KJM5250 - Organic NMR Spectroscopy

Laboratory Experiments - part 1

Pulse width, T1, Decoupling, DEPT, NOE Difference Spectroscopy

(Xwinnmr on PC, version 3.5)

on

DPX 200

and

DPX 300

Department of Chemistry

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Version I

90 Degree pulse width optimization.

The aim of this test is to determine the **90 degree pulse with** (in microseconds) for the **proton** channel of the instrument/probe combination in use and to get used to the concept of an arrayed experiment. You will also get somewhat acquainted to the concept of delay time **D1** between the pulses. The sample to be used in the experiment is 0.1% ethylbenzene in $CDCl_3$.

In principle this manual can be used to determine the 90 degree pulse width of the carbon channel with a little intelligent substitution of the appropriate parameters (Different pulse program, delay time, different setup, different number of transients, and different approximate value for the pulse angle).

More information about this experiment can be found in The Bruker Avance Manual on page 41 and onwards. Be aware that the Avance manual is written for an earlier version of the software - and you can get nasty surprises trying to follow all that is written there.

A prerequisite for using this manual is that you know how to: **Login**, enter **password** and start the **xwinnmr** program**,** remove the old sample, insert your sample, lock the field on the actual solvent and shim the magnetic field with $\mathbf{Z^1}$ (**Z** on DPX 300), $\mathbf{Z^2}$, $\mathbf{Z^3}$ and $\mathbf{Z^4}$, if needed, stop and start the spinning of the sample and start and stop the automatic shimming and giving the experiment a file name with **edc**.

After doing all this continue with:

1. 1. Type **edc↵** Type in the name of the experiment. Either the name of your synthesis product (FR7653) or for instance GROUP1PW. **Expno** is **1** and **procno** is **1**. Select disk **D:** or **M:.**

2. Type **rpar PROTON↵** Click on **Copy all**.

3. Type **getprosol↵**

4. Type **ii↵** Initialize interface.

5. Type **rga↵** Receiver gain adjustment.

6. Type **zg↵.**

   When the acquisition is finished type **ef↵** and phase manually; click on **phase**, then **biggest**, then **PH0** and use the mouse - left button - to phase around the biggest peak. Click on **PH1** and use the mouse to phase the rest of the spectrum. Click on **return** and **save & return.**

7. Now you are going to set the radio sender directly on the quartet of the ethylbenzene signal – the CH_2 - and also reduce the sweep width (width of the spectrum). Expand the spectrum either by using the arrow functions ><, <>, ->, <- or by clicking the mouse left button followed by the middle mouse button on both sides of the quartet.

8. Click on **utilities**.

9. Click on **O1** in the **O1 O2 O3** area and move the cursor to the middle of the quartet and click the middle mouse button to assign O1 to this position. **O1** is the position of the radio sender. Click on **return.**

10. Type **swh**↵ and change the value to 1000 Hz (just type **1000**↵) in the window that shows up.

11. Type **zg**↵. This starts the experiment.

12. Type **ef**↵ when the acquisition is finished. Phase manually so that the spectrum is positive; click on **phase**, then **biggest**, then **PH0** and use the mouse - left button - to phase around the biggest peak. Click on **PH1** and use the mouse to phase the rest of the spectrum. Click on **return** and **save & return.**

13. Type **eda**↵ Edit acquisition parameters window comes up. Enter the values in **table 6** on page 42 in the Avance manual. Do ***not*** change the values for **O1** and **SW** (you have already defined them in step 8 and 9). Click on **PROSOL** in the **eda** window and select **TRUE** to ensure correct pulses for the current probe. PL1 is automatically set to the correct value. **Save**.

14. Type **edp**↵ Edit processing parameters window comes up. Type in the values in **table 7** in the Avance manual on page 42. **Save**.

15. Type **zg**↵ Zero and go.

16. Type **ef**↵ and phase manually; click on **phase**, then **biggest**, then **PH0** and use the mouse - left button - to phase around the biggest peak. Click on **PH1** and use the mouse to phase the rest of the spectrum. Click on **return** and **save & return.**

17. Expand the region around the quartet so the signal fills about 1/4 of the screen. Click on **DP1** and confirm the three questions coming up by hitting return.

18. We are now ready with all the preparatory work and you can begin with the real experiment.

19. You will now start the automation program that will do the measurements.

20. Type **xau paropt↵**. Paropt means parameter optimization.

Answer the questions:

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

21. The acquisition starts automatically.

22. With these parameters the spectrometer will take 16 spectra with the pulse length from 2 μs to 32 μs. All spectra appear side-by-side in the dataset GROUP1PW 1 999 and the final result should be a sinusoidal curve showing the quartet with varied intensities over the 16 spectra. The curve should start with positive signals (alternatively. click on⁄<>⁄ if you don't see any spectra on the screen). At the end of the experiment the message "paropt finished" and a value for p1 are displayed. This value is an approximate **p1** 90 degree pulse length for the power level **pl1**.

23. The result can be plotted (se the general manual). Type **szero↵** and change the value to 7.5 cm before plotting or else the negative peaks will be cut by the ppm scale. Szero is the distance between the ppm scale and the baseline of the spectrum. Negative peaks will not be seen if the spectrum is too close to the ppm scale. Click on **utilities**, then on **CY**. Place the CY cursor just above the highest positive signal, click the left mouse button and set the requested value to 7.5. Expand the spectrum so that all signals are seen, click on **DP1** and confirm the three questions coming up by hitting return.

24. Type **re 1 1↵** (read experiment 1 process number 1) and you will get back to the first experiment. The spectrum is all negative here. Don't care and <u>don't phase</u>. The phase correction is taken from the last spectrum acquired in the array.

25. Type **edc↵** Change **expno** to **2**.

26. Type in **p1↵**. Change the value to approximately 360 degree based upon the results above.

27. Type **zg↵** You are now rerunning the experiment to find a more exact 360 degree estimate, and type **efp↵** when the acquisition is completed. Experiments that gives negative peaks

have pulse width of less than 360 degrees and those above 360 degrees results in positive peaks.

28. Plot if you want a printout of the results. Use the **view↵** command to take a quick look before actually plotting.

29. Continue manually with small changes of the **p1** pulse until you have found the correct 360 degree pulse (which of course does not give any signal at all). If you do not change **expno** with the help of **edc** between each run you will overwrite the previous run.

30. The 90^{o} pulse is of course 360^{o} pulse divided by 4. Why do we measure the 360^{o} pulse and not the 90 degree pulse?

T_1 relaxation time constant measurement.

In this experiment we will determine the T_1 relaxation constant (longitudinal relaxation) of the hydrogen atoms in a sample of a small molecule. In addition to learn how to obtain the T_1 value we will get training to use several parts of the complicated Bruker/Spectrospin software, and finally get more insight into the classical inversion recovery experiment. The inversion recovery experiment is a two pulse experiment. It is a very instructive example of what is going on when a spin system is perturbed (with one pulse P2) from equilibrium, allowed to evolve during a variable delay (VD) and finally detected with the observe pulse (P1) and acquired (ACQ). The central point is to understand what is going on during the evolution - during the variable delay VD. In the Bruker Avance users Guide on page 71 and onwards more detailed information can be found. This experiment can of course be performed on the carbon 13 atoms as well with the appropriate choice of parameters.

The nuclei will receive the following sequence of pulses and delays:

D1 Fixed delay to relax to equilibrium.

P2 Invert the spin population with an 180^o (Π) pulse. (If you don't know what inverting a spin population is - please read in the textbook - this is a good examination question).

D2 Variable delay time to moving (partly) towards equilibrium.

P1 Monitoring 90 degree ($\Pi/2$)pulse to measure peak height as a function of D2.

ACQ After the 90 degree pulse we will of course have the acquisition.

With a long delay the atoms are allowed to relax back to equilibrium before the 90 degree observation pulse. In this case we get maximum positive signals. With very short delays we get a situation very close to a 270 degree pulse from the addition of the 180 degree and the 90 degree pulses which are coming right after one another. In this case a maximum negative signal is observed. When there is an intermediate value of the delay the magnetic vectors along z-direction are just passing zero and the 90 degree pulse have no vector to work on, and zero signal is observed. This zero signal can be used to get a rough estimate of the T1 for that atom. From the relationship (see Friebolin page 166) - $Mz=M_0[1 - 2e\text{-}t/T_1]$ and $T_1=t_{zero}/\ln 2$ where t_{zero} = D2 when there is not detected any signal.

A prerequisite for using this manual to obtain T_1 values is that you know how to: **Login**, enter **password** and start the **xwinnmr** program**,** remove the old sample, insert your sample, lock

the field on the actual solvent and shim the magnetic field with **Z1** (**Z** on DPX 300), **Z2**, **Z3** and **Z4**, if needed, stop and start the spinning of the sample and start and stop the automatic shimming and giving the experiment a file name with **edc**.

After doing all this continue with:

1. Type **edc**↵ Type in the name of the experiment. Either the name of your synthesis product (FR7653) or for instance GROUP1T1. Select disk unit (DU) **C:** for local storage and **M:** for your nmr home directory.

2. Type **rpar PROTON**↵ Click on **Copy all**.

3. Type **getprosol**↵

4. Type **ii**↵ Initialize interface.

5. Type **rga**↵ Receiver gain adjustment.

6. Type **zg**↵ Zero and go.

7. Type **efp**↵ and **apk**↵ Exponential multiplication, Fourier transforming and automatic phasing.

8. Reduce the spectral width to the area which contains peaks by first reducing it on the screen with and then press **utilities** button and the press **sw-sfo1** button. Click on **return.**

9. Type **edc**↵ Change **expno** to **2** and keep the name the same.

10. Type **eda**↵ Edit acquisition parameters.

11. Find the parameter **parmod** on the new window which comes up. Change **parmod** to **2D**. click on **save**.

12. Answer **OK** on the questions concerning "deleting meta ext files".

13. Type **edlist**↵ in the main writing area. Select **vd** in the new window. Variable delay.

14. Type new name. Use for instance **GROUP1delay**.

15. A new white window comes up. Select the variable delays you want, for instance 15s, 10s, 5s, 2s, 1s, 0.5 s, 0.1s, 0.01s. Use a new line and press ↵ after each entry. Remember to write s for seconds behind the number. It should be no space between the number and the letter s. Start your list with the longest delay time. Be aware that this is 8 entries - remember how many you use in your experiment.

16. Select **save** and **exit** in the upper line of the window.

17. Type **eda**↵ Edit acquisition parameters.

18. Find the parameter **PULPROG** on the new window that comes up. Change **PULPROG** by clicking on the right hand side of PULPROG part of the window, a new list comes up, select **t1ir** (T1 inversion recovery pulse sequence).

19. Enter F1 and F2 parameters in the **eda** window according to table 24 in the Avance guide (page 73-74) except for **D1** that shall be set to 20 seconds. L4 under F2 and TD under F1 is the number of delays you have chosen - see 14. Use edlist if you don't remember. Under **VDLIST** enter the same name as you have chosen for the edlist in 13.

20. Click on **PROSOL** in the **eda** window and select **TRUE** to ensure correct pulses for the current probe.

21. Click on **save**.

22. Type **zg**↵ Starts the experiment. Ignore error messages concerning sizes. Click on **Seen**. Ignore the message that the residual experiment time is larger than you want.

23. Type **edp**↵ You are entering the **edit process parameter** mode/window.

24. Enter F1 and F2 parameters in the **edp** window according to table 25 in the Avance guide (page 75) except **BC_mod** in F2 dimension which shall be set to **no** and **ph_mod** to **pk**. **MC** in the list in the Avance manual is not relevant - don't do anything with it.

25. When the acquisition is over a message saying type xfb to process the data might appear - ignore this message. Type instead **rser 1**↵. Read serial file one - which is the experiment with the longest delay. (All peaks shall be positive here - why?)

26. Type **ef**↵ Exponential multiplication and Fourier transformation.

27. Do manual phase correction. Click on **phase**, then **biggest**, then **PH0** and use the mouse - left button - to phase. Click on **PH1** and use the mouse to phase the rest of the spectrum. **Save as 2D and return.**

28. You must define the areas containing the signals you want the T1 constants calculated for. Click on **integrate↵**.

29. Click with left button, followed by the middle button on left and right hand side of each region containing a peak. You MUST have one region for each peak you want the T1 for. Expand if needed with the arrows.

30. Click on **return**. Save as **intnrg & return**. Type **wmisc↵** A menu of different lists shows up. Select **intnrg** to select the integral range file type. Type in the new name at the bottom; for instance **Group1reg**. Remember this.

31. Expand the spectrum as much as possible but all peaks must be on the screen.

32. Click on **process**, click on **special processing,** click on **baseline correction operations** and finally on **interact. basel. definition for bcm/sab**.

33. Click on **def-pts↵** You are entering a subroutine for defining the peaks you want the instrument to use in the T1 calculations. Sometimes a CPR window comes up: baslpnts exits, append (a), overwrite (o) or quit. Select **o**.

34. Move the cursor on top of the leftmost peak, respectively in the midpoint of a multiplet and click the middle mouse button. The color of the cursor is transformed into another one. The cursur is fixed. You have now defined the first peak and you must do the same for the rest of them. Click with left button after having defined the last peak. The message: "baslpnts file written with **x** entries" (number of peaks you want to have calculated) should appear at the bottom of the screen. If not, click **def-pts**, select **o** and repeat the procedure described above. Click on **return**. **Save and return**. The message "bcm: finished" is shown at the bottom of the screen.

35. When all the peaks are selected type **wmisc↵** A menu of different lists shows up. Select **baslpnts** and type in the new name for the file and remember the name. Use for instance **Group1bas**.

36. If the question "Override Group1bas ?" comes up click on **OK**.

37. Click on **2D**. ***If you forget this everything goes wrong!!!*** Start from the beginning of the manual if this is forgotten.

38. Type **edt1**↵ Setup T1 parameter editing file comes up - changing **FITTYPE** to either **intensity** or **area** depending if you want intensity or area in the calculations. **Area** is reccomended **Save**.

39. Type **proc_t1**↵ The proc_t1 program starts and you must answer a series of questions. The answers are: **1**, **10000**, **-10000**, **20**, "**GROUP1bas"**, "**GROUP1reg"**, "**GROUP1delay"**, **1**. The three names for the three lists can of course be different. Think. Wait until message " T1 result is stored in t1r" appears. Click on **seen**. The display now shows you a crude version of the spectra one after another. Click on the +/- button until both positive and negative peaks/colors are seen.

40. Type **edo**↵ Edit output. Select curprin to **$HP LaserJet 4L**.

41. Type **dat1**↵ To plot all the T1 information on the **$HP LaserJet 4L**.

42. Click on **DefPlot**. Confirm the 3 questions without change by typing ↵. Type **stackT1**↵ Confirm all questions and error messages (there might be a lot, don't bother) by typing ↵, except for the question dealing with number of rows. Number of rows equals the number of delays in the VDLIST and this is of course 8 for the experiment described here. StackT1 plots all the spectra diagonally behind each other on one paper.

Try to think a little about the result. Why do the different protons have different relaxation times? Is there a correlation between structure and the T_1 values?

Homonuclear ^{1}H-decoupling.

Proton decoupling is a very fast and simple way to figure out the intricacies of a complicated proton spin-spin coupling system. If two protons are J-coupled, the result of irradiating one of them with high power during acquisition will simplify the pattern of the other one. The chemical shifts (δs) of the nuclei under investigation must however not be to close.

A prerequisite for using this manual is that you know how to: **Login**, enter **password** and start the **xwinnmr,** remove the old sample, insert your sample, lock on the actual solvent and shim the magnetic field with **Z^{1}** (**Z** on DPX 300), **Z^{2}**, **Z^{3}** and **Z^{4}**, if needed, stop and start the spinning of the sample and start and stop the automatic shimming.

After doing all this continue with:

1. Type **edc↵** Type in the name of the experiment. Either the name of your synthesis product (FR7653) or for instance GROUP1dec. Make sure **expno** is **1** and **procno** is also **1**. Select disk unit (DU) as **M:** for storage in your nmr home directory and **D:** for local storage. Click on **save**.

2. Type **rpar PROTON↵** Click on **Copy all**.

3. Type **getprosol↵**

4. Type **ii↵** Initialize interface.

5. Type **rga↵** Receiver gain adjustment.

6. Type **zg↵** Zero and go. You are doing this to get a spectrum without decoupling which is good to have as a reference.

7. Type **efp↵** and **apk↵** Exponential multiplication, Fourier transforming and automatic phasing. Plot if you want a hard copy.

8. Reduce the spectral width to the area which contains peaks by first reducing it on the screen and then press **utilities** button and the press **sw-sfo1** button. Click on **return.**

9. Type **edc↵** Change **expno** to **2** and **procno** to **1**, keep the name the same.

10. Type **rga↵** Receiver gain adjustment. Type **zg↵** Zero and go. You are doing this to get a spectrum with reduced spectral width without decoupling which is good to have as a

reference. Type **efp**↵ and **apk**↵ Exponential multiplication, Fourier transforming and automatic phasing. Plot if you want a hard copy.

11. Type **edasp**↵ The window for viewing and setting the radio senders comes up. Set both **NUC1** and **NUC2** to 1H. The point here is to select proton on both channels since you are only working on one nuclei. Click on **save**.

12. Type **eda**↵ and change/check the following parameters.

DIGMOD	**homodec-digital**
PULPROG	**zghd**
NS	**8**
DS	**4**
d12	**1.05 us**
PL24	**between 25 and 55 dB** Start with 55 on DPX 300 and 35 on DPX 200. This value later has to be changed up and down successively until the correct value is found, that is complete removal of the peak with as little power as possible. The higher db number the lower power. Change it in steps of 5 in the beginning and later in steps of 1-2 db. Click on **done**, then on **save**.

13. Click on **File** followed by **Copy** and **entire dataset to same disk**. Copy expno 2 to 3. You are copying the spectrum and FID in expno 2 to expno 3. Fill in the targetfile. Remember space between the entries. (ex: NMR300HOMO 3 1 C: brorjw).

14. Type **re 3 1**. You are now in experiment number 3 with process number 1.

15. Expand the area around the signal you want to decouple. Click on **utilities**. Click on **O2** button and move the cursor to the position you want to decouple. Press then the middle mouse button. Click on **return**.

16. Type **ii**↵ Initialize interface. You can never do this too often.

17. Type **zg**↵Zero and go.

18. Type **efp**↵ and **apk**↵ Exponential multiplication, Fourier transforming and automatic phasing is performed. If apk does not work use manual phasing (Phase, biggest, PH0, PH1, return). Look at the result and decide if any other peak is simplified. Determine if the power level for decoupling is correct. The irradiated peak is to be gone, or a spike is to be seen, but the nearby peaks are not to be affected. Change **PL24** up or down and repeat the process until a correct irradiation power is found. Remember that a higher dB number means lower power. Plot the spectrum when you have found the correct power level.

19. If you want to see the difference spectrum between the non irradiated spectrum and the irradiated you can do as follows: click on **Dual display** under **Display**. And select the correct expno **2** and procno **1** for spectrum number two. (The second dataset is the one without irradiation.). Click on **save**. By clicking on the arrows under spectrum 1 or 2 on the left side you can separate the spectra on the screen. This operation makes it much easier to compare the decoupled and the original spectra.

20. Click on **diff**. **Return**. The difference spectrum comes up. Plot if you want the difference spectrum.

21. Repeat irradiation on all other peaks of interest with the correct power level. The best way to do this is to go back to **expno 2 procno 1** and **copy** this (see 11) to **expno 4 procno 1**. Then you read expno 4 procno 1, **re 4 1↵** . Click on the utilities button, then on the **O2** button and move the cursor to the position you want to decouple. Click with the middle mouse button. Acquire the spectrum and process it (**zg** and **efp**). Plot if you want.

22. Repeat this for the rest of the interesting resonances and increase expno by 1 for each experiment.

23. The point is to figure out which protons are coupling with each other!!

DEPT
Distortionless enhancement by polarization transfer

The DEPT pulse sequences (distortionless enhancement by polarization transfer) followed by some data handling separate the **carbon resonances** in groups based on the numbers of attached protons, e.g. CH, CH_2 and CH_3. Resonances missing from this analysis belong to quaternary carbons. All the DEPT spectra is enhanced in intensity compared to a regular ^{13}C spectrum. If you are not interested in a spectrum containing the quaternary carbons you are generally better off with a DEPT spectrum. From this experiment - please determine if you should select DEPT45, DEPT90 or DEPT135 for this purpose.

A prerequisite for using this manual is that you know how to: **Login**, enter **password**, and start the **xwinnmr,** remove the old sample, insert your sample and shim the magnetic field with **Z**$^{\mathbf{1}}$ (**Z** on DPX 300), **Z**$^{\mathbf{2}}$, **Z**$^{\mathbf{3}}$ and **Z**$^{\mathbf{4}}$, if needed, stop and start the spinning of the sample and start and stop the automatic shimming.

After doing all this continue with:

1. Type **edc**↵ Type in the name of the experiment. Either the name of your synthesis product (FR7653) or for instance GROUP1DEPT. Make sure **expno** is 1 and **procno** is also 1. Select disk unit (DU) **M:** for storage of the data in your home directory and **D:** for local storage. Click on **save**.

2. Type **rpar**↵ Select a carbon file with proton decoupling (**C13CPD**) for the probe that is in the magnet. Click on **Copy all**.

3. Type **getprosol**↵

4. Type **eda**↵ Click on **PROSOL** and select **TRUE** to ensure correct pulses for the current probe. Set **D1** to 2s. **Save**.

5. Type **ii**↵ Initialize interface.

6. Type **rga**↵ Receiver gain adjustment.

7. Type **zg**↵ Zero and go. You are doing this to get a spectrum without the DEPT enhancement and editing. This is good to have as a reference. It is often not necessary to run through all the 1024 number of scans, especially if you have a highly concentrated sample. Type **tr**↵, **efp**↵ and **apk**↵ while running the experiment. When you are satisfied with the S/N ratio and all the expected signals are seen, stop the acquisition by typing

stop↵. Write down the NS-value and set it to the nearest value that is dividable by the number 4.

8. Type **efp**↵ and **apk**↵ Exponential multiplication, Fourier transforming and automatic phasing (you have in principle done this in the former point). Plot if you want a hard copy. Reduce the area on the screen to the area which contains the peaks. Click on the **Sw-sfo1** button, the following experiments which will be built upon this experiment will then have this reduced spectral width.

9. Type **edc**↵ Make sure **expno** is 2 and **procno** is also 1. Click on **save**.
   Reduce the sweep width on screen. Redo **ii**↵ Set **ns** = the number found in 7, then type ↵. **rga**↵ **zg**↵ **efp**↵ and **apk**↵.

DEPT90.

10. Type **edc**↵ Change **expno** to **3** and **procno** to **1**. Click on **save**.

11. Type **eda**↵ Change the pulse program to **dept90**. Set **P0** to the same value as **P1**. Use the same number of pulses as above. **Save**.

12. Type **ii**↵ Initialize interface.

13. Type **zg**↵ Zero and go.

14. Type **efp**↵ and **apk**↵ Exponential multiplication, Fourier transforming and automatic phasing. Move the spectrum up on the screen with the arrows on the left side. Type **szero**↵ and set it to 5.5 cm. In this way the spectrum will appear halfway up on the spectrum. Plot the spectrum. Which carbons are you seeing with **dept90**? Are the signal to noise equivalent to the regular ^{13}C spectrum?

DEPT45.

15. Type **edc**↵ Change **expno** to **4** and **procno** is **1**. Click on **save**.

16. Type **eda**↵ Change the pulse program to **dept45**. Set **P0** to half the value of **P1**. Use the same number of pulses as above. Save.

17. Type **ii**↵ Initialize interface.

18. Type **zg**↵

19. Type **efp**↵ and **apk**↵ Zero and go. Exponential multiplication, Fourier transforming and automatic phasing. Move the spectrum up on the screen with the arrows on the left side. Type **szero**↵ and set it to 5.5 cm. In this way the spectrum will appear halfway up on the spectrum. Plot the spectrum. Which carbons are you seeing with **dept45**? Are the signal to noise equivalent to the regular ^{13}C spectrum?

DEPT135.

20. Type **edc**↵ Change **expno** to **5** and **procno** is 1. Click on **save**.

21. Type **eda**↵ Change the pulse program to **dept135**. Set **P0** to 1.5 times the value of **P1**. Use the same number of pulses as above. Save.

22. Type **ii**↵ Initialize interface.

23. Type **zg**↵ Zero and go.

24. Type **efp**↵ and **apk**↵ Exponential multiplication, Fourier transforming and automatic phasing. Move the spectrum up on the screen with the arrows on the left side. Type **szero**↵ and set it to 5.5 cm. In this way the spectrum will appear halfway up on the spectrum. Plot the spectrum. Which carbons are you seeing with **dept135**? Are the signal to noise equivalent to the regular ^{13}C spectrum?

The various DEPT programs can be used to more or less automatically sort out C, CH, CH_2 and CH_3 groups in molecules. On the Varian Gemini 200 instrument this is best done with the DEPT and ADEPTS programs. On the Bruker DPX 200 and DPX 300 instruments there is currently no such program under the manual setup.

Nuclear Overhauser Difference Spectroscopy.

The one dimensional NOEDIF (nuclear Overhauser difference) experiment enable us to get insight into the spatial relationship between protons in a molecule. NOE´s are transferred through space and not via the bonds. Nuclear Overhauser effects can be both positive and negative. For very large molecules all effects are negative. Most molecules under investigation by organic chemists are small in this context. Direct effects transferred from one proton to another are positive ($A \Rightarrow B^+$) and effects from one proton via an intermediate proton to a third is negative ($A \Rightarrow B^+ \Rightarrow C^-$). Longer effects are alternating positive and negative and get successively smaller. Most effects described in organic literature are of the first kind, e.g. positive (since the parameter D1 described below has been set to a small value (4s), the long range NOE´s is built up very slowly and need long D1s). [We will only look for and see positive effects in this experiment.] NOE effects are inverse proportional to the distance between the nuclei ($1/r^{-6}$). The distance between the protons must be less than 3 Å. Ideally the parameter D1 should be set to 3 T1. Oxygen in the sample is detrimental if one is interested in measuring the maximum NOE effect. Oxygen behaves/is a diradical and is a very effective relaxation agent which "destroys" the buildup of the NOE effect. Samples are easily deoxygenated by blowing helium gas through the NMR sample for a minute or two.

If you are interested in the NOEs between a methyl group and a single proton the best way is to irradiate the methyl protons and measure the intensity for the single proton. The protons within a methyl group are effectively relaxed by themselves resulting in a very little intensity enhancement when irradiating a nearby single proton.

More information can be found in textbooks and in chapter 9 starting on page 101 in the Bruker Avance user's guide.

The experiment is built around **calibrating power level** on the radio sender and defining the exact frequencies to irradiate by creating a frequency list **fq2list** for each singlet or multiplet to irradiate. Then running the actual experiment with the help of the program **noemult.dmo** and then get the difference spectra plotted with the numerical NOE values on the paper.

A prerequisite for using this manual is that you know how to: **Login**, enter **password**, and start the **xwinnmr,** remove the old sample, insert your sample and shim the magnetic field with **Z1** (**Z** on DPX 300), **Z2**, **Z3** and **Z4**, if needed, stop and start the spinning of the sample and start and stop the automatic shimming. The NOE difference experiment is run without spinning.

After doing all this continue with:

1. Type **edc**↵ Type in the name of the experiment. Either the name of your synthesis product (FR7653) or for instance GROUP1NOE. Make sure **expno** is 1 and **procno** is also

Select disk unit (DU) as **M:** for storage in your nmr home directory and **D:** for local storage. Click on **save**.

2. Type **rpar PROTON↵** Click on **Copy all**.

3. Type **getprosol↵**

4. Type **edasp↵** The window for viewing and setting the radio senders comes up. Set both **NUC1** and **NUC2** to 1H. Click on save.

5. Type **ii↵** Initialize interface.

6. Type **rga↵** Receiver gain adjustment.

7. Type **zg↵** Zero and go.

8. Type **efp↵** and **apk↵** when efp is done. Exponential multiplication, Fourier transforming and automatic phasing is performed.

9. Reduce the spectral width to the area which contains peaks but leave a section of at least 3 ppm blank on one side. This empty area is to irradiated in the reference spectrum. Reduce the area on the screen and then press the **utilities** button and the press **sw-sfo1** button. Click on **return.**

10. Type **edc↵** Make sure **expno** is 2 and **procno** is 1. Click on **save**.

11. Type **rga↵** Receiver gain adjustment.

12. Type **zg↵** Zero and go.

13. Type **efp↵** and **apk↵** when efp is done. Exponential multiplication, Fourier transforming and automatic phasing is performed.

14. Expand the spectrum so that each multiplet covers 1/3 of the screen. Be aware that you cannot expand the spectrum with the mouse after entering **frqlist routine**. Move the spectrum on the screen so that the empty area where the reference irradiation is to be performed is visible.

15. The next step is to create a frequency list for each peak to be irradiated. Click on **utilities** and then on **frqlist.** Answer the questions: **f1**, **noedif.1**, **n**. If the f1 list already exists you

will answer if you want to append (a) , overwrite (o) or quit (q). Choose **o**. The f1 list has nothing to do with F1 and F2 radio senders.

16. Place the cursor with the mouse on the reference point of the spectrum and click with middle button and then with the left button to get out of the sub menu. Look for a red/green arrow/pointer. YOU MUST NOT CLICK WITH ANY MOUSE BUTTONS BEFORE THE POINTER IS AT THE CORRECT POSITION, IF YOU DO A MISTAKE HERE YOU MUST GO BACK TO 13. You have now made the first fq2list.

17. Use the arrows to get the first peak (leftmost) on the screen. Click on **frqlist.** Answer the questions: **f1**, **noedif.2**, **n**. If the f1 list is already existing, you must answer if you want to append (a) , overwrite (o) or quit (q). Choose **o.**

18. If the peak is a multiplet you must click with the middle mouse button on a selection of the local peaks - 5 is OK. Then click with left mouse button. You have now made the second fq2list.

19. Repeat 15 and 16 for all interesting peaks. Remember to answer **noedif.3**, **noedif.4** and so on! A maximum of 8 entries, including the one with irradiation on an empty space, must be observed. If you have more than 8 peaks to irradiate you should maybe run a NOESY experiment instead. If you do not want to do that you must run two separate NOE difference experiments.

20. When you have created all of the lists, you must optimize the power level for the radio sender. The main point is to have as low power as possible but still enough to cancel the signal almost completely. If the power level is too high complications may arise since the sender might hit a nearby proton. Type **edc**↵ and change **expno** to **3**. **Save**. Why is it a problem if the irradiation hits the nearby proton? If you do hit the nearby protons they become negative in the noedif spectrum and no useful information is obtained.

21. Type **eda**↵ and change/check the following parameters.

PULPROG	**noemul**
TD	**16 K**
NS	**8**
DS	**4**
PL14	**70dB** This has to be changed up and down later until the correct value is found.
D1	**30 ms**
D11	**30 ms**
D12	**20 μs**
D13	**3 μs**

D20 **50 ms**
L4 **50**
FQ2LIST **noedif.2** or noedif.3 Do not select noedif.1 since there is no signal for the noedif.1 region. Click on **Save**.

22. Type **zg**↵

23. Type **efp**↵ and **apk**↵ when the acquisition is over. Exponential multiplication, Fourier transforming and automatic phasing is performed.

24. If something goes wrong at this stage type **eda**↵ and all acquisition parameters are shown. Look for wrong parameters and correct them - go back to 16 if needed. If not go to 19.

25. Play around with the power level **PL14,** for instance **65, 70** and **75 dB** to get the best power level. You can compare this spectrum with the one in Expno 1 by clicking on **display** and **dual display.** Tell the instrument where the second spectrum is. When completed click **return**.

26. This point is optional. Sometimes it is good to turn off spinning and reshim Z^1 and Z^2 a little if the lock level falls.

27. Type **noemult.dmo**↵ on the 300 MHz or **noemult**↵ on the 200 MHz, and answer the questions:

base name of frequency lists: **noedif**
# of frequency lists: **enter the correct number inclusive reference list (irradiation of empty space).**
# of cycles through each list **50**
# of average cycles **10**
For stacked plot: scaling factor Cy: number comes up, press **enter.**
The experiment starts automatically.

28. While the experiment is running you must complete the setup of the processing parameters, type **edp**↵ Set **SI** to **8K**, **WDW** to **EM**, **LB** to **0.3 Hz**, **PKNL** to **TRUE**

29. When the experiment is completed multi Fourier transforming is done automatically. **A whole series of warnings / messages may come up on the screen due to an error in the program. (You should click OK on all of them. The program might stop if you are not doing this.** You might be doing this up to 40 times. A stacked plot of the result comes out

on the plotter/printer. No integrals are plotted though and you must manually go into each of the spectra and integrate them and plot them separately.

30. Your spectra are stored in two places now. The original proton spectrum is in **1 1**, the reduced spectrum is in **2 1** and the blank irradiated one is in **3 1**. The regularly irradiated spectra are in (expno procno) **4 1**, **5 1**, **6 1**, **7 1**, **8 1**, **9 1** and **10 1**. The difference spectra are in **11 1**, **12 1**, **13 1** and so forth. Use the **re XX X↵** command to get the spectrum you want.

31. Type **re 3 1↵** followed by **ef↵** (Which spectrum is this). Increase the spectrum to a sensible vertical scale and have a look on the phasing. Phase the spectrum manually if it is not good and **save and return**. If phasing is OK and you are not phasing manually, move to point 33 in this manual.

32. Type **multiefp↵** Multiefp will process the rest of the spectra correctly if you answer the following questions:
    Enter first expno to process: 4
    Enter number to process: enter the correct number (one less than #of frequency lists).

33. You must now subtract the control spectrum (the one with irradiation on a blank area - the first one in the **f1list** - that is **noedif.1**) from each of the real irradiation spectra. You can find the blank irradiation (noedif.1) spectrum in **EXPNO 3 Procno 1.** The first real irradiation spectrum (noedif.2) is found in **EXPNO 4 Procno 1.**

34. Type **re 4 1↵** The first irradiated spectrum comes up. Type **edc2↵** and define the **second data set** -the one with irradiation on an empty area. Set **EXPNO2 = 3** and **PROCNO2 = 1**. Do nothing with the third dataset!! Click on **Save**.

35. Click on **Display** and **dual display**.

36. Click on **diff**. And click on **Return**.

37. Click on **Save and return**. A window comes up and tells you where the result is stored. **expno 4 procno 2** is shown.

38. 36. Type **re 5 1↵** to get the second irradiated spectrum on screen. Type **edc 2↵** and define the second data set for this subtraction - the one with irradiation on an empty area. Set **EXPNO2 = 3** and **PROCNO2 = 1**. Do nothing with the third dataset!! Click on **Save**.

39. Click on **Display** and **dual display**. Click on **diff**. And click on **Return**. Click on **Save and return**. A window comes up and tells you where the result is stored. Write down the number **expno 5 procno 2** which is shown.

40. Type **re 6 1↵** and continue as described above for the rest of the irradiations.

41. **Integrate and plot each difference spectrum separately**. To do this you must remember how to integrate from the general manual and you must set the integral of the big negative peak to - 100 to ensure that the correct % number comes up for the NOE enhancements. Be aware that the distance between the ppm scale and the spectrum is defined by **szero↵** This is usually 1 cm and should be changed to a higher value (4-5 cm) just before plotting. Use the command **view↵** to see what is actually going to be plotted. Plot using the **plot↵** command.