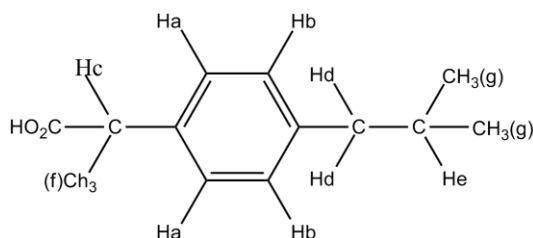


Transient H-1 1D- NOE with shape pulse (selective) excitation

UM protocol for H1 NMR: **Aselnoe**

Using selective irradiation of H-d in a sample compound, Ibuprofen, **Advil**[®], 2-3 mg in 0.6 ml DMSO.

To study the usage and essential parameters in 1D NOE application.



- The sign of NOE can be positive or negative (w.r.t. the non-related signals). Positive NOE typically derives from the proximity of nuclei, whereas negative NOE may be due to either the proximity of nuclei or chemical exchange among conformers.
- J coupling effect also may show up as dispersive phase signal in NOE spectrum.
- The magnitude of NOE obtained by the selective NOE pulse sequence depends on the mixing time, D8. Optimization should be made for best outcome.

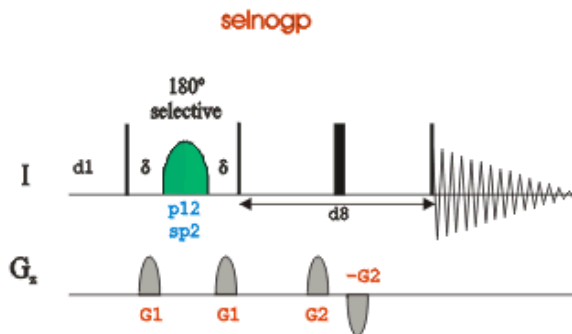
References:

- H. Kessler, H. Oschkinat, C. Griesinger & W. Bermel, J. Magn. Reson. 70, 106 (1986).
- J. Stonehouse, P. Adell, J. Keeler & A.J. Shaka, J. Am. Chem. Soc 116, 6037 (1994).
- K. Stott, J. Stonehouse, J. Keeler, T.L. Hwang & A.J. Shaka, J. Am. Chem. Soc 117, 4199-4200 (1995).

Bruker pulse sequence:

- Strong, shape and gradient pulses are used.
Refer Pulse program for details.

Summary: Four experiments are to be collected with the same sample, with both the 400 MHz and 600MHz spectrometers. Reserve ~ 2 hrs during day hours for your practice and notify staff for assistance.

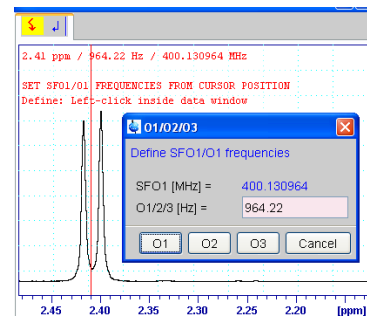



- 1) Locate the signal for selective NOE irradiation with a shape pulse.
- 2) Obtain NOE with the most commonly settings for small molecules (M.W. < 1,000).
- 3) Study the effect of incorrect short mixing time.
- 4) Study the effect of incorrect frequency offset of irradiation.

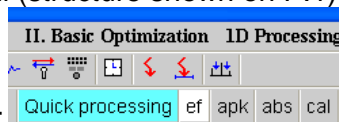
Procedure: Sample will be prepared for you and kept in the Lab.

Demo Sample: Ibuprofen, **Advil**[®], 2-3 mg in 0.6ml DMSO.

1. Create a new file "1DNOE expno 1".
 - a. RPAR Ah1; getprosol.
 - b. Critical: Tune H-1.**
 - c. Shim well, non-spinning.**
 - d. Set NS 8 and obtain a routine H1 spectrum, phased properly.
 - e. Calibrate shift using the solvent H-1 of DMSO signal (2.5ppm)
Expand the spectrum to locate the doublet between 2.48 and 2.46 ppm.
 - f. This is H-d in the structure of Advil (structure shown on P.1).



- g. Click the  in the top menu bar:



Place the mouse over the chemical shift of signal of interest (center in case it is a doublet). Then, left click to determine the exact location of the transmitter offset.

Write down the value of offset, o1 (Hz) from the display window.

Remark:

- *Typing o1p will also give the offset in ppm unit. It is not the chemical shift of the signal unless SR is set to zero.*
2. "iexpno" to create expno #2 with the same file name.
 - a. **RPAR Aselnoe** all, and type **getprosol**.
 - b. Type "**o1**" and enter the value of the offset obtained in step 1d.
 - c. Type **D8**, the mixing time, and set it to 0.6 (s). **Comment:** *Transient NOE is a dynamic process. It builds up during the period of mixing time, starting from zero and reaches its maximum level. Then, the NOE will start to decay gradually due to relaxation. Typically short mixing time e.g. 50ms is for large molecules such as proteins that have slow tumbling rates and short relaxation times.*
 - d. Adjust **D1 = 4 (s)** for sufficient relaxation delay.
 - e. **RGA.** Set **NS= 32 *** NS MUST be an even number.** Note: Increase NS for dilute sample or for sample with small NOE.

- f. In this exercise, you are going to set up two more experiment and use **multizg** to collect all three. *If there is only one signal to be studied, ZG to complete the measurement.*

3. To study the effect of mixing time:

- "IEXPNO" to create EXPNO #3.
- Change only the D8 to 0.05 (s). Do not "ZG"

4. To study the effect of excitation bandwidth for the shape pulse:

- "IEXPNO" to create EXPNO #4.
- Reset D8 value back to 0.6s.
- Change O1 = O1 (original value used in EXPNO #1) + 25 Hz.
- Don't "ZG".

5. Recall EXPNO #2 {NOT expno# 1}, short hand is "re 2 1".

Type **multizg and** enter the prompt with "3" to carry out experiments: #2, #3 and #4.

Processing:

Process all experiments: Calibrate chemical shifts with spectrum #1 and use its SR value for # 2,3 and 4. Remark on Phasing on NOE data presentation

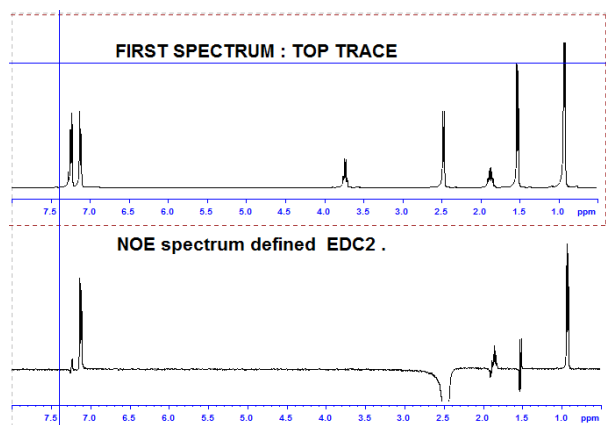
"Phase manually or automatically in such a way that the signal being irradiated negative with respect to the base line." Reason: When the spectrum is plot, the largest signal (that is being irradiated) can be intentionally clipped (as shown) so that the NOE of interests can be shown with suitable expansion.

Report: Submit three plots with proper title to indicate your observation.

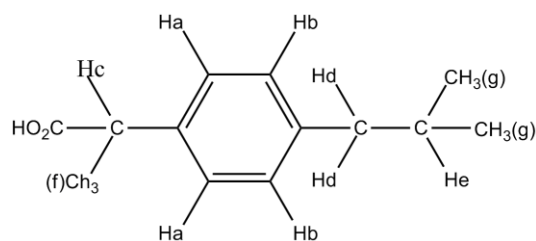
PLOT # ONE: Dual plot #1 (normal H1) and #2 (NOE)

- Recall spectrum of expno #1 in the display window.
- Type edc2 to define #2 as the second spectrum.
- Type or click "Print" and select a layout "+1D+1D+pp.xwp".
- For each spectrum, expand the same limits 8 to 0.5ppm.
- In the NOE spectrum, increase the vertical scale to show the NOE signal. Example plot:
- Indicate in your plot: the observed NOE as well as any artifacts.

PLOT



- Based on NOE, identify Ha, Hb and Hg in your printout



#TWO:

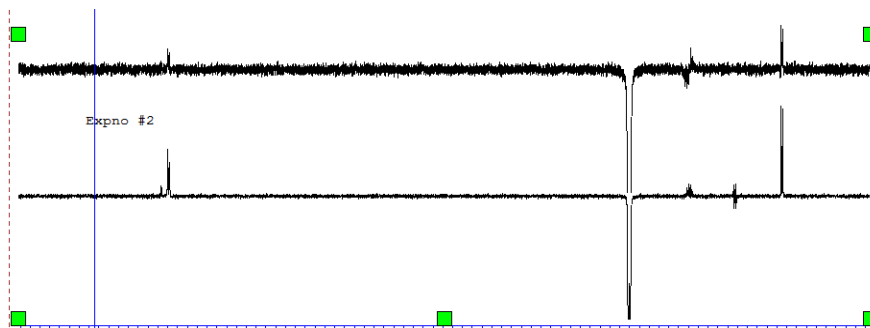
Compare the effect of mixing time on

the relative magnitudes of NOE between
Expno #2 (D8 0.6s) and expno#3 (D8 0.05s).

- Recall spectrum of expno #2 in the display window.
- Type edc2 to define expno#3 as the second spectrum.
- Type or click "Print" and select a layout "stack_2.xwp".
 - Label the plot the corresponding mixing time of the spectra.
 - Give a brief comment on your observation.

General feature of layout "stack_n.xwp" where n can be 2, 3, 4 etc.

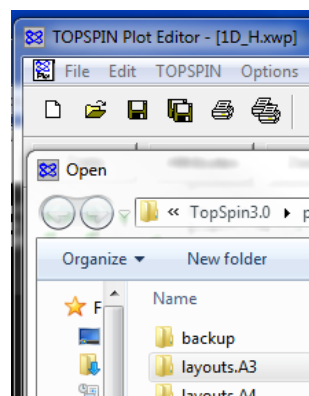
The spectrum initially in the display TOPSPIN window before you start the plot will be shown as the bottom trace and it controls all expansions for all the other spectra. This layout is used for quantitative presentation for NMR titration or kinetic studies, where absolute intensities or integrals are to be compared.



Further Remark of locating the layout :

If you cannot find the layout:

- Type or click "Print" and start with any
- In the Plot Editor, click "File" and double open a folder "layouts.A3."
- Double click the folder and you will see options for stack layouts.



1D layout.
clicks to
all the

PLOT #Three:

To compare the effect of offsets (EXPNO #2) and (EXPNO#4)

- Recall experiment #2 in the TOPSPIN window.
- Type edc2 to define filename for EXPNO #4.
- Use "stack_2.xwp" as layout for relative intensities comparison.