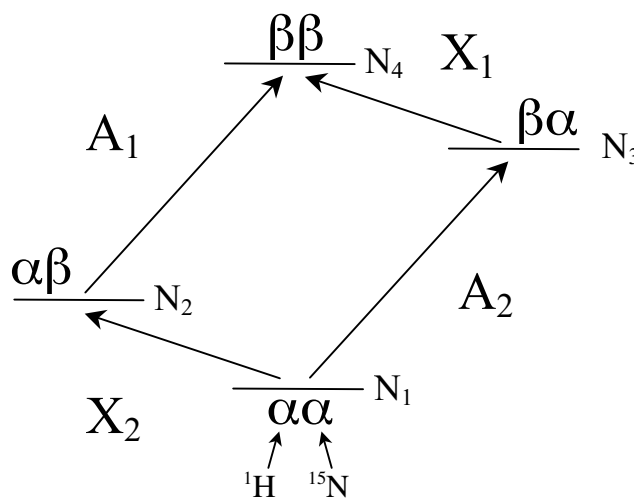


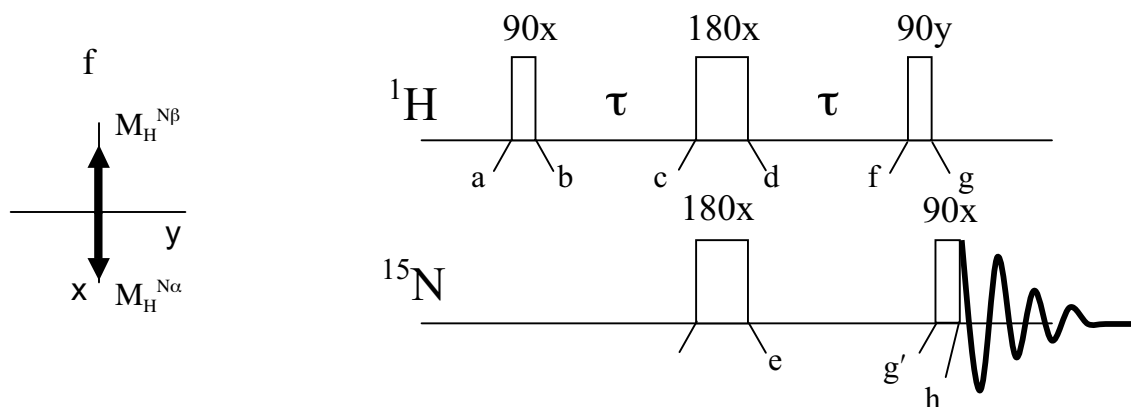
Problem Set #4, CHEM/BCMB 4190/6190/8189

1). Consider the populations N_1 , N_2 , N_3 and N_4 of the $\alpha\alpha$, $\alpha\beta$, $\beta\alpha$ and $\beta\beta$ states respectively for a ^1H - ^{15}N spin system. The energy diagram for this system is depicted (right), where A_1 and A_2 are the ^1H transitions, and X_1 and X_2 are the ^{15}N transitions. We will define ΔH as the difference in the number of spins in α and β states for ^1H , and ΔX as the difference in the number of spins in α and β states for ^{15}N .



- Write down the equilibrium values for N_1 - N_4 . Assume that $N_4 = N$.
- What is the relationship between ΔH and ΔX ?
- What are the population differences for the transitions A_1 , A_2 , X_1 and X_2 ?
- In the SPI experiment, either the A_1 or A_2 transition can be selectively excited (by a selective 180° pulse) to enhance the signals from the X_1 and X_2 transitions. If the A_1 transition is selectively excited, what are the new values for N_1 - N_4 ?
- After selective excitation of A_1 , what are the population differences for the ^{15}N (X_1 and X_2) and ^1H (A_1 and A_2) transitions?
- Based on your answers to 'c' and 'e', draw vector diagrams depicting the bulk magnetization vectors corresponding to the A and X transitions before and after selective excitation of the A_1 transition. Make sure to label properly the individual vectors and indicate their magnitudes.

2). Consider the effect of the INEPT pulse sequence (below) on the ^1H - ^{15}N spin system from problem 1 (above). From Problem Set #3, question #9, we know that at point f, the magnetization vectors ($M_{\text{H}}^{N\alpha}$ and $M_{\text{H}}^{N\beta}$) will be oriented as shown below.



- Using vector diagrams, show the effect of the ^1H 90° y pulse on the vectors $M_{\text{H}}^{N\alpha}$ and $M_{\text{H}}^{N\beta}$ (what do they look like at g).

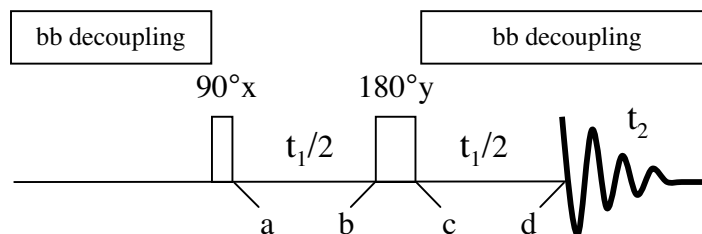
- b. What are the values for the populations N_1 - N_4 at point g? What are the population differences for the ^1H (A_1 and A_2) and ^{15}N (X_1 and X_2) transitions?
- c. What are the values for the populations N_1 - N_4 at point g, and what are the population differences for the ^1H (A_1 and A_2) and ^{15}N (X_1 and X_2) transitions, if the phase of the final ^1H 90° pulse is changed from 'y' to '-y'?

3). The following questions concern the DEPT technique:

- a. What does 'DEPT' stand for?
- b. Sketch the DEPT pulse sequence. Include delays, delay times, pulse widths, the axes about which pulses are applied, and any other pertinent elements.
- c. Using DEPT, what would be the simplest way to determine which signals in a normal ^1H decoupled 1D ^{13}C spectrum of an organic compound were from $-\text{CH}$ groups?
- d. The chemical shifts and carbon types for a particular organic compound are shown in the table below. Sketch the ^1H decoupled ^{13}C spectrum, the DEPT(90) spectrum, and the DEPT(135) spectrum for this compound.

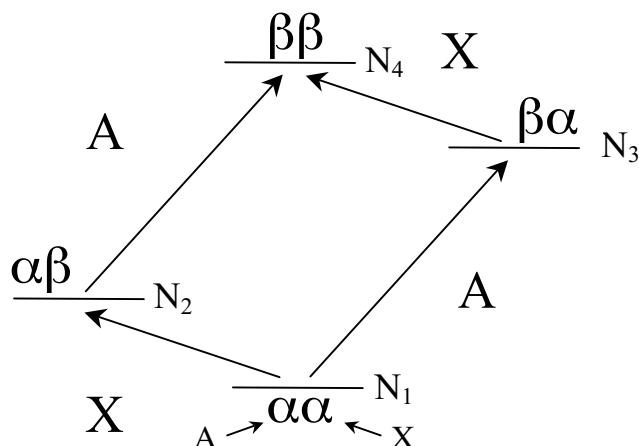
carbon	type	δ	J_{CH}
1	$-\text{CH}_2$	20 ppm	130 Hz
2	$-\text{CH}_3$	24 ppm	120 Hz
3	$-\text{CH}$	35 ppm	140 Hz
4	$-\text{CH}_2$	39 ppm	130 Hz
5	$-\text{CH}$	45 ppm	140 Hz

4). The following questions concern heteronuclear two-dimensional J -resolved spectroscopy:



- a. The 90-delay-180-delay-detect sequence is the normal spin-echo sequence. In the normal spin-echo sequence, J -couplings are refocused (at the end of the second delay, the vectors representing the individual multiplet components are all pointed along the same axis). For the pulse sequence above, the vectors are not refocused at 'd'. Why?
- b. For the compound in question 3 (above), sketch the 2D heteronuclear two-dimensional J -resolved spectrum expected for the compound. Label the axes appropriately, also make sure to add the appropriate units to the axes.

5). Consider the populations N_1 , N_2 , N_3 and N_4 of the $\alpha\alpha$, $\alpha\beta$, $\beta\alpha$ and $\beta\beta$ states respectively for a ^1H - ^1H spin system (spins 'A' and 'X') without J coupling (no coupling between A and X). The energy diagram for this system is depicted (right), where the A's represent the transitions of one of the ^1H nuclei, and the X's represent the transitions of the other ^1H nucleus. We will define ΔH as the difference in the number of spins in α and β states for A, and ΔH will also represent the difference in the number of spins in α and β states for X.



- Write down the equilibrium values for N_1 - N_4 and the population differences for the A and X transitions. Assume that $N_4 = N$.
 - If we perform an NOE experiment by selective **saturation** of the A transitions, what are the new values for N_1 - N_4 and the population differences for the A and X transitions after saturation (but before any relaxation takes place).
 - If we perform an NOE experiment by selective **inversion** of the A transitions, what are the new values for N_1 - N_4 and the population differences for the A and X transitions after saturation (but before any relaxation takes place).
 - For a small molecule, after either saturation or inversion, a number of $\beta\beta$ spins (δ) will convert to $\alpha\alpha$ spins via the W_2 (double quantum) pathway. Show the resulting population differences for the X transitions for both saturation and inversion.
- 6). The following questions concern the 2D NOESY experiment and the NOE:
- Sketch the 2D NOESY pulse sequence. On the sketch, draw vertical, dashed horizontal lines to delineate the detection, evolution, preparation and mixing periods, and label each of these periods. Make sure to label properly all delay periods and chemical shift evolution periods.
 - For a NOESY experiment, how should the mixing time be set? Should it be larger or smaller for very large molecules?
 - The intensity of a crosspeak for a pair of protons in a NOESY spectrum is X. If the distance between these two protons somehow increases by a factor of 2, what will be the new intensity of the crosspeak?
 - For a small molecule, if we consider a simple ^1H - ^1H two-spin system with no coupling, what is the maximum theoretical NOE enhancement of the NMR signal of one of the ^1H nuclei by saturation of the other?