Review Article

STRUCTURE-ACTIVITY RELATIONSHIPS OF VANCOMYCIN-TYPE GLYCOPEPTIDE ANTIBIOTICS†

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(Received for publication March 9, 1993)

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Introduction

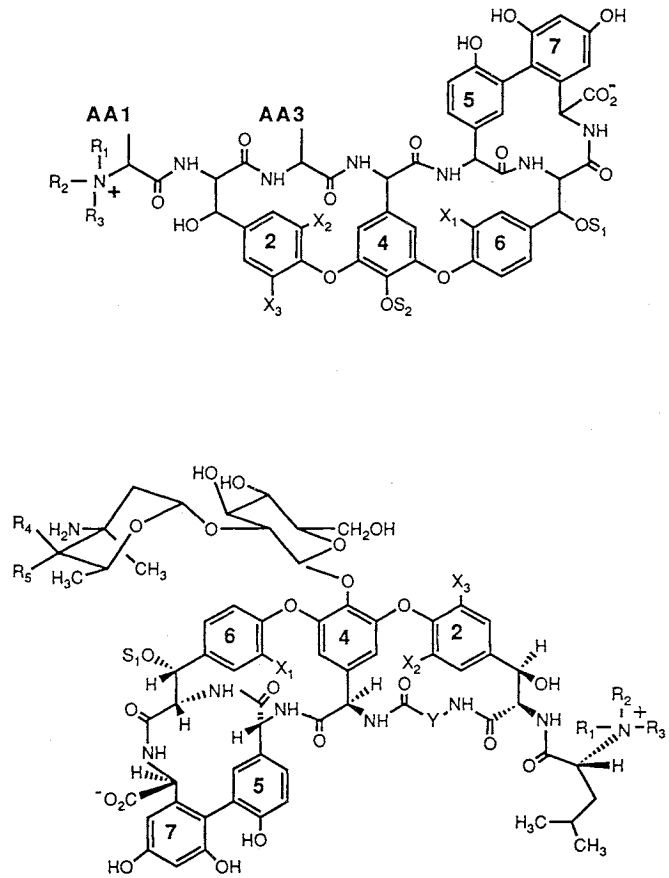
Vancomycin[1] has been marketed for the past 35 years, as the hydrochloride, to treat deep-seated Gram-positive infections. It is the drug of choice for infections caused by *Staphylococcus aureus*, especially those caused by methicillin-resistant and more importantly the multi-resistant strains. Vancomycin is bactericidal to most Gram-positive organisms, but Gram-negative organisms are resistant. Vancomycin is not absorbed from the gastrointestinal tract and the antibiotic is used to treat enterocolitis, especially that caused by *Clostridium difficile*. Vancomycin is in clinical use worldwide. In recent years there is both a medical and scientific resurgence in vancomycin in particular and glycopeptide antibiotics in general. Teicoplanin[2,3] was recently launched in Italy and France and is under clinical trials in Europe and the United States of America.

Vancomycin is produced by *Amycolatopsis orientalis*[4] (previously designated *Norcardia orientalis* and *Streptomyces orientalis*). There are 45 organisms that have been reported to produce this class of antibiotics.

All glycopeptide antibiotics contain a central core heptapeptide. This heptapeptide (Fig. 1) written in

† Portions of this Review Article are excerpted from a chapter in the forthcoming book "Glycopeptide Antibiotics" to be published by Marcd Dekker and edited by R. NAGARAJAN.

Fig. 1. General glycopeptide antibiotic structure.



In the structural representation of these glycopeptide antibiotics (top and bottom), R_1, R_2 and R_3 represent H or CH_3. R_4 and R_5 are H or OH. X_1, X_2 and X_3 are H or Cl. S_1 and S_2 are sugars. Y represents an asparagine, aspartic acid, or isoaspartic acid moiety. Numbers 1 to 7 represent the amino acids of the antibiotic heptapeptide (top), starting at the amino terminal. AA1 and AA3 refer to amino acids 1 and 3, respectively.

the conventional peptide structure, has a high degree of homology in the aromatic amino acids 2 and 4 to 7 for this class of antibiotics. In several glycopeptide antibiotics, the remaining amino acids 1 and 3, are also aromatic amino acids. Vancomycin-type glycopeptide antibiotics are structurally unique in that amino acids 1 and 3 are aliphatic amino acids. The classification of glycopeptide antibiotics into four classes is based on these and other structural differences.

As is common in microbial secondary metabolites, the glycopeptide-producing organisms elaborate a complex of antibiotics with minor chemical modifications. For example, the HPLC of partially purified vancomycin shows no less than nine other structurally closely related factors (Figs. 2 and 3)[5]. If we take such differences in the four classes of glycopeptides into consideration, well over 200 glycopeptides are known.

Scope of the SAR of Glycopeptides

Of the four classes of glycopeptide antibiotics, clearly the vancomycin-type is clinically the most important group. Several close structural analogs of vancomycin have been reported recently, and these

Fig. 2. HPLC profile of partially purified vancomycin hydrochloride using a linear gradient from 7 to 28% acetonitrile in triethylammonium phosphate buffer at pH 3.2 as described in ref 44.

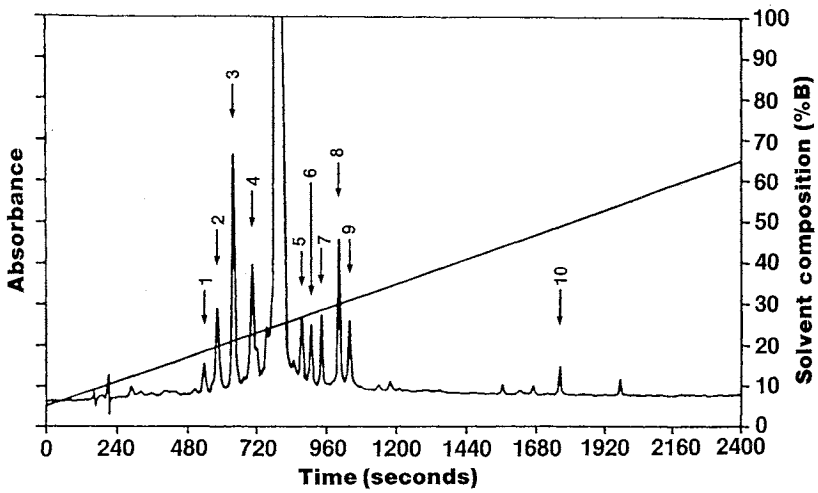
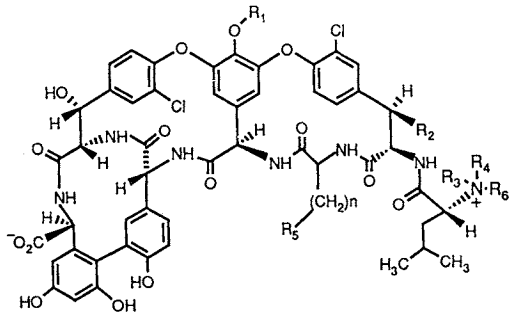
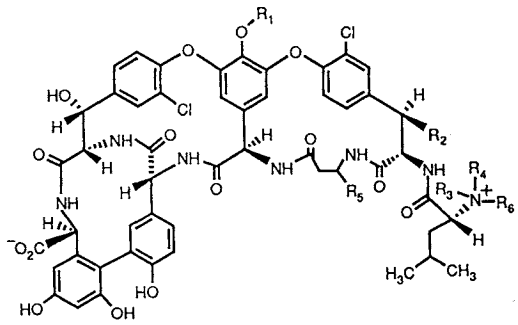


Fig. 3. Structural assignments proposed for vancomycin and related compounds in HPLC chromatogram in Fig. 2.



General vancomycin structure (unrearranged)



Compounds 1 and 7 (rearranged)

Compound	Compound No.	T_r(s)	n	R_1	R_2	R_3	R_4	R_5	R_6
Vancomycin	Main	800	1	Vancosaminyl-*O*-glucosyl-	–OH	–H	$-CH_3$	$-CONH_2$	–H
CDP-1	1	570		Vancosaminyl-*O*-glucosyl	–OH	–H	$-CH_3$	–COOH	–H
	2	600		Structure unknown					
M43G	3	650	2	Vancosaminyl-*O*-glucosyl-	–OH	–H	$-CH_3$	$-CONH_2$	–H
A51568A	4	723	1	Vancosaminyl-*O*-glucosyl-	–OH	–H	–H	$-CONH_2$	–H
M43F	5	857	1	Vancosaminyl-*O*-glucosyl-	–OH	–H	$-CH_3$	–COOH	–H
M43E	6	875	1	Vancosaminyl-*O*-glucosyl-	–H	–H	$-CH_3$	$-CONH_2$	–H
CDP-1	7	911		Vancosaminyl-*O*-glucosyl-	–OH	–H	$-CH_3$	–COOH	–H
M43D	8	994	1	Vancosaminyl-*O*-glucosyl-	–OH	$-CH_3$	$-CH_3$	$-CONH_2$	–H
Desvancosamine vancomycin	9	1024	1	Glucosyl-	–OH	–H	$-CH_3$	$-CONH_2$	–H
Aglucovancomycin	10	1767	1	–H	–OH	–H	$-CH_3$	$-CONH_2$	–H

include the M43 group of antibiotics[6], A42867[7], A51568[8], A82846[9], A83850[10], chloroorienticin[11], decaplanin[12], eremomycin[13], MM 45289[14], MM 47761[15], OA-7653[16,17], orienticin[18] and UK72051[19].

Secondly, several degradative and chemical interconversions of some of the above-mentioned antibiotics have been reported over the past 10 years[20~25].

Finally, recent reports have described glycopeptides with long chain acylamido side chains on a sugar residue[26~33]. One of these, teicoplanin, has been claimed to have more favorable antibacterial and pharmacokinetic properties than vancomycin[34]. Synthesis of several *N*-acyl and *N*-alkyl vancomycins and A82846 antibiotics[35~39] and their evaluation revealed that the *N*-alkyl derivatives have substantial advantage over the parent antibiotics.

We[38,39] have examined the *in vitro* antimicrobial activities of these compounds against benzylpenicillin-susceptible *S. aureus* X1.1 (SA1), benzylpenicillin-resistant *S. aureus* V41 (SA2), methicillin-resistant *S. aureus* X400 (SA3), methicillin-resistant *S. aureus* S13E (SA4), macrolide-resistant *Staphylococcus epidermidis* 270 (SE1), macrolide-susceptible *S. epidermidis* 222 (SE2), *Streptococcus pyogenes* C203 (SPy), *Streptococcus pneumoniae* Park (SPn), *Streptococcus* (*Enterococcus*) *faecium* X66 (SD1) and *Streptococcus* (*Enterococcus*) *feacalis* 2041 (SD2)[36].

In addition, all the compounds included in this chapter were evaluated against *Staphylococcus aureus* X1.1, *Streptococcus pyogenes* C203 and *Streptococcus pneumoniae* Park infections in the *in vivo* mice model[36]. Even though actual values for all compounds are not included in this review, the results are summarised in the SAR (Structure-Activity Relationships).

With the above data in hand, a SAR can now be delineated with reference to antibacterial activity of the vancomycin class of glycopeptide antibiotics. We will examine how changes in each functional group of the vancomycin class antibiotic affects the antibacterial activity.

SAR of the Core Hexapeptide's Amino Acids

Amino Acid 1

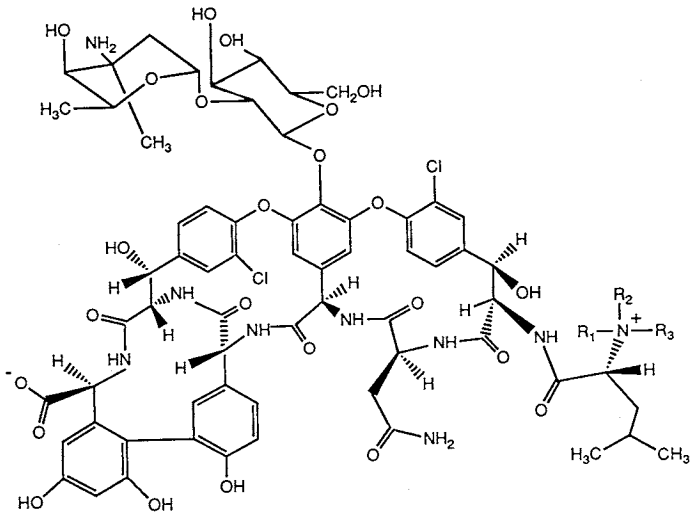
All the naturally occurring vancomycin-type glycopeptide antibiotics reported until now contain leucine or its methylated analogs[6~15]. The antibiotic activity of vancomycin, A51568A and M43 factors A and D are similar, suggesting that the state of methylation of the leucine residue does not affect the antibacterial activity. Antibiotic OA-7563, factors A and B, have *N*,*N*-dimethyl alanine[16,17] as the *N*-terminal amino acid in place of the *N*-methylleucine and both factors A and B of OA-7563 are less active than vancomycin. However, the removal of this important terminal amino acid leucine (Edman degradation product) needed for the crucial binding of the *N*-acyl-D-alanyl-D-alanine (acyl-D-ala-D-ala) carboxy terminus of UDP-acetylmuramylpentapeptide to the *N*-terminal leucine of vancomycin, completely destroys the antibacterial activity of vancomycin. Acylation of the Edman degradation product of vancomycin with both aliphatic and aromatic amino acids afforded vancomycin modified at amino acid 1. None of these modified derivatives showed better activity than vancomycin[40].

Amino Acid 2

An analog of vancomycin in which the benzylic hydroxyl group of amino acid 2 is replaced by hydrogen (M43E) has been isolated from a strain of *A. orientalis*[39]. This naturally occurring deoxyvancomycin is half as active as vancomycin.

Amino acid 2 contains a chlorine in the aromatic ring, and catalytic dechlorination of vancomycin[23]

Table 1. Vancomycin modified at amino acid 1.



Vancomycin: $R_1=R_2=H$; $R_3=CH_3$
A51568A: $R_1=R_2=R_3=H$
M43D: $R_1=H$; $R_2=R_3=CH_3$
M43A: $R_1=R_2=R_3=CH_3$
Edman degradation product = Leucine removed

Compound	MIC (μg/ml)									
	SA1	SA2	SA3	SA4	SE1	SE2	SPy	SPn	SD1	SD2
Vancomycin	0.5	0.5	1	1	2	1	0.5	0.5	1	2
A51568A	0.5	1	1	1	2	1	2	0.5	1	4
M43D	1	1	2	1	4	1	0.5	0.25	2	4
M43A	1	1	2	1	4	2	1	0.5	2	4
Edman product	128+	128+	128+	128+	128+	128+	128+	128+	128+	128+

removes this chlorine to afford the monodechlorovancomycin. Vancomycin is twice as active as this monodechlorovancomycin[39].

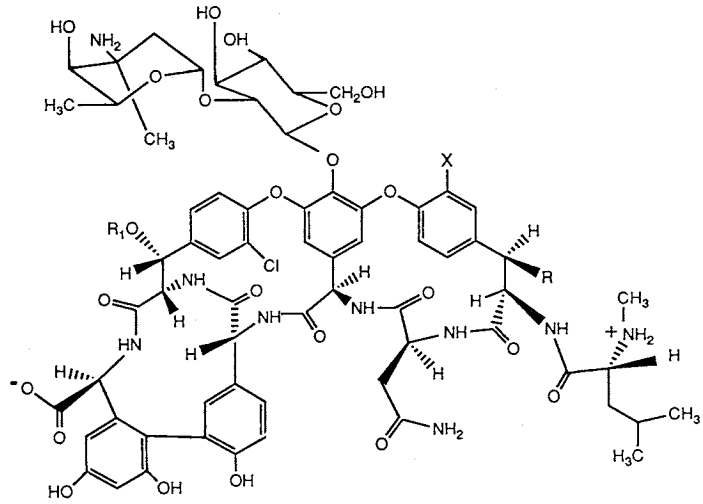
To establish a similar SAR for A82846 antibiotics, A82846B was catalytically dechlorinated to its monodechloro A82846B analog, and this monodechloro A82846B was shown to be identical to orienticin A[9,18]. The removal of chlorine in the aromatic amino acid 2 reduces the activity by ten-fold[39]. We will discuss the effect of antibacterial activity on the removal of the second chlorine from vancomycin when we examine the SAR of amino acid 6.

Amino Acid 3

The third amino acid of the heptapeptide core of the vancomycin class of antibiotic is the other aliphatic amino acid asparagine. Several modifications, both naturally occurring analogs and the uniquely rearranged CDP analogs, are known.

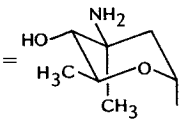
The naturally occurring A51568B[8] and M43G[39] are the glutamine analogs of A51568A[8] and vancomycin, respectively. These two antibiotics have an additional methylene on the amino acid 3 (asparagine) of vancomycin. A51568B is four-fold less active than vancomycin, and M43G is half as active

Table 2. Glycopeptide antibiotics modified at amino acid 2.

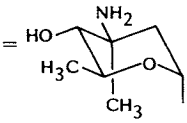


M43E: R=R$_1$=H; X=Cl
Monodechlorovancomycin: R=OH; R$_1$=X=H

A82846B: R=OH; X=Cl; R$_1$=



Orienticin A: R=OH; X=H; R$_1$=



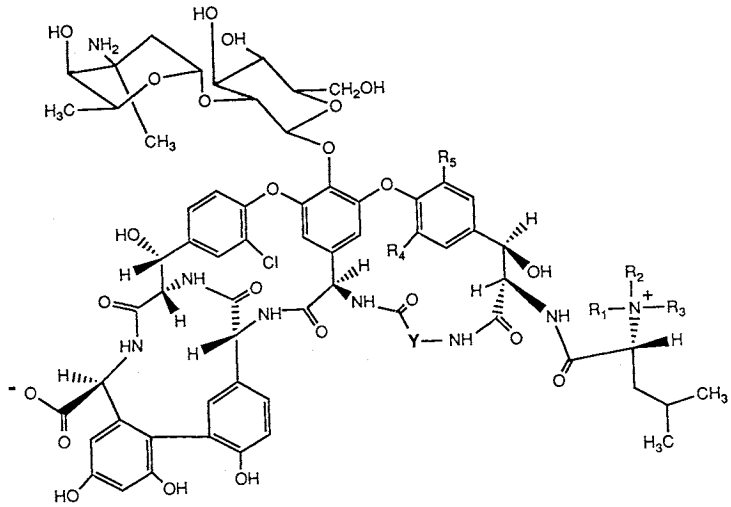
Compound	MIC (μg/ml)									
	SA1	SA2	SA3	SA4	SE1	SE2	SPy	SPn	SD1	SD2
M43E	1	2	2	2	4	2	1	0.5	2	4
Monodechloro-vancomycin	1	2	2	2	2	2	1	1	2	8
A82846B	0.25	0.25	0.5	0.25	0.5	0.25	0.25	0.25	0.5	0.5
Orienticin A	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	2	2

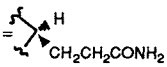
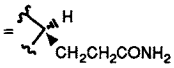
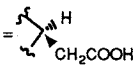
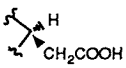
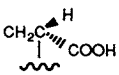
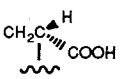
as vancomycin. Consequently, the increase in the chain length of asparagine to glutamine reduces the activity of vancomycin.

The naturally occurring antibiotics M43F and M43B are the aspartic acid analogs of vancomycin and M43A, respectively[6]. These two desamido analogs are at least 10 times less active than vancomycin. The rearranged CDP-1[41], the isoaspartic acid analog of vancomycin and the corresponding isoaspartic analog of M43A[6], are devoid of antibacterial activity[39].

The negative charges of the aspartate and isoaspartate moieties in M43B, M43F and in the rearranged analogs near the binding site and the changed conformational geometry[24] in the rearranged derivatives, seems to hinder the binding of the acyl-D-ala-D-ala carboxy terminus of UDP-*N*-acetylmuramyl-pentapeptide to the antibiotic and contributes dramatically to the diminution of the antibacterial activity.

Table 3. Glycopeptide antibiotics modified at amino acid 3.



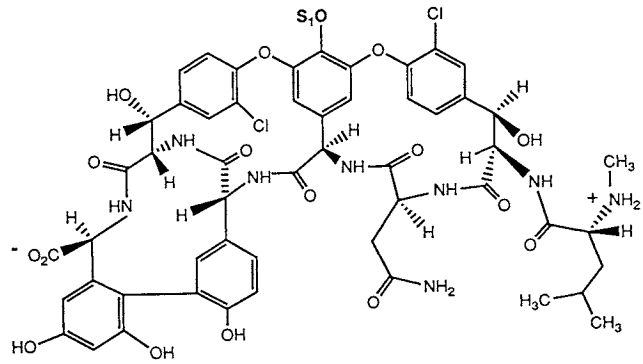
1. A51568B: $R_1 = R_2 = R_3 = R_4 = H$; $R_5 = Cl$; Y = 
2. M43G: $R_1 = R_2 = R_4 = H$; $R_3 = CH_3$; $R_5 = Cl$; Y = 
3. M43F: $R_1 = R_2 = R_4 = H$; $R_3 = CH_3$; $R_5 = Cl$; Y = 
4. M43B: $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$; $R_5 = Cl$; Y = 
5. CDP-1: $R_1 = R_2 = R_5 = H$; $R_3 = CH_3$; $R_4 = Cl$; Y = 
6. $R_1 = R_2 = R_3 = CH_3$; $R_4 = Cl$; $R_5 = H$; Y = 

Compound	MIC (μg/ml)									
	SA1	SA2	SA3	SA4	SE1	SE2	SPy	SPn	SD1	SD2
A51568B	2	2	2	2	2	2	0.5	0.06	1	2
M43G	2	4	4	4	4	4	1	0.125	2	4
M43F	4	4	8	8	16	8	4	2	8	16
M43B	16	32	32	32	32	32	16	2	32	32
CDP-1	128+	128+	128+	128+	128+	128+	128+	128+	128+	128+
6	128+	128+	128+	128+	128+	128+	128+	128+	128+	128+

Amino Acid 4

The only SAR of this amino acid pertains to the sugar moiety at the phenolic hydroxyl group. In several naturally occurring vancomycin-type glycopeptide antibiotics, the phenolic hydroxyl group carries a disaccharide unit. In many cases, the first sugar is D-glucose linked β-glycosidally and the second sugar, usually an amino sugar, is either L-vancosamine, or 4-L-*epi*-vancosamine, linked to glucose α-glycosidally.

Table 4. Glycopeptide antibiotics modified at amino acid 4.



1. Desvancosamine vancomycin $S_1 = \beta$-*O*-D-glucosyl
2. Aglucovancomycin $S_1 = H$
3. A83850B $S_1 = \alpha$-*O*-4-L-ketovancosaminyl-β-*O*-D-glucosyl
4. Reduced A83850B $S_1 = \alpha$-*O*-4-L-*epi*-vancosaminyl-β-*O*-D-glucosyl

Compound	MIC (μg/ml)									
	SA1	SA2	SA3	SA4	SE1	SE2	SPy	SPn	SD1	SD2
1	8	8	8	8	16	8	4	4	8	32
2	1	1	1	1	2	2	0.5	1	2	4
3	0.5	1	1	0.5	1	1	0.5	0.125	1	4
4	1	1	1	1	2	2	0.5	0.125	1	4

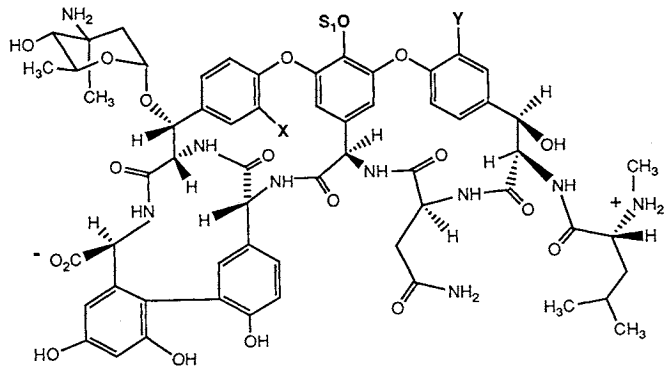
In the case of A83850B[10], the amino sugar is α-4-keto-L-*epi*-vancosamine. These two sugars were cleaved sequentially[20] and their antibacterial activity is shown in Table 4. In addition, similar analogs for A51568A, M43A and A83850A (the dimethylleucine analog of A83850B) were prepared and the SAR of these derivatives also support the conclusions discussed below.

The removal of the amino sugar, vancosamine, reduces the activity of the parent antibiotic by two- to five-fold. The removal of the second sugar, glucose, restores the activity, only in so far as the *in vitro* activity, but the *in vivo* activity is reduced five-fold. We have examined the *in vitro* and *in vivo* activities (in infections in mice) and they always parallel one another. The only exception seems to be the agluco derivatives. The sugars seem to play an important role in imparting enhanced pharmacokinetic properties for the vancomycin-type glycopeptide antibiotics. The keto group at the 4 position of L-vancosamine does not seem to affect the antibacterial activity. Reduction of A83850B affords the 4-*epi*-vancomycin analog. This compound, the 4-*epi* analog of vancomycin, is less active than vancomycin.

Amino Acid 6

Recently, several glycopeptide antibiotics were isolated, which have made possible the delineation of the effect of chlorine on the benzene ring of the amino acid 6 has on the antibacterial activity. Accordingly, antibiotic A82846B (identical with chloroorienticin A) has two chlorines, one on the aromatic amino acid 2 and the other on aromatic amino acid 6. Antibiotic A82846A (identical with eremomycin, MM 45289) has a chlorine on amino acid 2. Orienticin A (identical with UK-72,051) possesses a chlorine on amino acid 6 and A82846C (identical with orienticin C, MM 47756) is devoid of any chlorine substituent. In all other aspects, these four antibiotics are identical.

Table 5. Glycopeptide antibiotics modified at amino acid 6.



1. A82846B (chloroorienticin A) S_1 = α-*O*-4-L-*epi*-vancosaminyl-β-*O*-D-glucose; X = Y = Cl
2. A82846A S_1 = α-*O*-4-L-*epi*-vancosaminyl-β-*O*-D-glucose; X = H; Y = Cl
3. Orienticin A S_1 = α-*O*-4-L-*epi*-vancosaminyl-β-*O*-D-glucose; X = Cl; Y = H
4. A82846C (orienticin C) S_1 = α-*O*-4-L-*epi*-vancosaminyl-β-*O*-D-glucose; X = Y = H
5. Chloroorienticin B S_1 = β-*O*-D-glucose; X = Y = Cl
6. Chloroorienticin C S_1 = H; X = Y = Cl

Compound No.	MIC (μg/ml)									
	SA1	SA2	SA3	SA4	SE1	SE2	SPy	SPn	SD1	SD2
1	0.125	0.125	0.125	0.125	0.25	0.125	0.06	0.06	0.5	0.25
2	0.125	0.125	0.25	0.125	0.125	0.125	0.25	0.125	0.5	2
3	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	2	2
4	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	4	4
5	0.25	0.25	0.25	0.25	0.5	0.25	0.5	1	1	1
6	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.25	1	1

The examination of both *in vitro* and *in vivo* (not included) antibacterial activities show that A82846A and A82846B are about two to ten times more active than vancomycin, while orienticin A and A82846C are two-fold less active than vancomycin. (The differences in activities become clearer in the *in vivo* experiments). These data suggest that the removal of chlorine in the aromatic amino acid 6 has slight, if any, effect on the antibacterial activity. However, removal of chlorine of the aromatic amino acid 2 diminishes the activity 10-fold.

Finally, let us consider the effect of the amino sugar on the benzylic hydroxyl group of the aromatic amino acid 6. The selective and sequential removal of L-4-*epi*-vancosamine and then the second β-glucose from both A82846A and A82846B were accomplished and their antibacterial activity evaluated. The SAR is identical in both cases, but we will discuss here only the A82846B derivatives.

Removal of the L-4-*epi*-vancosamine on amino acid 4 from A82846B affords chloroorienticin B, and hydrolysis of the D-glucose affords chloroorienticin C. These compounds have also been isolated from natural sources[11]. These two compounds are two to five times more active than vancomycin, as is A82846B itself. Consequently, the presence of this benzylic amino sugar, L-4-*epi*-vancosamine, on aromatic amino acid 6 dramatically increases the activity of vancomycin by two- to five-fold.

Amino Acids 5 and 7

No naturally occurring or chemically modified analogs of the amino acids 5 and 7 are known. However,

the carboxyl group on amino acid 7 affords an opportunity for chemical modification.

SAR of Semisynthetic Glycopeptide Antibiotics

N-Acyl Derivatives

Several glycopeptides containing a long-chain aliphatic acyl residue on the amino sugar attached to the phenolic group of the amino acid 4 have been reported[26~33]. Teicoplanin, an antibiotic belonging to the above long chain acylamido glycopeptide class, has been claimed to have better antibacterial and more favorable pharmacokinetic properties than vancomycin[34]. We undertook a systematic SAR of *N*-acyl vancomycins and the more active A82846 antibiotics, especially the derivatives acylated on the amino sugar of the amino acid 4.

Thirty-two *N*-acyl vancomycins[35] and seven *N*-acyl A82846[39] derivatives were prepared and evaluated. In the case of vancomycin, acylation yielded two mono-*N*-acyl derivatives substituted at the amino groups of the vancosamine and *N*-methylleucine moieties, respectively, and one di-*N*-acyl derivative. However, in the case of A82846A and A82846B antibiotics, three mono-*N*-acyl, three di-*N*-acyl and one tri-*N*-acyl derivatives can be obtained. The ratio of three *N*-acyl derivatives in the case of vancomycin, and seven *N*-acyl derivatives in the case of A82846A and A82846B derivatives, depended on the reaction conditions[35,39].

In the aliphatic *N*-acyl vancomycin derivatives, the mono-*N*-acyl derivatives functionalized on the vancosamine amino group is more active than the mono-*N*-acyl vancomycin substituted on the amino acid, *N*-methylleucine. Both the mono-*N*-acyl vancomycins are more active than the di-*N*-acyl vancomycins. With the limited number of derivatives prepared, the SAR of *N*-acyl A82846 derivatives seems to be similar to the vancomycin series. Accordingly, the mono-*N*-acyl derivatives are more active than the di-*N*-acyl or tri-*N*-acyl A82846 derivatives.

A comparison of the more active aliphatic mono-*N*-acyl vancomycins substituted on the amino group of vancosamine reveals that increasing the length of the side chain increases activity. The optimum activity is found when the side chain is C_9 to C_{11} straight chain fatty acid residue. When the carbon chain length is greater than 11, the activity drops off. Modification of the side chain with a branched chain, or introduction of an amino, bromo, carboxyl, or insertion of an oxygen or sulfur does not enhance the activity.

The SAR of the *N*-aracyl vancomycins follows a pattern similar to those of the aliphatic *N*-acyl vancomycins. Accordingly, the mono-*N*-aracyl vancomycins substituted on the vancosamine amino moiety are more active than the mono-*N*-acyl derivatives functionalized on the amino acid, *N*-methyl leucine; and both the above-mentioned mono-*N*-aracyl vancomycins are more active than the corresponding di-*N*-aracyl vancomycins.

The aromatic mono-*N*-aracyl vancomycins substituted on the vancosamine sugar are more active than the corresponding aliphatic mono-*N*-acyl derivatives. The most active compounds in the mono-*N*-aracyl series are the *p*-octylbenzoyl and *p*-octyloxybenzoyl vancomycin derivatives, with an aliphatic hydrocarbon chain attached to the aromatic ring.

Finally, a comparison of the antibacterial activities of the parent vancomycin with the *N*-acyl derivatives show that, even though in some cases there is an increase in the *in vitro* spectrum of the mono-*N*-acyl derivatives, the *in vivo* activities do not reveal any great increase over that of vancomycin.

N-Alkyl Derivatives

As an extension of the above-mentioned SAR of *N*-acyl glycopeptide derivatives, over eighty *N*-aklyl

Table 6. Comparison of the SAR of C_{10} *N*-acyl and *N*-alkyl vancomycins.

Compound	MIC (μg/ml)									
	SA1	SA2	SA3	SA4	SE1	SE2	SPy	SPn	SD1	SD2
Vancomycin	0.5	0.5	1	1	2	1	0.5	0.5	1	2
Mono-*N*-decanoyl (A.A. 4)	0.5	0.5	0.5	0.5	2	1	0.5	1	0.5	1
Mono-*N*-decanoyl (A.A. 1)	0.5	0.5	0.5	1	4	2	4	4	2	4
Di-*N*-decanoyl	2	4	4	8	32	8	4	16	8	8
Mono-*N*-decyl (A.A. 4)	0.13	0.13	0.13	0.13	0.25	0.13	0.06	0.13	0.25	0.25
Mono-*N*-decyl (A.A. 1)	0.5	0.5	0.5	0.5	2	0.5	0.5	0.5	0.5	1
Di-*N*-decyl	2	4	4	4	16	4	2	4	4	4

Table 7. SAR of *N*-octyl derivatives of A82846B.

Compound	MIC (μg/ml)			ED_{50} (mg/kg) × 2; sc		
	SA1	SPy	SPn	SA1	SPy	SPn
A82846B	0.25	0.25	0.25	0.20	0.18	0.20
Mono-*N*-octyl (A.A. 4)	0.5	0.06	0.25	0.43	0.11	0.06
Mono-*N*-octyl (A.A. 1)	0.5	0.06	0.13	1.2	0.18	0.34
Di-*N*-octyl (A.A. 4 and A.A. 6)	1	0.5	0.5	1.2	0.18	0.32
Di-*N*-octyl (A.A. 1 and A.A. 6)	4	0.5	4	—	—	—

vancomycins[36] and seventy *N*-alkyl A82846 derivatives[38,39] were synthesized and evaluated.

Some derivatives in this category are the most active compounds known to date and exhibit interesting biological properties like longer half-life even though not highly serum-bound, and activity against vancomycin-resistant *Enterococcus* strains at clinically relevant MICs.

A comparison of the antibacterial activities of the *N*-decyl vancomycins with the *N*-decanoyl vancomycins show that the C_{10} alkyl analogs are more active than the corresponding alkanoyl series. Furthermore, the mono-*N*-decyl vancomycin substituted on the vancosamine sugar is more active *in vitro* than the parent vancomycin, is equivalent in activity to vancomycin *in vivo*, and shows a longer elimination half-life in rats. This was our first indication that the *N*-alkyl vancomycin derivative showed interesting biological properties and it triggered our interest in actively pursuing the SAR of *N*-alkyl vancomycin-type glycopeptide antibiotics.

As in the SAR of the *N*-acyl vancomycin series, the general SAR trend is that the *N*-alkyl derivatives substituted on the vancosamine sugar are more active than those substituted on the *N*-methylleucine; and both mono-substituted vancomycins are more active than the corresponding di-*N*-alkyl vancomycins.

In the *N*-alkyl A82846A and A82846B series, the SAR trend is similar to vancomycin. Accordingly, in general the mono-*N*-alkyl A82846A and A82846B derivatives substituted on either 4-*epi*-vancosamine sugar are more active than when substituted on the amino acid, *N*-methylleucine; and the mono-*N*-alkyl derivatives are more active than the di-*N*-alkyl A82846 derivatives.

Table 8. SAR of *N*-alkyl vancomycins.

Compound	MIC (μg/ml)			ED_{50} (mg/kg) × 2; sc			Serum; iv rat	
	SA1	SPy	SPn	SA1	SPy	SPn	$T_{1/2}$ (hours)	5 minutes concentration (μg/ml)
Vancomycin	0.5	0.5	0.5	1.8	0.8	0.9	0.75	160
$C_6H_5CH_2$	0.06	0.06	0.015	0.8	1.0	0.9	1.8	89
p-$C_4H_9C_6H_4CH_2$	0.125	0.125	0.125	0.7	0.4	0.8	5.4	156
p-$C_8H_{17}C_6H_4CH_2$	0.5	0.25	0.25	0.19	0.62	0.23	—	—
p-$C_4H_9OC_6H_4CH_2$	0.125	0.06	0.06	0.7	0.5	0.6	2.4	205
p-$C_8H_{17}OC_6H_4CH_2$	0.25	0.25	0.25	0.2	0.2	0.2	—	—

As in the case of *N*-acyl vancomycins, increasing the aliphatic straight-chain in the *N*-alkyl vancomycins enhanced activity. The optimum length seems to be C_{10}. Branching the side chain or substituting with oxygen or sulfur did not increase the activity. In the *N*-aralkyl vancomycin series, the benzyl derivatives and the benzyl substituted at the 4-position with an aliphatic side chain were more active than the aliphatic series in the *in vivo* models. The most active vancomycin derivatives were the *N*-octylbenzyl and the *N*-octyloxybenzyl vancomycins. Substitutions at the *para* positions of the benzyl moiety with hetero atoms such as oxygen, sulfur, nitrogen, or halogens did not seem to enhance activity. Several compounds in the *N*-alkyl vancomycin series were more active than the parent vancomycin. Accordingly, benzyl, octylbenzyl, octyloxybenzyl, butylbenzyl and butyloxybenzyl derivatives were up to five times more active than vancomycin.

Table 9. SAR of *N*-acyl and *N*-alkyl vancomycins against resistant enterococci.

Compound	MIC's (μg/ml)		
	Resistant enterococci ($n=26$)	Typical enterococci ($n=34$)	Ratio
Vancomycin	263	1.0	261
Teicoplanin	22	0.24	92
A82846B	11	0.46	24
Mono-*N*-decyl	4.8	0.56	8.1
Mono-*N*-*p*-octylbenzyl	5.6	0.74	7.6
Mono-*N*-*p*-octyloxybenzyl	4.6	0.62	7.4

The most active compounds in the *N*-alkyl A82846B series were the mono-*N*-octylbenzyl, mono-*N*-*p*-chlorobenzyl and mono-*N*-*p*-bromobenzyl derivatives, substituted on the 4-*epi*-vancosamine linked to amino acid 4.

Activity against Resistant Enterococci

Since the recent reported isolation of clinical isolates of *E. facium* and *E. faecalis* resistant to vancomycin[42], several glycopeptides and their derivatives were tested against 34 susceptible and 26 resistant enterococcal strains[37,39]. Some of the semi-synthetic *N*-alkylvancomycin and A82846B derivatives exhibited excellent activity against these resistant strains.

Whereas the geometric mean MICs of vancomycin were 1.0 and 263 μg/ml against 34 susceptible and 26 resistant enteroeocci strains, respectively, the corresponding values for teicoplanin were 0.24 and 22 μg/ml. However, for the most active mono-*N*-alkyl vancomycins the values ranged between 0.5 to 0.75 μg/ml for susceptible strains and 4 to 6 μg/ml for resistant strains. For the most active mono-*N*-alkyl A82846B derivatives, the corresponding values were between 0.65 to 0.75 μg/ml for the susceptible strains and between 2.1 and 3.6 μg/ml for the resistant strains, respectively. Thus, the *N*-alkyl vancomycin and A82846B derivatives are not only up to five times more active than vancomycin against Gram-positive bacteria, but

Table 10. SAR of mono-*N*-alkyl A82846B derivatives.

Compound	ED_{50} (mg/kg) × 2			Geometric mean MIC (μg/ml)		
	SA1	SPy	SPn	Resistant strains[a] ($n=25$)	Susceptible strains[b] ($n=34$)	Ratio
A82846B	0.2	0.18	0.2	10	0.46	21
Mono-*N*-octyl	0.43	0.11	0.06	2.1	0.73	2.1
Mono-*N*-*p*-chlorobenzyl	0.25	0.06	0.08	3.6	0.65	5.3
Mono-*N*-*p*-bromobenzyl	0.38	0.05	0.08	3.1	0.67	4.6

[a] Susceptibility of vancomycin-resistant and vancomycin-susceptible *Enterococcus faecium and E. faecalis.*
[b] All alkyl substituents are on *epi*-vancosamine in amino acid 4.

more importantly, these semi-synthetic *N*-alkyl derivatives are more active against the vancomycin-resistant enterococci strains at clinically relevant MICs.

Conclusions

The delineation of the SAR for the vancomycin-type class of antibiotics is entirely consistent with the above mode of action for this class of antibiotics. There are two sites where chemical modification close to the binding area of the vancomycin type molecule markedly affect the antibacterial activity of the glycopeptide antibiotic. Accordingly, firstly the removal of the crucial *N*-terminal leucine, that is involved in the binding, completely destroys the antibacterial activity of the antibiotic. Secondly, the introduction of a new carboxylate moiety, by substituting the carboxamide moiety of asparagine of the vancomycin-type antibiotic to aspartic acid as in M43F, or rearrangement of the asparagine to isoaspartic acid as in CDP-1, reduces the antibacterial activity dramatically. These two examples show clearly that changes near the vancomycin *N*-terminus that hinder the binding of the acyl-D-ala-D-ala carboxy terminus of UDP-*N*-acetylmuramylpentapeptide drastically diminish the antibacterial activity of the vancomycin-type class of glycopeptide antibiotics.

Chemical modification of other functional groups of vancomycin-type class of antibiotics far removed from the binding site do not seem to alter the antibacterial activity greatly.

Two types of modifications seem to decidedly enhance the antibacterial activity and even impart better pharmacokinetic properties. First, the additional α-L-4-*epi*-vancosamine amino sugar on the benzylic hydroxyl group of the aromatic amino acid 6, enhances the antibacterial activity. Secondly, the alkylation of the amino group of amino sugars on aromatic amino acids 4 and 6, especially amino acid 4, enhances antibacterial activity and imparts better pharmacokinetic properties. More importantly, some *N*-alkyl derivatives show excellent activity against vancomycin-resistant enterococci (Tables 9 and 10). The three *N*-alkyl derivatives, mono-*N*-octyl, mono-*N*-*p*-chlorobenzyl, and mono-*N*-*p*-bromobenzyl A82846B derivatives (Table 10) incorporate the above two modifications that enhance antibacterial activity in these glycopeptide antibiotics, and represent the most active compounds.

Structure-activity relationships of antibiotics are influenced by several parameters, and vancomycin-type antibiotics are no exception. The above SAR analysis of the vancomycin-type glycopeptides is an attempt to rationalize the SAR based on one, but an important parameter, that is the mode of action.

Accordingly, it should be pointed out that although A82846A is five- to ten-fold more active than vancomycin against Gram-positive bacteria, its affinity to the model peptidoglycan diacetyl-L-lysyl-D-alanine-D-alanine is 23-fold lower[43].

Acknowledgments

I am pleased to acknowledge my colleagues N. E. ALLEN, D. R. BERRY, F. T. COUNTER, M. DEBONO, A. FELTY-DUCKWORTH, R. L. HAMILL, H. HIGGINS, Jr., M. M. HOEHN, A. H. HUNT, N. D. JONES, G. G. MARCONI, K. MERKEL, K. MICHEL, W. NAKATSUKASA, T. I. NICAS, J. L. OCCOLOWITZ, J. L. OTT, A. A. SCHABEL and R. C. YAO for collaboration on glycopeptide research and for helpful discussions.

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