Multi-Nuclei detection.

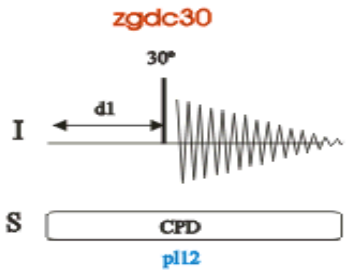
Objectives:

1. Signal enhancement and C-13-Proton multiplicity with CPD and DEPT.
2. Hands-on operation 500 MHz and 600 MHz spectrometers.
3. Use of "multizg"- to link multiple measurements.
4. Produce different types of plot for comparing "absolute or relative" spectra.
    a. Old software "1D+1D+1D.xwp" and "stack_2.xwp".
    b. Latest software: "layouts.multidisp/1D_3.xwp" and "layouts.stacks/stack_2.xwp".

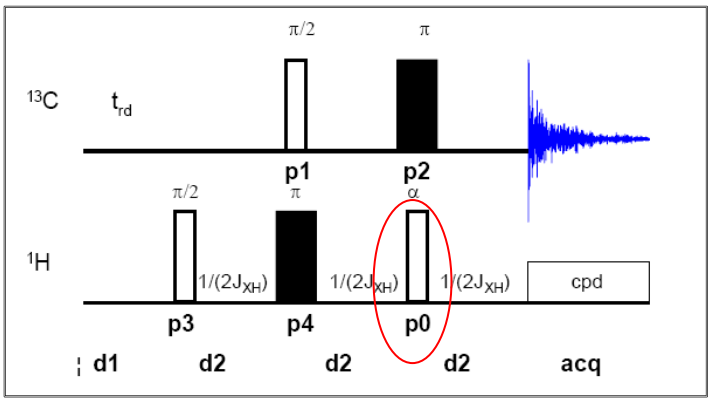
Pulse diagram:

Ac13_cpd

C13 with H-1 composite pulse decoupled.
CPD is a class of broad band H-1 decoupling pulses,
The most classical class is "WALT-16".



Ac13_dept135 **C13 with DEPT 135**



DEPT

Distortion-less **E**nhancement by **P**olarization **T**ransfer

A very efficient method for detect the presence of primary, secondary and tertiary carbon nuclei. The DEPT pulse sequence differentiates between CH, CH_2 and CH_3 groups by variation of the selection angle parameter (the tip angle of the final ^1H pulse):

- 90° angle gives only CH groups, the others being suppressed
- 135° angle gives all CH and CH_3 in a phase opposite to CH_2.

Reference: M.R. Bendall, D.M. Doddrell, and D.T. Pegg; J. Am. Chem. Soc. 103, 4603-4605 (1981).

Demo Sample (prepared for you):

A 40 mg Cholesteryl Acetate in 0.5ml D-solvent CDCL3. M.W. 428 $C_{29}H_{48}O_2$

Summary of the lab work:

There are two parts of this exercise: Part A is to be done with the 500MHz spectrometer, part B is to be carried out with the 600MHz spectrometer.

Part A: 500MHz Spectrometer with BBO probe:

500MHz (reserve about 3 hours for data acquisition, set up time is ~ 30 minutes).

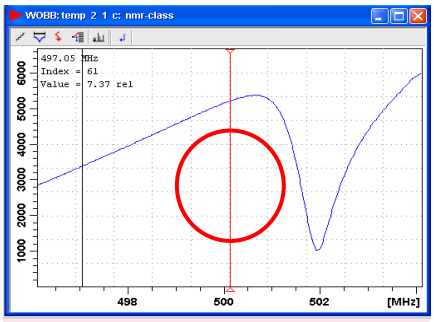
Perform three experiments with the same file name and consecutive experiment numbers.

1) Basic C13 with H_1 decoupled with NOE. {Ac13_cpd }.
2) Detect mainly carbons with single bonded proton. CH_1 {Ac13-dept90 }.
3) Detect majorly CH_3, CH_2 and CH_1 carbons (multiplicity). {Ac13-dept135}.

Procedure:

1. Create a new data set, name the file as "C13exercise" in your folder.
2. *Before inserting your sample, check temperature (**edte**), suitable for your sample!.*
3. **RPAR Ac13*** and select **Ac13_cpd**; copy all and [getprosol].
4. **Lock** to CDCL3.
5. **Tune probe: Critical and essential step!**

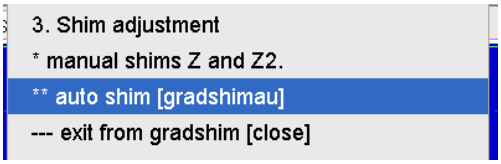
   - Click or type **[WOBB] to start the tune probe software.**
   - First-- adjust the capacitor in the flat panels at the bottom of the probe on C13 (Request TA present the first time).
     If no absorption is observed at the screen,
     Look up the Tune and Match capacitor settings (their values are post on the wall besides the magnet).



   - Use the fine slider (far right) to adjust the WOBB curve:
     - T panel for centering the positioning; M panel for maximize the absorption.
     - Note the T and M adjustments could interact slightly with each other, cycle T and M a couple of times to get the best fit.
     - Once C-13 adjustment is done, click  in the wobble window to switch to H-1 frequency.
     - Screen will change to H-1 WOBB curve in a few seconds; adjust alternatively the T and the M the capacitors similar conditions as for C13.
     - When adjust is complete, click  or type "**halt**".

6. **SHIM:**
   a. Use your basic know-how to roughly adjust the homogeneity with the keypad gradients (Z2 and Z1).

b. **Click "gradshimau" (500MHz) to use an automatic gradient shimming.**



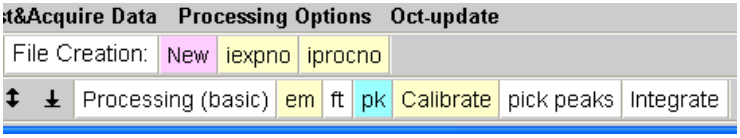
c. Wait when the lower keyboard entry shows "setshim complete".

7. Set **NS= 512** and **TDO = 1** (TDO is the count of saving NS onto hard drive).

- ✓ RGA
- ✓ Record the title page the acquisition time of this measurement and the values of NS, D1, and name of the pulse sequence. These critical values can be found by typing "ased".
- ✓ **DO NOT collect data with the command "ZG"** (as you are going to set up several experiments and collect all data sets, with a macro "multizg").

8. Type "**iexpno" or click the "iexpno icon":**

This command automatically creates a new file with one increment in EXPNO but with the same Data name.



- RPAR **Ac13_dept90**; getprosol
- Set NS = 256 Remark: Typical half the number of NS is needed as compared to those used for a regular C-13 _CPD experiment.
- RGA.
- Record the title page with values of NS and CNST2 (One bond coupling constant) and the pulse sequence used. These critical values can be found by typing "ased".
- **DO NOT collect data with the command "ZG"** (as you are going to set up several experiments and collect all data sets, with a macro "multizg").

9. Type "**iexpno" or click the icon to create EXPNO 3**.

- RPAR **Ac13_dept135**; getprosol
- Set NS =256, same as dept135
- RGA.
- Record the title page with values of NS, CNST2, and pulse sequence used.
- **DO NOT collect data with the command "ZG"** (as you are going to set up several experiments and collect all data sets, with a macro "multizg").

To collect all three spectra, follow the next two steps.

10. Return to the data file with the ***first experiment number*** that you would like to start.
In this lab session, type **"re 1"** to read (load) the EXPNO 1 data file onto the active spectrum window.

11. Type "**multizg**" and enter "**3**".
Automation will carry the experiments in consecutive manner.
Caution: "multizg" only works on consecutive experiments with the SAME file name!

Part B: Using the 600MHz, BBO probe (same sample).

600MHz (reserve ~ 1.5 hrs.)

1. **Notify staff** so that we will be showing you the new hardware. Read the general instruction on how to use the sample changer.

2. Follow the same procedure of part A, **except:**
   a. Step 5, use "ATMA" to tune probe instead of "wobb" for BBO probe.
   b. Step 6, type or click "topshim" instead of "gradshim".

3. Carry out only Step 7, namely, measure only Ac13_cpd with NS 512 and TDO=1.

Data processing

Process the data from the 500 MHz using the C-13-CPD (EXPNO # 1) to begin with.

Calibrate the Chemical Shift with the $CDCl_3$ carbon, set as 77.0ppm.

- Type SR. and note down its value (It will be used to calibrate the chemical shifts of the spectra #2,and 3).

1) Process *all the other data sets (2 and 3).*
2) For Spectrum from #2, DEPT-90 gives mainly CH carbons, phase the signals positively. Note: other carbons signals may also show up with reduced intensities as compared to a regular C13_cpd spectrum.
3) For Spectrum from #3, the DEPT135, phase CH and CH_3 positively, whereas those of CH_2 negatively. Note: The most high field C-13 signal (lowest ppm) should be phased upward.
4) Remark: **apk** may not work in some cases, use manual adjustment via the icon  .
5) *Recall the other two data sets individually; import the shift calibration SR from expno#1.*

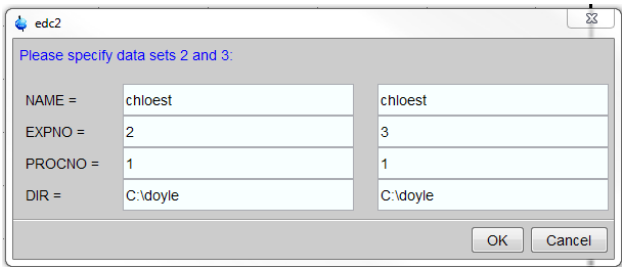
Summary of Report: You are expect to turn in

- Two hard copies of stacked plot (using the 500MHz result).
- Your observation on the number of CH3, CH2, CH and Q carbons in the sample.
- Finally, a dual plot to compare the signal to noise ratio of C13_CPD spectra between 500MHz and 600MHz spectrometer**.**

For plot in your report: explore two different procedures (A and B)

A) <u>When you use TOPSPIN (2.x ro 3.1)</u>

a. Load and display the C13-cpd spectrum (EXPNO#1) of the 500MHz spectrum. This will be the first spectrum appears as the top trace in the stack.



b. Type "edc2". Define the 2nd (middle trace DEPT90) and 3rd (bottom trace, DEPT135, all from the 500MHz spectrometer.

c. File > Print and select the LAYOUT **"+/1D+1D+1D.xwp";** plot two expansions.
   a. 180 to 45ppm.
   b. 45 to 10 ppm.
   Remark: ensure to adjust the baseline of the spectra (DEPT-135) so that the negative phased signals can be seen.

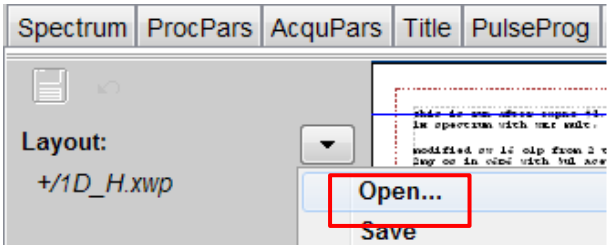
B) Using TOPSPIN 3.2 for multiple plot:

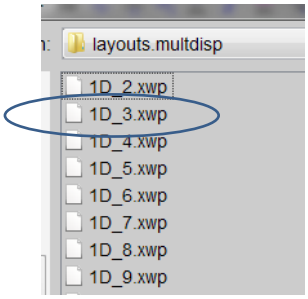
i. Load and display the C13-cpd spectrum (EXPNO#1) of the 500MHz spectrum

ii. Click  multiple display icon to define the other two spectra (dpet90 and dept135).

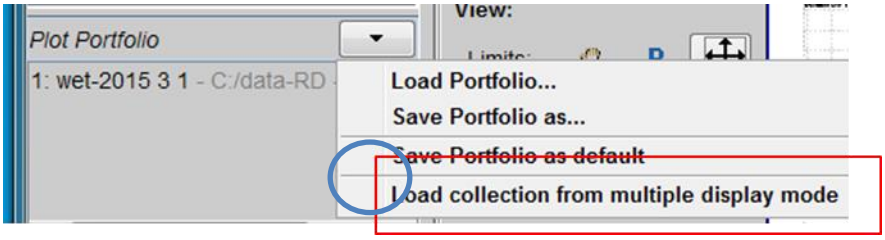
iii. Exit the multidisplay window, and click plot.

iv. Click Layout, open and select the **multidisplay** folder, then select "1D_3.xwp"





v. To import all the spectra from step 1, click Plot Portfolio;
vi. Select "Load collection from multiple display mode".

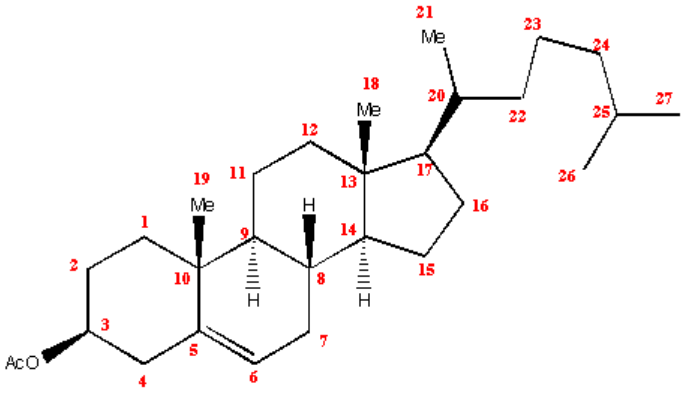


vii. Refer to general procedure how to define the plot limits and scaling.

2nd report:

Answer: ***Determination carbon multiplicity of*** **Cholesteryl Acetate**

Evaluate the carbon head counts for CH_3, CH_2, CH, and quad-carbon, based on the phased differences in your plot. Do they agree with the structure as shown below?



3rd report:

Turn in a hard copy of stack plot and a brief comment to compare the sensitivity of 500 and 600 spectra.

Use the layouts of your choice a or b:

a. Old version TOPSPIN: “stack_2.xwp”
b. New version TOPSPIN: “layouts.stack/stack_2.xwp”.

Plot limits: 110 to 0 ppm.