The synthesis and binding of *N*-terminal derivatives of vancomycin to a bacterial cell wall analogue

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We report the synthesis of novel derivatives of the glycopeptide antibiotic vancomycin, modified at the *N*-terminus. Binding constants were measured for the association of these derivatives with the tripeptide analogue *N*,*N*-diacetyl-L-lysyl-D-alanyl-D-alanine. Replacement of the sp^3 centre of the leucine residue of vancomycin with an sp^2 centre resulted in weaker binding in all cases. These findings contrast with the relatively strong binding of some of the analogous derivatives previously obtained from ristocetin A. The reduction in the binding affinities of the vancomycin derivatives is attributed to a conformational change in the antibiotic which is not possible in the analogous derivatives of the aglycone of ristocetin.

Introduction

Vancomycin is currently the antibiotic of last resort against multiply-resistant, pathogenic, Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA). The last ten years has seen the emergence of bacterial resistance even to vancomycin[1,2] and the search for new and more potent glycopeptide derivatives has intensified accordingly. Members of the vancomycin group of antibiotics function by inhibiting the biosynthesis of bacterial cell wall components in susceptible Gram-positive bacteria. These antibiotics block the approach of the enzymes involved in either the trans-glycosylation or trans-peptidation steps of peptidoglycan synthesis or in some cases, both. This arises because of strong binding between the antibiotic and pentapeptide precursors of the cell wall which terminate in the sequence L-lysyl-D-alanyl-D-alanine.[3,4] The inhibition of cross-linking results in loss of mechanical strength in the cell wall and leads to bacterial death by osmotic shock. ^{1}H NMR NOESY experiments have shown[5,6] that glycopeptides bind to tripeptide mimics ('ligands') such as *N*,*N*-diacetyl-L-lysyl-D-alanyl-D-alanine (Ac_2L-Lys-D-Ala-D-Ala) through a co-operative array of hydrogen bonds and hydrophobic interactions (Fig. 1).

Resistant bacteria synthesise cell wall precursors terminating in the depsipeptide sequence L-lysyl-D-alanyl-D-lactate, a modification which reduces the binding affinity (and hence potency) of clinically-used antibiotics, by a factor of *ca.* 1000.[7,8] To gain insight into the subtle factors dictating the favourable free energy of the antibiotic–ligand association, we focused on the 'binding pocket' of the antibiotic, a cavity lined with hydrophobic residues which contains three antibiotic amide protons that form hydrogen bonds with the carboxylate anion of the ligand (Fig. 1). Specifically, we have modified an area adjacent to the binding pocket (Fig. 1) by replacing the amino group of the leucine residue of *N*-demethylvancomycin,[9,10] a vancomycin analogue with convenient functionality for further modification.

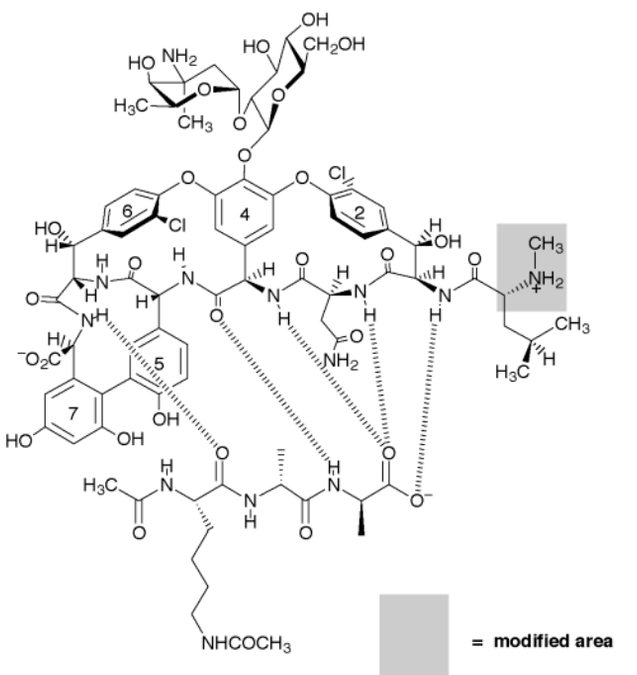

Fig. 1 Exploded view of the complex formed between Ac_2L-Lys-D-Ala-D-Ala and vancomycin.

Results and discussion

Synthesis

Derivatives of vancomycin (see Fig. 1) were synthesised by methods analogous to those used by Herrin and co-workers[11] to obtain derivatives of the aglycone of the glycopeptide ristocetin (Fig. 2). All reactions were carried out overnight under ambient conditions, and without the use of protecting groups (Fig. 3). Reaction of *N*-demethylvancomycin (**1**) with glyoxylic acid[12] in acetate buffer at pH 5 yielded the ketone (**2**) which was the starting material for the synthesis of the remaining derivatives. *N*-Demethylvancomycin (**1**) was chosen as the initial substrate since vancomycin itself is not susceptible to such a functional group interconversion because an intermediate imine cannot form.

Reduction of the ketone (**2**) with sodium cyanoborohydride at 0 °C afforded the alcohol (**3**). ^{1}H NMR NOESY revealed a strong cross peak between w_2 and the proton of the OH moiety, and a weaker one between the protons x_1 and w_2 (Fig. 4). However a secure assignment of the stereochemistry of the alcohol could not be made. Condensation of the ketone with malononitrile in methanol in the presence of piperidine afforded the

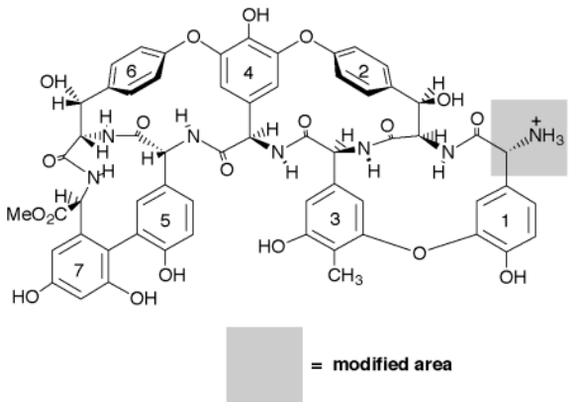

Fig. 2 The structure of ristocetin aglycone.

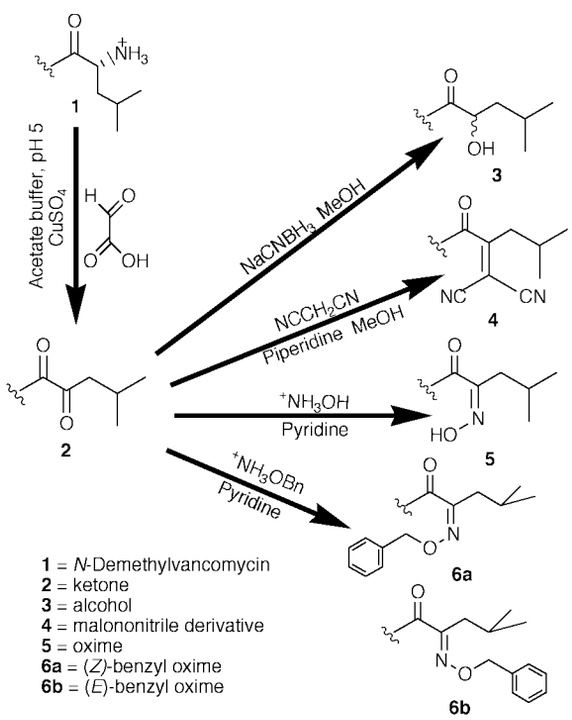

Fig. 3 Scheme for the synthesis of derivatives of *N*-demethylvancomycin.

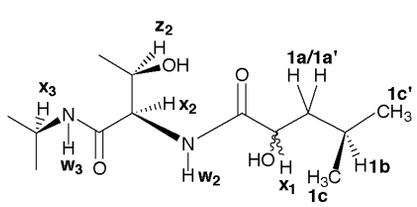

Fig. 4 The assignment of protons discussed in the text.

malononitrile derivative (**4**). The presence of the malononitrile moiety of **4** was seen to affect the chemical shifts of several protons on the peptide backbone (Table 1). In comparison to the values found in the other derivatives, the malononitrile displayed an upfield shift of $\delta \approx 0.35$ ppm for the proton x_3, and downfield shifts of $\delta \approx 0.70$ ppm and at least $\delta \approx 0.60$ ppm for the protons x_2 and w_2, respectively. These effects are attributed to the close proximity of these protons to the malononitrile moiety and are the result of shielding and deshielding by the conjugated π-system of the malononitrile.

The analogous reactions with both cyanoacetamide and malondiamide did not proceed, possibly due to the poorer reactivity or increased bulk of these reagents.

Oximes (**5**, **6a** and **6b**) were obtained by treatment of the ketone (**2**) with the corresponding hydroxylamine and benzyloxyamine hydrochlorides in pyridine. HPLC analysis showed that the oxime (**5**) was formed as a mixture of isomers (ratio *Z* to *E*; 4 : 1). Full conversion of the *E* to the thermodynamically favoured *Z* isomer was achieved after evaporation and stirring of the residue in methanol for 2 h. The benzyloximes (**6a**, **6b**) were formed as a mixture of isomers (ratio 3 : 2), as determined by reversed phase HPLC. A ^{1}H NMR NOESY recorded cross peaks between two of the benzyl protons and the protons x_2 and z_2 of the peptide backbone.

Table 1 Comparison of the chemical shifts of x_2, x_3, and w_2 in the derivatives **2**–**5** and **6b**

Compound	x_2	x_3	w_2
2	4.86	4.40	7.90
3	4.74	4.43	7.61
4	5.60	4.06	8.53
5	4.87	4.52	7.46
6b	4.81	4.50	7.25

Table 2 Binding constants for the association of Ac_2L-Lys-D-Ala-D-Ala with glycopeptides, as measured by UV difference spectrophotometry

Glycopeptide	K_{lig}/M^{-1} (*N*,*N*)Ac_2KDADA
Vancomycin	$8 \pm 2 \times 10^5$
N-Demethylvancomycin	$1.7 \pm 0.7 \times 10^6$
Ristocetin aglycone [11]	6.7×10^5
Ristocetin ketone [11]	5.6×10^5
2	$4.2 \pm 0.3 \times 10^3$
3	$1.6 \pm 0.1 \times 10^4$
4	Negligible
5	$1.0 \pm 0.6 \times 10^4$
6a, **6b**	$2.0 \pm 0.4 \times 10^4$

The binding of the cell wall precursor analogue Ac_2L-Lys-D-Ala-D-Ala to vancomycin (Fig. 1), *N*-demethylvancomycin (**1**) and the newly synthesised derivatives **2**–**6** was measured by UV difference spectrophotometry (Table 2). Ligand affinities for vancomycin and *N*-demethylvancomycin were, within experimental error, in agreement with previous studies.[7,10,13] All of the new derivatives displayed binding constants (K_{lig}) of the order of 10^4 M^{-1}, corresponding to significantly reduced binding.

The replacement of the sp^3-hybridised carbon bearing the amino group by an sp^2 centre leads to a conjugated system with less flexibility. This change effectively introduces a "spacer" which prevents the C_4H_9-sidechain from bending towards the binding pocket. Furthermore, the chain will most likely adopt the conformation in which the two carbonyl groups are in an s-*trans* arrangement, relieving dipole–dipole repulsion. This arrangement significantly withdraws the leucine side chain from the binding pocket and hence removes a large part of the hydrophobic casing around the binding pocket. The presence of hydrophobicity at the ligand–antibiotic interface enhances ligand binding by providing an entropic driving force to the association (due to liberation of ordered water at the hydrocarbon surface), and creates a region of low relative permittivity which enhances the electrostatic interaction.[14,15] Indeed, deletion of the leucine side chain results in a 20-fold reduction of the binding constant.[16] The ketone (**2**), which exhibits the weakest measurable binding affinity ($K_{lig} = 4.2 \times 10^3$ M^{-1}), and the malononitrile (**4**), which apparently fails to bind, seem to behave in accord with this model. In the latter case the complete loss of binding is likely to be due to the steric bulk of the additional planar conjugated malononitrile and the antiperiplanar conformation that the moiety adopts to relieve dipole–dipole interactions.

The reduced binding of these new derivatives to Ac_2L-Lys-D-

Ala-D-Ala is in contrast to the trend observed among analogous derivatives of ristocetin aglycone which all bind with affinities similar to that of the parent antibiotic[11,17] ($K_{lig} = 6.7 \times 10^5$ M^{-1}, Table 2). Indeed, it was the high binding affinities of these ristocetin analogues which had encouraged us to undertake the present work. We believe the difference in binding can be attributed to a conformational disruption of the binding pocket of vancomycin which is not possible with ristocetin. Ristocetin has a rigid structure due to crosslinking between two aromatic residues; therefore, alterations to the *N*-terminus result only in minimal conformational changes of the antibiotic binding pocket so that binding affinity is retained.

Conclusions

We observe weak binding between the bacterial cell wall precursor mimic Ac_2L-Lys-D-Ala-D-Ala and new derivatives of vancomycin. Alteration of the *N*-terminus of *N*-demethylvancomycin to give an sp^2 centre at the α-carbon of residue 1, creates a "spacer" which does not permit the hydrophobic side chain of leucine to approach the ligand interface, as required for strong binding. Dipole–dipole repulsion in derivatives with sp^2 functionality is believed to give rise to antiperiplanar geometry and hence to disrupt the binding pocket.

Experimental

All chemicals used were purchased from Aldrich and used without further purification. *N*-Demethylvancomycin was obtained from Dr Yan Husheng of Nankai University. Ac_2L-Lys-D-Ala-D-Ala was synthesised according to standard literature methods.[18]

^{1}H NMR spectra were recorded on a Bruker DRX-500 spectrometer at 300 K; *J* values are given in Hz and the letter code used for proton assignments is as previously described.[19] The standard nomenclature for the assignment of protons was used in agreement with previous publications (Fig. 4). High resolution mass spectra were obtained on a Fourier transform ion cyclotron resonance mass spectrometer equipped with a 4.7 T superconducting magnet and an external electrospray ionisation source (Analytica of Branford, Branford, USA). In reversed phase HPLC; solvent A: water with 0.1% TFA modifier; solvent B: acetonitrile with 0.1% TFA modifier. The gradient used with analytical HPLC (Phenomenex Jupiter column 5μ, C-18, 300 Å, 250 × 4.6 mm) was 0 min, 0% B; 15 min, 30% B; 23 min, 30% B; 25 min, 80% B; 30 min, 80% B; 32 min, 0% B. A Phenomenex Primesphere (5μ, C-18-MC, 300 Å, 150 × 21.2 mm) column was used for preparative HPLC. All the compounds **2**–**6** were indicated to be pure by the criterion of analytical HPLC after initial isolation by preparative HPLC.

Measurement of binding constants

Binding constants for the association between the vancomycin derivatives and Ac_2L-Lys-D-Ala-D-Ala were determined by UV difference spectrophotometry. All binding measurements were performed using a double beam 940 spectrophotometer equipped with a thermocirculator to maintain the temperature at 300 K. The light path was 1 cm. All antibiotic solutions were 50 mM, using KH_2PO_4 (50 mM, buffered to pH 7) as the diluent. The tripeptide ligand was dissolved in this antibiotic solution to maintain a constant concentration of antibiotic. Appropriate increments (10–40 μl) of the ligand solution (typically 5 mM) were added to the antibiotic solution in the titration cell, until the antibiotic was ~95% bound. In all cases the large change in absorbance at ~293 nm was monitored by obtaining $2A_{293} - (A_{283} + A_{303})$ throughout the titration (A_x is the difference in absorbance at *x* nm between the titration cell and the reference cell). The quoted wavelengths varied by several nm between derivatives. The large change in absorbance near 242 nm was not used in these determinations to avoid overlap with peaks due to the free tripeptide. Association constants were obtained using a least-squares curve-fitting programme in Kaleidagraph 3.0.5 (Adelbeck Software) by plotting $2A_{293} - (A_{283} + A_{303})$ against ligand concentration for each titration. Titrations were repeated to ensure reproducibility, and cases of large error (*e.g.* benzyl oxime) were the result of small absorbance changes on formation of the complex.

Vancomycin ketone (2)

Glyoxylic acid (50 mg, 0.71 mmol) and a catalytic amount of copper(II) sulfate were added to a stirred solution of *N*-demethylvancomycin (430 mg, 0.30 mmol) in acetate buffer (pH 5, 20 ml) and acetonitrile (4 ml), and the solution was stirred for 18 h. HPLC analysis revealed nearly complete conversion of the starting material. The mixture was concentrated to one third of the original volume and subjected to RP-column chromatography eluting with an acetonitrile gradient. The fractions containing the ketone (HPLC analysis or staining with cerium molybdate solution) were pooled and lyophilised to yield (347 mg, 81%) of the ketone **2** as a brownish solid. Analytically pure ketone was obtained by preparative HPLC.

t_R = 16.6 min; δ_H (500 MHz; DMSO-d_6) 0.93 (6H, d, *J* 6.9, 1c, 1c′), 1.10 (3H, V_6), 1.76 (1H, d, *J* 13.2, V_{2a}), 1.92 (1H, br s, V_{2e}), 2.09 (1H, sept, *J* 6.9, 1b), 2.15–2.28 (2H, m, 1a, 1a′), 2.74–2.84 (2H, m, 1a, 1a′), 4.20 (1H, d, *J* 10.7, x_6), 4.40–4.52 (3H, m, x_3, x_5, x_7), 4.70 (1H, d, *J* 5.9, V_5), 4.86 (1H, dd, *J* 9.7, 4.2, x_2), 5.23–5.27 (2H, m, V_1, z_2), 5.73 (1H, d, *J* 7.8, x_4), 5.89 (1H, d, *J* 4.4, 2-OH), 6.71 (1H, d, *J* 9.3, w_6), 7.90 (1H, d, *J* 9.7, w_2), 8.03 (1H, br s, w_4), 8.44 (1H, br s, w_5), 8.57 (1H, br s, w_7), 12.23 (1H, br s, COO*H*); *m*/*z* (ESI) $C_{65}H_{70}Cl_2N_8O_{25}$, (717.1932 $[M + 2H]^{2+}$ requires 717.1987).

Vancomycin alcohol (3)

To a solution of the ketone **2** (50 mg, 35 μmol) in methanol (2.5 ml) was added sodium cyanoborohydride (15 mg, 240 μmol) and glacial acetic acid (50 μl, 875 μmol) at 0 °C. The solution was then allowed to warm to ambient temperature. HPLC analysis after 4 h revealed complete conversion of the ketone. The reaction was quenched by the addition of 4 drops of 1 M hydrochloric acid. The residue was purified by preparative HPLC and lyophilised to afford the alcohol **3** (31 mg, 62%) as white solid.

t_R = 14.1 min; δ_H (800 MHz; DMSO-d_6) 0.88 (3H, d, *J* 6.8, 1c), 0.91 (3H, d, *J* 6.8, 1c′), 1.06 (3H, d, *J* 5.8, V_6), 1.30 (3H, s, V_7), 1.48–1.52 (1H, m, 1a), 1.55–1.59 (1H, m, 1a′), 1.85 (1H, sept, *J* 6.2, 1b), 1.90 (1H, d, *J* 10.9, V_{2a}), 2.13–2.17 (1H, m, V_{2e}), 3.18 (1H, d, *J* 5.8, V_4), 3.41–3.46 (1H, m, G_3), 3.52 (1H, dd, *J* 11.9, 4.7, G_6), 3.56 (1H, t, *J* 8.2, G_2), 3.66–3.69 (1H, m, $G_{6'}$), 3.98 (1H, br s, x_1), 4.00–4.03 (1H, m, G_5), 4.17–4.21 (1H, m, x_6), 4.43 (3H, br s, x_3, x_5, x_7), 4.68 (1H, d, *J* 5.4, V_5), 4.74 (1H, br s, x_2), 5.08–5.12 (2H, m, z_2, y_6), 5.12–5.16 (1H, m, V_1), 5.28 (1H, d, *J* 6.4, G_1), 5.70 (1H, d, *J* 5.0, 1-OH), 5.74 (1H, d, *J* 8.2, x_4), 5.82 (1H, br s, 2-OH), 5.94 (1H, br s, z_6), 6.70 (1H, br d, *J* 5.8, w_6), 7.20 (1H, d, *J* 7.9, 2e), 7.55 (1H, d, *J* 8.0, 2f), 7.33 (1H, d, *J* 7.0, 6e), 7.45 (1H, d, *J* 7.0, 6f), 7.61 (1H, br s, w_2), 7.83 (1H, d, *J* 7.0, 6b), 8.31 (1H, br s, w_4), 8.49 (1H, br s, w_7), 8.67 (1H, br s, w_5), 12.75 (1H, br s, COO*H*); *m*/*z* (ESI) $C_{65}H_{72}Cl_2N_8O_{25}$, (718.2005 $[M + 2H]^{2+}$ requires 718.2066).

Vancomycin malononitrile (4)

To a solution of the ketone **2** (50 mg, 35 μmol) in methanol (1 ml) was added 420 ml (1.2 equiv.) of a 0.1 M solution of malononitrile in methanol, followed by piperidine (35 μl, 360 μmol) and the solution was stirred for 16 h at ambient temperature. Preparative HPLC followed by lyophilisation yielded the malononitrile **4** (21 mg, 41%) as a yellow solid.

t_R = 19.5 min; δ_H (500 MHz; DMSO-d_6) 1.02 (3H, d, *J* 6.4, 1c), 1.07 (6H, d, *J* 6.4, 1c′, V_6), 1.34 (3H, s, V_7), 1.73 (1H, d, *J* 13.4, V_{2a}), 1.91 (1H, d, *J* 9.7, V_{2e}), 2.60–2.70 (2H, m, 3a, 3a′), 3.06–3.12 (1H, m, 1b), 3.54–3.59 (2H, m, G_2, G_6), 4.00–4.09 (2H, m, x_3, G_5), 4.16–4.22 (1H, m, x_6), 4.45 (1H, d, *J* 5.6, x_7), 4.48 (1H, m, x_5), 4.72 (1H, d, *J* 6.6, V_5), 5.25–5.29 (2H, m, G_1, V_1), 5.60–5.66 (1H, m, x_2, x_4), 6.26 (1H, s, 7d), 6.41 (1H, s, 7f), 6.65 (1H, br s, w_6), 8.53–8.68 (4H, m, w_2, w_4, w_5, w_7); *m*/*z* (ESI) $C_{68}H_{70}Cl_2N_{10}O_{24}$, (714.2033 $[M + 2H]^{2+}$ requires 714.2043).

Vancomycin oxime (5)

To a solution of the ketone **2** (40 mg, 27 μmol) in pyridine (2 ml) was added hydroxylamine hydrochloride (19 mg, 270 μmol), and the solution was stirred for 18 h at ambient temperature. The residual pyridine was azeotropically removed with toluene (3 × 10 ml). The residue was taken up in 2 ml of methanol and stirred for 2 h at ambient temperature. Preparative HPLC followed by lyophilisation yielded the oxime **5** (26 mg, 67%) as a white solid.

t_R = 14.6 min (*E*-form), t_R = 16.7 min (*Z*-form); (*Z*-form) δ_H (500 MHz; DMSO-d_6) 0.83 (3H, d, *J* 6.8, 1c), 0.87 (3H, d, *J* 6.8, 1c′), 1.10 (3H, d, *J* 6.8, V_6), 1.29, (3H, s, V_7), 1.74 (1H, d, *J* 13.6, V_{2a}), 1.89–1.94 (1H, m, V_{2e}), 1.98 (1H, sept, *J* 6.8, 1b), 2.10 (2H, m, 3a, 3a′), 2.20–2.28 (1H, m, 1a), 2.48 (1H, dd, *J* 11.9, 6.4, 1a′), 4.21 (1H, d, *J* 11.5, x_6), 4.45 (2H, br s, x_5, x_7), 4.52 (1H, br s, x_3), 4.71 (1H, q, *J* 6.8, V_5), 4.87 (1H, dd, *J* 9.4, 4.6, x_2), 5.23–5.26 (3H, m, V_1, z_2, G_1), 5.75 (1H, d, *J* 6.0, x_4), 5.85 (1H, d, *J* 3.4, 2-OH), 6.69 (1H, br s, w_6), 7.04 (1H, br s, w_3), 7.46 (1H, br s, w_2), 8.18 (1H, br s, w_4), 8.46 (1H, br s, w_7), 8.64 (1H, br s, w_5), 11.80 (1H, s, NO*H*), 12.70 (1H, br s, COO*H*); *m*/*z* (ESI) $C_{65}H_{71}Cl_2N_9O_{25}$, (724.7054 $[M + 2H]^{2+}$ requires 724.7097).

Vancomycin benzyloxime (6a, 6b)

To a solution of the ketone **2** (40 mg, 27 μmol) in pyridine (2 ml) was added benzylhydroxylamine hydrochloride (43 mg, 270 μmol) and the solution was stirred for 18 h at ambient temperature. The residual pyridine was azeotropically removed with toluene (3 × 10 ml). Preparative HPLC followed by lyophilisation yielded the oximes **6a** and **6b** (19 mg, 47%, *E*:*Z*, 2:3) as a white solid.

t_R = 21.4 min (*E* isomer), t_R = 22.5 min (*Z* isomer); (*Z* isomer) δ_H (500 MHz; DMSO-d_6) 0.82 (3H, d, *J* 6.8, 1c), 0.85 (3H, br s, 1c′), 1.09 (3H, br s, V_6), 1.28 (3H, s, V_7), 1.70–1.75 (1H, m, V_{2a}), 1.90 (1H, br s, V_{2e}), 1.96 (1H, sept, *J* 6.8, 1b), 2.08–2.24 (2H, m, 3a, 3a′), 2.37 (1H, d, *J* 13.6, 1a), 2.41 (1H, d, *J* 6.8, 1a′), 3.14 (1H, br s, V_4), 3.42–3.59 (3H, m, G_2, G_3, G_6), 3.63–3.73 (2H, m, G_4, G_6), 3.97–4.01 (1H, m, G_5), 4.16–4.22 (2H, m, x_6, y_6), 4.45–4.47 (2H, m, x_5, x_7), 4.50 (1H, br s, x_3), 4.67 (1H, br s, V_5), 4.81 (1H, br s, x_2), 5.09 (1H, br s, z_6), 5.19–5.35 (3H, m, V_1, G_1, z_2), 5.74 (2H, s, OCH_2Ph), 5.85 (1H, br s, 2-OH), 5.94 (1H, d, *J* 6.8, y_6), 6.24 (1H, s, 7d), 6.39 (1H, s, 7f), 6.67–6.69 (1H, m, w_6), 6.98 (1H, br s, w_3), 7.25–7.42 (7H, m, w_2, AsnN*H*, PhH), 8.42 (1H, br s, w_7), 8.63 (1H, br s, w_5); *m*/*z* (ESI) $C_{72}H_{76}Cl_2N_9O_{25}$, (769.218 $[M + 2H]^{2+}$ requires 769.2243).

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References

1 H. C. Neu, *Science*, 1992, **257**, 1064.
2 P. Courvalin, *Antimicrob. Agents Chemother.*, 1990, **34**, 2291.
3 D. H. Williams, *Nat. Prod. Rep.*, 1996, 469.
4 C. T. Walsh, S. L. Fisher, I.-S. Park, M. Prahalad and Z. Wu, *Chem. Biol.*, 1996, **3**, 21.
5 J. P. Waltho, J. Cavanagh and D. H. Williams, *J. Chem. Soc.*, *Chem. Commun.*, 1988, 707.
6 G. J. Sharman, M. S. Searle, B. Benhamu, P. Groves and D. H. Williams, *Angew. Chem.*, 1995, **34**, 1483.
7 N. E. Allen, D. L. LeTourneau and J. N. Hobbs, Jr, *J. Antibiot.*, 1997, **50**, 677.
8 R. J. Dancer, A. C. Try, G. J. Sharman and D. H. Williams, *Chem. Commun.*, 1996, 1445.
9 L. V. D. Boeck, F. P. Mertz, R. K. Wolter and C. E. Higgens, *J. Antibiot.*, 1984, **37**, 446.
10 H. Yan, D. Qi, X. Cheng, Z. Song, W. Li and B. He, *J. Antibiot.*, 1998, **8**, 750.
11 T. R. Herrin, A. M. Thomas, T. J. Perun, J. C. Mao and S. W. Fesik, *J. Med. Chem.*, 1985, **28**, 1371.
12 H. B. F. Dixon, *J. Protein Chem.*, 1984, **3**, 99.
13 R. Kannan, C. Harris, T. M. Harris, J. P. Waltho, N. J. Skelton and D. H. Williams, *J. Am. Chem. Soc.*, 1988, **110**, 2946.
14 M. P. Williamson, D. H. Williams and S. J. Hammond, *Tetrahedron*, 1984, **40**, 569.
15 M. S. Searle, D. H. Williams and U. Gerhard, *J. Am. Chem. Soc.*, 1992, **114**, 10697.
16 M. F. Cristofaro, D. A. Beauregard, H. Yan, N. J. Osborn and D. H. Williams, *J. Antibiot.*, 1995, **48**, 805.
17 M. B. Kobrin, H. S. Katrukha and G. B. Fedorova, *J. Antibiot.*, 1989, **XLII**, 1441.
18 M. Bodanszky and A. Bodanszky, *The Practice of Peptide Synthesis*, Springer-Verlag, Berlin, 1994.
19 J. P. Mackay, U. Gerhard, D. A. Beauregard, R. A. Maplestone and D. H. Williams, *J. Am. Chem. Soc.*, 1994, **116**, 4573.

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