BIOCATALYTIC APPROACHES FOR THE STEREOSELECTIVE SYNTHESIS OF CHIRAL BUILDING BLOCKS

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Abstract

This thesis presents a selection of the scientific activity gained by the candidate over the course of the last 14 years, including exclusively scientific results obtained after the public defence of her PhD thesis, as well as the brief description of some maine future research directions. The abilitation thesis contains 5 chapters. After the first introduction chapter, in the second (Enantiotope selective biotransformations) and in the third (Enantiomer selective processes) chapters, the main achieved scientific results are presented. In the chapter 4 some applicative results are described.The fifth chapter contains new approaches regarding biocatalysis.

Chapter 2 presents the synthesis of heteroaryl-ethanols and ethanediols with *Saccharomyces cerevisiae* cells, under the action of the present dehydrogenases (Y-ADHs) and hydrolases. While the reduction of methyl-heteroaryl prochiral ketones undergoes selectively, resulting in the substituted (*S*)-ethanols, by an appropriate α-derivatization and subsequent biotransformation with baker's yeast cells, both enantiomers of heteroaryl-ethane diols were prepared. With the aim to synthesized the opposite enantiomer of the biocatalytically obtained enantiopure ethanols, the Mitsunobu reaction was used as a tool to perform a controled stereoinversion, whithout significant loss in enantiomeric excess and with high yields.

Chapter 3 contains selected results obtained in the field of enzymatic kinetic resolution of racemic heteroaryl-ethanols, -hydroxy acids and -amines. As target compounds, important structures in drug synthesis like phenothiazin, (benzo)furan and (benzo)thiophene, as well as thiazole based chiral building blocks, were used. To develope efficient procedures several optimization procedures were investigated. The effects of the biocatalyst, reaction temperature, type of solvent and acylating reagent upon the efficacy of each developed procedure was studied.

In our first attempt, both enantiomerically enriched stereoisomers of various heteroaryl-1-ethanols were prepared by lipase mediated kinetic resolutions of the racemic mixtures. The optimal conditions (enzyme, solvent, acylating agent, *ratio*, etc) were found in each case and used succesfully also in preparative scale scale biotransformations.

Mechanism-based competition between the (*R*)-acetate (enzymatic acylation product), vinyl acetate (added acylating reagent) and acetic acid (enzymatic hydrolysis product) toward CAL-B, together with the residual water of the lipase were shown to be potential reasons for side reactions, which affected the course of the kinetic resolution of various furan-2-yl-ethanols.

The versatility of β-hydroxycarboxylic acids and their esters make them valuable intermediates for the synthesis of many bioactive compounds. Multienzymatic procedures were developed for the synthesis of both enantiomers of some new optically pure heteroaryl-3-hydroxypropanoic acids with *N*-substituted phenothiazinyl-, benzofuranyl and benzothiophenyl moyeties. As an important theoretical approach, the enantiopreference of lipases A and B from *Candida antarctica* in the kinetic resolutions of these derivatives was also presented.

Next, the enzymatic resolution of some racemic heteroaryl-ethane amines was is presented.

Starting from the racemic cyanohydrins as substrates, a chemo-enzymatic strategy was developed for the synthesis of optically pure heteroaromatic α-hydroxy acids.

Optically active cyanohydrins are important and versatile intermediates since they can be easily transformed into other optically active compounds such as α-amino and α-hydroxycarboxylic acids, β-aminoethanols or ethane diols, etc. Finally, the lipase mediated dynamic kinetic resolution of novel phenothiazin-cyanohydrins is described.

Chapter 4 presents some applicative aspects regarding the use of biocatalysis in the synthesis of pharmaceuticals and of chiral building blocks. Lipase AK from *Pseudomonas fluorescens* (PFL) was adsorbed on Celite (diatomaceous earth) in the presence of sucrose, cross-linked as CLEAs or encapsulated in sol–gels and finally used as biocatalysts for the EKR of a serie of racemic ethyl 3-(hetero)aryl-3-hydroxypropanoates. The immobilized preparations were characterized and optimized using constant protein contents.

As the final subject, two enzymatic alternative biocatalytic approaches for the synthesis of both enantiomers of Bufuralol, a widely studied potent, nonselective, β-adrenergic receptor antagonist is presented. Through the biotransformation of prochiral α-substituted 7-ethyl-benzofuran-2-yl-ethanones and by the lipase catalyzed enantiomer selective *O*-acylation kinetic resolution of (7-ethyl-benzofuran-2-yl)-bromohydrin, highly enantiomerically enriched intermediates were obtained and subsenquently chemically transformed in the desired compounds.

Part II, New strategies for the future, include the following research directions: chiral switching in drug synthesis using biocatalytic methodologies, taylor-made biocatalysts in drug synthesis and the development of an efficient isolation method of chiral molecules from complex mixtures using click reaction based procedures.