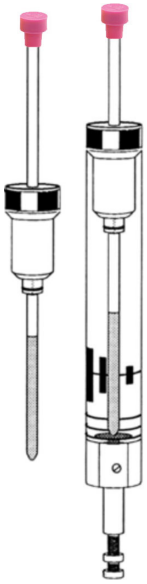
Bruker Fourier300 NMR Spectrometer

*Sample solution height **4.5 cm** with sample fully dissolved in a **deuterated solvent.***
Sign log book. Open Bruker TopSpin software. (Icon on desktop, user NMR.)
Type means click on bottom left command line, type command, then press enter/return.



Inserting Sample (Check that the black dust cap has been removed and ro off.)

1. To eject previous sample, type ***ej*** or click on *Acquire* in the top menubar, then select *Sample* in the next line, then select *Turn on sample lift air (ej).*
2. Remove the previous sample and spinner floating at the top center of the NMR. Slide the old sample out of the spinner, and slide your sample into the spinner.
← Use the plastic depth gauge to set the appropriate solution height by pushing the sample tube through the spinner gently to the bottom of the gauge. Put your tube with spinner into the NMR opening. It should float.
3. To lower the sample, type ***ij*** (inject) or click on *Acquire* in the top menubar, then select *Sample* in the next line, then select *Turn off sample lift air (ij).*

You will hear a click as the sample is properly positioned in probe.

Defining Sample and Experiment

1. Type ***new*** or CTRL N or click on *Start* in the top menubar, then select *Create Dataset* in the next line.
2. Enter information for your sample in the boxes at the far right of the window.
NAME – Folder name should be **your last name**. This will create or add to a folder.
EXPNO – **Increment this number** each time you run a sample.
PROCNO – This value must remain "1".
Experiment – For ^{1}H, use **PROTON**. For ^{13}C, use **C13CPD** (decoupled protons with NOE) or **C13DEPT135** (CH and CH_3 positive, CH_2 negative) or **C13DEPT90** (only CH) or **C13GD** (coupled protons with NOE "gated decoupling").
Set solvent – Choose **solvent** from the drop down menu, usually CDCl3.
Leave *Execute getprosol* and *Keep Parameters* unchecked.
DIR – C:\Bruker\TopSpin3.2\data**coursename** from menu.
Leave *Receivers* set to 1.
TITLE – Enter **sample name** for the printed spectrum.
3. Click **OK** at bottom right. If you get an error, be sure you incremented EXPNO.

Locking, Spinning, and Shimming

1. Look at bottom row Sample to see if already spinning. If not spinning
Type ***ro on*** to spin (rotate) sample or click on *Acquire* in the top menubar, then select *Spin* in the next line, then select *Turn sample rotation on (ro on).*
Wait for *SPIN ON* in the bottom left of main window.
2. Look at bottom row Lock to see if already locked. If solvent changed or if not locked
Type **lock** or **lock cdcl3** or click on *Acquire* in the top menubar, then select *Lock* in the next line.
The *Solvents Table* window will pop up. Choose your solvent, usually CDCl3.
The trace will scan back and forth in the lock window at far right. Higher is better. If lock window is missing, type *lockdisp*. If offscale, type *bsmsdisp* and adjust lock gain.
Wait for *lockn finished* in the bottom left of main window but moving the cursor clears the message. At the bottom right a green check after the word "locked" also indicates success. If the lock is way off, type *flock*.

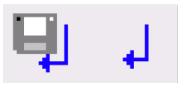
3. Shimming should be done once a week or if solvent has changed.
   Type *gradshim* (solvent with single deuterium peak, lock, ro on) or click on *Acquire* in the top menubar, then click *Shim* in the next line.
   Wait for *gradshim: finished.* This may take minutes. If you get a good shim set that you want to remember, type *wsh* to save or *rsh* to recall. Include date in filename.
   Longer option: Type *bsmsdisp* or double left click on *BSMS status* at window bottom to manually adjust shims (starting with z^1 and z^2) by clicking on *Step+* or *Step–* to maximize lock signal. Type *ro off* before adjusting *x* or *y*.

Collecting Data

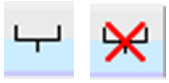
1. Update pulse width and power levels. Type ***getprosol*** or click on *Acquire* in the top menubar, then select *Prosol* in the next line.
2. Type ***ns*** to set the number of scans or select *AcquPars* in the spectrum window. For samples that dissolve well, use 4 or 8 for proton, 64 for DEPT, 128 or 256 for carbon.
   Options: Type *ased* or select *AcquPars* to modify parameters like delay time (D1).
   Type *expt* to see time required. Type *showpp* to view the pulse sequence.
3. Type ***xaua*** or click on *Acquire* in the top menubar, then select *Gain*, wait, then select *Go*.
   Watch progress at bottom left (*rga*, *zg*) and number of scans at bottom center.
4. Option: To see the FID, click on *Acquire* in the top menubar, then click on *Fid* in the Spectrum window. Type *prnt* in bottom command line to print the active window.
5. Option: If *ns* is large, you might type *tr* to transfer collected data to a temporary file. Process Spectra as listed next. If enough data has been acquired, type *halt* to stop.

Processing Spectra

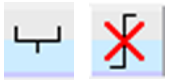
1. Select ***Process/Proc. Spectrum*** to Fourier transform, phase, and reference (*ef, apk, sref*).
   Frequent Option: Right click on the spectrum to select *Show Full Spectrum*.
2. When you enter a process mode, different icons appear at the top of the spectrum window. To exit a mode, click on the Save+Return icon to keep changes or the Return icon to discard changes.



   Optional: Type ***.ph*** or select *Process/Adjust Phase* or type . Right click at one end of the spectrum and select *Set Pivot Point*. Click on the 0 icon in the upper left and left drag vertically to adjust the phase of peaks near the pivot point; click on the 1 icon in the upper left and left drag vertically to adjust the phase of the rest. Exit the mode.
   Optional: Type ***.cal*** or select on *Process/Calib. Axis*. Left click on TMS or known (solvent) peak. Enter the chemical shift value for that peak.
   Optional: Type ***.pp*** or select *Process/Pick Peaks*. Select the field goal icon, then left drag a box that includes peak tops. To delete all, click the X icon. Exit the mode.



   Optional: Type ***.int*** or select *Process/Integrate*. Select the field goal icon, then left drag across each peak to integrate. To set an integral value, right click on a peak and select *Calibrate Current Integral*. To delete all, click the X icon. Exit the mode.



3. Type *plot* or click ***Plot*** on the Spectrum toolbar (if *Plot* is absent, did you exit any previous mode?) A useful layout file to open is *Beloit.xwp*. Click on spectrum then at left *Axes Grids, Curve*. Set *Plot limits x*: 12 –0.5 for proton or 250 –10 for carbon.
4. To create **inset plots**, click outside the dotted margins of the plot. *Insert new elements* by clicking at left on the NMR drop down and selecting 1D spectrum. Left drag to create a new plot. Click on the new plot and edit its *Plot limits*.
5. Type *prnt* or CTRL P to print Use *Landscape* orientation. or click on ***Publish*** in the top menubar, then select ***Print*** in the next line.

When finished, type *ro off*. See inserting sample to swap sample tubes.
Always store a sample in spectrometer! Complete your entry in the log book.
Replace the black plastic dust cap at end of lab.

The **COSY** experiment relies on J-coupling where cross peaks indicate which protons are close to which other protons through bonds.
The **NOESY** experiment relies on direct dipolar couplings where cross peaks indicate which protons are close to which protons through space.

1. First run the ^{1}H spectrum and remember which EXPNO.
2. Then type *new* or *CTRL N* or select *Start/Create Dataset*.
   Select Experiment **COSYGPSW or NOESYGPPHSW**.
   Increment EXPNO. Modify TITLE. OK.
   Type ***ro off*** or *Acquire/Spin/Turn sample rotation off (ro off)* since 2-D experiments should always be run without rotation.
   Type ***getprosol*** or select *Acquire/Prosol*.
3. Select *Acquire/**Set Limits***. Left drag the previous ^{1}H spectrum name in the browser window into the spectrum window. Left drag over the entire peaks with 0.2 ppm of baseline at both ends. *OK*. Verify SW and O1P are properly set. *Close*.
4. For NOESY type *ased* or select *AcquPars* in spectrum window and click  . Change D8 to 0.450.
5. Type *rga*, wait, then *zg* or select *Acquire/Gain*, wait, then select *Acquire/Go*.
   COSY takes about 5 minutes with 1 scan and 128 increments.
   NOESY takes about 50 minutes with 4 scans and 256 increments
6. Select ***Process/Proc. Spectrum***.
   Use mouse scroll wheel to adjust the contour levels. Type *.ls* to save levels.
7. Type *plot* or click ***Plot*** on the Spectrum toolbar. A useful layout file to open is *Beloit2D.xwp*.
8. Type *prnt* or *CTRL P* or select *Publish/Print*.

The **HSQC** experiment relies on the large J_{HC} coupling constant to correlate the chemical shift of a proton with the chemical shift of its directly bonded carbon.

1. First run the ^{1}H spectrum and remember which EXPNO.
2. Then type *new* or *CTRL N* or select *Start/Create Dataset*.
   Select Experiment **HSQCGPPH**. Increment EXPNO. Modify TITLE. OK.
   Type ***ro off*** or *Acquire/Spin/Turn sample rotation off (ro off)* since 2-D experiments should always be run without rotation.
   Type ***getprosol*** or select *Acquire/Prosol*.
3. Select *Acquire/**Set Limits***. Left drag the previous ^{1}H spectrum name in the browser window into the spectrum window. Left drag over the entire peaks with 0.2 ppm of baseline at both ends. *OK*. Verify SW and O1P are properly set. *Close*.
4. Type *rga*, wait, then *zg* or select *Acquire/Gain*, wait, then select *Acquire/Go*.
   This takes about 15 minutes with 2 scans and 256 increments.
5. Select ***Process/Proc. Spectrum.*** Low resolution 1D projections are displayed at the top (^{1}H) and left (^{13}C) of your spectrum. If you have full spectra, right click on the projection and select *External Projection*. Select the appropriate EXPNO. *Show Spectra Thumbnails* in the Browser window for the *Last50* might help.
6. Use mouse scroll wheel to adjust the contour levels. Type *.ls* to save levels.
7. Type *prnt* or *CTRL P* or select *Publish/Print*.

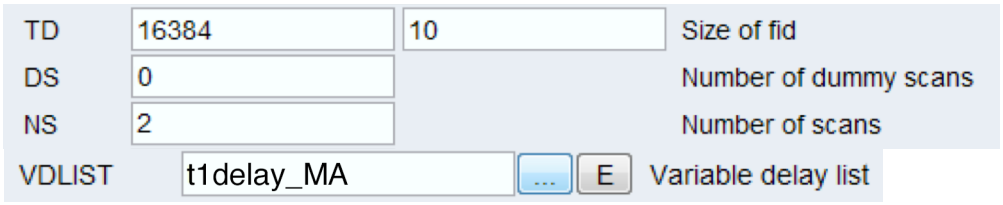
Some calculations

Type *sinocal* to calculate the **signal to noise ratio** for a spectrum.
Type *hwcal* to calculate the **width of a peak at half height**.
Type *diffe* to calculate the **difference spectra** between expnos.

The inversion-recovery experiment measures **spin-lattice T1 relaxation** times where a 180° pulse is followed by spectra collected at various delay times,

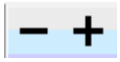
1. First run the ^{1}H spectrum.
2. Then type *new* or *CTRL N* or select *Start/Create Dataset*.
   Select Experiment **PROTONT1**. Increment EXPNO. Modify TITLE.
   Type ***ro off*** or *Acquire/Spin/Turn sample rotation off (ro off)*.
   Type ***getprosol*** or select *Acquire/Prosol*.
3. Select *Acquire/**Set Limits***. Do not answer the dialog. Left drag the previous ^{1}H spectrum name in the browser window into the spectrum window. Left drag over just the peaks with 1.0 ppm of baseline at both ends. The solvent peak may be excluded if it falls outside of the region of interest. *OK*. Verify SW and O1P are properly set. *Close*.
4. Select *AcquPars* in the spectrum window and perhaps click  or  .

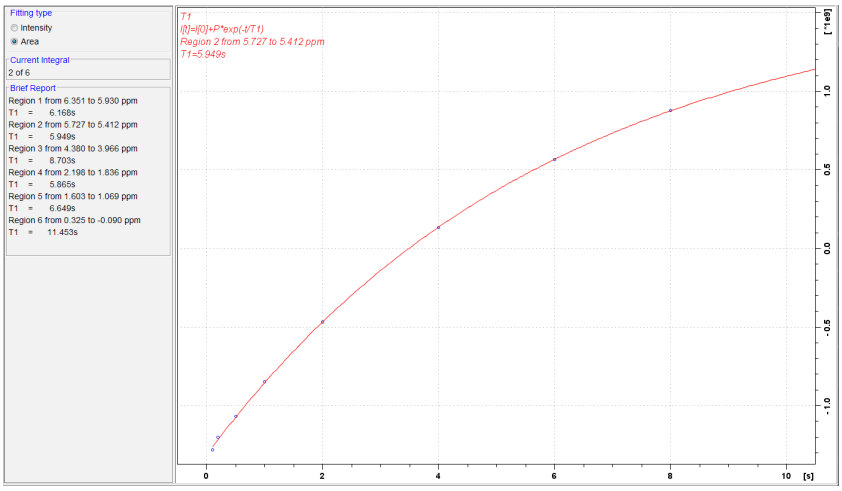
   Set up for 10 data points:

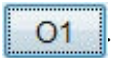
   Click E to edit list:
   0.1, 0.2, 0.5, 1, 2, 4, 6, 8, 12, 16. Select *File/Save*. Select *File/Close*.
   Also check D1 value. The default is 15. D1 + AQ should be greater than 10 x T1.
5. Select *Acquire/Gain*, wait, then select *Acquire/Go*. This takes about 8 minutes.
6. Type *rser 10* (last data row, if successful screen will say 1d raw data available).
   Type *ef* (Fourier transform). Type *apk* (phase).
   Type **.**2d or click  . (If successful screen will say 2D raw data available. Click on Fid in the spectrum window to see the 10 sets of data.)
   Type *xf2*. Type *abs2*.
7. Type *t1t2* or select *Analyse/TopSpin T1/T2 Module*.

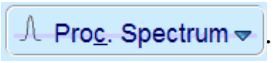
   Click ***Fid***. Enter maximum slice number *10* since 10 values in VDLIST. *OK*.
   Click ***Peaks/Ranges***.
   Click *Manual Integration*. *OK*.
   Define regions by dragging over each peak of interest.
   Click  . *Export Regions To Relaxation Module and .ref*.
   Click ***Relaxation***. *OK*.
   Click ***Fitting***.
   Select fitting type *Area*.
   Close then *OK*.
   Click ***Calculation***. *Close* then click  .
   Use  to step through all the peaks.
   Type *prnt* or *CTRL P* for each peak.
   Click *Report* if you want to see data table.



Proton 90 Degree Pulse p1

1. Run a one scan ^{1}H spectrum. Expand to include one peak (can be a multiplet).
2. Click on  . Left click on the peak center to set RF. Click on 

.
3. Right click on the expanded peak. and **Save Display Region to...**'
   Enable **Parameters F1/2**. OK.
4. Select *ProcPars* in the spectrum window
   SI = 8192
   LB [Hz] = 1
   PH_mod = pk
5. Select *AcquPars* in the spectrum window and perhaps click  or 

.
   PULPROG = zg (not the normal zg30)
   TD = 16384
   SW [ppm] =10
   D1 [sec] = 20 (long value so completely relaxes before next pulse)
   DS = 0
   NS = 1
6. Type ***ro off*** or *Acquire/Spin/Turn sample rotation off (ro off).*
7. Select *Acquire/Gain*, wait, then select *Acquire/Go.*
8. Click on the down arrow in *Process/* 

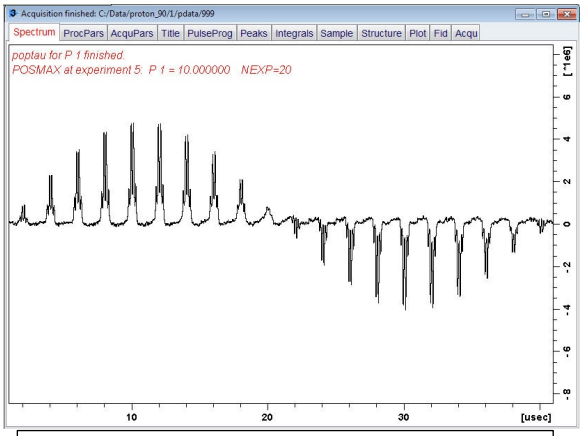
.
   Select **Configure Standard Processing (proc1d)**.

Select:	Deselect:
Exponential Multiply (em)	Set Spectrum Reference (sref)
Auto-Phasing (apk)	Auto-Baseline correction (absn)
	Warn if Processed data exist

   Execute.
9. Type *popt* or click on the down arrow in *Acquire/* 

.
   Choose *Optimize Acquisition Params (popt).*
   OPTIMIZE = Step by step
   GROUP
   PARAMETER = p1
   OPTIMUM = POSMAX
   STARTVAL = 10
   ENDVAL
   NEXP = 20
   VARMOD = LIN
   INC = 2

   Obtain 20 spectra incrementing 90° pulse width p1 from 10 usec by 2 usec.



   The zoomed region is plotted side-by-side in procno 999. Look for the spectrum closest to the null point. This is 180 or 360°. Divide time to get the 90° pulse.

10. Click on *Start Optimize. Save. y. OK.*
    Data is collected 20 times with a long wait between scans. Takes about 8 minutes.
11. When *trf: finished*, switch to *Spectrum* view.
    Click  to move baseline to center. Use mouse wheel to adjust the vertical height.
12. Repeat *popt*, changing values to pin down the zero crossing.
13. Type *prnt* or *CTRL P* or select *Publish/Print.*