Turun yliopisto
University of Turku

LIPASE AND ω-TRANSAMINASE CATALYSIS IN PREPARATION OF ALCOHOL AND AMINE ENANTIOMERS

Mari Päiviö

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1126 | Medica - Odontologica | Turku 2014

University of Turku

Faculty of Medicine
Institute of Biomedicine
Department of Pharmacology, Drug Development and Therapeutics
Laboratory of Synthetic Drug Chemistry
Drug Research Doctoral Programme

Supervisor and custos

Professor Liisa T. Kanerva, PhD
University of Turku
Turku, Finland

Reviewed by

Associate Professor Lucia Gardossi, PhD
University of Trieste
Trieste, Italy

Professor Jari Yli-Kauhaluoma, PhD
University of Helsinki
Helsinki, Finland

Opponent

Professor Per Berglund, PhD
Royal Institute of Technology
Stockholm, Sweden

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-5819-1 (PRINT)
ISBN 978-951-29-5820-7 (PDF)
ISSN 0355-9483
Painosalama Oy - Turku, Finland 2014

"*Education is an admirable thing,*

but it is well to remember from time to time that nothing that is worth knowing can be taught."

Oscar Wilde

To my mother

ABSTRACT

Mari Päiviö

Lipase and ω-transaminase catalysis in preparation of alcohol and amine enantiomers

Department of Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry, University of Turku

Annales Universitatis Turkuensis, Painosalama Oy, Turku, Finland, 2014

Enantiopure intermediates are of high value in drug synthesis. Biocatalysis alone or combined with chemical synthesis provides powerful tools to access enantiopure compounds. In biocatalysis, chemo-, regio- and enantioselectivity of enzymes are combined with their inherent environmentally benign nature. Enzymes can be applied in versatile chemical reactions with non-natural substrates under synthesis conditions. Immobilization of an enzyme is a crucial part of an efficient biocatalytic synthesis method. Successful immobilization enhances the catalytic performance of an enzyme and enables its reuse in successive reactions.

This thesis demonstrates the feasibility of biocatalysis in the preparation of enantiopure secondary alcohols and primary amines. Viability and synthetic usability of the studied biocatalytic methods have been addressed throughout this thesis. *Candida antarctica* lipase B (CAL-B) catalyzed enantioselective *O*-acylation of racemic secondary alcohols was successfully incorporated with *in situ* racemization in the dynamic kinetic resolution, affording the (*R*)-esters in high yields and enantiopurities. Side reactions causing decrease in yield and enantiopurity were suppressed. CAL-B was also utilized in the solvent-free kinetic resolution of racemic primary amines. This method produced the enantiomers as (*R*)-amides and (*S*)-amines under ambient conditions. An in-house sol-gel entrapment increased the reusability of CAL-B. *Arthrobacter* sp. ω-transaminase was entrapped in sol-gel matrices to obtain a reusable catalyst for the preparation enantiopure primary amines in an aqueous medium. The obtained heterogeneous ω-transaminase catalyst enabled the enantiomeric enrichment of the racemic amines to their (*S*)-enantiomers. The synthetic usability of the sol-gel catalyst was demonstrated in five successive preparative kinetic resolutions.

Keywords: biocatalysis, kinetic resolution, dynamic kinetic resolution, lipase, ω-transaminase, enzyme immobilization, secondary alcohols, primary amines

TIIVISTELMÄ

Mari Päiviö

Lipaasi- ja ω-transaminaasi katalyysi alkoholi- ja amiinienantiomeerien valmistuksessa

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

Annales Universitatis Turkuensis, Painosalama Oy, Turku, Finland, 2014

Lääkeainesynteesissä enantiopuhtaat intermediaatit ovat olennaisen tärkeitä. Biokatalyysi sellaisenaan tai yhdistettynä kemialliseen synteesiin mahdollistaa enantiopuhtaiden yhdisteiden valmistamisen. Biokatalyysissä yhdistyy entsyymien kemo-, regio- ja enantioselektiivisyys sekä entsyymien luontainen ympäristöystävällisyys. Entsyymejä voidaan hyödyntää lukuisten niiden tavanomaisista substraateista poikkeavien yhdisteiden reaktioissa vaativissa olosuhteissa. Entsyymien immobilisointi on olennainen osa tehokasta biokatalyyttistä synteesimenetelmää. Entsyymi-immobilisoinilla voidaan parantaa entsyymin ominaisuuksia ja mahdollistaa entsyymin uudelleenkäyttö useammissa peräkkäisissä reaktioissa.

Väitöskirjassa tarkastellaan biokatalyysin käyttökelpoisuutta enantiopuhtaiden sekundaaristen alkoholien ja primaaristen amiinien valmistuksessa. Sekundaaristen alkoholien dynaamisessa kineettisessa resoluutiossa *Candida antarctica*n lipaasin B (CAL-B) katalysoima enantioselektiivinen *O*-asylaatio yhdistettiin *in situ* rasemointiin. Menetelmä mahdollisti useiden (*R*)-esterien valmistamisen korkealla saannolla. Saantoon ja tuotteen enantiopuhtauteen vaikuttavien sivureaktioiden osuutta pienennettiin merkittävästi. CAL-B katalysoi myös primaaristen amiinien kineettistä resoluutiota liuotinvapaissa reaktio-olosuhteissa. Menetelmän avulla enantiomeerit valmistettiin (*S*)-amiineina ja (*R*)-amideina ympäristön olosuhteissa. Sooligeeli-immobilisointi lisäsi entsyymin kierrätettävyyttä. *Arthrobacter*-lajin ω-transaminaasi immobilisoitiin sooligeeliin. Tällöin saatiin heterogeeninen katalyytti, jota voitiin käyttää primaaristen amiinien valmistamiseksi (*S*)-enantiomeereina vesiliuoksissa. Immobilisointi mahdollisti entsyymin tehokkaan uudelleenkäytön viidessä peräkkäisessä preparatiivisessa reaktiossa.

Avainsanat: biokatalyysi, kineettinen resoluutio, dynaaminen kineettinen resoluutio, lipaasi, ω-transaminaasi, entsyymi-immobilisointi, sekundaariset alkoholit, primaariset amiinit

CONTENTS

ABBREVIATIONS

AADH	amino acid dehydrogenase
Asp	aspartic acid
Boc	*tert*-butyloxycarbonyl
*t*BuOK	potassium *tert*-butoxide
CAL-B	*Candida antarctica* Lipase B
DKR	dynamic kinetic resolution
c	conversion
CLEA	Cross Linked Enzyme Aggregate
DMSO	dimethyl sulfoxide
E	enantiomeric ratio
EC	Enzyme Commision
ee	enantiomeric excess
ES	enzyme substrate complex
GC	gas chromatography
GDH	glucose dehydrogenase
hex	*n*-hexane
His	histidine
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
iBuTMS	isobutyl trimethoxysilane
IPA	isopropyl alcohol
KR	kinetic resolution
L	large group
LDH	lactate dehydrogenase
Lys	lysine
M	medium-sized group
MeOH	methanol
MeTMS	methyl trimethoxysilane
MOM	methoxymethyl ether
NMR	nuclear magnetic resonance
Nu	nucleophile
PDC	pyruvate decarboxylase
PLP	pyridoxal-5'-phosphate
PMP	pyridoxamine-5'-phosphate
PrTMS	propyl trimethoxysilane
PVA	polyvinyl alcohol
RTMS	alkyl trimethoxysilane
Ser	serine
TMOS	tetramethoxysilane
TBME	*tert*-butyl methyl ether

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following original publications referred to in the text by their Roman numerals (I-III).

I. Päiviö, M.; Mavrynsky, D.; Leino, R.; Kanerva, L. T. Dynamic kinetic resolution of a wide range of secondary alcohols: cooperation of dicarbonylchlorido(pentabenzyl-cyclopentadienyl)ruthenium and CAL-B. *Eur. J. Org. Chem*. **2011**, 1452-1457.

II. Päiviö, M.; Perkiö, P.; Kanerva, L. T. Solvent-free kinetic resolution of primary amines catalyzed by *Candida antarctica* lipase B: effect of immobilization and recycling stability. *Tetrahedron: Asymm.* **2012**, *23*, 230-236.

III. Päiviö, M.; Kanerva, L. T. Reusable ω-transaminase sol-gel catalyst for the preparation of amine enantiomers. *Process Biochem.* **2013**, *48*, 1488-1494.

The original publications have been reproduced with the permission of the copyright holders.

1. INTRODUCTION

Enzymes are catalytic proteins found in living organisms. Catalysis takes place at the active site of the enzyme. Since enzymes consist of L-α-amino acids their active site is able to recognize chirality in a molecule, and only a molecule which is bound to the active site is transformed into a product. Chirality is an ability of a molecule to exist as two enantiomers. The two enantiomers are non-superimposable mirror images of one another and have the same physical properties apart from ability to rotate plane polarized light to opposite directions, a property used to distinguish the enantiomers of a compound. As a result, the enantiomers behave similarly until transferred into a chiral environment. Enantiomerism is often connected with the biological activity of a molecule. Thus, a pair of physiologically active enantiomers often possess different biological activities which can lead to even dramatically different effects in their biological targets (receptors, enzymes, transporters).

After the groundbreaking results on the applicability of enzyme catalysis in organic solvents published in the 1980s,[1,2] the prospects provided by enzyme catalysis have gradually been appreciated in organic and medicinal chemistry. In the following years, researchers have mapped the synthetic possibilities of biocatalysis. On one hand, the focus has been on finding novel enzymes to broaden the toolbox of biocatalysis, and on the other hand, the interest has been in developing viable biocatalytic synthetic methods to produce molecules of complex stereochemical structures. A combination of various modern techniques has been successfully utilized, for example, in the engineering of a robust ω-transaminase catalyst which considerably facilitated the manufacture of the anti-diabetic drug sitagliptin as its (*R*)-enantiomer (trade name Januvia, Figure 1).[3,4] The implementation of the economically viable manufacturing process, published in 2010, has for one established biocatalysis as a worthy methodology and an alternative to the conventional synthetic strategies.

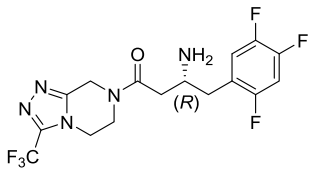


Figure 1. Structure of the anti-diabetic drug sitagliptin.

The present thesis discusses biocatalysis in the context of secondary alcohols and primary amines, and their preparation in enantiopure form. The discussion is restricted to the use of

lipases and ω-transaminases as biocatalysts. Stabilization of the biocatalysts via immobilization is also discussed. Present biocatalytic synthesis methods for the preparation of enantiopure secondary alcohols and primary amines are presented utilizing 1-phenylethanol and 1-phenylethylamine as model compounds. The literature review provides a background to the research conducted in the thesis. In the research, biocatalytic kinetic resolution techniques were studied and applied to the preparation of secondary alcohols **1**-**31** and primary amines **32**-**40** as their pure enantiomers (Structures are presented in Section 3, Figure 5). The emphasis of the studies was on the synthetic usability of the methods. The research results have been published as three original papers in international journals (Papers I-III).

2. REVIEW OF LITERATURE

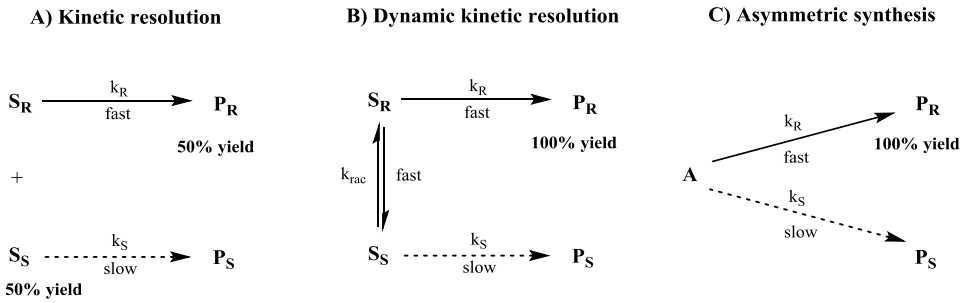
2.1. Biocatalysis

Catalysis is a process where a catalyst increases the rate of a chemical reaction by reducing the activation energy of the reaction. The catalyst itself is not consumed or altered in the process. Biocatalysis is traditionally connected to different life processes. The present thesis concerns biocatalysis in terms of its applicability in chemical reactions with non-natural substrates under synthesis conditions. Biocatalysis covers the areas of catalysis which utilize enzymes in whole cells, individual enzymes, catalytic antibodies and ribozymes as catalysts. This thesis focuses on reactions catalyzed by individual enzymes and, with ω-transaminases, also enzyme containing cells are included in the discussion. According to the types of reactions they catalyze, enzymes are divided into six classes: 1. Oxidoreductases, 2. Transferases, 3. Hydrolases, 4. Lyases, 5. Isomerases and 6. Ligases.[5] Of the two enzyme types discussed herein, lipases belong to the class of hydrolases and ω-transaminases to the class of transferases.

Enantiopure compounds are of high value in pharmaceutical, fine chemical and agrochemical industries. Consequently, enormous efforts have been invested in the development of viable methods for their production. Biocatalysis provides powerful tools to produce versatile enantiopure compounds under ambient conditions at the temperature range of 20-40 °C and at normal pressure and in aqueous environments at neutral or close to neutral pH. Biocatalysts alone or combined with chemical reaction steps (chemoenzymatic synthesis) are widely exploited in academic research and, also increasingly, in industrial applications. Several strategies have been utilized to accomplish new efficient biocatalytic methods for the preparation of secondary alcohol and primary amine enantiomers. The herein reported biocatalytic synthesis strategies cover different kinetic resolution and dynamic kinetic resolution methods, as well as methods utilizing asymmetric synthesis. When the viability and synthetic usability of a biocatalytic method is estimated, several factors should be considered. In addition to the chemo-, regio- and enantioselectivities and the substrate specificity of a biocatalyst, the factors also include catalyst availability, expense, ease of handling and reusability, as well as substrate and product solubilities and ease of product recovery.

2.2. Synthetic methods employing biocatalysis

Biocatalysis is widely applied in the kinetic resolution (KR) of racemic mixtures. Kinetic resolution results in the separation of the substrate enantiomers (S_R and S_S) as the fast-reacting enantiomer (here S_R) reacts to the product (here P_R) and the slow-reacting enantiomer (here S_S) remains unreacted (Scheme 1A). Accordingly, the method provides both enantiomers of the racemate but, at the same time, the maximum theoretical yield of an enantiomer is only 50% of the racemate.



Scheme 1. Kinetic resolution (A), dynamic kinetic resolution (B) and asymmetric synthesis (C).

When only one enantiomer of a racemate is desired the 50% yield of kinetic resolution can be considered as a drawback. To overcome the limitation, dynamic kinetic resolution (DKR) processes have been developed (Scheme 1B). In DKR, the slow-reacting substrate enantiomer (here S_S) is racemized *in situ* and the reaction ideally results in the formation of one product enantiomer (here P_R) according to the enantiopreference of the enzyme used. The continuous racemization between the substrate enantiomers (S_R and S_S) enables in theory the maximum yield of 100% (Scheme 1B). The KR and DKR methods are based on the enzymatic discrimination between the two substrate enantiomers, whereas in asymmetric synthesis the chirality is introduced to a prochiral substrate (A) according to the enantioprefence of an enzyme (Scheme 1C). Thus, asymmetric synthesis provides an enantiopure product (here P_R) with the theoretical yield of 100%. As both DKR and asymmetric synthesis provide merely one enantiomer, enantiocomplementary enzymes are needed when both enantiomers are of interest.

2.3. Considerations on kinetic resolution and dynamic kinetic resolution

Enantiopurity of the unreacted substrate and the obtained product enantiomers in a kinetic resolution can be determined using substrate and product enantiomeric excess values (ee_s and ee_p, respectively for an (*S*)-selective reaction) according to Equations 1 and 2, where [S_R] and

$[S_S]$ denote the concentrations of the substrate enantiomers and $[P_R]$ and $[P_S]$ the concentrations of the product enantiomers.[6] The *ee* values (ee_s and ee_p) are related to the reaction conversion (c) through Equation 3. The enantiomeric ratio of a kinetic resolution (E, Equations 4 and 5) as a constant value independent of reaction conversion is a measure of enantioselectivity. Equations 4 and 5 can only be applied when a reaction is irreversible and no side-reactions occur. For reversible reactions, E values can be determined when the equilibrium constant K of the reaction is known,[7] however, such reactions are rarely of synthetic interest.

$$ee_s = \frac{[S_R]-[S_S]}{[S_R]+[S_S]} \qquad \text{(Eq. 1)}$$

$$ee_p = \frac{[P_S]-[P_R]}{[P_R]+[P_S]} \qquad \text{(Eq. 2)}$$

$$c = \frac{ee_s}{ee_s + ee_p} \qquad \text{(Eq. 3)}$$

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \qquad \text{(Eq. 4)}$$

$$E = \frac{\ln[1-c(1+ee_P)]}{\ln[1-c(1-ee_P)]} \qquad \text{(Eq. 5)}$$

In theory, a kinetic resolution (Scheme 1A) with an E value >800 provides both enantiomers (ee_s and ee_p =100%, with three significant figures) of a racemic mixture at 50% conversion. In practice, the enantiomers are obtained in high enantiopurity (*ee* >96%) at 50% conversion when $E \geq 200$. The slow-reacting substrate enantiomer can always be obtained with high enantiopurity when the reaction is allowed to proceed to sufficiently high conversions (c >50%), although, the yield of the enantiomer decreases with a decreasing E value (Figures 2A-F, ee_s marked with blue line). At the same time, the enantiopurity of the fast-reacting substrate enantiomer decreases rapidly when the E value decreases (Figures 2A-F, ee_p marked with red line). As an example, a kinetic resolution with E=5 has the maximum ee_p value of 66% and provides the unreacted enantiomer with an ee_s >98% with the maximum yield of 16% (Figure 2A).

In an ideal DKR (Scheme 1B), the substrate remains racemic throughout the reaction, and hence the initial state of the corresponding kinetic resolution depicts the DKR reaction and provides means to estimate its feasibility. Consequently, the *ee* value of the DKR product (marked with green dashed line in Figures 2A-F) is equal to the ee_p value of the corresponding KR reaction (marked with red line in Figures 2A-F) at zero conversion. Thus, when a kinetic resolution with

a low *E* value (*E* = 5-10) is transformed into the corresponding DKR, the result is unsatisfactory. To obtain an enantiomer product with *ee* ≥95% *E* value ≥50 is required (Figures 2D-F), and when aiming at *ee* ≥99% DKR needs to be based on a highly enantioselective kinetic resolution method (*E* ≥200).

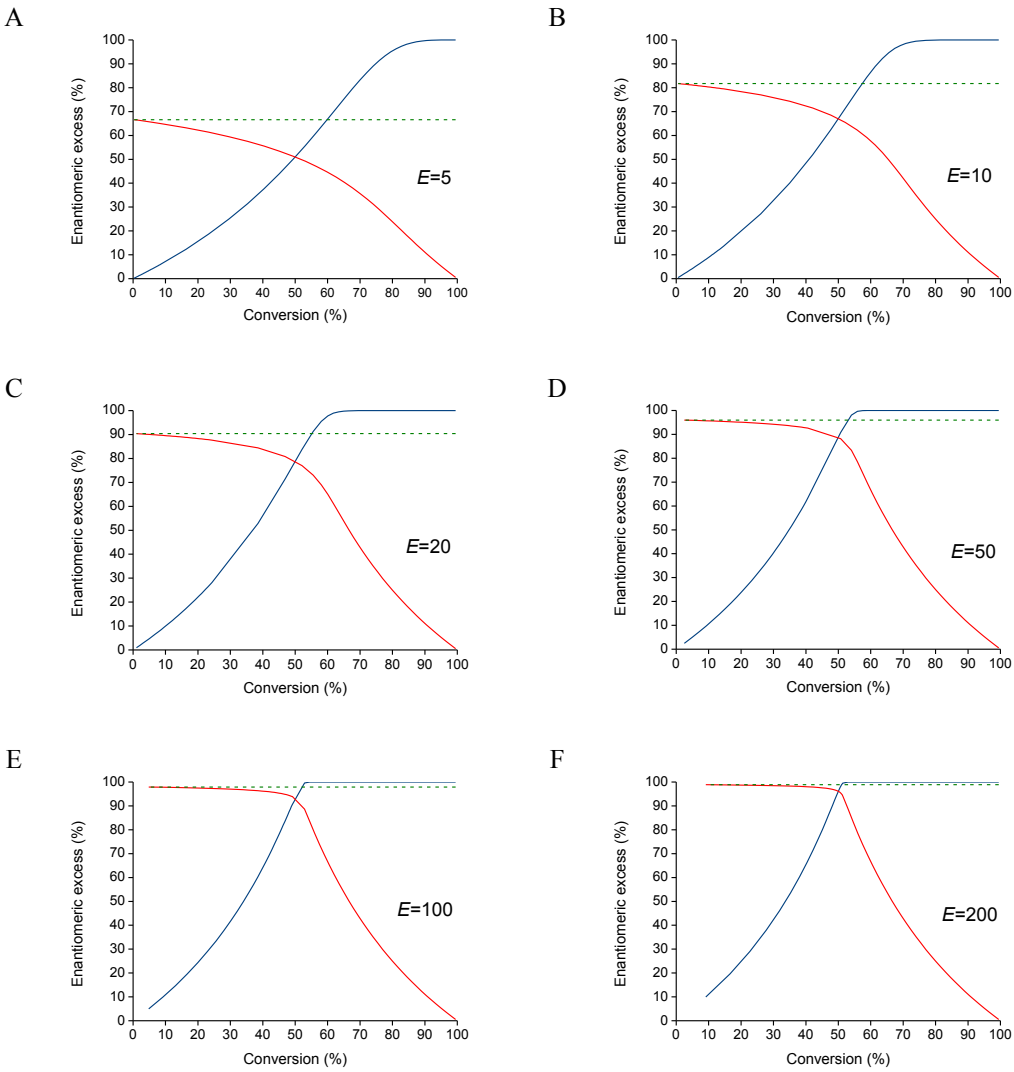
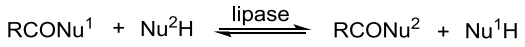


Figure 2. Enantiomeric excess (ee_s marked with blue line and ee_p with red line, product *ee* in dynamic kinetic resolution marked with dashed green line) vs. conversion in kinetic resolution and dynamic kinetic resolution: *E*=5 (A), *E*=10 (B), *E*=20 (C), *E*=50 (D), *E*=100 (E) and *E*=200 (F).

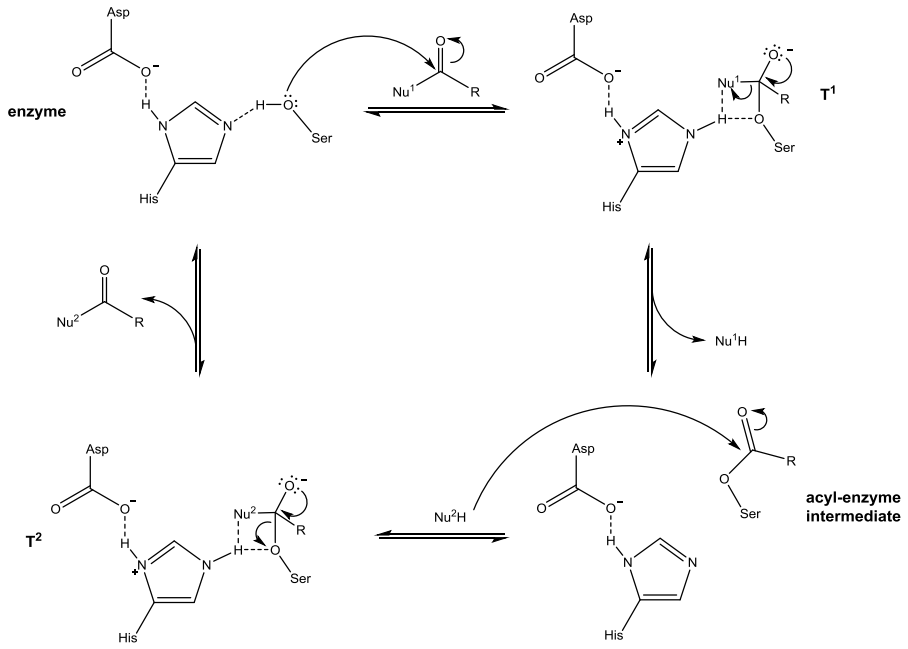
2.4. Lipase catalysis

Lipases (EC 3.1.1.3) belong to the group of hydrolytic enzymes, more accurately to serine hydrolases, and in nature they catalyze the hydrolysis of triacylglycerols to give glycerol and fatty acids in aqueous environment. Lipases do not require co-factors. Since the discovery of their usability as catalysts in organic solvents,[1,2] the synthetic use of lipases has broadened significantly, and the catalytic mechanism has been exploited in various reactions over the years. In general, lipases catalyze the acyl-transfer between an acyl donor ($RCONu^1$) and a nucleophile (Nu^2H) in the formation of a new acyl-product ($RCONu^2$) and a new nucleophile (Nu^1H) as the nucleophilic moieties (Nu^1 and Nu^2) are switched (Scheme 2). When used for racemic substrates the enantioselective reaction is often considered either as the acylation of a racemic nucleophile (Nu^2H) or as the deacylation of a racemic acyl donor ($RCONu^1$). In most cases, the acyl donor is an ester, whereas different nucleophiles such as alcohols, amines and thiols can be used in the place of water in non-aqueous media.[8] Herein, secondary alcohols and primary amines are discussed more precisely as they are the target compounds investigated in this thesis.



Scheme 2. Lipase-catalyzed reaction.

Lipase catalysis takes place according to the so-called ping-pong bi-bi -mechanism, according to which two substrates ($RCONu^1$ and Nu^2H) react to give two products ($RCONu^2$ and Nu^1H) in such a way that the first product (Nu^1H) is released prior to the binding of the second substrate (Nu^2H).[9] In the active site of all lipases, serine, histidine and aspartate or glutamate residues form the so-called catalytic triad which initiates the catalysis (Scheme 3). The orientation of these amino acid residues decreases the pK_a-value of the serine hydroxyl, enabling its action as a nucleophile which then attacks the carbonyl carbon of the acyl donor ($RCONu^2$) in the first step of the reaction. The reaction proceeds via a tetrahedral transition state (T^1) which reacts further releasing the first product (Nu^1H) and forms the acyl-enzyme -intermediate. In the second reaction step, the nucleophile (Nu^2H) attacks the carbonyl carbon of the acyl-enzyme intermediate and the reaction proceeds again via a tetrahedral transition state (T^2) in the formation of the second product ($RCONu^2$) and the regeneration of the free enzyme.



Scheme 3. Mechanism of the lipase-catalyzed reaction.

2.4.1. Lipases in synthetic chemistry

Lipases are among the most widely used enzymes in synthetic chemistry due to their broad substrate specificity, ease of accessibility and effortless handling. Due to their thermostability and robustness, lipases can even be utilized in synthetic methods which require harsh reaction conditions. As a disadvantage, most wild-type lipases possess the same enantiopreference in the reactions they catalyze. As a result, the faster reacting enantiomer of a racemic secondary alcohol can often be predicted on the basis of the so-called Kazlauskas rule (Figure 3, L and M denote large- and medium-sized substituents, respectively).[10] A representative example to the rule is 1-phenylethanol (*rac*-**1**, (*R*)-**1** being the fast reacting enantiomer) which is used as a model substrate throughout of this thesis (Figure 3).

Figure 3. The faster reacting enantiomer of racemic secondary alcohols and primary amines.

Accordingly, a lipase can be predicted to be (*R*)-enantioselective towards secondary alcohols which have the same CIP-sequence priorities (OH>L>M) as **1**. Primary amines of the type $R^1R^2CH\text{-}NH_2$ are isosteric with secondary alcohols, thus the faster reacting amine enantiomer [here (*R*)-**32**] can be predicted according to the same rule (Figure 3). Racemic 1-phenylethylamine (*rac*-**32**), the amine counterpart of *rac*-**1**, is used as a model substrate in the discussions concerning primary amines.

2.4.2. *Candida antarctica* lipase B

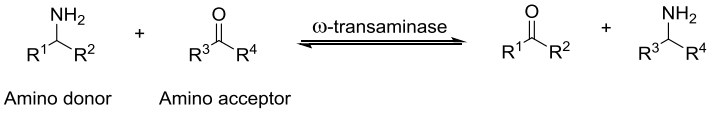
Lipase B from yeast *Candida antarctica* (CAL-B) is probably the most widely used biocatalyst in synthetic chemistry due to its robustness, activity and enantioselectivity. The yeast strain was isolated in Antarctica when enzymes with extreme properties were sought. CAL-B, having a pH optimum at 7, is stable in aqueous media in the pH range of 3.5–9.5.[11] The denaturation temperature of the enzyme in aqueous media varies between 50 °C and 60 °C depending on the pH of the medium. The use of organic solvents and immobilization further enhances the thermostability of the enzyme. CAL-B is commercially available as a free enzyme and as different immobilized preparations (Table 1).

The crystal structure of CAL-B was reported 20 years ago, and with help of the structure and modeling the features of the enzyme have been elucidated.[12-14] The investigations have provided insights into the active site of CAL-B explaining the substrate specificity and enantioselectivity of the enzyme. The studies have also indicated the existence of a so-called water tunnel from the surface of the enzyme to the active site. The water tunnel enables the entrance and easy participation of water molecules in organic solvents in competing catalytic events.[15]

2.5. ω-Transaminase catalysis

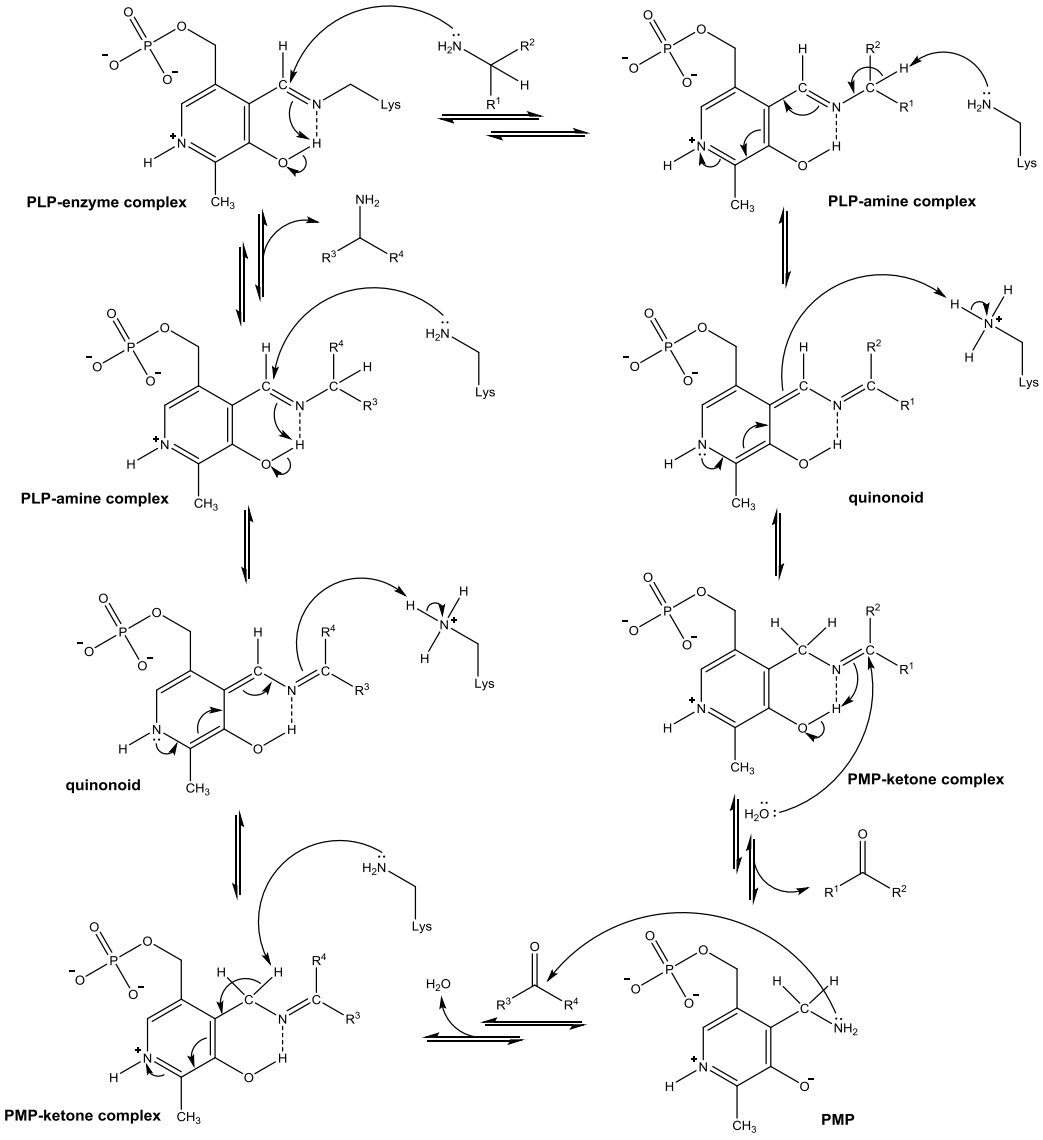
Transaminases (EC 2.6.1.n) are pyridoxal-5'-phosphate (PLP) -dependent enzymes which catalyze the transfer of an amino group from an amino donor to an amino acceptor. ω-Transaminases belong to the group of transaminases and they catalyze the transfer of an amino group between an amine and a ketone or an aldehyde without the requirement of an adjacent carboxylic acid moiety (Scheme 4). Thus, ω-transaminases have relatively broad substrate specificity and their use enables the preparation of versatile amines. Two synthetic approaches, kinetic resolution and asymmetric synthesis, can be utilized in the transamination reaction for the preparation of enantiopure primary amines (Scheme 4). In kinetic resolution, a racemic amino donor is enantioselectively deaminated resulting in enantiomeric enrichment of the slow-

reacting substrate enantiomer. In asymmetric synthesis, the asymmetric transamination transforms a prochiral amino acceptor into a single amine enantiomer. Both (*R*)- and (*S*)-selective enzymes are available, enabling enantiocomplementary synthesis strategies.



Scheme 4. ω-Transaminase catalyzed transamination reaction.

Transaminase catalysis takes place according to the ping-pong bi-bi –mechanism. The two substrates ($R^1R^2CH\text{-}NH_2$ and R^3R^4CO) react to give two products (R^1R^2CO and $R^3R^4CH\text{-}NH_2$) in such a way that the first product (R^1R^2CO) is released before the second substrate (R^3R^4CO) is bound. As any detailed mechanism for the ω-transaminase-catalyzed reaction has not been reported to date, a general PLP-mediated mechanism of a transamination reaction is depicted herein (Scheme 5).[16,17] In the resting state of the enzyme, the PLP aldehyde group is covalently bound to an amino group of a lysine residue (Lys) of the transaminase via imine linkage (PLP-enzyme complex, Scheme 5). The transamination reaction begins with a nucleophilic addition of the amino group of the amino donor ($R^1R^2CH\text{-}NH_2$) to the C=N bond of the PLP-enzyme complex in the first reaction step, which gives a PLP-amine complex and releases the amino group of Lys. The reaction proceeds via deprotonation of the PLP-amine complex and reprotonation of the formed quinonoid, and the C=N bond is tautomerized accordingly. Hydrolysis of the PMP-ketone complex gives the first product (R^1R^2CO) and pyridoxamine-5'-phosphate (PMP). In the second step of the reaction, the amino group of PMP attacks the carbonyl carbon of the amino acceptor (R^3R^4CO). After release of water, the ketone and PMP form an imine (PMP-ketone complex). After deprotonation a quinonoid is formed, to which Lys donates a proton. The protonation initiates the tautomerization of the C=N bond to give a PLP-amine complex. Nucleophilic addition of the Lys amino group to the complex releases the second product ($R^3R^4CH\text{-}NH_2$) and completes the catalytic cycle.



Scheme 5. Mechanism of the transaminase-catalyzed reaction.

2.5.1. ω-Transaminases in synthetic chemistry

During the last decade ω-transaminases have been intensely investigated due to their high potential in the preparation of valuable amine enantiomers. Due to the novelty of the enzymes in synthetic chemistry, the research first focused on enzyme discovery and method development. In recent years, the focus has shifted towards synthetic usability of ω-transaminase catalysis. Most ω-transaminase enzymes show (*S*)-enantioselectivity which has

led to extensive searches to find novel (*R*)-selective ω-transaminases.[18,19] At first, identification of novel ω-transaminases relied on the classical, relatively laborious approach which utilizes enrichment cultures in the screening of ω-transaminase activity in microorganisms. Recently, computational methods exploiting public database search in discovery of novel ω-transaminases have emerged as a viable substitute.[19-21] The increase in biological data during the last decades together with prediction of protein function based on sequence homology has enabled these cost-efficient and less time-consuming *in silico* screenings. Only few suppliers offer selections of ω-transaminases and, as mentioned above, a significant part of ω-transaminases used are in-house recombinant enzymes.

Transamination reactions are mainly performed in aqueous media which often means relatively low substrate concentrations and reaction efficiencies due to the poor solubility of the substrates and products. Accordingly, water-miscible organic solvents have been used as co-solvents to improve solubility.[3] The recently published methods, operable in organic solvents, have provided new opportunities for the efficient utilization of ω-transaminase catalysis.[22,23]

2.5.2. *Arthrobacter* sp. ω-transaminase

The ω-transaminase from *Arthrobacter* sp. KNK168 is one of the few identified (*R*)-selective ω-transaminases. The enzyme originates from a micro-organism isolated from soil and is commercially available as an enzyme lyophilizate (from Codexis as ATA-117 with the substitution of I306V).[24] The lyophilizate has been utilized in experimental work of this thesis. The crystal structure of the enzyme has not been reported. The enzyme has been used as whole-cell preparations, cell-free extracts, enzyme lyophilizates and immobilizates.[III,25-30] Studies on optimal reaction conditions for the *Arthrobacter* sp. ω-transaminase catalysis, utilizing whole-cell[26] and free enzyme preparations,[27-29] have been reported. The observed temperature optimum was 30 °C after which a sharp decrease in enzyme activity and stability was detected. The optimum pH range of the ω-transaminase catalysis depended on the catalysis formulation.[26-29] The whole-cell preparation has an optimum under weak alkaline conditions (pH 8.5–9),[26] whereas the enzyme lyophilizates started to lose activity after pH 8 and stability after pH 7.[27-29] The stability of the free enzyme also suffered from the introduction of organic co-solvents, however, activity enhancements were detected in the presence of MeOH and DMSO (5-15%, v/v).[28,29] The wild-type enzyme has been successfully modified to obtain improved catalyst activity and stability under harsh synthesis conditions.[3] Immobilization of

the modified enzyme further enhanced the feasibility of the synthesis method by allowing the use of neat isopropyl acetate as a solvent.[23]

2.6. Immobilization of enzyme catalysts

A great deal of effort has been focused to improve or alter enzyme characteristics via immobilization.[31-35] The choice of an appropriate immobilization method requires a careful consideration of the particularities of the chosen enzyme and the eventual synthetic application. When operating in aqueous reaction media, immobilization of an enzyme transforms a homogeneous catalyst into a heterogeneous catalyst enabling its recovery and reuse. In organic solvents, enzyme immobilization is used to improve dispersion and stability of the enzyme and accordingly, to make the enzyme more tolerant towards harsh reaction conditions, often a requirement for a feasible synthesis method. Successful enzyme immobilization enhances the catalytic performance of an enzyme, however, the real synthetic feasibility of the immobilization is tested in reuse of the immobilizate. When these criteria are met, biocatalytic synthesis method economics can be greatly enhanced by enzyme immobilization. Increasingly today, enzyme immobilization combined with protein engineering is a case-specific quest for optimum enzyme performance under such reaction conditions which enable the efficient synthesis of a given high-value target molecule.[3]

Enzyme immobilization methods can be divided into different categories on the basis of the method characteristics (Figure 4). In adsorption of enzymes, either hydrophobic or hydrophilic solid supports are employed depending on the enzyme and the application (Figure 4A).[32] Non-covalent interactions involved in the adsorption are van der Waals interactions, entropy changes and hydrogen bonding. Enzymes can also be non-covalently attached on versatile charged carriers via ionic interactions (Figure 4B). When covalent binding is used, enzyme molecules are tightly fixed to a solid support (Figure 4C).[33] In the process enzyme molecules are, however, chemically modified which can harm their activity. Covalent binding is also utilized in carrier-free enzyme immobilization. Cross Linked Enzyme Aggregates (CLEA) are formed when enzyme molecules are aggregated and cross-linked in the presence of a difunctional agent such as glutaraldehyde (Figure 4D).[34] The confinement of an enzyme in a carrier material is less likely to interfere with the activity of the enzyme. Such methods include immobilization via entrapment and encapsulation, which also protect the enzyme from the surrounding environment. In entrapment, enzyme molecules are confined into a rigid network of carrier

material (Figure 4E), whereas encapsulation encloses enzyme molecules in carrier capsules (Figure 4F).[35]

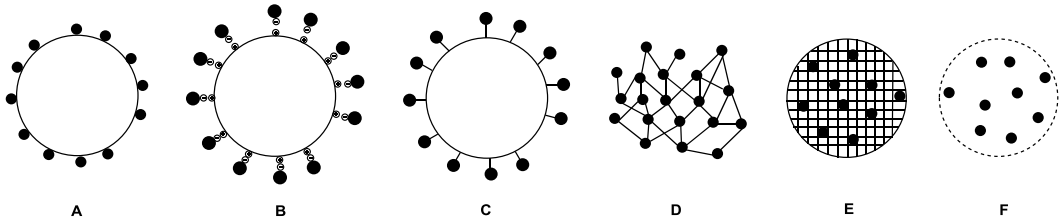


Figure 4. Schematic illustration of methods utilized in enzyme immobilization: Adsorption (A), Attachment via ionic interactions (B), Covalent binding (C), CLEA (D), Entrapment (E) and Encapsulation (F).

2.6.1. Immobilization of lipases

Due to the growing interest over the years, the commercial availability has increased to cover a wide variety of lipases. Along with the development of enzyme immobilization techniques, the variety of commercially available immobilizates has also expanded. Examples of different commercial CAL-B preparations are presented in Table 1. Nevertheless, novel immobilization methods are constantly being developed and optimized for academic studies and for industrial applications. A case-specific enzyme immobilization, although being somewhat laborious, affords an enzyme catalyst meeting the demands of a given reaction system which explains the steady interest in in-house immobilization of lipases over the years. Due to the synthetic usability of lipase catalysis, immobilization of lipases is a broad research area which is under constant development. Thus, only an overview to the research area is provided herein.

With lipases, adsorption[36,37] on hydrophobic and hydrophilic supports has been the most widely used immobilization technique owing to its cost-efficiency and simplicity (Table 1, Entries 3-6). As a drawback, the attachment of an enzyme to a solid support by relatively weak adsorption forces can cause enzyme leaching under harsh conditions and in aqueous media. Under such conditions, encapsulation and entrapment methods can be applied to exclude the enzyme molecules from the reaction medium and to prevent their leaching.[38,39] These methods have been typically utilized in recently reported in-house lipase immobilizations. Some sol-gel entrapped lipases are available on the market (Table 1, Entry 10). Also covalent attachment[40] and CLEA techniques[41] have been used in lipase immobilization, and some preparations are commercially available as well (Entries 7-9).

Table 1. Commercially available CAL-B preparations.

Entry	Lipase preparation	Form	Activity[a]	Supplier
1	CV-CALBY	non-immobilized dry powder	43000 TBU/g	Chiral Vision
2	Novozymes CALB L	non-immobilized aqueous solution	≥5000 LU/g	Sigma
3	Novozym 435	adsorbed on macroporous acrylic resin	≥5000 PLU/g	Sigma
4	IMMCALB-T1-350	adsorbed on acrylic beads	4000 TBU/g 10000 PLU/g	Chiral Vision
5	SPRIN liposorb CALB	adsorbed on acrylic resin	>9000 PLU/g	Sprin technologies
6	SPRIN actisorb CALB	adsorbed on polystyrene resin	>7000 PLU/g	Sprin technologies
7	IMMCALB-T2-350	covalently attached to acrylic beads	2500 TBU/g 5000 PLU/g	Chiral Vision
8	SPRIN epobond	covalently immobilized on epoxy acrylic resin	>1500 PLU/g	Sprin technologies
9	CaLB CLEA-ST	cross-linked enzyme aggregate as powder	10000 TBU/g	CLEA technologies
10	Sol-Gel-AK	immobilized on Sol-Gel-AK on pumice as beads	1.5 TBU/g	Sigma

[a] Activity of enzyme in tributyrin units per gram enzyme (TBU/g) or in propyl laurate units per gram enzyme (PLU/g).

2.6.2. Immobilization of ω-transaminases

With increasing number of novel ω-transaminases with synthetic potential, the need to stabilize the enzymes to ensure their activity and selectivity, and to enable their reuse has become crucial. To date, only non-immobilized ω-transaminase lyophilizates are commercially available by few suppliers such as Codexis and Syncozymes. Consequently, when an immobilized ω-transaminase catalyst is required, one usually relies on an in-house enzyme immobilizate.

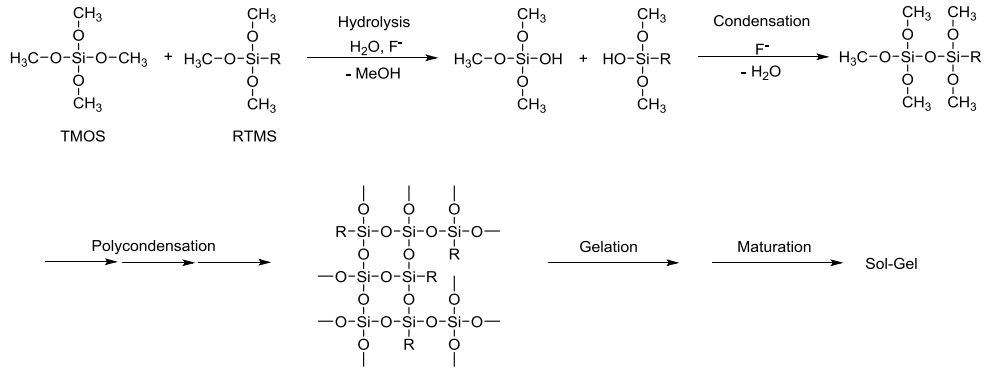
The reported in-house immobilization methods for ω-transaminases include methods for individual ω-transaminases and ω-transaminase containing cells. In the most commonly applied immobilization techniques, enzymes and whole cells are excluded from an aqueous reaction medium by confining them in different matrices.[III,30,42-46] These less invasive immobilization methods constitute entrapment of the enzyme into a sol-gel matrix[III,30,42] and encapsulation of

the enzyme containing cells into calcium alginate beads[43,44] or a polyvinyl alcohol hydrogel,[45] as well as incorporation of whole-cells in a chitosan precipitate.[46] Covalent immobilization of ω-transaminase on chitosan beads has also been reported.[47,48] The reported ω-transaminase catalyzed reactions in organic solvents utilize crude preparations of free ω-transaminases[22] or ω-transaminases adsorbed on hydrophobic supports[23].

2.6.3. Sol-gel entrapment in enzyme immobilization

Two different literature methods are commonly utilized in sol-gel entrapment of enzymes.[49,50] The methods rely on acid and base catalyzed hydrolysis and condensation of the silane precursors followed by gelation and maturation of the sol-gel (Scheme 6). Since the method utilizing fluoride-ion as a Lewis base catalyst has been applied to the entrapment of CAL-B[II] and *Arthrobacter* sp. ω-transaminase[III] in this thesis, the discussion herein is restricted to this method.

Versatile sol-gel precursors have been applied in different mixtures and combinations. A common feature has been the use of the hydrophilic tetramethyl orthosilicate [$Si(OMe)_4$, TMOS] together with more hydrophobic alkyltrimethoxysilanes [$RSi(OMe)_3$, RTMS; where R is a non-hydrolyzable alkyl moiety] such as methyl-, ethyl-, propyl or isobutyltrimethoxysilane (Scheme 6).[51] The resulting inert sol-gel network protects the entrapped enzyme from the external environment and prevents enzyme leaching, but can also cause diffusion limitations. Composition of the matrix has a substantial effect on the activity and stability of the entrapped enzyme. Characteristics of the enzyme determine the hydrophobicity of the sol-gel matrix. The hydrophobicity is tailored by the proportion of the alkyltrialkoxysilane component in the matrix. The alkyl-groups of the component provide hydrophobicity to the microenvironment of the entrapped enzyme and to the external surface facing the reaction medium. As a result, aqueous reaction medium does not effectively wet the sol-gel surface which can cause sluggish reaction progression.[50]



Scheme 6. Fluoride-ion catalyzed sol-gel synthesis.

A known way of improving the properties of the sol-gel catalysts is the use of additives such as polyvinyl alcohol (PVA), isopropyl alcohol (IPA), crown ethers, surfactants and Celite.[50] Entrapment of an additive or additives together with the enzyme can dramatically enhance the catalytic performance of the enzyme,[52] but the effects depend on the enzyme, substrates, reaction environment and the composition of the sol-gel. Considerable case-specific activity and enantioselectivity enhancements upon introduction of additives have been reported, however, any universal conclusions of their effects on sol-gel entrapment are difficult to make.

Sol-gel entrapped lipases have displayed relative activities up to 800-1000% (with respect to free enzyme powder) in esterification reactions in organic solvents,[37,38] whereas application of sol-gel lipases to ester hydrolysis in aqueous solutions have mostly led to relative activities below 40%.[53] Sol-gel CAL-B catalysts have also been successfully reused in organic solvents giving excellent retention of activity and enantioselectivity.[52] Sol-gel entrapment of ω-transaminases has afforded heterogeneous catalysts for reactions in aqueous environments.[III,30] The use of sol-gel ω-transaminases enabled successful catalyst reuse in terms of retention of enantioselectivity and synthetic usability (measured as a retention of conversion).

2.7. Preparation of secondary alcohol enantiomers utilizing lipase catalysis

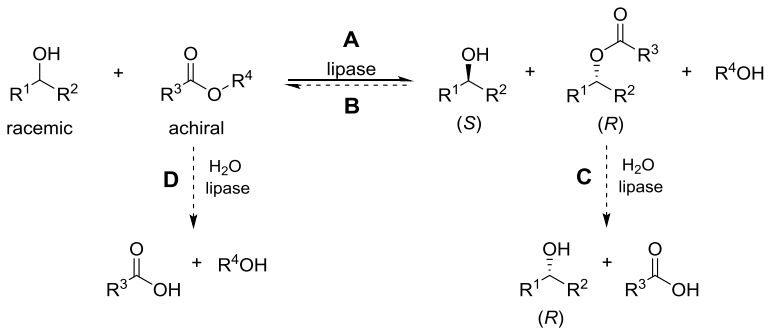
2.7.1. Kinetic resolution of secondary alcohols

There are several possibilities to utilize lipase-catalyzed kinetic resolution in the preparation of enantiomers of a racemic secondary alcohol.[54] In addition to the conventional lipase-catalyzed hydrolysis of racemic esters in aqueous environments, alcoholysis and aminolysis reactions have been extensively studied in non-aqueous media. Furthermore, lipase-catalyzed *O*-acylation of racemic alcohols performed in dry organic solvents[55] has been widely applied. The

discussion of this section is limited to the lipase-catalyzed *O*-acylation -based kinetic resolutions of racemic secondary alcohols (Scheme 7, Route A). Kinetic resolution of secondary alcohols described herein affords a basis for the corresponding dynamic kinetic resolution methods discussed in Section 2.7.2.

Lipase-catalyzed *O*-acylation

The kinetic resolution methods consist of esterification (Scheme 7, Route A, R^4 = H, a reaction between an alcohol and a carboxylic acid) and transesterification (Route A, R^4 = alkyl or aryl, a reaction between an alcohol and an ester) depending on the nature of the co-substrate. Esterification of the reacting alcohol enantiomer with a carboxylic acid results in a stoichiometric formation of water alongside of the desired ester enantiomer formation, which increases the water content of the reaction system (Route A, R^4 = H). If not removed from the reaction medium, water can act as a nucleophile in the reverse lipase catalyzed hydrolysis causing the esterification to stop at an equilibrium (Route B, R^4=H). Lipase-catalyzed transesterification occurs between the reacting enantiomer of a racemic alcohol and an achiral ester and forms alcohol instead of water (Route A, R^4 = alkyl or aryl). Any accessible water can lead to lipase-catalyzed hydrolytic side-reactions (Routes C and D). Depending on the character of the alcohol released (R^4OH, R^4 = alkyl or aryl), a reverse alcoholysis reaction can also take place (Route B).



Scheme 7. Lipase-catalyzed *O*-acylation of secondary alcohols (R^1>R^2, CIP-priority).

Achiral acyl donors in *O*-acylation

Activation of the acyl donor is often required to facilitate fast lipase-catalyzed transesterification (Scheme 7, Route A, R^4 = alkyl or aryl). However, activated acyl donors are susceptible to hydrolysis which leads to their partial consumption (Route D).[56] Degree of the

hydrolysis can be decreased by thorough drying of the substrates and the organic solvent used. Another important feature of an acyl donor is the nature of the alcohol liberated in transesterification (Route A, R^4OH, R^4 = alkyl or aryl). In order to prevent the reverse alcoholysis (Route B), the liberated alcohol should be a weak nucleophile or it should tautomerize into a compound not able to act as a nucleophile. In order to meet the demands, versatile acyl donors have been studied.[57] The widely used 2-halo esters, such as 2,2,2-trifluoroethyl acetate and butanoate have a high activation degree and they release 2,2,2-trifluoroethanol, which is a weak nucleophile rendering the transesterification "quasi-irreversible". Enol esters, vinyl and isopropenyl acetate, are probably the most widely used acyl donors in kinetic resolution of secondary alcohols. Enol acetates are highly activated and release unstable ethenol and propen-2-ol which tautomerize to give acetaldehyde and acetone, respectively, thus making the *O*-acylation irreversible.[58]

2.7.2. Dynamic kinetic resolution of secondary alcohols

The first reported DKR processes for secondary alcohols (cyanohydrins) utilized resins in combination with lipases.[59,60] The concept of DKR combining an enzyme with a transition-metal catalyst in the preparation of enantiopure secondary alcohols was first introduced in the late 1990s.[61,62] The discussion herein is restricted to transition metal complexes meeting the crucial requirements of sufficient catalytic activity and stability in the presence of an enzymatic kinetic resolution process. Furthermore, only complexes acting on secondary alcohols which have no other reactive functionalities, in addition to the hydroxyl-group, are discussed.

Hitherto, the most successful catalyst design is based on half-sandwich ruthenium complexes **41**-**47** (Figure 4).[63,64] The initially employed Shvo's catalyst **41** requires elevated temperature for generating the active monoruthenium species,[62,65] whereas the base-activated monomeric ruthenium complexes **42**-**47** can be utilized at ambient temperature.[I,66-73] In addition to homogeneous transition metal catalysis, heterogeneous zeolites and acid resins have been utilized in DKR of non-functionalized secondary alcohols.[74-77] The herein discussed DKR methods are based on CAL-B (the Novozym 435 preparation) -catalyzed kinetic resolution. As mentioned before, *rac*-**1** is utilized as a model compound in the following discussion (Scheme 8).

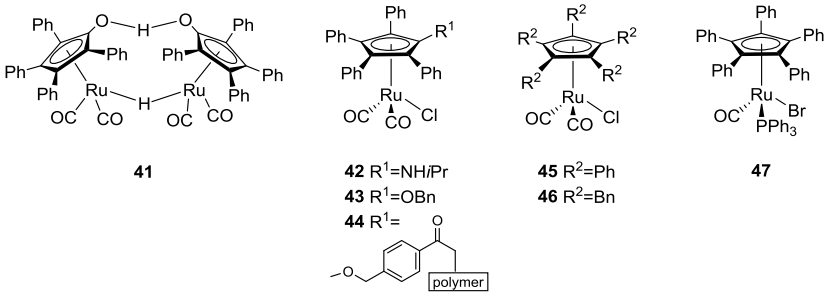
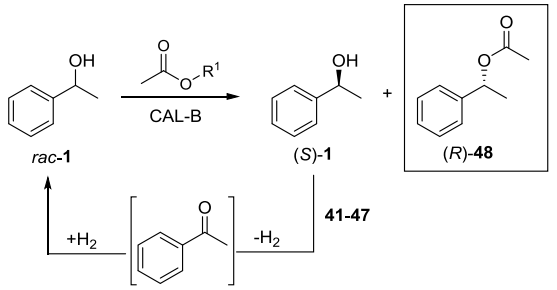


Figure 4. Ruthenium complexes **41**-**47**.

Dynamic kinetic resolution with CAL-B and ruthenium complexes

The racemization of secondary alcohols catalyzed by ruthenium complexes **41**-**47** is based on hydrogen-transfer occurring via oxidation of an alcohol [here (*S*)-**1**] to a ketone and the subsequent non-selective reduction of the ketone back to the alcohol (Scheme 8). The *in situ* racemization occurs alongside the CAL-B catalyzed *O*-acylation which produces the more reactive enantiomer [here (*R*)-**1**] as an ester [here (*R*)-**48**].

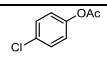


Scheme 8. Metalloenzymatic dynamic kinetic resolution of *rac*-**1**.

The first successful method employed a high loading of CAL-B (30 mg/1 mmol substrate) and binuclear **41** (2 mol-% in proportion to *rac*-**1**) in the DKR of *rac*-**1** with *para*-chlorophenyl acetate (3 equiv.) in toluene at 70 °C (Table 2, Entry 1).[62] After 46 hours, (*R*)-**48** was isolated in 80% yield and 99% *ee*. However, the (*R*)-acetate, obtained after purification, was contaminated with the similarly structured acyl donor. The significant amount of acetophenone formed via oxidation of *rac*-**1** decreased the yield of (*R*)-**48**. The scope of this method was extended to the preparation of 13 enantiopure (*R*)-acetates (mostly *ee*>99%) in yields of 63-88%.[65]

The first published monomeric ruthenium complex (**42**, 4 mol-%) together with a significantly lower CAL-B loading (3 mg $mmol^{-1}$) enabled the DKR of *rac*-**1** with isopropenyl acetate (1.5 equiv.) to (*R*)-**48** at 25 °C in toluene after 1.3 days (Table 2, Entry 2).[66,67] The presence of Na_2CO_3 (1 equiv.), neutralizing the acetic acid formed in the hydrolysis of the acyl donor, and lesser extent of acetophenone formation, favored by elevated temperature, contributed to the high yield (95%).[66] Use of the analogous ruthenium complex **45** (4 mol-%) and CAL-B (6 mg $mmol^{-1}$) under otherwise similar conditions enhanced the method substantially, affording (*R*)-**48** in 92% isolated yield after three hours (Entry 3).[69,70] Complex **46** (2 mol-%), bearing benzyl substituents on the cyclopentadienyl ligand, together with CAL-B provided (*R*)-**48** in 95% isolated yield in equal efficiency in terms of reaction time (3 h, Entry 7).[I] The reaction efficiency was enhanced due to the further optimized conditions [isopropenyl acetate (1.2 equiv.) and Na_2CO_3 (0.5 equiv.)] in addition to the lower racemization catalyst loading (2 mol-%), although a slightly higher CAL-B loading (10 mg $mmol^{-1}$) was used. The wide substrate scope, consisting of approximately 30 aromatic and aliphatic secondary alcohols resolved mainly in high yields and high enantiopurities, demonstrates the general usability of catalysts **42**, **45** and **46** in DKR.[I,66-71] Racemization catalyst **46** was developed for the DKR method discussed in Section 5.1.

Table 2. CAL-B and ruthenium complex **41**-**47** catalyzed dynamic kinetic resolution of 1-phenylethanol (*rac*-**1**) in toluene under inert atmosphere.

Entry	CAL-B (mg $mmol^{-1}$)	Racemization cat.[a] (mol-%)	Temp (°C)	Acyl donor (equiv.)		Time	Yield (%)	*ee* (%)	Ref.
1	30	**41** (2)	70		(3)	46 h	80	99	65
2	3	**42** (4)	25		(1.5)	1.3 d	95	>99	66
3	6	**45** (4)	25		(1.5)	3 h	92	>99	69
4[b]	8	**43** (4)	25		(1.5)	20 h	>99	>99	72
5[b]	8	**44** (4)	25		(1.5)	20 h	>99	>99	72
6[b]	8	**47** (4)	25		(2)	6 h	98	>99	73
7	10	**46** (2)	23		(1.2)	3 h	95	>99	I

[a] See Figure 4 for structures. [b] Performed in air.

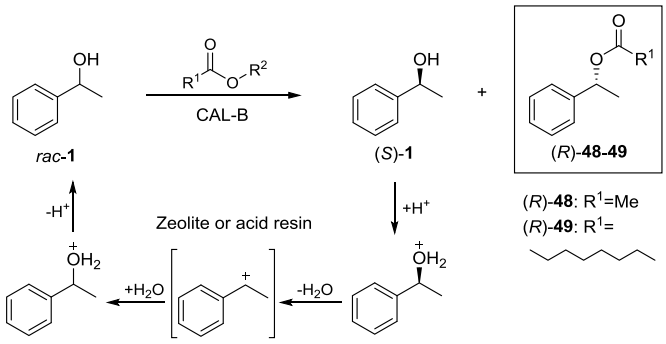
The shortcoming of catalysts **41**, **42**, **45** and **46** is their sensitivity towards air and moisture under DKR conditions. However, with an increase in the DKR scale from 1 mmol to 1 mol the process becomes less sensitive allowing a substantial decrease in the loading of catalysts **45** and

46 (0.05 mol-% vs. 4 and 2 mol-%, Table 2, Entries 3 and 7) and enhancing the economic viability of these methods.[78,79]

The ruthenium complexes **43** and **44** (4 mol-%) activated by K_3PO_4 (1 equiv.) catalyzed the DKR of *rac*-**1** with isopropenyl acetate (1.5 equiv.) with CAL-B (8 mg $mmol^{-1}$) in air (Table 2, Entries 4 and 5).[72] These methods afforded (*R*)-**48** in >99% yield after 20 hours. The catalyst system consisting of the polymer bound **44** and CAL-B was reusable giving full retention of conversion and enantioselectivity in up to three reaction cycles. The substrate scope of this method was extended to 10 secondary alcohols. An elevated temperature (50 °C) was required with aliphatic alcohols. Complex **47** was also stable under aerobic conditions, although it required the presence of Ag_2O (1 equiv.) to afford a high yield (98%) in the DKR of *rac*-**1** with isopropenyl acetate (2 equiv.) with CAL-B (8 mg $mmol^{-1}$, Entry 6).[73] This method was successfully applied in the DKR of six secondary alcohols.

Dynamic kinetic resolution with CAL-B and acid catalysts

Various acid catalysts have been explored in the racemization of secondary alcohols.[74-77] The resulting successful DKR methods have employed different zeolites and an acid resin. The acid catalyzed *in situ* racemization occurs via protonation, water loss, sp^2 carbenium ion formation, subsequent non-selective water addition and deprotonation (Scheme 9).



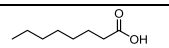
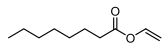
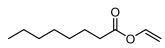
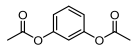
Scheme 9. Dynamic kinetic resolution of *rac*-**1** catalyzed by CAL-B and an acid catalyst.

These DKR methods benefit from the low cost, effortless handling and high stability of the racemization catalysts. At the same time, side products are often formed in significant amounts. The main side products reported are alkene (here styrene) formed via an elimination reaction and ether (here 1-phenylethyl ether) formed via an addition reaction. Also, the scope of

applicable secondary alcohols has been limited (1-8 aromatic secondary alcohols) in comparison with the scope of the metalloenzymatic DKR methods.

The first reported acid catalyzed DKR utilized a commercial zeolite preparation (325 mg $mmol^{-1}$) and CAL-B (87 mg $mmol^{-1}$) at 60 °C in a biphasic reaction system consisting of water and octane (Table 3, Entry 1).[74,75] The esterification of *rac*-**1** with octanoic acid (20 equiv.) resulted in 78% yield and 98% *ee* after 22 hours. Reduced amounts of octanoic acid (≤5 equiv.) gave yields comparable to those of kinetic resolution. The use of lower amount of an in-house prepared zeolite catalyst (25 mg $mmol^{-1}$) and CAL-B (15 mg $mmol^{-1}$) with vinyl octanoate (1 equiv.) afforded (*R*)-**49** in 72% yield and 97.7% *ee* in toluene at 60 °C (Entry 2).[75] Toluene as a reaction medium, instead of a biphasic reaction system, enabled the yield enhancement in the presence of an equivalent amount of the activated acyl donor.

Table 3. CAL-B and zeolite or acid resin catalyzed dynamic kinetic resolution of 1-phenylethanol (*rac*-**1**).

Entry	CAL-B (mg $mmol^{-1}$)	Racemization cat. (mg $mmol^{-1}$)	Temp. (°C)	Acyl donor (equiv.)		Time (h)	Yield (%)	*ee* (%)	Ref.
1	87	H-Beta zeolite 325	60		(20)	22	78	98	74
2	15	Zeolite beta 25	60		(1)	6	72	97.7	75
3	80	H-β nanozeolite 40	50		(2)	1	89	>99	76
4	100	acid resin 200	40		(3)	24	>99	95.8	77

Another in-house prepared zeolite catalyst (40 mg $mmol^{-1}$) and a higher loading of CAL-B (80 mg $mmol^{-1}$) enabled the fast reaction of *rac*-**1** with vinyl octanoate (2 equiv.) in cyclohexane at 50 °C. The combination provided (*R*)-**49** in 89% yield (Table 3, Entry 3).[76] In reusability studies, the catalysts gave reasonable retention of yield (>70%) within the studied five successive reactions. However, in order to reduce the extent of the side-reactions and to enable high yields and *ee* values, the zeolite preparations were added into the reaction mixture after the CAL-B catalyzed kinetic resolution was completed. Thus, the above mentioned methods do not fully meet the criteria of DKR.

Acid resins have also been studied as racemization catalysts in DKR of secondary alcohols. A commercial acid resin (200 mg $mmol^{-1}$) in combination with CAL-B (100 mg $mmol^{-1}$) catalyzed the DKR of *rac*-**1** with 1,3-diacetoxybenzene in toluene at 40 °C (Table 3, Entry 4).[77] After 24 hours, (*R*)-**48** was obtained in yield high (>99%), however, in slightly lower *ee* (95.8%) compared with the DKR methods based on zeolite catalyzed racemization. CAL-B and

the acid resin were successfully reused in ten successive reactions giving full retention of yield (>99%) and only a slight drop in *ee* to 92%.

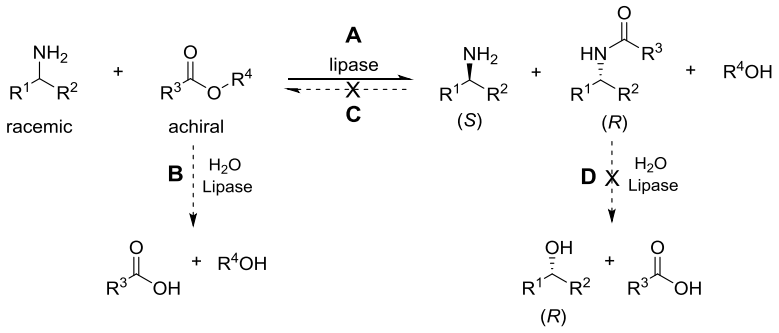
2.8. Preparation of primary amine enantiomers utilizing lipase catalysis

2.8.1. Kinetic resolution of primary amines

Racemic primary amines can be resolved by lipase-catalyzed kinetic resolutions. Lipase-catalyzed *N*-acylation of racemic primary amines in dry organic solvents has been most widely applied.[80,81] In such reactions, *N*-acylation, analogous to *O*-acylation of alcohols, occurs between the reacting enantiomer of a racemic amine and an achiral ester (Scheme 10, Route A). Similar to the kinetic resolution of secondary alcohols in Section 2.7.1., kinetic resolution of primary amines is discussed herein to provide the basis for the DKR methods presented in Section 2.8.2.

Lipase-catalyzed *N*-acylation

As already mentioned with secondary alcohols (Scheme 7), residual water often leads to lipase-catalyzed hydrolysis of an acyl donor in kinetic resolution (Scheme 10, Route B).[82] The acid formed in the hydrolysis can form a salt ion-pair with the substrate amine causing amine precipitation. Accordingly, thorough drying of the substrates and the organic solvent can substantially decrease the extent of the hydrolysis. Also molecular sieves are used to control the amount of water present in the reaction system.



Scheme 10. Lipase-catalyzed *N*-acylation of primary amines (R^1>R^2, CIP-priority).

Because cleavage of the formed amide bond via lipase-catalyzed alcoholysis (Route C) or hydrolysis (Route D) is generally not feasible in dry organic solvents, apart from the amine bond of β-lactams,[83,84] hydrolysis of the acyl donor can be considered as the only side-

reaction. In rare cases, CAL-B has been reported to catalyze the amide hydrolysis in aqueous solutions, however, the bond cleavage required elevated temperatures and long reaction times.[85,86]

Achiral acyl donors in *N*-acylation

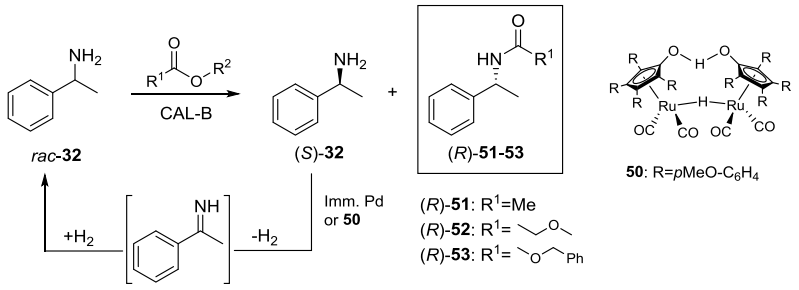
The choice of an achiral acyl donor in the kinetic resolution of amines is usually based on two strategies.[57] Non-activated acyl donors, such as ethyl and isopropyl esters, are utilized in large excess to enable the resolution. Sometimes problems with substrate solubility and sluggish reaction progression have been tackled by using the non-activated acyl donor as the reaction solvent.[87] Alternatively, activated acyl donors can be used in equimolar amounts with respect to the racemic amine substrate. However, the activation in the acyl donor may lead to non-selective chemical *N*-acylation. Thus, the acyl donor needs to be carefully selected. The enol esters, applied in *O*-acylation of alcohols, cannot be utilized due to the potential imine formation of the amine with aldehyde and ketone formed in the reaction. Methoxyacetates, in most cases ethyl or isopropyl methoxyacetate, are probably the most widely used activated acyl donors in the kinetic resolution of primary amines. Their use commonly leads to significant reactivity enhancements when used instead of the non-activated acyl donors.[88,89,II]

2.8.2. Dynamic kinetic resolution of primary amines

Versatile dynamic kinetic resolution processes for the preparation of amino-group containing compounds in enantiopure form have been reported.[64,90] Herein, the discussion is limited to DKR methods of such primary amines which have no other reactive functionality apart from the amino group. The methods combine different transition metal catalysts and CAL-B. As mentioned before, *rac*-**32** (Scheme 11) is utilized as a model compound in the following discussion.

Compatibility of a transition metal catalyst and an enzyme under the same reaction conditions has proven to be challenging in DKR of primary amines. Amine racemization, occurring via imine intermediate, is analogous to the racemization of secondary alcohols, however, not as facile (Scheme 8). Due to the stability of the imine and the relatively low activity of the transition metal catalysts, the racemization requires an elevated reaction temperature while enzymes work best at an ambient temperature. The *in situ* racemization of the slower-reacting amine enantiomer [here (*S*)-**32**)] is accomplished by heterogeneous catalysis using immobilized palladium particles or ruthenium complex **50**-based homogeneous catalysis (Scheme 11).[91-102]

CAL-B (as Novozym 435 preparation), used in the herein discussed methods, affords the fast-reacting enantiomer as (*R*)-amide [here (*R*)-**51**-**53**].



Scheme 11. Dynamic kinetic resolution of *rac*-**32** catalyzed by CAL-B and transition metal catalyst.

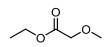
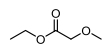
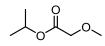
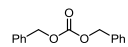
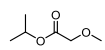
Selectivity of a transition metal catalyst towards amine racemization is a key issue. Under racemization conditions, side product formation has been reported with all the herein discussed racemization processes.[91-98] The main side reactions are the formation of an imine [here α-methyl-*N*-(1-phenylethylidene)benzylamine] and the corresponding secondary amine [here bis(1-phenylethyl)amine]. The latter is formed by condensation of the imine intermediate with an amine (here **32**). Hydrolysis of the imine to the corresponding ketone (here acetophenone) has also been reported.[96,97] These side products are often formed in considerable amounts decreasing the eventual yield of the amide product [here (*R*)-**51**-**53**].

Dynamic kinetic resolution with CAL-B and palladium catalysts

In the first published metalloenzymatic DKR of amines, CAL-B (100 mg/1 mmol of substrate) and palladium on charcoal (Pd/C, 0.9 mol-%) catalyzed the *N*-acylation of *rac*-**32** with ethyl acetate to (*R*)-**51** (*ee*=99%, Table 4, Entry 1).[91] After eight days at 50-55 °C, the yield was modest (64%) yet clearly exceeded the 50% limit of kinetic resolution. Later, palladium nanoparticles immobilized on alkaline supports were successfully used as racemization catalysts.[92,93] After 24 hours, CAL-B (300 mg/mmol) and $Pd/BaSO_4$ (5.4 mol-%) with isopropyl acetate (1 equiv.) gave (*R*)-**51** in 86% yield and *ee*>99% at 70 °C (Entry 2). The catalysts were reused in three successive DKR reactions.[93] Temperature elevation (100 °C) and the switch of the acyl donor to ethyl methoxyacetate decreased the reaction time to 55 min (Entry 3). However, a lower yield (74%) was obtained (entry 2 vs. entry 3). The use of microwave irradiation (MW) to selectively heat the palladium catalysts resulted in higher yield

after the same reaction time (88%, Entry 4).[94] $Pd/BaSO_4$ and CAL-B were successfully utilized in DKR of several aromatic primary amines.[92-95]

Table 4. CAL-B and transition metal catalyzed dynamic kinetic resolution of 1-phenylethylamine (*rac*-**32**) in toluene.

Entry	CAL-B loading (mg $mmol^{-1}$)	Racemization catalyst (mol-%)	Temp. (°C)	Acyl donor (equiv.)		Time	Yield (%)	*ee* (%)	Ref.
1	100	Pd/C (0.9)[b]	50-55		(4)	8 d	64	99	91
2	300	$Pd/BaSO_4$ (5.4)[c]	70		(1)	24 h	86	>99	92
3	300	$Pd/BaSO_4$ (5.4)[c]	100		(1.1)	50 min	74	99	93
4	300	$Pd/BaSO_4$ (5.4)[c]	100 (MW)[d]		(1.1)	50 min	88	99	94
5	50	Pd/AlO(OH) (1)	70		(3)	3 d	92	98	96
6	50	Pd/AlO(OH) (1)	70		(1.7)	6 h	92	99	97
7	40	**50**[e] (4)	90		(7)	3 d	90	98	99
8	40	**50**[e] (4)	90		(2.5)	3 d	90	93	100
9	40	**50**[e] (4)	100		(1.1)	26 h	68	98	102

[a] Triethylamine as solvent. [b] 10% Pd/C (10 mg). [c] 5% $Pd/BaSO_4$ (40 mg). [d] Microwave irradiation. [e] See Scheme 11 for structure.

Palladium particles entrapped in aluminium hydroxide [Pd/AlO(OH), 1 mol-%] and a significantly lower CAL-B loading (50 mg $mmol^{-1}$ vs. 300 mg $mmol^{-1}$) catalyzed the reaction of *rac*-**32** with ethyl acetate (3 equiv.) at 70 °C. After three days, (*R*)-**51** was obtained in 92% yield and 98% *ee* (Table 4, Entry 5).[96] The change of ethyl acetate to isopropyl methoxyacetate (1.7 equiv.) reduced the reaction time to six hours (Entry 6).[97] This method was applied to a series of aromatic primary amines and to two aliphatic primary amines. A higher temperature (100 °C) and a higher racemization catalyst loading (12 mol-%) were required to facilitate the DKR of aliphatic amines. Pd/AlO(OH) and CAL-B were highly reusable showing decrease in yield after eighth and in *ee* after tenth successive reactions. Methods with Raney cobalt and nickel as heterogeneous racemization catalysts have also been published. However, their combination in DKR with CAL-B catalyzed kinetic resolution gave only moderate results.[103]

Dynamic kinetic resolution with CAL-B and ruthenium complex 50

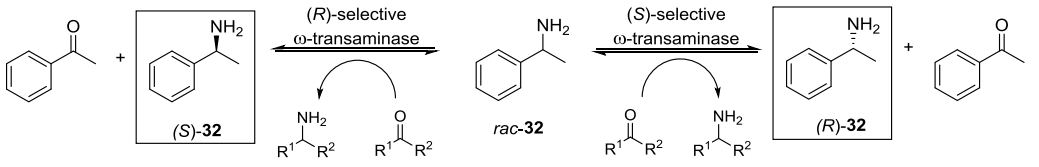
Shvo-type organometallic ruthenium complexes are the only homogeneous transition metal catalysts successfully applied to DKR of primary amines.[99-102] The original Shvo's catalyst,

utilized in the racemization of secondary alcohols, showed lower selectivity towards racemization with primary amines than the *para*-methoxy-substituted Shvo's catalyst (**50**, Scheme 11).[98] CAL-B (40 mg $mmol^{-1}$) and **50** (4 mol-%) catalyzed the DKR of *rac*-**32** with isopropyl acetate (7 equiv.) at 90 °C (Table 4, Entry 7).[99] After three days, (*R*)-**51** was obtained in 90% yield and 98% *ee*. The same reaction conditions with dibenzyl carbonate (2.5 equiv.) gave benzyl carbamate (*R*)-**53** in 90% yield and 93% *ee* (Entry 8).[100] The advantage of dibenzyl carbonate is that the obtained carbamate (*R*)-**53** can be easily deprotected under mild conditions. When isopropyl methoxyacetate (1.1 equiv.) was used as the acyl donor at 100 °C, (*R*)-**52** was obtained in a lower 68% yield after 26 hours (Entry 9 vs. Entries 7 and 8).[102] The use of **50** and CAL-B was successfully extended to a wide variety of primary amines including both aromatic and aliphatic amines.[96,97] Scale-up of the DKR of *rac*-**32** from 0.5 mmol to 45 mmol enabled the use of lower catalyst loading of 1.25 mol-% (instead of 4 mol-%).[104]

2.9. Preparation of primary amine enantiomers utilizing ω-transaminase catalysis

2.9.1. Kinetic resolution of primary amines

Enantiomeric enrichment of a racemic amine (here *rac*-**32**) accomplished by ω-transaminase catalyzed kinetic resolution provides the slow-reacting enantiomer in up to 50% yield, whereas the fast-reacting amine is transformed into the ketone (Scheme 12).[105,106] ω-Transaminase catalyzed kinetic resolution represents a relatively effortless way to access enantiopure amines without the requirement to shift the reaction equilibrium. The most commonly used co-substrate is pyruvate which is accepted well by many ω-transaminases. Availability of enantiocomplementary ω-transaminases allows access to both enantiomers of a racemic amine adding to the usability of the kinetic resolution. The use of both commercial and in-house ω-transaminase preparations, also in immobilized forms, has been reported. As before, the discussion and comparison of the kinetic resolution methods are limited to the reactions of the model compound *rac*-**32**.



Scheme 12. Enantiocomplementary kinetic resolutions of *rac*-**32** by ω-transaminases.

Kinetic resolution with non-commercial ω-transaminases

Two potential ω-transaminases, discovered by screening soil samples, have been reported.[43,107-109] These (*S*)-enantioselective enzymes were utilized in the kinetic resolution of *rac*-**32** (100-500 mM) with pyruvate (0.75 and 0.6 equiv.) yielding (*R*)-**32**. ω-Transaminase from *Bacillus thuringiensis* as whole-cells[107] and ω-transaminase from *Vibrio fluvialis* as cells enclosed in calcium alginate beads,[43] and as cell-free extracts,[108,109] catalyzed the enantiomeric enrichment of (*R*)-**32** giving *ee* >95% at conversions close to 50% (Table 5, Entries 1-4). The removal of co-product acetophenone, observed to cause enzyme inhibition, enabled the preparation of (*R*)-**32** in high concentrations. The removal, based on extraction, was carried out using a biphasic reaction system consisting of buffer and cyclohexanone in one vessel (Entry 1)[107] and in a flow-reactor containing buffer and isooctane equipped with a membrane to retain the enzyme and to allow free circulation of substrates and products (Entries 2 and 3).[43,108] In flow-reactor, substrate inhibition could be minimized by feeding *rac*-**32** over the course of the reaction process (Entry 2).[43] The procedure, utilizing the whole-cell immobilizate, allowed the large-scale enantiomeric enrichment of *rac*-**32** (40 mmol) in 70 hours. Also, an alcohol dehydrogenase was successfully applied to consume the acetophenone formed in the kinetic resolution of *rac*-**32** (Entry 4).[109] Two methods employing ω-transaminase from *Vibrio fluvialis* (Entries 2 and 3) were applied to the enantioselective kinetic resolution of three additional aromatic primary amines.[43,108] Recombinant ω-transaminase from *Arthrobacter* species (mutant CNB05-01), as lyophilized cells, catalyzed the kinetic resolution of *rac*-**32** (50 mM) affording (*R*)-**32** at 57% conversion (Entry 5).[110] Use of this method enabled the enantiomeric enrichment of two aromatic and two aliphatic primary amines.

Table 5. Kinetic resolution of 1-phenylethylamine (*rac*-**32**) with pyruvate catalyzed by ω-transaminase at 30 °C and pH 7.0-8.0.

Entry	ω-Transaminase (mg mL^{-1})	*rac*-**32** (mM)	Pyruvate (equiv.)	Time (h)	Conv. (%)	ee (%)	Ref.
1	whole-cell preparation[a] *Bacillus thuringiensis*	500	0.75	18	51.3	95.4 (*R*)	107
2	whole-cell immobilizate[a] (calcium alginate) *Vibrio fluvialis*	400	-	70	50[b]	96 (*R*)	43
3	cell-free extract[a] *Vibrio fluvialis*	200	0.75	47	50[b]	98 (*R*)	108
4[c]	cell-free extract (2.5 U/mL) *Vibrio fluvialis*	100	0.6	18	52	>98 (*R*)	109
5	whole-cell preparation (20) *Arthrobacter* sp (mutant)	50	1	3	57	>99 (*R*)	110
6	enzyme lyophilizate (6) ATA-113	50	1	24	51	>99 (*R*)	111
7	enzyme lyophilizate (6) ATA-114	50	1	24	53	>99 (*R*)	111
8	enzyme lyophilizate (6) *Arthrobacter* sp. (ATA-117)	50	1	24	50	>99 (*S*)	111
9	enzyme lyophilizate (1) ATA-113	25	0.08	3	50	>99 (*R*)	112
10	enzyme lyophilizate (1) *Arthrobacter* sp. (ATA-117)	100	0.08	12	50	>99 (*S*)	112
11	sol-gel/celite immobilizate (12.5) *Arthrobacter* sp. (ATA-117)	41	1	24	61	>99 (*S*)	30
12	sol-gel immobilizate (50) *Arthrobacter* sp. (ATA-117)	100	1	24	54	>99 (*S*)	III

[a] Information on enzyme loading not given. [b] Value extracted from a plot. [c] Temperature 37 °C.

Kinetic resolution with commercial ω-transaminases

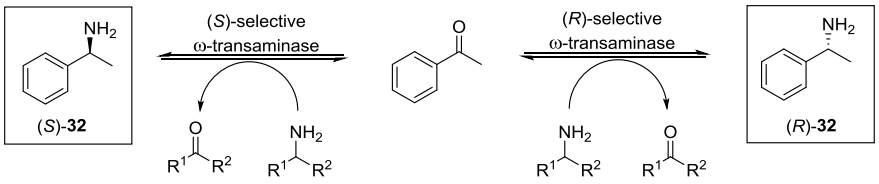
The launch of a selection of ω-transaminases by Codexis has enabled wide utilization of ω-transaminase catalysis in synthetic chemistry. These ω-transaminases have been applied to the kinetic resolution of *rac*-**32** as enzyme lyophilizates[111,112] and as immobilized enzymes [30,III] (Table 5, Entries 6-12). Two (*S*)-selective (ATA-113 and ATA-114, 6 mg mL^{-1}) and one (*R*)-selective ω-transaminases (ATA-117, ω-transaminase from *Arthrobacter* sp., 6 mg mL^{-1}) were active and enantioselective in the kinetic resolution of *rac*-**32** (50 mM) with pyruvate (1 equiv.) affording (*R*)-**32** at 51-53% and (*S*)-**32** at 50% conversions (entries 6-8).[106] The ω-

transaminases (ATA-113 and ATA-117; 1 mg mL^{-1}) were also employed in the kinetic resolution of *rac*-**32** (25 and 100 mM) using a catalytic amount of pyruvate (0.08 equiv.) which was efficiently recycled by an amino acid oxidase. This method provided (*R*)-**32** and (*S*)-**32** at 50% conversion (Entries 9 and 10).[112] These enantiocomplementary kinetic resolutions were successfully extended to several aromatic and aliphatic primary amines.[111,112]

ATA-117 from *Arthrobacter* sp. entrapped in sol-gel/Celite[30] and sol-gel matrices[III] were successfully used in the kinetic resolution of *rac*-**32** (41 and 100 mM) to yield (*S*)-**32** at 61% and 54% conversions (Table 5, Entries 11 and 12). In the case of the sol-gel ω-transaminase, the higher conversions (>60%) were explained by the adsorption of the amine on the hydrophobic sol-gel matrix.[III] The conversions could be decreased by the use of DMSO as a co-solvent. Entrapment of the ω-transaminase allowed the easy recovery and reuse of the catalyst in successive kinetic resolutions in both cases. The ATA-117 immobilizates were also successfully utilized in the preparation of other aromatic (*S*)-amines.[30,III]

2.9.2. Asymmetric synthesis of primary amines

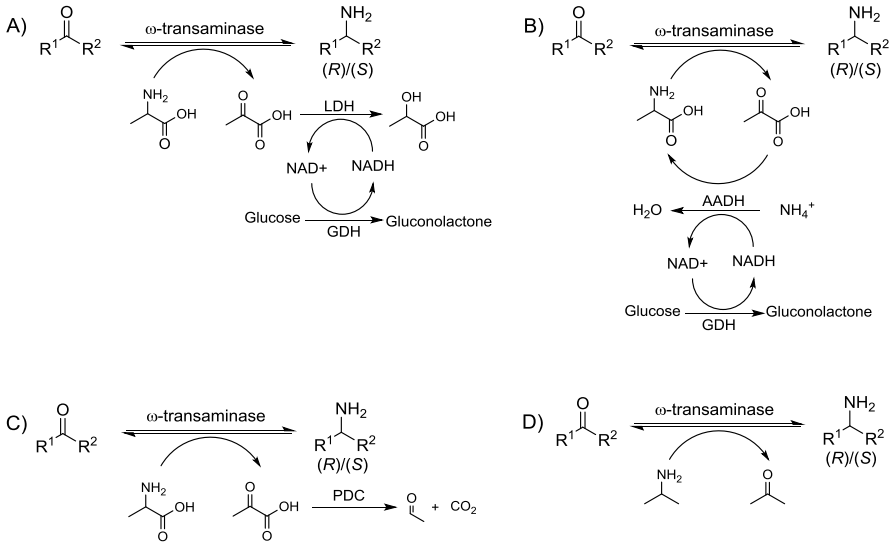
ω-Transaminase catalyzed asymmetric synthesis is an attractive approach for the preparation of enantiopure amines due to the maximum theoretical yield of 100%. Asymmetric synthesis employs a prochiral ketone as a substrate and ideally results in the formation of one amine enantiomer [here (*S*)- or (*R*)-**32**] according to the enantiopreference of the ω-transaminase (Scheme 13).[105,106] The main obstacle hampering the synthetic utilization of the method is the unfavourable reaction equilibrium which is on the side of the substrates. Various methods have been employed to shift the equilibrium to the side of the products.[113-116] Removal of co-product ketone has been accomplished via enzymatic degradation as well as via enzymatic recycling and subsequent reuse of the prosessed ketone as the amino donor.[113-115] The ketone removal serves the dual purpose of driving the reaction to completion and preventing potential ketone inhibition of the ω-transaminase. A straightforward strategy applied to shift the reaction equilibrium is the use of the amino donor in substantial excess in proportion to the reacting ketone in a so-called single enzyme system.[116] Also, enzyme inhibition caused by the amine substrate has been reported to hinder the synthetic usability of ω-transaminase catalyzed asymmetric synthesis.[113]



Scheme 13. Enantiocomplementary asymmetric synthesis of (*R*)- and (*S*)-**32** by ω-transaminases.

Methods utilized to shift the reaction equilibrium

In the reported enzymatic methods, the ω-transaminase catalyzed asymmetric synthesis has been performed in a cascade with other enzymatic reactions (Scheme 14A-C).[113-115] Alanine, forming pyruvate upon deamination, has commonly been used as the amino donor since it is readily available as both enantiomers. Transformation of pyruvate into non-inhibitory lactate has often been accomplished by action of lactate dehydrogenase (LDH, Scheme 14A).[113] The co-factor (NADH) is recycled by the use of glucose dehydrogenase (GDH) and glucose.



Scheme 14. Methods utilized to shift the reaction equilibrium of ω-transaminase catalyzed asymmetric synthesis of amines.[113-116]

Asymmetric synthesis has also been performed in combination with an amino acid dehydrogenase (AADH, Scheme 14B).[114,116] The dehydrogenase, consuming ammonium and using glucose or formate dehydrogenase for co-factor regeneration, enables the recycling and reuse of the amino donor alanine. Also an example of the use of pyruvate decarboxylase (PDC) has been reported.[115] The use of PDC allowed the transformation of pyruvate into volatile

carbon dioxide and acetaldehyde without the need of co-factor recycling (Scheme 14C). However, this method required a substantial excess of the amino donor alanine because ω-transaminase catalyzed amination of acetaldehyde formed consumed part of the alanine. Isopropylamine, forming acetone upon deamination, has been utilized in the single-enzyme methods based on excess use of amino donor (Scheme 14D).[116]

Asymmetric transamination of ketones

Cells containing ω-transaminase from *Vibrio fluvialis* catalyzed the (*S*)-selective transamination of acetophenone to (*S*)-**32** with L-alanine in 90.2% yield without the requirement for additional pyruvate removal (Table 6, Entry 1).[113] In the case of the commercial enzyme lyophilizates [*Vibrio fluvialis* (ATA-103) and *Arthrobacter* sp. (ATA-117)], three methods to shift the unfavourable equilibrium were investigated (Entries 2-8).[116] Coupling of the ω-transaminase catalyzed reaction with LDH and GDH resulted in fast reactions and afforded (*S*)- and (*R*)-**32** in excellent yields (>99%, Entries 2 and 3). Reaction efficiency of this method was increased by using only 0.5 equivalents of L-alanine (instead of 10 equiv., Entry 4). The reaction system required AADH for the recycling of the amino donor and addition of pyruvate (25 mM) to initiate the *in situ* generation of L-alanine. This method produced (*S*)-**32** in 96% yield after 48 hours. Use of an ion-exchange resin for amine removal decreased the amine product inhibition allowing significantly higher acetophenone concentrations to be used (416 mM vs. 30-50 mM, Entries 5 and 6).[117] The ω-transaminases (ATA-113 and ATA-117), in combination with LDH and GDH, produced (*S*)- and (*R*)-**32** in >90% yields. The enantiocomplementary ω-transaminases catalyzed also the transamination of acetophenone (20 mM) with a large excess of isopropylamine (50 equiv.) to (*S*)- and (*R*)-**32** in 95% yields (Entries 7 and 8).[116]

Table 6. Asymmetric synthesis of (*S*)- and (*R*)-1-phenylethylamine [(*S*)- and (*R*)-**32**] by ω-transaminase.

Entry	ω-Transaminase (mg mL^{-1})	Acetophenone (mM)	Amino donor (equiv.)	Time	*c* (%)	*ee* (%)	Ref.
1	whole-cell preparation (21.3 U mL^{-1}) *Vibrio fluvialis*	30	L-alanine (10)	1 d	90.2	>99 (*S*)	113
2	enzyme lyophilizate (5) *Vibrio fluvialis* (ATA-103)	50	L-alanine (10)	12 h[a]	>99	>99 (*S*)	116
3	enzyme lyophilizate (5) *Arthrobacter sp*. (ATA-117)	50	D-alanine (10)	9 h[a]	>99	>99 (*R*)	116
4	enzyme lyophilizate (5) *Vibrio fluvialis* (ATA-103)	50	L-alanine (0.5)[b]	48 h[a]	96	>99 (*S*)	116
5[c]	enzyme lyophilizate (5) ATA-113	416[d]	L-alanine (2.4)[e]	-	>90	>99 (*S*)	117
6[c]	enzyme lyophilizate (5) *Arthrobacter sp*. (ATA-117)	416[d]	D-alanine (2.4)[e]	-	>90	>99 (*R*)	117
7	enzyme lyophilizate (5) ATA-113	20	isopropylamine (50)	16 h[a]	95	>99 (*S*)	116
8	enzyme lyophilizate (5) *Arthrobacter sp*. (ATA-117)	20	isopropylamine (50)	24 h[a]	95	>99 (*R*)	116
9	whole-cell preparation (20) *Paracoccus denitrificans*[f]	50	L-alanine (5)	24 h	80	>99 (*S*)	118
10	whole-cell preparation(20) *Pseudomonas fluoresens*	50	L-alanine (5)	24 h	92	>99 (*S*)	118
11	enzyme lyophilizate (0.16) *Arthrobacter citreus* (variant S9)	1.8	isopropylamine (311)	24 h	>99	>99 (*S*)	119
12[g]	crude enzyme preparation (20) *Arthrobacter citreus*[h]	50	isopropylamine (3)	72 h	39	>99 (*S*)	22
13[g]	crude enzyme preparation (20) *Arthrobacter sp.*	50	isopropylamine (3)	72 h	43	>99 (*R*)	22
14	immobilizate (200) CDX-017 (mutant from Codexis)	250[j]	isopropylamine (3.8)[k]	24 h	87	>99 (*R*)	23

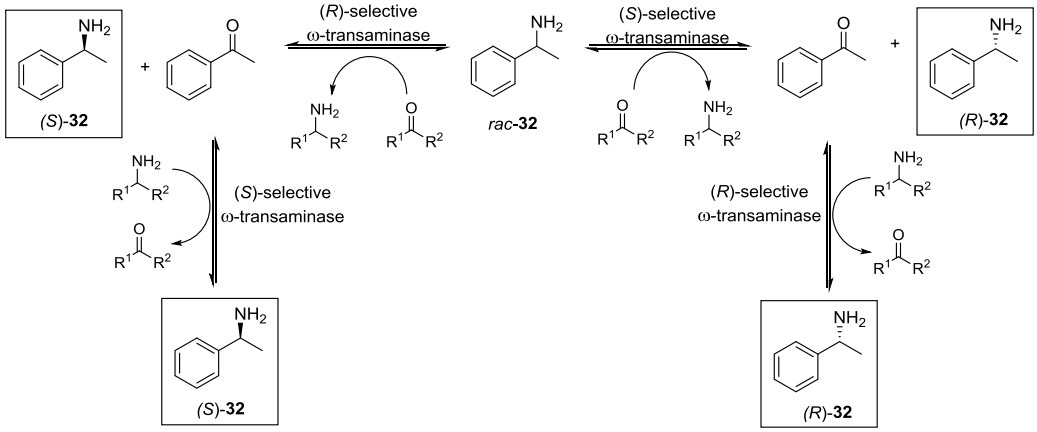
[a] Value extracted from a plot. [b] 25 mM of pyruvate added. [c] Ion-exchange resin 200 g L^{-1}. [d] 50 g L^{-1} [e]90 g L^{-1}. [f] Mutant with Strep-tag II. [g] In TBME. [h] Mutant with His-tag. [i] Adsorbed on a hydrophobic polymethacrylate resin. [j] 30 g L^{-1}. [k] 80 mL L^{-1}.

Two novel (*S*)-selective ω-transaminases from *Paracoccus denitrificans* and *Pseudomonas fluorescens*, used as whole-cell preparations, catalyzed the transamination of acetophenone with L-alanine to (*S*)-**32** in 80% and 92% yields (Table 6, Entries 9 and 10).[118] An (*S*)-selective ω-transaminase from *Arthrobacter citreus* was highly active and enantioselective in the transamination acetophenone with isopropylamine (Entry 11).[119] However, the enzyme

suffered from severe inhibition of acetophenone hampering its synthetic utilization in the preparation of (*S*)-**32**. Enantiocomplementary ω-transaminases from *Arthrobacter citreus* and *Arthrobacter* sp. as crude enzyme preparations were utilized in the asymmetric transamination of ketones in *tert*-butyl methyl ether (TBME) without any enzyme immobilization.[22] These enzymes were highly active and enantioselective with some ketones, however, the activity in the transamination of acetophenone was only moderate resulting in 39% and 43% conversions after 72 hours (Entries 12 and 13). The ω-transaminase, originally engineered for the manufacture of sitagliptin, was also applied to the synthesis of (*R*)-**32**.[23] The use of the enzyme, adsorbed on a hydrophobic resin, afforded (*R*)-**32** in neat isopropyl acetate at 60 °C at 87% conversion after 24 hours (Entry 14).

2.9.3. Deracemization of primary amines

Deracemization of primary amines, catalyzed by ω-transaminase, employs kinetic resolution of a racemic amine and asymmetric transamination of a ketone formed in the kinetic resolution in sequential manner.[30,111,120] In theory, this method allows the transformation of a racemic primary amine (here *rac*-**32**) into one enantiomer [(*R*)-**32** or (*S*)-**32**] in an overall yield of 100% (Scheme 15).[101] This method has the benefit of using racemic amines as starting material and resulting in one amine enantiomer. The use of two ω-transaminases with opposite enantiopreferences requires, however, the removal of the enzyme acting in the first step before the addition of the second enzyme. Such removal has been accomplished by denaturation of the first ω-transaminase rendering the method somewhat laborious and uneconomical.[111,120] The use of an immobilized ω-transaminase in the first step, enabling the recovery and further reuse of the enzyme, is a significant improvement to the method.[30] Moreover, the asymmetric transamination step of the deracemization has the same obstacles to overcome as the methods relying solely on asymmetric synthesis. When *rac*-**32** was used as starting material in deracemization, (*S*)- and (*R*)-**32** (*ee*>99) were obtained in 60% and 82% yields, respectively.[111]



Scheme 15. Enantiocomplementary deracemization of *rac*-**32** by ω-transaminases.

3. AIMS OF THE STUDY

The primary aim of this study was to discover viable biocatalytic synthesis methods for the preparation of secondary alcohols and primary amines as single enantiomers. The aim was to establish new synthetic methods to enable high reaction efficiencies in terms of green and sustainable chemistry. The research commenced with studies on lipase-catalyzed kinetic resolutions of secondary alcohols and primary amines in organic solvents. The aim was to establish effective and general kinetic resolution methods for racemic secondary alcohols and primary amines, and to transform these methods into the corresponding dynamic kinetic resolution methods using enantioselective lipase-catalyzed acylation and transition metal catalyzed *in situ* racemization of the less reactive substrate enantiomer. The research combined the lipase catalysis knowledge of Liisa Kanerva's research group (University of Turku) and the expertise in transition metal catalysis of Reko Leino's group (Åbo Akademi University). A method for the dynamic kinetic resolution of secondary alcohols is described in Paper I. Racemic secondary alcohols **1**-**31** were studied as substrates (Figure 5). However, studies to find alternative methods to enhance the classical kinetic resolution of primary amines soon replaced the efforts to develop the dynamic kinetic resolution of amines. Thereafter, the studies were focused on development of a lipase-catalyzed kinetic resolution method for racemic primary amines under solvent-free conditions (Paper II) and on the kinetic resolution of racemic primary amines by ω-transaminases in aqueous medium (Paper III). Racemic amines **32**-**40** were studied in the lipase-catalyzed solvent-free *N*-acylation while racemic amines **32**, **33**, **37** and **38** were included in the substrate studies of the ω-transaminase catalyzed kinetic resolution (Figure 5). Immobilization of the lipase and ω-transaminase catalysts was studied in order to obtain catalysts with improved properties for synthetic purposes (Papers II and III). Furthermore, the aim was to develop chromatographic analysis methods for the determination of reaction conversions and product enantiopurities (Papers I-III).

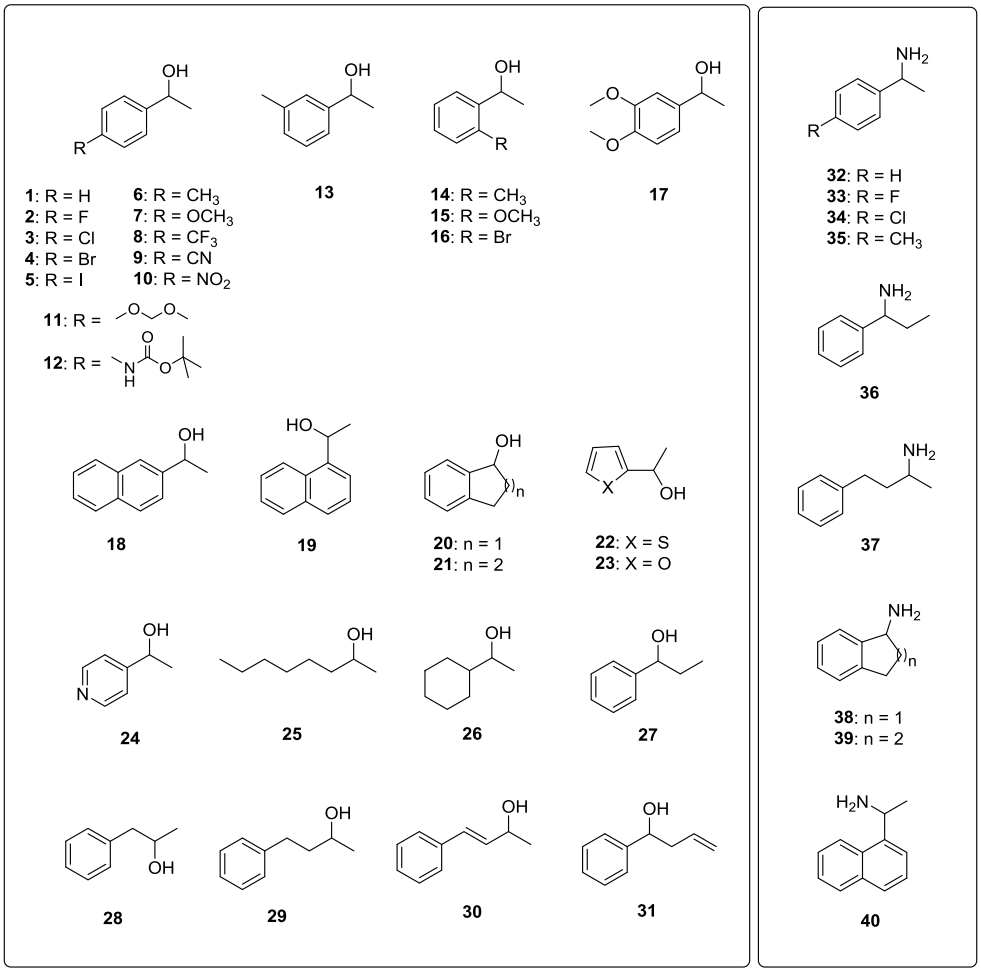


Figure 5. Secondary alcohols and primary amines studied in this thesis.

4. MATERIALS AND METHODS

4.1. Materials

Solvents, substrates and reagents were purchased from standard suppliers. To avoid unnecessary repetition, more detailed information on the suppliers and used procedures is available in the original publications (Papers I-III). The commercially unavailable racemic secondary alcohols were prepared by reduction of the corresponding ketones with sodium borohydride. Isopropyl methoxyacetate was prepared from the corresponding acyl chloride and isopropyl alcohol.

Lipase B from *Candida antarctica* (CAL-B, Novozym 435) was purchased from Novozymes. Free CAL-B powder was a product of Biocatalytics and free ω-transaminase powder (ATA-117) a product of Codexis. Both were used as received in the immobilizations. The protein contents of the free CAL-B and ω-transaminase powders were determined using the bicinchoninic acid assay with bovine serum albumin as the standard protein.[121,122]

4.2. In-house immobilized enzyme preparations

4.2.1. Sol-gel entrapment of *Candida antarctica* lipase B

Phosphate buffer (0.1 M, pH 7.5, 390 µL producing gel/water with the ratio 1:10) was added to a vial containing the CAL-B powder (75 mg) and possible solid additives [Celite 50 mg, Celite 50 mg together with sucrose 5 mg, 18-crown-6 (130 mg, 0.5 mmol) or 18-crown-6 (130 mg, 0.5 mmol) together with Celite 50 mg] were added to a vial containing the CAL-B powder (75 mg). The mixture was stirred on a Vortex-mixer at room temperature. After addition of possible liquid additives [IPA (isopropyl alcohol, 50 µL) and the aqueous PVA (polyvinyl alcohol, 4% w/v, 100 µL)] the aqueous NaF (1 M, 50 µL) was added to the solution under constant agitation. TMOS and MeTMS (total amount of 3 mmol) were added and the mixture was stirred until heat-formation was observed. The vial was cooled on an ice bath and the mixture was gently shaken until an increase in viscosity was observed. Closed vials were stored in a desiccator overnight for maturation. Sol-gel CAL-B preparations were dried in a lyophilizator and gently crushed with a spatula before use.

4.2.2. Sol-gel entrapment of *Arthrobacter* sp. ω-transaminase

Phosphate buffer (0.1 M, pH 7.5, 390 µL or 490 µL producing a sol-gel/water ratio of 1:10) was added to a vial containing ω-transaminase powder (50 or 100 mg) and possible solid additives

(Celite 50 mg or Celite 50 mg together with sucrose 5 mg). After addition of possible liquid additives [IPA (isopropyl alcohol, 50 μL) and/or the aqueous PVA (polyvinyl alcohol, 4% w/v, 100 μL)], the aqueous NaF (1 M, 50 μL) was added to the solution under constant agitation. TMOS and RTMS (total amount of 3 mmol) were added and the mixture was stirred until heat-formation was observed. The vial was cooled on an ice bath and the mixture was gently shaken until increase in viscosity was observed. Closed vials were stored at room temperature overnight for maturation. The ω-transaminase sol-gel preparations were gently crushed with spatula and sequentially washed (IPA 10 mL, water 10 mL, *n*-hexane 10 mL), dried at room temperature for 72 h and stored at 4 °C. The protein contents of the washing solutions were determined using the bicinchoninic acid assay with bovine serum albumin as the standard protein.[121,122]

4.3. Dynamic kinetic resolution of secondary alcohols

Ruthenium complex **46** (14 mg, 20 μmol), CAL-B (10 mg) and Na_2CO_3 (53 mg, 0.5 mmol) were weighed into a pre-dried test tube, 2 mL of toluene was added in a glovebox and the mixture was stirred for 2 min before a solution of *t*BuOK (0.25 M in THF; 100 μL, 0.025 mol) was added. After stirring 5 min one of the substrates **1**-**31** (1 mmol) was introduced and the mixture was stirred for another 5 min before the addition of isopropenyl acetate (135 μL, 1.2 mmol). The reactions were monitored by taking samples (10 μL), derivatizing them with propionic anhydride in the presence of DMAP in pyridine (1%), filtering the samples through silica using ethyl acetate as an eluent and analyzing them by GC. Isolation of the products was performed by silica gel column chromatography.

4.4. Lipase catalyzed kinetic resolution of primary amines

An acyl donor (0.2 M; isopropyl acetate, isopropyl butanoate, ethyl or isopropyl methoxyacetate) was added into a reaction vessel containing molecular sieves (4 Å, 50 mg) and the suspension of Novozym 435 (10 mg mL^{-1}), toluene (1 mL) and *rac*-**32** (0.1 M), or *rac*-**32**-**40** (2 mmol) and an acyl donor (2 mmol) were added into a reaction vessel containing Novozym 435 (10-50 mg) and molecular sieves (4 Å, 50 mg). The reaction mixture was shaken (170 rpm) at room temperature (23 ºC) or at 47 ºC. The reactions were monitored by taking samples which were derivatized with acetic or propionic anhydride and analyzed by GC or HPLC. When required, the reactions were stopped by filtering off the enzyme at (50±0.5)% conversion. Isolation of the products was performed by silica gel column chromatography.

Reuse of the CAL-B preparations

Coumpound *rac*-**32** (12.7 μL, 0.1 M in toluene or 2 mmol), isopropyl methoxyacetate (27.2 μL, 0.2 M in toluene or 2 mmol) were added to a vial containing Novozym 435 or sol-gel CAL-B preparation, and the reaction mixture was shaken at 23 ^{o}C. The reactions were stopped at 50% conversion by centrifuging gently the catalyst and pipetting off the liquid phase. New reagents (and toluene for solvent reactions) were added to start the next reaction cycle. Reuse was repeated 5 times under identical conditions.

4.5. ω-Transaminase catalyzed kinetic resolution of primary amines

Primary amines *rac*-**32**, **33**, **37** or **38** (50 mM), sodium pyruvate (50 mM) and pyridoxal-5'-phosphate monohydrate (0.2 mg mL^{-1}) in phosphate buffer (1 mL, 0.1 M, pH 7.5) with IPA or DMSO as a possible co-solvent (10%, v/v) were added to a 2 mL Eppendorf tube containing the ω-transaminase sol-gel catalyst (25 or 50 mg). The reaction mixture was shaken (170 rpm) at 30 °C. After 24 h, the reaction was stopped by centrifuging the reaction mixture and removing the reaction solution by pipette. Conversion was monitored by taking a sample (5 μL) from the reaction solution and diluting it with the HPLC eluent (500 μL). The obtained sample was filtered and analyzed by HPLC. A sample (400 μL) for *ee* analysis was taken and the addition of aqueous NaOH (2 M, 50 μL) followed by extraction with ethyl acetate (400 μL). The organic phase (300 μL) was dried with Na_2SO_4, and after filtration, the amine in the sample (200 μL) was derivatized with acetic anhydride (10 μL) to obtain *ee* by GC.

Reuse of ω-transaminase sol-gel catalyst in preparative-scale kinetic resolution

Phosphate buffer (4 mL, 0.1 M, pH 7.5, DMSO, 10%-v/v) containing *rac*-**32** (100 mM), sodium pyruvate (100 mM) and pyridoxal-5'-phosphate monohydrate (0.2 mg mL^{-1}) was added to a reaction vessel containing the ω-transaminase sol-gel catalyst (200 mg). The reaction mixture was shaken (170 rpm) at 30 °C. After 24 hours the enzyme preparation was recovered by centrifuging the reaction mixture and the reaction solution was replaced by fresh solution. After five successive reaction cycles aqueous HCl (6 M) was added to the collected reaction solutions, and the obtained solution was extracted with dichloromethane (4×10 mL) to remove acetophenone. Addition of aqueous NaOH (6 M) to the solution was followed by extraction with dichloromethane (4×15 mL). The organic phase was dried with Na_2SO_4 and evaporated to obtain the product.

4.6. Analytical methods

4.6.1. Methods utilized to monitor the enzymatic reactions

Chiral GC analysis was performed with a gas chromatograph equipped with a Varian CP7502 column or a Chrompack CP-Chirasil-DEX CB column and chiral HPLC analysis with an instrument equipped with a Chiralcel OD-H column using a mixture of *n*-hexane and 2-propanol as the eluent. The values of enantiomeric excess (ee_s and ee_p) were obtained using Equations 1 and 2, and the values of conversions (c) using Equation 3 (See Section 2.3.) The values of enantiomeric ratio (E) were obtained using Equations 4 and 5. With the ω-transaminase catalyzed reactions, the quantitative achiral HPLC analysis was performed using a liquid chromatograph equipped with a C-18 column eluting with a mixture of phosphate buffer (25 mM, pH 7) and acetonitrile.

4.6.2. Methods utilized to characterize the compounds

Optical rotations were determined using a Perkin-Elmer 341 polarimeter at sodium D line. The $[\alpha]_D^{25}$-values are given in units of 10^{-1}deg cm^2 g^{-1}. Melting points were recorded with a Gallenkamp apparatus. NMR spectra were recorded with a Bruker Avance 500 or a Bruker Avance 600 spectrometer using tetramethylsilane as an internal standard. HRMS were measured in ESI$^+$ mode with Bruker Daltonics micrOTOF-Q spectrometer.

5. RESULTS AND DISCUSSION

5.1. Dynamic kinetic resolution of secondary alcohols (Paper I)

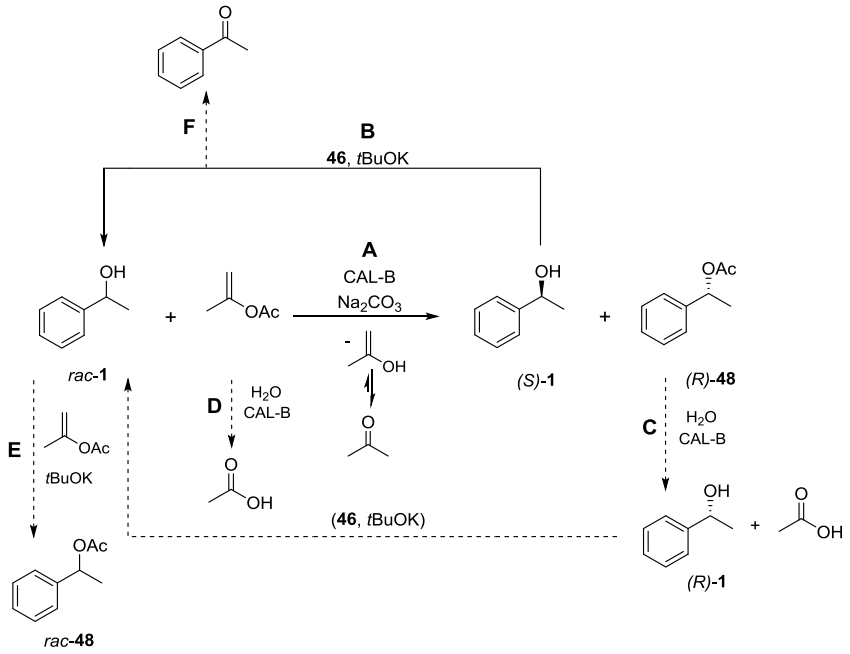
The relatively facile racemization of secondary alcohols has encouraged the incorporation of the racemization process with lipase catalyzed *O*-acylation into dynamic kinetic resolution. Various metal catalysts have been reported to racemize secondary alcohols, however, the crucial requirements of catalytic activity and stability in the presence of an enzymatic reaction have been fulfilled by only a few catalysts.[63,64] Accordingly, a metalloenzymatic dynamic kinetic resolution method was studied to effectively transform racemic secondary alcohols into ester enantiomers. Following the method development, the general viability of the method was studied with 31 different secondary alcohols.

5.1.1. Characterization of reaction system

A new racemization catalyst, dicarbonylchloro(pentabenzylcyclopentadienyl)ruthenium (**46**, synthesized by collaborators at Åbo Akademi University)[71,79] and CAL-B (Novozym 435 preparation, adsorbed on macroporous acrylic resin), known for its highly robust character and broad substrate specificity, were utilized in the dynamic kinetic resolution method. For the method development, compatibility of the two catalytic systems needed to be established and potential side reactions assessed and eliminated to the extent possible. The overall reaction system for the dynamic kinetic resolution of racemic 1-phenylethanol (*rac*-**1**, a model compound) is presented in Scheme 19. The kinetic resolution is based on the highly enantioselective CAL-B catalyzed *O*-acylation of (*R*)-**1** with isopropenyl acetate to (*R*)-**48** in toluene (Scheme 16, Route A; see also Table 7, Entry 1). Isopropenyl acetate was chosen as an acyl donor owing to its suitable character providing sufficient activity and irreversibility to the *O*-acylation (as discussed in section 2.7.1.2.). The slow-reacting substrate enantiomer [(*S*)-**1**] is racemized *in situ* by **46** (Scheme 19, Route B). Other reaction routes C-F in Scheme 16 represent side-reaction pathways.

Elimination of water plays a key role when combining these two catalytic systems. In the presence of CAL-B, water can act as a competing nucleophile to (*R*)-**1** and cause both hydrolysis of formed (*R*)-**48** to (*R*)-**1** (Scheme 16, Route C) as well as hydrolysis of isopropenyl acetate to acetic acid (Route D). CAL-B catalyzed hydrolysis of (*R*)-**48** can result in a synthetically unfavourable equilibrium between the reverse reactions [acylation of (*R*)-**1** (Route A) and hydrolysis of (*R*)-**48** (Route C)] preventing the DKR reaction from proceeding to completion. The hydrolysis (*R*)-**48** can also cause the depletion of the enantiomeric excess of

(*R*)-**48** when the enantiodiscrimination of the acylation is not perfect. Consequently, the acylation produces a minor amount of (*S*)-**48** which is then accumulated as a result of the hydrolysis of (*R*)-**48** (Route C). Potential for CAL-B catalyzed hydrolysis of the activated acyl donor is high in the presence of water. As a result, isopropenyl acetate is partly consumed in the hydrolysis and acetic acid is formed (Route D).



Scheme 16. CAL-B and ruthenium complex **46** catalyzed dynamic kinetic resolution of *rac*-**1**.

As **46** is instable in the presence of acids, racemization of **1** is easily affected by the hydrolytic formation of acetic acid through Route D (Scheme 16). Accordingly, the acetic acid formed has to be neutralized *in situ* with sodium carbonate (Na_2CO_3). The amount of Na_2CO_3 needed is dependent on the extent of the CAL-B catalyzed hydrolysis of isopropenyl acetate. Complete activation of the ruthenium catalyst **46** with potassium *tert*-butoxide (*t*BuOK) to give the active catalyst species is required for efficient racemization. However, *t*BuOK can only be used in a slight excess (1.25 equiv. in proportion to **46**) because higher amounts can leave *t*BuOK unreacted and lead to base catalyzed non-enantioselective acylation of *rac*-**1** to *rac*-**48** (Scheme 16, Route E). This side reaction reduces the enantiomeric excess of (*R*)-**48**. Yet another possible side reaction arises from an incomplete racemization process where the ketone (here acetophenone), formed by the oxidation of an alcohol enantiomer [(*S*)- or (*R*)-**1**], is not reduced

back to alcohol (Route F). Consequently, *rac*-**1** is consumed and the eventual yield of (*R*)-**48** is lowered.

5.1.2. Optimization of dynamic kinetic resolution method

Racemization catalyst **46** was developed and preliminary results published in a preceding study.[71] The DKR method was further developed in Paper I because the *in situ* racemization was not sufficiently efficient. Consequently, a combination of kinetic resolution and dynamic kinetic resolution, rather than in an efficient dynamic kinetic resolution, was observed. The optimization of the method is described herein.

The first experiments showed that racemization of (*R*)- and (*S*)-**1** [0.5 M, in toluene (1 mL), in the presence of **46** (4 mol-%) and tBuOK (5 mol-%) in an argon atmosphere] occurred within two minutes. However, when the racemization was combined *in situ* with the CAL-B catalyzed acylation of *rac*-**1** (0.5 M) with isopropenyl acetate (1.5 equiv.) in the presence of Na_2CO_3 (1 equiv.), the racemization seized quickly after the initiation of the reaction. This was revealed by the chromatographic analysis showing enrichment of (*S*)-**1** in comparison with (*R*)-**1**. Due to the enrichment of the slow-reacting enantiomer, the situation resembled conventional enzymatic kinetic resolution and led only to 56% conversion after three hours (Table 7, Entry 4).[71] After 24 hours, the reaction had proceeded to an acceptable 84% conversion and >99% *ee* demonstrating that there was some racemization activity left. When more Na_2CO_3 (3 equiv.) was used to neutralize the acetic acid side product, without making any other changes to the reaction system, a steady increase in conversions with time was observed (Entry 5). The increase resulted in 94% conversion and >99% *ee* after 24 hours (Entry 5). As another way to enhance the overall reaction rate, the concentration of *rac*-**1** was reduced from 0.5 M to 0.2 M, and the concentration of isopropenyl acetate accordingly, without changing the absolute amount of **46** (now 10 mol-%) or Na_2CO_3 (now 2.5 equiv., Entry 3). After three hours, conversion of 79% was reached demonstrating better activity and stability of **46**. The highest observed conversion under these conditions was reached after seven hours (Entry 3). However, conversion after 24 hours indicated a reverse hydrolysis reaction with a drop of conversion from 84% (7 h) to 72% (24 h) (Scheme 16, Route C). Higher amount of Na_2CO_3 (5 equiv.) and lower concentrations of *rac*-**1** (0.2 M) and isopropenyl acetate (0.3 M, 1.5 equiv.) together gave lower conversions (Entry 3). In addition to the conversion decrease, also a decrease in enantiomeric excess was observed. The decrease in *ee* indicated reduced enantiodiscrimination in the acylation (Scheme 16, Route A), as well as hydrolysis of (*R*)-**46** (Route C), causing (*S*)-**46** to accumulate. Due to the somewhat inefficient racemization, up to 5% of acetophenone was

produced in the above described reactions. The addition of tBuOK (as a solid together with **46** instead of solution added after dissolving of the catalyst) might have caused only partial activation of **46**. Later it was noticed that the low reaction scale of 1 mL potentially contributes to the inefficiency of the racemization, a factor which was eliminated in the further studies. Nevertheless, the DKR reaction afforded (*R*)-**48** in high yield (94%) and enantiomeric excess (>99%, Entry 5).[71]

Table 7. CAL-B and **46** catalyzed dynamic kinetic resolution of 1-phenylethanol (*rac*-**1**) with isopropenyl acetate in the presence of Na_2CO_3 in toluene in an inert atmosphere at 23°C.[71]

Entry	*rac*-**1** (M)	Isopropyl acetate (equiv.)	**46** (mol-%)	*t*BuOK (mol-%)	Na_2CO_3 (equiv.)	Time (h)	*c*[a] (%)	ee (%)
1[b,c]	0.5	1.5	-	-	-	4	50	>99
2[c]	0.2	1.5	10	12.5	2.5	3/7/24	79/84/72	>99/>99/99
3[c]	0.2	1.5	10	12.5	5	4/16/24	71/80/73	98/98/97
4[c]	0.5	1.5	4	5	1	3/24	56/84	>99/>99
5[c]	0.5	1.5	4	5	3	3/7/24	69/85/94	>99/>99/>99
6[d,e]	0.5	1.2	2	2.5	0.5	3	>99	>99

[a] Determined by chiral GC. [b] Kinetic resolution. [c] 1 mL scale, 3 mg mL^{-1} of Novozym 435. [d] 2 mL scale, 5 mg mL^{-1} of Novozym 435. [e] Paper I.

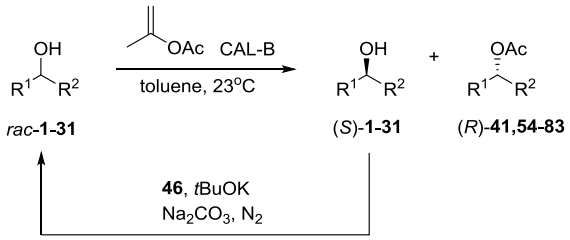
It was evident that the racemization process suffered from the suboptimal reaction system. In order to improve the system, water and molecular oxygen were eliminated from the solvent toluene. Also *rac*-**1** and isopropenyl acetate were distilled under argon prior to use to remove water. For more complete catalyst activation, a freshly prepared solution of sublimated tBuOK was used in the activation of **46**. To further improve the reaction efficiency, the reaction system was transferred into a glove box in nitrogen atmosphere. The water and molecular oxygen levels were monitored and kept below 1 ppm. Under these dry and oxygen free reaction conditions, the amount of isopropenyl acetate and Na_2CO_3 were decreased to 1.2 and 0.5 equiv., respectively. With these changes, the racemization by **46** turned highly efficient allowing the reduction in the content of **46** by half to 2 mol-%. Finally, no traces of acetophenone were detected by GC.

When the *in situ* racemization is fast the substrate remains racemic throughout the resolution reaction and the situation resembles that at the zero conversion of an enzyme catalyzed kinetic resolution (See Section 2.3.). Thus, the initial rate of the kinetic resolution, and the specific activity of the enzyme catalyst derived thereof, depicts the rate of the enzymatic reaction in

DKR. For the rate of the kinetic resolution to be in accordance with the fast racemization, the amount of CAL-B with the specific activity of 2.6 mmol min^{-1} g^{-1} [derived from the kinetic resolution of *rac*-**1** (0.5 M) with isopropenyl acetate (1.2 equiv.) in toluene at 23°C] was set to 10 mg $mmol^{-1}$ (5 mg mL^{-1}). As a result, such enzyme activity resulting in full conversion (>99%) of DKR of *rac*-**1** in three hours was obtained (Table 7, Entry 6).

5.1.3. Secondary alcohols in dynamic kinetic resolution

The general usability of the established dynamic kinetic resolution method utilizing **46** and CAL-B was studied with a wide range of structurally different secondary alcohols (Scheme 17). The racemic alcohols (*rac*-**1-31**, 1 mmol, 0.5 M) were subjected to acylation with isopropenyl acetate (1.2 equiv.) under the previously optimized reaction conditions [**46** (2 mol-%), tBuOK (2.5 mol-%), Na_2CO_3 (0.5 equiv.), nitrogen atmosphere, toluene (2 mL), 23 °C]. In order to ensure reproducibility and reliability of the reaction system, parallel reactions were performed and the acylated product was isolated with each substrate. The reactions entered as to have reached full conversion (>99%) do not show any substrate left in the reaction mixture according to GC-analysis. The possible formation of the corresponding ketone was closely monitored by GC, and if any formation was observed it was at negligible level (≤ 1%).



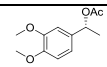
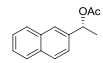
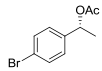
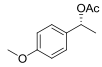
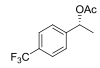
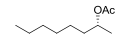
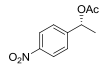
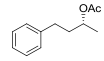
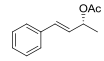
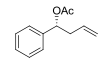
Scheme 17. Dynamic kinetic resolution of secondary alcohols.

When compared to the DKR reaction of *rac*-**1**, electron withdrawing halogen-substitution at the *para*-position of the phenyl ring had no effect on the outcome of the reaction as the reactions of *rac*-**2-5** afforded the corresponding (*R*)-acetates in quantitative yields in three hours (Table 8, Entries 2-5 vs. Entry 1). The same was true for *rac*-**6-7** bearing electron donating methyl- or methoxy-substituents at the *para*-position (Entries 6 and 7 vs. Entry 1). However, the introduction of an additional methoxy-group to the *meta*-position resulted in a slower reaction (Entry 17 vs. Entry 7). With *rac*-**8-10**, the strongly electron withdrawing substituents at the *para*-position (trifluoromethyl, cyano- and nitro-substituents, respectively) resulted in controversial results. In the case of *rac*-**8**, (*R*)-**8** was obtained in quantitative yield in six hours

(Entry 8). However, *rac*-**9**-**10** showed slow racemization and required prolonged reaction times to reach high conversions (Entries 9 and 10). Also, a slight decrease in the *ee* values was observed. The reactions of protected polyfunctional alcohols *rac*-**11** and *rac*-**12** (methoxymethyl ether, MOM and *tert*-butyloxycarbonyl, Boc; respectively) gave the corresponding (*R*)-acetates in high yields (Entries 11-12). The reaction time was dependent on the protected second functionality. The *ortho*-substituted alcohols *rac*-1**4**-**16** were successfully transformed into (*R*)-acetates in quantitative yields (Entries 14-16). The substituent had a substantial effect on the reaction rate. The reactions of *rac*-**14** and *rac*-**15** with methyl- and methoxy-substituents proceeded relatively smoothly (Entries 14 and 15), whereas the bromine-substitution on *rac*-**16** led to an exceptionally slow reaction (Entry 16). The conversion of *rac*-**14** to the corresponding (*R*)-acetate resulted in a lower *ee* value in comparison with the reactions of *rac*-**15** and *rac*-**16** (Entry 14 vs. Entries 15 and 16). Again in the reaction of *rac*-**13**, the bromine-substitution at the *meta*-position led to a fast reaction providing the (*R*)-acetate in quantitative yield after six hours (Entry 16 vs. Entry 13).

The use of the reaction system enabled the transformation of bicyclic alcohols *rac*-**18**-**21** into the corresponding (*R*)-acetates in high yields with feasible reaction times, with the exception of *rac*-**19** requiring a longer reaction time (Table 8, Entries 18-21). Also heterocyclic alcohols *rac*-**22**-**24** were converted into (*R*)-acetates in reasonable reaction times (Entries 22-24). In the case of *rac*-**24**, an elevated reaction temperature (60 °C) was needed (Entry 24). The aliphatic alcohols *rac*-**25** and *rac*-**26** gave fast reactions, however, a slightly lower *ee* value was observed with *rac*-**25** (Entries 25 and 26). The reactions of aromatic alcohols *rac*-**27**-**29**, bearing a longer aliphatic chain and the reacting hydroxyl group at different positions of the chain, gave fast reactions and excellent yields, although, lower *ee* values were observed with *rac*-**28** and *rac*-**29** (Entries 27-29). Alcohols *rac*-**30** and *rac*-**31**, differing in the position of the double bond and the hydroxyl group, were converted into the corresponding (*R*)-acetates in high yields and enantiopurities, yet there was a notable difference between the reaction times (Entries 30 and 31). The reaction of *rac*-**31**, bearing a terminal double bond, required a significantly longer reaction time and a higher amount of CAL-B (40 mg) to reach full conversion. Also, a somewhat lower *ee* value was detected (Entry 31 vs. Entry 30).

Table 8. CAL-B (10 mg) and **46** (2 mol-%) catalyzed dynamic kinetic resolution of *rac*-**1**-**31** (1 mmol, 0.5 M) with isopropenyl acetate (1.2 equiv.) in the presence of *t*BuOK (2.5 mol-%) and Na_2CO_3 (0.5 equiv.) under nitrogen in toluene (2 mL) at 23 °C.

Entry	Substr.	Time (h)	Product	Yield[a,b] (%)	*ee*[a] (%)	Entry	Substr.	Time (h)	Product	Yield[a,b] (%)	*ee*[a] (%)
1	*rac*-**1**	3		>99 (95)	>99	17	*rac*-**17**	18		>99 (98)	>99
2	*rac*-**2**	3		>99 (99)	>99	18[f]	*rac*-**18**	6		99 (96)	>99
3	*rac*-**3**	3		>99 (93)	>99	19[f]	*rac*-**19**	120		>99 (94)	99[e]
4	*rac*-**4**	3		>99 (94)	>99	20	*rac*-**20**	36		97 (96)	>99
5	*rac*-**5**	3		>99 (96)	>99	21	*rac*-**21**	24		95 (92)	98
6	*rac*-**6**	3		>99 (97)	>99	22	*rac*-**22**	24		99 (90)	>99
7	*rac*-**7**	3		>99 (99)	>99	23	*rac*-**23**	9		98 (94)	>99
8	*rac*-**8**	6		>99 (95)	>99	24[g,h]	*rac*-**24**	24		>99 (90)	>99
9	*rac*-**9**	30		99 (92)	>99	25	*rac*-**25**	3		>99 (99)	97
10	*rac*-**10**	96		98 (95)	98	26	*rac*-**26**	3		98 (94)	>99
11	*rac*-**11**	3		99 (95)	>99	27	*rac*-**27**	9		>99 (99)	>99
12[c]	*rac*-**12**	24		>95[d] (98)	98[e]	28	*rac*-**28**	6		>99 (96)	93
13	*rac*-**13**	6		>99 (95)	>99	29	*rac*-**29**	3		>99 (96)	94
14	*rac*-**14**	24		>99 (93)	96	30	*rac*-**30**	3		>99 (99)	98
15	*rac*-**15**	9		>99 (97)	>99	31[i]	*rac*-**31**	168		99 (96)	95
16	*rac*-**16**	168		>99 (91)	>99						

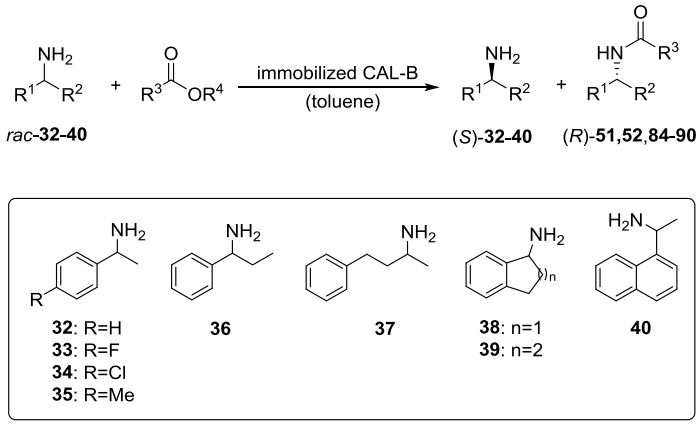
[a] Unless otherwise noted, determined by chiral GC. [b] Isolated yield in parentheses determined for single reaction. [c] 5 mL of toluene-THF -mixture (4:1). [d] Determined by NMR [e] Determined by NMR of the corresponding Mosher's ester. [f] 4 mL of toluene. [g] 2.5 mL of toluene-THF -mixture (4:1). [h] 60 °C. [i] 40 mg of CAL-B.

5.2. Lipase catalyzed kinetic resolution of primary amines (Paper II)

Aromatic amines often dissolve poorly in organic solvents tolerated well by enzymes. Accordingly, the synthetic applications of enantioselective acylation reactions of aromatic primary amines in organic solvents are limited to the use of low substrate concentrations. Moreover, the used enzyme loadings, enabling efficient reactions, have been relatively high rendering the kinetic resolution methods based on *N*-acylation less attractive. Thus, the focus herein was on developing a solvent-free reaction system which would allow higher reaction efficiency, in terms of substrate concentrations, and greener chemistry since solvents represent large part of the waste formed in organic synthesis. The previously reported solvent-free reaction systems enable the enantioseparation of racemic primary amines by CAL-B -catalyzed *N*-acylation.[123-125] However, these reactions required elevated temperatures (39-90 °C) and reduced pressures (5-45 mmHg). Consequently, the aim was to develop a simple and straightforward solvent-free method utilizing substrates and acyl donors in equivalent amounts under ambient conditions. In order to make enzymatic kinetic resolution effective and economical, the reuse possibility of the catalyst becomes important. Thus application of sol-gel entrapment to CAL-B was investigated along with the optimization of the solvent-free kinetic resolution.

5.2.1. Optimization of solvent-free kinetic resolution

Optimization was begun with acyl donor screening, after which the reaction conditions were optimized with respect to temperature, acyl donor amount and enzyme loading. The optimization of kinetic resolution was performed using the Novozym 435 preparation (CAL-B adsorbed on macroporous acrylic resin) as a catalyst and 1-phenyl ethylamine (*rac*-**32**) as a model compound (Scheme 18). The Novozym 435 preparation was chosen as a catalyst due to its well established performance in the kinetic resolutions of amines in organic solvents. The reaction progression to 50% conversion was used as a measure of enzyme activity and stability. Reaction progression was used rather than specific activity, derived from initial rate, because initial rate depicts only the initial state of a reaction. Consequently, a reaction with high specific activity can be completely stopped before its completion and thus, unsuitable for synthetic applications. All the used amines, acyl donors and possible solvents were thoroughly dried prior to use, and the amount of residual water was further controlled by the use of molecular sieves (4Å).



Scheme 18. CAL-B catalyzed kinetic resolution of primary amines **32**-**40**.

In order to find a suitable acyl donor, isopropyl acetate and butanoate as well as ethyl and isopropyl methoxyacetates were screened for the acylation of *rac*-**32** in toluene (Table 9, Entries 1-4). Isopropyl acetate gave only moderate enantioselectivity (*E*=49) in toluene while the use of isopropyl butanoate and methoxyacetates, with longer acyl moieties, resulted in excellent enantioselectivity (Entries 1-4). The reaction rates varied significantly, and the time needed to reach 50% conversion depended on the degree of the activation of the acyl donor. The activated methoxyacetates gave fast reactions (Entries 1 and 2) while the use of the non-activated isopropyl acetate and butanoate resulted in significantly slower reactions (24 h and 120 h to 50% conversion, respectively).

Table 9. CAL-B -catalyzed kinetic resolution of 1-phenyl ethylamine (*rac*-**32**, 0.1 M or 2 mmol) with acyl donor (0.2 M or 1 equiv.) in the presence of molecular sieves (4 Å, 50 mg) at 23 °C after 6 h.

Entry	Solvent	Acyl donor	CAL-B[a]	*c* (%)	ee_s (%)	ee_p (%)	*E*
1	toluene[b]	Isopropyl methoxyacetate (0.2 M)	10 mg mL^{-1}	50	96	98	>200
2	toluene[b]	Ethyl methoxyacetate (0.2 M)	10 mg mL^{-1}	49	94	99	>200
3	toluene[b]	Isopropyl acetate (0.2 M)	10 mg mL^{-1}	25	32	95	49
4	toluene[b]	Isopropyl butanoate (0.2 M)	10 mg mL^{-1}	20	25	>99	>200
5	-	Isopropyl methoxyacetate (1 equiv.)	25 mg	50	98	99	>200
6	-	Ethyl methoxyacetate (1 equiv.)	25 mg	45	81	99	>200

[a] Novozyme 435. [b] 1 mL.

Remarkably in the presence of isopropyl methoxy acetate (1 equiv.), the CAL-B catalyzed solvent-free kinetic resolution of *rac*-**32** did not differ from the reaction in toluene in terms of reaction time (Table 9, Entry 1 vs. Entry 5). This was true even when the enzyme/substrate ratio

(mg $mmol^{-1}$) was significantly decreased (100 mg enzyme/1 mmol of substrate vs. 12.5 mg enzyme/1 mmol of substrate) increasing substantially the enzyme efficiency of the method. The use of ethyl methoxyacetate under the solvent-free conditions led to significantly longer reaction time requiring 24 hours to reach 50% conversion (Entry 2 vs. Entry 6).

As satisfactory results were not obtained with other acyl donors (isopropyl acetate, isopropyl butanoate and ethyl methoxyacetate), isopropyl methoxyacetate was chosen for optimization of the reaction conditions (Table 10). Increase in the reaction temperature to 47 °C had no effect on catalyst activity (Entry 2). At 70 °C, stability of CAL-B was gradually lost and the reaction did not proceed to 50% conversion (Entry 3). Further optimization, concerning the amount of isopropyl methoxyacetate and enzyme loading, was carried out at room temperature (23 °C). Increase in the amount of isopropyl methoxyacetate (Entry 4) and CAL-B loading (Entry 7) had only a minor effect on the outcome of the reaction. Again, the decrease in the amount of acyl donor (Entries 5 and 6) and catalyst loading (Entry 8) led to significantly slower reactions. The use of isopropyl methoxyacetate in 1 equiv. to *rac*-**32** and CAL-B in 25 mg was optimal and reasonable in terms of both costs and reaction efficiency.

Table 10. CAL-B catalyzed kinetic resolution of 1-phenyl ethylamine (*rac*-**32**, 2 mmol) with isopropyl methoxyacetate (0.6-1.25 equiv.) in the presence of molecular sieves (4 Å, 50 mg).

Entry	Isopropyl methoxyacetate (equiv.)	Temp. (°C)	CAL-B[a] (mg)	Time[b] (h)	c (%)	ee_s (%)	ee_p (%)	*E*
1	(1)	23	25	6	50	98	99	>200
2	(1)	47	25	6	50	97	98	>200
3	(1)	70	25	6[c]	34	71	96	-[d]
4	(1.25)	23	25	4	50	99	99	>200
5	(0.75)	23	25	6 (24)	47	87	>99	>200
6	(0.6)	23	25	6 (56)	43	75	>99	>200
7	(1)	23	50	4	50	98	>99	>200
8	(1)	23	10	6 (24)	47	87	99	>200

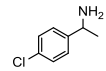
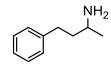
[a] Novozyme 435. [b] In parentheses time to reach 50 % conversion. [c] Reaction stopped before 50 % conversion. [d] *E* value not determined.

5.2.2. Primary amines in solvent-free kinetic resolution

The substrate scope of the CAL-B catalyzed solvent-free kinetic resolution was extended to nine structurally different aromatic primary amines (Scheme 18). The reaction conditions optimized for *rac*-**32** were applied. In the case of *rac*-**40**, higher reaction temperature (47 °C) was required to increase the solubility of the substrate amine. The results obtained in the kinetic

resolutions are presented in Table 11. The times required to reach 50% conversion in the kinetic resolutions of *rac*-**32**-**40** in toluene are presented in parentheses.

Table 11. CAL-B (Novozym 435, 25 mg) catalyzed solvent-free kinetic resolution of *rac*-**32**-**40** (2 mmol) with isopropyl methoxyacetate (2 mmol) in the presence of molecular sieves (4 Å, 50 mg) at 23 °C.

Entry	Substrate	Time[a] (h)	Recovered (*S*)-amine			Produced (*R*)-amide		
			Yield (%)	*ee* (%)	$[\alpha]_D^{25}$ [b]	Yield (%)	*ee* (%)	$[\alpha]_D^{25}$ [b]
1	 *rac*-**32**	6 (6)	35	98	-30.6	48	97	+101
2	 *rac*-**33**	3 (6)	37	>99	-24.4	39	97	+84.7
3	 *rac*-**34**	2 (6)	46	>99	-28.4	47	98	+102
4	 *rac*-**35**	3 (2)	45	>99	-30.5	45	98	+105/+120[c]
5	 *rac*-**36**	24 (48)	41	99	-9.4	45	99	+93.5/+103[c]
6	 *rac*-**37**	1 (1)	45	>99	+4.1	48	98	+34.1
7	 *rac*-**38**	2 (1)	39	>99	+20.8	44	95	+62.2/+105[c]
8	 *rac*-**39**	2 (24)	34	98	+34.7	45	99	+75.7
9[d]	 *rac*-**40**	6 (48)	35	>99	-59.2	37	99	+30.0[c]

[a] Time to reach 50±0.5% conversion; in parentheses time to reach 50% conversion in toluene [amine (0.1 M), isopropyl methoxyacetate (0.2 M), Novozym 435 (10 mg mL^{-1})]. [b] (*c* 1, $CHCl_3$). [c] (*c* 2, MeOH). [d] Reaction temperature 47 °C.

Reactions rates of the solvent-free reactions were either comparable or faster than the corresponding reactions in toluene. The enantiomers were isolated at 50% conversion. A fast column purification used for separation afforded the unreacted (*S*)-amines and produced (*R*)-amides in high yields and enantiomeric excesses ≥95%.

5.2.3. Sol-gel entrapment of *Candida antarctica* lipase B

In addition to efficient reaction conditions, reusability of the catalyst becomes important when aiming at increasing the efficiency of a synthesis method. Accordingly, CAL-B was entrapped in sol-gel matrices. The behavior of the obtained preparations were studied in the acylation of *rac*-**32** in toluene and under solvent-free conditions alongside the Novozym 435 preparation. Sol-gel entrapment was chosen because the immobilization method had proven to be applicable in the lipase *Pseudomonas fluorescens* catalyzed *N*-acylation.[126]

Free CAL-B was entrapped into sol-gel matrices following a literature protocol.[38,39,126] The sol-gel formation was based on fluoride ion -catalyzed hydrolysis of a mixture of tetramethoxysilane (TMOS) and methyltrimethoxysilane (MeTMS) followed by condensation, gelation and maturation. Each sol-gel catalyst batch (3 mmol mixture of TMOS and MeTMS) had the same enzyme loading of 75 mg. After maturation, the sol-gel preparations were dried by lyophilization to remove volatile impurities and to ensure maximum dryness. The catalytic performance of the preparations was investigated in the kinetic resolution of *rac*-**32** (0.1 M) with isopropyl methoxyacetate (0.2 M) in toluene at 23 °C. Kinetic resolutions in toluene were preferred in order to eliminate possible diffusional constraints present in a solvent-free reaction system. The influence of matrix hydrophobicity was studied by varying the precursor ratio TMOS/MeTMS (Table 12).

Table 12. The additive-free sol-gel CAL-B[a] (10 mg mL^{-1}) catalyzed kinetic resolution of 1-phenyl ethylamine (*rac*-**32**, 0.1 M) with isopropyl methoxyacetate (0.2 M) in toluene at 23 °C after 24 h.

Entry	Sol-gel CAL-B		Kinetic resolution			
	[TMOS]/[MeTMS]	Washing procedure	c (%)	ee_s (%)	ee_p (%)	*E*
1	1 : 0	IPA, water, IPA, hex	1	-	-	-
2	1 : 1	IPA, water, IPA, hex	39	63	>99	>200
3	1 : 5	IPA, water, IPA, hex	43	75	>99	>200
4	0 : 1	IPA, water, IPA, hex	37	58	>99	>200
5	1 : 5	-	50	>99	>99	>200

[a] 75 mg of CAL-B powder against a 3 mmol mixture TMOS and MeTMS.

The sol-gel consisting of TMOS resulted in practically zero conversion after 24 hours (Table 12, Entry 1). The TMOS/MeTMS ratio of 1:1 as well as pure MeTMS gave reasonable conversions and high enantioselectivities (Entries 2 and 4) while the TMOS/MeTMS ratio of 1:5 was optimal (Entry 3). The sol-gel catalysts were washed with isopropyl alcohol, water and *n*-hexane. When the entrapment utilizing the optimal ratio (TMOS/MeTMS, 1:5) was performed without washing, the catalyst obtained gave the highest activity hitherto (Entry 3 vs. entry 5). This high activity indicated that the washing had a deleterious effect on the catalytic performance of the entrapped CAL-B. Thus, the following sol-gel catalysts were prepared without washing.

The optimization was continued by studying the influence of additives in the enzyme entrapment (Figure 6). The protein content of the commercially available free CAL-B was determined (36%, w/w) allowing catalyst comparison based on matching protein contents. The enzyme contents of the sol-gel CAL-B –catalyzed reactions were set to correspond to the enzyme content (2.6 mg mL^{-1}, protein content 36%) of the most active additive-free sol-gel (Table 12, Entry 5).

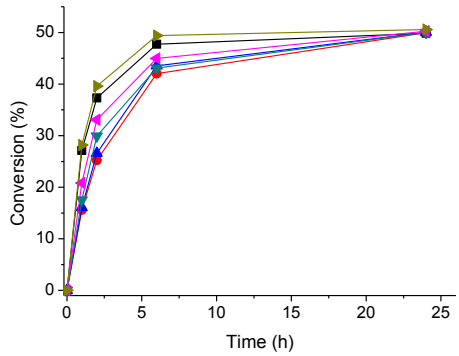


Figure 6. Effect of additives on the sol-gel CAL-B catalysts. Progression curves for the *N*-acylation of racemic 1-phenylethylamine (*rac*-**32**, 0.1 M) with isopropyl methoxyacetate (0.2 M) in toluene at 23 °C: Additive-free (■), Celite (●), Celite and sucrose (▲), 18C6 (▼), 18C6 and Celite (◀) and PVA and IPA (▶).

Addition of Celite, Celite together with sucrose, 18-crown-6 or 18-crown-6 together with sucrose led to reduced activities (Figure 6). When isopropyl alcohol (IPA) and polyvinyl alcohol (PVA) were added together, the activity increased. Nevertheless, the studied reactions reached 50% conversion in 24 hours allowing the highly enantioselective kinetic resolution of

rac-**32** (*E*>200). As introduction of additives did not lead to any significant improvements on the catalytic performance, the studies were continued with the additive-free sol-gel catalyst.

5.2.4. Reuse of catalysts in successive kinetic resolutions

Reuse of Novozym 435 and the optimized sol-gel CAL-B was studied in successive kinetic resolutions of *rac*-**32**. The progression of the reactions during the first 24 hours was used as a means of comparison. The reuse experiment with Novozym 435 (10 mg mL^{-1}) in toluene resulted in full retention of activity and enantioselectivity (*E*>200). The reuse of the enzyme enabled the acylation of *rac*-**32** (0.1 M) with isopropyl methoxyacetate (0.2 M) in 24 hours in the five successive reactions investigated (Figure 7).

Thereafter, the successive *N*-acylations catalyzed by Novozym 435 and sol-gel CAL-B were performed under the optimized solvent-free conditions [*rac*-**32** (2 mmol), isopropyl methoxyacetate (2 mmol), molecular sieves (4 Å, 50 mg), 23 °C]. Since the protein content of Novozym 435 is not known, the specific activities (initial rate divided by mass of CAL-B preparation) were determined (0.25 μmol min^{-1} mg^{-1} for Novozym 435 and 0.41 μmol min^{-1} mg^{-1} for sol-gel CAL-B) to ensure the comparability of the reactions. In order to match the specific activity obtained with 25 mg of the Novozym 435 preparation, the loading of sol-gel CAL-B was set to 16 mg.

Under the solvent-free conditions, the activity of Novozym 435 (25 mg) decreased substantially in every reuse cycle (Figure 8A). In the second reaction cycle, the reaction time to 50% conversion was already significantly increased when compared to that of the first cycle (48 h vs. 6 h). Further activity decrease in the following cycles foiled any feasible reuse of the catalyst. Activity of the sol-gel CAL-B (16 mg) also gradually decreased in the five successive solvent-free reactions (Figure 8B). Although, the first reaction cycle required 16 hours to reach 50% conversion, the decrease in activity in the following cycles was not as pronounced as in the case of Novozym 435 making the reuse of the catalyst still feasible. Although the activity of Novozym 435 and sol-gel CAL-B was partially lost, the enantioselectivity of the catalysts remained high (*E*>200) throughout the five reuse cycles.

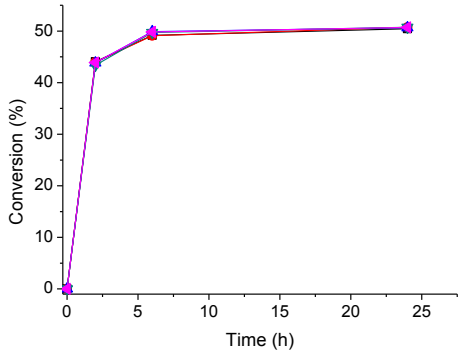


Figure 7. Progression curves for the kinetic resolution of 1-phenylethylamine (*rac*-**32**, 0.1 M) with isopropyl methoxyacetate (0.2 M) in toluene (2 mL) with Novozym 435 (10 mg mL^{-1}) at 23 °C in reuse: cycle 1 (■) (*E*>200), cycle 2 (●) (*E*>200), cycle 3 (▲) (*E*>200), cycle 4 (▼) (*E*>200) and cycle 5 (◀) (*E*>200).

A B

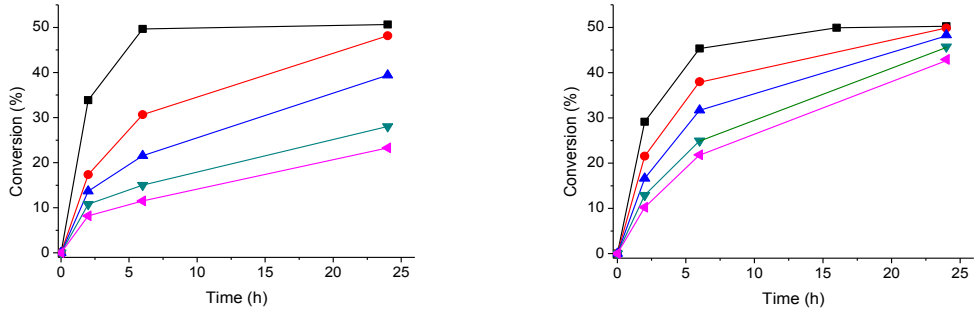


Figure 8. Progression curves for the solvent-free kinetic resolution of 1-phenylethylamine (*rac*-**32**, 2 mmol) with isopropyl methoxyacetate (2 mmol) with (A) Novozym 435 (25 mg) and (B) sol-gel CAL-B (16 mg) at 23 °C in reuse: cycle 1 (■) (*E*>200), cycle 2 (●) (*E*>200), cycle 3 (▲) (*E*>200), cycle 4 (▼) (*E*>200) and cycle 5 (◀) (*E*>200).

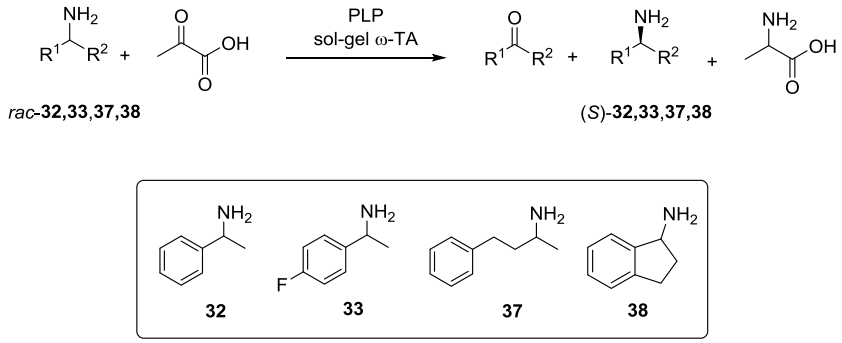
5.3. Immobilization of ω-transaminase for preparation of primary amines (Paper III)

Synthetic potential of ω-transaminases has not been fully appreciated. The focus of the research field has mostly been on the discovery of enzymes, and only in recent years been shifted towards synthetic usability. One aspect of feasibility in biocatalytic synthesis is catalyst reuse in successive reactions. However, the commercial availability of ω-transaminases is restricted to free enzyme powders. Thus, the studies herein concentrated on stabilizing the ω-transaminase to obtain a reusable catalyst for the preparation of enantiopure primary amines in an aqueous reaction system. Due to the success in the sol-gel entrapment of CAL-B (Paper II and Section 5.2.3.), the method was chosen for the immobilization of the ω-transaminase. Moreover, sol-gel immobilization was suitable for the purpose because the method entraps soluble enzyme molecules in an insoluble matrix, thus producing a heterogeneous catalyst workable in the required aqueous medium.

5.3.1. Sol-gel entrapment of *Arthrobacter* sp. ω-transaminase

The method used to entrap CAL-B in Section 5.2.3. was optimized for the (*R*)-enantioselective ω-transaminase from *Arthrobacter* sp. KNK168 (commercially available as ATA-117).[24] The ω-transaminase loading of each catalyst batch (3 mmol mixture of TMOS and RTMS, if not otherwise stated) was 50 mg. After gelation and maturation, the sol-gel preparations were washed and the washing solutions were collected for protein content determination. The protein contents of the free ω-transaminase powder (86%, w/w) and the collected washing solutions were determined in order to obtain immobilization degrees and loading capacities of the preparations. The sol-gel ω-transaminase catalysts were characterized in terms of immobilization degree, loading capacity and ability to catalyze the successive kinetic resolutions of racemic 1-phenyl ethylamine (*rac*-**32**, model compound, 50 mM) with sodium pyruvate (50 mM) in the presence of pyridoxal-5'-phosphate (0.2 mg mL^{-1}) in phosphate buffer (100 mM, pH 7.5) at 30 °C (Scheme 19). Kinetic resolution was chosen for catalyst characterization to keep the reaction system as simple as possible. Asymmetric synthesis would have required means to shift the reaction equilibrium, and any such additions to the reaction system could have distorted the evaluation of the ω-transaminase catalyst itself. Progression of the reactions over 24 hours was used as a measure of activity of the catalysts. A competing adsorption process, occurring alongside the deamination of (*R*)-**32**, distorted the *E* values of the

reactions (See section 2.3.). Thus enantiomeric excess values of the remaining (*S*)-**32** were used to describe enantioselectivity.



Scheme 19. ω-Transaminase catalyzed kinetic resolution of primary amines **32**, **33**, **37** and **38**.

Washing procedure of the sol-gel catalyst

Precursors TMOS and MeTMS in the ratio of 1:5, previously applicable in the entrapment of CAL-B (Paper II and Section 5.2.2.), were used as a starting point to optimize the washing protocol for the maturated sol-gels. The method used to wash the sol-gels (water, isopropyl alcohol and hexane in different combinations) proved to have a significant effect on the activity of the sol-gel catalysts (50 mg mL^{-1}, corresponding protein content of 8 mg mL^{-1}, Table 13). The use of IPA as the final washing solvent was beneficial to catalyst activity which was not the case when *n*-hexane or water was used (Entries 1-4). Immobilization degrees of the obtained catalysts were high (>90%) and independent of the washing procedure used.

Table 13. Sol-gel ω-transaminase[a] (50 mg mL^{-1}) catalyzed kinetic resolution of 1-phenylethylamine (*rac*-**32**, 50 mM) with sodium pyruvate (50 mM) in the presence of pyridoxal-5'-phosphate (0.2 mg mL^{-1}) in phosphate buffer (100 mM, pH 7.5) at 30 °C after 24 h.

Entry	Sol-gel ω-transaminase			Kinetic resolution	
	Washing procedure	Immobilization degree (%)	Loading capacity (%, w/w)	Conversion (%)	*ee* (%)
1	IPA, water, IPA, hex	-[b]	-[b]	49	76
2	IPA, water, IPA	93	16	58	>99
3	IPA, water	94	12	38	54
4	water, IPA	93	17	59	>99

[a] 50 mg of ω-transaminase (ATA-117) against a 3 mmol sol-gel (TMOS/MeTMS = 1:5). [b] Values were not obtained.

Loading capacities were of the same order, however, a somewhat lower value was obtained after the successive use of IPA and water (Entry 3). Water as the final solvent left the sol-gel visibly moist increasing the mass of the sol-gel matrix and decreasing the value of loading capacity of the catalyst. In order to avoid unnecessary complexity, the washing procedure utilizing water and IPA in succession (Entry 4) was adopted for further method optimization.

Precursor composition of the sol-gel catalyst

ω-Transaminase catalysts with variable sol-gel precursor compositions were prepared and examined (Table 14). First, the effect of the TMOS/MeTMS -ratio on the sol-gel ω-transaminase catalysis (50 mg mL^{-1}, protein content of 8 mg mL^{-1}) was studied in three successive kinetic resolutions of *rac*-**32** (Entries 1-4). Immobilization degrees remained high (>90%) throughout the series of the sol-gels. A slight decrease with increasing proportion of MeTMS, leading to increasing hydrophobicity of the sol-gel, was observed. Variation in loading capacities (14-17%) was more inconsistent. The most promising sol-gel catalyst had the precursor ratio of TMOS/MeTMS = 1:5, giving full conversions and enantiopure (*S*)-**32** in the three reuse cycles (Entry 3). The ratio of TMOS/MeTMS = 1:7 proved to be the limit in terms of feasible sol-gel formation (Entry 4).

Table 14. Additive-free sol-gel ω-transaminase[a] (50 mg mL^{-1}) catalyzed successive kinetic resolution of 1-phenylethylamine (*rac*-**32**, 50 mM) with sodium pyruvate (50 mM) in the presence of pyridoxal-5'-phosphate (0.2 mg mL^{-1}) in phosphate buffer (100 mM, pH 7.5) at 30 °C after 24 h.

Entry	Sol-gel ω-transaminase				Conversion (%)/*ee* (%)		
	R	[TMOS]/ [RTMS]	Immobilization degree (%)	Loading capacity (%, w/w)	Cycle 1	Cycle 2	Cycle 3
1	Me	1:1	97	17	45/45	34/40	28/38
2	Me	1:3	97	14	47/62	40/65	36/63
3	Me	1:5	93	17	59/>99	50/>99	50/>99
4	Me	1:7	91	15	42/57	29/52	26/45
5	Pr	1:5	93	16	39/39	33/41	30/41
6[b]	*i*-Bu	1:5	92	31	51/83	47/83	43/79

[a] 50 mg of ω-transaminase powder (ATA-117)/ 3 mmol sol-gel. [b] 25 mg catalyst content.

The optimal precursor ratio of 1:5 was applied to the preparation of sol-gels consisting of either propyltrimethoxysilane (PrTMS) or isobutyltrimethoxysilane (iBuTMS) together with TMOS using the same ω-transaminase loading (50 mg/ 3 mmol of TMOS and RTMS). The use of PrTMS did not change the immobilization degree or the loading capacity of the catalyst but led

to insufficient enzyme activity (Table 14, Entry 5). With iBuTMS, the immobilization degree remained at the same level but the loading capacity of the catalyst was significantly higher (Entry 6). The high value was due to a slow condensation reaction. As a result of the slow condensation, a considerable part of the sol-gel mixture was washed off as liquid precursors which resulted in low yield of the sol-gel matrix. When the protein content of the catalyst was kept at 8 mg mL^{-1} (corresponding to the catalyst content of 25 mg mL^{-1}), lower activity and reusability was observed in comparison with that of the corresponding sol-gel containing MeTMS (Entry 6 vs. Entry 3).

Introduction of additives to the sol-gel catalyst

Effects of additives on sol-gel ω-transaminase (25 mg mL^{-1}, protein content of 4 mg mL^{-1}) catalyzed kinetic resolution of *rac*-**32** were investigated using the optimal precursor ratio of TMOS/MeTMS = 1:5 (Table 15, Entries 2-6). The obtained results were compared to the results of the corresponding additive-free sol-gel catalyst (25 mg mL^{-1}, protein content of 4 mg mL^{-1}) catalyzed reactions (Entry 1). The presence of Celite and Celite with sucrose led to reduced conversions (Entries 2 and 3). Both IPA and polyvinyl alcohol (PVA) as sole additives gave higher conversions (Entries 4 and 5). However, these reactions did not reach completion within the monitored 24 hours (Entries 4 and 5). Simultaneous use of IPA and PVA enhanced the catalyst activity significantly and the first reaction cycle proceeded to completion within 24 hours. Nevertheless, the reusability of the catalyst was still not satisfactory (Entry 6).

To further enhance the catalytic properties of the sol-gel catalyst, ω-transaminase loading of the sol-gel preparation (3 mmol mixture of TMOS and MeTMS) was increased to 100 mg (instead of 50 mg). The immobilization degree of the catalyst remained high (Table 15, Entry 7). Thus, the loading capacity was significantly higher in comparison with catalyst having the lower ω-transaminase loading (Entry 7 vs. Entry 1). The two-fold increase in enzyme loading also led to enhanced catalyst activity and reusability. The benefits obtained from the use of IPA and PVA combined with the higher ω-transaminase loading produced a catalyst yielding (*S*)-**32** in three successive reuse cycles (Entry 8).

The simultaneous introduction of IPA and PVA was applied to the preparation of the TMOS/iBuTMS = 1:5 sol-gel catalyst to improve the properties of the catalyst (Entry 10). The introduction did not lead to desired results. Rather, reduced conversions were measured in comparison with those of the additive-free sol-gel catalyst (Entry 10 vs. Entry 9). The presence of PVA and IPA resulted in a similar immobilization degree, yet in a lower loading capacity.

The lower value was due to a more complete condensation process and, accordingly, a higher yield of the sol-gel matrix. The mere increase in the ω-transaminase loading of the catalyst (3 mmol mixture of TMOS and iBuTMS in ratio of 1:5) to 100 mg resulted in increased activity and reusability (Entry 11). However, the high loading capacity value was again a proof of an incomplete condensation process rendering the catalyst less economical when the protein content was compared to the catalyst consisting of precursors TMOS and MeTMS in the same ratio (42% and 27%, respectively). The optimized sol-gel catalyst [ω-transaminase (100 mg), silanes (3 mmol), TMOS/MeTMS = 1:5, IPA and PVA as additives, washed with water and IPA] was subjected to further studies concerning the scope and limitations of the catalyst.

Table 15. Sol-gel ω-transaminase[a] (25 mg mL^{-1}) catalyzed successive kinetic resolution of 1-phenylethylamine (*rac*-**32**, 50 mM) with sodium pyruvate (50 mM) in the presence of pyridoxal-5'-phosphate (0.2 mg mL^{-1}) in phosphate buffer (100 mM, pH 7.5) at 30 °C after 24 h.

Entry	Sol-gel ω-transaminase				Conversion (%)/*ee* (%)		
	R	Additive(s)	Immobilization degree (%)	Loading capacity (%, w/w)	Cycle 1	Cycle 2	Cycle 3
1	Me	-	93	17	34/44	24/34	22/31
2	Me	Celite	93	13	21/18	13/20	14/18
3	Me	Celite + sucrose	93	13	15/11	16/19	20/25
4	Me	IPA	93	15	53/94	40/82	37/68
5	Me	PVA	92	15	46/65	31/49	26/40
6	Me	IPA + PVA	93	16	55/>99	47/91	40/74
7[b]	Me	-	93	27	55/>99	50/>99	48/95
8[b]	Me	IPA + PVA	94	26	55/>99	52/>99	51/>99
9	*i*-Bu	-	92	31	51/83	47/83	43/79
10	*i*-Bu	IPA + PVA	90	23	23/23	18/23	17/21
11[b]	*i*-Bu	-	92	42	54/>99	49/>99	50/>99

[a] 50 mg of ω-transaminase powder (ATA-117)/ 3 mmol sol-gel (TMOS/RTMS=1:5). [b] 100 mg of ω-transaminase powder (ATA-117) against a 3 mmol sol-gel (TMOS/RTMS=1:5).

5.3.2. Insights into sol-gel ω-transaminase catalyzed kinetic resolution

Conversions of the successive kinetic resolutions, determined by the disappearance of *rac*-**32**, revealed a minor background process occurring in the presence of the sol-gel matrix. While all three successive kinetic resolutions catalyzed by the additive-free sol-gel ω-transaminase (50 mg mL^{-1}, TMOS/MeTMS=1:5) produced (*S*)-**32** with *ee*>99%, the conversion of the first reaction cycle was substantially higher than that of the following two cycles (Table 14, Entry 3). In the presence of an additive-free sol-gel matrix (TMOS/MeTMS = 1:5, in the absence of

ω-transaminase), 20 % of *rac*-**32** disappeared under the kinetic resolution conditions in two hours. The situation remained unaltered for the monitored 24 hours. These results gave the impression that the excess consumption was caused by adsorption of the amine on the hydrophobic sol-gel matrix.

The degree of the proposed adsorption in the first reaction cycle could be lowered by reducing the loading of the catalyst (Figure 9 and Table 16, Entry 2 vs. Entry 1). Further decrease in the adsorption was gained when the reduced catalyst loading was applied together with organic co-solvents DMSO or IPA (Figure 9).

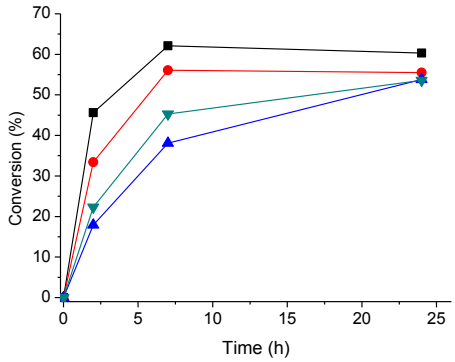


Figure 9. Sol-gel ω-transaminase [ω-transaminase (100 mg), silanes (3 mmol), TMOS/MeTMS = 1:5, IPA and PVA as additives, washed with water and IPA] catalyzed kinetic resolution of 1-phenylethylamine (*rac*-**32**, 50 mM) with sodium pyruvate (50 mM) in the presence of pyridoxal-5'-phosphate (0.2 mg mL^{-1}) in phosphate buffer (100 mM, pH 7.5) at 30 °C: 50 mg mL^{-1} of catalyst (■), 25 mg mL^{-1} of catalyst (●), 25 mg mL^{-1} of catalyst and 10% IPA (▲) and 25 mg mL^{-1} of catalyst and 10% DMSO (▼).

Table 16. Sol-gel ω-transaminase[a] catalyzed successive kinetic resolutions of 1-phenylethylamine (*rac*-**32**, 50 mM) with sodium pyruvate (50 mM) in the presence of pyridoxal-5'-phosphate (0.2 mg mL^{-1}) in phosphate buffer (100 mM, pH 7.5) at 30 °C after 24 h.

Entry	Sol-gel catalyst loading (mg)	Co-solvent (v/v)	Conversion (%)/*ee* (%)		
			Cycle 1	Cycle 2	Cycle 3
1	50	-	60/>99	52/>99	51/>99
2	25	-	56/>99	52/>99	51/>99
3	25	10% DMSO	54/>99	52/>99	50/>99
4	25	10% IPA	54/>99	49/99.5	49/96

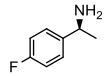
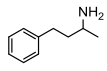
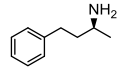
[a] 100 mg of ω-transaminase powder (ATA-117)/ 3 mmol sol-gel (TMOS/MeTMS=1:5, IPA + PVA, washed with water and IPA).

DMSO was chosen for further studies because IPA was not found as suitable when catalyst performance in reuse was considered (Table 16, Entries 3 and 4).

5.3.3. Primary amines in sol-gel ω-transaminase catalyzed kinetic resolution

The substrate scope of the optimized sol-gel ω-transaminase catalyst [ω-transaminase (100 mg), silanes (3 mmol), TMOS/MeTMS = 1:5, IPA and PVA as additives, washed with water and IPA] was investigated in the kinetic resolution of *rac*-**32**, **33**, **37** and **38** (Table 17). DMSO (10%, v/v) was utilized as a co-solvent in order to alleviate the proposed adsorption process and to ensure the solubility of the substrates and products. Sol-gel ω-transaminase (25 mg mL^{-1}) catalyzed kinetic resolution afforded (*S*)-**32**, **34** and **37** at 54-56% conversion after 24 hours (Entries 1-3). With *rac*-**38**, a two-fold higher catalyst loading (50 mg mL^{-1}) was needed to produce (*S*)-**38** in enantiopure form after 24 hours (Entry 4).

Table 17. Sol-gel ω-transaminase[a] (25 mg mL^{-1}) catalyzed kinetic resolution of *rac*-**32**, **33**, **37** and **38** (50 mM) with sodium pyruvate (50 mM) in the presence of pyridoxal-5'-phosphate (0.2 mg mL^{-1}) in phosphate buffer (100 mM, pH 7.5, 10 v/v-% DMSO) at 30 °C after 24 h.

Entry	Substrate	(*S*)-Product	Conversion (%)	*ee* (%)
1			54	>99
	rac-**32**	(*S*)-**32**		
4			56	>99
	rac-**33**	(*S*)-**33**		
5			54	>99
	rac-**37**	(*S*)-**37**		
6			44/64[b]	80/>99[b]
	rac-**38**	(*S*)-**38**		

[a] 100 mg of ω-transaminase powder (ATA-117)/ 3 mmol sol-gel (TMOS/MeTMS=1:5, IPA and PVA, washed with water and IPA). [b] Sol-gel ω-transaminase content 50 mg mL^{-1}.

5.3.4. Reuse of sol-gel ω-transaminase on preparative scale

The synthetic potential of the sol-gel ω-transaminase catalyst [50 mg mL^{-1}, ω-transaminase (100 mg), silanes (3 mmol), TMOS/MeTMS = 1:5, IPA and PVA as additives, washed with water and IPA] was investigated in five successive preparative kinetic resolutions of *rac*-**32**

(100 mM, Figure 10). DMSO was used to decrease the extent of the amine adsorption and to enable a higher substrate concentration (100 mM instead of 50 mM). Each reaction was let to proceed for 24 hours before the reaction mixture was removed by centrifugation. After the removal, a fresh reagent solution was added to initiate the next reaction cycle. After five cycles, (*S*)-**32** was isolated by adjustment of pH of the combined reaction solutions followed by extraction and evaporation. The five successive reactions reached close to 50% conversions affording (*S*)-**32** in 41% isolated yield and 98% *ee*. A slight decrease in the sol-gel catalyst activity can be observed (Figure 10). Part of the observed decrease in activity might be due to catalyst recovery losses (centrifugation and removal of the reaction mixture) which have accumulated over the reuse cycles.

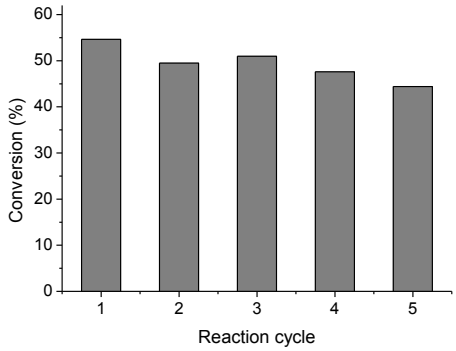


Figure 10. Sol-gel ω-transaminase (50 mg) catalyzed successive kinetic resolutions of 1-phenylethylamine (*rac*-**32**, 100 mM) with sodium pyruvate (100 mM) in the presence of pyridoxal-5'-phosphate (0.2 mg mL^{-1}) in phosphate buffer (100 mM, pH 7.5, 10 v/v-% DMSO) at 30 °C after 24 h.

6. SUMMARY

The literature review of this thesis introduces the concept of biocatalysis and its synthetic usability in the preparation of enantiopure compounds. A particular focus of the review is on lipase and ω-transaminase catalysis as well as on methods used in the immobilization of these enzymes. Discussions on synthetic utilization of lipases and ω-transaminases concentrate on the preparation of the two target compound groups of this thesis, secondary alcohols and primary amines, as single enantiomers. The feasibility of relevant biocatalytic synthesis methods is demonstrated by utilizing the reactions of the selected model compounds 1-phenylethanol (*rac*-**1**) and 1-phenylethylamine (*rac*-**32**). The lipase-catalyzed synthesis methods include acylation-based kinetic resolutions and dynamic kinetic resolutions of racemic secondary alcohols and racemic primary amines. The ω-transaminase catalyzed methods are discussed in the context of synthesis of primary amine enantiomers via enantioselective deamination racemic amines and via asymmetric amination of corresponding ketones.

The results and discussion section presents the research conducted on this thesis. In the first part of the section, the successful optimization and general usability of a dynamic kinetic resolution method for secondary alcohols are demonstrated. The method combines *Candida antarctica* lipase B (CAL-B) catalyzed *O*-acylation with isopropenyl acetate and ruthenium complex **46** catalyzed *in situ* racemization in the presence of neutralizing agent, Na_2CO_3. In order to enable a productive co-operation of CAL-B and complex **46**, the overall reaction system was assessed and potential side reactions were suppressed. The stable and controlled reaction conditions of glove-box (nitrogen atmosphere, water and molecular oxygen < 1 ppm) allowed high reaction efficiency in terms of **46** loading (2 mol-%) and amounts of isopropenyl acetate (1.2 equiv.) and Na_2CO_3 (0.5 equiv.). The smooth co-operation of CAL-B and **46** enabled the acylation of racemic alcohols **1**-**31** to the corresponding (*R*)-acetates in 90-99% yield and mostly in >99% *ee*. The described method provides the (*R*)-acetates of a range of structurally different secondary alcohols in excellent yields. As a disadvantage, the method cannot be easily extended to enable the preparation of (*S*)-acetates from racemic secondary alcohols. Switch of enantiopreference cannot be accomplished by the use of a wild-type lipase but would require a modified lipase with preference towards (*S*)-alcohols.

The second part of the section describes an efficient solvent-free kinetic resolution of primary amines under ambient reaction conditions (room temperature and normal pressure). The

optimized CAL-B catalyzed *N*-acylation, performed with equimolar amounts (2 mmol) of substrate and isopropyl methoxyacetate, afforded the enantiomers of racemic primary amines **32**-**40** at 50% conversion as (*R*)-amides and (*S*)-amines in 35-48% yield and ≥95% *ee*. When compared to the *N*-acylation of *rac*-**32** (0.2 M) in toluene (1 mL), the increase in reaction efficiency was evident. However, the use of this solvent-free reaction system was restricted to liquid substrates or substrates soluble in the acyl donor used. An increase in enzyme efficiency, measured as an enzyme/substrate –ratio, was attained in comparison with the corresponding kinetic resolution in toluene (12.5 mg/mmol vs. 100 mg/mmol). An in-house sol-gel immobilization increased the reusability of CAL-B when compared to a widely used commercial immobilizate, Novozyme 435. If the solvent-free *N*-acylation could be combined with racemization of the (*S*)-enantiomer *in situ*, an efficient dynamic kinetic resolution method for the production of the (*R*)-amide would be obtained.

The third part concentrates on the development of a sol-gel entrapment method for a commercial ω-transaminase lyophilizate (ATA-117, *Arthrobacter* sp.). After optimization of the entrapment, a heterogeneous ω-transaminase catalyst suitable for the kinetic resolution of primary amines in aqueous environment was obtained. The use of the sol-gel ω-transaminase enabled the enantiomeric enrichment of racemic amines **32**, **33**, **37** and **38** (50 mM) to their (*S*)-enantiomers at 55-64 % conversions. The kinetic resolution method suffered from the adsorption of the substrate amines on the hydrophobic sol-gel matrix. The degree of the adsorption could be lowered by using DMSO (10%, v/v) as a co-solvent. The presence of DMSO enabled the use of higher substrate concentration in the preparative kinetic resolutions of *rac*-**32** (100 mM). Reuse of the sol-gel catalyst in five successive kinetic resolutions, affording (*S*)-**32** in 41% yield 98% *ee*, demonstrates the synthetic usability of the catalyst. Further synthetic benefit of the heterogeneous ω-transaminase catalysis would be obtained by applying the catalyst to the asymmetric synthesis of the target amines utilizing the corresponding ketones as achiral substrates.

ACKNOWLEDGEMENTS

The experimental work of this thesis was carried out at the Laboratory of Synthetic Drug Chemistry, Institute of Biomedicine, Faculty of Medicine, University of Turku berween the years 2007 and 2013. Finacial support from the Finnish Funding Agency for Technology and Innovation (TEKES) and Graduate School of Organic Chemistry and Chemical Biology is gratefully acknowledged.

I am thankful to my supervisor, Professor Liisa Kanerva, for giving me the opportunity to join her research group and pursue a doctoral thesis. I thank Professor Reko Leino for the privilege to work in his group during the years 2009 and 2010 and for the critical reading of the manuscript of this thesis. Professor Jan-Erling Bäckvall and the members of his research group are thanked for sharing their knowledge in the field of dynamic kinetic resolution.

I wish to thank Professor Lucia Gardossi and Professor Jari Yli-Kauhaluoma for reviewing this thesis and presenting their constructive criticism and valuable comments. I also wish to take this opportunity to thank my opponent Professor Per Berglund.

This work would not have been possible without my collaborators. I am very thankful to Dr. Denys Mavrynsky, Dr. Katri Lundell and MSc. Päivi Perkiö for their contributions. Dr. Katri Lundell is also thanked for the careful reading of the manuscript of this thesis.

I also want to thank all my former and current colleagus at the Laboratory of Synthetic Drug Chemistry and Laboratory of Organic Chemistry, Åbo Akademi University.

A warm thank you goes to my colleagus abroad for all the fun times shared on conferences and graduate school courses over these years.

I feel very fortuned and privileged to have so many great friends in my life. I thank my childhood friends for their friendship which has lasted all these years. I am equally thankful to all my friends in Turku for making me feel at home.

I am thankful to Pia Roering for her friendship and support during these years. Our trips and hobbies have provided a much needed distraction from science. I want to thank my partners in chemistry Anna Kirjavainen and Anna Leisvuori for sharing this journey with me. I am very grateful to Anna Kirjavainen for all her support and especially for answering the phone whenever and wherever. I feel overwhelmed by the kindness Anna Leisvuori and her family

have shown me. Special thanks go to Oiva Tuomi for showing me the meaning of a true friendship.

Finally, I would like to thank my family. I am grateful to my brother Antti and his wife Pia for watching out for me, especially during my first years in Turku. I feel extra fortuned to have my nieces Ella and Iiris to remind me what really matters in life. I express my deepest gratitude to my parents Ritva and Antero for encouraging me to pursue a doctoral thesis and for supporting me in everything that came along with it. I feel immense gratitude to my mother for believing in me and standing by me through all the hardships I encountered.

Turku, August 2014

Mari Päiviö

REFERENCES

[1] Zaks, A.; Klibanov, A. M. Enzymatic catalysis in organic media at 100 °C. Science 1984, 224, 1249-1251.

[2] Zaks, A.; Klibanov, A. M. Enzyme-catalyzed processes in organic solvents. *PNAS* **1985**, *82*, 3192-3196.

[3] Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvie, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughhe, G. J. Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture. *Science* **2010**, *329*, 305-309.

[4] Desai, A. A. Sitagliptin Manufacture: A compelling tale of green chemistry, process intensification, and industrial asymmetric synthesis. *Angew. Chem. Int. Ed.* **2011**, *50*, 1974-1976.

[5] Tipton, K.; Boyce, S. History of the enzyme nomenclature system. *Bioinformatics* **2000**, *16*, 34-40.

[6] 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.

[7] Chen, C.-S.; Wu, S.-H.; Girdaukas, G., Sih, C. J. Quantitative analyses of biochemical kinetic resolution of Eenantiomers. 2. Enzyme-catalyzed esterifications in water-organic solvent biphasic systems. *J. Am. Chem. Soc.* **1987**, *109*, 2812-2817.

[8] Kanerva, L. T.; Liljeblad, A. *Transesterification – Biological* in *Encyclopedia of catalysis*, Ed. Horváth, I. T.; 1st Ed.; Wiley, **2010**.

[9] Kazlauskas, R. J. Elucidating structure-mechanism relationships in lipases: prospects for predicting and engineering catalytic properties. *TIBTECH* **1994**, *12*, 464-472.

[10] Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, A. L. 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.

[11] Anderson, E. M.; Larsson, K. M.; Kirk, O. One biocatalyst – many applications: The use of *Candida antarctica* B-lipase in organic synthesis. *Biocatal. Biotransform.* **1998**, *16*, 181-204.

[12] Uppenberg, J.; Hansen, M. T.; Patjar, S.; Jones, A. The sequence, crystal structure determination and refinement of two crystal forms of lipase B from *Candida antarctica*. *Structure* **1994**, *2*, 293-308.

[13] Uppenberg, J.; Öhrner, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T. A. Crystallographic and molecular-modeling studies of lipase B from *Candida antarctica* reveal a Stereospecificity pocket for secondary alcohols. *Biochem.* **1995**, *34*, 16838-16851.

[14] Orrenius, C.; Hæffner, F.; Rotticci, D.; Öhrner, N.; Norin, T.; Hult, K. Chiral recognition of alcohol enantiomers in acyl transfer reactions catalyzed by *Candida antarctica* lipase B. *Biocatal. Biotransform.* **1998**, *16*, 1-15.

[15] Larsen, M. W.; Zielinska, D. F.; Martinelle, M.; Hodalgo, A.; Jensen, L. J.; Bornsheuer, U. T.; Hult, K. Suppression of water as a nucleophile in *Candida antarctica* lipase B catalysis. *ChemBioChem* **2010**, *11*, 796-801.

[16] McMurry, J. *Organic Chemistry with Biological Applications 2e*, 2nd Ed.; Brooks/Cole, **2011**, pp. 836-840.

[17] Svedendahl, M.; Branneby, C.; Lindberg, L.; Berglund P. Reversed Enantiopreference of an ω-Transaminase by a Single-Point Mutation. *ChemCatChem* **2010**, *2*, 976-980.

[18] Mutti, F. G.; Fuchs, C. S.; Pressnitz, D.; Sattler, J. H.; Kroutil W. Stereoselectivity of four (*R*)-selective transaminases for the asymmetric amination of ketones. *Adv. Synth. Catal.* **2011**, *353*, 3227-3233.

[19] Höhne, M.; Schätzle, S.; Jochens, H.; Robins, K.; Bornscheuer, U. T. Rational assignment of key motifs for function guides *in silico* enzyme identification. *Nat. Chem. Biol.* **2010**, *11*, 807-813.

[20] Kaulmann, U.; Smithies, K.; Smith M. E. B.; Hailes, H. C.; Ward, J. M. Substrate spectrum of ω-transaminase from *Chromobacterium violaceum* DSM30191 and its potential for biocatalysis. *Enzyme and Microb. Technol.* **2007**, *41*, 628-637.

[21] Park, E.-S.; Kim, M.; Shin, J.-S. Molecular determinants for substrate selectivity of ω-transaminases. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 2425-2435.

[22] Mutti, F. G.; Kroutil, W. Asymmetric bio-amination of ketones in organic solvents. *Adv. Synth. Catal.* **2012**, *354*, 3409-3413.

[23] Truppo, M. D.; Strotman, H.; Hughes G. Development of an immobilized transaminase capable of operating in organic solvent. *ChemCatChem* **2012**, *4*, 1071-1074.

[24] Savile, C.; Mundorff, E.; Moore, J. C.; Devine, P. N.; Janey, J. M. (Codexis, Inc.). Transaminase biocatalysts. *US Patent 8,293,507 B2*; Oct. 23, **2012**.

[25] Iwasaki, A.; Yamada, Y.; Ikenaka, Y.; Hasegawa, J. Microbial synthesis of (*R*)- and (*S*)-3,4,-dimethoxyamphetamines through stereoselective transamination. *Biotechnol. Lett.* **2003**, 25, 1843-1846.

[26] Iwasaki, A.; Yamada, Y.; Kizaki, N.; Ikenaka, Y.; Hasegawa, J. Microbial synthesis of chiral amines by (*R*)-specific transamination with *Arthrobacter* sp. KNK168. *Appl. Microbiol. Biotechnol.* **2006**, *69*, 499-505.

[27] Iwasaki, A.; Matsumoto, K.; Hasegawa, J.; Yasohara, Y. A novel transaminase, (*R*)-amine:pyruvate aminotransferase, from *Arthrobacter* sp. KNK168 (FERM BP-5228): purification, characterization and gene cloning. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1563-1573.

[28] Koszelewski, D.; Lavandera, I.; Clay, D.; Rozzell, D.; Kroutil, W. Asymmetric synthesis of optically pure pharmacologically relevant amines employing ω-transaminases. *Adv. Synth. Catal.* **2008**, *350*, 2761-2766.

[29] Truppo, M. D.; Turner, N. J. Micro-scale process development of transaminase catalyzed reactions. *Org. Biomol. Chem.* **2010**, *8*, 1280-1283.

[30] Koszelewski, D.; Müller, N.; Schrittwieser, J. H.; Faber, K.; Kroutil, W. Immobilization of ω-transaminases by encapsulation in a sol-gel/celite matrix. *J. Mol. Cat. B: Enz.* **2010**, *63*, 39-44.

[31] Klibanov, A. M. Immobilized enzymes and cell as practical catalysts. *Science* **1983**, *11*, 722-727.

[32] Hanefeld, U.; Gardossi, L.; Magner, E. Understanding enzyme immobilization. *Chem. Soc. Rev.* **2009**, *38*, 453-468.

[33] Mateo, C.; Palomo, J. M.; Fernandez-Lorente, G.; Guisan, J. M.; Fernandez-Lafuente, R. Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzyme Microb. Technol.* **2007**, *40*, 1451–1463.

[34] Sheldon, R. A. Enzyme immobilization: The quest for optimum performance. *Adv. Synth. Catal.* **2007**, *349*, 1289-1307.

[35] Brady, D.; Jordaan, J. Advances in enzyme immobilization. *Biotechnol. Lett.* **2009**, *31*, 1639-1650.

[36] Marlot, C.; Langrand G.; Triantaphylides, C.; Baratti, J. Ester synthesis in organic solvent catalyzed by lipases immobilized on hydrophilic supports. *Biotechnol. Lett.* **1985**, *7*, 647-650.

[37] Norin, M.; Boutelje, J.; Holmberg, E.; Hult, K. Lipase immobilization by adsorption: Effect of support hydrophobicity on the reaction rate of ester synthesis in cyclohexane. *Appl. Microbiol. Biotechnol.* **1988**, *28*, 527-530.

[38] Reetz, M. T.; Zonta, A.; Simpelkamp, J. Efficient Heterogenous biocatalysts by entrapment of lipases in hydrophobic sol-gel materials. *Angew. Chem. Int. Ed.* **1995**, *34*, 301-303.

[39] Reetz, M. T.; Zonta, A.; Simpelkamp, J. Efficient immobilization of lipases by entrapment in hydrophobic sol-gel materials. *Biotechnol. Bioeng.* **1996**, *49*, 527-534.

[40] Ivanov, A. E.; Schneider, M. P. Methods for the immobilization of lipases and their use for ester synthesis *J. Mol. Cat. B: Enz.* **1997**, *3*, 303-309.

[41] Serrano-López, P.; Cao, L.; van Rantwijk, F.; Sheldon, R. A. Cross-linked enzyme aggregates with enhanced activity: application to lipases. *Biotechnol. Lett.* **2002**, *24*, 1379-1383.

[42] Lee, C.-w.; Yi, S.-S.; Kim, J.; Lee, Y.-S.; Kim, B.-G. Improved immobilized enzyme systems using spherical micro silica sol-gel enzyme beads. *Biotechnol. Bioprocess Eng.* **2006**, *11*, 277-281.

[43] Shin, J.-S.; Kim, B.-G.; Shin, D.-H. Kinetic resolution of chiral amines using packed-bed reactor. *Enzyme Microb. Technol.* **2001**, *29*, 232-239.

[44] Martin, A. M.; Shonnard, D.; Pannuri, S.; Kamat, S. Characterization of free and immobilized (*S*)-aminotransferase for acetophenone production. *Appl. Microbiol. Biotechnol*. **2007**, *76*, 843-851.

[45] Cárdenas-Fernández, M.; Neto, W.; López C.; Álvaro, G.; Tufvesson, P.; Woodley, J. M. Immobilization of *Escherichia coli* containing ω-transaminase activity in LentiKats®. *Biotechnol. Prog.* **2012**, *28*, 693-698.

[46] Rehn, G.; Grey, C.; Branneby, C., Lindberg, L.; Adlercreuzt, P. Activity and stability of different immobilized preparations of recombinant *E. coli* cells containing ω-transaminase. *Process Biochem.* **2012**, *47*, 1129-1134.

[47] Yi, S.-S.; Lee, C.-G.; Kim, J.; Kyung, D.; Kim, B.-G.; Lee, Y.-S. Covalent immobilization of ω-transaminase from *Vibrio fluvialis* JS17 on chitosan beads. *Process Biochem.* **2007**, *42*, 895-898.

[48] Mallin, H.; Menyes, U.; Vorhaben, T.; Höhne, M.; Bornscheuer, U. T. Immobilization of two (*R*)-amine transaminases on an optimized chitosan support for the enzymatic synthesis of optically pure amines. *ChemCatChem* **2013**, *5*, 588-593.

[49] Jin, W.; Brennan, J. D. Properties and applications of protein encapsulated within sol-gel derived materials. *Anal. Chim. Acta* **2002**, *461*, 1-36.

[50] Pierre, A. C. The sol-gel encapsulation of enzymes. *Biocatal. Biotransform.* **2004**, *22*, 145-170.

[51] Buthe, A. Entrapment of enzymes in nanoporous sol-gels in Nanoscale biocatalysis, Methods in molecular biology, Ed. Wang, P.; Springer, **2011**.

[52] Reetz, M. T.; Tielmann, P.; Wiesenhöfer, W.; Könen, W.; Zonta, A. Second generation sol-gel encapsulated lipases: robust heterogeneous biocatalysts. *Adv. Synth. Catal.* **2003**, *345*, 717-728.

[53] Reetz, M. T.; Wenkel, R., Avnir, D. Entrapment of lipases in hydrophobic sol-gel materials: Efficient heterogeneous biocatalysts in aqueous medium. *Synthesis* **2000**, *6*, 781-783.

[54] Kanerva, L. T. Hydrolase-catalyzed asymmetric and other transformations of synthetic interest in Enzymatic reactions in organic media, Ed. Koskinen, A. M. P. and Klibanov, A. M.; 1st Ed.; Blackie Academic & Professional, **1996**.

[55] Frykman, H.; Öhrner, N.; Norin, T.; Hult, K. S-Ethyl Thiooctanoate as acyl donor in lipase catalysed resolution of secondary alcohols. *Tetrahedron Lett.* **1993**, *34*, 1367-1370.

[56] Weber, H. K.; Weber, H.; Kazlauskas, R. J. 'Watching' lipase-catalyzed acylations using 1H NMR: competing hydrolysis of vinyl acetate in dry organic solvents. *Tetrahedron: Asymm.* **1999**, *10*, 2635-2638.

[57] Hanefeld, U. Reagents for (ir)reversible enzymatic acylations. *Org. Biomol. Chem.* **2003**, *1*, 2405-2415.

[58] Degueil-Castaing, M.; De Jeso, B.; Drouillard, S.; Maillaird, B. Enzymatic reactions in organic synthesis: 2-ester interchange of vinyl esters. *Tetrahedron Lett.* **1987**, *28*, 953-954.

[59] Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda J. One-pot synthesis of optically active cyanohydrin acetates from aldehydes via lipase-catalyzed kinetic resolution coupled with in situ formation and racemization of cyanohydrins. *J. Org. Chem.* **1992**, *57*, 5643-5649.

[60] Kanerva, L. T.; Rahiala, K.; Sundholm, O. Optically active cyanohydrins and enzyme catalysis. *Biocatal.* **1994**, *10*, 169-180.

[61] Dinh, P. M.; Howarth, J. A.; Hudnott, A. R.; Williams, J. M. J.; Harris W. Catalytic racemization of alcohols: Applications to enzymatic resolution reactions. *Tetrahedron Lett.* **1996**, *37*, 7623-7623.

[62] 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*. **1997**, *36*, 1211-1212.

[63] Lee, J. H.; Han, K.; Kim, M.-J.; Park, J. Chemoenzymatic dynamic kinetic resolution of alcohols and amines. *Eur. J. Org. Chem.* **2010**, 999-1015.

[64] Hoyos, P.; Pace, V.; Alcántara, A. R. Dynamic kinetic resolution *via* hydrolase-metal combo catalysis in stereoselective synthesis of bioactive compounds. *Adv. Synth. Catal.* **2012**, *354*, 2585-2611.

[65] Persson, B. A.; Larsson, A. L- E.; Le Ray, M.; Bäckvall, J.-E. Ruthenium- and enzyme-catalyzed kinetic resolution of secondary alcohols. *J. Am. Chem. Soc.* **1999**, *121*, 1645-1650.

[66] Choi, J. H.; Kim, Y. H.; Nam, S. H.; Shin, S. T.; Kim, M.-J., Park, J. Aminocyclopentadinyl ruthenium chloride: Catalytic racemization and dynamic kinetic resolution of alcohols at ambient temperature. *Angew. Chem. Int. Ed*. **2002**, *41*, 2373-2376.

[67] Choi, J. H.; Choi Y. K.; Kim, Y. H.; Park, E. S.; Kim, E. J.; Kim, M.-J., Park, J. Aminocyclopentadinyl ruthenium complexes as racemization catalysts for dynamic kinetic resolution of secondary alcohols at ambient temperature. *J. Am. Chem. Soc.* **2004**, *69*, 1972-1977.

[68] Csjernyik, G.; Bogár, K.; Bäckvall, J.-E. New efficient ruthenium catalysts for racemization of alcohols at room temperature. *Tetrahedron Lett.* **2004**, *45*, 6799-6802.

[69] Martin-Matute, B.; Edin, M.; Bogár, K.; Bäckvall, J.-E. Highly compatible metal and enzyme catalysts for efficient dynamic kinetic resolution of alcohols at ambient temperature. *Angew. Chem. Int. Ed*. **2004**, *43*, 6535-6539.

[70] Martin-Matute, B.; Edin, M.; Bogár, K.; Kaynak, F. B.; Bäckvall, J.-E. Combined ruthenium (II) and lipase catalysis for efficient dynamic kinetic resolution of secondary alcohols. Insight into the racemization mechanism. *J. Am. Chem. Soc.* **2005**, *127*, 8817-8825.

[71] 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.

[72] 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.

[73] Ko, S.-B.; Baburaj, B.; Kim, M.-J.; Park, J. Air-stable catalyst for dynamic kinetic resolution of secondary alcohols. *J. Org. Chem.* **2007**, *72*, 6960-6964.

[74] Wuyts, S.; De Temmermann K.; De Vos, D. E.; Jacobs, P. A. Acid zeolites as alcohol racemization catalysts: Screening and application in biphasic dynamic kinetic resolution. *Chem. Eur. J.* **2005**, *11*, 386-397.

[75] Zhu, Y.; Fow, K.-L.; Chuah, G.-K.; Jaenicke, S. Dynamic kinetic resolution of secondary alcohols combining enzyme-catalyzed transesterification and zeolite-catalyzed racemization. *Chem. Eur. J.* **2007**, *13*, 541-547.

[76] Li, X.; Shi, Y.; Wang, Z.; Zhang, Y.; Tang, Y. Catalytic performance of H-β nanozeolite microspheres in one-pot dynamic kinetic resolution of aromatic *sec*-alcohols. *J. Catal.* **2012**, *288*, 24-32.

[77] Cheng, Y.; Xu, G.; Wu, J.; Zhang, C.; Yang, L. Highly efficient dynamic kinetic resolution of secondary aromatic alcohols with low-cost and easily available acid resins as racemization catalysts. *Tetrahedron Lett.* **2010**, *51*, 2366-2369.

[78] Bogár, K.; Martin-Matute, B.; Bäckvall, J.-E. Large-scale ruthenium- and enzyme-catalyzed dynamic kinetic resolution of (*rac*)-1-phenylethanol. *Beilstein J. Org. Chem.* **2007**, *3*:50.

[79] Mavrynsky, D.; Kanerva, L. T.; Sillanpää, R.; Leino, R. Improved synthesis of $Bn_5CpRu(CO_2)Cl$ and its application as racemization catalyst in preparative-scale metalloenzymatic dynamic kinetic resolution of 1-phenylethanol. *Pure Appl. Chem.* **2011**, *83*, 479-487.

[80] Reetz, M. T.; Dreisbach, C. Highly efficient lipase catalyzed kinetic resolution of chiral amines. *Chimia* **1994**, *48*, 570.

[81] Gotor-Fernándes, V.; Gotor, V. Biocatalytic routes to chiral amines and amino acids. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 784-797.

[82] Léonard-Nevers, V-; Marton, Z.; Lamare, S.; Hult, K.; Braber, M. Understanding water effect on *Candida antarctica* lipase B activity and enantioselectivity towards secondary alcohols. *J. Mol. Cat. B: Enz* **2009**, *59*, 90-95.

[83] Li, X.-G.; Lähitie, M.; Päiviö, M.; Kanerva, L. T. Enantioselective acylation of alcohols with fluorinated β-phenyl-β-lactams in the presence of *Burkholderia cepacia* lipase. *Tetrahedron: Asymm.***2007**, *18*, 1567-1573.

[84] Forró, E.; Fülöp, F. Lipase-catalyzed enantioselective ring opening of unactivated alicyclic-fused β-lactams in an organic solvent. *Org. Lett.* **2003**, *5*, 1209-1212.

[85] Smidt, H.; Fischer, A.; Fischer, P.; Schimd, R. D. Preparation of optically pure chiral amines by lipase-catalyzed enantioselective hydrolysis of *N*-acyl-amines. *Biotechnol. Tech.* **1996**, *10*, 335-338.

[86] Ismail, H.; Lau, R. M.; van Rantwijk, F.; Sheldon, R. A. Fully enzymatic resolution of chiral amines: Acylation and deacylation in the presence of *Candida antarctica* lipase B. *Adv. Synth. Catal.* **2008**, *350*, 1511-1516.

[87] Hietanen, A.; Salonen, T.; Rosenberg, S.; Laitinen, E.; Leino, R.; Kanerva, L. T. Synthesis of enantipure benzyl homoallylamines by indium-mediated barbier-type allylation combined with enzymatic kinetic resolution: Towards the chemoenzymatic synthetsis of *N*-containing heterocycles. *Eur. J. Org. Chem.* **2010**, 909-919.

[88] Wagegg, T.; Enzelberger, M. M.; Bornscheuer, U. T.; Schmid, R. D. The use of methoxy acetoxy esters significantly enhances reaction rates in the llipase-catalyzed preparation of enantiopure 1-(4-chloro phenyl) ethyl amines. *J. Biotechnol.* **1998**, *61*, 75-78.

[89] González-Sabín, J.; Gotor, V.; Rebolledo, F. Kinetic resolution of (±)-*trans*- and (±)-*cis*-2-phenylcyclopentanamine by CALB-catalyzed aminolysis of esters: the key role of the leaving group. *Tetrahedron: Asymm.* **2004**, *15*, 481-488.

[90] Kim, Y.; Park, J.; Kim, M.-J. Dynamic kinetic resolution of amines and amino acids by enzyme-metal cocatalysis. *ChemCatChem* **2011**, *3*, 271-277.

[91] Reetz, M. T.; Schimmosek, K. Lipase-catalyzed dynamic kinetic resolution of chiral amines: Use of palladium as the racemization catalyst. *Chimia* **1996**, *50*, 668-669.

[92] Parvulescu, A.; De Vos, D.; Jacobs, P. Efficient dynamic kinetic resolution of secondary amines with Pd on alkaline earth salts and a lipase. *Chem. Commun.* **2005**, 5307-5309.

[93] Parvulescu, A. N.; Jacobs, P. A.; De Vos, D. E. Palladium catalysts on alkaline-earth supports for racemization and dynamic kinetic resolution of benzylic amines. *Chem. Eur. J.* **2007**, *13*, 2034-2043.

[94] Parvulescu, A. N.; Van der Eycken, E.; Jacobs, P. A.; De Vos, D. E. Microwave-promoted racemization and dynamic kinetic resolution of chiral amines over Pd on alkaline earth supports and lipases. *J. Catal.* **2008**, *255*, 206-212.

[95] Parvulescu, A. N.; Jacobs, P. A.; De Vos, D. E. Support influences in the Pd-catalyzed racemization and dynamic kinetic resolution of chiral benzylic amines. *Appl. Catal., A:* **2009**, *368*, 9-16.

[96] Kim, M.-J.; Kim, W.-H.; Han, K.; Choi, Y. K.; Park, J. Dynamic kinetic resolution of primary amines with a recyclable Pd nanocatalyst for racemization. *Org. Lett.* **2007**, *9*, 1157-1159.

[97] Kim, Y.; Park, J.; Kim, M.-J. Fast racemization and dynamic kinetic resolution of primary benzyl amines. *Tetrahedron Lett.* **2010**, *51*, 5581-5584.

[98] Pàmies, O.; Éll, A. H.; Samec J. S. M.; Hermanns, N.; Bäckvall, J.-B. An efficient and mild ruthenium-catalyzed racemization of amines: application to the synthesis of enantiomerically pure amines. *Tetrahedron Lett.* **2002**, *43*, 4699-4702.

[99] Paetzold, J.; Bäckvall, J.-E. Chemoenzymatic dynamic kinetic resolution of primary amines. *J. Am. Chem. Soc.* **2005**, *127*, 17620-17621.

[100] Hoben, C. E.; Kanupp, L.; Bäckvall, J.-E. Practical chemoenzymatic dynamic kinetic resolution of primary amines via transfer of a readily removable benzylcarbonyl group. *Tetrahedron Lett.* **2008**, *49*, 977-979.

[101] Thalén, L. K.; Zhao, D.; Sortais, J.-B.; Paetzold, J.; Hoben, C.; Bäckvall, J.-E. A chemoenzymatic approach to enantiomerically pure amines using dynamic kinetic resolution: Application to the synthesis of norsertraline. *Chem. Eur. J.* **2009**, *15*, 3403-3410.

[102] Veld, M. A. J.; Hult, K.; Palmans, A. R. A.; Meijer, E. W. Fast DKR of amines using isopropyl 2-methoxyacetate as acyl donor. *Eur. J. Org. Chem.* **2007**, *32*, 5416-5421.

[103] Parvulescu, A. N.; Jacobs, P. A.; De Vos, D. E. Heterogeneous raney nickel and cobalt catalysts for racemization and dynamic kinetic resolution of amines. *Adv. Synth. Catal.* **2008**, *350*, 113-121.

[104] Thalén, L. K.; Bäckvall, J.-E. Development of dynamic kinetic resolution on large scale for (±)-1-phenylethylamine. *Beilstein J.Org. Chem.* **2010**, *6*, 823-829.

[105] Koszelewski, D.; Tauber, K.; Faber, K.; Kroutil, W. ω-Transaminases for the synthesis of non-racemic α-chiral primary amines. *Trends Biotechnol.* **2010**, *28*, 324-332.

[106] Mathew, S.; Yun, H. ω-Transaminases for the production of optically pure amines and unnatural amino acids. *ACS Catal.* **2012**, *2*, 993-1001.

[107] Shin, J.-S.; Kim, B.-G. Kinetic resolution of α-methylbenzylamine with ω-transaminase screened from soil microorganisms: Application of a biphasic system to overcome product inhibition. *Biotechnol. Bioeng.* **1997**, *55*, 348-358.

[108] Shin, J.-S.; Kim, B.-G.; Liese, A.; Wandrey, C. Kinetic resolution of chiral amines with ω-transaminase using an enzyme-membrane reactor. *Biotechnol. Bioeng.* **2001**, *73*, 179-187.

[109] Yun, H.; Yang, Y.-H.; Cho, B.-K.; Hwang, B.-Y.; Kim, B.-G. Simultaneous synthesis of enantiomerically pure (*R*)-1-phenylethanol and (*R*)-α-methylbenzylamine from racemic α-methylbenzylamine using ω-transaminase/alcohol dehydrogenase/glucose dehydrogenase coupling reaction. *Biotechnol. Lett.* **2003**, *25*, 809-814.

[110] Koszelewski, D.; Göritzer, M.; Clay, D.; Seisser, B.; Kroutil, W. Synthesis of optically active amines employing recombinant ω-transaminases in *E. coli* cells. *ChemCatChem* **2010**, *2*, 73-77.

[111] Koszelewski, D.; Clay, D.; Rozzell, D.; Kroutil, W. Deracemization of α-chiral pimary amines by a one-pot, two-step cascade reaction catalysed by ω-transaminases. *Eur. J. Org. Chem.* **2009**, 2289-2292.

[112] Truppo, M. D.; Turner, H. J.; Rozzell, D. Efficient kinetic resolution of racemic amines using a transaminase in combination with an amino acid oxidase. *Chem. Commun.* **2009**, 2127-2129.

[113] Shin, J.-S.; Kim, B.-G. Asymmetric synthesis of chiral amines with ω-transaminase. *Biotechnol. Bioeng.* **1999**, *65*, 206-211.

[114] Koszelewski, D.; Lavandera, I.; Clay, D.; Guebitz, G. M.; Rozzell, D.; Kroutil, W. Formal asymmetric biocatalytic reductive amination. *Angew. Chem. Int. Ed.* **2008**, *47*, 9337-9340.

[115] Höhne, M.; Kühl, S.; Robins, K.; Borscheuer, U. T. Efficient asymmetric synthesis of chiral amines by combining transaminase and pyruvate decarboxylase. *ChemBioChem* **2008**, *9*, 363-365.

[116] Truppo, M. D.; Rozzell, J. D.; Moore J. C.; Turner, N. J. Rapid screening and scale-up of transaminase catalyzed reactions. *Org. Biomol. Chem.* **2009**, *7*, 395-398.

[117] Truppo, M. D.; Rozzell, J. D.; Turner, N. J. Efficient production of enantiomerically pure chiral amines at concentrations of 50 g/L using transaminases. *Org. Process Res. Dev.* **2010**, *14*, 234-237.

[118] Mutti, F. G.; Fuchs, C. S.; Pressnitz, D.; Turrini, N. G.; Sattler, J. H.; Lerchner, A.; Skerra, A.; Kroutil W. Amination of ketones by employing two new (*S*)-selective ω-transaminases and the His-tagged ω-TA from *Vibrio fluvialis*. *Eur. J. Org. Chem.* **2012**, 1003-1007.

[119] Engelmark, K. C.; Branneby, C.; Abedi, V.; Wells, A.; Berglund, P. Transaminations with isopropyl amine: equilibrium displacement with yeast dehydrogenase coupled to *in situ* cofactor regeneration. *Chem. Commun*. **2010**, *46*, 5569-5571.

[120] Koszelewski, D.; Pressnitz, D.; Clay, D.; Kroutil W. Deracemization of mexiletine biocatalyzed by ω-transaminases. *Org. Lett.* **2009**, *11*, 4810-4812.

[121] Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **1985**, *150*, 76–85.

[122] Wiechelman, K. J.; Braun, R. D.; Fitzpatrick, J. D. Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. *Anal. Biochem.* **1988**, *175*, 231–237.

[123] Öhrner, N.; Orrenius, C.; Mattson, A.; Norin, T.; Hult, K. Kinetic resolutions of amine and thiol analogues of secondary alcohols catalyzed by the *Candida antarctica* lipase B. *Enzyme Microb. Technol.* **1996**, *19*, 328-331.

[124] Irimescu, R.; Kato, K. Lipase-catalyzed enantioselective reaction of amines with carboxylic acids under reduced pressure in non-solvent system and in ionic liquids. *Tetrahedron Lett.* **2004**, *45*, 523-525.

[125] Prasad, A. K.; Husain, M.; Singh, B. K.; Gupta, R. K.; Manchanda, V. K.; Olsen, C. E.; Parmar, V. S. Solvent-free biocatalytic amidation of carboxylic acids. *Tetrahedron Lett.* **2005**, *46*, 4511-4514.

[126] Brem, J.; Turcu, M. C.; Paizs, C.; Lundell, K.; Toşa, M.-I.; Irimie, F.-D.; Kanerva, L. T. Immobilization to improve the properties of *Pseudomonas fluorescens* lipase for the kinetic resolution of 3-aryl-3-hydroxy esters. *Process Biochem.***2012**, *47*, 119-126.