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Invited review

Glycopeptide resistance in multiple antibiotic-resistant Gram-positive bacteria: a current challenge for novel semi-synthetic glycopeptide derivatives

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Summary — Resistance to the glycopeptide antibiotics vancomycin (V) and teicoplanin (T) in multi-resistant Gram-positive pathogens, particularly enterococci, is becoming a dramatic nosocomial problem. Besides the current lack of efficacious alternative therapeutic options, a major concern is the possibility of spreading of high glycopeptide resistance from VanA enterococci to methicillin-resistant *Staphylococcus aureus* (MRSA) and to coagulase-negative staphylococci (CNS), for which glycopeptides are still drugs of choice. In past years much effort was made in pursuit of new glycopeptide derivatives with enhanced efficacy against clinical isolates of MRSA and CNS with decreased susceptibility to T and occasionally to V. Promising results have been obtained by structural changes which did not affect binding to the glycopeptide's target peptide D-alanyl-D-alanine (D-Ala-D-Ala). The structure–activity relationships (SAR) of some modified T- and V-type compounds also indicated the possibility of achieving activity against highly glycopeptide-resistant enterococci by chemical derivatization of naturally occurring glycopeptides while maintaining unmodified the structure of the binding site. Recently, it has been found that the glycopeptide resistance in VanA enterococci is due to the replacement of target D-Ala-D-Ala by D-Ala-D-lactate depsipeptide in the peptidoglycan precursor. As glycopeptide derivatives active against resistant enterococci have not been shown to have enhanced binding to the target depsipeptide, a mode of action has been hypothesized that relates to their ability to dimerize and interact with membranes. The understanding of the mechanism of glycopeptide resistance in VanA enterococci, and the discovery of a selective procedure for the removal of amino acids 1 and 3 from natural glycopeptides also suggested new strategies which, based on molecular modeling studies, aim at obtaining glycopeptide-derived compounds suitably modified in their heptapeptide structure to allow simultaneous molecular interaction with both susceptible and resistant targets. As a preliminary approach, novel non-natural glycopeptides in which amino acids 1 and 3 are replaced by new amino acids have been prepared.

glycopeptide resistance / Gram-positive bacteria / multi-resistant pathogen

Introduction

Vancomycin (V, Eli Lilly; fig 1a) and teicoplanin (T, Lepetit; fig 1b), the glycopeptide antibiotics currently used in clinical practice, are an important part of the clinician's armamentarium against nosocomial infections due to Gram-positive bacteria. These drugs have increased in importance as the incidence of hospital-acquired infections by Gram-positive bacteria has increased, and multi-drug resistance has become more common [1–3]. V, and in some countries T, are drugs of choice for infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci (CNS), and are widely used in the therapy of severe enterococcal infections. Both V and T are also effective alternatives to beta-lactam antibiotics against other multi-resistant Gram-positive pathogens such as penicillin-resistant *Streptococcus pneumoniae*. For MRSA and methicillin-resistant CNS strains there is no uniformly effective therapeutic alternative to glycopeptides: these isolates are resistant to all penicillins and cephalosporins, and resistance to other agents, such as quinolones, is widespread [3]. Treatment of enterococcal infections is still more challenging [4]. Enterococci are intrinsically resistant to many agents including cephalosporins and aminoglycosides. Combination of aminoglycosides with either a penicillin or glycopeptides is the preferred therapy for serious enterococcal infections, and emerging resistance to penicillins and high-level aminoglycoside resistance is eliminating these therapeutic options. The appearance of glycopeptide resistance in the enterococci and the possibility of its spread to other pathogens brings the threat of further loss of effective treatments.

* Correspondence and reprints

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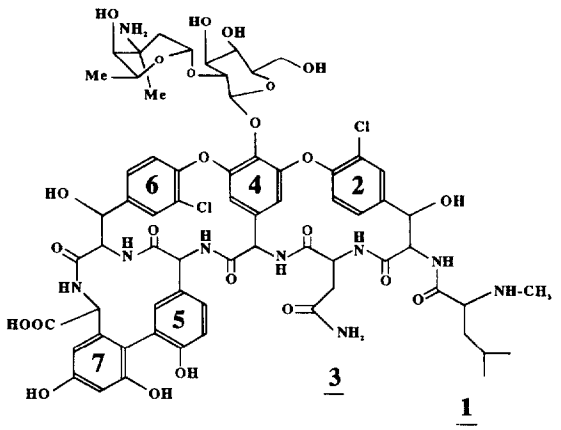
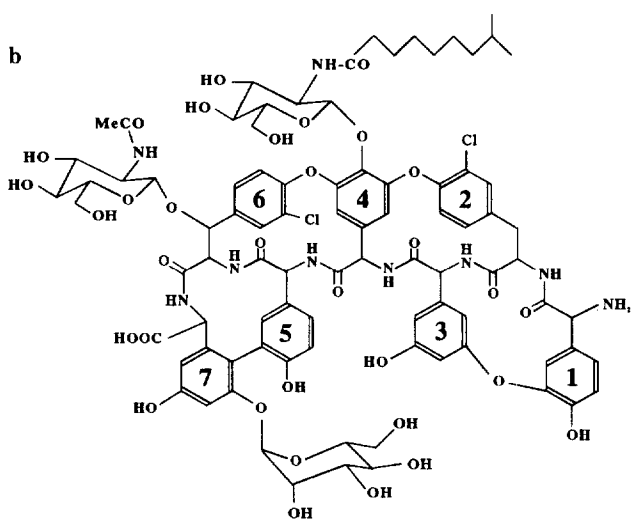


Vancomycin (V)

b



Teicoplanin (T)
(Structure of component T-A2-2 of T-complex)

Fig 1. Structure of commercially available glycopeptides V and T.

Resistance to V in pathogenic Gram-positive bacteria was not documented until 1988, over three decades after introduction of the drug, with the first reports of plasmid-mediated resistance to high levels of V and T in enterococci [5, 6]. Since then, resistant enterococci carrying the VanA and VanB gene clusters that confer resistance have become widespread in American and European hospitals [7, 8]. VanB genes typically confer resistance to V but not to T, although variants resistant to both are known. VanA genes confer high-level resistance to both glycopeptides. In recent years clinical isolates of staphylococci with some degree of resistance to glycopeptides have also been identified: these are most commonly CNS isolates, although rare examples of MRSA with intermediate levels of glycopeptide resistance have been noted [9]. Resistance is usually of intermediate level and typically affects T-susceptibility more than V. This form of resistance does not appear to be transferable among strains, and is unrelated to VanA or VanB resistance. Transfer of VanA resistance into staphylococci has been shown in the laboratory [10], but has not appeared in the clinic. Transfer of glycopeptide resistance into staphylococci could have disastrous consequences, as there is no currently available agent that would provide effective defense against glycopeptide-resistant MRSA.

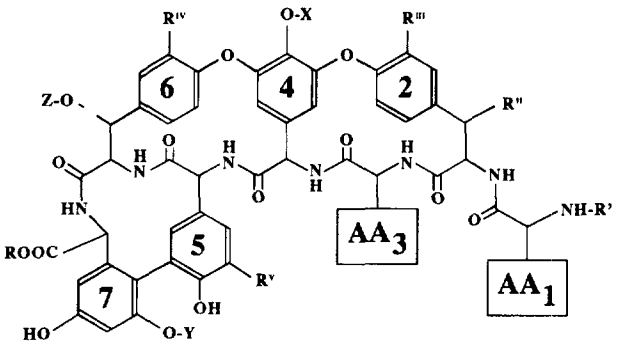
Overall, the possible spread of resistance to staphylococci and the urgent need for new agents have contributed to a renewed interest in glycopeptides. In this review, we will examine several approaches that are yielding interesting new glycopeptide antibiotics: (1) modifications of T-type glycopeptides that appear to effectively address the shortcomings of T and V with regard to activity against staphylococci. Some modifications of T-type glycopeptides also extend the glycopeptide spectrum to Gram-negative bacteria; (2) modifications of T- and V-type antibiotics leading to 'second generation' semisynthetic glycopeptides that show promise for their activity against glycopeptide-resistant enterococci and for improvement on V and T in potency. One of these agents, a V-type derivative is now in clinical trials; (3) modifications of the binding site of glycopeptides: application of knowledge of the biochemical basis of glycopeptide resistance to allow systematic structure–activity relationships (SAR) to alter antibiotic interaction with its target. The development of chemistry that allows removal and replacement of portions of the heptapeptide backbone is facilitating this approach.

Structure and mode of action of the glycopeptide antibiotics and mechanism of glycopeptide resistance

Core structure

The glycopeptides V and T are the most representative members of the dalbaheptide group of antibiotics [11–13], a large family of natural inhibitors of the bacterial cell wall.

General structure



For X, Y, Z, R, R^{I}, R^{II}, R^{III}, and R^{IV}, as well as for W_1 to W_7, see Ref. 13.

Glycopeptide families

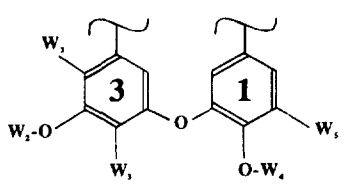
CLASS	AA_3	AA_1
VANCOMYCIN	ALIPHATIC	ALIPHATIC
SYNMONICIN	ALIPHATIC	AROMATIC
AVOPARCIN	AROMATIC	AROMATIC
RISTOCETIN (Teicoplanin)		

Fig 2. Naturally occurring glycopeptides.

Naturally-occurring dalbaheptides (fig 2) are highly modified linear heptapeptides of which amino acids 2, 4, 5, 6, and 7 are aryl amino acids and are common to all members of the group. The primary structural elements differentiating dalbaheptides are amino acids 1 and 3, which can be either aliphatic or aromatic. Other important elements of differentiation are the number, the structure and position of the sugars, the different degree of methylation or hydroxylation, and the number and position of chlorine atoms. The dalbaheptides are arbitrarily classified into four main classes according to variations at amino acids 1 and 3. In V-like glycopeptides these amino acids are both aliphatic. In synmonicin, amino acid 1 is aromatic and amino acid 3 is a methionine. The avoparcin family has both amino acids 1 and 3 aromatic. In ristocetin-like glycopeptides, aromatic amino acids 1 and 3 are linked together through a diphenyl ether bridge. The ristocetin family also includes T-type acylamido lipoglycopeptides in which the amino sugar on amino acid 4 is acylated with fatty acids.

Mode of action

The glycopeptide antibiotics exert their antibacterial action by inhibition of biosynthesis of peptidoglycan, the major component of the bacterial cell wall [14, 15]. The dalbaheptide core structure has been suitably designed by nature to specifically bind to D-alanyl-D-alanine (D-Ala-D-Ala). The antibiotics selectively bind to the peptidoglycan precursor, lipid-bound *N*-acetyl-muramyl-pentapeptide, interacting with the terminal D-Ala-D-Ala. The formation of a complex with D-Ala-D-Ala residues of the precursor prevents both transglycosylation and transpeptidation, blocking its addition to the growing peptidoglycan chain. This dipeptide target is present in both Gram-positive and Gram-negative bacteria, but the latter organisms are resistant to glycopeptides because the outer membrane of the cell envelope limits the access of these antibiotics to their target [16].

At a molecular level, glycopeptide interactions have been elucidated in modeling studies using short cell wall peptides [14, 17]. These studies have shown that primary binding involves hydrogen bonds between peptide-NH groups of amino acid 2, 3, and 4, and the carboxylate anion of terminal D-Ala, and the D-Ala-NH and the carbonyl-oxygen of residue 4 (fig 3a). Secondary hydrogen bonding systems, such as that between the peptide-NH of residue 7 and the Lys-CO of model tripeptide Di-Ac-Lys-D-Ala-D-Ala strengthen the antibiotic-dipeptide complex. An important role in the initial binding formation is played by the positively charged amino group of amino acid 1 in the electrostatic approach to the negatively charged carboxylate anion of target peptide. It follows that the binding properties of glycopeptides are in part dependant on the structure of amino acids 1 and 3.

Mechanism of glycopeptide resistance in enterococci

The biochemical basis of glycopeptide resistance has been extensively studied for VanA resistance [18, 22]. The resistance genes allow the synthesis of an alterna-

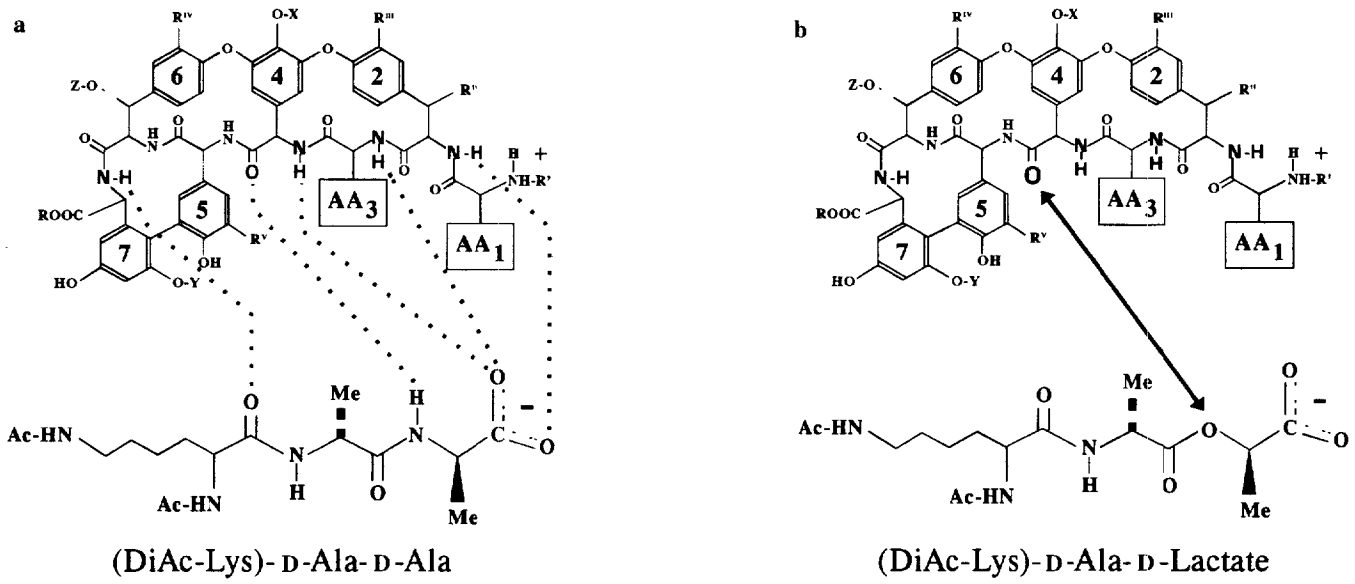


Fig 3. Molecular interactions with **a)** susceptible and **b)** resistant targets.

tive peptidoglycan precursor in which the normal D-Ala-D-Ala terminus of the peptide is replaced with depsipeptide D-alanyl-D-lactate (D-Ala-D-Lac). The genes required for this replacement are (fig 4): (i) *vanH*, encoding a reductase that converts pyruvate to D-lactate; (ii) *vanA*, encoding a ligase that links D-alanine and D-lactate: the resulting depsipeptide is then used in place of the normally synthesized D-Ala-D-Ala; and (iii) *vanX*, encoding a peptidase that degrades D-Ala-D-Ala, affording the preferential utilization of D-Ala-D-Lac. A second peptidase, the *vanY* gene product, acts on any residual D-Ala-D-Ala-containing pentapeptide. Although *vanY* is not essential for resistance, it does increase the level of resistance. The *vanZ* gene is of unknown function, but can confer low-level resistance to T in the absence of the other resistance genes [23]. The replacement of the terminal D-alanine with D-lactate and the resultant substitution of an ester for an amide linkage removes one of the key hydrogen bonding sites in the glycopeptide–precursor complex. This lowers the binding affinity by at least 1000-fold and resistance results [24].

Other Gram-positive bacteria resistant to glycopeptides also synthesize cell wall precursors with alternative amino acids [25, 26]. VanB genes also afford the synthesis of D-Ala-D-Lac-containing precursors. However, in typical VanB isolates, expression of resistance is regulated by a signal-transduction system with a sensor that appears to recognize only V and not T, and these isolates are usually susceptible to T; the regulatory system of VanA isolates recognizes both glycopeptides and possibly certain other inhibitors of the cell wall synthesis [27]. Enterococci of the species *E cassiflavus*, *E gallinarum* and *E flavescens*, the VanC enterococci, use D-alanyl-D-serine (D-Ala-D-Ser) precursor. Glycopeptide-resistant Gram-positive species such as pediococci, leuconostocs and some lactobacilli are now known to synthesize precursors with alternative amino acids or 2-hydroxy acids in place of D-Ala [25, 26].

Some differences in the activity of T and V against glycopeptide-resistant bacteria can be accounted for by binding differences. In T, the binding pocket generated by the diphenyl ether moiety allows the formation of a complex with other dipeptides such as D-Ala-D-Ser, which is weakly bound by V [28]. As D-Ala-D-Ser is the terminal dipeptide of VanC isolates, this could explain why these enterococci are susceptible to T but relatively resistant to V. In contrast, in highly glycopeptide-resistant VanB and VanA enterococci, where the target is the D-Ala-D-Lac depsipeptide, the presence of the depsipeptide–oxygen in place of the D-Ala-NH not only removes a hydrogen bonding site, but also allows a repulsion between the depsipeptide oxygen and the carbonyl-oxygen of residue 4 (fig 3b) [29]. An ideal new glycopeptide should afford the possibility of interaction with both resistant and susceptible targets.

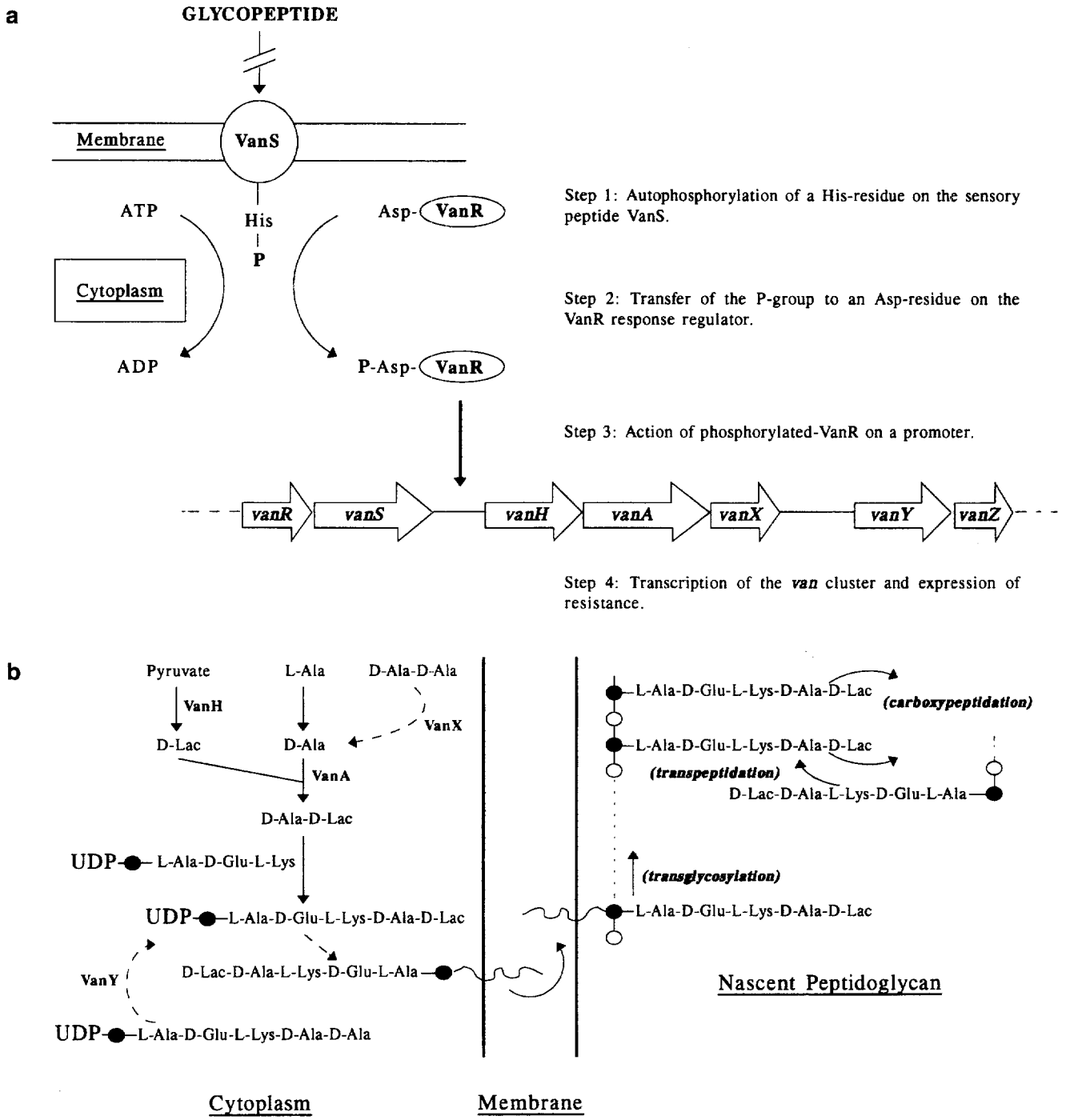


Fig 4. a. Regulation of the *van* gene cluster in *E faecium* BM4147 (VanA) in the presence of a glycopeptide (adapted from [8]). **b.** Schematic representation of peptidoglycan biosynthesis in VanA enterococci (modified from Arthur M, Couvalin P 1993, *Antimicrob Agents Chemother* 37, 1563–1571). ●: *N*-acetylmuramic acid; ○: *N*-acetylglucosamine; ~~~ : undecaprenyl lipid carrier.

Modulation of the antibacterial activity of glycopeptides by chemical derivatization of functional groups

In the last 15 years, the increasing incidence of glycopeptide resistance and the limited choice of effective antibiotics for the treatment of serious infections caused by multi-resistant staphylococci stimulated the search for new and more potent glycopeptides with improved activity against MRSA and CNS. The emergence of VanA enterococci currently presents the greatest challenge for a new glycopeptide. However, a successful new agent of wide clinical interest should possess both activity against VanA enterococci and excellent activity against staphylococci. The ability to bind specifically with both the modified target of resistant strains and the D-Ala-D-Ala-containing peptides of susceptible bacteria is desirable. In addition, it may also be possible to pursue activity against Gram-negative bacteria. Here we describe work that has achieved some success in all of these pursuits.

SAR of derivatives of teicoplanin-type glycopeptides

The lipoglycopeptide teicoplanin (T; fig 1b) was particularly suitable for chemical and biological transformations aimed at pursuing the above objectives. Compared to vancomycin (V; fig 1a), the opportunity to achieve further relevant SAR was provided by the presence of two additional sugars in different positions of the core heptapeptide and one N-acylated amino sugar on ring 4. The role of the sugars and the effect on the in vitro and in vivo activity of T-derivatives caused by variations of the isoelectric point were determined. Some antibacterial and physico-chemical properties, as well as chemical stability and reactivity of T were affected by the strained structure of the 14-membered 1,2,3-macrocyclic ring in the active site region.

Preliminary SAR of T-antibiotics were established by stepwise removal of the sugar moieties from the T-complex (T-A2, CTA)[1], and by modification of each of the functional groups of CTA, its acidic hydrolysis de(acylglucosaminyl) (TB) and acetylglucosaminyl (TC) pseudoaglycons, and aglycon (TD). Further SAR, which are discussed in *New synthetic approaches*, were achieved by selective hydrolysis of amide bonds of the heptapeptide backbone that allowed changings in the structure of the active site by either replacement of amino acids 1 and 3 with new amino acids, or enlargement of the 1,2,3-macrocyclic ring.

Deglycosylation and mannosylation

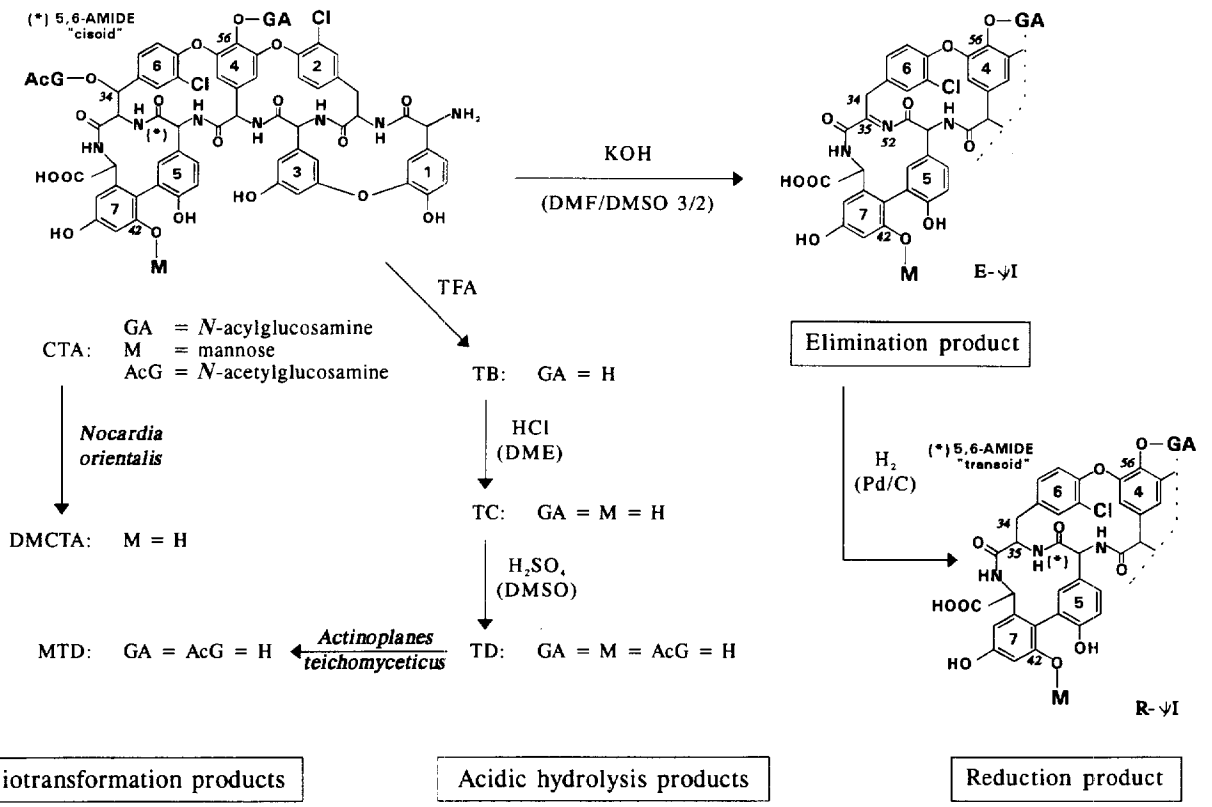
The influence of the sugar moieties on the antibacterial properties of T was investigated in detail. T-derived pseudoaglycons and aglycons (scheme 1) were prepared under selective acidic [30, 31], basic [32] or reductive [33] conditions, or by biotransformation [34] methods.

As summarized in previous reviews [35, 36], the loss of the *N*-acylglucosamine reduces the activity of T, particularly against streptococci and enterococci. The *N*-acylglucosamine is also responsible for the long plasma half-life and favorable pharmacokinetics (PK) of T, while the position of mannose near the binding pocket enhances the affinity for the target peptide and also provides T with good solubility in water at physiological pH. However, the presence of mannose has a negative effect on the in vitro activity of T-antibiotics against CNS strains. The role of these sugars on the antibacterial activity of T was determined by comparing the in vitro and in vivo activity, and the binding properties of CTA with those of its de(acylglucosaminyl) (TB) and de-mannosyl (DMCTA) pseudoaglycons, and of the aglycon (TD) with those of its mannosyl pseudoaglycon (MTD). Pseudoaglycons TB [30] and DMCTA [34] were obtained from CTA by selective acidolysis of the *N*-acylglucosamine under mild conditions (TFA) and by enzymatic hydrolysis of the mannose, respectively. Microbial mannosylation of the 42-phenolic-OH of TD yielded pseudoaglycon MTD [34].

In contrast, the influence of *N*-acetylglucosamine on T-activity could not be established since this sugar was stable to biotransformation treatments and more stable than *N*-acylglucosamine and mannose to acidic hydrolysis. The selective removal of *N*-acetylglucosamine from CTA was achieved only under basic [32] or reductive [33] conditions which affected the T-core structure. Reaction of CTA with strong alkali in polar aprotic solvents yielded an unsaturated de(acetylglucosaminyl) compound (E-ψI) containing 35,52-C=N bonds which could be hydrogenated to give the de(acetylglucosaminyl)-deoxy-pseudoaglycon (R-ψI). This reduction product was also prepared upon treatment of CTA with sodium borohydride in DMF/MeOH solution. In both compounds the conformation of the 5,6,7-macrocyclic ring was different from that of CTA. The role of this sugar in benzylic position remained unclear until the preparation of de(acetylglucosaminyl)-deoxy derivatives of secondary amides of CTA (see later).

The sugar-free T-aglycon (TD) was prepared from CTA, TB, or TC under relatively strong acidic condi-

[1] T (marketed as Targocid®, Lepetit) is a physical mixture of T-complex (CTA), as the main product, and its de(acylglucosaminyl) pseudoaglycon (TB). CTA is formed by five strictly related components, namely T-A2-1 to 5 (the structure of T-A2-2 is given in fig 1b), only differentiated by the structure of fatty acid acylating the glucosamine-NH_2 on ring 4.



Scheme 1. Teicoplanin-derived pseudoaglycons and aglycon.

tions (H_2SO_4 in DMSO) [31]. Although marginal, the activity of TD against T-resistant strains of *E coli* was a very interesting result, which indicated that the sugars have a negative effect on the permeability of T through the Gram-negative outer membrane.

Teicoplanin derivatives with enhanced activity against methicillin-resistant staphylococci and Gram-negative bacteria

From chemical derivatization of functional groups of most of the above compounds, it was observed that changes near the region of the active site generally affected the binding properties of T-derivatives and often resulted in reduction of the antibacterial activity (fig 5). In contrast, the conversion of the carboxy group into an ester, an amide or hydrazide did not modify binding to the target peptide, but generally improved the antibacterial activity of the resulting compounds to a different degree mostly depending on their ionic character. The most promising results were obtained with basic positively charged amides of CTA and TD that were significantly more active than CTA against staphylococci [37, 38]. The majority of the basic amides of TD also had encouraging in vitro activity against Gram-negative isolates [38].

Among the CTA-amides, the most active compound against CNS was the dimethylaminopropyl amide (MDL 62,873, Mideplanin; fig 6a), while among the TD-amides, one of the most interesting compounds was MDL 62,766 (fig 6b), a basic amide with a linear polyamine (table I). It has been demonstrated that the amides of the TD have the same mode of action as other glycopeptides but they are able to pass through the outer membrane of Gram-negative bacteria by a mechanism of self-promoted uptake [39]. However, these compounds were not active enough in vivo in animal models of Gram-negative infections to be considered for further development. As expected, corresponding basic amides of CTA were poorly active against Gram-negative bacteria [38]. This result further confirmed that the activity of T-antibiotics against these organisms is strongly affected by the presence of the sugars.

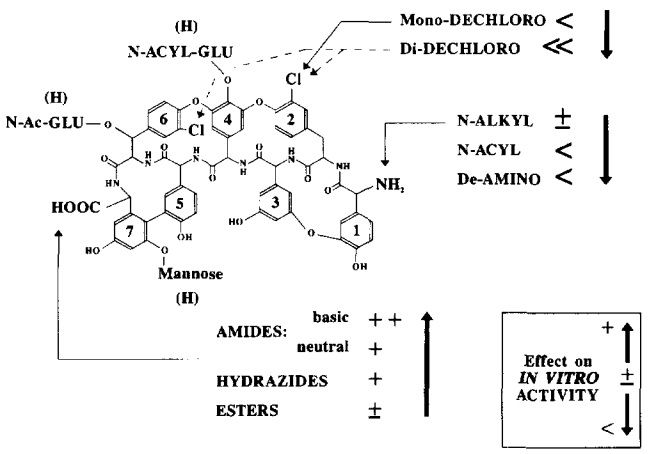


Fig 5. SAR of teicoplanin derivatives.

Teicoplanin derivatives active against VanA enterococci

As previously mentioned, the role of *N*-acetylglucosamine on T-activity was in part understood after removal of this sugar from secondary CTA-amides. In contrast to pseudoaglycon R-ψI, the secondary amides of de(acetylglucosaminyl)-deoxy-CTA retained the core structural features of CTA-amides, with the only exception due to the absence of the benzylic hydroxy group. The 'natural' conformation of the 5,6,7-macrocyclic ring was stabilized by the presence of the amide-NH proton.

The acetylglucosamine-less amide MDL 62,600 (fig 7a) was obtained upon treatment of Mideplanin with $NaBH_4$ in a DMF/MeOH solution (scheme 2) [33]. Under these basic reaction conditions, the acetylglucosamine is initially displaced according to a beta-elimination mechanism. The resulting enamine is in equilibrium with the imine form which is readily reduced by sodium borohydride. This mechanism was demonstrated by isolation of both enamine and imine derivatives under different basic conditions (scheme 2).

Table I. In vitro activity of compounds investigated (MIC range, mg/L).

Compound	*Organism (N°)*			
	Coagulase-negative staphylococci (95)	*E Coli (7)*	*P aeruginosa (5)*	*Other Gram-negative bacteria (17)*
Teicoplanin	0.13–32	> 128	> 128	> 128
MDL 62,873	< 0.03–2	> 128	> 128	> 128
MDL 62,766	0.03–0.5 (Five strains)	0.5–4	2–64	0.25–128

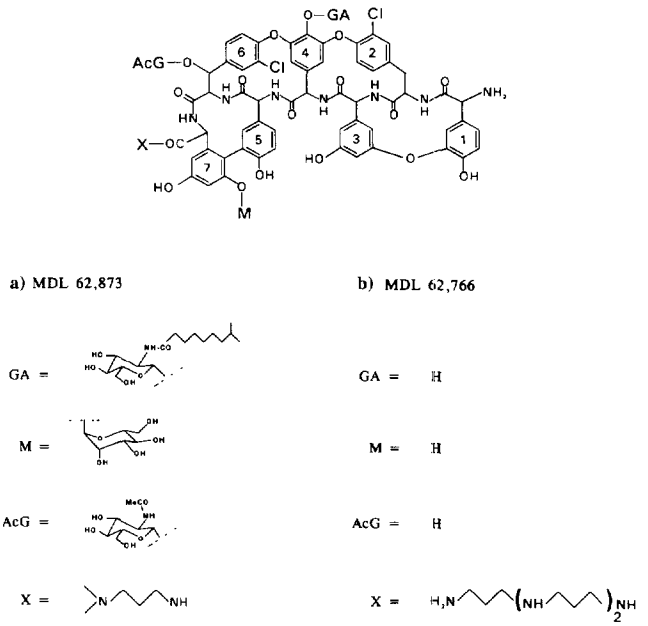


Fig 6. Structure of **a)** CTA-amide MDL 62,873; and **b)** TD-amide MDL 62,766.

As for CTA, treatment of Mideplanin with strong bases in polar aprotic solvents led to the formation of an enamine intermediate which tautomerized to the imine derivative under acidic conditions. Catalytic hydrogenation of the newly formed double bonds produced the same final reduced compound MDL 62,600.

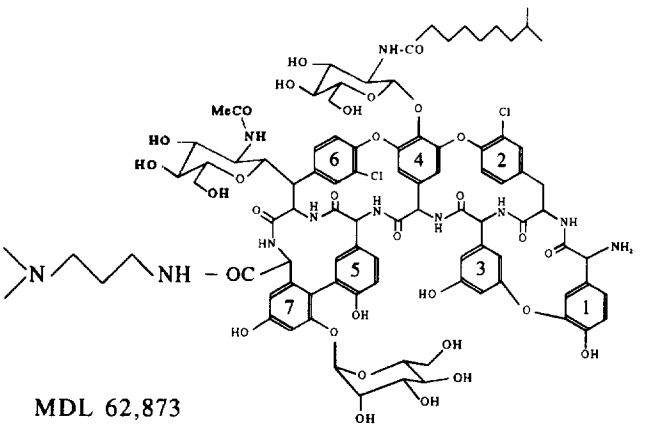
The most important effect caused by the reductive displacement of the acetylglucosamine from Mideplanin was that MDL 62,600 was somewhat active against few isolates of VanA enterococci (table II).

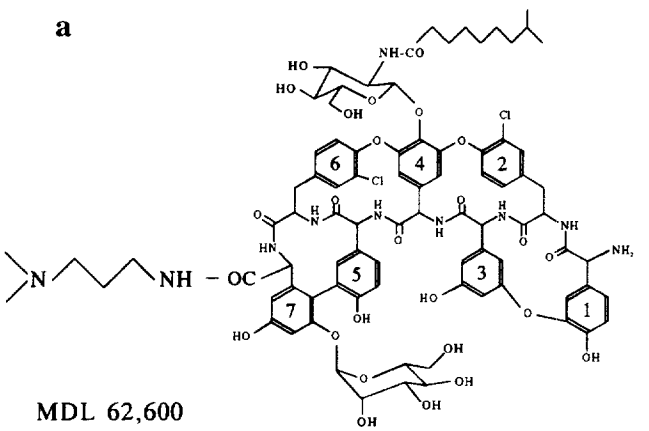
Derivatives of T-like glycopeptide A-40,926 active against VanA enterococci

Based on these findings, the next step was to determine if the activity against VanA enterococci was due only to the loss of the acetylglucosamine, or if it was

Table II. In vitro activity against VanA enterococci (5 isolates).

Compound	*MIC range (mg/L)*
MDL 62,873	64 – > 1,024
MDL 62,600	16 – 32
A-40,926	64 – 512
Teicoplanin	64 – > 1,024





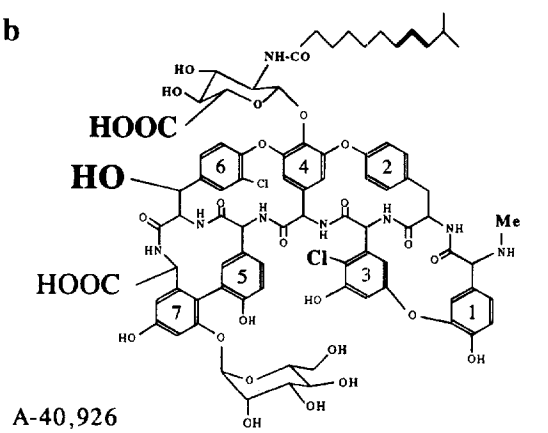
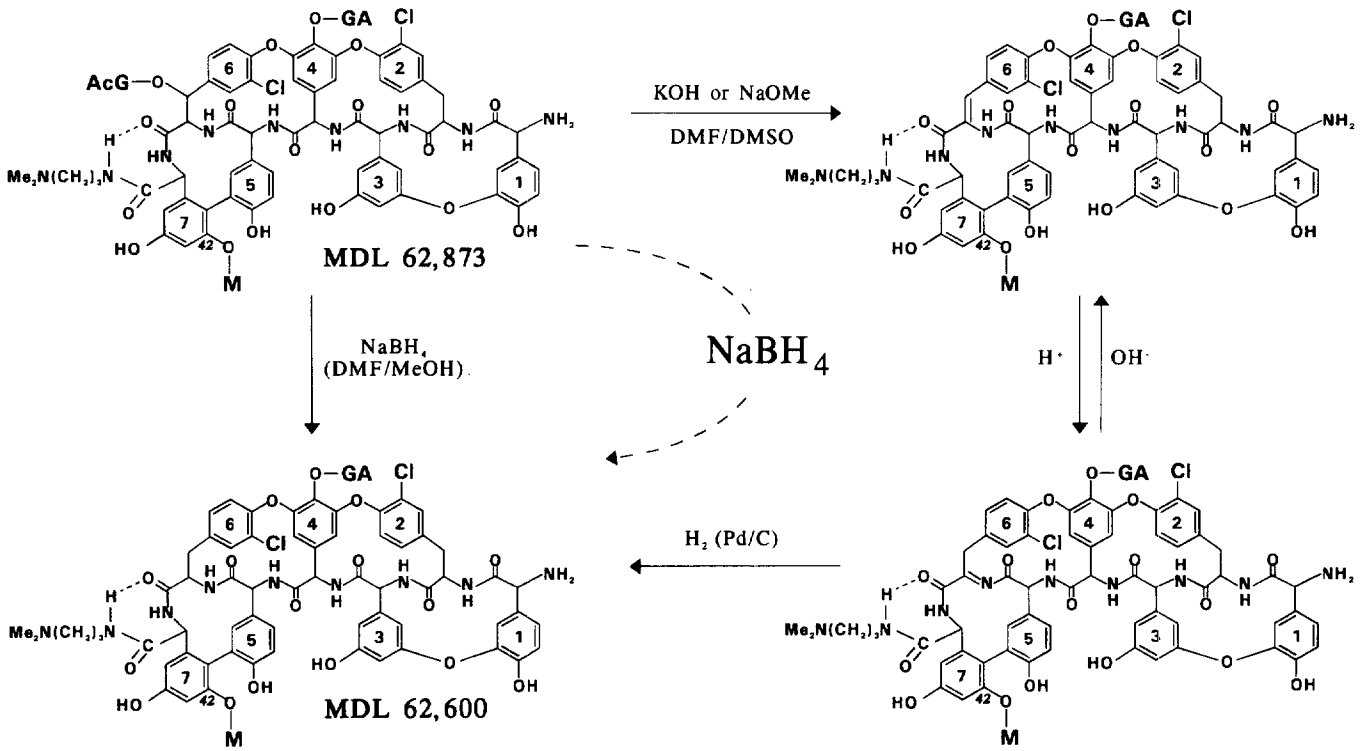


Fig 7. Structure of **a)** MDL 62,600; and **b)** A-40,926 in comparison with MDL 62,873.

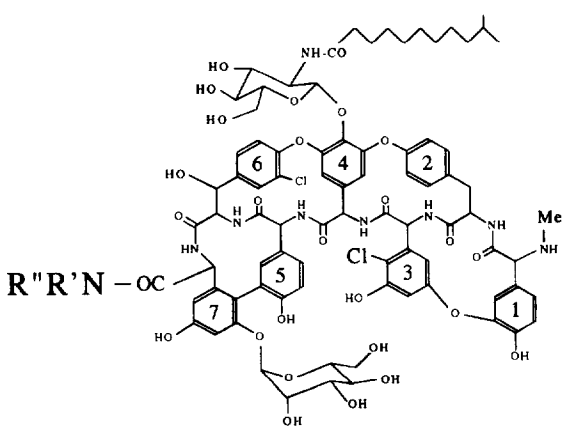
also related to the absence of the benzylic hydroxy group, a function which is present in the core structure of all glycopeptides. This opportunity was provided by the availability of T-like glycopeptide A-40,926 (fig 7b) [40], which is structurally related to T but lacks the acetylglucosamine in benzylic position. Another significant difference between A-40,926 and T is the presence of an acylaminoglucuronic acid on amino acid 4 instead of the acylglucosamine. Additional differences are the presence in A-40,926 of a terminal methylamino group, the position of one chlorine atom and the slightly longer fatty acid chain.

By reduction of the sugar-carboxy group to alcohol followed by amidation of the peptide-carboxy function, a series of amide derivatives structurally related to MDL 62,600, but possessing the natural benzylic hydroxy group, was obtained [41]. The higher reactivity of the sugar-carboxy group to acid-catalyzed esterification allowed the selective synthesis of the mono-methyl ester that was reduced to hydroxymethyl with sodium borohydride. Final amide derivatives were prepared by coupling the peptide carboxy group with amines under classical conditions (scheme 3).

A number of amide derivatives of reduced A-40,926 have been recently synthesized [41]. Among them, the most active compounds MDL 63,246 and MDL 63,042 (fig 8) had very interesting in vitro activity against a number of VanA enterococci highly



Scheme 2. Mechanism of reductive elimination of the *N*-acetylglycosamine (AcG) from MDL 62,873 (GA = *N*-acylglucosamine, M = mannose).



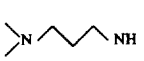
MDL	R"R'N
63,246	
63,042	

Fig 8. Structure of MDL 63,246 and MDL 63,042.

resistant to T and V (table III), but they were poorly efficacious in vivo against these enterococci. However, the most important characteristic of these derivatives was their outstanding in vitro and in vivo activity against almost all other Gram-positive bacteria, including Meth-R staphylococci, penicillin-R pneumococci, and VanB and VanC enterococci (table IV). MDL 63,246 is currently under pre-clinical development as the most active glycopeptide for MRSA and CNS infections [42–44].

SAR of derivatives of vancomycin-type glycopeptides

Modifications of V-type glycopeptides have not been as extensively explored as the T-like antibiotics as a source of new agents. However, some recent efforts have been quite productive especially in generating agents active against V-resistant enterococci (VRE).

N-Terminal modifications

V-Type glycopeptides possess a free N-terminal amino acid, in contrast to T-like compounds where amino acid 1 is linked to amino acid 3 through a diphenyl ether bridge. The presence of *N*-methylleucine

Table III. In vitro activity against VanA enterococci (20 isolates).

Compound	*MIC range (mg/L)*
MDL 63,246	4 – 64
MDL 63,042	0.5 – 32
MDL 62,600	16 – > 128
Teicoplanin	64 – > 1,024
Vancomycin	512 – > 1,024

(MeLeu) in position 1 of V allowed SAR to be established by removal (Edman degradation) and subsequent substitution of this amino acid [45, 46]. The hexapeptide (VHP) resulting from removal of MeLeu was inactive, and the replacement of MeLeu with other aliphatic or aromatic amino acids generally yielded analogs less active than V. In studies of modification of the N-terminus of V and the V-like glycopeptide eremomycin (fig 9) [47], nitrosation, acylation, or carbamylation all afforded compounds less active than their respective parents [48].

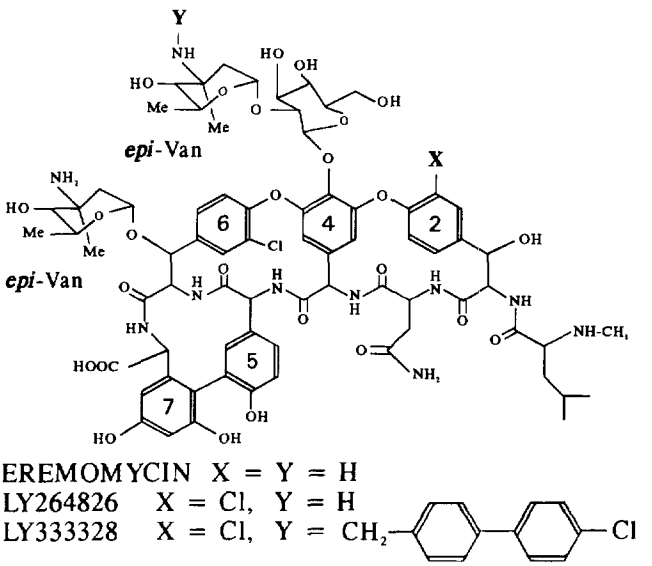
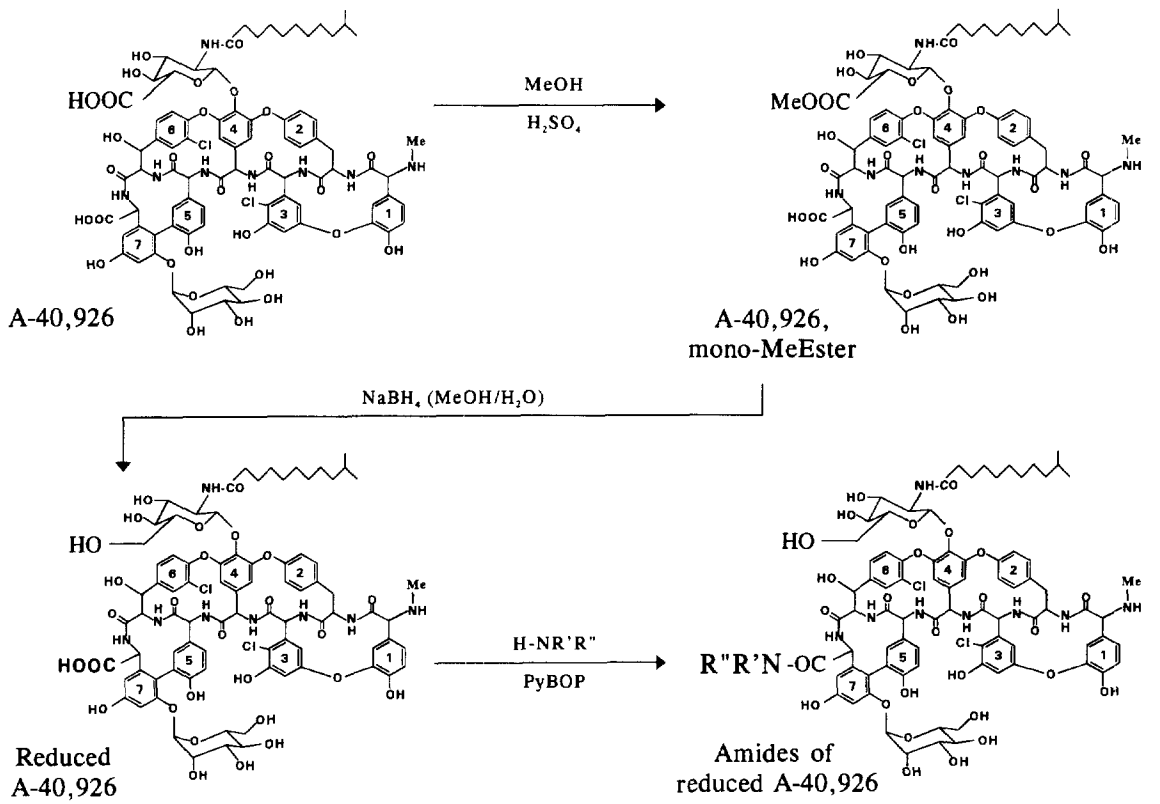


Fig 9. Structure of vancomycin-type glycopeptides eremomycin, LY264826 and its derivative LY333328.



Scheme 3. Synthesis of amides of reduced A-40,926.

Table IV. In vitro activity against other Gram-positive bacteria.

Organism (No)	*MIC_{90} (MIC range) (mg/L)*			
	MDL 63,246	*MDL 63,042*	*Teicoplanin*	*Vancomycin*
Staphylococcus aureus (20)	0.25 (0.125–0.5)	0.125 (< 0.03–0.125)	0.5 (0.25–0.5)	2 (0.5–4)
Coagulase-negative staphylococci (95)	1 (< 0.03–2)	0.5 (< 0.03–1)	32 (0.25–64)	2 (0.5–4)
Enterococci* (44)	1 (0.06–1)	0.25 (0.03–0.5)	0.5 (0.125–1)	2 (0.25–4)
Enterococci VanB (25)	0.5 (0.125–0.5)	0.25 (0.06–0.5)	1 (0.125–4)	256 (8–1024)
Streptococcus pyogenes (11)	< 0.03	< 0.03	0.03 (< 0.03–0.06)	0.5 (0.25–0.5)
Streptococcus pneumoniae (9)	< 0.03	< 0.03	0.06 (< 0.03–0.06)	0.5 (0.25–0.5)

* Vancomycin-susceptible.

For the V-like compounds, further SAR have been established by comparing the activity of V with that of naturally-occurring V-homolog and V-desamido analog glycopeptides in which the side chain of amino acid 3, asparagine, was replaced by glutamine or aspartic acid [49, 50]. These compounds were uniformly less active than V. In other studies, attempts to hydrolyze the amide group of asparagine to aspartic acid led to a rearrangement of amino acid 3 to iso-aspartic acid with loss of antibacterial activity [51].

Modification by addition of lipophilic groups

Some early programs of chemical modification of V were focused on inprovements in PK [46, 51, 53]. Relative to V, T has longer plasma half-life, which was believed to be a desirable property. In the early 80s much effort was put into the introduction of lipophilic side chains into the V-structure by acylation and alkylation of one or both of the two amino groups, that of vancosamine sugar and that of the MeLeu moiety. The most interesting compounds were mono-*N*-acyl and mono-*N*-alkyl derivatives at the vancosamine. These types of substitution generally enhanced both antibacterial activity and plasma half-life [46, 53]. As in the case of T, changes near the N-terminus which could be expected to affect binding to the target peptide generally resulted in loss of antibacterial activity.

Modifications of the terminal carboxy region and amino acid 6

In contrast to the T-class of glycopeptides, only recently a systematic program of derivatization of the carboxy group has been undertaken as an alternative strategy to enhance the activity of V. This approach was aimed at the design and preparation of derivatives with catalytic activity, for example cleavage of the peptidoglycan precursor by introduction of suitable functional groups linked to the C-terminus. A few carboxamides have been reported, but no biological activity was given [54, 55]. A small number of amides of eremomycin have also been prepared; most showed antibacterial activity comparable to the parent, with some improvements in anti-CNS activity; possible improvements in allergenicity were also noted [56].

The SAR of T-type glycopeptides indicated that the most active derivatives were those in which the acetylglucosamine in benzylic position was absent, and the C-terminal carboxy group was converted into basic amides. In contrast, in studies reported up to 1993, the V-type glycopeptide with the most significant improvement over V was observed in natural products where one additional sugar, 4-*epi*-vancosamine, was present on amino acid 6, with no alteration of the C-terminus. One of these, the natural glycopeptide LY264826 (fig 9)[2] in which the disaccharide vancosamine is also replaced by 4-*epi*-vancosamine, was uniformly 4–8-fold more active than V [46].

Covalent dimers of vancomycin

Recently, a striking extension of studies of carboxy modifications has been the synthesis of bis(V) carboxamides, covalent dimers of V tethered by

[2] Also known as A82846B, chloro-orienticin and chloro-eremomycin.

amide bridges [57]. Some of these compounds showed up to 60-fold enhancement of activity against VRE isolates. These dimers are unchanged in the V-binding pocket, but nonetheless appear to demonstrate some enhanced binding to target precursor. Activity against V-susceptible strains was not improved. It is of note that the point of attachment between the two V-molecules is quite different from the region intermolecular contact seen in natural dimer formation of glycopeptides such as LY264826 and eremomycin [58]. This approach to semisynthetic modification of glycopeptides is a promising new direction. However, it remains to be seen if both anti-VRE and anti-MRSA activity can be simultaneously improved by this approach.

Other approaches

An innovative approach to achieving activity against Gram-negative bacteria is by the addition of iron-binding groups that might be recognized by bacterial pathways that transport iron across the outer membrane. Compounds of this type have been prepared by selective acylation of the vancosamine-amino function with spermine-based catechol- and hydroxamate-containing conjugates. Although their antibacterial activity is reduced relative to V, marginal activity against the Gram-negative pathogen *Pseudomonas aeruginosa* in low iron-medium suggests that the compounds may be transported as hypothesized [59].

The modification of eremomycin, a natural glycopeptide related to LY264826 but lacking the Cl atom of amino acid 2 (fig 9), has also been pursued. In addition to the carboxy and MeLeu modifications mentioned above, modification by the action of alkyl halides resulted in mono- and di-alkylated derivatives at the disaccharide *epi*-vancosamine and MeLeu, and at the carboxy group, but none exhibited enhanced activity [60]. Studies of hydrazides were also pursued, again without improved activity [56].

Derivatives of vancomycin-like compounds active against VanA enterococci

When the first VRE strains appeared in 1988, both natural glycopeptides and their derivatives were tested against these isolates [46, 61]. Surprisingly, some V-derivatives alkylated at the amino group of vancosamine showed modest anti-VRE activity. It was also noted that LY264826 was more active than V against VRE. A number of *N*-alkyl and *N*-aralkyl derivatives of LY264826 modified at the disaccharide *epi*-vancosamine were also evaluated, and some were shown to possess unanticipated activity against VRE [46, 62]. Many such derivatives were prepared [63, 64]. The *para*-chlorophenylbenzyl derivative LY333328 (fig 9) was identified as one of the most active members of this family. This compound has been shown to be uniformly active against V- and T-resistant enterococci, including the highly resistant VanA strains (table V) [65, 66]. It also possesses potent activity against penicillin-resistant *Streptococcus pneumoniae* [67]. Its in vitro activity against *Staphylococcus aureus*, including MRSA, is similar to that of V, but it is more efficacious than V in animal models, possibly because of relatively greater bactericidal activity. The in vitro activity against the CNS of LY333328 is greater than that of T, but it is not as active as MDL 63,246[3]. The efficacy of LY333328 against VRE has been shown in a non-lethal organ recovery model in the mouse [68]; more definitive studies in models such as endocarditis have not yet been reported. This compound is currently undergoing phase I clinical trials in man.

Table V. In vitro activity against VanA enterococci (26 isolates).

Compound	*MIC range (mg/L)*
LY333328	0.25 – 2
LY264826	16 – > 128
Eremomycin	64 – > 512
Vancomycin	128 – > 1,024
Teicoplanin	16 – > 1,024

Mechanism studies

The mechanism of activity of LY333328 and related compounds is interesting in that studies using model substrates have not shown enhanced binding to the target depsipeptide of resistant bacteria, D-Ala-D-Lac [69, 70]. This might suggest an alternative mode of action. However, it has been shown that these compounds do act by the same mechanism of action as other glycopeptides [70]: precursor accumulation studies indicate blockage of the same step in peptidoglycan synthesis as V, and studies of inhibition of cell wall synthesis show that inhibition requires the presence of a pentapeptide or pentadepsipeptide precursor containing terminal D-Ala-D-Ala or D-Ala-D-Lac; polymerization with tetrapeptide precursor is not inhibited. Overall, this may suggest that models using small peptides in solution do not afford a complete picture of activity in whole cells at the site of peptidoglycan synthesis in vivo where intermolecular effects occurring at the subcellular target site come into play [17, 69].

[3] Unpublished observations; Lepetit Research Laboratories and Lilly Research Laboratories.

Recently, Williams has brought forward a model of molecular recognition functions of glycopeptides which could explain the activity of these compounds [21, 58]. While D-Ala-D-Lac binds weakly to the glycopeptide binding pocket, the main source of binding affinity remains the binding of the D-Ala-carboxylate anion into a pocket containing 3 NHs (fig 3); despite the greatly decreased affinity relative to D-Ala-D-Ala, a basic affinity remains that may be increased by neighboring interactions [29]. Increased affinity to the target depsipeptide by cooperative interactions may be brought about by the combined effects of membrane anchoring and self-association into homodimers. It has been demonstrated that glycopeptide dimerization can be highly favorable and cooperative with the binding of ligands [58, 71]. In the model, the decreased motion of a ligand in a binding site afforded by membrane anchoring also works to improve the functional affinity of neighboring binding sites. Experimental evidence shows both dimerization and membrane binding are enhanced in N-alkylated derivatives of LY264826, such as LY333328. Studies using capillary electrophoresis to measure dimerization constants have shown that the derivatives most active against VRE also have measurably higher dimerization affinities [69]. Enhanced ability to interact with bacterial membranes has been demonstrated experimentally using bacterial protoplasts [69]. While the possibility that interactions with membrane may also have a direct antibacterial effect cannot be excluded, the Williams' model may be adequate to explain activity of these potent compounds.

New synthetic approaches: modifications of the glycopeptide core structure

As previously discussed, the binding properties of the glycopeptide antibiotics depend primarily on the structure and conformation of the heptapeptide backbone. Although there is no strict correlation between binding strength and antibacterial activity, the mechanism of action of all glycopeptides is based on the formation of a complex with D-Ala-D-Ala-containing peptide precursors of peptidoglycan. Changes in binding properties aimed at improving the intrinsic activity of glycopeptides and possibly allowing simultaneous interaction with the modified D-Ala-D-Lac target in VanA enterococci could be effected by modifications in the structure of the binding site. One approach is to replace at minimum amino acid 3 with new amino acids or other chemical entities potentially more suitable for interacting with both glycopeptide-susceptible and resistant targets. Although the substitution of terminal MeLeu with other amino acids did not enhance the antibacterial activity in V-type glycopeptide derivatives (see previous section), the replacement of aryl amino acid 1 in T-type antibiotics with MeLeu or other aliphatic amino acids might change, and possibly improve, the binding properties of these glycopeptides. A chemoselective process to displace amino acid 3 by Edman degradation implies preliminary hydrolysis of the peptide bond between amino acids 2 and 3. Aimed at pursuing this objective, T-type antibiotics were submitted to several different acidic and basic treatments, achieving the very interesting results that are reviewed below.

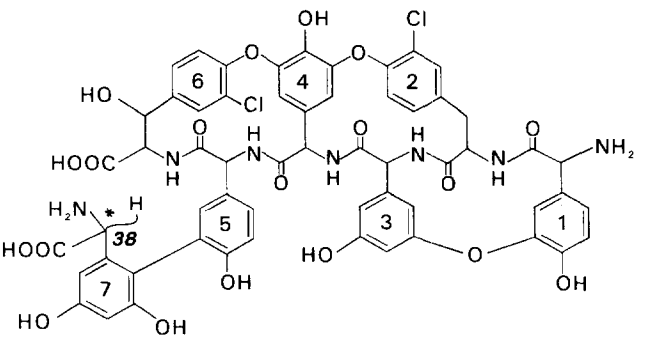


Fig 10. Structure of TD-derived acidic hydrolysis diastereoisomers AH-I and AH-II.

Table VI. In vitro activity of compounds investigated.

Organism	*MIC (mg/L)*		
	AH-I	*AH-II*	*TD*
Staphylococcus aureus Tour	0.25	2	0.063
Staphylococcus epidermidis ATCC 12228	0.125	0.25	0.016
Streptococcus pyogenes C 203	2	8	0.125
Streptococcus pneumoniae UC 41	1	8	0.125
Enterococcus faecalis ATCC 7080 (V-susceptible)	1	16	0.125

Table VII. In vitro activity of RH–TD, TDHPA, RC-1 and RC-2 in comparison with TD; MIC (mg/L)[a].

Compound	*Organism*					
	Staphylococcus aureus *Tour*	*Staphylococcus epidermidis* *ATCC 12228*	*Staphylococcus haemolyticus* *L 602*	*Streptococcus pyogenes* *C 203*	*Enterococcus faecalis*[b] *ATCC 7080*	*Escherichia coli* *SKF 12140*
RH–TD	32 (4)	8 (2)	16 (8)	32 (8)	16	> 128
TDHPA	2	0.5	16	8	8	> 128
RC-1	1	1	8	4	4	> 128
RC-2	16	32	> 128	32	32	> 128
TD	0.063	0.063 (0.016)	0.25	0.25 (0.13)	0.125	64

[a]Inocula: 5 x 10^5 cfu/mL (values in parentheses refer to inocula of 10^4 cfu/mL); [b]V-susceptible; data adapted from [74].

Selective hydrolysis of amide bonds of the heptapeptide backbone

Under acidic conditions [72], only the 6,7-amide of T-aglycon (TD) could be selectively hydrolyzed. The resulting hydrolysis products (AH-I and AH-II; fig 10) were identified as diastereoisomers at the chiral C-38 center. Although the structure of these open aglycon derivatives was disturbed in the left-hand part of the molecule, the right-hand side was almost unchanged as compared to TD. In particular, the geometry of the 1,2,3- and 2,3,4-macrocyclic rings which determine the structure of the binding site was not affected by this modification. However, a 10-fold decrease in the affinity for target dipeptide was observed for both compounds. Though to a different extent, this was reflected in the decreased antibacterial activity of AH-I and AH-II relative to TD (table VI).

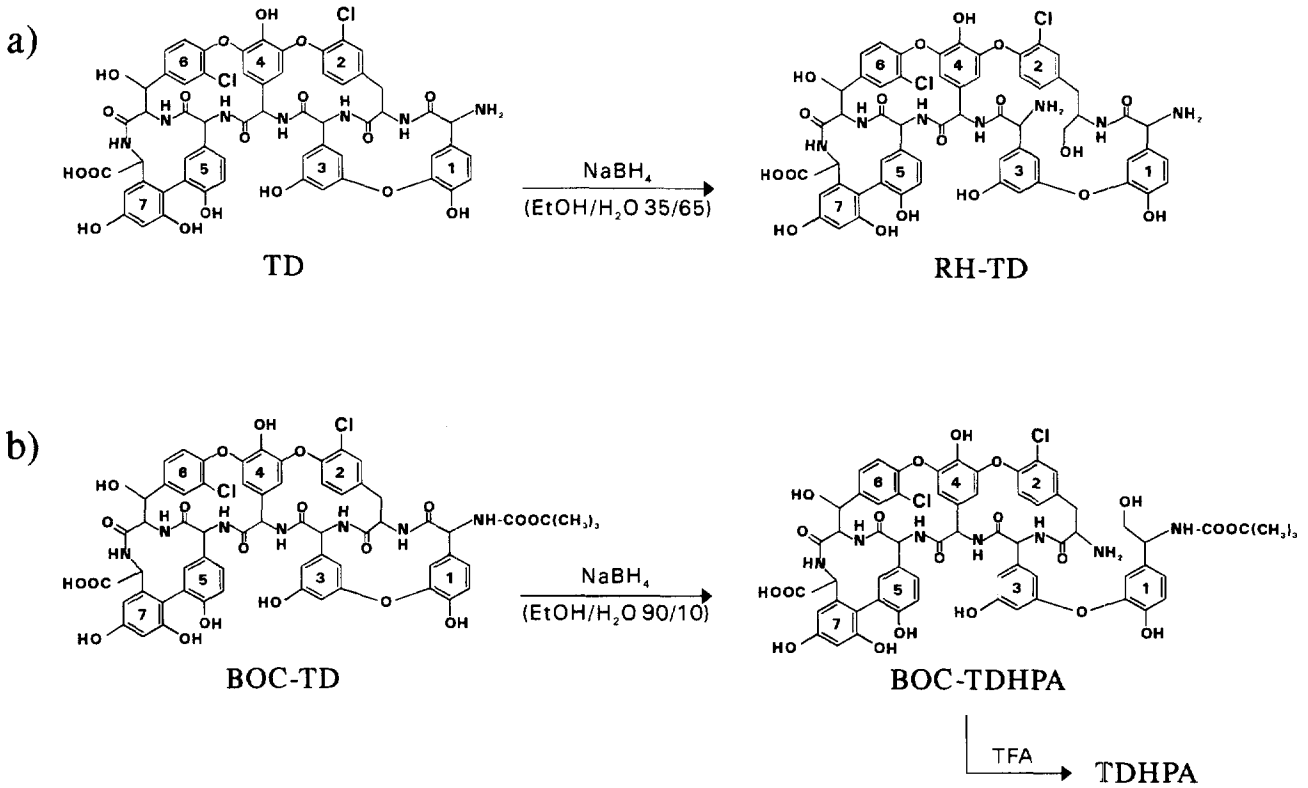
Under basic conditions, either epimerization at C-3 [73] or, as previously described, elimination of the acetylglucosamine from CTA, TB, and TC occurred dependent on the reaction solvents and temperature. In T-epimers, the change from the 'natural' *S* to the *R* configuration of amino acid 3 causes a rotation of the 2,3-amide bond by 180°. This results in the destruction of the binding pocket, and consequently in the loss of affinity for the dalbaheptide's target dipeptide. It is presumed that epimerization of teicoplanins is driven to completion by the greater thermodynamic stability of their epimer derivatives. As expected, the epimers of T-glycopeptides were devoid of antibacterial activity.

The selective hydrolysis of the 2,3-peptide bond was achieved only recently upon treatment of T-glycopeptides with sodium borohydride in aqueous ethanol solutions. Under these reductive conditions open pentapeptides were obtained in which the carbonyl group of amino acid 2 was reduced to hydroxymethyl [74]. In particular, the reductive-hydrolysis product RH-TD was obtained from TD (scheme 4a). Almost all glycopeptides were susceptible to reductive hydrolysis (RH) of the 2,3-amide under the above conditions. This was the key step which allowed removal of amino acids 1 and 3 by Edman degradation of the resulting glycopeptide-derived pentapeptides.

Treatment of carbamate derivatives of TD with $NaBH_4$ under RH conditions[4] produced open hexapeptide compounds in which the 1,2-amide bond was hydrolyzed and the carboxy group of amino acid 1 was reduced to primary alcohol (scheme 4b) [74]. In particular, the mono-N-protected hexapeptide-alcohol BOC-TDHPA was obtained from BOC-TD. This compound was used in the synthesis of TD-homolog derivatives with enlarged macrocyclic ring 1,2,3. Acidolysis (TFA) of the carbamate moiety yielded unprotected TDHPA.

The drastic structural modification of the binding pocket of TD accounts for the decreased though still interesting in vitro activity of the 1,2- and 2,3-RH-derivatives against Gram-positive bacteria (table VII). From peptide-binding studies of RH–TD using 2-D NMR spectroscopy, no interaction was observed between the 1,2- and 3,4-amide-NH groups and the free NH_2 of residue 3 with the carboxylate anion of target peptide model Di-Ac-Lys-D-Ala-D-Ala. However, it was seen that a weak binding still existed that involved the amide-NH proton of residue 7 and the Lys-CO of the synthetic tripeptide. This could explain the residual activity of RH–TD. Although less active than TD, TDHPA was significantly more active than

[4] The highest yields in 2,3-RH compounds from unprotected glycopeptides were obtained in EtOH/H_2O, 35:65 solution, whilst for the 1,2-RH carbamate-derivatives the best solvent composition was EtOH/H_2O, 9:1.



Scheme 4. Synthesis of TD-derived open pentapeptide (RH–TD) and hexapeptide (TDHPA) alcohols.

RH–TD against the majority of strains tested. The activity of TDHPA was unexpected considering that the hexapeptide (VAHP) derived from the V-aglycon (VA) was devoid of antibacterial activity [75]. This is very likely due to the different conformation of their hexapeptide backbone in the active site region. While TDHPA almost maintains the original conformation of the peptide chain (fig 5) and in particular the orientation of the 2,3-amide as TD, in VAHP the 2,3-*trans* peptide unit exhibits a 180° flipping motion, from having the NH-proton at the front of the molecule, as in VA, TD, and the other active glycopeptides, to having it at the rear face. This results in the loss of the ability by VAHP to bind to dalbaheptide's target peptide and hence in the loss of the antibiotic activity. One possibility is the presence in TDHPA of an hydrogen bonding, favored at the neutral/physiological pH of the in vitro tests, between the peptide N-terminal amino group of residue 2 and the newly formed hydroxy group of residue 1 (fig 11).

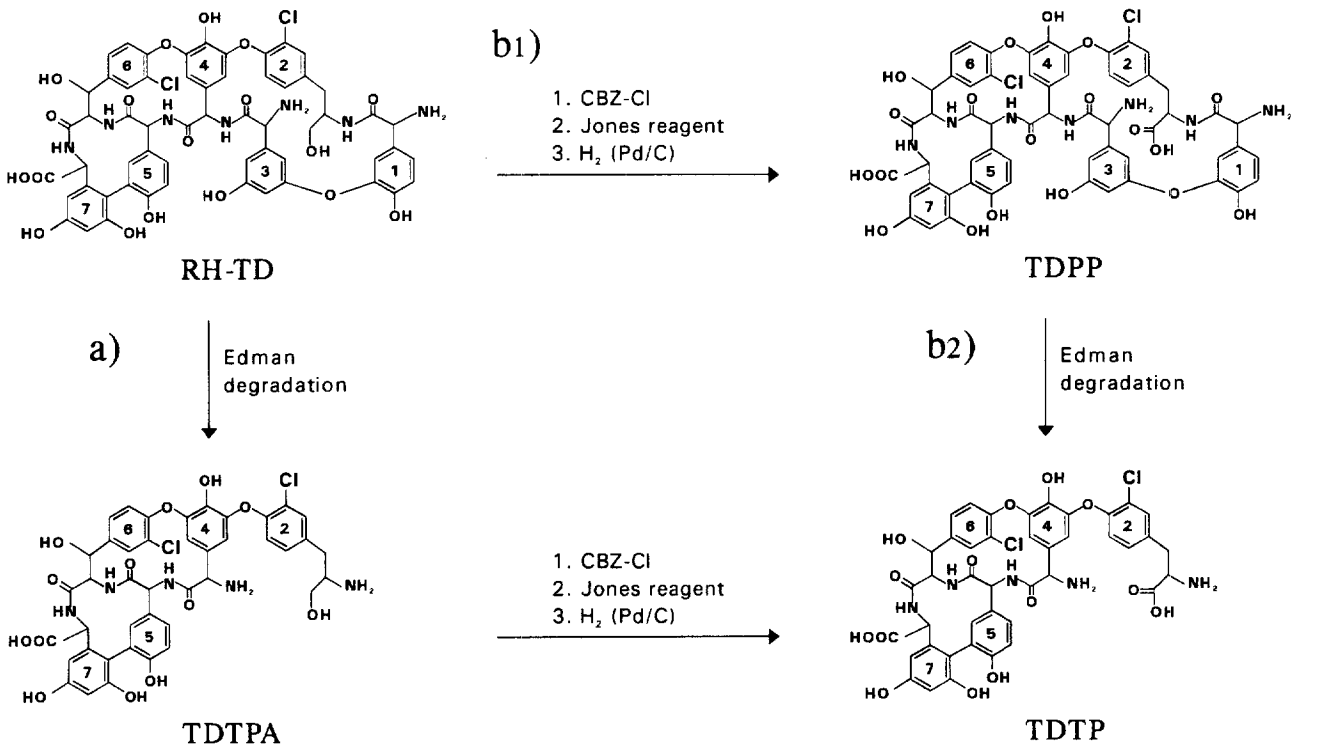
Substitution of amino acids 1 and 3 in T-aglycon

The synthesis of the TD-derived tetrapeptide alcohol (TDTPA, scheme 5a) following removal of amino acids 1 and 3 from RH–TD was reported recently [76]. After protection of the amino and phenolic groups, oxidation of the hydroxymethyl group to carboxy and fully deprotection, the TD-derived tetrapeptide (TDTP) was obtained (scheme 5b). This compound could also be prepared by Edman degradation of oxidized TD-derived pentapeptide (TDPP). Glycopeptide-derived tetrapeptide-aglycons are currently the most useful synthons for the introduction of new amino acids 1 and 3. This possibility has already been demonstrated with the synthesis of three new glycopeptide-aglycons.

Novel non-natural glycopeptide-aglycons

The possibility of producing structurally modified dalbaheptides of non-natural origin from glycopeptide-derived tetrapeptides was demonstrated with the synthesis of MDL 63,166, MDL 64,945, and MDL 64,468 (fig 12) [77].

The former glycopeptide-aglycon methyl ester, that is the first member of a novel class of dalbaheptides in which amino acid 1 (D-Lys) is aliphatic and amino acid 3 (L-Phe) has a benzylic side-chain, was prepared



Scheme 5. Synthesis of TD-derived tetrapeptide alcohol (TDTPA) and tetrapeptide (TDTP).

by a six-step procedure (scheme 6) as follows: (1) condensation of residue 4-NH_2 of di-protected tetrapeptide N^2-CBZ-TDTP-Me (**1**) [76] with the activated *N*-hydroxysuccinimide (NSu) ester of *N*-BOC-L-Phe; (2) removal of the BOC protective group from resulting L-Phe-containing open pentapeptide (**2**); (3) macrocyclization of the open pentapeptide (**3**) to the N^2-protected hexapeptide (HP) N^2-CBZ-Phe^3-TDHP-Me (**4**), carried out (8.5 mM solution in DMF/CH_2Cl_2 1:1), at room temperature, in the presence of equimolecular amounts of hydroxybenzotriazole (HBT), *N*-methylmorpholine and dicyclohexylcarbodiimide (DCC); (4) hydrogenolysis of the CBZ group; (5) condensation of the residue 2-NH_2 of the free hexapeptide (**5**) with *N'*,*N''*-di-BOC-D-Lys NSu ester; (6) final deprotection of the resulting di-BOC heptapeptide (**6**) to give MDL 63,166. Syntheses of MDL 64,945 and MDL 64,468 were performed following correct analog procedures.

The antibacterial activity of the three new dalbaheptides (table VIII) was particularly interesting, considering that MDL 63,166 and MDL 64,468 had

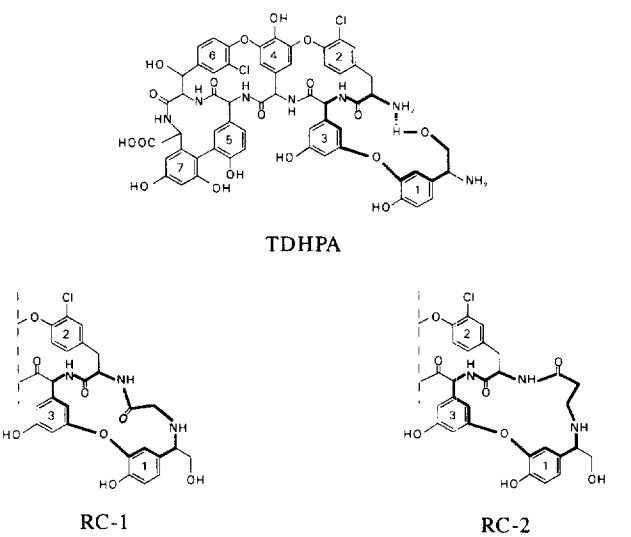
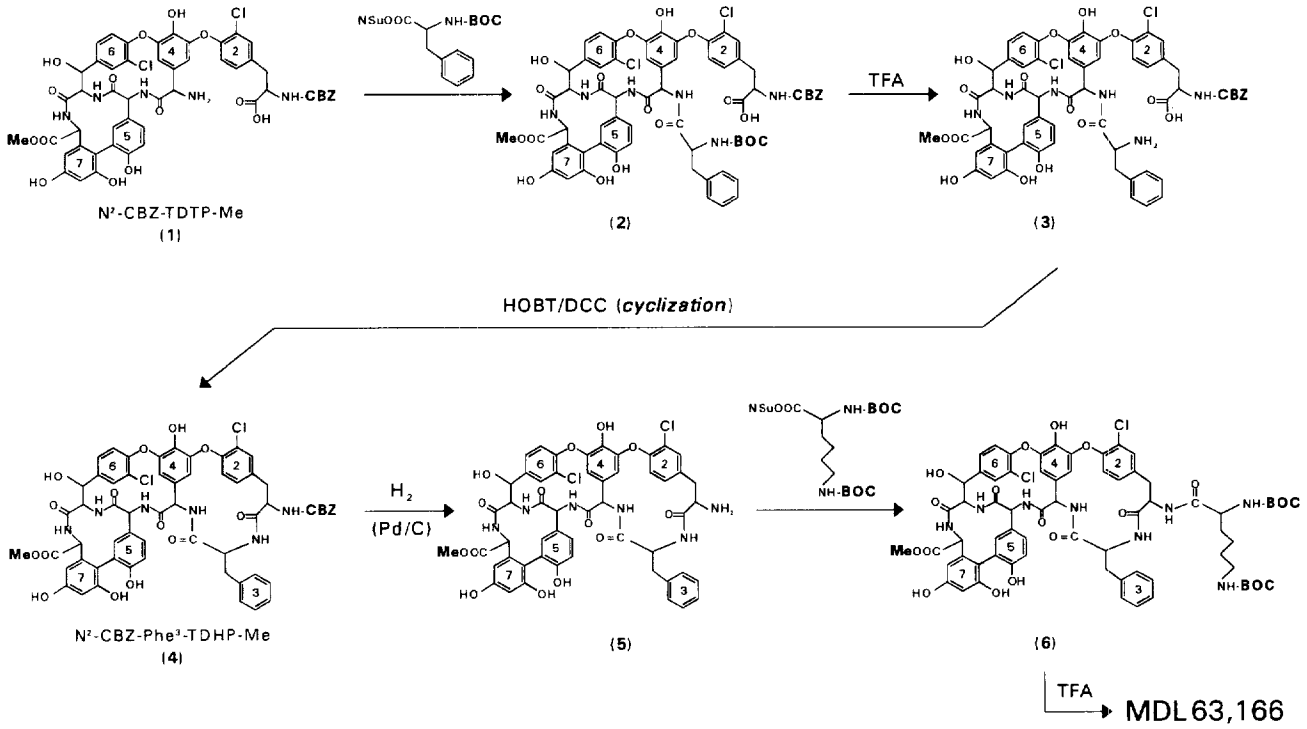


Fig 11. Structure of the 1,2,3-macrocyclic ring in cyclic compounds RC-1 and RC-2 compared with that generated by a hydrogen bonding system in TDHPA.



Scheme 6. Synthesis of MDL 63,166 (adapted from [77]).

moderate activity against VanA enterococci. A common property of these three compounds was their excellent activity against other Gram-positive isolates. Of particular interest was the in vitro anti-staphylococcal activity of MDL 64,945 which proved to be significantly superior to reference dalbaheptides against *S haemolyticus*.

These results clearly indicate that enhanced activity against glycopeptide-resistant enterococci and other Gram-positive pathogens could be pursued by replacement of amino acids 1 and 3 in the heptapeptide structure and also confirm the importance of the structure of these amino acids for the activity of dalbaheptides.

Enlargement of the 1,2,3-macrocyclic ring in T-aglycon

Further SAR have been drawn from the synthesis of two ring-closed compounds (RC-1 and RC-2; fig 11) in which the original 1,2,3-macrocyclic ring of TD was enlarged by a spacer of 1 and 2 methylene groups, respectively [74].

The in vitro activity of RC-1 was comparable to that of TDHPA, while homolog cyclic derivative RC-2 was markedly less active (table VII). The comparable activity between TDHPA and its cyclic derivative RC-1 was expected considering that the overall conformation of the two compounds is very similar. However,

Table VIII. In vitro activity (MIC, mg/L) of compounds investigated.

Organism	*MDL 63,166*	*MDL 64,945*	*MDL 64,468*	*Teicoplanin*	*Vancomycin*
MRSA	0.125	0.063	0.25	0.125	0.25
Staphylococcus epidermidis	0.125	0.063	0.125	0.25	0.125
Staphylococcus haemolyticus	0.5	0.063	0.125	8	1
Enterococcus faecalis (VanA)	16	> 128	32	512	> 1024

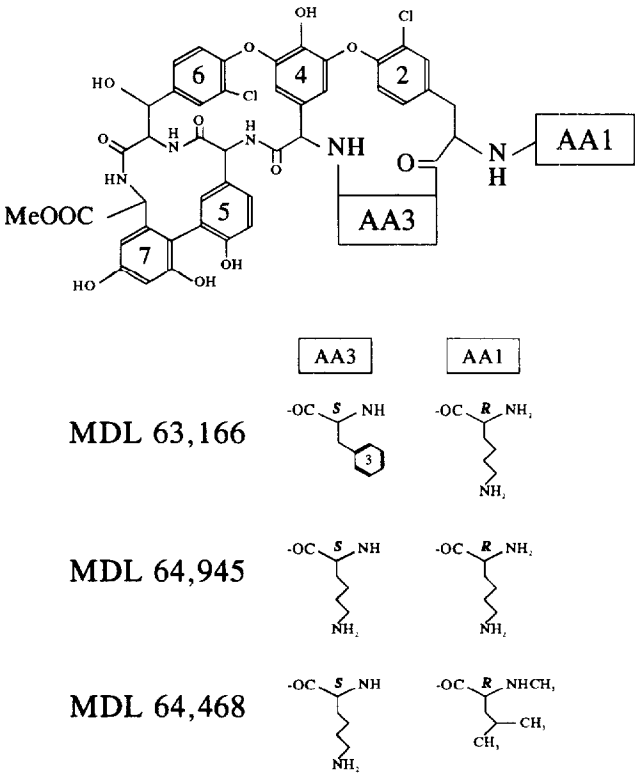


Fig 12. Structure of first non-natural glycopeptide-aglycons.

their lower activity compared with that of TD indicates that the enlargement of the 1,2,3-macrocyclic ring has a negative effect on their binding properties to the target dipeptide. This is even more evident with a further ring enlargement by one additional methylene group, as shown by the poor activity of RC-2.

Concluding remarks

This review of SAR of glycopeptide derivatives clearly indicates that by chemical derivatization there is the possibility to improve the activity of dalbaheptides. Where the target peptide contains terminal D-Ala-D-Ala, both enhanced activity against Gram-positive pathogens and in some cases activity against Gram-negative bacteria have been pursued with success, especially with derivatives of teicoplanin-like glycopeptides. The most active amide derivatives of reduced A-40,926, such as MDL 63,246, stand out as exceptionally potent agents for MRSA and CNS. Interesting activity against vancomycin- and teicoplanin-resistant organisms has also been achieved in compounds such as aralkylated vancomycin-type glycopeptides like LY333328, in bis(vancomycin) carboxamides, and to a moderate degree in acetylglucosamine-less teicoplanin-type compounds. LY333328 also has interesting activity against VRE and other multi-resistant Gram-positive pathogens, and is currently in clinical trial. However, none of these compounds is altered in the binding pocket for target peptide, and none has been shown to possess improvement in affinity for D-Ala-D-Lac that could account for their activity.

In working to overcome the serious clinical problem of enterococcal resistance with a glycopeptide, it may be most desirable to directly change binding properties. This objective could be pursued by a suitable modification of the structure of the heptapeptide backbone in the active site region so as to allow interaction with both vancomycin-susceptible and -resistant targets. An inkling of this possibility has been obtained with the preparation of glycopeptide-derived tetrapeptides that are the most useful synthons for the introduction of new chemical entities in positions 1 and 3. The work based on this approach began recently, and much effort is still needed to establish SAR of new families of 1,3-modified glycopeptides. These key intermediates might also be useful in preparing new families of glycopeptides further modified in their heptapeptide structure. The actual possibility of pursuing simultaneous interaction with both glycopeptide-susceptible and resistant targets by appropriate modifications in the core structure was recently confirmed by molecular modeling studies[5]. This strategy, possibly combined with the modification of the structure of amino acids 1 and 3, could provide a definitive solution to the dramatic clinical problem of glycopeptide resistance in enterococci and other multi-resistant Gram-positive pathogens.

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