Using Band-Selective HSQCNOESY to Resolve Overlapping Homonuclear Resonances

Application Note

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Abstract

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

Introduction

Homonuclear Nuclear Overhauser Enhancement (NOE) experiments are routinely used to probe through-space interactions between proton nuclei. These experiments allow both the assignment of relative stereochemistry within a given molecule and the investigation of intermolecular interactions between tightly bound compounds. One of the major stumbling blocks encountered when using these experiments is the overlap of homonuclear resonances. When this occurs, assignment of any observed NOE responses becomes ambiguous and subject to interpretation.

One approach that can be used to resolve this ambiguity is the incorporation of a heteronuclear editing step in the NOESY pulse sequence, yielding experiments such as the heteronuclear single quantum coherence NOE spectroscopy (HSQCNOESY) pulse sequence. This experiment provides homonuclear NOE responses that are dispersed into the heteronuclear frequency domain, thereby removing the problems caused by homonuclear overlap. The standard HSQCNOESY sequence, however, is quite insensitive, necessitating a difficult trade off between the number of transients collected per increment and the desired number of F1 increments to moderate the total experiment time.

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The addition of band selection in the F1 dimension saves time and improves resolution in any 2D experiment by reducing the number of increments needed for adequate resolution while allowing the data collection to focus exclusively on the region of interest. In the band-selective HSQCNOESY (bsHSQCNOESY) experiment, through-space connectivity information is encoded via ^{1}H-^{1}H NOE, but the responses are displayed using the chemical shifts of both ^{1}H and ^{13}C. The bsHSQCNOESY experiment particularly benefits from F1 band selection by a reduction in the number of F1 increments required to resolve the chemical shifts in the ^{13}C dimension of the experiment. This time savings can then be used to offset the inherently low sensitivity by increasing the number of transients acquired in each increment. It is important to note that the experiment can be done using many different F2/F1 spin pairs; however, $^{1}H/^{13}C$ and $^{1}H/^{15}N$ are the most common choices for an organic chemistry structural problem. In the traditional broadband experiment, such high resolution could only be obtained at the cost of a large number of increments with a concomitant increase in the spectrometer time required to collect the data.

A bsHSQCNOESY Example

To illustrate the utility of the bsHSQCNOESY experiment, a 15 mg sample of prednisone (Figure 1) in deuterochloroform was chosen as a model. In this particular example, the focus will be on the problem of deducing the relative stereochemistry between a single methine resonance (H8) and two methyl groups (H18 and H19). Figure 2 displays the HSQC spectrum of the molecule with the resonances in question labeled. Note that in the ^{1}H dimension there is severe overlap between these resonances and other proton resonances, rendering the homonuclear NOESY experiment inconclusive for determining the relative stereochemistry of the molecule. Because there is good separation in the F1 ^{13}C dimension, however, the bsHSQCNOESY experiment can be used to exploit the resolution of the ^{13}C chemical shifts to unambiguously assign the ^{1}H-^{1}H NOE responses.

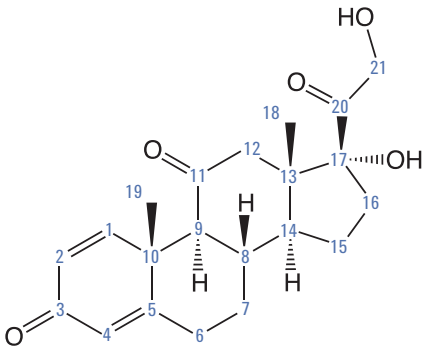

Figure 1. The chemical structure of prednisone.

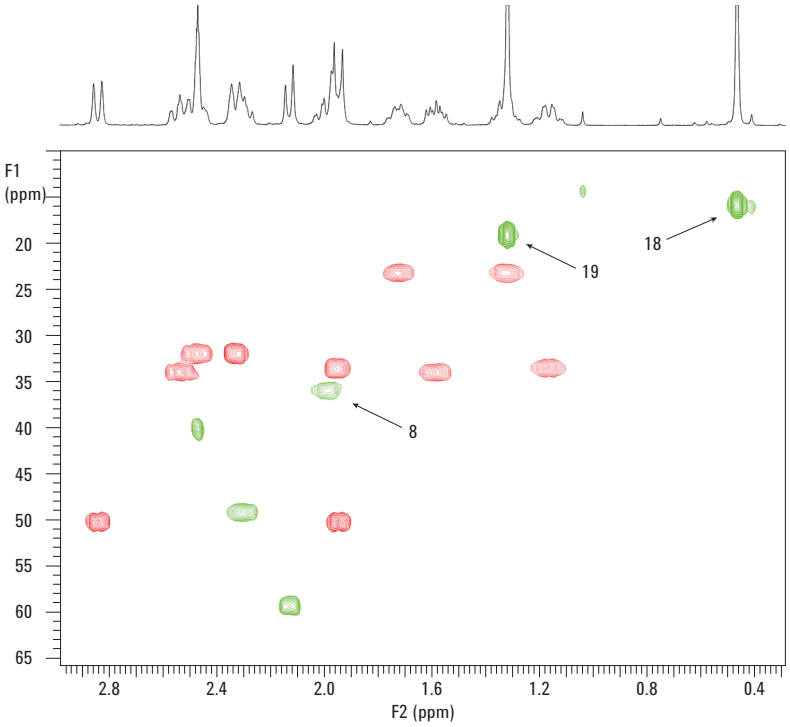

Figure 2. An expansion of the HSQC spectrum of prednisone, showing the responses for H8, H18, and H19. Note the overlap with other resonances in the proton dimension.

Figure 3 shows the bsHSQCNOESY spectrum acquired in an overnight experiment. The relevant NOE responses for the signals of interest are indicated. A one-dimensional experiment, such as a stepNOESY, would be of little use in this case because the methyls are each individual spin systems and thus the magnetization cannot be relayed to them from any of the resolved resonances. The bsHSQCNOESY experiment, however, yields clear and unambiguous NOE responses from the methyls at approximately 0.45 and approximately 1.35 ppm to the methine carbon at approximately 35.5 ppm, confirming the relative β stereochemistry for all three protons as drawn.

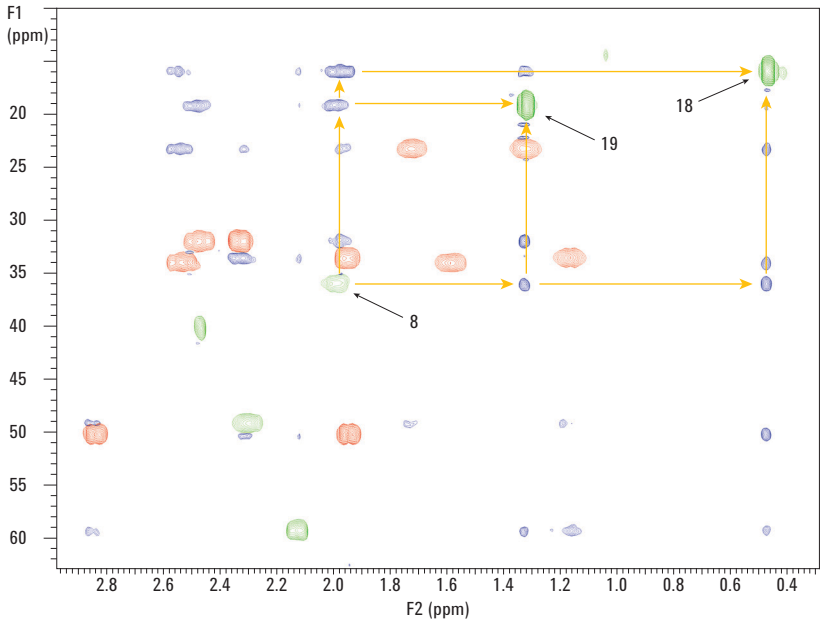

Figure 3. The bsHSQCNOESY spectrum of prednisone overlaid on the corresponding HSQC spectrum. Note that the NOESY responses from H8 to both H18 and H19 are fully resolved in the carbon dimension as shown by the yellow arrows.

Experimental Method

This method makes a very sophisticated F1 band-selected bsHSQCNOESY experiment accessible to chemists without requiring in the intricacies of manually creating the band-selected shapes in the carbon dimension or setting special parameters in the experimental workspace.

1. Select **New Study** under the Study Queue and choose **bsHSQCNOESY** from the **Sel2D** tab in the experiment selector. After this selection, the panel shown in Figure 4 will appear. The experiment is setup using proton and carbon by default, with ^{13}C in the band-selected F1 dimension.

2. The F1 window can be chosen by entering the desired region into the parameter entry boxes circled in Figure 4. All required shaped pulses will be created automatically. The number of scans and the NOE mixing time should also be adjusted as appropriate.

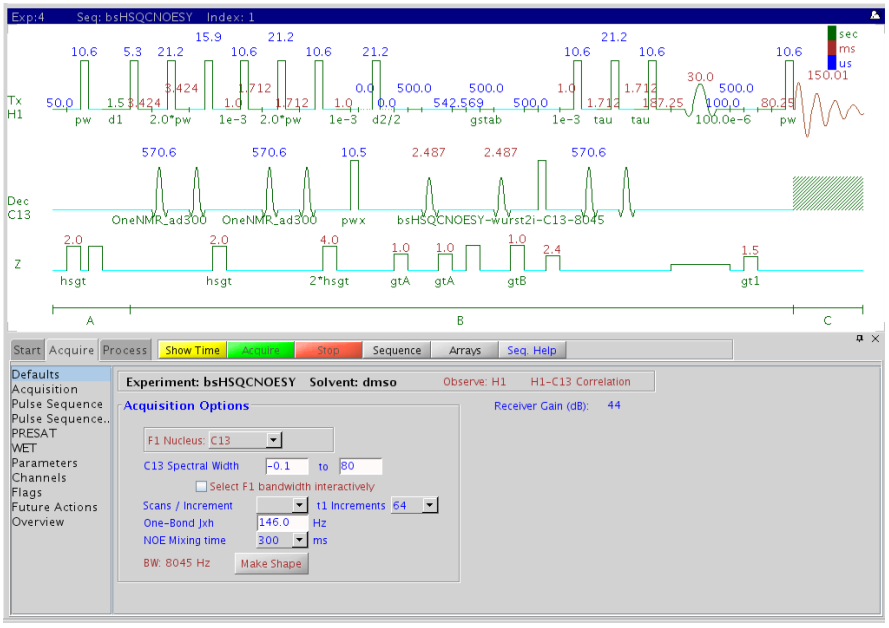

Figure 4. Setting up the bsHSQCNOESY experiment.

3. Save the parameter changes back to the Study Queue and click **Submit**.

4. If the bsHSQCNOESY experiment is setup from a traditional 2D data set of the same type, for example HSQC or HMBC, the operator can choose the band-selected frequencies with cursors from the existing spectra. In this scenario, select **Continue Study**, and the software will recognize that the appropriate experiment, for example an HSQCAD, if one is present in the current Study. The user can then choose the desired region interactively with the cursors in the graphics window, as shown in Figure 5.

5. After setting the desired number of scans and other basic parameters, simply save the changes to the Study Queue and submit the experiment.

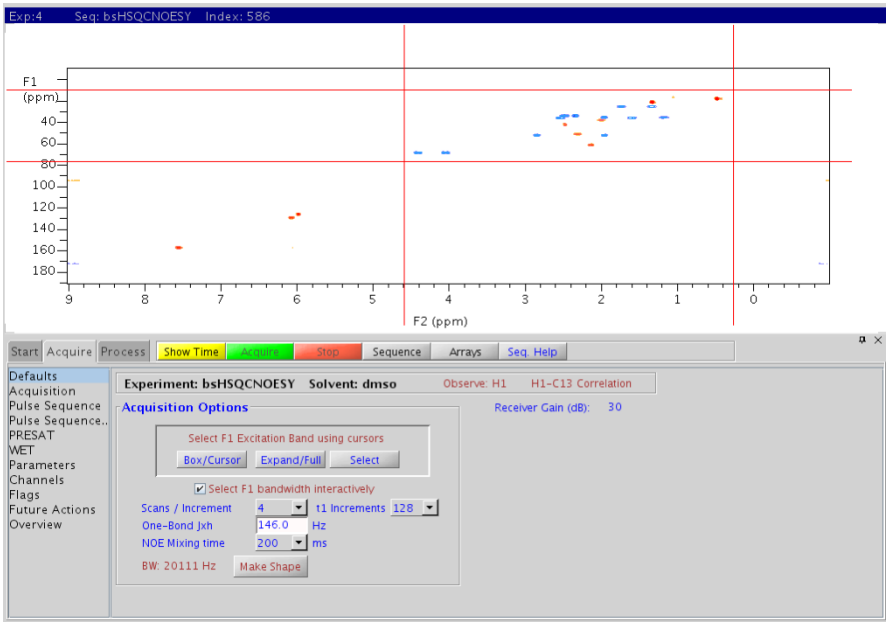

Figure 5. Choosing the F1 band-selection.

Conclusions

The utility of the HSQCNOESY experiment is severely restricted by its comparatively poor sensitivity. The band selective version, bsHSQCNOESY, ameliorates this sensitivity problem by reducing the number of F1 increments necessary for adequate resolution, thereby allowing an increase in the number of transients per increment without generating a prohibitively long experiment time. This experiment allows the power of heteronuclear editing to be applied as a routine tool for small molecule structural problems in situations where NOE investigations are hindered by spectral overlap in the homonuclear dimension.

References

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