StepNOESY – Overcoming Spectral Overlap in NOESY1D

Application Note

Abstract

VnmrJ 3 software provides easy-to-use, interactive tools for setting up advanced experiments. This allows even novice users to obtain critical information about their research samples using the most advanced NMR experiments available. This application note is one of a series designed to provide step-by-step guidance for setting up sophisticated experiments to collect the exact data you need for your analyses.
Introduction

A challenging step in many pharmaceutical, organic chemistry, and natural products structure elucidation investigations is the assignment of relative stereochemistry. Through-space, dipolar relaxation information collected using the Nuclear Overhauser Effect Spectroscopy (NOESY) experiment has been a primary tool for these analyses for many years. One common challenge for the interpretation of both the classic 2D NOESY and selective NOESY1D data is the ambiguity caused by spectral overlap.

A recent paper by Hu, et al. describes Selective TOCSY Edited Preparation NOESY (stepNOESY) as a novel approach that can be used to overcome the problems caused by overlapped peaks. The method uses a Total Correlation Spectroscopy One Dimensional (TOCSY1D) transfer preparation step to excite a single resonance in a region of overlap, thereby removing the overlap. The key element for the effective TOCSY1D preparation period is the zero quantum suppression methodology developed by Thrippleton and Keeler. Once magnetization is transferred to the resonance of interest, a selective excitation pulse is used on that resonance to initiate the NOESY1D experiment. Taken together this is a doubly-selective, two-step transfer of magnetization.

Figure 1. The chemical structure of brucine. The arrows indicate both the coupling pathway used to selectively transfer magnetization to H11α and the NOEs observed in the final stepNOESY1D experiment.
A stepNOESY Example

As an example of how the stepNOESY experiment might be used to solve a real-world problem, consider the stereochemical assignment of the H11 proton resonances in brucine (Figure 1). These methylene protons are observed as a geminal pair located at 3.03 and 2.58 ppm (Figure 2). Unfortunately, both of these resonances overlap other peaks in the spectrum, rendering any results from a typical homonuclear NOESY1D experiment ambiguous (Figure 3). While the goal of this experiment was to confirm the relative stereochemistry of the H11α proton, most of the observed NOE responses are derived from the other signals in the excitation band. These unwanted signals obscure the resonances of interest and make unequivocal interpretation of the H11α responses impossible.

Figure 2. An expansion of the proton NMR spectrum of brucine in deuterochloroform (CDCl₃). Note that the complex pattern between 2.80 and 3.15 ppm represents the overlapped resonances from H14 and H18, and the H11α proton resonances.

Figure 3. The 1D NOESY spectrum of brucine with the primary excitation band focused on the resonances between 2.80 and 3.15 ppm.
A TOCSY1D data set demonstrates that, despite the overlap, the resonances at 2.58 ppm are only associated with the resonances at 3.02 ppm via the H11 two-bond coupling pathway (Figure 4). This allows us to use the H11 proton signal at 2.58 ppm as a jumping-off point for stepNOESY.

First, the proton resonances at 2.58 ppm are selectively excited and the resulting coherence is efficiently transferred to the H11 proton at 3.03 ppm via the large geminal coupling. The resonance at 3.03 ppm is then selectively excited using a second shaped pulse and Nuclear Overhauser Effect (NOE) responses are allowed to evolve through a 500 ms mixing time. The only signals observed are those created by NOE coherence transfer from H11α to the protons within a ~4.5 Å radius. Observation of responses from H12 and H13 confirm the assignment of H11α at 3.05 ppm (Figure 5).

Figure 4. The 1D TOCSY spectrum of brucine, with the primary excitation band at 2.58 ppm. Note the single signal observed for the cluster of peaks at ~3.05 ppm despite multiple resonances present at 2.58 ppm.

Figure 5. The stepNOESY spectrum of brucine. The initial excitation window was centered at 2.58 ppm and the 1D NOESY excitation was centered at 3.05 ppm.
Experimental Method

The stepNOESY experiment requires two unique sets of frequency-selective shaped pulses to be calculated every time the experiment is used. While this might sound like a complicated task, in practice it is a simple operation because the necessary tools have been provided in the experiment panels. The frequency bands for the selective pulses are chosen interactively from the 1D PROTON spectrum and each shaped pulse is then created automatically by clicking the Select and makeshape buttons:

1. Collect a PROTON spectrum as a study in the Study Queue.

2. Load the PROTON spectrum into the current workspace, select Continue Study, and then select stepNOESY1D from the Experiment Selector (Figure 6).

Figure 6. Setting up the stepNOESY pulse sequence. After collecting a PROTON spectrum, stepNOESY1D is added to the Study Queue.
3. Select the region for the initial TOCSY transfer step by placing cursors on each side of the desired peak(s) and clicking the **Select and makeshape** button for the STEP band (Figure 7). Since stepNOESY is a comparatively less sensitive experiment, the TOCSY mixing time can be optimized to achieve the most efficient energy transfer to the peak of interest before the NOE portion of the sequence is enabled. This is easily accomplished by deselecting the **NOE ON/OFF** checkbox, thereby temporarily converting the experiment to a simple TOCSY1D pulse sequence (Figure 8). The value for the TOCSY mixing time can now be iteratively adjusted to optimize the desired transfer.

![Figure 7. Setting up the stepNOESY pulse sequence. Selecting the TOCSY excitation band is accomplished by placing cursors on each side of the desired peak and clicking the STEP band Select and makeshape button.](image1)

![Figure 8. Setting up the stepNOESY pulse sequence. By turning off the NOESY mixing period, the TOCSY transfer step can be iteratively optimized specifically for the experiment at-hand. Note that the NOE ON/OFF checkbox is deselected.](image2)
4. Once the TOCSY conditions have been optimized, ensure that the NOE ON/OFF check box is selected. Next, place cursors around the peak(s) to be used for the NOE transfer step and click the Select and makeshape button for the NOE band (Figure 9).

5. The experiment is now ready to acquire stepNOESY data. Use the Submit button in the Study Queue to initiate data collection.

Figure 9. Setting up the stepNOESY pulse sequence. Once the TOCSY transfer step has been optimized, the NOESY band is selected by placing the cursors on each side of the desired resonance and selecting the NOE band Select and makeshape button. The experiment is now ready to be submitted to acquisition.
Conclusions

The stepNOESY is a very powerful tool for structure elucidation. While it does suffer from low sensitivity due to the multiple selective transfer steps and the nature of the NOE itself, the data available from stepNOESY is unique and can make the difference between assumption and certainty when crucial resonances are obscured.

References


