

NOESY

NOESY

NOESY (Nuclear Overhauser Effect Spectroscopy) spectra provide information about protons that are 5 Angstroms or less apart in space. The information is through space and not through bond, like a COSY or TOCSY. The presence of a NOE peak is direct evidence that two protons are within 5 Angstroms through space. The absence of a NOE peak between protons does not necessarily mean that they are not within 5 Angstroms since other factors can reduce a NOE peak even if the protons are close in space. A mid-size molecule (~1000-1500MW range) may have NOEs that are close to zero and a ROESY may be required to see them. Large molecules generally give better NOEs at higher field, but small molecules may actually give better NOEs at lower field. A 2-D NOESY of a small molecule will have cross peaks of opposite phase to the diagonal. A 2-D NOESY of a large molecule will have cross peaks of the same phase as the diagonal. Theoretically, these experiments should be symmetrical, but it is typical to see more intense peaks on one side of the diagonal than the other.

Basic NOESY

NOESY - (all upper case) utilizes a probe file and gradients, no solvent suppression

If a probes file is not set up in the account, see documentation on setting up a probes file or ask for help. This version allows as few as two transients allowing a faster acquisition for concentrated samples. The macro is called by **>NOESY**. The top window should have "seq:NOESY".

1. Make sure **probe='HCN'**.
2. Find the 90-degree pulse width of the sample. Set **pw=pw90**. Collect a 1-D proton and adjust the sweep width and **tof**. Users may also want to determine an approximate T1 value so an optimal delay time between acquisitions can be set.
3. Call the macro by typing **>NOESY** in the command line. Use **>dps** to view the pulse sequence.
4. Check that **pw** is set to the 90-degree pulse width, set **d1** and **np**. Set the **sw1=sw**. Set **phase=1,2**. Also, set **nt** and the **gain** using **ni=1**. The number of transients (**nt**) required for this version can be as little as 2.
5. One parameter requiring forethought is the **mix** time. The **mix** is usually determined by the size of the molecule under study. Small molecules require longer mix times, 0.5 to 1 second (suggested **mix=0.5**). Large molecules generally range from 0.05-0.3 seconds (suggested **mix=0.15**).
6. Set the **ni** and check **>time**. Adjust the delays, transients and/or increments to fit the experiment into the time available. Use **>go** to start the experiment.

The all-caps versions of the NOESY includes linear prediction in the standard parameter sets. To do simple processing of these spectra:

```
>setLP1  
>gaussian  
>wft2da
```

To turn off linear prediction set **proc='ft'** and **proc1='ft'**. Turning off linear prediction is recommended for larger data sets because it can increase processing time dramatically.

Adding presaturation to the basic NOESY

Instructions for this can be found in the manual page for the NOESY pulse sequence (**man('NOESY')**). Set **d1=0**, set **satflag='yynn'**, **satdly=1.5**, **satfrq=tof**, **satpwr=2**. The **satfrq** is normally equal the **tof**, but is not required. Values of **satdly** and **satpwr** vary with the sample.

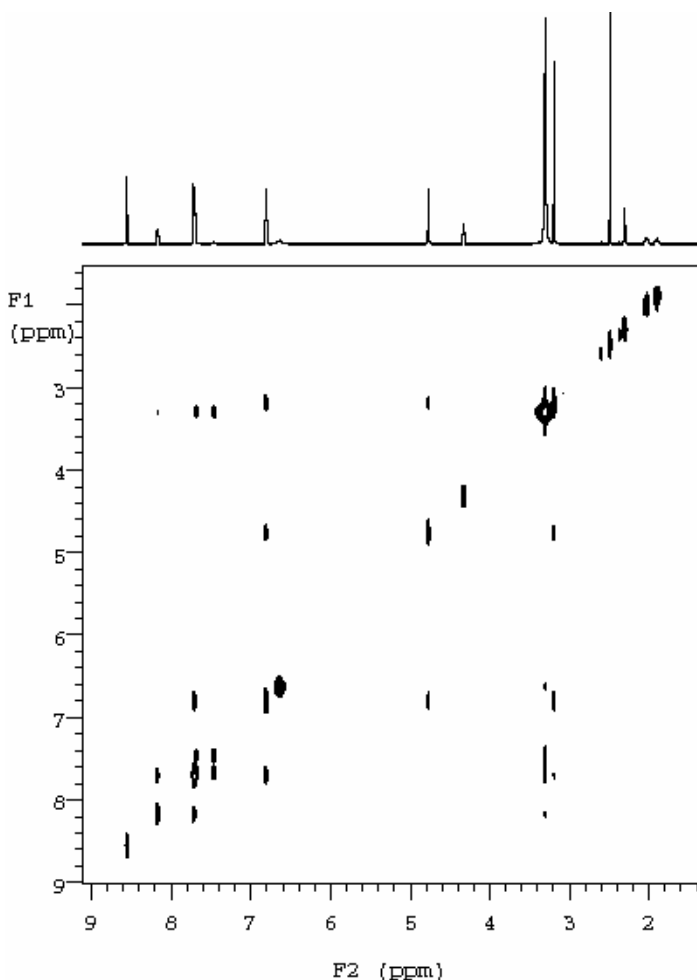


Figure 26: A simple NOESY spectrum of a small molecule should be relatively symmetrical and gives proton-proton through space correlations.

Versions of NOESY

Multiple pulse programs exist for NOESY spectra in this facility. The other most commonly used versions are included below.

a. **noesy** - (all lower case) no gradients, no water suppression

The macro is called by **>noesy**. The top window will have "seq:noesy". Parameters are similar to the basic NOESY.

b. **tnnoesy** - (all lower case) no gradients, presaturation with transmitter

This macro is the same as "noesy" but includes the option of water suppression with the transmitter. The macro is called by **>tnnoesy**.

"tnnoesy" is the same as "noesy" except includes set up for the presaturation parameters. When acquiring the 1-D spectrum, move the transmitter onto the water peak (**>nl movetof**). Also, be sure to determine the 90-degree pulse width and set the parameter **pw90=pw**. Move the parameters the 1-D to a new experiment (i.e. **mp(1,2)**). Call the macro **>tnnoesy**.

Set **d1=0**. Do not use a delay between acquisition and presaturation that would allow the water signal to relax back. Set **satmode='ynn'**, **satdly=1.5**, **satfrq=tof**, **satpwr=2**. Start with the **satdly** at 1.5 sec and the **satpwr** at 2. Adjust these values but do not use a **satpwr** > 20 ever and typical values are 10 or below. Be sure to increase the **gain** after adjusting the water suppression.

c. **gnoesywg** - gradient NOESY with watergate suppression

This macro can be used with the macro "**autowatergatenoesy**". The parameters can be loaded from the parameter set in **/vnmr/parlib**.

Run a 1-D spectrum and move the transmitter onto the water peak (**nl movetof**). Find the 90-deg pulse width and set **pw90=pw**. Two options exist for setting up at this point: First, use the "**autowatergatenoesy**" macro. After setting the above parameters, type

>autowatergatenoesy. The macro will then set up a series of 1-D arrayed experiments to find the best watergate parameters and run one increment of the final parameters.

Second, if a user has already run gwatergate 1-D, load the parameters for gnoesywg from **/vnmr/parlib**. The parameters are called **watergatenoesy.par**. Then enter the watergate parameters from the 1-D into the 2-D parameter set. These parameters are: **p180**, **p1**, **p1lv1**, **phincr1**. Run one increment to check to see that the water suppression is working and increase the receiver gain (i.e. **gain=40**).

Set the mix time, **d1**, **at**, **sw =sw1**, **nt**, **ni**, and **phase=1,2**. Check the amount of time the experiment will take. Adjust the delay, **nt** or **ni** accordingly to fit the time allowed for the experiment.

d. **wgnoesy** - gradient NOESY with watergate suppression

This version is from the BioPack pulse sequences and requires BioPack activated in the account before using it. This version can be autocalibrated from the setup menu. Be sure to check the linear prediction settings in this version.

This version of watergate noesy is supplied through the Varian package, BioPack. If BioPack is not in the account, it must be first activated. Activating BioPack takes a few

minutes – see section on BioPack activation. BioPack will do an autocalibration for the experiment. Make sure **probe='HCN'**. Use the Menu Bar and select: **[Setup] [Water] [Auto Calibration] [Watergates]** then **[3-9-19 NOESY]**

A user will be prompted to use the values from the standard parameter sets. Answer 'n' and put in the values for the **tpwr** and **pw90**. The operator will also be asked for a **d1** time, **mix** time, and for a **flippw** time (typically between 1-2 ms).

The macro will then run a series of arrayed 1-D experiments finding the optimal values for the water suppression including an optimized **tof** value. The last thing it will do is run one increment at the optimized parameters. Set the **sw, sw1, nt, ni**, and **at**. Be sure to turn the receiver gain up before starting the experiment. Check the amount of time the experiment will take using the macro **>time**.

e. WET NOESY

WET NOESY can be set up manually for multiple solvent suppression, please refer to the section on Water Suppression, under WET. To run WET NOESY for water suppression only, the experiment can be set up with auto calibration in BioPack by:

[SetUp] [Water] [AutoCalibration] [WET NOESY]

Answer the questions, use a calibrated **pw** instead of the standard parameter set and be ready to supply a mixing time in sec.

Processing a NOESY

Most NOESY spectra can be processed through the menu system as follows:

[ProcMenu]

[Phase F2]

The first increment will appear (will possibly have a 180-degree phase shift). Phase the baseline as a 1-D spectrum.

[Adjust Weighting]

Use any weighting function desired, but a common one for a NOESY is a gaussian (**gf**).

[Return] [Transform F2]

A spectrum will appear. Place the cursor on a FID (not solvent) that has a reasonable signal and **[Adjust Weighting]**. Again use a gaussian function or other function.

[Return] [Transform F1]

The spectrum should appear on the screen. If an error about being outside of range occurs, use **>f full dconi**. To further correct phase on the spectrum and/or do a drift correct. For drift correct:

>dc('f1') or **dc('f2')**

or

>abc

Alternatively, process the spectrum as follows:

>wft(1) Phase the spectrum. **>wti**

Adjust the weighting function for the FID and use **>wft1da**.

Select a trace and use **>wti**. Apply the second weighting function and use **>wft2da**.

