Reg. No: 2023/49/N/NZ1/03081; Principal Investigator: mgr in . Agata Ewa Matera

Steroids are a group of compounds with a broad and well-documented spectrum of biological activities. They exhibit among others anti-inflammatory, anti-cancer, and sedative effects and are one of the most important classes of drugs used in medicine. Unfortunately, due to the wide spectrum of action, steroids cause a variety of undesirable side effects, especially during prolonged usage. Accordingly, research focused on modifying and obtaining new steroid compounds may result in the development of pharmaceuticals with better therapeutic properties.

One of the modification methods that steroids may undergo is glycosylation, i.e. attachment of a sugar moiety. This process enhances stability of various chemical compounds, improves solubility, and may modify the biological properties of the molecule. Chemical glycosylation of compounds with such complex structure as steroids is a complicated, time-consuming, and expensive process. Using enzymes may be a good alternative, especially enzymatic cascades, which autonomise the process from the addition of expensive and low-available cofactors.

The proposed project aims to develop an innovative enzymatic platform for the glucosylation of steroids. The project implementation will involve the selection of steroid glucosyltransferase, assessment of their substrate specificity and their ability to cooperate with sucrose synthase is self-sufficient glucosylation cascade.

Research conducted within this project will combine synthetic biology and organic chemistry. The base for the production of tested enzymes will be genetically modified *Escherichia coli* strains. The activity and specificity of the enzymes will be assessed *in vitro* using chromatographic and colorimetric techniques. In addition, the selected enzymes will be immobilized to assess their utility for larger-scale processes.

The cascade developed within this project will be a convenient and low-cost platform for the glucosylation of a broad range of steroid compounds, which will increase their availability, facilitate the evaluation of their medical activities and also production of such desirable pharmaceuticals. The project findings will be shared through presentations at scientific conferences and successively published in international scientific journals, ensuring the wider scientific community can benefit from and build upon our research.