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Combining biocatalysis and chemoselective chemistries for glycopeptide antibiotics modification

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Glycopeptide antibiotics are clinically important medicines to treat serious Gram-positive bacterial infections. The emergence of glycopeptide resistance among pathogens has motivated considerable interest in expanding structural diversity of glycopeptide to counteract resistance. The complex structure of glycopeptide poses substantial barriers to conventional chemical methods for structural modifications. By contrast, biochemical approaches have attracted great attention because ample biosynthetic information and sophisticated toolboxes have been made available to change reaction specificity through protein engineering, domain swapping, pathway engineering, addition of substrate analogs, and mutagenesis.

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Introduction

As lack of complexity from conventional combinatorial chemistry, 'diversity-oriented synthesis' or 'synthesis on privileged compounds' has been a growing trend in the field of medicinal chemistry [1•,2]. This is simply because natural products are rich in structural diversity and possess a wealth of biological activities (i.e. antimicrobial, antitumor, immunosuppression), making them superb lead compounds. Owing to the strong correlation of natural products to pharmacological functionality, efforts to expand chemical diversity have employed multidisciplinary strategies to leverage natural products in drug discovery. The combination of synthetic chemical tools and recombinant enzymes, particularly through manipulation/engineering of genes/enzymes for secondary metabolites, has an edge over others. Such an approach also appears to be environmentally friendly, having attracted great interest.

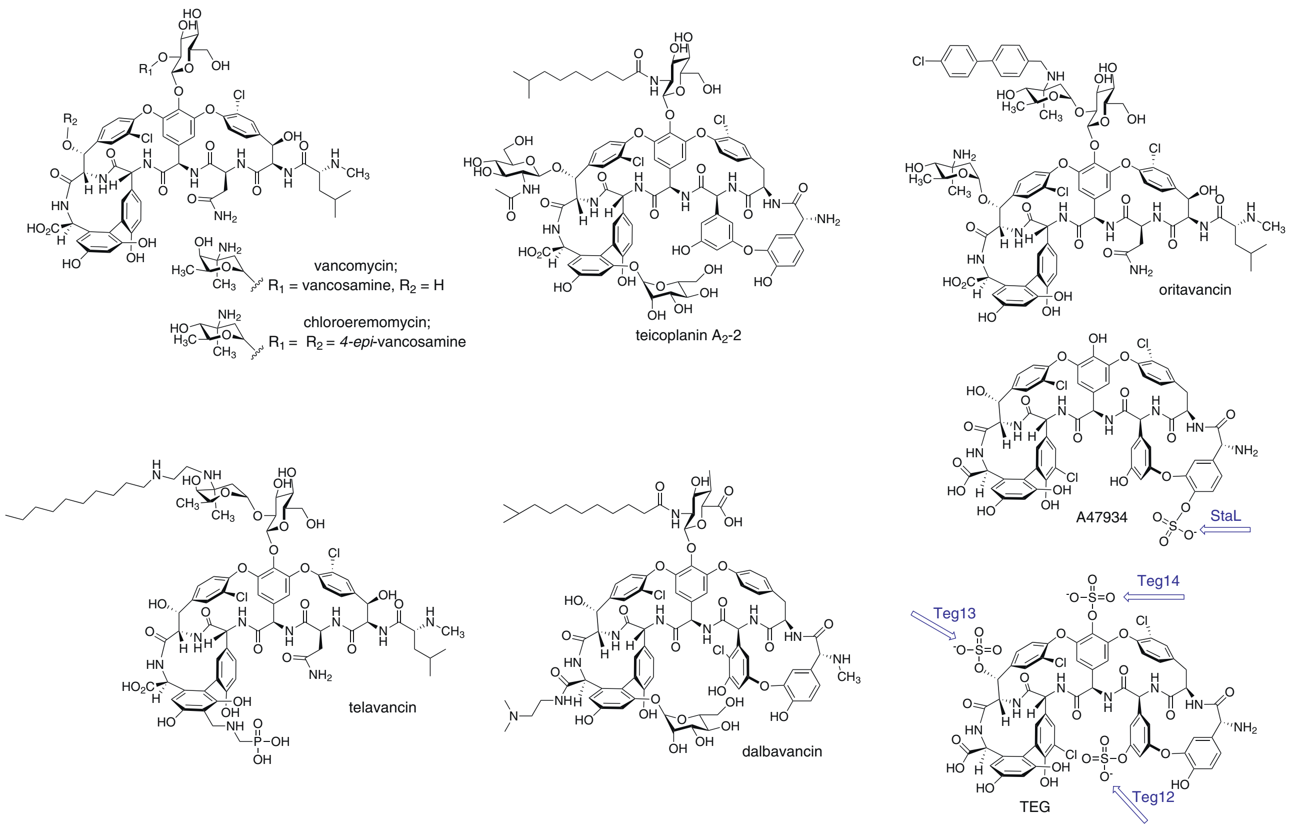
Glycopeptide antibiotics are an important class of natural products. For example, vancomycin and teicoplanin (Figure 1) are currently drugs of last resort for life-threatening infections caused by multi-drug resistant Gram-positive pathogens [3]. While methicillin-resistant *Staphylococcus aureus* (MRSA) can only be successfully treated with vancomycin, a strain of *S. aureus* (VRSA) immune to vancomycin has emerged [4]. Recently, three semisynthetic glycopeptides – oritavancin, dalbavancin and telavancin, have been introduced into the clinic to combat Gram-positive pathogens (Figure 1) [5–7]. These analogs were modified from their individual parent scaffolds but more effective against some drug-resistant pathogens, probably resulting from new modes of action (i.e. disruption of cell wall synthesis and/or cell membrane integrity). However, new analogs with better efficacy are still in high demand as drug resistance continues to keep pace with introduction of new antibiotics into clinical settings [8,9]. In this review we will discuss enzymes and chemoenzymatic strategies to expand glycopeptide structural diversity.

Glycopeptide antibiotics

Glycopeptide antibiotics are classified into five structural subtypes, type I–V [10]. Of the varying structural subtypes, type I structures, such as vancomycin, contain aliphatic side chains Leu and Asn, whereas types II, III, and IV include aromatic side chains in place of the aliphatic residues. Types III and IV contain an extra ring system. Type IV compounds have an additional long fatty-acid chain attached to the sugar appended to the central 4-hydroxyphenylglycine (Hpg). Type V, such as complestatin and kistamicin A and B, contains the characteristic tryptophan moiety linked to the central Hpg.

To date, six gene clusters involved in the biosynthesis of balhimycin (*bal*), chloroeremomycin (*cep*), A47934 (*sta*), A40926 (*dbv*), teicoplanin (*tcp*/*tei*) and sulfo-teicoplanin (*teg*) are available in GeneBank, providing the basis for elucidating most of the biosynthesis steps [11–17,18•]. Readers can refer to several excellent reviews for relevant biosynthetic information [19••,20–23]. Given that members of the glycopeptide antibiotics family are similar but not identical, it is likely the cross-pollination of biosynthetic enzymes and/or substrates among species can be used to create new glycopeptide scaffolds. By virtue of the traits of the relevant substrates/enzymes and the deciphered biosynthetic information, one now is in a position to reprogram natural glycopeptide antibiotics at the level of heterogeneous backbone unit and post-assembly modification through sulfation, glycosylation, acylation, methylation, halogenation, oxidation, and so

Figure 1



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Chemical structures of selected natural and semi-synthetic glycopeptides: oritavancin, analog of chloroeremomycin; telavacin, analog of vancomycin; dalbavancin, analog of A40926. Blue arrows indicate where sulfation takes place for a given sulfotransferase.

on. Progress has been made in recent years, of which major achievements are discussed in following sections.

Sulfation and sulforandomization

A47934 contains a sulfate group on the *N*-terminal Hpg (Figure 1). *In vivo* and *in vitro* experiments demonstrated the involvement of the sulfotransferase StaL catalyzing the final step in A47934 biosynthesis [14]. Not only does StaL regiospecifically sulfate the A47934 heptapeptide aglycone but also teicoplanin to generate A47934 and sulfated teicoplanin. Thereby, enzymatic sulfation modification of glycopeptide becomes feasible.

The biosynthetic gene cluster *teg* that encodes the biosynthesis of a polysulfated glycopeptide congener was recently identified. Three closely related PAPS-dependent sulfotransferases (Teg12, Teg13 and Teg14) sulfate the teicoplanin heptapeptide core at three unique sites (Figure 1) [18•]. By virtue of combination of these enzymes, seven new sulfated glycopeptides (without glycomodification) at various degrees of sulfation were produced. Whether naturally occurring glycopeptides other than the teicoplanin heptapeptide core suit as substrates of these enzymes remains awaiting further examination. Nevertheless, crystal structures of StaL, Teg12 and Teg14 have been solved, which should enable the use of sulfotransferases toward a new level of diversity for this class of antibiotics [24–26].

Glycosylation and glycorandomization

Glycosylation considerably extends the diversity of naturally occurring glycopeptides [27•,28,29]. For example, chloroeremomycin adorns the central Hpg, the penultimate β-hydroxytyrosine (Hty) and the 2′-OH group of the glucosyl moiety with D-glucose, L-*epi*-vancosamine and L-*epi*-vancosamine through three sequential regiospecific glycosylation reactions catalyzed by three glycosyltransferases (GTs) GtfB, A and C, respectively (Figure 2a) [30]. Vancomycin is different from chloroeremomycin by lacking a sugar moiety at the penultimate Hty. Two glycosyltransferases GtfE and GtfD that correspond to GtfB and GtfC finish the glycosylation but using L-vancosamine as a replacement for L-*epi*-vancosamine [31]. In teicoplanin, GtfB (Orf10*) and GtfA (Orf1) respectively