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STUDIES ON LIPASE SELECTIVITY FOR PREPARATION OF CYANOHYDRINS, SUGAR CONJUGATES AND SECONDARY ALCOHOLS

Riku Sundell

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ABSTRACT

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Studies in selectivity of lipases in preparation of cyanohydrins, sugar conjugates and secondary alcohols

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Biocatalysis can be applied in organic synthetic chemistry to counter challenges posed by increased demands towards chemo-, regio- and stereoselectivity, not forgetting the need for greener chemistry. During the last 30 years, biocatalysis with the use of enzymes as chiral catalysts has become more common in chemistry laboratories and industrial processes. In this thesis, the use of lipases as versatile biocatalysts in the acylation of alcohols is examined both in the light of literature examples and four original publications.

In the first part of the work presented in this thesis lipases were utilized in two examples concerning secondary alcohols. First, the kinetic resolution of heterocyclic aromatic secondary alcohols through transesterification was thoroughly examined including the studies of competing hydrolysis and esterification reactions. In another example, lipases were utilized in the formation of a dynamic systemic resolution (DSR) process which in turn was used as a developmental tool in the optimization of the dynamic kinetic resolution (DKR) of five heterocyclic aromatic cyanohydrins in one pot for the preparation of cyanohydrin esters as single enantiomers.

In the second part of the work, the regio- and stereoselectivity of lipases was used to form sugar conjugates of glyceric and β-amino acids. The primary hydroxyl groups of methyl α-D-galacto-, -gluco- and -mannopyranosides were now acylated trough lipase-catalyzed transesterification and enantioselective lipase-catalyzed ring-opening of β-lactams, respectively.

Keywords: biocatalysis, lipase, kinetic resolution, dynamic kinetic resolution, dynamic systemic resolution, sugar conjugate, β-lactam.

TIIVISTELMÄ

Riku Sundell

Tutkimuksia lipaasien selektiivisyyteen syanohydriinien, sokerikonjugaattien ja sekundääristen alkoholien valmistamisessa

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

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Biokatalyysiä voidaan soveltaa orgaanisessa synteettisessä kemiassa vastaamaan niihin haasteisiin, joita kasvaneet kemo-, regio- ja stereoselektiivisyyden sekä vihreän kemian tarpeet ovat aiheuttaneet. Viimeisten 30 vuoden aikana entsyymien käyttö kiraalisina katalyytteinä on yleistynyt kemian laboratorioissa ja teollisissa prosesseissa. Tutkielmassa tarkastellaan lipaasien hyödyntämistä monikäyttöisinä biokatalyytteinä alkoholien asylaatiossa sekä kirjallisuusesimerkkien että neljän alkuperäisjulkaisun kautta.

Tutkielmassa esitettävän työn ensimmäisessä osiossa lipaaseja hyödynnettiin kahdessa sekundäärisiä alkoholeja koskevassa tapauksessa. Ensimmäisessä esimerkissä tarkasteltiin heterosyklisten aromaattisten alkoholien kineettistä resoluutiota, ja halutun transesterifikaatioreaktion rinnalla mahdollisesti kilpailevia hydrolyysi- ja esterifikaatioreaktioita. Toisessa esimerkissä lipaaseja hyödynnettiin muodostettaessa dynaaminen systeeminen resoluutioprosessi (DSR), jota puolestaan hyödynnettiin viiden heterosyklisen aromaattisen syanohydriinin dynaamisen kineettisen resoluutiomenetelmän (DKR) kehittämisessä ja optimoinnissa samanaikaisesti syanohydriiniestereiden enantiomeerien valmistamiseksi.

Työn toisessa osiossa lipaasien regio- ja stereoselektiivisyyttä hyödynnettiin muodostettaessa glyserolihapon ja β-aminohappojen sokerikonjugaatteja. Metyyli α-D-galakto-, -gluko- ja -mannopyranosidien primäärisiä hydroksyyliryhmiä voitiin asyloida sekä lipaasin katalysoiman transesterifikaation että enantioselektiivisen β-laktaamien renkaanaukeamisen kautta.

Avainsanat: biokatalyysi, lipaasi, kineettinen resoluutio, dynaaminen kineettinen resoluutio, dynaaminen systeeminen resoluutio, sokerikonjugaatti, β-laktaami.

CONTENTS

ABBREVIATIONS

Ac	acetyl
ATP	adenosine triphosphate
BCL	*Burkholderia cepacia* lipase
Boc	*tert*-butoxycarbonyl
BTAH	benzyltrimethylammonium hydroxide
CAL-A	*Candida antarctica* lipase A
CAL-B	*Candida antarctica* lipase B
CAD	Charged aerosol detection
CAN	ceric ammonium nitrate
COSY	correlation spectroscopy
DCC	dynamic combinatorial chemistry
DCL	dynamic combinatorial library
DCM	dichloromethane
de	diastereomeric excess
DIPE	diisopropyl ether
DKR	dynamic kinetic resolution
DMAP	4-methylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMP	2,2-dimethoxypropane
DSR	dynamic systemic resolution
E	enantioselectivity
ee	enantiomeric excess
EI	electron ionization
ESI	electrospray ionization
GC	gas chromatography
HMBC	heteronuclear multiple-bond correlation spectroscopy
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectroscopy
HSQC	heteronuclear single-quantum correlation spectroscopy
NMR	nuclear magnetic resonance
PFA	paraformaldehyde
PFL	*Pseudomonas fluorescens* lipase
PPL	porcine pancreatic lipase
RML	*Rhizomucor miehei* lipase
RP-HPLC	Reverse phase HPLC
RT	Room temperature
t-AmOH	*tert*-amyl alcohol
TBME	*tert*-butyl methyl ether

TEA	triethylamine
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
TFEB	trifluoroethyl butanoate
THF	tetrahydrofuran
TLL	*Thermomyces lanuginosus* lipase
TMSCN	trimethylsilyl cyanide
TOF	time-of-flight
TsOH	*p*-toluenesulfonic acid

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I. Hara, P.; Turcu, M.; Sundell, R.; Toşa, M.; Paizs, C.; Irimie, F.-D.; Kanerva, L. T. Lipase-catalyzed Asymmetric Acylation in the Chemoenzymatic Synthesis of Furan-Based Alcohols. *Tetrahedron: Asymmetry* **2013**, *24*, 142–150.

II. Sundell, R.; Turcu, M.; Kanerva, L. T. Lipase-Catalyzed Dynamic Combinatorial Resolution and the Synthesis of Heteroaromatic Cyanohydrin Ester Enantiomers. *Curr. Org. Chem.* **2013**, *17*, 672–681.

III. Sundell, R.; Kanerva, L. T. Lipases in the Regioselective Preparation of Glyceric Acid Esters of Methyl Glycosides. *Eur. J. Org. Chem.* **2013**, 4971–4978.

IV. Sundell, R.; Siirola, E.; Kanerva, L. T. Regio- and Stereoselective Lipase-Catalysed Acylation of Methyl α-D-Glycopyranosides with Fluorinated β-Lactams. *Eur. J. Org. Chem.* **2014**, *accepted*.

DEFINITIONS

Biocatalysis	Chemical conversion of a substrate into a desired product with the aid of a free or immobilized enzyme or enzymes (or antibodies or ribozymes) inside whole cells.[1]
Chirality	The geometric property of a rigid object (or spatial arrangement of points or atoms) of being non-superimposable on its mirror image; such an object has no symmetry elements of the second kind (a mirror plane, center of inversion, a rotation-reflection axis). An object, superimposable with its mirror, is described as being *achiral*.[2]
Conformation	The spatial arrangement of the atoms affording distinction between stereoisomers which can be interconverted by rotations about formally single bonds.[2]
Diastereomer	Diastereomers are stereoisomers not related as mirror images, characterized by differences in physical properties and by some differences in chemical behavior towards chiral and achiral reagents.[2]
Enantiomer	One of a pair of molecular entities which are mirror images of each other and non-superimposable.[2]
Enantioselectivity	Preferential formation in a chemical reaction of one enantiomer over another. [2]
Racemate	An equimolar mixture of a pair of enantiomers.[2]
Stereochemistry	The area of chemistry that deals with spatial arrangements of atoms in molecules and the effects of these arrangements on the chemical and physical properties of substances.

1. INTRODUCTION

Biocatalysis, the use of enzymes (as isolated or whole-cell preparations), catalytic antibodies and ribozymes, has proven to be a valuable tool for organic synthesis.[1] Enzymes are catalytic proteins produced by organisms for the chemical transformations of life to occur, usually on the basis of one enzyme per reaction. This transpired 120 years ago when Emil Fischer postulated his "lock-and-key" analogue describing the interactions of enzymes towards their substrates.[3] Since then, we have learnt a great deal more and today synthetic methods have been developed that utilize the wide array of possibilities presented by these catalysts under conditions far from the natural cellular environment of their origin. More recently, the modification of proteins on the scale of single amino acid residues has brought us the possibility of building new enzyme catalysts to counter the challenges posed by *e.g.* the growing demand of sustainability and the need to construct ever more elaborate compounds for a variety of uses.[4,5]

Chemical and pharmaceutical industries have taken up using enzymes in the preparation of fine chemicals and pharmaceuticals with many examples where the process productivity has been improved, resource economics have been enhanced and above all, substantial improvements towards greener, more environmentally sustainable synthesis have been taken.[5–7] For instance, in the field of asymmetric synthesis the use of transition-metal catalysts in such classic reactions as Sharpless epoxidation,[8] Sharpless asymmetric dihydroxylation[9] and Noyori asymmetric hydrogenation[10] have given a multitude of possibilities for the synthesis of countless compounds. Advances in biocatalysis are now giving alternative and complementary routes for organic synthesis. For instance, the production of antidiabetic sitagliptin (**1**) was enormously enhanced through the development of an engineered *R*-selective transaminase enabling higher productivity and enantiomeric purity.[11,12] Another example of the use of biocatalysis has been the various approaches developed for the synthesis of the side-chain in the cholesterol-lowering drugs, such as atorvastatin (**2**).[13–16] In a third example, the synthesis of a key intermediate of montelukast (**3**, a leukotriene receptor antagonist for treating asthma and allergies) was greatly improved by the development of a ketoreductase-dependent synthetic pathway.[17] Finally, the development of an amine oxidase-catalyzed desymmetrization *via* a Strecker-type reaction was presented and

used in the synthesis of a bicyclic [3.1.0]proline moiety, an important intermediate in the synthesis of boceprevir (**4**, a drug used for treating hepatitis C).[18] By 2002, biotransformations being carried out on an industrial scale numbered 134.[19] While the impact of biocatalysis on the production of fine chemicals and pharmaceuticals has been significant during 2001–10, it is expected to be even more substantial towards 2020.[20,21]

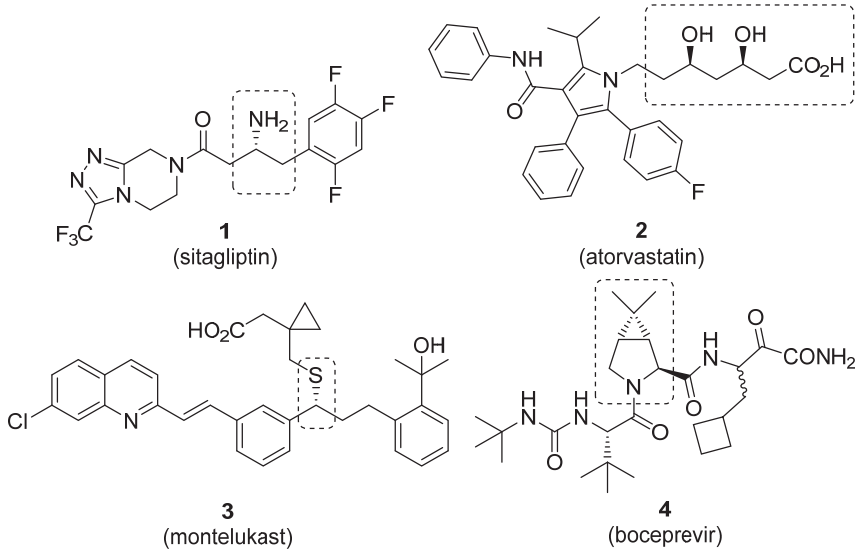


Figure 1. Structures of sitagliptin (**1**), atorvastatin (**2**), montelukast (**3**) and boceprevir (**4**); structural subunits prepared by biocatalysis are highlighted.

The examples presented above constitute only a small fraction of results obtained during the decades of work directed to biocatalysis. This holds true also for this thesis, focusing on the use of lipases as catalysts in organic synthesis. The utilization of these enzymes as practical catalysts is probed in four cases (Papers I–IV) where the advantages and disadvantages encountered are addressed. Ultimately, significantly differing alcohol substrates (heterocyclic secondary alcohols and cyanohydrins together with simple glycopyranosides) were transformed into ester products by using lipases as selective biocatalysts.

2. LITERATURE REVIEW

2.1. Enzymes in organic synthesis

2.1.1. General

For the chemical and pharmaceutical industries, different forms of catalysis play important roles for the synthesis of the numerous compounds they produce.[7] In addition to the vital roles they play for life, enzymes have also been exploited in organic synthesis as practical alternatives in chemo-, regio- and stereoselective reactions.[5,7,15,19,20] In their natural cellular environment, enzymes typically act in aqueous conditions, at neutral pH and at temperatures ranging from 20–40°C, often with minute amounts of substrate. They work with high catalytic efficiency (k_{cat}/K_m, ~10^7 s^{-1} M^{-1}) and offer an increase in reaction rates that spans many orders of magnitude when compared to non-catalyzed chemical reactions (k_{cat}/k_{non}, 10^6–10^{26}).[22,23] A generalized presentation of enzyme catalysis is shown in Figure 2 below. Before the enzyme can perform the chemical transformation from substrate (S) to product (P), the formation of a substrate-enzyme complex (S•Enz) must occur. The effectiveness of this formation is governed by the interactions between the enzyme and the substrate. The Michaelis–Menten constant $[K_m = (k_{-1} + k_{cat})/k_1]$ inversely measures the magnitude of the affinity between the substrate and the enzyme.[24,25]

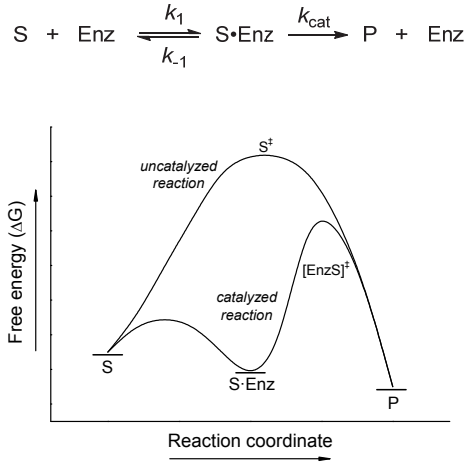


Figure 2. A generalized view of enzyme catalysis: S, Enz and P denote substrate, enzyme and product, respectively. The rate of complex formation (k_1) and dissociation (k_{-1}) together with rate of catalysis (k_{cat}) are presented.

Previously, many of the commonly utilized enzymes have been isolated from natural sources or used as whole-cell preparations. However, during the last few decades significant progress in research areas, such as molecular biology, bioinformatics and protein engineering have prompted the possibility of designing novel fit-for-purpose enzymes for organic synthesis.[4,5,26] For instance, progress in the development of protein engineering allows the changing and removing specific groups on enzymes enabling the optimization of catalysts one amino acid residue at a time, increasing expectations for better enzyme thermostability, changes in selectivity and overall activity.[27,28]

Enzymes are categorized by the Enzyme Commission number (EC A.B.C.D) based on the chemical transformations they catalyze (Table 1).[29] Number A refers to the reaction type catalyzed by the enzyme, B denotes the reaction subtype, while C indicates the nature of the co-substrate and D is the individual number for the enzyme. Many open sources offer information on enzymes. For instance, information on proteins, their sequences and structures can be found from such free sources as the Protein Data Bank (PDB)[30], UniProt[31] and BRENDA (BRaunschweig Enzyme Database)[32].

Table 1. The EC numbers (1–6).[29]

Entry	Group	Reaction catalyzed by the enzyme
1	EC 1 Oxidoreductases	Transfer of H and O atoms or electrons from one substrate to another in oxidation/reduction reactions.
2	EC 2 Transferases	Transfer of a functional group from one substrate to another (*e.g.* methyl, acyl, amino or phosphate group).
3	EC 3 Hydrolases	Hydrolysis of a given bond in the formation of two products from the substrate.
4	EC 4 Lyases	Non-hydrolytic addition or removal of groups from substrates.
5	EC 5 Isomerases	Intramolecular rearrangement.
6	EC 6 Ligases	Formation of new bonds in reactions that consume ATP.

From the six classes of enzymes in Table 1, hydrolases and among them lipases are industrially one of the most widely utilized enzymes.[1,19]

2.1.2. Lipases

Lipases (EC 3.1.1.3; EC 3.x.x.x hydrolase, EC 3.1.x.x acting on ester bonds, EC 3.1.1.x carboxylic ester hydrolase, EC 3.1.1.3 triacylglycerol lipase) represent a robust class of enzymes frequently utilized in organic synthesis. In nature, these extracellular enzymes are responsible for the hydrolysis of long-chain triacylglycerols to glycerol and free fatty acid moieties at water/oil interfaces and are important for the catabolic activity of the organisms of their origin (Figure 3). Despite the diversity in origin, lipases display a rather conserved α/β hydrolase fold, consisting of a central β sheet of six to eight mostly parallel strands (with several flanking α-helices).[33] In the active site of lipases, the catalytic triad can be found on top of the β-sheet consisting of a nucleophilic residue (Ser), a catalytic acid residue (Asp) and a basic residue (His).[34]

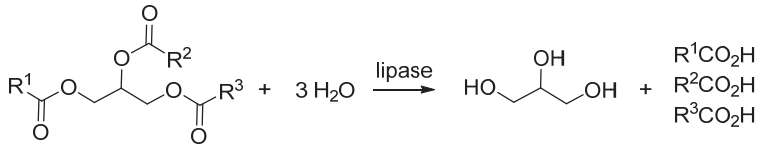


Figure 3. The hydrolysis of triacylglycerols by lipases.

The ping-pong bi-bi mechanism [two substrates are transformed into two products (bi-bi) with the first product leaving the active site before the second substrate enters (ping-pong)] of serine hydrolases such as lipases (and proteases) has been established (Figure 4a) with the catalytic cycle being presented in more detail in Figure 4b.[35,36] In this simplified view of the catalytic cycle for a transesterification reaction the reacting species are first brought together in a geometry (proximity and orientation) that favors the formation of the S·Enz complex. This may require the active-site of the lipase to adjust to allow binding of the specific substrate which can also adopt a reactive conformation within the active-site.[37–39] In the mechanistic first step, the attack of the hydroxyl group of the nucleophilic serine residue with an acyl donor (vinyl acetate in the present case) forms a tetrahedral intermediate (**TI1**), which is stabilized by hydrogen bonding with nearby NH-groups located in the so-called oxyanion hole (in gray in Figure 4b). Next, **TI1** undergoes the elimination of the alcohol moiety of the acyl donor to form the acyl-enzyme intermediate (**Enz-acyl**). An attack by a nucleophile [which can be *e.g.* water (hydrolysis), ammonia (ammonolysis), an amine (aminolysis), a thiol (thiolysis), a carboxylic acid (acidolysis) or as the case of Figure 4, an alcohol substrate (alcoholysis)] to **Enz-acyl** leads to the formation of a second tetrahedral intermediate

(**TI2**). In the final step, liberation of the formed product (in this case, CH_3CO_2R) from **TI2** leaves the enzyme ready for another catalytic cycle.

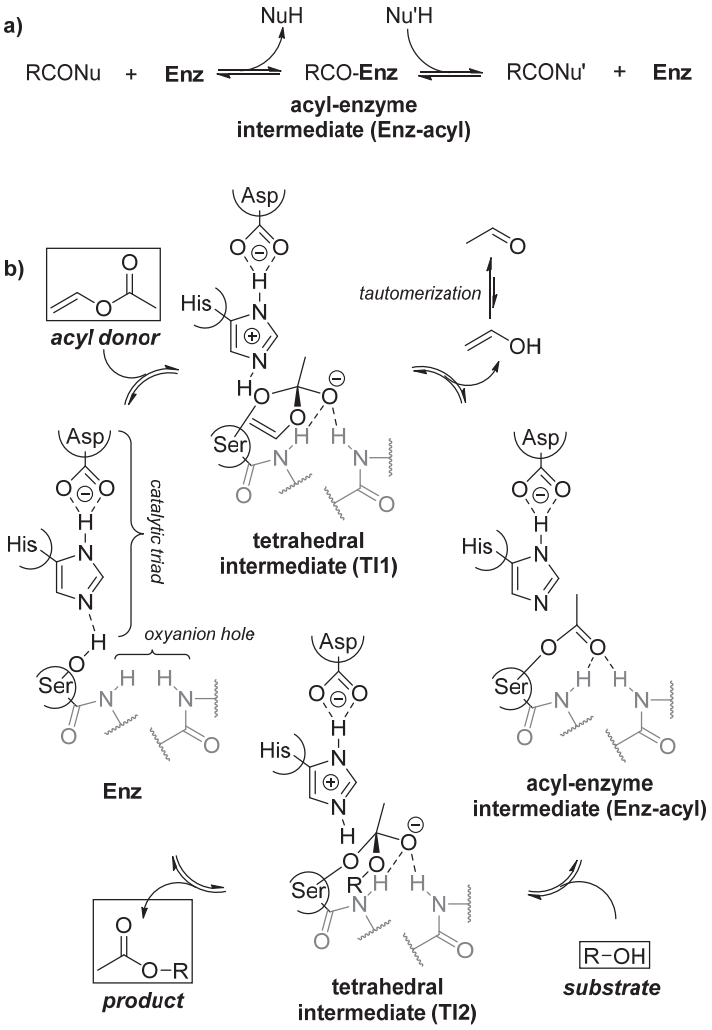


Figure 4. a) The ping-pong bi-bi mechanism and b) the catalytic cycle of a lipase in a transesterification reaction.[35,36]

The stereoselectivity of lipase catalysis stems from recognition at the molecular level: the geometry and the three-dimensional asymmetric structure of the active site of the enzyme, and of the substrate, intermediates and product, all affect the outcome. In Figure 4b, enantioselectivity of the reaction is determined during the formation of **TI2** (as it is formed through the binding of the preferred enantiomer of ROH). As the enzyme provides a chiral environment, the individual substrate enantiomers (A and B) would form diastereomeric complexes with the substrate with differing free energies

([EnzA]‡ and [EnzB]‡).[1] This energy difference ($\Delta\Delta G^{\ddagger}$) determines the degree of enantioselectivity (Figure 5).

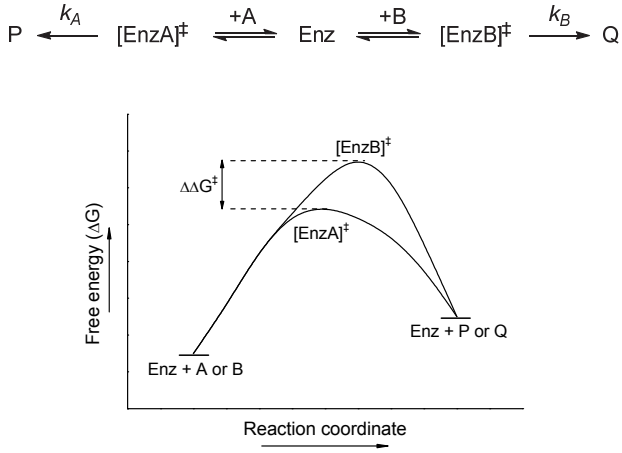


Figure 5. A free-energy diagram for an enantioselective reaction (k_A>k_B). A and B denote the substrate enantiomers and P and Q the product enantiomers, respectively.

Empirically, the stereopreference of lipase-catalyzed kinetic resolution of racemic secondary alcohols has been portrayed by the so-called Kazlauskas' rule for *Burkholderia cepacia* lipase (BCL, formerly known as *Pseudomonas cepacia*; a Gram-negative bacterium) catalyzed reactions (Figure 6a).[40] The rule was later complemented by a similar observation for reactions concerning primary alcohols showing inverted selectivity (Figure 6b), although it was deemed to be less accurate especially when an oxygen atom is present in the stereocenter.[41] The rule is applicable to lipase-catalysis in general. However, as exceptions can occur, the absolute conformation should always be proved experimentally.

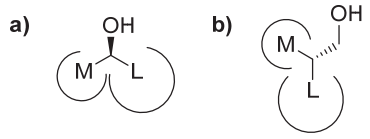


Figure 6. Enantiopreference of lipase-catalyzed reactions according to the empirical Kazlauskas' rule: the more reactive enantiomer shown for a) secondary alcohols and b) primary alcohols.[40,41] M represents a medium-sized and L a large substituent at the asymmetric center.

While lipases catalyze the acylation and deacylation reactions discussed above, they have also been characterized as rather promiscuous enzymes. The machinery harbored by the active site of some lipases enable the occurrence of reactions not usually attributed to those enzymes.[42] For instance, lipase B from the yeast *Candida antarctica*

(CAL-B) has been found to catalyze Michael additions, aldol reactions, together with racemization and epoxidation reactions–all traits further developed through mutations or already encompassed by the wild-type enzymes.[43]

2.1.3. Common lipase catalysts and preparations

Most lipases used in synthetic applications have a microbial origin (both bacteria and fungi are well represented) and many are commercially available. Although lipases, and isolated enzymes in general, can be utilized in free form (or as whole-cell preparations), immobilized enzymes are frequently favored. Immobilization ensures better handling, recovery and reuse of the biocatalyst, and it also tends to increase the stability of enzymes towards non-natural conditions such as the use of organic media and elevated temperatures.[44] Immobilization has also been reported to enhance enzyme activity, specificity and enantioselectivity.[45,46] Many issues can be addressed by immobilization, but it can also have adverse effects such as enzyme inactivation due to an incompatible immobilization technique and decrease in specific activity due to mass transfer limitations.[47] Lipases have been immobilized by adsorption or through covalent bonding to various inert materials [such as Celite (diatomaceous earth), Toyonite (ceramic) and silica], entrapped within different matrices (*e.g.* encapsulation in a sol-gel) and transformed into cross-linked enzyme crystals (CLECs) or cross-linked enzyme aggregates (CLEAs) (Figure 7).[44–49] A list of common commercially available lipase preparations are listed in Table 2.

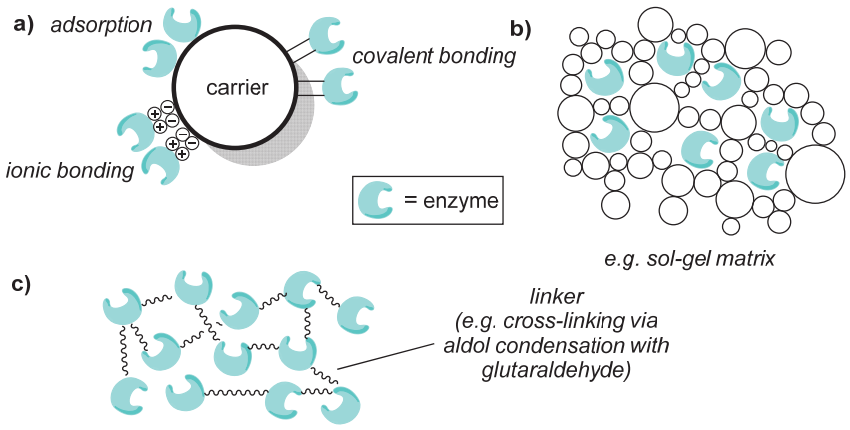


Figure 7. Immobilization techniques: a) binding to carrier (adsorption, ionic or covalent bonding), b) entrapment in a matrix, c) cross-linking (CLEC or CLEA).[44–49]

Table 2. A list of selected commercially available immobilized lipase preparations.

Entry	Preparation	Source	Immobilization	Producer
1	Lipase PS-D	*Burkholderia cepacia*	Adsorbed on diatomaceous earth (Celite).	Amano
2	Lipase PS-C II		Adsorbed on porous ceramic beads (Toyonite 200).	Amano
3	IMMABC-T2-150		Covalently bound on polyacrylic beads.	ChiralVision
4	Novozym 435[a]	*Candida antarctica*	Adsorbed on macroporous metacrylic beads.	Novozymes
5	IMMCAL-B-T1-1500[a]		Adsorbed on polypropylene.	ChiralVision
6	IMMCAL-A-T2-150[b]		Covalently bound on polyacrylic beads.	ChiralVision
7	IMMAPF-T2-150	*Pseudomonas fluorescens*	Covalently bound on polyacrylic beads.	ChiralVision
8	Sol-Gel-AK PFL		Entrapped in sol-gel-AK.	Fluka
9	Lipozyme RM IM	*Rhizomucor miehei*	Adsorbed on a weak anion exchange resin (Duolite A568).	Novozymes
10	Lipozyme TL IM	*Thermomyces lanuginosus*	Adsorbed on granulated silica.	Novozymes
11	TLL CLEA		Cross-linked enzyme aggregate.	Fluka

[a]Contains *C. antarctica* lipase B (CAL-B). [b]Contains *C. antarctica* lipase A (CAL-A).

One of the most frequently used lipases is the already mentioned CAL-B as the Novozym 435 preparation. The support material in the preparation is a divinylbenzene-crosslinked hydrophobic macroporous polymer based on methyl and butyl methacrylic esters, and the enzyme is adsorbed on the support by hydrophobic interactions.[50] It was also one of the lipase preparations used in the experimental work described in the thesis and original publications.[I–IV] CAL-B consists of 317 amino acid residues with a molecular weight of 33 kDa and the crystal structure has been resolved.[51,52] The catalytic triad consists of Ser105, Asp187, and His224; and the oxyanion hole bears a GX motif (G being glycine and X any other residue; comprising of Gln106 and Thr40). As an important structural element, most lipases include a so-called lid moiety (consisting of one or two short α-helixes) that covers the active site in aqueous media preventing access to the active site in the closed inactive form of the enzyme. It is opened when the enzyme is exposed to hydrophobic elements, such as organic solvents or the lipid/water interface, to attain the active conformation of the enzyme.[53] CAL-B is peculiar among lipases as it is missing the lid and consequently it does not require

interfacial activation. As another peculiarity, the existence of a water-tunnel has been described. The tunnel, described for some other enzymes as well, could give the water nucleophile an alternative direct pathway to the active site.[54] While the use of CAL-B is rather widespread, the utilization of *Candida antarctica* lipase A (CAL-A) is less frequent. It shares common origin with CAL-B, but the characteristics, activities and specificities of this enzyme are different. CAL-A is a highly thermostable lipase with a molecular weight of 45 kDa.[55] CAL-A (as crystal structure 2VEO in PDB) comprises of 431 amino acid residues in the X-ray crystal structure (actually 441 amino acid residues with 10 residues missing probably due to an endogenous protease) and shares a 14% amino acid sequence similarity with the C-terminal domain of dipeptidyl peptidase IV/CD26.[56] The catalytic triad consists of the Ser184, Asp334 and His366 residues, while the oxyanion hole is apparently constructed by the Asp95 and Gly185 residues–a structure reminiscent of various peptidases.

From the enzymes listed in Table 2, the fungal *Thermomyces lanuginosus* lipase (TLL, formerly known as *Humicola lanuginosa*) is a proper lipase incorporating a mobile lid structure covering the active site, whereas CAL-B has been considered as an esterase due to the missing (or smaller than usual) lid and CAL-A as an intermediary enzyme with regard to interfacial activation.[57] The enzyme consists of 269 amino acid residues with a molecular weight of 31.7 kDa and is rather thermostable (maintains activity at 55–60°C).[58] Many crystallographic structures have been solved for TLL both in open and closed conformation.[59,60] The catalytic triad consists of the Ser146, His256 and Asp199 residues, the oxyanion hole being formed by Ser83. The three-dimensional crystal structure of fungal *Rhizomucor miehei* lipase (RML, formerly known as *Mucor miehei*) was one of the first lipases to be elucidated and where the existence of a lid covering the entrance to the active site was also first described.[61–65] The catalytic triad of this protein consisting of 269 amino acid residues is comprised of Ser144, Asp203 and His257, and the oxyanion hole by Ser82 and Leu145. Molecular mass is calculated to be 29.5 kD.[65] Interestingly, no crystal structure is available for *Pseudomonas fluorescens* lipase (PFL), although a preliminary report on this 33.5 kDa protein with 317 amino acid residues was published already in 1991.[66]

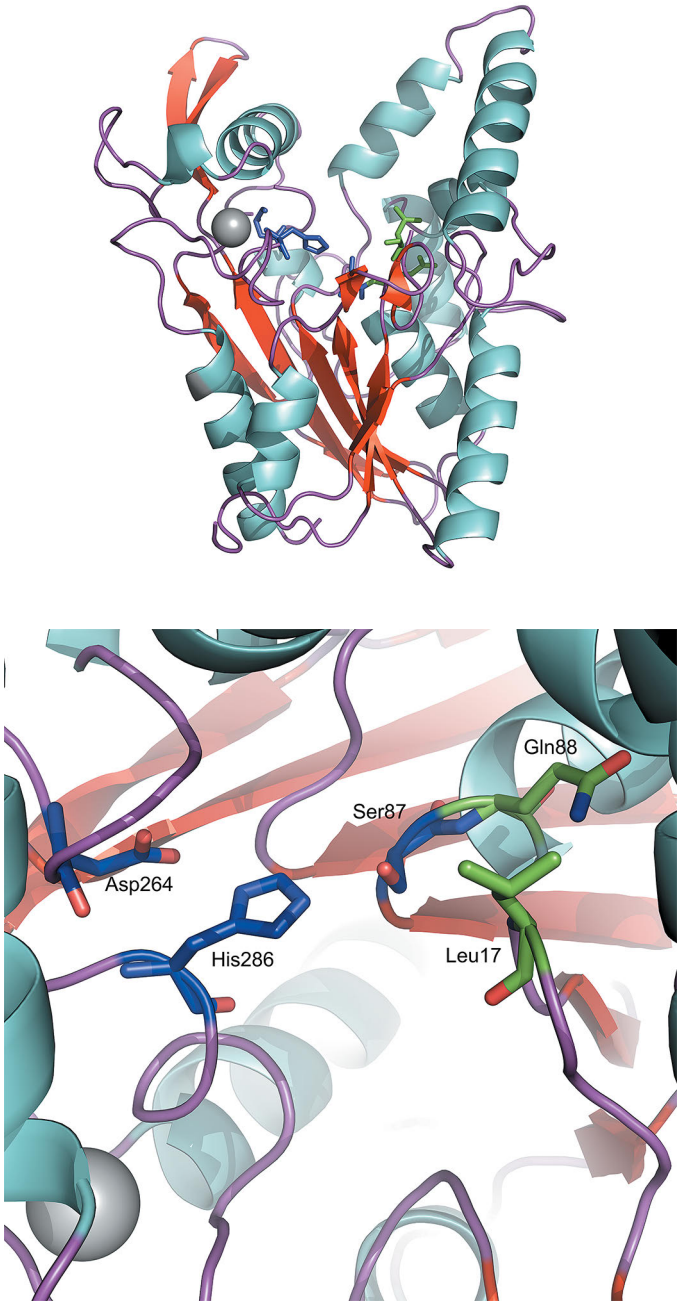


Figure 8. The X-ray crystal structure of BCL in open conformation (PDB: 3LIP[67]) as a visualization with PyMOL[74]; top: a view of the whole protein, bottom: a top-down view of the active site showing both the catalytic triad (Ser87, Asp264 and His286; dyed blue) and the oxyanion hole (Gln88 and Leu17, dyed green). Structural Ca^{2+} represented as a sphere.

Finally, another widely used lipase is the already mentioned *Burkholderia cepacia* lipase (BCL, Figure 8). Alongside CAL-B, BCL is one of the most widely used lipases in organic synthesis. The single chain protein consists of 320 amino acids and has a 33.8 kDa molecular mass with several crystal structures published.[67–71] The catalytic triad comprises of Ser87, Asp264 and His286. The oxyanion hole consists of Gln88 and Leu17 thus representing the typical GX-motif. GX-type lipases have the carbonyl oxygen atom of the oxyanion hole X residue facing towards the binding pocket incorporating the C_α-atom of the alcohol whereas GGGX-type hydrolases have more room for bulkier substrates (For instance, the GGGX-type CAL-A can catalyze reactions with tertiary alcohols as substrates).[72,73] As already mentioned, the stereoselectivity of lipase catalysis has been probed in conjunction with the structural examination of BCL. In addition to the oxyanion hole, inhibitor studies and phosphorous-containing transition state analogue complexes have revealed three binding pockets have been described for BCL [with triacylglycerols, the large hydrophobic pocket (HA) binds the *sn*-3 acyl chain, the *sn*-2 acyl chain fits inside a smaller hydrophilic pocket (HH) and the *sn*-1 acyl chain is loosely bound within a slightly hydrophobic pocket (HB)].[68,69,71] Differences in the sizes of binding pockets can explain differences in selectivity between lipases of different origin.

2.1.4. From aqueous to non-aqueous conditions

Contrary to the widely held view that enzymes can only work in the aqueous conditions of the cellular environment, Klibanov showed that lipases (together with esterases) could retain catalytic activity even in anhydrous organic solvents in the 1980s.[75–77] The use of anhydrous conditions often improves stability of enzymes in general and enables the reaction temperature to be elevated up to 100°C in some cases.[76] This increase in thermal stability can be attributed to increased rigidity of the protein. It should be noted that thermal tolerance relates to thermodynamic stability (the ability of the protein to refold after exposure to heat) whereas thermostability refers to kinetic stability, or the ability of a protein to avoid irreversible denaturation.[44] The change to anhydrous organic media will often result in lower activity than what is observed in aqueous conditions. However, the measurement of such activity for certain reactions can be difficult. For instance, studying lipase-catalyzed acylation reactions in aqueous conditions would be practically impossible as the backwards reaction is notably faster. Nevertheless, enzymes require some water in their microenvironment (called essential,

residual or structural water) to remain active: a layer of water covers the protein, lubricating it and enabling catalytic activity. The water available for the enzyme varies with the solvent in question as some solvents (with sufficient hydrophilicity) can remove or distort the essential layer of water thus leading to inactivation of the enzyme.[78,79] Also, the enantioselectivity of lipase-catalyzed reactions has been noted to change from solvent to another.[80]

Generally, the effects stemming from solvents can be categorized in three groups: 1) change in enzyme conformation and/or flexibility due to solvent, 2) changes in substrate [and product(s)] solubility and 3) competitive inhibition by the solvent.[81] A number of organic solvents used together with lipases are listed in Table 3 below. Log *P*, a measure of hydrophobicity, is the participation coefficient for a solute (solvent herein) between water and 1-octanol. Negative values denote higher solubility in water (hydrophilic) while positive values refer to preference towards 1-octanol (hydrophobic).

Table 3. Selected organic solvents and their hydrophobicity (as log *P*).[82]

Entry	Solvent	log *P*[a]	Entry	Solvent	log *P*[a]
1	Dimethylsulfoxide	-1.3	8	Isopropanol	0.8
2	*N*,*N*-Dimethylformamide	-1.0	9	*tert*-Butyl methyl ether	1.35
3	Acetonitrile	-0.33	10	Diisopropyl ether	1.9
4	Ethanol	-0.24	11	Toluene	2.5
5	Acetone	-0.23	12	Cyclohexane	3.2
6	Tetrahydrofuran	0.49	13	Hexane	3.5
7	Ethyl acetate	0.68	14	Heptane	4

[a]log P = log ($[A]_{Oct}/[A]_{Water}$), where [A] denotes the concentration of a solute A.

A solvent can be dried to anhydrous state but the enzyme cannot (essential water is necessary as already mentioned). One way to study the effect of water for reactions in organic solvents is to examine the activity of water (a_w) under the reaction conditions.[78,79] When the reaction system is at equilibrium, a_w is at equilibrium between the different parts of the system (bulk solvent, essential water, water in the atmosphere etc.) (Figure 9). In essence, a_w will determine the amount of water that is associated with the enzyme and this water will be available to the catalytic machinery of the enzyme to some extent depending on the reaction conditions (*e.g.* the nature of the solvent or the presence of salts capable of forming hydrates) and the enzyme in question.

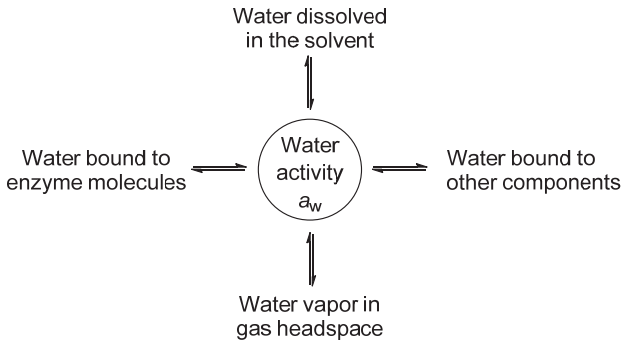


Figure 9. Water activity mediates the equilibrium of water between various states and phases.[79]

Residual water (or water otherwise in the system) can affect lipase-catalyzed reactions negatively. In addition to water acting as a competitive inhibitor,[81,83] water can be a competing nucleophile in lipase-catalyzed acylation reactions in organic solvents, thus forming carboxylic acid as a side product (Figure 10, where the acylation of R^2OH with $R^1CO_2CH{=}CH_2$ can lead to formation of R^1CO_2H as a side product through hydrolysis of the acyl donor or formed product ester $R^1CO_2R^2$).[84] This in turn, can affect the overall enantioselectivity of the reaction as the esterification and transesterification reactions can progress with a difference in enantioselectivity. The formed acid can also affect the pH of the microenvironment near the enzyme and its active site.[85]

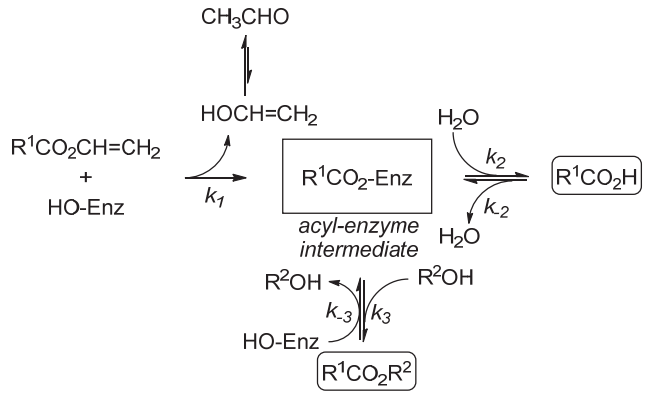


Figure 10. Acylation of an alcohol (R^2OH) with an acyl donor ($R^1CO_2CH{=}CH_2$) and competing pathways in lipase catalysis.

2.2. Enzymatic kinetic resolution

2.2.1. General

Kinetic resolution (KR), separation of the enantiomers of a racemic substrate through the difference of reactivity between the enantiomers, serves as an important tool for the preparation of single enantiomers (Figure 11).[1,19,40,47,86,87] While the theoretical yield for one enantiomer is only 50% of the racemate, it should be noted that the lipase-catalyzed KR gives an opportunity of obtaining both enantiomers (one as the product P and the other as the unreacted substrate S) with a theoretical yield of 50% for each.

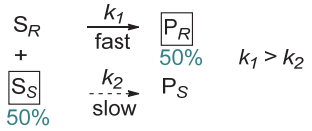


Figure 11. Principle of kinetic resolution.

In principle, KR can be achieved through any kind of reaction were the substrate enantiomers react differently. One well recognized and widely applied biocatalytic method is the use of lipase catalysis to achieve the resolution through acylation (esterification, transesterification) or deacylation (alcoholysis, ammonolysis, hydrolysis) reactions.[88] The use of an interesterification reaction (reactions between esters) is also plausible although less common.[89] From the mathematical basis of chemical KR, such as the KR of prochiral allylic alcohols by titanium alkoxide tartrate-catalyzed epoxidation,[8,90] Sih *et al.*[91,92] presented three key parameters to describe the outcome of a biocatalytic KR: *the extent of transformation* of the racemic substrate to product enantiomer [as conversion (c or conv.)], *enantiomeric purity* of the product and remaining substrate (as enantiomeric excess, *ee*), and *enantioselectivity* (as the enantiomeric ratio, *E*). These parameters are valid to enzymatic KRs when no racemization or other side reactions occur. The value of *E* is independent of substrate concentration and conversion. In equation 1, the thermodynamic connection between the differences of free energy states of the diastereomeric complexes formed during catalysis and the enantiomeric ratio is shown (see Section 2.1.2, Figure 5). It also refers that *E* can be affected by changes in temperature (as $\Delta\Delta G^{\ddagger} = \Delta\Delta H^{\ddagger} - T\Delta\Delta S^{\ddagger}$).[93] In equations 2–4, the key relationships between the parameters are presented.

$$\Delta\Delta G^{\ddagger} = -RT \ln E \quad (1)$$

$$c = \frac{ee_S}{(ee_S + ee_P)} \quad (2)$$

$$ee = \frac{[A]-[B]}{[A]+[B]} \quad (3)$$

$$E = \frac{\ln[(1-c)(1-ee_S)]}{\ln[(1-c)(1+ee_S)]} \quad (4)$$

$$E = \frac{\ln[(1-c)(1+ee_P)]}{\ln[(1-c)(1-ee_P)]} \quad (5)$$

In the absence of side reactions, the conversion for a KR can be calculated from the *ee*-values of the product (ee_P) and substrate (ee_S) (equations 2 and 3). In equation 3, *A* represents the amount (*e.g.* concentration or peak area on chromatogram in practice) of the faster reacting enantiomer and *B* the amount of the slower reacting enantiomer. Equations 4 and 5 express *E* as derived from ee_S and ee_P, respectively. In Figure 12, enantiomeric excess (ee_S and ee_P) is presented as a function of conversion with different values of *E*.

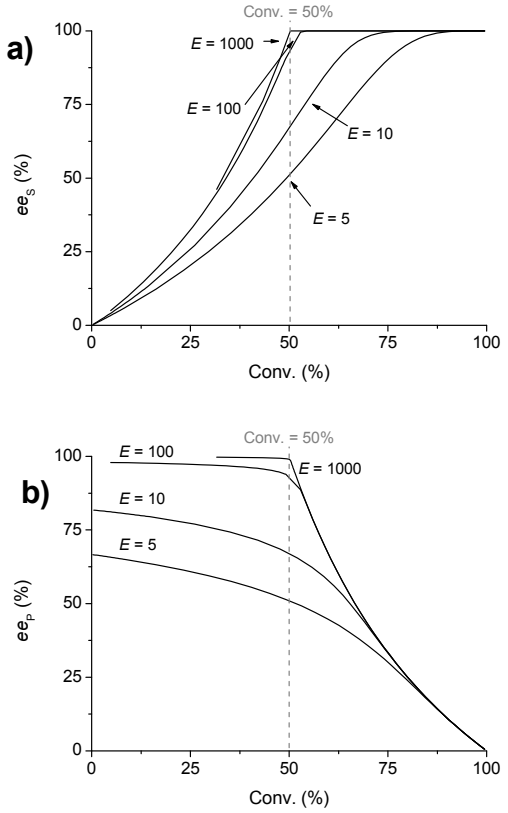


Figure 12. Dependence of conversion and enantiomeric excess of a) substrate and b) product on *E* (*E* = 5, 10, 100 and 1000).

Clearly, the product enantiomer is obtained with high enantiopurity only at high *E* values (*E* of the order of 100 or higher) while the unreacted substrate enantiomer is always obtained in highly enantiopure form although at the expense of yield. At very high *E* values (such as *E* =1000), the reaction stops at 50% conversion with both enantiomers appearing in highly enantiopure form.

2.2.2. Esters and activated esters as acyl donors

One of the main requirements for a successful KR of a racemic alcohol through lipase-catalyzed acylation is irreversibility of the reaction (basic structures of different types of acyl donors are presented in Figure 13).[85] One way to achieve this and drive the reaction forward is to use an irreversible acyl donor, such as an enol ester, that has a good leaving group (enols will tautomerize to give the ketoform as presented in Figure 4 earlier).[94] The use of acid anhydrides[95] as irreversible acyl donors has also been presented, although removal of the formed acid may be required[96]. The use of enol lactones has been presented as an option combining the good aspects of enol esters and acid anhydrides.[97] With reversible acyl donors, the formation of the acyl-enzyme intermediate leads to the liberation of a competing nucleophile and a possible side reaction.

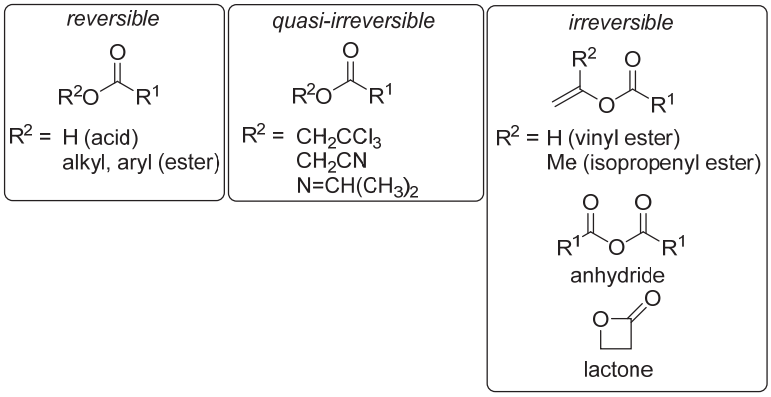


Figure 13. Examples of reversible, quasi-reversible and irreversible acyl donors.[85]

Unlike irreversible acyl donors, the leaving group of a quasi-irreversible acyl donor can be considered as a weak nucleophile that the backwards reaction can be typically dismissed.[85] For instance, as 2,2,2-trifluoroethanol is a good leaving group and a poor nucleophile, it is a possible alcohol moiety for a quasi-irreversible acyl donor. These activated esters appear already in the early examples describing lipase-catalyzed KR.[98]

In conclusion, the selected acyl donor can affect the outcome of a lipase-catalyzed reaction just as much as the choice of substrate or catalyst. It can significantly affect, for example, the productivity (reactivity), stereo- and regioselectivity of the lipase-catalyzed reaction.[99,100]

2.2.3. β-lactams as acyl donors

While most esters can be cleaved by lipases, amides tend to remain unattainable for them. Like lipases, amidases (EC 3.5.1.4, acylamide amidohydrolases are enzymes that cleave amide bonds) are serine hydrolases. Similarities between the two groups of enzymes are evident, but they differ in their ability to cleave amide bonds.[101] As an exception to the rule, lipases have been found to cleave certain cyclic amides, azetidin-2-ones (β-lactams), often with high enantioselectivity. The use of lipases in the preparation of β-amino acids, β-amino esters and β-dipeptides from β-lactams is well documented.[102–105] In the first step of lipase-catalyzed β-lactam ring-opening, the formation of the acyl-enzyme intermediate occurs analogously to what is seen in a reaction with an ester Now, the difference to the reaction with an ester is that nothing is eliminated during the formation of the acyl-enzyme intermediate, enabling the use of β-lactams as irreversible acyl donors (Figure 14).

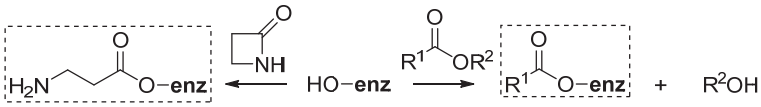
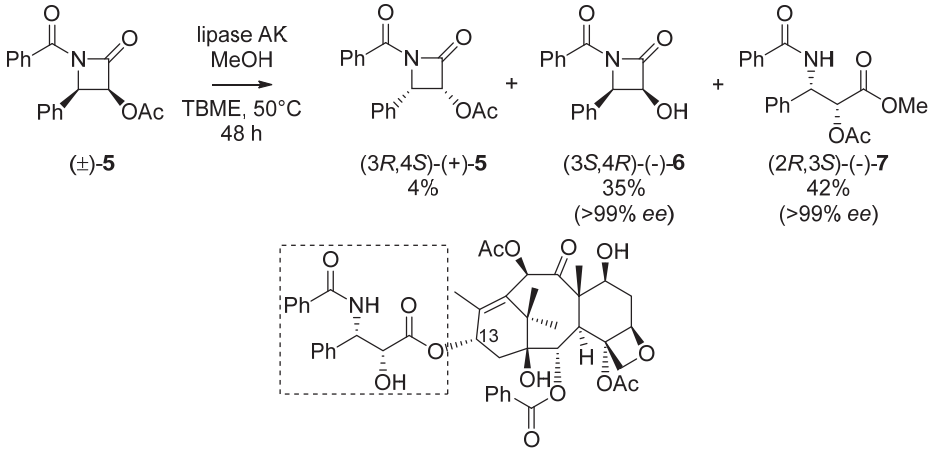


Figure 14. Formation of an acyl-enzyme intermediate (Enz-acyl) with lipases from esters and β-lactams.

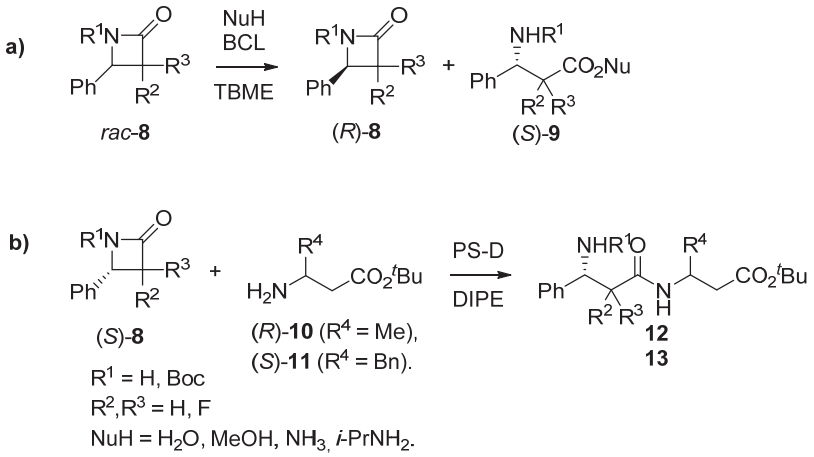
Previously, this approach has been utilized synthetically in a number of interesting examples. For instance, in the first reported ring-opening of β-lactams by lipases in the synthesis of (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine, the C-13 acyl side chain of the important cancer drug paclitaxel [Taxol, a natural product first isolated from the Western Yew (*Taxus brevifolia*)], was accomplished by lipase P-30 (*Pseudomonas sp.* lipase, apparently BCL, immobilized on diatomaceous earth) and lipase AK (*Pseudomonas fluorescens* lipase) (Scheme 1).[106] Both methanol and water served as nucleophiles in the ring-opening of a protected 3-hydroxy-4-phenyl-β-lactam (±5), yielding highly enantiopure products such as (2*R*,3*S*)-(-)-**7** (>99% *ee*).



Scheme 1. Preparation of the C-13 acyl side chain of paclitaxel (Taxol) through the lipase AK-catalyzed ring-opening of (±)-**5**.[106]

In the many examples presented since the Taxol example, CAL-B has been found as a prominent catalyst for the hydrolysis of β-lactams.[107–116] For instance, the CAL-B–catalyzed hydrolysis of the strained 1,4-ethyl- and 1,4-ethylene-bridged cispentacin in diisopropyl ether (DIPE) at 70°C reached 50% conversion in 8–11 days with high enantioselectivity (E >200).[109] However, the development of acylation of alcohols through the lipase-catalyzed ring-opening of β-lactams has been less studied as it tends to demand further activation of the β-lactam ring. This has been achieved by the incorporation of electron withdrawing substituents, such as fluorine, to the lactam ring. For example, BCL as the lipase PS-D preparation has been used in the enantioselective ring-opening of 3-mono- and 3,3-difluoro-substituted β-lactams for the preparation of corresponding β-amino acids, esters, amides and β-dipeptides (Scheme 2).[117,118] For instance, the lipase PS-D–catalyzed methanolysis (NuH = MeOH) of *rac*-3,3-difluoro-4-phenylazetidin-2-one (*rac*-**8a**; R^1 = H, R^2, R^3 = F) proceeded with excellent enantioselectivity (E>200) whereas the corresponding non-fluorine analogue (*rac*-**8d**; R^1,R^2, R^3 = H) would remain inert (Scheme 2a).[117] Substituting lipase PS-D with CAL-B enables the enantioselective hydrolysis of *rac*-**8d**.[110,117] Additionally, the formation of dipeptide **12** through lipase PS-D–catalyzed ring-opening (*S*)-**8a** with amino acid ester (*R*)-**10** and proceeded efficiently, reaching 100% conversion in 15 h [(*R*)-**8a** was inactive] and by further activating the non-fluorine **8d** with a *N*-protective group (R^1 =

Boc), enzymatic ring-opening by lipase PS-D could be achieved (significant chemical ring-opening was also observed as a side reaction) (Scheme 2b).[118]

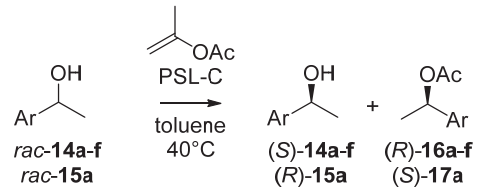


Scheme 2. a) BCL-catalyzed reactions for the synthesis of β-amino acids, esters and amides and b) lipase PS-D –catalyzed synthesis of β-dipeptides.[117,118]

2.2.4. Acylation of secondary alcohols

The use of KR in the preparation of single enantiomers of secondary alcohols is well established.[1,40,86,87] All forms of lipase-catalyzed KR are commonly utilized, but here the emphasis will be in acylation reactions of alcohols. Many aspects, like the structure of a substrate alcohol, affect the outcome of lipase-catalyzed KRs. This is a direct result of the subtle interactions between the substrate and the catalyst during the formation of the S•Enz complex (Figure 2). For instance, slight differences in the substitution pattern of an aromatic ring [*e.g. ortho*- (E = 16), *meta*- (E = 78) or *para*-methyl (E = 29) substituent in the phenyl moiety in *rac*-**14c-e**, respectively] or the change from a phenyl (*rac*-**14a**, E = 93) to a heteroaryl ring (*rac*-**15a**, E = 4) can have significant effects under otherwise identical conditions (Table 4).[119] Nevertheless, even with poor enantioselectivity at times, the unreacted enantiomer [(*S*)-**14a-f** and (*R*)-**15a**] could be obtained in high enantiomeric purity (>99% *ee*) as long as the reaction was allowed to proceed long enough.

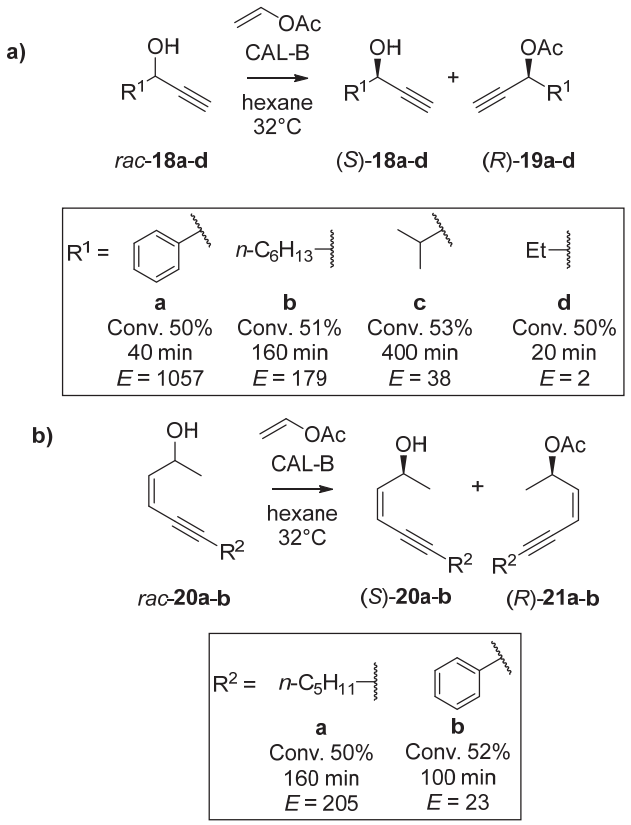
Table 4. PSL-C–catalyzed transesterification of secondary alcohols with isopropenyl acetate in toluene at 40°C.[119]



rac-**14a-f** *rac*-**15a** (*S*)-**14a-f** (*R*)-**15a** (*R*)-**16a-f** (*S*)-**17a**

Entry	Substrate (Ar =)	Time (h)	Conv. (%)	$ee^{(S)\text{-}14\text{-}15}$ (%)	$ee^{(R)\text{-}16\text{-}17}$ (%)	*E*
1	*rac*-**14a**	4	56	>99	78	93
2	*rac*-**14b**	4	51	>99	93	>300
3	*rac*-**14c**	15	73	>99	36	16
4	*rac*-**14d**	4	56	>99	78	78
5	*rac*-**14e**	24	67	>99	49	23
6	*rac*-**14f**	4	53	>99	87	141
7	*rac*-**15a**	4	94	>99	5	4

The effect of solvent for the enantioselectivity of a lipase-catalyzed acylation reaction became clear in the KR of racemic propargylic (*rac*-**18a-d**) and allylic alcohols (*rac*-**20a-b**) by CAL-B–catalyzed acylation with vinyl acetate (Scheme 3).[120] The use of hexane as solvent was found beneficiary over THF, Et_2O and benzene, for the preparation of both the free alcohols and the acetate products. The structure of the substrate affected both the reactivity (as seen in the reaction times) and enantioselectivity.



Scheme 3. CAL-B–catalyzed acylation of a) propargylic and b) allylic alcohols with vinyl acetate in hexane at 32°C.[120]

2.3. Dynamic kinetic resolution

2.3.1. General

The preparation of single enantiomers *via* KR and subsequent inversion of stereochemistry of the unwanted enantiomer, for instance by the Mitsunobu reaction represents an option, but is rather laborious.[121] Another way would be to recycle the unwanted enantiomer, for instance through a minor enantiomer recycling (MER) process.[122] Alternatively, one can resort to some form of asymmetric synthesis such as dynamic kinetic asymmetric transformation (DYKAT): stereoinversion, enantio-convergent process (ECP) or cyclic deracemization (CycD) (Figure 15a-c).[123,124] Herein, the focus is on dynamic kinetic resolution (DKR), a combination of KR with *in situ* racemization of the slow or non-reacting enantiomer, and the theoretical maximal yield for the product enantiomer is quantitative. (Figure 15d).[125–128] This makes DKR a powerful method if the product enantiomer is the only compound that is sought after.

Further synthetic steps or catalysts of complementary stereoselectivity are needed if the non-reacting enantiomer is wanted. In DKR, the substrate enantiomers (S_R and S_S) are in rapid equilibrium with each other and react at different rates to form the product (P_R and P_S). For a DKR process to be most effective in practice, the two parallel catalyzed steps (racemization and kinetic resolution) have to be compatible with each other, the racemization between S_R and S_S has to be equally effective and the resolution step has to be inherently irreversible. Thus, the ratios k_R/k_S and k_{inv}/k_R affect the outcome of the process significantly (Figure 16).[129]

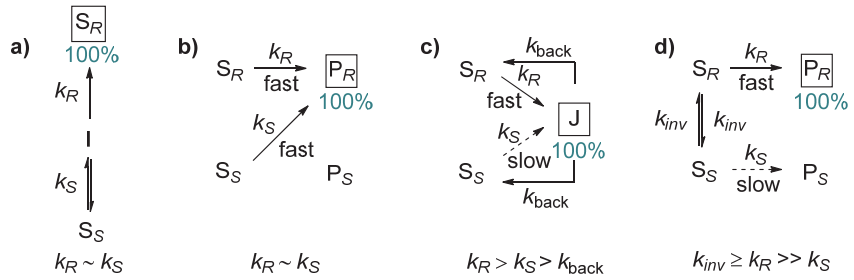


Figure 15. Preparing single enantiomers by: a) stereoinversion, b) enantio-convergent process (ECP), c) cyclic deracemization (CycD) or d) DKR.[123,124] Herein, S_R and S_S, I, P_R and P_S represent substrate enantiomers, achiral intermediate and product enantiomers, respectively.

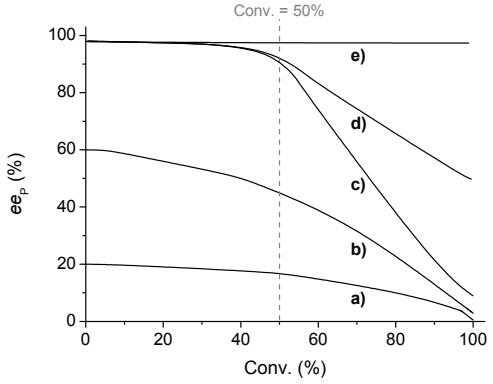


Figure 16. Dependence of enantiomeric purity of the product (ee_P) and conversion on the ratios of the individual rates k_R (fast), k_S (slow) and k_{inv} in DKR: a) with $k_R/k_S = 1.5$ and $k_{inv}/k_R = 0.01$, b) with $k_R/k_S = 4$ and $k_{inv}/k_R = 0.01$, c) with $k_R/k_S = 99$ and $k_{inv}/k_R = 0.001$, d) with $k_R/k_S = 99$ and $k_{inv}/k_R = 0.01$ and e) with $k_R/k_S = 99$ and $k_{inv}/k_R = 1$.[129]

In essence, DKR can be based on the combination of any compatible racemization and resolutions steps (*i.e.* chemical, chemoenzymatic and enzymatic methods are all plausible when applicable: one of the most widely studied approach is the use of transition-metal complexes as racemization catalysts in combination with lipase catalysts in the metalloenzymatic DKR of secondary alcohols.[127,128,130,131] However,

the emphasis in the thesis will be in an earlier dynamic process, the DKR of cyanohydrins (α-hydroxy nitriles).

2.3.2. Preparing single enantiomers of cyanohydrins

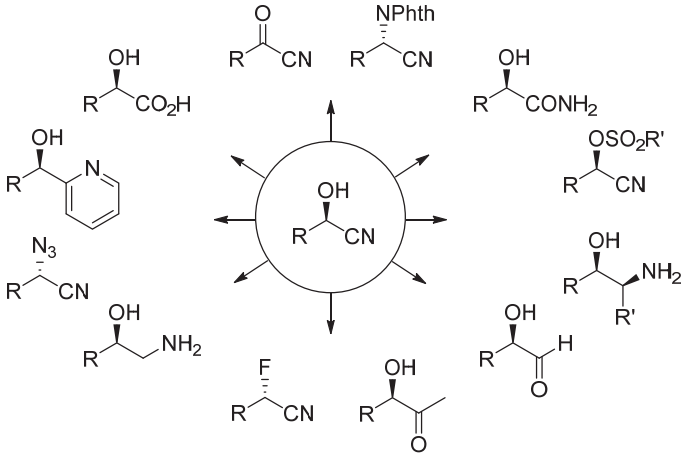
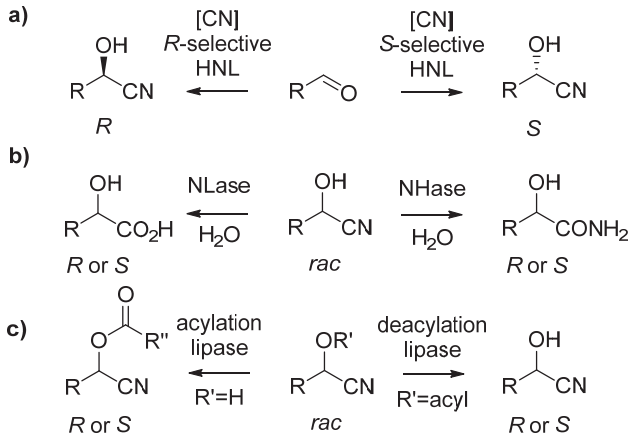


Figure 17. Synthetic possibilities presented by cyanohydrins.[132]

Cyanohydrins with their two independently modifiable functionalities (HO- and CN-groups) are important for the synthesis of such valuable intermediates as α-hydroxy acids,[133,134] α-hydroxy aldehydes,[135] α-hydroxy ketones,[136] β-hydroxy amines,[137] and β-hydroxy amides[138,139] (Figure 17). In addition, other synthetic steps can be undertaken. For instance, certain cyanohydrin esters can be cyclized through an intramolecular Blaise reaction giving furanone derivatives.[122] In the classic cyanohydrin reaction, a new C–C bond is created when cyanide is added to a carbonyl compound (aldehyde or ketone). While the reaction requires the use of cyanide, safer options for the use of hydrogen cyanide (HCN) are available. For instance, the use of trimethylsilyl cyanide (TMSCN) in the synthesis of racemic cyanohydrins is an efficient possibility.[140] Approaches for chemical asymmetric synthesis of cyanohydrins have also been developed, utilizing transition metal catalysts and organocatalysts,[141,142] but will not be considered here any further as enzymatic methods will be emphasized.

Three enzymatic routes to enantioselective synthesis of cyanohydrins have been described.[132] The first enzymatic approach utilizes hydroxynitrile lyases (HNLs, also known as oxynitrilases) from different plant sources [*e.g. Hevea brasiliensis* (rubber tree; *Hb*HNL), *Manihot esculenta* (cassava; *Me*HNL), *Sorghum bicolor* (a cane-like grass; *Sb*HNL) and *Prunus amygdalus* (almond; *Pa*HNL)] that have evolved for

defensive purposes against herbivores (cyanogenesis) or have been retained from earlier ancestors (Scheme 4a). In a HNL-catalyzed reaction, cyanide is added to a prochiral aldehyde or ketone thus forming either an (*R*)- or (*S*)-cyanohydrin, depending on the HNL and the substituents in the substrate (according to the Cahn–Ingold–Prelog rules). Both isolated enzymes and whole-cell preparations have been successfully used.[132,143–148]

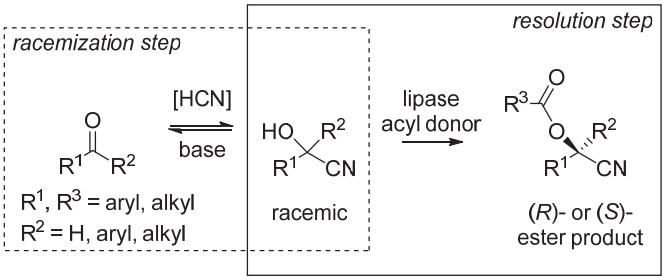


Scheme 4. Preparation of cyanohydrin enantiomers through a) HNL catalysis, b) NLase or NHase –catalyzed hydrolysis, and c) lipase-catalyzed acylation and deacylation. [CN] = cyanide source.

The second (chemo)enzymatic method relies of the use of nitrilases (NLase, nitrile aminohydrolase, EC 3.5.5.1) or nitrile hydratases (NHase, EC 4.2.1.84) which enantioselectively hydrolyze the nitrile group of a racemic cyanohydrin to carboxylic acid or amide, respectively (Scheme 4b).[149,150] While both approaches presented in Scheme 3a and b are important for the synthesis of enantiomers of cyanohydrins, amides and carboxylic acids, they will not be considered further in this thesis. The third (chemo)enzymatic method for the enantioselective synthesis of cyanohydrins relies on lipase-catalysis. Here, both the lipase-catalyzed acylation of racemic cyanohydrins[151] and the deacylation of racemic cyanohydrin esters[152] can be considered (Scheme 4c). Although the KR of racemic cyanohydrins through lipase catalysis is as plausible as with any other secondary alcohol, there is promise for much more.

The formation of a new C-C bond through the cyanohydrin reaction from carbonyl compounds and the lability of cyanohydrins in the presence of bases allows the development of DKR processes (Scheme 5). The *in situ* formed racemic cyanohydrin is

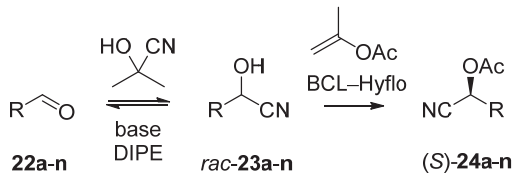
subjected to enantioselective lipase-catalyzed acylation with the other enantiomer being racemized *via* the carbonyl compound by base catalysis.

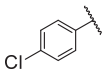
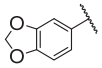
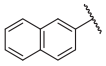
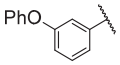
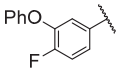
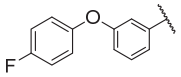
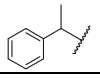


Scheme 5. Formation of cyanohydrin ester enantiomers *via* lipase-catalyzed DKR.

In the groundbreaking work presented by Inagaki *et al.*,[153,154] a number of aromatic cyanohydrin acetate enantiomers were formed in a one-pot reaction from the corresponding aldehydes in the presence of a lipase and a base catalyst. Basic resins like Amberlite IRA-904 [OH^-, CO_3^{2-}, CN^-, HCO_3^-, AcO^-, TsO^-, Cl^-, (*R*)-binaphthol and (*S*)-binapthnol forms], Amberlite IRA-400 (Type I), Amberlyst A-21 (tertiary amino group), Amberlyst A-27 (OH^- form), Duolite A-162 (OH^- and AcO^- forms) and Amberlyst A-21 were examined for the formation of racemic cyanohydrins *rac*-**23a-n** from the corresponding aldehydes **22a-n** and acetone cyanohydrin, with Amberlite IRA-904 (OH^-, CO_3^{2-}, CN^-, HCO_3^-, AcO^- forms gave comparable performances) outperforming the others.[154] When immobilized *Burkholderia cepacia* lipase (BCL–Hyflo, immobilized on Hyflo Super Cel, a certain treated form of diatomaceous earth) together with isopropenyl acetate in dry DIPE were combined with Amberlite IRA-904 (OH^- form), enantiomerically enriched (*S*)-**24a-n** could be prepared from the aldehydes **22a-n** (Table 5).

Table 5. Preparation of (*S*)-cyanohydrin acetates (*S*)-**24a-n** with BCL–Hyflo and Amberlite IRA-904 (OH^- form).[153,154]

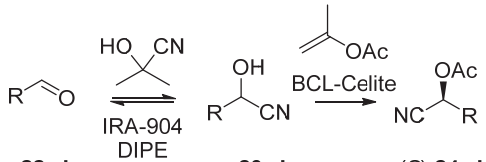


Entry	**22**–**24** (R =)	Time (d)	Conv. (%)[a]	*ee*$^{(S)\text{-}20}$ (%)[b]	Isolated yield (%)[c]
1	**a** 	6.3	100	84	96
2	**b** 	2.5	82	91	64
3	**c** 	3.8	93	84	83
4	**d** 	6.5	96	91	81
5	**e** 	6.1	98	85	88
6	**f** 	6.1	79	70	70
7	**g** 	2.9	88	89	80
8	**h** 	3.0	93	91	88
9	**i** 	3.0	98	87	92
10	**j** 	6.0	76	47[d]	57
11	**k** $(CH_3)_2CH$—	9.3	63	51	47
12	**l** $CH_3(CH_2)_4$—	6.0	88	15	83
13	**m** 	3.0	84	78	68
14	**n** 	7.6	43/41	82/85	32/20

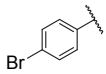
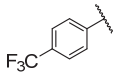
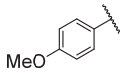
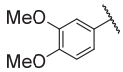
[a]Conversion of racemic cyanohydrin to acylated product. [b]Of the cyanohydrin acetate. [c]Yield from corresponding aldehyde. [d](*R*)-cyanohydrin acetate.

Later, the same one-pot approach was adopted by Kanerva *et al.*, together with the use of Celite-immobilized BCL (BCL-Celite; Celite is a form of diatomaceous earth similar to Hyflo).[155] The results (Table 6) from the DKRs of a number of aromatic and aliphatic cyanohydrins were comparable to the previous example (Table 5) with slight improvements.

Table 6. One-pot synthesis of cyanohydrin acetates (*S*)-**20a,b**, (*S*)-**20o-s**.[155]



22a,b **22o-s** *rac*-**23a,b** *rac*-**23o-s** (*S*)-**24a,b** (*S*)-**24o-s**

Entry	**22**–**24** (R =)	Time (h)	Conv. (%)	$ee^{(S)\text{-}20}$ (%)
1	**a** 	96	99	91
2	**b** 	168	99	94
3	**o** 	95	94	92
4	**p** 	168	95	41
5	**q** 	165	90	92
6	**r** 	168	84	90
7	**s** 	72	72	49

Moreover, it was found that while the aromatic cyanohydrins could be easily racemized *in situ* by Amberlite IRA-904 (OH^- form, 5 mg mL^{-1}) in DIPE [*e.g.* (*R*)-mandelonitrile (*R*)-**23a** (80% *ee*) was fully racemized within 5 h whereas the corresponding (*S*)-acetate (*S*)-**24a** (79% *ee*) proved relatively stable (77% *ee* after 24 h)]. This was not the case with aliphatic cyanohydrins and their acetates which proved hard to racemize under the same conditions. (*S*)-**24p**, with the *para*-CF_3 substituent would racemize slowly in the presence of Amberlite IRA-904 (OH^- form) (from 95% *ee* to 47% *ee* in 22 h).

Kanerva *et al.* continued to use the developed system also in further examples. For instance, the (*R*)-furylbenzothiazole-based cyanohydrin acetates (*R*)-**25a-d** were prepared by using CAL-A immobilized on Celite (CAL-A–Cel) and Amberlite IRA-904 (OH^- form) as catalysts (Figure 18a).[156] CAL-A–Cel was also found to be a superior catalyst for the preparation of phenothiazine-based cyanohydrin acetates (*S*)-**26a-e** *via*

chemoenzymatic DKR (Figure 18b).[157] Now, depending from the size of the *N*-substituent, excellent enantioselectivity (>99% *ee* with the largest substituents) and isolated yield (92–94%) was observed in every case. When phenylfuran-based cyanohydrin esters (*R*)-**27a-e** were prepared with BCL immobilized in Celite (BCL–Cel), the use of vinyl butanoate as acyl donor was seen preferable as the corresponding acetate enantiomers could not be analyzed successfully by chiral HPLC (Figure 18c).[158]

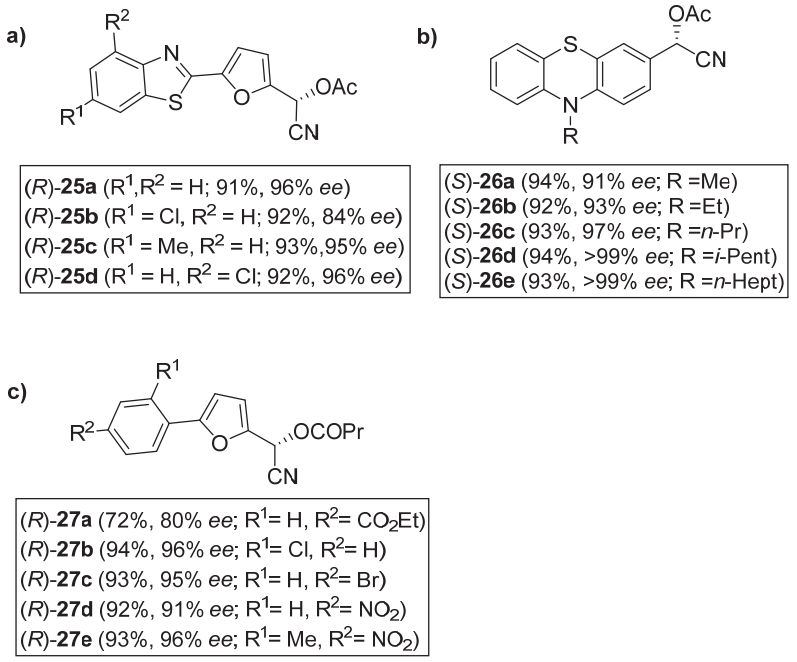
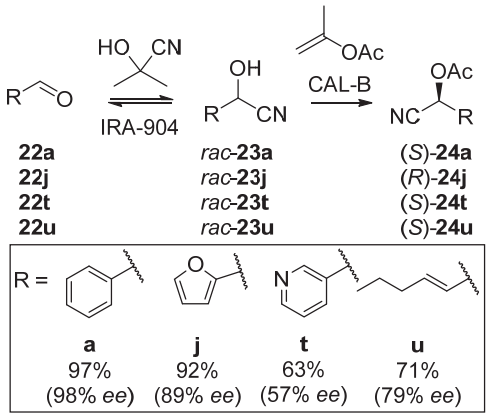


Figure 18. Structures of a) furylbenzothiazole-based cyanohydrins (*R*)-**25a-d**, b) phenothiazine-based cyanohydrins (*S*)-**26a-e** and c) phenylfuran-based cyanohydrins (*R*)-**27a-e** prepared by DKR.[156–158]

Long reaction times, such as the ones presented in Table 5 and 6 above, are problematic as they give more time for possible side reactions to occur. One reason for the long reaction times might be side reactions as both the formed product ester and acyl donor are susceptible to hydrolysis in the presence of a lipase (and residual water in the system). The produced carboxylic acid might in turn neutralize the base catalyst thus retarding the reaction (if the racemization step is not efficient, the DKR process can regress into a KR). Secondly, while the reaction conditions are such that the cyanohydrin esters are stable against base-catalyzed racemization (in most cases), base-

catalyzed acylation of the free cyanohydrin could in turn decrease the eventual enantiomeric purity of the product (as it would be an enantiodiscriminant reaction).

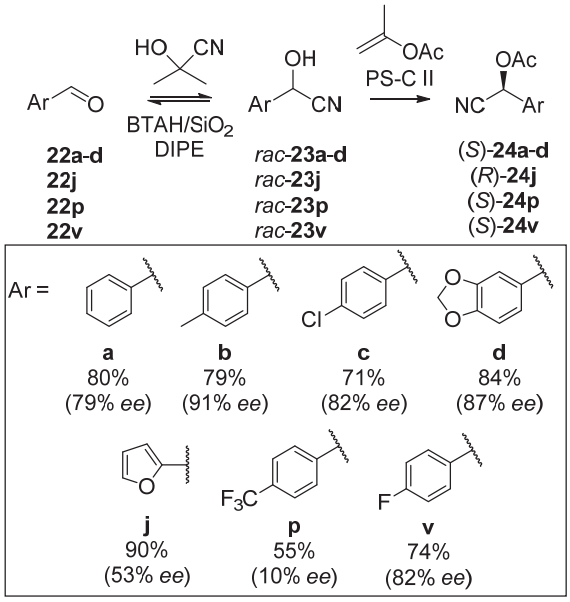
While studying the CAL-B (Novozym 435)-catalyzed DKR of mandelonitrile (*rac*-**23a**), Hanefeld *et al.* found that the DKR did not work although the KR under the same conditions in the absence of the base proceeded with excellent enantioselectivity (*E*>400) (Scheme 6).[159–161] It turned out that the carrier material used in the lipase preparation had a significant effect on the DKR process. For example, when a polymethacrylate immobilized CAL-B preparation [CAL-Bpma (similar to Novozym 435)] was used, the conversion of the DKR remained low (<50%) even after 3 days with >92% *ee*. By changing the carrier material to Celite R-633, CAL-B (CAL-Bcel), (*S*)-**24a** could be obtained in 97% isolated yield and 98% *ee* in 4 days. This effect relates to the hydrophobic qualities of the carrier material: the polymethacrylate material will expel any water included in the preparation thus enabling hydrolytic side reactions. The hydrophilic Celite-material (also known as diatomaceous earth, a natural silicate capable of binding water *via* hydrogen bonding) was expected to hold on to any water encountered in the reaction mixture and play down hydrolytic side reactions. The same conditions yielded (*R*)-**24j** in 92% isolated yield with 89% *ee*, a clear improvement to the results seen before (Table 4, entry 10).



Scheme 6. The synthesis of (*S*)-cyanohydrin acetates [(*S*)-**24a,t,u** and (*R*)-**24j**] by DKR.[159–161]

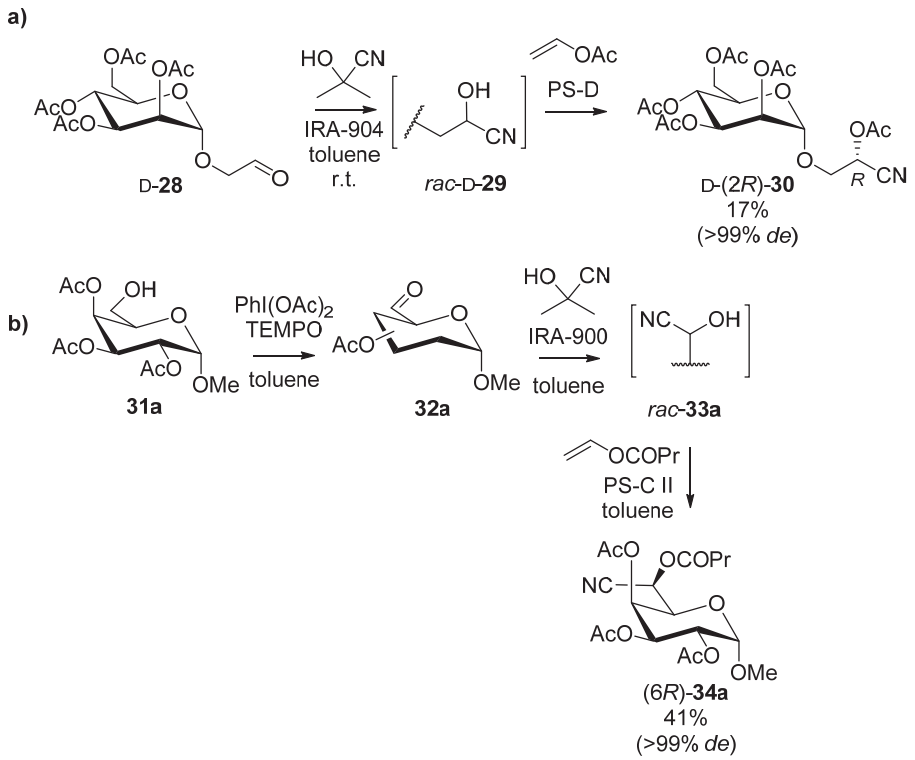
Changing the basic Amberlite IRA-904 (OH- form) resin to something more stable has been proposed to improve the yield, enantiomeric purity of the products and the time required for the DKR. By using a silica-supported benzyltrimethylammonium hydroxide (BTAH-SiO_2) catalyst together with lipase PS-C II, significant reduction in

reaction time (now 15–71 hours) was seen in every case (Scheme 7).[162] Unfortunately, the isolated yields and enantiomeric purities for the products **24** were lower than previously presented (Tables 5 and 6, Scheme 6).



Scheme 7. The preparation of (*S*)-**24a-d**, (*R*)-**24j**, (*S*)-**24p** and (*S*)-**24v** by silica-supported benzyltrimethyl-ammonium hydroxide (BTAH-SiO_2) and lipase PS-C II.[162]

Recently, the cyanohydrin DKR process has been utilized in the synthesis of glycopyranoside-based compounds. For example, the 2,3,4,6-tetra-*O*-acetyl mannosyl aldehydes D-**28** and L-**28** were used as a starting point in the synthesis of cyanohydrin acetates D-(2*R*)-**30** and L-(2*R*)-**30** (Scheme 8a).[163] Depending on the mannoside moiety, up to >99% *de* with 17% isolated yield [for D-(2*R*)-**26**] could be obtained. A three-step one-pot cascade synthesis of sugar cyanohydrin esters through the combination of TEMPO-catalyzed oxidation and classical cyanohydrin DKR (asymmetric synthesis with lipase catalysis in combination with the use of a basic resin [Amberlite IRA-900]) (Scheme 8b).[164] Preparative-scale reactions beginning from methyl 2,3,4-triacetyl-α-D-galactopyranoside (**31a**) enabled the one-pot synthesis of butanoate (6*R*)-**34a** in 41% isolated yield and excellent diastereomeric purity (>99% *de*). Reactions starting with methyl 2,3,4-triacetyl-α-D-gluco- and mannopyranosides (**31b** and **31c**, respectively) showed noticeable lower stereoselectivity and were subsequently not attempted at preparative scale.



Scheme 8. a) Syntheses of a) D-(2*R*)-**30** and b) a three-step one-pot formation of cyanohydrin butanoate (6*R*)-**34a**.[163,164]

2.3.3. Dynamic systemic resolution

The synthesis of individual structurally similar compounds en masse can be laborious depending on the case, but necessary for use in screening processes. The strength of combinatorial chemistry (CC) can be attributed in its ability to synthesize pools or libraries of structurally similar compounds. One combinatorial approach, dynamic combinatorial chemistry (DCC), enables the use of adaptive compound libraries, so-called dynamic combinatorial libraries (DCLs).[165,166,167] A given DCL will contain the real combinations of constituents present at any time, which is only a subset of all those being potential and virtually available (virtual combinatorial library).[167] The reversible nature of DCC enables the construction of such molecular systems which can balance themselves and adapt to, for example, changes in physical (such as temperature[168], light[169] or electric field[170]) or chemical conditions (for example, addition of metal cations[171], change in pH[168] or phase changing[172]) and amplify the fittest compound of all possible library constituents. It also permits the utilization of more subtle interactions, such as hydrogen bonding[173] or CH/π stacking[174] with regard to studying

molecular recognition processes important for chemical biology. As a further development to DCC, dynamic systemic resolution (DSR) [also known as dynamic combinatorial resolution (DCR) earlier] combines the thermodynamic step forming the DCL from the initial building blocks and a following (irreversible) selection step of this library mixture to give the resolution products (Figure 19).[175] Depending on the conditions of library formation and the nature of the selection under the reaction conditions, individual system components can be favored at the expense of the others. The approach enables the studying of target enzymes; the thermodynamically controlled system produces the substrates for the studied enzymes.

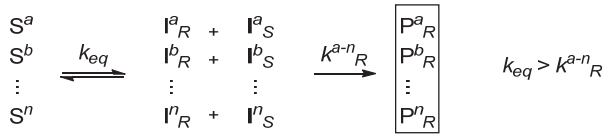
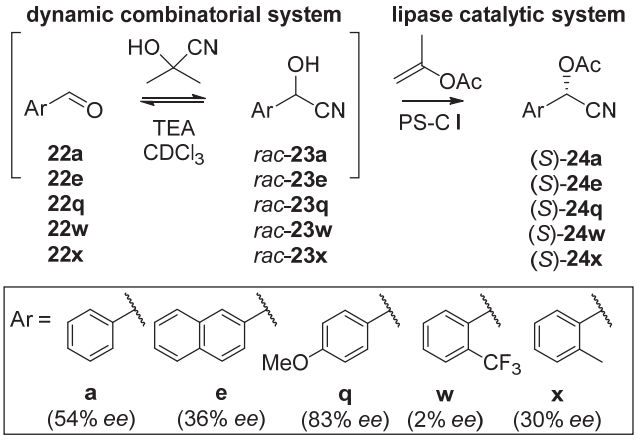


Figure 19. The principle of dynamic systemic resolution (DSR).[175]

The DSR approach has been applied in diverse systems based on the nitroaldol (Henry) reaction,[176–178] domino thia-Michael-Henry reaction,[179] hemithioacetal lactonization,[180] thiolester exchange,[181,182] 1,3-cycloaddition of azomethine ylides[183] and in a dual dynamic combinatorial resolution system based on the Strecker reaction[184]. In 2011, the use of a cyanohydrin DSR as an *in situ* evaluation system for lipase catalysts and reaction conditions was described (Scheme 9).[185] In the experiments, five aldehydes (**22**) together with equimolar amounts of acetone cyanohydrin and a base [triethylamine (TEA) was found best suited over pyridine, *N*,*N*-diisopropylethylamine or 4-dimethylaminopyridine (DMAP)] were left to form the racemic cyanohydrin *rac*-**23** overnight in the presence of a lipase preparation (lipase PS-C I, BCL immobilized on ceramic beads, was chosen). Then, the acyl donor (5 equiv.) was added and the reaction was followed by ^{1}H NMR [the *ee*-values for the formed products (*S*)-**24** were established by chiral HPLC after a bisulfite treatment and subsequent removal of remaining **22** as adducts from the mixture by filtration]. Now, (*S*)-**23a** was found to be the most preferable substrate for lipase PS-C I–catalyzed acylation as (*S*)-**24a** (with up to 54% *ee*) was the product that was most formed. The effect of acyl donor, temperature and solvent were also probed with chloroform together with isopropenyl acetate at 0°C giving the best results. The highest enantioselectivity under the reaction conditions (83% *ee*), was measured for (*S*)-**24q**. TEA was as the

selected base, catalyzed the reasonable fast equilibrium (3 h) between the mixture of aldehydes **22** and racemic cyanohydrins *rac*-**23**.



Scheme 9. Cyanohydrin DSR process.[185]

2.4. Lipases in regioselective modification of carbohydrates

2.4.1. Regioselectivity of lipase catalyzed reactions

In the realm of lipase-catalyzed reactions with substrates containing multiple similar groups, aspects such as regioselectivity become important. This holds true also for reactions catalyzed by lipases in nature: reactions towards the primary positions of a triacylglycerol (*sn*-1,3) or at all positions (*sn*-1,2 and 3) are favored with the existence of a pure *sn*-2 selective enzyme being rare (Figure 20).[186] The occasions where *sn*-2 selectivity is suspected, the possibility of acyl migration should be considered (acyl migration from *sn*-2 to free primary hydroxyl groups at positions *sn*-1,3 can also explain the total hydrolysis of triglycerides by lipases in many cases).[187] When the number of similar functional groups increases, the need to understand the selectivity of the catalyst grows. For instance, regioselectivity of lipase-catalyzed acylation and deacylation of carbohydrates and derivatives thereof represents an intriguing landscape as several similar functional groups are present and can be affected in different manners. Additionally, other functionalities may also be present in the substrate compound as sugar moieties are frequently encountered in natural products.

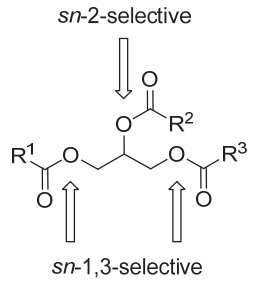


Figure 20. Regioselectivity with triacylglycerols.

Monosaccharides [$C_n(H_2O)_m$], both aldehydes and ketoses in their open chain form, are the simplest of carbohydrates (which are also commonly referred as sugars). They are also the smallest repeating units in larger di-, oligo- and polysaccharides. In Figure 21, the structures of three typical aldohexose monosaccharides [D-glucose (D-Glc, **35**), D-galactose (D-Gal, **36**) and D-mannose (D-Man, **37**)] in their cyclic hemiacetal form (hexopyranose) are presented. The chemical reactivity of the different hydroxyl groups in the six-member hexopyranose ring tends to follow the order: 6-OH > 1-OH > 2-OH > 3-OH > 4-OH. Differences arise, when the orientation (axial and equatorial) of the hydroxyl groups in the sugar ring differs. For lipase-catalyzed acylation reactions, both the catalyst and the substrate in question affect the regioselectivity. For instance, the 2-OH in D-Man (**37**) as an axially oriented hydroxyl group can be a difficult target for lipase catalysis while with the equatorially oriented 2-OH-group in D-Glc (**35**) can be acylated by a lipase catalyst [naturally, only after the most reactive position(s) have either reacted or been otherwise blocked by protective groups; for preparation of esters of secondary OH-groups].[188,189] For hexopyranoses, lipase-catalyzed acylation with reactive acyl donors (such as enol esters, oximes and trihaloethyl esters) produces 6-*O*-acylated monoesters whereas reactions with alkyl (or aryl) glycopyranosides (Figure 21) will produce further acylated products as well (*e.g.* 2,6-di-*O*-acylated or 3,6-di-*O*-acylated products).[190]

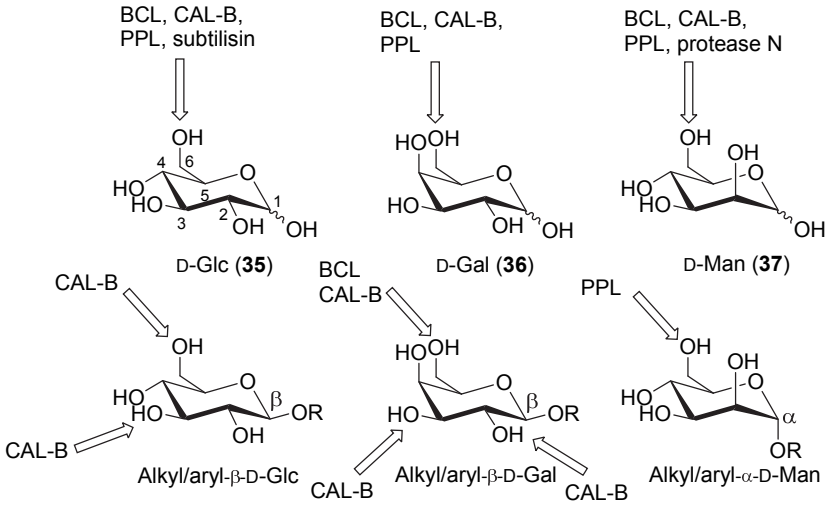


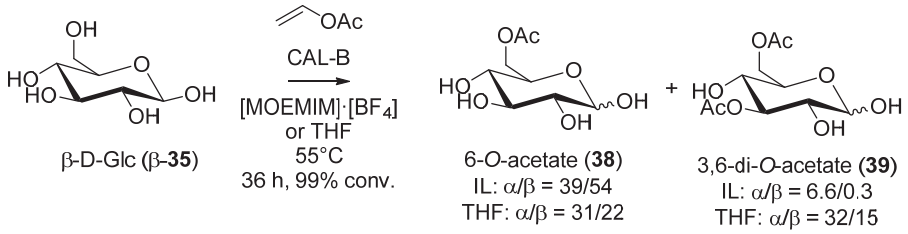
Figure 21. Hexopyranose structures of D-glucose (D-Glc, **35**), D-galactose (D-Gal, **36**) and D-mannose (D-Man, **37**) with corresponding alkyl/aryl D-glycopyranosides (as β- or α-anomers). Preferential positions for lipase-catalyzed acylation reactions highlighted.[190]

2.4.2. O-acylation of glycopyranosides and functional acyl donors

In the modification of natural products and use in protective group chemistry, the regioselectivity of lipase catalysis has been utilized in the preparation of esters of mono-, di- and oligosaccharides, alkaloids, steroids and phenols in organic solvents.[190] One aspect that has deeply affected the use of carbohydrates as targets for lipase-catalyzed acylation reactions has been the poor solubility of sugars to organic solvents, especially the solvents that are generally used in lipase-catalyzed reactions. To address this problem, circumventive approaches have been developed. The first examples of regioselective biocatalytic acylation of sugars were presented by Klibanov,[188,191,192] where porcine pancreatic lipase (PPL) and *Bacillus subtilis* protease (subtilisin) were used in polar solvents enabling full solvability of sugars, such as in DMF and pyridine. But as these solvents are incompatible with many enzymes, circumventing methods have been developed. Firstly, the use of solvents (and mixtures thereof) enabling (at least partial) solvability, such as THF[188,193,194], *tert*-butanol[195,196], *tert*-amyl alcohol (2-methyl-2-butanol, *t*-AmOH)[197,198], acetone[199,200], acetonitrile[201,202] have found use in many occations. Another simple solution has been to use alkyl (or aryl) glycopyranosides which offer higher solubility to organic solvents instead of free monosaccharides.[190] Finally, more unconventional approaches have been developed to

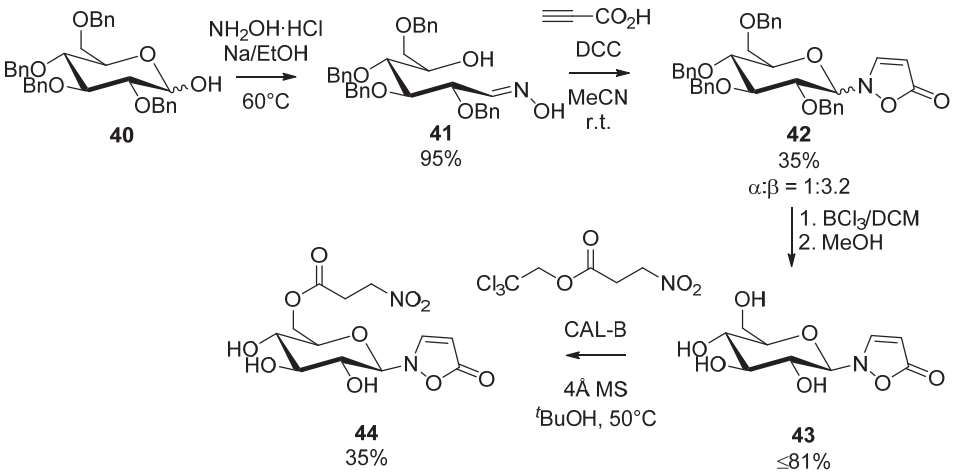
increase solubility. For instance, the use of boronic acid complexation to enhance solubility of sugars has been presented.[203]

Both the nature of the solvent and the acyl donor will affect the outcome. For instance, the CAL-B–catalyzed synthesis of 6-*O*-acetyl β-D-glucose (**38**) together with the formation of further acylated 3,6-di-*O*-acetyl β-D-glucose (**39**) was achieved with [MOEMIM]·[BF_4] (3-methoxyethyl-1-methylimidazolium tetrafluoroborate), an ionic liquid (IL) exhibiting notably better capabilities in dissolving glucose than, for instance, THF (Scheme 10).[193] The degree of mono *vs.* diacylation and extent of anomerization was clearly affected by the solvent system used, the use of IL leading to the formation of less **39** and less anomerization (ratio of α- and β-sugars).



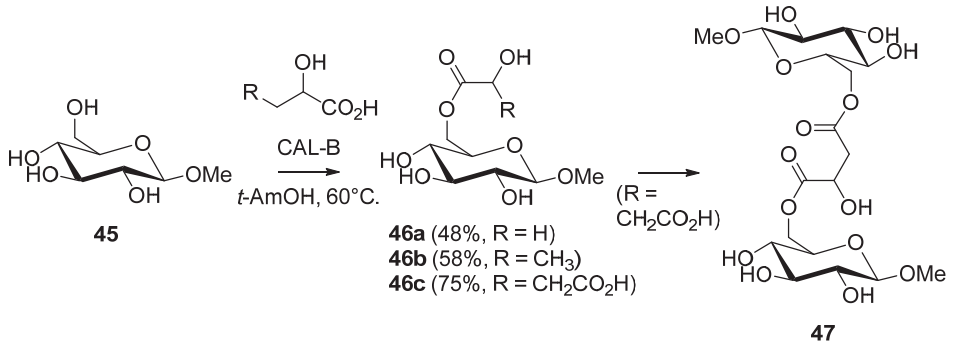
Scheme 10. Product distribution in the regioselective CAL-B catalyzed acylation of β-D-Glc (**35**) with vinyl acetate in [MOEMIM]·[BF_4] (IL) and THF.[193]

In addition to the more typical acyl donors such as acetates and butanoates, polyfunctional acyl donors have also been used. Selected examples of the use of such acyl donors, with more than one ester/acid functionality (*i.e.* polyfunctional acyl donors), are next presented. For instance, the selectivity of lipase-catalyzed acylation reactions has been exploited in the synthesis of 2-[6'-(3''-nitropropanoyl)-β-D-glucopyranosyl]-3-isoxazolin-5-one (**44**), a component of the defensive secretions of adult leaf beetles (Chrysomelidae). In the last step, CAL-B–catalyzed regioselective (95% 6-*O*-acylated product) acylation with the activated acyl donor gave **44** with 35% yield (Scheme 11).[204]



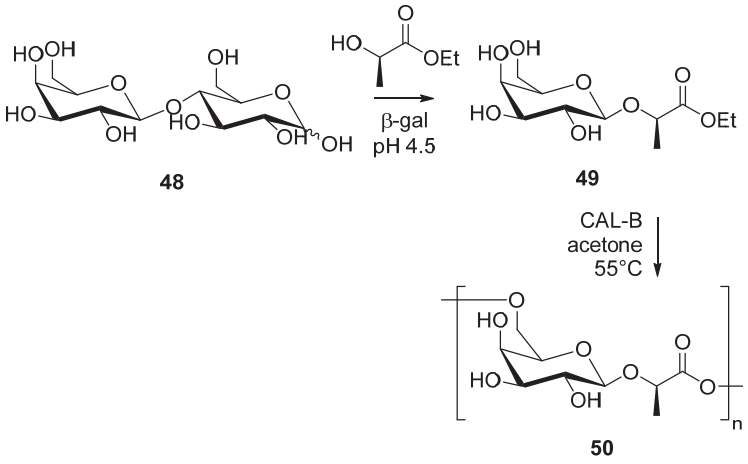
Scheme 11. The chemoenzymatic synthesis of 2-[6'-(3''-nitropropanoyl)-β-D-glucopyranosyl]-3-isoxazolin-5-one (**44**).[204]

With regard to the target compounds, the synthesis of surfactants and emulsifiers has been an important part of lipase-catalyzed acylations of carbohydrates.[195,196,199] The use of α-hydroxy acids (such as lactic acid) as acyl donors in the acylation of carbohydrates and alkyl glycosides in the presence of CAL-B enabled the preparation of hydrating and moisturizing agents.[205,206] When three α-hydroxy acids (glycolic, lactic and malic acids) were reacted with methyl β-D-glucopyranoside (**45**) in the presence of CAL-B in *t*-AmOH, conversions of up to 75% (with malic acid) were observed (Scheme 12).[207] The use of malic acid, a dicarboxylic acid, would also lead to the formation of diester **47** (80:20 monoester/diester), linking two sugar moieties together with a malic linker.



Scheme 12. The CAL-B–catalyzed preparation of α-hydroxy acid esters **46a-c**.

In addition to use as acyl donors, polyfunctional acyl donors, such as α-hydroxy acids, can enable further synthetic steps as well. For example, the combination of β-galactosidase (β-gal) and CAL-B catalysis enabled the preparation of 800–2000 MW polymer **50** from D-lactose (**48**) and ethyl L-lactate (Scheme 13).[208] First, the β-gal–catalyzed step formed β-D-galactosyl-L-lactic acid ethyl ester **49** (up to 27% isolated yield) which was subsequently polymerized to **50** by CAL-B–catalyzed with up to 60% conversion after 4 days. The C6-acylation site was presumed based on previous work.



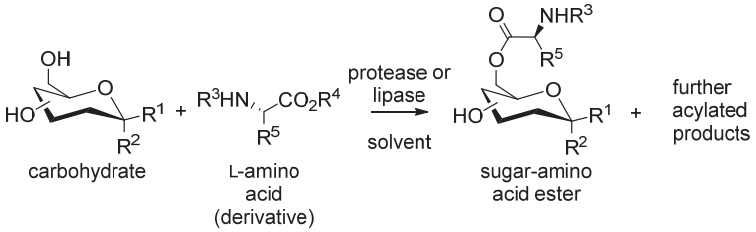
Scheme 13. Using β-D-galactosyl-L-lactic acid ethyl ester (**49**) as an intramolecular acyl donor in the CAL-B–catalyzed polymerization reaction to produce polymer **50**.[208]

2.4.3. Sugar amino acid esters by enzymatic synthesis

Glycosylated proteins, sugar peptides and other conjugates of carbohydrates and amino acids represent a wide area of biologically active compounds. Instead of lipases being used to prepare amino acid carbohydrate esters, the research area has been dominated by another type of hydrolases, proteases. In the first such published example, Riva *et al.* showed the effectiveness of *Bacillus subtilis* protease (subtilisin, EC 3.4.21.62) in the preparation of sugar amino acid conjugates from *N*-acetyl amino acid chloroethyl esters with D-glucose, D-fructose, D-sorbitol and methyl β-D-galactopyranoside in dry DMF (Table 7, entries 1–4).[192] A commercial protease preparation, Optimase M-440 (contains subtilisin from *Bacillus licheniformis*), was used as a catalyst in the preparation of 6-*O*-(*N*-Boc-L-phenylalanyl) sugars from the *N*-Boc-protected amino acid trifluoroethyl ester and a variety of carbohydrates in anhydrous pyridine showing up to 96% conversions (entries 5–7).[209] Later, the regioselective acylation of disaccharides (sucrose and trehalose) and carbohydrates containing two primary hydroxyl groups

(such as D-sorbitol, D-xylitol and D-mannitol) with *N*-protected activated amino acid esters (*e.g.* trifluoroethyl esters of *N*-Boc-L-phenylalanine, *N*-Boc-L-leucine and *N*-Boc-L-methionine) could also be effectively achieved in the presence of Optimase M-440 in pyridine.[210,211] Finally, a comparison of enzyme-surfactant preparations of a number of lipases (*Candida rugosa*, *Mucor javanicus*, *Pseudomonas cepacia* and *Pseudomonas fluorescens*) and proteases (subtilisin Carlsberg, *Aspergillus melleus*, *Aspergillus oryzae* and *Bacillus* sp.) showed a clear preference for the use of subtilisin in the synthesis of *N*-acetyl-amino acid esters of carbohydrates with up to 71% conversions in 24 h when cyanomethyl esters of amino acids are used as acyl donors in polar solvent systems (10% DMSO in *tert*-butanol).[212] However, when unprotected carbohydrates (including D-Glc, D-Gal, D-Man and others) and amino acids (L-proline, L-phenylalanine, L-tryptophan and L-histidine) were reacted in the presence of *Candida rugosa* lipase (CRL) in 9:1 dichloromethane (DCM)/DMF, both mono- and diacylated ester products (total yields ranged from 7–79% depending on the case) could be identified (entries 9–11).[213] Similar results have been obtained for RML-or PPL-catalyzed reactions between D-Glc and L-alanine, L-phenylalanine and L-leucine (entries 12 and 13).[214–216]

Table 7. Selected examples of the use of amino acids (and esters thereof) as acyl donors in the regioselective acylation of monosaccharides.



Entry	Sugar	Catalyst	Acyl donor	Solvent	Conv.[a] (%)	Position[b]	Ref.
1	D-Glc (**35**)	subtilisin	Ac-L-Phe-OCH_2Cl	DMF	73 (40)	6 (80) 3 (15) 2 (5)	[192]
2	Me β-D-Gal (**51**)	subtilisin	Ac-L-Phe-OCH_2Cl	DMF	66 (36)	6 (95)	[192]
3	Me β-D-Gal (**51**)	subtilisin	Ac-L-Met-OCH_2Cl	DMF	70 (54)	6 (87) 3 (8)	[192]
4	Me β-D-Gal (**51**)	subtilisin	Ac-L-Ala-OCH_2Cl	DMF	70 (49)	6 (84) 4 (8)	[192]
5	D-Glc (**35**)	Optimase M-440	Boc-L-Phe-OCH_2CF_3	pyridine	96 (-)	6 (100)	[209]

Table 7 continued

6	D-Gal (**36**)	Optimase M-440	Boc-L-Phe-OCH_2CF_3	pyridine	50 (-)	6 (100)	[209]
7	Me α-D-Glc (**52b**)	Optimase M-440	Boc-L-Phe-OCH_2CF_3	pyridine	74 (-)	6 (100)	[209]
8	D-Glc (**35**)	CRL	L-Pro	9:1 DCM/ DMF[c]	60 (-)	6 (48) 3 (26) 2 (26)	[213]
9	D-Glc (**35**)	CRL	L-Phe	9:1 DCM/ DMF[c]	79 (-)	6 (25) 3 (23) 2 (19) 2,6 (17) 3,6 (16)	[213]
10	D-Glc (**35**)	CRL	L-Trp	9:1 DCM/ DMF[c]	42 (-)	6 (38) 2 (22) 3 (21) 2,6 (10) 3,6 (9)	[213]
11	D-Glc (**35**)	CRL	L-His	9:1 DCM/ DMF[c]	32 (-)	6 (28) 2 (25) 3 (24) 2,6 (12) 3,6 (11)	[213]
12	D-Glc (**35**)	RML	L-Phe	9:1 DCM/ DMF	>99 (-)	6 (-) 2 (-) 3 (-) 2,6 (-) 3,6 (-)	[214]
13	D-Glc (**35**)	PPL	L-Phe	9:1 DCM/ DMF[c]	87 (-)	6 (-) 2 (-) 3 (-) 2,6 (-) 3,6 (-)	[214]

[a]Conversion (and isolated yield in parenthesis). [b]Acylation position (% of product mixture in parenthesis). [c]With added 0.001–0.1 M acetate buffer, pH 4–7.

3. AIMS OF THE STUDY

The primary aim of the thesis was to study the applicability of lipases in chemoenzymatic synthesis. The stereo- and regioselectivity offered by lipases was exploited in the synthesis of a number of challenging synthetic scenarios. One challenge was posed by the similarity (and the polyfunctional nature) of the used substrates, especially when analyzing reaction mixtures. Secondly, control over the hydrolytic nature of the lipase catalyst under the reaction conditions has been of the essence. Accordingly, the main focus has been in the optimization of lipase-catalyzed reactions and in the development of suitable analysis methods (even when analyzing complex mixtures of structurally similar compounds). The examples presented herein include the preparation of enantiomerically enriched heterocyclic aromatic secondary alcohols through kinetic and dynamic kinetic resolution (Papers I and II), together with the regioselective preparation of methyl α-D-glycopyranoside glyceric acid esters (Paper III) and β-amino acid esters (Paper IV). All formed products and developed methods can be considered important as such, or as part of developing compounds and synthetic pathways of pharmaceutical importance. The main challenges encountered during the experimental work for Papers I–IV:

A. Applicability of combinatorial chemistry and especially dynamic systemic resolution (DSR) in the development and optimization of dynamic kinetic resolution (DKR) of cyanohydrins.
B. Transesterification, hydrolysis (alcoholysis) and esterification as competing catalytic pathways in lipase-catalyzed acylation reactions.
C. Exploitation of the regioselectivity of lipase catalysis in order to avoid protective group chemistry.
D. Analysis of structurally similar compounds (and mixtures thereof).

4. MATERIALS AND METHODS

4.1. Materials

All chemical reagents were either purchased from commercial sources (Sigma-Aldrich, TCI Europe or Carbosynth Ltd) or were synthesized as presented in the original publications.[I-IV] Certain reagents, such as acetone cyanohydrin and vinyl or isopropenyl esters were freshly distilled prior to use. The solvents used were dried prior to use either by refluxing over CaH_2 followed by distillation, or with molecular sieves (3Å) unless otherwise stated. Water-contents of the solvents used in the enzymatic reactions were determined by Karl Fischer titration using a Metrohm 831 KF Coulometer (when the reaction mixtures did not include desiccants such as molecular sieves). The lipase preparations were obtained from commercial sources: Lipase PS-D and Lipase PS-C II preparations were from Amano Europe containing *Burkholderia cepacia* lipase (BCL) immobilized by adsorption to diatomaceous earth and covalently to ceramic beads (Toyonite 200), respectively. *Candida antarctica* lipase B (CAL-B) as the Novozym 435 preparation (immobilized on a polyacrylic resin), *Thermomyces lanuginosus* lipase (TLL) as the Lipozyme TL IM preparation (immobilized on silica), *Rhizomucor miehei* lipase (RML) as the Lipozyme RM IM preparation (immobilized on silica) were products of Novozymes. *Pseudomonas fluorescens* lipase (PFL) as the IMMPF-T2-150 preparation (immobilized covalently on polyacrylic beads) was a product of ChiralVision, while *Candida antarctica* lipase A (CAL-A) as the NZL-101-IMB preparation was from Biocatalytics. Some lipase preparations were also made in-house (BCL, CAL-A or PFL immobilized on Celite, referred as BCL-Cel, CAL-A-Cel and PFL-Cel).[217] More details on immobilized lipase preparations can be seen in Section 2.1.3.

4.2. Small-scale enzymatic reactions

Small-scale enzymatic reactions (reaction volume 1–2 mL) were typically performed in 4 mL corkscrew-capped vials and the reaction mixtures were shaken (*ca.* 170 rpm) at the given temperatures [47°C or 23°C (RT)].. Reactions were followed by taking samples (50–100 μL) from the reaction mixtures at times and by analyzing the samples with the developed GC- or HPLC-methods after derivatization and possible dilution. For reactions with furan-based secondary alcohols (**15**, **17**) and the reactions of cyanohydrins (**56-58**), the samples (100 μL) were drawn by syringe and filtered

(Millex-FH, 4 mm × 0.45 μm, PTFE) before derivatization by the addition of an acid anhydride (acetic, propanoic or butanoic anhydride, 5–10 μL) and 1% DMAP/Py (5 μL) for analysis as described in Papers I and II. To detect carboxylic acid contents with the furan-based reactions (Paper I), non-derivatized samples were analyzed also by GC. For reactions concerning reactions with sugars (**52a-c** in Papers III and IV), the samples (50 μL) were drawn from the reaction mixtures, filtered, diluted with dry acetonitrile (total sample volume 150 μL) and analyzed by RP-HPLC. For reactions with β-lactams *rac*-**8a-d** (Paper IV), an additional sample (50 μL) was also taken, filtered and diluted with hexane (150 μL) and analyzed by chiral HPLC.

In the studies concerning the furan-based secondary alcohols and cyanohydrins (Papers I and II), an Agilent 6850 GC/FID or a HP 5890 Series II GC/FID equipped with a Chrompack CP-Chiralsil-DEX CB (25 m × 0.25 mm × 0.25 μm) –column was used. For following the reactions with sugars (Papers III and IV), a RP-HPLC method was used utilizing a Waters 2690 Separations Module equipped with an ESA Corona CAD detector, Waters 2990 DAD detector (for samples containing chromophoric compounds) and an Agilent Technologies ZORBAX Eclipse XDB-C8 (4.6 mm × 150 mm × 5 μm) –column. The eluents used in the developed HPLC methods were mixtures of water and methanol as specified in the original publications and supplementary materials together with retention times for individual compounds. Resolutions of β-lactam enantiomers (Paper IV) were accomplished either with a HP 1090 HPLC/DAD equipped with a Daicel Chiralcel OD-H (250 mm × 4.6 mm × 5 μm) -column or a Agilent 6850 GC/FID equipped with a Chrompack CP-Chiralsil-DEX CB (25 m × 0.25 mm × 0.25 μm) –column. More detailed descriptions of the analysis methods are presented in the original publications and in the available associated supplementary information. Conversion of substrates to products was determined as disappearance against an internal standard or calculated from the *ee*-values (see equation 2, section 2.2.1) when stated. The values for the enantiomeric ratio (*E*) were calculated by the use of linear regression (*E* as the slope of $\ln[(1-c)(1-ee_S)]$ *vs.* $\ln[(1-c)(1+ee_S)]$.

4.3. Preparative scale enzymatic reactions

Up-scaled versions of the small-scale enzymatic reactions, or so-called preparative scale reactions, were performed under the optimized conditions (*e.g.* concentration, temperature and time) established in the small-scale reactions (reaction volume typically

10–30 mL). The reactions would be terminated at a given time by enzyme filtration and concentration of the filtrate. The products were purified by column chromatography [Merck Kieselgel 60 (0.063–0.200 μm)].

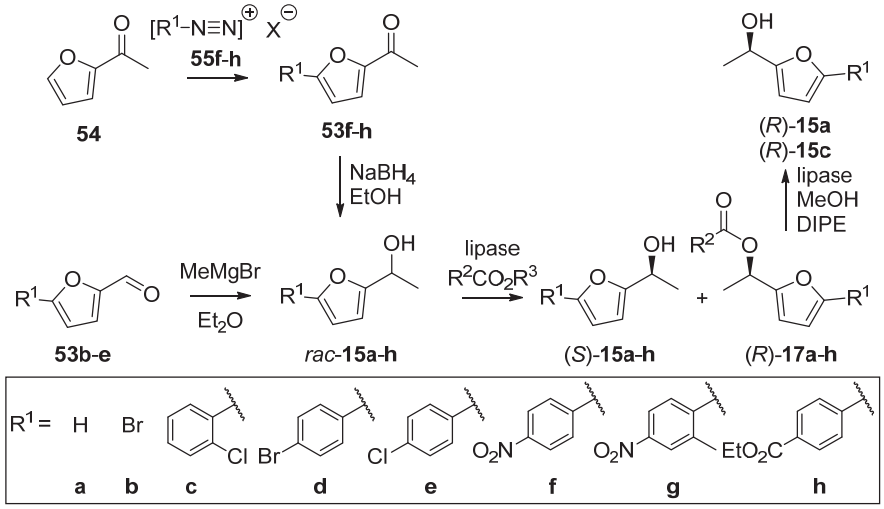
4.4. Product characterization

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded with a Bruker Avance 500 NMR spectrometer (working at 500, 471 and 126 MHz, respectively) equipped with a BBI 5mm-Zgrad ATM or BBO 5mm-Zgrad probe at 298K. 2D NMR experiments such as ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC were conducted when required for assignation of signals. High resolution mass spectra (HRMS) were recorded with a Bruker micrOTOF-Q (ESI) spectrometer on positive mode or with a VG ZabSpec 7070E (EI) on positive mode. Merck Kieselgel $60F_{254}$ sheets were used in analytical thin-layer chromatography (TLC). Melting points of solids were measured with a Sanyo Gallenkamp device and are uncorrected. Optical rotations were measured with a Perkin-Elmer 341 Polarimeter and are presented in units of $[\alpha]_T^D = 10^{-1}$ deg cm^{-2} g^{-1} in the original publications.

5. RESULTS AND DISCUSSION

5.1. Kinetic resolution of 1-(furanyl)ethanols (Paper I)

In paper I, the chemoenzymatic preparation of the enantiomers of furan-based secondary alcohols **15a-h** was examined (Scheme 14). The furan ring represents a reactive aromatic moiety for the synthesis biologically active compounds.[218] The racemic substrate alcohols *rac*-**15b-g** were prepared by a Grignard reaction from the corresponding aldehydes **53b-e** or by reduction with $NaBH_4$ from the corresponding ketones **53f-h** (*rac*-**15a** was commercially available). The ketones in turn were synthesized by a variation of the Meerwein method[219] from 1-(furan-2-yl)ethanone (**54**) and the corresponding diazonium salts (**55f-h**).



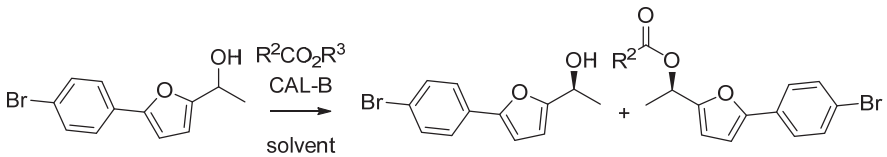
Scheme 14. Chemoenzymatic preparation of the enantiomers of 1-furylethanols **15a-h**.

Optimization

From a number of commercial lipase preparations (containing BCL and CAL-B) and lipases immobilized in-house (BCL-Cel, CAL-A-Cel and PFL-Cel), CAL-B (10 mg mL^{-1}) was found the most enantioselective (E = 27) in the enzymatic KR of *rac*-**15d** (50 mM) as a model substrate with vinyl butanoate (2 equiv.) as an acyl donor in undried DIPE (directly from a bottle) at room temperature. In comparison, BCL as the PSL-C preparation showed poor enantioselectivity (E = 4) in the KR of *rac*-**15a** also earlier (Section 2.2.4, Table 4).[119] The amount of CAL-B was screened (from 2–20 mg mL^{-1}) and the optimal quantity was found to be 5 mg mL^{-1}. Optimization was performed by screening acyl donors and solvents (Table 8), using the acylation of *rac*-**15d** (50 mM)

with an acyl donor (2 equiv.) in the presence of CAL-B (5 mg mL^{-1}) as a model reaction. The best results close to 50% conversion were obtained with the combination of vinyl acetate and DIPE (entry 7). However, the lipase-catalyzed acylation ceased before 50% conversion, the value of *E* varied notably with conversion and the remaining (*S*)-**15d** was not being purified further (from 92% *ee*) at late conversion stages. This leads to conclude that side reactions are involved in the acylation. Accordingly, the *E*-values were deemed unreliable (are marked as *E'* in the Table below).

Table 8. Acylation of *rac*-**15d** (50 mM) with an acyl donor (2 equiv.) in dried organic solvents in the presence of CAL-B (5 mg mL^{-1}).



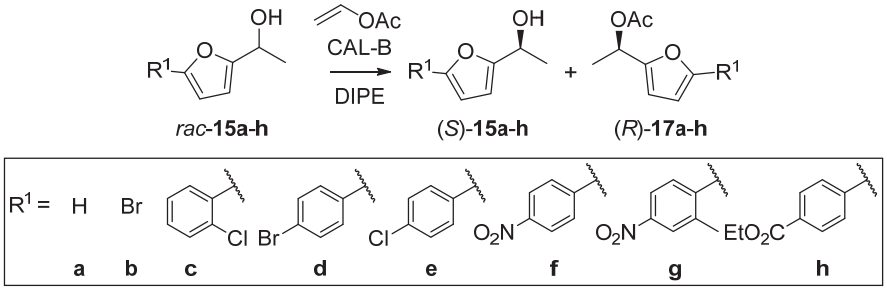
rac-**15d** (*S*)-**15d** (*R*)-**17d**

Entry	Solvent	Acyl donor	Time (h)	Conv. (%)[a]	$ee^{(S)\text{-}15}$ (%)	$ee^{(R)\text{-}17}$ (%)	*E*'[b]
1	DIPE	Vinyl butanoate	2.5	49	86	90	50
2	Toluene	Vinyl butanoate	3.5	48	82	86	35
3	TBME	Vinyl butanoate	3.5	49	83	85	35
4	Hexane	Vinyl butanoate	0.5	48	82	87	25
5	DIPE	TFEB[c]	3.5	47	74	85	26
6	Ethyl butanoate[d]		3.5	40	54	80	15
7	DIPE	Vinyl acetate	1.5	50	92	93	90
8	Toluene	Vinyl acetate	1.5	44	73	93	60
9	TBME	Vinyl acetate	1.5	47	82	92	60
10	Hexane	Vinyl acetate	1.5	51	94	92	80

[a]Calculated from the *ee*-values. [b]Marked as *E'* due to inaccuracy following possibility of side reactions. [c]TFEB = 2,2,2-trifluoroethyl butanoate. [d]Ethyl butanoate acting both as a solvent and an acyl donor.

The established reaction conditions were next applied to the other substrates *rac*-**15a-h** (Table 9). Now, similar behavior for *rac*-**15c** (entry 3) was observed as for *rac*-**15d** before. Poor results (*E* = 25) led to discontinuation of studies with *rac*-**15e** (entry 5).

Table 9. Acylation of *rac*-**15a-h** (50 mM) with vinyl acetate (2 equiv.) in the presence of CAL-B (5 mg mL^{-1}) in DIPE at room temperature.



Entry	Substrate	Time (h)	Conv. (%)	$ee^{(S)\text{-}15}$ (%)	$ee^{(R)\text{-}17}$ (%)	*E*
1	*rac*-**48a**[a]	2	50	98	99	>200
2	*rac*-**48b**[a]	2	50	98	99	>200
3	*rac*-**48c**[a]	2	39	60	96	-[b]
4	*rac*-**48d**	1.5	49	91	93	-[b]
5	*rac*-**48e**	1.5	48	78	83	25±5
6	*rac*-**48f**	1.5	44	76	97	177±21
7	*rac*-**48g**	1.5	50	95	95	150±10
8	*rac*-**48h**	1.5	50	94	94	90±13

[a]With *rac*-**15** (100 mM), vinyl acetate (2 equiv.) and CAL-B (1 mg mL^{-1}). [b]Unreliable value of *E* as discussed above.

Examining possible esterification side reaction

The hydrolysis of the acyl donor (CH_2=CHOAc) and the formed product ester [(*R*)-**17**] in the presence of lipase catalyst are potential side reactions and possible explanations for the behavior observed above for the acylation of *rac*-**15d** in DIPE (Table 8, see also Section 2.1.4. Figure 10). Accordingly, the amount of acetic or butanoic acid in the reaction mixtures was examined by GC from underivatized samples (Figure 22). The amount of the acid increased almost linearly with conversion until the KR had reached 50% conversion. Thereafter, the formation of the acid turned faster. As the esterification of *rac*-**15** can progress with a difference in enantioselectivity to the acylation reaction (transesterification) and the lipase-catalyzed hydrolysis of formed product ester evidently favors the hydrolysis of the (*R*)-ester, they both can have implications for the end result as a whole.

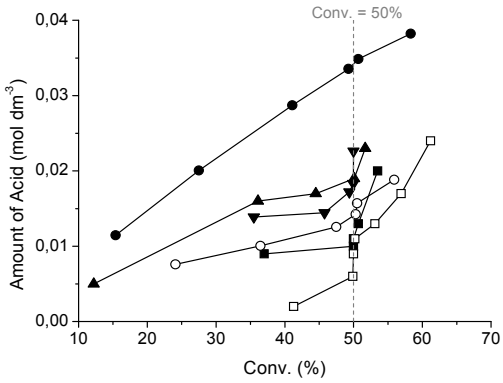
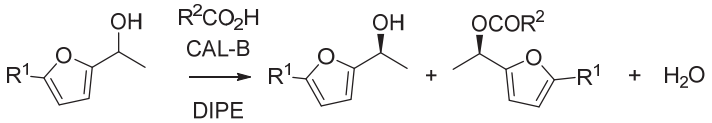


Figure 22. Acid formation during the acylation of *rac*-**15a-c** [100 mM; *rac*-**15a** (■), *rac*-**15b** (●) and *rac*-**15c** (▲)] and *rac*-**15d** (50 mM, ▼) with vinyl acetate (2 equiv., filled signs) or vinyl butanoate (2 equiv., open signs) in DIPE in the presence of CAL-B (1 mg mL^{-1} for *rac*-**15a-c** and 5 mg mL^{-1} for *rac*-**15d**).

Next, the esterification of *rac*-**15b** (100 mM) and *rac*-**15d** (50 mM) with acetic and butanoic acid was studied separately in DIPE in the presence of CAL-B (1 mg mL^{-1}) at room temperature. The rather enantioselective esterification of *rac*-**15b** (94–99% *ee* at 1–11% conversion) reached equilibrium within 2 h (Table 10, entries 1–4 and 6–9). As expected, initial rates (v_0) for the esterification as well as the amount of (*R*)-**17b** increased with increasing acid concentration (although the acid concentration here is relatively small in comparison to the amount of vinyl ester used above). However, the esterification of *rac*-**15d** (50 mM) with the carboxylic acids (1 equiv.) gave (*R*)-**17d** with only moderate enantioselectivity (entries 5 and 10). Overall, the esterification result suggests that when the carboxylic acid is present in the acylation mixture, it can take part in the formation of the acylated product at some level. Enantioselectivity of the reaction depends on the structure of the acylated alcohol and the acyl donor.

Table 10. Esterification of *rac*-**15b** (100 mM) and *rac*-**15d** (50 mM) with acetic and butanoic acid in DIPE in the presence of CAL-B (1 mg mL^{-1}) at room temperature.



rac-**15b** (*S*)-**15b** (*R*)-**17b**
rac-**15d** (*S*)-**15d** (*R*)-**17d**

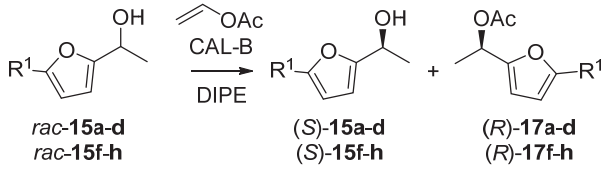
Entry	Alcohol	Acid (mM)	v_o (μmol min^{-1} mg^{-1})	[(*R*)-**17**][a] (%)	$ee^{(R)\text{-}17}$[b] (%)
1	*rac*-**15b**	AcOH (50)	13.6±0.7	10	98
2	*rac*-**15b**	AcOH (20)	6.9±0.1	7	97
3	*rac*-**15b**	AcOH (10)	5.0±0.4	4	95
4	*rac*-**15b**	AcOH (5)	2.5±0.3	3	94
5	*rac*-**15d**	AcOH (50)	-	26	79
6	*rac*-**15b**	$PrCO_2H$ (50)	13.5±0.3	11	98
7	*rac*-**15b**	$PrCO_2H$ (20)	4.4±0.5	6	95
8	*rac*-**15b**	$PrCO_2H$ (10)	1.7±0.3	3	98
9	*rac*-**15b**	$PrCO_2H$ (5)	0.7±0.1	1	99
10	*rac*-**15d**[c]	$PrCO_2H$ (50)	-	22	85

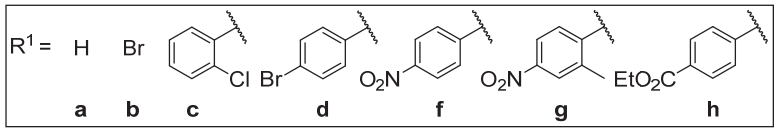
[a]Amount of (*R*)-**17** after 2 h. [b]*ee* after 2 h. [c]10 mg mL^{-1} CAL-B; reaction time 1.5 h.

Preparative-scale reactions

The preparative scale KR of *rac*-**15a-d** and *rac*-**15f-h** (100 mM for *rac*-**15a-c**, 50 mM *rac*-**15d** and **f-h**) with vinyl acetate (2 equiv.) in the presence of CAL-B (1 mg mL^{-1} for *rac*-**15a-c** and 5 mg mL^{-1} for *rac*-**15d** and **f-h**) resulted in good isolated yields at 50% conversion (91–99% *ee*) (Table 11). Racemization during silica column purification was observed that affected mainly the ester products [*e.g.* (*R*)-**17d**, entry 4]. For instance, when (*R*)-**17d** (50 mM, 56% *ee*) and silica (100 mg mL^{-1}) were mixed with dichloromethane (the solvent used as eluent), 0% *ee* was detected after 1 h. To prevent loss of enantiomeric purity during purification, TEA (0.5–1%) was added to the eluent to neutralize the silica. Another intriguing possibility is that self-disproportionation of enantiomers during column purification might affect the end result with these compounds.[220] The possibility for further enantiomeric purification of (*R*)-**17** by lipase-catalyzed alcoholysis was examined by subjecting *rac*-**17a** (100 mM) and *rac*-**17c** (100 mM) with methanol (5 equiv.) in the presence of CAL-B (5 mg mL^{-1}) in DIPE at RT, giving good results (E >200 and E = 96, respectively). Accordingly, (*R*)-**17a** (92% *ee*) and (*R*)-**17c** (90% *ee*) were transformed to (*R*)-**15a** (83% isolated yield, 99% *ee*) and (*R*)-**15c** (76% isolated yield, 99% *ee*) in preparative scale.

Table 11. Preparative scale KR of *rac*-**15a-d** and *rac*-**15f-h** with vinyl acetate (2 equiv.) in the presence of CAL-B (1 or 5 mg mL^{-1}) in DIPE.





Entry	Substrate	Time (h)	Conv. (%)[c]	$ee^{(S)\text{-}15}$ (%)[d]	$ee^{(R)\text{-}17}$ (%)[d]	Isolated yield (%)[e]	
						(*S*)-**15**	(*R*)-**17**
1	*rac*-**15a**[a]	5	50	99	98	42 (98)	43 (92)
2	*rac*-**15b**[a]	4	50	96	95	45 (95)	45 (94)
3	*rac*-**15c**[a]	23	50	99	98	48 (98)	49 (90)
4	*rac*-**15d**[b]	2	50	92	91	21 (91)	55 (75)
5	*rac*-**15f**[b]	10	49	94	96	28 (89)	27 (90)
6	*rac*-**15g**[b]	5	51	98	94	46 (95)	46 (88)
7	*rac*-**15h**[b]	4	50	93	94	48 (93)	43 (86)

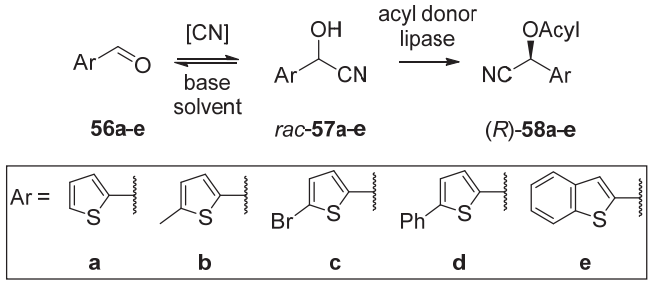
[a]*rac*-**15** (100 mM), vinyl acetate (2 equiv.), CAL-B (1 mg mL^{-1}). [b]*rac*-**15** (50 mM), vinyl acetate (2 equiv.), CAL-B (5 mg mL^{-1}). [c]As calculated from the *ee*-values in the reaction mixture. [d]*ee* in the resolution mixture. [e]As calculated from the racemic mixture [*ee* (%) after purification shown in parenthesis].

The results in Table 11 show that the examining potential side reactions and minimizing them by optimization of reaction conditions allows the KR of *rac*-**15a-d** and *rac*-**15f-h**. Clearly, some of the substrate alcohols (*rac*-**15c** and *rac*-**15d**) were more prone towards hydrolytic side reactions whereas others were not. Also, the loss of enantiomeric purity during purification was significant at times. As a potential remedy, the subsequent lipase-catalyzed methanolysis of formed product acetates was presented.

5.2. Dynamic systemic resolution in optimization of a dynamic kinetic resolution of cyanohydrins (Paper II)

The effectiveness of screenings in the optimization of biocatalytic reactions suffers from the fact that they are typically performed using a model substrate. However, it is characteristic to biocatalysis that catalysts are often selective to changes in the substrate structure, *i.e.* the selected conditions may be less attractive to some (related) substrates. In 2011, Ramström *et al.* presented the DSR of cyanohydrins,[185] being used in the evaluation of lipase performance in asymmetric synthesis (as discussed earlier in Section 2.3.3). In paper II, the DSR of five thiophene-based cyanohydrins *rac*-**57a-e** is

presented as a tool in evaluating optimal reaction conditions for each individual cyanohydrin rather than using the DKR of one selected model substrate (Scheme 15). Finally, the method was validated by preparing each cyanohydrin ester (*R*)-**58a-e** with the one-pot lipase-catalyzed acylation reaction including the *in situ* base-catalyzed preparation of the free racemic cyanohydrin from the corresponding aldehyde (**56a-e**).

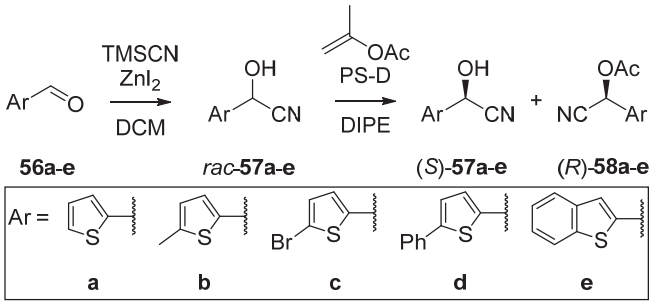


Scheme 15. General scheme for the DSR (with all five aldehydes at the same time)/DKR (one aldehyde at a time) process in Paper II.

Kinetic resolution of racemic cyanohydrins

In order to have a point of reference and to be able to evaluate lipase performance during DSR experiments, a conventional lipase screening was conducted. For this purpose, each *rac*-**57a-e** was prepared by ZnI_2-catalyzed reaction[221] from **56a-e** and trimethylsilyl cyanide (TMSCN) in dichloromethane, giving the corresponding *rac*-**57a-e** at 88–99% isolated yields. Acylation of *rac*-**57a** (100 mM) with isopropenyl acetate (2 equiv.) in DIPE at RT as a model reaction was first screened with six commercial lipase preparations (50 mg mL^{-1}, containing BCL, CAL-B, CAL-A, PFL, RML and TLL). Lipase PS-D preparation (50 mg mL^{-1}), as the most successful catalyst, was thereafter used to examine the acylation of the other racemic cyanohydrins *rac*-**57b-e** under the same conditions (Table 12). The ee_P^0-value at zero conversion for a given KR represents the highest possible value for ee_P in the corresponding successful DKR process,[129,222] and the ee_P-values can be deduced from the *E* values of the KRs. The use of toluene instead of DIPE resulted in slightly higher enantioselectivity (entries 1 and 2).

Table 12. Chemical synthesis and the acylation of *rac*-**57a-e** (100 mM) with isopropenyl acetate (2 equiv.) in DIPE in the presence of lipase PS-D (25 mg mL^{-1}) at RT after 4 h.



Entry	Compound	Yield (%)[a]	Conv. (%)[b]	$ee^{(S)\text{-}57}$ (%)	$ee^{(R)\text{-}58}$ (%)	*E*	$ee^{(R)\text{-}58}$ (%)[c]
1	*rac*-**57a**	97	33	44	88	23±2	92
2	*rac*-**57a**[d]	-	17	19	95	28±2	93
3	*rac*-**57b**	95	32	45	97	127±9	98
4	*rac*-**57c**	99	20	23	93	36±3	94
5	*rac*-**57d**	90	32	42	90	27±3	93
6	*rac*-**57e**	88	27	26	72	8±1	78

[a]Isolated yield of *rac*-**57a-e** prepared from **56a-e**. [b]Conversion calculated from the *ee*-values. [c]Theoretical $ee_P{}^0 = (E\text{-}1)/(E\text{+}1)$.[222] [d]Toluene as the solvent.

Formation of a library of racemic cyanohydrins

The formation of the mixture of *rac*-**57a-e** from **56a-e** (20 mM each, total aldehyde content 100 mM) in a thermodynamically controlled equilibrium reaction in toluene in the presence of different bases at various amounts [Amberlite IRA-904 (OH^- form; 10 mg mL^{-1}), Amberlite IRA-900 (OH^- form; 10, 5 and 2 mg mL^{-1}), TEA (10 equiv., 1 equiv. and 0.1 equiv. to total aldehyde content), anhydrous Na_2CO_3 (10 equiv.) and anhydrous K_2CO_3 (10 equiv.)] was studied for initial evaluation (Figure 23a). From the bases, Amberlite IRA-904 has been a classical choice for cyanohydrin DKR,[153–161,163] but has since then been commercially discontinued. For this reason, Amberlite IRA-900, another strongly basic quaternary ammonium anion exchange resin with apparent similar properties to Amberlite IRA-904, was chosen as an alternative. TEA, on the other hand, was a soluble base utilized by Ramström *et al.* in their publication.[185] Both anhydrous Na_2CO_3 and K_2CO_3 are solid salts and potential desiccants (10 equiv. to total aldehyde content was found as a maximum amount due to mixing problems). As the source of cyanide, acetone cyanohydrin (1 equiv. and 3 equiv.) was preferred as it has been regarded as a safer option to HCN. Samples from the mixtures were analyzed by a developed chiral GC method enabling the determination of both conversion (disappearance of **56**) and enantiomeric purity of formed cyanohydrins (**57** and **58**).

In Figure 23a, the effect of the base on the degree of conversion (from **56a-e** to *rac*-**57a-e**) and in Figure 23b on the relative amount of formed *rac*-**58a-e** (in the presence of isopropenyl acetate) is presented in increasingly darker color. The amounts of formed *rac*-**57a-e** were observed to follow the order: **57e** > **57c** > **57a** > **57d** > **57b** (regardless of amount or type of base used; changes in acetone cyanohydrin content did not change the order). While the use of TEA (0.1 equiv.) led to the rapid formation of racemic cyanohydrins *rac*-**57a-e** (<0.5 h to reach equilibrium-like plateau), increasingly darker color and precipitation appeared with increasing TEA content (1 equiv. and 10 equiv.), reminiscent of HCN-polymerization was observed. Similar observations were seen when Amberlite IRA-900 (10 and 5 mg mL^{-1}) was used. With Na_2CO_3 (10 equiv.), the equilibrium settled within 30 minutes without the formation of any noticeable precipitate or change in color. K_2CO_3 (10 equiv.) proved inefficient and was discontinued.

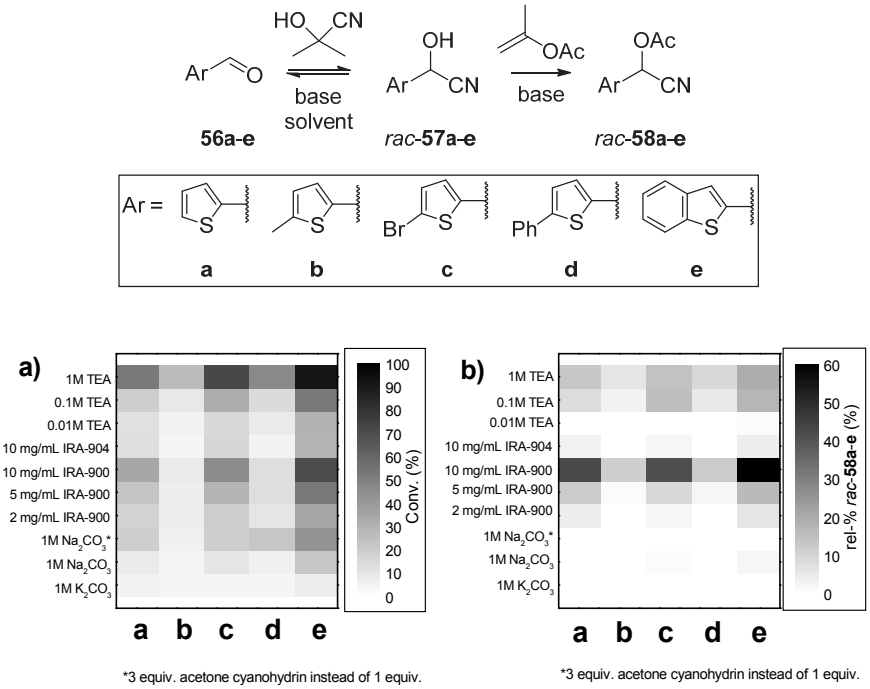


Figure 23. a) Formation of *rac*-**57a-e** from **56a-e** (20 mM each, 100 mM in total) and acetone cyanohydrin (1 equiv. to total aldehyde) in the presence of a base in toluene at 47°C (24 h). b) Estimation for the base-catalyzed formation of *rac*-**58a-e** under the conditions of Figure 24a when isopropenyl acetate (3 equiv. to total aldehyde) is present (24 h).

Possible base-catalyzed acylation of *rac*-**57a-e** can disturb the outcome of the lipase-catalyzed formation of (*R*)-**58a-e**. Thus, the mixture of **56a-e** (20 mM each, 100 mM in

total), acetone cyanohydrin (1 equiv.) and isopropenyl acetate (3 equiv.) was incubated in the presence a base, both in DIPE and toluene. After 24 h, the formation of *rac*-**58a-e** was observed especially when Amberlite IRA-900 (5 or 10 mg mL^{-1}) or TEA (1 equiv. or 10 equiv.) was present (Figure 23b). Base-catalyzed acylation was less prominent when 0.1 equiv. TEA, 10 equiv. K_2CO_3 or 10 equiv. Na_2CO_3 was used (with 3 equiv. of acetone cyanohydrin). Results obtained with Amberlite IRA-904 (10 mg mL^{-1}) demonstrate the capabilities of this resin, originally used in cyanohydrin DKR. The formation of *rac*-**58a-e** by base-catalyzed acylation is one possible side reaction observed in the presence of a base, the others being the polymerization of HCN and aldol condensation of **56a-e**. In conclusion, the use of a higher amount of acetone cyanohydrin (3 equiv.) is beneficial for the formation of *rac*-**57a-e**, and the nature of the base and its content should be sufficient in producing *rac*-**57a-e** without leading to base-catalyzed acylation.

Dynamic systemic resolution with cyanohydrins 57a-e

In the DSR experiments, aldehydes **56a-e** (20 mM each, 100 mM in total), acetone cyanohydrin (1 equiv.), Amberlite IRA-900 (2 mg mL^{-1}), TEA (1 equiv.) or Na_2CO_3 (10 equiv.), isopropenyl acetate (1 or 3 equiv.) and one of the lipase preparations (PS-D, CAL-B or IMMAPF, 10 or 25 mg mL^{-1}) were mixed together in toluene at either room temperature or 47°C (Figures 24a-e). Clearly, both the conversion and enantiomeric purity of the formed products were deeply affected by the choice of catalysts and reaction conditions. Thus, the reaction at RT (Figure 24d) led to accumulation of free cyanohydrins (*S*)-**57a-e** indicating a poorly functioning racemization step. Product formation was minimal with lipase IMMAPF (4–28%, with 14–90% *ee* in 24 h) leading to discontinuation with this lipase (Figure 24a-c). The use of either Amberlite IRA-900 or TEA (Figures 24a and b) resulted in low conversions [and poor enantioselectivity, *e.g.* (*R*)-**58e** only 12–18% *ee* with TEA]. Moreover, even with low base content (2 mg mL^{-1}), Amberlite IRA-900 led to apparent HCN condensation with the resin being dyed black during the reaction. When optimization was continued with Na_2CO_3 (10 equiv.) together with a higher amount of acetone cyanohydrin (3 equiv.), the use of lipase PS-D led to a clear increase in amounts of produced products (*R*)-**58a-e**, while with CAL-B the amount of products stayed unchanged (Figure 24c). Increasing the amount of lipase catalyst from 10 to 25 mg mL^{-1} increased the relative yields with both lipase preparations.

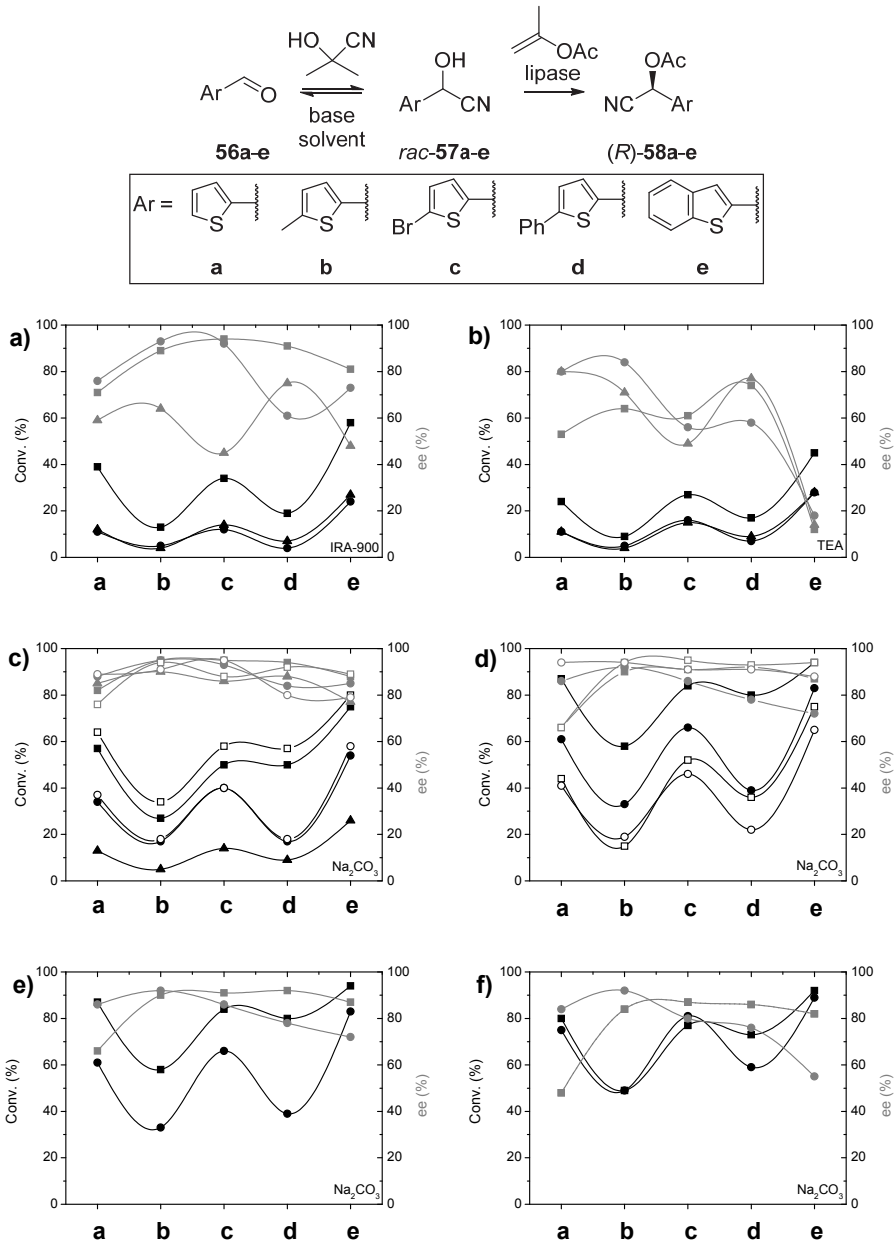


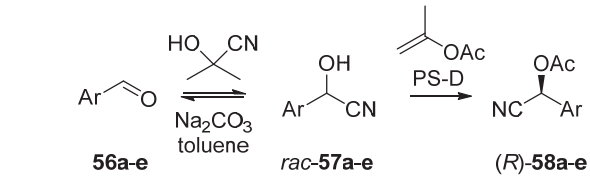
Figure 24. DSR results after 24 h in the mixture of **56a-e** (20 mM each, 100 mM in total), acetone cyanohydrin (1 equiv.), isopropenyl acetate (3 equiv.), a lipase preparation (10 mg mL^{-1}) and a base in toluene at 47°C: a) Amberlite IRA-900 (2 mg mL^{-1}), b) TEA (0.1 equiv.), c) Na_2CO_3 (10 equiv.) with acetone cyanohydrin [1 (solid signs) or 3 equiv. (open signs)], d) Na_2CO_3 (10 equiv.) with acetone cyanohydrin (3 equiv.) and a lipase preparation (25 mg mL^{-1}) at 47°C (solid) or RT (hollow), e) Na_2CO_3 (10 equiv.) with acetone cyanohydrin (3 equiv.) and a lipase preparation (25 mg mL^{-1}) in DIPE, f) Na_2CO_3 (10 equiv.) with acetone cyanohydrin (3 equiv.) and a lipase preparation (25 mg mL^{-1}). Conversion (black) and *ee* (gray) with lipase PS-D (■), CAL-B (●), lipase IMMPF (▲) are presented.

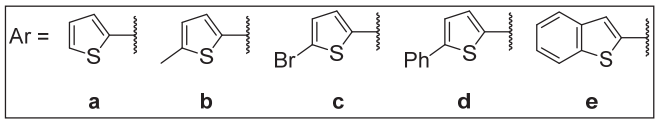
While the changes in the *ee*-values were negligible in toluene (Figure 24e), a clear difference was observed when DIPE was used as the solvent (Figure 24f). The results for lipase PS-D are in accordance with the estimated ee_P^0 from the independent KR experiments (Table 12) with the exception of products (*R*)-**58a** showing lower ee_P (48% *ee vs.* 93% *ee*; Table 12, entry 2) than was expected.

Preparing cyanohydrin esters (R)-58a-e by DKR

Preparative scale DKR of each of *rac*-**57a-e** were made by mixing one of the aldehydes **56a-e** (100 mM) together with acetone cyanohydrin (3 equiv.), isopropenyl acetate (3 equiv.), lipase PS-D (25 mg mL^{-1}) and Na_2CO_3 (10 equiv.) in toluene at 47°C (Table 13). The reactions proceeded for 1–4 days before halting. In every case, a longer reaction time tended to increase the yield at the expense of enantiomeric purity indicating a secondary process at work. For instance, (*R*)-**58e** could be isolated with 71% yield and 57% *ee* after a four day reaction, whereas a one day reaction ensured higher enantiopurity (91% *ee*) with a similar yield (68%) (entry 6). This behavior is most likely due to chemical acylation or the sum of competing ester hydrolysis, esterification and/or transesterification taking place concurrently as was detected with the furan-based alcohols earlier (section 5.1). As activated esters, the cyanohydrin ester products (*R*)-**58a-e** may be hydrolyzed by the lipase preparation (and the residual water it contains). To study this potential, (*R*)-**58a** (100 mM, 66% *ee*) was mixed with lipase PS-D (10 mg mL^{-1}), leading to the formation of (*R*)-**57a** both in toluene (85% *ee*, 6% conversion) and DIPE (62% *ee*, 42% conversion) in 24 h. As expected, the addition of Na_2CO_3 (10 equiv.) to these conditions only drove the reactions further to **56a** (18% and 57% conversions in toluene and DIPE, respectively) at the same time. Results obtained from the DCR experiments hinted to the capabilities of CAL-B in the case of **56a** and indeed, the enantiomeric purity of synthesized (*R*)-**58a** could be notably improved by selecting CAL-B instead of lipase PS-D (entry 1 *vs.* 2).

Table 13. Preparative scale DKR of cyanohydrins *rac*-**57a-e** (100 mM) with acetone cyanohydrin (3 equiv.) and isopropenyl acetate (3 equiv.) in the presence of Na_2CO_3 (10 equiv.) and lipase PS-D (25 mg mL^{-1}) in toluene at 47°C.





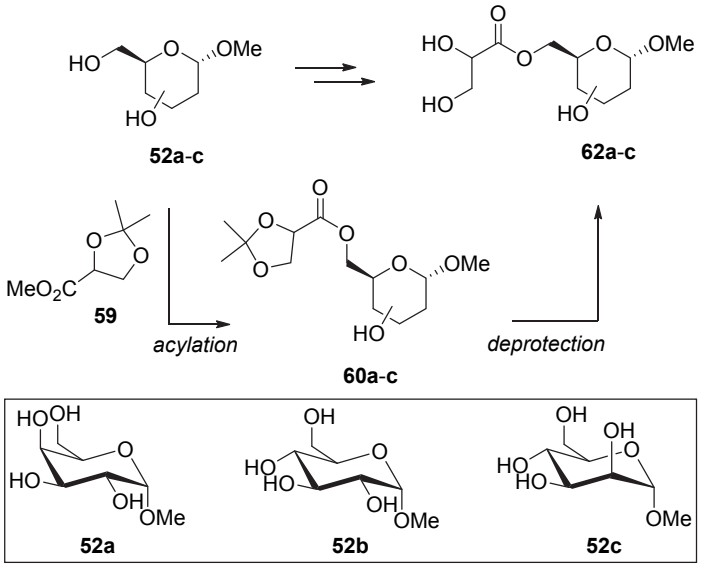
Entry	Aldehyde	Product	Time (d)[b]	Isolated yield (%)[b]	$ee^{(R)\text{-}58}$ (%)[b]
1	**56a**	(*R*)-**58a**	2 (4)	63 (99)	68 (62)
2	**56a**[a]	(*R*)-**58a**	4	88	82
3	**56b**	(*R*)-**58b**	3 (4)	92 (>99)	89 (86)
4	**56c**	(*R*)-**58c**	2 (4)	74 (88)	89 (82)
5	**56d**	(*R*)-**58d**	2 (4)	73 (86)	90 (85)
6	**56e**	(*R*)-**58e**	1 (4)	68 (71)	91 (57)

[a]CAL-B (25 mg mL^{-1}) used instead of lipase PS-D. [b]Values in parenthesis present results for preparative DKR after 4 days.

The DSR approach proved to be a valuable screening method for optimizing reaction conditions for chemoenzymatic DKR of cyanohydrins. However, the development of a successful DSR system can be limited by the need for analysis methods capable to give the essential information effectively.

5.3. Regioselective preparation of glyceric acid esters of methyl α-D-glycosides (Paper III)

The regioselective acylation of methyl α-D-galacto-, -gluco- and –mannopyranoside (**52a-c**, respectively) with a polyfunctional acyl donor in the presence of a lipase catalyst, was studied with the aim of synthesizing glyceric acid esters **62a-c** (Scheme 16). Similar compounds have been previously synthesized through the lipase-catalyzed esterification between sugars and free α-hydroxy acids (glycolic, lactic and malic acids).[205–207] The chosen acyl donors herein were the commercially available enantiomers of the isopropylidene-protected glyceric acid methyl ester [(*S*)- and (*R*)-**59**], the protection preventing the acyl donor to react with itself. Moreover, the use of (*S*)- and (*R*)-**59** ensured diastereomeric purity of the formed product.



Scheme 16. Preparation of glyceric acid esters **62a-c** *via* lipase-catalyzed regioselective acylation of methyl α-D-glycopyranosides **52a-c** with ester (*R*)- and (*S*)-**59**.

Optimization of solubility and regioselective acylation

Before small-scale screening experiments, the solubility of one of the substrates [methyl α-D-galactopyranoside (**52a**)] into selected organic solvents was evaluated (Figure 25). Clearly, solvents such as DMF and pyridine previously utilized by Klibanov *et al.*[188,191,192] in the acylation of sugars with PPL and subtilisin, are capable of dissolving **52a** but can lead to inactivation of lipase (as was observed herein as well). Moreover, solvents frequently utilized in lipase-catalyzed acylation reactions such as DIPE, TBME and toluene, were found unsuited in dissolving **52a**. Finally, *t*-AmOH was chosen for our studies. *t*-AmOH has been reported to dissolve crystalline α-glucose relatively well, solubility rising from 0.6 to 9.0 g L^{-1} with rising temperature (30–102°C), and the solubility of the sugar could be facilitated by the presence of sugar esters.[223,224]

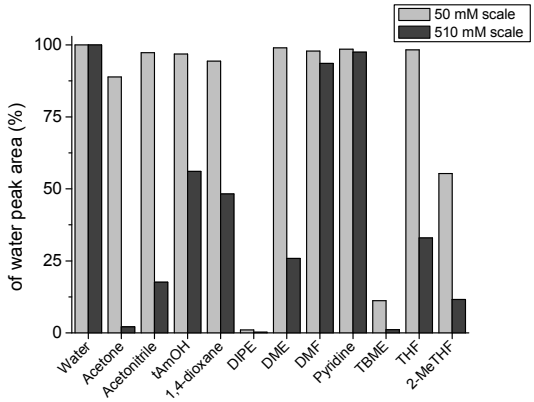
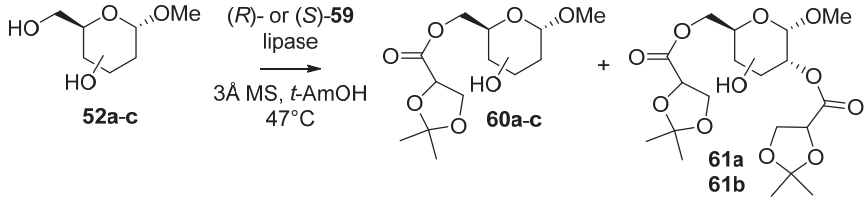


Figure 25. Solvability of methyl α-D-galactopyranoside (**52a**) into selected organic solvents *vs.* water.

In the screening of catalysts, a number of commercially available immobilized lipase preparations were evaluated in reaction between **52a-c**, (*S*)- and (*R*)-**59** in *t*-AmOH (Table 14). The reactions were performed in the presence of 3Å molecular sieves (50 mg mL^{-1}) in order to suppress hydrolysis side reactions. Generally, (*S*)- and (*R*)-**59** reacted similarly with the lipases with the (*R*)-enantiomer being somewhat more reactive (entries 1–8 and 11–15), lipase RM IM being an exception (entries 9 and 10). With the galacto- and glucopyranosides (**52a-b**), the formation of both 6-*O*-acylated (**60a-b**) and 2,6-*O*-diacylated products (**61a-b**) were observed whereas reactions with the mannopyranoside (**52c**) led only to the formation of the 6-*O*-acylated product **60c**. As lipase TL IM (entries 11, 12, 14 and 15) gave generally the highest productivity, it was chosen for further evaluation and reaction condition optimizing.

Table 14. Screening of lipases for the acylation of **52a-c** (50 mM) with (*R*)- or (*S*)-**59** (5 equiv.) in the presence of a lipase preparation (15 mg mL^{-1}) in *t*-AmOH at 47°C, after 24 h.



Entry	Lipase	(*R*)-/(*S*)-**59**	Amount of product (%)[a]				
			60a	**61a**	**60b**	**61b**	**60c**
1	CAL-B	*R*	51	6	71	12	71
2	CAL-B	*S*	24	1	55	1	41
3	CAL-A	*R*	3	<1	<1	-	<1
4	CAL-A	*S*	<1	-	<1	-	-
5	PS-D	*R*	48	4	53	17	43
6	PS-D	*S*	38	<1	23	1	17
7	PS-C II	*R*	45	4	42	9	36
8	PS-C II	*S*	33	<1	21	1	11
9	RM IM	*R*	26	<1	10	-	13
10	RM IM	*S*	38	<1	24	<1	35
11	TL IM	*R*	54	7	75	5	70
12	TL IM[b]	*R*	51	5	53	3	47
13	TL IM[c]	*R*	40	1	-	-	-
14	TL IM	*S*	52	9	65	3	62
15	TL IM[b]	*S*	40	3	-	-	-

[a]([**60**] or [**61**])/$[\mathbf{52}]_0$. [b]**59** (3 equiv.) with lipase TL IM (30 mg mL^{-1}). [c]Methyl β-D-galactopyranoside (**51**, 50 mM) in the place of **52a**; with **59** (3 equiv.) and lipase TL IM (30 mg mL^{-1}).

Studies with different lipase TL IM contents (5–50 mg mL^{-1}) in the reaction between **52a** (50 mM) and (*R*)-**59** (3 equiv.) in *t*-AmOH were performed to attain optimal catalyst content for the preparation of **60a-c** (together with **61a** and **61b**) (Figure 26a). With 50 mg mL^{-1} of lipase TL IM, the formation of **60a** was highest, but the disappearance (apparent hydrolysis by the residual water) of this 6-*O*-acylated product would also be accelerated (the formation of the 2,6-di-*O*-acylated product **61a** remained low, <5% by 48 h). Finally, 30 mg mL^{-1} for lipase TL IM was chosen as both the 6-*O*-acylated **60a** and 2,6-di-*O*-acylated **61a** products were wanted for further studying (the latter especially for analytical studies). When the amount of the acyl donor (*R*)-**59** was probed (1.2–7.0 equiv.) in the presence of lipase TL IM (30 mg mL^{-1}), it was concluded that while a higher acyl donor content would ensure a faster reaction (Figure 26b), the use of a more moderate amount of the reagent would be justified for economic reasons. With

this in mind, 3 equiv. of acyl donor would be used in the subsequent reactions with unprotected methyl α-D-glycopyranosides (**52a-c**).

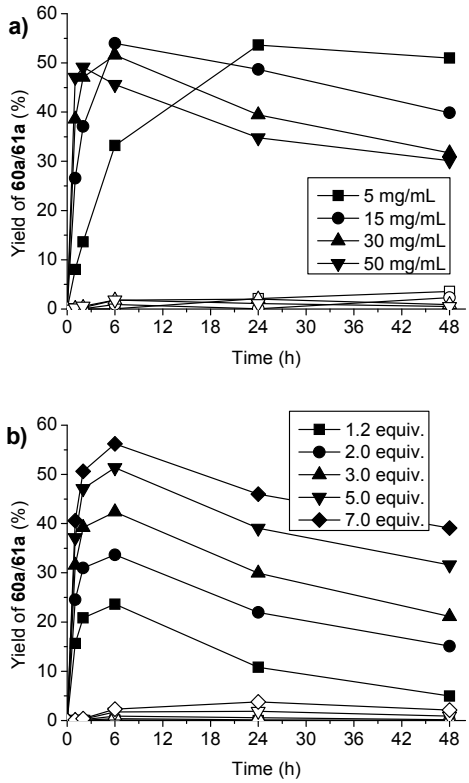
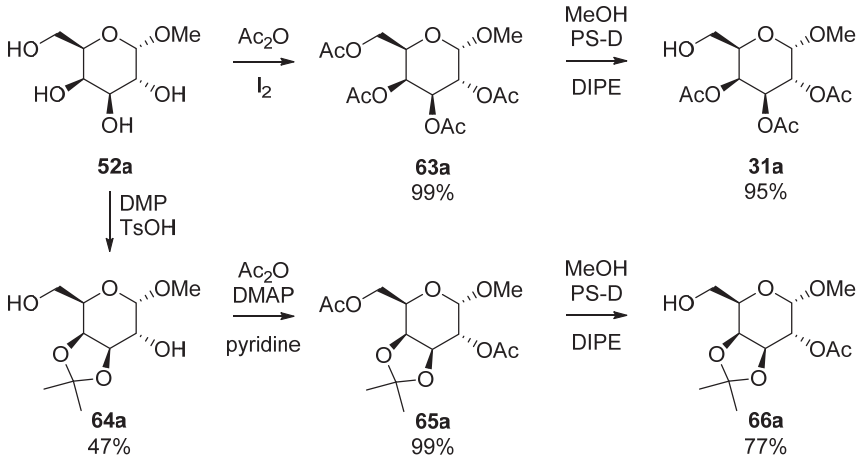


Figure 26. Acylation of **52a** (50 mM) with (*R*)-**59** in *t*-AmOH: a) effect of lipase TL IM content [5–50 mg mL^{-1}, with 5 equiv. (*R*)-**59**]; b) effect of amount of (*R*)-**59** (1.2–7.0 equiv., with 30 mg mL^{-1} lipase TL IM). The amount of **60a** (solid signs) and **61a** (open signs).

Studies with protected sugars

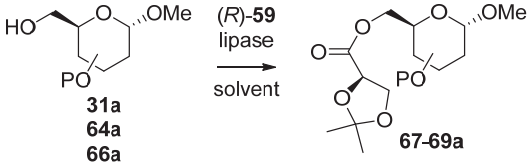
To facilitate solubility and to ensure only C6 acylation, selected protective group schemes were undertaken (Scheme 17). To attain the triacetylgalactose **31a**, **52a** was first peracetylated by I_2/Ac_2O to yield the tetraacetate **63a** (99% isolated yield),[225] which was in turn regioselectively deacetylated by lipase-catalyzed methanolysis to give **31a** in 95% yield.[164] The 3,4-*O*-isopropylidenation of **52a** was achieved with a treatment with 2,2-dimethoxypropane (DMP) in the presence of a catalytic amount of *para*-toluenesulfonyl acid (TsOH) giving the protected sugar **64a** (47% isolated yield).[226] Subsequent acetylation of **64a** and deprotection of **65a** by lipase-catalyzed methanolysis yielded the 2-*O*-acetyl-3,4-*O*-isopropylidene protected **66a**.



Scheme 17. The synthesis of partially protected methyl α-D-galactopyranosides **31a**, **64a** and **66a**.

The acylation of the partially protected **31a**, **64a** and **66a** with (*R*)-**59** in the presence of a lipase was next studied (Table 15). In addition to *t*-AmOH, the protected substrates could be dissolved in other solvents as well (such as DIPE and THF). Moreover, protected sugar derivatives allowed the use of up to 10 times higher sugar contents than with the unprotected **52a** (50 mM) (entry 10). The combination of **64a** and CAL-B in THF appeared to give highest productivity (entries 7–10).

Table 15. Acylation of protected methyl α-D-galactopyranosides (**31a**, **64a** and **66a**) with (*R*)-**59**; 24 h.



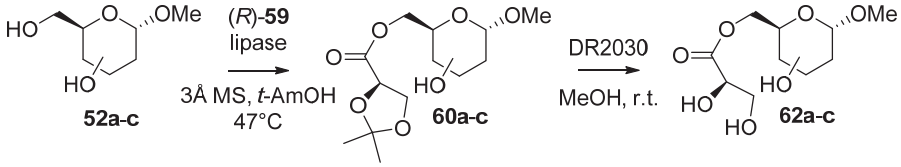
Entry	Substrate (mM)	Lipase (mg mL^{-1})	(*R*)-**59** (equiv.)	Solvent	Product (%)[a]
1	**31a** (50)	TL IM (30)	3	*t*-AmOH	**67a** (41)
2	**31a** (50)	TL IM (30)	3	DIPE	**67a** (44)
3	**31a** (50)	CAL-B (30)	3	DIPE	**67a** (16)
4	**31a** (50)	PS-D (30)	3	DIPE	**67a** (53)
5	**64a** (50)	TL IM (30)	3	*t*-AmOH	**68a** (63)
6	**64a** (50)	TL IM (15)	5	THF	**68a** (2)
7	**64a** (50)	CAL-B (15)	5	THF	**68a** (23)
8	**64a** (50)	CAL-B (30)	3	THF	**68a** (79)
9	**64a** (100)	CAL-B (30)	2	THF	**68a** (79)
10	**64a** (500)	CAL-B (30)	2	THF	**68a** (77)
11	**66a** (50)	TL IM (30)	3	DIPE	**69a** (29)
12	**66a** (50)	TL IM (30)	3	*t*-AmOH	**69a** (14)

[a]Relative amount according to RP-HPLC in parenthesis.

Preparative-scale synthesis of 62a-c and deprotection in the acyl side chain

Preparative-scale reactions under the established conditions gave the 6-*O*-acylated products **60a-c** in 40–57% isolated yields, with the diacylated **61a** and **61b** being also obtained after purification (Table 16, entries 1–3). By using the diisopropylidene protected **64a**, the 6-*O*-acylated **68a** could be isolated with a >99% yield (entry 4). The isopropylidene protected 6-*O*-acylated sugars **60a-c** and **68a** could be deprotected by treatment with acidic Dowex DR2030 resin (H^+ form)[227] in methanol to give **62a-c** with up to 93% yield.

Table 16. Preparative-scale acylation of **52a-c** (50 mM) with (*R*)-**59** (3 equiv.) in the presence of lipase TL IM (30 mg mL^{-1}) in *t*-AmOH at 47°C and subsequent deprotection of **61a-c** with Dowex DR2030 in methanol.



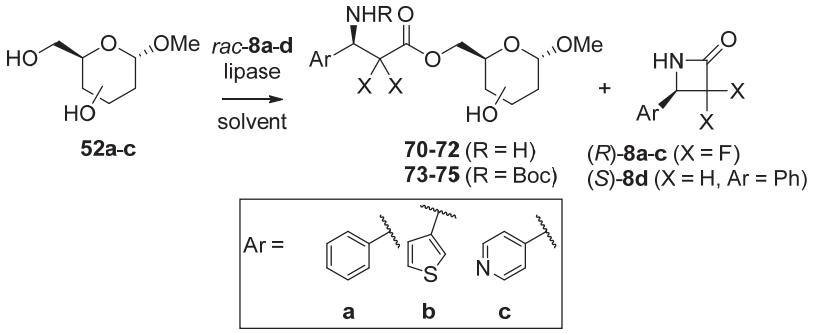
Entry	Substrate	Time (h)	Isolated yield (%)		
			60[a]	**61**[a]	**62**[b]
1	**52a**	48	42	8	93
2	**52b**	48	57	11	81
3	**52c**	24	40	-	83
4	**64a**[c]	72	>99[d]	-	76[e]

[a]Isolated yield from **52**. [b]Isolated yield from **60**. [c]**64a** (50 mM), (*R*)-**59** (2 equiv.), CAL-B (30 mg mL^{-1}) in THF at 47°C. [d]Product is **68a**. [e]Isolated yield from **68a**.

It can be concluded from the initial optimization and the experiments with the protected galactopyranosides that while the use of unprotected glycopyranosides is plausible given that a combination of a catalyst and a solvent with sufficient dissolving capabilities can be determined, the use of protected substrates can enable better solubility and subsequent use of higher substrate concentrations. However, the use of protective groups requires additional synthetic steps and the protected substrates might require further screening to attain good catalysts for them (the protected form of a substrate is in fact a new substrate for the catalyst altogether). Eventually, the combination of lipase-catalysis and protective group chemistry enabled the synthesis of three glyceric acid monoesters of methyl α-D-glycopyranosides.

5.4. Lipase-catalyzed ring-opening of β-lactams in preparation of β-amino acid esters of methyl α-D-glycosides (Paper IV)

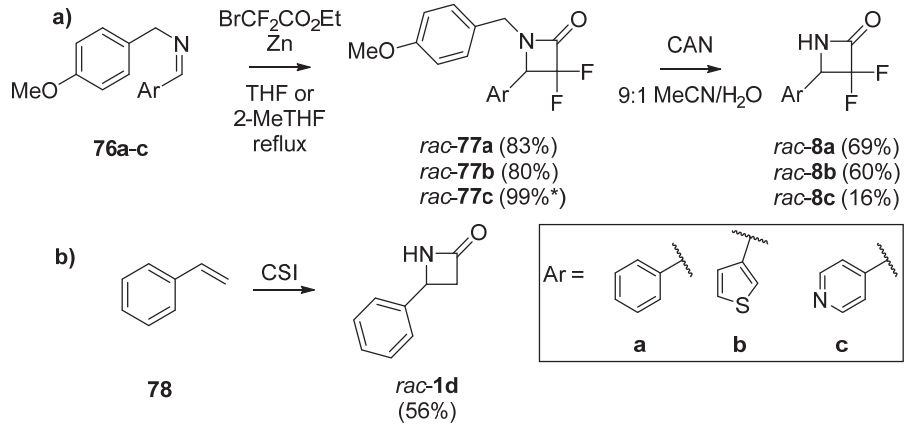
In paper IV, the lipase-catalyzed regioselective synthesis of 6-*O*-acylated sugar β-amino acid conjugates **70–75** through the enantioselective ring-opening of racemic 3,3-difluoro-4-aryl-β-lactams (*rac*-**8a-c**) was examined (Scheme 18). Previously, the synthesis of α-amino acid-sugar esters has utilized protease-[192,209–,211] or lipase-catalyzed[213–216] reactions between (protected) amino acids and carbohydrates (as discussed in Section 2.4.3.). Herein, activated β-lactams were exploited as irreversible acyl donors.



Scheme 18. Formation of sugar β-amino acid conjugates **70-75**.

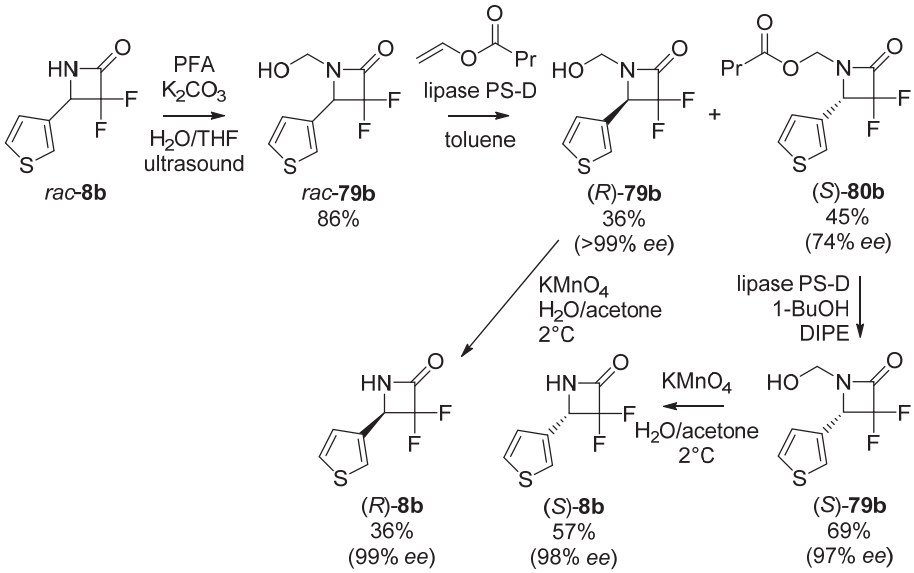
Synthesis of racemic β-lactams and method for enantioseparation

N-(*para*-Methoxybenzyl)-protected *rac*-**10a-c** were synthesized by a variation of the Reformatsky reaction from the corresponding aldimines **9a-c** with further oxidative deprotection by a treatment with ceric ammonium nitrate (CAN) yielding the racemic β-lactams *rac*-**8a-c** (Scheme 19).[228–230] The aldimines **9a-c** were prepared from the corresponding aldehydes in a reaction with *para*-methoxybenzylamine in the presence of $MgSO_4$. In the case of *rac*-**10c**, the obtained product was a 1/3 mixture with the corresponding ethyl ester but it was used as such for the CAN procedure. For comparative reasons, a non-fluorinated β-lactam (*rac*-**8d**) was also synthesized, now by a 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) to styrene (**45**) (Scheme 20).[231,232]



Scheme 19. Synthesis of a) 3,3-difluoro-4-aryl-β-lactams *rac*-**8a-c** and non-fluorine analogue *rac*-**8d**.

Enantiomers (*R*)- and (*S*)-**8b** were prepared through the lipase PS-D–catalyzed *O*-acylation of *N*-hydroxymethylated *rac*-**79b** (Scheme 20).[232–234] First, *N*-hydroxymethylation of *rac*-**8b** with paraformaldehyde (PFA) was followed by lipase PS-D–catalyzed *O*-acylation of *rac*-**79b** with vinyl butanoate. The resolution product (*S*)-**80b** was subjected to butanolysis in the presence of lipase PS-D, improving the enantiomeric purity significantly (from 74% *ee* to 97% *ee*). Finally, the *N*-hydroxymethyl side chain was oxidatively cleaved from both enantiomers (*S*)- and (*R*)-**79b** yielding (*S*)- and (*R*)-**1b** (with 98% and 99% *ee*, respectively).

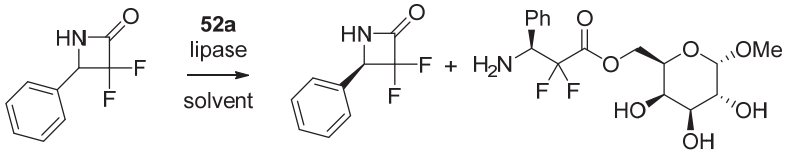


Scheme 20. Reaction route from *rac*-**8b** to (*R*)- and (*S*)-**8b**.

Optimization of reaction conditions

The lipase-catalyzed reaction between methyl α-D-galactopyranoside (**52a**) and *rac*-**8a** was chosen as a model reaction for the screening of lipases and solvents (Table 17). As a number of solvents were already screened for their abilities in dissolving methyl α-D-glycopyranosides in Paper III, three solvents were selected for closer examination: TBME, 2-methyltetrahydrofuran (2-MeTHF) and *t*-AmOH. As in Paper III, the reactions were again performed in the presence of 3Å molecular sieves (50 mg mL^{-1}) in order to suppress hydrolysis side reactions and at 47°C to increase solubility. From the selected lipases, only CAL-B and lipase PS-D showed practical enantioselectivity (entries 1 and 2). However, as the tendency of the CAL-B preparation used was to release any water contained within was expected to cause problems [as also evident in entry 1, TBME (57% conversion)], work was continued with lipase PS-D. Despite the presence of molecular sieves, the small-scale experiments with *rac*-**8a** (50 mM) under the conditions of Table 17 with lipase PS-D in the absence of the sugar revealed that 62% in TBME, 45% in 2-MeTHF and 35% in *t*-AmOH of the lactam had hydrolyzed in 24 h. These observations left *t*-AmOH and lipase PS-D as the most preferred combination in the light of enantioselectivity, conversion and lesser tendency for hydrolytic side reactions.

Table 17. Lipase screening for the enantioselective ring opening of *rac*-**8a** (50 mM) with **52a** (1 equiv.) in the presence of a lipase preparation (30 mg mL^{-1}) and 3Å molecular sieves (50 mg mL^{-1}) in dried solvents; reaction time 24 h at 47°C.



rac-**8a** (*R*)-**8a** (*S*)-**70a**

Entry	Lipase	TBME		2-MeTHF		*t*-AmOH	
		Conv. (%)[a]	ee^{8a} (%)	Conv. (%)[a]	ee^{8a} (%)	Conv. (%)[a]	ee^{8a} (%)
1	CAL-B	57	>99	26	37	43	79
2	PS-D	53	98	50	93	44	78
3	RM IM	33	50	10	12	7	5
4	TL IM	26	18	11	10	25	4[b]

[a]Conversion as the disappearance of *rac*-**8a** against internal standard. [b]Opposite enantioselectivity observed.

In contrast to the use of amino acids as acyl donors in enzymatic reactions, β-lactams can be well utilized without *N*-protective groups. However, by incorporating Boc_2O (1

equiv.) in the mixture of *rac*-**8a** (50 mM) and **52a** (1 equiv.) in the presence of lipase PS-D (30 mg mL^{-1}) and 3Å molecular sieves (50 mg mL^{-1}), *in situ N*-Boc protection of the formed product could be performed. This in turn helped in HPLC analysis of the reaction mixture as the indefinite peak shape of **70a** was now replaced with a sharper, more definite peak of **73a** (Figure 27).

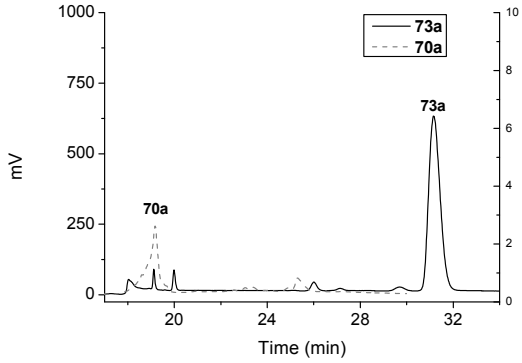


Figure 27. Overlaid RP-HPLC/CAD chromatograms showing the indefinite peak shape of **70a** (dashed) and the *N*-Boc-protected **73a** (solid).

Another aspect was the amounts of the reacting species, the sugar and the β-lactam. Increasing amounts of **52a** (1, 2 and 5 equiv. to *rac*-**8a**) in the presence of lipase PS-D (30 mg mL^{-1}) did not change the conversion of *rac*-**8a** during 24 h (53% in every case). By doubling the amount of *rac*-**8a** (from 50 to 100 mM) and keeping the amount of **52a** the same (50 mM), the rel. amount of formed **70a** increased (from 53 to 75%) during 24 h. However, due to low availability of β-lactam, further experiments were conducted with 50 mM *rac*-**8a**.

Thirdly, the amount of lipase PS-D (5–50 mg mL^{-1}) was screened in the reaction between *rac*-**8a** (50 mM) and **52a** (1 equiv.) in the presence of 3Å molecular sieves (50 mg mL^{-1}) in *t*-AmOH (Figure 28). When the conversion of *rac*-**8a** was plotted together with the amount of formed **73a**, it became evident that the difference between the two would increase with increasing lipase content. This in turn can be connected to possible hydrolysis side reaction.

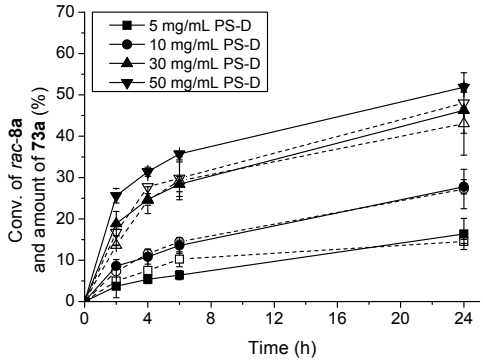


Figure 28. Conversion of *rac*-**8a** (50 mM, filled signs) and the amount of formed product **73a** (open sings) in the reaction with **52a** (1 equiv.) in the presence of Boc_2O (1 equiv.), lipase PS-D (5–50 mg mL^{-1}) and 3Å molecular sieves (50 mg mL^{-1}) in *t*-AmOH.

As the enantiomers (*R*)- and (*S*)-**8b** became available, they were studied independently as acyl donors (Figure 29). Previously, the ring-opening of *rac*-**8a** through lipase PS-D–catalyzed methanolysis exhibited excellent enantioselectivity (*E*>200).[117] Moreover, the reaction between (*R*)-**8a** and β-amino esters was not catalyzed by lipase PS-D while (*S*)-**8a** was highly reactive in the synthesis of β-dipeptides.[118] Now, the reactivity of (*S*)-**8b** was clearly higher, reaching 58% conversion within 24 h whereas the disappearance of (*R*)-**8b** during the same time was consistently *ca.* 30% regardless of presence of sugar. Even in the absence of lipase and sugar, the conversion for (*R*)-**8b** reached noticeable levels (<10% conversion in 48 h) suggesting chemical reactivity to some extent.

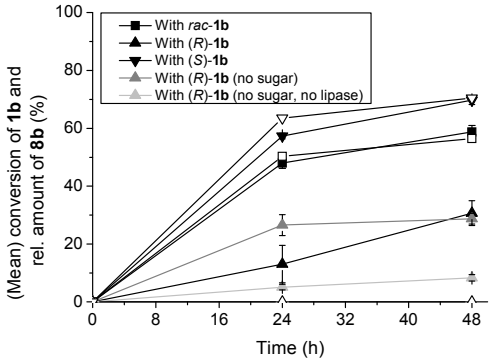
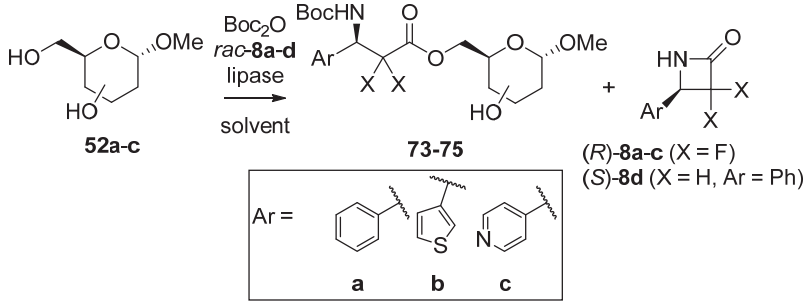


Figure 29. Conversion of (*S*)-, (*R*)- and *rac*-**8b** (50 mM, filled signs) and the rel. amount of formed **73b** (open signs) in the reaction with **52a** (1 equiv.) in the presence of Boc_2O (1 equiv.), lipase PS-D (30 mg mL^{-1}) and 3Å molecular sieves (50 mg mL^{-1}) in *t*-AmOH.

Racemic β-lactams as acyl donors–towards preparative scale reactions

After the reaction conditions were established for **52a** and *rac*-**8a**, the use of methyl α-D-gluco- and mannopyranosides (**52b** and **52c**) together with the variations in the aryl moiety and extent of activation of the lactam (*rac*-**8b-d**) were examined (Table 18).

Table 18. Reaction between **8a-d** (50 mM) and **52a-c** (1 equiv.) and between *rac*-**8a** and **64a** (1 equiv.) in the presence of lipase PS-D (30 mg mL^{-1}), Boc_2O (1 equiv.) and 3Å molecular sieves (50 mg mL^{-1}) in *t*-AmOH at 47°C; reaction time 24 h.



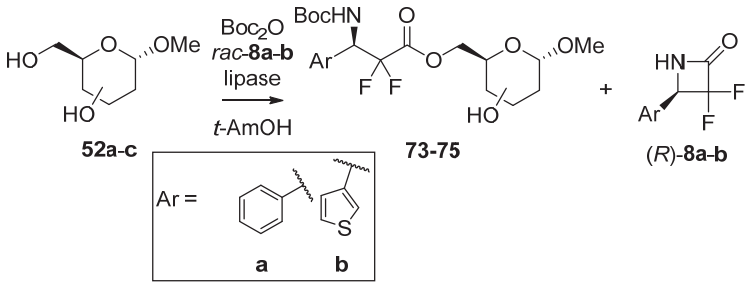
Entry	Sugar	Lactam	Conv. (%)[a]	*ee*[1] (%)	Amount (%)[b]	
					52a-c	**73-75**
1	**52a**	*rac*-**8a**	44	75	49	46
2	**52a**	*rac*-**8b**	46	69	55	22
3	**52a**	(*R*)-**8b**	13	99	100	-
4	**52a**	(*S*)-**8b**	58	99	42	39
5	**52a**	*rac*-**8c**	83[d]	16	64	-[e]
6	**52a**	*rac*-**8d**	0	-	100	-
7	**52b**	*rac*-**8a**	30	30	70	24[c]
8	**52b**	*rac*-**8b**	26	33	47	< 1[c]
9	**52b**	*rac*-**8c**	83[d]	20	52	-[e]
10	**52c**	*rac*-**8a**	15	15	22	< 1[c]
11	**52c**	*rac*-**8b**	22	16	20	< 1[c]
12	**52c**	*rac*-**8c**	76[d]	6	19	-[e]
13	**64a**[f]	*rac*-**8a**	31	13	96[c]	4[c]
14	**64a**[f,g]	*rac*-**8a**	21	22	53[c]	47[c]

[a]Conversion according to the disappearance of *rac*-**8a-d** against internal standard (mean value from chiral and achiral analyses). [b]From the RP-HPLC-chromatograms. [c]Relative amounts. [d]According to achiral analysis only. [e]Not determined due to extensive amount of peaks (see text). [f]Methyl α-D-3,4-*O*-isopropylidenegalactopyranoside (**64a**). [g]With CAL-B (30 mg mL^{-1}).

The activation brought by fluorine substitution proved important: lactams *rac*-**8a-c** all were reactive, while *rac*-**8d** was not. However, the highly reactive nature of *rac*-**8c** appeared problematic as the amount of new peaks observed with the RP-HPLC method proved excessive (>10 formed new peaks observed after 24 h reaction) and the conversion reached *ca.* 80% regardless of the sugar present (entries 5, 9 and 12). This

led to discontinuation with *rac*-**8c**. With **8a** and **8b** as acyl donors, sugars **52a** and **52b** (entries 1–3, 7 and 8) were both forming products, while the amount of formed products with **52c** (entries 10 and 11) were too small to measure. As already examined (Figure 29), no product formation was detected with (*R*)-**8b** while (*S*)-**8b** gave 39% of **73b** in a 24 h reaction. The use of methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside (**64a**) as the sugar nucleophile that enabled high yields in previously[III] did not prove especially good in this perspective (entries 13 and 14). Finally, preparative scale reactions were performed (Table 19). Now, *N*-Boc-protected sugar β-amino acid conjugates **73a**-**75a** and **73b** could be isolated in 3–20% yields. As the results in Table 18 already showed, products derived from **52a** were more abundant than with **52b** and **52c** (Table 19, entries 1 and 2 *vs.* 3 and 4). One way to increase the amount of formed product would be to use (*S*)-**8** as an acyl donor instead of *rac*-**8**. However, this would come at a cost of a longer synthesis pathway.

Table 19. Preparative-scale synthesis of **73-75**.[a]



Entry	Lactam	Sugar	Time (h)	Conv. (%)[b]	$ee^{(R)\text{-}8}$ (%)	Amount of **73-75** (%)[c]	Yield of **73-75** (%)[d]
1	*rac*-**8a**	**52a**	24	57	>99	25	20
2	*rac*-**8b**	**52a**	24	44	33	23	18
3	*rac*-**8a**	**52b**	48	52	26	18	10
4	*rac*-**8a**	**52c**	48	80	13	-[e]	3

[a]With *rac*-**8** (50 mM), **52a-c** (1 equiv.), Boc_2O (1 equiv.), lipase PS-D (30 mg mL^{-1}), 3Å molecular sieves (50 mg mL^{-1}), *t*-AmOH, 47°C. [b]Conversion as 1-([**52a-c**]$_t$/[**52a-c**]$_0$). [c][**73-75**]$_t$/[**52a-c**]$_0$. [d]Isolated yield. [e]Peak of **75a** was too small for detection.

In conclusion, a novel approach for the preparation of β-amino acid sugar esters based on the enantioselective lipase-catalyzed ring-opening of β-lactams was presented. However, as the reaction requires high activation for the already strained β-lactams which act as acyl donors, it is also sensitive towards side reactions (such as hydrolysis).

6. CONCLUSIONS

Lipases are versatile catalysts for organic synthesis. The four cases presented in this thesis (Papers I–IV), all rely strongly on lipase catalysis and the selectivity they represent. The power and possibilities of biocatalysis in general were presented in the literary review of the thesis spanning the topics encountered during the experimental eork. In paper I, while studying the KR of furan-based secondary alcohols, competing esterification and transesterification were observed. This drew attention to possible hydrolytic side reactions occurring that may hamper the outcome of lipase-catalyzed reactions and prompted the use of desiccants (salts or molecular sieves) with the following topics (in addition to standard drying of solvents prior to experimental work). Moreover, the formed 1-(furanyl)ethyl acetates proved sensitive towards the conditions of column purification, lowering the obtained enantiomeric purity. The loss of enantiopurity could eventually be regained through lipase-catalyzed methanolysis of the product esters in two example cases. The DSR and DKR of thiophene-based cyanohydrins in Paper II showed that lipases can be successfully combined with chemical catalysts in one-pot processes to yield enantiomerically enriched products. Here, the problematic role of biased specificity of biocatalysts towards certain substrates during screening and optimization was also discussed. In Papers III and IV, the synthesis of sugar conjugates through lipase catalysis in organic solvents was addressed. First, the synthesis of sugar glycerates (α,β-hydroxypropanoic acid esters) through a combination of lipase-catalyzed regioselective acylation of methyl α-D-glycopyranosides and subsequent deprotection at the introduced acyl side chain was presented in Paper III. After examining possible protective group schemes for methyl α-D-galactopyranoside, a clear improvement to the lipase-catalyzed step was identified. In Paper IV, the remarkable feature of lipases towards activated β-lactams was exploited. It enabled the regioselective preparation of novel β-amino esters of methyl α-D-glycopyranosides through the enantioselective ring-opening. However, the activated nature of the β-lactams made them susceptible towards hydrolysis side reactions at times. This role of water was a theme that followed through every example presented in this thesis: the careful balance of esterification and transesterification, effect of hydrolysis, the effect of desiccants such as molecular sieves, to lipase catalysis were found most important.

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8. REFERENCES

[1] Faber, K. *Biotransformations in Organic Chemistry*, 6th ed., **2011**, Springer-Verlag Berlin Heidelberg.

[2] Moss, G. P. Basic terminology of stereochemistry. *Pure & Appl. Chem.* **1996**, *68*, 2193–2222.

[3] Fischer, E. Einfluss der Configuration auf die Wirkung den Enzyme. *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 2985–2993.

[4] Bommarius, A. S.; Blum, J. K.; Abrahamson, M. J. Status of protein engineering for biocatalysts: how to design an industrially useful biocatalyst. *Curr. Opin. Chem. Biol.* **2011**, *15*, 194–200.

[5] Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. Engineering the third wave of biocatalysis. *Nature* **2012**, *485*, 185–194.

[6] Constable, D. J. C.; Dunn, P. J.; Hayler, J. D.; Humphrey, G. R.; Leazer, J. L.; Linderman, R. J.; Lorenz, K.; Manley, J.; Pearlman, B. A.; Wells, A.; Zaks, A.; Zhang, T. Y. Key green chemistry research areas–a perspective from pharmaceutical manufacturers. *Green. Chem.* **2007**, *9*, 411–420.

[7] Busacca, C. A.; Fandrick, D. R.; Song, J. J.; Senanayake, C. H. The Growing Impact of Catalysis in the Pharmaceutical Industry. *Adv. Synth. Catal.* **2011**, *353*, 1825–1864.

[8] Katsuki, T.; Sharpless, K. B. The First Practical Method for Asymmetric Epoxidation. *J. Am. Chem. Soc.* **1980**, *102*, 5974–5976.

[9] Jacobsen, E. N.; Markó, I.; Mungall, W. S.; Schröder, G.; Sharpless, K. B. Asymmetric Dihydroxylation via Ligand-Accelerated Catalysis. *J. Am. Chem. Soc.* **1988**, *110*, 1968–1970.

[10] Noyori, R.; Ohkuma, T.; Kitamura, M.; Takaya, H.; Sayo, N.; Kumobayashi, H.; Akutagawa, S. Asymmetric Hydrogenation of β-Keto Carboxylic Esters. A Practical, Purely Chemical Access to β-Hydroxy Esters in High Enantiomeric Purity. *J. Am. Chem. Soc.* **1987**, *109*, 5856–5858.

[11] Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. Biocatalytic Asymmetric Synthesis of Chiral Amines from Ketones Applied to Sitagliptin Manufacture. *Science* **2010**, *329*, 305–309.

[12] Desai, A. A. Sitagliptin manufacture: a compelling tale of green chemistry, process intensification, and industrial asymmetric catalysis. *Angew. Chem. Int. Ed.* **2011**, *50*, 1974–1976.

[13] DeSantis, G.; Wong, K.; Farwell, B.; Chatman, K.; Zhu, Z.; Tomlinson, G.; Huang, H.; Tan, X.; Bibbs, L.; Chen, P.; Kretz, K.; Burk, M. J. Creation of a Productive, Highly Enantioselective Nitrilase through Gene Site Saturation Mutagenesis (GSSM). *J. Am. Chem. Soc.* **2003**, *125*, 11476–11477.

[14] Greenberg, W. A.; Varvak, A.; Hanson, S. R.; Wong, K.; Huang, H.; Chen, P.; Burk, M. J. Development of an efficient, scalable, aldolase-catalyzed process for enantioselective synthesis of statin intermediates. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5788–5793.

[15] Liljeblad, A.; Kallinen, A.; Kanerva, L. T. Biocatalysis in the Preparation of the Statin Side Chain. *Curr. Org. Synth.* **2009**, *6*, 362–379.

[16] Ma, S. K.; Gruber, J.; Davis, C.; Newman, L.; Gray, D.; Wang, A.; Grate, J.; Huisman, G. W.; Sheldon, R. A. A green-by-design biocatalytic process for atorvastatin intermediate. *Green Chem.* **2010**, *12*, 81–86.

[17] Liang, J.; Lalonde, J.; Borup, B.; Mitchell, V.; Mundorff, E.; Trinh, N.; Kochrekar, D. A.; Cherat, R. N.; Pai, G. G. Development of a Biocatalytic Process as an Alternative to the (-)-DIP-Cl-Mediated Asymmetric Reduction of a Key Intermediate of Montelukast. *Org. Proc. Res. Dev.* **2010**, *14*, 193–198.

[18] Li, T.; Liang, J.; Ambrogelly, A.; Brennan, T.; Gloor, G.; Huisman, G.; Lalonde, J.; Lekhal, A.; Mijts, B.; Muley, S.; Newman, L.; Tobin, M.; Wong, G.; Zaks, A.; Zhang, X. Efficient, Chemoenzymatic Process for Manufacture of the Boceprevir Bicyclic

[3.1.0]Proline Intermediate Based on Amine Oxidase-Catalyzed Desymmetrization. *J. Am. Chem. Soc.* **2012**, *134*, 6467–6472.
[19] Straathof, A. J. J.; Panke, S.; Schmid, A. The Production of fine chemicals by biotransformations. *Curr. Opin. Biotechnol.* **2002**, *13*, 548–556.
[20] Ghisalba, O.; Meyer, H.-P.; Wohlgemuth, R. Industrial Biotransformation. *Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology* (ed. Flickinger, M. C.), **2010**, 1–17, John Wiley & Sons, Inc. DOI: 10.1002/9780470054581.eib174
[21] OECD. *Future Prospects for Industrial Biotechnology*, **2011**, OECD Publishing. DOI: 10.1787/9789264126633-en
[22] Wolfenden, R. Degrees of Difficulty of Water-Consuming Reactions in the Absence of Enzymes. *Chem. Rev.* **2006**, *106*, 3379–3396.
[23] Edwards, D. R.; Lohman, D. C.; Wolfenden, R. Catalytic Proficiency: The Extreme Case of S-O Cleaving Sulfatases. *J. Am. Chem. Soc.* **2012**, *134*, 525–531.
[24] Michaelis, L.; Menten, M. L. Die Kinetik der Invertinwirkung. *Biochem. Z.* **1913**, *49*, 333–369.
[25] Johnson, K. A.; Goody, R. S. The Original Michaelis Constant: Translation of the 1913 Michaelis–Menten Paper. *Biochem.* **2011**, *50*, 8264–8269.
[26] Behrens, G. A.; Hummel, A.; Padhi, S. K.; Schätzle, S.; Bornscheuer, U. T. Discovery and Protein Engineering of Biocatalysts for Organic Synthesis. *Adv. Synt. Catal.* **2011**, *353*, 2191–2215.
[27] Reetz, M. T.; Carballeira, J. D.; Vogel, A. Iterative Saturation Mutagenesis on the Basis of B Factors as a Strategy for Increasing Protein Thermostability. *Angew. Chem. Int. Ed.* **2006**, *45*, 7745–7751.
[28] Wu, Q.; Soni, P.; Reetz, M. T. Laboratory Evolution of Enantiocomplementary Candida antarctica lipase B Mutants with Broad Substrate Scope. *J. Am. Chem. Soc.* **2013**, *135*, 1872–1881.
[29] The EC-numbers: http://www.chem.qmul.ac.uk/iubmb/enzyme/
[30] The Protein Data Bank, http://www.pdb.org
[31] UniProt, http://www.uniprot.org
[32] BRENDA, http://www.brenda-enzymes.org
[33] Jaeger, K.-E.; Dijkstra, B. W.; Reetz, M. T. Bacterial Biocatalysts: Molecular Biology, Three-Dimensional Structures, and Biotechnological Applications of Lipases. *Annu. Rev. Microb.* **1999**, *53*, 315–351.
[34] Ollis, D. L.; Cheah, E.; Cygler, M.; Dijkstra, B.; Frolow, F.; Franken, S. M.; Harel, M.; Remington, S. J.; Silman, I.; Schrag, J.; Sussman, J. L.; Verschueren, K. H. G.; Goldman, A. The α/β hydrolase fold. *Prot. Eng.* **1992**, *5*, 197–211.
[35] Kraut, J. Serine proteases: Structure and Mechanism of Catalysis. *Ann. Rev. Biochem.* **1977**, *46*, 331–358.
[36] Ema, T. Mechanism of Enantioselectivity of Lipases and Other Synthetically Useful Hydrolases. *Curr. Org. Chem.* **2004**, *8*, 1009–1025.
[37] Ringe, D.; Petsko, G. A. How Enzymes Work. *Science* **2008**, *320*, 1428–1429.
[38] Koshland, D. E. Application of a Theory of Enzyme Specificity to Protein Synthesis. *Proc. Natl. Acad. Sci. USA* **1958**, *44*, 98–104.
[39] Koshland, D. R. The Key-Lock Theory and the Induced Fit Theory. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2375–2378.
[40] Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. A Rule To Predict Which Enantiomer of a Secondary Alcohol Reacts Faster in Reactions Catalyzed by Cholesterol Esterase, Lipase from Pseudomonas cepacia, and Lipase from Candida rugosa. *J. Org. Chem.* **1991**, *56*, 2656–2665.
[41] Tuomi, W. V.; Kazlauskas, R. J. Molecular Basis for Enantioselectivity of Lipase from Pseudomonas cepacia toward Primary Alcohols. Modeling, Kinetics, and Chemical Modification of Tyr29 to Increase or Decrease Enantioselectivity. *J. Org. Chem.* **1999**, *64*, 2638–2647.

[42] Khersonsky, O.; Roodveldt, C.; Tawfik, D. S. Enzyme promiscuity: evolutionary and mechanistic aspects. *Curr. Opin. Chem. Biol.* **2006**, *10*, 498–508.

[43] Humble, M. S.; Berglund, P. Biocatalytic Promiscuity. *Eur. J. Org. Chem.* **2011**, 3391–3401.

[44] Polizzi, K. M.; Bommarius, A. S.; Broering, J. M.; Chaparro-Riggers, J. F. Stability of biocatalysts. *Curr. Opin. Chem. Biol.* **2007**, *11*, 220–225.

[45] Rodrigues, R. C.; Ortiz, C.; Berenguer-Murcia, Á.; Torres, R.; Fernández-Lafuente, R. Modifying enzyme activity and selectivity by immobilization. *Chem. Soc. Rev.* **2013**, *42*, 6290–6307.

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

[47] Adlercreutz, P. Immobilization and applications of lipases in organic media. *Chem. Soc. Rev.* **2013**, *42*, 6406–6436.

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

[49] Pierre, A. C. The Sol-Gel Encapsulation of Enzymes. *Biocat. Biotransform.* **2004**, *22*, 145–170.

[50] Heinsman, N. W. J. T.; Schroën, X. G. P. H.; van der Padt, A.; Franssen, M. C. R.; Boom, R. M.; van't Riet, K. Substrate sorption into the polymer matrix of Novozym 435® and its effect on the enantiomeric ratio determination. *Tetrahedron: Asymmetry* **2003**, *14*, 2699–2704.

[51] Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jonas, T. A. The sequence determination and refinement of two crystal forms of lipase B from *Candida antarctica*. *Structure* **1994**, *2*, 293–308.

[52] Uppenberg, J.; Öhrner, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T. A. Crystallographic and Molecular-Modeling Studies of Lipase B from *Candida antarctica* Reveal a Stereospecificity Pocket for Secondary Alcohols. *Biochem.* **1995**, *34*, 16838–16851.

[53] Schmid, R. D.; Verger, R. Lipases: Interfacial Enzymes with Attractive Applications. *Angew. Chem. Int. Ed.* **1998**, *37*, 1609–1633.

[54] Larsen, M.W.; Zielinska, D. F.; Martinelle, M.; Hidalgo, A.; Jensen, L.J.; Bornscheuer, U.T.; Hult, K. Suppression of Water as a Nucleophile in *Candida Antarctica* Lipase B Catalysis. *ChemBioChem* **2010**, *11*, 796–801.

[55] Kirk, O.; Christensen, M. W. Lipases from *Candida antarctica*: Unique Biocatalysts from a Unique Origin. *Org. Proc. Res. Dev.* **2002**, *6*, 446–451.

[56] Ericsson, D. J.; Kasrayan, A.; Johansson, P.; Bergfors, T.; Sandström, A. G.; Bäckvall, J.-E.; Mowbray, S. L. X-ray Structure of *Candida antarctica* Lipase A Shows a Novel Lid Structure and a Likely Mode of Interfacial Activation. *J. Mol. Biol.* **2008**, *376*, 109–119.

[57] Martinelle, M.; Holmquist, M.; Hult, K. On the interfacial activation of *Candida antarctica* lipase A and B as compared with *Humicola lanuginosa* lipase. *Biochim. Biophys. Acta* **1995**, *1258*, 272–276.

[58] Fernandez-Lafuente, R. Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. *J. Mol. Catal. B: Enz.* **2010**, *62*, 197–212.]

[59] Derewenda, U.; Swenson, L.; Wei, Y.; Green, R.; Kobos, P. M.; Joerger, R.; Haas, M. J.; Derewenda, Z. S. Conformational lability of lipases observed in the absence of an oil–water interface: crystallographic studies of enzymes from the fungi *Humicola lanuginosa* and *Rhizopus delemar*. *J. Lipid. Res.* **1994**, *35*, 524–534.

[60] Brzozowski, A. M.; Savage, H.; Verma, C. S.; Turkenburg, J. P.; Lawson, D. M.; Svendsen, A.; Patkar, S. Structural Origins of the Interfacial Activation in *Thermomyces* (*Humicola*) lanuginosa Lipase. *Biochem.* **2000**, *39*, 15071–15082.

[61] Brady, R. L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge,

U. A serine protease triad forms the catalytic centre of a triacylglycerol lipase. *Nature* **1990**, *343*, 767–770.

[62] Brzozowski, A. M.; Derewenda, U.; Derewenda, Z. S.; Dodson, G. G.; Lawson, D. W.; Turkenburg, J. P.; Bjorkling, F.; Huge-Jensen, B.; Patkar, S. A.; Thim, L. A model for interfacial activation in lipases from the structure of a fungal lipase-inhibitor complex. *Nature* **1991**, *351*, 491–494.

[63] Derewenda, U.; Brzozowski, A. M.; Lawson, D. M.; Derewenda, Z. S. Catalysis at the Interface: The Anatomy of a Conformational Change in a Triacylglyceride Lipase. *Biochem.* **1992**, *31*, 1532–1541.

[64] Vasel, B.; Hecht, H.-J.; Schmid, R. D.; Schomburg, D. 3D-Structures of the lipase from *Rhizomucor miehei* at different temperatures and computer modelling of a complex of the lipase with trilaurylglycerol. *J. Biotechnol.* **1993**, *28*, 99–115.

[65] Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E. J.; Dodson, G. G.; Turkenburg, J. P. Structure and Molecular Model Refinement of *Rhizomucor miehei* Triacylglyceride Lipase: a Case Study of the Use of Simulated Annealing in Partial Model Refinement. *Acta Cryst. B* **1992**, *48*, 307–319.

[66] Larson, S.; Day, J.; Greenwood, A.; Oliver, J.; Rubingh, D.; McPherson, A. Preliminary Investigation of Crystals of the Neutral Lipase from *Pseudomonas fluorescens*. *J. Mol. Biol.* **1991**, *222*, 21–22.

[67] 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. The open conformation of a *Pseudomonas* lipase. *Structure* **1997**, *5*, 187–202.

[68] Lang, D. A.; Mannesse, M. L. M.; De Haas, G.; Verheij, H. M.; Dijkstra, B. W. Structural basis of the chiral selectivity of *Pseudomonas cepacia* lipase. *Eur. J. Biochem.* **1998**, *254*, 333–340.

[69] Luić, M.; Tomić, S.; Leščić, I.; Ljubović, E.; Šepac, D.; Šunjić, V.; Vitale, L.; Saenger, W.; Kojić-Prodić, B. Complex of *Burkholderia cepacia* lipase with transition state analogue of 1-phenoxy-2-acetoxybutane. *Eur. J. Biochem.* **2001**, *268*, 3964–3973.

[70] Mezzetti, A.; Schrag, J. D.; Cheong, C. S.; Kazlauskas, R. J. Mirror-Image Packing in Enantiomer Discrimination: Molecular Basis for the Enantioselectivity of *B. cepacia* Lipase toward 2-Methyl-3-Phenyl-1-Propanol. *Chem. Biol.* **2005**, *12*, 427–437.

[71] Luić, M.; Štefanić, Z.; Ceilinger, I.; Hodošček, M.; Janežič, D.; Lenac, T.; Ašler, I. L.; Šepac, D.; Tomić, S. Combined X-ray Diffraction and QM/MM Study of the *Burkholderia cepacia* Lipase-Catalyzed Secondary Alcohol Esterification. *J. Phys. Chem. B.* **2008**, *112*, 4876–4883.

[72] Pleiss, J.; Fischer, M.; Peiker, M.; Thiele, C.; Schmid, R. D. Lipase engineering database Understanding and exploiting sequence–structure–function relationships. *J. Mol. Cat. B: Enz.* **2000**, *10*, 491–508.

[73] Henke, E.; Pleiss, J.; Bornscheuer, U. T. Activity of Lipases and Esterases towards Tertiary Alcohols: Insights into Structure–Function Relationships. *Angew. Chem. Int. Ed.* **2002**, *41*, 3211–3213.

[74] http://www.pymol.org; The PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC.

[75] Cambou, B.; Klibanov, A. M. Preparative Production of Optically Active Esters and Alcohols Using Esterase-Catalyzed Stereospecific Transesterification in Organic Media. *J. Am. Chem. Soc.* **1984**, *106*, 2687–2692.

[76] Zaks, A.; Klibanov, A. M. Enzymatic Catalysis in Organic Media at 100°C. *Science* **1984**, *224*, 1249–1251.

[77] Zaks, A.; Klibanov, A. M. Enzyme-catalyzed processes in organic solvents. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 3192–3196.

[78] Zaks, A.; Klibanov, A. M. The Effect of Water on Enzyme Action in Organic Media. *J. Biol. Chem.* **1988**, *263*, 8017–8021.

[79] Halling, P. J. Thermodynamic predictions for biocatalysis in nonconventional media: Theory, tests, and recommendations for experimental design and analysis. *Enzyme Microb. Technol.* **1994**, *16*, 178–206.

[80] Overbeeke, P. L. A.; Jongejan, J. A.; Heijnen, J. J. Solvent Effect on Lipase Enantioselectivity. Evidence for the Presence of Two Thermodynamic States. *Biotechnol. Bioeng.* **2000**, *70*, 278–290.

[81] Graber, M.; Irague, R.; Rosenfeld, E.; Lamare, S.; Franson, L.; Hult, K. Solvent as a competitive inhibitor for *Candida antarctica* lipase B. *Biochim. Biophys. Acta* **2007**, *1774*, 1052–1057.

[82] Laane, C.; Boeren, S.; Vos, K.; Veeger, C. Rules for Optimization of Biocatalysis in Organic Solvents. *Biotech. Bioeng.* **1987**, *30*, 81–87.

[83] Bousquet-Dubouch, M.-P.; Graber, M.; Sousa, N.; Lamare, S.; Legoy, M.-D. Alcoholysis catalyzed by *Candida antarctica* lipase B in a gas/solid system obeys a Ping Pong Bi Bi mechanism with competitive inhibition by the alcohol substrate and water. *Biochim. Biophys. Acta* **2001**, *1550*, 90–99.

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

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

[86] Ghanem, A. Trends in lipase-catalyzed asymmetric access to enantiomerically pure/enriched compounds. *Tetrahedron* **2007**, *63*, 1721–1754.

[87] Ahmed, M.; Kelly, T.; Ghanem, A. Applications of enzymatic and non-enzymatic methods to access enantiomerically pure compounds using kinetic resolution and racemization. *Tetrahedron* **2012**, *68*, 6781–6802.

[88] Kanerva, L. T.; Liljeblad, A. *Encyclopedia of Catalysis: Transesterification—Biological. Fundamentals of Enzymatic Transesterification*, Wiley Online Library, **2010**; doi: http://dx.doi.org/10.1002/0471227617.eoc197.

[89] Li, X.-G.; Rantapaju, M.; Kanerva, L. T. *Candida antarctica* Lipase B in a Chemoenzymatic Route to Cyclic α-Quaternary α-Amino Acid Enantiomers. *Eur. J. Org. Chem.* **2011**, 1755–1762.

[90] Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. Kinetic Resolution of Racemic Allylic Alcohols by Enantioselective Epoxidation. A Route to Substances of Absolute Enantiomeric Purity? *J. Am. Chem. Soc.* **1981**, *103*, 6237–6240.

[91] Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. Quantitative Analyses of Biochemical Kinetic Resolutions of Enantiomers. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.

[92] Chen, C.-S.; Sih, C. J. General Aspects and Optimization of Enantioselective Biocatalysis in Organic Solvents: The Use of Lipases. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 695–707.

[93] Sakai, T.; Kishimoto, T.; Tanaka, Y.; Ema, T.; Utaka, M. Low-Temperature Method for Enhancement of Enantioselectivity in the Lipase-Catalyzed Kinetic Resolution of Solketal and Some Chiral Alcohols. *Tetrahedron Lett.* **1998**, *39*, 7881–7884.

[94] Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. Lipase-Catalyzed Irreversible Transesterification Using Enol Esters as Acylating Reagents: Preparative Enantio- and Regioselective Syntheses of Alcohols, Glycerol Derivatives, Sugars, and Organometallics. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.

[95] Bianchi, D.; Cesti, P.; Battistel, E. Anhydrides as Acylating Agents in Lipase-Catalyzed Stereoselective Esterification of Racemic Alcohols. *J. Org. Chem.* **1988**, *53*, 5531–5534.

[96] Berger, B.; Rabiller, C. G.; Königsberger, K.; Faber, K.; Griengl, H. Enzymatic acylation using acid anhydrides: crucial removal of acid. *Tetrahedron: Asymmetry* **1990**, *1*, 541–546.

[97] Suginaka, K.; Hayashi, Y.; Yamamoto, Y. Highly Selective Resolution of Secondary Alcohols and Acetoacetates with Lipases and Diketene in Organic Media. *Tetrahedron: Asymmetry* **1996**, *7*, 1153–1158.

[98] Kirchner, G.; Scollar, M. P.; Klibanov, A. M. Resolution of Racemic Mixtures via Lipase Catalysis in Organic Solvents. *J. Am. Chem. Soc.* **1985**, *107*, 7072–7076.

[99] Armesto, N.; Ferrero, M.; Fernández, S.; Gotor, V. Regioselective Enzymatic Acylation of Methyl Shikimate. Influence of Acyl Chain Length and Solvent Polarity on Enzyme Specificity. *J. Org. Chem.* **2002**, *67*, 4978–4981.

[100] Chênevert, R.; Pelchat, N.; Morin, P. Lipase-mediated enantioselective acylation of alcohols with functionalized vinyl esters: acyl donor tolerance and applications. *Tetrahedron: Asymmetry* **2009**, *20*, 1191–1196.

[101] Syrén, P.-O. The solution of nitrogen inversion of amidases. *FEBS J.* **2013**, *280*, 3069–3083.

[102] Liljeblad, A.; Kanerva, L. T. Biocatalysis as a profound tool in the preparation of highly enantiopure β-amino acids. *Tetrahedron* **2006**, *62*, 5831–5854.

[103] Alcaide, B.; Almendros, P.; Aragoncillo, C. β-Lactams: Versatile Building Blocks for the Stereoselective Synthesis of Non-β-Lactam Products. *Chem. Rev.* **2007**, *107*, 4437–4492.

[104] Busto, E.; Gotor-Fernández, V.; Gotor, V. Hydrolases in the Stereoselective Synthesis of N-Heterocyclic Amines and Amino Acid Derivatives. *Chem. Rev.* **2011**, *111*, 3998–4035.

[105] Forró, E.; Fülöp, F. Recent Lipase-Catalyzed Hydrolytic Approaches to Pharmacologically Important β- and γ-Amino Acids. *Curr. Med. Chem.* **2012**, *19*, 6178–6187.

[106] Brieva, R.; Crich, J. Z.; Sih, C. J. Chemoenzymatic Synthesis of the C-13 Side Chain of Taxol: Optically-Active 3-Hydroxy-4-phenyl β-Lactam Derivatives. *J. Org. Chem.* **1993**, *58*, 1068–1075.

[107] Forró, E.; Fülöp, F. Lipase-Catalyzed Enantioselective Ring Opening of Unactivated Alicyclic-Fused β-Lactams in an Organic Solvent. *Org. Lett.* **2003**, *5*, 1209–1212.

[108] Forró, E.; Fülöp, F. Advanced procedure for the enzymatic ring opening of unsaturated alicyclic β-lactams. *Tetrahedron: Asymm.* **2004**, *15*, 2875–2880.

[109] Forró, E.; Fülöp, F. Synthesis of enantiopure 1,4-ethyl- and 1,4-ethylene-bridged cispentacin by lipase-catalyzed enantioselective ring opening of β-lactams. *Tetrahedron: Asymm.* **2004**, *15*, 573–575.

[110] Forró, E.; Paál, T.; Tasnádi, G.; Fülöp, F. A New Route to Enantiopure β-Aryl-Substituted β-Amino Acids and 4-Aryl-Substituted β-Lactams through Lipase-Catalyzed Enantioselective Ring Cleavage of β-Lactams. *Adv. Synth. Catal.* **2006**, *348*, 917–923.

[111] Forró, E.; Fülöp, F. Do lipases also catalyse the ring cleavage of inactivated cyclic trans-β-lactams. *Tetrahedron: Asymm.* **2006**, *17*, 3193–3196.

[112] Tasnádi, G.; Forró, E.; Fülöp, F. *Candida antarctica* lipase B-catalyzed ring opening of 4-arylalkyl-substituted β-lactams. *Tetrahedron: Asymm.* **2007**, *18*, 2841–2844.

[113] Forró, F.; Fülöp, F. Vapour-assisted enzymatic hydrolysis of β-lactams in a solvent-free system. *Tetrahedron: Asymm.* **2008**, *19*, 1005–1009.

[114] Kazi, B.; Kiss, L.; Forró, E.; Fülöp, F. Synthesis of orthogonally protected azepane β-amino ester enantiomers. *Tetrahedron Lett.* **2010**, *51*, 82–85.

[115] Kiss, L.; Cherepanova, M.; Forró, E.; Fülöp, F. A New Access Route to Functionalized Cispentacins from Norbornene β-Amino Acids. *Chem. Eur. J.* **2013**, *19*, 2102–2107.

[116] Cherepanova, M.; Kiss, L.; Forró, E.; Fülöp, F. A De Novo Stereocontrolled Approach to *syn*- and *anti*-Disubstituted Acyclic $\beta^{2,3}$-Amino Acid Enantiomers. *Eur. J. Org. Chem.* **2014**, 403–409.

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

[118] Li, X.-G.; Lähitie, M.; Kanerva, L. T. *Burkholderia cepacia* lipase and activated β-lactams in β-dipeptide and β-amino amide synthesis. *Tetrahedron: Asymm.* **2008**, *19*, 1857–1861.
[119] Ghanem, A.; Schurig, V. Lipase-catalyzed Irreversible Transesterification of Secondary Alcohols Using Isopropenyl Acetate. *Monatsh. Chem.* **2003**, *134*, 1151–1157.
[120] Raminelli, C.; Comasseto, J. V.; Andrade, L. H.; Porto, A. L. M. Kinetic resolution of propargylic and allylic alcohols by *Candida antractica* lipase (Novozyme 435). *Tetrahedron: Asymmetry* **2004**, *15*, 3117–3122.
[121] Vänttinen, E.; Kanerva, L. T. Combination of the Lipase-Catalysed Resolution with the Mitsunobu Esterification in One Pot. *Tetrahedron: Asymm.* **1995**, *6*, 1779–1786.
[122] Herzberg, R.; Moberg, C. One-Step Preparation of *O*-(α-Bromoacyl) Cyanohydrins by Minor Enantiomer Recycling: Synthesis of 4-Amino-2(5*H*)-furanones. *J. Org. Chem.* **2013**, *78*, 9174–9180.
[123] Faber, K. Non-Sequential Processes for the Transformation of a Racemate into a Single Stereoisomer Product: Proposal for Stereochemical Classification. *Chem. Eur. J.* **2001**, *7*, 5004–5010.
[124] Steinreiber, J.; Faber, K.; Griengl, H. De-racemization of Enantiomers versus De-epimerization of Diastereomers- Classification of Dynamic Kinetic Asymmetric Transformations (DYKAT). *Chem. Eur. J.* **2008**, *14*, 8060–8072.
[125] Ward, R. S. Dynamic Kinetic Resolution. *Tetrahedron: Asymm.* **1995**, *6*, 1475–1490.
[126] Pellissier, H. Dynamic kinetic resolution. *Tetrahedron* **2003**, *59*, 8291–8327.
[127] Pellissier, H. Recent developments in dynamic kinetic resolution. *Tetrahedron* **2008**, *64*, 1563–1601.
[128] Pellissier, H. Recent developments in dynamic kinetic resolution. *Tetrahedron* **2011**, *67*, 3769–3802.
[129] Kitamura, M.; Tokunaga, M.; Noyori, R. Mathematical Treatment of Kinetic Resolution of Chirally Labile Substrates. *Tetrahedron* **1993**, *49*, 1853–1860.
[130] Kim, M.-J.; Ahn, Y.; Park, J. Dynamic kinetic resolutions and asymmetric transformations by enzymes coupled with metal catalysis. *Curr. Opin. Biotechnol.* **2002**, *13*, 578587.
[131] Pàmies, O.; Bäckvall, J.-E. Combination of Enzymes and Metal Catalysts. A Powerful Approach in Asymmetric Catalysis. *Chem. Rev.* **2003**, *103*, 3247–3261.
[132] Holt, J.; Hanefeld, U. Enantioselective Enzyme-Catalyzed Synthesis of Cyanohydrins. *Curr. Org. Synth.* **2009**, *6*, 15–37.
[133] Effenberger, R.; Hörsch, B.; Weingart, F. Enzyme-Catalyzed Synthesis of (R)-Ketone-Cyanohydrins and Their Hydrolysis to (R)-α-Hydroxy-α-Methyl-Carboxylic Acids. *Tetrahedron Lett.* **1991**, *32*, 2605–2608.
[134] Effenberger, F.; Heid, S. (R)-Oxynitrilase Catalyzed Synthesis of (R)-Ketone Cyanohydrins. *Tetrahedron: Asymm.* **1995**, *6*, 2945–2952.
[135] Hayashi, M.; Yoshiga, T.; Nakatani, K.; Ono, K.; Oguni, N. Reduction of α-Trialkylsiloxy Nitriles with Diisobutylaluminium Hydride (DIBAH): A Facile Preparation of α-Trialkylsiloxy Aldehydes an Their Derivatives. *Tetrahedron* **1994**, *50*, 2821–2830.
[136] Brussee, J.; Roos, E. C.; Van Der Gen, A. Bio-Organic Synthesis of Optically Active Cyanohydrins and Acyloins. *Tetrahedron Lett.* **1988**, *29*, 4485–488.
[137] Effenberger, F.; Gutterer, B.; Jäger, J. Stereoselective Synthesis of (1*R*)- and (1*R*,2*S*)-1-aryl-2-alkylamino alcohols from (*R*)-Cyanohydrins. *Tetrahedron: Asymm.* **1997**, *8*, 459–467.
[138] Brown, R. F. C.; Jackson, W. R.; McCarthy, T. D. The Synthesis of Homochiral Naturally Occurring Hydroxy Amides. *Tetrahedron: Asymm.* **1993**, *4*, 205–206.
[139] Brown, R. F. C.; Donohue, A. C.; Jackson, W. R.; McCarthy, T. D. Synthetic Applications of Optically Active Cyanohydrins. Enantioselective Syntheses of the Hydroxyamides Tembamide and Aegeline, the Cardiac Drug Denopamine, and Some Analogues of the Bronchodilator Salbutamol. *Tetrahedron* **1994**, *50*, 13739–13752.

[140] Gassman, P. G.; Talley, J. J. Cyanohydrins – a General Synthesis. *Tetrahedron Lett.* **1978**, *40*, 3773–3776.

[141] Brunel, J.-M.; Holmes, I. P. Chemically Catalyzed Asymmetric Cyanohydrin Syntheses. *Angew. Chem.Int. Ed.* **2004**, *43*, 2752–2778.

[142] North, M.; Usanov, D. L.; Young, C. Lewis Acid Catalyzed Asymmetric Cyanohydrin Synthesis. *Chem. Rev.* **2008**, *108*, 5146–5226.

[143] Griengl, H.; Klempier, N.; Pöchlauer, P.; Schmidt, M.; Shi, N.; Zabelinskaja-Mackova, A. A. Enzyme Catalysed Formation of (*S*)-Cyanohydrins Derived from Aldehydes and Ketones in a Biphasic Solvent System. *Tetrahedron* **1998**, *54*, 14477–14486.

[144] Purkarthofer, T.; Pabst, T.; van den Broeck, C.; Griengl, H.; Maurer, O.; Skranc, W. Large-Scale Synthesis of (*R*)-2-Amino-1-(2-furyl)ethanol via a Chemoenzymatic Approach. *Org. Proc. Res. Dev.* **2006**, *10*, 618–621.

[145] Torrelo, G.; van Midden, N.; Stloukal, R.; Hanefeld, U. Immobilized Hydroxynitrile Lyase: A Comparative Study of Recyclability. *ChemCatChem* **2014**, DOI: 10.1002/cctc.201300892.

[146] Andexer, J.; von Langermann, J.; Mell, A.; Bocola, M.; Kragl, U.; Eggert, T.; Pohl, M. An *R*-Selective Hydroxynitrile Lyase from *Arabidopsis thaliana* with an α/β-Hydrolase Fold. *Angew. Chem. Int. Ed.* **2007**, *46*, 8679–8681.

[147] Okrob, D.; Paravidino, M.; Orru, R. V. A.; Wiechert, W.; Hanefeld, U.; Pohl, M. Hydroxynitrile Lyase from *Arabidobsis thaliana*: Identification of Reaction Parameters for Enantiopure Cyanohydrin Synthesis by Pure and Immobilized Catalyst. *Adv. Synth. Catal.* **2011**, *353*, 2399–2408.

[148] Kiljunen, E.; Kanerva, L. T. (*R*)- and (*S*)-Cyanohydrins Using Oxynitrilases in Whole Cells. *Tetrahedron: Asymmetry* **1996**, *7*, 1105–1116.

[149] Martínková, L.; Mylerova, V. Synthetic Applications of Nitrile-Converting Enzymes. *Curr. Org. Chem.* **2003**, *7*, 1279–1295.

[150] Martínková, L.; Křen, V. Biotransformations with nitrilases. *Curr. Opin. Chem. Biol.* **2010**, *14*, 130–137.

[151] Wang, Y.-F.; Chen, S.-T.; Liu, K. K.-C.; Wong, C.-H. Lipase-Catalyzed Irreversible Transesterification using Enol Esters: Resolution of Cyanohydrins and Syntheses of Ethyl (R)-2-Hydroxy-4-phenylbutyrate and (S)-Propranolol. *Tetrahedron Lett.* **1989**, *30*, 1917–1920.

[152] Veum, L.; Kuster, M.; Telalovic, Hanefeld, U.; Maschmeyer, T. Enantioselective Synthesis of Protected Cyanohydrins. *Eur. J. Org. Chem.* **2002**, 1516–1522.

[153] Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, J. Lipase-Catalyzed Kinetic Resolution with in Situ Racemization: One-Pot Synthesis of Optically Active Cyanohydrin Acetates from Aldehydes. *J. Am. Chem. Soc.* **1991**, *113*, 9360–9361.

[154] Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, J. One-Pot Synthesis of Optically Active Cyanohydrin Acetates from Aldehydes via Lipase-Catalyzed Kinetic Resolution Coupled with in Situ Formation and Racemization of Cyanohydrins. *J. Org. Chem.* **1992**, *57*, 5643–5649.

[155] Kanerva, L. T.; Rahiala, K.; Sundholm, O. Optically Active Cyanohydrins and Enzyme Catalysis. *Biocat.* **1994**, *10*, 169–180.

[156] Paizs, C.; Toşa, M.; Majdik, C.; Tähtinen, P.; Irimie, F. D.; Kanerva, L. T. *Candida antarctica* lipase A in the dynamic resolution of novel furylbenzotiazol-based cyanohydrin acetates. *Tetrahedron: Asymm.* **2003**, *14*, 619–627.

[157] Paizs, C.; Tähtinen, P.; Toşa, M.; Majdik, C.; Irimie, F.-D.; Kanerva, L. T. Biocatalytic enantioselective preparation of phenothiazine-based cyanohydrin acetates: kinetic and dynamic kinetic resolution. *Tetrahedron* **2004**, *60*, 10533–10540.

[158] Paizs, C.; Tähtinen, P.; Lundell, K.; Poppe, L.; Irimie, F.-D.; Kanerva, L. T. Preparation of novel phenylfuran-based cyanohydrin esters: lipase-catalyzed kinetic and dynamic resolution. *Tetrahydron: Asymm.* **2003**, *14*, 1895–1904.

[159] Li, Y.-X.; Straathof, A. J. J.; Hanefeld, U. Enantioselective formation of mandelonitrile acetate-investigation of a dynamic kinetic resolution. *Tetrahedron: Asymm.* **2002**, *13*, 739–743.

[160] Veum, L.; Hanefeld, U. Enantioselective formation of mandelonitrile acetate: investigation of a dynamic kinetic resolution II. *Tetrahedron: Asymm.* **2004**, *15*, 3707–3709.

[161] Veum, L.; Kanerva, L. T.; Halling, P. J.; Maschmeyer, T.; Hanefeld, U. Optimisation of the Enantioselective Synthesis of Cyanohydrin Esters. *Adv. Synth. Catal.* **2005**, *347*, 1015–1021.

[162] Sakai, T.; Wang, K.; Ema, T. Lipase-catalyzed dynamic kinetic resolution giving optically active cyanohydrins: use of silica-supported ammonium hydroxide and porous ceramic-immobilized lipase. *Tetrahedron* **2008**, *64*, 2178–2183.

[163] Hietanen, A.; Ekholm, F. S.; Leino, R.; Kanerva, L. T. Applying biocatalysis to the synthesis of diastereomerically enriched cyanohydrin mannosides. *Eur. J. Org. Chem.* **2010**, 6974–6980.

[164] Hietanen, A.; Kanerva, L. T. One-Pot Oxidation-Hydrocyanation Sequence Coupled to Lipase-Catalyzed Diastereoresolution in the Chemoenzymatic Synthesis of Sugar Cyanohydrin Esters. *Eur. J. Org. Chem.* **2012**, 2729–2737.

[165] Ramström, O.; Lehn, J.-M. Drug Discovery by Dynamic Combinatorial Libraries. *Nat. Rev. Drug Discov.* **2002**, *1*, 26–36.

[166] Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. Dynamic Combinatorial Chemistry. *Chem. Rev.* **2006**, *106*, 3652–3711.

[167] Lehn, J.-M. Dynamic Combinatorial Chemistry and Virtual Combinatorial Libraries. *Chem. Eur. J.* **1999**, *5*, 2455–2463.

[168] Giuseppone, N.; Lehn, J.-M. Protonic and Temperature Modulation of Constituent Expression by Component Selection in a Dynamic Combinatorial Library of Imines. *Chem. Eur. J.* **2006**, *12*, 1715–1722.

[169] Ingerman, L. A.; Waters, M. L. Photoswitchable Dynamic Combinatorial Libraries: Coupling Azobenzene Photoisomerization with Hydrazone Exchange. *J. Org. Chem.* **2009**, *74*, 111–117.

[170] Giuseppone, N.; Lehn, J.-M. Electric-Field Modulation of Component Exchange in Constitutional Dynamic Liquid Crystals. *Angew. Chem. Int. Ed.* **2006**, *45*, 4619–4624.

[171] Giuseppone, N.; Schmitt, J.-L.; Lehn, J.-M. Driven Evolution of a Constitutional Dynamic Library of Molecular Helices Toward the Selective Generation of [2×2] Gridlike Arrays under the Pressure of Metal Ion Coordination. *J. Am. Chem. Soc.* **2006**, *128*, 16748–16763.

[172] Hafezi, N.; Lehn, J.-M. Adaptation of Dynamic Covalent Systems of Imine Constituents to Medium Change by Component Redistribution under Reversible Phase Separation. *J. Am. Chem. Soc.* **2012**, *134*, 12861–12868.

[173] Berl, V.; Huc, I.; Lehn, J.-M.; DeCian, A.; Fischer, J. Induced Fit Selection of a Barbiturate Receptor from a Dynamic Structural and Conformational/Configurational Library. *Eur. J. Org. Chem.* **1999**, 3089–3094.

[174] Santana, A. G.; Jiménez-Moreno, E.; Gómez, A. M.; Corzana, F.; González, C.; Jiménez-Oses, G.; Jiménez-Barbero, J.; Asensio, J. L. A Dynamic Combinatorial Approach for the Analysis of Weak Carbohydrate/Aromatic Complexes: Dissecting Facial Selectivity in CH/π Stacking Interactions. *J. Am. Chem. Soc.* **2013**, *135*, 3347–3350.

[175] Sakulsombat, M.; Zhang, Y.; Ramström, O. Dynamic Systemic Resolution. *Top. Curr. Chem.* **2012**, *322*, 55–86.

[176] Vongvilai, P.; Angelin, M.; Larsson, R.; Ramström, O. Dynamic Combinatorial Resolution: Direct Asymmetric Lipase-Mediated Screening of a Dynamic Nitroaldol Library. *Angew. Chem. Int. Ed.* **2007**, *46*, 948–950.

[177] Angelin, M.; Fischer, A.; Ramström, O. Crystallization-Induced Secondary Selection from a Tandem Driven Dynamic Combinatorial Resolution Process. *J. Org. Chem.* **2008**, *73*, 3593–3595.

[178] Angelin, M.; Vongvilai, P.; Fischer, A.; Ramström, O. Crystallization-Driven Asymmetric Synthesis of Pyridine-β-nitroalcohols via Discovery-Oriented Self-Resolution of a Dynamic System. *Eur. J. Org. Chem.* **2010**, 6315–6318.

[179] Zhang, Y.; Vongvilai, P.; Sakulsombat, M.; Fischer, A.; Ramström, O. Asymmetric Synthesis of Substituted Thiolanes through Domino Thia-Michael-Henry Dynamic Covalent Systemic Resolution using Lipase Catalysis. *Adv. Synth. Catal.* **2014**, *356*, 987–992.

[180] Sakulsombat, M.; Zhang, Y.; Ramström, O. Dynamic Asymmetric Hemithioacetal Transformation by Lipase-Catalyzed γ-Lactonization: In Situ Tandem Formation of 1,3-Oxathiolan-5-one Derivatives. *Chem. Eur. J.* **2012**, *18*, 6129–6132.

[181] Larsson, R.; Pei, Z.; Ramström, O. Catalytic Self-Screening of Cholinesterase Substrates from a Dynamic Combinatorial Thioester Library. *Angew. Chem. Int. Ed.* **2004**, *43*, 3716–3718.

[182] Larsson, R.; Ramström, O. Dynamic Combinatorial Thiolester Libraries for Efficient Catalytic Self-Screening of Hydrolase Substrates. *Eur. J. Org. Chem.* **2006**, 285–291.

[183] Hu, L.; Ramström, O. Silver-catalyzed dynamic systemic resolution of α-iminonitriles in a 1,3-dipolar cycloaddition process. *Chem. Commun.* **2014**, DOI:10.1039/c4cc00944d.

[184] Vongvilai, P.; Ramström, O. Dynamic Asymmetric Multicomponent Resolution: Lipase-Mediated Amidation of a Double Dynamic Covalent System. *J. Am. Chem. Soc.* **2009**, *131*, 14419–14425.

[185] Sakulsombat, M.; Vongvilai, P.; Ramström, O. *In Situ* Evaluation of Lipase Performances Through Dynamic Asymmetric Cyanohydrin Resolution. *Org. Biomol. Chem.* **2011**, *9*, 1112–1117.

[186] Chandler, I. C. Determining the Regioselectivity of Immobilized Lipases in Triacylglycerol Acidolysis Reactions. *J. Am. Oil Chem. Soc.* **2001**, *78*, 737–742.

[187] Liljeblad, A.; Vänttinen, E.; Kanerva, L. T. Lipase-Catalyzed Resolution of Glycerol-Based Racemates. *Chirality* **1999**, *11*, 432–439.

[188] Therisod, M.; Klibanov, A. M. Regioselective Acylation of Secondary Hydroxyl Groups in Sugars Catalyzed by Lipases in Organic Solvents. *J. Am. Chem. Soc.* **1987**, *109*, 3977–3981.

[189] MacManus, D. A.; Vulfson, E. N. Substituent effects on the regioselectivity of enzymatic acylation of 6-*O*-alkylglucopyranosides using *Pseudomonas cepacia* lipase. *Carbohydr. Res.* **1995**, *279*, 281–291.

[190] Kadereit, D.; Waldmann, H. Enzymatic Protecting Group Techniques. *Chem. Rev.* **2001**, *101*, 3367–3396.

[191] Therisod, M.; Klibanov, A. M. Facile Enzymatic Preparation of Monoacylated Sugars in Pyridine. *J. Am. Chem. Soc.* **1986**, *108*, 5638–5640.

[192] Riva, S.; Chopineau, J.; Kieboom, A. P. G.; Klibanov, A. M. Protease-Catalyzed Regioselective Esterification of Sugars and Related Compounds in Anhydrous Dimethylformamide. *J. Am. Chem. Soc.* **1988**, *110*, 584–589.

[193] Park, S.; Kazlauskas, R. J. Improved Preparation and Use of Room-Temperature Ionic Liquids in Lipase-Catalyzed Enantio- and Regioselective Acylations. *J. Org. Chem.* **2001**, *66*, 8395–8401.

[194] Afach, G.; Kawanami, Y.; Cheetangdee, N.; Fukada, K.; Izumori, K. Lipase-Catalyzed Synthesis of D-Psicose Fatty Acid Diesters and their Emulsification Activities. *J. Am. Oil Chem. Soc.* **2008**, *85*, 755–760.

[195] Otto, R. T.; Bornscheuer, U. T.; Syldatk, C.; Schmid, R. D. Lipase-catalyzed synthesis of arylaliphatic esters of β-d-glucose, n-alkyl- and arylglucosides and characterization of their surfactant properties. *J. Biotechnol.* **1998**, *64*, 231–237.

[196] Gao, C.; Whitcombe, M. J.; Vulfson, E. N. Enzymatic synthesis of dimeric and trimeric sugar-fatty acid esters. *Enz. Microb. Technol.* **1999**, *25*, 264–270.

[197] Flores, M. V.; Naraghi, K.; Engasser, J.-M.; Halling, P. J. Influence of Glucose Solubility and Dissolution Rate on the Kinetics of Lipase Catalyzed Synthesis of Glucose Laurate in 2-Methyl 2-Butanol. *Biotechnol. Bioeng.* **2002**, *78*, 815–821.

[198] Park, D.-W.; Haam, S.; Ahn, I.-S.; Lee, T. G.; Kim, H.-S.; Kim, W.-S. Enzymatic esterification of β-methylglucoside with acrylic/methacrylic acid in organic solvents. *J. Biotechnol.* **2004**, *107*, 151–160.

[199] Arcos, J. A.; Bernabé, M.; Otero, C. Quantitative Enzymatic Production of 6-*O*-Acylglucose Esters. *Biotechnol. Bioeng.* **1998**, *57*, 505–509.

[200] Arcos, J. A.; Bernabé, M.; Otero, C. Different Strategies for Selective Monoacylation of Hexoaldoses in Acetone. *J. Surfactants Detergents* **1998**, *1*, 345–352.

[201] Córdova, A.; Hult, K.; Iversen, T. Esterification of methyl glycoside mixtures by lipase catalysis. *Biotechnol. Lett.* **1997**, *19*, 15–18.

[202] Afach, G.; Kawanami, Y.; Izumori, K. Synthesis of D-Allose Fatty Acid Esters via Lipase-Catalyzed Regioselective Transesterification. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 833–835.

[203] Ikeda, I.; Klibanov, A. M. Lipase-Catalyzed Acylation of Sugars Solubilized in Hydrophobic Solvents by Complexation. *Biotechnol. Bioeng.* **1993**, *42*, 788–791.

[204] Becker, T.; Görls, H.; Pauls, G.; Wedekind, R.;Kai, M.; von Reuß, S. H.; Boland, W. Synthesis of Isoxazolin-5-one Glucosides by a Cascade Reaction. *J. Org. Chem.* **2013**, *78*, 12779–12783.

[205] Bousquet, M.-P.; Willemot, R.-M.; Monsan, P.; Boures, E. Enzymatic Synthesis of α-Butylglucoside Lactate: A New α-Hydroxy Acid Derivative. *Biotechnol. Bioeng.* **1999**, *62*, 225–234.

[206] Wei, D.-Z.; Zou, P.; Tu, M.-B.; Zheng, H. Enzymatic synthesis of ethyl glucoside lactate in non-aqueous system. *J. Mol. Catal. B: Enz.* **2002**, *18*, 273–278.

[207] Park, D.-W.; Kim, J.-S.; Haam, S.; Kim, H.-S.; Kim, W.-S. Lipase-catalyzed synthesis of β-methylglucoside esters containing an α-hydroxy acid. *Biotechnol. Lett.* **2001**, *23*, 1947–1952.

[208] Jia, H.; Wang, P. Enzymatic synthesis of galactosyl lactic ethyl ester and its polymer for use as biomaterials. *J. Biotechnol.* **2007**, *132*, 314–317.

[209] Park, O.-J.; Park, H. G.; Yang, J.-W. Enzymatic Transesterification of Monosaccharides and Amino Acid Esters in Organic Solvents. *Biotechnol. Lett.* **1996**, *18*, 473–478.

[210] Park, O.-J.; Jeon, G.-J.; Yang, J.-W. Protease-catalyzed synthesis of disaccharide amino acid esters in organic media. *Enz. Microb. Technol.* **1999**, *25*, 455–462.

[211] Jeon, G.-J.; Park, O.-J.; Hur, B.-K.; Yang, J.-W. Enzymatic synthesis of amino acid-sugar alcohol conjugates in organic media. *Biotechnol. Lett.* **2001**, *23*, 929–934.

[212] Maruyama, T.; Nagasawa, S.-I.; Goto, M. Enzymatic Synthesis of Sugar Amino Acid Esters in Organic Solvents. *J. Biosci. Bioeng.* **2002**, *94*, 357–361.

[213] Lohith, K.; Divakar, S. *Candida rugosa* lipase catalysed preparation of L-prolyl, L-phenylalanyl, L-tryptophanyl and L-histidyl esters of carbohydrates. *Biochem. Eng. J.* **2007**, *34*, 28–43.

[214] Vijayakumar, G. R.; Lohith, K.; Somashekar, B. R, Divakar, S. Lipase catalyzed synthesis of L-alanyl, L-leucyl and L-phenylalanyl esters of D-glucose using unprotected amino acids. *Biotechnol. Lett.* **2004**, *26*, 1323–1328.

[215] Lohith, K.; Manohar, B.; Divakar, S. Influence of buffer and L-phenylalanine concentration in the *Rhizomucor miehei* lipase catalysed synthesis of L-phenylalanyl-D-glucose ester investigated through response surface methodology. *Eur. Food. Res. Technol.* **2006**, *224*, 219–224.

[216] Somashekar, B. R.; Divakar, S. Lipase catalyzed synthesis of L-alanyl esters of carbohydrates. *Enz. Microb. Technol.* **2007**, *40*, 299–309.

[217] Kanerva, L. T.; Sundholm, O. Enzymatic Acylation in the Resolution of Methyl *threo*-2-Hydroxy-3-(4-methoxyphenyl)-3-(2-X-phenylthio)propionates in Organic Solvents. *J. Chem. Soc. Perkin Trans. 1* **1993**, 2407–2410.

[218] Lipshutz, B. H. Five-Membered Heteroaromatic Rings as Intermediates in Organic Synthesis. *Chem. Rev.* **1986**, *86*, 795–819.

[219] Bencze, L. C.; Paizs, C.; Toşa, M.; Irimie, F.-D. Substituent effects on the stereochemical outcome of the baker's yeast-mediated biotransformation of α-hydroxy- and α-acetoxymethyl- 5-phenylfuran-2-yl-ethanones. *Tetrahedron: Asymmetry* **2010**, *21*, 356–364.

[220] Soloshonok, V. A.; Roussel, C.; Kitagawa, O.; Sorochinsky, A. E. Self-disproportionation of enantiomers via achiral chromatography: a warning and an extra dimension in optical purifications. *Chem. Soc. Rev.* **2012**, *41*, 4180–4188.

[221] Gassman, P. G.; Talley, J. J. Cyanohydrins – a General Synthesis. Tetrahedron Lett. **1978**, *40*, 3773–3776.

[222] Straathof, A. J. J.; Jongejan, J. A. The enantiomeric ratio: origin, determination and prediction. *Enz. Microb. Technol.* **1997**, *21*, 559–571.

[223] Tsavas, P.; Polydorou, S.; Faflia, I.; Voutsas, E. C.; Tassios, D.; Flores, M. V.; Naraghi, K.; Halling, P. J.; Chamouleau, F.; Ghoul, M.; Engasser, J.-M.; Ferrer, M.; Plou, F. J. Solubility of Glucose in Mixtures Containing 2-Methyl-2-butanol, Dimethyl Sulfoxide, Acids, Esters, and Water. *J. Chem. Eng. Data* **2002**, *47*, 807–810.

[224] Voutsas, E. C.; Tsavas, P.; Magoulas, K.; Tassios, D.; Ferrer, M.; Plou, F. J.; Ballesteros, A. Solubility Measurements of Fatty Acid Glucose and Sucrose Esters in 2-Methyl-2-butanol and Mixtures of 2-Methyl-2-butanol with Dimethyl Sulfoxide. *J. Chem. Eng. Data* **2002**, *47*, 1517–1520.

[225] Kartha, K. P. R.; Field, R. A. Iodine: A Versatile Reagent in Carbohydrate Chemistry IV. Per-O-Acetylation, Regioselective Acylation and Acetolysis. *Tetrahedron* **1997**, *53*, 11753–11766.

[226] Rashid, A.; Mackie, W.; Colquhoun, I. J.; Lamba, D. *Can. J. Chem.* **1990**, *68*, 1122–1127.

[227] Lehtilä, R. L.; Lehtilä, J. O.; Roslund, M. U.; Leino, R. Selectively protected galactose derivatives for the synthesis of branched oligosaccharides. *Tetrahedron* **2004**, *60*, 3653–3661.

[228] Marcotte, S.; Pannecoucke, X.; Feasson, C.; Quirion, J.-C. Enantioselective Synthesis of α,α-Difluoro-β-amino Acid and 3,3-Difluoroazetidin-2-one via the Reformatsky-Type Reaction of Ethyl Bromodifluoroacetate with Chiral 1,3-Oxazolidines. *J. Org. Chem.* **1999**, *64*, 8461–8464.

[229] Boyer, N.; Gloanec, P.; De Nanteuil, G.; Jubault, P.; Quirion, J.-C. Chemoselective and stereoselective synthesis of *gem*-difluoro-β-aminoesters or *gem*-difluoro-β-lactams from ethylbromodifluoroacetate and imines during Reformatsky reaction. *Tetrahedron* **2007**, *63*, 12352–12366.

[230] Boyer, N.; Gloanec, P.; De Nanteuil, G.; Jubault, P.; Quirion, J.-C. Synthesis of α,α-Difluoro-β-amino Esters or *gem*-Difluoro-β-lactams as Potential Metallocarboxypeptidase Inhibitors. *Eur. J. Org. Chem.* **2008**, 4277–4295.

[231] Palomo, C.; Oiarbide, M.; Bindi, S. A Concise β-Lactam Route to Short Peptide Segments Containing β,β-Disubstituted β-Amino Acids. *J. Org. Chem.* **1998**, *63*, 2469–2474.

[232] Forró, E.; Fülop, F. Synthesis of 4-aryl-substituted β-lactam enantiomers by enzyme-catalyzed kinetic resolution. *Tetrahedron: Asymmetry* **2001**, *12*, 2351–2358.

[233] Csomós, P.; Kanerva, L. T.; Bernáth, G.; Fülöp, F. Biocatalysis for the Preparation of Optically Active β-Lactam Precursors of Amino Acids. *Tetrahedron: Asymmetry* **1996**, *7*, 1789–1796.

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