HETCOR on the U400

PRELIMINARY INFORMATION

1) ***pw90:*** The default *pw90* is usually accurate enough for this experiment, unless the physical properties of your sample are very different from those of the standard due to, for example, the presence of ionic or paramagnetic materials or the use of a very different solvent. Refer to handout UVU423 for the determination of *pw90*, if it is required. If a new one is determined, remember to change the parameter *pw90* to the newly determined value.

2) ***1H T1:*** If you can not make a reasonable guess of the longest 1H T1 of your sample, it may be necessary to measure it in order to set the relaxation delay *d1* correctly. Refer to handout UVU 438 for instructions on the measurement of T1.

3) ***Sample spinning:*** non-spinning of the sample is recommended to minimize T1 noise and other artifacts in the HETCOR spectrum.

4) ***Optimization of acquisition parameters — sw, sw1, np, ni, nt, and d1:***
   a) ***sw:*** Resolution in the F2 (carbon) dimension is usually not a problem. In that case, the default *sw* of 2500 Hz (250ppm) may be used. However, if resolution in this dimension is a concern, you should optimize *sw* to include only the *protonated* carbon signals plus 10% of empty baseline. Of course, if you are running a long-range HETCOR, all peaks should be included, especially the non-protonated or quaternary ones.
   b) ***sw1:*** It is essential that *sw1* be optimized in order to maximize digital resolution in the F1 (proton) dimesion and to save time.
   c) ***np:*** Along with sw, *np* determines the digital resolution on the F2 dimension (Hz/pt = 2xsw/np). Normally, for a given *sw*, *np* is set so that Hz/pt(F2) ≈ 20. Typical setting for *np* is between 4096 and 1024 (see table below for some reference values).

sw (ppm)	250	200	150	100
np (for Hz/pt=20)	2500	2000	1500	1000

   d) ***ni:*** Along with *sw1*, ni determines the digital resolution on the F1 dimension (Hz/pt = sw1/ni). Normally, for a given *sw1*, *ni* is set so that Hz/pt(F2) ≈ 15. Typical setting for *ni* is between 512 and 128 (see table below for some reference values).

sw1 (ppm)	20	15	10	8	6	4
ni (for Hz/pt=15)	533	400	267	213	160	107

   e) ***nt:*** Set *nt* to the smallest multiple of 4 that would give reasonable signal-to-noise in the 1D 13C spectrum. Note that doubling *ni*, *nt*, or *d1* will approximately double the experiment time.
   f) ***d1:*** Ideally *d1* = {1.3 x T1(longest) - *at*}. *d1* is usually set to 1 to 2 sec because for most medium-size molecules, their proton's T1's are typically around 1 sec, and *at* for HETCOR is usually very small (< 0.1 sec). If these assumptions do not hold for your compound, change the *d1* accordingly.

5) ***VT:*** With long acquisitions (longer than 4 hours), consider running with VT on at 25 - 30˚C to avoid or minimize artifacts due to room temperature fluctuation.

6) ***Calculation of experiment time:*** The time calculated by the command **time** and the *Time Remaining* in the Acquisition Status window (shown only after the acquisition is started) may not be accurate, and could underestimate the length of a 2D experiment by as much as 25%. You should take this into account when setting up the experiment.

1. COLLECT A 1D 1H SPECTRUM

chdir <rtn> — enter your username at the prompt
jexp1 <rtn> — collect the 1D 1H spectrum in exp1

Select a 1H standard parameter set and retrieve an appropriate shim file.
Lock and shim on the sample as usual. When done with shimming, enter:

nt=1 <rtn> — set *nt* for a spectrum that can be phased and referenced properly; *nt*=1 is usually sufficient
ga <rtn> — start acquisition with autogain
f full aph <rtn> — full display and autophase
nl rl(__) <rtn> — set reference

Optimize sw1 for the HETCOR experiment *place the two cursors to include the signals of interest plus 10% of empty baseline on either side of the spectrum, then enter:*

movesw <rtn> — reset *sw* and *tof* as defined by the two cursors
gain='y' <rtn> — set gain value to that selected by autogain above
nt=_____ <rtn> — set *nt* for a reasonable S/N 1D 1H spectrum
ga <rtn> — start acquisition
f full aph <rtn> — full display and autophase

Inspect the spectrum to make sure that it contains all the signals of interest and that folding over, if any, does not cause a problem. Also check and make sure that the spectrum is phased and referenced correctly. If everything is OK, save the data as usual.

2. COLLECT A 1D 13C SPECTRUM

jexp2 <rtn> (If doesn't exist, create it with **cexp(2)**) — collect the 1D 13C spectrum in exp2

Select an appropriate 13C standard parameter set, then enter:

nt=_____ <rtn> — set *nt* to 1 if sw is to be optimized; otherwise, set it to obtain a 13C spectrum with acceptable S/N
ga <rtn> — start acquisition with autogain
f full aph <rtn> — full display and autophase
nl rl(__) <rtn> — set reference

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If sw needs to be optimized *place the two cursors to include the signals of interest plus 10% of empty baseline on either side of the spectrum, then enter:*

movesw <rtn> — reset *sw* and *tof* as defined by the two cursors
gain='y' <rtn> — set gain value to that selected by autogain above
nt=_____ <rtn> — set *nt* for a reasonable S/N 13C spectrum
ga <rtn> — start acquisition
f full aph <rtn> — full display and autophase

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Inspect the spectrum to make sure that it contains all the signals of interest and that it is phased and referenced correctly. If everything is OK, save the data as usual.

3. SET UP THE HETCOR EXPERIMENT — dohetcor

NOTE: **dohetcor** is an interactive macro that will take you through the setup of a HETCOR experiment. In this handout, the dialog will appear **`in this font`**, and the symbol ¶ signifies that a response is required. Most messages will appear in the Status Window, while inputs are entered *via* the Input Window. Window arrangement on the left side of the screen is, from top to bottom, Status, Input, Menu, Graphics, and Text.

3A. Enter the Macro

In the experiment where the 1D 13C spectrum has been acquired (usually exp2), enter:

dohetcor <rtn> set up a HETCOR experiment in exp3 with the 1H parameters in exp1

NOTE: If you wish to do the HETCOR in a different experiment (with 1H parameters still in exp1), enter **dohetcor(*2D_exp#*)**, where ***2D_exp#*** is the experiment number for the HETCOR. If the 1H exp# is not 1, then you must enter **dohetcor(*2D_exp#,1H_exp#*)**, where ***1H_exp#*** is the experiment number containing the 1H parameters. The **dohetcor** macro requires that all three experiment numbers be different and between 1 and 9, excluding 5.

After entering the macro, the following menu will be displayed in the text window at the bottom of the screen:

```
HETCOR MENU

1) np=2048, ni=128, nt=16, d1=1, time=40min
2) np=2048, ni=256, nt=16, d1=1, time=1hr20min
3) np=2048, ni=128, nt=64, d1=1, time=2hr30min
4) np=2048, ni=256, nt=64, d1=1, time=5hr
5) Manual entry of np, ni, nt, and d1
```

The suggested **np** and **ni** value will also be shown in the Status Window, which is calculated using the current sw and sw1 to give a digital resolution of 20 Hz/pt in the F2 dimension and 15 Hz/pt in the F1 dimension. This is for your reference in choosing a suitable HETCOR setup.

3B. Choose a Setup

Enter your choice of setup at the following prompt in the Input Window:

```
Enter your choice (1-5) from the menu below: ¶
```

If 1 to 4 is chosen, the parameters will be set automatically.
If 5 is chosen, the following dialog will appear:

```
Enter the following parameters:
np= ¶
ni= ¶
nt= ¶
d1= ¶
Is everything OK? (y/n) ¶
```

If answered no, you will be prompted to re-enter these parameters.

NOTE: **nt** will be rounded off to the nearest multiple of 4.

3C. Save the Data

Finally, you are given a choice of saving the HETCOR data automatically when it is finished:

```
Save HETCOR data when done? (y/n) ¶
```

If answered yes, the current directory will be shown in the Status Window and the dialog will appear:

```
Are you in the correct directory? (y/n) ¶
```

If answered no, you will be prompted to enter your username, and the working directory will be changed to your fids directory. Then, the dialog continues:

Enter file name to save: ¶

If the file already exists:

File exists! overwrite? (y/n) ¶

If answered no, you will be prompted to enter a new file name.

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The **dohetcor** macro will terminate at this point and allow you to check the parameters before starting the experiment.

If you wish to run a long-range HETCOR, set the following additional parameters before starting the experiment:

jnxh=_____ <rtn> (typically around 10)	averaged two and three-bond C-H coupling constants
hmult='y' <rtn>	hmult='n' is not allowed when jnxh≠0
nt=_____ <rtn>	at least 4 times that of the normal HETCOR

Reminder: Your 13C spectral window must include all the signals of interest, especially the quaternary ones.

3D. Start the Experiment

You may start the HETCOR experiment with either the **au** or **go** command:

au — start HETCOR experiment, and if you answered yes to **Save HETCOR data when done?**, it will save and process the data at the conclusion of the experiment. If you answered no, it will process the data only, and no data will be saved. **IMPORTANT**: If the experiment is terminated with **aa** or **sa** (see below) before it is finished, you will have to manually save and/or process the data.

go — start HETCOR experiment, with no actions taken at the conclusion of the experiment.

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NOTE: Once the experiment is started, it may be aborted by clicking the Abort Acq button or typing the **aa** command *if no data is to be retained*, such as when you discover that a mistake has been made in the setup of the experiment, or when it has become apparent that the experiment is useless. *If data retention is desired*, such as when sufficient signal-to-noise has been obtained or when you have run out of time, you should use the **sa('fid')** command which will stop the acquisition at the next complete FID. At this point, you should save the data manually because automation will not work when the experiment is terminated before its completion.

NOTE: You may examine the data in the middle of the acquisition and in the same experiment the data is being acquired by typing **hetcorproc** (see Part 3A below). Be aware that a 2D spectrum processed before its completion will have a lower resolution in the *f1* dimension (or both dimensions if data is symmetrized) because fewer FID's are being used. Therefore, it is recommended that you wait until at least half of the number of FID's have been collected before processing and examining the data.

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When the experiment is finished, save and/or process the data if it is not yet saved and/or processed. Then proceed with the displaying and plotting of the data as described below.

4. PROCESSING, DISPLAYING, AND PLOTTING OF HETCOR DATA

NOTE: Since processing and plotting of 2D data require a significant amount of time, it is recommended that you move these tasks to the SPARC2 data station. This will not only free up the spectrometer for other users, but will also save you time and research money, because the SPARC2 has a faster computer and a much lower charge rate (approximately half that of the U400).

4A. Processing

hetcorproc <rtn>	macro to process HETCOR data

This macro will do a weighted 2D Fourier transformation. It will also attempt to adjust the vertical scale and the threshold properly and display a rectangular color map with room for traces or projections. It gives the same display as **hetcordisp** or **hetcords** below.

To process the HETCOR data manually, enter:

wft2d <rtn>	weighted 2D Fourier transformation

4B. Displaying

a. Adjust the dimensions of the 2D display: (not needed if data is processed with **hetcorproc**)

hetcordisp <rtn>	macro to adjust *vs* and *th* and display a rectangular
- OR -	2D color map with room for projections
hetcords <rtn>	macro to display a rectangular 2D color map without changing *vs* or *th*

NOTE: If a different display is desired, see Part 3.C.c of handout UVU448 (*COSY on the U400*).

b. Scaling the 2D display manually:

Option 1: place cursor on the weakest signal you want to see and click the middle mouse button. This will rescale and update the 2D display so that the selected signal is just visible. Then adjust the threshold with the middle mouse button on the colored threshold vertical bar to obtain the desired display.

Option 2:

vs=____ <rtn>	enter an estimated *vs* value
dconi <rtn>	redisplay the 2D spectrum using the new *vs* value

then adjust the threshold as above to obtain the desired display.

c. Expanding a region:

LC (at lower left-hand corner of the region of interest)
RC (at upper right-hand corner of the region of interest)

LC `3:Expand`	expand the enclosed region

4C. Plotting

NOTE: If you are plotting to the Zeta plotter, set the pen to about 1 cm from the perforation.

a. hetcorplot — quick and easy (Fig. 1):

hetcorplot(#,#) <rtn>	macro to plot HETCOR data with levels=5, spacing=2, 1D 1H in exp1, and 13C in exp2

The vertical scale of the 1D plots can be adjusted by providing the two optional arguments with the command:
† First argument — vs scaling factor for the 1H plot on the left side of the 2D plot, *i.e.*, the vertical scale of the 1H spectrum as adjusted by **vsadjh** will be multiplied by this factor. Default is 1.
† Second argument — vs scaling factor for the 13C plot (vertical scale adjusted by **vsadjc**) on the top of the 2D plot, default is 1.

You will be prompted to confirm if the 1D spectra are in the right place:

```
Is the 1H spectrum in exp1 and 13C in exp2? (y/n) ¶
```

If answered no, the macro will be terminated. You should then either reload the data into the correct experiments or use the **hetcorpl** macro instead (see below).

If the 1D data are in the right place, You will be prompmpted to enter a choice from the following:

```
plot with: 1)text & parameters, 2)text only, or 3)none? ¶
```

Then the plotting will begin. The positioning and the dimesions of the plot depend on the plotter:

For the LaserJet_150R:	wc = 180, wc2 = 120, sc = 10, sc2 = 0
For the Zeta:	wc = 288, wc2 = 160, sc = 10, sc2 = 0
For the Zeta_L:	wc = 576, wc2 = 160, sc = 20, sc2 = 0

b. hetcorpl — comprehensive and flexible (Fig. 3):

hetcorpl(#,#,#,#,#,#) <rtn> — macro to plot HETCOR data with user's input of contour levels, spacing, and 1D 1H and 13C exp#.

† First argument — number of contour levels to be plotted, default is 5.
† Second argument — spacing (in mm) between contour levels, default is 2.
† Third argument — 1D 1H experiment number. If not provided, the following dialog will appear:

Is 1H spectrum in exp1? (y/n) ¶
If answered no:
plot without 1H spectrum on the side? (y/n) ¶
If answered no, enter the 1H exp# at the following prompt:
exp# containing 1H spectrum: ¶

† Fourth argument — 1D 13C experiment number. If not provided, a similar dialog to the one above will appear.
† Fifth argument — vs scaling factor for the 1D 1H plot on the left side of the 2D plot. If not provided, the following dialog will appear unless you had specified earlier that no 1D spectra are to be plotted on the edges:

1D vs: 1)auto adjustment or 2)plot as displayed? ¶

† sixth argument — vs scaling factor for the 1D 13C plot on the top of the 2D contour, default is 1.

With the **hetcorpl** macro, you will have the following options:
1) plot the 2D contours with any specified levels and spacing
2) plot the 2D contours either as default or as displayed
3) plot with both 1D spectra on the edges, with 13C on the top only, with 1H on the side only, or with no 1D
4) plot the 1D spectra either with automatic adjustment of vertical scales or as displayed
5) 1D 1H and 13C data can be in experiments other than 1 and 2 repectively

NOTE: Normally, you would want to provide the first four arguments with the **hetcorpl** command, unless you don't want either or both of the 1D spectra to be plotted.

c. Customized plotting:
You may position and/or size the HETCOR plot to your own specification by changing **wc**, **wc2**, **sc**, and **sc2** first, then redraw the display with the **dconi** command, and plot it using the **hetcorpl** macro with the **as displayed** option. Make sure you have enough room for the 1D plots if you want them. See part 3.C.c of handout UVU448 (*COSY on the U400*) for more details.

d. Plotting with grid (Fig. 2):

grid <rtn> — display grid (NOTE: **grid(#)** will change spacing between grid lines to # cm, default is 1)
dcon <rtn> (without it, the cursor cross will be plotted along with the grid lines)
plgrid <rtn> — plot grid (*plgrid(#)* works the same way as *grid(#)*)
hetcorpl <rtn> (**IMPORTANT**: you must choose the **as displayed** plotting option)

f. Plotting with projections (Fig. 2):
i) Plot the 2D contours: adjust the 2D display as before and enter:

pcon <rtn> — plot the 2D contours
dconi <rtn> — enter interactive 2D display

ii) Plot the horizontal projection:

LC `3:Proj` — select projection menu
LC `1:Hproj(max)` — select horizontal projection
CC (on the projection) — adjust vertical scale of projection, if necessary
LC `5:Plot` — plot horizontal projection
(Make sure the `Plot` button blinks with the click, otherwise the command may not have been taken.)

iii) Plot the vertical projection:

LC `3:Proj` — select projection menu

LC 3:Vproj(max)	select vertical projection
CC (on the projection)	adjust vertical scale of projection, if necessary
LC 5:Plot	plot vertical projection
page <rtn>	send plots to the plotter

g. Plotting traces from the 2D dataset:

dconi <rtn>	enter interactive 2D display mode
LC 2: Trace	select trace menu
LC (on peak of interest)	select a trace
CC (on the trace)	adjust vertical scale of the trace, if necessary
ds <rtn>	display the trace

The trace can now be displayed and plotted just like a normal 1D spectrum. Adjust **wc**, **sc**, and **vs**, then plot it as usual with **pl pscale page** <rtn>, for example.

Hidden text:

Fig. 1. ***Typical output from*** **hetcorplot**

Fig. 2. ***Plot with grid and projections***

Fig. 3. ***Output from*** **hetcorpl** ***with text and no 1D***