

EXPERIMENTS TO DO:

1. Acquire, process, and assign 1D ^1H spectrum
2. Calibration the ^1H 90 degree pulse for your sample
3. Acquire, process, and assign 1D ^{13}C spectrum
4. Acquire, process, and assign 1D ^{13}C DEPT spectra

STANDARDS TO USE THIS WEEK: 2% 2-ethyl-1-indanone for ^1H , 1 M 2-ethyl-1-indanone with $\text{Cr}(\text{acac})_3$ to reduce T_1 relaxation times for ^{13}C and ^{13}C DEPT. Both are in deuterated chloroform (CDCl_3).

Basic Data Acquisition:

For solution NMR, the general data acquisition procedure is the same, whether you use a Bruker, Varian or other brand of spectrometer: insert your sample, read in acquisition parameters i.e. set-up the experiment, lock to deuterated solvent, tune the probe, shim the magnetic field, edit acquisition parameters, set the receiver gain, acquire data. The details and commands for acquisition will differ, depending on what spectrometer/software you utilize, but the general procedure is the same.

Data Processing:

MestReNova is free of charge for University of Oregon students and researchers. You will need to be connected to the UO network when activating the license. The MestReNova License should not be shared with outside parties as the license is only available to UO students and researchers. From a campus network-connected computer, go to <http://hotwax.uoregon.edu/mnova> to download the Mnova license and installation files. Follow the installation instructions PDF if you run into difficulties.

Bruker also offers a free academic license for TopSpin. Go to <http://bruker.com/freetopspin> to download your own copy.

You can also remotely access your data from a computer connected to the campus network or VPN connected. Use an SSH or SFTP program to connect to the NMR data server hotwax.uoregon.edu using the **NMR class username (ch610)** and **password (ch610-2021)**. Examples of SSH/SFTP software for Windows are Putty, CuteFTP, WinSCP, Cyberduck and FileZilla. Mac software includes Cyberduck and Fetch.

Booking NMR Time:

You can navigate to <http://hotwax.uoregon.edu> and log in using the NMR class username and password to make an NMR reservation. The NMR class has priority access to the spectrometer(s) during Thursday afternoons. If you have any difficulty getting spectrometer time, please contact Nanette.

Experiments

Insert your sample into the instrument via the SampleCase loading system. Then, log on to the computer and launch the TopSpin software. Refer to the Routine 1D Acquisition on Bruker handout for more detailed information on how to acquire data on Bruker spectrometers.

1. ^1H NMR Spectrum

^1H NMR Spectrum – Data Acquisition

- 1) Type **new** to set up a new experiment and read in the proton parameters (**1h.av600**).
- 2) **Lock** on to your deuterated solvent.
- 3) Tune the probe by typing **atma**.
- 4) Read in the default shim file: **rsh LAST**. Then run **topshim**.
- 5) Type **ased** to check your acquisition conditions. You could use **ns = 1** scans as the sample is pretty concentrated.
- 6) Set the receiver gain by typing **rga**.
- 7) Type **zg** to acquire your data.
- 8) Process your data: **ef** apodizes and Fourier transforms the time domain data, **apk** automatically phases the data and **abs n** corrects the baseline for more accurate integrations.

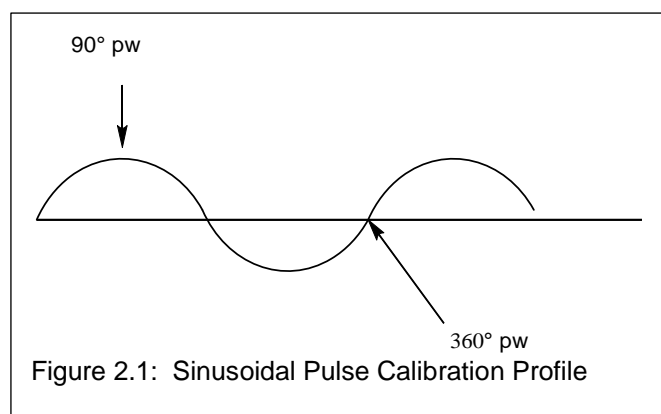
^1H NMR Spectrum – Data Processing

Additional data processing can be done offline via MestReNova. Please integrate your data and assign the peaks.

2. ^1H 90 degree ($\pi/2$) Pulse Calibration

Occasionally, some experiments require a careful pulse calibration. There are two ways to calibrate the 90 degree pulse on a Bruker spectrometer. In general, you can either use the automatic calibration or the manual calibration. Also in general, the automatic calibration does not always work... so you will likely need to do it by hand.

** For this assignment, you will need to turn in a complete pulse calibration array (like Figure 2.1), which requires a manual calibration. **



Automatic Pulse Calibration – Data Acquisition

- 1) Type **new** to set up a new experiment and read in the proton parameters (**1h.av600**).
- 2) Execute the automatic 90° pulse calculation by running the command **pulsecal**. Pulsecal returns three values. Take note of the bottom one, your 90° pulse (μs) and the power level (dB) (Figure 2.2.) Pulsecal often fails (gives the error "too many entries in peaklist").

Manual Pulse Calibration – Data Acquisition

1) Follow step 1 above. Type **getprosol** to read in the default pulse length.

2) Set the pulse program **pulprog** to **zg**. (Should have been set as **zg30** before). Also set **ns = 1**.

3) Set **p1** to $p1/2$ (e.g., if $p1 = 14$ after **getprosol**, set $p1 = 7$).

4) Acquire a spectrum with **zg**, then **ef; apk**.

5) Zoom in on the peak you wish to calculate the 90° pulse for.

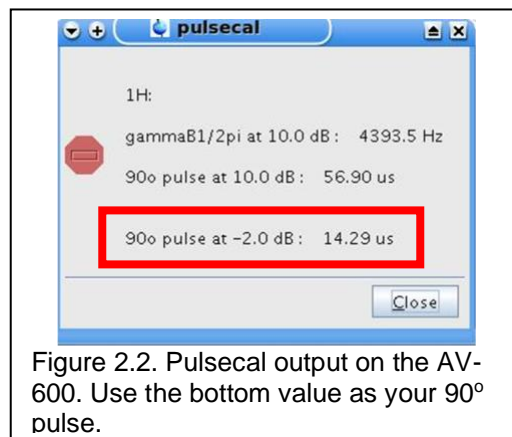


Figure 2.2. Pulsecal output on the AV-600. Use the bottom value as your 90° pulse.

6) Type **dpl1**. This will set the left and right x-coordinates (F1/F2 or F1P/F2P) and the vertical scaling of the result.

7) Type **paropt** and enter $p1$, the first value, the increment, and the number of measurements in the series. Increments of a few microseconds is usually good enough.

a. In this class, we can use the values **P1, 2, 4, 16** in the 4 pop-up windows.

8) Each time **paropt** runs, you are left in **procno 999**. You can return to the **expno** and **procno** from which you started by using the **re** command (i.e., if you started in **expno 1 procno 1**, type **re 1 1**).

9) Expand the vertical scale as necessary to judge the null point as the point with symmetrically distributed residual signal. You can either use the scaling buttons on the left or the equivalent command line commands to rescale the display while **paropt** is running. You'll have to mentally count the number of increments over to the target peak, so choose start values and increments that make the arithmetic easy.

a. If you do not want to wait for the full array to run, you can **kill** the experiment.

Type **kill**, and a window of all of the running processes will pop-up. Select **paropt** and hit the "Kill" button. Exit out of the pop-up window.

Pulse Calibration Data Processing:

1) Generate a PDF of your **paropt** output. When you turn in your assignment, please note where the 90, 180, 270, and 360 degree pulses are as well as their approximate times.

3. ¹³C NMR Spectrum

¹³C NMR Spectrum – Data Acquisition

Typically you will run a ¹³C NMR experiment after acquiring a ¹H dataset on a particular sample (so you would skip locking and shimming). However, for the sake of time, we are switching to a highly concentrated ¹³C NMR sample so you must make sure to lock and shim again.

1) Insert the 1 M 2-ethyl-1-indanone sample. Type **new** to set up a new experiment and read in the carbon parameters (**13c.av600**).

2) **Lock** on to your deuterated solvent.

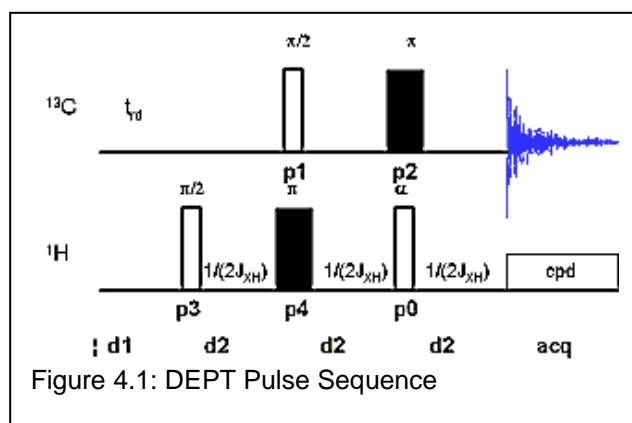
- 3) Tune the probe by typing **atma**.
- 4) Read in the default shim file: **rsh LAST**. Then run **topshim**.
- 5) Type **ased** to check your acquisition conditions. You can use **ns = 32** for 32 scans.
- 6) Due to its naturally low sensitivity (compared to ^1H), the receiver gain is already set to the maximum value. Do NOT type rga!
- 7) Type **zg** to acquire your data.
- 8) Process your data: **ef** apodizes and Fourier transforms the time domain data, **apk** automatically corrects the baseline, and **abs n** corrects the baseline for more accurate integrations.

^{13}C NMR Spectrum – Data Processing

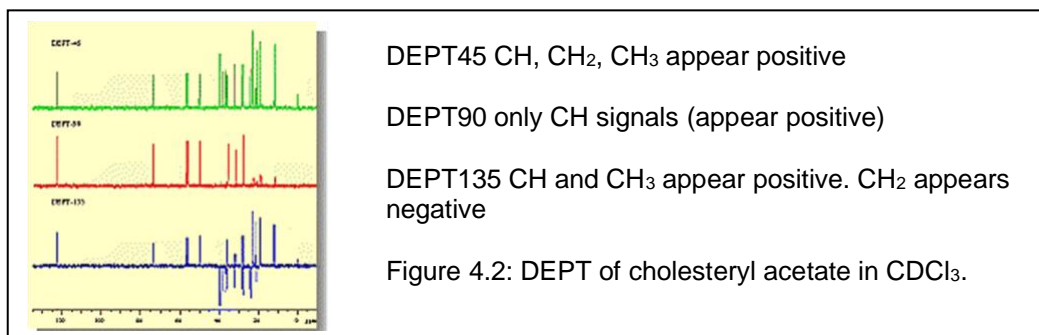
Additional data processing can be done offline via MestReNova. Please assign the spectrum, using the DEPT spectra if necessary to help make the assignments.

4. ^{13}C DEPT NMR Spectrum

1. Distortionless Enhancement by Polarization Transfer is a polarization transfer technique used to observe nuclei with a small gyromagnetic ratio, which are J-coupled to ^1H . DEPT makes use of this polarization transfer to differentiate different types of ^{13}C signals [methyl (CH_3), methylene (CH_2), and methine (CH)]. Quaternary carbons are missing from DEPT spectra because the one-bond heteronuclear J-coupling (J_{XH}) is used for polarization transfer.



2. DEPT may be run with or without ^1H -decoupling and it is relatively insensitive to the precise matching of delays with coupling constants, and so is much easier to use than the closely related INEPT sequence. DEPT, on the other hand, is more sensitive to pulse imperfections than INEPT.



3. The DEPT pulse sequence is shown in Figure 4.1. The final ^1H pulse with flip angle θ selects for the CH_3 , CH_2 or CH signals. This angle is set to 45° in the DEPT-45 sequence, which yields spectra with positive CH , CH_2 , and CH_3 signals; to 90° in DEPT-90, which

yields spectra with only CH signals; and to 135° in DEPT-135, which yields spectra with positive CH and CH₃ signals and negative CH₂ signals. If you compare the standard ¹H-decoupled ¹³C, and DEPT-45, -90, and -135 spectra, it is possible to determine which signals arise from primary, secondary, tertiary, and quaternary ¹³C's (Figure 4.2).

4. The full DEPT experiment involves all three: DEPT45, DEPT90, & DEPT135. If the multiplicities of some of the signals are already known from previous structural information, one can in principal acquire only the DEPT135 and correctly phase the known signals. The unknown signals should then be either positive or negative depending on their multiplicities. While this is not complete information, one can usually distinguish CH's from CH₃'s on the basis of the chemical shifts.

¹³C DEPT Data Acquisition:

- 1) We've already acquired a ¹³C spectrum. Drag and drop that dataset into the TopSpin window and type **ixpno** to create a new experiment with the same parameters as the carbon. You will need to change the **pulprog** to the DEPT experiment you wish to run. The names of the pulse sequences are as follows:
 - a. **dept45** – DEPT-45 experiment
 - b. **dept90** – DEPT-90 experiment
 - c. **dept135** – DEPT-135 experiment
- 2) You may want to edit some acquisition parameters in **ased**.
 - a. **cnst2 = 145** (an "average" value for a one-bond C-H J-coupling constant).
 - b. **p3** = the 90 degree pulse you calibrated in step 1
 - c. For routine use (and this class), we suggest **ns = 32**, **ds = 8**, but you may have to increase **td0** for low concentration samples.
- 3) Make sure that you are tuned to both proton and carbon. Type **zg** to start your data acquisition. If you set up three experiments in a row (e.g., EXPNO 3-5 are DEPT45, 90, and 135), you can use the command **multizg 3** in the active window of the first experiment.

¹³C DEPT Data Processing:

- 1) Process with your data and plot the spectra so the negative peaks can be seen. The DEPT45 can be used to determine the phase to be used for the rest of the DEPT spectra. The phase constants of the DEPT45 are applied to the DEPT90 and DEPT135 spectra with the **pk** command (usually in the combined **efp** command).
 - a. For DEPT45: you can use **ef**; **apk**; **abs n**. Note the **phc0** and **phc1** values.
 - b. For DEPT90 & DEPT135: set **phc0** and **phc1** to the same value as in your DEPT45 experiment. Then use the **efp** and **abs n** commands.
- 2) If the multiplicities of some of the signals are already known from previous structural information, you can in principal acquire only the DEPT135 spectrum and correctly phase the known signals. The unknown signals should then be either positive or negative depending on their multiplicities. While this is not complete information, one can usually distinguish CH's from CH₃'s on the basis of the chemical shifts.

When you are done in the lab, make sure to log out. Type **CTRL + ALT + BACKSPACE** to log out of the computer.

What to turn in:

- Processed ^1H spectrum of ethyl-indanone, properly phased, and integrated with each peak assigned.
- Plot of the ^1H pulse calibration array. Label the 90, 180, 270, and 360 degree points on the sinusoidal nutation curve, as well as their approximate times.
- Processed spectra for each of the four ^{13}C experiments on ethyl-indanone, properly phased. Also include one comparison plot where you stack them horizontally. Assign the peaks (do this for the original standard ^{13}C NMR spectrum, not for each one). Food for thought: Compare the differences between DEPT135 and "standard" ^{13}C NMR—aside from the number of peaks, there is signal intensity difference even though you used the same number of scans. Why?
- Answers to the following questions:

Questions

1. What purpose does locking serve?
2. What purpose does tuning the probe serve?
3. List a few ways you can tell if your sample is well shimmed.
4. In the case of our current experiment, we make the assumption that our ^1H integrals are reasonably accurate. Why do we not make the same assumption for the ^{13}C spectra?
5. If you play around with your data in MestReNova, you can try different window functions (Processing > Apodization **w**). What is the effect of increasing the exponential line broadening parameter **lb**? What about changing the apodization function from exponential to something else?
6. What is zero-filling and what is the purpose of zero-filling data?
7. NMR spectroscopists use the term "resolution" to mean different things in different contexts. What is the difference between "spectral resolution," "digital resolution", and resolution in terms of magnetic field homogeneity?