**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**)
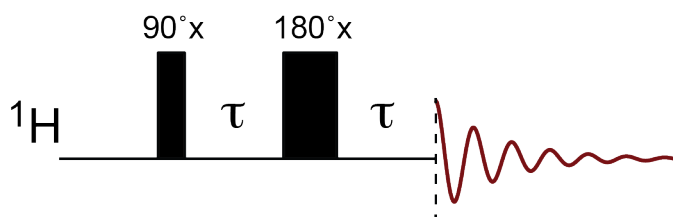
$$\mathbf{I}_{1z} + \mathbf{I}_{2z} \xrightarrow{\;\;\pi\!/_2\, \mathbf{I}1x\;\;} -\mathbf{I}_{1y} + \mathbf{I}_{2z}$$

$$\xrightarrow{\;\;2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}t\;\;} -\mathbf{I}_{1y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z}\sin(\pi J_{1,2}\tau)$$

$$\xrightarrow{\;\;\pi\mathbf{I}1x\;\;} +\mathbf{I}_{1y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z}\sin(\pi J_{1,2}\tau)$$

$$\xrightarrow{\;\;2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}t\;\;} +\mathbf{I}_{1y}\cos^2(\pi J_{1,2}\tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2z}\cos(\pi J_{1,2}\tau)\sin(\pi J_{1,2}\tau)$$

$$+2\mathbf{I}_{1x}\mathbf{I}_{2z}\cos(\pi J_{1,2}\tau)\sin(\pi J_{1,2}\tau) + \mathbf{I}_{1y}\sin^2(\pi J_{1,2}t) + \mathbf{I}_{2z}$$

$$\xrightarrow{\;\;simplify\;\;} \mathbf{I}_{1y} + \mathbf{I}_{2z}$$

The observable ^{1}H magnetization is $-\mathbf{I}_{1y}$ at the beginning of the spin echo (following the initial 90° pulse), and $\mathbf{I}_{1y}$ at the end, with no net modulation by $^1J_{HN}$.

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 ^{1}H-^{15}N coupling constant ($^{1}J_{HN}$). 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 $\tau = 1/(2J)$). 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**)
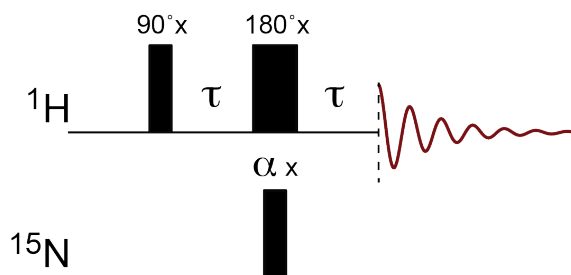
Here is the result for the = 90° x pulse.

$$\mathbf{I}_{1z} + \mathbf{I}_{2z} \xrightarrow{\frac{\pi}{2}\mathbf{I}1x} -\mathbf{I}_{1y} + \mathbf{I}_{2z}$$

$$\xrightarrow{2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}t} -\mathbf{I}_{1y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z}\sin(\pi J_{1,2}\tau) + \mathbf{I}_{2z}$$

$$\xrightarrow{\pi\mathbf{I}1x} +\mathbf{I}_{1y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z}\sin(\pi J_{1,2}\tau) + \mathbf{I}_{2z}$$

$$\xrightarrow{\frac{\pi}{2}\mathbf{I}2x} +\mathbf{I}_{1y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z}\cos(\pi J_{1,2}\tau)\sin(\pi J_{1,2}\tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2y}\sin^2(\pi J_{1,2}\tau) - \mathbf{I}_{2y}$$

$$\xrightarrow{2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}t} +\mathbf{I}_{1y}\cos^2(\pi J_{1,2}\tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2z}\cos(\pi J_{1,2}\tau)\sin(\pi J_{1,2}\tau)$$
$$+2\mathbf{I}_{1x}\mathbf{I}_{2z}\cos(\pi J_{1,2}\tau)\sin(\pi J_{1,2}\tau) + \mathbf{I}_{1y}\sin^2(\pi J_{1,2}t)$$
$$-2\mathbf{I}_{1x}\mathbf{I}_{2y}\sin^2(\pi J_{1,2}\tau) - \mathbf{I}_{2y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1z}\mathbf{I}_{2x}\sin(\pi J_{1,2}\tau)$$

$$\xrightarrow{simplify} \mathbf{I}_{1y}\cos^2(\pi J_{1,2}\tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2y}\sin^2(\pi J_{1,2}\tau) - \mathbf{I}_{2y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1z}\mathbf{I}_{2x}\sin(\pi J_{1,2}\tau)$$

$$\xrightarrow{\tau = 1/(2J)} -2\mathbf{I}_{1x}\mathbf{I}_{2y} + 2\mathbf{I}_{1z}\mathbf{I}_{2x}$$

first term is multiple quantum, so is not observed

second term is antiphase for spin 2, which is not observed if observing spin 1

Here is the result for the = 180° x pulse.

$$\mathbf{I}_{1z} + \mathbf{I}_{2z} \xrightarrow{\pi/2\, \mathbf{I}1x} -\mathbf{I}_{1y} + \mathbf{I}_{2z}$$

$$\xrightarrow{2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}t} -\mathbf{I}_{1y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z}\sin(\pi J_{1,2}\tau)$$

$$\xrightarrow{\pi \mathbf{I}1x} +\mathbf{I}_{1y}\cos(\pi J_{1,2}\tau) + 2\mathbf{I}_{1x}\mathbf{I}_{2z}\sin(\pi J_{1,2}\tau)$$

$$\xrightarrow{\pi \mathbf{I}2x} +\mathbf{I}_{1y}\cos(\pi J_{1,2}\tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2z}\sin(\pi J_{1,2}\tau)$$

$$\xrightarrow{2\pi J_{1,2}\mathbf{I}_{1z}\mathbf{I}_{2z}t} +\mathbf{I}_{1y}\cos^2(\pi J_{1,2}\tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2z}\cos(\pi J_{1,2}\tau)\sin(\pi J_{1,2}\tau)$$

$$-2\mathbf{I}_{1x}\mathbf{I}_{2z}\cos(\pi J_{1,2}\tau)\sin(\pi J_{1,2}\tau) - \mathbf{I}_{1y}\sin^2(\pi J_{1,2}t) + \mathbf{I}_{2z}$$

$$\xrightarrow{simplify} \mathbf{I}_{1y}\cos(2\pi J_{1,2}\tau) - 2\mathbf{I}_{1x}\mathbf{I}_{2z}\sin(2\pi J_{1,2}\tau) + \mathbf{I}_{2z}$$

$$\xrightarrow{\tau = 1/(2J)} -\mathbf{I}_{1y} + \mathbf{I}_{2z}$$

first term is transverse 1H magnetization

second term is 15N z magnetization (not observed)

So, for the 0° x pulse, the result is $\mathbf{I}_{1y}$, for the 90° x pulse there is no signal (signal of intensity = 0), and for the 180° pulse, the result is $-\mathbf{I}_{1y}$. The results show that the signal varies from a maximum intensity at 0°, through an intensity of zero at 90°, and to a maximum negative signal at 180°. The suggestion is that the intensity will continue back to zero intensity at 270° and to maximum intensity again at 360°. So, starting at 0°, as the pulse angle is slowly increased (either by changing the power of the pulse holding the length of the pulse constant, or by changing the length of the pulse holding the power constant), the intensity will reach zero when a 90° pulse is reached, thus calibrating a 90° pulse width. When the signal reaches a maximum negative value, that is the 180° pulse width (which should be twice the 90° pulse width).

Name _____

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			j	degeneracy	total
	protons	neutrons	(j = l ± ½)	(2j + 1)	
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

According to the shell model rules, because all neutrons are paired (an even number), their contributions are not considered. However, there is 1 unpaired proton in a level with j=3/2. According to the rules, the spin is 3/2.

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**)

The actual spin of ^{31}P is ½. According to the shell model rules that were laid out, "usually" higher values for j indicate lower energies. However, in this case, most likely the energy of the 2s level is somewhat lower than the 1d level with j=3/2 (the 2s level energy is between the 1d levels with j=3/2 and j=5/2). Thus, the unpaired proton would then populate the 2s level, where j=1/2, so the spin would then be ½.

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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**)

> *If the frequency difference, $\Delta\nu$, between coupled nuclei is large compared with the coupling constants, (i.e. if $\Delta\nu \gg J$), then the spectra/signals are considered to be first order. As a result, they can usually be analyzed by first order methods and usually display first order characteristics. Given the dependence of frequency on magnetic field strength, B_0 ($\Delta\nu = \gamma B_0/(2\pi)$), the frequency difference, $\Delta\nu$, between the ring protons in benzene and derivatives can be increased by increasing the resonance frequency, i.e. by increasing the magnetic field strength. This of course has no effect on J, thus the difference between $\Delta\nu$ and J is increased as is the first-order character.*

5) For a first order, two spin AX (^{1}H, ^{1}H) system, calculate the transition probability for the $\beta\beta \to \alpha\alpha$ 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**)

$$\hat{H}' = \gamma B_1(\hat{I}_{x1} + \hat{I}_{x2}) \qquad (\text{recall } \hat{I}_x|\alpha\rangle = 1/2\,\beta \quad \hat{I}_x|\beta\rangle = 1/2\,\alpha)$$

$$\rho_{\beta\beta\to\alpha\beta} \propto \left|\langle\beta\beta|\gamma B_1(I_{x1} + I_{x2})|\alpha\alpha\rangle\right|^2$$

$$= \left|\gamma B_1\left[\langle\beta\beta|(I_{x1})|\alpha\alpha\rangle + \langle\beta\beta|(I_{x2})|\alpha\alpha\rangle\right]\right|^2$$

$$= \left|\gamma B_1\left[1/2\langle\beta\beta|\beta\alpha\rangle + 1/2\langle\beta\beta|\alpha\beta\rangle\right]\right|^2$$

$$= \left|\gamma B_1\left[0 + 0\right]\right|^2 = 0$$

> *The transition probability is zero, indicating that this particular transition cannot be observed (the intensity is zero).*

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**)
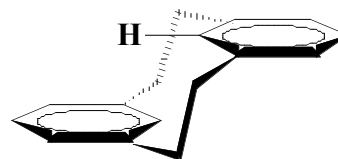
The typical chemical shift for a hydrogen in benzene is ~7.3 ppm. So, this hydrogen is about 4 ppm more shielded. From the "isoshielding" plot for benzene (Johnson and Bovey), this would suggest that the hydrogen is approximately 1.5 angstroms above the ring below it.

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.
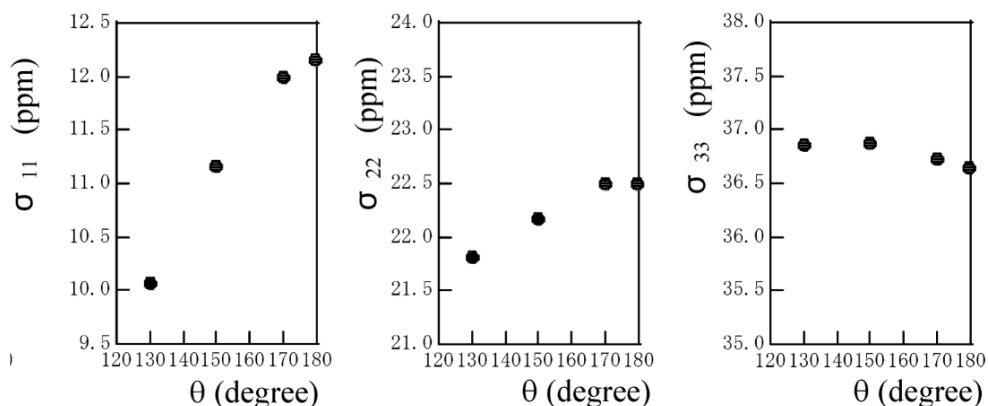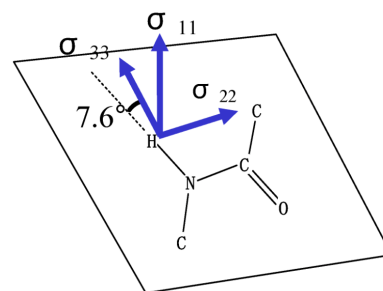
If, in solution, the hydrogen bond angle is 180°, and the chemical shift of this amide ^1H nucleus is 8.0, what would be the chemical shift of this ^1H nucleus in solution if the hydrogen bond angle was 130° instead of 180°? You will have to show your work for credit. (**8 points**)

The isotropic (solution) tensors (in ppm, as in the plots) are:

$$\sigma_{iso,180} = \mathrm{Tr}\begin{bmatrix}12.2 & & \\ & 22.5 & \\ & & 36.6\end{bmatrix} = (12.2 + 22.5 + 36.6)/3 = 23.8 \qquad \sigma_{iso,130} = \mathrm{Tr}\begin{bmatrix}10.1 & & \\ & 21.8 & \\ & & 36.8\end{bmatrix} = (10.1 + 21.8 + 36.8)/3 = 22.9$$

The chemical shift difference is then 23.8 - 22.9 = 0.9 ppm. So, at 130° the hydrogen is 0.9 ppm less shielded, so its chemical shift is 8.9 ppm.

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.
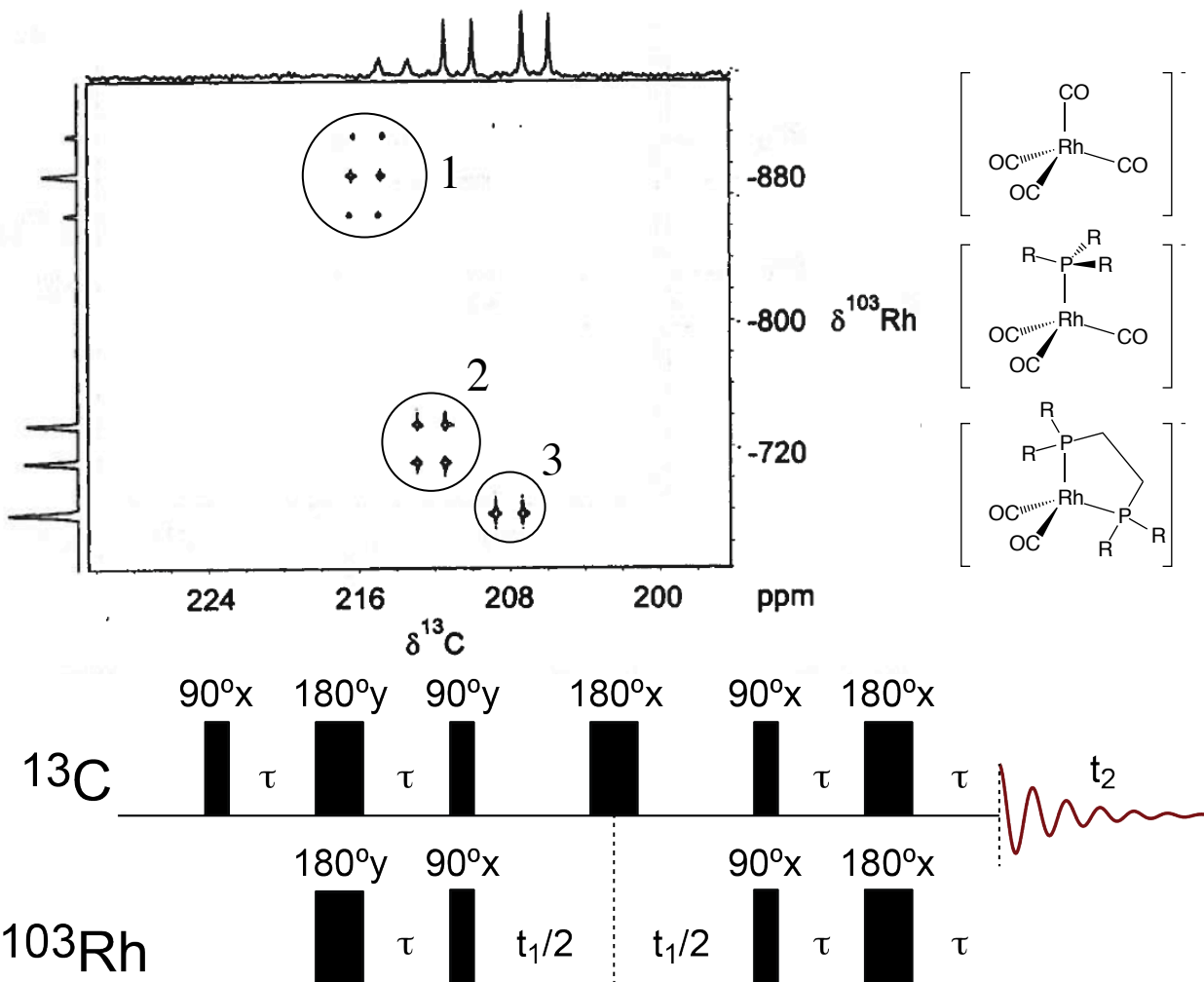
a) There are three signals in the HSQC spectrum, each split into multiple peaks. Circle each of the three signals. Then label the signal from the least shielded ^{13}C nucleus/nuclei '1', the most shielded '3', and the remaining signal '2'. (**4 points**)

see above

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

All are doublets in the ^{13}C dimension.

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

1 is a triplet, 2 is a doublet, and 3 is a singlet in the ^{103}Rh dimension.

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**)

First, ^{103}Rh is a spin ½ nucleus, as is ^{31}P, and these isotopes represent essentially 100% of these elements at natural isotopic abundance. ^{13}C is also spin ½, with about 1.1% ^{13}C in carbon at natural isotopic abundance.

The pulse sequence shows that in the ^{13}C dimension (t_2, directly detected dimension), there is no decoupling of ^{103}Rh, so all ^{13}C signals should show splitting by the directly bonded ^{103}Rh nucleus. Each signal is a doublet in the ^{13}C dimension, suggesting that the splitting is due to the 1-bond ^{13}C-^{103}Rh coupling. This must be the case, because one of the molecules (Rh-(CO)$_4$) has four equivalent CO groups bound to a single ^{103}Rh nucleus, and the ^{13}Rh nucleus is the only nucleus in the molecule that could produce splitting. At natural isotopic abundance, even if the CO groups were not equivalent, there would not be any observed 2-bond ^{13}C-^{13}C splitting (in any given molecule, if one of the CO groups has ^{13}C the average likelihood of any other carbon atom being ^{13}C is low, so the splitting would not be observed). So, for this molecule, the splitting in the ^{13}C dimension must be from the 1-bond ^{13}C-^{103}Rh coupling. The same reasoning applies for the other two molecules.

In the indirectly detected (t_1) dimension, there is a 180° ^{13}C decoupling pulse centered in the t_1 evolution period, so the observed splitting of the signals in the ^{103}Rh dimension is not due to ^{13}C, and must be due to ^{31}P (1-bond ^{103}Rh-^{31}P coupling). One of the molecules (Rh-(CO)$_4$) has no ^{31}P, and there is no splitting of one of the signals (signal #3) in the ^{103}Rh dimension, so that signal must correspond to that molecule. One of the remaining molecules has a single ^{31}P nuclei directly bonded to the single ^{103}Rh atom, so this would be expected to give a doublet, as is the case for signal 2, and thus identifies this molecule as giving rise to signal 2. The remaining molecule has two (equivalent) ^{31}P nuclei directly bonded to the single ^{103}Rh atom, so this would be expected to give a triplet, as is the case for signal 3, and thus identifies this last molecule as giving rise to signal 3.

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

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

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**)

Relaxation in the transverse plane includes contributions from both magnetic field inhomogeneity includes contributions from both magnetic field inhomogeneity and spin-spin relaxation. The time constant T_2^* is typically used to describe the net relaxation stemming from both of these contributions, whereas T_2 is often used to describe only the spin-spin contribution. This is not always the case, however, especially when spin-spin relaxation dominates, as is the case for large molecules. In such cases, where the contribution from magnetic field inhomogeneity is small/insignificant, then T_2^* is often replaced with, simply, T_2.

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**)

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

Two matrices (B_i and B_j) are orthogonal to one another if Tr{$B_i^t B_j$}=0, where $B^t = B^{T*}$. So, for matrix A, $A^t = A^{T*}$:

$$A = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix}, \quad A^T = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix}, \quad A^{T*} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix}$$

$$A^{T*}B = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix}\begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

The trace of $A^t B$=0 (1+(-1)+(-1)+1=0), so the matrices are orthogonal.

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. (**8 points**)

For a 90° ($\pi/2$) pulse, the operators reduce to:

$$\hat{\mathbf{R}}_y = \frac{\sqrt{2}}{2}\begin{bmatrix} 1 & -1 \\ 1 & 1 \end{bmatrix} \qquad \hat{\mathbf{R}}_y^{-1} = \frac{\sqrt{2}}{2}\begin{bmatrix} 1 & 1 \\ -1 & 1 \end{bmatrix}$$

The equilibrium deviation density matrix is:

$$\hat{\sigma}_{eq} = \frac{1}{2}\begin{bmatrix} \gamma\hbar B_0/2k_B T & 0 \\ 0 & -\gamma\hbar B_0/2k_B T \end{bmatrix} \equiv \frac{1}{2}\begin{bmatrix} \delta & 0 \\ 0 & -\delta \end{bmatrix} \propto M_z(eq)$$

The rotation operation is as follows:

$$\hat{\sigma}(t) = \hat{\mathbf{R}}_{y,\pi/2}\hat{\sigma}_{eq}\hat{\mathbf{R}}_{y,\pi/2}^{-1} = \frac{\sqrt{2}}{2}\begin{bmatrix} 1 & -1 \\ 1 & 1 \end{bmatrix}\frac{1}{2}\begin{bmatrix} \delta & 0 \\ 0 & -\delta \end{bmatrix}\frac{\sqrt{2}}{2}\begin{bmatrix} 1 & 1 \\ -1 & 1 \end{bmatrix} = \frac{1}{4}\begin{bmatrix} 1 & -1 \\ 1 & 1 \end{bmatrix}\begin{bmatrix} \delta & \delta \\ \delta & -\delta \end{bmatrix} = \frac{1}{4}\begin{bmatrix} 0 & 2\delta \\ 2\delta & 0 \end{bmatrix} = \delta\frac{1}{2}\begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix}$$

The matrix representation for the I_x operator is:

$$\hat{I}_x = \frac{1}{2}\begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix}$$

Thus, rotation of equilibrium z-magnetization about the y-axis results in x-magnetization

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**)

The ^{1}H chemical shift difference between the two signals is 13.2 ppm. Thus, the frequency difference in Hz at a 600 MHz field strength is:

$$\Delta\delta = \frac{\Delta\upsilon}{600 \text{ MHz}} \times 10^6, \text{ so } \Delta\upsilon = \Delta\delta \frac{600 \times 10^6 \text{ Hz}}{10^6} = 13.2 \times 600 \text{ Hz} = 7920 \text{ Hz}$$

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**)

The Larmor frequency depends on the gyromagnetic ratio (γ) and the static field strength (B_0):

$$\upsilon_L = \left|\frac{\gamma}{2\pi}\right| B_0$$

The ratio of the ^{1}H Larmor frequency and the ^{13}C frequency at the same field strength is simply the ratio of the gyromagnetic ratios. With the values of γ for ^{13}C and ^{1}H or their ratio (about ¼) the frequency is calculated to be about 150 MHz.

$$\frac{\upsilon_{L,^1H}}{\upsilon_{L,^{13}C}} = \frac{\left|\frac{\gamma_{^1H}}{2\pi}\right| B_0}{\left|\frac{\gamma_{^{13}C}}{2\pi}\right| B_0} = \frac{|\gamma_{^1H}|}{|\gamma_{^{13}C}|} \qquad \text{so, } \upsilon_{L,^{13}C} = \upsilon_{L,^1H} \frac{|\gamma_{^{13}C}|}{|\gamma_{^1H}|}$$

$$\upsilon_{L,^{13}C} = 600 \text{ MHz} \frac{6.7283 \times 10^7 \text{ rad / T / s}}{26.7519 \times 10^7 \text{ rad / T / s}} = 150.09 \text{ MHz} \approx 150 \text{ MHz}$$

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**)

We assume that the relaxation process is first order,

$$M_z = M_0(1 - e^{-\tau/T_1}) \qquad \frac{M_z}{M_0} = 1 - e^{-\tau/T_1} \qquad \frac{M_z}{M_0} - 1 = -e^{-\tau/T_1} \qquad \ln\left(1 - \frac{M_z}{M_0}\right) = -\tau/T_1 \qquad T_1 = -\frac{\tau}{\ln\left(1 - \dfrac{M_z}{M_0}\right)}$$

and that $M_z = M_0(1 - e^{-\tau/T_1})$:

$$T_1 = -\frac{5}{\ln(1 - 0.1)} = 47.46 \ \text{seconds}$$

So, T_1 is about 47 seconds.

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×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×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**)
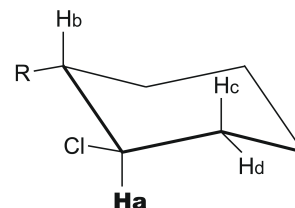
In this case, the return to equilibrium is governed by $M_z = M_0(1 - e^{-t/T_1})$.
For t=5×T_1=50 s;

$$\frac{M_z}{M_0} = 1 - e^{-50/10} = 1 - 0.006737947 = 0.9933.$$

*So, after 5×T_1, the equilibration process is > 99% complete*

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**)

> *The dihedral angle between two axial hydrogens in a six member ring is approximately 180 degrees. The dihedral angle between an axial hydrogen and an equatorial hydrogen is about 60 degrees, as is the angle between two adjacent equatorial hydrogens. The Karplus relationship tells us that the coupling constant ($^3J_{H1,H2}$) is dependent on this angle. The largest coupling constants are observed when the dihedral angle is 0 or 180 degrees, and the smallest for 90 degrees. For the compound above, $^3J_{Ha,Hb}$ and $^3J_{Ha,Hc}$ are large because of the 180 degree dihedral angles between Ha and Hb, and between Ha and Hc. The dihedral angle of 60 degrees between Ha and Hd results in the smaller coupling constant.*

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**)
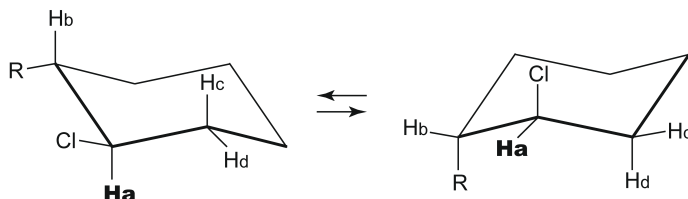
> *If the molecule converts to the other chair form shown above, the hydrogens that were axial (Ha, Hb, Hc) now become equatorial, and the equatorial hydrogen (Hd) becomes axial. In this instance, the dihedral/torsion angle between Ha and Hb is ~60°, the angle between Ha and Hc is ~60°, and the angle between Ha and Hd is ~60°. Thus, according to the Karplus relationship, all of the couplings ($^3J_{Ha,Hb}$, $^3J_{Ha,Hc}$, $^3J_{Ha,Hd}$) will be ~5 Hz. Because the equilibrium concentrations of the two chair forms are approximately equal, the observed coupling constants will be approximately the average of those for the two forms. Thus, $^3J_{Ha,Hb} \approx (5+10)/2 \approx 7.5$ Hz, $^3J_{Ha,Hc} \approx (5+10)/2 \approx 7.5$ Hz, and $^3J_{Ha,Hd} \approx (5+5)/2 \approx 5$ Hz.*

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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