CHEM / BCMB  4190/6190/8189

Introductory NMR

Lab 5
Laboratory #5:

Homonuclear Chemical Shift Correlation: TOCSY
and
Nuclear Overhauser Effect Spectroscopy: NOESY

Objectives:

1. Acquire 2D $^1$H, $^1$H-TOCSY spectra (“TOCSY”) at two different mixing times on our standard menthol sample (in CDCl$_3$), and process, plot and analyze the data.
2. Acquire 1D selective NOESY spectra (“NOESY-1D”) on our standard menthol sample, and process, plot and analyze the data.
3. Analyze 2D NOESY spectra (“NOESY”) collected on our standard menthol sample.

The basics of the setup of the 400 MHz instrument are the same as for the previous four laboratories. If you think that you need help remembering the basics, please bring with you the old lab handouts. Only the aspects/parameters specific for the TOCSY and NOESY experiments will be outlined below.

1. 2D TOCSY data acquisition

You will collect 2 2D TOCSY data sets:
- one with a TOCSY mixing time of 150 ms
- one with a TOCSY mixing time of 30 ms.

- select sample location
- select solvent (cdcl3)
- select user name (chem6190)
- select TOCSY (NOT TOCSY-1D !!!)

click **Customize Exp.**

click **Proton**

spectral width - 0.5 - 9.5
minimize sweep width **auto**
proton scans 8
proton recycle delay 1
proton pulse angle **default**

click **select, time, save & exit**

click **TOCSY**

TOCSY scans per inc. 2
TOCSY number if inc. 128
TOCSY mixing time 150 (or 30 milliseconds)

click **select, time, save & exit**

click **Done**

- select an input file name
2. Processing/Reprocessing the TOCSY spectra

The TOCSY data set consists of 3 separate data sets. The first (filename.1) is a 1D \(^1\text{H}\) spectrum with the default (large) spectral width. The second (filename.2) is a 1D \(^1\text{H}\) spectrum with a “minimized” spectral width. The third is the 2D TOCSY data set. The second (filename.2) will be used, if necessary to rephase the TOCSY 2D data set if the automated phasing routines do not work properly.

**Retreive Data**
- Refer to Laboratory #1: 1D \(^1\text{H}\) NMR Spectroscopy
  
  Type `jexp2`, then load 1D \(^1\text{H}\) data (data in filename.2)
  
  Type `wft`, `aph`

  Type `jexp3`, then load TOCSY data (filename.3)

**Process 2D TOCSY**
- Click `process`
  
  Click **Full 2D transform**
  
  - Type `foldt`

  -the displayed region can be changed using the cursors much like the 1D displays. The contours can be changed with the `-20vs/+20vs` buttons

**Plot the spectrum**
- first, just click **Autoplot**. Wait until the plot is finished to see what it looks like
  
  -you can also try the following:
    
    - Type `decon`
    
    - Click `return` -> `plot` -> **pos. only**
    
    - Type `page`

  -another alternative way to plot:
    
    Type `plcosy('pos', 10, 1.3, 2)`

    The last number is the experiment number where 1D spectrum is located (exp2, see above). This method uses the phase information from the processed 1D spectrum to aid in proper phasing of the 2D spectrum

***Make sure to plot the 2D TOCSY contour plots at several contour levels***

3. 1D Selective NOESY Data Acquisition

Collection of this data is very similar to collection of the homonuclear 1D decoupling data. Selected signals (3.41 ppm, 2.18 ppm, 1.97 ppm, 1.42 ppm, 1.12 ppm, and 0.81 ppm) in the menthol spectrum will be sequentially inverted (one at a time) with a long, selective pulse, and enhancements of other signals will be detected by observing the difference spectra.
-select sample location
-select solvent (cdcl3)
-select user name (chem6190)
-select NOESY-1D (NOT NOESY !!!)
click Customise Exp.
click Proton
  spectral width - 0.5 - 9.5
  minimize sweep width auto
  proton scans 8
  proton recycle delay 1
  proton pulse angle default
click select, time, save & exit
click NOESY-1D
  selective peaks 1
  frequency (ppm) 3.41 (2.18, 1.97, 1.42, 1.12, 0.81)
  width (Hz) 50
  NOESY 1D scans 64
  NOESY mixing time 500 ms
click select, time, save & exit
click Done
-select an input file name

4. Processing the 1D Selective NOESY Data

The 1D NOESY data set consists of 3 separate data sets. The first (filename.1) is a 1D $^1$H spectrum with the default (large) spectral width. The second (filename.2) is a 1D $^1$H spectrum with a “minimized” spectral width. The third (filename.3) is the 1D NOESY difference data set, and is processed like any ordinary 1D data set.

General Statement Concerning Lab Reports:

While I encourage students to work together in terms of discussing the laboratory materials, including the laboratory results, and discussing answers to questions for the laboratory reports, the reports themselves should be original works of a single individual, should not be written by teams, and should not be the products of joint efforts between two or more students.
Questions for Lab 5:

Please collect all the figures showing your 2D TOCSY data and include them in your report. Please provide a typed report.

On page 6 (next page) is the 1D $^1$H spectrum of menthol with the peaks labeled. The labels correspond to the protons on the menthol molecule shown on the page. These are the correct assignments that most of you arrived at using the decoupling data from lab #2.

1. (10 pts) Examine the TOCSY spectrum with the 150 ms mixing time. For several reasonably well resolved signals (3.41, proton 10; 2.18, proton 5; 1.97, proton 8 ‘a’; 1.42, proton 6; 1.12, proton 9, and 0.81, proton(s) 1), make a table indicating the protons to which each of these shares a cross-peak. In cases of chemical shift degeneracy and crowding, if ambiguity exists, make footnotes to your table explaining the ambiguities, etc. Do all of the crosspeaks make sense based on what you know about how the TOCSY experiment works? Remember, you should plot the 2D TOCSY contour plots at several different contour levels to permit you to see very small peaks (very low contour level, near baseline) and also to permit you to remove most of the noise and look only at strong signals (very high contour level).

2. (10 pts) Examine the TOCSY spectrum with the 30 ms mixing time. You will notice immediately that there are many fewer peaks in this spectrum, and that this is precisely what you would expect based on the fact that the mixing time is much shorter. In the table that you made for question #1, circle entries (corresponding to peaks) that are missing in the TOCSY spectrum with the 30 ms mixing time (but are present in the 150 ms mixing time TOCSY spectrum). Do you have any cases (in either the 150 or 30 ms spectrum) where a crosspeak from a neighboring proton is missing but one from a more distant proton still shows up? If so describe them. Also, include in your report a copy of your 150 ms TOCSY spectrum plotted near the baseline, and a copy of your 30 ms TOCSY plotted near the baseline. Circle in the 150 ms TOCSY all of the crosspeaks involving a given well-resolved signal/proton (for instance, the proton at 3.41 ppm) that appear in the 150 ms TOCSY but that do not appear in the 30 ms TOCSY.

3. (30 pts) On page 7 is shown two views of the x-ray crystal structure (model) of menthol. Our standard identifiers are shown, as are representative distances. On page 8 is the 1D $^1$H spectrum of menthol indicating the signals that you chose for selective inversion in order to elicit NOE perturbation of the signals from neighboring protons. Pages 9-11 show the 1D NOE data (difference spectra) when each of these signals is inverted. Here are your jobs. You must assign the axial and equatorial positions at 4, 7, and 8, i.e. we know that one of the protons at C8 gives rise to a signal at 1.97, and the other gives rise to a signal at about 0.95. Which (axial, equatorial) gives rise to the signal at 1.97, and which (axial, equatorial) gives rise to the signal at 0.95 (do the same for 4 and 7). You must carefully analyze the NOE spectra on pages 9-11 do do this. You will not merely supply me with an answer, but you will describe your logic and rationale, and you will refer to specific peaks in the spectra on pages 9-11 to make you points. You will also refer to the figures on page 7 and the measured distances shown there, and you will use these in your detailed explanations. Also, based on the NOE spectra, is the assignment of methyl groups 1 and 2 on page 7 correct? Why (explain in detail, using the NOE spectra, etc.). Next, on page 12 is shown 2 plots (at different contour levels) of a 2D NOESY spectrum of menthol. The lower plot was plotted at a higher contour level, and only shows very large crosspeaks (disregard diagonal peaks). In the lower spectrum circle 3 or 4 unique crosspeaks (do not pick a crosspeak and its symmetrically related crosspeak) and tell me why they should be large, based on the crystal structure of menthol in figure 7, the distances, etc. Then, in the top figure (plotted at a lower contour level, showing strong and weaker crosspeaks), pick (circle) some weak crosspeaks (those that do not show up in the lower plot) and explain to me, based on the structure on page 7, why the crosspeaks should be weaker. Finally, there appears to be an artifact in the 2D NOESY spectrum…..a vertical “streak” at about 1.9 ppm. What do you suppose this is due to?