2-D HMBC / CIGAR

IU NMR Facility - August 31, 2004

Summary:

HMBC and its variants are some of the most powerful 2-D NMR experiments for structure elucidation. HMBC is related to HMQC but one bond H-C correlations are suppressed and a much longer delay is used to yield only long range H-C correlations - primarily 2 and 3 bond.

As a $^1$H detected (also called indirect or inverse detected) experiment it is dependent on $^1$H relaxation and has better sensitivity than $^{13}$C detected long range correlation experiments such as COLOC.

The CIGAR experiment is a variant of the gradient HMBC experiment that uses a variable HMBC delay to help avoid missing correlations. For detailed work however it is better to run multiple gHMBCs with different long range delays (jnxh). CIGAR with accord='n' is a standard gHMBC experiment.

Running HMBC experiments in phase sensitive mode (phase=1,2) and processing in mixed mode (av ph1) will give a square root of 2 advantage in signal to noise over pure absolute value (phase=0 or 1). In addition the phase cycled HMBC has a square root 2 advantage in signal to noise over the gradient version. So the best signal to noise is obtained by running the mixed mode phase cycled HMBC, but usually the cleanliness of the gHMBC or CIGAR more than makes up for the difference in signal to noise.

Experiment Overview:

1) Set up is similar to other 2D indirect detection experiments.
2) Acquire a $^1$H spectrum with a 90 degree pulse width, phase and reference it. Optionally copy the parameters to another experiment. Do not spin the sample and autogain must be off (gain='y' or a number).
3) For the phase cycled experiment type iuhmhc or use the Other/2D menu and select hmhc. They are the same. For the gradient version type iucigar or select cigar from the Other/2D menu. For a longer or shorter experiment vary either ni, nt or both. nt should be a multiple of 8 for the phase cycled experiment.
4) For cigar make sure pfgon='nny' and gain can usually be set to 60.
5) After acquiring the data, process with any of these options: 1) Type iuhmhcproc or use the Other/2D menu under Processing and select hmhc – both are the same and also work for cigar. The macro assumes phase=1,2. 2) Use the general 2D processing command wft2da (wft2d for absolute value).

Procedure:
1) Set up the $^1$H experiment as normal in EXP1. Verify that pw = 90 degree pulse. This is a critical parameter and will typically be 20-25 μs for quad and broadband probes and 8-10 μs for proton detection probes.

2) Lock, shim and obtain $^1$H spectrum as normal except DO NOT SPIN THE SAMPLE. It is usually worth checking the non-spin shims (x,y, etc.) also.

3) Set the cursors just outside either ends of the spectrum and type movesw. This command adjusts sw and tof to observe just the region between the cursors.

4) Obtain another $^1$H spectrum with the new sw and tof. Autogain is not allowed with 2D experiments. A good way to set the gain is set gain='n' and obtain a 1D $^1$H spectrum with autogain, then type gain='y'.

5) It is convenient to keep the 1D spectrum in EXP1 and run the 2D experiment in EXP2. To do this type mp(1,2) jexp2 to transfer the 1D info then set up the hmbc in EXP2 by typing iuhmbc or use the Other/2D menu under Setup and select hmbc to run the same macro. For cigar use iucigar or select cigar from the menu.

6) With the default parameters of nt=8 ni=96 and d1=1 phase=1,2 the experiment will take about 32 minutes. For a quick check of the experiment type phase=1 and ni=1 to obtain just the first increment of the 2-D experiment. It should look like kind of an outline of the 1-D $^1$H spectrum.

7) Type ga to begin the experiment.

Processing:

1) Type iuhmbcproc or use the Other/2D menu under processing and select hmbc (for any phase cycled or gradient HMBC variant). You can do nearly the same by typing fn=2k fn1=2k sinebell lp2d(512) wft2da for phase=1,2. lp2d is a macro that sets up linear prediction and a window function for the F1 dimension. You will usually want to answer y to use linear prediction.

2) If you are not using the processing macro for absolute value experiments make sure pmode='partial' and type av av1 wft2d. For mixed mode processing use pmode='full' and av ph1 wft2da.

3) If the 2-D spectrum appears weak or not at all (black screen) right click anywhere in the spectrum to increase the 2-D vertical scale (vs2d). Once you can see peaks you will usually want to right click on a medium strength crosspeak to set vs2d, or you can always adjust it manually. There are menu options to adjust vs2d as well and most other 2-D display options can be found in the display menus.

4) The macros plot2d or iuplhxcor can be used to plot the 2-D data with the 1-D spectrum on the sides. We recommend typing plot2d and following the prompts. Learn more about either with man('plot2d') or man('iuplhxcor') help files.

Notes:

It is often useful to save your data again after processing (overwrite the original data) to save the processing parameters so that the next time you call up the data you can simply type wft2da or wft2d to view it.