

Attempted introduction of a fourth amide NH into the carboxylate-binding pocket of glycopeptide antibiotics

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We report the synthesis of a novel derivative of the glycopeptide antibiotic vancomycin, modified at the *N*-terminus. This incorporates a fourth amide NH into the antibiotic, at the position which was formerly the *N*-terminus of the antibiotic-binding pocket. Although this modification gives the potential to form an extra hydrogen bond to the carboxylate anion of the bacterial cell-wall precursor analogue (*N,N*-diacetyl-L-lysyl-D-alanyl-D-alanine), the modified antibiotic does not show enhanced binding to this precursor. This lack of enhanced binding is associated with an unfavourable conformational change (relative to the desired conformation) in the antibiotic.

Introduction

The glycopeptide antibiotics vancomycin and teicoplanin find widespread clinical use against infections due to multi-resistant bacteria such as strains of *Staphylococcus aureus* (MRSA) and are often the antibiotics of last resort.^{1,2} Vancomycin-group antibiotics bind to bacterial cell-wall mucopeptide precursors terminating in the sequence L-lysyl-D-alanyl-D-alanine,² thereby inhibiting the crosslinking enzymes.^{3,4} The resulting interference with peptidoglycan synthesis causes a loss of mechanical strength of the cell wall and leads ultimately to cell lysis.

In the binding of bacterial cell-wall analogues terminating in D-Ala-D-Ala to vancomycin (**2**) and ristocetin A,⁵ the main source of the binding energy is to be found in the binding of the carboxylate of the terminal D-Ala residue into a pocket of three adjacent NHs of the antibiotic (the NHs of residues 1, 2, and 3, Fig. 1a).⁶ We reasoned that if this pocket could be extended to one of four adjacent NHs (Fig. 1b), then a dramatic increase in the binding affinity might occur. The covalent connectivity which can allow, in principle, such an extension does not simply involve the acylation (*e.g.*, *N*-acetylation) of the *N*-terminal amino group (residue 1) of, for example *N*-demethylvancomycin (**3**) (Scheme 1). This is because the D-stereochemistry at this centre causes the *N*-terminal ammonium ion to point out of the carboxylate anion-binding pocket, and the α -CH of residue 1 to point into this pocket. Thus an inversion of the stereochemistry of residue 1 is required, prior to its acetylation, to give *N*-acetyl-*N*-demethyl-L-leucyl-vancomycin (**7**). In principle, the required modifications can be achieved either by inverting the stereochemistry of the existing *N*-terminal D-leucine of *N*-demethylvancomycin (**3**) and subsequently acetylating this material, or alternatively by removing this residue, and then replacing it by *N*-acetyl-L-leucine. Both methods were attempted.

Results and discussion

Initial attempts to obtain **7** involved introduction of sp²-hybridisation (in the form of a ketone) at the centre which originally constituted the α -CH centre of D-leucine.⁷ In the case of ristocetin aglycone, there is precedence for reduction of such a ketone (**1**), using ammonium acetate and cyanoborohydride, to give a product with the required stereochemistry.⁸ However, the application of this method to the vancomycin ketone (**10**)

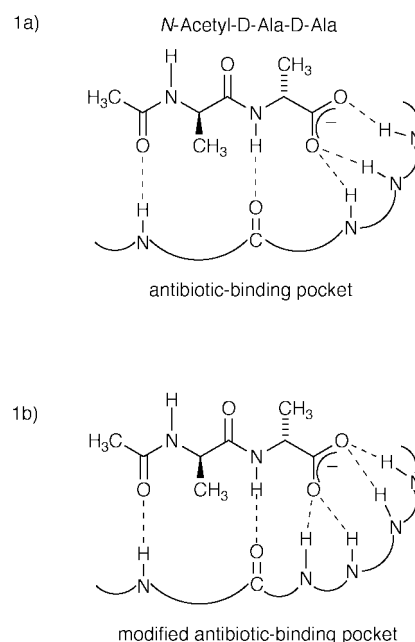
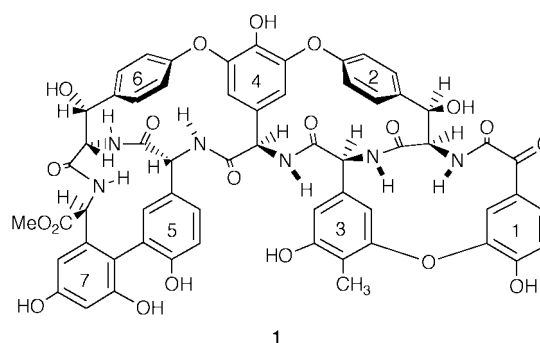
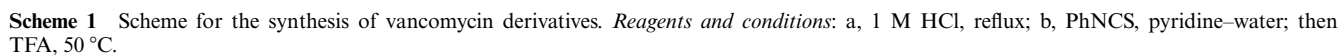


Fig. 1 The cooperative array of hydrogen bonds is responsible for ligand binding.



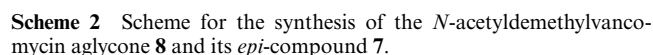
resulted in very low yields of the desired product, despite a broad variation of the reaction conditions. Therefore, the alternative strategy involving the removal of the *N*-methyl-D-leucine from vancomycin by an Edman degradation⁹ and subsequent



Derivative	$K_{\text{lig}}/\text{M}^{-1}$ (N,N)Ac ₂ KDADA
1 ⁶	5.6×10^5
2 ⁵	8.0×10^5
3 ⁵	1.7×10^6
4 ⁹	4.6×10^5
5	1.5×10^5
6 ¹⁰	<i>a</i>
7	3.6×10^4
8	1.0×10^4
9 ⁹	5.0×10^3
10 ⁵	4.2×10^3
11	<i>a</i>
12	<i>a</i>
13	2.0×10^4

attachment of *N*-acetyl-L-leucine was employed.¹⁰ Since the vancomycin sugar residues do not contribute largely to the binding of cell-wall precursors in *in vitro* experiments, we decided to remove them in order to avoid problems with the regioselectivity in the coupling step.

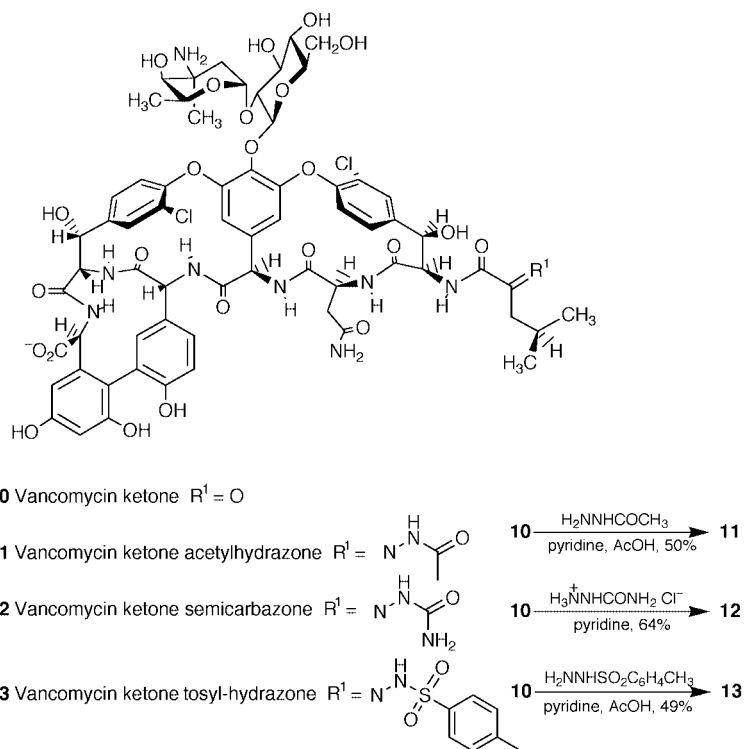
A second approach to the incorporation of an extra amide bond involved the reaction of acetylated or sulfonylated hydrazines with vancomycin ketone (**10**). CPK models suggested that



the extra NH proton of such derivatives might be suitably located for interaction with the carboxylate residue of the ligand. The ketone **10**⁷ was condensed, in turn, with acetylhydrazine, semicarbazide hydrochloride and tosylhydrazine in pyridine to give the corresponding hydrazones **11**, **12** and **13**, respectively, in yields between 49 and 64% (Scheme 3).

The binding of the cell-wall precursor analogue Ac₂-L-Lys-D-Ala-D-Ala to *N*-demethylvancomycin aglycone (**5**) and the newly synthesised derivatives **7**, **8** and **11–13** were measured by UV difference spectrophotometry (Table 1). The affinity of ligands for vancomycin aglycone **4** was, within experimental error, in agreement with previous studies,¹¹ and *N*-demethylvancomycin aglycone **5** showed a comparable binding constant of $1.5 \times 10^5 \text{ M}^{-1}$.

The binding constant obtained for **7** ($K_{\text{lig}} = 3.6 \times 10^4 \text{ M}^{-1}$) strongly suggests that a fourth hydrogen bond does not form to the carboxylate anion of Ac₂-L-Lys-D-Ala-D-Ala (which requires the binding pocket conformation shown in Fig. 2a). It seems possible that its formation might be precluded by **7**



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

undergoing $\approx 120^\circ$ rotation about the α -CH-to-carbonyl bond of the leucine residue (Fig. 2b). This conclusion is supported NMR data; a 1H NOESY spectrum shows cross-peaks between the methyl protons of the *N*-acetyl group at residue 1 and the NH protons of the asparagine side-chain. This observation is consistent with the proposed 120° rotation, since an X-ray study of vancomycin¹⁶ shows that the asparagine NH_2 group occupies the 'lower face of the vancomycin structure', a conformation which would bring this NH_2 close to the *N*-acetyl moiety (Fig. 2b).¹⁷ Most convincingly, a strong NOE cross-peak between x_1 (the α -CH of the L-leucine residue) and w_2 (the NH of the β -hydroxy-chloro-tyrosine residue) can be observed (Fig. 2b). We note that such a conformation might be favoured because it avoids the additional electrostatic repulsion of bringing a fourth positive end of an NH-CO dipole parallel to the existing three parallel dipoles (constituted from the NHs of residues 2, 3, and 4). We further note that the tolerance of these latter three parallel dipoles in vancomycin (despite their intrinsic electrostatic repulsion) seems to be occasioned by the cross-linking of the side-chains of residues 2 and 4.

The acetylated derivative **8** displayed only weak binding ($K_{lig} = 1 \times 10^4 M^{-1}$). This is comparable to binding affinities of compound **9**, derived from vancomycin, reported previously.¹¹ Therefore the substitution of an NH proton in **8** for a methyl group in **9** does not make a significant difference to binding.

No measurable binding was observed for the derivatives **11** and **12**, which suggests that the additional NH is not pointing into the binding pocket. The tosyl hydrazone **13** showed moderate binding ($K_{lig} = 2 \times 10^4 M^{-1}$), and significantly enhanced solubility of this derivative was observed.

Conclusions

We observe only weak binding between the cell-wall precursor analogue (*N,N*)-diacetyl-L-lysyl-D-alanyl-D-alanine and vancomycin derivatives which carry a fourth amide proton at the *N*-terminus. There is at least a 10-fold decrease of binding compared with vancomycin aglycone. Unfavourable conformational changes relative to the geometry required for enhanced binding appear likely to be the cause of this behaviour.

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_2 -L-Lys-D-Ala-D-Ala was synthesised according to standard literature methods.¹⁸

1H 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 assignment is as previously described.¹⁹ The descriptor G refers to protons in the glucose and V refers to protons of the vancosamine sugar. 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 was water with 0.1% TFA modifier, and solvent B was acetonitrile with 0.1% TFA modifier. The gradient used with analytical HPLC (Phenomenex Jupiter column 5 μ , C-18; 300 Å; 250 $\times$ 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 (bead size 5 μ , C-18-MC; pore size 300 Å; 150 $\times$ 21.2 mm) column was used for preparative HPLC.

Measurement of binding constants

Binding constants for the association between the vancomycin derivatives and Ac_2 -L-Lys-D-Ala-D-Ala were determined by UV difference spectrophotometry. All binding measurements were performed using a double-beam UVIKON 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 μ l) of the ligand solution (typically 5 mM) were added to antibiotic solution in the titration cell, until the antibiotic was $\approx 95\%$ 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

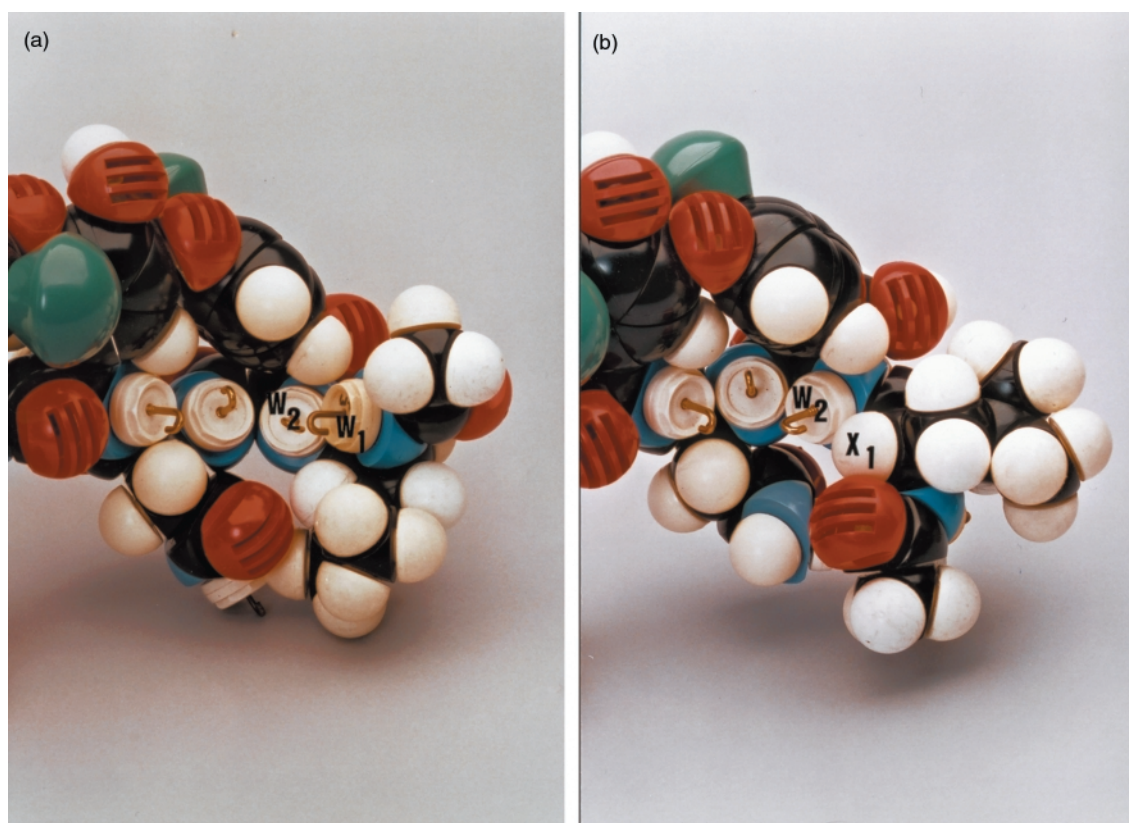


Fig. 2 CPK models showing (a) the desired conformation of a carboxylate-binding pocket containing four amide NHs, (b) the conformation which is indicated experimentally by the observation of proton NOEs. Protons, white; nitrogens, blue; carbons, black; oxygens, red (see text for proton code).

(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 were the result of small absorbance changes on formation of the complex. All compounds **2** and **4–6**, **8**, **10–13** were indicated to be pure by the criterion of analytical HPLC after initial isolation by preparative HPLC.

N-Demethylglycovancomycin **5**

N-Demethylvancomycin **3** (1.0 g, 0.96 mmol) was dissolved in 25 ml of 1 M HCl and heated in a boiling water-bath for 4 min. The mixture was cooled and filtered. Preparative HPLC followed by lyophilisation yielded *N*-demethylglycovancomycin **5** (0.79 g, 66%) as a white solid, t_R 16.2 min; δ_H (500 MHz; DMSO- d_6) 0.92 (3H, d, J 6.2, 1c), 0.93 (3H, d, J 6.2, 1c'), 1.54–1.64 (2H, m, 1a, 1a'), 1.70 (1H, ap. sext, J 6.6, 1b), 2.14 (1H, dd, J 16.4 and 8.5, 3a), 2.68–2.74 (1H, m, 3a'), 4.07 (1H, br s, x_1), 4.13–4.21 (2H, m, x_3 , x_6), 4.44–4.47 (2H, m, x_5 , x_7), 4.82 (1H, dd, J 7.2 and 3.6, x_2), 5.12 (1H, br d, J 7.2, z_2), 5.14–5.16 (1H, m, z_6), 5.67 (1H, br s, z_6OH), 5.76 (1H, d, J 8.1, x_4), 5.85 (1H, br s, x_2), 6.27 (1H, d, J 1.9, 7d), 6.41 (1H, d, J 1.5, 7f), 6.45 (1H, br s, w_3), 6.70 (1H, d, J 1.3, w_6), 6.72 (1H, d, J 8.5, 5e), 6.79 (1H, dd, J 8.8 and 1.3, 5f), 7.09–7.12 (1H, br s, Asn_dNH), 7.14 (1H, br s, 5b), 7.25 (1H, d, J 8.3, 6e), 7.47 (1H, dd, J 8.3 and 1.3, Asn_dNH), 7.50–7.53 (1H, m, 2f), 7.86 (1H, d, J 1.3, 6b), 8.21 (1H, br s, w_1), 8.25 (1H, br s, w_4), 8.53 (1H, br s, w_2), 8.61 (1H, d, J 5.5, w_7), 8.71 (1H, d, J 4.6, w_5), 12.75 (1H, br s, COOH); m/z (ESI) ($C_{52}H_{57}Cl_2N_8O_{17}$ m/z 1129.2785. $[M + H]^+$ requires m/z 1129.2748).

L-*N*-Acetyldemethylvancomycin aglycone **7**

To an ice-cold solution of *N*-acetyl-L-leucine (27 mg, 152 mmol) in DMF (1 ml) were added TDBTU (105 mg, 0.3 mmol) and diisopropylethylamine (DIEA) (33 μ l, 195 mmol) and the mixture was stirred for 15 min at this temperature. Des-leucylglycovancomycin **6** (40 mg, 40 mmol) in DMF (1 ml) was cooled to 0 °C, added to the other solution, and the mixture was stirred for 1 h at 0 °C and for 5 h at ambient temperature, and evaporated to dryness. The residue was brought to pH 10 with aq. NH_3 and incubated at 37 °C for 1 h. Preparative HPLC followed by lyophilisation yielded *L*-*N*-acetyldemethylglycovancomycin **7** (19 mg, 41%) as a white solid, t_R 21.5 min; δ_H (500 MHz; DMSO- d_6) 0.93 (3H, d, J 6.4, 1c), 1.03 (3H, d, J 6.8, 1c'), 1.49 (1H, dd, J 13.2 and 6.6, 1a), 1.65 (1H, dd, J 13.2 and 6.4, 1a'), 1.77 (3H, s, Ac), 1.82 (1H, hept, J 6.4, 1b), 2.20 (1H, dd, J 14.4 and 7.0, 3a), 2.42–2.50 (1H, m, 3a'), 4.08–4.13 (1H, m, x_1), 4.17 (1H, d, J 12.0, x_6), 4.38 (1H, d, J 4.7, x_5), 4.45 (1H, d, J 5.5, x_7), 4.48–4.54 (1H, m, x_3), 4.90 (1H, dd, J 8.5 and 4.7, x_2), 5.09 (1H, br s, z_6), 5.19–5.23 (1H, m, z_2), 5.41 (2H, br s, 4b, z_2), 5.61–5.65 (1H, br s, 4f), 5.68 (1H, d, J 7.7, x_4), 5.92–5.97 (1H, m, z_6OH), 6.25 (1H, s, 7f), 6.40 (1H, s, 7d), 6.68 (1H, d, J 8.2, Asn_dNH), 6.73 (1H, d, J 8.8, 5e), 6.79 (1H, d, J 8.5, 5f), 7.01–7.04 (1H, m, Asn_dNH), 7.07–7.11 (1H, m, w_3), 7.18 (1H, s, 5b), 7.25 (1H, d, J 8.5, 6e), 7.29 (1H, d, J 8.1, 2e), 7.45–7.49 (1H, m, 2f, 6f), 7.73 (1H, d, J 9.4, w_2), 7.84 (1H, s, 6b), 7.87–7.90 (1H, m, w_4), 8.29 (1H, d, J 5.1, w_1), 8.43 (1H, br d, J 5.5, w_7), 8.48 (1H, br s, w_5), 12.74 (1H, br s, COOH); m/z (ESI) ($C_{54}H_{53}Cl_2N_8O_{18}$ m/z 1171.2511. $[M + H]^+$ requires m/z 1171.2545).

N-Acetyldemethylvancomycin aglycone **8**

N-Demethylglycovancomycin **5** (100 mg, 80 mmol) was dissolved in a mixture of MeOH–Ac $_2$ O (4 : 1; 5 ml) and the solution was stirred at ambient temperature for 4 h and evaporated to dryness. The residue was brought to pH 10 with aq. NH_3 ,

and incubated at 37 °C for 1 h. Preparative HPLC followed by lyophilisation yielded *N*-acetyldemethylglycovancomycin **8** (73 mg, 78%) as a white solid, t_R 18.0 min; δ_H (500 MHz; DMSO- d_6) 0.83 (3H, d, J 6.4, 1c), 0.93 (3H, d, J 6.4, 1c'), 1.46–1.58 (2H, m, 1a/1a'), 1.71 (1H, d, J 7.2 and 6.4, 1b), 1.71 (3H, s, Ac), 2.12 (1H, dd, J 15.7 and 5.5, 3a), 2.18–2.24 (1H, m, 3a'), 4.18 (1H, d, J 11.9, x_6), 4.24 (1H, dd, J 14.0 and 6.4, x_2), 4.49 (1H, d, J 5.5, x_5), 4.45 (1H, d, J 6.0, x_7), 4.54 (1H, br s, x_3), 4.68 (1H, d, J 8.5 and 4.7, x_2), 5.11 (1H, br s, z_6), 5.20 (1H, br s, z_2), 5.23 (1H, s, 4f), 5.54 (1H, s, 4b), 5.71 (1H, br s, z_2 OH), 5.73 (1H, br s, x_4), 5.94 (1H, br s, z_6 OH), 6.26 (1H, s, 7f), 6.41 (1H, s, 7d), 6.71 (1H, d, J 13.2, Asn_4 NH), 6.73 (1H, d, J 8.5, 5e), 6.78 (1H, d, J 8.5, 5f), 6.89 (1H, br s, w_3), 7.01 (1H, br s, w_2), 7.15 (1H, s, 5b), 7.22 (1H, d, J 8.5, 2e), 7.23 (1H, d, J 13.2, Asn_4 NH), 7.28 (1H, s, 2b), 7.28–7.30 (1H, m, 6e), 7.47 (1H, d, J 8.1, 6f), 7.68 (1H, d, J 8.5, 2f), 7.84 (1H, s, 6b), 7.99 (1H, br s, w_4), 8.46 (1H, d, J 6.0, w_1), 8.53 (1H, d, J 5.6, w_7), 8.64 (1H, d, J 5.5, w_5), 12.76 (1H, br s, COOH); m/z (ESI) ($C_{54}H_{53}Cl_2N_8O_{18}$ m/z 1171.2553. $[M + H]^+$ requires m/z 1171.2545).

Vancomycin ketone acetylhydrazone **11**

To a solution of the ketone **10** (65 mg, 45 μ mol) in pyridine (1 ml) were added acetylhydrazine (37 mg, 0.5 mmol) and glacial acetic acid (40 ml, 0.7 mmol), 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 acetylhydrazone **11** (43 mg, 64%) as a white solid, t_R 14.3 min; δ_H (500 MHz; DMSO- d_6) 0.83 (3H, d, J 6.4, 1c), 0.89 (3H, d, J 6.4, 1c'), 1.12 (3H, d, J 6.8, V_6), 1.34 (3H, s, V_7), 1.74–1.79 (1H, m, V_{2e}), 1.89–1.94 (2H, m, V_{2e} , 1b), 2.08 (3H, s, NAc), 2.07–2.12 (2H, m, 3a/3a'), 3.16–3.20 (1H, m, V_4), 3.44–3.50 (1H, m, G_3), 3.55–3.61 (2H, m, G_2 , G_6), 3.66–3.71 (1H, m, G_6), 3.94–3.99 (1H, m, G_5), 4.20–4.24 (1H, m, x_6), 4.45 (1H, d, J 6.0, x_7), 4.49–4.51 (1H, m, x_5), 4.54–4.58 (1H, m, x_3), 4.70 (1H, dd, J 6.8 and 5.5, V_5), 4.78–4.82 (1H, m, x_2), 5.23–5.30 (4H, m, G_1 , V_1 , 4f, z_2), 5.71–5.74 (2H, m, x_4 , z_2 OH), 6.25 (1H, s, 7d), 6.41 (1H, s, 7f), 6.67–6.70 (1H, m, w_6), 7.66–7.69 (1H, m, w_2), 8.23 (1H, br s, w_4), 8.47 (1H, br s, w_4), 8.61 (1H, br s, w_4), 10.80 (1H, br s, hydrazoneNH), 12.74 (1H, br s, COOH); m/z (ESI) ($C_{67}H_{75}Cl_2N_{10}O_{25}$ m/z 1489.3978. $[M + H]^+$ requires m/z 1489.4281).

Vancomycin ketone semicarbazone **12**

To a solution of the ketone **10** (50 mg, 35 μ mol) in pyridine (1 ml) was added semicarbazide hydrochloride (40 mg, 358 μ mol), 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 semicarbazone **12** (26 mg, 50%) as a white solid, t_R 14.6 min; δ_H (500 MHz; DMSO- d_6) 0.83 (3H, d, J 6.4, 1c), 0.84 (3H, d, J 6.4, 1c'), 1.11 (3H, d, J 6.8, V_6), 1.31 (3H, s, V_7), 1.75–1.79 (1H, d, J 13.6, V_{2e}), 1.87–1.95 (2H, m, V_{2a} , 1b), 2.10–2.15 (1H, m, 3a), 2.25 (1H, br s, 3a'), 2.41–2.47 (1H, m, 1a), 2.51–2.55 (1H, m, 1a'), 4.21 (1H, d, J 11.5, x_6), 4.45 (1H, d, J 6.0, x_7), 4.47–4.49 (2H, m, x_3 , x_5), 4.69 (1H, d, J 6.8, V_5), 4.81 (1H, d, J 3.8, x_2), 5.22–5.29 (3H, m, G_1 , V_1 , z_2), 5.57 (1H, m, z_2 OH), 5.75 (1H, d, J 7.7, x_4), 6.26 (1H, d, J 1.7, 7f), 6.41 (1H, d, J 1.7, 7d), 6.73 (1H, d, J 8.5, w_6), 7.63 (1H, br s, w_2), 8.27 (1H, br s, w_4), 8.48 (1H, br s, w_7), 8.60 (1H, br s, w_5), 9.88 (1H, br s, hydrazoneNH); m/z (ESI) ($C_{66}H_{74}Cl_2N_{11}O_{25}$ m/z 745.7126. $[M + H]^+$ requires m/z 745.7151).

Vancomycin ketone tosylhydrazone **13**

To a solution of the ketone **10** (40 mg, 27 μ mol) in pyridine (1 ml) were added tosylhydrazine (52 mg, 280 μ mol) and acetic acid (35 μ l, 0.6 mmol), 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 tosylhydrazone **13** (21 mg, 49%) as a white solid, t_R 17.8 min; δ_H (600 MHz; DMSO- d_6) 0.76 (3H, d, J 6.4, 1c), 0.87 (3H, d, J 6.4, 1c'), 1.11 (3H, d, J 6.0, V_6), 1.30 (3H, s, V_7), 1.75 (1H, d, J 12.8, V_{2a}), 1.83 (2H, m, 1b, V_{2e}), 2.10–2.15 (2H, m, 3a, 3a'), 2.30–2.44 (1H, m, 1a), 2.38 (3H, s, C_6H_4Me), 2.50–2.54 (1H, m, 1a'), 3.17 (1H, d, J 7.3, V_4), 3.45 (1H, dd, J 14.3 and 6.0, G_3), 3.55–3.60 (2H, m, G_2 , G_6), 3.65–3.70 (1H, m, G_6), 3.92 (1H, t_{app} , J 5.5, G_5), 4.45 (1H, d, J 5.5, x_6), 4.49–4.51 (1H, m, x_7), 4.52–4.59 (2H, m, x_5 , x_3), 4.68–4.75 (1H, m, x_2 , V_5), 5.12 (1H, d, J 6.8, z_6), 5.20–5.26 (3H, m, G_1 , V_1 , z_2), 5.34 (1H, d, J 5.1, G_3 OH), 5.43 (1H, d, J 6.8, V_4 OH), 5.53 (1H, s, 4b), 5.75–5.78 (1H, m, x_4), 5.95 (1H, d, J 6.4, z_6 OH), 6.26 (1H, d, J 1.3, 7f), 6.48 (1H, d, J 1.3, 7d), 6.64–6.72 (2H, m, w_6 , Ans_4 NH), 6.74 (1H, d, J 8.1, 5e), 6.79 (1H, d, J 8.1, 5f), 7.09–7.14 (2H, m, w_3 , Ans_4 NH), 7.22 (1H, s, 5b), 7.28–7.33 (1H, m, 2e), 7.39–7.42 (3H, m, 6e, 3,5-ArH), 7.47–7.52 (2H, m, 6f, 2b), 7.62 (1H, m, w_2), 7.81 (1H, d, J 8.1, 2f), 7.83 (2H, d, J 8.1, 2,6-ArH), 7.86 (1H, s, 6b), 8.19 (1H, br s, w_4), 8.45–8.48 (1H, m, w_5), 8.65–8.67 (1H, m, w_7), 11.22 (1H, s, hydrazineNH); m/z (ESI) ($C_{72}H_{79}Cl_2N_{10}O_{26}S$ m/z 801.2086. $[M + H]^+$ requires m/z 801.2088).

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