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 [(1R,2S)-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).
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 $\beta$-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 ($E > 200$).

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 (1S,2R)-11, (1S,2R)-12, (1R,2R)-13 and (1R,2R)-14 and the unreacted enantiomers with high enantiopurity (ee ≥ 98%), which were separated by column chromatography. We demonstrated that, through reduction by LAH, (1R,2S)-2 and (1S,2S)-4 can be transformed to the corresponding diamines (1S,2S)-15 and (1S,2R)-16 with only a slight drop in ee, and these results proved the R selectivity of the enzymatic acylation (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).
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 ($1R,2S$)-5, ($1R,2S$)-6, ($1S,2S$)-7 and ($1S,2S$)-8 and the opposite enantiomers as butyramides were obtained with ee $\geq 95\%$, after separation on silica. The reduction of ($1R,2S$)-6 by LAH resulted in ($1S,2S$)-15, verifying the $R$ selectivity of CAL-B (Scheme 4).

![Scheme 4.](image)

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; $E = 63$).
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·H$_2$O 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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CAL-A</td>
<td><em>Candida antarctica</em> lipase A</td>
</tr>
<tr>
<td>CAL-B</td>
<td><em>Candida antarctica</em> lipase B</td>
</tr>
<tr>
<td>DIPE</td>
<td>diisopropyl ether</td>
</tr>
<tr>
<td>LAH</td>
<td>lithium aluminium hydride</td>
</tr>
<tr>
<td>lipase PS</td>
<td><em>Burkholderia cepacia</em> lipase</td>
</tr>
<tr>
<td>TAA</td>
<td>tert-amyl alcohol</td>
</tr>
<tr>
<td>TBME</td>
<td>tert-butyl methyl ether</td>
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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  
i.f.: 2.468

II. Fitz, M.; Lundell, K.; Fülöp, F.; Kanerva, L. T.  
Lipase-catalysed kinetic resolution of 2-aminocyclopentane- and 2-aminocyclohexanecarboxamides  
i.f.: 2.468

III. Fitz, M.; Forró, E.; Vigóczki, E.; Lázár, L.; Fülöp, F.  
Lipase-catalysed N-acylation of β²-amino esters  
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-ciklopendánt és -ciklohexánkarboxamidok enzim-katalizált kinetikus rezolválása

V. Fitz, M.
A cisz- és transz-2-amino-ciklopendánt és -ciklohexánkarboxamidok enzim-katalizált kinetikus rezolválása

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,

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