

KJM5250 - Organic NMR Spectroscopy

Laboratory Experiments - part 2

Homonuclear 2D NMR Spectroscopy

COSY, TOCSY, NOESY, ROESY

(Xwinnmr 3.5 patchlevel 6, Windows XP)

**DPX200
DPX300
DRX500**



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COSY45 and COSYGPSW

CORrelation Spectroscop**Y** is a homonuclear 2D technique which is used to correlate the chemical shifts of ^1H nuclei which are J-coupled to each other. The information obtained is in principle the same as you get from 1D homodecoupling experiments. The 1D decoupling experiment is faster for small molecules but the COSY experiment is easier to set up and run. Before you can perform the COSY you must acquire a 1D ^1H NMR reference spectrum of your sample. More information is found in chapter 12 in the black Bruker Avance manual (pages 133-148).

A prerequisite for using this manual is that you know how to: **Login**, enter **password** and start the **xwinnmr**, remove the old sample, insert your sample, lock the sample on the actual solvent and shim the magnetic field with **Z¹** (**Z** on Lucy), **Z²**, **Z³** and **Z⁴**, if needed, stop and start the spinning of the sample and start and stop the automatic shimming.

If you need to reduce or increase the ppm scale (sweep width) of this experiment there is a section describing this for all of the experiments in this manual at the end of this manual.

After doing all this continue with:

1. Type **edc**↵ Type in the name of the experiment. Either the name of your synthesis product (FR7653) or for instance GROUP1PW. **Expno** is **1** and **procno** is **1**. Select disk unit **M:** or **D:**. **D:** is the local disk and M: is your home directory.
2. Type **rpar PROTON**↵ Choose **Copy all**.
3. Type **getprosol**↵
4. Type **ii**↵ Initialize interface. The computer checks that all parts of the spectrometer is on line.
5. Type **rga**↵ Receiver gain adjustment.
6. Type **zg**↵ Zero and go, starts the experiment.
7. Type **efp**↵ and **apk**↵ when efp is finished. Exponential multiplication, Fourier trans-forming and automatic phasing. Plot if you want a hard copy.
8. Type **rpar COSY***↵ A list of available COSY experiments comes up. Select **COSY45SW** followed by **getprosol**↵ on DPX200 and DPX300 . Select **COSYGPSW** or **COSY45SW** followed by **getprosol**↵ on the DRX500 instrument. If you are using the DRX 500 instrument

you should do both experiments. The teaching assistant will show you how to optimize the “offset” at the PGPA 10 unit (field gradient unit) on top of the DRX500 console.

9. Type **rga**↵ and wait until the automatic receiver gain adjustment is finished, then type **zg**↵ Starts the experiment. Type **expt**↵ and you are told how long the experiment takes. Change **ns** and or **TD/td** in F1 dimension (in **eda**) to reduce the time the experiment takes.
10. When the acquisition is finished type **xfb**↵ to Fourier transform the data in both dimensions. Click on +/- button until only positive peaks are seen. The commands **abs1**↵ and **abs2**↵ might improve the quality of the baseline in the two dimensions.
11. Another but dangerous way to improve the look of the spectrum is to type **sym**↵ in the command line.
12. Press **calibrate** place the pointer at the solvent diagonal peak and press the middle mouse button. Type in the shift value for the solvent and press ↵, you will do this two times.
13. The threshold value of the peaks on the screen can be changed by using the ***2** and **/2** buttons or the **◇** button together with the mouse. The spectrum can be displayed in three modes on the screen. **Intensities**, **contours** and **oblique**. Try them.
14. After defining the correct intensities (the spectrum does look ok on the screen) click on **defplot** to ensure that the chosen intensities are stored. Press ↵ for all three questions you are asked and do not change anything.
15. Type **edg**↵ The graphic parameter menu comes up. Click on **EDPROJ1**. Remember in which file the 1D Proton spectrum is located and fill in the corresponding **PF1DU (D:)**, **PF1USER**, **PF1NAME**, **PF1EXP** and **PF1PROC**. Click on **save**. Do the same for **EDPROJ2** and click on **save**.
16. Type **setti**↵ and type a title for the plot. Save and exit.
17. Type **view**↵ to get a preview on the screen of what is coming on the paper. Press **quit**.
18. If you need to increase the signal intensities of the 1D spectra you have to enter **edg** again and change **PF1CY** in **EDPROJ1** and **PF2CY** in **EDPROJ2**.
19. Type **plot**↵ if you are satisfied.

20. If you want expansions of the 2D spectra, enclose the area you want to expand with the help of the square button located on the left hand side of the panel with the help of the mouse. Click with the left mouse button on the upper left corner of the area to be expanded, keep the button depressed and drag the mouse down and to the right and let go of the button when the area is correct. Click with the right mouse button and the selected area is expanded. If you want the whole spectrum back - click on the **exp** button and then the **all** button. Ask for help if you don't understand this. After expanding and increasing or decreasing the threshold (by the ***2**, **/2** or the \diamond button and hold left mouse button pressed down and move the mouse up and down to increase or decrease the intensity of the peaks) click on **defplot** and answer the questions and type **plot** to get the spectrum out.

TOCSY.

Total **C**orrelation **S**pectroscop**Y** is a homonuclear 2D technique which is used to identify ^1H spin systems. In principle all members of a J coupled spin system couples with all the other members regardless of how many bonds there are between the protons as long as there are no breaks in the chain of CH, CH₂ and CH₃ groups. In practice you will only see coupling over a distance of five groups of protons. The longer the so-called spin-lock period/tocsy mixing time (d9) the more couplings are seen.

A prerequisite for using this manual is that you know how to: **Login**, enter **password** and start the **xwinnmr**, remove the old sample, insert your sample and shim the magnetic field with **Z¹** (**Z** on DPX300), **Z²**, **Z³** and **Z⁴**, if needed, stop and start the spinning of the sample and start and stop the automatic shimming and giving the experiment a file name with **edc**.

If you need to reduce or increase the ppm scale (sweep width) of this experiment there is a section describing this for all of the experiments in this manual at the end of this manual.

1. Type **edc** ↵ Type in the name of the experiment. Either the name of your synthesis product (FR7653) or for instance GROUP1TOCSY. **Expno** is **1** and **procno** is **1**. Select disk unit D: .
2. Type **rpar PROTON** ↵ **Copy all**.
3. Type **getprosol**.
4. Type **ii** ↵ Initialize interface.
5. Type **rga** ↵ Receiver gain adjustment.
6. Type **zg** ↵ Remove whatever is in the experiment and start.
7. Type **efp** ↵ and **apk** ↵ Exponential multiplication, Fourier transforming and automatic phasing. Plot if you want a hard copy.
8. Type **edc** ↵ Change **expno** to **2**. Click on **save**. Type **rga** ↵ acquire with **zg** ↵ and process **with efp** ↵ and **apk** ↵ to get the spectrum with reduced width.
9. Type **rpar MLEV*** A list of the available TOCSY experiments comes up. Choose **MLEVPHSW** for the DPX200 and DPX300 instruments. Choose **MLEVPHSW** or

MLEVGPPH19SW if you are using the DRX500 instrument. (As for the COSY experiments the GP (gradient experiment) is much faster.)

10. The teaching assistant will show you how to optimize the “offset” at the PGPA 10 unit (field gradient unit) on to of the DRX500 console.
11. Type **rga**↵ and wait until the receiver gain adjustment is done, then type **zg**↵ which starts the experiment. The acquisition time can be found with the command **expt**↵.
12. While the instrument is acquiring data you can set up the correct processing parameters in the **edp** menu. Type **edp**↵ Edit processing parameters.
13. When the acquisition is finished type **xfb**↵ to Fourier transform in both dimensions. When this is completed click on the +/- button and watch the difference between positive, negative and both positive and negative peaks. If all signals are displayed in only one color, click on colors and move the mouse upwards. Red colors represent positive values and violet represent negative values.
14. The threshold value of the peaks on the screen can be changed by using the ***2** and **/2** buttons or the **◇** button together with the mouse. The spectrum can be displayed in three modes on the screen. **Intensities**, **contours** and **oblique**. Try them and end up with intensities.
15. The TOCSY spectrum must be phased. Phasing of a 2D spectra is complicated and you must be careful and somewhat intelligent to succeed. Type **rser 2**↵ This means read serial file 2. A 1D FID comes up and is stored temporarily in ~TEMP 1 1.
(It is only for TOCSY and ROESY spectra that you use the serial file 2 for phasing, for all other 2Ds to be phased the **rser 1**↵ command shall be used to get the serial file 1.) Read more about this in the Avance manual page 171.
16. Type **sinm**↵ to enter a sine bell window function.
What is a sine bell window function? This is a good examination question.
17. Type **ft**↵ To Fourier transform the spectrum.
18. Manually phase the 1D spectrum. Click on **Phase**, Cursor, and tie the cursor on the spectrum by clicking on **biggest**. Click on **PH0** and use the mouse to phase the spectrum. Then click on **PH1** to phase the rest of the spectrum. Click on **return**, and also on **Save as 2D & return**. In this way the phase corrections you have made are stored in the TOCSY 2D data file.

19. Click on **2D** in left lower corner and the last 2D comes back (The TOCSY presumably). Type **xfb** to Fourier transform once more. The phasing along the F2 axis should now be better.
20. You must now perform phase correction in the F1 dimension. This is done in a different way. Click on the **phase** button. A new window with several small sub windows comes up. Click on the +/- button to get both positive and negative colors in the upper left window.
21. Click on **col** for columns, a new moveable cross comes up. The cross shall be used to define which columns are to be moved into the three windows on the right. The three columns moved shall then be used to get correct phase corrections in the other dimension.
22. Select a **col** to the right of the small spectrum by moving the mouse to a position, (where there are peaks outside the diagonal) click with the middle button to define the column. Click then with the left mouse button to get out of this submenu. Then click on **move: 1** to move the spectrum into the upper of the three windows.
23. Repeat this for a column in the middle of the spectrum with peaks outside the diagonal and move that to window 2. Continue with a column in the left side with crosspeaks outside the diagonal and move it to window 3. When all are moved scale them to a correct size with the ***2, /2** buttons at lower left corner. You must click on 1, 2 or 3 in the green windows to get the scaling to work.
24. Click then on window 1. Use **ph0** to phase (click on ph1 with the left mouse button, don't let go and drag the mouse up or down) the spectrum right side of the upper spectrum manually. Click then on window 3 and use **ph1** to phase the left side of the lower spectrum. Watch that all three spectra are phased at the same time and select the phasing corrections that give best overall result. All peaks shall be positive or as positive as possible. Click on **return. Save and return.** Confirm **xf1p** by clicking **OK**. The computer Fourier transforms in one dimension. The resulting spectrum should look better. If it not looking better try to move three **rows** into the green windows and phase them manually. Confirm **xf2p**. Then try **xfb** to phase in both dimensions. If still not OK go back to columns and then to rows. If you are phasing while still acquiring you must most probably phase again when the whole data set is acquired.
25. **abs1** and **abs2** can also be used to improve the baseline in the two dimensions.
26. Press **calibrate** place the pointer at the solvent diagonal peak and press the middle mouse button. Type in the shift value for the solvent and press **↵**, you will do this two times.

27. The threshold value of the peaks on the screen can be changed by using the ***2** and **/2** buttons or the \diamond button together with the mouse. The spectrum can be displayed in three modes on the screen. **Intensities, contours** and **oblique**. Try them.
28. After defining the correct intensities with ***2**, **/2** buttons or the \diamond button, click on **defplot** to ensure that the chosen intensities are stored. Press \downarrow for all of the three questions you are asked and change nothing.
29. Type **edg** \downarrow The graphic parameter menu comes up. Click on **EDPROJ1**. Remember in which file the 1D Proton spectrum is located and fill in the corresponding **PF1DU (D:)**, **PF1USER**, **PF1NAME**, **PF1EXP** and **PF1PROC**. Click on **save**. Do the same for **EDPROJ2** and click on **save**.
30. Type **setti** \downarrow , and write a title for your spectrum in the notepad that appears. Select **file-save** and **file-exit** to save and exit.
31. Type **view** \downarrow to get a preview on the screen of what is coming on the paper. Press **quit**.
32. If you need to increase the signal intensities of the 1D spectra you have to enter **edg** again and change **PF1CY** in **EDPROJ1** and **PF2CY** in **EDPROJ2**
33. Type **plot** \downarrow if you are satisfied.
34. If you want expansions of the 2D spectra, enclose the area you want to expand with the help of the square button located on the left hand side of the panel with the help of the mouse. Click with the left mouse button on the upper left corner of the area to be expanded, keep the button depressed and drag the mouse down and to the right and let go of the button when the area is correct. Click with the right mouse button and the selected area is expanded. If you want the whole spectrum back - click on the **exp** button and then the **all** button. Ask for help if you don't understand this. After expanding and increasing or decreasing the threshold (by the ***2**, **/2** or the \diamond button and hold left mouse button pressed down and move the mouse up and down to increase or decrease the intensity of the peaks) click on **defplot** and answer the questions and type **plot** \downarrow to get the spectrum out.
35. If you have time you ought to run the experiment twice with the tocsy mixing time delay **d9** to **60 ms** (default) and **200 ms** to see the difference this makes.

NOESY

Nuclear Overhauser Effect Spectroscopy identifies spin pairs undergoing cross-relaxation. The most common cross-relaxation "method" is magnetic dipolar coupling. The NOE "goes" through space and is extremely dependent upon distance. Noesy therefore gives information about which nuclei are close in space. The same information is obtainable from the 1D experiment NOEDIFF. Noediff is faster for small molecules. NOESY is used for large molecules with hundreds and thousands of correlations. For the regular user NOESY is simpler to set up and run since the Bruker NOEDIFF experiment is not easy to perform.

A prerequisite for using this manual is that you know how to: **Login**, enter **password** and start **xwinmr**, remove the old sample, insert your sample and shim the magnetic field with **Z¹** (**Z** on DPX300), **Z²**, **Z³** and **Z⁴**, if needed, stop and start the spinning of the sample and start and stop the automatic shimming and giving the experiment a file name with **edc**.

If you need to reduce or increase the ppm scale (sweep width) of this experiment there is a section describing this for all of the experiments in this manual at the end of this manual.

1. Type **edc** ↵ Type in the name of the experiment. Either the name of your synthesis product (FR7653) or for instance GROUP1NOE. **Expno** is **1** and **procno** is **1**. Select disk unit **D:** or **M:**. D: is the local disk and M: is your home directory.
2. Type **rpar PROTON** ↵ **Copy all.**
3. Type **getprosol** ↵
4. Type **ii** ↵ Initialize interface.
5. Type **rga** ↵ Receiver gain adjustment.
6. Type **zg** ↵ Zero and go.
7. Type **efp** ↵ and **apk** ↵ Exponential multiplication, Fourier transforming and automatic phasing. Plot if you want a hard copy.
8. Expand the area around the solvent peak, click on **calibrate**, place the arrow on top of the central peak, click the middle mouse button and enter the exact chemical shift value.
9. Type **edc** ↵ Change **Expno** to **2** and **Procno** to **1**. Click on **save**.
10. Type **rpar NOESY*** ↵

11. Select **NOESYPHSW** for **DPX200** and DPX300 spectrometers. Select **NOESPHSW** or **NOESYGPPH19SW** for the DRX500 spectrometer.
12. Optimization of **d8**. **d8** is the so called **mixing period** when the NOE is building up. This time is around one T1. **d8** is optimized in a 1D experiment below. Type **edc** and change **Expno** to **4** and **Procno** to **1**. Click on **save**.
13. Type **eda** and change **PARMODE** to **1D** and click on **save** and **OK** to delete some files.
14. Type **eda** again and the new 1D **eda** window shows up. Change **PULPROG** to **zg**, **NS** to **1** and **DS** to **0** and **TD** to **16k** (if you do not change this last parameter the 1D spectra will have to low resolution.). The pulse program is changed to **zg**, the number of scans to one and the dummy scans to zero. Click on **save**.
15. Type **rga** and wait until the adjustment of the receiver is finished, followed by typing **zg** and Fourier transform the FID with **ef**. Phase the spectrum manually (Phase, Biggest, PH0, PH1) and **save** the phasing corrections. Be aware that this phasing must be performed correctly since this phasing is to be used later on. **Return** followed by **Save and return**.
16. Type **eda** again and the new 1D **eda** window shows up. Change **PULPROG** to **t1ir1d** which is a T1 inversion recovery pulse program. Change **d7** (D7) to **500 ms**. The **d7** value here corresponds to the **d8** value used in the 2D experiment later on. Click on **done**, then on **save**.
17. Click on **Acquire** and **observe fid window** and type **gs**. The go setup routine is started, in which a whole lot of parameters can be optimized interactively. A new frame for a window comes up - click on it to fix it on the screen. Select the correct button for optimizing delays (**d7**).
18. Click on **Display** then **Show frequency domain** then **Display** once more, **Frequency domain phasing** and finally **Pk phasing**. You are watching the Fourier transformed data instantly during the **gs** routine.
19. While monitoring the Fourier transformed data on screen change **d7** by typing **d7** and change the value in the window that is showing up. With short **d7**'s all peaks are negative. With long **d7**'s all peaks are positive. Why? Find the value where the signals just have passed zero and are slightly positive. Type **stop** to get out of the go setup routine. Or use the command **kill** and select the **gs** process.

20. The **d7** value just found shall be used as **d8** in the NOESY experiment. Type **re 3 1**, the NOESY experiment setup returns. Type **d8** and enter the chosen value (for instance 750ms) followed by **↵**.
21. Press the **SPIN ON/OFF** button on the shim console to stop spinning the sample.
22. Type **rga** to allow the instrument to adjust the receiver for the chosen d8 value. Then type **zg** which starts the experiment. Type **expt** to see how long time it will take. The acquisition will take several hours depending on how long **d8** is. Take a 15 minutes break before continuing with the next point.
23. While the instrument is acquiring data you can set up the correct processing parameters in the **edp** menu. Type **edp** and the edit processing parameters window comes up.
24. When the acquisition is finished type **xfb** to Fourier transform in both dimensions.
25. The threshold value of the peaks on the screen can be changed by using the ***2** and **/2** buttons or the **◇** button together with the mouse. The spectrum can be displayed in three modes on the screen. **Intensities**, **contours** and **oblique**. Try them. When you are in the **Intensities** mode click on the **+/-** button to become used to see negative peaks, positive peaks, both positive and negative peaks and none. A common beginner's error for people working with small molecules is to phase and plot the NOESY with only negative or only positive peaks, getting a completely meaningless result plotted. Select both positive and negative values before going on.
26. The NOESY spectrum must be phased. Phasing of 2D spectra are complicated and you must be careful and somewhat intelligent to succeed. Type **rser 1** (It is only for TOCSY and ROESY spectra that you use the serial file 2 for phasing, for all other 2Ds to be phased the **rser 1** command shall be used to get the serial file 1.) Read more about this in the Avance manual page 171.
27. Type **sinm** to enter a sine bell window function.
What is a sine bell window function? This is a good examination question.
28. Type **ft** to Fourier transform the spectrum.
29. Manually phase the 1D spectrum. Click on **Phase**, **Biggest**, and phase around the biggest peak by clicking **PH0** and use the mouse to phase the spectrum. Then click on **PH1** to phase the rest of the spectrum. Click on **return**, **Save as 2D & return**. In this way the phase corrections you have made are stored in the NOESY 2D data file.

30. Click on 2D in left lower corner and the last 2D comes back (The NOESY presumably). Type **xfb** ↵ to Fourier transform once more. The phasing along the F2 axis should now be better.
31. You must now perform phase correction in the F1 dimension. This is done in a different way. Click on the **phase** button. A new window with several small sub windows comes up.
32. Click on **col** for columns, a new cross comes up. The cross shall be used to define which columns are to be moved into the three windows on the right. The three columns moved shall then be used to get correct phase corrections in the other dimension.
33. Select a **col** to the right of the small spectrum by moving the mouse to a position, (containing a diagonal peak) click with the middle button to define the column. Click then with the left mouse button to get out of this submenu. Then click on **1** to move the spectrum into the upper of the three windows.
34. Repeat this for a column in the middle of the spectrum with a diagonal peak and move that to window 2. Continue with a column in the left side containing diagonal peaks and move it to window 3.
35. Click then on **window 1** (the upper one). Click on **Biggest** then click on **PH0** and phase the spectrum manually. Click then on **PH1** and phase the rest of the spectrum, especially the part which is farthest away from the diagonal in window 1, that is the diagonal peak in window 3. Watch that all three spectra are phased at the same time and select the phasing corrections that gives best overall result. Click on **Save and return**. Confirm **xf1p** by clicking **OK**.

If you are not satisfied please phase the rows manually just as you phased the columns.

If you are still not satisfied read on pages 154 - 155 in the Avance manual for instructions about phasing **rows**. (F2 dimension- xf2p). Be aware that the Avance manual is written for an earlier version of the software and some symbols might have different names. When NOESY is phased correctly all diagonal peaks will all be either negative or all positive. Crosspeaks can be both negative and positive. Why? There can also be COSY artifacts which is seen as anti phase peaks.

36. After defining the correct intensities with ***2**, **/2** buttons or the **◇** button, click on **defplot** to ensure that the chosen intensities are stored. Press ↵ for all the questions you are asked and do not change anything. At this stage it is time to stop for a moment and think, were you asked about both positive and negative levels and the number of contours? If not you must have done something wrong. Both positive and negative contours must be defined - and for instance 15 different contour level chosen for positive peaks and 3 for negative to ensure easy selection

between them when plotted on a black and white plotter/printer. Click on the +/- button followed by **defplot**, repeat this two step procedure until both negative and positive contours are defined.

37. Type **edg**↵ The graphic parameter menu comes up. Click on **EDPROJ1**. Remember in which file the 1D Proton spectrum is located and fill in the corresponding PF1 Name, PF1 Experiment no. and PF1 Processno. (For instance GR1NOESY, 2, 1). Click on **save**. Do the same for **EDPROJ2** and click on **save**.
38. Type **setti**↵, and write a title for your spectrum in the notepad that appears. Select **file-save** and **file-exit** to save and exit.
39. Type **view**↵ to get a preview on the screen of what is coming on the paper. Press **quit**.
40. Type **edg**↵ The graphic parameter menu comes up. Click on **EDPROJ1**. Remember in which file the 1D Proton spectrum is located and fill in the corresponding **PF1DU (D:)**, **PF1USER**, **PF1NAME**, **PF1EXP** and **PF1PROC**. Click on **save**. Do the same for **EDPROJ2** and click on **save**.
41. If you need to increase the signal intensities of the 1D spectra you have to enter **edg** again and change **PF1CY** in **EDPROJ1** and **PF2CY** in **EDPROJ2**
42. Type **plot**↵ if you are satisfied.
43. If you want expansions of the 2D spectrum, enclose the area you want to expand with the help of the square button located on the left hand side of the panel with the help of the mouse. Click with the left mouse button on the upper left corner of the area to be expanded, keep the button depressed and drag the mouse down and to the right and let go of the button when the area is correct. Click with the right mouse button and the selected area is expanded. If you want the whole spectrum back - click on the **exp** button and then the **all** button. Ask for help if you don't understand this. After expanding and increasing or decreasing the threshold (by the ***2**, **/2** or the \diamond button and hold left mouse button pressed down and move the mouse up and down to increase or decrease the intensity of the peaks) click on **defplot** and answer the questions and type **plot**↵ to get the spectrum out.

ROESY

Rotating-frame Overhauser Effect Spectroscopy measures the homonuclear NOE effects under "spin-lock" conditions. ROESY is especially valuable since ROESY crosspeaks are always positive and there is no risk of getting crosspeaks of zero intensities. In NOESY there exist the possibility that $\omega \cdot t_c$ is about 1 and if this condition is fulfilled the NOESY crosspeaks are zero even if the protons are close in space. Both COSY and TOCSY peaks can sometimes be seen and if you don't realize it you might be in trouble. COSY peaks in the ROESY experiment have anti phase appearance, which means that there exist positive and negative parts of the same peak. ROESY peaks are only positive. Peaks impossible to phase (see several pages later) are therefore probably COSY peaks. TOCSY cross peaks are identified most easily since they have the same phase as the diagonal peaks which are negative relatively to the ROESY cross peaks.

A prerequisite for using this manual is that you know how to: **Login**, enter **password** and start **xwinnmr**, remove the old sample, insert your sample and shim the magnetic field with **Z¹** (**Z** on Lucy), **Z²**, **Z³** and **Z⁴**, if needed, stop and start the spinning of the sample and start and stop the automatic shimming and giving the experiment a file name with **edc**.

If you need to reduce or increase the ppm scale (sweep width) of this experiment there is a section describing this for all of the experiments in this manual at the end of this manual.

1. Type **edc** ↵ Type in the name of the experiment. Either the name of your synthesis product (FR8764) or for instance GR1ROESY. **Expno** and **procno** are both **1**.
2. Type **rpar PROTON** ↵ **Copy all**.
3. Type **getprosol** ↵
4. Type **ii** ↵ Initialize interface.
5. Type **rga** ↵ Receiver gain adjustment.
6. Type **zg** ↵ Zero and go.
7. Type **efp** ↵ and **apk** ↵ Exponential multiplication, Fourier transforming and automatic phasing. Plot if you want a hard copy.
8. Expand the area around the solvent peak, click on **calibrate**, place the arrow on top of the central peak, click the middle mouse button and enter the exact chemical shift value.

9. Type **edc** and Change **Expno** to **2** and keep **Procno** as **1**. Click on **save**.
10. Type **rpar ROESY*** Select ROESYPHSW.
11. Check the value of **p15** either in eda or by typing **p15** It should be of the same length as **d8** in the NOESY experiment. If this experiment is run on a sample where you have not acquired a NOESY spectrum please look at the NOESY manual to see how you shall determine d8 (d7 value in a t1ir experiment).
12. Type **rga** and wait until the receiver is adjusted for the selected parameter then type **zg** which starts the experiment. The acquisition will take several hours depending especially upon the length of **p15** and the values for **ns** and **td**.
13. While the instrument is acquiring data you can set up the correct processing parameters in the **edp** menu. Type **edp** and the edit processing parameters window shows up.
14. When the acquisition is finished type **xfb** to perform the Fourier transformation in both dimensions.
15. The threshold value of the peaks on the screen can be changed by using the ***2** and **/2** buttons or the \diamond button together with the mouse. The spectrum can be displayed in three modes on the screen. **Intensities**, **contours** and **oblique**, the last option gives you the possibility to look at the spectrum from many sides by changing the Θ and Φ angles. Try them. Click on the **+/-** button until both positive and negative peaks are displayed. This must be done since the diagonal peaks have opposite sign of the crosspeaks. Go back to **intensities**, both positive and negative contours must be defined to ensure good phasing below.
16. The ROESY spectrum must be phased. Phasing of 2D spectra are complicated and you must be careful and somewhat intelligent to succeed. Type **rser 2** (It is only for TOCSY and ROESY spectra that you use the serial file 2 for phasing, for all other 2Ds to be phased the **rser 1** command shall be used to get the serial file 1.) Read more about this in the Avance manual page 171.
17. Type **sinm** to enter a sine bell window function.
What is a sine bell window function? This is a good examination question.
18. Type **ft** To Fourier transform the spectrum.
19. Manually phase the 1D spectrum. Click on **Phase, Biggest**, and phase the biggest peak. By clicking on **ph0** and use the mouse to phase the spectrum. Then click on **ph1** to phase the rest

- of the spectrum. Click on **return**, **Save as 2D & return**. In this way the phase corrections you have made are stored in the ROESY 2D data file.
20. Click on 2D in left lower corner and the last 2D comes back (The ROESY presumably). Type **xfb** to Fourier transform once more. The phasing along the F2 axis should now be better.
 21. You must now perform phase correction in the F1 dimension. This is done in a different way. Click on the **phase** button. A new window with several small sub windows comes up.
 22. Click on **col** for columns, a new cross comes up. The cross shall be used to define which columns are to be moved into the three windows on the right. The three columns moved shall then be used to get correct phase corrections in the other dimension.
 23. Select a **col** to the right of the small spectrum by moving the mouse to a position, containing a diagonal peak) click with the middle button to define the column. Click then with the left mouse button to get out of this submenu. Then click on **1** to move the spectrum into the upper of the three windows.
 24. Repeat this for a column in the middle of the spectrum with diagonal peak(s) and move that to window 2. Continue with a column in the left side with diagonal peak(s) and move it to window 3.
 25. Click then on window 1. Click on **PH0** and phase the spectrum manually. The diagonal peak in window one shall be phased correctly. Click then on **PH1** and phase the rest of the spectrum, the diagonal peak in window 3 farthest from the diagonal shall be phased correctly. Watch that all three spectra are phased at the same time and select the phasing corrections that give best overall result. Click on **return** in the lower left corner and **Save and return** in the little window that comes up. Confirm **xf1p** by clicking **OK**. If you are not satisfied read on page 164 in the Avance manual about how to continue phasing of the **rows**.
 26. Type **edg** The graphic parameter menu comes up. Click on **EDPROJ1**. Remember in which file the 1D Proton spectrum is located and fill in the corresponding **PF1DU (D:)**, **PF1USER**, **PF1NAME**, **PF1EXP** and **PF1PROC**. Click on **save**. Do the same for **EDPROJ2** and click on **save**.
 27. Type **setti**, and write a title for your spectrum in the notepad that appears. Select **file-save** and **file-exit** to save and exit.
 28. Type **view** to get a preview on the screen of what is coming on the paper. Press **quit**.

29. If you need to increase the signal intensities of the 1D spectra you have to enter **edg** again and change **PF1CY in EDPROJ1 and PF2CY in EDPROJ2**
30. Type **plot** if you are satisfied.
31. If you want expansions of the 2D spectrum, enclose the area you want to expand with the help of the square button located on the left hand side of the panel with the help of the mouse. Click with the left mouse button on the upper left corner of the area to be expanded, keep the button depressed and drag the mouse down and to the right and let go of the button when the area is correct. Click with the right mouse button and the selected area is expanded. If you want the whole spectrum back - click on the **exp** button and then the **all** button. Ask for help if you don't understand this. After expanding and increasing or decreasing the threshold (by the ***2**, **/2** or the \diamond button and hold left mouse button pressed down and move the mouse up and down to increase or decrease the intensity of the peaks) click on **defplot** and answer the questions and type **plot** to get the spectrum out.

If you need to reduce (or in some rare instances increase) the ppm scale (sweep width) of the Homonuclear experiments read here:

1. Acquire a 1D ^1H experiment as described many times before.
2. Calibrate the spectrum. Expand the area around the solvent peak, click on **calibrate**, place the arrow on top of the central peak, click the middle mouse button and enter the exact chemical shift value.
3. Reduce the spectral width so that the ^1H signals almost covers the whole window. Click on the **sw-sfo1** button twice – due to a bug in Xwinnmr it must be done twice, if you do it only once no change takes place. You have now reduced the spectral width to what was selected on the screen and set the radio sender to the middle of the new spectral window.
4. Write down the two values that are shown underneath the xwinnmr command line: SW=?, O1=??
5. Increase the experiment number and load the 2D parameter set (rpar “something”, getprosol and so forth).
6. Type **sw** in the xwinnmr command line and enter the new value in the window that comes up.

7. Type **o1** in the xwinmr command line and enter the new value in the window that comes up.
8. You have now changed parameters for the F2 dimension and have to move on to the F1 dimension. Type **eda** and change **sw** or **swh** in the F1 column to the same value as in the F2 column (should be the value you entered in #6 above).
9. Observe that FIDRES (digital resolution) in F1 changes when you enter the new value of sw. FIDRES is given as the ratio sw/td. The digital resolution in F2 is much better (smaller) than in F1. Why is it so?
10. The acquisition time has to be checked. Type **aq** in the command line. Check the **aq** time in a standard non changed experiment and change accordingly if the acquisition time seems wrong. TD changes as AQ changes. What is the relationship between TD and AQ?
11. Before running the experiment an new **rga** must be performed.
12. If the ppm scale wide enough but is covering too much space on one side and too little on the other you can move **o1/o1p** to the left or right to set a new center. Do this in **eda**
13. If the **sw** is too small you have to increase it in **eda**.
14. If changing anything related to **sw** and **o1** values in **eda** the ppm scales when processing will most likely be wrong on the spectrum (and in **edp**).
15. The simplest way to correct this is when you know the ppm value (the value is the same in both dimensions in homonuclear experiments) of one peak from the 1D ¹H spectrum. Simply click on **calibrate** and use the cursor and the middle mouse button to set the value.
16. If you have no idea about the shift values you should open **edp** and find the parameter **offset**. Offset is the value of the left hand side of the ¹H spectrum. Find this value from a calibrated ¹H spectrum and enter it.
17. If you know how to do it, you might now adjust the acquisition **aq** time and the **si** to appropriate values.