ELECTRONIC SUPPLEMENTARY INFORMATION

Solution structures of thiopeptide antibiotics†

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Table. Observed ^3J coupling constants and predicted torsion angles for amythiamicin D **2** compared to observed torsion angles for the bound GE2270A **3** X-ray structure.

Compound	Protons	NH9-H10	NH19-H19	NH27-H27(proR)	NH27-H27(proS)	NH29-H29
Amythiamicin D	Observed J (Hz)	9.5	7.9	9.5	3.1	6.1
	Predicted torsion (º)	±180	±152	±180	±53 ±114	±21 ±138
GE2270A	Observed torsion (º)	-163	153	-146	-27	-147

Experimental Section

Synthesis and purification of amythiamicin D

Amythiamicin D **2** was obtained by total synthesis as previously described.[1,2] Chromatography on silica gel (elution with 0-10% methanol-chloroform) followed by preparative reverse phase HPLC (19 x 50 mm ODS column; 3:2 acetonitrile:water as eluant; 20 mLmin^{-1}) gave pure amythiamicin D.

NMR Measurements

All NMR experiments were performed on a Bruker Avance spectrometer at a proton frequency of 600 MHz with the exception of the NMR assay which was performed on a Varian UnityInova spectrometer at a proton frequency of 500 MHz. Samples were prepared in chloroform-*d* (Fluorochem, UK) and chemical shifts were referenced to tetramethylsilane at all temperatures. The NMR assay[3] of amythiamicin D (as monomer) used the ERETIC technique[4] and an external standard of tetrachloronitrobenzene. The samples used to determine the concentration dependence of the chemical shifts were prepared by serial dilution of this sample. Errors in concentration were estimated as ±5% and in chemical shift ±0.005 ppm. Published assignments for amythiamicin D[5] were used and no inconsistencies with our 1D and 2D NMR data were noted. There is however slight disagreement with our previously reported results for the concentration dependence of the chemical shift of NH29.[1,2] We believe the currently reported results to be the more accurate as data is based on assayed concentrations of amythiamicin D. The standard (non-quantitative) ROESY experiment used the Bruker pulse sequence roesyph and

was run at a mixing time of 300 ms. The quantitative 2D-ROESY experiments used the Bruker

pulse sequence troesyph[6] modified slightly to switch the spinlock between +4500 Hz and –4500

Hz off-resonance on alternate scans. Data were collected at mixing times of 50, 75, 100, 125 and

150 ms. The ROE's were quantified by summing rows of the 2D dataset containing the peaks of

interest and performing integration on the resulting 1D spectrum. In most cases the reported

results are the average obtained from the two quadrants. The H27 geminal methylene protons

were used as a distance reference (1.78 Å). Signal overlap prevented use of the *ortho* aromatic

protons H2 and H3 as a reference.

A least squares fit of initial rate data was performed and forced through the origin. No significant

curvature was detected, and all points were used for the fit. The 95% confidence intervals quoted

are dominated by the fit to the reference data and the relatively large confidence intervals reflects

the difference in magnitude between the reference and calculated distances.

Dimerization Model

The concentration-dependent chemical shift data for proton NH29 were fitted to the dimerization

model below.

$$\delta_{obs} = \frac{2[Monomer]\delta_{Monomer} + (C_t - [Monomer])\delta_{Dimer}}{C_t + [Monomer]}$$

Where

$$2[Monomer]^2 + k_D[Monomer] - k_D C_t = 0$$

and k_D is the dissociation constant for dimerization; C_t is the total concentration of macrolide assuming all monomer (*i.e.* $C_t = [Monomer] + 2[Dimer]$); δ_{obs} is the observed chemical shift of NH29 and $\delta_{Monomer}$ and δ_{Dimer} are the chemical shifts for pure monomer and dimer species respectively.

The data for NH29 were fitted to the above equations using the Microsoft Excel solver utility, solving for k_D and δ_{Dimer} but assuming $\delta_{Monomer} = 6.118$ ppm (the observed shift in the most dilute sample). The fitted values were $\delta_{Dimer} = 9.36 \pm 0.02$ ppm, $k_D = 36.4 \pm 0.2$ mM and $R^2 = 0.9997$. The SOLVSTAT macro was used to generate the 95% confidence limits shown.[7] Once the value of the dimerization constant had been established, the observed chemical shift for NH29 could be used to determine the concentrations of the samples used for the ROESY and NH temperature coefficient measurements.

References

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