Fast and simple NMR Assignments of Antibiotic

Introduction

Carbohydrate-based antibiotics are common and potent tools for fighting a large variety of infections. Repeating carbohydrate fragments often result in significantly overlapping regions of the ^{1}H NMR spectra, this complicates NMR data analysis. Utilization of the added chemical shift dispersion of the ^{13}C nuclei can simplify the assignment process, at a cost of reduced sensitivity caused by low ^{13}C natural abundance. A boost in sensitivity, offered by modern JEOL's cryogenically cooled NMR probe technology, dramatically reduces time and effort required to assign NMR signals using only small quantities of sample compound.

In this Application Note we present an example of how to efficiently obtain a set of full ^{13}C&^{1}H assignments of Kanamycin-A disulfate (MW ~880 Da), using only 1mg of the antibiotic, four basic NMR experiments and utilizing JEOL's new nitrogen-cooled ***SuperCool™ MARVEL™*** probe.

Methodology

A time-proven simple approach to dealing with overlap in ^{1}H NMR spectra is to pull the protons apart in a 2nd, ^{13}C dimension. The use of edited HSQC at good resolution can often provide a near automatic creation of a table of correlated proton and carbon atoms. From this point it becomes a matter of establishing atom-atom connectivities. Commonly used experiments to achieve this can be composite two-dimensional methods such as HSQC-TOCSY, HSQC-COSY. However, in the proton-rich situation here with the possibility for a range of ^{1}H-^{1}H coupling constant values, H2BC provides a clear path to assignments. For this note, we used HSQC and H2BC in a powerful and concerted fashion to build a complete structural assignment for Kanamycin disulfate in D_2O solution. Long-range ^{1}H-^{13}C connectivity from HMBC experiments in a problem like this provide an easy means to bridge intra-ring carbohydrate connectivities.

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The JEOL NMR system used in this work was the **500 MHz ECZ Luminous Console G** equipped with the liquid-nitrogen cooled **SuperCool MARVEL** probe.

A CRISIS-enhanced gHSQCAD, recorded with 4 scans on a sample of 1mg of Kanamycin di-sulfate (MW ~880), is shown in **Figure 1**. From this data the first four columns of the table of assignments are obtained. The gH2BCAD and gHSQCAD are overlaid together in **Figure 2** and **Figure 3**, with connectivities drawn showing assignments for Ring-A and Ring-B respectively. The gHSQCAD was linear sampled in F1. The H2BC and HMBC were acquired with 50% NUS. All data reflect excessive signal to noise sufficient to trace some of the connectivity for the very minor anomers. The F1 axis (^{13}C) for each dataset is from a projection to illustrate the data quality.

Figure 1. Plot of the gHSQCAD for 1mg Kanamycin di-sulfate in D_2O solution.

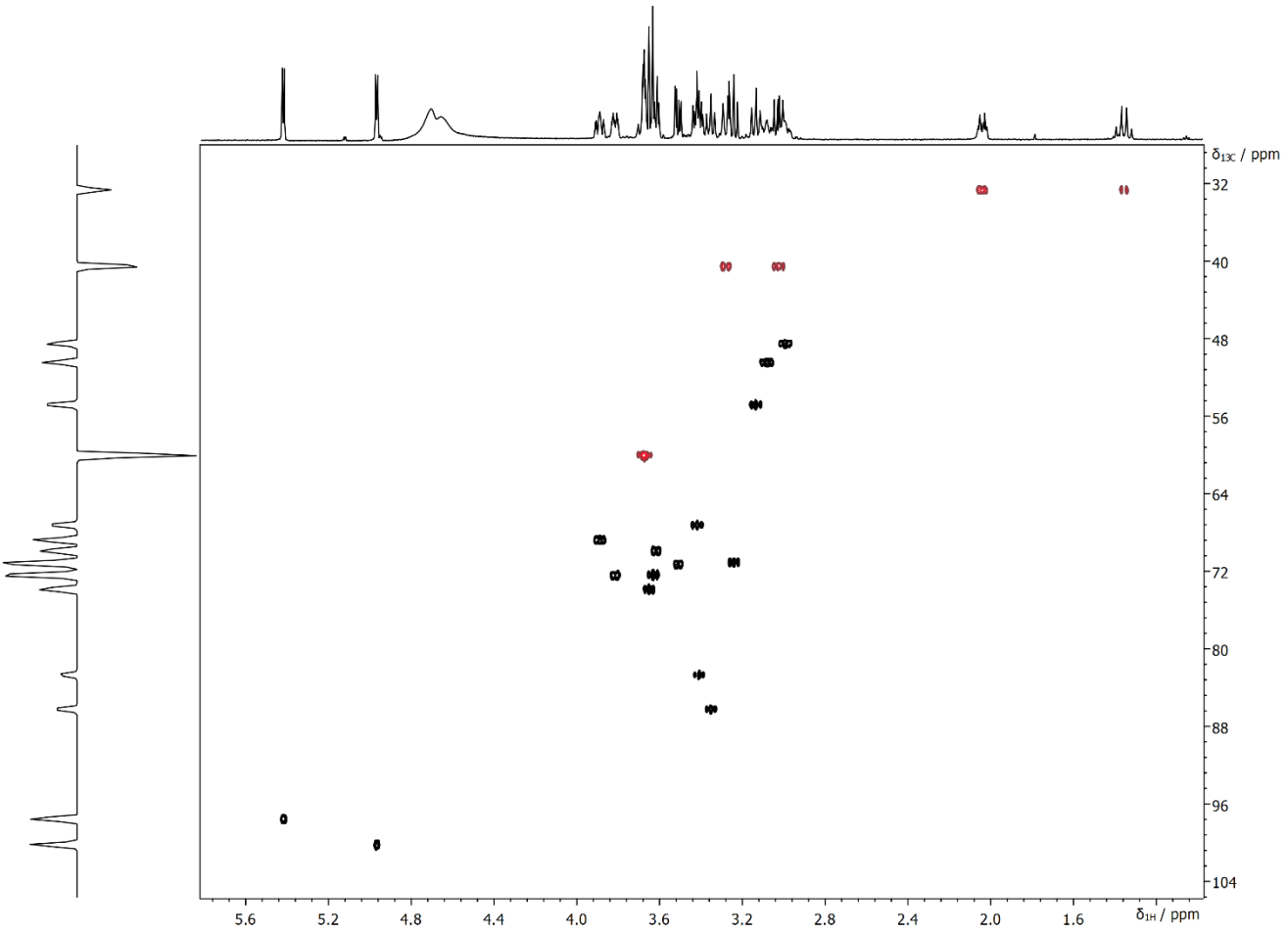


Figure 2. Overlay plot of the gHSQCAD and gH2BCAD with connectivities drawn assigning Ring-A

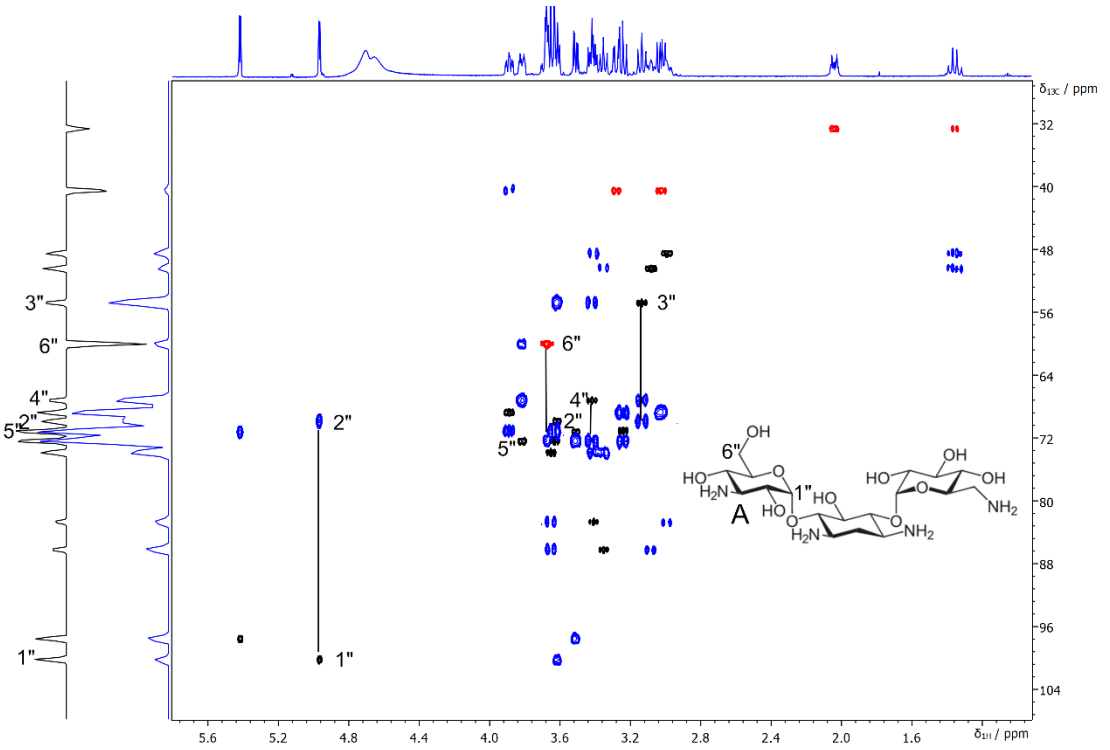


Figure 3. Overlay plot of the gHSQCAD and gH2BCAD with connectivities drawn showing assignments for the central carbohydrate moiety of center Ring-B

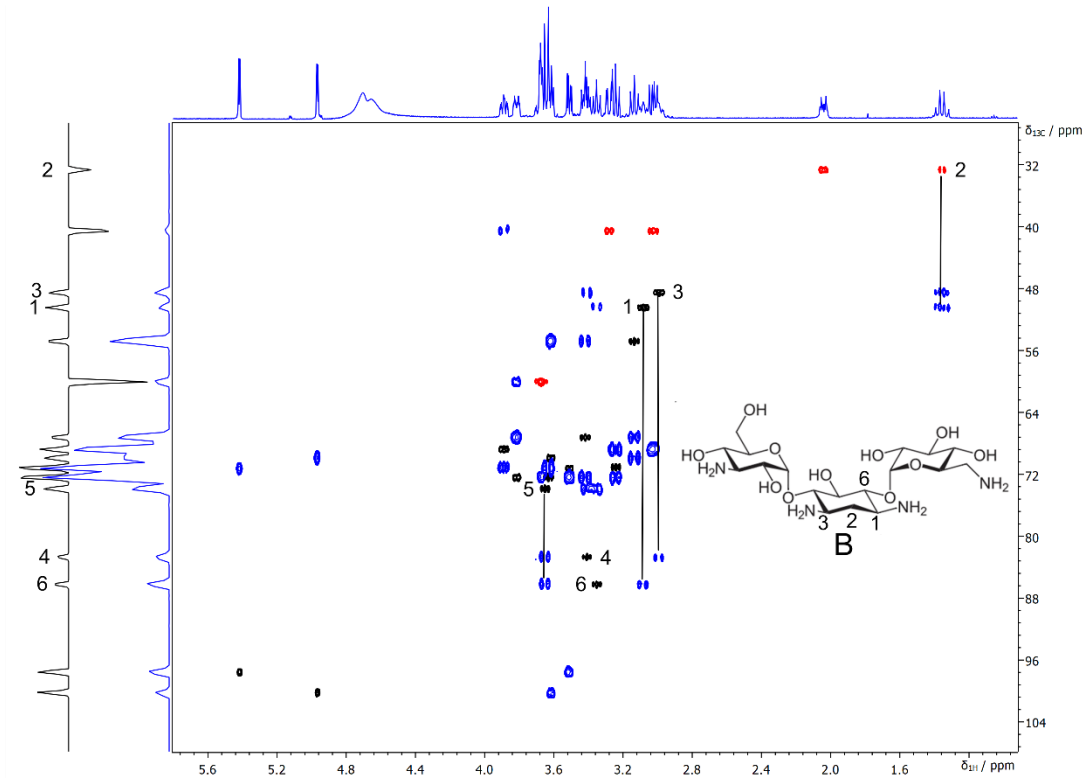
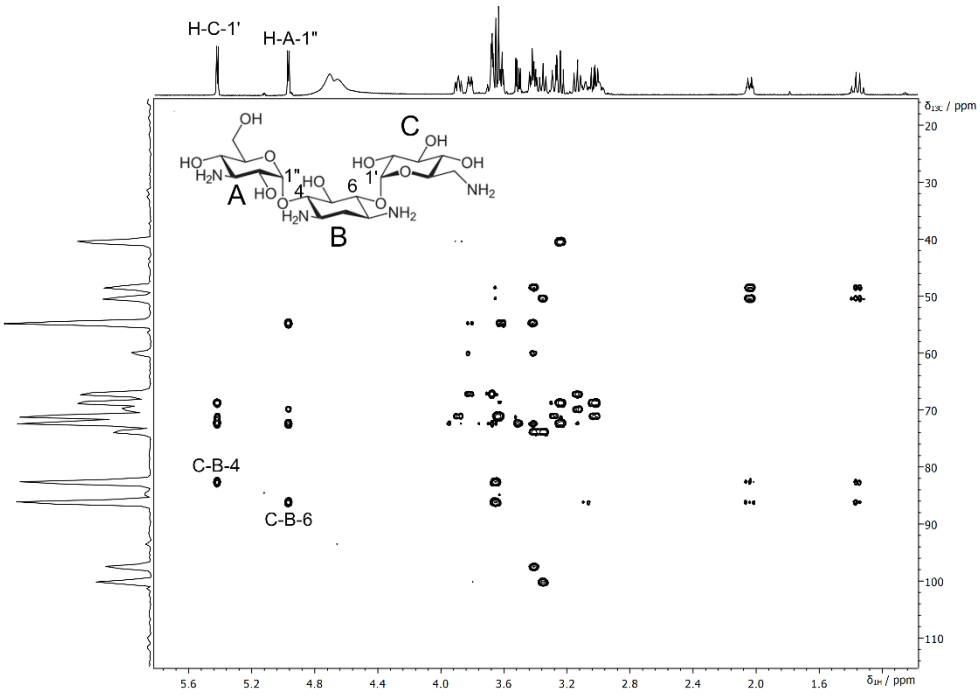


Figure 4. Plot of the gHMBCAD results for the 1mg Kanamycin di-sulfate labeled with the $^{3}J_{HC}$ connectivities revealing attachment points for Ring-A and Ring-C to the central Ring-B carbohydrate.



Results

The work of completing the assignments in the **Table 1** was achieved by overlaying the HSQC and H2BC to facilitate assigning connectivity paths some of which are drawn and labeled with vertical lines to illustrate the technique. **Figure 2**, **Figure 3** and **Figure 4** are labeled to show assignments for rings A and B respectively.

Table 1 Table of Assignments for 1mg sample of Kanamycin di-sulfate in D2O solution.

13C shifts	1H shifts	1H shifts	multiplicity	Ring A		Ring B		Ring C	
100.167	4.951		CH	1”					
97.508	5.405		CH					1’	
86.265	3.331		CH			6			
82.652	3.393		CH			4			
73.883	3.625		CH			5			
72.315	3.791		CH	5”					
72.304	3.614		CH					3’	
71.246	3.489		CH					2’	
71.083	3.22		CH					4’	
70.099	3.572		CH	2”					
68.863	3.859		CH					5’	
67.467	3.371		CH	4”					
60.04	3.633		CH_2	6”					
54.653	3.084		CH	3”					
50.413	3.038		CH			1			
48.524	2.962		CH			3			
40.536	3.25	2.989	CH_2					6’	
32.736	2.012	1.335	CH_2			2			