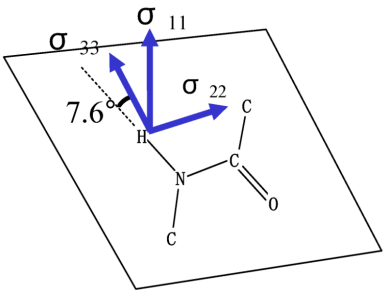
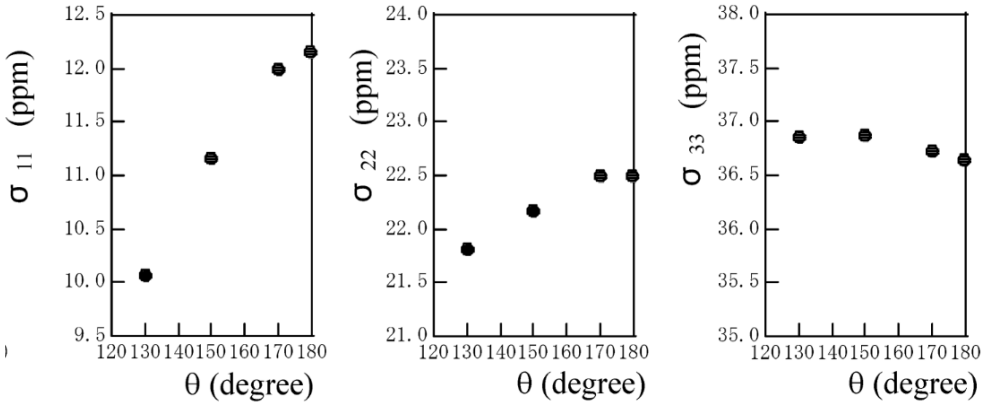
Name _____

Final Exam: CHEM/BCMB 8190 (228 points) Monday, 1 May, 2017

INSTRUCTIONS: You will have three hours to work on this exam. You can use any notes or books that you bring with you to assist you in answering the questions. You cannot leave the examination room to retrieve additional notes or books. No electronic devices of any kind are allowed, except for a hand-held calculator. No access of any kind to the internet is allowed. Please write your answers on this exam in the space provided. Make certain to write your name on the exam. Please do not detach any pages from the exam (leave it stapled and intact). *If a question asks for a calculation, simply 'setting up' the calculation or writing down an equation is not adequate. You must complete the calculation for credit.* There is a table at the end of the exam with information you may find useful.

1) The shielding tensor elements for the hydrogen-bonded amide proton of N-methylacetamide (hydrogen bonded to formamide) were calculated using *ab initio* molecular orbital methods as a function of the hydrogen bond angle (θ). The directions of the chemical shielding tensor components (σ_{11}, σ_{22} and σ_{33}), along with the chemical structure, are shown in the figure (right). The values of the tensor elements as a function of θ are shown in the plots below.





If, in solution, the hydrogen bond angle is 150°, and the chemical shift of this amide ^{1}H nucleus is 9.0, what would be the chemical shift of this ^{1}H nucleus in solution if the hydrogen bond angle was 170° instead of 150°? You will have to show your work for credit. (**8 points**)

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2) A protein contains the following unassigned amino acid sequence:

….. N P T E A E L Q D M I N E V D A …..

You have data from several triple resonance experiments for the residues in this unassigned region, and the data is complete (no missing peaks). For each amide $^{1}H_{i}$, $^{15}N_{i}$ pair, you have correlated the $^{13}C\alpha_{i}$, $^{13}C\beta_{i}$, $^{13}C\alpha_{i-1}$, $^{13}C\beta_{i-1}$, $^{1}H\alpha_{i-1}$, $^{1}H\beta_{i-1}$, and $^{13}C'_{i-1}$ (carbonyl) chemical shifts.

a) For residue *j*, the following correlations are observed:

$^{13}C\alpha_{j}$ / $^{13}C\beta_{j}$ = 64.1 ppm / 39.1 ppm

$^{13}C\alpha_{j-1}$ / $^{13}C\beta_{j-1}$ / $^{13}C'_{j-1}$ = 57.0 ppm / 35.8 ppm / 178.3 ppm

Which amino acid in the sequence is residue *j* ? You will have to provide a proper explanation for credit. (**6 points**)

b) What do you think is the secondary structure of the region of the protein that residue *j* is in? Why (you will have to justify your answer for credit)? (**4 points**)

c) For amino acid *k*, you have correlated $^{1}H_{k}$, $^{15}N_{k}$, $^{13}C\alpha_{k-1}$, $^{13}C\beta_{k-1}$ chemical shifts. In order to link residue *k* to the preceding residue in the sequence, you need to find a ^{1}H, ^{15}N pair with intraresidue $^{13}C\alpha$ and $^{13}C\beta$ correlated chemical shifts that match $^{13}C\alpha_{k-1}$, $^{13}C\beta_{k-1}$. Even though your data is complete (no missing peaks), you cannot find these matches. What is the identity of residue *k*? You will have to provide a proper explanation for credit. (**6 points**)

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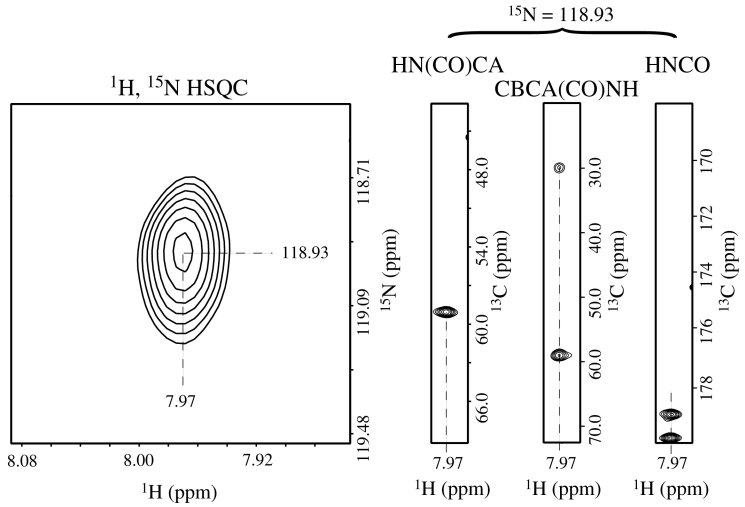
3) Regions from two- and three-dimensional heteronuclear and triple resonance experiments for a particular protein are shown. The displayed regions are plotted near the baseline, and the entire ^{13}C spectral widths are shown for the triple resonance data sets (i.e. no peaks are "missing" from the spectra). Please provide a good explanation for why two signals are observed in the displayed region of the HNCO spectrum. (**6 points**)



4) When assigning the side chain resonances for a particular Val residue in a protein using amide resolved TOCSY spectra (C(CO)NH and H(CCO)NH), peaks were found with the following chemical shifts:

C(CO)NH: ^{13}C = 21.0 ppm, 22.0 ppm H(CCO)NH: ^{1}H = 0.8 ppm, 0.9 ppm

a) What nuclei of the Val residue can these chemical shifts be assigned to? Please explain for credit? (**4 points**)

b) What additional information could potentially be obtained for these shifts/nuclei from an HCCH-TOCSY experiment? (**4 points**)

5) In a recent publication, Arora et al. (*Nat. Struct. Biol*. **8**, 334-338 (2001)), determined the three-dimensional fold (low-resolution structure) of a membrane protein (19 kDa, 177 amino acids) in micelles. The protein (transmembrane domain of OmpA) that they used was nearly completely (~98%) deuterated at nonexchangeable sites.

a) Why, in general, are proteins sometimes deuterated for NMR studies? For credit you must explain any assertions you make. (**4 points**)

b) Given the relatively small size of OmpA, why did the researchers use deuterated OmpA? (**6 points**)

c) In their assignment procedure, the researchers used an experiment called HN(CA)CB. What nuclei are correlated in this experiment, and how does this differ from the HNCACB experiment? (**4 points**)

d). The authors did not use the CBCA(CO)NH experiment in their assignment strategy. Why? (**6 points**)

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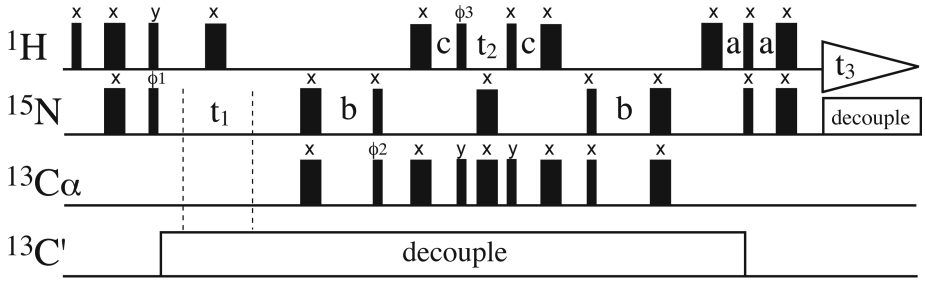
6) Consider the three-dimensional, triple resonance pulse sequence for correlating chemical shifts in isotopically labeled proteins shown below:



a) For amino acid *i* in a protein, the chemical shifts for what nuclei in *i*, or adjacent amino acids, are correlated, or might be correlated, by this experiment? You will have to provide a proper explanation for credit. (**8 points**)

b) In the pulse sequence are two time periods labeled 'a' and 'c'. What is the ratio of 'a' to 'c'? Please explain for credit. (**6 points**)

c) What would be a good name (acronym) for this experiment? Please explain why. (**4 points**)

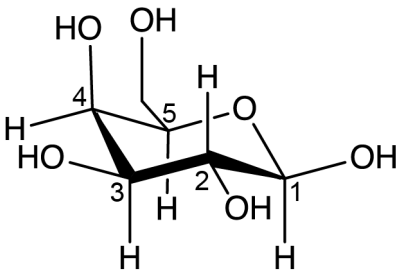
d) Would you expect this experiment to be more or less sensitive than the HNCO experiment? than the HNCA experiment? Please explain for credit. (**4 points**)

e) Triple resonance assignment experiments often come in pairs, for reasons we discussed in class. Examples are HNCA/HN(CO)CA, HNCACB/CBCA(CO)NH, for instance. Among those triple resonance experiments that we discussed in class, which one could serve to pair with the experiment above? (**4 points**)

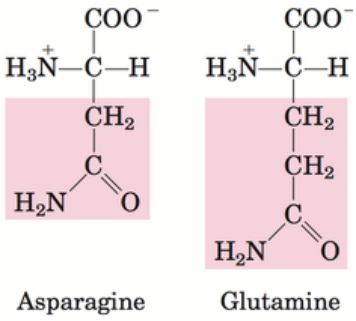
Name _____

7) A hydrogen exchange experiment is conducted observing the intensity of amide proton resonances in a protein in H_2O as a function of time after dilution with D_2O. At pH 7.5 a proton has a half-life of 3 hr. Assuming an EX2 mechanism, what half-life do you expect at pH 6.5? You will have to explain for credit. (**8 points**)

8) Residual dipolar coupling contributions to splitting of carbon signals in the indirect dimension of a coupled ^{13}C-^{1}H HSQC spectrum of a galactose (below) containing oligosaccharide show values of +3.0 Hz, -5.0 Hz, -5.1 Hz and -5.3 Hz for cross peaks that will eventually be assigned to the H2, H3, H4 and H5 cross peaks (not necessarily in this order). Based on these data, what can you say about the relative orientations of the four C-H vectors, and which RDC is from H4? You will have to explain your reasoning for credit. (**6 points**)



9) Which triple resonance experiment(s) could be used to correlate the ^{15}N chemical shifts of side chain $–CH_2$-(C=O)-NH_2 groups of glutamine or asparagine residues in proteins with side chain ^{13}C chemical shifts? Explain. (**6 points**)



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10) The one bond ^{15}N-$^{13}C\alpha$ coupling constant in a peptide is 7 Hz. What do you expect the ^{15}N=^{13}C one bond coupling constant in a histidine sidechain ring to be? Provide a calculation and explanation for credit. (**8 points**)

11) Would you expect the ^{1}H signal of CHF_3 to be up field or down field of a CH_4 resonance? Please explain why for credit. (**4 points**)

12) Sketch the ^{1}H NMR signal for $CHCF_3$. If it is a singlet, please explain why. If it is a multiplet, please explain the multiplet pattern, the relative intensities of the peaks, and what the significance is of the distance between the peaks. (**6 points**)

Name _____

13) Calculate the direct product (Kronecker product) of the two matrices below. (**4 points**)

$$A = \begin{bmatrix} 1 & -2 \\ -1 & 0 \end{bmatrix} \qquad B = \begin{bmatrix} 4 & -3 \\ 2 & 3 \end{bmatrix}$$

14) Schemes based on relaxation interference mechanisms (cross-correlation effects) can dramatically improve resolution for large molecules at high magnetic field strengths. It has been reported that signals from aromatic rings (phenylalanine) can benefit significantly from these effects (otherwise, these signals are broad, and due to chemical shift degeneracy are often overlapped and difficult to interpret). For C-H groups in phenylaline rings, provide an explanation for these effects. You should include a discussion of what effects are involved, how they interact (interfere), and what the result is. (**8 points**)

Name _____

15) A molecule with a correlation time of 3 ns is found to have an ^{15}N spin lattice relaxation time of 0.5 s at 18.7 T (800 MHz for ^{1}H) for a protonated amide site. Will the relaxation time increase or decrease as we lower the temperature? You will have to do a calculation (show your work) and justify your reasoning for credit. (**8 points**)

16) The following is the density matrix for a pair of ^{1}H nuclei at a time just prior to acquisition. What is the value of the *y*-magnetization at the beginning of acquisition. You will have to do the appropriate calculation and show your work for credit (you can leave out the constants). (**8 points**)

$$\sigma(t) = \begin{bmatrix} 0.5 & 0.1 & 0.1i & 0.5 \\ 0.1 & 0 & 0.2 & 0.1i \\ -0.1i & 0.2 & 0 & 0.1 \\ 0.5 & -0.1i & 0.1 & -0.5 \end{bmatrix}$$

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17) The T_2 of ^{15}N in an HSQC experiment conducted using a 500 MHz magnet (11.75 T) is 0.1s. Signals in the ^{15}N dimension are spread over 30 ppm. How many complex t_1 points should you collect? (**6 points**)

18) In an SAR by NMR two ligands are identified that bind to adjacent sites on a protein with binding constants of 1500 M^{-1} and 2000 M^{-1} respectively. They are then successfully linked for form a single ligand. What would you expect for an approximate binding constant? (**4 points**)

19) A TROSY version of an ^{15}N-^{1}H HSQC spectrum is collected on a large protein. How do the positions of peaks in this spectrum relate to positions in a normal HSQC? For credit you should draw a sketch and indicate distances between signals/peaks that are relevant. (**6 points**)

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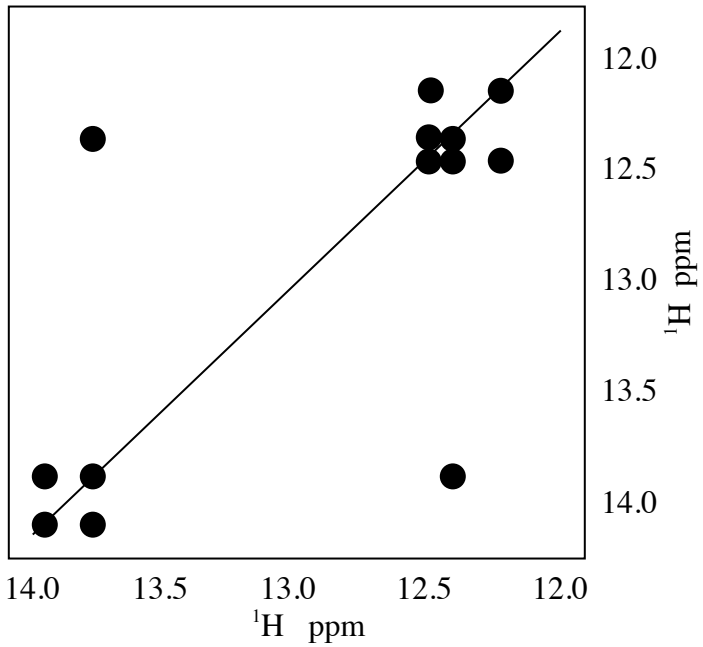
20) The spectrum (right) represents the imino portion of a ^{1}H-^{1}H NOESY on the following RNA molecule that dimerizes to form an A form helix. From left to right, the first 5 bases are labeled/numbered (as shown below):

1 2 3 4 5
GGCUUAAGCC



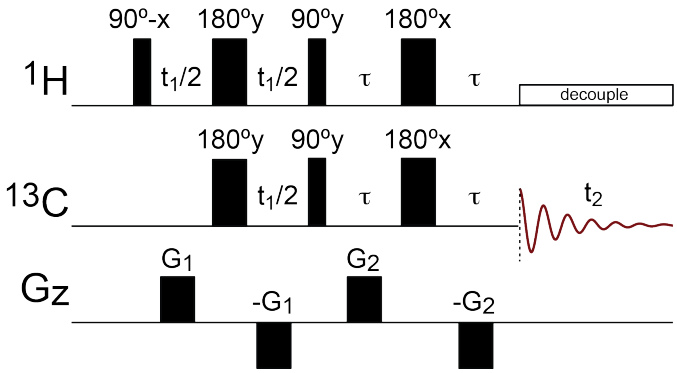
a) Give a probable assignment for the five signals shown in the NOESY spectrum. You should label the diagonal peaks with the correct assignment (1, 2, 3, 4, or 5). For credit, you must also explain your reasoning. (**10 points**)

b) How do chemical shift positions help in assignment for RNA molecules such as this? (**4 points**)

c) How would information from a ^{15}N-^{1}H HSQC help in assignment for RNA molecules such as this? (**4 points**)

Name _____

21) The pulse sequence shown (right) is of a 2D experiment used for molecules with ^{1}H bound directly to ^{13}C (^{1}H-^{13}C). The delay τ is set to $1/(4J_{CH})$.



a) Just after the final 90° pulse on ^{13}C, from a product operator analysis, what are the important terms (ignore the pulsed field gradient pulses). (**8 points**)

b) Based on your answer to 'a', what is measured in t_1 of this experiment? (**6 points**)

c) The pulsed field gradients are used to select for a pathway from ^{1}H to ^{13}C magnetization, while suppressing signals from ^{1}H nuclei attached to ^{12}C. The magnitudes of the first two gradient pulses are G_1 and the magnitudes of the second two are G_2. What should be the ratio of G_2 to G_1 for this pulse sequence to work as designed? (**6 points**)

d) What is the purpose of the final τ-(^{1}H 180°x/^{13}C 180°x)-τ element of the pulse sequence? Please explain what is accomplished during this element and why it is included. (**6 points**)

Name _____

22) The chemical shift range for ^{19}F (γ = 25.1815 x 10^7 rad Tesla^{-1} s^{-1}) is quite large; ~1300 ppm. For a static magnetic field strength of 9.4 Tesla, what is the frequency difference between two ^{19}F signals separated by 1300 ppm? You must do a calculation and show your work for credit. (**6 points**)

23) Proton line widths for non-exchanging amide protons in myoglobin are approximately 15 Hz. Hemoglobin is a tetramer of subunits that are approximately the same size as myoglobin. What would you expect for line widths of non-exchangeable amide protons in hemoglobin? Provide a complete justification of your answer for credit. (**6 points**)

24) Five seconds after application of a 90° pulse, only 10% of the original (equilibrium) magnetization has returned to *z*. What is the approximate T_1 relaxation time constant? You must do a calculation and show your work for credit. (**6 points**)

Name _____

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You may find some of the information in this table useful:

Nuclide	Spin *I*	Natural abundance (%)	Relative sensitivity	Gyromagnetic ratio, γ (10^7 rad T^{-1} s^{-1})	NMR frequency (MHz @ B_0 = 2.3488 T)
^{1}H	1/2	99.985	1.00	26.7519	100
^{2}H	1	0.015	9.65×10^{3}	4.1066	15.351
^{3}H	1/2	~0	1.21	28.5350	106.664
^{10}B	3	19.58	1.99×10^{-2}	2.8747	10.746
^{11}B	3/2	80.42	0.17	8.5847	32.084
^{12}C	0	98.9	-	-	-
^{13}C	1/2	1.108	1.59×10^{-2}	6.7283	25.144
^{14}N	1	99.63	1.01×10^{-3}	1.9338	7.224
^{15}N	1/2	0.37	1.04×10^{-3}	-2.7126	10.133
^{19}F	1/2	100	0.83	25.1815	94.077
^{31}P	1/2	100	6.63×10^{-2}	10.8394	40.481
^{103}Rh	1/2	100	3.11×10^{-5}	-0.846	3.1474
^{195}Pt	1/2	33.8	9.94×10^{-3}	5.8383	21.499