

## Carbon and Heteronuclear NMR using Topspin

There are several different types of carbon spectra such as a normal qualitative spectrum, UDEFT, DEPT, coupled, selectively decoupled, and those with and without NOE. Routine 1D carbon spectra are qualitative spectra, i.e., the intensities do not reflect the number of carbons in the molecule. Read this handout for more information.

IMPORTANT NOTE: when doing carbon spectra, one should not use the rga command (or start). The appropriate value of rg is already set. Simply start the acquisition with zg.

### How to take 13C spectra


1. **Read in the carbon parameters:** Define a new data set (edc). Type **rpar carbon.top**. (Modify for different nuclei. See list at the end of this handout.) Lock and shim.
2. **Tune the Probe:** The next step is to tune the carbon channel of the probe. This is essential since if the probe is not tuned, no signal may be observed. Type **wobb**. On the screen, a tuning curve with a "dip" will be displayed. Tuning and matching capacitors are now adjusted to make the "dip" both centered and as deep as possible. The tuning capacitors are different on the 300wb and 300nb. On the 300wb they are gold sliders with numbers indicating positions. There is a chart near the probe that lists for each nucleus an approximate set of numbers for the tuning and matching. Adjust the least significant bit until the tuning curve is centered and as deep as possible. If you have not done this before, ask for assistance. You should also tune the proton channel if you intend to do DEPT. To do so, push the channel select button on the pre-amp box and wait until the wobble curve changes to proton (this takes a few seconds) and tune using the yellow proton rods. Type **stop** to end this tuning procedure.
3. **Choose experiment and set parameters:** By default, rpar carbon.top reads in parameters for the standard 1D 13C experiment. Set the following parameters if you want to do an experiment different from the standard 1D. DEPT135 is the recommended experiment.

experiment	pulprog	d1	ns	ds	l4	aq	cnst12
Standard 1D	zpgg30	0.7	n	0	-	0.7	-
UDEFT	udeft.jd	2	1	0	n	0.18	-
DEPT135	deptqsp	2	4*n	4	-	0.7	1.5
DEPT90	deptqsp	2	4*n	4	-	0.7	1
Inverse-gated	zgig30	0.7-30 sec	n	0	-	0.7	-
gated	zggd30	0.7	n	0	-	0.7	-

4. **Start the acquisition by typing zg (do not type start or rga).** To look at the spectrum without stopping the acquisition, type **tr**, (NOT FOR UDEFT) and then after the next scan is completed, type **ef**. To stop scanning, type **halt**.

### UDEFT and standard 1D

Standard 1D 13C spectra are usually taken with a simple one-pulse experiment. UDEFT, (Uniform Driven Equilibrium) however, gives higher signal-to-noise. For UDEFT, the number of scans is the parameter l4 and **ns MUST BE SET to 1**. Aq must be set to 0.18 sec or so. Ds must be zero. The tr command does not work with UDEFT. You can, however, see the FT spectrum while still scanning by doing this: type

acqu and then click  which gives a real-time FT spectrum. **UDEFT only runs on the 300wb at this time.**

### Determining Multiplicity - DEPT

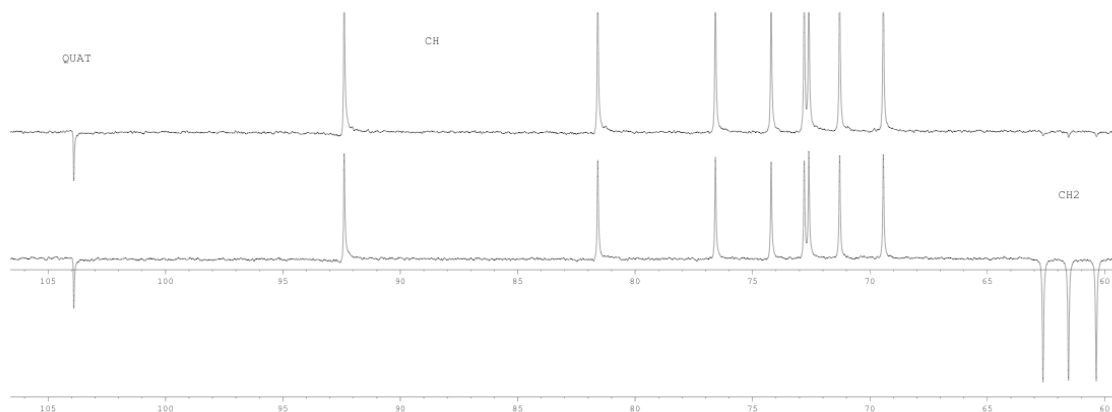
DEPT is the recommended standard carbon experiment. For maximum sensitivity for quats, however, either UDEFT or the standard 1D is superior. The multiplicity refers to the number of directly attached protons, i.e., whether the carbon is a methine, methylene, or methyl. In the version of DEPT at Columbia, the quats are not suppressed. There are two useful versions of DEPT spectra. The version is determined by the parameter `cnst12`.

<b>experiment</b>	<b>cnst12</b>	<b>information</b>
dept90	1	CH, Q only
dept135	1.5	CH, CH <sub>3</sub> up, CH <sub>2</sub> , Q down

The dept135 experiment is the most useful. The *phase* of the peak gives the multiplicity - the CH and CH<sub>3</sub> peaks will point in one direction and the CH<sub>2</sub> and quat peaks will be pointing in the opposite direction. The dept90 ideally gives only CH peaks but in practice this means that CH peaks are enhanced relative to others.

It is important to tune both the carbon and proton channels of the probe prior to taking a dept spectrum. The DEPT technique is a little more sensitive than a qualitative carbon spectra. Edited HSQC give the same info as DEPT but are much more sensitive.

Shown below is the DEPT135 (bottom) and DEPT90 (top) spectrum of sucrose. Note the quats have the same phase as CH<sub>2</sub>s.



## About sample size and tubes

When sample quantity is very limited, it is advantageous to limit the amount of solvent in which it is dissolved. If a normal 5mm tube is used, however, this cannot be less than about 500 $\mu$ L for proton spectra or 300 $\mu$ L for carbon spectra without causing serious lineshape problems (shimming problems) and the attendant loss of signal-to-noise. When one reduces the solvent quantity in a normal 5mm tube, it is important that the sample be centered within the coil. To do this, center the sample about the scored line on the plastic depth gauge.

There are special tubes made by Shigemi that can be used to restrict the active volume and, hence, reduce the amount of solvent without causing lineshape problems.

## Signal-to-noise

The signal-to-noise improves as the square-root of the number of scans and thus the square-root of the time of the acquisition. An approximate guide to experiment length for the standard 1D (based on strychnine, MW=334. It is the moles that are important so scale according your molecule) is as follows:

sample quantity (per ml)	Molarity (mM)	time
50mg	150	5min
15mg	45	30min
6mg	18	3hrs
3mg	9	12hrs

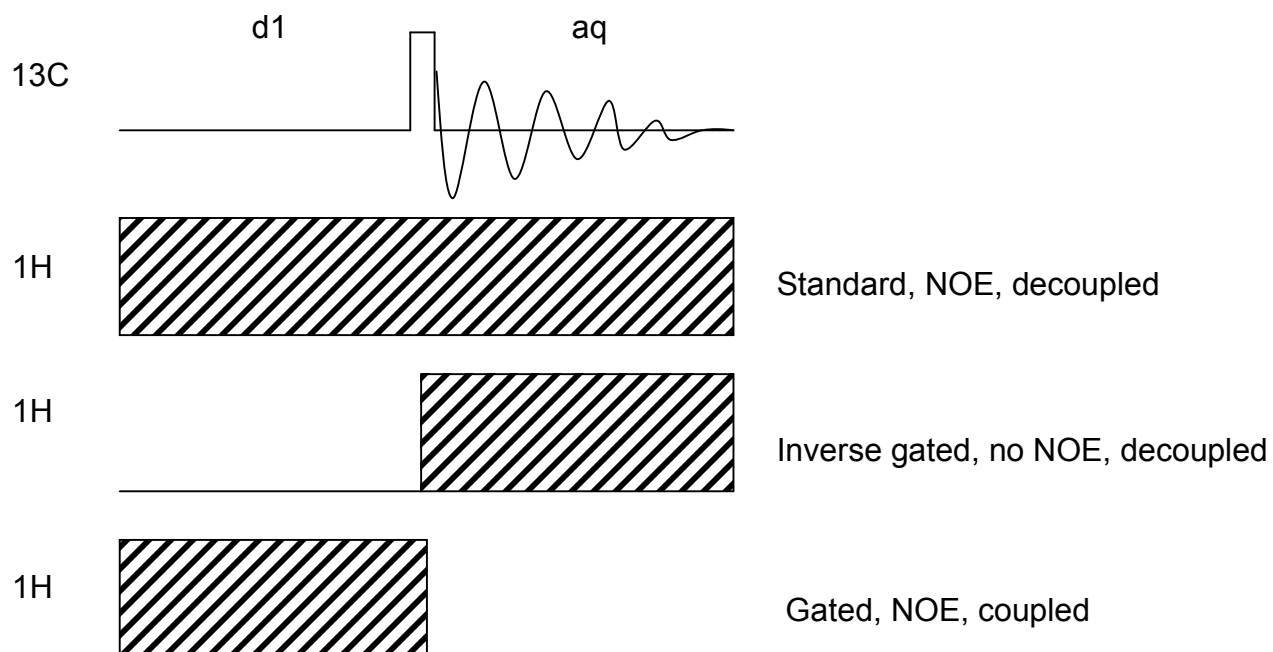
Only sign-up for the time you need. Spectra that show no baseline noise have used too much spectrometer time.

The minimum amount for an overnight run in a shigemi tube is about 1 mg for a 300 amu small molecule.

Carbon spectra may be essential but they are very time-consuming. A 2D HSQC requires much less time than a single carbon spectrum. If you are only interested in protonated carbons, the HSQC is to be greatly preferred over the 1D carbon.

### Decoupling modes

The decoupling mode can be controlled to give quantitative or coupled spectra. The pulse sequence for standard 1D experiment is divided into 2 periods, d1 and aq, separated by the pulse. D1 is the relaxation delay and aq is the time the FID is sampled.



Shown here are the standard experiments using different decoupling modes. A filled rectangle indicates the  $^1\text{H}$  decoupler is on. When the decoupler is on during the relaxation delay, the NOE effect is developed. When the decoupler is on during acquisition, a decoupled spectrum is acquired. These two effects are largely independent, i.e., one can have a coupled spectrum with NOE or a decoupled spectrum without NOE. The normal qualitative carbon spectrum is decoupled with NOE and hence, the decoupler is on all the time.

On Bruker NMR instruments, the pulse program must be changed to change the decoupler mode. The variable **pulprog** contains the name of the pulse program to be used for acquisition. See the table on the first page.

## **Quantitative Spectra – Inverse-gated decoupling**

Normal carbon spectra are qualitative in that the intensities do not correspond to the number of carbons present. There are two reasons for this: some carbons may not receive the full NOE and different carbons have different relaxation times ( $T_1$ ); carbons with long  $T_1$ 's experience saturation which results in reduced intensity. In general, carbons with no directly bonded protons suffer most from reduced NOE and long  $T_1$ 's. Quantitative spectra are taken without NOE, decoupled, and with a very long relaxation delay,  $d1$ , ranging from 2 -30 sec. Use the inverse-gated mode for quantitative spectra.

$^{13}\text{C}$  quantitative spectra take an extremely long time to acquire and should only be done overnight on concentrated samples. This mode is much more useful for  $^{31}\text{P}$  spectra.

## **Coupled Spectra- gated decoupling**

The simplest approach to measuring carbon-proton coupling constants is to take the carbon spectra coupled. For sensitivity reasons, these spectra are usually acquired with NOE. Use the gated decoupling mode.

Since the peak intensity is now divided into multiplets instead of singlets, this experiment must run approximately 10 times longer than the qualitative, decoupled one, in order to get equivalent signal-to-noise spectra.

## **Line Broadening**

The processing parameter, **lb**, controls the amount of exponential multiplication applied to the FID when it is transformed with the **ef** command (or the **em** command). The **ft** command does not apply exponential multiplication. Carbon spectra are always processed with some amount of line broadening and, thus, the **ef** command should always be used to transform carbon and other hetero-nuclear spectra. The normal value for carbon spectra is 3. Larger values, up to about 6, result in broader lines but higher signal-to-noise, while smaller values decrease line width (and increase resolution) but increase the noise.

## **Automating the process**

It is sometimes desired to be able to do a qualitative carbon spectrum as well as both dept spectra in series without being present at the spectrometer to start each separate experiment. The command **xau multizg** solves this problem. It executes multiple acquisitions on sequential experiment numbers. For example, assume your first experiment is < mycarbodata 2 1 x jimmy > while the second is <mycarbodata 3 1 x jimmy > and the third is <mycarbodata 4 1 x jimmy >. When you type **xau multizg** it will ask you how many experiments you wish to perform. If your current data set is <mycarbodata 2 1 x jimmy> and you enter 3, it will collect data for <mycarbodata 2 1 x jimmy>, <mycarbodata 3 1 x jimmy>, and <mycarbodata 4 1 x jimmy> in this order. Note that the data sets must already exist and have the correct parameters set. Thus, to use this method, do the following:

- a. setup for a carbon experiment in the current data set.
- b. Type edc and increment the experiment number (expno) by one. This creates a new data set and carries over the current parameters (those for the carbon experiment). Change parameters appropriately for a dept spectrum, if desired.
- c. Repeat step b if desired

d. Return to the first experiment number (lowest numerically) in this series of experiments, by typing re #, where # is the number. Alternatively, you may use the search command.

e. Type **xau multizg** and enter the number of experiments to be performed.

## Nuclei other than carbon

Most heteronuclear NMR is done on the 300wb, although the 300nb can do fluorine, carbon, and phosphorus. To see a list of heteronuclear parameter sets on the 300wb, type **rpar \*.5mm**. The current list is:

- boron11.5mm
- carbon.5mm
- deuterium.5mm
- lead.5mm
- lithium.5mm
- mercury.5mm
- n15.5mm
- phosphorus.5mm
- platinum.5mm
- Potassium.5mm
- selenium.5mm
- silicon.5mm
- sodium.5mm
- tellurium.5mm
- thallium.5mm
- tin.5mm

Others are possible. Ask John Decatur.

Some nuclei ( $^{15}\text{N}$ , Sn, Te, Si) have a negative gyro-magnetic ratio. This has implications for the pulse sequence one should use. When the gyro-magnetic ratio is negative, the NOE from protons is opposite in sign to its natural signal. If it happens that the NOE signal is nearly equal to the natural signal, they will cancel and give no apparent signal. Inverse gated decoupling mode (pulprog = zsig30, see above, or DEPT) is preferred to avoid the buildup of NOE for these nuclei. Ask for details.

For some nuclei, the INEPT or DEPT experiment can provide much higher signal-to-noise than the standard 1D experiment. This is true only for peaks that have observable couplings to proton. For example, for  $^{29}\text{Si}$  and  $^{15}\text{N}$ , the DEPT provides 2.5 times more signal than the standard 1D with NOE.

## External Referencing

Often in heteronuclear NMR, one uses an external reference. This refers to having a chemical shift reference in a separate NMR tube from your sample. Use internal referencing, if possible, since it is always superior. To insure correct external referencing, proper procedures must be followed.

The NMR lock controls the frequency calibration. **The lock must know the identity of the lock solvent at all times.**


For each sample, one must lock and choose the correct solvent. For instance, if the reference is in  $\text{CDCl}_3$ , and the sample is in acetone, after the reference is placed in the magnet, one must lock and choose  $\text{CDCl}_3$ , and then take the spectrum. For the sample, one must first lock and choose acetone, and then take the spectrum. Sometimes, the lock will simply “come on”, meaning that it locked without being told which solvent to lock on. This is wrong and will lead to incorrect referencing. Always re-lock when changing samples when working with external references.

An additional complication arises when the solvent has more than one resonance. Toluene, for example, has two sets of peaks. Normally the methyl group is chosen by the lock. The lock, however, may find the aromatic peaks first and lock on these. If this occurs, all referencing will be off by the difference in

chemical shift of the two solvent peaks. For this reason, when working in solvents with multiple resonances, one must manually check that the lock has found the correct peak. Ask for a quick demonstration of this procedure.

## Selective Decoupling of Protons

To selectively decouple protons while observing carbon, or more commonly, phosphorus, follow this procedure (written assuming phosphorus observation):

1. Take a 1D spectrum of proton. Obtain the frequency of the proton to be decoupled by clicking on , then adjusting the red line to the desired frequency, and click the left mouse button. Choose o2 and note the frequency.
2. Take a standard phosphorus spectrum. Change the data set and retake spectrum after changing the following parameters to the following values:
  - pulprog – zgcw
  - O2 – value determined in step one
  - pl26 – power for selectively decoupling protons
3. Power level pl26 depends on the breadth of the multiplet. A multiplet that covers 200 Hz needs much more power than one cover 20 Hz. Higher powers ensure complete decoupling but at higher powers, neighboring protons may also show decoupling effects. Several different powers may need to be investigated to ensure good results. Power levels are in decibels of attenuation. **Lower numbers mean higher powers. Never use a level less than 25 dB or probe damage may result!** Use the table below as a guide:

<u>Instrument</u>	<u>high power for 200 Hz multiplet</u>	<u>power for 20 Hz multiplet</u>
300nb	30	43
300wb	44	57
400	47	50

4. Finally, you may want to compare decoupled and coupled spectra. Use dual display to do so. Type edc2 and define the second data set. Click save and then click Dual to enter dual display.