Advanced NMR Experiments: Routine Procedures

This document assumes you are familiar with the basic operation of the NMR. If you are unsure or have not yet been trained, see Kyle in 907

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A note on notation: Single quotation marks like 'this' refer to commands you type into Topspin or for which there is a button you can press. Double quotation marks like "this" refer to text or information you may see or type inside the window. Brackets like [this] tell you to type the number corresponding to that term.

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Basic Techniques

1D NMR Experiments

2D NMR Experiments

Finding the 90° Pulse

Description: To get the best spectra, you should always find the 90° pulse for your compound.

Relevant Experiments: All

Instructions:
1. Create a new data set ('new')
2. Load the ^{1}H parameters ('rpar...')
3. Type 'ased'
4. Change the pulse program name from "zg30" to "zg"
5. Type 'rga' (If you are running a NOESY, take note of this value)
6. Set ns = 1 and ds = 0
7. Type 'zg'
8. When done, type 'efp' then 'apk'
9. Type 'ased'
10. Multiply the current value for "p1" by 4 (in order to determine the 360° pulse, from which we can deduce the 90° pulse). You may need to expand the "channel f1" header to find it. (Note: while you are here, take note of the value for pl1. You won't need to change it, but you will need to know what it is)
11. Type 'zg'
12. When done, type 'efp'. Do NOT do apk!
13. Modify p1, then type 'zg' and 'efp', until the spectrum gives a null signal (either zero or equally positive and negative)
14. Divide this value of p1 by 4 to obtain the 90° pulse. Also note the value for pl1.

Quick T_1

Description: Knowing the relaxation time of your signals is an important parameter in setting up a proper NOESY

Relevant Experiments: NOESY, 1D NOE

Instructions:
1. Create a new data set ('new')
2. Load the ^{1}H parameters ('rpar...')
3. Type 'rga'
4. Type 'ased'
5. Change the pulse program to "quickt1"
6. Enter your 90^o pulse for p1
7. Use the same receiver gain (rg) that you did when determining the 90^o pulse.
8. Set ns = 1
9. Set d7 = 0.001s (1.0ms)
10. Set d1 = 15s
11. Type 'zg'
12. When done, type 'efp'
13. Type '.ph' and phase the spectrum manually upside down (with the "0" and "1" buttons)
14. Type 'ased'
15. Set d7 = 1s
16. 'zg' and 'efp'
17. Modify d7, then type 'zg' and 'efp', until the peaks of interest are nulled (equally positive and negative; different peaks will have different T1 relaxation times)
18. When you are satisfied, calculate T_1 using the equation: $\mathbf{T_1 = 1.44*d7}$

^{13}C-DEPT

Description: A series of 1D-^{13}C experiments that allow you to differentiate primary, secondary, tertiary, and quaternary carbons:

DEPT135: CH and CH_3 opposite phase from CH_2
DEPT90: only CH carbons
DEPT45: CH, CH_2, and CH_3, all in phase

Best Instruments: AV-600 and DRX-500

Run time: same as for a typical ^{13}C experiment

Instructions:
1. Create a new data set ('new')
2. Set up a normal ^{13}C experiment
3. Take a few scans until you see solvent signal
4. Process the spectrum with 'efp' and 'apk'
5. Change the pulse program for the desired DEPT experiment by typing: 'pulprog dept135', 'pulprog dept90', or 'pulprog dept45'
6. Type 'getprosol 1H [p1value] [pl1 value]'
7. Type 'ased'
8. Set ns = 64, ds = 4.
9. Change the value of td0 to alter the length of the experiment
10. Type 'expt' to check the length of the experiment
11. Type 'zg' to begin the experiment

Processing: same as for a typical ^{13}C experiment

COSY

Description: ^{1}H-^{1}H Homonuclear Experiment; provides information on which protons couple to one another

Best instrument: AV-500

Run time: ~6 minutes

Instructions:
1. Create a new data set ('new')
2. Type 'rpar gcosy.av500' (If you are using a different instrument, simply change the portion after the period i.e. 'rpar gcosy.avb400')
3. Type 'eda'
4. Check the values for SW and O1P (these usually do not need to be changed)
5. Set TD(F1) = 128
6. Type 'ased'
7. Type 'getprosol 1H [p1value] [pl1 value]'
8. Set ns = 1, ds = 16, and d1 = 2s
9. Type 'rga'
10. Type 'expt' to check the length of the experiment
11. Type 'zg' to begin the experiment

Processing:
1. Type 'xfb'
2. Click the "ProcPars" tab and set both Window Functions to "SINE"
3. Check that the SI values are at least as large as the corresponding TD values (you can change SI(F1) to 128 if you want)
4. If you already have a ^{1}H spectrum, you can insert it into the horizontal and vertical traces. Simply right click on the trace, click "External projection" and type in the name and number of the data set for your proton spectrum. Do this for both traces.

NOESY

Description: ^{1}H-^{1}H Homonuclear Experiment; provides through-space information on which protons are near one another

Best instrument: AV-500

Run time: ~20 minutes

Instructions:
1. Create a new data set ('new')
2. Type 'rpar gnoesy.av500' (If you are using a different instrument, simply change the portion after the period i.e. 'rpar gnoesy.avb400')
3. Type 'eda'
4. Check the values for SW and O1P (these usually do not need to be changed)
5. Set TD(F1) = 128
6. Type 'ased'
7. Type 'getprosol 1H [p1value] [pl1 value]'
8. Set ns = 2
9. Set d1 = 3 * T_1
10. Set d8 = T_1
11. Type 'rga'
12. Type 'expt' to check the length of the experiment
13. Type 'zg' to begin the experiment

Processing:
1. Type 'xfb'
2. Click the "ProcPars" tab. Set the F2 Window Function to "EM" and the F1 Window Function to "QSINE"
3. Check that the SI values are at least as large as the corresponding TD values (you can change SI(F1) to 128 if you want)
4. If you see more than one color in a single peak, the spectrum needs phasing:
    a. Type '.ph'
    b. Select 3 peaks to phase by right-clicking on each peak and selecting "add"
    c. Click the "R" button and phase the peaks with the "0" and "1" buttons
    d. Click the save and return button
    e. Click the "C" button and phase the peaks with the "0" and "1" buttons
    f. Click the save and return button
    g. Type 'absb'
5. Type 'xfb'
6. If you already have a ^{1}H spectrum, you can insert it into the horizontal and vertical traces. Simply right click on the trace, click "External projection" and type in the name and number of the data set for your proton spectrum. Do this for both traces.

1D NOE Difference Experiment

Description: ^{1}H-^{1}H Homonuclear Experiment; provides through-space information on which protons are near one another. Much faster than 2D experiment, because data is only collected in 1 dimension.

Best instrument: AV-500

Run time: ~5 minutes

Instructions:
1. In the original proton spectrum, click on the ProcPars tab
2. Change SR[Hz] to 0
3. Place cursor over peak of interest. **Record absolute frequency *in Hz*** (shown on the upper left hand portion of the screen)
4. Create a new data set ('new')
5. Type 'rpar 1dnoe.av500' (if you are using a different instrument, simply change the portion after the period i.e. 'rpar 1dnoe.av600')
6. Type 'eda'
7. Change the value of O1 to the absolute frequency (in Hz) of the ^{1}H of interest.
8. Check value for SW (does not usually need to be changed but should be large enough to see all protons of interest)
9. Type 'ased'
10. Type 'getprosol 1H [p1 value] [pl1 value]'
11. Set ns = 8
12. Set d1 = 3 * T_1
13. Set d8 = T_1
14. Type 'rga'
15. Type 'expt' to check the length of the experiment
16. Type 'zg' to begin the experiment

Processing:
1. Type 'efp'
2. Type '.ph' and phase the peak you irradiated down. All peaks that show an NOE to this peak should be phased up. All other peaks give a null signal (half up/half down)

HSQC

Description: ^{1}H-^{13}C Heteronuclear Experiment; provides information on which protons are directly bonded to which carbons

Best instruments: AV-600 and DRX-500

Run time: ~30 minutes

Instructions:
1. Create a new data set ('new')
2. Type 'rpar ghsqc.av600' (If you are using a different instrument, simply change the portion after the period i.e. 'rpar ghsqc.avb400')
3. Type 'eda'
4. Check the values for SW and O1P (these usually do not need to be changed)
5. Set TD(F2) = 4000
6. Set TD(F1) = 128
7. Type 'ased'
8. Set ns = 4
9. Set ds = 16
10. Set d1 = 1
11. Type 'getprosol 1H [p1value] [pl1 value]'
12. Type 'rga'
13. Type 'expt' to check the length of the experiment
14. Type 'zg' to begin the experiment

Processing:
1. Type 'xfb'
2. If you see more than one color in a single peak, the spectrum needs phasing:
    a. Type '.ph'
    b. Select 3 peaks to phase by right-clicking on each peak and selecting "add"
    c. Click the "R" button and phase the peaks with the "0" and "1" buttons
    d. Click the save and return button
    e. Click the "C" button and phase the peaks with the "0" and "1" buttons
    f. Click the save and return button
    g. Type 'absb'
3. Click the "ProcPars" tab. Set the F2 Window Function to "EM" and the F1 Window Function to "QSINE"
4. Make sure the SI(F2) is at least equal to TD(F2) and SI(F1) is at least 2 times larger than TD(F1)
5. Type 'xfb'
5. If you already have ^{1}H and ^{13}C spectra, you can insert them into the appropriate horizontal and vertical traces. Simply right click on the trace, click "External projection" and type in the name and number of the data set for the corresponding spectrum. Do this for both traces.

HMBC

Description: ^{1}H-^{13}C Heteronuclear Experiment; provides correlations between protons and carbons separated by several (2-3) bonds

Best instruments: AV-600 and DRX-500

Run time: ~50 minutes

Instructions:
1. Create a new data set ('new')
2. Type 'rpar ghmbccar.av600' (Note: for the AVB-400, it seems you need to type: 'rpar ghmbc-car.avb400')
3. Type 'eda'
4. Check the values for SW and O1P (these usually do not need to be changed)
5. Set TD(F2) = 4000
6. Set TD(F1) = 128
7. Type 'ased'
8. Set ns = 16
9. Set ds = 16
10. Set d1 = 1
11. Type 'getprosol 1H [p1value] [pl1 value]'
12. Type 'rga'
13. Type 'expt' to check the length of the experiment
14. Type 'zg' to begin the experiment

Processing:
1. Type 'xfb'
2. If you see more than one color in a single peak, the spectrum needs phasing:
    a. Type '.ph'
    b. Select 3 peaks to phase by right-clicking on each peak and selecting "add"
    c. Click the "R" button and phase the peaks with the "0" and "1" buttons
    d. Click the save and return button
    e. Click the "C" button and phase the peaks with the "0" and "1" buttons
    f. Click the save and return button
    g. Type 'absb'
3. Click the "ProcPars" tab. Set the F2 Window Function to "EM" and the F1 Window Function to "QSINE"
4. Make sure the SI(F2) is at least equal to TD(F2) and SI(F1) is at least 2 times larger than TD(F1)
5. Type 'xfb'
5. If you already have ^{1}H and ^{13}C spectra, you can insert them into the appropriate horizontal and vertical traces. Simply right click on the trace, click "External projection" and type in the name and number of the data set for the corresponding spectrum. Do this for both traces.