PINMRF

Bruker AV-III / Avance DRX NMR Spectrometers running TopSpin

Training Supplement for Advanced 1D NMR Spectroscopy

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

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06-07-2011: First Edition - JSH.

Advanced 1D Spectra using Standard Acquisition Macros - Overview

This booklet provides the information necessary to run some advanced 1D NMR spectra on routine samples using PINMRF's Bruker Avance and AV-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 are set up using customized setup macros designed to work with PINMRF's dedicated parameter sets. These should 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 is unusual or demanding in some way, please contact PINMRF staff for assistance with more highly specialised experiments.

The experiments presented here include the following:

1. The ^{13}C DEPT experiment;
2. ^{1}H homonuclear decoupling;
3. ^{1}H nOe-difference spectroscopy;
4. peak suppression in ^{1}H NMR using presaturation.

Each experiment section includes all relevant instructions so that each one can be followed without referring to another experiment in this booklet. Note that some of these experiments require that probe tuning should be carried out on all relevant nuclei. See pp. 16-17 of the PINMRF manual **Bruker AV-III / Avance DRX NMR Spectrometers - 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 Avance and AV-series Bruker spectrometers running TopSpin.

Bruker AV-III / Avance DRX NMR Spectrometers - ^{13}C DEPT Instructions

For an overview of the DEPT experiment, see NOTE a) at the end of the instructions.

1. Run the conventional proton spectrum using the standard parameters as described elsewhere.
2. Process and phase the proton spectrum as normal.
3. If the proton spectrum is OK, it is advantageous to run the carbon-13 spectrum prior to running the DEPT spectrum. If time permits, run the carbon-13 spectrum using the standard parameters.
4. Process and phase the carbon-13 spectrum as normal.
5. Type `wrpa` followed by a new experiment number to copy the current dataset to a new dataset with the requested experiment number. For example, if the current dataset has the name "smellythiol" and the experiment number "2", typing `wrpa 3` will copy "smellythiol / 2" to "smellythiol / 3".
6. Type `re` followed by the new experiment number to go to the new dataset. Following the example above, type `re 3` to go to experiment number 3.
7. Check the tuning of both the proton and carbon-13 coils as described elsewhere.
8. Run the experiment with sample spinning ON.
9. In the new dataset, type `deptjsh` to convert the current experiment into the DEPT experiment. NOTE: if you did not run the carbon-13 spectrum as described above, you must read the correct C13 parameter set (using `rpar`) in the current experiment before executing the `deptjsh` command. A reminder message will show, click OK if you are ready to start. At this point, a dialog begins, and you should enter the following:

a) Enter type of DEPT experiment (45, 90 or 135): 135 (see NOTE a) below)
e) Enter number of scans: 16 (or any multiple of 8)
f) Enter number of dummy scans: 4
g) Enter average J value: 150 (default value, see NOTE c))

The data will be acquired automatically after the parameter input is complete (don't type `zg`).

10. After the acquisition completes process the data in the normal fashion. Keep in mind that the DEPT-135 will have both positive and negative peaks (see NOTE a)).

11. **COMPARING SPECTRA USING THE MULTIPLE DISPLAY MODE**: Go to the expno of the DEPT spectrum (e.g., `re 3`). To compare the reference ^{13}C and the DEPT spectra, click the multiple-display icon in the icon bar, or type `.md` . This will enter the multiple display mode with the current spectrum shown. Use the data browser to load the second data set by opening the directory icons for (1) the system NMR data directory, (2) your username's data directory, then (3) the name of the current dataset. Now drag the icon representing the expno of the reference ^{13}C dataset into the multiple-display window, where it will now be displayed along with the DEPT spectrum. Click the overlay icon to change the display between showing the spectra overlaid or separated. You can expand and manipulate both spectra together with the normal spectrum manipulation icons, or manipulate one independently of the other by separating their display, highlighting one spectrum's icon, then using the manipulation icons in the multiple-display icon bar. Please see the annotated screenshot on the next page.

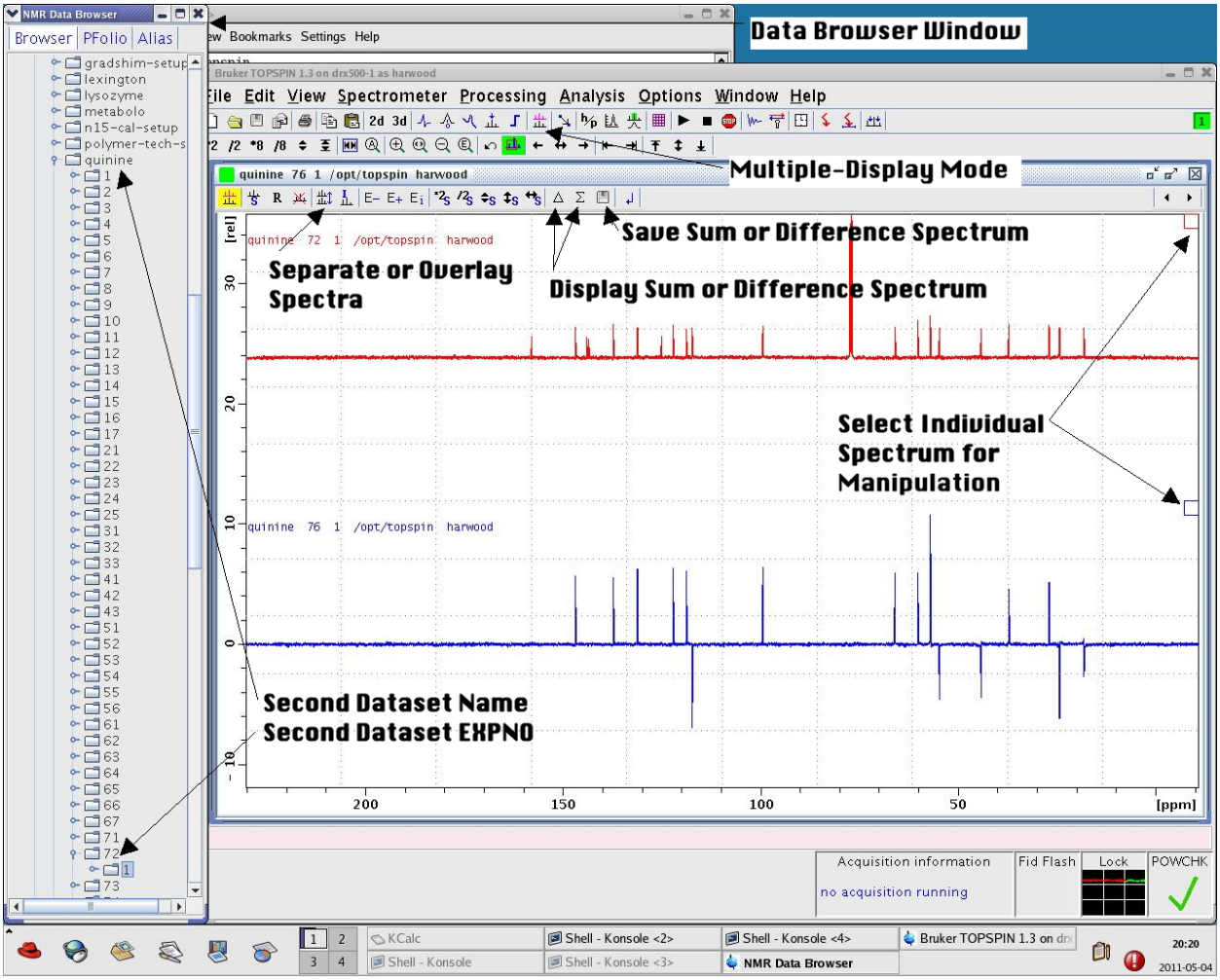
12. Repeat steps 5, 6 and 9, above, to acquire other DEPT (e.g. 45 or 90) spectra.

NOTES - DEPT:

a) The DEPT (Distortionless Enhancement by Polarization Transfer) experiment is a multipulse experiment that allows the determination of the different types of carbon atoms present in a molecule. The number at the end of the DEPT string (e.g. DEPT-45, DEPT-135) refers to the duration in degrees of the last ^{1}H pulse in the pulse sequence, which is the part of the pulse sequence which actually differentiates between the types of carbon atoms. The DEPT-45 experiment shows all of the protonated carbons with positive phase. The DEPT-90 experiment shows only the -CH- carbons, with positive phase. The DEPT-135 experiment shows $-CH_3$ and -CH- carbons with positive phase and $-CH_2-$ carbons with negative phase. Quaternary carbons do not appear in any DEPT spectrum. Appropriate combinations of the different DEPT spectra on a given molecule can provide for subspectra of each different type of carbon atom to be generated.

b) The sensitivity of the DEPT experiment is higher than that of a normal ^{1}H-decoupled ^{13}C spectrum, up to a factor of two. DEPT spectra by default are also ^{1}H-decoupled.

c) Other than the ^{1}H pulse duration, the only other variable in the DEPT pulse sequence is the one-bond ^{1}H-^{13}C J-coupling. Usually an approximate average value is used, which for most small organic molecules is on the order of 130 - 160 Hz.



Above: Fullscreen Screenshot of TopSpin 1.3 in Multiple-Display Mode

Bruker Avance / AV NMR Spectrometers - Proton Homonuclear Decoupling Instructions

21. Run the conventional proton spectrum using the standard parameters as described elsewhere.

22. Process and phase the proton spectrum as normal.

23. Type `wrpa` followed by a new experiment number to copy the current dataset to a new dataset with the requested experiment number. For example, if the current dataset has the name "smellythiol" and the experiment number "1", typing `wrpa 2` will copy "smellythiol / 1" to "smellythiol / 2".

24. Type `re` followed by the new experiment number to go to the new dataset. Following the example above, type `re 2` to go to experiment number 2.

25. **SET THE IRRADIATION FREQUENCY**: Expand the spectrum about the peak you want to irradiate. Click with the left mouse button on the set RF icon in the icon bar, or type `.set0123` . Then move the mouse pointer to the spectrum area (the pointer will jump to the spectrum) and place the cursor on the center of the peak you want to remove (saturate). Click the left mouse button to define this frequency as the decoupler frequency (parameter O2), then select O2 from the popup menu. Then click the return/save icon on the top of the spectrum window to exit.

26. Check the tuning of the proton coil as described elsewhere.

27. Run the experiment with sample spinning ON.

28. In the new dataset, type `homodecjsh` to convert the current experiment into the homodecoupling experiment. A reminder message will show, click OK if you are ready to start. Enter/confirm the parameters that are requested – usually the default values will work fine. The data will be acquired automatically after the parameter input is complete (i.e., there is no need to type `zg`).

29. After the acquisition completes process the data in the normal fashion.

30. Expand the decoupled peak to check for complete decoupling. If decoupling is not complete, adjust the decoupler power. To do this, type `pl24` and enter a new value that is 3 - 6 units lower numerically than the current value (i.e., if the current value is 45, enter 42 or 39). Do not enter a `pl24` value less (numerically lower) than 30! Repeat the experiment by re-running it using the `zg` command, then re-check for complete decoupling.

31. **COMPARING SPECTRA USING THE MULTIPLE DISPLAY MODE**: See step 11 for the DEPT experiment, above.

32. Repeat steps 3, 4 and 5, above, followed by the command `zg`, to acquire spectra with other peaks decoupled.

Bruker AV-III / Avance DRX Spectrometers - Proton nOe-Difference Experiment Instructions

Please read the notes at the end of these instructions for some discussion and hints regarding this experiment.

41. Run the conventional proton spectrum using the standard parameters as described elsewhere.
42. Process and phase the proton spectrum as normal.
43. Type `wrpa` followed by a new experiment number to copy the current dataset to a new dataset with the requested experiment number. For example, if the current dataset has the name "smellythiol" and the experiment number "1", typing `wrpa 2` will copy "smellythiol / 1" to "smellythiol / 2".
44. Type `re` followed by the new experiment number to go to the new dataset. Following the example above, type `re 2` to go to experiment number 2.
45. **PUT THE IRRADIATION FREQUENCIES INTO A FREQUENCY LIST**: Expand the spectrum about the peak(s) you want to irradiate. Click on the frequency list icon with the left mouse button, or type `.freqlist` . At this point, a popup window appears, which has two input fields (user input is underlined):

Left-hand side field	**Right-hand side field**
type of list (f1, f2, f3, etc)	name of list file
FQ1LIST (choose from pulldown)	usernoediff (e.g., "user" = your login ID)

Now move the mouse pointer to the spectrum area (the pointer will jump to the spectrum) and place the cursor in an area of the spectrum close to the peak(s) you want to irradiate, but not on any peak (i.e., in a baseline region). Click the left mouse button to define this frequency - a red box showing the frequency offset will show up above the spectrum at this point. This frequency will be used to define the reference spectrum that the other irradiated spectra will be compared to when generating the difference spectra. Now move the mouse pointer to the center of a peak you want to irradiate then click the left button again to add this frequency to the list. Repeat this

process for all the peaks of interest. When you have added all the peaks you want irradiated, click on the return/save icon above the spectrum window to terminate the frequency list input and save the list. Then, click Yes to save the list name to the current experiment. See NOTE a), below, for more information.

46. Check the tuning of the proton coil as described elsewhere.

47. Turn off sample spinning. Turn on the VT controller if your data acquisition will last longer than ca. 30 minutes.

48. In the new dataset (expno 2, in our example), type noediffjsh to convert the current experiment into the nOe difference experiment. A reminder message will show, click OK if you are ready to start. At this point, another dialog begins, and you should enter the following:

a) Name of frequency list:	usernoediff (as above)
	(where "user" = your login ID)
b) Irradiation time in seconds (= d1):	4 (default should be OK)
c) Irradiation power level	78 - 39 (see NOTE b) below)
d) # of irradiation points:	2 or more - check it's correct
e) # of scans:	8
f) # of dummy scans:	4
g) # of time average cycles:	1 (or more, see NOTE c) below)

The data will be acquired automatically after the parameter input is complete (there is no need to type zg). Various informational messages will show on the screen. Each irradiation frequency will be acquired in a new, sequential, expno.

49. After the acquisition completes process the data in the first expno in the normal fashion. Unless you have specifically moved between different expno's, this will be the current expno.

50. Type multiefp to start the processing macro. When requested, enter the current expno and the number of expno's to process (this should be the same as the number of irradiation points, above). The macro will carry out Fourier transformation and phase correction on all of the spectra in sequential expno's.

51. Go to the expno of one of the spectra with a peak that has been irradiated (e.g., re 3) Expand the irradiated peak to check for complete saturation (i.e., the selected peak should be

gone). If saturation is not complete, you will need to go back to the first expno of the nOe difference series (e.g. `re 2`) and re-run the experiment. When the "Irradiation power level:" question appears, enter a new value that is 3 - 6 units lower numerically than the initial value (i.e., if the current value is 60, enter 57 or 54). Do not enter a value less (numerically lower) than 30! Repeat the experiment to check the completeness of saturation. See NOTE b) for further discussion.

52. **COMPARING SPECTRA USING THE MULTIPLE DISPLAY MODE**: See step 11 for the DEPT experiment, above.

53. **OBTAINING THE DIFFERENCE SPECTRUM**: While still in the multiple display mode, click the difference icon (Δ) on the multiple-display icon bar left to display the difference of the two spectra displayed. The difference spectrum should show the irradiated peak with full negative intensity, and any peaks that show a nOe to this peak will show up as weak positive peaks. Peaks that are dispersive (up and down) are subtraction artifacts and cannot reliably be considered to be the result of a nOe interaction. If these are present they sometimes can be minimized by left-clicking on the horizontal-shift icon in the icon bar (if the spectra are separated) and moving the mouse left to right. This shifts the spectra relative to one another and sometimes can improve the quality of the subtraction. If you want to save the difference spectrum, click the save icon in the icon bar of the multiple display window, then enter the procno in which to save the difference spectrum (usually 2) in the popup window that appears. Click the return icon to leave the multiple display mode.

54. The difference spectrum can be manipulated like any spectrum. The dataset title is not stored with it so that information will have to be re-entered (`setti` command, or click the Title tab above the spectrum) prior to printing. The difference spectrum can be integrated, and I recommend setting the value of the integral of the irradiated peak to be -100 for peaks comprising one proton, -200 for those from two protons and -300 for those from three protons (e.g. methyl groups). The remaining integrals can then be read as percentages. This spectrum can be printed, but the plot settings in XWinPlot will need to be checked and/or adjusted.

55. To go back to one of the other spectra of the nOe difference series the correct procno must be specified. Thus, to go back to expno 2, enter `re 2 1` (read expno 2 procno 1) or `rep 1` (read procno 1 of current experiment number). See NOTE d), below, for more about moving between datasets when multiple procno's are in use.

NOTES:

a) In general, an nOe difference measurement will require at least two, ideally more, irradiation frequencies: the reference (off-resonance) frequency, and the peak of interest and its nOe partner(s). It is usually beneficial to obtain difference spectra with irradiation of all nOe-interacting partners.

b) When setting the irradiation power, keep in mind the following. Broader peaks require more power than do narrow ones, and peaks that are very close to another resonance should be irradiated with a minimum amount of power in order to reduce the possibility of partly irradiating another peak. In this case a longer irradiation time may be beneficial.

c) The nOe difference experiment set up presented in this discussion allows the entire series of irradiations to be repeated for signal averaging. For example, if you needed to run 64 scans for each irradiation, it could be done in one of two ways: the number of scans could be set to 64 (step 8e), and the number of cycles set to one (step 8g), or the number of scans could be set to eight (step 8e) and the number of cycles set to eight (step 8g). The latter setup (8 x 8) is recommended, as it will reduce the effects of any drifting or instability that might be present.

d) When working with datasets that have multiple expno's and multiple procno's within each expno, keep in mind that when using the `re` or `edc` (the latter is not recommended) commands to move between datasets, the correct procno for the target dataset must be set. My recommendation is to always use procno 1 for the target (e.g., type `re 3 1` instead of just `re 3`), which will guarantee that you will end up in the correct target expno. The command `rep` will read the requested procno within the current expno.

e) The sample quality is particularly important for nOe-based experiments. For more information on this, please see pp. 10 - 12 of the PINMRF manual Bruker Avance / AV NMR Spectrometers - Routine/Survey 2D Spectra using Standard Parameter Sets.

Bruker AV-III / Avance DRX Spectrometers - Proton Presaturation

61. Run the conventional proton spectrum using the standard parameters as described elsewhere.
62. Process and phase the proton spectrum as normal.

63. Type `wrpa` followed by a new experiment number to copy the current dataset to a new dataset with the requested experiment number. For example, if the current dataset has the name "smellythiol" and the experiment number "1", typing `wrpa 2` will copy "smellythiol / 1" to "smellythiol / 2".

64. Type `re` followed by the new experiment number to go to the new dataset. Following the example above, type `re 2` to go to experiment number 2.

65. **SET THE IRRADIATION FREQUENCY**: Expand the spectrum about the peak you want to irradiate. Click with the left mouse button on the set RF icon in the icon bar, or type `.set0123` . Then move the mouse pointer to the spectrum area (the pointer will jump to the spectrum) and place the cursor on the center of the peak you want to remove (saturate). Click the left mouse button to define this frequency as the decoupler frequency (parameter O2), then select O2 from the popup menu. Then click the return/save icon on the top of the spectrum window to exit.

66. Check the tuning of the proton coil as described elsewhere.

67. Depending on the intensity and broadness of the peak to be saturated, sample spinning may need to be ON or OFF. You will have to check this yourself by experimentation.

68. In the new dataset, type `presatjsh` to convert the current experiment into the presaturation experiment. A reminder message will show, click OK if you are ready to start. Enter/confirm the parameters that are requested – usually the default values will work fine. The data will be acquired automatically after the parameter input is complete (i.e., there is no need to type `zg`).

69. After the acquisition completes process the data in the normal fashion.

70. Expand the irradiated peak to check for complete saturation. If saturation is not complete (enough), adjust the decoupler power. To do this, type `pl14` and enter a new value that is 3 units lower numerically than the current value (i.e., if the current value is 45, enter 42). Do not enter a `pl14` value less (numerically lower) than 30! Repeat the experiment by re-running it using the `zg` command, then re-check the saturation efficiency.

71. Repeat steps 3, 4 and 5, above, followed by the command `zg,` to acquire spectra with other peaks irradiated.