

University of Szeged
Doctoral School of Pharmaceutical Sciences

Educational Program: Pharmaceutical Chemistry and Drug Research

Programme director: Dr. István Szatmári

Institute: Institute of Pharmaceutical Chemistry

Supervisors: Prof. Dr. Enikő Forró

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Sayeh Shahmohammadi

**New enzymatic strategies for the preparation of
pharmaceutically important enantiomeric β -amino acid
derivatives**



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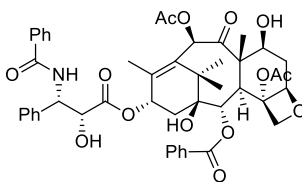
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A. INTRODUCTION AND AIMS

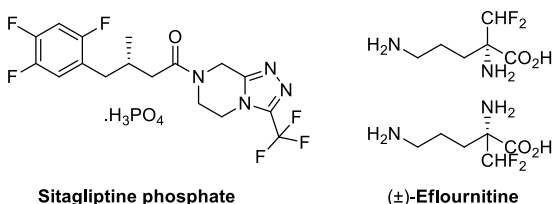
In recent years, optically pure β -aryl-substituted β -amino acids have acquired considerable interest due to their pharmacological importance, unique and remarkable biological activity, their utility in synthetic chemistry and drug research. Therefore, this class of compounds has been described as a crucial scaffold in the design and synthesis of feasible pharmaceutical drugs. For instance, (*S*)- β -phenylalanine can find application as a fundamental component in the synthesis of novel antibiotics. 3-Amino-3-phenylpropionic acid, a key pharmaceutical building block, is present in anticancer agents such as **Taxol** (Scheme 1).



Scheme 1.

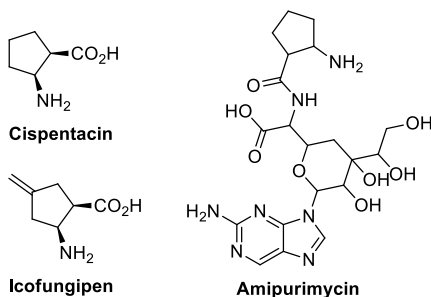
Enormous attainments in the development of fluorinated β -amino acid drugs proved the high significance of this group of compounds in pharmaceutical chemistry. The continuous rise is associated with the unique properties of the fluorine atom with respect to its high electronegativity and the polarity of the C–F bond. Additionally, the pK_a , affinity, dipole moment, stability, lipophilicity, and bioavailability of groups adjacent to fluorine can be altered. Therefore, fluorine-containing compounds are providing stronger activity and stability, longer half-life, and better bioabsorbability, especially in the fields of pharmaceutical intermediates, cancer treatment, antiviral agents, photovoltaics, diagnostic probes, and bioinspired materials. In addition, natural proteins incorporating fluorinated amino acids also showed many unique characteristics. These compounds are employed in the biotherapeutics protein–protein interaction and in the synthesis of chemicals with better quality. For example, (\pm)-**Eflornitine**, a fluorine-incorporated β -amino acid drug was used for the treatment of trypanomiasis and against facial hirsutism

in women. Januvia™ (**Sitagliptin phosphate**) an antidiabetic agent contains an (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid subunit (Scheme 2).



Scheme 2.

Another class of molecules with valuable therapeutic effects are carbocyclic β -amino acids. The most obvious examples are **Cispentacin** [(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid] and **Icfofungipen** [(1*R*,2*S*)-2-amino-4-methylenecyclopentanecarboxylic acid] both exhibiting antifungal activity. For instance, **Amipurimycin**, a peptidyl nucleoside antibiotic, bears a cispentacin subunit (Scheme 3). Carbocyclic β -amino acids can be employed as building blocks for the synthesis of modified peptides and self-organizing foldameric structures with increased activity and stability. They can also be applicable in heterocyclic and combinatorial chemistry.

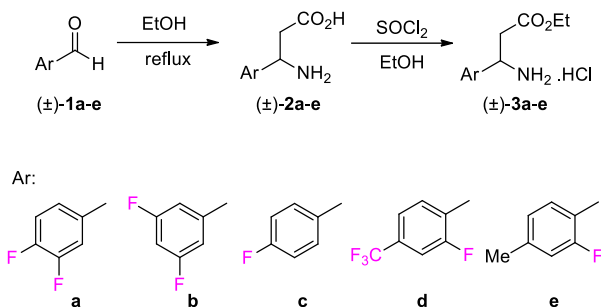


Scheme 3.

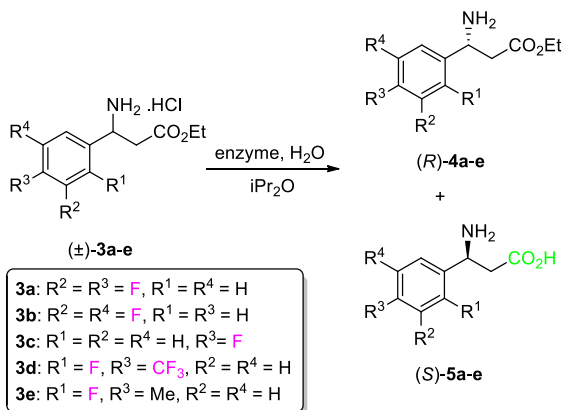
Besides, different approaches for the synthesis of β -aryl- β -amino acid enantiomers and numerous enzymatic procedures were developed for the synthesis of enantiomerically pure β -amino acids through enantioselective lipase-catalyzed hydrolysis of β -amino esters

or ring opening of β -lactams in an organic medium. In recent years, due to stringent requirements for the development of environmentally benign strategies, green approaches like solvent-free systems and application of mechanochemical forces with the recovery and reusability of catalysts have gained increasing attention.

The present Ph.D. work has been planned to accomplish two major goals. In view of the significance of fluorine-substituted compounds, the first aim was to synthesize a selection of (\pm)- β -amino carboxylic ester hydrochloride salts **3a–e** (Scheme 4), then to generate an appropriate lipase-catalyzed method for their resolution through hydrolysis, furnishing enantiopure new β -fluorophenyl-substituted β -amino acids (*S*)-**5a–e** and unreacted β -amino esters (*R*)-**4a–e** (Scheme 5).

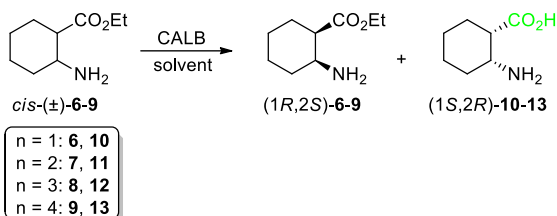


Scheme 4.



Scheme 5.

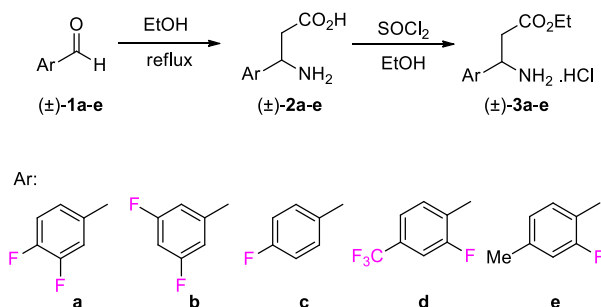
The second objective of my work was a comparative investigation of different green strategies and then to build an environmentally benign CALB-catalyzed hydrolysis of 5–8-membered *cis* carbocyclic amino esters **6–9** (Scheme 6).



Scheme 6.

B. RESULTS AND DISCUSSION

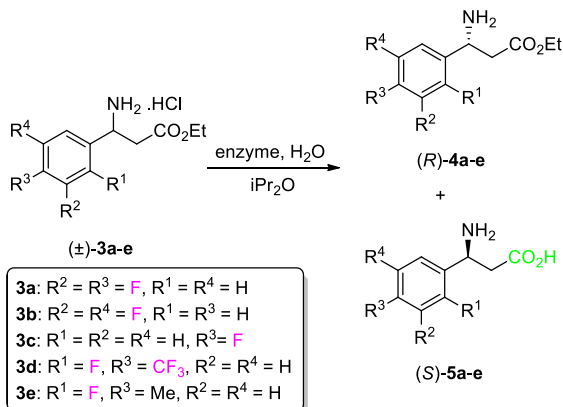
1. Based on the modified Rodionov synthesis, racemic fluorophenyl-substituted β -amino acids (\pm)-**2a-e** were synthesized through the reaction of the corresponding aldehydes **1a-e** in the presence of NH_4OAc with malonic acid in EtOH at reflux temperature. The white crystals formed were filtered off and washed with acetone and then they were recrystallized from H_2O and acetone with yields ranging from 15% to 51% (Scheme 4).
2. β -Amino carboxylic esters (\pm)-**3a-e.HCl** were prepared in the presence of SOCl_2 , EtOH, and the corresponding (\pm)-**2a-e** by esterification. The resulting white salts were recrystallized from EtOH and Et_2O with yields ranging from 76% to 98% (Scheme 4).



Scheme 4.

3. In order to determine the optimized conditions for the resolution of (\pm)-**3a-e.HCl**, a set of preliminary screenings including enzyme, solvent, temperature, and concentration of enzyme were conducted.
4. The preparative-scale hydrolysis of racemic β -amino carboxylic ester hydrochloride salts **3a-e** was carried out under optimized conditions: in $i\text{Pr}_2\text{O}$ with lipase PSIM, in the presence of Et_3N with H_2O as a nucleophile at 45°C (Scheme 5). Reactions were stopped by filtering off the enzyme at a conversion close to 50% with excellent enantioselectivities

($E > 200$). The enantiomers could be easily separated by Et₂O/H₂O extraction. Both unreacted amino carboxylic esters (*R*)-**4a–e** with high *ee* ($\geq 94\%$) and good yields ($\geq 38\%$) as well as product amino acids (*S*)-**5a–e** with excellent *ee* ($>99\%$) and yields ($\geq 48\%$) were isolated (Table 1).



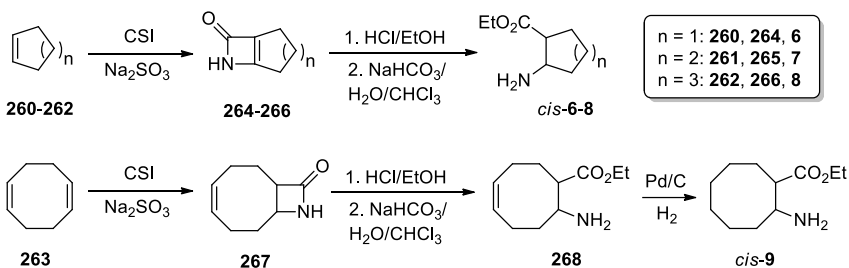
Scheme 5.

Table 1. Lipase PSIM-catalyzed hydrolysis of (±)-**3a–e**^a

(±)	Rt (h)	Conv. (%)	<i>E</i>	β-Amino acid (5a–e)				β-Amino ester (4a–e)			
				Yield (%)	Isomer	<i>ee</i> ^b (%)	[α] _D ²⁵ (H ₂ O)	Yield (%)	Isomer	<i>ee</i> ^c (%)	[α] _D ²⁵ (CHCl ₃)
3a	8	50	>200	48	(<i>S</i>)	>99	−3.1 ^d	49	(<i>R</i>)	97	+17.9 ^e
3b	72	49	>200	48	(<i>S</i>)	>99	−5 ^f	38	(<i>R</i>)	94	+9 ^g
3c	18	50	>200	49	(<i>S</i>)	>99	−3 ^h	49	(<i>R</i>)	>99	+18.9 ⁱ
3d	26	49	>200	49	(<i>S</i>)	>99	−11 ^j	48	(<i>R</i>)	>99	+20.3 ^k
3e	23	50	>200	48	(<i>S</i>)	>99	−13 ^l	47	(<i>R</i>)	>99	+16 ^m

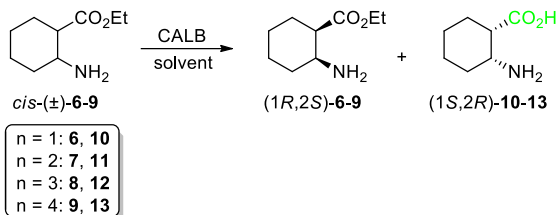
^a30 mg mL^{−1} PSIM in iPr₂O, 5 equiv. Et₃N, 0.5 equiv. H₂O, at 45 °C. ^bAccording to GC after derivatization. ^cAccording to GC after double derivatization. ^d*c* = 0.28. ^e*c* = 0.44. ^f*c* = 0.26. ^g*c* = 0.29. ^h*c* = 0.28. ⁱ*c* = 0.41. ^j*c* = 0.19 (MeOH). ^k*c* = 0.53. ^l*c* = 0.21 (MeOH). ^m*c* = 0.13.

5. In accordance with the Markovnikov orientation, the regioselective 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) to cyclopentene **260**, cyclohexene **261**, cycloheptene **262**, and 1,5-cyclooctadiene **263** delivered racemic *cis* β -lactams **264**, **265**, **266**, and **267**. Ring cleavage of *cis* lactams **264–266** and **267** with 22% ethanolic HCl yielded the desired *cis* carbocyclic amino esters **6–8** and unsaturated ethyl *cis*-2-aminocyclooct-5-ene carboxylate **268**. Then the latter was subjected to catalytic reduction to give saturated ethyl *cis*-2 aminocyclooctanecarboxylate **9** (Scheme 7).



Scheme 7.

6. Preliminary experiments including green solvent, enzyme quantity, temperature and frequency screening, as well as enzyme reusability testing were revealed the best reaction conditions.
7. Sustainable CALB-catalyzed strategies have been demonstrated for the resolution of 5–8-membered (\pm)-*cis*-**6–9** carbocyclic β -amino esters through hydrolysis in green organic solvent *t*BuOMe, under solvent-free conditions and using ball milling for compound. In the frame of preliminary experiments, the best combination of enantioselectivity and reaction rate was observed in green organic solvent, therefore, preparative-scale resolutions were performed with CALB in *t*BuOMe as a green solvent at 65 °C (Scheme 6) resulting in the desired enantiomeric unreacted β -amino esters (1*R*,2*S*)-**6–9** with yields ranging from 27% to 31% and *ee*_s values of $\geq 91\%$, and product β -amino acids (1*S*,2*R*)-**10–13** with yields ranging from 25% to 33% and high *ee*_p values ($\geq 96\%$) (Table 2).



Scheme 6.

Table 2. CALB catalyzed preparative-scale hydrolysis of carbocyclic *cis* β -amino esters **6–9**

(\pm)	Rt (h)	Conv. (%)	β -Amino acid (10–13)				β -Amino ester (6–9)			
			Yield (%)	Isomer	ee^b (%)	$[\alpha]_D^{25}$ (H ₂ O)	Yield (%)	Isomer	ee^c (%)	$[\alpha]_D^{25}$ (EtOH)
6	4(24)	36(75)	25	(1 <i>S</i> ,2 <i>R</i>)	96	+9.41 ^g	31	(1 <i>R</i> ,2 <i>S</i>)	98	-6.94 ^g
7	23	50	33	(1 <i>S</i> ,2 <i>R</i>)	>99	+19.84 ^h	27	(1 <i>R</i> ,2 <i>S</i>)	96	-11.13 ^g
8	23(3d)	20(69)	32	(1 <i>S</i> ,2 <i>R</i>)	98	+6.54 ^h	30	(1 <i>R</i> ,2 <i>S</i>)	91	-4.09 ⁱ
9	23(20d)	20(62)	28	(1 <i>S</i> ,2 <i>R</i>)	>99	-19.15 ^k	27	(1 <i>R</i> ,2 <i>S</i>)	62	+20.92 ^j

^a100 mg substrate, 30 mg mL⁻¹ CALB, (S/E, 1:4.5), in 15 mL *t*BuOMe, at 65 °C. ^b100 mg substrate, 30 mg mL⁻¹ CALB, (S/E, 1:4.5), in 15 mL *t*BuOMe, at 65 °C. ^c100 mg substrate, 50 mg mL⁻¹ CALB, (S/E, 1:7.5), in 15 mL *t*BuOMe, at 65 °C. ^d100 mg substrate, 50 mg mL⁻¹ CALB, (S/E, 1:7.5), in 15 mL *t*BuOMe, at 65 °C. ^eAccording to GC after derivatization. ^fAccording to GC after double derivatization. ^g $c = 0.20$. ^h $c = 0.25$. ⁱ $c = 0.23$. ^j $c = 0.19$. ^k $c = 0.22$.

- 8.** It is worth mentioning that, the lipase-catalyzed hydrolysis of 7- and 8-membered carbocyclic β -amino esters was described for the first time.
- 9.** The resulted enantiomers were characterized by GC measurements, optical rotations, ¹H, ¹³C, and ¹⁹F NMR, HRMS analysis and melting point values.

C. PUBLICATIONS

1. **Shahmohammadi, S.**; Fülöp, F.; Forró, E.
Efficient synthesis of new fluorinated β -amino acid enantiomers through lipase-catalyzed hydrolysis.
Molecules. **2020**, *25*, 5990, DOI: 10.3390/molecules25245990 **IF.:4.412**
2. **Shahmohammadi, S.**; Faragó, T.; Palkó, M.; Forró, E.
Green strategies for the preparation of enantiomeric 5-8-membered carbocyclic amino acid derivatives through CALB-catalyzed hydrolysis.
Molecules. **2022**, *27*, 2600, DOI: 10.3390/molecules27082600 **IF.:4.412**

Other

3. Némethi, G.; Berkecz, R.; **Shahmohammadi, S.**; Forró, E.; Lindner, W.; Péter, A.; Ilisz, I.
Enantioselective high-performance liquid chromatographic separation of fluorinated β -phenylalanines utilizing *Cinchonane* alkaloid-based ion exchanger chiral stationary phases.
J. Chromatogr. A. **2022**, *1670*, 462974, DOI: 10.1016/j.chroma.2022.462974 **IF.:4.759**

D. CONFERENCE LECTURES

1. **Sayeh Shahmohammadi**, Ferenc Fülöp and Enikő Forró
Synthesis of new fluorine-substituted β -amino acid enantiomers through lipase catalyzed hydrolysis.
25th International Symposium on Analytical and Environmental Problems.
October 7, 8. 2019. Szeged, Hungary
2. **Sayeh Shahmohammadi**, Ferenc Fülöp and Enikő Forró
Lipase catalyzed hydrolysis of fluorine-substituted beta-amino esters.
Virtual conference held by Hungarian foundation to Support Selectively the Organic Chemists.
May 20. 2020. Szeged, Hungary
3. **Sayeh Shahmohammadi**, Tünde Faragó, Márta Palkó and Enikő Forró
Green strategies to prepare amino acid enantiomers by CALB catalyzed hydrolysis of carbocyclic amino esters.
II.FKF Szimpozium.
June 16-18. 2021. Virtual. Hungary
4. **Sayeh Shahmohammadi**, Tünde Faragó, Márta Palkó and Enikő Forró
Green strategies for the preparation of enantiomeric carbocyclic β -amino acid derivatives.
4th International Green Catalysis Symposium.
April 19-22. 2022. Rennes, France

