

University of Szeged

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**KINETIC AND DYNAMIC KINETIC ENZYMATIC
RESOLUTIONS OF TETRAHYDRO- β -CARBOLINE AND
TETRAHYDROISOQUINOLINE DERIVATIVES**

Ph.D. Thesis

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Publications

Papers related to the thesis:

- I. **R. Megyesi**, E. Forró, F. Ferenc
Enzymatic strategy for the resolution of new 1-hydroxymethyl tetrahydro- β -carboline derivatives in batch and continuous-flow systems
ChemistryOpen **2016**, 5, 254-260.
- II. E. Forró, **R. Megyesi**, T. A. Paál, F. Ferenc
Efficient dynamic kinetic resolution method for the synthesis of enantiopure 6-hydroxy- and 6-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid
Tetrahedron: Asymmetry **2016**, 27, 1213-1216.
- III. **R. Megyesi**, A. Mándi, T. Kurtán, E. Forró, F. Fülöp
Dynamic kinetic resolution of ethyl 1,2,3,4-tetrahydro- β -carboline-1-carboxylate. Use of different hydrolases for stereocomplementary processes.
Eur. J. Org. Chem. **2017**, 4713-4718.
- IV. **R. Megyesi**, E. Forró, F. Fülöp
Substrate engineering: Effects of different *N*-protecting groups in the CAL-B catalysed asymmetric *O*-acylation of 1-hydroxymethyl-tetrahydro- β -carboline.
Tetrahedron **2018**, 74, 2634-2640.

Other papers:

- I. G. Lajkó, N. GreCsó, **R. Megyesi**, E. Forró, F. Fülöp, D. Wolrab, W. Lindner, A. Péter, I. Ilisz
Enantioseparation of β -carboline derivatives on polysaccharide- and strong cation exchanger-based chiral stationary phases. A comparative study
J. Chromatogr. A **2016**, 1467, 188-198.
- II. B. Kovács, **R. Megyesi**, E. Forró, F. Fülöp
Efficient lipase-catalysed route for the kinetic resolution of salsolidine and its β -carboline analogue
Tetrahedron: Asymmetry **2017**, 28, 1829-1833.

Lectures

- a. **Megyesi R.**, Forró E., Fülöp F.
Új enzimes módszer gyógyszerkémiail jelentőségű tetrahydro- β -karbolinvázis amino-alkoholok rezolválására
MTA Alkaloidkémiail és Flavonoidkémiail munkabizottság, Balatonalmádi, május 12-13, **2014** (orális előadás)
- b. **R. Megyesi**, E. Forró, **F. Fülöp**
Enzyme-catalysed kinetic resolution of 1-hydroxymethyl-2,3,4,9-tetrahydro- β -carbolines in batch and continuous flow reactions
Chirality, Prague, 27-30 July, **2014** (poster)
- c. **R. Megyesi**, E. Forró, F. Fülöp
New enzymatic strategy for the resolution of tetrahydro- β -carboline amino-alcohol derivatives in batch and continuous-flow system
Biotrans, Vienna, 26-30 July, **2015** (poster).
- d. **R. Megyesi**, A. Mándi, T. Kurtán, E. Forró, F. Fülöp
Directed dynamic kinetic enzymatic strategy for the preparation of both enantiomers of 1,2,3,4-tetrahydro- β -carboline-1-carboxylic acid
SECAT17, Oviedo, 26-28 June, **2017** (poster)

Abbreviations

AK lipase	lipase from <i>Pseudomonas fluorescens</i>
Alcalase	protease from <i>Subtilisin Carlsberg</i>
BEMP	<i>tert</i> -butylimino-2-diethylamino-1,3-dimethylperhydro-1,2,3-diazaphosphorine
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
Bz	benzoyl
CAL-A	<i>Candida antarctica</i> lipase A
CAL-B	<i>Candida antarctica</i> lipase B
Cbz	benzyloxycarbonyl
CF	continuous-flow
conv.	conversion
DBN	1,5-diazabicyclo[4.3.0]non-5-ene
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DEA	diethylamine
DIPE	diisopropyl ether
DKR	dynamic kinetic resolution
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
<i>E</i>	enantioselectivity
<i>ee</i>	enantiomeric excess
<i>ee</i> _p	enantiomeric excess of the product
<i>ee</i> _s	enantiomeric excess of the substrate
equiv.	equivalent
Et ₃ N	triethylamine
Fmoc	9-fluorenylmethyloxycarbonyl
HPLC	high-performance liquid chromatography
IM	immobilised
KR	kinetic resolution
MeCN	acetonitrile
MW	microwave
NAD	nicotinamide adenine dinucleotide
Oct ₃ N	trioctylamine
PG	protecting group
PPL	porcine pancreas lipase
Pr ₂ NH	dipropylamine
PSL	<i>Burkholderia cepacia</i> lipase
SE	substrate engineering
t	reaction time
T	temperature
TBD	1,4,5-triazabicyclo[4.4.0]dec-5-ene
TBME	<i>tert</i> -butyl methyl ether
TFA	trifluoroacetic acid
THIQ	tetrahydroisoquinoline
TH β C	tetrahydro- β -carboline
Ts	toluenesulfonyl

1. Introductions and aims

Many scientific research has focused on the isolation, synthesis and biological investigation of enantiopure compounds containing tetrahydroisoquinoline (THIQ) or tetrahydro- β -carboline (TH β C) frameworks. The reason for this high interest is their potential pharmaceutical activities.^[1-4]

Derivatives containing the **THIQ** core are an important part of alkaloids, *e.g.* expectorant emetine (*Ipecacuanhe*)^[5] and antitussive noscapine (*Papaver somniferum*)^[6,7] (Figure 1). The anticancer effect of the latter has been intensively investigated in recent years.^[8] Trabectedine (Figure 1) was isolated from a marine tunicate (*Ecteinascidia turbinate*) and it was approved as Yondelis[®] for cancer treatment.^[9] Other natural products such as liensinine (*Nelumbo nucifera*)^[10] and saframycin A (*Myxococcus xanthus*)^[11,12] (Figure 1) as well as other synthetic THIQ alkaloid analogues, such as Zalypsis[®],^[13-15] showed promising pharmaceutical activities toward HIV or cancer.

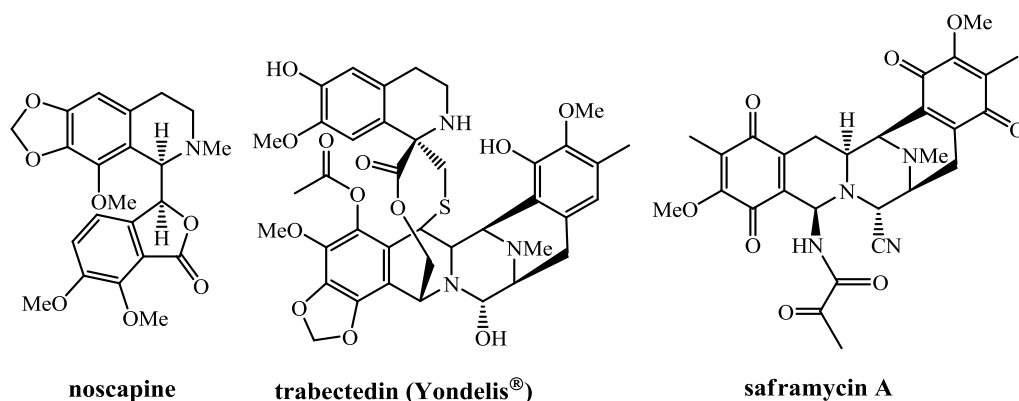


Figure 1.

TH β C alkaloids such as vincristine, vinblastine^[16] and reserpine^[17] are well known about their valuable medicinal applications in the therapy of cancer or hypertension. Several new members of the TH β C family were isolated from natural sources such as vincamajorins A and B (*Vinca-major*),^[18] mappiodines A-C (Figure 2) and mappiodosines A-G (*Mappianthus iodoides*).^[19] Some of them have important biological effects, *e.g.* cytotoxic callophycine A (*Callophycus oppositifolius*)^[20] and (3*S*)-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (*Chicorium endivia*),^[21] antinociceptive harmicine (*Kopsia Griffithii*)^[22,23] (Figure 2) or antimalarial (+)-7-bromotypargine (*Ancorina sp.*)^[24] Besides natural products, synthetic TH β Cs also contributed to modern drug research. Tadalafil

(Cialis[®]) was successfully applied in the treatment of erectile dysfunction.^[25,26] MK-4256 (Figure 2) is an antagonist of somatostatin subtype receptor 3 and it was a promising preclinical candidate for the medication of Type II diabetes mellitus.^[27,28] Significant proliferation inhibitor effect of pyridoxal TH β C derivatives against *Plasmodium falciparum* was also reported.^[29]

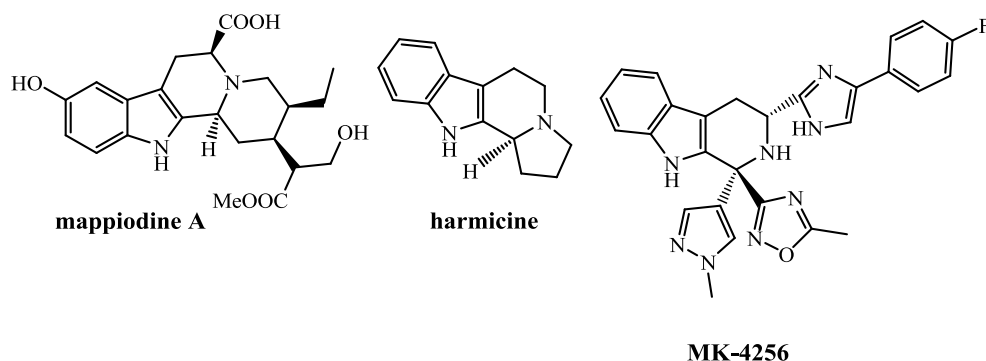
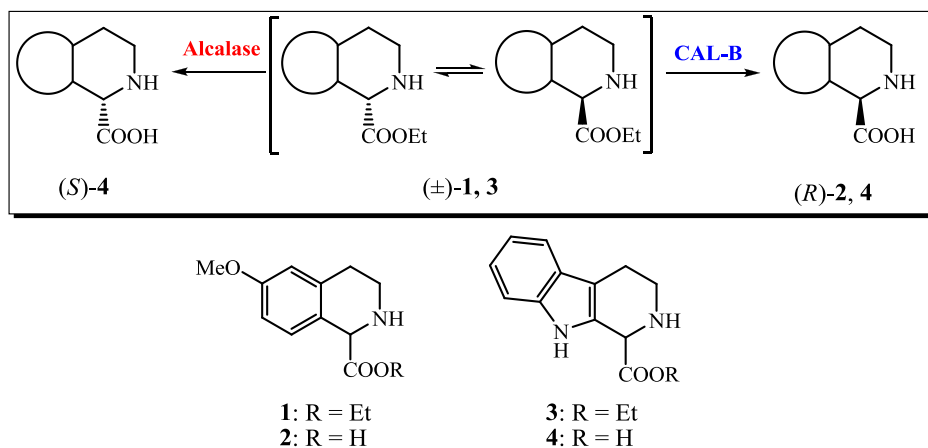


Figure 2.

(*R*)-6-Methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid [(*R*)-**2**] can be transformed into (*R*)-2-*tert*-butyl-1-methyl-6-hydroxy-3,4-dihydroisoquinoline-1,2(1*H*)-dicarboxylate, which is the key intermediate of a Liver X Receptor agonist.^[30] The *N*-Boc-protected form of 1,2,3,4-tetrahydro- β -carboline-1-carboxylic acid [(\pm)-**4**] is a building-block in the synthesis of diketopiperazine-fused TH β Cs through Ugi four-component reaction.^[31]

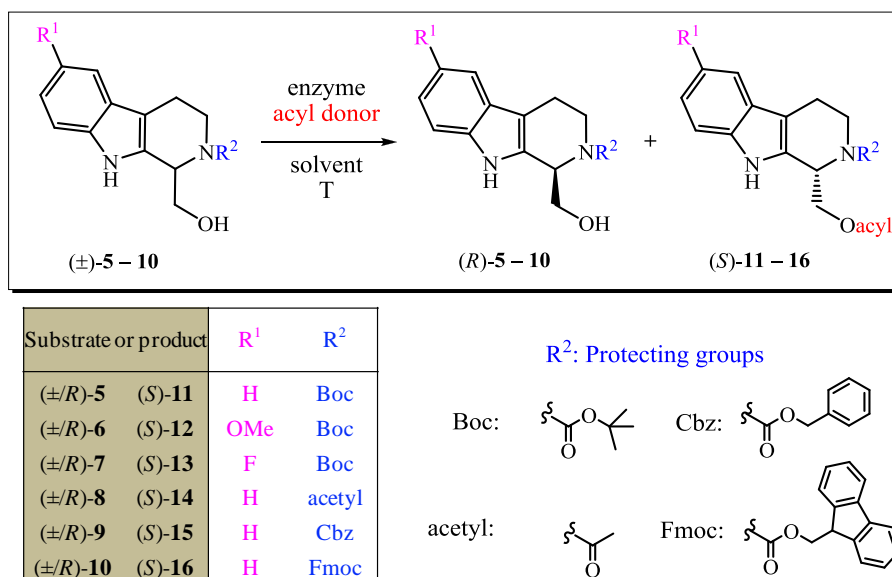
One of our aims was to work out a new strategy for the preparation of TH β C- and THIQ-type amino acids through enzyme-catalysed dynamic kinetic resolution (DKR) of the corresponding ethyl esters [(\pm)-**1** and (\pm)-**3**] (Scheme 1). We also intended to develop a directed DKR of (\pm)-**3** by the use of hydrolases with different enantiopreferences.



Scheme 1.

A further aim was to devise a method for the kinetic resolutions (KR) of *N*-protected 1-hydroxymethyl-substituted TH β C derivatives (\pm)-**5**–(\pm)-**10** through enzyme-catalysed asymmetric acylations of the primary hydroxy group (Scheme 2).

In the frame of substrate engineering (SE), we planned to investigate the effect of different substituents at position 6 and *N*-protecting groups (PG) at position 2 on the outcome of the enzymatic transformation (Scheme 2).



Scheme 2.

2. Literature

2.1. Enzyme-catalysed kinetic and dynamic kinetic resolutions

Effective ways for the preparation of enantiopure compounds are the enzymatic KR and DKR. In **KR**, only one enantiomer of the racemic compound is transformed into product, while the opposite enantiomer remains unreacted (Figure 3). The enzyme can catalyse the transformation of one enantiomer of the racemic mixture with significantly higher reaction rate (fast-reacting enantiomer), than its mirror image pair (slow-reacting enantiomer) ($k_1 \gg k_2$). From the aspect of selectivity, KR is a very effective method for the preparation of enantiopure compounds. The limiting factor of the use of KR is that the desired enantiomer is produced with the maximum yield of 50% at the end of reaction.^[32-35]

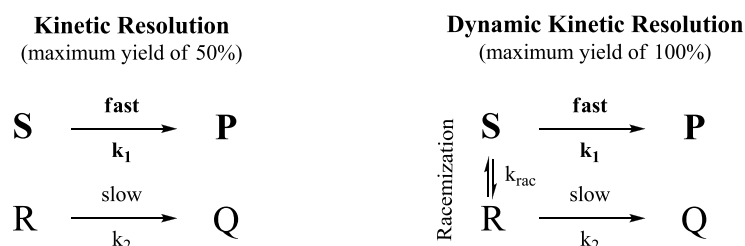


Figure 3.

DKR is a possible route to prepare enantiomers with theoretical yields of 100%. The enzymatic transformation is the same as in the KR, with the difference that the fast-reacting enantiomer is continuously reformed, as a result of the racemisation of the slow-reacting enantiomer (Figure 3). Requirements of an efficient DKR are that the rate of the racemisation (k_{rac}) should be (at least 10 times) faster than the reaction rate of the resolution of the slow-reacting enantiomer ($k_{\text{rac}} \gg k_2$). The KR part of the reaction should be selective (enantioselectivity >20) and irreversible. One of the most challenging steps of the DKR is the racemisation of the starting compound, because in most cases racemisation needs harsh conditions or special catalysts, which are often incompatible with the enzymatic reaction. On the basis of the racemising agent, DKR resolutions can be classified into two main groups metal-catalysed or induced by non-metal agents.^[32-39]

Metal catalysts, *e.g.* ruthenium, rhodium, palladium, iridium and iron, are commonly used as racemising agents in numerous DKRs, such as the resolution of

secondary alcohols.^[40-45] In most cases, the racemisation of alcohols goes through hydrogen transfer or involves the formation of π -allylmetal complexes.^[46] Metal-catalysed racemisations of primary^[47-50] and secondary amines^[51] were also described through hydrogen transfer between their amine and imine forms. DKRs, in which the enzymatic resolution is induced with a metal catalyst as racemising agent, have been discussed in several reviews^[32-39,52-56] and these reactions are no longer treated in the thesis.

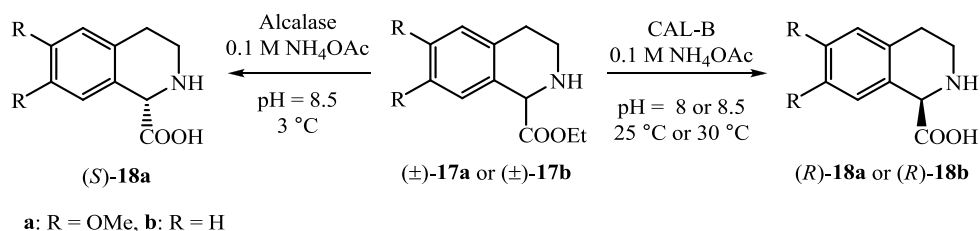
Non-metal catalysts, such as base additives, enzymes, *etc.*, can also catalyse racemisation of the substrate usually through proton transfer, oxidation/reduction, addition/elimination, nucleophilic substitution or free-radical mechanism.^[32,33,35,37,39] The following part of the thesis focuses on enzymatic DKRs in which *in situ* racemisation was achieved by non-metal catalysts or it took place spontaneously in the reaction medium without any external additives. The following enzyme-catalysed DKRs are classified according to the type of racemising agents.

2.1.1. Spontaneous racemisation in the reaction medium without addition of external auxiliaries

Some DKR reactions have been known in the literature, where spontaneous racemisation of the starting compound was observed in the reaction medium without addition of any external auxiliaries, such as organic base/acid, ion-exchange resin, enzyme, *etc.*

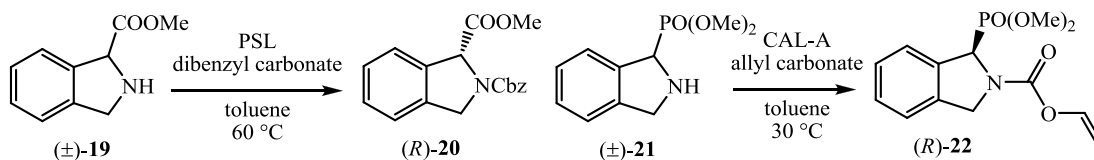
DKR of THIQ esters (\pm)-**17a** and (\pm)-**17b** was described through their spontaneous racemisation in the presence of hydrolases (Scheme 3).^[57] In the *R*-selective resolution of (\pm)-**17a**, catalysed by *Candida antarctica* lipase B (CAL-B), slow hydrolysis and racemisation were observed with the use of base additives such as diisopropylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or Amberlite IRA resin in organic solvents. When the reaction medium was changed into basic aqueous buffers, fast racemisations of (\pm)-**17a** were achieved without further addition of any other external base. The desired *R*-enantiomer [(*R*)-**18a**] was prepared with an *ee* of 92% and a yield of 85% after 7 h with CAL-B enzyme at 25 °C and pH 8.5. The Alcalase-catalysed resolution of (\pm)-**17a** with opposite enantioselectivity gave (*S*)-**18a** with excellent results (*ee* = 93%, yield = 92%,

after 3 days at 3 °C). The preparation of (*R*)-**18b** was also described with CAL-B enzyme (*ee* of 98%, 85% yield, 1 day, 3 °C).



Scheme 3.

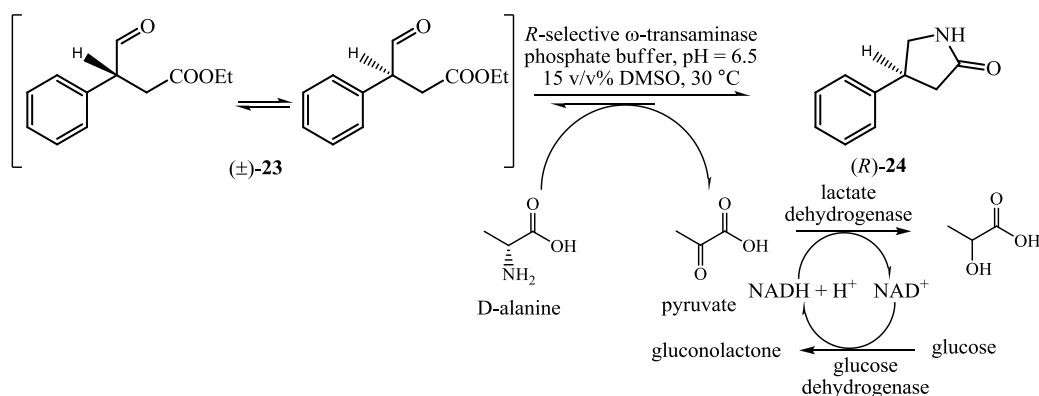
Gotor *et al.* described PSL *Burkholderia cepacia* lipase (PSL) and *Candida antarctica* lipase A (CAL-A) catalysed DKRs of **19**⁵⁸ (Scheme 4) and **21**⁵⁹ (Scheme 5) through proton transfer racemisation, using dibenzyl and allyl carbonates as acyl donors. The isoindole carbamate products [(*R*)-**20** and (*R*)-**22**] were isolated with yields of 63% and 58% (conv. $\geq 77\%$) and *ee* of $\geq 96\%$. The transformation of 3-hydroxy-2-pyvaloyl-2,3-dihydroisoindolin-1-one and other *N*-acylhemiaminals⁶⁰ into enantiopure acetates was achieved by lipase-catalysed *O*-acylation. The substrates were probably racemised through a ring-opening procedure under the reaction conditions (isopropenyl acetate as acyl donor, *n*-hexane as solvent, 60-70 °C). The product acetates were isolated with quantitative yields and most of them with *ee* values of 99%.



Scheme 4.

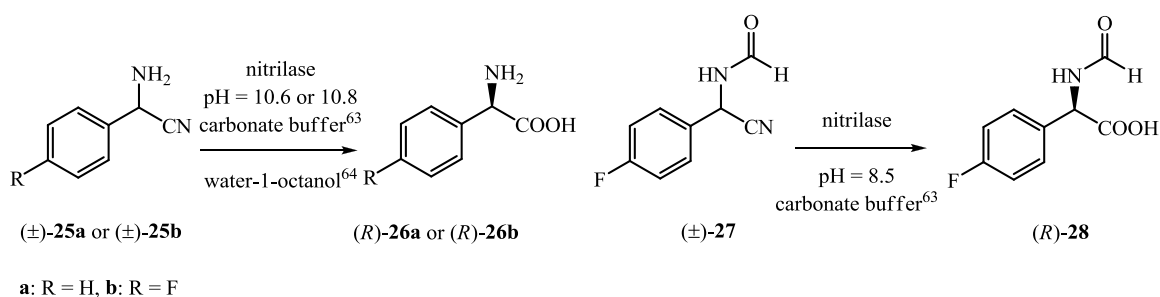
Scheme 5.

Bioaminations of 2-phenylpropanal derivatives with various *R*- and *S*-selective ω -transaminases were described at pH 7.0 and 30 °C in the presence of pyridoxal-5-phosphate co-factor.^[61] The preparative-scale transformations of α -chiral aldehydes resulted in the formation of the corresponding 2-phenyl-1-propylamine derivatives with yields of 50-86% and *ee* of 75-99%. Selective amination of 4-oxo-3-phenylbutyric acid ethyl ester (\pm)-**23** catalysed by ω -transaminases afforded γ -aminobutyrolactam (*R*)-**24** with an *ee* of 68% and a yield of 92% (Scheme 6).^[62] In the reaction, D-alanine was used as amine donor, lactate and glucose dehydrogenases were applied to remove pyruvate and shift the thermodynamic equilibrium to the product side.



Scheme 6.

Nitrilase-catalysed DKR of phenylglycinonitrile [(±)-**25a**]^[63,64] and its 4-fluoro derivative [(±)-**25b**]^[63] into amino acids [(*R*)-**26a**, (*R*)-**26b**] was described (Scheme 7) through their pH-dependent spontaneous racemisation. The reaction with nitrilase at pH 10.6 and 18 °C gave (*R*)-**26a** with a yield of 60% and an *ee* of 91%. The corresponding values for (*R*)-**26b** are 80% yield and 96% *ee* at pH 10.8.^[63] One of the reasons of the moderated yields was the decomposition of the substrates. Therefore, Burk *et al.* prepared *N*-acetylamino nitrile (±)-**27**, a derivative of **25b**, which was more stable under the reaction conditions (Scheme 8). The transformation of (±)-**27** provided (*R*)-*N*-formyl-4-fluorophenylglycine (*R*)-**28** (87% yield, *ee* 98%, Tris-HCl buffer, pH 8.5). Wei *et al.* used water:1-octanol biphasic reaction medium and overexpressed nitrilase containing whole-cell *Escherichia coli* for the hydrolysis of (±)-**25a** to synthesise product (*R*)-**26a** (81% yield, *ee* 95%).^[64]



Scheme 7.

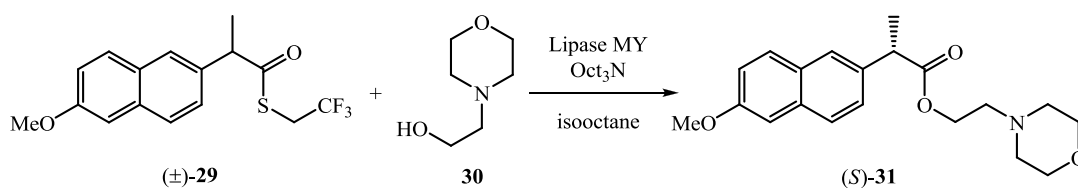
Scheme 8.

2.1.2. Racemisation catalysed by organic base additives

In several cases, the racemisation of the substrate in the enzymatic DKR reaction occurred in the presence of a strong organic base such as triethylamine (Et₃N), trioctylamine (Oct₃N), DBU, 1,5-diazabicyclo[4.3.0]non-5-ene (DBN),

1,4,5-triazabicyclo[4.4.0]dec-5-ene (TBD) or *tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,2,3-diazaphosphorine (BEMP).

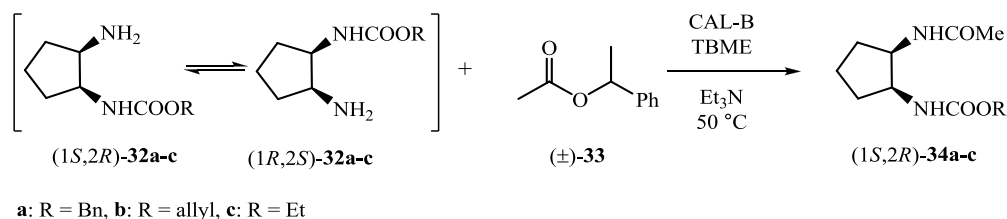
(*R*)-1,2,3,4-Tetrahydroisoquinoline-1-carboxylic acid was obtained with an *ee* of 96% and a yield of 80% in the CAL-B-catalysed DKR hydrolysis of its corresponding ethyl ester in an organic reaction mixture [toluene:acetonitrile (4:1), dipropylamine, 1 equiv. water, 25 °C, 6 days].^[65] Berkessel *et al.* reported the lipase-catalysed synthesis of optically pure β^2 -amino acid allyl esters through ring-opening reaction of the corresponding aryl-substituted β -lactams.^[66] The DKR furnished the amino acids with *ee* values of >99% and in quantitative yields in the presence of a catalytic amount DBN and allyl alcohol as nucleophile. CAL-B-catalysed alcoholic ring-opening of substituted oxazinones^[67] and oxazolones^[68] was also described with the use of Et₃N as racemising agent. Enzymatic resolution of methyl 2,3-dihydro-1*H*-indene-1-carboxylate^[69] and 3-substituted 2,3-dihydrobenzo[*b*]furan^[70] enantiomers was carried out through hydrolysis in biphasic system with CAL-B and a strong base (TBD or BEMP). Enzymatic dynamic kinetic hydrolysis of thioesters was intensively investigated because they can provide great opportunities for the preparation of amino acid enantiomers.^[71-74] The reaction of naproxen thioester (\pm)-**29** with 4-morpholine ethanol (**30**) proceeded with lipase MY from *Candida rugosa* and product (*S*)-**31** was isolated with a yield of 65% and an *ee* of 95% (Scheme 9).^[75] Added Oct₃N facilitated the *in situ* racemisation of (*R*)-**29** and the possible reason for the medium yield was the enzyme inhibitory effect of the formed alcohol.



Scheme 9.

Rebolledo *et al.* described CAL-B-catalysed DKR of diamines (\pm)-**32a-c** in the mixture of *tert*-butyl methyl ether (TBME) and Et₃N (9:1) with the use of racemic 1-phenylethyl acetate (\pm)-**33** as acyl donor (Scheme 10).^[76] The *in situ* racemisation of the substrates took place by intramolecular migration of the alkoxycarbonyl group; however, the asymmetric centres did not change in the reaction. Note that no intramolecular migration was observed with the use of *N*-

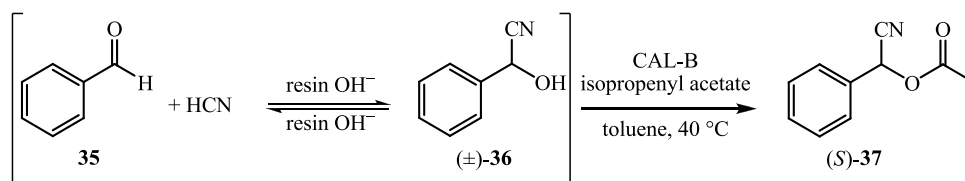
Boc protecting group. The resulting products [(1*S*,2*R*)-**34a-c**] were isolated with yields of 70-94% and excellent *ee* values ($\geq 95\%$).



Scheme 10.

2.1.3. Heterogeneous base- or acid-catalysed racemisation

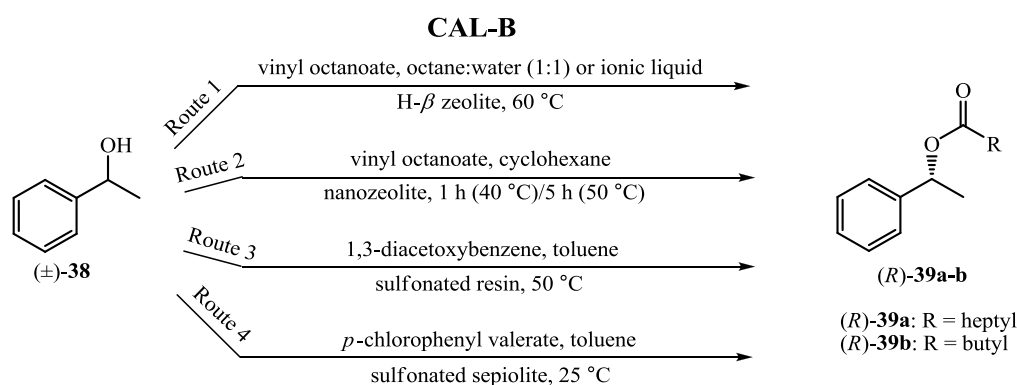
In some cases, in the enzymatic DKR the less-reactive enantiomer was racemised by heterogeneous catalysts such as basic or acidic ion-exchange resins, silicon dioxide, zeolite, other minerals and their functionalized derivatives. As an example, Hanefeld *et al.* described the CAL-B-catalysed preparation of (*S*)-mandelonitrile acetate (*S*)-**37** with an excellent yield of 97% and *ee* of 98% after 4 days (Scheme 11).^[77,78] For the base-catalysed racemisation, Amberlite OH[−] resin was used instead of other organic bases or buffers to prevent the polymerization of HCN.^[79] PSL-catalysed enantiorich preparation of (*S*)-**37** was also published by Ema *et al.*^[80] Silica-supported benzyltrimethylammonium hydroxide was used as racemising agent instead of Amberlite resin to avoid the problem of the resin decomposition during the long reaction. CAL-B-catalysed acylation of (±)-**36** with PSL in diisopropyl ether (DIPE) at room temperature gave product (*S*)-**37** with a yield of 80% and an *ee* of 79% after 26 h.



Scheme 11.

DKR of secondary alcohols, in most cases, is difficult because they are stereostable in normal reaction conditions.^[81] Several heterogeneous acidic racemising agents were successfully applied in CAL-B-catalysed stereoselective acylations of (±)-1-phenylethanol [(±)-**38**], which could allow a comparison of the different racemisation methods (Scheme 12). The use of H-β-zeolite in a biphasic reaction system resulted in (*R*)-**39a** (Route 1; 78% yield, *ee* 98%, 22 h).^[82] In ionic liquid the reaction gave almost the same results but in shorter time (75% yield, *ee*

99%, 6 h)^[83] (Route 1). Immobilization of CAL-B to a H- β -acidic nanozeolite core provided a bi-functional catalyst with an added polydiallyldimethylammonium chloride interlayer. It gave (*R*)-**39a** with excellent results after 5 h (92% yield and *ee* >99%) (Route 2).^[84] In Route 3 the application of sulfonated resin and 1,3-diacetoxybenzene acyl donor furnished (*R*)-**39a** with a yield of >99% and an *ee* of 96% in 3 h.^[85] Sulfonated sepiolite was an efficient racemisation catalyst in the DKR of (\pm)-**38** in the presence of *p*-chlorophenyl valerate acyl donor (Route 4). The product [(*R*)-**39b**] was isolated with an *ee* of >99% and a yield of 95% after 24 h.^[86]

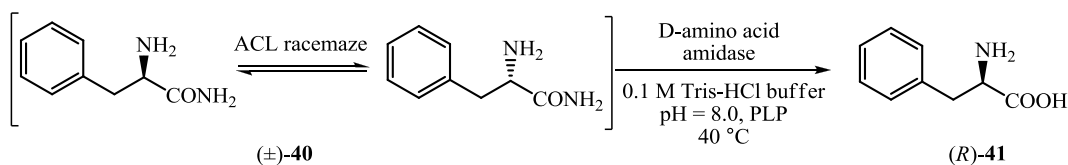


Scheme 12.

2.1.4. Enzyme-catalysed racemisation

Racemases (EC 5.1) form a small group in the large and diverse family of enzymes. They have great biocatalytic potential because of their ability to catalyse racemisation at mild reaction conditions.^[87] However, only a few reactions are known in the literature where enzyme-catalysed *in situ* racemisation are combined with enzyme-catalysed resolution.^[88,89]

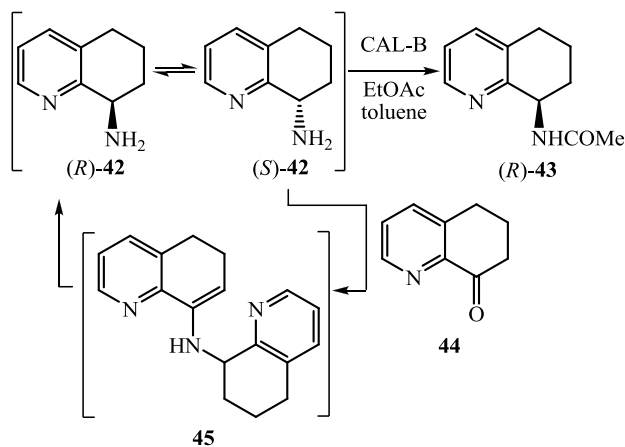
A combination of D-amino acid amidase and α -amino- ϵ -caprolactam racemase (ACL) was successfully applied in the enantioselective preparation of phenylalanine (*R*)-**41** (Scheme 13) and its non-natural derivatives.^[90] The mutant form of ACL was used in resolutions to extend the racemase narrow substrate specificity. DKR of (\pm)-**40** gave (*R*)-**41** with an *ee* of 88% and a conversion of >99% after 24 h.



Scheme 13.

Both kinetic resolution and simultaneous racemisation catalysed by halohydrin dehalogenase were applied in the synthesis of oxazolidinones.^[89] Racemisation occurred through an enzyme-catalysed ring-opening transformation of the starting epoxides in the presence of halide ion. The product 5-substituted 2-oxazolidinones were isolated with high *ee* (>98%) and yields (>87%) after 5 h.

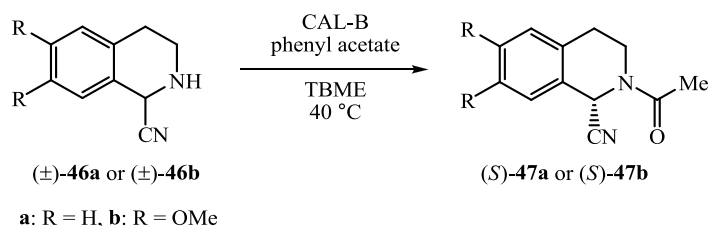
Beside racemases, lipases have also been applied in the racemisation during DKRs because of their catalytic promiscuity.^[91-93] Racemisation of 8-amino-5,6,7,8-tetrahydroquinoline (\pm)-**42** was observed by Crawford and *et al.*^[91] in the CAL-B-catalysed *N*-acylation reaction (Scheme 14). The key step of the racemisation was the spontaneous formation and condensation of ketone **44** with the slow-reacting amine enantiomer (*S*)-**42** into enamine intermediate **45**. This transformation only proceeded in the presence of the enzyme. Addition of 5 mol% **44** into the reaction mixture could increase the conversion of the reaction from 60% to 78% in 48 h. In this case, (*R*)-**42** was isolated with an *ee* of 95% after the hydrolysis of (*R*)-**43**.



Scheme 14.

PSL racemase activity was proved in the enantioselective preparation of optically active aminonitrile derivatives with the presumed lipase-catalysed retro-Strecker reaction leading to racemisation.^[92] CAL-B catalysed both the enantioselective *N*-acylation and racemisation of 1-cyano-1,2,3,4-tetrahydroisoquinolines (\pm)-**46a** and (\pm)-**46b** in the presence of phenyl acetate as

acyl donor (Scheme 15).^[93] The DKR reaction gave the amide products [(*S*)-**47a** and (*S*)-**47b**] with high *ee* ($\geq 95\%$) at a conversion of $\geq 92\%$.



Scheme 15.

2.2. Substrate engineering in enzymatic transformations

The use of enzymes as catalyst is restricted mainly by their limited reaction scope, narrow substrate range towards non-natural substrates, unsatisfactory selectivity, their sensitivity to non-physiological reaction medium (such as organic solvent) and drastic reaction conditions. To overcome these drawbacks, medium, enzyme (or protein) and substrate engineering can be possible devices to improve enzyme features like activity, stability, including thermostability, specificity and stereoselectivity.^[94-98]

Medium engineering is a potential way to optimize the enzymatic processes. Enzymes' catalytic optimum is mainly higher in aqueous medium, but they can also function in organic solvents, biphasic systems, in supercritical carbon dioxide or ionic liquids.^[95] The physicochemical properties of the solvent (dipole moment, solubility, *etc.*) have significant influence on enzyme-catalysed reactions.^[99] Altering the enzyme microenvironment, such as temperature, pH or direct immobilization, can cause drastic change in the conformation of enzymes. In the case of **conformational engineering**, physicochemical tools were applied to modify the shape of the catalytic pocket and this principle was used to change CAL-B enantioselectivity by Palomo *et al.*^[100]

Enzyme engineering is an effective way to produce improved enzymes with the desired catalytic properties. Mutant enzyme libraries can be created by direct evolution from which the enzyme with the desired function was selected with effective screening techniques. A more targeted way of enzyme evolution is rational design. It is based on the background knowledge of the three-dimensional structure of the enzyme, its function and catalytic mechanism including the exact binding interaction between the catalytic pocket and the substrate. Controlled

changes in the composition of the active site formed by amino acids can lead to a mutant enzyme with suitable catalytic properties for our biotransformation aims.^[95,98,101-107]

Substrate engineering (SE) is a versatile method to directly or indirectly influence the results of biocatalytic reactions.

- The correct choice of substrate structure can modify the outcome of the enzymatic transformation by influencing the binding mode of the substrate into the active site.^[95,108-110] For instance, altering the position of the double bond of unsaturated aldehydes can cause a switch in the enantioselectivity of ene-reductases.^[109-111] The effect of protecting groups on the selectivity of the desymmetrization reaction of hydroxyaziridines catalysed by pig pancreatic lipase (PPL) was also described.^[112] CAL-B enantiopreference toward the production of carboxylic acids in view of the location of the stereocenter have been reviewed by Tsai.^[113]
- SE enables to extend the substrate specificity of biotransformation reactions. On the basis of docking and protecting group strategy,^[114,115] 2-butanone was masked into thio derivatives in cyanohydrin reaction to overcome the enzyme's insufficiency to differentiate between two small groups.^[116]
- The definition of the enzymes' substrate scope can be approached by the SE concept. Knowing the typical substrate map of the enzymes, in particular, in the case of new enzymes, is essential for their subsequent utilization and it can help the understanding of binding interactions, which is the first step toward an enzyme evolution.^[117,118] Xu *et al.* developed a method to characterize substrate 'fingerprinting' of the activity lipases (*Candida rugosa* and pig liver lipase) in the hydrolytic reaction of acetylated alcohols and analyzed the diversity of enzyme substrate spectrum in a quantitative way (with Shannon–Weiner index).^[119]
- SE is a potential way to find new reaction types of the enzymes beyond their biological function (mainly with non-natural substrates) known as enzyme promiscuity.^[95,120]

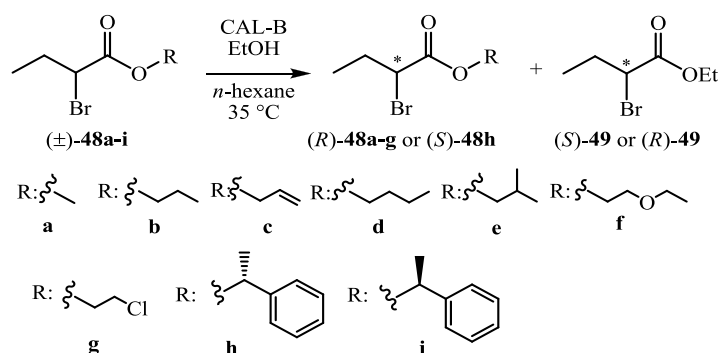
SE can be used on its own to modify the results of enzymatic transformations. SE can also be a real alternative instead of using resource-rich

enzyme engineering processes or complement and support the enzyme evolution to create tailor-made enzymes by the understanding of their catalytic mechanism.^[109,118,121]

In the following section the literature background of the application of the SE concept in hydrolase-catalysed KR of esters, alcohols and amines are surveyed. The reactions have been classified according to the type of the starting compound.

2.2.1. Resolution of esters

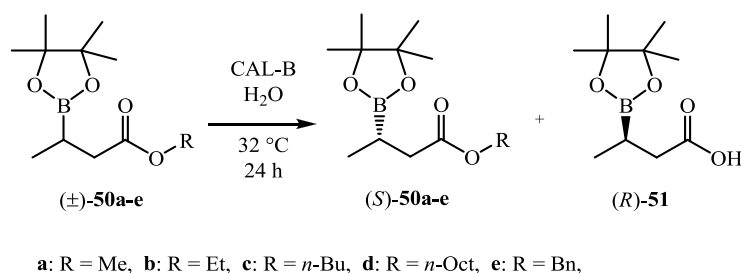
Piovan *et al.* applied the SE concept to overcome CAL-B's low enantioselectivity, which is often observed in the preparation of carboxylic acids (Scheme 16).^[122] Effects of the hydrocarbon chain length and branching, the presence of a double bond or heteroatom in the alcohol moiety of 2-bromobutyric esters [(±)-**48a-i**] on the enantioselectivity (*E*) and reaction rate in enzymatic transesterifications were investigated. In most cases, no significant improvement on the enantioselectivity was achieved (*E* < 5). However, when the starting compound bears the (*R*)-1-phenylethanol moiety [(±)-**48h**] as the leaving group instead of an achiral group, inversion in CAL-B enantiopreference from *R* to *S* was observed [*ee* 19% for (*S*)-**48h**, *ee* 47% for (*R*)-**49**, conv. 13%]. When the ester group contained the (*S*)-1-phenylethanol moiety [(±)-**48i**] no reaction was observed after 36 h.



Scheme 16.

Transesterification and hydrolysis of boron derivatives were described by Andrade and Reis (Scheme 17).^[123] When the esterifying moiety was a short chain [methyl or ethyl, (±)-**50a** and **b**], the hydrolysis gave rather similar results (*E* = 19 and 24, conv. ~ 50%). The presence of longer or branched alkyl chains and an aralkyl moiety in the ester group [(±)-**50c-e**] induced decreases in the *E* or no hydrolytic reaction was observed. When the reaction temperature was raised from

30 °C to 70 °C, both the methyl [(*S*)-**50a**] and ethyl ester [(*S*)-**50b**] were recovered with *ee* >99% after 3 h, while the *ee* of the amino acid [(*R*)-**51**] was lower than 62%.

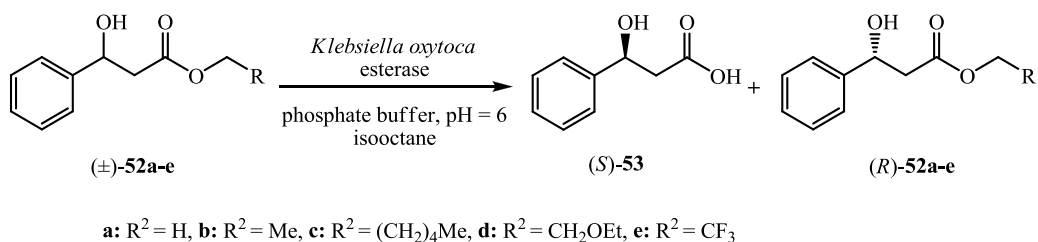


Scheme 17.

Effects of the leaving alcohol group on the lipase-catalysed hydrolysis of naproxen esters^[96] or α -chlorophenyl acetates^[124] were also investigated. The ethyl group proved to be the best leaving moiety in the KR of α -chlorophenyl acetates among electron-withdrawing groups (such as ethoxy or 2,2,2-trifluoro) of different strength considering the *E* of the reactions.^[124] Steric and electronic effects of the substituents at position C3 and on the phenyl ring on the enantioselective hydrolysis of ethyl esters of 3-aryl-substituted alkanolic acids were investigated.^[125] Alkyl groups larger than methyl at position C3 (Et, *i*-Pr, *t*-Bu) induced decreases in both the reaction rate and *E*. Altering the substituent on the aromatic ring (*p*-Me, *m*-Me, *o*-Me, *p*-OMe), however, did not cause drastic changes in catalytic properties.

2.2.2. Resolution of hydroxy esters

Resolution of hydroxyesters catalysed by *Klebsiella oxytoca* esterase was studied in detail by Tsai *et al.* in view of the substrate structure focusing, in particular, on the type of the leaving alcohol moiety.^[97,126,127] (\pm)-2-Chloromandelates were used as model compounds to investigate the effect of electron-withdrawing ester groups of different strengths.^[126] In view of the reactivity and *E*, methyl was found to be the best ester group. The kinetic study was extended to different (\pm)-3-hydroxycarboxylic acid derivatives and the SE concept was combined with covalent immobilization for further enhancement of the enzyme features.^[97] Hydrolysis of (\pm)-3-hydroxy-3-phenylpropionates [(\pm)-**52a-e**] in biphasic reaction medium was also evaluated considering the effect of leaving alcohol moieties, which could help to confirm the reaction mechanism and optimize the *E* (Scheme 18).^[127]



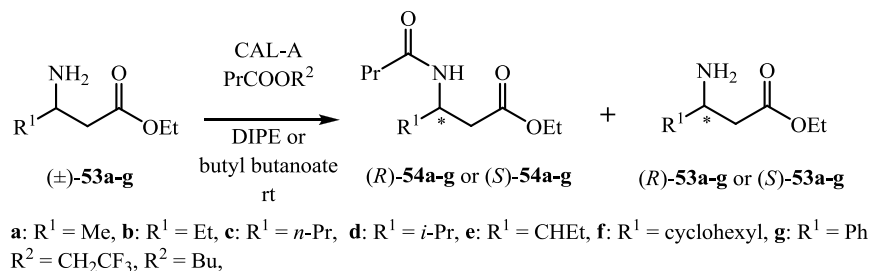
Scheme 18.

2.2.3. Resolution of amino esters

(*S*)-*N*-(2-ethyl-6-methylphenyl)alanine, the key intermediate of (*S*)-metachlor, was prepared through lipase-catalysed two-step resolution of its corresponding ester.^[128] Several alkyl esters were tested to investigate the effect of the alcohol leaving group on conversion and *E*. Short non-branched alkyl chains (from methyl to butyl ester) brought about only minor differences in the *E* (>100, at conv. >42%). The hydrolysis proceeded with the lowest *E* when the alkyl group was *n*-Oct (*E* = 9, at conv. 1%). Larger ester groups had negative effect on the reaction rate, probably caused by their steric inhibition effect. Preparative KR of the corresponding methyl ester was carried out with PSL in aqueous buffer allowing the separation of the product amino acid and amino ester. The desired *S*-enantiomer was obtained as its methyl ester, and a second CAL-B-catalysed hydrolysis was used to obtain the free *S*-amino acid product with a conversion of 48% and an *ee* of 98%. In the Alcalase-catalysed hydrolysis of *N*-unsubstituted α -amino esters, the change of the alcohol moiety from methyl to bulkier *i*-Bu caused an enhancement in the *E* from 3 to 130.^[129]

Resolution of β -amino esters can also be carried out by the selective acylation of their free amino group.^[130-131] KR of β -substituted β -amino esters was performed in butyl butanoate, which served as both the acyl donor and solvent, or with 2,2,2-trifluoroethyl butanoate as acyl donor in DIPE (Scheme 19).^[131] The effect of the alteration of substrate structure on the *E* followed the same tendency in both reaction media. When the R^1 was a methyl group [(±)-**53a**] or a group containing unsaturation, low *E* values were measured (≤ 38). The changing of the methyl group to ethyl [(±)-**53b**], however, caused a high increase in *E* of the reactions (≥ 168 , at conv. ~ 50%). In the case of cyclic compounds [(±)-**53f** and (±)-**53g**], higher *E* values were observed in butyl butanoate reaction medium [*E*

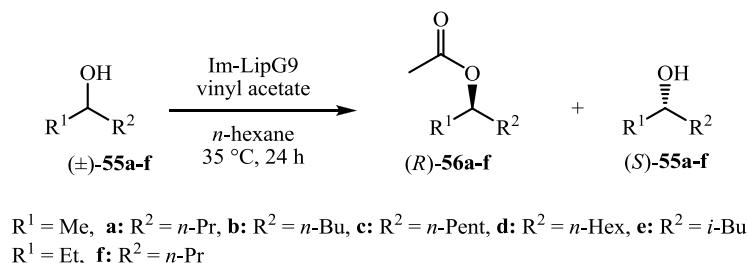
>100 for (±)-**53f** and 75 for (±)-**53g**]. It could also be concluded that the fast-reacting enantiomer of the racemic mixture depended on the substrate structure.



Scheme 19.

2.2.4. Resolution of primary and secondary alcohols

LipG9 is a new lipase, which was isolated from metagenomic protein library. Substrate scope of the immobilized LipG9 towards the acylation of aliphatic secondary alcohols was screened by Piovan *et al.* (Scheme 20), which can help future application of the enzyme.^[132] For alcohols with lower alkyl chains [(±)-**55a** and (±)-**55b**] better *E* values were observed (39 and 19 at conv. of 49-54%), while larger aliphatic chains [(±)-**55c**, (±)-**55d**, (±)-**55f**] had a negative effect on the *E* (~4 at conv. of 52-59%). The highest enantioselectivity was observed when the substrate contained *i*-Bu group [(±)-**55d**] but in this case the reaction rate was lower (*E* = 63 at a conv. of 19%).



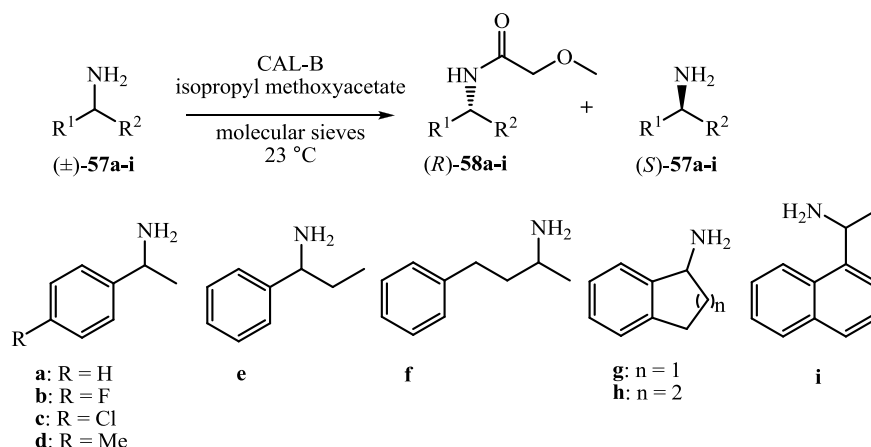
Scheme 20.

The effect of heteroaryl moieties on the acylation of primary^[133] and secondary alcohols^[134,135] was also investigated.^[133,134] Acylation of 1-(benzofuran-2-yl)ethanols containing different substituents at the aromatic ring catalysed by TUB 3b lipase (isolated from thermophilic fungi) was carried out.^[135] The results showed that the electron-withdrawing nitro group at position 5 had no significant effect on the reaction rate of the enzymatic acylation (conv. 50% after 24 h), while the 5-Br and 7-OMe substituents caused lower reaction rates (at a conv. of ~50% after 168 h).

Hydrolysis of 2-*O*-acetyl-1-*O*-alkyl-3-*O*-tosyl-*sn*-glycerol^[136] or acrylates of secondary aliphatic and aryl-substituted aliphatic alcohols^[137] was performed with the use of lipase in phosphate buffer. The *E* of the *Pseudomonas fluorescens* lipase (AK) catalysed KR strongly depend on the aromatic ring substitution.^[137] In the case of 1-(4-methoxyphenyl)ethyl acrylate, the hydrolysis proceeded with higher *E* (>200) than the reaction of the unsubstituted compound (*E* = 52, conv. 50%). Halogens at position 2 or the nitro substituent at position 4 decreased the *E* of hydrolysis (<35, conv. = 42-52%). The reaction of acrylates without an aryl substituent gave the opposite enantiomers of the alcohols (*R*-selective) than the hydrolysis of benzyl derivatives (*S*-selective).

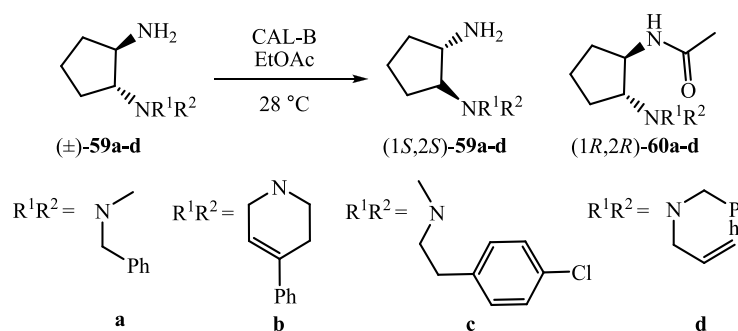
2.2.5. Resolution of primary amines

Resolutions of 1-phenylethylamine and its analogues were performed through lipase-catalysed acylation of their amino group in both batch mode^[138] and in the CF system.^[139] CAL-B-catalysed acylation of substrates (±)-**57a-i** proceeded with significantly different reaction rates. In all cases, however, the reactions took place with excellent *E* (>200, 50% conv.) and all products could be isolated with *ee* ≥ 95% (Scheme 21).^[138] The lowest reaction rate was observed in the resolution of (±)-**57e** (50% conv. after 24 h).



Scheme 21.

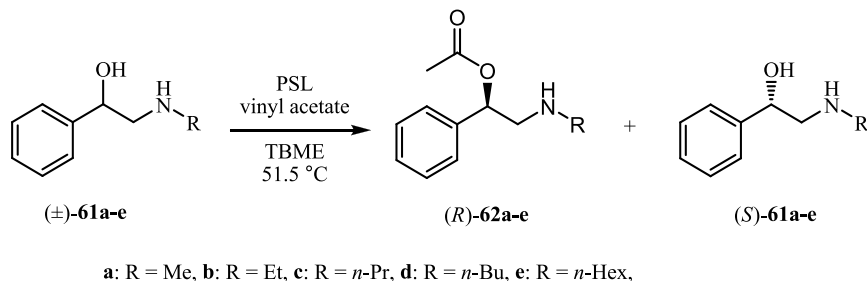
Enantioselective preparation of *trans*-*N,N*-dialkylcyclopentane-1,2-diamines was performed through CAL-B-catalysed selective *N*-acylation in EtOAc (Scheme 22).^[140] The best result was achieved when the substrate was (±)-**59a** (*E* of 86, 47% conv., 3 h). Acylation of (±)-**59b** took place at a lower reaction rate but with *E* of 65 (conv. of 51%, 13 h). The lowest *E* was observed in the resolution of (±)-**59d** (*E* = 20 at a conv. of 55%).



Scheme 22.

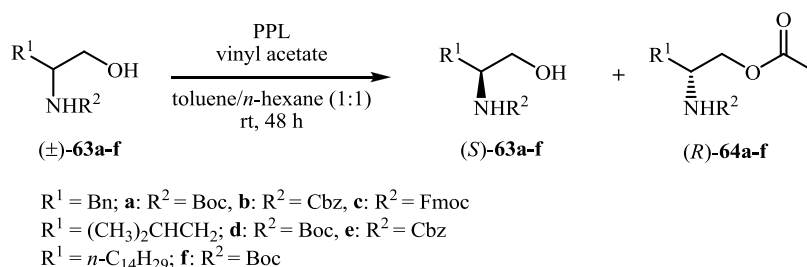
2.2.6. Resolution of amino alcohols

Response surface methodology was applied to optimize reaction conditions of the KR of (±)-2-amino-1-phenylethanols (Scheme 23).^[141] Immobilized PSL-catalysed acylation reactions of (±)-**61a-e** were performed with excellent results ($E > 200$). The isolated yields of the product enantiomers increased with the increasing length of the nitrogen alkyl substituent [44% for (*S*)-**61a** and 50% for (*S*)-**61e**]. CAL-B-catalysed hydrolysis of β -amino alcohols related to (±)-2-amino-1-phenylethanols was described by Allala *et al.*^[142] The authors concluded that the structure of 2-acetamido-1-arylacetates exerted significant effect on both the reaction rate and the E .



Scheme 23.

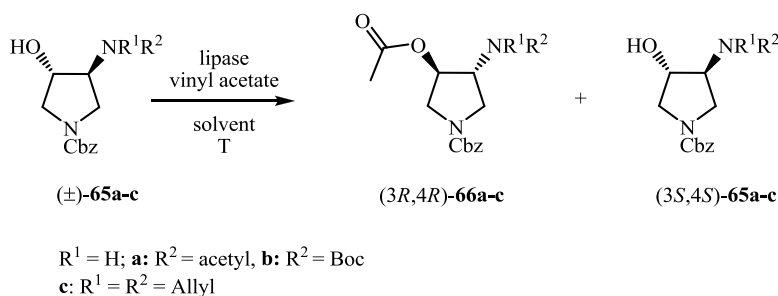
The effect of *N*-protecting groups on the PPL-catalysed *O*-acylation of β -amino alcohols [(±)-**63a-f**] was investigated by Kokotos *et al* (Scheme 24).^[143] The results showed that the type of the protecting group had a marked effect on the E and reaction rate of the enzymatic resolution, while the side chain of the amino alcohol had a lower impact on the acylation. The resolution gave the products with higher ee values [97% for (*R*)-**64a**] and yields [24% for (*R*)-**64a** and 28% for (*R*)-**64d**], when the *N*-Boc protecting group was used. Lower ee (74-63%) and yields (~10%) were found in the acylation of compounds containing the *N*-Cbz and *N*-Fmoc groups [(±)-**63b,c,e**]. This indicated that the bulkier aromatic rings inhibited the interaction between the substrate and the catalytic pocket of the enzyme.



Scheme 24.

CAL-B-catalysed KR of benzimidazolyl ethanol derivatives bearing different substituent at the nitrogen atom (H, Me, Bn, Ts, Bz) was performed.^[144] Electron-withdrawing groups (Ts, Bz) were shown to diminish the *ee* ($E \leq 25$), while the Me and Bn substituent increased the selectivity of the reactions ($E \geq 165$) at a conversion of ~50%.

Resolutions of orthogonally-protected *trans*-3-amino-4-hydroxypyrrolidines [(±)-**65a-c**] were performed through the selective acylation of their secondary hydroxy group, while their amino group was substituted with different protecting groups (Scheme 25).^[145] The best reaction medium for the *O*-acylation of (±)-**65c** was TBME with PSL-IM lipase ($E > 200$, 48% conv., 30 °C after 3 h). In contrast, the resolution of *N*-Boc-protected substrate (±)-**65b** under the same reaction conditions gave very poor results ($E = 8$ at a conv. of 73%). The best result was achieved in the resolution of (±)-**65b** when CAL-B was used as catalyst in 1,4-dioxane ($E = 120$, 50% conv.). Reactions of (±)-**65a** proceeded with poor *E* under the investigated conditions. The results showed that the resolutions of (±)-**65a-c** strongly depended on the applied protecting group.



Scheme 25.

3. Materials and methods

3.1. Materials and instruments

All enzymes used were commercially available. Thus, CAL-B (lipase B from *Candida antarctica*, Cat. N.: L4777, adsorbed on a macroporous resin) was purchased from Sigma, lipase AK (*Pseudomonas fluorescens*) from Amano Pharmaceuticals, PSL-IM (*Burkholderia cepacia* immobilized on diatomaceous earth) from Amano Enzyme Europe Ltd, CAL-A (lipase A from *Candida antarctica*) from Novo Nordisk and Alcalase (cross-linked immobilized protease aggregation from *Subtilisin Carlsberg*) was from Fluka. All solvents and reagents for the experiments were of the highest analytical grades.

H-Cube[®] (purchased from ThalesNano Inc.) in ‘Controlled’ mode (at 1 bar) was used for the removal of *N*-Cbz protecting group. Hydrogenation was performed using a cartridge (30 mm long, also bought from ThalesNano Inc.) filled with 10% Pd/C. ¹H NMR and ¹³C NMR spectra were recorded with Bruker Avance DRX 400 and Bruker Ascend[™] 400 instruments. Elemental analyses were determined by Perkin-Elmer CHNS-2400 Ser II Elemental Analyzer. Optical rotations were measured in a Perkin-Elmer 341 polarimeter. MW reactions were carried out in a CEM Discover MW reactor. Orion 210 A+ pH meter was used for determination of pH of buffer solutions. Melting points were measured on a Kofler apparatus. Batch reactions were performed in New Brunswick Scientific Innova 4000 incubator shaker with shaking speed of 150 rpm. Enzymatic reactions were followed by Jasco high-performance liquid chromatography (HPLC) equipped with a multiwavelength detector and Daicel Chiralpak IA or OD-H chiral columns (4.6×250 mm) at room temperature.

3.2. Enzymatic experiments

The preliminary reactions of the KR resolution of (±)-**5** were carried out in a continuous-flow reactor (CF system, in H-Cube[®], in ‘no H₂’ mode) to utilize its advantages such as high reproducibility, precise process control and easy and rapid implementation of the reaction.^[146,147] In a typical CF reaction the starting compound was dissolved in the solvent, then the acyl donor was added to the solution and the mixture was pumped through a pressure- and temperature-

resistant column (CatCart[®], 70 mm long, 4 mm internal diameter), which was previously filled with the enzyme. The flow of the reaction mixture was provided by a HPLC pump (0.1 mL min⁻¹) and the reaction samples were collected after a single cycle at the end of the CF reactor (Figure 4).

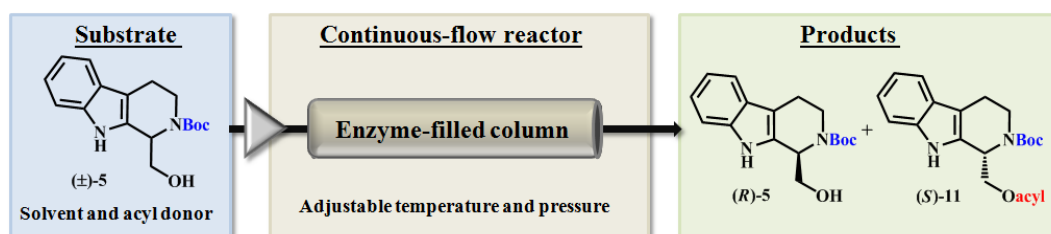
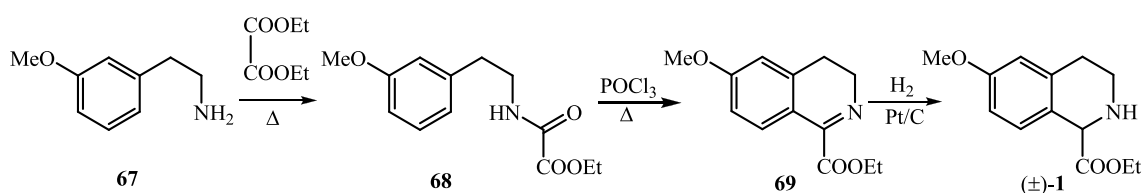


Figure 4.

In a typical batch reaction, the enzyme was added to the solution of racemic compound followed by adding other required additives and the mixture was shaken in the incubator shaker at given temperatures. Preparative-scale resolutions were performed under the optimized reaction conditions. The product enantiomers were characterized with ¹H NMR and ¹³C NMR spectroscopy, melting point, elemental analysis and optical rotation.

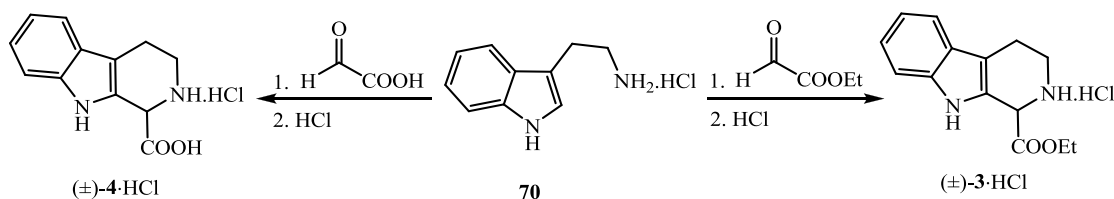
3.3. Synthesis of starting compounds

Heating of 3-methoxyphenethylamine (**67**) and diethyl oxalate gave 3-methoxyphenethylamine hemioxalamide ethyl ester (**68**). Bischler–Napieralski cyclisation of **68** followed by hydrogenation resulted in racemic 6-methoxy-1-tetrahydroisoquinoline carboxylic acid ethyl ester (±)-**1** (Scheme 26).^[148]



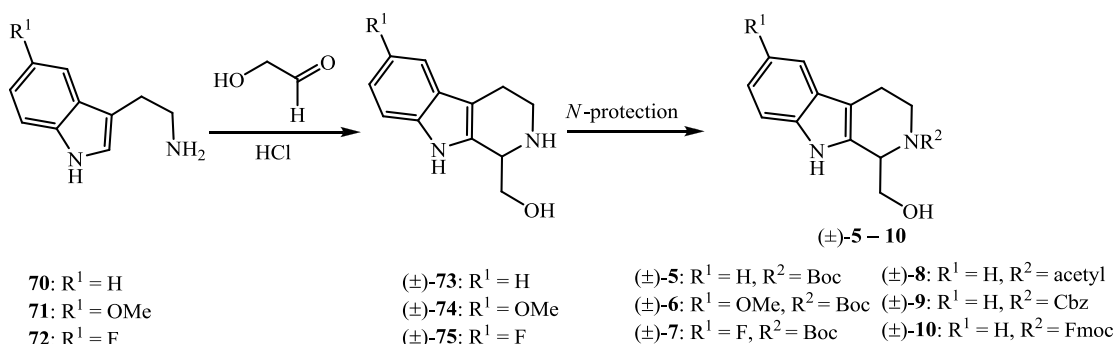
Scheme 26.

Ethyl 1,2,3,4-tetrahydro- β -carboline-1-carboxylate [(±)-**3**] and racemic 1,2,3,4-tetrahydro- β -carboline-1-carboxylic acid [(±)-**4**] were prepared by Pictet–Spengler ring-closing procedure of tryptamine hydrochloride (**70**) and ethyl glyoxylate^[149] or glyoxylic acid (Scheme 27).^[150]



Scheme 27.

Protected amino alcohols $(\pm)\text{-5}$ – $(\pm)\text{-10}$ were synthesized by Pictet–Spengler cyclisation of the corresponding tryptamine derivatives and glycolaldehyde according to a known literature method^[151] followed by protection of the amino group at position 2 with Boc, acetyl,^[152] Cbz and Fmoc (Scheme 28).



Scheme 28.

3.4. Analytical method

Based on HPLC chromatograms, enantiomer excesses, enantioselectivity and conversion values were calculated by using equations 1-4 where A₁, A₂, A₃, A₄ are peak areas of the enantiomers and A₂ > A₁, A₃ > A₄:

1. $ee_s = (A_2 - A_1) / (A_1 + A_2)$
2. $ee_p = (A_3 - A_4) / (A_3 + A_4)$
3. $conv. = ee_s / (ee_s + ee_p)$
4. $E = \{\ln[(1 - ee_s) / (1 + ee_s / ee_p)]\} / \{\ln[(1 + ee_s) / (1 + ee_s / ee_p)]\}$.

In the case of DKR, an external standard was used for the calculation of the conversions via equations 5-7^[153] where A_{st} is the peak area of the standard:

5. $k^0 = A_{st}^0 / (A_1^0 + A_2^0)$
6. $k = A_{st} / (A_1 + A_2)$
7. $conv. = 100 \times (k - k^0) / k$.

The *ee* values of **1** and **3** were determined in the presence of diethylamine (DEA 0.1 %) in the eluent *n*-hexane:isopropyl alcohol (60:40) by using Chiralpak IA column, while the *ee* for product amino acids **2** and **4** measured after derivatization of the samples with Boc anhydride, in the presence of trifluoroacetic

acid (TFA 0.1 %) in the eluent *n*-hexane:isopropyl alcohol (85:15) by using Chiralpak IA column. Amino alcohols **5–10** and amino esters **11–16** were injected directly into the corresponding chiral column to determine the *ee* values [eluent *n*-hexane:isopropyl alcohol (93:7), Chiralpak OD-H column].

Conditions of the measurements in detail such as flow rates, retention times and derivatization methods can be found in the original papers.^{I-IV}

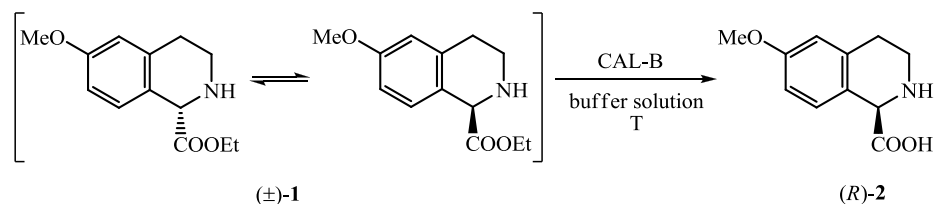
4. Results and discussion

4.1. Dynamic kinetic resolutions of tetrahydroisoquinoline and tetrahydro- β -carboline amino acid ethyl esters^{II,III}

4.1.1. Preliminary experiments

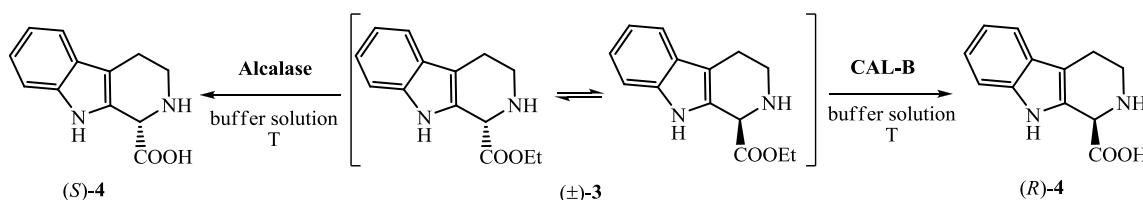
(*R*)-1,2,3,4-Tetrahydroisoquinoline carboxylic acid with an *ee* of 96% and a yield of 80% was prepared earlier through DKR hydrolysis of its corresponding ethyl ester with CAL-B in toluene:MeCN (4:1), in the presence of dipropylamine (Pr_2NH) and 1 equiv. water, at 25 °C in 6 days.^[65] Both enantiomers of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline carboxylic acid [(*R*)-**18a** with an *ee* of 92% and a yield of 85% and (*S*)-**18a** with an *ee* of 93% and a yield of 92%] were successfully resolved with different hydrolases (*R*-selective CAL-B and *S*-selective Alcalase) in aqueous reaction medium (0.1 M NH_4OAc buffer, pH 8.5, at 25 °C or 3 °C) (Scheme 3).^[57] 6-Hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid was obtained with 87% yield and *ee* >99% in CAL-B-catalysed hydrolysis in DIPE/MeCN (3:2) solvent mixture at 25 °C with Pr_2NH racemising agent.^{II} On the basis of these results, the hydrolysis of (\pm)-**1** (Scheme 29) was first carried out under the optimized conditions [2 equiv. of H_2O , 0.25 equiv. of Pr_2NH , DIPE/MeCN (3:2) at 25 °C] in an incubator shaker. Unfortunately, only a trace amount of (*R*)-**2** was produced after 2 days (*ee* 91%, 35% conv.).

Next, the DKR of (\pm)-**1** was carried out under mild basic reaction conditions (aqueous buffer of NH_4OAc at pH 8.5, at 25 °C). The racemisation of (\pm)-**1** took place but the desired amino acid product (*R*)-**2** was detected only in traces (*ee* of 66%) and formation of numerous impurities was observed in the reaction mixture at a conversion of 90% after 2 days. The reason of this poor result was the fast decomposition of (\pm)-**1** used in its free base form. To decrease the decomposition of (\pm)-**1**, reactions were started from its more stable hydrochloride salt [(\pm)-**1**·HCl]. Under the same reaction conditions (NH_4OAc buffer, pH 8.5, 25 °C) the desired amino acid (*R*)-**2** was produced as the major product with an excellent *ee* of >99% at a conversion of 90% after 3 h. The presumable reason of the improvement is the result of the slow *in situ* liberation of substrate (\pm)-**1** from its hydrochloride salt under the mild basic reaction conditions.



Scheme 29.

Encouraged by the DKR of $(\pm)\text{-1}$, we next turned to develop an efficient enzymatic method for the preparation of both enantiomers of 1,2,3,4-tetrahydro- β -carboline-1-carboxylic acid [$(R)\text{-4}$ and $(S)\text{-4}$] through the directed DKR hydrolysis of its corresponding ethyl ester $(\pm)\text{-3}$ (Scheme 30). Preliminary experiments were started with CAL-B catalysis expecting R -selective hydrolysis of $(\pm)\text{-3}$. Substrate $(\pm)\text{-3}$ underwent a similar strong decomposition observed previously in the case of $(\pm)\text{-1}$. Therefore, enzymatic resolutions were performed with its hydrochloride salt $[(\pm)\text{-3}\cdot\text{HCl}]$.



Scheme 30.

The small-scale reactions of $(\pm)\text{-3}\cdot\text{HCl}$ were carried out in batch mode. Resolution of $(\pm)\text{-3}$ was started in aqueous NH_4OAc buffer (pH 8.0 at 30 °C) with CAL-B (Table 1, entry 1). Amino acid product $(R)\text{-4}$ was obtained with an excellent ee of 97% and a high conversion of 97% only in 5 min (entry 1). The increase of the pH of the reaction medium from 8.0 to 9.0 led to a decrease in the ee of $(R)\text{-4}$ to 87% from 99% at a conversion of 99% (entry 2 vs. 1). The amino acid [$(R)\text{-4}$] was produced with a similar high conversion and ee of ~ 90% (entry 2 vs. 3) after reducing the temperature of the reaction from 30 °C to 4 °C. When NH_4OAc buffer was replaced with 0.1 M borate buffer at pH 8.0, a high reaction rate and ee_p (90%) were observed at 30 °C (entry 4). At pH 9.0 the reaction rate of the hydrolysis slightly decreased (>99% in 5 min), while the ee of $(R)\text{-4}$ remained excellent (94%) (entry 5).

Table 1. CAL-B-catalysed hydrolysis of (\pm)-**3**·HCl^a

Entry	Buffer	pH ^b	T (°C)	Time (min)	<i>ee</i> _s (%) ^c	<i>ee</i> _p (%) ^c	Conv. (%) ^c
1	NH ₄ OAc	8.0	30	5	21	97	97
				15	6	97	99
2	NH ₄ OAc	9.0	30	5	rac.	89	95
				15	rac.	87	99
3	NH ₄ OAc	9.0	4	5	rac.	90	94
				15	rac.	90	99
4	borate	8.0	30	5	14	89	96
				15	66	90	99
5	borate	9.0	30	5	-	94	>99

^a(\pm)-**3**·HCl (0.0175 mmol, 4.9 mg), 1 mL 0.1 M buffer solution, 30 mg mL⁻¹ CAL-B. ^bpH was adjusted with aqueous solution of NH₄OH or NaOH. ^cAccording to HPLC.

It is known from the literature that Alcalase can catalyse enzymatic transformations with opposite enantiopreference compared to most of the lipases.^[57,154] Therefore, Alcalase (cross-linked immobilized protease aggregation from *Subtilisin Carlsberg*) was selected for the preparation of the *S*-enantiomer of the desired amino acid [(*S*)-**4**].

At first, Alcalase-catalysed DKR of (\pm)-**3**·HCl was carried out under the same conditions as in the case of CAL-B-catalysed resolution of (\pm)-**3**·HCl (NH₄OAc buffer, pH 8.0, 30 °C) (Table 2, entry 1). According to our assumption, Alcalase catalysed the hydrolysis of (\pm)-**3**·HCl with complementary stereoselectivity in comparison with CAL-B. Indeed, the hydrolysis gave the *S*-product amino acid [(*S*)-**4**]. Unfortunately, however, it was obtained with a lower *ee* (53%) and a conversion of 99% after 24 h (Table 2, entry 1). To increase the selectivity and the reaction rate, the amount of Alcalase was increased from 30 to 60 mg mL⁻¹. The *ee* of (*S*)-**4** decreased greatly, while the reaction rate did not change (*ee* 42%, conv. 31% after 3 h; *ee* 12%, conv. >99% after 24 h). It is known that the type of the buffer and the pH can affect the selectivity of the reaction directly (by conformational engineering of the enzyme)^[100] or indirectly (by chemical hydrolysis and modulating the racemisation rate of the substrate).^[57] Therefore, the pH of the reaction was changed from 8.0 to 9.0 (entry 2), and a significantly higher reaction rate (99% conv. reached after 3 h instead of 24 h), but only a slight increase of *ee* (entry 2 vs. 1) were observed.

Hydrolysis performed at lower temperature (4 °C instead of 30 °C) resulted in an enhancement in the *ee* of (*S*)-**4** (66%, entry 3) with the concomitant decrease

in the reaction rate (conv. of 30% after 24 h). Unfortunately, the *ee* of (*S*)-**4** decreased to 46% at higher conversion (90%) (entry 3). When the pH was changed to 9.0 from 8.0 at 4 °C, the *ee* of the product [(*S*)-**4**] diminished to 40% at a conversion of 99% (entry 4 vs. 3).

Altering the NH₄OAc buffer to borate (pH 8.0 at 30 °C) provided (*S*)-**4** with an increased *ee* of 65% at high conversion (>99%, entry 5 vs. 1). When the pH was set to 9.0, a decrease in *ee* of (*S*)-**4** (48%) was observed (entry 6 vs. 5).

Table 2. Alcalase-catalysed hydrolysis of (±)-**3**·HCl^a

Entry	Buffer	pH ^b	T (°C)	Time (h)	<i>ee</i> _s (%) ^c	<i>ee</i> _p (%) ^c	Conv. (%) ^c
1	NH ₄ OAc	8.0	30	1	rac.	42	40
				24	33	53	99
2	NH ₄ OAc	9.0	30	1	rac.	55	97
				3	-	47	>99
3	NH ₄ OAc	8.0	4	24	rac.	66	31
				72	rac.	46	90
4	NH ₄ OAc	9.0	4	48	rac.	48	57
				72	rac.	40	99
5	borate	8.0	30	6	rac.	65	32
				24	rac.	65	99
6	borate	9.0	30	4	rac.	60	46
				24	-	48	>99

^a(±)-**3**·HCl (0.0175 mmol, 4.9 mg), 1 mL 0.1 M buffer solution, 30 mg mL⁻¹ Alcalase. ^bpH was adjusted with aqueous solution of NH₄OH or NaOH. ^cAccording to HPLC.

Alcalase-catalysed hydrolysis of amino esters in organic solvent–aqueous buffer mixtures with excellent results can be found in the literature.^[155,156] Therefore, the hydrolysis of (±)-**3**·HCl was attempted in the mixture of *t*-BuOH and 0.1 M NH₄OAc aqueous buffer (pH 8.5, 95:5) at 40 °C^[156] in a hope to increase the *ee* of (*S*)-**4** (Table 3). In this case, the reaction reached a conversion of 98% after 24 h; however, the amino acid product [(*S*)-**4**] could not be detected in the reaction mixture (entry 1). Another organic solvent TBME was also applied in the hydrolysis of (±)-**3**·HCl. It was used in the presence of Et₃N as an added base to promote the liberation of the substrate from its HCl salt and using water (2 equiv.) as a nucleophile for the hydrolysis (entry 2). In this case, (*S*)-**4** was not detected in the reaction mixture neither after 24 h at a conversion of 48% (entry 2).

Table 3. Alcalase-catalysed hydrolysis of (\pm)-**3**·HCl in organic solvent^a

Entry	Solvent	Time (h)	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%) ^c
1	<i>t</i> -BuOH/ NH ₄ OAc buffer ^c	3	rac.	-	18
		24	rac.	-	98
2	TBME ^d	3	4	-	17
		24	64	-	48

^a(\pm)-**3**·HCl (0.0175 mmol, 4.9 mg), 30 mg mL⁻¹ Alcalase. ^bAccording to HPLC. ^c1 mL mixture of *t*-BuOH and NH₄OAc buffer (95:5), at 40 °C. ^d1 mL TBME with added Et₃N (10 μ L) and H₂O (2 equiv.), at 30 °C.

4.1.2. Preparative-scale resolutions

The CAL-B-catalysed preparative-scale resolution of (\pm)-**1** was carried out using its hydrochloride salt [(\pm)-**1**·HCl] in NH₄OAc buffer, at pH 8.5, 25 °C (Table 4, entry 1). The scaled-up reaction led to (*R*)-**2**·HCl with an excellent *ee* of 99% and a yield of 91% after 3 h. CAL-B-catalysed DKR of (\pm)-**3**·HCl was performed under similar reaction conditions (NH₄OAc buffer, pH 8.0, 30 °C) and (*R*)-**4**·HCl was isolated with an *ee* of 98% and a yield of 90% after 20 min (entry 2). The *S*-selective preparative hydrolysis of (\pm)-**3**·HCl was carried out with Alcalase in 0.1 M aqueous borate buffer (pH 8.0, 30 °C) and product (*S*)-**4**·HCl was obtained with an *ee* of 60% and a yield of 66% (99% conv., entry 3). Results of preparative resolutions are summarized in Table 4.

Table 4. Preparative-scale resolutions of (\pm)-**1** HCl and (\pm)-**3**·HCl^a

Entry	Substrate	Enzyme	Time	Product	<i>ee</i> _p (%) ^b	Yield (%)	[α] _D ²⁵
1	(\pm)- 1	CAL-B ^c	3 h	(<i>R</i>)- 2 ·HCl	99	91	-58 ^f
2	(\pm)- 3	CAL-B ^d	20 min	(<i>R</i>)- 4 ·HCl	98	90	-12 ^g
3	(\pm)- 3	Alcalase ^e	45 h	(<i>S</i>)- 4 ·HCl	60	66	+6.5 ^h

^a100 mg (\pm)-**1** HCl (0.36 mmol) and (\pm)-**3**·HCl (0.37 mmol), 30 mg mL⁻¹ enzyme.

^bAccording to HPLC. ^c25 mL 0.1 M NH₄OAc buffer, pH 8.5, 25 °C. ^d15 mL 0.1 M NH₄OAc buffer, pH 8.0, 30 °C. ^e15 mL 0.1 M borate buffer, pH 8.0, 30 °C. ^f*c* = 0.2 (H₂O).

^g*c* = 0.2 (EtOH). ^h*c* = 0.32 (EtOH).

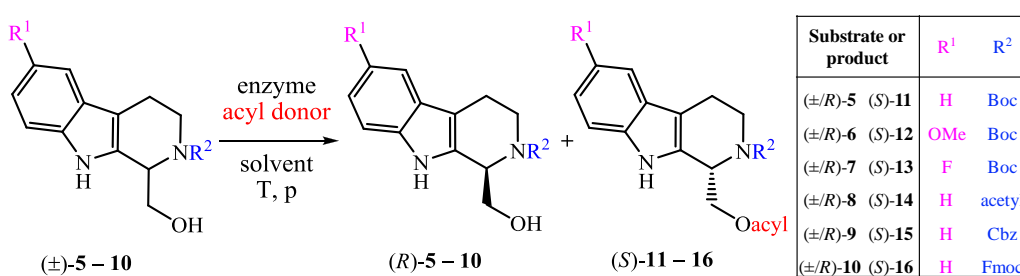
4.1.3. Determination of absolute configurations

Evaluation of the HPLC chromatograms showed that the CAL-B-catalysed hydrolysis of (\pm)-**1** took place with the same enantioselectivity as previously observed in the cases of ethyl 1,2,3,4-tetrahydroisoquinoline carboxylate and ethyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline carboxylate. These results indicated that the CAL-B-catalysed resolution of (\pm)-**1** occurs with *R*-selectivity.

Enantiomers of (\pm)-**4** were earlier separated by chiral HPLC^[157,158] but the enantiomers had not been characterized by specific rotation or electronic circular dichroism. In cooperation with the University of Debrecen, Department of Organic Chemistry, the absolute configurations of the enantiomers of **4** were determined by electronic circular dichroism and optical rotation calculations. Thus, CAL-B *R*-, while Alcalase *S*-selectivity in the DKR of (\pm)-**3** were confirmed.

4.2. Kinetic resolutions of 1-hydroxymethyl-substituted tetrahydro- β -carboline derivatives^{I,IV}

High *E* values were observed in the lipase-catalysed asymmetric *O*-acylation of *N*-Boc-protected tetrahydroisoquinolines such as intermediate of crispine A (*E* ≥ 52),^[159] homocalycotomine (*E* = 88)^[160] and calycotomine (*E* >200).^[161] These findings led us to develop an enzymatic method for the preparation of the enantiomers of tetrahydro- β -carbolines: 1-hydroxymethyl-1,2,3,4-tetrahydro- β -carboline [(\pm)-**73**], 1-hydroxymethyl-6-methoxy-1,2,3,4-tetrahydro- β -carboline [(\pm)-**74**] and 1-hydroxymethyl-6-fluoro-1,2,3,4-tetrahydro- β -carboline [(\pm)-**75**] (Scheme 34). To ensure the *O*-acylation of the primary hydroxy group, KR was carried out through their *N*-Boc-protected forms [(\pm)-**5**, (\pm)-**6** and (\pm)-**7**] (Scheme 31). The effect of the various protecting groups (*N*-Boc, *N*-acetyl, *N*-Cbz, *N*-Fmoc) on the enantioselectivity and reaction rate was also investigated when R¹ was H [(\pm)-**5**, (\pm)-**8**, (\pm)-**9** and (\pm)-**10**] (Scheme 31).



Scheme 31.

4.2.1. Preliminary experiments of (\pm)-**5**, (\pm)-**6** and (\pm)-**7** – different substituents at the aromatic ring at position 6

Preliminary experiments for the resolution of (\pm)-**5** were carried out in a CF reactor starting with enzyme screening (Table 5). When the KR of (\pm)-**5** was performed with CAL-B in the presence of vinyl acetate as acyl donor, in toluene at 45 °C^[161] (entry 1), the reaction showed excellent *E* (>200) but gave only a low

conversion (4%) after single cycle. PSL-IM-catalysed *O*-acylation, again, underwent with excellent *E* (>200), while the reaction rate remained very low (1.5% conv., entry 2). Next, AK lipase was tested without product formation after one cycle (entry 3). Lipase CAL-A was also tried in the reaction and it catalysed the resolution with the highest conversion (11%) but with a very low *E* (entry 4). In view of the *E* and reaction rate of the KRs, CAL-B was selected because of its slightly higher reaction rate compared to that of PSL-IM (entry 1 vs. 2).

Table 5. Enzyme screening for the resolution of (±)-**5**^a

Entry	Enzyme	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	CAL-B	4	99	4	>200
2	PSL-IM	1.5	99	1.5	>200
3	AK	No reaction			
4	CAL-A	3	24	11	1.6

^a(±)-**5** 0.0125 mmol (3.7 mg); 230 mg CAL-B, 248 mg PSL-IM, 338 mg AK lipase, 231 mg CAL-A (70-mm long column); 1 mL toluene; 1.1 equiv. vinyl acetate (1.2 μL); 45 °C; 0.1 mL min⁻¹ flow rate; 1 bar; 1 cycle. ^bAccording to HPLC.

In an effort to achieve higher conversion in the resolution of (±)-**5**, in addition to vinyl acetate (Table 6, entry 1), five other acyl donors were probed in the KR. No activity in the *O*-acylation reaction was observed with the use of ethyl acetate and isopropenyl acetate (entries 2 and 3). Another activated ester, 2,2,2-trifluoroethyl butyrate, was also tested, but despite the relatively good reaction rate (conv. of 22%), only a low *E* of 3 was measured (entry 4). Besides esters, anhydride acyl donors such as acetic and butyric anhydride were also used in the enzymatic reaction, despite of their known ability to chemical esterification, which can cause decrease in the *ee* of the product.^[162] KR of (±)-**5** was carried out with butyric anhydride affording low *E* and moderate conversion (entry 5). When acetic anhydride was applied (entry 6), a relatively fast reaction took place (conv. = 17%) with excellent enantioselectivity (*E* >200). Therefore, acetic anhydride was selected as acyl donor for further optimization.

Table 6. Acyl donor screening for the resolution of (±)-**5**^a

Entry	Enzyme	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	vinyl acetate	4	99	4	>200
2	ethyl acetate		No reaction		
3	isopropenyl acetate		No reaction		
4	2,2,2-trifluoroethyl butyrate	12	42	22	3
5	butyric anhydride	31	63	32	6
6	acetic anhydride	20	99	17	>200

^a(±)-**5** 0.0125 mmol (3.7 mg); 230 mg CAL-B (70-mm long cartridge); 1 mL toluene, 1.1 equiv. acyl donor; 60 °C, 0.1 mL min⁻¹ flow rate; 1 bar; 1 cycle. ^bAccording to HPLC.

To increase the reaction rate, the acylation of (±)-**5** was performed at different temperatures. Correlations were found between the temperature, the reaction rate and the *E* (Table 7). With the increase in temperature from 60 °C to 70 °C and then to 80 °C, conversions also increased (from 17% to 26%), but in parallel, large reduction in the *E* was observed (from >200 to 96). Because of the loss in the *E*, 60 °C was used in further studies.

Table 7. Temperature effect to the resolution of (±)-**5**^a

Entry	Temperature	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	60	20	99	17	>200
2	70	23	98	19	124
3	80	35	97	26	92

^a(±)-**5** 0.0125 mmol (3.7 mg); 230 mg CAL-B (70-mm long cartridge); 1 mL toluene; 1.1 equiv. acetic anhydride (1.2 μL); 0.1 mL min⁻¹ flow rate; 1 bar; 1 cycle. ^bAccording to HPLC.

Next, preliminary experiments were carried out in different solvents, such as toluene, TBME, DIPE, MeCN, chloroform and 1,4-dioxane (Table 8). In MeCN and 1,4-dioxane, low *E* (≤ 39) was observed (entries 4 and 5), whereas excellent *E* (>200) was achieved in the other solvents. However, reactions in TBME and 1,4-dioxane (entries 2 and 6) showed very low reaction rates (≤ 4% after one cycle). Relatively good conversions (17% after one cycle) were observed in toluene and DIPE (entries 1 and 3). Taking into account the above results, DIPE was chosen for further enzymatic reactions.

Table 8. Solvent effect in the resolution of (±)-**5**^a

Entry	Enzyme	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	toluene	20	99	17	>200
2	TBME	4	99	4	>200
3	DIPE	20	99	17	>200
4	MeCN	1	95	1	39
5	chloroform	2	73	3	7
6	1,4-dioxane	3	99	3	>200

^a(±)-**5** 0.0125 mmol (3.7 mg); 230 mg CAL-B (70-mm long cartridge); 1 mL solvent, 1.1 equiv. acetic anhydride; 60 °C, 0.1 mL min⁻¹ flow rate; 1 bar; 1 cycle. ^bAccording to HPLC.

In the next step, the optimized reaction conditions determined in the CF system (CAL-B, DIPE, acetic anhydride, 60 °C) were implemented in the batch mode in incubator shaker. CAL-B-catalysed acylation of (±)-**5** in batch was achieved with good conversion (43%) after 3 h, but the *E* was lower than in H-Cube[®] (*E* = 36 vs. >200). Therefore, DIPE was changed to toluene, which also proved to be as good reaction medium as DIPE in the reactions (Table 8, entry 1 vs. 4). Batch reaction of (±)-**5** in toluene provided excellent results (*E* >200, 48% conv. after 3 h). To achieve the maximum conversion of the KR, the amount of the acyl donor was increased from 1.1 equiv. to 2 equiv. Thus, the selective *O*-acylation occurred with the maximum conversion of 50% after 3 h while the *E* remained excellent (>200).

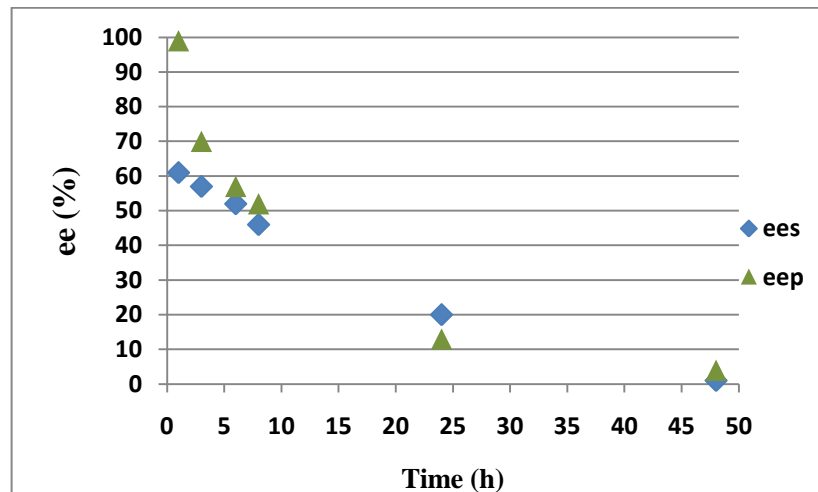
Next the optimized conditions for (±)-**5** (CAL-B, 2 equiv. acetic anhydride, toluene, 60 °C) were implemented to the *O*-acylations of (±)-**6** and (±)-**7**. However, the lower reaction rate and the resulting chemical esterification side-reaction led to a decrease in the *E* of the reactions of (±)-**6** and (±)-**7** [*E* = 82 at conv. = 42% for (±)-**6** and *E* = 39 at conv. = 45% for (±)-**7** after 7 h]. To eliminate the negative effect of the low reaction rates, the amount of acetic anhydride was increased from 2 equiv. to 8 equiv. [(±)-**6**] and to 6 equiv. [(±)-**7**] in the KR. The small-scale reactions proceeded with excellent *E* (>200) and conversions of 50% after 2.5 h and 2 h, respectively.

4.2.2. Preliminary experiments of (±)-**5**, (±)-**8**–(±)-**10** – different *N*-protecting groups at position 2

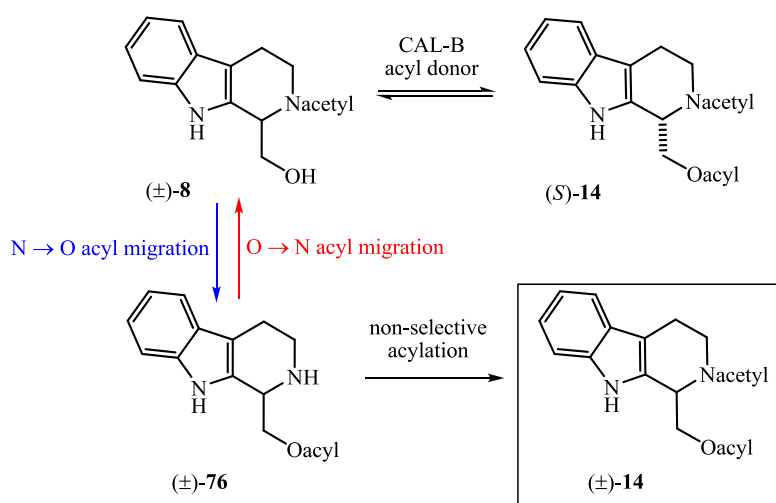
The effect of different *N*-protecting groups on the features of the enzymatic transformation was investigated in the case of 1-hydroxymethyl-1,2,3,4-

The CAL-B-catalysed *O*-acylation of *N*-acetyl-protected substrate (\pm)-**8** with vinyl acetate gave interesting results. Because of the fast enzymatic *O*-acylation, excellent results were observed in 30 min [$E > 200$, *ee* 65% for (*R*)-**8**, *ee* 99% for (*S*)-**14**]. However, with the progress of the reaction, decreased *ee* values were found (Figure 5) after 48 h for both **8** and product **14**. The results were the same when acetic anhydride or 2,2,2-trifluoroethyl butyrate was used in the resolution instead of vinyl acetate. These findings suggested that $N \rightarrow O^{[163]}$ and $O \rightarrow N^{[164-166]}$ acyl migrations occurred during resolution. When the acylation was performed in the presence of CAL-B but without an acyl donor, the formation of *N,O*-diacylated product **14** was detected in the reaction mixture [*ee* = 22% for (*S*)-**14** and *ee* = 11% for (*R*)-**8**, after 8 h]. Next, without added acyl donor and enzyme [(\pm)-**8** shaken in toluene at 60 °C], **14** was not produced after 2 days. These phenomena suggested that besides acyl migration, CAL-B reversibility^[166-168] also influenced the results of the reaction (Scheme 33). In view of the above results, the acetyl group appears to be unsuitable for the protection of (\pm)-**73** in the enzymatic *O*-acylation under the reaction conditions applied.

Figure 5: *O*-acylation of (\pm)-**8**^a



^a(\pm)-**8**: 3.1 mg (0.0125 mmol); 30 mg mL⁻¹ CAL-B; 2 equiv. vinyl acetate; 1 mL toluene; 60 °C



Scheme 33.

When the enzymatic resolution of *N*-Cbz-protected substrate (\pm)-**9** was performed with acetic anhydride in toluene at 60 °C, an excellent *E* (>200) was measured (Table 10, entry 1) when the reaction was stopped at a conversion of ~ 41% in 1 h. After continuing the reaction, only a small increase in the reaction rate was observed with a concomitant large drop of *E* (entry 1). The same observation was made when the reaction was carried out with vinyl acetate as acyl donor (entry 2) instead of acetic anhydride. Resolution with 2,2,2-trifluoroethyl butyrate, again, gave an excellent *E* at a conversion of 26% (entry 3), but *E* decreased at higher conversion (*E* = 35 at a conv. of 36%). To increase the rate of resolution of *N*-Cbz-protected substrate (\pm)-**9**, the amount of acetic anhydride was doubled (from 2 to 4 equiv.). The reaction furnished the remaining alcohol (*R*)-**9** and product ester (*S*)-**15** with excellent *ee* values ($\geq 97\%$, after 20 min, at a conv. of 51%).

Table 10: *O*-acylation of (\pm)-**9**^a

Entry	Acyl donor	t (min)	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	Acetic anhydride	60	69	99	41	>200
		180	69	93	43	57
2	Vinyl acetate	30	58	99	37	>200
		60	59	97	38	121
		180	59	83	42	20
3	2,2,2-Trifluoroethyl butyrate	30	35	99	26	>200
		60	48	98	33	160
		180	52	91	36	35

^a(\pm)-**9** 4.2 mg (0.0125 mmol); 30 mg mL⁻¹ CAL-B; 2 equiv. acyl donor; 1 mL toluene; 60 °C.

^bAccording to HPLC.

Resolution of *N*-Fmoc-protected substrate (\pm)-**10** with acetic anhydride (Table 11, entry 1) gave the product amino ester [(*S*)-**16**] with a high *ee* of $\geq 98\%$ (toluene, 60 °C, 45% conv., 60 min). Acylation with vinyl acetate also resulted in a high *E* but with a lower reaction rate (entry 2). In the resolution with 2,2,2-trifluoroethyl butyrate, both lower conversion (29%) and *E* were observed after 60 min (entry 3). Similar to the observation mentioned above, 50% conversion and an excellent *E* (>200) of the KR of (\pm)-**10** could be achieved when the amount of acetic anhydride was increased from 2 to 4 equiv. after 30 min.

Table 11: *O*-acylation of (\pm)-**10**^a

Entry	Acyl donor	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	Acetic anhydride	79	98	45	>200
2	Vinyl acetate	66	98	40	>200
3	2,2,2-Trifluoroethyl butyrate	39	98	29	145

^a(\pm)-**10** 5.3 mg (0.0125 mmol); 30 mg mL⁻¹ CAL-B; 2 equiv. acyl donor; 1 mL toluene; 60 °C after 60 min. ^bAccording to HPLC.

4.2.3. Comparison of the effect of different substituents on the enzymatic *O*-acylation

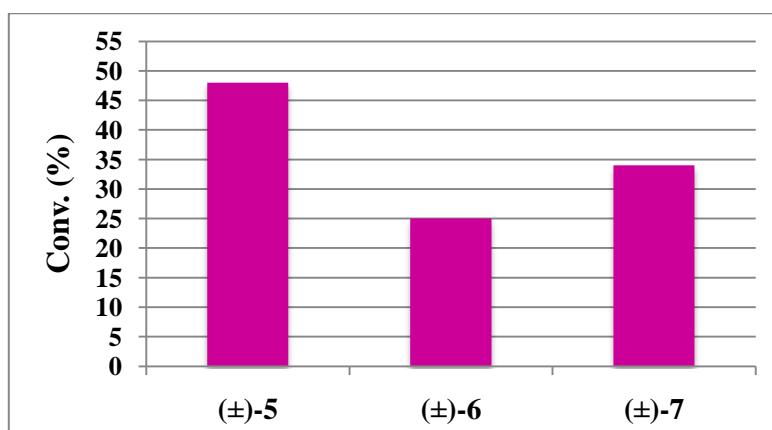
A systematic study was performed to investigate the effect of different substituents at position 6 [(\pm)-**5**, (\pm)-**6** and (\pm)-**7**] and *N*-protecting groups at position 2 [(\pm)-**5**, (\pm)-**9** and (\pm)-**10**] on the conversion and *E* of the CAL-B-catalysed resolution under identical reaction conditions (toluene, 2 equiv. acetic anhydride, 60 °C, batch mode; results were evaluated after 60 min).

In the case of *N*-Boc-protected compound (\pm)-**5** unsubstituted at the aromatic ring, the enzymatic reaction gave 48% conversion and a high *E* in 60 min (Figure 6). When the hydrogen atom was replaced with methoxy group at position 6 [(\pm)-**6**], the reaction rate decreased significantly (the conversion changed from 45% to 25% after 60 min), while the *E* of the reaction remained high ($E > 200$). The same phenomena were observed in the KR of 6-fluoro-substituted compound (\pm)-**7**: after 60 min, the conversion was only 34% and the *E* of the reaction, again, was excellent (>200).

We also evaluated the effect of different *N*-protecting groups on the stereoselectivity of *O*-acylation (Figure 7). Excellent *E* (>200) but varied conversions were found in the acylation of *N*-Boc- [(\pm)-**5**], *N*-Cbz- [(\pm)-**9**] and *N*-

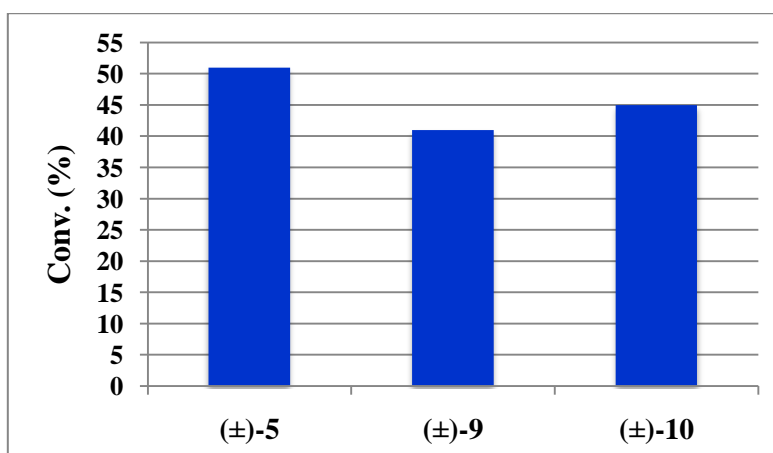
Fmoc-protected [(±)-**10**] substrates in the presence of CAL-B with acetic anhydride (toluene, 60 °C, 60 min). The resolution of (±)-**5** took place with a conversion of 51% in 60 min with an of $E > 200$. With bulkier aromatic carbonate PGs [(±)-**9** and (±)-**10**], conversions remained below 50% [41% for (±)-**9** and 46% for (±)-**10**], while selectivities were still excellent ($E > 200$). We also noted that, of all investigated acyl donors – in view of conversion and E – acetic anhydride proved to be the best in the resolution of derivatives with different protecting groups [(±)-**5**, (±)-**9** and (±)-**10**].

Figure 6. Effect of the substituent at position 6 on the conversion in the resolution of (±)-**5**–(±)-**7**^a



^a30 mg mL⁻¹ CAL-B; 2 equiv. acetic anhydride; 1 mL toluene; 60 °C after 60 min. In all cases E values were >200 at the indicated conversion levels.

Figure 7. Effect of the different *N*-protecting group on the conversion in the resolution of (±)-**5**, (±)-**9** and (±)-**10**^a



^a30 mg mL⁻¹ CAL-B; 2 equiv. acetic anhydride; 1 mL toluene; 60 °C after 60 min. In all cases E values were >200 at the indicated conversion levels.

The above results showed that the *E* values of the CAL-B-catalysed selective *O*-acylations were almost independent (with *E* >200 in all cases) of the type of substituents at position 6 [(±)-**5**, (±)-**6** and (±)-**7**] and *N*-protecting groups at position 2 [(±)-**5**, (±)-**9** and (±)-**10**]. Considering enantioselectivity, the enzyme seems to be very tolerant towards the substrate structure with modifications at position 2 or 6 at the THβC ring. The reaction rate of *O*-acylation, however, was strongly dependent on the structure of substrates studied (Figure 6 and 7). We could note that the decrease in reaction rate of substrates, bearing a substituent at the aromatic ring at position 6, was higher (difference between the highest and the lowest conv. was 14%, Figure 6). In contrast, the PGs at position 2 induced a lower difference of 6% (Figure 7). We should also mention that no correlation was found between the size of the PG and the reaction rate. We could conclude that *N*-Boc [(±)-**5**] was a suitable protecting group in the KR of (±)-**73** regarding *E* and reaction rate.

4.2.4. Preparative-scale resolutions

The preparative-scale enzymatic *O*-acylations of (±)-**5**–(±)-**7**, (±)-**9** and (±)-**10** were performed under the optimized reaction conditions with CAL-B enzyme and acetic anhydride as acyl donor [2 equiv. for (±)-**5**, 8 equiv. for (±)-**6**, 6 equiv. for (±)-**7** and 4 equiv. for (±)-**9** and (±)-**10**, toluene, 60 °C, incubator shaker] (Table 12). All scale-up reactions, with the exception of the resolution of (±)-**9**, gave excellent *E* values (>200) and yields (≥43%). The preparative-scale resolution of (±)-**9** occurred with a lower *E* (94) (entry 4) than that observed previously in the small-scale experiment (*E* >200, *ee* ≥97%, 20 min, 51% conv.). Results of the preparative-scale resolutions are summarized in Table 12.

Table 12. Preparative-scale resolutions of (±)-**5**–(±)-**7**, (±)-**9** and (±)-**10**^a

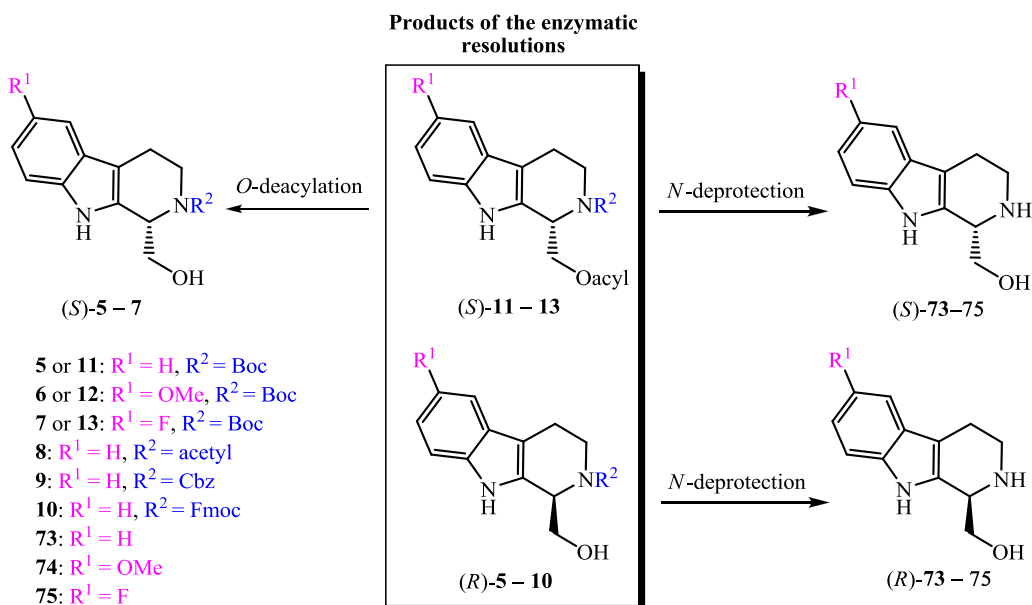
Entry	Substrate	Time	Amino alcohols [(<i>R</i>)- 5 –(<i>R</i>)- 7 , (<i>R</i>)- 9 , (<i>R</i>)- 10]			Amino esters [(<i>S</i>)- 11 –(<i>S</i>)- 13 , (<i>S</i>)- 15 , (<i>S</i>)- 16]		
			<i>ee</i> _s (%) ^b	Yield (%)	[α] _D ²⁵ (EtOH)	<i>ee</i> _p (%) ^b	Yield (%)	[α] _D ²⁵ (EtOH)
1	(±)- 5	1.5 h	98	47	+107.5 ^c	98	46	-102.2 ^d
2	(±)- 6	2.5 h	98	47	+82 ^e	98	43	-92.3 ^f
3	(±)- 7	2 h	96	47	+97 ^g	98	46	-131.8 ^h
4	(±)- 9	15 min	88	48	+77.2 ⁱ	94	45	-84.3 ^j
5	(±)- 10	50 min	99	44	+54 ^k	96	44	-57.8 ^l

^a30 mg mL⁻¹ CAL-B; acetic anhydride; toluene; 60 °C. ^bAccording to HPLC. ^c*c* = 0.34. ^d*c* = 0.32. ^e*c* = 0.23. ^f*c* = 0.61. ^g*c* = 0.21. ^h*c* = 0.38. ⁱ*c* = 0.52. ^j*c* = 0.625. ^k*c* = 0.28. ^l*c* = 0.80.

4.2.5. Further transformations

The amino ester products [(*S*)-**11**–(*S*)-**13**] of preparative resolutions of (\pm)-**5**–(\pm)-**7** were transformed into their corresponding amino alcohol enantiomers [(*S*)-**5**–(*S*)-**7**] through methanolysis (K_2CO_3 in MeOH at 60 °C) without changes in *ee* values (*ee* 98%, Scheme 34).

The *N*-Boc protecting group of (*R*)-**5**–(*R*)-**7** and (*S*)-**11**–(*S*)-**13** enantiomers was removed with an MW-assisted method in water at 100 °C (Scheme 34).^[169-171] The PG removal gave the desired unprotected enantiomers [(*R*)-**73**–**75** and (*S*)-**73**–**75**] with high *ee* values ($\geq 96\%$). Removal of Cbz PG of (*R*)-**9** was performed by catalytic hydrogenation in the CF reactor (H-Cube[®]) with the use of 10% Pd/C using a filled cartridge.^[172] In this case, however, both a significant decrease in the *ee* of (*R*)-**73** (from 88% to 78%) and a low yield of 50% were experienced. The *N*-Fmoc group of (*R*)-**10** was removed in DMF mixed with 20% piperidine. The reaction provided (*R*)-**73** without change in the *ee* (99%) and with a yield of 75% in 8 h.

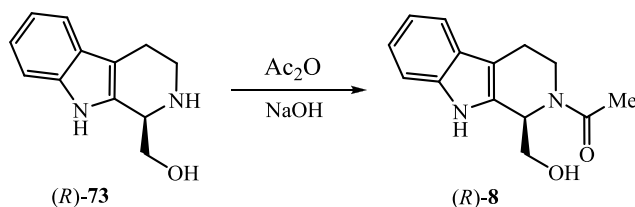


Scheme 34.

4.2.6. Determination of absolute configuration

To determine the absolute configuration, the enantiomer of **73** was transformed by a known literature method^[150] into its *N*-acetyl derivative (**8**) (Scheme 35) with a known absolute configuration and optical rotation reported earlier.^[173] A comparison of the optical rotation value of **8** measured $\{[\alpha]_{\text{D}}^{25} =$

+164 ($c = 0.2$ in EtOH)} with the literature data for (*R*)-**8** { $ee = 98\%$, lit.^[173] $[\alpha]_D^{25} = +17.3$ ($c = 0.2$ in ethanol) showed a considerable difference. The reason for this difference in specific rotations was an error in the placement of the decimal point}. The direction of the optical rotation indicated that the CAL-B-catalysed *O*-acylation of (\pm)-**5** is *S*-selective.



Scheme 35.

To determine the stereochemistry of the *N*-Cbz (**9**) and *N*-Fmoc (**10**) enantiomers, the optical rotations of their unprotected form (**73**) { $[\alpha]_D^{25} = -22$ and -35.4 in EtOH} were compared with the specific rotation of (*R*)-**73** {lit.^[39], $[\alpha]_D^{25}$: -37.8 in EtOH}. Thus, CAL-B catalysed *S*-selectively the *O*-acylation of *N*-Cbz- and *N*-Fmoc-protected substrates [(\pm)-**9** and (\pm)-**10**] similar to that of *N*-Boc protected derivative (\pm)-**5**.

5. Summary

CAL-B-catalysed DKR hydrolysis of 6-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid ethyl ester (\pm)-**1** was performed in DIPE/MeCN (3:2) in the presence of 2 equiv. of H₂O, 0.25 equiv. of Pr₂NH at 25 °C. (*R*)-**2** was produced with an *ee* of 91% at a conversion of 35% after 2 days. When DKR of (\pm)-**1** was carried out in aqueous NH₄OAc buffer at pH 8.5 only traces of (*R*)-**2** were obtained (*ee* of 66% at a conv. of 90% after 2 days). To circumvent the problem of the decomposition of substrate (\pm)-**1**, the DKR was performed from its hydrochloride salt [(\pm)-**1**·HCl] in NH₄OAc buffer, at pH 8.5, at 25 °C and the desired amino acid (*R*)-**2** was isolated with an excellent *ee* of >99% and yield of 91% after 3 h.

R-Selective DKR hydrolysis of the hydrochloride salt of 1,2,3,4-tetrahydro- β -carboline-1-carboxylic acid ethyl ester [(\pm)-**3**·HCl] with CAL-B in 0.1 M NH₄OAc buffer (pH 8.0, 30 °C) afforded the product amino acid [(*R*)-**4**·HCl] with an *ee* of 98% and a yield of 90% after 20 min. Alcalase-catalysed DKR of (\pm)-**3**·HCl for the preparation of the corresponding *S*-amino acid was carried out in 0.1 M NH₄OAc buffer, pH 8.0 at 30 °C, but low selectivity was observed (*ee* = 53% at a conv. of 99% after 24 h). Changing the pH from 8.0 to 9.0 caused an increase in the reaction rate but resulted in a decrease in the *ee*. Lowering the temperature, in turn, had an opposite effect on the reaction. A change of the NH₄OAc buffer to borate buffer led an optimized reaction medium (Alcalase, 0.1 M borate buffer, pH 8.0, 30 °C). This allowed preparative-scale resolution and isolation of (*S*)-**4**·HCl with an *ee* of 60% and a yield of 66% after 45 h.

The KR of (\pm)-**5** with CAL-B, in toluene, with 2 equiv. of acetic anhydride as acyl donor in a batch process at 60 °C afforded an excellent *E* (>200) and products (*R*)-**5** and (*S*)-**11** were isolated with an *ee* of 98% and a yield of \geq 46% after 1.5 h. The optimized reaction conditions, after slight modification, could be extended to compounds (\pm)-**6** and (\pm)-**7** in a batch system (CAL-B, 8 equiv. acetic anhydride for (\pm)-**6** and 6 equiv. acetic anhydride for (\pm)-**7**, toluene, 60 °C). KR gave (*R*)-**6** and (*S*)-**12** with an *ee* of 98% and a yield of \geq 43% after 2.5 h. (*R*)-**7** and (*S*)-**13** were also isolated with high *ee* (\geq 96%) and yields (\geq 45%) after 2 h.

The KR of *N*-acetyl- [(±)-**8**], *N*-Cbz- [(±)-**9**] and *N*-Fmoc-protected [(±)-**10**] substrates was carried out with CAL-B, 2 equiv. acyl donor, in toluene, at 60 °C. We found that *N*-acetyl [(±)-**8**] was an inadequate PG because of *N*→*O* and *O*→*N* acyl migrations. Preparative-scale resolutions of (±)-**9** and (±)-**10** (with 4 equiv. acetic anhydride) gave amino alcohol enantiomers (*R*)-**9** and (*R*)-**10** and esters (*S*)-**15** and (*S*)-**16** with good *ee* (≥88%) and high yields (≥44%).

A systematic study under the same reaction conditions (CAL-B, 2 equiv. acetic anhydride, toluene, 60 °C, 60 min) was performed to investigate the effect of different substituents at position 6 [(±)-**5**, (±)-**6** and (±)-**7**] and *N*-protecting groups at position 2 [(±)-**5**, (±)-**9** and (±)-**10**] on the reaction rate and *E*. The use of the SE concept revealed that substituents at position 2 and 6 had a significant effect on the reaction rate but only limited effect on the *E* of the reactions.

Amino esters of (*S*)-**11**–(*S*)-**13** were transformed into (*S*)-**5**–(*S*)-**7** via methanolysis (in the presence of K₂CO₃ in MeOH at 60 °C) without changes in the *ee* (98%) and yields of 75-90%. MW-assisted *N*-Boc PG removals for (*R*)-**5**–(*R*)-**7** and (*S*)-**11**–(*S*)-**13** were carried out in water at 100 °C and product enantiomers (*R*)-**73**–**75** and (*S*)-**73**–**75** were isolated maintaining high *ee* values (≥96%) and with yields of 38-79%. The *N*-Cbz group of (*R*)-**9** was removed by catalytic hydrogenation in a CF reactor and (*R*)-**73** was isolated with an *ee* of 78% (decreased from 88%) and a yield of 50%. The removal of the *N*-Fmoc group of (*R*)-**10** was performed in DMF containing 20% piperidine and product (*R*)-**73** was obtained with an *ee* of 99% and with a yield of 75% after 8 h.

19 new enantiomers were prepared and characterized by *ee*, optical rotation, ¹H and ¹³C NMR, elemental analysis and melting point measurements.^{I-IV}

6. References

- [1.] P. Roszkowski, S. Czarnocki, J. K. Maurin, A. Siwicka, A. Zawadzka, J. Szawkało, A. Leniewski, Z. Czarnocki, *Int. Congr. Ser.* **2007**, 1304, 46-59.
- [2.] M. Dembitsky, T. A. Glorizova, V. V. Poroikov, *Phytomedicine* **2015**, 22, 183-202.
- [3.] C. E. P. Galvis, V. V. Kouznetsov, *Synthesis* **2017**, 49, 4535-4561.
- [4.] I. Chakraborty, S. Jana, *Synthesis* **2013**, 45, 3325-3331.
- [5.] E. M. Boyd, L. M. Knight, *J. Pharm. Pharmacol.* **1964**, 16, 118-124.
- [6.] M. A. Al-Yahya, M. M. A. Hassan, *Anal. Profiles Drug Subst.* **1982**, 11, 407-461.
- [7.] X. Chen, P. J. Facchini, *Plant J.* **2014**, 77, 173-184.
- [8.] Z.-Z. Fang, K. W. Krausz, F. Li, J. Cheng, N. Tanaka, F. J. Gonzalez, *Br. J. Pharmacol.* **2012**, 167, 1271-1286.
- [9.] A. Poveda, I. Ray-Coquard, I. Romero, J. A. Lopez-Guerrero, N. Colombo, *Cancer Treat. Rev.* **2014**, 40, 366-375.
- [10.] Y. Kashiwada, A. Aoshima, Y. Ikeshiro, Y.-P. Chen, H. Furukawa, M. Itoigawa, T. Fujioka, K. Mihashi, L. M. Cosentino, S. L. Morris-Natschke, K.-H. Lee, *Bioorg. Med. Chem.* **2005**, 13, 443-448.
- [11.] A. T. Plowright, S. E. Schaus, A. G. Myers, *Chem. Biol.* **2002**, 9, 607-618.
- [12.] B. Silakowski, G. Nordsiek, B. Kunze, H. Blöcker, R. Müller, *Chem. Biol.* **2001**, 8, 59-69.
- [13.] J. W. Lane, A. Estevez, K. Mortara, O. Callan, J. R. Spencer, R. M. Williams, *Bioorganic Med. Chem. Lett.* **2006**, 16, 3180-3183.
- [14.] Z.-Z. Liu, Y. Wang, Y.-F. Tang, S.-Z. Chen, X.-G. Chen, H.-Y. Li, *Bioorganic Med. Chem. Lett.* **2006**, 16, 1282-1285.
- [15.] J. F. M. Leal, V. García-Hernández, V. Moneo, A. Domingo, J. A. Bueren-Calabuig, A. Negri, F. Gago, M. J. Guillén-Navarro, P. Avilés, C. Cuevas, L. F. García-Fernández, C. M. Galmarini, *Biochem. Pharmacol.* **2009**, 78, 162-170.
- [16.] N. Neuss, M. Gorman, W. Hargrove, N. J. Cone, K. Biemann, G. Buchi, R. E. Manning, *J. Am. Chem. Soc.* **1964**, 86, 1440-1442.
- [17.] L. Garrett, O. Carrier Jr., B. H. Douglas, *Eur. J. Pharmacol.* **1967**, 2, 236-238.
- [18.] Z.-J. Zhang, J. Yang, J. He, X.-D. Wu, L.-D. Shao, Y. Li, S.-X. Huang, R.-T. Li, Q.-S. Zhao, *Tetrahedron Lett.* **2014**, 55, 6490-6494.
- [19.] H.-J. Cong, Q. Zhao, S.-W. Zhang, J.-J. Wei, W.-Q. Wang, L.-J. Xuan, *Phytochemistry* **2014**, 100, 76-85.
- [20.] S. P. B. Ovenden, J. L. Nielson, C. H. Liptrot, R. H. Willis, D. M. Tapiolas, A. D. Wright, C. A. Motti, *Phytochem. Lett.* **2011**, 4, 69-71.
- [21.] F. X. Wang, A.-J. Deng, M. Li, J.-F. Wei, H.-L. Qin, A.-P. Wang, *Molecules* **2013**, 18, 418-429.
- [22.] T.-S. Kam, K.-M. Sim, *Phytochemistry* **1998**, 47, 145-147.
- [23.] H. M. Spindola, D. B. Vendramini-Costa, M. T. Rodrigues Jr., M. A. Foglio, R. A. Pilli, J. E. Carvalho, *Pharmacol. Biochem. Behav.* **2012**, 102, 133-138.
- [24.] R. A. Davis, S. Duffy, V. M. Avery, D. Camp, J. N. A. Hooper, R. J. Quinn, *Tetrahedron Lett.* **2010**, 51, 583-585.

- [25.] G. Brock, X. Ni, M. Oelke, J. Mulhall, M. Rosenberg, A. Seftel, D. D'Souza, J. Barry, *J. Sex. Med.* **2016**, *13*, 860-875.
- [26.] S. Xiao, X.-X. Shi, J. Xing, J.-J. Yan, S.-L. Liu, W.-D. Lu, *Tetrahedron: Asymmetry* **2009**, *20*, 2090-2096.
- [27.] S. He, Z. Lai, Z. Ye, P. H. Dobbelaar, S. K. Shah, Q. Truong, W. Du, L. Guo, J. Liu, T. Jian, H. Qi, R. K. Bakshi, Q. Hong, J. Dellureficio, M. Reibarkh, K. Samuel, V. B. Reddy, S. Mitelman, S. X. Tong, G. G. Chicchi, K.-L. Tsao, D. Trusca, M. Wu, Q. Shao, M. E. Trujillo, G. Fernandez, D. Nelson, P. Bunting, J. Kerr, P. Fitzgerald, P. Morissette, S. Volksdorf, G. J. Eiermann, C. Li, B. Zhang, A. D. Howard, Y.-P. Zhou, R. P. Nargund, W. K. Hagmann, *ACS. Med. Chem. Lett.* **2015**, *6*, 513-517.
- [28.] S. He, P. H. Dobbelaar, L. Guo, Z. Ye, J. Liu, T. Jian, Q. Truong, S. K. Shah, W. Du, H. Qi, R. K. Bakshi, Q. Hong, J. D. Dellureficio, E. Sherer, A. Pasternak, Z. Feng, M. Reibarkh, M. Lin, K. Samuel, V. B. Reddy, S. Mitelman, S. X. Tong, G. G. Chicchi, K.-L. Tsao, D. Trusca, M. Wu, Q. Shao, M. E. Trujillo, G. Fernandez, D. Nelson, P. Bunting, J. Kerr, P. Fitzgerald, P. Morissette, S. Volksdorf, G. J. Eiermann, C. Li, B. B. Zhang, A. D. Howard, Y.-P. Zhou, R. P. Nargund, W. K. Hagmann, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1529-1535.
- [29.] R. Brokamp, B. Bergmann, I. B. Müller, S. Bienz, *Bioorg. Med. Chem.* **2014**, *22*, 1832-1837.
- [30.] I. S. Grill, E. Kick, K. Richlin-Zack, W. Yang, Y. Wang, R. N. Patel, *Tetrahedron: Asymmetry* **2007**, *18*, 2147-2154.
- [31.] I. Khan, S. Khan, V. Tyagi, P. S. Chouhan, P. M. S. Chauhan, *RSC Adv.* **2015**, *5*, 102713-102722.
- [32.] A. Kamal, M. A. Azhar, T. Krishnaji, M. S. Malik, S. Azeza, *Coord. Chem. Rev.* **2008**, *252*, 569-592.
- [33.] M. Ahmed, T. Kelly, A. Ghanem, *Tetrahedron* **2012**, *68*, 6781-6802.
- [34.] Z. S. Seddigi, M. S. Malik, S. A. Ahmed, A. O. Babalghith, A. Kamal, *Coord. Chem. Rev.* **2017**, *348*, 54-70.
- [35.] C. E. Humphrey, M. Ahmed, A. Ghanem, N. J. Turner, Separation Of Enantiomers: Synthetic Methods 1st. ed., Chapter 4., John Wiley and Sons, INC., **2014**, pp. 123-159.
- [36.] O. Pàmies, J.-E. Bäckvall, *Trends Biotechnol.* **2004**, *22*, 130-135.
- [37.] A. H. Kamaruddin, M. H. Uzir, H. Y. Aboul-Enein, H. N. A. Halim, *Chirality* **2009**, *29*, 449-467.
- [38.] R. S. Ward, *Tetrahedron: Asymmetry* **1995**, *6*, 1475-1490.
- [39.] R. Azerad, D. Buisson, *Curr. Opin. Biotechnol.* **2000**, *11*, 565-571.
- [40.] P. Hoyos, M. A. Quezada, J. V. Sinisterra, A. R. Alcàntare, *J. Mol. Catal. B: Enzym.* **2011**, *72*, 20-24.
- [41.] K. P. J. Gustafson, A. Guðmundsson, K. Lewis, J.-E. Bäckvall, *Chem. Eur. J.* **2017**, 1048-1051.
- [42.] I. A. Shuklov, N. V. Dubrovina, J. Schulze, W. Tietz, A. Börner, *Tetrahedron* **2014**, *55*, 3495-3497.
- [43.] J. Cho, J. Lee, J. Park, M.-J. Kim, *Tetrahedron: Asymmetry* **2015**, *26*, 840-845.
- [44.] J.-S. Im, S.-H. Ahn, J.-H. Park, *Chem. Eng. J.* **2013**, *234*, 49-56.
- [45.] K. Michalak, J. Wicha, J. Wójcik, *Tetrahedron* **2016**, *72*, 4813-4820.
- [46.] A. Lumbroso, M. L. Cooke, B. Breit, *Angew. Chem. Int. Ed.* **2013**, *52*, 1890-1932.

- [47.] L. K. Thalén, D. Zhao, J.-B. Sortais, J. Paetzold, C. Hoben, J.-E. Bäckvall, *Chem. Eur. J.* **2009**, *15*, 3403-3410.
- [48.] K. Engström, M. Shakeri, J.-E. Bäckvall, *Eur. J. Org. Chem.* **2011**, 1827-1830.
- [49.] M. Shakeri, K. Engström, A. G. Sandström, J.-E. Bäckvall, *ChemCatChem* **2010**, *2*, 534-538.
- [50.] Y. Kim, J. Park, M.-J. Kim, *Tetrahedron Lett.* **2010**, *51*, 5581-5584.
- [51.] M. Stirling, J. Blacker, M. I. Page, *Tetrahedron Lett.* **2007**, *48*, 1247-1250.
- [52.] O. Verho, J.-E. Bäckvall, *J. Am. Chem. Soc.* **2015**, *12*, 3996-4009.
- [53.] P. Hoyos, V. Pace, A. R. Alcántara, *Adv. Synth. Catal.* **2012**, *354*, 2585-2611.
- [54.] Y. Kim, J. Park, M.-J. Kim, *ChemCatChem* **2011**, *3*, 271-277.
- [55.] Y. Ahn, S.-B. Ko, M.-J. Kim, J. Park, *Coord. Chem. Rev.* **2008**, *252*, 647-658.
- [56.] O. Långvik, T. Saloranta, D. Y. Murzin, R. Leino, *ChemCatChem* **2015**, *7*, 4004-4015.
- [57.] T. A. Paál, A. Liljeblad, L. T. Kanerva, E. Forró, F. Fülöp, *Eur. J. Org. Chem.* **2008**, 5269-5276.
- [58.] R. Morán-Ramallal, V. Gotor-Fernández, P. Laborda, F. J. Sayago, C. Cativiela, V. Gotor, *Org. Lett.* **2012**, *14*, 1696-1699.
- [59.] M. López-Iglesias, A. Arizpe, F. J. Sayago, V. Gotor, C. Cativiela, V. Gotor-Fernández, *Tetrahedron* **2016**, *72*, 7311-7316.
- [60.] M. Sharfuddin, A. Narumi, Y. Iwai, K. Miyazawa, S. Yamada, T. Kakuchic, H. Kaga, *Tetrahedron: Asymmetry* **2003**, *14*, 1581-1585.
- [61.] C. S. Fuchs, M. Hollauf, M. Meissner, R. C. Simon, T. Besset, J. N. H. Reek, W. Riethorst, F. Zepeck, W. Kroutil, *Adv. Synth. Catal.* **2014**, *356*, 2257-2265.
- [62.] D. Koszelewski, D. Clay, K. Faber, W. Kroutil, *J. Mol. Catal. B: Enzym.* **2009**, *60*, 191-194.
- [63.] J. A. Chaplin, M. D. Levin, B. Morgan, N. Farid, J. Li, Z. Zhu, J. McQuaid, L. W. Nicholson, C. A. Rand, M. J. Burk, *Tetrahedron: Asymmetry* **2004**, *15*, 2793-2796.
- [64.] J. Qui, E. Su, W. Wang, D. Wei, *Tetrahedron Lett.* **2014**, *55*, 1448-1451.
- [65.] T. A. Paál, E. Forró, A. Liljeblad, L. T. Kanerva, F. Fülöp, *Tetrahedron: Asymmetry* **2007**, *18*, 1428-1433.
- [66.] E. Gianolio, R. Mohan, A. Berkessel, *Adv. Synth. Catal.* **2016**, *358*, 30-33.
- [67.] A. Berkessel, I. Jurkiewicz, R. Mohan, *ChemCatChem* **2011**, *3*, 319-330.
- [68.] S. A. Brown, M.-C. Parker, N. J. Turner, *Tetrahedron: Asymmetry* **2000**, *11*, 1687-1690.
- [69.] J. Pietruszka, R. C. Simon, F. Kruska, M. Braun, *Eur. J. Org. Chem.* **2009**, 6217-6224.
- [70.] P. Bongen, J. Pietruszka, R. C. Simon, *Chem. Eur. J.* **2012**, *18*, 11063-11070.
- [71.] C.-S. Chang, S.-W. Tsai, J. Kuo, *Biotechnol. Bioeng.* **1999**, *5*, 120-126.
- [72.] P. D'Arrigo, L. Cerioli, A. Fiorati, S. Servi, F. Viani, D. Tessaro, *Tetrahedron: Asymmetry* **2012**, *23*, 938-944.
- [73.] D. Arosio, A. Caligiuri, P. D'Arrigo, G. Pedrocchi-Fantoni, C. Rossi, C. Saraceno, S. Servi, D. Tessaro, *Adv. Synth. Catal.* **2007**, *349*, 1345-1348.
- [74.] C.-N. Lin, S.-W. Tsai, *Biotechnol. Bioeng.* **2000**, *5*, 31-38.
- [75.] C.-S. Chang, S.-W. Tsai, *Biochem. Eng. J.* **1999**, *3*, 239-242.

- [76.] F. J. Quijada, V. Gotor, F. Rebolledo, *Org. Lett.* **2010**, *12*, 3602-3605.
- [77.] Y.-X. Li, A. J. J. Straathof, U. Hanefeld, *Tetrahedron: Asymmetry* **2002**, *13*, 739-743.
- [78.] L. Veum, U. Hanefeld, *Tetrahedron: Asymmetry* **2004**, *15*, 3707-3709.
- [79.] N. Harper, M. Dolman, B. D. Moore, P. J. Halling, *Chem. Eur. J.* **2000**, *6*, 1923-1929.
- [80.] T. Sakai, K. Wang, T. Ema, *Tetrahedron: Asymmetry* **2008**, *64*, 2178-2183.
- [81.] Y. Zhu, K.-L. Fow, G.-K. Chuah, S. Jaenicke, *Chem. Eur. J.* **2007**, *13*, 541-547.
- [82.] S. Wuyts, K. De Temmerman, D. E. De Vos, P. A. Jacobs, *Chem. Eur. J.* **2005**, *11*, 386-397.
- [83.] K.-I. Shimomura, H. Harami, Y. Matsubara, T. Nokami, N. Katada, T. Itoh, *Catal. Today* **2015**, *255*, 41-48.
- [84.] X. Li, Y. Yan, W. Wang, Y. Zhang, Y. Tang, *J. Colloid Interface Sci.* **2015**, *438*, 22-28.
- [85.] Y. Cheng, G. Xu, J. Wu, C. Zhang, L. Yang, *Tetrahedron Lett.* **2010**, *51*, 2366-2369.
- [86.] J.-P. Wu, X. Meng, L. Wang, G. Xu, L.-R. Yang, *Tetrahedron Lett.* **2014**, *55*, 5129-5132.
- [87.] B. Schnell, K. Faber, W. Kroutil, *Adv. Synth. Catal.* **2003**, *345*, 653-666.
- [88.] N. J. Turner, *Curr. Opin. Biotechnol.* **2004**, *8*, 114-119.
- [89.] A. Mikleušević, Z. Hameršak, B. Salopek-Sondi, L. Tang, D. B. Janssen, M. M. Elenkov, *Adv. Synth. Catal.* **2015**, *357*, 1709-1714.
- [90.] K. Yasukawa, Y. Asano, *Adv. Synth. Catal.* **2012**, *354*, 3327-3332.
- [91.] J. B. Crawford, R. T. Skerlj, G. J. Bridger, *J. Org. Chem.* **2007**, *72*, 669-671.
- [92.] P. Vongvilai, M. Linder, M. Sakulsombat, M. S. Humble, P. Berglund, T. Brinck, O. Ramström, *Angew. Chem. Int. Ed.* **2011**, *50*, 6592-6595.
- [93.] M. Sakulsombat, P. Vongvilai, O. Ramström, *Chem. Eur. J.* **2014**, *20*, 11322-11325.
- [94.] M. Avi, R. M. Wiedner, H. Griengl, H. Schwab, *Chem. Eur. J.* **2008**, *14*, 11415-11422.
- [95.] R. A. Sheldon, P. C. Pereira, *Chem. Soc. Rev.* **2017**, *46*, 2678-2691.
- [96.] S.-W. Tsai, C.-C. Chen, H.-S. Yang, I.-S. Ng, T.-L. Chen, *Biochim. Biophys. Acta* **2006**, *1764*, 1424-1428.
- [97.] P.-Y. Wang, S.-W. Tsai, T.-L. Chen, *Biotechnol. Bioeng.* **2008**, *101*, 460-469.
- [98.] G. Li, H. Zhang, Z. Sum, X. Liu, M. T. Reetz, *ACS Catal.* **2016**, *6*, 3679-3687.
- [99.] K. Faber, *Biotransformations in organic chemistry*, 2nd ed., Springer-Verlag, **1995**, pp. 309-310.
- [100.] J. M. Paloma, G. Fernández-Lorente, C. Mateo, M. Fuentes, R. Fernández-Lafuente, J. M. Guisan, *Tetrahedron: Asymmetry* **2002**, *13*, 1337-1345.
- [101.] S. Gåsaidnes, B. Synstad, J. E. Nielsen, V. G. H. Eijsink, *J. Mol. Catal. B: Enzym.* **2003**, *21*, 3-8.
- [102.] J. Zhang, M. Xu, X. Zhang, T. Yang, Z. Xu, Z. Rao, *J. Mol. Catal. B: Enzym.* **2016**, *113*, 360-370.
- [103.] S. Yoon, S. Kim, S. Park, E. Hong, J. Kim, S. Kim, T. H. Yoo, Y. Ryu, *J. Mol. Catal. B: Enzym.* **2014**, *100*, 25-31.

- [104.] M. S. Newton, V. L. Arcus, M. L. Gerth, W. M. Patrick, *Curr. Opin. Biotechnol.* **2018**, *48*, 110-116.
- [105.] M. Widmann, J. Pleiss, A. K. Samland, *Comput. Struct. Biotechnol. J.* **2012**, *2*, e201209016.
- [106.] P. Baker, S. Y. K. Seah, *Comput. Struct. Biotechnol. J.* **2012**, *2*, e201209003.
- [107.] A. Li, L. Ye, X. Yang, B. Wang, C. Yang, J. Gu, H. Yu, *ChemCatChem* **2016**, *8*, 1-6.
- [108.] Y. Shimada, H. Sato, S. Mochizuki, K. Matsumoto, *Synlett* **2008**, *19*, 2981-2984.
- [109.] E. Brenna, F. G. Gatti, D. Monti, F. Parmeggiani, A. Sacchetti, J. Valoti, *J. Mol. Catal. B: Enzym.* **2015**, *114*, 77-85.
- [110.] E. Brenna, G. Fronza, C. Fuganti, F. G. Gatti, A. Manfredi, F. Parmeggiani, P. Ronchi, *J. Mol. Catal. B: Enzym.* **2012**, *84*, 94-101.
- [111.] E. Brenna, M. Crotti, F. G. Gatti, A. Manfredi, D. Monti, F. Parmeggiani, A. Pugliese, D. Zampieri, *J. Mol. Catal. B: Enzym.* **2014**, *101*, 67-72.
- [112.] J. Y. Choi, R. F. Borch, *Org. Lett.* **2007**, *9*, 215-218.
- [113.] S.-W. Tsai, *J. Mol. Catal. B: Enzym.* **2016**, *127*, 98-116.
- [114.] A. de Raadt, H. Griengl, H. Weber, *Chem. Eur. J.* **2001**, *7*, 27-31.
- [115.] A. de Raadt, B. Fetz, H. Griengl, M. F. Klingler, B. Krenn, K. Mereiter, D. F. Münzer, P. Plachota, H. Weber, R. Saf, *Tetrahedron* **2001**, *57*, 8151-8157.
- [116.] M. H. Fechter, K. Gruber, M. Avi, W. Skranc, C. Schuster, P. Pöchlauer, K. O. Klepp, H. Griengl, *Chem. Eur. J.* **2007**, *13*, 3369-3376.
- [117.] D. Zhu, H. T. Malik, L. Hua, *Tetrahedron: Asymmetry* **2006**, *17*, 3010-3014.
- [118.] B. Wilding, M. Winkler, B. Petschacher, R. Kratzer, S. Egger, G. Steinkellner, A. Lyskowski, B. Nidetzky, K. Gruber, N. Klempier, *Chem. Eur. J.* **2013**, *19*, 7007-7012.
- [119.] J. Y. Liu, G.-W. Zheng, C.-X. Li, H.-L. Yu, J. Pan, J.-H. Xu, *J. Mol. Catal. B: Enzym.* **2013**, *89*, 41-47.
- [120.] P. J. O'Brien, D. Herschlag, *Chem. Biol.* **1999**, *6*, 91-105.
- [121.] R. Beine, R. Moraru, M. Nimtz, S. Na'amnieh, A. Pawlowski, K. Buchholz, J. Seibel, *J. Biotechnol.* **2008**, *138*, 33-41.
- [122.] T. S. da Silva, S. K. Campos, A. R. M. de Oliveira, L. Piovan, *J. Mol. Catal. B: Enzym.* **2016**, *133*, S317-S323.
- [123.] J. S. Reis, L. H. Andrade, *Tetrahedron: Asymmetry* **2012**, *23*, 1294-1300.
- [124.] P.-Y. Wang, T.-L. Chen, S.-W. Tsai, *J. Mol. Catal. B: Enzym.* **2007**, *48*, 16-22.
- [125.] R. E. Deasy, M. Brossat, T. S. Moody, A. R. Maguire, *Tetrahedron: Asymmetry* **2011**, *22*, 47-61.
- [126.] P.-Y. Wang, T.-L. Chen, S.-W. Tsai, W. Kroutil, *Biotechnol. Bioeng.* **2007**, *98*, 30-38.
- [127.] P.-Y. Wang, S.-W. Tsai, *J. Mol. Catal. B: Enzym.* **2009**, *59*, 70-75.
- [128.] L. Zheng, S. Zhang, L. Zhao, G. Zhu, X. Yang, G. Gao, S. Cao, *J. Mol. Catal. B: Enzym.* **2006**, *38*, 119-125.
- [129.] T. Miyazawa, H. Minowa, T. Yamada, *Biotechnol. Lett.* **2006**, *28*, 295-299.
- [130.] H. Mäenpää, L. T. Kanerva, A. Liljeblad, *ChemCatChem* **2016**, *8*, 1226-1232.
- [131.] S. Gedey, A. Liljeblad, L. Lázár, F. Fülöp, L. T. Kanerva, *Tetrahedron: Asymmetry* **2001**, *12*, 105-107.

- [132.] P. T. Bandeira, R. C. Alnoch, A. R. M. de Oliveira, E. M. de Souza, F. de O. Pedrosa, N. Krieger, L. Piovan, *J. Mol. Catal. B: Enzym.* **2016**, *125*, 58-63.
- [133.] T. Miyazawa, E. Kaito, T. Yukawa, T. Murashima, T. Yamada, *J. Mol. Catal. B: Enzym.* **2005**, *37*, 63-67.
- [134.] O. V. Kucner, A. O. Kolodiazhnaya, O. B. Smolii, D. V. Prisuazhnyk, K. A. Tolmacheva, O. A. Zaporozhets, Y. S. Moroz, P. K. Mykhailiuk, A. A. Tomachev, *Eur. J. Org. Chem.* **2014**, *34*, 7692-7698.
- [135.] C. Paizs, M. Toşa, V. Bóдай, G. Szakács, I. Kmecz, B. Simándi, C. Majdik, L. Novák, F.-D. Irimie, L. Poppe, *Tetrahedron: Asymmetry* **2003**, *14*, 1943-1949.
- [136.] Y. Shimada, H. Sato, S. Mochizuki, K. Matsumoto, *Synlett* **2008**, *19*, 2981-2984.
- [137.] P. P. Bora, G. Bez, J. M. H. Anal, *J. Mol. Catal. B: Enzym.* **2011**, *72*, 270-275.
- [138.] M. Päivöi, P. Perkiö, L. T. Kanerva, *Tetrahedron: Asymmetry* **2012**, *23*, 230-236.
- [139.] Z. Boros, P. Falus, M. Márkus, D. Weiser, M. Oláh, G. Hornyánszky, J. Nagy, L. Poppe, *J. Mol. Catal. B: Enzym.* **2013**, *85-86*, 119-125.
- [140.] J. González-Sabín, V. Gotor, F. Rebolledo, *J. Org. Chem.* **2007**, *72*, 1309-1314.
- [141.] D. Yildirim, S. S. Tükel, *Process. Biochem.* **2013**, *48*, 819-830.
- [142.] A. Alalla, M. Merabet-Khalessi, O. Riant, L. Aribi-Zouiouche, *Tetrahedron: Asymmetry* **2016**, *27*, 1253-1259.
- [143.] V. Magrioti, I. Fotakopoulou, N. Athinaios, P. Anastasopoulou, V. Constantinou-Kokotou, G. Kokotos, *Lett. Org. Chem.* **2010**, *7*, 159-162.
- [144.] R. K. Cheedra, R. Sachwani, P. D. Krishna, *Tetrahedron: Asymmetry* **2008**, *19*, 901-905.
- [145.] J. A. Rodríguez-Rodríguez, F. J. Quijada, R. Brieva, F. Rebolledo, *Tetrahedron*, **2013**, *69*, 5407-5412.
- [146.] J. Wegner, S. Ceylan, A. Kirschning, *Chem. Commun.* **2011**, *47*, 4583-4592.
- [147.] C. Wiles, P. Watts, *Green Chem.* **2012**, *14*, 38-54.
- [148.] Z. Zalán, T. A. Martinek, R. Sillanpää, F. Fülöp, *Tetrahedron* **2006**, *62*, 2883-2891.
- [149.] L. F. Tietze, Y. Zhou, E. Töpken, *Eur. J. Org. Chem.* **2000**, 2247-2254.
- [150.] Z. J. Vejdělek, V. Trčka, M. Protiva, *J. Med. Chem.* **1961**, *3*, 427-440.
- [151.] I. D. Spenser, *Can. J. Chem.* **1959**, *37*, 1851-1858.
- [152.] Z. Arazny, Z. Czarnocki, K. Wojtasiewicz, J. K. Maurin, *Tetrahedron: Asymmetry* **2000**, *11*, 1623-1628.
- [153.] E. Forró, F. Fülöp, *Org. Lett.* **2003**, *5*, 1209-1212.
- [154.] L.-C. Lo, T.-C. Chou, J.-J. Shie, *Chirality* **2004**, *16*, 267-271.
- [155.] D. A. Schichl, S. Enthaler, W. Holla, T. Riermeier, U. Kragl, M. Beller, *Eur. J. Org. Chem.* **2008**, *73*, 3506-3512.
- [156.] S.-T. Chen, K.-T. Wang, *Chin. Chan. Soc.* **1999**, *46*, 301-311.
- [157.] A. Péter, E. Vékes, G. Tóth, D. Tourwé, F. Borremans, *J. Chromatogr. A* **2002**, *948*, 283-294.
- [158.] A. Péter, G. Török, D. W. Armstrong, G. Tóth, D. Tourwé, *J. Chromatogr. A* **2000**, *904*, 1-15.

- [159.] E. Forró, L. Schönstein, F. Fülöp, *Tetrahedron: Asymmetry* **2011**, 22, 1255-1260.
- [160.] L. Schönstein, E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2013**, 24, 1059-1062.
- [161.] L. Schönstein, E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2013**, 24, 202-206.
- [162.] E. Forró, K. Lundell, F. Fülöp, L. T. Kanerva, *Tetrahedron: Asymmetry* **1997**, 8, 3095-3099.
- [163.] G. Fodor, J. Kiss, *J. Am. Chem. Soc.* **1950**, 72, 3495-3497.
- [164.] T. Furutani, M. Furui, H. Ooshima, J. Kato, *Enzym. Microb. Technol.* **1996**, 19, 578-584.
- [165.] R. B. Martin, A. Parcell, *J. Am. Chem. Soc.* **1961**, 83, 4835-4838.
- [166.] L. T. Kanerva, M. Kosonen, E. Vättinen, T. T. Huuhtanen, M. Dahlqvist, *Acta Chem. Scand.* **1992**, 46, 1101-1105.
- [167.] F. Le Joubioux, N. Bridiau, Y. B. Henda, O. Achour, M. Graber, T. Maugard, *J. Mol. Catal. B: Enzym.* **2013**, 95, 99-110.
- [168.] F. Le Joubioux, Y. B. Henda, N. Bridiau, O. Achour, M. Graber, T. Maugard, *J. Mol. Catal. B: Enzym.* **2013**, 85-86, 193-199.
- [169.] G. Wang, C. Li, J. Li, X. Jia, *Tetrahedron Lett.* **2009**, 50, 1438-1440.
- [170.] S. R. Dandepally, A. L. Williams, *Tetrahedron Lett.* **2009**, 50, 1071-1074.
- [171.] G. Benedek, M. Palkó, E. Wéber, T. A. Martinek, E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2009**, 20, 2220-2225.
- [172.] K. R. Knudsen, J. Holden, S. V. Ley, M. Ladlow, *Adv Synth Catal.* **2007**, 349, 535-538.

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ANNEX