Enzyme-catalyzed kinetic resolution and heterogeneous metal-catalyzed racemization of 1-substituted tetrahydroisoquinoline and tetrahydro-ß-carboline derivatives

Ph.D. Thesis

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List of abbreviations

CAL-B	*Candida Antarctica* lipase B
CAL-A	*Candida Antarctica* lipase A
AY	*Candida rugosa*
PS-IM	*Burkholderia (Pseudomonas) cepacia* lipase
PPL	*Porcine pancreas* lipase
CCL	*Candida cylindracea* lipase
DKR	dynamic kinetic resolution
KR	kinetic resolution
conv.	conversion
E	enantioselectivity
ee	enantiomeric excess
ee_s	enantiomeric excess of substrate
ee_p	enantiomeric excess of product
equiv.	equivalent
subst.	substrate
t	reaction time
T	temperature
RT	room temperature
$[IrCp^*I_2]_2$	pentamethylcyclopentadienyliridium(III) iodide dimer
DIPE	diisopropyl ether
Et_3N	triethylamine
***t*-BuOMe**	*tert*-butyl methyl ether
THF	tetrahydrofuran
TFA	trifluoroacetic acid
EtOAc	ethyl acetate
HCOOH	formic acid
PPh_3	triphenylphosphine
$Pd_2(dba)_3.CHCl_3$	tris(dibenzylideneacetone)dipalladium-chloroform adduct
HPLC	high-performance liquid chromatography
NPs	nanoparticles
CH_3COOK	potassium acetate
LiO*t*Bu	lithium *tert*-butoxide
AMP-MCF	silica-based mesocellular foam functionalized with aminopropylsilane
AlO(OH)	aluminum hydroxide oxide
TBDPS	*tert*-butyldiphenylsilyl
TBDMS	*tert*-butyldimethylsilyl
DCE	dichloroethane
DFT	density functional theory
MAO A	monoamine oxidase A
Alloc	allyloxycarbonyl
Ser	serine
His	histidine
Asp	asparagine
TBAB	tetrabutylammonium bromide

1. INTRODUCTION AND AIMS

Enantiomerically pure amines are frequent research subjects due their exquisite biological activity as building blocks of natural products and pharmaceutical compounds.[1–5] Numerous applications are known as important drugs, including levocetirizine, esomeprazole or tadalafil, illustrated below (Figure 1).[4,6,7]

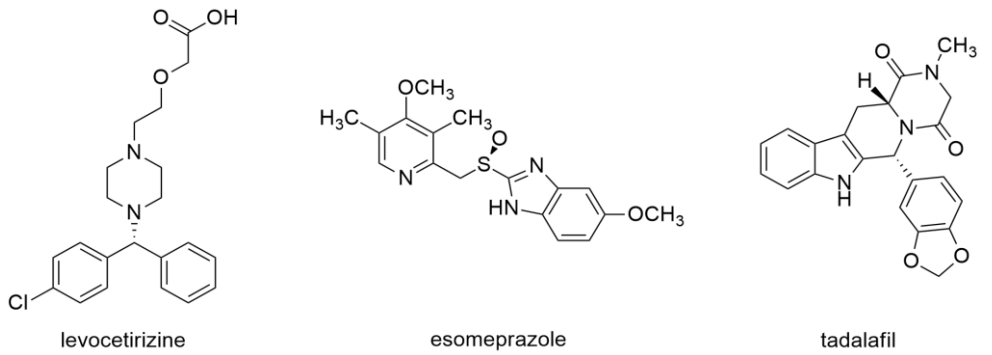


Figure 1. Pharmaceutically important enantiopure amines

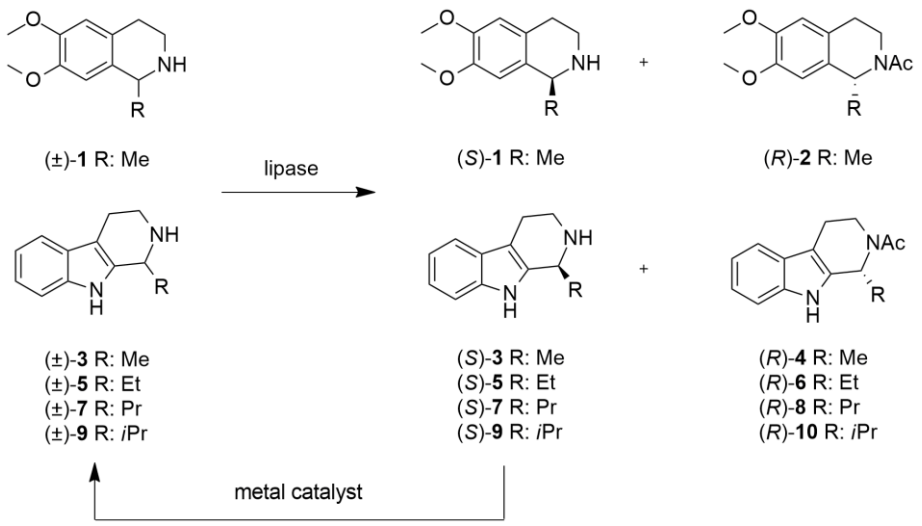
Many synthetic pathways exist for producing optically pure amines, such as diastereomeric crystallization,[1,5,8] nucleophilic addition,[9,10] enantioselective reduction of imines[11–14] or kinetic resolution using biocatalysts.[1,8,15–18]

Catalysis is a popular route to explore and establish new pathways, because it is able to speed up reactions through lower energy barriers.[19] Among the different types of catalytic processes,[20–23] enzymes and transition-metal catalysts have been widely used in recent years.[24–29] Protein catalysts or enzymes have several diversified biological functions in Nature, such as breaking down food in the stomach, catalyzing metabolism, DNA replication, *etc*. Their useful employment in organic syntheses as ultimate green catalysts has been widespread. Enzymes are non-toxic, reusable, commercially available, and they are capable of catalyzing different types of reactions under mild conditions. Further features include broad substrate specificity and high stereoselectivity.[30] Thus, KR using enzymes is a favorable route to separate and produce pure enantiomers.[31–34]

In addition to biocatalysis, transition-metal catalysis is another way to design new synthetic routes. The field of catalysis is divided into two parts, homogeneous and heterogeneous catalysis.[35] Heterogeneous catalysts are often used in industry,[36] since these can be recycled and characteristically show high stability. Furthermore, their simple

separation from the reaction mixture is also an important characteristic.[35,37] Among the many different types of heterogeneous metal-catalyzed reactions,[26] racemization is also widely studied to overcome the drawback of KR, namely its limited maximum yield of 50%.[38,39] When combined with KR, the reaction system is turned to DKR, which is a potential solution to obtain a single product enantiomer with the theoretically yield of 100%.[40,41]

To continue our previous laboratory experiments dealing with the enantioseparation of tetrahydroisoquinolines and tetrahydro-ß-carbolines,[42–46] my PhD work focused on the KR of secondary amines, specifically that of 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (±)-**1** and 1-substituted tetrahydro-ß-carbolines [1-methyl (±)-**3**, 1-ethyl (±)-**5**, 1-propyl (±)-**7**, 1-isopropyl (±)-**9**] (Scheme 1). We planned to devise an enzymatic strategy for the asymmetric synthesis of (±)-**1** in a continuous flow system. This method has many advantages, including short reaction time, rapid heating and pressure screening.[47] Several examples for resolution in continuous flow reactor are known in the literature.[48–51] Besides the development of a suitable KR method for the preparation of enantiomers, we also planned to examine the substrate specificity of CAL-B. Specifically, we wanted to explore how the substituents on C1 affect the *E* and reaction rate in the asymmetric *N*-acylation of (±)-**3**,**5**,**7**,**9**. The combination of KR and racemization can be a powerful process for asymmetric synthesis. Thus, a further aim was to investigate the racemization of model substrates 1-Me-substituted (*S*)-**1** and (*S*)-**3** using heterogeneous metal catalysts (Scheme 1).



Scheme 1. KR and racemization of secondary amines

2. LITERATURE BACKGROUND

2.1. Importance of tetrahydroisoquinolines and tetrahydro-ß-carbolines

A large number of 1-substituted tetrahydroisoquinolines display a huge variety of pharmacologically useful biological effects. Both natural products and synthetic compounds represent popular research fields thanks to their wide range of structural diversity and bioactivity. Naturally occurring compounds have been isolated as racemates or pure enantiomers from different natural sources. Chirality has an enormous role in medicine. Illustrative examples include bicuculline (Figure 2), which is a GABA receptor antagonist, but capnoidine, its *threo* isomer (Figure 2), is inactive.[52] Naturally occurring (*S*)-norcoclaurine (Figure 2) is an intermediate in the synthesis of three pharmaceutically important derivatives, papaverine, morphine or the antibacterial berberine.[53,54] Racemic norcoclaurine shows α- and ß-adrenoreceptor activity.[53] Noscapine (Figure 2) has been used as an antitussive since the 19th century, whereas its anticancer activity through mitochondrial-mediated apoptotic process was recently reported.[55,56] Numerous tetrahydroisoquinoline derivatives, such as renieramycin G (Figure 2), safracins, saframycins, lemonomycin, and ecteinascidins, exhibit potent antitumor and antimicrobial effects.[57] The synthetic compound solifenacin (Figure 2) shows urinary antispasmodic effect.[55]

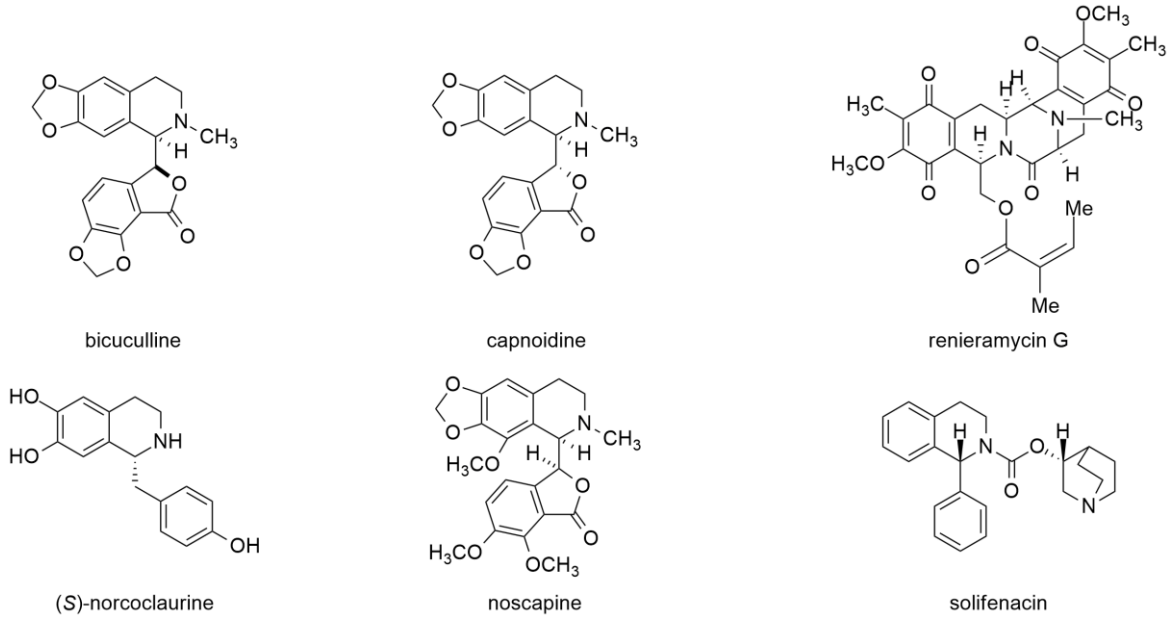


Figure 2. Compounds bearing the tetrahydroisoquinoline skeleton

The enantiomers of salsolidine [(±)-**1**] are naturally occurring compounds: the (*S*)-enantiomer was isolated from *Salsola richteri*, while the (*R*)-enantiomer was found in *Genista pungens*.[58] The (*R*)-enantiomer also has a MAO A inhibitory effect. As the core of a trimethoprim analogue, it has dihydrofolate reductase inhibitory effect. Furthermore, it inhibits the uptake of serotonine by human blood platelets.[58]

The medicinal importance of compounds containing the tetrahydro-ß-carboline moiety is as significant as that of tetrahydroisoquinoline derivatives mentioned above. Two alkaloids, vincristine and vinblastine exhibiting cytotoxic activity, have been used for cancer chemotherapy for a long time.[59–61] Reserpine (Figure 3) shows both antihypertensive effect and antitumor activity.[62–64] Yohimbine is used for the treatment of erectile disfunction.[61,64] Synthetic 1-substituted *N*-acylated tetrahydro-ß-carbolines exhibit inhibitory effect against the Breast Cancer Resistance Protein (ABCG2).[65]

The naturally occurring eleagnine [(±)-**3**] was isolated from *Petalostyles labicheoides* and *Eleagnus angustifolia*.[66] It is an inverse agonist on $GABA_A$ receptors.[67] More important alkaloids, such as ajmalicine (Figure 3) or yohimbine, contain the (*S*)-1-ethyl-1,2,3,4-tetrahydro-ß-carboline core [(*S*)-**5**].[68,69] Strictosidine (Figure 3) bearing this skeleton has antifungal activity.[69] Komaroidine [(±)-**7**] was isolated from *Nitraria species*.[70] The protected form of (*S*)-1-isopropyl-1,2,3,4-tetrahydro-ß-carboline [(*S*)-**9**] was used in the synthesis of (*S*)-quinolactacin-B (Figure 3) by the Winterfeldt reaction. The latter compound shows activity against tumor necrosis factor.[71]

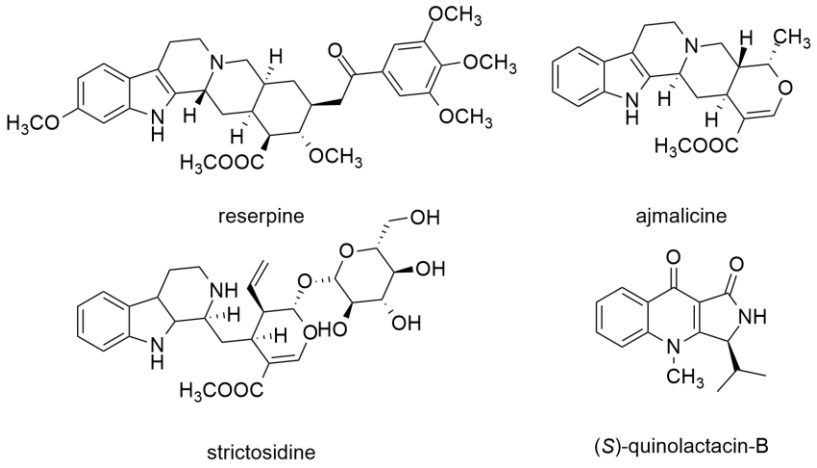
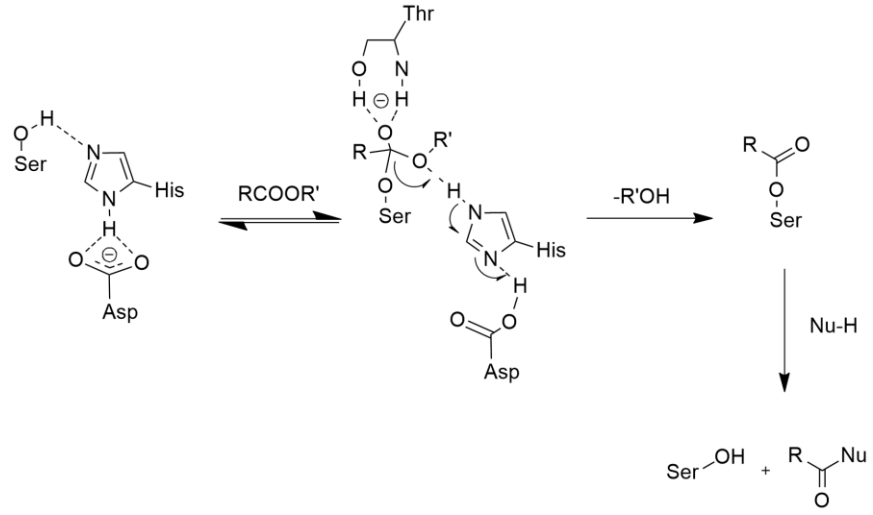


Figure 3. Tetrahydro-ß-carboline alkaloids

2.2. The mechanism of CAL-B-catalyzed *N*-acylation of amines

In nature, lipases are responsible for the hydrolysis of lipids. The mechanism of these biocatalytic processes is the same as that of serine proteases, which represent one third of all existing proteases.[72,73] and they have important role, for example, in coagulation or in inflammatory responses in the human body.[74] Lipases show high regio- and enantio-selectivity and are able to resolve chiral amines under mild conditions. The active site of CAL-B, a commonly used lipase, contains a Ser residue activated by His and Asp residues and these form the catalytic triad.[75,76] The *N*-acylation mechanism starts with the attack of the OH group of Ser on the carbonyl C of the acyl donor. A tetrahedral intermediate is formed and the negative charge of the carbonyl O is stabilized by the amino acids of the oxyanion hole. With the elimination of the alcohol the acyl–enzyme complex is formed. The amine as a nucleophile attacks the acyl–enzyme complex affording the corresponding amide (Scheme 2).[72,75,77,78]



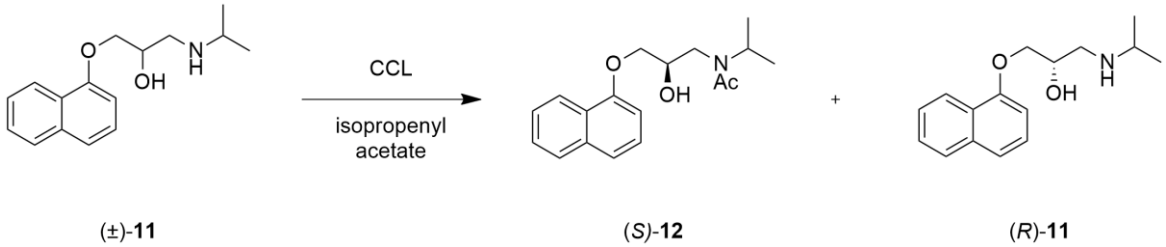
Scheme 2. Reaction mechanism of lipase-catalyzed *N*-acylation of amines

2.3. KR of secondary amines through lipase-catalyzed asymmetric *N*-acylation

There are only a limited number of publications in the literature about the enzyme-catalyzed *N*-acylation of secondary amines. The majority of examples are about primary amines and the use of aryl alkyl amines as model substrates.[3,79–82]

2.3.1. KR of an aliphatic secondary amino group

(*S*)-Propranolol shows higher binding affinity to ß$_1$ and ß$_2$ receptors than the (*R*)-enantiomer, that is the preparation of the enantiomers was an obvious necessity. The asymmetric *O*-acylation of racemic **11** was studied. However, because the amino group possesses higher nucleophilicity, it reacts with the acylating reagents, thus the enzymatic *N*-acylation had to be optimized (Scheme 3).[83]

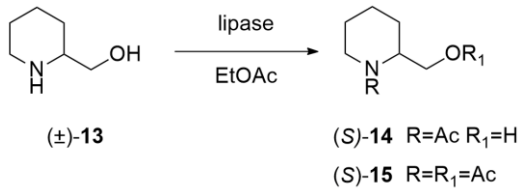


Scheme 3. Enantioselective *N*-acylation of (±)-**11** using CCL lipase

The use of solvents with higher hydrophobicity (DIPE, *t*-BuOMe) was found to give better selectivity and reactivity. (±)-**11** was dissolved in DIPE then isopropenyl acetate as acyl donor and CCL were added and the reaction mixture was shaken at 37 °C for 4 days. The resolution was characterized with an *E* of 16.6 affording the products with good *ee* values (ee_s = 64%, ee_p = 72%). Changing the concentration of the substrate from 0.05 M to 0.025 M improved the outcome of the reaction with an *E* = 21 (ee_s = 67%, ee_p = 73.4%). Gram-scale preparation was not attempted.

2.3.2. Enantioselective *N*-acylation of piperidine derivatives

Asensio et al. have investigated the KR of (±)-**13** using two types of enzymes, lipase Amano P or PPL lipase, in EtOAc at different temperatures (Scheme 4).[84] The highest *ee* values (ee_s = 10%, ee_p = 92%) were obtained with the use of PPL at low temperature (0–5 °C) in 4 h with yields of 90% for (*R*)-**13** and 9% for (*S*)-**14**. Lipase Amano P at higher temperature (40 °C) gave *N,O*-diacylated product (*S*)-**15** with a yield of 9% (*ee* = 85%), while (*R*)-**13** was formed in 13% yield (ee_s = 81%) and (*S*)-**14** with 75% yield (ee_p = 15%). As a conclusion, the enzymes exhibited the same chiral preference with selectivity for the (*S*)-enantiomer.

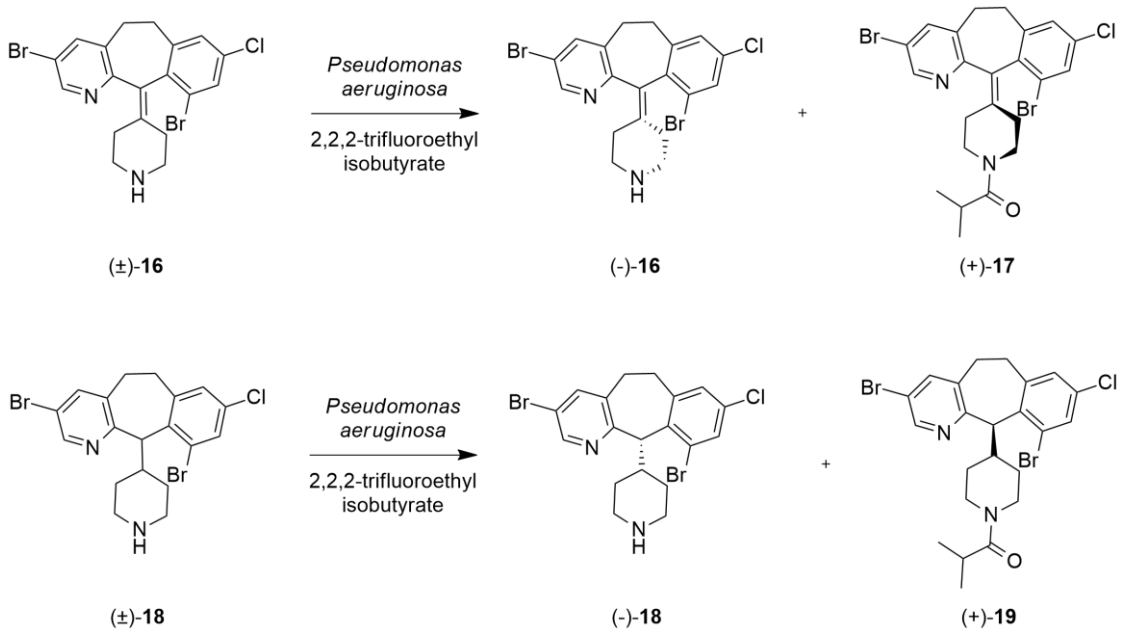


(±)-**13** (*S*)-**14** R=Ac R_1=H

(*S*)-**15** R=R_1=Ac

Scheme 4. Asymmetric *N*-acylation of amino alcohol (±)-**13**

Morgan and his co-workers have described the enantioselective synthesis of the key intermediate of the farnesyl protein transferase inhibitor, as a potential target for anticancer therapy.[85] (±)-**16** has atropisomeric conformational enantiomers, thus it was subjected to enzymatic KR via isobutyrylation (Scheme 5). The same experiment was repeated with (±)-**18**. In the optimization phase, increased reactivity and selectivity were observed, when bulkier acylating reagents were used and the moisture was removed. During preparative-scale resolutions, racemic substrates **16** and **18** were dissolved in *t*-BuOMe and then the mixtures were dried by azeotropic distillation. 2,2,2-Trifluoroethyl isobutyrate was used as acylating reagent in the presence of *Pseudomonas aeruginosa* at RT. The enantiomers [(+)-**17** and (-)-**16**] were obtained with excellent *ee* (> 96%) and yield (> 42%) in 26 h. Compound (+)-**19** was isolated with 98% *ee* and 36% yield, while (-)-**18** with an *ee* of 94% was separated in a yield of 36% in 20 h.

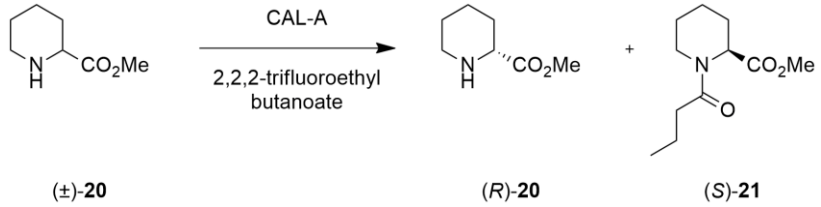


(±)-**16** (-)-**16** (+)-**17**

(±)-**18** (-)-**18** (+)-**19**

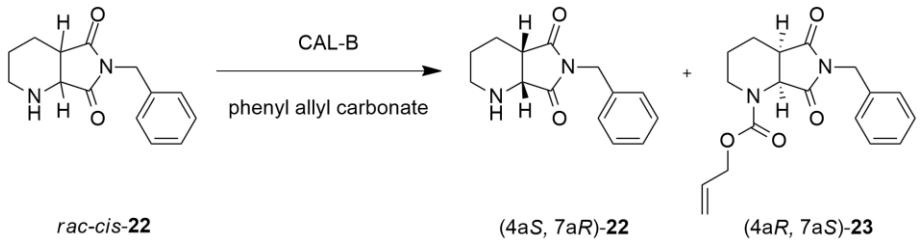
Scheme 5. Enzymatic isobutyrylation of (±)-**16** and (±)-**18**

The KR of methyl pipecolinate (±)-**20** was reported by Liljeblad et al. (Scheme 6).[86] The enzymatic reaction was performed in *t*-BuOMe with CAL-A and 2,2,2-trifluoroethyl butanoate at RT and the product enantiomers were obtained with good *ee* [90% for (*R*)-**20** and 98% for (*S*)-**21**].



Scheme 6. KR of methyl pipecolinate catalyzed by CAL-A

The enantiopure form of compound **22** is an important building block of the key intermediate in the synthesis of moxifloxacin. Thus, Li et al. have investigated the enzyme-catalyzed KR of *rac-cis*-**22** (Scheme 7).[87] In this case, the use of 2,2,2-trifluoroethyl butanoate was not successful. However, when replaced by phenyl allyl carbonate, an excellent *E* (> 200) characterized the reaction in *t*-BuOMe with CAL-B, in the presence of Et_3N and 4Å molecular sieves at 45 °C in 22 h (conv. = 49%) (Scheme 7). The product enantiomers were obtained with a yield of > 39% and an *ee* of > 97%. Furthermore, the carbamate enantiomer was subjected to hydrolysis to obtain the target enantiomer (4a*R*, 7a*S*)-**22** (*ee* = 99.98%).

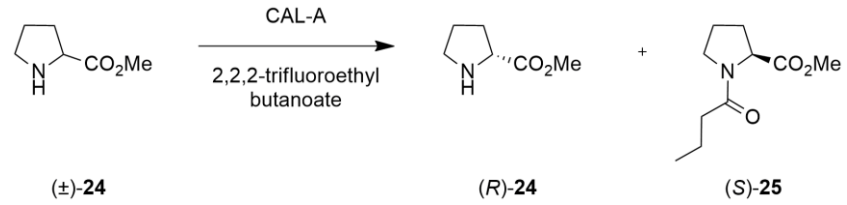


Scheme 7. Enzymatic *N*-alkoxycarbonylation of racemic compound **22**

2.3.3. Asymmetric *N*-acylation of pyrrolidine and indoline derivatives

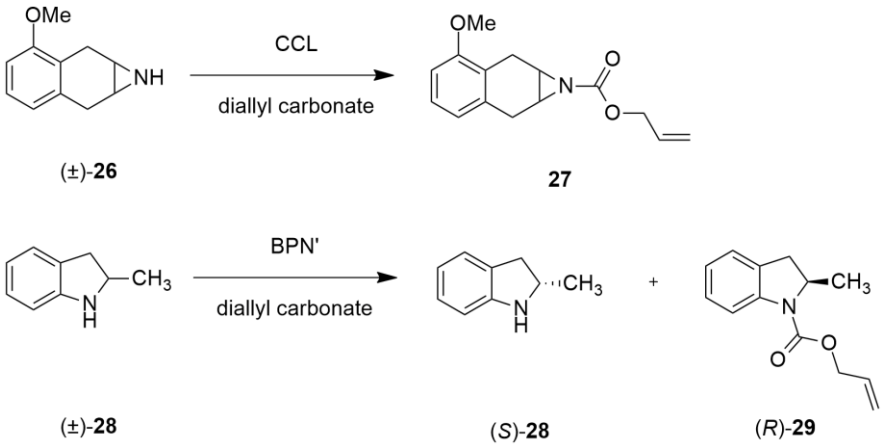
After reporting the KR of methyl pipecolinate (±)-**20**,[86] Liljeblad et al. have described the CAL-A-catalyzed resolution of (±)-**24** as well (Scheme 8).[88] Many primary amines were also subjected to KR using the conditions optimized previously (CAL-A, 2,2,2-trifluoroethyl butanoate, *t*-BuOMe, RT).[86] However, only substrates (±)-**20** (Scheme 6) and

(±)-**24** containing the sterically hindered secondary amino group (Scheme 8) proved to be highly selective (*E* > 100). The same results were observed under different conditions as well.



Scheme 8. CAL-A-catalyzed *N*-acylation of (±)-**24**

The KR of (±)-**26** and (±)-**28** was carried out by Orsat et al. (Scheme 9).[89] Racemic aziridine **26** was resolved by CCL using diallyl carbonate and phosphate buffer at RT. Carbamate **27** was isolated in 49% yield with an *ee* of 84% in 45 h. The same conditions, except the use of subtilisin BPN' (Nagarse, type XXVII) as enzyme, were applied to the KR of (±)-**28**. (*R*)-**29** was formed with 93% *ee* but only with a low yield of 6% in 92 h.

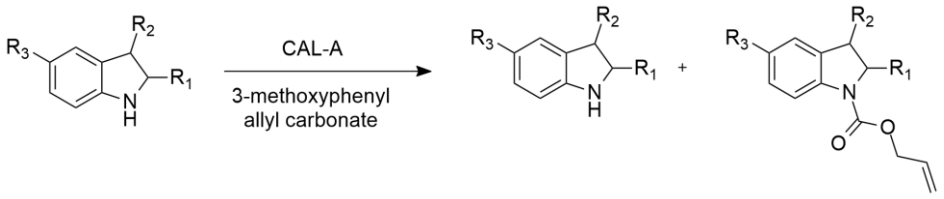


Scheme 9. Asymmetric *N*-alkoxycarbonylation of racemic **26** and **28**

The enzymatic KR of (±)-**28** was revisited by Gotor et al. (Table 1, entry 1).[90] The enzyme screening with (±)-**28** was first investigated with the use of diallyl carbonate in DIPE at 30 °C. As an ideal catalyst for the resolution of sterically hindered compounds, CAL-A showed high selectivity (*E* > 200, conv. = 13% in 136 h). Further screening tests (changing the temperature, solvents, acylating reagents, amine/enzyme/acyl donor ratios) resulted in higher reaction rate. A conversion of 50% (after 66 h) and an excellent *E* (> 200) were observed in *t*-BuOMe in the presence of 3-methoxyphenyl allyl carbonate at 45 °C (Table 1,

entry 1). Additional data acquired by exploring a wider substrate scope of substituted indolines are collected in entries 2–5 of Table 1.

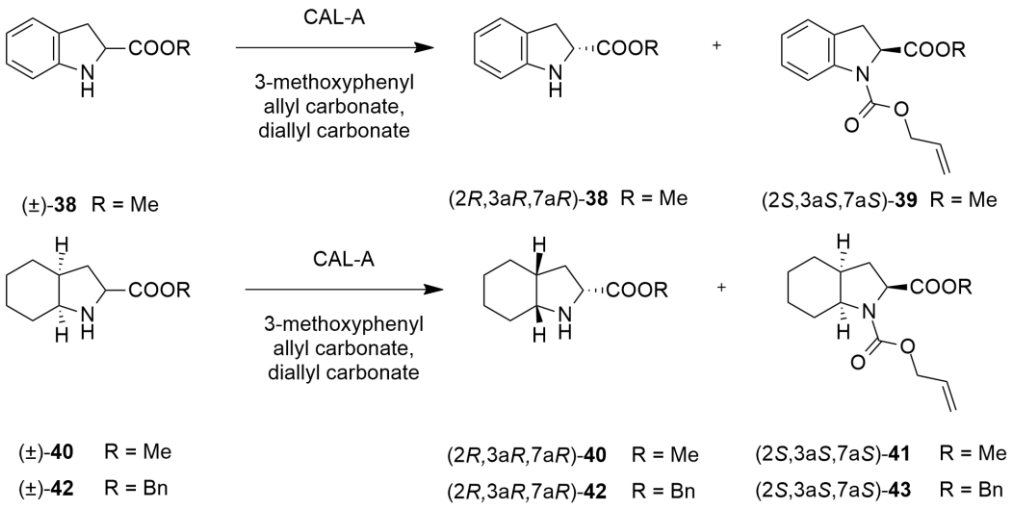
Table 1. KR of indoline derivatives catalyzed by CAL-A in *t*-BuOMe with 3-methoxyphenyl allyl carbonate at 45 °C



(±)-**28,30,32,34,36** (*S*)-**28,30,32,34,36** (*R*)-**29,31,33,35,37**

entry	subst.	R_1	R_2	R_3	t (h)	ee_s (%)	ee_p (%)	conv. (%)	*E*
1	(±)-**28**	Me	H	H	66	99	99	50	>200
2	(±)-**30**	Ph	H	H	20	97	99	50	>200
3	(±)-**32**	Me	H	OMe	20	99	95	51	>200
4	(±)-**34**	Me	H	F	20	99	99	50	>200
5	(±)-**36**	H	Me	H	4	99	23	81	6

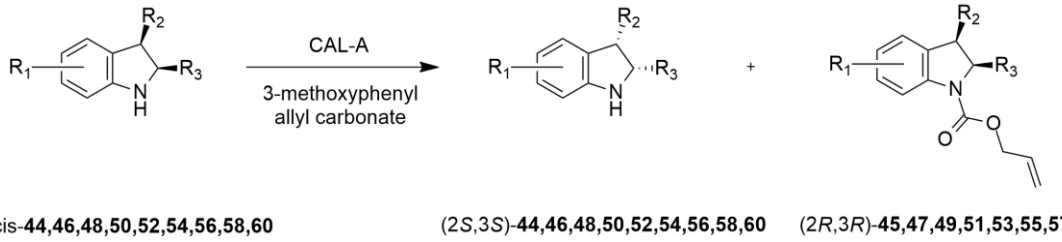
The Gotor group continued the investigation of enantioselective transformation of indoline-2-carboxylic acid derivatives using CAL-A from different sources (Scheme 10).[91] In *t*-BuOMe with 3-methoxyphenyl allyl carbonate at 30 °C, 50% conversion was detected in 4 h (*E* > 200) in the case of (±)-**38**, when CAL-A from Codexis was used. By using CAL-A from Biocatalytics, the same high *E* (> 200) but slower reaction (conv. = 50% in 20 h) were observed. With racemic **40** – but with CAL-A from Codexis – no enantioselectivity was detected under the same conditions. However, replacing the acylating reagent with diallyl carbonate afforded an excellent *E* (> 200), albeit in a slower reaction (20% conv. in 23 h). With CAL-A from Biocatalytics and with diallyl carbonate at 30 °C, the KR of racemic **42** was completed in 72 h with excellent *E* (> 200).



Scheme 10. CAL-A-catalyzed KR of (±)-**38**, (±)-**40** and (±)-**42**

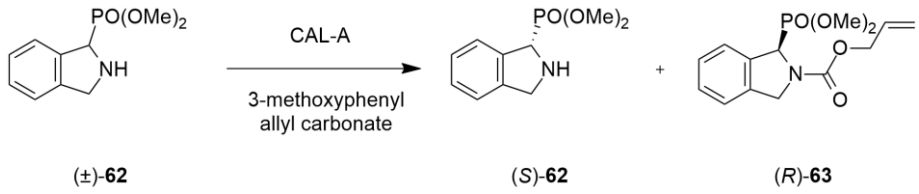
A further study by Gotor-Fernández and his co-workers focused on the KR of indolines varying the substituents at C-2, C-3, C-5 or C-7 (Table 2).[92] Excellent *E* value (> 200) was found in every case, when CAL-A was used as enzyme and 3-methoxyphenyl allyl carbonate as acyl donor in *t*-BuOMe at 30 °C.

Table 2. KR of multi-substituted indolines using CAL-A



entry	subst.	R_1	R_2	R_3	t (h)	ee_s (%)	ee_p (%)	c (%)	*E*
1	(±)-**44**	H	Me	Me	24	99	98	50	>200
2	(±)-**46**	5-Me	Me	Me	6,5	99	97	50	>200
3	(±)-**48**	5-MeO	Me	Me	4	99	97	50	>200
4	(±)-**50**	5-F	Me	Me	24	99	99	50	>200
5	(±)-**52**	7-Me	Me	Me	111	-	-	-	-
6	(±)-**54**	H	Me	Et	67	45	99	31	>200
7	(±)-**56**	H	Et	Me	71	25	99	20	>200
8	(±)-**58**	H	$-(CH_2)_4-$	$-(CH_2)_4-$	34	97	98	50	>200
9	(±)-**60**	H	Ph	Me	72	96	99	49	>200

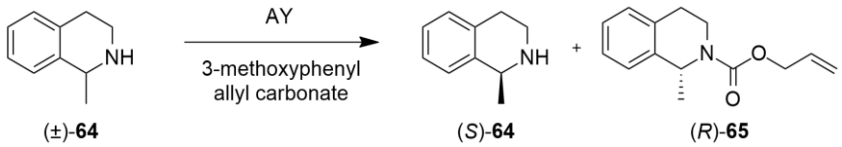
Recently, Gotor-Fernández and his co-workers completed their study with respect to the substrate scope of the indoline core performing the enzyme-catalyzed reaction of dimethyl (1,3-dihydro-2*H*-isoindol-1-yl)phosphonate (±)-**62** (Scheme 11).[93] The aminophosphonate was subjected to enantioselective alkoxycarbonylation under the conditions applied previously. Like above, CAL-A and 3-methoxyphenyl allyl carbonate proved to be adequate for KR combining an *in situ* racemization leading to DKR. A conversion of 77% was reached in toluene at 30 °C in 47 h. 96% *ee* and 58% yield characterized the formation of allyl carbamate enantiomer (*R*)-**63**. The remaining aminophosphonate (*S*)-**62** showed only 9% *ee*. This reaction did not need any external auxiliaries for racemization. They presumed a deprotonation–protonation equilibrium via an achiral enolate intermediate.



Scheme 11. Lipase-catalyzed DKR of racemic aminophosphonate (±)-**62**

2.3.4. Tetrahydroisoquinolines in KR

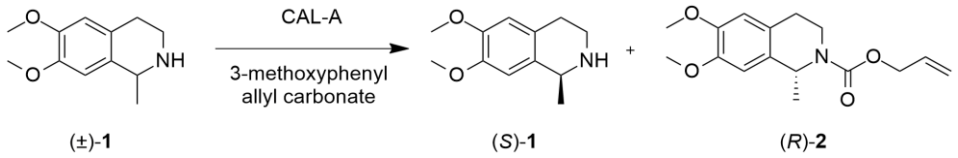
Breen and his co-workers investigated various acylating reagents on the KR of (±)-**64** (Scheme 12), since most of the common acyl donors cannot be used with amines, because of low reaction rates and selectivities.[94] When 3-methoxyphenyl allyl carbonate was used with AY in water-saturated toluene at 30 °C connected with a flask containing a saturated sodium chloride solution at 50 °C, (*S*)-**64** was obtained with 99% *ee* and 46% yield after 8 h, while (*R*)-**65** showed 98% *ee* and a 47% yield.



Scheme 12. KR of 1-methyl-1,2,3,4-tetrahydroisoquinoline

Ding et al. examined the KR of (±)-**1** using CAL-A as enzyme (Scheme 13).[95] Many acyl donors were screened without detecting any enantioselective reaction with the

commonly used EtOAc or methyl acetate. In contrast, phenyl allyl carbonate derivatives showed high selectivities (E > 200). 3-Methoxyphenyl allyl carbonate proved to be particularly adequate, providing 50% conversion in 72 h in toluene at 40 °C. Under the same conditions, the commercially available phenyl allyl carbonate was less selective [50% conversion in 96 h and a low E (26)].



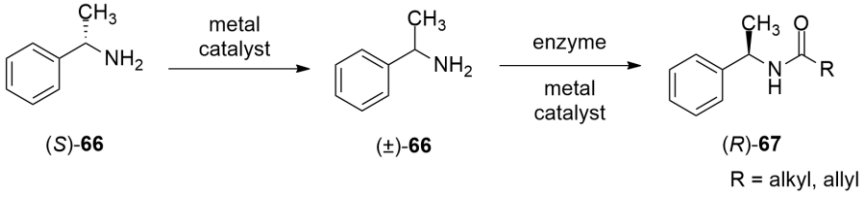
Scheme 13. Asymmetric *N*-alkoxycarbonylation of (±)-**1**

2.4. Racemization of amines

The six metals of the platinoid group (Pd, Pt, Rh, Ru, Ir, and Os) are the most versatile catalysts. They are able to catalyze different reactions including cross-coupling reactions, C–H activations, oxidations, reductions, polymerizations, cyclizations, izomerizations, racemizations, *etc*.[26] Numerous possibilities may be used to carry out racemizations, for example, acid- or base-catalyzed processes, redox or radical reactions, photochemical or thermal methods.[96]

In this thesis, results acquired with the use of metal catalysts employed for racemization of secondary amines involving a transfer hydrogenation process are presented. First, the substrate undergoes dehydrogenation followed by the re-addition of hydrogen to the imine from the catalyst. In general, the conversion to imine requires much harsher reaction conditions, than in the dehydrogenation of secondary alcohols, since the formation of ketones is usually a low-barrier process.[97,98]

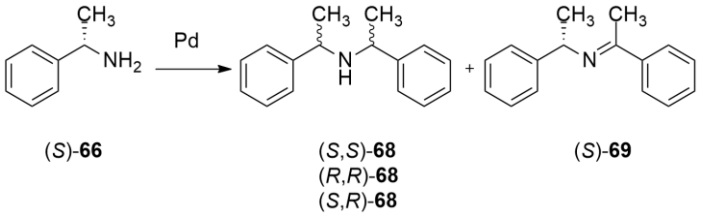
(*S*)-1-Phenylethylamine (*S*)-**66** is a commonly used starting compound for testing the effectiveness of metals as racemization catalysts (Scheme 14).[99–117]



Scheme 14. Racemization and DKR of (*S*)-1-phenylethylamine

2.4.1. Racemization using Pd catalysts

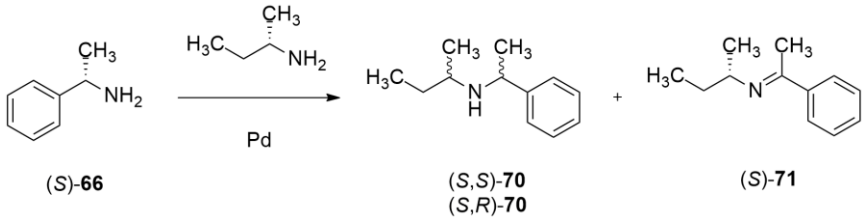
The use of Pd as a racemization catalyst was first suggested by the Murahashi group. They have studied the catalytic alkyl group exchange of primary and secondary amines by metals and their complexes (Scheme 15).[99]



Scheme 15. Alkyl group exchange reaction of (*S*)-**66** by Pd black

The Pd-catalyzed alkyl group exchange reaction of optically pure (*S*)-phenylethylamine was carried out at 100 °C. It was found that (*S*)-**66** undergoes racemization to form compound **68** with 1.9% *ee* (yield = 77%), while (*S*)-**69** was obtained with 6.6% *ee* (yield = 20%) (Scheme 15).

When (*S*)-*sec*-α-butylamine was added to the reaction mixture, isomers (*S,S*)-**70** and (*S,R*)-**70** were detected with the ratio 53:47 (yield = 38%), while (*S*)-**71** retained its optical purity (*ee* = 93%, yield 51%) (Scheme 16).



Scheme 16. Racemization of (*S*)-**66** in the presence of (*S*)-*sec*-α-butylamine

Reetz and his co-workers have investigated the DKR of (±)-**66** using Pd/C and Novozym SP 435 with EtOAc as acylating reagent in Et_3N at 50–55 °C (Scheme 14).[100] The conversion reached 75–77% in 8 h and the *N*-acylated (*R*)-enantiomer was isolated in 64% yield with 99% *ee*.

Palladium catalysts on alkaline earth supports were applied in the racemization of (*S*)-**66** by Parvulescu and his co-workers (Scheme 14).[101] Because condensation of the amine

and imine was observed with Pd/C, basic supports ($BaSO_4$, $CaCO_3$, $SrCO_3$, $BaCO_3$) were used to suppress the condensation reaction. When using basic supports, the main side product was ethylbenzene. 56% conversion and 2% *ee* of the amine were reached in 24 h with $Pd/BaSO_4$ at 70 °C in toluene under a H_2 pressures of 0.1–2 bar. The DKR was performed with CAL-B and EtOAc was used as acylating reagent under 0.2 bar H_2 pressure (Scheme 14). All catalysts showed satisfactory performance, since the amide yields, in all cases, were above the limited yield of KR (50%) with *ee* 99% in 24 h. Pd/C was the less selective, while $Pd/BaSO_4$ showed the highest selectivity with an appropriate activity. The formation of by-products in DKR was lower than in racemization, since the amine turned to amide before it could be irreversibly disappearing by hydrogenolysis.

Other catalysts, such as Ir, Pt, Ru, and Rh were also investigated in the racemization of (*S*)-1-phenylethylamine, but Pd proved to be the most suitable, especially Pd on alkaline-earth supports contrary to Pd/C.[102] 50% conversion and 1% *ee* of the amine were detected in 1 h in Et_3N using $Pd/CaCO_3$, when optimum hydrogen pressure (0.01 MPa) was used and the catalyst was pretreated by washing with NaOH.

Subsequently, the range of basic supports was expanded with amine-functionalized silica and layered double hydroxides for offering efficient racemization in the DKR of 1-phenylethylamine with Pd catalysts (Scheme 14).[103] > 90% conversion and 99% *ee* of (*R*)-amide was observed in 24 h with CAL-B over all Pd catalysts in toluene in the presence of isopropyl acetate or ethyl methoxyacetate at 70 °C under 0.01 MPa H_2 pressure.

Kim and his co-workers have reported a fast DKR of (±)-**66** with a Pd nanocatalyst (Scheme 14).[104] The racemization completed in 3 h, when Pd on AlO(OH) was used in toluene at 70 °C. The application of the catalyst in DKR was then examined and the (*R*)-amide was obtained (*ee* = 98%) with good yield (92%) in 6 h (conv. = 97%) in the presence of CAL-B, isopropyl methoxyacetate and Na_2CO_3 in toluene at 70 °C. The catalyst proved to be adequate for the DKR of other benzylamines as well.

Bäckvall and his co-workers have synthesized a silica-based mesocellular foam and then functionalized it with aminopropylsilane (AmP-MCF).[105] The Pd-AmP-MCF catalyst had high activity in the racemization of (*S*)-**66** in toluene under H_2 flow in the temperature range of 40–100 °C (Scheme 14). Racemic **66** was obtained in 1 h at 100 °C by using 0.2 mol% Pd. Using 1.5 mol% Pd at lower temperature (40 °C), the racemic product was detected in 60 h. The catalyst was successfully reused in four runs without activity loss.

Later, a Pd–CAL-B hybrid catalyst was synthesized modifying Pd-AmP-MCF developed previously.[106] The comparison of the two catalyst systems, namely Pd NPs and CAL-B co-immobilized in the same cavity of MCF as well as the mixture of Pd-AmP-MCF and CAL-B/AmP-MCF, was carried out in the DKR of (±)-**66** in toluene in the presence of ethyl methoxyacetate, Na_2CO_3, and pentadecane at 70 °C under H_2 in 16 h (Scheme 14). 99% *ee* characterized the product (*R*)-amide in both cases. In contrast, 99% yield was reached with the hybrid catalyst; and the product was obtained with a yield of 66% when using the mixture of the two catalyst components.

The DKR of (±)-**66** using Pd-AmP-MCF was then investigated in toluene in the presence of CAL-B, ethyl methoxyacetate, molecular sieves (4 Å), and pentadecane at 70 °C under H_2 (Scheme 14).[107] With 1.25 mol% Pd loading, 99% conversion in 6 h was detected and the product (*R*)-amide was observed with 99% *ee*. Furthermore, the same results were found (conv. = 99%, *ee* = 99%) at 50 °C using 5 mol% catalyst in 24 h. Both Na_2CO_3 and molecular sieves (4 Å) were successfully used as additives to reach full conversions and excellent *ee* values. With lipase PS and 5 mol% Pd catalyst, 82% conversion (*ee* = 99%) was obtained in 36 h in toluene with Na_2CO_3 under H_2 flow at 50 °C. Finally, the substrate scope of the DKR at 70 °C using Pd-AmP-MCF and CAL-B was exploited and excellent *ee* (> 97%) and yields (> 87%) were observed.

The effect of inorganic salts on catalytic activity and selectivity of Pd-AmP-MCF was examined in the racemization of (*S*)-1-phenylethylamine **66**.[108] 42% selectivity over Pd-AmP-MCF was detected in toluene in the presence of hexadecane (H_2, 70 °C in 4 h). Using basic additives K_3PO_4, K_2CO_3, and CH_3COOK afforded selectivities of 97%, 96%, and 91%, respectively. It seems that they are able to decrease the formation of side reactions, which depends on the basicity of the inorganic salts. The more basic salt was added, the more selective reaction was observed. K_2CO_3 was selected for further investigations in combination with Pd catalysts with different supports (Pd/SBA-15, Pd/FDU-12, Pd/SiO_2, Pd/C). In all cases, high selectivities (> 90%) were observed. Substituted (*S*)-1-phenylethylamines were also successfully subjected to racemization using K_2CO_3–Pd-AmP-MCF. Furthermore, the DKR of (*S*)-**66** was carried out using all Pd catalysts in the presence of K_2CO_3, CAL-B, H_2, and ethyl methoxyacetate in toluene at 70 °C (Pd-AmP-MCF, Pd/SBA-15, Pd/FDU-12, Pd/SiO_2: *ee* > 98%, yield > 90% in 9 h; Pd/C: *ee* = 99%, yield = 96% in 6 h).

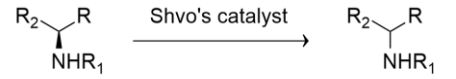
Bäckvall and his co-workers reported the DKR of benzylic amines using a biohybrid Pd NPs catalyst immobilized on cross-linked enzyme aggregates of CAL-B.[109] This catalyst was successfully used in the DKR of (±)-**66** (Scheme 14) in THF at 60 °C under H_2 flow by adding different additives (Na_2CO_3, K_2CO_3, NaOAc) affording the amide enantiomer with an *ee* of 99% and in yields of 45–65% after 18 h. They reached excellent *ee* (> 95%) and yield (> 81%) in toluene or in 1,4-dioxane with Na_2CO_3 as well. The recyclability of the catalyst was then investigated and a small drop in *ee* from 98% to 92% was found in toluene in the 3rd cycle at 90 °C, while in 1,4-dioxane the *ee* decreased from 98% to 86% in the 2nd cycle. Moreover, *ee* values dropped further during the next cycles in both cases.

Hybrid biocatalyst Glt-Pd-SiO2@APTES was applied as support for CAL-B immobilization and the product was used in the DKR of (±)-**66** in toluene in the presence of methyl methoxyacetate, molecular sieves, Na_2CO_3, and ammonium formate at 70 °C (Scheme 14).[110] 98% *ee* of (*R*)-**67** and 76% conversion were reached in 17 h.

2.4.2. Ru-based catalysts for the racemization of amines

The Bäckvall group described the racemization of primary amines by using the homogeneous Shvo catalyst in toluene at 110 °C (Table 3).[111]

Table 3. Racemization of primary amines using the Shvo catalyst



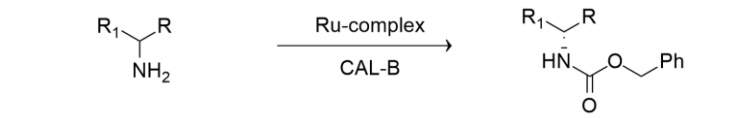
(*S*)-**66,72-81** (±)-**66, 72-81**

entry	amine	R	R_1	R_2	t (h)	yield (%)	*ee* (%)
1	**66**	Ph	H	Me	24	98	3
2	**72**	4-$MeOC_6H_4$	H	Me	9	98	3
3	**73**	4-FC_6H_4	H	Me	24	95	2
4	**74**	4-MeC_6H_4	H	Me	9	92	2
5	**75**	2-Naphthyl	H	Me	36	99	5
6	**76**	Ph	Me	Me	1	99	0
7	**77**	Ph	Ph	Me	24	98	1
8	**78**	4-$MeOC_6H_4$	Ph	Me	12	99	3
9	**79**	Ph	H	COOMe	12	98	1
10	**80**	Ph	H	CH_2OTBDPS	48	95	57
11	**81**	Ph	H	CH_2OTBDMS	48	95	32

The *para*-fluoro- and *para*-methoxy-substituted analogues of the Shvo catalyst were then subjected to test reactions. The best results in the DKR of primary amines were observed with the *para*-methoxy-substituted catalyst in toluene in the presence of CAL-B, isopropyl acetate, and Na_2CO_3 at 90 °C in 3 days. For example, using **66**, **72–76** as starting compounds, the (*R*)-acylated products were formed with a > 69% yield and > 98% *ee*.[112]

Then, additional acyl donors, such as diallyl, dibenzyl, and di-*tert*-butyl carbonates, were investigated.[113] The DKR of primary amines was carried out using the *para*-methoxy-substituted Shvo catalyst and CAL-B in toluene in the presence of dibenzyl carbonate and Na_2CO_3 at 90 °C for 3 days (Table 4).

Table 4. DKR of primary amines using a Ru-complex and CAL-B

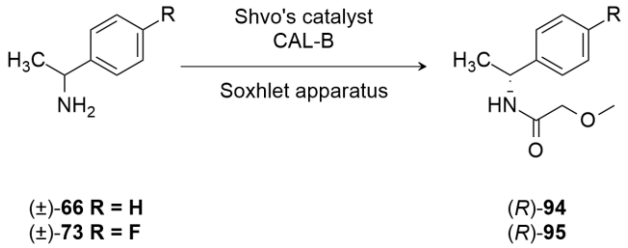


(±)-**66,72,73,84,86,88,90,92** (*R*)-**67,82,83,85,87,89,91,93**

entry	amine	(*R*)-amide	R	R_1	yield (%)	*ee* (%)
1	**66**	**67**	Ph	Me	90	93
2	**72**	**82**	4-$MeOC_6H_4$	Me	74	97
3	**73**	**83**	4-FC_6H_4	Me	72	99
4	**84**	**85**	4-BrC_6H_4	Me	95	98
5	**86**	**87**	dihydro-indenyl	Me	64	99
6	**88**	**89**	cyclohexyl	Me	92	96
7	**90**	**91**	heptyl	Me	89	90
8	**92**	**93**	*i*Pr	Me	60	99

A Soxhlet reactor was used for the DKR of 1-arylethylamines in the presence of Shvo's catalyst and CAL-B (Scheme 17).[114] Most of the amines have high boiling points (>150 °C). In order to achieve suitable circulation and condensation of all compounds under reflux condition and reduced pressure, the solvent and acyl donor had to be choosen accordingly. Specifically, compounds with boiling points close to the boiling point of the amines were used. Glymes (di-, tri-, tetra-) were selected as solvents and isopropyl

methoxyacetate, ethyl caprylate or ethyl laurate as acyl donors. From the heated racemization flask (105–120 °C) the compounds with high boiling points evaporated to a side arm, where the reaction mixture cooled down to the appropriate temperature. After reaching the reflux condenser, the solution was collected in the chamber containing thermolabile enzyme. Finally, the reaction mixture returned to the racemization flask. After 48 h, full conversions were reached in diglyme with isopropyl methoxyacetate at 105 °C in the DKR of (±)-**66** (*ee* = 99%), while 97% *ee* for the *para*-fluoro-substituted analogue [(±)-**73**] was detected. The yield was 65% in both cases.

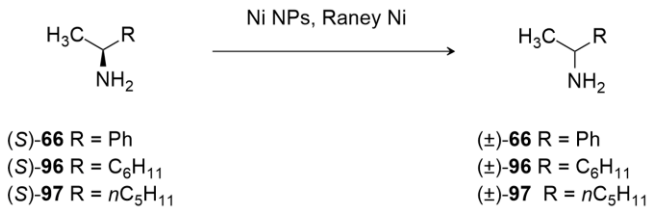


Scheme 17. DKR in Soxhlet apparatus in the presence of Shvo's catalyst and CAL-B

2.4.3. Ni- and Co-catalyzed racemization of primary amines

De Vos and his co-workers have investigated Ni NPs and Raney Ni as racemization catalysts.[115] In addition to (*S*)-**66**, (*S*)-1-cyclohexylethylamine [(*S*)-**96**] and (*S*)-2-aminoheptane [(*S*)-**97**] were also tested in toluene or in the ionic liquid TBAB at 110 °C (Table 5). When $NiBr_2$ was used, the addition of NaH and Li*O*t*Bu to the reaction mixture gave the most active NPs. High selectivity of Ni NPs in TBAB was observed using other phenyl-substituted amines or naphthyl analogues. The corresponding data, however, are not summarized in Table 5.

Table 5. Racemization of primary amines using Ni NPs or Raney Ni



(*S*)-**66** R = Ph
(*S*)-**96** R = C_6H_{11}
(*S*)-**97** R = nC_5H_{11}

(±)-**66** R = Ph
(±)-**96** R = C_6H_{11}
(±)-**97** R = nC_5H_{11}

entry	catalyst	(*S*)-amine	solvent	t (h)	conv. (%)	*ee* (%)	sel. (%)
1	Ni NPs	**66**	toluene	1	54	0	87
2	Ni NPs	**96**	toluene	4	47	7	99
3	Ni NPs	**97**	toluene	5	40	24	96
4	Raney Ni	**66**	toluene	1	64	7	49
5	Ni NPs	**66**	TBAB	1	49	6	95
6	Ni NPs	**96**	TBAB	2	49	2	99
7	Ni NPs	**97**	TBAB	5	41	17	99

The racemization of (*S*)-**66** was further examined using Raney Ni in comparison with Raney Co catalyst.[116] Ni is more active than Co and, consequently, lower catalyst concentration (40 mg Ni, 80 mg Co) needed to reach 50% conversion in toluene at 70 °C (24 h by Ni and 72 h by Co). Furthermore, since the Raney Ni catalyst contains a considerable amount of adsorbed hydrogen contrary to Raney Co, a lower hydrogen pressure (0.01 MPa) was also satisfactory with Raney Ni (0.02 MPa for Raney Co). Additional benzylic and aliphatic amines were then tested for both catalysts. In general, racemizations were completed in 24 h with 100% selectivity with the use of Ni, while longer reaction time was needed with Co. The racemization of aliphatic amines was more selective over both catalysts. In the case of 2-methylpiperidine, a secondary amine, the reaction was slower, but selective with Ni, while no reaction was observed with Co. The results of DKR using Raney Ni are summarized in Table 6. Raney Co showed low activity in DKR, because of oxidative corrosion resulting in catalyst deactivation.

Table 6. DKR of primary amines using Raney Ni and CAL-B

entry	substrate	t (h)	T (°C)	conv. (%)	ee_p (%)	sel.$_{(R)\text{-amide}}$ (%)
1	(*S*)-**66**[a]	120	80	62	94	98
2	1-*p*-tolylethyl-amine[b]	72	70	72	80	90
3	1-amino-tetralin[b]	72	70	65	87	94
4	2-hexylamine[b]	96	70	98	97	97
5	2-heptylamine[b]	48	80	78	94	97
6	2-octylamine[b]	96	80	75	82	92
7	1-cyclohexyl-ethylamine[b]	96	80	70	96	98
8	1-methyl-3-phenyl-propylamine[b]	96	80	79	98	100

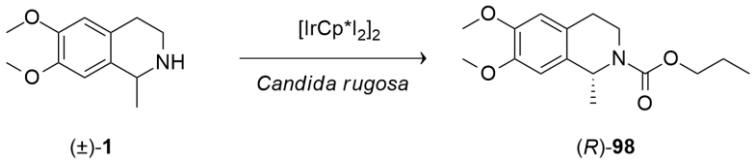
[a] methyl decanoate, H_2; [b] ethyl methoxyacetate, H_2

2.4.4. Pt and Ir as racemization catalysts in the DKR of amines

A zeolitic microcapsule with encapsulated Pt NPs and a thin shell of silicalite-1 is applied as racemization catalyst in the DKR of (±)-**66** in toluene in the presence of CAL-B and vinyl octanoate at 70 °C under H_2 (Scheme 14).[117] 80% conversion, 93% selectivity, and excellent *ee* (> 99%) were observed in 35 h. The reusability of the catalyst was also investigated with 76% conversion, 87% selectivity, and 99% *ee* detected in the 5th run.

Stirling and his co-workers have investigated the DKR of 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (±)-**1** by $[IrCp^*I_2]_2$ and *Candida rugosa* in toluene in the

presence of 3-methoxyphenyl propyl carbonate at 40 °C.[118,119] 90% conversion was observed and 96% *ee* characterized the formation of the (*R*)-carbamate (82% yield, 23 h) (Scheme 18).



(±)-**1** (*R*)-**98**

Scheme 18. DKR of a secondary amine using a homogeneous Ir catalyst

3. MATERIALS AND METHODS

3.1. Materials and instruments

Enzymes were commercially available. CAL-B immobilized on acrylic resin was purchased from Sigma. Lipase AY was a preparation by Fluka. Lipase PS IM immobilized on diatomaceus earth was from Amano Enzyme Europe Ltd. and CAL-A was from Novo Nordisk.

Pt/Al_2O_3 (type 94, 5 wt% Pt) and Pt/Al_2O_3 (type 123, 5 wt% Pt) were purchased from Johnson Matthey. Pd/C (5 wt%) and Pt/C (5 wt%) were purchased from Degussa, while Ir/C was prepared according to a reported method.[120] The particle size and metal dispersion of catalysts supported on Al_2O_3[121] and C[122] were published previously. Ni/Al_2O_3 (HTC-500) with ~21% Ni loading and 8.2 nm particle size was purchased from Crossfield. Pd black was from Sigma Aldrich. Ir supported on mesoporous MCM-41 (2 wt%), zeolite beta-25 (1 wt%), and γ-alumina (1 wt%) were prepared by impregnating the supports with aqueous iridium chloride solution in a Büchi rotavapor, followed by drying, calcination at 500 °C, and reduction under hydrogen. The specific metal surface of 5 wt% Ir/C was measured by CO chemisorption.[120] The reactions with metal catalysts were carried out using hydrogen gas from Linde Gas-AGA (99.999%).

Some preliminary reactions were performed in an *H*-Cube system by using 'No H_2 mode'. This continuous flow reactor is equipped with a stainless-steel enzyme-charged cartridge (70 mm length, 4 mm inside diameter). Batch reactions were performed in an incubator shaker (Innova 4000).

A four-necked 100 mL round-bottom flask equipped with a gas inlet (7 μm), a rubber septum (for sampling), and a condenser connected to an oil bubbler for gas outlet was used for racemization experiments. Some reactions, carried out in the absence of H_2, were performed in a Schlenk tube.

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. ^{1}H NMR spectra were recorded on a Bruker Avance DRX 400 MHz or a Bruker Avance 500 MHz NMR spectrometer. Melting points were determined on a Kofler apparatus. Microwave (MW) reactions were performed in a CEM Discover MW reactor (Matthews, NC, USA). The

elemental analysis was carried out by means of a Perkin-Elmer CHNS-2400 Ser II Elemental Analyzer.

3.2. Enzymatic experiments

The enzymatic work was generally performed in batch mode by adding the enzyme (30 mg mL^{-1}) to the reaction solution containing the racemic starting compound (0.025 M), the solvent (1 mL), the acyl donor (4 equiv.), and the additive (1 or 10 μL Et_3N). The reaction mixture was shaken in an incubator shaker at given temperatures (40–80 °C). Some reactions were shaken in an ultrasound bath for 1 min to liberate the active site of the enzyme from the forming compounds.

With model substrate (±)-**1**, preliminary reactions were performed in *H*-Cube (in 'no H_2 mode'). This system allows to test the effect of pressure on *E* and reaction rate. In a typical continuous flow reaction, the starting material (0.025 M) was dissolved in the solvent (1 mL) followed by the addition of the acyl donor (4 equiv.). The reaction mixture was pumped through the enzyme-filled pressure- and temperature-resistant column (CatCart®, 70 mm long, 4 mm internal diameter) (244 mg CAL-B or 250 mg AY). The flow was provided by an HPLC pump (0.1 mL min^{-1}). The reaction mixture was collected in a vial after each cycle and then it was pumped through the system again during more cycles.

During preparative-scale reactions, the racemic substrates (0.47–0.51 M) were dissolved in the solvent (25 mL) and then, after adding the acyl donor (4 equiv.) and Et_3N (50 μl), the reaction mixtures were stirred in the incubator shaker at 40–60 °C. The unreacted amine and the carbamate were separated by column chromatography (conditions can be found in the original papers).[I,III]

During hydrolysis reactions, the (*R*)-carbamates (0.19–0.22 M) in a Schlenk tube were dissolved in THF (2 mL), then PPh_3 (0.04 M), HCOOH (0.74 M), and $Pd_2(dba)_3.CHCl_3$ (0.014 M) were added. The reaction mixtures were stirred in an oil bath at 40 °C under Ar.

The product enantiomers were characterized by *ee*, optical rotation, ^1H NMR, melting point, absolute configuration, and elemental analysis. Absolute configurations were compared to literature data.

3.3. Racemization experiments

During a typical reaction, the reaction flask was first evacuated and purged twice with Ar. Then the solvent (40 mL) was added into the flask containing the substrate (10 mg) followed by the addition of the metal catalyst (2–20 mol%) and immediate bubbling of H_2. The reaction mixtures were stirred at given temperatures (50–90 °C). During recyclability investigation, after a racemization cycle, the catalyst was filtered off, washed with the solvent, and dried in air overnight before reuse. When the inhibitory effect of the imine was investigated under H_2 flow, the imine (10 mg), prepared separately, and the catalyst (10 mol%) were first stirred for 10 min in toluene (40 mL) at 80 °C under Ar. Then the reaction was started by the addition of the substrate enantiomer (10 mg) and immediate bubbling of H_2. When investigating the leaching of the metal species, the racemization was stopped after 45 min by filtering off the catalyst, then the liquid was treated further under H_2 flow and the *ee* was followed for an additional 1 h. Preparative-scale reactions were run in 50-mg scale in 40 mL solvent at 80 °C under H_2 flow. After completion the catalyst was filtered off and washed, then the solvent was evaporated affording the racemic product. They were characterized by ^{1}H NMR and *ee*. Reactions in the absence of H_2 were performed in Schlenk tube and were started with evacuation and filling with Ar, then the substrate (10 mg), the solvent (4 mL), and the metal catalyst (10–20 mol%) were added into the flask followed by treatment at 80–90 °C.

3.4. Analytical methods

Reactions were followed by HPLC equipped with a chiral column to determine the *ee* values of the compounds.

The conversion and *E* were calculated using the following equations:

$$ee_s = (A_2 - A_1)/(A_1 + A_2)$$

$$ee_p = (A_3 - A_4)/(A_3 + A_4)$$

$$\text{conv.} = ee_s/(ee_s + ee_p)$$

$$E = \{\ln[(1 - ee_s)/1 + ee_s/ee_p)]\}/\{\ln[(1 + ee_s)/(1 + ee_s/ee_p)]\}$$

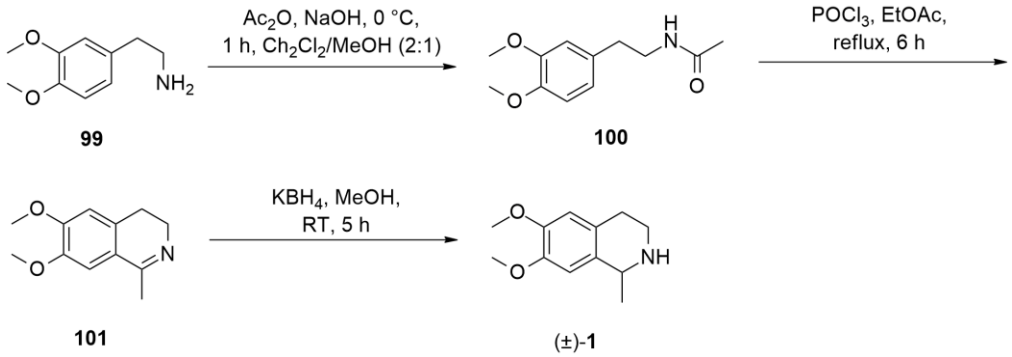
where A_1, A_2, A_3, and A_4 are the peak areas and $A_2 > A_1$, $A_3 > A_4$.

The *ee* values of compounds (±)-**1**–(±)-**9** were determined by HPLC equipped with Chiralpak IA column. The exact conditions containing the eluent, flow rate, detection, and retention times can be found in the original papers.[I-III]

3.5. Syntheses of the racemic starting compounds [(±)-1–(±)-9]

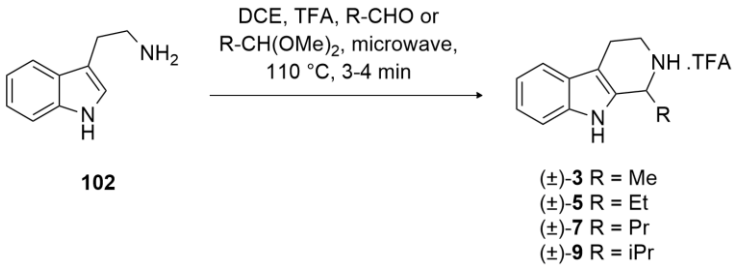
The syntheses of the substrates were carried out according to literature methods.[123,124]

In the preparation of (±)-**1** (Scheme 19),[123] homoveratrylamine was dissolved in CH_2Cl_2/H_2O (2 : 1) in the presence of NaOH and then Ac_2O was added. The product amide was treated with $POCl_3$ in EtOAc at reflux temperature resulting in the dihydroisoquinoline analogue via the Bischler–Napieralski reaction. Then the product was reduced by KBH_4 in MeOH at RT. In this three-step process, pure (±)-**1** was obtained as white solid in a yield of 36%.



Scheme 19. Synthesis of racemic **1**

Racemic **3**,**5**,**7**, and **9** were prepared via a microwave-assisted procedure through the Pictet–Spengler reaction (Scheme 20).[124] In a microwave vial, tryptamine was dissolved in DCE followed by the addition of the corresponding acetal or aldehyde and TFA. The reaction mixture was put into the reactor and was heated to 110 °C for 3 or 4 min. The racemic products, after the liberation from TFA salts with saturated $NaHCO_3$ solution, were obtained as pale yellow solids in good yields [(±)-**3**: 80%, (±)-**5**: 74%, (±)-**7**: 85%, (±)-**9**:72%].



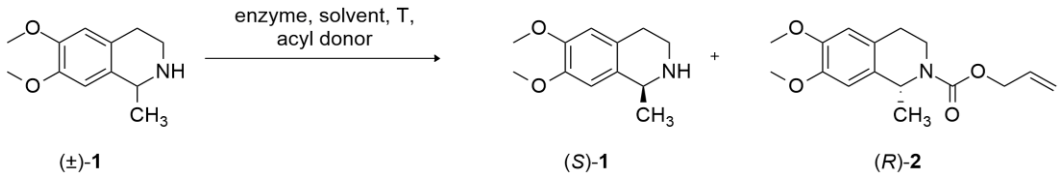
Scheme 20. Syntheses of tetrahydro-ß-carbolines in microwave reactor

4. RESULTS AND DISCUSSION

4.1. KR of racemic 1,3,5,7,9

4.1.1. Small-scale resolutions in batch for the preparation of (*S*)-1 and (*R*)-2

Preliminary small-scale reactions were carried out in order to find the optimum conditions to obtain the enantiomers of (±)-**1**, (±)-**3**, (±)-**5**, (±)-**7**, and (±)-**9**. (±)-**1** was selected first to find an efficient and mild method on the KR of secondary amines (Scheme 21). Ding et al. have previously published the asymmetric *N*-alkoxycarbonylation of salsolidine [(±)-**1**] (*E* > 200, conv. = 50% in 72 h, yield > 46%) using CAL-A, 3-methoxyphenyl allyl carbonate in toluene at 40 °C.[95] Reaction conditions reported in this communication were applied in our experiments.



Scheme 21. Lipase-catalyzed KR of salsolidine [(±)-**1**]

Preliminary reactions were first performed in batch and started with enzyme screening (Table 7, entries 1–4). Resolutions were carried out with commercially available phenyl allyl carbonate in toluene at 40 °C. When the reaction was catalyzed by CAL-A, low *E* (= 9) was observed (entry 1) just as with PS-IM (*E* = 2) (entry 2). In the CAL-B-catalyzed reaction, *E* = 46 and 49% conversion were found in 96 h (entry 4). AY showed optimum activity with 50% conversion in 72 h with excellent *E* (> 200) (entry 3).

Table 7. Enzyme-catalyzed *N*-alkoxycarbonylation of (±)-**1** in batch[a]

entry	enzyme	T (°C)	solvent	reaction time (h)	conv. (%)	ee_s[b](%)	ee_p[c](%)	*E*
1	CAL-A	40	toluene	96	42	50	68	9
2	PS-IM	40	toluene	96	3	13	25	2
3	AY	40	toluene	72	50	99	98	>200
4	CAL-B	40	toluene	96	49	85	89	46
5	CAL-B	50	toluene	48	50	96	95	154
6	CAL-B	60	toluene	48	51	96	92	94
7	CAL-B	50	*t*-BuOMe	2.5	50	99	97	>200

[a] 5 mg (±)-**1**, phenyl allyl carbonate. [b] According to HPLC after derivatization with Ac_2O. [c] According to HPLC.

Since CAL-B is an immobilized enzyme and, from the point of view of its potential use in continuous flow system, it was selected for further optimization. First, the temperature was increased from 40 °C to 50 °C then to 60 °C (Table 7, entries 4–6) with the concomitant increase in reaction rates. However, the highest *E* (154) was found at 50 °C (entry 5). When toluene was replaced by *t*-BuOMe, the reaction completed in 2.5 h (*E* > 200, conv. = 50%) (entry 7).

4.1.2. Optimization in continuous flow reactor

These optimized conditions (CAL-B, phenyl allyl carbonate, *t*-BuOMe, 50 °C, 1 bar) in batch seemed to be appropriate to perform a reaction in a continuous flow reactor (an *H*-Cube in 'no H_2 mode'). A 70-mm long heat- and pressure-resistant stainless steel CatCart® was filled with CAL-B. Using the optimized conditions determined in batch mode, only 20% conversion but an excellent *E* (>200) were detected in a single cycle (Table 8, entry 1).

Table 8. Temperature and pressure optimization in continuous flow system in the *N*-alkoxycarbonylation of (±)-**1**[a]

entry	p (bar)	T (°C)	conv. (%)	ee_s[b](%)	ee_p[c](%)	*E*
1	1	50	20	24	99	>200
2	30	50	3	3	99	>200
3	60	50	9	10	99	>200
4	1	60	21	26	99	>200
5	1	70	25	33	99	>200
6	1	80	7	7	99	>200

[a] 5 mg substrate, 244 mg CAL-B, *t*-BuOMe, 4 equiv. phenyl allyl carbonate, 0.1 mL min^{-1}. [b] According to HPLC after derivatization with Ac_2O. [c] According to HPLC.

Next, reactions at increased pressure (at 30 bar then 60 bar) were performed, in order to improve the reaction rate (entries 2 and 3). Unfortunately, conversions decreased significantly compared to the reaction carried out at 1 bar (entry 1). In the hope of increasing the reaction rate, the temperature was increased from 50 °C to 60 °C, then to 70 °C (entries 1, 4 and 5). Indeed, it was found, that the higher the temperature, the higher the conversion. Note, however, that the reaction rate decreased drastically at 80 °C (entry 6) in the first cycle.

The best combination of reaction rate and *E* was detected at 70 °C and at 1 bar. Thus, using these conditions, the reaction mixture was pumped through the same CAL-B-filled CatCart® of the reactor five times consecutively. In parallel with the increasing number of cycles, the reaction rate increased as well (Figure 4). 41% conversion was reached after the

5th cycle with excellent *E* (>200). It has to be noted, that the activity of the enzyme decreased after the 2nd cycle.

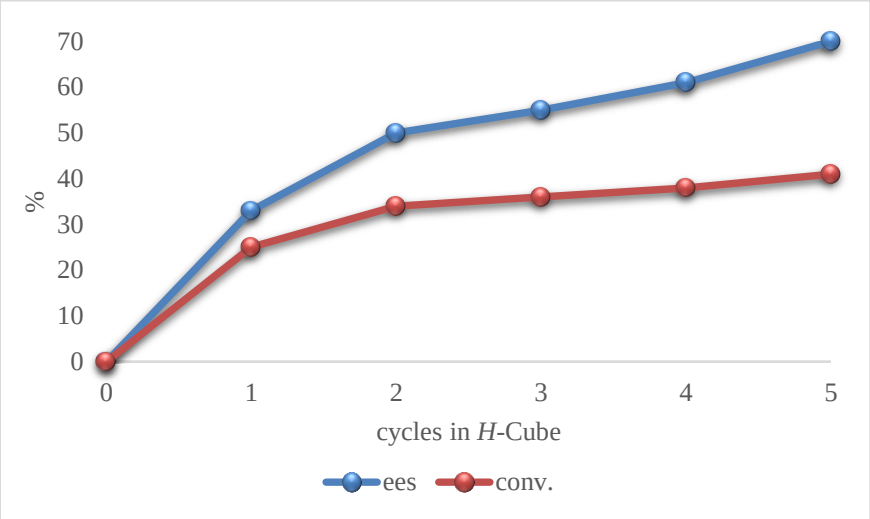


Figure 4. KR of (±)-**1** in the continuous flow system through 5 cycles

Finally, the KR was carried out with AY, phenyl allyl carbonate in toluene at 40 °C, at 1 bar in *H*-Cube. Both the conversion and the *E* were low in a single cycle (ee_s = 3%, ee_p = 53%, conv. = 5%, *E* = 3), since the enzyme was compressed because its powdery structure.

4.1.3. Preparative-scale resolution of (±)-1

The preparative-scale resolution of (±)-**1** was performed under the optimized conditions in batch with both enzymes used successfully. Excellent *E* (> 200) was detected with CAL-B (conv. = 50%, *ee* > 97%) (Figure 5) and with AY (conv. = 50%, *ee* = 99%) (Table 9).

Table 9. Preparative-scale resolution of salsolidine [(±)-**1**]

entry	reaction time (h)	enzyme	conv. (%)	*E*	enantiomer	*ee* (%)	yield (%)	$[\alpha]_D^{25}$
1[a]	24	AY	50	>200	(*S*)-**1**[c]	99	40	-59[e]
					(*R*)-**2**[d]	99	39	-104[e]
2[b]	49	CAL-B	50	>200	(*S*)-**1**[c]	99	41	-58.9[f]
					(*R*)-**2**[d]	97	38	-102[e]

[a] 100 mg substrate, 30 mg mL^{-1} enzyme, 30 mL toluene, 4 equiv. phenyl allyl carbonate, 40 °C. [b] 100 mg substrate, 30 mg mL^{-1} enzyme, 30 mL *t*-BuOMe, 4 equiv. phenyl allyl carbonate, 50 °C. [c] According to HPLC after derivatization with Ac_2O. [d] According to HPLC. [e] *c* 0.3 EtOH. [f] *c* 0.55 EtOH.

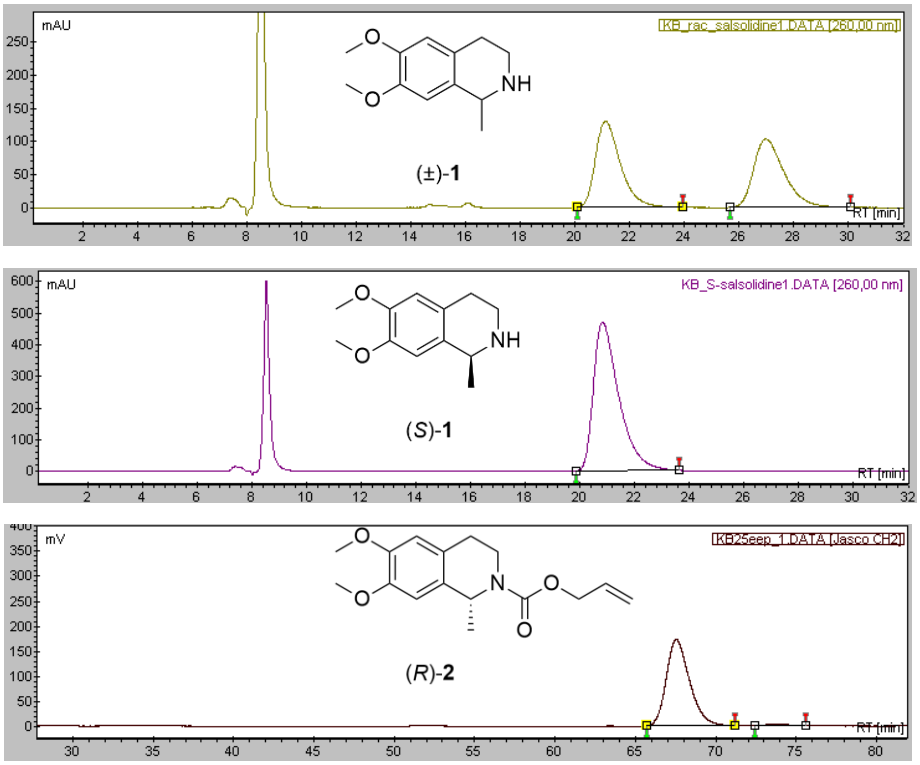
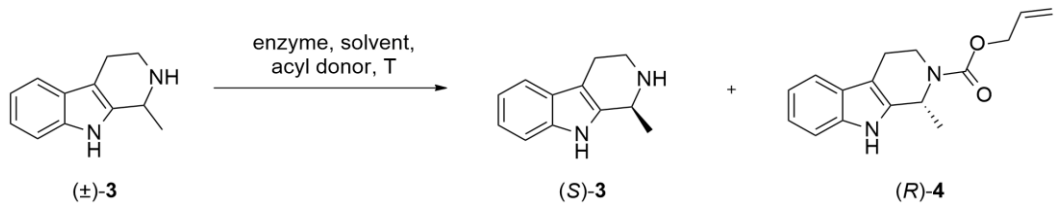


Figure 5. HPLC chromatograms of (±)-**1**, (*S*)-**1** (*ee* = 99%) and (*R*)-**2** (*ee* = 97%) of the preparative-scale resolution with CAL-B

4.1.4. Small-scale KR of (±)-3,5,7,9 in batch mode

On the basis of the above-mentioned results, the CAL-B-catalyzed enantioselective *N*-alkoxycarbonylation of (±)-**3** (Scheme 22) was carried out with phenyl allyl carbonate in *t*-BuOMe at 50 °C in incubator shaker. Although an excellent *E* (> 200) was detected, the reaction had to be stopped at a 47% conversion after a week (ee_s = 89%, ee_p = 99%).



Scheme 22. CAL-B-catalyzed resolution of (±)-**3**

To overcome the problem, 3-methoxyphenyl allyl carbonate, used by Ding et al. in the KR of salsolidine [(±)-**1**],[95] was tested. The reaction rate was low (4% conversion was

detected after a week), although an excellent *E* (> 200) was observed. Thus, we tested phenyl allyl carbonate used previously.

A faster reaction was found with the addition of catalytic amount of Et_3N[42] (conv. = 50% after 36 h, ee_s = 97%, ee_p = 98%) without deactivation before 50% conversion.

As in the case of (±)-**1**, lipase AY was also used in toluene with phenyl allyl carbonate at 40 °C for the *N*-acylation of (±)-**3**. Unluckily, racemic **3** was only detected even after a week, that is the enzyme seemed to be inactive.

The KR of (±)-**5**,**7**,**9** was carried out using the reaction conditions applied above (CAL-B, phenyl allyl carbonate, 50 °C, *t*-BuOMe, Et_3N). All test reactions gave excellent *E* (> 200); however, the reactions stopped at low conversions [27% for (±)-**5**, 29% for (±)-**7**, and 10% for (±)-**9** after a week].

First, (±)-**5** was further tested in order to avoid the stoppage of the reactions. Higher amount of Et_3N was added and the reaction rate increased significantly (47% conv. after a week). An increased reaction rate was also observed when the temperature was increased to 60 °C (49% conv. after a week, *E* > 200). However, a slightly lower conversion (41%) but still an excellent *E* (> 200) were reached at 70 °C. Highly enantioselective (*E* > 200) reaction was also observed in DIPE at 60 °C (48% conversion after a week).

We surmised that the product carbamate blocked the activity of the enzyme by adsorbing on the active sites; therefore, the reaction mixtures were shaken daily in an ultrasound bath for 1 min. The reactions in *t*-BuOMe and DIPE at 60 °C and with higher amount of Et_3N were repeated and were subjected to ultrasound shaking each day affording increased reaction rates with excellent *E* (> 200) with 44% conversion after a week in *t*-BuOMe and 50% conversion in 3 days in DIPE.

Under the best conditions (CAL-B, phenyl allyl carbonate, DIPE, Et_3N, 60 °C, ultrasound shaking), the small-scale reactions of (±)-**7**,**9** and racemic **3** were also performed. The reaction rate increased significantly in the case of (±)-**3** (50% in 1 days) and (±)-**7** (50% in 5 days), With respect to (±)-**9**, the same low conversion was observed (21% after a week).

Further reactions at 70 and 80 °C were carried out with (±)-**9** in order to increase the conversion, but it remained under 25% even after a week in both cases. With 3-methoxyphenyl allyl carbonate at 70 °C, the same low reaction rate was detected (23% after a week, *E* > 200).

4.1.5. Preparative-scale CAL-B-catalyzed resolutions of (±)-3,5,7

First, the enantioselective alkoxycarbonylation of racemic **3** was carried out in the presence of CAL-B with phenyl allyl carbonate and Et_3N in *t*-BuOMe at 60 °C. 50% conversion was reached in 48 h (*E* > 200, *ee* > 97%) (Table 10, entry 1).

On the basis of the results found in the preliminary experiments of (±)-**5**, the preparative-scale KR of (±)-**3** using CAL-B and phenyl allyl carbonate was repeated in DIPE at 60 °C with a higher amount of Et_3N. 50% conversion was reached in 24 h (*E* > 200, *ee* > 97%) (Table 10, entry 2).

Next, under the same conditions, the preparative-scale resolution of (±)-**5** was completed in 5 days using daily ultrasound shaking for 1 min. Excellent *E* (> 200) characterized the reaction (*ee* > 99%) (Table 10, entry 3).

In the case of (±)-**7**, 50% conversion was observed in 7 days with excellent *E* (> 200, *ee* > 99%) (Table 10, entry 4).

Due to the low reaction rate reached in the KR of (±)-**9**, preparative-scale resolution was not attempted.

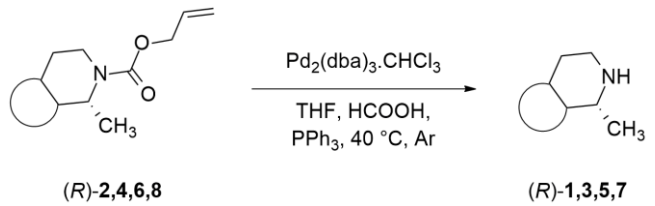
Table 10. Summary of the preparative-scale resolutions of (±)-**3**,**5**,**7**

entry	solvent	reaction time (day)	conv. (%)	*E*	enantio-mer	*ee* (%)	yield (%)	$[\alpha]_D^{25}$
1[a]	*t*-BuOMe	2	50	>200	(*S*)-**3**[c]	98	40	-63[e]
					(*R*)-**4**[d]	97	41	-99[e]
2[b]	DIPE	1	50	>200	(*S*)-**3**[c]	98	42	-62[f]
					(*R*)-**4**[d]	97	40	-98.5[g]
3[b]	DIPE	5	50	>200	(*S*)-**5**[c]	99	43	-89.8[h]
					(*R*)-**6**[d]	99	43	-60[i]
4[b]	DIPE	7	50	>200	(*S*)-**7**[c]	99	41	-70[g]
					(*R*)-**8**[d]	99	43	-71[j]

[a] 100 mg substrate, 25 mL solvent, 30 mg mL^{-1} CAL-B, 4 equiv. phenyl allyl carbonate, 50 µL Et_3N, 60 °C. [b] 100 mg substrate, 25 mL solvent, 30 mg mL^{-1} CAL-B, 4 equiv. phenyl allyl carbonate, 50 µL Et_3N, 60 °C, daily ultrasound shaking. [c] According to HPLC for the *N*-butanoyl-derivatized form. [d] According to HPLC. [e] *c* 0.3 EtOH. [f] *c* 0.22 EtOH. [g] *c* 0.95 EtOH. [h] *c* 0.45 EtOH. [i] *c* 0.37 EtOH. [j] *c* 0.28 EtOH.

4.1.6. Hydrolysis of the (*R*)-carbamate enantiomers

The removal of the Alloc moiety from the (*R*)-carbamate enantiomers (**2**,**4**,**6**,**8**) was then tested (Scheme 23).



Scheme 23. Removal of the Alloc group from the (*R*)-carbamates

First, (*R*)-**2** and (*R*)-**4** were treated with triethanolamine in 50% aqueous NaOH solution at 120 °C.[95,125] The removal of the protecting group did not succeed even after a one-week treatment. Then iodine, a non-transition metal catalyst, was used for deprotection in dry acetonitrile, in the presence of water at RT.[126] A small amount of (*R*)-**3** was detected in 3 days (yield = 24%, *ee* = 92%), but no reaction was found in the case of (*R*)-**2**.

As a further attempt, a homogeneous Pd catalyst [$Pd_2(dba)_3.CHCl_3$] was used in order to remove the Alloc moiety (Scheme 23).[127] The reaction was performed with all prepared carbamate enantiomers [(*R*)-**2**,**4**,**6**,**8**] in the presence of HCOOH and PPh_3 in THF at 40 °C, under Ar. In every case, the enantiomer of the free amines (*R*)-**1**,**3**,**5**,**7** were detected without the loss of enantiopurity (*ee* > 99%) and with good yields (60–85%) (Table 11).

Table 11. Removal of the Alloc moiety of (*R*)-**2**,**4**,**6**,**8** by a homogeneous Pd catalyst[a]

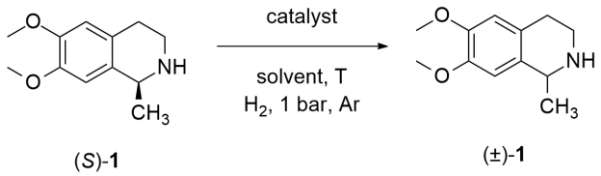
entry	(*R*)-enantiomer	reaction time (h)	*ee* (%)	yield (%)	$[\alpha]_D^{25}$
1	**1**	16	99[b]	60	+60[d]
2	**3**	2	99[c]	85	+62[d]
3	**5**	2	99[c]	78	+87.2[e]
4	**7**	4	99[c]	79	+72.7[e]

[a] 55–60 mg (*R*)-carbamate, 2 mL THF, 0.04 mmol PPh_3, 0.74 mmol HCOOH, 0.014 mmol $Pd_2(dba)_3.CHCl_3$, 40 °C. [b] According to HPLC after derivatization with Ac_2O. [c] According to HPLC for the *N*-butanoyl-derivatized form. [d] *c* 0.3 EtOH. [e] *c* 0.75 EtOH.

4.2. Racemization of (*S*)-1-methyl-substituted tetrahydroisoquinoline and tetrahydro-ß-carboline

4.2.1. Small-scale racemizations of the compounds (*S*)-1 and (*S*)-3

In order to develop mild conditions for racemization, which might be efficient for DKR, (*S*)-**1** was first subjected to test reactions (Scheme 24).



Scheme 24. Racemization of (*S*)-**1**

The first set of experiments were carried out with high catalyst loading (20 mol%) to determine the initial reaction rate. Commercially available heterogeneous hydrogenation catalysts – Pd black, Pd, Pt, Ir, and Ni catalysts supported on microporous active carbon or mesoporous materials – were selected. Furthermore, some heterogeneous, colloidally prepared Ir catalysts were also screened.

Figure 6 is illustrative of the racemization of (*S*)-**1** by using the selected catalysts in toluene at 80 °C under H_2 flow at 1 bar, under Ar.

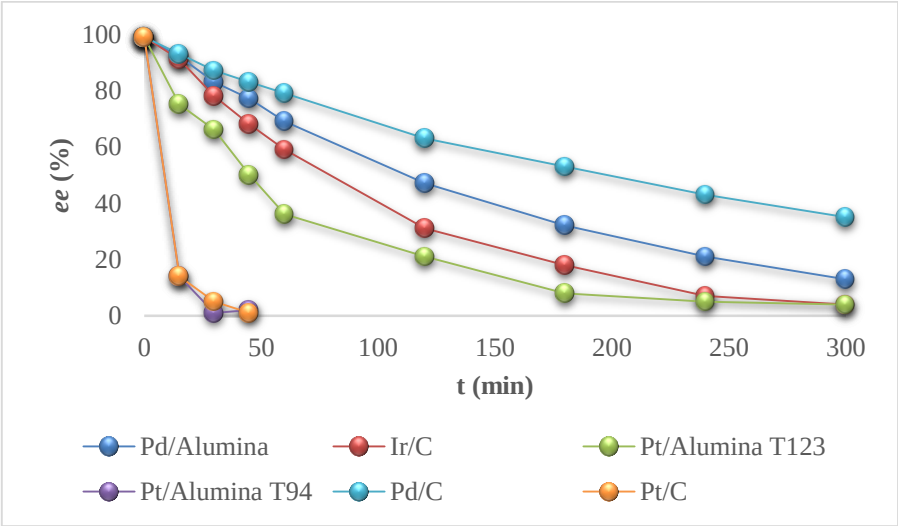


Figure 6. Drops in *ee* in the racemization of (*S*)-**1** (10 mg) using 20 mol% of different heterogeneous catalysts in toluene (40 mL) at 80 °C under H_2 flow at 1 bar

Racemizations were not detected even at 90 °C using Ni/Al_2O_3, Pd black or Ir prepared by colloidal route.

Contrary to our expectations, racemization with heterogeneous Pd catalysts turned out to be slower than with Ir and Pt catalysts. Faster reactions were detected using Pt/Al_2O_3 (T94) or Pt/C. Specifically, they racemized (*S*)-**1** in 30 min (Figure 6). Pt/Al_2O_3 (T94) was selected for further experiments, because of easier pelletization and better reproducibility of this support material in comparison with active carbon.

However, Ir/C showed lower activity than Pt catalysts, but still complete racemization took place in 300 min, thus it was also subjected to further kinetic studies. A commercially available homogeneous Ir catalyst $[IrCp^*I_2]_2$ reported by Page and co-workers was also tested for both starting materials (*S*)-**1** and (*S*)-**3** in toluene at 40 °C.[118] The racemization of the compounds completed in 1 h, motivating us for further investigation with heterogeneous Ir catalyst.

Pt/Al_2O_3 (T94) with different loadings was tested on (*S*)-**1** (Figure 7). Decreasing loadings from 20 mol% to 5 mol% resulted in decreasing reaction rates, although racemic **1** was still obtained in a few hours (20 mol%: complete racemization in 45 min, 5 mol%: complete racemization in 240 min). When 3 mol% catalyst was applied, a reasonably longer reaction time was observed (29% *ee* in 300 min), suggesting that the amine or the imine form of the compound are able to inhibit the catalyst, thus affecting the reaction rate at lower catalyst amount.

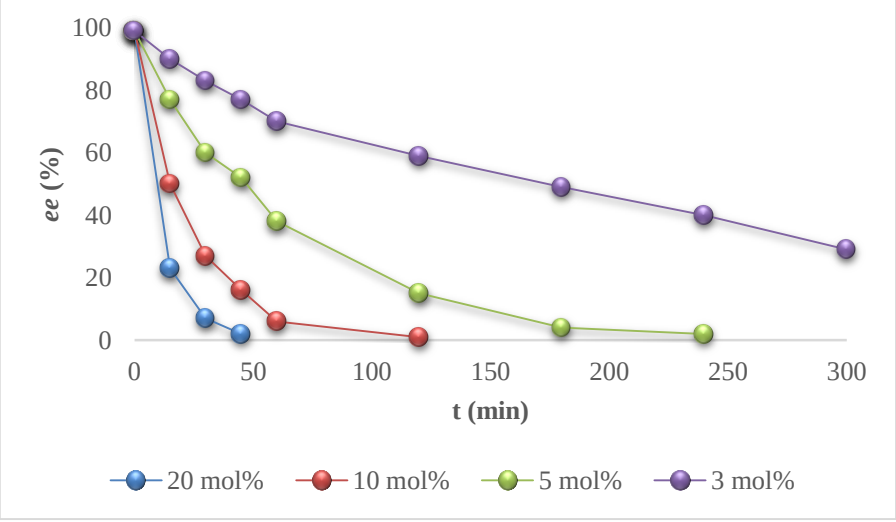


Figure 7. Reaction rates of racemization of (*S*)-**1** (10 mg) in the presence of Pt/Al_2O_3 (T94) in toluene (40 mL), at 80 °C under H_2 flow at 1 bar

Kinetic studies carried out by Professor Murzin allowed the calculation of kinetic constants, which data should be independent from catalyst concentration. However, a close to first-order dependence on catalyst concentration was determined (see Figure 3 in the original publication).[II] These results are in line with the significant catalyst inhibition observed at low catalyst loading.

The Ir/C catalyst with different loadings was also screened (Figure 8). The reaction rate decreased simultaneously with reduced Ir/C catalyst concentration (20 mol%: *ee* = 2%, 15 mol%: *ee* = 13%, 10 mol%: *ee* = 24% in 300 min), but catalyst deactivation, observed previously with Pt catalyst, was not found at these concentrations.

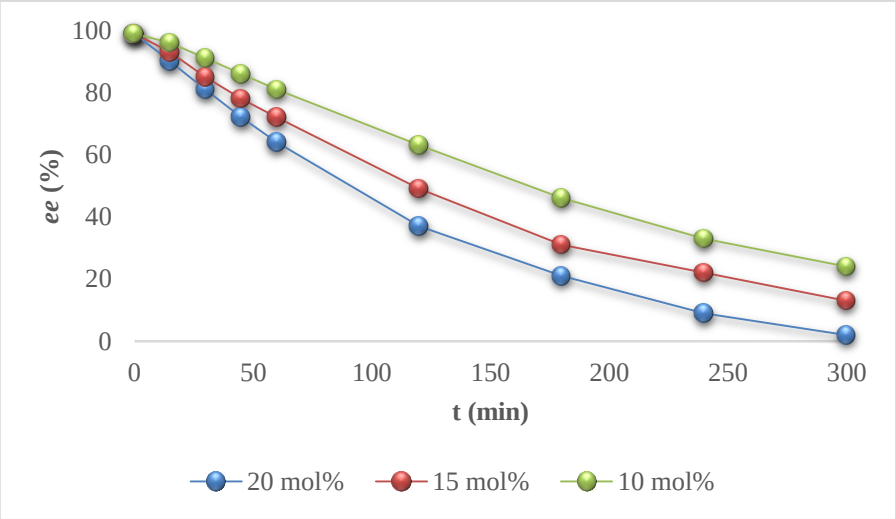


Figure 8. Racemization of (*S*)-**1** (10 mg) in the presence of Ir/C in toluene (40 mL) at 80 °C under H_2 flow at 1 bar

To compare the activation energies, additional experiments were carried out at different temperatures with 10 mol% Pt/Al_2O_3 (T94) (Figure 9) or 15 mol% Ir/C (Figure 10). At higher temperatures, faster reactions were observed with both catalysts. With 10 mol% Pt/Al_2O_3 (T94), racemization completed at 80 °C in 120 min, while at 50 °C *ee* = 17% was detected in 300 min. Using 15 mol% Ir/C, complete racemization took place at 90 °C and *ee* = 35% was found at 70 °C in 300 min.

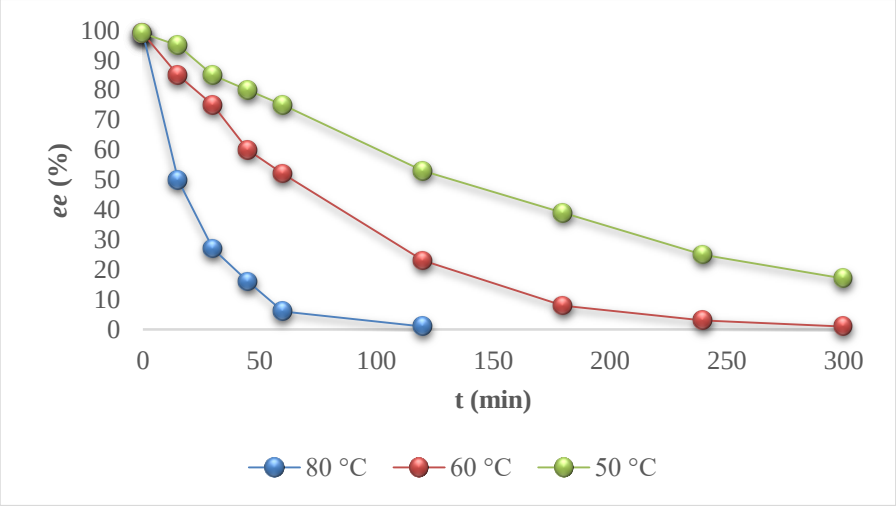


Figure 9. Racemization of (*S*)-**1** (10 mg) in the presence of Pt/Al_2O_3 (T94) (10 mol%) at different temperatures in toluene (40 mL) under H_2 flow at 1 bar

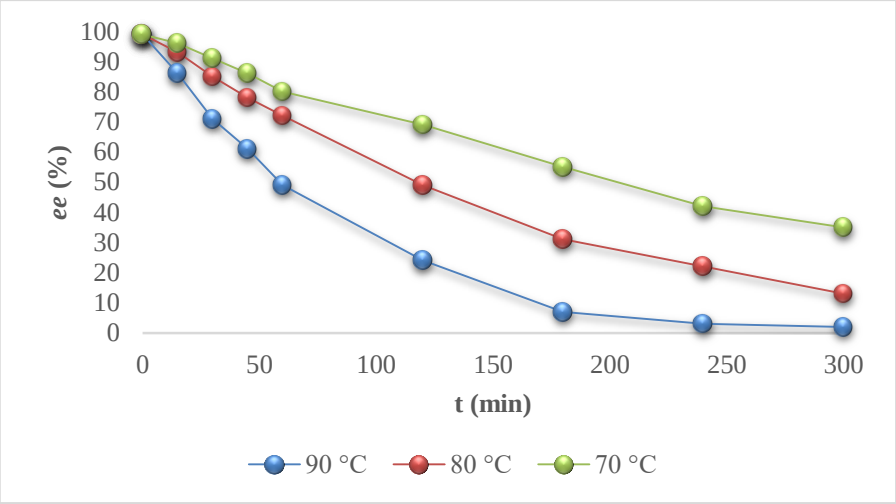
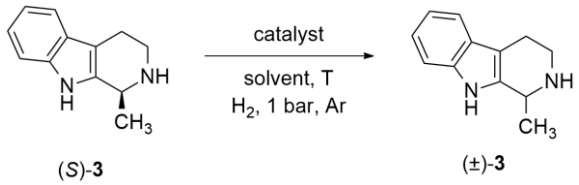


Figure 10. Racemization of (*S*)-**1** (10 mg) in the presence of Ir/C (15 mol%) at different temperatures in toluene (40 mL) under H_2 flow at 1 bar

An activation energy of 27 kJ mol^{-1} was determined for Pt/Al_2O_3 (T94) and a much higher value, 65 kJ mol^{-1} for Ir/C was found from kinetic constants by Professor Murzin. These data are in line with the experimental results; namely, the racemization is faster over Pt.

As a remark, in the test reactions different (*S*)-**1** substrate batches were used; nevertheless, the figures are totally coherent. Under the same reaction conditions, minor differences in *ee* values (within a 10% margin) were observed at the same reaction time for the different substrate batches in sample analysis.

First, racemization of (*S*)-**3** (Scheme 25) was carried out with the use of Pt/Al_2O_3 (T94) in toluene at 80 °C under H_2 flow at 1 bar under Ar.



Scheme 25. Racemization of (*S*)-**3**

The reaction completed in 2 h with 10 mol% catalyst loading, thus in the next step the catalyst loading was reduced to 2 mol% (Figure 11). As in the case of (*S*)-**1**, the activity of the catalyst significantly decreased at lower catalyst concentration resulting in only 47% *ee* after 5 h. The probable reason is catalyst deactivation brought about by the amine and imine form. When 20 mol% Ir/C was used under the same conditions, 14% *ee* was reached in 5 h (Figure 11).

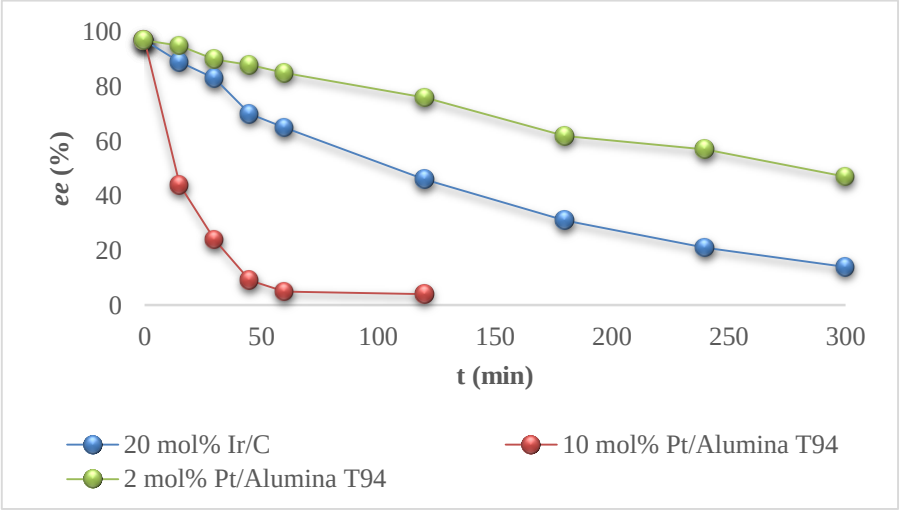


Figure 11. Reaction rates of racemization of (*S*)-**3** (10 mg) catalyzed by different heterogeneous catalysts in toluene (40 mL) at 80 °C under H_2 flow at 1 bar

Additionally, racemization of (*S*)-**3** catalyzed by Pt/Al_2O_3 (T94) was performed in *t*-BuOMe instead of toluene. Only a moderate decrease in *ee* was observed from 98 to 91% in 5 h, when 10 mol% catalyst at 80 °C was used.

4.2.2. Isolations of racemic products 1 and 3

Racemization reactions of (*S*)-**1** and (*S*)-**3** in larger scale (50 mg) were executed using 10 mol% Pt/Al_2O_3 (T94) in toluene at 80 °C under H_2 flow at 1 bar, under Ar. Racemic **1** was obtained in 2 h with excellent yield (94%) (Figure 12), while racemic **3** was detected in 1 h and it was isolated in a yield of 90% (Figure 13) after filtering off the catalyst, solvent evaporation, HPLC, and ^1H NMR analysis.

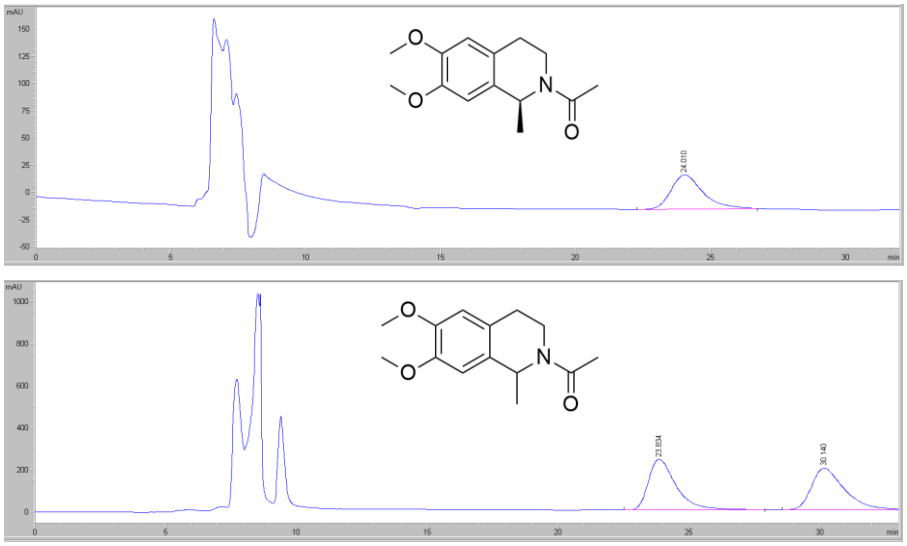


Figure 12. HPLC chromatogram of the *N*-acetyl derivatives of (*S*)-**1** (*ee* = 99%) and (±)-**1** from the preparative-scale racemization induced by Pt/Al_2O_3 (T94)

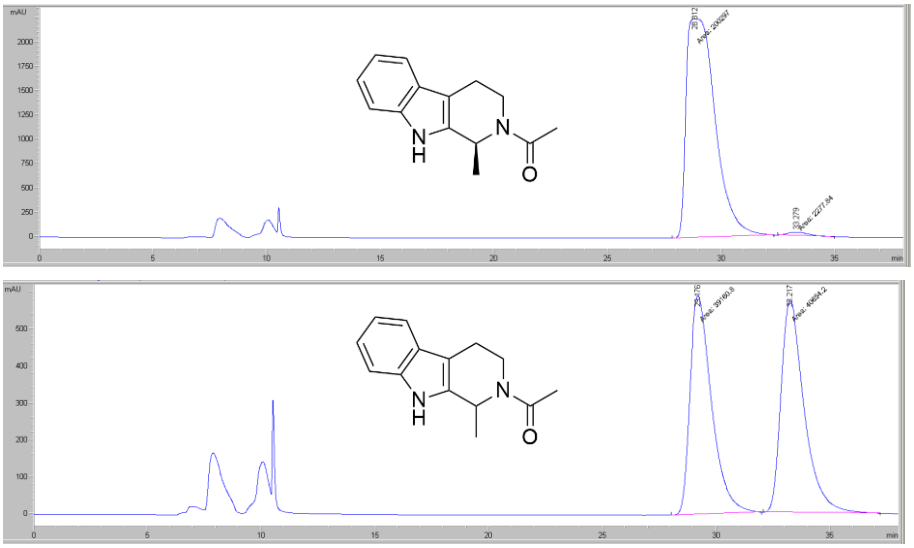


Figure 13. HPLC chromatogram of the *N*-acetyl derivatives of (*S*)-**3** (*ee* = 98%) and (±)-**3** from the preparative-scale racemization induced by Pt/Al_2O_3 (T94)

4.2.3. Additional experiments to characterize the catalysts

Figure 14 shows the results found in catalyst recycling studies. After a racemization cycle, the catalysts were reused under the same conditions. Decreased reaction rate was detected in the 2nd cycle when 10 mol% Pt/Al_2O_3 (T94) was used. The *ee* of 50% after 15 min in the 1st cycle changed to 67% in the 2nd cycle in similar duration. No such effect was found with Ir/C.

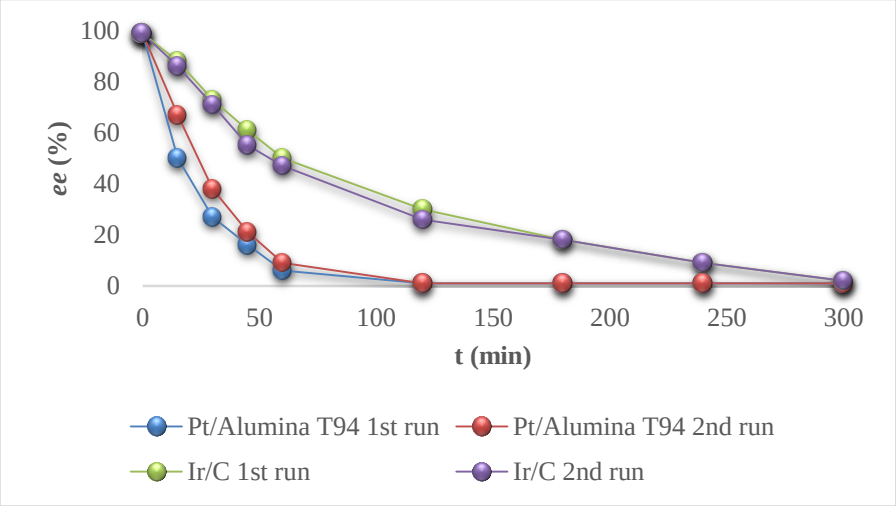


Figure 14. Recyclability experiments utilizing 20 mol% Ir/C and 10 mol% Pt/Al_2O_3 (T94) catalysts for the racemization of (*S*)-salsolidine at 80 °C under H_2 flow at 1 bar

Next, the deactivation of the Pt catalyst by the imine intermediate formed in the reaction was explored. First, the imine formation by the catalyst was investigated using racemic **1** as substrate at elevated temperature in the absence of H_2. The transformation of (±)-**1** into the imine underwent rapidly in the presence of Ir/C (Figure 15), while the dehydrogenation rate was much slower with the use of Pt/Al_2O_3 (T94) (Figure 16).

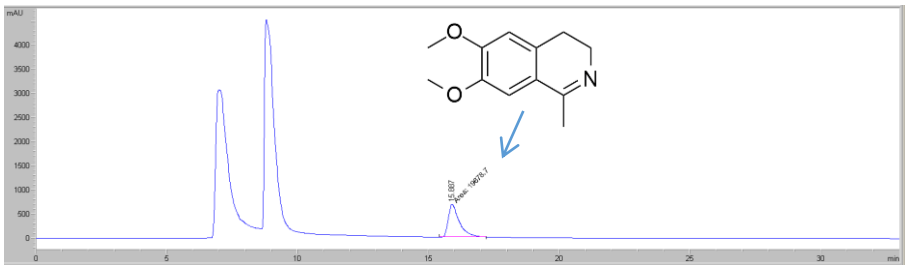


Figure 15. HPLC chromatogram of the corresponding imine from the reaction of (±)-**1** using 20 mol% Ir/C in toluene after 5 hours

Figure 16. HPLC chromatogram of the *N*-acetyl derivative of (±)-**1** and the corresponding imine using 20 mol% Pt/Al_2O_3 (T94) in toluene after 5 hours

A significant reduction in the reaction rate was also detected, when the imine prepared separately was added to the reaction mixture in the racemization of (*S*)-**1** using Pt/Al_2O_3 (T94) (Figure 17). Without imine the racemization completed in 120 min, while 29% *ee* was detected with the imine in 120 min.

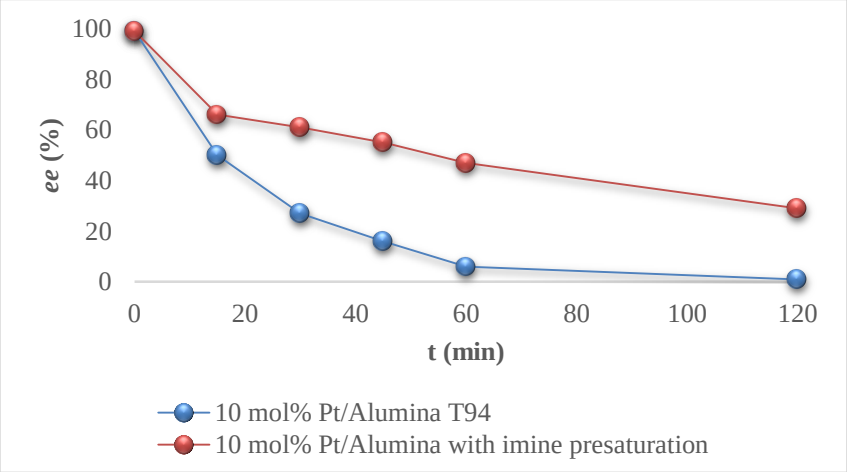


Figure 17. Investigation of the inhibitory effect of imine in the racemization of (*S*)-**1** on Pt/Al_2O_3 (T94) in toluene under H_2 flow at 1 bar

The leaching of the active metal species from the heterogeneous catalysts [Pt/Al_2O_3 (T94), Ir/C] was excluded. The racemization of (*S*)-**1** was set up with 5 mol% Pt/Al_2O_3 (T94) or with 15 mol% Ir/C in toluene at 80 °C under H_2 flow at 1 bar, under Ar. After 45 min, the catalyst was filtered off and, without the catalyst, the *ee* did not decrease further [*ee* = 20% with Pt/Al_2O_3 (T94) and *ee* = 57% with Ir/C in 45 h]. It is a clear indication, that the racemization stopped. Note, the reaction mixture did not contain metal particles.

DFT calculations were carried out by Professor Karoliina Honkala, who determined the relative stability of (*S*)-**1** and its imine form on Pt(111) and Ir(111) surfaces. The

adsorption of (*S*)-**1** is strongly exothermic; however, the physisorption energy on Pt(111) is more exothermic than that on Ir(111) and the imine form also prefers the Pt(111) face. These results suggest that Pt has better activity than Ir, which is in harmony with the experimental data (faster reactions over Pt).

4.2.4. DKR investigation

A test reaction was performed using Ir/C and CAL-B in toluene for the DKR of (±)-**1** at 80 °C with phenyl allyl carbonate under H_2 bubbling and under Ar without significant activity. The optimum conditions of KR and racemization have to be similar before merging them into a successful DKR. Furthermore, according to literature data, the successful DKR of amines were usually carried out utilizing a racemization catalyst and an enzyme supported on the same carrier material.[106] Here, the two parts of DKR were optimized separately and a successful DKR needs further studies.

5. SUMMARY

- KR of (±)-**1** was carried out using AY in toluene with phenyl allyl carbonate at 40 °C. 50% conversion was reached in 24 h, and (*S*)-**1** and (*R*)-**2** were produced with an *ee* of 99% and a yield of > 39%.
- KR of (±)-**1** with CAL-B in *t*-BuOMe with phenyl allyl carbonate at 50 °C in batch afforded (*S*)-**1** and (*R*)-**2** with an *ee* of > 97% and a yield of > 38% in 49 h.
- In the continuous flow reactor, 25% conversion was detected (*E* > 200) under the same conditions, but at 70 °C. The reaction mixture was pumped through the same CAL-B-filled column five times. 41% conversion with excellent *E* was finally reached in the last cycle.
- (±)-**3** was subjected to KR in batch mode using CAL-B in *t*-BuOMe with phenyl allyl carbonate and Et_3N at 50 °C. (*S*)-**3** was isolated with 98% *ee* and (*R*)-**4** with 97% *ee* in a yield of > 40% in 2 days. In DIPE at 60 °C, the reaction completed in 1 day (*ee* > 99%, yield > 40%). Enantioselective reaction was not observed by using AY.
- The preparative-scale CAL-B-catalyzed reaction of (±)-**5** was carried out in DIPE with phenyl allyl carbonate and Et_3N at 60 °C by shaking the reaction mixture in an ultrasound bath each day. 50% conversion was reached in 5 days and compounds (*S*)-**5** and (*R*)-**6** were isolated with > 99% *ee* and in 43% yield.
- The KR of (±)-**7** was carried out using the same conditions affording 50% conversion in 7 days. (*S*)-**7** and (*R*)-**8** were isolated with an *ee* of 99% and in a yield of > 41%.
- 21% conversion (*E* > 200) was detected after a week, when (±)-**9** was subjected to KR under the best conditions. Modifications, however, did not result in any significant increase in conversion (25% conversion after a week at 80 °C). Preparative-scale resolution, consequently, was not carried out.
- Substituents with different sizes at C1 affected the reaction rate in the KR of tetrahydro-ß-carbolines. Specifically, conversions decreased in parallel with increasing substituent size.
- The removal of the Alloc moiety from (*R*)-**2**,**4**,**6**,**8** was carried out using $Pd_2(dba)_3.CHCl_3$ in THF in the presence of PPh_3, HCOOH at 40 °C under Ar. (*R*)-**1**,**3**,**5**,**7** were obtained with 99% *ee* and > 60% yield in < 16 h.
- Racemization reactions of (*S*)-**1** were studied with heterogeneous Pt and Ir catalysts in toluene at 80 °C under H_2 flow at 1 bar, under Ar.

- 20 mol% Pt/Al_2O_3 (T94) racemized (*S*)-**1** in 30 min, while with 3 mol% Pt/Al_2O_3 (T94) 29% *ee* was detected in 300 min. Catalyst deactivation was observed at low catalyst concentration.
- The catalyst deactivation ability of the imine intermediate was proved, when the imine prepared separately was added to the reaction mixture catalyzed by 10 mol% Pt/Al_2O_3 (T94). The reaction completed in 120 min without the imine, while the *ee* was 29% in 120 min with the imine.
- With 20 mol% Ir/C, the racemization completed in 300 min, whereas with 10 mol% catalyst *ee* = 24% was observed in the same reaction time. In these cases, catalyst deactivation was not found.
- Temperature screening was carried out to determine activation energies. The values are 27 kJ mol^{-1} for Pt/Al_2O_3 (T94) and 65 kJ mol^{-1} for Ir/C. These results are in line with the experiments, that is racemization catalyzed by Pt is faster.
- The racemization of (*S*)-**3** with 10 mol% Pt/Al_2O_3 (T94) completed in 120 min, while with 2 mol% catalyst loading 47% *ee* was observed after 5 h. Catalyst deactivation was detected as well. When 20 mol% Ir/C was used, the *ee* was 14% in 5 h. In *t*-BuOMe, no significant decrease in *ee* was detected.
- The synthesis of racemic **1** and **3** were carried out using 10 mol% Pt/Al_2O_3 (T94) in toluene at 80 °C under H_2 flow at 1 bar, under Ar. (±)-**1** was obtained with 94% yield in 2 h and (±)-**3** was isolated with 90% yield in 1 h.
- The reuse of the two catalysts was studied in the racemization of (*S*)-**1**. The decrease in *ee* was lower in the 2nd cycle by using 10 mol% Pt/Al_2O_3 (T94): 17% difference in *ee* was found after 15 min, while no decline in the reaction rate was detected with Ir/C.
- Additionally, the metal leaching from the catalysts was ruled out. When the catalysts were filtered off, racemizations stopped, and the *ee* did not decrease further.
- The DKR reaction of (±)-**1** was performed with Ir/C and CAL-B in toluene with phenyl allyl carbonate at 80 °C under H_2 flow, under Ar. No significant activity was observed.

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ANNEX

I.

Tetrahedron: *Asymmetry* 28 (2017) 1829–1833

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Efficient lipase-catalysed route for the kinetic resolution of salsolidine and its ß-carboline analogue

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ABSTRACT

Racemic 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline **1** and 1-methyl-1,2,3,4-tetrahydro-ß-carboline **3** were resolved through lipase-catalysed asymmetric acylation on the secondary amino group. High enantioselectivities (*E* >200) were observed when the acylation of racemic **1** was performed with phenyl allyl carbonate in the presence of *Candida rugosa* lipase in toluene at 40 °C or with *Candida antarctica* lipase B in *tert*-butyl methyl ether at 50 °C. Excellent enantioselectivity (*E* >200) characterised the CAL-B-catalysed acylation of racemic **3** with phenyl allyl carbonate in the presence of triethylamine in *tert*-butyl methyl ether at 50 °C. The product (*R*)-carbamates (*ee* >97%) were hydrolysed into the corresponding (*R*)-enantiomers of the free amines **1** and **3** (*ee* = 99%) with the use of $Pd_2(dba)_3{\cdot}CHCl_3$ catalyst.

1. Introduction

Interest in the research of compounds containing the 1-substituted 1,2,3,4-tetrahydroisoquinoline ring system has recently come into view, since compounds bearing this skeleton exert major biological activities. Several derivatives possess a common nucleus of synthetic structures, while other compounds are extracted from natural sources. All of them are associated with important pharmacological effects. The naturally occurring (*S*)-norcoclaurine [(1*S*)-1-(4-hydroxybenzyl)-1,2,3,4-tetrahydro-6,7-isochinolindiol] is an intermediate in the synthesis of morphine, papaverine or the antibacterial berberine.[1] Racemic norcoclaurine has α- and ß-adrenoreceptor activity.[2] Solifenacin [(1*S*,3′*R*)-3′quinuclidinyl-1-phenyl-1,2,3,4-tetrahydro-2-isoquinolinecarboxylate] containing a 1-phenyl-substituted tetrahydroisoquinoline core is an example of synthetic compounds. It shows urinary antispasmodic effect.[3] Both enantiomers of 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline **1** (salsolidine) are naturally occurring compounds.[4] The (*R*)-enantiomer was isolated from *Genista pungens*,[4] while the (*S*)-enantiomer was found in *Salsola richteri*.[4] Many pharmacological properties have been described to salsolidine; *e.g.*, it inhibits the uptake of 5-hydroxytryptamine by human blood platelets.[4] The (*R*)-enantiomer has a monoamine oxidase A (MAO A) inhibitoring effect.[4] On the other hand, as a structural feature of a trimethoprim analogue, it acts as a dihydrofolate reductase inhibitor.[4] The pharmaceutically valuable 1-substituted 1,2,3,4-tetrahydro-ß-carboline skeleton is also a common building block of several alkaloids, including the naturally occurring reserpine, which shows antitumor activity, as well as antihypertensive and neuroprotective effects.[5] Synthetic 1-substituted *N*-acylated tetrahydro-ß-carbolines have inhibitory activity against the Breast Cancer Resistance Protein (ABCG2).[6] The (*S*)-enantiomer of salsolidine analogue 1-methyl-1,2,3,4-tetrahydro-ß-carboline **3** (eleagnine) was isolated from *Eleagnus angustifolia* and *Petalostyles labicheoides*.[7] The racemic form can bind to the $GABA_A$ receptors in the benzodiazepine binding site, but it has an inverse agonist effect.[8]

It is not surprising, therefore, that a large number of synthetic routes have been developed for the preparation of **1** and **3**, in particular, in enantiomeric forms.[7,9–13] As an example, enantiomeric **1** was synthesized by Ding and et al. through enantioselective acylation of the racemic mixture in a batch process.[14]

In this work, our aim was to devise a new enzymatic strategy for the preparation of enantiomeric **1** and **3**, through lipase-catalysed kinetic resolution (Scheme 1). In addition to the batch reactions, we planned to examine the possibilities for their enzymatic reactions in a continuous-flow system. The use of this novel method has clear advantages, such as short reaction times, rapid heating and pressure screening.[15] In the literature, there are various examples using this innovative technique for resolution. For example, the asymmetric acylation of 1-phenylethylamine[16,17] and an imidazole derivative[18] as well as the esterification of flurbiprofen[19] have been reported.

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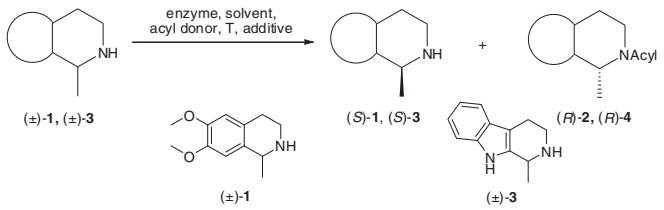


Scheme 1. Kinetic resolution of (±)-**1** and (±)-**3** through lipase-catalysed *N*-acylation on the secondary amino group.

2. Results and discussion

2.1. Synthesis of (±)-1 and (±)-3

1-Methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (±)-**1** was prepared from 3,4-dimethoxyphenylethylamine and acetic anhydride through Bischler–Napieralski cyclization followed by reduction, while racemic 1-methyl-1,2,3,4-tetrahydro-ß-carboline (±)-**3** was synthesized utilizing the Pictet–Spengler reaction via a microwave-assisted procedure according to known literature methods.[9,11]

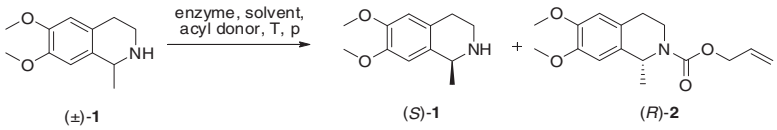
2.2. Enzymatic resolution of (±)-1 and (±)-3

Ding et al. reported the asymmetric *N*-acylation of racemic **1** ($E > 200$, conv. = 50% in 72 h, yield > 46%) by using CAL-A (*Candida antarctica* lipase A), 3-methoxyphenyl allyl carbonate in toluene at 40 °C.[14] The dynamic kinetic resolution of (±)-**1** was also described by Page *et al.* using a combination of catalysts AY (*Candida rugosa* lipase) and pentamethylcyclopentadienyliridium(III) iodide dimer in the presence of 3-methoxyphenyl propyl carbonate at 40 °C (conv. = 90% in 23 h, *ee* = 96%, yield = 82%).[20]

We started the resolution of (±)-**1** by an enzyme screening. First, the reaction was performed in batch with phenyl allyl carbonate in toluene at 40 °C (Scheme 2, Table 1, entries 1–4). Low enantioselectivity was observed with CAL-A ($E = 9$, entry 1) and with PS-IM (*Burkholderia cepacia* lipase) ($E = 2$, entry 2). In contrast, an improved $E = 46$ was found with CAL-B (*Candida antarctica* lipase B) 49% conversion in 4 days (entry 4). Lipase AY was the best catalyst with a conversion of 50% in 72 h and an excellent E (>200) (entry 3). Since the tested CAL-B was purchased as an immobilized enzyme (from Sigma) and also in view of its potential use in continuous-flow system, CAL-B was chosen for further optimization.

Next, the CAL-B-catalysed reaction was performed at 50 and then 60 °C (Table 1, entries 5 and 6). As the temperature was increased, the reaction rate increased considerably (entries 4–6). However, the best combination of reaction rate and enantioselectivity was found at 50 °C (entry 5). When toluene was replaced with *t*-BuOMe, a much faster reaction with excellent E (>200) was observed (entry 7).

Having the optimized conditions in the batch process (CAL-B, phenyl allyl carbonate, *t*-BuOMe, 50 °C, 1 bar), we decided to perform a reaction under these conditions in a continuous-flow reactor (an *H*-Cube in 'no H_2 mode'). A 70-mm-long heat- and pressure-resistant stainless-steel CatCart was filled with CAL-B. Unfortunately, only 20% conversion was reached after a cycle, although an excellent E (>200) was observed (Table 2, entry 1).



Scheme 2. Kinetic resolution of (±)-**1** through enantioselective *N*-acylation.

Table 1
N-Acylation of (±)-**1** in batch[a]

Entry	Enzyme	T (°C)	Solvent	Reaction time (h)	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	CAL-A	40	Toluene	96	42	50	68	9
2	PS-IM	40	Toluene	96	3	13	25	2
3	AY	40	Toluene	72	50	99	98	>200
4	CAL-B	40	Toluene	96	49	85	89	46
5	CAL-B	50	Toluene	48	50	96	95	154
6	CAL-B	60	Toluene	48	51	96	92	94
7	CAL-B	50	*t*-BuOMe	2.5	50	99	97	>200

[a] 0.025 M (±)-**1**, phenyl allyl carbonate.
[b] According to HPLC after a derivatisation with Ac_2O.
[c] According to HPLC.

Table 2
Effect of pressure and temperature on the acylation of (±)-**1** with phenyl allyl carbonate in a continuous-flow system[a]

Entry	p (bar)	T (°C)	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	1	50	20	24	99	>200
2	30	50	3	3	99	>200
3	60	50	9	10	99	>200
4	1	60	21	26	99	>200
5	1	70	25	33	99	>200
6	1	80	7	7	99	>200

[a] 0.025 M (±)-**1**, 244 mg CAL-B (70 mm CatCart); *t*-BuOMe, 0.1 mL min^{-1}.
[b] According to HPLC after a derivatisation with Ac_2O.
[c] According to HPLC.

In order to improve the reaction rate, the pressure was increased from 1 to 30, and then 60 bar. The *E* remained excellent (>200), but the reaction rate decreased significantly (compare entries 2 and 3 to entry 1). In a further attempt to increase the rate, we examined the effect of temperature on the reaction. As the temperature was increased from 50 °C to 60 °C and then 70 °C, the rate increased slightly (entries 1, 4 and 5), while at 80 °C the activity of the enzyme decreased drastically (entry 6) after a single cycle. Thus, 70 °C was selected as the optimal temperature for further experiments.

Because of the relatively modest conversion of 25% obtained after a cycle (Table 2, entry 5), the enzymatic mixture was pumped through the reactor five times consecutively, using the same CatCart filled with CAL-B. As the number of the cycles increased, the conversion increased progressively (Fig. 1) reaching 41% with an excellent final *E* (>200) after the 5th cycle. However, it is noteworthy, that the activity of the enzyme decreased significantly after the 2nd cycle.

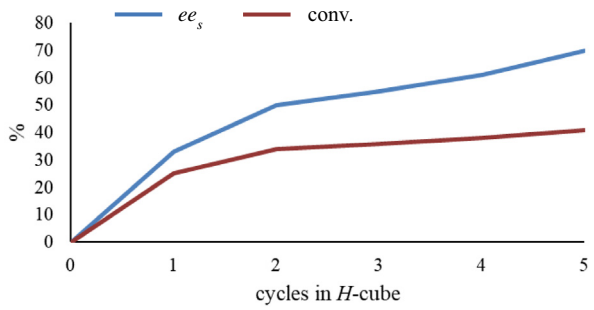
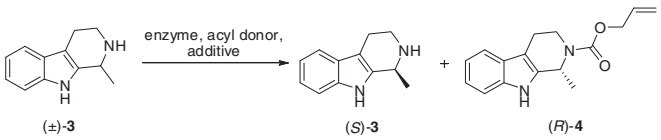


Figure 1. The influence of the number of cycles on reaction rate in the case of (±)-**1**, 1st cycle: ee_s = 33%, conv. = 25%, 2nd cycle: ee_s = 50%, conv. = 34%, 3rd cycle: ee_s = 55%, conv. = 36%, 4th cycle: ee_s = 61%, conv. = 38%, 5th ee_s = 70%, conv. = 41%, ee_p = 99% in each cycle.

Finally, the kinetic resolution of (±)-**1** was also tested with lipase AY and phenyl allyl carbonate in *H*-Cube (toluene, 40 °C, 1 bar). Both the reaction rate and the enantioselectivity were rather low after a cycle (ee_s = 3%, ee_p = 53%, conv. = 5%, *E* = 3), since the enzyme was compressed due to its powdery structure.

On the basis of the preliminary experiments, the preparative-scale resolution of (±)-**1** was performed with both lipase AY (conv. = 50%, *E* > 200, *ee* = 99%) and CAL-B (conv. = 50%, *E* > 200, *ee* > 97%) enzymes under the optimized conditions in batch (Table 3).

In view of the above results, *N*-acylation of racemic **3** (Scheme 3) was performed with phenyl allyl carbonate in the presence of CAL-B in *t*-BuOMe at 50 °C. Excellent *E* (>200) was observed but the reaction stopped after a long run (conv. = 47% after a week, ee_s = 89%, ee_p = 99%). To solve this problem, 3-methoxyphenyl allyl carbonate[21] was tested in the reaction. Excellent *E* (> 200), but rather low reaction rate (conv. = 4% after a week) was observed. Therefore, phenyl allyl carbonate was used in further reactions.



Scheme 3. Kinetic resolution of (±)-**3** through lipase-catalysed enantioselective *N*-acylation.

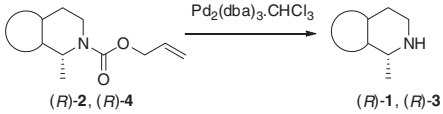
When a catalytic amount of Et_3N was added to the reaction mixture,[22] a relatively fast reaction (conv. = 50% after 36 h) was observed without the earlier-mentioned deactivation stop before 50% (ee_s = 97%, ee_p = 98% at a conversion of 50%).

Lipase AY was also tested for the acylation of racemic **3** with phenyl allyl carbonate in toluene at 40 °C. Unfortunately, the enzyme proved to be inactive and only racemic **3** was detected in the reaction media even after a week.

In view of these preliminary results, the CAL-B-catalysed preparative-scale resolution of racemic **3** was performed (phenyl allyl carbonate, *t*-BuOMe, Et_3N, 50 °C) and 50% conversion was observed in 48 h (*E* > 200, *ee* > 97%) (Table 3).

2.3. Hydrolysis of (*R*)-2 and (*R*)-4

The removal of the *N*-allyloxycarbonyl (Alloc) moiety from carbamates (*R*)-**2** and (*R*)-**4** was also investigated (Scheme 4).



Scheme 4. Hydrolysis of (*R*)-**2** and (*R*)-**4**.

First, the hydrolysis was carried out with triethanolamine in 50% aqueous NaOH solution at 120 °C,[14,23] but the removal of the protecting groups was not observed even after a one-week treat-

Table 3
Preparative-scale resolution of (±)-**1** and (±)-**3**

Substrate	Reaction time (h)	Enzyme	Conv. (%)	*E*	Enantiomer	*ee* (%)	Yield (%)	$[\alpha]_D^{25}$
(±)-**1**[a]	24	AY	50	>200	(*S*)-**1**	99[d]	40	−59[g]
					(*R*)-**2**	99[e]	39	−104[g]
(±)-**1**[b]	49	CAL-B	50	>200	(*S*)-**1**	99[d]	41	−58.9[h]
					(*R*)-**2**	97[e]	38	−102[g]
(±)-**3**[c]	48	CAL-B	50	>200	(*S*)-**3**	98[f]	40	−63[g]
					(*R*)-**4**	97[e]	41	−99[g]

[a] 0.48 M (±)-**1**, 30 mg mL^{-1} AY, 30 mL toluene, 4 equiv. of phenyl allyl carbonate, 40 °C.
[b] 0.48 M (±)-**1**, 30 mg mL^{-1} CAL-B, 30 mL *t*-BuOMe, 4 equiv. phenyl allyl carbonate, 50 °C.
[c] 0.54 M (±)-**3**, 30 mg mL^{-1} CAL-B, 15 mL toluene, 4 equiv. phenyl allyl carbonate, 5 μl Et_3N, 50 °C.
[d] According to HPLC after derivatisation with Ac_2O.
[e] According to HPLC.
[f] According to HPLC after derivatisation with $(CH_3CH_2CH_2CO)_2O$.
[g] *c* 0.3, EtOH.
[h] *c* 0.55, EtOH.

ment. Then iodine, a non-transition metal catalyst, was tested for deprotection,[24] in dry acetonitrile, in the presence of water at room temperature. After 3 days, a relatively small amount of (*R*)-**3** (yield = 24%, *ee* = 92%) and no (*R*)-**1** were detected. Next, a Pd catalyst [tris(dibenzylideneacetone)dipalladium-chloroform adduct, $Pd_2(dba)_3{\cdot}CHCl_3$] was used for the removal of the Alloc moiety,[25] in the presence of formic acid (HCOOH) and triphenylphosphine (PPh_3) in tetrahydrofuran (THF) at 40 °C, under Ar. In both cases (*R*)-**1** and (*R*)-**3** were formed without any loss in enantiopurity (*ee* = 99%) and in good yields [85% in 2 h for (*R*)-**3** and 60% in 16 h for (*R*)-**1**].

2.4. Absolute configurations

The stereochemistry of the enantiomers was determined by comparing the specific rotation values of the prepared free amines **1** and **3** with literature data for (*R*)-salsolidine and the (*R*)-enantiomer of its ß-carboline analogue (Experimental). Both enzymes were found to display (*R*)-selectivity during acylation reactions.

3. Conclusions

Efficient new enzymatic methods have been developed for the synthesis of the enantiomers of salsolidine and its ß-carboline analogue. Excellent selectivity *E* (>200) was observed upon performing the acylation of (±)-**1** in the presence of CAL-B with phenyl allyl carbonate in *t*-BuOMe in both batch mode and a continuous-flow system. The same high *E* (>200) characterised the resolution of (±)-**1**, when the acylation was carried out in the presence of lipase AY with phenyl allyl carbonate in toluene at 40 °C. CAL-B catalysed the enantioselective acylation of (±)-**3** with excellent *E* (>200), when the reaction was performed with phenyl allyl carbonate, in the presence of Et_3N in *t*-BuOMe at 50 °C. To the best of our knowledge, (±)-**3** was resolved for the first time by using enzymes. Carbamate (*R*)-**2** and (*R*)-**4** were hydrolysed into the corresponding amines with $Pd_2(dba)_3{\cdot}CHCl_3$ as catalyst in THF, in the presence of PPh_3 and HCOOH. The products were formed with excellent enantiopurity [*ee* = 99% for both (*R*)-**1** and (*R*)-**3**] and in good yields [60% of (*R*)-**1** and 85% of (*R*)-**3**].

4. Experimental

4.1. Materials and methods

CAL-B (lipase B from *Candida antarctica*) immobilized on acrylic resin was purchased from Sigma, and lipase PS-IM (*Burkholderia Cepacia*) immobilized on diatomaceous earth was from Amano Enzyme Europe Ltd. Lipase AY (*Candida rugosa*) was from Fluka and CAL-A (lipase A from *Candida antarctica*) from Novo Nordisk.

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. ^{1}H NMR spectra were recorded on a Burker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. Microwave (MW) reactions were performed in a CEM Discover MW reactor (Matthews, NC, USA). The elemental analysis was measured by means of a Perkin-Elmer CHNS-2400 Ser II Elemental Analyzer. The *H*-Cube reactor used in 'no H_2 mode' and equipped with a stainless steel enzyme-charged cartridge (70 mm length, 4 mm inside diameter) was from ThalesNano Inc.

The *ee* values of the enantiomers were determined by HPLC [Chiralpak IA column (4.6 mm × 250 mm)]. Eluent: *n*-hexane/*i*Pa (80:20), flow rate: 0.5 mL min^{-1}, 260 nm; retention times (min) for Ac_2O-derivatised form of (*S*)-**1**: 21.10, for (*R*)-**1**: 26.96 and eluent: *n*-hexane/*i*Pa (96:4), flow rate: 0.5 mL min^{-1}, 220 nm; retention times (min) for (*R*)-**2**: 67.53, (*S*)-**2**: 73.73. In the case of (±)-**3**, the eluent ratio was *n*-hexane/*i*Pa (90:10), 240 nm; retention times (min) for the $(CH_3CH_2CH_2CO)_2O$-derivatised form of (*R*)-**3**: 23.71, (*S*)-**3**: 26.89 and for (*R*)-**4**: 28.43 and (*S*)-**4**: 32.43.

4.2. Syntheses of (±)-1 and (±)-3

Racemic **1** was synthesized from 3,4-dimethoxyphenylethylamine (18.12 g, 99.98 mmol) in three steps according to a known literature method.[10] Pure (±)-**1** was obtained as a white solid (7.40 g, 36% yield, mp: 48 °C, lit.:[9] 48–49 °C).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.41–1.51 (d, *J* = 6.8 Hz, 3H, CH-C*H*$_3$); 1.60–1.75 (br s, 1H, N*H*); 2.62–2.72, 2.77–2.87 (2 m, 2 × 1H, NH-CH$_2$-C*H*$_2$); 2.96–3.08, 3.23–3.33 (2 m, 2 × 1H, NH-C*H*$_2$-CH$_2$); 3.81–3.93 (d, *J* = 1.8 Hz, 6H, 2 × CH-O-C*H*$_3$); 4.04–4.12 (q, *J* = 6.4 Hz, 1H, NH-C*H*-CH$_3$) 6.60 (s, 1H, Ar); 6.66 (s, 1H, Ar). Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54, H 8.27, N 6.76. Found: C, 69.52, H 8.20, N 6.81.

Racemic **3** was synthesized from tryptamine (600 mg, 3.75 mmol) in a microwave reactor.[13] (±)-**3** was isolated in good yield (560 mg, 80% yield, mp: 177–179 °C, lit.:[26] 177–178 °C) as a pale yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.41–1.53 (d, *J* = 3.3 Hz, 3H, CH-C*H*$_3$); 1.54–1.67 (br s, 1H, CH-N*H*); 2.67–2.83 (m, 2H, NH-CH$_2$-C*H*$_2$); 2.99–3.11, 3.31–3.43 (2 m, 2 × 1H, NH-C*H*$_2$-CH$_2$), 4.14–4.24 (m, 1H, NH-C*H*-CH$_3$), 7.06–7.18 (m, 2H, Ar); 7.28–7.35 (d, *J* = 8 Hz, 1H, Ar) 7.45–7.51 (d, *J* = 8 Hz, 1H, Ar); 7.68–7.85 (br s, 1H, Ar-N*H*). Anal. Calcd for $C_{12}H_{14}N_2$: C, 77.38, H 7.58N 15.04. Found: C, 77.33, H 7.62, N 15. 09.

4.3. Small-scale enzymatic reactions

Preliminary small-scale experiments were carried out in both batch and continuous-flow systems. In batch, racemic (±)-**1** or (±)-**3** (0.025 mmol) was dissolved in an organic solvent (1 mL). 4 equiv. acyl donor, 30 mg enzyme and 1 μL Et_3N used as an additive in the case of (±)-**3** were added and the reaction mixtures were shaken in an incubator shaker at 40–60 °C. During continuous-flow investigations, (±)-**1** (0.025 mmol) was dissolved in *t*-BuOMe (1 mL) and phenyl allyl carbonate (4 equiv.) was added. The solution was pumped through the compressed (1–60 bar) and heated (50–80 °C) CAL-B-filled cartridge (244 mg) in *H*-Cube in 'no H_2 mode' with 0.1 mL min^{-1} flow rate. In the case of the AY-catalysed reaction (250 mg), the solution was pumped through the CatCart cartridge (1 bar, 40 °C) with 1.8 mL min^{-1} flow rate.

4.4. Preparative-scale resolution of (±)-1

Racemic **1** (100 mg, 0.48 mmol) was dissolved in toluene (30 mL). After adding lipase AY (900 mg, 30 mg mL^{-1}) and phenyl allyl carbonate (0.31 mL, 1.91 mmol, 4 equiv.), the reaction mixture was shaken in an incubator shaker at 40 °C for 24 h. The reaction was stopped at 50% conversion by filtering off the enzyme and washing it with toluene (2 × 30 mL) followed evaporation of the solvent. The products [(*S*)-**1**, (*R*)-**2**] were separated by column chromatography on silica, with the elution of CH_2Cl_2/MeOH (1:1), affording carbamate (*R*)-**2** {55 mg, 39%, $[\alpha]_D^{25}$ = −104 (*c* 0.3, EtOH) colourless oil, *ee* = 99%}. The free amine (*S*)-**1** was crystallized from hexane/EtOAc (2:1) {40 mg, 40%, $[\alpha]_D^{25}$ = −59 (*c* 0.3, EtOH) lit.:[14] $[\alpha]_D^{25}$ = −58.5 (*c* 0.5, EtOH) white crystalline product, mp: 47 °C, lit.:[14] 47–48 °C, *ee* = 99%}.

(±)-**1** (100 mg, 0.48 mmol) was dissolved in *t*-BuOMe (30 mL). After adding CAL-B (900 mg, 30 mg mL^{-1}) and phenyl allyl carbonate (0.31 mL, 1.91 mmol, 4 equiv.), the reaction was carried out in an incubator shaker at 50 °C in 49 h. Separation of the enantiomers was performed as above, affording carbamate (*R*)-**2** {53 mg, 38%, $[\alpha]_D^{25}$ = −102 (*c* 0.3, EtOH), colourless oil, *ee* = 97%} and free amine

(*S*)-**1** crystallized in hexane/EtOAc (2:1) {41 mg, 41%, $[\alpha]_D^{25}$ = −58.9 (*c* 0.55, EtOH), white crystalline product, mp: 46–47 °C, *ee* = 99%}.

^{1}H NMR (400 MHz, $CDCl_3$) for (*R*)-**2**: δ (ppm): 1.42–1.49 (d, *J* = 6.0 Hz, 3H, CH-C*H*$_3$); 2.58–2.72, 2.79–2.96 (2m, 2 × 1H, NH-CH$_2$-C*H*$_2$); 3.12–3.37, 3.49–3.69 (2 m, 2 × 1H, NH-C*H*$_2$-CH$_2$); 3.80–3.89 (d, *J* = 1.1 Hz, 6H, 2 × CH-O-C*H*$_3$), 4.04–4.31 (m, 1H, NH-C*H*-CH$_3$), 4.54–4.73 (d, *J* = 5.2 Hz, 2H, C*H*$_2$-CH=CH$_2$); 5.17–5.26 (dd, *J* = 1.3 Hz, 10.4 Hz, 1H, CH=C*H*$_2$); 5.28–5.37 (dd, *J* = 1.5 Hz, 17.5 Hz, 1H, CH=C*H*$_2$); 5.91–6.02 (m, 1H, C*H*=CH$_2$); 6.59 (br s, 2H, Ar). Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96, H 7.27, N 4.81. Found: C, 65.92, H 7.21, N 4.86.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*S*)-**1** were similar to those for (±)-**1**.

4.5. Preparative-scale resolution of (±)-3

(±)-**3** (100 mg, 0.54 mmol) dissolved in *t*-BuOMe (15 mL) was mixed with CAL-B (450 mg, 30 mg mL^{-1}), phenyl allyl carbonate (0.35 mL, 2.15 mmol, 4 equiv.) and Et_3N (5 μL). The reaction mixture was shaken for 48 h in an incubator shaker at 50 °C. When a conversion of 50% was observed, the enzyme was filtered off and washed with *t*-BuOMe (2 × 15 mL). Carbamate (*R*)-**4** was separated from free amine (*S*)-**3** by column cromatography with CH_2Cl_2/MeOH (1:1). Carbamate (*R*)-**4** was obtained as a colourless oil {60 mg, 41%, $[\alpha]_D^{25}$ = −99 (c = 0.3, EtOH), *ee* = 97%}, and secondary amine (*S*)-**3** was crystallized in hexane {40 mg, 40%, $[\alpha]_D^{25}$ = −63 (*c* 0.3, EtOH), lit.:[7] $[\alpha]_D^{25}$ = −65.8 (*c* 2.0, EtOH), lit.:[27] $[\alpha]_D^{25}$ = −56.8 (*c* 2.0, EtOH), pale yellow solid, mp: 177–178 °C, lit.:[27] 179–181 °C, *ee* = 98%}.

^{1}H NMR (400 MHz, $CDCl_3$) for (*R*)-**4**: δ (ppm): 1.46–1.53 (d, *J* = 6.8 Hz, 3H, CH-C*H*$_3$); 2.67–2.89 (m, 2 × 1H, NH-CH$_2$-C*H*$_2$); 3.11–3.28, 4.29–4.56 (m, 2 × 1H, NH-C*H*$_2$-CH$_2$); 4.6–4.72 (m, 2H, C*H*$_2$-CH=CH$_2$); 5.17–5.26 (dd, *J* = 1.1 Hz, 10.5 Hz, 1H, CH$_3$-C*H*-NH); 5.27–5.43 (dd, *J* = 0.8 Hz, 17.2 Hz, 1H, CH=C*H*$_2$); 5.91–6.04 (m, 1H, C*H*=CH$_2$); 7.05–7.20 (m, 2H, Ar), 7.28–7.34, 7.41–7.5 (d, J = 8.0 Hz, 2H, Ar); 7.66–7.95 (br s, 1H, Ar-N*H*). Anal. Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09, H 6.71, N 10.36. Found: C, 71.13, H 6.75, N 10.29.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*S*)-**3** were similar to those for (±)-**3**.

4.6. Hydrolysis of (*R*)-2 and (*R*)-4

(*R*)-**4** (60 mg, 0.22 mmol) was dissolved in dry acetonitrile (0.72 ml) and water (12 μL, 0.66 mmol, 3 equiv.) and then iodine (167.6 mg, 0.66 mmol, 3 equiv.) were added to the reaction mixture. After stirring during 72 h at room temperature, it was cooled down to 4 °C and a 20% solution of Na_2SO_3 was added, followed by extraction with CH_2Cl_2 (3 × 5 mL). Product (*R*)-**3** was purified by column chromatography with CH_2Cl_2/MeOH (1:1) and it was crystallized in hexane giving a pale yellow solid with a low yield {10 mg, 24%, $[\alpha]_D^{25}$ = +50, (*c* 0.3, EtOH), lit.:[27] $[\alpha]_D^{25}$ = +55.6 (*c* 2.0, EtOH), mp: 177–178 °C, lit.:[27] 179–181 °C, *ee* = 92%}.

Carbamate (*R*)-**2** (55 mg, 0.19 mmol) was dissolved in THF (2 mL). PPh_3 (10 mg, 0.04 mmol), HCOOH (28 μL, 0.74 mmol) and $Pd_2(dba)_3$·$CHCl_3$ (14.5 mg, 0.014 mmol) were added and the solution was stirred overnight at 40 °C. After evaporation the catalyst was removed on a celite column with MeOH. Product (*R*)-**1** was purified by column chromatography with CH_2Cl_2/MeOH/Et_3N (89:10:1). The amine was dissolved in 5 mL H_2O and saturated $NaHCO_3$ solution was added until pH > 9. The aqueous media was extracted with $CHCl_3$ (2 × 7 mL). After evaporation of the organic media, (*R*)-**1** was crystallized from hexane/EtOAc (2:1) as a white solid {23.4 mg, 60%, $[\alpha]_D^{25}$ = +60, (c 0.3, EtOH), lit.:[14] $[\alpha]_D^{25}$ = +59.2 (*c* 1.0, EtOH), mp: 46 °C, lit.:[14] 47–48 °C, *ee* = 99%}. The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*R*)-**1** were similar to those for (±)-**1**.

In the case of (*R*)-**4** (60 mg, 0.22 mmol), the reaction was completed in 2 h, providing (*R*)-**3** as a pale yellow solid (crystallized from hexane) {34.9 mg, 85%, $[\alpha]_D^{25}$ = +62, (*c* 0.3, EtOH), lit.:[27] $[\alpha]_D^{25}$ = +55.6 (*c* 2.0, EtOH), mp: 178–180 °C, lit.:[27] 179–181 °C, *ee* = 99%}. The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*R*)-**3** were similar to those for (±)-**3**.

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Racemization of Secondary-Amine-Containing Natural Products Using Heterogeneous Metal Catalysts

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Heterogeneously catalyzed racemization reactions of the secondary amines (*S*)-1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (salsolidine) and (*S*)-1-methyl-1,2,3,4-tetrahydro-ß-carboline were investigated using Pd, Pt, and Ir on carbon or Al_2O_3 supports. A comparison of kinetics and deactivation on selected platinum and iridium catalysts was performed. Furthermore, the relative stabilities of (*S*)-salsolidine and the corresponding imine on Pt(111) and Ir(111) surfaces were analyzed by density functional theory calculations. The racemization was faster on platinum and took place without detectable byproduct formation. Iridium, however, proved reusable and, in contrast to the platinum catalyst, deactivation at low catalyst concentration was not observed. Also, the physisorption of (*S*)-salsolidine and the imine was stronger on platinum than on iridium.

Introduction

Enantiomerically pure amines are in great demand owing to their importance as building blocks for many pharmaceutically active compounds.[1,2] Among the commonly employed preparative pathways, which include diastereomeric crystallization,[2] enantioselective reduction of imines,[3] and nucleophilic addition,[4] kinetic resolution (KR) using biocatalysts has become a favored separation route.[5] Conventional KR, however, is limited by the maximum theoretical yield of 50% of the desired pure enantiomer. In situ catalytic racemization of the slower reacting enantiomer of the KR process is a potential solution to the yield problem, turning the reaction system into a dynamic kinetic resolution (DKR) by which 100% yield of the single product enantiomer can theoretically be obtained.

In contrast to the racemization of *sec*-alcohols, the development of efficient approaches for DKR and racemization of amines has remained challenging.[6–21] Mechanistically, the racemization of amines typically involves dehydrogenation followed by re-addition of hydrogen to the initially formed imine. Although the oxidation of an alcohol to the corresponding ketone is often a low-barrier process, the conversion of an amine to the corresponding imine requires much harsher reaction conditions and temperatures, which may be detrimental to the enzyme catalyst used for the KR reaction.[22] Amines may also bind strongly to metal-based catalysts.[22]

Earlier, Murahashi and co-workers have described the racemization of amines by palladium black,[6] which also was used by Reetz and co-workers for the DKR of racemic 1-phenylethylamine in combination with *Candida antarctica* lipase B (CAL-B).[7] Similar Pd catalysts were successfully used for primary amines by Jacobs et al. by changing the support to alkaline earth salts[8a,b] and later to modified silica.[8c] Bäckvall et al. described an efficient racemization procedure for primary amines using the homogeneous Shvo catalyst.[9] The *para*-fluoro and *para*-methoxy substituted analogues of the Shvo catalyst have also been used in the DKR of primary amines with the best results obtained by combining the *para*-methoxy substituted catalyst with CAL-B.[10] An efficient immobilized nanoparticle Pd catalyst has been developed by Kim, Park, and co-workers,[11] used also by Bäckvall and co-workers in combination with CAL-A immobilized on functionalized mesocellular foam (MCF) for DKR of β-amino esters.[12] Bäckvall and co-workers have also developed a highly effective racemization catalyst for primary amines consisting of Pd nanoparticles on siliceous amino-functionalized MCF.[13] Also, co-immobilization of the

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catalyst with CAL-B on the same cavities of the MCF has been described and used successfully for DKR of 1-phenylethylamine.[14] Li and co-workers have demonstrated that the addition of alkaline salts, especially K_2CO_3, improve the selectivity of the Pd/MCF catalyst.[15] Raney nickel has been shown to racemize aliphatic primary amines with higher activity and stability than cobalt, although requiring large catalyst quantities and long reaction times,[16a] which could however be circumvented by use of nickel nanoparticles.[16b] Also the combination of Raney Ni and CAL-B for DKR of mexiletine has been described.[17] Faber and co-workers have investigated the racemization of primary amines based on a reversible deamination/amination sequence by use of ω-transaminases in the presence of symmetric ketones.[18] Highly efficient DKR of 1-methyl-1,2,3,4-tetrahydroisoquinoline, a secondary amine, using (pentamethylcyclopentadienyl)iridium (III) iodide dimer as a homogeneous racemization catalyst in combination with lipase *Candida rugosa* has also been reported.[19] Platinum-encapsulated zeolitic microcapsular catalyst has been used in the DKR of 1-phenylethylamine.[20] Recently, a palladium catalyst Glt-Pd-SiO_2@APTES was used as a support for CAL-B immobilization and this hybrid biocatalyst successfully catalyzed the DKR of α-methylbenzylamine.[21]

In the literature, most of the examples on DKRs of amines involve racemizations and resolutions only of primary amines. Furthermore, for developing industrially viable one-pot DKR procedures, heterogeneous or immobilized catalyst systems for both the racemization and enzymatic resolution would be preferred.[23,24] To the best of our knowledge, the only heterogeneously catalyzed examples reported in the literature to date for racemization of secondary amines are those by Murahashi[6] and De Vos and co-workers.[8c] In the first case, the reaction temperatures far exceeded the conditions typically compatible with most standard enzymes, and in the latter the Raney catalysts employed displayed only limited reactivity towards secondary amines. Examples on homogeneously catalyzed racemizations or DKRs of secondary amines are likewise scarce.[19]

Here, we report the efficient racemization of two secondary amine model compounds, (*S*)-1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1**) and (*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline (**2**) (Figure 1) using heterogeneous metal catalysts based on palladium, iridium, and platinum on different supports. In addition, kinetic analysis of the racemization reactions supported by DFT calculations is presented.

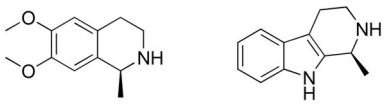
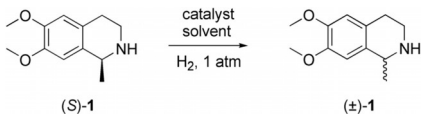


Figure 1. The structures of (*S*)-**1** and (*S*)-**2**.

Results and Discussion

For developing mild and efficient racemization conditions, (*S*)-**1** was first subjected to test reaction screening (Scheme 1).



Scheme 1. Racemization of (*S*)-**1**.

For finding the suitable catalyst and for determining the initial reaction rate, the first set of experiments was performed by using high catalyst loadings (20 mol%). As a starting point, selected commercially available hydrogenation catalysts were investigated. In addition to Pd black, different heterogeneous palladium, platinum, iridium, and nickel catalysts supported on mesoporous materials or microporous active carbon were screened. In addition, some heterogeneous iridium catalysts prepared by the colloidal route were investigated. Selected racemization curves are illustrated in Figure 2. Values of

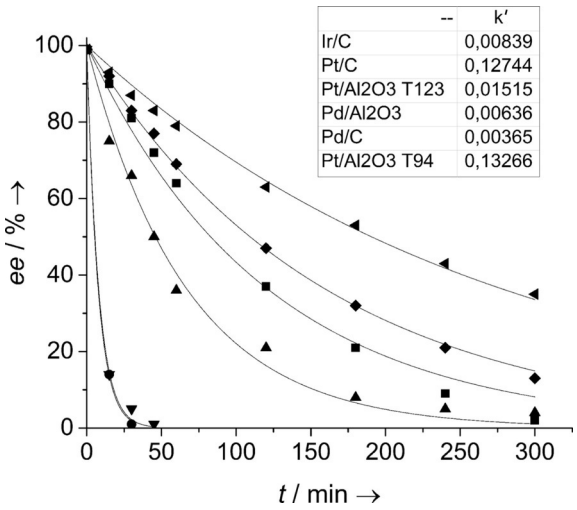


Figure 2. Reaction rates of racemization of (*S*)-**1** (10 mg) using 20 mol% of different heterogeneous catalysts in toluene (40 mL) at 80 °C, under H_2 flow and at 1 bar (■ Ir/C, ● Pt/Al_2O_3 (T94), ▲ Pt/Al_2O_3 (T123), ▼ Pt/C, ◆ Pd/Al_2O_3, ◀ Pd/C).

lumped kinetic constants are also included, calculated using the Equation (1):

$$ee = S_o e^{-2kc_{cat}t} = S_o e^{-k't} \quad (1)$$

where *ee* is the enantiomeric excess, *k* is the racemization constant, S_0 is the initial concentration of *S*, equal to 100%, c_{cat} is the molar concentration, and *k'* is the lumped constant.

With the colloidally prepared iridium catalysts, nickel on alumina, and Pd black, racemizations were not observed even at 90 °C. In contrast to initial expectations, however, conventional, commercially available Ir and Pt catalysts showed higher racemization activities than Pd. Both Pt/Al_2O_3 (T94) and Pt/C racemized (*S*)-**1** in 30 minutes. Of these two, the Pt/Al_2O_3 (T94) catalyst was selected for further studies because of the better reproducibility and easier pelletization of the support material

compared to active carbon. Although Ir/C proved slower than the Pt catalysts, showing complete racemization after 300 min, it was also included in further kinetic studies. For verification of the experimental setup, racemization test reactions on (*S*)-**1** and (*S*)-**2** were also performed using the commercially available, homogeneous iridium catalyst $[IrCp^*I_2]_2$ reported earlier by Page and co-workers for racemization of secondary amines.[19] For both model compounds, racemization under homogeneous conditions took place in 1 hour, further motivating the studies on the heterogeneous Ir catalyst, pursued here in more detail.

Next, different loadings of the selected heterogeneous catalysts were investigated. If the amount of the Pt/Al_2O_3 (T94) was reduced from 20 mol% to 5 mol%, a decrease in the reaction rate was observed (Figure 3). Nevertheless, racemic (±)-**1** was

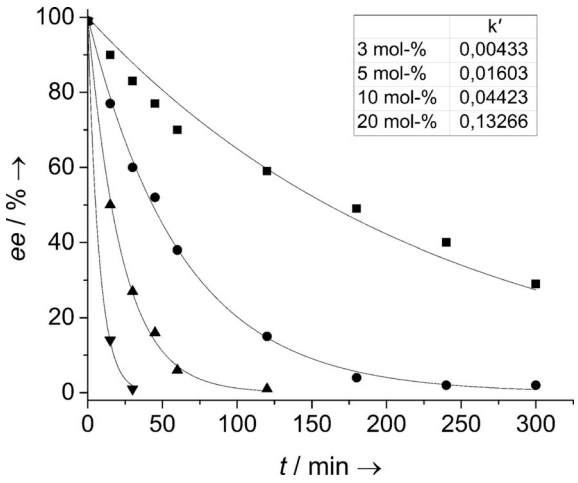


Figure 3. Racemization of (*S*)-**1** (10 mg) in the presence of Pt/Al_2O_3 (T94) in toluene (40 mL), at 80 °C under H_2 flow at 1 bar (■ 3 mol%, ● 5 mol%, ▲ 10 mol%, ▼ 20 mol%).

still formed in a few hours. A further reduction of the Pt loading to 3 mol% resulted in considerably longer reaction time, suggesting that either the amine or imine form of the substrate could block the catalyst and thereby influence the racemization rate at lower catalyst concentrations.

The dependence of the enantiomeric excess on the catalyst concentration was incorporated in Equation (1) explicitly, which means that the racemization rate constants (*k*) should be independent of the amount of the catalyst. Interestingly, analysis of the racemization rate constants for experimental data presented in Figure 3 reveals that this was not the case, and a close to first-order dependence on the catalyst concentration was observed (Figure 4). Such observations are consistent with significant catalyst deactivation at low catalyst loadings.

Similar racemization test reactions were also performed using different concentrations of the Ir/C catalyst (10–20 mol%). Although here the racemization rate again consistently decreased in parallel with a decrease in the Ir loading, an inhibitory effect similar to that of Pt was not observed at the concentrations studied (Figure 5).

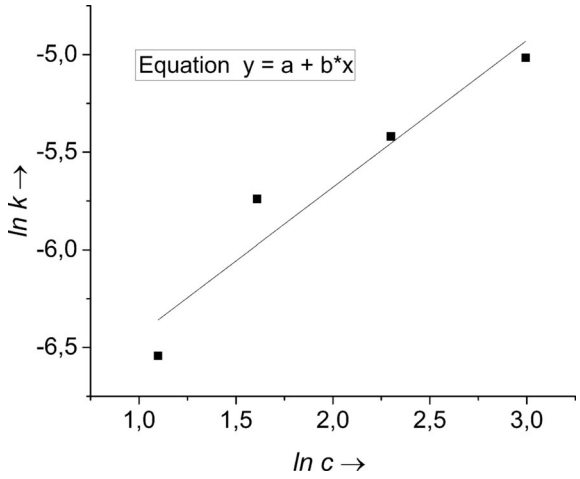


Figure 4. Dependence of the racemization rate constants (*k*) on the catalyst concentration.

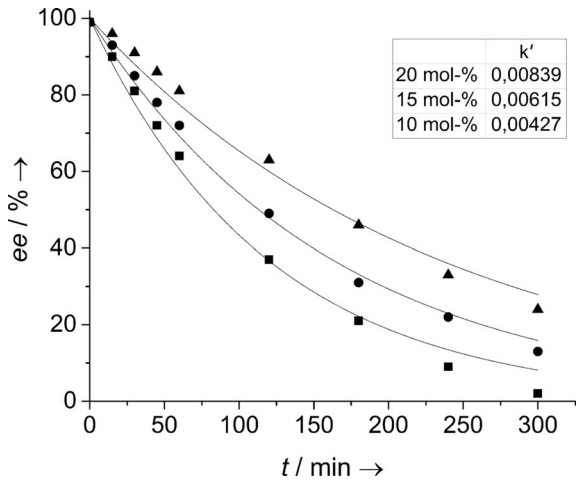


Figure 5. Racemization of (*S*)-**1** (10 mg) in the presence of Ir/C in toluene (40 mL) at 80 °C under H_2 flow at 1 bar (▲ 10 mol%, ● 15 mol%, ■ 20 mol%).

For a comparison of the activation energies, additional experiments at different temperatures using 10 mol% Pt/Al_2O_3 (T94) (Figure 6) and 15 mol% Ir/C (Figure 7) were performed. As expected, faster racemizations were recorded with both catalysts at higher temperatures. The activation energy calculated from the values of constants (Figure 6) equals 27 kJ mol^{-1} for Pt/Al_2O_3 (T94). A much higher value of the activation energy (65 kJ mol^{-1}) was observed for Ir/C.

Notably, although the racemization test reactions of (*S*)-**1** with Pt/Al_2O_3 (T94) and Ir/C were performed by using different substrate batches, each figure is internally fully coherent. Under similar reaction conditions, slightly different *ee* values at the same reaction times, although within a 10% margin, were obtained for the different substrate batches upon sample analysis.

The racemization of (*S*)-**2** (Scheme 2) was first studied using Pt/Al_2O_3 (T94) (Figure 8). Complete racemization was observed in 2 h with 10 mol% catalyst loading. Consequently, the amount of Pt catalyst loading was reduced to 2 mol%. Similar-

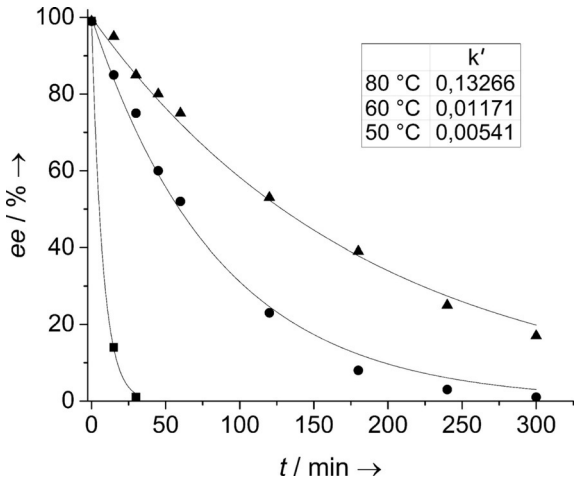


Figure 6. Racemization of (*S*)-**1** (10 mg) in the presence of Pt/Al_2O_3 (T94) (10 mol%) at different temperatures in toluene (40 mL) under H_2 flow at 1 bar (▲ 50 °C, ● 60 °C, ■ 80 °C).

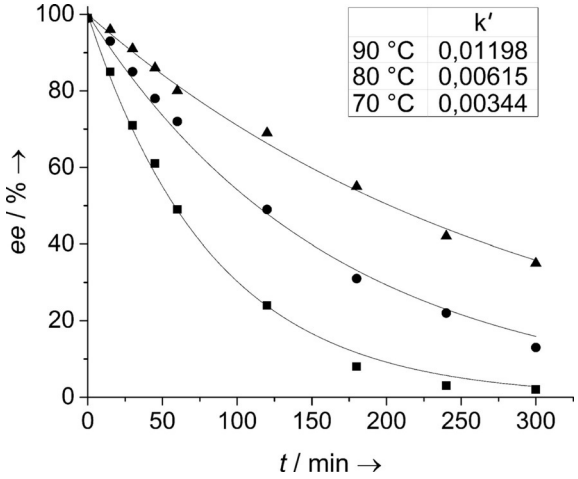
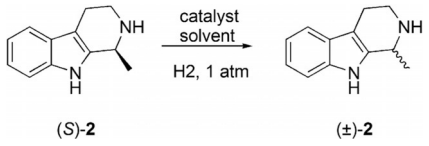


Figure 7. Racemization of (*S*)-**1** (10 mg) in the presence of Ir/C (15 mol-%) at different temperatures in toluene (40 mL) under H_2 flow at 1 bar (▲ 70 °C, ● 80 °C, ■ 90 °C).



Scheme 2. Racemization of (*S*)-**2**.

ly to the case of (*S*)-**1**, the activity of the catalyst strongly decreased at the lower catalyst concentration, presumably because of the amine or imine inhibitory effect, resulting in only 47% *ee* after 5 h. If 20 mol% Ir/C were used, 14% *ee* was observed in 5 h. In addition, *tert*-butyl methyl ether was tested as a solvent instead of toluene, resulting however in a slight decrease of *ee* from 98 to 91% in 5 h if 10 mol% Pt/Al_2O_3 (T94) was used as the catalyst.

Finally, for the isolation of the racemic products **1** and **2**, racemization experiments on a 50 mg scale were performed by

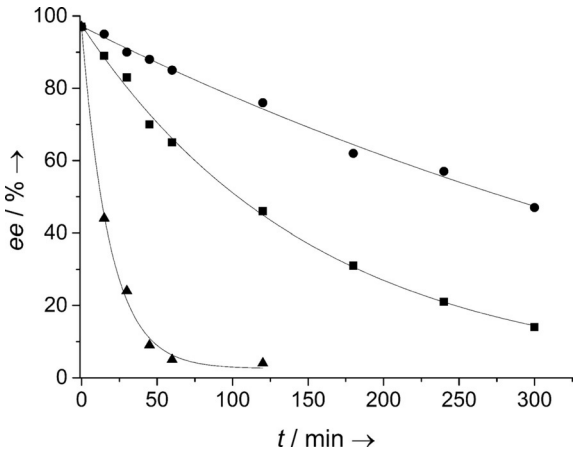


Figure 8. Racemization of (*S*)-**2** (10 mg) catalyzed by different heterogeneous catalysts in toluene (40 mL) at 80 °C under H_2 flow at 1 bar (■ Ir/C 20 mol%, ● Pt/Al_2O_3 T94 2 mol%, ▲ Pt/Al_2O_3 T94 10 mol%).

using 10 mol% Pt/Al_2O_3 (T94) in toluene at 80 °C under H_2 flow at 1 bar. Racemic (±)-**1** was detected in 2 h and was isolated in excellent yield (94%), whereas (±)-**2** was obtained in 1 h in 90% yield after filtration of the catalyst followed by solvent evaporation and NMR analysis.

Additional experiments were also performed to study the recyclability of the racemization catalysts (Figure 9). The catalysts were first separated from the reaction mixture by filtration and washing, followed by drying in air overnight before reuse. The racemization rate decreased slightly during the 2nd cycle using Pt/Al_2O_3 (T94), whereas no such effect was observed for Ir/C.

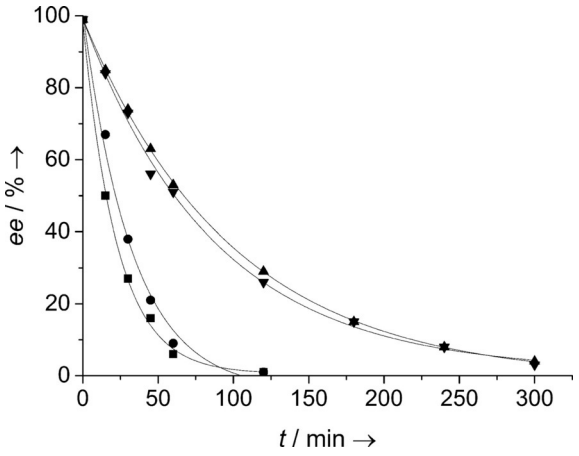


Figure 9. Reuse of Ir/C and Pt/Al_2O_3 (T94) catalysts for racemization of (*S*)-**1** at 80 °C under H_2 flow (Ir/C 1st run ▲; 2nd run ▼. Pt/Al_2O_3 (T94) 1st run ■; 2nd run ●).

A significant culprit for deactivation of the platinum catalyst might be an imine intermediate formed in the reaction. First the formation of imine intermediate was verified by allowing (±)-**1** to interact with the catalysts at elevated temperature in the absence of hydrogen. If Ir/C was used as a catalyst, all (±)-**1** was rapidly converted into imine, whereas with Pt/Al_2O_3

(T94) the rate of dehydrogenation was considerably slower. If separately prepared imine[25] was added to the Pt-catalyzed racemization reaction of (*S*)-**1**, a significant decrease in the reaction rate was observed (Figure 10).

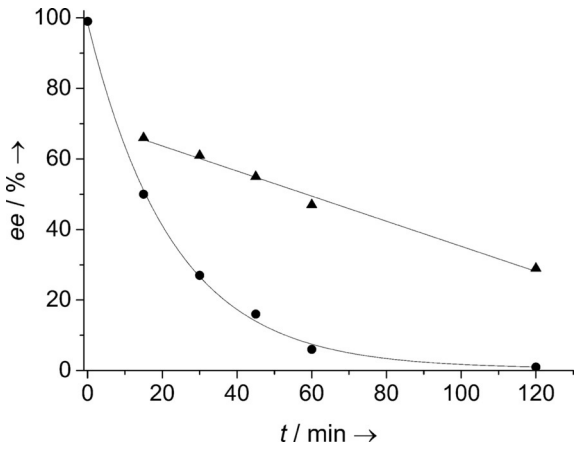


Figure 10. Inhibitory effect of imine on the racemization of (*S*)-**1** on Pt/Al_2O_3 (● Pt/Al_2O_3 T94 10 mol%, ▲ Pt/Al_2O_3 T94 10 mol% with imine presaturation).

The possible leaching of active species of metal from the heterogeneous catalyst was studied and ruled out. After filtration of the catalyst from the reaction mixture (*ee* = 20% at Pt/Al_2O_3 (T94) and *ee* = 57% at Ir/C after 45 min), the *ee* did not decrease further and the racemization reaction was stopped.

Next, density functional theory calculations were performed to analyze the relative stability of (*S*)-**1** species and its racemization product on Pt(111) and Ir(111) surfaces, which mimic the most stable facets of the Pt and Ir nanoparticles. As a saturated compound, (*S*)-**1** does not form a chemical bond with the metal surfaces. This is seen as a planar adsorption geometry and the long adsorbate–surface distance, which indicates that the (*S*)-**1**-metal interaction is dominated by the van der Waals interactions responsible for physisorption. Despite imine being an unsaturated species, it also adopts a planar adsorption geometry on both surfaces and the minimum adsorbate–surface distance is always at least 3.6 Å. This is probably the result of the internal rigidity of the molecule and the steric hindrance between the imine and the surface. On Pt(111), an adsorption geometry deviating from the planar structure was however located. In that case, the methoxy groups are tilted away from the surface plane, and the C atom in between the N atom and the CH_3 group is closest to the surface with the C–Pt distance of 2.36 Å. This geometry is, however, energetically equally favorable to the planar one. The thermodynamic potential energy surface for (*S*)-**1** and imine on Pt(111) and Ir(111) surfaces is displayed in Figure 11. The plot shows that on both surfaces (*S*)-**1** adsorption is strongly exothermic but on Pt(111) the physisorption energy is approximately 53 kJ mol^{-1} more exothermic than on Ir(111). Also, the imine prefers Pt(111) to Ir(111) with the energy difference of 58 kJ mol^{-1}. More exothermic physisorption on Pt(111) than on Ir(111) suggests that Pt is more active than Ir, assuming that the reaction forms a Brønsted–Evans–Polanyi correlation.[26]

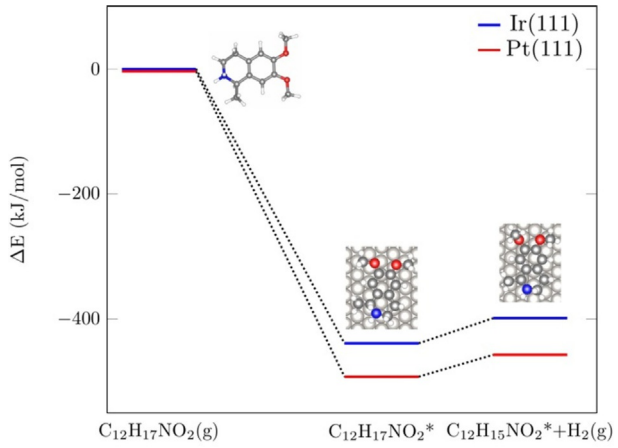


Figure 11. Adsorption energies of (*S*)-**1** and the corresponding imine on Ir(111) and Pt(111) surfaces. Inset figures show (*S*)-**1** in gas phase and on both (*S*)-**1** and its imine on the Pt(111) surface. Pt atoms are light gray, C atoms are darker gray, N and O atoms are shown in blue and red, respectively, and H atoms are white.

This interpretation is in line with the experiments, which show that racemization is faster on Pt than on Ir.

In successful DKR applications reported in the literature for amines, the racemization catalyst and the enzyme are typically supported on the same carrier material. Here, for initial insights, a test reaction was performed by using a mechanical mixture of Ir/C and CAL-B on acrylic resin for DKR of (±)-**1** at 80 °C under H_2 bubbling, albeit without significant activity. It is evident that after optimization of the heterogeneously catalyzed racemization reaction, as performed here, comprehensive studies and similar optimization of the kinetic resolution and the catalyst combination are needed before merging the two reactions into a successful DKR. Such extensive studies are, however, beyond the scope of the present investigation.

Conclusions

Pd, Pt, and Ir immobilized on carbon or Al_2O_3 function as heterogeneous catalysts for the racemization of model secondary amines studied in this work. Both Pt/Al_2O_3 (T94) and Ir/C proved to be efficient racemization catalysts without detectable formation of byproducts. Pt/Al_2O_3 (T94) showed the highest catalytic activity (at par with Pt/C) but also suffered from deactivation by the intermediate imine at low catalyst loadings and on recycling of the catalyst. In contrast, Ir/C displayed lower racemization activity but without the inhibitory effects observed with Pt/Al_2O_3 (T94). DFT analyses of both catalysts were performed and were in line with the experimental and kinetic observations. The remarkably different catalyst supports in Pt/Al_2O_3 (T94) and Ir/C are likely to contribute to the observed differences.

The racemization rates of both Pt/Al_2O_3 (T94) and Ir/C at reaction temperatures applicable for supported enzymes used for kinetic resolutions are fully sufficient with regard to possible future dynamic kinetic resolution applications of these systems. In comparison to previously described heterogeneous

methods for racemization of primary amines, the observed racemization kinetics at the temperatures investigated enable similarly efficient methods to be developed with comparable reaction times and temperatures. In future work, we aim to further investigate solid-supported iridium as a racemization catalyst and to expand this work towards fully heterogeneously catalyzed, metalloenzymatic dynamic kinetic resolutions of secondary amines.

Experimental Section

Synthesis of the starting material

The enantiomerically enriched starting compounds (*S*)-**1** (*ee* = 99%) and (*S*)-**2** (*ee* = 98%) were prepared by CAL-B catalysed N-acylation ($E > 200$) of the corresponding racemic amines **1** and **2**.[27]

Reaction setup

The racemization experiments were conducted in a four-necked 100 mL round-bottom flask equipped with a gas inlet (7 μm), rubber septa for sampling, and a condenser connected to an oil bubbler for gas outlet. Pt/Al_2O_3 (type 123; 5 wt% Pt) and Pt/Al_2O_3 (type 94; 5 wt% Pt) were purchased from Johnson Matthey. Pt/C (5 wt%) and Pd/C (5 wt%) were obtained from Degussa, and Ir/C (5 wt%) was prepared according to a previously published method.[28] Particle sizes and metal dispersions of the alumina[29] and active carbon[30] supported catalysts have been reported earlier. Ni/Al_2O_3 (HTC-500) catalyst from Crossfield contains ≈21% Ni and has the metal particle size of 8.2 nm. Palladium black and the CAL-B enzyme supported on acrylic resin were obtained from Sigma Aldrich. Iridium catalysts supported on mesoporous MCM-41 (2 wt%), zeolite beta-25 (1 wt%), and γ-alumina (1 wt%) were prepared by impregnating the corresponding supports with aqueous iridium chloride solution in a Büchi rotavapor, followed by drying, calcination at 500 °C, and reduction under hydrogen. The specific metal surface of 5 wt% Ir/C was measured by using CO chemisorption.[28] Data on all of the catalysts is collected in the Supporting Information. All catalysts were used as wet basis. The reactions were performed using hydrogen gas (Linde Gas-AGA, 99.999%) under atmospheric pressure.

In a typical small-scale racemization experiment, the reaction vessel was first evacuated and subsequently purged twice with argon. The reaction was started by introducing the solvent (40 mL) into the flask containing the starting material (10 mg) followed by the catalyst (3–20 mol%) and immediate bubbling with H_2. The reaction mixtures were stirred at different temperatures (50–80 °C) for 5 h. For isolation of the products, larger-scale racemization (50 mg substrate) was performed by use of Pt/Al_2O_3 (T94) in toluene (40 mL) at 80 °C under H_2 flow at 1 bar. After completed reaction, the catalyst was filtered off, washed, and the liquid phase was then evaporated providing racemic **1** or **2** as white crystals. For recycling of the catalysts, racemization of (*S*)-**1** was repeated by reuse of 10 mol% Pt/Al_2O_3 (T94) or 20 mol% Ir/C at 80 °C in toluene (40 mL) under H_2 flow at 1 bar. Some of the reactions were performed in a Schlenk tube, which was first evacuated and then purged twice with argon. The racemization was started by addition of the substrate (10 mg) followed by the catalyst (10–20 mol%) and solvent (4 mL) at different temperatures (80–90 °C) under argon and without H_2 flow. For investigation of the imine formation with (±)-**1**, the reactions were set up in a Schlenk tube using 20 mol% catalyst at 80 °C in the absence of H_2 flow. The inhibitory effect was further investigated under H_2. In these experiments, the imine (10 mg) and 10 mol% Pt/Al_2O_3 (T94) were stirred for 10 min in toluene (40 mL) at 80 °C. The reaction was then started by addition of (*S*)-**1** (10 mg) immediately followed by bubbling with H_2. For excluding the possibility of metal leaching, (*S*)-**1** was subjected to racemization for 45 min under 5 mol% Pt/Al_2O_3 (T94) or 15 mol% Ir/C in toluene (40 mL) under H_2 flow at 80 °C. The reactions were thereafter quenched by filtering off the catalyst and returning the liquid phase back under H_2. Afterwards, the *ee* was followed for additional 1 hour.

Analysis of compounds 1 and 2

Samples were periodically taken through rubber septa during 5 h. The *ee* values were determined by HPLC [Chiralpak IA column (4.6 mm × 250 mm), eluent: *n*-hexane/*i*Pa (80:20), flow rate: 0.5 mL min^{-1}, 220 nm]; retention times (min) for Ac_2O derivatized form of (*S*)-**1**: 23.83, of (*R*)-**1**: 30.14. For compound **2**, the eluent ratio was *n*-hexane/*i*Pa (90:10), flow rate: 0.5 mL min^{-1}, 220 nm, retention times (min) for the Ac_2O derivatized form of (*S*)-**2**: 28.81, of (*R*)-**2**: 33.27. The NMR spectra of the isolated products were recorded on a Bruker Avance 500 MHz NMR spectrometer equipped with a BBi-5 mm-Zgrad-ATM probe at 25 °C operating at 500.13 MHz for 1 H.

Computational methods

DFT calculations were performed in the real-space grid implementation of the PAW formalism employing the GPAW code.[31] Kohn–Sham equations were solved self-consistently using the BEEF-vdW functional[32] to describe exchange and correlation effects. The grid spacing of 0.2 Å was used throughout the study and the k-point sampling of (2 × 2 × 1) was employed for the surface systems. Pt(111) and Ir(111) surfaces were modelled with a three-monolayers thick slab, a 4 × 4 surface cell, and the optimized lattice parameters of 3.981 Å and 3.890 Å, respectively. In all calculations, at least a 5 Å thick vacuum region separates the atoms from the nonperiodic unit cell edges. The atomic positions were relaxed until residual forces were below 0.05 eV $Å^{-1}$, and the bottom metal layer was frozen to the bulk positions.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: amines · racemization · iridium · platinum · supported catalysts

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Candida antarctica lipase B catalysed kinetic resolution of 1,2,3,4-tetrahydro-ß-carbolines: Substrate specificity

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ABSTRACT

In the frame of substrate specificity, CAL-B-catalysed asymmetric *N*-alkoxycarbonylations of 1-substituted tetrahydro-ß-carbolines (Me, Et, Pr, iPr) have been studied. High enantioselectivities (>200) were observed, when alkoxycarbonylation of racemic compounds (±)-**1,3,5,7** were performed in DIPE in the presence of phenyl allyl carbonate and Et_3N at 60 °C using ultrasound shaking method. The reaction time increased considerably with increasing substituent size on C1; however, the isopropyl-substituted compound proved to be too bulky for the optimum activity of CAL-B. The (*R*)-carbamate enantiomers were hydrolysed using $Pd_2(dba)_3.CHCl_3$ and the enantiomers of the free amines were obtained with excellent *ee* (>99%).

1. Introduction

Alkaloids containing the tetrahydro-ß-carboline core represent great interest because of their importance as valuable bioactive compounds, including the antihypertensive agents reserpine [1,2] and ajmalicine [3] or yohimbine used in the therapy of erectile dysfunction [2,3]. 1-Methyl-1,2,3,4-tetrahydro-ß-carboline (eleagnine) isolated from *Eleagnus angustifolia* has inverse agonist effect on $GABA_A$ receptors [4,5]. The 1-propyl-substituted substrate (komaroidine) was found in *Nitraria species* [6]. Strictosidine bearing the (*S*)-1-ethyl-1,2,3,4-tetrahydro-ß-carboline moiety shows antifungal activity [7]. Furthermore, it is a key intermediate in the synthesis of other alkaloids, such as ajmalicine or cathenamine [8]. (*S*)-Quinolactacin-B exhibits activity against the tumor necrosis factor and it is synthetized from protected (*S*)-1-isopropyl-1,2,3,4-tetrahydro-ß-carboline by Winterfeldt reaction [9].

Kinetic resolution is a favoured route [10] to produce enantiomerically pure cyclic secondary amines [11–14]. Due to the structural diversity of chiral compounds, in the frame of substrate specificity, numerous enzymes were recently used for enantioseparation to determine their activity and selectivity in the kinetic resolution of substrates having the same skeleton with different substituents. *Candida antarctica* lipase A (CAL-A) prefers sterically hindered substrates [15], which was proved by Gotor and his coworkers in the kinetic resolution of indolines [16]. *Candida antarctica* lipase B (CAL-B) has a preference for smaller substituents [17]. Consequently, *E* values decreasing from 200 to 1 were detected in the CAL-B-catalysed *O*- alkoxycarbonylation of *N*-Boc-protected tetrahydroisoquinolines, when the distance between the active hydroxy group and the stereogenic centre increased [18]. CAL-B-catalysed ring cleavage of ß-lactams were investigated and faster resolution was found, when *N*-hydroxymethyl-substituted ß-lactam was used instead of their corresponding unsubstituted counterparts [19]. Müller and his coworkers carried out the biocatalytic reduction of 1-methyl- and 1-ethyl-3,4-dihydroisoquinolines and their ß-carboline analogues using imine reductases, which are the preferred catalysts for methyl-substituted compounds [20]. Zheng et al. investigated imine reductases using more bulky 1-substituted dihydroisoquinolines [21]. Imine reductase from *S. nassauensis* was found to be promising in the asymmetric reduction of sterically hindered cyclic amines. The asymmetric *N*-alkoxycarbonylation of 2-substituted piperidines with achiral *N*-heterocyclic carbene and chiral hydroxamic acid cocatalyst has been described [22]. Good selectivities were observed in every case. More bulky 2-substituted starting materials, in turn, afforded decreasing conversions and enantiomeric ratios. Turner et al. have investigated the deracemization of 1-substituted tetrahydro-ß-carbolines using monoamine oxidase enzymes [23]. High enantiomeric excess (*ee* = 99%) was

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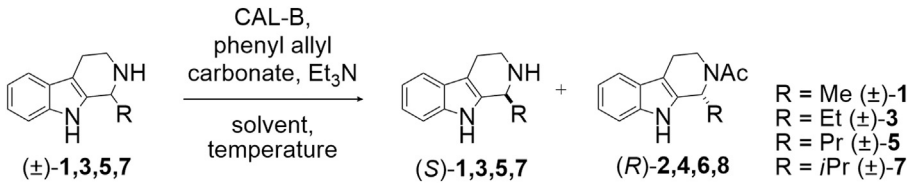


Scheme 1. CAL-B-catalysed asymmetric *N*-alkoxycarbonylation of 1-alkyl-substituted tetrahydro-ß-carbolines.

observed for (*R*)-1-methyl-1,2,3,4-tetrahydro-ß-carboline and moderate *ee* (25–36%) for the ethyl-substituted compound, while a switch in enantioselectivity was detected with ß-carbolines bearing a longer alkyl chain and aryl substituent. Excellent *ee* (99%) for (*S*)-1-propyl-1,2,3,4-tetrahydro-ß-carboline was detected, although lower *ee* values were observed for sterically more hindered substrates (e.g. *tert*-butyl, phenyl substituents) with the use of MAO-N D9.

In the frame of substrate specificity, our aim in the present work was to investigate how substituents (Me, Et, Pr, *i*Pr) at position 1 affect the *E* and reaction rate in the enzymatic *N*-alkoxycarbonylation of (±)-**1**, (±)-**3**, (±)-**5** and (±)-**7** (Scheme 1).

2. Results and discussion

2.1. Syntheses of 1-methyl-1,2,3,4-tetrahydro-ß-carboline (±)-1, 1-ethyl-1,2,3,4-tetrahydro-ß-carboline (±)-3, 1-propyl-1,2,3,4-tetrahydro-ß-carboline (±)-5, and 1-isopropyl-1,2,3,4-tetrahydro-ß-carboline (±)-7

Racemic 1-substituted tetrahydro-ß-carbolines [(±)-**1,3,5** and **7**] were prepared through Pictet–Spengler reaction under microwave irradiation according to a known literature method [24].

2.2. Kinetic resolution of (±)-1,3,5 and 7

On the basis of our earlier results on the CAL-B-catalysed kinetic resolution of 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline and its analogue 1-methyl-1,2,3,4-tetrahydro-ß-carboline [25], important from both biological and chemical aspects, *N*-alkoxycarbonylation of (±)-**1,3,5** and **7** were performed under the same reaction conditions: with CAL-B in the presence of phenyl allyl carbonate and Et_3N at 50 °C in batch conditions. A conversion of 50% was reached in 7 days for (±)-**1** with excellent *E* (>200). The kinetic resolution of (±)-**3,5** and **7** showed also excellent *E* (>200), but with different reaction rates. Furthermore, the reactions stopped at low conversions (maximal conversions = 27% for (±)-**3**, 29% for (±)-**5**, and 10% for (±)-**7** after 7 days).

In an attempt to avoid the stoppage of the reactions, a set of preliminary experiments were performed for the alkoxycarbonylation of (±)-**3** (Table 1). When a higher amount of Et_3N was used, the reaction rate increased significantly (entries 1 and 2). With the increase of temperature, a further increase in reaction rate was observed with conversions of 49% at 60 °C and 41% at 70 °C, while maintaining excellent *E* (>200) values (entries 3 and 4). Finally, the solvent (*t*-BuOMe) was replaced by DIPE resulting in a slightly slower but highly enantioselective (*E* > 200) reaction (entry 5).

To further optimize the reaction rate, the reaction mixture was subjected to ultrasound shaking, since we surmised that the carbamate produced hindered the activity of the enzyme by adsorbing on the active sites. Thus the reactions were repeated with CAL-B in DIPE or *t*-BuOMe in the presence of phenyl allyl carbonate, Et_3N, and 60 °C with ultrasound shaking every 24th h (Table 2). Faster reactions with excellent *E* (>200) were observed: 44% conversion after 7 days in *t*-BuOMe (entry 1) and 50% conversion after 3 days in DIPE (entry 2).

Small-scale reactions of (±)-**1,5** and **7** were then performed under the above conditions (CAL-B, DIPE, phenyl allyl carbonate, Et_3N, 60 °C, ultrasound shaking). Significant increases in rates without stoppage in the reaction of (±)-**1** and **5** (entries 4 and 7) and unchanged low reaction rate for (±)-**7** (entry 6) were observed.

Due to the rather low reaction rate for (±)-**7**, further reactions at 70 and 80 °C were performed. Unfortunately, reaction rates could

Table 1
Kinetic resolution of (±)-**3**.[a]

entry	Et_3N (μL)	Temperature (°C)	Solvent	ee_s (%)	ee_p (%)	conv. (%)	*E*
1	1	50	*t*-BuOMe	40	99	28	>200
2	10	50	*t*-BuOMe	89	99	47	>200
3	10	60	*t*-BuOMe	96	99	49	>200
4	10	70	*t*-BuOMe	70	99	41	>200
5	10	60	DIPE	91	99	48	>200

[a] Substrate (0.025 M), CAL-B (30 mg/mL), solvent (1 mL), phenyl allyl carbonate (4 equiv.), Et_3N, 7 days.

Table 2
N- alkoxycarbonylation of (±)-**1, 3, 5** and **7**.[a]

entry	Substrate	Solvent	Reaction time (day)	ee_s (%)	ee_p (%)	conv. (%)	*E*
1	(±)-**3**	*t*-BuOMe	7	80	99	44	>200
2	(±)-**3**	DIPE	3	99	99	50	>200
3	(±)-**5**	*t*-BuOMe	7	84	99	46	>200
4	(±)-**5**	DIPE	5	99	99	50	>200
5	(±)-**7**	*t*-BuOMe	7	16	99	14	>200
6	(±)-**7**	DIPE	7	27	99	21	>200
7	(±)-**1**	DIPE	1	99	97	50	>200

[a] Substrate (0.025 M), CAL-B (30 mg/mL), solvent (1 mL), phenyl allyl carbonate (4 equiv.), Et_3N (10 μL), 60 °C, daily ultrasound shaking.

Table 3
Preparative-scale resolution of (±)-**1**, (±)-**3**, and (±)-**5**.

Reaction time (day)	conv. (%)	*E*	Enantiomer	*ee*	yield (%)	$[\alpha]_D^{25}$
1	50	>200	(*S*)-**1**	98	42	−62[a]
			(*R*)-**2**	97	40	−98.5[b]
5	50	>200	(*S*)-**3**	99	43	−89.8[c]
			(*R*)-**4**	99	43	−60[d]
7	50	>200	(*S*)-**5**	99	41	−70[c]
			(*R*)-**6**	99	43	−71[e]

Substrate (100 mg); CAL-B (30 mg/mL); DIPE (25 mL); phenyl allyl carbonate (4 equiv.); 60 °C, Et_3N (50 μL), daily ultrasound shaking.
[a] c = 0.22 EtOH.
[b] c = 0.95 EtOH.
[c] c = 0.45 EtOH.
[d] c = 0.37 EtOH.
[e] c = 0.28 EtOH.

not be increased (conversion <25% after 7 days). When 3-methoxyphenyl allyl carbonate was used as acyl donor at 70 °C, practically the same low conversion (23% after 7 days, $E > 200$) was achieved.

On the basis of preliminary experiments, preparative-scale resolution of (±)-**1**, (±)-**3** and (±)-**5** was performed under the optimized conditions (CAL-B, DIPE, phenyl allyl carbonate, Et_3N, 60 °C). Due to the rather low reaction rate (max. conv. <25% achieved after 7 days), no preparative-scale resolution of (±)-**7** was carried out. The results are summarized in Table 3.

2.3. Hydrolysis of (R)-4 and (R)-6

Recently, we described the removal of the *N*-allyloxycarbonyl (Alloc) moiety from carbamate enantiomer (*R*)-**2** using tris(dibenzylideneacetone)dipalladium-chloroform adduct ($Pd_2(dba)_3.CHCl_3$) in tetrahydrofuran (THF) in the presence of formic acid (HCOOH) and triphenylposphine (PPh_3) at 40 °C, under Ar. (*R*)-**1** was isolated with an *ee* of 99% and a yield of 60% (after 16 h) [25]. A slightly modified literature method was used to remove the *N*-allyloxycarbonyl (Alloc) moiety from carbamate enantiomers (*R*)-**4** and (*R*)-**6** (Scheme 2) [25,26].

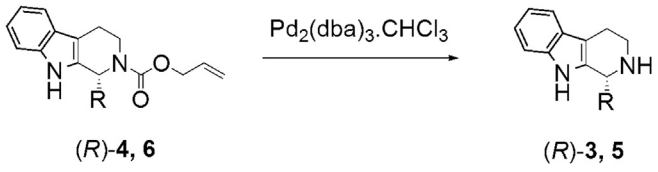
The Pd catalyst [$Pd_2(dba)_3.CHCl_3$] was used in THF in the presence of HCOOH and PPh_3 at 40 °C, under Ar. (*R*)-**3** and **5** were formed in good yields (78% for (*R*)-**3** and 79% for (*R*)-**5**) and without the loss of enantiopurity (*ee* = 99%).

2.4. Absolute configurations

The specific optical rotation values of free amine enantiomers **1**, **3** and **5** were compared with the literature data for the (*S*)-1-methyl- [27], (*S*)-1-ethyl- [28], and (*S*)-1-propyl-substituted [27] tetrahydro-ß-carbolines (Experimental). Thus, CAL-B showed (*R*)-selectivity for the alkoxycarbonylation of 1-methyl, 1-ethyl, 1-propyl, and 1-isopropyl substituted tetrahydro-ß-carbolines.

3. Conclusions

In the frame of substrate specificity, CAL-B-catalysed *N*-alkoxycarbonylation of 1-substituted tetrahydro-ß-carboline derivatives [(±)-**1,3,5,7**] were carried out in order to investigate the influence of different alkyl chain substituents (Me, Et, Pr, *i*Pr) on enantioselectivity and reaction rate. Whereas excellent enantioselectivities (>200) were found for all compounds, reaction rates decreased considerably with increasing alkyl chain lengths, when CAL-B was used as enzyme in DIPE, in the presence of phenyl allyl carbonate and Et_3N at 60 °C in batch conditions. (±)-**1** furnished (*S*)-**1** and (*R*)-**2** with excellent *ee* (>97%) and yields (>40%) after 1 day. (±)-**3** gave (*S*)-**3** and (*R*)-**4** with excellent *ee* (>99%) and yields (>41%) after 5 days. (±)-**5** resulted in the formation of (*S*)-**5** and (*R*)-**6** with the same excellent *ee* values (>99%) and good yields (43%) after 7 days. To the best of our knowledge, (±)-**3** and (±)-**5** were resolved for the first time by enzymes. The preparative-scale resolution of (±)-**7** was not performed, since it proved to be too bulky for the optimum enzyme activity. Carbamate (*R*)-**4** and (*R*)-**6** were hydrolysed into the corresponding amines by using $Pd_2(dba)_3.CHCl_3$ in THF, in the presence of PPh_3 and HCOOH. The enantiomers were obtained with good yields (>78%) and without the loss of enantiopurity (= 99%).



(*R*)-**4, 6** (*R*)-**3, 5**

Scheme 2. Removal of the Alloc moiety from (*R*)-**4** and (*R*)-**6**.

4. Experimental

4.1. Material and methods

CAL-B (lipase B from Candida antarctica) immobilized on acrylic resin was purchased from Sigma.

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. The ^1H NMR spectra were recorded on a Burker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. Microwave (MW) reactions were performed in a CEM Discover MW reactor (Matthews, NC, USA). The elemental analysis was carried out by means of a Perkin-Elmer CHNS-2400 Ser II Elemental Analyzer.

The preliminary small-scale experiments were performed as batch reactions. The racemic tetrahydro-ß-carbolines (0.025 mmol) were dissolved in an organic solvent (1 mL) followed by the addition of CAL-B (30 mg), acyl donor (4 equiv.), and Et_3N (1 or 10 μL). The reaction mixture was shaken in an incubator shaker at 50–80 °C, supported with an ultrasound shaking every 24th h.

The *ee* values of the enantiomers were determined by HPLC [Chiralpak IA column (4.6 mm × 250 mm)]. The eluent was *n*-hexane/IPA (90: 10), 240 nm; retention times (min) for the *N*-butanoyl-derivatized form of (*R*)-**1**: 24.61, (*S*)-**1**: 27.95 and for (*R*)-**2**: 28.43 and (*S*)-**2**: 32.43. The eluent ratio was *n*-hexane/IPA (80: 20) for the *N*-butanoyl-derivatized form of (*R*)-**3**: 15.93, (*S*)-**3**: 20.12; and for (*R*)-**4**: 17.33, for (*S*)-**4**: 21.57; for the *N*-butanoyl-derivatized form of (*R*)-**5**: 17.13, (*S*)-**5** 22.49; and for (*R*)-**6**: 18.98, for (*S*)-**6**: 22.82. The eluent ratio was *n*-hexane + 0.1% Et_2NH/IPA (90: 10) for (*R*)-**7**: 18.41, for (*S*)-**7**: 21.73, and for (*R*)-**8**: 41.23, for (*S*)-**8**: 47.36.

4.2. Syntheses of (±)-1, (±)-3, (±)-5 and (±)-7

Racemic 1-alkyl-1,2,3,4-tetrahydro-ß-carboline derivatives were synthesized from tryptamine (600 mg tryptamine, 3.75 mmol) in a microwave reactor [24]. (±)-**1** was isolated as a pale yellow solid in good yield (560 mg, 80%, mp: 177–179 °C, lit. [28]: 179–181 °C).

The ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.41–1.53 (d, $J = 3.3$ Hz, 3H); 1.54–1.67 (br s, 1H); 2.67–2.83 (m, 2H); 2.99–3.11, 3.31–3.43 (2 m, 2 × 1H), 4.14–4.24 (m, 1H), 7.06–7.18 (m, 2H); 7.28–7.35 (d, $J = 8$ Hz, 1H) 7.45–7.51 (d, $J = 8$ Hz, 1H); 7.68–7.85 (br s, 1H). Anal. calcd. for $C_{12}H_{14}N_2$: C 77.38, H 7.58 N 15.04, found: C 77.33, H 7.62, N 15. 09.

(±)-**3** (556 mg, 74%, mp: 117 °C, lit. [29]: 115–117 °C) is a pale yellow solid.

The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.02–1.13 (t, J = 7.2 Hz, 3H); 1.65–1.78 (m, 1H); 1.88–1.99 (m, 1H); 2.66–2.82 (m, 2H), 2.97–3.10, 3.32–3.44 (2 × m, 2 × 1H), 3.96–4.1 (m, 1H); 7.05–7.19 (m, 2H) 7.28–7.36 (d, J = 7.6 Hz, 1H); 7.45–7.53 (d, J = 7.6 Hz, 1H). Anal. calcd. for $C_{13}H_{16}N_2$: C 77.96, H 8.05, N 13.99, found: C 77.92, H 8.08, N 13.95.

(±)-**5** is a pale yellow solid (680 mg, 85%, mp: 183 °C, lit. [30]: 180–182 °C).

The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.95–1.05 (t, J = 7.2 Hz, 3H); 1.43–1.61 (m, 2H); 1.66–1.75, 1.79–1.90 (2 × m, 2 × 1H); 2.66–2.81 (m, 2H), 2.98–3.08, 3.30–3.42 (2 × m, 2 × 1H), 4.02–4.13 (m, 1H); 7.04–7.17 (m, 2H) 7.28–7.34 (d, J = 7.6 Hz, 1H); 7.43–7.52 (d, J = 7.6 Hz, 1H), 7.63–7.76 (br, 1H). Anal. calcd. for $C_{14}H_{18}N_2$: C 78.46, H 8.47, N 13.07, found: C 78.42, H 8.51, N 13.11.

(±)-**7** (580 mg, 72%, mp: 120 °C, lit. [31]: 117 °C, 118–120 °C) is a pale yellow solid.

The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.86–0.93, 1.12–1.19 (2 × d, J = 7.6 Hz, 2 × 3H); 2.11–2.25 (m, 1H); 2.65–2.82 (m, 2H); 2.93–3.06, 3.34–3.45 (2 × m, 2 × 1H), 3.97–4.06 (m, 1H); 7.03–7.19 (m, 2H) 7.28–7.37 (d, J = 8 Hz, 1H); 7.44–7.53 (d, J = 8 Hz, 1H), 7.64–7.8 (br, 1 H). Anal. calcd. for $C_{14}H_{18}N_2$: C 78.46, H 8.47, N 13.07, found: C 78.43, H 8.49, N 13.12.

4.3. Preparative-scale resolution of (±)-1

(±)-**1** (100 mg, 0.54 mmol) was dissolved in DIPE (25 mL), then CAL-B (30 mg/mL), phenyl allyl carbonate (0.35 mL, 2.15 mmol, 4 equiv.) and Et_3N (50 μL) were added. The reaction mixture was shaken for 1 day in an incubator shaker at 60 °C. The reaction was stopped by filtering off CAL-B and washing with DIPE (2 × 25 mL). After evaporation of the solvent, the enantiomers were separated by column cromatography on silica with CH_2Cl_2/MeOH (25: 1), affording carbamate (*R*)-**2** {59 mg, 40%, $[\alpha]_D^{25} = -98.5$ (*c* 0.95 EtOH), colourless oil, *ee* = 99%}. The free amine (*S*)-**1** was crystallized in hexane {42 mg, 42%, $[\alpha]_D^{25} = -62$ (*c* 0.22, EtOH), lit. [32]: $[\alpha]_D^{25} = -65.8$ (c 2.0 EtOH), lit. [27]: $[\alpha]_D^{25} = -56.8$ (c 2.0 EtOH), pale yellow solid, mp: 177 °C, lit. [27]: 179–181 °C, *ee* = 98%}.

The ^{1}H NMR (400 MHz, $CDCl_3$) for (*R*)-**2**: δ (ppm): 1.46–1.53 (d, J = 6.8 Hz, 3H); 2.67–2.89 (m, 2 × 1H); 3.11–3.28, 4.29–4.56 (m, 2 × 1H); 4.6–4.72 (m, 2H); 5.17–5.26 (dd, J = 1.1 Hz, 10.5 Hz, 1H); 5.27–5.43 (dd, J = 0.8 Hz, 17.2 Hz, 1H); 5.91–6.04 (m, 1H); 7.05–7.20 (m, 2H), 7.28–7.34, 7.41–7.5 (d, J = 8.0 Hz, 2H); 7.66–7.95 (br s, 1H). Anal. calcd. for $C_{16}H_{18}N_2O_2$: C 71.09, H 6.71, N 10.36, found: C 71.13, H 6.74, N 10.32.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*S*)-**1** were similar to those for (±)-**1**.

4.4. Preparative-scale resolution of (±)-3

(±)-**3** (100 mg, 0.49 mmol) was dissolved in DIPE (25 mL) and mixed with CAL-B (30 mg/mL), phenyl allyl carbonate (0.32 mL, 1.96 mmol, 4 equiv.) and Et_3N (50 μL). The reaction mixture was shaken for 5 days in an incubator shaker at 60 °C. Each day, the mixture was shaken for 15 s in an ultrasound bath. The reaction was stopped after reaching 50% conversion by filtering off CAL-B and washing with DIPE (2 × 25 mL). After evaporation of the solvent, the enantiomers were separated by column cromatography on silica with CH_2Cl_2/MeOH (25: 1), affording carbamate (*R*)-**4** {60 mg, 43%, $[\alpha]_D^{25} = -60$ (*c* 0.45 EtOH), colourless oil, *ee* = 99%}. The free amine (*S*)-**3** was crystallized in hexane {43 mg, 43%, $[\alpha]_D^{25} = -89.8$ (*c* 0.37, EtOH), lit. [28]: $[\alpha]_D^{25} = -87.9$ (c 1.61 EtOH), pale yellow solid, mp: 117 °C, lit. [29]: 115–117 °C, *ee* = 99%}.

The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for (*R*)-**4**: δ (ppm): 0.99–1.14 (t, J = 7.4 Hz, 3H); 1.76–2.00 (m, 2H); 2.62–2.93 (m, 2H); 3.08–3.29, 4.33–4.58 (m, 2 × 1H); 4.59–4.75 (m, 2H); 5.08–5.25, 5.26–5.41 (m, 2H + 1H); 5.85–6.07 (m, 1H); 7.04–7.22 (m, 2H), 7.27–7.33, 7.42–7.54 (d, J = 8.0 Hz, 2H); 7.73–8.02 (br s, 1H). Anal. calcd. for $C_{17}H_{20}N_2O_2$: C 71.81, H 7.09, N 9.85, found: C 71.76, H 7.11, N 9.78.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*S*)-**3** were similar to those for (±)-**3**.

4.5. Preparative-scale resolution of (±)-5

Racemic **5** (100 mg, 0.47 mmol) was subjected to preparative-scale resolution applying the above procedure, affording carbamate (*R*)-**6** {61 mg, 43%, $[\alpha]_D^{25} = -71$ (*c* 0.28 EtOH), colourless oil, *ee* = 99%}. (*S*)-**5** was crystallized in hexane {41 mg, 41%, $[\alpha]_D^{25} = -70$ (*c* 0.45 EtOH), lit. [27]: $[\alpha]_D^{25} = -73.5$ (*c* 1.0 EtOH), mp. 182 °C, lit. [30]: 180–182 °C, pale yellow solid, *ee* = 99%}.

The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for (*R*)-6: δ (ppm): 0.92–1.04 (t, J = 7.2 Hz, 3H); 1.19–1.30 (m, 2H); 1.45–1.65, 1.75–1.88 (m, 2 × 1H); 2.63–2.95 (m, 2H), 3.12–3.32, 4.33–4.56 (m, 2 × 1H); 4.58–4.74 (m, 2H); 5.15–5.27, 5.28–5.42 (m, 2H + 1H); 5.88–6.07 (m, 1H); 7.04–7.21 (m, 2H), 7.27–7.34, 7.42–7.55 (d, J = 7.8 Hz, 2H); 7.73–7.91 (br s, 1H). Anal. calcd. for $C_{18}H_{22}N_2O_2$: C 72.46, H 7.43, N 9.39, found: C 72.40, H 7.39, N 9.42.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*S*)-**5** were similar to those for (±)-**5**.

4.6. Hydrolysis of (R)-4 and (R)-6

(*R*)-**4** (58 mg, 0.20 mmol) was dissolved in THF (2 mL). PPh_3 (10 mg, 0.04 mmol) and HCOOH (28 μL, 0.74 mmol) were added immediately followed by $Pd_2(dba)_3.CHCl_3$ (14.5 mg, 0.014 mmol), and the solution was stirred for 2 h at 40 °C under Ar. After evaporation the catalyst was removed on a celite column with MeOH. Product amine (*R*)-**3** was purified by column chromatography with the eluent CH_2Cl_2/MeOH (25: 1). The TFA salt of (*R*)-**3** was dissolved in 5 mL H_2O and saturated $NaHCO_3$ solution was added until pH > 9. The aqueous media was extracted using $CHCl_3$ (2 × 7 mL). After drying with Na_2SO_4 and evaporation of the organic media, (*R*)-**3** was crystallized from hexane as a pale yellow solid {32 mg, 78%, $[\alpha]_D^{25} = +87.2$, (c = 0.75, EtOH), lit. [28]: $[\alpha]_D^{25} = -87.9$ (c 1.61 EtOH) for (*S*)-isomer, mp. 115 °C, lit. [29]: 115–117 °C, *ee* = 99%}. The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*R*)-**3** were similar to those for (±)-**3**.

(*R*)-**6** (61 mg, 0.20 mmol) was hydrolysed using the above procedure. The reaction was completed in 4 h, providing (*R*)-**5** as a pale yellow solid {34 mg, 79%, $[\alpha]_D^{25} = +71.5$, (c = 0.75, EtOH), lit. [27]: $[\alpha]_D^{25} = +72.7$ (*c* 1.0 EtOH), mp. 182 °C, lit. [30]: 180–182 °C, *ee* = 99%}. The ^{1}H NMR (400 MHz, CDCl3) spectroscopic data for (*R*)-**5** were similar to those for (±)-**5**.

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