Carbon, Deuterium and Heteronuclear NMR using Topspin

1. Carbon

There are several different types of carbon spectra such as a normal qualitative spectrum, DEPT, coupled, selectively decoupled, and those with and without NOE. A special capability, found only on the 400SL, is the ability to decouple 19F and 1H simultaneously while observing carbon.

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.

a. How to take 13C spectra

1. **Read in the carbon parameters:** Type **new** to define a new data set. Type **rpar carbon.top**. (If you are on the 400SL and you want to decouple 19F as well as 1H, see notes in section below on 19F decoupling,)
2. Lock and shim.
3. **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 **atma** and wait; tuning is automatic.

4. **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.

experiment	pulprog	d1	ns	ds	l4	aq	cnst12
Standard 1D	zgpg30	0.7	n	0	-	0.7	-
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	-

5. **Start the acquisition by typing start**. To look at the spectrum without stopping the acquisition, type **tr**, and then after the next scan is completed, type **ef**. To stop scanning, type **halt**.

b. Determining Multiplicity - DEPT

DEPT is the recommended standard carbon experiment. For maximum sensitivity for quats, however, 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.

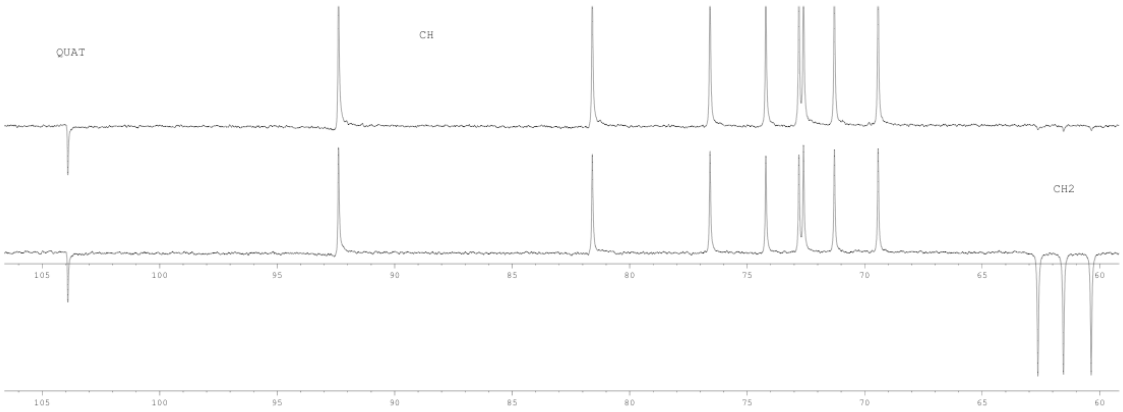
experiment	**cnst12**	**information**
dept90	1	CH, Q only
dept135	1.5	CH, CH_3 up, CH_2,Q down

The dept135 experiment is the most useful. The *phase* of the peak gives the multiplicity - the CH and CH_3 peaks will point in one direction and the CH_2 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. 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 CH2s.



c. 13C with 19F and 1H decoupling

Carbon observation with 13C and 19F decoupling is only possible on the 400SL with the TBO probe. Instead of rpar carbon.top, you must type **rpar carbon_19F_1H_dec_sp.top.** Type **atma** to tune the probe.

Decoupling the entire 19F shift range is not possible since it is so large. It is only possible to decouple a partial range (approximately+- 75 PPM) on the 400SL. You must center the decoupler on your peaks of interest. If there are multiple 19F peaks with very large shift differences, you may have to take two spectra. The parameter o2p defines the center of the decoupling in ppm. It is possible to decouple only 19F and not 1H. Ask for details.

d. 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 500μL for proton spectra or 300μL 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. There are also susceptibility plugs, available from Wilmad, that accomplish the same thing.

e. 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 for the

500 room-temperature probe (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
25mg	75	5min
7.5mg	22.5	30min
3mg	9	3hrs
1.5mg	4.5	12hrs

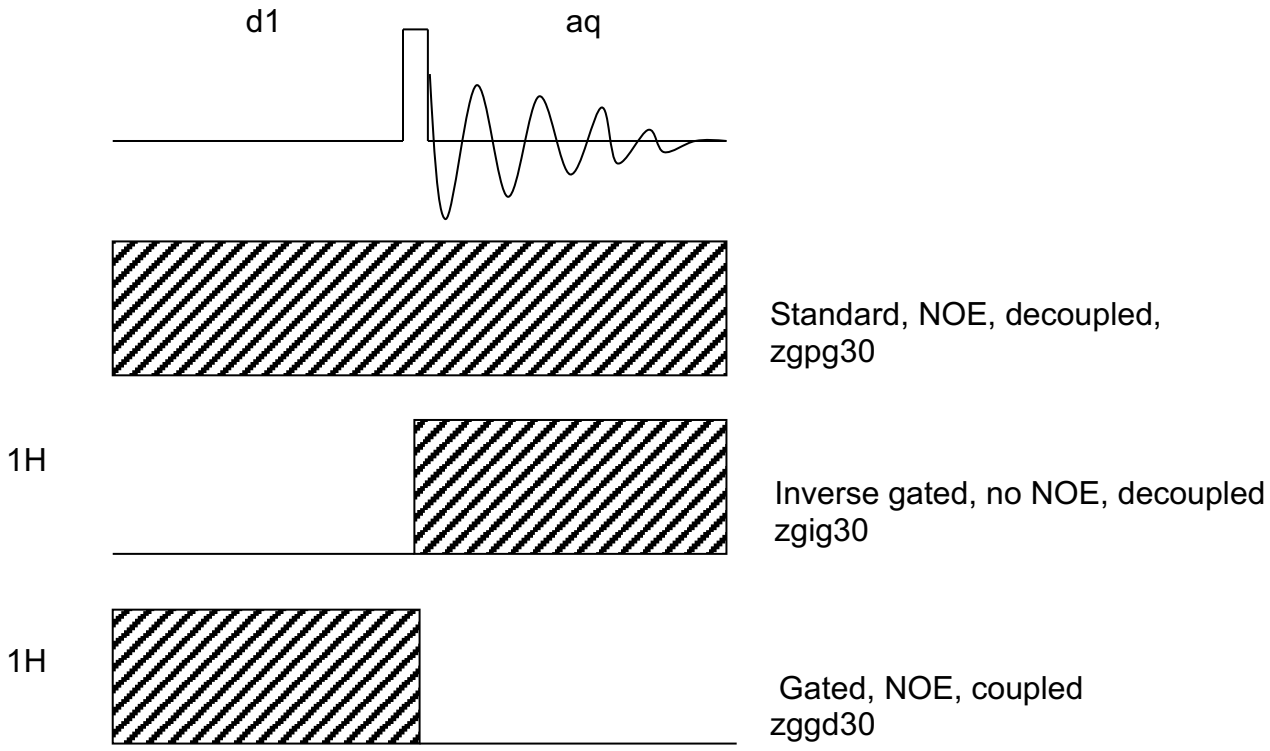
The 500 cryoprobe has about 6x better sensitivity for carbon. Thus, the limit of detection for 12 hour scanning time is about 0.75 mM on the cryoprobe.

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 0.75 mg for a 300 amu small molecule. If your molecular weight is much larger than 300, say 1000 amu, then the optimum S/N requires a longer pulse width (Ernst Angle). To effect this change, simply change the value of pulprog to zgpg instead of zgpg30. 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.

f. 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.

13C



Shown here are the standard experiments using different decoupling modes and the Bruker pulse sequence. A filled rectangle indicates the 1H 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.

g. 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 -60 sec. Use the inverse-gated mode for quantitative spectra.

13C 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 31P spectra.

h. 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.

2. Nuclei other than carbon

The best place for most heteronuclear NMR is the 400SL, 500, or 500 Ascend. All can do 19F. To see a list of heteronuclear parameter sets, type **rpar *.top.** A partial list is:

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

The NMR web site has a table of nuclei that are setup:
https://nmr.chem.columbia.edu/content/x-nuclei
Others are possible. Ask John Decatur.

To setup for 7Li, for example, you simply need to type rpar **lithium.top**, tune the probe, shim, and type zg.

Some nuclei (^{15}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 = zgig30, 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}Si and ^{15}N, the DEPT provides 2.5 times more signal than the standard 1D with NOE.

Removing Baseline distortion:
For some nuclei, such as ^{29}Si, ^{11}B, or ^{19}F, the spectrum baseline is often rolling or large broad peaks due to background from the glass in NMR tubes or probe materials. To remove most of it, one can make the parameter, de, equal to 100us, prior to taking the spectrum. A better approach, however, is backward linear prediction (BLP). For this, do not alter the parameter de. During processing type convdta and give a new experiment number. Then in Topspin, in Procpars, set ME_mod to LPbc, TDoff to about 32, and NCOEF to value anywhere from 128 to 1024. Then, type ef. There is a command, cryoproc1d, that automatically performs BLP. MestreNova can also perform backward LP. Ask.

a. Deuterium

Deuterium can be observed on all the NMRs. You must use normal protonated solvents and NOT deuterated solvents. There cannot be a lock and thus, one cannot shim on your sample normally. Since there is no lock, the magnetic field may not be in its usually place and thus a chemical shift reference is essential. For a reference, one can add a drop or two of the corresponding deuterated version of your solvent.

If you want the best possible spectrum you need to topshim on the solvent's proton peak. Since there is no lock, the solvent peak will not be in its usual position and thus you must take a proton spectrum prior to topshim and measure the chemical shift of your solvent peak. To do so:

1. The lock and sweep must be off. After your sample is inserted, do not type lock, but instead type lopo. In the BSMS window, select the [Lock/Level] tab and turn off both lock and sweep. The sweep and lock buttons should NOT be green. If they are green, click the relevant On-Off button.
2. Take a normal 1D spectrum, but with ns=1 and rg=1. (Type rpar protonstd all and then ns=1 and rg=1 and then zg or start). Process and note the shift(s) of your solvent peak(s).
3. To shim, you can use the 1H selective Topshim function. Type topshim lockoff 1h o1p=# in the command line where # is the ppm of largest solvent peak. If there is more than one peak, append selwid=0.5 to the command above.

Then change the dataset and follow these steps to observe 2H:
1. rpar deuterium.top. (rpar deuterium_lockamp.top on the 400SL)
2. type ii. This turns the lock off.
3. Type start.
4. When finished, you must change the data set (new), read in proton parameters, and type ii. This turns the lock back on.

b. Fluorine

The 400SL, 500, and 500 Ascend are capable of observing 19F. To setup, type **rpar fluorine.top**. Then type **atma**, and **start** to begin. Decoupling protons is also an option. To setup, type **rpar fluorine_1Hdec.top**. You will need to perform backward linear prediction to remove the large rolling peaks.

For quantitating ^{19}F, it is important to use a 30 degree pulse instead of 90 degree. This improves the excitation bandwidth. To use a 30 degree pulse change pulprog to zg30.

It is also possible to observe 1H while decoupling 19F. Ask John Decatur.

3. 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
   - pldb26 – power for selectively decoupling protons
3. Power level pldb26 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!** User the table below as a guide:

Instrument	high power for 200 Hz multiplet	power for 20 Hz multiplet
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.

4. 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.

5. 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. On ALL NMRs there is a queuing system. If the NMR is currently collecting spectra, then typing zg puts an experiment into a queue that will be executed when the current job is complete.

6. Chemical Shift Referencing

Often in heteronuclear NMR, there are conflicting primary references. In 2001, IUPAC set new definitions and standards for NMR referencing,[1] and updated these in 2008[2]. A significant change

from past conventions is the introduction of a "unified scale," with a single primary reference of the 1H resonance of TMS for all nuclei. The unified scale relies on Ξ values, stated as percentages:

$$\Xi_x \equiv 100\times(\nu_x/\nu_{TMS})$$

where ν_x = the absolute frequency for the 0 ppm position in the X spectrum, and ν_{TMS} = the TMS absolute frequency for ^{1}H of 0.1% TMS in CDCL3. For example, on the 500, ν_{TMS} =500.130013. The Ξ_x value is found in a table in reference 2 and is specific for each nucleus. ***ν_x is equal to the parameter SF in Bruker's software***. Below is a table with the proper SF values already calculated according to the equation above. These SF values set the zero ppm scale. With Topspin software, follow this procedure:

> The value of SF must be set indirectly. Using the following relationship: SR_x= $SF\text{-}BF1_x$. Find the value of SF from the table below, and the value of BF1 from Topspin's acqpar. Then, calculate SR and enter this value. Note that SR is in Hz and SF and BF1 are in MHz so you must convert prior to subtraction. Then you must check that SF matches the value in the table.

Mestrenova also allows the use of absolute frequency referencing. You must have a properly referenced proton spectrum (ideally TMS in CDCL3) loaded along with the X nuclei spectrum. Then in the X nuclei window, click the 'Absolute Reference button of the toolbar and then select 1H experiment as the reference and check the boxes of the other experiments that you want to reference. The procedure works for 2D as well.

The NMR lock controls the frequency calibration**. <u>The lock must know the identity of the lock solvent at all times</u>.** For each sample, one must lock and choose the correct solvent.

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.
Table of SF values for common nuclei based on IUPAC method for our NMRs. See Harris, et al (2001) for the actual molecule that was used to choose the 0 PPM. Note that the 400L and 400SL have slightly different frequencies. This is also true of the 500 and 500ASC.

Nucleus		300	400SL	400L	500	500asc
1H		300.1300065	400.1300092	399.9200134	500.1300130	500.0530004
7Li	38.863797	116.6419165	155.5057145	155.4241022	194.369513	194.339583
11B	32.083974	96.29363325	128.3776081	128.3102331	160.4615833	160.4368746
13C	25.14502	75.46775016	100.6127708	100.5599674	125.7577918	125.738427
15N	10.136767	30.42347946	40.56024673	40.53895994	50.69701411	50.68920753
17O	13.556457	40.68699528	54.24345264	54.21498465	67.79991016	67.78946998
19F	94.094011	282.4043613	376.4983749	376.3007814	470.5923894	470.5199252
23Na	26.4519	79.39008919	105.8419899	105.786442	132.2938909	132.2735196
29Si	19.867187	59.62738963	79.49457717	79.45285691	99.36176493	99.34646469
31P	40.480742	121.4948536	161.9755967	161.8905888	202.4563402	202.425165
39K	4.666373	14.00518559	18.67155871	18.66175953	23.33793189	23.3343382
57Fe	3.237778	9.717543322	12.95532141	12.94852221	16.19309953	16.19060604
77Se	19.071513	57.23933321	76.31084672	76.27079735	95.38236045	95.36767298

Nucleus		300	400SL	400L	500	500asc
89Y	4.900198	14.70696458	19.60716271	19.5968725	24.50736089	24.50358712
103Rh	3.186447	9.563483588	12.74993067	12.74323927	15.9363778	15.93392383
109Ag	4.653533	13.9666489	18.62018202	18.6104098	23.2737152	23.27013139
113Cd	22.193175	66.60837757	88.80155317	88.75494843	110.994729	110.9776375
117Sn	35.632259	106.9431013	142.5753612	142.500535	178.2076216	178.1801802
125Te	31.549769	94.69032375	126.2400936	126.1738404	157.7898638	157.7655665
129W	4.166387	12.50457757	16.67096469	16.66221545	20.83735184	20.8341432
133Cs	13.116142		52.48162019			65.58766161
195Pt	21.496784	64.51829922	86.0150838	85.96994145	107.5118686	107.4953134
199Hg	17.910822	53.75575123	71.66657372	71.62896174	89.5773964	89.56360281
203Tl	57.1232	171.4438639	228.5670654	228.4471091	285.6902676	285.6462755
207Pb	20.920599	62.78899514	83.7095947	83.66566232	104.6301945	104.614083

REFERENCES

1. R.K. Harris, E.D. Becker, S.M. Cabral de Menezes, R. Goodfellow, and P. Granger, "NMR Nomenclature. Nuclear Spin Properties and Conventions for Chemical Shifts (IUPAC Recommendations 2001)", Pure and Applied Chemistry 73, 1795-1818 (2001). The paper is available on-line at: http://www.iupac.org/publications/pac/2001/7311/7311x1795.html

2. R.K. Harris, E.D. Becker, S.M. Cabral de Menezes, P. Granger, R.E Hoffman, K.W. Zilm, "Further conventions for NMR shielding and chemical shifts," *Pure Appl. Chem.* **80**, 59-84 (2008). Available at: http://www.iupac.org/publications/pac/80/1/0059

Chemical Shift Issues for Nitrogen.

The chemical shift ranges are the same for both nitrogen isotopes ^{14}N and ^{15}N. IUPAC recommends CH3NO2(90% in CDCl3) as the chemical shift standard for both nuclei. However, most spectroscopists reference nitrogen spectra to liquid NH3 and Topspin, by default, uses it as well. To convert 15N chemical shifts to the IUPAC CH3NO2 standard, subtract 380.5 ppm and for 14N, subtract 381.6 ppm.