

1D Transient NOE on the Bruker DRX-500 and DRX-600

Reference: Stott, K., Stonehouse, J., Keeler, T.L. and Shaka, A.J., J. Amer. Chem. Soc. **1995**, *117* (14), pp. 4199-4200.

At thermal equilibrium in a strong magnetic field there is a slight excess of population of nuclei in the lower energy (aligned with the magnetic field) state and a slight depletion of nuclei in the higher energy state (opposed to the magnetic field). If this equilibrium is perturbed for one group of nuclei (corresponding to a peak in the ^1H spectrum), this perturbation is propagated to nearby nuclei in the molecule due to the Nuclear Overhauser Effect (NOE). Because the intensity of a peak in an NMR spectrum is directly proportional to this population difference, the perturbation can be measured by simply recording a spectrum.

The traditional 1D NOE experiment involves irradiating with low power radio frequency at the resonant frequency of one peak in the ^1H in order to equalize the populations of the two states ("saturation"). This saturated state is maintained by continued irradiation until the perturbation populations of nearby nuclei in the molecule reaches a steady-state and does not change any further. Then a 90° pulse is applied and an FID is recorded to measure the amount of perturbation on the nearby nuclei. Since the enhancement of signals is quite small (a few percent), it is necessary to record a control spectrum with irradiation away from any peaks in the spectrum, and then subtract the control spectrum from the NOE spectrum. There are a number of disadvantages to this approach:

1. In any difference spectrum the conditions (temperature, RF power, sensitivity, magnetic field, vibration) must be identical in the two experiments in order to get perfect subtraction of the signals which are not affected. This subtraction is always imperfect since the two spectra are recorded at different times, so there are always big subtraction artefacts in the difference spectrum.

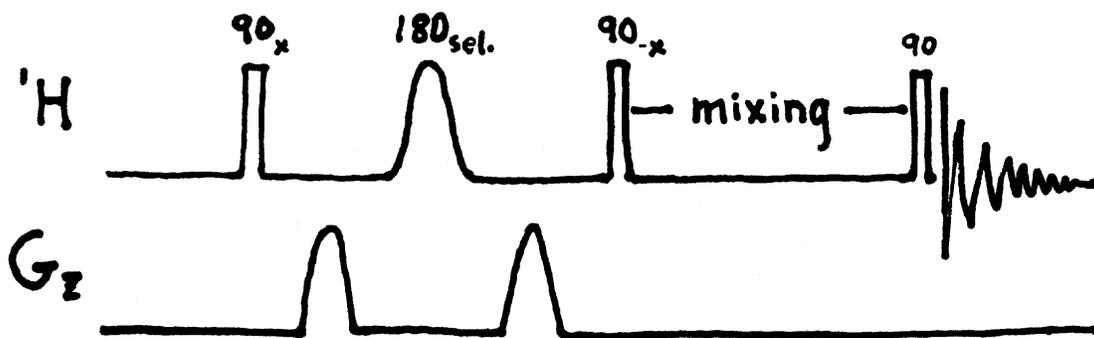
2. The magnitude of the NOE is proportional to inverse sixth power of the distance between two nuclei only for very short times between the perturbation and the measurement of the effect on other nuclei. The magnitude of the steady-state NOE is dependent on many other competing relaxation processes so it cannot be used as an accurate measure of distance. To accurately measure distances you need to measure the NOE with a number of different times between perturbation and measurement ("mixing times") and measure the initial slope of the curve as the effect increases with time.

3. The selectivity of irradiation is limited, and in crowded regions of the spectrum nearby peaks are also affected. This sometimes makes the results ambiguous.

With shaped (selective) pulses we can apply a 180° pulse specifically to a single peak in the spectrum. This is the most dramatic perturbation you can create, since the excess population in the lower energy level is now in the higher energy level and the depleted population is now in the lower energy level. If we then wait a short time for this

perturbation to propagate to nearby nuclei, a 90° pulse will "read" the effect on the other nuclei in the form of a spectrum with enhanced peak areas. To avoid having to subtract two spectra, we can also use gradients to kill any magnetization on other nuclei at the same time that we invert the desired peak. Thus the only thing that will be detected with the 90° "read" pulse is the perturbation due to the NOE. In principle, an NOE spectrum similar to an NOE difference spectrum could be obtained with one scan, but for improved signal-to-noise ratio we will average the result of many scans. This method relies only on the transient NOE and does not involve the subtraction of spectra. The results are excellent and there are practically no artefacts.

The pulse sequence is as follows: A non-selective 90° pulse rotates all of the sample magnetization onto the y axis. Then a gradient "twists" the magnetization into a helix. The selective (shaped) 180° pulse is applied to invert the magnetization of the peak of interest, so that its "twist" is now in the reverse direction. A second gradient of equal intensity and duration to the first now unwinds the twist for the peak of interest. But all the other peaks in the spectrum are just twisted twice as far, since their magnetization helix was not reversed by the selective 180° pulse. This destroys this magnetization and leaves only one thing in the sample: the peak of interest with its magnetization aligned along the -y axis. A second non-selective 90° pulse is now applied to rotate this magnetization from the -y axis to the -z axis. Thus we have accomplished two things: the peak of interest has been inverted (population inversion) and the rest of the peaks have been destroyed. This approach of destroying all of the unwanted magnetization to "reveal" the desired magnetization was developed by A. J. Shaka at the University of California, Irvine, who called it "excitation sculpting" by analogy to a sculptor revealing a work of art by chipping away pieces of stone.

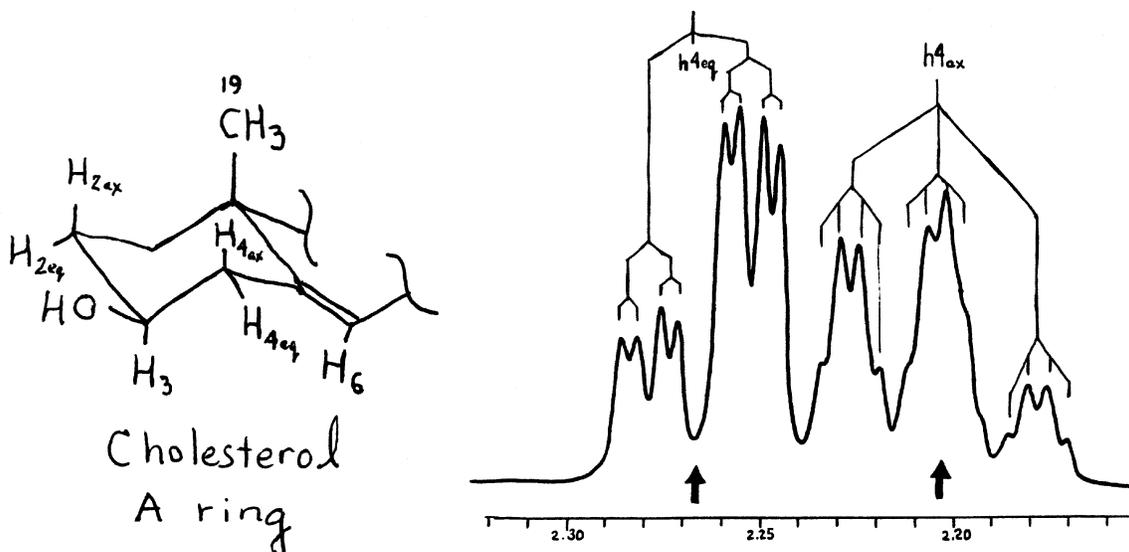


Setting Up for the Selective 1D NOE Experiment

Insert your sample, lock and shim. Do not spin the sample for this experiment. Make sure the temperature is stable at the regulated value (**temon**) and re-shim if necessary after the temperature has stabilized. Get parameters for a simple 1D ^1H experiment (**rpar**) and record a ^1H spectrum. Transform and phase, and set **SR** (spectral reference) to the same value as **O1**. This makes the scale read directly in units of absolute audio frequency in Hz relative to the center of the spectral window. Click the left mouse button to get the vertical arrow cursor, and move the cursor to the position of the peak you wish to select. Read off the cursor frequency from the small window above the spectrum and write down this precise value. This is the offset frequency (**SPOFF1**) for the shaped pulse. If this value were set to zero you would get selective excitation at the center of the spectral window (that is, at the **O1** frequency). By setting an offset frequency you can shift the frequency of excitation to any desired peak in the spectrum.

For example, let's say you enter **O1** and find that the **O1** value is 2858.20 Hz. Then you enter **SR** and enter 2858.20 for **SR**. Place the vertical arrow cursor on the peak of interest in your spectrum and read -1706.13 Hz for the cursor frequency. This is the value you need for the shaped pulse frequency offset (**SPOFF1**). Write this down and enter it when you run **ased**. (Do not use this exact number, of course, yours will be different!!!!)

For the cholesterol sample, you will see that the two proton signal at 2.25 which represents the $\text{h}4_{\text{ax}}$ and $\text{h}4_{\text{eq}}$ is beginning to be resolved into two signals at 500 MHz. This is the signal which gave NOEs to m19 (the 19-Me group) and h6 (the olefinic proton) in the steady-state NOE difference experiment on the Unity-300. Looking at the structure of cholesterol, it seems likely that the NOE to the angular methyl group is primarily from $\text{h}4_{\text{ax}}$, and the NOE to h6 is primarily from $\text{h}4_{\text{eq}}$. With shaped pulses (and at higher field) we may be able to separate these two effects. In the expanded plot below,



if you ignore the small couplings, you can see a strongly skewed downfield doublet ($h_{4_{eq}}$) and a skewed upfield triplet ($h_{4_{ax}}$). They are leaning toward each other because their chemical shifts are so close. The triplet structure of $h_{4_{ax}}$ is due to the fact that it has two large couplings: one to $h_{4_{eq}}$ (geminal) and one to h_3 (antiperiplanar). The $h_{4_{ax}}$ signal has only one large coupling, the geminal coupling to $h_{4_{eq}}$. The two smaller couplings observed in the upfield ($h_{4_{eq}}$) signal are due to h_3 (vicinal axial-equatorial) and $h_{2_{eq}}$ (W-coupling).

Get the offset values for the center of the $h_{4_{eq}}$ signal and for the center of the $h_{4_{ax}}$ signal. These two values will be used for two separate 1D NOE experiments. Note that there is no need to record a "control" frequency since this is a direct NOE measurement and not a difference spectrum.

This offset value and the mixing time are the only parameters you need to edit! Everything else is set for you in the standard parameter set for this experiment. "Clone" your 1D proton experiment to a new area so you don't overwrite your proton spectrum:

enter **edc**

change experiment number to a new experiment (e.g., 2)

click on *SAVE*

You should now be in a new 1D experiment with no FID data, but with standard ^1H parameters. Now get the standard parameters for this experiment using **rpar**. Scroll down to the bottom of the list where the parameter sets starting with "std" are found, and select **std-1DNOE-n3**. Select **Copy All** to load the parameters.

You must have the Nalorac Triple-Resonance Gradient Probe installed to run this experiment!!!

To check the parameters, enter **eda** and make sure the O1 and SW_h values are set the same as they were for your 1D ^1H experiment. Click on *SAVE* and enter **ased**. Scroll to the bottom half of the list and enter the value you calculated for the shaped pulse frequency offset (**SPOFF1**). Check the value of the delay time for NOE transfer (the mixing time) which is **d8** in this experiment. A value of 0.35 ms should be optimal for a molecule the size of cholesterol (larger molecules need shorter mixing times and smaller ones need longer). Click on *SAVE*.

To adjust the receiver gain, go to the acquisition window (enter **a** or **acqu**) and enter **gs**. You should see the lock signal periodically fall and then recover as the gradients fire. Adjust the vertical scale (using the *2, /2, *8 and /8 buttons) so that the 100,000 value in absolute units is near the top of the scale.

If the vertical scale is in centimeters, you need to exit the acquisition window (click *Return*) and select *Utilities* and *YU*, then return to the acquisition window (**a**).

If the FID at any point exceeds the limits from -100,000 to +100,000 on this absolute scale, you need to reduce the RG value. You can do so by entering RG at any time, even

while *gs* is running. If the FID is significantly smaller than +/-100,000 you should increase the value of *RG*. When you are satisfied with the receiver gain, enter **halt** (DO NOT click on *STOP* - this can lock up the gradient amplifier and the lock display!!!).

You are now ready to start the experiment. For this concentrated sample (43 mg cholesterol) you can set **NS** to 16 and **DS** to 4 scans. Start the experiment with **zg**. Set up the second experiment in the next consecutive experiment number (use *edc* to clone) and edit the value of **SPOFF1**. When the first NOE experiment is done you can start the second one. If you are really being efficient you can set up both experiments and queue them using the command **multizg**.

Data Processing

The selective 1D NOE experiment is just a 1D ¹H spectrum so you can process it normally. Phase correct so that the selected peak is upside-down (negative) and you will see NOE peaks as normal (positive) peaks. To compare to the normal 1D spectrum, click on **Display / Dual Display**. Select the second spectrum to display by editing the experiment number and setting the processing number (**PROCNO**) to 1.

If you don't see the second spectrum, its color may be the same as the background. Enter **setres** and select **Second Spectrum**, using the sliders to change the color to 100% red (or any color that stands out). Click on **Apply** and then exit the User Interface Settings window. Again select **Display / Dual Display** and you should see the second spectrum displayed in red.

In this mode you can change the vertical scale of the second spectrum (*2, /2, etc.) or shift it horizontally relative to the first spectrum. This makes it easy to compare two 1D spectra. When finished, click on return to exit the Dual Display mode.

Compare the two 1D NOE spectra and verify that the NOEs are very different (and make sense in terms of distances) for h_{4ax} and h_{4eq} .