Problem Set 2

1) The triplet is coupling between the N14 and H1. The side peaks are most likely isotope effect from the mixture in slow exchange of NH4, NH3D, NH2D2, NHD3 in the 90%H2O/10%D20 solvent. An alternative explanation is some sort of shimming issue or maybe a poorly mixed sample or the lock frequency moving during the experiment. To check this explanation, I would add some sort of small molecule and see if that peak is a clean singlet. Alternatively, using a different lock solvent so 90%H2O/10%D6 DMSO would also help as the peaks come from exchange with D2O. In reality, that is what was done and the side bands go away suggesting that side bands are related to D2O.

The conditions are important for the side bands for exchange with D, as the more acidic conditions slow exchange and make the NH easier to see. In addition, the exchange between NH4 to NH3 is slow, so not just exchange with H2O/D2O. This has 2 effects there is no blur of frequencies between the NH4 and NH3 shift. Also, since it is NH4 slow on the NMR time scale the N14 relaxation is slowed, presumably it is tetrahedral rather than trigonal now slow on the NMR time scale. Since quadrupolar relaxation is affected by symmetry, the N14 relaxation is now slow which leads to splitting on the H. Since N14 is I = 1, the splitting is a 1:1:1 triplet with roughly equal intensity. For comparison, the student had acquired at higher pH and only 1 resonance was observed and the peak was broadened due to exchange.
2) 1) 90 degree angle/3bond coupling, 3JNH = 0 so no HMBC peak
2) 2JNH can also be ~0, especially aromatic 2JCH so no HMBC peak
3) long T1 relaxation time for relevant proton/d1 too short in either HSQC or HMBC or both
4) short 1H T2, broad line, so peak is suppressed; could be issue in either spectrum (likely due to H1 exchange for NH)
5) short 15N T2, could be issue in either spectrum, but especially hmbc due to long delay for multiple bond transfer (protonated Nitrogens have shorter T2 relative to ones without attached protons, so overall spectrum could be good, but NH or especially NH2 nitrogens may not show up, see NHMBC for amides)
6) Multiple bond coupling constant not close in one direction or other relative to input jnxh value. Attempt to measure coupling by 1D 1H and look for 3JNH coupling in baseline or estimate 3JNH based upon structural model/estimate based upon model compounds/Karplus curve. Reacquire HMBC with different JNXH value
7) There is no 2 or 3-bond JNH possible in the structure. Probably should not have acquired the HMBC in the first place (unless you were looking for other correlations)
8) The 1H or 15N resonance are at the very edge of the spectrum, so the 1H is more than ~10ppm or less than 0 the N15 is more than 100ppm from the center. Either the 1H or 15N or off-resonance and suppressed because of this as they are not feeling full 90/180 pulses.. Reacquire with different tof/dof to fit the chemical shift. (see NHSQC spectrum from project and 2nd attempt)

Also-
9) Long 1H T2 for relevant proton; normally long T2 is good, but possibly suppressing signal to noise a little bit by short at so peak is broader than it could be so smaller in height relative to noise; increase at, reacquire HMBC or simply add in zero-fill (see 15N HMBC peak 8.15/~150; much more intense with zero fill or with longer at)
9) Long 15N T2 for relevant carbon; increase ni and reacquire HMBC or add in zero-fill, linear prediction and weight over LP points
11) sample degraded during experiment so the 2 parts are not linked
12) 1H/1H couplings during t1- HMQC type experiments have observable 1H/1H couplings and that causes observed splitting in t1 from the homonuclear coupling- could use CT-HMBC [note in general CT-HMBC is a poorer experiment- but can be an issue with gHMBC]
13) Resolution is too poor to resolve correlation, more ni or np or change temp/solvent
3) Long T1/T2, should be downfield resonance/double bond, probably choose one of the 2 resonances from nucleoside. The relaxation times are long as the ring is rigid, and not close to any other protons.

Long T1/short T2, should be exchangeable peak as the amides do have reasonably long T1s, not that close to a lot of other protons, fairly rigid, but they exchange with each other and with H2O which leads to a short T2.

Short T1/T2, should be upfield resonance, especially a sugar resonance, as they are very close to multiple other protons which leads to short relaxation times both T1 and T2.
4) As temperature is increased the rotation around N-C bond on the NH2 increases, leading to a single NH2 resonance at higher temperature. Also, the H2O resonance shifts upfield as temperature is increased, and any resonance that exchanges with H2O also shifts, so the OH resonances and the NH2. DMSO briefly gets sharper as it tumbles faster at higher temperature; at the highest temperature it broadens somewhat, presumably due to either shimming or temperature instability. Some of the CH resonances shift very slightly presumably due to slight structural changes, and increased motion around the glycosidic bond and the conformation of the sugar ring. Slight changes are observed for the CH resonances, of ~0.01-0.03ppm, which could be related to structural changes caused by Hydrogen bonding, or rotation of the base or change in sugar conformation.

The 300 MHz and 600 MHz are different time scales, so the chemical shift between the resonances is the same in ppm, but half as much in Hz. The rotation is the same at the same temperature on both instruments, but how that relates to the frequency difference in Hz is different- what is fast at 300 is not fast at 600.

Also, note the chemical shift is the same at 45c on both instruments, but downfield at 38c (see Part A).
PART B.
The rotation of the NH2 is faster on adenosine than cytidine, presumably due to partial double bond in cytidine from resonance structures in contrast to adenosine, which does not have them, so it is a single bond.
PART C.
The rotation is slower in the RNA helix of the amino because it is Hydrogen bonded to the carbonyl of uridine, which slows the rotation. Thus, 2 separate shifts are observed for the amino. Also, the difference in chemical shift is much greater than in the free nucleoside because of the Hydrogen bonding and because of different ring current shifts from the base stacking above and below the adenosine in the helix.
The integration is affected by a combination of T1, NOE and off-resonance effects.

Turning off the NOE will decrease the signal significantly for the ribose resonances. It will also decrease the signal for the CH on the base. There is some NOE for the 2 ring carbons without attached protons, but that is minimal.

Increasing the delay will increase the signal per scan for every resonance; however, the 2 ring carbons without attached protons will increase the most per scan as they have the longest T1. The other 2 downfield carbons- the CH and CF would have comparable relaxation times, longer than the ribose carbons, but shorter than the other 2. Increasing the delay will help them somewhat per scan.

In the initial scan the CF was weaker than the CH as the CH has significant NOE, but the CF does not have CH NOE; instead, the CF could have a large CF NOE, but no F19 decoupling is possible simultaneous to the H1 decoupling.

Overall signal to noise is way worse as the loss of NOE is significant and the loss of about a factor of 4-8 in scans, so about a factor of 5-10 loss for the ribose carbons, but only about a factor of 2 or so for the carbonyl/CNH2.
Suppression of the water suppresses anything which exchanges with water on the NMR time scale. The OHs are suppressed as they exchange with water faster than the NH2 does at room temp. None of the peaks are completely suppressed because the T1 is short relative to the exchange rates so you can never completely suppress the OHs or NH2 by saturating water under these conditions. At higher temperature, you would see more suppression of the OHs and NH2 as the exchange rate increases without much change in T1. The gain value should be higher than in a normal 1D as the largest peak in the spectrum is no longer H2O, so the instrument can amplify the other signals more in the receiver/analog-to-digital converter without overflowing which leads to somewhat higher signal to noise.
7) The sample is 5-Fluorocytidine, and the nucleotide is β. To determine, you need to acquire 1D NOE. You should see NOEs to the H4’, H6 and H2’. The H2’ is on the opposite side but is not that far away in space as the angle between them is not perfectly trans. The H4’ NOE is what tells you that the H1’/H4’ are on the same side. At higher temp, you might get a little better data as the molecule tumbles faster in solution so you get further away from the NOE crossover point where NOE is 0. At 600 at 20C, it is very close to the crossover point, so there is little to no NOE or even somewhat negative NOE. Thus, at 600 the tumbling of the molecule in viscous solvent is slow relative to the magnetic field, while it is fast relative to the magnetic field on the 300 especially at 50C. In less viscous solvent, NOE would be positive at 600 for ~300MW molecule but not in DMSO/D2O; also positive at 50C on 600. Thus, for DMSO/D2O 1DNOE should be acquired at higher temperature, or should use ROE type experiment.
8) The D2O sample is TSP, so it has Na and Si 
Molecule 1 and has C13 spectrum 2 
Molecule 2 and C13 spectrum 1

The correlation to C13 data:
The sharp Boron peak means that its T2 is long, so that will cause the C13 to see Boron10 and 
Boron11 coupling; since B11 is spin 3/2, that leads to 2*I+1 lines = 2*3/2+1 = 4; since B10 is spin 
= 3, then 2*3+1 = 7. You could predict that would be the sharp Boron resonance because the 
Boron will be tetrahedral and has 4 equivalent groups attached to it, so highly symmetric which 
leads to sharp lines on quadrupolar nuclei.

The broader Boron peak means its T2 is shorter so the C13 sees an average coupling. However, 
it is not so fast that the C13 is sharp with no B-C coupling, it is intermediate so broad C13, not 
sharp and not split. You could predict that molecule 2 would fit that because it will be trigonal, 
so faster relaxation; overall a small molecule and somewhat symmetric on what the Boron is 
bound to, so not a super broad Boron peak, but moderately broad.

If you look closely at the C13 of the molecule 1, you will see that there is an isotope effect, so 
the B11-C13 chemical shift is not the same as the B10-C13 chemical shift. Also if you look real 
closely at the coupling constants, you will see that they are not the same but are actually the 
nearly exact ratio of the B11:B10 gyromagnetic ratio, ~50 Hz: 16.5 Hz in 1JCB and 3:1 in 
gyromagnetic ratio

For Silicon, you needed to have a long d1 and a number of scans to observe the peak, and dm = 
‘nny’ as Silicon gyromagnetic ratio is negative. Silicon could also be done as a DEPT, that would 
help a lot. To do that, you would have needed to acquire a 1D 1H to see if there is coupling to 
1H, then measure that coupling, and use it as the JXH parameter in DEPT. You could have also 
done HMBC for this. While the sample was moderately concentrated, the signal to noise for 
silicon is not that good- moderately low abundance and low gyromagnetic ratio, also no NOE, 
long T1… If the parameters were suboptimal, then you probably observed nothing.

Boron and Sodium are both quadrupolar, so shorter acquisition time, and short d1 could help 
you see the resonances faster, although both are sensitive enough that it does not matter 
much. For Boron, the large peak from the glass gets in the way of observing real peaks, but the 
real peaks are either very sharp relative to the glass or moderately sharp relative to the glass. 
Since the glass a longer T1 than the real peak, you could improve the spectra by using a large 
number of ss to saturate it, before acquiring real data. Also, acquiring data with an empty NMR 
tube or a tube with a sample an no Boron (so the CDCl3 standard would work), you can see the 
glass peaks and compare to the spectra that you get.

Chloroform:
The solvent is mostly CDCl₃, but there is some residual CHCl₃. The small peak is the CHCl₃ peak, and there is an isotope shift of about 0.2 ppm between H1 and H2. The CHCl₃ peak is a singlet as the H1 is decoupled. The CDCl₃ peak is a triplet as deuterium is spin = 1, so 2NI + 1 = 2*1*1+1 = 3. The ratio is 1:1:1 as a spin = 1 triplet is 1:1:1.

There are a large number of lines that could be observed but are not because of T2 relaxation issues. The 2 separate reasons are isotope shift from the chlorine as in Part A, and quadrupole splitting from chlorine as in this part to Deuterium.

Both Cl35 and Cl37 are spin = 3/2, so 2NI + 1 = 2*3*3/2 + 1 = 10. The ratio would be: 1:3:6:10:12:12:10:6:3:1 for both Cl35 and Cl37. You do not see these lines as the chlorine relaxation is fast on the NMR time scale so the carbon sees an average, so just one line to chlorine. You do observe the splitting to the quadrupolar Deuterium, but Deuterium relaxation is much slower than Chlorine. The difference relative to the Boron is that Cl35 or 37 are both much shorter T2 than either Boron, so there is not much broadening observed for CDCl₃ or CHCl₃. The Deuterium is comparable to the tetrahedral Boron, so you do see splitting. The isotope shift of CHCl₃ relative to CDCl₃ is bigger than the Boron shift, as H is smaller than B.

The other possibility is to observe isotope shift from the chlorine. This isotope shift would be more complicated than in Part A as there are 3 Chlorines, so you could have 3 Cl35s, 3 Cl37s, 2 Cl35s and 1 Cl37, or 1Cl35 and 2 Cl37s, so instead of 2 lines as in Part A, there would be 4. Instead of a ratio of 3:1, the ratio would be: 27:27:9:1 [Cl35:2Cl35/1Cl37:1Cl35/2Cl37:Cl37]. The isotope shift is not observed because the Carbon line is too broad to observe them. The shift is only ~0.01 ppm, but the linewidth at half height is ~0.04 ppm. While acquisition/processing/shimming parameters might not have been optimal to observe the isotope effect, the primary reason for not observing it is the line width/T2 of CDCl₃. The Carbon resonance is broad relative to Part A because the Carbon on CDCl₃ is coupled to 4 quadrupolar nuclei. While the coupling to the 3 Chlorines is not observed, the coupling is still there and induces more rapid relaxation of the Carbon. In addition, the coupling to Deuterium also increase the relaxation of the Carbon due to the quadrupole effect. The difference in Part A was that carbon was bonded to 2 spin = 0 nuclei (Carbon), 1 spin = ½ (Hydrogen) and the 1 Chlorine, so T2 relaxation of it was much slower than CDCl₃, so sharper line, more observable. In addition the large number of potential lines also broaden the observed resonance for CDCl₃.

The total number of possible lines would be: 4 (deuterium splitting + CHCl₃(if H1 decoupling on)) * 10 (chlorine splitting) * 4 (isotope shift chlorine) = 160.

If the Cl35/C13 coupling were different than Cl37/C13, I think it would be 240 (the CD/HCl35(3) or CD/HCl37(3) would have 40 lines, the CD/HCl35(2)Cl37(1) and CD/HCl35(1)Cl37(1) would have 80 each.
If H1 decoupling were turned off, then $5 \times 10^4 = 200$ or 280 with different Cl/C coupling constants.