# Direct enzymatic route for the preparation of novel enantiomerically enriched hydroxylated β-amino ester stereoisomers


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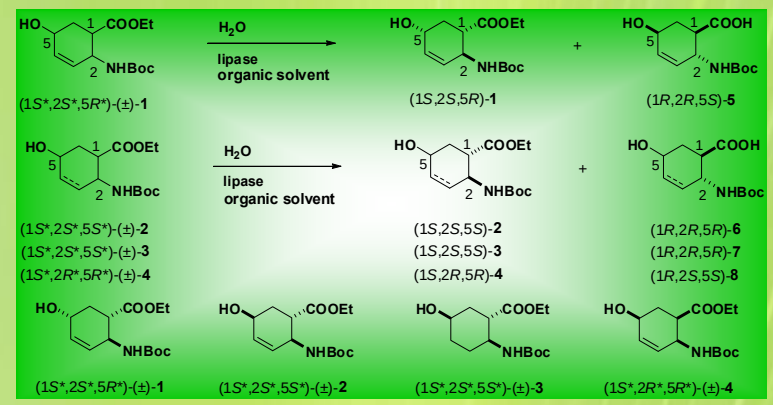
## Introduction

Hydroxylated *β*-amino acids represent a valuable class of amino acids, because they are important key elements of many biologically relevant compounds. For example taxol and taxotere having a hydroxylated *β*-amino acid residue in their structure are effective clinically available chemotherapeutic agents for the treatment of cancer diseases [1]. Among the cyclic analogues several hydroxylated *β*-amino acid derivatives have antibiotic (e.g. oryzoxymycin [2]) or antifungal activities, and are building blocks for pharmaceutically important natural substances such as fortamine, chryscandin, pentopyranamine, gougerotin and blasticidin [3]. The cyclic, conformationally restricted *β*-amino acids have been reported as building blocks in the construction of novel peptides [4]. The hydroxylated *β*-amino acids might be interesting components for the synthesis of potential biologically active peptides having also a relevant contribution in their secondary structure.

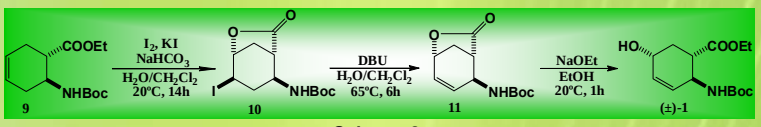
## Results and discussion

Our earlier results on enzymatic hydrolysis of alicyclic *β*-amino esters [2] suggested the possibility of the lipase-catalysed enantioselective hydrolysis of hydroxylated *β*-amino esters (±)-1 - (±)-4, in an organic solvent (Scheme 1).




Scheme 1

Racemic hydroxylated *β*-amino ester (±)-1 was prepared starting from *N*-Boc-protected amino ester (9) via iodolactonization reaction (10), followed by HI elimination in the presence of DBU as base and opening of the resulted lactone (11) with NaOEt in EtOH (Scheme 2).




Scheme 2

In order to determine the optimal conditions for the enzymatic resolutions, extensive enzyme screening was performed for the hydrolysis of (±)-1 with 0.5 equiv. of $H_2O$ in *i*$Pr_2O$ at 45 °C or 60 °C (Table 1).

Table 1. Conversion and enantioselectivity of hydrolysis of (±)-1[a]

| Entry | Enzyme (30 mg mL$^{-1}$) | $H_2O$ (equiv.) | *T* (°C) | *t* (h) | Conv. (%) | $ee_s$[b] (%) | $ee_p$[b] (%) | *E* |
|---|---|---|---|---|---|---|---|---|
| 1 | CAL-B | 0.5 | 60 | 48 | 37 | 48 | 82 | 16 |
| 2 | CAL-A | 0.5 | 60 | 48 | 44 | 7 | 9 | 1 |
| 3 | lipase PS | 0.5 | 45 | 48 | 22 | 10 | 35 | 2 |
| 4 | lipase AY | 0.5 | 45 | 48 | 11 | 2 | 16 | 1 |
| 5 | lipase AK | 0.5 | 45 | 48 | 25 | 1 | 3 | 1 |
| 6 | PPL | 0.5 | 45 | 48 | 20 | 5 | 20 | 2 |

[a] 0.05 M substrate, 1 mL *i*$Pr_2O$. [b] According to HPLC.

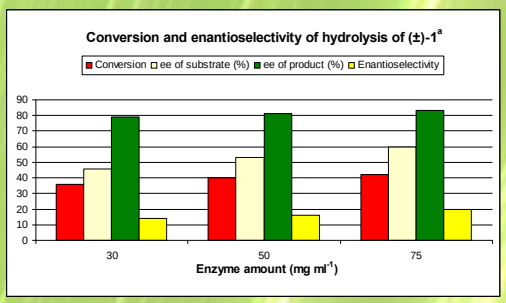
In an attempt to increase the *E* and reaction rate, a set of experiments were performed in different solvents and at different temperatures. Relatively high enantioselectivity (*E* = 48) was observed when the CAL-B (*Candida antarctica* lipase B)-catalysed hydrolysis of (±)-1 was performed with $H_2O$ (0.5 equiv.) in *t*-BuOMe at 60 °C (Table 2, entry 5).

Table 2. Conversion and enantioselectivity of hydrolysis of (±)-1[a]

| Entry | T (°C) | Solvent | Conv. (%) | $ee_s$[b] (%) | $ee_p$[b] (%) | *E* |
|---|---|---|---|---|---|---|
| 1 | 60 | *i*$Pr_2O$ | 37 | 48 | 82 | 16 |
| 2 | 60 | *n*-hexane | 24 | 18 | 58 | 4 |
| 3 | 60 | toluene | 35 | 48 | 91 | 34 |
| 4 | 60 | 1,4-dioxane | 25 | 16 | 48 | 3 |
| 5 | 60 | *t*-BuOMe | 45 | 75 | 91 | 48 |
| 6 | 50 | *t*-BuOMe | 35 | 47 | 86 | 21 |
| 7 | 40 | *t*-BuOMe | 36 | 46 | 81 | 15 |

[a] 0.05 M substrate, 30 mg ml$^{-1}$ CAL-B, 48 h. [b] According to HPLC.

The *E* and reaction rate for the hydrolysis of (±)-1 clearly increased as the amount of the enzyme was increased (Scheme 3).




Scheme 3

On the basis of these preliminary results, preparative-scale resolutions of (1*S**,2*S**,5*R**)-(±)-1, (1*S**,2*S**,5*S**)-(±)-2, (1*S**,2*S**,5*S**)-(±)-3 and (1*S**,2*R**,5*R**)-(±)-4 were performed with 0.5 equiv. of $H_2O$, in the presence of CAL-B (30 mg mL$^{-1}$), in *t*-BuOMe, at 60 °C. The results are presented in Table 3.

Table 3. CAL-B-catalysed hydrolysis of (±)-1 - (±)-4[a]

| Racemate | Time (days) | Conv. (%) | Unreacted enantiomer | | | |
|---|---|---|---|---|---|---|
| | | | Yield (%) | Isomer | $ee$[b] (%) | $[\alpha]_D^{25}$ (EtOH) |
| (1*S**,2*S**,5*R**)-(±)-**1** | 10 | 43 | 32 | (1*S*,2*S*,5*R*)-**1** | 68 | +34 (*c* 0.19) |
| (1*S**,2*S**,5*S**)-(±)-**2** | 10 | 41 | 34 | (1*S*,2*S*,5*S*)-**2** | 78 | +16 (*c* 0.165) |
| (1*S**,2*S**,5*S**)-(±)-**3** | 10 | 36 | 29 | (1*S*,2*S*,5*S*)-**3** | 54 | -4 (*c* 0.24) |
| (1*S**,2*R**,5*R**)-(±)-**4** | 7 | 43 | 41 | (1*S*,2*R*,5*R*)-**4** | 90 | +100 (*c* 0.155) |

[a] 50 mg mL$^{-1}$ CAL-B in *t*-BuOMe 0.5 equiv. $H_2O$, 60 °C. [b] According to HPLC.

The hydroxy-substituted *β*-amino acid enantiomers (5-8) are missing from the Table 3, as the *β*-amino acids (1*R*,2*R*,5*S*)-5, (1*R*,2*R*,5*R*)-6, (1*R*,2*R*,5*R*)-7 and (1*R*,2*S*,5*S*)-8 underwent polymerization (the side-products were not characterized).

## Conclusions

Four hydroxy-substituted *β*-amino ester stereoisomers were resolved through a simple direct enzymatic method, involving CAL-B-catalyzed enantioselective hydrolysis in organic media. Moderate to good enantiomeric excess values (*ee* ≥ 52%) were obtained for the unreacted amino esters when the reactions were performed with 0.5 equiv. of added $H_2O$, in *t*-BuOMe, at 60 °C. Due to polymerization, the supposed *β*-amino acid enantiomers practically could not be isolated.

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