Biotechnological methodologies for BamA Inhibitor derivatization

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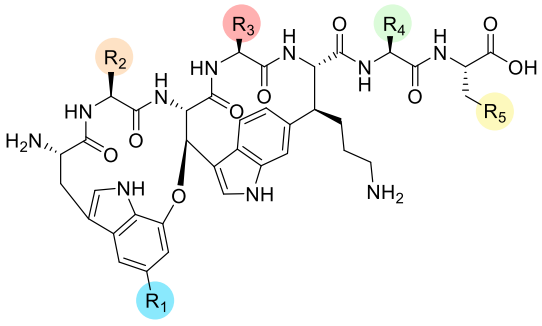
The development of novel antibiotics against the WHO's top-priority or "critical" Gram-negative pathogens, continues to challenge the scientific community. A promising discovery are darobactins (DARs), a group of specialized bacterial metabolites that arrest the functionality of the essential outer membrane protein insertase BamA. Here, we want to present our biotechnological repertoire for DAR modification and optimization.

Firstly, we were able to randomize amino acid side-chains of the peptidic backbone by side-saturation mutagenesis of the precursor peptide encoding gene *darA* to yield a heterologous producer library harboring the biosynthetic information for ~16.000 DAR-like heptapeptides. Independently, we discovered the halogenase gene (*darH*) in a darobactin-like gene cluster of a marine *Pseudoalteromonas* strain. Purified DarH was used to produce brominated darobactins in a cell free reaction. Catalysis by DarH was specific to bromination and iodation and limited to N-terminal tryptophane of the final product. Lastly, we applied amber stop codon suppression to introduce non-canonical amino acids at the C-terminus of the DAR peptide sequence. In a first attempt, we were able to exchange the native phenylalanine with modified variants such as 4-fluoro-phenylalanine, 4-methoxy-L-phenylalanine or 2,3,4,5,6-pentafluoro-L-phenylalanine.

Certainly, not all newly engineered derivatives exhibited increased potency compared to our frontrunner darobactin B, but we believe that the diversification of the biotechnological toolbox for modification of DAR-like peptides is of great value. Using these technologies in combination with computational chemistry, we will continue our endeavor to expand the chemical space of DAR-like peptides to ultimately deduce a clear LO strategy



R_1 = H, Br, I

R_2 - R_4 = residues corresponding to natural amino acid side chains

R_5 = C_6H_5, 4-FC_6H_4, 4-$(OCH_3)C_6H_4$, 4-IC_6H_4, C_6F_5