Development of new, nature-friendly biotrasformations to pharmaceutically and/or chemically important enantiomeric compounds, in batch and continuous-flow systems
Project 129049 -closing report-

In the frame of substrate engineering, the steric effect of different *N*-protecting groups (*N*-Boc, *N*-Cbz and *N*-Fmoc) on the enantioselectivity and reaction rate of CAL-B-catalyzed (*S*)-selective *O*-acylation of *N*-protected 1-hydroxymethyl-tetrahydro-ß-carbolines has been investigated. Excellent enantioselectivities ($E > 200$) were observed when the acylation of *N*-Boc, *N*-Cbz and *N*-Fmoc-protected substrates was performed with the use of CAL-B and acetic anhydride in toluene at 60 °C. The resolution of *N*-acetyl protected substrate showed excellent *E* (>200) after 30 min, but as the reaction progressed, *E* started decreasing after 2 days, because of *N*/*O* and *O*/*N* acyl migrations[1].

Our work with regard to the enantioselective hydrolysis of *N*-Boc-protected 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-ethyl-propionate affording the desired enantiomeric aminoacid and unrected amino ester has not been successful. No reaction observed in spite of a laborious preliminary experiments (enzyme screening, reactions in different solvents, different temperatures, different substrate:enzyme and substrate:H_2O ratio, *etc.*). As a possible alternative route to prepare the desired enantiomers, enzymatic amidation of racemic amino-acid with benzyl-amine in the presence of molecular sieves was investigated. CAL-B in *t*BuOMe at 60 °C showed activity (comv. 60% after 1 day) but quite many products formed.

In the frame of substrate specificity, CAL-B-catalyzed asymmetric *N*-alkoxycarbonylations of 1-substituted (Me, Et, Pr, *i*Pr) tetrahydro-ß-carbolines have been studied. High enantioselectivities (>200) were observed, when the rEactions were performed in iPr_2O in the presence of phenyl allyl carbonate and Et_3N at 60 °C using ultrasound shaking method. The reaction time increased considerably with increasing substituent size on C1; however, the isopropyl-substituted compound proved to be too bulky for the optimum activity of CAL-B.[2]

Heterogeneously catalyzed racemization reactions of secondary amines (*S*)-1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline and (*S*)-1-methyl-1,2,3,4-tetrahydro-ß-carboline were investigated using Pd, Pt and Ir on carbon or Al_2O_3 supports. The racemization was faster on platinum and took place without detectable by-product formation. Iridium, however, proved reusable and, in contrast to the platinum catalyst, deactivation at low catalyst concentration was not observed. Ir on carbon is a potential racemization catalyst for further studies and in future expansion of this work towards fully heterogeneously catalyzed metalloenzymatic dynamic kinetic resolutions of secondary amines.[3]

An efficient synthetic approach for the construction of fluorine-containing piperidine γ-amino acid derivative (chiral ones being of them) has been developed. The synthetic concept was based on oxidative ring opening of an unsaturated bicyclic γ-lactam (Vince-lactam) through its ring C=C bond, followed by double reductive amination of the diformyl intermediate performed with various fluoroalkylamines. The method has been extended towards the access of alkylated and perfluoroalkylated substances and for γ-lactam derivatives.[4]

A new HPLC technique has been devised for the enantioseparation of cationic tetrahydro-β-carboline and 1,2,3,4-tetrahydroisoquinoline analogs on Cinchona-alkaloid-based zwitterionic ion-exchanger type chiral stationary phases applying MeOH/THF and MeOH/MeCN mobile phases containing TEA and FA (or NH_4OAc) additives. The influence of the nature and concentration of the organic components of the mobile phase, organic salt additives on the retention behavior and enantioseparation were studied. Investigations on the structure–retention relationships were also performed.[5]

An efficient new enzymatic strategy for the preparation of novel β-aryl-fluorine-containing β-amino carboxylic acid enantiomers (*ee* > 99%) through lipase PSIM-catalyzed hydrolysis (E > 200) of the corresponding racemic β-aryl-fluorine-containing β-amino carboxylic ester hydrochlorides has been devised. The reactions performed in iPr$_2$O at 45°C in the presence of Et_3N and H_2O furnished unreacted *R*-amino esters and product *S*-amino acids in good chemical yields (> 48%).[6]

A simple synthetic procedure with high stereocontrol has been devised for the preparation of novel fluorine-containing six- and seven-membered *N*-heterocyclic racemic β-amino esters, based on ring olefin bond transformation of some cycloalkene amine esters or lactams. Further experiments for the access of enantiomerically pure substances is ongoing.[7]

Efficient enzymatic strategies have been developed for the enzymatic resolution of 5–8-membered carbocyclic β-amino esters through hydrolysis in green organic media, under solvent-free conditions and using ball milling. In view of the best enantioselectivity, preparative-scale resolutions were performed in tBuOMe at 65 °C, resulting in the desired enantiomeric unreacted (1*R*,2*S*)-β-amino esters and product (1*S*,2*R*)-β-amino acids with high enantiomeric excess values (>96%). Easy separation of the enantiomers could be achieved since the unreacted β-amino esters were soluble in organic solvent and the product β-amino acids in H_2O. To the best of our knowledge, the lipase-catalyzed hydrolysis of 7- and 8-membered carbocyclic β-amino esters was described for the first time.[8]

In view of the importance of chiral fluorinated compounds, high-performance liquid chromatographic enantioseparation techniques for our earlier prepared fluorinated ß-phenylalanine derivatives have been developed by using Cinchona alkaloid-based zwitterionic and macrocyclic glycopeptides- and derivatized cyclofructan-based chiral stationary phases.[9,0]

A mini-review, discussing the most relevant lipase-catalyzed strategies for the synthesis of pharmaceuticlly important cyclic and acyclic α-, β- or γ-amino carboxylic acid enantiomers (iantermediates for the synthesis of Abacavir, CatHA inhibitors, Anatoxin-a, AZD6564, Moxifloxacin, modulators of nuclear receptors, Cispentacin, Taxol side-chain, CEP-28122), through hydrolysis of the corresponding amino carboxylic esters and lactams, over the last decades has been prepared. The methods were classified as kinetic, dynamic kinetic and sequential kinetic resolution. Mechanistic information of the lipase-catalyzed transformations discussed is available at the end of this overview.[11]

In spite of the promising preliminary results (E > 200) obtained for CALB-catalyzed hydrolysis of racemic ethyl *cis*-4-aminocyclopent-2-enecarboxylate, when continued working with newly synthetized starting γ-amino ester the above excellent E could not be reproduced. To overcome the possible degradation of amino ester during the enzymatic reactions, we started to investigate the enzymatic hydrolysis of the more stable hydrochloric salt of amino ester in the presence of Et_3N. The max E obtained under the optimized conditions (2M2B, pyridine, 25°C) was only 15. We also investigated the possibility of enzymatic amidation of racemic γ-amino acid with benzyl-amine but the results so far are not as good as to be published.

The most relevant lipase-catalyzed strategies for the preparation of pharmaceutically and chemically important tetrahydroisoquinoline and tetrahydro-*β*-carboline enantiomers through *O*-acylation, *N*-acylation or CO*OEt*-hydrolysis of the corresponding racemic substrates, over the last decade are collected in a mini-review, which is currently under preparation.[12]

A very efficient CAL-B-catalyzed green strategy for the preparation of amides through amidation of carbocyclic acids, with different primary and secondary amines has been developed. The reactions carried out in the presence of molecular sieves in a green organic solvent such as *t*BuOMe, 1-MeTHF or propylene carbonate at 60 °C resulted the desired products with excellent yields (> 93%) in 1-2 days. In order to follow the progress of the reactions, an adequate HPLC-MS analytical method has also been devised. The article with the above-results is under preparation.[13]

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During the project years a number of 11 papers (ΣIF: 39.937) have been published, from which 9 papers with acknowledgement toward OTKA (ΣIF: 37.490). The most important results have been presented at conferences held abroad or in Hungary.

The new results achieved in the project also served as the basis of valuable sections of PhD dissertations; 3 PhD students, with highly productive research activities in the project have written their PhD theses; Rita Megyesi defended it in 2018, Barbara Kovács in 2021 and Sayeh Shahmohammadi in 2022.