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# Previews

## Sweetening Cyclic Peptide Libraries

**Enzymatic macrolactamization of linear glycosidated peptides provides access to an important class of drug-like molecules. The work presented in this issue [1] shows that it may be possible to make complex libraries of glycosidated cyclic peptides by incorporating glycosidated amino acids into linear peptides via solid-phase peptide synthesis followed by thioesterase-mediated peptide cyclization.**

From a drug discovery standpoint, cyclic peptides possess many advantageous properties. Large peptide libraries can be readily assembled from the vast array of available amino acids. The cyclic nature of the compounds allows the molecules to populate a defined molecular structure. These two factors permit sampling of a large amount of conformational space, increasing the likelihood of identifying or optimizing biological activity. Cyclic peptides are also predisposed to favorable pharmacokinetic properties, including metabolism and excretion. Additionally, they are more resistant to proteases than their linear analogs, increasing their serum stability. The combination of these factors makes cyclic peptides a useful core structure for drug development via combinatorial library screening [2].

However, cyclic peptides have serious limitations as a platform for drug discovery. Critically, they are poor at crossing biologically relevant membranes, severely limiting their utility as pharmaceutical agents. Additionally, chemical cyclization of linear peptides is challenging and sequence dependent, limiting library size and biasing libraries toward particular regions of conformational space.

Glycosidation substantially improves the pharmacological characteristics of cyclic peptides. Cellular uptake and solubility can be enhanced by glycosidation. Glycosidation provides a potential mechanism to target cyclic peptides to specific cell types. The promise of glycosidated cyclic peptide libraries, with their intrinsically favorable pharmacokinetics and high levels of molecular diversity, is tempered only by the difficulty of constructing these compounds.

Construction of cyclic peptide libraries is a challenging task due to the inherent difficulty in cyclizing linear peptides. The *trans* configuration of the amide bonds in linear peptides prevents the reactive C and N termini from reaching each other. A number of chemical methods are available for the formation of cyclic peptides [3]. These approaches require linear peptide conformations that place the C and N termini in close proximity. This proximity is enforced via specific amino acid sequences (e.g., gly- or pro-rich sequences, mixtures of D and L amino acids), limiting the applicability of these methodologies to large library construction.

Enzymatic approaches have proved effective at generating diverse libraries of cyclic peptides. Split intein-based approaches are capable of generating cyclic peptide libraries without the structural biases required in chemical approaches [4]. This methodology however has two serious limitations. Since the inteins are expressed in vivo, purification of the cyclic peptide products is challenging and the peptide diversity is limited to proteinogenic amino acids.

Walsh and coworkers have successfully used recombinant thioesterase (TE) from the tyrocidine biosynthetic pathway to construct a library of cyclic peptides [5]. The cyclic decapeptide tyrocidine is generated biosynthetically by a nonribosomal peptide synthetase (NRPS) system [6]. These multienzyme systems, which also generate molecules like gramicidin and vancomycin, catalyze the formation of a linear peptide that is linked as a covalent acyl-enzyme intermediate to the enzyme. The final enzyme in the pathways is the TE, which catalyzes cyclization and release of the product.

Purified recombinant tyrocidine TE can convert linear peptides into cyclic peptide products efficiently in vitro. Studies have shown that the TE is remarkably tolerant to changes in the linear peptide [7]. Linear peptides of various lengths are cyclized (6–14 amino acids) and replacement of most amino acids is tolerated by the TE. The crucial structural elements that permit enzymatic recognition and cyclization of the substrate appear to be the N-terminal amino acid residue and the penultimate C-terminal amino acid. Changing either of these amino acids dramatically attenuates enzymatic cyclization. The otherwise broad substrate tolerance makes it possible to use the TE-mediated cyclization in library formation since one can be confident that cyclic peptide products are generated for the large majority of library members [5]. This is important in large library synthesis since each library member cannot be assayed for chemical structure or purity. An additional advantage is that the linear peptide substrates are synthesized by solid-phase peptide synthesis (SPPS), allowing for the incorporation of nonproteinogenic amino acids and increasing the potential library scope.

Glycosidation of cyclic peptides is not currently useful for accessing large libraries of glycosidated cyclic peptides. While chemical glycosidation of cyclic peptides is an appealing approach to carbohydrate introduction, this method suffers from multiple drawbacks in a library context [8]. In particular, the peptide substrates are highly insoluble in the anhydrous organic solvents required for sucessful glycosidation [9]. Additionally, there is little regiochemical control of glycosylation, which leads to heterogeneity of each library member, complicating library screening and evaulation.

Enzymatic glycosidation of cyclic peptides is also not suited for large library synthesis. The limiting factor in enzymatic glycosidation is the substrate specificity of the glycotransferases. Glycotransferases are highly specific to both their sugar and aglycon substrates. For effective synthesis of a diverse library, this specificity needs to be dramatically attenuated. Unfortunately, no

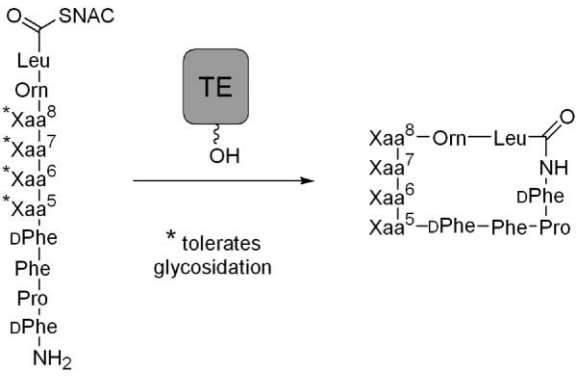


Figure 1. Linear Glycosidated Peptides Were Synthesized by FMOC SPPS and Successfully Cyclized by Purified Recombinant Tyrocidine TE

multipurpose glycotransferase has been characterized to date [10].

Recognizing the difficulties associated with glycosidation of cyclic peptides, Lin et al. [1] opted to explore the TE-mediated cyclization of glycosidated linear peptides. One advantage of this approach is the well-documented literature detailing the regioselective incorporation of sugars into linear peptides via SPPS [8]. Since the tyrocidine TE domain was known to be tolerant to substantial modification of the linear peptide, it was anticipated that glycosidation would not adversely effect the cyclization activity.

A variety of linear *O*-linked glycosidated peptides were prepared by FMOC SPPS, using glycosidated serine or tyrosine residues. The TE domain catalyzed cyclization of all the glycosidated peptides tested (Figure 1). The TE was tolerant of glycosidation at amino acids 5–7. Cyclization kinetics were essentially unaffected by the composition of the carbohydrate (Glc, GlcNAc, $GlcNH_2$, Gal). Fully protected carbohydrates (tetraacetates) were also processed by the TE domain. Reduced cyclization efficiency was observed when amino acid 8 was glycosidated; however, these reactions still generated the desired product.

These results suggest that TE-mediated cyclization of glycosidated linear peptides may be an effective route for generating libraries. Introduction of the sugar residue by SPPS allows carbohydrate complexity to be generated into linear peptides. Independence of cyclization kinetics from carbohydrate identity and regiochemistry suggest that this method may faithfully incorporate the diversity of a linear peptide library into cyclized compounds.

This work raises a number of interesting questions that will need to be addressed before the scope of the methodology can be determined. Cyclization of the protected carbohydrates suggests that the TE domain might also cyclize oligosaccharide decorated compounds. Will the TE domain also tolerate multiple sugar attachment sites? Will the TE domain tolerate glycosidation at the other amino acid residues? Will it be possible to introduce depsipeptide linkages or peptidomimetic scaffolds into these systems? These questions will help define the limits of carbohydrate and peptide complexity that can be introduced via the tyrocidine system. There are also a host of other TE domains known and many more still to be identified. Will these TE also exhibit relaxed substrate specificity and will they tolerate gycosidation of their linear substrate? If so, these enzymatic domains may provide a way to access glycosidated cyclic peptides with different enzyme recognition elements, dramatically different ring sizes, or with different types of cyclization (i.e., through side chain rather than backbone functionality). This work opens the door to an exciting and promising new direction for the synthesis of complex libraries of glycosidated cyclic peptides.

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**Selected Reading**

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