Problem set 2: Chem-232
Due December 13 in the box outside my office, room #6 Stauffer I

1) Use the T1 data from the NMR project (you will likely need to know the structures or at least part of the structures to answer this). Find 4 resonances (not DMSO/H2O):

A) The one that has the longest T1
B) The one that is directly adjacent to that one in chemical shift
C) One that has a T1 that is one of the shortest in the mixture and also has a T2* that is one of the shortest
D) One that has a T1 that is not one of the shortest but has a T2* that is one of the shortest

Part I. Calculate T2* for each of these resonances

Part II. Explain why the T1/T2 values are short/long based upon what Hydrogen it is in the molecule; in particular, compare Peak A and Peak B and explain this.
2) For the 1D C13 spectrum from the project, find these resonances:

- A) A resonance at ~210ppm
- B) Any CH2 resonance
- C) Any CH resonance that is less than 90ppm
- D) The Carbon resonance bound to the H1 with the longest T1
- E) The Carbon resonance bound to the H1 adjacent in H1 shift to the H1 with the longest T1
- F) Both Carbon-Fluorine resonances
- G) A quarternary Carbon resonance somewhat near the middle of the spectrum (so between ~40 and 150ppm)

Integrate these as accurately as possible.

Part I) Explain why the integrations do not match each other

Part II) Explain what you would need to do increase the signal to noise of the Carbon-Fluorine resonances.

Part III) Explain how you would re-acquire the data to get accurate integration.
3) Looking at the 1D C13 data as in Question #2. There are several Carbon resonances that appear broader than the large majority of them, see the peaks at 184.3, 85.9, and 47.3 relative to DMSO at 39.5ppm. In VNMR, you can type ft rather than wft to see the linewidth difference; in MNova, turn off line broadening in Apodization option.

Part A. Why are each of these 3 peaks broader than other resonances in the same sample/molecule? [Note, they are not all the same reason].

Part B. There are 2 other major reasons (to some extent 3 as 1 is similar/related to Part A) some peaks can appear broader in normally acquired 1D C13 spectra, but are hopefully not the explanations here (one is instrumental setup, the other 2 are sample related). Some but not all peaks appear broader than others (so not shim values which would affect all resonances, not a large molecule which would affect nearly all resonances).
4) An H1, F19, and P31 1D were acquired on the merc400 on 1 sample. Explain what the peaks are in the F19 and P31 spectra, and determine the relative amount for each molecule. Explain the potential problems with the accuracy of this, and what you would do to get a better estimate.

The spectra are in your accounts on nmr1, and nmr300 as previously, labeled Problem_set_2_H.fid, Problem_set_2_F.fid and Problem_set_2_P.fid.
5) A small portion of a 1D 1H experiment acquired on a small peptide (~5 amino acids) is shown below. The peptide is a normal peptide, all amino acids are among the 20 standard amino acids. The sample was not isotopically labeled, so the C13 is ~1%, the N15 ~0.4%, the O17 ~0.04%. The sample was dissolved in DMSO.

Part A) Explain the three peaks below (to help you out these are NOT 3 separate singlets). You should assign them to a specific hydrogen(s) in a peptide, such as this is the alpha proton on Glycine or this the acid proton on aspartic acid or one of the backbone amide protons, etc. Explain why they appear as such. Also, what is special about them so that they are being observed and being observed as below, i.e. what special conditions is the sample in to create such resonances?

Part B) If you could theoretically acquire C13, N14, and N15 1D data for this sample (so the instrument hardware exists to do that, and the concentration was sufficient), what would you observe for the C13/N14/N15 closest through-bond to this H1. [so if this is an alpha Carbon, then that alpha Carbon and the N14 or N15 bonded to it; if this were an NH, then that N14 or N15 and then the Carbon bonded to that Nitrogen, etc.]

Part C) If you replaced the D6-DMSO with 90% H2O/10% D2O, but otherwise the same conditions, describe/draw what this part of the H1 spectrum would look like.
6) You need to acquire 1D $^1$H spectra at 25c, 40c, and 50c on the 300 on 5-Fluorocytidine. Explain any temperature dependent changes you observe. Also, explain any differences you observe on the data at 300 versus the data at 600 (shown below) [note before you change temperatures see questions #7 and 8]. You can work in groups of a few if you want for this and all acquisition questions. Also, at the end of each acquisition (or at least 25C and 50C), type: gain? Then, record the gain value. You will need this in Question 7.
PART B.
Below is a spectrum on adenosine on the 600 at 25C. Why is the NH2 resonance a single peak at 25C in contrast to the modified cytidine?
PART C.
Below is a 2D NOESY of an RNA helix acquired in 90% H2O/10% D2O, the highlighted crosspeaks are to the NH2 of one adenosine (residue 3 in the sequence) from the NH of uridine (residue 18 in the sequence). Why are 2 separate shifts for the NH2 observed in RNA at 25C in contrast to adenosine alone?
7) Acquire a 1D 1H experiment on the same 5 Fluoro Cytidine sample on the 300 that suppresses the water peak at 25C and 50C. Compare the relative integrations of the resonances and explain why they are no longer the correct values. You can use a saturation power = -6. For each spectrum, set:

gain = ‘n’

Then after processing the data with wft, type:

gain?

Compare this to the gain value from Question 4. Explain the difference.
8) You need to determine whether the nucleoside is α or β, so what is the stereochemistry on the anomeric carbon for 5Fluoro Cytidine in a 1D spectrum. Acquire the data at both 25°C and at 50°C. Compare the results between the 2 spectra and does the result fit the expected result. Also, compare to the same data shown below acquired on the 600 at 25°C.
9) Part A. There are 3 samples by the 300, one contains Silicon, the other 2 Boron, 2 of the 3 contain sodium. You need to show which sample has Silicon, which 2 have Boron with a positive result for each, and which 2 have sodium. You do not have to find optimal parameters, you only need to observe a signal or not- but that might require choosing decent parameters. What parameters should you change (or default to significantly different values) for each of Silicon, Boron and Sodium relative to Carbon? Why are they different? [2 of the samples are in DMSO, 1 in D2O]. You can use the 300 or 500 for these [before trying, you might figure out which instrument you want to use]. Also, the sample that has Si, has a JH-Si coupling constant of ~6.5 Hz. You can assume that pw90 calibrations are basically correct, at least close enough if the probe is tuned.
Part B. The aromatic portion of the C13 spectrum acquired on the 300 (so about 40 ppm wide) for the 2 samples that contain Boron are shown below. The referencing on the spectra are not correct- both were set to 0 ppm for the middle of the displayed region.

Explain:
1) Which carbon spectrum belongs to which sample from Part A, and how you could determine this with the data from Part A alone. Explain what is observed for the carbon(s) attached to Boron for the 2 spectra (note- you need to look at the data very closely).

2) Which carbon spectrum belongs to which of these 2 molecules (these are the 2 samples in Part A), and again how would know which spectrum was which without using chemical shift data or how many carbon resonances you observe.
Spectrum 1:

[Note small peaks at ~1200Hz are probably impurities]

Zoom on downfield of above spectrum
Spectrum 2 processed with $lb = 0.2$:

Pulse Sequence: algpul

Spectrum 2 processed with $lb = 9$:

Pulse Sequence: algpul
Molecule 1:

Molecule 2
Part C. Below is Chloroform, CDCl₃ solvent, acquired on a sample that does not have any resonances between 70 and 80 ppm for 16 hours on the 500 MHz NMR. Explain why you see the resonances that you do see below and do not see others? Compare what you see with the C13 of Chloroform to the C13 of the Boron complexes above.