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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Protons</th>
<th>$J$ (Hz)</th>
<th>NH9-H10</th>
<th>NH19-H19</th>
<th>NH27-H27(proR)</th>
<th>NH27-H27(proS)</th>
<th>NH29-H29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amythiamicin D</td>
<td>Observed $J$</td>
<td>9.5</td>
<td>7.9</td>
<td>9.5</td>
<td>3.1</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Predicted torsion (°)</td>
<td>±180</td>
<td>±152</td>
<td>±180</td>
<td>±53</td>
<td>±114</td>
<td>±138</td>
</tr>
<tr>
<td>GE2270A</td>
<td>Observed torsion (°)</td>
<td>-163</td>
<td>153</td>
<td>-146</td>
<td>-27</td>
<td>-147</td>
<td></td>
</tr>
</tbody>
</table>
Experimental Section

_Synthesis and purification of amythiamicin D_

Amythiamicin D 2 was obtained by total synthesis as previously described.\textsuperscript{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 mL min\textsuperscript{-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-\textit{d} (Fluorochem, UK) and chemical shifts were referenced to tetramethylsilane at all temperatures. The NMR assay\textsuperscript{3} of amythiamicin D (as monomer) used the ERETIC technique\textsuperscript{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\textsuperscript{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.\textsuperscript{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.

\[ \Delta_{\text{obs}} = \frac{2[\text{Monomer}] \cdot \Delta_{\text{Monomer}} + (C_t \cdot [\text{Monomer}]) \cdot \Delta_{\text{Dimer}}}{C_t + [\text{Monomer}]} \]

Where

\[ 2[\text{Monomer}]^2 + k_d[\text{Monomer}] \cdot k_dC_t = 0 \]
and \( k_D \) is the dissociation constant for dimerization; \( C_t \) is the total concentration of macrolide assuming all monomer (i.e. \( C_t = [\text{Monomer}] + 2[\text{Dimer}] \)); \( \text{d}_{\text{obs}} \) is the observed chemical shift of NH29 and \( \text{d}_{\text{Monomer}} \) and \( \text{d}_{\text{Dimer}} \) 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 \( \text{d}_{\text{Dimer}} \) but assuming \( \text{d}_{\text{Monomer}} = 6.118 \text{ ppm} \) (the observed shift in the most dilute sample). The fitted values were \( \text{d}_{\text{Dimer}} = 9.36 \pm 0.02 \text{ ppm} \), \( k_D = 36.4 \pm 0.2 \text{ mM} \) 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**