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Kinetic and dynamic kinetic enzymatic resolutions of tetrahydro-*β*-carboline and tetrahydroisoquinoline derivatives

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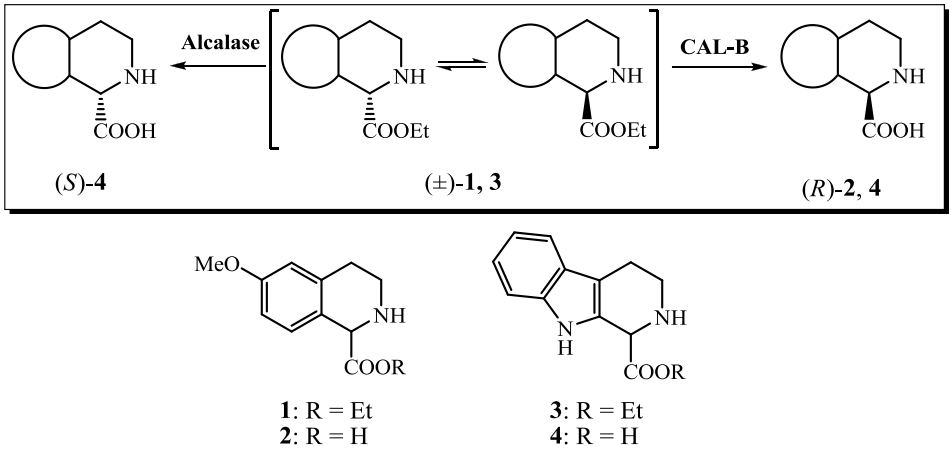
1. Introduction and aims:

Many scientific research has focused on the isolation, synthesis and biological investigation of enantiopure compounds containing tetrahydroisoquinoline or tetrahydro-*β*-carboline frameworks. The reason for this high interest is their potential pharmaceutical activities. Derivatives containing the tetrahydroisoquinoline core are an important part of alkaloids, *e.g.* expectorant emetine (*Ipecacuanhe*) and antitussive noscapine (*Papaver somniferum*). Other natural products such as liensinine (*Nelumbo nucifera*) and saframycine A (*Myxococcus xanthus*) as well as other synthetic tetrahydroisoquinoline alkaloid analogues, such as Zalypsis®, showed promising pharmaceutical activities toward HIV or cancer. Tetrahydro-*β*-carboline alkaloids such as vincristine, vinblastine and reserpine are well known about their valuable medicinal applications in the therapy of cancer or hypertension.

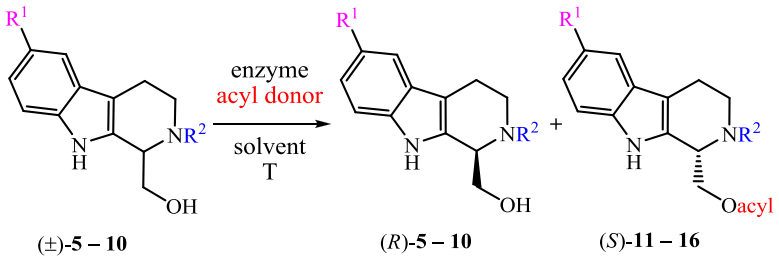
One of our aims was to work out a new strategy for the enantioselective preparation of 6-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid and 1,2,3,4-tetrahydro-*β*-carboline-1-carboxylic acid through enzyme-catalysed dynamic kinetic resolution (DKR) of the corresponding ethyl esters [(±)-**1** and (±)-**3**] (Scheme 1). We also intended to develop a directed DKR of (±)-**3** by the use of hydrolases with different enantiopreferences.



Scheme 1.

A further aim was to device a method for the kinetic resolutions (KR) of *N*-protected 1-hydroxymethyl-substituted tetrahydro-*β*-carboline derivatives (±)-**5**–

(±)-**10** through enzyme-catalysed asymmetric acylations of the primary hydroxy group (Scheme 2). In the frame of substrate engineering, we planned to investigate the effect of different substituents at position 6 and *N*-protecting groups at position 2 on the outcome of the enzymatic transformation.



Substrate or product		R^1	R^2
(±/*R*)-**5**	(*S*)-**11**	H	Boc
(±/*R*)-**6**	(*S*)-**12**	OMe	Boc
(±/*R*)-**7**	(*S*)-**13**	F	Boc
(±/*R*)-**8**	(*S*)-**14**	H	acetyl
(±/*R*)-**9**	(*S*)-**15**	H	Cbz
(±/*R*)-**10**	(*S*)-**16**	H	Fmoc

Scheme 2.

2. Methods:

The racemic starting compounds were prepared according to known literature methods. Enzyme catalysed preliminary reactions were carried out milligram-scale in batch (incubator-shaker) or in continuous-flow reactor (H-Cube®). In a typical continuous-flow reaction the starting compound was dissolved in the solvent, then the acyl donor was added to the solution and the mixture was pumped through a pressure- and temperature-resistant column, which was previously filled with the enzyme. The flow of the reaction mixture was provided by a HPLC pump (Figure 1).

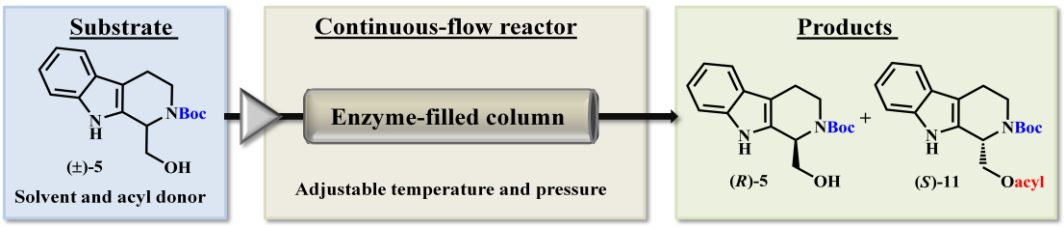


Figure 1.

Preparative-scale resolutions were performed in batch under optimized reaction conditions. The progress of the reactions were followed by HPLC equipped with chiral column.

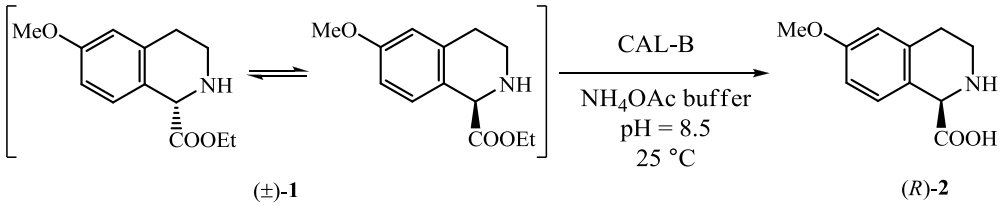
The product enantiomers were characterized with ^{1}H NMR and ^{13}C NMR spectroscopy, melting point, elemental analysis and optical rotation.

3. Results

3.1. *Dynamic kinetic resolutions of tetrahydroisoquinoline and tetrahydro-β-carboline amino acid ethyl esters*[II,III]

3.1.1. CAL-B (*Candida antarctica* lipase B) catalysed DKR of 6-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid ethyl ester (±)-**1** was performed in aqueous NH_4OAc buffer at pH 8.5, but only traces of (*R*)-**2** were obtained (*ee* of 66% at a conv. of 90% after 2 days) (Scheme 3).

3.1.2. The preparative-scale resolution of (±)-**1**·HCl was performed with CAL-B, in NH_4OAc buffer, at pH 8.5 and at 25 °C. (*R*)-**2**·HCl was isolated with an excellent *ee* of 99% and a yield of 91%.

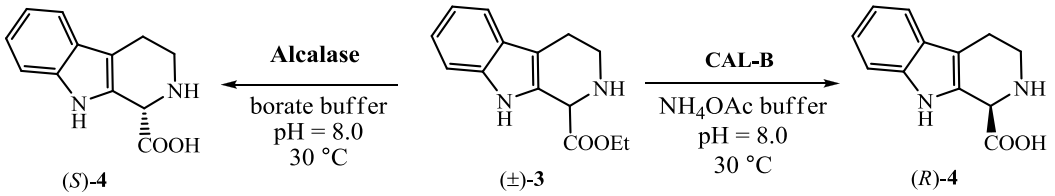


(±)-**1** (*R*)-**2**

Scheme 3.

3.1.3. Resolution of 1,2,3,4-tetrahydro-*β*-carboline-1-carboxylic acid ethyl ester [(±)-**3**·HCl] was performed in aqueous NH_4OAc buffer (pH 8.0 at 30 °C) with CAL-B and (*R*)-**4** was obtained with an excellent *ee* of 97% and a high conversion of 97% only in 5 min. Optimization of the reaction conditions (*e.g.* buffer type, pH, temperature) did not led notable changes in the reaction rate or selectivity (Scheme 4).

3.1.4. Alcalase-catalysed DKR of (±)-**3**·HCl for the preparation of the corresponding *S*-amino acid was carried out in NH_4OAc buffer, pH 8.0 at 30 °C, but low selectivity was observed (*ee* = 53% at a conv. of 99% after 24 h) (Scheme 4). Changing the pH from 8.0 to 9.0 caused an increase in the reaction rate but resulted in a decrease in the *ee* (47%). A change of the NH_4OAc buffer to borate buffer gave (*S*)-**4** with *ee* of 65% at conversion of 99% after 24 h (Scheme 4).



Scheme 4.

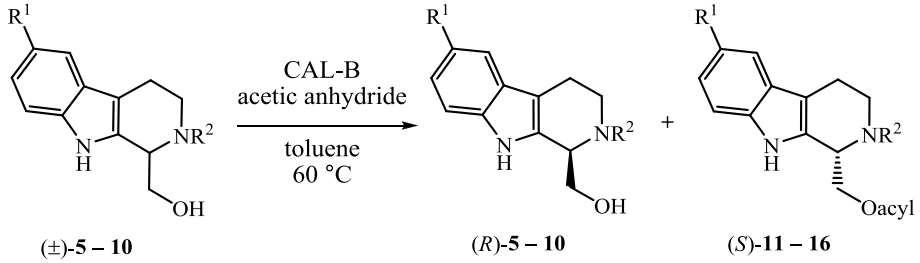
3.1.5. Alcalase-catalysed DKR of (±)-**3**·HCl was attempted in organic solvents (in *t*-BuOMe and in the mixture of *t*-BuOH and NH_4OAc aqueous buffer). However, the reactions reached conversion of 98% and 48% after 24 h, the (*S*)-**4** could not be detected in the reaction mixtures.

3.1.6. CAL-B-catalysed *R*-selective preparative resolution of (±)-**3**·HCl (NH_4OAc buffer, pH 8.0, 30 °C) afforded the product amino acid [(*R*)-**4**·HCl] with an *ee* of 98% and a yield of 90% (Scheme 4).

3.1.7. Alcalase-catalysed *S*-selective preparative resolution of (±)-**3**·HCl was carried out under optimized reaction condition (borate buffer, pH 8.0, 30 °C) (Scheme 4). The product (*S*)-**4**·HCl was isolated with an *ee* of 60% and a yield of 66%.

3.2. *Kinetic resolutions of 1-hydroxymethyl-substituted tetrahydro-β-carboline derivatives*[I,IV]

3.2.1. The optimized reaction conditions were determined, for the resolution of *N*-Boc-protected 1-hydroxymethyl-1,2,3,4-tetrahydro-*β*-carboline [(±)-**5**], in the continuous-flow system (CAL-B, *i*Pr_2O, acetic anhydride, 60 °C) (Figure 1). In the batch mode the acylation of (±)-**5** was achieved with good conversion (43%) but lower enantioselectivity (E = 36 *vs.* >200), therefore *i*Pr_2O was changed to toluene (Scheme 5).



5 and **11**: R^1 = H, R^2 = Boc; **6** and **12**: R^1 = OMe, R^2 = Boc; **7** and **13**: R^1 = F, R^2 = Boc

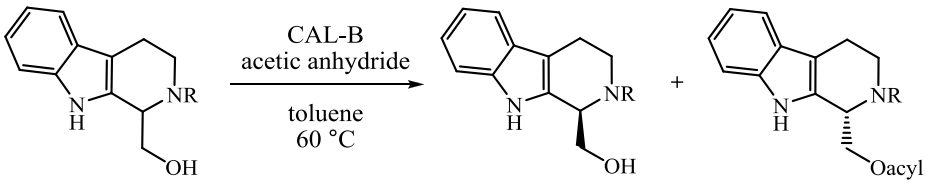
Scheme 5.

3.2.2. Maximum conversion of the KR of (±)-**5** was achieved when the amount of the acyl donor was increased from 1.1 equiv. to 2 equiv after 3 h while the *E* remained excellent (>200).

3.2.3. The optimized conditions for (±)-**5** (CAL-B, 2 equiv. acetic anhydride, toluene, 60 °C) were implemented to the *O*-acylations of (±)-**6** and (±)-**7**. To eliminate the negative effect of the low reaction rates [*E* = 82 at conv. = 42% for (±)-**6** and *E* = 39 at conv. = 45% for (±)-**7** after 7 h], the amount of acetic anhydride was increased from 2 equiv. to 6 equiv or 8 equiv.

3.2.4. The preparative-scale KR of (±)-**5**–(±)-**7** with CAL-B, in toluene, with acetic anhydride in a batch process at 60 °C afforded an excellent *E* (>200) and products (*R*)-**5**–(*R*)-**7** and (*S*)-**11**–(*S*)-**13** were isolated with *ee* of ≥96% and a yields of ≥45%.

3.2.5. The KR of *N*-acetyl- [(±)-**8**], *N*-Cbz- [(±)-**9**] and *N*-Fmoc-protected [(±)-**10**] substrates were carried out with CAL-B, acetic anhydride, in toluene, at 60 °C (Scheme 6). We found that *N*-acetyl [(±)-**8**] was an inadequate protecting group because of *N*→*O* and *O*→*N* acyl migrations.



(±)-**5**, **8 – 10**

(±)-**5**: R = Boc
(±)-**8**: R = acetyl
(±)-**9**: R = Cbz
(±)-**10**: R = Fmoc

(*R*)-**5**, **8 – 10**

(*R*)-**5**: R = Boc
(*R*)-**8**: R = acetyl
(*R*)-**9**: R = Cbz
(*R*)-**10**: R = Fmoc

(*S*)-**11**, **14 – 16**

(*S*)-**11**: R = Boc
(*S*)-**14**: R = acetyl
(*S*)-**15**: R = Cbz
(*S*)-**16**: R = Fmoc

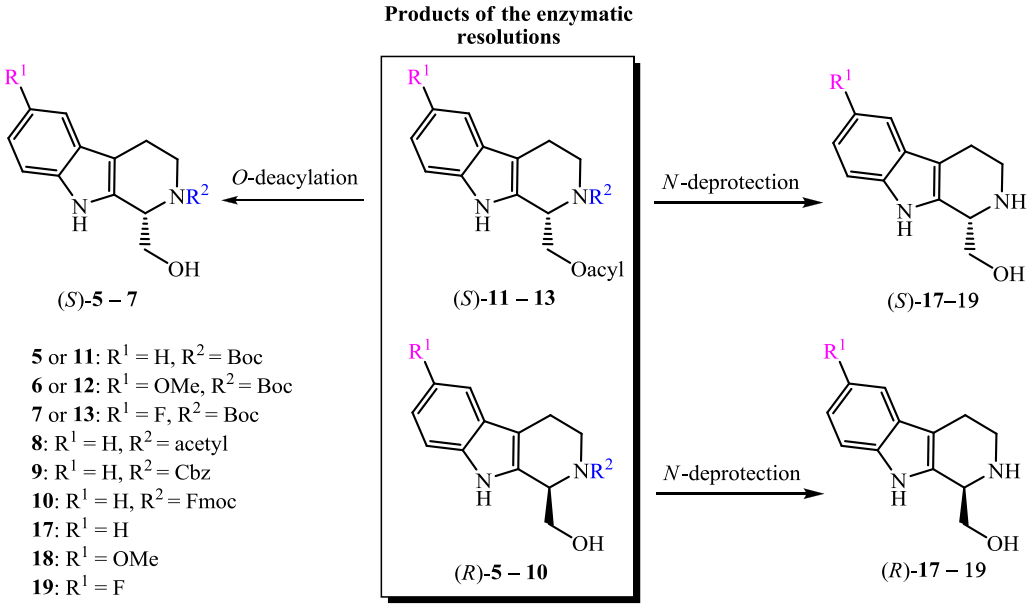
Scheme 6.

3.2.6. When the enzymatic resolution of (±)-**9** and (±)-**10** were performed, the reactions stopped at conversion of 41-45%. To increase the rate of resolution, the amount of acetic anhydride was doubled (from 2 to 4 equiv.)

3.2.7. Preparative-scale resolutions of (±)-**9** and (±)-**10** (CAL-B, 4 equiv. acetic anhydride, toluene, 60 °C) gave amino alcohol enantiomers (*R*)-**9** and (*R*)-**10** and esters (*S*)-**15** and (*S*)-**16** with good *ee* (≥88%) and high yields (≥44%).

3.2.8. The amino ester products [(*S*)-**11**–(*S*)-**13**] of preparative resolutions were transformed into their corresponding amino alcohol enantiomers [(*S*)-**5**–(*S*)-**7**] through methanolysis (K_2CO_3 in MeOH at 60 °C) without changes in *ee* values (*ee* 98%). Protecting-group removals [(*S*)-**5**–(*S*)-**7,** (*S*)-**11**–(*S*)-**13**, (*R*)-**9** and (*R*)-**10**] were also performed (Scheme 7). In case of *N*-Cbz-protecting group [(*R*)-**9**] removal, significant decrease in the *ee* was observed (decreased from 88% to 77%).

3.2.9. A systematic study under the same reaction conditions (CAL-B, 2 equiv. acetic anhydride, toluene, 60 °C, 60 min) was performed to investigate the effect of different substituents at position 6 [(±)-**5,** (±)-**6** and (±)-**7**] and *N*-protecting group at position 2 [(±)-**5,** (±)-**9** and (±)-**10**] on the reaction rate and enantioselectivity. Considering enantioselectivity, CAL-B seems to be very tolerant towards the substrate structure with modifications at position 2 or 6 at the tetrahydro-*β*-carboline ring. Substituents at position 2 and 6 had a significant effect on the reaction rate. We should also mention that no correlation was found between the size of the protecting group and the reaction rate.



Scheme 7.

Publications

Papers related to the thesis:

I. **R. Megyesi**, E. Forró, F. Ferenc

Enzymatic strategy for the resolution of new 1-hydroxymethyl tetrahydro-*β*-carboline derivatives in batch and continuous-flow systems

ChemistryOpen **2016**, *5*, 254-260. **IF: 2.801***

II. E. Forró, **R. Megyesi**, T.A. Paál, F. Ferenc

Efficient dynamic kinetic resolution method for the synthesis of enantiopure 6-hydroxy- and 6-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid

Tetrahedron: Asymmetry **2016**, *27*, 1213-1216. **IF: 2.126****

III. **R. Megyesi**, A. Mándi, T. Kurtán, E. Forró, F. Fülöp

Dynamic kinetic resolution of ethyl 1,2,3,4-tetrahydro-*β*-carboline-1-carboxylate. Use of different hydrolases for stereocomplementary processes.

Eur. J. Org. Chem. **2017**, 4713-4718. **IF: 2.882***

IV. **R. Megyesi**, E. Forró, F. Fülöp

Substrate engineering: Effects of different *N*-protecting groups in the CAL-B catalysed asymmetric *O*-acylation of 1-hydroxymethyl-tetrahydro-β-carbolines.

Tetrahedron **2018**, *74*, 2634-2640. **IF: 2.377***

Other papers:

I. G. Lajkó, N. Grecsó, **R. Megyesi**, E. Forró, F. Fülöp, D. Wolrab, W. Lindner, A. Péter, I. Ilisz

Enantioseparation of *β*-carboline derivatives on polysaccharide- and strong cation exchanger-based chiral stationary phases. A comparative study

J. Chromatogr. A **2016**, *1467*, 188-198. **IF: 3.716***

II. B. Kovács, **R. Megyesi**, E. Forró, F. Fülöp

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

Tetrahedron: Asymmetry **2017**, *28*, 1829-1833. **IF: 2.126****

Cumulative impact factor: 16.028

*The impact factors for the year 2017 are presented.

**The impact factors for the year 2016 are presented.

Lectures

a. **Megyesi R.**, Forró E., Fülöp F.
Új enzimes módszer gyógyszerkémiai jelentőségű tetrahidro-*β*-karbolinvázas amino-alkoholok rezolválására
MTA Alkaloidkémiai és Flavonoidkémiai munkabizottság, Balatonalmádi, május 12-13, **2014** (orális előadás)

b. **R. Megyesi**, E. Forró, F. Fülöp
Enzyme-catalysed kinetic resolution of 1-hydroxymethyl-2,3,4,9-tetrahydro-*β*-carbolines in batch and continuous flow reactions
Chirality, Prague, 27-30 July, **2014** (poster)

c. **R. Megyesi**, E. Forró, F. Fülöp
New enzymatic strategy for the resolution of tetrahydro-*β*-carboline amino-alcohol derivatives in batch and continuous-flow system
Biotrans, Vienna, 26-30 July, **2015** (poster).

d. **R. Megyesi**, A. Mándi, T. Kurtán, E. Forró, F. Fülöp
Directed dynamic kinetic enzymatic strategy for the preparation of both enantiomers of 1,2,3,4-tetrahydro-*β*-carboline-1-carboxylic acid
SECAT17, Oviedo, 26-28 June, **2017** (poster)