2D HMBC and CIGAR (VnmrJ ChemPack)

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Summary:

HMBC and its variants are some of the most powerful 2D NMR experiments for structure elucidation. HMBC is related to HMQC and direct heteronuclear J coupling correlations are observed, but one bond ^{1}H-^{13}C correlations are suppressed and a longer delay is used to yield primarily 2 and 3 bond ^{1}H-^{13}C correlations. 4 and even 5 bond long range correlations are also observed.

As ^{1}H detected (also called proton, indirect or inverse detected) experiments they are dependent on ^{1}H relaxation and have better sensitivity than ^{13}C detected long range correlation experiments such as COLOC.

There are many variations of HMBC. Gradient versions (gHMBC) are preferred to give cleanest results and allow for maximum gain. The CIGAR experiment is a popular variant of gradient HMBC experiment that uses a variable HMBC delay to help avoid null correlations and constant-time ^{13}C evolution for better resolution and less ^{1}H -^{1}H coupling issues. For most work it is recommended to run multiple gHMBC experiments with different long range delays or at least both a gHMBC and a CIGAR. The I500 versions of gHMBC that use broadband adiabatic ^{13}C pulses are also recommended.

HMBC experiments are often run in absolute value mode but can be acquired phase sensitive (phase=1,2) and processed in mixed mode (av ph1) to give sharper peaks in the 13C dimension. We use the mixed mode HMBC versions by default. The non-gradient HMBC has a square root 2 advantage in S/N over the gradient version, but usually the cleanliness of gradient HMBC or CIGAR more than makes up for the difference in signal to noise.

Experiment Overview:

1) Optional: Lock, shim, setup a 1D proton experiment, choose solvent, acquire a quick 1D proton spectrum, reference and save it. This step is helpful but not required. You can also optionally determine pw90 (^{1}H) and pwx (^{13}C) for your sample to get best results. See the VnmrJ 2D Guide for these procedures.
2) Use the command "iuhmbc" or "iucigar" on any spectrometer to setup an optimized experiment for that spectrometer. Or, from VnmrJ pulldown menu Experiments -> Setup NEW Parameters to do … -> 1H-13C Indirect Het. Corr. (Basic) -> HMBC, Gradient HMBCAD, Gradient HMBC or CIGAR.
3) Optional: You can run a test 1D HMBC by setting **ni=1** and **phase=1**. This will confirm that the experiment is working and that you have

enough signal to run a 2D experiment. Reset **ni=64-96** for a 2D experiment.

4) For a longer or shorter experiment change **nt**, **ni** and/or **d1**. **nt** should be a multiple of 8 and increasing it improves signal to noise. Increasing **ni** gives better resolution in the 2D dimension and a little better signal to noise.
5) Save your data after acquisition is complete. Optionally type "**lp2d**" to setup linear prediction or from the "Process" -> "Default" template use the "Auto LP F1" button with "F1" checked. Uncheck "F1" box to turn linear prediction off. The 2D Fourier transforms can then be done using the VnmrJ "Process" -> "Basic" template or the "**wft2da**" command.
6) Use the "Process" -> "Default" template for referencing and optional baseline correction. Use the 2D toolbar on the right side to adjust the display.
7) Use the VnmrJ "Process" -> "Plot" template for plotting or type "**plot2d**" to use the IU macro for plotting that allows you to plot 1D spectra along the edges of the 2D spectrum.
8) If you like the results, you can save the data again (overwrite) to save your modified processing parameters with the data.

Notes:

Common artifacts in HMBC experiments are one bond ^{1}H-^{13}C correlation peaks that are not fully suppressed. They are usually easy to identify as they will appear as pairs of peaks (proton split by ~130 – 180 Hz one bond J-coupling).

T1 noise ridges along big peaks are also often seen in HMBC experiments. Not spinning the sample, good temperature control and using gradient experiments can reduce or eliminate these.

Mixed mode is recommended for the HMBC experiments but CIGAR and gHMBC can be run in absolute value mode if desired. To run AV CIGAR or gHMBC: Setup mixed mode experiments as per above then type "**phase=1 f1coef = '1 0 0 -1' pmode='partial' av av1**". VnmrJ HMBC (non-gradient) does not work correctly in absolute value mode.

For reference to set mixed mode: "**phase=1,2 f1coef = '1 0 1 0 0 -1 0 1' pmode='full' av ph1**".

As mentioned previously single delay HMBC can result in null signals at certain J values so a few correlations that should exist can be (and often are) missing. Solutions are, try CIGAR or run multiple HMBC experiments with different delays. A standard delay is jnxh=8(Hz), a good two HMBC combination is jnxh=5 and 8.

Linear prediction can be very useful for improving HMBC results – for lower concentration samples increase nt and use linear prediction in f1.