

BARE BONES GUIDE TO gradientHMBC on the Inova-400&500 (w/ PFG probe installed)

This guide is written assuming proficiency in basic operation of the Varian NMR instrument. You should be experienced in performing basic 1-dimensional NMR experiments before attempting to perform 2D experiments on your own. Please ask for help the first time you perform this, to minimize your frustration (if for no other reason).

September 6, 2007

Conventions in this manual:

Boldface text indicates commands to be typed at the computer

<angle brackets> are used to designate a key to be pressed (i.e. **<Ret>** for Return/Enter)

[square brackets] designate an icon/button in the VNMR menu to be *clicked*

Mouse Conventions: *click*, by default, refers to the Left Mouse Button.

LMB will be used to designate the Left Mouse Button

MMB will be used to designate the Middle Mouse Button

RMB will be used to designate the Right Mouse Button

Sometimes you will need to *hold*, rather than *click* the mouse button. This means that you should press and hold the button down throughout the operation.

Note: All **commands** in boldface assume that you press **<Return>** afterwards.

Important Considerations before starting:

- You can perform this experiment in either the 4-nucleus (n4n/asw) probes, or the indirect-detection (NTR/3mmID) probes; however, the sensitivity is MUCH better indirect-detect probe. If you need a probe-change on either the 400 or 500, please schedule with Dr. Shoemaker in advance, and reserve an extra 10-20 minutes.
- If you have acquired a ^{13}C spectrum of this sample, it is useful to know the transmitter offset (tof) and spectral width (sw), and chemical-shift reference parameters (rfl & rfp) used for this spectrum. Otherwise, the default spectral window is usually adequate.

- 1) Make sure you are working in Exp:1 (type **jexp1<Ret>** if necessary).
- 2) Acquire a normal ^1H NMR spectrum, setting nt=4.
- 3) Zoom-In on the region containing peaks of interest (Note: if you zoom-in on a region with peaks outside that region, you will have *folded* peaks in your spectrum...this can be OK, or it can be a problem; depending upon where the folded peaks land).
- 4) Type **movesw**, and this will set the spectral width and offset to match your selected window.
- 5) Type **ga** to acquire a new spectrum, then phase it.
- 6) Save this spectrum (if you wish) in your directory, as a 1-D trace for plotting beside your 2D contour plot.
- 7) Optimizing the ^{13}C spectral window (this only works for solvents containing carbon).
 - a) If you don't know where to expect your protonated carbon resonances, skip this step and use the defaults.
 - b) If you DO know where your ^{13}C peaks are, you need to join an unused experiment (i.e. Exp:2) to setup the carbon window:
 - i) Type **jexp2 <Ret>** (assuming that the ^1H data is in Exp:1, we'll use Exp:2 for ^{13}C)
 - ii) Setup for ^{13}C observe in your solvent, set nt=4, then do **ga <Ret>**. You will probably only see the solvent peak in the ^{13}C spectrum, but that's all you need.
 - iii) Set your ^{13}C chemical shift using the solvent peak, turn on the PPM axis (dscale), and zoom-in on the desired ^{13}C window (using the cursors and shift-axis for reference), then type **movesw <Ret>**... then do **ga** to collect another 4-scan ^{13}C spectrum. Be sure that the full ^{13}C spectral window contains your desired window.
 - iv) Remember the experiment number that contains this optimized ^{13}C window (i.e. Experiment #2 in this example).
- 8) Type **mp(1,100) jexp100** (remember, your narrowed ^1H spectrum is located in Exp: 1). You should now be in Exp:100 (note the top of the VNMR window). This copies ^1H parameters from Exp1 to Exp100, then joins Exp100.

- 9) Type **gHMBC** to load the parameters, the gHMBC parameters should load, and you should see the pulse sequence on the screen (don't worry about it if you don't see the sequence).
- 10) If you skipped step #7, skip this step also (use the default ^{13}C spectral window). If you have acquired an optimized ^{13}C spectral window (in step #7 above), type **setcarbonsw** <Ret>. You will be prompted to enter the experiment number containing the narrowed & referenced ^{13}C spectrum (Exp:2 in this example); therefore, enter **2** <Ret>. (this macro sets "sw1, dof, rfl1, & rfp1" to match "sw, tof, rfl, & rfp" in the referenced & narrowed ^{13}C spectrum acquired in step #7).
- 11) The parameter "**jnxh**" determines the long-range C-H coupling constant for optimal signal-selection. The default is 8.0 Hz. You can increase/decrease as necessary to select shorter-range/longer-range couplings.
 - a) $\text{jnxh}=8.0$ or 9.0 is usually appropriate for optimizing J^3CH (J_{ccch}) couplings.
 - b) setting "**jnxh**" to a lower value (i.e. less than 7) will emphasize weaker, long-range couplings, but there will be a loss in overall signal:noise ratio.
 - c) setting "**jnxh**" to a higher value (i.e. 12 or greater) will emphasize stronger long-range C-H couplings, which will result in a more simplified 2D spectrum; however, it is possible that key correlations might not be observable.
- 12) Set **nt** (# of transients): (the default will usually be 8, but you often don't need that many scans)
 - a) Usually **nt=2** works fine (and takes the minimum amount of time).
 - b) Use **nt=4** for more dilute samples (will take 2x as long).
 - c) For very dilute samples (< 5mg) you may need as many as 8 or 16 scans; however, 16 scans per block, with **ni=512** will require an overnight run.
- 13) Set the number of increments in the *t1* dimension:
 - a) Type **ni?**, and note the current value for **ni**... **ni=400** is normally the default, and usually works well, and will give fairly high resolution for routine spectra.
 - b) To improve resolution in the ^{13}C dimension, increase **ni**: type **ni=512** (or larger if you have more time).
 - i) This experiment gives limited resolution in the ^{13}C dimension. If you wish to improve resolution in the indirect (^{13}C) dimension you must increase "ni", which will increase the total experiment time linearly.
 - ii) To save time, you can decrease "ni" (i.e. **ni=256**); however, the resolution in the ^{13}C dimension will suffer (but is often good enough to resolve what you need to see).
 - c) Note that gHMBC usually covers a larger spectral width (because the shift of non-protonated carbons, like carbonyls, must be included in the ^{13}C window); therefore, it requires a larger value of "ni" to get high resolution. However, **ni=256** for HMBC is equivalent to **ni=128** for HSQC or HMQC (because gHMBC is not acquired in phase-sensitive mode).
- 14) Type **time** to see how long the experiment will take,... adjust parameters if necessary.
- 15) Type **go** to start the experiment.
 - a) After ~36 or more blocks in *t1*, you can type **setLP1**, then **sinebell**, followed by **wft2d** to see if your spectrum is coming along nicely. Otherwise, you can wait until it is done if you wish.
 - b) If you must stop the acquisition before it is done (i.e., you run out of time), always stop the 2D experiment by typing: **sa('nt')**. This will stop the experiment at the end of the current FID.
 - c) When the acquisition is done, you should type **setLP1** to setup linear prediction parameters
 - i) Type **sinebell** to set the apodization window function then type **wft2d**.
 - ii) Note: the order should always be: **setLP1**, then **sinebell**, then **wft2d**.
 - iii) Click on **[Full]** (or type **f full**), then type **dconi** if you don't see the whole spectrum
- 16) Type **svf**, followed by your filename, to save the data for processing. (be sure you are in your data directory).
- 17) Quitting:
 - a) Type **jexp1**, eject your sample, and re-insert the reference.
 - b) Be sure to turn the spinner back on in **[Acqi]**.
 - c) Lock and shim on the reference, and sign-out in the logbook.

Processing and Plotting the 2D-HMBC spectrum:

As of January 18, 2005 the instructions for processing and plotting the data have been moved to a separate manual. Please see the manual on Processing & Plotting gHMBC NMR for further instructions.