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Preparation of chiral derivatives of phosphonates using biocatalytic methods

Potential biological activity of phosphonic acids is a major reason of great interest in the bioconversion of phosphonic analogues of amino acids, which can be considered as mimetics in enzymatic reactions. The main aim of the research is the application of the biocatalysis as a synthetic tool for the obtaining valuable compounds. Also, bioconversion processes are using advantages of microbiology, biochemistry and bioorganic chemistry. The biotransformations, carried out not only on the laboratory scale, but also in the industry, with use of whole- cells biocatalysts has gained a great importance. They are selective towards different substrates, usually, easy to cultivate and biodegradable.

Presented thesis discusses the results of the application of the fungal biocatalysts for the synthesis of optically pure organophosphorus compounds. At the beginning, the experiments were devoted to the laboratory scale studies with the free fungal mycelium applied as biocatalyst. From the tested aminophosphonic substrates, the following compounds were converted: 1-amino-2-methylpropylphosphonic acid **21**, 1-aminophenylmethanephosphonic acid **22**, 1-amino-2-methylbutylphosphonic acid **59** and 1-amino-2-methylpiridynphosphonic acid **63**. The path of bioconversion was defined and the products and intermediates of biotransformation were identified. Biological conversions of aliphatic α-aminophosphonates: 1-amino-2-methylpropylphosphonic acid **21**, 1-amino-2-methylbutylphosphonic acid **59** and aromatic α-aminophosphonates 1-amino-2-methylpiridynphosphonic acid **63** were performed with the use of *Penicillium funiculosum*, which was the most effective biocatalyst. Aminophosphonates were transformed starting from the oxidative deamination of the substrates– ketones formation, which were subsequently bioreduced in the next step of the process to α- hydroxyphosphonates. It allows obtaining final products: 1-hydroxy-2-methylpropylphosphonic acid **73**, 1-hydroxy-2-methylbutylphosphonic acid **74** and 1-hydroxy-2-methylpiridynphosphonic acid **78**. Also, unreacted enantiomer of *R*-1-amino-2-methylpropylphosphonic acid **21** (100% *e.e.*) was obtained.

An analogous scheme of the bioconversion was observed for 1-aminophenylmethanephosphonic acid **22**, but in this case *Fusarium oxysporum* (UW1) was applied. The cascade bioprocess of stereoselective oxidation and further non-stereoselective reduction of intermediate α- ketophosphonic acid leads to racemic mixture of

1-hydroxyphenylmethanephopshonic acid **75** and resulted in the receiving of unreacted enantiomer of aminophosphonic acid **22** of *S* configuration (100% *e.e.*).

Also, hydrolysis of *O,O*-dimethyl-4-oxoazetidin-2-ylphosphonate **69** was studied and the results proved that this compound is converted according to enantioconvergent pattern to optically pure *O*-methyl-4-oxoazetidin-2-ylphosphonate **86** (100% *e.e.*). The activity of the enzymatic system of *P. minioluteum* allowed to obtain 30% of conversion of racemic mixture of substrate **69** and optically pure product **86**. Using whole-cells biocatalyst *O*-methyl-4-oxoazetidin-2-ylphosphonate **86** was transformed, but after the amide bond hydrolysis, the racemization was noticed. Therefore, also hydrolytic enzymes were studied as catalysts towards this substrate. The application of the isolated enzymes, namely penicillinase from *Enterobacter cloacae*, which was active toward substrate **69**, resulted in the only one product **86,** obtained with 100% of *e.e*. and conversion degree of 35%. In this case, hydrolysis of the amide bond and racemization was not observed.

Immobilized on polyurethane foams biocatalysts were also applied to increase the scale of the process and also to increase the amount of obtained products. Bioconversion of 1-amino-2-methylpiridynphosphonic acid **63** led to very interesting result, which was different than previous studies. The use of biocatalysts immobilized on polyurethane foams (*P. funiculosum*) in continuous process led to obtain optically pure product- *S*-1-amino-2-methylpiridynphosphonic acid **63** (100% *e.e.*). It is also kind of enantioconvergent process- optically pure product was obtained after the non-stereoselective conversion of the chiral substrate.

In order to analyze the results of the study, it was necessary to develop a method of isolating valuable bioconversion products to confirm the structures and the postulated path of biotransformation process. In addition, the analytical conditions for the enantiomeric excesses evaluation were elaborated for all compounds. It allowed to check the progress of the reaction using the ^{31}P NMR spectroscopy technique with the addition of α-cyclodextrin as the chiral solvating reagent.