# Binding of D-serine-terminating cell-wall analogues to glycopeptide antibiotics

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**Binding studies of –L-Lys-D-Ala-D-Ser-terminating ligands, used as model systems for cell-wall precursors found in VanC resistant bacteria, with vancomycin, chloroeremomycin and teicoplanin show that they bind to these glycopeptides in a similar manner as –L-Lys-D-Ala-D-Ala-terminating ligands, but that the binding constants are one to two orders of magnitude lower.**

The glycopeptide antibiotics vancomycin and teicoplanin are well-known for their theurapeutic use as antibiotics of last resort against methicillin-resistant *Staphylococcus aureus* (MRSA).[1] The antibacterial activity of the glycopeptides results from interference of the synthesis of the growing bacterial cell wall by binding to the precursor peptide sequence terminating in –L-Lys-D-Ala-D-Ala.[2,3] The molecular features responsible for the antibacterial activity of these antibiotics are well understood. Important features promoting activity include dimerisation of the antibiotics, in the cases of vancomycin and chloroeremomycin (also known as A82846B and LY264826), and the presence of a membrane anchor (a $C_{11}$ alkyl chain) in the case of teicoplanin.[2,3]

Recently there has been a rise in the number of cases of vancomycin-resistant enterococcal infections in hospitals.[4] In most cases this resistance is caused by the presence of peptidoglycan precursors terminating in –D-Ala-D-Lac rather than –D-Ala-D-Ala; so-called VanA and VanB resistance.[5] It has been demonstrated that the glycopeptides bind –D-Ala-D-Lac-terminating cell-wall analogues in a similar way[6] to the corresponding –D-Ala-D-Ala-terminating ones but considerably weaker.[6,7] A different form of vancomycin resistance seems to be specifically found in *E. gallinarum* and *E. casseliflavus*.[8–10] In these bacteria cell-wall precursors terminating in –D-Ala-D-Ser have been found.[11] Vancomycin shows reduced activity against these VanC resistant bacteria (MIC 2–32 μg ml$^{-1}$), whereas teicoplanin remains more or less active (MIC $\leqslant$ 0.5 μg ml$^{-1}$).[12,13]

Although binding constants for *N*-acetyl-D-Ala-D-Ser towards vancomycin and teicoplanin have been determined,[14] no evidence is provided that these ligands bind in a similar way to the –D-Ala- and –D-Lac-terminating peptides. Therefore, we decided to investigate the molecular details of the binding of –D-Ser-terminating cell-wall analogues using di-*N*-acetyl-L-Lys-D-Ala-D-Ser (synthesised in our laboratory by solid phase peptide synthesis). The tripeptide was used as we believe that this tripeptide is a better model system for the cell-wall precursors than the dipeptide. Her we report our initial results on the binding of –D-Ser-terminating peptides to vancomycin, chloroeremomycin and teicoplanin.

UV difference spectrophotometry was used to determine the binding constants of the different antibiotics to di-*N*-acetyl-L-Lys-D-Ala-D-Ser.‡ The results are summarised in Table 1.

It is clear that vancomycin and chloroeremomycin show reduced binding to the –D-Ser-terminating tripeptide compared to binding the –D-Ala-terminating cell-wall analogue. Significantly, vancomycin binds more weakly to the tripeptide terminating in –D-Ser relative to that terminating in –D-Ala by a factor of 20; this difference is larger than that between the corresponding dipeptides (a factor of 7 difference).[14] The decrease is, however, not as dramatic as that observed for the –D-Lac-terminating cell-wall analogue. This may explain the increased activity of vancomycin against bacteria showing VanC resistance compared to bacteria with VanA or VanB resistance. Furthermore, the results indicate that the difference in activity of vancomycin and teicoplanin against VanC resistant bacteria may be caused by the difference in binding strength to the –D-Ser-terminating peptide, which is about one order of magnitude greater for teicoplanin. In general, the results indicate that the introduction of a hydroxymethyl group, present on the serine residue, compared to the methyl group on the alanine residue does not result in as large a change in binding energy as to the change of the amide group to an ester group (as found in lactate-terminating peptides).

In complexes with ligands terminating in –D-Ala[15] and –D-Lac[6] a large downfield shift occurs in NMR spectra of the signal corresponding to the amide proton of residue 2 ($w_2$), due to the formation of a hydrogen bond between the ligand carboxylate and the amide proton. In case of the –D-Ser-terminating ligand the chemical shift of $w_2$ of chloroeremomycin moves from $\delta$ 8.48 (free antibiotic) to *ca.* 11.2 in the fully bound complex.§ This clearly indicates the formation of hydrogen bond from the amide hydrogen to the carboxylate of the D-Ser residue.

To gain evidence that not only was the carboxylate anion of the –D-Ser tripeptide bound in the manner typical of a cell-wall analogue to a glycopeptide antibiotic, but also that the whole peptide was anchored in the usual way, two-dimensional NOESY and TOCSY spectra were obtained.¶ The experiments establish that the serine sidechain is buried against ring 4, that the alanine methyl group points towards proton 7d (see Fig. 1), that the lysine sidechain is situated over ring 7 of the antibiotic, and that the lysine backbone Ac group is adjacent to the *epi*-vancosamine sugar attached to residue 6 (Table 2). A schematic representation of the inferred binding of the serine tripeptide to the glycopeptide antibiotics is represented in Fig. 1.

In conclusion, we have demonstrated that glycopeptides bind –D-Ala-D-Ser-terminating cell-wall analogues, found in bacteria with VanC resistance, in a similar way as –D-Ala-D-Ala- and –D-Ala-D-Lac-terminating cell-wall analogues. The binding strength of the –D-serine-terminating tripeptides is lower than the corresponding alanine ones, but not as low as the –D-lactate-terminating peptides, which can account for the less severe nature of the resistance observed in bacteria exhibiting VanC resistance.

**Table 1** Summary of binding constants ($K_{ass}$) to glycopeptide antibiotics with tripeptide cell-wall analogues, di-*N*-acetyl-L-Lys-D-Ala-X, determined by UV difference spectrophotometry

| | $K_{ass}$/dm$^3$ mol$^{-1}$ | | |
|---|---|---|---|
| Antibiotic | X = D-Ala | X = D-Lac | X = D-Ser |
| Vancomycin | $1.5 \times 10^6$ [a] | 410 [b] | $(7 \pm 2) \times 10^4$ |
| Chloroeremomycin | $1.3 \times 10^6$ [c] | 245,[d] $1.6 \times 10^3$ [b] | $(8 \pm 1) \times 10^4$ |
| Teicoplanin | $1.6 \times 10^6$ [e] | n.d.[f] | $(5 \pm 1) \times 10^5$ |

[a] Ref. 17. [b] Ref. 7. [c] Ref. 16. [d] Ref. 6. [e] Ref. 18. [f] n.d. = not determined.

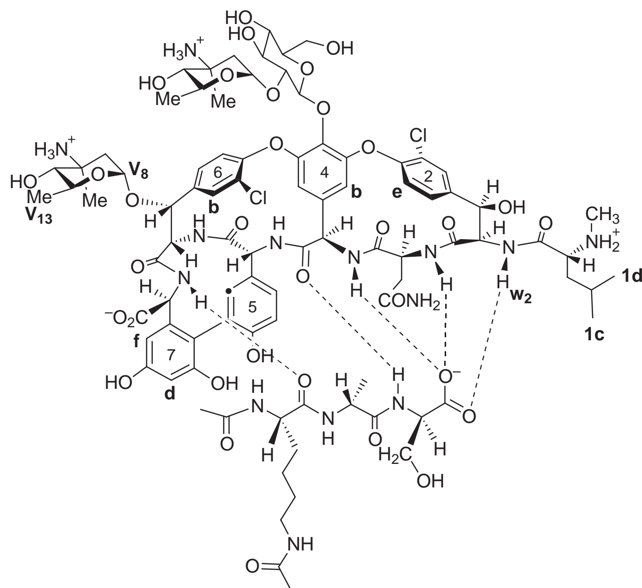


**Fig. 1** Exploded view of the binding of di-*N*-acetyl-L-Lys-D-Ala-D-Ser to chloroeremomycin. Dashed lines indicate hydrogen bonds.

**Table 2** Observed NOEs between di-*N*-acetyl-L-Lys-D-Ala-D-Ser and chloroeremomycin

| Assignment | δ (ppm) | Observed NOEs |
|---|---|---|
| D-Ser-α | 4.33, 4.45[a] | 1c, d |
| D-Ser-β | 2.53, 2.54[a] | 2e, 4b |
| | 3.38, 3.65[a] | 2e |
| D-Ala-α | 4.84 | 6b |
| D-Ala-β | 1.15 | 7d |
| Lys-α-Ac | 2.06 | 6b, $V_8$, $V_{13}$ |
| Lys-β | 1.66 | 7f |
| Lys-γ | 1.48 | 7f |

[a] Two chemical shifts observed due to the different environments in each half of the asymmetric antibiotic dimer.

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## Notes and References

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‡ UV spectrophotometry was carried out on a UVIKON 940 dual beam spectrophotometer. Both the reference and sample cells contained 50 μM antibiotic in 0.1 mol $dm^{-3}$ phosphate buffer (pH 4.5, 298 K). Aliquots of a ligand solution containing 50 μM antibiotic were added to the sample cell. The solution was stirred after each addition, and the absorbance at both *ca.* 240 and *ca.* 280 nm was measured repeatedly until stable. The data at *ca.* 240 nm were subtracted from those at *ca.* 280 nm and analysed as previously described (ref. 16).

§ The limiting chemical shift of $w_2$ for the vancomycin complex could not be determined due to low solubility of the complex.

¶ The spectra were obtained from samples containing chloroeremomycin (15 mmol $dm^{-3}$) and di-*N*-acetyl-L-Lys-D-Ala-D-Ser (15 mmol $dm^{-3}$) in $D_2O$ and 10% $D_2O$–$H_2O$ at 290 K (pH 4.5). The NOESY and TOCSY spectra were recorded on a Bruker DRX 500 spectrometer in phase-sensitive mode using Time Proportional Phase Increment (TPPI) to give quadrature detection in $f_1$. 2048 complex data points were recorded in $f_2$ and 512 real points in $f_1$ using mixing times of 100–150 ms. Zero filling was used to give a final transformed matrix of 2048 × 2048 real points.

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