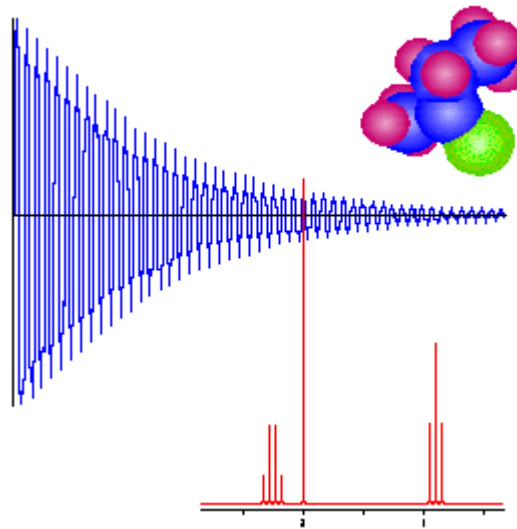


# COURSE#1022: Biochemical Applications of NMR Spectroscopy

<http://www.bioc.aecom.yu.edu/labs/girvlab/nmr/course/>

## **Lab 2: 1D Homonuclear and Heteronuclear Experiments**



LAST UPDATE: 2/7/2007

## Reading

### **Selected Readings for Lab 2:**

- refer to lecture on “One Dimensional Methods”
- AECOM Reference Guide for the DRX300
- Bruker Avance User Guide (BAUG)

## Outline

- data set handling
- spectral phasing
- spectral referencing
- integration
- 1D NMR experiments
  - 1D  $^1\text{H}$  spectrum
  - 1D  $^1\text{H}$  spectrum with Selective Homonuclear Decoupling
  - 1D  $^1\text{H}$  spectrum with Selective nOe
  - 1D  $^{13}\text{C}$  Spectrum w/ Broadband  $^1\text{H}$ -Decoupling
  - 1D  $^{13}\text{C}$  Spectrum w/out  $^1\text{H}$ -Decoupling ( $^1\text{H}$ -coupled  $^{13}\text{C}$  spectrum)
  - 1D  $^{13}\text{C}$  DEPT  $135^\circ$  Spectrum
  - 1D  $^{13}\text{C}$  Spectrum w/ Selective  $^1\text{H}$ -Decoupling
- Ernst Angle relation

## Data Set Handling

**Data Set File Name = NAME EXPNO PROCNO DISK USER**

(note: available disks are /u300 for data sets on drx300, /u600 for data sets on drx600 and /unmr0 for data sets on nmr0)

- edc (File -> New)** creates a new data set using parameters from the current data set and switches to new data set (note: if target data set exists, will simply switch to target data set)
- wrd** asks for new data set name, expno, procno, disk, username and will create new data set but will not switch to target data set (note: if target data set exists, will ask to overwrite)
- wrpa** same as wrd but must supply data set name info on command line
- re** allows you to switch to another data set by specifying appropriate combination of filename, expno, etc.
- dir (\*)** allows you to list data sets in current disk unit and to switch between data sets
- browse** allows you to list data sets on any disk unit and to switch between data sets
- search** graphical user interface for switching between data sets
- dela (\*)** deletes acquisition data in selected data sets (use mode to switch between deleting only data & data+parameters)
- delp (\*)** deletes processed data in selected data sets

\* - indicates that filename can be specified using complete name or with wildcards

## Phase Correction of a 1D Spectrum

Phase correction can be performed automatically or manually. Automatic correction is executed with the command *apk*.

Further small adjustments are often required which may be carried out with manual interaction, or it may be just as quick to perform the whole phasing process by manual operation alone. For most simple 1D experiments, it should be possible to adjust the phase of the spectrum so that all peaks are positive.

Click on **phase** to enter the phase correction submenu.

Click on **biggest**. This selects the biggest peak of the spectrum as the reference peak for the 0th-order phase correction. Notice that the phase of the biggest peak is automatically adjusted. To adjust the 0th-order phase manually, place the cursor on **PH0**, hold down the left mouse button, and move the mouse until the reference peak is positive and the baseline on either side is as flat as possible.

Most likely, at this point, peaks to the left and right of the reference peak are not yet phased correctly. These require a 1st-order phase correction. To adjust the 1st-order phase correction, place the cursor on **PH1**, hold down the left mouse button, and move the mouse until the peaks far from the reference point are also positive.



## Phase Correction of a 1D Spectrum, cont.

Note that it is advisable to select the 0th-order phase correction reference peak to be near one end of the spectrum. For some samples, the biggest peak is towards the middle of the spectrum. When this is the case, click on **cursor** rather than **biggest**. This ties the cursor to the spectrum, and the user can then define the reference peak by moving the cursor to the desired peak and clicking the middle mouse button.

Once the spectrum is phased correctly, click on **return** to exit the submenu and save the phase corrections by selecting **Save & return**. The 0th- and 1st-order phase corrections are stored as processing parameters **phc0** and **phc1**, respectively. To quit the phase correction submenu without saving the corrections, simply click on **return** and select **return**. In either case, the display returns to the main menu and the spectrum appears on the screen.



## Chemical Shift Referencing of a 1D Spectrum

With the digital lock, provided parameters are set correctly in the edlock table and that lock-in was achieved using the command lock, the magnetic field value is very nearly the same regardless of the lock solvent and so the spectra should be automatically calibrated - there may be an error of a few Hz.

To reference spectrum manually:  $^1\text{H}$  NMR spectra are customarily calibrated by setting the TMS (or DSS or TSP for aqueous samples) peak to 0 ppm. First expand the spectrum about this peak. To do this, move the cursor so that it is anywhere within the data field and click the left mouse button to tie the cursor to the spectrum. Now moving the mouse causes the cursor to move along the spectrum and the precise frequency of its position to be displayed in the small window entitled "Data Set". Move the cursor to the left of the TMS peak (the peak farthest to the right in the spectrum). Click the middle mouse button. Move the cursor to the right of the TMS peak and click the middle mouse button again. The spectrum is now expanded about the TMS peak. Click the left mouse button again to release the cursor from the spectrum. Now calibrate the TMS peak. Click on **calibrate** button on left menu, tie the cursor to the spectrum as described above. Position the cursor on the top of the TMS peak. Click the middle mouse button and at the bottom of the window the prompt "Cursor frequency in ppm" appears. Enter **0**. The TMS peak is now calibrated to 0 ppm. Click on **return**.

Note that a solvent peak can be also used for referencing – refer to list of solvent  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts in back of DRX300 Reference Guide (note that  $\text{H}_2\text{O}$  and HOD have a significant proton shift temperature dependence  $\sim 0.01\text{ppm}/^\circ\text{C}$ ).

## Integration of a 1D Spectrum

It is often useful to integrate  $^1\text{H}$  spectra. The simplest way to define the integral range is by entering *abs*. The command *abs* performs an automatic baseline correction and also automatically defines the integral range.

Use the following procedure to modify the integrals manually: Click on the **integrate** button on the left of the screen. Click on the **File** menu and choose **Read intrng** to load the automatic peak integrations. You will now see the peak integrations above the peaks.

- Select a peak to modify by clicking near the right edge of the integral. A star will appear when the integral is selected. Delete the integral by clicking the **delete** button in the 'current' grouping at the left of the screen.
- Integrate a region by clicking the left mouse button which places a moveable cursor on the spectrum baseline. Move the cursor to where the integral should start, then click the middle mouse button. Click the middle mouse button again where the integral should end. The new integral will appear above the peak. Repeat this process for all peaks which require re-integration. The integrated value of a selected peak can be specified by clicking on the **calibrate** button on the left of the screen and typing in a new value. All other peaks will be automatically recalculated.
- The integrals can be resized by clicking and holding on the **resize** button (both up and down arrows) and moving the mouse vertically. Resizing can be done in either the 'current' or 'all' button groupings.
- When the integrations are satisfactory, click on simply click on **return** and choose **Save as integr & return** to exit the integration tool.



## 1D <sup>1</sup>H Spectrum

See BAUG Chapter 3

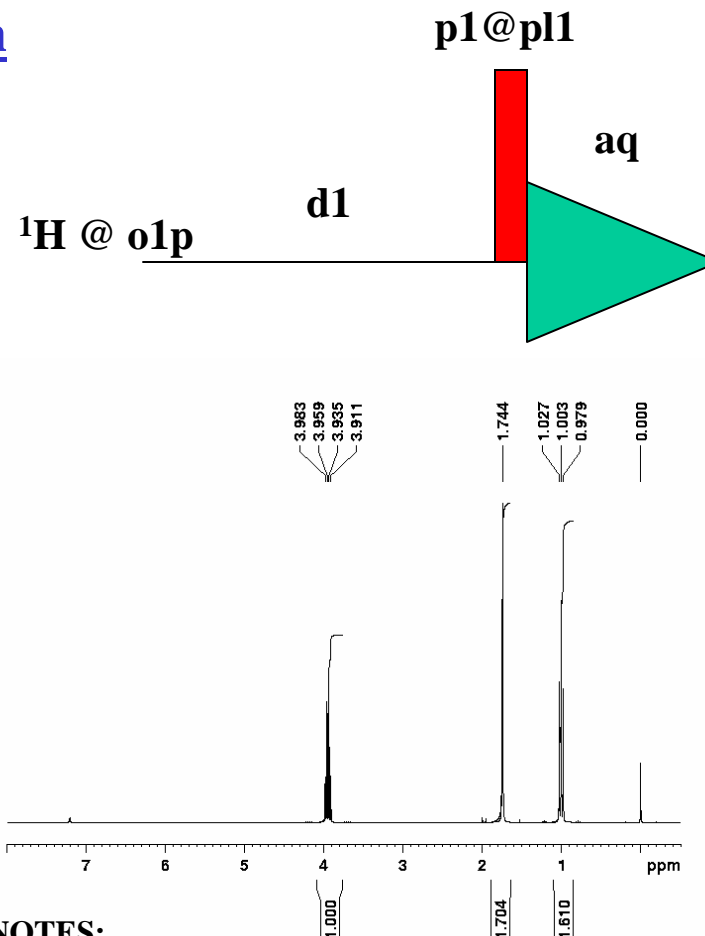
macro = h1zg

acquisition parameters:

pulprog	zg30	basic pulse sequence w/ 30 degree pulse
td	16k	number of data points (time domain size)
ns	8*n	number of scans
sw	20.5 ppm	sweep width
aq	1.33 s	acquisition time – determined by sweep width and number of data points
o1p	4.64 ppm	center of spectrum
d1	1.5 s	delay parameter in seconds – if pulse is <90°, don't need to wait as long – (Ernst Angle relation)

processing parameters:

si	16K	real spectrum size, si = td (zero fill 1x)
wdw	EM	exponential window function
lb	0.30 hz	exponential weighing factor



### NOTES:

- make sure proton channel is tuned
- integration should be accurate (represents *ratio* of number of protons)
- residual solvent signal will show splitting to <sup>2</sup>H if CHD<sub>n</sub>
- deuterated organic solvents contain small amounts of D<sub>2</sub>O (by-product of process) so exchangeable protons may have lower than expected integral
- Exchangeable protons can be identified by adding small amount of D<sub>2</sub>O to deuterated solvent and following signals that disappear

## 1D $^1\text{H}$ Spectrum w/ Selective Homonuclear Decoupling

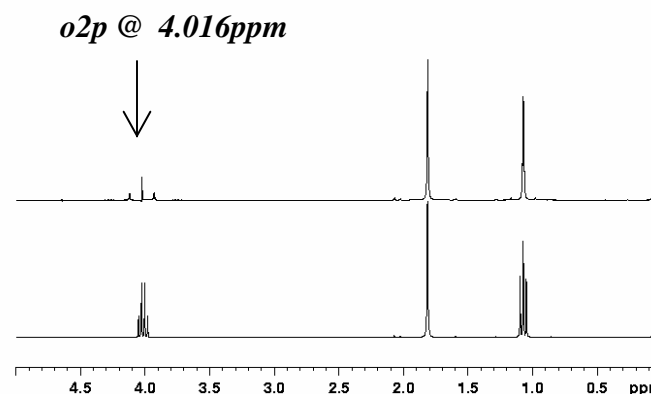
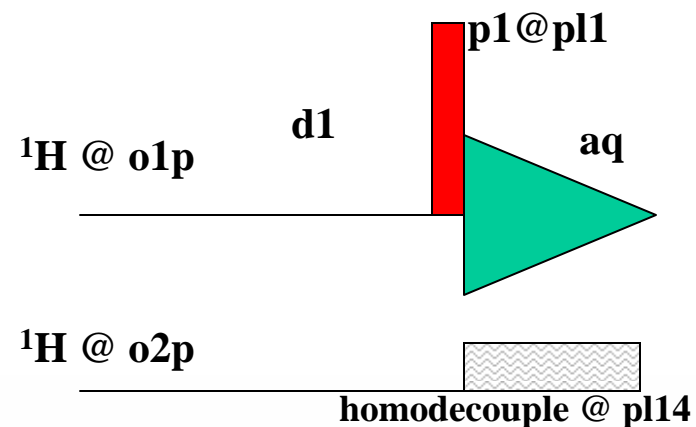
macro = h1zghd

### acquisition parameters:

pulprog	zg30hd	pulse sequence
td	16k	number of data points (time domain size)
ns	8*n	number of scans
sw	20.5 ppm	sweep width
aq	1.33 s	acquisition time – determined by sweep width and number of data points
o1p	4.64 ppm	center of spectrum
o2p	user defined	see procedure on next page
pl14	47dB	power level for homodecoupling
d1	1.5 s	delay parameter in seconds – if pulse is $<90^\circ$ , don't need to wait as long (Ernst Angle relation)

### processing parameters:

si	16K	real spectrum size, si = td (zero fill 1x)
wdw	EM	exponential window function
lb	0.30 hz	exponential weighing factor



### NOTES:

- make sure proton channel is tuned
- see procedure on next page for setting o2p

## Procedure for 1D <sup>1</sup>H Spectrum w/ Homonuclear Decoupling

1. create a new data set (this will contain the "reference" spectrum).
2. type `h1zg` to automatically acquire and process a proton spectrum.
3. click on utilities button to enter utilities menu.
4. click on O2 button, then drag mouse pointer to top of signal you wish to decouple and click the middle mouse button to define.
5. type `o2p` and a window will appear indicating frequency of selected proton signal in ppm. Remember value and then hit return to close window.
6. click on return to exit from utilities menu.
7. create a new data set using the command **edc** (this will contain the decoupled spectrum).
8. type `o2p` and set value to position of signal you wish to decouple in ppm.
9. type `zg` to acquire a new spectrum.
10. after data is collected, type `trf` to transform data
11. compare the decoupled spectrum with the reference spectrum as follows: type the command **edc2** and input name2, expno2 and procno2 of reference spectrum then click on save. Click on dual button or type the command **dual**. Two spectra should be displayed, one green (the current decoupled spectrum) and one pink (the reference spectrum). Zoom in on individual signals to see if they were affected by proton decoupling.
12. to select another signal for decoupling, return to reference spectrum using the `edc` command and repeats steps 3 – 11.

## 1D $^1\text{H}$ Spectrum w/ Selective nOe

### See BAUG Chapter 9

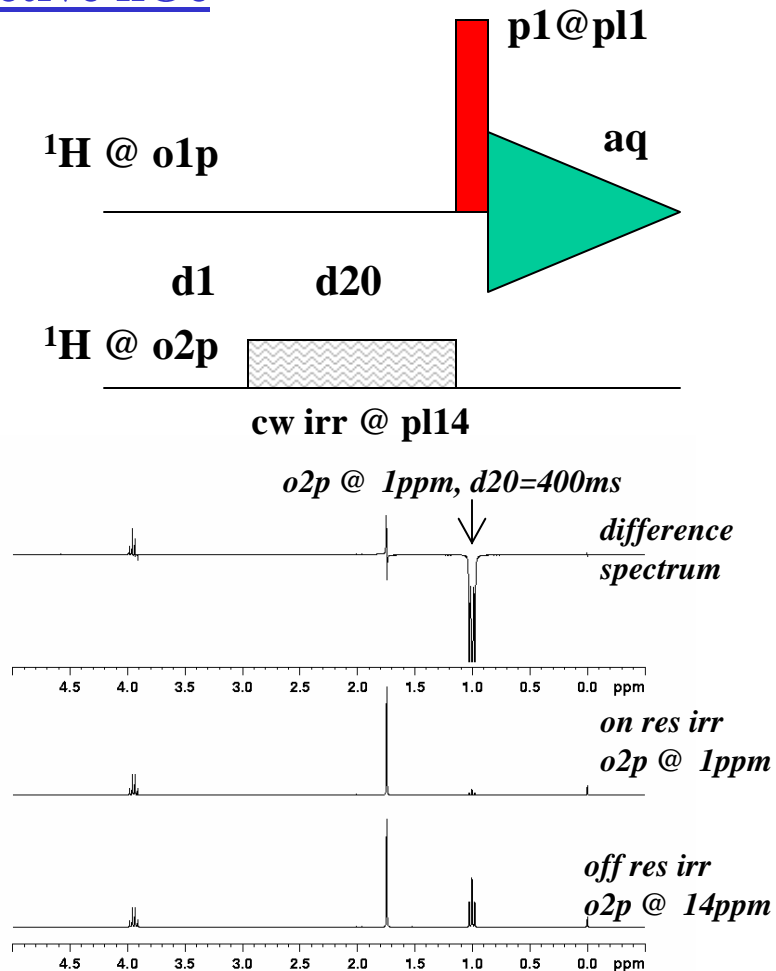
macro = h1selnoe

#### acquisition parameters:

pulprog	noedifld	pulse sequence
td	16k	number of data points (time domain size)
ns	8*n	number of scans
sw	20.5 ppm	sweep width
aq	1.33 s	acquisition time – determined by sweep width and number of data points
o1p	4.64 ppm	center of spectrum
o2p	user defined	see procedure for homonuclear decoupling but use macro h1selnoe
pl14	64dB	power level for cw saturation at o2p
d20	0-5xT <sub>1</sub>	time for cw saturation at o2p
d1	varies ~5s	delay parameter in seconds – need to wait 5xT <sub>1</sub> for accurate measurement of nOe

#### processing parameters:

si	16K	real spectrum size, si = td (zero fill 1x)
wdw	EM	exponential window function
lb	0.30 hz	exponential weighing factor



#### NOTES:

- make sure proton channel is tuned
- see procedure for setting o2p
- for steady state nOe,  $d20 \sim 5xT_1$ , not very useful. For truncated-driven nOe (TOE),  $d20 < T_1$ , useful for assignments.
- other relax mech. will hurt nOe!

## 1D $^{13}\text{C}$ Spectrum w/ Broadband $^1\text{H}$ -Decoupling

See BAUG Chapter 4

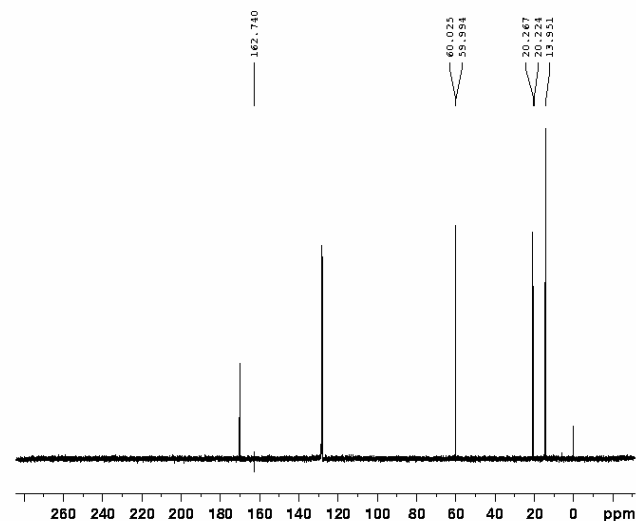
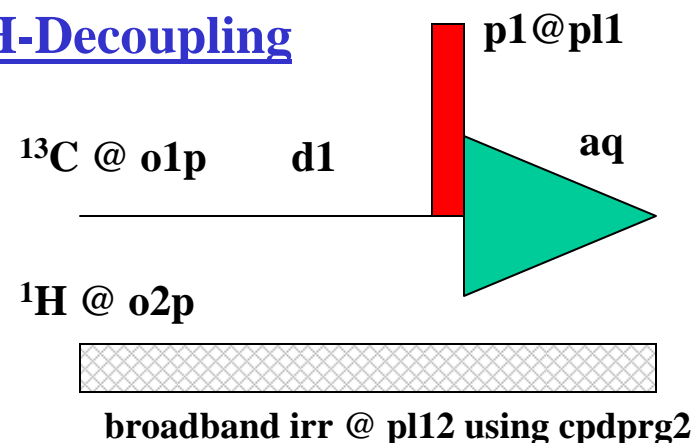
macro = c13zgdc

acquisition parameters:

pulprog	zgdc30	basic pulse sequence w/ 30 degree pulse
td	64k	number of data points (time domain size)
ns	8*n	number of scans
sw	315.5 ppm	sweep width
aq	1.38 s	acquisition time – determined by sweep width and number of data points
o1p	126.3 ppm	center of spectrum
o2p	4ppm	center for broadband $^1\text{H}$ decoupling
pl12	21dB	power level for broadband $^1\text{H}$ decoupling
cpdprg2	waltz16	composite pulse decoupling program
pcpd2	100 $\mu\text{s}$	$^1\text{H}$ 90 degree pulse @ pl12 for cpdprg2
d1	2 s	delay parameter in seconds – if pulse is $<90^\circ$ , don't need to wait as long (Ernst Angle relation)

processing parameters:

si	32K	real spectrum size
wdw	EM	exponential window function
lb	1 hz	exponential weighing factor



### NOTES:

- make sure proton and carbon channels are tuned
- $^1\text{H}$ -decoupling will generate  $^{13}\text{C}\{^1\text{H}\}$ -nOe (maximum intensity enhancement will be 3-fold) so sensitivity of spectrum will be enhanced but integration will not be accurate.
- $T_1$ 's of  $^{13}\text{C}$  can be quite long, especially quaternary C
- deuterated solvent will show signal(s) split by  $^2\text{H}$

## 1D $^{13}\text{C}$ Spectrum w/out $^1\text{H}$ -Decoupling ( $^1\text{H}$ -coupled $^{13}\text{C}$ spectrum)

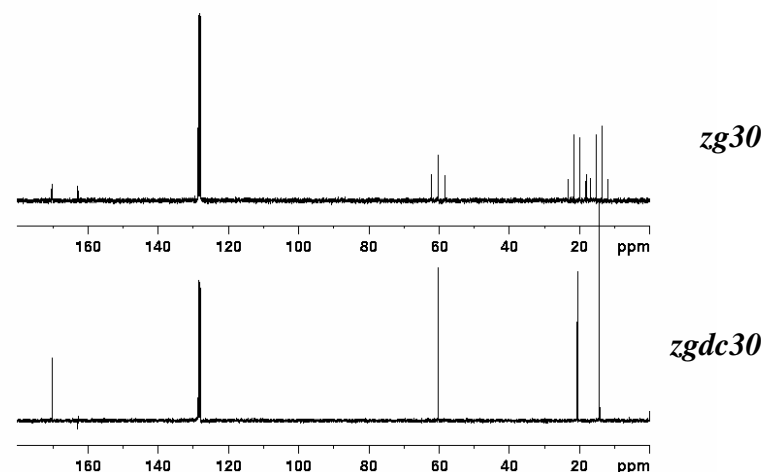
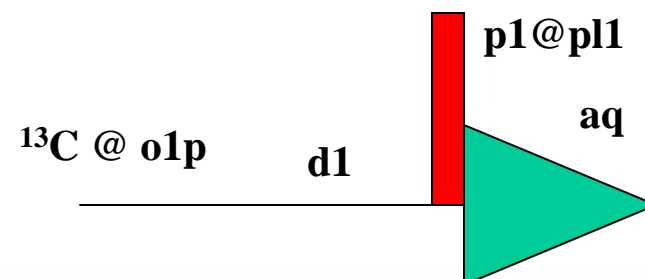
macro = c13zg

### acquisition parameters:

pulprog	zg30	basic pulse sequence w/ 30 degree pulse
td	64k	number of data points (time domain size)
ns	8*n	number of scans
sw	315.5 ppm	sweep width
aq	1.38 s	acquisition time – determined by sweep width and number of data points
o1p	126.3 ppm	center of spectrum
d1	2 s	delay parameter in seconds – if pulse is $<90^\circ$ , don't need to wait as long (Ernst Angle relation)

### processing parameters:

si	32K	real spectrum size
wdw	EM	exponential window function
lb	1 hz	exponential weighing factor



### NOTES:

- make sure proton and carbon channels are tuned
- much lower sensitivity than 1D  $^{13}\text{C}$  with broadband proton decoupling because no nOe enhancement and because of proton splitting
- proton splitting from  $^n\text{J}_{\text{CH}}$  ( $n=1,2$  or  $3$ ) can make spectrum very complex

## 1D <sup>13</sup>C DEPT 135° Spectrum

See BAUG Chapter 6

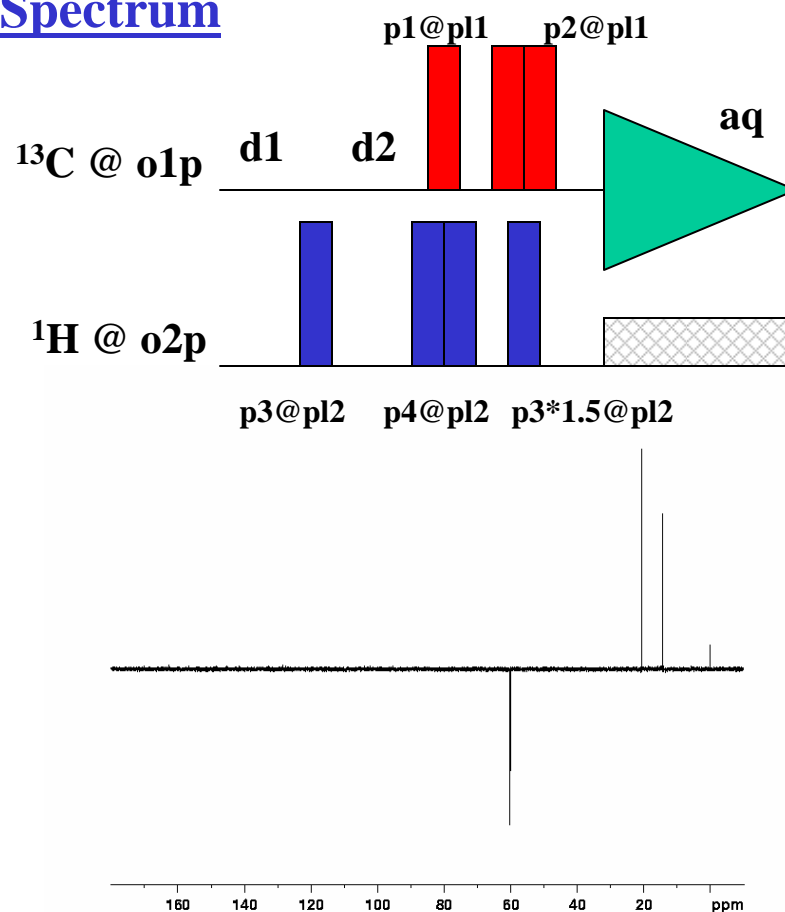
macro = c13dept135

### acquisition parameters:

pulprog	dept135	pulse sequence
td	64k	number of data points (time domain size)
ns	4*n	number of scans
sw	315.5 ppm	sweep width
aq	1.38 s	acquisition time – determined by sweep width and number of data points
o1p	126.3 ppm	center of spectrum
o2p	4ppm	center for <sup>1</sup> H pulses
pl2	0dB	power level for <sup>1</sup> H hard pulse
p3	10μs	<sup>1</sup> H 90 degree pulse @ pl2
d2	3.57ms	1/(cnst2*2) where cnst2= <sup>1</sup> J <sub>CH</sub> ~140Hz
d1	2 s	delay parameter in seconds

### processing parameters:

si	32K	real spectrum size
wdw	EM	exponential window function
lb	1 hz	exponential weighing factor



### NOTES:

- make sure proton and carbon channels are tuned
- NO quaternary carbons will appear in spectrum, methyl and methine carbons will have phase opposite to that of methylene (note: methyl carbons generally are most upfield signals)
- dept sequence can be used to vary last <sup>1</sup>H pulse in order to “edit” <sup>13</sup>C spectrum with only CH, CH<sub>2</sub> or CH<sub>3</sub>
- because of polarization xfer, recycle time determined by <sup>1</sup>H T<sub>1</sub>'s, not carbon.

# 1D $^{13}\text{C}$ Spectrum w/ Selective $^1\text{H}$ -Decoupling

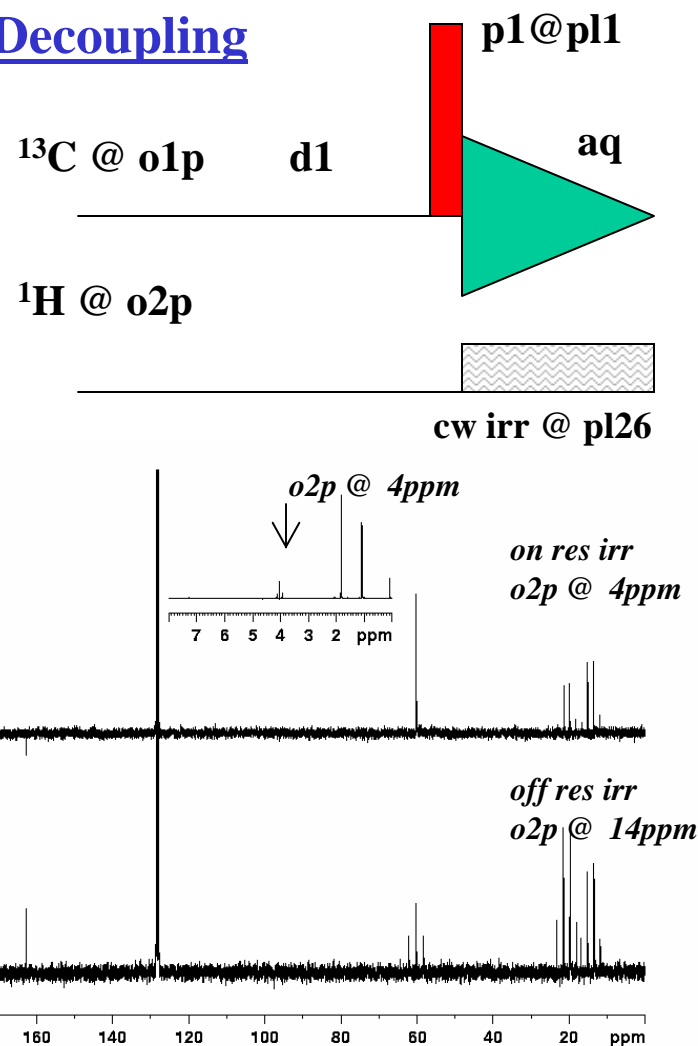
macro = c13zgcw

## acquisition parameters:

pulprog	zgcw30	basic pulse sequence w/ 30 degree pulse
td	64k	number of data points (time domain size)
ns	8*n	number of scans
sw	315.5 ppm	sweep width
aq	1.38 s	acquisition time – determined by sweep width and number of data points
o1p	126.3 ppm	center of spectrum
o2p	user defined	frequency for selective $^1\text{H}$ decoupling see procedure on next page for setting o2p
pl26	40dB	power level for selective $^1\text{H}$ decoupling
d1	2 s	delay parameter in seconds – if pulse is $<90^\circ$ , don't need to wait as long (Ernst Angle relation)

## processing parameters:

si	32K	real spectrum size
wdw	EM	exponential window function
lb	1 hz	exponential weighing factor



## NOTES:

- make sure proton and carbon channels are tuned
- much lower sensitivity than 1D  $^{13}\text{C}$  with broadband proton decoupling because no nOe enhancement and because of proton splitting
- proton splitting from  $^n\text{J}_{\text{CH}}$  ( $n=1,2$  or  $3$ ) can make spectrum very complex
- see procedure on next page for setting o2p

## Procedure for 1D $^{13}\text{C}$ Spectrum with Selective $^1\text{H}$ -Decoupling

1. create a new data set (this will contain the "reference" spectrum).
2. type `c13zgcw` to automatically acquire and process a coupled carbon spectrum.
3. create a new data set using the command **edc** (this will contain the spectrum with selective proton decoupling).
4. type `o2p` and set value to position of proton signal you wish to decouple in ppm (refer to steps 3 – 5 in the procedure for collecting proton spectra with homonuclear decoupling for determining the `o2p` value).
5. type `zg` to acquire a new spectrum.
6. after data is collected, type `trf` to transform data
7. compare the decoupled spectrum with the reference spectrum as follows: type the command **edc2** and input `name2`, `expno2` and `procno2` of reference spectrum then click on save. Click on dual button or type the command **dual**. Two spectra should be displayed, one green (the current decoupled spectrum) and one pink (the reference spectrum). Zoom in on individual signals to see if they were affected by proton decoupling.
8. to select another signal for decoupling, return to reference spectrum using the `re` command and repeats steps 3 – 7.

## The Ernst Angle

- Assume the steady state has been reached.
- Use a flip angle of  $\theta$  degrees.
- Find a condition where the transverse magnetization following the flip is maximized.

$$\cos \theta = \exp\left(-\frac{TR}{T_1}\right)$$

Relaxation time (sec)	Ernst Angle (with 1 sec repetition time)
100 (very slow T1)	8 degree
10	25 degree
4	33 degree
2	53 degree
1	68 degree
0.4	86 degree
0.1 (rapid T1)	90 degree