Ph.D. Thesis

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

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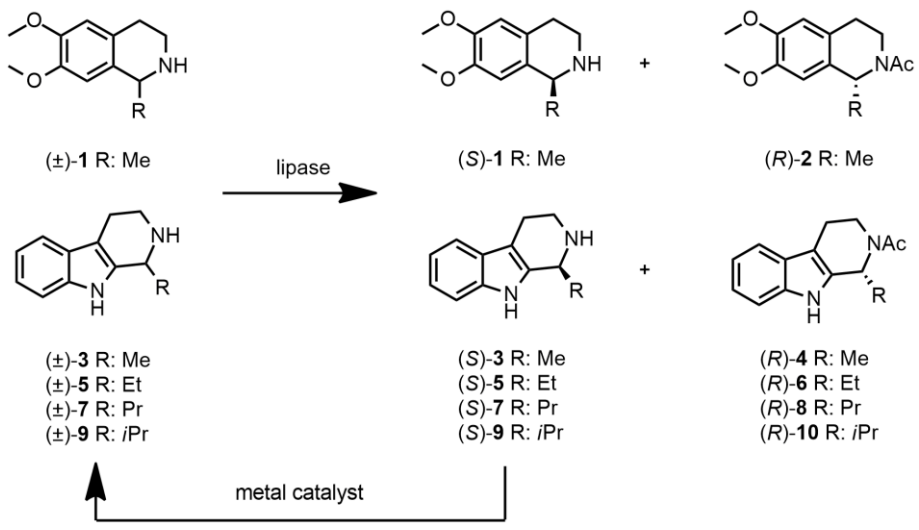
1. Introduction and aims

Enantiopure compounds containing the tetrahydroisoquinoline or tetrahydro-ß-carboline core represent high interest due their potential pharmaceutical activities. Both natural products and their synthetic derivatives are widely examined, because they possess major biological effects. The naturally occurring emetine (*Ipecacuanha*) having the tetrahydroisoquinoline skeleton is an expectorant, while noscapine (*Papaver Somniferum*) bearing the same skeleton is used as an antitussive since the 19th century. As an example of synthetic tetrahydroisoquinoline compounds, solifenacin shows urinary antispasmodic effect. Compounds containing the tetrahydro-ß-carboline moiety also have enormous role in medicine. For example, two alkaloids, vincristine and vinblastine, have been used for a long time in cancer chemotherapy. Another example is yohimbine, which is effective in erectile disfunction.

In our work we first focused on the preparation of enantiopure 1-substituted tetrahydroisoquinoline and tetrahydro-ß-carbolines [(±)-**1**, (±)-**3**, (±)-**5**, (±)-**7**, (±)-**9**] through lipase-catalyzed kinetic resolution (Scheme 1).



Scheme 1.

A further aim was to develop an enzymatic strategy for the asymmetric *N*-alkoxycarbonylation of (±)-**1** in a continuous flow system (*H*-Cube).

In the frame of substrate specificity, we also planned to examine the effect of C1 substituents (Me, Et, Pr, *i*Pr) on the *E* value and reaction rate in the asymmetric *N*-alkoxycarbonylation of (±)-**3**,**5**,**7**,**9** using CAL-B.

The combination of kinetic resolution and racemization can lead to a powerful asymmetric synthesis, thus we worked on the racemization of 1-Me-substituted (*S*)-**1** and (*S*)-**3** using heterogeneous metal catalysts (Scheme 1).

2. Methods

Racemic starting compounds were synthesized according to known literature methods. Preliminary reactions were carried out in batch mode or in a continuous flow reactor in a milligram scale. In a typical batch reaction, the substrate was dissolved in the solvent followed by the addition of the enzyme, the acyl donor, and the additives. Reaction mixtures were shaken in an incubator shaker at different temperatures. In continuous flow reactions, the starting compound was dissolved in a solvent and after adding the acyl donor, the reaction mixture was pumped through the enzyme-filled pressure- and temperature-resistant column by an HPLC pump. Preparative-scale resolutions were performed in batch under the optimized conditions. The hydrolysis reactions of the (*R*)-carbamate products were executed in Schlenk tube, in which the reaction mixture was stirred in an oil bath under argon.

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

During a typical racemization process, the reaction flask was evacuated and purged with argon. Then the substrate, the solvent and the metal catalyst were introduced followed by immediate bubbling of H_2. Reaction mixtures in the Schlenk tube were evacuated, the tube was filled with argon, followed by adding the substrate, the solvent, and the metal catalyst.

The racemic products were characterized by ^{1}H NMR and melting point.

All reactions were followed by measuring *ee* on HPLC equipped with chiral IA column.

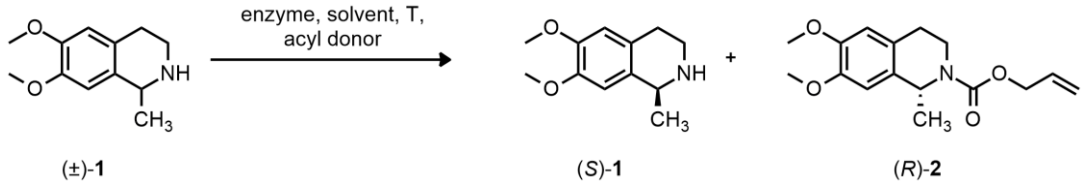
3. Results

3.1. Kinetic resolution of racemic 1,3,5,7,9[I,III]

Kinetic resolution of (±)-**1** was carried out in toluene with AY (*Candida rugosa*) and phenyl allyl carbonate at 40 °C in batch mode (Scheme 2). (*S*)-**1** and (*R*)-**2** were obtained with excellent *ee* (> 99%) and yield (> 39%) in 24 h.

Asymmetric *N*-alkoxycarbonylation of (±)-**1** was further investigated using CAL-B in *t*-BuOMe with phenyl allyl carbonate at 50 °C and high *E* (> 200) was detected in 49 h (Scheme 2). The product enantiomers were obtained with *ee* values of > 97% and in a yield of > 38%.

Using the optimized conditions determined in the batch process, the kinetic resolution of (±)-**1** was also performed in a continuous flow reactor. When CatCart® filled with AY was used, no enantioselective reaction was detected. Using CAL-B-filled CatCart® in *t*-BuOMe with phenyl allyl carbonate at 70 °C and at 1 bar, 25% conversion was detected (*E* > 200) after a single cycle. To enhance the yield, the reaction mixture was pumped through the CAL-B-filled column five times consecutively. In this case, 41% conversion was reached with excellent *E* (> 200) after the 5th cycle.



Scheme 2.

After optimization, asymmetric *N*-alkoxycarbonylation of (±)-**3** was carried out in the presence of CAL-B in *t*-BuOMe with phenyl allyl carbonate and Et_3N at 60 °C. The reaction, completed in 48 h (*E* > 200), afforded enantiomers (*S*)-**3** and (*R*)-**4** with an *ee* of > 97% and in a yield of > 40%. The reaction was repeated in DIPE and the maximum conversion (50%) was detected in 24 h. > 97% *ee* and > 40% yield characterized the product enantiomers (Scheme 3).

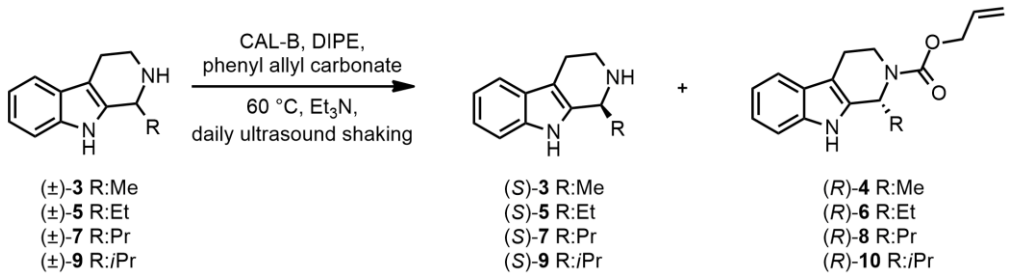
The preparative-scale *N*-alkoxycarbonylation of (±)-**5** under the same reaction conditions but with daily ultrasound shaking for 1 min was completed in 5 days and the

enantiomers [(*S*)-**5** and (*R*)-**6**] were obtained with excellent *ee* (> 99%) and good yield (43%) (Scheme 3).

The CAL-B-catalyzed (*R*)-selective preparative resolution of (±)-**7** was then performed in DIPE (Scheme 3). The reaction was completed in 7 days providing the products [(*S*)-**7** and (*R*)-**8**] with excellent *ee* (> 99%) and > 41% yield.

Under the same conditions only 21% conversion was detected in the kinetic resolution of (±)-**9**, thus preparative-scale resolution was not attempted (Scheme 3).

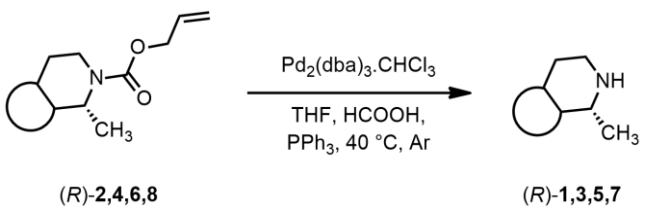
During the kinetic resolution of (±)-**3**,**5**,**7**,**9**, the effect of different C1 substituents [Me: (±)-**3**, Et: (±)-**5**, Pr: (±)-**7**, *i*Pr: (±)-**9**] on *E* and reaction rate was investigated. We have found excellent *E* values (> 200) in every case, but the reaction rate decreased drastically with the increasing substituent size.



Scheme 3.

3.2. Hydrolysis of the (*R*)-carbamate enantiomers[I,III]

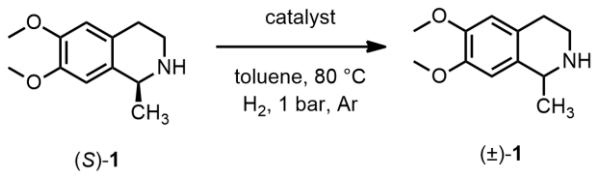
In order to remove the Alloc moiety from the (*R*)-carbamates [(*R*)-**2**,**4**,**6**,**8**], a homogeneous Pd catalyst [$Pd_2(dba)_3.CHCl_3$] was used (Scheme 4). The reactions were accomplished with the carbamate enantiomers in THF with HCOOH and PPh_3 at 40 °C under argon. The enantiomers of the free amines [(*R*)-**1**,**3**,**5**,**7**] were obtained without the loss of enantiopurity (*ee* > 99%) and with good yields [(*R*)-**1**: 60%, (*R*)-**3**: 85%, (*R*)-**5**: 78%, (*R*)-**7**: 79%].



Scheme 4.

3.3. Racemization of (*S*)-1-methyl-tetrahydroisoquinoline and (*S*)-1-methyl-tetrahydro-ß-carboline[II]

The first racemization experiments of (*S*)-**1** were performed with 20 mol% catalyst loading [Pd/Al_2O_3, Pd/C, Ir/C, Pt/Al_2O_3 (T94), Pt/Al_2O_3 (T123), Pt/C] (Scheme 5). Pt/Al_2O_3 (T94) and Pt/C racemized (*S*)-**1** in 30 min. Ir/C showed lower activity than the Pt catalysts, but racemization still took place in 5 h. In contrast, racemizations were not observed even at 90 °C, when Ni/Al_2O_3, Pd black or Ir prepared by colloidal route was used. $[IrCp^*I_2]_2$ (0.2 mol%) reported previously by Page and co-workers was also investigated for (*S*)-**1** and (*S*)-**3** in toluene at 40 °C with full conversion in 1 h. Pt/Al_2O_3 (T94) and Ir/C were selected for further experiments.



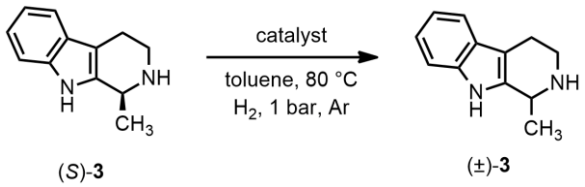
Scheme 5.

Using the same conditions, the effect of varied catalyst loadings on the reaction rate of the racemization of (*S*)-**1** was examined. Using 20 mol% Pt/Al_2O_3 (T94), the racemic product was detected in 45 min, while the use of 3 mol% catalyst resulted in an *ee* of 29% in 5 h. With 20 mol% Ir/C the reaction completed in 5 h, while with 10 mol% catalyst the *ee* decreased to 24 mol% in 5 h. Catalyst deactivation at low catalyst loading took place with Pt/Al_2O_3 (T94). Similar phenomenon, in turn, was not observed with Ir/C.

To compare the activation energies of the two catalysts, test reactions at different temperatures were carried out. When 10 mol% Pt/Al_2O_3 (T94) was used in toluene at 1 bar under H_2 flow and under argon, the reaction completed in 120 min at 80 °C, while 17% *ee* was detected at 50 °C in 5 h. Using 15 mol% Ir/C, full racemization occurred in 5 h at 90 °C, while 35% *ee* was observed at 70 °C. The calculated activation energies [27 kJ mol^{-1} for Pt/Al_2O_3 (T94) and 65 kJ mol^{-1} for Ir/C] are in line with the experimental results, that is the Pt catalyst is more suitable to induce racemization.

The racemization of (*S*)-**3** completed in 2 h using 10 mol% Pt/Al_2O_3 (T94) (Scheme 6). With 20 mol% Ir/C 14% *ee* was reached in 5 h (Scheme 6). Catalyst deactivation, because of the inhibitory effect of the amine and imine form, was also detected when a lower amount

of Pt/Al_2O_3 (T94) (2 mol%) was used (*ee* = 47%, 5 h). When toluene was replaced by *t*-BuOMe in the racemization of (*S*)-**3** catalyzed by Pt/Al_2O_3 (T94), only a moderate decrease in *ee* was observed from 98% to 91% in 5 h (10 mol% catalyst, 80 °C in H_2 flow, 1 bar, under argon).



Scheme 6.

Preparative-scale racemizations were performed using 10 mol% Pt/Al_2O_3 (T94) in toluene at 80 °C under H_2 flow at 1 bar, under argon. Racemic **1** was detected in 2 h with excellent yield (94%) and racemic **3** was obtained in 1 h with a yield of 90%.

Next, catalyst recyclability, the inhibitory effect of the imine and the metal leaching from the catalysts were investigated in the racemization of (*S*)-**1** using Pt/Al_2O_3 (T94) or Ir/C in toluene at 80 °C in H_2 flow at 1 bar and under argon.

During recyclability experiments, after a racemization cycle, the catalyst was reused under the same reaction conditions. When 10 mol% Pt/Al_2O_3 (T94) was applied, 50% *ee* was detected after 15 min in the 1st cycle, while the *ee* was 67% in the 2nd cycle indicating a lower rate. The reduction of the reaction rate, however, was not observed with 20 mol% Ir/C.

The deactivation of the Pt catalyst by the imine intermediate formed in the reaction was observed. In the absence of H_2, (±)-**1** converted into imine immediately by 20 mol% Ir/C, while a much slower dehydrogenation rate was observed under the same conditions with the use of 20 mol% Pt/Al_2O_3 (T94). A significant reduction in the racemization rate was detected, when the prepared imine was added to the reaction mixture using 10 mol% Pt/Al_2O_3 (T94) (*ee* = 29% after 2 h with imine addition, *ee* = 1% after 2 h without imine).

Metal leaching from the heterogeneous catalysts was ruled out. The catalyst was filtered off from the reaction mixture after 45 min with *ee* values of 20% using 5 mol% Pt/Al_2O_3 (T94) and *ee* = 57% applying 15 mol% Ir/C. After the removal of the catalyst, the sampling of the reaction mixture was continued without any further decrease in *ee* values. These results clearly indicated that the mixture did not contain metal species.

3.4. Dynamic kinetic resolution investigation

A test reaction using Ir/C and CAL-B was carried out in toluene for the dynamic kinetic resolution of (±)-**1** at 80 °C with phenyl allyl carbonate under H_2 bubbling and under argon without any significant activity.

PUBLICATION LIST

Papers related to the thesis:

I. **Kovács, B.**; Megyesi, R.; Forró, E.; Fülöp, F.
Efficient lipase-catalyzed route for the kinetic resolution of salsolidine and its ß-carboline analogue
Tetrahedron: Asymmetry, **2017**, *28*, 1829-1833
IF: 1.77

II. **Kovács, B.**; Savela, R.; Honkala, K.; Yu. Murzin, D.; Forró, E.; Fülöp, F.; Leino, R.
Racemization of secondary amine containing natural products using heterogeneous metal catalysts
ChemCatChem, **2018**, *10*, 2893-2899
IF: 4.495

III. **Kovács, B.**; Forró, E.; Fülöp, F.
Candida antarctica lipase B catalyzed kinetic resolution of 1,2,3,4-tetrahydro-ß-carbolines: Substrate specificity
Tetrahedron, **2018**, *74*, 6873-6877
IF: 1.92

Conference lectures:

I. **Kovács, B.**; Forró, E.; Fülöp, F.
Szalszolidin és ß-karbolin vázas analógjának enzim katalizált *N*-acilezése szakaszos és folyamatos üzemmódban
A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány és a SZAB Szerves és Gyógyszerkémiai Munkabizottsága 15. tudományos előadóülés
Szeged, Hungary, 12 May, 2016, oral presentation

II. **B. Kovács**; R. Savela; E. Forró; F. Fülöp; R. Leino
Heterogeneous dynamic kinetic resolution of secondary amines
Winter school Conference Cruise Helsinki – Stockholm 2017
Helsinki-Stockholm, Finland-Sweden, 12-14 January, 2017, oral presentation

III. **Kovács, B.**; Savela, R.; Honkala, K.; Yu. Murzin, D.; Forró, E.; Fülöp, F.; Leino, R.

Szekunder aminok kinetikus rezolválása és kísérletek a dinamikus kinetikus rezolválás megvalósítására

Vegyészkonferencia

Hajdúszoboszló, Hungary, 19-21 June, 2017, poster presentation P-25

IV. **B. Kovács**; R. Savela; K. Honkala; D. Yu. Murzin; E. Forró; F. Fülöp; R. Leino
Kinetic resolution and racemization of secondary amines
13th International Symposium on Biocatalysis and Biotransformations
Budapest, Hungary, 9-13 July, 2017, poster presentation P-065

V. **Kovács, B.**; Forró, E.; Fülöp, F.
Szalszolidin és 1-alkilszubsztituált tetrahidro-ß-karbolinok lipáz-katalizált kinetikus rezolválása és kísérletek a dinamikus kinetikus rezolválás megvalósítására
MTA Heterociklusos és Elemorganikus Kémiai Munkabizottság Ülése
Balatonszemes, Hungary, 6-8 June, 2018, oral presentation