TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 1036

MEDICA - ODONTOLOGICA

STUDIES ON CHEMOENZYMATIC SYNTHESIS:

LIPASE-CATALYZED ACYLATION IN MULTISTEP ORGANIC SYNTHESIS

by

Ari Hietanen

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2012

Department of Pharmacology, Drug Development and Therapeutics
Laboratory of Synthetic Drug Chemistry
Institute of Biomedicine, Faculty of Medicine
University of Turku
Turku, Finland

Supervisor and custos:
Professor Liisa T. Kanerva, PhD
University of Turku
Turku, Finland

Reviewers:
Professor Petri Pihko, PhD
University of Jyväskylä
Jyväskylä, Finland

and

Professor Patrick Adlercreutz, PhD
Lund University
Lund, Sweden

Opponent:
Professor Romano Orru, PhD
VU University
Amsterdam, The Netherlands

ISBN 978-951-29-5138-3 (PRINT)
ISBN 978-951-29-5139-0 (PDF)
ISSN 0355-9483
Painosalama Oy - Turku, Finland 2012

ABSTRACT

Ari Hietanen

Studies on chemoenzymatic synthesis: Lipase-catalyzed acylation in multistep organic synthesis

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

Annales Universitatis Turkuensis, Painosalama Oy, Turku, Finland, 2012

In this thesis, biocatalysis is defined as the science of using enzymes as catalysts in organic synthesis. Environmental aspects and the continuously expanding repertoire of available enzymes have firmly established biocatalysis as a prominent means of chemo-, regio- and stereoselective synthesis. Yet, no single methodology can solve all the challenges faced by a synthetic chemist. Therefore, the knowledge and the skills to combine different synthetic methods are relevant.

Lipases are highly useful enzymes in organic synthesis. In this thesis, an effort is being made to form a coherent picture of when and how can lipases be incorporated into non-enzymatic synthesis. This is attempted both in the literature review and in the discussion of the results presented in the original publications contained in the thesis. In addition to lipases, oxynitrilases were also used in the work. The experimental part of the thesis comprises of the results reported in four peer-reviewed publications and one manuscript. Selected amines, amino acids and sugar-derived cyanohydrins or their acylated derivatives were each prepared in enantio- or diastereomerically enriched form. Where applicable, attempts were made to combine the enzymatic reactions to other synthetic steps either by the application of completely separate sequential reactions with isolated intermediates (kinetic and functional kinetic resolution of amines), simultaneously occurring reactions without intermediate isolation (dynamic kinetic resolution of amino acid esters) or sequential reactions but without isolating the intermediates (hydrocyanation of sugar aldehydes with subsequent diastereoresolution). In all cases, lipase-catalyzed acylation was the key step by which stereoselectivity was achieved. Lipase from *Burkholderia cepacia* was a highly selective enzyme with each substrate category, but careful selection of the acyl donor and the solvent was important as well.

Keywords: biocatalysis, chemoenzymatic, kinetic resolution, lipase, oxynitrilase, amines, amino acids, cyanohydrins.

TIIVISTELMÄ

Ari Hietanen

Tutkimuksia kemoentsymaattisista synteeseistä: Lipaasien katalysoima asylaatio monivaiheisessa orgaanisessa synteesissä

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

Annales Universitatis Turkuensis, Painosalama Oy, Turku, Finland, 2012

Tässä väitöskirjassa biokatalyysillä tarkoitetaan entsyymien käyttöä katalyytteinä orgaanisessa synteesissä. Ympäristöystävällisyys ja jatkuvasti laajeneva saatavilla olevien entsyymien kirjo ovat vakiinnuttaneet biokatalyysin kemo-, regio- ja stereoselektiivisen synteesin keskeiseksi keinoksi. Kuitenkaan mikään yksittäinen menetelmä ei pysty ratkaisemaan kaikkia synteesikemistin kohtaamia haasteita. Näin ollen eri synteesimenetelmien yhdistämiseen tarvittavat tiedot ja taidot ovat olennaisia.

Lipaasit ovat orgaaniseen synteesiin erityisen hyvin soveltuvia entsyymejä. Väitöskirjassa pyritään muodostamaan kokonaisvaltainen kuva siitä, milloin ja miten lipaaseja voidaan hyödyntää perinteisen synteesin osana. Tätä päämäärää tavoitellaan sekä kirjallisuus-katsauksessa että väitöskirjaan sisältyvissä alkuperäisjulkaisuissa. Lipaasien lisäksi työssä hyödynnettiin oksinitrilaasi-entsyymejä. Väitöstyön kokeellinen osa koostuu tuloksista, jotka on raportoitu neljässä vertaisarvioidussa tutkimusartikkelissa ja yhdessä toistaiseksi julkaisemattomassa käsikirjoituksessa. Tutkimuskohteiksi valitut amiinit, aminohapot ja sokeriperäiset syanohydriinit tai niiden asyloidut johdannaiset valmistettiin enantio- tai diastereomeerisesti rikastuneessa muodossa. Soveltuvin osin tutkittiin mahdollisuutta yhdistää entsymaattisia reaktioita synteesin muihin vaiheisiin joko suorittamalla reaktiot täysin erillisinä välituotteet eristäen (amiinien kineettinen ja funktionaalinen kineettinen resoluutio), samanaikaisina reaktioina eristämättä välituotteita (aminohappoestereiden dynaaminen kineettinen resoluutio) tai erillisinä reaktioina mutta eristämättä välituotteita (sokerialdehydien hydrosyanaatio ja tätä seuraava diastereoresoluutio). Kussakin tapauksessa lipaasin katalysoima asylaatio oli keskeinen stereoselektiivisyyden määräävä reaktio. *Burkholderia cepacia* -bakteerista eristetty lipaasi oli hyvin selektiivinen entsyymi kullekin lähtöaineluokalle, mutta myös asyylinluovuttajan ja liuottimen huolellinen valitseminen oli tärkeää.

Avainsanat: biokatalyysi, kemoentsymaattinen, kineettinen resoluutio, lipaasi, oksinitrilaasi, amiinit, aminohapot, syanohydriinit.

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ABBREVIATIONS

ANL	Lipase from *Aspergillus niger*
API	Active pharmaceutical ingredient
BCL	Lipase from *Burkholderia cepacia*
Boc	*tert*-Butyloxycarbonyl
CAL-A	Lipase A from *Candida antarctica*
CAL-B	Lipase B from *Candida antarctica*
CRL	Lipase from *Candida rugosa*
CSP	Chiral stationary phase
Conv.	Conversion
D	Diastereomeric ratio
de	Diastereomeric excess
DIPE	Diisopropyl ether
DKR	Dynamic kinetic resolution
E	Enantiomeric ratio
ee	Enantiomeric excess
EtOAc	Ethyl acetate
equiv., eq.	Number of molar equivalents
GC	Gas chromatography
HNL	Hydroxynitrile lyase (oxynitrilase)
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
IMDA	Intramolecular Diels-Alder reaction
KR	Kinetic resolution
MeOH	Methanol
M.S.	Molecular sieves
n.d.	Not determined
NMR	Nuclear magnetic resonance
NMO	*N*-Methylmorpholine *N*-oxide
PFL	Lipase from *Pseudomonas fluorescens*
PPL	Porcine pancreatic lipase
PS-D	Lipase from *Burkholderia cepacia* immobilized on diatomaceous earth
PS-C II	Lipase from *Burkholderia cepacia* immobilized on ceramic
*p*TsA	*para*-Toluenesulfonic acid
RCM	Ring-closing metathesis
RDL	Lipase from *Rhizopus delemar*
r.t.	Room temperature (~21 - 24 ^{o}C)
TBME	*tert*-Butyl methyl ether
TCA(A)	Trichloroacetic acid (anhydride)
TLL	Lipase from *Thermomyces lanuginosus*

LIST OF ORIGINAL PUBLICATIONS

I Hietanen, A.; Saloranta, T.; Rosenberg, S.; Laitinen, E.; Leino, R.; Kanerva, L. T. Synthesis of enantiopure benzyl homoallylamines by indium-mediated Barbier-type allylation combined with enzymatic kinetic resolution: Towards the chemoenzymatic synthesis of N-containing heterocycles. *Eur. J. Org. Chem.* **2010**, 909-919.

II Hietanen, A.; Saloranta, T.; Leino, R.; Kanerva, L. T. Kinetic resolution of a pyridylamine by lipase-catalyzed acylation. *Manuscript in preparation.*

III Hietanen, A.; Lundell, K.; Kanerva, L. T.; Liljeblad, A. Advances in the kinetic and dynamic kinetic resolution of piperazine-2-carboxylic acid derivatives with *Candida antarctica* lipase A; structural requirements for enantioselective N-acylation. *Arkivoc* **2012**, (*v*), 60-74.

IV 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.

V 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.

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1. INTRODUCTION

Enzymes are catalytic proteins in living organisms, which promote the chemical transformation of a particular chemical entity (the substrate) into another one (the product). In nature, enzymes have evolved to be highly efficient in aqueous solution and often highly selective, catalyzing the transformation of only a single substrate. In the 1980s it was noted that enzymes can be taken out of their natural surroundings and applied in organic solvents, the environment most useful for organic synthesis, where many enzymes remained active and accepted non-natural compounds as substrates. The substrates were transformed into products with high selectivity so that the preparation of, for example, pure stereoisomers was possible. Enzymes were thus established as useful tools for synthetic organic chemistry. Today, the efficiency, selectivity and non-toxicity to humans or the environment are the most important motivations for developing new and improved enzymatic syntheses.

Lipases are particularly suitable enzymes for organic synthesis. They are tolerant toward organic solvents, accept a broad substrate range and are often selective, stable and readily available. The main part of this thesis is to present the results of my research on lipase catalysis in organic synthesis accompanied by the original publications. The main chapters of the thesis are organized according to the requirements of the faculty of medicine. Literature review serves to put the experimental section in proper context. Relevant stereochemical terminology is defined in section 2.1. The role of chirality in pharmacology is surveyed in section 2.2. Strategies of preparing single stereoisomers are introduced in section 2.3. The theoretical and the practical framework for enzyme-mediated synthesis and particularly for enzymatic kinetic resolution are presented in section 2.4. A part of the research (within the experimental section 5.2) included the synthesis of cyanohydrins by oxynitrilase catalysis and the relevant literature is reviewed in section 2.5. The main body of the work comprises of kinetic resolution and diastereoresolution by lipase-catalyzed acylation. The literature on this topic is thoroughly reviewed in section 2.6. Throughout the literature review, the original research results, when relevant to a particular topic, are noted but details are not presented since this would result in unnecessary repetition of the results chapter.

The aims of the study are stated in chapter 3. Materials and methods are presented in chapter 4. More experimental details can be found from the original publications. Part of the work was done in collaboration with the department of organic chemistry at Åbo Academy University. Chapter 4 also specifies their contribution. Results of the work are covered in chapter 5. The thesis is summarized and concluded in chapter 6.

2. LITERATURE REVIEW

2.1 Stereochemical concepts and definitions

Achiral molecules have superimposable mirror images and do not rotate plane–polarized light.

Asymmetric induction refers to the source of discrimination between the possible chiral products in a given stereoselective process. Asymmetric induction can be either external or internal. In *external asymmetric induction*, chiral discrimination is brought about by a molecule not to be incorporated into the final product (reagent control). In *internal asymmetric control*, the substrate molecule itself is already chiral and guides the formation of the new stereogenic center (substrate control).

Chirality is the property of an object, such as a molecule, to have a non-superimposable mirror image. The most common, but not the only, feature making a molecule *chiral* is it to have a *stereogenic center*, for example an *asymmetric* carbon atom, a carbon with four unequal substituents.

Configuration is the arrangement of substituent groups around a central atom. The configuration of a steregenic center can be designated according to Cahn-Ingold-Prelog convention either as (*R*) or (*S*). The configuration of a certain stereogenic center can not be changed without bond-breakage.

Diastereomers are stereoisomers that are not enantiomers. When chiral, a pair of diastereomers has at least one stereogenic center of equal and one with opposite configuration. The chemical and physical properties of diastereomers differ in general from each other. Diastereomers can also be achiral (for example, *E*/*Z*-alkenes).

Enantiomers are a pair of chiral molecules with all of their stereogenic centers having opposite configuration. The chemical and physical properties of enantiomers are identical apart from their ability to rotate plane-polarized light in opposite directions, clockwise (+) or anticlockwise (-). Enantiomers are also called optical isomers.

Enantiomeric excess (*ee*) of a solution of enantiomers is calculated as $ee = (c_M - c_m)/(c_M + c_m)$ where c_M and c_m represent the concentrations of the major and the minor enantiomers. *Ee* thus represents the excess of one enantiomer over a racemic solution. Therefore, a compound with an *ee* of 98% can be said to have two percent of a racemate and 98% of the major enantiomer. Equivalently, the compound consists of 99% of the major and 1% of the minor enantiomer (and has *enantiomer ratio* 99:1). *Diastereomeric excess* (*de*) can be similarly defined but is applicable only when two

diastereomers are present. With more than two diastereomers present, *diastereomer ratio* can be applied.

Enantiomerically enriched is a non-racemic mixture of enantiomers and synonymous to *optically active*. *Enantiopure* is enantiomerically highly enriched, strictly speaking, a compound/solution where the minor enantiomer can not be detected (in this thesis, when the minor enantiomer was not detected, *ee* >99% is claimed).

Epimers are diastereomers with more than one stereocenter and which have opposite configurations only at one stereocenter. For example, methyl α-D-galactoside and methyl β-D-galactoside are epimers.

Racemate is an equimolar mixture of a pair of enantiomers (or all diastereomers) so that the optical rotation of a *racemic* solution is zero.

Stereoisomers are molecules which have identical atomic composition (amount and connectivity of the atoms) but differ in the spatial arrangement of those atoms. Stereoisomers thus include both enantiomers and diastereomers.

Stereoselectivity is the property of a chemical reaction to form one stereoisomer in excess over other possible stereoisomers. For example, an addition reaction into a carbonyl group may be stereoselective if the substrate or a catalyst contains features promoting the addition to occur on one of the sides of the planar carbonyl group instead of the other.

Stereospecificity is the property of a chemical reaction to exclusively form one stereoisomer due to the mechanism of the reaction. In a stereospecific reaction, a molecule of a certain configuration will always react to give a molecule of a certain configuration but not the other. A stereospecific reaction, for example a bimolecular nucleophilic substitution (S_N2) reaction, will give a racemic product if the substrate is a racemate and an enantiopure product if the substrate is enantiopure and no competing reactions occur.

2.2 Chirality in pharmacology and drug discovery

Active pharmaceutical ingredients (APIs, drug molecules) exert their effect by interacting with a target molecule (receptor, enzyme, transporter protein or other proteins or occasionally nucleic acids) in a three-dimensional manner. The target is homochiral (always expressed as a single stereoisomer), and when the API is chiral too, the interaction will be stereoselective. One stereoisomer (enantiomer or diastereomer) of the API will be bound to the receptor more strongly and has a stronger effect than the others. The other stereoisomers might instead bind to other proteins causing undesirable clinical effects. Further, the different stereoisomers might also bind differently to metabolizing enzymes or tranporter proteins, thus having different pharmacokinetic behaviour. For example, formoterol (Figure 1), which is a β_2-adrenoceptor agonist useful in the management of asthma, has two chiral centers and thus four diastereomers. (*R*,*R*)-formoterol is approximately 1000 times more potent than its (*S*,*S*)-enantiomer, while the (*R*,*S*)- and (*S*,*R*)-diastereomers have intermediate and approximately equal potencies.[1]

Another important example on the importance of single-enantiomer drugs is praziquantel (Figure 1), an antihelmintic agent used in the therapy and profylaxis of schistosomiasis in the developing world. Due to cost and low profit, the drug is manufactured in a racemic form. However, the (*R*)-enantiomer is the therapeutically active one (and tasteless), while the (*S*)-enantiomer only contributes to side-effects and tastes bitter. The use of a racemate makes the pill large and difficult to swallow, particularly as many of the patients are children. As a result, often only subtherapeutic blood concentrations of the effective enantiomer are achieved. Thus the use of the racemate has lead to inefficient treatment/profylaxis while still causing side effects, a situation that can be improved now that a cost-efficient manufacturing route to the clinically effective enantiomer has been developed.[2]

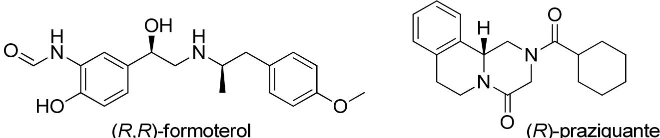


Figure 1. The biologically effective stereoisomers of formoterol and praziquantel.

In light of these and many other examples, both the enantiomers and the diastereomers of a drug molecule can be considered as different chemical entities in their pharmacologic effect. Drug regulatory agencies, such as the US FDA, require that the pharmacological and toxicological assays should monitor all stereoisomers contained

in the drug product.[3] Thus, there is also a clear regulatory and cost incentive to apply approval for a single-enantiomer drug. The replacement of a racemic drug with an enantiopure drug may also give extended patent protection for an already approved drug substance (chiral switch).[4] Already in the early 2000s, more than 50% of the FDA approved new molecular entities were chiral and almost all of them were single enantiomers.[5] Two trends in drug discovery literature support the notion that single-enantiomer drugs will continue to increase in proportion of newly developed drugs. First, there is a correlation between sets of compounds of increased stereochemical and shape complexity and protein-binding profiles of those compounds so that molecules with high proportion of stereogenic and sp^3-hybridized carbon atoms, such as natural products, show tighter and more specific protein binding.[6,7] This favors the inclusion of more chiral molecules in compound libraries. The presence of the non-active enantiomer in racemic compounds would result in lower potencies and increased toxicities in screening assays. There is therefore a clear advantage to use enantiopure compounds early on in the drug discovery process. Secondly, computational drug design produces lead structures with exact orientation of functional groups, meaning requirements for strict stereochemical control.[8] My analysis of the US FDA approved new molecular entities (NME) between 2005 - 2010 shows that of the 93 small-molecule NMEs, 67% were chiral with an average of 4 stereogenic centers per a chiral drug. The chiral drugs were also on average larger, more polar, had more sites of hydrogen bonding and were more complex than the achiral ones (Figure 2).[9]

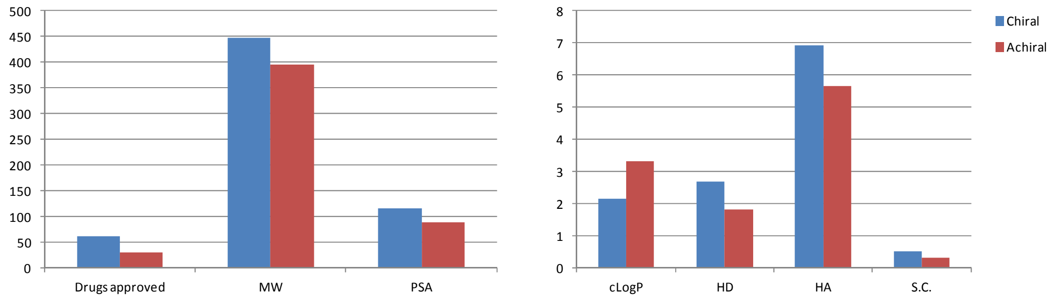


Figure 2. Comparison of average properties of chiral (blue) and achiral (red) small molecule drugs approved by the US FDA between 2005 - 2010. MW = molecular weight; PSA = polar surface area; cLogP = calculated partition coefficient; HD and HA = number of hydrogen bond donors and acceptors; S.C. = scaffold complexity (proportion of sp^3-hybridized carbons of all carbon atoms).

2.3 Methods for preparing single stereoisomers

Different approaches to prepare single stereoisomers exist. In stereoselective (asymmetric, enantioselective) synthesis an achiral starting material is transformed into a single enantiomer product directly during the chemical reaction. Theoretically, 100% yield can be obtained. In the resolution of enantiomers, the chiral product is first formed as a racemic mixture from the achiral substrate and the two enantiomers are then separated with an external chiral reagent. This will limit the yield of one enantiomer to 50%. In favorable cases the undesired enantiomer can be racemized and the resolution can be repeated to improve the total yield beyond 50%. In kinetic resolution, one substrate enantiomer of a racemate reacts to another chemical compound. The product and the substrate, now consisting of distinct enantiomers, are then separated. The same principles can be applied to the preparation of diastereomers when more than one chiral center is present in the substrate. Which one of the methods is to be preferred varies on a case-by-case basis, most importantly the decision is based on cost (bulk chemical manufacturing) and development time (synthesis for drug development).[10]

2.3.1 Asymmetric synthesis

Stereoselective synthesis results in the generation of the product in an enantiomerically or diastereomerically enriched form. Stereoselective synthesis has three broad subcategories depending on how the chirality is introduced into the molecule. These are synthetic strategies relying on the use of chiral pool, chiral auxiliaries or catalytic asymmetric synthesis (Figure 3).

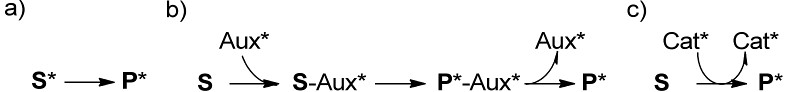


Figure 3. The principles of asymmetric synthesis by a) chiral pool strategy; b) by using a chiral auxiliary; and c) by catalytic asymmetric synthesis. **S** = substrate for the desired synthetic reaction, **P** = product, Aux = chiral auxiliary, Cat = chiral catalyst, * designates chirality.

In cases when the already existing stereogenic center in the substrate remains in the end product with the newly created stereogenic center, the approach is called chiral pool strategy (Figure 3a). Tartaric acid, natural amino acids and simple carbohydrates are perhaps the most often utilized sources of chirality in chiral pool-based synthesis. Another case of internal asymmetric control is the use of chiral auxiliaries (Figure 3b). These are chiral fragments which are first introduced to the achiral substrate to guide the stereoselective transformation leading to the new stereogenic center and are finally

removed from the now-chiral product. Thus, chiral auxiliaries behave analoguously to protecting groups serving only a temporary function at a certain phase of the synthesis. Notable examples of chiral auxiliaries are the enantiomers of substituted oxazolidinones (Evans' auxiliaries).[11] Catalytic asymmetric synthesis is a form of external asymmetric induction in which the catalyst is itself a single enantiomer and prefers to form only one product stereoisomer over other possible stereoisomers (Figure 3c).[12] The chiral catalyst can be a transition metal complex[13], an organic small molecule (organocatalysis)[14] or an enzyme (biocatalysis).

2.3.2 Resolution of enantiomers

Resolution of a racemic mixture of enantiomers to afford optically pure compounds may be based on chiral chromatography, diastereomeric salt formation or kinetic resolution. Chiral chromatography columns are coated with a stereochemically defined material to serve as chiral stationary phase (CSP) while the racemate is eluted along the mobile phase. As the enantiomers interact differently with the chiral coating, they are separated from each other. Chiral chromatography is of fundamental importance to stereochemical studies as an analytical method, but it has traditionally been of limited importance in process scale. However, it has importance for early-stage drug discovery since it enables rapid access to pure enantiomers. [15] Additionally, simulated moving bed (SMB) chromatography with a CSP is an expanding process technology, making chiral column chromatography a feasible alternative to scale-up. [16]

Racemic mixtures can be resolved also by crystallization. It is estimated that approximately 5-10% of organic compounds can crystallize as single-enantiomer conglomerates, crystals composing of single enantiomers.[17] This is how Louis Pasteur first observed chirality when D-(-)-tartaric acid and L-(+)-tartaric acid spontaneously crystallized as different-looking conglomerates which could be picked up separately.[18] A more general method is crystallization by diastereomeric salt formation. Racemic mixture is treated with a single enantiomer of a molecule, which forms the counterion to the racemic compound upon solvation. A diasteromeric salt is formed, and since diastereomers have different physical properties, conditions can be found where one diastereomeric salt pair is crystallized while the other is not. The process thus enables the recollection of both enantiomers, one as a crystallized salt and the other dissolved in the liquid phase. This technique is quite often used in industry since it is straightforward to do with standard equipment, can be scaled-up easily and yields pure products.[10] Enantiomeric crystallization is also often performed to enrich the products of asymmetric synthesis, which are not always acquired in sufficient high *ee*, to the required level of enantiomeric purity.[17]

2.3.3 Kinetic resolution

A third way to resolve a racemic mixture is by kinetic resolution. The two enantiomers of a racemic substrate react with different rates in a reaction promoted by a chiral catalyst. The other enantiomer is transformed into a new chemical species while the other remains intact. Thus, kinetic resolution lies halfway between a conventional resolution and a stereoselective synthesis. While the substrate of a kinetic resolution is always chiral, the product can be either chiral or achiral. Examples of such kinetic resolutions of alcohols are shown in Figure 4. Kinetic resolution was pioneered by Sharpless with the kinetic resolution of allylic alcohols through titanium alkoxide tartrate catalyzed oxidation of the alkene functionality into the corresponding epoxide.[19] Later developments have relied on many different types of chemical transformations applied to a wide range of substrate types, the hydrolysis of terminal epoxides catalyzed by (salen)Co complexes[20], oxidation and acylation with chiral catalysts being among the most extensively studied non-enzymatic processes.[21] A large part of published research on kinetic resolution is based on enzymatic catalysis.

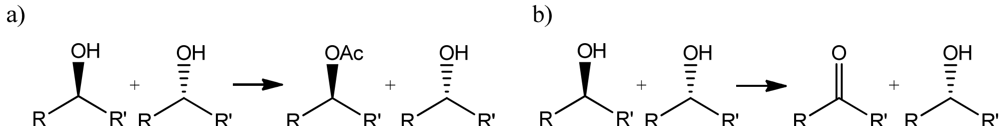


Figure 4. Examples of kinetic resolutions of alcohols with a) formation of a chiral product (selective acylation) and b) formation of an achiral product (selective oxidation).

A prerequirement for a successful enzymatic kinetic resolution is that the spontaneous (or non-enzymatic) reaction is negligibly slow. Ideally, the transformation of the preferred enantiomer is catalyzed while that of the non-preferred enantiomer is not so that the product and the substrate are enriched with the respective enantiomers. In practice, the two enantiomers often compete for binding to the catalyst. The ratio of the initial reaction rates of the two enantiomers (enantiomeric ratio, *E*), will then determine the enantioselectivity of the resolution. Enantiomeric ratio is dependent on the ability of the catalyst to discriminate between the two enantiomers according to equation *(1)*, and, in case of enzymatic catalysis, can be expressed as a ratio of the respective specificity constants (k_{cat}/K_M) according to equation *(2)*. The enantiomeric excess (*ee*) of both the product (when chiral) and the substrate will depend not only on the *E* value but also on the conversion (Conv.) of the resolution. Initially high ee_P (enantiomeric excess of the product) decreases and the initially low ee_S (enantiomeric excess of the substrate) improves as conversion increases. In an ideal process, the kinetic resolution should completely halt at 50% conversion. Supposing that the reaction is irreversible and that no side-reactions take place, the parameters of kinetic resolution (ee_P, ee_S,

Conv. and E) are all interrelated according to equations *(3)-(4)*.[22,23] When both the substrate and the product are chiral, combining equations *(3)* and *(4)* gives equation (5).[24]

(1) $\Delta\Delta G^{\ddagger} = -RT \ln E$, where
R is the universal gas constant and T is the absolute temperature

(2) $E = (k_{cat}/K_M)_{(R)} / (k_{cat}/K_M)_{(S)}$, where
k_{cat} is catalytic rate constant and K_M is the Michaelis-Menten constant for *R*- and *S*-enantiomers (*R*-selective reaction presumed).

(3) $\text{Conv.} = ee_S / (ee_S + ee_P)$

(4) $E = \ln [1 - \text{Conv.} (1 + ee_P)] / \ln [1 - \text{Conv.}(1 - ee_P)]$
and
$E = \ln [(1 - \text{Conv.})(1 - ee_S)] / \ln [(1 - \text{Conv.})(1 + ee_S)]$

(5) $E = \{\ln [ee_P(1 - ee_S) / (ee_S + ee_P)]\} / \{\ln [ee_P(1 + ee_S) / (ee_S + ee_P)]\}$

Using these equations, enantiomeric excess of the substrate and the product can be graphically represented as a function of conversion for a given E value. Figure 5 shows three such curves for $E = 10$, 100 and 200. At 25 ^{o}C, these correspond to $\Delta\Delta G^{\ddagger}$ of 1.36 kcal mol^{-1}, 2.73 kcal mol^{-1}, and 3.14 kcal mol^{-1}, respectively. Since enantiomeric ratio is highly sensitive to the measured ee_S and ee_P values, a common notation is that kinetic resolutions with $E > 200$ are considered highly selective.

Kinetic resolution can be applied not only to enantiomers but also to diastereomers. Where a pair of diastereomers forms a non-racemic mixture (such as epimers) the process in this thesis is called diastereoresolution (relevant to section 5.2). In these cases, *ee* and E are replaced by diastereomeric excess (*de*) and diastereomer ratio (*D*).

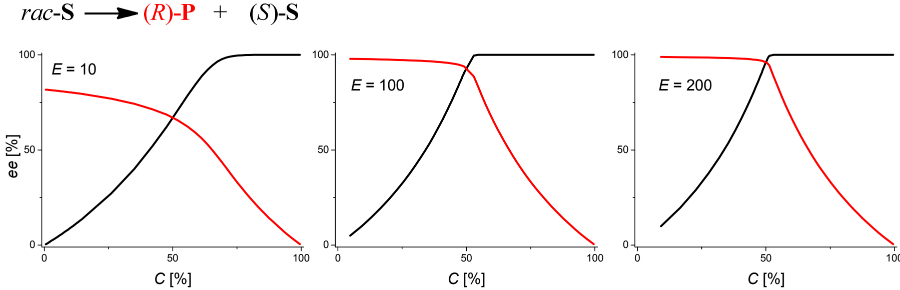


Figure 5. Change of the *ee* of the substrate (black lines) and the product (red lines) as a function of conversion in a kinetic resolution having different enantiomeric ratios.

2.4 Enzymatic catalysis

2.4.1 Fundamentals of enzymatic catalysis

Enzymes are proteins produced by living organisms that catalyze the necessary chemical reactions to occur within a suitable time-scale for the organism to sustain and reproduce. Enzymes are macromolecular catalysts, the functional structure of which is held together mainly by weak non-covalent interactions between different parts of the polymer. In living systems, enzymes operate in complex aqueous solutions dilute in substrate (typically $\leq 10^{-5}$ M).[25] Many enzymes have evolved to achieve or approach the maximum achievable rate, namely that limited by diffusion (with rate constants at $\sim 10^{9}$ $s^{-1}M^{-1}$). To achieve these rates, very large rate acceleration by enzymes is required, with k_{cat}/k_{uncat} typically varying from 10^{7} to 10^{19} -fold.

According to transition state theory, enzymes catalyze reactions by stabilizing the transition state of the reaction so that the Gibbs' free energy of activation ($\Delta G^{\ddagger}$) is lower for the catalyzed than the non-catalytic reaction (Figure 6a).[26,27] To pass through the catalytic cycle, the enzyme must *i*) bind the substrate within the active site of the enzyme to form an enzyme-substrate complex, *ii*) transform the substrate via the stabilized transition state to *iii)* form an enzyme-product complex and *iv*) release the product to the solution and regenerate the intact enzyme to start the catalytic cycle again (Figure 6b). The theory requires the enzyme to bind the transition state tighter than the substrate. A central mechanism for this is the induced fit, in which the enzyme is preorganized to have partial complementarity between the substrate and the enzyme active site. The complementarity is strengthened as the substrate is bound, achieves its maximum at transition state and reduces back to the initial state as the product is released.[28] Substrate binding among many enzymes is estimated to occur with a Michaelis constant K_M of $10^{3.7\pm1.3}$ M while a similar computational binding constant for transition states has values of K_{tx} $10^{16.0\pm4.0}$ M, the latter being the largest association constants among the known host-guest complexes.[29] This differential binding of enzymes between the substrate and the transition state has been estimated to afford 11-38 kcal mol^{-1} difference in transition state binding energy ($\Delta\Delta G^{\ddagger}$).[30]

Multiple specific physical mechanisms may transmit catalysis. The enzyme active site has a preorganized cavity encompassing the substrate in such a way that substrate bonds are strained to conformations resembling the transition state. Thus, a substrate molecule spends a much larger amount of time in these near attack conformers (NAC) inside the enzyme than in solution.[31] Often the transition state is more polar than the ground state and electrostatic stabilization, such as hydrogen bonding or ionic interactions, contribute a major part to catalysis. For example, many enzymes have

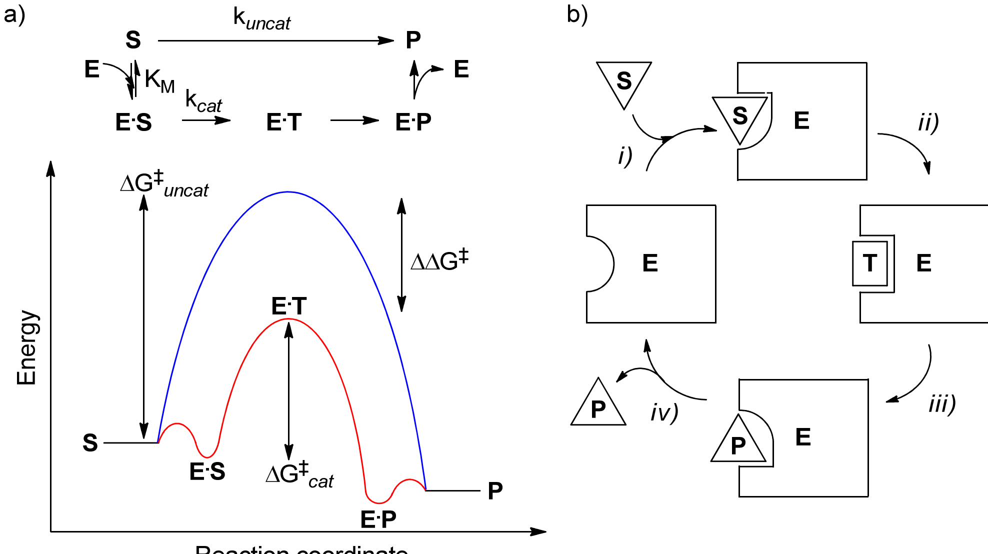


Figure 6. a) Schematic presentation of a general one-substrate catalytic mechanism of enzymes with the associated energetic diagram; b) Representation of the induced fit at enzyme active site during the catalytic cycle. **S** = substrate, **P** = product, **T** = substrate component of enzyme-substrate transition state complex, **E** = enzyme.

oxyanion holes, cavities that donate 2-3 hydrogen bonds from the peptide backbone to a negatively charged oxygen in a tetrahedral transition state derived from a carbonyl substrate. The oxyanion hole hydrogen bond donors are arranged in such a way that the ratio of transition state binding affinity over ground state binding affinity is at its maximum.[32]

It is estimated that most enzymes exert their catalytic effect not solely by non-covalent interactions but by forming transient covalent bonds.[30] This will thus change the reaction mechanism fundamentally from that of the uncatalyzed reaction and is expected to be involved whenever >15 kcal mol^{-1} transition state stabilization is observed. Covalent reaction mechanism requires the precise and proximal positioning of the catalytically active amino acid residues of the enzyme with respect to the substrate. This will reduce the entropy of the reagents so that the reaction kinetics resemble an intramolecular reaction.[33] Examples include the acyl-enzyme intermediate formation in serine proteases and Schiff base intermediate in aldolases. Acid-base (proton transfer) catalysis is similarly achieved with proximally positioned proton acceptor/donor, often a His residue, the pK_a of which can be altered within the enzyme compared to the solution-state pK_a of that residue.[34] Both oxynitrilases and lipases use also this mechanism of catalysis (see later sections). Metal ion catalysis (Lewis acid catalysis) is important for some proteases and many oxidizing enzymes.[35]

It should be noted that many enzymes use several mechanisms in the elementary steps of their overall catalytic cycles. Lipases provide a good example (see Scheme 7) as they use electrostatic stabilization of the transition state (oxyanion hole stabilization of the tetrahedral intermediate), proximity and covalent catalysis (acyl-enzyme intermediate formation) and acid-base catalysis (Asp/Glu-His-Ser proton transfers).

2.4.2 Application of enzymes in synthesis

High selectivity and mild operating conditions make enzymes lucrative reagents for chemical synthesis beyond what they do in nature. However, several issues must be addressed for the application of enzymes in synthetic settings.[36,37] The enzyme should not be too specific so that it also accepts other substrates than the natural one(s). Some enzymes do not tolerate high temperatures and other solvents than water, restricting laboratory synthesis to aqueous media at moderate temperatures. Substrates cannot always be used at high concentrations since the enzymes are prone to substrate and/or product inhibition. Substrate solubility, enzyme stability, processability, availability and cost may pose additional challenges for synthetic applications.[38] Many of these challenges can be solved by either protein engineering approaches (for example by directed evolution) or by process development approaches (enzyme immobilization and recycling, solvent and reactor type selection, in situ product removal etc.). [39,40] Another aspect is that many useful enzymes require cofactors, such as NAD(P)H. Since cofactors are expensive, the use of stoichiometric amounts of them is not feasible and systems for cofactor recycling are required. This field is, however, rather advanced and often a suitable method for in situ cofactor regeneration can be found.[41] One solution is to use whole cells instead of isolated enzymes so that cofactor regenerating enzymes are present within the same biocatalyst. Several reviews cover the practical aspects of the use of enzymes in organic synthesis and applications in industrial synthesis of drugs.[42-45]

Enzyme Commission system classifies enzymes in six main classes based on the reaction type they catalyze in nature.[46] A four-digit E.C. number is given for each enzyme, in which the first digit determines the main class and subsequent digits specify the substrate(s) in the reaction. The main classes together with examples of synthetic importance are listed in Table 1. The most applied class in industrial organic synthesis are hydrolases, followed by oxidoreductases and certain types of lyases and transferases.[47,48] Synthesis with isomerases is mainly restricted to substrate racemization in repeated and dynamic kinetic resolution reactions. Ligases have relatively few synthetic applications but research on them is on-going, for example, for the synthesis of various bioconjugates.[49]

Table 1. Enzyme Commission (E.C.) classification of enzymes with selected examples.

1 Oxidoreductases Class definition: Oxidation and reduction. Examples: Alcohol dehydrogenases, oxidases, monooxygenases, peroxidases, laccases, reductases. Applications: Oxidation of alcohols, C-H oxidation, phenolic coupling, reduction of carbonyl compounds.
2 Transferases Class definition: Transfer of functional groups (methyl-, acyl-, amino-, phosphate-). Examples: Methyl transferases, transaminases, glycosyl transferases. Applications: Methylation, asymmetric reductive amination, oligosaccharide synthesis.
3 Hydrolases Class definition: Hydrolysis. Examples: Proteases, lipases, esterases, nitrilase. Applications: Hydrolytic kinetic resolution, transesterification, hydrolysis of nitriles to carboxylic acids.
4 Lyases Class definition: Addition or elimination of molecules with C-C, C-O, C-N or C-S bond cleavage/formation. Examples: Aldolases, hydroxynitrile lyases (oxynitrilases), benzaldehyde lyases, decarboxylases. Applications: Synthesis of polyhydroxy compounds, α-hydroxy ketones and cyanohydrins.
5 Isomerases Class definition: Isomerization (intramolecular rearrangement). Examples: Racemases (of α-hydroxy acids, α-amino acids, hydantoins). Applications: Racemization of substrate in repetitive and dynamic kinetic resolution.
6 Ligases Class definition: Coupling of two molecules by C-C, C-O, C-N or C-S bond formation with simultaneous breakdown of ATP. Examples: DNA/RNA ligases. Applications: Synthesis of oligonucleotides and their conjugates.

The E.C. number specifies the reaction that is enzyme-catalyzed, but not the individual enzyme per se. For example, triacylglycerol lipases from different organisms each have the E.C. code 3.1.1.3, though the enzymes are different in their amino acid sequence and in structure. Individual proteins are specified in UniProt database.[50] and structures for visualization can be found from Protein Data Bank.[51] BRENDA is an enzyme specific database and is extensive in its information content.[52]

The ability of enzymes to discriminate between the enantiomers of a racemic compound has found utility in a wide array of kinetic resolution applications. Examples of enzymatic kinetic resolutions of synthetic importance are presented in Table 2. Of these, hydrolase-catalyzed acylations and deacylations are the most widely applied. Many of the enzymatic reactions in Table 2 can also be applied in asymmetric synthesis, starting from achiral substrates and yielding chiral products.

Table 2. Examples of enzymatic kinetic resolutions.

Enzyme class	Substrates	Products	Enzyme
Oxidoreductases	*sec*-Alcohols	Ketones	Alcohol dehydrogenases
	Cyclic ketones	Lactones	Bayer-Villiger monooxygenases
	Alkanes	Alcohols	Cytochrome P450 monooxygenases
	Amines	Imines	Monoamine oxidases
Transferases	Amines, amino acids	Ketones	Transaminases
Hydrolases	*sec*-Alcohols	Esters	Esterases, lipases
	tert-Alcohols	Esters	Esterases, lipases (rare)
	Esters	Carboxylic acids, Alcohols	Esterases, lipases
	Amides	Amines	Proteases, amidases, lipases
	Amines	Amides	Proteases, lipases
	Carboxylic acids	Esters, amides	Lipases, esterases, proteases
	Epoxides	*sec*-Alcohols	Epoxide hydrolases
Lyases	β-Hydroxy ketones	Aldehydes	Aldolases

2.4.3 Enzymatic desymmetrization, stereoconvergent processes, deracemization and dynamic kinetic resolution

In classical kinetic resolution, the product yield is limited to 50%. Sometimes the ability of an enzyme to discriminate between the two enantiomers of a racemate or the two faces of a prochiral compound can be utilized so that the product can be obtained in 100% theoretical yield. These techniques comprise desymmetrization, stereoconvergent processes, deracemization and dynamic kinetic resolution (Figure 7). Apart from desymmetrization, they combine a kinetic resolution step by an enantioselective enzyme and a racemization/stereoinverting reaction to result in an overall asymmetric synthesis.

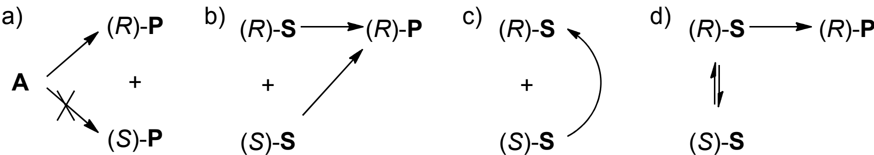


Figure 7. The principles of a) desymmetrization; b) stereoconvergent processes; c) deracemization and d) dynamic kinetic resolution. **A** = achiral substrate (planar symmetric or *meso* compound), **S** = chiral substrate, **P** = chiral product. *R*-selectivity is arbitrarily chosen.

Meso compounds have one or more asymmetric centers, but contain a plane of symmetry and are therefore achiral. Modification of one of the symmetrically positioned groups in the molecule but not the other will result in a chiral product. This process is called desymmetrization (Figure 7a). Mechanistically it is identical to kinetic resolution, the only difference being that the molecular symmetry allows the process to overcome the 50% yield limitation. Hydrolytic enzymes are often applied in desymmetrizations via acylation of a diol or deacylation of diesters.[53] For example, a symmetrical diol **2** was selectively hydrolyzed with pig liver esterase to yield monoacid **3** in a total synthesis of oseltamivir (**5**), while hydrolysis of the precursor **1** with a lipase from *Aspergillus oryzae* followed by cleavage of the methyl ethers in **4** gave the enantiomer *ent*-**3** (Scheme 1).[54]

Scheme 1. Pig liver esterase (PLE) and a lipase-catalyzed deracemizations as part of a total synthesis of oseltamivir phosphate (Tamiflu, **5**) and its enantiomer (*ent*-**5**).

If the stereoinversion of one of the resolved enantiomers can be accomplished in a separate reaction, then only one enantiomer of the product is obtained after the two steps. These stereoconvergent processes are based on a stereoselective catalytic step followed by a stereospecific reaction (Figure 7b). As an example, a kinetic resolution of an alcohol by lipase-catalyzed acylation followed by Mitsunobu stereoinversion of the unreacted alcohol with a carboxylic acid as a nucleophile, both reactions in one pot, yields the homochiral ester enantiomer (Scheme 2).[55]

Scheme 2. A stereoconvergent process for the preparation of enantiopure esters.

In deracemization a chiral racemic compound is transformed to a single substrate enantiomer via an achiral intermediate (Figure 7c). Racemic secondary alcohols can be oxidized with an enantioselective oxidizing enzyme to afford a kinetic resolution yielding a ketone and an unreacted alcohol enantiomer. If a reducing enzyme with an opposite enantiopreference can be introduced to the system, the ketone is reduced back to the alcohol. This yields the same configuration to the newly created alohol as what the unreacted alcohol had. A wide array of racemic alcohols have been deracemized with two pairs of enantiocomplementary alcohol dehydrogenases.[56] Analogously, chiral amines can be deracemized with *R*- and *S*-selective transaminases. Racemic mexiletine (*rac*-**6**) was deracemized to (*R*)-mexiletine (biologically active enantiomer) by applying complementarily selective ω-transaminases (ωTA) in a one-pot two-step synthesis in the presence of amino donor/acceptor system (Scheme 3).[57] Amines[58,59] and amino acids[60] can be deracemized also with a combination of an enantioselective monoamine/amino acid oxidase and a non-selective chemical reduction.

Scheme 3. Deracemization of a chiral amine **6**, mexiletine, with two ω-transaminases applied in sequence in the presence of amino donor and acceptor (L/ D-alanine and pyruvate).

If the product of a kinetic resolution is the desired one, the unreacted substrate enantiomer can be racemized in a separate step and then be subjected to another round of kinetic resolution. After 2 cycles of resolution-racemization, 75% theoretical yield is obtained and after overall 5 cycles, the theoretical yield approximates to 97%. In dynamic kinetic resolution (DKR), the racemization of the unreacted substrate takes place simultaneously as the desired enantiomer reacts in a kinetic resolution reaction (Figure 7d). This will keep the substrate racemic all the time, provided that the racemization is fast enough, so that 100% yields are possible. In all cases the unreacted chiral substrate is racemized via a planar intermediate in a reversible reaction. The racemization reaction must be such that it only racemizes the substrate, not the product. Moreover, the reagents needed for racemization must be compatible with the enzyme needed in the kinetic resolution. This makes, for example, thermal racemization and racemization with strongly acidic or basic conditions rarely possible. Methods of dynamic kinetic resolution are reviewed in section 2.6.6.

2.5 Asymmetric synthesis of cyanohydrins by oxynitrilase catalysis

Cyanogenesis, generation of hydrogen cyanide, is a defence mechanism of many plants. A cyanohydrin aglycone is first cleaved from its carbohydrate carrier by a glycosidase and then HCN is released from the aglycone by an oxynitrilase (hydroxynitrile lyases, HNLs). The reverse reaction, addition of hydrogen cyanide to an aldehyde or a ketone, yields a tetrahedral carbon in the cyanohydrin (α-hydroxynitrile) product. The product, when derived from an aldehyde or unsymmetrical ketone, is chiral. Oxynitrilases catalyze the reaction in both directions and enantioselective cyanohydrin synthesis can thus be obtained. This is a highly useful reaction since non-natural cyanohydrins are versatile intermediates toward other products.[61] Both *R*- and *S*-selective oxynitrilases are now available for synthetic purposes. Table 3 summarizes some properties of commercially available HNLs.

Table 3. Some properties of synthetically important oxynitrilases (HNLs).[a]

HNL[62-67]	Source (Latin)	Source (English)	Selectivity	pH optimum
*Pa*HNL	*Prunus amygdalus*	Bitter almond	*R*	5.5
*Lu*HNL	*Linum usitatissimum*	Flax	*R*	5.5
*At*HNL	*Arabidopsis thaliana*	Mouse ear cress	*R*	5.7
*Me*HNL	*Manihot esculenta*	Cassava	*S*	5.6
*Hb*HNL	*Hevea brasiliensis*	Para rubber tree	*S*	5.4
*Sb*HNL	*Sorghum bicolour*	Millet	*S*	5.0

[a] Data compiled from the cited references and from the BRENDA database.

In aqueous solution most oxynitrilases have a pH optimum at around pH 5.5, and buffers are typically used in synthesis. In aqueous solution with pH $\geq$5 the addition of HCN to aldehydes or ketones may occur also non-enzymatically leading to a racemic product.[68] To suppress the non-enzymatic background reaction, pH <5.5 is often used, even though this is suboptimal for the enzyme (HNLs retain some of their activity down to pH ~3.5). Typically, the best balance between enzyme activity and background suppression in terms of yield and obtained *ee* of the product lies between pH 5.0 - 5.5. An alternative way to minimize chemical hydrocyanation is to use low temperature, although this method may also reduce enzymatic activity. The background hydrocyanation can be reduced also by using organic solvents rather than aqueous solution as the reaction medium. A small amount of water nevertheless needs to be present since oxynitrilases are not active in fully anhydrous solvents.[69]

Cyanide can be introduced to the reaction system in various ways. In buffered aqueous solutions, salts of cyanide can be used but low solubility makes this method unsuitable for reactions in organic solvents.[70] Hydrogen cyanide can be directly added as a condensed liquid[71] or applied as a dissolved gas for example in DIPE[72] or as a gas

diffusing from another compartment of a reaction vessel into the reaction solution in another compartment.[73] A third alternative is to use an organic cyanohydrin as a cyanide donor.[74] Acetone cyanohydrin (**7**) is among the safest sources of cyanide. The transhydrocyanation reaction is based on the equilibrium of the carbonyl compound and free HCN with the cyanohydrin product. In general, the equilibrium favours the carbonyl compound in the case of ketones and the cyanohydrin in the case of aldehydes. Thus, an oxynitrilase first cleaves the cyanide from acetone cyanohydrin and then adds it to the aldehyde substrate (Scheme 4a). This may also keep the concentration of free cyanide, which can act as an inhibitor to the enzyme, low in solution and suppress the non-enzymatic hydrocyanation. With *Hb*HNL and 0.2 M substrate concentration, Costes *et al* have shown that the highest enzyme activity was obtained with 1.0 M HCN, beyond which activity sharply dropped.[75] However, this HCN concentration resulted in a small drop in product *ee* which could be retained at its highest level when 0.2 - 0.6 M HCN was used. Thus, literature examples support the use of 1-5 equivalents of HCN, and this range was used in the experimental part of the thesis (section 5.2).

The *R*-selective oxynitrilase from almonds (*Pa*HNL) is a commonly used oxynitrilase and used also in this thesis. Mechanistically, the reaction relies on a histidine residue which deprotonates HCN (Scheme 4b).[76] The cyanide is activated by a positively charged enzyme pocket and the substrate by hydrogen bonding between the carbonyl and a tyrosine and a cysteine residue. *Pa*HNL displays interfacial activation, that is, its activity is substantially increased once the enzyme faces a water-hydrophobic interface.[77]

Scheme 4. a) Oxynitrilase-catalyzed transhydrocyanation between acetone cyanohydrin (**7**) and an aldehyde substrate and b) mechanism for the *Pa*HNL-catalyzed hydrocyanation.

*Pa*HNL is available in variously immobilized forms but also a whole-cell defatted preparation, almond meal, can be used. Although the latter contains other enzymes and isoenzymes, it is cheap compared to the purified enzyme. Moreover, the oxynitrilase is stabilized by its natural matrix. Suitable reaction conditions and substrate scope for *Pa*HNL-catalyzed asymmetric synthesis has been extensively studied. Both with immobilized *Pa*HNL[75] and with almond meal[73], 2 vol.-% of buffer in DIPE has been the optimal amount of water present. For *Pa*HNL and *Sb*HNL 0.1 M buffer concentration gave the best balance between the enzyme activity and selectivity. While acetate, phosphate, tartrate and citrate buffers gave comparable enantioselectivity, the activity was highest with tartrate buffer.[73] In that study, slightly better conversions and *ee*s were obtained with *Pa*HNL/*Sb*HNL at 5 °C in DIPE than in toluene. However, in a systematic solvent screening at 25 °C there was a direct correlation between the conversion and increasing solvent hydrophobicity, whereas enantioselectivity was maximal in toluene (logP 2.5) and lower both in DIPE (logP 1.9) and in hexane (logP 3.5).[78]

The synthesis of tertiary cyanohydrins from ketones is possible, although, ketones are more difficult substrates for oxynitrilases than aldehydes due to thermodynamic and steric reasons.[79] Mandelonitrile (**13**) is the natural substrate of *Pa*HNL. The enzyme is particularly active and selective toward aromatic substrates but also toward a wide range of aliphatic aldehydes[80] As an example, 2-chlorobenzaldehyde (**8**) was used as a substrate for almond meal-catalyzed hydrocyanation and the corresponding cyanohydrin (*R*)-**9** was synthesized in high yield (96% conversion) and good selectivity (91% *ee*, Scheme 5).[81] Acidic hydrolysis then afforded the α-hydroxyacid **10**, an important precursor for the anti-thrombotic agent chlopidogrel (Plavix).

Scheme 5. Almond meal-catalyzed asymmetric synthesis of a chlopidogrel intermediate **10**.

Compared to aromatic aldehydes, aldehydes with electron-rich alicyclic rings seem to yield the products in lower yields and *ee* values. For instance, benzaldehyde (**11**) and cyclohexylcarbaldehyde gave the corresponding cyanohydrins in high yield and selectivity (95% conv., 99% *ee* and 90% conv., 99% *ee*, respectively) whereas the

results are clearly worse with 3-cyclohexenylcarbaldehyde (86% conv. and 55% *ee*).[80] Cyclic substrates with α-oxygen substituents have given particularly low *ee* values and the enantiopreference for the enzyme may also switch.[82, 83] In accordance with those observations, very low diastereoselectivity was obtained with the α-oxygen substituted aldehydes used in this work (section 5.2).

An example of the use of alternative cyanide sources is a synthesis where excess ethyl cyanoformate (**12**) was used simultaneously both as a source of HCN and as a protecting group donor.[84] The reagent first decomposes to liberate HCN, which is enantioselectively added to benzaldehyde via oxynitrilase catalysis (Scheme 6). Another molecule of ethyl cyanoformate then liberates a carbonate group to protect the labile cyanohydrin **13** as cyanohydrin carbonate **14**.

O NC O 12 CO_2, EtOH
*Pa*HNL, $PhCH_3$-buffer
11
OH CN
(*R*)-**13**
12
O O O CN
(*R*)-**14**
47%, 99% *ee*

Scheme 6. Ethyl cyanoformate and an oxynitrilase in an asymmetric synthesis of (*R*)-**14**.

2.6 Lipase catalysis in kinetic resolution

2.6.1 Catalytic mechanism and reaction types

Triacylglycerol lipases are enzymes which in nature hydrolyze triacylglycerols to glycerol and carboxylic acids. The reaction is regio- and stereoselective and these features, together with the applicability of non-natural substrates, have made lipases among the most synthetically useful enzymes both in laboratory-scale synthesis of biologically active compounds[85] and in industrial manufacturing processes.[86]

Lipases belong to α/β-hydrolases and operate using a bisubstrate mechanism called ping-pong bi-bi mechanism.[87] Lipases are serine hydrolases with three amino acid residues, serine, histidine and aspartate (or glutamate) forming a catalytic triad (Scheme 7).[88] Many lipases display interfacial activation, substantial enhancement of catalytic activity once the reaction medium is changed from fully aqueous to biphasic aqueous-hydrophobic medium. Interfacial activation of lipases occurs once a lid-motif in the enzyme structure is shifted to an open conformation, exposing the enzyme active site for the substrate to enter.[89] In ester hydrolysis, the ester enters the active site (**I**). An aspartate residue deprotonates histidine τ-nitrogen, and the π-nitrogen deprotonates the serine residue. Nucleophilic attack of the deprotonated serine to the ester carbonyl forms a tetrahedral intermediate (**II**). Upon reformation of the carbonyl double bond the proton transfers within the substrate and the catalytic triad occur in reverse, except that now the alcohol moiety is cleaved from the ester. The serine residue is thus acylated, forming an acyl-enzyme intermediate. The newly formed alcohol exits the cycle and water enters the active site (**III**). Proton transfers reoccur to form another oxyanion hole-stabilized tetrahedral intermediate, now made of the acylated serine and water (**IV**). The regeneration of the intact enzyme liberates the carboxylic acid (**V**).

In synthesis, water can be replaced by other nucleophiles. The ester substrate may be decomposed by an alcohol (alcoholysis, transesterification) or amine (aminolysis).[90] In addition to carboxylic acid esters, lipases catalyze the deacylation of thioesters.[91] The alcoholysis of amides is not catalyzed by lipases, although certain amide bonds can be hydrolyzed.[92,93] Alcohols and amines can be acylated by lipases.[94] Primary alcohols tend to be acylated with lower enantioselectivity than secondary ones.[91] Tertiary alcohols can be acylated only with certain lipases. Typically, only primary amines can be acylated with lipase catalysis, although some examples of the acylation of secondary amines exist.[95-97] With aminoalcohol substrates (primary NH_2 and secondary OH) N-acylation is often observed with or without O-acylation. This may result from faster N-acylation or from O→N acyl migration.[98-100] Thiols are less potent acyl acceptors and can rarely be acylated with lipase catalysis.[101]

Scheme 7. Catalytic mechanism of a lipase-catalyzed ester hydrolysis. In synthesis, the water molecule is replaced by another nucleophile such as an alcohol or an amine and conditions are arranged so that the equilibrium favors the acylation of the nucleophile.

Lipases have promiscuous activity for aldol[102], Michael[103] and related addition reactions.[104] This is because in the reaction, an unsaturated carbonyl compound or an enolate forms a similar tetrahedral intermediate with a negatively charged oxygen as is formed in acyl transfer reactions. The catalytic activities toward these reactions are usually low. The reactions may occur as side reactions in kinetic resolution processes (see section 5.1) and may serve as starting points for directed evolution toward the use of lipases in asymmetric conjugate addition reactions.[105]

2.6.2 Properties of selected lipases

Apart from porcine pancreatic lipase (PPL), most lipases used in synthetic applications are derived from microbial sources. These have several biotechnological advantages as they are readily expressed and produced in host organisms and are rather easy targets for genetic engineering.[106,107] Lipases used in the experimental part of the thesis were selected on the basis of availability and complementarity of their general properties and a short summary of the selected lipases is presented below.

Lipase from *Burkholderia cepacia* (BCL, previously known as *Pseudomonas cepacia*, commercially available as lipase PS) has its active site within an elliptical funnel.[108,109] This lipase is among the most widely used and is the prototype for substrate-based prediction of enantiopreference in the kinetic resolution of secondary alcohols and analogous primary amines.[110] In the model, the ligands for the secondary alcohol are, besides OH and H groups, labeled as large (L) or medium (M) (Figure 8a). When typical Cahn-Ingold-Prelog priorities are assumed (1 = OH, 2 = L, 3 = M, 4 = H), this will lead the *R*-isomer to be the fast reacting one. With primary alcohols (with no O-substituent at the asymmetric carbon) the OH group is positioned to point to the same direction as with secondary alcohols but opposite enantiopreference will result due to the kink imposed by the methylene group (Figure 8b).[111] The model for BCL active site has been further elaborated by other studies later on.[112,113] It is considered that the larger the size difference between the L and M groups is, particularly when the large substituent is aromatic, the better the enantioselectivity will be.

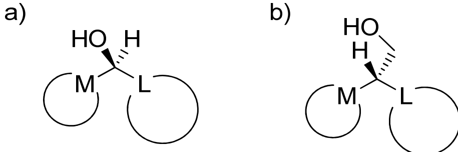


Figure 8. The preferred enantiomers for *Burkholderia cepacia* lipase-catalyzed acylation of a) secondary and b) primary alcohols.

Candida antarctica lipase B (CAL-B, commercially available as Novozym 435) is a structurally similar lipase with BCL but has somewhat steeper and narrower funnel incorporating the active site.[108] CAL-B does not have a typical lid like most lipases have.[114] Rather, a short helix exists in its place and CAL-B does not display interfacial activation. Both BCL and CAL-B are thought to have relatively large acyl binding but narrow alcohol binding clefts.[115] CAL-B, generally speaking, obeys the predictive models for substrate enantiopreference. Notably, proteases (such as subtilisin) have a mirror image active site compared to CAL-B/BCL and often display the opposite stereopreference.[116]

Lipase from *Candida rugosa* (CRL, previously known as *Candida cylindracea*) has a characteristic GGGF motif in its active site whereas most lipases have GX motif (X = any amino acid).[117] This makes the active site of CRL large and the lipase can accept acetates of tertiary alcohols as substrates[118] and to be able to acylate secondary amines.[119] Although the basic structure of the active site of CRL is similar to that of other lipases, differences in the residues surrounding the active site[120] cause CRL often to disobey the substrate model above, for example with cyanohydrins and certain

halohydrins.[121] Therefore, the model above cannot be considered a reliable predictor for CRL-catalyzed reactions.

Candida antarctica lipase A (CAL-A) shares a very low sequence homology with other lipases and is the most thermostable lipase known.[122] Its active site is also exceptional.[123] CAL-A has an active site flap made of glycine residues and it displays slow interfacial activation. The acyl-binding cleft is 30 Å long and narrow making an approximately 90° kink on its way. CAL-A prefers long acyl chains over short ones and, when unsaturated carboxylic acids are present, it prefers to esterify *trans*-fatty acids rather than *cis*-fatty acids that are preferred by other lipases.[124] The nucleophile binding cleft of the active site lies close to the surface of the enzyme. Once the enzyme is in the open conformation, the nucleophile-binding area is virtually unrestricted in steric accessibility. CAL-A is often able to acylate sterically bulky alcohols, including tertiary alcohols.[125] It rarely shows good enantioselectivity toward secondary alcohols but is highly selective toward many β-amino esters.[93,97] CAL-A is also among the few enzymes that are able to hydrolyze amide bonds (other than activated β-lactams) and can also be used to deprotect Boc-protected amines.[95,124]

Lipase from *Thermomyces lanuginosus* (TLL, formerly known as *Humicola lanuginosa*, commercially available preparations Lipolase and Lipozyme TL IM) is a typical lipase as it shows strong interfacial activation with a lid covering the active site.[126] A characteristic feature of TLL is its tendency to form bimolecular aggregates where the active sites of the two enzyme molecules face each other. This will reduce its activity and moderate amounts of detergents have been found to improve the activity.

2.6.3 Solvent, immobilization and temperature in lipase-catalyzed acylation

The use of organic solvents as a reaction medium instead of water is essential for lipase-catalyzed acylation, otherwise hydrolysis of the acyl-enzyme intermediate would prevent the acylation of the substrate. Lipases are particularly suitable enzymes to be applied in organic solvents since they naturally operate at the oil-water interface. In organic solvents the stability of lipases is improved through restricted conformational flexibility of the enzyme, which improves their thermostability, and through low water content, which suppresses degradative hydrolytic reactions on the protein.[127] However, lipases do not function in absolutely anhydrous conditions but require a hydration layer of water and structurally necessary water embedded within the enzyme. Hydrophobic solvents (toluene, hexane and ethers) generally provide better lipase activity than water-misciple solvents (acetonitrile, DMSO, DMF) since the latter may denature the enzyme or strip the essential water from the lipase. Exceptions to this tendency do exist. In general, water activity determines balance between lipase-

catalyzed acylation and hydrolysis so that maximum transesterification is obtained when water activity is high enough to support enzymatic activity but low enough to suppress hydrolysis. The absolute value of optimal water activity (a_w) depends on the solvent, the enzyme and the substrate concentration.[128] In addition to conventional organic solvents, ionic liquids and supercritical carbon dioxide can be used as these enable good solubility of polar substrates while still behave like hydrophobic solvents toward the enzyme.[129]

Lyophilized enzyme powders are soluble in water and stay as insoluble precipitates in organic solvents. The precipitated enzymes tend to aggregate and this can be prevented by enzyme immobilization. The immobilization may be based on covalent attachment of the enzyme and the carrier, ionic bonding with ion exchange resins, weak interactions (van der Waals interactions and hydrogen bonding) with for example diatomaceous earth (Celite) or encapsulation or entrapment within a gel, such as sol-gels.[130,131] Covalent attachment of lipases onto hydrophobic carrier is believed to result in the opening of the lid structure and therefore in greater enzymatic activity. A particular form of immobilization is cross-linked enzyme aggregates (CLEAs) where the precipitated enzyme molecules are attached together via small linkers, such as glutaraldehyde.

Both organic solvents and immobilization can also change the stability and activity of enzymes. Moreover, chemo-, regio- and enantioselectivities of lipases can depend drastically upon solvent selection and immobilization. For example, the preference of lipases to either O- or N-acylation of bifunctional substrates can be solvent dependent.[132] The effects of solvent and immobilization are difficult to predict and in most cases the best solution is found by screening.

The dependence of enantioselectivity of lipases on temperature can be understood on thermodynamic basis.[133] It can be shown that there is a racemic temperature T_r (where $E = 1$) according to $T_r = \Delta\Delta H^{\ddagger} / \Delta\Delta S^{\ddagger}$. Below the racemic temperature, enantioselectivity is controlled by activation enthalpy and selectivity decreases as the temperature is raised. Above T_r, enansioselectivity is reversed, governed by activation entropy and increases as the temperature is raised. For most lipase-catalyzed transesterifications the racemic temperature lies well above room temperature and the enantioselectivity improves as the reaction temperature is decreased.[134] However, with more thermostable lipases at higher temperatures the opposite may occur, so that selectivity can improve as the temperature is raised.

2.6.4 Acyl donors in lipase-catalyzed acylation

In lipase-catalyzed acylation, a carboxylic acid derivative, the acyl donor, donates its acyl group for the substrate while the leaving group of the acyl donor is a co-product of the reaction. The co-product may then react further to secondary products. The acyl part forms an acyl-enzyme intermediate while the leaving group in some cases, contrary to the hydrolytic mechanism in Scheme 7, can remain within the enzyme active site when the substrate is acylated.[135] Thus, both parts of the acyl donor may affect the rate and the selectivity of the reaction. Another factor is that the acyl donor and its concentration must be selected so that the reaction becomes essentially irreversible. In other words, the acylated product must not be deacylated back to the substrate. With this respect, acyl donors have been classified as reversible, quasi-irreversible and irreversible reagents (Table 4).[136] Too reactive acyl donors may also react spontaneously, resulting in lowered product enantiopurities. Since the acyl donor may have specific interactions with the lipase, there is not always a direct correlation between enzymatic and non-enzymatic reactivity of the acyl donor.

Reversible acyl donors have a leaving group that can act as a nucleophile and thus react reversibly. In practice, these acyl donors are non-activated esters or, more rarely, thioesters, which release alcohols or thiols, respectively. Irreversibility is achieved through the use of excess of the acyl donor. Common, relatively small esters such as ethyl or isopropyl acetate are often used as solvents and acyl donor is in high excess. This may be problematic with longer-chain esters due to viscosity or with more expensive or otherwise less readily available acyl donors. Since amide bonds are more stable than ester bonds, the acylation of an amine by an ester is irreversible and less excess of the acyl donor is in principle required. Non-activated esters are usually inert toward spontaneous O-acylation of alcohols. Amines are more nucleophilic and non-catalytic N-acylation can take place, particularly when the leaving group of the acyl donor is good. With this respect, Wong *et al* have classified acyl donors for N-acylation based on C=O bond strengths as measured by IR absorption maxima.[137] Acyl donors with a low C=O wave number (strong bond) are in general more useful for enantioselective acylation of amines. Acyl donors of particular interest within this category are methoxyacetate esters that have been found to be particularly reactive while still selective for amine acylation with CAL-B. This has been proposed to result from a specific hydrogen bonding between amine hydrogen and methoxy oxygen atoms at the enzyme active site.[138] However, a comparison of ethyl methoxyacetate with its carba, aza and thio analogues has shown that the effect is more complex, and likely a combination of hydrogen bonding and electronic activation of the ester by the methoxy group is involved.[139]

Table 4. Classification of acyl donors in lipase-catalyzed transesterification reactions.[136]

R−XH + R'C(=O)OR'' —lipase→ R−X−C(=O)R' + R''OH —spont.→ Secondary product

Substrate (X = O, NH); Acyl donor; Product; Co-product

Acyl donor(s)	Co-product(s)	Secondary product(s)
Reversible acyl donors:		
esters (R''O−C(=O)R')	R''OH	-
Quasi-irreversible acyl donors:		
2,2,2-trihaloethyl esters (Cl_3C−CH_2−O−C(=O)R', F_3C−CH_2−O−C(=O)R')	Cl_3C−CH_2OH, F_3C−CH_2OH	-
oxime esters (R''R''C=N−O−C(=O)R')	R''R''C=N−OH	-
cyanomethyl esters (N≡C−CH_2−O−C(=O)R')	N≡C−CH_2OH	CH_2O + HCN
Irreversible acyl donors:		
anhydrides (R'C(=O)−O−C(=O)R')	R'C(=O)OH	-
carbonates (R'O−C(=O)−OR')	R'OH	-
enol esters: (vinyl), (isopropenyl), (ethoxyvinyl)	vinyl alcohol, isopropenyl alcohol, ethoxyvinyl alcohol (OH)	Vinyl acetate, acetone, EtOAc

Quasi-irreversible acyl donors have a leaving group that is a weak nucleophile. The most used acyl donors in this category are 2,2,2-trifluoroethyl esters, in which the electron-withdrawing effects of the fluorines ensure high reactivity toward acylation and weak nucleophilicity of the resulting alcohol, trifluoroethanol. The trichloroanalogues are much less commonly used. As a both acyl and alkyl activated

acyl donor, 2,2,2-trifluoroethyl chloroacetate has been found to be a very reactive and yet a quite selective reagent for the N-acylation of amino acids[140] and amines (see section 5.1). Besides trihaloalkyl esters, oxime esters and cyanomethyl esters can be classified as quasi-irreversible acyl donors. Oxime esters have been used in particular in enzyme-catalyzed protection of sugars and nucleosides and cyanomethyl esters in enzyme-mediated dipeptide synthesis. However, the resulting oxime, which may be difficult to purify, and the formation of HCN, respectively, limit their usefulness.

Irreversible acyl donors include anhydrides, carbonates and enol esters. Anhydrides are often too reactive for selective N-acylation, although succinic anhydride has been used in the kinetic resolution of secondary alcohols.[141] The advantage is that the product now contains a carboxylic acid moiety and can be easily separated from the unreacted substrate. Carbonates instead are not practical acylating reagents for alcohols although they are suitable for N-acylation. The resulting carbamate bond can also be cleaved under milder conditions than amide bonds. By far the most applied acyl donors are enol esters, namely vinyl, isopropenyl and 1-ethoxyvinyl esters. Irreversibility is ensured by that the immediate products are vinyl alcohols, which rapidly tautomerize to acetaldehyde, acetone and ethyl acetate, respectively. Since aldehydes and ketones will react with amines to form imines, vinyl and isopropenyl esters cannot in general be used in the kinetic resolution of amines. However, the imine formation can be utilized for racemization in the case of amino acids (section 2.6.6). Although vinyl and less reactive isopropenyl esters are irreversible acyl donors, they are prone to hydrolysis with the resulting formation of acetic acid. In addition, acetaldehyde can react with the surface-exposed lysine residues of the lipase and thus inactivate the lipase.[142] 1-Ethoxyvinyl esters are useful alternatives since the coproduct ethyl acetate is non-harmful to lipases.[143]

Beyond this classification, direct acylation of a nucleophile with a carboxylic acid is rare although examples of amine acylation with long-chain carboxylic acids do exist.[144]

2.6.5 Kinetic resolution of amines and cyanohydrins by lipase-catalyzed acylation

Recent reviews for the enzymatic preparation of amines and amino acids are available.[145,146] Biocatalytic methods for the preparation of optically active cyanohydrins have also been reviewed.[61] Sterically unhindered amines, such as phenethylamine, can be easily acylated with for example CAL-B catalysis.[139,147] Many other examples of highly selective acylative kinetic resolutions of amines containing aryl and heteroaryl groups do exist, most with CAL-B catalysis.[148-150] For example, CAL-B-catalyzed transesterification of 1-aryl-2-propanamines were very selective for

naphthyl and N-heteroarylsubstituents apart from a pyridine-based compound, for which the selectivity and the isolated yield were much lower (Scheme 8a).[149] Other studies with CAL-B and ethyl acetate have resulted in rather low selectivity with pyridine-based amines.[150,151] Variable selectivities in the kinetic resolution of a set of aromatic substrates with benzylic primary amino group as the site of acylation were obtained (Scheme 8b).[150] In that study, both the sterical effects of the aliphatic chain (substituents and flexibility) and electronic effects of the aromatic moiety (substitution pattern and position of the pyridine nitrogen) affected selectivity.

Scheme 8. Examples of kinetic resolution of a) 1-aryl-2-propanamines[149] and b) 1-aryl-1-alkylamines[150] by CAL-B-catalyzed acylation.

Cyanohydrin esters have been resolved by either alcoholysis or hydrolysis [152-155], and even ketone cyanohydrins have been enzymatically hydrolyzed.[156-158] The reversed strategy, the acylation of cyanohydrins by lipases, is useful as it allows the avoidance of water in the case of labile cyanohydrins. It is also the basis of the dynamic kinetic resolution to prepare cyanohydrin esters (section 2.6.6). Some examples of lipase-catalyzed acylation of aliphatic[159] and aromatic[160] cyanohydrins are shown is Scheme 9. With BCL, aromatic substrates apart from pyridine gave, generally speaking, better selectivity than aliphatic ones.

Scheme 9. Examples of kinetic resolution of cyanohydrins by a) PPL[159] and b) BCL[160]-catalyzed acylation.

2.6.6 Lipase catalysis in dynamic kinetic resolution

A multitude of dynamic kinetic resolution methods have been developed for different substrate classes (Table 5). Ruthenium-catalyzed racemization of secondary alcohols and primary amines combined with lipase-catalyzed acylation is perhaps the most general DKR method. α-Amino acids and cyanohydrins can be racemized with aldehydes and bases, respectively, lipase-catalyzed acylation completing the DKR. These methodologies are shortly reviewed below. Details of other DKR methods can be found from the cited references in Table 5.

Table 5. Compilation of enzymatic dynamic kinetic resolution methods.

Racemic substrates	Achiral intermediate	Enantiomeric Product	Racemization catalyst	Enzyme for kinetic resolution
sec-Alcohols	Ketones	Esters	Ru(II) catalyst	Lipase[161-168]
Allyl alcohols	Allyl-V complex	Allyl acetates	V=O catalyst	Lipase[169]
Allyl acetates	Allyl-Pd complex	*sec*-Alcohols	Pd(0) catalyst	Lipase[170]
Allyl alcohol	Allyl alcohol (chiral)	Allyl acetate	Spontaneous rearrangement	Lipase[171]
sec-Alcohols	Carbocations	Esters	Acids	Lipase[172,173]
Thioesters	Enolates	Carboxylic acids, esters	Bases	Lipases[174,175]
Primary amines	Imines	Amides	Ru(II) catalyst	Lipase[176-178]
Primary amines	Imines	Amides	Pd(0), Ni, Co catalysts	Lipase[179-183]
Primary amines	α-Amino radical	Amides	Thiyl radicals, AIBN, hν	Lipase, Protease[184-187]
Primary amines	Enamine	Amides	Spontaneous	Lipase[188]
Secondary amines	Imines	Amides	Ir catalyst	Lipase[189]
Halohydrins	α-Haloketones	Epoxides	Ir catalyst	Haloalcohol dehalogenase[190]
Hydantoins	Schiff base	α-Amino acids	Hydantoin racemase	Carbamoylase[191]
N-acetyl α-amino acids	Schiff base	α-Amino acids	*N*-acetyl-α-amino acid racemase	Acylase[191]
α-Amino acid esters	Schiff base	*N*-acyl α-amino acid esters	Aldehydes	Lipase[192]
Cyanohydrins	Aldehydes	Cyanohydrin esters	Bases	Lipase[193-204]
α-Nitroalcohols	Aldehydes	α-Nitroalcohol esters	Bases	Lipase[205]

The most general method for the DKR of alcohols and amines is achieved by combining transition-metal and enzyme catalysis. The DKR of secondary alcohols is based on Ruthenium (II) catalysts, where the racemization takes place via a reversible hydrogen transfer redox process.[161] A so-called Shvo's catalyst is able to continuously racemize the substrate alcohol by reversible oxidation to ketone while one of the alcohol enantiomers is acylated by a lipase and an acyl donor. This affords enantiomerically enriched acetates in high yield and optical purity. Shvo's catalyst requires hydrogen mediators and thermal activation (70 ^{o}C).[162] Typically, vinyl acetate is the acyl donor and CAL-B (Novozym 435) the lipase, although with α-hydroxy acids the temperature can be reduced to 60 ^{o}C and BCL is applicable.[163] The method can also be used for the DKR of primary amines with analogous redox cycle between the amine and imine but even higher temperatures (100 - 110 ^{o}C) are needed.[176] A pentaphenylcyclopentadienyl ligand in the Ru complex (Bäckvall's catalyst) transforms the catalyst to become base-activated and efficient DKR of secondary alcohols is achieved at ambient temperature. For example, (*R*)-1-phenylethylacetate was obtained from racemic 1-phenylethanol in 98% yield and >99% *ee* at 25 ^{o}C (Scheme 10).[164] The DKR of primary amines requires 90 ^{o}C reaction temperature with the Bäckvall's catalyst.[177] As an example, Ru complex/Novozym 435 system has been used in the synthesis of norsertraline.[178] Further catalyst development is a contemporary field of research and the groups of Park[165-167] and Leino[168] have developed new catalysts with improved features.

Scheme 10. Dynamic kinetic resolution of 1-phenylethanol with CAL-B and Bäckvall's catalyst. The mechanism of racemization is also shown.

The kinetic resolution of α-amino acids can be based on either acylation of an amino group or hydrolysis of an amino acid ester. Chiral amino acids can be racemized by both acids and bases but the conditions are usually so harsh that they cannot be applied in the presence of enzymes. Racemization in the DKR of amino acids is based on deprotonation at the α-carbon. The acidity of the α-proton can be substantially increased if the amino group is first transformed into an imino group, such as in Schiff bases and oxazolones, or into a hydantoin. For example, formation of a Schiff base of glycine methyl ester has been shown to decrease the pK_a of the α-proton by 7 units.[206] This has been utilized in a kinetic resolution and a dynamic kinetic resolution based on enzymatic ester hydrolysis.[207,208] The method has been fuether developed in such a way that acetaldehyde, responsible for racemization, is formed in situ from the breakdown of the acyl donor (vinyl ester) and the resolution step is based on lipase-catalyzed N-acylation.[192] The proposed mechanism of racemization involves an iminium ion formation between acetaldehyde and the unacylated amino ester, which activates the substrate for racemization (Scheme 23, section 5.1). The N-acylated product is unable to react with the aldehyde and does not racemize. The method has been applied to the methyl esters of cyclic amino acids using CAL-A due to its ability to acylate secondary amines (Table 6). With suitable substrates, such as proline methyl ester, both high yields and *ee* values can be obtained with short reaction times.

Table 6. DKR of secondary α-amino acids by acetaldehyde and CAL-A catalysis.

rac-amino acid methyl esters → (CAL-A, Et_3N, TBME) → (*S*)-butanamides

Substrate	T [°C]	Time [h]	Product yield [%]	Product *ee* [%]	Reference
proline methyl ester (CO_2Me)	25	1	86	97	[192]
pipecolic acid methyl ester (CO_2Me)	56	24	69	97	[192]
N-Boc-piperazine-2-carboxylic acid methyl ester (CO_2Me)	48	48	61	97	Paper III

Cyanohydrins can be readily racemized by base catalysis (see chapter 2.5). Dynamic kinetic resolution of cyanohydrins can be performed once an aldehyde substrate is mixed with a cyanide source, a base, a lipase and an acyl donor as the base catalyzes both the formation and decomposition of cyanohydrins. One of the stereoisomers of the racemic cyanohydrin can then be acylated by a lipase (Scheme 11). Once acylated, the cyanohydrin ester is stable toward racemization. The challenge in the method is to find such conditions where the cyanohydrin is efficiently racemized while neither the cyanohydrin ester nor the acyl donor is hydrolyzed. Hydrolysis of the acyl donor would liberate acid into the solution, which would then neutralize the base. Therefore, strictly anhydrous conditions or the application of drying or neutralizing agents are required.[193] Moreover, the lipase carrier may play a significant role as it may either liberate or absorb water from the solution.[194] With acetone cyanohydrin as the source of cyanide, catalytical amounts of an anion-exchange resin in OH^- form are typically used as the racemizing base.[195] Examples with *Cinchona* alkaloids[196] and silica-supported ammonium hydroxide as the base exist.[197] Stoichiometric amounts of cyanide salts, sodium cyanide in particular, can be used as bases and are attractive in that the cyanide, being present at sufficient quantities, can neutralize any acid formed in the solution.[198] The use of oxynitrilases instead of bases as cyanohydrin-forming catalysts would be beneficial since the acyl donor would not be hydrolyzed. Thus far, this has not been possible in practice since oxynitrilases require some water to be active and cannot be used in one-pot DKR processes with lipases.[199]

Scheme 11. Base and lipase-catalyzed dynamic kinetic resolution of cyanohydrins.

The dynamic kinetic resolution of cyanohydrins is particularly suitable for aromatic aldehyde substrates, likely because benzylic cyanohydrins are rather unstable, thus affording efficient racemization and the aromatic ring enables good stereo discrimination by the lipase.[200-203] Aliphatic cyanohydrin esters are usually obtained in lower yields and/or selectivities and the use of cyanide bases has been reported to be beneficial as compared to a basic resin in terms of yield while selectivity varies depending on the lipase preparation.[200,204] These findings are shown in Table 7.

Table 7. Substrate scope and conditions of dynamic kinetic resolution of cyanohydrins.[a]

Substrate	Lipase	Acyl donor/ Solvent[b]	Conv. [%]	Yield [%]	*ee* [%]	Ref.
	BCL CAL-B	IPA/DIPE IPA/$PhCH_3$	100 100	96 97	84 98	[200] [198]
	BCL	IPA/DIPE	R=Cl: 97 R=Me: 88	 83 64	 84 91	[200]
	BCL	IPA/DIPE	89	81	91	[200]
	BCL	IPA/DIPE	R^1=H, R^2=H: 95 R^1=F, R^2=H: 96 R^1=H, R^2=F: 98	 80 88 92	 89 91 87	[200]
	BCL	IPA/DIPE	98	88	85	[200]
	BCL	IPA/DIPE	91	70	70	[200]
	BCL	IPA/DIPE	100	68	78	[200]
	BCL	IPA/DIPE	100	32	82	[200]
	BCL CAL-B	IPA/DIPE IPA/$PhCH_3$	96 100	57 92	47 89	[200] [198]
	CAL-B	IPA/$PhCH_3$	63	n.d.	57	[198]
	BCL	VB/ $PhCH_3$	R^1=H, R^2=H: 86 R^1=Cl, R^2=H: >99	 73 94	 79 96	[201]

Substrate	Lipase	Acyl donor/ Solvent[b]	Conv. [%]	Yield [%]	*ee* [%]	Ref.
	BCL	VB/ $PhCH_3$	R^1=H, R^2=Br:			[201]
			>99	92	95	
			R^1=H, R^2=NO_2:			
			>99	92	91	
			R^1=Me, R^2=NO_2:			
			>99	93	96	
	CAL-A	VA/MeCN	R^1=H, R^2=H:			[202]
			>99	91	96	
			R^1=H, R^2=Cl:			
			>99	92	84	
			R^1=H, R^2=Me:			
			>99	93	95	
			R^1=Cl, R^2=H:			
			>99	92	96	
	CAL-A	VA/MeCN	R=Me:			[203]
			>99	94	91	
			R=Et:			
			>99	92	93	
			R=^{n}Pr:			
			>99	93	97	
			R=*iso*-pentyl:			
			>99	94	>99	
			R=*n*-hexyl:			
			>99	93	>99	
	BCL	IPA/DIPE	100	47	51	[200]
	CAL-B	IPA/DIPE	71	n.d.	79	[198]
	BCL	IPA/DIPE	100	83	15	[200]
	CAL-B	IPA/ $PhCH_3$	100	74	50	[204][c]
	CAL-B	IPA/ $PhCH_3$	99	92	78	[204][c]

[a] Acetone cyanohydrin was used as the cyanide source and Amberlite OH^- as the base unless otherwise noted. [b] IPA = isopropenyl acetate; VA = vinyl acetate; VB = vinyl butanoate. [c] NaCN used both as the cyanide source and the base.

2.6.7 Lipase-catalyzed acylation in multistep synthesis

Though lipase-catalyzed acylation is a versatile tool in kinetic resolution, acylation is also a useful synthetic reaction in its own right. Regioselective protection of polyhydroxylated compounds by lipase-catalyzed acylation is an important area of application of lipases. Regioselective acylation of carbohydrates was an early example

of lipase-catalyzed synthesis[209-211] and enzymatic protection of carbohydrates is a continuing field of research.[212] In general, the primary hydroxyl group reacts first while the reactivity of the secondary hydroxyl groups and also the α/β-selectivity at the anomeric position depends on the sugar configuration, the lipase and also on the solvent, immobilization and the carrier used in enzyme immobilization.[213,214] The case is similar with polyhydroxylated steroids where the primary hydroxyl group (if present) is acylated first, but the preference for the secondary hydroxy groups depends on the substrate, the lipase and its immobilization and the acyl donor.[215,216]

In conventional kinetic and dynamic kinetic resolution and regioselective protection, the acyl group of the acyl donor is chosen mainly on the basis of its effect on selectivity and reactivity. However, there are cases where the acyl donor selection is determined by the structural or functional groups that it introduces to the product. The acyl group in these cases contains a moiety which either is present in the final target molecule or reacts in subsequent steps to form the end product. In this thesis, this is referred to as functional acylation. Through such functional acylation, the regio- and enantioselectivity of lipases can be directly made use of in synthesis without deacetylation of the product. Of course, this requires that the product contains an acyl functionality and that the enantiopreference is the desired one. Functional kinetic resolution of amines was performed in paper I of this thesis (section 5.1). Below, several examples from literature are reviewed to illustrate the concept. By far, the most often used lipases in these cases are CAL-B (Novozym 435) and BCL (lipase PS).

Selective acylation with lipases can be used to introduce desired structural fragments into complex products. Monoacylation of protected glycerol **15** was achieved with tetracosahexaenoic acid trifluoroethylester **16** via BCL-catalyzed acylation (Scheme 12).[217] Product **17** was ultimately transformed to phosphatidylcholine **18**.

Scheme 12. BCL-catalyzed acylation with a long-chain acyl donor as part of a phosphatidylcholine synthesis.[217]

10-deacetyl baccatin III (10-DAB, **19**, Scheme 13) is a relatively well available natural precursor of anticancer drug paclitaxel, which is obtained by regioselectively acylating the 13-OH of **19**. This OH group is the chemically least reactive hydroxyl group in **19** whereas the 7-OH is the most reactive. The difference in reactivity is small and difficult to make use of by chemical means. BCL-catalyzed acylation of **19** with trichloroacetic anhydride (TCAA) in THF gave 7,10-diacylated product (**20**, Scheme 13a). When using a 7-silyl protected derivative of **19**, BCL acylated 13-OH instead of 10-OH.[218] Surprisingly, the acylation of **19** with a lipase from *Rhizopus delemar* (RDL) in THF gave a rearranged product **21** (Scheme 13b).[219] Apparently, the lipase has preference to the 13-OH and the acyl-enzyme intermediate reacts in a concerted fashion to trigger a scaffold rearrangement of the substrate with a concomitant elimination of water and trichloroacetic acid (TCA).

Scheme 13. a) Regioselective acylation of 10-DAB (**19**) by BCL[218] and b) scaffold rearrangement to **21** caused by selective acylation with RDL.[219]

Both CAL-B (as Novozym 435) and BCL (lipase PS-C) have been found to be highly selective in the acylation of rapamycin (**22**) at 42-OH instead of non-selective acylation of both 42- and 31-OH by non-enzymatic means.[220] Acylation of **22** with a custom-made vinyl ester **23** gave the desired product **24** in high yield (98%) and selectivity (Scheme 14). Acidic deprotection of the acetonide in **24** while retaining the ester group intact gave temsirolimus (**25**), an inhibitor of the mTOR signaling pathway and approved for the treatment of renal cell carcinoma. Several other 42-hemiesters of rapamycin were also prepared, including a hemisuccinate and a hemiadipate, which can be used for the synthesis of rapamycin-protein conjugates.

Scheme 14. Regioselective acylation of rapamycin (22) in the synthesis of temsirolimus.[220]

Beyond forming a part of the final structure, a suitably functionalized acyl moiety can also be used to react in further steps. The latter steps may take place in one-pot with lipase-catalyzed acylation or in a fully separate reaction. In literature, the follow-on step is usually a C=C -bond manipulation. The group of Kita introduced such functional acylation with an enantioselective lipase-catalyzed acylation – intramolecular Diels-Alder (IMDA) domino sequence.[221] Furfuryl alcohols **26a,b** were acylated with ethoxyvinyl methyl fumarate **27a** catalyzed by an immobilized form of *P. aeruginosa* lipase (TOYOBO-LIP, Scheme 15a). Since the acyl donor, prepared from monomethyl fumarate and ethoxy acetylene, could not be purified, enzymatic acylation was performed in acetone in situ. Whereas the acylation of **26a** (kinetic resolution) to **28a** took some hours, extending the reaction time up to 6 days allowed the intramolecular Diels-Alder reaction to take place. Furan as a diene and fumarate moiety as a dienophile reacted to give cycloadduct **29a** in moderate overall yield (18%) and diastereoselectivity (26% *de*, *syn* products formed preferentially) but with good enantioselectivity (86% *ee*). Curiously, better diastereoselectivity in the cyclization of **28a** was obtained in the presence than in the absence of the lipase. Methyl substituent at the position 3 of the furan ring in **26b** gave improved reactivity (43% yield) and improved diastereoselectivity (100% *de*) while enantioselectivity (84% *ee*) remained similar. The reaction scope was studied further in a follow-up study with a substituent at furan ring at 3- and at 5-positions and with methyl or ethyl substituents at the aliphatic methylene.[222] In general, close to perfect

diastereoselectivity for *syn* products was obtained with >90% *ee* and yields around 35-45%. A 3-bromo substituent at furan (**26c**) enabled the cycloadduct **29c** to be used in subsequent Suzuki and Sonogashira couplings to prepare products **30** and **31**, respectively (Scheme 15b).

The domino process was further extended by introducing a ruthenium-catalyzed racemization step into the procedure, allowing yields above 50%.[223] Alcohols **32a-c** were used as diene substrates and acylated with either methyl or ethyl ethoxyvinyl maleate (**33a**,**b**) using CAL-B catalysis (Scheme 16). A ruthenium-mesitylene complex racemized the unreacted (*S*)-substrates via redox mechanism while the acylated (*R*)-products **34a-c** went through intramolecular Diels-Alder reaction to give *syn*-cycloadducts **35a-c** in good yields (69-83%) and with high diastereo- and enantioselectivity (100% *de*, 91-93% *ee*). The work represents the first example where such a functional lipase-catalyzed acylation has been turned into a dynamic kinetic resolution.

a) ≡—OEt + HO_2C–CH=CH–CO_2Me → $[RuCl_2(p\text{-cymene})]_2$

26a: R=H; **26b**: R=Me — **27a** (EtO(C=CH₂)O–CO–CH=CH–CO_2Me), TOYOBO-LIP, acetone, 30 °C, **26a**: 6 d; **26b**: 1 d → [**28a-b**] → IMDA → *syn*-**29a-b** + *anti*-**57a-b**

29a: 18%, 26% *de*, 86% *ee*
29b: 43%, 100% *de*, 84% *ee*

b) **26c** — **27b**, TOYOBO-LIP, acetone, 30 °C, 6 days → *syn*-**29c** 43%, 96% *ee*

to **30**: $PhB(OH)_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3, THF, 65°C, 26 h
to **31**: TMS–≡, $Pd(PPh_3)_4$, CuI, Et_2NH, DMF, 50 °C, 45 min

30: 61% (R = Ph)
31: 60% (R = $CCSiMe_3$)

Scheme 15. a) *P.aeruginosa* (TOYOBO-LIP) catalyzed acylation of furyl alcohols followed by intramolecular Diels-Alder cycloaddition.[221] b) Br substituent allows the Diels-Alder cycloadduct react further in cross-coupling reactions.[222]

OH
EtO O O CO_2R^2
33a-b
CAL-B
[{$RuCl_2$(mesitylene)}$_2$]
acetone, Et_3N, 3Å M.S.
25-35 °C, 3-7.5 days
IMDA
32a: R^1 = H
32b: R^1 = Me
32c: R^1-R^1 = -S(CH_2)$_3$S-
(*R*)-**34a**: R^2 = Me
(*R*)-**34b**: R^2 = Me
(*R*)-**34c**: R^2 = Et
35a: 83%, 93% *ee*
35b: 81%, 91% *ee*
35c: 69%, 93% *ee*

Scheme 16. CAL-B and Ru complex catalyzed dynamic kinetic resolution and intramolecular Diels-Alder reaction to prepare cycloadducts **35a-c**.[223]

In a sequential acylation-cyclization synthesis, a diene and diol functionalized silyl compound **36** was used as a substrate in a *Candida rugosa* -catalyzed desymmetrization (Scheme 17).[224] Acetoxime ester of acrylic acid (**37**) under cryogenic conditions afforded monoester **38** in good yield (71%) but with moderate enantioselectivity (40% *ee*). This compound was then subjected to intramolecular Diels-Alder reaction at thermal and Lewis acid ($EtAlCl_2$) catalyzed conditions. The thermal cycloaddition afforded cycloadduct **39** with very low diastereoselectivity (9% *de*). Although the Lewis acid -catalyzed cycloaddition was more selective, it eventually resulted in product decomposition.

36
37
CRL, DIPE
-20 °C, 28 h
38
71%
40% *ee*
IMDA
$PhCH_3$
150 °C, 48 h
39
44%
9% *de*

Scheme 17. Desymmetrization of a silicon-centered diol **36** with a separate intramolecular Diels-Alder cyclization.[224]

Other than Diels-Alder cycloaddition, the strategy of functional lipase-catalyzed acylation has been used with subsequent metathesis reactions. The (*S*)-enantiomer of γ-jasmolactone, a fruity flavor compound, was prepared by an intramolecular desymmetrization reaction.[225] Ketodiacid **40** was first transformed to dibenzylester **41** (Scheme 18). Porcine pancreatic lipase catalyzed intramolecular acylation of **41** to afford lactone **42** in 74% yield and 90% *ee*. Hydrogenation deprotected the remaining benzyl ester in high yield while it retained the lactone moiety intact. Carboxylic acid **43** was then transformed to aldehyde **44** with redox steps, and a Wittig reaction finalized the synthesis of (*S*)-(-)-γ-jasmolactone (**45**).

Scheme 18. An intramolecular acylation by PPL in a synthesis of (*S*)-(-)-jasmolactone (**45**).[225]

The bioactive natural product (+)-(*R*)-goniothalamin (**48**) has been synthesized by two groups (routes a) and b) in Scheme 19) using functional lipase-catalyzed acylation with a ring-closing metathesis reaction (RCM). Grignard reaction between allylmagnesium bromide and cinnamaldehyde afforded racemic alcohol **46** as a substrate for enzymatic acylation. Both CAL-B[226] and BCL[227] were effective lipases for enantioselective acylation with vinyl acrylate. Notably, the latter worked well in dichloromethane, an unusual solvent for biocatalysis. A separate RCM of **47** with Grubbs' first generation ruthenium catalyst then finalized the synthesis of (*R*)-**48** in good yields (92% and 85%). (*S*)-**48** was chemically synthesized from unacylated (*S*)-**46** with acryloyl chloride and subjected similarly to RCM by one of the groups.[226] Notably, enantiomeric enrichment of the product during metathesis ring-closure was observed with both (*R*)-**47** (from 93% to >99% *ee*) and (*S*)-**47** (from 74% to 85% *ee*).

Scheme 19. Acylation - ring-closing metathesis sequence in the synthesis of goniothalamin enantiomers by two research groups based on either a) CAL-B or b) BCL.[226,227]

Grubbs' second generation catalyst (structure shown in Scheme 22) with an N-heterocyclic carbene ligand instead of one of the tricyclohexylphosphine ligands displays improved air and moisture stability and higher activity than the first generation catalyst with two $P(Cy)_3$ ligands.[228] This catalyst was utilized in the chemoenzymatic synthesis of γ-alkyl-γ-butenolides.[229] Racemic alkyl substituted allyl alcohols **49** were acylated by CAL-B with vinyl crotonate as the acyl donor and to give (*S*)-esters **50** in high *ee* and in high yields based on 50% conversions achieved (Scheme

20). The isolated esters were then subjected to a ring-closing metathesis reaction to complete the synthesis. The reaction failed with the first generation Grubbs' catalyst but high yields (89-95%) of lactones **51** were obtained with the second generation catalyst. The difference between the success of the catalysts is explained by that the carbonyl oxygen is able to coordinate to ruthenium and thus inhibit its catalytic activity in the case of the first but not in the case of the second generation catalyst.

OH
R
O
O
CAL-B, hexane
38 °C, 2.5-4 days
50% conv.
rac-**49**
R = Bu, Pr, pentyl, hexyl
O
O
R
(*S*)-**50**
38-94% (based on conv.)
98->99% ee
Grubbs' 2st gen. catalyst
CH_2Cl_2, reflux, 24 h
O
O
R
(*S*)-**51**
89-95%

Scheme 20. Grubbs' 2nd generation catalyst in the synthesis of (*S*)-γ-alkyl-γ-butenolides.[229]

Chênevert et al have studied the concept of functional acylation with a variety of vinyl ester acyl donors containing either double bonds or other functional groups in protected forms.[230] Protein kinase C ligand (*R*)-**53** was prepared by *Aspergillus niger* lipase (ANL) catalyzed desymmetrization of diol **52** with a bulky acyl donor vinyl pivalate (96% yield, >98% *ee*, Scheme 21a). In the same study, phoracantholide natural products **57** and **58** were prepared by first acylating the unsaturated alcohol **54** in a kinetic resolution with CAL-B and ethoxyvinyl ester **55** to give product (*R*)-**56** in 44% yield and 95% *ee* (Scheme 21b). RCM with Grubbs' second generation catalyst afforded **57** in 34% yield and **58** (73%) was obtained by subsequent hydrogenation.

a)
OBn
HO OH
52
O
O
ANL, Et_2O
r.t., 13 days
OBn
HO O
O
(*R*)-**53**
96%, >98% *ee*
O
O
(*R*)-**58**
73%
H_2, Pd-C
b)
OH
rac-**54**
OEt O
O
55
CAL-B, $PhCH_3$
r.t., 50% conv.
O
O
(*R*)-**56**
44%, 95% *ee*
Grubbs' 2st
gen. catalyst
CH_2Cl_2, reflux
3 days
O
O
(*R*)-**57**
34%

Scheme 21. Lipase-catalyzed acylation in a) desymmetrization and b) kinetic resolution as part of the synthesis of protein kinase C ligand (*R*)-**53** and natural products (*R*)-**57** and (*R*)-**58**.[230]

3. AIMS OF THE STUDY

The primary aim was to study the preparation of the target compounds in enantiopure and/or diastereopure form by combining biocatalysis with non-enzymatic organic synthesis (Figure 9). Lipase-catalyzed acylation formed a key step in the synthetic sequences applied. Motivated by the fact that chiral amines are prevalent fragments in pharmaceuticals, all target compounds either contained an amino group (amines and amino acids in section 5.1) or its precursor (cyanohydrins in section 5.2). With each category one or more model compounds was used. These included benzylic amines with an alkene functionality, cyclic secondary amino esters and protected monosaccharides.

Various types of enzyme-mediated methods were studied depending on how they were integrated to non-enzymatic steps:

- Preparing the enantioenriched products of kinetic resolution of primary amines as substrates for subsequent synthetic steps (traditional kinetic resolution).
- Subjecting amine substrates to kinetic resolution with double bond-containing acyl donors, thus allowing the acylated products to be used as substrates in subsequent ring-closing metathesis reaction (functional kinetic resolution)
- Developing kinetic and dynamic kinetic resolution methods for amino acids, the latter with aldehyde-promoted racemization.
- Combining chemical oxidation with subsequent hydrocyanation (chemical or enzymatic) sequentially in one-pot with minimal intermediate work-up steps. To this, lipase-catalyzed acylation was combined to stabilize and further stereoenrich the products.

Figure 9. General structures of the targeted amines, amino acids and cyanohydrins.

4. MATERIALS AND METHODS

Materials

The sources of the used enzyme preparations are described in the original publications and mentioned when relevant in the discussion below. Substrates for enzymatic reactions were either from commercial suppliers or prepared by collaborators (**59a-e**, **67** and **76**). Chemical reagents from commercial sources (Aldrich, Fluka, Fluorochem) were used as such unless mentioned otherwise. Acyl donors were either from commercial sources (ethyl/isopropyl/vinyl/isopropenyl acetate, propanoate, butanoate and methoxyacetate, ethyl acrylate) or prepared within the laboratory (2,2,2-trifluoroethyl esters, isopropyl acrylate and 3-butenoylic esters). A representative synthesis is given for isopropyl 3-butenoate: Vinyl acetic acid (10 mL, 0.118 mol) and ^{i}PrOH (13.6 mL, 0.177 mol) were cooled to +4 ^{o}C and thionyl chloride (9.5 mL, 0.130 mmol) was added dropwise. The mixture was slowly heated and refluxed overnight. The mixture was cooled to r.t. and washed with sat. $NaHCO_3$ (3 × 20 mL) and brine (1 × 20 mL). Organic phase was dried with $NaSO_4$ to yield pure isopropyl 3-butenoate (7.06 g, 0.055 mol, 47%). Trifluoroethyl esters and isopropyl acrylate were distilled after the synthesis. The purity of the acyl donors was checked by NMR before use in enzymatic reactions. The NMR spectra of the acyl donors were identical with reference spectra.

Small-scale enzymatic reactions and reaction analysis

Enzymatic reactions were performed mixing in a shaker (set approximately at 170 rpm) at the given temperature. Small-scale analytical reactions were typically at a scale of 1 - 3 mL. The reactions were followed by taking samples for GC, HPLC or NMR. Samples from enzymatic reactions of benzyl homoallylamines (**59a-e**) were derivatized with acetic, propanoic or butanoic anhydride and analyzed with a gas chromatograph (GC) equipped with a Varian CP Chirasil-Dex CP chiral column. Samples from enzymatic reactions of pyridyl homoallylamine **67** were also derivatized with acetic or butanoic anhydride and analyzed with HPLC equipped with a Daicel Chiralcel OD-H chiral column at 40 ^{o}C and eluted with 10% ^{i}PrOH in hexane at a flow of 0.8 mL min^{-1} and a UV detector set at 235 nm. Samples from enzymatic reactions of cyclic amino acid esters (**71**, **73**, **74**) were derivatized with acetic or butanoic anhydride and analyzed with GC equipped with a Varian CP Chirasil-Dex CP or J & W Scientific Cyclosil-B chiral column or with HPLC equipped with Daicel Chiralcel OD-H chiral column (conditions as above). Hexadecane was used as an internal standard for the determination of conversion. Enzymatic reactions and chemoenzymatic reaction sequences of sugar-derived cyanohydrins and their esters and precursors (**76-83**) were

analyzed either with HPLC equipped with a Daicel Chiralcel OD-H chiral column at 23 °C and eluted with 10% ^{i}PrOH in hexane at a flow of 0.8 mL min^{-1} and a Corona charged aerosol detector or by ^{1}H NMR following the anomeric or methoxy peak integrals.

In all cases, calculation of *E* was based on equation *(5)* (section 2.3.3). When reactions were monitored with more than one time point, *E* was calculated by linear regression (*E* as the slope of the line $\ln[(1-c)(1-ee_S)]$ versus $\ln[(1-c)(1+ee_S)]$). *Ee* was calculated as defined in section 2.1 on the basis of the peak integrals of the respective enantiomers in GC/HPLC chromatograms since the integrals are directly proportional to the concentrations of the enantiomers. Where the analysis used GC/HPLC and a standard compound was not used, conversion was calculated from equation *(3).* In the case of secondary amino ester substrates, where a standard was used, the conversion/yield was interpolated from a precreated standard curve. In reactions monitored by HNMR, the *ee* values and yields were calculated from the peak integrals as they are directly proportional to concentration even when the compounds are not enantiomers.

Preparative-scale enzymatic reactions and compound characterization

Preparative chemical synthesis was performed with standard laboratory equipment. Enzymatic reactions were agitated by shaking, worked up by filtration followed by concentration and purification by column chromatography (EtOAC/hexane, silica gel 60 Å, Merck, 230–400 mesh, enriched with 0.1% Ca). NMR spectra were recorded with Bruker Avance 500 MHz or 600 MHz spectrometers and HRMS measured with Bruker micrOTOF-Q quadrupole-TOF spectrometer. Melting points were measured with a Gallenkamp apparatus and are uncorrected. Optical rotations were determined with a PerkinElmer 241 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Full experimental details including synthesis descriptions and NMR, HRMS, GC and HPLC characterization of the prepared compounds can be found in the original publications and associated supporting informations (available online).

Statement of contribution

Substrates **59a-e**, **67** and **76** (Scheme 24) were prepared by the collaborators at Åbo Academy University. Allylation and ring-closing metathesis reactions of (*S*)-**59a** and ring-closing metathesis of (*R*)-**62** and (*R*)-**63** (Scheme 22) were performed and analyzed by the collaborators. All other experiments were done by the author. The authors of the original publications took part in the preparation of the manuscripts for the parts that conserned their own research.

5. RESULTS AND DISCUSSION

5.1 Amino compounds as substrates for kinetic and dynamic kinetic resolution by lipase-catalyzed N-acylation (papers I-III)

α-Chiral amines are ubiquitously present in pharmaceuticals and natural products.[231] They are also important compounds as enantioselective organocatalysts.[232] As was described in the literature review, the most important biocatalytic methods for the preparation of enantiomerically enriched chiral amines are kinetic resolution of a racemate by hydrolase-catalyzed (lipase or protease) acylation/deacylation, transamination of a carbonyl precursor and deracemization of a racemate by selective oxidation with in situ reduction.[145]

In this part of the work, racemic α-chiral homoallylamines were resolved by enzymatic acylation and the products thereof used in subsequent synthesis. Conditions for a selective kinetic resolution by lipase-catalyzed acylation of racemic substrate **59a** were searched to give amine (*S*)-**59a** and acetamide (*R*)-**60a** or methoxyacetamide (*R*)-**61a**. The method was then applied to a series of homoallylamines *rac*-**59b**-**e**. The key for success was to find such a combination of reaction variables (lipase, acyl donor, reaction medium, temperature) that the rate and selectivity of the enzyme-catalyzed reaction is maximized while the non-enzymatic acylation of the substrate and the hydrolysis of the acyl donor are prevented (see sections 2.6.2-2.6.4). To extend the concept of functional acylation to amines (see section 2.6.7), the model compound **59a** was further used as a substrate to give amides (*R*)-**62** or (*R*)-**63**. These amides and the unreacted substrate (*S*)-**59a** were utilized in subsequent chemical syntheses to give cyclic amine (*S*)-**64** and lactams (*R*)-**65** and (*R*)-**66**. The developed kinetic resolution by lipase-catalyzed acylation was also applied to pyridylamine **67**. The substrate proved more challenging than the parent compound **59a** (see section 2.6.5) and further method development was needed to yield amides (*R*)-**68**-**70**. The structures of the resolved compounds and the products thereof are presented in Figure 10.

Another class of important chiral amino compounds studied in this thesis is secondary α-amino acids. As described in section 2.6.6, α-amino esters can be subjected to lipase-catalyzed dynamic kinetic resolution, where acetaldehyde acts as a racemizing agent, AND CAL-A (see section 2.6.2) enantioselectively acylates the secondary amino groups. The results of the kinetic resolution of piperazine-2-carboxylic acid methyl esters by CAL A-catalyzed acylation are presented (Figure 11). The substrate was used both as its N-4 (**71**) and N-1 (**73**) Boc-protected derivatives. Dynamic kinetic

resolution of the former and its 4-C analogue pipecolic acid methyl ester (**74**) was studied with different CAL-A preparations.

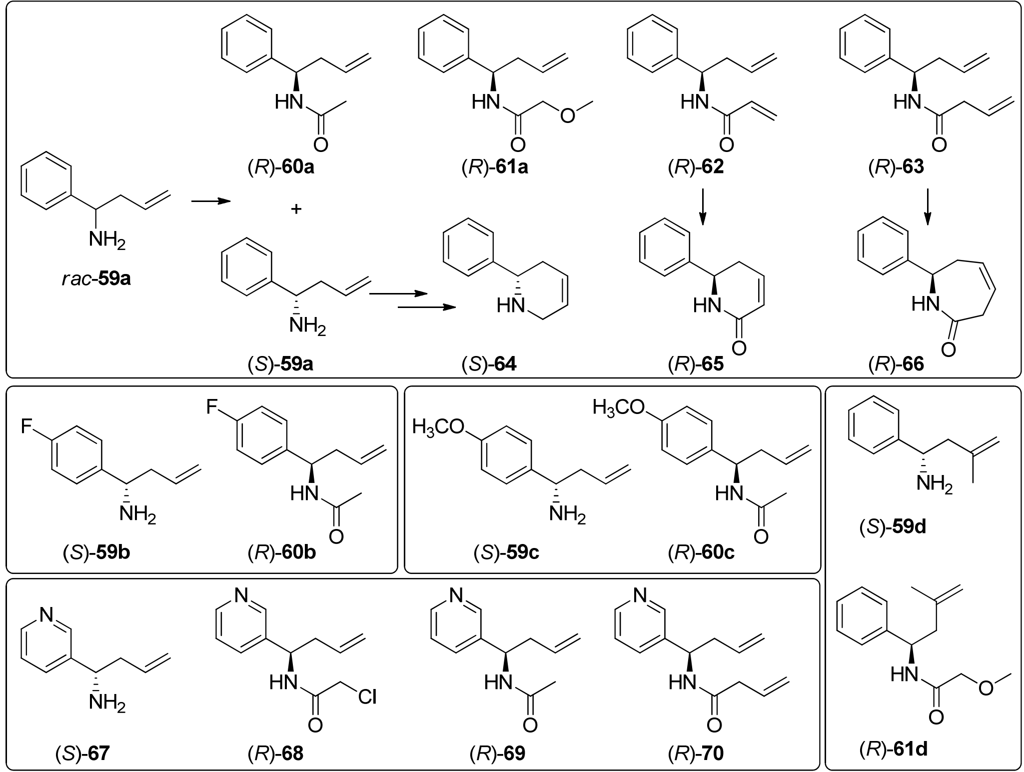


Figure 10. Chemical structures of the resolved primary amines and derivatives thereof.

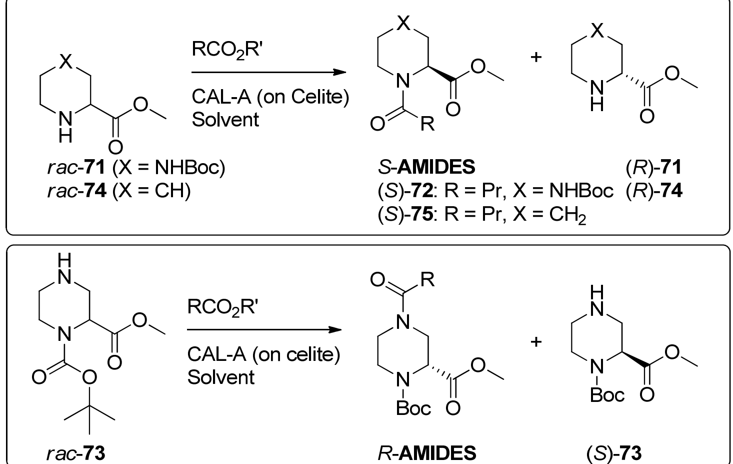


Figure 11. Kinetic resolution of secondary α-amino acid methyl esters **71**, **73** and **74**.

Kinetic resolution of 59a-e

Amine **59a** was used as a model compound for the development of kinetic resolution conditions. A set of microbial lipases, an acylase (acylase I) and a protease (Subtilisin Carlsberg) were screened for activity and enantioselectivity with *rac*-**59a** in *tert*-butyl methyl ether (TBME) and ethyl methoxyacetate as an acyl donor (Table 8). Lipase PS-D (entry 1) and Novozym 435 (entry 2) showed most selectivity while CAL-A adsorbed on Celite (entry 3) was the most reactive of the enzymes leading to enantioselective catalysis. Other enzymes had negligible reactivity. Strictly anhydrous conditions were required and the solvent was changed to dried toluene for subsequent reactions due to better solubility.

Table 8. Screening of enzyme activity in the kinetic resolution of amine **59a**.[a]

Entry	Enzyme	Conv. [%]	ee_P [%]	ee_S [%]	*E*
1	Lipase PS-D (BCL)	27	95	35	52
2	Novozym 435 (CAL-B)	35	92	50	39
3	CAL-A on Celite	74	5	15	1
4	Lipozyme TL IM (TLL)	5	72	4	6
5	CRL on Celite	2	30	1	2
6	Acylase I	3	11	0	1
7	Subtilisin Carlsberg	1	9	0	1

[a] [*rac*-**59a**] = 0.10 M with 6 vol.-% (5 equiv.) ethyl methoxyacetate and 25 mg mL^{-1} enzyme.

Acyl donors were screened for lipase PS-D -catalyzed acylation of *rac*-**59a** in toluene (Table 9). Optimization of the reaction conditions showed that isopropyl acetate, when used as a neat solvent, and ethyl methoxyacetate (6 vol.-% in toluene) gave the best combination of reactivity and enantioselectivity. Although the initial selectivity was moderate, the use of 4 Å molecular sieves considerably improved it with both acyl donors (entries 9 vs. 7 and 15 vs. 13). Thus, 50 mg mL^{-1} of lipase PS-D in the presence of 4 Å molecular sieves at room temperature in dry isopropyl acetate gave the best enantioselectivity (entry 11). However, 6 vol.-% ethyl methoxyacetate in anhydrous toluene at + 48 ^{o}C was required to push the conversion to 50% while still affording high *ee* for both the product and the substrate (entry 18).

Table 9. Development of the kinetic resolution conditions of **59a** with lipase PS-D.[a]

rac-**59a** + R[1] = H or OCH_3; R^2 = H, CH_3, CH_2CH_3 or OCH_3 → (Lipase PS-D, $PhCH_3$, 72 h) → (*R*)-**60a** (R^2 = H), (*R*)-**61a** (R^2 = OCH_3) + (*S*)-**59a**

Entry	Acyl donor, content [vol.-%]	T [° C]	PS-D [mg mL^{-1}]	Additives	Conv. [%]	ee_P [%]	ee_S [%]	*E*
1	ethyl acetate, 6	r.t.	25	-	11	10	1	1
2	ethyl propanoate, 6	r.t.	25	-	1	43	<1	n.d.
3	ethyl butanoate, 6	r.t.	25	-	6	2	<1	n.d.
4	isopropyl acetate, 6	r.t.	25	-	3	78	2	8
5	isopropyl acetate, 20	r.t.	25	-	5	87	5	15
6	isopropyl acetate, 50	r.t.	25	-	19	95	23	49
7	isopropyl acetate, neat	r.t.	25	-	31	95	42	59
8	isopropyl acetate, neat	r.t.	25	Et_3N[b]	32	95	44	60
9	isopropyl acetate, neat	r.t.	25	4Å M.S.[c]	31	99	45	>200
10	isopropyl acetate, neat	+4	50	4Å M.S.[d]	38	98	59	>200
11	isopropyl acetate, neat	r.t.	50	4Å M.S.[d]	45	99	82	>200
12	isopropyl acetate, neat	+48	50	4Å M.S.[d]	48	96	87	>100
13	ethyl methoxyacetate, 6	r.t.	25	-	14	95	16	46
14	ethyl methoxyacetate, 6	r.t.	25	Et_3N[b]	17	96	20	60
15	ethyl methoxyacetate, 6	r.t.	25	4Å M.S.[c]	44	99	76	>200
16	ethyl methoxyacetate, 6	+4	50	4Å M.S.[d]	42	99	73	>200
17	ethyl methoxyacetate, 6	r.t.	50	4Å M.S.[d]	45	99	82	>200
18[e]	ethyl methoxyacetate, 6	+48	50	4Å M.S.[d]	50	97	97	>200
19	ethyl methoxyacetate, 20	r.t.	25	-	29	91	37	30
20	ethyl methoxyacetate, 20	r.t.	25	Et_3N[b]	38	94	58	58
21	ethyl methoxyacetate, 20	r.t.	25	4Å M.S.[c]	57	75	99	35
22	ethyl methoxyacetate, 50	r.t.	25	-	34	76	39	11

[a] [*rac*-**59a**] = 0.10 M; [b] [Et_3N] = 0.10 M; [c] 25 mg mL^{-1}; [d] 50 mg mL^{-1}; [e] 48 h reaction time.

The developed methods for kinetic resolution were applied to substrates **59a-e** on preparative scale (Table 10). **59a** gave its acetate ester (*R*)-**60a** with good yield at 99% *ee* but the unreacted amine retained relativey low *ee* of 84%. The kinetic resolution was repeated to give (*S*)-**59a** in 99% *ee*, although the repeated resolution caused reduction in the overall yield. When *rac*-**59a** was subjected to the kinetic resolution with ethyl methoxyacetate (entry 2), reactivity and selectivity were slightly inferior to those obtained in the development phase (entry 18 in Table 9) and longer reaction time was needed. Substituted substrates **59b** and **59c** were also resolved in neat isopropyl acetate with lipase PS-D as a catalyst (entries 3 and 4) and, after enantiomeric enrichment for **59b**, both the recovered (*R*)-amides **60b** and **60c** and (*S*)-amines were

obtained with good *ee* values. The amines with steric bulk in the aliphatic chain were much more challenging substrates. Substrate **59d** did not react in isopropyl acetate although did give product (*R*)-**61d** when 20 vol.-% ethyl mehoxyacetate was used. Reactivity was still low and 7 days was required to give 31% conversion (entry 5). Substrate **59e** did not react under any of the experimented reaction conditions (entry 6).

Table 10. Preparative scale kinetic resolutions of substrates **87a-e** (0.10 M).

Entry	Substrate (R groups)	Product	Time [h]	Conv. [%]	(*R*)-amide Yield[a] [%]	*ee* [%]	(*S*)-amine Yield[a] [%]	*ee* [%]
1	**59a** (R^1=R^2=R^3=R^4=H)	**60a**[b]	72	46	92	99	66	84/99[f]
2	**59a**	**61a**[c]	96	48	83	94	58	87
3	**59b** (R^1=F; R^2=R^3=R^4=H)	**60b**[b]	96	33	75	>99	75	48/95[f]
4	**59c** (R^1=OMe; R^2=R^3=R^4=H)	**60c**[b]	96	49	65	>99	93	94
5	**59d** (R^1=R^2=R^3=H; R^4=Me)	**61d**[d]	168	31	quant.	>99	35	44/72[f]
6	**59e** (R^1=R^4=H ; R^2=R^3=Me)	-[e]	-	-	-	-	-	-

[a] Isolated yields based on conversion. [b] Neat isopropyl acetate at r.t. with 50 mg mL^{-1} lipase PS-D. [c] 6 vol.-% ethyl methoxyacetate in toluene at +48 °C with 50 mg mL^{-1} lipase PS-D. [d] 20 vol.-% ethyl methoxyacetate in toluene at +48 °C with 50 mg mL^{-1} Novozym 435. [e] No reaction with any of the applied methods. [f] *ee* after first/second round of kinetic resolution.

Acetamide (*R*)-**60a** and methoxyacetamide (*R*)-**61a** were hydrolyzed under acidic conditions in order to confirm the absolute configuration of the products. Derivatization of both (*S*)- and (*R*)-**59a** with (*S*)-enantiomer of Mosher's acid chloride [(*S*)-MTPA-Cl] gave a pair of diastereomers.[233] Chemical shift differences in ^{1}H NMR spectra of the diastereomers showed that (*R*)-**59a** was the preferentially reacting enantiomer with lipase PS-D, as predicted by the Kazlauskas' model. Supposing the same elution order in GC, the (*R*)-enantiomers of the other substrates were also preferentially acylated.

Kinetic resolution was performed also with olefinic acyl donors to afford functionalized (*R*)-amides. Ethyl and isopropyl acrylate and isopropyl 3-butenoate were used in the kinetic resolution of **59a** based on lipase PS-D -catalyzed acylation

(Table 11). The resolution was highly enantioselective (*E* >100) when ethyl acrylate (entries 1-3) was used but completely non-selective with isopropyl acrylate (entries 4-6). In both cases, acylation was slow and could not be accelerated by increasing the acyl donor concentration. In a preparative reaction, 5 days was required to reach 19% conversion under conditions of entry 2 and (*R*)-**62** was obtained in 65% yield (calculated from conversion) with 65% *ee*. When the reaction was allowed to proceed further, aza-Michael reaction between **59a** and acrylic ester began to take place. Lipases have been shown to be able to catalyze Michael-addition reactions[103], and as the concentration of the preferentially acylated enantiomer diminishes, 1,4-conjugate addition starts to get favoured. Isopropyl 3- butenoate, not being a Michael acceptor, much improved reactivity and good enantioselectivity (entries 7-9). Thus, in a preparative reaction under conditions of entry 8, 47% conversion was reached in 5 days and (*R*)-**63** was obtained in 85% yield (calculated from conversion) with >99% *ee*.

Table 11. Kinetic resolution of **59a** (0.10 M) with olefinic acyl donors.

rac-**59a** + R, O, n — Lipase PS-D, $PhCH_3$, 4Å M.S., r.t., 72 h → (*R*)-**62** (*n* = 0) (*R*)-**63** (*n* = 1) + (*S*)-**59a**

Entry	R	*n*	Product	Content of acyl donor [vol.-%]	*Conv.* [%]	ee_P [%]	ee_S [%]	*E*
1	H	0	(*R*)-**62**	5	17	98	21	>100
2	H	0	(*R*)-**62**	10	16	98	19	>100
3	H	0	(*R*)-**62**	20	18	98	21	>100
4	CH_3	0	(*R*)-**62**	5	7	3	<1	1
5	CH_3	0	(*R*)-**62**	10	1	23	<1	2
6	CH_3	0	(*R*)-**62**	20	2	13	<1	1
7	CH_3	1	(*R*)-**63**	5	40	>99	67	>200
8	CH_3	1	(*R*)-**63**	10	45	>99	83	>200
9	CH_3	1	(*R*)-**63**	20	48	>99	91	>200

Synthesis of nitrogen heterocycles from the resolved amine 59a

(*S*)-**59a** and its amides (*R*)-**62** and (*R*)-**63** were used in the synthesis of N-heterocyclic compounds. The resolved (*S*)-**59a** was N-allylated (67% yield) and, following amino protection as a quaternary salt, ring-closing metathesis (RCM) reaction with Grubbs' 2nd generation catalyst in dichloromethane gave the dehydropiperidine (*S*)-**64** (70% yield, Scheme 22). Similarly, the olefinic amides (*R*)-**62** and (*R*)-**63** were subjected to RCM to afford the six- and seven-membered lactams (*R*)-**65** (82% yield) and (*R*)-**66** (22% yield). The large difference in yield is likely explained by the more difficult formation of medium-sized rings vs. a six-membered ring.[234]

Scheme 22. Synthesis of N-heterocycles from the products of the kinetic resolution of **59a**. Reagents and conditions: a) allyl bromide (1 equiv.), KOH (3 equiv.), DMF, r.t.; b) Grubb's 2nd generation catalyst (0.1 equiv.), *p*TsA (1 equiv.), CH_2Cl_2, 40 °C; c) Grubb's 2nd generation catalyst (0.1 equiv.), CH_2Cl_2, 40 °C.

Kinetic resolution of 67

The enzymatic kinetic resolution of the 3-substituted pyridine derivative *rac*-**67** was investigated with activated and non-activated acyl donors. As an initial set-up based on the results in paper I, lipase PS-D was used as the catalyst and toluene as the solvent in the kinetic resolution of **67** in the presence of 4Å M.S. and activated acyl donors were screened (Table 12). 2,2,2-Trifluoroethyl esters were all very unreactive, reactivity decreasing as the acyl chain elongated (entries 1-3). Acyl activation with ethyl and particularly with isopropyl methoxyacetate improved reactivity, but enantioselectivity was still very low (entries 4 and 5). Further improvement in conversion was achieved by substituting methoxy group with chlorine in the acyl moiety resulting also in excellent enantioselectivity (entry 6). A highly reactive acyl donor, 2,2,2-trifluoroethyl chloroacetate, afforded somewhat lowered *ee* values due to concomitant non-enzymatic acylation (entry 7). Nevertheless, the high reactivity encouraged the conditions to be further developed.

The effects of temperature, additives and acyl donor concentration were studied to suppress chemical with respect to the enzymatic acylation with 2,2,2-trifluoroethyl chloroacetate as an acyl donor (Table 13). Reactions were sampled at 10 min to determine initial rate differences and at 60 min to determine the extent and selectivity at or around 50% conversion (beyond which the reaction proceeded very slowly or not at all). The best results were obtained with 0.75-1 equiv. of 2,2,2-trifluoroethyl chloroacetate at +4 °C in the presence of 4Å molecular sieves or $NaSO_4$ as drying agents.

Table 12. Kinetic resolution of **67** (0.10 M) with activated acyl donors and lipase PS-D (50 mg mL^{-1}).

rac-**67** — Acyl donor; Lipase PS-D, 4Å M.S., $PhCH_3$, r.t. → (*R*)-**AMIDES** + (*S*)-**67**

Entry	Acyl donor	Acyl donor [equiv.]	Time[a] [h]	Conv. [%]	ee_P [%]	ee_S [%]	*E*
1		2	30	13	30	5	1
2		2	30	5	<1	2	1
3		2	30	0	-	-	-
4		2	30	12	n.d.[b]	12	<10
5		2	30	30	n.d.[b]	19	<10
6		2	72	52	90	99	>100
7		1	1	51	89	91	n.d.[c]

[a] The reaction did not proceed after the given time point. [b] Enantiomers did not fully separate in HPLC. [c] Not determined due to chemical acylation.

Table 13. Kinetic resolution of **67** (0.10 M) with 2,2,2-trifluoroethyl chloroacetate and lipase PS-D (50 mg mL^{-1}).

rac-**67** + 2,2,2-trifluoroethyl chloroacetate — Lipase PS-D, $PhCH_3$ → (*R*)-**68** + (*S*)-**67**

Entry	Temp. [°C]	Additives[a]	Acyl donor [equiv.]	Conv.^{10min} [%]	Conv.^{60min} [%]	ee_P^{60min} [%]	ee_S^{60min} [%]	*E*
1	+22	4Å M.S.	1	35	51	93	66	52
2	+4	4Å M.S.	1	35	50	94	94	>100
3	+4	$MgSO_4$	1	n.d.	35	95	50	69
4	+4	Na_2SO_4	1	n.d.	47	96	84	>100
5	+4	4Å M.S.	2	37	50	92	90	72
6	+4	4Å M.S.	0.75	30	46	97	82	>100
7	+4	4Å M.S.	0.5	-	35[b]	97	53	>100

[a] 50 mg mL^{-1}. [b] The reaction did not proceed after the given time point.

The kinetic resolution of *rac*-**67** was then performed on preparative scale under optimized conditions (entry 6, Table 13). Reaction analysis after 75 min (Conv. 48%) indicated 90% *ee* for the unreacted amine and 97% *ee* for the produced amide (*E* >100). However, a large part of the material was lost in the work-up and both the recovered amine (*S*)-**67** and amide (*R*)-**68** had lowered *ee* (82% and ~50%, respectively). This implies that part of the activated amide (*R*)-**68** was hydrolyzed during the work-up and/or there were side reactions that took place. Other acyl donors were needed for a preparative resolution.

Kinetic resolution of **67** with non-activated acyl donors was studied in order to yield more stable amides (*R*)-**69** and (*R*)-**70** (Table 14). Ethyl acetate as a neat solvent (entry 1) gave lower reactivity and selectivity than its isopropyl congener (entry 2a). The reaction was still slow and halted below 50% conversion (entry 2b). A higher temperature could push the conversion to 50% (entry 3), but this reduced selectivity. Increase of the enzyme content up to 100 mg mL^{-1} (entries 4 and 5) improved the maximum conversion obtained. A raise in temperature reduced selectivity (entry 6) and reduction of acyl donor content slowed down the reaction without significant improvement in selectivity (entry 7). CAL-B (Novozym 435) was both a less active and a less selective catalyst (entry 8) in comparison to lipase PS-D. With isopropyl 3-butenoate the reaction was both faster and more selective (entry 9), as both amide (*R*)-**70** and amine (*S*)-**67** were obtained in 98% *ee* at 50% conversion.

Table 14. Kinetic resolution of *rac*-**67** (0.10 M) with non-activated acyl donors.[a]

rac-**67** + isopropyl ester (R^1, R^2) → Lipase PS-D, 4Å M.S. (PhCH$_3$) → (*R*)-**69** / (*R*)-**70** + (*S*)-**67**

Entry	R^1	R^2	Temp. [°C]	Lipase [mg mL^{-1}]	Time [h]	Conv. [%]	ee_P [%]	ee_S [%]	*E*
1	H	H	22	PS-D (50)	72	12	64	9	4
2a	CH_3	H	22	PS-D (50)	72	32	97	46	>100
2b	CH_3	H	22	PS-D (50)	96[b]	43	96	71	>100
3	CH_3	H	48	PS-D (50)	72	50	87	86	38
4	CH_3	H	22	PS-D (75)	96[b]	48	96	87	>100
5	CH_3	H	22	PS-D (100)	96[b]	50	95	94	>100
6	CH_3	H	48	PS-D (100)	96[b]	53	87	97	58
7[c]	CH_3	H	22	PS-D (100)	96[b]	39	97	63	>100
8	CH_3	H	22	CAL-B (100)	96[b]	33	92	45	37
9	CH_3	CH=CH_2	22	PS-D (100)	24[b]	50	98	98	>200

[a] 50 mg mL^{-1} 4Å M.S. [b] The reaction did not proceed after the given time point. [c] 50 vol.-% ^{i}PrOAc in PhCH$_3$.

On preparative scale, kinetic resolution of *rac*-**67** in neat isopropyl acetate with lipase PS-D (100 mg mL^{-1}) at room temperature proceeded to 47% conversion in 96 h. Amide (*R*)-**69** was obtained in 94% yield with 97% *ee* and amine (*S*)-**67** in 74% yield with 88% *ee* (Table 15, entry 1, both yields based on conversion). (*R*)-**69** was subjected to acidic hydrolysis under microwave irradiation. (*R*)-**67** was obtained in 78% yield and in 85% *ee*. Isopropyl 3-butenoate gave high selectivity for the kinetic resolution of **67** and after 24 h (51% conversion), amide (*R*)-**70** was collected in 88% yield (based on conversion) with >99% *ee* (Table 15, entry 2). However, amine (*S*)-**67** was obtained in low yield (24% yield based on conversion) although with high *ee* (97%). (*R*)-**70** gave a lower yield for (*R*)-**67** than (*R*)-**69** upon acidic hydrolysis and a significant drop in *ee* occurred (from >99% to 84%).

Table 15. Preparative kinetic resolution of *rac*-**67** (0.10 M) with non-activated acyl donors followed by hydrolysis.[a]

Entry	R	Kinetic resolution			(*S*)-**67**		(*R*)-**69**/**70**		(*R*)-**67**	
		Time [h]	*Conv* [%]	*E*	Yield [%][b]	*ee* [%]	Yield [%][b]	*ee* [%]	Yield [%][c]	*ee* [%]
1	H	96	47	>100	39 (74)	88	44 (94)	97	78	85
2	$CH=CH_2$	24	51	>200	12 (24)	97	45 (88)	>99	50	84

[a] Reagents and conditions: a) 0.10 M *rac*-**67** in neat isopropyl acetate/3-butenoate, lipase PS-D (100 mg mL^{-1}), 4Å M.S. (100 mg mL^{-1}), r.t.; b) 0.05 M (*R*)-**69**/**70**, 2 M aq. HCl, MW 165 °C, 15 min; c) aq. NH_4OH, then extracted to EtOAc, dried with Na_2SO_4 and concentrated to yield (*R*)-**67**; [b] Isolated yield based on racemic starting material (yield based on conversion in parenthesis); [c] Isolated yield of the hydrolysis step.

Kinetic resolution of 71 and 73

Kinetic resolution of *rac*-**71** and *rac*-**73** was studied with various acyl donors and CAL-A immobilized on Celite in DIPE and TBME (Table 16). The important difference between the compounds is that the former reacts as an α-amino ester whereas the latter reacts as a β-amino ester. Kinetic resolution of **71** with 2,2,2-trifluoroethyl acetate in DIPE afforded only low conversion after 1 h (entry 1), whereas the corresponding butanoate ester gave highly efficient kinetic resolution (entry 2). The difference between the two activated acyl donors can be explained by the butanoate better fitting the long and narrow acyl-binding pocket of CAL-A (see section 2.6.2).[123] Under these conditions, 50% conversion was reached within an hour giving both (*S*)-**72** and (*R*)-**71** in >99% *ee*. Identical reaction in TBME gave a somewhat lower conversion

after 1 h (entry 3) and substituting trifluoroethyl moiety in the acyl donor with an ethyl group reduced reactivity (entry 4). Like with trifluoroethyl esters, vinyl butanoate (entry 5) gave much more effective resolution than its acetate counterpart (entry 6). However, the system operates under dynamic conditions with vinyl esters and selectivities are therefore not comparable to those of trifluoroesters. Preparative reaction with 2,2,2-trifluoroethylbutanoate (2 equiv.) afforded 50% conversion in 3 h and both (*S*)-**72** (50% isolated yield) and (*R*)-**71** (40% isolated yield) were obtained with >99% *ee*.

Racemic **73** was more difficult to resolve than **71**. Again, trifluoroethyl butanoate (entry 8) was to be preferred over the acetate (entry 7), but now a higher conversion was achieved in TBME than in DIPE (entry 9 vs. entry 7). Ethyl butanoate, at this concentration, was non-effective. Comparison with commercially available pure enantiomer showed that (*R*)-**73** was always acylated under CAL-A catalysis although a preparative reaction was not done due to the low rates of acylation.

Table 16. Kinetic resolution of rac-**71** and rac-**73** (0.05 M) with CAL-A (25 mg mL^{-1}) catalysis.

rac-**71** → (Acyl donor, CAL-A, Solvent, +48 °C, 1 h) → *S*-**AMIDE** + (*R*)-**71** or *rac*-**73** → (Acyl donor, CAL-A, Solvent, +48 °C, 1 h) → *R*-**AMIDE** + (*S*)-**73**

Entry	Substrate	Acyl donor [a]	Solvent	Conv. [%]	ee_P [%]	ee_S [%]	*E*
1	*rac*-**71**	2,2,2-trifluoroethyl acetate	DIPE	6	>99	6	>200
2	*rac*-**71**	2,2,2-trifluoroethyl butanoate	DIPE	50	>99	>99	>200
3	*rac*-**71**	2,2,2-trifluoroethyl butanoate	TBME	47	>99	88	>200
4	*rac*-**71**	ethyl butanoate	TBME	6	>99	6	>200
5	*rac*-**71**	vinyl butanoate	TBME	48	>99	93	n.d.
6	*rac*-**71**	vinyl acetate	TBME	2	>99	2	n.d.
7	*rac*-**73**	2,2,2-trifluoroethyl acetate	DIPE	6	>99	6	45
8	*rac*-**73**	2,2,2-trifluoroethyl butanoate	DIPE	23	>99	30	140
9	*rac*-**73**	2,2,2-trifluoroethyl acetate	TBME	16	>99	18	200
10	*rac*-**73**	ethyl butanoate	TBME	<1	>99	<1	-

[a] 0.10 M acyl donor.

Dynamic kinetic resolution of 71 and 74

Kinetic resolution with vinyl butanoate affords vinyl alcohol co-product which rapidly tautomerizes to acetaldehyde (section 2.6.4). As was mentioned in section 2.6.6, acetaldehyde can racemize the unreacted α-amino ester via the suggested mechanism in Scheme 23. The racemization is based on imine formation through an initial intermediate **I**. The acidity of α-H is markedly increased in the iminium ion intermediate **II** which is converted to the planar intermediate **III**. Addition of water completes the cycle and gives back the now racemic α-amino ester. Water in the system affects the position of the equilibria and may also hydrolyze the acyl donor.

Scheme 23. The mechanism of the dynamic kinetic resolution of α-amino acid esters **71** and **74**.

The mechanism of racemization requires sufficient acidity for the α-H in intermediate **II**. For α-amino acids this is enhanced, in addition to the carbonyl group, by the positively charged iminium group. For β- amino acids the stabilization of the negative charge by the iminium group in the betaine intermediate **III** is much weaker. Calculated pK_a values for hypothetical intermediates of **71** and **73** show that the former experiences approximately 5 unit drop in pK_a upon iminium formation whereas the latter of only 1 unit (Figure 12). The racemization of **73** would thus likely to be too slow for practical application in the dynamic kinetic resolution.

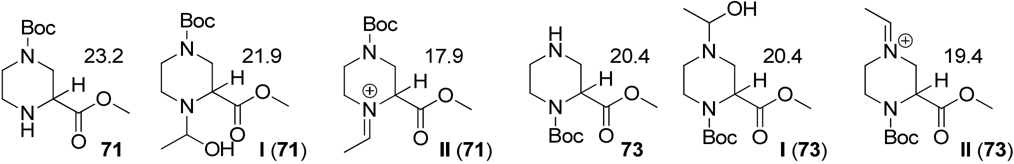


Figure 12. Calculated pK_a values for the α-H in amino acids **71** and **73** and their intermediates **I** and **II**. Calculated with ACE pK_a calculator, available online at http://aceorganic.pearsoncmg.com/epoch-plugin/public/pKa.jsp.

The dynamic kinetic resolution of α-amino acid ester **71** and and its C-analogue pipecolic acid methyl ester **74** was studied. Since the initial specific activity and water content may vary between enzyme preparations, CAL-A was used in different preparations. Free lipase (Novozym 735, Chirazyme L5 and Cat#ICR112) was purified by dialysis and immobilized on Celite in the presence of sucrose. In addition, CAL-A covalently attached on acrylic beds (IMMCALA-T2-150) and CAL-A as a cross-linked enzyme aggregate (CAL-A CLEA) were used. An earlier work had established triethylamine as a useful additive, possibly by neutralizing any acid from hydrolytic side reactions.[192] Triethylamine was therefore introduced to the reaction and also the effect of molecular sieves was studied. TBME was chosen as a hydrophobic solvent. Additionally, acetonitrile as a polar solvent was used since it may be able to dissolve the substrate even if it has formed a salt pair with acid. The results of these experiments are presented in Table 17.

Reactions of **71** with CAL-A on Celite in TBME afforded 48% yield for Novozym 735 -derived enzyme (entry 3) and 61% yield for Chirazyme L5 -derived enzyme (entry 6). Covalently immobilized preparation (entry 1) gave slightly reduced yield and CLEA preparation was clearly less active (entry 2). In these reactions, the substrate remained almost racemic. With CAL-A on Celite similar yields were obtained when 3Å molecular sieves were present as without them (entry 4), but 4Å M.S. reduced the yield (entry 5). Both types of molecular sieves, however, did lower the efficiency of acetaldehyde-catalyzed racemization since the substrate was enantiomerically enriched. The yields of (*S*)-**72**, although obtained with high *ee*, did not surpass 61%. Also in all cases, the total yields of the product and substrate were less than 100%, suggesting that some of the amino acid substrate is lost out of the reaction system.

Dynamic kinetic resolution of pipecolic acid methyl ester **74** was studied in acetonitrile (entries 8-10). Yields over 50% with high enantiopurity were obtained with CAL-A on Celite. Cat#ICR112-derived preparation gave the best yield of 75% and >99% *ee* (entry 10). The issue of less than 100% total yield remained. Plausible causes for this are *i*) acetaldehyde and its condensation products have formed imines with the amino groups of the basic amino acid residues of the enzyme, thereby inhibiting the enzyme and removing acetaldehyde from the solution[142]; *ii*) the imine intermediate (**II**) during the racemization cycle may has been too stable so that the substrate was lost from the resolution system and *iii*) butanoic acid, if formed from hydrolysis of the acyl donor, has formed a salt pair with the amine and precipitated it out of solution. That the substrate loss was lower in the presence of 3Å molecular sieves suggests that the latter explanation is at least partly effective. On the other hand, color intensification during the reactions implied imine accumulation.

Table 17. Dynamic kinetic resolution of **71** (0.05 M) and **74** (0.10 M) with vinyl butanoate (4 equiv.) and CAL-A catalysis in the presence of triethylamine (1 equiv.) at +48 °C for 48 h.

CAL-A (50 mg mL-1) Solvent, Et_3N: *rac*-**71** → (*S*)-**72**; or *rac*-**74** → CAL-A (75 mg mL-1) Solvent, Et_3N → (*S*)-**75**

Entry	Substrate	CAL-A preparation (source)	Solvent	Substrate		Product	
				Yield [%]	*ee* [%]	Yield [%]	*ee* [%]
1	*rac*-**71**	IMMCALA-T2-150	TBME	10	3	43	98
2	*rac*-**71**	CAL-A CLEA	TBME	34	3	19	95
3	*rac*-**71**	CAL-A on Celite (Novozymes 735)	TBME	13	7	48	98
4[a]	*rac*-**71**	CAL-A on Celite (Novozymes 735)	TBME	42	62	45	97
5[b]	*rac*-**71**	CAL-A on Celite (Novozymes 735)	TBME	14	70	26	97
6	*rac*-**71**	CAL-A on Celite (Chirazyme L5)	TBME	-	-	61	97
7	*rac*-**71**	CAL-A on Celite (Novozymes 735)	CH_3CN	10	6	60	98
8	*rac*-**74**	CAL-A on Celite (Novozymes 735)	CH_3CN	24	7	59	>99
9	*rac*-**74**	CAL-A on Celite (Chirazyme L5)	CH_3CN	traces	-	69	>99
10	*rac*-**74**	CAL-A on Celite (Cat#ICR112)	CH_3CN	traces	-	75	>99

[a] In the presence of 3Å M.S.; [b] In the presence of 4Å M.S.

5.2 Oxidation and hydrocyanation of glycosides with subsequent diastereoresolution by lipase-catalyzed O-acylation (papers IV and V)

Cyanohydrins are versatile intermediates toward other polyfunctional molecules (section 2.5).[61] For example, hydrolysis will produce α-hydroxy carboxylic acids and reduction will yield 1,2-aminoalcohols, both important structural fragments in pharmaceuticals. Carbohydrate-derived leads are emerging as highly potential molecules for drug discovery.[235] This is in part due to the importance of specific oligosaccharides in cellular recognition related to cancerous and infective diseases and also due to the high functional group density with defined stereochemistry offered by carbohydrates as synthetic precursors. For example, carbohydrates can be used as precursors for complex natural product synthesis and sugar-amino acid conjugates as building blocks for oligosaccharide mimetics and peptidomimetics.[236,237] Motivated by such arguments, research concentrating on diastereoselective hydrocyanation of glycoside aldehydes was studied.

Mannosylacetaldehyde α-D-**76** was prepared by the collaborators and used as a substrate for enzymatic and non-enzymatic hydrocyanation. Both non-enzymatic hydrocyanation with basic resin and enzymatic hydrocyanation with oxynitrilase yielded cyanohydrin α-D-**77** without diastereomeric enrichment for the newly generated stereogenic center. Therefore, a lipase was applied together with vinyl acetate in a diastereomeric resolution to afford cyanohydrin acetate (2*R*)-**78** and cyanohydrin (2*S*)-**77**, both with high diastereomeric excess. The method was also applied to the corresponding α-L-mannoside (α-L-**76**).

A similar strategy was applied to a selectively deprotected galactoside α-D-**79a**. The primary alcohol was oxidized to α-D-**80a**, hydrocyanated to α-D-**81a** and resolved with lipase-catalyzed acylation. Cyanohydrin butanoate α-D-**82a** and cyanohydrin acetate α-D-**83a** were isolated in high diastereomeric purity. Similar synthetic experiments with the corresponding mannoside (α-L-**79b**) and glucoside (α-L-**79c**) resulted in lower selectivity, indicating the importance of the axial substituent at position 4.

The synthetic schemes described above are presented in Figure 13. With both substrate classes the intermediates were used in subsequent steps in either one-pot synthesis or by filtering heterogeneous reagents off and thus simplifying the overall process. Dynamic kinetic resolution from the aldehydes to the cyanohydrin esters were studied but side reactions made the process less efficient. The stereochemical assignments of the newly created cyanohydrins and their ester epimers were correlated to the known behavior of the used enzymes and confirmed by NMR analysis.

Figure 13. Overview of the chemoenzymatic synthesis of sugar-derived cyanohydrins and lipase-catalyzed diastereoresolution to give cyanohydrin esters in high optical purity.

Synthesis of aldehydes α-D-76, α-L-76 and alcohol α-D-79a-c

Aldehydes α-D/L-**76** were prepared from allylglycoside **84** by a two-step oxidation by the collaborators (Scheme 24). Dihydroxylation with catalytic osmium tetroxide and stoichiometric *N*-methylmorpholine *N*-oxide (NMO) was followed by oxidative cleavage of diol **85** with sodium periodate. Aldehydes α-D-**76** and α-L-**76** were obtained in 91% and 77% yields, respectively.

Scheme 24. Synthesis of aldehyde α-D-**76**. The L-enantiomer was synthesized similarly.

The synthesis of alcohols α-D-**79a-c** first required selective deprotection of the corresponding peracetylated methyl glycosides. A set of lipases was investigated for the regioselective methanolysis of peracetylated methyl α-D-galactoside, -mannoside and glucoside (α-D-**86a-c**) in DIPE to afford the primary alcohols α-D-**89a-c** (Table 18). Lipase from *Burkholderia cepacia* was able to produce galactoside **79a** but did not accept other substrates under these conditions. CAL-B, CAL-A and CRL showed varied but generally low activity. TLL was very active on all three substrates and

although deacylation proceeded beyond position 6, good isolated yields were obtained, provided the reactions were stopped at 24 h.

Table 18. Synthesis of α-D-**79a-c** in the lipase-catalyzed methanolysis of peracetylated methyl α-D-glycopyranosides.[a]

MeOH, lipase / DIPE, +48 °C

α-D-**86a-c** → α-D-**79a**, α-D-**79b**, α-D-**79c**

Entry	Lipase	Yield[79a] [%]	Yield[79b] [%]	Yield[79c] [%]
1	Lipase PS-D (BCL)	80	0	6
2	Lipase PS-C II (BCL)	72	0	6
3	Novozym 435 (CAL-B)	14	36	0
4	CAL-A on Celite	10	0	17
5	CRL on Celite	15	28[b]	64
6	Lipozyme TL IM (TLL)	95 (81[c])	92 (84[c])	69 (64[c])

[a] [Substrate] = 0.05 M, [MeOH] = 0.25 M, lipase content 50 mg mL^{-1}; reaction time 24h; [b] Reaction time 72 h.; [c] Isolated yield.

Oxidation of α-D-**79a** was performed with diacetoxyiodobenzene and catalytic 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) in organic solvents (Table 19). Dichloromethane, a common solvent in $PhI(OAc)_2$-based oxidations, afforded 62% yield for the desired aldehyde α-D-**80a** but also 7% of undesired aldehyde **87** (entry 1). Oxidation in DIPE gave very low yield for α-D-**80a** (entry 2). In toluene, 75% yield for α-D-**80a** was obtained without any detectable formation of the unsaturated aldehyde **87** (entry 3). However, α-D-**80a** decomposed upon column chromatography and was subsequently used in solution without any work-up or purification.

Table 19. Oxidation of alcohol α-D-**79a** in different organic solvents.[a]

$PhI(OAc)_2$ (1.1 equiv.), TEMPO (0.1 equiv.) / Solvent, r.t., 12 h

α-D-**79a** → α-D-**80a** + α-D-**87**

Entry	Solvent	α-D-**79a** [%]	α-D-**80a** [%]	α-D-**87** [%]
1	CH_2Cl_2	0	62	7
2	DIPE	0	75	0
3	$PhCH_3$	81	4	0

[a] [Substrate] = 0.05 M.

Hydrocyanation of α-D/L-76 and diastereoresolution of α-D/L-77 by lipase-catalyzed acylation

The hydrocyanation of the prepared aldehydes was accomplished by using oxynitrilases or by chemical catalysis using Amberlite IRA-900 or 904 ion exchange resin conditioned to the OH^- form. The (*R*)-oxynitrilases used were almond meal, covalently immobilized *Pa*HNL (from almonds, ChiralVision) and aqueous solution of *At*HNL (oxynitrilase from *Arabidopsis thaliana*, Evocatal). Hydrocyanation of α-D-**76** to give cyanohydrin α-D-**77** by (*R*)-oxynitrilase catalysis was studied in buffered (tartrate buffer) mixtures of toluene varying the amount and pH of the water present (Table 20). Reactions were done in the presence and in the absence of the enzyme to check the extent of the non-enzymatic hydrocyanation. The results show that with 5 equiv. acetone cyanohydrin (**7**), almond meal catalyzed the hydrocyanation on the pH range 4.00 - 5.50 at 2 vol.-% (entries 5-7) or higher water content (entries 1-3). However, non-enzymatic hydrocyanation took place at similar or close to similar extent at these water contents whenever the pH of the buffer exceeded 4.00. No diastereopreference for α-D-**77** was obtained in any of the enzyme-promoted reactions (*de* ≤5%), even under conditions where the non-enzymatic hydrocyanation was suppressed (entry 7). The use of one equivalent acetone cyanohydrin only slowed down the reaction but did not provide diastereomerically enriched product (entry 4). Toluene saturated with water (entry 8) and fully anhydrous toluene (entry 9) suppressed the enzymatic activity to low levels, again with no gains in stereoselectivity. Immobilized *Pa*HNL behaved similarly but with lower activity (total mass basis) compared to almond meal under otherwise identical conditions (entries 10 vs. 7 and 11 vs. 9). An aqueous solution of *At*HNL gave no diastereoselectivity either (entry 12), though this might be explained by the high pH of the enzyme solution. In short, almond meal, under microaqueous conditions, was able to promote the formation of cyanohydrin α-D-**77** but without any diastereoselectivity.

α-D-**77** could be isolated in 82% yield under conditions of entry 5 (Table 20) but the two diastereomers were inseparable in column chromatography. To enable the separation of the diasteromers, α-D-**77** was subjected to lipase-catalyzed O-acylation. Lipase PS-D (50 mg mL^{-1}) and vinyl acetate (3 equiv.) were found to selectively afford cyanohydrin acetate α-D-(2*R*)-**78**. Before adding the acylation reagents, the reaction mixture of α-D-**77** was dried with $NaSO_4$, filtrated and 4Å molecular sieves were applied to ensure fully anhydrous conditions for the acylation. Column chromatography was now able to separate α-D-(2*R*)-**78** and α-D-(2*S*)-**77** (Table 21). The method was also applied to the L- enantiomer of **76**, and a similar pair of diastereomers was obtained after diastereoresolution.

Table 20. Hydrocyanation of α-D-**76** to yield α-D-**77** in toluene.[a]

Entry	Enzyme (amount)	Buffer (vol.-%)	Buffer pH	Yield [%] (with enzyme)	Yield [%] (without enzyme)
1	almond meal (20 mg mL^{-1})	5	5.50	quant.	quant.
2	almond meal (20 mg mL^{-1})	5	4.75	quant.	quant.
3	almond meal (20 mg mL^{-1})	5	4.00	quant.	44
4[b]	almond meal (20 mg mL^{-1})	2	5.50	61	37
5	almond meal (20 mg mL^{-1})	2	5.50	100	83
6	almond meal (20 mg mL^{-1})	2	4.75	100	77
7	almond meal (20 mg mL^{-1})	2	4.00	100	8
8[c]	almond meal (20 mg mL^{-1})	<1	4.00	7	0
9[d]	almond meal (20 mg mL^{-1})	0	-	11	0
10	immobilized *Pa*HNL (20 mg mL^{-1})	2	4.00	39	8
11[d]	immobilized *Pa*HNL (20 mg mL^{-1})	0	-	0	0
12	aq. *At*HNL (5 vol.-%)	-	8.0	100	-

[a] [Substrate] = 0.05 M, [**7**] = 0.25 M, buffer = 0.10 M tartrate buffer at the given pH. [b] [**7**] = 0.05 M. [c] toluene saturated with the buffer. [d] anhydrous toluene (≤20 ppm water).

Table 21. Preparative diastereoresolution of α-D- and α-L-**77** in anhydrous toluene after hydrocyanation.[a]

Substrate	Conv. [%]	Time [h]	*D*	(2*R*)-**78** Yield [%]	(2*R*)-**78** Purity [%]	(2*R*)-**78** *de* [%]	(2*S*)-**77** Yield [%]	(2*S*)-**77** Purity [%]	(2*S*)-**77** *de* [%]
α-D-**76**	48	3	>100	35	>99	>99	34	>99	93
α-L-**76**	51	24	62	31	89[b]	89	25	82[c]	93

[a] Reagents and conditions: a) [α-**76**] = 0.05 M, [**7**] = 0.25 M, almond meal 20 mg mL^{-1}, $PhCH_3$, 2 vol.-% 0.1 M tartrate buffer pH 5.50, r.t., 24 h, then Na_2SO_4 and filtration; b) [vinyl acetate] = 0.15 M, lipase PS-D 50 mg mL^{-1}, 4Å M.S. [b] Contains α-L-(2*S*)-**77** as impurity. [c] Contains α-L-(2*R*)-**78** as impurity.

Sequential oxidation and hydrocyanation with subsequent diastereoresolution of α-D-81a-c

Aldehyde α-D-**80a** was unstable toward the attempted efforts of purification and its subsequent hydrocyanation was performed in a one-pot manner, so that the reagents for hydrocyanation were added directly to the reaction vessel once oxidation had occurred. Almond meal and Amberlite IRA-900 OH^- resin were used as catalysts for hydrocyanation. Cyanide was introduced to the reaction either in the form of acetone cyanohydrin (**7**) or directly as HCN diffusing to the reaction vessel from another compartment through an open tunnel connecting the headspaces of the two compartments (Table 22). In microaqueous toluene, almond meal yielded cyanohydrin α-D-**81a** only in 17% yield (all yields calculated from α-D-**79a**) and 27% *de* (entry 1) when acetone cyanohydrin was used, but 74% yield and 37% *de* when HCN acted as a cyanide source (entry 2). In a preparative synthesis under the conditions of entry 2, cyanohydrin α-D-**81a** partially decomposed during column chromatography, mainly to **87**, so that reaction yield >80% afforded only 35% isolated yield and the two diastereomers of α-D-**81a** were inseparable. When anhydrous toluene alone was used as a solvent, almond meal did not catalyze the reaction (entry 3) but basic resin Amberlite IRA-900 OH^- afforded the desired cyanohydrin in 85% yield and 54% *de* (entry 4). The latter method was developed by optimizing the resin content (Table 23). While there was no non-enzymatic hydrocyanation (entry 1), under basic resin catalysis hydrocyanation (to give α-D-**81a**) competed with elimination (to give **87**) with a low resin content (entry 2). Higher resin contents favoured hydrocyanation without any noticeable effect on diastereoselectivity (entries 3 and 4). Accordingly, at 20 mg mL^{-1} resin content cyanohydrin α-D-**81a** was obtained in 92% reaction yield and 52% *de* (entry 4).

Table 22. Enzyme-catalyzed hydrocyanation of α-D-**80a** to yield α-D-**81a** in toluene.[a]

Entry	Catalyst (amount, mg mL^{-1})	Buffer [vol.-%]	CN^- source (equiv.)	Yield [%] (with enzyme)	*de* [%] (with enzyme)
1	almond meal (50)	4	**7** (2)[b]	17	27
2	almond meal (50)	4	HCN (5)[c]	74	37
3	almond meal (50)	0	**7** (2)[b]	0	-
4	Amberlite IRA-900 OH^- (10)	0	**7** (2)[b]	85	54

[a] [Substrate] = 0.05 M, 0.10 M tartrate buffer, pH 5.4. [b] [**7**] = 0.10 M. [c] [HCN] = 0.25 M.

Table 23. Effect of Amberlite IRA-900 OH^- content for one-pot hydrocyanation of α-D-**80a**.[a]

α-D-**80a** → **7** (2 equiv.), Amberlite IRA-900 OH^-, $PhCH_3$, r.t., 24 h → α-D-**81a**

Entry	Resin [mg mL^{-1}]	**80a** [%]	**81a** [%]	de^{81a} [%]
1	0	75	0	-
2	5	22	43	52
3	10	0	85	54
4	20	0	92	52

[a] 0.05 M α-D-**80a** as oxidized with $PhI(OAc)_2$ and TEMPO according to Table 19, entry 3.

A further analysis with 20 mg mL^{-1} resin content showed that while the initial hydrocyanation took place rapidly (α-D-**80a** had completely reacted within about 2 hours), the yield for α-D-**81a** continued to increase from 83% to 92% (Figure 14, black symbols). This implies that the aldehyde went through condensation reactions which were gradually reversed as the equilibrium was shifted toward cyanohydrin products. However, when the reagents both for oxidation of alcohol α-D-**79a** and for subsequent hydrocyanation were added simultaneously (Figure 14, open symbols), the reaction cascade stopped at less <20% yield for α-D-**81a** while the aldehyde intermediate was observed only in trace quantities. The early halt of the reaction might be due to the basic resin deactivating diacetoxyiodobenzene by cleaving acetate groups off or due to acetic acid neutralizing the basic resin. The two reactions thus could be performed without isolating the aldehyde intermediate by add-on reagent addition.

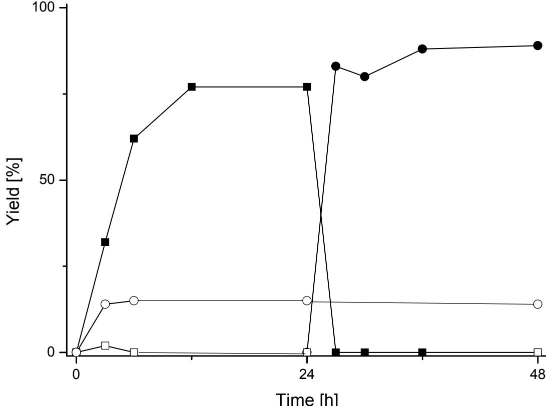


Figure 14. Yields of α-D-**80a** (■,□) and α-D-**81a** (●,○) of the oxidation of α-D-**79a** with a subsequent base-catalyzed one-pot hydrocyanation by either add-on (filled symbols) or at once (open symbols) reagent addition.

The oxidation-hydrocyanation sequence was applied to all three methyl glycosides α-D-**79a-c**. The galactosyl derivative was obtained in 92% reaction yield, but lower yields were obtained for the corresponding mannosyl (77%) and glucosyl derivatives (73%). The stereoselectivity was also lower in the latter two cases, which implies of the importance of the axial substituent in the galactoside scaffold. The obtained cyanohydrins were subjected to lipase-catalyzed acylation with vinyl butanoate as the acyl donor (Table 24). Lipases PS-D (entry 1) and PS-C II (entry 2) were active toward each α-D-**81a-c**. Good selectivity was obtained only for galactoside α-D-**82a**, for which lipase PS-C II showed higher activity than PS-D. Contrary to these two enzyme preparations, CAL-B (Novozym 435, entry 3) did not show activity to the galactosyl cyanohydrin α-D-**81a** but accepted the mannosyl and glucosyl derivatives α-D-**81b** and α-D-**81c**. CAL-A and CRL (both on Celite, entries 4 and 5) were most active toward mannoside α-D-**81b**. There is therefore substrate complementarity within the set of lipases employed depending on the sugar configuration of the cyanohydrin. However, both CAL-B and CAL-A displayed very low stereoselectivity. CRL showed good stereoselectivity only toward glucoside α-D-**81c** with a seemingly opposite stereopreference compared to other lipases. TLL (Lipozyme TL IM, entry 6) was inactive towards the acylation of any of the cyanohydrins α-D-**81a-c**.

Table 24. Reaction yields of lipase-catalyzed acylation of α-D-**81a-c** with vinyl butanoate to yield cyanohydrin esters α-D-**82a-c**. Absolute configuration at C-6 of **82b-c** is unconfirmed.[a]

α-D-**81a**: 92%, 52% *de*
α-D-**81b**: 77%, 19% *de*
α-D-**81c**: 73%, 5% *de*

Entry	Lipase	**82a**[%]	*de*^{82a} [%]	**82b**[%]	*de*^{82b} [%]	**82c**[%]	*de*^{82c} [%]
1	Lipase PS-D	42	87	65	30	69	19
2	Lipase PS-C II	75	85	65	33	56	42
3	Novozym 435	<1	-	76	12	71	15
4	CAL-A on Celite	10	83	69	25	5	-
5	CRL on Celite	0	-	77	16	27	99[b]
6	Lipozyme TL IM	4	-	<1	-	0	-

[a] Reagents and conditions: step a) α-D-**79a-c** (0.05 M), $PhI(OAc)_2$ (1.1 equiv.), TEMPO (0.1 equiv.), $PhCH_3$, r.t., 12 h; b) acetone cyanohydrin (2 equiv.), Amberlite IRA-900 OH^- (20 mg mL^{-1}), r.t., 6 h, then filtration; step c) vinyl butanoate (3 equiv.), lipase (50 mg mL^{-1}), r.t., 72 h. [b] Opposite stereopreference.

Variation of enzyme content and acyl donor concentration in the diastereoresolution showed that acylation is initially faster when lipase PS-C II amount is doubled from 50 to 100 mg mL^{-1} content while rate difference between 3 and 6 equivalents of vinyl butanoate was somewhat smaller (Figure 15a). However, close to 90% final yield of cyanohydrin butanoate α-D-**82a** was achieved in all cases. Even in the early phase of the reaction the diastereomeric excess of the product was not higher than 91% and it remained at about 90% up to yield of about 60%, beyond which *de* rapidly started to drop (Figure 15b). This corresponds to the point when all of the preferentially reacting 6*R*-diastereomer has been acylated and the less preferred 6*S*-diasteromer starts to react extensively. The intrinsic selectivity of the resolution was therefore not very high. The use of a short-chain acyl donor vinyl acetate gave a faster reaction but the selectivity was very similar (45% yield with 91% *de* for cyanohydrin acetate α-D-**83a** vs. 34% yield with 90% *de* for cyanohydrin butanoate α-D-**82a** in 24 h with 3 equiv. acyl donor and 50 mg mL^{-1} lipase PS-C II).

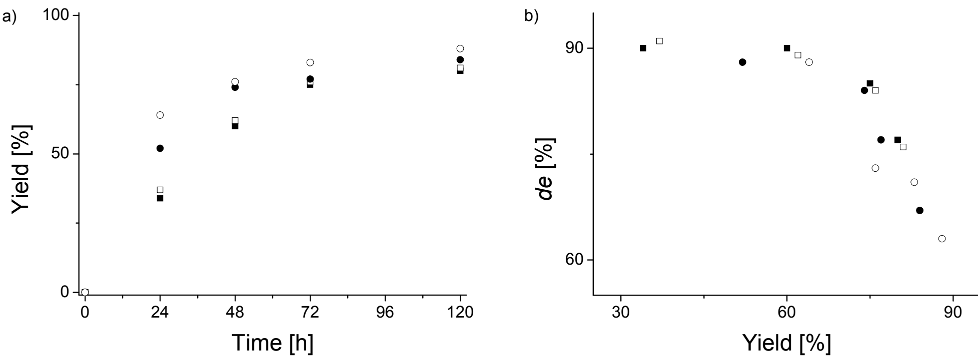


Figure 15. Yields (a) and *de* (b) of α-D-**82a** in lipase PS-C II -catalyzed acylation of α-D-**81a** (92%, *de* 51%) with vinyl butanoate in $PhCH_3$ at r.t.: 50 mg mL^{-1} lipase and 3 equiv. acyl donor (■); 50 mg mL^{-1} lipase and 6 equiv. acyl donor (□); 100 mg mL^{-1} lipase and 3 equiv. acyl donor (●); 100 mg mL^{-1} lipase and 6 equiv. acyl donor (○).

In a preparative reaction, cyanohydrin butanoate α-D-**82a** was formed in 75% yield and 85% *de* in 72 h using 50 mg mL^{-1} lipase PS-C II and 3 equiv. vinyl butanoate. Similar results were obtained for acetate α-D-**83a** (71% yield, 83% *de* in 24 h) when 6 equiv. vinyl acetate and 100 mg mL^{-1} lipase PS-C II were used. The cyanohydrin esters could be diastereomerically enriched upon chromatography although a part of the products were lost in later fractions. Both α-D-**82a** and α-D-**83a** were thus obtained in approximately 40% overall yield in optically pure form (Table 25).

Table 25. Preparative diastereoresolution of α-D-**81a** with vinyl butanoate and vinyl acetate.[a]

Product	Acyl donor (n), equiv.	Lipase PS-D [mg mL^{-1}]	Time [h]	Reaction analysis Yield [%]	Reaction analysis *de* [%]	Isolated products Yield [%]	Isolated products *de* [%]
α-D-**82a**	Vinyl butanoate (2), 3	50	72	75	85	41	>99
α-D-**83a**	Vinyl acetate (0), 6	100	24	71	83	39	>99

[a] Oxidation (a) and hydrocyanation (b) conditions as in Table 24. Yields are calculated from α-D-**79a**.

Purified cyanohydrin acetate α-D-**83a** was subjected to lipase PS-C II -catalyzed methanolysis in DIPE (conditions as in Table 18) to yield selectively deprotected α-D-(6*R*)-**81a** in 73% yield without loss of stereopurity. Thus, even though the stereoselectivity was low in the hydrocyanation reaction and the diastereomers of the cyanohydrin were inseparable and labile in silica gel chromatography, enzymatic acylation-deacylation sequence allowed the isolation of the optically pure (6*R*)-cyanohydrin.

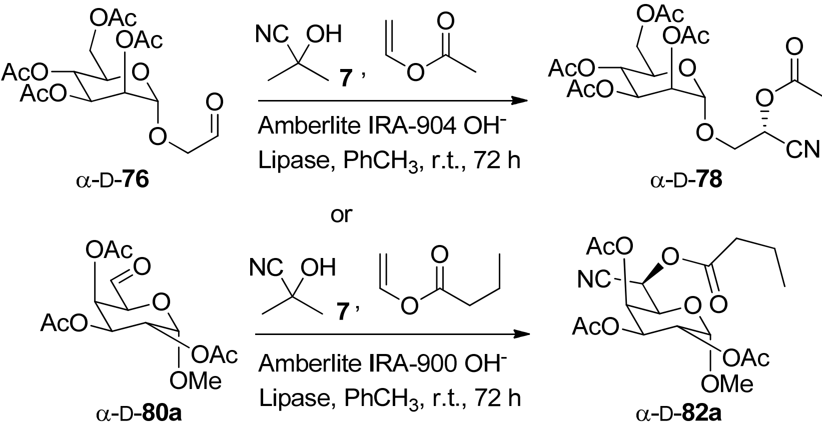
Dynamic kinetic diastereoresolution of cyanohydrins α-D-77 and α-D-81a

The aldehydes α-D-**76** and α-D-**80a** were subjected to dynamic conditions to afford the corresponding cyanohydin esters α-D-**78** and α-D-**82a**. In the synthesis, the reagents for hydrocyanation (acetone cyanohydrin and Amberlite IRA-904/900 OH^{-}) and acylation (vinyl acetate/butanoate and lipase) are added simultaneously to solutions of aldehyde substrates in anhydrous toluene, the latter together with the remaining oxidation reagents (see section 2.6.6).

The synthesis of α-D-**78** was developed in terms of resin amount, lipase and concentrations of acetone cyanohydrin and vinyl acetate (Table 26, entries 1-6). Very good selectivity was obtained with CAL-A, lipase PS-C II and lipase PS-D but the yield remained very low, highest with lipase PS-D at 34% (entry 4). Although the yield could eventually be raised to 51% (entry 6), this came at a cost of reduced selectivity and the system remained inferior compared to the sequential approach.

The synthesis of α-D-**82a** also produced low yields (Table 26, entries 7-10). Although a similar selectivity to that of the sequential approach was achieved (*de* 91%, entry 9), the yield remained very low. The main problem was the elimination side reaction to give aldehyde **87**, which then underwent further reactions.

Table 26. Dynamic kinetic resolution to yield cyanohydrin esters α-D-**78** and α-D-**82a**.[a]



Entry	Substrate (α-D-)	**7** [eq.]	Resin [mg mL^{-1}]	Acyl donor [eq.]	Lipase (mg mL^{-1})	Yield [%]	*de* [%]
1	**76**	2	4	3	Novozym 435 (50)	14	2
2	**76**	2	4	3	CAL-A on Celite (50)	15	>99
3	**76**	2	4	3	Lipase PS-C II (50)	27	>99
4	**76**	2	4	3	Lipase PS-D (50)	34	>99
5	**76**	4	4	6	Lipase PS-D (50)	25	>99
6	**76**	4	20	6	Lipase PS-D (50)	51	88
7	**80a**	2	20	3	Lipase PS-C II (50)	0	-
8 [b]	**80a**	2	20	3	Lipase PS-C II (50)	12	61
9 [b]	**80a**	2	20	6	Lipase PS-C II (100)	18	91
10 [b, c]	**80a**	2	20	6	Lipase PS-C II (100)	14	64

[a] [Substrate] = 0.05 M. [b] in the presence of 3Å M.S. (50 mg mL^{-1}). [c] Temperature +48 °C

Stereochemical considerations

The absolute configuration of the newly created stereogenic centers of the cyanohydrins were solved by NMR analysis and correlated to the known preferences of the used enzymes. When the natural stereopreference of an (*R*)-oxynitrilase (see section 2.5) is applied to the sugar derived substrates used in this work, the acetal oxygen changes Cahn-Ingold-Prelog priorities so that the (2/6*S*)-diastereomer of **77** and **81a** are the expected products (Figure 16a). Likewise, when Kazlauskas' model for lipase PS reactivity is applied (see section 2.6.2), the (*R*)-cyanohydrin esters are the expected products in the diastereomeric resolution (Figure 16b).

Figure 16. Reactivity models for a) (*R*)-oxynitrilase from almonds and b) lipase from *Burkholderia cepacia* as applied to sugar cyanohydrins. L = Large substituent (sugar ring), M = Medium-size substituent (nitrile group).

Almond meal -catalyzed synthesis of cyanohydrin α-D-**77** resulted in ~1:1 mixture (*de* ≤5%) of 2*S*- and 2*R*-diastereomers as did the chemical background hydrocyanation. Enzymatic resolution then afforded the diastereomerically pure α-D-**77**. Instead, 27-37% *de* for α-D-**81a** was obtained from almond meal catalysis and 52% *de* from chemical catalysis. In the latter case, the diastereomer that was formed in excess was the same in both enzymatic and non-enzymatic reactions. This was also the diastereomer that was preferentially acylated by lipase PS-D/-C II. The cyanohydrins thus obtained (α-D-**77** and α-D-**81** of 37% *de*) were analyzed in complex with (*R*)-mandelic acid - DMAP and (*S*)-mandelic acid - DMAP to solve the absolute configuration of the newly created stereogenic center (Figure 17).[238] In line with literature description[239], with the (*S*)-cyanohydrins **77** and **81a** the phenyl ring of the (*R*)-mandelate complex points away from the α-proton of the cyanohydrin (Figure 17a) whereas the phenyl ring of the (S)-mandelate complex shields the α-proton (Figure 17b). Therefore, downfield shift of the optically pure α-D-**77** ($\Delta\delta^{RS}$ = +0.066 ppm) and the minor diastereomer of the mixture of α-D-**81a** ($\Delta\delta^{RS}$ = +0.032 ppm) have the *S*-configuration. The major diastereomer of α-D-**81a** did not have a noticeable chemical shift difference between the two mandelate complexes. Either the complex formation is disturbed in this case by oxygen atoms in the sugar scaffold or the α-proton of the *R*-cyanohydrins, which points away from the phenyl ring of the mandelate complex, is too far from it to have large enough chemical shift differences between the diastereomeric complexes.

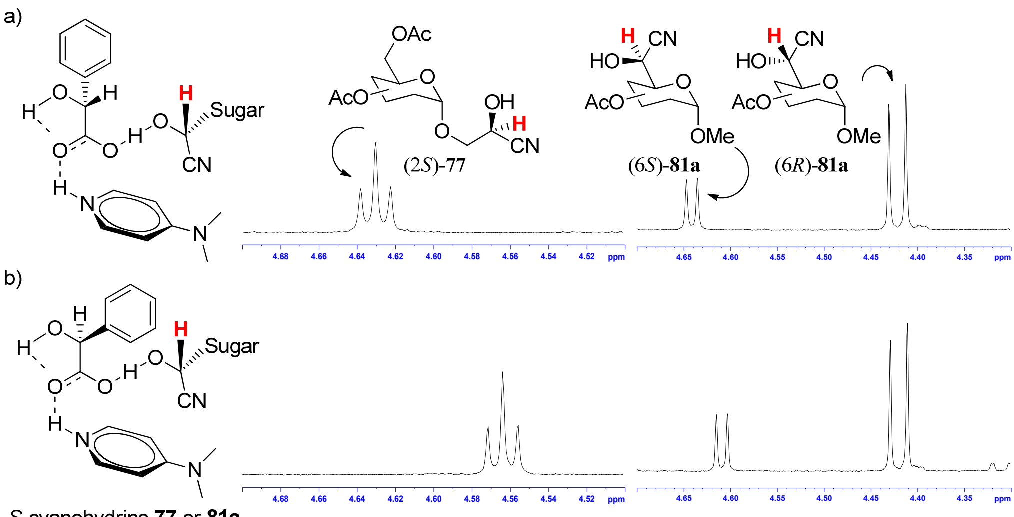


Figure 17. Models of (2*S*)-**77** and (6*S*)-**81a** in complex with (*R*)-mandelate - DMAP (a) and (*S*)-mandelate - DMAP (b) together with α-proton subsections of the measured ^{1}H NMR spectra.

Further analysis of the diastereomeric mixture of α-D-**81a** showed that the minor diastereomer has a coupling constant $^3J_{5,6}$ = 5.4 Hz and the major diastereomer $^3J_{5,6}$ = 8.7 Hz. Both 6*R*- and 6*S*-diastereomers have a minimum energy conformation where H-5 and H-6 are in *anti* relation. MM2 molecular mechanics simulation showed that both have also a local minimum energy conformation where the two protons are in *gauche* orientation. The *gauche* conformation lies about 5 kcal mol^{-1} higher in energy than the *anti* conformation in the case of (6*R*)-**81a** but only about 2 kcal mol^{-1} higher in (6*S*)-**81a**. Thus, (6*S*)-**81a** should be less strictly bound to *anti* conformation and, according to Karplus equation, have a smaller coupling constant between H-5 and H-6 than the 6*R*-diastereomer.

Overall, the use of (*R*)- and (*S*)-mandelate - DMAP in complex with α-D-(2*S*)-**77** and with α-D-(6*R*/*S*)-**81a** and the coupling constant analysis of the latter both support the stereochemical assignment presented in this section. Almond meal was completely non-selective for the synthesis of α-D-**77** and there was no internal asymmetric control either. The synthesis of α-D-**81a** slightly favoured the (6*R*)-stereomer in both almond meal catalysis (*de* 27-37%) and chemical catalysis (*de* 52-54%). The reason why the oxynitrilase favoured the same diastereomer as the lipases, yet less selectively than internal asymmetric control alone, cannot be explicitly stated with the available data. As predicted, lipase PS preferred the acylation of 2*R*- and 6*R*-diastereomers with α-D-**77** and α-D-**81a**, respectively.

6. SUMMARY

The literature review introduces the concepts and state of modern stereoselective synthesis with a particular focus on biocatalysis. Regio- and stereoselective acylation with lipases are covered in detail from a point of view relevant to the experimental part of the thesis. As was demonstrated through chosen examples, lipases perform the regio- and stereoselective acylation of alcohols and amines with structural and functional variety in both the nucleophile and the acyl donor. Compatibility of lipases with other reagents has been shown to enable the coupling of lipase-catalyzed acylation with other synthetic steps yielding advanced synthetic procedures such as dynamic kinetic resolution and functional acylation with in situ cyclization. High selectivity of acylation-based kinetic resolution does require careful choice of the acyl donor, the enzyme and the solvent as well as other reaction conditions. These requirements are even more stringent when other steps are incorporated to the overall process.

In the first part of the experimental section of the thesis (chapter 5.1), the usability of lipases in the acylation of primary and secondary amino compounds has been shown. A series of aryl homoallylamines was prepared and subjected to lipase-catalyzed kinetic resolution. The applicability of the method was demonstrated by synthesizing N-containing heterocycles from the resolved compounds. The enantiomerically enriched amides were recovered in high yields and *ee* values whereas the amines were collected in both lower yields and enantiopurity. A heteroaromatic amine (**67**) was a particularly challenging substrate, likely due to the basicity of the free pyridine nitrogen. The results support the notion that the electronic properties of the benzyl moiety (whether a substituted phenyl or a pyridyl) and the steric properties of the alkyl moiety greatly affect the conditions of a succesful resolution of benzylic amines. Perhaps the greatest potential of the work comes from using kinetic resolution as a synthetic step. In this work, alkene-functionalized acyl donors provided substrates for ring-closing metathesis reaction as the products of kinetic resolution, but one could envision other reaction combinations as well. In principle, any functional group not disturbing the enzymatic reaction could be applied. If such a kinetic resolution could be combined with a racemization step, a dynamic kinetic resolution system where the product could be directly used for the next synthetic step would be achieved.

CAL-A -catalyzed N-acylation as a kinetic resolution of partially protected piperazine 2-carboxylic acid was studied and found to be both effective and selective when the substrate reacted as an α-amino ester (**71**) with trifluoroethyl butanoate. When the substrate reacted as a β-amino acid ester, reactivity was much lower. Dynamic kinetic resolution of the α-amino ester **71** in acetonitrile afforded butanamide **72** in 60% yield

and 98% *ee*. Similarly, pipecolic acid methyl ester (**74**) gave its butanamide **75** in 75% yield and >99% *ee*. The aldehyde-induced racemization coupled to lipase-catalyzed acylation is sensitive to reaction conditions and enzyme properties, organic solvent, water activity and acyl donor selection play important roles in the reaction outcome.

In the second part of the experimental section (chapter 5.2), monosaccharide substrates were transformed into the corresponding aldehydes and subjected to enzymatic and non-enzymatic hydrocyanation. Although the hydrocyanation was either completely non-selective or only modestly selective, coupling the synthesis to lipase-catalyzed kinetic diastereoresolution afforded the cyanohydrins, either as unprotected alcohols or in acylated form, in essentially diastereomerically pure form. Intermediate work-up steps were kept at minimum whenever possible, performing the reactions either in a one-pot manner or filtrating heterogeneous catalysts. Column chromatographic purification was required only to separate the products of the final diastereoresolution step. Possibility to combine different steps into a cascade process was investigated both for the oxidation-hydrocyanation sequence and for the hydrocyanation-acylation sequence but reagent incompatibility prevented the efficient application of this strategy. However, with different protecting groups and different reagent selection this target should be achievable as well. For example, the base and/or cyanide source can play an important role in the hydrocyanation-acylation sequence under dynamic conditions and mutually compatible reagents with other suitable conditions for cascade or one-pot oxidation-hydrocyanation-acylation sequence can eventually be found.

In summary, the work has shown that lipases are efficient catalysts for selective preparation of chiral nitrogenated compounds, whether based on N-acylation of amino groups or O-acylation of polyfunctional compounds with a masked amino group. Lipases are robust enzymes and future might include a more integrated use of lipases with other synthetic steps. This might take the form of functional acylation, new dynamic kinetic resolution methods or novel one-pot sequences. For example, azido- or boronate-substituted acyl donors in lipase-catalyzed acylation could afford substrates for click conjugation (via azide - alkyne cycloaddition reaction) or for Pd-catalyzed cross-coupling reactions, respectively. Complex products could be stereoselectively obtained if rearrangement reactions could be combined with acylation of one of the tautomers. Stereoselective acylation could also drive the equilibrium forward in multistep cascade processes with several labile intermediates.

ACKNOWLEDGEMENTS

The laboratory work resulting to this thesis was done between the years 2008-2011 within the laboratory of synthetic drug chemistry, institute of biomedicine, faculty of medicine at the University of Turku. The Academy of Finland is gratefully acknowledged for funding the research. I also thank the Graduate School of Organic Chemistry and Chemical Biology for financially supporting my participation in courses and conferences in Finland and abroad.

Professor Romano Orru is gratefully thanked for all his efforts to read the thesis, travel and present his criticism in public. I am equally grateful to Professor Petri Pihko and Professor Patrick Adlercreutz for reviewing the thesis and presenting their comments. I feel immensely honoured that these distinguished scientists saw the effort worthwhile.

Professor Liisa Kanerva supervised me throughout these years and I fully appreciate her support and guidance in all aspects related to the work. I also thank Professor Reko Leino and PhD Filip Ekholm, PhD Tiina Saloranta, PhD Sara Rosenberg and Ms. Eveliina Laitinen at Åbo Academy University for fruitful collaboration.

I am grateful for valuable discussions and help for my long-term collaborators PhD Arto Liljeblad and PhD Katri Lundell and Ms. Mari Päiviö, Mr. Riku Sundell, PhD Xiang-Guo Li, PhD Mihaela-Claudia Turcu, Ms. Päivi Perkiö and PhD Piia Hara (in approximate order of shared laboratory time). I also appreciate the time I have spent with other current and former researchers at the laboratory, including PhD Jürgen Brem, Mr. Toni Kurki, Ms. Meeri Käkelä, Mr. Niko Laaksovirta, Ms. Outi Lehtovirta, Mr. Harri Mäenpää, Mr. Juuso Nästi, Ms. Maria Rantapaju, Ms. Heli Savolainen and Ms. Elina Siirola (in alphabetical order).

PhD Jari Sinkkonen and PhD Olli Martiskainen at the Department of Chemistry, University of Turku, are acknowledged for technical support with NMR and HRMS instruments.

Finally, I shall humbly thank my wife Julia for supporting my decision to pursue a PhD graduation and accepting all its consequences.

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ORIGINAL PUBLICATIONS I-V

FULL PAPER

I

DOI: 10.1002/ejoc.200901216

Synthesis of Enantiopure Benzyl Homoallylamines by Indium-Mediated Barbier-Type Allylation Combined with Enzymatic Kinetic Resolution: Towards the Chemoenzymatic Synthesis of N-Containing Heterocycles

Ari Hietanen,[a][‡] Tiina Saloranta,[b][‡] Sara Rosenberg,[b] Evelina Laitinen,[b] Reko Leino,*[b] and Liisa T. Kanerva*[a]

Keywords: Amines / Allylation / Enzyme catalysis / Kinetic resolution / Ring-closing metathesis

Barbier-type indium-mediated allylations of different *N*,*N*-(dimethylsulfamoyl)-protected aldimines with a number of allyl bromides followed by high-yielding deprotection afforded allylic amines in good to excellent yields. The racemic amines were then subjected to enzymatic kinetic resolution in order to obtain the corresponding (*S*)-amines and (*R*)-amides. When acyl donors with a terminal double bond were applied in the enzymatic kinetic resolution, the product amide could be converted into unsaturated lactams in a straightforward manner by utilizing ring-closing metathesis. Furthermore, the enantiopure (*S*)-1-phenylbut-3-enylamine was converted into the corresponding diallylamine, which was subjected to ring-closing metathesis to yield a substituted dehydropiperidine mimicking a number of natural products.

Introduction

Chiral benzylamines are important intermediates in the synthesis of biologically and pharmaceutically relevant targets. Thus, the development of synthetic strategies towards such moieties is of considerable academic as well as industrial importance in both fine chemical and pharmaceutical industries. In particular, chiral benzylamines have emerged as fragments in some neuroactive pharmaceutical ingredients. For example, the parasympathomimetic (*S*)-rivastigmine has been adopted in the clinical treatment of dementia associated with Alzheimer's disease.[1] Moreover, the synthesis of N-containing heterocycles such as piperidines,[2] lactams[3] and derivatives thereof is of topical interest due to the frequent occurrence of such moieties in biologically active small molecules, whether of natural or synthetic origin, and peptidomimetics. Figure 1 illustrates representative examples of each of these compound classes.[4]

Recently, we briefly described an efficient and high-yielding procedure for the synthesis of racemic homoallylamines by utilization of Barbier-type allylation of an *N*,*N*-dimethylsulfamoyl-protected aldimine followed by subsequent deprotection by transamination.[5] A series of *N*,*N*-dimethylsulfamoyl-protected aldimines have also been utilized in Pd-catalyzed allylations to produce various protected amines in good to excellent yields.[6] In the present work, we further examine the Barbier-type allylation of the *N*,*N*-dimethylsulfamoyl-protected aldimines and extend the product scope to amines with different electronic and steric properties (Figure 2).

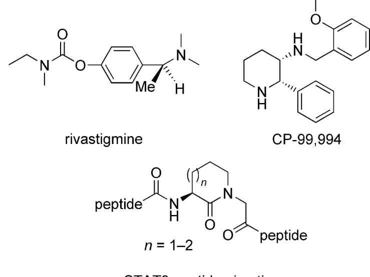

Figure 1. Examples of bioactive chiral benzylamines, piperidines and lactams.

Similar homoallylic amines have been synthesized previously in an enantioselective manner by utilizing chiral induction from the protective group,[7a] enantiodifferentiation by addition of cinchona alkaloids,[7b] or in the presence of a chiral ligand in catalytic allylations.[7c] Herein, we report a methodology to produce the enantiomers of bifunctional

[a] Institute of Biomedicine/Department of Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry,
University of Turku, 20014 Turku, Finland
Fax: +358-2-333-7955
E-mail: lkanerva@utu.fi
[b] Laboratory of Organic Chemistry
Åbo Akademi Univeristy, 20500 Åbo, Finland
Fax: +358-2-215-4866
E-mail: reko.leino@abo.fi
[‡] A. Hietanen and T. Saloranta contributed equally to this work
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200901216.

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R' = electron-withdrawing or -donating substituent

R'' = H or Me

Figure 2. General structure of homoallylamines prepared in present work with variations in electronic and steric properties.

homoallylbenzylamines *rac*-**3a**–**e** (Figure 3) using the lipase-catalyzed *N*-acylation of the more reactive (*R*)-amines in racemates. Racemic 1-phenylbut-3-en-1-amine (*rac*-**3a**) was selected as a model substrate for the enzymatic studies throughout the work.

Figure 3. Homoallylbenzylamines prepared in the present work for enzymatic kinetic resolution.

Finally, we have also explored the utilization of the enantiopure homoallylic amines for the synthesis of N-containing heterocycles by olefin metathesis. The approach is schematically illustrated in Scheme 1. By utilization of acyl donors with a terminal double bond, the enzymatically produced (*R*)-amides can be converted into the corresponding unsaturated lactams by ring-closing metathesis, a strategy previously used in the synthesis of lactones.[8] Therefore, this approach directly utilizes the enhanced molecular functionality and increases the attractiveness of the kinetic resolution method. Furthermore, conversion of the enzymatically less reactive (*S*)-amines into the corresponding diallylic compounds provides precursors for ring-closing metathesis to yield substituted dehydropiperidines.

Scheme 1. Synthesis of enantiopure N-containing heterocycles via a chemoenzymatic pathway.

Results and Discussion

Synthesis of Homoallylic Amines

In our previous work, the synthesis of racemic homoallylbenzylamine *rac*-**3a** via Barbier-type allylation of the precursor aldimine was briefly explored.[5] In the present work, the substrate scope of this reaction was successfully extended to cover also substituted benzaldimines with electron-withdrawing (**1b**) and electron-donating (**1c**) *para*-substituents to prepare the racemic amines **3b** and **3c** (Figure 3, Table 1). In addition, sterically demanding allylating agents methallyl bromide and prenyl bromide were utilized to synthesize the products **3d** and **3e**, respectively. The indium-mediated allylations of aldimines **1a**–**c** were performed in anhydrous THF giving full conversions in all cases except with the bulkier prenyl bromide reagent as allylating agent (Table 1, Entry 5, step 1).

Table 1. Synthesis of homoallylic amine series.[a]

Entry	Product	R^1	R^2	R^3	R^4	Conv. [%][b]	Yield [%][c]
1	*rac*-**3a**	H	H	H	H	100/100	71/91
2	*rac*-**3b**	F	H	H	H	100/100	68/68
3	*rac*-**3c**	OMe	H	H	H	100/100	68/90
4	*rac*-**3d**	H	H	H	Me	100/100	65/76
5	*rac*-**3e**	H	Me	Me	H	75/100	27/83

[a] Allylations were performed at room temp. on 4–11-mmol scale with 3 equiv. of indium and allylating agent, respectively. [b] Conversion as determined by ^{1}H NMR spectroscopy (step 1/step 2). [c] Isolated yields (step 1/step 2).

In our previous work,[5] it was shown that the *N*,*N*-dimethylsulfamoyl group is smoothly removed by transamination with 1,3-diaminopropane under conventional reflux conditions. Here, the same protocol was utilized to deprotect all modified substrates **2b**–**e**. The method proved its feasibility and the desired allylic amines **3a**–**e** were obtained in good to excellent yields (Table 1, step 2).

Enzymatic Kinetic Resolution of Homoallylic Amines

Enzymatic kinetic resolution of racemic amines is typically based on reactions between a primary or, less frequently, a secondary amine and a suitable acyl donor in organic solvents.[9,10] Lipases (E.C. 3.1.1.3) in general, and *Burkholderia cepacia* lipase (as a lipase PS-D preparation on Celite) and *Candida antarctica* lipases A (as adsorbed on Celite in the presence of sucrose[11]) and B (CAL-B as a Novozym 435 preparation) in particular, are useful biocatalysts for the enantioselective *N*-acylation of amino groups since they only rarely can split an amide bond other than

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that in β-lactams, and accordingly cause the transformation of the amide back into the amine.[12,13] It has been suggested that the resonance stabilization of normal amide functionalities makes amides stable toward lipases.[13c] Another issue related to amide stability is the intrinsic reactivity of the acyl donor. High enough reactivity is pivotal to enable reasonable reaction rates while too high reactivity can lead to chemical *N*-acylation parallel with the enzymatic one. Moreover, irreversible acyl donors, vinyl and isopropenyl esters (common with alcoholic substrates), are inappropriate with amine substrates due to possible imine formation with acetaldehyde and acetone, tautomerization products from the liberated vinyl and isopropenyl alcohols, respectively.

For developing a procedure for the enzymatic kinetic resolution, *rac*-**3a** (0.1 M) was first subjected to lipase screening (see the lipases in the Exp. Section) using commercial lipase preparations (25 $mg\,mL^{-1}$) and ethyl methoxyacetate (5 equiv. corresponding to 6 vol.-% of the acyl donor) in *tert*-butyl methyl ether (TBME) dried with molecular sieves (4 Å). The potential of Subtilisin Carlsberg (a serine protease) and acylase I to acylate *rac*-**3a** was also tested. Acyl activated ethyl methoxyacetate was chosen as it was reported to work well in the CAL-B-catalyzed acylation of amines.[14] The activation is mainly due to a weak hydrogen bond between the β-oxygen atom of the methoxyacetate moiety of the acyl-enzyme intermediate and the amine hydrogen of the reactive amine counterpart on the mechanistic pathway of serine hydrolases.[15] CAL-A was by far the most reactive but virtually a non-enantioselective catalyst while lipase PS-D [$ee^{(R)\text{-amide}}$ = 95% at 30% conversion] and CAL-B [$ee^{(R)\text{-amide}}$ = 92% at 35% conversion] both showed reactivity with relatively good enantioselectivity. Other enzymes in the test set were completely inefficient. A possible chemical background reaction was excluded by performing the reaction under the same conditions except in the absence of an enzyme. However, the ammonium salt between the substrate and methoxyacetic acid (hydrolysis product of the acyl donor) precipitated and lowered the rate and selectivity of the resolution. Changing the solvent from ethers (TBME or diisopropyl ether) to toluene (dried on molecular sieves) more than halved the water content (from more than 90 ppm to about 40 ppm), and precipitation was not visually detectable any more with lipase PS-D. This effect is in accordance with our earlier experience of lipase PS-D causing less hydrolysis of hydrolyzable compounds than CAL-B (Novozym 435) when used in organic solvents,[16] and might explain the better selectivity of lipase PS-D vs. CAL-B.

At this point, attention was paid to the nature of the acyl donor and to the reaction conditions in order to increase reactivity and enantioselectivity. The commonly used alkyl-activated acyl donors, 2,2,2-trifluoroethyl acetate and butanoate, resulted in fast but completely non-selective acylation of *rac*-**3a** (0.1 M) with lipase PS-D (25 $mg\,mL^{-1}$) in toluene. The ability of normal esters (6 vol.-%, Entries 1–4) to enantioselectively acylate *rac*-**3a** indicated isopropyl acetate to have some potential (Entry 4) (Table 2). With increasing isopropyl acetate content enantioselectivity increased (Entries 4–7). When ethyl methoxyacetate content was increased, conversion after 3 d was enhanced at the expense of enantioselectivity (Entries 13, 19 and 22). Accordingly, working at low ethyl methoxyacetate content or in neat isopropyl acetate was shown to be favorable. Attempts to neutralize the possibly formed methoxyacetic (Entries 14 and 20) or acetic acid (Entry 8) by performing the reaction in the presence of triethylamine affected the outcome only at high ethyl methoxyacetate contents. Attempts to keep the system dry by adding molecular sieves into the reaction mixture were more productive, and excellent *E* values could be calculated when the reaction proceeded in neat isopropyl acetate (water content 60 ppm) or with ethyl methoxyacetate (6 vol.-%, equals to 5 equiv.) in toluene (water content 40 ppm) (Entries 9 and 15). In addition, considerable reactivity enhancement was gained. In order to further increase reactivity, the enzyme content was increased from 25 $mg\,mL^{-1}$ to 50 $mg\,mL^{-1}$ (Entries 11 and 17). Larger amounts of the enzyme did not boost reactivity higher. Increasing the temperature above room temperature negatively affected enantioselectivity with isopropyl acetate (Entries 16–18).

Table 2. Screening of acyl donors (0.5 M) for the acylation of *rac*-**3a** (0.1 M) with lipase PS-D (25 $mg\,mL^{-1}$) in toluene at room temperature; reaction time 3 d.

Entry	Acyl donor/[vol.-%]	Conv. [%]	$ee^{(R)\text{-amide}}$ [%]	$ee^{(S)\text{-3}}$ [%] (E)[g]
1	ethyl acetate/6	11	10	1
2	ethyl propanoate/6	1	43	<1
3	ethyl butanoate/6	6	2	<1
4	isopropyl acetate/6	3	78	2
5	isopropyl acetate/20	5	87	5
6	isopropyl acetate/50	19	95	23
7	isopropyl acetate/100	31	95	42
8	isopropyl acetate/100[a]	32	95	44
9	isopropyl acetate/100[b]	31	99	45 (200)
10	isopropyl acetate/100[c,d]	38	98	59 (200)
11	isopropyl acetate/100[c]	45	99	82 (200)
12	isopropyl acetate/100[c,e]	48	96	87 (100)
13	ethyl methoxyacetate/6	14	95	16
14	ethyl methoxyacetate/6[a]	17	96	20
15	ethyl methoxyacetate/6[b]	44	99	76 (200)
16	ethyl methoxyacetate/6[c,d]	42	99	73 (200)
17	ethyl methoxyacetate/6[c]	45	99	82 (200)
18	ethyl methoxyacetate/6[c,e,f]	50	97	97 (200)
19	ethyl methoxyacetate/20	29	91	37
20	ethyl methoxyacetate/20[a]	38	94	58
21	ethyl methoxyacetate/20[b]	57	75	99
22	ethyl methoxyacetate/50	34	76	39

[a] Et_3N (0.1 M) was added. [b] Molecular sieves (4 Å) were added. [c] 50 $mg\,mL^{-1}$ of lipase PS-D in the presence of molecular sieves (4 Å). [d] Reaction temperature 4 °C. [e] Reaction temperature 48 °C. [f] Reaction time 2 d. [g] *E* given only with clearly excellent enantioselectivity.

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With this data available, the preparative-scale kinetic resolution of *rac*-**3a** was performed with ethyl methoxyacetate (6 vol.-%) in toluene in the presence of lipase PS-D (50 mg mL^{-1}) and molecular sieves (4 Å) at 48 **°C** (Entry 1, Table 3) to afford (*R*)-**4a** and (*S*)-**3a**. The outcome was slightly less satisfactory with $E = 98$ than what was expected on the basis of a small-scale reaction (Entry 18, Table 2). For that reason, the kinetic resolutions of *rac*-**3a**–**c** were next performed in isopropyl acetate in the presence of lipase PS-D (50 mg mL^{-1}) and molecular sieves (4 Å) at room temperature (Entries 2–4, Table 3). While the kinetic resolution of *rac*-**3c** gave the resolution products (*S*)-**3c** and (*R*)-**5c** with excellent enantiopurities after 4 d at 49% conversion, the reactions with substrates **3a** and **3b** slowed down beyond about 30–40% of conversion. For instance, the resolution of *rac*-**3a** was at 46% conversion after 3 d, but 6 more days were required to reach 49% conversion. Lipase PS-D, CAL-B and CAL-A were all unable to acylate *rac*-**3d** and *rac*-**3e** in neat isopropyl acetate. On the other hand, when the acyl donor was changed to ethyl methoxyacetate (20 vol.-% was required) in toluene, acylation of **3d** slowly proceeded in the presence of CAL-B, and after 7 d (*R*)-**4d** was obtained enantiopure (*ee* > 99%) at 31% conversion (Entry 5). *Rac*-**3e** stayed unreacted. The enantiomerically enriched unreacted (*S*)-**3a** (*ee* = 84%), (*S*)-**3b** (*ee* = 48%) and (*S*)-**3d** (*ee* = 44%) were further purified by subjecting them under the kinetic resolution conditions, yielding 99%, 95% and 72% *ee*, respectively. In general, the isolated yields for the unreacted (*S*)-amines stayed somewhat low and the need to further enantiomerically purify the products by repeating the enzymatic acylation lowered the yields. Our efforts to hydrolyze (*R*)-**4a** and (*R*)-**5a** gave also low yields for (*R*)-**3a** due to by-product formation (see Exp. Sect.).

According to the Kazlauskas rule, the (*R*)-enantio-preference was expected for the lipase PS-D in the present *N*-acylations.[17] This was confirmed when (*S*)-**3a** and (*S*)-**3c** were at our hands after the kinetic resolution allowing the sign of the $[\alpha]_D$ values to be compared to those given for the (*S*)-enantiomers in literature.[7c] In order to further support this suggestion, both enantiomers of **3a** were derivatized with (*S*)-(+)-*α*-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)-MTPA-Cl] giving a pair of diastereomeric amides that were characterized by ^{1}H NMR spectroscopy. The comparison of the $\Delta\delta$ values further corroborates the reacting stereoisomer of the amine to have the (*R*)-configuration.[18]

To extend the scope of the resolution, we then used ethyl and isopropyl acrylates and isopropyl 3-butenoate as functionalized acyl donors in the kinetic resolution of *rac*-**3a** in toluene (Table 4). We envisioned that the amides (*R*)-**6** and (*R*)-**7** could yield the corresponding enantiopure six- and seven-membered unsaturated lactams when used as substrates for ring-closing metathesis. The resolution with isopropyl acrylate was completely non-enantioselective (Entries 4–6) while excellent enantioselectivities were observed with ethyl acrylate (Entries 1–3) and with isopropyl 3-butenoate (Entries 7–9). The reactions with acrylate esters remained very slow, and it was not possible to affect reactivity by increasing the content of the acyl donor from 5 to 20 vol.-% (Entries 1–6). Finally, preparative-scale kinetic resolution produced (*R*)-**6** with 65% isolated yield (calculated from conversion) when 19% of the racemate had reacted (Scheme 3). The lipase-catalyzed Michael addition started to compete seriously with the *N*-acylation at this point.[19] Lacking Michael acceptor properties, isopropyl 3-butenoate (10 vol.-%) successfully afforded the amide (*R*)-**7** at 47% conversion with 85% isolated yield and *ee* > 99%.

Table 3. Preparative-scale kinetic resolution of *rac*-**3a**–**e** (0.1 M) in isopropyl acetate (neat) at room temperature or with ethyl methoxyacetate in toluene in the presence of molecular sieves (4 Å).

lipase PS-D (50 mg/mL); *rac*-**3a–e**; (*R*)-**4a**,**d**: R = CH_3OCH_3; (*R*)-**5a–c**: R = CH_3; (*S*)-**3a–d**

Entry	Substrate	Time [d]	Conv. [%]	(*R*)-Amide Yield [%][a]	(*R*)-Amide *ee* [%]	(*S*)-Amine Yield [%][a]	(*S*)-Amine *ee* [%]
1	**3a**[b]	4	48	83	94	58	87
2	**3a**	3	46	92	99	66	84/99[e]
3	**3b**	4	33	75	>99	75	48/95[e]
4	**3c**	4	49	65	>99	93	94
5	**3d** [c]	7	31	quant.	>99	35	44/72[e]
6	**3e** [d]	–	–	–	–	–	–

[a] Isolated yields based on conversion. [b] Ethyl methoxyacetate (6 vol.-%) in toluene at 48 °C. [c] CAL-B (50 mg mL^{-1}) in the place of lipase PS-D and ethyl methoxyacetate (20 vol.-%) in toluene at room temp. [d] No reaction with any applied method. [e] *ee* after enantiomeric enrichments.

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Table 4. Kinetic resolution of *rac*-**3a** (0.1 M) with ethyl and isopropyl acrylate and isopropyl 3-butenoate (toluene was used as cosolvent) in the presence of lipase PS-D (50 mg mL^{-1}) and molecular sieves (4 Å) at room temperature; reaction time 3 d.

Entry	R	*n*	Acyl donor [vol.-%]	Conv. [%]	$ee^{(R)\text{-amide}}$ [%]	$ee^{(S)\text{-3a}}$ [%]	*E*
1	H	0	5	17	98	21	>100
2	H	0	10	16	98	19	>100
3	H	0	20	18	98	21	>100
4	CH_3	0	5	7	3	<1	1
5	CH_3	0	10	1	23	<1	2
6	CH_3	0	20	2	13	<1	1
7	CH_3	1	5	40	>99	67	>200
8	CH_3	1	10	45	>99	83	>200
9	CH_3	1	20	48	>99	91	>200

Synthesis of N-Containing Heterocycles

In our previous work, the protected crotylated amine was converted into the corresponding diallylic sulfamide that was then ring-closed using Grubbs' 2nd generation catalyst to yield a substituted dehydropiperidine in excellent overall yield.[5] Unfortunately, the removal of the *N*,*N*-dimethylsulfamoyl protective group from this tertiary amine proved unsuccessful. In the present work, having the enantiopure amine at hand, we were encouraged to redesign the synthesis strategy in order to prepare enantiopure dehydropiperidines in a similar fashion. Accordingly, the unprotected amine (*S*)-**3a** was converted into the corresponding diallylic amine (*S*)-**8** by deprotonation with KOH and subsequent reaction with one equivalent of allyl bromide in 67% yield. The diallylic amine obtained was then subjected to ring-closing metathesis. As known from the literature, free amines are detrimental to the Grubbs-type ruthenium catalysts.[20] When, however, the diallylic amine was converted into the corresponding salt by adding one equivalent of *p*-toluenesulfonic acid (*p*TsA) the ring-closure proceeded smoothly affording the enantiopure dehydropiperidine (*S*)-**9** in 70% isolated yield (Scheme 2).

Scheme 2. Synthesis of cyclic secondary amine (*S*)-**9**. i) allyl bromide (1 equiv.), KOH (3 equiv.), DMF. ii) Grubbs' 2nd generation cat. (10 mol-%), *p*TsA (1 equiv.), CH_2Cl_2, 40 °C.

Finally, the diallylic resolution products (*R*)-**6** and (*R*)-**7** were subjected to ring-closing metathesis using Grubbs' 2nd generation catalyst. A similar type of approach, although based on a longer synthetic route for preparation of the enantiopure diallyl precursors, has been described by Fiorelli and Savoia.[21] In their work, a number of substituted diallylic amides were successfully ring-closed to yield the corresponding α,β-unsaturated δ-lactams in good to excellent yields. In accordance with this published procedure,[21] the δ-lactam (*R*)-**10** was synthesized from (*R*)-**6** in 83% isolated yield (Scheme 3). In our hands, however, the corresponding seven-membered lactam (*R*)-**11** was formed in low yield only (22%) when starting from the diallylic amide (*R*)-**9**.

Scheme 3. Kinetic resolution of **3a** to yield amide (*R*)-**6** and the synthesis of the lactam (*R*)-**10**.

Nevertheless, this work has shown that a number of biologically relevant N-containing heterocycles could be produced by combining the elegancy of enzymatic and metal-catalyzed reactions. Currently, we are investigating the possibilities to extend the scope of this protocol to synthesis of some natural products and analogues.

Conclusions

We have shown that the indium-mediated allylation of *N*,*N*-dimethylsulfamoyl-protected aldimines and the subse-

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quent deprotection by transamination is a reliable procedure for production of homoallylic amines with different electronic and steric properties. Moreover, our study has shown that the enzymatic kinetic resolution of the sterically more demanding benzyl homoallylamines (allyl as the medium-sized group) is possible by carefully adjusting the most critical reaction parameters. As the steric crowding is transferred closer to the reaction center, the resolution becomes more challenging. Furthermore, by utilizing acyl donors with a terminal double bond, the *N*-acylation has been used as a synthetic step, as the resolution product can be directly subjected to ring-closing metathesis yielding unsaturated lactam products as exemplified by the straightforward synthesis of an enantiopure α,β-unsaturated δ-lactam. Finally, an enantiopure amine has been transferred into its diallylic counterpart, which has been subjected to ring-closing metathesis to afford a substituted enantiopure dehydropiperidine product. The obtained N-containing heterocycles represent an attractive class of moieties found in a number of biologically and pharmaceutically relevant targets.

Experimental Section

Materials and Methods: The allylation and ring-closing reactions were performed under dry argon using anhydrous solvents. THF was distilled from Na/benzophenone and CH_2Cl_2 was distilled from CaH_2. The anhydrous solvents were then stored under argon. *N,N*-dimethylsulfamide was readily prepared from commercial dimethylsulfamoyl chloride and 30% aqueous ammonia. All other reagents were purchased and used as received. Solvents and acyl donors for enzymatic reactions were obtained from commercial sources and stored over molecular sieves unless stated otherwise. Isopropyl acrylate and isopropyl 3-butenoate were prepared according to literature procedures.[22] *Burcholderia cepacia* lipase (lipase PS-D) was acquired from Amano Europe, *Candida antarctica* lipase B (CAL-B, Novozym 435), *Thermomyces lanuginosus* lipase (Lipozyme TL IM) and *Rhizopus miehei* lipase (lipase RM IM) were purchased from Novozymes, *Candida antarctica* lipase A (CAL-A, immobilized) was from Biocatalytics, *Candida rugosa* lipase (CRL) and Protease (Subtilisin Carlsberg) were from Sigma and Acylase I (on Eupergit C) was from Fluka. Enantiomeric excesses of the amines **3a**–**e** as the corresponding acet-, propan- or butanamides (amines derivatized with the corresponding anhydride) were determined with a HP1090 gas chromatograph equipped with a Varian CP Chirasil-Dex CP chiral column. For retention times and temperature programs, see Table 5. NMR spectra were recorded with Bruker Avance 500 MHz or 600 MHz spectrometers. ^{1}H NMR spectra were analyzed by PERCH software with spin simulation/iteration techniques.[23] HRMS were measured in ESI$^+$ mode with Bruker micrOTOF-Q quadrupole-TOF or Fisons ZABSpec-oaTOF spectrometers. Melting points were recorded with a Gallenkamp apparatus and are uncorrected. Optical rotations were determined with a PerkinElmer 241 or 341 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Enzymatic reactions were performed at room temperature (23–24 °C) unless indicated otherwise. The determination of *E* was based on equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$ with $c = ee_S/(ee_S + ee_P)$ using linear regression (*E* as the slope of the line $\ln[(1-c)(1-ee_S)]$ vs. $\ln[(1-c)(1+ee_S)]$).[24] Flash chromatography was performed using silica gel (60 Å, Merck, 230–400 mesh, enriched with 0.1% Ca).

Table 5. Gas chromatographic analysis of the resolved compounds.

Amine	Amide derivative	Oven temp. [°C]	$t_r[(S)/(R)]$ [min]
3a[a]	acetamide	130	37.7/39.3
3a[a]	acetamide	130	43.1/45.5
3a	butanamide	130	85.3/87.3
3a	methoxyacetamide	130	57.0/58.5
3a	acrylamide	160, then 130	27.2/28.3
3a	3-butenamide	160, then 130	36.4/37.2
3b	acetamide	130	53.0/54.4
3c	acetamide	130	147.1/147.4
3d	acetamide	130	54.1/56.3
3d	methoxyacetamide	130	84.6/89.7

[a] The acetamides were analyzed with two columns, the retention times depending on the column specifics.

Synthesis of *N*,*N*-(Dimethylsulfamoyl)benzaldimines 1a–c: Compounds **1a**–**c** were synthesized according to a literature procedure.[25] The corresponding benzaldehyde (20 mmol) and *N,N*-dimethylsulfamide (2.54 g, 20.5 mmol) were dissolved in toluene (80 mL) and water was azeotropically distilled for 16 h using a Dean–Stark apparatus. After removal of the solvent under reduced pressure, the residue was dissolved in CH_2Cl_2 and filtered. Solvents were evaporated under reduced pressure and the crude product was used as such in the allylation step.

Standard Procedure for the Synthesis of Homoallylic Amines (Table 1): In a Schlenk tube flushed with argon, substrate **1a**–**c** was dissolved in anhydrous THF (0.10–0.18 M solution). Indium (3 equiv.) and allylating agent (3 equiv.) were added and the resulting mixture was stirred overnight at room temperature. The reaction was quenched by adding 1 M HCl until pH ≈ 3 and extracted with diethyl ether. The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, and dried with anhydrous Na_2SO_4. After evaporation of ether, the crude product was subjected to analysis and purified by flash chromatography (eluent: hexane/EtOAc, 4:1) affording **2a**–**e** as a white solid. Compound **2a**–**e** was then dissolved in 1,3-diaminopropane (24-fold molar excess), the mixture was heated to 140 °C and refluxed for 2 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2 and washed with water. The organic phase was dried with anhydrous Na_2SO_4 and concentrated to yield **3a**–**e**.

1-Phenylbut-3-en-1-amine (3a): Step 1: starting from **1a** (1.95 g, 9.18 mmol) to yield **2a** (1.67 g, 6.56 mmol, 71%). Step 2: starting from **2a** (2.14 g, 8.41 mmol) to yield **3a** (1.13 g, 7.68 mmol, 91%) as light yellow oil. ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.38–7.33 (m, 4 H, arom. H), 7.28–7.23 (m, 1 H, arom. H), 5.75 (dddd, $J_{CH=,CH2a}$ = 6.3, $J_{CH=,CH2b}$ = 8.0, $J_{CH=,CH2cis}$ = 10.2, $J_{CH=,CH2trans}$ = 17.1 Hz, 1 H, CH_2-C*H*=CH_2), 5.12 (dddd, $J_{CH2trans,CH2b}$ = –1.2, $J_{CH2trans,CH2a}$ = –1.6, $J_{CH2trans,CH2cis}$ = –2.0, $J_{CH2trans,CH}$ = 17.1 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.08 (dddd, $J_{CH2cis,CH2b}$ = –0.8, $J_{CH2cis,CH2a}$ = –1.2, $J_{CH2cis,CH2trans}$ = –2.0, $J_{CHcis,CH}$ = 10.2 Hz, 1 H, CH_2-CH=CH_{2cis}), 3.99 (dd, $J_{CH,CH2a}$ = 5.2, $J_{CH,CH2b}$ = 8.2 Hz, 1 H, C*H*NH_2), 2.46 (ddddd, $J_{CH2a,CH2cis}$ = –1.2, $J_{CH2a,CH2trans}$ = –1.6, $J_{CH2a,CH}$ = 5.2, $J_{CH2a,CH}$ = 6.2, $J_{CH2a,CH2b}$ = –13.8 Hz, 1 H, CH_{2a}-CH=CH_2), 2.36 (ddddd, $J_{CH2b,CH2cis}$ = –0.8, $J_{CH2b,CH2trans}$ = –1.2, $J_{CH2b,CH}$ = 8.0, $J_{CH2b,CH}$ = 8.2, $J_{CH2b,CH2a}$ = –13.8 Hz, 1 H, CH_{2b}-CH=CH_2), 1.53 (br. s., 2 H, NH_2) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 145.9 (arom. C), 135.5 (*C*H=CH_2), 128.4 (2 arom. C), 126.9 (arom. C), 126.4 (2 arom. C), 117.6 (CH=*C*H_2), 55.4 (*C*HNH_2), 44.1 (*C*H_2-

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CH=CH_2) ppm. HRMS: calcd. for $C_{10}H_{13}N$ $[M]^+$ 147.1071; found 147.1048.

1-(4-Fluorophenyl)but-3-en-1-amine (3b): Step 1: starting from **1b** (1.59 g, 6.91 mmol) to yield **2b** (1.28 g, 4.69 mmol, 68%). Step 2: starting from **2b** (1.28 g, 4.69 mmol) to yield **3b** (0.53 g, 3.21 mmol, 68%) as yellow oil. ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.33–7.29 (m, 2 H, arom. H), 7.04–6.99 (m, 2 H, arom. H), 5.73 (dddd, $J_{CH=,CH2a}$ = 6.2, $J_{CH=,CH2b}$ = 8.0, $J_{CH=,CH2cis}$ = 10.1, $J_{CH=,CH2trans}$ = 17.1 Hz, 1 H, CH_2-C*H*=CH_2), 5.11 (dddd, $J_{CH2trans,CH2b}$ = –1.2, $J_{CH2trans,CH2a}$ = –1.6, $J_{CH2trans,CH2cis}$ = –2.0, $J_{CH2trans,CH}$ = 17.1 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.08 (dddd, $J_{CH2cis,CH2b}$ = –0.9, $J_{CH2cis,CH2a}$ = –1.2, $J_{CH2cis,CH2trans}$ = –2.0, $J_{CHcis,CH2}$ = 10.1 Hz, 1 H, CH_2-CH=CH_{2cis}), 3.99 (dd, $J_{CH,CH2a}$ = 5.3, $J_{CH,CH2b}$ = 8.1 Hz, 1 H, C*H*NH_2), 2.42 (ddddd, $J_{CH2a,CH2cis}$ = –1.2, $J_{CH2a,CH2trans}$ = –1.6, $J_{CH2a,CH}$ = 5.3, $J_{CH2a,CH}$ = 6.2, $J_{CH2a,CH2b}$ = –13.8 Hz, 1 H, CH_{2a}-CH=CH_2), 2.33 (ddddd, $J_{CH2b,CH2cis}$ = –0.9, $J_{CH2b,CH2trans}$ = –1.2, $J_{CH2b,CH}$ = 8.0, $J_{CH2b,CH}$ = 8.1, $J_{CH2b,CH2a}$ = –13.8 Hz, 1 H, CH_{2b}-CH=CH_2), 1.47 (br. s, 2 H NH_2) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 161.8 (d, $^1J_{C,F}$ = 244.7 Hz, arom. *C*-F), 141.5 (d, $^4J_{C,F}$ = 3.3 Hz, arom. C), 135.2 (*C*H=CH_2), 127.8 (d, $^3J_{C,F}$ = 7.7 Hz, 2 arom. C), 117.8 (CH=*C*H_2), 115.1 (d, $^2J_{C,F}$ = 20.9 Hz, 2 arom. C), 54.7 (*C*HNH_2), 44.3 (*C*H_2-CH=CH_2) ppm. HRMS: calcd. for $C_{10}H_{12}NF$ $[M]^+$ 165.0954; found 165.0929.

1-(4-Methoxyphenyl)but-3-en-1-amine (3c): Step 1: starting from **1c** (0.93 g, 4.42 mmol) to yield **2c** (0.85 g, 3.00 mmol, 68%). Step 2: starting from **2c** (0.85 g, 3.00 mmol) to yield **3c** (0.48 g, 2.70 mmol, 90%) as colorless oil. ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.28–7.24 (m, 2 H, arom. H), 6.89–6.85 (m, 2 H, arom. H), 5.75 (dddd, $J_{CH=,CH2a}$ = 6.2, $J_{CH=,CH2b}$ = 8.0, $J_{CH=,CH2cis}$ = 10.2, $J_{CH=,CH2trans}$ = 17.1 Hz, 1 H, CH_2-C*H*=CH_2), 5.11 (dddd, $J_{CH2trans,CH2b}$ = –1.3, $J_{CH2trans,CH2a}$ = –1.6, $J_{CH2trans,CH2cis}$ = –2.1, $J_{CH2trans,CH}$ = 17.1 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.07 (dddd, $J_{CH2cis,CH2b}$ = –0.9, $J_{CH2cis,CH2a}$ = –1.1, $J_{CH2cis,CH2trans}$ = –2.1, $J_{CH2cis,CH2}$ = 10.2 Hz, 1 H, CH_2-CH=CH_{2cis}), 3.95 (dd, $J_{CH,CH2a}$ = 5.3, $J_{CH,CH2b}$ = 8.1 Hz, 1 H, C*H*NH_2), 3.80 (s, 3 H, OCH_3), 2.43 (ddddd, $J_{CH2a,CH2cis}$ = –1.1, $J_{CH2a,CH2trans}$ = –1.6, $J_{CH2a,CH}$ = 5.3, $J_{CH2a,CH}$ = 6.2, $J_{CH2a,CH2b}$ = –13.8 Hz, 1 H, CH_{2a}-CH=CH_2), 2.34 (ddddd, $J_{CH2b,CH2cis}$ = –0.9, $J_{CH2b,CH2trans}$ = –1.2, $J_{CH2b,CH}$ = 8.0, $J_{CH2b,CH}$ = 8.1, $J_{CH2b,CH2a}$ = –13.8 Hz, 1 H, CH_{2b}-CH=CH_2), 1.46 (br. s, 2 H NH_2) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 158.5 (arom. C), 138.0 (arom. C), 135.6 (*C*H=CH_2), 127.3 (2 arom. C), 117.5 (CH=*C*H_2), 113.7 (2 arom. C), 55.3 (O*C*H_3), 54.8 (*C*HNH_2), 44.3 (*C*H_2-CH=CH_2) ppm. HRMS: calcd. for $C_{11}H_{15}NO$ $[M]^+$ 177.1154; found 177.1112.

2-Methyl-1-phenylbut-3-en-1-amine (3d): Step 1: starting from **1a** (2.42 g, 11.4 mmol) to yield **2d** (1.92 g, 7.15 mmol, 65%). Step 2: starting from **2d** (1.60 g, 5.86 mmol) to yield **3d** (0.72 g, 4.44 mmol, 76%) as light yellow oil. ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.38–7.35 (m, 2 H, arom. H), 7.35–7.31 (m, 2 H, arom. H), 7.26–7.22 (m, 1 H, arom. H), 4.85 [ddq, $J_{CHtrans,CHa}$ = –0.5, $J_{CH2trans,CH3}$ = 1.5, $J_{CHtrans,CHcis}$ = –2.2 Hz, 1 H, CH_2-C(CH_3)=CH_{2trans}], 4.80 [dddq, $J_{CH2cis,CH3}$ = 0.9, $J_{CHcis,CHb}$ = –0.9, $J_{CHcis,CHa}$ = –1.4, $J_{CHcis,CHtrans}$ = –2.2 Hz, 1 H, CH_2-C(CH_3)=CH_{2cis}], 4.10 (dd, $J_{CH,CH2a}$ = 4.5, $J_{CH,CH2b}$ = 9.5 Hz, 1 H, C*H*NH_2), 2.36 (dddd, $J_{CH2a,CH2trans}$ = –0.5, $J_{CH2a,CH2cis}$ = –1.4, $J_{H2a,CH}$ = 4.5, $J_{CH2a,CH2b}$ = –13.6 Hz, 1 H, CH_{2a}-CH=CH_2), 2.31 (ddd, $J_{CH2b,CH2cis}$ = –0.9, $J_{H2b,CH}$ = 9.5, $J_{CH2a,CH2b}$ = –13.7 Hz, 1 H, CH_{2b}-CH=CH_2), 1.76 (dd, $J_{CH3,CH2cis}$ = 0.9, $J_{CH3,CH2trans}$ = 1.5 Hz, 3 H, CH_3), 1.48 (br. s, 2 H NH_2) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 146.2 (arom. C), 142.9 [*C*(CH_3)=CH_2], 128.4 (2 arom. C), 126.9 (arom. C), 126.3 (2 arom. C), 113.3 [CH(CH_3)=*C*H_2], 53.4 (*C*HNH_2), 48.6 [*C*H_2-C(CH_3)=CH_2], 22.2 (*C*H_3) ppm. HRMS: calcd. for $C_{11}H_{15}N$ $[M]^+$ 161.1205; found 161.1223.

2,2-Dimethyl-1-phenylbut-3-en-1-amine (3e): Step 1: starting from **1a** (2.12 g, 9.99 mmol) to yield **2e** (0.76 g, 2.69 mmol, 27%) after repeated purification by column chromatography. Step 2: starting from **2e** (1.25 g, 4.42 mmol) to yield **3e** (0.64 g, 3.67 mmol, 83%) as yellow oil. ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.30–7.27 (m, 4 H, arom. H), 7.25–7.22 (m, 1 H arom. H), 5.87 (dd, $J_{CH=,CH2cis}$ = 10.8, $J_{CH=,CH2trans}$ = 17.5 Hz, 1 H, C*H*=CH_2), 5.09 (dd, $J_{CH2cis,CH2trans}$ = –1.4, $J_{CHcis,CH=}$ = 10.8 Hz, 1 H, CH=CH_{2cis}), 5.03 (dd, $J_{CH2trans,CH2cis}$ = –1.4, $J_{CH2trans,CH}$ = 17.5 Hz, 1 H, CH=CH_{2trans}), 3.75 (s, 1 H, C*H*NH_2), 1.43 (br. s, 2 H, NH_2), 0.99 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 145.8 (*C*H=CH_2), 142.9 (arom. C), 128.5 (2 arom. C), 127.5 (2 arom. C), 126.9 (arom. C), 113.0 (CH=*C*H_2), 64.1 (*C*HNH_2), 41.5 [*C*(CH_3)$_2$], 25.5 (*C*H_3), 21.7 (*C*H_3) ppm. HRMS: calcd. for $C_{12}H_{17}N$ $[M]^+$ 175.1361; found 175.1311.

Standard Procedure for the Small-Scale Enzymatic Kinetic Resolution: Lipase PS-D and molecular sieves (4 Å) were weighed in a reaction vial before *rac*-**3a** (0.1 M, 1.0 mL) in dry isopropyl acetate (or an acyl donor in an organic solvent) was added. Reactions proceeded under shaking at 170 rpm. The progress was followed by taking samples (100 µL) at intervals, filtering off the enzyme and analyzing the samples (diluted with hexane after derivatization of the amine with an appropriate acid anhydride) by GC.

Procedures for the Preparative-Scale Enzymatic Kinetic Resolutions

1-Phenylbut-3-en-1-amine (3a) with Ethyl Methoxyacetate: Lipase PS-D (0.93 g, 50 mg mL^{-1}) and molecular sieves (4 Å, 0.93 g) were weighed in the reaction vessel before *rac*-**3a** (273 mg, 1.85 mmol) in toluene (17 mL) containing ethyl methoxyacetate [6% (v/v)] was added. After 4 d at +48 °C the reaction was stopped at 48% conversion. The enzyme was filtered, washed with CH_2Cl_2 and the combined organic phases were concentrated in vacuo. The crude product was purified by silica gel chromatography (5% Et_3N in EtOAc) to yield (*S*)-**3a** as a pale yellow oil [82 mg, 0.56 mmol, 58%, *ee* = 87%, $[\alpha]_D^{25}$ = –30 (*c* = 1.0, $CHCl_3$)] and (*R*)-**4a** as a white solid [162 mg, 0.74 mmol, 83%, *ee* = 94%, $[\alpha]_D^{25}$ = +43 (*c* = 1.0, $CHCl_3$), m.p. 60 ± 1 °C]. (*R*)-**4a**: ^{1}H NMR ($CDCl_3$, 600.13 MHz, 25 °C): δ = 7.36–7.23 (m, 5 H, arom. H), 6.85 (d, $J_{NH,CH}$ = 8.6 Hz, 1 H, NH), 5.70 (dddd, $J_{CH=,CH2a}$ = 7.0, $J_{CH=,CH2b}$ = 7.0, $J_{CH=,CH2cis}$ = 10.2, $J_{CH=,CH2trans}$ = 17.1 Hz, 1 H, CH_2-C*H*=CH_2), 5.13 (ddd, $J_{CH,CH2a}$ = 5.8, $J_{CH,CH2b}$ = 8.0, $J_{CH,NH}$ = 8.6 Hz, 1 H, C*H*NH), 5.12 (dddd, $J_{CH2trans,CHa}$ = –1.4, $J_{CH2trans,CH2b}$ = –1.5, $J_{CH2trans,CH2cis}$ = –1.9, $J_{CH2trans,CH}$ = 17.1 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.08 (dddd, $J_{CH2cis,CHa}$ = –1.0, $J_{CH2cis,CH2b}$ = –1.1, $J_{CH2cis,CH2trans}$ = –1.9, $J_{CH2cis,CH}$ = 10.2 Hz, 1 H, CH_2-CH=CH_{2cis}), 3.91 (d, $J_{CH2c,CH2d}$ = –15.1 Hz, 1 H, CH_{2c}OCH_3), 3.88 (d, $J_{CH2d,CH2c}$ = –15.1 Hz, 1 H, CH_{2d}OCH_3), 3.41 (s, 3 H, CH_2OCH_3), 2.59 (ddddd, $J_{CH2a,CH2cis}$ = –1.0, $J_{CH2a,CH2trans}$ = –1.4, $J_{CH2a,CH}$ = 5.8, $J_{CH2a,CH}$ = 7.0, $J_{CH2a,CH2b}$ = –14.0 Hz, 1 H, CH_{2a}-CH=CH_2), 2.59 (ddddd, $J_{CH2b,CH2cis}$ = –1.1, $J_{CH2b,CH2trans}$ = –1.5, $J_{CH2b,CH}$ = 7.0, $J_{CH2b,CH}$ = 8.0,, $J_{CH2b,CH2a}$ = –14.0 Hz, 1 H, CH_{2b}-CH=CH_2) ppm. ^{13}C NMR ($CDCl_3$,150.9 MHz, 25 °C): δ = 168.8 (*C*O), 141.4 (arom. C), 133.8 (*C*H=CH_2), 128.6 (2 arom. C), 127.4 (arom. C), 126.5 (2 arom. C), 118.3 (CH=*C*H_2), 72.0 (*C*H_2OCH_3), 59.2 (CH_2O*C*H_3), 51.8 (*C*HNH), 40.6 (*C*H_2-CH=CH_2) ppm. HRMS: calcd. for $C_{13}H_{17}NO_2Na$ $[M + Na]^+$ 242.1152; found 242.1171.

1-Phenylbut-3-en-1-amine (3a) with Isopropyl Acetate: Lipase PS-D (1.00 g, 50 mg mL^{-1}) and molecular sieves (4 Å, 1.00 g) were weighed in the reaction vessel before *rac*-**3a** (300 mg, 2.04 mmol) in neat isopropyl acetate (20 mL) was added. After 3 d the reaction was stopped at 46% conversion. The crude product was purified

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by silica gel chromatography (EtOAc) to yield (*S*)-**3a** as a colorless oil (108 mg, 0.73 mmol, 66%, *ee* = 84%) and (*R*)-**5a** as white solid [163 mg, 0.86 mmol, 92%, *ee* = 99%, $[\alpha]_D^{25}$ = +115 (*c* = 1.0, $CHCl_3$), m.p. 74 ± 1 °C]. A fraction of the enantiomerically enriched (*S*)-**3a** (86 mg, 0.59 mmol) was purified under kinetic resolution conditions, yielding (*S*)-**3a** as a colorless oil [38 mg, 0.26 mmol, 44%, *ee* = 99%, $[\alpha]_D^{25}$ = –51 (*c* = 1.0, $CHCl_3$), $[\alpha]_D^{25}$ (lit.) = –50.0 (*c* = 1.13, $CHCl_3$, *ee* = 96%)[7c]]. (*R*)-**5a**: ^1H NMR ($CDCl_3$, 500.13 MHz, 25 °C): δ = 7.39–7.23 (m, 5 H, arom. H), 5.99 (d, $J_{NH,CH}$ = 8.2 Hz, 1 H, NH), 5.68 (dddd, $J_{CH=,CH2b}$ = 6.7, $J_{CH=,CH2a}$ = 7.3, $J_{CH=,CH2cis}$ = 10.1, $J_{CH=,CH2trans}$ = 17.2 Hz, 1 H, CH_2-C*H*=CH_2), 5.10 (dddd, $J_{CH2trans,CHa}$ = –1.4, $J_{CH2trans,CH2b}$ = –1.4, $J_{CH2trans,CH2cis}$ = –1.9, $J_{CH2trans,CH=}$ = 17.2 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.07 (dddd, $J_{CH2cis,CHa}$ = –1.1, $J_{CH2cis,CH2b}$ = –1.1, $J_{CH2cis,CH2trans}$ = –1.9, $J_{CH2cis,CH}$ = 10.1 Hz, 1 H, CH_2-CH=CH_{2cis}), 5.07 (ddd, $J_{CH,CH2b}$ = 5.9, $J_{CH,CH2a}$ = 7.9, $J_{CH,NH}$ = 8.2 Hz, 1 H, C*H*NH), 2.57 (ddddd, $J_{CH2a,CH2cis}$ = –1.1, $J_{CH2a,CH2trans}$ = –1.4, $J_{CH2a,CH}$ = 7.3, $J_{CH2a,CH}$ = 7.9, $J_{CH2a,CH2b}$ = –13.8 Hz, 1 H, CH_{2a}-CH=CH_2), 2.56 (ddddd, $J_{CH2b,CH2cis}$ = –1.1, $J_{CH2b,CH2trans}$ = –1.4, $J_{CH2b,CH}$ = 5.9, $J_{CH2b,CH}$ = 6.7, $J_{CH2b,CH2a}$ = –13.8 Hz, 1 H, CH_{2b}-CH=CH_2), 1.99 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 125.8 MHz, 25 °C): δ = 169.3 (*C*O), 141.6 (arom. C), 134.0 (*C*H=CH_2), 128.6 (2 arom. C), 127.4 (arom. C), 126.5 (2 arom. C), 125.9 (C_{arom}), 118.2 (CH=*C*H_2), 52.5 (*C*HNH), 40.5 (*C*H_2-CH=CH_2), 23.4 (CO*C*H_3) ppm. HRMS:: calcd. for $C_{12}H_{15}NONa$ $[M + Na]^+$ 212.1046; found 212.1039.

1-Phenylbut-3-en-1-amine (3a) with Ethyl Acrylate: *rac*-**3a** (296 mg, 2.01 mmol) in 1:9 (v/v) mixture of ethyl acrylate in toluene was added on lipase PS-D (1.00 g, 50 $mg\,mL^{-1}$) and molecular sieves (4 Å, 1.00 g). After 5 d the reaction was stopped at 19% conversion. The crude product was dissolved in H_2O (5 mL) and the solution was acidified with 2 M HCl (5 mL) and extracted with EtOAc (3 × 10 mL). The organic phases were combined, dried with Na_2SO_4, concentrated and purified by silica pad filtration (EtOAc) to yield (*R*)-**6** as a white solid [50 mg, 0.25 mmol, 65%, *ee* = 95%, $[\alpha]_D^{25}$ = +133 (*c* = 1.0, $CHCl_3$), m.p. 83 ± 1 °C]. ^1H NMR ($CDCl_3$, 600.13 MHz, 25 °C): δ = 7.36–7.24 (m, 5 H, arom. H), 6.28 (dd, $J_{CH2trans',CH2cis'}$ = –1.5, $J_{CH2trans',CH='}$ = 17.0 Hz, 1 H, CO-CH=CH_{2trans}), 6.11 (dd, $J_{CH=',CH2cis'}$ = 10.4, $J_{CH=',CH2trans'}$ = 17.0 Hz, 1 H, CO-C*H*=CH_2), 5.87 (d, $J_{NH,CH}$ = 8.1 Hz, 1 H, NH), 5.70 (dddd, $J_{CH=,CH2b}$ = 6.9, $J_{CH=,CH2a}$ = 7.1, $J_{CH=,CH2cis}$ = 10.2, $J_{CH=,CH2trans}$ = 17.1 Hz, 1 H, CH_2-C*H*=CH_2), 5.64 (dd, $J_{CH2cis',CH2trans'}$ = –1.5, $J_{CH2cis',CH}$ = 10.4 Hz, 1 H, CO-CH=CH_{2cis}), 5.17 (dd, $J_{CH,CH2b}$ = 6.4, $J_{CH,CH2a}$ = 7.3 Hz, 1 H, C*H*NH), 5.12 (dddd, $J_{CH2trans,CH2a}$ = –1.4, $J_{CH2trans,CH2b}$ = –1.5, $J_{CH2trans,CH2cis}$ = –1.9, $J_{CH2trans,CH}$ = 17.1 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.09 (dddd, $J_{CH2cis,CH2a}$ = –1.0, $J_{CH2cis,CH2b}$ = –1.2, $J_{CH2cis,CH2trans}$ = –1.9, $J_{CH2cis,CH}$ = 10.2 Hz, 1 H, CH_2-CH=CH_{2cis}), 2.62 (ddddd, $J_{CH2a,CH2cis}$ = –1.0, $J_{CH2a,CH2trans}$ = –1.4, $J_{CH2a,CH}$ = 7.1, $J_{CH2a,CH}$ = 7.3, $J_{CH2a,CH2b}$ = –13.7 Hz, 1 H, CH_{2a}-CH=CH_2), 2.61 (ddddd, $J_{CH2b,CH2cis}$ = –1.2, $J_{CH2b,CH2trans}$ = –1.5, $J_{CH2b,CH}$ = 6.4, $J_{CH2b,CH}$ = 6.9, $J_{CH2b,CH2a}$ = –13.7 Hz, 1 H, CH_{2b}-CH=CH_2) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz, 25 °C): δ = 164.7 (*C*O), 141.4 (arom. C), 133.9 (*C*H=CH_2), 130.7 (CO-*C*H=CH_2), 128.7 (2 arom. C), 127.4 (arom. C), 126.8 (CO-CH=*C*H_2), 126.5 (2 arom. C), 118.3 (CH=*C*H_2), 52.5 (*C*HNH), 40.4 (*C*H_2-CH=CH_2) ppm. HRMS: calcd. for $C_{13}H_{15}NONa$ $[M + Na]^+$ 224.1046; found 224.1144.

1-Phenylbut-3-en-1-amine (3a) with Isopropyl 3-Butenoate: *rac*-**3a** (693 mg, 4.71 mmol) in the 1:9 (v/v) mixture of isopropyl 3-butenoate in toluene was added on lipase PS-D (2.36 g, 50 $mg\,mL^{-1}$) and molecular sieves (4 Å, 2.40 g). After 5 d the reaction was stopped at 47% conversion. The crude product was dissolved in H_2O (10 mL) and the solution was acidified with 4 M HCl (4 mL) and extracted with EtOAc (3 × 20 mL). Purification as above yielded (*R*)-**7** as a white solid [504 mg, 1.88 mmol, 85%, *ee* > 99%, $[\alpha]_D^{25}$ = +63 (*c* = 1.0, $CHCl_3$), m.p. 55 ± 1 °C]. ^1H NMR ($CDCl_3$, 500.13 MHz, 25 °C): δ = 7.35–7.31 (m, 2 H, arom. H), 7.27–7.23 (m, 3 H, arom. H), 5.94 (dddd, $J_{CH=',CH2a'}$ = 7.1, $J_{CH=',CH2b'}$ = 7.2, $J_{CH=',CH2cis'}$ = 10.1, $J_{CH=',CH2trans'}$ = 17.1 Hz, 1 H, CO-CH_2'-CH'=CH_2'), 5.92 (d, $J_{NH,CH}$ = 8.2 Hz, 1 H, NH), 5.67 ($J_{CH=,CH2a}$ = 6.9, $J_{CH=,CH2b}$ = 7.3, $J_{CH=,CH2cis}$ = 10.1, $J_{CH=,CH2trans}$ = 17.1 Hz, 1 H, CH_2-C*H*=CH_2), 5.25 (dddd, $J_{CH2cis',CH2b'}$ = –1.0, $J_{CH2cis',CH2a'}$ = –1.1, $J_{CH2cis',CH2trans'}$ = –1.6, $J_{CH2cis',CH='}$ = 10.1 Hz, 1 H, CO-CH_2'-CH'=CH_{2cis}'), 5.23 (dddd, $J_{CH2trans',CH2b'}$ = –1.4, $J_{CH2trans',CH2a'}$ = –1.4, $J_{CH2trans',CH2cis'}$ = –1.6, $J_{CH2trans',CH='}$ = 17.1 Hz, 1 H, CO-CH_2'-CH'=CH_{2trans}'), 5.10 (dddd, $J_{CH2trans,CH2b}$ = –1.4, $J_{CH2trans,CH2a}$ = –1.4, $J_{CH2trans,CH2cis}$ = –1.9, $J_{CH2trans,CH}$ = 17.1 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.09 (ddd, $J_{CH,CH2a}$ = 5.9, $J_{CH,CH2b}$ = 7.6, $J_{CH,NH}$ = 8.2 Hz, 1 H, C*H*NH), 5.08 (dddd, $J_{CH2cis,CH2b}$ = –0.9, $J_{CH2cis,CH2a}$ = –1.2, $J_{CH2cis,CH2trans}$ = –1.9, $J_{CH2cis',CH='}$ = 10.1 Hz, 1 H, CH_2-CH=CH_{2cis}), 3.03 (dddd, $J_{CH2a',CH2cis'}$ = –1.1, $J_{CH2a',CH2trans'}$ = –1.4, $J_{CH2a',CH='}$ = 7.1, $J_{CH2a',CH2b'}$ = –14.1 Hz, 1 H, CO-CH_{2a}'-CH'=CH_2'), 3.02 (dddd, $J_{CH2b',CH2cis'}$ = –1.0, $J_{CH2b',CH2trans'}$ = –1.4, $J_{CH2b',CH='}$ = 7.2, $J_{CH2b,CH2a'}$ = –14.1 Hz, 1 H, CO-CH_{2b}'-CH'=CH_2'), 2.56 (ddddd, $J_{CH2a,CH2cis}$ = –1.2, $J_{CH2a,CH2trans}$ = –1.4, $J_{CH2a,CH}$ = 5.9, $J_{CH2a,CH}$ = 6.9, $J_{CH2a,CH2b}$ = –14.2 Hz, 1 H, CH_{2a}-CH=CH_2), 2.55 (ddddd, $J_{CH2b,CH2cis}$ = –0.9, $J_{CH2b,CH2trans}$ = –1.4, $J_{CH2b,CH}$ = 7.3, $J_{CH2b,CH}$ = 7.6, $J_{CH2b,CH2a}$ = –14.2 Hz, 1 H, CH_{2b}-CH=CH_2) ppm. ^{13}C NMR ($CDCl_3$, 125.8 MHz, 25 °C): δ = 169.6 (CO), 141.5 (arom. C), 133.9 (*C*H=CH_2), 131.3 (C'H=$C'H_2$), 128.6 (2 arom. C), 127.4 (arom. C), 126.4 (2 arom. C), 120.0 (C'H=*C*$'H_2$), 118.3 (CH=CH_2), 52.2 (*C*HNH), 41.7 ($C'H_2$-C'H=$C'H_2$), 40.5 (CH_2-CH=CH_2) ppm. HRMS: calcd. for $C_{14}H_{17}NONa$ $[M + Na]^+$ 238.1202; found 238.1187.

1-(4-Fluorophenyl)but-3-en-1-amine (3b) with Isopropyl Acetate: *rac*-**3b** (429 mg, 2.60 mmol) was resolved as above. After 4 d the reaction was stopped at 33% conversion. The work-up gave (*S*)-**3b** as a pale yellow oil (216 mg, 1.31 mmol, 75%, *ee* = 48%). The enzymatic purification gave (*S*)-**3b** as a pale yellow oil [71 mg, 0.43 mmol, 33%, *ee* = 95%, $[\alpha]_D^{25}$ = –35 (*c* = 1.0, $CHCl_3$)]. (*R*)-**5b** was a white solid [133 mg, 0.64 mmol, 75%, *ee* > 99%, $[\alpha]_D^{25}$ = +109.0 (*c* = 1.0, $CHCl_3$), m.p. 120 ± 1 °C]. (*R*)-**5b**: ^1H NMR ($CDCl_3$, 500.13 MHz, 25 °C): δ = 7.26–7.22 (m, 2 H, arom. H), 7.04–6.99 (m, 2 H, arom. H), 5.75 (d, $J_{NH,CH}$ = 8.0 Hz, 1 H, NH), 5.66 (dddd, $J_{CH=,CH2b}$ = 6.8, $J_{CH=,CH2a}$ = 7.2, $J_{CH=,CH2cis}$ = 10.1, $J_{CH=,CH2trans}$ = 17.2 Hz, 1 H, CH_2-C*H*=CH_2), 5.11 (dddd, $J_{CH2trans,CHa}$ = –1.4, $J_{CH2trans,CH2b}$ = –1.5, $J_{CH2trans,CH2cis}$ = –1.9, $J_{CH2trans,CH=}$ = 17.2 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.09 (dddd, $J_{CH2cis,CHa}$ = –1.1, $J_{CH2cis,CH2b}$ = –1.1, $J_{CH2cis,CH2trans}$ = –1.9, $J_{CH2cis,CH=}$ = 10.1 Hz, 1 H, CH_2-CH=CH_{2cis}), 5.05 (ddd, $J_{CH,CH2b}$ = 6.6, $J_{CH,CH2a}$ = 7.2, $J_{CH,NH}$ = 8.0 Hz, 1 H, C*H*NH), 2.54 (ddddd, $J_{CH2a,CH2cis}$ = –1.1, $J_{CH2a,CH2trans}$ = –1.4, $J_{CH2a,CH}$ = 7.2, $J_{CH2a,CH}$ = 7.2, $J_{CH2a,CH2b}$ = –13.9 Hz, 1 H, CH_{2a}-CH=CH_2), 2.54 (ddddd, $J_{CH2b,CH2cis}$ = –1.1, $J_{CH2b,CH2trans}$ = –1.5, $J_{CH2b,CH}$ = 6.6, $J_{CH2b,CH}$ = 6.8, $J_{CH2b,CH2a}$ = –13.9 Hz, 1 H, CH_{2b}-CH=CH_2), 1.99 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 125.8 MHz, 25 °C): δ = 169.3 (*C*O), 162.0 (d, $^1J_{C,F}$ = 245.8 Hz, arom. C), 137.5 (d, $^4J_{C,F}$ = 2.8 Hz, arom. C), 133.7 (*C*H=CH_2), 128.0 (d, $^3J_{C,F}$ = 8.3 Hz, 2 arom. C), 118.5 (CH=*C*H_2), 115.4 (d, $^2J_{C,F}$ = 21.1 Hz, 2 arom. C), 51.9 (*C*HNH), 40.5 (*C*H_2-CH=CH_2), 23.4 (CO*C*H_3) ppm. HRMS: calcd. for $C_{12}H_{14}FNONa$ $[M + Na]^+$ 230.0952; found 230.0949.

1-(4-Methoxyphenyl)but-3-en-1-amine (3c) with Isopropyl Acetate: *rac*-**3c** (227 mg, 1.28 mmol) was resolved as above. After 4 d the reaction was stopped at 49% conversion. The work-up gave (*S*)-**3c**

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as a colorless oil [0.108 mg, 0.61 mmol, 93%, *ee* = 94%, $[\alpha]_D^{25}$ = –34 (*c* = 1.0, $CHCl_3$), $[\alpha]_D^{25}$ (lit.) = –42.3 (*c* = 0.81, $CHCl_3$, *ee* = 97%)[7c]] and (*R*)-**5c** as a white solid [91 mg, 0.41 mmol, 65%, *ee* >99%, $[\alpha]_D^{25}$ = +110 (*c* = 1.0, $CHCl_3$), m.p. 122 ± 1 °C]. (*R*)-**5c**: ^{1}H NMR ($CDCl_3$, 500.13 MHz, 25 °C): δ = 7.22–7.18 (m, 2 H, arom. H), 6.88–6.84 (m, 2 H, arom. H), 5.84 (d, $J_{NH,CH}$ = 8.1 Hz, 1 H, NH), 5.68 (dddd, $J_{CH=,CH2b}$ = 6.9, $J_{CH=,CH2a}$ = 7.1, $J_{CH=,CH2cis}$ = 10.2, $J_{CH=,CH2trans}$ = 17.2 Hz, 1 H, CH_2-C*H*=CH_2), 5.09 (dddd, $J_{CH2trans,CHa}$ = –1.4, $J_{CH2trans,CH2b}$ = –1.5, $J_{CH2trans,CH2cis}$ = –1.9, $J_{CH2trans,CH}$ = 17.2 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.06 (dddd, $J_{CH2cis,CHa}$ = –1.1, $J_{CH2cis,CH2b}$ = –1.1, $J_{CH2cis,CH2trans}$ = –1.9, $J_{CH2cis,CH}$ = 10.2 Hz, 1 H, CH_2-CH=CH_{2cis}), 5.03 (ddd, $J_{CH,CH2b}$ = 6.7, $J_{CH,CH2a}$ = 7.3, $J_{CH,NH}$ = 8.1 Hz, 1 H, C*H*NH), 2.56 (ddddd, $J_{CH2a,CH2cis}$ = –1.1, $J_{CH2a,CH2trans}$ = –1.4, $J_{CH2a,CH}$ = 7.1, $J_{CH2a,CH}$ = 7.3, $J_{CH2a,CH2b}$ = –14.2 Hz, 1 H, CH_{2a}-CH=CH_2), 2.53 (ddddd, $J_{CH2b,CH2cis}$ = –1.1, $J_{CH2b,CH2trans}$ = –1.5, $J_{CH2b,CH}$ = 6.7, $J_{CH2b,CH}$ = 6.9, $J_{CH2b,CH2a}$ = –14.2 Hz, 1 H, CH_{2b}-CH=CH_2), 1.97 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 125.8 MHz, 25 °C): δ = 169.2 (*C*O), 158.8 (arom. C), 134.2 (*C*H=CH_2), 133.7 (arom. C), 127.7 (2 arom. C), 118.0 (CH=*C*H_2), 114.0 (2 arom. C), 55.3 (O*C*H_3), 52.0 (*C*HNH), 40.4 (*C*H_2-CH=CH_2), 23.4 (CO*C*H_3) ppm. HRMS: calcd. for $C_{13}H_{17}NO_2Na$ $[M + Na]^+$ 242,1152; found 242,1257.

3-Methyl-1-phenylbut-3-en-1-amine (3d) with Ethyl Methoxyacetate: *rac*-**3d** (380 mg, 2.36 mmol) was resolved as above except that CAL-B (Novozym 435, 1.22 g, 50 mg mL^{-1}) was used in the place of lipase PS-D. After 7 d the reaction was stopped at 31% conversion. The crude product was dissolved in H_2O (10 mL) and the solution was acidified with 4 M HCl (4 mL) and extracted with EtOAc (3 × 20 mL). The aqueous phase was alkalized with 4 M NaOH (4 mL) and extracted with EtOAc (3 × 20 mL). The organic phases were combined, dried with Na_2SO_4 and concentrated to yield (*S*)-**3d** as a colorless oil (93 mg, 0.57 mmol, 35%, *ee* = 44%). The enantiomerically enriched (*S*)-**3d** was purified under kinetic resolution conditions, yielding (*S*)-**3d** as a colorless oil [20 mg, 0.12 mmol, 21%, *ee* = 72%, $[\alpha]_D^{25}$ = –31 (*c* = 1.0, $CHCl_3$)]. The combined organic phases containing (*R*)-**4d** was treated in the same way and purified by silica pad filtration (EtOAc) to yield (*R*)-**4d** as a white solid [184 mg, 0.79 mmol, quant. yield, *ee* > 99%, $[\alpha]_D^{25}$ = +45 (*c* = 1.0, $CHCl_3$), m.p. 53 ± 1 °C]. (*R*)-**4d**: ^{1}H NMR ($CDCl_3$, 500.13 MHz, 25 °C): δ = 7.35–7.23 (m, 5 H, arom. H), 6.78 (d, $J_{NH,CH}$ = 8.4 Hz, 1 H, NH), 5.20 (ddd, $J_{CH,CH2a}$ = 5.8, $J_{CH,NH}$ = 8.4, $J_{CH,CH2b}$ = 9.4 Hz, 1 H, C*H*NH), 4.82 (ddq, $J_{CH2trans,CHa}$ = –0.6, $J_{CH2trans,CH3}$ = 1.4, $J_{CH2trans,CH2cis}$ = –2.0 Hz, 1 H, CH_2-CH=CH_{2trans}), 4.74 (dddq, $J_{CH2cis,CH3}$ = 0.9, $J_{CH2cis,CH2b}$ = –1.0, $J_{CH2cis,CHa}$ = –1.3, $J_{CH2cis,CH2trans}$ = –2.0 Hz, 1 H, CH_2-CH=CH_{2cis}), 3.88 (s, 2 H, CH_2OCH_3), 3.40 (s, 3 H, CH_2OCH_3), 2.54 (dddd, $J_{CH2a,CH2trans}$ = –0.6, $J_{CH2a,CH2cis}$ = –1.3, $J_{CH2a,CH}$ = 5.8, $J_{CH2a,CH2b}$ = –14.2 Hz, 1 H, CH_{2a}-CH=CH_2), 2.50 (ddd, $J_{CH2b,CH2cis}$ = –1.0, $J_{CH2b,CH}$ = 9.4, $J_{CH2b,CH2a}$ = –14.1 Hz, 1 H, CH_{2b}-CH=CH_2), 1.74 (s, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 125.8 MHz, 25 °C): δ = 168.8 (*C*O), 142.1 (arom. C), 141.8 [*C*(CH_3)=CH_2], 128.6 (2 arom. C), 127.3 (arom. C), 126.3 (2 arom. C), 113.8 [C(CH_3)=*C*H_2], 72.0 (*C*H_2OCH_3), 59.3 (CH_2O*C*H_3), 50.5 (*C*HNH), 45.1 [*C*H_2-C(CH_3)=CH_2], 22.1 (*C*H_3) ppm. HRMS: calcd. for $C_{14}H_{19}NO_2Na$ $[M + Na]^+$ 256.1308; found 256.1396.

Procedure for the Hydrolysis of (*R*)-5a and (*R*)-4a: (*R*)-**5a** (169 mg, 0.89 mmol, *ee* = 97%) was refluxed in 4 M HCl for 19 h, and thereafter the solution was cooled to room temperature, alkalized with 4 M NaOH and extracted with EtOAc (3 × 25 mL). The combined organic layer was concentrated in vacuo and the residue purified through column chromatography (EtOAc) to yield (*R*)-**3a** (24 mg, 0.16 mmol, 18%, *ee* = 97%.). (*R*)-**4a** (74 mg, 0.34 mmol, *ee* = 94%) was refluxed in 6 M HCl for one hour, and thereafter handled identical to above except that the system was alkalized with 6 M NaOH and extracted with EtOAc (4 × 20 mL). Purification yielded (*R*)-**3a** (11 mg, 0.07 mmol, 21%, *ee* = 91%).

Synthesis of (1*S*)-1-Phenyl-*N*-(prop-2-en-1-yl)but-3-en-1-amine [(*S*)-8]: In a Schlenk tube flushed with argon, (*S*)-**3a** (60 mg, 0.41 mmol, *ee* 99%) was dissolved in anhydrous DMF (1.5 mL). KOH (70.2 mg, 1.25 mmol, 3 equiv.) was added and the mixture was stirred for 15 min. Allyl bromide (35 µL, 0.40 mmol, 1 equiv.) was added and the mixture was stirred at room temperature for 3 h. The reaction was quenched by adding H_2O (5 mL) and extracted with Et_2O (3 × 5 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated to yield the crude product that was purified by flash chromatography (eluent hexane/EtOAc, 1:1 + 1% Et_3N; silica enriched with ca. 0.1% Ca) yielding of (1*S*)-1-phenyl-*N*-(prop-2-en-1-yl)but-3-en-1-amine (51.5 mg, 0.27 mmol, 67%) as a colorless oil. $[\alpha]_D^{20}$ = –27 (*c* = 0.01, $CHCl_3$). R_f = 0.65 (hexane/EtOAc, 1:1). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.35–7.29 (m, 4 H, arom. H), 7.26–7.22 (m, 1 H, arom. H), 5.86 (dddd, $J_{CH=',CH2a'}$ = 5.4, $J_{CH=',CH2b'}$ = 6.7, $J_{CH=',CH2cis'}$ = 10.2, $J_{CH=',CH2trans'}$ = 17.2 Hz, 1 H, CH_2'-C*H*′=CH_2'), 5.72 (dddd, $J_{CH=,CH2a}$ = 5.9, $J_{CH=,CH2b}$ = 8.3, $J_{CH=,CH2cis}$ = 10.1, $J_{CH=,CH2trans}$ = 17.1 Hz, 1 H, CH_2-C*H*=CH_2), 5.10 (dddd, $J_{CH2trans',CH2b'}$ = –1.4, $J_{CH2trans',CH2a'}$ = –1.7, $J_{CH2trans',CH2cis'}$ = –1.9, $J_{CH2trans',CH='}$ = 17.2 Hz, 1 H, CH_2'-CH′=CH_{2trans}'), 5.09 (dddd, $J_{CH2trans,CH2b}$ = –1.2, $J_{CH2trans,CH2a}$ = –1.7, $J_{CH2trans,CH2cis}$ = –2.0, $J_{CH2trans,CH}$ = 17.1 Hz, 1 H, CH_2-CH=CH_{2trans}), 5.06 (dddd, $J_{CH2cis',CH2b'}$ = –1.2, $J_{CH2cis',CH2a'}$ = –1.4, $J_{CH2cis',CH2trans'}$ = –1.9, $J_{CH2cis',CH='}$ = 10.2 Hz, 1 H, CH_2'-CH′=CH_{2cis}'), 5.05 (dddd, $J_{CH2cis,CH2b}$ = –0.9, $J_{CH2cis,CH2a}$ = –1.3, $J_{CH2cis,CH2trans}$ = –2.0, $J_{CH2cis,CH=}$ = 10.1 Hz, 1 H, CH_2-CH=CH_{2cis}), 3.70 (dd, $J_{CH,CH2a}$ = 5.8, $J_{CH,CH2b}$ = 7.9 Hz, 1 H, C*H*NH), 3.11 (dddd, $J_{CH2a',CH2cis'}$ = –1.4, $J_{CH2a',CH2trans'}$ = –1.7, $J_{CH2a',CH='}$ = 5.4, $J_{CH2a',CH2b'}$ = –14.2 Hz, 1 H, C*H*2a′-CH′=CH2′), 3.02 (dddd, $J_{CH2b',CH2cis'}$ = –1.2, $J_{CH2b',CH2trans'}$ = –1.4, $J_{CH2b',CH='}$ = 6.7, $J_{CH2b',CH2a'}$ = –14.2 Hz, 1 H, CH_{2b}'-CH′=CH_2'), 2.43 (ddddd, $J_{CH2a,CH2cis}$ = –1.3, $J_{CH2a,CH2trans}$ = –1.7, $J_{CH2a,CH}$ = 5.8, $J_{CH2a,CH}$ = 5.9, $J_{CH2a,CH2b}$ = –13.9 Hz, 1 H, CH_{2a}-CH=CH_2), 2.40 (ddddd, $J_{CH2b,CH2cis}$ = –0.9, $J_{CH2b,CH2trans}$ = –1.2, $J_{CH2b,CH}$ = 7.9, $J_{CH2a,CH=}$ = 8.3, $J_{CH2a,CH2b}$ = –13.9 Hz, 1 H, CH_{2a}-CH=CH_2) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 143.7 (arom. C), 136.9 (*C*′H=C′H_2) 135.4 (*C*H=CH_2), 128.3 (2 arom. C) 127.2 (2 arom. C.), 127.0 (arom. C), 117.5 (CH=*C*H_2), 115.7 (CH′=*C*H_2'), 61.7 (*C*HNH), 50.0 (*C*′H_2-C′H=C′H_2), 43.0 (*C*H_2-CH=CH_2) ppm. HRMS: calcd. for $C_{13}H_{17}N$ $[M]^+$ 187.1361; found 187.1350.

Synthesis of (2*S*)-2-Phenyl-1,2,3,6-tetrahydropyridine [(*S*)-9]: In a Schlenk tube flushed with argon, (1*S*)-1-phenyl-*N*-(prop-2-en-1-yl) but-3-en-1-amine (*S*)-**8** (22.0 mg, 0.12 mmol) was dissolved in anhydrous CH_2Cl_2 (1.2 mL). *p*-toluenesulfonic acid monohydrate (23.4 mg, 0.12 mmol) of was added and the mixture was stirred for 10 min. Grubbs' second-generation catalyst (NHC)(PCy_3)-Cl_2Ru=CHR (10.2 mg, 0.012 mmol, 10 mol-%) was added and the resulting mixture was stirred at room temperature for 17 h. The solvent was evaporated and the residue dissolved in EtOAc (15 mL). The organic layer was extracted with 1 M HCl (4 × 5 mL). The combined aqueous layers were alkalized (pH = 14) by adding NaOH (s). The basic solution was extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried with anhydrous Na_2SO_4, filtered and concentrated to yield the crude product that was purified by flash chromatography (eluent hexane/EtOAc, 1:1 + 1% Et_3N; silica enriched with ca. 0.1% Ca) yielding of (2*S*)-2-phenyl-1,2,3,6-tetrahydropyridine (13.4 mg, 0.084 mmol, 70%) as yellowish oil. $[\alpha]_D^{20}$ = –88 (*c* = 0.01, $CHCl_3$). R_f = 0.15 (hexane/EtOAc, 1:1). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.40–7.37

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(m, 2 H arom. H), 7.36–7.32 (m, 2 H, arom. H), 7.28–7.25 (m, 1 H, arom. H), 5.87 (ddddd, $J_{CH=,CH2a'}$ = –1.8, $J_{CH=,CH2a}$ = 1.8, $J_{CH=,CH2b}$ = 2.5, $J_{CH=,CH2b'}$ = –5.3, $J_{CH=,CH='}$ = 10.1 Hz, 1 H, C*H*′=CH′), 5.79 (ddddd, $J_{CH=',CH2b'}$ = 1.5, $J_{CH=',CH2b}$ = –1.9, $J_{CH=',CH2a'}$ = 2.7, $J_{CH=',CH2a}$ = –4.4, $J_{CH=',CH=}$ = 10.1 Hz, 1 H, CH′=C*H*′), 3.85 (dd, $J_{CH,CH2b'}$ = 3.8, $J_{CH,CH2a'}$ = 10.3 Hz, 1 H, C*H*), 3.62 (ddddd, $J_{CH2a,CH}$ = 1.8, $J_{CH2a,CH2a'}$ = –2.5, $J_{CH2a,CH2b'}$ = –3.4, $J_{CH2a,CH='}$ = –4.4, $J_{CH2a,CH2b}$ = –16.9 Hz, 1 H, C*H*2a-CH=CH′), 3.50 (ddddd, $J_{CH2b,CH2b'}$ = –0.9, $J_{CH2b,CH='}$ = –1.9, $J_{CH2b,CH}$ = 2.5, $J_{CH2b,CH2a'}$ = –4.7, $J_{CH2b,CH2a}$ = –16.9 Hz, 1 H, C*H*2b-CH=CH′), 2.29 (dddddd, $J_{CH2a',CH}$ = –1.8, $J_{CH2a',CH2a}$ = –2.5, $J_{CH2a',CH='}$ = 2.7, $J_{CH2a',CH2b}$ = –4.7, $J_{CH2a',CH}$ = 10.3, $J_{CH2a',CH2b'}$ = –17.3 Hz, 1 H, C*H*2a′-CH′=CH′), 2.25 (dddddd, $J_{CH2b',CH2b}$ = –0.9, $J_{CH2b',CH='}$ = 1.5, $J_{CH2b',CH2a}$ = –3.4, $J_{CH2b',CH}$ = 3.8, $J_{CH2b',CH}$ = –5.3, $J_{CH2b',CH2a'}$ = –17.3 Hz, 1 H, C*H*2b′-CH′=CH′) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 144.4 (arom. C), 128.6 (2 arom. C), 127.2 (arom. C), 126.6 (2 arom. C) 125.9 (CH=*C*H′), 125.6 (*C*H=CH′), 57.7 (*C*H), 46.1 (*C*H$_2$), 34.1 (*C*H$_2$′) ppm. HRMS: calcd. for $C_{11}H_{13}N$ $[M]^+$ 159.1048; found 159.1046.

Synthesis of (6*R*)-6-Phenyl-5,6-dihydropyridin-2(1*H*)-one [(*R*)-10]: In a Schlenk tube flushed with argon, *(R)-N*-(1-phenylbut-3-enyl) acrylamide (*R*)-**6** (20.2 mg, 0.10 mmol, *ee* 95%) was dissolved in anhydrous CH_2Cl_2 (1.5 mL). Grubbs' second-generation catalyst $(NHC)(PCy_3)Cl_2Ru{=}CHR$ (3.6 mg, 0.0042 mmol) dissolved in anhydrous CH_2Cl_2 (0.5 mL) was added and the resulting mixture was stirred at room temperature for 30 min and then refluxed (+40 °C) for 4 h after which Grubbs' second-generation catalyst (3.9 mg, 0.0046 mmol) dissolved in anhydrous CH_2Cl_2 (0.5 mL) was added and stirring at reflux was continued for 18.5 h. The crude product was purified by flash chromatography (eluent hexane/EtOAc, 1:1 + 1% Et_3N; silica enriched with ca. 0.1% Ca) yielding (6*R*)-6-phenyl-5,6-dihydropyridin-2(1*H*)-one (14.4 mg, 0.083 mmol, 83%) as a off-white solid. $[a]_D^{20}$ = +210 (*c* = 0.01, $CHCl_3$). R_f = 0.09 (hexane/EtOAc, 1:1). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.41–7.33 (m, 5 H, arom. H), 6.65 (ddd, $J_{CH=,CH2b}$ = –2.8, $J_{CH=,CH2a}$ = –5.5, $J_{CH=,CH='}$ = 10.0 Hz, 1 H, CO-C*H*=CH), 6.03 (dddd, $J_{CH=',CH}$ = –1.1, $J_{CH=',CH2a}$ = 1.4, $J_{CH=',CH2b}$ = 2.6, $J_{CH=',CH}$ = 10.0 Hz, 1 H, CO-CH=C*H*), 5.58 (br. s., 1 H, N*H*), 4.75 (dd, $J_{CH,CH2a}$ = 5.5, $J_{CH,CH2b}$ = 11.8 Hz, 1 H, C*H*), 2.59 (dddd, $J_{CH2a,CH='}$ = 1.4, $J_{CH2a,CH}$ = 5.5, $J_{CH2a,CH}$ = –5.5, $J_{CH2a,CH2b}$ = –17.8 Hz, 1 H, C*H*$_{2a}$), 2.52 (dddd, $J_{CH2b,CH='}$ = 2.6, $J_{CH2b,CH}$ = –2.8, $J_{CH2b,CH}$ = 11.8, $J_{CH2b,CH2b}$ = –17.8 Hz, 1 H, C*H*$_{2b}$) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 166.5 (CO), 141.0 (arom. C), 140.3 (CO-*C*H=CH′), 129.0 (2 arom. C), 128.4 (arom. C), 126.4 (2 arom. C), 124.5 (CO-CH=*C*H′), 56.0 (*C*H), 33.1 (*C*H$_2$) ppm. HRMS: calcd. for $C_{11}H_{11}NONa$ $[M + Na]^+$ 196.0738; found 196.0750.

Synthesis (7*R*)-7-Phenyl-1,3,6,7-tetrahydro-2*H*-azepin-2-one [(*R*)-11]: Procedure as above starting from (*R*)-**7** (16.6 mg, 0.08 mmol, *ee* > 99%). Yield (3.2 mg, 0.017 mmol, 22%). The amount of purified material was not sufficient for reliable measurement of the optical rotation. R_f = 0.15 (hexane/EtOAc, 1:1), ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.42–7.38 (m, 2 H, arom. H), 7.37–7.33 (m, 3 H, arom. H), 5.76 (dd, $J_{NH=,CH2b'}$ = –2.3, $J_{NH=,CH}$ = 5.8 Hz, 1 H, N*H*), 5.74 (dddd, $J_{CH=,CH2a'}$ = 2.9, $J_{CH=,CH2a}$ = 3.1, $J_{CH=,CH2b'}$ = 5.1, $J_{CH=,CH='}$ = 11.6 Hz, 1 H, COCH$_2$C*H*=CH′), 5.66 (ddddd, $J_{CH=',CH2b'}$ = 1.8, $J_{CH=',CH2a'}$ = 2.4, $J_{CH=',CH2a}$ = 2.8, $J_{CH=',CH2b}$ = 8.6, $J_{CH=',CH}$ = 11.6 Hz, 1 H, COCH$_2$CH=C*H*′), 4.95 (ddd, $J_{CH,CH2b'}$ = 2.4, $J_{CH,NH}$ = 5.8, $J_{CH,CH2a'}$ = 12.1 Hz, 1 H, C*H*), 3.66 (ddddd, $J_{CH2a,CH='}$ = 2.8, $J_{CH2a,CH}$ = 3.1, $J_{CH2a,CH2b'}$ = –3.6, $J_{CH2a,CH2a'}$ = –4.3, $J_{CH2a,CH2b}$ = –16.7 Hz, 1 H, CO-CH$_{2a}$), 2.92 (dddd, $J_{CH2b,CH2a'}$ = –1.0, $J_{CH2b,CH2b'}$ = –1.9, $J_{CH2b,CH='}$ = 8.6, $J_{CH2b,CH2a}$ = –16.7 Hz, 1 H, CO-CH$_{2b}$), 2.71 (dddddd, $J_{CH2a',CH2b}$ = –1.0, $J_{CH2a',CH='}$ = 2.4, $J_{CH2a',CH}$ = 2.9, $J_{CH2a',CH2a}$ = –4.3, $J_{CH2a',CH}$ = 12.1, $J_{CH2a',CH2b'}$ = –18.2 Hz, 1 H, CH-CH$_{2a'}$), 2.48 (ddddddd, $J_{CH2b',CH='}$ = 1.8, $J_{CH2b',CH2b}$ = –1.9, $J_{CH2b',NH}$ = –2.3, $J_{CH2b',CH}$ = 2.4, $J_{CH2b',CH2a}$ = –3.6, $J_{CH2b',CH}$ = 5.1, $J_{CH2b',CH2a'}$ = –18.2 Hz) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 174.2 (CO), 140.4 (arom. C), 129.2 (2 arom. C), 128.7 (*C*H=CH′), 128.4 (arom. C), 126.3 (2 arom. C), 120.3 (CH=*C*H′), 55.0 (*C*H), 37.1 (*C*H$_2$′), 35.4 (*C*H$_2$) ppm. HRMS: calcd. for $C_{12}H_{13}NO$ $[M]^+$ 187.0997; found 187.0993.

Supporting Information (see also the footnote on the first page of this article): Gas chromatograms of the resolved compounds and ^1H and ^{13}C NMR spectra of the prepared compounds.

Acknowledgments

The authors thank the Academy of Finland for financial support [research grants to L. K. (#121983) and R. L. (#121334); project: *Fusing Biocatalytic and Chemocatalyzed Reaction Technologies*]. Stipendiary support from the Swedish Academy of Engineering Sciences in Finland (to T. S.), the Waldemar von Frenckell Foundation and the Rector of Åbo Akademi University (to S. R.) is also gratefully acknowledged. Markku Reunanen is thanked for HRMS analysis.

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Received: October 26, 2009
Published Online: December 22, 2009

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Manuscript in preparation

Kinetic resolution of a pyridylamine by lipase-catalyzed acylation

Ari Hietanen,[a] Tiina Saloranta,[b] Reko Leino[b] and Liisa T. Kanerva*[a]

[a] Institute of Biomedicine/Department of Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry, University of Turku, FI-20014 Turku, Finland
Fax: +358 2 333 7955
E-mail: lkanerva@utu.fi
[b] Laboratory of Organic Chemistry
Åbo Akademi University, FI-20500 Åbo, Finland

II

Abstract. Chiral pyridine-based primary amines are useful building blocks for complex molecule synthesis. One such compound, 3-(1-amino-3-butenyl)pyridine (**1**) was enzymatically resolved by *Burkholderia cepacia* lipase (lipase PS-D) -catalyzed acylation. 2,2,2-Trifluoroethyl chloroacetate and isopropyl 3-butenoate were the most efficient and selective acyl donors, the latter being practically more applicable and yielding (*S*)-**1** in 97% *ee* and the 3-butenamide of (*R*)-**1** in >99% *ee*.

Nitrogen heterocycles are frequent moieties in chiral pharmaceuticals and natural products. For example, pyridylhomoallylamine **1** was used as a starting material in a simple synthesis of tobacco alkaloids (±)-anatabine and (±)-anabasine.[1] To obtain pure enantiomers, lipase-promoted acylation of the primary amino group in **1** seemed alluring, since both enantiomers could be obtained using kinetic resolution and the subsequent hydrolysis of the acylated product (Scheme 1).

In literature, pyridine-based primary amines have been found to be difficult substrates for lipases. For example, the acylation of 2-(1-aminoethyl)-3-chloro-5-(substituted)pyridines resolved by *Candida antarctica* lipase B (CAL-B) resulted in low enantiomeric ratios (*E* = 27 at most, Scheme 2a).[2] Aminoalkyl-substituted pyridines were resolved under similar conditions, enantioselectivity ranging from excellent (*E* >500) to negligible (*E* = 5, Scheme 2b).[3] CAL-B and ethyl methoxyacetate were used in the acylation of 3-(hetero)aromatic-2-propanamines.[4] Whereas aromatic, indole and histidine rings as substituents gave high enantioselectivity in the kinetic resolution (*E* >200), the same conditions gave much lower selectivity with 3-substituted pyridine as the aromatic moiety (Scheme 2c). We previously found lipase PS-D (lipase from *Burkholderia cepacia* adsorbed on diatomaceous earth) to be an effective (and somewhat more selective than CAL-B) catalyst for the acylation of benzyl amines with, for example, isopropyl acetate or ethyl methoxyacetate as acyl donors.[5] We now report our efforts to resolve the substrate of interest, 3-(1-amino-3-butenyl)pyridine (**1**) by lipase PS-D catalyzed acylation.

Lipase-catalyzed acylation of amines differs from that of alcohols in two crucial aspects.[6] First, primary amines are more nucleophilic than secondary alcohols and non-enzymatic N-acylation, leading to a racemic product, is a potential side-reaction. Secondly, should any hydrolysis of the acyl donor take place, the substrate amine would be salted out of the solution by the released carboxylic acid. This issue is particularly actual in the case of

Scheme 1. Transformation of *rac*-**1** into (*R*)- and (*S*)-**1**.

Scheme 2. Literature examples for the kinetic resolution of pyridyl-substituted primary amines by CAL-B -catalyzed acylation.[2-4]

pyridine substrates since there are only very limited options for the N-protection of the pyridine ring. As an initial set-up, lipase PS-D was used to acylate *rac*-**1** in toluene in the presence of molecular sieves with variously activated acyl donors (Table 1). 2,2,2-Trifluoroethyl esters with activated alkyl ends were all very unreactive (entries 1-3). Acyl activation in the case of ethyl and particularly isopropyl methoxyacetate improved reactivity, but selectivity, as based on approximated E values, was still very low (entries 4-5). Ethyl chloroacetate afforded 50% conversion with excellent enantioselectivity (entry 6). With 2,2,2-trifluoroethyl chloroacetate (entry 7), very high reactivity was obtained accompanied by chemical acylation producing *rac*-**1** (entry 6).[9] High reactivity nevertheless encouraged the conditions to be further developed to achieve higher selectivity with 2,2,2-trifluoroethyl chloroacetate.

To improve selectivity, the effects of temperature, additives and acyl donor concentration were studied with 2,2,2-trifluoroethyl chloroacetate (Table 2). Reactions were sampled at 10 min to determine initial rate differences and at 60 min to determine the extent and selectivity at or around 50% conversion (beyond which the reaction proceeded very slowly or not at all). At room temperature (~22 ^{o}C) moderate selectivity was obtained with one equivalent of the acyl donor in the presence of molecular sieves (entry 1). Selectivity was improved when temperature was lowered to +4 ^{o}C and this occurred without any decrease in reaction rate (entry 2). Reaction outcome was worse when molecular sieves were substituted with magnesium sulfate (entry 3), but essentially unchanged when sodium sulfate was used (entry 4). A higher excess of the acyl donor (2 equiv.) reduced selectivity (entry 5) whereas lowering the concentration of 2,2,2-trifluoroethyl chloroacetate to 0.75 equiv. substantially improved it with only a small effect on reaction rate (entry 6). When only 0.5 equiv. of trifluoroethyl chloroacetate was used (i.e. 1 equiv. with respect to the preferred enantiomer), the reaction halted before 50% conversion and selectivity was lowered too. Thus, conditions of low acyl donor content and temperature at +4 ^{o}C gave the best selectivity among those tested with approximately 50% conversion in 1 hour (entry 6, no non-enzymatic acylation observed in control experiments).

The kinetic resolution of *rac*-**1** was then performed on preparative scale under the optimized conditions. Reaction analysis after 75 min (Conv. 48%) indicated 90% *ee* for the amine and 97% *ee* for the

Table 1. Kinetic resolution of **1** (0.10 M) with activated acyl donors and lipase PS-D (50 mg mL^{-1}).

rac-**1** → (Acyl donor, lipase PS-D; $PhCH_3$, 4Å M.S., r.t.) → (*R*)-AMIDES + (*S*)-**1**

Entry	Acyl donor	Acyl donor [equiv.]	Time[a] [h]	Conv. [%]	ee_P [%]	ee_S [%]	E
1	F_3C, O, O	2	30	13	30	5	1
2	F_3C, O, O	2	30	5	<1	2	1
3	F_3C, O, O	2	30	0	-	-	-
4	O, O, O	2	30	12	n.d.[b]	12	<10
5	O, O, O	2	30	30	n.d.[b]	19	<10
6	O, O, Cl	2	72	52	90	99	>100
7	F_3C, O, O, Cl	1	1	51	89	91	n.d.[c]

[a] The reaction did not proceed after the given time point. [b] Enantiomers did not fully separate in HPLC. [c] Not determined due to concomitant chemical acylation (conversion 4% in 1h without enzyme).

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Table 2. Kinetic resolution of **1** (0.10 M) with 2,2,2-trifluoroethyl chloroacetate and lipase PS-D (50 mg mL^{-1}).

Entry	Temp. [°C]	Additives[a]	Acyl donor [equiv.]	Conv.^{10min} [%]	Conv.^{60min} [%]	ee_P^{60min} [%]	ee_S^{60min} [%]	*E*
1	+22	4Å M.S.	1	35	42	93	66	n.d.
2	+4	4Å M.S.	1	35	50	94	94	>100
3	+4	$MgSO_4$	1	n.d.	35	95	50	69
4	+4	Na_2SO_4	1	n.d.	47	96	84	>100
5	+4	4Å M.S.	2	37	50	92	90	72
6	+4	4Å M.S.	0.75	30	46	97	82	>100
7	+4	4Å M.S.	0.5	-	35[b]	97	53	>100

[a] 50 mg mL^{-1}. [b] The reaction did not proceed or proceeded only slowly after the given time point.

amide (*E* >100). However, after column chromatography a large part of the material was lost and both amine (*S*)-**1** and amide (*R*)-**2** had lowered *ee* (82% and 50%, respectively). This implies that part of the activated amide (*R*)-**2** was hydrolyzed during the work-up and/or there were side reactions that took place in the concentrated solution.

Acylation of *rac*-**1** with non-activated acyl donors was next studied in order to yield more stable amides (*R*)-**3** and (*R*)-**4** (Table 3). The reaction of *rac*-**1** in neat ethyl acetate (entry 1) resulted in lower reactivity and selectivity than in isopropyl acetate (entry 2a) at room temperature. The reaction iPrOAc was still rather slow and practically halted below 50% conversion (entry 2b). Elevated temperature could be used to push the conversion to 50% with the cost of reduced enantioselectivity (entry 3). Increase of enzyme content up to 100 mg mL^{-1} of lipase PS-D was required to achieve 50% conversion (entries 4 and 5). As before, increase in temperature reduced selectivity (entry 6) and reduction of acyl donor content slowed down the reaction without significant improvement in selectivity (entry 7). CAL-B (Novozym 435) was both a less active and a less selective catalyst (entry 8) in comparison to lipase PS-D. Isopropyl 3-butenoate was also used as the acyl donor (entry 9). In comparison to kinetic resolution with isopropyl acetate, the reaction was both faster and more selective. Indeed, 50% conversion was reached in 24 h affording both amide (*R*)-**4** and amine (*S*)-**1** in 98% *ee*.

On preparative scale, kinetic resolution of *rac*-**1** in neat isopropyl acetate with lipase PS-D (100 mg mL^{-1}) proceeded in 96 h to 47% conversion at room temperature. After work-up and purification the amide (*R*)-**3** was obtained in 44% yield with 97% *ee* and amine (*S*)-**1** in 39% yield with 88% *ee* (Table 4, entry 1). (*R*)-**3** was subjected to acidic hydrolysis under microwave irradiation to afford (*R*)-**1** in 78%

Table 3. Kinetic resolution of *rac*-**1** (0.10 M) in neat non-activated acyl donors.[a]

Entry	Product	R^1	R^2	Temp. [°C]	Lipase [mg mL^{-1}]	Time [h]	Conv. [%]	ee_P [%]	ee_S [%]	*E*
1	(*R*)-**3**	H	H	22	PS-D (50)	72	12	64	9	4
2a	(*R*)-**3**	CH_3	H	22	PS-D (50)	72	32	97	46	>100
2b	(*R*)-**3**	CH_3	H	22	PS-D (50)	96[b]	43	96	71	>100
3	(*R*)-**3**	CH_3	H	48	PS-D (50)	72[b]	50	87	86	38
4	(*R*)-**3**	CH_3	H	22	PS-D (75)	96[b]	48	96	87	>100
5	(*R*)-**3**	CH_3	H	22	PS-D (100)	96[b]	50	95	94	>100
6	(*R*)-**3**	CH_3	H	48	PS-D (100)	96[b]	53	87	97	58
7[c]	(*R*)-**3**	CH_3	H	22	PS-D (100)	96[b]	39	97	63	>100
8	(*R*)-**3**	CH_3	H	22	CAL-B (100)	96[b]	33	92	45	37
9	(*R*)-**4**	CH_3	$CH=CH_2$	22	PS-D (100)	24[b]	50	98	98	>200

[a] 50 mg mL^{-1} 4Å M.S. [b] The reaction did not proceed after the given time point. [c] 50 vol.% iPrOAc in $PhCH_3$.

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Table 4. Preparative kinetic resolution of **1** (0.10 M) with non-activated acyl donors followed by hydrolysis.[a]

Entry	Product	R	Kinetic resolution Time [h]	Conv [%]	E	(S)-1 Yield[b] [%]	ee [%]	(R)-3/4 Yield[b] [%]	ee [%]	(R)-1 Yield[c] [%]	ee [%]
1	(*R*)-**3**	H	96	47	>100	39	88	44	97	78	85
2	(*R*)-**4**	$CH=CH_2$	24	51	>200	12	97	45	>99	50	84

[a] Reagents and conditions: a) 0.10 M *rac*-**1** in neat isopropyl acetate/3-butenoate, lipase PS-D (100 mg mL^{-1}), 4Å M.S. (100 mg mL^{-1}), r.t.; b) 0.05 M (*R*)-**3**/**4**, 2 M aq. HCl, MW 165 °C, 15 min; c) aq. NH_4OH, then extracted to EtOAc.; [b] Isolated yield from racemic starting material; [c] Isolated yield of the hydrolysis step.

yield. Isopropyl 3-butenoate gave high selectivity for the acylation of *rac*-**1** and after 24 h (51% conversion) the amide (*R*)-**4** was collected in 45% yield and >99% *ee* (Table 4, entry 2). The amine (*S*)-**1** was only obtained in low yield (12%) although with high *ee* (97%). (*R*)-**4** gave lower yield for (*R*)-**1** than (*R*)-**3** upon acidic hydrolysis and significant racemization took place (from *ee* >99% to 84%).

We have addressed the problematic enzymatic acylation of pyridine-based primary amines by the use of lipase PS and different acyl donors under anhydrous conditions. Activated acyl donor 3,3,3-trifluoroethyl chloroacetate was efficient for the resolution reaction but the product amide was unstable toward purification. Isopropyl acetate and isopropyl 3-butenoate had lower reactivities but the amide products were stable and obtained in high *ee*.

Experimental

Materials and methods. *rac*-**1** was prepared as described previously.[1] Lipase PS-D was acquired from Amano. Ethyl methoxyacetate and isopropyl acetate were from Aldrich. Isopropyl 3-butenoate was synthesized from isopropanol and vinyl acetic acid (see supporting information). 2,2,2-trifluoroethyl esters were synthesized with the same method previously in the laboratory and the purities were checked by NMR before use. Toluene was from J. T. Baker and dried before use.

Small-scale kinetic resolutions were done in vials with approximately 1 mL reaction volume. Agitation, both in small and preparative scale, was done by shaking. Reactions were followed by HPLC equipped with Daicel CHIRALCEL OD-H analytical column (40 °C) that was eluted with 10% iPrOH in hexane (0.8 mL min^{-1}) and detected with a UV detector at 235 nm. HPLC samples were derivatized either with acetic or butanoic anhydride. The determination of E was based on equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$, where $c = ee_S/(ee_S+ee_P)$.

NMR spectra were recorded with a Bruker Avance 600 MHz spectrometer. HRMS were measured in ESI^+ mode with a Bruker micrOTOF-Q quadrupole-TOF spectrometer. Optical rotations were determined with a PerkinElmer 241 or 341 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Flash chromatography was performed using silica gel 60 Å (Merck, 230–400 mesh, enriched with 0.1% Ca).

Kinetic resolution of *rac*-1 by lipase-catalyzed acylation. *rac*-**1** (113 mg, 0.764 mmol) was dissolved in iPrOAc (7.65 mL) and the solution was added on lipase PS-D (765 mg, 100 mg mL^{-1}) and 4 Å molecular sieves (770 mg). After 96 h of shaking at +22 °C (47% conversion) the reaction was filtered and the enzyme/M.S. was washed with iPrOAc. The organic phases were combined, concentrated and purified by column chromatography (5% Et_3N in EtOAc) to collect (*R*)-**3** (64 mg, 0.337 mmol, 44%, *ee* 97%, purity 94%) and (*S*)-**1** (44 mg, 0.297 mmol, 39%, *ee* 88%). (*R*)-**4** was prepared similarly from *rac*-**1** (78 mg, 0.526 mmol) but isopropyl 3-butenoate (5.26 mL) was used as the solvent/acyl donor instead of iPrOAc. 24 h reaction time (51% conversion) was followed by work-up and column chromatography to afford (*R*)-**4** (52 mg, 0.238 mmol, 45%, *ee* >99%) and (*S*)-**3** (10 mg, 0.064 mmol, 12%, *ee* 97%). (*S*)-**1**: $[\alpha]_D^{25}$ = -39 (c = 0.5, $CHCl_3$, *ee* 97%). NMR spectra were in accordance to those reported in literature.[1] (*R*)-**3**: $[\alpha]_D^{25}$ = +82 (c = 1.0, $CHCl_3$, *ee* 97%). ^{1}H-NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 8.76 (s, 1H, H_{Ar2}); 8.52 (d, $J_{Ar6-Ar5}$ = 3.0 Hz, 1H, H_{Ar6}); 7.75 (d, $J_{Ar4-Ar5}$ = 8.40 Hz, 1H, H_{Ar4}); 7.38 (dd, 1H, H_{Ar5}); 6.27 (d, J_{NH-CH} = 6.60 Hz, 1H, NH); 5.68 (m, 1H, CH=); 5.16 (m, 1H, CH); 5.13 (m, 2H, $=CH_2$); 2.61 (m, 2H, $-CH_2-$); 2.03 (s, 3H, $COCH_3$) ppm. HRMS: calcd. for $C_{11}H_{14}N_2ONa^+$ $[M+Na]^+$ 213.0998, found 213.1071. (*R*)-**4**: $[\alpha]_D^{25}$ = +88 (c = 1.0, $CHCl_3$, *ee* >99%). 8.54 (s, 1H, H_{Ar2}); 8.49 (d, $J_{Ar6-Ar5}$ = 4.50 Hz, 1H, H_{Ar6}); 7.59 (m, 1H, H_{Ar4}); 7.25 (m, 1H, H_{Ar5}); 6.57 (d, J_{NH-CH} = 7.80 Hz, 1H, NH); 5.91 (m, 1H, $=CH^{acyl}$); 5.66 (m, 1H, $=CH^{alkyl}$); 5.21 (m, 2H, $=CH_2^{acyl}$); 5.12 (m, 1H, CH); 5.09 (m, 2H, $=CH_2^{alkyl}$); 3.01 (m, 2H, $-CH_2-^{acyl}$); 2.56 (m, 2H, $-CH_2-^{alkyl}$) ppm. ^{13}C-NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.1 (C=O); 148.5 (C_{Ar6}); 148.1 (C_{Ar2}); 137.4 (C_{Ar3}); 134.3 (C_{Ar4}); 133.2 ($=CH_2^{alkyl}$); 131.2 ($=CH_2^{acyl}$); 123.5(C_{Ar5}); 119.8 ($=CH_2^{acyl}$); 119.0 ($=CH^{alkyl}$); 50.5 (CH); 41.4 ($-CH_2-^{acyl}$); 40.1 ($-CH_2-^{alkyl}$) ppm. HRMS: calcd. for $C_{13}H_{16}N_2ONa$ $[M+Na]^+$ 239.1155, found 239.1124.

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Hydrolysis of (*R*)-3 and (*R*)-4 to give (*R*)-1. (*R*)-**3** (26 mg, 0.138 mmol, *ee* 88%) was stirred in 2 N aq. HCl (2.6 mL) under microwave heating at 165 ^{o}C for 15 min. After cooling to r.t. the reaction was treated with aq. NH_4OH (24.5 %, 6 mL) and the basic solution was extracted with EtOAc (3 × 10 mL). The organic phases were combined, dried with $NaSO_4$ and concentrated to yield (*R*)-**1** (16 mg, 0.109 mmol, 78%, *ee* 85%). (*R*)-**4** (55 mg, 0.254 mmol, *ee* >99%) was hydrolyzed identically to yield (*R*)-**1** (19 mg, 0.128 mmol, 50%, *ee* 84%). (*R*)-**1**: $[\alpha]_D^{25}$ = +38 (c = 0.5, $CHCl_3$, *ee* 84%). NMR spectra were in accordance to those reported in literature.[1]

Acknowledgements

The authors thank the Academy of Finland for financial support (research grants #121983 for LK and #121334 for RL/Fusing Biocatalytic and Chemocatalyzed Reaction Technologies).

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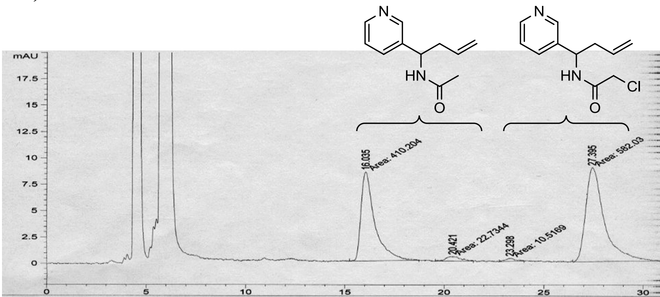
Supporting information for

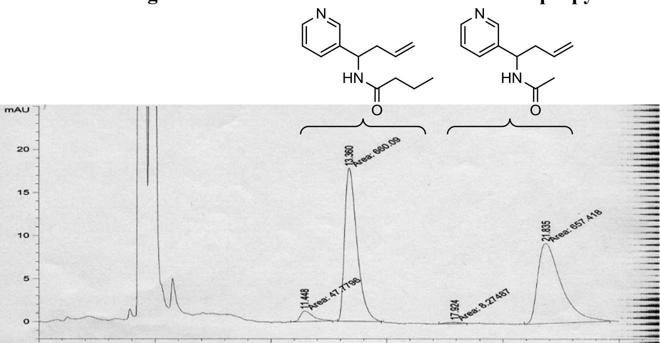
Kinetic resolution of a pyridylamine by lipase-catalyzed acylation

Ari Hietanen,[a] Tiina Saloranta,[b] Reko Leino[b] and Liisa T. Kanerva*[a]

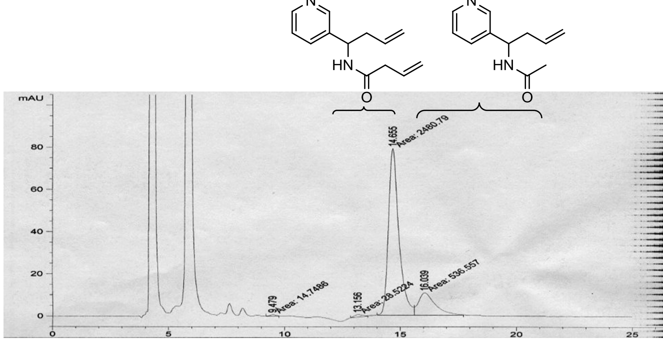
HPLC chromatograms of the kinetic resolution of 1 with 2,2,2-trifluoroethyl chloroacetate as the acyl donor (75 min):



HPLC chromatograms of the kinetic resolution of 1 with isopropyl acetate as the acyl donor (96 h):



HPLC chromatograms of the kinetic resolution of 1 with isopropyl 3-butenoate as the acyl donor (24 h):



Synthesis of isopropyl 3-butenoate. Vinyl acetic acid (10 mL, 0.118 mol) and iPrOH (13.6 mL, 0.177 mol) were cooled to +4 ^{o}C and thionyl chloride (9.5 mL, 0.130 mmol) was added dropwise. The mixture was slowly heated and refluxed overnight. The mixture was cooled to r.t. and washed with sat. $NaHCO_3$(3 × 20 mL) and brine (1 × 20 mL). Organic phase was dried with $NaSO_4$ to yield pure isopropyl 3-butenoate (7.06 g, 0.055 mol, 47%). NMR spectra were identical with reference spectra.

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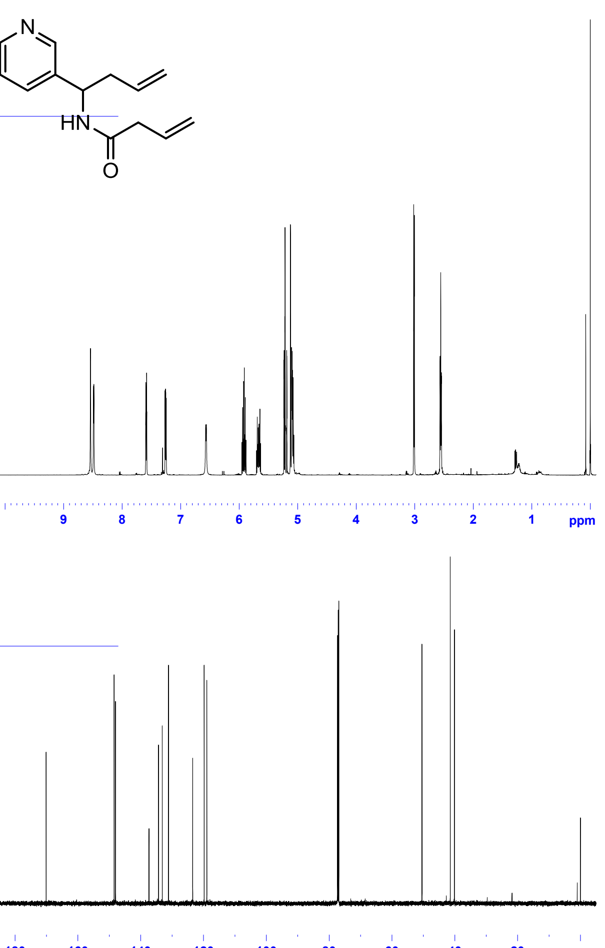
^{1}H and ^{13}C NMR spectra of (*R*)-4.



Issue in Honor of Prof. Ferenc Fülöp ARKIVOC 2012 (v) 60-74

Advances in the kinetic and dynamic kinetic resolution of piperazine-2-carboxylic acid derivatives with *Candida antarctica* lipase A; structural requirements for enantioselective N-acylation

Ari Hietanen, Katri Lundell, Liisa T. Kanerva, and *Arto Liljeblad

Institute of Biomedicine, Department of Pharmacology, Drug Development and Therapeutics / Laboratory of Synthetic Drug Chemistry and Department of Chemistry, University of Turku, Lemminkäisenkatu 5 C, FIN-20520, Turku, Finland
E-mail: artlilje@utu.fi

Dedicated to Professor Ferenc Fülöp on his 60th birthday

Abstract
The kinetic resolution of piperazine-2-carboxylic acid was studied as its *N*-4- and *N*-1-Boc-piperazine-2-carboxylic acid methyl esters, *rac*-**1** and -**2**, respectively. Lipase A from *Candida antarctica* (CAL-A) catalyzed highly enantioselective (*E*>200 for *rac*-**1** and 200 for *rac*-**2**) N-acylation of both compounds with 2,2,2-trifluoroethyl butanoate in TBME. Aldehyde-based dynamic kinetic resolution of *rac*-**1** with vinyl butanoate in acetonitrile gave up to 75% product yield with *S*-absolute configuration in 48 hours. The present results together with literature data suggest that CAL-A –catalyzed N-acylation is enhanced in terms of reaction rate or enantioselectivity when an electron rich structure, such as carboxylic ester or aromatic ring, is attached to the asymmetric center next to the secondary ring nitrogen.

Keywords: *Candida antarctica* lipase A, CAL-A, kinetic resolution, dynamic kinetic resolution, N-acylation, piperazine-2-carboxylic acid

Introduction

Piperazine-2-carboxylic acid (A, Scheme 1) is a valuable building block and constituent of many current and potential drug molecules. Such molecules include antagonists for *N*-methyl-D-aspartic acid (NMDA) type glutamate receptors[1], indinavir[2] used to treat HIV infections and inhibitors for TNF-α converting enzyme (TACE)[3] and farnesyl protein transferase[4]. Various methods have been developed for the preparation of the enantiomers of piperazine-2-carboxylic acid. In addition to traditional crystallization of diastereomeric salts,[5] kinetic resolution (KR) methods based on the enzymatic hydrolysis of an ester or amide functionality at the 2-position of

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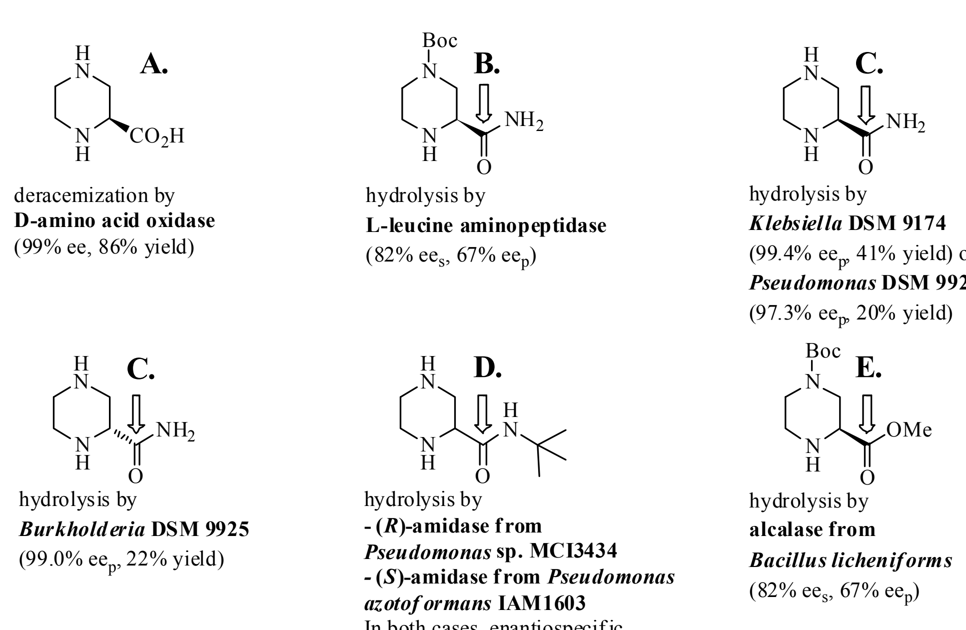
the piperazine ring exist (Scheme 1). Thus, hydrolyses of piperazine-2-carboxamide by L-leucine aminopeptidase (B)[6] and amidases (C)[7] in a bacterial whole cell system have been developed. Moreover, (*R*)- and (*S*)-specific amidases, hydrolyzing piperazine-2-*tert*-butylcarboxamides (D), have been isolated,[8,9] and alcalase (*Bacillus licheniformis* protease) has been reported to hydrolyze 4-*tert*-butoxycarbonylpiperazine-2-carboxylic acid methyl ester (E)[10]. Both enantiomers can be obtained by the KR of a racemate. However, dynamic kinetic resolution (DKR) methods, usually combining KR with the *in situ* racemization of the less reactive enantiomer, have been considered preferable as in the best case a racemate can be transformed into one enantiomer with 100% yield. Methods based on the DKR of piperazine-2-carboxylic acid or its derivatives have not been published so far. In addition to KR and DKR methods, deracemization is an interesting third option applied to transform D,L-α-amino acids like piperazine-2-carboxylic acid (A) into the L-enantiomer with D-amino acid oxidase.[11]



Scheme 1. Biocatalytic preparation of piperazine-2-carboxylic acid derivatives of high enantiopurity. The more reactive enantiomer has been presented.[6-11]

In this paper, we have studied the possibility to resolve racemic piperazine-2-carboxylic acid methyl ester with *Candida antarctica* lipase A (CAL-A) relying on the previously established N-acylation method for proline and pipecolic acid methyl esters.[12a,b] However, piperazine-2-carboxylic acid methyl ester with two secondary amino groups provides two N-acylation sites, making the KR challenging. Accordingly, we decided to study one N-acylation in time, the other

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nitrogen being Boc-protected (*rac*-**1** and -**2**; Scheme 2). We have also studied the possibility to transform the KR of *rac*-**1** into DKR using the previously described aldehyde-based *in situ* racemization of the less reactive (*R*)-**1** (Scheme 3).[12b] It is worth noting that the DKR of *rac*-**2** by the same method unlikely takes place since the N-acylation position is not directly attached to the asymmetric centre. We finally consider the results obtained in this work and found in literature to establish structural requirements for the CAL-A –catalyzed secondary N-acylations of heterocyclic amines. Additionally, purification of CAL-A from a glycerol solution of Novozym 735 and its immobilization as a CAL-A on Celite preparation is described.

Lipase A from *Candida antarctica* (CAL-A) has proven to own many interesting and exceptional properties usable in synthetic applications.[13] Related to the present study, CAL-A catalyzes highly enantioselective N-acylations of various α- and β-amino esters and, atypically to most lipases, N-acylations of several secondary amines.[12] This can be explained by the wide nucleophile binding site of the enzyme. According to the crystal structure of CAL-A and molecular modeling, CAL-A has been proposed to undergo a conformational change upon substrate binding, where the active-site "flap" (Gly426-Gly440) moves around the active site, widening the available space large for the nucleophile to be bound.[14] On the other hand, the acyl binding pocket is long and narrow, making the enzyme to prefer long-chain carboxylic acid derivatives as acyl donors.

Results and Discussion

Kinetic resolution

Scheme 2. Enantioselective N-acylation of *rac*-**1** and -**2** with CAL-A.

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The kinetic resolution of 4- and 1-*N*-Boc-protected *rac*-**1** and -**2** was studied with 2,2,2-trifluoroethyl, vinyl and ethyl esters in diisopropyl ether (DIPE) and *tert*-butyl methyl ether (TBME) in the presence of CAL-A (Scheme 2). The KR studies were performed using Novozym 735 –based CAL-A on Celite preparation prepared as described in the Experimental Section.

Table 1. CAL-A[a] –catalyzed (25 mg mL^{-1}) N-acylation of *rac*-**1** and -**2** (0.050 M) with various acyl donors (0.100 M; t=60 min)

Entry	Substrate	Acyl donor	Solvent	c (%)	ee_s (%)	ee_p (%)	*E*
1	*rac*-**1**	2,2,2-trifluoro-ethyl acetate	DIPE	6	6	>99	>200
2	*rac*-**1**	2,2,2-trifluoro-ethyl butanoate	DIPE	50	>99	>99	>200
3	*rac*-**1**	2,2,2-trifluoro-ethyl butanoate	TBME	47	88	>99	>200
4	*rac*-**1**	ethyl butanoate	TBME	6	6	>99	>200
5	*rac*-**1**	vinyl butanoate	TBME	48	93	>99	-[b]
6	*rac*-**1**	vinyl acetate	TBME	2	2	>99	-[b]
7	*rac*-**2**	2,2,2-trifluoro-ethyl acetate	DIPE	6	6	>99	45
8	*rac*-**2**	2,2,2-trifluoro-ethyl butanoate	DIPE	23	30	>99	140
9	*rac*-**2**	2,2,2-trifluoro-ethyl butanoate	TBME	16	18	>99	200
10	*rac*-**2**	ethyl butanoate	TBME	<1	<1	>99	-[c]

[a] CAL-A isolated by dialysis from Novozym 735 and immobilized on Celite in the presence of sucrose as described in the Experimental Section. [b] not calculated; racemization of (*R*)-**1** is possible due to released acetaldehyde. [c] not detected.

As expected on the basis of our previous studies for the N-acylations of proline and pipecolic acid esters,[12a,b] the KR of *rac*-**1** (an α-amino ester) with all acyl donors tested took place with exquisite enantioselectivity (*E*>200), the *S*-enantiomer being the reactive one (Table 1; entries 1-6). On the other hand, N-acylations proceeded efficiently only when alkyl-activated (trifluoroethyl or vinyl) esters were used as acyl donors (compare entries 2, 3 and 5 to 4). Additionally, despite activated alkyl part, corresponding reactions with acyl donors based on acetate as a short-chain carboxylic acid took place slowly (compare entries 1 to 2 and 5 to 6). It is worth noting that vinyl esters give vinyl alcohol as another product of enzymatic N-acylation, leading to acetaldehyde as a spontaneous decomposition product. This causes a risk for imine (Schiff base) formation between **1** and acetaldehyde and, accordingly, a risk for reduced

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enantiomeric excess values (ee_S) of the unreacted substrate enantiomer. For this reason *E* values are not given when vinyl esters have been used as acyl donors (entries 5 and 6).

The N-acylation of *rac*-**2** (a β-amino ester) proceeded considerably slower (Table 1; entries 7-10), and the reaction in DIPE displayed clearly lower enantioselectivity (entries 7 and 8) than the reaction of *rac*-**1** under the same conditions (entries 1 and 2). Excellent enantioselectivity for *rac*-**2** (*E*=200) was detected only in the reaction with 2,2,2-trifluoroethyl butanoate in TBME (entry 9). However, with extended reaction time signs of the formation of the other enantiomer appeared in the HPLC chromatogram (ee_p 98% at 42% conversion after 6 h), whereas ee_p >99% was detected with *rac*-**1** even after 24 h, indicating higher enantioselectivity in the latter case. Interestingly, opposite enantiomers reacted with CAL-A in the N-acylations of *rac*-**1** and -**2**. The determination of the reactive enantiomer (*S*)-**3** is based on the optical rotation as described in the Experimental Section. The *S*-enantiopreference of CAL-A is also in accordance with the N-acylations of proline and pipecolic acid methyl esters.[12a,b] Commercial (*R*)-**2** as a reference compound indicated (*R*)-**2** to be the reactive enantiomer.

In order to show the synthetic value of the kinetic resolution method, *rac*-**1** was subjected to gram-scale KR with 2,2,2-trifluoroethyl butanoate in TBME. When the reaction was stopped at 50% conversion, (*R*)-**1** (ee=99%) and (*S*)-**3** (ee>99%) were successfully isolated by column chromatography. In the same way, the enantiomers of *rac*-**2** can be separated in a preparative scale. However, it is not possible obtain both (*R*)-**4** (ee_P=98% at 42% conversion) and (*S*)-**2** (ee_S=97% at 52% conversion) at high enantiopurities in a single resolution step. Accordingly, the gram-scale resolution of *rac*-**2** was not performed here.

Dynamic kinetic resolution

Dynamic kinetic resolution of amines can be achieved by using aldehydes,[12b,15] metals,[16] radical initiators[17] or enzymes[18] as racemization catalysts in an enzymatic kinetic resolution mixture. As the kinetic resolution of *rac*-**1** with vinyl butanoate proceeded with excellent enantioselectivity, producing the corresponding (*S*)-**3** with high efficiency (Table 1, entry 5), we found it interesting to subject *rac*-**1** to the previous simple and inexpensive DKR conditions[12b]. According to this method, vinyl butanoate is used as an acyl donor for the N-acylation of the secondary amino group of an N-heterocyclic α-amino ester. Hence, no added aldehyde is needed for the racemization of the unreacted enantiomer, because in the formation of the N-acylated product (*S*)-**3** or -**6** the vinyl alcohol released from the acyl donor (vinyl butanoate) is isomerized *in situ* into the racemization agent acetaldehyde (Scheme 3). Acetaldehyde can be also formed by enzymatic hydrolysis of vinyl butanoate by enzyme-bound water. Hydrolysis also yields butanoic acid which may further form salt **10** with the starting material.

It is astonishing that with N-heterocyclic α-amino esters like *rac*-**1** (or *rac*-**5**) the formation of the intermediate Schiff base **8** is reversible under the mild enzymatic reaction conditions, since generally the hydrolysis of a Schiff base requires acidic conditions at elevated temperatures.[19] Although the exact racemization mechanism is not established, we propose that the acidic α-hydrogen at the asymmetric centre is involved in the racemization through the

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formation of **8**. As a support to this, the calculated pK_a values of the α-protons of *rac*-**1**, **7** (Y=*N*-Boc) and **8** (Y=*N*-Boc) decrease in the order of 23.2, 21.9 and 17.9, respectively, while the corresponding pK_a values for *rac*-**5**, **7** (Y=CH_2) and **8** (Y=CH_2) are 24.8, 23.5 and 19.4.[20] As a support to the calculated descending trend, the iminium ion formation has been experimentally shown to correspond to the effect of 7 pK_a units when the α-proton of glycine methyl ester is compared to that of the iminium ion.[21]

Scheme 3. DKR of *rac*-**1** and *rac*-**5**.

When *rac*-**1** was subjected to acylation with vinyl butanoate by Novozym 735-based CAL-A on Celite under the previous DKR conditions[12b] disappointing results were observed (the results not shown herein). Thus, the aldehyde-based racemization step seemed to be slow compared to the KR step, and the enzymatic N-acylation had virtually stopped while slightly racemized starting material **1** was still present. In the previous work, lyophilized Chirazyme L5 powder (former SP 526 from Novo Nordisk) rather than aqueous glycerol solution (Novozym 735) needing dialysis before adsorption on Celite was used. Possibly the Novozym 735 –based CAL-A on Celite (although perfectly worked in the KR, Table 1) was not in the same stable stage as the earlier Chirazyme L5 –based preparation had been. For this reason, the DKR of pipecolic acid methyl ester *rac*-**5** was reinvestigated with different CAL-A preparations in TBME (Table 2, entries 1-5 and 7). Indeed, the previously observed 61% product yield with Chirazyme L5-based preparation[12b] and the disappearance of the substrate at this stage (entry 6) were not achieved with the Novozym 735-based CAL-A preparation (entry 3), confirming different behaviors of the two enzyme preparations. The DKR results of *rac*-**5** with other CAL-A-preparations, CLEA (cross-linked enzyme aggregate) and covalently immobilized CAL-A-T2-150 (entries 1 and 2), did not give better results either, and the reactions stopped at early

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conversions. Interestingly, the DKR of *rac*-**5** with Novozym 735 –based CAL-A on Celite in acetonitrile allowed the preparation of (*S*)-**6** at 60% yield (entry 7), although even then still 10% of slightly enantiomerically enriched **5** was present in the reaction mixture. The difference between the reactions in TBME (entry 3) and acetonitrile (entry 7) may reflect for instance solubility differences of the ammonium salt **10** between the amino ester substrate and butanoic acid into these solvents.

Finally, the N-acylation of *rac*-**1** in acetonitrile was studied with three different CAL-A-immobilizates (Novozym 735-, Chirazyme L5- and lyophilized Cat#ICR-112-based) on Celite (entries 8-10). As with *rac*-**5** (entry 7), the product yields reached the levels of 59-75%. Moreover, *rac*-**1** was transformed into (*S*)-**3** with excellent enantiopurity (ee>99%). However, also for the reaction in acetonitrile considerable amount of the unreacted substrate (24%, ee_S 7%, entry 8) stayed in the reaction mixture with Novozym 735 –based CAL-A on Celite, indicating that this preparation is less stable than the other commercial enzyme powders under the DKR conditions (entries 9 and 10).

Table 2. Acylation of *rac*-**1** (0.050 M) and *rac*-**5** (0.100 M) with vinyl butanoate (4 equiv.) by CAL-A preparations (50 mg mL^{-1} for *rac*-**1;** 75 mg mL^{-1} for *rac*-**5**) in the presence of triethylamine (1 eq.) at 48 °C (t=48 h)

Entry	Substrate	CAL-A preparation	solvent	Substrate		Product	
				ee_s (%)	Yield (%)	ee_p (%)	Yield (%)[a]
1	*rac*-**5**	CAL-A-T2-150	TBME	3	10	98	43
2	*rac*-**5**	CAL-A-CLEA	TBME	3	34	95	19
3	*rac*-**5**	CAL-A on Celite[b]	TBME	7	13	98	48
4	*rac*-**5**	CAL-A on Celite[b]	TBME+3Å mol. sieves	62	42	97	45
5	*rac*-**5**	CAL-A on Celite[b]	TBME+4Å mol. sieves	70	14	97	26
6	*rac*-**5**	CAL-A on Celite[c]	TBME	-	-	97	61
7	*rac*-**5**	CAL-A on Celite[b]	CH_3CN	6	10	98	60
8	*rac*-**1**	CAL-A on Celite[b]	CH_3CN	7	24	>99	59
9	*rac*-**1**	CAL-A on Celite[d]	CH_3CN	-	traces	>99	69
10	*rac*-**1**	CAL-A on Celite[e]	CH_3CN	-	traces	>99	75

[a] Yield at the conversion where the reaction has stopped. [b] CAL-A dialyzed from Novozym 735 solution before immobilized on Celite. See Experimental Section. [c] Chirazyme L5 (20%) in the presence of sucrose (12%) and Celite (68%). See ref. 12b. [d] Chirazyme L5 (20%) in the presence of sucrose (12%) and Celite (68%). [e] Cat#ICR-112 (20%) in the presence of sucrose (12%) and Celite (68%).

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At least three reasons can be addressed to cause problems in the present DKR with CAL-A: (*i*) acetaldehyde or its condensation products which tend to form adducts with basic amino acid residues of the enzyme, (*ii*) stability of the Schiff base **8** or some of its adduct and (*iii*) ion pair formation of the substrate with an acid obtained when an acyl donor is enzymatically hydrolyzed by the residual water of the seemingly dry enzyme preparation. As to alternatives (*i*) and (*ii*), the previous results with five microbial lipases suggest that acetaldehyde forms Schiff bases with basic amino acid residues of the enzymes and reacts into α,β-unsaturated polyenals which may subsequently form stable Michael-adducts with the enzymes.[22] Even though CAL-A was not included in the study, these results are evidently general to lipases. Indeed, small amounts of unidentified products were detected in the GC- or HPLC-chromatograms of the present DKRs with CAL-A. However, stopping of the acylations of *rac*-**1** or **-5** after 48 h with the Novozym 735 –based CAL-A on Celite cannot be attributed to acetaldehyde alone as in the previous work[12b] Chirazyme L5 –based CAL-A on Celite effectively catalyzed especially the DKR of proline methyl ester (ee_P = 97%, 86% yield after 1 h, 25 ^oC) under the otherwise same conditions.

On the other hand, it is apparent that the residual water may cause significant background hydrolysis of ester substrates and products. The ester functionalities in **1** and **5** are unlikely substrates to enzymatic hydrolysis as the narrow acyl binding pocket of CAL-A hardly accommodates the acyl part of the substrates.[14] In the present work, the residual water hydrolyzed vinyl butanoate producing butanoic acid. The acid can form salt **10**, precipitating the amine substrate out of the reaction mixture, and the salt pair with the amino groups of basic amino acid residues in the protein structure itself. The latter effect, affecting conformational changes, can cause differences in the CAL-A preparations obtained from Chirazyme L5 and Novozym 735. To minimize the salt pair formation one equivalent of triethylamine was added into the reaction mixture to capture the acid. On the other hand, to minimize the hydrolysis of vinyl butanoate and accordingly to better control the amount of releasing acetaldehyde, the DKR was performed in the presence of molecular sieves (3 Å). Considerably less substrate now disappeared (the sum 87% of the substrate and product yields compared to the sum 61%, entries 3 and 4) while the product outcome otherwise was unchanged. When the reaction was repeated in the presence of larger pore size molecular sieves (4 Å) both the substrate and product yields stayed low (entry 5).

As a conclusion, the exceptional property of CAL-A to catalyze N-acylations of secondary amino groups in N-heterocyclic α-amino acids connected to aldehyde-based racemization affords a potential DKR method. The method allows the transformations of racemic proline, pipecolic acid and 4-*N*-Boc-piperazine-2-carboxylic acid methyl esters into the N-acylated *S*-enantiomers at 86-88[12b], 60-61[12b] and 60-75% yields, respectively. The present results address the importance of immobilization for the successful DKR. On the other hand, the history of the CAL-A is evidently important, even if the preparations are finally immobilized on Celite by the same method as in the present work.

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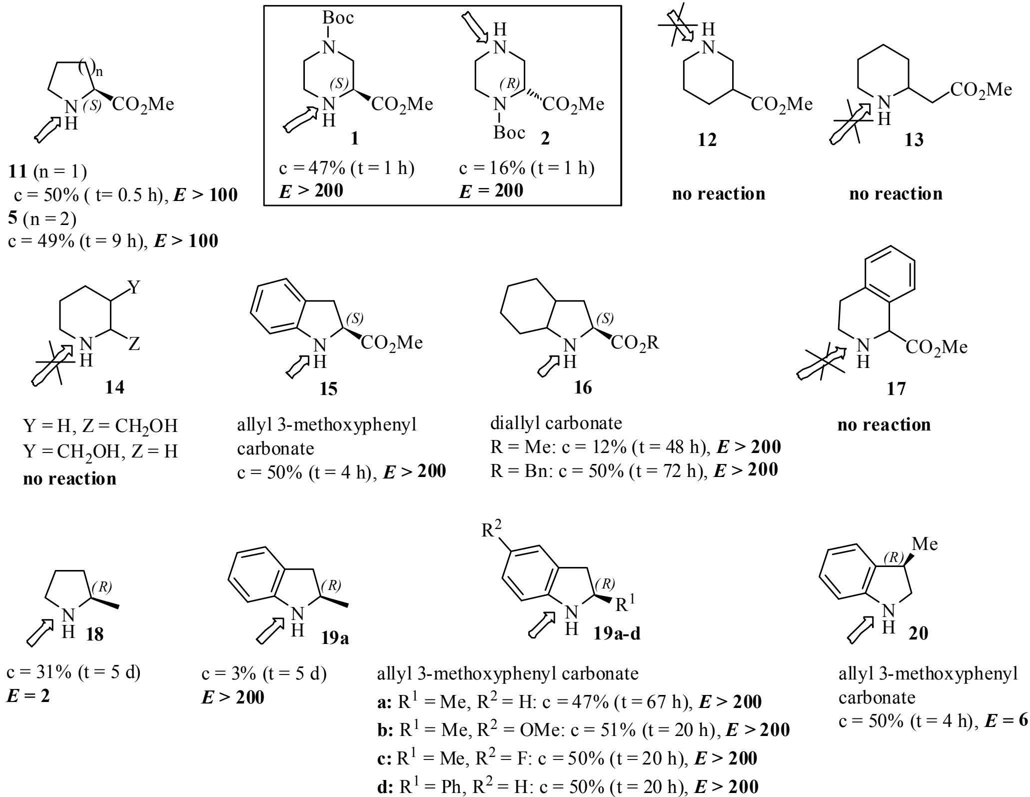
Substrate requirements for CAL-A



Scheme 4. KR of of *N*-heterocyclic amines with 2,2,2-trifluoroethyl butanoate (unless otherwise stated) in TBME. Faster reacting enantiomers have been presented.[12a-g]

Since the first papers on the CAL-A-catalyzed secondary N-acylation of proline methyl ester **11** and pipecolic acid methyl ester **5**,[12a,b] many studies have widened the knowledge of the substrate scope of the enzyme related to the KR of compounds with heterocyclic secondary ring nitrogen (Scheme 4).[12c-g] These substrates include α-amino esters **1**, **5**, **11** and **15-17**, β-amino esters **2**, **12** and **13** as well as other secondary amines **14** and **18-20**. The substrate of the present paper, 4-*N*-Boc-piperazine-2-carboxylic acid methyl ester **1**, together with structurally similar α-amino esters **5**, **11**, **15** and **16** with the *S*-configuration are effectively accepted by CAL-A. The results also show that bicyclic indoline **15** and octahydroindole **16**[12c] rings are allowed but not bicyclic **17,** methyl 1,2,3,4-tetrahydroisoquinoline-1-carboxylate (TIC-1)[12d]. When piperidine-ring contains –CH_2OH **14** either in 2- or 3-positions, secondary N-acylation does not take place.[12e] Although the N-acylated product was observed, it was formed through O→N acyl

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migration after the enzymatic O-acylation. When carboxylic group is one carbon away from the amine nitrogen in β–amino esters **12** and **13**, the N-acylation does not take place.[12f] Therefore, the enantioselective N-acylation of *rac*-**2** observed in this work was not expected. Evidently the presence of the Boc protected α-amino group with possibilities to participate in hydrogen bonding or hydrophobic interactions with CAL-A makes the compound a good substrate. It is interesting to note that the *R*-enantiomer of *rac*-**2** is reactive, whereas in all other cases of Scheme 4 the opposite stereostructure reacts.

The KR of 2-methylpyrrolidine **18** and its analog 2-methylindoline **19a** with 2,2,2-trifluoroethyl butanoate in TBME was investigated in the current study using both Novozym 735 and Chirazyme L5 –based CAL-A on Celite as catalysts to compare the behavior of the secondary amines to α-amino esters **11** and **15**. When the ester functionality of **11** was replaced by methyl **18**, both enantioselectivity and the reaction rate drastically dropped (E=2, c=31% in 5 days) while the aromatic ring in **19a** contributed to the high enantioselectivity of the reaction. This indicates the importance of an electron rich structure (carbonyl group or benzene ring fused with pyrrolidine) in the substrate structure. The group of Gotor managed to enhance the reaction rate of **19a-d** by using various carbonates as acyl donors.[12g] The shift of the asymmetric centre one carbon away from the ring nitrogen **20** causes a marked decrease on enantioselectivity (E = 6).[12g]

Conclusions

The present study describes an efficient CAL-A-catalyzed kinetic resolution of *N*-4-Boc-piperazine-2-carboxylic acid methyl ester (*rac*-**1**) where highly *S*-enantioselective N-acylation of the secondary amino group with trifluoroethyl butanoate yielded both the substrate and the product enantiomers with >99% ee. Similarly, the N-acylation of *N*-1-Boc-2-piperazinecarboxylic acid methyl ester (*rac*-**2**) took place with high enantioselectivity (E=200) under the same conditions in spite of the opposite enantiopreference observed. The transformation of the kinetic resolution of *rac*-**1** into the aldehyde-based dynamic one showed some potential, forming the amide product (*S*)-**3** (ee>99%) at 75% yield from *rac*-**1** in acetonitrile. By-product formation due to the use of acetaldehyde as a racemization catalyst lowered the yield from the theoretical 100% yield. However, potential of the method comes from the fact that acetaldehyde, the racemization catalyst, is formed *in situ* in the reaction mixture from the acyl donor. Thus, mostly used expensive chemical racemization catalysts which often need extremely dry and air-free reaction conditions can be avoided.

The obtained results with the literature data aid conceptualizing the substrate requirements for the secondary N-acylation by CAL-A. The structures so far studied encompass a pyrrolidine, piperidine or piperazine ring. Systematic studies with smaller or larger heterocyclic ring structures or with alicyclic secondary amines have not yet been reported. However, according to the present understanding, reaction rate and enantioselectivity of N-acylation is clearly enhanced

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when carboxylic ester is attached to the asymmetric center next to the secondary nitrogen of the heterocyclic ring. Also the aromatic ring attached to the pyrrolidine ring may aid improving the enantioselectivity. As a conclusion, CAL-A is an appealing catalyst when resolutions based on the N-acylation of the secondary ring nitrogens should be carried out.

Experimental Section

General. *N*-4- and *N*-1-Boc-piperazine-2-carboxylic acid methyl esters (*rac*-**1** and -**2**) and enantiopure (*R*)-**2** were obtained from Fluorochem. Vinyl butanoate and diisopropyl ether were purchased from Fluka and J. T. Baker, respectively. Celite (filter agent, high-purity analytical grade, 167436), (*R*)- and *rac*-2-methylpyrrolidine **18**, 2-methylindoline **19a**, methyl pipecolinate hydrochloride, ethyl butanoate, acetonitrile, vinyl acetate and *tert*-butyl methyl ether were products of Aldrich. CAL-A solution (Novozym® 735) was obtained from Novozymes A/S. Lyophilized CAL-A preparations Chirazyme L5 and Cat#ICR-112 were products of Roche and Biocatalytics, respectively. BCA solution, copper(II) sulfate perhydrate solution (4%) and bovine serum albumin used for the determination of the protein content of Novozym 735 solution were products of Sigma. 2,2,2-Trifluoroethyl butanoate and acetate were synthesized from 2,2,2-trifluoroethanol and the corresponding acid chloride or anhydride by the usual procedures.
The solution state ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using Bruker Avance 500 MHz spectrometers. HRMS was measured in ESI$^+$ mode with a Bruker micrOTOF-Q quadrupole-TOF spectrometer. Optical rotations were determined by Perkin Elmer Polarimeter 341. The protein concentrations were determined by Perkin Elmer Lambda 650 UV/VIS spectrometer.

Purification and immobilization of CAL-A
The CAL-A solution (Novozym 735; 25 mL) containing 40% of glycerol was diluted with phosphate buffer (20 mM, pH 7, 1:1) and dialyzed against it (1 × 15 L and 1 × 7.5 L) and tris buffer (20 mM, pH 7.8, 1 × 7.5 L). The volume of the obtained glycerol-free solution was 110 mL. For the determination of the protein content, the BCA reagent solution (one volume of copper(II) sulfate solution and 50 volumes of BCA solution) and the lipase sample were mixed with vortex and incubated for 30 minutes at 35 °C.[23] After cooling to room temperature (23 °C) the absorbance at 562 nm was measured using bovine serum albumin (0-100 µg/assay) as a standard. The protein content 0.0229 mg ml^{-1} corresponded to 2.52 mg of protein for the total volume of 110 ml. Thus, Novozym 735 contained 10% of protein (2.52 mg protein/25 mL). However, all commercial CAL-A formulations contain protein impurities, which hampers determination of the real CAL-A content.[13e] Therefore, the amounts of Celite and sucrose were calculated based on the lyophilization of 5 mL of glycerol-free lipase solution which gave 0.228 g of solid. To adsorb the lipase, Celite (3.88 g; 68%) and sucrose (0.684 g; 12%) were subsequently added into 110 mL of purified Novozym 735 solution containing 1.14 g (20%) of

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solid, and water was let to evaporate. Thus, 20% proportion of CAL-A used in our preparations contains contaminant proteins, buffer salts and possible stabilizers.

Chirazyme L5 and Cat#ICR-112 were immobilized by dissolving 3.374 g of lyophilized CAL-A (20%), 2.025 of sucrose (12%) and 11.473 g of Celite (68%) into 100 mL of tris buffer (0.014 M; pH 7.8). The slurry was poured into a pot with large area followed by evaporation to dryness (3 days).

Enzymatic reactions

Typical reaction volume for enzymatic reactions was 1-3 mL. Substrate (0.050 M) and an acyl donor (0.10-0.40 M) were dissolved in a solvent. In DKR studies, triethylamine (0.050 M) was added as well. The addition of CAL-A preparation (25-75 mg mL^{-1}) started the reaction. Quantitative analysis of the DKR reactions was performed by using hexadecane as an internal standard (0.010 M). The progress of the reactions was followed by GC with Varian CP-Chirasil-DEX CB (*rac*-**5** and -**19a**) or J&W Scientific Cyclosil-B (*rac*-**18**) columns, or by HPLC with Daicel Chiralcel OD-H column (*rac*-**1** and -**2**). The samples (0.1 ml) were taken at intervals and derivatized with acetic or trifluoroacetic anhydride (butanoate as an acyl donor) or butanoic anhydride (acetate as an acyl donor).

The determination of *E* was based on the equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$.[24] Using linear regression *E* was achieved as the slope of a line. Unless otherwise stated, the calculation of KR conversion was based on the equation $c = ee_S/(ee_S+ee_P)$.

Preparative scale KR

2,2,2-Trifluoroethyl butanoate (618 μL; 4.09 mmol) and *rac*-**1** (0.5005 g; 2.05 mmol) were dissolved in TBME (19.4 mL). Addition of CAL-A (0.5010 g; 25 mg mL^{-1}) started the reaction. The enzyme was filtered off after 3 hours at 50% conversion. Purification by silicagel column chromatography (hexane/EtOAc 1:1 as an eluent) yielded (*R*)-**1** (0.20 g; 0.82 mmol; ee 99%; $[\alpha]_D^{25}$ –39.6° (c 1.0, $CHCl_3$)) and (*S*)-**3** (0.32 g; 1.02 mmol; ee > 99%; $[\alpha]_D^{25}$ –22.4° (c 1.0, $CHCl_3$)).

(*R*)-4-*tert*-butyloxypiperazine-2-carboxylic acid methyl ester [(*R*)-1]. ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 3.75 (s, 3 H, OCH_3), 3.72 (dd, $J_{3a\text{-}3b}$ = –4.0 Hz, $J_{3a\text{-}2}$ = 3.5 Hz, 1 H, H-3a), 3.69 (dd, $J_{3b\text{-}2}$ = 2.9 Hz, 1 H, H-3b), 3.46 (m, 1 H, H-2), 3.08 (m, 1 H, H-6a), 3.04 (dd, $J_{5a\text{-}5b}$ = –4.0 Hz, $J_{5a\text{-}6}$ = 3.5 Hz, 1 H, H-5a), 3.01 (dd, $J_{5b\text{-}6}$ = 3.5 Hz, 1 H, H-5b), 2.75 (m, 1 H, H-6b), 2.15 (s, 1 H, NH), 1.47 (s, 9 H, $C(CH_3)_3$) ppm. ^{13}C NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 187.4 ($C{=}O_{ester}$), 154.6 ($C{=}O_{carbamate}$), 80.0 ($\mathit{C}(CH_3)_3$), 56.8 (C-2), 52.2 (OCH_3), 51.5 (C-5), 44.2 (C-3), 43.2 (C-6), 28.4 ($C(\mathit{C}H_3)_3$) ppm.

(*S*)-1-Butanoyl-4-*tert*-butyloxypiperazine-2-carboxylic acid methyl ester [(*S*)-3]. ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.20 (d, $J_{2\text{-}3}$ = 3.5 Hz, 1 H, H-2), 4.58 (dd, $J_{3a\text{-}3b}$ = –14.0 Hz, 1 H, H-3a), 4.43 (m, 1 H, H-6a), 3.73 (s, 3 H, OCH_3), 3.67 (m, 1 H, H-6b), 3.50 (m, 1 H, H-5a), 3.05 (dd, 1 H, H-3b), 2.85 (m, 1 H, H-5b), 2.37 (dd, $J_{CH2\text{-}CH2CH3}$ = 7.5 Hz, 2 H, $C\mathit{H}_2CH_2CH_3$), 1.68 (m, 2 H, $CH_2C\mathit{H}_2CH_3$), 0.98 (m, 3 H, $CH_2CH_2C\mathit{H}_3$) ppm. ^{13}C NMR (500.13 MHz, $CDCl_3$,

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25 °C): δ = 173.5 (C=O_{ester}), 171.2 (C=O_{amide}), 154.1 (C=$O_{carbamate}$), 80.4 ($C(CH_3)_3$), 55.9 (C-2), 52.8 (C-5), 52.4 (OCH_3), 51.9 (C-3), 42.6 (C-6), 35.3 ($CH_2CH_2CH_3$), 28.3 ($C(CH_3)_3$), 18.4 ($CH_2CH_2CH_3$), 13.9 ($CH_2CH_2CH_3$) ppm. HRMS: calcd. for $C_{15}H_{26}N_2O_5Na^+$ = $[M + Na]^+$ 337.1734; found 337.1734.

Absolute configurations

For the determination of absolute configuration of **1**, (*R*)-**1** (0.19 g; 0.78 mmol; ee 99%) obtained from the KR was dissolved in the mixture of water (15 mL) and 37% HCl (3.2 mL). The reaction took place overnight at room temperature and was thereafter refluxed for 4 hours. The product amino acid was filtered off, dried in a desiccator and characterized (42.4 mg; 0.21 mmol; $[\alpha]_D^{25}$ +4.6° (c 1.0, H_2O)). The corresponding literature value has been reported ($[\alpha]_D^{20}$ +4.74° (c 1.2, H_2O); ee 99.2%).[7]

In the KR of *rac*-**2,** enantiopreference was based on the chiral GC chromatograms observed compared to that of the commercial reference compound, (*R*)-**2**.

Acknowledgements

M.Sc. Paula Herttola is acknowledged for performing part of the experimental work**.** The authors thank the Academy of Finland for financial support (research grant #121983, Fusing Biocatalytic and Chemocatalyzed Reaction Technologies).

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FULL PAPER

DOI: 10.1002/ejoc.201001131

Applying Biocatalysis to the Synthesis of Diastereomerically Enriched Cyanohydrin Mannosides

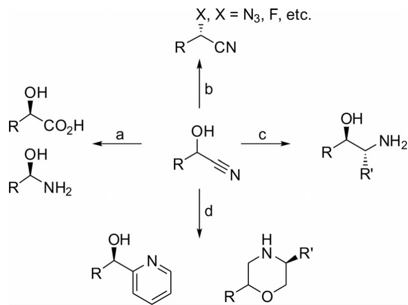
Ari Hietanen,[a] Filip S. Ekholm,[b] Reko Leino,*[b] and Liisa T. Kanerva*[a]

Keywords: Enzyme catalysis / Kinetic resolution / Diastereoselectivity / Carbohydrates

Fully acetylated D- and L-α-mannosylacetaldehydes have been prepared and used as substrates to produce the corresponding cyanohydrins or cyanohydrin acetates with (2*S*) or (2*R*) configuration, respectively, at the cyanohydrin moiety. The (*R*)-oxynitrilase-catalysed synthesis and lipase-catalysed diastereomeric kinetic and dynamic kinetic resolutions were investigated. Sequential catalysis with almond meal [an economic source of (*R*)-oxynitrilase] and *Burkholderia cepacia* lipase was shown to be a straightforward method that yielded the four diastereomeric target cyanohydrins, the absolute configurations of which were confirmed by ^{1}H NMR analysis.

Introduction

Glycosides, both natural and non-natural, currently have many applications in synthetic, medicinal and biological chemistry. Non-natural glycosides display significant potential in several developing areas, including asymmetric synthesis[1a] and carbohydrate-based drug discovery.[1b] They also serve as probes for chemical biology studies.[1c] A good monosaccharide building block for the synthesis of non-natural glycosides should bear an aglycon from which a large number of variations in structure and functional groups can be easily generated. It is also essential that the aglycon structure is accessible in its enantiomeric/diastereomeric forms. Cyanohydrin (α-hydroxy nitrile) glycosides can be considered to be versatile building blocks that fulfil many of the desired properties mentioned above (Scheme 1).[2]



Scheme 1. Funtional groups readily accessible from cyanohydrins: (a) α-hydroxy carboxylic acids and amides, (b) α-functionalized nitriles, (c) 1,2-amino alcohols, (d) N-heterocycles.

Traditional chemical catalysts for the asymmetric synthesis of cyanohydrins from hydrogen cyanide or, more often, from trimethylsilyl cyanide and an aldehyde or ketone include chiral complexes of transition metals (such as titanium alkoxides), other Lewis acids and organocatalysts (such as histidine-derived dioxopiperazines).[2a–2e] In addition, two enzymatic protocols have been thoroughly reviewed.[2f,2g] In more detail, these methods include the enantiofacial addition of hydrogen cyanide to aldehydes (Scheme 2a, routes 1 and 2) and ketones by oxynitrilases (also known as hydroxynitrile lyases, HNLs, E.C. 4.1.2.x) and the kinetic resolution of racemic cyanohydrins (Scheme 2b, route 3) by lipases (E.C. 3.1.1.3). Moreover, the reversible addition of cyanide to carbonyl compounds in the presence of a base has been exploited to convert enzymatic kinetic resolution by acylation into dynamic kinetic resolution (Scheme 2b, routes 3 + 4). In oxynitrilase-catalysed transcyanation (Scheme 2a), acetone cyanohydrin[3] can be used as the cyanide source in reversible reactions in which the enzyme first releases HCN from acetone cyanohydrin, the equilibrium being on the acetone side. This is followed by the enzyme-catalysed addition of HCN to the carbonyl group of the aldehyde. The whole process is based on the higher stability of aldehyde cyanohydrins over the ketone cyanohydrins, which, together with the initial excess of acetone cyanohydrin, drives the system towards the desired chiral product **3**. Accordingly, the use of highly hazardous hydrogen or trimethylsilyl cyanides used in chemical

[a] Institute of Biomedicine/Department of Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry, University of Turku, 20014 Turku, Finland
Fax: +358-2-333-7955
E-mail: lkanerva@utu.fi
[b] Laboratory of Organic Chemistry, Åbo Akademi University, 20500 Åbo, Finland
Fax: +358-2-215-4866
E-mail: reko.leino@abo.fi
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201001131.

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Scheme 2. Enzymatic strategies to diastereomerically enriched cyanohydrins (α-D-mannoside-based cyanohydrin formation shown): (a) oxynitrilase (HNL) catalysed asymmetric synthesis (routes 1 and 2), (b) lipase-catalysed diastereomeric kinetic (route 3) and dynamic kinetic resolution (routes 3 + 4). In this work, the CN^- donor is acetone cyanohydrin.

synthesis is avoided. These biocatalytic methods have been shown to have several attractive features, such as the commercial availability of the biocatalysts from different sources, high stereoselectivity and large variations in accepted substrates.

In this work (*R*)-oxynitrilases and lipases have been used to study the possibility of preparing the diastereomers of α-(2-cyano-2-hydroxy/acetoxy)mannosides (1′*S*,2′*S*,3′*S*,4′*R*,5′*R*,2*S*)-**3**, (1′*S*,2′*S*,3′*S*,4′*R*,5′*R*,2*R*)-**4**, (1′*R*,2′*R*,3′*R*,4′*S*,5′*S*,2*S*)-**3** and (1′*R*,2′*R*,3′*R*,4′*S*,5′*S*,2*R*)-**4** from the corresponding aldehydes D- and L-**2** (Scheme 2). An (*R*)-oxynitrilase [(*R*)-HNL] enzyme was expected to yield through route 2 (Scheme 2) the products D- and L-**3** possessing the absolute configuration (*S*) at C-2. Although stereochemically similar to (*R*)-mandelonitrile (the natural substrate of the oxynitrilases used),[2e,2g] the application of CIP rules now reverses the order of priority of the substituents and, accordingly, the notation of absolute configuration from (*R*) to (*S*). Lipases, on the other hand, show the opposite enantiopreference, and therefore the (*R*)-cyanohydrin acetates D- and L-(2*R*)-**4** were the expected products of route 3. These stereochemical predictions were experimentally confirmed in this work. Fully acetylated sugar aldehydes **2** were obtained in four steps with high yields from D- and L-mannose.

Results and Discussion

Preparation of Aldehydes 2

We have previously described the synthesis of allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**1**), and this substrate was used as the precursor to prepare aldehydes D- and L-**2** (Scheme 3).[4] The synthesis commenced by using slightly modified literature procedures.[5] In short, the alkene functionality was dihydroxylated with a catalytic amount of OsO_4 followed by fragmentation with $NaIO_4$ to give aldehyde D-**2** in almost quantitative yield over two steps. It was noticed that when following the procedure of Binder et al. the yield of the final fragmentation was only 50%.[5b] The main problem was identified as the extraction step for which the reported procedure used only Et_2O. When performing additional extractions with EtOAc and CH_2Cl_2 the isolated yield could be significantly improved. For the preparation of L-**2** a slightly modified glycosylation procedure was used to shorten the reaction time. It was observed that upon heating the reaction mixture at reflux the reaction is essentially complete within 3 h instead of the 24 h required in the absence of heating, and the product can be isolated in good yield (60%). The product was converted into L-**2** by the methods described above and in similar yields. Hence, both enantiomers of the substrate aldehyde were available for the hydrocyanation step.

Scheme 3. Synthesis of aldehydes **2** (α-D-mannoside-based synthesis shown here): Reagents and conditions: (i) OsO_4, NMO, acetone/H_2O (4:1; 91%); (ii) $NaIO_4$, CH_3CN/H_2O (2:1.2; quant.).

(*R*)-Oxynitrilase-Catalysed C–C Bond Formation

The transcyanation of D- and L-**2** with acetone cyanohydrin was investigated. Three (*R*)-oxynitrilase preparations, namely crude almond enzyme known as almond meal, covalently immobilized (*R*)-oxynitrilase from *Prunus amygdalus* (*Pa*HNL) and an aqueous solution of (*R*)-oxynitrilase from *Arabidopsis thaliana* (*At*HNL) were first studied by using D-**2** as substrate. The almond enzyme has a pH optimum at around 5.5–6.0 and has been successfully used at a pH as low as 3.75.[6,7a] Applications of *At*HNL, an α/β-hydrolase fold based (*R*)-oxynitrilase, have proceeded at pH = 5 and possibly enlarged the tolerated substrate scope compared with the almond enzyme alone.[8] Low pH and low water content are beneficial both for the stability of the free cyanohydrin and for the suppression of its chemical formation, which tends to take place in parallel with the enzymatic synthesis. However, the oxynitrilase enzymes are usually inactive under anhydrous conditions. In some cases, immobilization has been shown to render enzymatic activity at negligible water contents and even in neat organic sol-

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vents.[9] *Pa*HNL specifically has been shown to retain its activity for extended periods at the water/diisopropyl ether (DIPE) interface.[10]

Our previous work on transcyanation reactions were based on the use of almond meal [a rich, readily available and inexpensive source of (*R*)-oxynitrilase] in DIPE containing just small amounts (usually 2% v/v) of added water.[7] Almond meal also turned out to be the preferred catalyst for the transcyanation of D-**2** with acetone cyanohydrin (Table 1). Owing to the low solubility of the present glycosides in DIPE, ethyl acetate and toluene were used as solvents. The transcyanation reactions were studied in four sets of reaction media by using two biphasic microaqueous media with 5 and 2 vol.-% of tartrate buffer (0.1 M, pH = 4.0–5.5), toluene saturated with buffer (0.03 vol.-% water) and anhydrous toluene (water content 20 ppm). Initial comparisons indicated much better cyanohydrin formation in toluene than in EtOAc. With regard to the two potential side-reactions under the employed reaction conditions, the α-glycosidic bonds were stable, whereas the spontaneous formation of D-**3** was significant in the absence of the enzyme. Lowering the amount of acetone cyanohydrin from 5 to 1 equiv. suppressed both the enzymatic and non-enzymatic reactions (Entry 5 vs. 4). As expected, the formation of D-**3** in the absence of almond meal considerably decreased when the water content was reduced at a given pH (e.g., Entries 3, 7 and 8) and when the pH was lowered at constant water content (e.g., Entries 5–7). Independent of the pH, cyanohydrin formation was completed in the presence of almond meal at a buffer content ≥2 vol.-% in 2 d. Under these conditions L-**2** behaved nearly identically to its D isomer. However, all the reactions proceeded with negligible diastereoselectivity (*de* ≤ 5%). Changing from a biphasic medium to water-saturated toluene (Entry 8) and further to anhydrous conditions (Entry 9) nearly completely suppressed the reactivity. The selectivity was not improved when almond meal was replaced by covalently immobilized *Pa*HNL or an aqueous solution of *At*HNL (Entries 10–12).

Table 1. Formation of D-**3** by (*R*)-oxynitrilase-catalysed transcyanation in toluene at room temp. after 2 d.

Entry	Biocatalyst	Buffer[a] vol.-%/pH	Yield[b] [%] Enzyme present	Yield[b] [%] Enzyme absent
1	meal	5/5.50	100	100
2	meal	5/4.75	100	100
3	meal	5/4.00	100	44
4[c]	meal	2/5.50	61	37
5	meal	2/5.50	100[d]	83
6	meal	2/4.75	100	77
7	meal	2/4.00	100	8
8[e]	meal	<1/4.00	7	0
9[f]	meal	0/–	11	0
10	*Pa*HNL	2/4.00	39	8
11	*Pa*HNL	0/–	0	0
12	*At*HNL	2/–	100	–

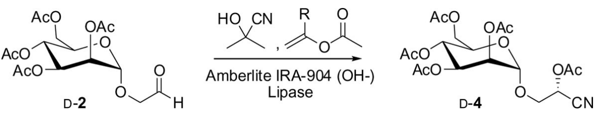
[a] 0.1 M Tartrate buffer. [b] Yields according to ^{1}H NMR spectroscopy by using the peak integrals of the α-H atom of the newly formed chiral centre at C-2. [c] 1 equiv. of acetone cyanohydrin, otherwise 5 equiv. [d] Yield already 100% after 1 d. [e] Toluene saturated with buffer. [f] Dry toluene (water content 20 ppm).

To summarize, although the (*R*)-oxynitrilases used accelerate the formation of cyanohydrins D- and L-**3**, the selectivity is negligible, and chemical transformations accompany the enzymatic reaction.

Lipase-Catalysed Acylation Under Dynamic Conditions

As (*R*)-oxynitrilase preparations were unable to give a sufficient selectivity for the synthesis of D- and L-**3**, attention was focused on the lipase-catalysed diastereomeric dynamic kinetic resolution in toluene (routes 3 + 4, Scheme 2b). Encouraged by the results of previous work,[11]

Table 2. Formation of D-**4** from D-**2** (0.05 M) by diastereomeric dynamic kinetic resolution with lipases in toluene at room temp. after 3 d.



Entry	R	Resin [mg mL^{-1}]	Acetone cyanohydrin [M]	Acyl donor [M]	Lipase	Yield[a] [%]	*de*[a] [%]
1	H	4	0.10	0.15	CAL-B	14	2
2	H	4	0.10	0.15	CAL-A[b]	14	48
3	H	4	0.10	0.15	CAL-A[c]	15	>99
4	H	4	0.10	0.15	Lipase PS C-II	27	>99
5	H	4	0.10	0.15	Lipase PS-D	34/17[d]	>99
6[e]	H	4	0.10	0.15	Lipase PS-D	18	>99
7	Me	4	0.10	0.15	Lipase PS-D	14	>99
8	H	4	0.20	0.30	Lipase PS-D	25	>99
9	H	20	0.20	0.30	Lipase PS-D	51	88

[a] Yield and *de* of D-**4** as determined from HPLC chromatograms. [b] Covalently immobilized CAL-A. [c] CAL-A adsorbed on Celite in the presence of sucrose.[12] [d] Isolated yield. [e] EtOAc instead of toluene.

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reactions involving D-**2**, acetone cyanohydrin (the cyanide donor, 2 equiv.) and vinyl acetate (the acyl donor, 3 equiv.) were studied in toluene in the presence of Amberlite IRA-904 basic resin and one of the lipases from *Candida antarctica* (CAL-A and -B) and *Burcholderia cepacia* (lipase PS-D and PS C-II). In line with these works,[11] D-(2*R*)-**3** reacted with each lipase giving the product D-(2*R*)-**4** (route 3, Scheme 2b). The results are shown in Table 2. Lipase PS-D afforded the highest yields (Entry 5 vs. 1–4), and toluene was superior to EtOAc (Entry 5 vs. 6). Although the selectivity was excellent (*de* > 99%), except with CAL-B and covalently immobilized CAL-A (Entries 1 and 2), the general problem of low yields persisted. Neither replacement of vinyl acetate by isopropenyl acetate (Entry 7) nor increasing of the contents of acetone cyanohydrin and vinyl acetate (Entry 8) improved the yield. Only with a high resin content (20 instead of 4 mg mL^{-1}) was a clear effect on the yield detected but at the cost of selectivity (Entry 9). Separate epimerization experiments with diastereomerically pure D-(2*S*)-**3** indicated that the asymmetric centre at C-2 was completely transformed into a mixture of diastereomers D-**3**. Accordingly, slow epimerization did not explain the low yields observed. Rather the HPLC analysis implied the formation of base-induced condensation side-products.

To summarize, although the diastereomeric dynamic kinetic resolution method is able to produce D-(2*R*)-**4** in a diastereopure form, the need to use a base for racemization renders the yield impractical.

Combination of Almond Meal and Lipase PS-D Catalyses

Because the synthesis of D- and L-**3** with (*R*)-oxynitrilase was high-yielding (but non-selective), whereas the acylation of D-**3** with lipase PS-D was highly diastereoselective (but low-yielding), a combination of the two methods was seen as a fascinating alternative to explore. Note that dry reaction conditions are necessary for lipase-catalysed acylation to prevent the enzymatic hydrolysis of the ester substrate and product and that – although the hydrocyanation step proceeds without an added catalyst – it is notably slower at low water content (e.g., conversion is 61% after 1 d and 83% after 2 d with 2 vol.-% of water: Entry 5, Table 1). Thus, almond meal, as a cheap source of (*R*)-oxynitrilase, provides kinetic rather than selectivity advantage over the uncatalysed alternative when working at this minimal water content. In practice, two methods were studied to achieve our goal. Cyanohydrin D-**3** was first isolated in 82% yield from the transcyanation mixture of D-**2**, acetone cyanohydrin and almond meal in toluene (buffer content 2 vol.-%, pH = 5.5) after 24 h. In this case, the cyanohydrin intermediate can well be prepared at some higher water content without an enzyme, unless one wants to avoid the isolation of the labile cyanohydrin. The isolated product was then subjected to classic kinetic diastereomeric resolution with vinyl acetate and lipase PS-D in toluene to yield cyanohydrin acetate D-(2*R*)-**4** (*de* 93%) and unreacted D-(2*S*)-**3** (*de* 92%). The diastereomeric excesses were somewhat lower than expected on the basis of the results in Table 2. When the resolution was performed in the presence of molecular sieves (4 Å), the *de* values increased to afford diastereomeric ratios *D* (as the analogue of enantiomeric ratio *E*) of over 100.

By another method, the procedure was simplified by leaving the intermediate D-**3** unpurified. Almond meal, in which most of the water (2 vol.-%, pH = 5.5 was used) was impregnated, was removed after completing the transcyanation, and the resulting reaction mixture was dried with Na_2SO_4 before it was subjected to lipase PS-D-catalysed acylation with vinyl acetate in the presence of molecular sieves. This method was applied to both D- and L-**2** and yielded the acetate products D- and L-(2*R*)-**4** and the unreacted cyanohydrins D- and L-(2*S*)-**3** with good stereopurity (Table 3). The L diastereomers gave lower selectivity and were more difficult to separate by conventional column chromatography than the D diastereomers, consistent with the reduced separation also observed on TLC. The isolated, seemingly low yields in Table 3 were calculated with respect to the achiral starting material **2**, but when considering the maximum theoretical yield of 50%, the yields obtained after two steps are more satisfactory.

Determination of Absolute Configurations

The absolute configurations of the unreacted cyanohydrin diastereomers D- and L-**3** were determined by the NMR method developed by Moon et al. using (*R*)- and (*S*)-man-

Table 3. Sequential synthesis and diastereomeric kinetic resolution for the formation of D- and L-(2*R*)-**4** and D- and L-(2*S*)-**3** at room temp. (R corresponds to the peracetylated α-mannoside moiety).

D- or L-**2** → (HO CN acetone cyanohydrin; Almond meal, buffer, $PhCH_3$) → D- or L-**3** → (vinyl acetate; Lipase PS-D, 4Å MS, $PhCH_3$) → D- or L-(2*R*)-**4** + D- or L-(2*S*)-**3**

Substrate	Conversion [%][a]	Time [h]	*D*[b]	(2*R*)-**4** Yield [%][c]	(2*R*)-**4** Purity [%][d]	(2*R*)-**4** *de* [%]	(2*S*)-**3** Yield [%][c]	(2*S*)-**3** Purity [%][e]	(2*S*)-**3** *de* [%]
D-**2**	48	3	>100	35	>99	>99	34	>99	93
L-**2**	51	24	62	31	89	89	25	82	93

[a] Conversion was calculated as $c = de_3/(de_4 + de_3)$. [b] $D = \ln[(1 - c)(1 - de_3)]/\ln[(1 - c)(1 + de_3)]$. [c] Isolated yield as calculated from aldehyde **2**. [d] Contains (2*S*)-**3** as impurity. [e] Contains (2*R*)-**4** as impurity.

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delic acids (in separate samples) in the presence of 4-(dimethylamino)pyridine (DMAP) as chiral shift reagent.[13] In the complex with (*R*)-mandelate the α-proton signal of both D- and L-**3** experiences a larger downfield shift than that of the complex with (*S*)-mandelate [$\Delta\delta^{RS}$ = +0.066 for D-**3** (a) and +0.063 for L-**3** (b), Figure 1]. The positive sign indicates that D- and L-**3** have a configuration similar to (*R*)-mandelonitrile. Bearing the CIP rules in mind, this leads to the assignment of D-(2*S*)-**3** and L-(2*S*)-**3**, respectively.

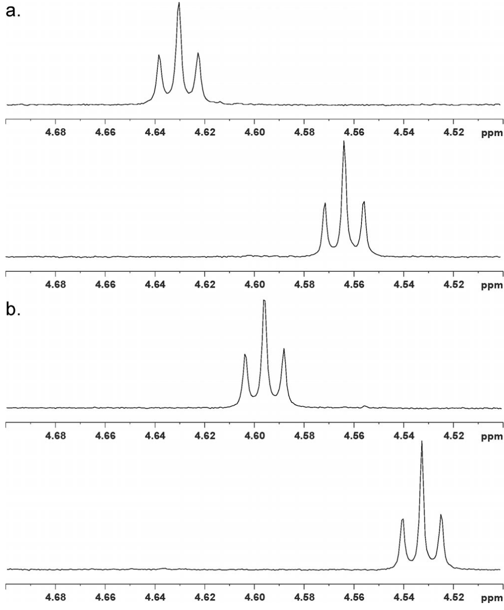

Figure 1. H NMR signals for the α-H of D-**3** (a) and L-**3** (b) complexed with (*R*)-mandelic acid (top) and (*S*)-mandelic acid (bottom) in the presence of DMAP (in $CDCl_3$, calibrated with TMS).

Conclusions

Four mannoside products [D- and L-(2*S*)-**3** and D- and L-(2*R*)-**4**] have been prepared from aldehydes D- and L-**2** by a sequential two-step method with almond meal as the source of (*R*)-oxynitrilase for the transcyanation reaction and lipase PS-D for diastereomeric kinetic resolution by acylation. Although exemplified here with mannosides, the synthetic methodology presented should be equally applicable to other carbohydrates. Kinetic resolution also has the advantage of providing both stereoisomers of the target compound, an important aspect considering its applications, for example, in the biological screening of new molecular entities. Conversion of the carbohydrate cyanohydrins prepared herein to biologically important building blocks by transformations analogous to those depicted in Scheme 1 will be investigated in future work.

Experimental Section

Materials and Methods: All reagents were purchased from commercial sources and used as received. Almond meal was acquired from Sigma as β-glucosidase from almonds, *Pa*HNL (170 U g^{-1}) and CAL-A, both covalently immobilized, were from ChiralVision, *At*HNL (aqueous solution, 39.5 U mg^{-1}, 32 mg mL^{-1} protein) from Evocatal, CAL-B (Novozym 435) and CAL-A powder from Novozymes and lipase PS-D and PS C-II from Amano Europe. Before use CAL-A powder was adsorbed on Celite in the presence of sucrose as described previously, the final lipase content in the preparation being 20% (w/w).[12] Amberlite IRA-904 was purchased from Acros and was conditioned as described previously.[14] HPLC analysis was performed with a Waters 2690 liquid chromatograph equipped with a Daicel Chiralcel OD-H column (0.46 × 25 cm at 23–24 °C) with *i*PrOH (10 vol.-%) in hexane (flow 0.8 mL min^{-1}) as eluent and with a Corona charged aerosol detector. NMR spectra were recorded with a Bruker Avance 600 MHz spectrometer and analysed with PERCH software by using spin simulation/iteration techniques.[15] HR mass spectra were measured in ESI^+ mode with a Bruker micro-TOF-Q quadrupole-TOF spectrometer. Optical rotations were obtained with a Perkin Elmer 241 polarimeter and $[\alpha]_D$ values are given in units of 10^{-1} deg $cm^2 g^{-1}$ at 25 °C unless otherwise indicated. Enzymatic reactions were performed at room temperature (23–24 °C). Column chromatography was performed on silica gel (60 Å, Merck, 230–400 mesh, enriched with 0.1% Ca). All reactions with appreciable amounts of HCN or acetone cyanohydrin were performed in the presence of an HCN sensor and waste containing cyanides was treated with bleach at least overnight followed by acidic destruction of the residues.

Synthesis of Aldehydes D-2 and L-2: As a general procedure for the synthesis of aldehydes, the protected allyl glycoside **1** (1 equiv.) was dissolved in a mixture of acetone/H_2O (4:1, 2 mL/100 mg), and 4-methylmorpholine *N*-oxide (NMO, 2 equiv.) and a catalytic amount of OsO_4 (2.5 wt.-% solution) were added. The mixture was stirred for 4–7 h, diluted with CH_2Cl_2 (30 mL) and washed with brine (30 mL). The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried with Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography with EtOAc as eluent to give a 1:1 mixture of diastereomers. The dihydroxylated glycoside was dissolved in a mixture of CH_3CN/H_2O (2:1.2, 6 mL/100 mg), and $NaIO_4$ (2 equiv.) was added. The reaction mixture was stirred for 1–3 h, diluted with Et_2O (30 mL) and washed with H_2O (30 mL). The aqueous layer was extracted with EtOAc (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine (40 mL), dried with Na_2SO_4, filtered and concentrated to give pure aldehyde.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-α-D-mannopyranosyl)acetaldehyde (D-2): The compound was synthesized from allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**1**; 1.1 g, 2.9 mmol) according to the above general procedure for the synthesis of aldehydes. The dihydroxylated glycoside was obtained as a colourless oil (1.1 g, 91%). Part of this material (230 mg, 0.5 mmol) was used in the fragmentation step to provide the title compound as a colourless oil (210 mg, quant.). $[\alpha]_D^{23}$ = +53 (*c* = 0.33, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 9.74 (dd, $J_{CHO,CH2a}$ = 0.7, $J_{CHO,CH2b}$ = 1.0 Hz, 1 H, C*H*O), 5.40 (dd, $J_{3,2}$ = 3.5, $J_{3,4}$ = 10.1 Hz, 1 H, 3-H), 5.38 (dd, $J_{2,1}$ = 1.8, $J_{2,3}$ = 3.5 Hz, 1 H, 2-H), 5.31 (dd, $J_{4,3}$ = 10.1, $J_{4,5}$ = 10.2 Hz, 1 H, 4-H), 4.28 (dd, $J_{6a,5}$ = 5.5, $J_{6a,6b}$ = –12.2 Hz, 1 H, 6a-H), 4.26 (dd, $J_{CH2a,CHO}$ = 0.7, $J_{CH2aCHO,CH2bCHO}$ = –17.7 Hz, 1 H, CH_{2a}CHO), 4.22 (dd, $J_{CH2b,CHO}$ = 1.0, $J_{CH2bCHO,CH2aCHO}$ = –17.7 Hz, 1 H, CH_{2b}CHO), 4.11 (dd, $J_{6b,6a}$ = –12.2, $J_{6b,5}$ = 2.6 Hz,

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1 H, 6b-H), 4.11 (ddd, $J_{5,4}$ = 10.2, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 2.6 Hz, 1 H, 5-H), 2.17 (s, 3 H, 3-OCOCH_3), 2.10 (s, 3 H, 6-OCOCH_3), 2.06 (s, 3 H, 2-OCOCH_3), 2.01 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 198.2 (*C*HO), 170.6 (6-O*C*OCH$_3$), 169.9 (3-O*C*OCH$_3$, 2-O*C*OCH$_3$), 169.7 (4-O*C*OCH$_3$), 98.3 (C-1), 73.2 (*C*H$_2$CHO), 69.3 (C-5), 69.2 (C-2), 68.8 (C-3), 65.9 (C-4), 62.4 (C-6), 20.9 (3-OCO*C*H$_3$), 20.7 (2-OCO*C*H$_3$, 4-OCO*C*H$_3$, 6-OCO*C*H$_3$) ppm. HRMS: calcd. for $C_{16}H_{22}NO_{11}Na$ [M + Na]$^+$ 413.1054; found 413.1025.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-α-L-mannopyranosyl)acetaldehyde (L-2): The compound was synthesized from allyl 2,3,4,6-tetra-*O*-acetyl-α-L-mannopyranoside (**1**; 350 mg, 0.9 mmol) according to the general procedure for the synthesis of aldehydes to provide the title compound as a colourless oil (270 mg, 77%). $[a]_D^{23}$ = –57 (*c* = 0.45, $CHCl_3$). The ^{1}H and ^{13}C NMR chemical shifts and coupling constants are identical to those of D-**2**. HRMS: calcd. for $C_{16}H_{22}NO_{11}Na$ [M + Na]$^+$ 413.1060; found 413.1061.

Synthesis of D-3 and L-3 with (*R*)-Oxynitrilase: Table 1. Almond meal or *Pa*HNL (20 mg mL^{-1}) was weighed into a reaction vial, tartrate buffer (0.1 M) was added, and the enzyme was allowed to wet for 15–30 min before one of the substrates D-**2** or L-**2** (0.05 M) in toluene or ethyl acetate was added. *At*HNL was added as an aqueous solution (2 vol.-%, 1500 units/mmol substrate). The addition of acetone cyanohydrin (0.25 M) initiated the reaction. The mixture was shaken at room temp. at 172 rpm. Samples (100 µL) were removed through a septum, concentrated and analysed in $CDCl_3$ by ^{1}H NMR spectroscopy.

Diastereomeric Dynamic Kinetic Resolution: Table 2. Amberlite IRA-904 (OH$^-$, 4 or 20 mg mL^{-1}) and an appropriate lipase (50 mg mL^{-1}) were weighed into a reaction vial, and D-**2** (0.05 M) in toluene was added. Vinyl acetate (0.15 or 0.30 M) or isopropenyl acetate (0.15 M) and acetone cyanohydrin (0.10 M or 0.20 M) were added. The reaction mixture was shaken at room temp. at 172 rpm. Samples were removed through a septum and analysed by HPLC without derivatization.

Synthesis and Diastereomeric Kinetic Resolution to Yield (2*R*)-4 and (2*S*)-3: Compound D-**3** was synthesized from D-**2** (821 mg, 2.10 mmol) in toluene (40.29 mL) containing tartrate buffer (0.1 M, 822 µL, 2 vol.-%, pH = 5.5), almond meal (840 mg, 20 mg mL^{-1}) and acetone cyanohydrin (959 µL, 10.50 mmol) by applying the above general procedure. The reaction mixture was shaken at room temp. at 172 rpm. After 24 h at 98% conversion, $NaSO_4$ (2.50 g) was added to dry the reaction mixture The enzyme was removed, washed with toluene (10 mL), and the combined organic phases were added to lipase PS-D (2.50 g, approximately 50 mg mL^{-1}) and molecular sieves (2.50 g, 4 Å). After 3 h (48% conversion), the lipase was filtered off and the filtrate concentrated and purified by column chromatography by using 60% EtOAc in hexane as eluent to yield the product D-(2*R*)-**4** (340 mg, 0.74 mmol, 35%, *de* >99%) and unreacted D-(2*S*)-**3** (296 mg, 0.71 mmol, 34%, *de* 93%). The L diastereomers were prepared similarly to the D isomers from L-**2** (124 mg, 0.32 mmol). Lipase PS-D catalysed diastereomeric kinetic resolution was stopped after 24 h at 51% conversion to yield L-(2*R*)-**4** (47 mg, 0.10 mmol, 31%, *de* 89%) and L-(2*S*)-**3** (34 mg, 0.08 mmol, 25%, *de* 93%).

(2*S*)-2-Hydroxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-propionitrile [D-(2*S*)-3]: Pale-yellow oil. $[a]_D^{25}$ = +68 (*c* = 0.5, $CHCl_3$, *de* 93%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.33 (dd, $J_{2,1}$ = 1.7, $J_{2,3}$ = 3.0 Hz, 1 H, 2-H), 5.31 (dd, $J_{3,2}$ = 3.0, $J_{3,4}$ = 10.1 Hz, 1 H, 3-H), 5.31 (dd, $J_{4,3}$ = 10.1, $J_{4,5}$ = 10.1 Hz, 1 H, 4-H), 4.92 (d, $J_{1,2}$ = 1.7 Hz, 1 H, 1-H), 4.69 (dd, $J_{CHCN,CH2b}$ = 3.8, $J_{CHCN,CH2a}$ = 4.9 Hz, 1 H, C*H*CN), 4.28 (dd, $J_{6a,5}$ = 5.2, $J_{6a,6b}$ = –12.3 Hz, 1 H, 6a-H), 4.16 (dd, $J_{6b,6a}$ = –12.3, $J_{6b,5}$ = 2.2 Hz, 1 H, 6b-H), 4.12 (ddd, $J_{5,4}$ = 10.1, $J_{5,6a}$ = 5.2, $J_{5,6b}$ = 2.2 Hz, 1 H, 5-H), 3.95 (dd, $J_{CH2a,CH2b}$ = –10.6, $J_{CH2a,CHCN}$ = 4.9 Hz, 1 H, CH_{2a}), 3.89 (br., 1 H, OH), 3.84 (dd, $J_{CH2b,CH2a}$ = –10.6, $J_{CH2b,CHCN}$ = 3.8 Hz, 1 H, CH_{2b}), 2.18 (s, 3 H, 3-OCOCH_3), 2.13 (s, 3 H, 6-OCOCH_3), 2.07 (s, 3 H, 2-OCOCH_3), 2.00 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.7 (6-O*C*OCH$_3$), 170.3 (2-O*C*OCH$_3$), 170.1 (3-O*C*OCH$_3$), 169.8 (4-O*C*OCH$_3$), 117.6 (C≡N), 97.9 (C-1), 69.3 (C-5, OCH$_2$), 69.0 (C-2), 65.7 (C-3, C-4), 62.4 (C-6), 60.6 (*C*HCN), 20.8 (2 × OCO*C*H$_3$), 20.7 (2 × OCO*C*H$_3$) ppm. HRMS: calcd. for $C_{17}H_{23}NO_{11}Na$ [M + Na]$^+$ 440.1163; found 440.1202.

(2*R*)-2-Acetoxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-propionitrile [D-(2*R*)-4]: Colourless oil. $[a]_D^{25}$ = +23 (*c* = 0.5, $CHCl_3$, *de* > 99%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.52 (dd, $J_{CHCN,OCH2b}$ = 4.7, $J_{CHCN,OCH2a}$ = 6.8 Hz, 1 H, C*H*CN), 5.30 (dd, $J_{3,2}$ = 3.2, $J_{3,4}$ = 10.2 Hz, 1 H, 3-H), 5.30 (dd, $J_{4,3}$ = 10.2, $J_{4,5}$ = 9.8 Hz, 1 H, 4-H), 5.27 (dd, $J_{2,1}$ = 1.8, $J_{2,3}$ = 3.2 Hz, 1 H, 2-H), 4.92 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1-H), 4.28 (dd, $J_{6a,5}$ = 5.5, $J_{6a,6b}$ = –12.3 Hz, 1 H, 6a-H), 4.13 (dd, $J_{6b,5}$ = 2.4, $J_{6b,6a}$ = –12.3 Hz, 1 H, 6b-H), 4.02 (ddd, $J_{5,4}$ = 9.8, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 2.4 Hz, 1 H, 5-H), 4.02 (dd, $J_{OCH2a,OCH2b}$ = –11.0, $J_{OCH2a,CHCN}$ = 6.8 Hz, 1 H, OCH_{2a}), 3.91 (dd, $J_{OCH2b,OCH2a}$ = –11.0, $J_{OCH2b,CHCN}$ = 4.7 Hz, 1 H, OCH_{2b}), 2.21 [s, 3 H, CH(CN)OCOCH_3], 2.17 (s, 3 H, 3-OCOCH_3), 2.11 (s, 3 H, 6-OCOCH_3), 2.06 (s, 3 H, 2-OCOCH_3), 2.00 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.6 (6-O*C*OCH$_3$), 170.0 (2-O*C*OCH$_3$), 169.8 (3-O*C*OCH$_3$), 169.7 (4-O*C*OCH$_3$), 168.8 [CH(CN)O*C*OCH$_3$], 114.7 (C≡N), 98.2 (C-1), 69.4 (C-5), 69.1 (C-2), 68.6 (C-4), 66.5 (O*C*H$_2$), 65.8 (C-3), 62.3 (C-6), 60.0 (*C*HCN), 20.8 (OCO*C*H$_3$), 20.7 (3 × OCO*C*H$_3$), 20.3 (OCO*C*H$_3$) ppm. HRMS: calcd. for $C_{19}H_{25}NO_{12}Na$ [M + Na]$^+$ 482.1269; found 482.1316.

(2*S*)-2-Hydroxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-α-L-mannopyranosyl)-propionitrile [L-(2*S*)-3]: Pale-yellow oil. $[a]_D^{25}$ = –36 (*c* = 0.5, $CHCl_3$, *de* 93%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.34 (dd, $J_{3,2}$ = 3.5, $J_{3,4}$ = 9.8 Hz, 1 H, 3-H), 5.33 (dd, $J_{2,1}$ = 1.7, $J_{2,3}$ = 3.5 Hz, 1 H, 2-H), 5.28 (dd, $J_{4,3}$ = 9.8, $J_{4,5}$ = 10.5 Hz, 1 H, 4-H), 4.93 (d, $J_{1,2}$ = 1.7 Hz, 1 H, 1-H), 4.64 (dd, $J_{CHCN,OCH2b}$ = 3.4, $J_{CHCN,OCH2a}$ = 4.5 Hz, 1 H, C*H*CN), 4.23 (ddd, $J_{5,4}$ = 10.5, $J_{5,6b}$ = 3.9, $J_{5,6a}$ = 4.8 Hz, 1 H, 5-H), 4.22 (dd, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –11.7 Hz, 1 H, 6a-H), 4.21 (dd, $J_{6b,5}$ = 3.9, $J_{6b,6a}$ = –11.7 Hz, 1 H, 6b-H), 4.04 (dd, $J_{OCH2a,CHCN}$ = 4.5, $J_{OCH2a,OCH2b}$ = –11.9 Hz, 1 H, OCH_{2a}), 4.00 (br., 1 H, OH), 3.92 (dd, $J_{OCH2b,CHCN}$ = 3.4, $J_{OCH2b,OCH2a}$ = –11.9 Hz, 1 H, OCH_{2b}), 2.17 (s, 3 H, 3-OCOCH_3), 2.13 (s, 3 H, 6-OCOCH_3), 2.07 (s, 3 H, 2-OCOCH_3), 2.01 (s, 3 H, 4-OCOCH_3) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.6 (6-O*C*OCH$_3$, 2-O*C*OCH$_3$), 170.0 (3-O*C*OCH$_3$), 169.8 (4-O*C*OCH$_3$), 117.5 (C≡N), 99.2 (C-1), 71.8 (O*C*H$_2$), 69.8 (C-5), 69.2 (C-2), 68.6 (C-3), 65.8 (C-4), 62.6 (C-6), 61.1 (*C*HCN), 20.8 (2 × OCO*C*H$_3$), 20.7 (2 × OCO*C*H$_3$) ppm. HRMS: calcd. for $C_{17}H_{23}NO_{11}Na$ [M + Na]$^+$ 440.1163; found 440.1180.

(2*R*)-2-Acetoxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-α-L-mannopyranosyl)-propionitrile [L-(2*R*)-4]: Pale-yellow oil. $[a]_D^{25}$ = –55 (*c* = 0.5, $CHCl_3$, *de* 89%). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 5.57 (dd, $J_{CHCN,OCH2b}$ = 4.8, $J_{CHCN,OCH2a}$ = 5.5 Hz, 1 H, C*H*CN), 5.32 (dd, $J_{3,2}$ = 3.4, $J_{3,4}$ = 10.1 Hz, 1 H, 3-H), 5.30 (dd, $J_{2,1}$ = 1.8, $J_{2,3}$ = 3.4 Hz, 1 H, 2-H), 5.30 (dd, $J_{4,3}$ = 10.1, $J_{4,5}$ = 10.2 Hz, 1 H, 4-H), 4.92 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1-H), 4.27 (dd, $J_{6a,5}$ = 5.6, $J_{6a,6b}$ = –12.3 Hz, 1 H, 6a-H), 4.15 (dd, $J_{6b,5}$ = 2.3, $J_{6b,6a}$ = –12.3 Hz, 1 H, 6b-H), 4.07 (ddd, $J_{5,4}$ = 10.2, $J_{5,6a}$ = 5.6, $J_{5,6b}$ = 2.3 Hz, 1 H, 5-H), 4.00 (dd, $J_{OCH2a,CHCN}$ = 5.5, $J_{OCH2a,OCH2b}$ = –11.3 Hz, 1 H, OCH_{2a}), 3.93 (dd, $J_{OCH2b,CHCN}$ = 4.8, $J_{OCH2b,OCH2a}$ = –11.3 Hz, 1

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H, OCH_{2b}), 2.20 [s, 3 H, $CH(CN)OCOCH_3$], 2.17 (s, 3 H, 3-$OCOCH_3$), 2.12 (s, 3 H, 6-$OCOCH_3$), 2.06 (s, 3 H, 2-$OCOCH_3$), 2.01 (s, 3 H, 4-$OCOCH_3$) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ = 170.6 (6-$OCOCH_3$), 169.9 (2-$OCOCH_3$), 169.8 (3-$OCOCH_3$), 169.7 (4-$OCOCH_3$), 168.8 [$CH(CN)OCOCH_3$], 114.8 (C≡N), 98.0 (C-1), 69.5 (C-5), 69.1 (C-2), 68.7 (C-3), 66.5 (OCH_2), 65.8 (C-4), 62.4 (C-6), 59.7 (*C*HCN), 20.8 ($OCOCH_3$), 20.7 ($3\times OCOCH_3$), 20.2 ($OCOCH_3$) ppm. HRMS: calcd. for $C_{19}H_{25}NO_{12}Na$ $[M + Na]^+$ 482.1269; found 482.1240.

Supporting Information (see footnote on the first page of this article): ^1H and ^{13}C NMR spectra and HPLC chromatograms of the prepared compounds.

Acknowledgments

The authors thank the Academy of Finland for financial support (research grants #121983 for L. K. and #121334 for R. L., Fusing Biocatalytic and Chemocatalyzed Reaction Technologies).

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Received: August 12, 2010
Published Online: November 12, 2010

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FULL PAPER

DOI: 10.1002/ejoc.201200086

One-Pot Oxidation–Hydrocyanation Sequence Coupled to Lipase-Catalyzed Diastereoresolution in the Chemoenzymatic Synthesis of Sugar Cyanohydrin Esters

Ari Hietanen[a] and Liisa T. Kanerva*[a]

Keywords: Carbohydrates / Enzyme catalysis / Heterogeneous catalysis / Hydrolases / Chiral resolution / Oxidation

A three-step, one-pot synthesis and diastereoresolution sequence is described in anhydrous toluene starting from methyl α-D-2,3,4-tri-*O*-acetylgalacto- (**1a**), -manno- (**1b**) and -glucopyranosides (**1c**). The reaction sequence, including consecutive transformations through the aldehyde [PhI(OAc)$_2$, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)] and cyanohydrin [basic resin or (*R*)-oxynitrilase] into the (6*R*)-cyanohydrin ester (lipase) is shown to proceed in a one-pot cascade, except that the basic resin (when used) should be removed before the addition of the enzymatic acylation reagents. We have shown that the effective transformation of **1a** (75 % reaction yield) through labile intermediates gives the stable (6*R*)-cyanohydrin butanoate (85 % *de*). Further diastereomeric purification by chromatography is possible, although the product is already of high diastereopurity. (6*R*)-Cyanohydrin esters are obtained through acylation with *Burkholderia cepacia* lipase. The (6*S*)-ester (*de* 99 %) is produced by *Candida rugosa* lipase when the sequence is started from **1c** whereas the other sugar derivatives are less suited to the reaction with lipase.

V

Introduction

Modification of natural carbohydrates is a powerful approach both for synthetic objectives and to the elucidation of carbohydrate function in chemical biology studies.[1] When such modifications are required to generate new asymmetric centers, high stereochemical control, in addition to the control caused by the stereostructure of the carbohydrate itself, is necessary. The nonreducing end of a monosaccharide is an important site for synthetic modifications, for instance, for biological recognition processes. On the other hand, the C-6 position of aldohexoses is attractive for structural modifications that allow specific interactions with external substrates. For instance, cyclodextrins modified with cyanohydrin moieties at C-6 can function as efficient hydrolase-like catalysts.[2]

A strategy often employed in C-6 modifications of sugar derivatives consists of initial oxidation of the primary alcohol functionality to the corresponding aldehyde followed by the desired addition reaction. Examples of such reactions include a Staudinger reaction initiated process,[3] reductive amination,[4] Diels–Alder reaction,[5] and Grignard reaction.[6] The addition of formaldehyde dialkylhydrazones to sugar aldehydes followed by oxidative cleavage to yield free or benzyl-protected sugar cyanohydrins has also been reported.[7] Selectivity in these reactions is caused by internal asymmetric induction and leads to diastereomerically enriched products.

Cyanohydrins (α-hydroxynitriles) form a versatile class of compounds for synthetic chemistry as they allow easy access to important compounds, such as α-hydroxy acids and amides, α-functionalized nitriles, 1,2-amino alcohols, and N-heterocycles.[8] In our previous work, a cyanohydrin moiety was introduced into the aglycon part of peracetylated α-D- and α-L-mannosylacetaldehydes to afford novel mannosylglycosides.[9] Stereoselectivity was then achieved through lipase catalyzed acylation after a nonselective hydrocyanation step, affording the diastereomerically enriched epimers of the sugar cyanohydrin and cyanohydrin acetate. We now report the results of our studies on the introduction of a cyanohydrin moiety at the C-6 position of monosaccharides. The chemoenzymatic synthesis includes the deprotection of peracetylated methyl α-D-glycosides (formation of compound **1**, Step a), followed by a one-pot reaction sequence with oxidation (aldehyde **2** formation, Step b), hydrocyanation (C–C bond formation, Step c or c′), and acylation (Step d) at the C-6 position of a sugar pyranoside **3** under mild reaction conditions (Scheme 1). The synthesis was optimized for methyl α-D-galactopyranoside, and the method was then applied to the corresponding α-D-mannose- and α-D-glucose-based glycosides with ^{1}H NMR spectroscopic analysis of the reaction.

The absolute configurations of the cyanohydrin products in Scheme 1 are depicted as was expected on the basis of the models for (*R*)-oxynitrilase [(*R*)-HNL] and many lipase enzymes. Accordingly, the (6*S*)-cyanohydrin was expected

[a] Institute of Biomedicine/Department of Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry, University of Turku, 20014 Turku, Finland
E-mail: liisa.kanerva@utu.fi
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201200086.

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Scheme 1. Synthetic strategy from carbohydrates: (a) regioselective deprotection, (b) oxidation, (c) enzymatic hydrocyanation, (c′) chemical hydrocyanation, (d) enzymatic acylation, and (e) enzymatic deacylation.

to be the major diastereomer obtained from the hydrocyanation reaction catalyzed by (*R*)-oxynitrilase (Scheme 2, a), whereas the formation of the (6*R*)-cyanohydrin ester was suggested to be the major diastereomer with lipase catalyzed *O*-acylation (Scheme 2, b).[10] NMR analysis was used to examine the presumed absolute configurations and it was found that these did not quite follow the expectations with (*R*)-oxynitrilases.

Results and Discussion

Chemoenzymatic Preparation of 1a–c (Step a)

Our approach to the target cyanohydrins and cyanohydrin esters required the secondary alcohol functionalities in the sugar moieties to be protected to block their reactivity later in the lipase catalyzed acylation and to promote solubility in organic solvents, whereas the primary alcohol group at C-6 was to remain unprotected so that the desired cyanohydrin functionality could be introduced (Scheme 1). After peracetylation by using literature methods,[11] a set of readily available lipase preparations was screened to assess their ability to deprotect position C-6 by regioselective methanolysis in diisopropyl ether (DIPE), affording compounds **1a**–**c** (Table 1). Lipase PS-D and PS-C II preparations (*Burkholderia cepacia* lipase immobilized on diatomaceous earth and ceramic, respectively) were effective with the galactoside substrate but displayed very low or no activity toward the other sugar acetates (Table 1, entries 1 and 2). Lipases A (CAL-A adsorbed on Celite) and B (CAL-B as a commercial Novozym 435 catalyst) from *Candida antarctica* and *Candida rugosa* lipase (CRL, adsorbed on Celite) gave generally low product yields for **1a**–**c** (Table 1, entries 3–5). *Thermomyces lanuginosus* lipase (Lipozyme TL IM) allowed highly efficient deprotection irrespective of which sugar was used (Table 1, entry 6), although the reaction was not perfectly selective for C-6, particularly with substrate **1c**. Nevertheless, Lipozyme TL IM was used for the preparative syntheses of **1a**–**c** in good isolated yields (64–84%).

Table 1. Formation of **1a**–**c** by lipase-catalyzed methanolysis of peracetylated methyl α-D-glycopyranosides.[a]

a) MeOH, Lipase, DIPE, 48 °C, 24 h → **1a** (Gal), **1b** (Man), **1c** (Glc)

Entry	Lipase	**1a**[%]	**1b** [%]	**1c** [%]
1	Lipase PS-D	80	0	6
2	Lipase PS-C II	72	0	6
3	Novozym 435	14	36	0
4	CAL-A on Celite	10	0	17
5	CRL on Celite	15	28[b]	64
6	Lipozyme TL IM	95 (81[c])	92 (84[c])	69 (64[c])

[a] [Substrate] = 0.05 M, [MeOH] = 0.25 M, lipase content 50 mg mL^{-1} in DIPE. [b] Reaction time 72 h. [c] Isolated yields.

Oxidation of 1a (Step b)

Both biocatalytic and chemical oxidation are potential methods for the preparation of aldehydes **2a**–**c** from the corresponding alcohols **1a**–**c** (Step b of Scheme 1). The bio-

Scheme 2. Suggested configurations at C-6 based on (a) the benzaldehyde-mandelonitrile model for (*R*)-HNL and (b) the secondary alcohol model for acylation by lipase PS catalysis.

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catalytic oxidation of the primary alcohol group of hexose sugars at C-6 reported in the literature largely rely on the use of galactose oxidase in aqueous solutions, restricting the studies to galactosyl moieties[12] and predisposing the product to side reactions.[13] Methods that rely on the oxidation of a primary alcohol to the aldehyde stage by chloroperoxidase from *Caldariomyces fumago* in organic solvents have been extensively studied,[14] but our efforts to use this approach failed with both unprotected methyl α-D-galactopyranoside and **1a** as substrates.

Literature reports concerning chemical oxidation can be be classified into three broad categories. Dimethyl sulfoxide-based oxidation of acetyl-protected sugars gave α,β-unsaturated aldehyde **7a** as the main product.[15] Catalytic 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) coupled with a stoichiometric oxidant[16] or with a O_2/laccase system[17] for unprotected sugars carries the possibility of overoxidation, affording the carboxylic acid rather than the aldehyde, particularly when water or a base is present. On the other hand, Dess–Martin oxidation is an effective method for sugar oxidation, but the method is typically used in solvents such as dimethyl sulfoxide (DMSO), MeCN, or CH_2Cl_2 that are less suitable for subsequent enzymatic steps.[5,18] The method based on iodine(III) reagents with catalytic TEMPO, also related to the Dess–Martin oxidation, is particularly attractive because the isomerization of chirally labile aldehydes and overoxidation to carboxylic acids should be completely avoided.[18,19] For example, a diacetoxyiodobenzene/TEMPO oxidation of γ,δ-unsaturated primary alcohols coupled to enzymatic hydrocyanation is reported to provide advantages of better atom economy and a more facile work-up compared to the use of Dess–Martin periodinane alone.[20] Accordingly, the oxidation of **1a** with diacetoxyiodobenzene and catalytic TEMPO in dichloromethane resulted in 62% reaction yield for the desired aldehyde **2a** with one major impurity among other unidentified minor impurities (Table 2, entry 1). Whereas **2a** was unstable upon chromatographic purification, the main impurity was separated and shown to be the unsaturated aldehyde **7a**. Toluene and DIPE are solvents commonly used with oxynitrilases and lipases.[21] Oxidation in DIPE was not achieved, evidently due to the low solubility of the hypervalent iodine oxidant (Table 2, entry 3), whereas a yield of 75% for **2a** in toluene was accomplished without the formation of **7a** (Table 2, entry 2). Compared to soluble TEMPO, the use of TEMPO immobilized on polystyrene gave low product yields (15%); thus, soluble TEMPO was used throughout the work. In addition to purification by column chromatography, aqueous work-up was also deleterious for the produced aldehyde, causing product loss to the aqueous phase and the formation of impurities. Therefore, subsequent steps from **1a** in the reaction cascade were performed without isolating the aldehyde.

Table 2. Solvent effects on the oxidation of **1a** (0.050 M) with $PhI(OAc)_2$ (0.055 M)/TEMPO (0.05 M).

Entry	Solvent	**1a** [%]	**2a** [%]	**7a** [%]
1	CH_2Cl_2	0	62	7
2	toluene	0	75	0
3	DIPE	81	4	0

Hydrocyanation of 2a–c (Steps c and c′)

A set of (*R*)-oxynitrilases were first studied for the transhydrocyanation between **2a** and acetone cyanohydrin in buffered microaqueous toluene containing all the reaction components from the previous oxidation Step b (Step c in Scheme 1). The reaction was performed (1) in a one-compartment reactor (all reagents and catalysts present in situ), and (2) in a two-compartment reactor (acetone cyanohydrin decomposes to HCN in the presence of Amberlite IRA-900 HO^- ion exchange resin in one of the compartments, thereafter diffusing freely into the enzymatic reaction mixture in the second compartment).[21] In Method 1, (*R*)-oxynitrilase in toluene containing tartrate buffer (4 vol.-%, 0.10 M, pH 5.4) was responsible for the decomposition of acetone cyanohydrin and for the formation of the sugar cyanohydrin **3a**. Low diastereomeric excess (27% *de*) and product yield (17%) for (6*R*)-**3a** obtained by almond meal catalysis (a rich source of (*R*)-oxynitrilase) were found to be drawbacks of the reaction (Table 3, entry 1). Similar results were obtained with commercial (*R*)-oxynitrilase catalysts from *Prunus amygdalis* (*Pa*HNL) and *Arabidopsis thaliana* (*At*HNL). Evidently, the rapid rate of HCN generation (and resulting in high HCN concentration) in Method 1 caused enzyme inhibition and resulted in low yield in the one-compartment reactor. In Method 2, the reaction yield of (6*R*)-**3a** increased considerably with a moderate improvement in the diastereomeric excess (Table 3, entry 2). Labile **3a** was isolated in 35% yield (37% *de*) by column chromatography, the rest of the product decomposing to

Table 3. Effect of catalyst and cyanide source on the (trans)hydrocyanation of **2a**.

Entry	Catalyst (buffer vol.-%)	CN^- source	Yield [%]	*de* [%]
1	Almond meal[a] (4)	ACH[c]	17[d]	27
2	Almond meal[a] (4)	HCN[d]	74[d]	37
3	– (4)	ACH[c]	0	–
4	Almond meal[a] (0)	ACH[c]	0[d]	–
5	IRA-900 OH^-[b] (0)	ACH[c]	85	52

[a] 50 mg mL^{-1}. [b] 10 mg mL^{-1}. [c] 2 equiv. of acetone cyanohydrin (one-compartment reactor). [d] HCN formed from acetone cyanohydrin (5 equiv., two-compartment reactor).

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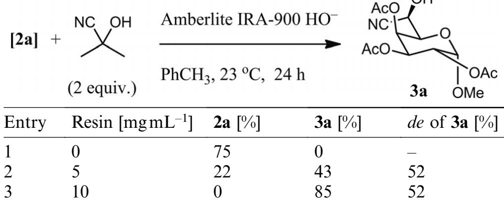
aldehyde **7a** and other side products. Surprisingly, the prevailing diastereomer had the 6*R* rather than the originally expected 6*S* configuration (Scheme 2, a). There was no reaction in the absence of the enzyme under otherwise identical conditions, indicating low enzymatic diastereoselectivity (Table 3, entry 3). The stereostructure of the galactose ring evidently steers the stereochemical outcome of **3a**. The presence of some water is necessary for (*R*)-oxynitrilase catalysis (Table 3, entry 4) and, accordingly, the use of almond meal is preferred over other (*R*)-oxynitrilase catalysts because the water is mainly impregnated in the meal and thus easy to remove for the next step in the reaction cascade.[9]

In addition to the decomposition of acetone cyanohydrin (in the two-compartment reactor case above), the formation of cyanohydrins was also catalyzed by bases (Scheme 1, Step c′). Thus, Amberlite IRA-900 in the HO^- form was used to transform **2a** in the reaction mixture into diastereomerically enriched (6*R*)-**3a** (52% *de*) in high yield in 24 hours using acetone cyanohydrin as the cyanide source (Table 3, entry 5). Thus, asymmetric induction from the galactopyranose ring gave a diastereomeric mixture containing 76 mol-% (6*R*)-**3a** and 24 mol-% (6*S*)-**3a**.

Increasing the resin content from 5 to 20 $mg\,mL^{-1}$ increased the yield of **3a** from 43 to 92% in 24 hours without affecting the diastereomeric excess (Table 4). On the other hand, the formation of **7a** (3% in 3 h and 20% in 24 h) was also clear in the absence of acetone cyanohydrin. Accordingly, the base catalyzes hydrocyanation considerably faster than elimination. When acetone cyanohydrin and the basic resin were both introduced after the oxidation step (add-on synthesis), hydrocyanation proceeded rapidly to 83% yield (2–3 h), and thereafter slowly increased to 92% (Figure 1, filled symbols). To explain the slight increase in yield at this point, we suggest that some condensation side-products might decompose back to the aldehyde when the aldehyde content in the reaction mixture is lowered due to hydrocyanation. However, when the oxidation and hydrocyanation reagents were introduced simultaneously into the toluene solution of **1a**, only a trace amount of aldehyde **2a** was detected and the reaction quickly halted at approximately 20% yield for **3a** (Figure 1, open circles). The reagents used in Steps b and c′ are clearly not fully compatible. Apparently, the base consumes active iodine oxidant and induces condensation reactions.

Table 4. Effect of Amberlite IRA-900 HO^- content on the trans-hydrocyanation of **2a**.



Entry	Resin [$mg\,mL^{-1}$]	**2a** [%]	**3a** [%]	*de* of **3a** [%]
1	0	75	0	–
2	5	22	43	52
3	10	0	85	52
4	20	0	92	52

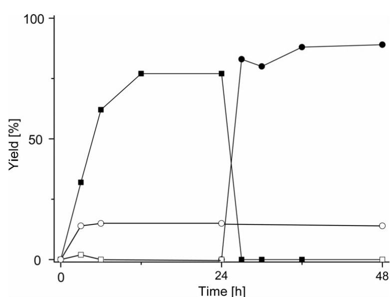


Figure 1. Yields of **2a** (■,□) and **3a** (●,○) with time scale; oxidation/hydrocyanation (20 $mg\,mL^{-1}$ Amberlite IRA-900 HO^-) in one-pot by add-on (filled symbols) and at once (open symbols) reagent additions.

To conclude, chemical hydrocyanation under anhydrous conditions by the add-on principle is most suitable for the production of (6*R*)-**3a** (52% *de* in favor of the 6*R*-diastereomer), whereas the almond meal method gives the product with somewhat lower reaction yield (37% *de*). Pyranoside derivatives **1b** and **1c** were also subjected to the chemical oxidation/hydrocyanation conditions, but the reactions were prone to generate side reactions, producing the cyanohydrins **3b** and **3c** in 77 and 73% reaction yields, respectively, with negligible diastereoselectivity (19 and 5% *de*, respectively). These results indicate the importance of the axial substituent at the C-4 position of the carbohydrate scaffold as a stereocontrol element and the need to protect **2** against acetic acid elimination (formation of **7**).

Lipase-Catalyzed Acylation of 3a–c (Step d)

The production of cyanohydrin esters under basic conditions from aldehydes in the presence of acetone cyanohydrin, a lipase, and an acyl donor is among the oldest dynamic kinetic resolution methods.[22,23a–23c] However, when acetone cyanohydrin, the basic resin, lipase PS-C II, and vinyl butanoate were introduced simultaneously into the reaction mixture obtained through Step b (Scheme 1), the formation of **4a** did not exceed 16%, with the main product being **7a**. The base-catalyzed formation of **7a** means that the dynamic equilibrium between **2a** and **3a** had turned towards the aldehyde. As a possible explanation, the so-called residual water introduced with the seemingly dry lipase favors hydrolysis of vinyl butanoate, disfavoring acylation,[23d] and reduces the stability of **3a**.

The one-pot reaction from **1a**–**c** to diastereomerically enriched **3a**–**c** through Steps a→b→c′ in anhydrous toluene was repeated according to the above principles at 23 °C to carry out the next lipase-catalyzed Step d (and e) of the reaction cascade (Scheme 1). On the basis of the diastereomeric excess values, diastereomerically enriched **3a**–**c** contained 76, 60 and 52 mol-% of galactosyl-, mannosyl- and glucosylpyranoside derivatives as the major dia-

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Table 5. Lipase screening for the acylation of **3a**–**c** with vinyl butanoate in the reaction sequence starting from **1a**–**c**.[a]

Entry	Lipase	**4a** Yield [%]	**4a** *de* [%]	**4b** Yield [%]	**4b** *de* [%]	**4c** Yield [%]	**4c** *de* [%]
1	Lipase PS-D	42	87	65	30	69	19
2	Lipase PS-C II	75	85	65	33	56	42
3	Novozym 435	<1	–	76	12	71	15
4	CAL-A on Celite	10	83	69	25	5	–
5	CRL on Celite	0	–	77	16	27	99[b]
6	Lipozyme TL IM	4	–	<1	–	0	–

[a] Reagents and conditions: Step b: **1a**–**c** (0.05 M), $PhI(OAc)_2$ (1.1 equiv.), TEMPO (0.1 equiv.), toluene, 23 °C, 12 h; Step c′: acetone cyanohydrin (2 equiv.), Amberlite IRA-900 HO^- (20 $mg\,mL^{-1}$), one-pot reactor, 23 °C, 6 h, followed by decantation; Step d: vinyl butanoate (3 equiv.), lipase (50 $mg\,mL^{-1}$), 23 °C, 72 h, absolute configurations at C-6 of **4b** and **4c** unconfirmed. [b] Opposite diastereomeric preference to other lipases.

stereomers (evidently 6*R* also with **3b** and **3c**), respectively. A set of commonly employed microbial lipases and vinyl butanoate was studied next for the acylation of cyanohydrins **3a**–**c** in toluene in the one-pot reaction mixtures obtained after first removing the basic resin by filtration (Table 5, entries 1–6). Lipases PS-D and PS-C II accepted all three cyanohydrin pyranosides as substrates, although the diastereoselectivity was good only for **4a** (85–87% *de*, Table 5, entries 1 and 2). Novozym 435 did not accept galactoside **3a**, and the enzyme displayed very low diastereoselectivities with **3b** and **3c** (Table 5, entry 3). CAL-A (Table 5, entry 4) and CRL (Table 5, entry 5) were markedly active only with mannoside **3b**. The diastereoselectivity of CRL to produce **4c** was excellent (99% *de*) although opposite [evidently producing (*S*)-**4c**] to that of the other lipases studied. In acylation, the stereoselectivity of CRL is known to depend strongly on the structure of the racemic alcohol, with reversed enantioselectivity to common lipases (like lipase PS) being observed, for instance, with cyanohydrin substrates.[24] Lipozyme TL IM was completely inactive for the acylation of cyanohydrins **3a**–**c** (Table 5, entry 6). At this point, investigations with **3b** and **3c** were discontinued because of the disapointing results.

Next, optimization for the preparation of **4a** was continued by studying the effects of lipase PS-C II and vinyl butanoate concentration on the acylation of cyanohydrin **3a**. Acylation was faster when 100 $mg\,mL^{-1}$ of enzyme was used than with 50 $mg\,mL^{-1}$ with a set amount of the acyl donor, however, 80–90% yields were reached irrespective of the amount of acyl donor (Figure 2, a). At the same time, diastereomeric excess values depended only on conversion, allowing the formation of (6*R*)-**4a** at 89–92% diastereomeric excess up to a reaction yield of ca. 60% (Figure 2, b); at this point a sharp drop in the diastereomeric excess was observed as the less reactive (6*S*)-**3a** diastereomer became more active in product formation.

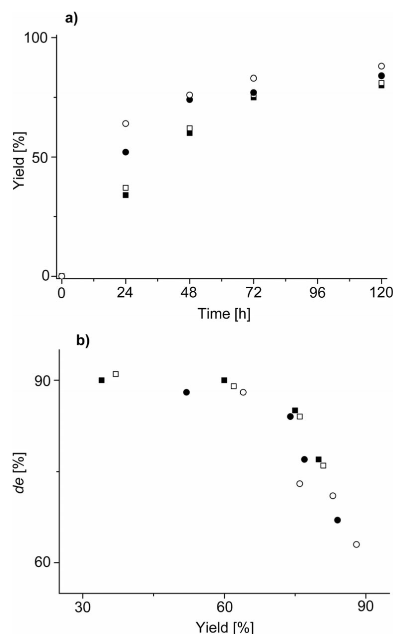

Figure 2. (a) Reaction yields of **4a** vs. time, and (b) diastereomeric excess vs. reaction yields for the lipase PS-C II-catalyzed acylation of **3a** (92%, 52% *de*) in toluene at 23 °C. (■) lipase (50 $mg\,mL^{-1}$) and vinyl butanoate (3 equiv.); (□) lipase (50 $mg\,mL^{-1}$) and vinyl butanoate (6 equiv.); (●) lipase (100 $mg\,mL^{-1}$) and vinyl butanoate (3 equiv.); (○) lipase (100 $mg\,mL^{-1}$) and vinyl butanoate (6 equiv.).

The length of the acyl part of the acyl donor clearly affected reactivity, whereas the diastereomeric excess of **4a**–**5a** mainly depended on conversion (Table 6). Thus, al-

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though the reaction was initially most effective with vinyl acetate (reaction yield 45% in 24 h, Table 6, entry 2), the reaction yield of 75% [approximately the theoretical yield for (6*R*)-**4a**] was reached only with vinyl butanoate in 72 h (Table 6, entry 1). Acylation with vinyl laurate (to give **6a**) was somewhat more selective but impractically slow for efficient application (Table 6, entry 3).

Table 6. Effect of an acyl donor (3 equiv.) on the acylation of **3a** with lipase PS-C II (50 mg mL^{-1}).

1a →(b) **[2a]** →(c') **3a** (92%, *de* 51%) →(d) vinyl ester, Lipase PS-C II, $PhCH_3$, 23 °C → **4a**: R=Pr; **5a**: R=Me; **6a**: R=$(CH_2)_{10}CH_3$

Entry	Vinyl ester	Product	24 h Yield [%]	*de* [%]	72 h Yield [%]	*de* [%]
1	butanoate	**4a**	34	90	75	85
2	acetate	**5a**	45	91	66	81
3	laurate	**6a**	13	96	34	96

Unlike free cyanohydrin **3a**, products **4a** and **5a** allowed the diastereomeric excess to be enhanced upon column chromatography. However, the recoverable yield of the pure diastereomer (de > 99%) remained low due to considerable tailing in the separation, even when highly diastereomerically enriched esters (*de* of the order of 85%) were purified. Thus, in the preparative synthesis, the fractions of both (6*R*)-**4a** and (6*R*)-**5a** were enriched to diastereomeric excesses of more than 99%, with isolated yields of 39–41% (reaction yield in the order of 75%) as calculated over the three steps from **1a**. The activity of lipase PS-C II was then used in the reverse direction by subjecting cyanohydrin acetate (6*R*)-**5a** to methanolysis (under the conditions detailed in Table 1, reaction time 24 h). Free cyanohydrin (6*R*)-**3a** was obtained by simple filtration of the enzyme without chromatography and epimerization in 73% yield.

Absolute Configuration

As described above, the (*R*)-oxynitrilase and lipase models predict different stereoisomeric preferences for the products **3** and **4** (Scheme 2). However, the same diastereomer preferentially forms in the base- (Step c′) as well as in the (*R*)-oxynitrilase-catalyzed (Step c) hydrocyanation, and this is also the preferentially reacting diastereomer in lipase PS-C II catalyzed acylation (Step d). Accordingly, NMR analysis of the diastereomeric mixture of **3a** could be used to confirm the absolute configuration of the obtained cyanohydrin esters.

First, both stereoisomers (6*R*)- and (6*S*)-**4a** have a minimum energy conformation in which H-5 and H-6 are in an *anti*-relationship along the C-5–C-6 bond. The coupling constants $^3J_{5,6}$ measured were 5.4 Hz for the minor and 8.7 Hz for the major diastereomer of **3a**. Thus, H-5 and H-6 of the major diastereomer are clearly in an *anti* orientation whereas there is more *gauche* character in the minor diastereomer. When the torsion angle energies were analyzed with MM2 molecular mechanics, the *gauche* conformations along the C-5–C-6 bond were found to be somewhat lower in energy with (6*S*)-**3a** (ca. 2 kcal mol^{-1} higher than *anti* conformation) than with (6*R*)-**3a** (ca. 5 kcal mol^{-1} higher than *anti* conformation), suggesting that (6*S*)-**3a** is the minor diastereomer.

Secondly, the diastereomeric mixture of **3a** (37% *de*, Table 3, entry 2) was dissolved with (*R*)- and (*S*)-mandelic acid, both in the presence of 4-(dimethylamino)pyridine (DMAP), and the ^{1}H NMR spectra were measured (Figure 3).[25] The downfield shift of H-6 of the minor diastereomer of **3a** in the complex with (*R*)-mandelate-DMAPH$^+$ compared to the shift with the (*S*)-mandelate-DMAPH$^+$ ($\Delta\delta^{RS}$ = +0.032 ppm) implies homochirality with (*R*)-mandelonitrile and, therefore, the assignment (6*S*)-**3a**; the major diastereomer of **3a** had nearly equal shift with both (*R*)- and (*S*)-mandelate-DMAPH$^+$. However, this was also observed with diastereomerically pure **3a** and full verification of the absolute configuration by this method could not be obtained.

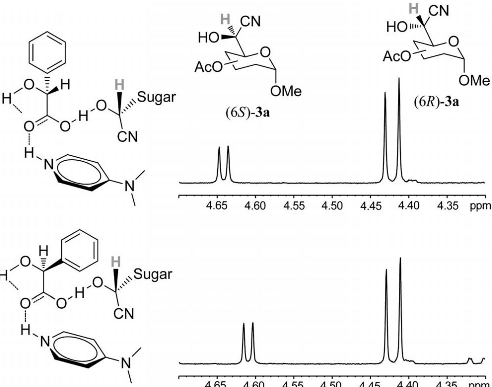

Figure 3. ^{1}H NMR signals of the minor (downfield) and major (upfield) diastereomer of **3a** (37% *de*) in complex with (*R*)-mandelic acid–DMAP (top) and (*S*)-mandelic acid–DMAP (bottom). The models for the respective complexes of (6*S*)-**3a** are shown on left.

Overall, the NMR experiments support, although do not unambiguously confirm, the assignment of (6*R*)-**3a** (contrary to the prediction of Scheme 2, a) and (6*R*)-**4a** configurations to be preferentially formed in the hydrocyanation and lipase PS-C II catalyzed acylation steps.

Conclusions

A straightforward, multistep synthesis and kinetic diastereoresolution sequence has been developed based on bio- and chemo-catalytic reaction steps, starting from peracetylated methyl α-D-glycosides. Essentially, three main reaction

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steps, oxidation [$PhI(OAc)_2$, TEMPO], hydrocyanation (basic Amberlite IRA-900 ion exchange resin and acetone cyanohydrin), and acylation (lipase PS-C II and a vinyl ester) taking place through a cascade sequence in anhydrous toluene turned out to be most applicable. It is essential to note that the sugar aldehydes and cyanohydrins are unstable during chromatographic separation, emphasizing the importance of proceeding without purifying the intermediate products and obtaining stable cyanohydrin esters. The butanoate ester of galactosyl cyanohydrin was prepared with high diastereoselectivity (85% *de*) and reaction yield (75%). Further diastereomeric purification of the ester product by column chromatography afforded fractions of cyanohydrin acetate **5a** and butanoate **4a** as (6*R*)-diastereomers with diastereomeric excesses of more than 99%. However, the yield remained low (31–41%) due to imperfect diastereomeric separation. When coupled to enzymatic deacylation of (6*R*)-**4a** with methanol, the corresponding relatively labile free (6*R*)-cyanohydrin (*de* > 99%) was obtained through simple filtration of the lipase. It has been shown that the selectivity of the method depends on the sugar configuration because mannose- and glucose-derived substrates gave inferior results under the same conditions. In these cases, the method was hampered partly due to side reactions triggered by acetate elimination at the C-4 position of the acetylated sugar aldehyde. Changes in protective group strategy might enable improvements in synthesis yield and selectivity.

Experimental Section

Materials and Methods: Chemical reagents were purchased from commercial sources and used as received unless otherwise mentioned. Methyl-α-D-glycosides, $PhI(OAc)_2$, TEMPO (soluble and on polystyrene, 1.0 $mmol\,g^{-1}$), and acetone cyanohydrin were purchased from Aldrich; vinyl acetate, butanoate, and laurate were purchased from Fluka. Almond meal (β-glucosidase from almonds) was acquired from Sigma, immobilized *Pa*HNL from ChiralVision, aq. *At*HNL from Evocatal, lipase PS-D and PS-C II (*Burkholderia cepacia* lipase) from Amano Europe, and Novozym 435 (*Candida antarctica* lipase B) and Lipozyme TL IM (*Thermomyces lanuginosus* lipase) from Novozymes. CAL-A (*Candida antarctica* lipase A, from Novozymes) and CRL (*Candida rugosa* lipase, from Sigma) powders were immobilized on Celite in the presence of sucrose.[26] Chloroperoxidase from *Caldariomyces fumago* (lyophilized and immobilized preparations) were kindly obtained from Bio-Research Products, Inc. (Iowa, USA). Amberlite IRA-900 ion exchange resin (Aldrich) was conditioned to the HO^- form before use.[23] NMR spectra were measured with a Bruker Avance 500 MHz spectrometer, and high-resolution mass spectra were recorded with a Bruker micro-TOF-Q quadrupole-TOF spectrometer operating in the ESI+ mode. Optical rotations were measured with a Perkin–Elmer 241 polarimeter, and $[\alpha]_D^{25}$ values are given in units of 10^{-1} $deg\,cm^2\,g^{-1}$. Analytical scale reactions were performed on 1–5 mL scale at room temp. (ca. 23 °C) or at 48 °C. Samples (100–200 µL) were analyzed by NMR (samples concentrated and redissolved in $CDCl_3$ with TMS as a calibrant). Whenever sampling resulted in more than ca. 10% loss in reaction volume, parallel reactions were run and sampled once each. Reactions were analyzed using assigned ^1H NMR signals according to Tables 7 and 8. Anomeric signals were assigned based on ^{13}C and HSQC spectra and 1-CH_3O signals correlated to those based on peak integrals. For galactoside derivatives (**Xa**) the signals were confirmed by full assignment of the prepared compounds, however, the assignments are tentative for mannosyl (**Xb**) and glucosyl (**Xc**) compounds. Column chromatography was performed on dried silica gel (60 Å, 230–400 mesh).

Table 7. Partial assignment and chemical shifts of the ^1H NMR signals used in the analysis of galactosides (**Xa**).

	H-1 [ppm]	H-6 [ppm]	OCH_3 [ppm]
1a	5.00	3.68, 3.52	3.42
2a	9.54	5.16	3.46
3a (*R*/*S*)	5.06/5.11	4.40/4.68	3.48/3.45
4a (*R*/*S*)	5.08/5.12	5.48/n.d.	3.49/3.43
5a (*R*/*S*)	5.08/5.12	5.46/n.d.	3.49/3.43
6a (*R*/*S*)	5.08/5.12	5.47/n.d.	3.49/3.43
7a	9.25	5.15	3.52

Table 8. Tentative assignment and chemical shifts of the ^1H NMR signals used in the analysis of mannosides (**Xb**) and glucosides (**Xc**).

	Mannosides (**Xb**)		Glucosides (**Xc**)	
	H-1 [ppm]	OCH_3 [ppm]	H-1 [ppm]	OCH_3 [ppm]
1	4.73	3.41	4.97	3.41
2	4.81	3.45	5.07	3.45
3 (*R*/*S*)	4.83/4.79	3.50/3.45	5.09/5.03	3.50/3.45
4 (*R*/*S*)	4.82/4.78	3.49/3.44	5.09/5.02	3.49/3.44

Syntheses of Alcohols 1a–c. Typical Procedure: Methyl α-D-galactopyranoside (1.01 g, 5.20 mmol) was stirred with I_2 (50 mg) in Ac_2O (5 mL) for 10 min at 23 °C. The reaction was diluted with EtOAc (10 mL) and washed with 10% aq. Na_2CO_3 (2 × 10 mL). The aqueous phases were combined and extracted with EtOAc (2 × 10 mL). The organic phases were combined, dried with Na_2SO_4, concentrated, and filtered through a silica pad (EtOAc/hexane, 4:1; R_f = 0.85) to collect the tetraacetylated product (1.79 g, 4.94 mmol, 95%).

One of the peracetylated sugars (450 mg, 1.24 mmol) and Lipozyme TL IM (620 mg) were shaken in diisopropyl ether (12.15 mL) and MeOH (251 µL, 6.20 mmol) at 48 °C. After 24 h, the enzyme was filtered off and the product was purified by column chromatography (EtOAc/hexane, 4:1; R_f = 0.55] to yield, for instance, **1a** (323 mg, 1.01 mmol, 81%).

The NMR spectra of **1a**–**c** are in accordance with those reported in ref.[27] and HRMS correspond to the sodium adducts of the molecular ion.

Almond Meal Catalyzed Synthesis of Cyanohydrin 3a: In a dried glass vessel with two compartments connected through a class tube between the headspace of the compartments, **1a** (141 mg, 0.440 mmol) was dissolved in anhydrous toluene (8.8 mL) in one of the compartments (compartment 1). $PhI(OAc)_2$ (156 mg, 0.484 mmol) and TEMPO (6.9 mg, 0.044 mmol) were added, and the reaction mixture was stirred at 23 °C. After 12 h, tartrate buffer (0.10 M, pH 5.4, 350 µL) and almond meal (435 mg) were added. For the production of HCN, in compartment 2, hexane (8.8 mL), Amberlite IRA-900 HO^- (88 mg) and acetone cyanohydrin (200 µL, 2.19 mmol) were introduced. The reaction mixtures in both compartments were stirred at 23 °C for 24 h. From the hydrocyanation mixture (compartment 1) the almond meal was filtered and the crude product (74%, 37% *de* by NMR analysis) was purified by column chromatography (EtOAc/hexane, 6:4; R_f = 0.50) to

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collect **3a** (54 mg, 0.156 mmol, 35%, 37% *de*). (6*R*)-**3a**: ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.51 (dd, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 1.0 Hz, 1 H, 4-H), 5.35 (dd, $J_{3,2}$ = 10.9 Hz, 1 H, 3-H), 5.18 (dd, $J_{2,1}$ = 3.7 Hz, 1 H, 2-H), 5.06 (d, 1 H, 1-H), 4.40 (d, $J_{6,5}$ = 8.7 Hz, 1 H, 6-H), 4.17 (dd, 1 H, 5-H), 3.7 (br., 1 H, OH), 3.48 (s, 3 H, OCH_3), 2.20 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.01 (s, 3 H, $COCH_3$) ppm. ^{13}C NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 170.5 (O*C*OCH$_3$), 170.3 (O*C*OCH$_3$), 170.1 (O*C*OCH$_3$), 118.2 (CN), 97.3 (1-C), 68.9 (5-C), 67.9 (2-C), 67.5 (4-C), 67.4 (3-C), 60.1 (6-C), 55.8 (OCH_3), 20.8 (OCO*C*H$_3$), 20.7 (OCO*C*H$_3$), 20.8 (OCO*C*H$_3$) ppm. (6*S*)-**3a**: ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.57 (dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.1 Hz, 1 H, 4-H), 5.37 (dd, $J_{3,2}$ = 10.9 Hz, 1 H, 3-H), 5.23 (dd, $J_{2,1}$ = 3.6 Hz, 1 H, 2-H), 5.11 (d, 1 H, 1-H), 4.68 (d, $J_{6,5}$ = 5.4 Hz, 1 H, 6-H), 4.17 (dd, 1 H, 5-H), 3.7 (br., 1 H, OH), 3.45 (s, 3 H, OCH_3), 2.23 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.03 (s, 3 H, $COCH_3$) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$, 25 °C): δ = 117.4 (CN), 97.5 (1-C), 67.8 (5-C), 67.8 (2-C), 67.8 (4-C), 67.0 (3-C), 60.4 (6-C), 56.1 (OCH_3), 21.1 (OCO*C*H$_3$), 20.9 (OCO*C*H$_3$) ppm (C=O signals too weak for detection). (6*R*/6*S*)-**3a**: HRMS: calcd. for $C_{14}H_{19}NO_9Na^+$ [M + Na]$^+$ 368.0952; found 368.0919.

One-Pot Synthesis of Cyanohydrin Butanoate (6*R*)-4a: Alcohol **1a** (438 mg, 1.37 mmol) was dissolved in anhydrous toluene (27.4 mL), $PhI(OAc)_2$ (485 mg, 1.51 mmol) and TEMPO (21.4 mg, 0.137 mmol) were added and **2a** was formed after 12 h as described above. Amberlite IRA-900 HO$^-$ resin (540 mg) and acetone cyanohydrin (248 µL, 2.72 mmol) were added into the reaction mixture. After 6 h the solution contained (6*R*)-**3a** (86%, 55% *de*). The basic resin was removed by decanting the solution part into a second vessel loaded with lipase PS-C II (1.35 g) followed by the addition of vinyl butanoate (495 µL, 3.90 mmol). The mixture was shaken at 23 °C. After 72 h, the lipase was filtered off from the mixture containing (6*R*)-**4a** (75%, 85% *de*), and the filtrate was washed with EtOAc (3 × 30 mL). The combined filtrates were concentrated and purified by column chromatography (EtOAc/hexane, 6:4; R_f = 0.62) to collect (6*R*)-**4a** (233 mg, 0.56 mmol, 41%, *de* > 99%). $[\alpha]_D^{25}$ = +95 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.48 (d, $J_{6,5}$ = 9.5 Hz, 1 H, 6-H), 5.42 (dd, $J_{4,5}$ = 1.0, $J_{4,3}$ = 3.5 Hz, 1 H, 4-H), 5.35 (dd, $J_{3,2}$ = 11.0 Hz, 1 H, 3-H), 5.14 (dd, $J_{2,1}$ = 3.5 Hz, 1 H, 2-H), 5.08 (d, 1 H, 1-H), 4.32 (dd, 1 H, 5-H), 3.49 (s, 3 H, OCH_3), 2.29 (m, 2 H, C*H*$_2$CH$_2$CH$_3$), 2.11 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 1.98 (s, 3 H, $COCH_3$), 1.65 (m, 2 H, CH$_2$C*H*$_2$H$_3$), 0.95 (t, $J_{CH2-CH3}$ = 7.5 Hz, 3 H, CH$_2$CH$_2$C*H*$_3$) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$, 25 °C): δ = 170.9 (OCOPr), 170.3 (O*C*OCH$_3$), 170.0 (O*C*OCH$_3$), 169.8 (O*C*OCH$_3$), 115.4 (CN), 97.5 (1-C), 67.7 (2-C), 67.4 (5-C), 67.0 (3-C), 66.1 (4-C), 58.1 (6-C), 56.0 (OCH_3), 35.1 (*C*H$_2$CH$_2$CH$_3$), 20.8 (OCO*C*H$_3$), 20.6 (OCO*C*H$_3$), 20.5 (OCO*C*H$_3$), 17.9 (CH$_2$*C*H$_2$CH$_3$), 13.4 (CH$_2$CH$_2$*C*H$_3$) ppm. HRMS: calcd. for $C_{18}H_{25}NO_{10}Na^+$ [M + Na]$^+$ 438.1371; found 438.1402.

One-Pot Synthesis of Cyanohydrin Acetate (6*R*)-5a: Oxidation of **1a** and its hydrocyanation were performed as described above. For acylation, vinyl acetate (6 equiv.) and lipase PS-C II (100 mg mL^{-1}) were applied. After 24 h, a reaction mixture containing (6*R*)-**5a** (71%, 83% *de*) was obtained. Purification by column chromatography (EtOAc/hexane, 6:4; R_f = 0.71) gave (6*R*)-**5a** (39%, *de* > 99%). $[\alpha]_D^{25}$ = +128 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500.13 MHz, $CDCl_3$, 25 °C): δ = 5.46 (d, $J_{6,5}$ = 9.5 Hz, 1 H, 6-H), 5.44 (dd, $J_{4,5}$ = 1.3, $J_{4,3}$ = 3.4 Hz, 1 H, 4-H), 5.35 (dd, $J_{3,2}$ = 10.8 Hz, 1 H, 3-H), 5.15 (dd, $J_{2,1}$ = 3.6 Hz, 1 H, 2-H), 5.08 (d, 1 H, 1-H), 4.32 (dd, 1 H, 5-H), 3.49 (s, 3 H, OC*H*$_3$), 2.12 (s, 3 H, COC*H*$_3$), 2.11 (s, 3 H, COC*H*$_3$), 2.10 (s, 3 H, COC*H*$_3$), 1.98 (s, 3 H, COC*H*$_3$) ppm. ^{13}C NMR (125.77 MHz, $CDCl_3$, 25 °C): δ = 170.3 (O*C*OCH$_3$), 170.1 (O*C*OCH$_3$), 169.8 (O*C*OCH$_3$), 168.2 (O*C*OCH$_3$), 115.3 (CN), 97.5 (1-C), 67.7 (2-C), 67.4 (5-C), 67.0 (3-C), 66.1 (4-C), 58.3 (6-C), 56.0 (OCH_3), 21.0 (OCO*C*H$_3$), 20.6 (OCO*C*H$_3$), 20.5 (OCO*C*H$_3$), 20.0 (OCO*C*H$_3$) ppm (contains traces of **7a**). HRMS: calcd. for $C_{16}H_{21}NO_{10}Na^+$ [M + Na]$^+$ 410.1058; found 410.1074.

Enzymatic Methanolysis of (6*R*)-5a to give (6*R*)-3a: Cyanohydrin acetate (*R*)-**5a** (106 mg, 0.274 mmol, 99% *de*) and lipase PS-C II (273 mg) were shaken in diisopropyl ether (5.42 mL) at 48 °C, and MeOH (55 µL, 1.36 mmol) was added. After 24 h the lipase was filtered to yield (*R*)-**3a** (69 mg, 0.200 mmol, 73%, *de* > 99%). $[\alpha]_D^{25}$ = +117 (*c* = 1.0, $CHCl_3$). NMR spectra and HRMS correspond to those given for (6*R*)-**3a**.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of the prepared compounds.

Acknowledgments

The authors thank the Academy of Finland for financial support (research grant number 121983). This work was sponsored by the European Union (EU) in the frame of the COST Action CM0701.

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Received: January 25, 2012
Published Online: March 19, 2012

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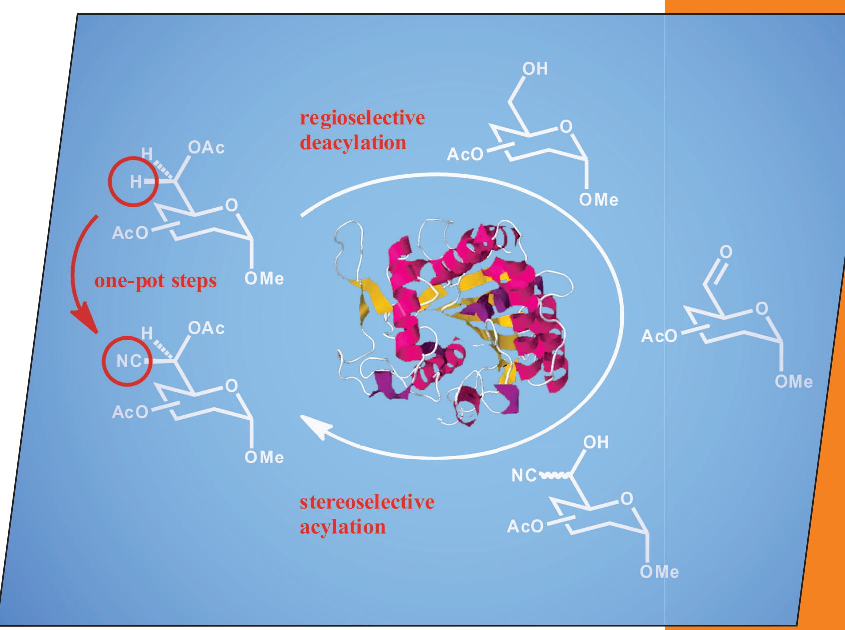

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Microreview

Klaus Ruhland

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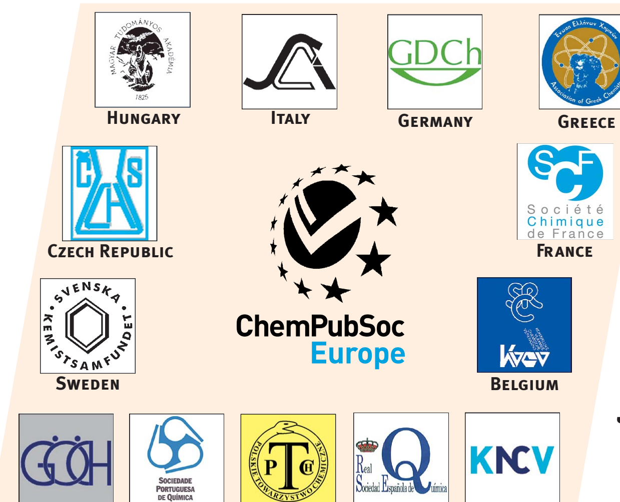

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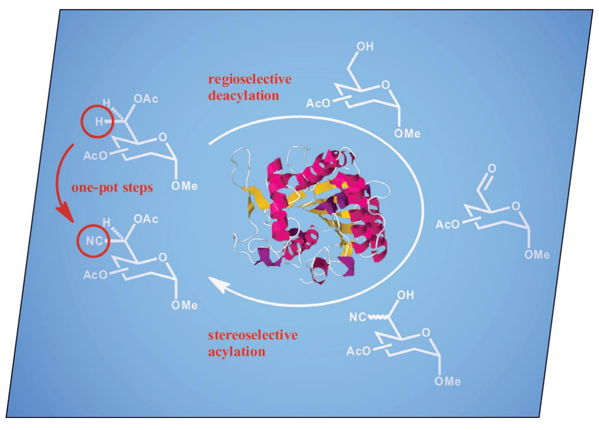
COVER PICTURE

The cover picture shows the key intermediates in the chemoenzymatic sequence in one-pot from peracetylated methyl α-D-glycosides into sugar cyanohydrin acetates. The method allows the stereoselective substitution of hydrogen with a nitrile group through labile intermediates that are not separated. Lipase from *Burkholderia cepacia*, a key enzyme responsible for selectivity in the sequence, is shown in the middle (adopted from pdb entry 1OIL). Details are discussed in the article by A. Hietanen and L. T. Kanerva on p. 2729ff.



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