SYNTHESIS OF NEW FLUORINE-CONTAINING β-AMINO ACID ENANTIOMERS THROUGH LIPASE CATALYZED HYDROLYSIS

Sayeh Shahmohammadi, Enikő Forró, and Ferenc Fülöp

Institute of Pharmaceutical Chemistry, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary
e-mail: sayeh.s@pharm.u-szeged.hu

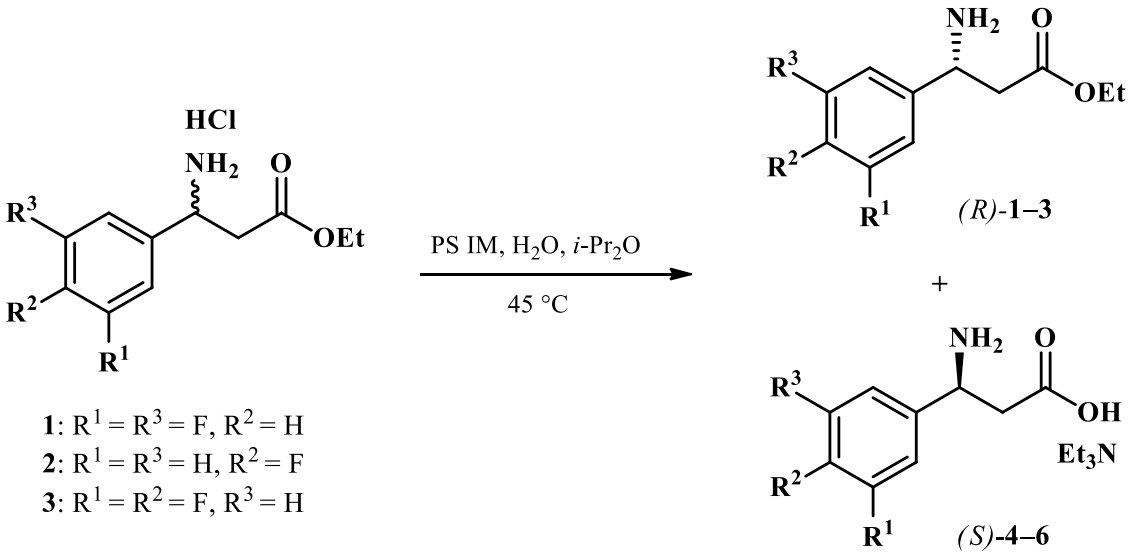
Abstract
An efficient and novel enzymatic method was developed for the synthesis of β-aryl-fluorine-containing β-amino acid enantiomers through lipase PS IM (*Burkholderia cepasia*) catalyzed hydrolysis in *i*-Pr_2O at 45 °C in the presence of Et_3N. In order to follow the enzymatic reactions, an adequate analytical method was devised for the enantioseparation of racemic β-amino esters and β-amino acids.

Introduction
In recent years, enantiomerically pure β-aryl-substituted β-amino acids have been intensively investigated due to their unique and remarkable biological properties [1] and their utility in drug research [2]. In particular, incorporation of fluorine into the structure of β-amino acids has become the core of interest of scientists in the last decades, because of unique properties of fluorine atom and its importance for the synthesis of pharmaceuticals [3]. Enzymatic hydrolysis of both cyclic [4] and acyclic [5] β-amino esters is known in the literature. Herein, our aim was to devise an enzymatic protocol for the preparation of new enantiomeric β-aryl-fluorine-containing β-amino acids, important from both pharmaceutical and chemical aspects.

Experimental
Lipase PS IM was from Amano Pharmaceuticals. Substituted benzaldehydes were from Sigma-Aldrich. Triethylamine was from Merck. The solvents were of the highest analytical grade and from Sigma-Aldrich. Optical rotations were measured with a Perkin-Elmer 341 Polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. The enantiomeric excess *ee* values for the unreacted β-amino easter and the β-amino acid enantiomers produced were determined by GC equipped with a Chirasil-L-Val column after double derivatization [6] with (i) diazomethane [**Caution!** the derivatization with diazomethane should be performed under a well-working hood]; (ii) acetic anhydride in the presence of 4-dimethylaminepyridine and pyridine [90 °C for 10 min → 170 °C (temperature rise 20 °C min^{-1}), 10 psi].

Results and discussion
Racemic β-amino acids were synthesized with good yields (42-75%) by Rodionov synthesis [7] through the reactions of aldehydes with malonic acid in the presence of NH_4OAc in EtOH at reflux. Subsequently, the β-amino esters were prepared with yields ranging from 76 to 87% by the esterification of corresponding β-amino acids in the presence of $SOCl_2$ in EtOH.

Preparative scale resolution of (±)-**1**-**3** were performed under the optimized conditions [lipase PS IM, H_2O, *i*-Pr_2O, Et_3N,], and the reaction mixture was shaken in an incubator shaker at 45 °C, 200 rpm. The progress of reaction was followed by taking samples from the reaction mixture at intervals and analyzing them by gas chromatography. The reaction was stopped by filtering off the enzyme at 50% conversion. The products were separated by column chromatography. Unreacted amino esters (*R*)-**1**-**3** and the product amino acids (*S*)-**4**-**6** were obtained with high *ee* (≥ 99%), and good yields (≥ 48%).



1: $R^1 = R^3 = F$, $R^2 = H$
2: $R^1 = R^3 = H$, $R^2 = F$
3: $R^1 = R^2 = F$, $R^3 = H$

Conclusion

Novel fluorine-containing amino acid enantiomers have been prepared through PS IM lipase catalyzed hydrolysis of racemic amino esters **1-3**. Excellent enantioselectivities (E>200) were obtained when the reactions were performed with H_2O as a nucleophile in *i*-Pr_2O at 45 °C, in the presence of Et_3N. Both the unreacted amino esters *(R)*-**1-3** and product amino acids *(S)*-**4-6** were isolated with high *ee* (≥ 99%), and promising yields (≥ 48%). To follow the enzymatic reactions and calculate the enantiomeric excess, conversion and enantioselectivities suitable analytical methods were optimized.

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