

# Pulse Calibration

## Introduction

5.1

This chapter describes pulse calibration procedures for  $^1\text{H}$  and  $^{13}\text{C}$  as both the observe and the decouple nuclei. It is assumed that the user is already familiar with basic acquisition and processing. Note that while working through this chapter, the user may find it helpful to refer to Appendix A ‘Data Sets and Selected Parameters’, and Appendix B ‘Pulse Calibration Results’. Appendix A lists data sets generated throughout the course of this manual and also provides a table in which the user can record the **o1**, **o2**, and **sw** values appropriate for the various samples used. Appendix B provides a table in which the user can record the pulse lengths and power levels determined during the pulse calibration procedures described in this chapter.

## $^1\text{H}$ Observe $90^\circ$ Pulse

5.2

To calibrate a  $^1\text{H}$   $90^\circ$  pulse using the observe channel (f1), the one-pulse sequence described in Chapter 3 ‘Basic  $^1\text{H}$  Acquisition and Processing’ is used. The carrier frequency (**sfo1**) is set to the resonance frequency of a peak in the  $^1\text{H}$  spectrum of an appropriate sample. That peak is monitored while the length (**p1**) and/or strength (**pl1**) of the RF pulse is adjusted to determine the exact conditions for a  $90^\circ$  pulse.

### Sample

A common sample to use for  $^1\text{H}$  pulse calibration is 0.1% Ethylbenzene in  $\text{CDCl}_3$ . Ethylbenzene has a simple  $^1\text{H}$  spectrum with well-separated signals, which makes it easy to select one signal for pulse calibration. The drawback of using this sample, however, is that due to the relatively long  $T_1$  of Ethylbenzene, a long recycle delay time must be used.

## Preparation

5.2.1

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and  $Z^2$  shims until the lock level is optimized. Tune and match the probehead for  $^1\text{H}$  observation.

A few preparatory spectra need to be collected to determine the correct carrier frequency, spectral width, phase correction, and plotting region to be used in the actual calibration experiment.

First create a new data set. Since this will be a  $^1\text{H}$  observe experiment, it is helpful to create the new data set starting from a previous  $^1\text{H}$  data set, for example proton/3/1 (which was created in Section 3.5.2 on page 28). Enter **re proton 3 1** to call up proton/3/1, then enter **edc** and change the following parameters:

|        |        |
|--------|--------|
| NAME   | test1h |
| EXPNO  | 1      |
| PROCNO | 1 .    |

Click on **SAVE** to create the data set test1h/1/1.

Enter **eda** and set the acquisition parameter values as shown in Table 6.

## Pulse Calibration

**Table 6. <sup>1</sup>H One-pulse Acquisition Parameters**

| Parameter | Value  | Comments  |
|-----------|--------|---|
| PULPROG   | zg     | see Figure 1 for pulse sequence diagram.  |
| TD        | 4k     |   |
| NS        | 1      |   |
| DS        | 0      |   |
| PL1       |        | high power level on f1 channel (see “An Important Note on Power Levels” on page 7). |
| P1        | 3 μsec | start with less than a 90° pulse.   |
| D1        | 10sec  | note long T <sub>1</sub> of ethylbenzene.   |
| SW        | 20ppm  | start with a large spectral width; this will be optimized later.                    |
| O1        |        | start with value from proton/3/1; this will be optimized later.                     |

Enter **rga** to perform an automatic receiver gain adjustment.

Enter **zg** to acquire the FID.

Enter **edp** and set the processing parameters as shown in Table 7.

**Table 7. <sup>1</sup>H One-pulse Processing Parameters**

| Parameter | Value  | Comments                           |
|-----------|--------|------------------------------------|
| SI        | 2k     |                                    |
| LB        | 1Hz    |                                    |
| PSCAL     | global | this will be used by paropt below. |

Add line broadening and then Fourier transform the spectrum with the command **ef**. Manually phase correct the spectrum and store the correction.

Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

### Set o1 and sw

Now it is necessary to move **o1** to the signal that will be used to calibrate the 90° pulse, which in this case is the quartet of the Ethylbenzene <sup>1</sup>H spectrum. Expand the spectrum so that only the quartet at 2.6ppm is displayed. Click on **utilities** to enter the calibration submenu. Click on **O1** with the left mouse button to select **o1** calibration. Move the cursor to the center of the quartet and click the middle mouse

**<sup>1</sup>H Observe 90° Pulse**

button to assign **o1** to this frequency. Click on **return** to exit the calibration submenu and return to the main window.

Reduce the spectral width by entering **swh** and changing the value to 1000Hz. Notice that with a digital filter, it is possible to reduce the spectral width this low and yet not have aliasing.

Enter **zg** to acquire a new FID using the new values of **o1** and **swh**. Process the spectrum with the command **ef**.

**Define phase correction and plot region**

Now it is necessary to define the phase correction and spectral region that will be plotted in the output file of the automation program used to determine the 90° pulse time. Phase correct the spectrum so that the quartet is positive. Expand the spectrum so that the quartet covers approximately the central quarter of the screen. Click on **DP1** with the left mouse button and hit return for the following 3 questions, or answer them as follows:

```
F1                2.8ppm
F2                2.4ppm
change y-scaling on display according to PSCAL? y .
```

At this point, preparations are complete and we are ready to begin the actual calibration experiment.




**Calibration: High Power****5.2.2**

A convenient way to calibrate a 90° pulse is with the automation program **paropt**. It is helpful to use **paropt** in the procedure outlined below when the user has no idea what the 90° time will be. Since it is somewhat time consuming, however, it is not the best procedure to follow if the user already has an idea of the correct pulse time and power level. (If this is the case, it is better to make educated guesses rather than to use **paropt** to check such a wide range of values).

To start the automation program, simply type **xau paropt** and answer the questions as follows:

```
Enter parameter to modify:    p1
Enter initial parameter value: 2
Enter parameter increment:    2
Enter # of experiments:      16 .
```

In this case, **paropt** acquires and processes 16 spectra while incrementing the parameter **p1** from 2μsec to 32μsec. For each value of **p1**, only the spectral region defined above is plotted. All 16 spectra appear side-by-side in **test1h/1/999**, and the results should resemble those shown in Figure 10. The intensity of the quartet should vary sinusoidally over the 16 spectra. At the end of the experiment, the message “**paropt finished**” and a value for **p1** are displayed. This value is approximately the 90° pulse length of the <sup>1</sup>H transmitter with the current power level **p11**. Write this value down and follow the procedure below to obtain a more accurate 90° time.

Note that the data set can be scaled horizontally with the  button, and vertically with the  and  buttons.

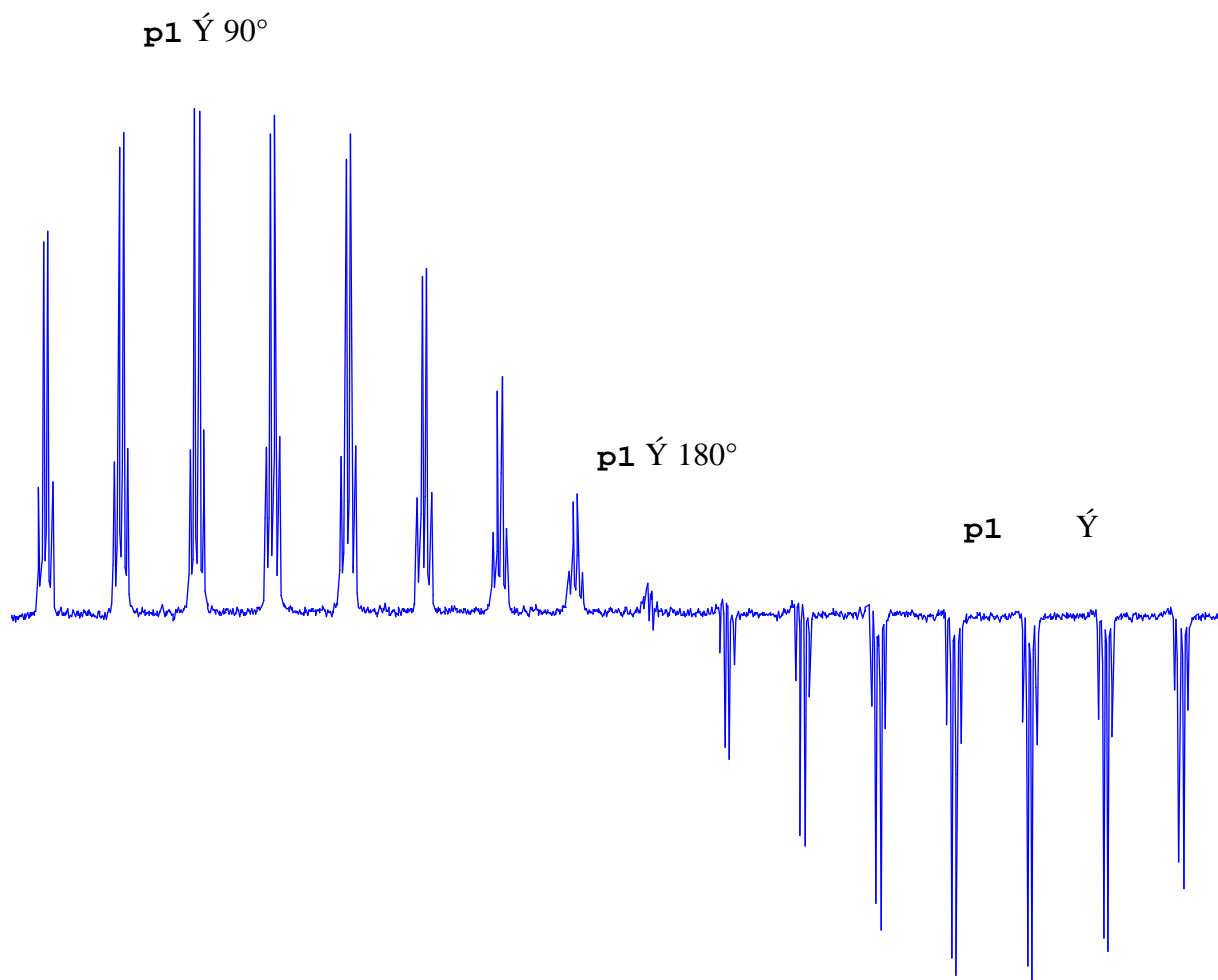
## Pulse Calibration

Return to the data set test1h/1/1 by entering **re 1 1**. Type **p1** and change the value to be approximately a  $360^\circ$  pulse (i.e., 4 times the  $90^\circ$  value determined by paropt).

Acquire and process another spectrum (**zg, efp**). Change **p1** by a small amount, acquire and process another spectrum, etc., until the quartet goes through a null, indicating a  $360^\circ$  pulse. Notice that when the phase correction is defined so that the quartet is positive for small pulse angles (as above), then the quartet will be negative when the pulse angle is slightly less than  $360^\circ$  and will be positive when the pulse angle is slightly more than  $360^\circ$ .

The  $360^\circ$  pulse time divided by 4 is the  $90^\circ$  pulse length for the  $^1\text{H}$  transmitter, with the current power level **p11**, and using the current probehead.

Figure 10: Paropt Results for  $^1\text{H}$   $90^\circ$  Pulse Calibration



**Calibration: Low Power for MLEV Spinlock****5.2.3**

The <sup>1</sup>H 90° pulses of the MLEV sequence used during the spinlock period of a TOCSY sequence should be 30 to 40 μsec, so it is necessary to find the corresponding power level. The procedure outlined below uses paropt to check a wide range of power levels. Alternatively, the user may make use of the rule of thumb that the pulse length should double, approximately, for every 6 dB decrease in power level. For example, say that it was determined that the 90° time (**p1**) for **p11** = -6 dB is 8 μsec. It could then be expected, roughly, that **p1** = 16 μsec for **p11** = 0 dB and **p1** = 32 μsec for **p11** = 6 dB. (Notice that **p11** is an attenuation level, so higher numbers correspond to lower power).

Assuming the user has just finished calibrating the <sup>1</sup>H 90° pulse for high power level, as described in Section 5.2.2, return to test1h/1/1 (**re 1 1**). Enter **p1** and change the value to 35 μsec. Use paropt to adjust the power level by typing **xau paropt** and answering the following questions (note that the appropriate initial power level will depend on the instrument):

```
Enter parameter to modify:  p11
Enter initial parameter value:  0
Enter parameter increment:  1
Enter # of experiments:      16 .
```

The results will be displayed in test1h/1/999. Notice that this time the result does not look like a simple decaying sinusoid. At the end of the experiment, the message “paropt finished” and a value for **p11** are displayed. This value is the <sup>1</sup>H transmitter power level for a 90° pulse time of approximately 35 μsec. Write down this value and follow the steps below to obtain the exact 90° time for this power level.

Return to test1h/1/1 (**re 1 1**). Type **p1** and change the value to be approximately a 360° pulse (i.e., 4 times 35 μsec). Acquire and process a spectrum (**zg, efp**) using the power level **p11** determined by paropt above. Change **p1** in small increments until the quartet goes through a null indicating a 360° pulse. Divide this 360° pulse time by 4 to get the 90° pulse time for this power level.

This is the power level and 90° pulse time for MLEV spinlocking with this probehead. Note that the parameters used by the TOCSY sequence are **p6** for the 90° pulse time and **p110** for the power level, rather than **p1** and **p11** as used here.

**Calibration: Low Power for ROESY Spinlock****5.2.4**

The power level required for the cw spinlock used during ROESY corresponds to a 90° pulse length of 100 to 120 μsec. (Note, however, that no 90° pulse is actually used during a ROESY spinlock). The procedure outlined below uses paropt to check a wide range of power levels. Alternatively, the user may make use of the rule of thumb that the pulse length should double, approximately, for every 6 dB decrease in power level. For example, say that it was determined that the 90° time (**p1**) for **p11** = -6 dB is 8 μsec. It could then be expected, roughly, that **p1** = 128 μsec for **p11** = 18 dB. (Notice that **p11** is an attenuation level, so higher numbers correspond to lower power).

Return to test1h/1/1 (**re 1 1**). Enter **p1** and change the value to 110 μsec. Use paropt to adjust the power level by typing **xau paropt** and answering the

## Pulse Calibration

following questions (note that the appropriate initial power level will depend on the instrument):

```

Enter parameter to modify:  p11
Enter initial parameter value:  10
Enter parameter increment:  1
Enter # of experiments:  16 .

```

The results will be displayed in test1h/1/999. At the end of the experiment, the message “paropt finished” and a value for **p11** are displayed. This value is the <sup>1</sup>H transmitter power level for a 90° pulse time of approximately 110µsec. Write down this value and follow the steps below to verify the 90° time for this power level.

Return to test1h/1/1 (**re 1 1**). Type **p1** and change the value to be approximately a 360° pulse (i.e., 4 times 110µsec). Acquire and process a spectrum (**zg, efp**) using the power level **p11** determined by paropt above. Change **p1** in small increments until the quartet goes through a null indicating a 360° pulse. Divide this 360° pulse time by 4 to get the 90° pulse time for this power level.

Notice that since ROESY uses cw spinlocking, only the power level, not the actual 90° time, is required. The above procedure is recommended simply to verify that the power level selected does in fact give a 90° time of 100 to 120µsec. Also, the parameter used by the ROESY sequence is **p111** for the cw power level, rather than **p11** as used here.

### <sup>13</sup>C Observe 90° Pulse

5.3

#### Sample

<sup>13</sup>C observe pulse calibration experiments require a sample with a strong <sup>13</sup>C signal. A good choice is 80% Benzene in Acetone-d6. If an appropriate sample is not available, it is possible to use the inverse mode <sup>13</sup>C pulse calibration procedure described in Section 5.5 instead.

#### Preparation

5.3.1

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z<sup>2</sup> shims until the lock level is optimized. Tune and match the probehead for <sup>13</sup>C observation, <sup>1</sup>H decoupling.

First create a new data set. Since this will be a <sup>13</sup>C observe experiment, it is helpful to create the new data set starting from a previous <sup>13</sup>C data set, for example carbon/2/1 (which was created in Section 4.2.3 on page 35). Enter **re carbon 2 1** to call up carbon/2/1, then enter **edc** and change the following parameters:

```

NAME          test13c
EXPNO         1
PROCNO        1 .

```

Click on **SAVE** to create the data set test13c/1/1.

Enter **eda** and set the acquisition parameters as shown in Table 8.

**Table 8. <sup>13</sup>C One-pulse Acquisition Parameters**

| Parameter | Value  | Comments  |
|-----------|--------|---|
| PULPROG   | zg     | see Figure 5 for pulse sequence diagram.  |
| TD        | 4k     |   |
| NS        | 1      |   |
| DS        | 0      |   |
| PL1       |        | high power level on f1 channel (see “An Important Note on Power Levels” on page 7). |
| P1        | 3 μsec | start with less than a 90° pulse.   |
| D1        | 20sec  | note long <sup>13</sup> C T <sub>1</sub> .  |
| SW        | 350ppm |   |
| O1        |        | start with value from carbon/2/1; this will be optimized later.                     |

Enter **rga** to perform an automatic receiver gain adjustment.

Enter **zg** to acquire the FID.

Enter **edp** and set the processing parameters as shown in Table 9.

**Table 9. <sup>13</sup>C One-pulse Processing Parameters**

| Parameter | Value  | Comments |
|-----------|--------|----------|
| SI        | 2k     |          |
| LB        | 3Hz    |          |
| PSCAL     | global |          |

Add line broadening and then Fourier transform the spectrum with the command **ef**. Manually phase correct the spectrum and store the correction.

Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

#### **Set o1 and sw**

Now it is necessary to move **o1** to the signal that will be used to calibrate the 90° pulse. Expand the spectrum so that only the doublet at 130ppm is displayed. Click on **utilities** to enter the calibration submenu. Click on **O1** with the left mouse button to select **o1** calibration. Move the cursor to the center of the doublet and click the middle mouse button to assign **o1** to this frequency. Click on **return** to exit the calibration submenu and return to the main window.

## Pulse Calibration

Reduce the spectral width by entering **swh** and changing the value to 1000Hz. Notice that with a digital filter, it is possible to reduce the spectral width this low and yet not have aliasing. Acquire and Fourier transform another spectrum (**zg**, **ef**).

### Define phase correction and plot region

Now it is necessary to define the phase correction and spectral region that will be plotted in the output file produced by **paropt**. Phase correct the spectrum so that the doublet is positive. Expand the spectrum so that the doublet covers approximately the central third of the screen. Click on **DP1** with the left mouse button and hit return for the following 3 questions, or answer them as follows:

```
F1                133 ppm
F2                127 ppm
change y-scaling on display according to PSCAL? y .
```

At this point, preparations are complete and we are ready to begin the actual calibration experiment.

## Calibration: High Power

### 5.3.2

Again, in the procedure outlined below, the automation program **paropt** is used to do the pulse calibration. This procedure is helpful when the user has no idea what the  $90^\circ$  time will be. Since it is somewhat time consuming, however, it is not the best procedure to follow if the user already has an idea of the correct pulse time. (If this is the case, it is better to make educated guesses rather than to use **paropt** to check such a wide range of pulse times).

To start the automation program, simply type **xau paropt** and answer the questions as follows:

```
Enter parameter to modify:  p1
Enter initial parameter value:  2
Enter parameter increment:  2
Enter # of experiments:  16 .
```

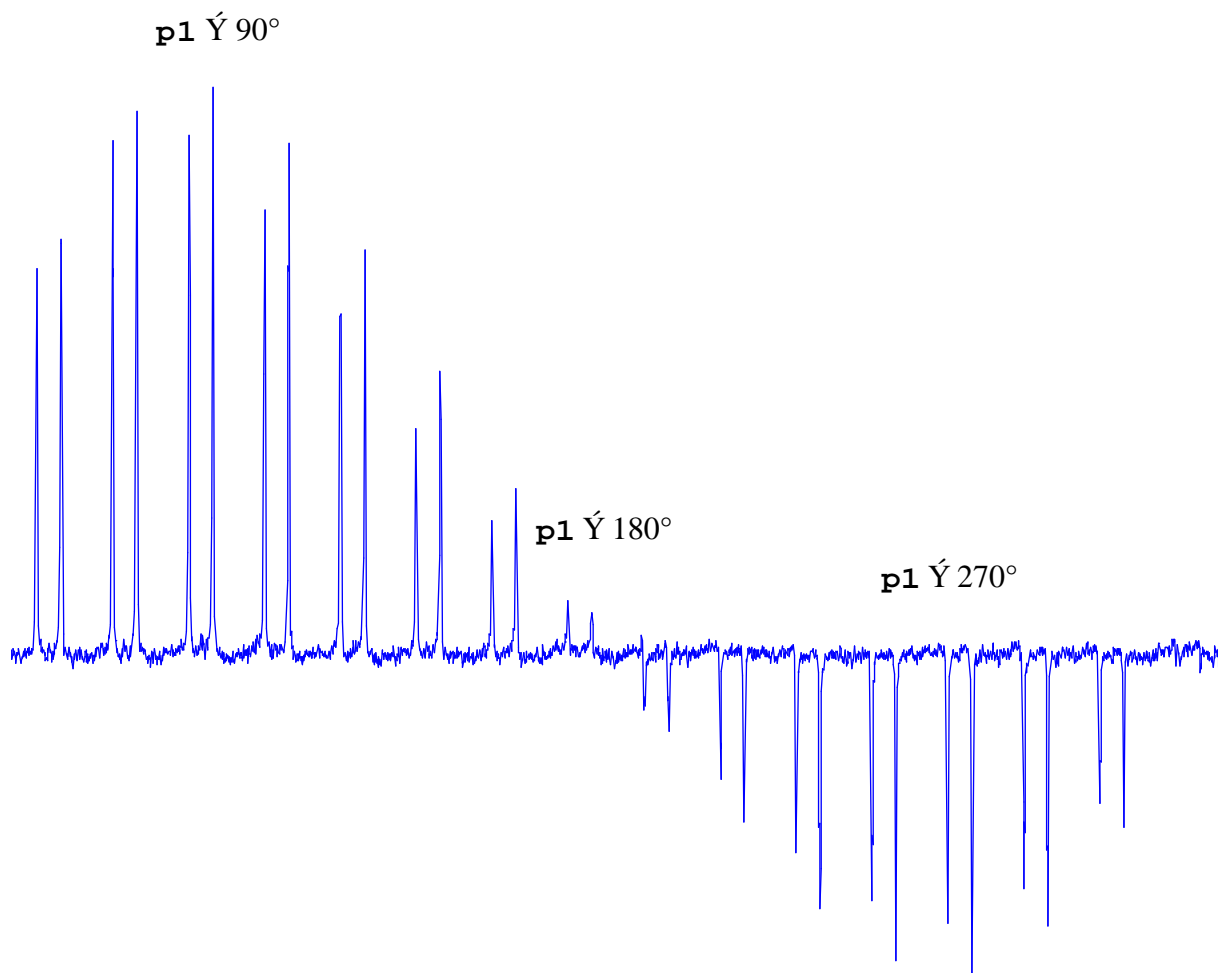
**Paropt** acquires and processes 16 spectra, while incrementing the parameter **p1** from  $2\mu\text{sec}$  to  $32\mu\text{sec}$ , and displays the results side-by-side in **test13c/1/999**. The results should resemble those shown in Figure 11. The intensity of the doublet should vary sinusoidally over the 16 spectra. At the end of the experiment, the message “**paropt finished**” and a value for **p1** are displayed. This value is approximately the  $90^\circ$  pulse length of the  $^{13}\text{C}$  transmitter with the current power level **p11**. Write this value down and follow the procedure below to obtain a more accurate  $90^\circ$  time.

Return to the data set **test13c/1/1** by entering **re 1 1**. Type **p1** and change the value to be approximately a  $360^\circ$  pulse (i.e., 4 times the  $90^\circ$  value determined by **paropt**).

Acquire and process another spectrum (**zg**, **efp**). Change **p1** by a small amount, acquire and process another spectrum, etc., until the doublet goes through a null, indicating a  $360^\circ$  pulse. The  $360^\circ$  pulse time divided by 4 is the  $90^\circ$  pulse length for the  $^{13}\text{C}$  transmitter, with the current power level **p11**, and using the current probehead.

Note that if the  $90^\circ$  pulse length is less than  $5\mu\text{sec}$  for 5 mm probes and less than  $10\mu\text{sec}$  for 10 mm probes, there is a chance that the probe may arc. To prevent this from happening, if the  $90^\circ$  is too short change **p11** to a higher value (i.e., increase the attenuation on the transmitter) and find the new  $90^\circ$  time.

Figure 11: Paropt Results for  $^{13}\text{C}$   $90^\circ$  Pulse Calibration



## Pulse Calibration

### $^1\text{H}$ Decouple $90^\circ$ Pulse

5.4

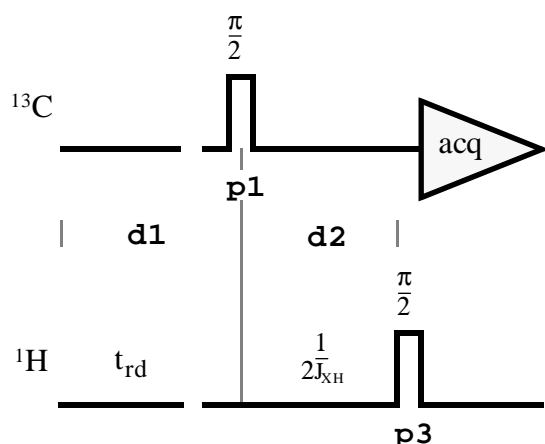
#### Sample

For  $^1\text{H}$  decoupling pulse calibrations, the sample must have a large  $^{13}\text{C}$  signal with detectable  $^1\text{H}$  coupling. A good choice is 80% Benzene in Acetone- $d_6$ .

#### Pulse sequence

The pulse sequence used in this procedure is the DECP90 sequence shown in Figure 12. This sequence consists of a recycle delay followed by a  $90^\circ$   $^{13}\text{C}$  pulse, a delay  $1/(2J_{\text{XH}})$  for the creation of antiphase magnetization, a  $^1\text{H}$  pulse, and finally detection of the  $^{13}\text{C}$  signal. During calibration, the length and/or strength of the  $^1\text{H}$  pulse is adjusted. When the  $^1\text{H}$  pulse is exactly  $90^\circ$ , the  $^{13}\text{C}$  magnetization exists purely as multiple quantum coherence and so the signal disappears.

Figure 12: DECP90 Pulse Sequence



### Preparation

5.4.1

Ideally, this procedure is carried out immediately following the  $^{13}\text{C}$  observe pulse calibration described above in Section 5.3, in which case the correct sample is already in the magnet, the magnet shimmed, the field locked, and the probehead tuned and matched for  $^{13}\text{C}$  and  $^1\text{H}$ .

#### Set o2

First create a new data set. Since this first spectrum will be a  $^1\text{H}$  observe experiment to determine the correct frequency for the DECP90  $^1\text{H}$  decoupling pulse, it is helpful to create the new data set starting from a previous  $^1\text{H}$  data set, for example proton/3/1 (which was created in Section 3.5.2 on page 28). Enter **re proton 3 1** to call up proton/3/1, then enter **edc** and change the following parameters:

|        |         |
|--------|---------|
| NAME   | testdec |
| EXPNO  | 1       |
| PROCNO | 1 .     |

Click on **SAVE** to create the data set testdec/1/1.

**<sup>1</sup>H Decouple 90° Pulse**

Enter **eda** and set the acquisition parameters as shown in Table 6.

**Table 10. <sup>1</sup>H One-pulse Acquisition Parameters**

| Parameter | Value | Comments  |
|-----------|-------|---|
| PULPROG   | zg    | see Figure 1 for pulse sequence diagram.  |
| TD        | 4k    |   |
| NS        | 1     |   |
| DS        | 0     |   |
| PL1       |       | high power level on f1 channel (see “An Important Note on Power Levels” on page 7). |
| P1        | 3μsec | start with less than a 90° pulse.   |
| D1        | 5 sec |   |
| SW        | 20ppm | start with a large spectral width until the appropriate value is known.             |
| O1        |       | start with value from proton/3/1.   |

Enter **rga** to perform an automatic receiver gain adjustment.

Enter **zg** to acquire an FID.

Enter **edp** and set the processing parameters as shown in Table 7.

**Table 11. <sup>1</sup>H One-pulse Processing Parameters**

| Parameter | Value  | Comments |
|-----------|--------|----------|
| SI        | 2k     |          |
| LB        | 1 Hz   |          |
| PSCAL     | global |          |

Add line broadening and Fourier transform the data with the command **ef**. Manually phase correct the spectrum and store the correction.

Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

With the cursor in the data field of the main window, click the left mouse button to tie the cursor to the spectrum. Move the cursor to the top of the Benzene peak at 7.3ppm. Note the value of the cursor position in Hz shown in the small ‘Info’ window. This will be the value of **o2** in the DECP90 experiment. Click the left mouse button to release the cursor from the spectrum.

## Pulse Calibration

### Set o1 and sw

This step was already carried out on test13c/1/1 in Section 5.3.1 on page 46. To transfer all the parameters from test13c/1/1 to the new data set, simply enter **re test13c 1 1**, then enter **edc** and change the following parameters:

```

NAME          testdec
EXPNO         2
PROCNO        1 .

```

Click on **SAVE** to create the data set testdec/2/1.

Here, however,  $^1\text{H}$  decoupling is required. Enter **edsp** and set NUC2 to 1H so that the spectrometer parameters are as follows:

```

NUC1          13C
OFSX1         o1 from test13c/1/1
NUC2          1H
OFSH1         o1 from testdec/1/1
NUC3          off .

```

Enter **eda** and set the acquisition parameters values as shown in Table 12. Notice that **o1** and **swh** should be set to the values used in test13c/1/1. **Be sure** to set **o2** to the  $^1\text{H}$  offset frequency determined in the subsection “Set o2” on page 50. The DECP90 experiment will not work as described below unless both **o1** and **o2** are set correctly.

**Table 12. DECP90 Acquisition Parameters**

| Parameter | Value             | Comments  |
|-----------|-------------------|---|
| PULPROG   | dec90             | see Figure 12 for pulse sequence diagram.   |
| TD        | 4k                |   |
| NS        | 1                 |   |
| DS        | 0                 |   |
| PL1       |                   | high power level on f1 channel (see “An Important Note on Power Levels” on page 7).             |
| PL2       |                   | high power level on f2 channel (see “An Important Note on Power Levels” on page 7).             |
| P1        |                   | $90^\circ$ $^{13}\text{C}$ pulse determined in Section 5.3.2 on page 48.                        |
| P3        | 2 $\mu\text{sec}$ | start with less than a $90^\circ$ pulse; this will be optimized.                                |
| D1        | 5 sec             | note long $^{13}\text{C}$ $T_1$ .   |
| D2        | 3.125 msec        | delay for creation of anti-phase magnetization ( $1/(2J_{\text{XH}})$ ); calculated internally. |
| CNST2     | 160Hz             | one-bond heteronuclear J-coupling ( $J_{\text{XH}}$ ); used to calculate d2.                    |
| SWH       | 1000Hz            |   |

**<sup>1</sup>H Decouple 90° Pulse**

|    |  |   |
|----|--|---|
| O1 |  | <sup>13</sup> C offset frequency of Benzene signal found in “Set o1 and sw” on page 47. |
| O2 |  | <sup>1</sup> H offset frequency of Benzene signal found in “Set o2” on page 50.         |

Enter **zg** to acquire the FID. The receiver gain should already be set appropriately.

Enter **edp** and verify that the processing parameters are as shown in Table 13.

**Table 13. DECP90 Processing Parameters**

| Parameter | Value  | Comments |
|-----------|--------|----------|
| SI        | 2k     |          |
| LB        | 1 Hz   |          |
| PSCAL     | global |          |

Add line broadening and then Fourier transform the spectrum with the command **ef**. Manually phase correct the spectrum so that the left peak is positive and the right peak is negative. Store the correction.

The spectrum is already calibrated if the current data set was created from test13c/1/1.

Since paropt is not going to be used for this calibration procedure, it is not necessary to define the phase correction and plot region as described in the previous calibration procedures.

At this point the preparations are complete and we are ready to begin the actual calibration experiment.

### Calibration: High Power

### 5.4.2

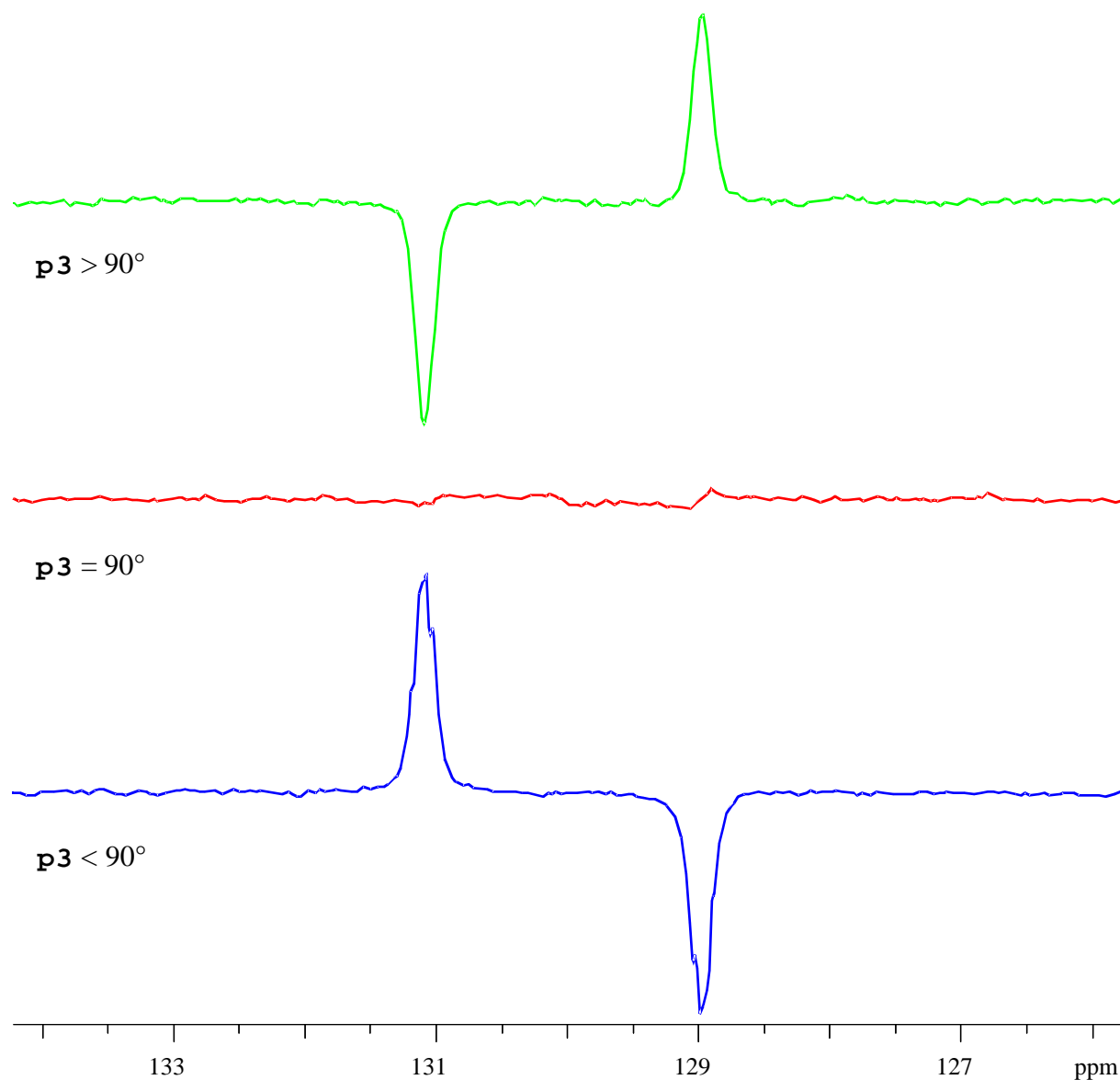
The <sup>1</sup>H decouple 90° pulse time should be close to the <sup>1</sup>H observe 90° pulse time for the same power level, so it makes sense to do this calibration by hand rather than by using paropt.

Increase **p3** to be the 90° time found in Section 5.2.2 on page 43. Acquire and process another spectrum (**zg, efp**). If the pulse angle is less than 90°, the left peak will remain positive and the right peak negative. If the pulse angle is greater than 90° but less than 270°, the left peak will be negative and the right peak positive. At 90°, the signals go through a null. This is shown in Figure 13.

Adjust **p3** slightly until the signals go through a null. This is the 90° pulse length for high power.

## Pulse Calibration

Figure 13:  $^1\text{H}$  Decouple  $90^\circ$  Pulse Calibration Results



### Calibration: Low Power for WALTZ-16 CPD

5.4.3

The WALTZ-16 composite pulse decoupling (cpd) sequence requires a  $90^\circ$  decoupling pulse length of 80 to 100  $\mu\text{sec}$ . Adjust **p12** and **p3** to determine the combination that gives a  $90^\circ$  pulse length in this range, keeping in mind that the  $90^\circ$  pulse time should approximately double for each 6 dB increase in **p12**.

This is the power level and  $90^\circ$  pulse time for WALTZ-16 cpd with this probehead. Note that the parameters used by cpd sequences are **pcpd2** for the  $90^\circ$  pulse time and **p112** for the decoupler power level, rather than **p3** and **p12** as used here.

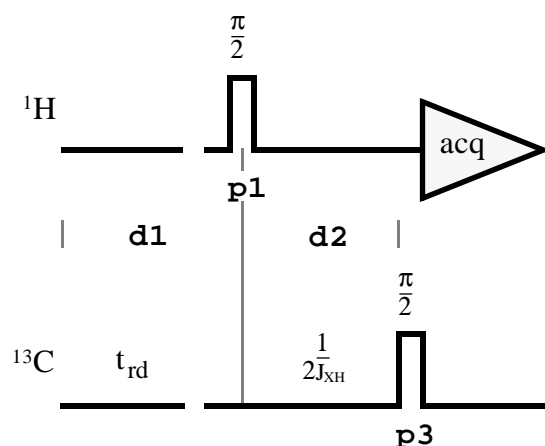
**$^{13}\text{C}$  Decouple  $90^\circ$  Pulse (Inverse Mode)****5.5**

This calibration procedure should yield nearly the same results as the  $^{13}\text{C}$  observe  $90^\circ$  pulse calibration procedure outlined in Section 5.3 on page 46; however, it may be more convenient to implement because  $^1\text{H}$  signals are easier to detect than  $^{13}\text{C}$  signals and  $^1\text{H}$   $T_1$ 's are shorter than  $^{13}\text{C}$   $T_1$ 's.

**Sample**

For inverse experiment pulse calibrations, the detected nucleus is  $^1\text{H}$ , but  $^{13}\text{C}$  satellites must be visible. A sample with easily detected  $^{13}\text{C}$  satellites in the  $^1\text{H}$  spectrum is the  $^1\text{H}$  Lineshape Sample (i.e., 10% Chloroform in Acetone- $d_6$  for frequencies  $\delta$ 300MHz, 3% Chloroform in Acetone- $d_6$  for frequencies between 400 and 500MHz, and 1% Chloroform in Acetone- $d_6$  for frequencies  $\delta$ 600MHz).

Figure 14: DECP90 Pulse Sequence



The pulse sequence used in this calibration procedure is DECP90 and is shown in Figure 12. This is the same pulse sequence as was used for  $^1\text{H}$  decouple  $90^\circ$  pulse calibration in 5.4, except that now the roles of  $^1\text{H}$  and  $^{13}\text{C}$  have been exchanged.

**Preparation****5.5.1**

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and  $Z^2$  shims until the lock level is optimized. Tune and match the probehead for  $^1\text{H}$  observation,  $^{13}\text{C}$  decoupling.

**Set o2**

The first preliminary spectrum is a  $^{13}\text{C}$  observe experiment to determine the correct offset for  $^{13}\text{C}$ , which is **o1** here but will be **o2** in the inverse calibration experiment.

Create a new data set. Since this first spectrum will be a  $^{13}\text{C}$  observe experiment to determine the correct frequency for the DECP90  $^{13}\text{C}$  decoupling pulse, it is helpful to create the new data set starting from a previous  $^{13}\text{C}$  data set. A good choice is

## Pulse Calibration

carbon/3/1 (which was created in Section 4.3.1 on page 37), since in this data set, the  $^{13}\text{C}$  **o1** was set to the Chloroform peak. Enter **re carbon 3 1** to call up carbon/3/1, then enter **edc** and change the following parameters:

```

NAME          testinv
EXPNO         1
PROCNO        1 .

```

Click on **SAVE** to create the data set testinv/1/1.

Enter **eda** and set the acquisition parameters values as shown in Table 14.

**Table 14.  $^{13}\text{C}$  One-pulse Acquisition Parameters**

| Parameter | Value             | Comments  |
|-----------|-------------------|---|
| PULPROG   | zgdc              | see Figure 8 for pulse sequence diagram.  |
| TD        | 4k                |   |
| NS        | 1                 |   |
| DS        | 0                 |   |
| PL1       |                   | high power level on f1 channel (see “An Important Note on Power Levels” on page 7).               |
| PL12      |                   | power level for cpd on f2 channel; use value determined in Section 5.4.3 on page 54.              |
| P1        | 3 $\mu\text{sec}$ | start with less than a $90^\circ$ pulse.  |
| PCPD2     |                   | $90^\circ$ $^1\text{H}$ pulse for cpd sequence; use value determined in Section 5.4.3 on page 54. |
| D1        | 5 sec             | note long $^{13}\text{C}$ $T_1$ .   |
| SWH       | 1000Hz            |   |
| RG        | 8k                | or use rga.   |
| O1        |                   | start with value from carbon/3/1.   |
| O2        |                   | start with value from carbon/3/1.   |
| CPDPRG2   | waltz16           | a common cpd sequence for $^1\text{H}$ decoupling.  |

Notice that the **swh** value listed above is much smaller than the value customarily used for  $^{13}\text{C}$  spectra. In this case it is ok to start with such a small value because we are only interested in the Chloroform signal and **o1** is already set to nearly the correct frequency.

In addition, for this first preliminary spectrum, we will just use an approximate value for **o2** taken from the data set carbon/3/1.

Enter **zg** to acquire the FID.

Enter **edp** and set the processing parameters as shown in Table 15.

**<sup>13</sup>C Decouple 90° Pulse (Inverse Mode)****Table 15. <sup>13</sup>C One-pulse Processing Parameters**

| Parameter | Value  | Comments |
|-----------|--------|----------|
| SI        | 2k     |          |
| LB        | 1 Hz   |          |
| PSCAL     | global |          |

Add line broadening and then Fourier transform the spectrum with the command **ef**. Manually phase correct the spectrum and store the correction.

Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

Now it is necessary to move **o1** to the Chloroform peak at 77 ppm. Expand the spectrum to display the Chloroform peak only. Click on **utilities** to enter the calibration submenu. Click on **O1** with the left mouse button to select **o1** calibration. Move the cursor to the center of the signal and click the middle mouse button to assign **o1** to this frequency. Click on **return** to exit the calibration submenu and return to the main window. This **o1** value will be the <sup>13</sup>C **o2** value for the DECP90 pulse sequence below.

**Set o1 and sw**

The second preliminary spectrum is a <sup>1</sup>H observe experiment to determine the correct offset for <sup>1</sup>H, which is **o1** both here and in the inverse calibration experiment.

First create a new data set. Since this will be a <sup>1</sup>H observe experiment, it is helpful to create the new data set starting from a previous <sup>1</sup>H data set, for example proton/3/1 (which was created in Section 3.5.2 on page 28). Enter **re proton 3 1** to call up proton/3/1, then enter **edc** and change the following parameters:

```

NAME          testinv
EXPNO         2
PROCNO        1 .

```

Click on **SAVE** to create the data set testinv/2/1.

Enter **eda** and change the acquisition parameters as shown in Table 16.

## Pulse Calibration

**Table 16. <sup>1</sup>H One-pulse Acquisition Parameters**

| Parameter | Value | Comments  |
|-----------|-------|---|
| PULPROG   | zg    | see Figure 1 for pulse sequence diagram.  |
| TD        | 8k    |   |
| NS        | 1     |   |
| DS        | 0     |   |
| PL1       |       | high power level on f1 channel (see “An Important Note on Power Levels” on page 7). |
| P1        |       | 90° <sup>1</sup> H pulse; use value determined in Section 5.2.2 on page 43.         |
| D1        | 5 sec |   |
| SW        | 20ppm | this will be reduced later.   |
| RG        |       | or use rga.   |
| O1        |       | start with value from proton/3/1; this will be optimized later.                     |

Enter **rga** to perform an automatic receiver gain adjustment.

Enter **zg** to acquire the FID.

Enter **edp** and set the processing parameters as shown in Table 17.

**Table 17. <sup>1</sup>H One-pulse Processing Parameters**

| Parameter | Value  | Comments |
|-----------|--------|----------|
| SI        | 4k     |          |
| LB        | 0.3Hz  |          |
| PSCAL     | global |          |

Add line broadening and then Fourier transform the spectrum with the command **ef**. Manually phase correct the spectrum.

Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

Now it is necessary to move **o1** to the Chloroform peak at 7.2ppm. Expand the spectrum to display the Chloroform peak only. Click on **utilities** to enter the calibration submenu. Click on **O1** with the left mouse button to select **o1** calibration. Move the cursor to the center of the signal and click the middle mouse button to assign **o1** to this frequency. Click on **return** to exit the calibration submenu and return to the main window. This **o1** value will be the <sup>1</sup>H **o1** value for the DECP90 pulse sequence below.

**<sup>13</sup>C Decouple 90° Pulse (Inverse Mode)**

It is now possible to reduce the spectral width. Enter **swh** and type in a value of 1000Hz at the prompt.

**Define phase correction**

The correct <sup>1</sup>H and <sup>13</sup>C frequencies have now been determined. The next preliminary spectrum required is a DECP90 spectrum to determine the appropriate phase correction.

Create a new data set. Enter **edc** and change EXPNO to 3. Click **SAVE** to create the data set testinv/3/1.

Next enable <sup>13</sup>C decoupling. Enter **edsp** and set NUC2 to 13C. Also set OFSH1 to the value of **o1** determined in testinv/2/1 (the preliminary <sup>1</sup>H spectrum) and OFSX1 to the value of **o1** determined in testinv/1/1 (the preliminary <sup>13</sup>C spectrum).

Enter **eda** and set the acquisition parameters as shown in Table 16.

**Table 18. DECP90 Acquisition Parameters**

| Parameter | Value    | Comments   |
|-----------|----------|--|
| PULPROG   | dec90    | see Figure 12 for pulse sequence diagram.  |
| TD        | 8k       |  |
| NS        | 1        |  |
| DS        | 0        |  |
| PL1       |          | high power level on f1 channel (see “An Important Note on Power Levels” on page 7).            |
| P2        |          | high power level on f2 channel.  |
| P1        |          | 90° <sup>1</sup> H pulse; use value determined in Section 5.2.2 on page 43.                    |
| P3        | 2μsec    | start with less than a 90° pulse.  |
| D1        | 5 sec    | relaxation delay; should be 1–5 *T <sub>1</sub> ( <sup>1</sup> H).                             |
| D2        | 2.34msec | delay for creation of anti-phase magnetization (1/(2J <sub>XH</sub> )); calculated internally. |
| CNST2     | 214Hz    | one-bond heteronuclear J-coupling (J <sub>XH</sub> ); used to calculate d2.                    |
| SWH       | 1000Hz   |  |
| RG        |          | use value from testinv/2/1.  |
| O1        |          | <sup>1</sup> H offset frequency of Chloroform peak; use o1 value from testinv/2/1.             |
| O2        |          | <sup>13</sup> C offset frequency of Chloroform peak; use o1 value from testinv/1/1.            |

## Pulse Calibration

Enter **zg** to acquire the FID.

Enter **edp** and set the processing parameters as shown in Table 19.

**Table 19. DECP90 Processing Parameters**

| Parameter | Value  | Comments |
|-----------|--------|----------|
| SI        | 4k     |          |
| LB        | 0.3Hz  |          |
| PSCAL     | global |          |

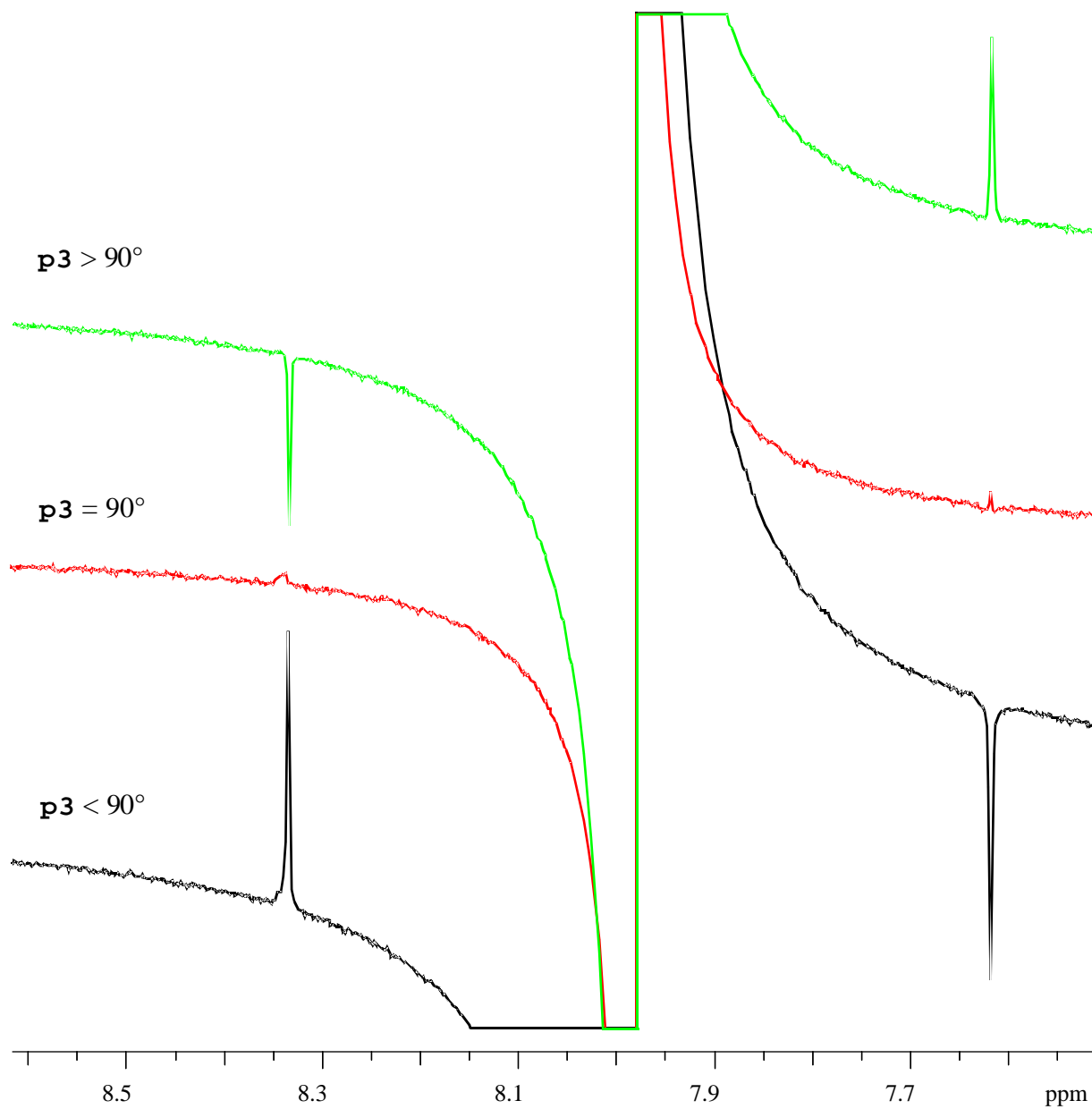
Apply line broadening and Fourier transform the spectrum (**zg**, **ef**). Expand the spectrum to display the region from about 8.5 to 7.5 ppm. This should include the Chloroform peak and its two  $^{13}\text{C}$  satellites. Correct the phase so that the left satellite is pointing up and the right satellite is pointing down.

### Calibration: High Power

### 5.5.2

Increase **p3** to be the  $^{13}\text{C}$  90° time found in Section 5.3.2 on page 48. Acquire and process another spectrum (**zg**, **efp**). If the pulse angle is less than 90°, the left satellite will remain positive and the right satellite negative. If the pulse angle is greater than 90° but less than 270°, the left satellite will be negative and the right satellite positive. At 90°, the satellites go through a null. This is shown in Figure 15.

Adjust **p3** slightly until the signals go through a null. This is the 90° pulse length for high power.

**$^{13}\text{C}$  Decouple  $90^\circ$  Pulse (Inverse Mode)**Figure 15:  $^{13}\text{C}$  Decouple  $90^\circ$  Pulse Calibration Results

## Pulse Calibration

### Calibration: Low Power for GARP CPD

5.5.3

The GARP composite pulse decoupling (cpd) sequence requires a  $90^\circ$  decoupling pulse length of 60 to  $70\ \mu\text{sec}$ . Adjust **p12** and **p3** to determine the combination that gives a  $90^\circ$  pulse length in this range, keeping in mind that the  $90^\circ$  pulse time should approximately double for each 6dB increase in **p12**.

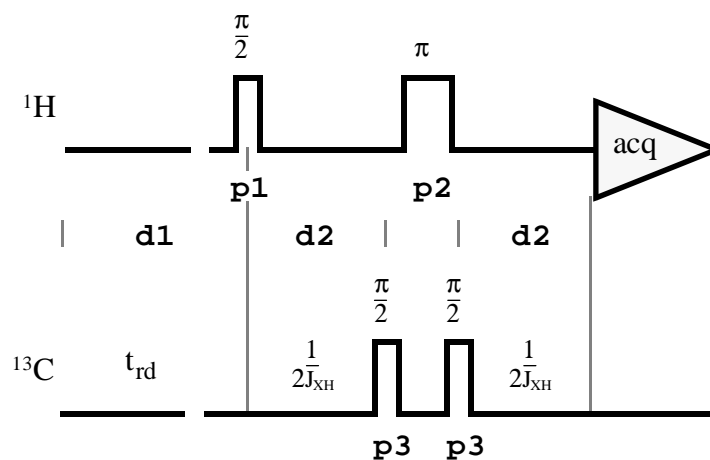
This is the power level and  $90^\circ$  pulse time for GARP cpd with this probehead. Note that the parameters used by cpd sequences are **pcpd2** for the  $90^\circ$  pulse time and **p112** for the decoupler power level, rather than **p3** and **p12** as used here.

### 1D Inverse Test Sequence

5.5.4

The 1D HMQC pulse sequence shown in Figure 16 may be used to determine how well parameters are set for inverse experiments. This is an inverse test experiment without  $^{13}\text{C}$  decoupling. The detected signal is from  $^1\text{H}$ 's bonded directly to  $^{13}\text{C}$ 's only. The signal from  $^1\text{H}$ 's bonded directly to  $^{12}\text{C}$ 's is canceled by phase cycling. Thus, the 1D HMQC spectrum of the current sample (10% Chloroform in Acetone- $d_6$ ), should consist of the  $^{13}\text{C}$  satellites only, without the large central peak.

Figure 16: 1D HMQC Pulse Sequence



Ideally, this procedure is carried out immediately following the experiments described in Sections 5.5.2 and 5.5.3, in which case the correct sample is already in the magnet, the magnet shimmed, the field locked, and the probehead tuned and matched for  $^{13}\text{C}$  and  $^1\text{H}$ .

Create a new data set starting from testinv/3/1, which was created in Section 5.5.1 on page 55. Enter **edc** and set EXPNO to 4. Click **SAVE** to create the data set testinv/4/1.

Enter **eda** and set the acquisition parameters as shown in Table 20. The value of **p1** should be that for a high power  $^1\text{H}$  observe  $90^\circ$  pulse as determined in Section 5.2.2

**<sup>13</sup>C Decouple 90° Pulse (Inverse Mode)**

on page 43, and the value of **p3** should be that for a high power <sup>13</sup>C decouple 90° pulse as determined in Section 5.5.2 on page 60.

**Table 20. 1D HMQC Acquisition Parameters**

| Parameter | Value      | Comments   |
|-----------|------------|--|
| PULPROG   | inv4ndrd1d | see Figure 16 for pulse sequence diagram.  |
| TD        | 8k         |  |
| NS        | 16         | the number of scans should be 4*n in order for the phase cycling to work properly.             |
| DS        | 16         | number of dummy scans.   |
| PL1       |            | high power level on f1 channel (see “An Important Note on Power Levels” on page 7).            |
| PL2       |            | high power level on f2 channel (see “An Important Note on Power Levels” on page 7).            |
| P1        |            | 90° <sup>1</sup> H high power pulse on f1 channel.   |
| P2        |            | 180° <sup>1</sup> H high power pulse on f1 channel; calculated internally.                     |
| P3        |            | 90° <sup>13</sup> C high power pulse on f2 channel.  |
| D1        | 20sec      | relaxation delay; should be 1–5*T <sub>1</sub> ( <sup>1</sup> H).                              |
| D2        | 2.34msec   | delay for creation of anti-phase magnetization (1/(2J <sub>XH</sub> )); calculated internally. |
| CNST2     | 214Hz      | one-bond heteronuclear J-coupling (J <sub>XH</sub> ); used to calculate d2.                    |
| D13       | 3µsec      | short delay.   |

Parameters such as **rg**, **o1**, and **swh** are already set correctly if this data set was created from testinv3/1.

Enter **zg** to acquire the FID.

Enter **edp** and set the processing parameters as shown in Table 21.

**Table 21. 1D HMQC Processing Parameters**

| Parameter | Value  | Comments                                 |
|-----------|--------|--|
| SI        | 4k     |  |
| WDW       | EM     |  |
| LB        | 0.30Hz |  |
| PKNL      | TRUE   | necessary when using the digital filter. |

## Pulse Calibration

Add line broadening and Fourier transform the spectrum with the command **ef**. Manually correct the phase and store the correction.

A 1D HMQC spectrum of Chloroform is shown in Figure 17. Notice that, due to technical limitations of the spectrometer, phase cycling is never enough to completely eliminate the parent signal (the central peak arising from  $^1\text{H}$ 's bonded directly to  $^{12}\text{C}$ ).

Figure 17: 1D HMQC Spectrum of 10% Chloroform in Acetone- $d_6$

