Stereoselective synthesis of quaternary α-amino acids using chemo- and biocatalysts

Stereoselektive Synthesen von quartären α-Aminosäuren unter Verwendung von Chemo- und Biokatalysatoren

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List of abbreviations

Ac	acetyl
AD	asymmetric dihydroxylation
ADH	alcohol dehydrogenase
AD mix α	commercially available mixture of reagents for performing the Sharpless asymmetric dihydroxylation of alkenes
Boc	*tert*-butoxy carbonyl
br s	broad singulett
CAL-B	*candida antarctica* lipase B
Cbz	carboxybenzyl
CDI	carbonyldiimidazole
config.	configuration
conv.	conversion
d	days
d	dublett
d. e.	diastereomeric excess
d. r.	diasteriomeric ratio
DCM	dichloromethane
DEA	diethyl amine
DMAP	4-dimethylaminopyridine
DMF	dimethyl formamide
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
DPPA	diphenylphosphoryl azide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	enantiomeric excess
EI	electron impact
eq.	equivalents
ETBE	ethyl *tert*-butyl ether
EtOAc	ethyl acetate
FAB	fast atom bombardement
h	hour(s)
HPLC	high performance liquid chromatography
IPA	isopropanol
IR	infrared
J	scalar coupling constant
Konfig.	Konfiguration
l	*like*
m	multiplett

MALDI	matrix assisted laser dissorption/ionisation
m/z	ratio of mass over charge
MS	mass spectrometry
Ms	methanesulfonyl
MTBE	methyl *tert*-butyl ether
NAD(P)H	Nicotinamide adenine dinucleotide (phosphate), reduced form
n-BuLi	*n*-butyl lithium
NMR	nuclear magnetic resonance
PLE	pig liver esterase
ppm	parts per million
Ppy	4-(1-Pyrrolidinyl)pyridine
q	quartett
quant.	quantitative
rac	racemic
r. t.	room temperature
RT	Raumtemperatur (in German "Zusammenfassung")
s	singulett
t	triplett
TA	threonine aldolase
TAA	*tert*-amyl alcohol
TBA	*tert*-butyl alcohol
THF	tetrahydrofurane
TMS	tetramethylsilane
u	*unlike*
U	unit

1. Introduction and motivation

1.1 Introduction

One of the first facts that students of molecular biology learn is that life builds with a set of 20 amino acids, the proteinogenic L-amino acids of type **1**: phenylalanine, leucine, isoleucine, methionine, valine, serine, proline, threonine, alanine, tyrosine, histidine, glutamine, lysine, asparagine, aspartic acid, glutamic acid, cysteine, tryptophane, arginine and glycine. All these amino acids bear at least one hydrogen-atom at the stereogenic α-carbon and all of them (except prochiral glycine) are chiral and have L-configuration. However, it must be pointed out that (like most foundational generalisations of biology) the simple statement that life builds with 20 amino acids is quite simply untrue. In fact it is true that most naturally occurring organisms directly genetically encode this standard set of 20 amino acids.[1] But despite these 20 proteinogenic amino acids thousands of non-proteinogenic amino acids are known to appear in nature. These amino acids can be classified into different sub-classes such as D-amino acids **2**, β-amino acids **3** and quaternary amino acids **4** (Figure 1).

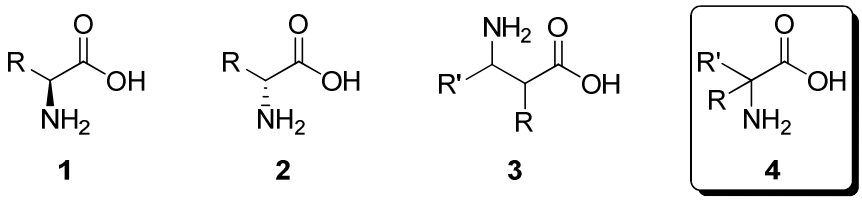


Figure 1. Classes of amino acids

Whereas many proteinogenic amino acids of type **1** are most easily prepared by fermentation[2] and many other elegant syntheses for them exist, synthesis of non-proteinogenic amino acids **2**-**4** is still challenging.[3] Especially ambitious is the synthesis of quaternary amino acids of type **4**, since the enantioselective construction of quaternary stereogenic centers is often arduous.[4] Nevertheless synthesis of quaternary amino acids (or α,α-dialkyl amino acids) **4** is worthwile since these amino acids have often superior properties when compared to other types of amino acids. As they lack the small hydrogen at the α-carbon, peptides containing them are sterical restricted, leading to stereochemical controlled peptide folding.[5] In addition the incorporation of such an amino acid often leads to slower hydrolysis under physiological conditions.[6]

Furthermore, many natural products containing these non-proteinogenic amino acids of type **4** as structural motifs are known.[7] Whereas the widespread oligopeptides called peptaibols,

which are from funghal origin, most often only bear the most simply achiral quaternary amino acid α-aminoisobutyric acid,[8] some other natural products such as lactacystin (**6**) or omuralide (**7**), once isolated from a *Streptomyces* strain,[9] or sphingofungins **9** from the fermentation of *Paecilomyces variotii*[10] are build on more complex chiral amino acid derivatives **8**,**9** (Figure 2).

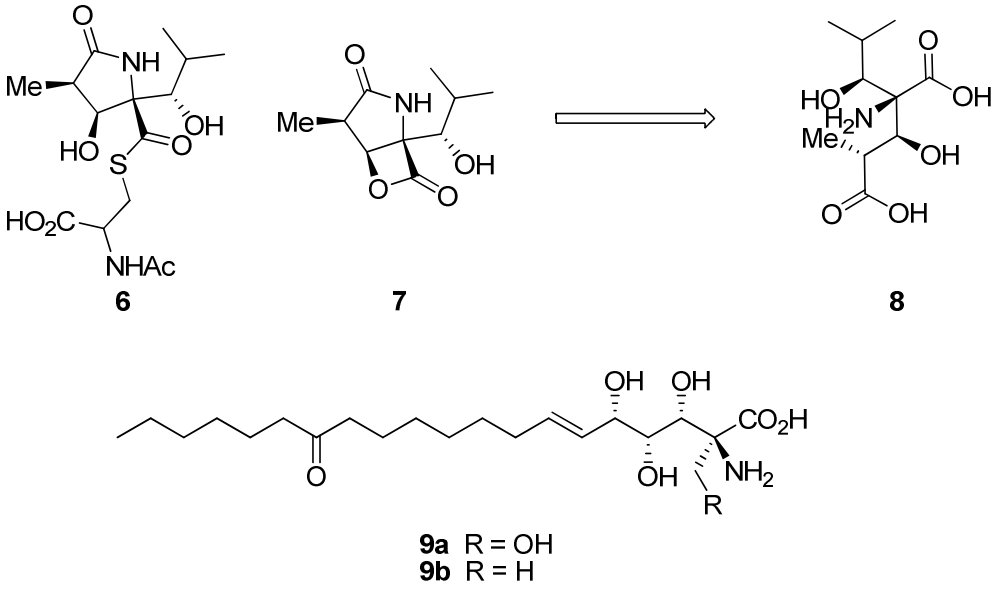


Figure 2. Natural products build on complex quaternary amino acids (**4**)

As many of these natural products show exciting properties such as antibiotic impact (e. g. peptaibols)[11] or enzyme deactivation (e. g. lactacystin (**6**), omuralide(**7**))[9], quaternary amino acids of type **4** and peptides containing them were not only conducted in folding studies of peptides and peptidomimetics,[5] but also in pharma-related studies, for example towards enzyme inhibitions.[6,12]

Nowadays many (potential) applications of quaternary amino acids **4** are known. Besides some potential applications in the nutrition industry,[13] they are more widely used in agricultural and pharmaceutical chemistry.

Fenamidone (**10**, Figure 3) is a fungizide developed by Bayer Crop Science and is known under different trade names such as Fenomen®, Censor®, Consento®, Fenomenal®, Mildex®, Reason® and Verita®. This fungizide is based on the quaternary amino acid (*S*)-α-methyl phenylglycine (**11**) and is used for a broad range of fungal infections in agriculture such as downy mildew and early and late blight.[14]

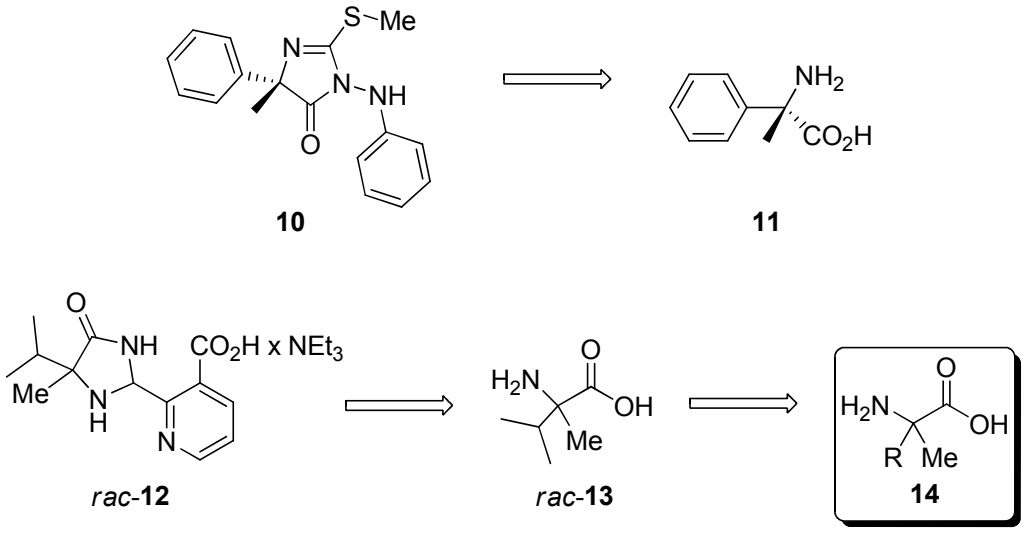


Figure 3. Derivatives of quaternary amino acids applied for agricultural purposes

A herbizide based on *rac*-α-methyl valine (*rac*-**13**) an α-methylated aliphatic amino acid of type **14** is imazapyr (*rac*-**12**), produced by BASF (Figure 3).[15] This non-selective herbizide is selled under various trade names such as Arsenal® or Strigaway® and applied as a spray for season long control of most annual and perennial grasses and broadleaf weeds on non-cropland areas.

In addition to these agricultural applications, some selected examples of applications in pharmaceutical industry are described in the following. Despite some studies of isotope labeled α-methylated tyrosine derivatives in tumor imaging[16] and some reports of α-methyl tryptophane acting as an enzyme inhibitor,[17] the incorporation of α-methyl tyrosine into angiotensin II was studied.[6b] This artificial hormone showed almost the same activity as the natural hormone, but was - in contrast to the non-methylated natural hormone - virtually not degraded by α-chymotrypsin.

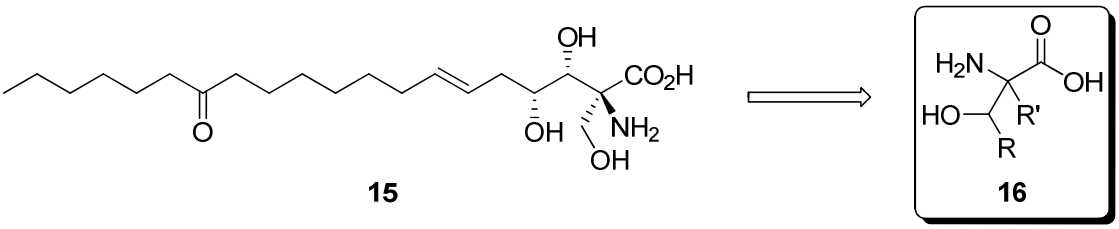


Figure 4. Myriocin (**15**) as a natural product with a β-hydroxy α,α-dialkyl amino acid **16** as structural motif

Myriocin (**15**, Figure 4) as well as the sphinfungins E and F (**9a**,**b**, Figure 2), which bear close resemblance to myriocin, are based on β-hydroxy α,α-dialkyl amino acids **16** as structural

motifs. They are remarkly potent immunorepresants and are furthermore potent serine palmitoyltransferase (SPT) inhibitors with antifungal activities against several human pathogenic fungi.[10,18]

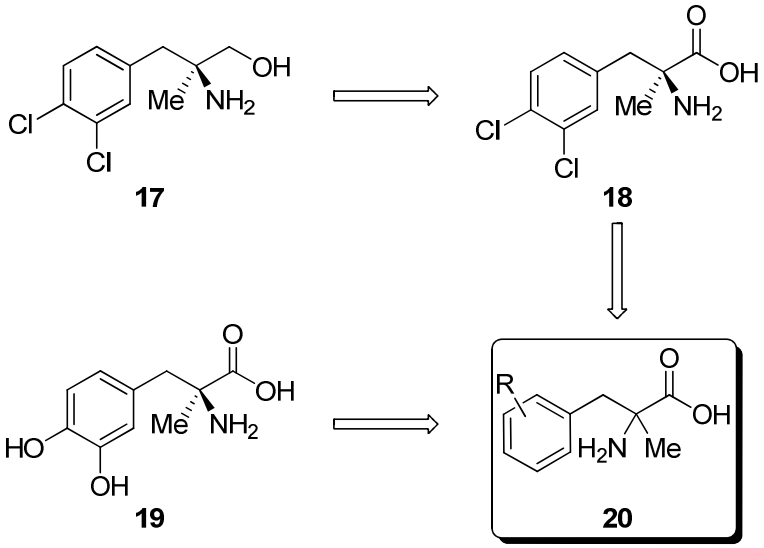


Figure 5. Pharmaceuticals based on α-methylated phenylalanines **20** as structural motif

A potent and selective synaptosomal 5-hydroxytryptamine (serotonin) uptake inhibitor under development is Cericlamine (**17**, Figure 5), an aminoalcohol which can be thought as the reduced form of the α-methylated phenylalaine derivative **18**.[19]

A pharmaceutical being applied today, based on the same structural motif of an α-methylated phenylalanine derivative **20** is L-α-methyl dopa (**19**, Figure 5), which can be purchased under various trade names such as Aldomet®, Aldoril®, Dopamet® and Dopegyt®. **19** is a psychoactive drug used as a sympatholytic or antihypertensive.[20] Even though its use is now deprecated following introduction of alternative safer classes of agents, it continues to have a role in otherwise difficult to treat hypertension and gestational hypertension (also known as pregnancy-induced hypertension (PIH)).[21]

1.2 Motivation

As α,α-dialkyl amino acids (**4**) are interesting structures especially for pharmaceutical purposes there is growing demand for new synthetic approaches. The aim of this thesis is to develop new enantioselective pathways for the synthesis of α,α-dialkyl amino acids (**4**). Three important sub-classes of α,α-dialkyl amino acids, namely β-hydroxy α,α-dialkyl amino acids **16**, α-methylated aliphatic amino acids **14** and α-methylated phenylalanines **20** should be synthesised in enantiomerically enriched form.

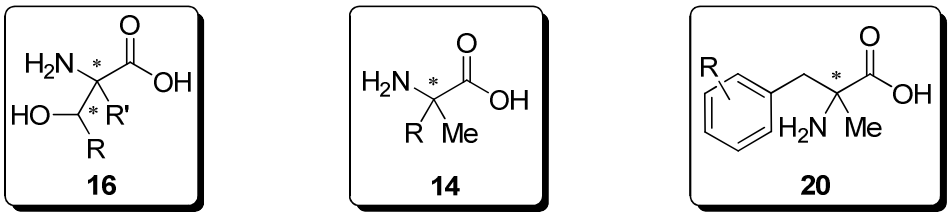


Figure 6. Interesting sub-classes of chiral α,α-dialkyl amino acids **4**

The methods used to access enantiomerically enriched compounds can be divided into three general categories depending on the type of starting material used (chiral pool, racemates or prochirals substrates, Figure 7).[22]

Chiral pool synthesis relies on enantiomerically pure natural products as starting materials. Even though enantiomerical purity is perfect in almost all cases and there is often no need for the construction of stereogenic centers, this method has some major disadvantages. As only a limited number of enantiomerical pure natural products (e. g. L-amino acids, sugars, tartaric acid) are readily available, synthesis of the desired final product often needs many synthetic steps for functional group interconversion. In addition, often only one specific stereoisomer of the product is accessible as only one enantiomer of the starting material is available. Nevertheless, chiral pool is still often the most economically possibility for the synthesis of chiral compounds. For this reason many applications on an industrial scale are known.[23]

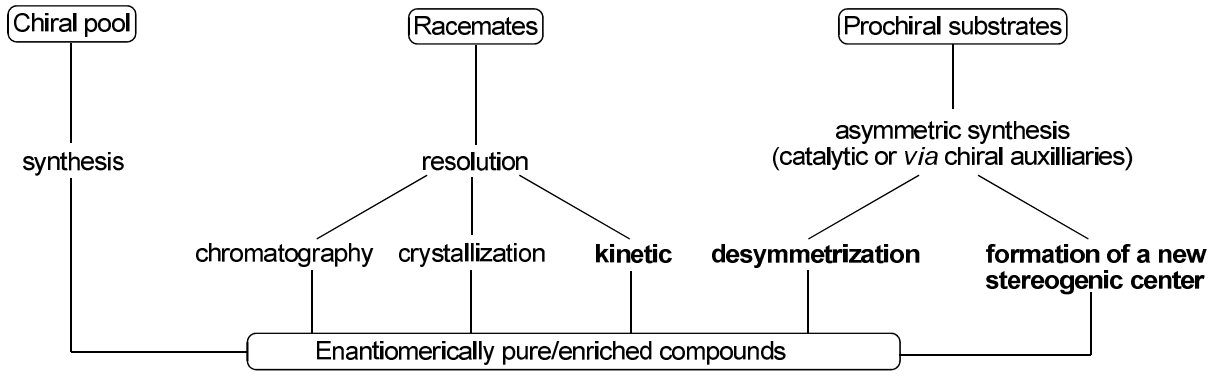


Figure 7. Methods to obtain enantiomerically pure/enriched compounds (methods with the potential to use enantioselective catalytic key steps are shown in bold)

Asymmetric synthesis using chiral auxiliaries has the drawback that an - often expensive - enantiomerically pure compound is needed in stoichiometric quantities. Even if this compound can be recycled, incorporation and removal of the chiral auxiliary means at least two additional synthetic steps. In addition sometimes the availability of the opposite enantiomer is limited since only one enantiomer of the auxiliary is available in some cases.

Classic resolution of racemates by crystallisation or chromatography is often cumbersome, but nevertheless leads to both enantiomers of the desired product. However, stationary (or mobile) phase for chromatography has to be chiral itself. Even though chiral column chromatography often leads to satisfying results, these chiral columns are very expensive and application on industrial scale is often not an appropriate method. In addition, the maximum theoretical yield for each enantiomer is limited to 50%.[24]

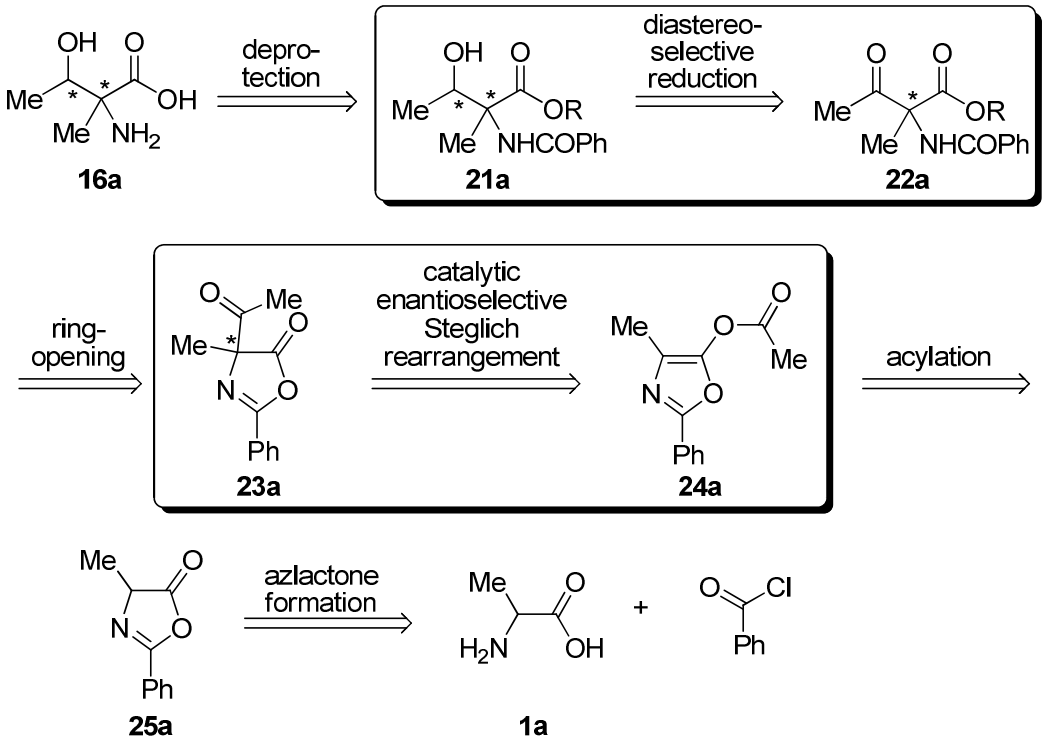
More favoured in modern chemistry are approaches using catalytic methodologies. These approaches have in common that only a small amount of an enantiomerically pure catalyst is needed. Since the catalyst retain unreacted after the reaction recycling of the catalyst is possible in principle. One of these catalytic approaches is kinetic resolution. Even though theoretical yield is limited to 50% for each enantiomer, kinetic resolution is often an attractive approach, especially if the racemic substrate is readily available, as both enantiomers are available in one step. An even more attractive approach is the catalytic desymmetrisation of prochiral substrates, as a theoretical yield of 100% is achievable. An elegant approach is the catalytical enantioselective generation of a new stereogenic center by formation of a new bond, for example *via* addition to a sp^2-hybridised carbon. Prochiral substrates are transferred to enantiomerically pure products and at the same time a new bond for the structure of the desired product is built up.

I wanted to focus on these three catalytic pathways, namely kinetic resolution, desymmetrisation and formation of a new stereogenic center by addition to a sp^2-hybridised carbon. A challenging goal of this thesis is to show that synthesis of all possible stereoisomers of an amino acid is possible by the same synthetic route. Besides this major criterion, these new synthetic routes should meet some other criterias such as:

- Readily available substrates and catalysts
  (commercially available or at least easy to synthesise within a few steps)
- High conversions, yields and selectivities
  (enantiomeric excess in the diastereomerically pure product of approx. 90% ee or higer)
- Few and easy to handle synthetic steps for the total synthesis of amino acids
  (at least fewer than 10 synthetic steps, preferably no inert-gas atmosphere needed)

For the synthesis of β-hydoxy α,α-dialkyl amino acids **16**, I chose the application of an enantioselective Steglich rearrangement as an example for the catalytic formation of a new

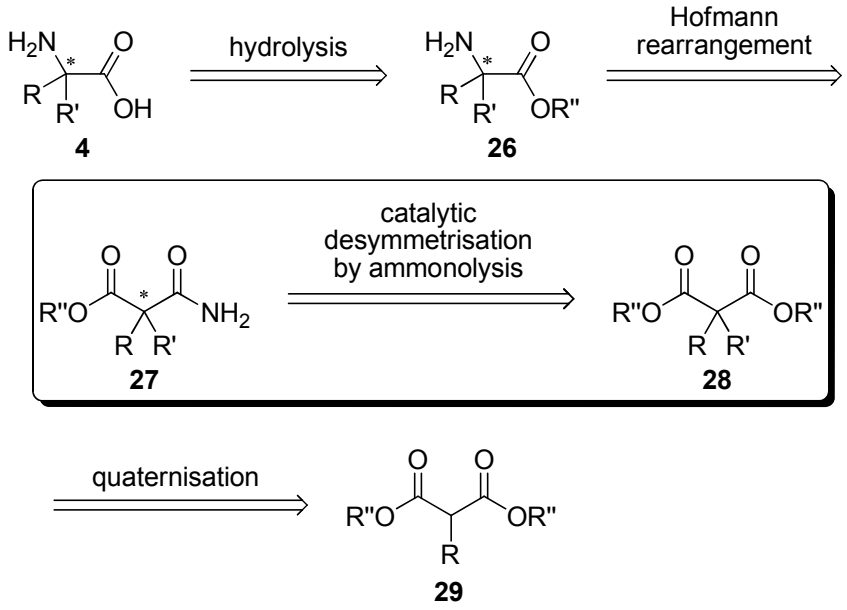
stereogenic center by C-C bond formation. The retrosynthetic concept, examplified for α-methyl threonine (**16a**) is shown in Scheme 1.



Scheme 1. Retrosynthetic analysis for the formation of β-hydroxy α,α-dialkyl amino acids **16** via enantioselective Steglich rearrangement, examplified for α-methyl threonine (**16a**)

Before final deprotection to free amino acid **16a**, a diastereoselective reduction of chiral ketone **22a** bearing a quaternary stereogenic center has to be done. Since numerous stereoselective asymmetric reductions are known, this step should enable the selective reduction of the C=O bond in either the *R*- or the *S*-direction, depending on the absolute configuration of the reducing agent or the catalyst. The chiral ketone **22a** was planned to be prepared by catalytic ring opening of the heterocycle **23a**, which is formed in an enantioselective catalytic Steglich rearrangement reaction starting from prochiral *O*-acylated azlactone derivative **24a**. Substrate **24a** can be easily prepared from azlactone **25a** *via* acetylation. The azlactone itself can be synthesised starting from readily available proteinogenic amino acids **1a** and benzoyl chloride.

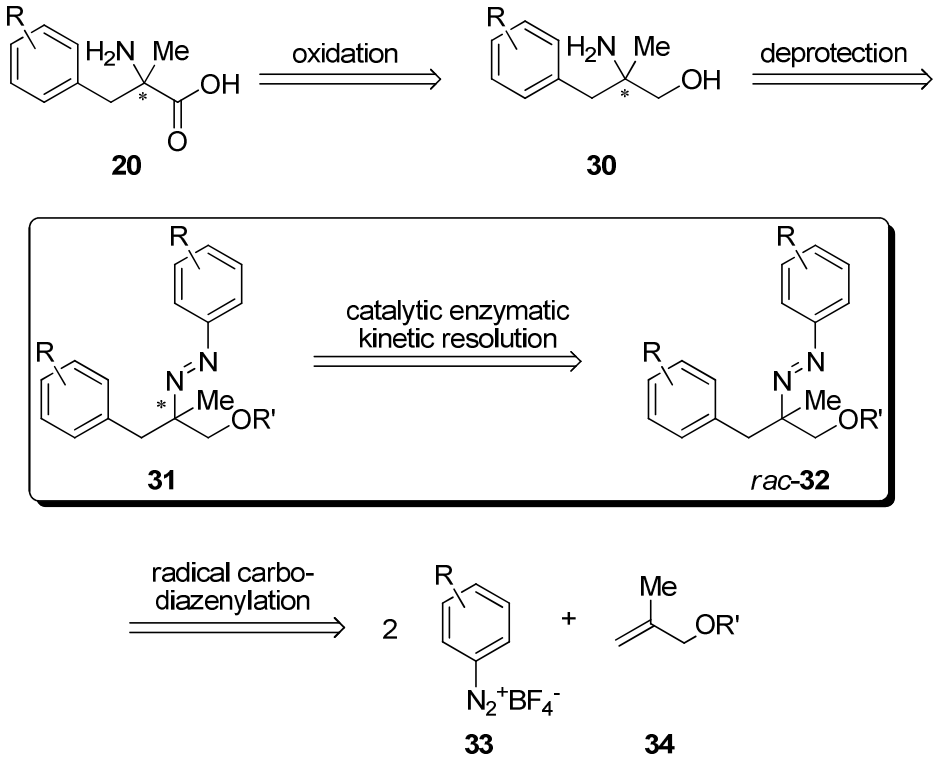
The synthesis of all three desired sub-classes of α,α-dialkyl amino acids **14**, **16**, **20** might be possible in principle by applying a hydrolase-catalysed desymmetrisation of appropriate malonates as the key step. The retrosynthetic concept is shown in Scheme 2 examplified for the synthesis of **4**.



Scheme 2. Retrosynthetic concept for the synthesis of α,α-dialkyl amino acids **4** applying desymmetrisation of malonates **28** as a key step

The desired α,α-dialkyl amino acids **4** are yielded after final hydrolysis of the Hofmann rearrangement product **26** which can be synthesised from chiral malonic acid amide **27**. The key step is the desymmetrisation of a prochiral malonic acid diester **28**, which should be conducted by means of an enzyme catalysed ammonolysis. If no active enzyme for the ammonolysis was found, enzyme catalysed hydrolysis and subsequent (chemical) amidation would be an alternative synthetic strategy. The synthesis of prochiral malonates **28** can be done by alkylation or acylation of malonates **29**. If β-hydroxy amino acids of type **16** are the desired products, an additional diastereoselective reduction step at an appropriate stage will be necessary.

Last, the synthesis of α-methylated phenylalanines of type **20** *via* kinetic resolution of azo compounds *rac*-**32** should be examined. The retrosynthetic concept for this approach is depicted in Scheme 3.



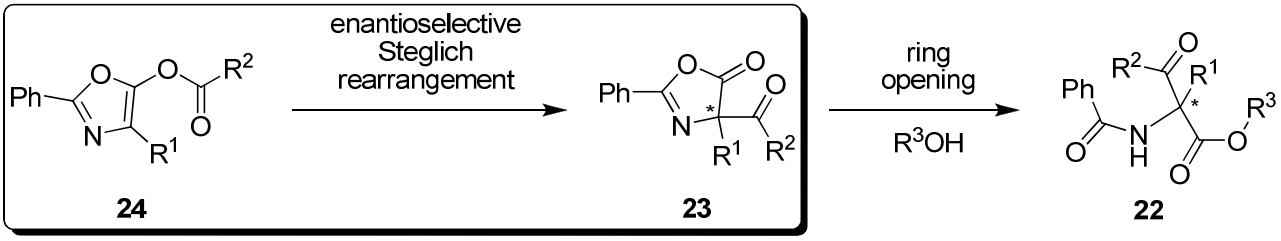
Scheme 3. Retrosynthetic concept for the synthesis of α-methyl phenylalaines **20**

Enantiomerically pure α-methylated phenylalanines **20** can be synthesised by the oxidation of the corresponding amino alcohol **30**, which itself is prepared by deprotection of azo compound **31**. The key step, the enzyme-catalysed kinetic resolution of racemic azo compound *rac*-**32** can be applied either by hydrolysis of an ester or by formation of an ester. The racemic azo compound **32** is formed by radical carbodiazenylation of the olefin **34** applying a method recently developed by the Heinrich group.[25]

In summation, three different catalytic approaches should be studied for the synthesis of three different sub-classes of α,α-dialkyl amino acids. The three shown retrosynthetic concepts have the potential to meet all criteria mentioned above.

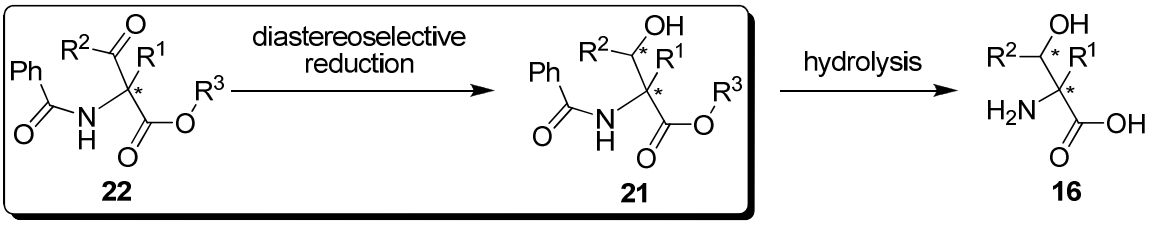
2 Addition to a sp^2-hybridised carbon: Enantioselective Steglich rearrangement

The first novel synthetic concept studied within this thesis is based on a catalytic enantioselective rearrangement of an acyl group such as acetyl from an *O*-acylated azlactone **24** (Steglich rearrangement, Scheme 4).



Scheme 4. Concept for the construction of the quaternary stereogenic center *via* Steglich rearrangement

Within this organocatalytic key step, a C-C-bond is formed, constructing a novel quaternary stereogenic center. Since the desired products, β-hydroxy α,α-dialkyl amino acids **16** in general bear two stereogenic centers, the formation of the second stereogenic center by diastereoselective reduction of the keto-moiety of the ring opened products of the rearrangement reaction **22** represents a second key step (Scheme 5).



Scheme 5. Concept for the construction of the second stereogenic center *via* diastereoselective reduction

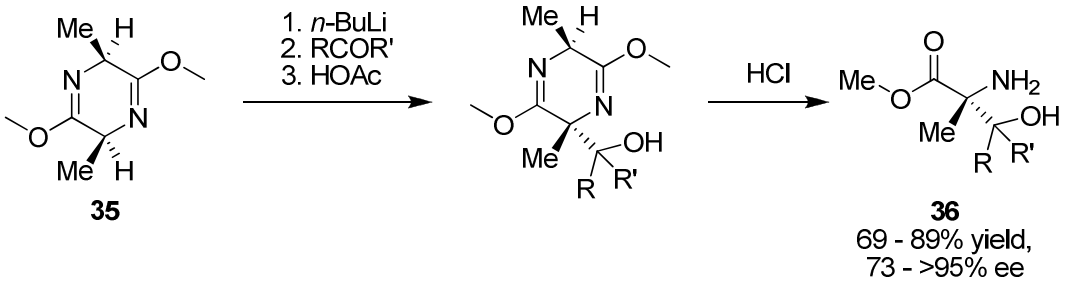
Hydrolysis of the products **21** would then directly furnish the desired β-hydroxy α,α-dialkyl amino acids **16**.

2.1 State of the art

2.1.1 General routes for the synthesis of β-hydroxy-α,α-dialkyl amino acids

Enantioselective synthesis of α,α-dialkyl amino acids **4** is in general difficult, as the formation of a quaternary stereogenic center is still a challenging task for synthetic chemists. The synthesis of β-hydroxy α,α-dialkyl amino acids **16** is even more cumbersome, as a third functional group and – in most cases - a second stereogenic center has to be introduced. However, many chemists faced this challenge in the last decades leading to a number of elegant approaches. Representative examples are described in the following.

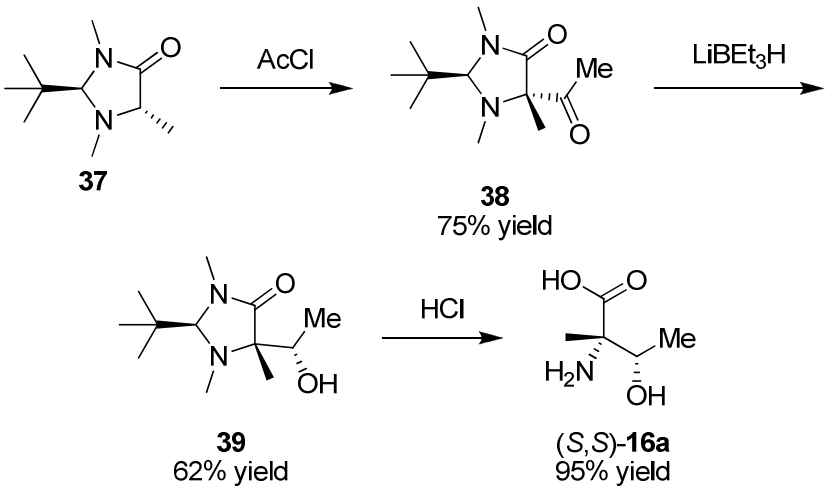
Intensive examination of diastereoselective approaches towards β-hydroxy α,α-dialkylamino acids **16** using chiral auxiliaries began in the early 1980s. In 1980 Schöllkopf *et al.* developed the synthesis of α-methylated β-hydroxy α-amino acid derrivatives **36** by base induced diastereoselective aldol reaction of pyrazine **35** derived from L-alanine (Scheme 6).[26]



Scheme 6. Synthesis of α-methylated β-hydroxy α-amino acids according to Schöllkopf *et al*.[26]

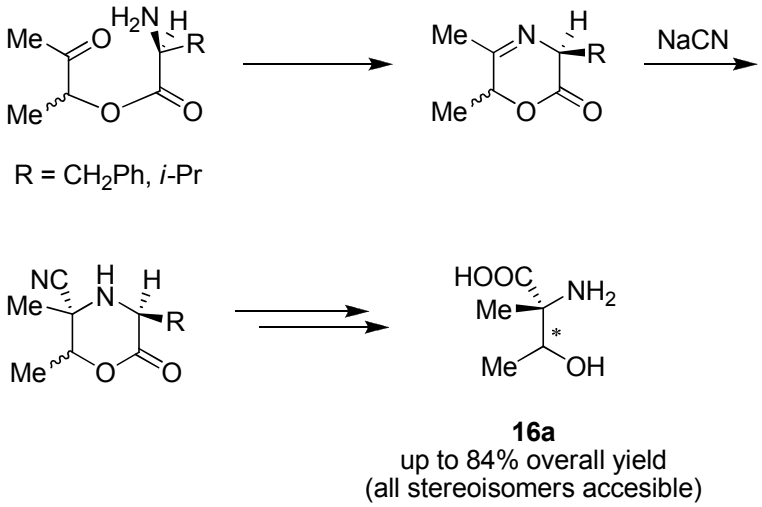
Despite the relatively broad substrate spectra of this short route towards α-methylated β-hydroxy α-amino acid derrivatives **36**, this method had some major drawbacks such as availability of only one stereoisomer of the product **36** and the need of two equivalents L-alaine. However, selectivities are excellent in most cases. Later on Schöllkopf *et al.* improved the diastereoselective synthesis of this type of amino acids by using L-valine instead of a second alanine subunit for the preparation of diketopiperazines, which were applied to aldol reactions.[27]

Seebach *et al.* who diastereoselectively alkylated oxazoline derivatives of threonine in the 1980s,[28] later developed an alternative approach, starting with an alanine-derived oxoimidazolidin derivative **37**. The key step is the diastereoselective acetylation of **37**, followed by a diastereoselective reduction of the keto moiety in **38** and hydrolysis, thus leading to (*S,S*)-α-methyl threonine ((*S,S*)-**16a**) (Scheme 7).[29]



Scheme 7. Synthesis of (*S,S*)-α-methyl threonine ((*S,S*)-**16a**) by diastereoselective acetylation[29]

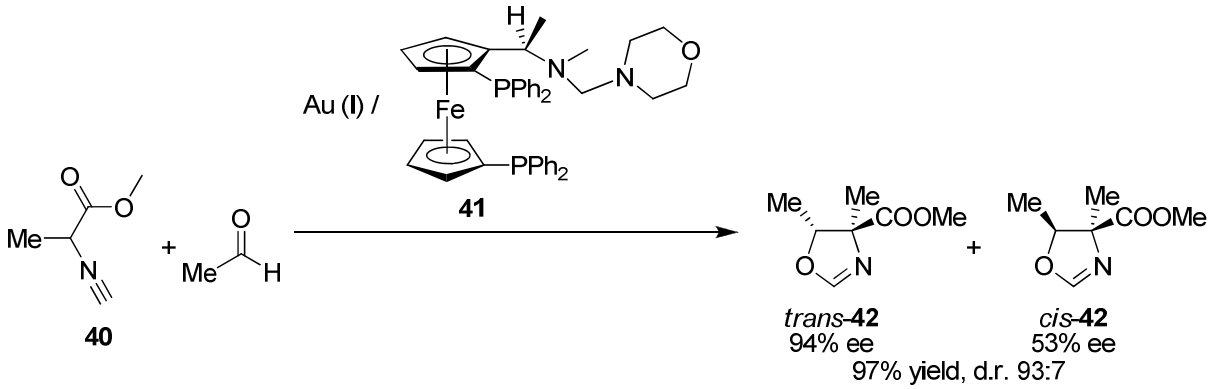
A diastereoselective Strecker reaction was used by Ohfune *et al.* in the synthesis of all stereoisomers of α-methyl threonine (**16a**) applying a phenylalanine or a valine derivative as chiral auxiliary (Scheme 8).[30]



Scheme 8. Diastereoselective strecker reaction towards α-methyl threonine (**16a**)

Even though numerous pathways for the synthesis of quaternary stereogenic centers are well known nowadays,[4] diastereoselective synthesis of β-hydroxy α,α-dialkyl amino acids is still an attractive goal for organic chemists, which can be shown by two recent examples, published within the last three years. An example for a chiral pool based synthesis was described by Wang and Lin, who developed a multistep synthesis towards spingofunghins based on L-(+)-tartaric acid as a chiral pool starting material.[10b] Another recent example is the methylation of a threonine derived bicyclic chrial building block published by Aydillo *et al.*.[31]

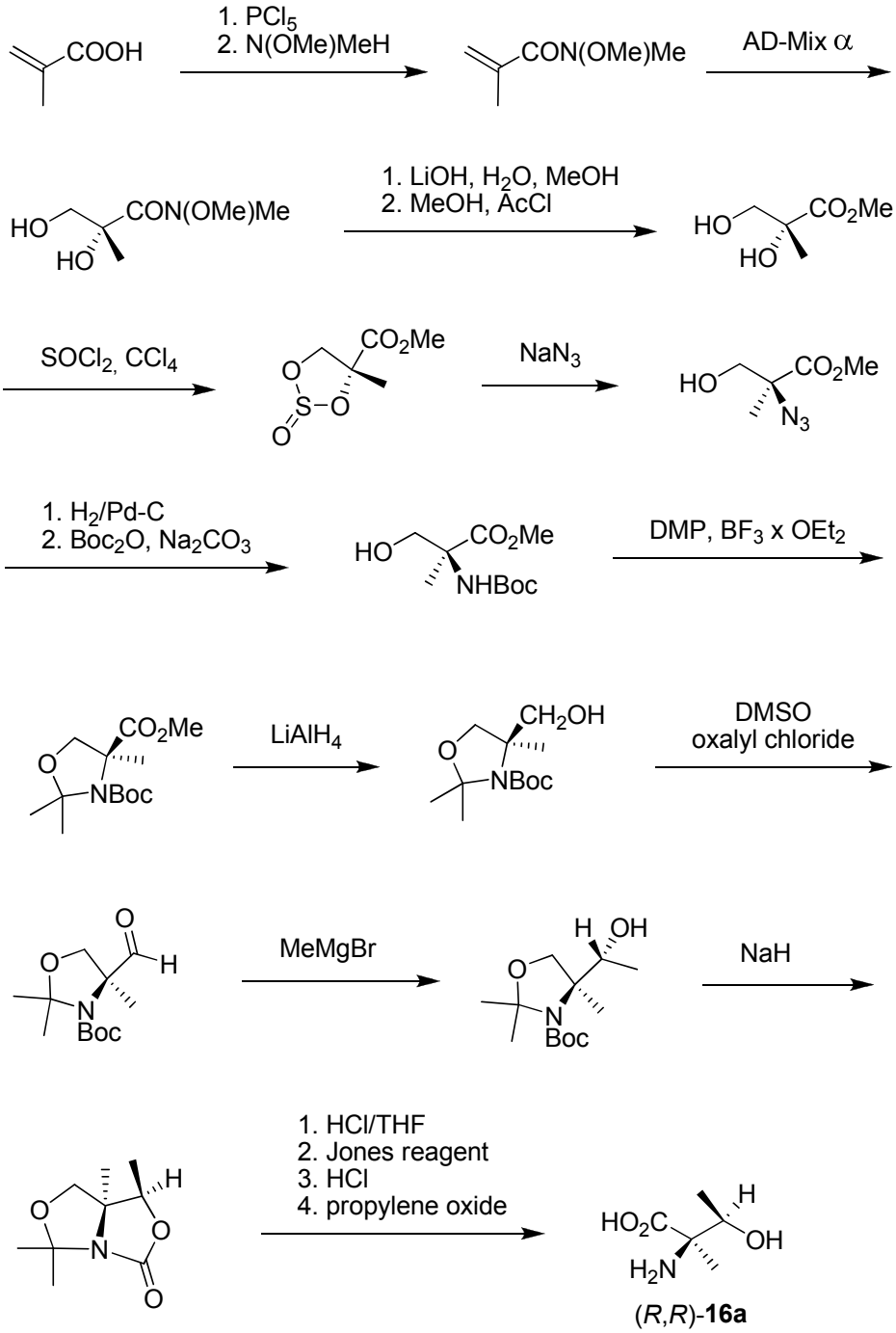
The first catalytic enantioselective synthesis was developed by Ito *et al.* in 1988 (Scheme 9).[32] The chiral gold-complex **41** was utilised to catalyse the asymmetric aldol reaction of an isocyanocarboxylate **40** and an aldehyde to yield oxazolines **42**, which could be easily converted to β-hydroxy α-amino acids such as α-methyl threonine (**16a**) and α-methyl β-phenyl serine (**16e**).



Scheme 9. Gold-catalysed aldol reaction described by Ito *et al.*[32]

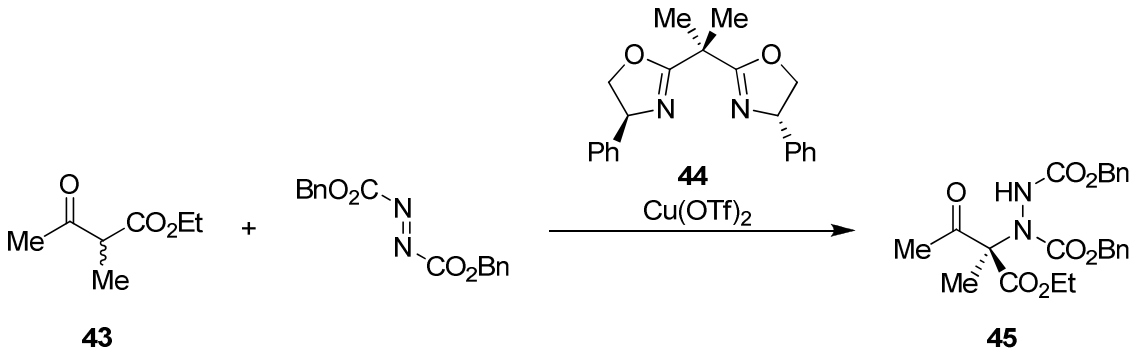
Yields and enantiomeric excesses of the *trans*-products were generally high. However, diasteriomeric ratios as well as enantiomeric excesses of the *cis*-products were often low. Besides the cumbersome synthesis of the catalyst, another drawback of this approach is the impossibility to gain the *cis*-products as major products.

More modern enantioselective synthesis of β-hydroxy α-amino acids of type **16** often rely on Sharpless asymmetric dihydroxylation (AD) to build up both stereogenic centers in one step.[33] Within these approaches the α-hydroxy residue is most widely converted into an amino residue by azide techniques. Enantiomeric excesses are excellent and this concept starts with easily accessible starting material but has the problem of the usage of toxic osmium for the dihydroxylation as at least one general major drawback. Often too many synthetic steps are necessary for this synthetic approach. An impressive example is shown in Scheme 10. The synthesis of (*R,R*)-α-methyl threonine ((*R,R*)-**16a**) by AD-technique needs altogether 18 synthetic steps, when applying the strategy of Avenoza *et al.*.[33b,34]



Scheme 10. Synthesis of (*R,R*)-α-methyl threonine ((*R*,*R*)-**16a**) according to Avenoza *et al.* [33b,34]

A more straightforeward route for the synthesis of β-hydroxy α-amino acids was introduced by the Jørgensen group in 2003. The enantioselective copper-catalysed amination of β-keto esters **43** proceeds with high enantiomeric excesses up to 99% ee even when applying only 0.5 mol% of a copper-catalyst and the ligand **44**.[35]



43 **45**

Scheme 11. Enantioselective amination of β-ketoesters according to Jørgensen *et al.*[35]

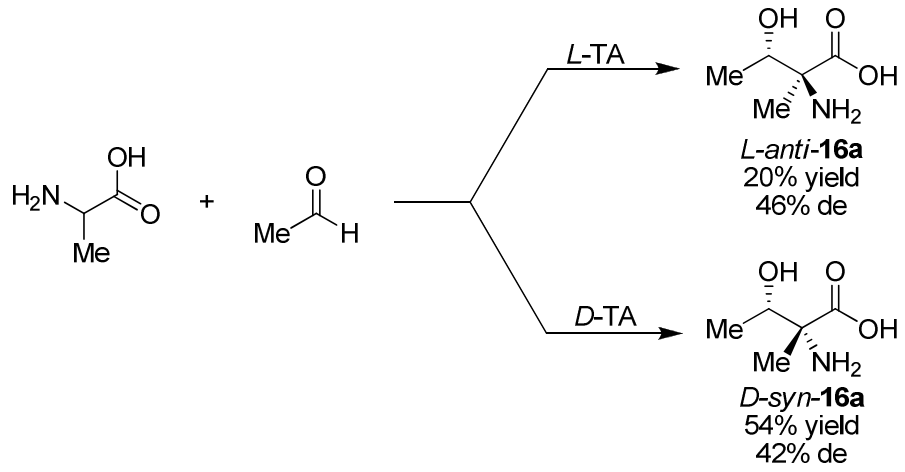
Diastereoselective reduction of the keto moiety in **45** was done applying L-selectride as a reducing agent, leading to an *N*-amino oxazolidinone which could be converted into a derivative of α-methyl threonine (**16a**) by zinc-induced N-N-bond cleavage.

Another elegant approach toward this class of amino acids is the silver-catalysed dia- and enantioselective Mannich reaction, which was described recently.[36] Allthough only very special amino acids are achievable by this approach until now, this type of reaction seems to be attractive, as only few synthetic steps are necessary for this approach.

All shown catalytic enantioselective pathways rely on metal catalysts in their key steps and most of these syntheses need lots of effort for the introduction and cleavage of protection groups or for the conversion of functional groups (e. g. conversion of an hydroxyl group into an amino group). A technique overcoming these problems delivers biocatalysis. Biocatalytic pathways to the desired amino acids of type **16** were examined intensively during the last five years.

One of the first biocatalytic approaches which allowed synthesis of β-hydroxy α,α-dialkyl amino acids was the kinetic resolution of oxiranenitriles by a *Rhodococcus sp.* whole-cell catalyst containg a nitrile hydratase as well as an amidase.[37] However many chemical transformations were needed to get access to the desired amino acids from the products of the kinetic resolution.

The most direct access to β-hydroxy α-amino acids of type **16** is the direct aldol reation of an amino acid and an aldehyde.[38] This aldolase-catalysed reaction was known for glycine as an aldol donor for long time.[39] However this concept could not be transferred to other amino acids such as alanine until a patent publication in 2006.[40] Since then, some applications of this strategy were published using, reengineered alanine racemase[41] as well as aldolases as biocatalysts.[42]

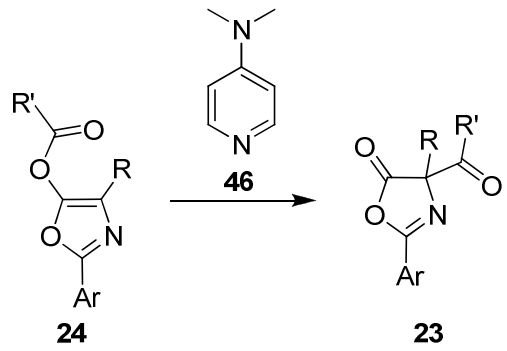


Scheme 12. Synthesis of two stereoisomers of α-methyl threonine (**16a**) by applying two different aldolases according to Griengl *et al.*[42b]

A number of α-methylated β-hydroxy α-amino acids were synthesised very recently by Griengl *et al.* in moderate yields when applying threonine aldolases and alanine as a donor.[42b] As an example, the synthesis of α-methyl threonine (**16a**) is shown in Scheme 12. Enantioselectivity was perfect for the stereogenic α-carbon but diastereoselectivity was comparable low.

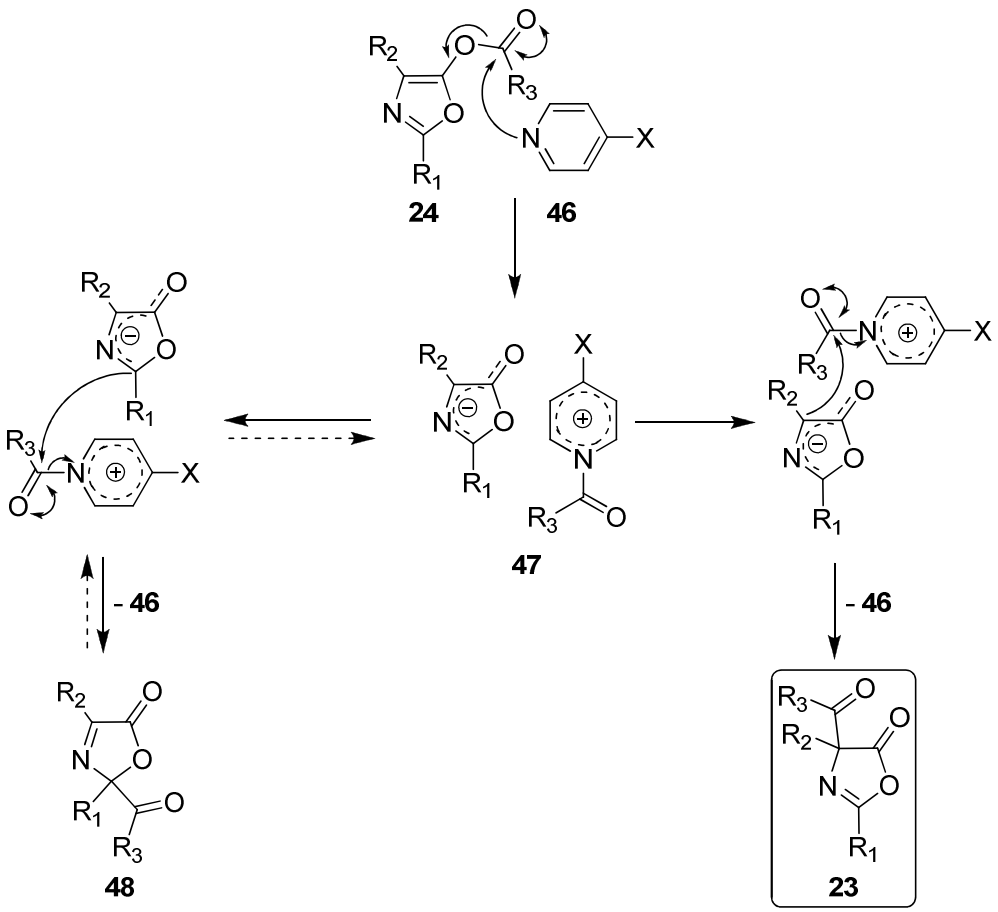
2.1.2 Steglich rearrangement in the synthesis of β-hydroxy-α,α-dialkyl amino acids

Rearrangement reactions involving carbon-carbon or carbon-heteroatom migrations constitute the basis of several well-established, powerful C-C and C-X bond-forming methodologies, which in many cases allow the construction of molecular frameworks not easily accessible by other approaches.[43] Hence, the construction of quaternary stereogenic centers is often possible using rearrangement reactions. An interesting rearrangement reaction forming a new C-C bond as well as a quaternary stereogenic center is the rearrangement of the acyl group of 5-acyloxy-2-aryl oxazoles **24** to form 4-acyl-2-aryl-oxazol-5-ones **23** (Scheme 13).



Scheme 13. General scheme for DMAP-catalysed Steglich rearrangements

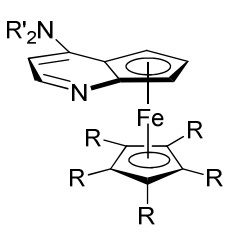
This rearrangement is named after Prof. Steglich, who introduced *N,N*-dimethyl aminopyridine DMAP (**46**), one of the most important nucleophilic catalysts, into organic chemistry.[44] He and his group found that the rearrangement of **24** into **23** is much faster, when applying DMAP as a catalyst compared to the pyridine-catalysed rearrangement.[45]



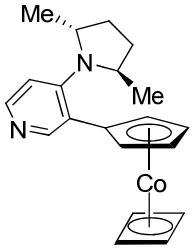
Scheme 14. Mechanism of the Steglich rearrangement

The mechanism (Scheme 14) proceeds over the proofed ion pair **47**, which is built up after nucleophilic attack of the catalyst **46** on the exocyclic carbonyl group of a 5-acyloxy oxazole **24**.[45b] This ion pair **47** has two possible reaction pathways. In almost all cases acylation of the 4-position leads to thermodynamically stable products **23**, which are formed rapidly if the residue in 4-position (R_2) is an unbranched alkyl chain. More sterical demanding residues such as isopropyl lead to a kinetic mixture of 2- and 4-acetylated products. Whereas 2-acetylated products **48** are rearranged under thermodynamical control to the 4-acylated products **23** for medium sterical demanding residues such as isopropyl, acylation in 2-position of the oxazole can be seen in the final product if the residue in 4-position (R_2) of the azlactone is as sterical demanding as a *tert*-butyl group.[45c] However, the nature of the catalyst has some influence on the product distribution.[45c]

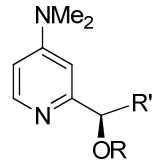
As nucleophilic catalysts such as DMAP (**46**) catalyse a broad range of reactions,[46] a huge number of chiral DMAP derivatives as well as some other chiral nucleophilic catalysts were prepared in the last two decades. Figure 8 shows some selected examples of different chiral DMAP-type catalysts designed in the working groups of Fu (**49**),[47] Richards (**50**),[48] Vedejs (**51a**,**b**),[49] Gotor (**51c**),[50] Spivey (**52**),[51] Iwasa (**53**)[52] and Fuji (**54**).[53]



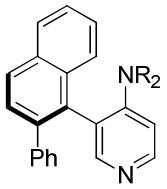
(-)-49a R = Ph, R' = Me
(-)-49b R = Ph, NR'$_2$ = pyrrolidino
(-)-49c R = Me, R' = Me
(-)-49d R = Me, NR'$_2$ = pyrrolidino



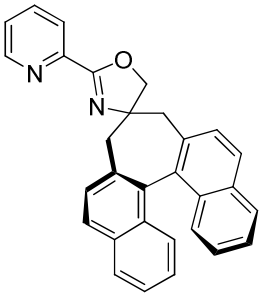
50



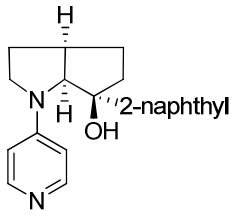
(*R*)-**51a** R = Ac, R' = CPh_3
(*R*)-**51b** R = Me, R' = *t*-Bu
(*R*)-**51c** R = Ac, R' = Ph



(*S*)-**52a** R = -(CH2)4-
(*S*)-**52b** R = Me
(*S*)-**52c** R = *n*-Bu



(*R*)-**53**

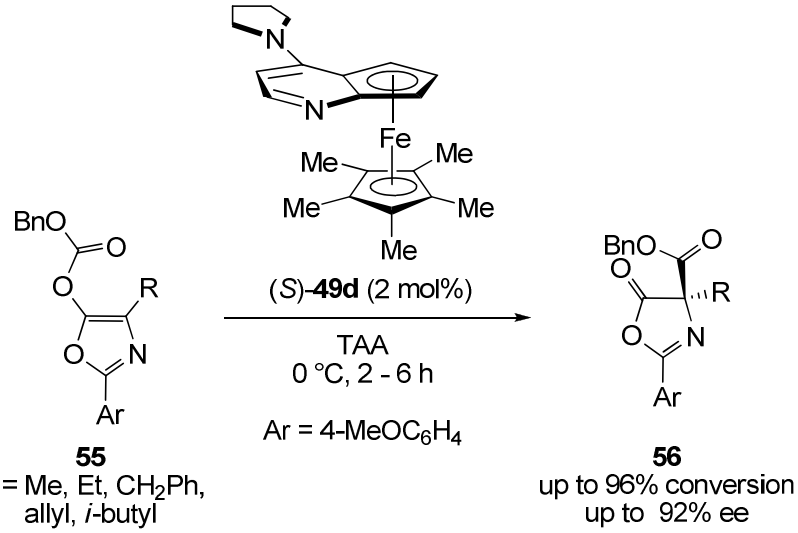


54

Figure 8. Overview of chiral DMAP-derivatives

Most of these catalysts, as well as some other DMAP-type catalysts not shown in Figure 8[54] and other nucleophilic catalysts such as chiral phosphines[55] or *N*-heterocyclic carbenes,[56] were once designed for application in kinetic resolution processes. However, potential applications are in a much wider field.[57] Many of them were applied to enantioselective Steglich rearrangement reactions.[47b,c,48,49b,c,50,54d,58]

The planar-chiral DMAP and PPy derivatives **49a**-**d**, developed in the Fu-group in the late 1990s, showed the best results in terms of enantioselectivity and conversion (Scheme 15).



Scheme 15. Enantioselective Steglich rearrangement according to Ruble and Fu[47c]

Very recently Birman *et al.* introduced two new classes of (chiral) nucleophilic catalysts, namely bicyclic amidines (**57**, **58**)[59] and bicyclic isothioureas (**59**-**64**)[60] (Figure 9). Relative reactivities of different amidines and isothioureas in acetylation reactions were recently examined and showed that some derivatives are actually more reactive than DMAP.[61]

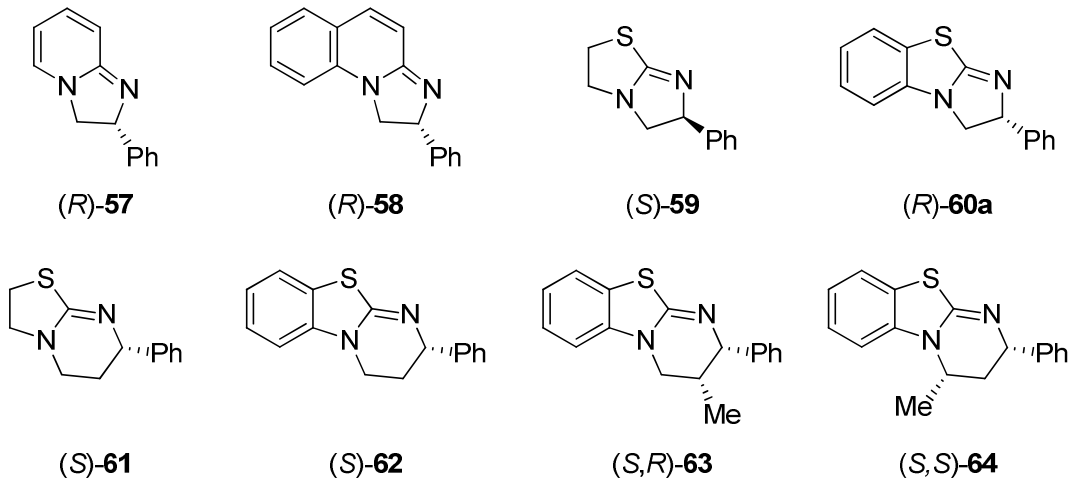
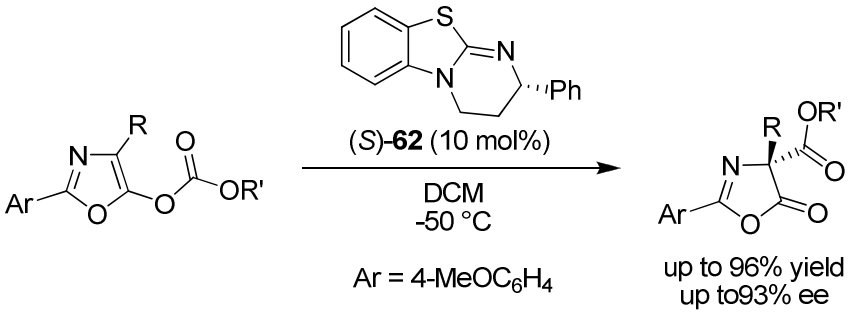


Figure 9. Chiral amidines and thioureas ("Birman-type catalysts")

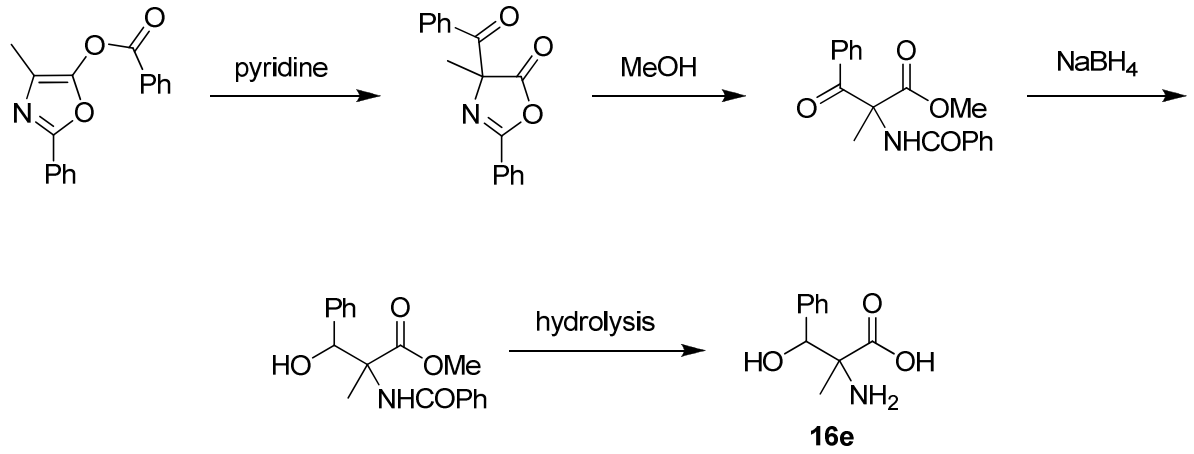
Homobenzotetramisole (**62**) and derivatives thereof were recently applied in enantioselective Steglich rearrangement reactions by Smith *et al*..[62]



Scheme 16. Steglich rearrangement catalysed by (*S*)-homobenzotetramisole ((*S*)-**62**)

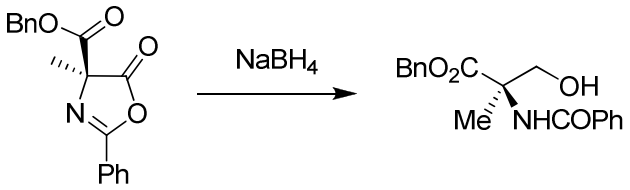
In 2009 they could show that homobenzotetramisole (**62**) and its derrivatives are efficient catalysts for the enantioselective rearrangement of carboxyl groups, leading to the desired products in good to excellent yields and enantiomeric excesses (Scheme 16).

The potential of Steglich rearrangements in the syntheses of β-hydroxy α,α-dialkyl amino acids **16** was shown as early as 1969 when Pines and Sletzinger applied a (racemic) Steglich rearrangement for the synthesis of α-methyl β-phenylserine (**16e**) (Scheme 17).[63]



Scheme 17. Synthesis of α-methyl β-phenylserine (**16e**) according to Pines and Sletzinger[63]

The application of this synthetic approach on products of enantioselective Steglich rearrangements was limited to the synthesis of α-alkylated serine derivatives (**66**, Scheme 18) for a long time as only alkyloxycarbonyl and benzyloxycarbonyl groups and not acyl groups such as acetyl were transferred in enantioselective Steglich rearrangements.

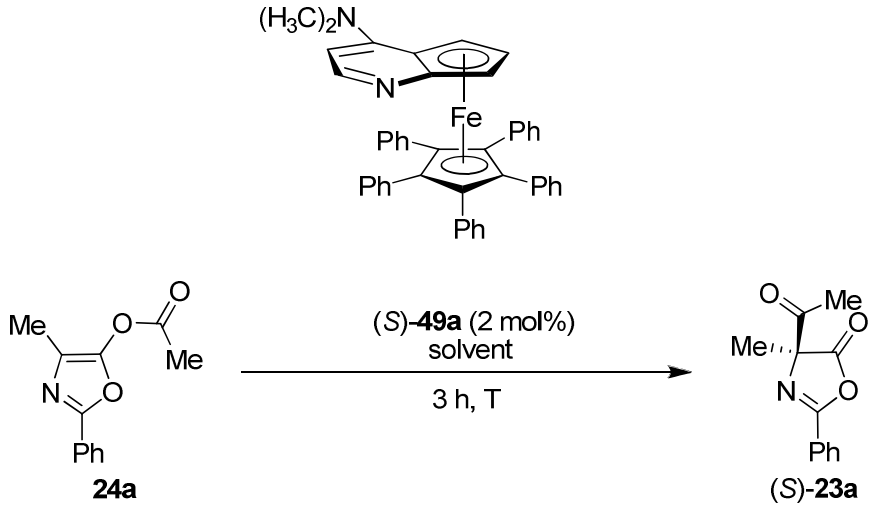


65 **66**
62% yield

Scheme 18. Synthesis of protected α-methyl serine (**66**) according to Ruble and Fu[47c]

In my diploma thesis I could show that enantioselective Steglich rearrangement of an acyl group such as acetyl is possible using Fu-type catalyst (*S*)-4-dimethylaminopyridinyl-(pentaphenylcyclopentadienyl)iron ((*S*)-**49a,** Table 1).[64]

Table 1: Solvent dependency of enantioselective Steglich rearrangement using Fu-type catalyst **49a**

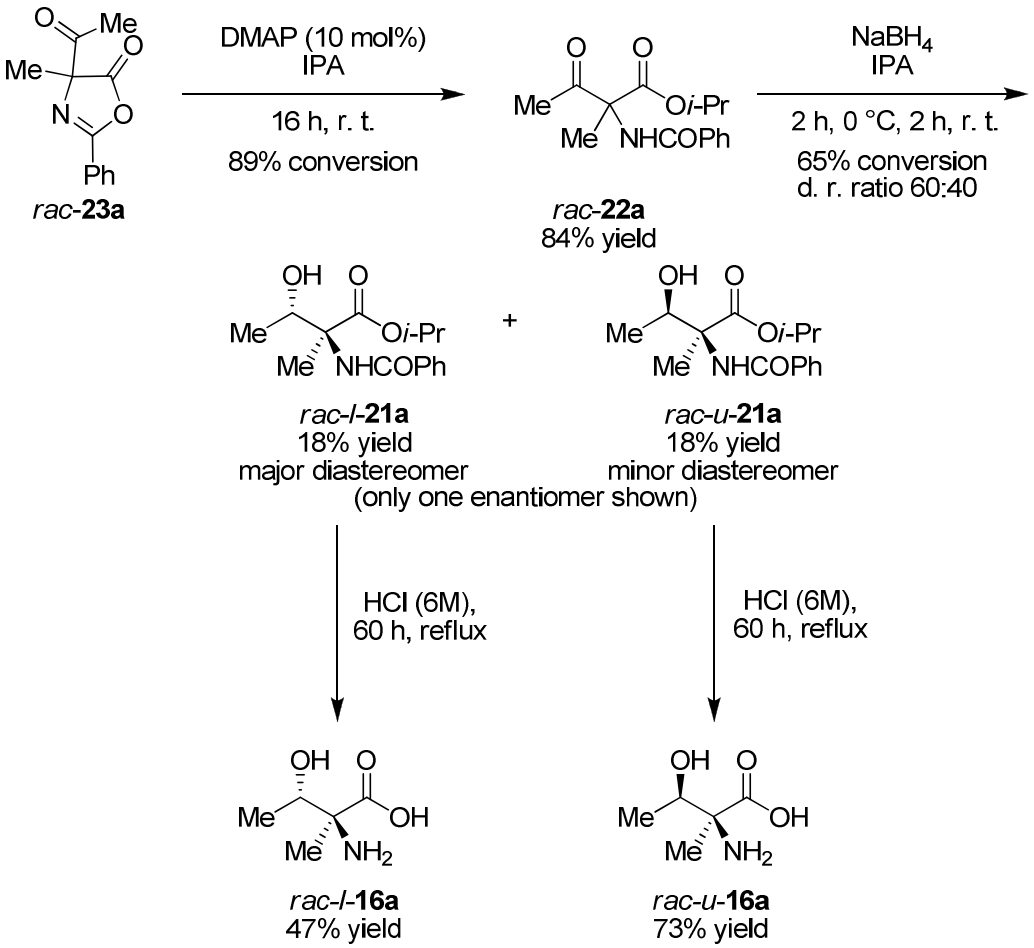


24a (*S*)-**23a**

entry	solvent	T	conversion (%)	ee (%)
1	DCM	r. t.	84	25
2	toluene	r. t.	80	23
3	MTBE	r. t.	78	24
4	THF	0 °C	71	18

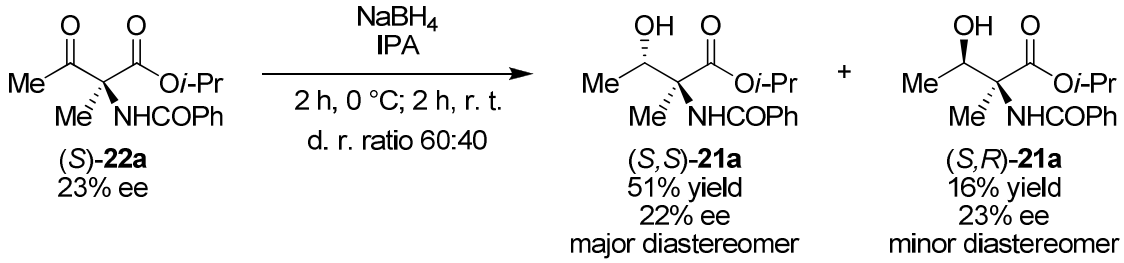
The best result was achieved in dichloromethane; in the presence of only 2 mol% (*S*)-**49a** the desired azlacton **23a** was generated with a high conversion of 84% after three hours reaction time (Table 1, entry 1). However enantiomeric excesses were comparable low in all tested solvents.

It was possible to synthesise both diastereomers of racemic α-methyl threonine (*rac*-**16a**) *via* ring opening, reduction of the keto moiety and deprotection, starting from racemic Steglich rearrangement product *rac*-**23a** (Scheme 19).



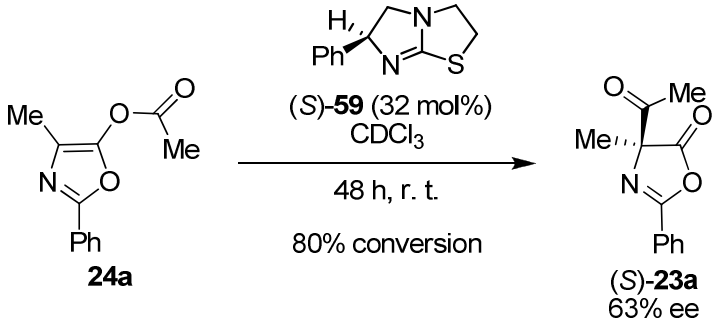
Scheme 19. Synthesis of both diasteromers of racemic α-methyl threonine (*rac*-**16a**) starting from Steglich rearrangement products

However, the overall yield for racemic α-methyl threonine (*rac-l*-**16a** and *rac-u*-**16a**, respectively) in this non-optimised synthesis was comparable low (7% and 11%, respectively). In addition it was shown that the enantiomeric excess retains constant during diastereoselective reduction of the keto moiety when enantiomerically enriched substrate **22a** is used, applying sodium borohydride in isopropanol. Unfortunately, diastereoselectivity of this reduction was comparable low (Scheme 20).[64a]



Scheme 20. Reduction of the keto moiety in (*S*)-**22a**

In the final stages of my diploma thesis, a first test of the chiral bicyclic isothiourea (*S*)-tetramisole ((*S*)-**59**), which is economically attractive as it is produced in large scale for medicinal and veterinary purposes and commercial available in enantiomerical pure form as a hydrochloride under the trade name Levamisol, was conducted.



Scheme 21. First enantioselective Steglich rearrangement by application of a Birman-type catalyst

The conversion proceeded much slower, when compared to reaction rates applying Fu-type catalyst (*S*)-**49a** leading to a conversion of 80% after two days, even when conducting the reaction at a high catalyst loading of 32 mol% (*S*)-tetramisole ((*S*)-**59**) (Scheme 21). Surprisingly a significant improvement in the enantioselectivity of the rearrangement was achieved when using this catalyst (*S*)-**59**, leading to the desired product in 63% ee.

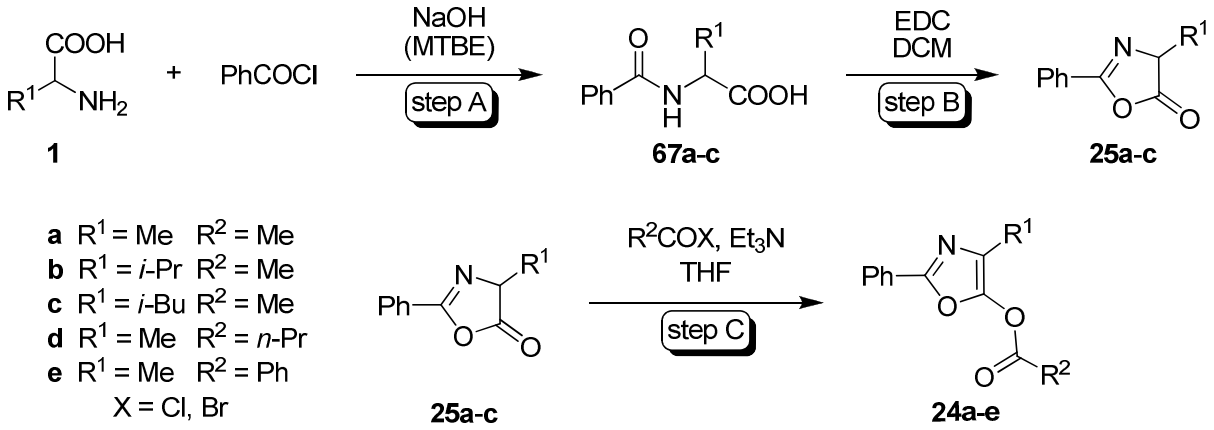
This work represents the proof of principle that enantioselective synthesis of β-hydroxy α,α-dialkyl amino acids bearing two stereogenic centers of type **16** *via* enantioselective Steglich rearrangement and subsequent reduction of the keto moiety is possible. However, enantio- and diastereoselectivities of the key steps were comparable low and the overall yield, even in the racemic synthesis, was low. Furthermore the substrate scope was not examined sufficiently.

2.2 Results and discussion

2.2.1 Substrate synthesis

Substrate syntheses were done in three steps starting from an amino acid and benzoyl chloride according to literature procedures (Table 2).[45b,65] Syntheses of azlactones **25a**-**c** as well as the syntheses of acetylated azlactones **24a**-**c** were previously described in detail in my diploma thesis.[64a] The new substrates **24d**,**e** were synthesised by the same methods, namely acylating **25a** with the appropriate acyl chloride or bromide, leading to the desired substrates in moderate overall yields.

Table 2: Substrate syntheses



entry	product	X	yield **67** (step A)	yield **25** (step B)	yield **24** (step C)	overall yield **24**[a]
1	**24a**	Cl	73%	74%	81%	44%
2	**24b**	Cl	91%	94%	42%	36%
3	**24c**	Cl	62%	75%	86%	40%
4	**24d**	Cl	73%	74%	72%	39%
5	**24e**	Br	73%	74%	55%	30%

[a] overall yield is the yield of product **24** related to the amount of amino acid **1** applied in step A

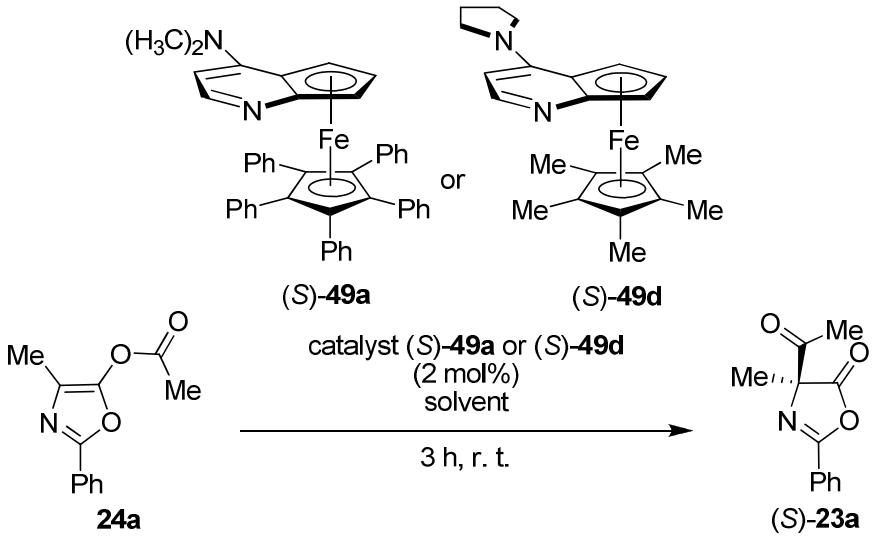
2.2.2 Key step: Catalytic Steglich rearrangement

Since first experiments during my diploma thesis led to promising but not yet satisfactory results in this key step, further experiments should be done to get access to the desired key intermediates of this synthetic approach, the Steglich rearrangement products **23a**-**e**, in high enantiomeric excesses and good yields. For this reason a catalyst screening was done as well as a process development.

2.2.2.1 Catalyst screening and process development

4-Methyl-2-phenyloxazol-5-yl acetate (**24a**) was chosen as a test substrate since this substrate showed the best results in the preliminary studies.[64a] To complete the examination of the previously examined catalyst (*S*)-**49a** this catalyst was examined in *t*-amyl alcohol (TAA), which was reported as an excellent solvent for enantioselective Steglich rearrangements of alkoxycarbonyl and benzyloxycarbonyl groups,[47c] leading to the best result for this catalyst (Table 3, entry 1 compared to Table 1). As Fu-type catalyst **49a** showed good reactivity but only minor enantioselectivity towards this substrate, a further commercial Fu-type catalyst, (*S*)-4-pyrrolidinopyrindinyl-penatmethylcyclopentadienyliron ((*S*)-**49d**), was examined.

Table 3: Enantioselective Steglich rearrangement of substrate **24a**, applying Fu-type catalysts (*S*)-**49a** and (*S*)-**49d**



entry	catalyst	solvent	conversion[a] (%)	ee (%)
1	(*S*)-**49a**	TAA	93	30
2	(*S*)-**49d**	DCM	95	41
3	(*S*)-**49d**	toluene	88	30
4	(*S*)-**49d**	MTBE	92	20
5	(*S*)-**49d**	THF	94	29
6	(*S*)-**49d**	TAA	94	50

[a] conversion means product related conversion to **23a**, consumption of substrate **24a** was quantitative for all entries

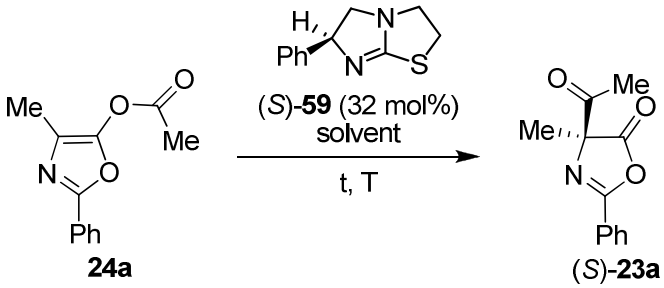
The use of this catalyst gave in general high conversions (up to 95%) at a low catalytic loading of only 2 mol% (Table 3). However, the resulting enantioselectivities remained in a moderate range and did not exceed 50% ee. In general both higher enantioselectivities and higher conversions were obtained with the Fu-catalyst (*S*)-**49d** (Table 3, entries 2-6) compared to the initially tested catalyst (*S*)-**49a** (Table 1, Table 3, entry 1). For example, in the presence of 2 mol% of (*S*)-**49a** in dichloromethane as a solvent azlactone **24a** rearranged to the desired key intermediate **23a** with 84% conversion and 25% ee (Table 1, entry 1), whereas under identical reaction conditions product (*S*)-**23a** was formed with 95% conversion and 41% ee when using catalyst (*S*)-**49d** (Table 3, entry 2). When carrying out the reaction in *t*-amyl alcohol the enantioselectivity further increased up to 50% ee when using the Fu-type catalyst (*S*)-**49d** (Table 3, entry 6).

To sum up the examination of Fu-type catalysts, (*S*)-**49d** was found to be the more efficient and more enantioselective catalyst compared to (*S*)-**49a**. In addition the best solvent for this reaction is *t*-amyl alcohol, leading to the desired key intermediate with up to 50% ee. High conversions even at short reaction times of three hours and a low catalytic loading of 2 mol% are ensured using both catalysts in all tested solvents.

As good experience was made when applying the chiral cyclic isothiourea (*S*)-tetramisole ((*S*)-**59**) as a catalyst for the enantioselective Steglich rearrangement of **24a** I decided to further examine this class of catalysts, which were originally introduced by the Birman group.[60]

As (*S*)-tetramisole ((*S*)-**59**) was the only Birman-type catalyst commercially available (nowadays (*R*)-benzotetramisole ((*R*)-**60a**) is commercially available as well), studies first concentrated on further examinations of this catalyst (Table 4).

Table 4: Enantioselective Steglich rearrangement of substrate **24a**, applying Birman-type catalyst (*S*)-**59**



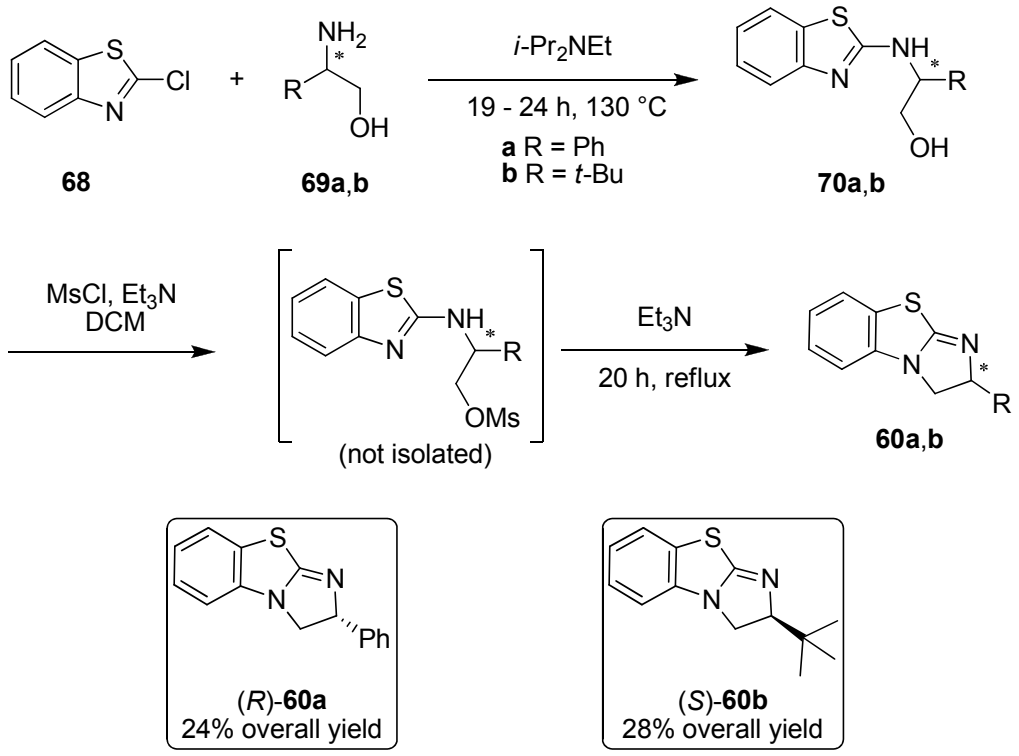
24a (*S*)-**23a**

entry	catalyst	solvent	T	t (h)	conversion[a] (%)	ee (%)
1	(*S*)-**59**	DCM	r. t.	68	82	59
2	(*S*)-**59**	DCM	40 °C	24	84	52
3	(*S*)-**59**	TAA	r. t.	24	74	60

[a] conversion means product related conversion to **23a**, consumption of substrate **24a** was quantitative for all entries

In the presence of a catalytic amount of 32 mol% of (*S*)-**59** an enantioselectivity of 59% ee in the asymmetric Steglich rearrangement of **24a** was observed (Table 4, entry 1) when applying dichloromethane as a solvent. In order to further improve the reaction rate, the reaction temperature was increased. Whereas at 40 °C the reaction is completed within one day enantioselectivity turned out to be somewhat lower (Table 4, entry 2). Consumption of the substrate in *t*-amyl alcohol was fast, leading to complete consumption in one day. However, conversion to the desired product (*S*)-**23a** was lower when compared to reactions in chlorinated hydrocarbons since more deacetylated side product was generated (Table 4, entry 3), presumably by the acetylation of the solvent alcohol. The best solvent for the enantioselective Steglich rearrangement in terms of selectivity was $CDCl_3$, leading to the desired key intermediate (*S*)-**23a** in 80% conversion and a comparable high enantiomeric excess of 63% ee at an acceptable reaction time of 48 hours (Scheme 21).

For further examination of this type of catalysts, catalyst synthesis was done. The synthesis of (*R*)-benzotetramisole ((*R*)-**60a**), a catalyst which showed superior results in kinetic resolution processes of alcohols when compared to (*S*)-tetramisole ((*S*)-**59**), was previously described by Birman and Li, starting from 2-chlorobenzothiazole (**68**) and enantiomerically pure (*R*)-(+)-phenylglycinole ((*R*)-**69a**) as commercially available starting materials.[60a] The first step of this synthesis is the substitution of the chloro moiety in **68** by the amino alcohol **69a**. Conversion of the alcohol moiety in **70a** into an appropriate leaving group and *in situ* ring closure completes this two step synthesis (Scheme 22). The synthesis was done according to this literature procedure, yielding the desired catalyst in an overall yield of 24%.

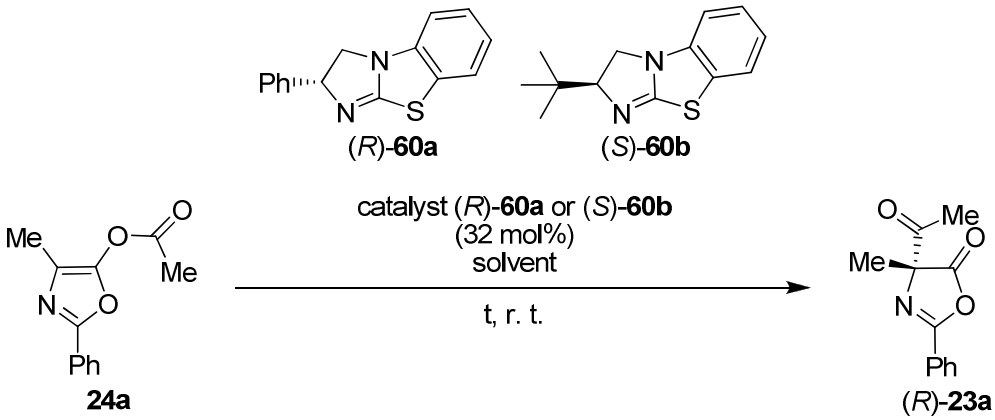


Scheme 22. Synthesis of Birman-type catalysts (*R*)-**60a** and (*S*)-**60b**

The introduction of a *tert*-butyl group into a chiral catalyst is a well known technology to improve selectivity.[66] For this reason, the new Birman-type compound *(S)*-2,3-Dihydro-2-*tert*-butyl-imidazo[2,1-*b*]benzothiazole ((*S*)-**60b**) was designed for the application as an organocatalyst. This molecule was synthesised in an analogous manner as (*R*)-**60a** when starting from (*S*)-*tert*-leucinol **69b** instead of (*R*)-phenylglycinol **69a**, leading to the desired isothiourea (*S*)-**60b** in 28% overall yield (Scheme 22).

Steglich rearrangement of substrate **24a** using the two synthesised catalysts was examined in three different solvents for each catalyst (Table 5).

Table 5: Enantioselective Steglich rearrangement of substrate **24a,** applying Birman-type catalysts **60a**,**b**



entry	catalyst	solvent	t (h)	conversion[a] (%)	ee (%)
1	(*R*)-**60a**	CH_2Cl_2	96	69	50
2	(*R*)-**60a**	$CDCl_3$	48	79	61
3	(*R*)-**60a**	TAA	24	84	52
4	(*S*)-**60b**	CH_2Cl_2	168	35	n.d.
5	(*S*)-**60b**	TAA	168	21	n.d.
6	(*S*)-**60b**	$CDCl_3$	168	29	n.d.

[a] conversion means product related conversion to **23a**, consumption of substrate **24a** was quantitative for entries 1-3

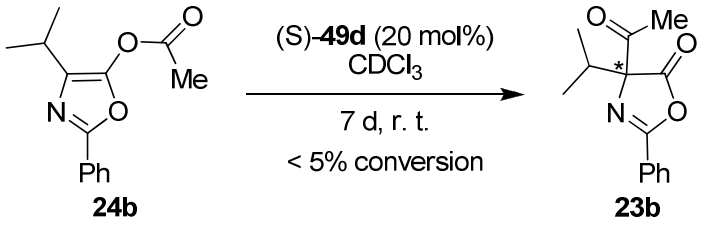
When using (*R*)-**60a** as an organocatalyst similar conversion and enantioselectivity was obtained (up to 61% ee, Table 5, entries 1-3) when compared to the application of (*S*)-**59**. The best solvent with respect to the enantiomeric excess of the product was $CDCl_3$ (Table 5, entry 2). The catalyst (*R*)-**60a** led to the opposite enantiomeric form of **23a** compared to the use of (*S*)-tetramisole ((*S*)-**59**). Since the (*R*)-enantiomer of tetramisole is not commercially available, (*R*)-benzotetramisole ((*R*)-**60a**) represents an advantageous complementary catalyst to (*S*)-**59**, thus offering access to both enantiomers of the desired product **23a** with enantiomeric excesses of about 60% ee by means of these organocatalysts (*S*)-**59** and (*R*)-**60a**.

(S)-2,3-Dihydro-2-*tert*-butyl-imidazo[2,1-*b*]benzothiazole ((*S*)-**60b**) showed a very low catalytic activity in the Steglich rearrangement of **24a** (Table 5, entries 4-6). Even after one week reaction time, conversion did not exceed 35% in any solvent examined. Complete consumption of the substrate **24a**, however, is essential, because the catalyst of the ring opening (DMAP) would otherwise rearrange the remaining substrate, leading to racemic product and thus decreasing the overall enantiomeric excess of the ring-opened product **22a**.

For this reason, the enantiomeric excess of Steglich rearrangement products **23a** was not determined.

2.2.2.2 Study of substrate range

The substrate range of this novel synthetic approach towards β–hydroxy α,α-dialkyl amino acids **16** was examined applying the four synthesised acylated azlactones **24b**-**e** as substrates. In these experiments I carried out the Steglich rearrangement of **24b**-**e** under in *situ* formation of **23b**-**e**, followed by (non-selective) ring-opening to β-keto α-amino esters **22c**-**e**. Whereas conversions were determined for **23b**-**e** yields and enantioselectivities were determined for **22c**-**e**.



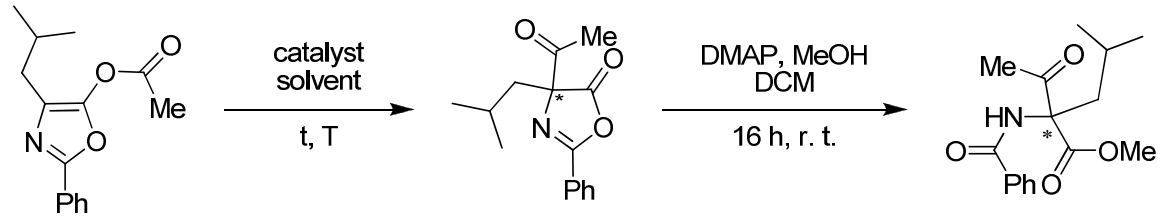
Scheme 23. Enantioselective Steglich rearrangement of **24b**

5-Acetyloxy-4-isopropyl-2-phenyl-oxazole (**24b**) displaying an azlactone bearing an α-branched side chain at C4, led to negligible conversions of lower than 5% even after one week reaction time when applying the most reactive catalyst (*S*)-**49d** at a high catalyst loading of 20 mol% (Scheme 23). This finding is in good accordance with literature. To the best of my knowledge, no enantioselective Steglich rearrangement of an oxycarbonyl group is known at an azlactone bearing an α-branched alkyl chain at C4. Shaw *et al.* argued that these alkyl chains cause a too strong steric hindrance for the acylation of this position with chiral catalyst which are sterically more demanding than non-chiral organocatalysts such as DMAP.[49c]

Next, the rearrangement of the acetyl group of 5-acetyloxy-4-isobutyl-2-phenyl-oxazole (**24c**) was examined. Applying only 2 mol% of catalyst (*S*)-**49d** in $CDCl_3$ led to complete consumption of this substrate bearing a β-branched alkyl-side chain at C4 within six hours reaction time (Table 6, entry 1). Unfortunately, enantiomeric excess of the product was only moderate (30 - 31% ee), even when applying twice the amount of the catalyst (*S*)-**49d** in *t*-amyl alcohol at low temperature of 0 °C (Table 6, entry 2).

Steglich rearrangement applying Birman-type catalysts (*S*)-**59** and (*R*)-**60a** was possible, when applying these catalysts in 32 mol% in $CDCl_3$. Unfortunately enantiomeric excesses of the products were comparable low (Table 6, entries 3, 5). Surprisingly a much higher enantiomeric excess of 85% ee was obtained when applying (*S*)-**59** in *t*-amyl alcohol as a solvent (Table 6, entry 4).To the best of my knowledge, this represents the highest ever observed enantioselectivity in a Steglich rearrangement of an acetyl group.

Table 6: Enantioselective Steglich rearrangement of **24c**

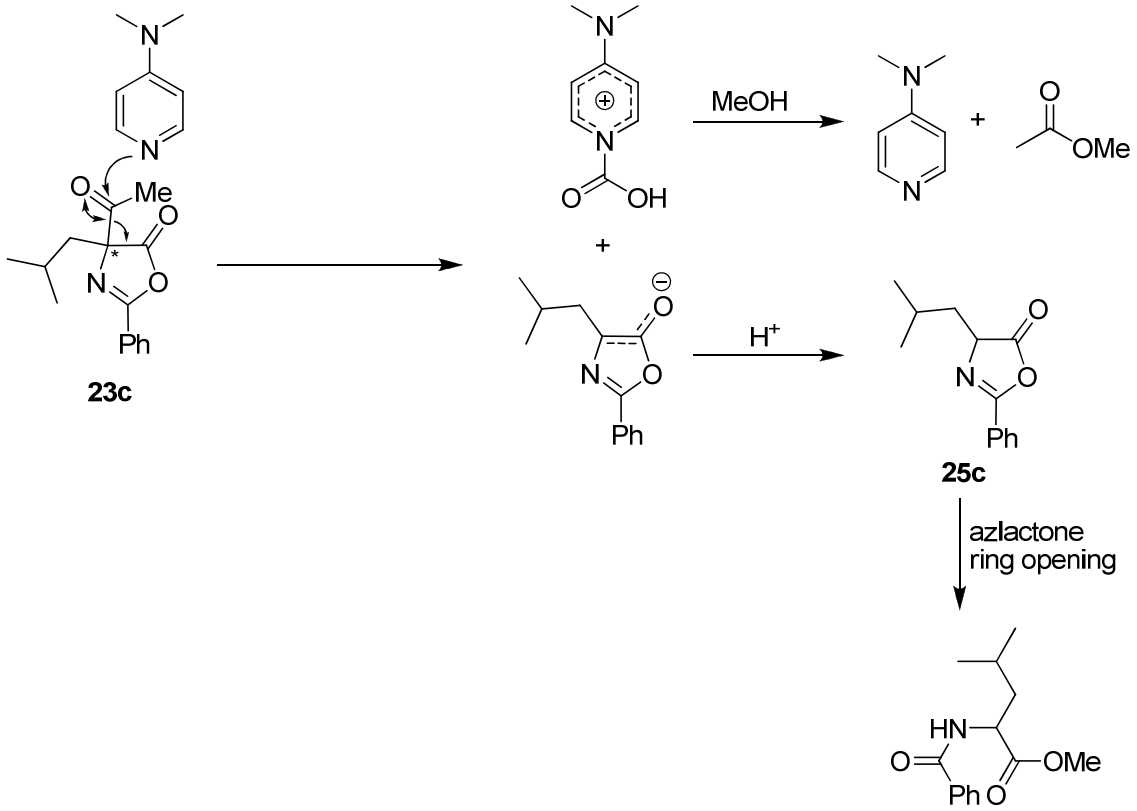


24c **23c** **22c**

entry	catalyst	solvent	t (h)	T	conversion **23c**[a] (%)	yield **22c** (%)	ee (%)
1	(*S*)-**49d** (2 mol%)	$CDCl_3$	6	r. t.	n. d.	n. d.	31
2	*(S)*-**49d** (4 mol%)	TAA	6	0 °C	72	n. d.	30
3	(*S*)-**59** (32 mol%)	$CDCl_3$	48	r. t.	61	n. d.	29
4	(*S*)-**59** (32 mol%)	TAA	75	r. t.	59	17	85
5	(*R*)-**60a** (32 mol%)	$CDCl_3$	72	r. t.	n. d.	n. d.	21

[a] conversion means product related conversion to **23c**, consumption of substrate **24c** was quantitative for all entries

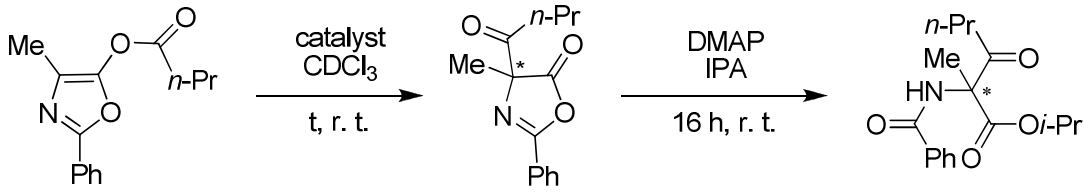
However, the yield of the desired ketone **22c** was only low (17%) due to deacetylation during DMAP catalysed ring opening (Scheme 24). As the azlactone bears two sterical demanding residues at 4-position, namely *iso*-butyl and acetyl, nucleophilic attack of DMAP at C-5 of the azlactone is disfavoured and nucleophilic attack at the acetyl moiety is a reasonable side reaction. This leads to the deacetylated azlactone **25c** which is then transformed into its ring opened derivative by standard azlacton ring-opening mechanism, lowering the yield of the desired product **22c**.



23c 25c

Scheme 24. Proposed mechanism for the deacetylation of **23c**

Table 7: Enantioselective Steglich rearrangement of **24d**



24d **23d** **22d**

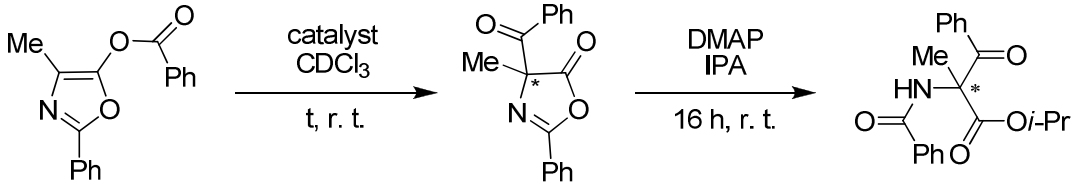
entry	catalyst	t	conversion[a] **23d** (%)	yield **22d** (%)	ee (%)
1[b]	(*S*)-**49d** (2 mol%)	5 d	18	n. d.	n. d.
2	*(S)*-**49d** (20 mol%)	6 h	73	n. d.	25
3	*(S)*-**49d** (20 mol%)	24 h	80	54	24
4	(*S*)-**49a** (20 mol%)	2 d	37	n. d.	18
5[c]	(*S*)-**59** (32 mol%)	3 d	38	n. d.	n. d.

[a] conversion means product related conversion to **23d**, consumption of substrate **24d** was quantitative for entries 2-4, [b] consumption of substrate **24d** 61%, [c] consumption of substrate **24d** 71%

A higher catalytic loading of 20 mol% was needed when examining the enantioselective Steglich rearrangement of a medium chain acyl group such as butanoyl since the application of 2 mol% of (*S*)-**49d** led to incomplete consumption of the substrate **24d** even after five days

reaction time in $CDCl_3$ as a solvent (Table 7, entry 1). Application of (*S*)-**49d** at this higher catalytic loading of 20 mol% led to complete consumption of **24d** within six hours and a good conversion of 73% (Table 7, entry 2). This conversion could even be raised by stirring the reaction mixture for 24 hours, leading to a conversion of 80%. The β-keto ester **22d** was isolated in 24% ee and 54% yield after column chromatography (Table 7, entry 3). In contrast, 20 mol% of catalyst (*S*)-**49a** led to an unsatisfactory low conversion of 37% and a low enantiomeric excess of 18% ee even after two days reaction time (Table 7, entry 4). Birman-type catalyst (*S*)-tetramisole ((*S*)-**59**) was not able to complete the reaction within three days when applying a catalytic loading of 32 mol% in $CDCl_3$ (Table 7, entry 5).

Table 8: Enantioselective Steglich rearrangement of **24e**



24e **23e** **22e**

entry	catalyst	t	consumption **24e** (%)	conversion **23e**[a] (%)	yield **22e** (%)	ee (%)
1	*(S)*-**49d** (10 mol%)	7 d	10	5	n. d.	n. d.
2	*(S)*-**49d** (20 mol%)	7 d	> 95	48	29	45
3	(*S*)-**49a** (20 mol%)	7 d	72	13	n. d.	n. d.
4	(*S*)-**59** (32 mol%)	8 d	67	9	n. d.	n. d.

[a] conversion means product related conversion to **23e**

Last, the enantioselective Steglich rearrangement of an aromatic acyl group such as benzoyl was examined by applying **24e** as a substrate. The application of 10 mol% of (*S*)-**49d** did not lead to significant consumption of the substrate, even after one week reaction time (Table 8, entry 1). For this reason a higher catalytic loading of 20 mol% was used for further examinations. The application of (*S*)-**49d** (20 mol%) led to complete consumption after one week of stirring at room temperature in $CDCl_3$. A conversion to **23e** of 48% was measured as well as an enantiomeric excess of 45% ee. The desired (protected) β-keto amino acid **22e** was isolated in 29% yield after column chromatography (Table 8, entry 2). In contrast, the application of (*S*)-**49a** (20 mol%) and (*S*)-tetramisole ((*S*)-**59**, 32 mol%) did not lead to complete consumption of **24e** and only low conversions were observed (Table 8, entries 3,4).

In summary, it was demonstrated that the Steglich rearrangement of several substrates proceeds enantioselectively with *O*-acylated azlactone **24a** being the most suitable substrate

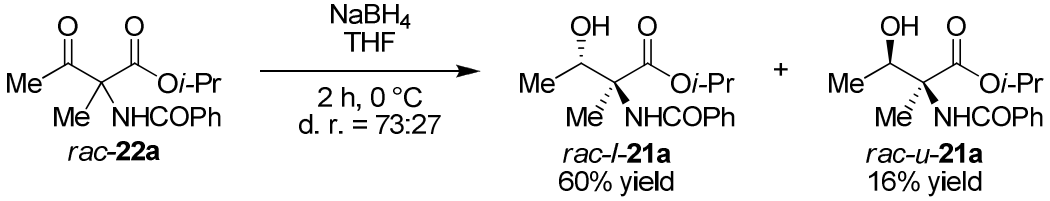
so far. Whereas the most active catalyst towards all tested substrates was the Fu-type catalyst (*S*)-**49d**, highest enantioselectivities were observed when applying Birman-type catalyst (*S*)-**59** and (*R*)-**60a**. However, these catalysts seemed to be effective catalysts only for the rearrangement of small acyl groups such as acetyl.

2.2.3 Process development for the derivatisation towards β-hydroxy-α,α-dialkyl amino acids

After successful constructing the first stereogenic center (at the amino moiety) by means of Steglich rearrangement, the next step towards the synthesis of all stereoisomers of a β-hydroxy α,α-dialkyl amino acid **16** consisted in the development of a method for the construction of the second stereogenic center in β-position *via* diastereoselective reduction of the keto moiety.

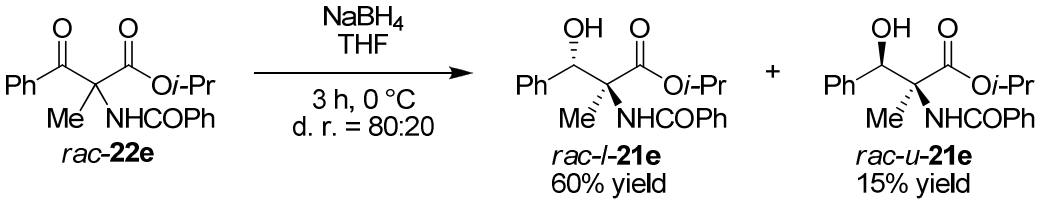
2.2.3.1 Diastereoselective reduction of Steglich rearrangement products

As initial experiments for the reduction of **22a** to protected α-methyl threonine **21a** applying sodium borohydride in isopropanol led to incomplete conversions and low diastereomeric ratios of d. r. = 60:40,[64a] this reduction methodology was first tried to be improved by applying an aprotic solvent such as THF instead of protic isopropanol (Scheme 25).



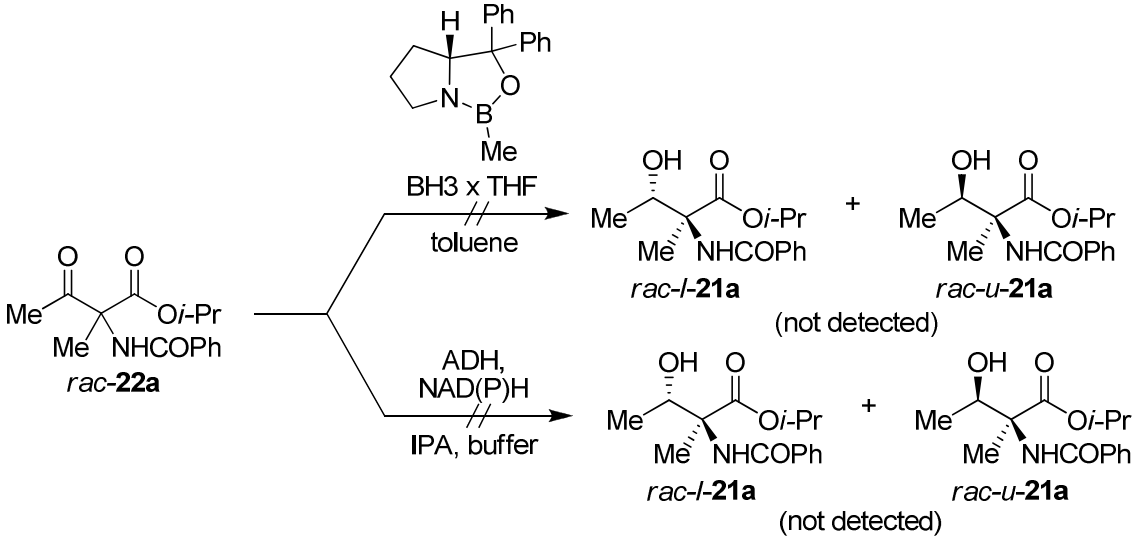
Scheme 25. Reduction of *rac*-**22a** applying sodium borohydride under optimised conditions

Reduction under these conditions proceeded more smoothly when compared to reduction in isopropanol (Scheme 19), leading to complete conversion and an improved diastereomeric ratio of d. r. = 73:27. Both racemic diastereomers were isolated in diastereomerically pure form in 60% resp. 16% yield after chromatography (Scheme 25). Reduction of *rac*-**22e** under these conditions furnished both diastereomers of protected α-methyl β-phenylserine **21e** diastereomerically pure in 60% and 16% yield, respectively (Scheme 26). The diastereomeric ratio of this reduction was slightly higher (d. r. = 80:20).



Scheme 26. Reduction of *rac*-**22e** applying sodium borohydride

Next, borane was tried to be used as reducing agent applying the CBS-reduction methodology (Scheme 27).[67,68] However, these reduction experiments turned out to be not successful leading to crude reaction mixtures due to side reactions (presumably, e.g., reduction of the ester moiety in *rac*-**22a**). Another well established methodology for the reduction of ketones, especially of aceto-moieties is the enzymatic reduction using an alcohol dehydrogenase as a biocatalyst.[69] Unfortunately no enzyme displaying activity towards the tested substrate *rac*-**22a** was found when applying alcohol dehydrogenases from *Rhodococcus sp*., *Lactobacillus kefir* and *Lactobacillus brevis* (Scheme 27). This might be due to the quaternary stereogenic center in α-position to the keto-moiety in **22a**, bearing three sterically demanding residues. This could make access to and the right orientation within the active site difficult hence leading to no activity.

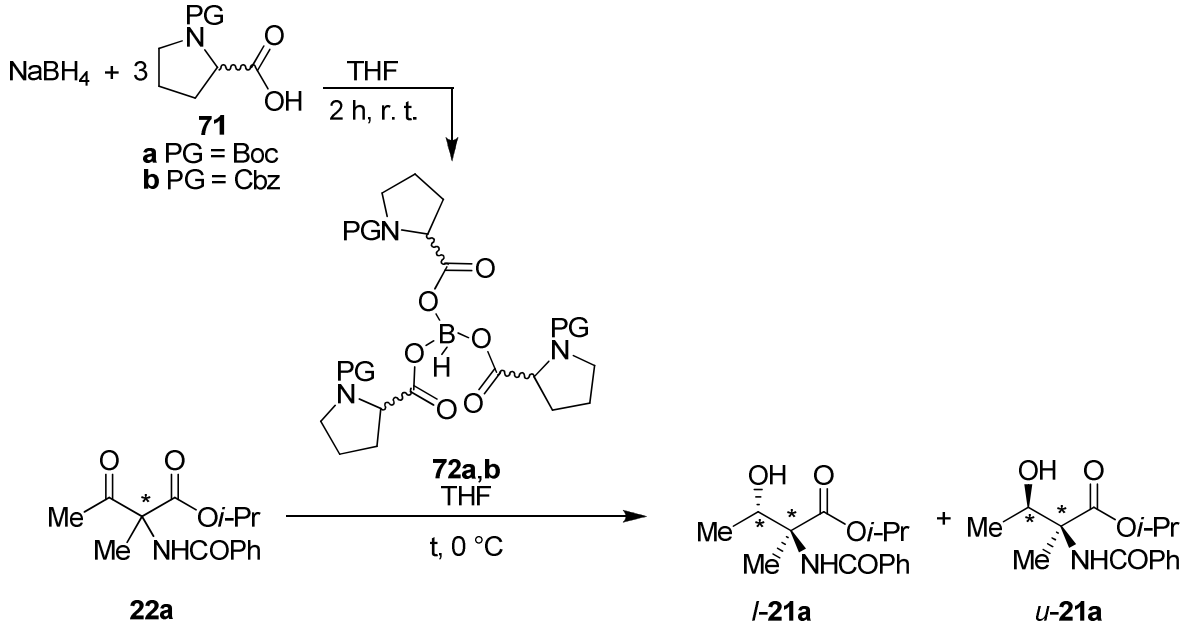


Scheme 27. Failed reductions applying CBS-technology and ADH-catalysed reduction

As sodium borohydride allowed chemoselective reduction of the keto moiety in presence of an ester and an amide moiety, I decided to stick to borohydrides as reducing agent. Since enantiomeric excesses for the construction of the first stereogenic center *via* enantioselective Steglich rearrangement were only moderate, a possibility to further increase the enantiomeric excess of the final products was needed.

In 1979 Umino *et al.* reported that the use of enantiomerically pure *N*-protected amino acids as chiral additives to sodium borohydride leads to an enantioselective reduction of ketones.[70] Later on this method was enhanced by using three equivalents of an *N*-protected amino acid.[71] As *N*-Boc-proline was reported to be one of the most efficient additives, β-keto-α-amino acid ester **22a** was reduced using sodium borohydride and three equivalents of L-*N*-Boc-proline as reducing agent (Table 9).

Table 9: Stereoselective reductiuon of **22a** with *N*-protected proline **71** as chiral auxiliary



entry	starting material	chiral auxilliary	t (h)	conversion (%)	d. r.	% ee **22a** (config.)	% ee *l*-**21a** (config.)	% ee *u*-**21a** (config.)
1	*rac*-**22a**	L-**71a** (1.3eq)	20	62	80:20	55 (*S*)	36 (*R*,*R*)	n. d.
2	*rac*-**22a**	L-**71a** (2.6eq)	70	62	80:20	56 (*S*)	40 (*R*,*R*)	31 (*R*,*S*)
3	(*R*)-**22a** (55% ee)	L-**71a** (1.3eq)	20	51	83:17	29 (*R*)	79 (*R*,*R*)	44 (*R*,*S*)
4	*rac*-**22a**	D-**71a** (1.3eq)	20	60	80:20	41 (*R*)	49 (*S*,*S*)	6 (*R*,*S*)
5	(*S*)-**22a** (62% ee)	D-**71a** (1.3eq)	20	55	88:12	46 (*S*)	79 (*S*,*S*)	53 (*S*,*R*)
6	*rac*-**22a**	L-**71b** (1.3 eq.)	20	30	86:14	28 (*S*)	59 (*R*,*R*)	22 (*S*,*R*)

The diastereomeric ratio of the reaction improved to d. r. = 80:20 (at 62% conversion) compared to d. r. = 73:27 (at 100% conversion) when using only borohydride, but even more important, the main product (*R,R)*-**21a** was obtained with a conversion of 62% in 40% yield

and with an enantiomeric excess of 36% ee, thus indicating an asymmetric induction of the reducing agent (Table 9, entry 1). The remaining unreacted substrate **22a** and the minor diastereomer *u*-**21a** showed small enantiomeric excesses as well. Unfortunately it was not possible to improve the relatively low conversions by applying longer reaction times or more equivalents of the reducing agent (Table 9, entry 2). Application of these reduction conditions on enantiomerically enriched (*R*)-**22a** with an enantiomeric excess of 55% ee led to a further improvement of the diastereomeric ratio to d. r. = 83:17. The enantiomeric excess of the yielded major diastereomer (*R*,*R*)-**21a** was determined to be 79% ee (Table 9, entry 3). Whereas the enantiomeric excess of the desired major product-diastereomer *l*-**21a** was significantly higher compared to the enantiomeric excess in the starting material, enantiomeric excess in the remaining substrate **22a** and the minor diastereomer *u*-**21a** decreased.

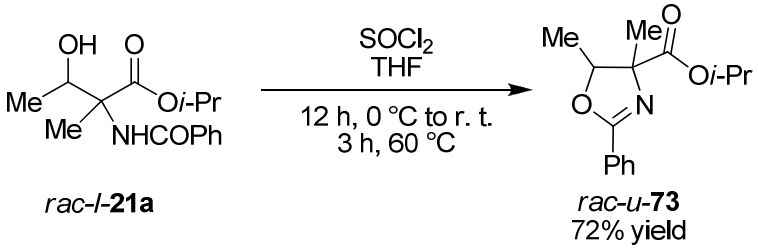
The application of the opposite enantiomer of the chiral auxiliary, D-*N*-Boc-proline (D-**71a**), on racemic β-keto amino ester *rac*-**22a** led to the same diastereomeric ratio of 80:20 and the same major diastereomer, the *like*-diastereomer. As planned, the opposite enantiomer (*S,S*)-**21a** was generated preferably (Table 9, entry 4). Enantiomerically enriched (*S*)-**22a** was reduced, applying D-*N*-Boc-proline (D-**71a**) as chiral auxiliary, yielding desired protected (*S,S*)-α-methyl threonine ((*S*,*S*)-**21a**) with an increased enantiomeric excess of 79% ee (Table 9, entry 5). In addition, L-*N*-Cbz-proline (L-**71b**) was examined as a chiral auxiliary for the reduction of *rac*-**22a**. Unfortunately conversion was even lower when compared to reductions applying *N*-Boc-proline as a chiral auxiliary, although diastereomeric ratio was good (Table 9, entry 6).

These results showed that combination of an enantioselective organocatalytic Steglich rearrangement and subsequent diastereoselective reduction using sodium borohydride and *N*-Boc-proline as reducing agent is a suitable and practical synthetic route towards (enantiomerically enriched) *l*-α-methyl threonine (*l*-**16a**). Furthermore, asymmetric induction is possible at both stages, thus offering the potential for obtaining a high “overall” enantiomeric excess.

2.2.3.2 Inversion of the stereogenic center in β-position

With respect to formation of the remaining two *u*-diastereomers, namely (*R*,*S*)- and (*S*,*R*)-stereoisomers of α-methyl threonine (**16a**), application of the diastereoselective reduction of **22a** turned out to be difficult. Those diastereomers represent the minor diastereomers and attempts to reverse the diastereoselectivity in the reducing step were not successful. Thus, I

focused on the preparation of the (*R*,*S*)- and (*S*,*R*)-diastereoisomers starting from the major (*R*,*R*)- and (*S*,*S*)-diastereoisomers, respectively, by inversion of the absolute configuration of the stereogenic centers in β-position. Such a concept was earlier successfully applied by Hamada *et al.* for the inversion of the configuration of the β-hydroxy group of a protected β-hydroxy α-amino acid in the synthesis of all four stereoisomers of 3-hydroxyleucine.[72]



rac-l-**21a**

rac-u-**73**
72% yield

Scheme 28. Inversion of the β-stereogenic center by formation of an oxazoline (**73**)

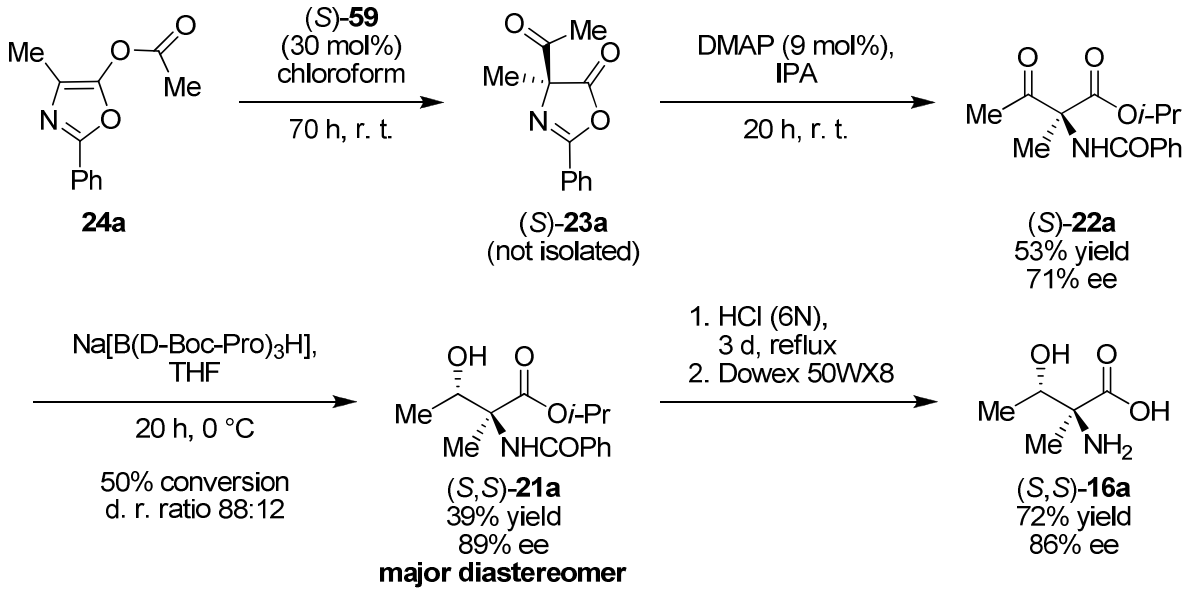
Application of this technique, thionyl chloride induced ring closure to form an oxazoline, on *rac-l*-**21a** furnished the desired racemic oxazoline *rac-u*-**73** in 72% yield (Scheme 28).

2.2.4 Total synthesis of all stereoisomers of α-methyl threonine

Since techniques for the preparation of all four stereoisomers of α-methyl threonine (**16a**) were investigated intensively, synthesis of them was done simply by applying optimised reaction conditions for each step.

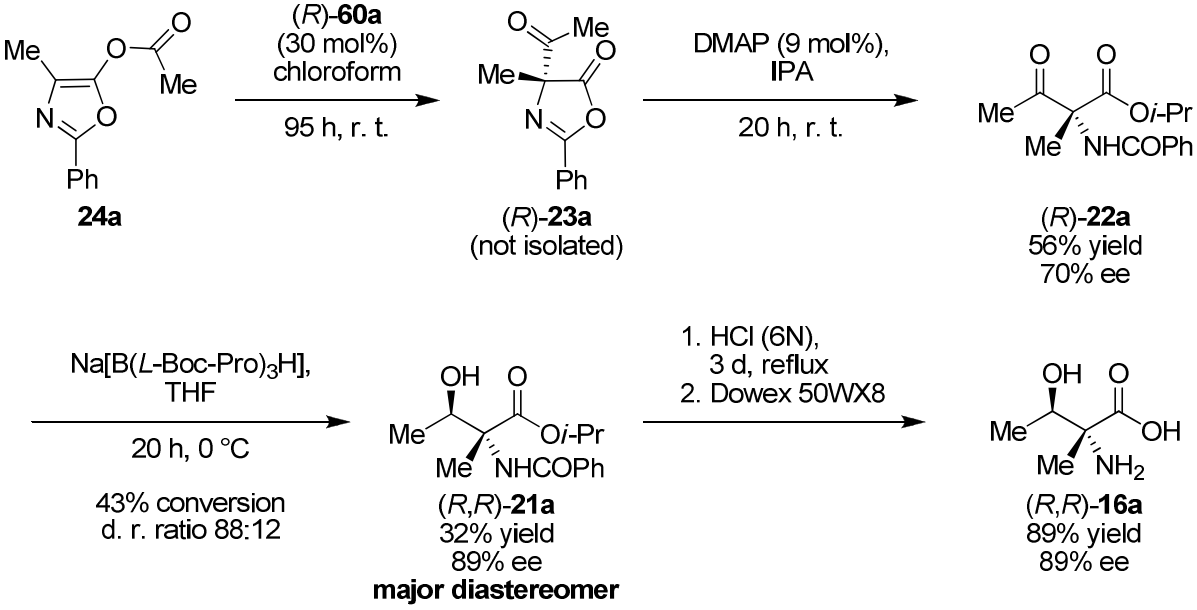
For the synthesis of (*S*,*S*)-α-methyl threonine ((*S*,*S*)-**16a**) (*S*)-tetramisole ((*S*)-**59**) was used in a catalytic amount of 30 mol% for initial enantioselective Steglich rearrangement in chloroform (Scheme 29). To ensure complete consumption of azlactone **24a**, a longer reaction time of 70 hours was applied. After subsequent ring opening in the presence of DMAP as a catalyst and subsequent column chromatography the β-keto ester (*S*)-**21a** was obtained in 53% yield with an enantiomeric excess of 71% ee. The enantiomeric excess of the ester (*S*)-**22a** in this experiment on a 4.0 mmol scale was higher compared to the enantiomeric excess in the small scale experiment (0.23 mmol scale; see Scheme 21), since reaction time has been enlarged to ensure complete consumption of the substrate **24a**. The reduction was carried out using sodium borohydride and D-Boc-Proline as a chiral additive, leading to (*S*,*S*)-2-benzoylamino-3-hydroxy-2-methyl-butyric acid isopropyl ester ((*S,S*)-**21a**) in 39% yield after chromatography. This reduction proceeds diastereoselectively with a diastereomeric ratio of d. r. = 88:12, yielding the product (*S,S*)-**21a** with an enantiomeric excess of 89% ee. Hydrolysis of diastereomerically pure and enantiomerically enriched ester

((*S,S*)-**21a**) with 86% ee gave diastereomerically pure (*S*,*S*)-α-methyl threonine ((*S*,*S*)-**16a**) in 72% yield and with 86% ee (Scheme 29). The overall yield for this multi step synthesis was 15%.



Scheme 29. Synthesis of (*S,S*)-α-methyl threonine ((*S,S*)-**16a**)

(*R*,*R*)-α-Methyl threonine ((*R*,*R*)-**16a**) was prepared in an analoguous manner using (*R*)-benzotetramisole ((*R*)-**60a**) as a catalyst for the Steglich rearrangement (Scheme 30).

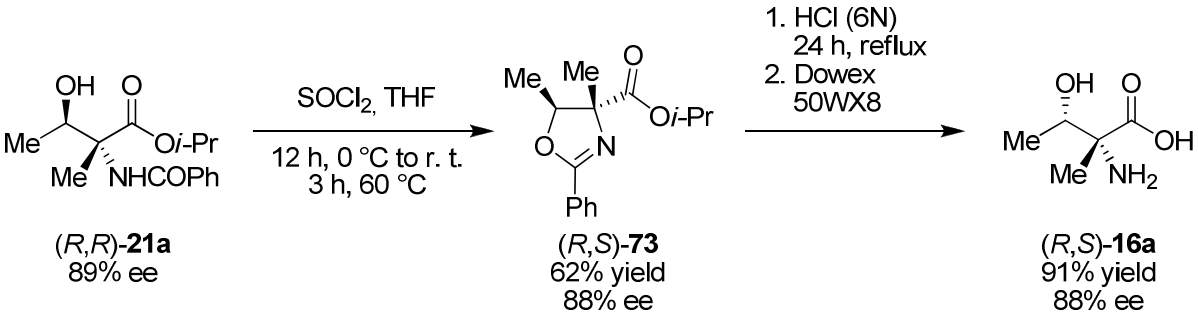


Scheme 30. Synthesis of (*R,R*)-α-methyl threonine ((*R,R*)-**16a**)

The intermediate (*R*)-2-benzoylamino-2-methyl-3-oxo-butyric acid isopropyl ester ((*R*)-**22a**) was obtained in 56% yield and with an enantiomeric excess of 70% ee after subsequent ring opening (Scheme 30). Reduction with a sodium borohydride complex bearing L-Boc-proline

as a ligand led to (*R*,*R*)-2-benzoylamino-3-hydroxy-2-methyl-butyric acid isopropyl ester ((*R,R*)-**21a**) in 32% yield as the major diastereomer in 89% ee after chromatography. Hydrolysis in hydrochloric acid and subsequent purification on ion exchange resin led to diastereomerically pure (*R*,*R*)-α-methyl threonine ((*R*,*R*)-**16a**) in 89% yield and with 89% ee (Scheme 30). The overall yield for (*R*,*R*)-α-methyl threonine ((*R*,*R*)-**16a**) was 16%.

Using the oxazoline technique for the inversion of the β-stereogenic center led to the desired remaining diastereoisomers (*R*,*S*)-α-methyl threonine ((*R*,*S*)-**16a**) and (*S*,*R*)-α-methyl threonine ((*S*,*R*)-**16a**).



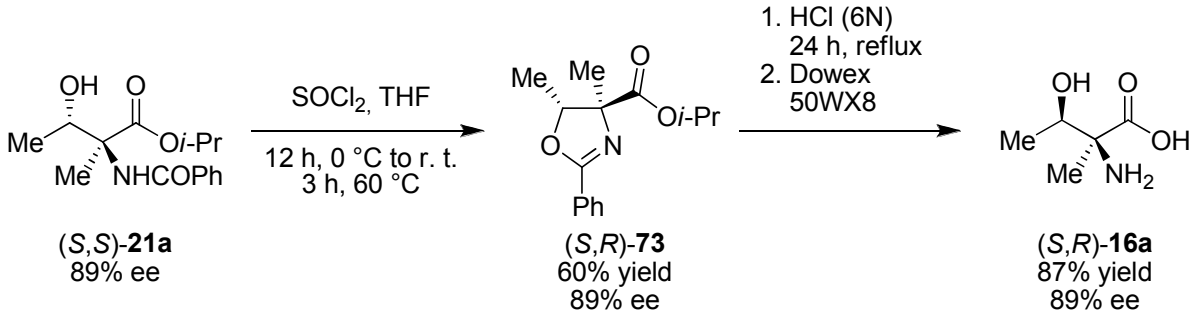
(*R,R*)-**21a**
89% ee

(*R,S*)-**73**
62% yield
88% ee

(*R,S*)-**16a**
91% yield
88% ee

Scheme 31. Synthesis of (*R,S*)-α-methyl threonine ((*R,S*)-**16a**)

The (*R,S*)-oxazole ((*R,S*)-**73**) could be isolated in 62% yield, when starting with (*R,R*)-**21a**. The enantiomeric excess of this product (88% ee) was nearly the same as the one of the starting material (89% ee). Hydrolysis in hydrochloric acid and work up using ion exchange resin led to diastereomerically pure (*R,S*)-α-methyl threonine ((*R,S*)-**16a**) in 91% yield and with 88% ee (Scheme 31). The overall yield related to azlactone **24a** for (*R,S*)-α-methyl threonine ((*R,S*)-**16a**) was 10%, thus indicating only a slight loss of overall yield by the inversion of the β-stereogenic center.



(*S,S*)-**21a**
89% ee

(*S,R*)-**73**
60% yield
89% ee

(*S,R*)-**16a**
87% yield
89% ee

Scheme 32. Synthesis of (*S,R*)-α-methyl threonine ((*S,R*)-**16a**)

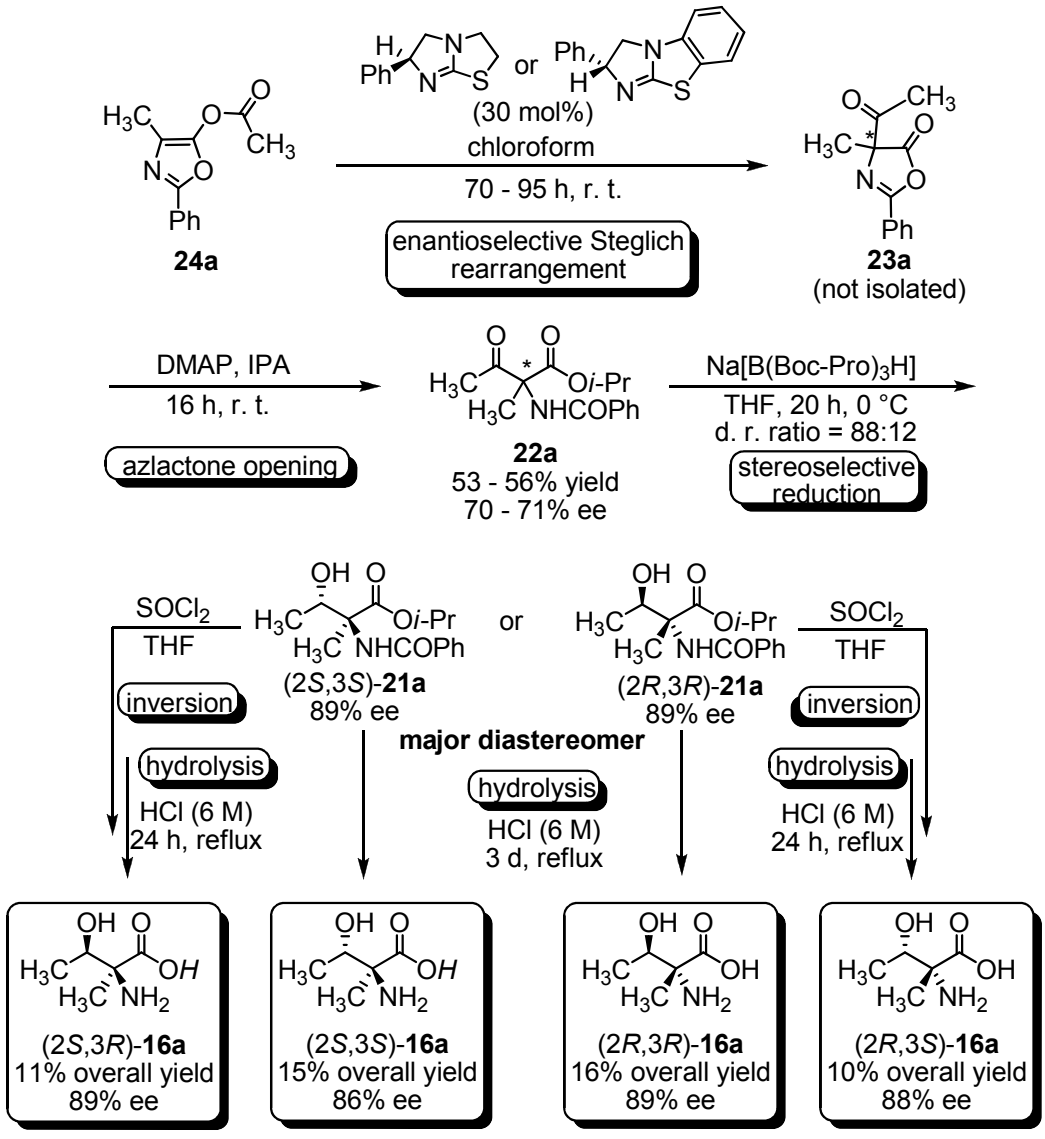
Starting from (*S,S*)-**21a** with 89% ee for the oxazoline formation led to diastereomerically pure (*S,R*)-**73** with 89% ee in 60% yield. Subsequent hydrolysis furnished diasteriomerically

pure (*S,R*)-α-methyl threonine ((*S,R*)-**16a**) in 87% yield and with 89% ee (Scheme 32). The overall yield related to azlactone **24a** was 11%.

2.3 Summary

In conclusion, an efficient synthetic route towards all four stereoisomers of α-methyl threonine (**16a**) was developed. The desired stereoisomers were obtained in diastereomerically pure form and with high enantiomeric excesses in the range of 86-89% ee (Scheme 33). The overall yields were in the range of 10% to 16%.

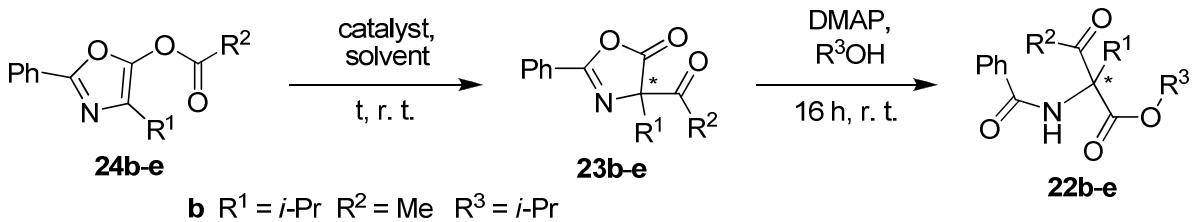
It has to be inflected that the stereoselective synthesis of each stereoisomer is possible by choosing the appropriate combination of catalyst and reducing agent. Key step in this multi-step synthesis is an enantioselective organocatalytic Steglich rearrangement reaction of *O*-acetylated azlactones **24a**.



Scheme 33. Overview of the synthesis of all four stereoisomers of α-methyl threonine (**16a**)

In addition, the substrate spectrum of this key step, the Steglich rearrangement of acyl groups, was studied (Table 10).

Table 10: Overview of the substrate scope of the enantioselective Steglich rearrangement



24b-e **23b-e** **22b-e**

b R^1 = *i*-Pr R^2 = Me R^3 = *i*-Pr
c R^1 = *i*-Bu R^2 = Me R^3 = Me
d R^1 = Me R^2 = *n*-Pr R^3 = *i*-Pr
e R^1 = Me R^2 = Ph R^3 = *i*-Pr

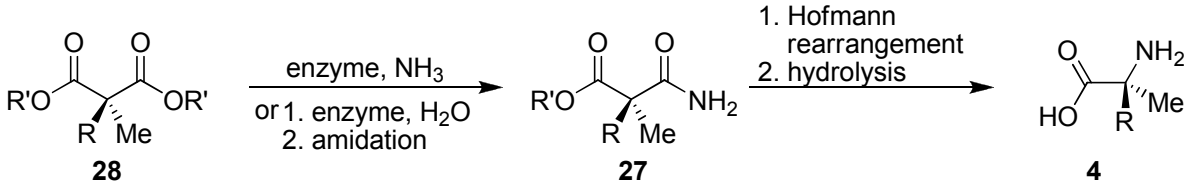
entry	substrate	catalyst	solvent	t	conversion[a] **23** (%)	yield **24** (%)	ee (%)
1	**24b**	(*S*)-**49d**	$CDCl_3$	7 d	< 5	n. d.	n. d.
2	**24c**	(*S*)-**59**	TAA	75 h	59	17	85
3	**24d**	(*S*)-**49d**	$CDCl_3$	24 h	80	54	24
4	**24e**	(*S*)-**49d**	$CDCl_3$	7 d	48	29	45

[a] conversion means product related conversion to product **23**, consumption of substrate **24** was quantitative for entry 2-4

This synthetic strategy, which allows access to β-hydroxy α,α-dialkyl amino acids of type **16**, may be further improved by the application of novel nucleophilic catalysts such as homobenzotetramisole (**62**) and its derivatives which showed improved properties in related studies when compared to tetramisole (**59**) and benzotetramisole (**60a**), which were applied in this study.[62]

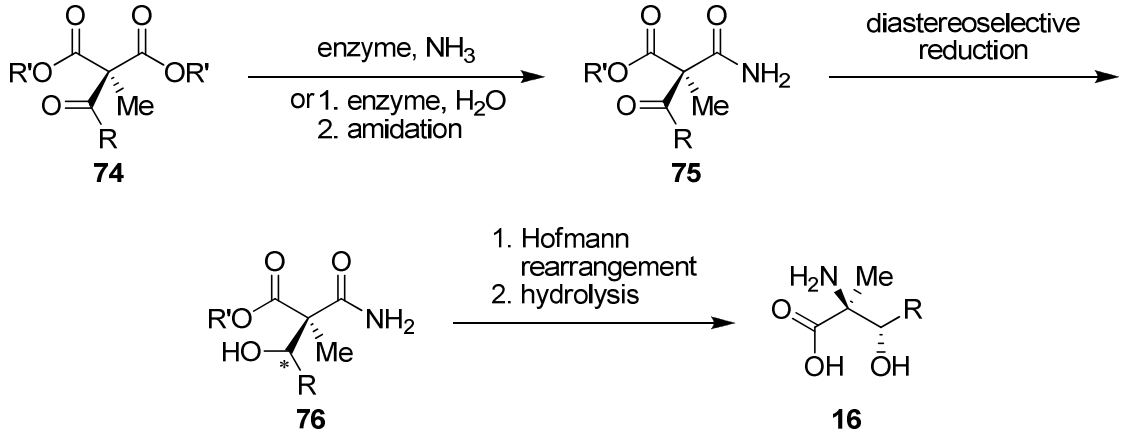
3 Desymmetrisation: PLE-catalysed hydrolysis of a dialkyl malonate

This concept is based on an enzymatic desymmetrisation of a dialkyl malonate, bearing two different alkyl substituents at C-2 as a key step. Enzymatic desymmetrisation is widely used in organic chemistry as it is a powerful tool for the production of enantiomerically enriched molecules. Unlike kinetic resolution desymmetrisation allows a theoretical yield of 100%.[73]



Scheme 34. Concept for the synthesis of α,α-dialkyl amino acids (**4**) by the desymmetrisation of malonates

Starting from easily accessible dialkyl malonates **28** hydrolysis or, more favorable, ammonolysis of one ester group would lead to chiral derivatives **27** (in case of hydrolysis an additional amidation step is necessary). Hofmann rearrangement of these amides **27** and hydrolysis of the products allows access to α,α-dialkyl amino acids **4** in a short and highly practicable synthetic route (Scheme 34).



Scheme 35. Concept for the synthesis of α-methylated β-hydroxy-α-amino acids (**16**) by the desymmetrisation of malonates

Furthermore, transfer of this concept on acylated malonates (**74**) furnishes a potential access to α-methylated β-hydroxy-α-amino acids of type **16**. As the first stereogenic center is generated by desymmetrisation, diastereoselective reduction of the keto-moiety in **75** and

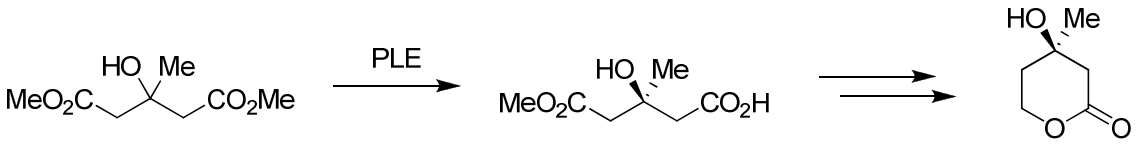
Hofmann rearrangement of the products **76** would lead to diastereomercally and enantiomerically pure protected amino acids **16** in a convenient way (Scheme 35).

3.1 State of the art

As enzymatic desymmetrisation of malonates is a standard technique for the synthesis of enantiomerically enriched molecules plenty of examples are literature-known. Most of these reactions are conducted using pig liver esterase as a catalyst.[74]

Pig liver esterase (PLE, EC 3.1.1.1) is the most widely used esterase in synthetic procedures.[74] As a serine-type esterase belonging to the class of (mammalian) carboxylester hydrolases,[75] PLE comprises several isoenzymes. These are normally organised as trimers of various subunits. The major subunits α, β and γ were isolated by Junge and Heymann in the late 1970s.[76] The most common combinations of subunits are $\alpha\alpha\alpha$, $\alpha\alpha\gamma$, $\alpha\gamma\gamma$ and $\gamma\gamma\gamma$.[76]

Since PLE is a mammalian enzyme, the availability of the enzyme is good due to the easy preparation by extraction of minced pig livers. For this reason the first asymmetric reaction catalysed by PLE appeared as early as 1903,[77] though the potential application remained without use until 1975, when Sih and co-workers revisited the use of PLE in organic synthesis.[78] In this article they applied PLE for the desymmetrisation of prochiral dimethyl 3-hydroxy-3-methyl glutarate (**77**) for the synthesis of (*R*)-mevalonic acid ((*R*)-**78**, Scheme 36).



77 (*R*)-**78**

Scheme 36. Synthetic application of PLE, desymmetrisation of dimethyl 3-hydroxy-3-methyl glutarate (**77**)[78]

Since then interest in the enzyme raised and it was intensively studied. Besides the discovery of many applications as a biocatalyst, especially in hydrolyses reactions for asymmetric kinetic resolutions and desymmetrisation of prochiral compounds,[74] active site models for pig liver esterase were established.[79] As no crystallographic data for this enzyme was available, the dimension of the pocket was determined by empirical approaches, using the data of hydrolyses of different substrates. The most widely used empirical model was

reported by Jones and co-workers, who examined hydrolyses of over 100 methyl esters.[79d] Examination of the hydrolyses of sterically demanding malonates, bearing bulky bicylcononyl, adamantyl or 3-biphenylyl groups led to further improvements and to the state of the art of this model (Figure 10).[79a]

The active site consists of two polar binding sites, $P_{F(ront)}$ and $P_{B(ack)}$, and two hydrophobic pockets, $H_{L(arge)}$ and $H_{S(mall)}$. The catalytically essential region is that encircling the serine residue that initiates hydrolysis by its attack on the carbonyl group of the susceptible ester function. [79d] This region is located near P_B, which interacts well with hydrogen bond donors like alcohol, ether and carbonyl functions. P_F's most common role is to bind the nonhydrolysed second ester function of diester substrates. In contrast to P_B, P_F is not too polar to accept hydrophobic moieties. [79d]

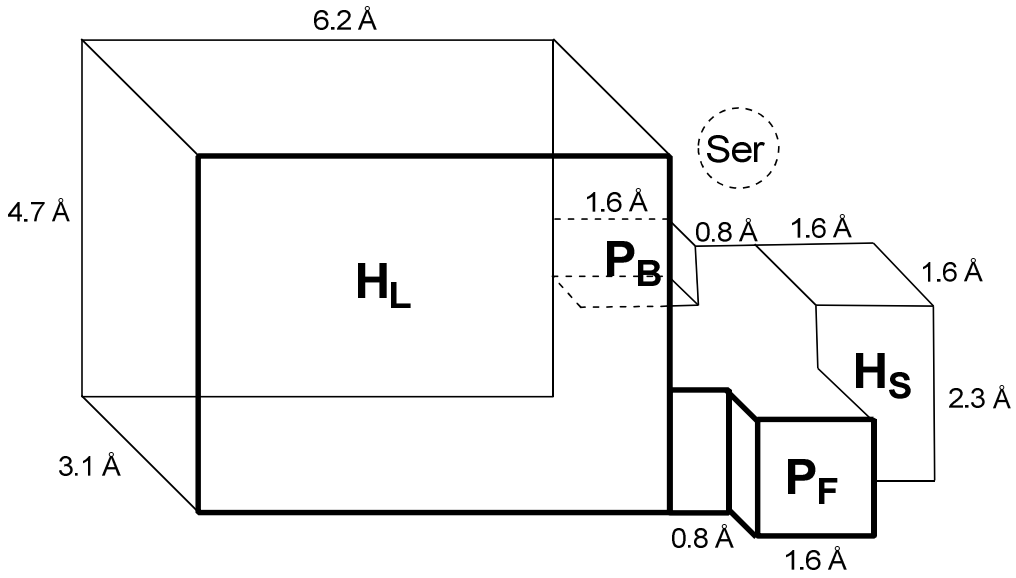


Figure 10. Model of the active site of PLE, according to Jones and coworkers[79a]

The two hydrophobic zones interact with the aliphatic or aromatic residues of a substrate. The larger one (H_L) has a volume of approximately 90 Å^3 (6.2 Å x 4.7 Å x 3.1 Å), the smaller one (H_s) has roughly the volume of 6 Å^3 (1.6 Å x 2.3 Å x 1.6 Å).[79a] Whereas polar groups such as hydroxy, amino, carbonyl and nitro are excluded from these areas, less polar heteroatom functions such as halogen, ether or ketal oxygen atoms can be accommodated if necessary. Most pockets seem to be closed, although there are some open faces of the model. As well as the rear boundary of the P_B-pocket is open, where hydrogen bonding or other polar groups may extend out beyond of this region, the area above the center of the model is open and completely accessible to any substrate moiety that needs to locate there.[79d]

Even though PLE is a well studied enzyme with many advantageous properties, some organic chemists, especially in the pharmaceutical industry, were reluctant to choose PLE as a biocatalyst for several reasons. One reason was the animal origin of PLE, which led to restrictions in some synthetic fields like pharmaceuticals. The mammalian enzyme could potentially introduce viruses in the process stream, which could lead to a contaminated product. This is still an important issue although recent studies showed a very low risk of viral contamination through the use of PLE in synthesis.[80]

Even more important is the lack of purity of commercial available PLE, which are actually crude mixtures of pig liver extractions with organic solvents. Therefore, not only proteins – and clearly not just esterases – will be present, leading to unwanted side-reactions as well as to reproducibility problems. These reproducibility problems are intensified by the fact that pig liver esterase produces several isoenzymes,[76,81] each displaying different catalytic activities and enantioselectivities towards a certain substrate.[82]

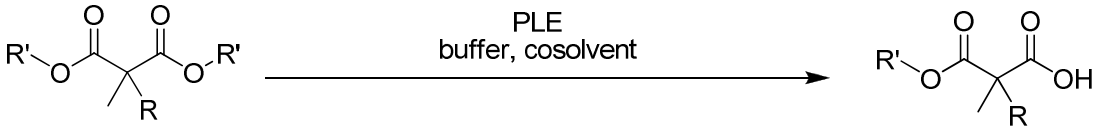
To overcome these problems, attempts to achieve the cloning and overexpression of PLE in microbial hosts have been extensively pursued since the 1990s. As PLE is an enzyme from animal origin, cloning was cumbersome and early attempts led to non-active enzymes.[83] After some efforts, functional PLE was first cloned in *Pichia pastoris* by the Bornscheuer group in 2001.[84] Whereas the recombinant enzyme named *r*-PLE or *rec*-PLE shows a similar substrate range compared to commercial available PLE, selectivities towards some substrates are somewhat better.[85] In the last decade some PLE-mutants were developed, often showing enhanced or even altered enantioselectivity compared to the wild type *r*-PLE.[86] Very recently another isoenzyme of PLE called APLE (for alternative PLE) was expressed in *Pichia pastoris.*[87] All these results are reopening the PLE field as PLE is now available from microbial origin in reproducible form, containing one single isoenzyme. In addition the possibility of mutagenesis could lead to “tailor-made” enzymes with enhanced properties to specific substrates.

Whereas many reports for the desymmetrisation of malonates by hydrolysis are known, the desymmetrisation of malonates by ammonolysis is, to the best of my knowledge, still unknown.[88] The first desymmetrisation of a malonate substrate using PLE as a catalyst was conducted by Schneider *et al*. in 1984.[89] Many other examples were added in the last decades.[79b,c,90] An overview is shown in Table 11.

Whereas substrates with one bulky substituent are converted with high enantioselectivities (Table 11, entries 1-6), enantioselecivities using less bulky substituents as isopropyl or

isobutyl are very low (Table 11, entries 8-9). Very recently substrates which would allow access to α-methyl serine were established (Table 11, entry 11), leading to the corresponding monoester in high enantiomeric excess when applying optimised conditions.

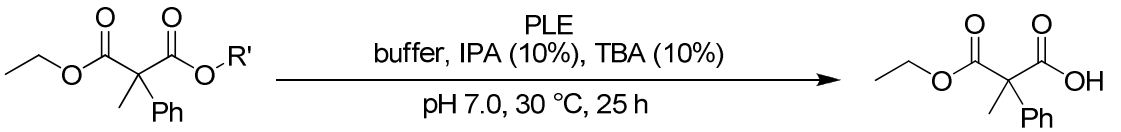
Table 11: Desymmetrisation of 2-methylated malonates in literature (for experimental details see references)



28 **79**

entry	R	Yield (%)	% ee	Ref.
1	*t*-Bu	90	96	[90d]
2	$TMSCH_2$	95	98	[90e]
3	naphtyl	95	> 95	[79c]
4	adamantylmethyl	80	> 98	[79a]
5	3-biphenyl	80	> 98	[79a]
6	4-*t*-Bu-C_6H_4	83	> 97	[79c]
7	Ph	90	81	[79c]
8	*i*-Pr	99	6	[79b]
9	*i*-Bu	98	7	[79b]
10	*i*-Pentyl	97	> 95	[79b]
11	Ch_2OBn	60	> 97	[91d]

It was demonstrated that the addition of cosolvents like DMSO or alcohols (preferable poor nucleophilic alcohols like isopropanol or *tert*-butanol) can influence the activity and enantioselectivity of PLE.[90b,j,91] An impressive example was given by Gröger *et al.* in 2005. They applied different amounts of alcohols for the hydrolysis of diethyl 2-methyl-2-phenylmalonate **28**. The best result was achieved when applying a ternary mixture consisting of buffer (80%), isopropanol (10%) and *tert*-butanol (10%), leading to an increased enantiomeric excess of 96% ee (compared to 81% ee, see Table 11, entry 7).[91a]



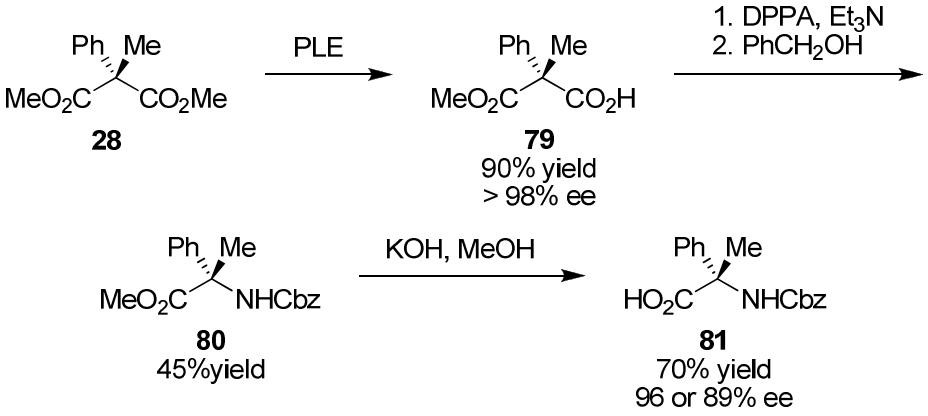
28
50 g/L
300 mL scale

79
83% yield (11.2g)
96% ee

Scheme 37. Optimised hydrolysis of diethyl 2-methyl-2-phenylmalonate in a multi gram scale[91a]

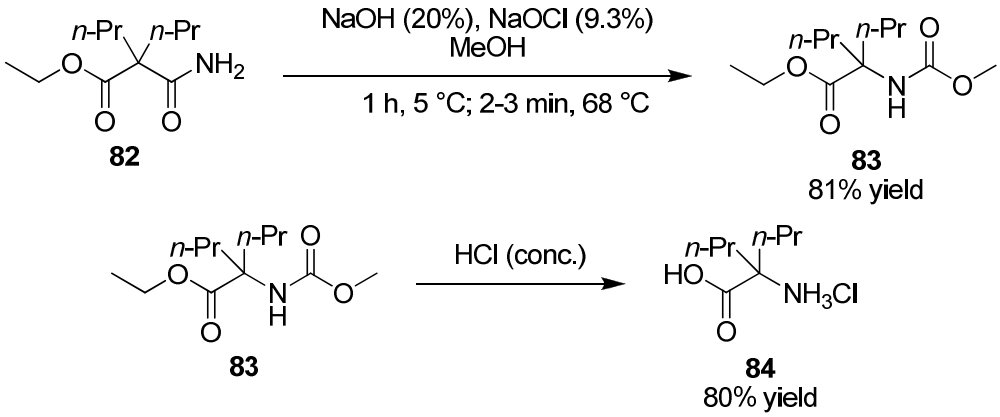
In addition they were able to show that this technique is applicable at a multi gram scale. Later on, they showed that catalytic amounts of water soluble ionic liquids could also improve the activity and selectivity of PLE.[92]

The idea to apply malonate monoesters for the synthesis of α,α-dialkyl α-amino acids was introduced by Schneider *et al.,* who reported the first PLE-catalysed malonate desymmetrisation.[89] The first synthetic example was described by Björkeling *et al.*, preparing substituted phenylalanines from malonates *via* azide formation and subsequent Curtius rearrangement.[90b] This rearrangement technique was used for the synthesis of several amino acids since then.[91b,93] In 2010 Back *et al.* used Curtius technique for the synthesis of (protected) α-methyl phenylglycine **81** and related compounds (Scheme 38).[93b]



Scheme 38. Synthesis of protected α-methyl phenylglycine **81** *via* Curtius rearrangement

Another possibility to convert acids into amines is the formation of an amide and subsequent Hofmann rearrangement to the corresponding amine. Hofmann rearrangement is a classic organic reaction and was first introduced by August W. von Hofmann in 1881.[94] The first application of this method to synthesise amino acids from malonic acid derivatives was described by Huang in 1948.[95] After the development of a Hofmann-type rearrangemenet using lead tetraacetate as an oxidizing agent in the 1970s,[96] this technique was used for the synthesis of some amino acids.[97] Since the intermediate isocyanate is not hydrated but alcoholated, leading to stable carbamates, soluble in organic solvents, this technique allows easy separation of the products. The drawback of this technique is the employment of toxic lead tetraacetate.



Scheme 39. Hofmann rearrangement in methanol[98]

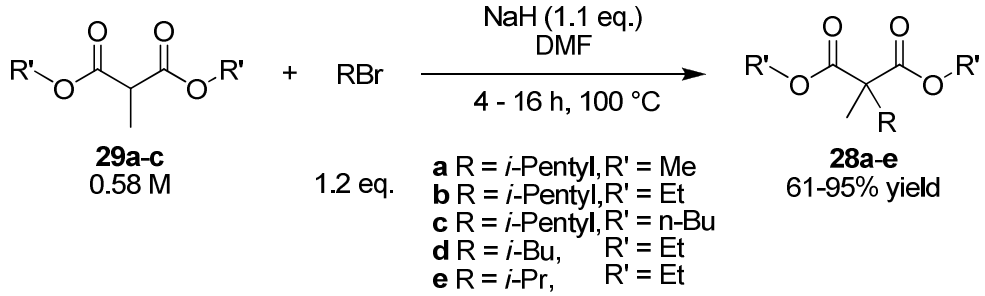
The state of the art in Hofmann rearrangement is described in a patent by Klemiss and Feld.[98] This work offers the possibility to easily isolate the rearranged product **83** avoiding toxic reagents. Like within the lead tetraacetate technique, the intermediate isocyanate is not hydrated which would lead to the loss of carbon dioxide. Instead of water, methanol, which is used as a solvent, adds to the C-N double bond of the intermediate isocyanate, leading to stable carbamates **83**, which are readily soluble in organic solvents and could be easily converted into free amino acids **84** (Scheme 39). Surprisingly, to the best of my knowledge, the potential of this sophisticated rearrangement technology in combination with PLE-catalysed hydrolysis has not been demonstrated until now by a practical example. A related approach, applying bromine and sodium methoxide as reagents was currently applied in the synthesis of α-methyldopa (**19**).[99]

3.2 Results and discussion

3.2.1 Substrate synthesis

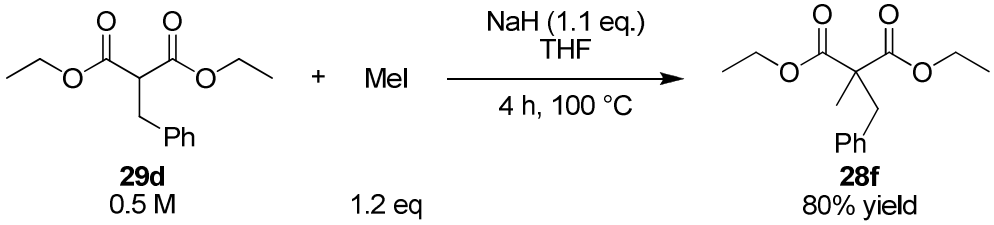
The substrates for this synthetic route towards α-methylated amino acids can be easily synthesised from readily commercial available monosubstituted malonates.

Syntheses of dialkyl substrates **28a**-**e** were done in DMF as a solvent using 1.1 equivalents of sodium hydride as a base. DMF seemed to be a more preferable solvent compared to ethers like MTBE or THF as the intermediate sodium salts are less soluble in the latter solvents – and for this reason readily precipitate – leading to stirring problems of the heterogeneous mixture. Alkylation was done using 1.2 equivalents of an isoalkyl bromide leading to the desired disubstituted malonates **28a**-**e** in moderate to good yields (Scheme 40, Figure 11).



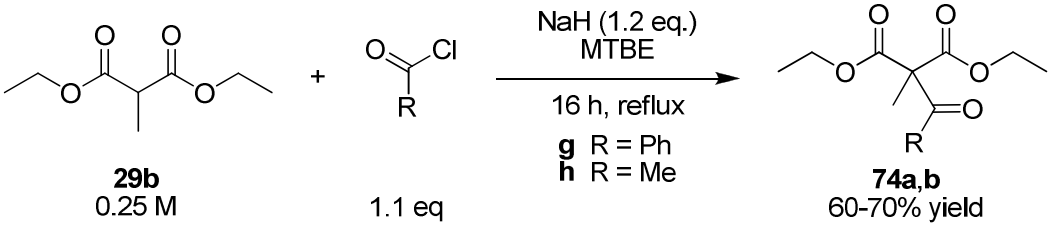
Scheme 40. Syntheses of dialkyl 2-isoalkyl-2-methylmalonates **28a**-**e**

Unlike most substrates, diethyl 2-benzyl-2-methylmalonate (**28f**) was synthesised starting from diethyl 2-benzylmalonate (**29d**), using methyl iodide as a methylating agent (Scheme 41, Figure 11).



Scheme 41. Synthesis of diethyl 2-benzyl-2-methylmalonate (**28f**)

β-Keto substrates **74a**,**b** were synthesised in a similar manner, starting from diethyl 2-methylmalonate **29b** applying 1.2 equivalents sodium hydride as a base in MTBE as a solvent. Acylation was done using 1.1 equivalents of the corresponding acyl chloride. The desired substrates were isolated in moderate yields (Scheme 42, Figure 11).



Scheme 42. Syntheses of β-keto substrates **74a**,**b**

An overview of the synthesised substrates is given in Figure 11.

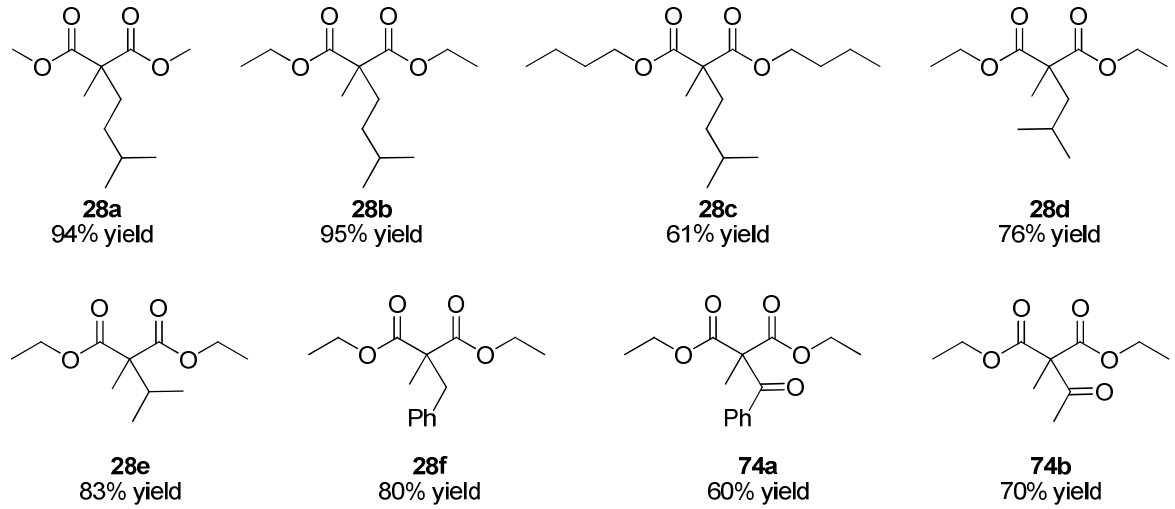
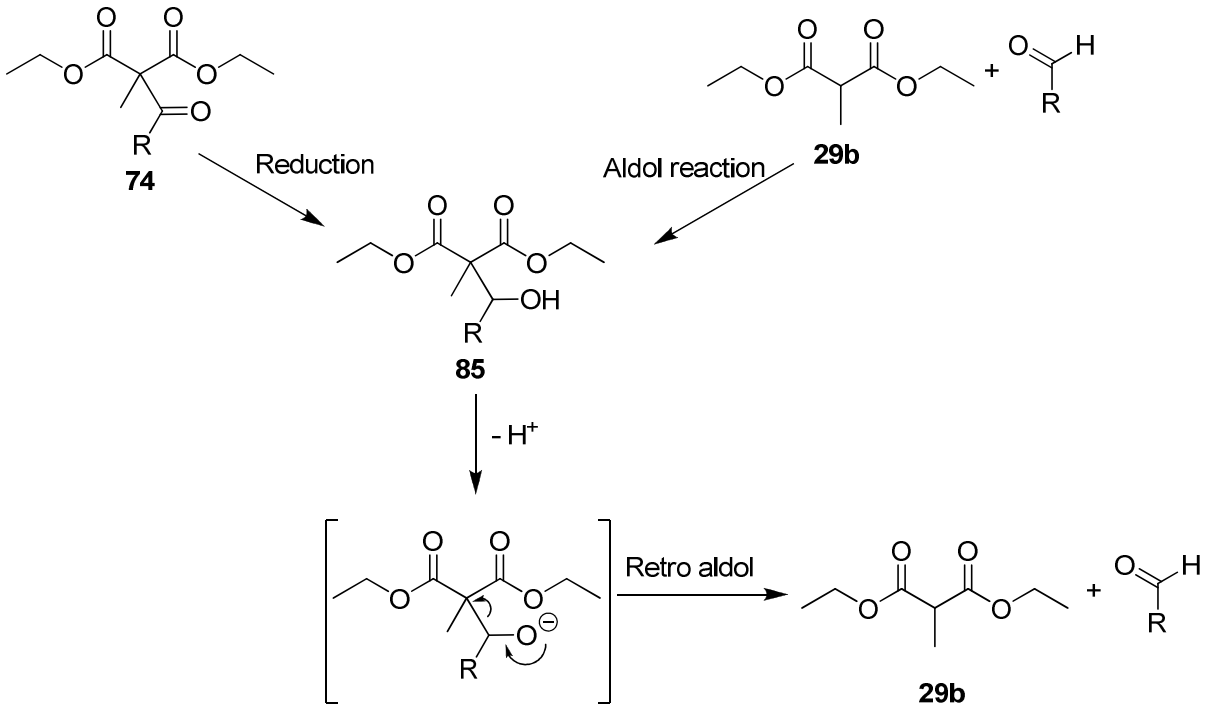


Figure 11. Overview of synthesised malonates

The synthesis of β-hydroxy substrates such as diethyl 2-(hydroxyphenyl)-2-methylmalonate **85** was not possible, neither starting from preformed keto substrates **74a** by subsequent reduction nor by aldol reaction between diethyl 2-methylmalonate **29b** and an aldehyde due to instability of the products. These products are known to decompose within minutes to hours by retro aldol reaction (Scheme 43).[100]

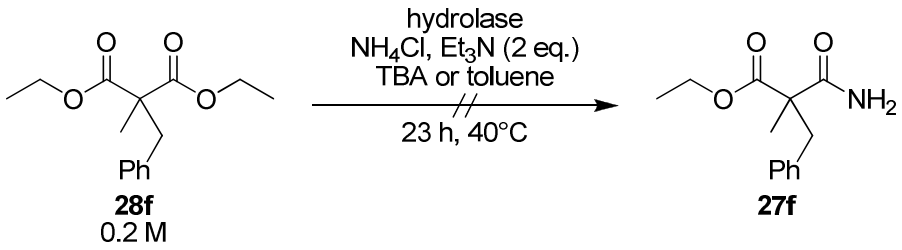


Scheme 43. Impossible synthesis of β-hydroxy substrates **85** due to retro aldol reaction

3.2.2 Key step: Desymmetrisation

3.2.2.1 Attempts towards an ammonolysis reaction

Enzymatic ammonolysis would be the most preferable way to desymmetrise malonates with a perspective to synthesise amino acids. As ammonolyses would lead directly to the desired amides **27** starting from malonates **28** Hofmann rearrangement could be conducted without any further previous chemical transformation. Thus, an enzyme screening, applying 16 different enzymes was conducted using diethyl 2-benzyl-2-methylmalonate (**28f**) as a substrate (Scheme 44). The conditions of the screening were chosen according to lipase-catalysed ammonolyses reactions in literature.[101] In addition to these conditions (triethylamine and ammonium chloride in *tert*-butanol), toluene was tested as a solvent.

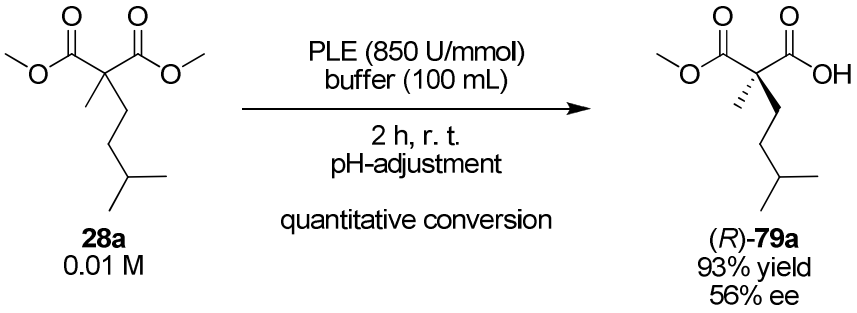


Scheme 44. Enzyme screening for the ammonolysis of diethyl 2-benzyl-2methylmalonate (**28f**)

None of the tested enzymes showed activity to ammonolyse diethyl 2-benzyl-2-methylmalonate, neither in *tert*-butanol nor in toluene. In all cases the substrate remained unreacted after work up.

3.2.2.2 Hydrolysis: reproducibility of literature reactions

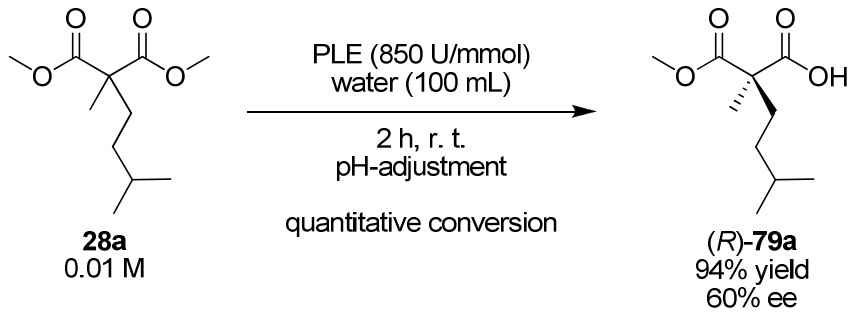
As ammonolyses of malonates **28** failed, well-established hydrolysis technique, applying PLE as a catalyst was chosen as an alternative. Since the hydrolysis of **28a** is described in literature,[90f] hydrolysis of this substrate under standard conditions was applied first using phosphate buffer (pH 7) as a solvent (Scheme 45).



Scheme 45. PLE-catalysed hydrolysis of **28a** in buffer

The pH of the reaction media was adjusted by automatic titration of sodium hydroxide (0.2M). This titration was done, applying a 702 SM Titrino™ from METROHM Inc.[102]

Under these conditions, hydrolysis of **28a** proceeded with quantitative conversion within two hours. The monoester **79a** was isolated in 93% yield but with only a moderate enantiomeric excess of 56% ee. Hydrolysis of dimethyl 2-isopentyl-2-methyl malonates in literature was conducted in water, yielding (*R*)-**79a** with an enantiomeric excess of > 95% ee. Thus, the reaction was conducted under comparable conditions. Unfortunately the enantiomeric excess was significantly poorer compared to literature (Scheme 46 compared to Table 11, entry 10)[89f] when applying reaction conditions according to literature.



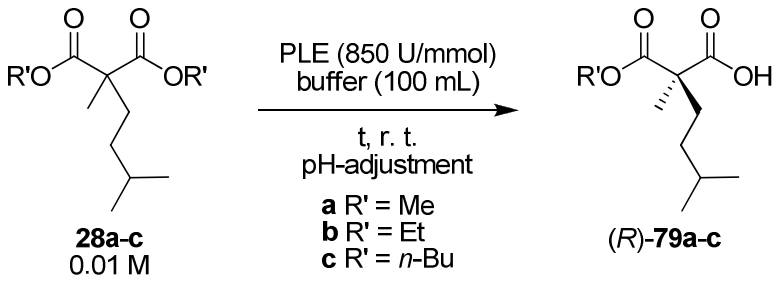
Scheme 46. PLE-catalysed hydrolysis of **28a** in water

Reproducibility problems often arise when applying PLE due to the heterogeneity of the catalyst being a crude extract from pig liver, containing fluctuate amounts of different isoenzymes of PLE besides impurities from other enzymes. Nevertheless the product was assigned to be the (*R*)-enantiomer as all related malonates were hydrolysed to the corresponding (*R*)-enantiomer as well (see references in Table 11).

3.2.2.3 Process development

Since PLE offered good reactivity towards **28a** but only a moderate selectivity of 56-60% ee, a purposeful process development to increase the enantiomeric excess of the monoester **79a** was needed. The influence of ester residues R' on the reaction was studied next, applying standard conditions (Table 12).

Table 12: Hydrolyses of different esters in buffer



28a-c
0.01 M

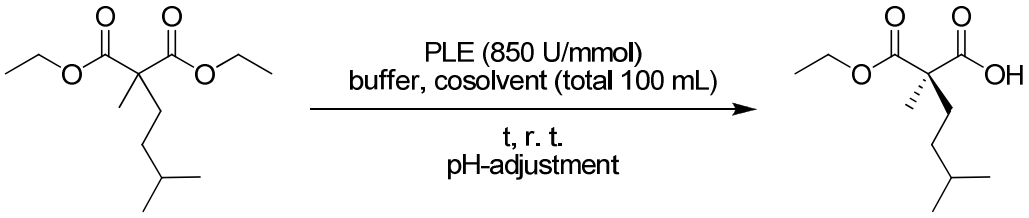
(*R*)-**79a-c**

entry	substrate	t (h)	conversion	yield (%)	ee (%)
1	**28a**	2	quant.	93	56
2	**28b**	19	quant.	84	63
3	**28c**	120	79	64	82

Whereas the methyl ester **28a** was hydrolysed very fast but with only a moderate enantioselectivity of 56% ee (Table 12, entry 1), the *n*-butyl ester **28c** was converted much slower, leading to a non-quantitative conversion of 79% even after 120 hours reaction time (Table 12, entry 3). However the enantiomeric excess of the *n*-butyl ester **79c** was notably higher (82% ee) than those of the methyl ester **79a**. The ethyl ester **28b** was converted quantitatively over night, yielding the desired monoester **79b** in 63% ee (Table 12, entry 2).

The ethyl ester **28b** seemed to be the most promising substrate, offering an improved selectivity compared to **28a** without significant loss of reactivity. As it is well known that water soluble poor nucleophilic alcohols such as *tert*-butanol and isopropanol could improve the enantioselectivity of PLE-catalysed hydrolyses of malonates[91] the influence of different amounts of these solvents on the hydrolysis of **28b** was examined.

Table 13: Influence of different amounts of alcohols on the desymmetrisation of substrate **28b**



28b
0.01 M

(*R*)-**79b**

entry	cosolvent	t (h)	conversion (%)	yield (%)	ee (%)
1	-	19	quant.	84	63
2	IPA (10%)	28	quant.	99	81
3	IPA (20%)	21	quant.	90	86
4	IPA (30%)	92	quant.	80	95
5	TBA (10%)	19	quant.	95	79
6	TBA (20%)	21	quant.	98	86
7	TBA (30%)	92	49	21	94

Complete conversion could be attained at almost all reaction conditions. As reactions were performed in two Titrino^{TM}-apparatus, two reactions were carried out in parallel without the possibility to stop the detection of the reaction cours of one reaction without stopping the other detection (this problem is generated by tiamo^{TM}-software). Thus, times given in Table 13 are not actually reaction times but times when the slower of both parallel reactions had a quantitative conversion. For this reason a comparison of the reaction rates is not possible by comparing the times given in Table 13.

However, tiamo^{TM} gives the possibility for online monitoring the reaction rate by the amount of sodium hydroxide added for pH-adjustment. This leads to the possibility to compare conversions for each reaction at any reaction time.

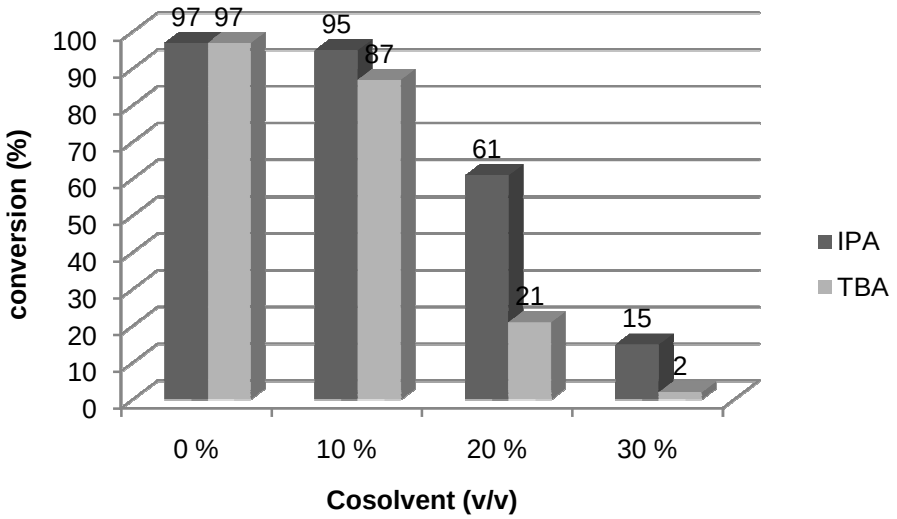


Figure 12. Conversion of **28b** after three hours reaction time

After three hours reaction time, the reaction in pure buffer seemed to be nearby complete. The conversion of the reaction conducted with 10% isopropanol also proceeded very fast resulting in a conversion of 95% after three hours. No significant differences were detectable when examining hydrolysis applying 10% of *tert*-butanol compared to 10% of isopropanol (87% conversion compared to 95% conversion). Huge differences between both solvents could be seen by comparing conversions of the reactions conducted with 20% and 30% of one of the cosolvents. Whereas the reaction in buffer / isopropanol (20% isopropanol) seemed to proceed fairly well (61% conversion), the rate of the reaction in buffer / *tert*-butanol (20% *tert*-butanol) was approximately one third (21% conversion) compared to the reaction conducted with isopropanol as a cosolvent. The lowest conversions were determined when applying 30% of an alcohol with the reaction conducted in buffer / *tert*-butanol having virtually no detectable conversion (2%).

Even more interesting were the results by comparing conversions after one hour, displaying approximately the initial reaction rates. It could be seen that even 10% of isopropanol slows down the reaction rate of hydrolyses from 78% conversion (no cosolvent) to 54% after one hour. The effect of *tert*-butanol was even more dramatic, lowering the conversion to 36% when applying just 10% of it as a cosolvent. Higher concentrations of alcohols led to a further decrease in reaction rates with *tert*-butanol having more effect on the reaction rate than isopropanol.

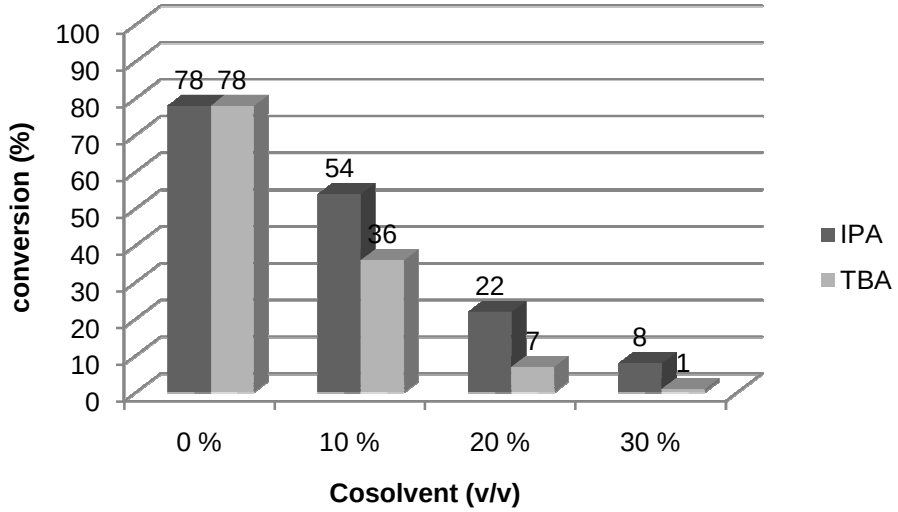


Figure 13. Conversion of **28b** after one hour reaction time

Even more important than the influence on the reaction rate is the influence on the enantioselectivity (Figure 14). Whereas enantioselectivity in pure buffer was only moderate (63% ee, Table 13, entry 1), the selectivity dramatically increased to 79-81% ee when applying 10% of an alcohol as a cosolvent (Table 13, entry 2,5). Application of higher amounts of alcohol led to further increase in enantioselectivity up to 95% ee when applying 30% of isopropanol (Table 13, entry 4). For this reason, the application of 30% isopropanol was chosen as the best condition, allowing access to the desired monoester (*R*)-**79b** in high enantiomeric excess of 95% ee within a reaction time of 92 hours.

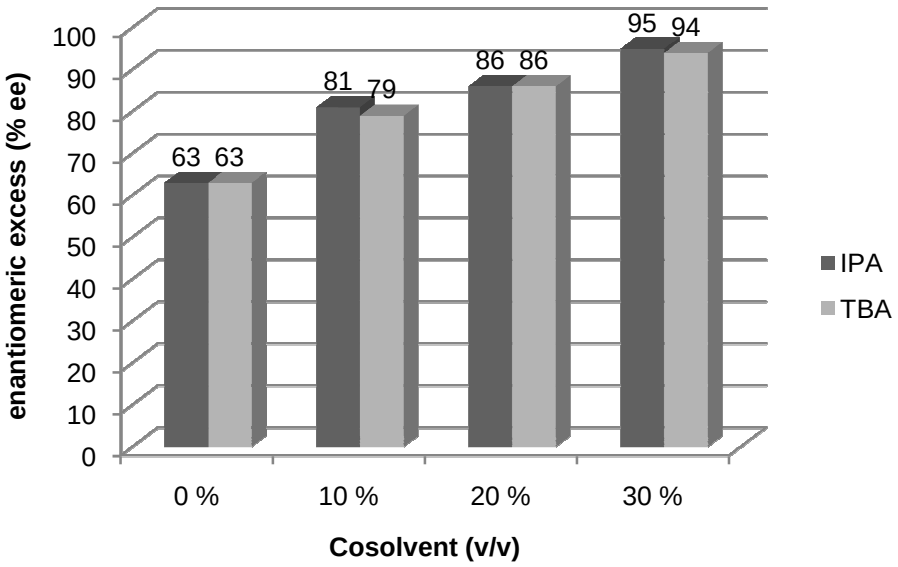
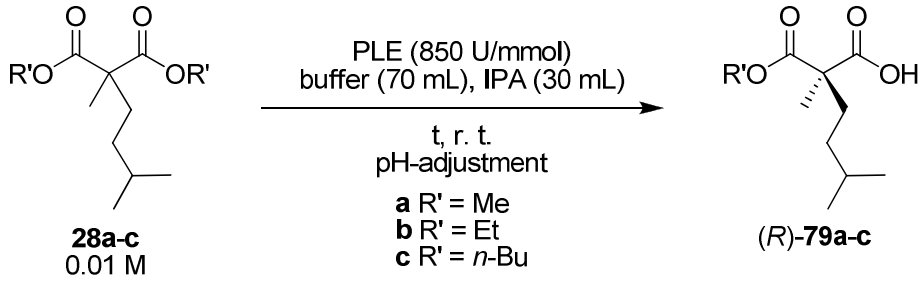


Figure 14. Enantioselectivity of hydrolyses dependent on the amount of cosolvent

Three major explanations for the solvent dependency of the enantiomeric excess should be discussed. First, it is known that solvents alter the 3D-structure of enzymes. The 3D-structure of the active site might thus be altered in a manner that one orientation of the substrate is more favoured, leading to an improved stereoselectivity. Second, as stated above, PLE consists of different isoenzymes, which are built up from three parts. It is possible that some trimers are more favoured to be formed, when a cosolvent is added to a once aqueous solution. If these trimers are more selective towards a specific substrate orientation the selectivity can rise. For this reason, pure γ-PLE, which is available recombinant from *P. pastoris* and so cans only build up γγγ-trimers, often shows superior selectivity compared to crude PLE.[85] The last issue to be addressed is the possibility that impurities in the enzyme formulation, especially lipases, might be deactivated by adding water soluble cosolvents. Unlike esterases, most lipases need two phases, an organic and an aqueous phase, to show a reasonable activity. As malonates are poorly soluble in pure water, two phases exist when carrying out the reaction in pure aqueous media. Solubility in mixtures of alcohol and water is much better, resulting in only one homogenous phase. Consequently, lipases are less active in this media. As lipases showing no or the "wrong" selectivity towards the substrate are deactivated the overall selectivity rises. It is likely that all of the above mentioned reasons have an influence on the stereochemical outcome of the reactions.

The optimised conditions were applied for the enantioselective enzymatic hydrolysis of the methyl ester **28a** and the *n*-butyl ester **28c** (Table 14).

Table 14: Hydrolyses of different esters under optimised conditions



28a-c
0.01 M

(*R*)-**79a-c**

entry	substrate	t (h)	conversion (%)	yield (%)	ee (%)
1	**28a**	168	quant.	94	93
2	**28b**	92	quant.	80	95
3	**28c**	168	76	58	94

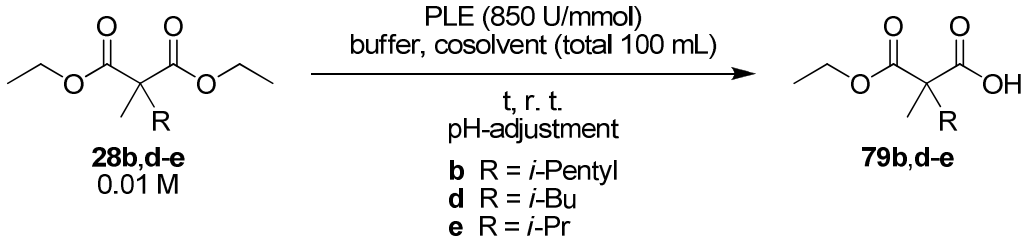
Whereas dimethyl 2-isopropyl-2-methylmalonate (**28a**) was converted quantitatively (Table 14, entry 1) the hydrolysis of the corresponding *n*-butyl ester **28c** under these conditions proceeded very slowly. Even after one week reaction was not completed (Table 14, entry 3).

The influence of the ester residues on the enantioselectivity under optimised conditions was negligible. Enantiomeric excesses were in the range of 93 to 95% ee, with ethyl ester **28b** being the best substrate.

3.2.2.4 Study of substrate range

To proof the generality of these optimised conditions, diethyl 2-isoalkyl-2-methyl malonates **28e**,**f** were tested as further substrates under these conditions and compared to hydrolysis under standard conditions, using no cosolvent (Table 15).

Table 15: Application of optimised condition on further 2-isoalkyl-2-methylmalonates



entry	substrate	cosolvent	t (h)	conversion (%)	yield (%)	ee (%)
1	**28b**	-	19	quant.	84	63
2	**28b**	IPA (30%)	92	quant.	80	95
3	**28d**	-	6	quant.	93	63
4	**28d**	IPA (30%)	168	92	n. d.	87
5	**28e**	-	6	quant.	86	14
6	**28e**	IPA (30%)	168	48	n. d.	14[a]

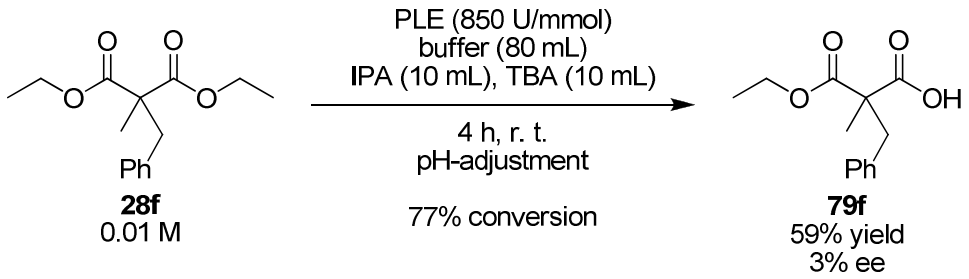
[a] opposite enantiomer is the major enantiomer.

Diethyl 2-isobutyl-2-methylmalonate (**28d**) was converted quantitatively within six hours, when applying standard conditions. The enantiomeric excess was determined to be 63% ee (Table 15, entry 3), which is a remarkably high selectivity compared to 7% ee, determined for the corresponding methyl ester in literature (Table 11, entry 9). The enantioselectivity could be further improved by applying 30% isopropanol as a cosolvent, leading to desired monoester **79d** in 87% ee (Table 15, entry 4).

Unfortunately enantioselectivity was very low when applying diethyl 2-isopropyl-2-methylmalonate (**28e**) as a substrate under standard conditions (Table 15, entry 5). In addition it was not possible to increase enantioselectivity by application of the optimised conditions (Table 15, entry 6). Interestingly the enantioselectivity of the enzyme was altered by addition of 30% isopropanol, leading to the opposite enantiomer as the major enantiomer.

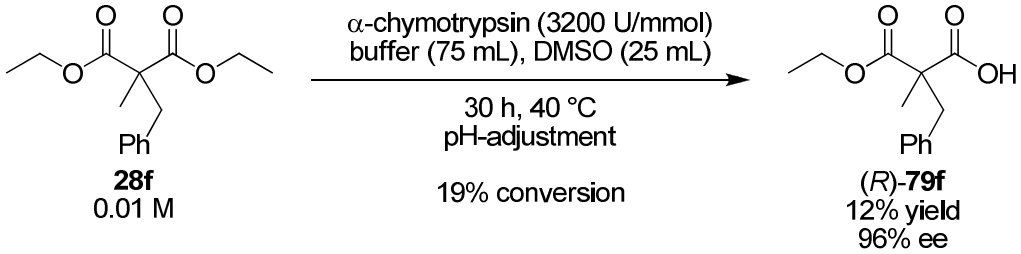
The relatively small isopropyl residue seems to fit well in both hydrophobic pockets H_L and H_S (Figure 10) leading to poor enantioselectivities.

Furthermore, diethyl 2-benzyl-2-methylmalonate (**28f**) was hydrolysed using optimised conditions for diethyl 2-methyl-2-phenylmalonate described in literature.[91a] Reaction under these conditions proceeded smoothly, leading to the desired monoester **79f** in 59% yield in four hours reaction time (Scheme 47).



Scheme 47. PLE-catalysed hydrolysis of **28f**

As PLE provided virtually no enantiomeric excess for this substrate, α-chymotrypsin was chosen as an alternative catalyst, which is known to hydrolyse **28f** in a very selective manner, when applying 25% DMSO as a cosolvent.[90c]

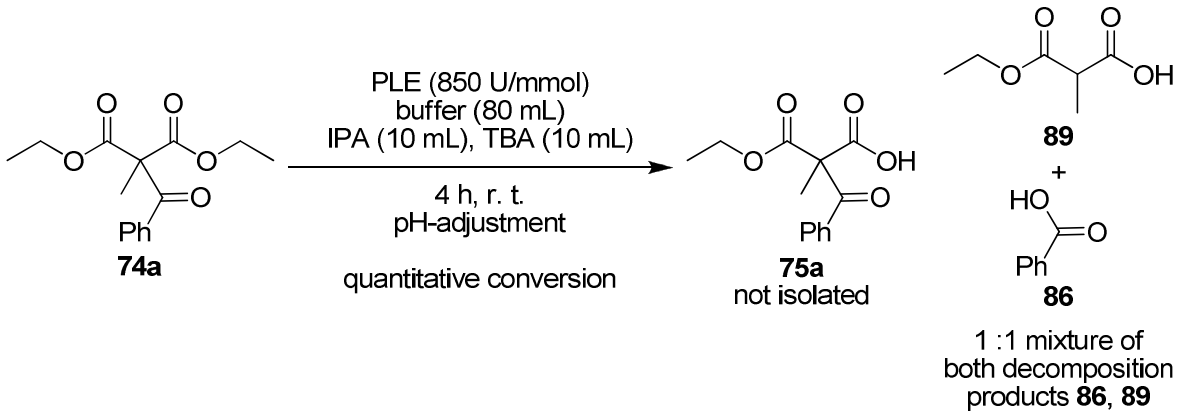


Scheme 48. α-chymotrypsin catalysed hydrolysis of **28f**

This excellent selectivity could be reproduced yielding (*R*)-**79f** with 96% ee, although the conversion was low even when applying a high enzyme loading of 3200 units per mmol at 40 °C (Scheme 48). Due to low conversions, an enzyme screening to identify further hydrolases with activity towards this substrate was done. Unfortunately none of the tested lipases showed significant activity towards **28f**.

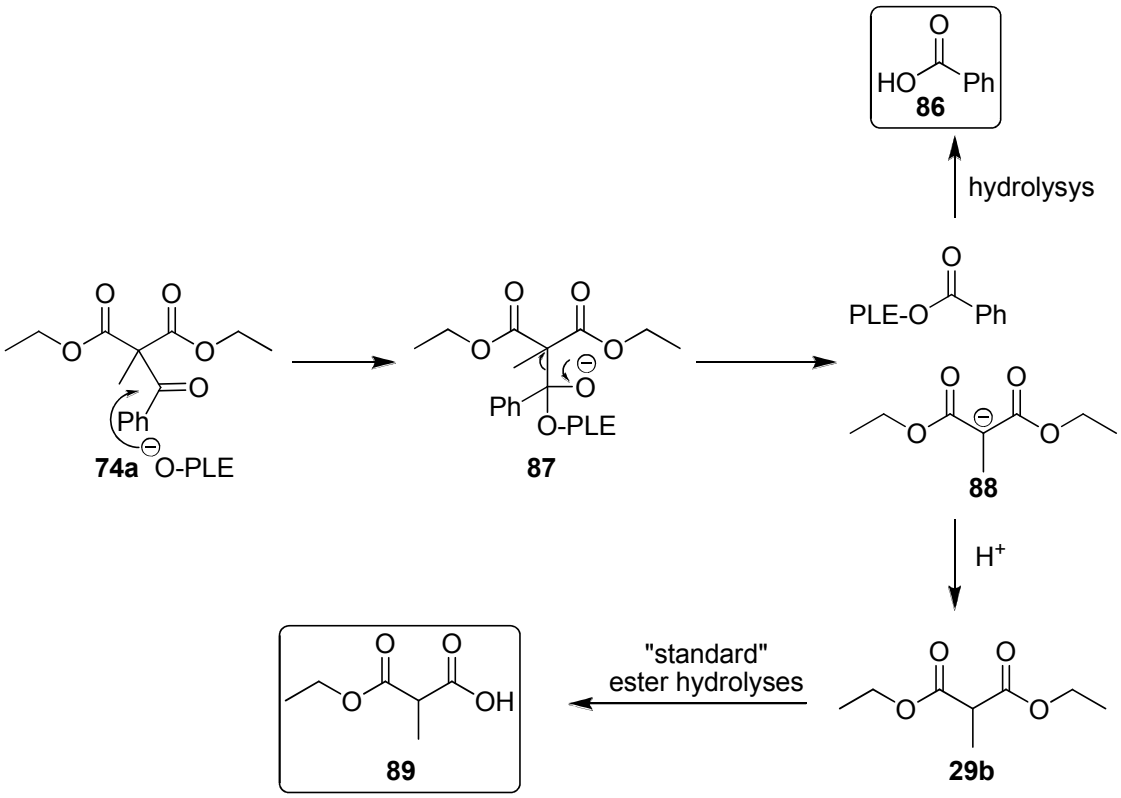
Finally desymmetrisation of β-keto substrate **74a** was examined. The reaction proceeded quite fast, completing the reaction within one day. Unfortunately it was not possible to isolate the desired monoester **75a**. Instead, benzoic acid **86** and deacylated monoethyl

2-methylmalonate **89** was found in the reaction mixture. Interestingly, the same products were described to be found in the non-enzymatic hydrolysis of this substrate.[103]



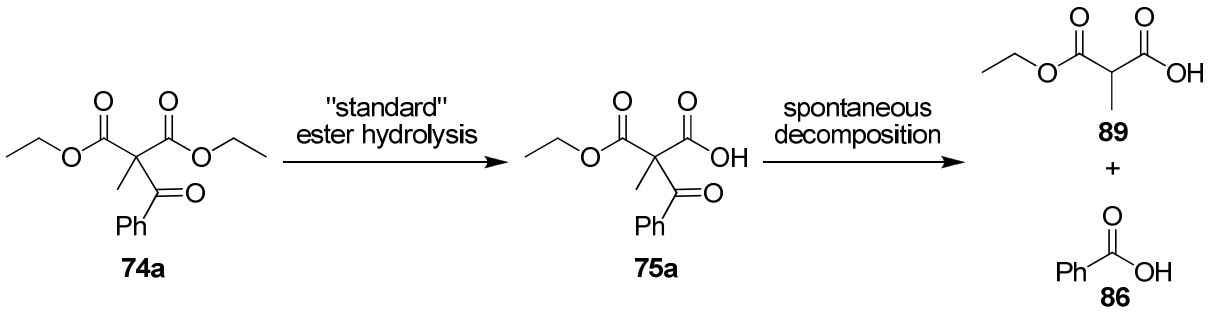
Scheme 49. Hydrolysis of β-keto substrate **74a**

As no conversion was seen under these conditions when conducting the reaction without enzyme, the reaction must be catalysed by the enzyme. Two possible mechanisms for this reaction were assumed. On the one hand, the serine residue could attack the keto-moiety of **74a** first, leading to the tetrahedral intermediate **87**. Esters of malonic acids are known to be C-H acidic. As a result of this acidity, malonate **88** is an acceptable leaving group. The protonated diethyl 2-methylmalonate **29b** is attacked a second time by serine, leading to the monoester **89** (Scheme 50).



Scheme 50. Possible mechanism for the deacetylation of **74a** I: Nucleophilic attack on keto-group

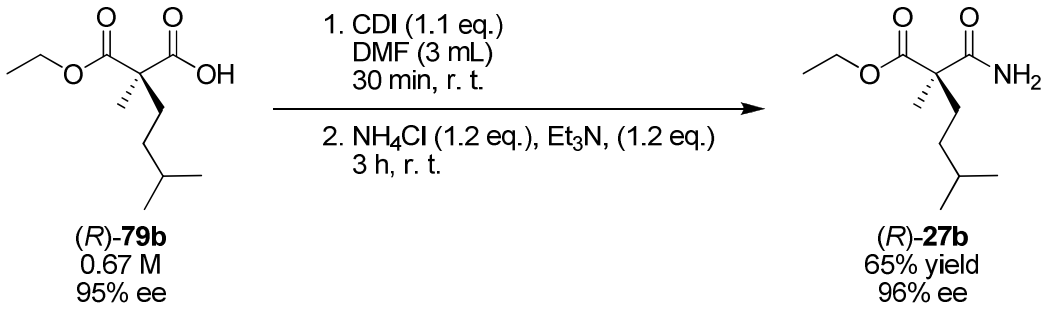
On the other hand, the first attack of the serine group might be directed towards an ester residue. The resulting monoester could be too unstable and might loose the benzoyl group spontaneously (Scheme 51).



Scheme 51. Possible mechanism for the deacetylation of **74a** II: Standard ester hydrolysis

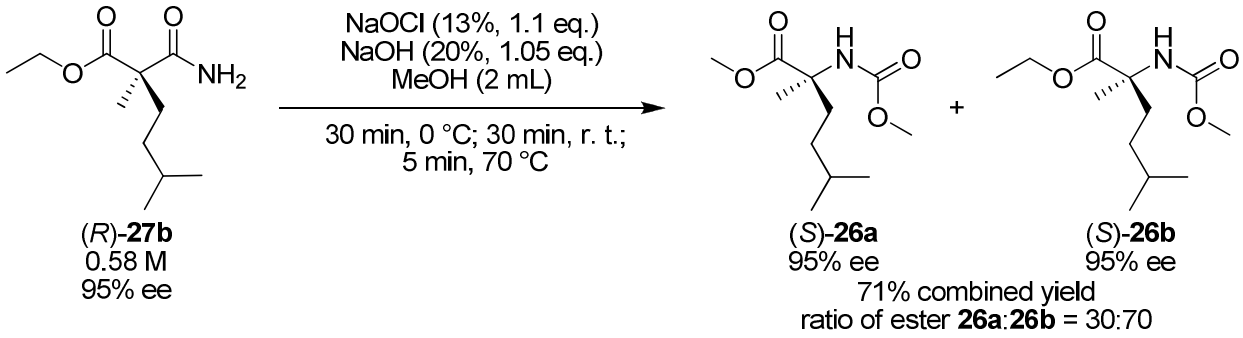
3.2.3 Total synthesis of 2,5-dimethyl norleucine

Since diethyl 2-isopentyl-2-methylmalonate (**28b**) was prooven to be a suitable substrate for PLE-catalysed desymmetrisation, the resulting monoester (*R*)-**79b** was applied for the synthesis of the α,α-dialkyl amino acid (*S*)-2,5-dimethyl norleucine ((*S*)-**14b**). A standard protocol for the syntheses of amides[104] was chosen to synthesise (*R*)-ethyl 2-carbamoyl-2,5-dimethylhexanoate ((*R*)-**27b**). The product could be isolated in 65% yield after chromatography. The enantiomeric excess was proven by HPLC-chromatography to retain unaltered during this transformation (Scheme 52).



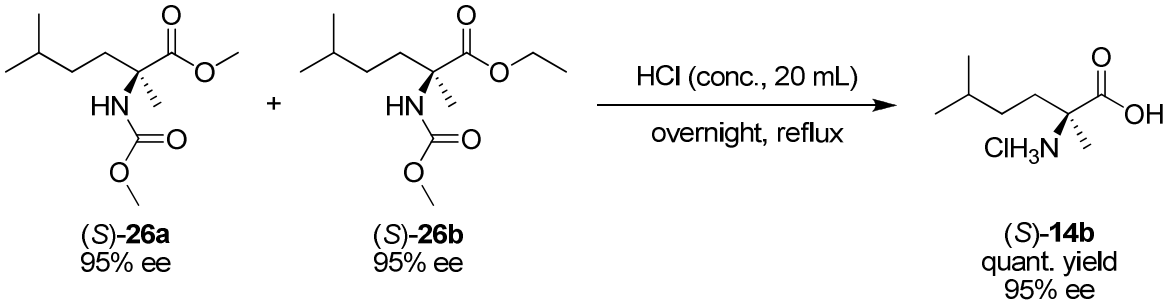
Scheme 52. Synthesis of (*R*)-ethyl 2-carbamoyl-2,5-dimethylhexanoate ((*R*)-**27b**)

After successful synthesis, the amide (*R*)-**27b** was applied to Hofmann rearrangement under conditions according to Kleemiss and Feld (Scheme 53).[98]



Scheme 53. Hofmann rearrangement of (*R*)-**27b**

The resulting protected amino acid was yielded in 71% as a mixture of the methyl ester (*S*)-**26a** and the ethyl ester (*S*)-**26b** in a ratio of 30:70. Both esters had an enantiomeric excess of 95% ee according to HPLC-analysis. The mixture of both esters was applied for hydrolysis to amino acid hydrochloride (*S*)-**14b** without further purification.



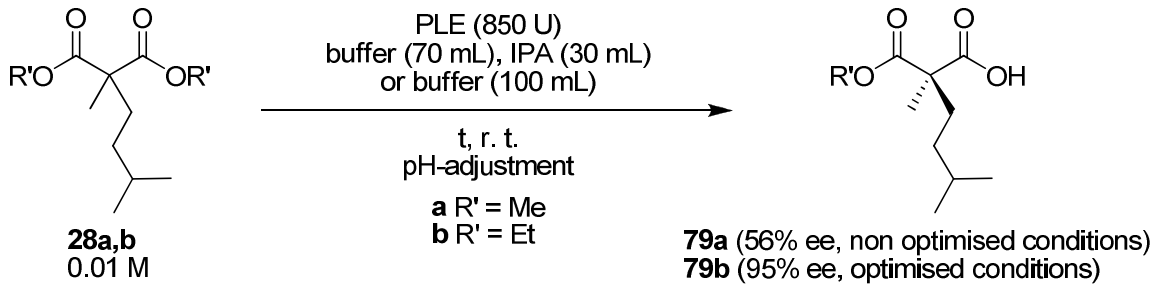
Scheme 54. Hydrolysis to (*S*)-2,5-dimethyl norleucine (*S*)-**14b**

Hydrolysis of the mixture, consisting of (*S*)-**26a** and (*S*)-**26b**, in concentrated hydrochloric acid delivered smoothly the desired amino acid (*S*)-2,5-dimethyl norleucine ((*S*)-**14b**) as a hydrochloride in quantitative yield (Scheme 54). The enantiomeric excess of the product was determined to be 95% ee by HPLC analysis of the corresponding ethyl ester, synthesised *via* standard technique.

Thus, Hofmann rearrangement technique offers a convenient way for the total synthesis of (*S*)-2,5-dimethyl norleucine ((*S*)-**14b**) starting from prochiral diethyl 2-isopentyl-2-methylmalonate (**28b**). The overall yield of the three step synthesis starting from monoethyl malonates **79b** is 46%. The Hofmann rearrangement technique appears to be superior to the Curtius rearrangement technique for two major reasons. First toxic azides are avoided, second the overall yield is higher compared to Curtius rearrangement technique (for an example of a Curtius rearrangement applied to synthesise amino acids from malonic acid monoesters see Scheme 38).[93b]

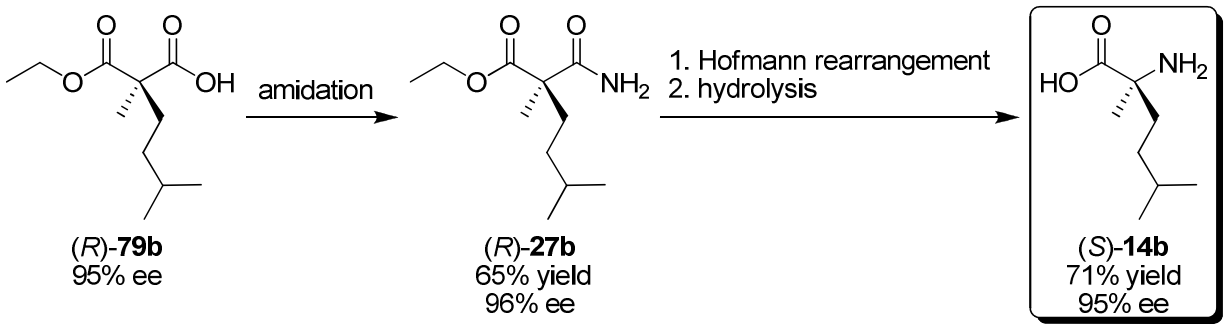
3.3 Summary

To sum up, it was shown that PLE-catalysed desymmetrisation of dialkylmalonates **28** and subsequent Hofmann reaction of a preformed amide **27** represents an efficient synthetic protocol for the synthesis of α,α-dialkyl amino acids of type **14**. Even though PLE often shows only moderate selectivity to a specific substrate, this selectivity can often be improved by process development (Scheme 55).



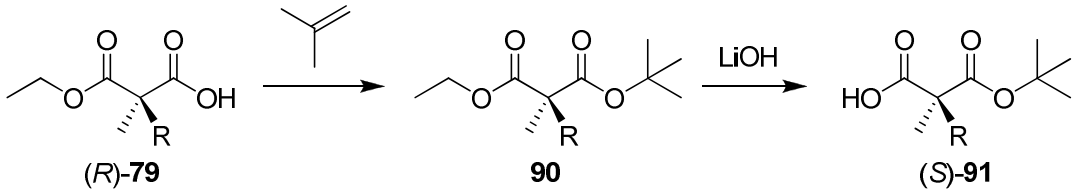
Scheme 55. Improvement of enantioselectivity by process development

Mono ester of malonates, key intermediates in this synthetic strategy, can be easily transformed into α,α-dialkyl amino acids (Scheme 56). Hofmann rearrangement appears to be superior to Curtius rearrangement.



Scheme 56. Synthesis of (*S*)-2,5-dimethyl norleucine ((*S*)-**14b**)

Furthermore, it is literature known that the absolute configuration of the desired amino acid can be altered by chemical transformation of the monoester into its *t*-butyl derivative **90** and subsequent (chemical) cleavage of the ethyl ester to monoester **91** (Scheme 57).[91b] The subsequent steps towards an amino acid could be done with the *t*-butyl ester in the same manner as mentioned above for the ethyl ester.



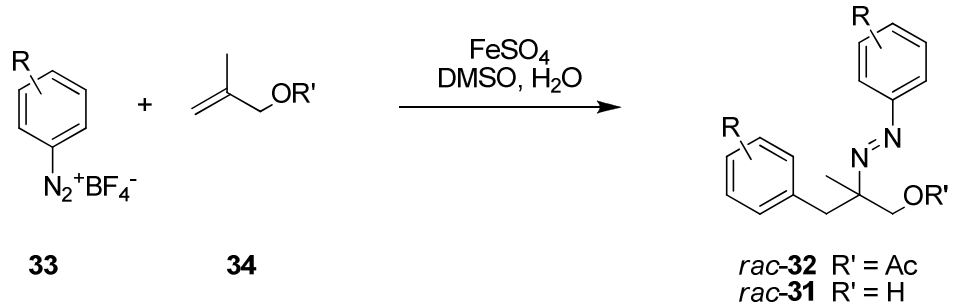
Scheme 57. Switching the absolute configuration of malonate monoesters

However this synthetic route seems to be restricted to methylated aliphatic amino acids, phenylglycine and aromatic amino acids bearing bulky substituents. It was shown that diethyl 2-benzyl-2-methylmalonate (**28f**), which would allow access to phenylalanine, is not a suitable substrate for PLE-catalysed hydrolysis. Furthermore, the synthesis of β-hydroxy α-amino acids of type **16** is not possible (at least not without much effort for the introduction and cleavage of protecting groups) due to instability of both, β-keto malonates **74** and β-hydroxy malonates **85**.

Apart from these restrictions PLE-catalysed desymmetrisation and subsequent Hofmann rearrangement of the corresponding amide offers an excellent access to both enantiomers of α,α-dialkyl amino acids of type **14** with high enantiomeric excess. The enzyme and the substrates are readily available, the yields are generally good and the synthetic effort is relatively low with altogether five synthetic steps.

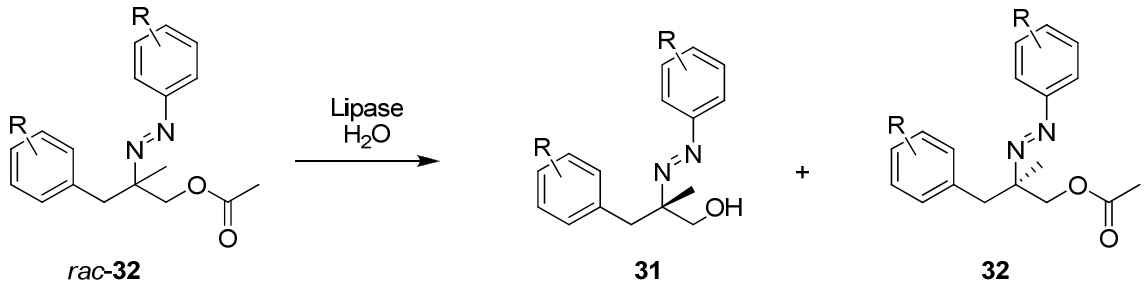
4 Kinetic resolution: CAL-B-catalysed hydrolysis of an azo compound

This concept for the synthesis of α,α-dialkyl amino acids applies enzymatic kinetic resolution of a (racemic) azo compound as the key step.[105] A drawback of kinetic resolution is that the theoretical yield is limited to 50%. However, this drawback can be neglected if the racemic substrate can be easily synthesised, starting from cheap and easily available starting materials. For this reason I chose racemic azo compounds of type *rac*-**31,32**, which can be easily synthesised by radical carbodiazenylation of olefins **34** as a starting material (Scheme 58).[25]



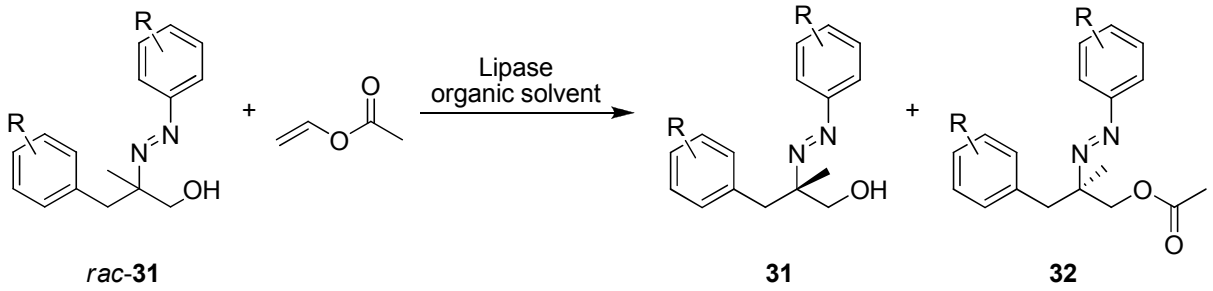
Scheme 58. Synthesis of starting materials for the kinetic resolution

Kinetic resolution should be done by applying a hydrolase, preferably a lipase, to hydrolyse the racemic ester *rac*-**32** leading to both, the product **31** and the substrate **32**, in an enatiomerically enriched form (Scheme 59).



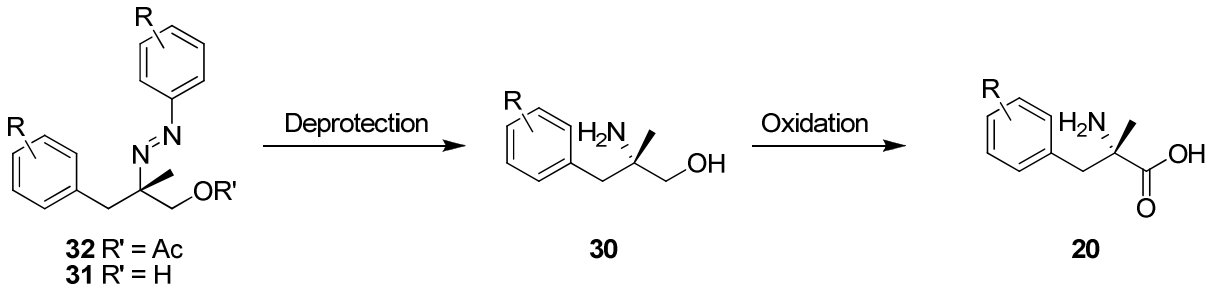
Scheme 59. Kinetic resolution by hydrolysis

Alternatively, a racemic azo alcohol *rac*-**31**, which can be synthesised in the same manner as racemic azo esters *rac*-**32**, can be used as starting material. Kinetic resolution can be achieved by lipase-catalysed transesterification in organic solvent using vinyl acetate as an acetyl source (Scheme 60).



Scheme 60. Kinetic resolution by transesterification

Both types of enantiomerically enriched compound, the esters **32** and the alcohols **31** can be easily separated by column chromatography. Deprotection and oxidation of either esters **32** or alcohols **31** would lead to enantiomerically enriched α-methylated phenylalanines of type **20** (Scheme 61).



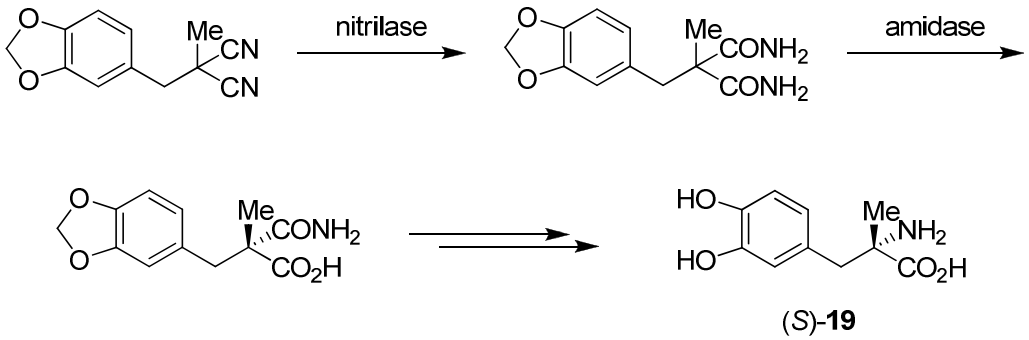
Scheme 61. Synthesis of α-methylated phenylalanines **20** by deprotection and oxidation

As only four synthetic steps – substrate synthesis, kinetic resolution, deprotection and oxidation – are necessary, this synthetic route would allow quick and easy access to α-methylated substituted phenylalanines **20**, by applying an enzymatic kinetic resolution of an azo compound as a key step. Both enantiomers of the desired amino acids would be accessible as both, the remaining substrate and the product of the kinetic resolution, bearing opposite absolute configuration, are applicable for the synthesis of amino acids.

4.1 State of the art

As α-methylated phenylalanine and its derivatives of type **20** are an interesting sub-class of α,α-dialkylated amino acids **4**, several synthetic approaches towards this class of amino acids were established. Apart from approaches using chiral auxiliaries[106] or Seebach's chiral self reproduction technique,[107] few syntheses using organocatalytic enantioselective key steps are described in literature.[108] However, all these approaches need much effort to synthesise the desired amino acids due to costly and time consuming synthesis and cleavage of either chiral auxiliaries or protection groups.

To overcome this problem, bioctalytic approaches towards these amino acids have been studied since the 1980s. Among these approaches is the desymmetrisation of malonate esters catalysed by PLE (see chapter 3) as well as the desymmetrisation of malonoamides catalysed by an amidase from *Rhodococcus spec*. (Scheme 62).[99,109] Besides these desymmetrisation approaches kinetic resolution utilizing different enzyme classes such as esterases, amdidases and acylases are known.[19b,110]



Scheme 62. Synthesis of (*S*)-α-methyldopa ((*S*)-2-amino-3-(3,4-dihydroxyphenyl)-2-methyl-propanoic acid, ((*S*)-**19**)) by desymmetrisation of amides[99]

Biocatalytic kinetic resolution is a standard approach for the synthesis of diverse chiral building blocks and is used in many industrial processes.[23,111] In an (enzyme-)catalysed kinetic resolution process of a racemic substrate one enantiomer will be converted preferentially. When the catalyst is absolutely selective, 50% of enantiopure product will be obtained while 50% enantiopure substrate will remain at the end of the reaction. For lower enantioselectivity, the required enantiomeric purity of substrate may still be reached at higher conversions; thus, the relationship between enantiomeric excess of substrate and the extent of conversion is of practical importance.[112] Since the work of Chen *et al.* in the early 1980s ratios of specificity constants for enantiomers are commonly expressed as the enantiomeric ratio E.[*113*] As this enantiomeric ratio E is the most important value to describe a kinetic resolution process, several equations for the calculation of E were established.[112] The most useful equations allow calculation using two of three easily measurable parameters, conversion (c), enantiomeric excess of the substrate (ee(S)) and enantiomeric excess of the product (ee(P)).

$$E = \frac{\ln\left[(1-c)\left(1-ee(S)\right)\right]}{\ln\left[(1-c)\left(1+ee(S)\right)\right]} \quad (1) \qquad E = \frac{\ln\left[1-c\left(1+ee(P)\right)\right]}{\ln\left[1-c\left(1+ee(P)\right)\right]} \quad (2)$$

Equations (1) and (2) were developed by Chen *et al.* and allow the calculation of the enantiomeric ratio E by determination of the conversion and one enantiomeric excess either of the substrate or of the product.[113]

$$E = \frac{\ln\left[\frac{1 - ee(s)}{1 + (ee(S)/ee(P)}\right]}{\ln\left[\frac{1 + ee(s)}{1 + (ee(S)/ee(P)}\right]} \quad (3)$$

Rakels *et al.* developed equation (3) allowing the calculation of the eantiomeric ratio E without determination of the conversion, using both enantiomeric excesses instead.[114] As determination of the enantiomeric excesses *via* HPLC using chiral stationary phases is often more precise than determination of the conversion, which is often conducted by ^{1}H-NMR, equation (3) often delivers the most precise result for the enantiomeric ratio E.

As the enantiomeric ratio E towards a specific substrate under specific conditions is an intrinsic value of an enzyme it will usually not change while reaction is proceeding. For this reason, the enantiomeric excesses of both substrate and product could be calculated at any conversion when the enantiomeric ratio E was once determined.[112] A practical computation program for the calculation of enantiomeric ratios was developed by Faber and Hoenig.[115] This program calculates the enantiomeric ratio based on equations (1) to (3). In addition, diagrams showing the course of both enantiomeric excesses for the calculated enantiomeric ratios E are available.

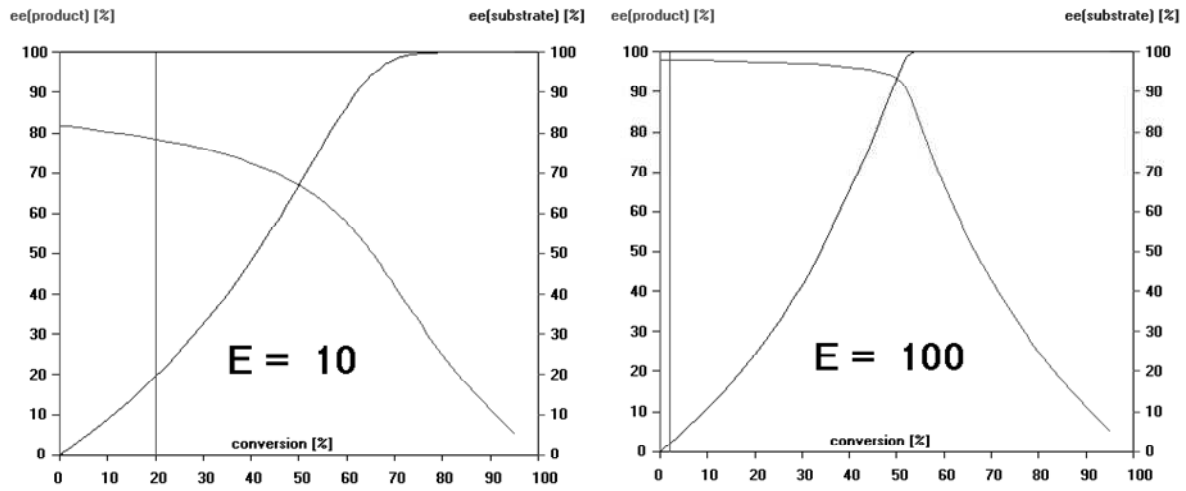
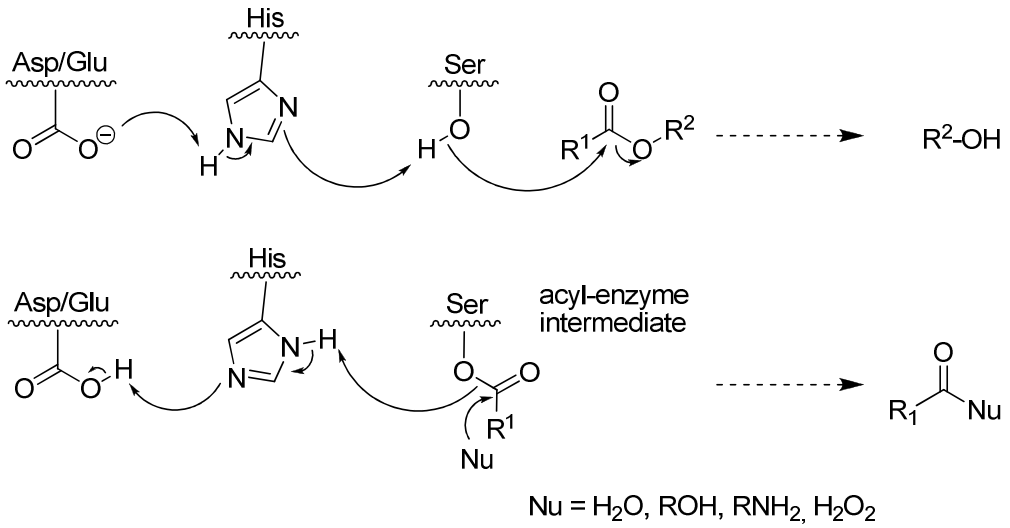


Figure 15. Course of enantiomeric excesses at different enantiomeric ratios [115]

A major advantage of kinetic resolutions can be seen in Figure 15. The substrate can be recovered in excellent enantiomeric excess at high conversion even when applying a kinetic resolution with a relatively low selectivity. However, kinetic resolutions with enantiomeric ratios smaller than 15 are considered less practical.

Most enzyme catalysed kinetic resolutions were conducted using a lipase as a catalyst. Lipases, belonging to the class of serine hydrolases, are readily available, inexpensive, highly stable and accept a broad range of substrate structures.[23] They are ubiquitous enzymes from microbial, plant or animal origin.[116] Differing widely in the number of amino acids and primary sequences,[23] the common feature of all lipases is that their active site is built up of three amino acids, serine, aspartate or glutamate and histidine, which are referred to as the catalytic triad.[23] In addition, the determined 3D structures of all lipases show an α/β-hydrolase fold as well as a nucleophilic elbow, were the catalytic serine is located.[117]



Scheme 63. Serine hydrolase mechanism

The mechanism of ester hydrolysing enzymes is very similar to the mechanism of chemical hydrolysis by a base. Serine from the active site of the enzyme acts as a nucleophilic group and attacks the carbonyl group of the ester substrate (Scheme 63).[121] Special arrangement within the catalytic triad effects a decrease of the serine's pK_a-value thus enabling it to perform a nucleophilc attack on the carbonyl group of the substrate. The acyl moiety of the substrate becomes covalently linked to the enzyme, forming the "acyl-enzyme intermediate" by liberating the leaving group. Then a nucleophile (in the case of hydrolysis water) can in turn attack the acyl-enzyme intermediate, thus regenerating the enzyme and releasing the product (in case of hydrolysis a carboxylic acid, Scheme 63).[121]

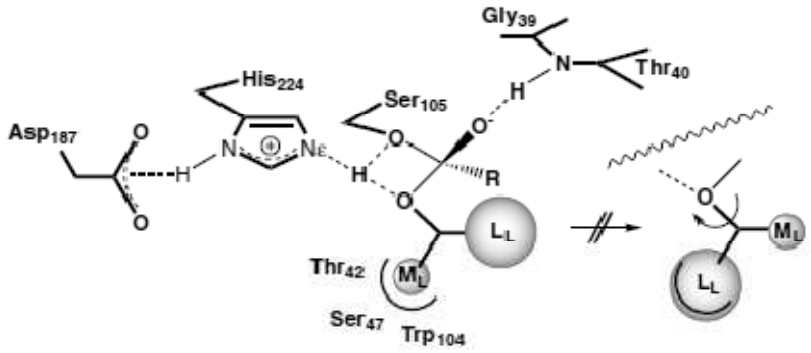


Figure 16. CAL-B's active site geometry, showing catalytic triad, oxyanion hole and a substrate in the transition state[122]

Both transition states for this mechanism contain an anionic tetrahedral transition state. The negative charge of these transition states is stabilised by so-called oxyanion holes (Figure 16).[118]

Lipases were previously defined in kinetic terms based on the "interfacial activation" phenomenon, which most lipases show.[23] These lipases show an increase in activity when a partially water-soluble substrate becomes water insoluble.[119] 3D structures of these lipases suggest that interfacial activation might be due to the presence of an amphiphilic peptidic loop covering the active site of the enzyme in solution, just like a lid.[120]

However all these common features of most lipases are not suitable criteria for classifying specific esterases as lipases since not all lipases show all of these characteristics.[105a] For this reason lipases can be more pragmatically redefined as carboxylesterases acting on long chain acylglycerols. In contrast to esterases, which prefer water soluble substrates, lipases prefer water insoluble substrates.[116]

The most widely used lipase used for kinetic resolutions is lipase B from *Candida antarctica* (CAL-B), which was once isolated from an Antarctic yeast and is now readily available in a recombinant form from *Aspergillus oryzae*.[121] Various preparations of this enzyme are produced by Novozymes (DK) in bulk quantities. Furthermore a number of rational and combinatorial protein engineering projects have focused on extending and tailoring CAL-B's catalytic and physical properties to generate customised catalysts.[122]

CAL-B is an exceptional robust enzyme which is deactivated only at 50-60 °C in free form and at 60-80 °C in immobilised form. In addition, it shows increased resistance towards organic solvents. As it shows virtually no lipase-typical interfacial activation,[123] selectivity enhancement by the addition of water miscible solvents such as DMSO or *tert*-butanol – a

technique rather common for esterases (see chapter 3) – is possible. CAL-B shows outstanding biocatalytic characteristics especially for the stereoselective conversion of secondary alcohols.[124]

The properties of CAL-B were intensively studied and its primary sequence is known as well as crystallographic data is available for this enzyme.[125] The 33 kDa enzyme has an α/β-hydrolase-like fold, with an active site serine triad that is accessible from the solvent through a narrow channel (Figure 17).

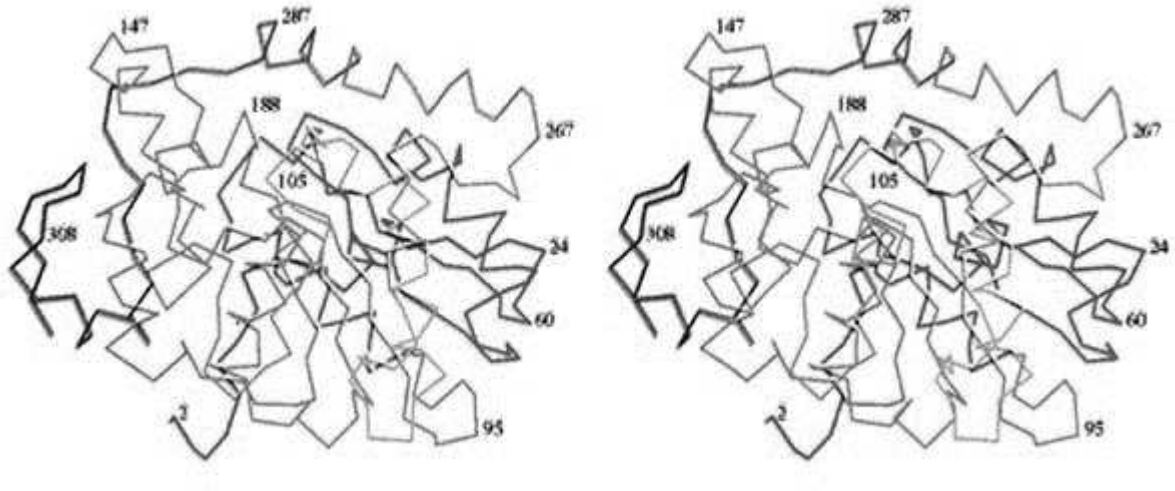
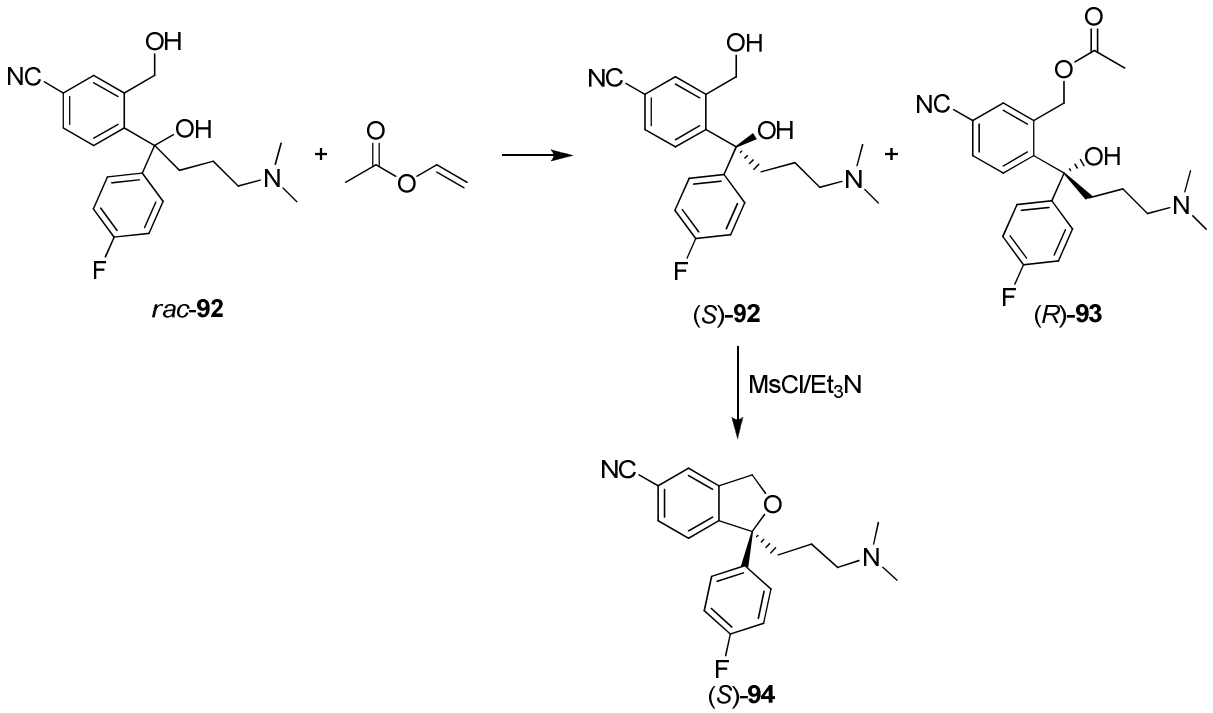


Figure 17. Stereo drawing of the Cα trace of CAL-B

A short helix was identified as a potential lid, which is supposed to be too small to effect notable interfacial activation.[116] An unusual feature of CAL-B is that it does not have the consensus amino acid sequence, GXSXG, around the active site serine that is common to the folding family of hydrolases. Instead, it has a threonine at the position of the first conserved glycine.[122] CAL-B has a high enantio-preference in the resolution of secondary alcohols since the selectivity is not only due to size limitations in the alcohol binding pocket but is further controlled through the optimal orientation of the nitrogen in ε-position of histidine 224 and the oxygen of the alcohol leaving group, thus minimizing the energy barrier for the required hydrogen transfer.[122]

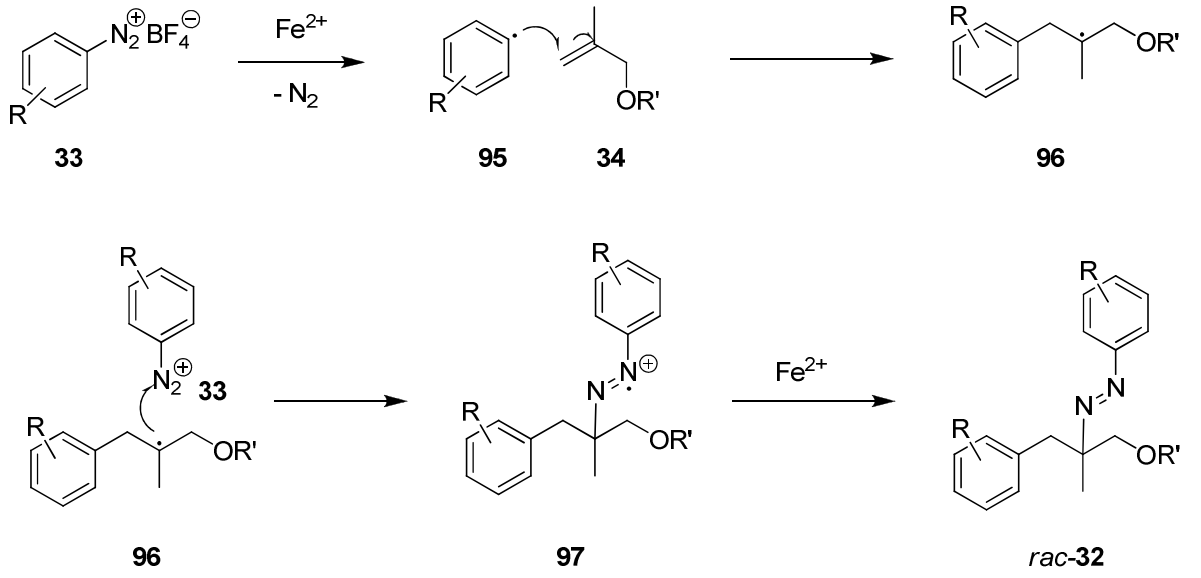
Whereas the kinetic resolution of tertiary stereogenic centers using a lipase as a biocatalyst is well studied, there are only few examples for the lipase-catalysed kinetic resolution of quaternary stereogenic centers.[126] One of the rare examples is the kinetic resolution of a quaternary stereogenic center in the synthesis of the serotonin (5-HT) reuptake inhibitor (*S*)-(+)-Citalopram ((*S*)-**94**, Scheme 64).[126c]



Scheme 64. Kinetic resolution in the synthesis of (*S*)-(+)-Citalopram ((*S*)-**94**)

To the best of my knowledge, a lipase catalysed kinetic resolution of a quaternary stereogenic center, bearing a functional group which allows easy transformation into an amino functionality is not known until now.

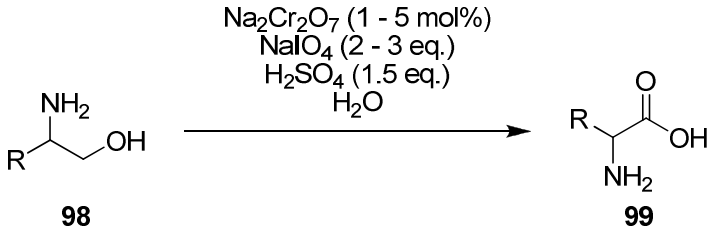
In contrast, the synthesis of the substrates for the kinetic resolution step by radical carbodiazenylation in aquatic solvents is well known and the mechanism is well studied.[25]



Scheme 65. Mechanism of the radical carbodiazenylation of olefines

A diazonium salt **33** is reduced by iron (II) and looses molecular nitrogen. The resulting radical **95** undergoes addition to an olefin **34**, forming a new radical **96**, which could attack another diazonium salt **33**. After reduction of this newly formed radical **97** a stable racemic aza compound *rac*-**32** can be obtained (Scheme 65). This type of reaction is suitable for the synthesis of various substrates as many different mono- and disubstituted diazoniumsalts and different olefins are accepted within this reaction.[25]

The reduction of azo compounds to yield amines (Scheme 61) is a standard technique in organic synthesis. For this reason many variants of this reaction are described in literature. Most reductions are conducted either using nascent hydrogen[25a,127] or catalytic hydrogenation.[128]



Scheme 66. Oxidation of amino alcohols **98** according to Alsters *et al*.[130]

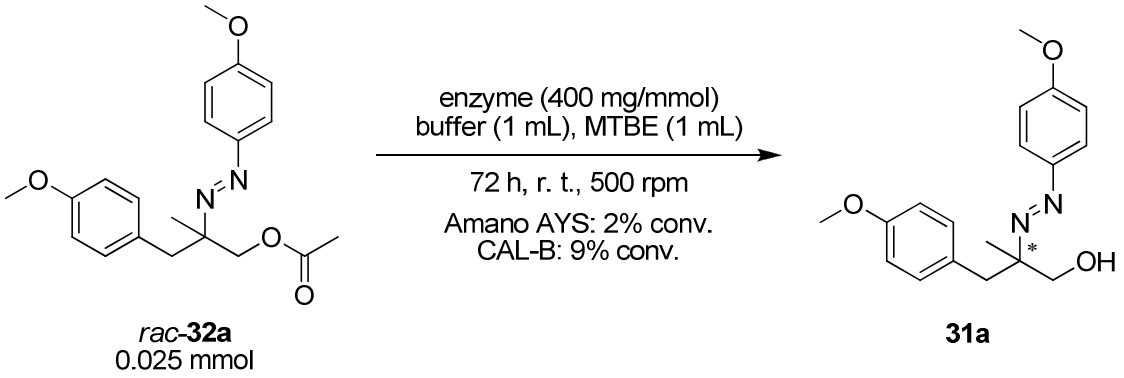
Even though the oxidation of primary alcohols is a standard reaction in organic chemistry with operating procedures available in textbooks for undergraduates,[129] the chemoselective oxidation of amino alcohols **98** to amino acids **99** required the implementation of protecting groups until Alsters *et al*. invented a new method for the oxidation of amino alcohols **98**, using sodium dichromate as a catalyst and stochiometric amounts of sodium periodate as an oxidizing agent in sulfur acidic water (Scheme 66).[130]

4.2 Results and discussion

4.2.1 Key step: Kinetic resolution by hydrolysis

4.2.1.1 Enzyme screening

To detect active enzymes, an enzyme screening on the hydrolysis of *rac*-**32a** was done, testing 14 enzyme formulations in a two-phase system consisting of buffer and MTBE (Scheme 67).



rac-**32a**
0.025 mmol

31a

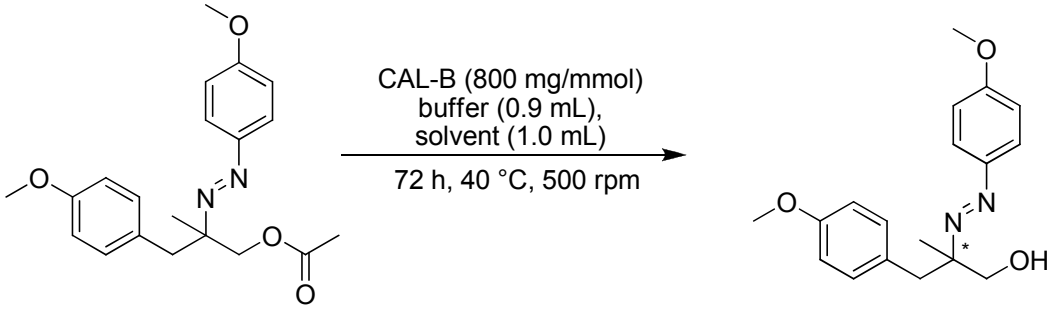
Scheme 67. Enzyme screening on the hydrolysis of **32a**

Fortunately, two of the tested enzymes showed activity towards the hydrolysis of *rac*-**32a**. Whereas lipase Amano AYS showed only reluctant activity of approximately 2% conversion after three days of shaking at room temperature, CAL-B's activity was slightly higher leading to a conversion of 9% after three days. All other tested enzymes as well as the blank test showed no conversion even after three days.

4.2.1.2 Process development

The reaction should be optimised by the examination of the influence of different conditions such as solvent, enzyme loading and temperature. At the beginning, the influence of five different solvents on the activity and selectivity of the enzyme was tested. Two different ethers (MTBE, ETBE) as well as an aromatic hydrocarbon (toluene) and two aliphatic hydrocarbons (*n*-octane, *i*-octane) were chosen as solvents for this examination (Table 16). No chlorinated solvent such as dichloromethane or chloroform was used, as **32a** turned out to be not stable in these solvents.

Table 16: Solvent screening on the hydrolysis of *rac*-**32a**



rac-**32a**
0.025 mmol

31a

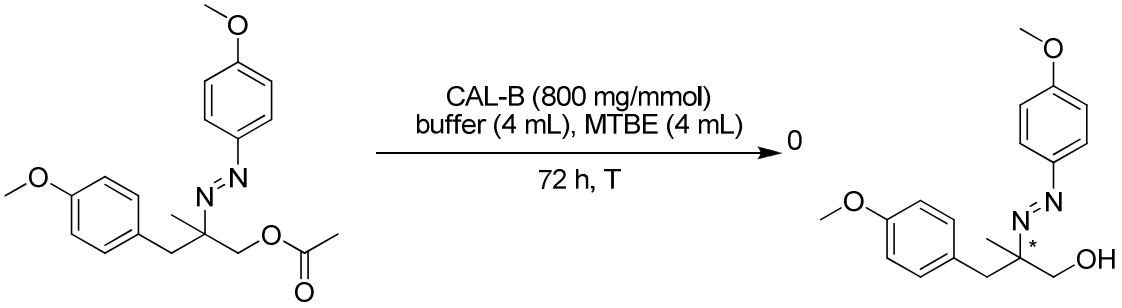
entry	solvent	conversion (%)	ee(P)	E (conv./ee(P))
1	MTBE	11	81	11
2	ETBE	5	78	9
3	toluene	< 2	n. d.	n. d.
4	*n*-octane	24	64	6
5	*i*-octane	29	48	3

Virtually no conversion of *rac*-**32a** was detected after three days of shaking at 40 ℃ in a mixture of toluene and buffer (Table 16, entry 3). In contrast to the situation when using this aromatic hydrocarbon, conversion is much higher in the presence of aliphatic hydrocarbons leading to the desired product **31a** in 24% and 29% conversion in *n*-octane and *i*-octane, respectively. Unfortunately selectivity in these solvents was relatively low (Table 16, entries 4,5).

A low conversion of only 5% was obtained when using ETBE as a solvent. However, selectivity was higher – with an enantiomeric ratio of E=9 – when compared to aliphatic hydrocarbons (Table 16, entry 2). Best results were achieved when applying MTBE as a solvent. Even though conversion was only moderate, the highest enantiomeric ratio of E = 11 was determined (Table 16, entry 1).

Due to these promising results in the solvent screening, the reaction was scaled up under these conditions (CAL-B as enzyme in MTBE - buffer as reaction medium, 72 hours reaction time), examining the influence of different temperatures next (Table 17).

Table 17: Influence of temperature on the hydrolysis of *rac*-**32a**



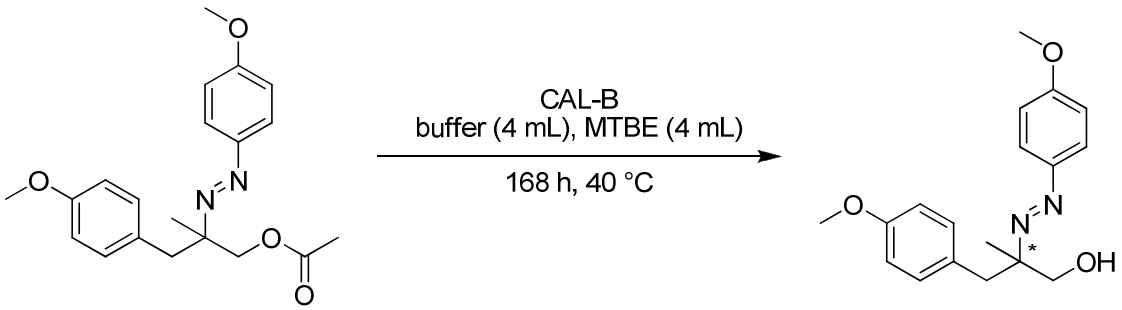
rac-**32a**
0.1 mmol

31a

entry	T	t	conv. (%)	ee(S)	ee(P)	E (conv./ee(P))	E (conv./ee(S))	E (ee(S)/ee(P))
1	r. t.	3d	13	n. d.	91	24	n. d.	n. d.
2	40 °C	3d	19	21	90	23	25	23
3	60 °C	3d	8	n. d.	87	16	n. d.	n. d.

The conversion at 40 °C was remarkably higher compared to the conversion at room temperature (Table 17, entries 1,2). Surprisingly conversion at 60 °C was the lowest conversion detected. Perhaps this was due to slow enzyme inactivation at this high temperature, even though immobilised CAL-B is known to be stable at even higher temperatures.[121] Whereas selectivities at room temperature and 40 °C appeared to be in the same range of about E=24 (Table 17, entries 1,2), the enantiomeric ratio at 60 °C was somewhat lower with E=16 (Table 17, enty 3).

Table 18: Influence of the enzyme loading on the hydrolysis of *rac*-**32a**



rac-**32a**
0.1 mmol

31a

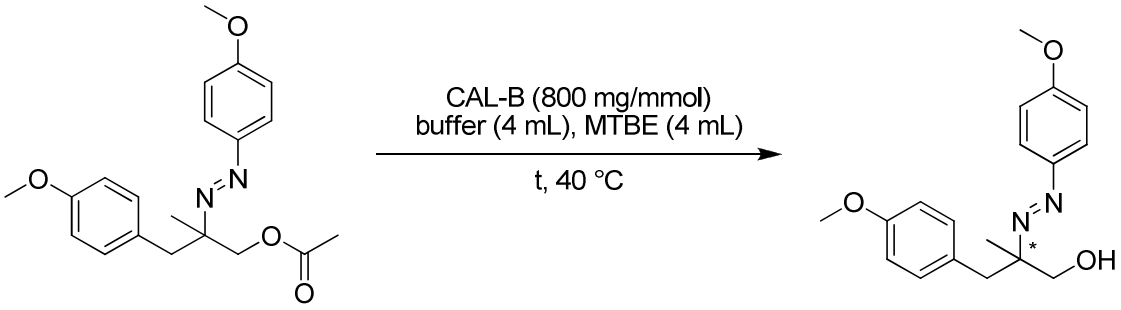
entry	m (CAL-B)	conversion (%)	ee(S)	ee(P)	E (conv./ee(P))	E (conv./ee(S))	E (ee(S)/ee(P))
1	80 mg	42	63	86	25	27	25
2	160 mg	46	n. d.	85	26	n. d.	n. d.

Since conversions higher than 50% are needed to yield the substrate with high enantiomeric excesses, I tried to increase the conversion by applying a longer reaction time of one week and examined the influence of enzyme loading on the conversion (Table 18).

Unfortunately, conversion did not exceed 50% even when applying twice the amount of enzyme (Table 18, entry 2). Even more, the enzyme loading seemed to have only reluctant influence on the conversion.

For an exact determination of the enantiomeric ratio E, several tests under the same optimised conditions ("standard reaction") were done, stopping the process at different reaction times (Table 19).

Table 19: Determination of the enantiomeric ratio E of the standard reaction



rac-**32a**
0.1 mmol

31a

entry	t	conversion (%)	ee(S)	ee(P)	E (conv./ee(P))	E (conv./ee(S))	E (ee(S)/ee(P))
1	3 d	19	21	90	23	25	23
2	3 d	20	22	90	24	24	24
3[a]	3 d	30	38	88	23	24	23
4	7 d	42	63	86	25	27	25
5	11 d	42	63	85	24	27	24

[a] new charge of CAL-B was used

The enantiomeric ratio E was determined to be roughly 24, even though some deviations were observed. The most accurate values for the enantiomeric ratio are the values determined for both enantiomeric excesses (Table 19, last column). These values were determined to be between 23 and 25 in all cases, regardless of reaction time. Interestingly, the conversion after 11 days of stirring is exactly the same as conversion after 7 days reaction time (Table 19, entries 4,5). This could be due to several reasons including enzyme deactivation and product inhibition of the enzyme.

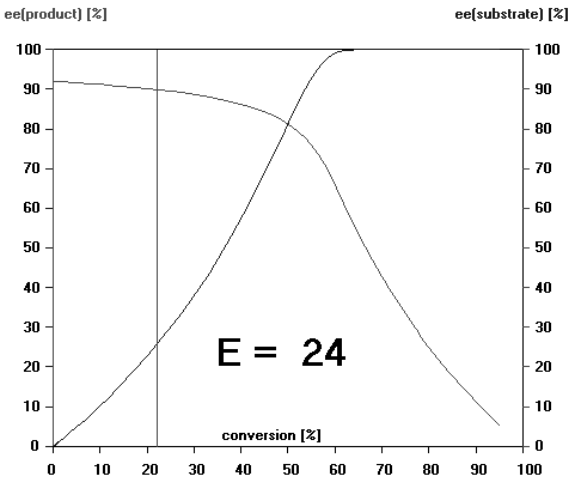
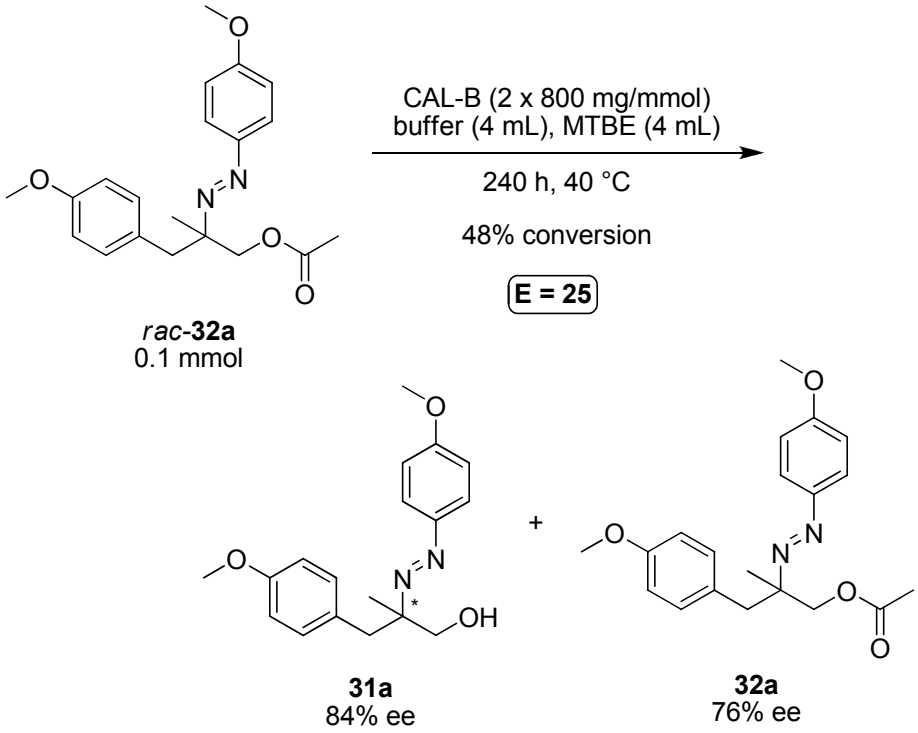


Figure 18. Course of ee-values within a kinetic resolution with an enantiomeric ratio of E=24

Whereas the enantiomeric excess of the product is limited to 92% ee, even when stopping the reaction at a very early stage, the substrate could be recovered in high enantiomeric excesses at high conversion. As it could be seen in Figure 18, a conversion of 60% would be enough to get access to the substrate **32a** in a nearby perfect enantiomeric excess of 99% ee. For this reason, a conversion of approximately 60% would be the best choice to get access to the substrate in an almost perfect enantiomeric excess and in good yields. A good compromise to yield the product **31a** in good enantiomeric excesses and acceptable yields would be to stop the reaction at approximately 20 to 25% conversion as the enantiomeric excess is in the range of 90% ee then.

When using a new charge of CAL-B the selectivity was in the same range as the selectivity of the original charge (Table 19, entry 3). However, the activity of the new charge was somewhat higher leading to a conversion of 30% after three days when compared to the previous one (Table 19, entry 3 compared to entries 1,2).

To overcome the possible enzyme deactivation in long term experiments a further long term reaction was conducted under standard conditions, adding additional CAL-B after six days reaction time (Scheme 68).



Scheme 68. Addition of fresh enzyme to standard reaction after 6 days

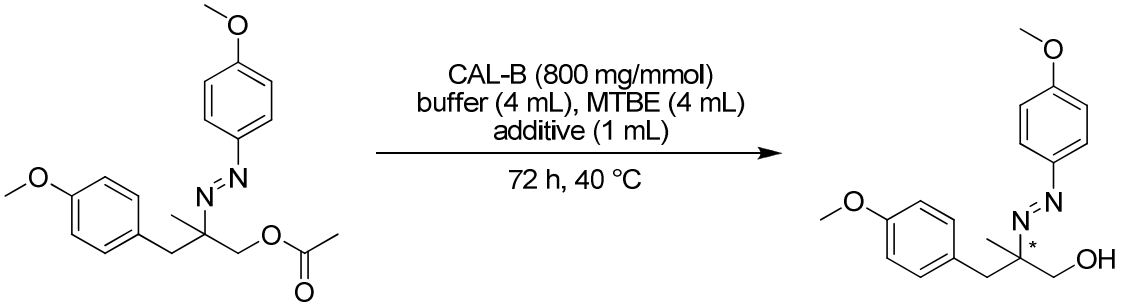
As conversion was not significantly higher when compared to standard reaction (Table 19, entries 4,5), enzyme deactivation could not be the major reason for the low conversions. Product inhibition seems to be a more appropriate explanation for the problems to achieve conversions of over 50%.

It is well known that the addition of water-soluble solvents can improve conversions of CAL-B catalysed reactions as CAL-B shows no interfacial activation and a water-miscible solvent can improve the solubility of the substrate and hence improve the availability of the substrate for the enzyme in the aqueous phase.[121] For this reason the dissatisfying conversions were tried to increase by adding small amounts of water soluble organic solvents to the biphasic system of the standard reaction.

Whereas conversion was in the same range as in the standard reaction when applying polar aprotic additives such as DMF or DMSO (Table 20, entries 7,8 compared to entry 1), the application of different alcohols led to low conversions (Table 20, entries 2-5). Interestingly, the lowest conversions were obtained when using the most hydrophilic alcohol. For example the application of ethanol led to a much lower conversion of 3% (Table 20, entry 2) compared to 9% conversion when applying *tert*-amyl alcohol (Table 20, entry 5). Virtually no conversion could be seen when applying acetonitrile as an additional solvent.

Enantiomeric ratio is slightly lower when applying DMF as an additive (Table 20, entry 7) compared to the standard reaction (Table 20, entry 1). In contrast, no effect on the enantiomeric ratio of the reaction was observed by the addition of DMSO (Table 20, entry 8).

Table 20: Influence of different additives on the hydrolysis of *rac*-**32a**



rac-**32a**
0.1 mmol

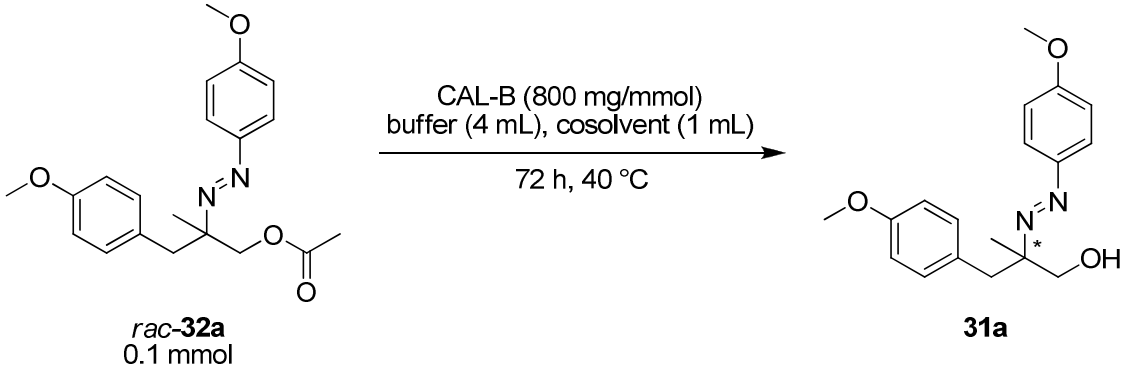
31a

entry	additive	conversion (%)	ee(S)	ee(P)	E (conv./ee(P))	E (conv./ee(S))	E (ee(S)/ee(P))
1	none	30	38	88	23	24	23
2	EtOH	3	n. d.	n. d.	n. d.	n. d.	n. d.
3	IPA	6	n. d.	n. d.	n. d.	n. d.	n. d.
4	TBA	8	n. d.	n. d.	n. d.	n. d.	n. d.
5	TAA	9	n. d.	n. d.	n. d.	n. d.	n. d.
6	CH_3CN	< 2	n. d.	n. d.	n. d.	n. d.	n. d.
7	DMF	25	28	86	17	18	17
8	DMSO	27	33	89	24	24	24

As some reproducibility problems appeared since MTBE vaporised during the reaction, especially within long reaction times, some MTBE-free reactions were conducted to examine the influence of MTBE on the reaction.

Whereas comparable conversions of 24% were achieved when using DMF or DMSO as additive in the absence of MTBE (Table 21, entries 2,3), a reaction conducted in pure buffer without any additive proceeds much slower than the standard reaction leading to a final conversion of only 13% (Table 21, entry 1). A reason for this observation could be the poor solubility of **32a** in this system. Selectivity was very low for all tested conditions. Hence a biphasic system seems to be crucial for the enantioselective hydrolysis of azo substrates like **32a**.

Table 21: Hydrolysis of *rac*-**32a** in one-phase systems

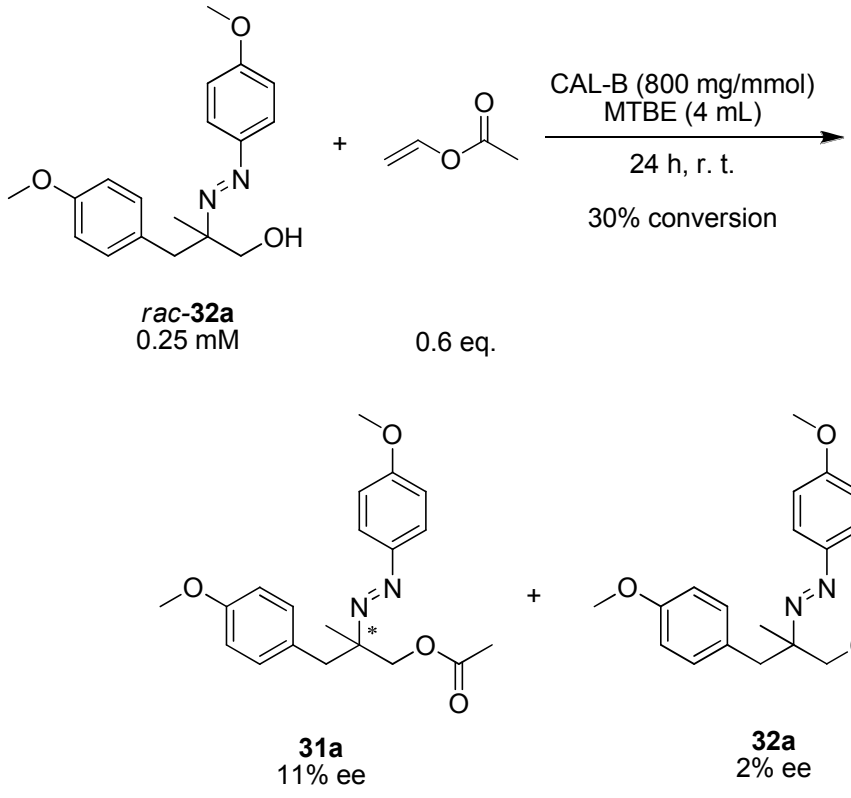


rac-**32a**
0.1 mmol

31a

entry	additive	conversion (%)	ee(P)	E (conv./ee(P))
1	none	13	36	2
2	DMF	24	56	4
3	DMSO	24	33	2

To exclude possible background reactions as a reason for this poor selectivity, the (uncatalysed) background reactions for the biphasic standard reaction, the reaction without any additive and with DMSO as an additive was examined. As there was no observable background reaction at any applied condition, background reaction is not the reason for the poor selectivities in one phase systems.



rac-**32a**
0.25 mM

0.6 eq.

31a
11% ee

32a
2% ee

Scheme 69. CAL-B catalysed esterification of *rac*-**31a** using vinyl acetate as an acetyl-source

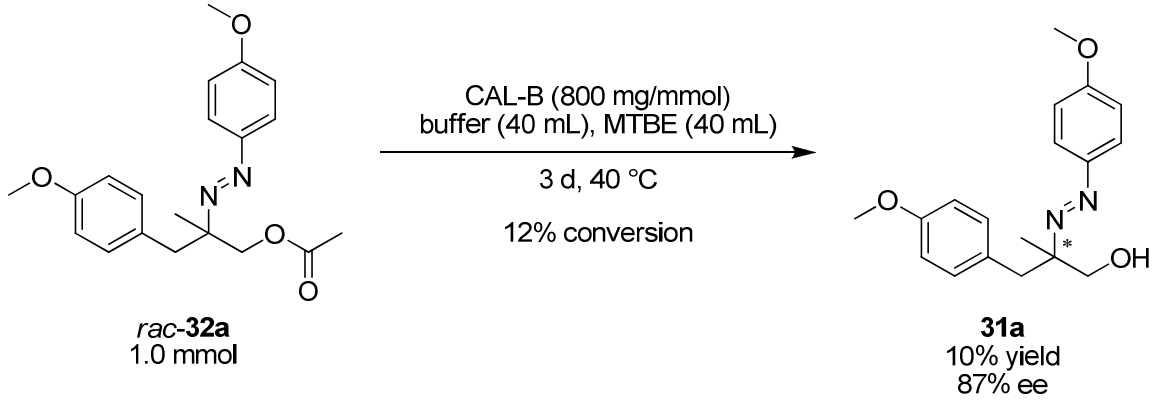
To test the second possible method for the resolution of these azo compounds, I conducted the transesterification of *rac*-**32a** using vinyl acetate as an acetyl donor in MTBE as a solvent. The reaction proceeded smoothly, leading to a conversion of 30% after 24 hours when stirring at room temperature (Scheme 69). Unfortunately virtually no enantioselectivity could be obtained by means of this system. Thus the transesterification of the primary alcohol *rac*-**32a** is not a suitable key step for the synthesis of enantiomerical enriched α,α-dialkyl amino acids.

4.2.2 Total synthesis of α,*O*-dimethyl tyrosine

To proof the practicability of the novel kinetic resolution of racemic azo compound *rac*-**32a** the corresponding amino acid α,*O*-dimethyl tyrosine (**20a**) was synthesised using kinetic resolution as the key step.

4.2.2.1 Kinetic resolution at an increased laboratory scale

The kinetic resolution was applied at an increased laboratory scale (Scheme 70) to get access to enough starting material **31a** for the subsequent steps towards the synthesis of α,*O*-dimethyl tyrosine (**20a**).



rac-**32a**
1.0 mmol

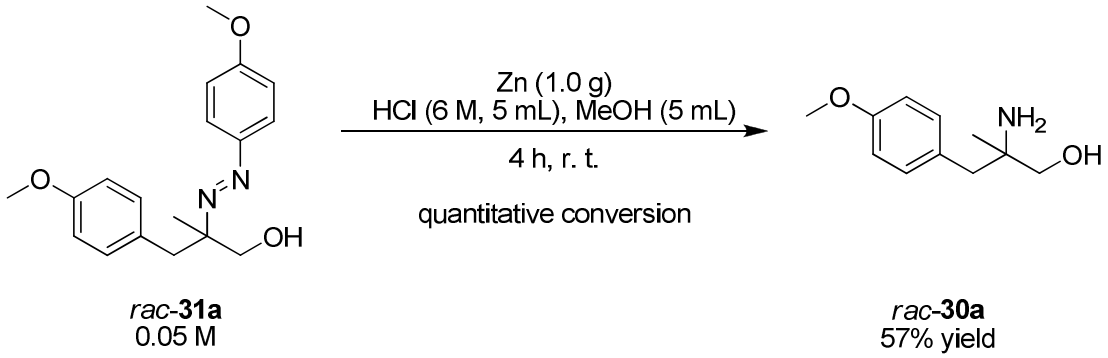
31a
10% yield
87% ee

Scheme 70. Kinetic resolution in an increased laboratory scale

Unfortunately conversion and selectivity dropped in this experiment (Scheme 70) when compared to the original experiment at a 0.1 mmol scale (Table 19). Nevertheless, **31a** was isolated in 10% yield with an enantiomeric excess of 87% ee.

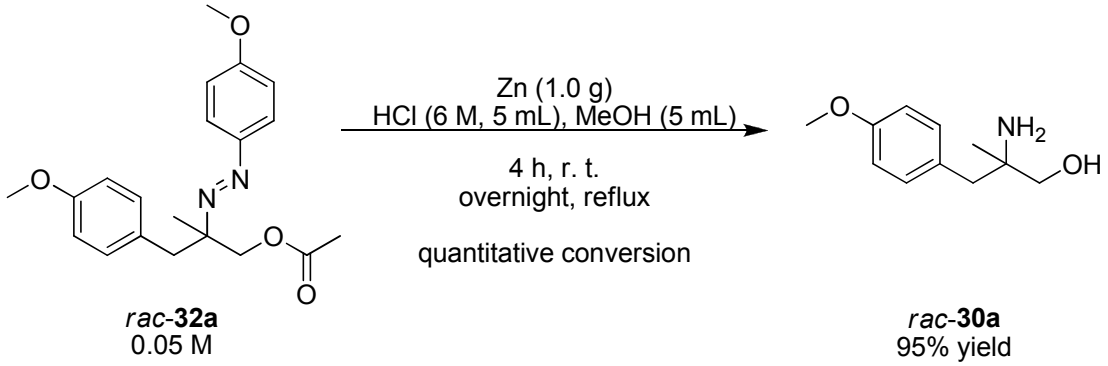
4.2.2.2 Deprotection and oxidation to free amino acid α,*O*-dimethyl tyrosine

As it was an aim of this project to show the possibility of yielding both enantiomers of the desired α-methylated amino acid **20a**, I had to show that deprotection of the azo alcohol **31a** to the amino alcohol **30a** is possible as well as those of the azo ester **32a** to the identical amino alcohol **30a**. This would allow access to both enantiomers since the product of the resolution step **31a** must have the opposite absolute configuration to that of the remaining substrate **32a**. To establish the deprotection to the free amino alcohol **30a**, both racemates were applied as substrates first.



Scheme 71. Deprotection of racemic azo alcohol *rac*-**31a**

Deprotection of the racemic azo alcohol *rac*-**31a** proceeded smoothly when applying standard conditions of zinc and hydrochloric acid in methanol (Scheme 71). After 4 hours, the yellow color of the azo compound disappeared and the conversion was determined to be quantitative by means of ^{1}H-NMR. The product was obtained in 57% yield after column chromatography.

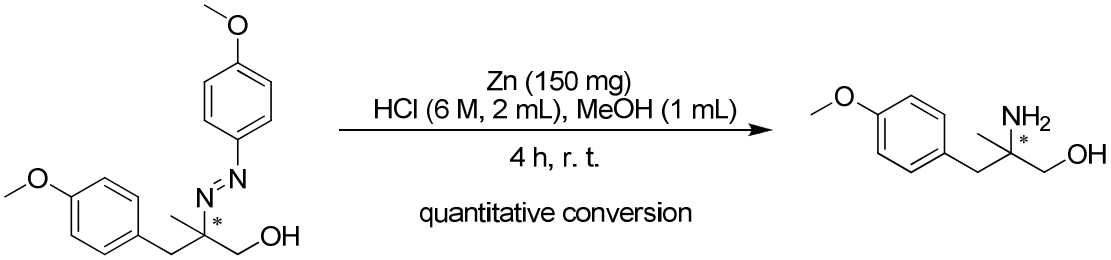


Scheme 72. Deprotection of racemic azo ester *rac*-**32a**

Encouraged by this success the deprotection of the ester *rac*-**32a** was conducted under the similar conditions (Scheme 72). The reduction of the azo group of **32a** proceeded as

smoothly as with the azo alcohol **31a**. However, the cleavage of the ester moiety was not completed under these conditions (according to TLC-analysis). To ensure quantitative ester cleavage, the reaction mixture was stirred overnight under reflux leading to the desired amino alcohol *rac*-**30a** in an excellent yield of 95%.

After deprotection of azo compounds **32a** and **31a** was established, I could proceed applying deprotection on the azo alcohol **31a** gained from kinetic resolution, bearing an enantiomeric excess of 87% ee (Scheme 73).



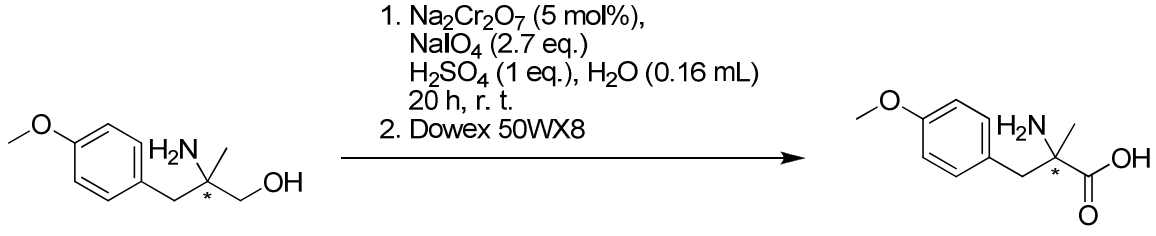
31a
0.04 M
87% ee

30a
89% yield
86% ee

Scheme 73. Deprotection of enantiomerically enriched **31a**

Standard deprotection protocol worked well and the product **30a** was isolated in 89% yield (Scheme 73). The enantiomeric excess of the product **30a** was slightly lower than that of the substrate **31a** (86% ee compared to 87% ee), as the starting material contained minor impurities of the corresponding ester **32a**.

The isolated amino alcohol **30a** was applied in an oxidation conducted under conditions described by Alsters *et al*. (Scheme 74).[130]



30a
0.2 M
86% ee

20a
quantitative yield
86% ee

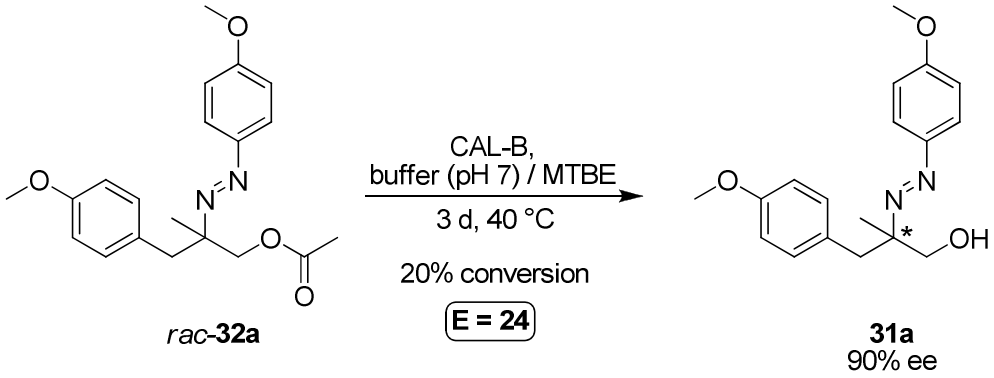
Scheme 74. Oxidation of amino alcohol **30a** to amino acid **20a**

The desired amino acid α,*O*-dimethyl tyrosine (**20a**) was yielded quantitatively after work up applying an ion exchange resin. The enantiomeric excess was proofed to retain stable at 86% ee during this reaction by analysis of the enantiomeric excess of the corresponding ethyl ester **100** by means of chiral HPLC-chromatography.

4.3 Summary

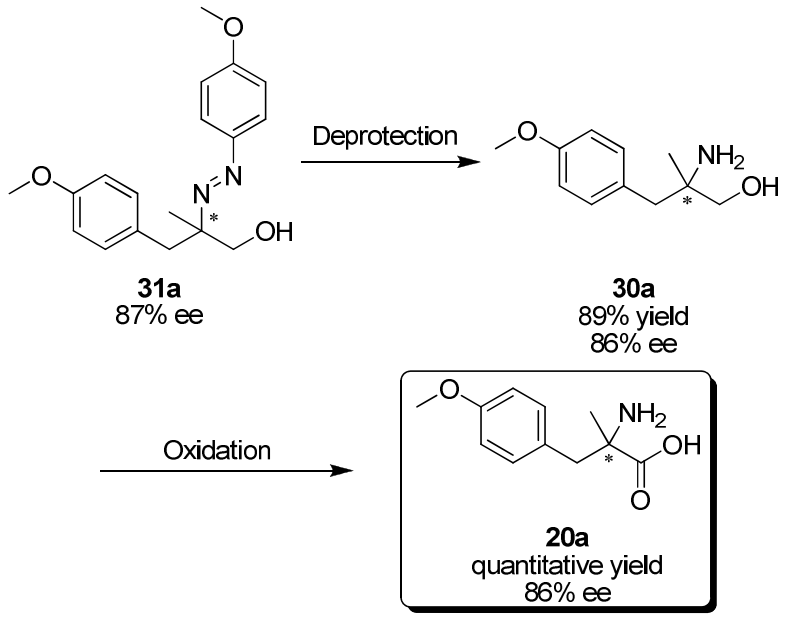
To sum up, a novel synthetic route towards α-methylated phenylalanines **20** was established. This approach has major advantages compared to other approaches as only four synthetic steps are necessary to obtain the desired amino acids in good enantiomeric excess, starting with cheap and easily available starting materials.

Furthermore a kinetic resolution was established as a key step of this synthetic strategy, using CAL-B, a readily available and widely applied lipase, as a catalyst. The enantiomeric ratio of this kinetic resolution was determined to be E=24 in the standard experiment (Scheme 75). This moderate to good selectivity is especially notable, since only few examples of lipase catalysed resolution of quaternary stereogenic centers are known and, to the best of my knowledge, this example is the first example of a lipase-catalysed kinetic resolution of a quaternary stereogenic center with a nitrogen residue attached to the stereogenic center.



Scheme 75. CAL-B-catalysed kinetic resolution of **32a** (Standard experiment)

The applicability of this novel synthetic route was demonstrated by means of the synthesis of one enantiomer of α,*O*-dimethyl tyrosine (**20a**) in 86% ee (Scheme 76). The overall yield of the subsequent steps is remarkably high (89%).



Scheme 76. Synthesis of α,*O*-dimethyl tyrosine (**20a**)

In addition, the synthesis of the second enantiomer in moderate enantiomeric excesses up to 76% ee is possible using techniques which are available now by applying deprotection conditions on the ester remaining after long time kinetic resolution (Scheme 68).

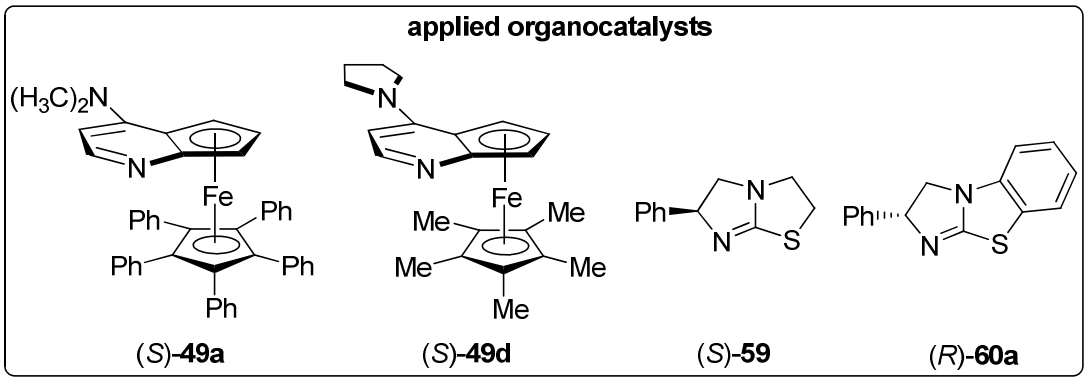
To further optimise the enantiomeric ratio of the kinetic resolution step and to ocvercome the problem of (potential) product inhibition leading to conversions of < 50% even after very long reaction times of over one week, the key step has to be studied further in the future. Many possible solutions for those problems are literature known, including *in situ* product isolation as well as enzyme engineering.[131] If the problem of low conversions was overcome, this novel synthetic route could become a strong synthetic platform for syntheses of α-methylated phenylalanines **20** as it is based on easy accessible materials and catalysts and comprises only 4 synthetic steps.

5 Summary

Aim of this Ph.D.-thesis was the synthesis of α,α-dialkyl amino acids of type **4** *via* novel synthetic approaches applying a catalytic key step for the enantioselective construction (or resolution) of the quaternary stereogenic center. To reach this aim, three different synthetic concepts based on three different catalytic key steps, namely addition to a sp^2-hybridised carbon, desymmetrisation and kinetic resolution, were examined intensively. Modern methods of organocatalysis and biocatalysis were applied in these key steps.

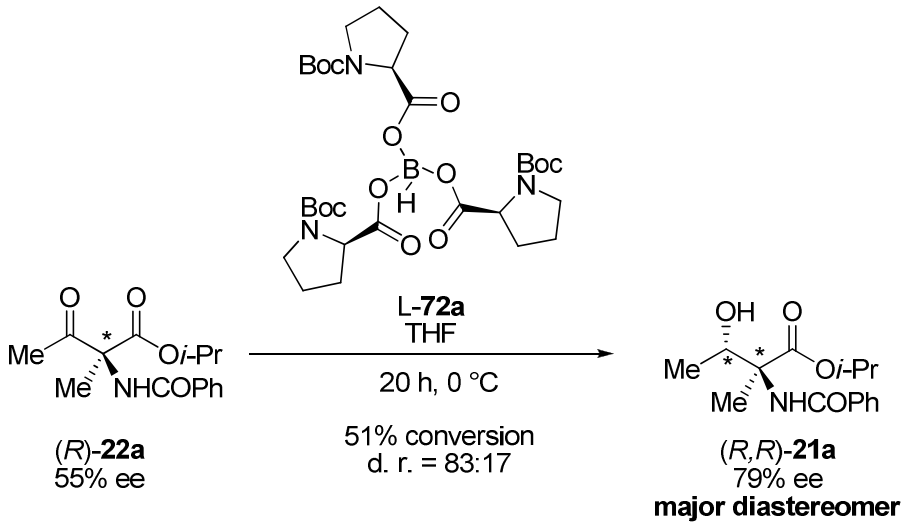
Steglich rearrangement was chosen as an example of an organocatalytic addition to a sp^2-hybridised carbon. Enantioselective Steglich rearrangement of acyl groups such as acetyl allows acces to β-hydroxy α,α-dialkyl amino acids of type **16**. In contrast, the well-known Steglich rearrangement of oxycarbonyl groups does not allow the synthesis of β-hydroxy α,α-dialkyl amino acids **16** apart from serine derivatives.

Table 22: Comparison of different catalysts for the enantioselective Steglich rearrangement



entry	catalyst	solvent	t (h)	conversion (%)	ee (%) (config.)
1	(*S*)-**49a** (2 mol%)	TAA	3	93	30 (*S*)
2	(*S*)-**49d** (2 mol%)	TAA	3	94	50 (*S*)
3	(*S*)-**59** (32 mol%)	$CDCl_3$	48	80	63 (*S*)
4	(*R*)-**60a** (32 mol%)	$CDCl_3$	48	79	61 (*R*)

The key step is catalysed most effectively by applying chiral cyclic isothioureas (“Birman-type catalyst”) such as (*S*)-tetramisole ((*S*)-**59**) or (*R*)-benzotetramisole ((*R*)-**60a**), which showed superior enantioselectivities when compared to planar chiral aminopyridine catalysts (“Fu-type catalysts”) such as (*S*)-**49a** or (*S*)-**49d** (Table 22).

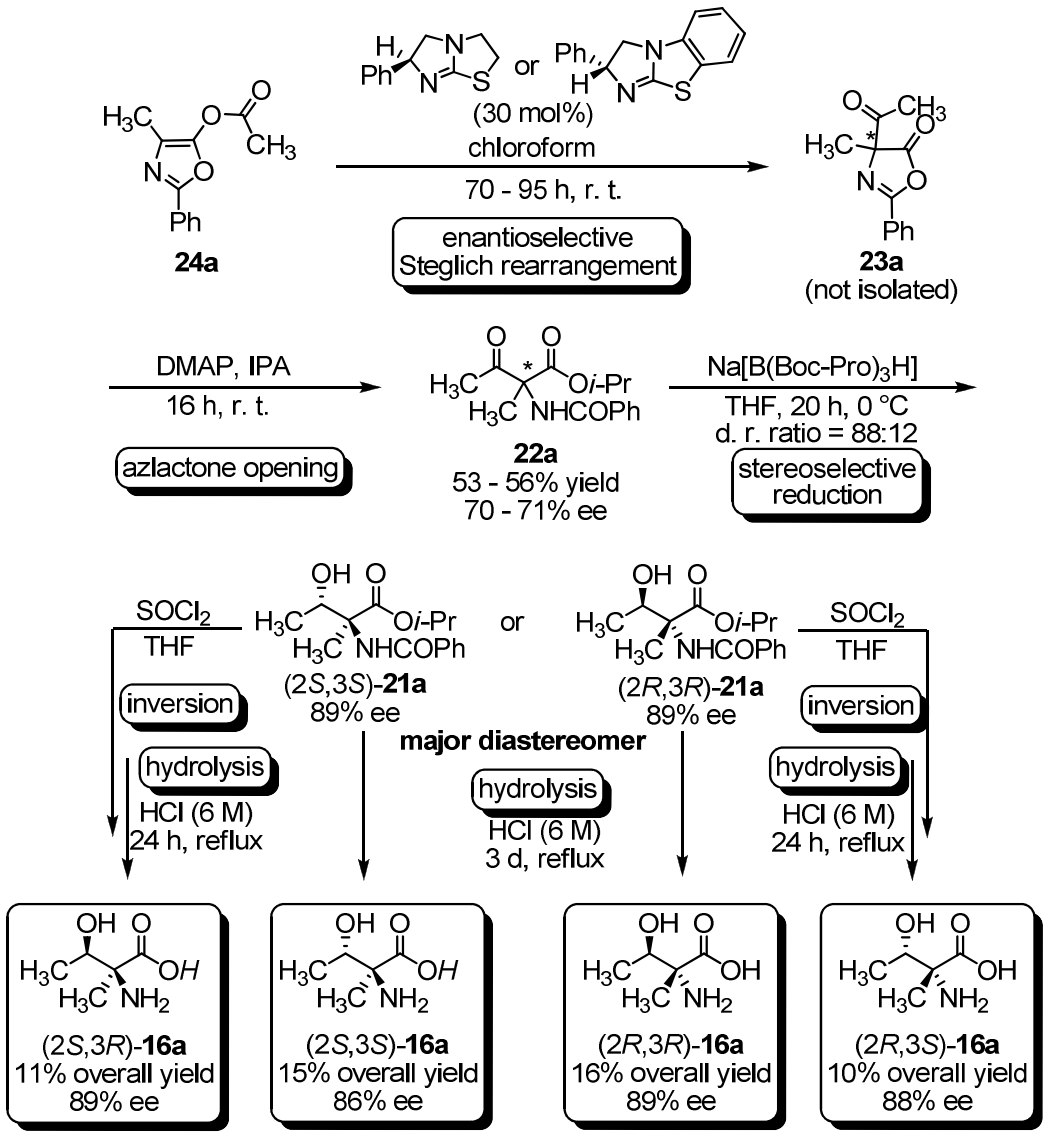


Scheme 77. Diastereoselective reduction to protected α-methyl threonine (**21a**)

As β-hydroxy α,α-dialkyl amino acids of type **16** in general bear a second stereogenic center, a second diastereoselective key step was needed to be conducted. As most reductive agents turned out to be less chemoselective (substrates bear ester and amide moieties beside the keto moiety), reduction applying sodium borohydride was optimised by the application of *N*-Boc-proline as a chiral auxiliary (Scheme 77). This led to an increased enantiomeric excess of the major product (*R*,*R*)-**21a**.

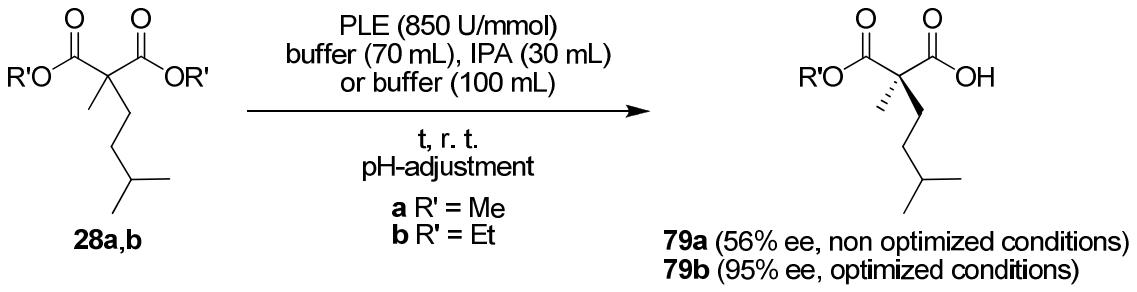
These key steps were applied in the multi step synthesis of α-methyl threonine (**16a**) an important representative of the class of β-hydroxy α,α-dialkyl amino acids **16** (Scheme 78).

It was shown that a combination of an enantioselective organocatalytic Steglich rearrangement and subsequent diastereoselective reduction using sodium borohydride and *N*-Boc-proline as reducing agent is a suitable and practical synthetic route towards all stereoisomers of (enantiomerically enriched) α-methyl threonine (**16a**). Furthermore, stereoselctive induction is possible at both stages, thus offering the potential for obtaining a high “overall” enantiomeric excess.



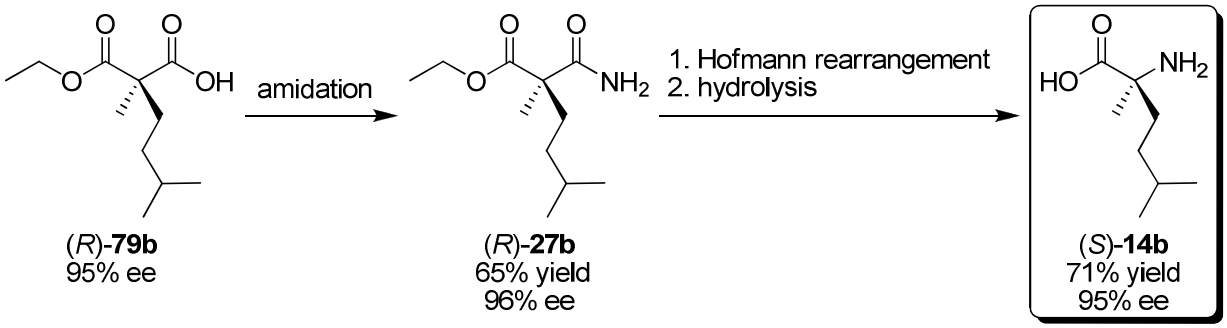
Scheme 78. Total synthesis of all stereoisomers of α-methyl threonine (**16a**)

As an example of desymmetrisation for the construction of quaternary stereogenic centers, PLE-catalysed hydrolysis of malonates was chosen, since no suitable enzyme for the ammonolysis of malonates could be identified. Even though this method was literature known in general, further examinations were needed for the optimisation of this biocatalytic key step.



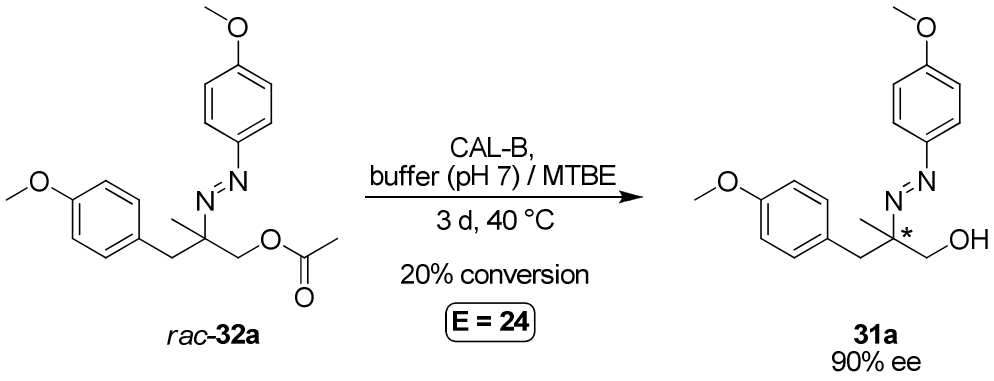
Scheme 79. Optimisation of the PLE-catalysed hydrolysis of malonates **28a**,**b**

Application of an optimised reaction media (consisting of buffer - isopropanol, 7:3) allowed access to the desired key intermediate **79b** with a high enantiomeric excess of 95% ee (Scheme 79). Monoester **79b** was converted into desired (*S*)-2,5-dimethyl norleucine ((*S*)-**14b**) as a representative of the product class of α-methylated aliphatic amino acids **14** (Scheme 80). A satisfactory overall yield of 37% related to malonate **28b** was obtained for this amino acid (*S*)-**14b**.



Scheme 80. Total synthesis of (*S*)-2,5-dimethyl norleucine ((*S*)-**14b**) from key intermediate (*R*)-**79b**

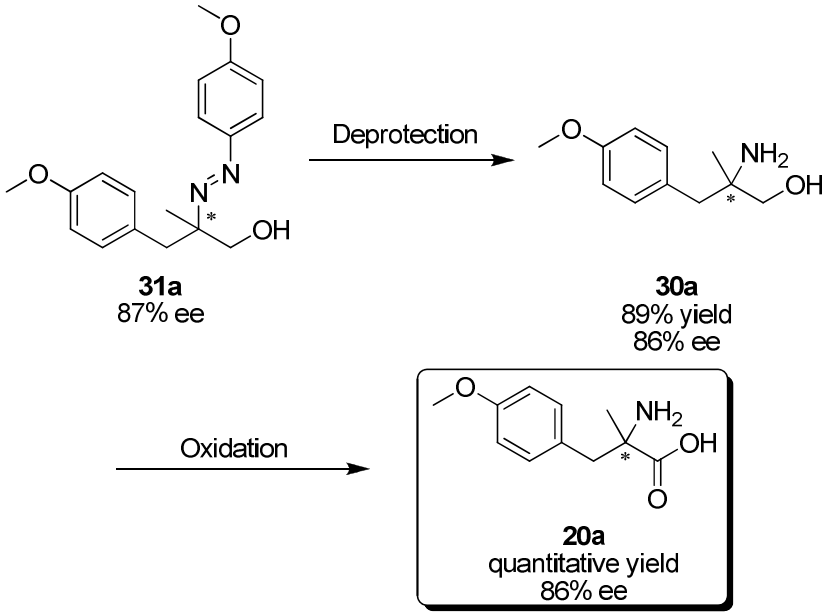
In addition, the inversion of the stereogenic center of malonate monoester is well known;[90b] hence the synthesis of both enantiomers is principally possible by this method. However, the synthesis of β-hydroxy α,α-dialkyl amino acids of type **16** as well as the synthesis of α-methylated phenylalanines of type **20** by this synthetic route turned out to be insufficient.



Scheme 81. Kinetic resolution of **32a** under optimised conditions

In contrast, the enantioselective synthesis of α-methylated substituted phenylalanines of type **20** was possible by kinetic resolution of a racemic azo compound *rac*-**32a** as a key step. The lipase CAL-B was identified as a suitable enzyme for the kinetic resolution of azo compound *rac*-**32a** (Scheme 81). An enantioselectivity of the resolution step of E=24 was achieved when using CAL-B as a biocatalyst in the optimised solvent mixture consisting of MTBE and buffer. The enantiomerically enriched product **31a** of this key step was converted into α,*O*-

dimethyl tyrosine (**20a**) as a representative of the product class of α-methylated phenylalanines **20** (Scheme 82).



Scheme 82. Synthesis of α,*O*-dimethyl tyrosine (**20a**) from key intermediate **31a**

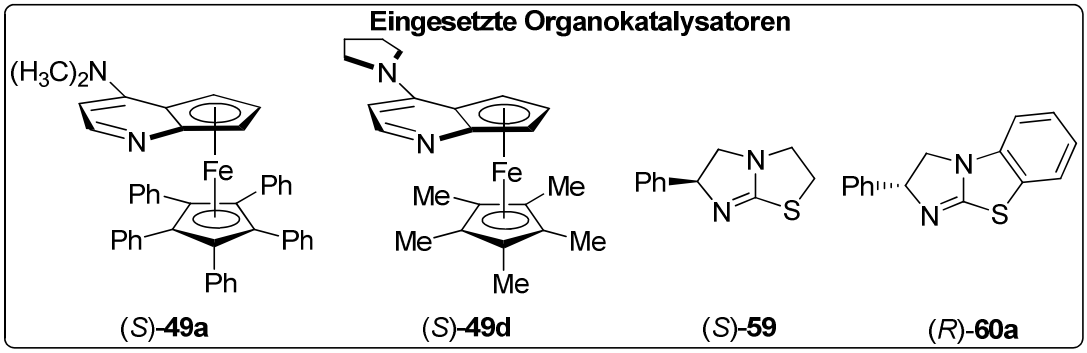
Altogether three new synthetic strategies towards enantiomerically enriched α,α-dialkyl amino acids of type **4** were investigated. Each strategy is based on a catalytic enantioselective key step. All three desired sub-classes, β-hydroxy α,α-dialkyl amino acids **16**, α-methylated aliphatic amino acids **14** and α-methylated substituted phenylalanines **20** are available by the application of one of these strategies. As none of these novel synthetic strategies is general (allowing access to all subclasses of α,α-dialkyl amino acids **4**), each strategy can be called “tailor made” for a specific subclass. Access to all stereoisomers of the desired products is possible, starting from easily available substrates and applying readily commercial available catalysts. The criterion of high conversion, yields and selectivities was met in most cases. However, further improvements with respect to e. g. yield and selectivity are needed in the future to increase the applicability of the new strategies. Since methods to improve the catalysts (either by synthesis of novel thioureas or by enzyme-engineering of the applied enzymes) are readily known,[60c,d,122] there is potential to achieve these improvements in the future. As all synthetic approaches meet the criterion of few and easy to handle synthetic steps, they represent a good basis for further investigations towards the development of highly effective synthesis of α,α-dialkyl amino acids **4**.

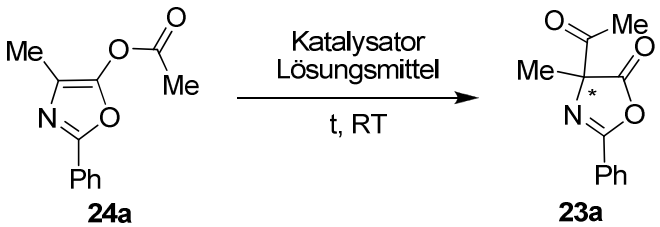
6 Zusammenfassung

Ziel dieser Doktorarbeit war die Synthese von α,α-Dialkylaminosäuren **4** über neuartige Synthesewege, welche einen katalytischen Schlüsselschritt für den enantioselektiven Aufbau (oder die Racematspaltung) eines quartären Kohlenstoffzentrums verwenden. Um dieses Ziel zu erreichen, wurden drei verschiedene synthetische Konzepte intensiv untersucht. Diese Konzepte basieren auf drei verschiedene katalytische Schlüsselschritte - Addition an ein sp^2-hybridisiertes Kohlenstoffatom, Desymmetrisierung und kinetische Racematspaltung. Moderne Methoden der Organo- und Biokatalyse sollten hierfür in den Schlüsselschritten angewendet werden.

Die Steglich-Umlagerung wurde als ein Beispiel für eine organokatalytische Addition an ein sp^2-hybridisiertes Kohlenstoffatom ausgewählt. Enantioselektive Steglich-Umlagerungen von Acylgruppen können dazu verwendet werden β-Hydroxy-α,α-dialkylaminosäuren vom Typ **16** zu synthetisieren. Im Gegensatz dazu ist über die wohlbekannte enantioselektive Steglich-Umlagerung von Oxycarbonylgruppen die Synthese von β-Hydroxy-α,α-dialkylaminosäuren **16**, abgesehen der von einigen Serinderivaten, nicht möglich.

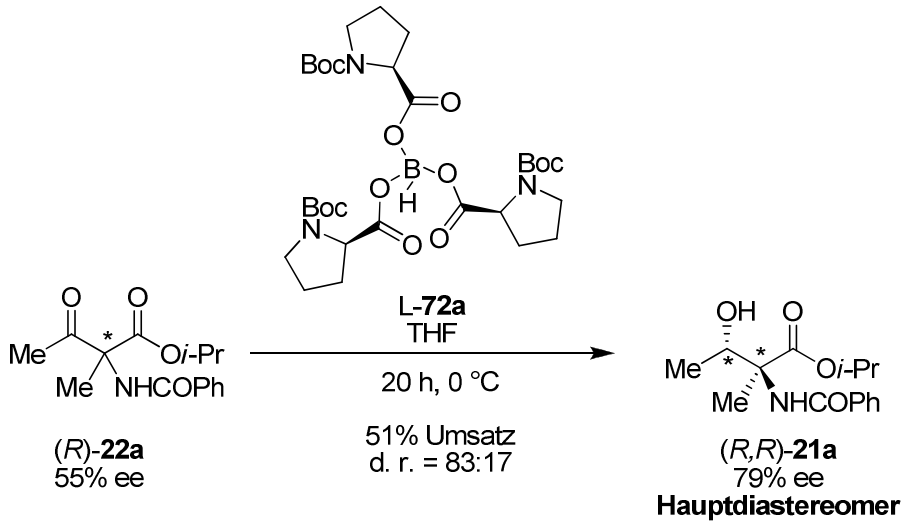
Tabelle 1: Vergleich verschiedener Katalysatoren für die enantioselektiven Steglich-Umlagerung





Eintrag	Katalysator	Lösungsmittel	t (h)	Umsatz (%)	ee (%) (Konfig.)
1	(*S*)-**49a** (2 mol%)	TAA	3	93	30 (*S*)
2	(*S*)-**49d** (2 mol%)	TAA	3	94	50 (*S*)
3	(*S*)-**59** (32 mol%)	$CDCl_3$	48	80	63 (*S*)
4	(*R*)-**60a** (32 mol%)	$CDCl_3$	48	79	61 (*R*)

Im Rahmen dieser Arbeit wurde gefunden, dass dieser Schlüsselschritt am effektivsten durch Verwendung chiraler cyclischer Isothioharnstoffe („Birman-Katalysatoren“), wie (*S*)-Tetramisol ((*S*)-**59**) oder (*R*)-Benzotetramisole ((*R*)-**60a**), bewerkstelligt wird. Diese Isothioharnstoffe erbrachten bessere Enantioselektivitäten, verglichen mit planar chiralen Aminopyridinderrivaten („Fu-Katalysatoren“) wie (*S*)-**49a** oder (*S*)-**49d** (Tabelle 1).

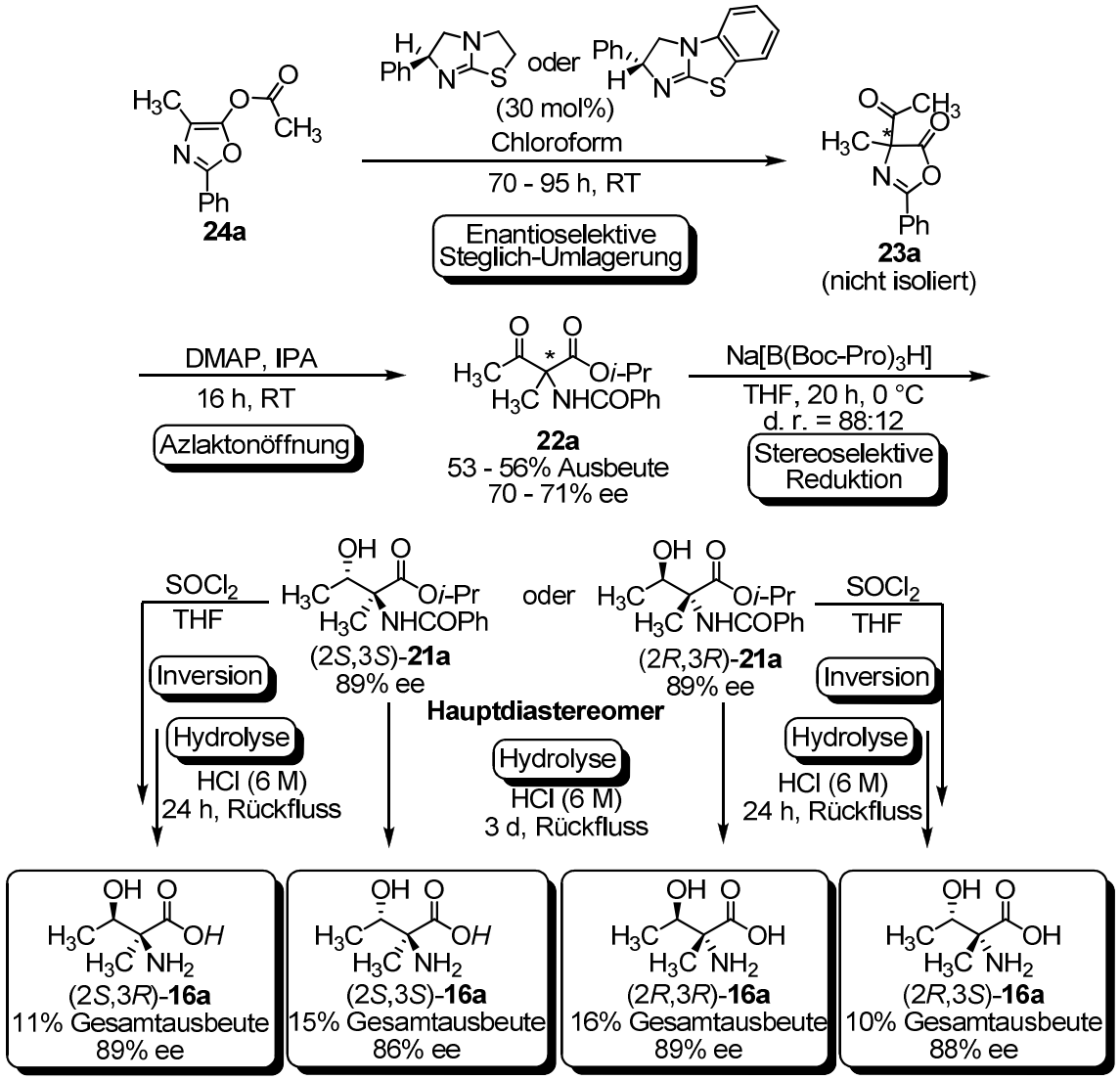


Schema 1. Diastereoselektive Reduktion zu geschütztem α-Methylthreonin (**21a**)

Weil β-Hydroxy-α,α-dialkylaminosäuren im Allgemeinen ein zweites Stereozentrum besitzen, muss ein zweiter, diastereoselektiver Schlüsselschritt durchgeführt werden. Da die meisten Reduktionsmittel sich als zu wenig chemoselektiv erwiesen (das Substrat besitzt einen Ester- und einen Amidrest neben der Ketofunktion), wurde die Reduktion unter Verwendung von Natriumborhydrid und den Einsatz von *N*-Boc-Prolin als chiralem Auxiliar optimiert (Schema 1). Dies führte zu einem gesteigerten Enantiomerenüberschuss im Hauptprodukt (*R,R*)-**21a**.

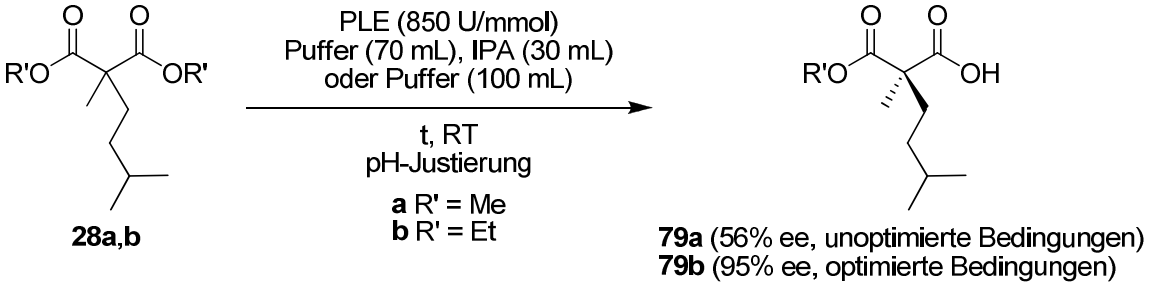
Diese beiden Schlüsselschritte wurden in der Mehrstufensynthese von α-Methylthreonin (**16a**), einem wichtigen Vertreter der Klasse der β-Hydroxy-α,α-dialkylaminosäuren vom Typ **16**, angewendet.

Es wurde gezeigt, dass die Kombination einer enantioselektiven organokatalytischen Steglich-Umlagerung und nachfolgender diastereoselektiven Reduktion unter Verwendung von Natriumborhydrid und *N*-Boc-Prolin als Reduktionsmittel eine sinnvolle und praktische Syntheseroute zur Synthese aller Stereoisomere von (enantiomerenangereicherten) α-Methlythreonin (**16a**) darstellt. Außerdem ist eine stereoselektive Induktion auf beiden Stufen möglich, so dass ein hoher „Gesamtenantiomerenüberschuss“ resultiert.



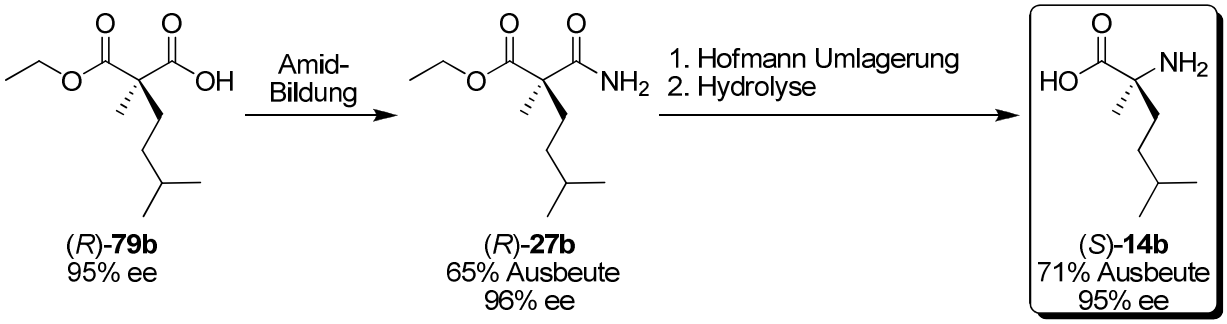
Schema 2. Totalsynthese aller Stereoisomere von α-Methylthreonin (**16a**)

Als Beispiel für eine Desymmetrisierung zum Aufbau des quartären Stereozentrums einer α,α-Dialkylaminosäure **4** wurde die PLE-katalysierte Hydrolyse von Malonaten **28** gewählt, da kein Enzym gefunden werden konnte, welches die Ammonolyse von diesen Malonaten katalysiert. Auch wenn diese Methode im Grunde bereits literaturbekannt war, waren weitere Untersuchungen nötig, um den biokatalytischen Schlüsselschritt zu optimieren.



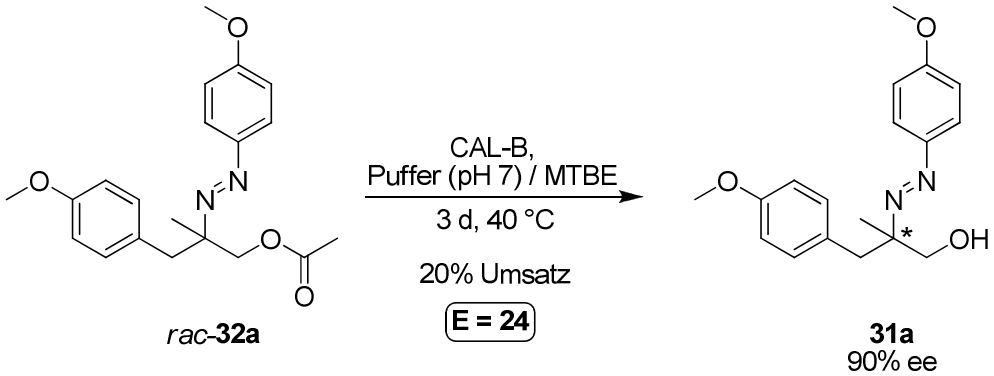
Schema 3. Optimierung der PLE-katalysierten Hydrolyse der Malonate **28a**,**b**

Die Anwendung eines optimierten Reaktionsmediums (bestehend aus Puffer - *iso*-Propanol, 7:3) erlaubte den Zugang zum gewünschten Schlüsselintermediat (*R*)-**79b** mit einem hohen Enantiomerenüberschuss von 95% ee (Schema 3). Der Monoester (*R*)-**79b** wurde in die gewünschte Zielverbindung (*S*)-2,5-Dimethylnorleucin ((*S*)-**14b**), einem Repräsentanten der Produktklasse der α-methylierten aliphatischen Aminosäuren **14**, umgesetzt (Schema 4). Für diese Aminosäure (*S*)-**14b** wurde eine zufriedenstellende Gesamtausbeute von 37%, bezogen auf das Malonat **28b**, erhalten.



Schema 4. Totalsynthese von (*S*)-2,5-Dimethylnorleucin (**14b**) ausgehend vom Schlüsselintermediat (*R*)-**79b**

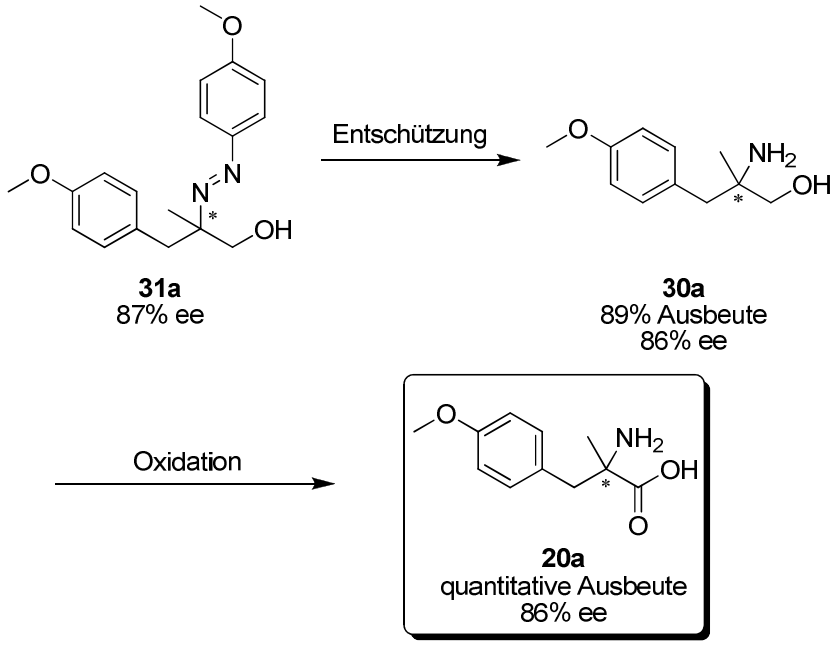
Außerdem ist auch die Inversion des Stereozentrums von Malonatestern wohlbekannt.[90b] Dadurch ist die Synthese beider Enantiomere durch diese Methode prinzipiell möglich. Die Synthese von β-Hydroxy-α,α-dialkylaminosäuren vom Typ **16** über diese Syntheseroute erwies sich jedoch als unbefriedigend, genauso wie die Synthese von α-methylierten Phenylalaninen vom Typ **20**.



Schema 5. Kinetische Racematspaltung von *rac*-**32a** unter optimierten Bedingungen

Im Gegensatz dazu ist die enantioselektive Synthese von α-methylierten substituierten Phenylalaninen vom Typ **20** unter Verwendung einer kinetischen Racematspaltung der racemischen Azoverbindung *rac*-**32a** als Schlüsselschritt möglich. Die Lipase CAL-B wurde

als ein nützlicher Biokatalysator für diese kinetische Racematspaltung identifiziert (Schema 5). Eine Enantioselektivität von E=24 wird erreicht, wenn CAL-B im optimierten Solvensgemisch aus Puffer und MTBE verwendet wird. Das enantiomerenangereicherte Produkt **31a** dieses Schlüsselschrittes wurde zu α,*O*-Dimethyltyrosin (**20a**), einem Vertreter der Produktklasse der α-methylierten Phenylalanine **20**, umgesetzt (Schema 6).



Schema 6. Synthese von α,O-Dimethyltyrosin (**20a**) ausgehend vom Schlüsselintermediat **31a**

Insgesamt wurden drei neue Synthesestrategien für enantiomerenangereicherte α,α-Dialkylaminosäuren vom Typ **4** untersucht. Dabei basiert jede Strategie auf einem katalytischen enantioselektiven Schlüsselschritt. Alle drei gewünschten Unterklassen, β-Hydroxy-α,α-dialkylaminosäuren **16**, α-methylierte aliphatische Aminosäuren **14** und α-methylierte substituierte Phenylalanine **20**, sind nun über eine dieser Strategien verfügbar. Da keine dieser neuartigen Synthesestrategien allgemein ist (Zugang zu allen Unterklassen von α,α-Dialkylaminosäuren **4**), kann man jede Strategie „maßgeschneidert“ für eine spezifische Produktklasse nennen. Dabei ist der Zugang zu allen Stereoisomeren möglich, ausgehend von einfach erhaltbaren Substraten unter Verwendung von kommerziell erhältlichen Katalysatoren. Das Kriterium hoher Umsätze, Ausbeuten und Selektivitäten wurde meist erfüllt. Trotz alledem werden weitere Verbesserungen im Hinblick auf beispielsweise Ausbeuten und Selektivitäten nötig sein, um die Anwendbarkeit der neuen Strategien zu verbessern. Nachdem Methoden zur Verbesserung der verwendeten Katalysatoren bekannt sind (Synthese neuer Thioharnstoffe bzw. Mutation der verwendeten Enzyme),[60c,d,122] sollten weitere Verbesserungen erreichbar sein. Da alle Syntheserouten das Kriterium weniger und leicht handhabbarer Syntheseschritte erfüllen, stellen diese eine

aussichtsreiche Basis für weitere Forschungsvorhaben mit dem Ziel der Entwicklung hocheffizienter Synthesen von α,α-Dialkylaminosäuren **4** dar.

7 Experimental section

7.1 Materials and reagents

Chemicals and materials were purchased from commercial suppliers ABCR, Fisher Scientific and Sigma-Aldrich and were used without further purification. MTBE was kindly supported by Evonik Oxeno GmbH. All solvents were distilled prior to usage. All used buffers were phosphate buffers.

All applied enzymes are commercial available and were purchased either from Sigma-Aldrich or Amano Enzymes. *Candida Antarctica* lipase B (CAL-B) in immobilised form is a commercial product from Novozymes A/S (product name: Novozym®435) and was kindly provided by Evonik Degussa GmbH.

^{1}HNMR and ^{13}CNMR spectra were recorded at room temperature on on a Bruker Avance 300, Bruker Avance 400, JEOL JNM GX 400 or JNM EX 400 spectrometer operating at 300 MHz or 400 MHz. Chemical shifts (δ) are reported in parts per million relative to tetramethylsilane (TMS). NMR raw data was analysed with the programme MestreC 4.9.9.9.

HPLC spectra were recorded on a Jasco HPLC system with a PU-1587 Intelligent Prep. Pump and a LC-Net II and ADC device or with a Shimadzu HPLC system (CBM-10A) with a LC-10AT Liquid Chromatograph pump at room temperature. As stationary phase the following Daicel® columns (4 mm, 250 mm) were used: OD, OJ-H, AD-H, IA and IB.

Mass spectra (MALDI) were recorded on a Shimadzu Biotech Axima Confidence spectrometer. Mass spectra (EI) were recorded on a Micromass Zabspec spectrometer with electron ionisation. A Micromass Zabspec spectrometer was used for FAB mass spectrometry measurements using 3-nitrobenzyl alcohol as matrix.

Elemental analyses were measured on an EA 1119 CHNS, CE instrument.

IR spectra were recorded on a Thermoscientific Nicolet IR-100 FT-IR spectrometer. The absorption is given in wavelength [cm^{-1}].

The optical rotation of chiral compounds was measured on a Perkin Elmer instruments polarimeter (model 341) with a Na-lamp (wavelenght *l* = 589 nm).

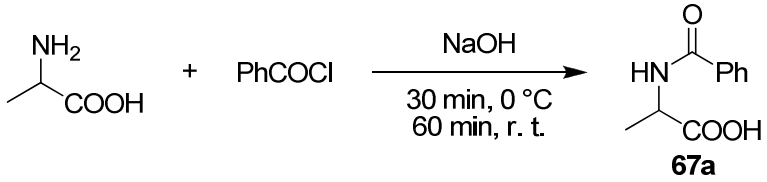
Preparative column chromatography was performed over Merck Silica gel 60 (230–400 mesh) as stationary phase. Thin layer chromatography (TLC) was performed on silica gel TLC cards (Alugramm® SIL G, layer thickness 0.20 mm, Fluka) with fluorescens indicator.

Online pH-adjustment was conducted applying a 702 SM Titrino from METROHM Inc. The data had been recorded and edited with the software tiamo 1.2.

7.2 Syntheses

7.2.1 Steglich rearrangement

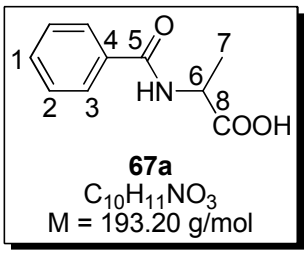
7.2.1.1 Standard operating procedure 1 (SOP1): Synthesis of *N*-benzoyl amino acids (67) in sodium hydroxide solution



Scheme 83. Synthesis of *N*-benzoyl amino acids (**67**) in sodium hydroxide solution

DL-Alanine (225 mmol) was dissolved in sodium hydroxide solution (2 M, 100 mL) in a 500 mL three-necked round bottom flask. The solution was cooled in an ice-bath and benzoyl chloride (1.1 eq., 247 mmol) and additional sodium hydroxide solution (2 M, 150 mL) were added through additional funnels over 10 minutes. The solution was stirred at ice-bath temperature for 30 minutes and additional 60 minutes at room temperature. Thereafter the solution was acidified using concentrated hydrochloric acid precipitating a white solid, which was filtered off and washed with ice-water and suspended in dichloromethane. After stirring this solution for one hour at room temperature, the solid was filtered off and dried *in vacuo*.

7.2.1.1.1 DL-*N*-Benzoylalanine (67a)



67a
$C_{10}H_{11}NO_3$
M = 193.20 g/mol

DL-Alanine (20.0 g, 225 mmol) was dissolved in sodium hydroxide solution (2 M, 100 mL) in a 500 mL three-necked round bottom flask. The solution was cooled in an ice-bath and benzoyl chloride (28.7 mL, 34.7 g, 247 mmol) and additional sodium hydroxide solution (2 M, 150 mL) were added through additional funnels over 10 minutes. The solution was stirred at ice-bath temperature for 30 minutes and additional 60

minutes at room temperature. Thereafter work up was done according to SOP1, yielding a white solid.

Yield: 31.74 g (73%).

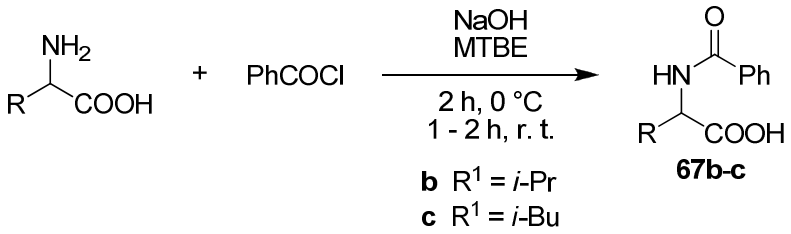
^{1}H-NMR (400 MHz, acetone-d_6): δ [ppm] = 1.51 (d, *J* = 7.3 Hz, 3H, H-C**7**), 4.68 (q, *J* = 7.3 Hz, 1H, H-C**6**), 7.44 - 7.48 (m, 2H, H-C**2**), 7.52 - 7.55 (m, 1H, H-C**1**), 7.92 - 7.94 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, acetone-d_6): δ [ppm] = 18.6 (C**7**), 50.0 (C**6**), 129.3 (C**3**), 130.3 (C**2**), 133.3 (C**1**), 136.4 (C**4**), 168.5 (C**5**), 175.5 (C**8**).

MS (FAB): m/z = 194 (MH+).

Spectroscopic data are in accordance with literature (see reference [65a])

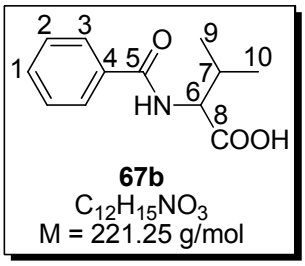
7.2.1.2 Standard operating procedure 2 (SOP2): Synthesis of *N*-benzoyl amino acids (67) in sodium hydroxide solution with additional MTBE



Scheme 84. Synthesis of *N*-benzoyl amino acids (**67**) in sodium hydroxide solution with additional MTBE

The amino acid (50 mmol) was dissolved in sodium hydroxide solution (2 M, 100 mL) and MTBE (100 mL) in a 500 mL three-necked round bottom flask. The solution was cooled in an ice-bath and benzoyl chloride (1.1 eq., 55 mmol) in MTBE (30 mL) was added through an additional funnel over 10 minutes. The solution was stirred at ice-bath temperature for 120 minutes and additional 60 - 120 minutes at room temperature. Thereafter the solution was acidified using concentrated hydrochloric acid. After separation of the organic phase, the aqueous phase was extracted with MTBE (2 x 50 mL). After evaporation of the solvent and washing of the residual white solid with ice-water, the white solid was dissolved in chloroform. After filtering off the insoluble the solution was dried over magnesium sulfate. The pure product was yielded after evaporation of the solvent.

7.2.1.2.1 DL-*N*-Benzoylvaline (67b)



67b
$C_{12}H_{15}NO_3$
M = 221.25 g/mol

DL-Valine (5.9 g, 50 mmol) was dissolved in sodium hydroxide solution (2 M, 100 mL) and MTBE (100 mL) in a 500 mL three-necked round bottom flask. The solution was cooled in an ice-bath and benzoyl chloride (6.4 mL, 7.7 g, 55 mmol) in MTBE (30 mL) was added through an additional funnel over 10 minutes. The solution was stirred at ice-bath temperature for 120 minutes and additional 60 minutes at room temperature. Thereafter the work up was done according to SOP2, yielding a white solid.

Yield: 10.1 g (91%).

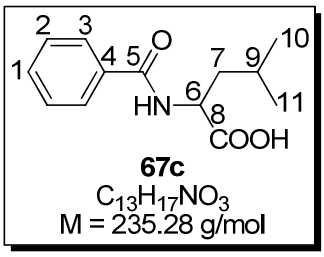
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.03, 1.05 (2d, *J* = 6.9 Hz, 6H, H-C**9**, H-C**10**), 2.30 - 2.38 (m, 1H, H-C**7**), 4.78 - 4.82 (m, 1H, H-C**6**), 6.75 (d, *J* = 8.6 Hz, 1H, NH), 7.40 - 7.52 (m, 3H, H-C**2**, H-C**1**), 7.78 - 7.80 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 17.7, 18.9 (C**9**, C**10**), 31.3 (C**7**), 57.5 (C**6**), 127.2 (C**3**), 128.7 (C**2**), 132.0 (C**1**), 133.8 (C**4**), 168.1 (C**5**), 176.5 (C**8**).

MS (FAB): *m/z* = 222 (MH^+), 443 ($M_2H^{+\bullet}$).

Spectroscopic data are in accordance with literature (see reference [65a]).

7.2.1.2.2 L-*N*-Benzoylleucine (67c)



67c
$C_{13}H_{17}NO_3$
M = 235.28 g/mol

L-Leucine (6.6 g, 50 mmol) was dissolved in sodium hydroxide solution (2 M, 100 mL) and MTBE (100 mL) in a 500 mL three-necked round bottom flask. The solution was cooled in an ice-bath and benzoyl chloride (6.4 mL, 7.7 g, 55 mmol) in MTBE (30 mL) was added through an additional funnel over 10 minutes. The solution was stirred at ice-bath temperature for 120 minutes and additional 60 minutes at room temperature. Thereafter the work up was done according to SOP2, yielding a white solid, containing benzoic acid besides the desired product.

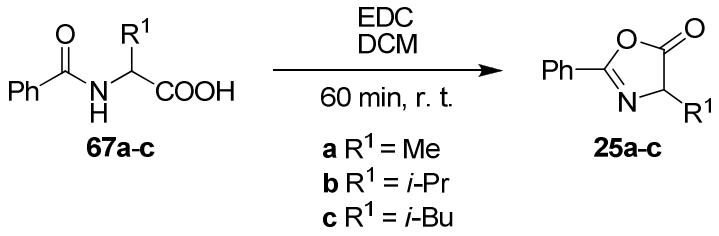
Yield: 10.1 g (purity > 85% (determined by means of 1H-NMR-spectroscopy), 62%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.97 - 0.99 (m, 6H, H-C**10**, H-C**11**), 1.70 - 1.83 (m, 3H, H-C**7**, H-C**9**), 4.79 - 4.87 (m, 1H, H-C**6**), 6.53 (d, *J* = 7.7 Hz, 1H, NH), 7.40 - 7.54 (m, 3H, H-C**1**, H-C**2**), 7.76 - 7.80 (m, 2H, H-C**3**), 8.87 (br s, 1H, COOH).

MS (FAB): m/z = 236 (MH+•), 471 (M_2H+).

Spectroscopic data are in accordance with literature (see reference [47b]).

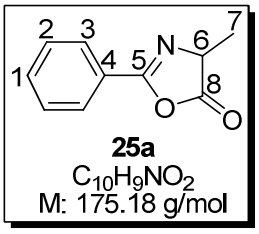
7.2.1.3 Standard operating procedure 3 (SOP3): Synthesis of azlactones (25)



Scheme 85. Synthesis of azlactones (**25a**-**c**)

The *N*-benzoyl amino acid **67** (10 - 31 mmol) was suspended in dry dichloromethane (100 - 250 mL) and cooled in an ice-bath. 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (1.0 eq., 10 - 31 mmol) was added slowly. The solution was stirred at ice-bath temperature for 60 minutes. Thereafter the solution was evaporated to dryness and the remaining solid was dissolved in MTBE (50 mL) and hydrochloric acid (1 M, 50 mL). After separation of the phases, the organic phase was washed with water and dried over magnesium sulfate. The pure products were yielded after evaporation of the solvent and drying *in vacuo.*

7.2.1.3.1 4-Methyl-2-phenyl-4H-oxazol-5-one (25a)



N-Benzoyl alanine (**67a**, 6.0 g, 31 mmol) was suspended in dry dichloromethane (250 mL) and cooled in an ice-bath. 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (6.0 g, 31 mmol) was added slowly. The solution was stirred at ice-bath temperature for 60 minutes. Thereafter the work up was done according to SOP3, yielding a white solid.

Yield: 4.03 g (74%).

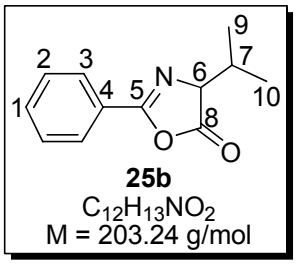
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.56 (d, *J* = 7.6 Hz, 3H, H-C**7**), 4.42 (q, *J* = 7.6 Hz, 1H, H-C**6**), 7.44 - 7.48 (m, 2H, H-C**2**), 7.53 - 7.57 (m, 1H, H-C**1**), 7.96 - 7.98 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 16.8 (C**7**), 61.0 (C**6**), 125.9 (C**4**), 127.9, 128.8 (C**2,** C**3**), 132.8 (C**1**), 161.6 (C**5**), 179.1 (C**8**).

MS (FAB): *m/z* = 176 (MH^+).

Spectroscopic data are in accordance with literature (see reference [47b]).

7.2.1.3.2 4-Isopropyl-2-phenyl-4H-oxazol-5-one (25b)



25b
$C_{12}H_{13}NO_2$
M = 203.24 g/mol

N-Benzoyl valine (**67b**, 2.21 g, 10 mmol) was suspended in dry dichloromethane (250 mL) and cooled in an ice-bath. 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (2.02 g, 10.5 mmol) was added slowly. The solution was stirred at ice-bath temperature for 60 minutes. Thereafter the work up was done according to SOP3, yielding a white solid.

Yield: 1.89 g (94%).

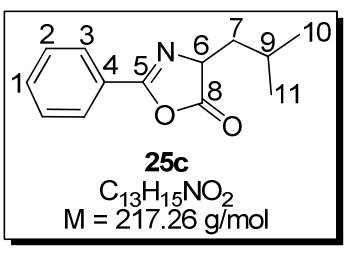
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.00 (d, *J* = 6.8 Hz, 3H, H-C**9**), 1.13 (d, *J* = 6.9 Hz, 3H, H-C**10**), 2.31 - 2.42 (m, 1H, H-C**7**), 4.27 (d, *J* = 4.5 Hz, 1H, H-C**6**), 7.45 - 7.48 (m, 2H, H-C**2**), 7.53 - 7.58 (m, 1H, H-C**1**), 7.98 - 8.00 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 17.4 (C**9**), 18.7 (C**10**), 31.2 (C**7**), 70.7 (C**6**), 126.0 (C**4**), 127.9, 128.8 (C**2,** C**3**), 132.7 (C**1**), 161.8 (C**5**), 177.9 (C**8**).

MS (FAB): *m/z* = 204 (MH^+).

Spectroscopic data are in accordance with literature (see reference [47b]).

7.2.1.3.3 4-Isobutyl-2-phenyl-4H-oxazol-5-one (25c)



25c
$C_{13}H_{15}NO_2$
M = 217.26 g/mol

N-Benzoyl leucine (**67c**, 3.1 g, 13.1 mmol) was suspended in dry dichloromethane (200 mL) and cooled in an ice-bath. 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimid hydrochloride (2.70 g, 13.8 mmol) was added slowly. The solution was stirred at ice-bath temperature for 60 minutes. Thereafter the work up was

done according to SOP3 applying an additional washing step with sodium bicarbonate solution to remove benzoic acid, yielding a white solid.

Yield: 2.07 g (75%).

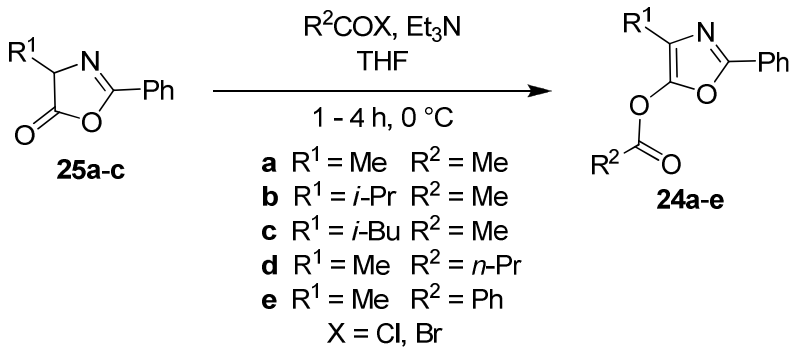
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.98 - 1.02 (m, 6H, H-C**10**, H-C**11**), 1.62 - 1.70 (m, 1H, H-C**7**), 1.79 - 1.86 (m, 1H, H-C**7**), 2.01 - 2.08 (m, 1H, H-C**9**), 4.39 (dd, *J* = 5.7 Hz, *J* = 8.9 Hz, 1H, H-C**6**), 7.44 - 7.57 (m, 3H, H-C**2**, H-C**1**), 7.96 - 7.99 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 22.0, 22.6 (C**10**, C**11**), 25.1 (C**9**), 40.7 (C**7**), 63.9 (C**6**), 126.1 (C**4**), 127.8, 128.9 (C**2,** C**3**), 132.7 (C**1**), 161.5 (C**5**), 179.1 (C**8**).

MS (FAB): *m/z* = 218 (MH^+).

Spectroscopic data are in accordance with literature (see reference [47b]).

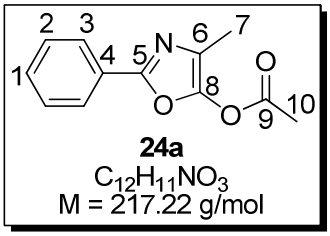
7.2.1.4 Standard operating procedure 4 (SOP4): Synthesis of 5-acyloxyoxazoles (24)



Scheme 86. Synthesis of 5-acyloxyoxazoles (**24**)

The azlactone **25a**-**c** (5.7 - 22.8 mmol) and the acyl halogenide (1.1 - 1.5 eq.) were dissolved in absolute THF (20 - 70 mL) and cooled in an ice-bath. Triethylamine (1.1 - 1.5 eq.) in THF (10 - 20 mL) was added dropwise over 10 minutes. The solution was stirred for one to four hours at 0 ℃ and the precipitated white solid was filtered off. The solvent was evaporated and the residue was dissolved in MTBE and washed with hydrochloric acid (1 M). The organic layer was dried over magnesium sulfate and the solvent evaporated.

7.2.1.4.1 4-Methyl-2-phenyloxazol-5-yl acetate (24a)



24a
$C_{12}H_{11}NO_3$
M = 217.22 g/mol

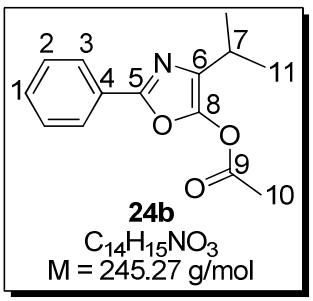
4-Methyl-2-phenyloxazol-5(4H)-one (**25a**, 4.0 g, 22.8 mmol) and acetyl chloride (2.5 mL, 2.7 g, 34.4 mmol) were dissolved in absolute THF (70 mL) and cooled in an ice-bath. Triethylamine (4.8 mL, 3.5 g, 34.6 mmol) in THF (20 mL) was added dropwise over 10 minutes. The solution was stirred for one hour at 0 °C. Work up was done according to SOP4, yielding a white solid.

Yield: 4.05 g (81%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 2.09 (s, 3H, H-C**10**), 2.35 (s, 3H, H-C**7**), 7.38 - 7.41 (m, 3H, H-C**1**, H-C**2**), 7.90 - 7.92 (m, 2H, H-C**3**).

Spectroscopic data are in accordance with literature (see reference [45b]).

7.2.1.4.2 4-Isopropyl-2-phenyloxazol-5-yl acetate (24b)



24b
$C_{14}H_{15}NO_3$
M = 245.27 g/mol

4-Isopropyl-2-phenyloxazol-5(4H)-one (**25b**, 2.5 g, 12.3 mmol) and acetyl chloride (960 µL, 1.06 g, 13.5 mmol) were dissolved in absolute THF (40 mL) and cooled in an ice-bath. Triethylamine (1.9 mL, 1.4 g, 13.5 mmol) in THF (10 mL) was added dropwise over 10 minutes. The solution was stirred for one hour at 0 °C. Work up was done according to SOP4, yielding an orange oil, which was purified by column chromatography (ethyl acetate - cyclohexane 1:10).

Yield: 1.28 g (42%), R_f = 0.45 (ethyl acetate - cyclohexane, 1:10).

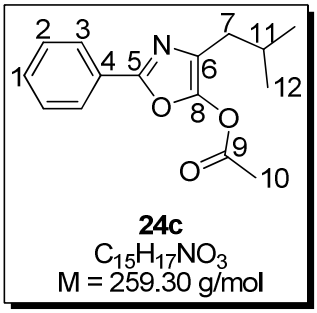
IR (powder film): 3066, 2966, 2881, 1784, 1653 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] = 1.25 (d, *J* = 7.0 Hz, 6H, H-C**11**), 2.32 (s, 3H, H-C**10**) 2.82 (septet, *J* = 7.0 Hz, 1H, H-C**7**), 7.38 - 7.40 (m, 3H, H-C**1**, H-C**2**), 7.91 - 7.94 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 20.1 (C**10**), 21.0 (C**11**), 25.4 (C**7**), 125.9 (C**3**), 127.6 (C**6**), 128.6 (C**2**), 129.8 (C**4**), 130.0 (C**1**), 144.2 (C**8**), 155.0 (C**5**), 167.8 (C**9**).

MS (FAB): *m/z* = 246 (MH^+), 204 (MH^+-$COCH_3$).

7.2.1.4.3 4-Isobutyl-2-phenyloxazol-5-yl acetate (24c)



24c
$C_{15}H_{17}NO_3$
M = 259.30 g/mol

4-Isobutyl-2-phenyloxazol-5(4H)-one (**25c**, 1.85 g, 8.5 mmol) and acetyl chloride (670 µL, 740 mg, 9.4 mmol) were dissolved in absolute THF (30 mL) and cooled in an ice-bath. Triethylamine (1.3 mL, 950 mg, 9.4 mmol) in THF (10 mL) was added dropwise over 10 minutes. The solution was stirred for one hour at 0 °C. Work up was done according to SOP4, yielding a white solid.

Yield: 1.91 g (86%); Mp 45 - 47 °C.

IR (powder film): 3061, 2961, 2868, 1791, 1729, 1660 cm^{-1}.

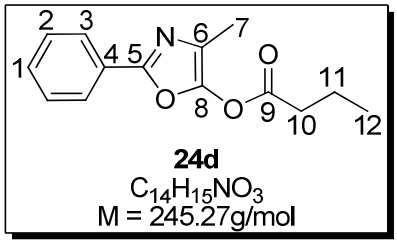
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.93 (d, *J* = 6.8 Hz, 6H, H-C**12**), 2.02 (m, 1H, H-C**11**), 2.29 (d, *J* = 6.8 Hz, 2H, H-C**7**), 2.33 (s, 3H, H-C**10**), 7.38 - 7.40 (m, 3H, H-C**1**, H-C**2**), 7.91 - 7.94 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 20.1 (C**10**), 22.2 (C**12**), 27.5 (C**7**), 33.9 (C**11**), 124.0 (C**6**), 125.9 (C**2**), 127.4 (C**4**), 128.7 (C**3**), 130.1 (C**1**), 146.2 (C**8**), 155.1 (C**5**), 167.5 (C**9**).

MS (FAB): *m/z* = 260 (MH^+), 218 (MH^+-$COCH_3$).

Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.65; H, 6.63; N, 5.17.

7.2.1.4.4 4-Methyl-2-phenyloxazol-5-yl butyrate (24d)



24d
$C_{14}H_{15}NO_3$
M = 245.27g/mol

4-Methyl-2-phenyloxazol-5(4H)-one (**25a**, 1.0 g, 5.7 mmol) and butanoyl chloride (600 µL, 610 mg, 5.7 mmol) were dissolved in absolute THF (20 mL) and cooled in an ice-bath. Triethylamine (791 µL, 575 mg, 5.7 mmol) in THF (5 mL) was added dropwise over 10 minutes. The solution was stirred for two hours at 0 °C. Work up was done acc ording to SOP4. The crude product was purified by column chromatography (ethyl acetate - cyclohexane, 1:3), yielding a colorless oil.

Yield: 1.0 g (72%); R_f = 0.38 (ethyl acetate - cyclohexane, 1:3).

IR (powder film): 3069, 2968, 2934, 2880, 1791, 1752, 1660 cm^{-1}.

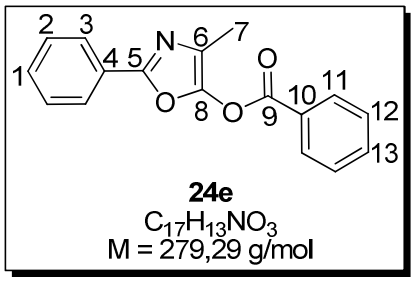
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.04 (t, *J* = 7.4 Hz, 3H, H-C**12**), 1.74-1.84 (m, 2H, H-C**11**), 2.08 (s, 3H, H-C**7**), 2.59 (t, *J* = 7.2 Hz, 2H, H-C**10**), 7.39 - 7.42 (m, 3H, H-C**1**, H-C**2**), 7.90 - 7.93 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 10.3 (C**7**), 13.4 (C**12**), 18.1 (C**11**), 35.3 (C**10**), 120.5 (C**6**), 125.8 (C**2**), 127.4 (C**4**), 128.7 (C**3**), 130.1 (C**1**), 145.9 (C**8**), 155.0 (C**5**), 170.0 (C**9**).

MS (FAB): *m/z* = 248 (MH^+), 177 (MH^+-$COCH_2CH_2CH_3$).

Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.92; H, 6.23; N, 5.62.

7.2.1.4.5 4-Methyl-2-phenyloxazol-5-yl benzoate (24e)



24e
$C_{17}H_{13}NO_3$
M = 279,29 g/mol

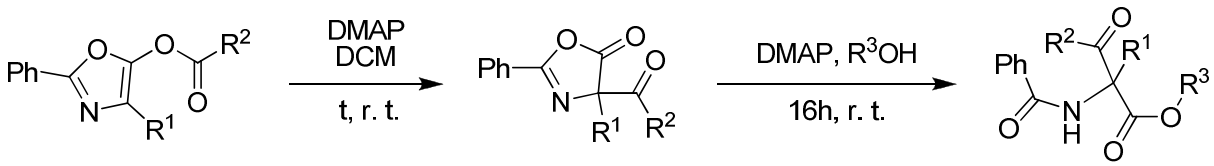
4-Methyl-2-phenyloxazol-5(4H)-one (**25a**, 1.0 g, 5.7 mmol) and benzoyl bromide (739 µL, 1.16 g, 6.3 mmol) were dissolved in absolute THF (20 mL) and cooled in an ice-bath. Triethylamine (870 µL, 632 mg, 6.3 mmol) in THF (10 mL) was added dropwise over 10 minutes. The solution was stirred for four hours at 0 °C. Work up was done according to SOP4. The crude product was purified by column chromatography (ethyl acetate - cyclohexane, 1:6), yielding a white solid.

Yield: 880 mg (55%); R_f = 0.33 (ethyl acetate - cyclohexane, 1:6).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 2.16 (s, 3H, H-C**7**), 7.40 - 7.42 (m, 3H, H-C**1**, H-C**2**), 7.51- 7.55 (m, 2H, H-C**12**), 7.66 - 7.70 (m, 1H, H-C**13**), 7.94 - 7.97 (m, 2H, H-C**3**), 8.19 - 8.21 (m, 2H, H-C**11**).

Spectroscopic data are in accordance with literature (see reference [45b]).

7.2.1.5 Standard operating procedure 5 (SOP5): Non-enantioselective Steglich rearrangement and ring opening (22, 23)



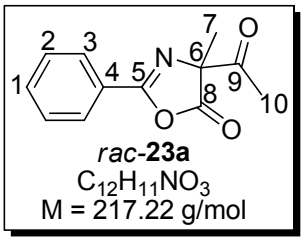
24a-e **23a-e** **22a-e**

a R^1 = Me, R^2 = Me, R^3 = *i-P*r
b R^1 = *i*-Pr, R^2 = Me, R^3 = *i*-Pr
c R^1 = *i*-Bu, R^2 = Me, R^3 = Me
d R^1 = Me, R^2 = *n*-Pr, R^3 = *i*-Pr
e R^1 = Me, R^2 = Ph, R^3 = *i*-Pr

Scheme 87. Non-enantioselective Steglich rearrangement and ring opening

The 5-acyloxy-4-methyl-2-phenyl-oxazole (**24**, 0.4 - 9.3 mmol) and DMAP (90 mg, 0.07 - 0.20 eq.) were dissolved in dichloromethane (5 - 50 mL) at room temperature. After three to five hours of stirring, the solvent was removed to give crude product **23**, which was used for the synthesis of **22** without further purification. The mixture was dissolved in isopropanol (5 - 40 mL). The reaction mixture was stirred for 16 hours and the excess of isopropanol was removed in vacuo. The resulting crude product was purified by column chromatography to give the pure product **22**.

7.2.1.5.1 *rac*-4-Acetyl-4-methyl-2-phenyl-oxazol-5-one (*rac*-23a)



rac-**23a**
$C_{12}H_{11}NO_3$
M = 217.22 g/mol

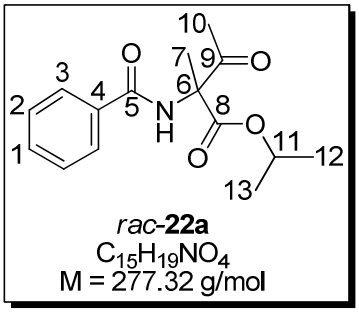
5-Acetyloxy-4-methyl-2-phenyl-oxazole (**24a**, 2.0 g, 9.3 mmol) and DMAP (90 mg, 0.7 mmol) were dissolved in dichloromethane (50 mL) at room temperature. After three hours of stirring, the solvent was removed to give *rac*-**23a** as yellow oil.

Yield: 2.05 g (quantitative, purity > 95% (determined by means of 1H-NMR-spectroscopy)).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.69 (s, 3H, H-C**7**), 2.26 (s, 3H, H-C**10**), 7.46 - 7.59 (m, 3H, H-C**1**, H-C**2**), 8.00 - 8.02 (m, 2H, H-C**3**).

Spectroscopic data are in accordance with literature (see reference [45b]).

7.2.1.5.2 Isopropyl *rac*-2-benzoylamino-2-methyl-3-oxo-butyrate (*rac*-22a)



rac-**22a**
$C_{15}H_{19}NO_4$
M = 277.32 g/mol

rac-4-Acetyl-4-methyl-2-phenyl-oxazol-5-one (*rac*-**23a**, 1.5 g, 6.9 mmol) and DMAP (90 mg, 0.7 mmol) were dissolved in isopropanol (40 mL). The reaction mixture was stirred for 16 hours and the excess of isopropanol was removed in vacuo. The resulting crude product was purified by column chromatography (ethyl acetate - cyclohexane, 1:3) to give colorless oil.

Yield: 1.62 g (84%); R_f = 0.36 (ethyl acetate - cyclohexane, 1:3).

HPLC (Chiralcel OD column; hexanes - *i*-PrOH, 97:3; 1.0 mL/min):
t_R = 9.0 (*R*), 10.8 (*S*) min.

IR (thin film): 3412, 3327, 2984, 1722, 1664 cm^{-1}.

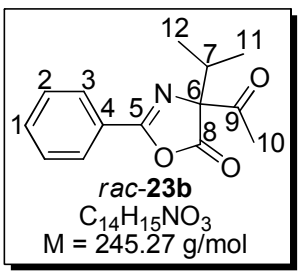
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.18 (d, *J* = 6.3 Hz, 3H, H-C**12**), 1.21 (d, *J* = 6.3 Hz, 3H, H-C**13**), 1.79 (s, 3H, H-C**7**), 2.21 (s, 3H, H-C**10**), 5.08 (septet, *J* = 6.3 Hz, 1H, H-C**11**), 7.40 - 7.44 (m, 2H, H-C**2**), 7.47 - 7.51 (m, 1H, H-C**2**), 7.71 (br s, 1H, NH), 7.78 - 7.81 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 19.9 (C**7**), 21.30, 21.32 (C**12**, C**13**), 23.9 (C**10**), 68.6 (C**11**), 70.5 (C**6**), 127.1, 128.6 (C**2**, C**3**), 131.9 (C**1**), 133.6 (C**4**), 166.0 (C**5**), 168.7 (C**8**), 200.4 (C**9**).

MS (EI): *m/z* = 278 (MH^+), 234 (MH^+-$COCH_3$).

Anal. Calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05; Found: C, 64.61; H, 6.88; N, 4.89.

7.2.1.5.3 *rac*-4-Acetyl-4-isopropyl-2-phenyl-oxazol-5-one (*rac*-23b)



rac-**23b**
$C_{14}H_{15}NO_3$
M = 245.27 g/mol

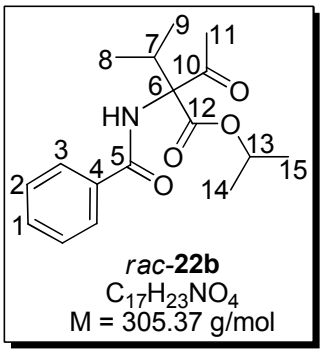
5-Acetyloxy-4-isopropyl-2-phenyl-oxazole (**24b**, 100 mg, 0.4 mmol) and DMAP (5 mg, 0.04 mmol) were dissolved in dichloromethane (5 mL) at room temperature. After three hours of stirring, the solvent was removed to give crude *rac*-**23b** as yellow oil, which was applied for ring opening to *rac*-**22b**.

Yield: 102 mg (quantitative, purity > 70% (determined by means of 1H-NMR-spectroscopy)).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.90 (d, *J* = 6.8 Hz, 3H, H-C**11**), 1.03 (d, *J* = 6.8 Hz, 3H, H-C**12**), 2.32 (s, 3H, H-C**10**), 2.76 (septet, *J* = 6.8 Hz, 1H, H-C**7**), 7.47 - 7.60 (m, 3H, H-C**1**, H-C**2**), 8.03 - 8.07 (m, 2H, H-C**3**).

Spectroscopic data are in accordance with literature (see reference [45b]).

7.2.1.5.4 Isopropyl *rac*-2-benzoylamino-2-isopropyl-3-oxo-butyrate (*rac*-22b)



rac-**22b**
$C_{17}H_{23}NO_4$
M = 305.37 g/mol

rac-4-Acetyl-4-isopropyl-2-phenyl-oxazol-5-one (*rac*-**23b**, 102 mg), containing DMAP and side products was dissolved in isopropanol (5 mL). The reaction mixture was stirred for 16 hours and the excess of isopropanol was removed in vacuo. The resulting crude product was purified by column chromatography (ethyl acetate - cyclohexane, 1:6) to give colorless oil.

Yield: 75 mg (61%); R_f = 0.20 (ethyl acetate - cyclohexane, 1:6).

IR (thin film): 3391, 2974, 2935, 2881, 1722, 1668 cm^{-1}.

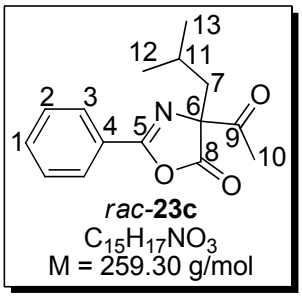
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.86 (d, *J* = 6.8 Hz, 3H, H-C**9**), 1.01 (d, *J* = 6.8 Hz, 3H, H-C**8**), 1.19 (d, *J* = 6.3 Hz, 3H H-C**14**), 1.22 (d, *J* = 6.3 Hz, 3H, H-C**15**), 2.14 (s, 3H, H-C**11**), 2.85 (septet, *J* = 6.8 Hz, 1H, H-C**7**), 5.11 (septet, *J* = 6.3 Hz, 1H, H-C**13**), 7.42 - 7.53 (m, 4H, H-C**1**, H-C**2**, NH), 7.80 - 7.82 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 17.7, 18.3 (C**8**, C**9**), 21.3, 21.4 (C**14**, C**15**), 24.7 (C**11**), 32.7 (C**7**), 70.3 (C**13**), 74.6 (C**6**), 127.1, 128.7 (C**2**, C**3**), 131.9 (C**1**), 133.9(C**4**), 166.4 (C**5**), 168.0 (C**12**), 199.1 (C**10**).

MS (EI): *m/z* = 306 (MH^+), 262 (MH^+-*i*-PrOH), 201 (MH^+-PhCO), 158 (MH^+- *i*-PrO - PhCO).

Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 67.58; H, 7.79; N, 4.57.

7.2.1.5.5 *rac*-4-Acetyl-4-isobutyl-2-phenyl-oxazol-5-one (*rac*-23c)



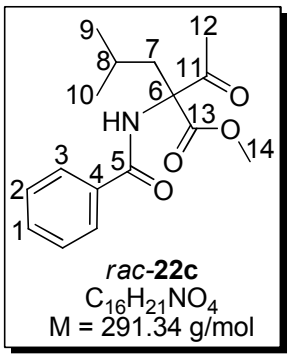
rac-**23c**
$C_{15}H_{17}NO_3$
M = 259.30 g/mol

5-Acetyloxy-4-isobutyl-2-phenyl-oxazole (**24c**, 400 mg, 1.4 mmol) and DMAP (15 mg, 0.1 mmol) were dissolved in dichloromethane (20 mL) at room temperature. After 4 hours of stirring, the solvent was removed to give crude *rac*-**23c** as yellow oil, which was applied for ring opening to *rac*-**22c**.

Yield: 402 mg (quantitative, purity > 85% (determined by means of 1H-NMR-spectroscopy)).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.86 - 0.91 (m, 6H, H-C**12**, H-C13), 1.61 - 1.73 (m, 1H, H-C**11**), 1.97 - 2.02 (m, 1H, H-C**7**), 2.19 - 2.25 (m, 1H, H-C**7**), 2.28 (s, 3H, H-C**10**), 7.48 - 7.51 (m, 2H, H-C**2**), 7.57 - 7.61 (m, 1H, H-C**1**), 8.02 - 8.05 (m, 2H, H-C**3**).

7.2.1.5.6 Methyl *rac*-2-benzoylamino-2-isobutyl-3-oxo-butyrate (*rac*-22c)



rac-**22c**
$C_{16}H_{21}NO_4$
M = 291.34 g/mol

For this synthesis, SOP5 was modified as follows: 4-acetyl-4-isopropyl-2-phenyl-oxazol-5-one (*rac*-**23c**, 380 mg), and DMAP (80 mg, 0.6 mmol) were dissolved in dichloromethane (50 mL). Methanol (5 mL) was added and the reaction mixture was stirred for 16 hours. The solvent and the excess of methanol were removed in vacuo. The resulting crude product was purified by column chromatography (ethyl acetate - cyclohexane, 1:3) to give pure *rac*-**22c** as colorless oil.

Yield: 137 mg (32%) R_f = 0.47 (ethyl acetate - cyclohexane, 1:3).

HPLC (Chiralcel OJ-H-column; hexanes - *i*-PrOH, 90:10; 1.0 mL/min): t_R = 12.2, 19.9 min.
HPLC (Chiralcel OD-column; hexanes - *i*-PrOH, 97:3; 1.0 mL/min): t_R = 8.5, 9.5 min.

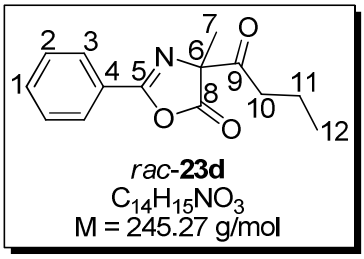
IR (thin film): 3408, 2961, 1749, 1722, 1664 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.83 - 0.86 (m, 6H, H-C**9**, H-C**10**), 1.46 - 1.55 (m, 1H, H-C**8**), 2.22 (s, 3H, H-C**12**), 2.37 - 2.42 (m, 1H, H-C**7**), 2.49 - 2.54 (m, 1H, H-C**7**), 3.76 (s, 3H, H-C**14**), 7.43 - 7.54 (m, 3H, H-C**1**, H-C**2**), 7.75 (br s, 1H, NH), 7.81 - 7.84 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 23.1, 23.6, 24.3, 24.4 (C**8**, C**9**, C**10**, C**12**), 39.5 (C**7**), 53.5 (C**14**), 72.1 (C**6**), 127.2, 128.8 (C**2**, C**3**), 132.1 (C**1**), 133.4 (C**4**), 165.9 (C**5**), 169.8 (C**13**), 200.6 (C**11**).
MS (FAB): *m/z* = 292 (MH^+).

Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 65.53; H, 7.36; N, 4.65.

7.2.1.5.7 *rac*-4-Butyryl-4-methyl-2-phenyloxazol-5-one (*rac*-23d)



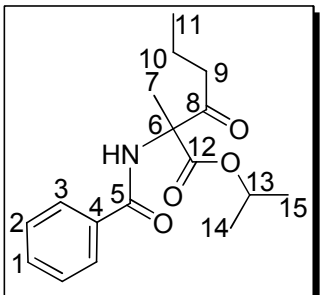
rac-**23d**
$C_{14}H_{15}NO_3$
M = 245.27 g/mol

4-Methyl-2-phenyloxazol-5-yl butyrate (**24d**, 200 mg, 0.8 mmol) and DMAP (20 mg, 0.16 mmol) were dissolved in dichloromethane (5 mL) at room temperature. After 3 hours of stirring, the solvent was removed to give crude *rac*-**23d** as yellow oil, which was applied for ring opening to *rac*-**22d**.

Yield: 210 mg (quantitative, purity approx. 90% (determined by means of 1H-NMR-spectroscopy)).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.85 - 0.90 (m, 3H, H-C**12**), 1.55 - 1.63 (m, 2H, H-C**11**), 1.70 (s, 3H, H-C**7**), 2.42 - 2.69 (m, 2H, H-C**10**), 7.46 - 7.62 (m, 3H, H-C**1**, H-C**2**), 8.01 - 8.04 (m, 2H, H-C**3**).

7.2.1.5.8 Isopropyl *rac*-2-benzoylamino-2-methyl-3-oxo-hexanoate (*rac*-22d)



rac-**22d**
$C_{17}H_{23}NO_4$
M = 305.37 g/mol

210 mg of the mixture from the reaction described in section 7.2.1.5.7, containing 4-butyryl-4-methyl-2-phenyloxazol-5-one (*rac*-**23d**, approx. 90%) and DMAP (approx. 10%) were dissolved in 5 mL isopropanol. The reaction mixture was stirred for 16 hours. The excess of alcohol was removed in *vacuo*. The resulting crude product was purified by column chromatography (ethyl acetate - cyclohexane, 1:3) to give pure *rac*-**22d** as colorless oil.

Yield: 140 mg (56%); R_f = 0.53 (ethyl acetate - cyclohexane, 1:3).

HPLC (Chiralpak AD-H-column; hexanes - *i*-PrOH, 97:3; 1.0 mL/min): t_R = 13.3, 14.9 min.

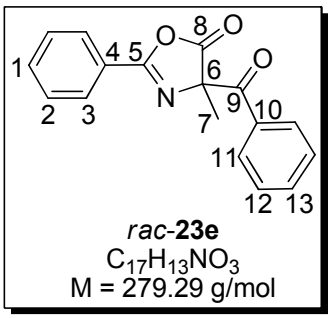
IR (thin film): 3408, 2968, 2937, 2880, 1745, 1718, 1664 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.86 (t, *J* = 7.4 Hz, 3H, H-C**11**), 1.16 (d, *J* = 6.3 Hz, 3H, H-C**14**), 1.19 (d, *J* = 6.3 Hz, 3H, H-C**15**), 1.57 - 1.66 (m, 2H, H-C**10**), 1.78 (s, 3H, H-C**7**), 2.41 - 2.56 (m, 2H, H-C**9**), 5.07 (septet, *J* = 6.3 Hz, 1H, H-C**13**), 7.39 - 7.43 (m, 2H, H-C**2**), 7.46 - 7.50 (m, 1H, H-C**1**), 7.76 (br s, 1H, NH), 7.77 - 7.80 (m, 2H, H-C**3**).
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 13.4 (C**11**), 17.0 (C**10**), 19.8 (C**7**), 21.32, 21.34 (C**14**, C**15**), 38.0 (C**9**), 68.5 (C**13**), 70.3 (C**6**), 127.0, 128.6 (C**2**, C**3**), 131.8 (C**1**), 133.7 (C**4**), 165.9 (C**5**), 168.7 (C**12**), 202.7 (C**8**).

MS (FAB): *m/z* = 307 (MH^+).

Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.39; H, 7.57; N, 4.39.

7.2.1.5.9 *rac*-4-Benzoyl-4-methyl-2-phenyloxazol-5-one (*rac*-23e)



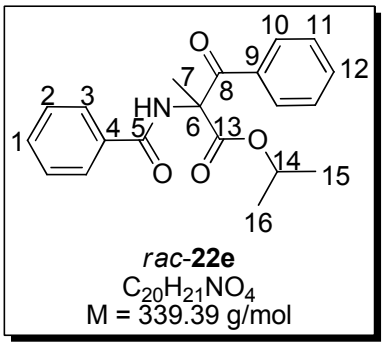
rac-**23e**
$C_{17}H_{13}NO_3$
M = 279.29 g/mol

4-Methyl-2-phenyloxazol-5-yl benzoate (**24e**, 260 mg, 0.93 mmol) and DMAP (23 mg, 0.19 mmol) were dissolved in dichloromethane (5 mL) at room temperature. After 5 hours of stirring, the solvent was removed to give crude **23e** as yellow oil, which was applied for ring opening to *rac*-**22e**.

Yield: 280 mg (quantitative, purity approx. 70% (determined by means of 1H-NMR-spectroscopy)).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.89 (s, 3H, H-C**7**), 7.43 - 7.59 (m, 6H, H-C**1**, H-C**2**, H-C**12**, H-C**13**), 8.01 - 8.20 (m, 4H, H-C**3**, H-C**11**).

7.2.1.5.10 Isopropyl *rac*-2-benzoylamino-2-methyl-3-oxo-3-phenylpropanoate (*rac*-22e)



rac-**22e**
$C_{20}H_{21}NO_4$
M = 339.39 g/mol

280 mg of the mixture from the reaction mentioned above, containing 4-benzoyl-4-methyl-2-phenyloxazol-5-one (*rac*-**23e**, approx. 70%), DMAP (approx. 10%) and deacylated side product, were dissolved in 20 mL isopropanol. The reaction mixture was stirred for 16 hours. The excess of alcohol was removed in *vacuo*. The resulting crude product was purified by column chromatography (ethyl acetate - cyclohexane, 1:3) to give pure *rac*-**22e** as white solid.

Yield: 100 mg (33%), Mp 128 - 130 °C; R_f = 0.46 (ethyl acetate - cyclohexane, 1:3).

HPLC (Chiralcel OJ-H-column; hexanes - *i*-PrOH, 90:10; 1.0 mL/min): t_R = 13.7, 29.4 min.

IR (powder film): 3408, 2984, 1737, 1698, 1652 cm^{-1}.

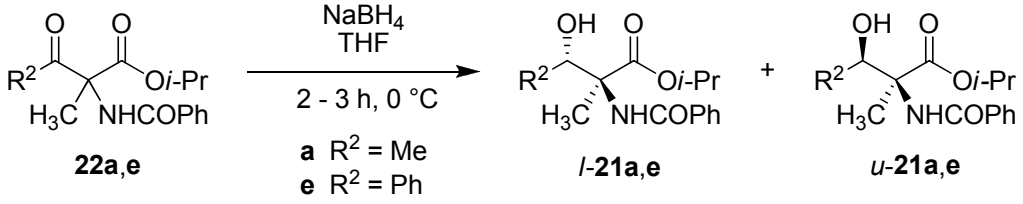
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.06 (d, *J* = 6.3 Hz, 3H, H-C**15**), 1.15 (d, *J* = 6.3 Hz, 3H, H-C**16**), 1.95 (s, 3H, H-C**7**), 5.12 (septet, *J* = 6.3 Hz, 1H, H-C**14**), 7.34 - 7.49 (m, 6H, H-C**1**, H-C**2**, H-C**11**, H-C**12**), 7.71 - 7.73 (m, 2H, H-C**3**), 7.93 - 7.95 (m, 3H, H-C**10**, NH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 21.2, 21.3, 22.0 (C**7**, C**15**, C**16**), 66.8, 71.0 (C**6**, C**14**), 127.0, 128.4, 128.56, 128.58, 131.8, 133.0, 133.6, 134.4 (aromatic C), 165.4 (C**5**), 170.3 (C**13**), 192.1 (C**8**).

MS (FAB): *m/z* = 341 (MH^+).

Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.71; H, 5.97; N, 3.92.

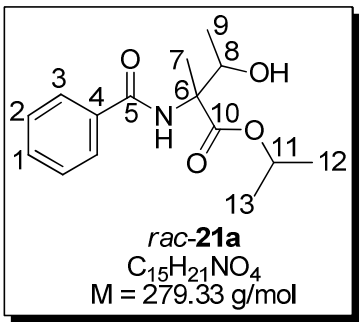
7.2.1.6 Standard operating procedure 6 (SOP6): Reduction to protected β-hydroxy-α-amino acids (21)



Scheme 88. Reduction to protected β-hydroxy-α-amino acids (only one enantiomer shown)

The protected β-keto-α-amino acid (**22a** or **22e**, 0.30 - 0.36 mmol) was dissolved in absolute THF (5 mL) and cooled in an ice-bath. After adding sodium borohydride (0.5 eq.) the mixture was stirred at ice-bath temperature for two to three hours. Subsequently hydrochloric acid (1 M) was added until no further hydrogen evolved. After addition of water the reaction mixture was extracted with MTBE (3 x 10 mL). Removal of the solvent furnished a crude product of the resulting racemic diastereomers, which were separated by column chromatography (ethyl acetate - cyclohexane, 1:3).

7.2.1.6.1 Isopropyl *rac*-2-benzoylamino-3-hydroxy-2-methyl-butyrate (*rac-l*-21a, *rac-u*-21a)



rac-**21a**
$C_{15}H_{21}NO_4$
M = 279.33 g/mol

Isoprpyl *rac*-2-benzoylamino-2-methyl-3-oxo-butyrate (*rac*-**22a**, 100 mg, 0.36 mmol) was dissolved in absolute THF (5 mL) and cooled in an ice-bath. After adding sodium borohydride (7.2 mg, 0.18 mmol) the mixture was stirred at ice-bath temperature for two hours. Subsequently work up was done according to SOP6 leading to both diastereomers as white solids. The diasteriomeric ratio was 73:27.

rac-l-**21a**:

Yield: 60 mg (60%), Mp 70 - 71 °C; R_f = 0.16 (ethyl acetate - cyclohexane, 1:3).

HPLC (Chiralcel OD column; hexanes - *i*-PrOH, 97:3; 1.0 mL/min): t_R = 9.9 (*R,R*), 12.5 (*S,S*) min.

IR (powder film): 3516, 3242, 2984, 1706, 1633 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.07 (d, *J* = 6.5 Hz, 3H, H-C**9**), 1.25 - 1.29 (m, 6H, H-C**12**, H-C**13**), 1.69 (s, 3H, H-C**7**), 4.14 - 4.22 (m, 1H, H-C**11**), 5.09 (septet, *J* = 6.4 Hz, 1H, H-C**8**), 5.49 (d, *J* = 10.1 Hz, 1H, OH), 7.41 - 7.45 (m, 2H, H-C**2**), 7.49 - 7.53 (m, 1H, H-C**1**), 7.61 (br s, 1H, NH), 7.79 - 7.81 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 18.8, 20.1 (C**7**, C**9**), 21.4 (C**12**, C**13**), 65.9 (C**6**), 70.5, 71.2 (C**8**, C**11**), 127.2, 128.7 (C**2**, C**3**), 132.1 (C**1**), 133.9 (C**4**), 168.0 (C**5**), 173.6 (C**10**).

MS (FAB): *m/z* = 280 (MH^+).

Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01; Found: C, 64.14; H, 7.58; N, 4.93.

rac-u-**21a**:

Yield: 16 mg (16%), Mp 111 - 112 °C; R_f = 0.06 (ethyl acetate - cyclohexane, 1:3).

IR (powder film): 3412, 3331, 2976, 1733, 1640 cm^{-1}.

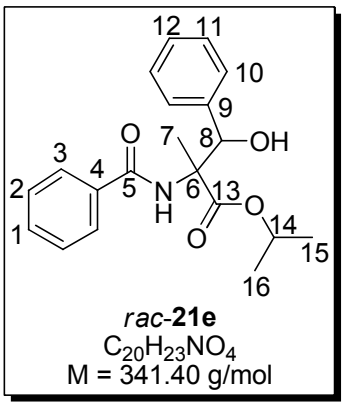
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.20 - 1.26 (m, 9H, H-C**12,** H-C**13**, H-C**9**), 1.57 (s, 3H, H-C**7**), 3.71 (br s, 1H, OH), 4.13 - 4.19 (m, 1H, H-C**11**), 5.09 (septet, *J* = 6.0 Hz, 1H, H-C**8**), 6.86 (br s, 1H, NH), 7.38 - 7.42 (m, 2H, H-C**2**), 7.46 - 7.50 (m, 1H, H-C**1**), 7.74 - 7.77 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 17.8, 18.5 (C**7**, C**9**), 21.56, 21.62 (C**12**, C**13**), 64.6 (C**6**), 69.6, 71.3 (C**8**, C**11**), 127.1, 128.6 (C**2**, C**3**), 131.7 (C**1**), 134.5 (C**4**), 167.9 (C**5**), 172.2 (C**10**).

MS (FAB): *m/z* = 280 (MH^+).

Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01; Found: C, 64.13; H, 7.61; N, 4.87.

7.2.1.6.2 Isopropyl *rac*-2-benzoylamino-3-hydroxy-2-methyl-3-phenylpropionate (*rac-l*-21e, *rac-u*-21e)



rac-**21e**
$C_{20}H_{23}NO_4$
M = 341.40 g/mol

Isopropyl *rac*-2-benzoylamino-2-methyl-3-oxo-3-phenylpropionate (*rac*-**22e**, 100 mg, 0.30 mmol) was dissolved in absolute THF (5 mL) and cooled in an ice-bath. After adding sodium borohydride (6.0 mg, 0.15 mmol) the mixture was stirred at ice-bath temperature for three hours. Subsequently work up was done according to SOP6 leading to both diastereomers as white solids. The diasteriomeric ratio was 80:20.

rac-l-**21e**:

Yield: 60 mg (60%), R_f = 0.36 (ethyl acetate - cyclohexane, 1:3).

HPLC (Chiralpak AD-H-column; hexanes - *i*-PrOH, 95:5; 1.0 mL/min): t_R = 16.7, 18.9 min.

IR (powder film): 3501, 3246, 3057, 2984, 1710, 1629 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.33 (d, *J* = 6.3 Hz, 3H, H-**15**) 1.40 (d, *J* = 6.3 Hz, 3H, H-C**16**), 1.84 (s, 3H, H-C**7**), 5.15 (septet, *J* = 6.3 Hz, 1H, H-C**14**), 5.33 (d, *J* = 8.9 Hz, 1H, H-C**8**), 6.47 (d, *J* = 8.9 Hz, 1H, OH), 7.17 - 7.26 (m, 6H, H-C**10**, H-C**11**, H-C**12**, NH), 7.38 - 7.52 (m, 3H, H-C**1**, H-C**2**), 7.65 - 7.68 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 20.0 (C**7**), 21.50, 21.55 (C**15**, C**16**), 65.9 (C**6**), 70.8 (C**14**), 77.2 (C**8**), 126.3, 127.0, 127.7, 128.0 128.7, 132.0, 133.8, 140.3 (ar. C), 168.7 (C**5**), 173.1(C**13**).

MS (MALDI): *m/z* = 342 (MH^+).

Anal. Calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10; Found: C, 70.01; H, 6.62; N, 3.92.

rac-*u*-**21e**:

Yield: 15 mg (15%), R_f = 0.19 (ethyl acetate - cyclohexane, 1:3).

HPLC (Chiralpak AD-H-column; hexanes - *i*-PrOH, 95:5; 1.0 mL/min): t_R = 18.3, 28.1 min.

IR (powder film): 3420, 3327, 3069, 2984, 1729, 1644 cm^{-1}.

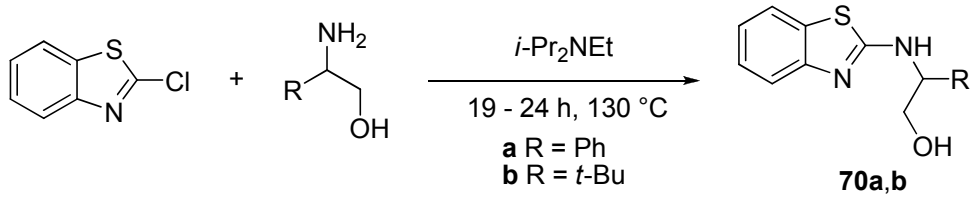
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.15 (d, *J* = 6.3 Hz, 3H, H-**15**) 1.25 (d, *J* = 6.3 Hz, 3H, H-C**15**), 1.54 (s, 3H, H-C**7**), 4.86 (d, *J* = 5.0 Hz, 1H, H-C**8**), 5.04 (septet, *J* = 6.3 Hz, 1H, H-C**14**), 5.19 (d, *J* = 5.0 Hz, 1H, OH), 6.69 (br s, 1H, NH), 7.27 - 7.33 (m, 5H, H-C**10**, C**11**, C**12**), 7.40 - 7.44 (m, 2H, H-C**2**), 7.48 - 7.52 (m, 1H, H-C**1**), 7.72 - 7.74 (m, 2H, H-C**3**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 20.0 (C**7**), 21.5, 21.7 (C**15**, C**16**), 65.2 (C**6**), 69.9 (C**14**), 78.2 (C**8**), 127.0, 127.4, 128.1, 128.2, 128.6, 131.8, 134.2, 139.1 (ar. C), 168.5 (C**5**), 171.6 (C**13**).

MS (MALDI): *m/z* = 342 (MH^+).

Anal. Calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10; Found: C, 69.29; H, 6.54; N, 4.05.

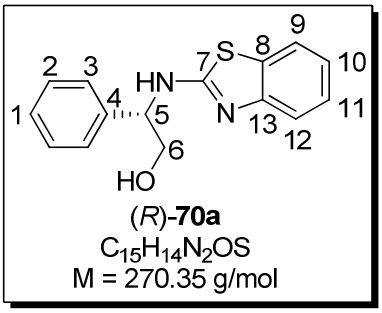
7.2.1.7 Standard operating procedure 7 (SOP7): Synthesis of *N*-(thiazolyl-2)-2-hydroxyethylamines (70)



Scheme 89. Synthesis of *N*-(thiazolyl-2)-2-hydroxyethylamines (**70**)

A pressure tube charged with 2-chlorobenzothiazole (10.1 mmol), an enantiomerically pure amino alcohol (10.4 mmol), *N*,*N*-diisopropylethylamine (15 mmol), and a stir bar was flushed with nitrogen several times, stoppered and heated at 130 °C for 19 to 24 hours. After cooling the tube to room temperature, the reaction mixture was treated with dichloromethane and left at room temperature to dissolve over night. The diluted reaction mixture was applied directly to a chromatographic column and eluted with isopropanol in dichloromethane.

7.2.1.7.1 (*R*)-*N*-(Thiazolyl-2)-2-hydroxy-1-phenylethylamine ((*R*)-70a)



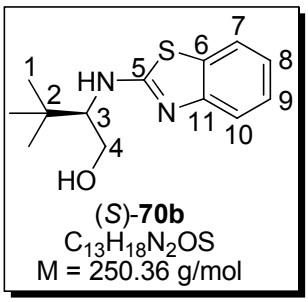
A pressure tube charged with 2-chlorobenzothiazole (1.32 mL, 1.72 g, 10.1 mmol), *R*-(+)-phenylglycinol (1.42 g, 10.4 mmo), *N*,*N*-diisopropylethylamine (2.7 mL, 15 mmol) and a stir bar was flushed with nitrogen several times, stoppered and heated at 130 °C for 19 hours. After cooling the tube to room temperature, a red colored viscous reaction mixture occurred. Work up was done according to SOP7 (solvent for column chromatography: dichloromethane – *i*-PrOH, 96:4), yielding a white solid.

Yield: 1.40 g (51%).

^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 3.78 - 3.88 (m, 2H, H-C**6**), 5.00 (dd, *J* = 7.1 Hz, *J* = 5.2 Hz 1H, H-C**5**), 7.03 (t, *J* = 7.3 Hz, 1H, H-C_{ar}), 7.19 - 7.45 (m, 7H, ar. H-C_{ar}), 7.55 (d, *J* = 7.9 Hz, 1H, H-C_{ar}).

Spectroscopic data are in accordance with literature (see reference [60a]).

7.2.1.7.2 *(S)-N*-(Thiazolyl-2)-2-hydroxy-1-*tert*-butylethylamine ((*S*)-70b)



(*S*)-**70b**
$C_{13}H_{18}N_2OS$
M = 250.36 g/mol

A pressure tube charged with 2-chlorobenzothiazole (1.32 mL, 1.72 g, 10.1 mmol), *(S)-tert*-leucinol (1.23 g, 10.4 mmol), *N*,*N*-diisopropylethylamine (2.7 mL, 15 mmol) and a stir bar was flushed with nitrogen several times, stoppered and heated at 130 °C for 24 hours. After cooling the tube to room temperature, the viscous reaction mixture was worked up according to SOP7 ((solvent for column chromatography: dichloromethane – *i*-PrOH, 97.5:2.5)) yielding a white solid.

Yield: 1.48 g (58%); Mp 155 - 157 °C; $[\alpha]_D$ = -34.8° (c = 1.0, MeOH).

IR (powder film): 3223, 3196, 2964, 2891, 1722, 1668, 1644, 1598, 1522 cm^{-1}.

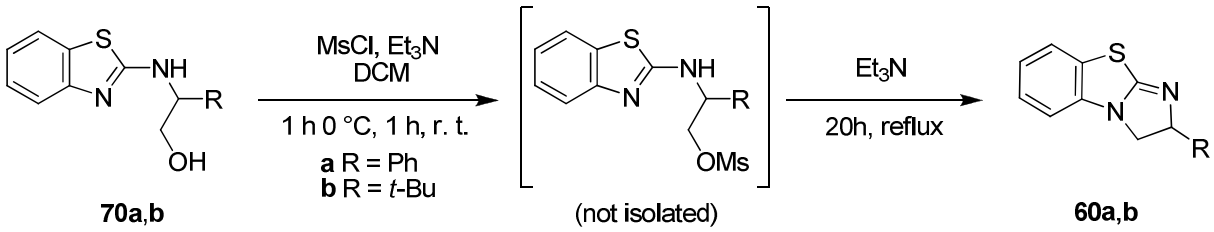
^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 0.97 (s, 9H, H-C**1**), 3.52 (dd, *J* = 8.1Hz, *J* = 11.3Hz, 1H, H-C**3**), 3.77 - 3.78 (m, 1H, H-C**3**), 3.85 (dd, *J* = 11.3Hz, *J* = 3.5Hz, 1H, H-C**4**), 6.95 - 7.00 (m, 1H, H-C**8**), 7.14 - 7.19 (m, 1H, H-C**9**), 7.32 - 7.34 (m, 1H, H-C**7**), 7.48 - 7.50 (m, 1H, H-C**10**).

^{13}C-NMR (100 MHz, CD_3OD): δ [ppm] = 27.4 (C**1**), 35.7 (C**2**), 63.0 (C**4**), 67.2 (C**3**), 118.8, 121.7, 122.5, 126.9 (C**7**, C**8**, C**9**, C**10**), 131.2 (C**6**), 153.3 (C**11**), 170.9 (C**5**).

MS (FAB): *m/z* = 251 (MH^+), 233 (MH^+-H_2O).

Anal. Calcd for $C_{13}H_{18}N_2OS$: C, 62.37; H, 7.25; N, 11.19; S, 12.81. Found: C, 62.24; H, 7.31; N, 11.09; S, 12.35.

7.2.1.8 Standard operating procedure 8 (SOP8): Synthesisis of Birman-type catalysts (60)

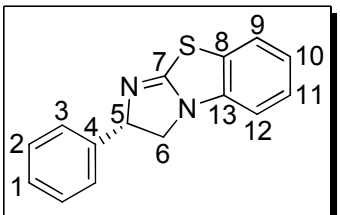


70a,**b** (not isolated) **60a**,**b**

Scheme 90. Synthesis of Birman-type catalysts **60**

The *N*-(thiazolyl-2)-2-hydroxyethylamine (**70**, 2.5 mmol) was suspended in anhydrous dichloromethane (25 mL) and cooled in an ice-bath. Triethylamine (7.5 mmol) and mesyl chloride (3.75 mmol) were added. The mixture was stirred for one hour at 0 °C and for one hour at room temperature. Methanol (0.15 mL) to quench the excess mesyl chloride and additional triethylamine (3.5 mL) were added. The mixture was heated to reflux for 20 hours and then cooled to room temperature. The cooled suspension was washed with a small amount of water, dried over sodium sulfate and evaporated *in vacuo*. The crude product was purified by chromatography (hexanes - *i*-PrOH 95:5, 1% Et_3N) to give a white solid as crude product. The compound was recrystallised from MTBE / hexanes, yielding a white solid.

7.2.1.8.1 (*R*)-2,3-Dihydro-2-phenyl-imidazo[2,1-*b*]benzothiazole ((*R*)-60a)



(*R*)-**60a**
$C_{15}H_{12}N_2S$
M = 252.33 g/mol

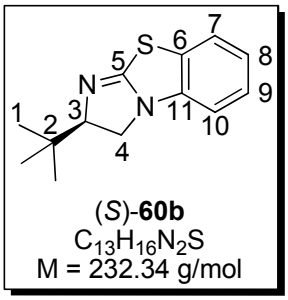
A suspension of (*R*)-N-(Thiazolyl-2)-2-hydroxy-1-phenylethylamine ((*R*)-**70a**, 0.68 g, 2.5 mmol) in anhydrous dichloromethane (25 mL) was cooled to 0 °C under nitrogen-atmosphere and treated with triethylamine (1.05 mL, 7.5 mmol) followed by mesyl chloride (0.29 mL, 3.75 mmol). The mixture was stirred for one hour at 0 °C and then allowed to warm to room temperature for one hour. Methanol (0.15 mL) was added to quench the excess mesyl chloride. Triethylamine (3.5 mL) was added and the mixture was refluxed for 20 hours. The work up was done according to SOP8, yielding a white solid.

Yield: 297 mg (47%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 3.70 (dd, *J* = 8.1 Hz, *J* = 8.9 Hz, 1H, H-C**5**,), 4.26 (dd, *J* = 8.9 Hz, *J* = 10.2 Hz, 1H, H-C**6**), 5.64 (dd, *J* = 8.2 Hz, *J* = 10.2 Hz, 1H, H-C**6**), 6.65 (dd, *J* = 7.9 Hz, *J* = 0.7 Hz, 1H, H-C**12**), 6.95 (td, *J* = 7.7 Hz, *J* = 1.2 Hz, 1H, H-C**10**), 7.16 (td, *J* = 7.7 Hz, *J* = 1.2 Hz, 1H, H-C**11**), 7.18 - 7.44 (m, 6H, H-C**9**, H-C**1**, H-C**2**, H-C**3**).

Spectroscopic data are in accordance with literature (see reference [60a]).

7.2.1.8.2 *(S)*-2,3-Dihydro-2-*tert*-butyl-imidazo[2,1-*b*]benzothiazole ((*S*)-60b)



(*S*)-**60b**
$C_{13}H_{16}N_2S$
M = 232.34 g/mol

A suspension of *(S)-N*-(thiazolyl-2)-2-hydroxy-1-*tert*-butylethyl-amine ((*S*)-**70b**, 0.63 g, 2.5 mmol) in anhydrous dichloromethane (25 mL) was cooled to 0 °C under nitrogen-atmosphere and treated with triethylamine (1.05 mL, 7.5 mmol) followed by mesyl chloride (0.29 mL, 3.75 mmol). The mixture was stirred at 0 °C for one hour and then allowed to warm to room temperature. Methanol (0.15 mL)

was added to quench the excess amount of mesyl chloride. Triethylamine (3.5 mL) was added and the mixture was refluxed for 20 hours. The cooled mixture was worked up according to SOP8, yielding a white solid.

Yield: 280 mg (48%); Mp 75 - 76 °C; $[\alpha]_D$ = -154.9° (c = 1.0, MeOH, 20 °C).

IR (powder film): 2957, 2903, 2864, 1606, 1475 cm^{-1}.

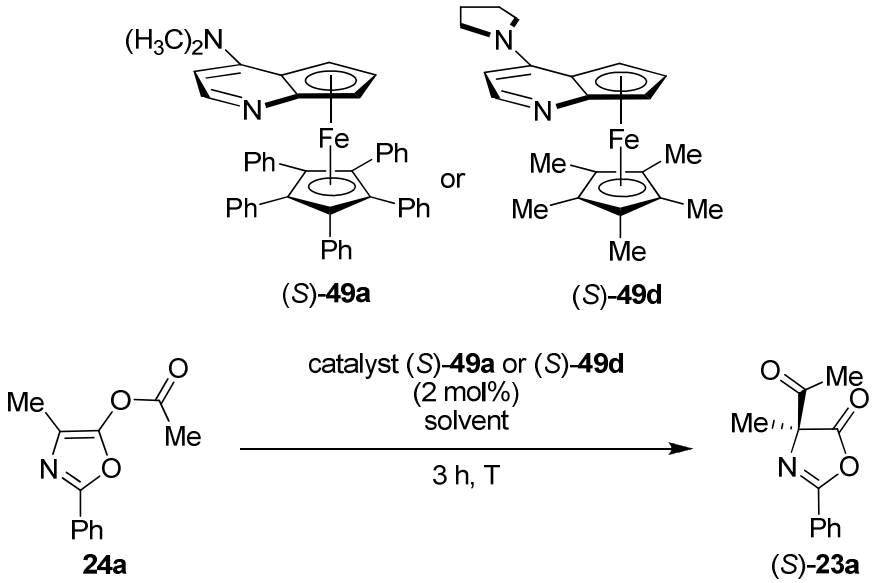
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.95 (s, 9H, H-C**1**), 3.52 - 3.56 (m, 1H, H-C**3**), 3.73 - 3.78 (m, 1H, H-C**4**), 4.29 - 4.34 (m, 1H, H-C**4**), 6.63 (d, *J* = 7.8 Hz, 1H, H-C**10**), 6.90 (dd, *J* = 7.7 Hz, *J* = 7.7 Hz, 1H, H-C**8**), 7.14 (dd, *J* = 7.7 Hz, *J* = 7.7 Hz, 1H, H-C**9**), 7.22 (d, *J* = 7.7 Hz, 1H, H-C**7**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 25.8 (C**1**), 34.3 (C**2**), 45.4 (C**4**), 82.7 (C**3**), 108.1 (C**10**), 121.1, 123.0, 126.4, (C**7**, C**8**, C**9**), 127.2 (C**6**), 137.3 (C**11**), 165.0 (C**5**).

MS (FAB): *m/z* = 233 (MH^+), 175 (MH^+-*t*-Bu).

Anal. Calcd for $C_{13}H_{16}N_2S$: C, 67.20; H, 6.94; N, 12.06; S, 13.80. Found: C, 66.91; H, 7.05; N, 11.96; S, 13.55.

7.2.1.9 Standard operating procedure 9 (SOP9): Enantioselective Steglich rearrangement of 24a using Fu-type catalysts (49a, 49d) (exemplified for reaction in Table 23, entry 1)



Scheme 91. Steglich rearrangement of **24a** applying Fu-type catalysts

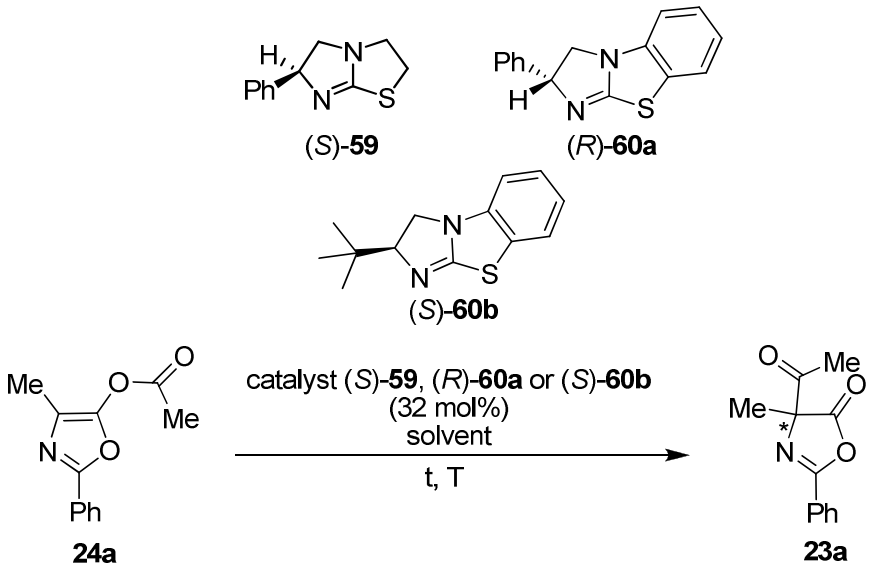
5-Acetyloxy-4-methyl-2-phenyl-oxazole (**24a**, 50 mg, 0.23 mmol) and (*S*)-4-dimethylamino-pyridinyl-(pentaphenyl-cyclopenta-dienyl)iron ((*S*)-**49a**, 4 mg, 0.005 mmol) were dissolved in dichloromethane (1 mL) and stirred at room temperature for three hours. Subsequently the solvent was removed to give 4-acetyl-4-methyl-2-phenyl-oxazol-5-one (**23a,** 52 mg) as an oily crude product. The conversion (84%) was determined by ^{1}H-NMR spectroscopy. The enantioselectivity (25% ee) was determined by chiral HPLC chromatography after derivatisation to isopropyl 2-benzoylamino-2-methyl-3-oxo-butyrate (**22a**), which was done as follows: the crude product was dissolved in isopropanol (5 mL) and DMAP (5 mg, 4.1 mmol) was added. After 16 hours of stirring, the solvent was evaporated *in vacuo*. The spectroscopic properties are in accordance with those of the racemic products *rac*-**23a** and *rac*-**22a**. Reactions were stirred until complete consumption of substrate **24a**, according to ^{1}H-NMR spectroscopy.

Table 23: Steglich rearrangement of **24a** applying Fu-type catalysts

entry	catalyst	solvent	T	conversion[a] (%)	ee (%)
1	(*S*)-**49a**	DCM	r. t.	84	25
2	(*S*)-**49a**	toluene	r. t.	80	23
3	(*S*)-**49a**	MTBE	r. t.	78	24
4	(*S*)-**49a**	THF	0 °C	71	18
5	(*S*)-**49a**	TAA	r. t.	93	30
6	(*S*)-**49d**	DCM	r. t.	95	41
7	(*S*)-**49d**	toluene	r. t.	88	30
8	(*S*)-**49d**	MTBE	r. t.	92	20
9	(*S*)-**49d**	THF	r. t.	94	29
10	(*S*)-**49d**	TAA	r. t.	94	50

[a] conversion means product related conversion to **23a**, consumption of substrate **24a** was quantitative for all entries

7.2.1.10 Enantioselective Steglich rearrangement of 24a using Birman-type catalysts (59, 60a, 60b) (exemplified for reaction in Table 24, entry 1)



Scheme 92. Steglich rearrangement of **24a** applying Birman-type catalysts

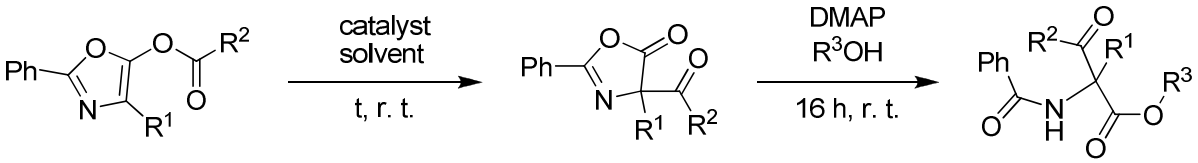
5-Acetyloxy-4-methyl-2-phenyl-oxazole (**24a**, 50 mg, 0.23 mmol) and (*S*)-tetramisole ((*S*)-**59**, 15 mg, 0.07 mmol) were dissolved in dichloromethane (1 mL) and stirred at room temperature for 68 hours. Subsequently the solvent was removed to give 4-acetyl-4-methyl-2-phenyl-oxazol-5-one **23a** (63 mg) as an oily crude product. The conversion (82%) was determined by ^{1}H-NMR spectroscopy. The enantioselectivity (59% ee) was determined by chiral HPLC chromatography after derivatisation to isopropyl 2-benzoylamino-2-methyl-3-oxo-butyrate (**22a**), which was done as follows: the crude product was dissolved in isopropanol (5 mL) and DMAP (5 mg, 4.1 mmol) was added. After 16 hours of stirring, the solvent was evaporated in *vacuo*. The spectroscopic properties are in accordance with those of the racemic products *rac*-**23a** and *rac*-**22a**. Reactions were stirred until complete consumption of substrate **24a** (except Table 24, entry 8 - 10). The absolute configuration was (*S*) for entries 1-4 and (*R*) for entries 5-7.

Table 24: Steglich rearrangement of **24a** applying Birman-type catalysts

entry	catalyst	solvent	T	t (h)	conversion[a] (%)	ee (%)
1	(*S*)-**59**	DCM	r.t.	68	82	59
2	(*S*)-**59**	$CDCl_3$	r.t.	48	80	63
3	(*S*)-**59**	DCM	40 °C	24	84	52
4	(*S*)-**59**	TAA	r.t.	24	74	60
5	(*R*)-**60a**	DCM	r.t.	96	69	50
6	(*R*)-**60a**	$CDCl_3$	r.t.	48	79	61
7	(*R*)-**60a**	TAA	r.t.	24	84	52
8	(*S*)-**60b**	DCM	r.t.	168	35	n.d.
9	(*S*)-**60b**	TAA	r.t.	168	21	n.d.
10	(*S*)-**60b**	$CDCl_3$	r.t.	168	29	n.d.

[a] conversion means product related conversion to **23a**, consumption of substrate **24a** was quantitative for entries 1-7

7.2.1.11 Standard operating procedure 11 (SOP11): Enantioselective Steglich rearrangement of substrates 24b-e



24b-e **23b-e** **22b-e**

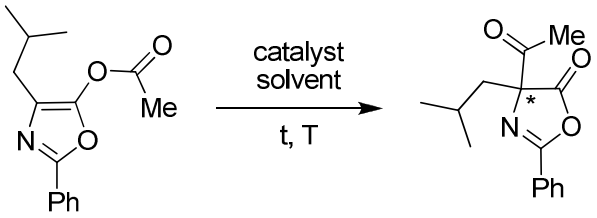
b R^1 = *i*-Pr R^2 = Me R^3 = *i*-Pr
c R^1 = *i*-Bu R^2 = Me R^3 = Me
d R^1 = Me R^2 = *n*-Pr R^3 = *i*-Pr
e R^1 = Me R^2 = Ph R^3 = *i*-Pr

Scheme 93. Enantioselective Steglich rearrangement of substrates **24b-e**

The 5-acyloxazole (**24b-e**, 0.18 mmol - 0.4 mmol) was dissolved in a solvent (1 - 2 mL). A Fu-type catalyst (**49a** or **49d**) or a Birman-type catalyst (**59** or **60a**, 32 mol%) was added. The mixture was shaken until complete consumption of the substrate (if not otherwise stated). Thereafter the solvent was evaporated *in vacuo* and the consumption and the conversion was determined by ^{1}H-NMR spectroscopy. The enantioselectivity was determined by chiral HPLC chromatography after derivatisation to protected β-keto-α-aminoacids (**22b-e**), which was done as follows: the crude product was dissolved in isopropanol (2 mL) and DMAP (0.04 - 0.08 mmol, 20 mol%) was added (in case of substrate **24c** dichloromethane (2 - 4 mL) and methanol (100 - 200 µL) was added instead of isopropanol). After

16 hours of stirring, the solvent was evaporated *in vacuo*. The spectroscopic properties are in accordance with those of the racemic products *rac*-**23b**-**e** and *rac*-**22b**-**e**. All results are shown in tables Table 25 -Table 27. A detailed working procedure for one result of each substrate is given below as representative examples (chapter 7.2.1.11.1 - 7.2.1.11.4).

Table 25: Enantioselective Steglich rearrangement of **24c**

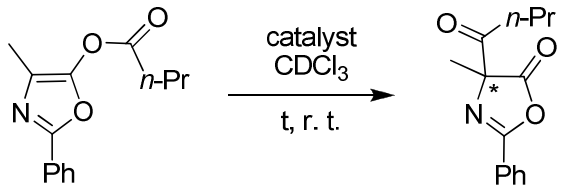


24c **23c**

entry	catalyst	solvent	t	T	conversion[a] (%)	% ee
1	(*S*)-**49d** (2 mol%)	$CDCl_3$	6 h	r. t.	n. d.	31
2	*(S)*-**49d** (4 mol%)	TAA	6 h	0 °C	72	30
3	(*S*)-**59** (32 mol%)	$CDCl_3$	48 h	r. t.	61	29
4	(*S*)-**59** (32 mol%)	TAA	75 h	r. t.	59	85
5	(*R*)-**60a** (32 mol%)	$CDCl_3$	72 h	r. t.	n. d.	21

[a] conversion means product related conversion to **23c**, consumption of substrate **24c** was quantitative for all entries

Table 26: Enantioselective Steglich rearrangement of **24d**

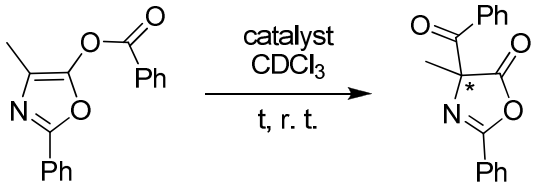


24d **23d**

entry	catalyst	t	conversion[a] (%)	% ee
1[b]	(*S*-)**49d** (2 mol%)	5 d	18	n. d.
2	*(S)*-**49d** (20 mol%)	6 h	73	25
3	*(S)*-**49d** (20 mol%)	24 h	80	24
4	(*S*)-**49a** (20 mol%)	2 d	37	18
5[c]	(*S*)-**59** (32 mol%)	3 d	38	n. d.

[a] conversion means product related conversion to **23d**, consumption of substrate **24d** was quantitative for entries 2-4, [b] consumption of substrate **24d** 61%, [c] consumption of substrate **24d** 71%.

Table 27: Enantioselective Steglich rearrangement of **24e**



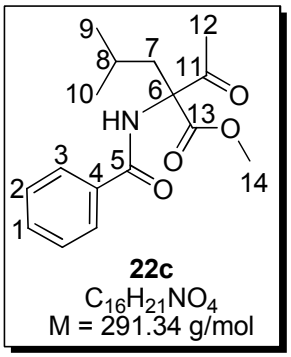
entry	catalyst	t	consumption (%)	conversion[a] (%)	% ee
1	*(S)*-**49d** (10 mol%)	7 d	10	5	n. d.
2	*(S)*-**49d** (20 mol%)	7 d	> 95	48	45
3	(*S*)-**49a** (20 mol%)	7 d	72	13	n. d.
4	(*S*)-**59** (32 mol%)	8 d	67	9	n. d.

[a] conversion means product related conversion to **23e**

7.2.1.11.1 Enantioselective Steglich rearrangement of 24b

5-Acetyloxy-4-isopropyl-2-phenyl-oxazole (**24b**, 50 mg, 0.20 mmol) and (*S*)-4-pyrrolidinylaminopyridinyl-(pentamethyl-cyclo-pentadienyl)iron ((*S*)-**49d**, 15 mg, 0.04 mmol) were dissolved in deuterochloroform (1 mL). After shaking this mixture for seven days, conversion was determined by ^{1}H-NMR-spectroscopy. As conversion was lower than 5%, no further reaction or purification was carried out.

7.2.1.11.2 Enantioselective Steglich rearrangement of substrate 24c: Synthesis of methyl 2-benzoylamino-2-isobutyl-3-oxo-butyrate (22c)

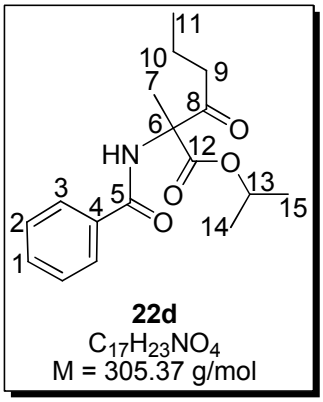


5-Acetyloxy-4-isobutyl-2-phenyl-oxazole (**24c**, 104 mg, 0.40 mmol) and (*S*)-tetramisole ((*S*)-**59**, 26 mg, 0.13 mmol) were dissolved in *tert*-amyl alcohol (2 mL). After stirring this mixture for 75 hours, solvent was removed to give crude **23c**. Conversion was determined by ^{1}H-NMR-spectroscopy. The mixture was dissolved in dichloromethane (4 mL) and DMAP (10 mg, 0.08 mmol) and methanol (200 µL) were added. After additional 16 hours of stirring, solvent was evaporated to give crude **22c**, which was purified by column chromatography (ethyl acetate - cyclohexane, 1:4). The spectroscopic properties are in accordance with those of the racemic compound *rac*-**22c**.

Yield: 21 mg (17%).

Enantiomeric excess: 85% ee.

7.2.1.11.3 Enantioselective Steglich rearrangement of 24d: Synthesis of isopropyl 2-benzoyl-amino-2-methyl-3-oxo-hexanoate (22d)



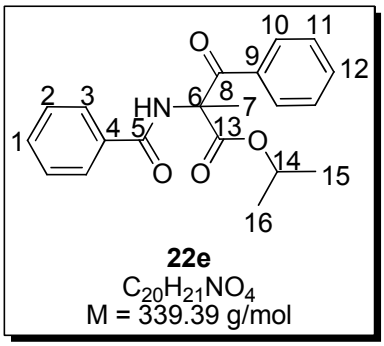
22d
$C_{17}H_{23}NO_4$
M = 305.37 g/mol

4-Methyl-2-phenyloxazol-5-yl butyrate (**24d**, 50 mg, 0.20 mmol) and (*S*)-4-pyrrolidinylaminopyridinyl-(pentamethyl-cyclopenta-dienyl)iron ((*S*)-**49d**, 15 mg, 0.04 mmol) were dissolved in deuterochloroform (1 mL). After shaking this mixture for 24 hours, conversion was determined by ^{1}H-NMR-spectroscopy. The mixture was evaporated in *vacuo*, dissolved in isopropanol (5 mL) and DMAP (10 mg, 0.08 mmol) was added. After additional 16 hours of stirring, solvent was evaporated to give crude **22d**, which was purified by column chromatography (ethyl acetate - cyclohexane, 1:3). The spectroscopic properties are in accordance with those of the racemic compound *rac*-**22d**.

Yield: 33 mg (54%).

Enantiomeric excess: 24% ee.

7.2.1.11.4 Enantioselective Steglich rearrangement of 24e: Synthesis of isopropyl 2-benzoylamino-2-methyl-3-oxo-3-phenyl-propanoate (22e)



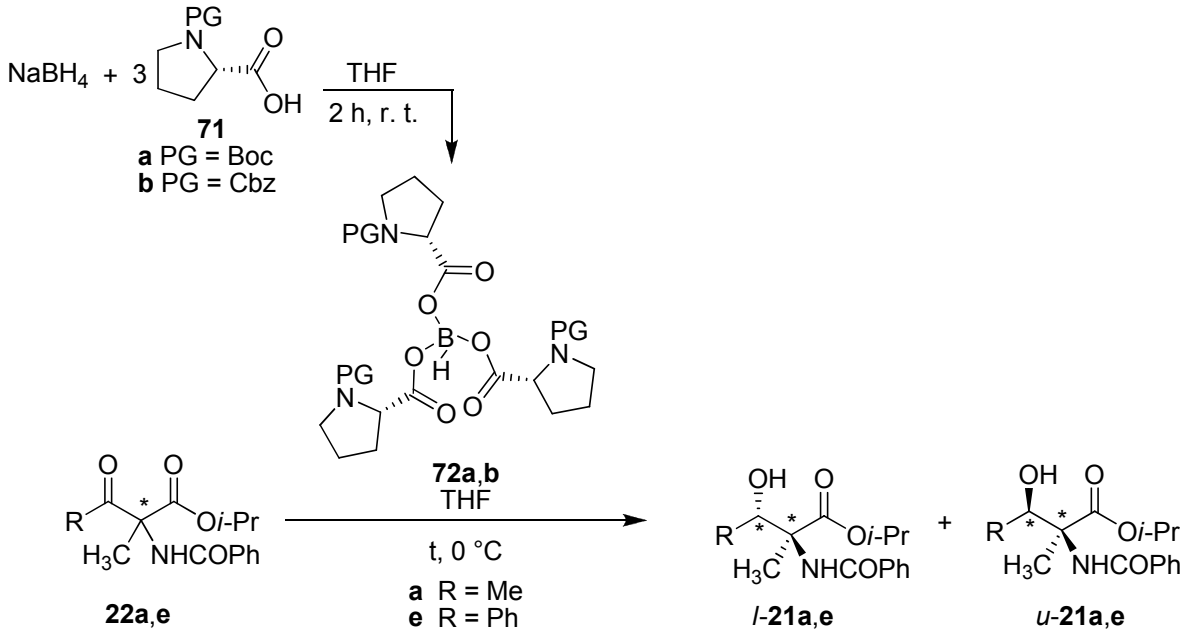
22e
$C_{20}H_{21}NO_4$
M = 339.39 g/mol

4-Methyl-2-phenyloxazol-5-yl benzoate (**24e**, 50 mg, 0.18 mmol) and (*S*)-4-pyrrolidinylaminopyridinyl-(penta-methylcyclopenta-dienyl)iron ((*S*)-**49d**, 14 mg, 0.036 mmol) were dissolved in deuterochloroform (1 mL). After shaking this mixture for seven days, conversion was determined by ^{1}H-NMR-spectroscopy. The mixture was evaporated in *vacuo*, dissolved in isopropanol (5 mL) and DMAP (10 mg, 0.08 mmol) was added. After additional 16 hours of stirring, solvent was evaporated to give crude **22e**, which was purified by column chromatography (cyclohexane - ethyl acetate, 3:1). The spectroscopic properties are in accordance with those of the racemic compound *rac*-**22e**.

Yield: 18 mg (29%).

Enantiomeric excess: 45% ee.

7.2.1.12 Standard operating procedure 12 (SOP12): Stereoselective reduction with *N*-protected proline (71) as chiral auxiliary



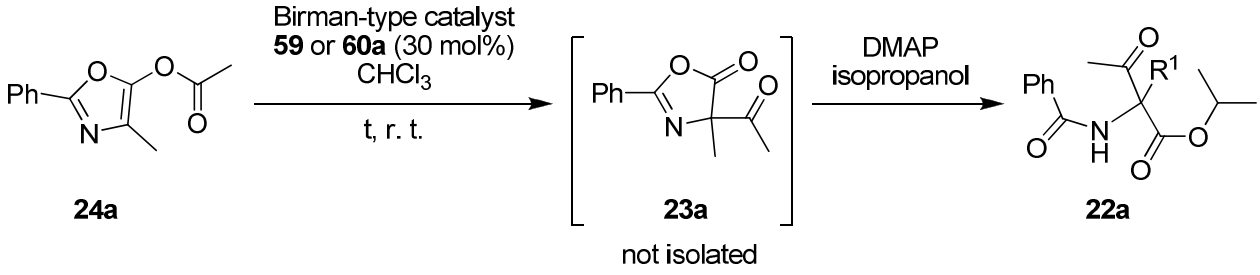
Scheme 94. Stereoselective reductiuon with *N*-protected proline (**71**) as chiral auxilliary

Sodium borohydride (0.48 - 0.96 mmol, 1.3 - 2.6 eq.) was suspended in absolute THF (2 - 4 mL) and cooled in an ice-bath. Enantiomerically pure *N*-protected proline (**71**, 1.44 - 2.88 mmol, 3.9 - 7.8 eq.) in THF (6 - 12 mL) was added through an additional funnel. After two hours of stirring at room temperature, the mixture was cooled in an ice-bath and isopropyl 2-benzoylamino-2-methyl-3-oxo-butyrate (**22a**, 0.36 mmol) and isopropyl 2-benzoylamino-2-methyl-3-oxo-3-phenylpropionate (**22e**, 0.30 mmol), respectively, in THF (6 mL) was added drop wise. After 20 to 70 hours of stirring at ice-bath temperature the excess of sodium borohydride was quenched by adding hydrochloric acid (2 M) and most of THF was evaporated. The resulting aqueous solution was basified with sodium bicarbonate and extracted with ethyl acetate (3 x 50 mL). After drying of the unified organic phases over magnesium sulfate, solvent was removed to give a crude mixture of starting material **22a** and **22e**, respectively and both diastereomers of product **21a** and **21e**, respectively. Diastereomerical ratio and conversion were determined by ^{1}H-NMR-spectroscopy. Enantiomeric excess was determined by HPLC-chromatography after column chromatography (cyclohexane - ethyl acetate, 3:1). The spectroscopic properties are in accordance with those of the racemic compounds *rac-l*-**21a**, *rac-u*-**21a**, *rac-l*-**21e** and *rac-u*-**21e**, respectively.

Table 28: Stereoselective reductiuon with *N*-protected proline (**71**) as chiral auxilliary

entry	starting material	chiral auxilliary	t (h)	conversion (%)	d. r.	% ee **22** (config.)	% ee *l*-**21** (config.)	% ee *u*-**21** (config.)
1	*rac*-**22a**	L-**71a** (1.3eq)	20	62	80:20	55 (*S*)	36 (*R*,*R*)	n. d.
2	*rac*-**22a**	L-**71a** (2.6eq)	70	62	80:20	56 (*S*)	40 (*R*,*R*)	31 (*R*,*S*)
3	(*R*)-**22a** (55% ee)	L-**71a** (1.3eq)	20	51	83:17	29 (*R*)	79 (*R*,*R*)	44 (*R*,*S*)
4	*rac*-**22a**	*D*-**71a** (1.3eq)	20	60	80:20	41 (*R*)	49 (*S*,*S*)	6 (*R*,*S*)
5	(*S*)-**22a** (62% ee)	*D*-**71a** (1.3eq)	20	55	88:12	46 (*S*)	79 (*S*,*S*)	53 (*S*,*R*)
6	*rac*-**22a**	L-**71b** (1.3 eq.)	20	30	86:14	28 (*S*)	59 (*R*,*R*)	22 (*S*,*R*)
7	*rac*-**22e**	L-**71a** (1.3eq)	20	38	86:14	n. d.	86	5

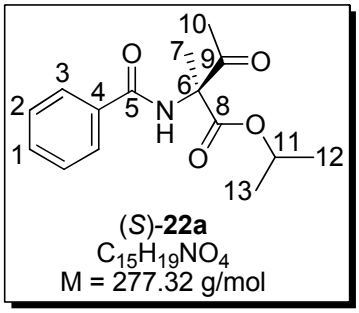
7.2.1.13 Standard operating procedure 13 (SOP13): Steglich rearrangement in an extended preparative scale



Scheme 95. Steglich rearrangement in extended scale

5-Acetyloxy-4-methyl-2-phenyl-oxazole (**24a**, 4.0 mmol) and a Birman-type catalyst ((*S*)-**59** or (*R*)-**60a**, 1.2 mmol) were dissolved in chloroform (17 mL). After stirring this mixture for 70 - 95 hours, isopropanol (21.5 mL) and DMAP (0.36 mmol) were added. After additional 16 hours of stirring, solvent was evaporated to give crude **22a**, which was purified by column chromatography (cyclohexane - ethyl acetate, 3:1).

7.2.1.13.1 Isopropyl (*S*)-2-benzoylamino-2-methyl-3-oxo-butyrate ((*S*)-22a)

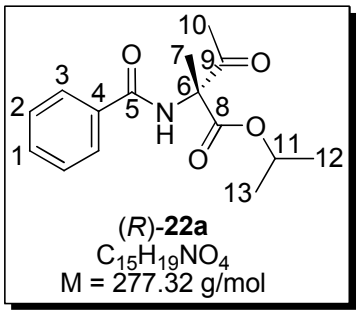


5-Acetyloxy-4-methyl-2-phenyl-oxazole (**24a**, 880 mg, 4.0 mmol) and (*S*)-tetramisole ((*S*)-**59**, 248 mg, 1.2 mmol) were dissolved in chloroform (17 mL). After stirring this mixture for 70 hours, isopropanol (21.5 mL) and DMAP (44 mg, 0.36 mmol) were added. After additional 16 hours of stirring, work up was done according to SOP13. The spectroscopic properties are in accordance with those of the racemic compound *rac*-**22a**.

Yield: 588 mg (53%).

Enantiomeric excess: 71% ee.

7.2.1.13.2 Isopropyl (*R*)-2-benzoylamino-2-methyl-3-oxo-butyrate ((*R*)-22a)

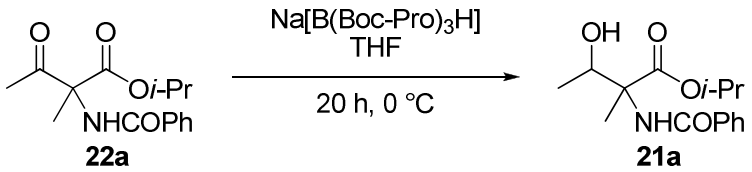


5-Acetyloxy-4-methyl-2-phenyl-oxazole (**24a**, 861 mg, 4.0 mmol) and (*R*)-benzotetramisole ((*R*)-**60a**, 300 mg, 1.2 mmol) were dissolved in chloroform (17 mL). After stirring this mixture for 95 hours, isopropanol (21.5 mL) and DMAP (43 mg, 0.35 mmol) were added. After additional 16 hours of stirring, work up was done according to SOP13. The spectroscopic properties are in accordance with those of the racemic compound *rac*-**22a**.

Yield: 615 mg (56%).

Enantiomeric excess: 70% ee.

7.2.1.14 Standard operating procedure 14 (SOP14): Stereoselective Reduction in an extended preparative scale

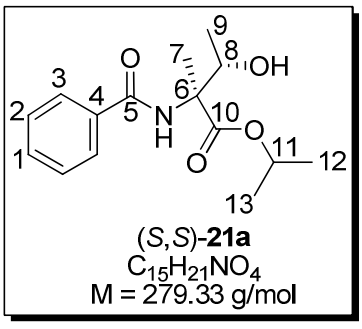


Scheme 96. Stereoselective reduction in an extended preparative scale

Sodium borohydride (2.79 - 2.89 mmol) was suspended in absolute THF (15 mL) and cooled in an ice-bath. Enantiomerically pure *N*-Boc-proline (8.36 - 8.68 mmol) in THF (30 mL) was

added through an additional funnel. After two hours of stirring at room temperature, the mixture was cooled in an ice-bath and enantiomerically enriched isopropyl 2-benzoylamino-2-methyl-3-oxo-butyrate (**22a**, 2.09 - 2.17 mmol, 70 - 71% ee) in THF (30 mL) was added drop wise. After 20 hours of stirring at ice-bath temperature the excess of sodium borohydride was quenched by adding hydrochloric acid (2 M) and most of THF was evaporated. The resulting aqueous solution was basified with sodium bicarbonate and extracted with ethyl acetate (3 x 50 mL). After drying of the unified organic phases over magnesium sulfate, solvent was removed to give a crude mixture of starting material **22a** and both diastereomers of product **21a**. Diastereomerical ratio and conversion were determined by ^{1}H-NMR-spectroscopy. Diastereomerically pure and enantiomerically enriched *l*-**21a** was obtained after column chromatography (cyclohexane - ethyl acetate, 3:1). The spectroscopic properties are in accordance with those of the racemic compound *rac-l*-**21a**.

7.2.1.14.1 Isopropyl (*S,S*)-2-benzoylamino-3-hydroxy-2-methyl-butyrate ((*S,S*)-21a)

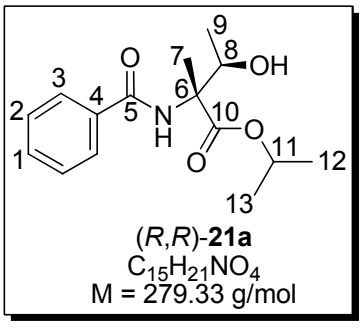


Sodium borohydride (106 mg, 2.79 mmol) was suspended in absolute THF (15 mL) and cooled in an ice-bath. *D-N*-Boc-proline (1800 mg, 8.36 mmol) in THF (30 mL) was added through an additional funnel. After two hours of stirring at room temperature, the mixture was cooled in an ice-bath and isopropyl (*S*)-2-benzoylamino-2-methyl-3-oxo-butyrate ((*S*)-**22a**, 580 mg, 2.09 mmol, 71% ee) in THF (30 mL) was added drop wise. After 20 hours of stirring at ice-bath temperature the work up was done according to SOP14. The spectroscopic properties are in accordance with those of the racemic compound *rac-l*-**21a**.

Yield: 230 mg (39%).

Enantiomeric excess: 89% ee.

7.2.1.14.2 Isopropyl (*R,R*)-2-benzoylamino-3-hydroxy-2-methyl-butyrate ((*R,R*)-21a)



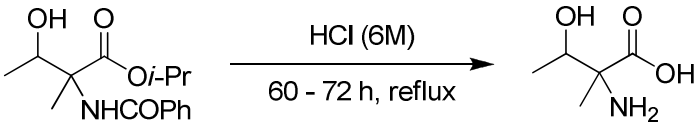
Sodium borohydride (109 mg, 2.89 mmol) was suspended in absolute THF (15 mL) and cooled in an ice-bath. L-*N*-Boc-proline (1868 mg, 8.68 mmol) in THF (30 mL) was added through an additional funnel. After two hours of stirring at room temperature, the mixture was cooled in an ice-bath and isopropyl (*R*)-2-benzoylamino-2-methyl-3-oxo-butyrate ((*R*)-**22a**, 600 mg, 2.17 mmol, 70% ee) in THF (30 mL) was

added drop wise. After 20 hours of stirring at ice-bath temperature the work up was done according to SOP14. The spectroscopic properties are in accordance with those of the racemic compound *rac-l*-**21a**.

Yield: 195 mg (32%).

Enantiomeric excess: 89% ee.

7.2.1.15 Standard operating procedure 15 (SOP15): Hydrolysis to α-methyl threonine (16a)

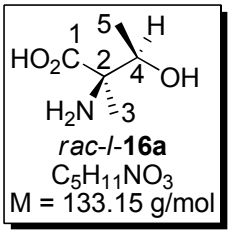


21a **16a**

Scheme 97. Hydrolysis to α-methyl threonine (**16a**)

Isopropyl 2-benzoylamino-3-hydroxy-2-methyl-butyrate (**21a**, 0.38 - 0.72 mmol) was dissolved in hydrochloric acid (6 M, 10 - 20 mL) and refluxed for 60 - 72 hours. After cooling to room temperature, the mixture was washed with MTBE (10 - 20 mL). The aqueous phase was evaporated to dryness. The resulting hydrochloride was converted into free **16a** by ion exchange resin (Dowex 50WX8, eluent: aqueous ammonia (1 M)).

7.2.1.15.1 *rac-l*-α-Methyl threonine (*rac-l*-16a)



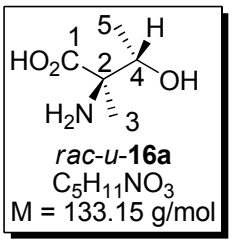
A mixture of isopropyl *rac-l*-2-benzoylamino-3-hydroxy-2-methyl-butyrate (*rac-l*-**21a**, 106 mg, 0.38 mmol) in hydrochloric acid (6 M, 10 mL) was refluxed for three days. After cooling to room temperature, work up was done according to SOP15.

Yield: 45 mg (89%).

^{1}H-NMR (400 MHz, D_2O): δ [ppm] = 1.14 (d, *J* = 6.6 Hz, 3H, H-C**5**), 1.36 (s, 3H, H-C**3**), 3.96 (q, *J* = 6.6 Hz, 1H, H-C**4**).

Spectroscopic properties are in accordance with literature (see reference [34]).

7.2.1.15.2 *rac-u*-α-Methyl threonine (*rac-u*-16a)



rac-u-**16a**
$C_5H_{11}NO_3$
M = 133.15 g/mol

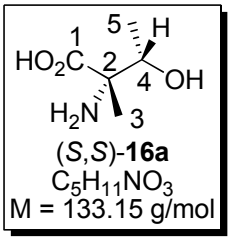
A mixture of isopropyl *rac-u*-2-benzoylamino-3-hydroxy-2-methyl-butyrate (*rac-u*-**21a**, 200 mg, 0.72 mmol) in hydrochloric acid (6 M, 20 mL) was refluxed for three days. After cooling to room temperature, work up was done according to SOP15.

Yield: 70 mg (73%).

^{1}H-NMR (400 MHz, D_2O): δ [ppm] = 1.27 (d, *J* = 6.6 Hz, 3H, H-C**5**), 1.42 (s, 3H, H-C**3**), 4.19 (q, *J* = 6.6 Hz, 1H, H-C**4**).

Spectroscopic properties are in accordance with literature (see reference [34]).

7.2.1.15.3 (*S,S*)-α-Methyl threonine ((*S,S*)-16a)



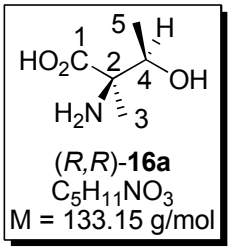
(*S,S*)-**16a**
$C_5H_{11}NO_3$
M = 133.15 g/mol

Isopropyl (*S,S*)-2-benzoylamino-3-hydroxy-2-methyl-butyrate ((*S,S*)-**21a**, 126 mg, 0.45 mmol, 86% ee) was dissolved in hydrochloric acid (6 M, 10 mL) and refluxed for 60 hours. After cooling to room temperature, work up was done according to SOP15. Spectroscopic properties are in accordance to those given above for the racemic compound *rac-l*-**16a** and those reported by Avenoza *et al*. (see reference [34]). Enantiomeric excess was determined by optical rotation.

Yield: 43 mg (72%); $[\alpha]_D^{20}$ = +10.0° (c = 0.85, H_2O).

Enantiomeric excess: 86% ee.

7.2.1.15.4 (*R,R*)-α-Methyl threonine ((*R,R*)-16a)



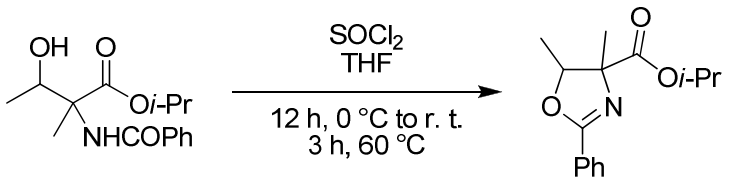
(*R,R*)-**16a**
$C_5H_{11}NO_3$
M = 133.15 g/mol

Isopropyl (*R,R*)-2-benzoylamino-3-hydroxy-2-methyl-butyrate ((*R,R*)-**21a**, 106 mg, 0.38 mmol, 89% ee) was dissolved in hydrochloric acid (6 M, 10 mL) and refluxed for 60 hours. After cooling to room temperature, work up was done according to SOP15. Spectroscopic properties are in accordance to those given above for the racemic compound *rac-l*-**16a** and those reported by Avenoza *et al*. (see reference [34]). Enantiomeric excess was determined by optical rotation.

Yield: 45 mg (89%); $[\alpha]_D^{20}$ = -10.4° (c = 0.85, H_2O).

Enantiomeric excess: 89% ee.

7.2.1.16 Standard operating procedure 16 (SOP16): Formation of an oxazoline (73)

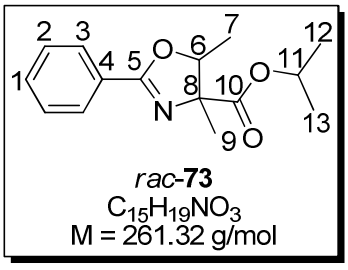


21a **73**

Scheme 98. Formation of an oxazoline **73** by dehydration

Isopropyl 2-benzoylamino-3-hydroxy-2-methyl-butyrate (**21a**, 0.5 - 0.8 mmol) was dissolved in THF (5 mL) and cooled in an ice-bath. After thionyl chloride (0.75 - 1.2 mmol) was added, the mixture was allowed to warm to room temperature over night. After additional three hours of stirring at 60 °C, the solution was cooled in an ice-bath and the excess of thionyl chloride was quenched with saturated sodium bicarbonate solution (30 mL). The mixture was extracted using ethyl acetate (3 x 20 - 30 ml). Unified organic phases were washed with water and brine, dried over sodium sulfate and evaporated. The crude product was purified by column chromatography (cyclohexan - ethyl acetate, 9:1) to yield colorless oil.

7.2.1.16.1 *rac-u*-4-Isopropoxycarbonyl-4,5-dimethyl-2-phenyl-1,3-oxazoline (*rac-u*-73)



rac-**73**
$C_{15}H_{19}NO_3$
M = 261.32 g/mol

Isopropyl *rac-l*-2-benzoylamino-3-hydroxy-2-methyl-butyrate (*rac-l*-**21a**, 140 mg, 0.5 mmol) was dissolved in THF (5 mL) and cooled in an ice-bath. After thionyl chloride (55 µL, 89 mg, 0.75 mmol) was added, the mixture was allowed to warm to room temperature over night. After additional 3 hours of stirring at 60 °C, work up was done according to SOP16.

Yield: 94 mg (72%).

HPLC (Chiralcel OJ-H-column; hexanes - *i*-PrOH, 90:10; 1.0 mL/min): t_R = 8.9 (*R,S*), 12.5 (*S,R*) min.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.23 (d, *J* = 6.3 Hz, 6H, H-C**12**, H-C**13**), 1.39 (s, 3H, H-C**9**), 1.40 (d, *J* = 6.7 Hz, 3H, H-C**7**), 4.95 (q, *J* = 6.7 Hz, 1H, H-C**6**), 5.03 (septet, *J* = 6.3 Hz, 1H, H-C**11**), 7.32 - 7.36 (m, 2H, H-C**2**), 7.40 - 7.44 (m, 1H, H-C**1**), 7.92 - 7.93 (m, 2H, H-C**3**).

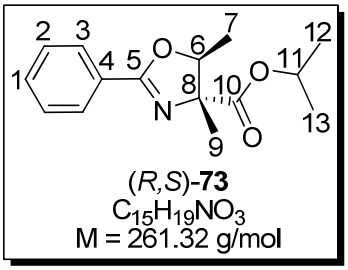
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 15.2, 19.2 (C**7**, C**9**), 21.4 (C**12**, C**13**), 68.8 (C**11**), 75.4, 80.7 (C**6**, C**8**), 127.6 (C**4**), 128.2, 128.4 (C**2**, C**3**), 131.5 (C**1**), 163.7 (C**5**), 173.3 (C**10**).

MS (FAB): *m/z* = 262 (MH^+), 220 (MH^+-*i*-Pr).

IR (thin film): 2984, 1725, 1644 cm^{-1}.

Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.50; H, 7.38; N, 5.33.

7.2.1.16.2 (*R,S*)-4-Isopropoxycarbonyl-4,5-dimethyl-2-phenyl-1,3-oxazoline ((*R,S*)-73)

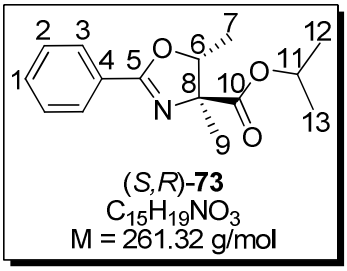


Isopropyl (*R,R*)-2-benzoylamino-3-hydroxy-2-methyl-butyrate ((*R,R*)-**21a**, 180 mg, 0.7 mmol, 89% ee) was dissolved in THF (5 mL) and cooled in an ice-bath. After thionyl chloride (73 µL, 119 mg, 1.0 mmol) was added, the mixture was allowed to warm to room temperature over night. After additional three hours of stirring at 60 °C, work up was done according to SO P16. Spectroscopic properties are in accordance with those of the racemic compound *rac-u*-**73**.

Yield: 105 mg (62%).

Enantiomeric excess: 88% ee.

7.2.1.16.3 (*S,R*)-4-Isopropoxycarbonyl-4,5-dimethyl-2-phenyl-1,3-oxazoline ((*S,R*)-73)

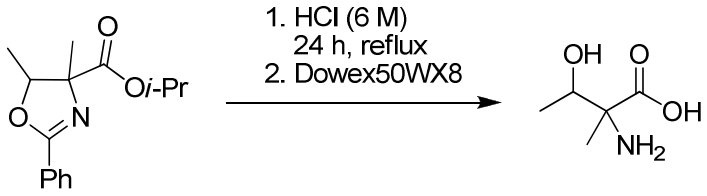


Isopropyl (*S,S*)-2-benzoylamino-3-hydroxy-2-methyl-butyrate ((*S,S*)-**21a**, 230 mg, 0.8 mmol, 89% ee) was dissolved in THF (8 mL) and cooled in an ice-bath. After thionyl chloride (91 µL, 143 mg, 1.2 mmol) was added, the mixture was allowed to warm to room temperature over night. After additional three hours of stirring at 60 °C, work up was done according to SO P16. Spectroscopic properties are in accordance with those of the racemic compound *rac-u*-**73**.

Yield: 127 mg (60%).

Enantiomeric excess: 89% ee.

7.2.1.17 Standard operating procedure 17 (SOP17): Hydrolysis of oxazoline to α-methyl threonine (16a)

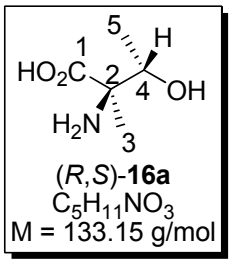


73 **16a**

Scheme 99. Hydrolysis of oxazoline **73** to α-methyl threonine (**16a**)

4-Isopropoxycarbonyl-4,5-dimethyl-2-phenyl-1,3-oxazoline (**73**, 0.38 - 0.49 mmol, 88 - 89% ee) was dissolved in hydrochloric acid (6 M, 15 mL) and refluxed for 24 hours. After cooling to room temperature, the mixture was washed with MTBE (2 x 10 mL). The aqueous phase was evaporated to dryness. The resulting hydrochloride was converted into free **16a** by ion exchange resin (Dowex 50WX8, eluent: aqueous ammonia (1M)).

7.2.1.17.1 (*R,S*)-α-Methyl threonine ((*R,S*)-16a)



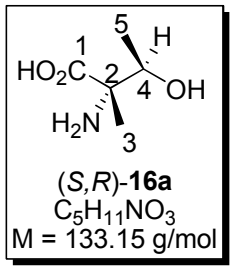
(*R,S*)-**16a**
$C_5H_{11}NO_3$
M = 133.15 g/mol

(*R,S*)-4-Isopropoxycarbonyl-4,5-dimethyl-2-phenyl-1,3-oxazoline ((*R,S*)-**73**, 100 mg, 0.38 mmol, 88% ee) was dissolved in hydrochloric acid (6 M, 15 mL) and refluxed for 24 hours. After cooling to room temperature, work up was done according to SOP17. Spectroscopic properties are in accordance to those given above for the racemic compound *rac-u*-**16a** and those reported by Avenoza *et al*. (see reference [34]). Enantiomeric excess was determined by optical rotation.

Yield: 46 mg (91%); $[\alpha]_D^{20}$ = +11.8° (c = 0.75, H_2O).

Enantiomeric excess: 88% ee.

7.2.1.17.2 (*S,R*)-α-Methyl threonine ((*S,R*)-16a)



(*S,R*)-**16a**
$C_5H_{11}NO_3$
M = 133.15 g/mol

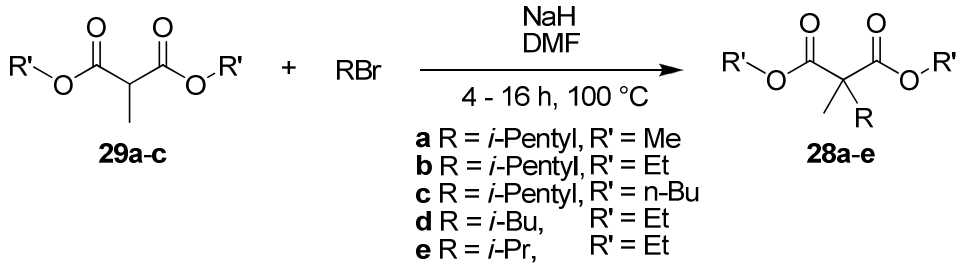
(*S,R*)-4-Isopropoxycarbonyl-4,5-dimethyl-2-phenyl-1,3-oxazoline ((*S,R*)-**73**, 127 mg, 0.49 mmol, 89% ee) was dissolved in hydrochloric acid (6M, 15 mL) and refluxed for 24 hours. After cooling to room temperature, work up was done according to SOP 17. Spectroscopic properties are in accordance to those given above for the racemic compound *rac-u*-**16a** and those reported by Avenoza *et al*. (see reference [34]). Enantiomeric excess was examined by optical rotation.

Yield: 56 mg (87%); $[\alpha]_D^{20}$ = -11.9°(c = 0.75, H_2O).

Enantiomeric excess: 89% ee.

7.2.2 Desymmetrisation of malonates

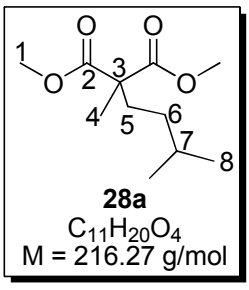
7.2.2.1 Standard operating procedure 18 (SOP18): Synthesis of dialkyl 2-alkyl-2-methylmalonates (28a-e)



Scheme 100. Synthesis of dialkyl 2-alkyl-2-methylmalonates **28**

The dialkyl 2-methylmalonate (**29**, 58 mmol) was dissolved in DMF (100 mL) and cooled in an ice-bath. Then sodium hydride (64 mmol, 60% in mineral oil) was added and the solution was stirred at ice-bath temperature until no further hydrogen evolved. The solution was allowed to warm to room temperature and an alkyl bromide (70 mmol) was added. The solution was stirred for 4 - 16 hours at 100 °C. After cooling to room temperature, ethyl acetate (200 mL) was added and the resulting mixture was washed with sodium thiosulfate (5%, 3 x 100 mL). After drying over magnesium sulfate, the solvent was removed to give the crude product, which was purified by column chromatography (cyclohexane - ethyl acetate, 95:5). All products are colorless oils.

7.2.2.1.1 Dimethyl 2-methyl-2-isopentylmalonate (28a)



Dimethyl 2-methylmalonate (**29a**, 2.93 g, 20 mmol) was dissolved in DMF (30 mL) and cooled in an ice-bath. Then sodium hydride (880 mg, 22 mmol, 60% in mineral oil) was added and the solution was stirred at ice-bath temperature until no further hydrogen evolved. The solution was allowed to warm to room temperature and isopentylbromide (3.0 mL, 3.6 g, 24 mmol) was added. The solution was stirred for 16 hours at 100 °C. After cooling to room temperature, work up was done according to SOP18.

Yield: 4.07 g (94%), R_f = 0.30.

IR (thin film): 2955, 2871, 1732 cm^{-1}.

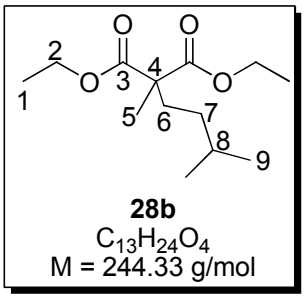
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.84 (d, *J* = 6.6 Hz, 6H, H-C**8**), 1.00 - 1.06 (m, 2H, H-C**6**), 1.35 (s, 3H, H-C**4**), 1.47 (septet, *J* = 6.6 Hz, 1H, H-C**7**), 1.79 - 1.83 (m, 2H, H-C**5**), 3.67 (s, 6H, H-C**1**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 19.8 (C**4**), 22.4 (C**8**), 28.2 (C**7**), 33.1, 33.5 (C**5**, C**6**), 52.3 (C**3**), 53.6 (C**1**), 172.9 (C**2**).

MS (EI): *m/z* = 217 (M^+).

Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 61.55; H, 9.43.

7.2.2.1.2 Diethyl 2-methyl-2-isopentylmalonate (28b)



28b
$C_{13}H_{24}O_4$
M = 244.33 g/mol

Diethyl 2-methylmalonate (**29b**, 10.0 mL, 10.1 g, 58 mmol) was dissolved in DMF (100 mL) and cooled in an ice-bath. Then sodium hydride (2.55 g, 64 mmol, 60% in mineral oil) was added and the solution was stirred at ice-bath temperature until no further hydrogen evolved. The solution was allowed to warm to room temperature and isopentylbromide (8.8 mL, 10.5 g, 70 mmol) was added. The solution was stirred for 16 hours at 100 °C. After cooling to room temperature, work up was done according to SOP18.

Yield: 13.5 g (95%), R_f = 0.34.

IR (thin film): 2957, 2873, 1730 cm^{-1}.

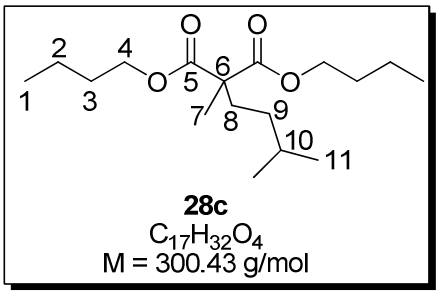
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.85 (d, *J* = 6.6 Hz, 6H, H-C**9**), 1.03 - 1.08 (m, 2H, H-C**7**), 1.21 (t, 6H, *J* = 7.1 Hz, H-C**1**), 1.35 (s, 3H, H-C**5**), 1.42 - 1.55 (m, 1H, H-C**8**), 1.80 - 1.84 (m, 2H, H-C**6**), 4.11 - 4.17 (m, 4H, H-C**2**).

^{13}C- NMR (100 MHz, $CDCl_3$): δ [ppm] = 13.9 (C**1**), 19.6 (C**5**), 22.4 (C**9**), 28.2 (C**8**), 33.1, 33.2 (C**6**, C**7**), 53.6 (C**4**), 61.0 (C**2**), 172.6 (C**3**).

MS (EI): *m/z* = 245, 243 (M^+), 201, 199 (M^+-OEt).

Anal. Calcd for $C_{13}H_{24}O_4$: C, 63.91; H, 9.90. Found: C, 63.62; H, 9.83.

7.2.2.1.3 Di-*n*-butyl 2-methyl-2-isopentylmalonate (28c)



28c
$C_{17}H_{32}O_4$
M = 300.43 g/mol

Di-*n*-butyl 2-methylmalonate (**29c**, 4.6 g, 20 mmol) was dissolved in DMF (30 mL) and cooled in an ice-bath. Then sodium hydride (880 mg, 22 mmol, 60% in mineral oil) was added and the solution was stirred at ice-bath temperature until no further hydrogen evolved. The solution was allowed to warm to room temperature and isopentylbromide (3.0 mL, 3.6 g, 24 mmol) was added. The solution was stirred for 16 hours at 100 °C. After cooling to room temperature, work up was done according to SOP18.

Yield: 3.67 g (61%), R_f = 0.39.

IR (thin film): 2957, 2873, 1730 cm^{-1}.

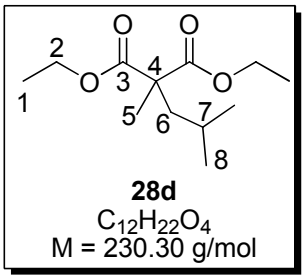
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.85 (d, *J* = 6.6 Hz, 6H, H-C**11**), 0.89 (t, *J* = 7.4 Hz, 6H, H-C**1**), 1.02 - 1.08 (m, 2H, H-C**9**), 1.29 - 1.38 (m, 4H, H-C**2**), 1.36 (s, 3H, H-C**7**), 1.43 - 1.52 (m, 1H, H-C**10**), 1.53 - 1.60 (m, 4H, H-C**3**) 1.80 - 1.84 (m, 2H, H-C**8**), 4.03 - 4.13 (m, 4H, H-C**4**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 13.5 (C**1**), 19.0 (C**2**), 19.7 (C**7**), 22.4 (C**11**), 28.3 (C**10**), 30.4 (C**3**), 33.1, 33.3 (C**8**, C**9**), 53.7 (C**6**), 64.9 (C**4**), 172.7 (C**5**).

MS (EI): *m/z* = 301 (M^+), 285 (M^+-Me), 245, 244 (M^+-O*n*-Bu), 230 (M^+-O*n*-Bu-Me).

Anal. Calcd for $C_{17}H_{32}O_4$: C, 67.96; H, 10.74. Found: C, 67.62; H, 10.48.

7.2.2.1.4 Diethyl 2-isobutyl-2-methylmalonate (28d)



28d
$C_{12}H_{22}O_4$
M = 230.30 g/mol

Diethyl 2-methylmalonate (**29b**, 10.0 mL, 10.1 g, 58 mmol) was dissolved in DMF (100 mL) and cooled in an ice-bath. Then sodium hydride (2.55 g, 64 mmol, 60% in mineral oil) was added and the solution was stirred at ice-bath temperature until no further hydrogen evolved. The solution was allowed to warm to room temperature and isobutylbromide (7.6 mL, 9.6 g, 70 mmol) was added. The solution was stirred for 16 hours at 100 °C. After cooling to room temperature, work up was done according to SOP18.

Yield: 10.1 g (76%), R_f = 0.31.

IR (thin film): 2959, 1729 cm^{-1}.

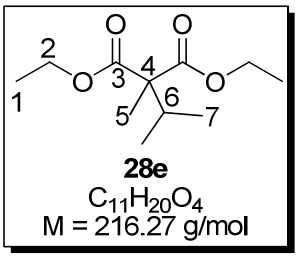
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.85 (d, *J* = 6.6 Hz, 6H, H-C**8**), 1.21 (t, 6H, *J* = 7.1 Hz, H-C**1**), 1.38 (s, 3H, H-C**5**), 1.58 - 1.71 (m, 1H, H-C**7**), 1.82 (d, 2H, *J* = 6.3 Hz, H-C**6**), 4.13 (q, 4H, *J* = 7.1 Hz, H-C**2**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 13.9 (C**1**), 19.9 (C**5**), 23.6 (C**8**), 24.3 (C**7**), 43.5 (C**6**), 53.3 (C**4**), 61.0 (C**2**), 172.9 (C**3**).

MS (EI): *m/z* = 231, 229 (M^+), 187, 185 (M^+-OEt).

Anal. Calcd for $C_{12}H_{22}O_4$: C, 62.58; H, 9.63. Found: C, 62.51; H, 9.61.

7.2.2.1.5 Diethyl 2-methyl-2-isopropylmalonate (28e)



28e
$C_{11}H_{20}O_4$
M = 216.27 g/mol

Diethyl 2-methylmalonate (**29b**, 10.0 mL, 10.1 g, 58 mmol) was dissolved in DMF (100 mL) and cooled in an ice-bath. Then sodium hydride (2.55 g, 64 mmol, 60% in mineral oil) was added and the solution was stirred at ice-bath temperature until no further hydrogen evolved. The solution was allowed to warm to room temperature and isopropylbromide (6.6 mL, 8.6 g, 70 mmol) was added. The solution was stirred for 16 hours at 100 °C. After cooling to room temperature, work up was done according to SOP18.

Yield: 4.9 g (40%, pure) and 5.9 g (43%, >90% pure (determined by means of ^{1}H-NMR-spectroscopy)), R_f = 0.28.

IR (thin film): 2976, 1729 cm^{-1}.

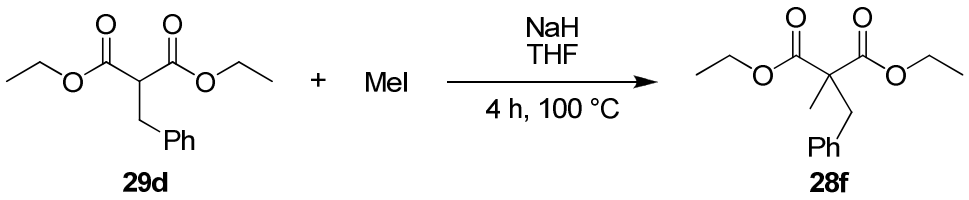
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.89 (d, *J* = 6.8 Hz, 6H, H-C**7**), 1.21 (t, 6H, *J* = 7.1 Hz, H-C**1**), 1.28 (s, 3H, H-C**5**), 2.45 (septet, 1H, *J* = 6.8 Hz, H-C**6**), 4.13 (q, 4H, *J* = 7.1 Hz, H-C**2**).

^{13}C- NMR (100 MHz, $CDCl_3$): δ [ppm] = 13.9 (C**1**), 15.3 (C**5**), 18.0 (C**7**), 32.1 (C**6**), 57.5 (C**4**), 60.9 (C**2**), 172.0 (C**3**).

MS (EI): *m/z* = 217 (M^+), 174 (M^+-OEt).

Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 60.89; H, 9.65.

7.2.2.2 Standard operating procedure 19 (SOP19): Synthesis of diethyl 2-benzyl-2-methylmalonate (28f)

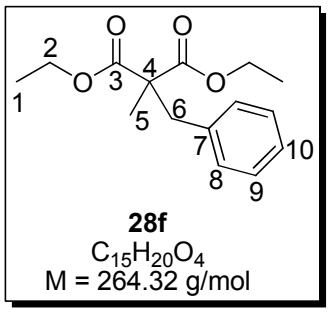


29d **28f**

Scheme 101. Synthesis of diethyl 2-benzyl-2-methylmalonate (**28f**)

Sodium hydride (27.5 mmol, 60% in mineral oil) was suspended in dry THF (50 mL) and cooled in an ice-bath. Diethyl 2-benzylmalonate (**29d**, 25 mmol) was added and the reaction mixture was allowed to warm to room temperature. After hydrogen evolution had ceased methyl iodide (30 mmol) was added. The reaction mixture was stirred at 100 °C for four hours. After cooling to room temperature, water was added to quench the excess sodium hydride. After evaporation of the bulk of THF at reduced pressure, the aqueous solution was extracted with ethyl acetate (3 x 100 mL). The unified organic phases were dried over magnesium sulfate and rotary evaporated, yielding crude **28f** which was purified by column chromatography (cyclohexane - ethyl acetate, 9:1) yielding a colorless oil.

7.2.2.2.1 Diethyl 2-benzyl-2-methylmalonate (28f)



28f
$C_{15}H_{20}O_4$
M = 264.32 g/mol

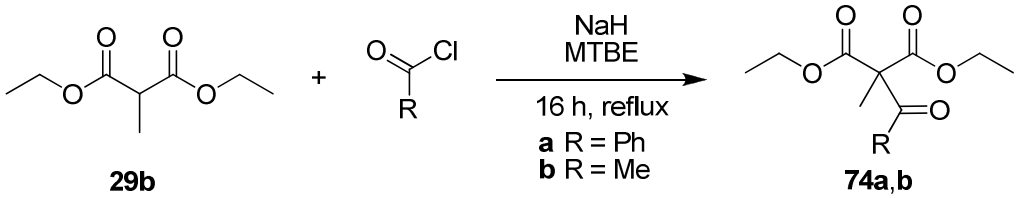
Sodium hydride (1.10 g, 27.5 mmol, 60% in mineral oil) was suspended in dry THF (50 mL) and cooled in an ice-bath. Diethyl 2-benzylmalonate (**29d**, 5.9 mL, 6.3 g, 25 mmol) was added and the reaction mixture was allowed to warm to room temperature. After hydrogen evolution had ceased methyl iodide (1.7 mL, 3.9 g, 30 mmol) was added. The reaction mixture was stirred at 100 °C for four hours. After cooling to room temperature, the work up was done according to SOP19 yielding a colorless oil.

Yield: 5.3g (80%), R_f = 0.45.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.23 (t, *J* = 7.1Hz, 6H, H-C**1**), 1.31 (s, 3H, H-C**5**), 3.21 (s, 2H, H-C**6**), 4.17 (q, 4H, *J* = 7.1 Hz, H-C**2**), 7.08 - 7.11 (m, 2H, H-C**9**), 7.19- 7.26 (m, 3H, H-C**8**, H-C**10**).

Spectroscopic data are in accordance with literature (see reference [132]).

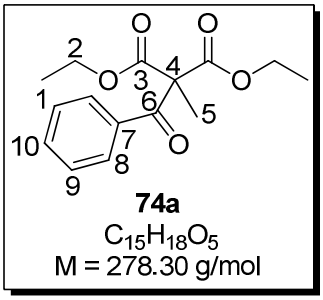
7.2.2.3 Standard operating procedure 20 (SOP20): Synthesis of dialkyl 2-acyl-2-methylmalonates (74a,b)



Scheme 102. Synthesis of dialkyl 2-acyl-2-methylmalonates (**74a**,**b**)

Sodium hydride (60 mmol, 60% in mineral oil) was suspended in dry MTBE (50 mL) and cooled in an ice-bath. Diethyl 2-methylmalonate (**29b**, 50 mmol) in MTBE (100 mL) was added and the reaction mixture was allowed to warm to room temperature and then refluxed for one to two hours. After hydrogen evolution had ceased an acyl chloride (55 mmol) in MTBE (50mL) was added. The reaction mixture was refluxed for 16 hours. After cooling in an ice-bath, hydrochloric acid (1M) was added to quench the excess sodium hydride. Phases were separated and the aqueous phase was extracted with MTBE (2 x 100 mL). The unified organic phases were washed with saturated sodium bicarbonate solution (3 x 150 mL). After drying over magnesium sulfate, the solvent was removed yielding yellowy oils as crude products. The pure products were yielded after distillation or column chromatography.

7.2.2.3.1 Diethyl 2-benzoyl-2-methylmalonate (74a)



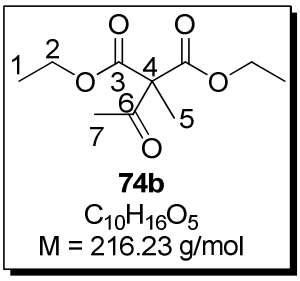
Sodium hydride (2.4 g, 60 mmol, 60% in mineral oil) was suspended in dry MTBE (50 mL) and cooled in an ice-bath. Diethyl 2-methylmalonate (**29b**, 8.5 mL, 8.7 g, 50 mmol) in MTBE (100 mL) was added and the reaction mixture was allowed to warm to room temperature and then refluxed for one hour. After hydrogen evolution had ceased, benzoyl chloride (6.4 mL, 7.7 g, 55 mmol) in MTBE (50 mL) was added. The reaction mixture was refluxed for 16 hours. The work up was done according to SOP20. Purification was done by distillation in vacuum yielding colorless oil, which crystallises slowly.

Yield: 8.36 g (60%), Bp: 120 °C at 0.05 mbar.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.16 (t, *J* = 7.2 Hz, 6H, H-C**1**), 1.81 (s, 3H, H-C**5**), 4.14 - 4.26 (m, 4H, H-C**2**), 7.37 - 7.41 (m, 2H, H-C**9**), 7.48 - 7.52 (m, 1H, H-C**10**), 7.77 - 7.80 (m, 2H, H-C**8**).

Spectroscopic data are in accordance with literature (see reference [100]).

7.2.2.3.2 Diethyl 2-acetyl-2-methylmalonate (74b)



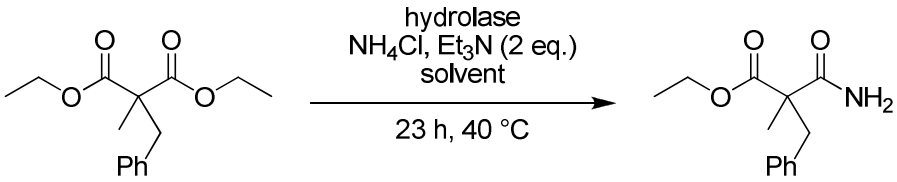
74b
$C_{10}H_{16}O_5$
M = 216.23 g/mol

Sodium hydride (2.4 g, 60 mmol, 60% in mineral oil) was suspended in dry MTBE (50 mL) and cooled in an ice-bath. Diethyl 2-methylmalonate (**29b**, 8.5 mL, 8.7 g, 50 mmol) in MTBE (100 mL) was added and the reaction mixture was allowed to warm to room temperature and then refluxed for two hours. After hydrogen evolution had ceased acetyl chloride (3.9 mL, 4.3 g, 55 mmol) in MTBE (50 mL) was added. The reaction mixture was refluxed for 16 hours. Work up was done according to SOP20. Purification was done by column chromatography (cyclohexane - ethyl acetate, 7:1), yielding a colorless oil.

Yield: 8.4 g (70%, > 90% (determined by means of ^{1}H-NMR-spectroscopy)), R_f = 0.34.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.25 (t, *J* = 7.2 Hz, 6H, H-C**1**), 1.58 (s, 3H, H-C**5**), 2.28 (s, 3H, H-C**7**), 4.21 (q, 4H, *J* = 7.2 Hz, H-C**2**).

7.2.2.4 Standard operating procedure 21 (SOP21): Enzyme screening on the ammonolysis of diethyl 2-benzyl-2-methylmalonate (28f)



28f **27f**

Scheme 103. Enzyme screening on ammonolysis

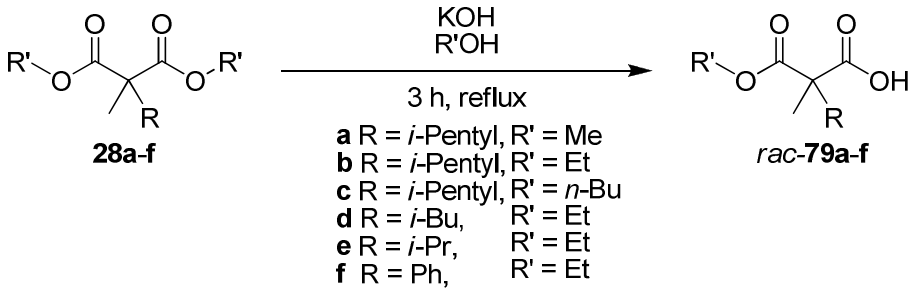
The screening was done under conditions similar to those described by Wang *et al.*.[101] Diethyl 2-benzyl-2-methylmalonate (**28f**, 50 µL, 53 mg, 0.2 mmol) was diluted in a suspension of ammonium chloride (21.4 mg, 0.4 mmol) in *tert*-butanol (1 mL) or toluene (1 mL) (each enzyme was tested in both solvents). Triethyl amine (59 µL, 41 mg, 0.4 mmol) and an enzyme formulation (20 mg) were added. The reaction mixture was stoppered and

heated for 23 hours at 40 °C while stirring. After this time, the mixture was filtered and washed with ethyl acetate (2 x 5 mL). The solvent was removed *in vacuo* and the resulting crude product was examined by means of ^{1}H-NMR-spectroscopy. No enzyme led to the desired product **27f**. The NMR-spectra consists mainly of the peaks of the substrate beside some residue of triethylamine hydrochloride.

List of tested enzymes:

- *Candida antarctica* lipase B
- *Candida rugosa* lipase
- *Candida cylindracea* lipase
- lipase Amano PS
- Amano acylase *Aspergillus mellus*
- lipase Amano AK 20
- lipase Amano AP6
- lipase Amano AS
- lipase Amano PS-IM
- lipase Amano PS-SD
- lipase Amano DF-DS
- lipase Amano AYS
- lipase Amano A
- lipase Amano M
- lipase Amano G
- porcine liver esterase (PLE)

7.2.2.5 Standard operating procedure 22 (SOP22): Synthesis of *rac*-monoalkyl 2-alkyl-2-methylmalonates (*rac*-79a-f)

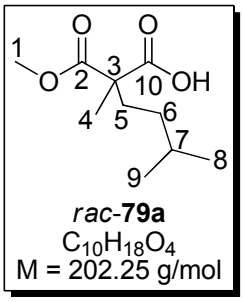


Scheme 104. Synthesis of *rac*-monoalkyl 2-alkyl-2-methylmalonates (*rac*-**79a**-**f**)

The dialkyl 2-alkyl-2-methylmalonate (**28a**-**f**, 1.0 mmol) and potassium hydroxide (1.1 mmol) were dissolved in the corresponding alcohol (10 mL). The mixture was refluxed for three

hours. The alcohol was removed *in vacuo* and the solid residue was dissolved in water. After extraction with dichloromethane (2 x 20 mL), the aqueous phase was acidified to pH 1 with hydrochloric acid (1 M). After extraction with dichloromethane (3 x 30 mL), the organic phase (extracted at pH 1) was dried over magnesium sulfate and the solvent was evaporated.

7.2.2.5.1 *rac*-2-(Methoxycarbonyl)-2,5-dimethylhexanoic acid (*rac*-79a)



rac-**79a**
$C_{10}H_{18}O_4$
M = 202.25 g/mol

Dimethyl 2-*iso*-pentyl-2-methylmalonate (**28a**, 216 mg, 1.0 mmol) and potassium hydroxide (62 mg, 1.1 mmol) were dissolved in methanol (10 mL). After refluxing this mixture for three hours, the work up was done according to SOP22 yielding colorless oil.

Yield: 144 mg (71%).

HPLC (Chiralcel OJ-H-column; hexanes - *i*-PrOH, 97:3, 0.1% formic acid; 1.0 mL/min): t_R = 6.3 (*R*), 6.9 (*S*) min.

IR (thin film): 2955, 2872, 1737, 1709 cm^{-1}.

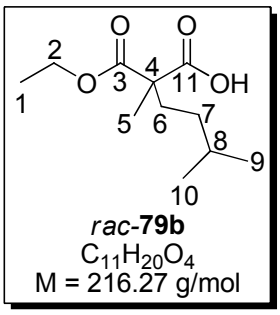
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.87 (d, *J* = 6.6 Hz, 6H, H-C**8**, H-C**9**), 1.07 - 1.14 (m, 2H, H-C**6**), 1.43 (s, 3H, H-C**4**), 1.48 - 1.53 (m, 1H, H-C**7**), 1.80 - 1.94 (m, 2H, H-C**5**), 3.74 (s, 3H, H-C**1**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 20.1 (C**4**), 22.3 (C**8**, C**9**), 28.2 (C**7**), 33.2, 33.8 (C**5**, C**6**), 52.6, 53.6 (C**1**, C**3**), 173.3 (C**2**), 177.8 (C**10**).

MS (EI) *m/z* = 224 ($M\text{-}Na^+$), 203 (M^+).

Spectroscopic data are in accordance with literature (see reference [90f]).

7.2.2.5.2 *rac*-2-(Ethoxycarbonyl)-2,5-dimethylhexanoic acid (*rac*-79b)



rac-**79b**
$C_{11}H_{20}O_4$
M = 216.27 g/mol

Diethyl 2-isopentyl-2-methylmalonate (**28b**, 244 mg, 1.0 mmol) and potassium hydroxide (62 mg, 1.1 mmol) were dissolved in ethanol (10 mL). After refluxing this mixture for three hours, the work up was done according to SOP22, yielding a colorless oil.

Yield: 157 mg (73%).

HPLC (Chiralcel OJ-H-column; hexanes - *i*-PrOH, 97:3, 0.1% formic acid; 1.0 mL/min): t_R = 5.1 (*R*), 5.5 (*S*) min.

IR (thin film): 2957, 2872, 2361, 1736, 1708 cm^{-1}.

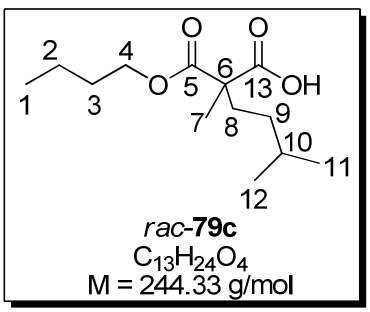
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.86 (d, *J* = 6.6 Hz, 6H, H-C**9**, C**10**), 1.07 - 1.13 (m, 2H, H-C**7**), 1.25 (t, 3H, *J* = 7.1 Hz, H-C**1**), 1.42 (s, 3H, H-C**5**), 1.46 - 1.55 (m, 1H, H-C**8**), 1.79 - 1.93 (m, 2H, H-C**6**), 4.17 - 4.22 (m, 2H, H-C**2**), 9.6 (br s, 1H, OH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 13.9 (C**1**), 20.1 (C**5**), 22.31, 22.33 (C**9**, C**10**), 28.2 (C**8**), 33.2, 33.8 (C**6**, C**7**), 53.5 (C**4**), 61.6 (C**2**), 172.9 (C**3**), 177.9 (C**11**).

MS (EI) *m/z* = 217, 216 (M^+).

Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 60.67; H, 9.38.

7.2.2.5.3 *rac*-2-(*n*-Butoxycarbonyl)-2,5-dimethylhexanoic acid (*rac*-79c)



rac-**79c**
$C_{13}H_{24}O_4$
M = 244.33 g/mol

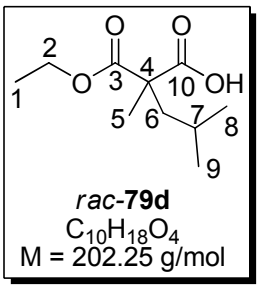
Di-*n*-butyl 2-*iso*-pentyl-2-methylmalonate (**28c**, 244 mg, 1.0 mmol) and potassium hydroxide (62 mg, 1.1 mmol) were dissolved in *n*-butanol (10 mL). After stirring this mixture for three hours at 100 °C, work up was done according to SOP22 but without extracting at basic pH, which was not possible due to frothing, yielding colorless oil that was a mixture of the monoester *rac*-**79c** and the diester **28c**.

Yield: 173 mg (35%, 50% pure (determined by means of ^{1}H-NMR-spectroscopy)).

HPLC (Chiralcel OJ-H-column; hexanes - *i*-PrOH, 97:3, 0.1% formic acid; 1.0 mL/min): t_R = 4.5 (*R*), 4.8 (*S*) min.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.86 (d, *J* = 6.6 Hz, 6H, H-C**11**, H-C**12**), 0.90 (t, *J* = 7.4 Hz, 6H, H-C**1**) 1.06 - 1.13 (m, 2H, H-C**9**), 1.32 - 1.38 (m, 2H, H-C**3**), 1.42 (s, 3H, H-C**7**), 1.46 - 1.64 (m, 3H, H-C**2**, H-C**10**), 1.78 - 1.92 (m, 2H, H-C**8**), 4.13 (t, *J* = 6.5 Hz, 2H, H-C**4**), 7.9 (br s, 1H, OH).

7.2.2.5.4 *rac*-2-(Ethoxycarbonyl)-2,4-dimethylpentanoic acid (*rac*-79d)



rac-**79d**
$C_{10}H_{18}O_4$
M = 202.25 g/mol

Diethyl 2-isobutyl-2-methylmalonate (**28d**, 230 mg, 1.0 mmol) and potassium hydroxide (62 mg, 1.1 mmol) were dissolved in ethanol (10 mL). After refluxing this mixture for three hours, work up was done according to SOP22, yielding a colorless oil.

Yield: 117 mg (58%).

HPLC (Chiralcel OJ-H-column; hexanes - *i*-PrOH, 97:3, 0.1% formic acid; 1.0 mL/min): t_R = 5.8 (*R*), 6.3 (*S*) min.

IR (thin film): 2959, 2874, 1735, 1709 cm^{-1}.

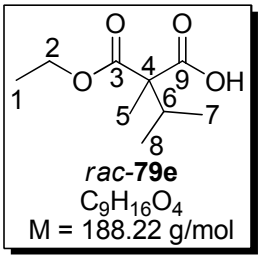
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.85 - 0.88 (m, 6H, H-C**8**, H-C**9**), 1.25 (t, 3H, *J* = 7.1 Hz, H-C**1**), 1.45 (s, 3H, H-C**5**), 1.61 - 1.71 (m, 1H, H-C**7**), 1.80 - 1.93 (m, 2H, H-C**6**), 4.19 (q, 2H, *J* = 7.1 Hz, H-C**2**), 9.5 (br s, 1H, OH).

^{13}C- NMR (100 MHz, $CDCl_3$): δ [ppm] = 13.9 (C**1**), 20.7 (C**5**), 23.5, 23.6 (C**8**, C**9**), 24.7 (C**7**), 44.0 (C**6**), 53.1 (C**4**), 61.7 (C**2**), 173.3 (C**3**), 178.1 (C**10**).

MS (EI) *m/z* = 203, 201 (M^+).

Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.39; H, 8.97. Found: C, 58.99; H, 8.97.

7.2.2.5.5 *rac*-2-(Ethoxycarbonyl)-2,3-dimethylbutanoic acid (*rac*-79e)



rac-**79e**
$C_9H_{16}O_4$
M = 188.22 g/mol

Diethyl 2-isopropyl-2-methylmalonate (**28e**, 216 mg, 1.0 mmol) and potassium hydroxide (62 mg, 1.1 mmol) were dissolved in ethanol (10 mL). After refluxing this mixture for three hours, work up was done according to SOP22, yielding a colorless oil.

Yield: 48 mg (29%).

HPLC (Chiralcel OJ-H-column; hexanes - *i*-PrOH, 97:3, 0.1% formic acid; 1.0 mL/min): t_R = 8.3, 9.5 min.

IR (thin film): 2973, 2880, 1731, 1705.

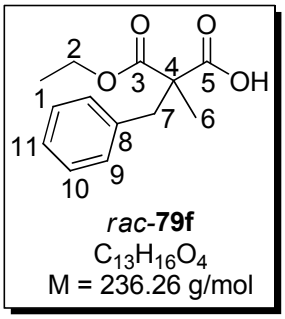
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.92 - 0.95 (m, 6H, H-C**7**, H-C**8**), 1.24 - 1.28 (m, 3H, H-C**1**), 1.35 (s, 3H, H-C**5**), 2.42 (sept, 1H, *J* = 6.8 Hz, H-C**6**), 4.17 - 4.23 (m, 2H, H-C**2**).

^{13}C- NMR (100 MHz, $CDCl_3$): δ [ppm] = 14.0 (C**1**), 15.7 (C**5**), 18.03, 18.04 (C**7**, C**8**), 32.9 (C**6**), 57.7 (C**4**), 61.5 (C**2**), 172.1 (C**3**), 177.4 (C**9**).

MS (EI) *m/z* = 189 (M^+).

Anal. Calcd for $C_9H_{16}O_4$: C, 57.43; H, 8.57. Found: C, 57.11; H, 8.55.

7.2.2.5.6 *rac*-2-Benzyl-3-ethoxy-2-methyl-3-oxopropanoic acid (79f)



rac-**79f**
$C_{13}H_{16}O_4$
M = 236.26 g/mol

Diethyl 2-benzyl-2-methylmalonate (**28f**, 265 mg, 1 mmol) and potassium hydroxide (62 mg, 1.1 mmol) were dissolved in ethanol (10 mL). After refluxing this mixture for three hours, work up was done according to SOP22, yielding a white solid.

Yield: 203 mg (86%).

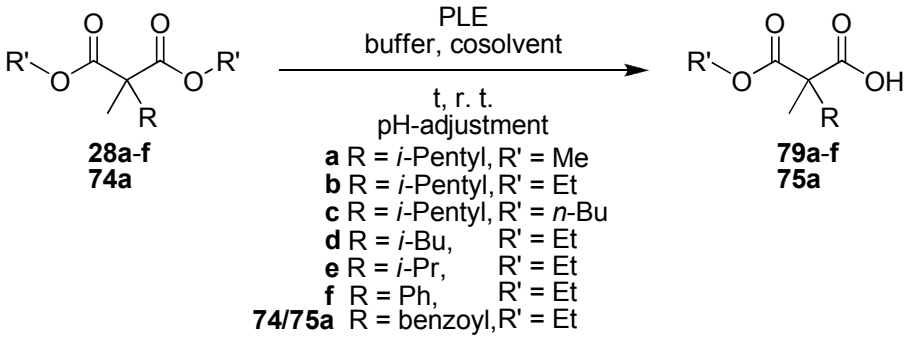
HPLC (Chiralpack IB-column; hexanes - *i*-PrOH, 95:5, 0.1% formic acid; 0.8 mL/min): t_R = 7.2 (*R*), 7.6 (*S*) min.

^{1}H-NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.17 (t, *J* = 7.1 Hz, 3H, H-C**1**), 1.17 (s, 3H, H-C**6**), 3.05 (d, 1H, *J* = 13.4 Hz, H-C**7**), 3.12 (d, 1H, *J* = 13.4 Hz, H-C**7**), 4.06 - 4.15 (m, 2H, H-C**2**), 7.11 - 7.28 (m, 5H, H-C**9**, H-C**10**, H-C**11**), 12.97 (br s, 1H, OH).

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.26 (t, *J* = 7.2 Hz, 3H, H-C**1**), 1.41 (s, 3H, H-C**6**), 3.18 (d, 1H, *J* = 13.7 Hz, H-C**7**), 3.28 (d, 1H, *J* = 13.7 Hz, H-C**7**), 4.20 (q, 2H, *J* = 7.2 Hz, H-C**2**), 7.12 - 7.14 (m, 2H, H-C**9**), 7.22 - 7.27 (m, 3H, H-C**8**, H-C**10**).

Spectroscopic properties in DMSO-d_6 are in accordance with literature (see reference [133]).

7.2.2.6 Standard operating procedure 23 (SOP23): Enzymatic desymmetrisation of dialkyl 2-isoalkyl-2-methylmalonates by hydrolysis



Scheme 105. Enzymatic desymmetrisation by hydrolysis

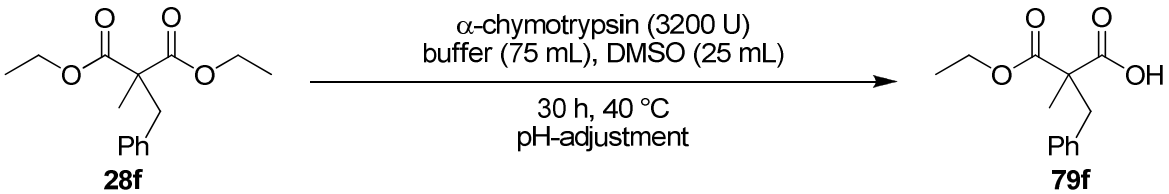
The dialkyl 2-methylmalonate (**28** or **74**, 1.0 mmol) and PLE (50 mg, 850 U) were added to phosphate buffer (pH 7, 50 mM, 70 – 100 mL) and additional solvent (0 - 30 mL). The mixture was vigorously stirred at room temperature. The pH was adjusted at the pH determined before the reaction (which is not measured as 7.0 due to effects from the cosolvents) by a Titrino apparatus (addition of 0.2 M sodium hydroxide). After the time given in Table 29, Titrino was stopped and the aqueous solution was worked up as follows:
The aqueous phase was acidified to pH 1 by adding hydrochloric acid (1 M) and extracted with dichloromethane (3 x 150 mL). As shaking the mixture led to frothing and non-separated phases, the emulsion was filtered through a glass frit (pore 3) to separate phases (often two filtrations were necessary). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated. The results are shown on Table 23. The spectral datas are in accordance with those of the racemic compounds *rac*-**79a**-**f**. In some cases some isopropylester side product could be seen.

Table 29: PLE-catalysed desymetrisation of malonates

entry	substrate	cosolvent	t (h)	conversion (%)	yield (%)	% ee
1	**28a**	-	2	quant.	93	56
2[a]	**28a**	-	2	quant.	94	60
3	**28b**	-	19	quant.	84	63
4	**28c**	-	120	79	64	82
5	**28b**	IPA (10%)	28	quant.	99	81
6	**28b**	IPA (20%)	21	quant.	90	86
7	**28b**	IPA (30%)	92	quant.	80	95
8	**28b**	TBA(10%)	19	quant.	95	79
9	**28b**	TBA(20%)	21	quant.	98	86
10	**28b**	TBA(30%)	92	40	21	94
11	**28a**	IPA (30%)	168	quant.	94	93
12	**28c**	IPA (30%)	168	76	58	94
13	**28d**	-	6	quant.	93	63
14	**28d**	IPA (30%)	168	92	n. d.	87
15	**28e**	-	6	quant.	86	14
16	**28e**	IPA (30%)	168	48.	n. d.	14[b]
17	**28f**	TBA(10%), IPA (10%)	4	77	59	3
18	**74a**	TBA(10%), IPA (10%)	24	quant.	0[c]	n. d.

[a] distilled water was used instead of buffer; [b] opposite enantiomer is the major enantiomer; [c] The expected product could not be isolated, instead benzoic acid and deacylatet monoethyl malonate were isolated.

7.2.2.7 Standard operating procedure 24 (SOP24): Hydrolysis applying α-chymotrypsin



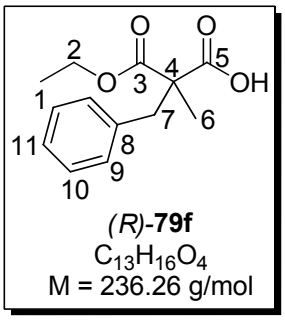
Scheme 106. Enzymatic desymmetrisation applying α-chymotrypsin

Diethyl 2-benzyl-2-methylmalonate (**28f**, 1.0 mmol) and α-chymotrypsin (3200 U) were added to phosphate buffer (pH 7, 50 mM, 75 mL) and DMSO (25 mL). The mixture was vigorously stirred at 40 °C. The pH was adjusted at the pH det ermined before the reaction (which is not measured as 7.0 due to effects from the cosolvent) by a Titrino apparatus (addition of 0.2 M

sodium hydroxide). After 30 hours of stirring the Titrino was stopped and the aqueous solution was worked up as follows:

The aqueous phase was acidified to pH1 by adding hydrochloric acid (1 M) and extracted with MTBE (3 x 100 mL). The unified organic phases were extracted with sodium bicarbonate solution (saturated, 3 x 50 mL). The unified organic phases were acidified to pH 1 by adding hydrochloric acid (1 M) and extracted with MTBE (3 x 100 mL). The product was yielded after drying over magnesium sulfate and evaporation of the solvent.

7.2.2.7.1 (*R*)-Monoethyl 2-benzyl-2-methylmalonate ((*R*)-79f)



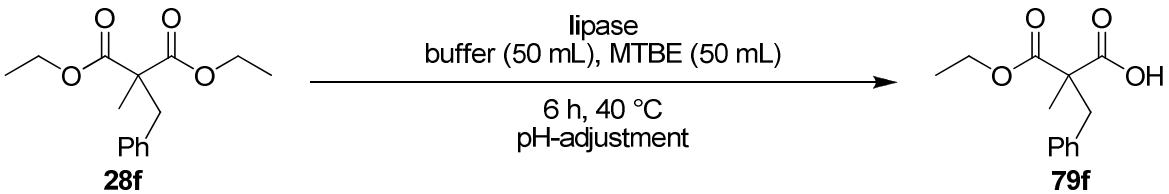
(R)-**79f**
$C_{13}H_{16}O_4$
M = 236.26 g/mol

Diethyl 2-benzyl-2-methylmalonate (**28f**, 265 mg, 1.0 mmol) and α-chymotrypsin (80 mg, 3200 U) were added to phosphate buffer (pH 7, 50 mM, 75 mL) and DMSO (25 mL). The mixture was vigorously stirred at 40 °C. The pH was adjusted at the pH determined before the reaction (which is not measured as 7.0 due to effects from the cosolvent) by a Titrino apparatus (addition of 0.2 M sodium hydroxide). After 30 hours of stirring the Titrino was stopped and the aqueous solution was worked up according to SOP24. Spectroscopic properties are in accordance with those of the racemic compound *rac*-**79f**.

Yield: 28 mg (12%).

Enantiomeric excess: 96% ee.

7.2.2.8 Standard operating procedure 25 (SOP25): Lipase screening on the hydrolysis of 28f



28f **79f**

Scheme 107. Lipase screnning on the hydrolysis of **28f**

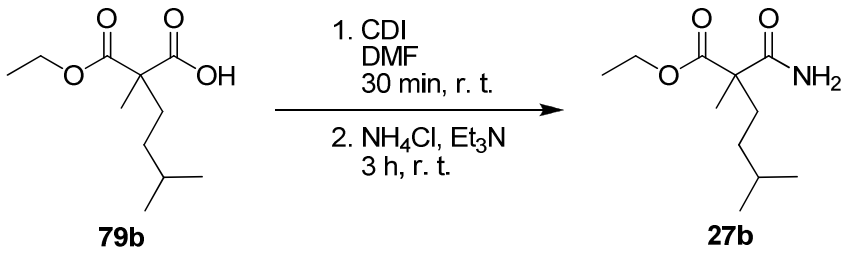
Diethyl 2-benzyl-2-methylmalonate (**28f**, 1.0 mmol) and a lipase (100 mg) were added to phosphate buffer (pH 7, 50 mM, 50 mL) and MTBE (50 mL). The mixture was vigorously stirred at room temperature. The pH was adjusted at the pH determined before the reaction (which is not measured as 7.0 due to effects from the cosolvent) by a Titrino apparatus

(addition of 0.2 M sodium hydroxide). The conversion was monitored by the volume of sodium hydroxide added. The conversion was lower than 2% for all tested enzymes.

List of tested enzymes:

- *Candida antarctica* lipase B
- *Candida rugosa* lipase
- *Candida cylindracea* lipase
- lipase Amano AK 20
- lipase Amano AP6
- lipase Amano AS
- lipase Amano PS-SD
- lipase Amano DF-DS
- lipase Amano AYS
- lipase Amano A
- lipase Amano M
- lipase Amano G

7.2.2.9 Standard operating procedure 26 (SOP26): Synthesis of amide (27b)

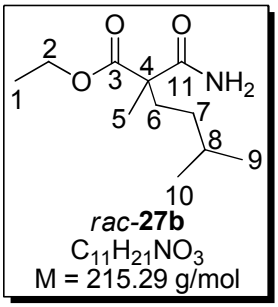


79b **27b**

Scheme 108. Synthesis of amide **27b**

Monoethyl 2-isopentyl-2-methylmalonate (**79b**, 0.58 - 2.0 mmol) was dissolved in DMF (1 - 3 mL) and carbonyldiimidazol (CDI, 1.1 eq.) was added. After stirring this mixture for 30 minutes at room temperature, ammonium chloride (1.2 eq.) and triethylamine (1.2 eq.) were added. The mixture was stirred for additional three hours at room temperature. Water was added and the aqueous phase was extracted with ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate and the solvent was evaporated. The pure product was yielded as colorless oil after column chromatography (cyclohexane - ethyl acetate, 7:3).

7.2.2.9.1 *rac*-Ethyl 2-carbamoyl-2,5-dimethylhexanoate (*rac*-27b)



rac-**27b**
$C_{11}H_{21}NO_3$
M = 215.29 g/mol

rac-2-(Ethoxycarbonyl)-2,5-dimethylhexanoic acid (*rac*-**79b**, 125 mg, 0.58 mmol) was dissolved in DMF (1 mL) and CDI (104 mg, 0.64 mmol) was added. After stirring this mixture for 30 minutes at room temperature, ammonium chloride (38 mg, 0.70 mmol) and triethylamine (97 µL, 71 mg, 0.70 mmol) were added. The mixture was stirred for additional three hours. Work up was done according to SOP26. The pure product was yielded as colorless oil.

Yield: 82 mg (66%), R_f = 0.15.

HPLC (Chiralcel IA-column; hexanes - *i*-PrOH, 97:3, 1.0 mL/min): t_R = 11.8 (*S*), 12.6 (*R*) min.

IR (thin film): 3447, 3352, 3204, 2956, 2871, 2361, 1720, 1667 cm^{-1}.

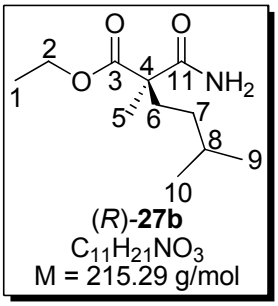
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.84 (d, *J* = 6.6 Hz, 6H, H-C**9**, C**10**), 1.03 - 1.13 (m, 2H, H-C**7**), 1.25 (t, 3H, *J* = 7.1 Hz, H-C**1**), 1.40 (s, 3H, H-C**5**), 1.48 (septet, *J* = 6.6 Hz, 1H, H-C**8**), 1.71 - 1.80 (m, 1H, H-C**6**), 1.88 - 1.95 (m, 1H, H-C**6**), 4.12 - 4.23 (m, 2H, H-C**2**), 5.71 (br s, 1H, NH), 6.88 (br s, 1H, NH).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 14.0 (C**1**), 20.8 (C**5**), 22.3, 22.5 (C**9**, C**10**), 28.2 (C**8**), 33.8, 36.0 (C**6**, C**7**), 53.7 (C**4**), 61.5 (C**2**), 174.1, 174.9 (C**3**, C**11**).

MS (EI) *m/z* = 216 (M^+).

Anal. Calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.83; H, 10.07; N, 6.42.

7.2.2.9.2 (*R*)-Ethyl 2-carbamoyl-2,5-dimethylhexanoate ((*R*)-27b)



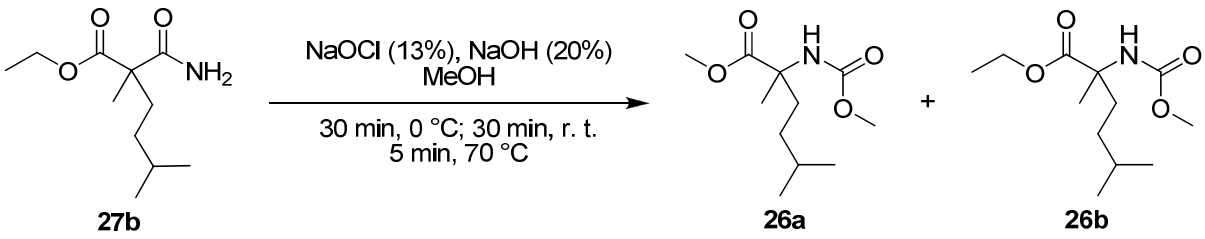
(*R*)-**27b**
$C_{11}H_{21}NO_3$
M = 215.29 g/mol

(*R*)-2-(Ethoxycarbonyl)-2,5-dimethylhexanoic acid ((*R*)-**79b**, 95% ee, 432 mg, 2.0 mmol) was dissolved in DMF (3 mL) and CDI (370 mg, 2.2 mmol) was added. After stirring this mixture for 60 minutes at room temperature, ammonium chloride (129 mg, 2.4 mmol) and triethylamine (333 µL, 243 mg, 2.4 mmol) were added. The mixture was stirred for additional three hours. Work up was done according to SOP26. The pure product was yielded as colorless oil. The spectroscopic properties are in accordance with those of the racemic compound *rac*-**27b**.

Yield: 278 mg (65%).

Enantiomeric excess: 96% ee.

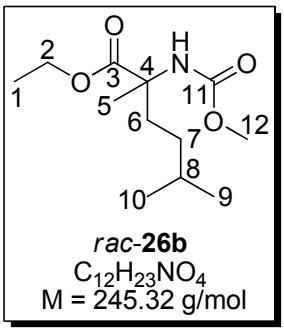
7.2.2.10 Standard operating procedure 27 (SOP27): Hofmann rearrangement



Scheme 109. Hofmann rearrangement

Ethyl 2-carbamoyl-2,5-dimethylhexanoate (**27b**, 0.5 - 1.15 mmol) was dissolved in methanol (1.0 - 2.0 mL) and cooled in an ice-bath. Sodium hydroxide solution (20%, 1.05 eq.) and sodium hypochlorite solution (approx. 13%, 1.1 eq.) were added and the solution was stirred for 30 minutes at ice-bath temperature and for 30 minutes at room temperature. The solution was separated from the precipitated solid and the solid was washed twice using the same amount of methanol as for the reaction. The clear solution was heated in an oil bath at 70 °C for five minutes. Thereafter, the methanol was evaporated. The aqueous solution was diluted and extracted with MTBE (4 x 10 mL). After drying over magnesium sulfate and evaporation of the solvent the crude ester products were obtained.

7.2.2.10.1 *rac*-Ethyl 2-(methoxycarbonylamino)-2,5-dimethylhexanoate (*rac*-26b)



rac-Ethyl 2-carbamoyl-2,5-dimethylhexanoate (*rac*-**27b**, 109 mg, 0.5 mmol) was dissolved in methanol (1.0 mL) and cooled in an ice-bath. Sodium hydroxide solution (20%, 90 µL, 0.53 mmol) and sodium hypochlorite solution (approx. 13%, 300 µL, 0.55 mmol) were added and the solution was stirred for one hour at ice-bath temperature. The solution was sucked off and the solid was washed with additional methanol (3 x 0.4 mL). The clear solution was heated in an oil bath at 70 °C for five minutes. Thereafter, work up was done according to SOP27 leading to a colorless oil. The crude product of the ester *rac*-**26b** also contains the corresponding methyl ester *rac*-**26a** and was used for the synthesis of *rac*-**14b** without further purification.

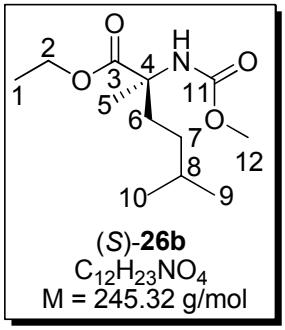
Yield: 77 mg (63%).

HPLC (Chiralcel IA-column; hexanes - *i*-PrOH, 97:3, 1.0 mL/min):;. t_R(**26a**) = 6.5 (*R*), 10.3 (*S*) min; t_R(**26b**) = 5.6 (*R*), 9.5 (*S*) min.

Ratio of esters: **26a** – **26b**, 44:56.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.82 (d, *J* = 6.6 Hz, 6H, H-C**9**, H-C**10**), 0.82 - 0.94 (m, 2H, H-C**7**), 1.07 - 1.18 (m, 1H, H-C**8**), 1.25 (t, 3H, *J* = 7.1 Hz, H-C**1**, **26b**), 1.41 - 1.50 (m, 1H, H-C**6**), 1.53 (s, 3H, H-C**5**), 1.68 - 1.75 (m, 1H, H-C**6**), 3.61 (s, 3H, H-C**12**), 3.73 (s, 3H, H-C**2**, **26a**), 4.12 - 4.25 (m, 2H, H-C**2**, **26b**), 5.48 - 5.53 (br d, 1H, NH).

7.2.2.10.2 (*S*)-Ethyl 2-(methoxycarbonylamino)-2,5-dimethylhexanoate ((*S*)-26b)



(*S*)-**26b**
$C_{12}H_{23}NO_4$
M = 245.32 g/mol

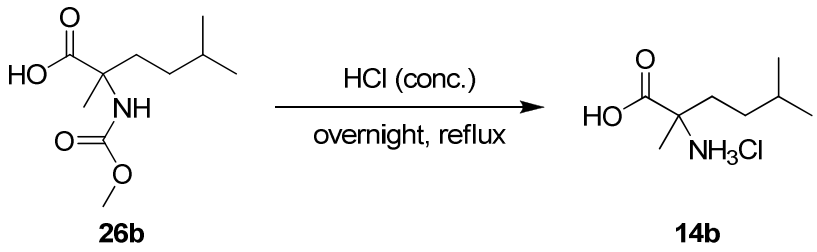
(*R*)-Ethyl 2-carbamoyl-2,5-dimethylhexanoate ((*R*)-**27b**, 96% ee, 249 mg, 1.15 mmol) was dissolved in methanol (2 mL) and cooled in an ice-bath. Sodium hydroxide solution (20%, 205 µL, 1.21 mmol) and sodium hypochlorite solution (approx. 13%, 693 µL, 1.27 mmol) were added and the solution was stirred for 30 minutes at ice-bath temperature and for 30 minutes at room temperature. The solution was sucked off and the solid was washed with additional methanol (3 x 0.7 mL). The clear solution was heated in an oil bath at 70 °C for five minutes. Thereafter, work up was done according to SOP27 leading to a colorless oil. The crude product of the ester (*S*)-**26b** also contains the corresponding methyl ester (*S*)-**26a** and was used for the synthesis of (*S*)-**14b** without further purification. The spectroscopic properties are in accordance with those of the racemic compound *rac*-**26b**.

Yield: 198 mg (71%).

Ratio of esters: **26a** - **26b**, 30:70.

Enantiomeric excess: 95% ee for both esters.

7.2.2.11 Standard operating procedure 28 (SOP28): Hydrolysis to amino acid hydrochloride (14b)

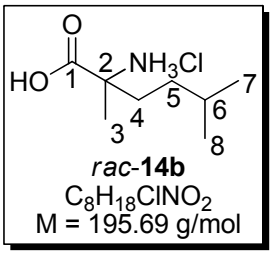


26b **14b**

Scheme 110. Hydrolysis to amino acid hydrochloride **14b**

Ethyl 2-(methoxycarbonylamino)-2,5-dimethylhexanoate (**26b**, 0.33 - 0.55 mmol) was dissolved in hydrochloric acid (conc., 15 - 20 mL) and stirred under reflux overnight. After cooling to room temperature, the mixture was extracted with MTBE (2 x 10 mL). The aqueous solution was evaporated to dryness yielding a white solid.

7.2.2.11.1 *rac*-2-Amino-2,5-dimethylhexanoic acid hydrochloride (*rac*-14b)



rac-**14b**
$C_8H_{18}ClNO_2$
M = 195.69 g/mol

rac-Ethyl 2-(methoxycarbonylamino)-2,5-dimethylhexanoate (*rac*-**26b**, 70 mg, 0.34 mmol) was dissolved in concentrated hydrochloric acid (15 mL) and stirred under reflux overnight. After cooling to room temperature, the work up was done according to SOP28.

Yield: 66mg (quantitative).

^{1}H-NMR (400 MHz, D_2O): δ [ppm] = 0.84 (d, *J* = 6.6 Hz, 6H, H-C**7**, H-C**8**), 1.05 - 1.14 (m, 1H, H-C**5**), 1.20 - 1.30 (m, 1H, H-C**5**), 1.49 - 1.55 (m, 1H, H-C**6**), 1.53 (s, 3H, H-C**3**), 1.76 - 1.84 (m, 1H, H-C**4**), 1.89 - 1.97 (m, 1H, H-C**4**).

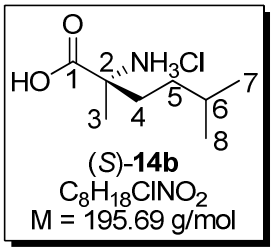
^{13}C-NMR (100 MHz, D_2O): δ [ppm] = 21.98, 22.03 (C**7**, C**8**), 22.4 (C**3**), 27.9 (C**5**), 32.3 (C**5**), 35.3 (C**6**), 61.1 (C**2**), 175.5 (C**1**).

IR (thin film): 3121, 3022, 2957, 2749, 1722, 1592, 1499 cm^{-1}.

MS (MALTI-TOF, sin-matrix): 160 (M^+).

Anal. Calcd for $C_8H_{18}ClNO_2$: C, 49.10; H, 9.27; N, 7.16. Found: C, 47.82; H, 9.18; N, 7.01.

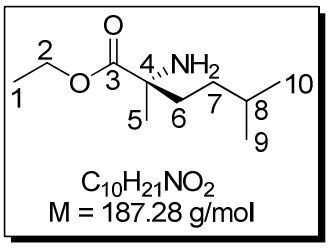
7.2.2.11.2 (*S*)-2-Amino-2,5-dimethylhexanoic acid hydrochloride ((*S*)-14b)



(*S*)-Ethyl 2-(methoxycarbonylamino)-2,5-dimethylhexanoate ((*S*)-**14b**, 95% ee, 122 mg, 0.5 mmol) was dissolved in concentrated hydrochloric acid (20 mL) and stirred under reflux overnight. After cooling to room temperature, work up was done according to SOP28. The spectroscopic properties are in accordance with those of the racemic compound *rac*-**14b**.

Yield: 98 mg (quantitative).

The enantiomeric excess was proofed to be >95% ee by synthesis of the corresponding ethyl ester and chiral HPLC of the ester, which was done as follows:



2-Amino-2,5-dimethylhexanoic acid hydrochloride (**14b**, 30 mg, 0.15 mmol) was suspended in ethanol (4.5 mL) and cooled in an ice-bath. After addition of thionyl chloride (16 µL, 24 mg, 0.22 mmol) the solution was allowed to warm to room temperature. The mixture was refluxed for three hours and cooled to room temperature. Then concentrated sodium bicarbonate solution (10 mL) was added and the mixture was extracted with dichloromethane (3 x 20 mL). After drying over magnesium sulfate and carefully evaporating the solvent the ethyl ester was obtained.

Enantiomeric excess: 95% ee

HPLC (Chiralcel AD-H-column; hexanes - *i*-PrOH, 99:1, 0.1% DEA, 1.0 mL/min): t_R = 11.8 (*S*), 12.6 (*R*) min.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.85 (2d, *J* = 6.6 Hz, 6H, H-C**9**, H-C**10**), 0.97 - 1.06 (m, 1H, H-C**7**), 1.12 - 1.20 (m, 1H, H-C**7**), 1.24 (t, *J* = 7.1 Hz, 3H, H-C**1**), 1.44 - 1.56 (m, 1H, H-C**8**), 1.28 (s, 3H, H-C**5**), 1.64 - 1.74 (m, 2H, H-C**6**), 4.07 - 4.17 (m, 2H, H-C**2**).

^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 14.2 (C**1**), 22.4, 22.5 (C**9**, C**10**), 26.4 (C**6**), 28.3 (C**5**), 33.1 (C**7**), 38.9 (C**8**), 57.7 (C**4**), 60.9 (C**2**), 177.8 (C**3**).

7.2.3 Kinetic resolution of azo compounds

7.2.3.1 Standard operating procedure 29 (SOP29): Analytic data for the kinetic resolution

The conversion was determined by ^{1}H-NMR spectroscopy, comparing the integrals of the signals of the aromatic protons at 7.85 ppm (**32a**, see spectral data below) and 7.72 ppm (**31a**, see spectral data below). Enantiomeric excesses of both compounds were examined by chiral HPLC using the methods given below.

7.2.3.1.1 *rac*-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-32a)

The racemic compound was synthesised by Prechter according to a literature protocol.[134] Spectroscopic data are in accordance with literature (see reference [25b]).

HPLC (Chiralcel AD-H-column; hexanes - *i*-PrOH, 99:1, 0.6 mL/min): t_R = 37.4, 39.1 min.

^{1}H-NMR (300 MHz, Benzol-d_6): δ [ppm] = 1.32 (s, 3H), 1.63 (s, 3H), 3.07 (d, *J* = 13.7 Hz, 1H), 3.16 (d, *J* = 13.3 Hz, 1H), 3.16 (s, 3H), 3.25 (s, 3H), 4.56 (d, *J* = 11.2 Hz, 1H), 4.62 (d, *J* = 11.2 Hz, 1H), 6.68 - 6.72 (m, 4H), 6.99 (d, *J* = 8.3 Hz, 2H), 7.85 (d, *J* = 8.6 Hz, 2H).

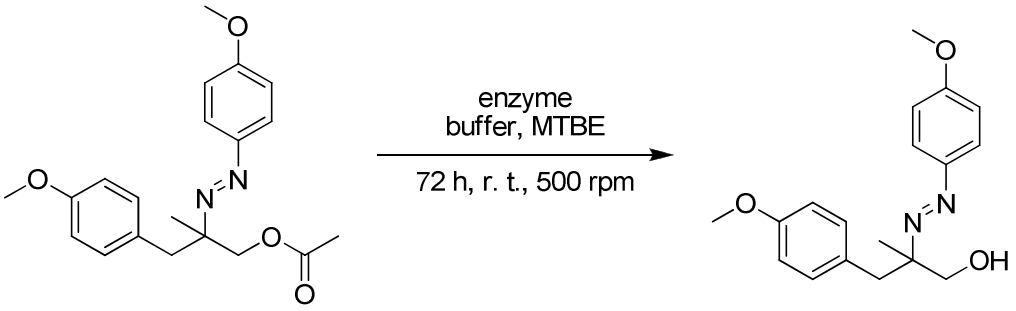
7.2.3.1.2 *rac*-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropan-1-ol (*rac*-31a)

The racemic compound was synthesised by Prechter according to a literature protocol.[134] Spectroscopic data are in accordance with literature (see reference [25b]).

HPLC (Chiralcel AD-H-column; hexanes - *i*-PrOH, 90:10, 0.8 mL/min): t_R = 14.5, 19.3 min.

^{1}H-NMR (300 MHz, Benzol-d_6): δ [ppm] = 1.27 (s, 3H), 2.34 (m, 1H), 3.02 (d, *J* = 13.3 Hz, 1H), 3.19 (d, *J* = 13.3 Hz, 1H), 3.19 (s, 3H), 3.29 (s, 3H), 6.70 - 6.76 (m, 4H), 7.17 (m, 2H), 7.72 (d, *J* = 9.0 Hz, 2H).

7.2.3.2 Standard operating procedure 30 (SOP30): Lipase screening



rac-**32a** **31a**

Scheme 111. Lipase screening

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-**32a**, 142 mg, 0.4 mmol) was dissolved in MTBE (16 mL). 1 mL of this solution (containing 8.9 mg, 0.25 mmol of *rac*-**32a**) was given to mixtures of an enzyme formulation (10 mg) in buffer (pH 7, 50 mM, 1 mL) in a test tube. The test tubes were stoppered and the mixtures shaken (500 rpm) at room temperature for 72 hours. The organic phase was examined by thin layer chromatography (cyclohexane - ethyl acetate, 3:1). The pure product (*rac*-**31a**, R_f = 0.29) and the pure substrate (*rac*-**32a**, R_f = 0.50) were added as references. The activities of the enzymes were determined by the appearance of product **31a**.

Table 30: Lipase Screening

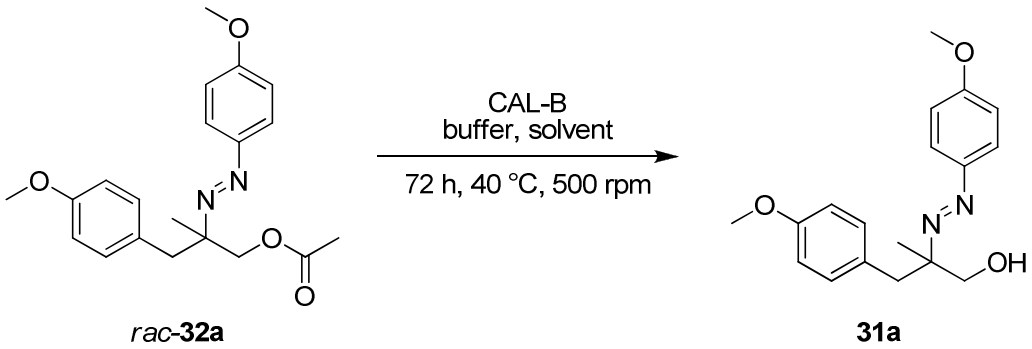
enzyme	substrate **32a**	product **31a**
none	yes	no
Candida antarctica lipase B	yes	yes
Candida rugosa lipase	yes	no
Candida cylindracea lipase	yes	no
Amano Acylase *Aspergillus mellus*	yes	no
lipase Amano AK 20	yes	no
lipase Amano AP6	yes	no
lipase Amano AS	yes	no
lipase Amano PS-IM	yes	no
lipase Amano PS-SD	yes	no
lipase Amano DF-DS	yes	no
lipase Amano AYS	yes	few
lipase Amano A	yes	no
lipase Amano M	yes	no
lipase Amano G	yes	no

The organic layers of both test tubes with product (CAL-B, Amano AYS) and of the reference without enzyme were separated and each aqueous phase was extracted once with ethyl acetate. Both organic phases of each test tube were unified, dried over magnesium sulfate and the solvent was evaporated. The conversion was determined by means of ^{1}H-NMR-spectroscopy, the enantiomeric excess of the product **31a** of the CAL-B-catalysed reaction was determined by HPLC-chromatography.

Conversion: without enzyme < 2%; Amano AYS 2%; CAL-B 9%.

Enantiomeric excess: CAL-B: 87% ee.

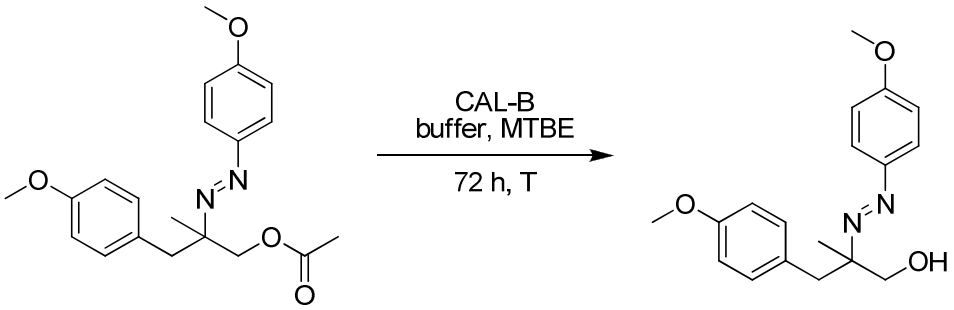
7.2.3.3 Standard operating procedure 31 (SOP31): Solvent screening



Scheme 112. Solvent screening

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-**32a**, 9 mg, 0.025 mmol) was dissolved in 0.9 mL of the solvent given in Table 16 in a test tube. CAL-B (20 mg) and buffer (pH 7, 50 mM, 0.9 mL) was added. The test tube was stoppered and the mixture shaken (500 rpm) at 40 °C for 72 hours. The organic phase was separated and the aqueous phase extracted with MTBE (1 mL). The unified organic phases were dried over magnesium sulfate and the solvent evaporated. The conversion was determined by means of ^{1}H-NMR-spectroscopy, the enantiomeric excess of the product **31a** was determined by chiral HPLC-chromatography. The results are shown in Table 16.

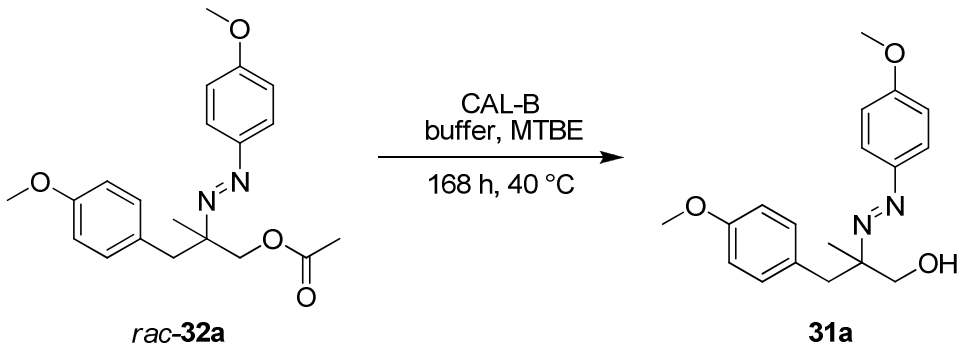
7.2.3.4 Standard operating procedure 32 (SOP32): Influence of temperature on the kinetic resolution



Scheme 113. Influence of temperature on the hydrolysis of **32a**

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-**32a**, 36 mg, 0.1 mmol) in MTBE (4 mL) was given to mixtures of CAL-B (80 mg) in buffer (pH 7, 50 mM, 4 mL) in a 25 mL round bottom flask. The flask was stoppered and the mixtures stirred at the temperatures given in Table 17 for 72 hours. The phases were separated and the aqueous phase was extracted with MTBE (2 x 15 mL). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated. The conversion was determined by means of ^{1}H-NMR-spectroscopy, the enantiomeric excesses of product **31a** and substrate **32a** were determined by chiral HPLC-chromatography after column chromatography (cyclohexane - ethyl acetate, 3:1). The results are shown in Table 17.

7.2.3.5 Standard operating procedure 33 (SOP33): Influence of enzyme loading on the kinetic resolution:

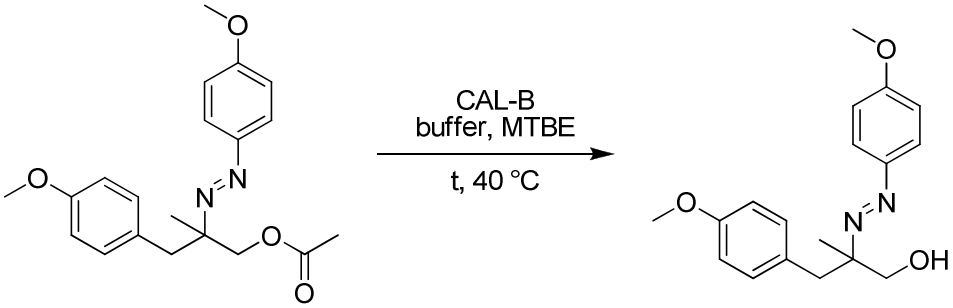


Scheme 114. Influence of enzyme loading

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-**32a**, 36 mg, 0.1 mmol) in MTBE (4 mL) was given to mixtures of CAL-B (amount given in Table 18) in buffer (pH 7, 50 mM, 4 mL) in a 25 mL round bottom flask. The flask was stoppered

and the mixtures stirred at 40 °C for 168 hours. The phases were separated and the aqueous phase was extracted with MTBE (2 x 15 mL). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated. The conversion was determined by means of ^{1}H-NMR-spectroscopy, the enantiomeric excesses of product **31a** and substrate **32a** were determined by chiral HPLC-chromatography after column chromatography (cyclohexane - ethyl acetate, 3:1). The results are shown in Table 18.

7.2.3.6 Standard operating procedure 34 (SOP34): Determination of the enantiomeric ratio E in the standard experiment (kinetic resolution)



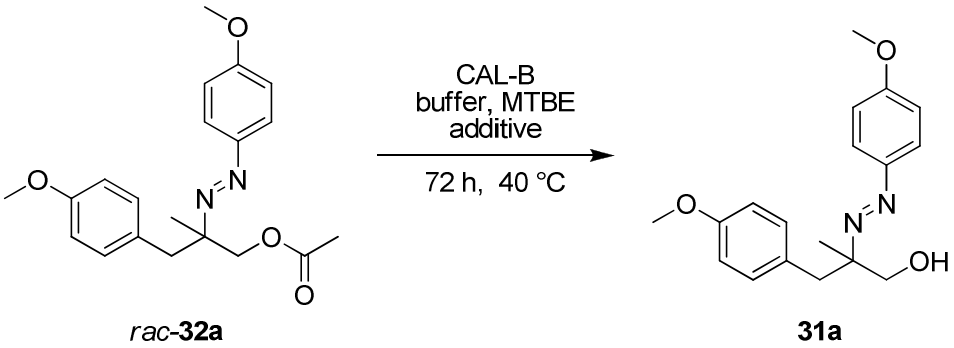
rac-**32a** **31a**

Scheme 115. Standard experiment

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-**32a**, 36 mg, 0.1 mmol) in MTBE (4 mL) was given to a mixture of CAL-B (80 mg) in buffer (pH 7, 50 mM, 4 mL) in a 25 mL round bottom flask. The flask was stoppered and the mixture stirred at 40 °C for the time given in Table 19 or Scheme 68, respectively. In one case additional CAL-B (80 mg) was added after 6 days of stirring. The phases were separated and the aqueous phase was extracted with MTBE (2 x 15 mL). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated. The conversion was determined by means of ^{1}H-NMR-spectroscopy, the enantiomeric excesses of product **31a** and substrate **32a** were determined by chiral HPLC-chromatography after column chromatography (cyclohexane - ethyl acetate, 3:1). The results are shown in Table 19 or Scheme 68, respectively.

The same reaction was applied in a 1.0 mmol scale. The results are shown in Scheme 70.

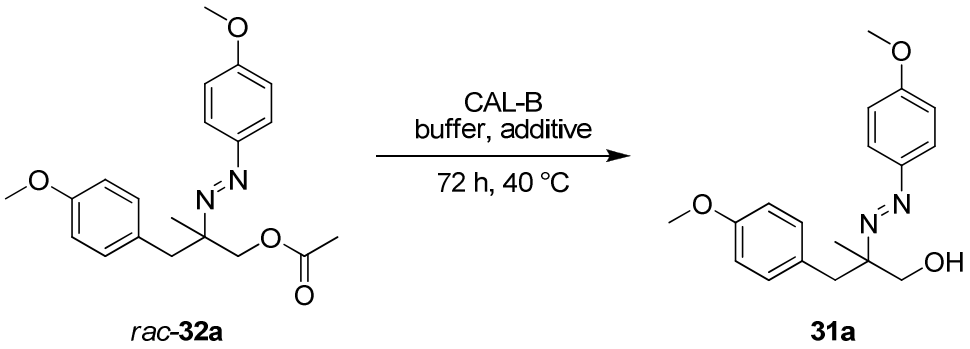
7.2.3.7 Standard operating procedure 35 (SOP35): Influence of additives on the kinetic resolution



Scheme 116. Influence of additives

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-**32a**, 36 mg, 0.1 mmol) in MTBE (4 mL) was given to mixtures of CAL-B (80 mg) in buffer (pH 7, 50 mM, 4 mL) in a 25 mL round bottom flask. The additional solvent given (1 mL) was added, the flask was stoppered and the mixture was stirred at 40 °C for 72 hours. The phases were separated and the aqueous phase was extracted with MTBE (2 x 15 mL). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated. The conversion was determined by means of ^{1}H-NMR-spectroscopy, the enantiomeric excesses of product **31a** and substrate **32a** were determined by chiral HPLC-chromatography after column chromatography (cyclohexane - ethyl acetate, 3:1). The results are shown in Table 20.

7.2.3.8 Standard operating procedure 36 (SOP36): MTBE-free kinetic resolution

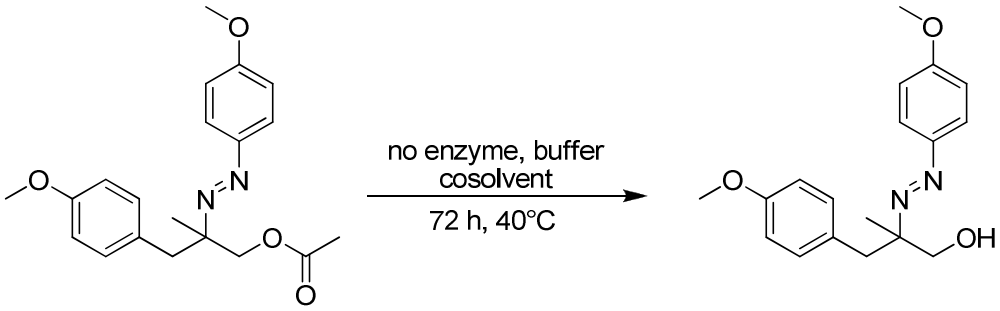


Scheme 117. MTBE-free kinetic resolution

The additive (1 mL, given in Table 21) was added to *rac*-3-(4-methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (**32a**, 36 mg, 0.1 mmol) in a 25 mL round bottom flask. CAL-B (80 mg) and buffer (pH 7, 50 mM, 4 mL) was added. The flask was stoppered and the mixtures stirred at 40 °C for 72 hours. The phases were separated and

the aqueous phase was extracted with MTBE (2 x 15 mL). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated. The conversion was determined by means of ^{1}H-NMR-spectroscopy, the enantiomeric excess of the product **31a** was determined by HPLC. The results are shown in Table 21.

7.2.3.9 Standard operating procedure 37 (SOP37): Examination of the background reaction of the kinetic resolution



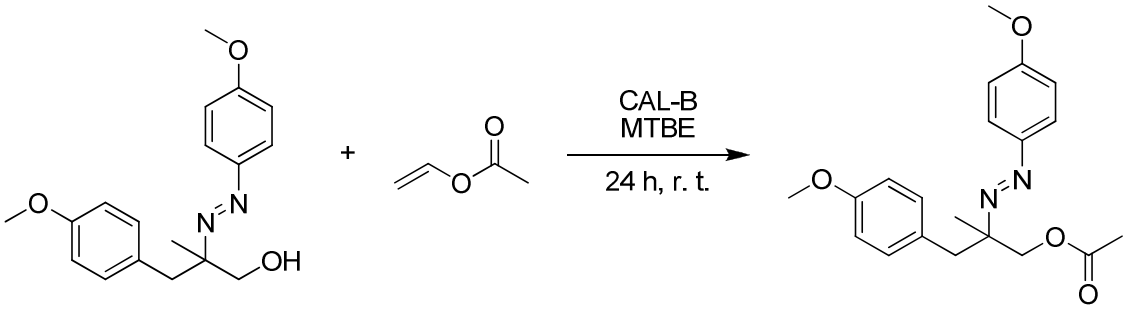
Scheme 118. Examination of the background reaction

The solvent (type and amount given in Table 31) was added to *rac*-3-(4-methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-**32a**, 36 mg, 0.1 mmol) in a 25 mL round bottom flask. Buffer (pH 7, 50 mM, 4 mL) was added, the flask was stoppered and the mixture was stirred at 40 °C for 72 hours. The phases were separated and the aqueous phase was extracted with MTBE (2 x 15 mL). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated. The conversion was determined by means of ^{1}H-NMR-spectroscopy. The results are shown in Table 31.

Table 31: Examination of the background reaction

entry	additive	conversion (%)
1	none	< 1
2	MTBE (4 mL)	< 1
3	DMSO (1 mL)	< 1

7.2.3.10 Standard operating procedure 38 (SOP38): CAL-B catalysed formation of the ester 32a

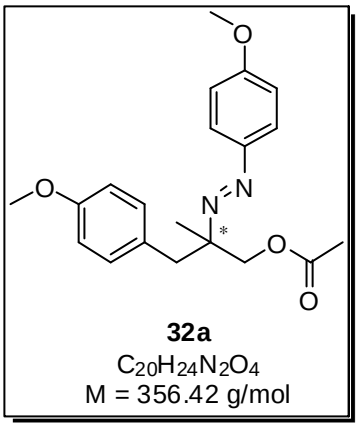


rac-**31a** **32a**

Scheme 119. CAL-B catalysed formation of the ester **32a**

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropan-1-ol (*rac*-**31a**, 31 mg 0.1 mmol) in MTBE (4 mL) was added to CAL-B (80 mg). After addition of vinyl acetate (5.6 µL, 5.2 mg, 0.06 mmol) the mixture was stirred at room temperature for 24 hours. The enzyme was removed by filtration and the solvent was evaporated, yielding yellow oil. The conversion was determined by means of ^{1}H-NMR spectroscopy. The enantiomeric excesses of product **32a** and substrate **31a** were determined by chiral HPLC-chromatography after column chromatography (cyclohexane - ethyl acetate, 3:1). The sperctroscopic properties are in accordance with those of the racemic compounds *rac*-**32a** and *rac*-**31a**.

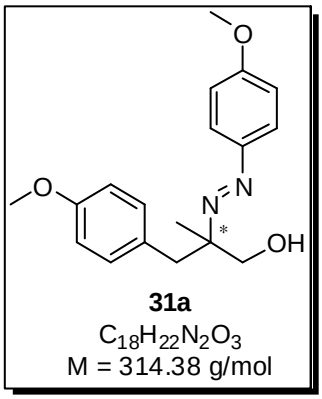
Conversion: 30%.



32a
$C_{20}H_{24}N_2O_4$
M = 356.42 g/mol

Yield: 4 mg (13%).

Enantiomeric excess: 11% ee.

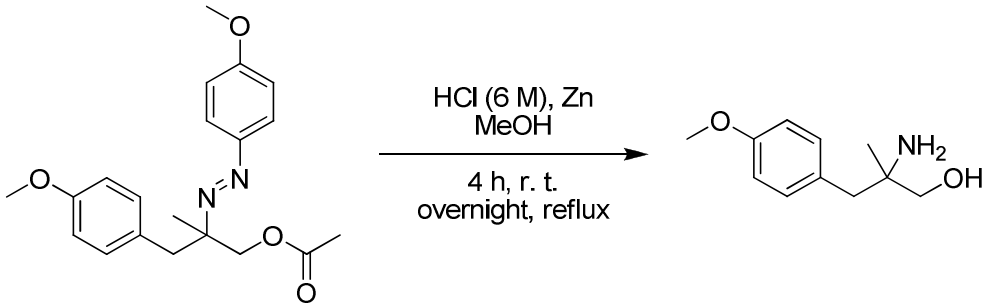


31a
$C_{18}H_{22}N_2O_3$
M = 314.38 g/mol

Yield 12 mg (39%).

Enantiomeric excess: 2% ee.

7.2.3.11 Standard operating procedure 39 (SOP39): Deprotection to amino alcohol 30a (ester 32a as starting material)

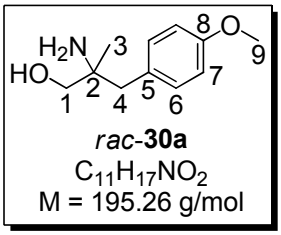


32a **30a**

Scheme 120. Deprotection of ester **32a**

3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (**32a**, 0.5 mmol) was dissolved in methanol (5 mL). Zinc-dust (1.0 g) and hydrochloric acid (6 M, 5 mL) were added. After one hour stirring at room temperature, additional hydrochloric acid (6 M, 5 mL) was added. The mixture was stirred for additional three hours at room temperature and was then refluxed overnight. The pH was adjusted with sodium hydroxide (20%) to pH >12 and the aqueous solution was extracted with dichloromethane (4 x 50 mL). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated. The pure product was yielded after column chromatography (dichloromethane - methanol, 5:1, 0.2% DEA).

7.2.3.11.1 *rac*-2-Amino-3-(4-methoxyphenyl)-2-methylpropan-1-ol (*rac*-30a)



rac-**30a**
$C_{11}H_{17}NO_2$
M = 195.26 g/mol

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropyl acetate (*rac*-**32a**, 178 mg, 0.5 mmol) was dissolved in methanol (5 mL). Zinc-dust (1.0 g) and hydrochloric acid (6 M, 5 mL) were added. After one hour stirring at room temperature, additional hydrochloric acid (6 M, 5 mL) was added. The mixture was stirred for

additional three hours at room temperature and then refluxed overnight. Work up was done according to SOP39, yielding a yellowish solid.

Yield: 93 mg (95%).

HPLC (Chiralcel AD-H-column; hexanes - *i*-PrOH, 95:5, 0.1% DEA; 1.0 mL/min): t_R = 21.0, 24.5 min.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.03 (s, 3H, H-C**3**), 2.07 (br s, 3H, OH, NH), 2.62 - 2.73 (m, 2H, H-C**4**), 3.31, 3.35 (2d, *J* = 10.6 Hz, 2H, H-C**1**), 3.79 (s, 3H, H-C**9**), 6.84 (d, *J* = 8.6 Hz, 2H, H-C**7**), 7.11 (d, *J* = 8.6 Hz, 2H, H-C**6**).

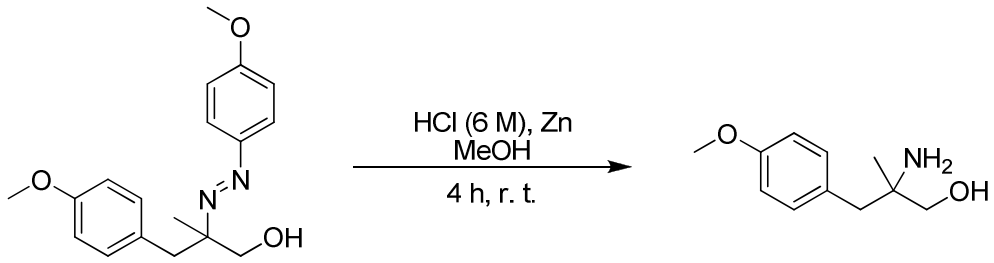
^{13}C-NMR (100 MHz, $CDCl_3$): δ [ppm] = 24.6 (C**3**), 44.7 (C**2**), 53.6 (C**4**), 55.2 (C**9**), 69.5 (C**1**), 113.6 (C**7**), 129.3 (C**5**), 131.4 (C**6**), 158.3 (C**8**).

MS (EI): 195 (M^+), 164 (M^+-OMe).

IR (thin film): 3335, 3279, 2933, 1746, 1669, 1610, 1511 cm^{-1}.

Anal. Calcd for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.76; H, 8.99; N, 7.24.

7.2.3.12 Standard operating procedure 40 (SOP40): Deprotection to amino alcohol 30a (alcohol 31a as starting material)



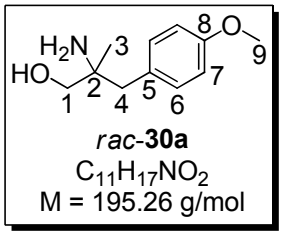
31a **30a**

Scheme 121. Deprotection of alcohol **31a**

3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropan-1-ol (**31a**, 0.1 mmol) was dissolved in methanol (1 mL). Zinc-dust (150 mg) and hydrochloric acid (6 M, 2 mL) were added. After four hours stirring at room temperature, the pH was adjusted with sodium hydroxide (20%) to pH >12 and the aqueous solution was extracted with dichloromethane (4 x 10 mL). The unified organic phases were dried over magnesium sulfate and the solvent

was evaporated. The pure product was yielded after column chromatography (dichloromethane - methanol, 5:1, 0.2% DEA).

7.2.3.12.1 *rac*-2-Amino-3-(4-methoxyphenyl)-2-methylpropan-1-ol (*rac*-30a)

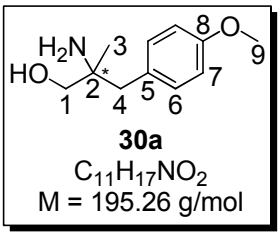


rac-**30a**
$C_{11}H_{17}NO_2$
M = 195.26 g/mol

rac-3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropan-1-ol (*rac*-**31a**, 629 mg, 2.0 mmol) was dissolved in methanol (20 mL). Zinc-dust (4.0 g) and hydrochloric acid (6 M, 20 mL) were added. After four hours stirring at room temperature, work up was done according to SOP40. Spectroscopic data are in accordance with spectroscopic data of the product obtained by deprotection of **32a**.

Yield: 223 mg (57%)

7.2.3.12.2 2-Amino-3-(4-methoxyphenyl)-2-methylpropan-1-ol (30a)



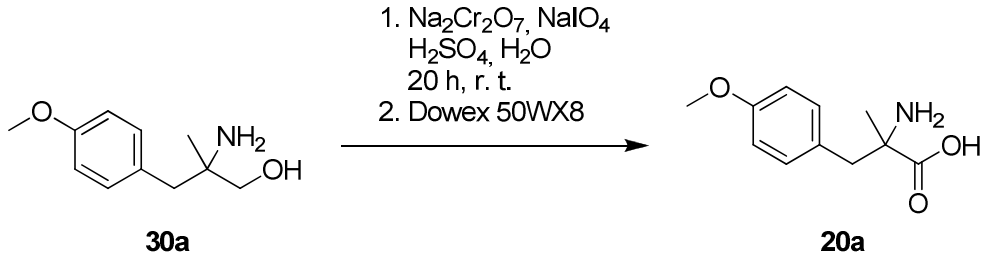
30a
$C_{11}H_{17}NO_2$
M = 195.26 g/mol

3-(4-Methoxyphenyl)-2-((4-methoxyphenyl)diazenyl)-2-methylpropan-1-ol (**31a**, 87% ee, 34 mg, 0.11 mmol) was dissolved in methanol (1 mL). Zinc-dust (150 mg) and hydrochloric acid (6 M, 2 mL) were added. After four hour stirring at room temperature, work up was done according to SOP 40, yielding a yellowish solid. Spectroscopic data are in accordance with spectroscopic data of the racemic compound *rac*-**30a**.

Yield: 19 mg (89%).

Enantiomeric excess: 86% ee.

7.2.3.13 Standard operating procedure 41 (SOP41): Oxidation to α,*O*-dimethyl tyrosine (20a)



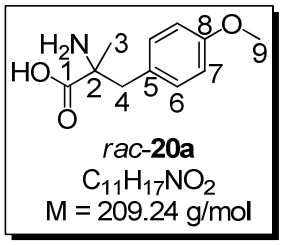
30a **20a**

Scheme 122. Oxidation to α,*O*-dimethyl tyrosine (**20a**)

Sodium dichromate (5 mol%), sodium periodate (2.7 eq.) and sulfuric acid (1.0 eq.) were dissolved in water (1.0 - 2.5 mL) and 2-amino-3-(4-methoxyphenyl)-2-methylpropanol

(0.03 - 0.25 mmol) were added at room temperature. After 20 hours of stirring at room temperature, Dowex 50WX8 in water was added to the reaction mixture and the suspension was stirred for additional 60 minutes at room temperature. Thereafter, the ion exchange resin was washed with water and the product was eluted applying aqueous ammonia (1 M) as an eluent. The product is yielded after evaporation of the water.

7.2.3.13.1 *rac*–α,*O*-Dimethyl tyrosine (*rac*-20a)



rac-**20a**
$C_{11}H_{17}NO_2$
M = 209.24 g/mol

Sodium dichromate (6 mg, 0.025 mmol), sodium periodate (290 mg, 1.35 mmol) and sulfuric acid (93 µL, 0.5 mmol) were dissolved in water (2.5 mL) and *rac*-2-amino-3-(4-methoxyphenyl)-2-methylpropanol (*rac*-**30a**, 98 mg, 0.25 mmol) was added at room temperature. After 20 hours of stirring at room temperature, Dowex 50WX8 in water was added to the reaction mixture and the suspension was stirred for additional 60 minutes at room temperature. Thereafter, work up was done according to SOP41.

Yield: 37 mg (71%).

^{1}H-NMR (400 MHz, D_2O): δ [ppm] = 1.49 (s, 3H, H-C**3**), 2.88, 3.20 (2d, *J* = 14.3, 2H, H-C**4**), 3.78 (s, 3H, H-C**9**), 6.94 (d, *J* = 8.5 Hz, 2H, H-C**7**), 7.17 (d, *J* = 8.5 Hz, 2H, H-C**6**).

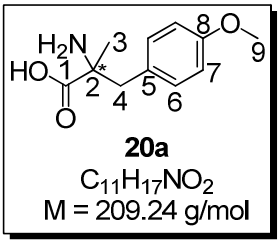
^{13}C-NMR (100 MHz, D_2O): δ [ppm] = 20.3 (C**3**), 39.9 (C**4**), 53.3 (C**9**), 60.1 (C**2**), 112.3 (C**7**), 124.8 (C**5**), 129.2 (C**6**), 156.3 (C**8**), 174.4 (C**1**).

MS (EI): 210 (MH^+), 232 (MNa^+), 248 (MK^+).

IR (thin film): 2931, 1596, 1512 cm^{-1}.

Spectroscopic data are in accordance with literature (see reference [135]).

7.2.3.13.2 α,*O*-Dimethyl tyrosine (20a)



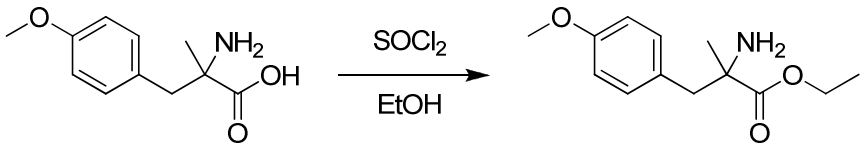
20a
$C_{11}H_{17}NO_2$
M = 209.24 g/mol

Sodium dichromate (2.4 mg, 0.01 mmol), sodium periodate (116 mg, 0.54 mmol) and sulfuric acid (37 µL, 0.2 mmol) were dissolved in water (1 mL). 162 µL of this mixture were added to 2-amino-3-(4-methoxyphenyl)-2-methylpropanol (**30a**, 86% ee, 6.3 mg, 0.03 mmol). After 20 hours of stirring at room temperature, Dowex 50WX8 in water was added to the reaction mixture and the suspension was stirred for

additional 60 minutes at room temperature. Thereafter, work up was done according to SOP41. Spectroscopic data are in accordance with those of the racemic product *rac*-**20a** and with literature (see reference [135]).
Yield: 7 mg (quantitative).

Enantiomeric excess: 86% ee (determined after conversion to the corresponding ethyl ester **100**, see below).

7.2.3.14 Standard operating procedure 42 (SOP42): Esterification of α,*O*-dimethyl tyrosine

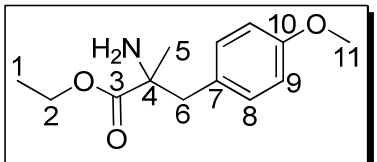


20a **100**

Scheme 123. Esterification of α,*O*-dimethyl tyrosine (**20a**)

α,*O*-Dimethyl tyrosine (**20a**, 0.025 - 0.05 mmol) was suspended in ethanol (2 mL) and cooled to -10 °C. Thionyl chloride (0.1 mmol) was added and the mixture was allowed to warm to room temperature. After three hours of refluxing and cooling to room temperature, sodium bicarbonate solution (saturated, 10 mL) was added. The aqueous solution was extracted with dichloromethane (3 x 10 mL). The unified organic phases were dried over magnesium sulfate and the solvent was evaporated, yielding a crude product containing the ethyl ester **100**.

7.2.3.14.1 *rac*-Ethyl α,*O*-dimethyl tyrosine (*rac*-100)



rac-**100**
$C_{13}H_{19}NO_3$
M = 237.29 g/mol

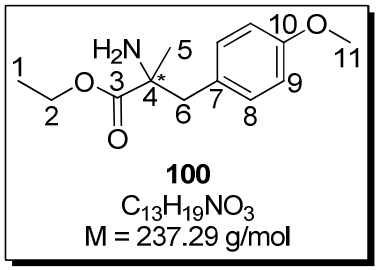
rac-α,*O*-Dimethyl tyrosine (*rac*-**20a**, 10 mg, 0.05 mmol) was suspended in ethanol (2 mL) and cooled to -10 °C. Thionyl chloride (8 µL, 12 mg, 0.1 mmol) was added and the mixture was allowed to warm to room temperature. After three hours of refluxing and cooling to room temperature, work up was done according to SOP42.

HPLC (Chiralcel AD-H-column; hexanes - *i*-PrOH, 99:1, 0.1% DEA; 1.0 mL/min): t_R = 30.4, 35.2 min.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.25 (t, *J* = 7.1 Hz, 3H, H-C**1**), 1.36 (s, 3H, H-C**5**), 1.62 (2H, br s, NH), 2.71, 3.06 (2d, *J* = 13.3, 2H, H-C**6**), 3.76 (s, 3H, H-C**11**), 4.14 (2H, *J* = 7.1 Hz, q, H-C**2**), 6.79 (d, *J* = 8.6 Hz, 2H, H-C**9**), 7.06 (d, *J* = 8.6 Hz, 2H, H-C**8**).

Spectroscopic data are in accordance with literature (see reference [136]).

7.2.3.14.2 Ethyl α,*O*-dimethyl tyrosine (100)



100
$C_{13}H_{19}NO_3$
M = 237.29 g/mol

α,*O*-Dimethyl tyrosine (**20a**, 86% ee, 5 mg, 0.025 mmol) was suspended in ethanol (2 mL) and cooled to -10 °C. Thionyl chloride (8 µL, 12 mg, 0.1 mmol) was added and the mixture was allowed to warm to room temperature. After three hours of refluxing and cooling to room temperature, work up was done according to SOP42. Spectroscopic data are in accordance with spectroscopic data of the racemic compound *rac*-**100** and with literature (see reference [136]).

Enantiomeric excess: 86% ee.

8 References

[1] For a recent review see: S. Freeland, *"Terrestrial" Amino Acids and their Evolution* in: *Amino Acids, Peptides and Proteins in Organic Chemistry – Origins and Synthesis of Amino Acids*, Wiley-VCH, Weinheim, Germany **2009**, *1*, 43-75.

[2] W. Leuchtenberger, K. Huthmacher, K. Drauz, *Appl. Microbiol. Biotechnol.* **2005**, *69*, 1-8.

[3] For recent reviews see: a) H. Gröger, F. R. Dietz, *Biocatalytic Synthesis of Natural and Non-natural α-Amino Acids* in: *Wiley Encyclopedia of Chemical Biology*, John Wiley & Sons, Hoboken, NJ, **2009**, *1*, 191-204; b) T. Sonke, B. Kaptein, H. E. Schoemaker, *Use of Enzymes in the Synthesis of Amino Acids* in: *Amino Acids, Peptides and Proteins in Organic Chemistry – Origins and Synthesis of Amino Acids*, Wiley-VCH, Weinheim, Germany, **2009**, *1*, 79-117; c) S. A. Habay, S. S. Park, S. M. Kennedy, A. R. Chamberlin, *Methods for the Chemical Synthesis of Noncoded α-Amino Acids found in Natural Product Peptides* in: *Amino Acids, Peptides and Proteins in Organic Chemistry – Origins and Synthesis of Amino Acids*, Wiley-VCH, Weinheim, Germany **2009**, *1*, 163-243; d) D. J. Ager, *Synthesis of Unnatural/Nonproteinogenic α-Amino Acids* in: *Amino Acids, Peptides and Proteins in Organic Chemistry – Origins and Synthesis of Amino Acids*, Wiley-VCH, Weinheim, Germany **2009**, *1*, 495-526.

[4] For reviews on the asymmetric formation of quaternary stereogenic centers see: a) S. F. Martin, *Tetrahedron* **1980**, *36*, 419-460; b) K. Fuji, *Chem. Rev.* **1993**, *93*, 2037-2066; c) E. J. Corey, A. Guzman-Perez, *Angew. Chem. Int. Ed.* **1998**, *37*, 388-401; d) J. Christoffers, A. Mann, *Angew. Chem. Int. Ed.* **2001**, *40*, 4591-4597; e) I. Denissova, L. Barriault, *Tetrahedron* **2003**, *59*, 10105-10146; f) D. J. Ramon, M. Yus, *Curr. Org. Chem.* **2004**, *8*, 149-183; g) J. Christoffers, A. Baro, *Adv. Synth. Catal.* **2005**, *347*, 1473-1482; h) B. M. Trost, C. Jiang, C. *Synthesis* **2006**, 369-395; i) P. G. Cozzi, R. Hilgraf, N. Zimmermann, *Eur. J. Org.Chem.* **2007**, 5969-5994; j) M. Bella, T. Gasperi, *Synthesis* **2009**, 1583-1614.

[5] For reviews on peptide folding of peptides containing quaternary amino acids see: a) R. Kaul, P. Balaram, *Bioorg. Med. Chem.* **1999**, *7*, 105-117; b) C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Pept. Sci.* **2001**, *60,* 396-419; c) M. Tanaka, *Chem. Pharm. Bull.* **2007**, *55*, 349-358.

[6] a) H. R. Almond, D. T. Manning, C. Niemann, *Biochemistry*, **1962**, *1*, 243-249; b) M. C. Khosla, K. Stachowiak, R. R. Smeby, F. M. Bumpus, F. Piriou, K. Lintner, S. Fermandjian, *Proc. Natl. Acad. Sci. USA*, **1981**, *78*, 757,760.

[7] For reviews see: a) S. H. Kang, S. Y. Kang, H.-S. Lee, A. J. Buglass, *Chem. Rev.* **2005**, *105*, 4537-4558; b) Y. Ohfune, T. Shinada, *Eur. J. Org. Chem.* **2005**, 5127-5143.

[8] T. Degenkolb, A. Berg, W. Gams, B. Schlegel, U. Gräfe, *J. Peptide. Sci.* **2003**, *9*, 666-678.

[9] a) G. Fenteany, R. F. Standaert, W. S. Lane, S. Choi, E. J. Corey, S. L. Schreiber, *Science* **1995**, *268*, 726-731; b) E. J. Corey, W.-D. Z. Li, *Chem. Pharm. Bull.* **1999**, *47*, 1-10.

[10] a) W. S. Horn, J. L. Smith, G. F. Bills, S. L. Raghoobar, G. L. Helms, M. B. Kurtz, J. A. Marrinan, B. R. Frommer, R. A. Thornton, S. M. Mandala, *J. Antibiot.* **1992**, *45*, 1692-1696; b) B. Wang, G.-Q. Lin, *Eur. J. Org. Chem.* **2009**, 5038-5046.

[11] D. Becker, M. Kiess, H. Brückner, *Liebigs Ann.* **1997**, 767-772.

[12] a) P. Fasella, A. Giartosio, G. G. Hammes, *Biochemistry* **1966**, *5*, 197-202; b) J. J. Walsh, D. E. Metzler, D. Powell, R. A. Jacobson, *J. Am. Chem. Soc.* **1980**, *102*, 7136-7138; c) D. Shirlin, F. Gerhart, J. M. Hornsperger, M. Hamon, J. Wagner, M. J. Jung, *J. Med. Chem.* **1988**, *31*, 30-36; d) M. Horikawa, Y. Shigeri, N. Yumoto, S. Yoshikawa, T. Nakajima, Y. Ohfune, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2027-2032; e) J. Watts, A. Benn, N. Flinn, T. Monk, M. Ramjee, P. Ray, Y. Wang, M. Quibell, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2903-2925; f) L. Wie, J. P. Steiner, G. S. Hamilton, Y.-Q. Wu, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4549-4551.

[13] a) R. H. Mazur, Ger. Offenlegungsschrift DE2321079, **1973**; b) S. Polinelli, Q. B. Broxterman, H. E. Schoemaker, W. H. J. Boesten, M. Crisma, G. Valle, C. Toniolo, J. Kamphuis, *Bioorg. Med. Chem. Lett.* **1992**, *2*, 453-456.

[14] a) P. Genix, J.-L. Guesnet, G. Lacroix, *Pflanzenschutz-Nachrichten Bayer* **2003**, *56*, 421-424; b) P. Genix, *Pflanzenschutz-Nachrichten Bayer* **2003**, *56*, 435-443.

[15] a) W. J. Stepek, M. M. Nigro, EP Patent, EP0123830, **1984**;
b) http://www.truenorthspecialty.com/english/Products/ABProducts/ArsenalCorner.pdf downloaded 10/08/2010;
c) http://www.truenorthspecialty.com/english/Products/Labels/arsenal_Label_Eng.pdf; downloaded 10/08/2010.

[16] J. McConathy, M. M. Goodman, *Cancer Metastasis Rev.* **2008**, *27*, 555-573.

[17] a) A. G. S. Blommaert, J.-H. Weng, A. Dorville, I. McCort, B. Ducos, C. Durieux, B. P. Roques, *J. Med. Chem.* **1993**, *36*, 2868-2877; b) D. C. Horwell, *Bioorg. Med. Chem.* **1996**, *4*, 1573-1576; c) A. G. S. Blommaert, H. Dhôtel, B. Ducos, C. Durieux, N. Goudreau, A. Bado, C. Garbay, B. P. Roques, *J. Med. Chem.* **1997**, *40*, 647-658;

d) B. Bellier, I. McCort-Tranchepain, B. Ducos, S. Danascimento, H. Meudal, F. Noble, C. Garbay, B. P. Roques, *J. Med. Chem*. **1997**, *40*, 3947-3956.

[18] Y. Miyake, Y. Kozutsumi, S. Nakamura, T. Fujita, T. Kawasaki, *Biochem. Biophys. Res. Comm.* **1995**, *211*, 396-403.

[19] a) C. J. Gouret, J. G. Wettstein, R. D. Porsolt, A. Puech, J. L. Junien, *Eur. J. Pharmacol.* **1990**, *183*, 1478; b) B. Kaptein, H. M. Moody, Q. B. Broxterman, J. Kamphuis, *Perkin Trans. 1* **1994**, 1495-1498.

[20] a) T. L. Sourkes, *Arch. Biochem. Biophys.* **1954**, *51*, 444-456; b) W. S. Saari, W. Halczenko, D. W. Cochran, M. R. Dobrinska, W. C. Vincek, D. C. Titus, S. L. Gaul, C. S. Sweet, *J. Med. Chem.* **1984**, *27*, 713-717.

[21] K. N. Bharathi, K. V. S. R. G. Prasa, P. Jagannath, B. C. Naik, *Pharmacologyonline* **2009**, *3*, 670-678.

[22] A. Ghanem, H. Y. Aboul-Enein, *Chirality* **2005**, *17*, 1-15.

[23] M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer, T. Zelinski, *Angew. Chem.* **2004**, *116*, 806-843; *Angew. Chem. Int. Ed.* **2004**, *43*, 788-824.

[24] J. Dingenen, J. N. Kinkel, *J. Chromatography A* **1994**, *666*, 627-650.

[25] a) O. Blank, A. Wetzel, D. Ullrich, M. R. Heinrich, *Eur. J. Org. Chem.* **2008**, 3179-3189; b) M. R. Heinrich, O. Blank, A. Wetzel, *J. Org. Chem.* **2007**, *72,* 476-484; c) Substrates for this thesis were kindly provided by A. Prechter from Prof. Heinrich's group

[26] U. Schöllkopf, W. Hartwig, U. Groth, *Angew. Chem.* **1980**, *92*, 205-206; *Angew. Chem. Int. Ed.* **1980**, *19*, 212-213.

[27] U. Schöllkopf, *Tetrahedron* **1983**, *39*, 2085-2091.

[28] a) D. Seebach, J. D. Aebi, *Tetrahedron Lett.* **1983**, *24*, 3311-3314; b) D. Seebach, J. D. Aebi, M. Gander-Coquoz, R. Naef, *Helv. Chim. Acta* **1987**, *70*, 1194-1216.

[29] S. Blank, D. Seebach, *Liebigs Ann. Chem.* **1993**, 889-896.

[30] a) S.-H. Moon, Y. Ohfune, *J. Am. Chem. Soc.* **1994**, *116*, 7405-7406; b) Y. Ohfune, S.-H. Moon, M. Horikawa, *Pure & Appl. Chem.* **1996**, *68*, 645-648.

[31] C. Aydillo, A. Avenoza, J. H. Busto, G. Jiménez-Osés, J. M. Peregrina, M. M. Zurbano, *Tetrahedron: Asymmetry* **2008**, *19*, 2829-2834.

[32] a) Y. Ito, M. Sawamura, E. Shirakawa, K. Hayashizaki, T. Hayashi, *Tetrahedron Lett.* **1988**, *29*, 235-238; b) Y. Ito, M. Sawamura, E. Shirakawa, K. Hayashizaki, T. Hayashi, *Tetrahedron*, **1988**, *44*, 5253-5262.

[33] a) H. Shao, J. K. Rueter, M. Goodman, *J. Org. Chem.* **1998**, *63,* 5240-5244; b) A. Avenoza, C. Cativiela, F. Corzana, J. M. Peregrina, D. Sucunza, M. M. Zurbano, *Tetrahedron: Asymmetry* **2001**, *12*, 949-957.

[34] A. Avenoza, J. H. Busto, F. Corzana, J. M. Peregrina, D. Sucunza, M. M. Zurbano, *Tetrahedron: Asymmetry* **2004**, *15*, 719-724.

[35] M. Marigo, K. Juhl, K. A. Jørgensen, *Angew. Chem.* **2003**, *115*, 1405-1407; *Angew. Chem. Int. Ed.* **2003**, *42*, 1367-1369.

[36] L. C. Wieland, E. M. Vieira, M. L. Snapper, A. H. Hoveyda, *J. Am. Chem. Soc.* **2009**, *131*, 570-576.

[37] M.-X. Wang, G. Deng, D.-X. Wang, Q.-Y. Zheng, *J. Org. Chem.* **2005**, *70*, 2439-2444.

[38] For a recent overview-article see: K. Baer, N. Dückers, W. Hummel, H. Gröger, *ChemCatChem* **2010**, *2*, 939-942.

[39] T. D. Machajewski, C.-H. Wong, *Angew. Chem.* **2000**, *112*, 1406-1430; *Angew. Chem Int. Ed.*, **2000**, *39*, 1352-1375.

[40] S. Kuroda, H. Nozaki, K. Watanabe, K. Yokozeki, Y. Imabayashi, WO Patent WO2006123745, **2006**.

[41] K. Fesko, L. Giger, D. Hilvert, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5987-5990.

[42] a) H. Nozaki, S. Kuroda, K. Watanabe, K. Yokozeki, *J. Mol. Catal. B* **2009**, *59*, 237-242; b) K. Fesko, M. Uhl, J. Steinreiber, K. Gruber, H. Griengl, *Angew. Chem.* **2010**, *122*, 125-128; *Angew. Chem Int Ed.* **2010**, *49*,121-124.

[43] For a recent review on asymmetric organocatalytic rearrangement reactions see: A. Moyano, N. El-Hamdouni, A. Atlamsani, *Chem. Eur. J.* **2010**, *16*, 5260-5273.

[44] W. Steglich, G. Höfle, *Angew. Chem.* **1969**, *81*, 1001; *Angew. Chem. Int. Ed.* **1969**, *8*, 981.

[45] a) W. Steglich, G. Höfle, *Angew. Chem.* **1968**, *80*, 78; *Angew. Chem. Int. Ed.* **1968**, *7*, 61; b) W. Steglich, G. Höfle, *Chem. Ber.* **1969**, *102*, 883-898; c) W. Steglich, G. Höfle, *Tetrahedron Lett.* **1970**, *54*, 4727-4730.

[46] For reviews on nucleophilic catalysis see: a) G. Höfle, W. Steglich, H. Vorbrüggen, *Angew. Chem.* **1978**, *90*, 602-615; *Angew. Chem. Int. Ed.* **1978**, *17*, 569-583; b) E. F. V. Scriven, *Chem. Soc. Rev.* **1983**, *83*, 129-161; c) A. C. Spivey, S. Arseniyadis, *Angew. Chem.* **2004**, *116*, 5552-5557; *Angew. Chem. Int. Ed.* **2004**, *43*, 5436-5441; d) S. E. Denmark, G. L. Beutner, *Angew. Chem.* **2008**, *120*, 1584-1663; *Angew. Chem. Int. Ed.* **2008**, *47*, 1560-1638.

[47] a) J. C. Ruble, G. C. Fu, *J. Org. Chem.* **1996**, *61*, 7230-7231; b) J. Liang, J. C. Ruble, G. C. Fu, *J. Org. Chem.* **1998**, *63*, 3154-3155; c) J. C. Ruble, G. C. Fu, *J. Am. Chem. Soc.* **1998**, *120*, 11532-11533.

[48] H. V. Nguyen, D. C. D. Butler, C. J. Richards, *Org. Lett.* **2006**, *8*, 769-772.

[49] a) E. Vedejs, X. Chen, *J. Am. Chem. Soc.* **1996**, *118*, 1809-1810; b) S. A. Shaw, P. Aleman, E. Vedejs, *J. Am. Chem. Soc.* **2003**, *125*, 13368-13369; c) S. A. Shaw, P.

Aleman, J. Christy, J. W. Kampf, P. Va, E. Vedejs, *J. Am. Chem. Soc.* **2006**, *128*, 925-934; d) T. A. Duffey S. A. Shaw, E. Vedejs, *J. Am. Chem. Soc.* **2009**, *131*, 14-15.

[50] E. Busto, V. Gotor-Fernández, V. Gotor, *Adv. Synth. Catal.* **2006**, *348*, 2626-2632.

[51] a) A. C. Spivey, T. Fekner, S. E. Spey, *J. Org. Chem.* **2000**, *65*, 3154-3159; b) A. C. Spivey, A. Maddaford, T. Fekner, A. J. Redgrave, C. S. Frampton, *J. Chem. Soc*., *Perkin Trans. 1*, **2000**, 3460-3468; c) A. C. Spivey, F. Zhu, M. B. Mitchell, S. G. Davey, R. L. Jarvest, *J. Org. Chem.* **2003**, *68*, 7379-7385; d) A. C. Spivey, D. P. Leese, F. Zhu, S. G. Davey, R. L. Jarvest, *Tetrahedron* **2004**, *60*, 4513-4525.

[52] K. Shibatomi, T. Muto, Y. Sumikawa, A. Narayama, S. Iwasa, *Synlett* **2009**, 241-244.

[53] a) T. Kawabata, M. Nagato, K. Takasu, K. Fuji, *J. Am. Chem. Soc.* **1997**, *119*, 3169-3170; b) T. Kawabata, K. Yamamoto, Y. Momose, H. Yoshida, Y. Nagaoka, K. Fuji, *Chem. Commun.* **2001**, 2700-2701; c) T. Kawabata, R. Stragies, T. Fukaya, Y. Nagaoka, H. Schedel, K. Fuji, *Tetrahedron Lett.* **2003**, *44*, 1545-1548; d) T. Kawabata, R. Stragies, T. Fukaya, K. Fuji, *Chirality* **2003**, *15*, 71-76.

[54] a) G. Priem, M. S. Anson, J. F. Macdonald, B. Pelotier, I. B. Campbell, *Tetrahedron Lett.* **2002**, *43*, 6001-6003; b) G. Priem, B. Pelotier, S. J. F. Macdonald, M. S. Anson, I. B. Campbell, *J. Org. Chem.* **2003**, *68*, 3844-3848; c) S. Tabanella, I. Valancogne, R. F. W. Jackson, *Org. Biomol. Chem.* **2003**, *1*, 4254-4261; d) J. G. Seitzberg, C. Dissing, I. Søtofte, P.-O. Norrby, M. Johannsen, *J. Org. Chem.* **2005**, *70*, 8332-8337.

[55] a) E. Vedejs, O. Daugulis, S. T. Diver, *J. Org. Chem.* **1996**, *61*, 430-431; b) E. Vedejs, O. Daugulis, *J. Am. Chem. Soc.* **1999**, *121*, 5813-5814; c) E. Vedejs, E. Rozners, *J. Am. Chem. Soc.* **2001**, *123*, 2428-2429; d) E. Vedejs, J. A. MacKay, *Org. Lett.* **2001**, *3*, 535-536; e) E. Vedejs, O. Daugulis, *J. Am. Chem. Soc.* **2003**, *125*, 4166-4173; f) E. Vedejs, O. Daugulis, L. A. Harper, J. A. MacKay, D. R. Powell, *J. Org. Chem.* **2003**, *68*, 5020-5027.

[56] T. Kano, K. Sasaki, K. Maruoka, *Org. Lett.* **2005**, *7*, 1347-1349.

[57] For reviews on the applications of planar chiral DMAP-derrivatives see: a) G. C. Fu, *Acc. Chem. Res.* **2000**, *33*, 412-420; b) G. C. Fu, *Acc. Chem. Res.* **2004**, *37*, 542-547.

[58] J. E. Thomson, K. Rix, A. D. Smith, *Org. Lett.* **2006**, *8*, 3785-3788.

[59] a) V. B. Birman, E. W. Uffman, H. Jiang, X. Li, C. J. Kilbane, *J. Am. Chem. Soc.* **2004**, *126*, 12226-12227; b) V. B. Birman, H. Jiang, *Org. Lett.* **2005**, *7*, 3445-3447; c) V. B. Birman, X. Li, H. Jiang, E. W. Uffman, *Tetrahedron* **2006**, *62*, 285-294.

[60] a) V. B. Birman, X. Li, *Org. Lett.* **2006**, *8*, 1351-1354; b) V. B. Birman, H. Jiang, X. Li, *Org. Lett.* **2007**, *9*, 3237-3240; c) V. B. Birman, X. Li, *Org. Lett.* **2008**, *10*, 1115-1118; d) X. Yang, G. Lu, V. B. Birman, *Org. Lett.* **2010**, *12*, 892-895.

[61] a) M. Kobayashi, S. Okamoto, *Tetrahedron Lett.* **2006**, *47*, 4347-4350; b) V. Birman, X. Li, Z. Han, *Org. Lett.* **2007**, *9*, 37-40.

[62] C. Joannesse, C. P. Johnston, C. Concellón, C. Simal, D. Philp, A. D. Smith, *Angew. Chem.* **2009**, *121*, 9076-9080; *Angew. Chem. Int. Ed* **2009**, *48*, 8914-8918.

[63] S. H. Pines, M. Sletzinger, *Tetrahedron Lett.* **1969**, *9*, 727-728.

[64 a) F. R. Dietz, *Diploma Thesis*, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany, **2007**; b) Enantioselective Steglich rearrangement of the acetyl group in **24a** was also studied within the Fu group, see: J. C. Ruble, *Ph.D. Thesis*, Massachusetts Institute of Technology, USA, **1999**.

[65] a) J. S. Davies, W. A. Thomas, *J. Chem. Soc., Perkin Trans. 2* **1978**, 1157-1163; b) F. M. F. Chen, K. Kuroda, N. L. Benoiton, *Synthesis* **1979**, *3*, 230-232.

[66] a) D. A. Evans, S. J. Miller, T. Lectka, *J. Am. Chem. Soc.* **1993**, *115*, 6460-6461; b) P. Ettmayer, M. Hubner, A. Billich, B. Rosenwirth, H. Gstach, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2851-2856; c) A. S. Bommarius, M. Schwarm, K. Stingl, M. Kottenhahn, K. Huthmacher, K. Drauz, *Tetrahedron: Asymmetry* **1995**, *6*, 2851-2888; d) N. S. Josephson, K. W. Kuntz, M. Snapper, A. H. Hoveyda, *J. Am. Chem. Soc.* **2001**, *123*, 11594-11599; e) P. Vachal, E. N. Jacobsen, *J. Am. Chem. Soc.* **2002**, *124,* 10012-10014; f) N. J. A. Martin, L. Ozores, B. List, *J. Am. Chem. Soc.* **2007**, *127*, 8976-8977.

[67] *Aldrich Technical Bulletin*, AL-218, **2004**.

[68] Review about asymmetric borane reduction: E. J. Corey, C. J. Helal, *Angew. Chem.* **1998**, *110*, 2092-2118; *Angew. Chem. Int. Ed.* **1998**, *37*, 1986-2012.

[69] For a recent review see: H. Gröger, S. Borchert, M. Kraußer, W. Hummel, *Enzyme-Catalyzed asymmetric reduction of ketones* in: *Encyclopedia of Industrial Biotechnology, Bioprocess, Bioseparation, and Cell Technology*, Wiley, Hoboken, NJ, **2010**.

[70] N. Umino, T. Iwakuma, N. Itoh, *Chem. Pharm. Bull.* **1979**, *27*, 1479-1481.

[71] a) K. Yamada, M. Takeda, T. Iwakuma, *J. Chem. Soc., Perkin Trans. 1* **1983**, 265-270; b) I. Reiners, H. Gröger, J. Martens, *J. Prakt. Chem.* **1997**, *339*, 541-546.

[72] K. Makino, N. Okamoto, O. Hara, Y. Hamada, *Tetrahedron: Asymmetry* **2001**, *12*, 1757-1762.

[73] For a review on enzymatic desymmetrisation see: E. Garcia-Urdiales, I. Alfonso, V. Gotor, *Chem. Rev.* **2005**, *105*, 313-354.

[74] For a recent review on PLE as a biocatalyst see: P. Dominguez de Maria, C. A. Gacia-Bugos, G. Bargeman, R. W. van Gemert, *Synthesis* **2007**, 1439-1452.

[75] For examples of enzymes closely related to PLE see: a) S. Smialowski-Fléter, A. Moulin, J. Perrier, A. Puigserver, *Eur. J. Biochem.* **2002**, *269*, 1109-1117; b) S. Smialowski-Fléter, A. Moulin, C. Villard, A. Puigserver, *Eur. J. Biochem.* **2000**, *267*, 2227-2234; c) H. Schwer, T. Langmann, R. Daig, A. Becker, C. Aslanidis, G. Schmitz, *Biochem. Biophys. Res. Commun.* **1997**, *233*, 117-120.

[76] a) E. Heymann, W. Junge, *Eur. J. Biochem.* **1979**, *95*, 509-518; b) W. Junge, E. Heymann, *Eur. J. Biochem.* **1979**, *95*, 519-525.

[77] a) H. D. Dakin, *J. Physiol.* **1903**, *30*, 253-263; b) H. D. Dakin, *J. Physiol.* **1905**, *32*, 199-206.

[78] F. C. Huang, L. F. Lee, R. S. D. Mittal, P. R. Ravikumar, J. A. Chan, C. J. Sih, E. Caspi, C. R. Eck, *J. Am. Chem. Soc.* **1975**, *97*, 4144-4145.

[79] a) L. Provencher, J. B. Jones, *J. Org. Chem.* **1994**, *59*, 2729-2732; b) L. Provencher, H. Wynn, J. B. Jones, A. Krawzcyk, *Tetrahedron: Asymmetry* **1993**, *4,* 2025-2040; c) E. J. Toone, J. B. Jones, *Tetrahedron: Asymmetry* **1991**, *2,* 1041-1052; d) E. J. Toone, M. J. Werth, J. B. Jones, *J. Am. Chem. Soc* **1990**, *112*, 4946-4952; e) J. Zemlicka, L. E. Craine, M. J. Heeg, J. P. Oliver, *J.Org. Chem.* **1988**, *53*, 937-942; f) L. K. P. Lam, R. A. Hui, J. B. Jones, *J. Org. Chem.* **1986**, *51*, 2047; g) M.Ohno, N. Waespe-Sarcevic, C. Tamm, K. Gawroska, J. K. Gawronski, *Helv. Chim. Acta* **1983**, *66*, 2501-2511.

[80] B. J. Gaede, C. A. Nardelli, *J. Org. Process Res. Dev.* **2005**, *9*, 23-29.

[81] D. Farb, W. D. Jencks, *Arch. Biochem. Biophys.* **1980**, *203*, 214-226.

[82] L. K. P. Lam, R. A. H. F. Hui, and J. B. Jones, *J. Org. Chem.* **1986**, *51*, 2047-2050.

[83] a) T. Takahashi, A. Ikai, K. Takahashi, *J. Biol. Chem.* **1989**, *264*, 11565-11571; b) T. Takahashi, M. Nishigai, A. Ikai, K. Takahashi, *FEBS Lett.* **1991**, *280*, 297-300; c) M. Matsushima, H. Inoue, M. Ichinose, S. Tsukada, K. Miki, K. Kurokawa, T. Takahashi, K. Takahashi, *FEBS Lett.* **1991**, *293*, 37-41; d) E. Heymann, K. Peter, *Biol. Chem. Hoppe-Seyler* **1993**, *374*, 1033-1036; e) L. David, X. J. Guo, C. Villard, A. Moulin, A. Puigserver, *Eur. J. Biochem.* **1998**, *257*, 142-148.

[84] a) S. Lange, A. Musidlowska, C. Schmidt-Dannert, J. Schmitt, U. T. Bornscheuer, *ChemBioChem* **2001**, *2*, 576-582; b) A. Musidlowska, S. Lange, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2001**, *40*, 2851-2853; *Angew. Chem.* **2001**, *113*, 2934-2936; c) U. Bornscheuer, A. Musidlowska, C. Schmidt-Dannert, S. Lange, WO Patent WO 02/48322 A2, **2002**.

[85] a) A. Musidlowska-Persson, U. T. Bornscheuer, *J. Mol. Catal. B: Enzym.* **2002**, *19-20*, 129-133; b) G. Scheid, E. Ruijter, M. Konarzycka-Bessler, U. T. Bornscheuer, L. A. Wessjohann, *Tetrahedron: Asymmetry* **2004**, *15*, 2861-2869.

[86] a) A. Musidlowska-Persson, U. T. Bornscheuer, *Tetrahedron: Asymmetry* **2003**, *14*, 1341-1344; b) A. Musidlowska-Persson, U. T. Bornscheuer, *Prot. Eng.* **2004**, *16*, 1139-1145; c) U. T. Bornscheuer, A. Musidlowska-Persson, H. Trauthwein, WO Patent WO2004055177, **2004**.

[87] M. Hermann, M. U. Kietzmann, M. Ivančić, C. Zenzmaier, R. G. M. Luiten, W. Skranc, M, Wubbolts, M. Winkler, R. Birner-Gruenberger, H. Pichler, H. Schwab, *J. Biotech.* **2008**, *133*, 301-310.

[88] For a review on lipase catalyzed ammonolysis see: V. Gotor, *Bioorg. Med. Chem.* **1999**, *7*, 2189-2197.

[89] M. Schneider, N. Engel, H. Boensmann, *Angew. Chem.* **1984**, *96,* 54-55; *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 66.

[90] a) T. Kitazume, T. Sato, T. Kobayashi, J. T. Lin, *J. Org. Chem.* **1986**, *51*, 1003-1006; b) F. Björkling, J. Boutelje, S. Gatenbeck, K. Hult, T. Norin, *Tetrahedron Lett.* **1985**, *26*, 4957-4958; c) F. Björkling, J. Boutelje, S. Gatenbeck, K. Hult, T. Norin, P. Szmulik, *Tetrahedron* **1985**, *41*, 1347-1352; d) M. Luyten, S. Mueller, B. Herzog, R. Keese, *Helv. Chim. Acta* **1987**, *70*, 1250-1254; e) B. de Jeso, N. Belair, H. Deleuze, M. C. Rascle, B. Maillard, *Tetrahedron Lett.* **1990**, *31*, 653-654; f) M. Breznik, D. Kikelj, *Tetrahedron: Asymmetry* **1997**, *8*, 425-434; g) N. Noguchi, M. Nakada, *Org. Lett.* **2006**, *8*, 2039-2042; h) B. Klotz-Berendes, W. Kleemiss, U. Jegelka, H. J. Schäfer, S. Kotila, *Tetrahedron: Asymmetry* **1997**, *8*, 1821-1823; i) H. Heidel, G. Huttner, R. Vogel, G. Helmchem, *Chem. Ber.* **1994**, *127*, 271-274; j) F. Björkling, J. Boutelje, S. Gatenbeck, K. Hult, T. Norin, P. Szmulik, *Bioorg. Chem.* **1986**, *14*, 176-181.

[91] a) P. Domínguez de María, B. Kossmann, N. Potgrave, S. Buchholz, H. Trauthwein, O. May, H. Gröger, *Synlett* **2005**, 1746-1748; b) D. S. Masterson, D. A. Rosado Jr., C. Nabors, *Tetrahedron: Asymmetry* **2009**, *20*, 1476-1486.

[92] a) S. Wallert, J. I. Grayson, K. Drauz, H. Gröger, C. Bolm, P. Domínguez de María, F. Chamouleau, WO Patent WO2006005409, **2006**; b) S. Wallert, K. Drauz, I. Grayson, H. Gröger, P. Domínguez de María, C. Bolm, *Green Chem.* **2005**, *7*, 602-605.

[93] a) A. D. Gee, B, Långström, *J. Chem. Soc. Perkin Trans. 1*, **1991**, 215-218; b) V. Iosub, A. R. Haberl, J. Leung, M. Tang, K. Vembaiyan, M. Parvez, T. G. Back, *J. Org. Chem.* **2010**, *75*, 1612-1619.

[94] A. W. Hofmann, *Ber. Dtsch. Chem. Ges.* **1881**, *14*, 2725-2736.

[95] Y.-T. Huang, *J. of the Chinese Chemical Society* **1948**, *15*, 224-232.

[96] H. E. Baumgarten, H. L. Smith, A. Staklis, *J. Org. Chem.* **1975**, *40*, 3554-3561.

[97] a) M. C. Pirrung, S. E. Dunlap, U. P. Trinks, *Helv. Chim. Acta* **1989**, *72*, 1301-1310; b) K. Burgess, K.-K. Ho, *Tetrahedron Lett.* **1992**, *33*, 5677-5680; c) K. Burgess, K.-K. Ho, *J. Org. Chem.* **1992***, 57*, 5931-5936.

[98] W. Kleemiss, M. Feld, EP Patent EP1046638A2, **1999**.

[99] M. Yokoyama, M. Kashiwagi, M. Iwasaki, K. Fuhshuku, H. Ohtab, T. Sugai, *Tetrahedron: Asymmetry* **2004**, *15*, 2817-2820.

[100] W. Mroczyk, *Roczniki Akademii Rolniczej w Poznaniu* **1988**, *192*, 79-83.

[101] S.-T. Chen, M.-K. Jang, K.-T. Wang, *Synthesis* **1993**, 858-860.

[102] For further informations on Titrino, see homepage of the manufacturer: www.metrohm.com

[103] R. Meyer, H. Lüders, *Liebigs Ann. Chem.* **1918**, *415*, 29-50.

[104] M. Gyoten, H. Nagaya, S. Fukuda, Y. Ashida, Y. Kawano, *Chem. Pharm. Bull.* **2003**, *51*, 122-133.

[105] For recent reviews on enzymatic kinetic resolution see: a) A. Ghanem, *Tetrahedron* **2007**, *63*, 1721-1754; b) see reference 22.

[106] a) A. G. Steinig, D. M. Spero, *J. Org. Chem.*, **1999**, *64*, 2406-2410; b) C. Cativiela, M. D. Diaz-de-Villegas, J. A. Galvez, *Tetrahedron: Asymmetry* **1994**, *5*, 261-268; c) G. I. Georg, X. Guan, J. Kant, *Tetrahedron Lett.* **1988**, *29*, 403-406; d) Y. N. Belokon, V. I. Bakhmutov, N. I. Chernoglazova, K. A. Kochetkov, S. V. Vitt, N. S. Garbalinskaya, V. M. Belikov, *J. Chem. Soc. Perkin Trans. 1* **1988**, 305-312; e) P. K. Subramanian, R. W. Woodard, *Synth. Comm.* **1986**, *16*, 337-342; f) Y. N. Belokon, N. I. Chernoglazova, C. A. Kochetkov, N. S. Garbalinskaya, V. M. Belikov, *Chem. Comm.* **1985**, 171-172; g) M. Kolb, J. Barth, *Liebigs Ann. Chem.* **1983**, 1668-1688; h) M. Kolb, J. Barth, *Angew. Chem.* **1980**, *92*, 753-754; *Angew. Chem. Int. Ed.* **1980**, *19*, 725-726.

[107] a) D. Seebach, M. Boes, R. Naef, W. B. Schweizer, *J. Am. Chem. Soc.* **1983**, *105,* 5390-5398; b) D. Seebach, A. Fadel, *Helv. Chim. Acta* **1985**, *68*, 1243-1250.

[108] a) S.-G. Kim, T.-H. Park, *Tetrahedron Lett.* **2006**, *47*, 9067-9071; b) H.-M. Guo, L. Cheng, L.-F. Cun, L.-Z. Gong, A.-Q. Mi, Y.-Z. Jiang, *Chem. Comm.* **2006**, 429-431; c) T. Ooi, M. Takeuchi, M. Kameda, K. Maruoka, *J. Am. Chem. Soc.* **2000**, *122,* 5228-5229.

[109] a) Z.-L. Wu, Z.-Y. Li, *Chem. Comm.* **2003**, 386-387; b) Z.-L. Wu, Z.-Y. Li, *J. Org. Chem.* **2003**, *68*, 2479-2482.

[110] a) W. Liu, P. Ray, S. A. Benezra, *J. Chem. Soc. Perkin Trans. 1* **1995**, 553-559; b) H. K. Chenault, J. Dahmer, G. M. Whitesides, *J. Am. Chem. Soc.* **1989**, *111,* 6354-6364.

[111] For recent reviews on the application of enzymes in industry see: a) V. Gotor, *Org. Proc. Res. Dev.* **2002**, *6,* 420-426; b) A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, *Nature* **2001**, *409*, 258-268.

[112] For a review on the determination of the enantiomeric ratio see: A. J. J. Straathof, J. A. Jongejan, *Enzyme Microb. Technol.* **1997**, *21*, 559-571.

[113] C.-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294-7299.

[114] J. L. L. Rakels, A. J. J. Straathof, J. J. Heijnen, *Enzyme Microb. Technol.* **1993**, *15*, 1051-1056.

[115] Program "Selectivity" by K. Faber and H. Hoenig, http://www.orgc.TUGraz.at/.

[116] R. D. Schmid, R. Verger*, Angew. Chem.* **1998**, *110*, 1694-1720; *Angew. Chem. Int. Ed.* **1998**, *37*, 1608-1633.

[117] a) D. L. Ollis, E. Cheah, M. Cygler, D. Dijkstra, F. Frolow, S. M. Franken, M. Harel, S. J. Remington, I. Silman, J. Schrag, J. L. Sussman, K. H. G. Vershueren, A. Goldman, *Protein Eng.* **1992**, *5*, 197-211; b) M. Cygler, J. D. Schrag, J. L. Sussman, M. Harel, I. Silman, M. K. Gentry, M. K.; B. P. Doctor, *Protein Sci.* **1993**, *2*, 366-382.

[118] J. Pleiss, M. Fischer, R. D. Schmid, *Chemistry and Physics of Lipids* **1998**, *93*, 67–80.

[119] L. Sarda, P. Desnuelle, *Biochim. Biophys. Acta* **1958**, *30*, 513-521.

[120] a) F. K. Winkler, A. D. Arcy, W. Hunziker, *Nature* **1990**, *343*, 771-774; b) L. Brady, A. M. Brzozowski, Z. S. Derewenda, E. Dodson, G. Dodson, S. Tolley, J. P. Turkenburg, L. Christiansen, B. Huge-Jensen, L. Norskov, L. Thim, U. Menge, *Nature* **1990**, *343*, 767-770.

[121] K. Faber, *Biotransformation in Organic Chemistry*, 5th ed.; Springer: Heidelberg, Germany, **2004**.

[122] For a review on Engineering of CAL-B see: S. Lutz, *Tetrahedron: Asymmetry* **2004**, *15*, 2743-2748.

[123] M. Martinelle, K. Hult, *Biochem. Biophy. Acta* **1995**, *1251*, 191-197.

[124] a) P. Berglund, *Biomol. Eng.* **2001**, *18*, 13-22; b) E. M. Anderson, M. Karin, O. Kirk, *Biocatal. Biotransform.* **1998**, *16*, 181-204.

[125] a) J. Uppenberg, N. Ohmer, M. Norin, K. Hult, G. J. Kleywegt, S. Patkar, V. Waagen, T. Anthomen, T. A. Jones, *Biochemistry* **1995**, *34*, 16838-16851; b) J. Uppenberg, S. Patkar, T. Bergfors, T. A. Jones, *J. Mol Biol.* **1994**, *235*, 790-792; c) J. Uppenberg, M. T. Hansen, S. Patkar, T. A. Jones, *Structure* **1994**, *2*, 293-308.

[126] a) R. Kourist, P. Dominguez de Maria, U. T. Bornscheuer, *ChemBioChem* **2008**, *9*, 491-498; b) J. Christoffers, T. Werner, W. Frey, A. Baro, *Chem. Eur. J.* **2004**, *10*, 1042-1045; c) L. F. Solares, R. Brieva, M. Quirós, I. Llorente, M. Bayod, V. Gotor,

Tetrahedron: Asymmetry **2004** ,*15*, 341-345; d) S. Acherar, G. Audran, N. Vanthuyne, H. Monti, *Tetrahedron: Asymmetry* **2003**, *14*, 2413-2418.

[127] a) L. H. Smith, P. Yates, *J. Am. Chem. Soc.*, **1954**, *76*, 6080-6084; b) W. A. Bolhofer, *J. Am. Chem. Soc.* **1952**, *74*, 5459-5461.

[128] a) T. Sakakura, M. Hara, M. Tanaka, *J. Chem. Soc., Perkin Trans. 1* **1994**, 289-293; b) G. Guanti, L. Banfi, E. Narisano, *Tetrahedron* **1988**, *44*, 5553-5562; c) T. Sakakura, M. Tanaka, *Chem. Comm.* **1985**, 1309-1310; d) J. J. Patroni, R. V. Stick, *Aust. J. Chem.* **1985**, *38*, 947-952; e) A. Albert, K. Ohta, *J. Chem. Soc. (C)* **1970**, 1540-1547; f) H. Henecka, H. Timmler, R. Lorenz, W. Geiger, *Chem. Ber.* **1957**, *90*, 1060-1069.

[129] K. Schwetlick, *Organikum*, 21st ed.; Wiley-VCH: Weinheim, Germany, **2001**.

[130] P. Alsters, S. Bouttemy, E. Schmieder-van de Vondervoort, J. M. Padron Carillo, PCT WO 01/53240A1, **2001**.

[131] D. R. Yazbeck, C. A. Martinez, S. Hu, J. Tao, *Tetrahedron: Asymmetry* **2004**, *15*, 2757-2763.

[132] H. S. Lee, D. H. Kim, *Bioorg. Med. Chem.* **2003**, *11*, 4685-4691.

[133] E. Graf von Roedern, H. Brandstetter, R. A. Engh, W. Bode, F. Grams, L. Moroder, *J. Med. Chem.* **1998,** *41,* 3041-3047.

[134] A. Prechter, *planned doctoral thesis*, Universität Erlangen-Nürnberg.

[135] D. Seebach, T. Gees, F. Schuler, *Liebigs Ann. Chem.* **1993**, 785-799.

[136] T. Harel, S. Rosen, *J. Org. Chem.* **2007**, *72,* 6500-6503.