PhD Thesis

# Enzymatic kinetic resolution of β-amino acid derivatives

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# A. Introduction and aims

In a consequence of their unique chemical and biological properties, aliphatic and alicyclic β-amino acids and their derivatives are important subjects of research. The most obvious examples are the naturally occurring cispentacin [(1*R*,2*S*)-2-aminocyclopentane-1-carboxylic acid] and its synthetic derivatives which is a potent antifungal antibiotics. β-Amino acids are important intermediates of β-lactams and heterocycles, and are constituents of many biologically active compounds, as antitumour taxoids. The incorporation of β-amino acids in α-peptides may modify the structure and the biological effect, and make the peptide resistant against proteolytic degradation. The secondary structure and folding properties of β-peptides have been studied deeply. β-Amino nitriles, -carboxamides and β-amino esters are intermediates of the corresponding β-amino acids.

The basic aim of this PhD work was to perform the enzymatic kinetic resolution of alicyclic *cis* and *trans*-β-aminocyclopentane- and -cyclo-hexanecarbonitriles (**1**-**4**) and the corresponding amino carboxamides (**5**-**8**; Figure 1). Besides the development of a suitable method for the preparation of enantiomerically pure β-amino acid derivatives, we planned to follow up the stereochemical preference of lipases in organic solvents. A further aim was to compare our results with those to be found in the literature for the enzymatic resolution of corresponding β-amino esters. It was also planned to develop an enzymatic method for the resolution of ethyl 3-amino-2-ethylpropanoate **9** and methyl 3-amino-2-isopropylpropanoate **10** through lipase-catalysed *N*-acylation (Figure 1).

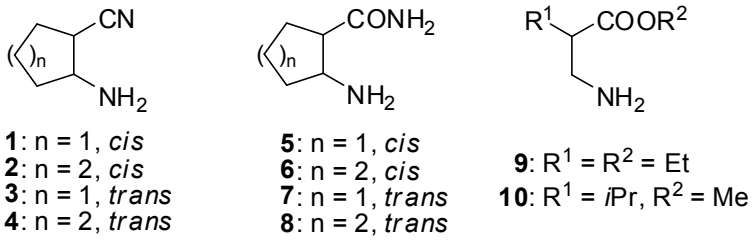
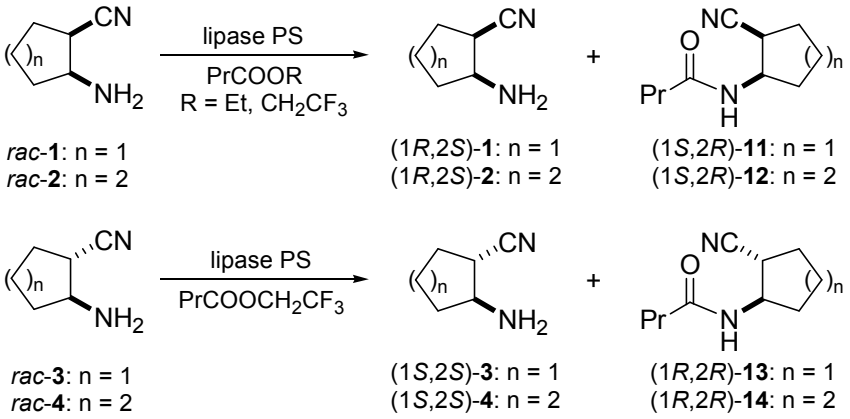



**Figure 1**

We set out to study the effects of the used enzyme, acyl donor and solvent on the reaction rate and enantioselectivity (*E*) in the frame of preliminary experiments, to summarize these results, and then to perform the gram-scale resolutions of the model compounds under the optimized conditions.

## B. Results and discussion

For the enantioselective *N*-acylation of alicyclic β-amino nitriles **1-4** (Scheme 1), lipase PS, CAL-A and CAL-B preparations were tested: the lipase PS preparations allowed the resolution of **1-4** in TBME with 2 equivalents of 2,2,2-trifluoroethyl butanoate ($E > 200$).

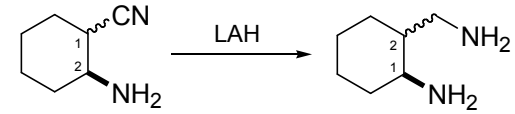



**Scheme 1**

We monitored the benefit of the activated ester (2,2,2-trifluoroethyl butanoate) in contrast with other acylating agents (*e.g.* ethyl butanoate) on the lipase PS-catalysed *N*-acylation of **2**. We decreased the rate of retardation of the enzymatic acylation to close to 50% conversion by using an increased amount of

lipase PS, which permitted the resolution of **2** in a reasonable time. The CAL-B-catalysed *N*-acylation of **2** in TBME and ionic liquids was not successful. We improved the solubility of *trans* compounds in TBME by the addition of TAA as co-solvent (for **3**) or by performing the reactions at elevated temperature (for **4**).

The gram-scale resolutions of **1**-**4** under the optimized conditions afforded the *N*-acylated (1*S*,2*R*)-**11**, (1*S*,2*R*)-**12**, (1*R*,2*R*)-**13** and (1*R*,2*R*)-**14** and the unreacted enantiomers with high enantiopurity (ee ≥ 98%), which were separated by column chromatography. We demonstrated that, through reduction by LAH, (1*R*,2*S*)-**2** and (1*S*,2*S*)-**4** can be transformed to the corresponding diamines (1*S*,2*S*)-**15** and (1*S*,2*R*)-**16** with only a slight drop in ee, and these results proved the *R* selectivity of the enzymatic acylation (Scheme 2).

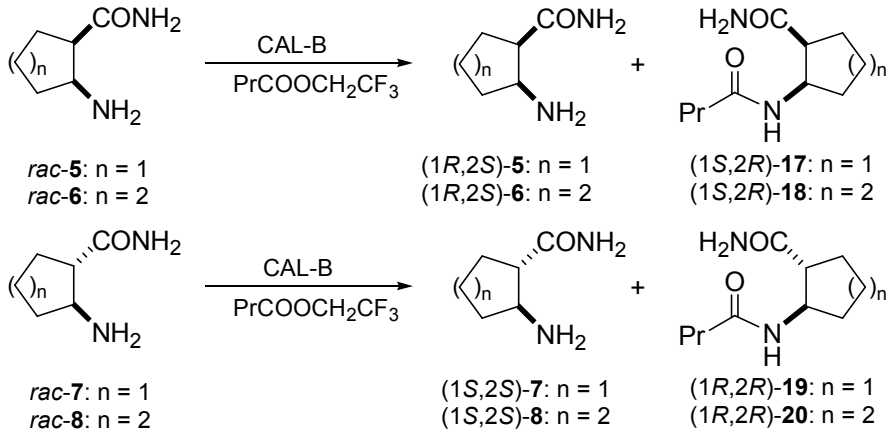



(1*R*,2*S*)-**2**: *cis*
(1*S*,2*S*)-**4**: *trans*

(1*S*,2*S*)-**15**: *cis*
(1*S*,2*R*)-**16**: *trans*

**Scheme 2**

The high polarity of alicyclic β-amino carboxamides **5**-**8** required the addition of co-solvents to TBME and elevated temperature (48 °C). CAL-B (50 mg/mL) proved to be a powerful catalyst for the enantioselective acylation of these compounds with 2 equivalents of 2,2,2-trifluoroethyl butanoate (Scheme 3).




*rac*-**5**: n = 1
*rac*-**6**: n = 2

(1*R*,2*S*)-**5**: n = 1
(1*R*,2*S*)-**6**: n = 2

(1*S*,2*R*)-**17**: n = 1
(1*S*,2*R*)-**18**: n = 2

*rac*-**7**: n = 1
*rac*-**8**: n = 2

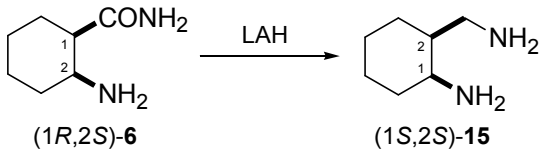
(1*S*,2*S*)-**7**: n = 1
(1*S*,2*S*)-**8**: n = 2

(1*R*,2*R*)-**19**: n = 1
(1*R*,2*R*)-**20**: n = 2

**Scheme 3**

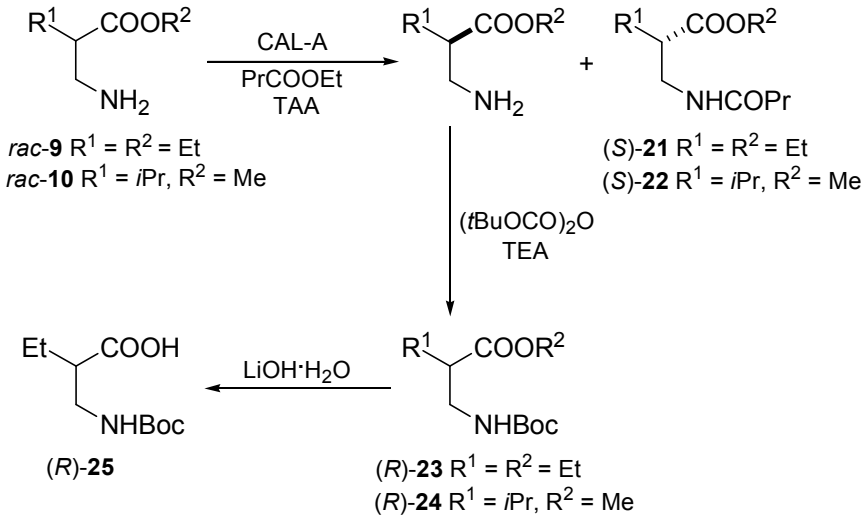
The effects of co-solvents were analysed, and it was found that TAA ensured the best results. Our experiments established that the reaction rate decreased with the elevation of the ratio of TAA. The *N*-acylations of **5**, **7** and **8** were highly selective ($E > 200$) in TBME-TAA (1:1); whereas for **6** a mixture of TBME-TAA (4:1) proved to be the optimum reaction medium ($E = 40$) and the resolution was performed in two stages.

The unreacted amino carboxamides (1*R*,2*S*)-**5**, (1*R*,2*S*)-**6**, (1*S*,2*S*)-**7** and (1*S*,2*S*)-**8** and the opposite enantiomers as butyramides were obtained with ee ≥ 95%, after separation on silica. The reduction of (1*R*,2*S*)-**6** by LAH resulted in (1*S*,2*S*)-**15**, verifying the *R* selectivity of CAL-B (Scheme 4).




**Scheme 4.**

On the basis of the results obtained for the CAL-A (50 mg/mL)-catalysed *N*-acylation of **9** (Scheme 5) with 2 equivalents of VA in DIPE at 25 °C ($E = 4$), further optimizations were developed; the use of lower temperature (4 °C), a reduced amount of CAL-A (25 mg/mL) and ethyl butanoate (0.55 equiv) as acyl donor led to a slightly better result ($E = 6.7$). Solvent screening revealed the influence of the solvent on the enzymatic acylation: in polar solvents (MeCN and TAA), the reactions were slow (26% and 40% conversion after 25 days, respectively) and highly enantioselective ($E > 200$). In order to resolve **9** in a reasonable time, the effects of temperature and the enzyme concentration were examined, and it was found that an elevated amount of CAL-A (50 mg/mL) in TAA at 4 °C offers a faster reaction with good selectivity (46% conversion after 15 days; $E = 63$).




**Scheme 5.**

When the optimal resolution conditions for **9** with a reduced enzyme amount (25 mg/mL) were applied to **10**, a low reaction rate (49% conversion after 35 days) and low selectivity ($E$ = 9) were obtained; the enantiomers of **10** were therefore prepared in two consecutive steps (Scheme 5).

After the gram-scale resolution reactions were stopped, the unreacted (*R*)-**9** and (*R*)-**10** were transformed to their Boc-protected forms (*R*)-**23** (ee = 95%) and (*R*)-**24** (ee = 78%), and separated from the butyramides (*S*)-**21** (ee = 85%) and (*S*)-**22** (ee = 76%) by column chromatography (Scheme 5). The *S*-enantiopreference of the enzyme was proved by the hydrolysis of (*R*)-**23** with $LiOH \cdot H_2O$ to the *N*-Boc-protected amino acid *R*-**25** (Scheme 5).

The 20 enantiomers prepared (19 among them new) were characterized by ee values, optical rotations, melting points and $^{1}$H-NMR. $^{13}$C-NMR and elemental analysis results were also established for β-amino nitrile and β-amino carboxamide enantiomers.

# Abbreviations

| | |
|---|---|
| CAL-A | *Candida antarctica* lipase A |
| CAL-B | *Candida antarctica* lipase B |
| DIPE | diisopropyl ether |
| LAH | lithium aluminium hydride |
| lipase PS | *Burkholderia cepacia* lipase |
| TAA | *tert*-amyl alcohol |
| TBME | *tert*-butyl methyl ether |

# C. Papers related to the thesis*

I. **Fitz, M**.; Lundell, K.; Lindroos, M.; Fülöp, F.; Kanerva L. T.
An effective approach to the enantiomers of alicyclic β-amino nitriles by using lipase catalysis
*Tetrahedron: Asymmetry* **2005,** *16*, 3690-3697. i.f.: 2.468

II. **Fitz, M**.; Lundell, K.; Fülöp, F.; Kanerva, L. T.
Lipase-catalysed kinetic resolution of 2-aminocyclopentane- and 2-aminocyclohexanecarboxamides
*Tetrahedron: Asymmetry* **2006,** *17*, 1129-1134. i.f.: 2.468

III. **Fitz, M**.; Forró, E.; Vigóczki, E.; Lázár, L.; Fülöp, F.
Lipase-catalysed *N*-acylation of $\beta^2$-amino esters
*Tetrahedron: Asymmetry* **2008,** *19*, 1114-1119. i.f.: 2.468

* Impact factors from the year 2006 are shown.

# D. Conference lectures related to the thesis

IV. **Fitz, M**.
A *cisz*- és *transz*-2-amino-ciklopentán- és -ciklohexánkarboxamidok enzim-katalizált kinetikus rezolválása
VII. Clauder Ottó Emlékverseny, Visegrád, 2004.

V. **Fitz, M**.
A *cisz*- és *transz*-2-amino-ciklopentán- és -ciklohexánkarboxamidok enzim-katalizált kinetikus rezolválása
„A Szegedi Ifjú Szerves Kémikusok Támogatásáért" Alapítvány tudományos előadóülése, 2005.

VI. **Fitz, M**.; Lundell, K.; Kanerva, L. T.; Fülöp, F.
Enzyme catalysed kinetic resolution of cyclic β-amino amides and β-amino nitriles
*7th International Symposium on Biocatalysis and Biotransformations*, Delft, The Netherlands, 2005.

VII. **Fitz M**.; Lundell, K.; Kanerva, L. T.; Fülöp F.
Ciklusos β-amino nitrilek és β-aminosavamidok enzim-katalizált kinetikus rezolválása
*Vegyészkonferencia*, Hajdúszoboszló, 2005.