INSTRUCTIONS FOR PERFORMING EXPERIMENTS BY BRUKER AVIII 300&500 FT NMR-SPECTROMETERS

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Sample preparation

- Deuterated solvents are invariably required for NMR spectroscopy. The solubility of the sample should first be checked by using a non-deuterated solvent, since the deuterated ones are expensive.
- $CDCl_3$ should be used whenever possible, since it is the cheapest of the NMR-solvents.
- The solvent should not be wasted – the solvent volume of 600 μL is sufficient. Also the solvent bottles should be closed tightly in order to prevent unnecessary evaporation.
- The solvents mustn't be taken away from the NMR laboratory without permission!
- Any precipitate should be filtered off.
- Immediately equip your sample with a Scotch-tape label (name of the sample, solvent, date, name of the person)
- If unnamed/unmarked sample tubes are found in the NMR laboratory, they will be destroyed.

Make sure that the spectrometer is free!

- If the spectrometer is in use you can see it on the Acquisition information box of the status bar below the command line: Name/Expno: | Scan Exp: | Residual time: If the spectrometer is not in use, the text: No acquisition running. appears in the Acquisition information box.
- The measurement can be ended by writing "halt [enter]" on the command line.
  Always ask before stopping any measurement!!

Replacement of the sample

- Using the left mouse button, press "**LOCK: On-Off**" in the digital BSMS control suite (lock off, button turns red). In the case of the 500 MHz NMR spectrometer either the BSMS keyboard (button LOCK) or the digital BSMS control suite can be used.
- Terminate spinning by clicking "**SPIN**". (Spinning is not in use in the 300 MHz NMR spectrometer.)
- Press "**LIFT**" (The sample tube rises. Red light next to the word "missing" is turned on).
- Transfer the NMR tube with the spinner to the sample height measure. DO NOT TOUCH THE SPINNER WITH YOUR FINGERS!! (Get hold of the NMR tube, not of the cap however.)
- Pull the previous sample out of the spinner and replace it with yours.
- Adjust the height of the NMR tube (2 cm) using the sample height measure.
- Take your NMR tube with the spinner on top of the bore, where the sample will "float in the airflow".
- Press "**LIFT**" (the sample falls into the magnet). Wait until the red "missing" light is turned off and the green "down" light on.
- Start spinning by clicking "**SPIN**". (Spinning is not in use in the 300 MHz NMR spectrometer. For 2D-measurements spinning is not started.)
- Write "**lock [enter]**" on the command line and choose the appropriate solvent from the list ($CDCl_3$).
- Wait until the text "locking: finished" appears under the command line.

Shimming (Adjusting the homogeneity of the magnetic field)

- Write "**topshim [enter]**" on the command line.
- Wait until the text "topshim completed successfully" appears under the command line.

- In the case of the 500 MHz NMR spectrometer you may need to shim by hand in order to obtain a desirable result. Shimming can be done by using either the BSMS keyboard or the digital BSMS control suite can be used.

Construction of the measurement file

- Go to your own measurement directory and open a measurement file to ensure that your current measurement will end up in the correct directory.
- Create a new measurement file by pressing the combination **Ctrl + N**.
- Change the **File name** and the **Experiment number** (if the experiment number in the ^{1}H measurement is 1, experiment numbers 101→ for the ^{13}C measurements and 201→ for the 2D measurements are commonly used) to correspond to your sample.
- Select the **<u>correct experiment</u>** from the drop-down menu (In the case of the 300 MHz NMR spectrometer use the following experiments: Oma_1H, Oma_13C, Oma_135DEPT, Oma_HH-COSY, Oma_NOESY, Oma_HSQC, Oma_HSQC_edited, Oma_HMBC, etc. In the case of the 500 MHz NMR spectrometer use the following experiments when equipped with the Prodigy probehead: P_Oma_1H, P_Oma_13C, P_Oma_DEPT135, P_Oma_HH-COSY, P_Oma_NOESY, P_Oma_HSQC_edited, Oma_HMBC, etc. and when equipped with the BBI&BBO probeheads the experiments: Oma_1H, Oma_13C, Oma_135DEPT, Oma_HH-COSY, Oma_NOESY, Oma_HSQC, Oma_HSQC_edited, Oma_HMBC, etc.).
- Choose Options:
  - **Set solvent** → select the correct solvent from the drop-down menu
  - **Execute getprosol** (= get probehead and solvent dependent parameters; has to be performed after changing the probehead or the solvent. For 2D-measurements don't select Execute getprosol)
- Change the **title** to correspond to your sample.

  (The text of the title can be of the following form:

  name_of_the_sample
  in $CDCl_3$ at 30 C
  1H NMR at 300 MHz/13C NMR at 75 MHz
  date name_of_the_measurer)

Before the measurement

- Write "**atma [enter]**" (= automatic tuning and matching; ensures that the channel is tuned on the right frequency) on the command line. Wait until the text "atma done on (X) state: OK in time x sec" appears under the command line.
- Write "**rga [enter]**" (= receiver gain adjustment) on the command line. Wait until the text "rga: finished" appears under the command line.
- You may check the following parameters (and adjust them if needed):
  - ns (=number of scans): in ^{1}H experiments typically 4 or 8, in ^{13}C experiments ns = 8, td0 = 10k (or ns = 10 k)
- (For 2D measurements check the spectral width on AcquPars leaflet. For example for COSY experiment the preset <u>spectral width (SW)</u> is –1.25 - 8.75 ppm. If your sample has e.g. an aldehyde proton resonating at $\delta \sim 10$ ppm you need to change the preset values of the spectral width. It can be done on line SW (= spectral width). Also remember to update the <u>center of the spectral area</u> on row <u>O1P</u>. Do the change for both F2- and F1-axes.)

Measurement

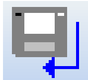
- The experiment is started by writing "**zg [enter]**" on the command line.
- If needed, the measurement can be cut off by writing command "halt [enter]" or by clicking "zg" in the pop-up window opened after writing command "kill [enter]" on the command line.

Copying and processing the data on your own workstation

- Workstations at the Department of Chemistry contain program TopSpin suitable for spectral processing. You can also download the program to your own computer from: https://www.bruker.com/service/support-upgrades/software-downloads/nmr.html
- Copy the data to a USB memory. You can find your measurement files from C:\Data\your own measurement directory.
- On your own computer paste the data to C:\Bruker\TopSpin 3.2\examdata.
- Open the TopSpin software. You will see the data on Browser leaflet from where it can be processed.

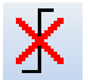
Spectral editing

- The Fourier transformation is conducted by writing **"efp [enter]"** on the command line (for 2D measurements "xfb [enter]"). Phase is corrected by the command **"apk [enter]"** and the baseline by the command **"abs [enter]"** (for 2D measurements baseline is corrected by using command "absb [enter]". For homonuclear 2D correlation spectra symmetrization can be applied by command "syma [enter]".).
- (If the automatic phase correction does not give a satisfying result, the phase can be adjusted manually. Use the mouse to choose the Adjust Phase functionality from the Process menu. By using the left mouse button and dragging (up/down) on top of the icons PH0 and PH1 in the phase correction mode, you can adjust the phase. When you are pleased with the result, click the "save & enter" icon.)

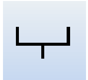
  

- Expand the spectrum in the vicinity of the resonance signal of the solvent by dragging with the left mouse button. Select the **Calib. Axis** functionality from the Process menu, set the cursor on top of the signal, and select using the left mouse button. Insert the chemical shift value of the reference signal to the pop-up window (can be found e.g. from the "NMR Solvent Data Chart" booklet located in the NMR laboratory. $CDCl_3$: ^{1}H $\delta = 7.26$ ppm, ^{13}C $\delta = 77.0$ ppm) and press [enter]. (For 2D measurements first project the previously measured 1D spectra to F2 and F1 axes by clicking the right mouse button on top of each axis, selecting "External projection", and finally choosing the correct measurement from the pop-up menu. Then decide the correlation peak that you will use for calibration. Expand the spectrum from the vicinity of the resonant frequency of the selected correlation peak area by dragging with the left mouse button down. Select the Calib. Axis functionality from the Process menu (equal to 1D processing), set the cursor in the middle of the contour chart of the signal (on top of the signal), and select using the left mouse button. Insert the corresponding chemical shift values from the 1D spectra.)

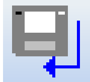
- Reset the whole spectrum on the screen (see functions of the icons below). Spread out the spectrum in the vicinity of the resonance signals of your sample and choose the **Integrate** functionality from the Process menu.
- In the integrate mode, first remove the integrals defined by the software as part of the abs command by clicking the icon:

  Then choose the integrate icon:

  and integrate the signals by dragging with the left mouse button.

- The default value for the first signal is 1.00. In the case you wish to change the value, click the right mouse button on top of the signal, choose calibrate from the pop-up menu, insert the desired value, and confirm by pressing [enter].
- When finished, leave the integrate mode by choosing "save & enter" icon.

- Select **Pick Peaks** functionality from the Process menu and further "Auto-pick All" from the drop-down menu, which opens by clicking the arrowhead on the frame of the Pick Peaks functionality. You can change the MI (minimum intensity) value (0 → e.g. 0.3) on the ProcPars leaflet by choosing "Show/Modify Picking Parameters" from the drop-down menu, which opens by clicking the arrowhead on the frame of the Pick Peaks functionality.

  (When you move the cursor on top of an icon and wait for a while, the text describing the functionality of that icon appears on the screen.)

Printing
- Select **Plot Layout** functionality from the Publish menu.
- LAYOUT can be changed by clicking the arrowhead next to the text on the grey background and selecting "Open" from the drop-down menu.

^{1}H:	+/1D_H.xwp
^{13}C & ^{13}C DEPT-135:	+/1D_X.xwp
^{1}H,^{1}H COSY:	+/2D_hom.xwp
^{1}H,^{13}C HMQC & HMBC:	+/2D_2pro.xwp

- Edit the spectrum the way you wish. There are separate instructions for using the PlotEditor software.
- The active window can be printed by selecting **Print** functionality from the Publish menu.

New experiment for the same sample (e.g. ^{13}C)
- Create a new measurement file by pressing the combination **Ctrl + N**.
- Change the experiment number (if the experiment number in the ^{1}H measurement is 1, an experiment number 101 is commonly used for the ^{13}C measurement).
- Select the correct experiment from the drop-down menu.
- From Options:
  - Check that the correct solvent is chosen
  - Check that Execute getprosol is chosen (in 1D experiments)
- Change the title of the measurement to correspond your experiment.
- Continue from ***"Before the measurement"***.

Ending
- Repeat ***"Replacement of the sample"***. Insert the solvent tube into the magnet and **<u>lock the field.</u>**
- Remember to fill in the log book.
- For washing the NMR tubes own guidelines are presented in this folder.