Aggregation, binding, and dimerisation studies of a teicoplanin aglycone analogue (LY154989) †

Ben Bardsley, Rosa Zerella and Dudley H. Williams *

Cambridge Centre for Molecular Recognition, Department of Chemistry, Lensfield Road, Cambridge, UK CB2 1EW

Received (in Cambridge, UK) 12th September 2001, Accepted 9th January 2002
First published as an Advance Article on the web 4th February 2002

LY154989 is a vancomycin group antibiotic closely related in structure to teicoplanin aglycone. In view of the clinical importance of teicoplanin, the dimerisation, aggregation, and binding of bacterial cell wall analogues by LY154989 are of interest. These properties have been studied by proton NMR spectroscopy. LY154989 has been shown to form concentration-dependent aggregates in aqueous solution, similar to those of teicoplanin, even though it does not possess a C_{11} acyl chain, which was hitherto thought to be the cause of this aggregation. The aggregation can be disrupted by the addition of bacterial cell wall precursor analogues such as Ac_2-KDADA, Ac-DADA or Ac-DA. Thus, growing bacteria may disrupt aggregates of teicoplanin and LY154989. LY154989 dimerises weakly in aqueous solution and the dimerisation is weakly cooperative with ligand binding.

Introduction

LY154989 (also known as A47934) is a naturally-occurring member of the vancomycin group which was isolated, in 1986, from a strain of *Streptomyces toyocaensis* present in a soil sample collected from a low-tide area in the state of Washington, USA.[1,2] Initial studies showed that LY154989 behaved like a typical member of the vancomycin group, showing activity against a broad spectrum of Gram-positive bacteria, including *Staphylococcus aureus*.[1]

The structure of LY154989 [Fig. 1(a)], determined by Hunt *et al.*, is an aglycone broadly similar to that of teicoplanin aglycone [Fig. 1(b)].[3] Common features include cross-linked aromatic residues at positions 1 and 3, and chlorine atoms attached to residues 2 and 6. The structure of LY154989 is also similar to teicoplanin aglycone in that it lacks a hydroxy group at the β-position of residue 2. The majority of members of the vancomycin group so far characterised possess a β-hydroxy tyrosine residue at this position.[4] The structure differs from teicoplanin aglycone only in the presence of a chlorine attached to the aromatic ring of residue 5 and a sulfate ester attached to the aromatic ring of residue 1 (Fig. 1). When LY154989 was first discovered, the presence of a sulfate ester was unique among the vancomycin group members then characterised, but since then another naturally-occurring member of the group, UK-69542, has also been shown to possess such a functional group.[5] In the case of UK-69542, the sulfate ester is attached to residue 5, at the position normally occupied by free hydroxy functionality in other members of the vancomycin group. This sulfate group of UK-69542 was shown to cause a *cis–trans* isomerisation of the amide bond formed between residues 5 and 6 of the antibiotic, in DMSO solution, thus resulting in the antibiotic adopting two conformations.[5]

It was therefore decided to investigate LY154989 to ascertain any possible aggregation, and to study ligand binding and dimerisation.

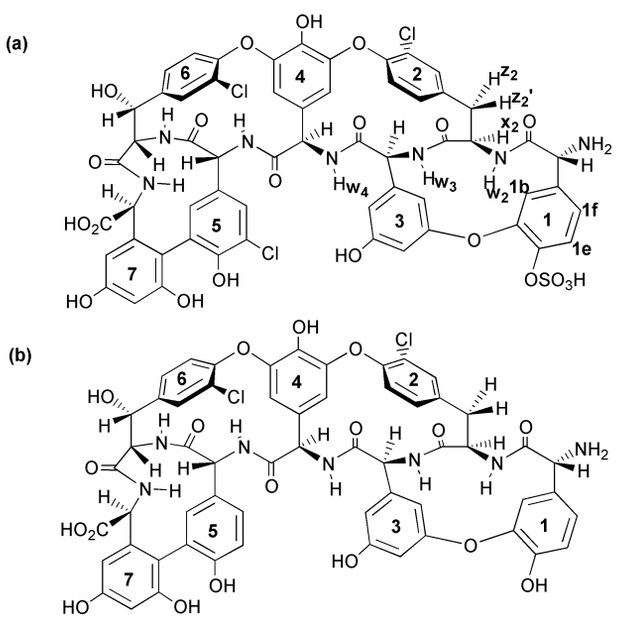

Fig. 1 Structures of (a) LY154989 and (b) teicoplanin aglycone. Protons of LY154989 referred to in the text are labelled.

Results and discussion

Assignment of the ^{1}H NMR spectrum in DMSO-d_6 and H_2O–CD_3CN

Assignment of LY154989 in DMSO-d_6. Prior to undertaking any structural studies on LY154989, its ^{1}H NMR spectrum was fully assigned in DMSO-d_6 and H_2O–CD_3CN solutions. DMSO-d_6 provides an environment where dimerisation (if this is possible) is suppressed, and therefore solely monomeric antibiotic is present. This effect of DMSO-d_6 results in greatly simplified spectra for many vancomycin group antibiotics, as only one set of antibiotic peaks is present in the spectrum.

Fig. 2 shows a 1-D NMR spectrum of a 20 mM sample of LY154989 in DMSO-d_6. The proton resonances were assigned through the use of 2-D NOESY and DQF-COSY experiments. The residue 2 β-protons z_2 and z_2' were used as a starting point, since these two signals are at the highest field in the spectrum and give rise to a very strong DQF-COSY cross peak between them. From this starting point, it was possible to assign all the

† Electronic supplementary information (ESI) available: tables of ^{1}H NMR assignments. See http://www.rsc.org/suppdata/p2/b1/b108273f/

 DOI: 10.1039/b108273f

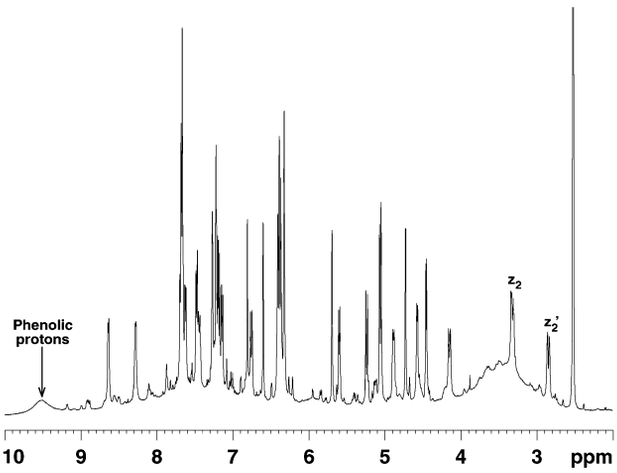

Fig. 2 1-D ^{1}H NMR spectrum of LY154989 in DMSO-d_6 at 300 K.

proton resonances, except for w_1 which was not observed, and the four phenolic protons which were only visible as a broad hump between 9.3 and 9.7 ppm (Fig. 2). Fig. 3 shows a portion

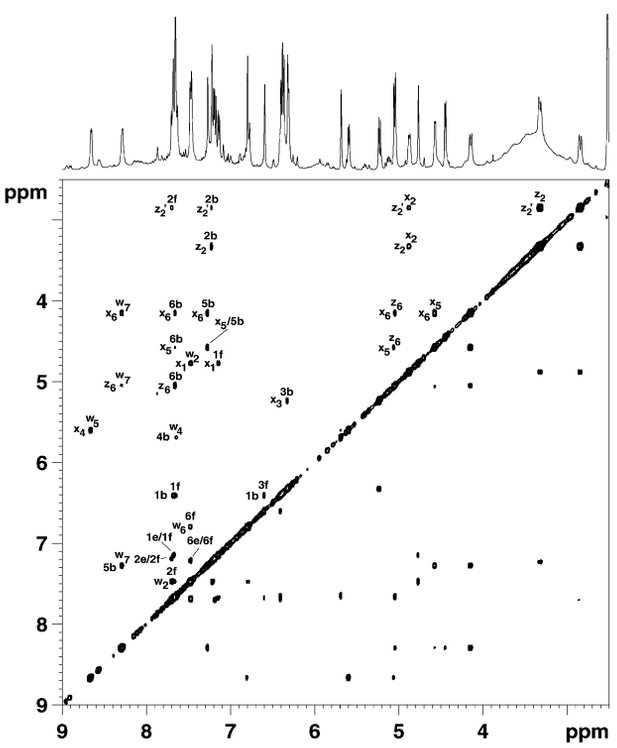

Fig. 3 Portion of the 120 ms 2-D NOESY spectrum, and the corresponding region of the 1-D spectrum, of LY154989 in DMSO-d_6 at 300 K. Cross peak assignments are labelled.

of the NOESY spectrum and some of the cross peak assignments. In the assignments, z_2 is defined as the proton in the same position as z_2 in other members of the vancomycin group, whilst z_2' is the proton in the position normally occupied by the β-hydroxy group. It was possible to distinguish between z_2 and z_2' on the basis of their coupling constants to x_2. Due to the relative rigidity of the three-dimensional structure, there is a relatively fixed geometric relationship between x_2, z_2 and z_2'. The coupling constants measured from x_2 to the two β-protons were 6.1 Hz to the proton at 3.33 ppm and <2 Hz (the coupling was not resolved) to the proton at 2.85 ppm. The approximate dihedral angles between x_2/z_2 and x_2/z_2' are 40° and 70°, respectively. Therefore, by comparison with the coupling constants predicted for such angles from the Karplus relationship, the proton at 3.33 ppm must be z_2 and the proton at 2.85 ppm due to z_2'.[6]

A marked feature of this spectrum (Fig. 2) is that, although the spectrum is well resolved, there are a large number of low intensity peaks present. HPLC analysis of LY154989 showed no significant impurities. Therefore, a possible origin for these peaks is that LY154989, like UK-69542, adopts two conformations in DMSO-d_6 solution, thus giving rise to two sets of resonances. The sulfate ester group of LY154989 is not in the same position as it is on UK-69542[5] (residue 1 as opposed to residue 5). Therefore, it is unlikely that a second conformation of LY154989 would arise from the same conformational change as that seen in UK-69542, *i.e.*, the *cis–trans* isomerisation of the residue 5–6 peptide bond. Due to the low intensity of the second set of peaks, we have been unable to characterise this putative second conformation. The assignment of the proton spectrum of LY154989 in DMSO-d_6 is given in the electronic supplementary information (ESI).

Assignment of LY154989 in H_2O–CD_3CN. An assignment of the ^{1}H NMR spectrum of LY154989 was also attempted in a solution of 9 : 1 H_2O–D_2O. Therefore, initially, 1 mM and 10 mM solutions of LY154989 were prepared at pH 7.0. However, the 1-D ^{1}H NMR spectra of these solutions at 300 K showed line broadening, particularly for the more concentrated sample (Fig. 4). Similar broadening has been observed previously for a

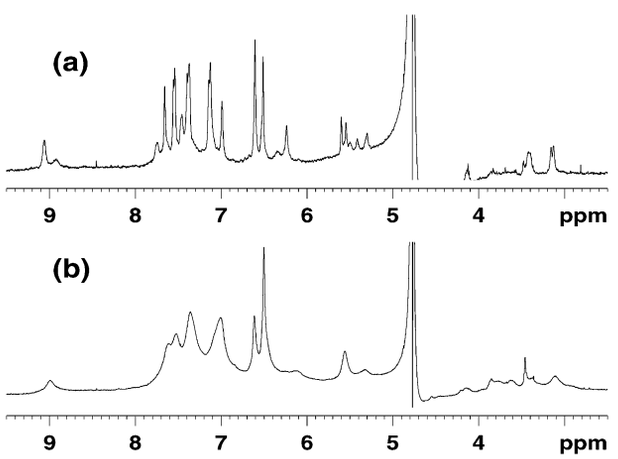

Fig. 4 1-D ^{1}H NMR spectra of LY154989 in 9 : 1 H_2O–D_2O at pH 4.5, 300 K at concentrations of (a) 1 mM and (b) 10 mM. The line broadening in (b) is caused by the formation of large, non-specific aggregates of LY154989.

solution of teicoplanin under similar conditions of pH and temperature.[7] This line broadening was attributed to the aggregation of the antibiotic in solution, resulting in longer correlation times for the individual molecules and resultant broad lines.[7] In the case of teicoplanin, its C_{11} acyl chain was postulated to be involved in this aggregation. Since this structural feature is not present on LY154989, it seems likely that the aggregation is caused simply by the non-specific association of regions of hydrophobic surface area. As the antibiotic concentration increases, so does the size of the aggregates and the extent of aggregation, and consequently, line broadening is more severe at higher concentrations.

It has been shown that the ^{1}H NMR spectra of antibiotics that form aggregates can be improved by the addition of a less polar solvent, such as acetonitrile-d_3 (CD_3CN) to the solution.[7] Therefore, a titration was carried out in which a solution of CD_3CN was titrated into an initial 10 mM solution of LY154989 in 9 : 1 H_2O–D_2O and 1-D spectra were recorded with different proportions of CD_3CN present. Fig. 5 shows the spectra obtained with increasing CD_3CN concentrations. It can be seen that the spectra sharpen considerably with increasing

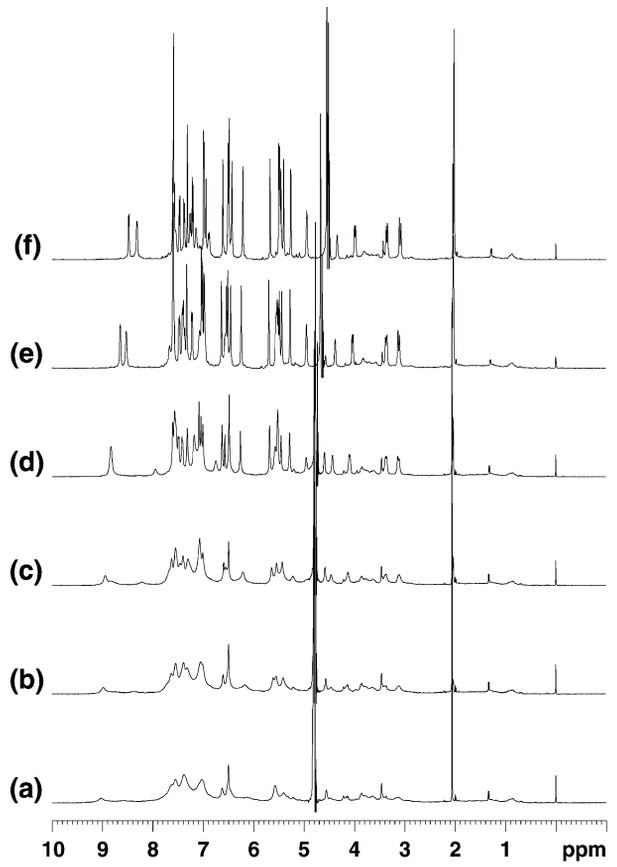

Fig. 5 1-D ^{1}H NMR spectra of a solution of LY154989 (initially 10 mM) in 9 : 1 H_2O–D_2O at pH 4.5, 300 K, recorded with increasing concentrations of added CD_3CN. The percentage of CD_3CN present (v/v) in each solution is (a) 0, (b) 1, (c) 2, (d) 5, (e) 15 and (f) 25%.

CD_3CN concentration, as the less polar solvent reduces the energetic benefit of forming hydrophobic aggregates. It can also be seen from Fig. 5 that, where the CD_3CN content is greatest, the peaks in the spectrum, while becoming narrower do not move to different chemical shift values (except for the amide protons at >8 ppm). Therefore, the ^{1}H NMR assignment of LY154989 was made from a solution of 10 mM LY154989 in 3 : 1 H_2O–CD_3CN. Later NMR experiments investigating dimerisation only employed a CD_3CN content of 10% v/v, in order to maximise the aqueous content of the solution and thus maintain conditions as close to those of physiological relevance as possible.

An almost complete ^{1}H NMR assignment of LY154989 as a 10 mM solution in 3 : 1 H_2O–CD_3CN at pH 4.5 and 298 K was possible. All the protons were assignable by analysis of 2-D NOESY and DQF-COSY experiments except for w_1 and the four phenolic protons, which were presumably exchanging rapidly with solvent protons on the NMR time-scale. As with the assignment in DMSO-d_6, it was possible to distinguish between z_2 and z_2' on the basis of the differing coupling constants of the resonances at 3.36 and 3.10 ppm to the x_2 resonance. As was expected, the z_2 resonance was the peak at 3.36 ppm, further downfield than z_2', at 3.10 ppm, similarly to the case in DMSO-d_6.

The full assignment of LY154989 in 3 : 1 H_2O–CD_3CN is given in the ESI.

Assignment of the LY154989–Ac_2-KDADA complex in H_2O

The ^{1}H NMR spectrum of LY154989 was also assigned when the antibiotic was bound to the bacterial cell wall analogue Ac_2-KDADA in aqueous solution. In this case, a solution of 10 mM LY154989 and 10 mM Ac_2-KDADA at pH 4.5 in 9 : 1 H_2O–D_2O was used [at these concentrations, >99% of the antibiotic is bound by ligand, based on the binding constant of (9.9 ± 1.5) × 10^5 M^{-1} shown in Table 1]. The 1-D ^{1}H NMR spectrum of this solution at 300 K gave sharp resonances and was almost identical to that obtained with 10% CD_3CN present (Fig. 6).

Table 1 Binding constants of cell wall precursor analogues to LY154989, and limiting chemical shifts of w_2 of LY154989 when complexed with the same precursor analogues, measured at pH 4.5, 300 K

Ligand	K_{bind}/M^{-1}	$\delta_{w_2}^{lim}$ (ppm)
Ac_2-KDADA	(9.9 ± 1.5) × 10^5	12.29
Ac-DADA	(5.2 ± 0.8) × 10^4	12.27
Ac-DA	(2.3 ± 0.3) × 10^3	11.77
Acetate	—[a]	9.80
Free	0.00	7.55

[a] The binding constant to acetate was not measured.

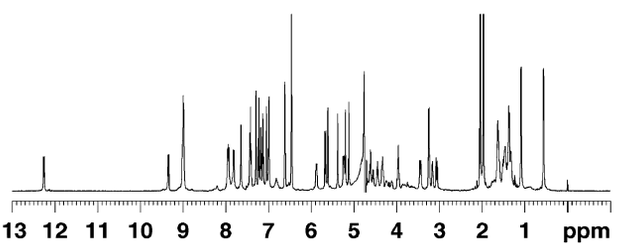

Fig. 6 1-D ^{1}H NMR spectra of the complex of LY154989 (10 mM) with Ac_2-KDADA (10 mM) in 9 : 1 H_2O–CD_3CN at pH 4.5, 300 K using presaturation to remove the water resonance.

Thus, unlike the case with the 10 mM solution of LY154989 with no ligand, the spectrum is sharp even without the addition of CD_3CN. This, as with the addition of CD_3CN to the initial solution, is similar to the situation observed for teicoplanin, where it was shown that the titration of a solution of a bacterial cell wall precursor analogue into a solution of the free antibiotic in 9 : 1 H_2O–D_2O resulted in the sharpening of the lines in the spectrum.[7,8] For teicoplanin, this was postulated to be due to a change in the relative populations of the two conformers formed by this antibiotic in solution: specifically, with a reduction in the population of the conformer associated with micelle formation and a concomitant increase in the population of the conformer associated with ligand binding.[8] In the case of LY154989, however, no such population of two conformers due to rotation of the ring 4 sugars is possible as it is an aglycone. It is thus likely that, in the cases of both LY154989 and teicoplanin, the binding of the ligand simply disrupts the aggregates formed by the antibiotics, rather than actually promoting a change from one conformer to another.

The ^{1}H NMR assignment of the LY154989–Ac_2-KDADA complex was again achieved using 2-D NOESY and DQF-COSY experiments. Assignments were made of all antibiotic and ligand protons except for w_1, the four phenolic protons, and 6e and 6f. The lack of observable resonances for 6e and 6f in the spectrum is significant. The resonances of these protons have been shown to undergo large upfield shifts (>2 ppm for 6e) in antibiotic dimers, relative to the monomers, due to the location of these protons over ring 4 of the antibiotic in the other half of the dimer.[9] It is therefore likely that LY154989 is dimerising in solution and that the exchange rate between the monomeric and dimeric forms of the antibiotic is approximately the same as the chemical shift differences between their monomeric and dimeric resonances. As such, these protons are in intermediate exchange between the monomeric and dimeric forms, and the resultant resonances are extremely broad, and thus not visible in either 1-D or 2-D spectra.

The full assignment for the LY154989–Ac_2-KDADA complex in 9 : 1 H_2O–D_2O is given in the ESI.

Ligand binding and dimerisation of LY154989

Ligand binding studies. Binding constants of LY154989 to the ligands Ac_2-KDADA, Ac-DADA and Ac-DA were measured by UV difference spectrophotometry at 300 K and in 100% H_2O. In addition, the limiting chemical shift of w_2 of LY154989, when bound to the above ligands (and also to acetate), was determined by ^1H NMR spectroscopy in 9 : 1 H_2O–D_2O and also with CD_3CN present.

1-D spectra of these complexes were acquired and the w_2 resonance assignments confirmed from 2-D NOESY spectra. Fig. 7 shows the regions of the spectra of LY154989 containing

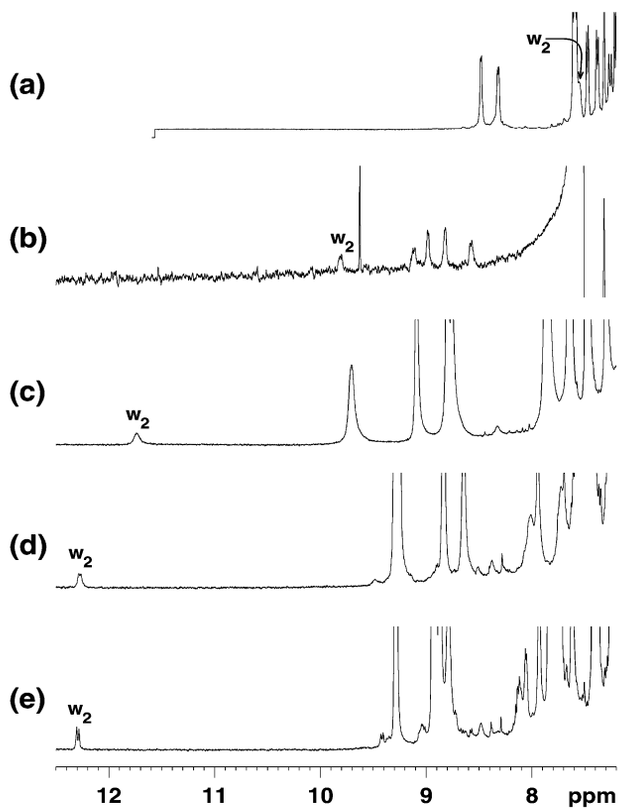

Fig. 7 1-D ^1H NMR spectra of 10 mM LY154989 when (a) free and when fully bound to (b) acetate, (c) Ac-DA, (d) Ac-DADA and (e) Ac_2-KDADA with the position of the w_2 resonance in each spectrum indicated. All solutions are at pH 4.5 and 300 K. The solutions used for (a), (d) and (e) are in 3 : 1 H_2O–CD_3CN, whilst those for (b) and (c) are in 9 : 1 H_2O–D_2O. Also, due to solubility problems, solution (b) contains only 1 mM LY154989. In all cases, the amount of ligand added is enough to ensure that the antibiotic is >90% bound by ligand.

w_2, with each of the above ligands bound and when free. For the binding of Ac_2-KDADA, Ac-DADA and Ac-DA, the w_2 resonance of the antibiotic was shifted far downfield due to hydrogen bonding to the ligand. Experiments with CD_3CN added showed that the spectra obtained were slightly sharper than those without CD_3CN, but that the limiting w_2 chemical shifts were approximately the same. For example, for the binding of Ac-DA, the limiting chemical shift of w_2 was 11.74 ppm in the absence of CD_3CN and 11.77 ppm in its presence.

Table 1 summarises the results from the above experiments showing binding constants and limiting w_2 chemical shifts for the four ligands binding to LY154989. Also included in Table 1 is the chemical shift of w_2 with no ligand present. As has been done previously for other antibiotics, the limiting chemical shifts of w_2 when bound to these different ligands were plotted against the free energy of binding of the ligands to LY154989 (Fig. 8).[10] The most noticeable feature of Table 1 and Fig. 8 is the exceptionally large downfield shift of w_2 when binding to Ac_2-KDADA, Ac-DADA and Ac-DA. In the case of Ac_2-KDADA binding to LY154989, this downfield shift of w_2 is >4.7

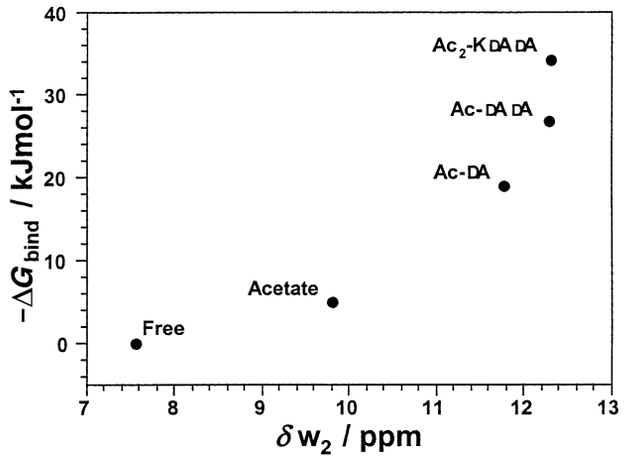

Fig. 8 Free energy of binding *vs.* limiting chemical shift of w_2 for a variety of bacterial cell wall precursor analogues binding to LY154989 at pH 4.5, 300 K. The free energy of binding of acetate to LY154989 was estimated as being similar to values measured previously for other antibiotics.

ppm. Previously, no limiting w_2 chemical shifts of >12 ppm have been observed, in aqueous solution, for the binding of any antibiotic to any ligand. LY154989, however, which binds ligands with similar binding constants to those of, for example, teicoplanin (the binding constant of teicoplanin to Ac_2-KDADA is 1.3×10^6 M^{-1}), induces greater downfield shifts even than those antibiotics which bind the ligands more strongly.[11]

With respect to the ligand binding constants to LY154989, Table 1 shows that the binding constant of LY154989 to Ac_2-KDADA of $(9.9 \pm 1.5) \times 10^5$ M^{-1} is approximately the same as the binding constant of Ac_2-KDADA to teicoplanin (1.3×10^6 M^{-1}). This similarity suggests that the sugars of teicoplanin play a relatively insignificant role in ligand binding. Binding constants of teicoplanin to Ac-DADA and Ac-DA have also been measured previously and they are 1.9×10^5 and 2.8×10^3 M^{-1}, respectively.[12] Both of these are only slightly greater than the corresponding values for binding to LY154989, confirming that the sugars of teicoplanin do not appear to exert a significant influence on ligand binding.

Dimerisation and cooperativity. Dimerisation constants were measured for LY154989, both in the absence and presence of ligand. Teicoplanin aglycone has also been investigated in this way, but no evidence of dimerisation was found.[7] Thus, it was expected that if LY154989 did indeed dimerise, then the dimerisation constant would be small.

Due to the expected weak dimerisation of LY154989, it was probable that the antibiotic dimers would exchange rapidly on the NMR time-scale resulting in spectra containing time-averaged population-weighted resonances. This was indeed the case, and dimerisation constants were therefore determined by following the chemical shift of the proton x_4 (the α-CH proton of residue 4) as a function of concentration, by titration of a concentrated solution of antibiotic into an NMR tube. This proton was used for the measurement as it was expected, on the basis of observations with other antibiotics, to suffer the greatest downfield shift upon dimerisation. All the experiments were performed in 9 : 1 D_2O–CD_3CN at pD 7.0, 300 K. The CD_3CN was present in order to sharpen up the spectra, in particular those of the antibiotic in the absence of ligand, but was kept to a low enough concentration that it was not anticipated to significantly affect the dimerisation constant. (Previously it has been demonstrated that a high concentration of CD_3CN increases the proportion of antibiotic dimer present in a solution, compared to the situation in a solution where no CD_3CN is present.)[13] For the determination of the dimeris-

ation constant in the presence of ligand, the ligand used was Ac-DADA and the concentration of ligand was such that, at all times during the titration, the antibiotic was >95% bound by ligand based on the binding constant shown in Table 1. Fig. 9

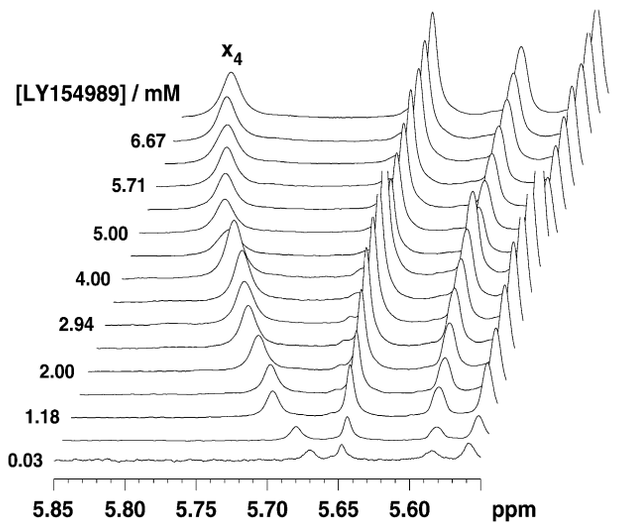

Fig. 9 Portions of the 1-D ^{1}H NMR spectra of the LY154989–Ac-DADA complex in 9 : 1 D_2O–CD_3CN, pD 7.0, 300 K, at various concentrations of antibiotic. The downfield movement of x_4 with increasing concentration of LY154989 can be seen. This downfield movement is due to the increasing proportion of dimer present at higher concentrations.

shows the downfield movement of x_4 in 1-D spectra recorded during the titration of LY154989, fully-bound to Ac-DADA, into an NMR tube.

The measured dimerisation constants were 20 M^{-1} for free LY154989, and 36 M^{-1} for LY154989 when fully bound by Ac-DADA. As expected, LY154989 dimerises weakly in solution, the value of 20 M^{-1} being the lowest recorded dimerisation constant for any member of the vancomycin group so far studied. The value in the presence of Ac-DADA is only slightly higher than the value for free antibiotic and thus the cooperativity expressed between dimerisation and ligand binding for LY154989 is low. This is not surprising given the lack of functional groups, such as sugars, which might provide cooperative enhancements. The fact that LY154989 dimerises at all, however, shows that the reason that teicoplanin cannot dimerise in solution is likely to be due to the nature of its sugar residues, since teicoplanin aglycone, which does not possess the sugar residues, is structurally almost identical to LY154989 (Fig. 1). In particular, the removal of the residue 6 sugar, and subsequent addition of a diamine to the C-terminus of teicoplanin, have been shown to result in compounds that do dimerise.[14–16]

Conclusions

LY154989 is an interesting antibiotic because of the presence of a sulfate ester functional group attached to ring 1 of its structure. A possible evolutionary role of this sulfate ester group is to increase the solubility of the antibiotic. This is because the aglycone portions of vancomycin group members are relatively insoluble compared to their glycosylated counterparts. Comparison of the solubilities of LY154989 and teicoplanin aglycone (which differ only by the presence of the sulfate ester group and a chlorine atom on LY154989) showed that LY154989 was considerably more soluble (by a factor of >20). This fact makes LY154989 a much more suitable antibiotic than teicoplanin aglycone for NMR studies, where high antibiotic concentrations (>10 mM) are desirable. The sulfate ester also affects the limiting chemical shift of the antibiotic proton w_2 when ligand is bound to the antibiotic. The downfield shift normally suffered by this proton upon complexation is reinforced, resulting in changes to w_2 of >4.7 ppm upon complexation with Ac-DADA and Ac_2-KDADA.

LY154989 has also been shown to form concentration-dependent aggregates in aqueous solution, similar to those of teicoplanin, even though it does not possess a C_{11} acyl chain, which was thought to be the cause of this aggregation. It is thus likely that the aggregation of LY154989 (and also teicoplanin) is simply due to non-specific burial of hydrophobic regions of the molecule, which may occur because the dimerisation constant is so low. The aggregation can be disrupted, however, by the addition of a less polar solvent, such as acetonitrile, or by the addition of a bacterial cell wall precursor analogue such as Ac_2-KDADA, Ac-DADA or Ac-DA.

Binding constants of these cell wall precursor analogues have been measured, and shown to be similar to the corresponding values for their binding to teicoplanin. Also, as with other antibiotics, the limiting chemical shift of w_2 when bound to these different ligands has been shown to correlate with the free energy of binding of the ligands. In the case of LY154989, due to the presence of the sulfate ester group, these limiting downfield shifts of w_2 are larger than have been observed for other antibiotics.

Finally, it has been shown that LY154989 dimerises weakly in aqueous solution and that dimerisation is weakly cooperative with ligand binding. The weak dimerisation behaviour of this antibiotic is consistent with the behaviour of other vancomcyin group antibiotics that carry aromatic amino acids at positions 1 and 3 of the peptide backbone (*e.g.*, teicoplanin and ristocetin A), which either dimerise weakly, or not at all.

Experimental

LY154989 was donated by Eli Lilly and Co. (Indianapolis, USA) and used without further purification.

^{1}H NMR spectroscopy

Samples were prepared by dissolution in DMSO-d_6 and aqueous solutions. CD_3CN was added to aqueous solutions in order to prevent aggregation of the antibiotic. For samples in H_2O–D_2O or D_2O, the pH was adjusted to the correct pH with NaOD or DCl using a Corning pH meter equipped with a combination glass electrode, and no corrections were made for isotope effects. Spectra were recorded at 300 K on Varian Unity 500 and 600 spectrometers. Trimethylsilyl propionate (TSP) was used as a reference for spectra in aqueous solutions, while spectra in DMSO-d_6 were referenced to the residual protio-solvent peak of DMSO-d_6. A standard WATERGATE pulse sequence[17] was used to suppress the water signal where necessary. Dimerisation constants were determined in 9 : 1 D_2O–CD_3CN at pD 7.0 and 300 K. After processing of the spectra, the chemical shift of the x_4 proton was plotted against the total concentration of the antibiotic for a range of antibiotic concentrations. Dimerisation constants were calculated by curve-fitting of the plotted data to the theoretical equations for a dimeric association using Kaleidagraph version 3.0.5 (Abelbeck Software).

UV difference spectrophotometry

Experiments were performed using a dual beam Uvikon 940 Spectrophotometer equipped with a thermocirculator maintaining a constant temperature of 300 K. To each cell were added 2.5 ml of a 50 μM solution of antibiotics in a buffer of sodium dihydrogen phosphate (0.1 M) at pH 4.5. A solution of ligand was then added to the front cell, the amount of which was calculated to give more than 90% bound antibiotic, based on the estimated binding constant for the association. Difference spectra were then taken. The association constants were

determined by curve-fitting a plot of change in absorbance *vs.* ligand concentration using Kaleidagraph version 3.0.5 (Abelbeck Software). Determinations were carried out in triplicate, the average result being quoted.

Acknowledgements

We thank Eli Lilly for the provision of a sample of LY154989, and EPSRC and BBSRC for financial support. The MRC NMR Centre at the National Institute for Drug Research (Mill Hill, London) is thanked for the provision of NMR facilities.

References

1 L. D. Boeck and F. P. Mertz, *J. Antibiot.*, 1986, **39**, 1533.
2 R. L. Hamill, L. D. Boeck, R. E. Kastner and R. M. Gale, *Program and Abstracts of 23rd Interscience Conf., Antimicrob. Agents Chemother.*, Las Vegas, 1983, 164.
3 A. H. Hunt, J. L. Occolowitz, M. Debono, R. M. Molloy and G. M. Maciak, *Program and Abstracts of 23rd Interscience Conf., Antimicrob. Agents Chemother.*, Las Vegas, 1983, 164.
4 B. Cavalleri and F. Parenti, in *Glycopeptides (dalbaheptides)*, ed. J. Wiley and Sons, Inc., New York, 1992, vol. 2, p. 995.
5 N. J. Skelton, D. H. Williams, M. J. Rance and J. C. Ruddock, *J. Am. Chem. Soc.*, 1991, **113**, 3757.
6 M. Karplus, *J. Am. Chem. Soc.*, 1963, **85**, 2870.
7 U. Gerhard, PhD Thesis, University of Cambridge, 1992.
8 M. S. Westwell, U. Gerhard and D. H. Williams, *J. Antibiot.*, 1995, **48**, 1292.
9 J. P. Waltho and D. H. Williams, *J. Am. Chem. Soc*, 1989, **111**, 2475.
10 M. S. Searle, G. J. Sharman, P. Groves, B. Benhamu, D. A. Beauregard, M. S. Westwell, R. J. Dancer, A. J. Maguire, A. C. Try and D. H. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2781.
11 A. Malabarba, F. Spreafico, P. Ferrari, J. Kettenring, P. Strazzolini, G. Tarzia, R. Pallanza, M. Berti and B. Cavalleri, *J. Antibiot.*, 1989, **42**, 1684.
12 M. S. Westwell, PhD Thesis, University of Cambridge, 1995.
13 P. Groves, M. S. Searle, J. P. Waltho and D. H. Williams, *J. Am. Chem. Soc.*, 1995, **117**, 7958.
14 A. Malabarba, R. Ciabatti, R. Scotti, B. P. Goldstein, P. Ferrari, M. Kurz, B. P. Andreini and M. Denaro, *J. Antibiot.*, 1995, **48**, 869.
15 A. Malabarba, R. Ciabatti, J. Kettenring, P. Ferrari, R. Scotti, B. P. Goldstein and M. Denaro, *J. Antibiot.*, 1994, **47**, 1493.
16 G. J. Sharman and D. H. Williams, *Chem. Commun.*, 1997, 723.
17 M. Piotto, V. Saudek and V. Sklenar, *J. Biomol. NMR*, 1992, **2**, 661.