Biocatalytic Oxygenations in Bioactive Compound Synthesis

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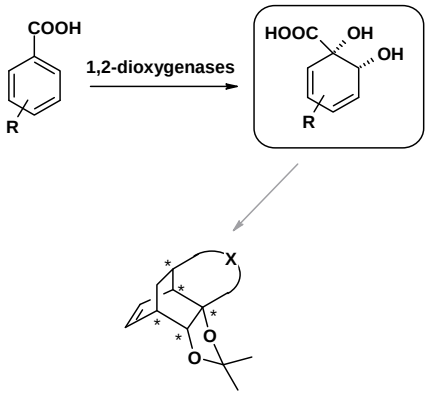
Introduction

Enzyme mediated oxygenations represent a highly environmentally benign class of transformations, which cannot be carried out enantioselectively using conventional synthetic approaches in many cases. However, wide-spread application among the community of synthetic chemists has so far been hampered be several obstacles. Our approach aims at the development of a biocatalyst based toolbox for enantioselective oxidation reactions. It is based on the utilization of whole-cells to overcome the problems of cofactor recycling and protein isolation and takes advantage of wild-type, mutant, and recombinant strains of living microorganisms.

1,2-Dihydroxylation of Aryl Carboxylates

While the potential of microbial 2,3-dihydroxylation has been demonstrated in recent years, the related enzymatic 1,2-dioxygenation of aryl carboxylates has been neglected to a greater extent. This biotransformation utilizes planar aromatic systems to generate quaternary and tertiary chiral centers. The biotransformation is performed with mutant microorganisms with a deficient metabolic pathway enabling the accumulation of highly functionalized intermediates in enantiomerically pure form. The obtained compounds represent versatile platforms that offer a great variety of options for subsequent functional group transformations.

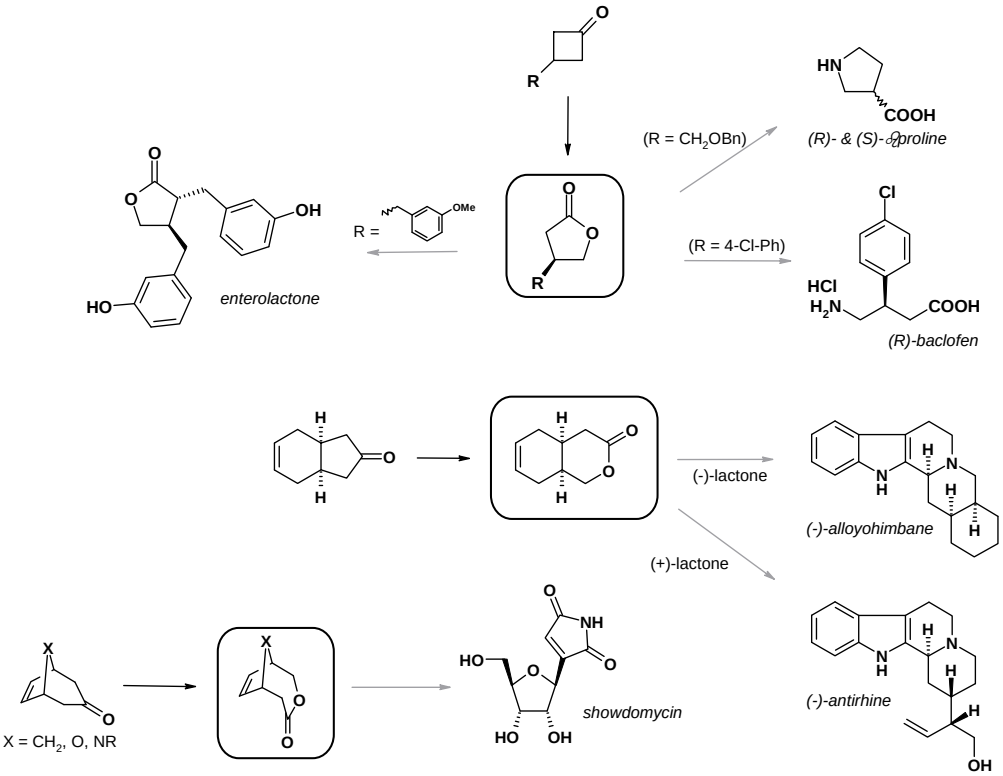
In this contribution our recent studies in the field intramolecular Diels-Alder cyclizations are presented. Utilizing microwave irradiation as sustainable energy source, polycyclic scaffolds were accessed in enantioselective manner with high control over several chiral centers in a minimum number of chemical steps (Scheme 1).[1]



Scheme 1. Dihydroxylation of aryl carboxylates by mutant whole-cells and subsequent synthetic elaboration implementing intramolecular cycloaddition reactions under microwave irradiation.

Microbial Baeyer-Villiger Oxidation

Baeyer-Villiger-type oxidation reactions using biocatalysts represent a powerful methodology for the one-step asymmetric synthesis of chiral lactones. Flavin dependent monooxygenases have been established as versatile chiral catalysts for this biooxidation and these enzymes are able to transform a large spectrum of non-natural substrates in high enantioselectivity.



Scheme 2. Microbial Baeyer-Villiger oxidation of prochiral ketones using recombinant whole-cells and subsequent synthetic elaboration towards the preparation of natural products and bioactive compounds.

The utilization of recombinant living cells circumvents the obstacle to recycle non-covalently bound cofactors such as NAD(P)H and provides easy to handle catalytic entities. According to recent reports, the stability of Baeyer-Villiger monooxygenases in isolated form is limited due to loss of cofactors and concomitant decrease of structural integrity. Hence, application of such enzymes in their natural environment inside living cells give superior results with respect to biocatalyst performance. In addition, the required proteins are produced in high concentration by strong promoters and become the dominant fraction in the cell's proteome. As a consequence, such high performance overexpression systems minimize the chance of unwanted side reactions caused by competing enzymes.[2]

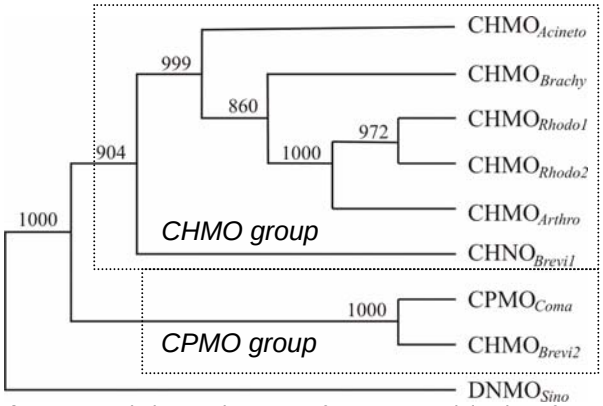


Figure 1. Phylogenetic tree of BVMOs originating from *Acinetobacter, Arthrobacter, Brachymonas, Brevibacterium, Comamonas,* and *Rhodococcus* species using the N^4-diaminopropane monoxygenase from *Sinorhizobium meliloti* (DNMO$_{Sino}$) as outgroup (1000 bootstraps).

Recently, our group has discovered the enantiodivergent biooxidation of a number of prochiral ketones by various Baeyer-Villigerases. In combination with novel data on the regiodivergent transformation of racemic substrates, the classification of a small library of 8 enzymes - available as recombinant whole-cell expression systems - into clusters is presented. These results represent the first connection of protein sequence information with stereopreference of such biocatalysts and suggests the existence of two distinct enzyme families (Figure 1).[3]

Applications of stereoselective Baeyer-Villiger biooxidations for the synthesis of natural products and bioactive compounds will be outlined on structurally diverse lactones starting from carbo- and heterocyclic precursor ketones using the above enzyme library (Scheme 2). A number of selected case studies will be presented to demonstrate the specific properties of some representatives of the library. The desymmetrization of prochiral ketone substrates will be emphasized as most efficient method to introduce chirality. With this approach up to four novel chiral centers could be generated in stereoselective Baeyer-Villiger biooxidations, moreover, allowing access to both antipodal lactone products in high optical purity.

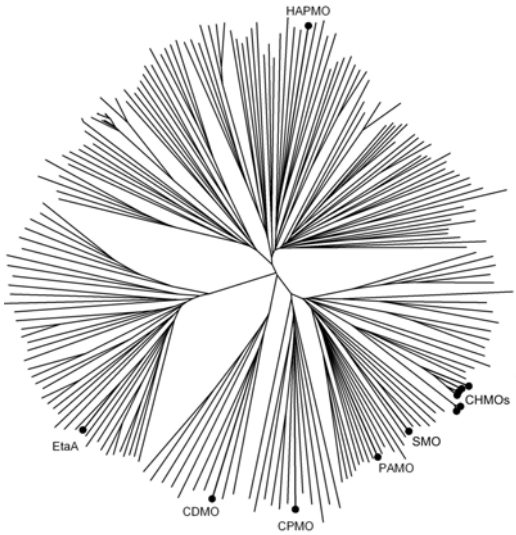


Figure 2. Phylogenetic tree representation of (putative) BVMO sequences, indicating their relative relationship based on sequence homology. Sequences were retrieved with the PHI-BLAST option using the sequence of HAPMO as seed and the BVMO-"fingerprint" sequence FXGXXXHXXXW as pattern. Characterized BVMOs are labeled with established acronyms.

Taking advantage of the natural diversity of Baeyer-Villiger monooxygenases available in our lab subcloned in recombinant overexpression systems (Figure 2), we are currently assessing substrate profiles in order to identify unique properties of this enzyme family. In this context we have developed a rapid parallel screening format, which provides information on the stereopreference and catalytic performance of recombinant microbial strains. In addition, modifications of the enzymes by random and knowledge-based mutagenesis and the effect on biotransformation efficiency and selectivity are currently investigated.

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