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ENZYMATIC RESOLUTION OF TETRAHYDROISOQUINOLINE DERIVATIVES IN BATCH AND CONTINUOUS-FLOW SYSTEMS

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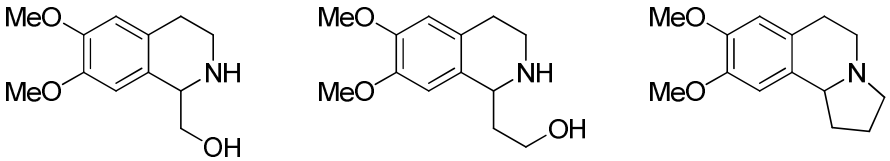
1. Introduction and aims

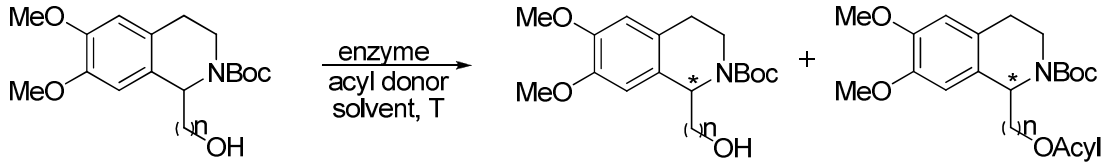
Tetrahydroisoquinoline derivatives are important members of the family of the naturally-occurring alkaloids, as well have a great importance in synthetic chemistry and drug research for their potential pharmaceutical activity. Some of the tetrahidroisoquinoline derivatives in enantiomerically pure form are important building blocks in drugs such as the antitussive noscapin, the antitumor agent trabectedin (as Yondelis®), the expectorant emetin. Some other drugs with tetrahydroisoquinoline skeleton such 1-methyl- and 1-phenyl-tetrahydroisoquinoline have an important role in the prevention of Parkinson's diseases. The tetrahydroisoquinoline skeleton is a basic structural unit in a large number of naturally-occurring alkaloids: calycotomine was isolated from *Calycotome spinosa* and crispine A from *Carduus crispus*. Crispine A has a high biological activity against SKOV3, KB and HeLa human cancer cell lines.



Calycotomine **1** Homocalycotomine **2** Crispine A **3**

Figure 1.

The primary aim of my thesis was to develop a new enzymatic strategy in batch and continuous-flow system for the preparation of both enantiomers of calycotomine, homocalycotomine and crispine A (Figure 1.). We planned to prepare the enantiopure intermediates of the desired compounds through an enzymatic *O*-acylation of the corresponding *N*-Boc protected primary alcohols [(±)-**4**–(±)-**6**] with a remote stereogenic centre.



(±)-**4**, n = 1
(±)-**5**, n = 2
(±)-**6**, n = 3

(*R*) or (*S*)-**4**, n = 1
(*R*) or (*S*)-**5**, n = 2
(*R*) or (*S*)-**6**, n = 3

(*R*) or (*S*)-**7**, n = 1
(*R*) or (*S*)-**8**, n = 2
(*R*) or (*S*)-**9**, n = 3

Scheme 1.

Another aim was to perform a systematic study on the effect of the remote stereogenic centre on the reaction rate and enantioselectivity through enzyme-catalysed acylation of primary alcohols (±)-**4**–(±)-**6**, when the distance between the reaction centre and stereogenic centre was one [(±)-**4**], two [(±)-**5**] and three [(±)-**6**] carbon atom (Scheme 1).

2. Methods

The starting compounds used in the enzyme catalysed kinetic resolutions were prepared according to literature methods. The enzym catalysed preliminary experiments were performed in milligram scale. The preliminary experiments were performed in a continuous-low reactor in an H-Cube system in 'No H_2' mode. (Figure 2). The main components of the flow reactor were the HPLC pump and the heat- and pressure-resistant holder equipped with a stainless-steel CatCart, filled with different enzymes. The HPLC pump pumped the reaction mixture through the CatCart and the products were collected and analysed on HPLC.

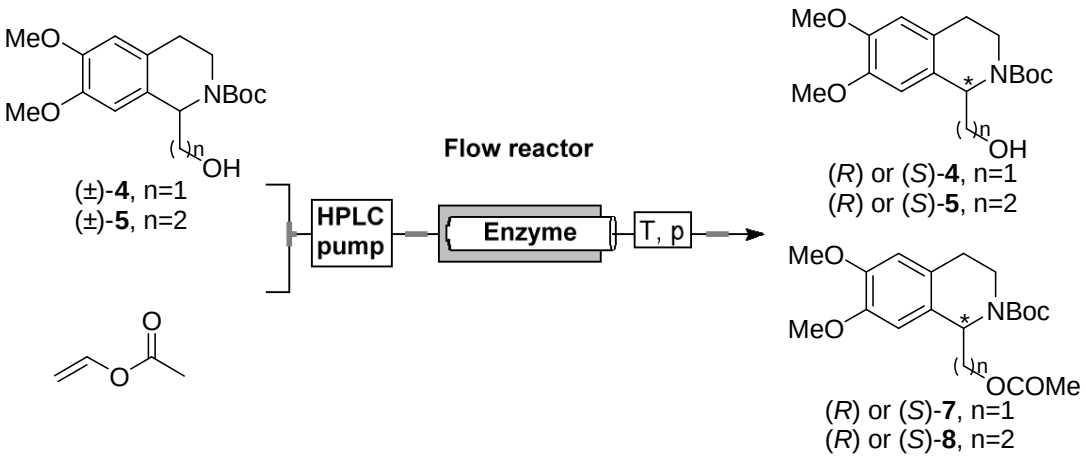


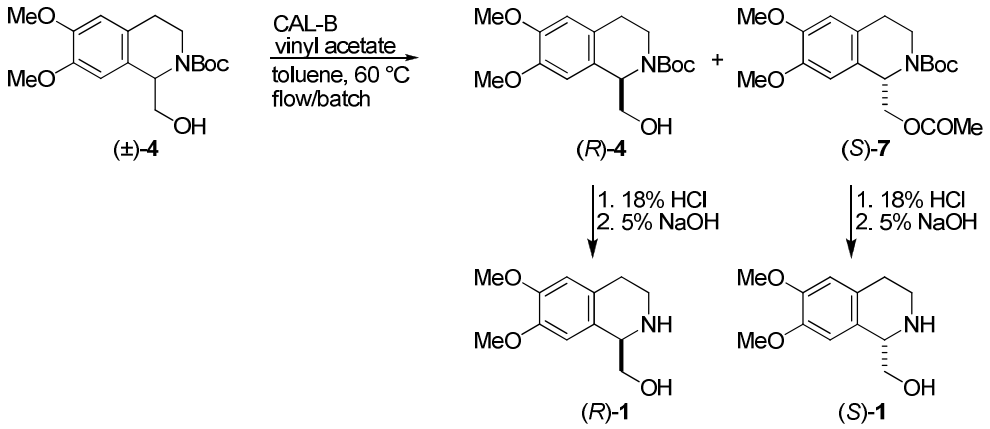
Figure 2. Enzymatic *O*-acylations in a flow reactor

The preparative scale resolutions were performed as a batch reaction and the enantiomeric excess of the obtained product ester and unreacted aminoalcohol were dermined by chiral column equipped HPLC.

In the preliminary experiments we tested the effect of the enzymes, solvent, acyl-donor, additive and temperature and in case of reaction performed in continuous-flow (CF) the effect of pressure on the reaction rate and enantioselectivity.

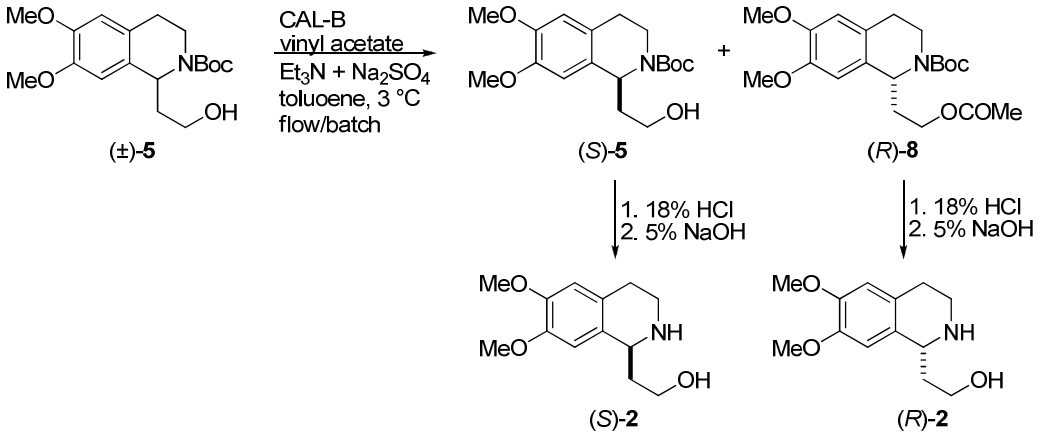
3. Results and discussion

3.1. We determinated the optimum conditions for the enzyme-catalysed *O*-acylation of (±)-**4** in CF system (E > 200). The preparative scale resolution of calycotomine was performed as a batch reaction under the optimal conditions [CAL-B (*Candida antarctica* lipase B), vinyl acetate, toluene, 60 °C]. We prepared the desired calycotomine enantiomers with Boc deprotection and ester hydrolyses of the enantiomeric products [(*R*)-**4** and (*S*)-**7**] obtained in preparative scale resolution (Scheme 2). Both calycotomine enantiomers were characterised with excellent enantiomeric excess (*ee* > 99%).



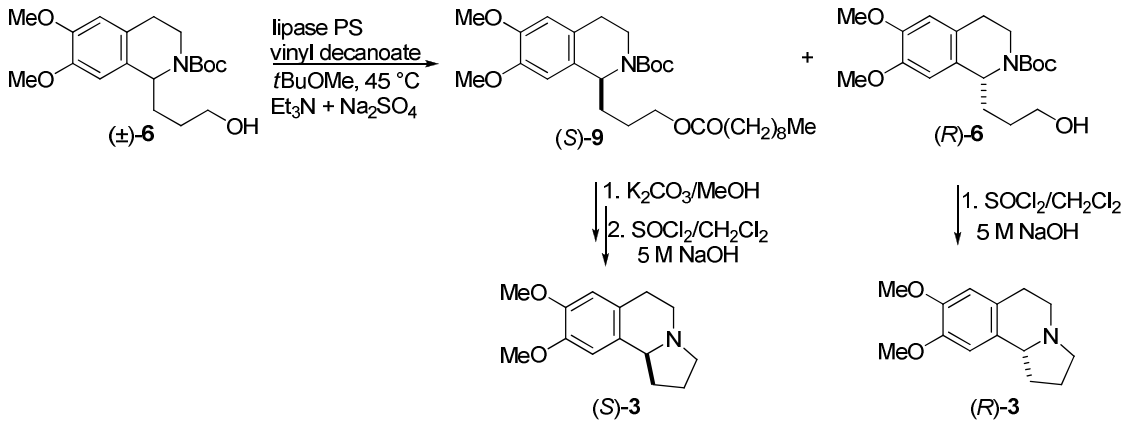
Scheme 2.

3.2. We combined the CF technique with batch in the phase of preliminary experiments for the enzyme catalysed *O*-acylation of (±)-**5**. The reaction preformed under the optimal conditions (CAL-B, vinyl acetate, Et_3N, Na_2SO_4, toluene, 3 °C) was characterised with a good enantioselectivity (*E* = 88). Homocalycotomine enantiomers were prepared with Boc deprotection and ester hydrolyses of the ester (*R*)-**8** and the unreacted alcohol (*S*)-**5** and the obtained enantiomers were characterised with good enantiomeric excess (*ee* > 94%).



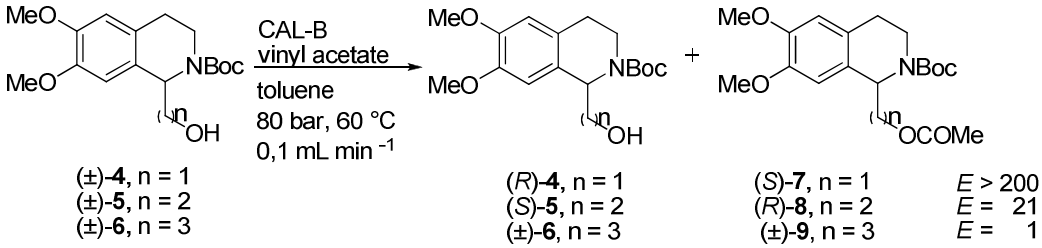
Scheme 3.

3.3. We have developed the first such total synthesic route for the preparation of crispine A enantiomers which contains an enzymatic key step, namely the *S*-selective *O*-acylation of *N*-Boc protected 1-(3-hydroxypropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (±)-**6** (E = 59). The reaction was performed with lipase PS (*Burkholderia cepacia*) as enzyme, vinyl decanoate as acyl donor, Et_3N and Na_2SO_4 as additive in *t*BuOMe at 45 °C. Boc deprotection and the cyclization of (*R*)-**6** and (*S*)-**6** were performed in one step. The obtained crispine A enantiomers [(*S*)-**3** és (*R*)-**3**] were characterised with good enantiomerically excess (*ee* ≥ 94%).



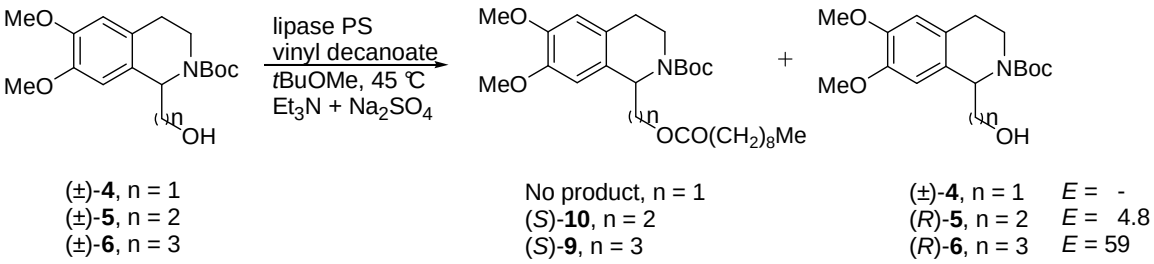
Scheme 4.

3.4. We have performed a systematic study to investigate the effects of the remote stereogenic centre (n = 1, 2, 3) on the conversion and E in case of six different enzymes. We have concluded that the enantioselectivity is strongly dependent on the used enzyme. In case of CAL-A (*Candida antarctica* lipase A), PPL (porcine pancreas lipase), lipase AY (*Candida rugosa*) and lipase AK (*Pseudomonas fluorescens*) the distance between the reaction centre and stereogenic centre did not have effect on enantioselectivity, while in case of CAL-B (vinyl acetate at 80 bar, 60 °C and 0.1 mL min^{-1} flow rate in toluene after one run) a significant decrease in enantioselectivity (E = 200 → 1) with increases of distance was observed.



Scheme 5.

In case of lipase PS (batch reaction, vinyl decanoate as acyl donor in *t*BuOMe at 45 °C and catalytic amount of Et_3N and Na_2SO_4) a significant decrease in enantioselectivity was observed from E = 59 (n = 3) to E = 4.8 (n = 2) and when n = 1 was no reaction observed with decrease of distance between the reaction and stereogenic centre.



Scheme 6.

4. Publications and lectures

Papers related to this thesis

I. E. Forró, **L. Schönstein**, F. Fülöp
Total synthesis of crispine A enantiomers through a *Burkholderia cepacia* lipase-catalysed kinetic resolution
Tetrahedron: Asymmetry **2011**, *22*, 1255-1260.

II. **L. Schönstein**, E. Forró, F. Fülöp
Continuous-flow enzymatic resolution strategy for the acylation of amino alcohols with a remote stereogenic centre: synthesis of calycotomine enantiomers
Tetrahedron: Asymmetry **2013**, *24*, 202-206.

III. **L. Schönstein**, E. Forró, F. Fülöp
Enzymatic reaction for the preparation of homocalycotomine enantiomers
Tetrahedron: Asymmetry **2013**, *24*, 1059-1062.

IV. **Schönstein L.**, Forró E., Fülöp F.
Tetrahidroizokinolin-vázas vegyületek enzimes rezolválása szakaszos és áramlásos kémiai módszerrel
Magy. Kém. Foly. **2014**, *120*, 26-31.

Other papers

V. E. Forró, **L. Schönstein**, L. Kiss, A. Vega-Peñaloza, E. Juaristi and F. Fülöp
Direct enzymatic route for the preparation of novel enantiomerically enriched hydroxylated β-amino ester stereoisomers
Molecules **2010**, *15*, 3998-4010.

Lectures related to this thesis

I. **Schönstein László**, Forró Enikő, Fülöp Ferenc
Kriszpin A enantiomerek szintézise enzim katalizált kinetikus rezolválással
"Szegedi Ifjú Szerves Kémikusok Támogatásáért" Alapítvány 10. tudományos előadó ülése, 5 May, 2010, Szeged, Hungary, oral presentation.

II. **Schönstein László**, Forró Enikő, Fülöp Ferenc
Kriszpin A enantiomrek totálszintézise enzim katalizált kinetikus rezolválással
"Szegedi Ifjú Szerves Kémikusok Támogatásáért" Alapítvány 11. tudományos előadó ülése, 18 April, 2011, Szeged, Hungary, oral presentation.

III. **Schönstein László**, Enikő Forró, Ferenc Fülöp
Kriszpin enantiomerek enzimes szintézise
MTA Alkaloidkémiai munkabizottságának ülése, 16-17 May, 2011, Balatonalmádi, Hungary, oral presentation.

IV. **László Schönstein**, Enikő Forró, Ferenc Fülöp
Total synthesis of crispine A enantiomers through a Burkholderia cepacia lipase catalysed kinetic resolution
XIVth Conference on Heterocycles in Bio-organic Chemistry, 4-8 September, 2011, Brno, Czech Republic, poster presentation (P-29).

V. **Schönstein László**, Forró Enikő, Fülöp Ferenc
Kalikotomin és homokalikotomin enzimes rezolválása áramlásos kémiai módszerrel
MTA Alkaloid- és Flavonoidkémiai Munkabizottsága ülése, 14-15 May, 2012, Balatonalmádi, Hungary, oral presentation.

VI. **Schönstein László**, Forró Enikő, Fülöp Ferenc
Kriszpin A enantiomerek totálszintézise enzimkatalizált kinetikus rezolválással
Kutatóegyetemi kiválósági központ létrehozása a szegedi tudományegyetemen, Molekulától a gyógyszerig, 24-25 May, 2012, Szeged, Hungary, poster presentation (P-03).

VII. **Schönstein László**, Forró Enikő, Fülöp Ferenc
Homokalikotomin enantiomerek enzimes előállítása folyamatos és szakaszos üzemmódban
MTA Alkaloid- és Flavonoidkémiai Munkabizottsága ülése, 13-14 May, 2013, Balatonalmádi, Hungary, oral presentation.

VIII. **László Schönstein**, Enikő Forró, Ferenc Fülöp
Continuous-flow enzymatic preparation of calycotomine enantiomers
15th Blue Danube Symposium on Heterocyclic Chemistry, 1-5 September, 2013, Olomouc, Czech Republic, poster presentation (P-67).

Other lectures

IX. **László Schönstein**, Enikő Forró, Loránd Kiss, Ferenc Fülöp
Enzymatic hydrolysis of hydroxylated alicyclic β-amino esters
Foldamers: building blocks, structure and function, 24-26 September, 2009, Szeged, Hungary, poster presentation (P-04).

X. **László Schönstein**, Enikő Forró, Lóránd Kiss, Alberto Vega Peñaloza, Eusebio Juaristi, Ferenc Fülöp
Direct enzymatic route for the preparation of novel enantiomerically enriched hydroxylated β-amino ester stereoisomers
Foldamers: Synthesis and Structure of Functional Materials, 7-9 April, 2011, Barcelona, Spain, poster presentation (P-06).