PINMRF

Bruker AV-III / Avance DRX NMR Spectrometers running TopSpin

Routine/Survey 2D Spectra using Standard Parameter Sets

INCLUDING:

AV-III-400-HD w/ 5mm BBFO SmartProbe – 369 WTHR

AV-III-500-HD w/ 5mm BBFO Cryoprobe Prodigy - B055 DRUG

AV-III-800 w/ 5mm QCI Cryoprobe - LB124 BRWN

Avance DRX500-1 w/ 5mm TXI Cryoprobe - 367 WTHR

Avance DRX500-2 w/ 5mm BBFO ATM Probe - G43 RHPH

Table of Contents

06-15-2017: Revised - JSH.

06-07-2012: Revised - JSH.

07-27-2011: First Edition - JSH.

Routine/Survey 2D Spectra using Standard Parameter Sets - Overview

This booklet provides the information necessary to obtain routine 2D NMR spectra on routine samples using the Bruker AV-III and Avance DRX series NMR spectrometers running TopSpin. These experiments require a minimum of operator intervention or optimization, and as such they are appropriate for the non-expert NMR user to carry out. Note that not all experiments are available for every instrument or probe.

These experiments encompass two general classes of 2D spectra. These are 1) proton homonuclear spectra, such as magnitude COSY, phase-sensitive TOCSY and NOESY, and 2) proton-detected ^{13}C-^{1}H gradient-enhanced magnitude-mode HMQC and HMBC experiments. These experiments are set up with dedicated parameter sets designed to provide good results, with minimal operator effort, for normal organic and organometallic samples in typical organic solvents or D_2O. However, if the ultimate in sensitivity or resolution is required in your work, or your sample falls into one of the categories mentioned below, please contact PINMRF staff for assistance with more highly specialised experiments.

The experiments presented here DO NOT encompass the following:

1. samples which need solvent or other peak suppression;
2. samples which need non-standard observation windows;
3. experiments involving selective or semi-selective pulses;
4. heteronuclear 2D experiments involving nuclei other than ^{13}C and ^{1}H;

Note that for all 2D experiments probe tuning should be carried out on all relevant. See pp. 16-17 of the PINMRF manual **Bruker AV-III / Avance DRX - Training Guide for Basic 1D NMR Spectroscopy** for information regarding probe tuning.

These notes assume that the reader is already checked out for basic 1D operation on PINMRF's Bruker spectrometers running TopSpin.

Data Obtained from the Experiments

The proton homonuclear spectra provide information on proton-proton coupling networks and the spatial proximity of protons to one another. The COSY spectrum shows couplings between protons when the J value between them is ca. 5 Hz. or higher; usually this results from 2-, 3- and 4-bond couplings. The TOCSY experiment shows couplings down to ca. 2 Hz., which can include 5- and occasionally 6-bond couplings. The NOESY experiment gives information about which protons are close together in space. The heteronuclear spectra relate information about proton-carbon J-couplings. The HMQC experiment correlates carbons with their directly attached protons while the HMBC experiment shows which carbons have 2- or 3-bond couplings (ca. 10 Hz.) to a given proton. Quaternary carbons don't show up in the HMQC/HMBC spectra.

NOTES

Routine/Survey 2D Spectra using Standard Parameter Sets - Acquisition and Processing Guidelines
Bruker AV-III / Avance DRX NMR Spectrometers running TopSpin
PINMRF

2D data acquisition using standard parameter sets

1. Create a new data set. All 1D and 2D files will be acquired using the SAME experiment NAME but DIFFERENT experiment NUMBERS.

2. Create/read experiment (expno) 1. Put in your sample, lock, spin (if desired) and shim.

3. Obtain a proton spectrum in expno 1 using the standard proton parameters (e.g. `h1.prob`, where `prob` refers to the probe: this will be either `cryo` or `bbo`). Check the probe tuning for proton.

4. If desired, obtain a ^{13}C spectrum in expno 2 using standard parameters. In addition, if desired, obtain a DEPT spectrum in expno 3. Check the probe tuning for ^{13}C.

NOTE: if you are running HMQC and/or HMBC experiments, a ^{13}C spectrum is recommended but not required. However, if you don't obtain a ^{13}C spectrum, you must still tune the probe for both ^{13}C and ^{1}H prior to starting the HMQC and/or HMBC experiment.

5. Create/read expno 4. We will run a COSY in this dataset. Type `rpar cosy.prob all` to load the COSY parameters.

6. If your sample is extremely dilute, increase the number of scans using the `ns` parameter. To examine/change this or any other acquisition parameter you can also use either the `eda` (edit acquistion parameters) or the `ased` (acquisition setup editor) commands, or choose the AcquPars tab from the main spectrum display.

7. Use the `setti` command or Title tab to enter your title (as discussed in the 1D operation guidelines).

8. Turn off sample spinning and check that the lock is stable; adjust lock power and/or gain if necessary. Touch up the Z1 and Z2 shims.

9. Type `acqu` to display the acquisition window and then use `rga` to set receiver gain - wait for message telling you it is finished.

10. Type `zg` to start acquisition. The default COSY experiment takes about 5 minutes. For some of the 2D experiments you will see the lock signal drop periodically - this is normal.
NOTE: to run other 2D experiments, create/read other expno's as needed. Repeat from step 5 above using the following parameter sets (instead of `cosy.prob all`):
For TOCSY: type `rpar tocsy.prob all` (default time: 20 min.);
For NOESY: type `rpar noesy.prob all` (default time: 2.5 hr.);
For HMQC: type `rpar hmqc.prob all` (default time: 20 min.);
For HMBC: type `rpar hmbc.prob all` (default time: 20 min.).

2D processing for magnitude experiments (COSY, HMQC/HMBC)

11. When the experiment is finished, or after enough increments are completed, type `xfb` to do the 2D Fourier transformation. The 2D spectrum will be displayed in intensity image mode.

2D processing for phase-sensitive experiments (TOCSY, NOESY)

11. Determine phase correction and do the 2D processing as follows:
a) Any time after the second FID has been completed and saved, type `rser 1` (for NOESY) or `rser 2` (for TOCSY) to read the first or second FID (as appropriate) into a separate 1D dataset.
b) Type `lb` and enter `8` into the window. Then type `ef` to apply exponential multiplication and do the Fourier transformation.
c) Now phase this spectrum as normal, EXCEPT instead of clicking the save/return choice when finished, click the save/return 2D choice instead.
d) Type `to2d` to go back to the 2D experiment, then `acqu` to go back to the acquisition window.
e) When the experiment is finished, or after enough increments are completed, type `xfb` to do the 2D Fourier transformation. The phasing parameters obtained from the 1D spectrum (above) will be used to phase the 2D data. The 2D spectrum will be displayed in phase-sensitive mode with positive intensity peaks one color and the negative intensity peaks another. The specific colors will depend on your TopSpin customization settings (Options pulldown, then select Preferences).

f) If additional phase correction is needed, then the 2D phase routine will be used. Please contact PINMRF staff for assistance with this routine, or read the appropriate section of the TopSpin software processing manual.

Displaying the projections

NOTE: Typically, when a 2D spectrum is displayed or plotted, the X and Y axes of the 2D matrix are flanked by 1D spectra that represent each axis. These 1D spectra are either the actual 1D spectrum of that axis, taken from a separate 1D experiment (called the "external" projection), or they are the actual skyline projection of the 2D data matrix in that dimension (called the "internal" projection). The external projection has the same resolution as the normal 1D spectrum, whereas the internal projection has the resolution of the 2D spectrum, which is a lot lower. The 2D parameter sets used in this procedure are set up to use internal projections by default. To change the internal projection to an external projection, follow the procedure below.

12. To select and display the correct spectra for the 1D projections of the 2D dataset, do the following:

a) First, right-click on the 2D spectrum. In the pop-up window, left-click on DISPLAY PROPERTIES. At the bottom of the new window there will be a set of three selections for Visible projections. Typically the normal selection is F1 + F2 (click the radio button), but for HMQC/HMBC spectra without a corresponding ^{13}C spectrum available you may want to select F2.

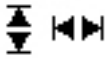
b) Now we will select the dataset to use for the F2 (top) projection. Right-click in the area of the top projection. In the pop-up window, left-click on EXTERNAL PROJECTION.... In the window that pops up, enter the correct information in the NAME, EXPNO and PROCNO fields for the 1D ^{1}H spectrum corresponding to the 2D dataset, then click OK to load that spectrum in the top projection field.

c) Now select the dataset to use for the F1 (left-side) projection. For homonuclear ^{1}H 2D spectra (COSY, NOESY, TOCSY) simply repeat step b), above. For heteronuclear 2D spectra (HMQC, HMBC), repeat step b), above, except enter the NAME, EXPNO and PROCNO data for the 1D ^{13}C spectrum corresponding to the 2D dataset.

Expansion and plotting

13. **SPECTRUM MANIPULATION**: To expand the 2D spectrum, move the mouse over the spectrum window. A crosshair will appear on the spectrum. Move the crosshair with the mouse to the lower left edge of the region you want to expand. Click and hold down the left mouse button while you move the mouse pointer from the lower left to the upper right edge of the region you want to expand. Release the mouse button to expand the selected region of the 2D spectrum. The 1D projection spectra will expand along with the 2D data. To go back to the full spectrum, click on the Show full spectrum (A) icon at the top of the spectrum window, or type `.all` . Clicking the  (Show last zoom) icon, or typing `.zl` , will re-expand to the previously chosen region.

Other manipulations:

- *2, /2, *8, /8: click these icons to adjust vertical scale up or down by factor 2 or 8;
-  : click this icon to re-display the entire spectrum;
-  : click these icons to display the entire spectrum on the vertical or horizontal axes;

14. **DEFINE PLOT REGION**: To define a region for plotting, expand the 2D spectrum and set the vertical scale as desired. Right-click on the displayed 2D spectrum. In the pop-up window, left click on "SAVE DISPLAY REGION TO F1 F2…" Parameters F1/F2 (radio button - requires a left-click).

15. **DEFINE CONTOUR LEVELS**: Right-click again on the 2D spectrum, and in the pop-up left-click on EDIT CONTOUR LEVELS. This will bring up a new window with several choices related to the plotting of the contour levels. We suggest the following:

For COSY, TOCSY, HMQC, HMBC: click the Positive radio button;

For NOESY: click the Positive & Negative radio button;

For all 2D spectra: Level increment: 1.5; Number of Levels: 9, then click the buttons Fill → Apply → OK.

16. **PLOTTING**:

To plot the spectrum on paper or as a PDF file using the default parameters, type `print .` In the pop-up menu, select the option Print with Layout - start Plot Editor (radio button). Check that the correct LAYOUT= is selected, as follows (these should default to the correct values):

For COSY, TOCSY, NOESY: +/2D_hom.xwp;

For HMQC, HMBC: +/2D_inv.xwp.

This will start the XWinPlot (TopSpinPlot) routine and the 2D printout will be shown. The appearance of the spectrum can be altered prior to plotting by right-clicking on the spectrum in the XWinPlot window and selecting the 1D/2D-edit choice. In the window that appears can be used to manipulate the scaling and expansion of the 2D spectrum and projections. To print the spectrum left-click on the File pulldown, followed by Print → Print (click-box). Please see the hardcopy manual "Plotting" for further details on the plotting routine.

NOTES

2D-NOESY Spectra of Small Molecules - Hints And Tips

One of the most common questions I hear is “why didn't I get a good NOESY spectrum?” This document aims to provide some comments on this experiment and its results along with some tips to help NMR users get the best possible 2D-NOESY data for small-organic-molecule samples. The discussion here assumes the reader is familiar both with the basics of 2D NMR and with obtaining routine 2D NMR spectra on our NMR Facility's Bruker spectrometers.

The NOESY spectrum uses the nuclear Overhauser effect (nOe) to provide information about which proton (typically) resonances are from protons which are close together in space. This is distinct from COSY-type spectra which use the J-coupling interaction to report on which proton resonances are located on the same or adjacent carbon nuclei. While it is true that most protons which are on adjacent carbons are close enough to give a NOESY cross-peak, it is not necessarily true that protons which give a NOESY cross-peak are J-coupled.

One of the main practical differences between NOESY spectra and COSY/TOCSY spectra is the fact that the nOe interaction is relatively weak. This results in NOESY spectra having much lower-intensity cross-peaks than one would expect from a COSY/TOCSY spectrum of the same sample, even if the NOESY spectrum is a “good” spectrum with no obvious problems. This results in a situation were there is not the same type of linear relationship between the “results” of a NOESY spectrum (usually considered to be the overall signal intensities of the cross-peaks) and the quality of the sample and experimental setup as there is for COSY-type spectra. In other words, if one runs a COSY experiment on a decent sample, barring instrument problems or gross errors in experiment setup, one can reasonably expect to see cross-peaks of decent intensity in the resulting spectrum. However, this is not always the case with NOESY spectra.

There are several steps one can take to maximize the chances of getting the best possible NOESY data. These steps involve both the sample itself and the experimental setup. We shall discuss these below.

Sample-Related Considerations

1. Purity. While it is possible to run NOESY experiments on impure samples or on mixtures, typically the best results will be obtained for pure, single-component, samples. This is in part due to the fact that because NOESY cross-peaks can be weak, it is easy for the desired cross-peaks to be obscured by those from an impurity or another mixture component. Also, if the components of a multi-component sample have many overlapping resonances, assigning the cross-peaks to the desired component may be difficult. Sample purity also relates to the issue of concentration: if a sample is only 50% pure in the desired component, the concentration of the desired component is only one-half of the entire sample concentration by weight.

2. Concentration. The optimum concentration for a NOESY sample is about the same as that needed to obtain a decent proton NMR spectrum in 8 - 16 scans. If the sample concentration is

too high, spectroscopic resolution can be degraded and the nOe interaction can be damped. If it is too low, weaker cross-peaks may not be found. A good estimate of a reasonable concentration is that which results in the sample peaks being of roughly (within 0.5 - 2.0 times) the same intensity as the residual proton signal from the solvent. If the sample can only be obtained in much lower concentration than that, then it would be of benefit to run the NOESY spectrum using an indirect-detection probe. If this is the case, contact the NMR Facility staff for assistance.

3. Quality. Foreign matter, such as silica gel, powder from molecular sieves, or other solids, in the sample can have a detrimental effect both upon spectroscopic resolution and upon the efficiency of the nOe interaction. Sample tube quality also can affect resolution. Thus, for NOESY spectra, always use a high-quality NMR tube (Wilmad 528PP or better) and always filter the sample (a plug of Kimwipe inserted into a Pasteur pipette is an easy way to do this) into the NMR tube. A good NOESY sample should be free of residual water or other extraneous peaks such as TMS, residual protonated solvent, etc. Finally, the sample volume should be at least 0.6 mL in order to minimize problems related to sample shimming. If you need to use a significantly smaller sample volume we recommend the use of a Shigemi-type NMR tube. Please contact PINMRF staff for assistance in this case.

4. Solvent. While $CDCl_3$ is usually the solvent of choice for organic samples, it often contains traces of acid which can cause reactions to occur with some samples, thus interfering with the stability of the sample. It has been my own experience that, when possible, the use of other solvents such as CD_3OD and acetone-d6 can give better spectroscopic dispersion and better NOESY spectra than that obtained with $CDCl_3$. Considerations such as solvent viscosity and the width of the solvent deuterium peak (relevant for spectrometer lock performance) sometimes are important also.

Experimental-Related Considerations

1. General. NOESY spectra should be run on samples that are well-shimmed and that have a strong, stable lock. The NOESY spectrum should be obtained without sample spinning. If the NOESY spectrum will be run for more than about four hours, use the sample temperature controller to keep the sample temperature constant; usually a setting of 298 - 300K works well. Be sure to take an adequate number of scans (NS, default is 16). It is better for a given amount of instrument time to take more scans and to acquire fewer increments (1_TD, default is 256). If you have to finish your experiment before all of the increments are completed don't worry, the software can handle this during the data processing. Finally, check the probe tuning on your NOESY sample prior to starting the experiment.

2. Delay values D1 and D8. The delay D1 is the time between each scan where relaxation of all the spins occurs. The delay D8 is the time period during each execution of the pulse sequence in the NOESY experiment where cross-relaxation (the actual nOe interaction) takes place. When you load the default NOESY parameters (e.g. `rpar noesy.cryo all`) these delays are set to 4.0 sec. and 0.5 sec., respectively. These values are approximations that should give acceptable

results for most good-quality solutions of small molecules, however, these values are almost certainly not going to be optimum for any given sample.

If you do not get a usable spectrum using the default values, you may find it advantageous to re-run the NOESY spectrum using a different value of D8; the typical range of D8 values for small organic molecules is 0.1 to 0.8 seconds, with the upper limit of D8 being on the order of the T1 (spin-lattice relaxation time) of the proton resonance in question. The D1 value is related to the T1 relaxation times of the protons in your sample; ideally D1 should be 2*T1 to 4*T1. Proton T1's are usually 1 second to a few seconds, if your sample has very long T1's it might be worth lengthening the D1 value and re-running the spectrum. It is possible to determine the T1 values for each proton resonance in your sample, but this is time-consuming and usually not necessary. The final point regarding these variables is that it is not possible to predict the best values for a given sample, so it is not unusual to have to run multiple NOESY spectra to obtain the best results.

3. Data processing. As with the delay values, the standard NOESY parameter set includes processing parameters that should give reasonable results for most good-quality samples. In order to change the windowing functions for data processing, one needs to open the processing parameters menu (command edp). The default window function for both dimensions is Gaussian multiplication (GM), you can see this shown in the pulldown menu adjacent to the WDW entry in the columns for each dimension. The parameters GB and LB also are relevant. The parameter GB varies between 0 and 1, with lower values favoring sensitivity over resolution. The LB parameter controls the "intensity" of the Gaussian function, making the value more negative increases the strength of the function. If your sample shows only weak cross-peaks, it can be advantageous to re-process the data with slightly smaller values of GB in both F1 and F2 (e.g., changing GB from 0.3 to 0.2) and using slightly larger negative values of LB (e.g. changing from -10 to -12). Note that if you change any of these parameters and reprocess the spectrum (command xfb), the original 2D FID is not disturbed - you can reprocess your data as many times as you want. You may also care to try different windowing functions, with sine-squared (QSINE in the WDW choice pulldown) often being useful to improve the resolution of the 2D NOESY data. This change will reduce the signal-to-noise ratio, however.

It can happen also that the phasing of the 2D spectrum might need additional work beyond the simple method giving in the 2D-Training Guide. This is accessed via the phasing icon in the top icon bar of the 2D spectrum display. It is beyond the scope of this article to discuss the 2D phasing routine, suffice it to say that the TopSpin processing software manual is recommended reading if you care to try this. Also, facility staff can assist with the 2D phasing routine. Finally, I do not recommend using symmetrization (command syma) as a general rule. It can make the 2D spectrum look better, but it actually often makes the interpretation process more difficult.

Additional Reading

The Nuclear Overhauser Effect in Structural and Conformational Analysis, D. Neuhaus and M. Williamson, VCH, New York 1989 (ISBN 1-56081-616-3, paper).