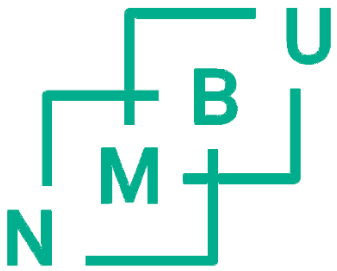


TopSpin – A practical guide

TopSpin 4.3

Version 1.0

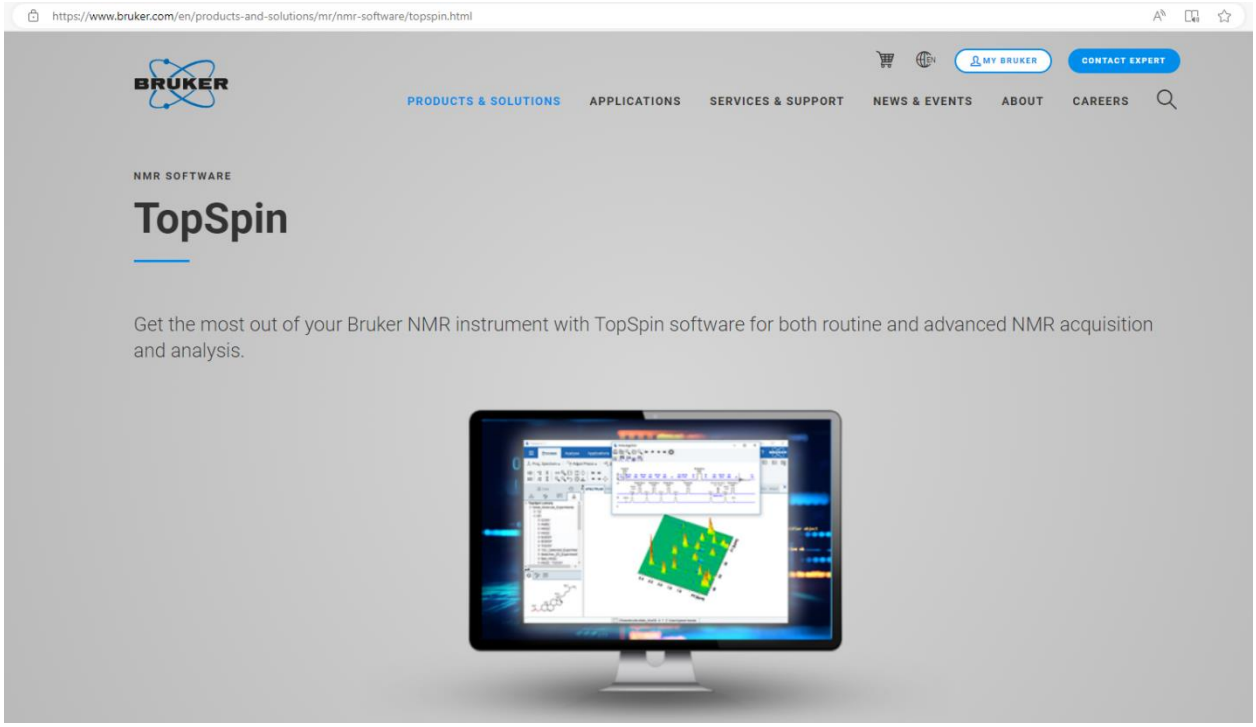
^{1}H-NMR and ^{13}C-NMR

English

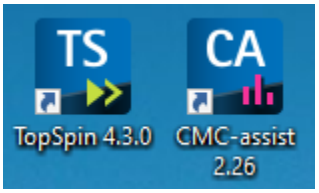
October 2023

Download TopSpin

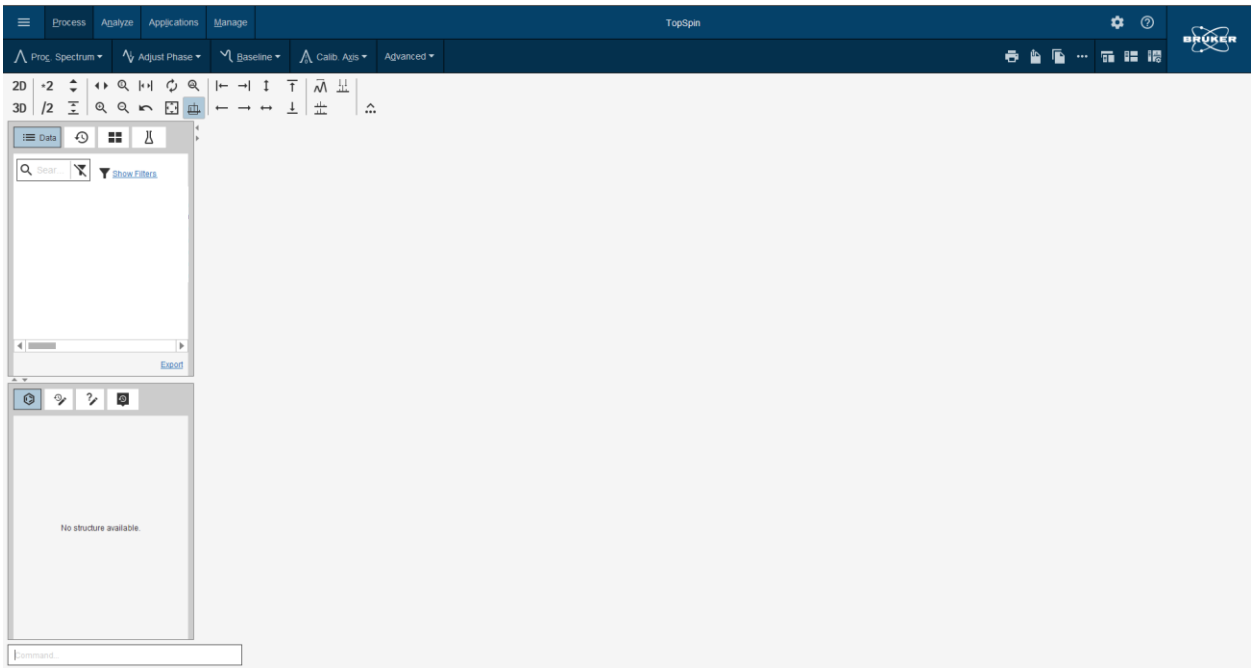
TopSpin can be downloaded from Bruker's webpage. If you have a university computer, the software is available on Software Center. On the webpage click → "Products & Solutions" → "NMR Software" → "TopSpin" or reach directly this webpage by typing *"Bruker topspin download"* on the browser.



Create your own account using your NMBU email address. NB! Do not use the same password as for your university email account. You will receive a confirmation email a couple of minutes after registering. Logg on to your Bruker account and request an academic free license: a personal code will then be generated, remember to save it. Download the version of the software compatible with your computer and install it (the file in the webpage should be called something like "TopSpin 4.3.0 & CMC-assist 2.26"). In the instillation process choose "Data process only", you will then be asked to create an NMR superuser: remember both the username you give and the password you set as it will be needed to use the software. Complete the installation.

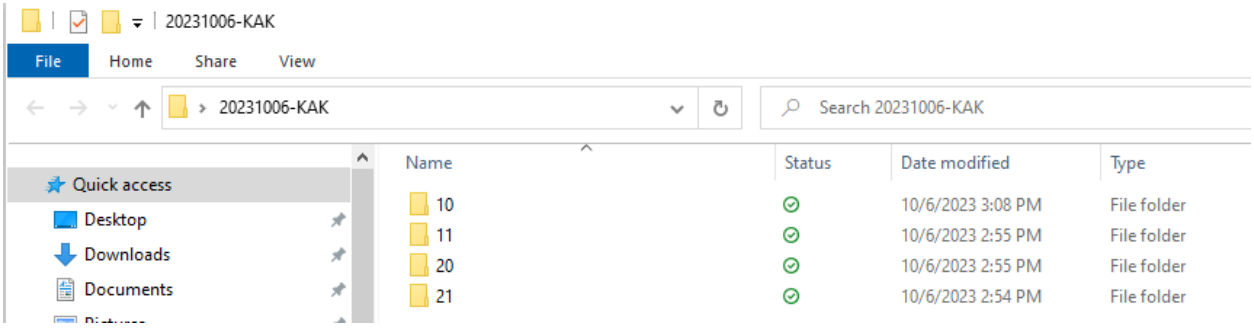


Click on the first icon "TopSpin 4.3.0" to open the program. An automatic black command window will be opened, don't close it, don't touch it, and leave it in the background. Right after a second window with the normal TopSpin layout will appear. It looks like in the picture below.



Download data

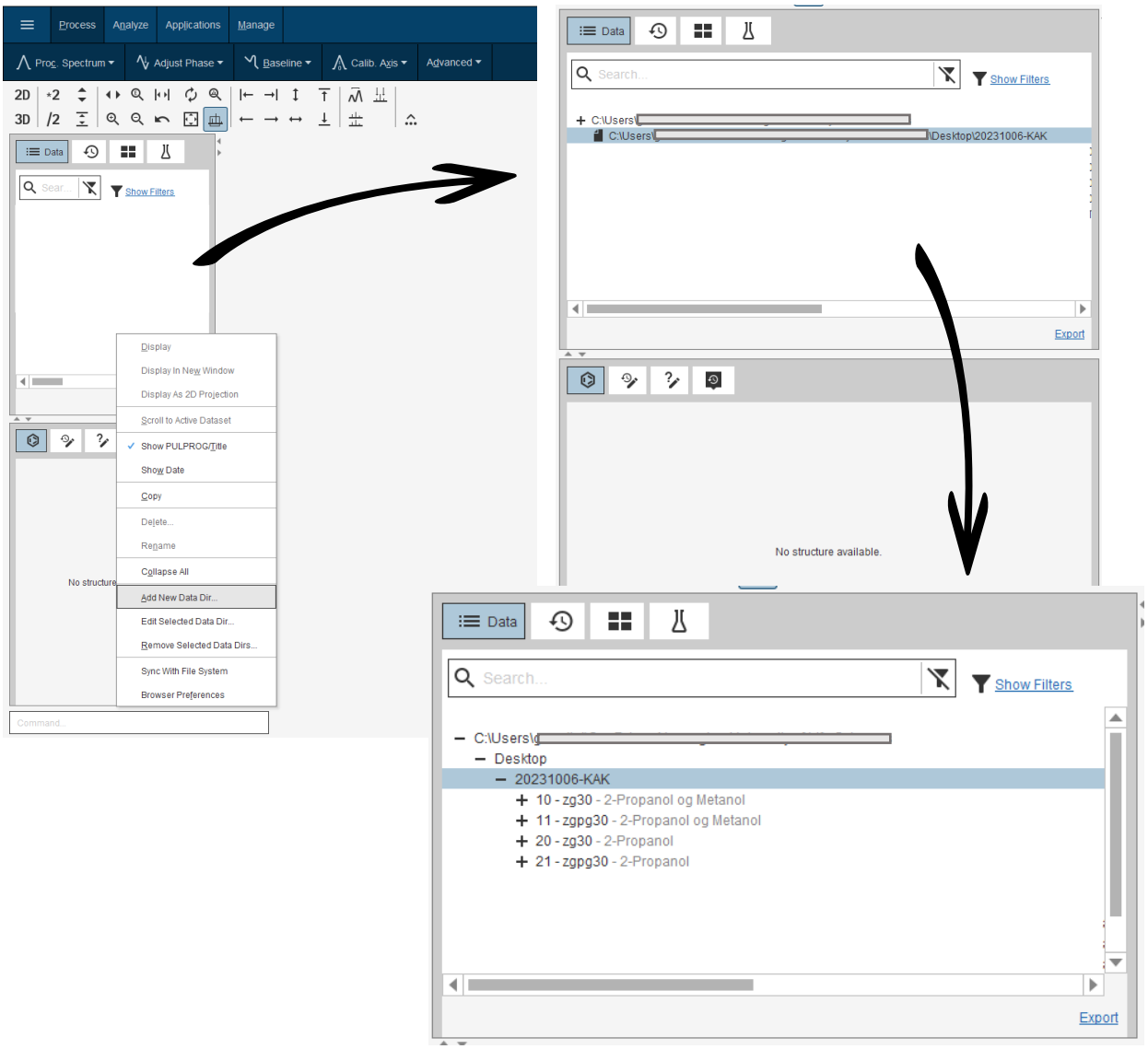
Open Canvas and download the NMR dataset to your computer. It should look something like this:



How to process ^{1}H-NMR data

Go back to TopSpin and create a shortcut to your spectra folder. Right click in the small “Data” window in an empty spot → “Add New Data Dir…” → Click on the […] and browse the location in which you have saved the folder. It will appear automatically among the available shortcuts. Click on the “+” and it will show the content of the path saved: inside the folder you see again the same files as before.

If you don’t see the title of the experiments (a short description with the PulsProgram and the Title) but only the name of the folders (numbers), you can right click to flag “Show PULPROG/Title”.



Double-click on the first spectrum (or if you do not want to create the shortcut, from menu (orange circle in the picture)→ "Open" → "Open the NMR data stored in standard Bruker format" → Browser type = "file chooser" → locate the folder in your computer → display. The spectrum will then opened like as shown underneath.

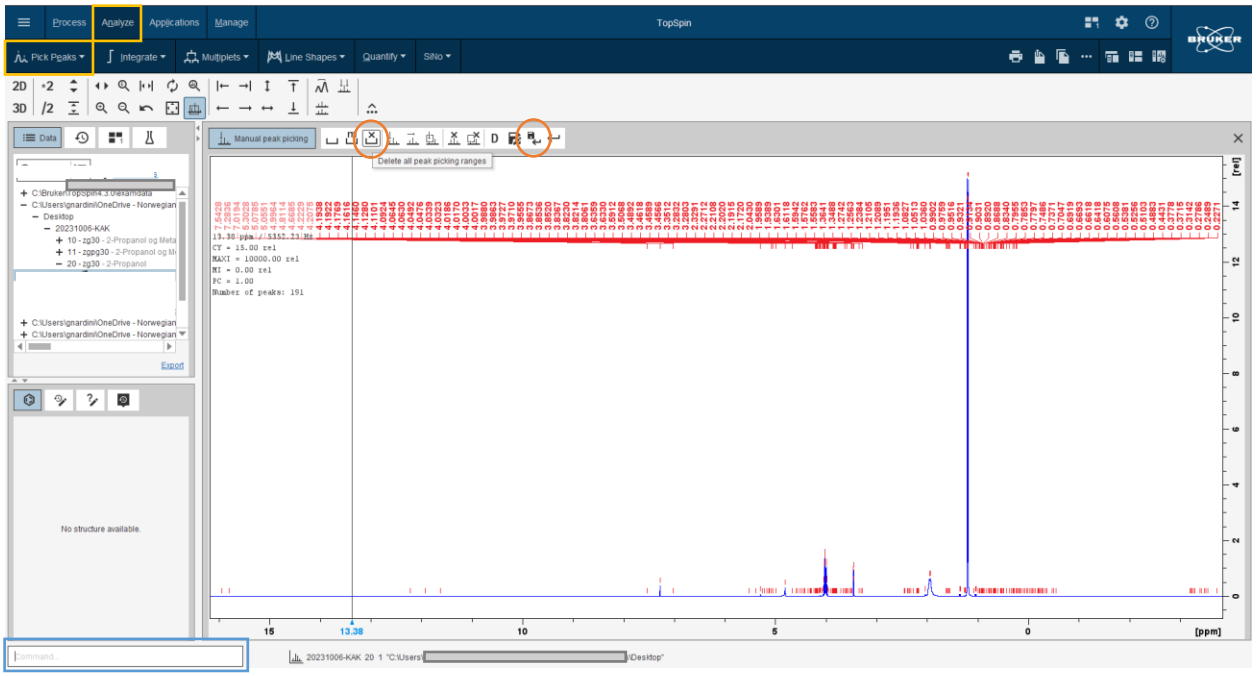
You should be in the "SPECTRUM" window. If you do not see the peaks of the spectrum, use the wheel of the mouse to increase/decrease the intensity of the relative signals (Y axis). If you want to zoom in and click with the left button and mark the region you want a closer look on. To go back at the comprehensive overview, use the button "Show full spectrum, reset intensity scale". This is done by clicking on the □ with four arrows inside, see green circle in the picture.

For more spectral details see the spectrum window (violet rectangle in the picture): PROCPARS (Processing Parameters), ACQUPARS (Acquisition Parameters), TITLE (short description of the sample in this case), PLUSEPROG (Pulse Program) and open also the "Graphical display of the pulse program"= Ω, PEAKS, INTEGRALS, SAMPLE, STRUCTURE, PLOT, FID (Free Induction Decay).

1. Start your spectrum processing deleting the automaticly generated peaks and integrals.

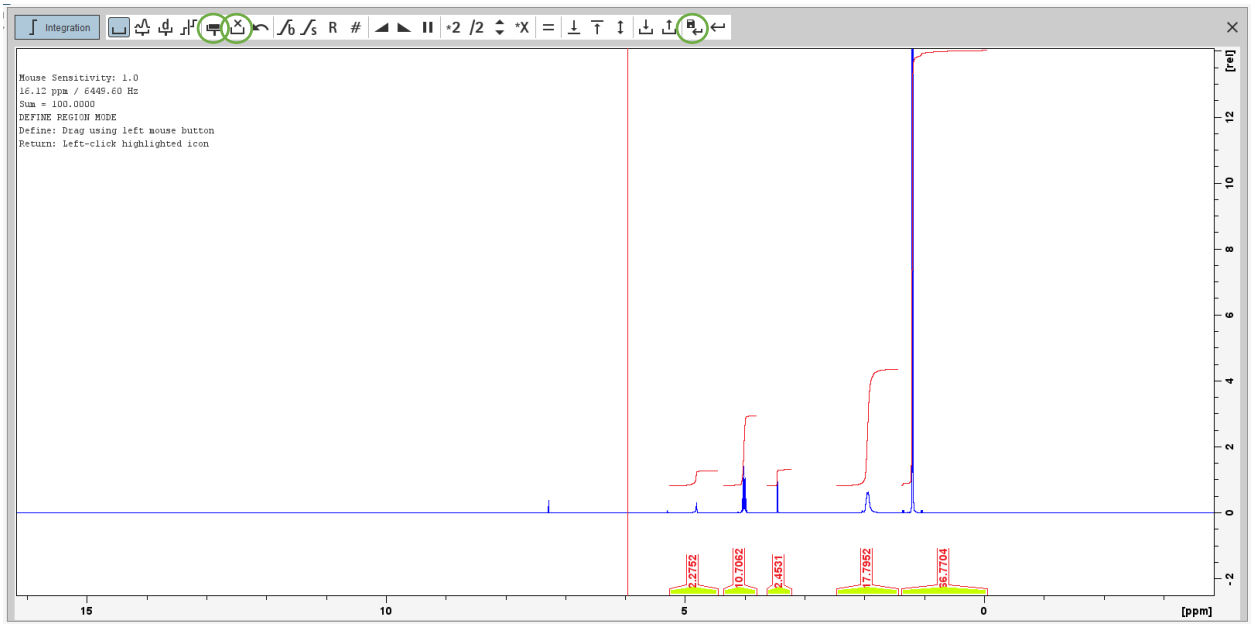
Click on “Analyze” in the top row of the TopSpin window (see yellow rectangles) → “Pick Peaks” → “Manual Peak Picking” (or type the command <.pp> in the command line (light blue rectangle)).

Click on the icon “Delete all peak picking ranges” and all the automatic peaks above the signal will disappear. Click on the button “Return, and save changes” (or type the command <.sret>).



Now, click on “Integrate” (the button next to “Pick Peaks”) → “Manual-Integrate” (or type the command <.int>). Click on the icon “Select/Deselect all regions” or manually select all the integrals holding down the

Ctrl key: they will become green. Then click on the icon “Delete selected regions” and all the automatic integrals under the baseline will disappear. Click on the button “Return, and save changes” (or type the command <.sret>).

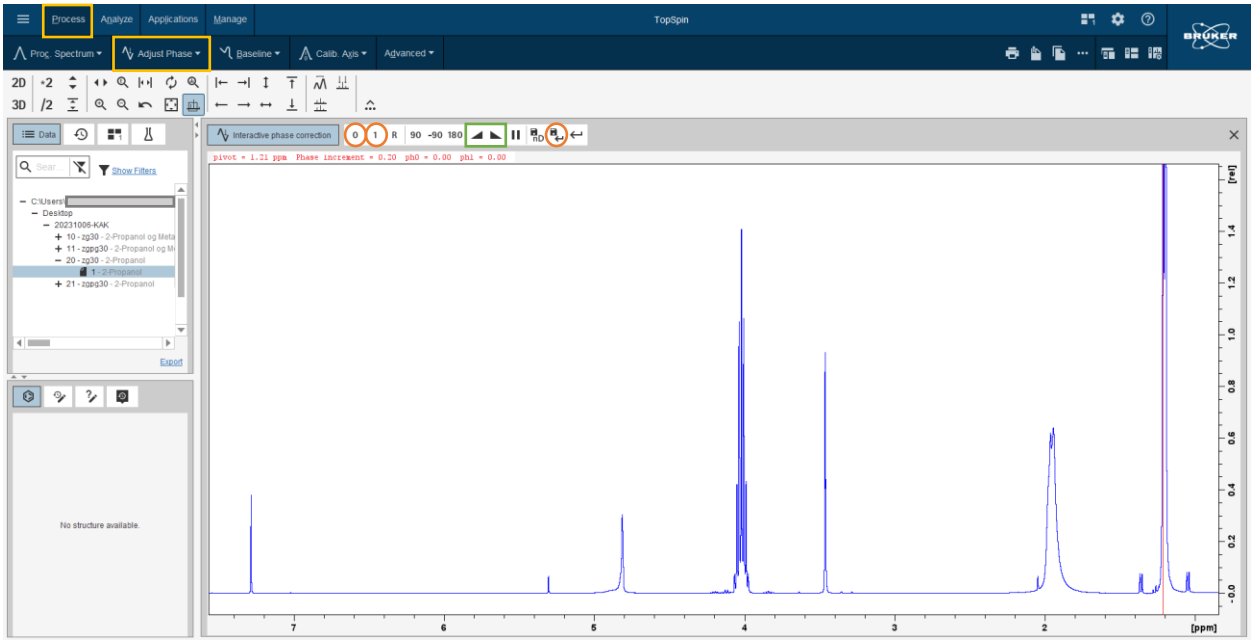


2. Phase correction

Zoom in your spectrum to see more clearly the peaks.

Go back to the “Process” window → “Adjust Phase” → “Adjust spectrum phase manually” (or type the command <.ph>).

The button “0” adjusts the spectrum at the pivot point (red vertical line) that locates the most intense NMR resonance, see figure underneath, while the “1” button phases the rest of the spectrum.



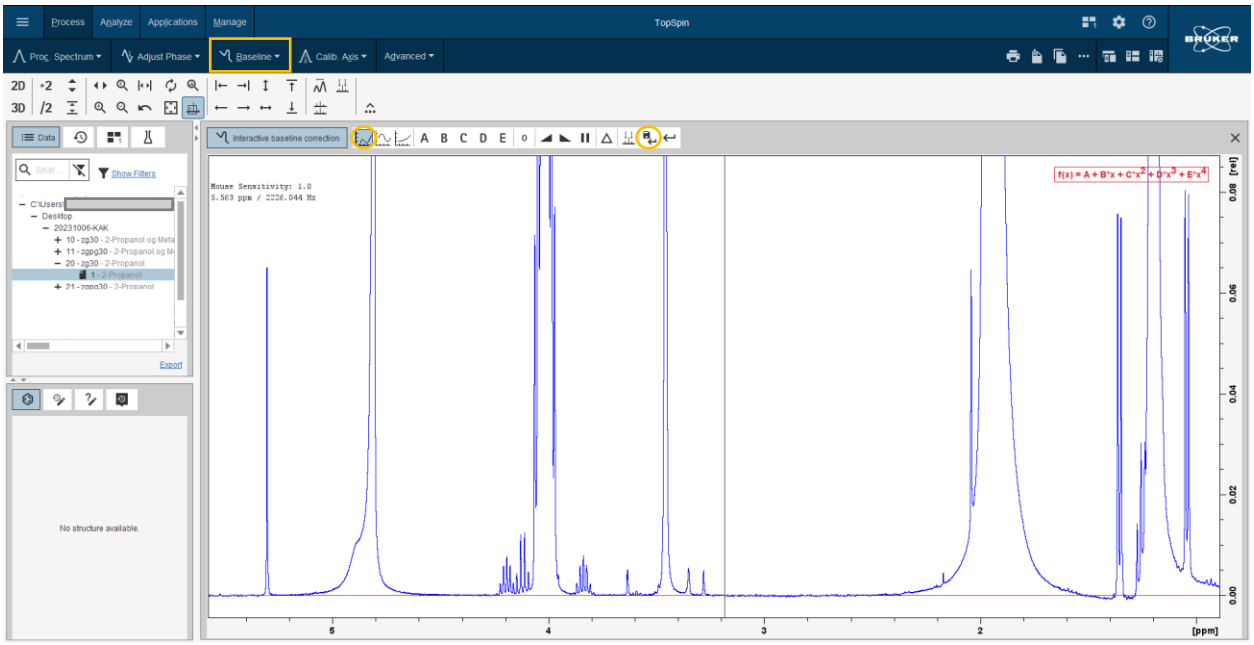
Click on the "0" button and drag it up and down to make the peak at the pivot point positive and as symmetrical as possible. After that, use the "1" button to make the rest of the peaks positive and as symmetrical as possible. If you move the mouse up and down without seeing changes or the changes are too fast to control, use the icon "Decrease/Increase mouse sensitivity" to adjust the sensitivity of your mouse movements. Click on the button "Return, and save phased spectrum" (or type the command <.sret>).

As you probably have noticed the spectrum seems already phased and it is difficult to improve the phasing manually. The manual phasing is mostly used in complicated situations in which the algorithm for automatic phasing is not able to succeed. So, click on "Adjust Phase" → "Automatic Phasing Options" → "0^{th} + 1^{st} order correction" (or type the command <apk>), and the algorithm will improve the phasing as much as possible.

3. Baseline correction

Click on "Baseline" and a red horizontal line will appear in your spectra at 0. Click again closer to the arrow and an option window will appear: choose "Automatic Using Polynomial of Degree ABSG", and click on the button "Return, and save regions" (or type the command <.sret>).

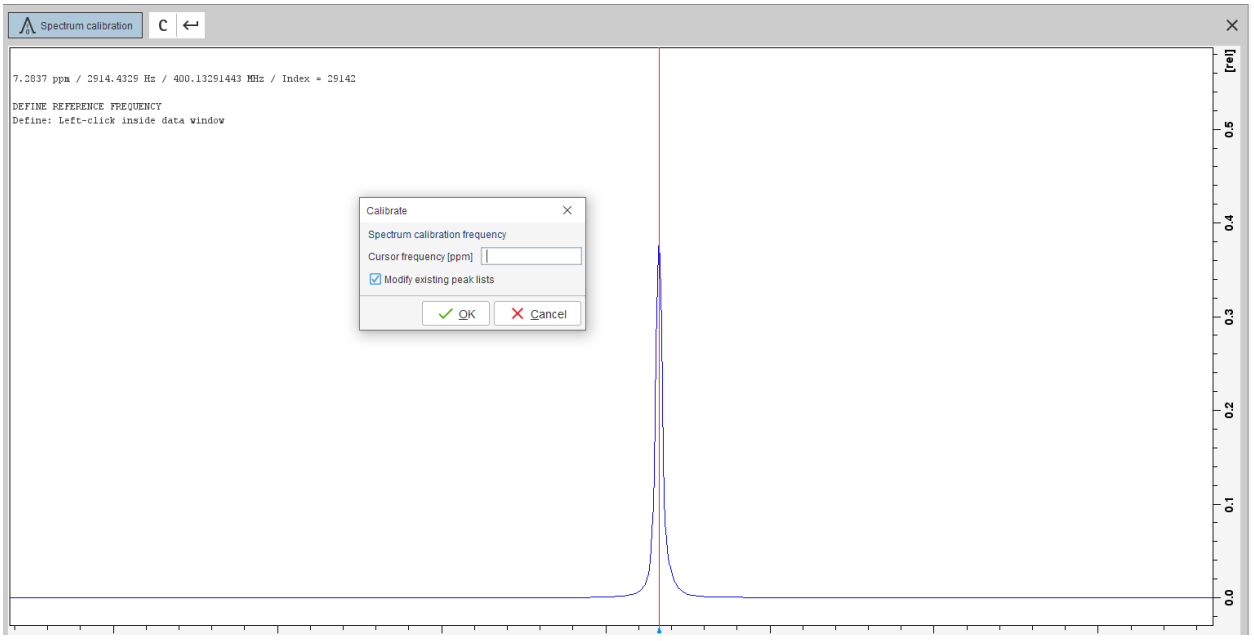
Otherwise is possible to type directly the command <abs> that performs automatically the baseline correction. If some integrals automatically appear delete them as described before. As before, in this type of spectrum it is difficult to notice the improvement.



4. Calibration of the ppm scale

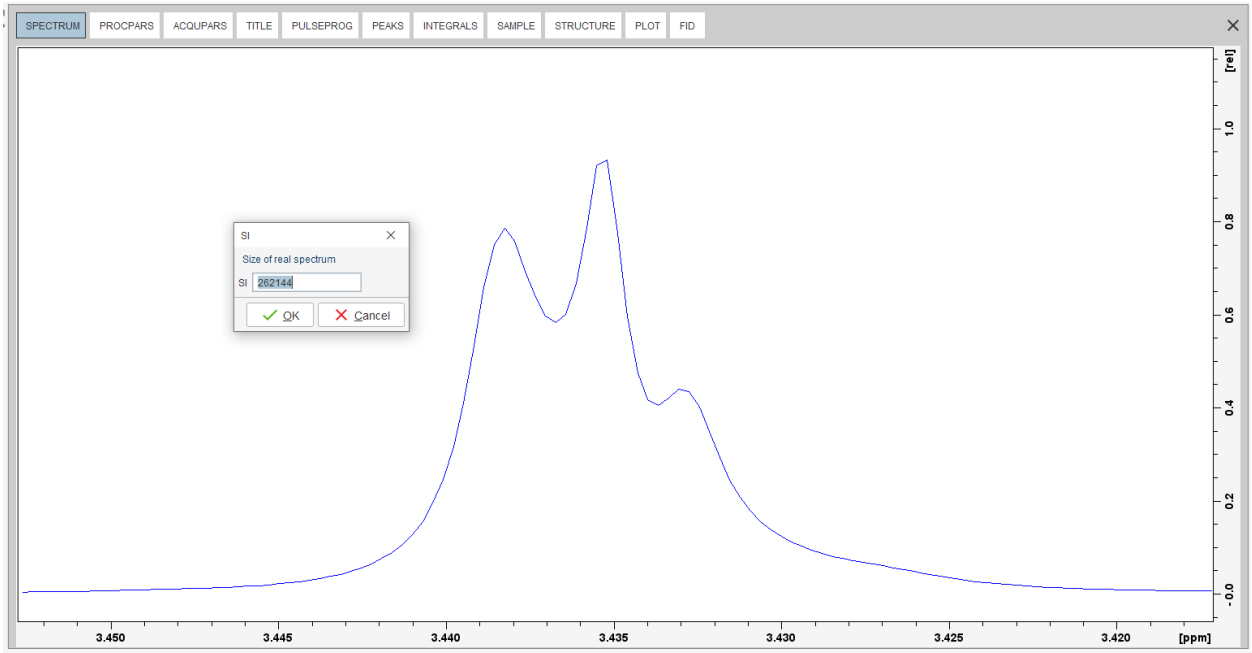
Zoom in on the solvent residual peak. Click on “Calib. Axis” → “Manual Axis Calibration” (or type the command <.cal>). Find the center of the solvent peak, click and enter the value of the solvent. For $CDCl_3$ the value is 7.26 ppm. Then click "OK" or use the Enter key.

Shift values for solvent peaks are found in literature: *“NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities”* (Hugo E. Gottlieb, Vadim Kotlyar, and Abraham Nudelman; *The Journal of Organic Chemistry* **1997** 62 (21), 7512-7515; DOI: 10.1021/jo971176v).

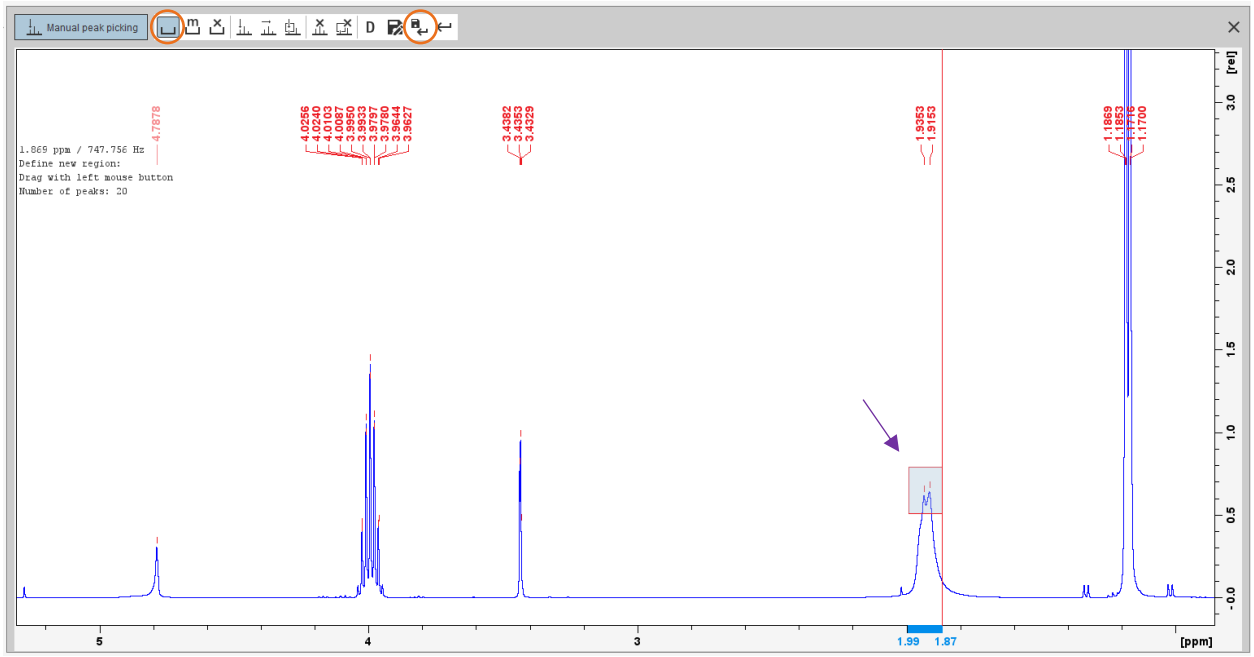


5. Manual peak picking

If the peaks are not satisfactory defined as shown below, you can change the number of data points by typing the command <si>. A window with “size of the real spectrum” will open and you can increase the number of data points to 256k (e.g.) and press OK. Then to visualize the result you have to type <efp> in the command line: the peaks should then look nicer.



Move from “Process” to the “Analyze” section. Click on “Analyze” → “Pick Peaks” → “Manual Peak Picking” (or type the command <.pp>). With the mouse you select the peaks as shown in the picture (violet arrow) and the value of the peaks will appear on top of them.

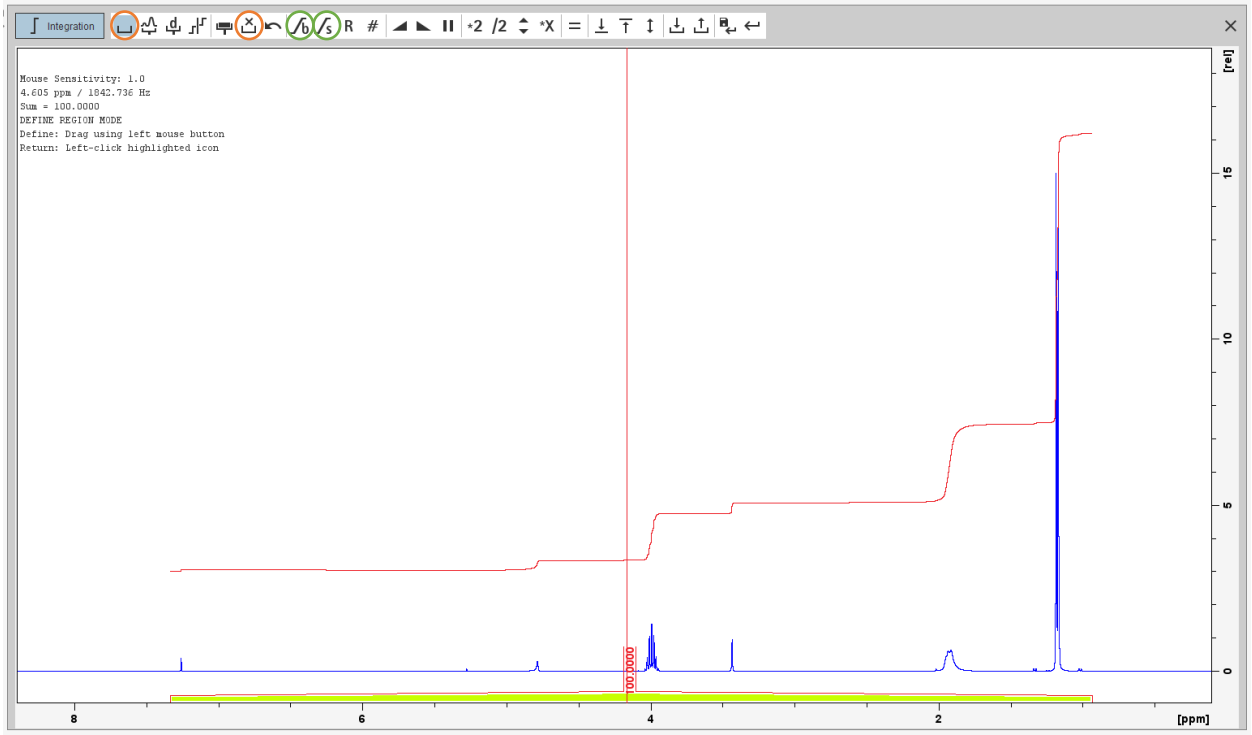


Exit by clicking on “Return, save changes” (or type the command <.sret>). The new list of peaks is available in the “PEAKS” window.

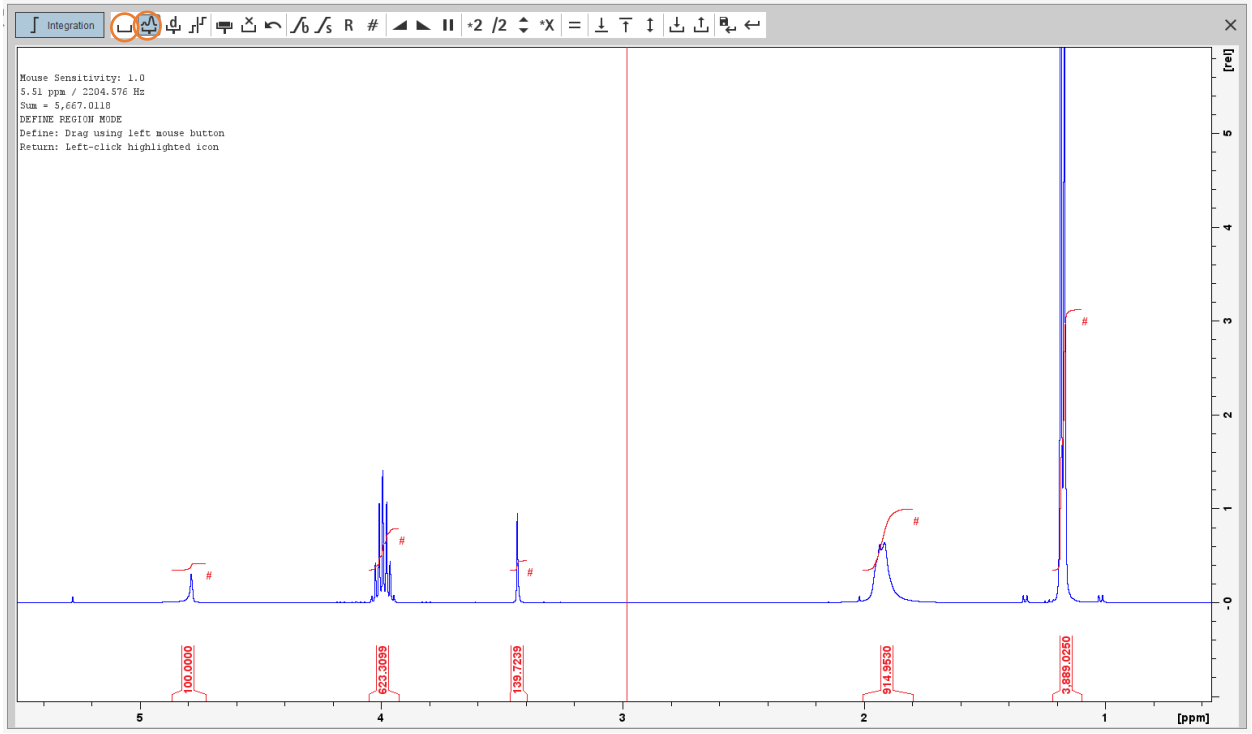
6. Manual integration

Inside the section “Analyze”, close to “Peak Pick” there is also “Integrate”: click on that button → “Manual-Integrate” (or type the command <.int>).

First, you have to integrate all the signals together (look at the picture below) and check that the horizontal part of the integral is absolutely flat and horizontal. If it is not right, you can correct it by selecting the integral so that it turns green as in the picture. Then use the buttons ∫b “Interactive bias correction” and ∫s “Interactive slope correction” (green circle in the picture). The buttons work by clicking on them and dragging up and down until you are happy with the result, it may be necessary to switch between them a couple of times to achieve an optimal result. After this you delete the integral by using the “Delete selected region” button.

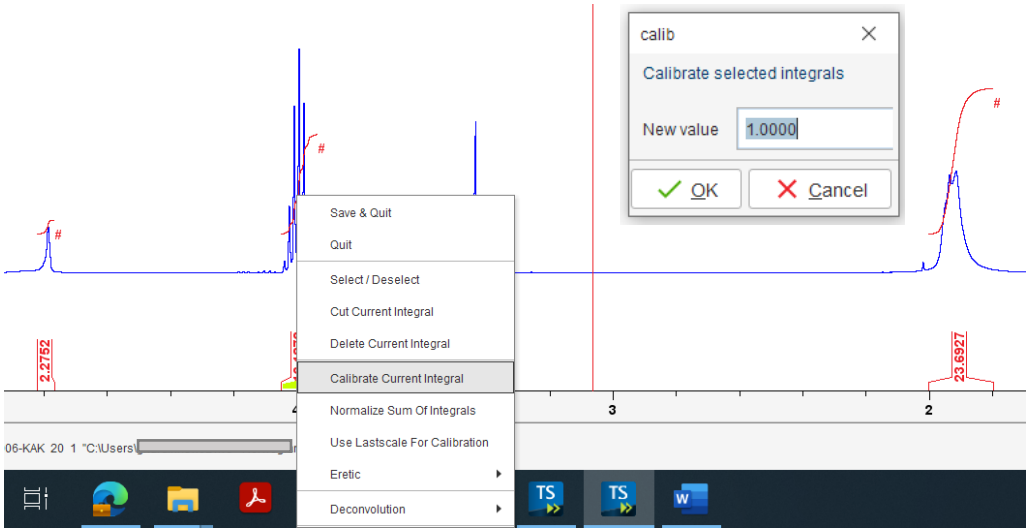


Then integrate each peak alone using the same procedure as before. If some peaks overlap and it is difficult to separate them, they can be integrated together.



When you get all your integrals, you have to normalize them. Choose a top/signal that you are quite sure of, e.g., a peak that should correspond to a single proton, right click, and select “Calibrate current integral”

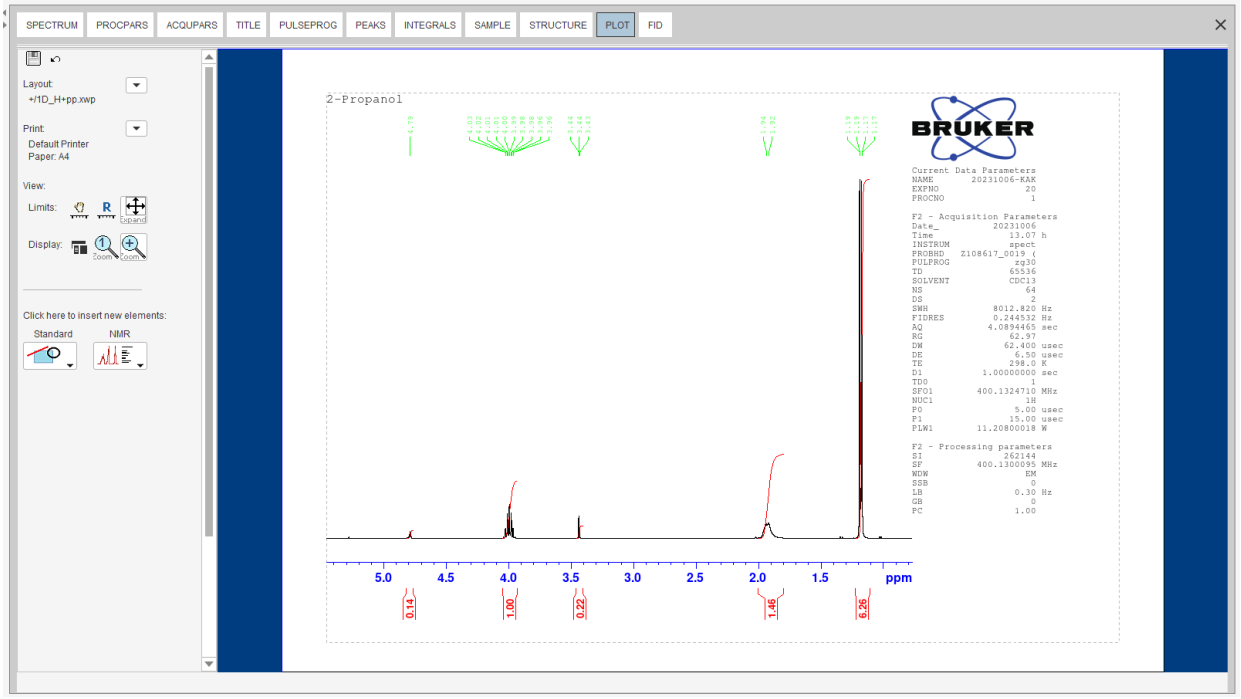
(do NOT use “Normalize sum of integrals”), change the value to the number of protons the peak corresponds to, in this case it is 1, and press OK (or click Enter key). All the other peaks are automatically adjusted. Exit by clicking on “Return, save regions” (or type the command <.sret>).



The new list of integrals with the updated relative values is available in the “INTEGRALS” window (or typing the command <li>). You can also use the command <lippf> which means “list integrals and peaks of the full spectrum (1D)”. If you have done everything correctly, all the modifications you have applied to the spectrum have been saved.

7. Plot window and print

You can plot your spectrum using the “PLOT” window. There you can adapt the plot to the way you want it to look. In the example below, all important parameters for the NMR experiment carried out are also shown.

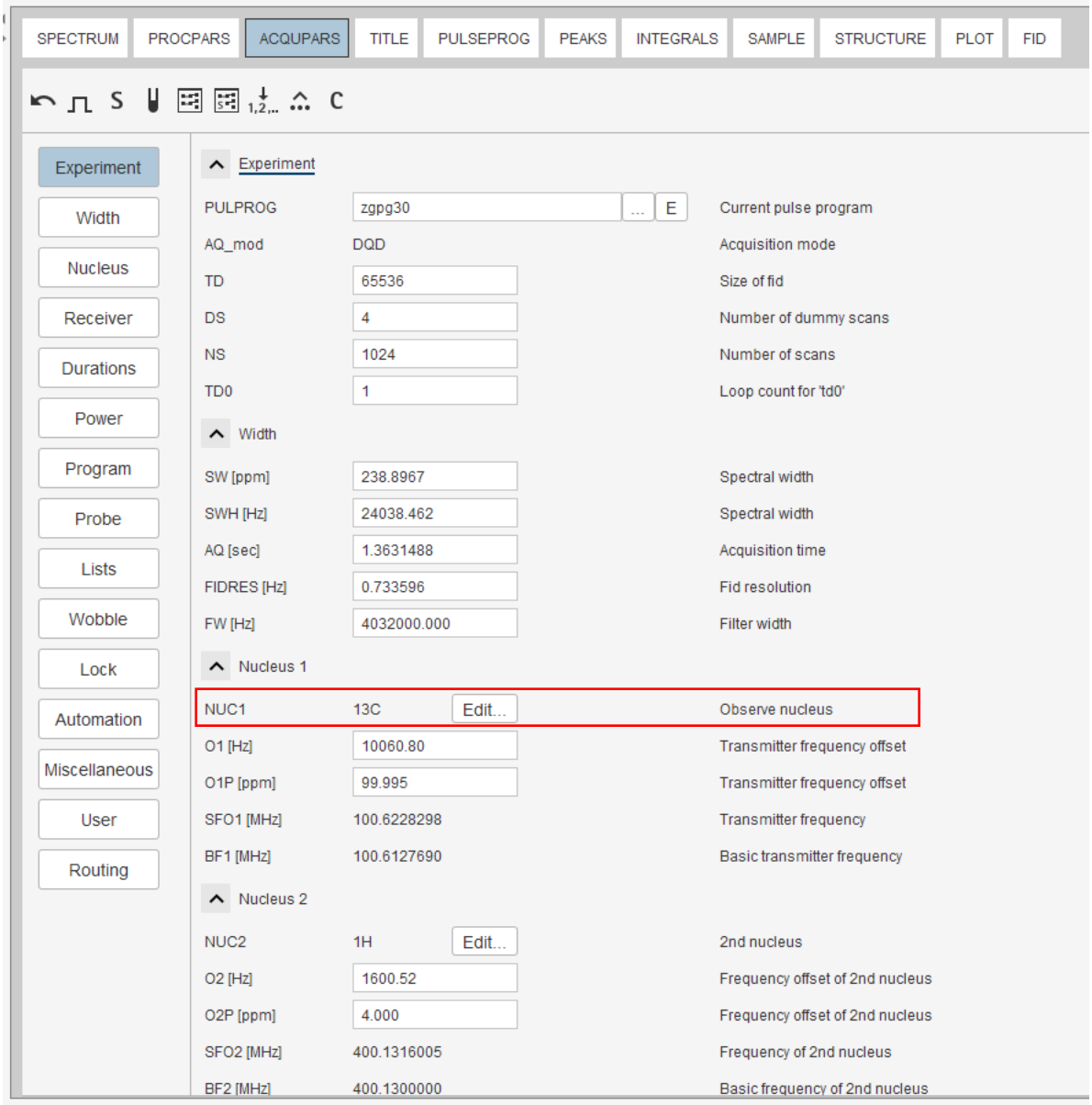


If you want to save the spectrum as it looks, use the buttons “Print active window” or “Export active data or plot window as PDF”. Remember to move the spectrum so that it there is no overlap of the integral values etc. This can be done using the interactive button “Shift baseline up/down while pressing left mouse button”. Display only want you want to be saved, right click and “Spectra display preferences”. This option is less formal, but can be used in the lab report.

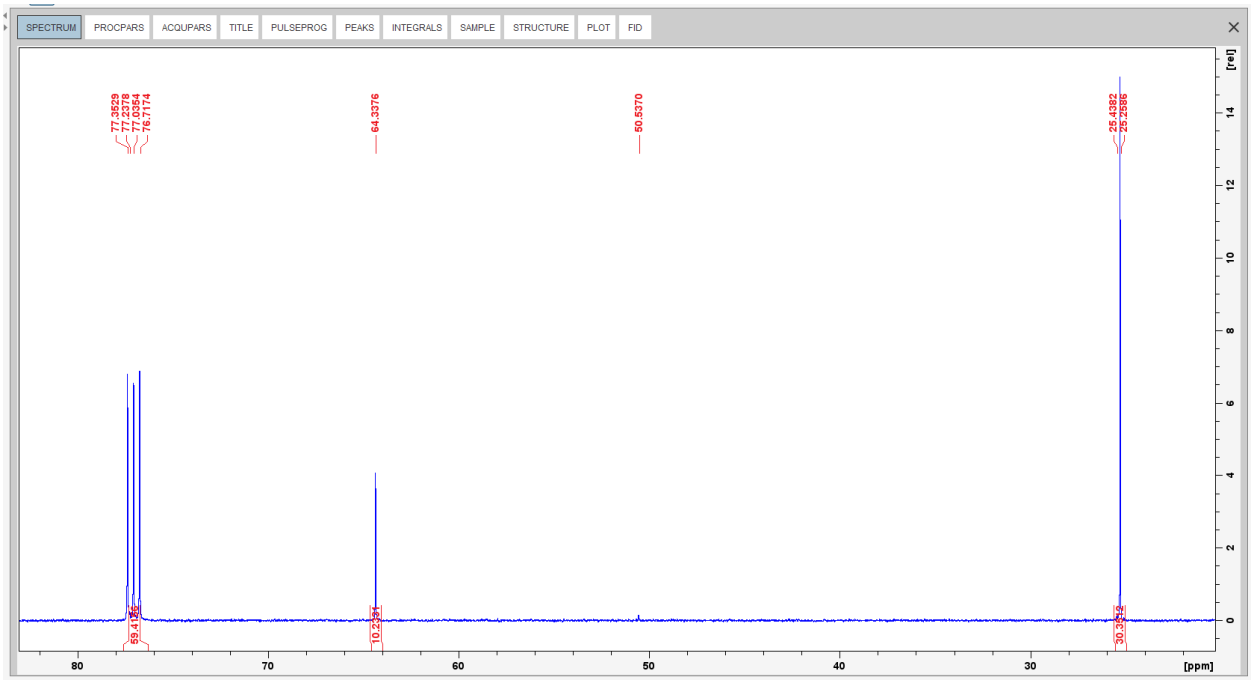
How to process ^{13}C-NMR data

^{13}C-NMR data is processed more or less the same way as for proton. The only difference is that we do not integrate the area underneath the peaks/signals. The reason for this is that area under a ^{13}C-NMR signal cannot easily be used to determine the number of carbons to which it corresponds. The signals for some types of carbons are inherently weaker than for other types – peaks corresponding to carbonyl carbons, for example, are much smaller than those for methyl or methylene (CH_2) peaks. For this reason, peak integration is generally not useful in ^{13}C-NMR spectroscopy.

Open the carbon spectrum, double-click on the spectrum in the shortcut or “Open” and “Brows” in the menu. If you are not sure that it is a carbon spectrum that you are looking at, you can open “AcquPars” and control the “Nucleus 1” which is the “Observed nucleus”.

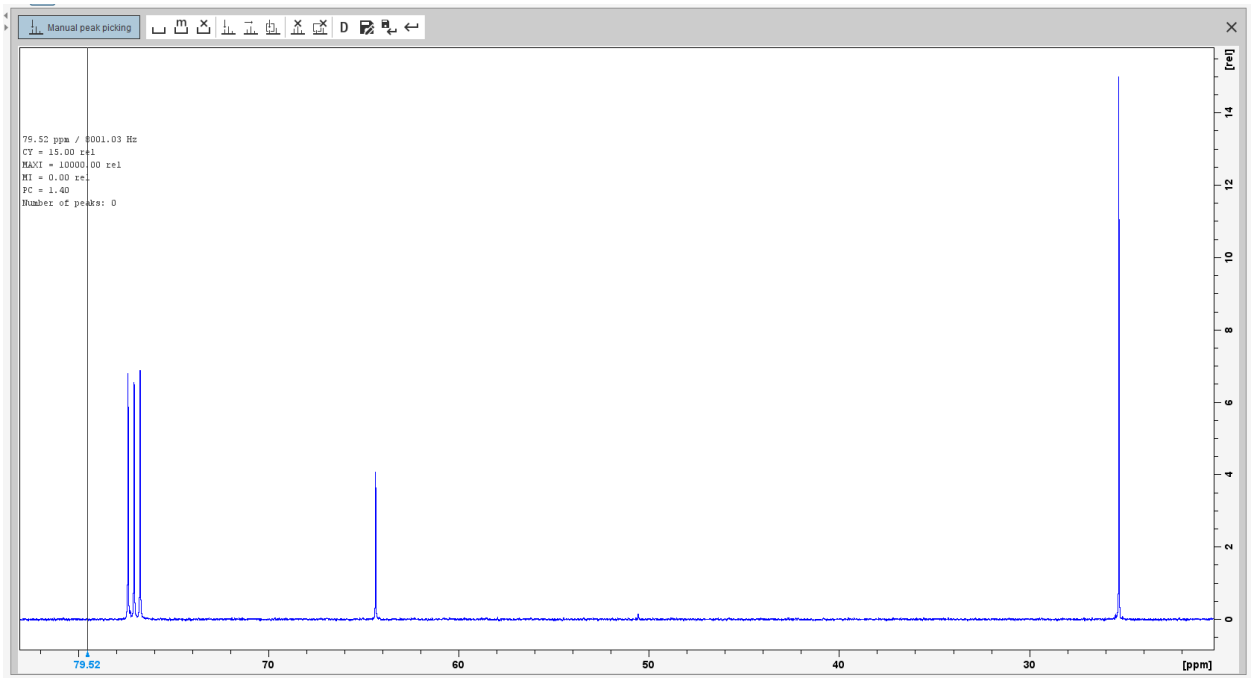


If you go back to the “SPECTRUM” window, the carbon spectrum will look something like in the one following picture.

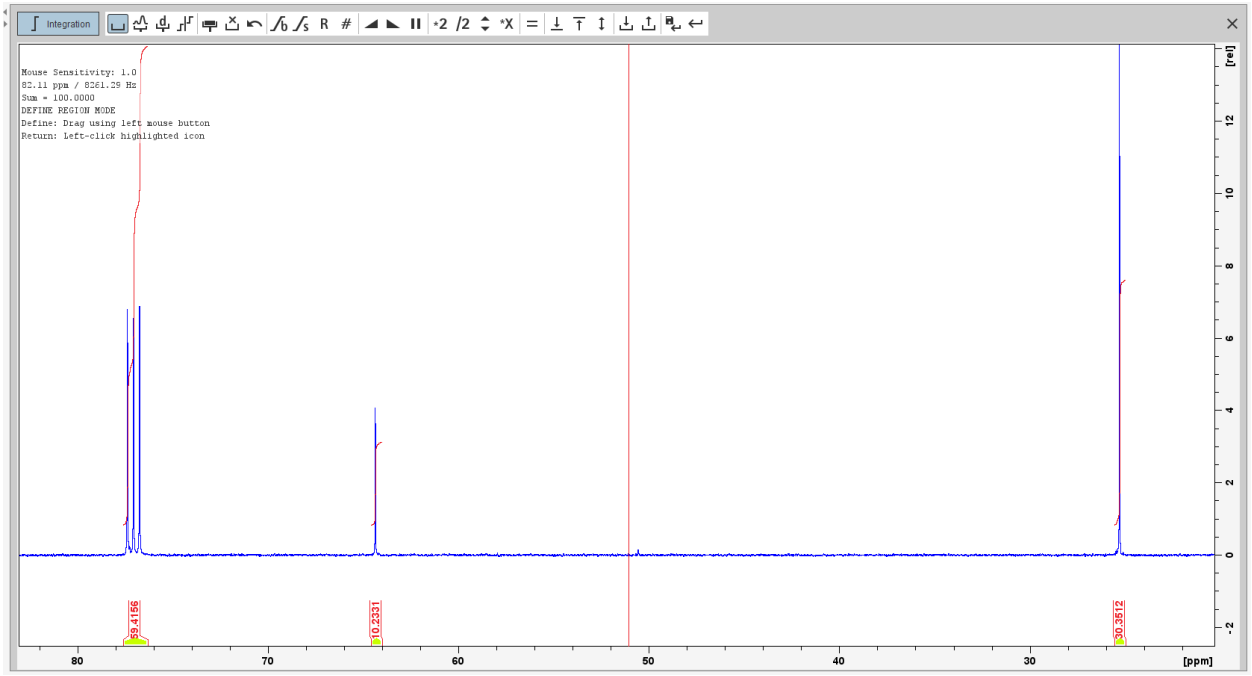


1. Delete automatically generated peaks and integrals.

Use the “Analyze” section and proceed as described before: “Pick Peaks” → “Delete all peak picking ranges” → “Return, and save changes”.

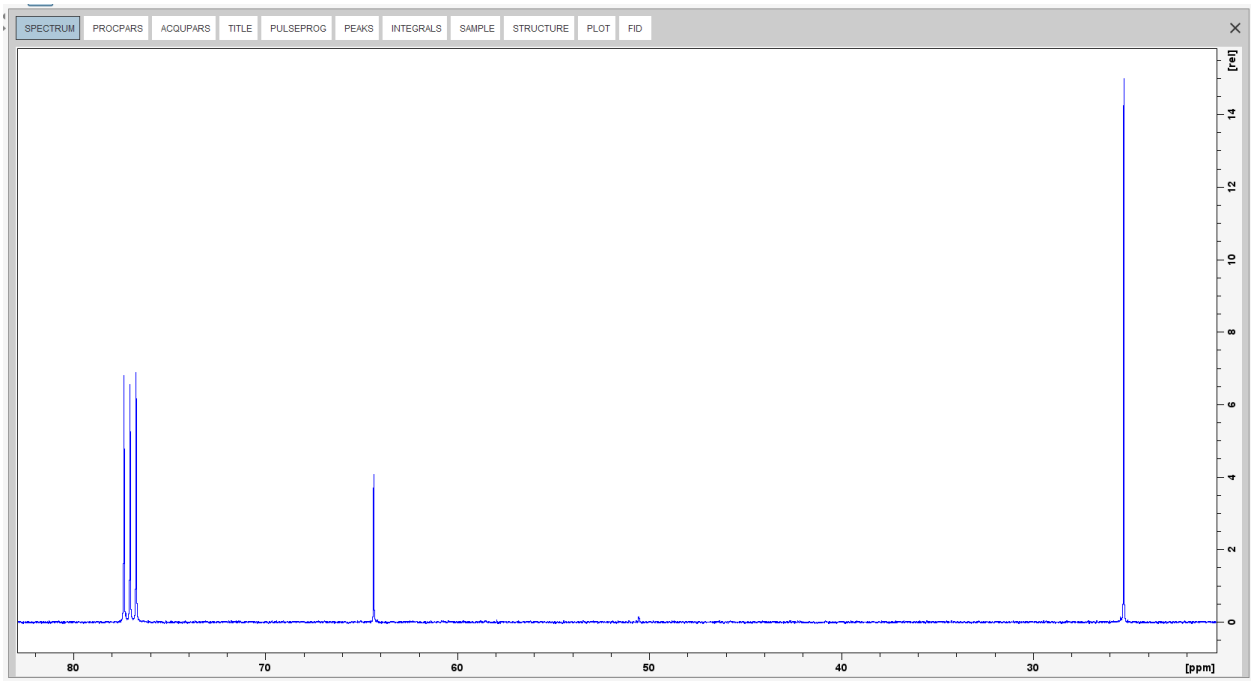


Move to “Integrate” → “Select/Deselect all regions” → “Delete selected regions” → “Return, and save changes”.



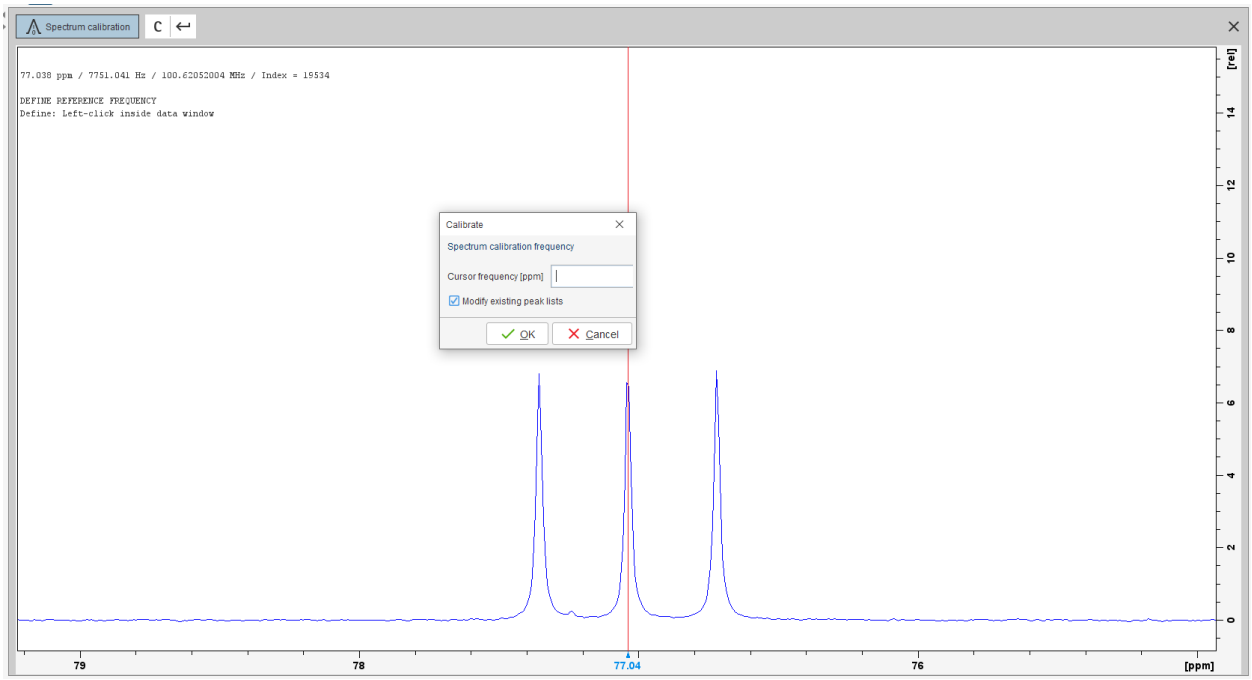
2. Phase and baseline correction

Type “apk” for the automatic phase correction, and type “abs” for the automatic baseline correction. If the spectrum does not look good as in the image below, the corrections can be made manually in the same way as for the proton spectrum.



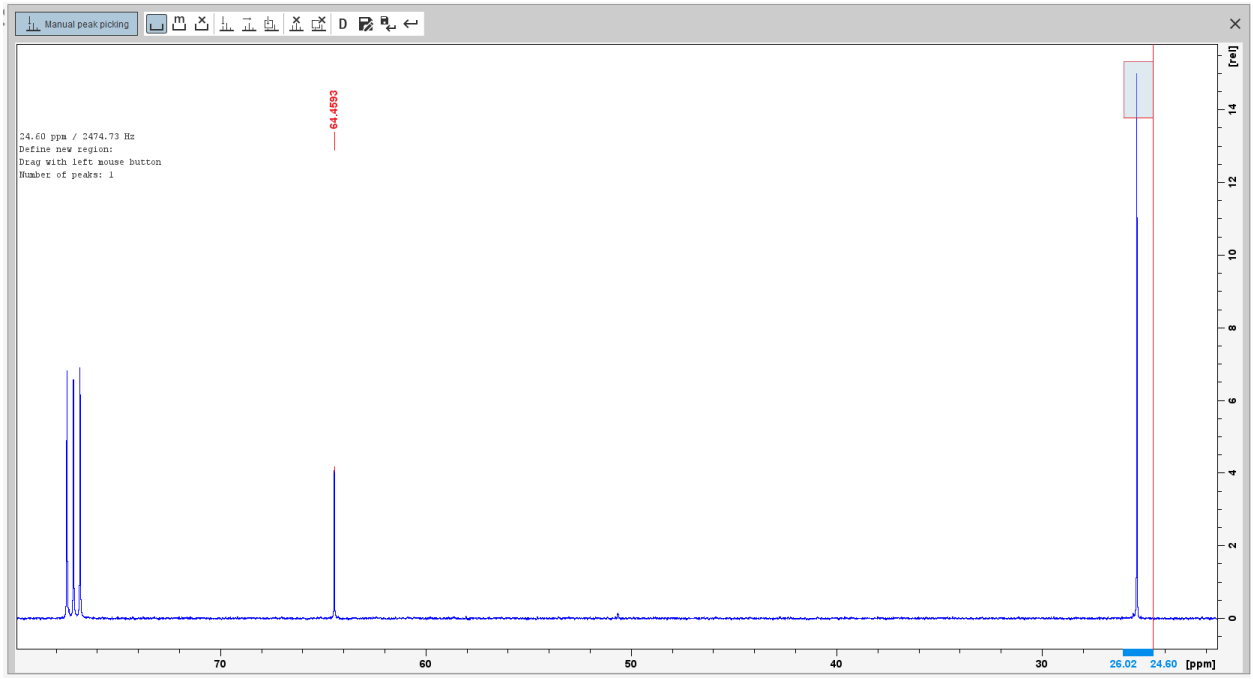
3. Calibration of the ppm scale

Zoom in on the solvent peak → “Manual Axis Calibration” → select the solvent peak and insert the new ppm reference value. $CDCl_3$ ^{13}C-NMR = 77.16.

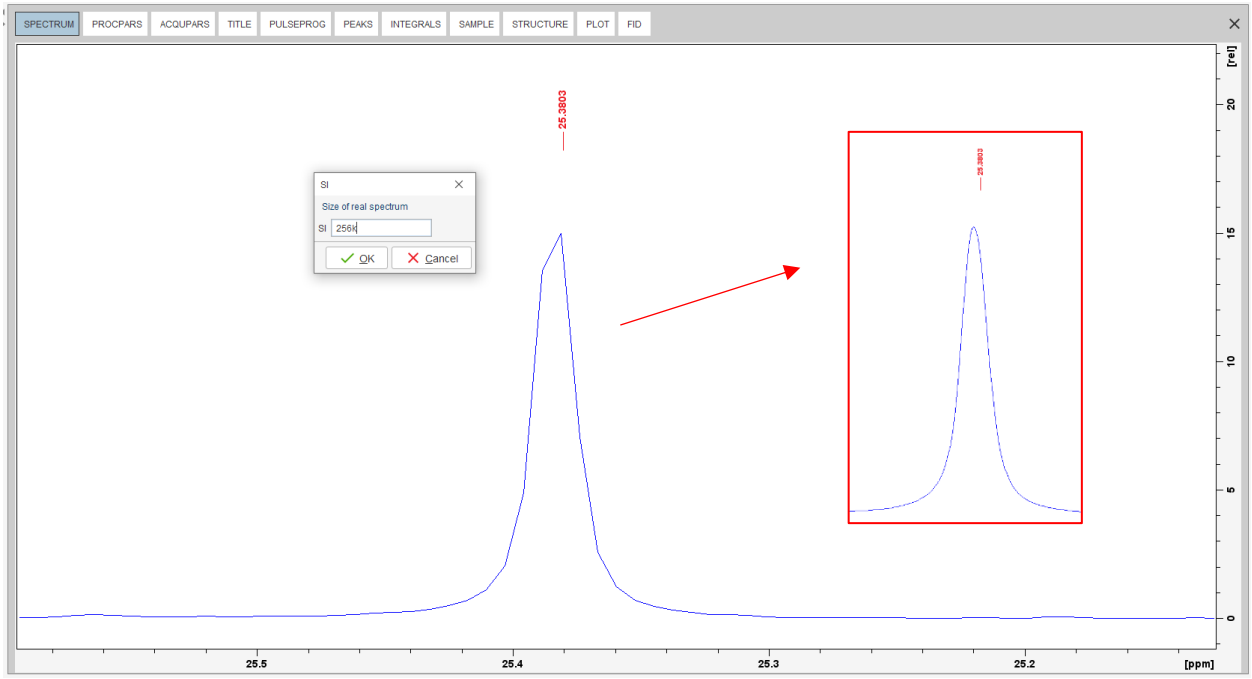


4. Manual peak picking

Move again to the “Analyze” window → “Manual Peak Picking” → select the peaks → “Return, save changes”.



If you want to improve the peak profiles, use the command <si> → increase to 256k → command <efp> and the peak profile will look more defined.



5. Plot window and print spectrum

Customize the plotting of your spectrum using the “PLOT” window and export it. Remember to fix overlapping details and amount of information if you want to use the buttons “Print active window” or “Export active data” or “plot window as PDF”.