# Selective NOE Experiments:
noesy1d, roesy1d in TopSpin

> **Use TopSpin on Callisto for this week's HW.**
> **Best to use the same sample as in HW#9-11.**

Reading – **noesy1d and roesy1d in Claridge chapter 8 (esp. 8.7.2, 8.7.3, 8.8.2, 8.8.3)**

Goals – **Utilize NOE measurements to assist with assignments, and understand how to estimate/measure ^1H T_1 and utilize that for setting d1 and repetition rates in a variety of NMR experiments.**

This week you will learn how to setup and acquire selective NOE-based 1D experiments using Bruker's TopSpin software. This set of experiments can provide significant advantages to the corresponding 2D experiment: (i) they are much faster to acquire; (2) they often provide superior data quality (e.g., unambiguous identification of much smaller NOEs); and (3) they provide significantly better resolution. They do require, however, resolved peaks/multiplets for the selection. Many small molecules fit the latter criterion, and noesy-1d and tocsy-1d experiments (in particular) have become mainstays of research performed in the Chemistry NMR facility.
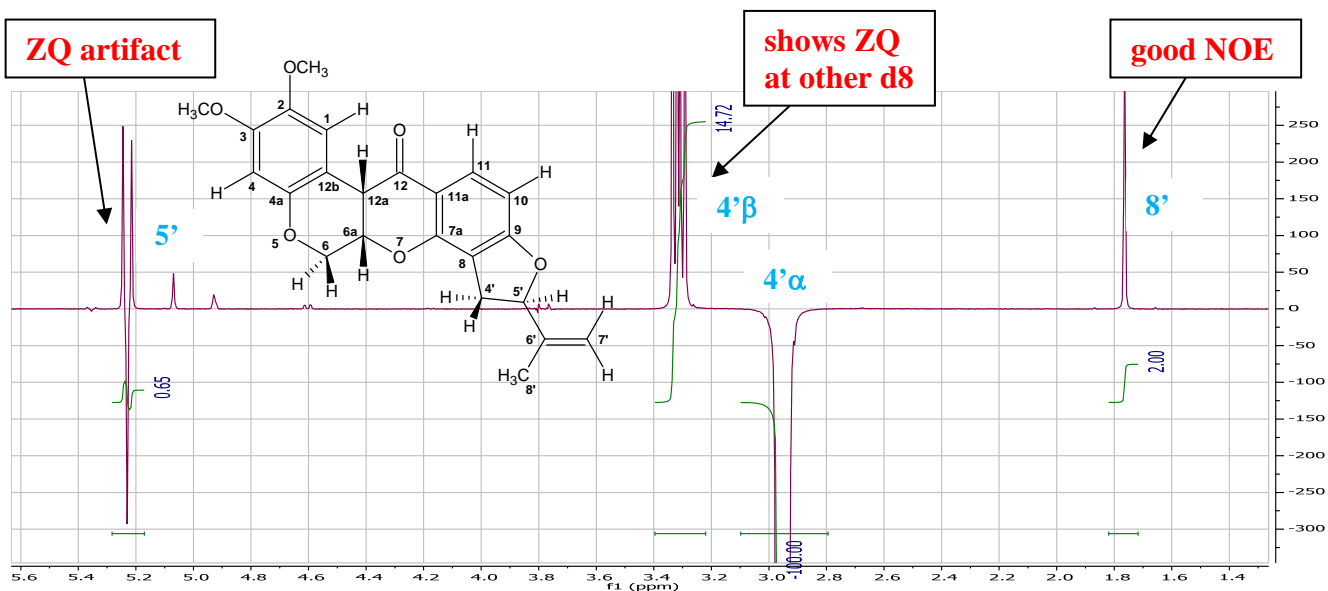
1 Plotting integrals of an NOE peak vs a set of (short; < 0.6 T_1) **mix** times produces a "linear build-up" curve. This data provides the strongest evidence that a presumed NOE is a true NOE. Doing so is critical for any NOE that is not obvious. [Linear buildup curves are particularly important for larger molecules, those that produce negative NOEs. Short mix times from 50 to 300ms are used in such cases.]

2 The selected multiplet should be shown with negative phase (a small phase correction is often needed). In TopSpin, **apk↵ invert↵** will usually work well.

3 It is important to correct the baseline to get accurate integrals. Use **absn** in TopSpin, or a good visual fit using manual correction **B** in MNova. It may be important to **Cut** the edges of the spectrum off to exclude Bruker "smiles" (arising from the digital filters) to get good baseline corrections.

4 Positive integrals of other peaks — once the spectrum is properly phased — are "positive NOEs", negative integrals are "negative NOEs" or "exchange peaks". Multiplets that have very small or zero integrals (i.e., are dispersive) are often observed due to zero-quantum (ZQ) artifacts (occurs for protons having J-coupling to the

selected multiplet). Using longer mix times will reduce the relative size of the J-coupling (ZQ) artifacts compared to the actual NOE.

✍5   roesy1d is usually not run unless noesy1d data suggest that the NOE is in the crossover region (usually for MW = 1000 to 3000 Da), so always run noesy1d first. For roesy1d, avoiding having the offset (**o1p**; center of the spectrum) centered on J-coupled proton pairs. Doing so can lead to TOCSY peaks showing up and appearing to be (false) ROE peaks.

Assignment: Perform noesy1d and roesy1d experiments on your compound. *T_1 estimates are required to properly run these experiments (as done last week).*

–   *Make certain that sample spinning is off:* TopSpin seems to always insure this, but best to make certain.
–   Sample spinning will degrade the quality of data to a point of it having no utility.
–   <u>Always acquire data as strict multiples of 8 scans.</u> The command **halt 128↵** is an example of the best way to stop an experiment where **ns** is set too large.

a)   Select two different proton multiplets to acquire data on.

b)   Use previously acquired T_1 data (from HW#11) to setup the noesy1d experiments with:

$$\mathbf{d1} \geq 3{\times}T_1 \text{ (longest of interest)} \quad \textbf{[repetition delay]}$$

$$\mathbf{d8} \leq T_1 \text{ (shortest of interest).} \quad \textbf{[mix time]}$$

c)   Acquire a noesy1d experiment for each multiplet with the largest **mix** time recommended for "normal" runs.

1+2☞ Plot the data for each spectrum as a normal 1D spectrum. Include integrals of all moderately to strongly enhanced multiplets, as well as the selected multiplet. Properly normalize the integrals such that the enhanced multiplets show η_{NOE} in percent.

d)   Choose one of the observed NOEs that you didn't easily predict, and is to a multiplet — let's call it proton B, where the selected multiplet selected in part c) we'll call proton A —that is reasonably resolved. Acquire the NOE going in the other direction: i.e., choose this multiplet and observe the NOE going to the A multiplet.

3☞   Plot the data for this new spectrum, and show η_{NOE} in percent.

Q1   You almost certainly will observe $\eta_{\text{NOE}}(A{\to}B) \neq \eta_{\text{NOE}}(B{\to}A)$. Explain briefly why this might happen.

e)   Choose another NOE that you're not completely confident about. Run at least 3 more NOESY1D spectra as part of a build-up experiment.

4☞   Plot the set of NOESY1D build-up data with the proton 1D spectrum as a stack plot. Indicate the η_{NOE} values and mix times for each of the NOESY1D spectra on the plot.

f)   Choose one of the NOE pairs, and rerun it now using roesy1d. Note that the mix time **p15** is now the spinlock length, and it typically set smaller — 100 to 300 ms would be typical — than **d8** in the noesy1d experiment. Since **p15** is a pulse length, it is stated in microseconds, μs.

5☞   Plot the ROESY1D spectrum in a stack with the NOESY1D analog and the 1H 1D. Include all proper annotations.

Q2   Is the ROE larger or smaller than the NOE? Explain what your expectations would be in general for this question.