Guide for ^{1}H NMR-60 MHz Anasazi Analysis

Preparation of Sample:

1.) Weigh 40-45 mg of **dried** acetyl salicylic acid sample and add to a 1.5mL GC-sample vial.

>   Note: Make sure to not weigh more than 45 mg, because acetyl salicylic acid is slightly soluble in $CDCl_3$.

2.) Only use a **clean** glass Pasteur pipet when dipping into the $CDCl_3$ solvent bottle, to avoid contamination! If the pipet gets contaminated, use a **new** pipet. Add 1.0 mL of $CDCl_3$ using a Pasteur pipet, this is about ¾ -full of the GC-sample vial.

3.) Using a Pasteur pipet, draw up solution into pipet then expel back into vial, repeat to mix a few times. This will aide in solvation of the solute in $CDCl_3$. Filter solution through a filter pipet prepared with glass wool to filter out undissolved solid. Sample must be neat, clean, and free of debris.

4.) Transfer enough of the prepared solution via pipet into a ^{1}H NMR tube filling to a height of 5 cm via Pasteur pipet. This can be checked with a ruler. If in excess, get a Kim-wipe and invert the tube so that some of the solution is absorbed. Left over solution is disposed in the $CDCl_3$-labeled waste jar. Dispose of glass pipet in the glass waste container.

5.) Top with plastic colored ^{1}H NMR cap.

6.) Place ^{1}H NMR tube into white plastic spinner and set into the plastic probe height measurement slot located in the front of the instrument. Gently push down on tube to lower until it stops (this aligns the tube for proper positioning/height in the probe). Check to see that the black air lever/switch is in "on" position (the "on" indicates that the switch is pulled up or in the horizontal position).

7.) Put ^{1}H NMR tube with spinner into probe column and release. The air flow in the column will keep the tube buoyant. Lower tube by pushing the air switch down to the "off" position.

8.) Pull air switch up to the "on" position by making slight up and down adjustments as needed with the switch to start spinning the ^{1}H NMR tube. Pull switch and leave it in the "on" position. The tube should be observed to be spinning fast.

9.) If spinning is not observed, eject sample by pressing black button next to air control switch/lever. Repeat steps 8-9.

Using PNMR and NUTS software to acquire proton spectrum:

1.) There are basic functions/commands that will be used in PNMR and NUTS.

-**Legend**: (>) = type into command line in NUTS ; (<>) = execute command to computer (i.e. - <Enter> means pressing the "Enter" key.)

a. **PNMR:**

- ns- number of scans

- nu- nucleus (should be H1 for proton nucleus)

- zg- zero go (initiates spectrum acquisition)

- Enter-execute command

b. **NUTS: Note-** when in this program, you must execute all commands from the **base level operation.** This means checking to see that you have exited a routine before beginning another routine.

- >zo- zoom

- >pe-trim phase

- >fb-fit baseline

- >id-integrate

- >dp-manual peak pick

- >pl-plot/print

- ><Enter>-exit routine

2.) Ensure that the sample is spinning.

3.) Open **PNMR** program. Verify that the number of scans is properly adjusted for experiment and that it is set to the right nucleus (i.e. ~16 scans for proton spectrum. Nucleus is H1 for proton and C13 for carbon and can be found in command line at the bottom left hand corner of the screen).

    a.   To adjust the number of scans:

        - Type "ns" then edit the number of scans, press <Enter> to close box.

    b.   To change the nucleus:

        - Type "nu" then edit with H1 or C13 depending on the type of spectrum you are running. Press <Enter> to close the box.

4.) Type "zg" then press <Enter>. (This executes the zero go command to acquire spectrum. A window asking for filename will be displayed. Select appropriate file name that the spectra will be saved under. Typically, this will be the default file name.) Press <Enter>.

5.) After acquisition is complete, a status window will alert you that the file has been written. Press <Alt+Tab> to toggle over to NUTS, to process data.

6.) **In NUTS**:

7.) Press <Cntrl+F2>. This will open your entire spectra referenced to the calibrated TMS line. Browse for your file name or select the default file. Click on <Open> to close window. A window containing parameters will be displayed. Edit as necessary or click on <OK> to exit.

8.) If noise to signal ratio is high then you can execute a command to reduce the noise before phasing.

    a. >2S- Performs 2-point smoothing for noise suppression

        - or

    b. >3S-Performs 3-point smoothing for noise suppression

9.) To set up phasing the zoom regions must first be defined.

    a. >zo

    b. Drag cursor over a **strong** peak on the left hand side to highlight it.

    c. Press <1>- this assigns your first strong peak to region one.

    d. Drag cursor over a **strong** peak on the right hand side to highlight it.

    e. Press <2>- this assigns your second peak to region 2.

    f. Exit zo by pressing <Enter>.

10.) Enter into the phase routine to trim phase.

    a. >pe

    b. Phase left peak you assigned to **region 1** by clicking the **left** mouse button and holding while dragging mouse left to right to even out the baseline.

    c. Phase the peak you assigned to **region 2** by clicking the **right** mouse button and holding while dragging mouse left to right to even out the baseline.

    d. Exit pe by pressing <Enter>.

11.)   Fit the baseline:

    a. >fb

    b. Remove stripes that are on or too close to peaks by clicking with the left mouse button. Press *<l>* to get a Least Squares fit.

    c. Press <Enter> to exit routine.

12.)   Integrate data:

    a. >id

    b. You will see a continuous integral that needs to be broken up for each individual signal. For each broken integral, click on the left mouse button twice on the left side of the peak(s) then once on the right side.

    c. To assign a relative integral value to a peak, click and hold the left mouse button on that peak then press <v> and enter a number in the display box.

    d. You can toggle the integrals on and off by <Cntrl+I>.

    e. Exit id by pressing <Enter>.

13.)   Pick Peaks- this enables you to get labeled peak shifts in PPM that will be displayed at the top of the screen.

    a. >dp

    b. The cursor becomes a crosshair with a DP label. Select each peak by clicking with the left mouse button.

    c. The peak label display of shifts in PPM can be toggled on and off with <Cntrl+P>.

    d. After you have selected your peaks, type <t> to write them to a table.

e. <Cntrl+B> toggles this table on and off the displayed screen.

f. Exit dp by pressing <Enter>.

14.) If necessary, you can expand a region of interest from the full screen by using the

zoom routine.

   a. >zo

   b. Select area of interest by clicking the left mouse button and highlighting desired

   region.

   c. <Cntrl+E> will jump to the expanded display.

   d. <Cntrl+F> will return to the full display.

   e. Edit the expanded region using dp and id.

   f. Print region if desired.

      - >pl   Note: In print menu, ensure that it is set to landscape view.

15.) Create a notes box with special instructions and identification information from

the full display.

   a. In task bar under the tools menu select NO.

   b. Left click on screen with mouse to open dialog box and type in pertinent

   information. Click <Enter> to close the box. The note now becomes displayed on

   your spectrum.

      - The note boxes can be moved by clicking the left mouse button and

         holding while dragging the box to the desired location.

   c. >P

      - This creates a note containing acquisition parameters for currently

         displayed data.

d. Exit notes by pressing <Enter>.

16.) Make sure peak labels, table, and integrals are displayed by toggling.

17.) Print spectra

a. >pl

- In print menu, ensure that it is set to landscape view

18.) Eject sample by pressing black button next to air control switch/lever. Dispose of

CDCl$_3$ solution into waste jar. Clean ^{1}H NMR tube by rinsing 3-4 times with acetone.

Invert and set clean tube into labeled container for drying.