Vancomycin Resistance: Small Molecule Approaches Targeting the Bacterial Cell Wall Biosynthesis

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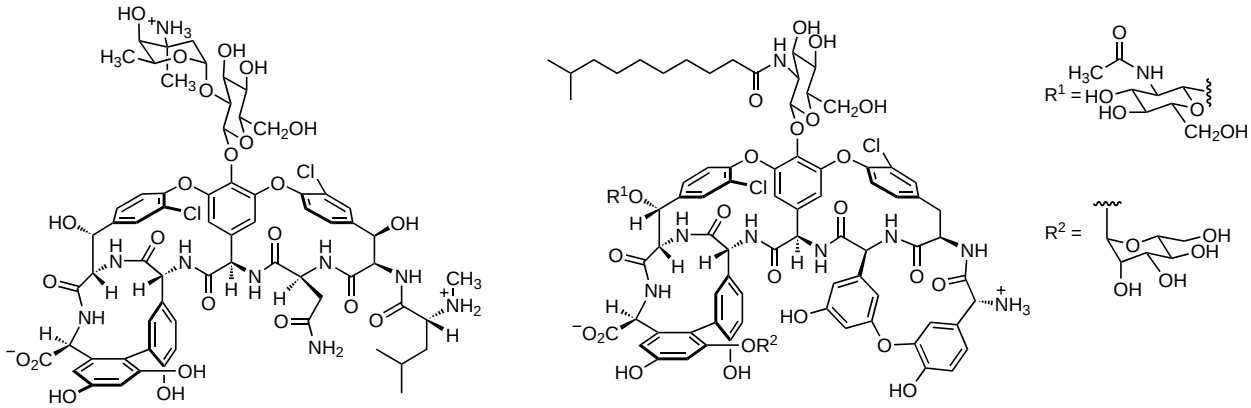
The development of bacterial resistance toward commonly used antibiotics represents an increasing threat to public health.[1] So far, resistances have been developed to all clinically used antimicrobial agents. Among the antibiotics, the glycopeptide vancomycin (**1**; Scheme 1) is of great clinical importance for the treatment of infections by Gram-positive bacteria such as Staphylococci, Enterococci, and Streptococci. In contrast to other antibiotics, it has been used for nearly 30 years without any observation of resistance.[1] Although relatively expensive and difficult to administer, vancomycin has gained the reputation as an antibiotic of last resort in the treatment of infections with methicillin-resistant *Staphylococcus aureus* (MRSA) strains.[1, 2] Major concerns evolved, however, when resistance against vancomycin was reported for strains of Enterococci in 1988[3] and was observed in staphylococcal isolates almost ten years later.[4]

The active principle of glycopeptides is a highly specific, noncovalent binding to the C-terminal D-Ala – D-Ala peptide motif of the UDP-muramyl pentapeptide that is a precursor of the bacterial cell wall biosynthesis (UDP = uridine diphosphate); this binding involves five hydrogen bonds. Subsequent transglycosylation (chain elongation) and transpeptidation (cross-linking) steps in the cell-wall assembly are inhibited and lethal cell lysis of the bacterium results (Figure 1).

also clinically used teicoplanin (**2**; Scheme 1) whereas VanB Enterococci are resistant to a wider range of vancomycin concentrations but remain susceptible to teicoplanin. The VanA and VanB resistances are both transposon-mediated and both rely on the alteration of the D-Ala – D-Ala peptide motif of the UDP-muramyl pentapeptide into the depsipeptide motif D-Ala – D-Lac. Thus, a 1000-fold decrease in affinity of the depsipeptide toward vancomycin confers antibiotic resistance. Similarly, intrinsically resistant VanC Enterococci (*E. gallinarum*, *E. casseliflavus*) use this principle in producing D-Ala – D-Ser altered peptidoglycan presursors (Table 1). The mechanisms of glycopeptide resistance in Staphylococci are far less understood than those in Enterococci. Although enterococcal resistance has been shown to be transferable to Staphylococci in lab experiments,[6] resistance



1 **2**

Scheme 1. *Structures of clinically used glycopeptide antibiotics vancomycin (**1**) and teicoplanin (**2**).*

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At present, in Enterococci three major resistance mechanisms (VanA/VanB/VanC) for glycopeptides have been described (Table 1).[2, 5] The VanA Enterococci (*E. faecium*, *E. faecalis*) are resistant to high concentrations of vancomycin and observed in Staphylococci thus far does not share the same mechanism as that of Enterococci.

A major part of the past chemical and biochemical research on glycopeptide antibiotics focused on a deeper under-

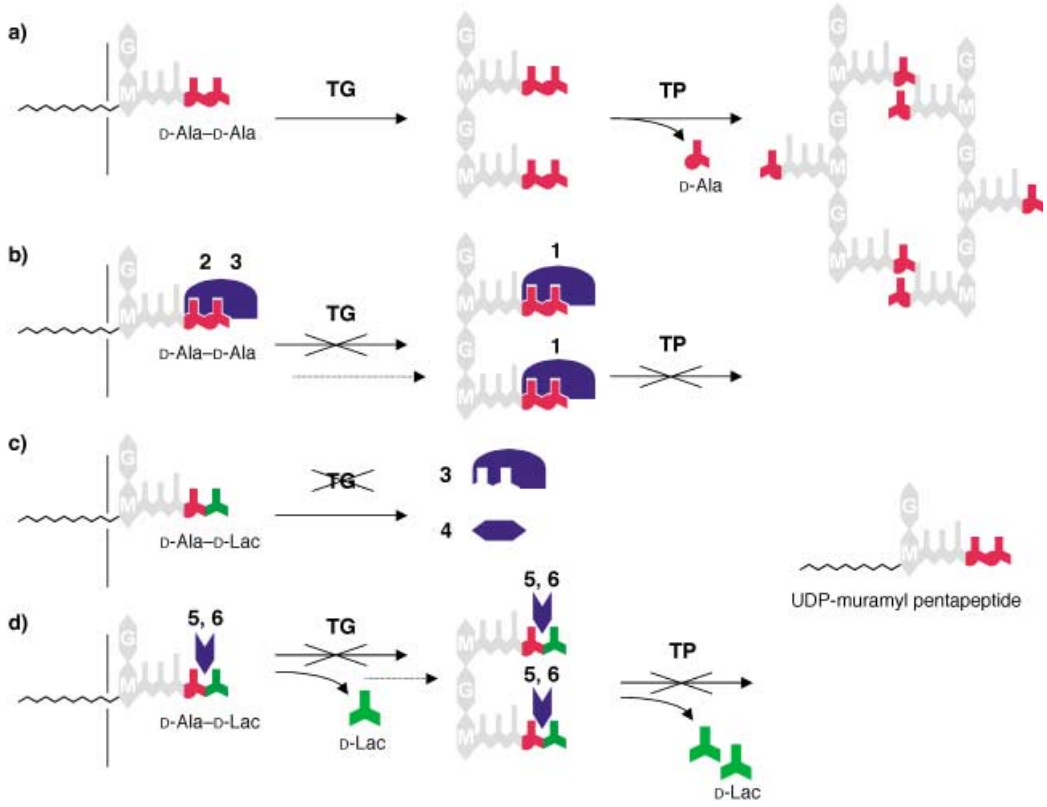


Figure 1. *a) Scheme of the cell wall biosynthesis of Gram-positive bacteria. Chain elongation of the UDP-muramyl pentapeptide is performed by a transglycosylase (TG) and further cross-linked by a transpeptidase (TP). The sequence of the UDP-muramyl pentapeptide precursors varies slightly dependant on the bacterium except for the C-terminal D-Ala – D-Ala motif. b) Vancomycin-sensitive strains: Inhibition of the cell wall biosynthesis by vancomycin (**1**), teicoplanin (**2**), or LY333328 (**3**). c) VanA/VanB-resistant Enterococci: Inhibition of transglycosylating enzymes by LY333328 (**3**) or **4**.[14] d) VanA/VanB-resistant Enterococci: Proposed location of the inhibition of cell wall biosynthesis by small molecule cleavers **5** and **6**.[16] The necessary presence of vancomycin suggests the concommitant occurrence of a mechanism according to (b).*

Table 1. *Major genotypes of vancomycin-resistant Enterococci and their resistance characteristics.*

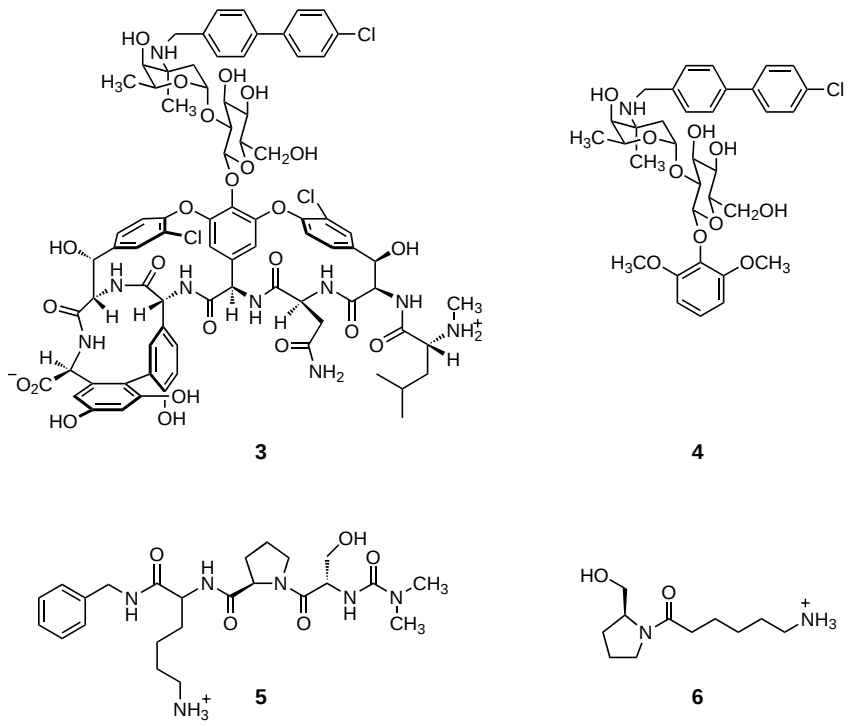
	VanA	VanB	VanC
alteration in cell wall biosynthesis	D-Ala – D-Lac	D-Ala – D-Lac	D-Ala – D-Ser
species	*E. faecium*	*E. faecium*	*E. gallinarum*
	E. faecalis	*E. faecalis*	*E. casseliflavus*
genetic determinant	acquired	acquired	intrinsic
transferable?	+	+	–
vancomycin MIC [μg ml^{-1}][a]	64 – > 1000	4 – 1024	2 – 32
teicoplanin MIC [μg ml^{-1}][a]	16 – 512	≤ 0.5	≤ 0.5

[a] MIC = minimal inhibitory concentration.

standing of the mode of action and on structure – activity relationship (SAR) studies in order to elucidate essential elements of molecular structure for antibiotic activity.[7, 8] With researchers already aware of the emergence of vancomycin-resistant Enterococci (VRE), simple chemical modification reactions of glycopeptides, such as deglycosylation, acylation, and alkylation reactions, predominated the first generation of studies in the early 1990s. This approach yielded considerable success in the form of the highly potent glycopeptide derivative LY333328 (**3**; Scheme 2), which displays high antibiotic activity against both MRSA and VRE and has since reached clinical phase studies.[9]

While the above-mentioned binding of D-Ala – D-Ala peptide motifs is crucial for antibiotic activity, the mode of action of the glycopeptides is still more sophisticated, and an ensemble of further structure-dependent effects, for example, dimerization (with chloroeremomycin) or membrane anchoring (teicoplanin),[10] have been suggested. These suggestions gave rise to the development of further semisynthetic glycopeptide derivatives, through the synthesis of dimers[11] and trimers, and through the usage of different tethers to cross-link the glycopeptide derivatives. Other methods break down the highly complex glycopeptide structure to a simpler and more easily synthetically accessible molecule in order to raise strong binders to the D-Ala – D-Ala and D-Ala – D-Lac peptide motifs.[12] An excellent review by Nicolaou and co-workers provides an overview of the developments in this field until 1999.[13]

However, the aforementioned contributions all consider vancomycin as a binding molecule and the investigations are led by the perception that an enhancement of binding to either the D-Ala – D-Ala or the D-Ala – D-Lac motif will lead to a stronger antibacterial effect in both glycopeptide-sensitive and -resistant bacteria. One recent contribution by Kahne and co-workers[14] questions this former view, by suggesting that antibac-



Scheme 2. *Structures of LY333328 (**3**) and chlorobiphenyl disaccharide **4**[14] and of D-Ala – D-Lac depsipeptide cleavers Bn-Lys – D-Pro – Ser dimethylurea (**5**)[16] and SProC5 (**6**).[16] Bn = benzyl.*

terial effects of some glycopeptide derivatives are not necessarily based on a strong peptide binding but rather on interactions with proteins involved in transglycosylation steps of the cell wall biosynthesis (Figure 1). The study shows that a modified disaccharide partial structural motif (**4**; Scheme 2) of the semisynthetic glycopeptide LY333328 (**3**) alone already displays antibiotic activity against both vancomycin-susceptible Enterococci as well as VRE. Although one might question that the investigation to locate the inhibited biosynthesis step was performed with Gram-negative *E. coli*, the Kahne group used a permeabilized vancomycin-sensitive strain and the observed antibacterial effects in Gram-positive Staphylococci seem to justify the extrapolation of results. A subsequent comparative study with the intact and damaged aglycon structure of vancomycin and teicoplanin supports these results.[15]

Consequently, in the opinion that binding is not necessarily required, another more recent contribution by Chiosis and Boneca[16] approaches the resistance problem posed by VanA/VanB-resistant Enterococci from another viewpoint. Herein the ester linkage of the D-Ala – D-Lac depsipeptide, characteristic of VanA/VanB-resistant Enterococci, is considered a structural motif cleavable by small molecules. Indeed, one has to agree that from the chemists' viewpoint, the cleavage of an ester is a priori a relatively easy exercise. Janda and co-workers employed similar considerations to those of Chiosis and Boneca in the isolation and characterization of catalytic antibodies as cleavers of the D-Ala – D-Lac peptidoglycan.[17] This is the first demonstration of an antibody using this substrate; however, the feasibility of advancement to a bacterial model might be impeded by the accessibility of the cell wall.

Starting with the above-mentioned hypothesis, Chiosis and Boneca tested different nonbiased tri- and tetrapeptide libraries in an on-bead color assay and selected some of the resulting best cleavers of the D-Ala – D-Lac peptide motif. As a basic requisite condition for a usable readout, the screening was performed in *N*,*N*-dimethylformamide in order to ensure the stability of the otherwise easily hydrolyzed transesterification intermediate. The concomittant emergence of Lys and Ser in positively tested beads led the authors to the suggestion that neighboring electrophilic and nucleophilic functional groups in the molecule's structure are required for an effective cleavage of the D-Ala – D-Lac motif, as found for structure **5** (Scheme 2). In further refinement studies for essential structural elements conferring bacterial activity, *N*-acylated prolinol derivatives were investigated, with ε-aminopentanoylated prolinol SProC5 (**6**) found to be the most effective compound (Scheme 2). The interaction of SProC5 with D-Ala – D-Lac is explained on the basis of a computer-generated model of a complex of both compounds. The authors showed in subsequent biological assays with a VanA-resistant *E. faecium* strain that concommitant administration of vancomycin and SProC5 indeed reduced the number of colony-forming units. An antibacterial effect of either SProC5 or vancomycin alone was not observed. Comparative studies with a vancomycin-sensitive *E. faecalis* strain showed no enhanced activity for vancomycin in the presence of SProC5, which supports their hypothesis. However, as the authors state, the SProC5 concentrations of 50 mM that are necessary to induce synergistic effects with vancomycin appear quite high and in this regard must be considered noncatalytic. Water solubility of the presumably hydrophobic compound SProC5 was not discussed as a problem in the performed experiments. Since it is known that VanA resistance is inducible by glycopeptides,[5] however, the authors give no explanation for the described resensitizing effect. A possible model would be that bacterial cell wall biosynthesis, because of the permanent cleavage of the D-Ala – D-Lac motif of the UDP-pentapeptide precursors, switches back to the original D-Ala – D-Ala synthesis and becomes sensitive to vancomycin again (Figure 1). A molecular interaction of vancomycin and SProC5, although not directly shown by experiments, can probably be excluded.

The contributions of Kahne and co-workers[14] and Chiosis and Boneca[16] set new horizons in the efforts to tackle the glycopeptide resistance problem. While the approach by Chiosis and Boneca selectively focuses on VanA- and VanB-resistant Enterococci, the approach by Kahne and co-workers appears more general and targets vancomycin-sensitive

and -resistant Gram-positive bacteria. Whereas the selective D-Ala – D-Lac cleavers still rely on glycopeptides as essential active agents, the findings of the Kahne group might reveal new target enzymes[18] and new antibacterial agents, which could point away from the use of glycopeptides. However, both approaches seem to have the potential for the development of new antibacterial compounds on the basis of small molecules. Lastly, considering the approach chosen by Chiosis and Boneca, this author is eager to stimulate work on the design of glycopeptide derivatives recognizing the D-Ala – D-Lac motif but devoid of tight binding. Such agents might induce D-Ala – D-Lac cleavage by acting as catalysts rather than as binders.

In light of these viewpoints one must pose the question of whether glycopeptides and their derivatives will still play a major role in drug development or if the recent surge of bacterial resistance heralds the end of the glycopeptide era. Indeed, rising stars, like the Food and Drug Administration (FDA) approved oxazolidinone-based antibiotic Linezolid,[19] are poised to take over the field, after having been proven as highly effective in the VRE strains *E. faecalis* and *E. faecium* as well as glycopeptide-intermediate *S. aureus* strains. However, in this context it is important to note that resistance has already been observed for Linezolid.[20] In the field of glycopeptides, the current investigations to understand their biosynthesis, especially the ring assembly of the aglycons, might shed light on new avenues for the synthesis of novel antibiotic agents. Next to an ongoing vigorous screening for new drugs that use these approaches, much more research must be aimed at an understanding of resistance mechanisms—particularly in Staphylococci—in order to reveal new targets for potential antibiotics.

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