"Development of new enzymatic strategies for the preparation of enantiomerically pure compounds, important from biological and chemical aspects" project (K 108943)

Project closing report

Very efficient, new, lipase-catalyzed kinetic, dynamic kinetic and sequential kinetic enzymatic routes for the synthesis of enantiomeric amino acids, amino esters, lactams and amino alcohols, through the enantioselective ring cleavage of the corresponding racemic lactams or the enantioselective hydrolysis of amino esters or the asymmetric acylation of the OH group of amino alcohols have been developed. The enzymatic reactions were performed either in a continuous-flow system or as batch reactions. A number of pharmaceutically important compounds have been prepared, as given below:

- Enantiomerically pure (*S*)-3-amino-3-(*o*-tolyl)propanoic acid, identified as the preferred enantiomeric form for the construction of novel *β*-amino acid derivatives as inhibitors of Cathepsin, was prepared through both indirect and direct enzymatic strategies (resolution of racemic hydroxymethylated 4-(*o*-tolyl)azetidin-2-one, ring cleavage of racemic 4-(*o*-tolyl)azetidin-2-one, hydrolysis of racemic ethyl 3-amino-3-(*o*-tolyl)propanoate)[1].

- New enzymatic methods in a continuous-flow or as batch reactions were developed for the enantioselective *O*-acylation of the primary hydroxy group in raccemic *N*-Boc-protected 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-yl)methanol[2] and *N*-Boc-protected 2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)ethanol[3]. The resulting ester and unreacted alcohol enantiomers were further transformed into the desired calycotomine and homocalycotomine enantiomers, respecively with high *ee* (> 94%). A systematic study in the continuous-flow system was performed on the *O*-acylation of amino alcohols with a remote stereogenic centre (when the distance between the hydroxy group and the stereogenic centre increased from one carbon atom to three carbon atoms)[4].

- It has demonstrated that he CAL-B-catalyzed enantioselective (*E* > 200) *O*-acylation of ethyl *cis*-2-hydroxycyclopentane-1-carboxylate and ethyl *cis*-5-hydroxycyclopent-1-ene-carboxylate was accompanied by hydrolysis, due to the presence of H_2O in the reaction mixture or at the surface of the enzyme[5]. Although the optimization of the hydrolyses of

model compounds did not lead to an efficient strategy for the preparation of enantiopure 2-hydroxycyclopentane-1-carboxylic acid and 5-hydroxycyclopent-1-enecarboxylic acid, a new method has been devised for the enantioseparation of *β*-hydroxy esters through enzyme-catalyzed hydrolysis. It was highlighted that, since many substrates selected for non-hydrolytic enzymatic transformation contain a hydrolysable function, the possibility of their hydrolysis needs to be carefully investigated.

- Some of the *β*-lactam ring-cleavage methods have been scaled up and the desired enantiomers prepared at multi-gram scale. Thus prepared enantiomers were subjected to further transformations in order to prepare chemically or pharmaceutically important compounds[6-9].

- High-performance liquid chromatographic methods were developed for the enantio-separation of four unnatural taxol precursor phenylisoserine analogs on chiral stationary phases containing macrocyclic glycopeptides and cyclofructans as chiral selectors[10].

- The crown ether (18-crown-6)-2,3,11,12-tetracarboxylic acid was evaluated as a chiral nuclear magnetic resonance (NMR) solvating agent for a series of diamines and bicyclic *β*-amino acids. Enantiomeric differentiation in the 1H NMR spectrum of one or more resonances of each compound was determined[11].

- A new enzyme-catalyzed strategy has been devised for the synthesis of enantiomeric (*ee* = 99%) 3-amino-3-phenyl-2-hydroxypropionic acid, a key intermediate for the taxol side-chain. *Burkholderia cepacia* lipase PS-IM catalyzed the *S*-selective acylation of *N*-hydroxymethylated (3*S**,4*R**)-3-acetoxy-4-phenylazetidin-2-one with 10 equiv. of VB in *i*Pr_2O at 25 ºC with excellent enantioselectivity ($E > 200$). The corresponding diester and unreacted monoester were obtained with excellent enantiomeric excess values (*ee* ≥ 98%). Since traces of undesirable enantiomeric diacetylated product and diol due to intramolecular acyl migration (~6% mole fractions) were also detected, it should be highlighted that the possibility of intramolecular acyl migration during an enzymatic transformation of substrates containing both OCOR and OH functions needs to be carefully investigated. Finally, (2*R*,3*S*)-3-phenylisoserine hydrochloride (*ee* = 99%), a key intermediate for the taxol side-chain, was prepared from the corresponding diester enantiomer through acidic hydrolysis[12].

- A new enzymatic method for the ring opening of 3,4-disubstituted *β*-lactams: 3-(benzyloxy)-4-(4-chlorophenyl)azetidin-2-one, 3-(benzyloxy)-4-phenylazetidin-2-one and 4-(4-chlorophenyl)-3-phenoxyazetidin-2-one was developed[13]. High enantioselectivities (*E* > 200) were obtained for ring-opening reactions of first two lactams, while a relatively moderate *E* of 12 for the third one, when used CAL-B as catalyst, 25 equiv. of H_2O as nucleophile and the reactions were performed in *t*BuOMe or *i*Pr_2O at 70 °C. The products could be easily separated. Present enzymatic method proved to be suitable for the preparation of (2*R*,3*S*)-3-amino-3-phenyl-2-hydroxypropanoic acid, a key intermediate for the Taxol® side-chain.

- An effective enzymatic method was developed for the enantioselective *O*-acylation of the primary hydroxy group of tetrahydro-*β*-carbolines *N*-Boc-protected 1-hydroxymethyl-1,2,3,4-tetrahydro-*β*-carboline, 1-hydroxymethyl-6-methoxy-1,2,3,4-tetrahydro-*β*-carboline and 1-hydroxymethyl-6-fluoro-1,2,3,4-tetrahydro-*β*-carboline[14]. Tacking advantage of the continuous-flow system, we carried out the preliminary experiments in a continuous-flow system, while the preparative-scale resolutions were performed as batch reactions (incubator shaker). Excellent *E* values (> 200) were observed when CAL-B and acetic anhydride were used in toluene at 60 °C. Enantiomeric *N*-Boc-protected amino alcohols and amino esters were obtained with high *ee* (≥ 96%) in good yields (≥ 43%). The transformations of enantiomers, obtained from enzymatic resolutions with MW-assisted Boc deprotection resulted in the desired tetrahydro-*β*-carboline amino alcohols without a drop in the *ee* values (≥ 96%).

- High-performance liquid chromatographic methods were described for the enantioseparation of free and *N*-protected *β*-carboline derivatives on six different polysaccharide- and two strong cation exchanger-type chiral stationary phases[15].

- A new, CAL-B-catalyzed two-step cascade procedure has been devised for rapid access to diverse amino acids from *N*-hydroxymethyl-*β*-lactams, such as the antifungal cispentacin and the intermediates for the taxol side-chain or cathepsin inhibitors. When the hydrolyses of racemic *N*-hydroxymethyl-*β*-lactams were performed with H_2O (0.5 equiv.) in *i*Pr_2O at 60 °C, enantioselective (*E* > 200) ring-cleavage reactions took place. As the ring-opened amino acids formed, the *N*-hydroxymethyl groups underwent spontaneous degradation, and the desired enantiomeric *β*-amino acid and unreacted *N*-hydroxymethyl-*β*-lactam enantiomers (*ee* > 95%) were formed. The formation of polymers induced by the liberation of formaldehyde was

successfully restricted by the addition of benzylamine as a capture in the enzymatic reaction mixtures[16]

- A very efficient enzymatic two-step cascade reaction has been devised ($E > 200$) for the resolution of activated γ-lactams. The *N*-hydroxymethyl group worked as a traceless activating group, when the reactions were performed with H_2O (0.5 equiv.) in the presence of benzylamine (1 equiv.) in $i Pr_2O$ at 60 °C. The ring-opened enantiomerically pure γ-amino acids (intermediate of abacavir being one of them) and unreacted lactams were obtained with very good *ee* ($\geq 96\%$)[17].

- A review article, which gives a brief insight into the most relevant enzymatic strategies for the synthesis of 25 year-old cispentacin and its close analogues containing the cispentacin subunit has been published[18]. The methods were classified as indirect or direct strategies. Some practical details for the preparative-scale preparation of cispentacin with excellent *ee* and very good yield were highlighted at the end of this overview.

- An efficient dynamic kinetic resolution technique was devised for the preparation of (1*R*)-6-hydroxy- and (1*R*)-6-methoxy-subsituted 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acids with *ee* > 99% (chemical yields > 87%), useful in the synthesis of modulators of nuclear receptors[19]. When *Candida antarctica* lipase B was used as catalyst and the reactions were performed in organic solvents or aqueous NH_4OAc buffer at pH 8.5, *R*-selective hydrolysis of the corresponding 1,2,3,4-tetrahydroisoquinoline-1-carboxylates took place.

- Covalently immobilized lipase AK from *Pseudomonas fluorescens* and lipase PS from *Burkholderia cepacia* on immobead T2-150 were successfully used for the kinetic resolution of new, exotic and variously substituted *rac*-(5-phenylfuran-2-yl)-alanine ethyl ester hydrochlorides, through enantioselective hydrolysis in NH_4OAc buffer (20 mM, pH 5.8) at 30 °C[20].

- Both enantiomers of 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid have been prepared by dynamic kinetic resolution, through enzymatic hydrolysis of the corresponding ethyl ester hydrochloride[21]. The CAL-B-catalyzed transformation in NH_4OAc buffer (pH 8.0, 30 °C) provided (*R*) amino acid·HCl with 98% *ee* and 90% yield in 20 min. The hydrolysis with Alcalase in borate buffer (pH 8.0, 30 °C) showed *S* selectivity and the product (*S*) amino acid HCl was obtained with 60% *ee* and 66% yield in 45 h. The absolute configuration of (*S*)

amino acid was determined by TDDFT, electronic circular dichroism and optical rotation calculations.

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A number of 28 papers (Σ IF: 72.589, number of independent citations: 61), from which 18 papers with acknowledgement toward OTKA (Σ IF: 42.394, number of independent citations: 32) have been published, during the project years. The most important results have been presented at conferences held abroad or in Hungary.

The new results achieved in the project also serve 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; László Schönstein has defended it (*summa cum laude*) in 2014, Zsolt Galla will defend it in this year (2017) and Rita Megyesi in next year (2018).