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CHEMOENZYMATIC ENANTIOSELECTIVE SYNTHESIS OF β-AMINO ACID DERIVATIVES USING LIPASES

by

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TURUN YLIOPISTO
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ABSTRACT

Demand for compounds of high enantiopurity in pharmaceutical industry is increasing since the two enantiomers of a chiral compound may have different physiological effects. To develop methods for preparing compounds in highly selective and environmentally benign ways is of high concern. One of the best strategies is to use enzymes in organic synthesis, as enzymes catalyze reactions in chemo-, regio- and enantioselective ways and with high efficiency. β-Amino acids and their derivatives are found in many natural products, and they often play important pharmacological roles. β-Peptides are potential peptide mimetics resisting undesired biodegradations.

This thesis demonstrates that it is highly feasible to integrate lipase catalysis in the synthesis of β-amino acid derivatives of high enantiopurity. Water as a competing nucleophile to added nucleophiles (ROH, RNH_2 and NH_3) has been discussed as an important issue in lipase catalysis in organic solvents throughout the thesis. The lipase from *Burkholderia cepacia* (lipase PS) was used for catalyzing the alcoholysis, aminolysis and ammonolysis of fluorinated β-lactams, thus leading to highly enantiopure β-amino esters, β-amino amides and β-lactams. The two-step lipase PS-catalyzed resolution of *N*-hydroxymethylated β-lactams afforded a method for the preparation of both enantiomers of β-lactams with high enantiopurity. The *Candida antarctica* lipase A (CAL-A)-catalyzed *N*-acylation of β-tryptophan ethyl ester as a heteroaryl-substituted β-amino ester and that of the corresponding β-amino nitrile was shown to proceed in highly enantio- and chemoselective ways. Two methods were developed for the preparation of enantio- and diastereopure β-dipeptides using some of the prepared β-amino esters and β-lactam enantiomers as monomeric components. As the first method, *Candida antarctica* lipase B (CAL-B) was used for preparing activated *N*-protected β-amino esters from the ethyl esters. The activated esters were then subjected to the subsequent CAL-A-catalyzed peptide formation with β-amino esters in an organic solvent. As the second method, lipase PS-D catalyzed the ring opening of activated β-lactams with β-amino esters, leading to the formation of β-dipeptides.

***Keywords**:* β-amino esters, β-dipeptides, enantioselectivity, fluorinated compounds, kinetic resolution, β-lactams, lipases

Xiang-Guo Li

β-AMINOHAPPOJOHDOSTEN KEMOENTSYMAATTINEN ENANTIOSELEKTIIVINEN SYNTEESI LIPAASEILLA

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

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TIIVISTELMÄ

Korkean enantioselektiivisyyden omaavien yhdisteiden tarve lääketeollisuudessa on kasvussa, koska kiraalisen yhdisteen enantiomeereilla saattaa olla hyvinkin erilaiset fysiologiset vaikutukset. Sellaisten menetelmien kehittely on hyvin tärkeää, joilla yhdisteitä voidaan valmistaa selektiivisesti ja ympäristöä säästävällä tavalla. Entsyymien käyttö orgaanisessa syntetiikassa on eräs parhaista menetelmistä, koska entsyymit katalysoivat reaktioita kemo-, regio- ja enantioselektiivisesti ja lisäksi hyvin tehokkaasti. β-aminohappoja ja niiden johdoksia esiintyy monissa luonnontuotteissa ja niillä on usein farmakologisesti merkittäviä tehtäviä. β-peptidit ovat potentiaalisia peptidomimeettejä, jotka kestävät biopilkkoutumista.

Väitöskirja osoittaa, että lipaasikatalyysi sopii hyvin enantiopuhtaiden β-aminohappojohdosten synteesiin. Työssä tarkastellaan kauttaaltaan veden merkitystä toimia kilpailevana nukleofiilina lisättyjen nukleofiilien (ROH, RNH_2 and NH_3) kanssa lipaasikatalyysissä orgaanisissa liuottimissa. *Burkholderia cepacia*n lipaasia (lipase PS) käytetään katalysoimaan fluoripitoisten β-laktaamien alkoholyysiä, aminolyysiä ja ammonolyysiä reaktioissa, jotka tuottavat korkean enantiopuhtauden omaavia β-aminoestereitä, β-aminoamideja ja β-laktaameja. *N*-hydroksimetyloitujen β-laktaamien kaksivaiheinen lipaasi PS:n katalysoima resoluutio esitellään menetelmänä, jolla voidaan valmistaa β-laktaamin molemmat enantiomeerit renkaan pilkkoutumatta. *Candida antarctica*n lipaasi A (CAL-A) katalysoi β-tryptofaanin etyyliesterin (esimerkki heteroaryylisubstituoidusta β-aminoesteristä) ja vastaavan β-aminonitriilin *N*-asylaatiota korkealla enantio- ja kemoselektiivisyydellä. Työssä on kehitetty myös kaksi merkittävää menetelmää enantio- ja diastereopuhtaiden β-dipeptidien valmistamiseksi käyttämällä joitakin valmistetuista β-aminoestereiden ja β-laktaamien enantiomeereistä monomeerisina yksiköinä. Toisessa menetelmässä *Candida antarctica*n lipaasia B (CAL-B) käytetään aktivoitujen, *N*-suojattujen β-aminoestereiden valmistamiseen etyyliestereistä. Aktivoidut esterit altistetaan sitten reagoimaan β-aminoestereiden kanssa CAL:n katalysoidessa reaktiota orgaanisessa liuottimessa. Toisena menetelmänä on lipaasi PS-D:n katalysoima aktivoidun β-laktaamin renkaan avaaminen β-aminoesterillä ja β-dipeptidin muodostuminen.

***Avainsanat**:* β-aminoesteri, β-dipeptidi, enantioselektiivisyys, fluoripitoinen yhdiste, kineettinen resoluutio, β-laktaami, lipaasi

ABBREVIATIONS

Asp	aspartic acid
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bu	butyl
Bz	benzoyl
c	conversion
CAL-A	*Candida antarctica* lipase A
CAL-B	*Candida antarctica* lipase B
CAN	cerium (V) ammonium nitrate
COSY	correlation spectroscopy
CRL	*Candida rugosa* lipase (previously *Candida cylindracea* lipase)
DIPE	diisopropyl ether
ee	enantiomeric excess
equiv	equivalent
E	enantiomeric ratio
EC	Enzymatic Commission
Et	ethyl
Et_2O	diethyl ether
GC	gas chromatography
Glu	glutamic acid
h	hour
His	histidine
HPLC	high performance liquid chromatography
HMBC	heteronuclear multiple bond correlation
HRMS	high resolution mass spectroscopy
HSQC	heteronuclear single quantum correlation
Lipase AK	*Pseudomonas fluorescens* lipase
Lipase PS	*Burkholderia cepacia* lipase (formerly lipase from *Pseudomonas cepacia*)
Lipase PS-C II	*Burkholderia cepacia* lipase immobilized on ceramics
Lipase PS-D	*Burkholderia cepacia* lipase immobilized on Celite
Me	methyl
min	minute
MS	mass spectroscopy
NMR	nuclear magnetic resonance
Ph	phenyl
PLE	porcine liver esterase
PPL	porcine pancreatic lipase
Pr	propyl
rac-	racemic
Ser	serine
t	time
TBME	*tert*-butyl methyl ether
THF	tetrahydrofuran
Z	benzyloxycarbonyl

LIST OF ORIGINAL PAPERS

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

I. 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: Asymmetry* **2007**, *18*, 1567-1573.

II. Li, X.-G.; Kanerva, L. T. Lipase-involved strategy to the enantiomers of 4-benzyl-β-lactams as a key intermediate in the preparation of β-phenylalanine derivatives. *Advanced Synthesis & Catal*ysis **2006**, *348*, 197 – 205.

III. Li, X.-G.; Kanerva, L. T. Chemoenzymatic preparation of fluorine-substituted β-lactam enantiomers exploiting *Burkholderia cepacia* lipase *Tetrahedron: Asymmetry* **2007**, *18*, 2468-2472.

IV. Li, X.-G.; Kanerva, L. T. Chemoenzymatic preparation of the enantiomers of β-tryptophan ethyl ester and the β-amino nitrile analogue *Tetrahedron: Asymmetry* **2005**, *16*, 1709-1714.

V. Li, X.-G.; Kanerva, L. T. Lipases in β-dipeptide synthesis in organic solvents *Organic Letters* **2006**, *8*, 5593-5596.

VI. Li, X.-G.; Lähitie, M.; Kanerva, L. T. *Burkholderia cepacia* lipase and activated β-lactams in β-dipeptide and β-amino amide synthesis *Tetrahedron: Asymmetry* **2008**, *19*, 1857-1861.

1 INTRODUCTION

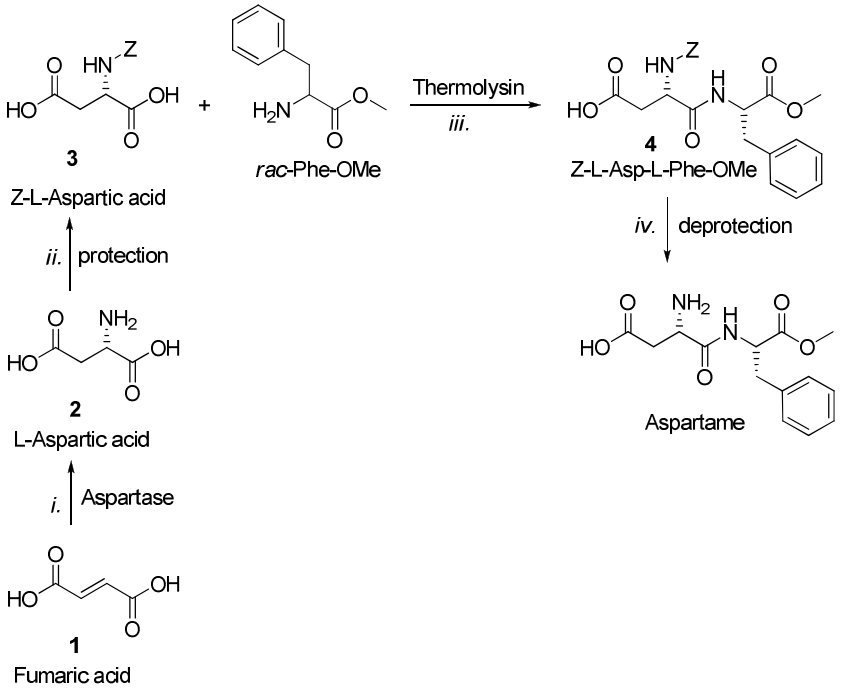
Nature is a perfect synthetic chemist. Plants, for example, have access only to air, soil and energy from the sun, and they produce countless amounts of compounds with enormous complexity. From nature we may get hints for developing cost-effective and sustainable methods to prepare biologically active compounds. Enzymes are biocatalysts responsible for chemical transformations in nature. According to the types of reactions they catalyze, enzymes can be grouped into six classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Each enzyme has a four-digit Enzyme Commission Number (EC A.B.C.D).[1] Number A denotes the main type of a reaction catalyzed by the enzyme, number B stands for the subtype, number C indicates the nature of the co-substrate, and number D is the individual number for the enzyme. For lipases, which belong to hydrolases, the EC Number is 3.1.1.3. The primary structure of an enzyme refers to the linear arrangement of L-amino acids in the protein, and the secondary structure is the local architecture of protein segments, such as an α-helix and a β-sheet. The overall three-dimensional architecture of the whole polypeptide chain is called the tertiary structure. The three-dimensional structure of an enzyme determines its function and is more conserved than the sequence during the evolution, and the same type of enzymes with different sequences from different species may have the same function.

Enzymes catalyze reactions by binding their substrates at their active sites located in the cavities of the enzymes. The shape of the cavity and the nature and the arrangement of the amino acid residues in the cavity have profound effects on the molecular recognition and catalysis. The rest of the enzyme might be important to channel the substrate into the active site and to keep the integrity of the active site correct for efficient catalysis.[2] Keeping the proper conformation is crucial for an enzyme to function. In general, enzymes operate by the same chemical and physical principles as non-biocatalysts do. Enzymes have been found to catalyze almost any reaction in organic chemistry. Rate acceleration and specificity are two distinct features of enzyme-catalyzed reactions. There are many hypotheses concerning the mechanism of enzyme catalysis, including lock and key theory, induced-fit theory and transition-state stabilization. Interactions between an enzyme and its substrate include hydrogen bonding, electrostatic forces, hydrophobic effects and other noncovalent weak interactions.

Since enzymes possess valuable features, such as chemoselectivity, regioselectivity and stereoselectivity, integrating enzymes into organic synthesis is supposed to fulfil the increasing demands for preparing compounds in selective manner. For instance, enzymes can interact differently with enantiomers which are non-superimposable mirror images, such as our left and right hands. This allows the kinetic resolution of the enantiomers of a racemate based on their different reactivities. This is shown in the case of lipase-catalyzed kinetic resolution of β-lactams and β-amino esters in this dissertation. Although the physico-chemical properties of the two enantiomers of a chiral molecule are the same in achiral media, except for their abilities to reverse plane-polarized light into different directions, the enantiomers can have totally different physiological effects. This is a driving force for synthetic chemists to turn attention to methods which allow the preparation of enantiopure chemicals. The availability, substrate scope and operational stability of enzymes are important factors governing the utility of enzymes in

organic synthesis. In line with the development of proteomics and genomics, more and more enzymes are available in large scale and of high quality than ever before. In addition to naturally existing enzymes, enzyme variants with broad substrate scope have been designed and produced for many kinds of synthetic purposes.

It has been shown that large-scale industrial productions by using enzymes are highly possible. Good examples include the synthesis of the low-calorie sweetener aspartame (L-aspartyl-L-phenylalanine methyl ester, DSM)[3] and the synthesis of amino acids (Tanabe Seiyaku[4] and DSM[5]).



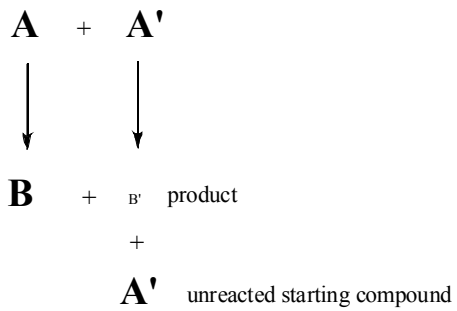
Scheme 1. Industrial production of aspartame by using enzymes (DSM, Z = benzyloxycarbonyl).[3]

As shown in Scheme **1**, the chemoenzymatic production of aspartame includes two enzymatic steps (*i.* and *iii.*) and two chemical steps (*ii.* and *iv*). The enzymatic steps are pivotal for obtaining enantiopure intermediates **2** and **4**, whereas the chemical steps afford the intermediate **3** and the final product, aspartame. In addition to aspartame, many pharmaceuticals are produced by the strategy of integrating biotransformations to organic synthesis. For instance, BioVerdant established ground-breaking chemoenzymatic processes for producing Atorvastatin® (Atorvastatin, a drug for lowering cholesterol) and other drug candidates.[6]

2 REVIEW OF THE LITERATURE

2.1 Kinetic resolution

Enzymatic kinetic resolution is an effective method for the preparation of compounds with high enantiopurity. In this procedure, one of the enantiomers (A) of a racemate (A + A') reacts into the product B faster than the other one (A' to B') does in the presence of an enzyme, as elucidated in Scheme 2. Thus, unequal proportions of the product enantiomers (B and B') are formed, leading in the most favorable case to the resolution mixture of the unreacted starting material A' and the product B in enantiopure forms at 50% conversion.[1,7,8]



Scheme 2. The principle of kinetic resolution.

Enantiomeric ratio (*E*) is generally used for indicating the enantioselectivity of an enzyme-catalyzed reaction. *E* can be calculated according to equation (1) (Scheme 3).[8,9] The Michaelis-Menten constant K_m is generally used for indicating how well a substrate interacts with an enzyme. The smaller the K_m is, the better is the binding. Thus, $K_m = 10^{-7}$ M indicates that the substrate has a greater affinity to the enzyme than when $K_m = 10^{-5}$ M. The turnover number k_{cat} indicates the number of molecules of the substrate converted to the product per unit time per molecule of an enzyme. Equations (2) and (3) can be derived from equation (1), which makes it possible to use conversion (c) and enantiomeric excesses (ee values for the product and unreacted starting compound) for the calculation of *E*.[8,9] Equations (2)-(4) can only be used when the reaction is irreversible, and racemization or side reactions do not take place. For a reversible reaction, the equations for *E* should contain the equilibrium constant of the reaction.[9,10] Values of ee are calculated according to equation (5), where [R] and [S] stand for the concentrations of the *R*- and *S*-enantiomers, respectively.

$$E = (k_{cat}/K_m)_A/(k_{cat}/K_m)_{A'} \quad (1)$$

$$E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)] \quad (2)$$

$$E = \ln[1-c(1+ee_p)]/\ln[1-c(1-ee_p)] \quad (3)$$

$$c = ee_s/(ee_s+ee_p) \quad (4)$$

$$ee = |[R]-[S]|/|[R]+[S]| \quad (5)$$

$$\text{Enz} + \text{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Enz}\bullet\text{S} \xrightarrow{k_2} \text{Enz}\bullet\text{P} \longrightarrow \text{Enz} + \text{P}$$

$K_m = (k_{-1} + k_2)/k_1$
$k_2 = k_{cat}$ when $k_2 << k_{-1}$

Scheme 3. Enzyme-catalyzed reaction where one substrate reacts into one product in an irreversible fashion.[8,9] Enz = enzyme, S = substrate and P = product.

In an ideal kinetic resolution, both the product and the substrate enantiomers are obtained in high enantiopurities (ee > 99%) at 50% conversion. This can be achieved with E = 1000 or higher as shown in the theoretical plot **a** in Figure **1**. With E values of 200, 50 and 10, the unreacted substrate can be obtained with high enantiopurity (ee > 99%) at 51%, 56% and 74% conversions, respectively (**b-d**). With E > 100, small experimental errors in the determination of enantiomeric excess values and conversion may lead to considerable errors in the E value. For this reason, high E-values are often marked as E > 200 (or sometimes E > 100) in literature.

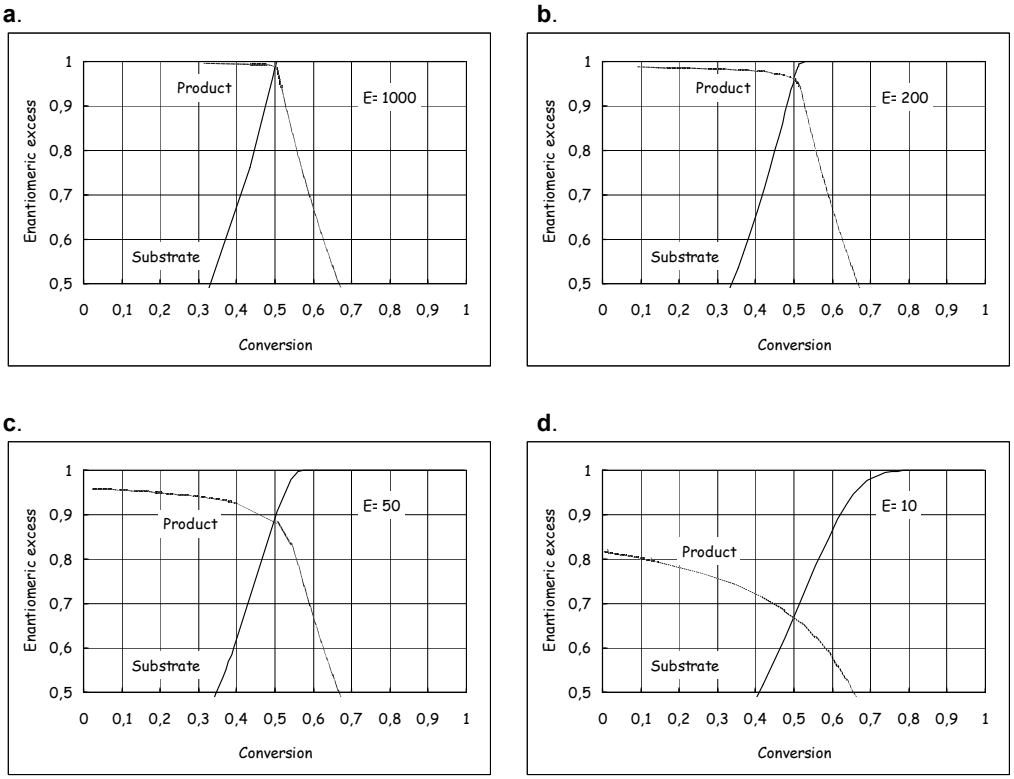


Figure 1. Theoretical plots of enantiomeric excesses (ee) of the product and the substrate vs. conversion for the kinetic resolution.

2.2 Lipase catalysis

2.2.1 Lipases

Lipases (EC 3.1.1.3) are ubiquitous triacylglycerol-hydrolases which catalyze the hydrolysis and synthesis of long-chain acylglycerols. Lipases belong to the α/β-fold hydrolases. In nature, lipases work at the water-lipid interface, and an interfacial activation of the lipase is usually necessary. Lipases are generally stable and function without cofactors. These features have made lipases favorable in industrial use. For example, BASF produces 2500 tons of (*S*)-(1-methoxy)-2-propylamine annually by lipase-catalyzed kinetic resolution.[11] Considering the lipophilic surroundings in which lipases naturally function, it is not surprising to see lipases to be used in non-aqueous media. In organic solvents, the enhanced solubility of reactants, the shift of an equilibrium towards synthesis and the easy work up of reactions may bring additional benefits. *Burkholderia cepacia* lipase (lipase PS, previously *Pseudomonas cepacia*) and *Candida antarctica* lipases A (CAL-A) and B (CAL-B) are the enzymes mainly used in this thesis. All the lipases were used in immobilized form, as the immobilization stabilizes the enzymes and facilitates their recycling when necessary.[12]

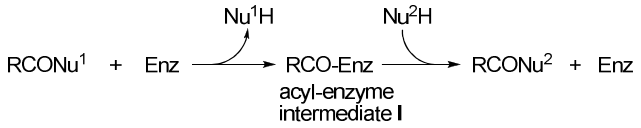
Lipase PS is a bacterial lipase which contains 320 amino acid residues. Its molecular mass is 33 kD.[13] A number of crystal structures of lipase PS are available.[14-21] The catalytic triad of this lipase is formed by Ser87, Asp264 and His286 residues. In addition to Asp264, Glu289 supposedly forms a hydrogen bond to the catalytic His286. The backbone nitrogens of Gln88 and Leu17 form the oxyanion hole which consists of a GX-motif (G denotes glycine and X is any other amino acid residue).[22]

The yeast *Candida antarctica* produces two lipases, CAL-A and CAL-B. CAL-A consists of 431 amino acid residues, and its crystal structure has been published recently (PDB entry code 2VEO).[23] A striking feature of CAL-A is its thermostability. The denaturation temperature is 93 ^{o}C at pH 7 and 96 ^{o}C at pH 4.5.[24] The catalytic triad is composed of Ser184, Asp334 and His366. Gly185 and Asp95 are supposed to form the oxyanion hole. It is unusual that such an acidic group as Asp95 can stabilize a negatively charged reaction intermediate, although the hydrophobic environment of Asp95 may promote an elevated pK_a value. It has been suggested that CAL-A contains a GGGX-motif and is able to accommodate such bulky substrates such as tertiary alcohols in the nucleophile site.[22] A discrete lid domain has been found in CAL-A. CAL-A is a special lipase in a way that its sequence similarity to other known lipases is very low. By multisequence alignment, CAL-A shows high similarity only to the lipases from *Kurtzmanomyces* sp. I-11 and *Ustilago maydis*. By structure comparison, all similar structures are peptidases.[25]

CAL-B is one of the most robust lipases commercially available. It has been broadly used in organic synthesis. The three-dimensional structure of CAL-B has been elucidated.[26,27] CAL-B is built up of 317 amino acid residues and has a molecular mass of 33 kD. The catalytic triad consists of Ser105, Asp187 and His224. The backbone nitrogens from Gln106, Thr40 and the side chain hydroxyl of Thr40 comprise the oxyanion hole which possesses a GX-motif. CAL-B lacks the property of interfacial activation and is considered to be an intermediate between a lipase and an esterase rather than being a true lipase.[28]

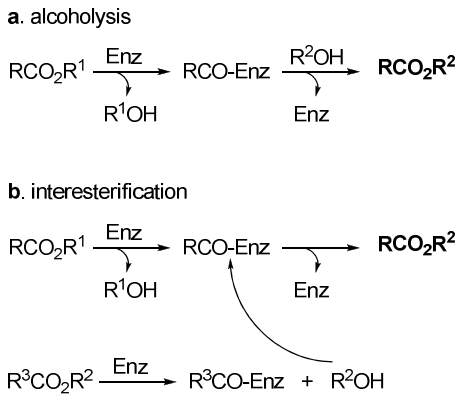
2.2.2 Mechanism

Lipase catalysis is supposed to follow the ping-pong bi-bi mechanism which is common to serine hydrolases, where two substrates are transformed into two products (bi-bi), and the first product leaves the active site before the second substrate enters (ping-pong) (Scheme 4).[29] Serine hydroxyl at the active site serves as a nucleophile in the formation of the acyl-enzyme intermediate I. The acyl donor ($RCONu^1$) can be a carboxylic ester, a carboxylic acid, an anhydride, a β-lactam or a lactone, except that in the last two cases there is no released product Nu^1H. The use of conventional amides as acyl donors in lipase-catalyzed reactions is rare, especially in dry organic solvents. Presumably it is difficult for the resonance stabilized amides to form the acyl-enzyme intermediate I (RCO-Enz), the formation of which is the prerequisite for the subsequent acyl-transfer to the added nucleophile Nu^2H. In Scheme 4, Nu^2H can be an alcohol (ROH in alcoholysis), an amine (RNH_2 in aminolysis), water (H_2O in hydrolysis), a thiol (RSH in thiolysis), an acid (RCO_2H in acidolysis) or hydrogen peroxide (H_2O_2 in peroxidation).

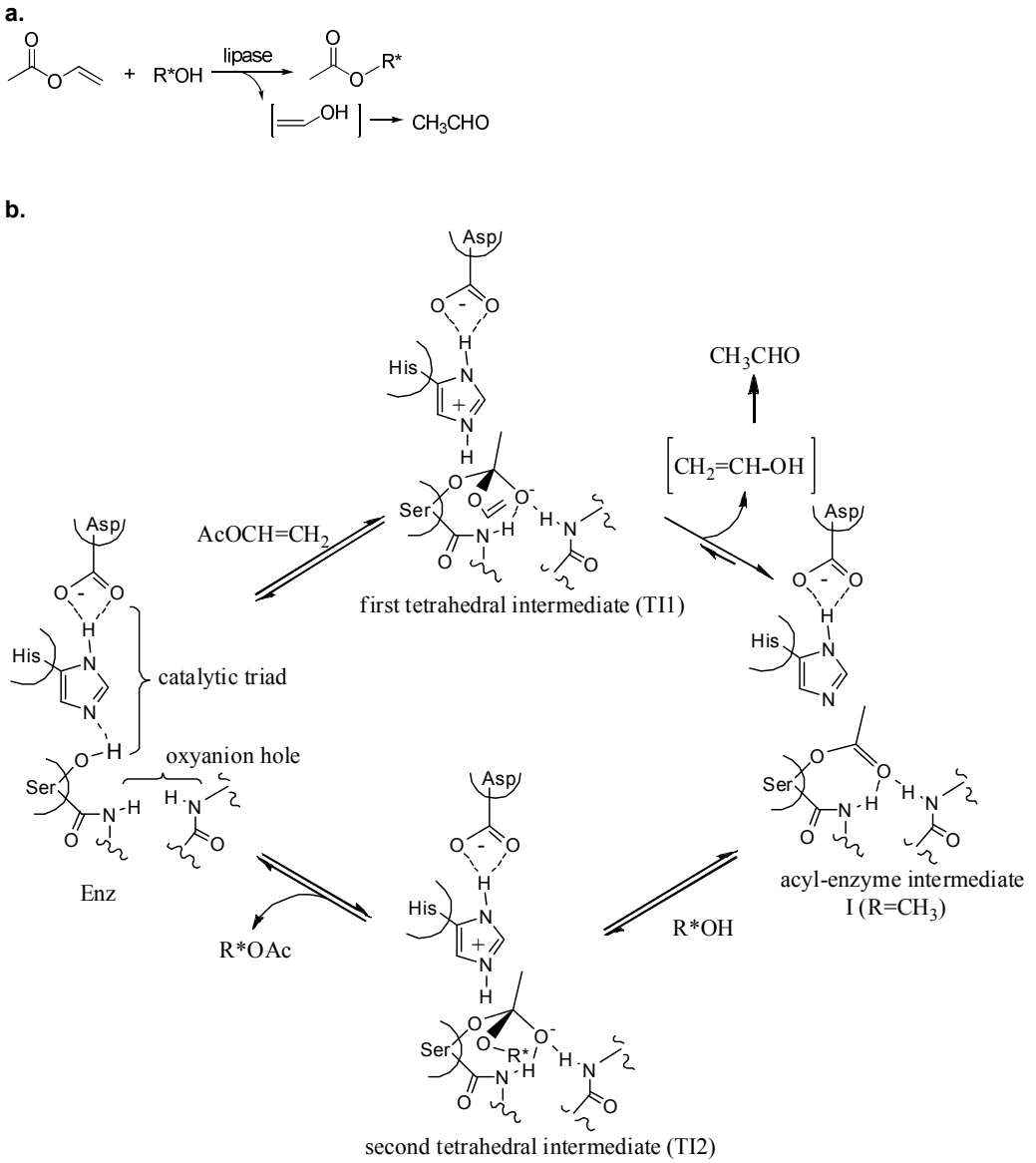


Scheme 4. Ping-pong bi-bi mechanism.

In this thesis, the reaction is called alcoholysis when an ester (RCO_2R^1) reacts with an alcohol (R^2OH), generating a new ester product (RCO_2R^2, Scheme 5, **a**). When the reaction of two esters leads to the formation of two new esters with exchanged alkyl groups, the reaction is called interesterification (Scheme 5, **b**). In route **b** an alcohol (R^2OH) is generated *in situ* from one of the esters $R^3CO_2R^2$, and it then reacts with an acyl-enzyme intermediate formed from another ester RCO_2R^1. Thus, the same product RCO_2R^2 as in route **a** is formed. From the mechanistic point of view, the interesterification reaction is actually an alcoholysis reaction.



Scheme 5. Lipase-catalyzed alcoholysis and interesterification (backward reaction is not presented in route **a** and **b**).



Scheme 6.[30] (a). Total reaction between a chiral alcohol and vinyl acetate in the presence of a lipase. (b). The serine hydrolase mechanism for the lipase-catalyzed acylation of a chiral alcohol with vinyl acetate is shown clockwise. By modifying the anticlockwise catalysis, the lipase-catalyzed hydrolysis/alcoholysis of an ester can be drawn. The abbreviations are as follows: Enz = enzyme; R*OH = chiral alcohol.

In lipase-catalyzed kinetic resolutions, chirality may reside in the acyl donor (in R or in Nu^1 of $RCONu^1$), in the nucleophile (Nu^2H), or in both of these (Scheme 4). The case where Nu^2H is chiral, such as a chiral alcohol (R*OH), its total reaction with an acyl donor, for example vinyl acetate, is shown in Scheme 6 **a**. The detailed mechanism is shown in Scheme 6 **b**. The hydroxyl group of serine attacks the carboxyl functionality of vinyl acetate and the generated oxyanion is stabilized by the oxyanion hole in the formation of the first tetrahedral intermediate (TI1). The release of the alcohol part (Nu^1H, in the form of acetaldehyde) of vinyl acetate leads

to the formation of an acyl-enzyme intermediate (Enz-Ac). When preferably one of the enantiomers of the chiral alcohol (Nu^2H, R*OH) enters the active site and attacks the acyl-enzyme intermediate, the second tetrahedral intermediate (TI2) results. Eventually, the other product (R*OAc) is released from the active site, leaving the free enzyme ready for the next cycle of reactions (Scheme 6 **b**).[30] The formation of TI2 is supposed to be the pivotal step for enantioselectivity. In a kinetic resolution, the faster reacting enantiomer shows a better binding to the active site than the slower reacting enantiomer does.

2.2.3 Competing nucleophiles

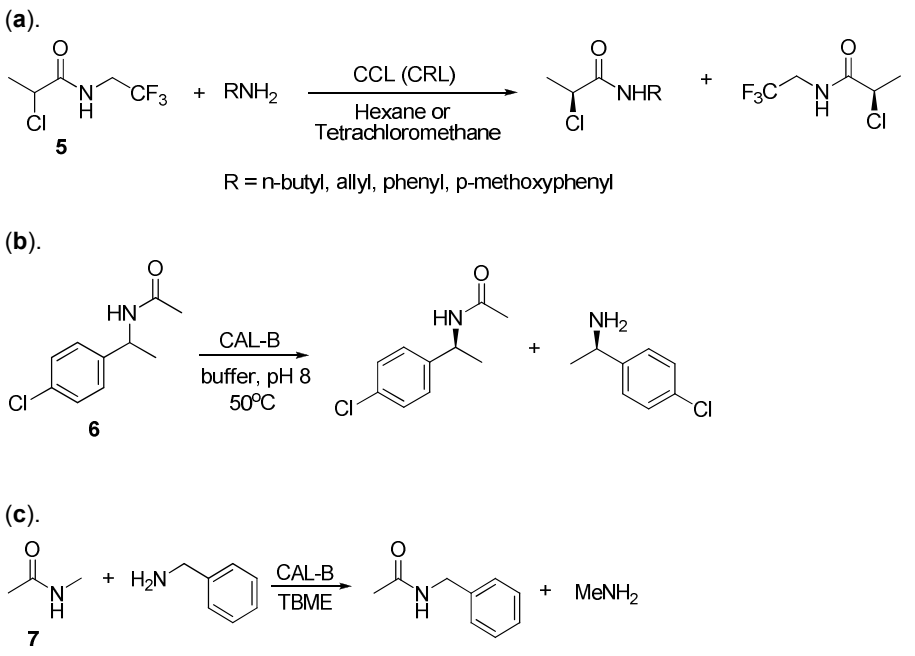
The acyl-enzyme intermediate I (RCO-Enz) formed in Scheme 4 can be attacked by any nucleophile present, in addition to the added Nu^2H. When a reaction is carried out in a dry organic solvent, the water present in the enzyme preparation is a common competing nucleophile which may cause undesired hydrolysis of hydrolysable substrates and products. Essential amount of water is, on the other hand, pivotal for enzyme activity. The water needed may help the enzyme to keep appropriate conformation for catalysis. In some cases progressive loss of enzyme activity can be observed in water-miscible solvents since the essential water molecules are stripped off from the protein.[31] Thus, exhaustive drying of the enzyme preparation is not appropriate.

In addition to water in the enzyme preparation, Nu^1H generated from the substrate ($RCONu^1$) in Scheme 4 can also be a competing nucleophile, causing the reverse reaction of the product back to the substrate and, accordingly, the reaction leads to an equilibrium. That is one of the reasons why it is important to choose an appropriate acyl donor for a lipase-catalyzed reaction. In *O*-acylation reactions such as the one presented in Scheme 6, the use of vinyl esters or isopropenyl esters as acyl donor is common since the enol generated is converted into acetaldehyde or acetone, respectively. For this reason, vinyl butanoate was in this thesis chosen for the *O*-acylation of *N*-hydroxymethylated β-lactams (section 5.2 and paper II & III). 2,2,2-Trifluoroethyl esters (for instance, 2,2,2-trifluoroethyl butanoate) were also used for *O*-acylations, as the liberated 2,2,2-trifluoroethanol is generally considered as a weak nucleophile. However, my work (section 5.4.2 and article V) shows that 2,2,2-trifluoroethanol is not as unreactive as often expected.

In lipase-catalyzed *N*-acylation reactions (Scheme 7), the R^2OH generated (corresponds to Nu^1H in Scheme 4) does not generally cleave conventional amide bonds, such as the amide bond in R^1CONHR^*. There are, however, some exceptions. The activated amide **5** is cleaved by *Candida rugosa* lipase [CRL, formerly *Candica cylindracea* lipase (CCL)] in hexane and tetrachloromethane in the presence of achiral amines [Scheme 8 (a)].[32] The hydrolysis of amide **6** by CAL-B has been achieved in aqueous medium at 50 °C [Bayer, Scheme 8 (b)].[33] The transamidation between the amide **7** and benzylamine has been performed in TBME in the presence of CAL-B, several analogues of amide **7** being also tested under similar conditions.[34]

$$R^1CO_2R^2 \quad + \quad R^*NH_2 \xrightarrow{\text{lipase}} R^1CONHR^* \quad + \quad R^2OH$$

Scheme 7. Lipase-catalyzed *N*-acylation of an amine.



Scheme 8. (**a**) CRL-catalyzed aminolysis of **5** in organic solvents.[32] (**b**) CAL-B-catalyzed hydrolysis of amide **6** (Bayer).[33] (**c**) CAL-B-catalyzed transamidation between **7** and benzyamine in *tert*-butyl methyl ether.[34]

It is worth mentioning that vinyl or isopropenyl esters are not suitable for *N*-acylation, since the generated acetaldehyde and acetone can form imines with amino groups of the substrates. In many publications, carboxylic acids have also been used as acyl donors for *N*-acylations, even though carboxylic acids and amines tend to form unreactive ionic pairs.[35-39]

2.3 Preparation of the enantiomers: β-amino acids and their derivatives

2.3.1 β-Amino acids in nature

In an α-amino acid, the carboxylic acid and the amino groups are attached to the same carbon (Figure 2, **d**). When there are two carbons between the groups, the molecule is called a β-amino acid (Figure 2, **a**-**c**). β-Amino acids can be further defined as β^2-amino acids, β^3-amino acids and $\beta^{2,3}$-amino acids according to the number and position of the substituents R, R^1 and R^2 (Figure 2). The diversity of β-amino acids is high in nature. They exist in free forms and as components of natural products.[40] For instance, the β-alanine fragment exists in a number of natural products including carnosine (Figure 3).[41] (2*R*,3*S*)-*N*-Benzoyl-3-phenylisoserine is a component of the medicinally important natural product, paclitaxel (Taxol®), isolated from the Western yew, *Taxus brevifolia*. Taxol® has been used for the treatment of a number of cancers, including breast and ovarian cancers (Figure 3).[41]

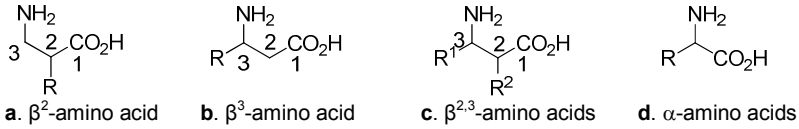


Figure 2. General structures of β-amino acids and α-amino acids.

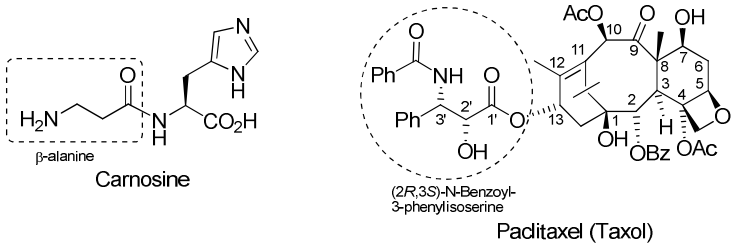


Figure 3. Two examples of natural products containing a β-amino acid: Carnosine and Taxol.

(2*R*,3*S*)-*N*-Acyl-3-phenylisoserine moieties of paclitaxel and docetaxel (an analogue of paclitaxel) are extremely important for their cytotoxicity and antitumor activity according to the structure-activity relationship (SAR) studies.[42,43] In order to develop Taxol-like compounds, much effort has been devoted to the asymmetric synthesis of the analogues of the side chain at the C-13 position of Taxol. Some exciting results have been reported by using fluorinated β-amino acids as the C-13 side chain (Figure 4). For instance, the cytotoxity of compounds **8a** and **8b** is more than two orders of magnitude higher than that of paclitaxel against the drug-resistant cell lines, MCF7-MDR and LCC6-MDR. The potency of compounds is several times higher than that of paclitaxel against the drug-sensitive cell lines, MCF7 and LCC6WT.[44]

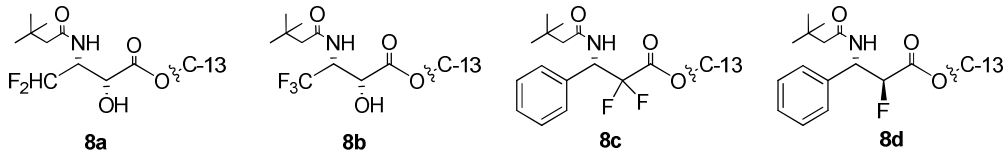


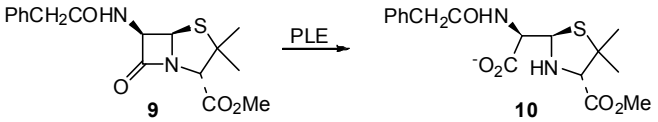
Figure 4. Analogues of the side chain to the C-13 position of Taxol.

Due to the importance of β-amino acids, increasing effort has been devoted to the enantioselective synthesis. Among the numerous methods for their preparation of enantiopure β-amino acids and their derivatives, the biocatalytic approach seems to be very effective and "green".[41, 45]

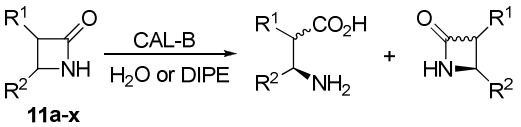
2.3.2 Enzyme-catalyzed ring opening of β-lactams

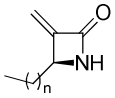
The enantiomers of β-lactams (2-azetidinones) are pharmaceutically interesting compounds as β-lactam antibiotics and as intermediates for various synthetic purposes including the preparation of β-amino acids.[41,46] In nature, β-lactamases are efficient biocatalysts for cleaving β-lactams. There are four classes of β-lactamases, classes A, C and D using serine hydrolase mechanisms and class B using a zinc hydrolase mechanism for catalysis.[47-49] The presence of β-

lactamases cause resistance to β-lactam antibiotics. In addition to β-lactamases, other types of enzymes have been found to have activity toward cleaving β-lactams. Pig liver esterase (PLE, EC 3.1.1.1) opens the β-lactam ring in the methyl ester of benzylpenicillin **9** leading to the methyl ester of benzylpenicilloate **10** (Scheme 9). PLE catalyzes the hydrolysis of the β-lactam ring more efficiently than the hydrolysis of the methyl ester of compound **9**.[50]

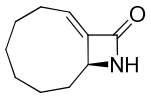


Scheme 9. Pig liver esterase-catalyzed hydolysis of the β-lactam ring.[50]

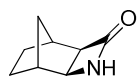




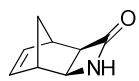
11a (n=0): *E*=74
t=86h, c=52%
11b (n=1): *E*>200
t=24h, c=50%



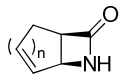
11c: *E*>200
t=68h, c=49%



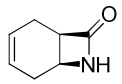
cis-**11d**: *E*>200
t=264h, c=50%



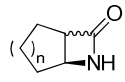
cis-**11e**: *E*>200
t=192h, c=50%



cis-**11f** (n=1): *E*>200
t=5h, c=51%
cis-**11g** (n=2): *E*>200
t=5h, c=50%

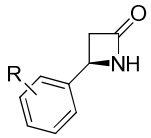


cis-**11h** (n=1): *E*>200
t=4.5h, c=50%
cis-**11i** (n=2): *E*>200
t=7h, c=51%



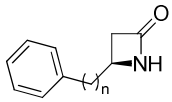
cis-**11j** (n=1): *E*>200
t=249h, c=48%
cis-**11k** (n=2): *E*>200
t=141h, c=50%
cis-**11l** (n=3): *E*>200
t=31h, c=50%

cis-**11m** (n=4): E>200
t=170h, c=50%
cis-**11n** (n=12): E>200
t=18h, c=50%
trans-**11o** (n=12): E>200
t=99h, c=50%



11p (R=H): *E*>200
t=24h, c=50%
11q (R=*p*-Me): *E*>200
t=30h, c=49%
11r (R=*o*-Cl): *E*>200
t=14h, c=50%
11s (R=*m*-Cl): E>200
t=11h, c=50%

11t (R=*p*-Cl): E>200
t=15h, c=50%
11u (R=*p*-Br): E>200
t=14h, c=49%
11v (R=*p*-F): E>200
t=13h, c=50%



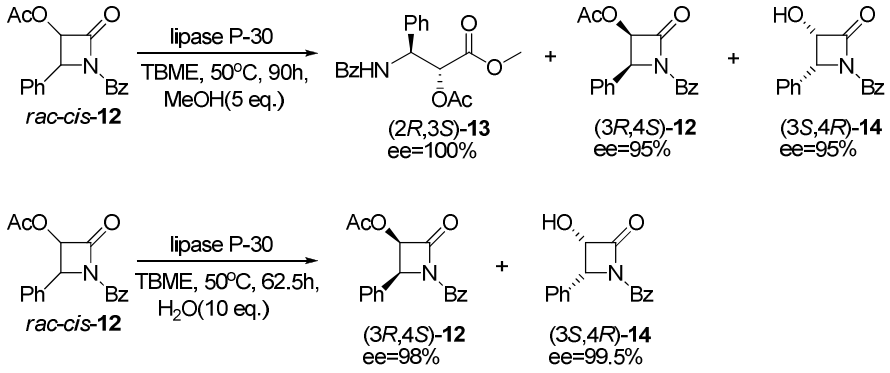
11w (n=1): *E*=4
t=13h, c=24%
11x (n=2): *E*=12
t=11h, c=29%

Scheme 10. CAL-B-catalyzed hydrolysis of β-lactams (more reactive isomers are presented).[51-60]

Although lipases generally do not catalyze the cleavage of stable amide bonds (section 2.2.3), lipases do catalyze the ring opening of β-lactams due to diminished resonance stabilization at the amide bonds of the strained four-membered rings.[45] CAL-B is an excellent catalyst for the

ring opening of unprotected β-lactams **11a-x**.[51-60] Adam *et al.* were the first who used CAL-B for opening the β-lactams **11a-c** in water.[51] The group of Fülöp in Szeged, Hungary, developed a method where CAL-B can be used for direct ring opening of β-lactams **11d-x** with one equivalent of water in diisopropyl ether (DIPE). For ring-fused β-lactams **11d-o** and 4-aryl-substituted β-lactams **11p-v**, enantioselectivity is generally high (Scheme 10).[52-59] Poor enantioselectivity is, however, observed when 4-arylalkyl-substituted β-lactams **11w** and **11x** are the substrates under otherwise similar resolution conditions as for compounds **11d-v**.[60] A recent study shows that CAL-B is capable of opening *trans*-**11o**, although the previous results have mainly focused on *cis*-isomers of ring-fused β-lactams.[59]

Sih *et al*. first succeeded to open an *N*-benzoyl activated β-lactam **12** with methanol in TBME in the presence of lipase P-30 (evidently *Burkholderia cepacia* lipase), ester **13** being formed in 19 % yield after 90 hours.[61] It is interesting that when methanol (5 eq.) is replaced by water (10 eq.) under the otherwise similar conditions, the ring opening does not occur, but only the hydrolysis of the C-3 acetoxy group (Scheme 11).



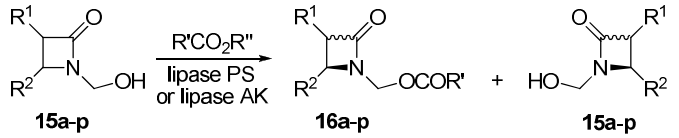
Scheme 11. Lipase P-30-catalyzed kinetic resolutions of Taxol C-13 side chain.[61]

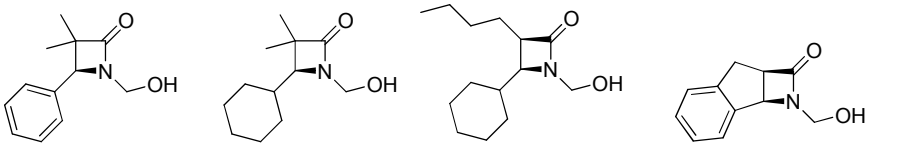
In a study where CAL-B was used for catalyzing the ring opening of *rac*-**11p** in the presence of 2-octanol and ethanol, the alcoholysis products were not observed at all. It was assumed that the esters are generated were hydrolyzed or polymerized (the polymers were not detected). It was observed that the hydrolysis rate of β-lactam *rac*-**11p** was enhanced by adding alcohols, especially when 2-octanol was added.[52] The molecular basis was suggested according to the molecular modeling study. The role of the added alcohol (2-octanol) was considered to assist the reaction by forming a network at the active site. When CAL-B was used for catalyzing the ring opening of *rac*-**11w** in the presence of methanol, both the alcoholysis and hydrolysis products were observed (paper II).

2.3.3 Lipase-catalyzed *O*-acylation of hydroxymethylated β-lactams

Attachment of a hydroxymethyl group at the nitrogen of a racemic β-lactam and resolving the racemate by acylation at the primary alcohol functionality leads to an alternative access to highly enantiopure β-lactams (Scheme 12).[62-70] The cleavage of the hydroxymethyl tail releases the enantiomers of the free β-lactam. Only few lipases have high enantioselectivity toward hydroxymethylated β-lactams due to the remote position of the stereogenic centres from the

reaction site.[62] According to the previous studies, lipase PS and lipase AK (from *Pseudomonas fluorescens*) are among the most applicable enzymes for the kinetic resolution of *N*-hydroxymethylated β-lactams.[45] Vinyl butanoate, vinyl acetate and 2,2,2-trifluoroethyl butanoate have served as acyl donors in these acylations in acetone. Toluene, dichloromethane and DIPE have also occasionally been used as solvents.





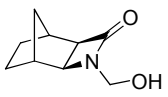
15a: *E*>200 t=9h, c=50% lipase PS

15b: *E*=60 t=84h, c=44% lipase PS

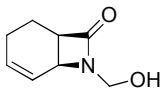
15c: *E*=40 t=72h, c=57% lipase PS

15d: *E*>200 t=7h, c=47% lipase AK

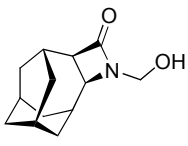
15d: *E*>200 t=7h, c=40% lipase PS



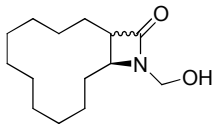
15e: *E*=62 t=3.5h, c=53% lipase AK



15f: *E*>100 t=6h, c=49% lipase PS

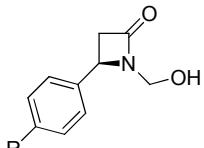


15g: *E*>200 t=3h, c=47% lipase PS

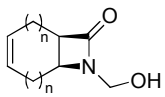


cis-**15h**: *E*=83 t=3h, c=48% lipase PS

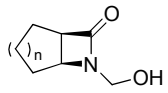
trans-**15h**: *E*=5 t=2h, c=39% lipase PS



15i (R=H): *E*=193 t=2h, c=50% lipase PS
15i (R=H): *E*=145 t=2h, c=50% lipase AK
15j(R=Me): *E*=58 t=1.5h, c=52% lipase PS



15k (n=1): *E*>200 t=4h, c=49% lipase PS
15l (n=2): *E*=16 t=2.5h, c=30% lipase AK
15l (n=2): *E*=25 t=7h, c=49% lipase PS



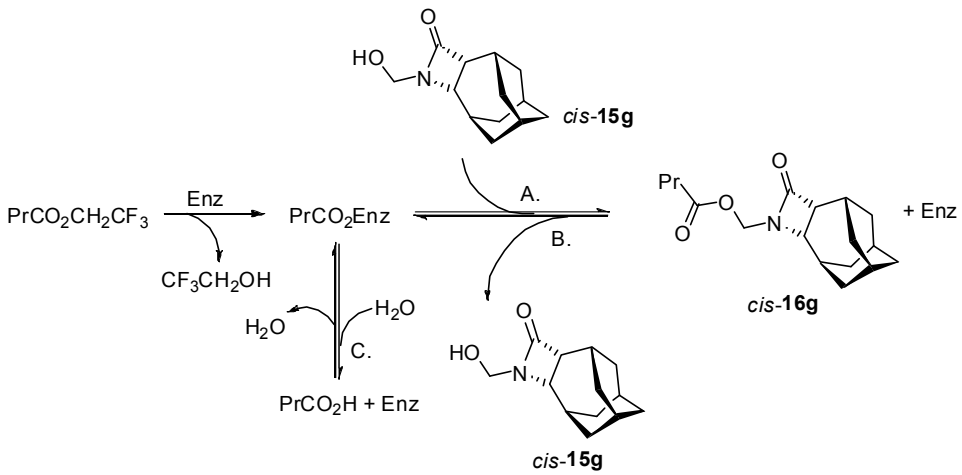
15m (n=1): *E*=90 t=2.7h, c=52% lipase AK
15n (n=2): *E*=136 t=2h, c=47% lipase AK
15n (n=2): *E*>200 t=2.7h, c=52% lipase PS

15o (n=3): *E*=82 t=1h, c=46% lipase AK
15o (n=3): *E*>200 t=1h, c=45% lipase PS
15p (n=4): *E*=27 t=2h, c=32% lipase PS

Scheme 12. Kinetic resolution of *N*-hydroxymethylated β-lactams by lipase PS and AK (more reactive isomers are presented).[62-70]

As discussed in section 2.2.3, water present in the enzyme preparation can act as a competing nucleophile, thus causing side reactions, especially in *O*-acylation reactions in organic solvent. A typical example is shown in Scheme 13, where in the presence of lipase PS the *O*-acylation of *cis*-**15g** afforded product *cis*-**16g** in high enantiopurity (ee>99%, route **A**). The reaction stopped, however, at around 45 % conversion. It was proposed that the hydrolysis of *cis*-**16g** (route B + C) caused the reaction to stop, when the hydrolysis became important at high conversion. Eventually the highly enantioselective reaction ended up in complex equilibria. The

racemization of the product *cis*-**16g** was not observed due to high enantioselectivity of the ester bond formation.[70]



Scheme 13. Route **A**: The *O*-acylation of *cis*-**15g** in the presence of lipase PS in TBME. Route **B** + **C**: the hydrolysis of *cis*-**16g** as a side reaction.[70]

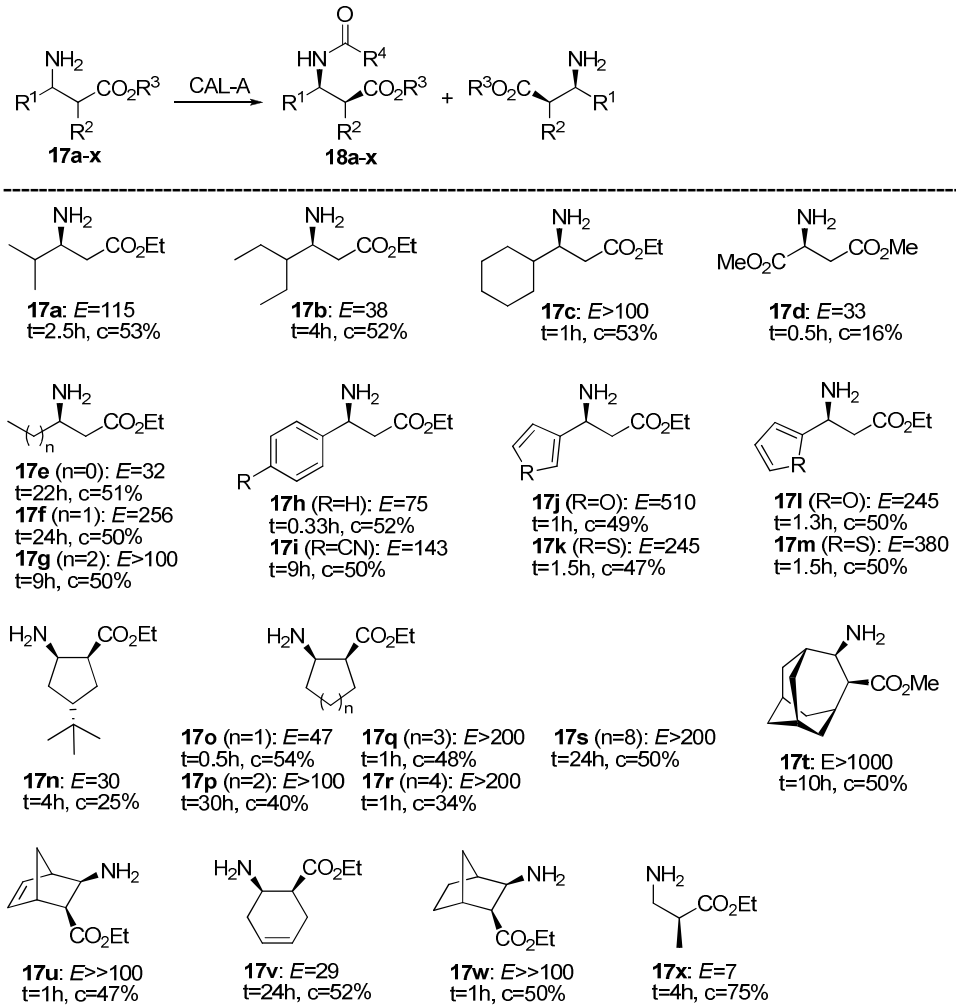
2.3.4 Lipase-catalyzed resolution of β-amino esters and β-amino nitriles

2.3.4.1 CAL-A-catalyzed N-acylation

Enzymatic *N*-acylation has been shown to be an effective approach to highly enantiopure β-amino esters. CAL-A is among the mostly used enzymes in resolving β-amino esters with *N*-acylation reactions. CAL-A is especially useful in the resolution of β^3- and $\beta^{2,3}$-amino esters **17a-w** (Scheme 14).[69-76] CAL-A was also applied to the enantioselective *N*-acylation of the β^2-amino ester **17x** and even two α-amino esters possessing secondary amines,[76-78] which are challenging with lipases in general.

CAL-A-catalyzed *N*-acylation of β-amino esters has usually been carried out in dry diisopropyl ether (DIPE), *tert*-butyl methyl ether (TBME), and diethyl ether. Butyl butanoate and 2,2,2-trifluoroethyl butanoate have been the most common acyl donors to achieve high enantioselectivity with reasonable reactivity. Carboxylic anhydrides as common acylation reagents in the chemical synthesis of amides are not appropriate acyl donors in CAL-A-catalyzed resolution of β-amino esters, because the uncatalyzed background reaction leads to the formation of racemic products.

Chemoselectivity of CAL-A toward the *N*-acylation of β-amino esters is very high (Figure 5). With the recently solved crystal structure of CAL-A, this can be easily understood.[23] The acyl binding site of CAL-A is long and narrow. This prefers binding of straight carboxylic acids over branched ones. Thus, β-amino esters with bulky side chains cannot be properly bound at the acyl donor cavity of CAL-A to form the acyl-enzyme intermediate I (section 2.2.2, Scheme 4). The nucleophile binding site is, however, relatively large and can accommodate bulky compounds such as **17t**.[23,70]



Scheme 14. CAL-A-catalyzed *N*-acylation of β^2-, β^3- and $\beta^{2,3}$-amino esters in organic solvent (the more reactive enantiomers are presented).[69-76]

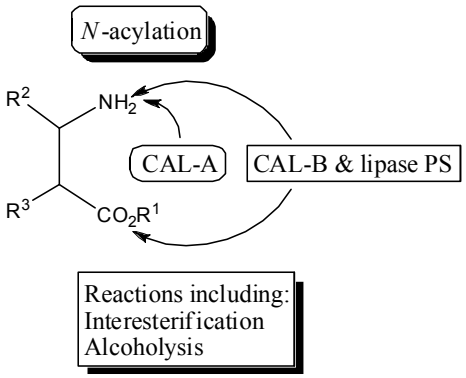


Figure 5. Chemoselectivity of CAL-A, lipase PS and CAL-B toward a β-amino ester.

2.3.4.2 CAL-B-catalyzed N-acylation, interesterification and alcoholysis

Only some β-amino esters have been resolved by CAL-B-catalyzed *N*-acylation reactions (Figure 6). Racemic ethyl 3-amino butanoate **17e** was resolved with CAL-B in neat ethyl acetate with *E* value of 80.[79] With isopropyl methoxyacetate as an acyl donor, excellent enantioselectivity was observed ($E > 1000$). Excellent enantioselectivity ($E > 1000$) was also obtained in the CAL-B-catalyzed *N*-acylation of alicyclic *trans*-**19** with isopropyl methoxyacetate as an acyl donor (Figure 6).[80] According to the molecular modeling studies, it was suggested that the acyl-chain oxygen in alkyl methoxyacetates can form a hydrogen bond with the amine nitrogen (O···H-N) at the transition state.[81]

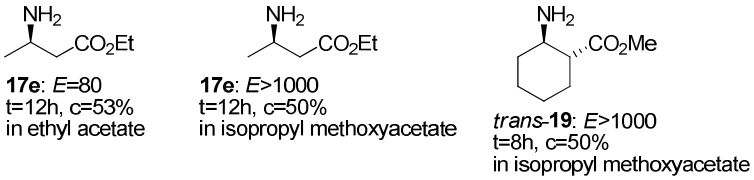


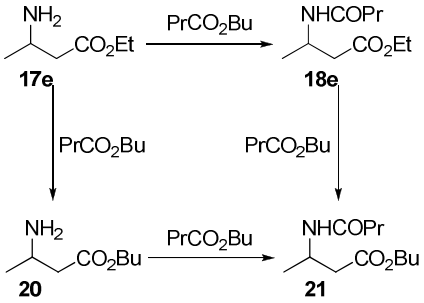
Figure 6. CAL-B-catalyzed *N*-acylation of **17e** and *trans*-**19**. The more reactive enantiomers are presented.[79,80]

Compared to CAL-A, CAL-B has been shown to catalyze reactions at the amino group (*N*-acylation) as well as at the carboxyl functionality of a β-amino ester. The ester group reacts through interesterification and alcoholysis (Scheme 5 and Figure 5). A good example is the sequential resolution of ethyl 3-aminobutanoate **17e** with butyl butanoate in the presence of CAL-B (Scheme 15).[82] Compound **18e** is the *N*-acylation product and **20** is the interesterification product. Both **18e** and **20** react further into product **21**. The relative amount of the three products depends on the conversion of the reaction. When the reaction reaches 65% conversion, **20** and **21** are the major products. Structural effects on the chemoselectivity of the CAL-B-catalyzed resolution of β-amino esters have also been investigated. The interesterification becomes favored over the *N*-acylation with the increasing size of the substituents at the β-position of the studied β^3-amino esters.[83]

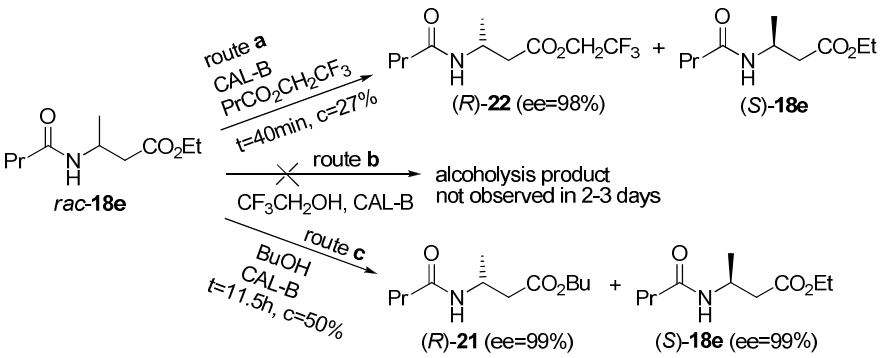
The *N*-protected compound *rac*-**18e** has been tested in CAL-B-catalyzed interesterification reaction in the presence of 2,2,2-trifluoroethyl butanoate (Scheme 16, route **a**). The reaction proceeds relatively fast with high enantioselectivity [27% conversion in 40 min, for (*R*)-22 ee = 98%].[82] This is probably the first lipase-catalyzed synthesis of 2,2,2-trifluoroethyl esters. This method has been applied to the preparation of activated β-amino esters for the synthesis of β-dipeptides in this thesis and will be discussed in more detail in section 5.4.2.

As already mentioned (section 2.2.2, Scheme 5), from the mechanistic point of view an interesterification reaction is actually an alcoholysis reaction. For instance, in the reaction of *rac*-**18e** and $PrCO_2CH_2CF_3$ (Scheme 16, route **a**), 2,2,2-trifluoroethanol is generated *in situ* from $PrCO_2CH_2CF_3$ by CAL-B. This 2,2,2-trifluoroethanol causes the alcoholysis of **18e** in the formation of the product (*R*)-**22**. The alcoholysis product **22** is not, however, observed when *rac*-**18e** is mixed with 2,2,2-trifluoroethanol in the presence of CAL-B in a separate reaction (route **b**). According to the proposed hypothesis, the liberated CF_3CH_2OH from $PrCO_2CH_2CF_3$

stays bound to the active site or its close vicinity rather than being liberated from the active site, which facilitates the subsequent alcoholysis reaction of an acyl-enzyme intermediate by CF_3CH_2OH.[82,83]



Scheme 15. Sequential resolution of compound **17e** with butyl butanoate by CAL-B.[82]



Scheme 16. CAL-B-catalyzed interesterification and alcoholysis of *rac*-**18e**.[82,83]

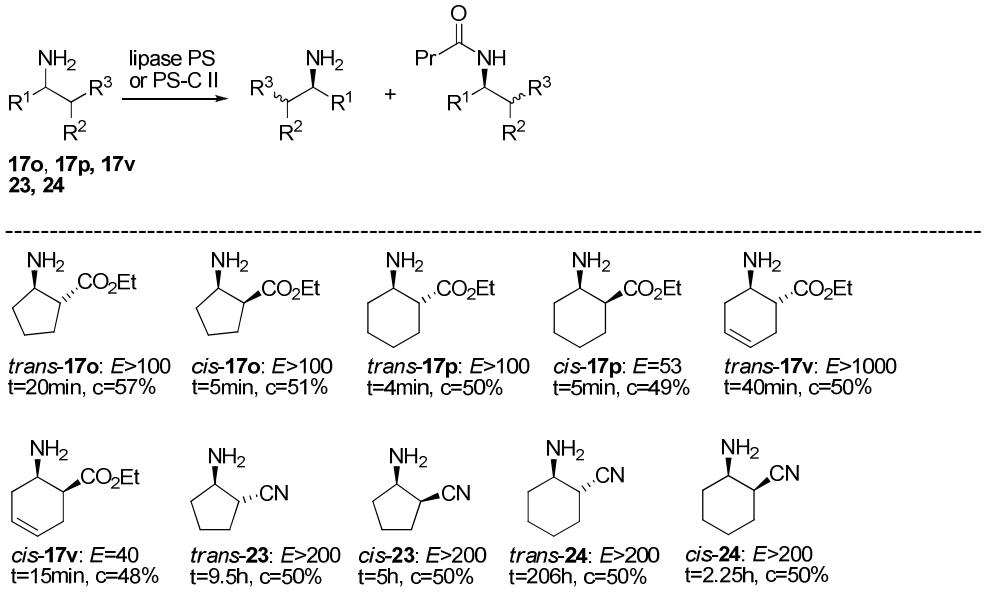
n-Butanol is more nucleophilic than 2,2,2-trifluoroethanol and evidently can also be accommodated at the active site. The alcoholysis of *rac*-**18e** with butanol proceeded very well (route **c**). At 50% conversion, highly enantiopure alcoholysis product (*R*)-**21** (ee=99%) and the unreacted substrate (*S*)-**18e** (ee=99%) were obtained.[83]

2.3.4.3 Lipase PS-catalyzed N-acylation

As described in section 2.3.3, lipase PS is an excellent biocatalyst for the *O*-acylation of various hydroxymethylated β-lactams. Lipase PS is capable of catalyzing *N*-acylation reactions as well as reactions at the carboxylic group of β-amino esters (Figure 5). In the case where compound **17e** reacts with 2,2,2-trifluoroethyl butanoate in the presence of lipase PS, the reaction proceeds in a similar way as in the CAL-B-catalyzed reaction of **17e** and butyl butanoate (Scheme 15), in which low chemoselectivity is observed.[82] On the other hand, several alicyclic β-amino esters **17o**, **17p** and **17v** were resolved by lipase PS-catalyzed *N*-acylation reactions in diethyl ether,

2,2,2-trifluoroethyl butanoate or 2,2,2-trifluoroethyl chloroacetate being the acyl donor with absolute chemoselectivity (Scheme 17).[75]

In addition to the β-amino esters, alicyclic β-amino nitriles **23** and **24** were effectively resolved on preparative scale by lipase PS-C II in TBME (Scheme 17).[84] Lipase PS-C II is a commercial preparation, where lipase PS is adsorbed on the surface of Toyonite 200 by hydrogen bonding.[85] In these cases, the enantiomer with the *R*-configuration reacted.



Scheme 17. *N*-acylation of β-amino esters and β-amino nitriles catalyzed by lipase PS or lipase PS-C II. The more reactive enantiomers are presented.[75,84]

2.4 Enzymatic synthesis of peptides

2.4.1 General about peptides

Peptides are oligomers of amino acids. α-, β- and γ-peptides are built up by α-, β- and γ-amino acids, respectively (Figure 7). Also "mixed" peptides exist which consist of different types of amino acid residues. For instance, the natural product carnosine is an α/β-dipeptide formed by β-alanine and α-histidine (section 2.3.1, Figure 3).

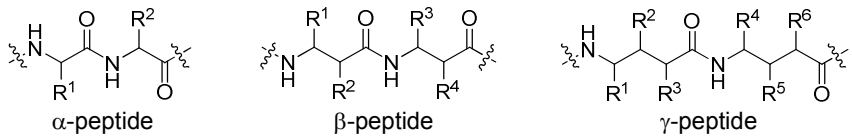


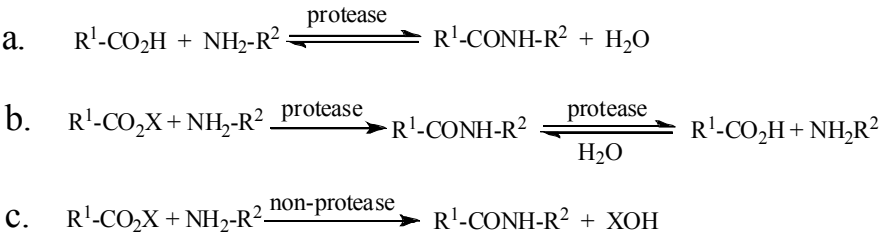
Figure 7. α-Peptide, β-peptide and γ-peptide.

Small- to medium-length peptides are generally synthesized in solution or on solid supports. For the synthesis of long peptides, chemical ligation is often used.[86-88] Chemical synthesis of α-peptides is a well developed method in organic chemistry, and has been thoroughly reviewed.[88] A number of drawbacks have been pointed out in the chemical approaches, which include racemization during the peptide bond formation and deprotection steps, the requirement to protect reactive groups at side chains, difficulties in recycling the coupling reagent used in excess to achieve effective coupling and the toxic nature of coupling reagents. For this reason, enzymatic synthesis is considered to be a "green" method, especially for the synthesis of small-sized peptides such as aspartame (Chapter 1, Scheme 1).[86,87]

A lot of research has been focused on the enzymatic synthesis of α-peptides. The publications about enzymatic synthesis of β-peptides are scarce.[89, paper V] For γ-peptides, there are no enzymatic procedures available.

2.4.2 Enzymatic preparation of α-peptides

Proteases are enzymes which typically hydrolyze α-peptide bonds. The hydrolysis reaction is reversible, which makes the use of proteases possible for synthetic direction. Protease-catalyzed peptide formation can be carried out in two ways. Thermodynamically controlled peptide synthesis is a reverse reaction for hydrolysis (Scheme 18, **a** and Figure 8, **a**). One of the serious problems with this method is the low equilibrium yield of the synthesis.[86] In order to achieve reasonable yields, many strategies have been applied to shift the equilibrium toward the peptide formation. Product precipitation is a good option. An example is the synthesis of aspartame (Chapter 1, Scheme 1). The thermolysin enzyme is used for incorporating the *N*-protected L-aspartic acid with the L-isomer of the racemic phenylalanine methyl ester, resulting in the protected dipeptide which generates aspartame after deprotection. An insoluble adduct is formed by the aspartame precursor with the unreacted D-isomer of phenylalanine methyl ester, leading to an increased yield. The adduct is, subsequently, broken down to liberate aspartame by changing the pH.



Scheme 18. a. thermodynamically controlled peptide synthesis; b. kinetically controlled peptide synthesis; c. peptide formation catalyzed by non-proteases. R^1 and R^2 represent the rest of the amino acids and X is an alkyl group.

In the kinetically controlled approach of protease-catalyzed peptide bond formation the acyl donor (R^1CO_2X) is employed in an activated form (Scheme 18. **b**), for instance as an ester.[90] The desired peptide accumulates as long as the acyl donor ester is not completely consumed. In

an ideal case, therefore, the reaction should be stopped at the kinetic maximum in order to get high yield. After that point, the hydrolysis draws the reaction to the same equilibrium as in the thermodynamically controlled approach (Figure 8, **b**). The kinetically controlled synthesis generally proceeds faster and requires smaller amounts of enzymes compared to the thermodynamically controlled synthesis. However, only serine or cysteine proteases can be used, because the formation of an acyl-enzyme intermediate with the acyl donor is necessary (R^1CO_2X). Subsequently, the nucleophile (NH_2R^2) attacks the acyl-enzyme intermediate, affording the peptide by the serine hydrolase mechanism similar to lipase catalysis (section 2.2.2).

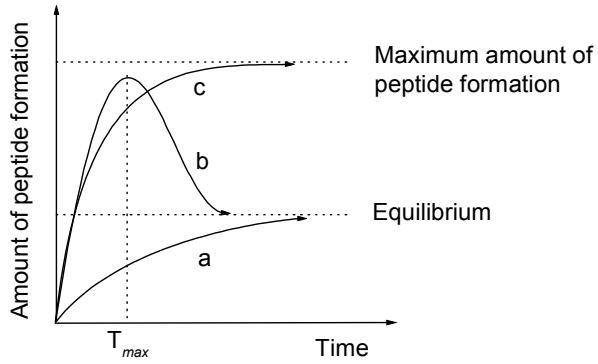
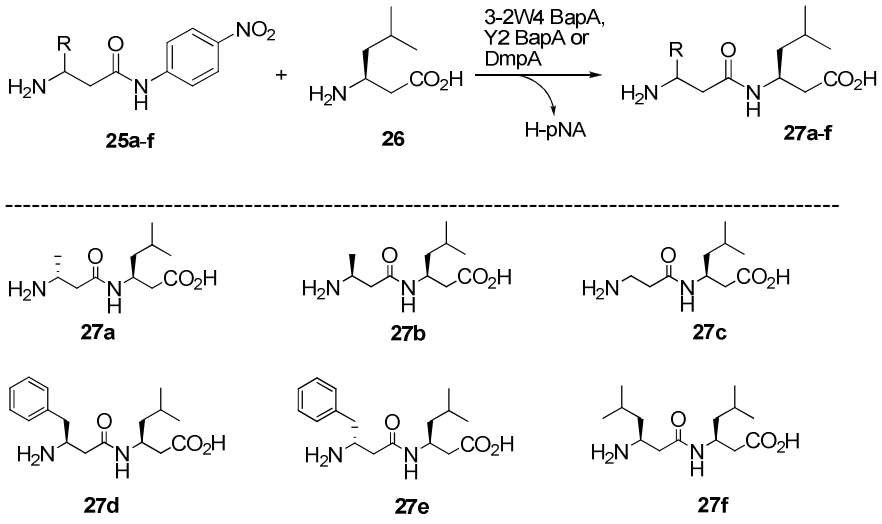


Figure 8. a. Protease-catalyzed thermodynamically controlled peptide synthesis. b. protease-catalyzed kinetically controlled peptide synthesis. c. nonprotease-catalyzed peptide synthesis.

As discussed above, proteases catalyze the reverse reaction during peptide synthesis. In addition to that, proteases generally have limited substrate scope. For this reason, enzymes which lack amidase activity and which have broad substrate scope seem to be ideal biocatalysts for peptide synthesis. In the previous studies, porcine pancreatic lipase (PPL) and a lipase from *Pseudomonas* species, PLE and CRL have been used for synthesizing a series of α-dipeptides in anhydrous organic solvents or in organic solvents with small amount of buffer.[91-96]

2.4.3 Enzymatic preparation of β-peptides

β-Peptides (oligomers of β-amino acids) are a class of potential peptidomimetics inducing pharmacological effects. Peptidomimetic is a general term for any designed compounds performing the function of peptides. β-Peptides display remarkable ability to fold into well-defined secondary structures on which biological functions of peptides are based. Thus far, biological studies carried out on β-peptides are rare. The preliminary results have shown that β-peptides function in ways similar to the corresponding α-peptides. They have low affinity to blood cells, no hemolytic effects (*in vitro*) being observed. The clearance is low, and the clearance proceeds through kidneys and intestinal tract, not through the liver or bile duct. There is almost no metabolism being observed, which means that β-peptides are resistant to the degradation by proteases and even P450-dependent enzymes.[40]

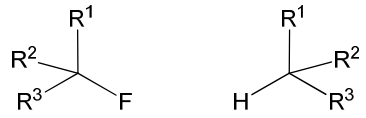


Scheme 19. β-peptide formation catalyzed by β-peptidyl aminopeptidases.[97]

When my work (paper V) on CAL-A-catalyzed β-dipeptide synthesis was published in 2006, no publications concerning enzymatic synthesis of β-peptides were available. When this thesis was in preparation, a research article where β-peptidyl aminopeptidases BapA from *Sphingosinicella xenopeptidilytica* 3-2W4, BapA from *Sphingosinicella microcystinivorans* Y2 and DmpA from *Ochrobactrum anthropi* LMG7991 were applied to the formation of β-peptides just appeared (Scheme 19).[89] BapA from *Sphingosinicella xenopeptidilytica* 3-2W4 seems to be a versatile catalyst capable of coupling various β-amino acids to the free *N*-termini of β- and α-amino acids and even to an α-tripeptide. These three enzymes are also able to catalyze the cleavage of *N*-terminal β-amino acid residues from β- and α/β-peptides.[97]

2.5 Fluorinated organic compounds

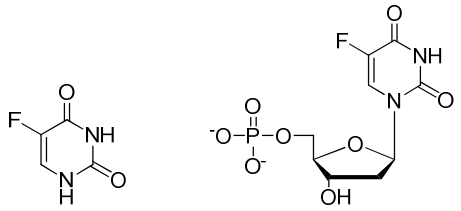
Fluorine is frequently used as a unique isostere to hydrogen. The fluorine atom is close to the hydrogen atom in size. Carbon-fluorine bond has high bond energy compared to carbon-hydrogen bond. Fluorine is the most electronegative element (Figure 9). Moreover, fluorine can receive hydrogen bonds from hydrogen bond donors.[98]



a. van der Waals radius: F 1.47 Å (H 1.2 Å)
b. Bond energy: C-F 116 kcal/mol (C-H 99 kcal/mol)
c. The most electronegative element
d. Hydrogen bond acceptor

Figure 9. Features of the fluorine atom and carbon-fluorine bond compared to the carbon-hydrogen bond. [98]

The introduction of fluorine into an organic molecule can cause profound effects on the chemical, physical and biological properties of the molecule. There are many drug molecules containing fluorine. The antitumor drug 5-fluorouracil **28** (the trade name in Finland is Flurablastin, Figure 10), which finds wide clinical use, is a good example. 5-Fluorouracil is converted into its active form, 5-fluoro-2´-deoxyuridylate **29** *in vivo*. The 5-fluoro-2´-deoxyuridylate generated inhibits the thymidylate synthase enzyme, thus inhibiting the DNA biosynthesis. The similarity of the *van der* Waals radius of fluorine to that of hydrogen makes 5-fluoro-2´-deoxyuridylate acceptable by the target enzyme acting on 2´-deoxyuridylate. The subsequent metabolism is prevented since there is no C-5 proton available.[2] As another example, edible acetic acid becomes poisonous by replacing one of the hydrogens of the acyl group with a fluorine atom. The importance of fluorinated compounds is also reflected by the fact that half of the top ten drugs sold in 2005 were fluorinated organic molecules and 20 % of the pharmaceuticals on the market were estimated to contain fluorine.[99]

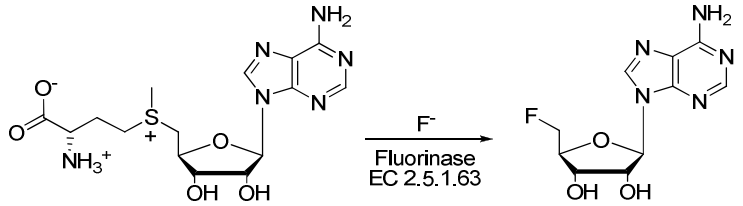


28 **29**

Figure 10. 5-Fluorouracil **28** and 5-fluoro-2´-deoxyuridylate **29**.[2]

Although fluorine is one of the most abundant elements on earth, fluorine-containing organics in nature are extremely rare. Most fluorine on earth crust is in the form of insoluble fluoride minerals, and the availability of fluorinated organic compounds relies on synthesis. There are two approaches to synthesize fluorinated organics: direct fluorinating approach and building block approach. Most published processes for the synthesis of fluorinated organic compounds are based on the building block approach. Efforts have been devoted to develop enantioselective fluorination reactions. The exploitation of novel catalysts for asymmetric synthesis has stimulated tremendous interests in the community of fluorine chemistry, leading to increasing number of organofluorine compounds.[100]

Untill now, fluorinase is the only enzyme which has been identified in nature as having the ability to fluorinate directly an organic compound (Scheme 20). The fluorinase enzyme is from *Streptomyces cattleya*, and its crystal structure is now available.[101,102] The discovery of the fluorinase is significant, since the structural information of the enzyme might offer hints for designing and producing novel biocatalysts to perform similar tasks as the fluorinase does.



Scheme 20. Fluorinase-catalyzed direct fluorination.

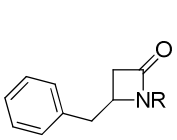
Incorporation of fluorine in the design of artificial amino acids has become a prominent strategy to induce special folding of peptides and proteins for structural studies.[103-105] The increased hydrophobicity of a fluorinated molecule can significantly increase diffusion across membranes, such as the blood-brain barrier, and improve the pharmacokinetic properties of peptide-based drugs.[40]

3 AIM OF THE STUDY

The aim of this work has been to study the possibilities to develop new and to improve the existing (section 2.3) lipase-catalyzed methods for the synthesis of β-amino acid derivatives of high enantiopurity (ee>98%). Lipase catalysis has been used throughout the experimental work. The lipases used in this study were the lipase from *Burkholderia cepacia* (lipase PS adsorbed on Celite in the presence of sucrose and lipase PS-D as the commercial preparation on Celite), *Candida antarctica* lipase A (CAL-A adsorbed on Celite in the presence of sucrose) and *Candida antarctica* lipase B (CAL-B as the commercial Novozym 435 preparation). The research is focused on the enzymatic steps of the chemoenzymatic routes which are the key steps in generating enantiopure compounds.

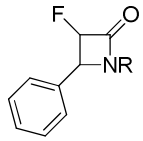
Figure 11 shows the compounds prepared in this work. As mentioned in section 2.3.2, the lipase-catalyzed hydrolysis of β-lactams has been thoroughly studied. The purpose here has been to develop the lipase-catalyzed enantioselective ring opening of β-lactams as a more general method where nucleophiles other than water can be used. Thus the present work is focused on the lipase PS-catalyzed alcoholysis of fluorinated β-lactams **33a** and **34a**, and on the ammonolysis and aminolysis of β-lactam **33a**, preparing highly enantiopure β-amino esters, β-amino amides and β-amino lactams (papers I and VI). I have also developed further the lipase-catalyzed method where the *O*-acylation of *N*-hydroxymethylated β-lactams is applied. The focus has been on the preparation of the both enantiomers as β-lactams, and accordingly to resolve the racemic compounds **15i**, **32a**, **33b** and **34b**. The lipase-catalyzed *O*-deacylation of the corresponding ester derivatives is introduced in order to cleave the ester bonds under mild conditions and at the same time to enhance the enantiopurity of the produced ester products in the cases where enantioselectivities are not high (papers II and III). By following the known method, heteroaryl-substituted β-amino ester *rac*-**38a** and β-amino nitrile *rac*-**38b** were resolved with CAL-A-catalyzed *N*-acylation reaction in order to study the enantio- and chemoselectivity (paper IV).

Based on the above mentioned work with β-amino acid derivatives, I have further focused on methods where β-peptides can be formed using lipase catalysis. Accordingly, part of the β-amino esters and β-lactams obtained were incorporated into β-dipeptides by two strategies. According to the first strategy, β-dipeptides **43a-g** were obtained by CAL-A-catalyzed aminolysis of *N*-protected β-amino esters with free β-amino esters (paper V). By use of the second strategy, β-dipeptides **43h-l** were obtained by lipase PS-D-catalyzed ring opening of β-lactams with β-amino esters (paper VI). All the enzymatic reactions were performed in organic solvents.

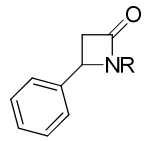


11w R=H
32a R=CH_2OH
32b R=CH_2OCOPr

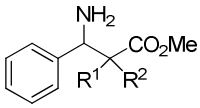
33a R=H
33b R=CH_2OH
33c R=CH_2OCOPr



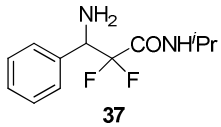
34a R=H
34b R=CH_2OH
34c R=CH_2OCOPr
34d R=Boc



11p R=H
15i R=CH_2OH
16i R=CH_2OCOPr
35 R=Boc



36a R^1=R^2=F
36b R^1=F, R^2=H
36c R^1=R^2=H



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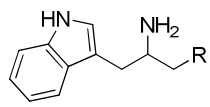
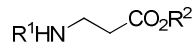
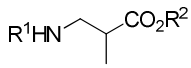


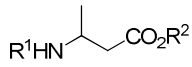
38a R=CO_2Et
38b R=CN



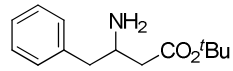
39a R^1=H, R^2=Et
39b R^1=H, R^2=Me
39c R^1=CH_3CO, R^2=Et
39d R^1=CH_3CO, R^2=CH_2CF_3



17x R^1=H, R^2=Et
40a R^1=CH_3CO, R^2=Et
40b R^1=CH_3CO, R^2=CH_2CF_3



17e R^1=H, R^2=Et
41a R^1=CH_3CO, R^2=Et
41b R^1=CH_3CO, R^2=CH_2CF_3
41c R^1=H, R^2=^{t}Bu



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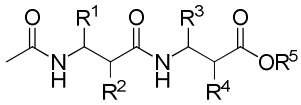
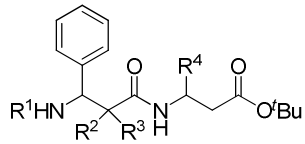


43a R^1=R^2=H, R^3=Ph, R^4=H, R^5=Me
43b R^1=R^2=H, R^3=Me, R^4=H, R^5=Et
43c R^1=R^2=R^3=H, R^4=Me, R^5=Et
43d R^1=R^2=R^3=R^4=H, R^5=Me
43e R^1=Me, R^2=R^3=R^4=H, R^5=Et
43f R^1=H, R^2=Me, R^3=R^4=H, R^5=Et
43g R^1=H, R^2=Me, R^3=Ph, R^4=H, R^5=Me



43h R^1=H, R^2=R^3=F, R^4=Me
43i R^1=R^2=H, R^3=F, R^4=Me
43j R^1=Boc, R^2=R^3=H, R^4=Me
43k R^1=Boc, R^2=R^3=H, R^4=Bn
43l R^1=Boc, R^2=H, R^3=F, R^4=Bn

Figure 11. Compounds prepared in this thesis.

4 MATERIALS AND METHODS

All the reagents and solvents used in this work were purchased from Aldrich or Fluka. The preparation of racemates and other chemical and enzymatic transformations have been described in detail in papers I-VI. The three enzymes mainly used in this thesis are lipase PS, CAL-A and CAL-B. All the enzymes were used in immobilized forms. Lipase PS as free enzyme powder or as immobilized lipase (lipase PS-D adsorbed on Celite) was purchased from Amano Europe, England. Before use, the free lipase PS powder was adsorbed on Celite by dissolving the enzyme (5 g) and sucrose (3 g) in Tris-HCl buffer (20 mL, 20 mM, pH 7.9) followed by the addition of Celite (17 g) and evaporation to dryness at room temperature.[12] In the same way, free CAL-A (product of Roche) was adsorbed on Celite. CAL-B (Novozym 435) immobilized on a methacrylate polymer was obtained from Novo Nordisk.

Preparative chromatographic separations were performed by column chromatography on Merck Kieselgel 60 (0.063-0.200 μm). TLC was carried out with Merck Kieselgel $60F_{254}$ sheets. Melting points were measured on a Sanyo instrument. Optical rotations were determined with a Perkin-Elmer polarimeter, and the calculated $[\alpha]_D$ values are given in the units of 10^{-1} deg cm^2 g^{-1}. The determination of E is based on the equation $E = \ln[(1-c)(1-ee_s)]/ \ln[(1-c)(1+ee_s)]$ with the use of linear regression, E being the slope of a line $\ln[(1-c)(1-ee_s)]$ *vs.* $\ln[(1-c)(1+ee_s)]$.[8]

The ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 spectrometer with tetramethylsilane (TMS) as an internal standard. ^1H-^1H COSY, ^1H-^{13}C HQSC and ^1H-^{13}C HMBC spectra were used for the assignment of the chemical shifts when necessary. 2,2,2-Trifluoroacetic acid was used as an external standard for measuring ^{19}F NMRs. Mass spectra were taken on a VG 7070E mass spectrometer. The progress of the reactions was followed by taking samples from the reaction mixture at intervals and analyzing them by HPLC on a CHIRACEL-OD column (0.46 × 25 cm) and GC on a Chrompack CP-Chirasil-DEX CB column or a Chrompack CP-Chirasil-L-Valine column.

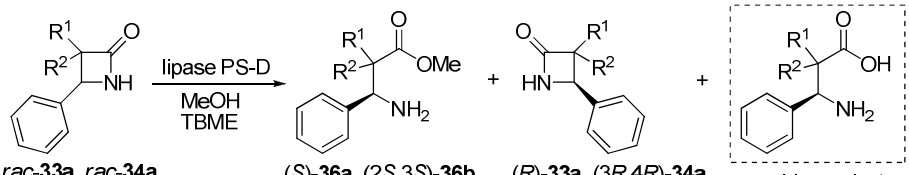
5 RESULTS AND DISCUSSION

5.1 Lipase-catalyzed ring opening of β-lactams

5.1.1 Enantioselective alcoholysis of β-lactams (paper I)

Lipase-catalyzed enantioselective direct ring opening of β-lactams is a useful method for preparing enantiopure β-lactams as less reactive enantiomers and β-amino acids, esters or amides as reactive enantiomers. CAL-B has been used as the catalyst for the enantioselective hydrolysis of β-lactams (section 2.3.2).[51-60] Lipase-catalyzed direct alcoholysis, aminolysis and ammonolysis of β-lactams has remained unexplored, except in one case where the alcoholysis of *N*-benzoyl protected β-lactam *rac-cis*-**12** has been briefly studied.[61] It is much more challenging to perform the alcoholysis than the hydrolysis of β-lactams, because in the former case the water in seemingly dry enzyme preparation tends to act as a competing nucleophile, leading to hydrolysis as the side reaction (sections 2.2.3 & 2.3.2).

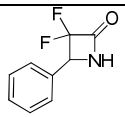
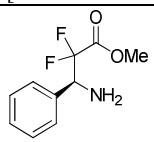
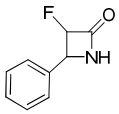
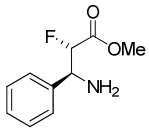
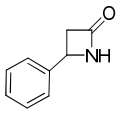
The interest here is to prepare fluorinated β-lactams and β-amino esters because of the importance of fluorinated compounds (section 2.5). For example, the (*S*)-enantiomer of 3,3-difluoro-4-phenyl-2-azetidinone (*S*)-**33a** has been coupled with the alcohol group of the taxane ring, giving a fluorotaxol analogue.[106] Accordingly, racemic compounds *rac*-**33a** and *rac*-**34a** were prepared via a Reformatsky addition followed by oxidative deprotection (paper I). The alcoholysis of difluorinated β-lactam *rac*-**33a** was carried out in the presence of lipase PS-D in dry *tert*-butyl methyl ether (TBME), product (*S*)-**36a** (ee > 99%) and unreacted substrate (*R*)-**33a** (ee > 99%) being produced at 50% conversion (Scheme 21 and Table 1, entry 1). In order to know the proportion of hydrolysis as the side reaction, quantitative analyses have been carried out. The amount of the resulted (*S*)-**36a** and (*R*)-**33a** were in accordance with the theoretical values based on stoichiometry, which excluded the possibility for hydrolysis as the side reaction by water in the enzyme preparation. When a single fluorine was at the carbon adjacent to the carbonyl group of *rac*-**34a**, the methanolysis product (2*S*,3*S*)-**36b** was obtained with excellent enantiopurity (ee > 99%), although 3% of hydrolysis was detected (entry 2). When lipase PS-D was incubated with nonfluorinated compound *rac*-**11p** under otherwise identical conditions, no reaction was observed (entry 3), indicating that the fluorine activation of the β-lactams was essential in facilitating the ring opening.



rac-**33a**, *rac*-**34a** (*S*)-**36a**, (2*S*,3*S*)-**36b** (*R*)-**33a**, (3*R*,4*R*)-**34a**

Scheme 21. Lipase PS-D-catalyzed alcoholysis of β-lactams: in *rac*-**33a**, (*R*)-**33a** and (*S*)-**36a** R^1=R^2=F, in *rac*-**34a**, (3*R*,4*R*)-**34a** and (2*S*,3*S*)-**36b** R^1=F and R^2=H.

Table 1. Lipase PS-D-catalyzed alcoholysis of β-lactams with methanol (5 equiv) in TBME at 23°C for 24 h.

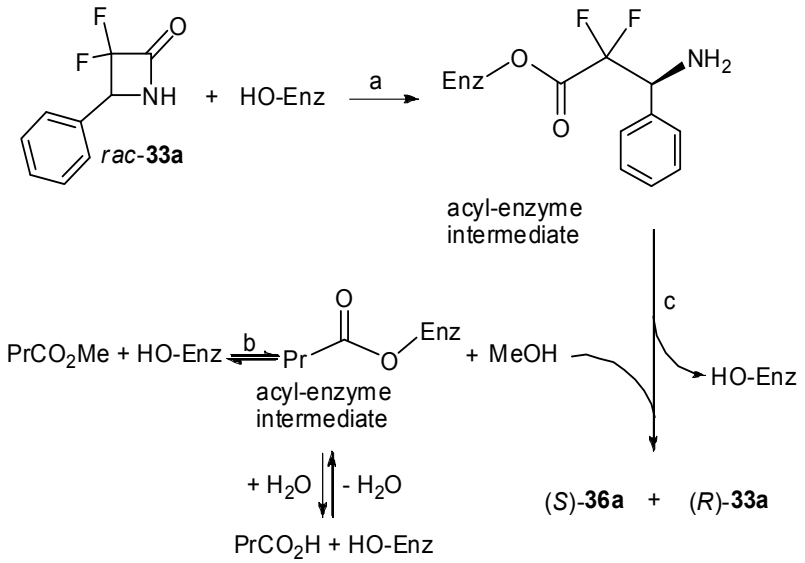
Entry	Racemates	Total conversion (%)	Proportion of hydrolysis (%)	Unreacted substrates [ee(%)]	Alcoholysis products [conversion & ee(%)]
1	 *rac*-**33a**	50	0	(*R*)-**33a** (ee>99%)	 (*S*)-**36a** (*c*=50%,ee>99)
2	 *rac*-**34a**	47	3	(3*R*,4*R*)-**34a** (ee=83%)	 (2*S*,3*S*)-**36b** (*c*=44%,ee>99)
3	 *rac*-**11p**	0	-	*rac*-**11p**	-

Finally, the preparative-scale alcoholyses of *rac*-**33a** and *rac*-**34a** were carried out in the presence of lipase PS-D and methanol in TBME at room temperature. Compounds (*S*)-**36a**, (2*S*,3*S*)-**36b**, (*R*)-**33a** and (3*R*,4*R*)-**34a** were prepared in high enantiopurity (Table 2).

Table 2. Preparative-scale resolution of *rac*-**33a** and *rac*-**34a** in the presence of PS-D and MeOH (5 equiv) in TBME at room temperature.

Racemates (mmol)	PS-D (mg mL^{-1})	Time (h)	Conversion (%)	Products (mmol)	Unreacted substrates (mmol)
rac-**33a** (1.09)	30	17.5	50	(*S*)-**36a** (0.43) ee= 99%	(*R*)-**33a** (0.53) ee= 99%
rac-**34a** (1.21)	50	48.0	50	(2*S*,3*S*)-**36b** (0.55) ee= 99%	(3*R*,4*R*)-**34a** (0.58) ee= 99%

As mentioned in section 2.2.2 (Scheme 5), mechanistically interesterification is an alcoholysis reaction leading to the same ester product as obtained in the presence of an added alcohol. This knowledge was applied to an alternative method in resolving *rac*-**33a** by replacing methanol with methyl butanoate. As expected, *rac*-**33a** and methyl butanoate formed the alcoholysis product (*S*)-**36a** in the presence of PS-D in TBME, enantioselectivity being very high ($E > 200$) (Scheme 22). In this case, the reaction was called "interesterification-type" rather than "interesterification" reaction, involving the reaction of a β-lactam and an ester rather than the reaction of two esters. Both the alcoholysis and the interesterification-type reaction can be used for the resolution of *rac*-**33a**.



Scheme 22. Ring opening by methanol generated *in situ* from $PrCO_2Me$. The water comes from the enzyme preparation.

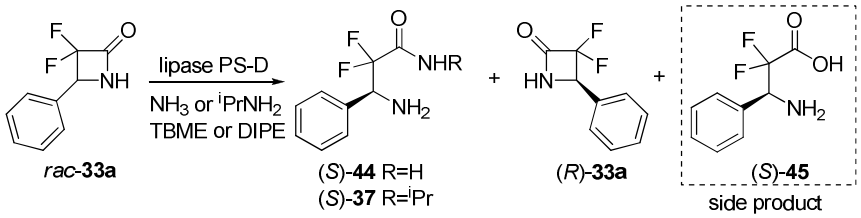
5.1.2 Enantioselective ammonolysis and aminolysis of β-lactams (paper VI)

As water and alcohols can react with β-lactams (section 2.3.2) , amines and ammonia can, naturally, be expected to open β-lactam rings in the presence of a lipase. Prior to this work there were no published examples of lipase-catalyzed aminolysis or ammonolysis of β-lactams, although lipase-catalyzed aminolysis has been used in the resolution of carboxylic acid derivatives[107] and in the desymmetrization of *meso*-dicarboxylic acid derivatives.[108] The success in the alcoholysis of β-lactams (section 5.1.1 & paper I) encouraged us to study the ring opening of β-lactams with ammonia and amines as the nucleophiles (Scheme 23).

Ammonia as the simplest amine was chosen for the initial study, where *rac*-**33a** was incubated with lipase PS-D in ammonia-saturated TBME at room temperature. The highly enantioselective formation of amide (*S*)-**44** and unreacted (*R*)-**33a** was observed at 50% conversion in 7 hours (Table 3, entry 1). As already mentioned (section 2.2.3 and section 5.1.1), water in the seemingly dry enzyme preparation competes with the added nucleophile (NH_3 in this case, corresponding to Nu^2H in Scheme 4), causing undesired hydrolysis as the side reaction. The hydrolysis product (*S*)-**45** was detected by HPLC. Accordingly, the stoichiometric analyses were applied to determine the proportion of the hydrolysis. It was observed that maintaining a proper concentration of ammonia was an important factor in this procedure. With the saturated concentration, the reaction proceeded without hydrolysis (entry 1). When ammonia was continuously bubbled into the reaction mixture, the ammonolysis of *rac*-**33a** became very slow, although no hydrolysis was observed. It has been proposed previously that some lipases do not maintain their full activity at high ammonia concentrations.[109] When a dilute solution of ammonia in TBME was used, hydrolysis as the side reaction became significant.

In the presence of one equivalent of isopropylamine, *rac*-**33a** afforded both unreacted (*R*)-**33a** and (*S*)-**37** with high enantiopurity (ee > 99%) by lipase PS-D in TBME, where hydrolysis caused approximately one fourth of the total conversion (entry 2). The addition of molecular sieves (4Å) as the drying agent suppressed the hydrolysis to some extent, leading to a decrease of the enantiopurity of the product. The proportion of hydrolysis was reduced when isopropylamine was used in excess to the β-lactam *rac*-**33a**. However, the increase of the concentration of isopropylamine (two equivalents to *rac*-**33a**) resulted in a minor decrease in reactivity and the enantiopurity of the product (entry 3). When DIPE (entry 4) was used as the solvent, the reaction proceeded somewhat faster compared to the corresponding reaction in TBME (entry 3). Eventually, the lipase PS-D-catalyzed gram-scale resolution of *rac*-**33a** in the presence of isopropylamine was performed under conditions similar to entry 2, exhibiting the synthetic usefulness of the enantioselective aminolysis (paper VI).

This work clearly shows that ammonia and amines can act as nucleophiles for opening the β-lactam ring with lipase catalysis in a highly enantioselective manner. This generates an alternative method for the preparation of enantiopure β-amino amides. Lipase-catalyzed amide bond formation takes place under mild reaction conditions. Most importantly, lipase catalysis leads to irreversible formation of the amides, because lipases do not usually catalyze the reverse reaction of the normal amide bond formations (section 2.2.3). The above results form the basis for developing a method for β-peptide synthesis by opening β-lactams with β-amino esters as nucleophiles (Chapter 5, section 5.4.3).



Scheme 23. Lipase PS-D-catalyzed ammonolysis and aminolysis of β-lactams *rac*-**33a**.

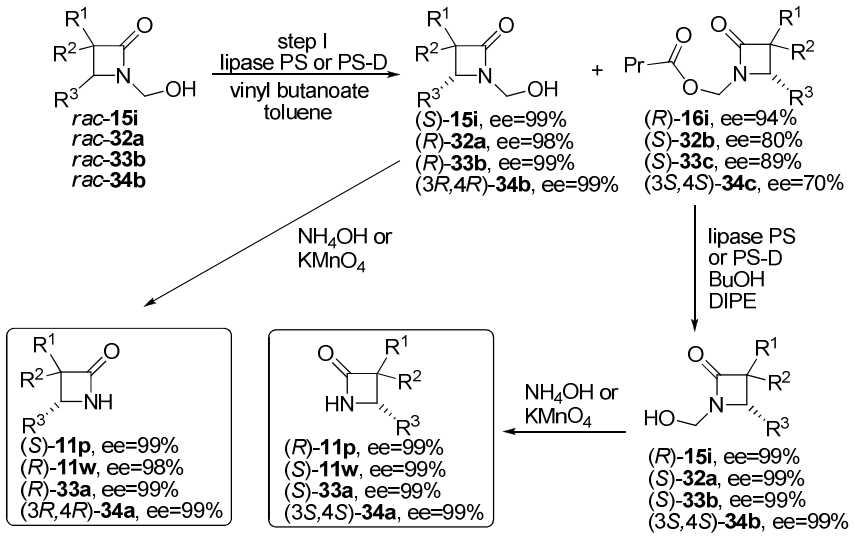
Table 3. Ammonolysis and aminolysis of β-lactams *rac*-**33a** (0.05 M) catalyzed by lipase PS-D (40 mg/ mL) at room temperature.

Entry	Solvent	Nucleophile (M)	Time (h)	Total conversion /(a)[a](%)	Unreacted (*R*)-**33a** [ee(%)]	Amides products [ee(%)]
1	TBME	NH_3[b]	7	50/(0)	>99	(*S*)-**44** (>99)
2	TBME	$^{i}PrNH_2$(0.05)	5	50/(12)	>99	(*S*)-**37** (>99)
3	TBME	$^{i}PrNH_2$(0.10)	5	42/(4)	68	(*S*)-**37** (97)
4	DIPE	$^{i}PrNH_2$(0.10)	2	50/(3)	87	(*S*)-**37** (96)

a. The proportion of hydrolysis (a). b. saturated in TBME.

5.2 Lipase-catalyzed *O*-acylation of *N*-hydroxymethylated β-lactams and *O*-deacylation of the corresponding ester derivatives (papers II & III)

The lipase-catalyzed ring opening of β-lactams is a powerful method to resolve these compounds with nucleophiles (H_2O, ROH and RNH_2). The less reactive isomer stays as a β-lactam and the reactive isomer forms the corresponding β-amino acid, β-amino ester or β-amino amide (sections 2.3.2 and 5.1). When both enantiomers of a β-lactam are needed to be kept intact during the resolution, the lipase-catalyzed *O*-acylation of *N*-hydroxymethylated β-lactams is highly applicable (section 2.3.3 and Scheme 24, step I).[62-70]



Scheme 24. Chemoenzymatic preparation of compounds of high enantiopurity: in **11p**, **15i** and **16i** R^1=R^2=H, R^3=Ph, in **11w**, **32a** and **32b**, R^1=R^2=H, R^3=Bn, in **33a-c** R^1=R^2=F, R^3=Ph, in **34a-c** R^1=F, R^2=H, R^3=Ph,.

Lipase PS and lipase AK are among the lipases which show enantioselectivity toward primary alcohols (section 2.3.3). In many cases, the enantioselectivity is not high enough to get both enantiomers of the racemate with high enantiopurity (ee > 98%) at 50% conversion. The double resolution method was, for this reason, introduced in this work (Scheme 24, Table 4). It can be conveniently carried out by performing the enzymatic acylation followed by enzymatic alcoholysis of the produced ester. This gives the both enantiomers in the forms of *N*-hydroxymethylated β-lactams. For instance, *rac*-**15i** was first acylated by lipase PS-D. When the reaction was allowed to reach 51% conversion, the unreacted (*S*)-**15i** was obtained with 99% ee, while the ester enantiomer showed 94% ee (Scheme 24, step I). Thereafter the PS-D-catalyzed *O*-deacylation of (*R*)-**16i** (ee = 94%) was performed, affording (*R*)-**15i** with high enantiopurity (ee = 99%) at 94% conversion. As the overall outcome, (*S*)-**15i** (ee = 99%) and (*R*)-**15i** (ee = 99%) were produced with 47% and 45% yields, respectively, which were close to the theoretical yields (50%) for each enantiomer (step II). The preparative-scale resolutions of *rac*-**15i**, *rac*-**32a**, *rac*-**33b** and *rac*-**34b** are shown in Table 4. This double resolution approach is particularly suitable for the resolution of alcohols, because the deacylation of the produced ester

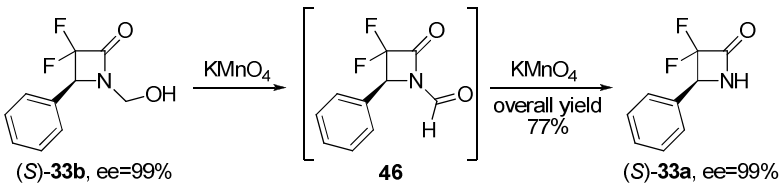
[e.g. (*R*)-**16i**] and the enhancement of the enantiopurity of the ester were accomplished in a single step.

Table 4. Results for the lipase-catalyzed two-step resolution of *N*-hydroxymethylated β-lactams at room temperature.

Entry	Racemate (mmol)	Lipase	Conversion (%)[a] at step I	Conversion (%)[a] at step II	More reactive enantiomer (ee & yield[b])	Less reactive enantiomer (ee & yield[c])
1	*rac*-**15i**	PS-D	51	94	(*R*)-**15i** (99&45)	(*S*)-**15i** (99&47)
2	*rac*-**32a**	Lipase PS	55	88	(*S*)-**32a**(99&45)	(*R*)-**32a**(98&46)
3	*rac*-**33b**	PS-D	53	92	(*S*)-**33b** (99&45)	(*R*)-**33b** (99&42)
4	*rac*-**34b**	PS-D	58	83	(3*S*,4*S*)-**34b** (99&42)	(3*R*,4*R*)-**34b** (99&41)

a. Determined by use of an internal standard; b. isolated yield over the two steps; c. isolated yield.

The next problem to be solved is how the free β-lactam enantiomers can be obtained from their *N*-hydroxymethylated counterparts. NH_4OH in methanol is usually used for this purpose,[68] and the method was shown to be usable for the preparation of the nonfluorinated compounds (*R*)-**11p**, (*S*)-**11p**, (*R*)-**11w**, and (*S*)-**11w** from (*R*)-**15i**, (*S*)-**15i**, (*R*)-**32a**, and (*S*)-**32a** respectively. However, when NH_4OH was applied to cleave the *N*-hydroxymethyl group of (*S*)-**33b**, compound (*S*)-**33a** was not obtained. It is possible that the amide bond in (*S*)-**33b** could not stand the reaction conditions, due to fluorine activation. Based on the knowledge that $KMnO_4$ can oxidize primary alcohols to aldehydes[110] and cleave the *N*-formyl group of a β-lactam,[111] it was assumed that oxidative *N*-deprotection of (*S*)-**33b** with $KMnO_4$ would be possible. In practice, the *N*-hydroxymethyl group of (*S*)-**33b** was successfully removed by using $KMnO_4$ in a mixture of acetone and H_2O at 2 °C, leading to (*S*)-**33a** without racemization (Scheme 25). According to previous work,[110] the formation of the intermediate **46** during the reaction was highly possible. Accordingly, this method had also been applied to the transformations from (*R*)-**33b**, (3*S*, 4*S*)-**34b** and (3*R*, 4*R*)-**34b** into (*R*)-**33a**, (3*S*, 4*S*)-**34a** and (3*R*, 4*R*)-**34a**, respectively, racemization being not observed in any of these cases.



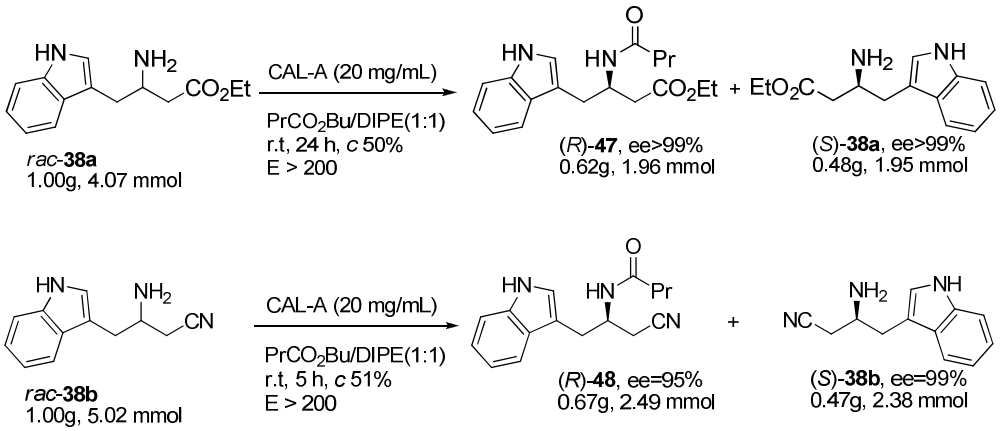
Scheme 25. Application of $KMnO_4$ for the cleavage of *N*-hydroxymethyl group of β-lactams.

5.3 CAL-A-catalyzed *N*-acylation (paper IV)

As described in section 2.3.4.1, CAL-A is an excellent lipase for the kinetic resolution of β-amino esters by *N*-acylation. CAL-A displays high chemoselectivity toward β-amino esters in organic solvents, with only the *N*-acylation of the amino group being observed and the ester functionality remaining intact (Figure 5 and Scheme 14).[69-78]

In this work, the β-amino ester *rac*-**38a** and β-amino nitrile *rac*-**38b** were used for studying the enantio- and chemoselectivity of CAL-A toward heteroaryl-substituted substrates. Accordingly, the kinetic resolution of 3-amino-4-(1*H*-3-indolyl)butanoate *rac*-**38a** was studied in the presence of CAL-A in dry DIPE at room temperature. There are three potential reaction sites in this compound (primary and secondary amino groups and the ester functionality) for lipase catalysis in the presence of an acyl donor. Butyl butanoate was chosen as the acyl donor according to the optimization of the reaction conditions (paper IV). There are two potential reaction sites in the β-aminonitrile *rac*-**38b** (primary and secondary amino groups), and it is the precursor for *rac*-**38a** in the chemoenzymatic preparation route (paper IV). Under reaction conditions similar to those employed for *rac*-**38a**, *rac*-**38b** was resolved with high enantioselectivity (*E*>200). This is the first case which shows that the β-aminonitrile can be resolved by the lipase with high enantioselectivity. Since lipases are generally inert toward the nitrile functionality, many types of lipases are supposed to have high chemoselectivities toward β-aminonitriles in *N*-acylation reactions. Accordingly, it has been shown that lipase PS-C II is able to resolve four alicyclic β-aminonitriles without any recognizable side reaction (section 2.3.4.3, Scheme 17). The CAL-A-catalyzed *N*-acylation of the indole amine group in **38a** or **38b** was not observed. Finally, the gram-scale resolutions of *rac*-**38a** and *rac*-**38b** were performed with high enantioselectivity (*E*>200) and good isolated yields (Scheme 26).

According to the above work, it becomes evident that CAL-A can accept bulky compounds at its nucleophilic site in the enzyme cavity and, furthermore, it displays high chemoselectivity toward β-amino esters in *N*-acylation reactions. This forms the basis for the further studies where CAL-A is used for catalyzing the formation of β-peptide bonds (section 5.4.2).



Scheme 26. CAL-A-catalyzed *N*-acylation reaction.

5.4 Lipase-catalyzed synthesis of β-dipeptides (paper V & VI)

5.4.1 Strategies

It is of interest to develop enzymatic methods for the preparation of β-peptides, as the work in our group has been long focused on the enzymatic preparation of enantiopure β-amino acids and their derivatives. In the present work, the proteolytic enzymes were not used, as they may cause undesired hydrolysis of peptides and they have limited substrate scope mainly for α-amino acids (section 2.4). Thus, lipases were chosen as the biocatalysts for the preparation of β-peptides.

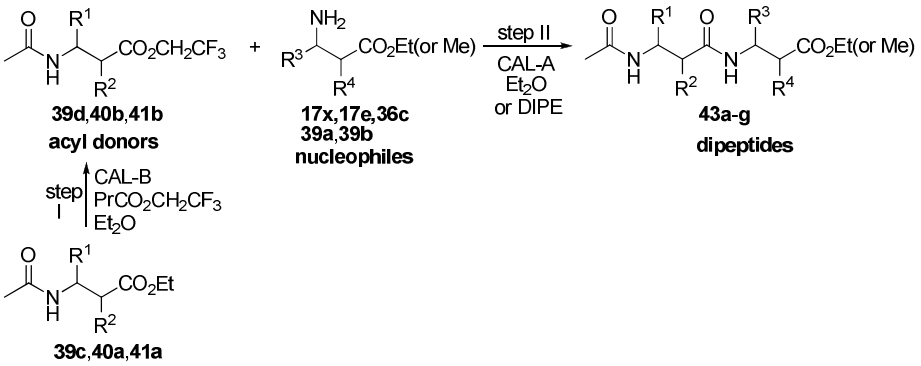
Two strategies were used for preparing β-dipeptides. By use of the first strategy (Scheme 28), 2,2,2-trifluoroethyl esters were prepared by CAL-B-catalyzed interesterification (step I). The subsequent CAL-A-catalyzed aminolysis (step II) resulted in the formation of the β-dipeptides **43a-g**. In the second strategy (Scheme 29), lipase PS-D was used for catalyzing the ring opening of β-lactams with β-amino esters, affording β-dipeptides **43h-l**.

5.4.2 Preparation of β-dipeptides by aminolysis of β-amino esters (paper V)

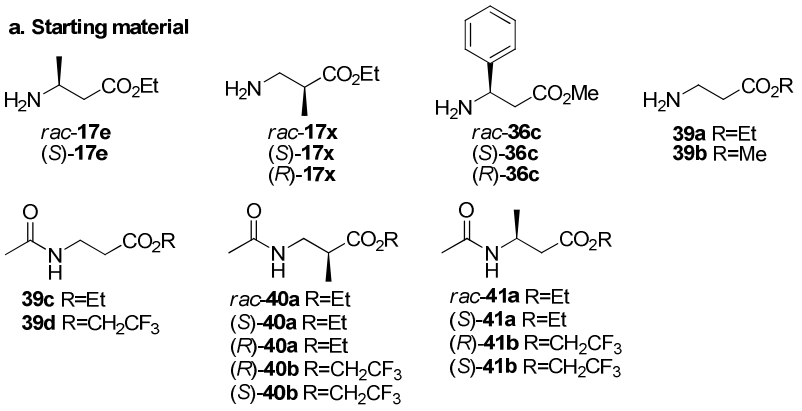
As discussed in section 2.3.4.1 and in section 5.3, CAL-A is an excellent lipase in catalyzing the amide bond formation in organic solvents (Scheme 27). CAL-A has excellent chemoselectivity in the *N*-acylation reactions of β-amino esters.[45] These features make CAL-A a potential biocatalyst for the preparation of β-peptides. In the initial study, structurally simple β-amino esters **39d**, **40b** and **41b** were chosen as the acyl donors (Scheme 28, step II). The idea to use 2,2,2-trifluoroethyl esters **39d**, **40b** and **41b** emerged from the previous results showing that amide bonds could be formed efficiently in the presence of 2,2,2-trifluoroethyl esters (for instance, 2,2,2-trifluoroethyl butanoate).

Scheme 27. CAL-A-catalyzed amide bond formation in an organic solvent.

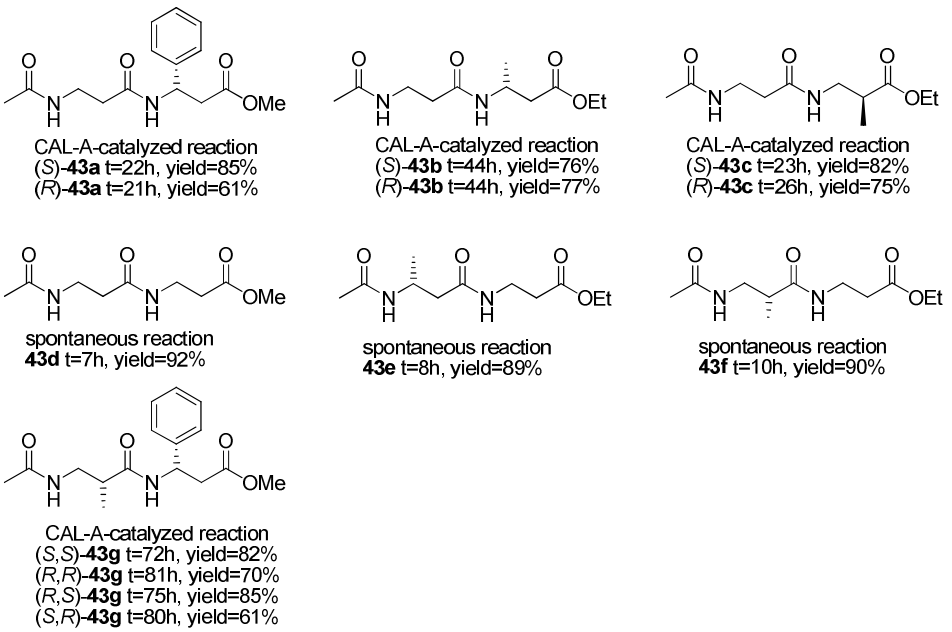
The interesterification of compound **39c** with 2,2,2-trifluoroethyl butanoate in Et_2O in the presence of CAL-B led to the formation of the activated ester **39d** (Scheme 28, step I and Table 5, entry 1). As already mentioned (section 2.2.2, section 2.3.4.2 and section 5.1.1), from the mechanistic point of view interesterification is actually an alcoholysis. In theory, compound **39d** can also be prepared by replacing 2,2,2-trifluoroethyl butanoate with 2,2,2-trifluoroethanol in the reaction. However, only trace amount (2% conversion) of **39d** was observed when **39c** was incubated with 2,2,2-trifluoroethanol in Et_2O (entry 2). This is in accordance with the previous results described in section 2.3.4.2. In another attempt, both 2,2,2-trifluoroethanol and 2,2,2-trifluoroethyl butanoate were added to the reaction mixture (entry 3), the outcome being the same as observed in entry 1. This experiment excluded the possibility that the 2,2,2-trifluoroethanol added separately would have inhibited the lipase.



a. Starting material



b. Prepared β-dipeptides



Scheme 28. **a**. Starting materials for the peptides. **b**. Prepared β-dipeptides **43a-g**. (only one isomer was drawn for the β-peptides.).

When molecular sieves (4 Å, 90 mg / mL) were added as the drying agent to the reaction mixture, **39c** was transformed into **39d** with 17 % conversion (entry 4). This indicated that the presence of molecular sieves could enhance the formation of **39d** to some extent. To exclude the possibility that the formation of **39d** was caused by molecular sieves, another experiment, in which no CAL-B was added, was carried out where upon **39d** was not observed at all (entry 5).

Table 5. Interesterification and alcoholysis of **39c** catalyzed by CAL-B (20 mg/mL) in dry Et_2O for 24 hours.

Entry	Concentration of **39c** (M)	Other reagent (M)	Total conversion(%)	Formation of **39d** (%)	Hydrolysis of **39c** (%)
1	0.1	$PrCO_2CH_2CF_3$(0.2)	60	60	0
2	0.1	CF_3CH_2OH(0.2)	28	2	26
3	0.1	$PrCO_2CH_2CF_3$(0.2) & CF_3CH_2OH(0.2)	60	60	0
4	0.1	CF_3CH_2OH(0.2) & 4Å MS (90 mg/ mL)[a]	36	17	19
5	0.1	CF_3CH_2OH(0.2) & 4Å MS (90 mg/ mL)[a]	0[b]	0	0
6	0.07	$PrCO_2CH_2CF_3$(0.14)	61	61	0
7	0.05	$PrCO_2CH_2CF_3$(0.1)	61	62	0
8	0.03	$PrCO_2CH_2CF_3$(0.06)	64	57	7
9	0.01	$PrCO_2CH_2CF_3$(0.02)	80	8	72
10	0.01	$PrCO_2CH_2CF_3$(0.2)	87	86	0

a. MS is molecular sieves. b. no CAL-B was added.

The water in the enzyme preparation may cause the hydrolysis of the formed 2,2,2-trifluoroethyl ester as an activated β-amino ester as well as that of the 2,2,2-trifluoroethyl butanoate (section 2.2.3). This was clearly shown when a dilute solution of **39c** was used (entries 8 & 9). As a trend, the hydrolysis of **39c** became less significant when more concentrated 2,2,2-trifluoroethyl butanoate was used (entries 1 and 6-10). The above results implied that the presence of an appropriate amount of hydrolysable 2,2,2-trifluoroethyl butanoate might make the hydrolyses of **39c** and **39d** less favored in dry organic solvents. Under conditions similar to those in entry 1, compounds **40a** and **41a** were eventually transformed into compounds **40b** and **41b** (step I), respectively. Next, **40b** and **41b** were subjected to CAL-A-catalyzed peptide formation via step II.

Compound **39d** was incubated with (*S*)-**36c** in the presence of CAL-A in dry Et_2O, leading to the formation of the β-dipeptide (*S*)-**43a**. As an activated ester, compound **39d** may also react spontaneously with (*S*)-**36c**. In a separate experiment where **39d** was mixed with (*S*)-**36c** in the absence of CAL-A, β-dipeptide (*S*)-**43a** was formed with 20% conversion. The proportion of uncatalyzed background reaction (chemical reaction) can, accordingly, be 20% or evidently less in the presence of CAL-A. For the reaction of **39d** with *rac*-**36c** and CAL-A, the formation of dipeptide (*S*)-**43a** with high enantiopurity (ee=92%) indicated that the lipase catalyzed formation of the dipeptide is predominant. The *S*-enantiopreference is the same as in the CAL-A-catalyzed resolution of *rac*-**36c** with 2,2,2-trifluoroethyl butanoate as the acyl donor. The water in CAL-A preparation competed with (*S*)-**36c** for the same acyl donor **39d**, causing hydrolysis as the side reaction (section 2.2.3). The proportion of the undesired hydrolysis of **39d** was around 5% according to the stoichiometry.

By utilization of the method employed for the preparation of **43a**, dipeptides **43b** and **43c** were prepared by the reactions of **39d** with free β-amino esters **17x** and **17e**, respectively. In the cases where free β-amino esters **39a** and **39b** were used as nucleophiles and **39d**, **40b** and **41b** as the acyl donors, the formation of dipeptides **43d-f** took place spontaneously, which made the procedure more economical.

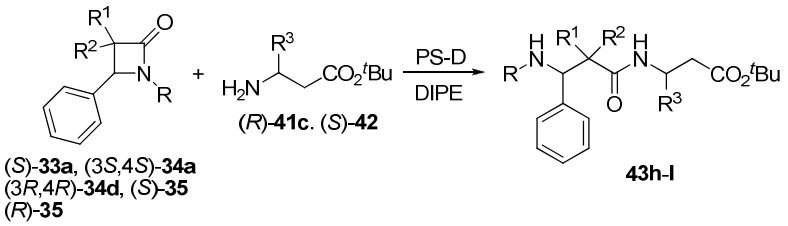
The reaction of **36c** and α-substituted acyl donor **40b** was very sluggish in the presence of CAL-A in Et_2O. The reaction rate was enhanced by replacing Et_2O with DIPE as the solvent, the two pairs of enantiomers of **43g** being prepared in 72-81 hours. As an explanation for the sluggishness of the reaction, CAL-A possesses a narrow acyl donor pocket which does not favor branched substrates. The reaction of β-substituted acyl donor **41b** was also tested, but no peptide formation was observed. It was noticed that the amount of **41b** remained the same for 168 hours, indicating that the hydrolysis as the side reaction did not take place either.

5.4.3 Preparation of β-dipeptides by aminolysis of β-lactams (paper VI)

As described above, the β-lactam ring of *rac*-**33a** can be opened with ammonia and isopropylamine in the presence of lipase PS-D, leading to the formation of the amides (*S*)-**44** and (*S*)-**37** (section 5.1.2), respectively. This led to the idea to study another method for β-peptide synthesis (Scheme 29), hoping to compensate for the limitation (CAL-A does not accept branched acyl donors) of the previous method (Scheme 28). According to the above results in the alcoholysis and aminolysis of β-lactams (sections 5.1.1 & 5.1.2), two points have to be kept in mind: activated β-lactams are needed as acyl donors in order to facilitate the ring opening, and the ester functionalities of the β-amino esters should be inert. Due to the inertness of *tert*-butyl ester functionality toward lipase catalysis, it was chosen as a protecting group.

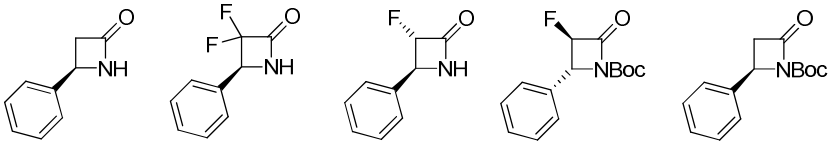
The difluorinated β-lactam (*S*)-**33a** reacted smoothly with (*R*)-**41c** in the presence of PS-D, affording the dipeptide **43h** in 96% yield (Scheme 29). The reaction of monofluorinated β-lactam (3*S*,4*S*)-**34a** with (*R*)-**41c** afforded dipeptide **43i** in 51% yield. When nonfluorinated β-lactam (*R*)-**11p** was incubated with (*R*)-**41c** in the presence of lipase PS-D, no peptide formation was observed. The β-lactam rings of (*S*)-**33a** and (3*S*,4*S*)-**34a** are activated by the fluorine and, thus, the activation is essential for the aminolysis by (*R*)-**41c**.

In order to enhance the ring opening of nonfluorinated β-lactam (*R*)-**11p**, another type of activation has to be planned. The *N*-activation has the benefit of simultaneous protection of the amino group. Thus, attention was paid to common protective groups in peptide synthesis. Accordingly, Boc-protected (*R*)-**35** was prepared from (*R*)-**11p** (Scheme 29). It was observed that the reactions of (*R*)-**35** and (*R*)-**41c** proceeded to some extent spontaneously in the absence of lipase PS-D. The addition of lipase PS-D and/or the increase of the temperature accelerated the reaction rate significantly. Accordingly, β-dipeptide **43j** was prepared from (*R*)-**35** and (*R*)-**41c** in the presence of lipase PS-D at 57 ^{o}C in good yield (82%). (*S*)-**35** was derivatized from the unfavored enantiomer (*S*)-**11p** by lipase PS-D catalysis, and the spontaneous reaction enabled its use in the synthesis of peptide **43k**. *N*-Boc-protected β-lactam (3*R*, 4*R*)-**34d** was activated both by the fluorine in the ring and by the *N*-Boc-protection. As expected, the reaction of (3*R*,4*R*)-**34d** with (*S*)-**42** took place spontaneously, leading to the formation of peptide **43l** in 97% yield.

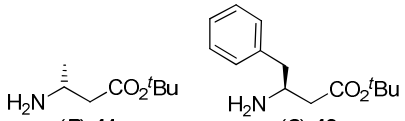


(*S*)-**33a**, (3*S*,4*S*)-**34a**
(3*R*,4*R*)-**34d**, (*S*)-**35**
(*R*)-**35**

a. Building blocks

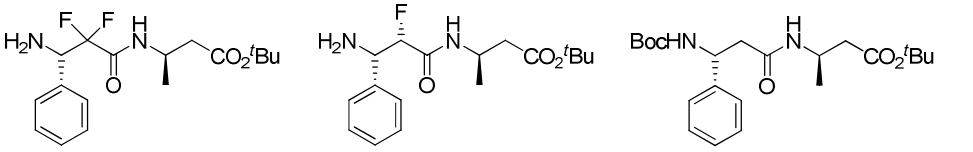


(*R*)-**11p** (*S*)-**33a** (3*S*,4*S*)-**34a** (3*R*,4*R*)-**34d** (*R*)-**35** (*S*)-**35**



(*R*)-**41c** (*S*)-**42**

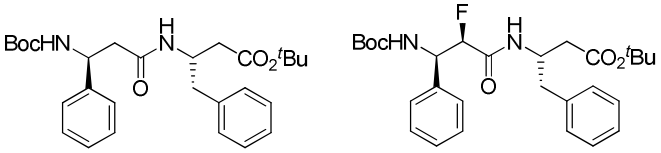
b. Prepared β-dipeptides



PS-D-catalyzed reaction, rt
43h, t=15 h, yield=96%

PS-D-catalyzed reaction, rt
43i, t=18 h, yield=51%

PS-D-catalyzed reaction, 57°C
43j, t=48 h, yield=82%

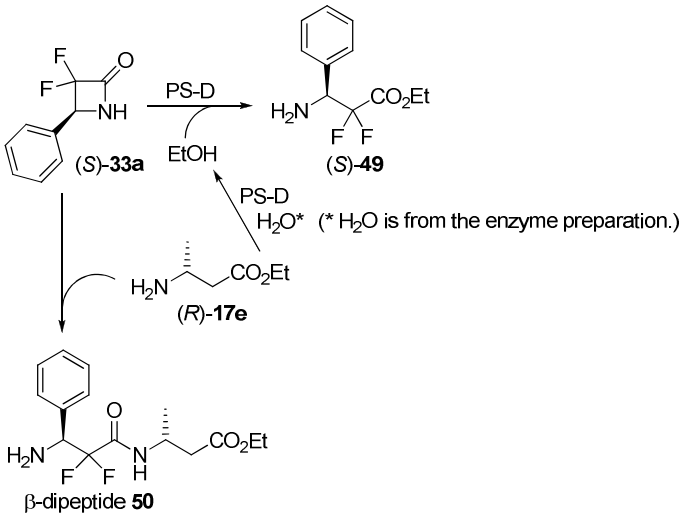


spontaneous reaction, rt
43k, t=144 h, yield=65%

spontaneous reaction, rt
43l, t=24 h, yield=97%

Scheme 29. **a**. Building blocks for peptide synthesis. **b**. prepared β-dipeptides with lipase PS-D catalysis (only one isomer is shown for each compound).

In the case where the ethyl ester (*R*)-**17e** rather than the *tert*-butyl ester (*R*)-**41c** was incubated with (*S*)-**33a** in the presence of lipase PS-D, the *in situ* generated EtOH acted as the competing nucleophile with the substrate (*R*)-**17e**, causing the formation of (*S*)-**49** as the predominant product (Scheme 30). This case is similar to the interesterification-type reaction described in Scheme 22 (section 5.1.1). The nucleophilicity of amines is generally stronger than that of alcohols. In the reaction shown in Scheme 30, the ethanol is generated *in situ* from (*R*)-**17e** by lipase PS-D. The ethanol may remain bound to the active site and be ready for the subseqent alcoholysis reaction of (*S*)-**33a**. This is again in accordance with the hypothesis formulated in section 2.3.4.2.



Scheme 30. A reaction caused by *in situ* generated ethanol from (*R*)-**17e**.

5.5 Future plans

The results described in sections 5.1-5.4 give rise to several possible studies to be continued from this thesis:

1. Lipase PS-catalyzed alcoholysis of β-lactams can be applied to the modification of natural products, and the work heading to modifications of sugars is already on going.

2. Lipase PS-catalyzed aminolysis of β-lactams will be extended to studies for the synthesis of α-/β-mixed peptides.

3. CAL-A is an excellent enzyme for the *N*-acylation of β-amino esters with high enantio- and chemoselectivity. A project has been started with a collaborator in order to obtain more information about the structure-reactivity relationships in CAL-A-catalysis. Possible mutants of CAL-A will be prepared with the purpose of being applied to organic synthesis.

6 SUMMARY

The results of the present work show that the enantiomers of β-amino acid derivatives can be conveniently prepared by strategies based on lipase catalysis.

It has been shown that the lipase from *Burkholderia cepacia* (lipase PS) effectively catalyzes the ring opening of fluorine activated β-lactams *rac*-**33a** and *rac*-**34a** with *S*-preference in the presence of methanol, leading to the resolution products (the formed amino esters and unreacted β-lactams) in high enantiopurity (ee=99%). The presence of fluorine in the ring is essential for the ring opening with lipase catalysis (paper I, Scheme 21). Ammonia and amines can replace methanol as a nucleophile. Thus, a β-lactam can be an acyl donor for an amine in the lipase catalysis (paper VI, Scheme 23).

The two-step kinetic resolution was introduced in order to prepare the both enantiomers of *N*-hydroxymethylated β-lactams with high enantiopurities. Thus, the less reactive isomers (*S*)-**15i**, (*R*)-**32a**, (*R*)-**33b** and (3*R*,4*R*)-**34b** were obtained in the lipase PS-catalyzed *O*-acylation of the corresponding racemic primary alcohols in organic solvents (papers II & III, Scheme 24). In the second step (*O*-deacylation), the enantiomerically enriched ester products (*R*)-**16i**, (*S*)-**32b**, (*S*)-**33c** and (3*S*,4S)-**34c** from the above kinetic resolution are transformed into the alcohol products using lipase PS-catalyzed alcoholysis with *n*-butanol. A benefit in resolving *N*-hydroxymethylated β-lactams relies on the fact that the rings in both enantiomers of a β-lactam remained intact during the reaction.

The studies on the chemoselectivity of *Candida antarctica* lipase A (CAL-A) for the reactions between β-tryptophan ethyl ester *rac*-**38a** and its nitrile precursor *rac*-**38b** with butyl butanoate affirm the idea of CAL-A as a highly enantioselective and chemoselective *N*-acylation catalyst. Only the acylation of the primary amino group could be detected, and resolution products in high enantiopurity (ee = 99%) were produced at 50% conversion (paper IV, Scheme 26).

CAL-A has been shown to catalyze the irreversible *N*-acylation reaction of β-amino esters. This offers a good possibility to prepare β-dipeptides **43a-g** (paper V, Scheme 28). The use of CAL-B-catalyzed interesterification reaction is a straightforward method to prepare activated esters **39d**, **40b** and **41b** which function as acyl donors for the CAL-A-catalyzed peptide formation. The lipase is simply changed without the need to purify the activated ester from the ethyl ester. This work appeared as the first publication on the enzymatic synthesis of β-peptides. As a limitation to this method, the acyl donor parts must be structurally simple compounds, which is in accordance with the previously published structure of CAL-A.[23]

Lipase PS-catalyzed aminolysis of β-lactams with β-amino esters has been developed as another method toward β-dipeptides, peptides **43h-l** being thus obtained in dry DIPE (paper VI, Scheme 29). With this method, structurally bulky acyl donors can be used for peptide synthesis. This method nicely complements the CAL-A-catalyzed peptide formation.

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Turku, Summer 2008

Xiang-Guo Li

REFERENCES

1. Faber, K. *Biotransformations in Organic Chemistry*, 4th Ed.; Springer-Verlag: Berlin, 2000; pp 21-22.
2. Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*, 2nd Ed.; Elsevier Academic Press: New York, 2004.
3. http://www.dsm.com/en_US/downloads/dfc/aspartame.pdf
4. Bommarius, A. S.; Schewarm, M.; Drauz, K. *Chimia* **2001**, *55*, 50.
5. Schoemaker, H. E.; Boesten, W. H. J.; Broxterman, Q. B.; Roos, E. C.; Kaptein, B.; Van Den Tweel, W. J. J.; Kamphuis, J.; Meijer, E. M.; Rutjes, F. P. J. T. *Chimia* **1997**, *51*, 308.
6. http://www.bioverdant.com/sci5.html
7. Kagan, H. B.; Fiaud, J. C. *Topics in stereochemistry* **1988**, *18*, 249-330.
8. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294-7299.
9. Chen, C. S.; Sih, C. J. *Angew. Chem. Int. Ed.* **1989**, *28*, 695-707.
10. Chen, C. S.; Wu, S. H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc*. **1987**, *109*, 2812-2817.
11. Breuer, M.; Ditrich, K.; Habicher, T.; Hauer, B.; Keβeler, M.; Sturmer, R.; Zelinski, T. *Angew. Chem. Int. Ed*. **2004**, *43*, 788-824.
12. Kanerva, L. T.; Sundholm, O. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407-2410.
13. Uyttenbroeck, W.; Hendriks, D.; Vriend, G.; de Baere, L.; Moens, L.; Scharpe, S. *J. Biol. Chem*. **1994**, *374*, 245-254.
14. Kim, K. K.; Hwang, K. Y.; Jeon, H. S.; Kim, S.; Sweet, R. M.; Yang, C. H.; Suh, S. W. J. *Mol. Biol*. **1992**, *227*, 1258–1262.
15. Kim, K. K.; Song, H. K.; Shin, D. H.; Hwang, K. Y.; Suh, S. W. *Structure* **1997**, *5*, 173-185.
16. Schrag, J. D.; Li, Y.; Cygler, M.; Lang, D.; Burgdorf, T.; Hecht, H.-J.; Schmid, R.; Schomburg, D.; Rydel, T. J.; Oliver, J. D.; Strickland, L. C.; Dunaway, M.; Larson, S. B.; Day, J.; McPherson, A. *Structure* **1997**, *5*, 187–202.
17. Lemke, K.; Lemke, M.; Theil, F. *J. Org. Chem*. **1997**, *62*, 6268-6273.
18. Lang, D. A.; Mannesse, M. L. M.; De Haas, G. H.; Verheij, H. M.; Dijkstra, B. W. *Eur. J. Biochem*. **1998**, *254*, 333-340.
19. Tafi, A.; van Almsick, A.; Corelli, F.; Crusco, M.; Laumen, K. E.; Schneider, M.; Botta, M. *J. Org. Chem*. **2000**, *65*, 3659-3665.
20. Schulz, T.; Pleiss, J.; Schmid, R. D. *Protein Sci*. **2000**, *9*, 1053-1062.
21. Luić, M.; Tomić, S.; Leščić, I.; Ljubović, E.; Šepac, D.; Šunjić, V.; Vitale, L.; Saenger, W.; Kojić-Prodić, B. *Eur. J. Biochem*. **2001**, *268*, 3964-3973.
22. Henke, E.; Pleiss, J.; Bornscheuer, U. T. *Angew. Chem., Int. Ed*. **2002**, *41*, 3211–3213.
23. Ericsson, D. J.; Kasrayan, A., Johansson, P.; Bergfors, T.; Sandström, A. G.; Bäckvall, J.-E.; Mowbray, S. L. *J. Mol. Biol*. **2008**, *376*, 109-119.
24. Svendsen, A.; Pathar, S. A.; Egel-Mitani, M.; Borch, K.; Clausen, I. G.; Hansen, M. T.,WO 94/01541.

25. Pfeffer, J.; Richter, S.; Nieveler, J.; Hansen, C.-E.; Bel Rhlid, R.; Schmid, R. D.; Rusnak, M. *Appl. Microbiol. Biotechnol*. **2006**, *72*, 931-938.
26. Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jones, T. A. *Structure* **1994**, *2*, 293–308.
27. Uppenberg, J.; Öhrner, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T. A. *Biochemistry* **1995**, *34*, 16838–16851.
28. Martinelle, M.; Holmquist, M.; Hult, K. *Biochim. Biophys. Acta* **1995**, *1258*, 272-276.
29. Kraut, J. *Ann. Rev. Biochem*. **1977**, *46*, 331-358.
30. Ema, T. *Curr. Org. Chem*. **2004**, *8*, 1009-1025.
31. Halling, P. Enzymic Conversions in Organic and Other Low-Water Media. In *Enzyme Catalysis in Organic Synthesis Vol. 1*; Drauz, K.; Waldmann, H., Ed.; Wiley-VCH: Weinheim 2002; pp 259-285.
32. Gotor, V.; Brieva, R.; Gonzalez, C.; Rebolledo, F. *Tetrahedron* **1991**, *47*, 9207-9214.
33. Smidt, H.; Fischer, A.; Fischer, P.; Schmidt, R. D.; Stelzer, U. EP 812363, 1995; *Chem. Abstr*. **1996**, *125*, 219744.
34. Sergeeva, M. V.; Mozhaev, V. V.; Rich, J. O.; Khmelnitsky, Y. L. *Biotechnol. Lett*. **2000**, *22*, 1419-1422.
35. Tuccio, B.; Ferré, E. ; Comeau, L. *Tetrahedron Lett*. **1991**, *32*, 2763-2764.
36. Maugard, T.; Remaud-Simon, M.; Petre, D.; Monsan, P. *Biotechnol. Lett*. **1997**, *19*, 751-753.
37. Litjens, M. J. J.; Straathof, A. J. J.; Jongejan, J. A.; Heijnen, J. J. *Chem. Commun*. **1999**, 1255-1256.
38. Litjens, M. J. J.; Sha, M.; Straathof, A. J. J.; Jongejan, J. A.; Heijnen, J. J. *Biotechnol. Bioeng*. **1999**, *65*, 347-356.
39. Maugard, T.; Remaud-Simon, M.; Petre, D.; Monsan, P. *Tetrahedron* **1997**, *53*, 5185-5194.
40. Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodivers*. **2004**, *1*, 1111-1239.
41. In *Enantioselective Synthesis of β-Amino Acids,* 2nd ed.; Juaristi, E.; Soloshonok, V., Eds.; Wiley-VHC: New York, 2005.
42. Kingston, D. G. I.; Jagtap, P. G.; Yuan, H.; Samala, L. *Prog. Chem. Org. Nat. Prod*. **2002**, *84*, 53-225.
43. Gerog, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol*®: *Science and Applications* Suffness, M. Ed.; CRC, New York, 1995, pp. 317-375.
44. Kuznetsova, L. V.; Ungureanu, I. M.; Pepe, A.; Zanardi, I.; Wu, X.; Ojima, I. *J. Fluor. Chem*. **2004**, *125*, 487-500.
45. Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831-5854.
46. Alcaide, B.; Almendros, P.; Aragoncillo, C. *Chem. Rev*. **2007**, *107*, 4437-4492.
47. Strynadka, N. C. J.; Adachi, H.; Jensen, S. E.; Johns, K.; Sielecki, A.; Betzel, C.; Sutoh, K.; James, M. N. G. *Nature* **1992**, *359*, 700–705.
48. Maveyraud, L.; Golemi, D.; Kotra, L. P.; Tranier, S.; Vakulenko, S.; Mobashery, S.; Samama, J.-P. *Structure* **2000**, *8*, 1289–1298.
49. Hall, B. G.; Salipante, S. J.; Barlow, M. *J. Mol. Evol*. **2003**, *57*, 249–254.
50. Jones, M.; Page, M. I. *J. Chem. Soc., Chem. Commun*. **1991**, 316–317.

51. Adam, W.; Groer, P.; Humpf, H.-U.; Saha-Möller, C. R. *J. Org. Chem*. **2000**, *65*, 4919–4922.
52. Park, S.; Forró, E.; Grewal, H.; Fülöp, F.; Kazlauskas, R. J. *Adv. Synth. Catal*. **2003**, *305*, 986–995.
53. Forró, E.; Fülöp, F. *Org. Lett*. **2003**, *5*, 1209–1212.
54. Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2004**, *15*, 573–575.
55. Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2004**, *15*, 2875–2880.
56. Forró, E.; Fülöp, F. *Mini-Rev. Org. Chem*. **2004**, *1*, 93-102.
57. Forró, E.; Paál, T.; Tasnádi, G.; Fülöp, F. *Adv. Synth. Catal*. **2006**, *348*, 917-923.
58. Forró, E.; Fülöp, F. *Chem. Eur. J*. **2006**, *12*, 2587–2592.
59. Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2006**, *17*, 3193-3196.
60. Tasnádi, G.; Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2007**, *18*, 2841–2844.
61. Brieva, R.; Crich, J. Z.; Sih, C. J. *J. Org. Chem*. **1993**, *58*, 1068–1075.
62. Csomós, P.; Kanerva, L. T.; Bernáth, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1789–1796.
63. Nagai, H.; Shiozawa, T.; Achiwa, K.; Terao, Y. *Chem. Pharm. Bull*. **1992**, *40*, 2227–2229.
64. Nagai, H.; Shiozawa, T.; Achiwa, K.; Terao, Y. *Chem. Pharm. Bull*. **1993**, *41*, 1933–1938.
65. Kámán, J.; Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2000**, *11*, 1593–1600.
66. Fülöp, F.; Palkó , M.; Kámán, J.; Lázár, L.; Sillanpää, R. *Tetrahedron: Asymmetry* **2000**, *11*, 4179–4187.
67. Forró , E.; Árva, J.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 643–649.
68. Forró , E.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 2351–2358.
69. Gyarmati, Z. Cs.; Liljeblad, A.; Rintola, M.; Bernáth, G.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2003**, *14*, 3805–3814.
70. Gyarmati,Z.Cs.; Liljeblad, A.; Argay, G.; Kálmán, A.; Bernáth, G.; Kanerva, L. T. *Adv. Synth. Catal*. **2004**, *346*, 566–572.
71. Liljeblad, A.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 4405–4415.
72. Gedey, S.; Liljeblad, A.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2001**, *12*, 105–110.
73. Solymár, M.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2002**, *13*, 2383–2388.
74. Solymár, M.; Kanerva, L. T.; Fülöp, F. *Tetrahedron: Asymmetry* **2004**, *15*, 1893–1897.
75. Kanerva, L. T.; Csomós, P.; Sundholm, O.; Bernáth, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1705–1716.
76. Solymár, M.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2002**, *13*, 1923–1928.
77. Liljeblad, A.; Lindborg, J.; Kanerva, A.; Katajisto, J.; Kanerva, L. T. *Tetrahedron Lett*. **2002**, *43*, 2471-2474.
78. Liljeblad, A.; Kiviniemi, A.; Kanerva, L. T. *Tetrahedron* **2004**, *60*, 671-677.
79. Sánchez, V. M.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **1997**, *8*, 37–40.

80. Stürmer, R.; Ditrich, K.; Siegel, W. U.S. Patent 6063615.

81. Cammenberg, M.; Hult, K.; Park, S. *ChemBioChem* **2006**, *7*, 1745-1749.

82. Gedey, S.; Liljeblad, A.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 2573–2581.

83. Gedey, S.; Lilkeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Can. J. Chem*. **2002**, *80*, 565-570.

84. Fitz, M.; Lundell, K.; Lindroos, M.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2005**, *16,* 3690-3697.

85. Sakai, T.; Hayashi, K.; Yano, F.; Takami, M.; Ino, M.; Korenago, T.; Ema, T. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 1441-1446.

86. Guzmán, F.; Barberis, S.; Illanes, A. *Electr. J. Biotech*. **2007**, *10*, 279-314.

87. Kumar, D.; Bhalla, T. C. *Appl. Microbiol. Biotechnol*. **2005**, *68*, 726-736.

88. Kimmerlin, T.; Seebach, D. *J. Peptide Res*. **2005**, *65*, 229-260.

89. Heck, T.; Kohler, H.-P. E.; Limbach, M.; Flögel, O.; Seebach, D.; Geueke, B. *Chem. Biodivers*. **2007**, *4*, 2016-2030.

90. Jakubke, H.-D. Hydrolysis and Formation of Peptides. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 2, pp 800–865.

91. West, J. B.; Wong, C.-H. *Tetrahedron Lett.* **1987**, *28*, 1629.

92. Kawashiro, K.; Kaiso, K.; Minato, D.; Sugiyama, S.; Hayashi, H. *Tetrahedron* **1993**, *49*, 4541.

93. Margolin, A. L.; Klibanov, A. M. *J. Am. Chem. Soc.* **1987**, *109*, 3802.

94. So, J.-E.; Kang, S.-H.; Kim, B.-G. *Enzyme Microb. Technol.* **1998**, *23*, 211.

95. Zhang, L.-Q.; Zhang, Y.-D.; Xu, L.; Li, X.-L.; Yang, X.-C.; Xu, G.-L.; Wu, X.-X.; Gao, H.-Y.; Du, W.-B.; Zhang, X.-T.; Zhang, X.-Z. *Enzyme Microb. Technol.* **2001**, *29*, 129.

96. Maruyama, T.; Nakajima, M.; Kondo, H.; Kawasaki, K.; Seki, M.; Goto, M. *Enzyme Microb. Technol.* **2003**, *32*, 655.

97. Heck, T.; Limbach, M.; Geueke, B.; Zacharlas, M.; Gardiner, J.; Kohler, H.-P. E.; Seebach, D. *Chem. Biodivers*. **2006**, *3*, 1325-1348.

98. Natarajan, R.; Azerad, R.; Badet, B.; Copin, E. *J. Fluorine Chem*. **2005**, *126*, 425-436.

99. Thayer, A. M. *Chem. Eng. News* **2006**, *84* (23), 15-24.

100. Nyffeler, P. T.; Durón, S. G.; Burkart, M. D.; Vincent, S. P.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2005**, *44*, 192-212.

101. Dong, C. J.; Huang, F. L.; Deng, H.; Schaffrath, C.; Spencer, J. B.; O'Hagan, D.; Naismith, J. H. *Nature* **2004**, *427*, 561-565.

102. ÓHagan, D.; Schaffrath, C.; Cobb, S. L.; Hamilton, J. T. G.; Murphy, C. D. *Nature* **2002**, *416*, 279.

103. Jäckel, C.; Koksch, B. *Eur. J. Org. Chem*. **2005**, 4483-4503.

104. Mikhailiuk, P. K.; Afonin, S.; Chernega, A. N.; Rusanov, E. B.; Platonov, M. O.; Dubinina, G. G.; Berditsch, M.; Ulrich, A. S.; Komarov, I. V. *Angew. Chem. Int. Ed*. **2006**, *45*, 5659-5661.

105. Son, S.; Tanrikulu, I. C.; Tirrell, D. A. *ChemBioChem* **2006**, *7*, 1251-1257.

106. Uoto, K.; Ohsuki, S.; Takenoshita, H.; Ishiyama, T.; Iimura, S.; Hirota, Y.; Mitsui, I.; Hirofumi, T.; Soga, T. *Chem. Pharm. Bull*. **1997**, *45*, 1793-1804.

107. van Rantwijk, F.; Hacking, M. A. P. J.; Sheldon, R. A. *Monatsh. Chem.* **2000**, *131*, 549-569.

108. Garcia-Urdiales, E.; Alfonso, I.; Gotor, V. *Chem. Rev*. **2005**, *105*, 313-345.

109. De Zoete, M. C.; Kock-van Dalen, A. C.; van Rantwijk, F.; Sheldon, R. A. *Biocata. Biotrans*. **1994**, *10*, 307-316.

110. Lou, J.-D.; Zhu, L.-Y.; Wang, L.-Z. *Monatsh. Chem*. **2004**, *135*, 31-34.

111. Georg, G. I.; Kant, J.; He, P.; Ly, A. M.; Lampe, L. *Tetrahedron Lett.* **1988**, *29*, 2409-2412.