

## DCIF: How to calibrate the $^1\text{H}$ $90^\circ$ Pulse on the Bruker NMRs

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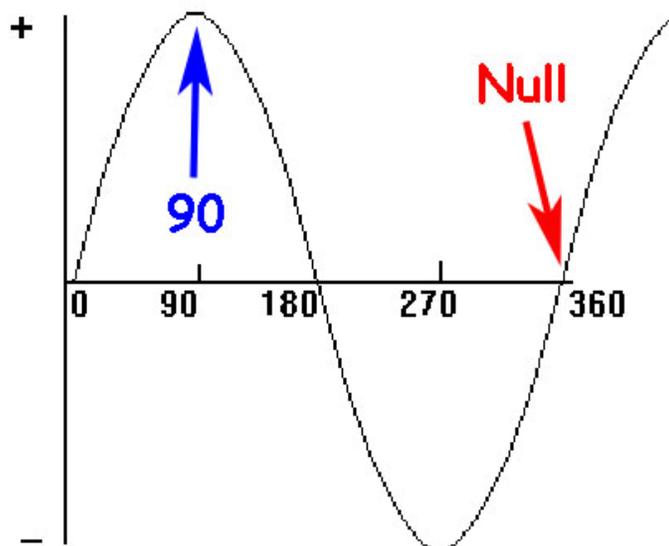
### Background Information:

For many NMR experiments such as DEPT, TOSCY, NOESY, and HMBC, the pulse sequence requires that many specific pulses or a series of pulses ( $90^\circ$ ,  $45^\circ$ ,  $180^\circ$ , etc.) be applied. Without properly calibrated pulses, many of these experiments will yield meaningless results, or most likely, fail outright.

Since each compound (and each nucleus) has a different chemical environment, each had a distinct  $90^\circ$  pulse width (p1). The  $90^\circ$  pulse is defined as the duration, in microseconds, that the rf signal must irradiate your sample in order to tilt the magnetizations into the XY-plane,  $90^\circ$  away from the Z-axis of the NMR's magnetic field. Another way to think of it is how long you must pulse in order to tip all the spins into the XY plane. This pulse is often referred to as the  $\pi/2$  pulse.

The  $90^\circ$  pulse width for proton NMR experiments is about 10-20 microseconds on most modern spectrometers. The exact value of the  $90^\circ$  pulse width depends on the sample (nucleus, solvent, etc.) as well as the instrument (probe, transmitter power, etc.). It may be 5 microseconds long, 17 microseconds, or 35 microseconds, or some other number determined experimentally. For this reason, it is necessary to measure the  $90^\circ$  pulse for every sample you need to perform 2D experiments on. Lucky for us, the proton  $90^\circ$  pulse is typically quite similar for all the protons in your sample.

Measuring the  $90^\circ$  pulse width is simple enough. Remember that the  $90^\circ$  pulse tilts the sample magnetization into the XY plane, which contains the detector. A simple pulse sequence of irradiate-observe should show a maximum for the pulse duration corresponding to a  $90^\circ$  pulse. Because it is difficult to discern maximum signal intensities by comparing similarly intense peaks (i.e. comparing an  $89^\circ$ , a  $90^\circ$ , and a  $91^\circ$  pulse.), we look at the  $180^\circ$  or the  $360^\circ$  pulse.



The  $360^\circ$  pulse corresponds to a 'null' - no signal is observed at this irradiation. Searching for this null is easier to determine and has the added advantage of minimizing the time required between pulses due to relaxation issues.

## The Bruker nitty gritty:

Please note that **all bold print** is input into the command window. Many steps are being skipped on the assumption that you already know how to acquire a simple 1D-proton spectrum.

- Tune the probe (if needed) and obtain a well-shimmed  $^1\text{H}$  spectrum.
- Use the cursors to zoom in on an area of interest and carefully phase this portion of the spectrum. If necessary, phase this portion by hand. Type **.setsw** to set this area of interest (sets the sweep width and o1). Write these values down!
- Type **p1**, hit enter and notice the current value for the  $90^\circ$  pulse. Record p1 and p11
- Type **pulprog zg**. Typically, Bruker uses a  $30^\circ$  pulse (zg30) for a proton 1D. This resets this to a  $90^\circ$  pulse.
- Change parameters (**ns 1; ds 0; d1 60**), reacquire, and phase. The value for d1 should be  $5 \times T_1$ , hence using a value of 60 here is an estimate. If you have a slow relaxer or know your value for  $T_1$ , you might want to set d1 to a larger value.
- Fourier transform (**ft**) and phase (**apk**). Type **dpl1** to set the display regions. Set **phmod** as **pk**
- Start the acquisition by executing the AU **popt** program.
  1. Check Optimize button
  2. Enter *parameter* to modify: p1
  3. Choose *optimum* value: zero
  4. Enter *startval* value: 8
  5. Enter *endval* value: 64
  6. Enter the number of experiments (*nexp*) 8
  7. Enter the increment variation mode (*varmod*) lin(ear)
  8. Enter parameter increment (*inc*) 8
  9. Click [start optimize]

<input type="checkbox"/>	store as 2D data (ser file)							
<input type="checkbox"/>	The AU program specified in AUNM will be executed							
<input checked="" type="checkbox"/>	Perform automatic baseline correction (ABSF)							
<input type="checkbox"/>	Overwrite existing files (disable confirmation Message)							
<input type="checkbox"/>	Run optimisation in background							
OPTIMIZE	PARAMETER	OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC	
<input checked="" type="checkbox"/>	p1	ZERO	8	64	8	LIN	8	
Start optimize		Halt optimize		Read protocol		Add parameter		Restore
Save		Read array file		Save array file as ...		Help		

- In PROCNO 999, the finished array will be displayed, similar to Figure 1.
- On the screen, you should see a series of spectra that start positive, pass through a null at  $180^\circ$ , become negative, and pass through a second null at  $360^\circ$ . Estimate the point where the signal goes from negative values through zero then become positive. This is the location of your  $360^\circ$  pulse. (If you do not see a clear null at  $360^\circ$ , you may need to run **popt** again, adjusting the entered values.)
- Run your array again, to determine the  $360^\circ$  pulse width  $\pm 0.5 \mu\text{s}$  (i.e. array 60 to 63 with an increment of 0.5)
- Calculate the  $90^\circ$  pulse by dividing the p1 value of the null by 4. Use this number for your p1 in your subsequent experiments on this sample.