Selective NOESY (NOESY1D) via CustomQ Interface

(January'09)

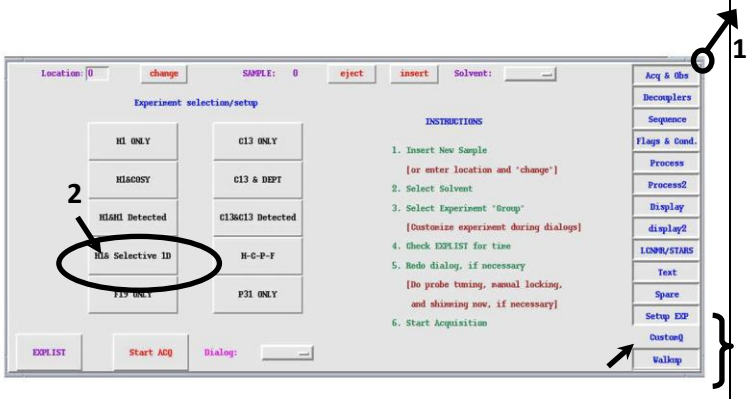
LOCK and SHIM (either automatically or manually) the sample. Type **spin=0 spin** *<enter>* or use the **Acqi** button to **STOP** spinning the sample

In all parameter display windows, one has to *left-hold* at the top right edge and re-size this Tcl/Ttk interface (see **1**) .

This allows the visualization of the three Tcl/Ttk tabs (**Setup EXP**, **CustomQ**, and **Walkup** – see bracket). They comprise the Varian's **Chempack** software.

Left-click once on the **CustomQ** tab (see arrow) to open up the window as shown in fig.

Left-click once on the **H1& Selective 1D** button as shown in **2**.

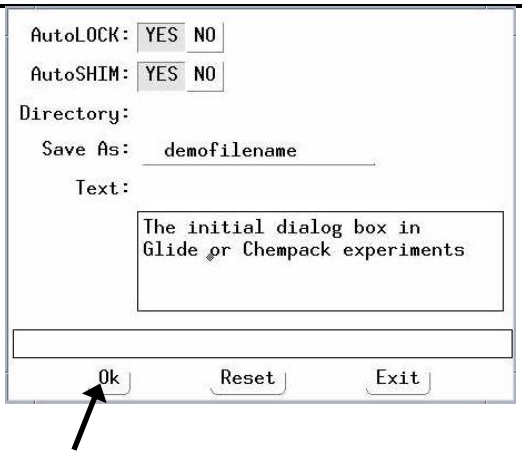


Lock, Shim, Save & Text Options:

The next window will prompt you to whether you want to **AutoLOCK** and **AutoSHIM** the sample. *Left-click* once on **NO** to both options, as you have previously LOCKED and SHIMMED the sample.

In "Save As", type the name of the sample, which will be the name of the directory into which all experiments will be saved (e.g. **PROTON.fid/, NOESY1D_2_12p.fid/**)

Left-click once on the **OK** button (see **arrow**)



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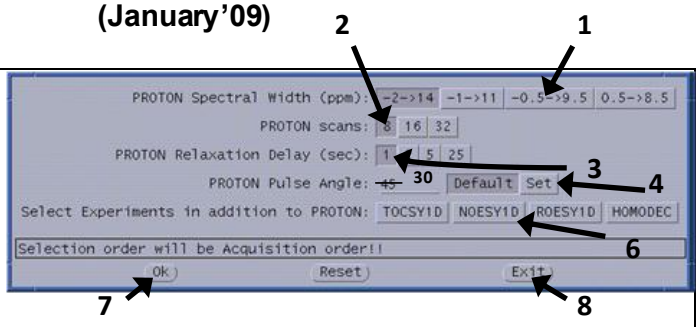
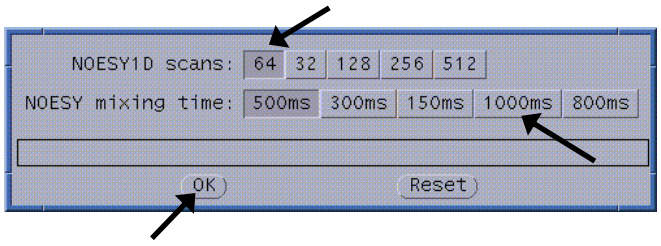
<table>
<tr><td>The next window (fig on the right column) will allow will to choose all parameters for the selective excitation experiment.</td><td> 
</td></tr>
<tr><td>(A) For example, if you know a priori that the -0.5-> 9.5 ppm will encompass all signals of your sample: then you left-click <u>once</u> on the button (1).</td><td>(B) Then, the choice is the number of scans for the reference ^{1}H spectrum. A normal reference ^{1}H spectrum may require less than 8 scans (particularly at high fields), but among the current options the minimum nt is 8. Left-click <u>once</u> on 16 and left-click <u>once</u> BACK on 8 (see arrow 2)</td></tr>
<tr><td colspan="2">(C) It is preferable to make an <u>active</u> choice of the parameter rather than leave it pressed, as presented in the default setting.</td></tr>
<tr><td>(D) Next choice is the relaxation delay (d_1). One normally choose d_1 = 1 sec as the ^{1}H spectrum will be a reference spectrum for the peaks to be selected for irradiation (Quantitation is not an issue in the selective experiments - (see arrow 3). Again, make an <u>active</u> choice of 1 sec.</td><td>(E) Choice of flip angle: the flip angle is the angle that you will tip the spins to acquire the reference ^{1}H spectrum. You may either leave it at 45 or you may left-click <u>once</u> on <u>Set button</u> and type 30 (see arrow 4).

(F) This seems like an open choice: however, because the short d_1 (as specified in D), it is better to <u>tip</u> the sample with small flip angle, allowing enough time for relaxation.</td></tr>
<tr><td>(G) Left-click <u>once</u> on the NOESY1D button (see 6).
(H) The window on the right will prompt, asking you how many NOESY1D scans you would like to run, and the mixing time (how many ms you will allow the cross-relaxation to occur).

(I) As a "rule of thumb", choose 64 scans to start, and for small molecules (MW ~350), choose 1000 ms (see arrows).</td><td> 

(J) Left-click <u>once</u> on the OK button.

(H) The window up in this page will return, then left-click <u>once</u> on the OK button (7) and left-click <u>once</u> on the Exit button (8)</td></tr>
</table>

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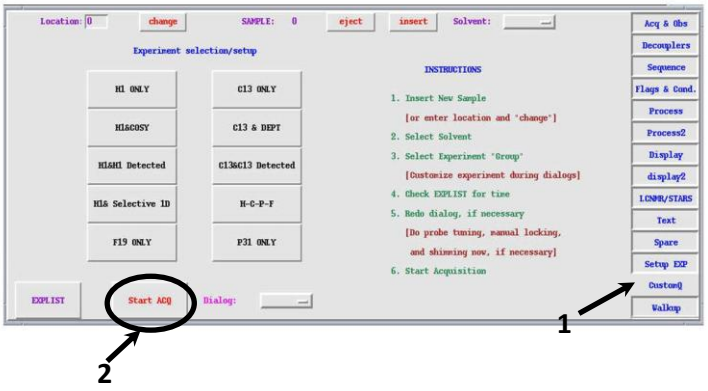
Left-click once on the **CustomQ** tab (**1**) and **Start ACQ** button (**2**).

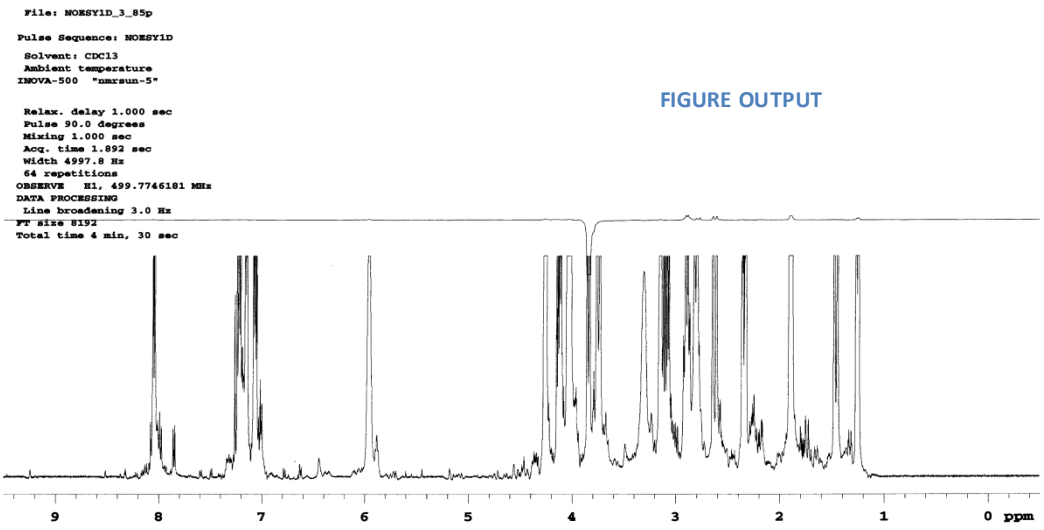
The acquisition of the ^{1}H spectrum will start. When it finishes, the ^{1}H 1D spectrum is presented (see **A** on the right side) and the following buttons at the selective excitation Menu: ***Box***, ***Expand***, ***Select***, ***Proceed***, ***Cancel***, ***Restart***, and ***Return***.



Left-click once the **Box** button and position the 2 cursors (*left-hold)* around the peak to be selectively excited, in this example, the H_{15a} at 2.35 ppm. *Left-click* once the ***Select*** button and in sequence *left-click* once the ***Proceed*** button to start the NOESY1D.

Wait for the NOESY1D to finish.

FIGURE OUTPUT



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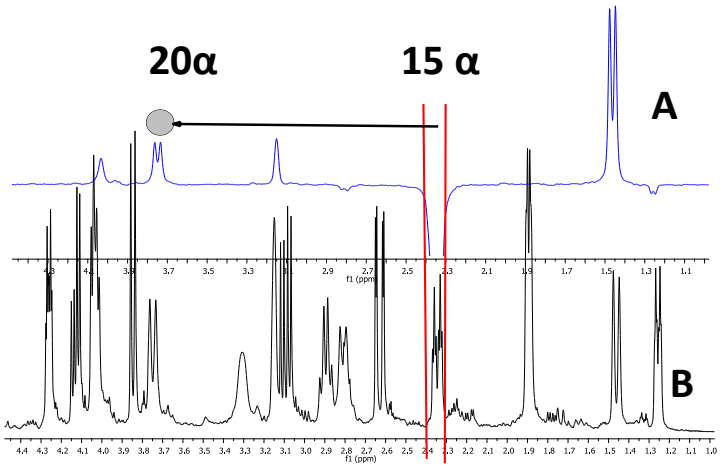
The **FIGURE OUTPUT** above is representative of the automatic printout of the NOESY1D, where the 1D ^{1}H spectrum appears below and the NOESY1D result(s) appear on top. The selectively excited peak appears inverted and cut (parameter *cutoff='y'*) one may see - in this example - small NOE peaks. Most of the time, because NOE peaks have intensities of 3-4% of the excited peak, one sees the inverted peak and the NOE responses are very weak, and not visible in the printout.

Hence, further processing is required for visualization of the NOE peaks.

- Type *lb*=10 *fn*=4k *cutoff='n' wft <enter>* to inspect the NOESY1D spectrum.
- *Middle-hold* to adjust the vertical scale (*vs*), and reposition the spectrum at ~50-60 mm with *vp*=50 (or *vp*=60) *<enter>* (do not use *vsadj*)

((In this example, it will be searched a through-space connectivity between one of the CH_2 protons (H_{15}) with one of the CH_2 protons (H_{20}). Due to the stereochemistry of the **strychnine** molecule, only one of the H_{15} connects to one of the H_{20} protons)).



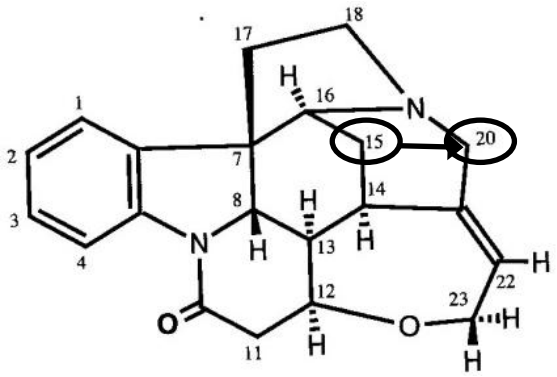
Obs – Important

1) If the Menu for selective excitation is lost (so you do not have Box, *Expand*, *Select*, *etc*), you should type:

➔ menu('ds_selfrq')

The *menu* command will restore the selective excitation menu and you can continue.

((..And REMEMBER: to get similar spectrum as **A** (blue) on the right, check *cutoff* parameter value with *cutoff? <enter>*. Parameter *cutoff* should be equal to **'n'** as mentioned above**))**



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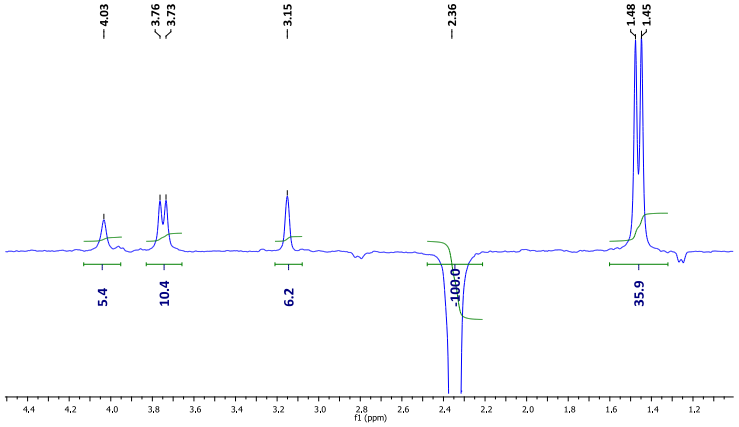
NOE data can be extracted from the 1D NOESY1D spectrum (right, blue).

Integrate the spectrum and correct the baseline with *bc <enter>* (which use the Reset points).

Set the integral of the selectively excited peak to **100** (with **SetInt** button) and all the other peaks **will display their NOE intensities**. It can be noticed that the geminal Hs of the CH_2 (H_{15}) give the most intense signal (~36%) as expected. The NOE at ~3.8 ppm (H_{20}) showed 10%, which was the purpose of the experiment (NOESY1D on ~1.45 ppm did not give NOE at 3.8 ppm – results not shown).



Plot the spectrum with:

pl pscale ppa pir ppf page *<enter>*,

which will give results similar to the NOESY1D spectrum above (with peak plotting after peak picking them too).

Obs – Anthony Mastracchio's (Chemistry, MacMillan group) comments to the NOESY1D description are acknowledged.