**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.
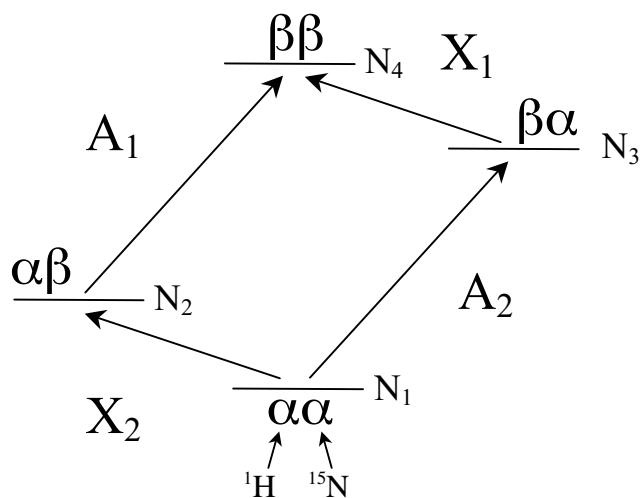
a. Write down the equilibrium values for N_1-N_4. Assume that $N_4 = N$.
b. What is the relationship between ΔH and ΔX?
c. What are the population differences for the transitions A_1, A_2, X_1 and X_2?
d. In the SPI experiment, either the A_1 or A_2 transition can be selectively excited (by a selective 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?
e. 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?
f. 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.

a. $N_4 = N$
       $N_3 = N + \Delta X$
       $N_2 = N + \Delta H$
       $N_1 = N + \Delta H + \Delta X$

b. The ratio $\Delta H/\Delta X$ is proportional to the ratio of the gyromagnetic ratios for ^1H and ^{15}N.
    $\gamma^1H/\gamma^{15}N = 26.7519/-2.7126 = -9.86 \approx -10$, so $\Delta H = -10\Delta X$.

c. $A_1 = N_2 - N_4 = \Delta H$
       $A_2 = N_1 - N_3 = \Delta H$
       $X_1 = N_3 - N_4 = \Delta X$
       $X_2 = N_1 - N_2 = \Delta X$

d. $N_4 = N + \Delta H$
       $N_3 = N + \Delta X$
       $N_2 = N$
       $N_1 = N + \Delta H + \Delta X$

e. $A_1 = N_2 - N_4 = -\Delta H$
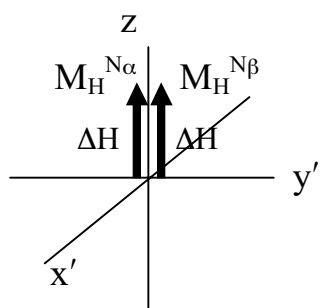 $A_2 = N_1 - N_3 = \Delta H$
       $X_1 = N_3 - N_4 = \Delta X - \Delta H = \Delta X - (-10\Delta X) = 11\Delta X$
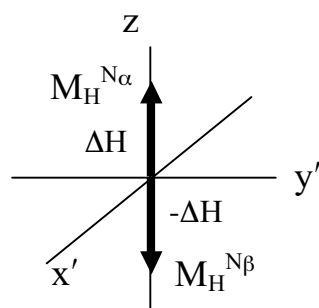 $X_2 = N_1 - N_2 = \Delta H + \Delta X = -10\,\Delta X + \Delta X = -9\Delta X$
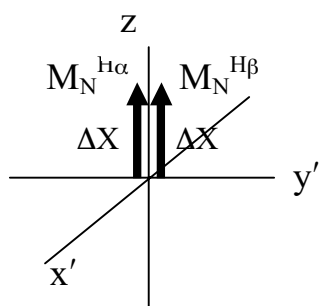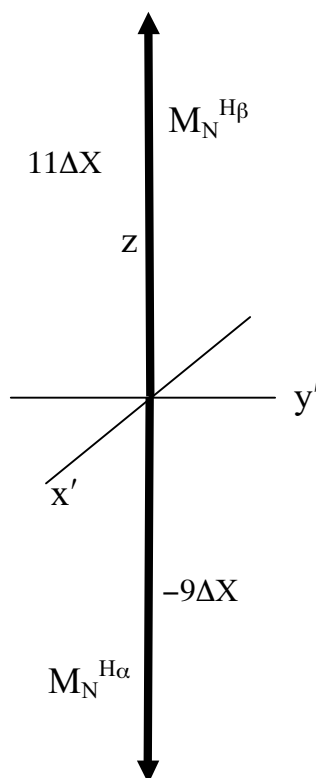
f.

A transitions before

z

$M_H{}^{N\alpha}$ $M_H{}^{N\beta}$

ΔH ΔH

y′

x′

A transitions after

z

$M_H{}^{N\alpha}$

ΔH

y′

$-\Delta H$

$M_H{}^{N\beta}$

x′

X transitions before

z

$M_N{}^{H\alpha}$ $M_N{}^{H\beta}$

ΔX ΔX

y′

x′

X transitions after

$M_N{}^{H\beta}$

$11\Delta X$

z

y′

x′

$-9\Delta X$

$M_N{}^{H\alpha}$

2). Consider the effect of the INEPT pulse sequence (below) on the ^{1}H-^{15}N spin system from problem 1 (above). From Problem Set #3, question #9, we know that at point f, the magnetization vectors ($M_H^{N\alpha}$ and $M_H^{N\beta}$) will be oriented as shown below.
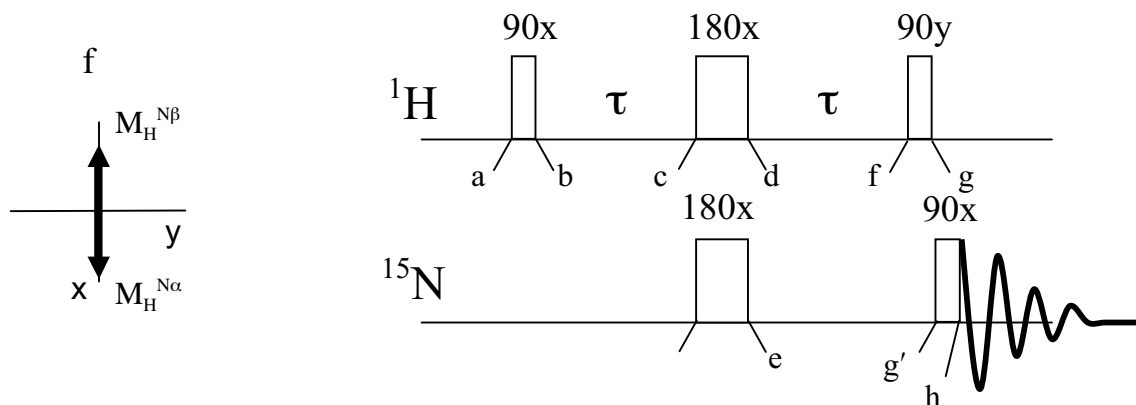
a. Using vector diagrams, show the effect of the ^{1}H 90° y pulse on the vectors $M_H^{N\alpha}$ and $M_H^{N\beta}$ (what do they look like at g).

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

a.
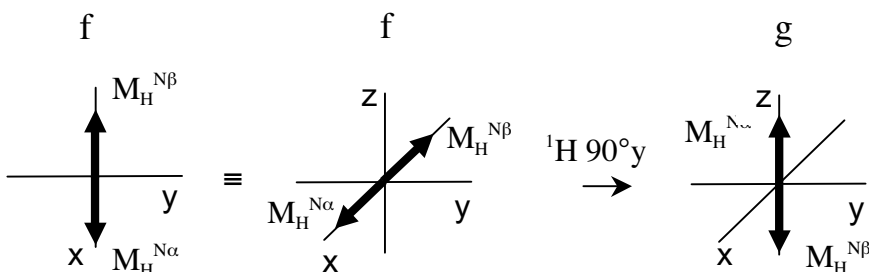
b. From the answer to 'a', we see that $M_H^{N\beta}$ is selectively inverted by the INEPT sequence. This is the ^{1}H transition with the β spin ^{15}N, A_1. This means that the levels N_2 and N_4 are exchanged (from their equilibrium values).

$N_4 = N + \Delta H$ $A_1 = N_2 - N_4 = -\Delta H$
$N_3 = N + \Delta X$ $A_2 = N_1 - N_3 = \Delta H$
$N_2 = N$ $X_1 = N_3 - N_4 = \Delta X - \Delta H = \Delta X - (-10\Delta X) = 11\Delta X$
$N_1 = N + \Delta H + \Delta X$ $X_2 = N_1 - N_2 = \Delta H + \Delta X = -10\ \Delta X + \Delta X = -9\Delta X$

c. In this case, $M_H^{N\alpha}$ will be selectively inverted by the INEPT sequence (at g, $M_H^{N\beta}$ will be poined along +z, and $M_H^{N\alpha}$ will be aligned along -z). Thus, N_1 and N_3 are exchanged (from their equilibrium values).

$N_4 = N$ $A_1 = N_2 - N_4 = \Delta H$
$N_3 = N + \Delta H + \Delta X$ $A_2 = N_1 - N_3 = -\Delta H$
$N_2 = N + \Delta H$ $X_1 = N_3 - N_4 = \Delta H + \Delta X = -10\ \Delta X + \Delta X = -9\Delta X$
$N_1 = N + \Delta X$ $X_2 = N_1 - N_2 = \Delta X - \Delta H = \Delta X - (-10\Delta X) = 11\Delta X$
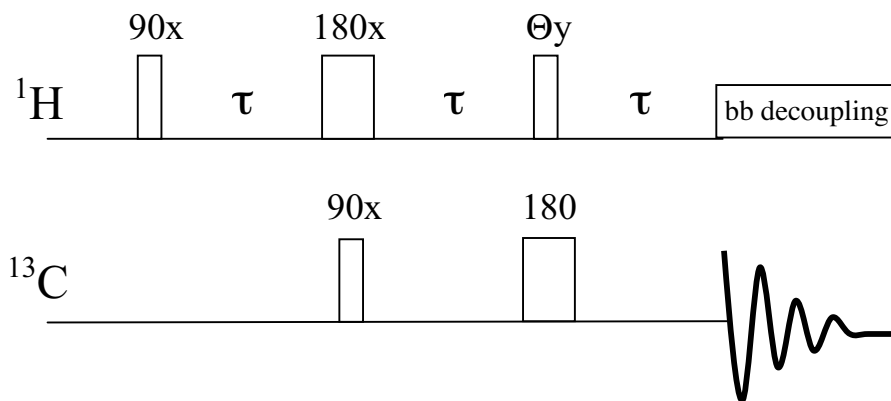
3). The following questions concern the DEPT technique:
   a. What does 'DEPT' stand for?
   b. Sketch the DEPT pulse sequence. Include delays, delay times, pulse widths, the axes about which pulses are applied, and any other pertinent elements.
   c. Using DEPT, what would be the simplest way to determine which signals in a normal ^{1}H decoupled 1D ^{13}C spectrum of an organic compound were from –CH groups?
   d. The chemical shifts and carbon types for a particular organic compound are shown in the table below. Sketch the ^{1}H decoupled ^{13}C spectrum, the DEPT(90) spectrum, and the DEPT(135) spectrum for this compound.

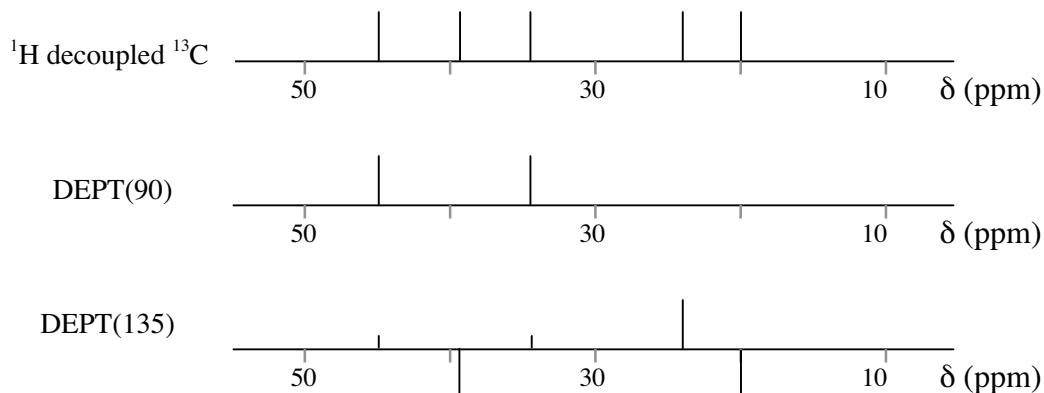| carbon	type	δ	J_{CH}
1	-CH$_2$	20 ppm	130 Hz
2	-CH$_3$	24 ppm	120 Hz
3	-CH	35 ppm	140 Hz
4	-CH$_2$	39 ppm	130 Hz
5	-CH	45 ppm	140 Hz

a. *Distortionless Enhancement by Polarization Transfer*

b.

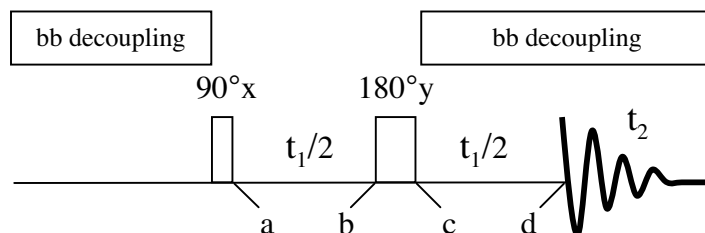*The τ delay is equal to 1/(2J$_{CH}$), and the pulse angle Θ is variable (usually 45, 90 or 135°)*

c. *DEPT(90) (Θ set to 90°) would give a spectrum showing signals only from –CH groups.*

d.

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.

a. At point 'c', the broadband ^1H decoupling is initiated. As such, the individual vectors (corresponding to the individual multiplet components) all precess at the Larmor frequency, because the reason for their differing precession rates (coupling) is removed by decoupling. Thus, the vectors do not refocus, and the broadband decoupling results in a signal (after Fourier tansformation) amplitude proportional to the vector sum of the components.

b.
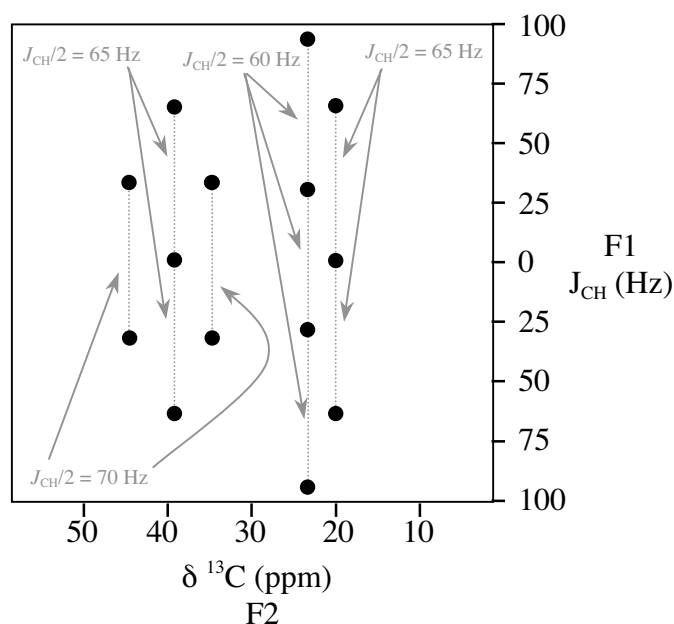
5). Consider the populations N_1, N_2, N_3 and N_4 of the $\alpha\alpha$, $\alpha\beta$, $\beta\alpha$ and $\beta\beta$ states respectively for a ^1H-^1H spin system (spins 'A' and 'X') without J coupling (no coupling between A and X). The energy diagram for this system is depicted (right), where the 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.
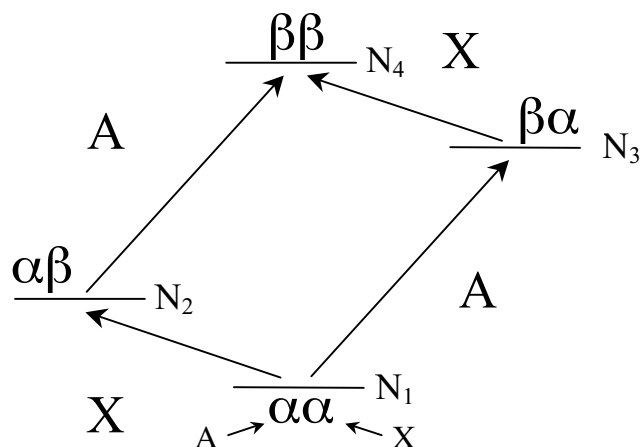
a. Write down the equilibrium values for N_1-N_4 and the population differences for the A and X transitions. Assume that $N_4 = N$.
b. If we perform an NOE experiment by selective **saturation** of the A transitions, what are the new values for N_1-N_4 and the population differences for the A and X transitions after saturation (but before any relaxation takes place).
c. 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).
d. 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.

a.   *$N_4 = N$*
        $N_3 = N + \Delta H$ *$A = N_2 - N_4 = N_1 - N_3 = \Delta H$*
        $N_2 = N + \Delta H$ *$X = N_3 - N_4 = N_1 - N_2 = \Delta H$*
        $N_1 = N + 2\Delta H$

b.   **Saturation** *of the A transitions equilibrates the spin populations of the N_1 and N_4 states, and of the N_2 and N_3 states:*
        $N_4 = N + \Delta H/2$
        $N_3 = N + 3\Delta H/2$ *$A = N_2 - N_4 = N_1 - N_3 = 0$*
        $N_2 = N + \Delta H/2$ *$X = N_3 - N_4 = N_1 - N_2 = \Delta H$*
        $N_1 = N + 3\Delta H/2$

c.   **Inversion** *of the A transitions inverts the spin populations of the N_1 and N_4 states, and of the N_2 and N_3 states:*
        $N_4 = N + \Delta H$
        $N_3 = N + 2\Delta H$ *$A = N_2 - N_4 = N_1 - N_3 = \Delta H$*
   *$N_2 = N$*   *$X = N_3 - N_4 = N_1 - N_2 = \Delta H$*
        $N_1 = N + \Delta H$

d.   *saturation*   *inversion*
        $N_4 = N + \Delta H/2 - \delta$ *$N_4 = N + \Delta H - \delta$*
        $N_3 = N + 3\Delta H/2$ *$N_3 = N + 2\Delta H$*
        $N_2 = N + \Delta H/2$ *$N_2 = N$*
        $N_1 = N + 3\Delta H/2 + \delta$ *$N_1 = N + \Delta H + \delta$*
        $X = N_3 - N_4 = N_1 - N_2 = \Delta H + \delta$ *$X = N_3 - N_4 = N_1 - N_2 = \Delta H + \delta$*

6). The following questions concern the 2D NOESY experiment and the NOE:
    a. 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.
    b. For a NOESY experiment, how should the mixing time be set? Should it be larger or smaller for very large molecules?
    c. 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?
    d. For a small molecule, if we consider a simple ^{1}H-^{1}H two-spin system with no coupling, what is the maximum theoretical NOE enhancement of the NMR signal of one of the ^{1}H nuclei by saturation of the other?

a.
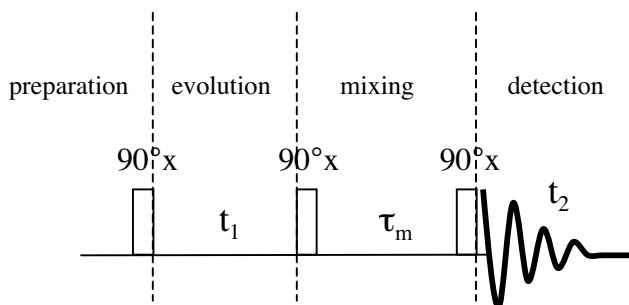
Here t_1 is the chemical shift evolution period for the indirectly detected dimension, t_2 is for the directly detected dimension, and τ_m is the mixing time

b. The mixing time, τ_m, should normally be set to approximately T_1. Of course, T_1 will vary for the individual ^{1}H spins in a molecule, so one has to consider if an average value should be used or if a value more similar to the largest or smallest values expected in the molecule should be used (if these values are known). Since T_1 is much smaller for very large malecules, the mixing time should be decreased accordingly.

c. The intensity of crosspeaks in NOESY spectra are dependent on $1/r^6$, where r is the distance between the ^{1}H nuclei that give rise to the crosspeak. If the original intensity is X, and the nuclear distance increases by a factor of 2, the intensity will decrease by a factor of 2^6 or 64, so the new intensity is $1/64^{th}$ of the original (0.015625X).

d. The maximal enhancement, η, is defined as $\eta = \gamma_a / (2\gamma_x)$ where γ_a and γ_x represent the gyromagnetic ratios of the saturated and observed nuclei, respectively. In this case, η will be 0.5, so the maximum enhancement will be 50%.