SP046

NMR of Glycolipids

Materials

Methanol-d_4
Choloroform-*d*
N_2 bath [SOP **SP**031]
NMR tubes
13 x 100 mm tubes + caps

Protocol

1._____Obtain 1-5 mg of dry sample in an acetone-rinsed 13 x 100 mm tube. Do the same for a control sample. (Note 1)

2._____Resuspend each sample in 1 ml $CDCl_3/CD_3OD$ (2:1), and dry down.

3._____Repeat step (2).

4._____Resuspend each sample in 1 ml deuterated solvents again, and transfer to NMR tubes. Parafilm tubes and keep at 4º C or -20º C before use. (Note 2)

5._____In the basement of the Chemistry building, clean each tube with a kimwipe and EtOH, and ensure tube is straight by inserting into the glass gauge.

6._____For JS-300 work, log in user name *jdw* and password *nmr4CSU*. In the command window enter **e**. Make certain the air is on, insert the spinner with the sample, and enter **i**.

7._____Click on **<setup>**, select **<$CDCl_3$>** as the solvent system, and enter **su** to initialize the hardware. (Note 3)

8._____To load the shim program enter **rts**, **shimfile**, then **setallshims**.

9._____Select **<acqui>** and click on **<lock>**. Ensure that the spin is at 20 Hz. Click **<lock off>** before adjusting any parameters. Maximize the *lock gain.* (Note 4)

10._____Begin obtaining a step function by increasing z_0 by 1 from about 300 until you see a plateau "step". (Note 5)

11._____Click **<lock on>** once a good step function is obtained.

12._____Select **<shim>** to fine tune the signal. Adjust z_1 and z_2 alternatively, reducing the *lock gain* if the signal reads above 100%. (Note 6)

13._____Enter **nt=128** or greater (a multiple of 4) as the number of scans, then **ga** to begin acquisition. (Note 7)

14._____Once the spectrum is finalized, save the data with the **svf** command followed by the file name at the next prompt, making sure to exclude spaces.

15._____To begin processing the spectrum, enter **aph** (phase correction), and **vsadj** to adjust peak height. Ensure <**Display**> and <**Interactive**> have been selected to enable further manipulation.

16._____Select <**Full**> and <**dscale**>. (Note 8)

17._____Expand a region of interest by setting the red borders with the left and right mouse buttons, then select <**Expand**>.

18._____Enter **atext(‘**experiment description**’)** for a general description of the plot. Type **atext(file)** to add the file name to the printout.

19._____Select <**th**> to set the level above which peaks will be labeled.

20._____Adjust peak height by clicking on the spectrum line with the middle button and slowly moving upward.

21._____To print, select <**main menu**>, <**display**>, and <**plot**>, then <**plot**>, <**scale**>, <**all parameters**>, <**peaks**>, and <**page**>.

22._____Enter **e**, remove sample, then enter **i**. Within the desktop click and hold the right mouse button, slide down to *log out*, and release.

23._____Transfer samples to 13 x 100 mm tubes and dry down.

24._____Resuspend each in 1 ml normal, non-deuterated 2:1 solvent, and dry down.

25._____Repeat step (24) once more. Samples should be stored at 4^{o} C until further QC.

26._____Resuspend in 1 ml 2:1 and transfer back to bulk sample for weight analysis.

Notes

(1) The Inova-400 can detect down to about 0.5 mg sample, but al least 3.0 mg will be needed for the JS-300 NMR.

(2) An NMR tube rack can be used, or the tubes may be held and transported in an Erlenmeyer flask stuffed with kimwipes to prevent jostling.

(3) There is not a selection for the dual solvent used for TDM or SL, but this is the closest.

(4) It may be more efficient to use parameters from the previous run, in which case the *lock gain* can be set to that value now. Leave *lock phase* alone. *Lock power* should not exceed 36 as excessive power will make it difficult to shim.

(5) Standard TDM will optimize between 300 and 350. Double check optimization by moving above and below this parameter by increments of 1. The best z_0 will be that with the highest variation between the upper and lower plateau, which also seems to remain constant. Record this number before moving on to shim.

(6) A signal which fluctuates between 60% and 100% is good, and between 80% and 100% is better. Continue to adjust *lock gain* down by 1 until the course signal will not exceed 100%, then click close. Record these parameters for future reference.

(7) The number of scans can be set to any multiple of the batch scan (BS) number. Monitor the spectrum as you go by entering **wft**.

(8) The tallest peak on the right is the tetramethyl silane (TMS) standard. Before clicking on **<dscale>**, expand that region, right click in center of peak, and click **<ref>**, then enter '0.' Once you click on **<dscale>** it will reset this peak as the zero reference.