rac-cis*-**103** with succinic anhydride

5.1.3. Access to the Enantiomers of *rac*-105



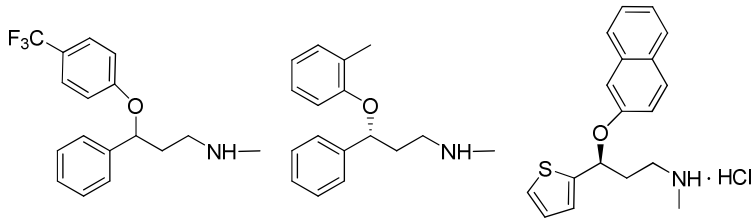
Scheme 31. Lipase-catalyzed reactions for *rac*-**105**

Rac-**105** was the only compound in the work where lipase catalysis was directed to an ester function, *i.e.*, where a chiral compound was an acyl donor. The kinetic resolution of *rac*-**105** by lipases can be performed by hydrolysis, interesterification, alcoholysis and aminolysis (Scheme 31).

The screening of lipases for the resolution of *rac*-**105** was done with commercial lipase preparations from different sources either in free or immobilized form (See Apendix 1). Lipase PS-C II showed the highest enantioselectivity (*E*=36) for the aminolysis of *rac*-**105** with *n*-hexylamine in DIPE. The reactivity was low for this enzyme, and only 20% conversion was achieved in 1 day with 100 mg mL^{-1} lipase PS-C II. The moderate enantioselectivity obtained with lipase PS-C II can be explained by the remote position of the ester group from the asymmetric center. CAL-B catalyzed also the aminolysis of *rac*-**105** in dioxane as a solvent with low enantioselectivity (*E*=6). The enantiopreference, estimated based on HPLC chromatograms, was opposite in the case of CAL-B compared with the rest of the lipases tested. The work with *rac*-**105** was limited to screening stage due to the low enantioselectivity obtained. For this reason the absolute configuration of the products from Scheme 31 are not confirmed, and the assignment in the scheme is just given in accordance with the enantiopreference of lipases for similar substrates.

The kinetic resolution of Dorzolamide intermediates, *rac*-**102**, *rac-cis*-**103**, *rac-cis*-**104**, and *rac*-**105** by lipases, has been studied. Lipases were not previously considered as chiral catalysts in the synthetic sequence of Dorzolamide although their benefits are numerous particularly in terms of enantiopurity of the products, easy of manipulation, no need of cofactors and the possibility to work with high substrate concentration, including a solvent free-system. Kinetic resolution of the aliphatic alcohol *rac*-**102** was performed with lipase CAL-B in a solvent-free system. The solvent-free system is preferred in the case of industrial applications due to reduced dimensions of the installation and reduced amount of waste generated. For the same substrate kinetic resolution was combined with inversion of configuration of the less reactive enantiomer to increase the yield of (*R*)-**102** (99% ee and 85% yield) needed in the synthesis of Dorzolamide. Lipase PS-D, a commercially available immobilized enzyme, was a highly enantioselective catalyst for the acylation with vinyl esters of *rac-cis*-**103** and *rac-cis*-**104**. By combining lipase PS-D catalysis with acid-catalyzed epimerization all stereoisomers of **103** were obtained with 96-99% ee and 98-99% de. Lipase-catalyzed kinetic resolution of the ester *rac*-**105** was less promising.

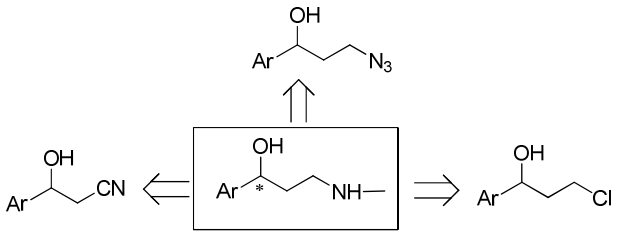
5.2. Lipase Catalysis Applied to β-Hydroxy nitriles as Potential Intermediates of Antidepressant Drugs (Papers III & IV)



Fluoxetine, *rac*-**125** Atomoxetine, (*R*)-**126** Duloxetine, (*S*)-**127**

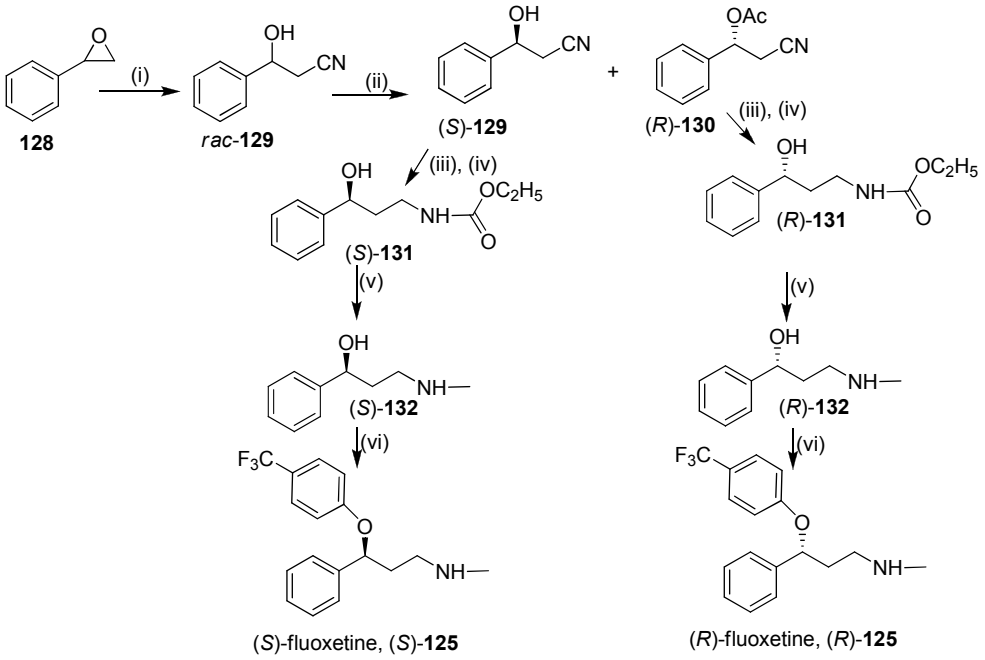
Scheme 32. Antidepressant drugs with 1-aryl-3-methylaminopropan-1-ol structure

Improved therapies for the treatment of depression are important considering the high number of people which experience depressive periods in their lives in modern world. The main class of drugs used in the treatment of depressive disorders is that of selective serotonin-reuptake inhibitors (SSRIs) such as fluoxetine, *rac*-**125**, (Prozac,™ Eli Lilly) which entered clinical use from the late 1980s onwards. Fluoxetine hydrochloride is sold as a racemate, but interest has been shown recently for the interactions of *S* and *R* forms of fluoxetine with serotonin neurotransmitter.[134] Atomoxetine, (*R*)-**126**, (Strattera,™ Eli Lilly) commercialized as an *R*-enantiomer, is a noradrenaline reuptake inhibitor approved for the treatment of attention-deficit hyperactivity disorder. Initially, atomoxetine was developed as a new antidepressant drug but it failed during the clinical trials. Duloxetine hydrochloride, (*S*)-**127**, (Cymbalta,™ Eli Lilly) is a selective inhibitor of serotonin and noradrenaline-reuptake inhibitor, for which improved efficiency and a faster onset of action is predicted.[135] These antidepressant drugs are derivatives of 1-aryl-3-methylaminopropan-1-ol. Retrosynthetic analysis reveals that enantiopure β-hydroxy nitriles are suitable intermediates for the synthesis of antidepressant drugs (Scheme 33). Enantiopure β-hydroxy nitriles may be obtained with high enantiopurity by lipase catalysis.[136,137] Lipase catalysis was also applied for the kinetic resolution of 3-chloro-1-arylpropan-1-ol[138] and 3-azido-1-arylpropan-1-ol[139] as possible intermediates for the synthesis of antidepressant drugs.



Scheme 33. Retrosynthetic scheme for antidepressant drugs with 1-aryl-3-methylaminopropan-1-ol structure

Chemoenzymatic synthesis of the both enantiomers of fluoxetine was proposed based on enantiopure β-hydroxy nitriles obtained by lipase catalysis by Kamal *et al.* (Scheme 34).[136]



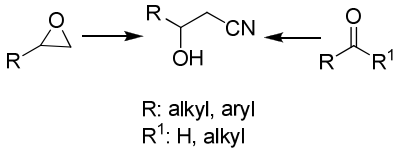
Reagents and conditions: (i) NaCN, H_2O - EtOH; (ii) Lipase PS-C II, VA, DIPE; (iii) BH_3-Me_2S, THF; (iv) ethyl chloroformate, K_2CO_3, DCM; (v) LAH, THF; (vi) 4-chlorobenzotrifluoride, NaH, dry DMSO.

Scheme 34. Chemoenzymatic synthesis of *R*- and *S*-fluoxetine[136]

Considering the potential of β-hydroxy nitriles as intermediates for the synthesis of antidepressant drugs, the lipase catalysis was applied to synthesize the both enantiomers of various heteroaromatic β-hydroxy nitriles. Two series of racemic β-hydroxy nitriles were prepared, thiophen-based (*rac*-**107(a-f)**, Scheme 25) and phenylfuran-based heterocycles (*rac*-**108(a-e)**, Scheme 25).

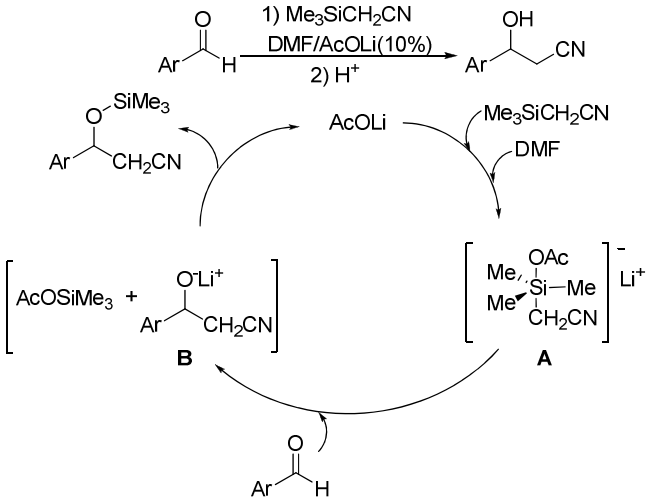
5.2.1. Synthesis of Racemic Heterocyclic β-Hydroxy nitriles

Two strategies are available for the synthesis of β-hydroxy nitriles using epoxides or carbonyl compounds as starting substrates (Scheme 35). The ring opening of epoxides with cyanide as nucleophile was performed with volatile and toxic HCN,[140] non-volatile alkali cyanides in the presence of perchlorate salts,[141] $Yb(CN)_3$,[142] acetone cyanohydrin in the presence of a base[143] or lanthanide alkoxides or alkyl aluminium cyanides[144] and $Ce(OTf)_4$.[145] Addition of acetonitrile to aldehyde or ketones was performed after its ionization with *n*-butyllithium or alkali amide.[146] When *n*-butyllithium was employed, a temperature of -80 °C was required to give the best yields (47-89%), and when alkali amides were employed a temperature of -33 °C was required to provide yields up to 93%.[147]



Scheme 35. Synthetic routes towards β-hydroxy nitriles

In this thesis, the synthesis of β-hydroxy nitriles was performed by cyanomethylation of the carbonyl compounds with (trimethylsilyl)acetonitrile ($TMSCH_2CN$) in the presence of lithium acetate (AcOLi) as a Lewis base at 0 °C.[148] This method has the advantage of high yields for the racemic β-hydroxy nitriles (Table 11) without requiring extreme temperatures. The mechanism of cyanometylation is not very clear yet. A mechanism proposed by Kawano *et al.*[148] is given in Scheme 36. The oxygen containing-anions generated from salts of carboxylic acids (AcOLi, AcONa, AcOK, AcOCs, AcO(*n*-NBu_4)) were considered effective Lewis base catalysts to activate the carbon-silicon bond in $TMSCH_2CN$.



Scheme 36. Mechanism of cyanomethylation for aromatic aldehydes[148]

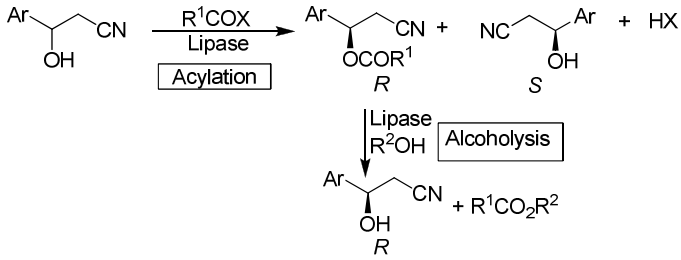
In the first mechanistic step a hypervalent silicate **A** is formed by coordination of the Lewis base catalyst to the silicon atom of $TMSCH_2CN$. The nucleophilicity of the silicate **A** is high enough to react with an aldehyde to form alkoxide **B** and TMSOAc. Then **B** is silylated by TMSOAc to the *O*-silyl ether and the catalyst is regenerated to continue the catalytic cycle. The yields obtained for synthesis of thiophen-based heterocyclic β-hydroxy nitriles was higher than 80% in most of the cases and in the range of 70-80% for the phenylfuran-based β-hydroxy nitriles depicted in Scheme 25 (Table 11).

Table 11. Yields for synthesis of β-hydroxy nitriles by cyanomethylation

Thiopen-based β-hydroxy nitriles		**Phenylfuran-based β-hydroxy nitriles**	
Compound	Yield (%)	Compound	Yield (%)
rac-**107a**	80	*rac*-**108a**	83
rac-**107b**	91	*rac*-**108b**	81
rac-**107c**	82	*rac*-**108c**	79
rac-**107d**	65	*rac*-**108d**	78
rac-**107e**	87	*rac*-**108e**	81
rac-**107f**	41		

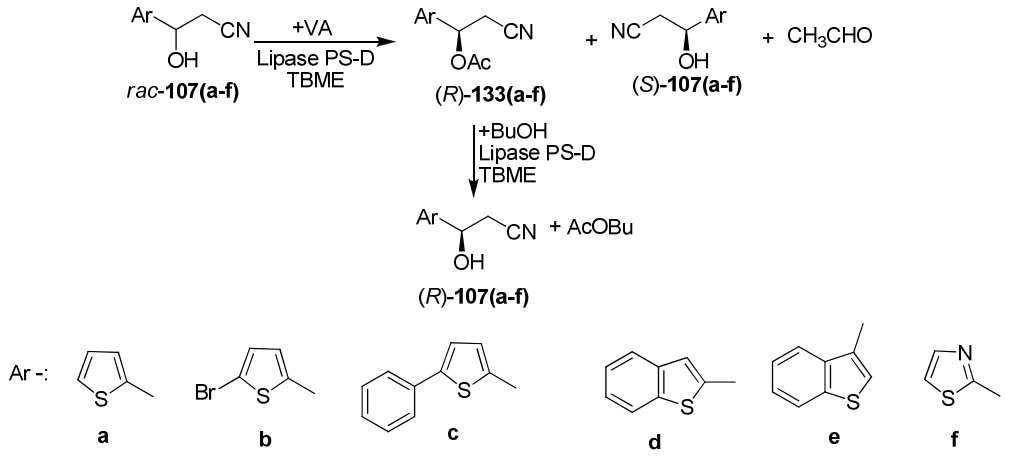
5.2.2. Preparation of the Enantiomers of Heterocyclic β-Hydroxy nitriles

A lipase-catalyzed two steps method was used to prepare the both enantiomers of heterocyclic β-hydroxy nitriles. The first step consists of the acylation by an enantioselective lipase of racemic β-hydroxy nitriles, followed by a second step consisting of the lipase-catalyzed alcoholysis of the ester enantiomers previously obtained (Scheme 37).



Scheme 37. Lipase catalysis applied for the synthesis of *R*- and *S*- β-hydroxy nitriles

Rac-**107c** was used as a pilot thiophen-based β-hydroxy nitrile for the enzyme screening using vinyl acetate as an acyl donor in TBME (Table 1, Paper III). Based on the screening results lipase PS-D was selected for the optimization and than for enantioselective preparation of the both enantiomers of thiophen-based β-hydroxy nitriles (Scheme 38).



Scheme 38. Lipase-catalyzed synthesis of thiophen-based β-hydroxy nitriles

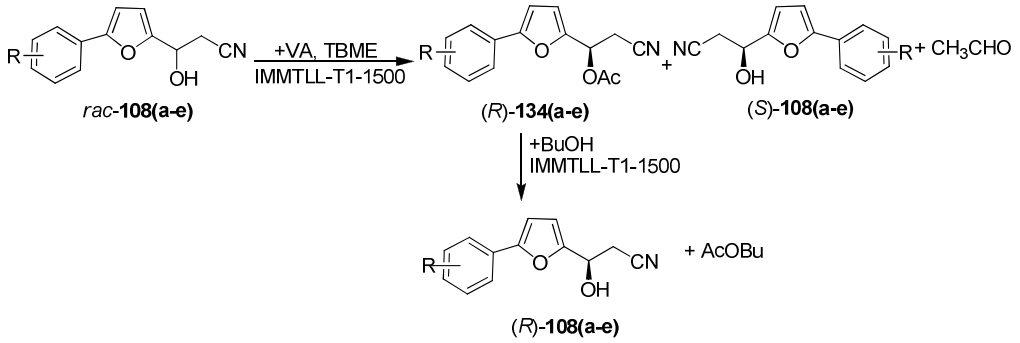
The enantioselectivity of lipase PS-D was excellent (E >>200) for the acylation with vinyl acetate in TBME of *rac*-**107(b-e)** (vinyl butanoate was the acyl donor due to analytical difficulties in detecting *rac*-**107e**). The enantioselectivity was very good also for *rac*-**107a** (E=126). Thiazol-based *rac*-**107f** was the only case with moderate enantioselectivity (E=15) for lipase PS-D catalysis. Optimization and preparative-scale synthesis was not continued for *rac*-**107f**. Lipase PS-D showed excellent enantioselectivity (E >>200) for alcoholysis with 1-butanol in TBME for all esters of *rac*-**107(a-e)**, as it is showed in Table 3, Paper III. Table 12 (Scheme 38) presents the yields and enantiopurities of the both enantiomers of thiophen-based heterocyclic β-hydroxy nitriles prepared by two-steps lipase catalysis.

Table 12. Enantiopure **107**(**a-e**) prepared by lipase PS-D-catalyzed kinetic resolution: the *R*-enantiomers obtained through acylation/alcoholysis sequence and the *S*-enantiomers through acylation of *rac*-**107**(**a-e**)

	(*R*)-**107**			(*S*)-**107**		
	ee (%)	**yield (%)**[a]	**Specific rotation**[b]	**ee (%)**	**yield (%)**[c]	**Specific rotation**[b]
a	99	72	+33.4	91	94	-28.3
b	99	90	+13.1	99	85	-13.7
c	99	84	+17.4	98	99	-16.3
d	99	77	+35.4	98	98	-33.9
e	95	65	+69.5	95	87	-66.5

[a]Overall yields from the enzymatic acylation/deacylation sequence.
[b](c 1, $CHCl_3$).
[c]Isolated yields based on the conversion of enzymatic acylation.

Rac-**108a** was selected as a pilot substrate for lipase-catalyzed acylation of phenylfuran-based β-hydroxy nitriles (Scheme 39).



Scheme 39. Lipase-catalyzed synthesis of phenylfuran-based β-hydroxy nitriles

Lipase screening for the acylation of *rac*-**108a** showed low enantioselectivity in the case of lipase PS-D, the most selective enzyme for the synthesis of thiophen-based β-hydroxy nitriles (entry 1, Table 13). This result emphasizes the importance of the substrate structure for binding to the active site of a lipase. Lipases from *Pseudomonas fluorescens* and *Thermomyces lanuginosus* showed better enantioselectivities for the acylation of *rac*-**108a** (entries 3, 4 and respectively entries 7, 8, 9). A relatively new commercial lipase preparation, IMMTLL-T1-1500, from ChiralVision was used for optimization and then for the preparative-scale preparation of the enantiomers of phenylfuran-based β-hydroxy nitriles. The entries 7, 8, 9 in the Table 13 refer to the use of the same enzyme, lipase from *Thermomyces lanuginosus*, but in different immobilization forms. The enantioselectivity varies for the three lipase preparations used, illustrating the effect of immobilization on lipase catalysis.

Table 13. Results for lipase screening (50 mg $mL^{-1)}$ for the acylation of *rac*-**108a** (0.05 M) with VA (0.1 M) in TBME

Entry	Enzyme preparation	Time (h)	ee$^{(S)\text{-}108a}$ (%)	ee$^{(R)\text{-}134a}$ (%)	c (%)	E^a
1	lipase PS-D	0.25	10	47	18	3±1
2	lipase PS-C II	0.25	11	43	20	3±1
3	lipase AK	3	50	93	35	50±3
4	lipase IMMAPF-T2-150	3	22	97	19	60±5
5	CAL-A	3	51	41	55	5±1
6	CAL-B	3	14	93	13	30±2
7	lipozyme TL IM[b]	0.5	14	99	13	>>200
8	lipase IMMTLL-T1-1500	3	87	93	48	79±3
9	lipase IMMTLL-T2-150	3	16	99	14	50±3

[a] Calculated from linear regression curve
[b] Hydrolysis was noticed after 16% conversion

Table 14 presents the enantioselectivity of lipase IMMTLL-T1-1500 for the acylation of *rac*-**108**(**a-e**) and respectively for the alcoholysis of *rac*-**134**(**a-e**). For the acylation the lowest enantioselectivity was obtained for *rac*-**108e**, E=46±12 (entry 5). This compound had reduced solubility in TBME and TBME/acetone (9:1) was used as a mixt solvent for the acylation. The highest enantioselectivity was obtained for the acylation of *rac*-**108b**, E=120±5 (entry 2). The enantioselectivity was higher for the alcoholysis of the acetate esters, *rac*-**134(a-e)**.

Table 14. Enantioselectivity of lipase IMMTLL-T1-1500 (50 mg mL^{-1}) for the acylation of *rac*-**108(a-e)** [*rac*-**108(a-e)** (0.1 M), VA (0.2 M), TBME] and for the alcoholysis of *rac*-**134(a-e)** [*rac*-**134(a-e)** (0.1 M), butan-1-ol (0.3 M), TBME]

Entry	Acylation		Alcoholysis	
	rac-**108**	E^a	*rac*-134	E^a
1	**a**	79±3	**a**	>200
2	**b**	120±5	**b**	162±16
3	**c**	115±11	**c**	>200
4	**d**	96±7	**d**	>200
5	**e**[b]	46±12	**e**[c]	72±19

[a]Enantiomer ratio, *E*, was determined using linear regression
[b]TBME/acetone (9/1)
[c]TBME/acetone (4/1)

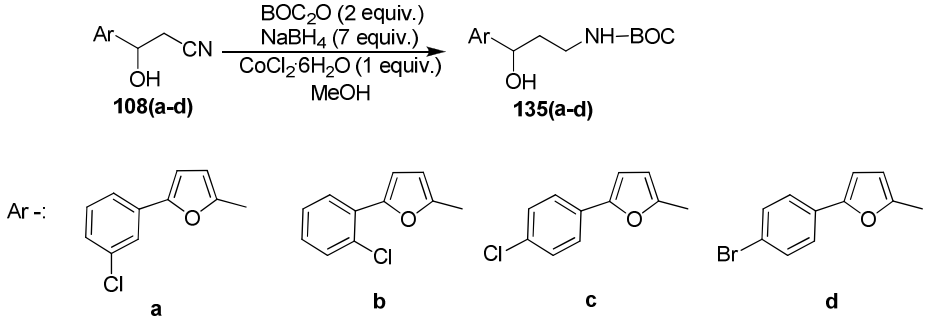
The synthesis of the enantiomers of phenylfuran-based β-hydroxy nitriles was done following the sequence acylation/deacylation described in Scheme 39. The acylation was let to proceed until 51-55% conversion to allow synthesis of (*S*)-**108(a-e)** with high enantiopurity. The enantiopurity of the (*R*)-**108(a-e)** was increased by lipase-catalyzed deacylation of the corresponding esters by alcoholysis (Table 15).

Table 15. Enantiopure **108**(**a-e**) prepared by lipase IMMTLL-T1-1500-catalyzed kinetic resolution: the *R*-enantiomers obtained through acylation/alcoholysis sequence and the *S*-enantiomers through acylation of *rac*-**108**(**a-e**)

	(*R*)-**108**			(*S*)-**108**		
	ee (%)	**yield (%)**[a]	**Specific rotation**[b]	**ee (%)**	**yield (%)**[c]	**Specific rotation**[b]
a	99	71	+46.7	98	83	-46.3
b	99	92	+44.7	99	97	-43.8
c	99	88	+45.5	99	91	-48.2
d	99	89	+39.4	99	89	-36.2
e	99	74	+55.4	97	95	-56.6

[a]Overall yields from the enzymatic acylation/deacylation sequence.
[b](*c* 1, $CHCl_3$)
[c]Isolated yields based on the conversion of enzymatic acylation.

5.2.3. Synthesis of Phenylfuran-based *N*-BOC-protected γ-amino alcohols



Scheme 40. Reduction of β-hydroxy nitriles

Chemical transformations of the nitrile group of enantiomerically enriched phenylfuran-based β-hydroxy nitriles were tested to develop a route towards γ-amino alcohols needed for the synthesis of antidepressant drugs with 1-aryl-3-methylaminopropan-1-ol structure (Scheme 40). Nitrile functionality is an unreactive group, thus the reduction protocols employ strong hydride donors, such as aluminium hydride, or make use of catalytic hydrogenation.[149] Sodium borohydride, a milder, easier-to-handle reduction reagent, alone is generally not strong enough to fulfill the reduction of nitriles. By addition of transition metal salts, such as $NiCl_2$ or $CoCl_2$, the reactivity of sodium borohydride is tuned up for reduction of the nitrile group.[150] It is not proved yet whether the metal halide co-reactant fulfills a true catalytic function or whether some transient, low-valent transition metal complex formed *in situ* is the actual reducing agent.[151] The reduction of racemic and enantiopure **108(a-d)** was performed with $NaBH_4$ in the presence of $CoCl_2 \cdot 6H_2O$. When the reduction of enantiopure β-hydroxy nitriles was performed no loss of enantiopurity was detected (Table 16). Reduction of **108e** was not successful possible due to the consumption of reducing species for the reduction of *p*-NO_2 group of the phenyl ring. *N*-Boc-protected amino-alcohols were prepared rather than free amino alcohols to avoide dimerization of primary free amines with an imine intermediate formed during reduction, as previously reported.[152,153]

Table 16. Prepared enantiopure γ-amino alcohols, **135(a-d)**

γ-amino alcohol	**ee (%)**	**Specific rotation**	**Yield[a] (%)**
(*S*)-**135a**	99	+1.40	59
(*S*)-**135b**	98	+0.24	68
(*R*)-**135b**	99	-0.18	70
(*S*)-**135c**	99	+1.88	57
(*S*)-**135d**	95	+1.22	61
(*R*)-**135d**	94	-1.08	68

[a]Yield for the reduction step

Kinetic resolution by lipases in organic solvents was successfully applied for the preparation of new enantiopure β-hydroxy nitriles. New racemic heterocyclic β-hydroxy nitriles were synthesized by cyanomethylation of commercially available aldehydes, procedure not applied before for this type of compounds. Two enantioselective lipases were found for the preparation of enantiopure β-hydroxy nitriles, lipase PS-D gave enantiopure thiophen-based heterocyclic β-hydroxy nitriles with 91-99% ee and lipase IMMTLL-T1-1500 gave enantiopure phenylfuran-based β-hydroxy nitriles with 98-99% ee. Lipase from *Thermomyces lanuginosus* has not as wide application in kinetic resolution of secondary alcohols as lipase PS-D and CAL-B. Reduction of nitrile group was achieved in mild conditions with $NaBH_4$ in the presence of $CoCl_2 \cdot 6H_2O$ to obtain enantiopure γ-amino alcohols. In the case that 1-substituted thiophen-3-methylaminopropan-ols or 1-phenylfuran-3-methylaminopropan-ols exhibit activity as serotonin-reuptake inhibitors than a chemoenzymatic method for their synthesis may be easily developed based on our research using commercially available aldehydes as starting substrates.

6. Summary

This thesis describes lipases as highly applicable chiral catalysts for the preparation of enantiopure secondary alcohols in general and for the preparation of enantiopure intermediates in drug synthesis, in particular. In my work I have targeted two types of drugs in order to study the potential of lipase catalysis to obtain enantiopure intermediates: Dorzolamide [(4*S*,6*S*)-**106**] and antidepressant drugs with 1-aryl-3-methylaminopropan-1-ol structure.

The enantiomers of ethyl 3-hydroxybutanoate, of *cis*-4-hydroxy-6-methyl-5,6-dihydro-4*H*-thieno-[2,3-*b*]thiopyran and of *cis*-4-hydroxy-6-methyl-5,6-dihydro-4*H*-thieno-[2,3-*b*]thiopyran 7,7-dioxide (**102-104**), which appear as intermediates in the synthetic path of Dorzolamide, were prepared with high enantiopurity by lipase catalysis. The enantiomer (*R*)-**102** was obtained with 99% ee and 85% overall yield starting from racemate by combining acylation with *Candida antarctica* lipase B in a solvent-free system and inversion of configuration of the unreacted alcohol enantiomer. The enantiomers of *rac-cis*-**103** and *rac-cis*-**104** were prepared with 96-99% ee and 98-99% de with the aid of lipase PS-D (*Burkholderia cepacia*). Efficient enzymatic acylation was developed for *rac-cis*-**103** (E>200), while only poor enantioselectivity was obtained for *rac-trans*-**103** (*E*=1.4). The preference of lipases to acylate hydroxyl groups orientated in equatorial position explains the difference in enantioselectivity observed for *cis*- and *trans*-**103**. The four possible stereoisomers of **103** were obtained by combining lipase catalysis and acid-catalyzed epimerization.

The enantiomers of thiophen-based and phenylfuran-based β-hydroxy nitriles, considered potential novel intermediates for antidepressant drugs, were also prepared by lipase catalysis. The enatiomers of thiophen-based β-hydroxy nitriles [**107(a-e)]** were prepared with 91-99% ee by lipase PS-D-catalyzed acylation/alcoholysis. The enantiomers of phenylfuran-based β-hydroxy nitriles [**108(a-e)**] were also prepared with 98-99% ee by acylation/alcoholysis sequence with *Thermomyces lanuginosus* lipase (IMMTLL-T1-1500). Lipase PS-D had poor enantioselectivity (*E*=3) for the acylation of phenylfuran-based β-hydroxy nitriles. Thus, substrate structure is important for its correct binding to the active site of the lipase. Reduction of racemic and enantiopure phenylfuran-based β-hydroxy nitriles **108(a-d)** to *N*-BOC-protected γ-amino alcohols **134(a-d)** was achieved with $NaBH_4$ in the presence of $CoCl_2 \cdot 6H_2O$. The enantiopurity of phenylfuran-based β-hydroxy nitriles enantiomers was not affected by the reduction.

The results obtained in this thesis encourage the integration of lipase-catalysis into chemoenzymatic synthesis of Dorzolamide and heterocyclic antidepressant drugs. The enantioselective lipases are identified and the kinetic resolution steps for the synthesis of alcohols as intermediates of these drug molecules are optimized, thus the incorporation of these new improvements into the synthetic strategies for these drugs should be straightforward.

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Mihaela Claudia Turcu

Appendix 1: Information about the lipases used in screenings

Lipases	Origin	Form	Activity	Producer
Lipase PS-C "Amano" II	*Burkholderia cepacia*	strong adsorption forces on ceramics (Toyonite 200)		Amano
Lipase PS-D "Amano" I	*Burkholderia cepacia*	absorbed on diatomaceous earth (celite)		Amano
Lipase AH "Amano"	*Burkholderia cepacia*	powder		Amano
IMMABC-T2-150	*Burkholderia cepacia*	immobilized covalently on polyacrylic beads	1500 U/g	ChiralVision
Novozym 435	*Candida antarctica* B	adsorption on macroporous polypropylene resin	10000U/g	Novozym
IMMCAL-B-T1-1500	*Candida antarctica B*	adsorption on polypropylene	1500 U/g	ChiralVision
Immozyme CALB-T2-150	*Candida antarctica B*	immobilized covalently on polyacrylic beads	2500 U/g	ChiralVision
IMMCAL-A-T2-150	*Candida antarctica lipase A*	immobilized covalently on polyacrylic beads	2500 U/g	ChiralVision
Lipase AK "Amano" 20	*Pseudomonas fluorescens*	powder	20000	Amano
Lipase AK –C "Amano"	*Pseudomonas fluorescens*	immobilized	600	Amano
IMMAPF-T2-150	*Pseudomonas fluorescens*	immobilized covalently on polyacrylic beads	1500 U/g	ChiralVision
Lipozyme RM IM	*Rhizomucor miehei* lipase	immobilized	150	Novozym
IMMRML-T2-150	*Rhizomucor miehei*	immobilized covalently on polyacrylic beads	1500 U/g	ChiralVision
Lipozyme TL IM	*Thermomyces lanuginosus*	immobilized on silica	250	Novozym
IMMTLL-T1-1500	*Thermomyces lanuginosus*	adsorption on polypropylene	4000 U/g	ChiralVision
IMMTLL-T2-150	*Thermomyces lanuginosus*	immobilized covalently on polyacrylic beads	16000 U/g	ChiralVision
Lipase OF	*Candida rugosa*	powder	400000	Meito Sangyo
Lipase AY "Amano"	*Candida rugosa*	powder		Amano
Lipase from CR	*Candida rugosa*	powder	943 U/mg	Sigma
IMMCRL-T2-150	*Candida rugosa*	immobilized covalently on polyacrylic beads	500 U/g	ChiralVision
Lipase CR	*Candida cylindracea*	Sol-Gel-AK	51.8 U/g	Fluka
Lipase M "Amano" 10	*Mucor javanicus*	powder	10000	Amano
IMMAMJ-T2-150	*Mucor javanicus*	immobilized covalently on polyacrylic beads	300 U/g	ChiralVision
Lipase Type II (PPL)	Crude from porcine pancreas	powder	50 U/mg	Sigma
Lipase Type II (PPL)	*Crude from porcine pancreas*	powder	370 U/mg	Sigma
Lipase R "Amano"	*Penicillium roquefortii*	powder		Amano
Lipase A "Amano" 6	Aspergillus niger	powder		Amano
Lipase G "Amano" 50	*Penicillium camembertii*	powder		Amano
Lipase L "Amano" 10	*Candida lipolytica*	powder		Amano
Lipase from CL	Candida lipolytica	powder		Biocatalytics
Lipase from RJ	*Rhizopus javanicus*	powder		Biocatalytics
IMMARO-T2-150	*Rhizopus oryzae*	immobilized covalently on polyacrylic beads	900 U/g	ChiralVision
IMMCCL-T2-150	*Candida cylindracea*	immobilized covalently on polyacrylic beads	600 U/g	ChiralVision

[1] Moss, G.P. Basic terminology of stereochemistry. *Pure & Appl. Chem.* **1996**, *68*, 2193-2222.
[2] Leresche, J. E.; Meyer, H.-P. Chemocatalysis and biocatalysis (biotransformation): Some thoughts of a chemist and of a biotechnologist *Org. Proc. Res. Dev.* **2006**, *10*, 572-580.
[3] Easson, L. H.; Stedman, E. CLXX. Studies on the relationship between chemical constitution and physiological action. V. Molecular dissymmetry and physiological activity. *Biochem. J.* **1933**, *27*, 1257-1266.
[4] Jones, J. B. *Applications of Biochemical Systems in organic chemistry*, part 1, edited by Jones, J. B.; Sih, C. J.; Perlman, D., John Wiley and Sons, New-York, **1976**, 1-46.
[5] McConathy, J.; Owens, M. J. Stereochemistry in drug action. *J. Clin. Psychiatry* **2003**, *5*, 70-73.
[6] http://www.chem.qmul.ac.uk/iubmb/enzyme/
[7] Zaks, A.; Klibanov, A. M. Enzymatic catalysis in nonaqueous solvents. *J. Biol. Chem.* **1988**, *263*, 3194-3201.
[8] Klibanov, A. M. Enzymatic catalysis in anhydrous organic solvents. *Trends Biochem. Sci.* **1989**, *14*, 141-144.
[9] Pellissier, H. Recent developments in dynamic kinetic resolution. *Tetrahedron* **2008**, *64*, 1563-1601.
[10] Schnell, B.; Faber, K.; Kroutil, W. Enzymatic racemisation and its application to synthetic biotransformations. *Adv. Synth. Catal.* **2003**, *345*, 653-666.
[11] Gladiali, S.; Alberico, E. Asymmetric transfer hydrogenation: chiral ligands and applications. *Chem. Soc. Rev.* **2006**, *35*, 226-236.
[12] Corey, E. J.; Helal, C. J. Reduction of carbonyl compounds with chiral oxazaborolidine catalysts: a new paradigm for enantioselective catalysis and a powerful new synthetic method. *Angew. Chem. Int. Ed.* **1998**, *37*,1986-2012.
[13] Soai, K.; Niwa, S. Enantioselective addition of organozinc reagents to aldehydes. *Chem. Rev.* **1992**, *92*, 833-856.
[14] Palomo, C.; Oiarbide, M.; García, J. M. Current progress in the asymmetric aldol addition reaction. *Chem. Soc. Rev.* **2004**, *33*, 65-75.
[15] Carey, J. S.; Laffan, D.; Thomson, C.; Williams, M. T. Analysis of the reactions used for the preparation of drug candidate molecules. *Org. Biomol. Chem.* **2006**, *4*, 2337-2347.
[16] Jaeger, K.-E.; Eggert, T. Lipases for biotechnology. *Curr. Opin. Biotech.* **2002**, *13*, 390-397.
[17] Lotti, M.; Alberghina, L. *Lipases: Molecular structure and function* in *Industrial enzymes: Structure, Function and Applications*, edited by Polaina, J. and MacCabe, A. P., Springer Netherlands, **2007**.
[18] Jaeger, K.-E.; Dijkstra, B. W., Reetz, M. T. Bacterial biocatalysts: Molecular biology, three-dimensional structures, and biotechnological applications of lipases. *Annu. Rev. Microbiol.* **1999**, *53*, 315-351.
[19] Schmid, R. D.; Verger, R. Lipases: Interfacial enzymes with attractive applications. *Angew. Chem. Int. Ed.* **1998**, *37*, 1608-1633.
[20] Ema, T. Mechanism of enantioselectivity of lipases and other synthetically useful hydrolases. *Curr. Org. Chem* **2004**, *8*, 1009-1025.
[21] Schrag, J. D.; Cygler, M. Lipases and α/β hydrolase fold. *Meth. Enzym.* **1997**, *284*, 85-107.
[22] Winkler, F.K.; D'Arcy, A.; Hunziker, W. Structure of human pancreatic lipase. *Nature* **1990**, *343*, 771-774.
[23] Martinelle, M.; Holmquist, M.; Hult, K. On the interfacial activation of *Candida antarctica* lipase A and B as compared with *Humicola lanuginosa* lipase *Biochim. Biophys. Acta* **1995**, *1258*, 272-276.
[24] Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*, Wiley-VCH, **1999**.
[25] Anderson, E.M; Larsson, K. M; Kirk, O. One biocatalyst – many applications: the use of *Candida antarctica* B-lipase in organic synthesis. *Biocat. Biotransf.* **1997,** *16*, 181-204.
[26] Uppenberg, J.; Patkar, S.; Bergfors, T.; Jones, T. A. Crystallization and preliminary X-ray studies of lipase B from *Candida antarctica*. *J. Mol. Biol.* **1994**, *235*, 790-792.
[27] Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jones, T. A. The sequence, crystal structure determination and refinement of two crystal forms of lipase B from *Candida antarctica*. *Structure* **1994**, *2*, 293-308.
[28] Kim, K. K.; Song, H. K.; Shin, D. H.; Hwang, K.Y.; Suh, S. W. The crystal structure of a triacylglycerol lipase from *Pseudomonas cepacia* reveals a highly open conformation in the absence of a bound inhibitor. *Structure* **1997**, *5*, 173-185.
[29] Schrag, J. D.; Li, Y.; Cygler, M.; Lang, D.; Burgdorf, T.; Hecht, H.-J.; Schmid, R.; Schomburg, D.; Rydel, T. J.; Oliver, J. D.; Strickland, L. C.; Dunaway, C. M.; Larson, S. B.; Day, J.; McPherson, A. The open conformation of a *Pseudomonas* lipase. *Structure* **1997**, *5*, 187-202.
[30] Derewenda, U.; Swenson, L.; Wei, Y.; Green, R.; Kobos, P. M.; Joerger, R.; Haas, M. J.; Derewenda, Z. S. Conformational lability of lipases observed in the absence of an oil-water interface: crystallographic studies of enzymes from the fungi *Humicola lanuginosa* and *Rhizopus delemar*. *J. Lipid Res.* **1994**, *35*, 524-534.
[31] Lawson, D. M.; Brzozowski, A. M.; Rety, S.; Verma, C.; Dodson, G. G. Probing the nature of substrate binding in *Humicola lanuginosa* lipase through X-ray crystallography and intuitive modeling. *Prot. Eng.* **1994**, *7*, 543-550.
[32] Santini, S.; Crowet, J. M; Thomas, A.; Paquot, M.; Vandenbol, M.; Thonart, P.; Wathelet, J. P.; Blecker, C.; Lognay, G.; Brasseur, R.; Lins, L.; Charloteaux, B. Study of *Thermomyces lanuginosa* lipase in the presence of tributyrylglycerol and water. *Biophys. J.* **2009**, *96*, 4814-4825.
[33] Lehtonen J. V., Still D. J., Rantanen V. V., Ekholm J., Björklund D., Iftikhar Z., Huhtala M., Repo S., Jussila A., Jaakkola J., Pentikäinen O., Nyrönen T., Salminen T., Gyllenberg M.; Johnson M. BODIL: a molecular modeling environment for structure-function analysis and drug design. *J. Comput. Aided Mol. Des*. **2004**, *18*, 401-419.

[34] Hasan, F.; Shah, A. A.; Hameed, A. Industrial applications of microbial lipases. *Enzy. Microb. Technol.* **2006,** *39*, 235-251.
[35] Hanefeld, U. *Immobilization as a Tool for Improving Enzymes* in *Modern Biocatalysis* edited by Fessner, W.-D.; Anthonsen, T., Wiley-VCH, **2009**.
[36] Zaks, A.; Klibanov, A. M. Enzymatic catalysis in organic media at 100 ^{o}C. *Science* **1984**, *224*, 1249-1251.
[37] Kanerva, L. T.; Liljeblad, A. *Transesterification- Biological* in *Encyclopedia of Catalysis*, 2nd edition, John Wiley & Sons, **2010,** DOI: 10.1002/0471227617.eoc197.
[38] Santaniello, E.; Casati, S.; Ciuffreda, P. Lipase-catalyzed deacylation by alcoholysis: a selective, useful transesterification reaction. *Curr. Org. Chem.* **2006**, *10*, 1095-1123.
[39] Chênevert, R.; Pelchat, N.; Jacques, F. Stereoselective enzymatic acylations (Transesterifications). *Curr. Org. Chem.* **2006**, *10*, 1067-1094.
[40] Lundell, K.; Lehtinen, P.; Kanerva, L. T. Chemo- and enantioselective acyl transfers by lipases and acylase I: Preparative applications in hydroxymethylpiperidine chemistry. *Adv. Synth. Catal.* **2003**, *345*, 790-796.
[41] Riva, S. Enzymatic modification of the sugar moieties of natural glycosides. *J. Mol. Cat. B: Enz.* **2002**, 43-54.
[42] Bornscheuer, U. T.; Kazlauskas, R. J. Catalytic promiscuity in biocatalysis: Using old enzymes to form new bonds and follow new pathways. *Angew. Chem. Int. Ed.* **2004**, *43*, 6032-6040.
[43] Hult, K.; Berglund, P. Enzyme promiscuity: mechanism and applications. *Trends in Biotechnology* **2007**, *25*, 231-238.
[44] Svedendahl, M.; Hult, K.; Berglund, P. Fast Carbon-Carbon bond formation by a promiscuous lipase. *J. Am. Chem. Soc.* **2005**, *127*, 17988-17989.
[45] Ghanem, A. Trends in lipase-catalyzed asymmetric access to enantiomerically pure/enriched compounds. *Tetrahedron* **2007**, *63*, 1721-1754.
[46] Kamal, A.; Azhar, M. A.; Krishnaji, T.; Malik, M. S.; Azeeza, S. Approaches based on enzyme mediated kinetic to dynamic kinetic resolutions: A versatile route for chiral intermediates. *Coordination Chemistry Reviews* **2008**, *252*, 569-592.
[47] Kanerva, L. T. *Hydrolase-catalyzed asymmetric and other transformations of synthetic* interest in *Enzymatic reactions in organic media* edited by Koskinen, A. M. P. and Klibanov, A. M., Blackie Academic & Professional, **1996**.
[48] Kazlauskas, R. J.; Weissfloch, N. E.; Rappaport, A. T.; Cuccia, L. A. A rule to predict which enantiomer of a secondary alcohol reacts faster in reactions catalyzed by cholesterol esterase, lipase from *Pseudomonas cepacia*, and lipase from *Candida rugosa*. *J. Org. Chem.* **1991**, *56*, 2656-2665.
[49] Carrea, G.; Riva, S. Properties and synthetic applications of enzymes in organic solvents. *Angew. Chem. Int. Ed.* **2000**, *39*, 2226-2254.
[50] Chen, C.-S.; Sih, C. J. General aspects and optimization of enantioselective biocatalysis in organic solvents: The use of lipases. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 695-707.
[51] Berglund, P. Controlling lipase enantioselectivity for organic synthesis. *Biomol. Eng.* **2001**, *18*, 13-22.
[52] Magnusson, A. O.; Takwa, M.; Hamberg, A.; Hult, K. An *S*-selective lipase was created by rational redisign and the enantioselectivity increased with temperature. *Angew. Chem. Int. Ed.* **2005**, *44*, 4582-4585.
[53] Lundh, M; Nordin, O.; Hedenström, E.; Högberg, H.-E. Enzyme catalysed irreversible transesterifications with vinyl acetate. Are they really irreversible? *Tetrahedron:Asymm.* **1995**, *6*, 2237-2244.
[54] Halling, P. J. Thermodynamic predictions for biocatalysis in nonconventional media: Theory, tests, and recommendations for experimental design and analysis. *Enzyme Microb. Technol.* **1994**, *16*, 178-206.
[55] Wescott, C. R.; Klibanov, A. M.The solvent dependence of enzyme specificity. *Biochim. Biophys. Acta* **1994**, *1206*, 1-9.
[56] Zaks, A.; Klibanov, A. The effect of water on enzyme action in organic media. *J. Biol. Chem.* **1988**, *263*, 8017-8021.
[57] Laane, C.; Boeren, S.; Vos, K.; Veeger, C. Rules for optimization of biocatalysis in organic solvents. *Biotechnol. Bioeng.* **1987**, *30*, 81-87.
[58] Forró, E.; Kanerva, L. T.; Fülöp, F. Lipase-catalysed resolution of 2-dialkylaminomethylcyclohexanols. *Tetrahedron: Asymm.* **1998**, *9*, 513-520.
[59] Sheldon, R. A. Green solvents for sustainable organic synthesis: state of the art. *Green Chem.* **2005**, *7*, 267-278.
[60] Dlugy, C.; Wolfson, A. Lipase catalyse glycerolysis for kinetic resolution of racemates. *Bioprocess. Biosyst. Eng.* **2007**, *30*, 327-330.
[61] Mesiano, A. J.; Beckman, E. J.; Russell, A. J. Supercritical biocatalysis. *Chem. Rev.* **1999**, *99*, 623-633.
[62] Sheldon, R. A.; Lau, R. M.; Sorgedrager, M. J.; van Rantwijk, F.; Seddon, K. R. Biocatalysis in ionic liquids. *Green Chem.* **2002**, *4*, 147-151.
[63] Saul, S.; Corr, S.; Micklefield, J. Biotransformations in low-boiling hydrofluorocarbon solvents. *Angew. Chem. Int. Ed.* **2004**, *43*, 5519-5523.
[64] Uhm, K.-N.; Lee, S.-J.; Kim, H.-K.; Kang, H.-Y.; Lee, Y. Enantioselective resolution of methyl 2-chloromandelate by *Candida antarctica* lipase A in a solvent-free transesterification reaction. *J. Mol. Cat. B: Enz.* **2007**, *45*, 34-38.
[65] Hanefeld, U. Reagents for (ir)reversible enzymatic acylations. *Org. Biomol. Chem.* **2003**, *1*, 2405-2415.

[66] Takayama, S.; Moree, W. J.; Wong, C.-H. Enzymatic resolution of amines and amino alcohols using pent-4-enoyl derivatives. *Tetrahedron Lett.* **1996**, *37*, 6287-6290.
[67] Moris, F.; Gotor, V. A useful and versatile procedure for the acylation of nucleosides through an enzymatic reaction. *J. Org. Chem.* **1993**, *58*, 653-660.
[68] Degueil-Castaing, M.; De Jeso, B.; Drouillard, S.; Maillard, B. Enzymatic reactions in organic synthesis: 2-ester interchange of vinyl esters. *Tetrahedron Lett.* **1987**, *28*, 953-954.
[69] Weber, H. K.; Zuegg, J.; Faber, K.; Pleiss, J. Molecular reasons for lipase-sensitivity against acetaldehyde. *J. Mol. Cat. B: Enz.* **1997**, *3*, 131-138.
[70] Bianchi, D.; Cesti, P.; Battistel, E. Anhydrides as acylating agents in lipase-catalyzed stereoselective esterification of racemic alcohols. *J. Org. Chem.* **1988**, *53*, 5531-5534.
[71] Gutman, A. L.; Brenner, D.; Boltanski, A. Convenient practical resolution of racemic alkyl-aryl alcohols via enzymatic acylation with succinic anhydride in organic solvents. *Tetrahedron: Asymm.* **1993**, *4*, 839-844.
[72] Chênevert, R.; Pelchat, N.; Morin, P. Lipase-mediated enantioselective acylation of alcohols with functionalized vinyl esters: acyl donor tolerance and applications. *Tetrahedron: Asymm.* **2009**, *20*, 1191-1196.
[73] Tanikaga, R.; Matsumoto, Y.; Sakaguchi, M.; Koyama, Y.; Ono, K. Conformational effects on lipase-mediated acylations of 2-substituted cyclohexanols. *Tetrahedron Lett.* **2003**, *44*, 6781-6783.
[74] Tanikaga, R.; Morita, A. Lipase-mediated diastereoselective and enantioselective acetylations of 3-substituted cyclohexanols. *Tetrahedron Lett.* **1998**, *39*, 635-638.
[75] Levy, L. M.; Lavandera, I.; Gotor, V. Is the ring conformation the most critical parameter in lipase-catalysed acylation of cycloalkanols. *Org. Biomol. Chem.* **2004**, *2*, 2572-2577.
[76] Toşa, M.; Pilbák, S.; Moldovan, P.; Paizs, C.; Szatzker, G.; Szakács, Novák, L.; Irimie, F.-D.; Poppe, L. Lipase-catalyzed kinetic resolution of racemic 1-heteroarylethanols-experimental and QM/MM study. *Tetrahedron: Asymm.* **2008**, *19*, 1844-1852.
[77] Paizs, C.; Toşa, M.; Bódai, V.; Szakács, G.; Kmecz, I.; Simándi, B.; Majdik, C.; Novák, L.; Irimie, F.-D.; Poppe, L. Kinetic resolution of 1-(benzofuran-2-yl)ethanols by lipase-catalyzed enantiomer selective reactions. *Tetrahedron: Asymm.* **2003**, *14*, 1943-1949.
[78] Cheedrala, R. K.; Sachwani, R.; Krishna, P. R. Lipase mediated kinetic resolution of benzimidazolyl ethanols. *Tetrahedron: Asymm.* **2008**, *19*, 901-905.
[79] Danda, H.; Maehara, A.; Umemura, T. Preparation of (4*S*)-4-hydroxy-3-methyl-2-(2`-propynyl)-2-cyclopentenone by combination of enzymatic hydrolysis and chemical transformation. *Tetrahedron Lett.* **1991**, *32*, 5119-5122.
[80] Danda, H.; Nagatomi, T.; Maehara, A.; Umemura, T. Preparation of optically active secondary alcohols by combination of enzymatic hydrolysis and chemical transformation. *Tetrahedron* **1991**, *47*, 8701-8716.
[81] Lemke, K.; Ballschuh, S.; Kunath, B.; Theil, F. An improved procedure for the lipase-catalysed kinetic resolution of *endo-endo-cis*-bicyclo[3.3.0]octane-2,6-diol - synthesis of potential C_2-symmetric enantiomerically pure bidentate auxiliaries. *Tetrahedron: Asymm.* **1997**, *8*, 2051-2055.
[82] Mitsuda, S.; Umemura, T.; Hirohara, H. Preparation of an optically pure secondary alcohol of synthetic pyrethroids using microbial lipases. *Appl. Microbiol. Biotechnol.* **1988**, *29*, 310-315.
[83] Takano, S.; Suzuki, M.; Ogasawara, K. Enantiocomplementary preparation of optically pure 2-trimethylsilylethynyl-2-cyclopentenol by homochiralization of racemic precursors: A new route to the key intermediate of 1,25-dihydroxycholecalciferol and vincamine. *Tetrahedron: Asymm.* **1993**, *4*, 1043-1046.
[84] Bouzemi, N.; Aribi-Zouioueche, L.; Fiaud, J.-C. Combined lipase-catalyzed resolution/Mitsunobu esterification for the production of enantiomerically enriched arylalkyl carbinols. *Tetrahedron: Asymm.* **2006**, *17*, 797-800.
[85] Väntinen, E.; Kanerva, L. T. Combination of the lipase-catalysed resolution with the Mitsunobu esterification in one pot. *Tetrahedron: Asymm.* **1995**, *6*, 1779-1786.
[86] Kim, M.-J.; Chung, Y. I.; Choi, Y. K.; Lee, H. K.; Kim, D.; Park, J. (*S*)-selective dynamic kinetic resolution of secondary alcohols by the combination of subtilisin and an aminocyclopentadienylruthenium complex as the catalysts. *J. Am. Chem. Soc.* **2003**, *125*, 11494-11495.
[87] Borén, L.; Martín-Matute, B.; Xu, Y.; Córdova, A.; Bäckvall, J.-E. (*S*)-selective kinetic resolution and chemoenzymatic dynamic kinetic resolution of secondary alcohols. *Chem. Eur. J.* **2006**, *12*, 225-232.
[88] Martin-Matute, B.; Bäckvall, J.-E. *Chemoenzymatic deracemization processes in Organic synthesis with enzymes in non-aqueous media* edited by Carrea, G.; Riva, S.; Wiley-VCH; **2008.**
[89] Pàmies, O.; Bäckvall, J.-E. Chemoenzymatic dynamic kinetic resolution. *Trends Biotechnol.* **2004**, *22*, 130-135.
[90] Dinh, P. M.; Howarth, J. A.; Hudnott, A. R.; Williams, J. M. J. Catalytic racemisation of alcohols: Applications to enzymatic resolution reactions. *Tetrahedron Lett.* **1996**, *37*, 7623-7626.
[91] Bäckvall, J.-E. Transition metal hydrides as active intermediates in hydrogen transfer reactions. *J. Organomet. Chem.* **2002**, *652*, 105-111.
[92] Pàmies, O.; Bäckvall, J.-E. Studies on the mechanism of metal-catalyzed hydrogen transfer from alcohols to ketones. *Chem. Eur. J.* **2001**, *7*, 5052-5058.
[93] Ito, M.; Osaku, A.; Kitahara, S.; Hirakawa, M.; Ikarriya, T. Rapid racemization of chiral non-racemic *sec*-alcohols catalyzed by (η^5-$C_5(CH_3)_5$)Ru complexes bearing tertiary phosphine-primary amine chelate ligands. *Tetrahedron Lett.* **2003**, *44*, 7521-7523.

[94] Huerta, F. F.; Mindis, A. B. E.; Bäckvall, J.-E. Racemisation in asymmetric synthesis. Dynamic kinetic resolution and related processes in enzyme and metal catalysis. *Chem. Soc. Rev.* **2001**, *30*, 321-331.
[95] Menashe, N.; Shvo, Y. Catalytic disproportionation of aldehydes with ruthenium complexes. *Organometallics* **1991**, *10*, 3885-3891.
[96] Larsson, A. L. E.; Persson, B. A.; Bäckvall, J.-E. Enzymatic resolution of alcohols coupled with ruthenium-catalyzed racemization of the substrate alcohol. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1211-1212.
[97] Martín-Matute, B.; Bäckvall, J.-E. Ruthenium- and enzyme-catalyzed dynamic kinetic asymmetric transformations of 1,4-diols: Synthesis of γ-hydroxy ketones. *J. Org. Chem.* **2004**, *69*, 9191-9195.
[98] Pámies, O.; Bäckvall, J.-E. Enzymatic kinetic resolution and chemoenzymatic dynamic kinetic resolution of δ-hydroxy esters. An efficient route to chiral δ-lactones. *J. Org. Chem.* **2002**, *67*, 1261-1265.
[99] Pámies, O.; Bäckvall, J.-E. Chemoenzymatic dynamic kinetic resolution of β-halo alcohols. An efficient route to chiral epoxides. *J. Org. Chem.* **2002**, *67*, 9006-9010.
[100] Pámies, O.; Bäckvall, J.-E. Dynamic kinetic resolution of β-azido alcohols. An efficient route to chiral aziridines and β-amino alcohols. *J. Org. Chem.* **2001**, *66*, 4022-4025.
[101] Pámies, O.; Bäckvall, J.-E. An efficient route to chiral α- and β-hydroxyalkanephosphonates. *J. Org. Chem.* **2003**, *68*, 4815-4818.
[102] Pàmies, O.; Bäckvall, J.-E. Efficient lipase-catalyzed kinetic resolution and dynamic kinetic resolution of β-hydroxy nitriles. A route to useful precursors for γ-amino alcohols. *Adv. Synth. Catal.* **2001**, *343*, 726-731.
[103] Pàmies, O.; Bäckvall, J.-E. Efficient lipase-catalyzed kinetic resolution and dynamic kinetic resolution of β-hydroxy nitriles. Correction of absolute configuration and transformation to chiral β-hydroxy acids and γ-amino alcohols. *Adv. Synth. Catal.* **2002**, *344*, 947-952.
[104] Kim, N.; Ko, S.-B.; Kwon, M. S.; Kim, M.-J.; Park, J. Air-stable racemization catalyst for dynamic kinetic resolution of secondary alcohols at room temperature. *Org. Lett.* **2005**, *7*, 4523-4526.
[105] Lee, D.; Huh, E. A.; Kim, M.-J.; Jung, H. M.; Koh, J. H.; Park, J. Dynamic kinetic resolution of allylic alcohols mediated by Ruthenium- and lipase-based catalysts. *Org. Lett.* **2000**, *2*, 2377-2379.
[106] Mavrynsky, D.; Sillanpää, R.; Leino, R. Cyclopenta[*l*]phenanthrenyl and cyclopenta[*a*]acenaphthylenyl half-sandwich complexes of ruthenium as racemization catalysts for secondary alcohols. *Organometallics* **2009**, *28*, 598-605.
[107] Mavrynsky, D.; Päiviö, M.; Lundell, K.; Sillanpää, R.; Kanerva, L. T.; Leino, R. Dicarbonylchloro(pentabenzylcyclopentadienyl)ruthenium as racemization catalyst in the dynamic kinetic resolution of secondary alcohols. *Eur. J. Org. Chem.* **2009**, 1317-1320.
[108] Ariëns, E. J. Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. *Eur. J. Clin. Pharmacol.* **1984**, *26*, 663-668.
[109] http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation
[110] Commission of the European Communities (1993) *CPMP Note for Guidance: Investigation of Chiral Active Substances*, London, UK (http://www.emea.europa.eu/).
[111] Agranat, I.; Caner, H. Intellectual property and chirality of drugs. *Drug Disc. Today* **1999**, *4*, 313-321.
[112] Yazbeck, D. R.; Martinez, C. A.; Hu, S.; Tao, J. Challenges in the development of an efficient enzymatic process in the pharmaceutical industry. *Tetrahedron: Asymm.* **2004**, *15*, 2757-2763.
[113] Patel, R. N. Synthesis of chiral pharmaceutical intermediates by biocatalysis. *Coord. Chem. Rev.* **2008**, *252*, 659-701.
[114] Gotor-Fernández, V.; Brieva, R.; Gotor, V. Lipases: Useful biocatalysts for the preparation of pharmaceuticals. *J. Mol. Cat. B: Enz.* **2006**, *40*, 111-120.
[115] Liljeblad, A.; Kallinen, A.; Kanerva, L. T. Biocatalysis in the preparation of the statin side chain. *Curr. Org. Synth.* **2009**, *6*, 362-379.
[116] Borah, J. C.; Boruwa, J.; Barua, N. C. Synthesis of the C-13 side chain of taxol. *Curr. Org. Synth.* **2007**, *4*, 175-199.
[117] Brieva, R.; Crich, J. Z.; Sih, C. J. Chemoenzymatic synthesis of the C-13 side chain of Taxol: Optically-active 3-hydroxy-4-phenyl β-lactam derivatives. *J. Org. Chem.* **1993**, *58*, 1068-1075.
[118] Anand, N.; Koul, S.; Taneja, S. C.; Parshad, R.; Manhas, K. S.; Sharma, R. L.; Qazi, G. N. WO 118750 A1, **2009**
[119] Hamamoto, H.; Mamedov, V. A., Kitamoto, M., Hayashi, N.; Tsuboi, S. Chemoenzymatic synthesis of the C-13 side chain of paclitaxel (Taxol) and docetaxel (Taxotere). *Tetrahedron: Asymm.* **2000**, *11*, 4485-4497.
[120] Gogoi, N.; Borah, J. C.; Boruwa, J.; Barua, N. C. Lipase catalysed kinetic resolution of 2-nitroalcohol: Total synthesis of Taxol side chain and (-)Bestatin. *Lett. Org. Chem.* **2007**, *4*, 234-235.
[121] Lee, D.; Kim, M.-J. Lipase-catalyzed transesterification as a practical route to homochiral *syn*-1,2-diols. The synthesis of the Taxol side chain. *Tetrahedron Lett.* **1998**, *39*, 2163-2166.
[122] Yu, R. H.; Polniaszek, R. P.; Becker, M. W.; Cook, C. M.; Yu, L. H. L. Research and development of an efficient synthesis of hexahydrofuro[2,3-*b*]furan-3-ol moiety- A key component of the HIV protease inhibitor candidates. *Org. Proc. Res. Dev.* **2007**, *11*, 972-980.
[123] Mangas-Sánchez, J.; Rodríguez-Mata, M.; Busto, E.; Gotor-Fernández, V.; Gotor, V. Chemoenzymatic synthesis of rivastigmine based on lipase-catalyzed processes. *J. Org. Chem.* **2009**, *74*, 5304-5310.
[124] Vaidyanathan, R.; Hesmondhalgh, L.; Hu, S. A chemoenzymatic synthesis of an androgen receptor antagonist. *Org. Proc. Res. Dev.* **2007**, *11*, 903-906.

[125] Forró, E.; Lundell, K.; Fülöp, F.; Kanerva, L. T. Preparation of the stereoisomers of 2-cyanocycloalkanols by lipase-catalysed acylation. *Tetrahedron: Asymm.* **1997**, *8*, 3095-3099.
[126] Kamal, A.; Krishnaji, T.; Khan, M. N. A. Lipase-catalysed resolution of *N*-(3-cyano-2-hydroxypropan-1-yl)phthalimide. Synthesis of (*R*)-GABOB and (*R*)-carnitine. *J. Mol. Catal. B: Enz.* **2007**, *47*, 1-5.
[127] Rebouche, C. J. Carnitine metabolism and function in humans. *Ann. Rev. Nutr.* **1986**, *6*, 41-66.
[128] Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. Quantitative analyses of biochemical kinetic resolutions of enantiomers. *J. Am. Chem. Soc.* **1982**, *104*, 7294-7299.
[129] Blacklock, T. J.; Sohar, P.; Butcher, J. W.; Lamanec, T.; Grabowski, E. J. J. An enantioselective synthesis of the topically-active carbonic anhydrase inhibitor MK-0507: 5,6-dihydro-(*S*)-4-(ethylamino)-(*S*)-6-methyl-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride. *J. Org. Chem.* **1993**, *58*, 1672-1679.
[130] Blacker, A. J.; Holt, R. A. *Development of a Multi-stage Chemical and Biological Process for an Optically Active Intermediate for an Anti-glaucoma Drug* in *Chirality in Industry II*, edited by Collins, A. N.; Sheldrake, G.N; Crosby, J., John Wiley and Sons, New York, **1997**.
[131] Fishman, A.; Eroshov, M.; Dee-Noor, S. S.; van Mil, J.; Cogan, U.; Effenberger, R. A two-step enzymatic resolution process for large-scale production of (*S*)- and (*R*)-ethyl-3-hydroxybutyrate. *Biotechnol. Bioeng.* **2001**, *74*, 256-263.
[132] Carnell, A. J.; Head, R.; Bassett, D.; Schneider, M. Efficient large scale stereoinversion of (*R*)-ethyl 3-hydroxybutyrate. *Tetrahedron: Asymm.* **2004**, *15*, 821-825.
[133] Shimizu, T.; Hiranuma, S.; Nakata, T. Efficient method for inversion of secondary alcohols by reaction of chloromethanesulfonates with cesium acetate. *Tetrahedron Lett.* **1996**, *37*, 6145-6148.
[134] Sahu, P. K.; Wang, C.-H.; Lee, S.-L. Interaction of Serotonin and Fluoxetine: Toward understanding the importance of the chirality of Fluoxetine (*S* form and *R* form). *J. Phys. Chem. B* **2009**, *113*, 14529-14535.
[135] Waitekus, A. B.; Kirkpatrick, P. Fresh from the pipeline. Duloxetine hydrochloride. *Nat. Rev.* **2004**, *3*, 907-908.
[136] Kamal, A.; Ramesh Khanna, G. B.; Ramu, R. Chemoenzymatic synthesis of both enantiomers of fluoxetine, tomoxetine and nisoxetine: lipase-catalyzed resolution of 3-aryl-3-hydroxypropanenitriles. *Tetrahedron: Asymm.* **2002**, *13*, 2039-2051.
[137] Kamal, A.; Ramesh Khanna, G. B.; Ramu, R.; Krishnaji, T. Chemoenzymatic synthesis of duloxetine and its enantiomer: lipase-catalyzed resolution of 3-hydroxy-3-(2-thienyl)propanenitrile. *Tetrahedron Lett.* **2003**, *44*, 4783-4787.
[138] Liu, H.-L.; Hoff, B.H.; Anthonsen, T. Chemoenzymatic synthesis of the non-tricyclic antidepressants Fluoxetine, Tomoxetine and Nisoxetine. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1767-1769.
[139] Kamal, A.; Malik, M. S.; Shaik, A. A.; Azeeza, S. Lipase mediated resolution of γ-azidoalcohols in aqueous and organic media: Synthesis of (*R*)- and (*S*)-fluoxetine and duloxetine. *J. Mol. Cat. B: Enz.* **2009**, *58*, 132-137.
[140] Smith, J. G. Synthetically useful reactions of epoxides. *Synthesis* **1984**, 629-656.
[141] Chini, M.; Crotti, P.; Favero, L.; Macchia, F. Easy direct stereo- and regioselective formation of β-hydroxy nitriles by reaction of 1,2-epoxides with potassium cyanide in the presence of metal salts. *Tetrahedron Lett*. **1990**, *32*, 4775-4778.
[142] Matsubara, S.; Onishi, H.; Utimoto, K. Reaction of cyanotrimethylsilane with oxiranes under $Yb(CN)_3$ catalysis. *Terahedron Lett*. **1990**, *31*, 6209-6212.
[143] Mitchell, D.; Koenig, T. M. Regiospecific opening of 1,2-epoxides with acetone cyanohydrin under mildly basic conditions. *Tetrahedron Lett*. **1992**, *33*, 3281-3284.
[144] Mullis, J. C.; Weber, W. P. Regiospecificity of reactions of epoxides and oxetanes with trimethylsilyl cyanide. *J. Org. Chem.* **1982**, *47*, 2873-2875.
[145] Iranpoor, N.; Shekarriz, M. Ring opening of epoxides with sodium cyanide catalyzed with $Ce(OTf)_4$. *Synth. Commun*. **1999**, *29*, 2249-2254.
[146] Kaiser, E. W.; Hauser, C. R. Ionization of an *α* hydrogen of acetonitrile by *n*-butyllithium and alkali amides. Condensations with ketones and aldehydes to form *β*-hydroxynitriles. *J. Org. Chem.* **1968**, *33*, 3402-3404.
[147] Kisanga, P.; McLeod, D.; D´Sa, B.; Verkade, J. $P(RNCH_2CH_2)_3N$-catalyzed synthesis of *β*-hydroxy nitriles. *J. Org. Chem.* **1999**, *64*, 3090-3094.
[148] Kawano, Y.; Kaneko, N.; Mukaiyama, T. Lewis base-catalyzed cyanomethylation of carbonyl compounds with (trimethylsilyl)acetonitrile. *Chem. Lett.* **2005**, *34*, 1508-1509.
[149] Brown, H. C.; Krishnamurthy, S. Forty years of hydride reductions. *Tetrahedron* **1979**, *35*, 567-607.
[150] Ganem, B.; Osby, J. O. Synthetically useful reactions with metal boride and aluminide catalysts. *Chem. Rev.* **1986**, *108*, 67-72.
[151] Osby, J. O.; Heinzman, S. W.; Ganem, B. Studies on the mechanism of transition-metal-assisted sodium borohydride and lithium aluminium hydride reductions. *J. Am. Chem. Soc.* **1986**, *108*, 67-72.
[152] Caddick, S.; De K. Haynes, A. K.; Judd, D. B.; Williams, M. R. V. Convenient synthesis of protected primary amines from nitriles. *Tetrahedron Lett.* **2000**, *41*, 3513-3516.
[153] Caddick, S.; De K. Haynes, A. K.; Judd, D. B.; de K. Lewis, A. K.; Reich, M. T.; Williams, M. R. V. Convenient synthesis of protected primary amines from nitriles. *Tetrahedron* **2003**, *59*, 5417-5423.