PhD Thesis

Direct enzymatic routes to β-substituted β-amino acid enantiomers

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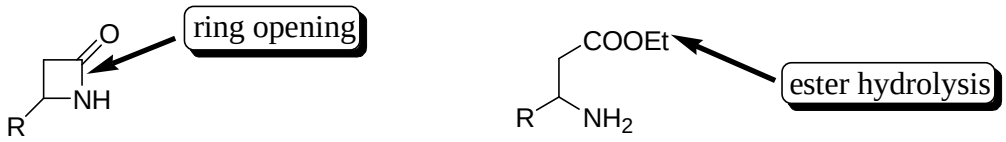
1. Introduction and aims

The significance of β-lactams and β-amino acids is well known in the literature. The natural product cispentacin [(1*R*,2*S*)-2-aminocyclopentane-1-carboxylic acid] and its synthetic derivatives have proved to be efficient antifungal agents. However, numerous biologically active, more complex compounds contain a β-aryl-, β-heteroaryl- or β-arylalkyl-β-amino acid enantiomer. Elarofiban, a derivative of (*S*)-3-amino-3-(3-pyridyl)propanoic acid, has progressed successfully through human phase II clinical trials as an antithrombotic agent. Januvia™ (sitagliptin phosphate) was the first approved drug for the inhibition of dipeptidyl peptidase IV, a new therapeutic target for the treatment of type 2 diabetes. Sitagliptin contains an (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid subunit. β-Amino acids can be used as building blocks for the synthesis of modified peptides with increased activity and stability. β-Amino acids can be prepared in enantiomerically pure form by asymmetric synthetic routes or by the resolution of racemic compounds. In the latter case, biocatalysts are widely investigated and have come into the focus of research.

Lipases have been successfully applied for the resolution of carbocyclic β-lactams and β-amino esters. However, the ring opening of acyclic β-lactams and the hydrolysis of acyclic β-amino esters in an organic medium have been less deeply investigated. The aim of my thesis was the enzymatic kinetic resolution of 4-aryl- and 4-arylalkyl-substituted β-lactams through opening of the lactam ring and development of the enzymatic hydrolysis of β-aryl-, β-heteroaryl- and β-arylalkyl-substituted β-amino esters (Scheme 1).



R = aryl, arylalkyl R = aryl, heteroaryl, arylalkyl

Scheme 1

A number of reaction conditions, *e.g.* the amounts and effects of enzymes, the nature of the solvent, the temperature, and the presence of additives, were investigated in order to achieve the best reaction rate and enantioselectivity. The optimum reaction conditions were then extended to preparative-scale resolutions.

2. Methods

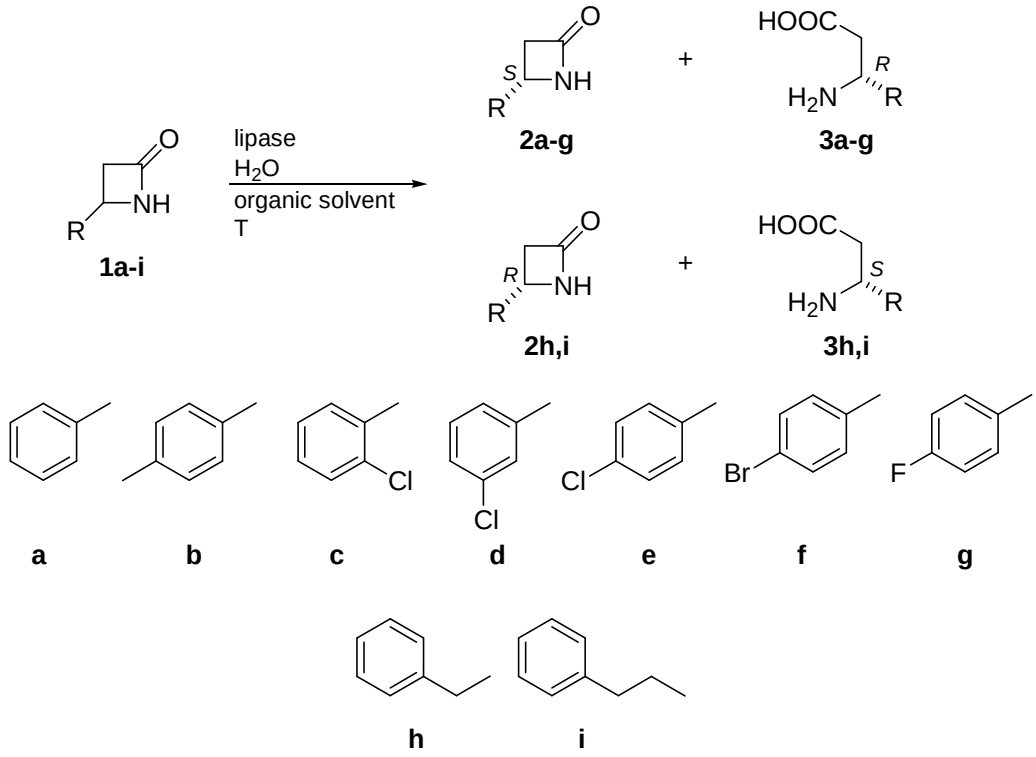
Racemic starting materials were synthetized according to literature methods. β-Lactams **1a-i** were prepared by the addition of chlorosulfonyl isocyanate to the corresponding alkenes, and β-amino esters **5a-o** by the modified Rodionov synthesis, followed by esterification; the reduction of enamines; or the ring opening of β-lactams with acidic EtOH. The preliminary enzymatic reaction experiments were performed on a milligram scale. The progress of reactions was followed by taking samples from the reaction mixture, and samples were injected directly or after derivatization into the chiral column of a GC or HPLC. Enantiomers prepared by preparative-scale resolution were characterized by NMR, melting point and specific rotation.

3. Results and discussion

3.1. Enzymatic ring opening of β-lactams[I,II,VII]

3.1.1. In the ring cleavage of 4-phenyl-2-azetidinone (**1a**) and 4-phenylethyl-2-azetidinone (**1i**) (Scheme 2), we found that differently immobilized forms of lipase B from *Candida antarctica* (CAL-B), *i.e.* Lipolase, Novozym 435 and Chyrazyme L-2, catalysed the ring opening of **1a** with excellent enantioselectivity ($E > 200$) in diisopropyl ether (*i*-Pr_2O) at 60 °C with 1 equiv. of H_2O. However, in the case of 4-phenylethyl-2-azetidinone (**1i**), low *E* values (~11) were observed in the presence of the above-mentioned CAL-B preparations and reaction conditions. Moreover, a decrease of the temperature from 60 °C to 45 °C did not result in an increase in *E*. Further enzymes, *e.g.* lipase A from *Candida antarctica* (CAL-A), lipase AK (from *Pseudomonas fluorescens*),

lipase AY (from *Candida rugosa*) and porcine pancreas lipase (PPL), displayed very low activities and selectivities for both model compounds (conv. ≤ 3%, $E \leq 2$).



Scheme 2

3.1.2. The ring opening of **1a** proved to be enantioselective (E > 200) in *i*-Pr_2O (conv. = 39% after 2 h) and toluene (conv. = 18% after 2 h), but a lower reaction rate was observed in toluene. In the case of the arylalykl-substituted compound **1i**, the nature of the solvent did not influence the reaction rate or enantioselectivity.

3.1.3. Addition of different additives, such as triethylamine (Et_3N), *N*,*N*-diisopropylethylamine (*i*-Pr_2EtN) or 2-octanol, to the reaction mixtures did not significantly affect either the enantioselectivity or the reaction rate of the ring cleavage of **1a,i**. Moreover, the hydrolysis was complete even without added H_2O in a small-scale resolution. The H_2O present in the reaction medium (< 0.1%) or on the surface of the enzyme preparation (< 5% w/w H_2O) was sufficient for the lactam ring opening.

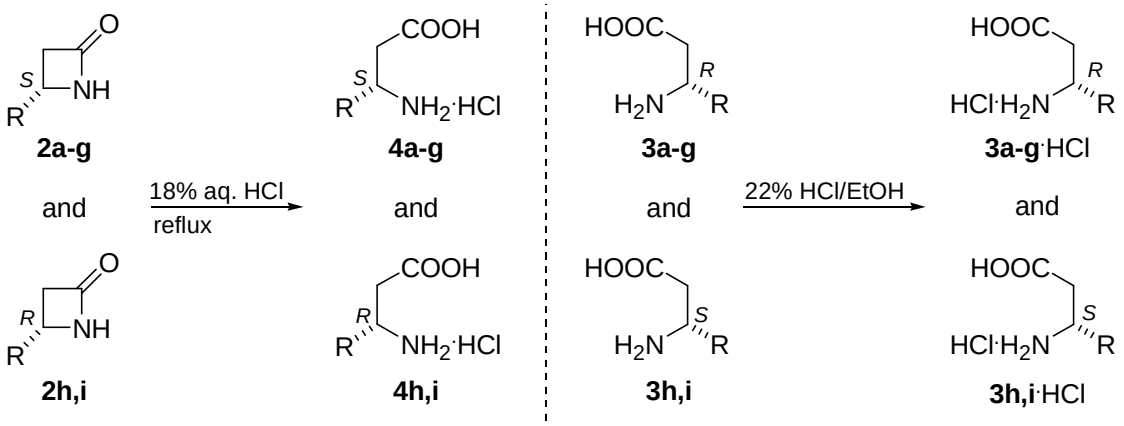
3.1.4. The reaction rate of the ring opening of 4-phenyl-2-azetidinone (**1a**) clearly increased as the amount of Lipolase was increased. The reaction rate was lowest in the presence of 10 mg mL^{-1} enzyme and needed 41 h to reach 49% conversion. Although the optimum enzyme quantity proved to be 75 mg mL^{-1}, preparative-scale reactions were performed with 30 mg mL^{-1} Lipolase for economic reasons.

3.1.5. When the Lipolase (50 mg/mL)-catalysed ring opening of *N*-Boc-4-phenylethyl-2-azetidinone (*N*-Boc-**1i**) was performed at 45 ºC, better results were not observed (conv. = 94% after 24 h, E = 3).

3.1.6. The Lipolase-catalysed ring opening of 4-phenyl-2-azetidinone (**1a**) in supercritical carbon dioxide ($scCO_2$) at 14 MPa and 70 °C ensured full conversion in 120 h. The resulting (*R*)-β-phenylalanine (**3a**) (*ee* ≥ 98%) and (*S*)-4-phenyl-2-azetidinone (**2a**) (*ee* ≥ 99%) were easily separated by $scCO_2$ extraction of the (*S*)-β-lactam and subsequent washing of the enzyme with hot H_2O to recover the amino acid.

3.1.7. When the Lipolase-catalysed preparative-scale resolutions of 4-aryl-substituted β-lactams (**1a-g**) were performed in *i*-Pr_2O with 1 equiv. of H_2O at 60 °C, excellent E (> 200) was observed. The unreacted lactams **2a-g** and the product amino acids **3a-g** were separated with H_2O/organic solvent extraction and isolated in good yields (41-49%) and with high enantiomeric excesses (*ee* ≥ 95%).
The preparative-scale resolutions of 4-benzyl-2-azetidinone (**1h**) and 4-phenylethyl-2-azetidinone (**1i**) were performed in two steps, in *i*-Pr_2O with 0.5 equiv. of H_2O, using Lipolase at 45 °C. This method resulted in products with good *ee* (≥ 87%), but relatively low yields (27-36%).

3.1.8. The transformations involving the ring opening of β-lactams **1a-i** with 18% aqueous HCl afforded β-amino acid hydrochlorides **4a-i**, while treatment of amino acids **3a-i** with 22% HCl/EtOH resulted in the corresponding β-amino acid hydrochlorides **3a-g**·HCl without a decrease in *ee* (Scheme 3).

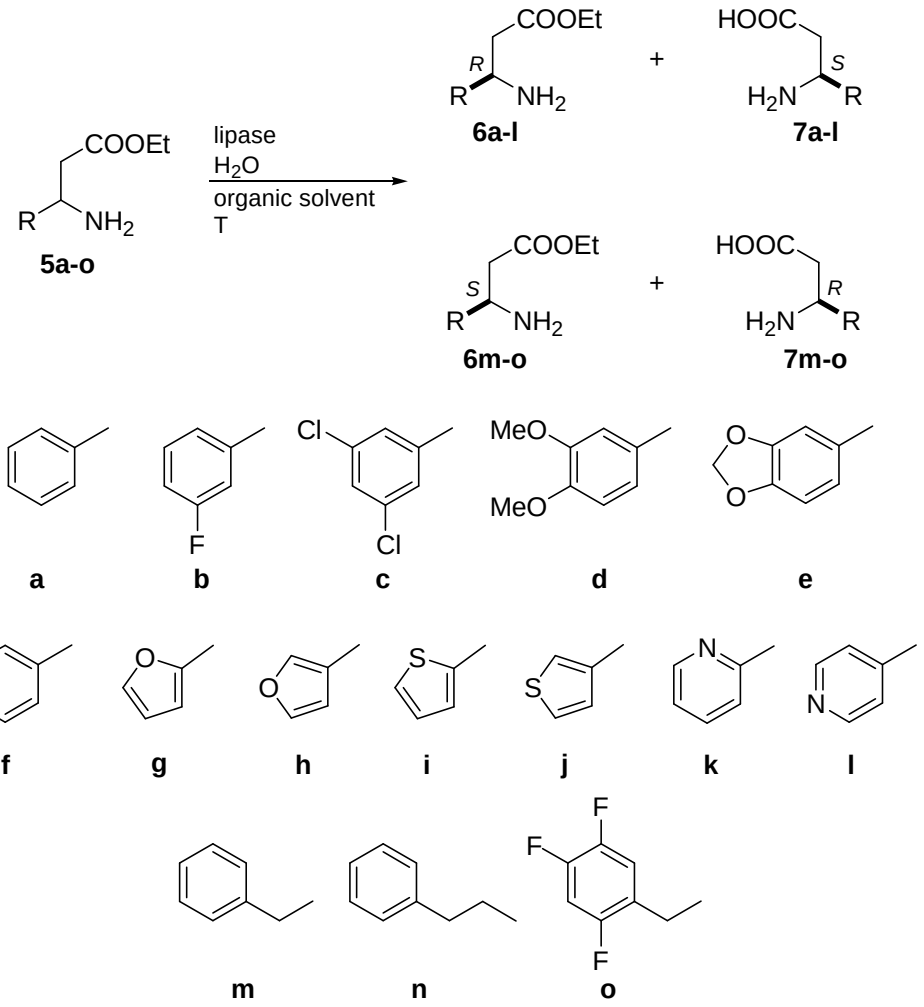


Scheme 3

3.1.9. The absolute configurations were proved by comparing the specific rotations of the enantiomers with literature data or, if this was not possible, the analysed chromatograms suggested the enantiopreference by analogy. Thus, the absolute configurations indicated *R*-selectivity for the ring opening of 4-aryl-substituted β-lactams **1a-g**, and *S*-selectivity for 4-arylalkyl-substituted β-lactams **1h,i**. (In the cases of the arylalkyl-substituted compounds, the priority sequence of the substituents varied according to the Cahn–Ingold–Prelog priority rules, which caused the seemingly opposite selectivity of Lipolase.)

3.2. Enzymatic hydrolyses of β-amino esters[III-VI]

3.2.1. In the hydrolyses of ethyl 3-amino-3-phenylpropionate (**5a**), ethyl 3-amino-3-(3-pyridyl)propionate (**5f**) and ethyl 3-amino-4-phenylbutanoate (**5m**) (Scheme 4), lipase PS (from *Burkholderia cepacia*) preparations exhibited high to excellent enantioselectivity (E > 200 for **5a,m**; E = 100 for **5f**) in *i*-Pr_2O (in the cases of **5a,f**) or *tert*-butyl methyl ether (*t*-BuOMe) (in the case of **5m**) with 0.5 equiv. of H_2O at 45 °C.



Scheme 4

The reaction time required to reach 50% conversion was several times longer for the arylalkyl-substituted model compound (**5m**, 72 h) than that for the aryl- and heteroaryl-substituted substrates (**5a**, 5 h; **5f**, 17 h). Decrease of the temperature to 25 °C decreased the reaction rate of the hydrolysis of ethyl 3-amino-4-phenylbutanoate (**5m**). In the case of ethyl 3-amino-3-(3-pyridyl)propionate (**5f**), an enhancement in the *E* (> 200) value was observed. In view of these results, further experiments were performed at 45 °C with the β-aryl- and β-arylalkyl-substituted models (**5a,m**) and at 25 °C with the β-heteroaryl-substituted model (**5f**).

3.2.2. *i*-Pr_2O, *t*-BuOMe, *n*-hexane and toluene proved to be suitable reaction media for the hydrolyses of the β-aryl- and β-heteroaryl-substituted substrates **5a,f**. However, noteworthy effects of the solvents were observed in the cases of the arylalkyl-substituted compounds **5m-o**: **5m** was hydrolysed with high *E* in *i*-Pr_2O and also in *t*-BuOMe, while higher reaction rates and better *E* values were obtained in *i*-Pr_2O than in *t*-BuOMe for ethyl 3-amino-5-phenylpentanoate (**5n**) and ethyl 3-amino-4-(2,4,5-trifluorophenyl)butanoate (**5o**).

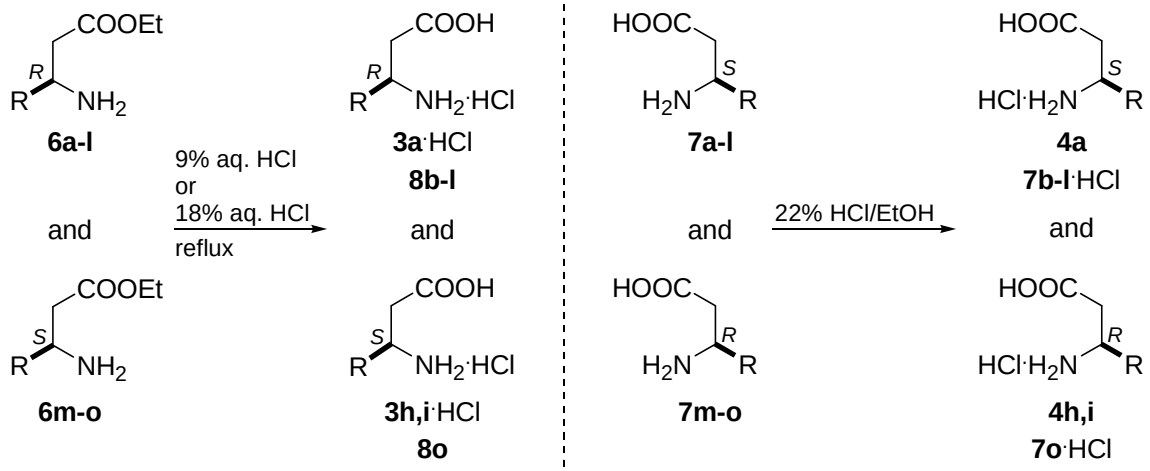
3.2.3. Increasing amounts of added H_2O slightly increased the rates of hydrolysis of β-aryl- and β-heteroaryl-substituted compounds **5a,f**, but slowed down the hydrolysis of β-arylalkyl-substituted compound **5m**. However, in the case of ethyl 3-amino-3-(3-pyridyl)propionate (**5f**), the *E* of the hydrolysis decreased, an effect which was not observed for **5a,m**. In good correlation with our results on the ring opening of β-lactams,[I,II] we could perform these hydrolyses without the addition of any H_2O in a small-scale experiment.

3.2.4. For each of the model compounds **5a,f,m**, the reaction rate clearly increased as the quantity of lipase was increased, while *E* did not change significantly. The highest reaction rates were observed in the presence of 75 mg mL^{-1} enzyme, but the preparative-scale resolutions were performed with 30 (for **5a,f**) or 50 (for **5m**) mg mL^{-1} lipase PS preparation.

3.2.5. The hydrolysis of ethyl 3-amino-4-phenylbutanoate (**5m**) was performed with the addition of 1 equiv. of Et_3N, *i*-Pr_2EtN or 2-octanol, but the conversions of the reactions with these additives were not better relative to that in H_2O.

3.2.6. The preparative-scale resolutions of β-aryl- (**5a-e**), β-heteroaryl- (**5f-l**) and β-arylalkyl- (**5m-o**) β-amino esters were performed in *i*-Pr_2O or *t*-BuOMe in the presence of a lipase PS preparation with 0.5 equiv. of H_2O at 45 °C or 25 °C. β-Amino acids **7a-o** were isolated with high enantiomeric excesses (≥ 96%) and in good yields (40-47%) at 50% conversions. The unreacted β-amino esters **6a-o** were immediately transformed to

the corresponding β-amino acid hydrochlorides **3a,h,i**·HCl and **8b-l,o** with aq. HCl (*ee* ≥ 96%, yield = 40-49%). The treatment of enantiomers **7a-o** with 22% HCl/EtOH resulted in the β-amino acid hydrochlorides **4a,h,i** and **7b-l,o**·HCl (*ee* ≥ 96%) (Scheme 5).



Scheme 5

3.2.7. The absolute configurations were proved by comparing the specific rotations of the enantiomers or their derivatives with literature data or, if this was not possible, the analysed chromatograms or the comparative specific rotations indicated the enantiopreference of the enzyme. Thus, the absolute configurations pointed to *S*-selectivity for the hydrolysis of β-aryl- and β-heteroaryl-β-amino esters **5a-l**, and to *R*-selectivity for β-arylalkyl-β-amino esters **5m-o**.

4. Publications on which this thesis is based

I. E. Forró, T. Paál, **G. Tasnádi**, F. Fülöp
A new route to enantiopure β-aryl-substituted β-amino acids and 4-aryl-substituted β-lactams through lipase-catalyzed enantioselective ring cleavage of β-lactams
Adv. Synth. Catal. **2006**, *348*, 917-923.
IF: 4.762

II. **G. Tasnádi**, E. Forró, F. Fülöp
Candida antarctica lipase B-catalyzed ring opening of 4-arylalkyl-substituted β-lactams
Tetrahedron: Asymmetry **2007**, *18*, 2841-2844.
IF: 2.634

III. **G. Tasnádi**, E. Forró, F. Fülöp
An efficient new enzymatic method for the preparation of β-aryl-β-amino acid enantiomers
Tetrahedron: Asymmetry **2008**, *19*, 2072-2077.
IF: 2.796

IV. **G. Tasnádi**, E. Forró, F. Fülöp
Burkholderia cepacia lipase as an excellent enzyme for the enantioselective hydrolysis of β-heteroaryl-β-amino esters
Tetrahedron: Asymmetry **2009**, *20*, 1771-1777.
IF: 2.625

V. **G. Tasnádi**, E. Forró, F. Fülöp
Improved enzymatic syntheses of valuable β-arylalkyl-β-amino acid enantiomers
Org. Biomol. Chem. **2010**, *8*, 793-799.
IF: 3.762 (2009)

VI. **G. Tasnádi**, E. Forró, F. Fülöp
β-Aril- és β-heteroaril-β-aminosav enantiomerek enzimatikus úton történő előállítása
Magy. Kém. Foly. **2010**, accepted manuscript.

VII. M. Utczás, E. Székely, **G. Tasnádi**, É. Monek, L. Vida, E. Forró, F. Fülöp, B. Simándi
Kinetic resolution of 4-phenyl-2-azetidinone in supercritical carbon dioxide
J. Supercrit. Fluids **2010**, submitted manuscript.

Sum of impact factors of the published papers: 16.579

5. Lectures related to this thesis

I. Chemical conference 28-30 June, 2005, Hajdúszoboszló, Hungary, poster presentation:
Enikő Forró, Tihamér Paál, **Gábor Tasnádi**, Ferenc Fülöp
Enantioselective ring cleavage of 4-aryl-substituted β-lactams (P-27) (III. award)

II. Multi-step Enzyme Catalysed Processes 18-21 April, 2006, Graz, Austria, poster presentation:
Gábor Tasnádi, Enikő Forró, Ferenc Fülöp
Enantioselective ring cleavage of 4-substituted β-lactams (P-65)

III. Clauder Ottó-memorial competition 12-13 April, 2007, Budapest, Hungary, oral presentation:
Gábor Tasnádi
Development of the enzyme-catalysed ring opening of 4-substituted β-lactams

IV. Training school in biocatalysis, 28 April - 3 May, 2007, Siena, Italy, oral presentation:
Gábor Tasnádi
Enzyme-catalysed ring-opening of 4-substituted-β-lactams

V. "Szegedi Ifjú Szerves Kémikusok Támogatásáért" Alapítvány 8. tudományos előadó ülése, 16 April, 2008, Szeged, Hungary, oral presentation:
Gábor Tasnádi
3-Amino-3-arilpropionsav enantiomerek enzimatikus úton történő előállítása

VI. 20th International Symposium on Chirality, 6-9 July, 2008, Geneva, Switzerland, poster presentation:
Gábor Tasnádi, Enikő Forró, Ferenc Fülöp
Enantioselective hydrolysis of ethyl 3-amino-3-arylpropionates (P-2)

VII. 16th European Symposium on Organic Chemistry, 12-16 July, 2009, Prague, Czech Republic, poster presentation:
Gábor Tasnádi, Enikő Forró, Ferenc Fülöp
Lipase PS-catalysed hydrolysis of β-heteroaryl-substituted β-amino esters (P2-198)

VIII. Foldamers: building blocks, structure and function, 24-26 September, 2009, Szeged, Hungary, poster presentation:
Gábor Tasnádi, Enikő Forró, Ferenc Fülöp
An improved enzymatic method for the preparation of valuable β-arylalkyl-β-amino acid enantiomers (P-11)