

# KJM4250 - Organic NMR Spectroscopy

## Laboratory Experiments - part 3

Heteronuclear  $^{13}\text{C}$  detected 2D NMR Spectroscopy.

XHCORR (HETCOR) and COLOC

Heteronuclear  $^1\text{H}$  detected 2D NMR Spectroscopy.

HMQC, HSQC, HMBC

H2BC (HSQC-COSY)

(Xwinmr 3.5 patchlevel 6, Windows XP)

**DPX200**  
**DPX300**  
**DRX500**



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## **XHCORR (HETCOR), COLOC, HMQC, HSQC, HMBC and H2BC (HSQC-“COSY”).**

**This manual is a general manual for a whole lot of different experiments. The main practical difference between the various experiments is the “rpar” file chosen and also variations in processing details.**

Heteronuclear (**X, H**) shift correlation NMR-spectroscopy is a 2D technique which is used to determine which  $^1\text{H}$ 's of a molecule that are bonded to which  $^{13}\text{C}$  atom (or another X nuclei) in the molecule. Those off you that are used to Varian terminology are probably used to the term **HETCOR** for this experiment. Bruker is using the acronym HCCOS name for this experiment. Before you can perform the **HCCOS** experiment you must acquire a 1D  $^1\text{H}$  NMR reference spectrum of your sample and a 1D  $^{13}\text{C}$  spectrum or a DEPT 45 spectrum as reference spectra.

**C**orrelation spectroscopy via **L**ong-range **C**oupling (**COLOC**) is a 2D NMR-technique which is used to determine which  $^1\text{H}$ 's of a molecule that are bonded to which  $^{13}\text{C}$  atoms (or another X nuclei ( $^{15}\text{N}$  for instance)) in the molecule. In XHCORR large one bond coupling constants are used to observe the correlation peaks. In COLOC the experiment is optimized for small coupling constants so that 2 and 3 bond couplings are observed as well as the one bond coupling. It is therefore not advisable to only run a COLOC spectrum - you must also have the regular hetcor spectrum to identify the one bond couplings seen in the COLOC experiment. Those off you that are used to Varian terminology are probably used to the term “long range HETCOR” for this experiment. Before you can perform the COLOC experiment you must acquire a 1D  $^1\text{H}$  NMR reference spectrum of your sample and a 1D  $^{13}\text{C}$  spectrum. (Why can you not use a DEPT 45 spectrum as the reference spectrum in this experiment?)

**H**eteronuclear **M**ultiple **Q**uantum **C**orrelation experiments (HMQC) and **H**eteronuclear **S**ingle **Q**uantum experiments (HSQC) are 2D techniques are used to obtain the same structural information as the XHCORR experiment. HMQC and HSQC experiments are however detected on the  $^1\text{H}$  atoms contrary to the HETCOR experiment where the detection is on  $^{13}\text{C}$ .  $^1\text{H}$  detection is much more sensitive than  $^{13}\text{C}$  detection. In addition are the HMQC and HSQC experiments described in this manual for the DRX500 instrument speeded up even more since they are used with **Gradient Pulses** (GP). Read more about this in textbooks. In gradient HMQC/HSQC no phase cycling is needed, this means that if you have lots of substance you need only one scan ( $\text{NS} = 1$ ) in the F2 dimension for each F1 increment. HSQC is much more sensitive to bad shim settings than HMQC. The indirect dimensions in both XHCORR ( $\text{F1} = ^1\text{H}$ ) and HMQC/HSQC ( $\text{F1} = ^{13}\text{C}$ ) is having low resolution. This means that for aromatic compounds with lots of resonances close to each other in the  $^{13}\text{C}$  dimension the XHCORR is the recommended experiment, if you have enough sample and instrument time.

HMBC is the  $^1\text{H}$  detected version of COLOC ( $^{13}\text{C}$  detected). As for the COLOC experiment you will also see the unwanted direct  $^1\text{H}$ - $^{13}\text{C}$  couplings in HMBC. H2BC experiments are optimized for two

bond correlations between  $^1\text{H}$  and  $^{13}\text{C}$ . H2BC is very insensitive and the experiment needs a long acquisition time. The parameter set and pulse program used in this manual is a modified version adapted from Bruker-Biospin in Switzerland as well as from the Carlsberg NMR laboratory <http://www.crc.dk/nmr/nmrpulse/experiments/small/h2bc/index.shtml> On the Carlsberg www pages it is stated: “A two-dimensional NMR-pulse sequence for long-range correlation of  $^1\text{H}$  and  $^{13}\text{C}$  nuclei. The experiment has several attractive features compared to the widely used HMBC experiment. Typically strong enhancement of correlations over two bonds while suppressing those over more bonds. Independence of long-range  $^1\text{H}$ - $^{13}\text{C}$  coupling constants, full homo- and heteronuclear decoupling in the indirect dimension and heteronuclear decoupling in the acquisition dimension, pure 2D absorption peak shapes, a pulse sequence duration significantly shorter than that of HMBC. The experiment is quite complementary to HMBC and does not effect correlations to quaternary carbons that must be obtained by HMBC.”

The information obtained from a H2BC spectrum together with the information from a HSQC (or HMQC) spectrum (one bond correlations) can be looked upon as a pseudo INADEQUATE spectrum, however only Carbons with protons attached will be included in the INADEQUATE “walk”. Please look for details and an example at the Calsberg www pages.

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 **Z<sup>1</sup>** (**Z** on Lucy), **Z<sup>2</sup>**, **Z<sup>3</sup>** and **Z<sup>4</sup>**, if needed, stop and start the spinning of the sample and start and stop the automatic shimming.

#### 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 **D:** or **M:**. **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.

7. Type **efp** and **apk** when **efp** is finished. 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 keep **procno** to **1**. Click on **save**.
10. Type **rpar** and select a carbon parameter set with proton decoupling (**C13CPD**) and click on **copy all**. Alternatively select a DEPT45 experiment. DEPT gives higher S/N and is faster but does not give the quaternary carbons. Do you think you need them in this experiment? If you are doing these experiments on the DRX500 instrument with the TXI probe in the magnet you have to obtain the  $^{13}\text{C}$  spectrum from the DPX300 instrument and move it over to the DRX500 instrument or use the **edg** menu to define the location of that spectrum on another computer when plotting.
11. Type **getprosol**
12. Type **ii** Initialize interface. The computer checks that all parts of the spectrometer is on line. Type **rga** Receiver gain adjustment. Type **zg** Zero and go. Type **efp** and **apk** when the Fourier transformation is finished. Plot if you want a hard copy.
13. 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.
14. Type **edc** Change **Expno** to **3** and keep **Procno** as **1**. Click on **save**.
15. Type **rpar** **HCCOSW** (DPX200 and DPX300), **HCCOLOCSW** (DPX200, DPX300) **HMQC** (DPX200 DPX300), **HMQCGP** (DRX500), **HMBCGPND** (DRX500), **HSQCGP** (DRX500), **HSQCETGP** (DRX500), **HSQCEDETGP** (DRX500) and when you have tested most of these you can test **H2BCETGP.dp** (DRX500) if you have at least 50 mg of your substance and 12 hours of instrument time. **ET** in these names “ means “phase sensitive using the Echo/Antiecho-TPPI-method”. **ED** means edited =  $\text{CH}_2$  negative blue colors and the rest positive with red colors. **HSQCGP** has only positive correlation peaks. The inverse  $^1\text{H}$  detected experiments, except **H2BCETGP.dp**, are fast, and both groups of students doing experiments on the same week day in the KJM5250 course must cooperate and exchange samples and perform experiments on both instruments. One dimensional  $^{13}\text{C}$  experiments are to be run on the DPX300 instrument and the inverse ( $^1\text{H}$  detected 2D experiments on the DRX500 instrument. The teaching assistant will perform tuning and matching on the DRX500

instrument each time a sample is changed, as well as showing you how to optimize the “offset” at the PGPA 10 unit (field gradient unit) on top of the DRX500 console. You **will** also perform **zero filling** and **linear prediction** on all of the 2D data sets (see separate manuals).

16. The acquisition time parameter sets above are defined with a minimum numbers of scans (NS) and a low number of increments in the indirect dimension. Use **expt**↵ to see the suggest experiment time and use **eda**↵ to see the actual numbers for **NS** and **TD (F1)** in each experiment. For **HMQCGP** ncreasing **NS** to **32** and **TD (in F1)** to 256 from the default value on the DRX500 instrument increases the time to 4h and 18 min. Similarly **HMBCGPND** takes 2h 9 min with NS 16 and TD (F1) 256. **HMQCGP** takes 1h and 51 min with NS 16 and TD (F1) 256. **HSQCEDETGP** with NS 16 and TD (F1) 256 takes 1h and 55 min. **HSQCETGP** takes 1h and 55min with NS 16 and TD (F1) 256. **HSQCGP** with NS 16 TD (F1) 256 takes 1h and 56min. **H2BCETGP.dp** takes 57min with the default values of NS 8 and TD (F1) 256. This experiment has a very low sensitivity and for instance NS 128 and TD (F1) 512 gives 1 day, 7h and 23 minutes as acquisition time.
17. Type **rga**↵ and wait until the receiver adjustment is performed, then type **zg**↵ which starts the experiment. The acquisition might take several hours.
18. When the acquisition is finished type **xfb**↵ to Fourier transform the data in both dimensions for all experiments except for H2BC experiments where the command **xf2m**↵ is used. Click on +/- button several times.
19. In this section *tips* and *tricks* for processing the various 2D experiments described in this maual are given. Processing can generally be done with the command **xfb**↵. **abs1**↵ and **abs2**↵ can often flatten rolling baselines in the two dimensions. For **HMQCGP** and **HMBCGPND** you should test out different window functions – **WDW – SINE and QSINE** and change **SSB (F2)** and **SSB (F1)** between the numbers 0, 1 and 2 to find the best processing parameters. These parameters can be seen in the **edp** window.
20. The three **HSQC** experiments: **HSQCEDETGP**, **HSQCETGP** and **HSQCGP** are a little complicated to process. All # from 20 through # 37 deals with these complications. 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. Select both positive and negative values before going on.

21. The **HSQC** spectra must be phased. Phasing of 2D spectra is complicated and you must be careful and somewhat intelligent to succeed. In module 1 in the course KJM-MEF 4010 you have already been trained in phasing HSQCs. HSQC's should be phased in both dimensions. You will first perform phase correction in the F2 dimension. Click on the **phase** button. A new window with several small sub windows comes up. Click on **row**, a new cross comes up. The cross shall be used to define which rows are to be moved into the three windows on the right. The three rows moved shall then be used to get correct phase corrections in the other dimension.
22. Select a **row** (containing a peak) high up in the small spectrum by moving the mouse to a position, and click with the middle button to define the row. Click then with the left mouse button to get out of this submenu. Find the area with a shaded move button and click on **1** to move the spectrum into the upper of the three windows.
23. Repeat this for a row in the middle of the spectrum with a peak and move that to window 2. Continue with a row in the lower part of the spectrum and move it to window 3.
24. If the three spectra to the right are of wrong intensity adjust with the \*2 and /2 buttons on the lower left side. After first clicking with the mouse in the corresponding window to define which spectrum you want to change.
25. Click then on **window 1** (the upper one). Click on the shaded button named big then click on **ph0** and phase the spectrum manually by moving the mouse up or down while holding the left mouse button depressed. Click then on **ph1** and phase the rest of the spectrum, especially the part which is farthest away from the spectrum in window 1. Watch that all three spectra are phased at the same time and select the phasing corrections that gives the best overall result. Click on **Return** and then **Save and return**. And OK start xf2p
26. Type **xfb** ↵ to Fourier transform in both dimensions. The spectrum should now look a lot better. You may have to adjust the intensities. Sometimes it is enough to phase the rows for a good result. Use your head when deciding this. If you are not pleased with the result, go on to phase the columns. This is done the same way as the rows, except that you select columns instead of rows.
27. You must now perform phase correction in the F1 dimension. Click on the **phase** button. A new window with several small sub windows comes up. Click +/- to get both positive and negative colors.

28. 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.
29. Select a **col** to the right of the small spectrum by moving the mouse to a position, (containing a 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.
30. 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 peaks and move it to window 3.
31. Click then on **window 1** (the upper one). Click on **big** then click on **ph0** and phase the spectrum manually. Click then on **ph1** and phase the rest of the spectrum, especially the peak in window 3. Watch that all three spectra are phased at the same time and select the phasing correction that gives best overall result. Click on return then click on **Save and return** and OK "start xfb?"
32. You must now perform phase correction in the F1 dimension. Click on the **phase** button. The window with several small sub windows comes up.
33. Click on **col** for columns, a 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.
34. Select a **col** to the right of the small spectrum by moving the mouse to a position, (containing a 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.
35. 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 peaks and move it to window 3.
36. 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 peak in window 3. Watch that all three spectra are phased at the same time and select the phase correction that gives best overall result. Click on **Save and return** and OK "start xfb2".
37. Type **xfb** ↵ to Fourier transform in both dimensions.

38. For the **H2BC** experiment (rpar file H2BCETGP.dp) use **xf2m** to Fourier transform in both dimensions. The **m** in **xf2m** means magnitude mode – all signal intensities regardless of sign (+ or -) are displayed in the positive direction.
39. 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. You must have the corresponding values ready from the calibrated <sup>1</sup>H and <sup>13</sup>C one dimensional spectra.
40. After defining the correct intensities click on **defplot** to ensure that the chosen intensities are stored before plotting.
41. Type **edg** The graphic parameter menu comes up. Click on **EDPROJ1**. Remember in which file the 1D Proton spectrum or the 1D <sup>13</sup>C or DEPT experiment is located and fill in the corresponding PF1 Name, PF1 Experiment no. and PF1 Processno. (For instance GR1HET, 2, 1). Click on **save**. Be aware that F2 is related to the acquisition nucleus (<sup>1</sup>H for inverse experiments and <sup>13</sup>C in the “older” COLOC and HETCOR experiments). In other word for the F1 axis you must define the location of the 1D spectrum related to the indirect dimension of your experiment.
42. Do the corresponding procedure for **EDPROJ2** (For instance GR1HET, 4, 1) and click on **save**.
43. Type **setti**, and write a title for your spectrum in the notepad document that appears. Select **file-save** and **file-exit** to save and exit.
44. Type **view** to get a preview on the screen of what is coming on the paper. Press **quit**.
45. Most likely the square containing the 2D spectrum is placed wrongly on the paper. See # 48 below.
46. Type **edo** to check which plotter to use.
47. Type **plot** if you are satisfied.
48. If you are not satisfied you have to enter **edg** to change whatever is wrong. Often the 2D spectrum does not have “space” on the left hand side to include the 1D spectrum and you have to make this space available. Suggested parameters to change; **PROJ1** (to yes), **EDPARM** (moving parameter section around), **EDCONT**P and change **CX2** from 20 cm to 15 cm. You have to try out a lot of things here.

49. 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  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.