With the advent of high throughput biomolecular engineering techniques, it has become possible to isolate short peptides that bind to a variety of targets ranging from inorganic materials to proteins. The application of peptides as therapeutics has been hampered by the inherent susceptibility of peptides to proteases present throughout the human body. One strategy to overcome this protease susceptibility is to fortify peptides via cyclization or other conformational constraints. Indeed, nature uses this strategy in several classes of peptides such as cyclotides and defensins which are stabilized by networks of disulfide bonds and in some cases head-to-tail cyclization. My group studies a class of peptides termed lasso peptides because of their unique slipknot-like structure. This highly entropically disfavored fold endows the peptides with tremendous stability; some lasso peptides can retain their structure and function even after boiling in 8 M urea. Lasso peptides are also resistant to proteolysis by digestive proteases such as pepsin and chymotrypsin. In this talk I will describe our efforts in understanding the biosynthesis of lasso peptides with a particular focus on the role of the leader peptide in lasso peptide biosynthesis. Insights into lasso peptide biosynthesis have enabled us to develop a new genome mining approach for lasso peptide discovery. I will also describe our efforts in lasso peptide engineering including a directed evolution study aimed at improving the antimicrobial efficacy of the lasso peptide microcin J25. Finally I will describe new work on the biosynthesis of “designer” lasso peptides in which we graft an arbitrary peptide sequence onto the hyperstable lasso peptide scaffold with an eye toward potential therapeutic applications.