Expanding Thiopeptide Chemical Space Through in Vitro Enzymatic Synthesis

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Thiopeptides are a class of macrocyclic, post-translationally modified peptide natural products that includes potent antibiotics such as thiostrepton, GE2270A, and thiomuracin. Thiopeptides routinely display nanomolar activity against a variety of clinically-relevant pathogens including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci. Despite their impressive in vitro profile, thiopeptides remain underutilized in clinical settings due to poor physicochemical properties and challenging chemical syntheses. We have reconstituted the core biosynthetic machinery from thiomuracin biosynthesis and have optimized this system to produce multi-milligram quantities of bioactive macrocycle. Reconstitution has enabled in-depth studies of each enzymatic transformation on this pathway to reveal reaction order, timing, and scope of substrate specificity. Armed with this information, we are now poised to harness this system to generate analogs with enhanced properties/potency as well as functionalizable moieties for chemo- and site-selective derivatization.