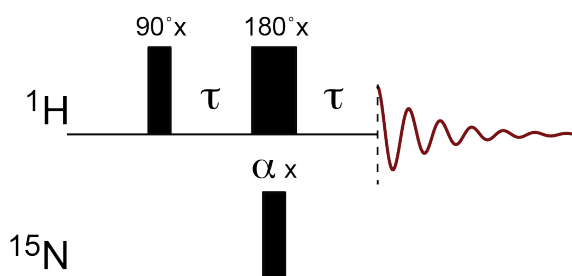Name _____

Midterm Exam: CHEM/BCMB 8190   (148 points)   Friday, 3 March, 2017

INSTRUCTIONS: You will have 50 minute 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) Assume you have a ^{1}H-^{15}N spin system. Using product operators, demonstrate that the spin-echo sequence below will refocus ^{1}H-^{15}N heteronuclear coupling. You can ignore chemical shift evolution (the spin echo refocuses chemical shift evolution). You must show your work for credit. (**10 points**)
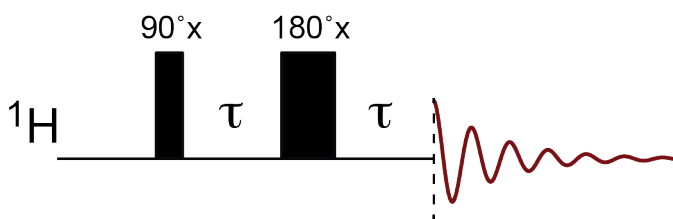
2) The pulse sequence below can be used to calibrate ^{15}N pulse widths indirectly. For this purpose, τ must be set to 1/(2J) where J is the one bond ^1H-^{15}N coupling constant ($^1J_{HN}$). The variable α_x represents a pulse on x whose pulse angle is varied. In problem #1 (above), you demonstrated what would happen if the pulse angle is 0 (i.e. $\alpha_x = 0°$ x, assuming τ =1/(2J)). Here, using product operators, you should examine the cases where $\alpha_x = 90°$ x and $\alpha_x = 180°$ x. Then, with that information, and the information from problem #1, explain how this pulse sequence is used to calibrate the ^{15}N pulse angle. You will have to show your work and provide an explanation for credit (there is also room on the following page for you to use if necessary). (**20 points**)

3) Below is an energy level diagram that can be used to determine the spin (spin quantum number) of a nucleus using the shell model.

a) For ^{31}P (atomic number 15), using the shell model rules, determine the spin of ^{31}P. Use the energy diagram below. Fill in the levels with the proton and neutron spins, and then state what the spin is, according to the model and the diagram, and why. (**6 points**)

n+1	protons	neutrons	j (j = l ± ½)	degeneracy (2j + 1)	total
2s (l=0)	—	—	1/2	2	20
1d (l=2)	— —	— —	3/2	4	
	— — —	— — —	5/2	6	
1p (l=1)	—	—	1/2	2	8
	— —	— —	3/2	4	
1s (l=0)	—	—	1/2	2	2

b) If your answer differs from the actual spin of ^{31}P, please explain the likely reason, and then make the appropriate adjustments to the model to get the correct answer. Please provide a good justification for credit. (**6 points**)

4) In the NMR text by Horst Friebolin text, the author discusses ^{1}H, ^{1}H couplings in benzene compounds:

*"In benzene and its derivatives, the ortho, meta, and para couplings are different, and by analyzing the aromatic region of the proton spectrum one can therefore determine the arrangement of the substituents. **It is often possible to analyze the spectrum by first-order methods, particularly if the spectra have been recorded at high resonance frequency**".*

Knowing that the chemical shift range of the (coupled) ring protons of benzene derivatives is small, please explain in detail the highlighted (**bolded**) sentence. (**6 points**)

5) For a first order, two spin AX (^{1}H, ^{1}H) system, calculate the transition probability for the $\beta\beta \rightarrow \alpha\alpha$ transition. Write down the Hamiltonian you will use, then do the calculation AND explain the meaning of your result. You will have to show your work for credit. (**6 points**)

Name _____

6) For the molecule shown, if the chemical shift of the indicated (bolded) hydrogen is 3.3 ppm, what is the distance between this hydrogen and the benzene ring below it. You will have to justify or otherwise explain your answer/reasoning for credit. (**4 points**)
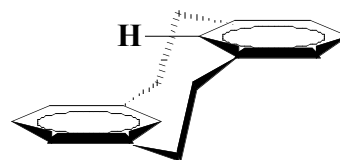
7) The shielding tensor elements for the hydrogen-bonded amide proton of N-methylacetamide (hydrogen bonded to formamide) were calculated using *ab initio* molecular orbital methods as a function of the hydrogen bond angle (θ). The directions of the chemical shielding tensor components (σ_{11}, σ_{22} 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.
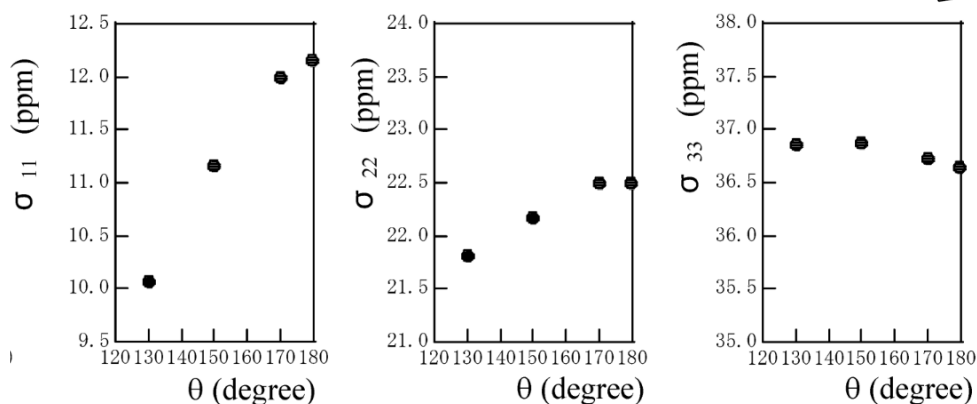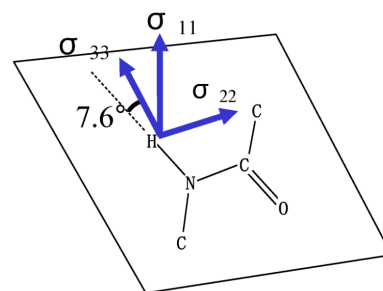
If, in solution, the hydrogen bond angle is 180°, and the chemical shift of this amide ^{1}H nucleus is 8.0, what would be the chemical shift of this ^{1}H nucleus in solution if the hydrogen bond angle was 130° instead of 180°? You will have to show your work for credit. (**8 points**)

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8) A 2D ^{13}C-^{103}Rh HSQC spectrum of a mixture (approximately equimolar) of three related compounds (below, right) at natural isotopic abundance is shown below. Projections along the x- and y-axes are shown above the 2D spectrum and on the left side of the 2D spectrum. The HSQC pulse sequence used to acquire the spectrum is shown below.
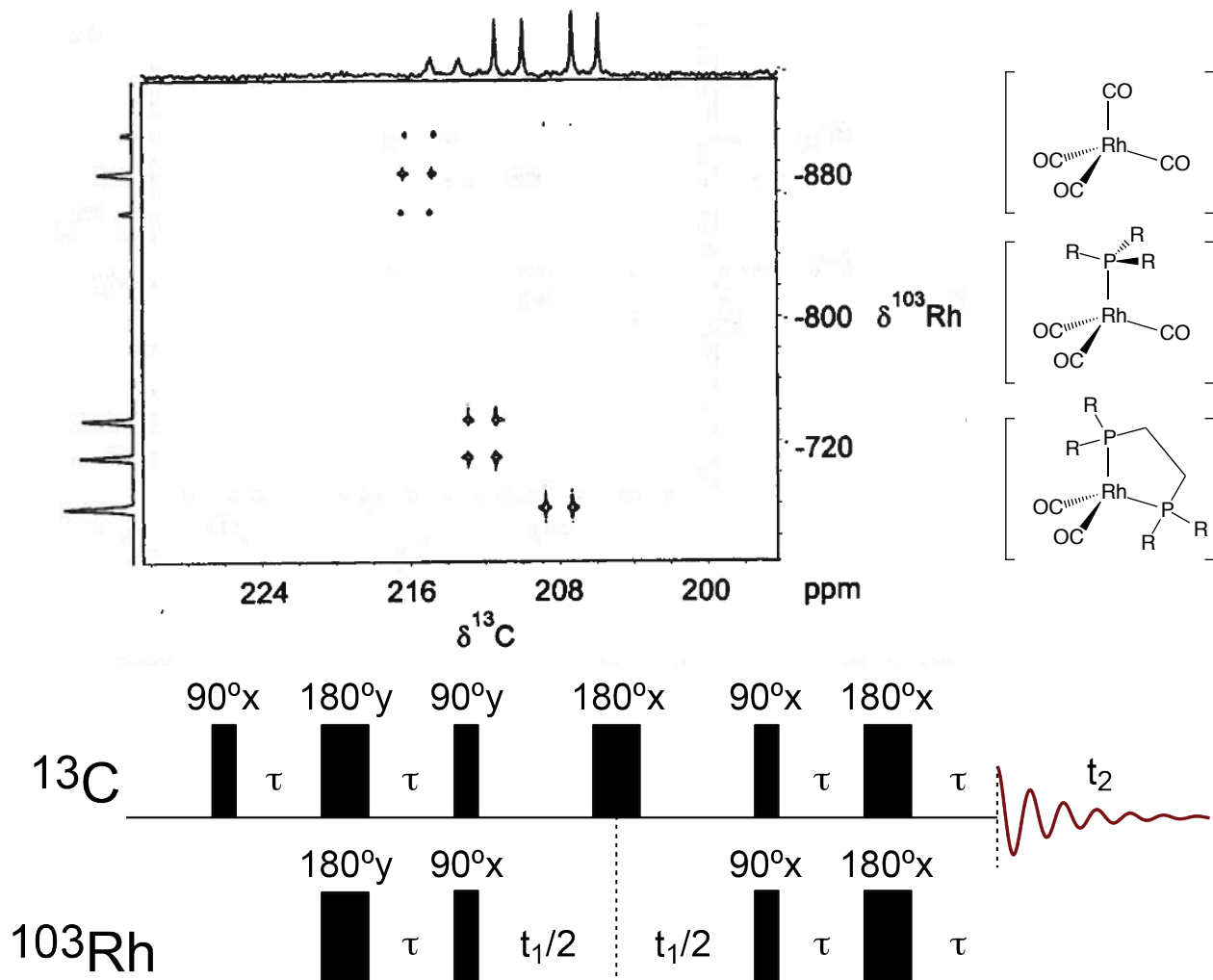
a) There are three signals in the HSQC spectrum, each split into multiple peaks. Circle each of the three signals. Then label the signal from the least shielded ^{13}C nucleus/nuclei '1', the most shielded '3', and the remaining signal '2'. (**4 points**)

b) For each signal, in the ^{13}C dimension, state what the multiplet structure is (singlet?, doublet?, septet?) (**4 points**)

c) For each signal, in the ^{103}Rh dimension, state what the multiplet structure is (singlet?, doublet?, septet?) (**4 points**)

d) For each signal, _explain in detail_ what is giving rise to each signal. Your answer must include:
-the compound or compounds responsible for each signal.
-an explanation of the coupling pattern in each dimension based on the compound or compounds responsible for producing the signal.
-an explanation as to how the HSQC pulse sequence shown is consistent, or not consistent, with your analysis of the splitting of each signal in each dimension. (**12 points**)

9) The linewidths of NMR signals are dependent on transverse relaxation:

$$\Delta \upsilon_{1/2} = 1/(\omega T_2) \text{ or } = 1/(\omega T_2^*)$$

For biomolecules, often T_2 is used instead of T_2^*. Why? Your answer must include an explanation of the difference between 'T_2' and 'T_2^*'. (**6 points**)

10) Are the matrices below orthogonal to one another? Please explain the definition of orthogonality for matrices in this context, and then do the appropriate calculations/operations that demonstrate whether the two matrices below are orthogonal to one another or not. You will have to show your work for credit. (**6 points**)

$$\mathbf{A} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix} \quad \mathbf{B} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix}$$

11) The rotation operators in matrix form for rotation about the *y*-axis are:

$$\hat{\mathbf{R}}_y = \begin{bmatrix} \cos(\omega_1 t/2) & -\sin(\omega_1 t/2) \\ \sin(\omega_1 t/2) & \cos(\omega_1 t/2) \end{bmatrix} \qquad \hat{\mathbf{R}}_y^{-1} = \begin{bmatrix} \cos(\omega_1 t/2) & \sin(\omega_1 t/2) \\ -\sin(\omega_1 t/2) & \cos(\omega_1 t/2) \end{bmatrix}$$

Using these operators and the appropriate matrix operations, show the result of applying a 90° pulse along the y-axis on equilibrium (*z*) magnetization. You will have to show your work and explain/interpret the final result for credit. (**10 points**)

12). Using a 400 MHz NMR instrument, the only two signals observed in the ^{1}H NMR spectrum of a particular compound are at -0.9 ppm and 12.3 ppm.

a) What is the frequency difference, in Hz, between these two signals when the ^{1}H NMR spectrum is acquired using a 600 MHz instrument? (you must show your work or clearly justify your answer for credit) (**6 points**)

b) What is the approximate resonance (Larmor) frequency for a normal ^{13}C nucleus using a 600 MHz (^{1}H) magnet? (you must show your work or clearly justify your answer for credit) (**6 points**)

13) A 90° pulse is applied to the equilibrium (*z*) magnetization. If, 5 seconds after the pulse is applied, only 10% of the bulk magnetization has returned to the *z*-axis, what is the magnitude of the T_1 relaxation time constant? You must do a calculation and show your work for credit. (**6 points**)

14) In a simple, one-dimensional NMR experiment, the experiment is repeated twice and the individual signals added together to improve the signal-to-noise. If only 90° pulses are used, the rule of thumb is to wait for a time equal to approximately $5 \times T_1$ (T_1 is the longitudinal relaxation time) between successive experiments in order to allow for "complete" re-equilibration of magnetization, and, thus, maximal signal-to-noise. If T_1 is 10 s, and the time between experiments is $5 \times T_1$, how complete is the re-equilibration (what percentage of the original, equilibrium magnetization will have returned to equilibrium)? You must do a calculation, and show your work, for credit. (**6 points**)

15) In the compound shown (right), the hydrogen **Ha** is coupled to Hb, Hc, and Hd. The approximate magnitudes of the coupling constants are: $^3J_{Ha,Hb} \approx 10$ Hz, $^3J_{Ha,Hc} \approx 10$ Hz, $^3J_{Ha,Hd} \approx 5$ Hz.
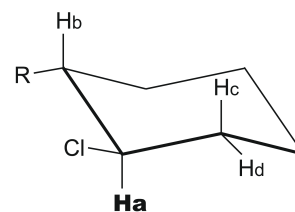
a) Please explain quantitatively the magnitudes of these coupling constants based on the Karplus relationship. (**6 points**)

b) If the molecule above can freely interconvert between two different chair conformations (below) on a fast time scale, and the equilibrium concentrations of the two forms are approximately equal, what will be the magnitudes of the coupling constants between **Ha** and neighboring hydrogens? To receive credit you will have to provide a complete and unambiguous explanation. (**6 points**)
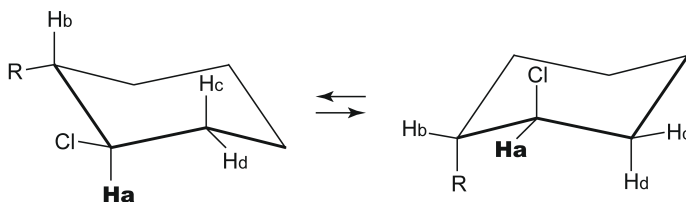
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You may find some of the information in this table useful:

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