An NMR Caveman's Guide to Quickly Acquiring Spectroscopic Data

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Disclaimer: this guide is meant to be a quick, routine means of obtaining characterization data for unknown compounds in the shortest possible spectrometer time (and user time). I have found that these parameters work well for the molecules I've encountered in my research when I have a substantial amount of sample (>1 mg of a <1000 Da molecule; ideally 30-100 mg). If you have a smaller sample or run into problems using the protocols herein, I recommend calibrating your pulsewidth, autoshimming, tuning the probe, and/or utilizing more rigorous parameters and procedures elaborated elsewhere.

Key: emboldened text refers to items to be typed in (e.g., **nt=4 ga** literally means type in "nt=4 ga") and hit the "enter/return" key, and text in brackets refers to items to be clicked on using the mouse (e.g., [Setup] → [Shim] literally means click on the "Setup" button and then click on the "Shim" button). Keep that in mind that typed commands are case-sensitive.

A. Taking a 1D ^{1}H (typically on the I500, I500B, or I600)
Minimum experimental time: 3 min

1. Insert your sample after adequately cleaning it and centering using the spin gauge (**e** for eject; **i** for inject)

2. **jexp1** If exp1 is locked, type **unlock(1)** and then **jexp1**

3. [Setup] → [1H,CDCl3] for $CDCl_3$ samples, or go to [Nuc,Solv] for other NMR solvents. For example, if you have a sample in d_6-DMSO, you would click [Setup] → [Nuc,Solv] → [1H] → [DMSO]. After choosing the NMR solvent, type **su**

4. Change your directory to the folder you wish to save your fid

5. Go to [Acqi], lock and shim your sample

   Detailed instructions: Use the red binder near the keyboard to find the appropriate lock power level and approximate z0 shim value for the solvent you're using. For example, if you're using $CDCl_3$ the lock power level should be set to 20, and you should start your lock signal search with a z0 value of about +2000 on the I600

   Crank the lock gain up to its maximum of 48 before you start lock-signal searching and gradually lower the gain value as you find lock signal and shim. I usually set my final lock value to around 60-70

   If you have problems shimming, [Close] the Acqi window, type **rts** and then **cdcl3** then **su** to reload the default shim values for $CDCl_3$. Sometimes a n00b will use the machine right before you and screw up all the shims (z0-6, xy, etc.), and reloading the shimfile helps with this

6. If gradient autoshimming is desired, **jexp2** then [Setup] → [Shim] → [Gradient Autoshim on Z]. This should take 30 s to 5 min depending on how well you manually shimmed (typically 1-6 iterations). If you hit the 10th iteration, autoshimming will stop, and I recommend adjusting your shim manually (Step 5) before attempting more gradient autoshimming. For whatever reason, gradient shimming on the I500 is 3x faster than on the I500B (gradient shimming is not available on any other machines)

7. **jexp1** then type **nt=9999 d1=0.4 gain=20 ss=0 bs=4 ga**

8. While this starts acquiring, type **wft text('SAMPLE NAME') aph f nl rl(7.26p) cz**, and when you see that ct ≥ 4 (or BS 1 complete) hit enter to automatically name your spectrum, autophase it, display the full spectrum, find the $CHCl_3$ reference line and set it to 7.26 ppm, and clear the previous user's integration intervals (note: the nl rl(7.26p) will only work for the $CHCl_3$ reference peak; for other solvents, reference after obtaining the fid)

9. Type **dscale** to display the horizontal ppm scale. [Part Int] to display partial integrals (note that this is the same button as [Full Int] and [No Int]), and [Resets] to set your integral intervals. **vsadj** can be used to adjust the vertical scale to the highest peak, **sp=*n*p wp=*m*p** can be used to adjust the horizontal scale in between *n* and *m* ppm (e.g., **sp=0.75p wp=1.25p** will zoom in on the 0.75-2 ppm portion of your spectrum), and **daereg** will automatically zoom to the –0.5-9 ppm range. If I need to change the vertical scale slowly, I usually type **vs=vs*2** or **vs=vs/2** successively (and quickly using the up arrow) instead of using the mouse or vsadj. **dpf** will display peak frequencies (based on your specified threshold)

10. Since nt=9999, you will be continuously taking a 1D ^{1}H. At any point, you can type **wft aph** to display an updated spectrum (note that you will have to re-reference the $CHCl_3$ peak in most cases)

11. Once a desired integrated spectrum is obtained, type **dc** then **bc** then **isadj** to drift correct, baseline correct, and adjust your integral scale. Put the red cursor on an integrated region, click [Set Int] and type what integration value you'd like for that specific peak. You can type **dpir** to display integral ranges

12. To print, either type **plot** or **daeplot** or I type **pl pscale pap pir page** pscale prints the horizontal scale, pap prints the parameters of your NMR experiment, pir prints your integral values, and page sends it to the printer. Add **ppf** before page if you wish to print peak frequencies (i.e., type **pl pscale pap pir ppf page**)

13. Save your file by typing **svf** following by your desired filename (don't use spaces or any punctuation aside from periods, underscores, and hyphens)

14. Type **aa** then **e** to stop your acquisition and eject your sample

B. Taking a 1D ^{13}C (typically on the I500C)
Minimum experimental time: 3 min

1. Insert your sample after adequately cleaning it and centering using the spin gauge (**e** for eject; **i** for inject)

2. [Setup] → [13C,CDCl3] for $CDCl_3$ samples, or go to [Nuc,Solv] for other NMR solvents, then **su**

3. Change the directory to the folder you wish to save your fid

4. Go to [Acqi], lock and shim your sample. Specifically for ^{13}C spectra in $CDCl_3$, I set the lock power to 36. After finding the lock signal and shimming, I adjust lock gain so that it is just below the "receiver overflow" warning (so that the lock level hovers just below 100).

   To check if you're getting receiver overflow, look at the console to the right of the I500C computer monitor. The "Receiver Overflow" red light will be blinking if your gain level is set too high. For solvents other than $CDCl_3$, increase the lock power and decrease the lock gain until the lock signal step-function starts to become unstable. You want to be just under that point.

5. Type **nt=99999 bs=8 ga** The default block size is 32, but I change it to 8 because I'm impatient and want to see my updated spectrum every 10 seconds instead of every 40 seconds

6. While this starts acquiring, I type **wft text('SAMPLE NAME') aph f**, and when you see that ct≥8 (or BS 1 complete) hit enter to automatically name your sample, autophase it, and display the full spectrum

7. Since nt=99999, you will be continuously taking a 1D ^{1}H. At any point, you can type **wft aph** to display an updated spectrum

8. You may have to manually phase your sample by clicking [Phase] and clicking on a portion of your spectrum, and scrolling up and down while holding the left mouse button down. This sometimes works better than autophasing (**aph**, **aph0**, etc.)

9. Once a desired spectrum is obtained, zoom in on the $CDCl_3$ triplet, put the red cursor on the center peak and type **nl rl(77.23p)**

10. Adjust the peak threshold by clicking [Th] and dragging the yellow line up and down to a desired level.

11. **pl pscale pap ppf page**

12. **svf** then type your sample filename

13. Type **aa** then **e** to stop your acquisition and eject your sample

C. Taking a gCOSY-45 (on the I500B or I600; alternatively you can take a regular gCOSY on the I500)
Minimum experimental time: 6 min

1. Follow steps 1-7 from Section A. In step 7, you can alternatively type **nt=1 gain=20 ga** to produce a 1-scan proton. Or alternatively, you can load a ^{1}H spectrum that was saved previously

2. Type **mf(1,2)** then type **jexp2** then type **wft aph**

3. Using the left- and right-mouse buttons, adjust the red vertical lines to leave about 1 ppm space on either side of your desired outermost peaks

4. Type **movesw** to move the spectral width

5. Type **gCOSY45** then **su** then **at=0.3 d1=0.8 ni=256**

6. Type **time** to see how long it'll take. The default (where nt=1) takes around 5 min, but for smaller amounts of sample (<3 mg), you'll probably need a longer experiment (nt>1). Adjust nt as necessary

7. Type **go**

8. While this is running, type **wft2da text('SAMPLE NAME') svf('SAMPLE_NAME')** and hit enter when the experiment is done to automatically name your spectrum and save it (I always forget to save it later)

9. Usually, you'll have to click [Full] to display to full spectrum, and adjust the peak intensities by clicking on [vs+20%] and [vs–20%]

10. When ready to print, type **plcosy(12,1.2,1,1)**

D. Taking a gHSQC (on the I500 or I500B)
Minimum experimental time: 25 min

1. Follow steps 1-4 from Section C.

2. Type **gHSQC** then **su** and for molecules <500 Da, type **at=0.2 d1=0.9 ni=128**, and for molecules >500 Da, type **at=0.1 d1=0.5 ni=128** The j1xh value should be 140, but you can check by typing **j1xh?**

3. Type **time** to see how long it'll take. The default (where nt=4) takes around 20 min, but for smaller amounts of sample (<15 mg), you'll probably need a longer experiment (nt>4, in multiples of 4). Adjust nt as necessary

4. Type **go**

5. While this is running, type **wft2da text('SAMPLE NAME') svf('SAMPLE_NAME')** and hit enter when the experiment is done to automatically name your spectrum and save it (I always forget to save it later)

6. Usually, you'll have to click [Full] to display to full spectrum, and adjust the peak intensities by clicking on [vs+20%] and [vs–20%]

7. When ready to print, type **plhxcor(12,1.2,1,1,1,1)** This will print your ^{1}H spectrum (saved in exp1) along both the f_1 and f_2 axes, but if you'd like to print the spectrum with the ^{13}C spectrum along the f_2 axis, use one of the computers next to the login computer. If you load your ^{1}H spectrum on exp1, and your ^{13}C spectrum on exp2, and your gHSQC in exp3, you can type **plhxcor(12,1.2,1,2,1,1)** to print this out.

E. Taking a gHMBC (on the I500 or I500B)
Minimum experimental time: 25 min

1. Follow steps 1-4 from Section C.

2. Type **gHMBC** then **su** and for molecules <500 Da, type **at=0.2 d1=0.9 ni=256**, and for molecules >500 Da, type **at=0.1 d1=0.5 ni=256** The j1xh value should be 140, but you can check by typing **j1xh?** The jnxh value should be 8, but this can be checked by typing **jnxh?**

3. Type **time** to see how long it'll take. The default (where nt=8) takes around 40 min, but I usually adjust **nt=4** to make it 20 min if I have a lot of material in the NMR sample. For smaller amounts of sample (<15 mg), you'll probably need a longer experiment (nt>4, in multiples of 4). Adjust nt as necessary

4. Type **go**

5. While this is running, type **wft2da text('SAMPLE NAME') svf('SAMPLE_NAME')** and hit enter when the experiment is done to automatically name your spectrum and save it (I always forget to save it later)

6. Usually, you'll have to click [Full] to display to full spectrum, and adjust the peak intensities by clicking on [vs+20%] and [vs–20%]

7. When ready to print, type **plhxcor(12,1.2,1,1,1,1)** This will print your ^{1}H spectrum (saved in exp1) along both the f_1 and f_2 axes, but if you'd like to print the spectrum with the ^{13}C spectrum along the f_2 axis, use one of the computers next to the login computer. If you load your ^{1}H spectrum on exp1, and

your ^{13}C spectrum on exp2, and your gHMBC in exp3, you can type **plhxcor(12,1.2,1,2,1,1)** to print this out.

F. Taking a 1D-NOESY (typically on the I500, I500B, or I600)
Minimum experimental time: 6 min/NOESY

1. First, determine which peaks you'd like to irradiate. This usually involves solving the flat structure or at least putting fragments together into proposed arrangements

2. Follow steps 1-7 from Section A. In step 7 you can alternatively type **nt=1 gain=20 ga** to produce a 1-scan proton. Or alternatively, you can load a ^{1}H spectrum that was saved previously

3. Make sure the window displaying your spectrum is as large as possible, and zoom your 1D ^{1}H in so that the region you wish to probe is blown up, with about 0.5 ppm space to the left and right of your furthest peaks. This will save you a lot of time if you're taking multiple NOESY's

4. **mf(1,2)** then type **jexp2** then type **wft aph**

5. Type **NOESY1D** You will be prompted to select a region to irradiate. Select your region using the left- and right-mouse buttons. Keep in mind that zooming in and out will change the NOESY spectral width

6. Once an irradiation region is selected, click [select] and [Proceed] in sequence. Your NOESY pulse sequence should then be displayed. You can change your parameters, but I typically don't unless I'm dealing with a compound whose molecule weight exceeds 750 Da. An experiment will take around 5 minutes using these default settings

7. Type **go** to start your acquisition

8. If you have to take multiple NOESY1D's, type **mf(1,3)** then **mf(1,4)** then **mf(1,5) mf(1,6)** then **mf(1,7)**…until you have enough experiments containing your 1D ^{1}H corresponding to the number of NOESY1D's you'd like to take

9. **jexp3** then **wft aph** then repeat steps 5-7. Until exp2 is finished, the NOESY1D in exp3 will be queued

10. Repeat steps 8-9 for every NOESY you need to take (given the time constraint of your reserved time block)

11. Once this is done, **jexp2** and type **wft text('SAMPLE NAME') svf('SAMPLE_NAME')** (DO NOT type **aph** – this will create a huge headache for you). You'll have to manually [Phase] your 1D NOESY

12. Zoom in on the pertinent region you are looking at, integrate all the peaks, adjust the peak-picking threshold, and adjust the vertical scale. Then **bc** then **dc** then **isadj**. I usually set the irradiated peak (the large negative peak) to –100

13. **pl pscale pap ppf pir page** I usually print the peak frequencies to make analysis more straightforward

14. Repeat steps 11-13 for all NOESY1D's as they complete, or just walk away and come back later, giving yourself enough time to save (and possibly process) all your collected NOESY data

G. Queuing up Multiple NMR Experiments

Situation: let's say you isolate 1.6 mg of an interesting unknown compound, and you wish to obtain full characterization data. You believe you can get good gHSQC and gHMBC data in about 5 hours apiece, and you have booked an 11 pm-9 am timeslot on the I500. How do you avoid returning to the NMR lab at 4 am to start a second NMR experiment on this single overnight time block?

1. Follow steps 1-3 of section D, and 1-3 of section E. Usually I have the ^{1}H in exp1, the gHSQC set up in exp2, and the gHMBC set up in exp3

2. Once these experiments are ready to go, **jexp2** and type **go**, and then **jexp3** and type **go** You'll see the gHSQC start running, and as soon as this is completed, the spectrometer will automatically start acquiring the gHMBC