

VII. Basic ¹³C 1-D NMR

Set-up

1. First acquire a proton spectrum of the sample in exp1. Move the transmitter offset (**movetof**) to the center of the proton signals. Note the **tof** (transmitter offset).
2. Join experiment 2. Re-cable the machine for direct detection of a heteronucleus (there should be diagrams on the magnet legs). On 5001 a switch changes the nucleus, no re-cabling is necessary.
3. Set up the experiment for carbon acquisition:
[Main Menu] [Set Up] [Nucleus, Solvent]
[C13] [solvent]
4. Check to be sure the temperature setting is correct. Check that **dm='nnn'** (Decoupler is OFF.). Make sure **alock='n'** and **wshim='n'**. Then **>su** to send the parameters to the console. At this point, carbon is on channel 1 (**tn**) and proton is on channel 2 (**dn**) for tuning purposes.
5. Tune the carbon side of the probe. Tuning for carbon is the same as proton except use the cables from the broadband preamp and use the carbon tune wand. Tuning 5001 for carbon is different, please ask facility personnel to demonstrate.

Proton coupled ¹³C spectrum

The decoupler should be off: **dm='nnn'**

Check parameters like sweep width (**sw**), transients (**nt**), pulse width (**pw**), and transmitter offset (**tof**). An approximate carbon pulse width can be found in the back of the log notebook. Acquire a single transient first. If the sample is dilute and unlabeled, many transients may be needed to see carbon peaks. Carbon spectra will almost always have more noise than a proton spectrum. A 90-degree pulse width determination can be performed on carbon in the same manner it is done for proton. Carbon peaks from carbons attached to protons will be split. This spectrum can be integrated. Be sure to increase the receiver gain (**gain=#**). On INOVA instruments, use **gain=60**.

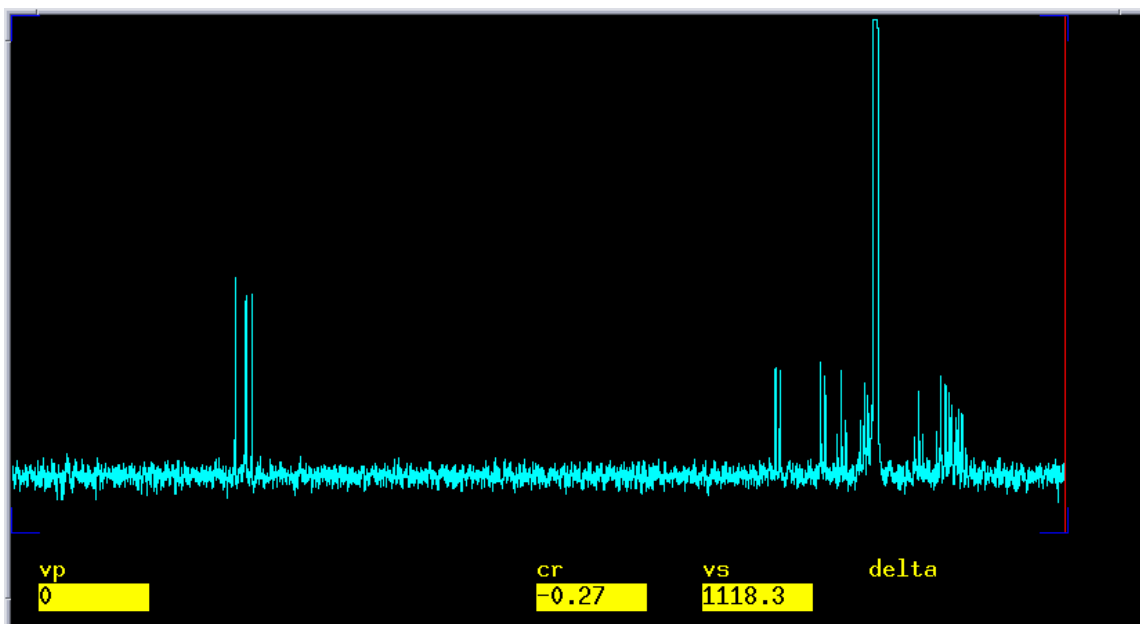


Figure 15: The carbon spectrum of a three-residue peptide in DMSO with no proton decoupling retains all of the coupling information in the carbon peaks.

Proton decoupled ^{13}C spectrum

Note the **tof** from the proton spectrum in exp1. Set the **dof** (decoupler offset) to decouple protons to the same number as the **tof** from the proton spectrum.

The decoupler power (**dpwr**) is typically set at 30 db for the dioxane sample. This number should stay at or below 40db for safety. The decoupler modulation mode (**dmm**) is the mode the decoupler is using. To decouple protons, use waltz decoupling for this example (**dmm='w'**); for garp decoupling use **dmm='g'**. When using waltz or garp decoupling, the decoupler modulation frequency (**dmf**) is set to $4X \gamma_{\text{H2}}$. The γ_{H2} value is the strength of the decoupler and a calibrated value can be found in the back of the logbook.

To decouple the protons, set the decoupler mode to **dm='nyy'**, which means the decoupler is off during the first time period and on during the other two. Use **dps** (display pulse sequence) to see when the decoupler is off and on. Set **d2=1.0** and **d1=0.001** and look at the pulse sequence again. These numbers can be varied, but the purpose in doing this is so that the decoupler will be turned off during the **d1** period and will be off at the end. Therefore, **d1** should be very small (just enough to turn it off) and **d2** to be larger so that the decoupler is not off very long for full decoupling. Be sure to increase the receiver gain (**gain=#**).

Now acquire the spectrum. The carbon peaks should be singlets if the decoupler is set up properly. Integration of this spectrum may not be accurate due to the carbon-proton NOE enhancement.

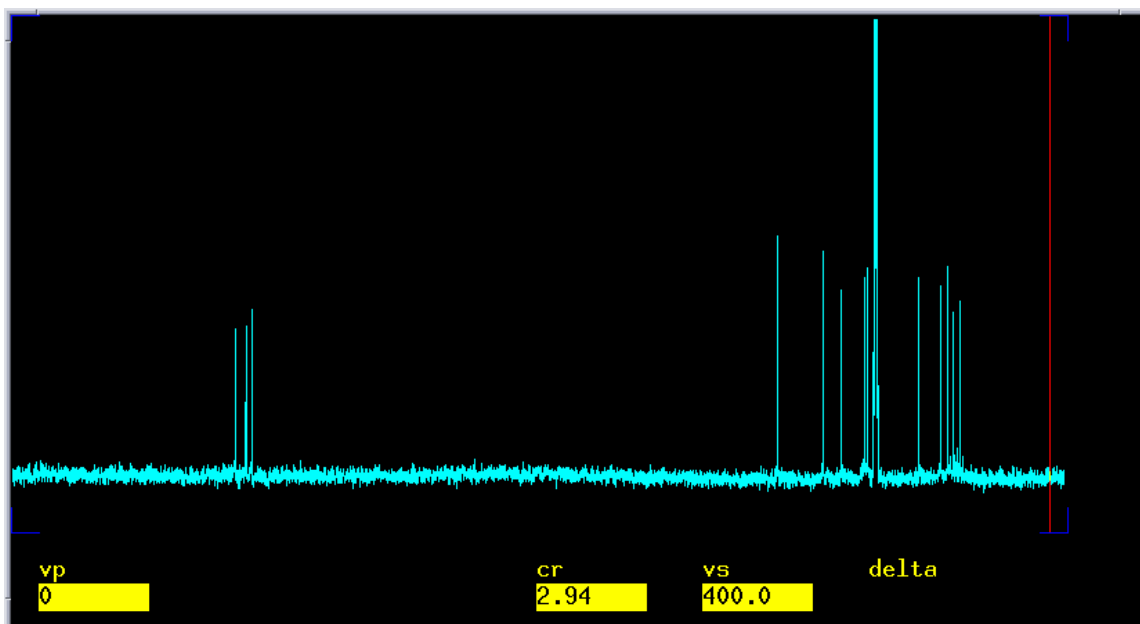


Figure 16: The carbon spectrum of the same three-residue peptide with the protons fully decoupled. The spectrum has a large increase in signal-to-noise from the decoupling but loses the coupling information.

Table 1

Decoupling Mode	S/N	Integration	Splitting
'nnn'	poor	yes	yes
'nny'	fair	yes	no
'nyn'	fair	no	yes
'nyy'	best	no	no

Different decoupling settings will allow for better or worse signal-to-noise and determines whether the spectrum can be accurately integrated and/or whether the coupling information (splitting) is retained.

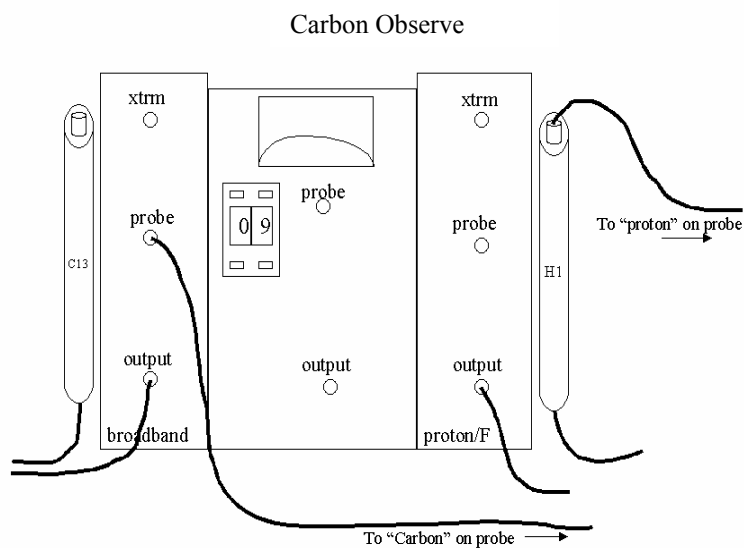
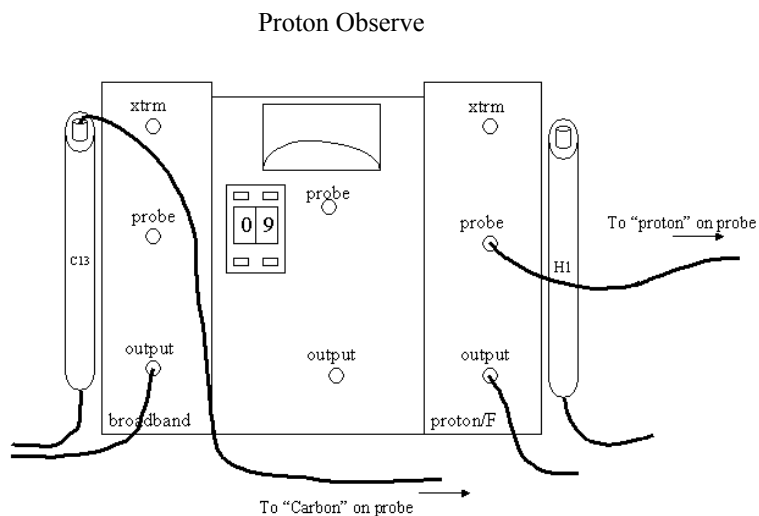


Figure 17: The 600s must be re-cabled to directly observe carbon. The top diagram is cabling to observe proton and the bottom for observing carbon.

Hints for obtaining good 1-D carbon data

Accept that a concentrated sample is required and/or acquire longer experiments. Using the broadband probe will provide the best signal-to-noise. If integration is required, try to use gated decoupling rather than no decoupling. Use a weighting function such as line broadening (**lb**) to help with s/n when viewing data. Also, do a baseline correction if the

baseline is rolling. Use longer **d1** values and a **pw** that is less **pw90** to cope with relaxation problems.

Finishing Up

When finished acquiring,

1. Save the data.
2. Make sure the decoupler is off (**dm='n' su**).
3. Set up a proton experiment. **[Setup] [Nucleus, Solvent] [H1] [solvent] su**
4. Re-cable the machine for proton detection. Always leave the machine cabled for proton detection.