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*New applications of biocatalytic methods in the asymmetric
synthesis of heteroorganic compounds using ene-reductases and
enzyme catalytic promiscuity*

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Abstract

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The following thesis presents new applications of ene-reductases in the synthesis of chiral organophosphorus compounds and a new example of enzyme catalytic promiscuity – enzymatic aza-Henry reaction. As part of the studies concerning ene-reductases, a new method of synthesis of (Z)- α,β -unsaturated phosphonates utilizing Still-Gennari olefination has been developed. Moreover, biotransformations using isolated ene-reductases were compared with the application of *Mucor circinelloides* whole cells for the same process, where the asymmetric carbonyl group reduction was also observed.

The most important achievements of the presented thesis are:

- Application of isolated ene-reductases in the asymmetric reduction of α,β -unsaturated phosphonates. Ene-reductases were proven to successfully catalyse the reduction of α,β -unsaturated phosphonates with excellent stereoselectivity. Moreover, an insight into the mechanism of the transformation was gained by a deuterium labelling experiment and molecular docking studies using AutoDock 4.2.
- Application of Still-Gennari olefination for the synthesis of (Z)- α,β -unsaturated phosphonates and confirmation of a stereocontrol possibility of ene-reductase catalysed reduction by an appropriate selection of substrate geometrical configuration. A series of yet unknown (Z)- α,β -unsaturated phosphonates was obtained using Still-Gennari olefination. Enzymatic OYE3 catalysed reduction of substrate (E)-**1a** afforded product (+)-**9a** while reduction of (Z)-**1a** gave (-)-**9a** proving the possibility of control of the stereochemical outcome of the reaction *via* selection of substrate geometrical isomers.
- Application of *Mucor circinelloides* in the asymmetric reduction of α,β -unsaturated γ -ketophosphonates. *M. circinelloides* whole cells were applied for the biotransformation of the abovementioned substrates. The asymmetric carbonyl group reduction was observed along with the C-C double bond reduction. All the biotransformations were highly stereoselective proving the high application potential of *M. circinelloides* in the asymmetric synthesis.
- The first example of the enzymatic aza-Henry reaction was presented. *Arabidopsis thaliana* oxynitrilase AtHNL and *Pseudomonas stutzeri* lipase TL were applied in the addition of nitromethane to imines. Experiments with denatured enzymes confirm the promiscuous activity of the enzymes, however all the products were obtained as racemic mixtures.