1. (15 pts) For ‘a’ and ‘b’ below, indicate relative intensities of signals in spectra, and indicate coupling constants (in terms of $J_{\text{CH}}$) where appropriate. Mark the Larmor frequencies on your spectra with $\nu_H$ and $\nu_C$. Identify individual transitions (i.e. $M_{C\alpha}^H$, etc.)

a. Sketch the 1D, $^1$H NMR spectrum of chloroform, CHCl$_3$ (no decoupling).

\[ J_{\text{CH}} \]
\[ ^1\text{H}-^{13}\text{C}- \]
\[ M_{C\alpha}^H \quad M_{C\beta}^H \]
\[ \nu_H \]

b. Sketch the 1D, $^{13}$C NMR spectrum of chloroform (no decoupling).

\[ J_{\text{CH}} \]
\[ M_{C\alpha}^{13\text{C}} \quad M_{C\beta}^{13\text{C}} \]
\[ \nu_C \]

c. If you were going to perform an SPI experiment to enhance the intensities in the $^{13}$C NMR spectrum, indicate (circle) one transition in one of the signals above that you might excite to accomplish this.

*In the SPI experiment, either the $M_{C\alpha}^H$ or $M_{C\beta}^H$ transition in the $^1$H NMR signal (but not both!) could be excited to enhance the intensities of the components of the $^{13}$C signal. I’ve arbitrarily circled one of them.*
2). (20 pts) In the DEPT experiment, the phase angle (pulse width) of the third \(^1\text{H}\) pulse (applied along the ‘y’ axis) can be set to any value, in order to achieve the desired result. Shown in the figure below are the intensities of signals from CH, CH\(_2\), and CH\(_3\) groups as a function of the phase angle \(\Theta_y\):

![Diagram showing intensities of signals from CH, CH\(_2\), and CH\(_3\) groups as a function of \(\Theta_y\).]

\[
\begin{align*}
\text{O} & \quad \| \\
\text{C}^1\text{H}_3\text{-C}^2\text{-O-C}^3\text{H}_2\text{-C}^4\text{H}_2\text{-C}^5\text{H}-\text{C}^6\text{H}_3 & \quad \| \\
\text{C}^7\text{H}_3
\end{align*}
\]

isopentyl acetate

(a) The DEPT(135) spectrum for isopentyl acetate is shown below. Draw the DEPT(45)-DEPT(135) spectrum for isopentyl acetate. Make sure to identify (label) the signals in the same manner that they are labeled in the DEPT(135) spectrum. Write a short explanation of why the DEPT(45)-DEPT(135) spectrum looks as it does.

![Diagram of DEPT(135) and DEPT(45)-DEPT(135) spectra with labeled signals A, B, C, and D.]

With the phase angle \(\Theta_y\) set at 45°, the intensities of –CH and –CH\(_3\) signals (DEPT(45)) are almost identical to their intensities when \(\Theta_y\) set at 135° (DEPT(135)). Thus in the DEPT(45)-DEPT(135) spectrum, signals from these groups will be absent. The intensities of signals from –CH\(_2\) groups in the DEPT(45) spectrum are very large and positive, whereas signals from –CH\(_2\) groups in DEPT(135) spectra are very large and negative. Subtracting the latter from the former results in very large positive signals (DEPT(45)-DEPT(135)).

Final Exam: CHEM/BCMB 4190/6190/8189
b. Indicate in the table below whether each signal (A-E) arises from a –CH₃, -CH₂, -CH, or quaternary carbon by placing an ‘X’ in each row in the appropriate column.

<table>
<thead>
<tr>
<th>signal</th>
<th>-CH₃</th>
<th>-CH₂</th>
<th>-CH</th>
<th>quaternary</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (21 ppm)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (23 ppm)</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (25 ppm)</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>D (35 ppm)</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>E (65 ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

c. In the table below, assign each signal in the DEPT(135) spectrum of isopentyl acetate by placing the appropriate integer (1-7) in the ‘assignment’ column, corresponding to the correct carbon atom in the structure of isopentyl acetate shown above. Give a concise but thorough rationale for the assignment.

<table>
<thead>
<tr>
<th>signal</th>
<th>assignment</th>
<th>rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (21 ppm)</td>
<td>1</td>
<td>Signals from E and D must be from –CH₂ groups (down in DEPT(135)). C must be from –CH (very small and up in DEPT(135)). A and B must be from –CH₃. B is twice as large as A, so must be due to equivalent –CH₃ groups 6 &amp; 7. Thus, A is from –CH₃ group 1.</td>
</tr>
<tr>
<td>B (23 ppm)</td>
<td>6 &amp; 7</td>
<td>Signals from A and B are due to –CH₃ groups (see above). B is twice as large a A, so B must be due to equivalent –CH₃ groups 6 &amp; 7</td>
</tr>
<tr>
<td>C (25 ppm)</td>
<td>5</td>
<td>C must be from –CH (very small and up in DEPT(135)). 5 is only –CH in molecule</td>
</tr>
<tr>
<td>D (35 ppm)</td>
<td>4</td>
<td>Signals from E and D must be from –CH₂ groups (down in DEPT(135)). 4 will be upfield from 3 because 3 next to very electronegative group. Thus, signal D is from 4.</td>
</tr>
<tr>
<td>E (65 ppm)</td>
<td>3</td>
<td>Signals from E and D must be from –CH₂ groups (down in DEPT(135)). 3 will be downfield from 4 because 3 is next to a very electronegative group. Thus, signal E is from 3.</td>
</tr>
</tbody>
</table>
3). (20 pts) Consider the effect of the normal spin-echo pulse sequence (right, top) and the effect of this pulse sequence on heteronuclear and homonuclear J coupling, ignoring the effects of chemical shift evolution and magnetic field inhomogeneity. Consider a simple heteronuclear spin system \(^{(1}\text{H}-^{13}\text{C}),\) i.e. CHCl\(_3\)) with a Larmor frequency equal to our reference frequency \((\nu_c = \nu_{\text{rf}})\), and a simple \(^1\text{H}-^1\text{H}\) homonuclear spin system (A-X) with a Larmor frequency equal to our reference frequency \((\nu_H = \nu_{\text{rf}})\).

a. Using vector diagrams, show the effect of the spin echo pulse sequence on the heteronuclear spin system with \(\tau = 1/(4J_{\text{CH}})\). Discuss the effect of the spin-echo pulse sequence on heteronuclear J coupling refocusing.

\[\begin{array}{c}
\text{transverse plane} \\
\tau \\
\theta = 90^\circ \\
\end{array}\]

The 180° pulse reflects the vectors through \(x\), and the second \(\tau\) period permits them to refocus along \(-y\). Thus, the pulse sequence promotes refocusing of heteronuclear J coupling.

b. Using vector diagrams, show the effect of the spin echo pulse sequence on the homonuclear spin system with \(\tau = 1/(4J_{\text{AX}})\). Discuss the effect of the spin-echo pulse sequence on homonuclear J coupling refocusing.

\[\begin{array}{c}
\text{transverse plane} \\
\tau \\
\theta = 90^\circ \\
\end{array}\]

The 180° pulse reflects the vectors through \(x\). This is in effect a pulse on the ‘A’ spin. The 180° pulse also exchanges the populations of the \(\alpha\) and \(\beta\) states of the ‘X’ spin, i.e. this is the effect of the pulse on the ‘X’ spin. Both operations cause the vectors to move away from one another during the second \(\tau\) period, becoming antiphase to one another at ‘e’. Thus, this pulse sequence does not promote refocusing of homonuclear J coupling.
4). **(15 pts)** The following diagram represents the pulse sequence used to measure $T_1$ for $^{13}$C nuclei:

![Diagram of pulse sequence](image)

At point ‘a’, just before the first ($180^\circ_x$) pulse, the magnitude of the longitudinal component of the bulk $^{13}$C magnetization ($M_z$) is equal to the magnitude of the equilibrium magnetization ($M_0$), as shown in the vector diagram at the right.

a. For some value of $\tau$, the magnitude of the longitudinal component of the bulk magnetization is zero ($M_z = 0$) at point ‘c’, and the Fourier transform of the FID reveals no signal. If $T_1$ is 10 seconds, at what value of $\tau$ does this occur?

We assume, as did Bloch, that this relaxation process is first order, and $M_z = M_0 (1 - 2e^{-\tau/T_1})$.

When $M_z = 0$:

$0 = M_0 (1 - 2e^{-\tau/T_1})$

$0 = M_0 - 2M_0 e^{-\tau/T_1}$

$M_0 = 2M_0 e^{-\tau/T_1}$

$1 = 2e^{-\tau/T_1}$

$\ln(1/2) = -\tau/T_1$

$T_1 \ln(2) = \tau$

b. You find that when $\tau$ is 10s, there is no signal at point ‘d’. What is $T_1$?

*From your answer to question ‘a’, you know that no signal is observed when $T_1 \ln(2) = \tau$. If $\tau = 10 s$, then $T_1 = 10 s/0.693 = 14.43 s$. *

c. In order to measure $T_1$ properly, we measure the amplitude of the signal (Fourier transform of the FID) as a function of $\tau$ and fit the data to the appropriate first order equation. We should wait for a time equal to at least $5T_1$ between successive experiments in order to allow for re-equilibration of the bulk magnetization. If $T_1$ is 10 s, and if we wait for $5T_1$ between experiments, how complete is the re-equilibration (assume $\tau=0$, the acquisition time=0 and the $90^\circ$ pulse width is also=0)?

*For $\tau=0$, from question ‘a’ we know that at point ‘d’ the vector diagram to the right describes the bulk magnetization. In this case, the equation that governs the return to equilibrium is $M_z = M_0 (1 - e^{-t/T_1})$. For $t=5T_1=50 s$,*

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

*So, after $5T_1$, the equilibration process is $> 99\%$ complete*. 

Final Exam: CHEM/BCMB 4190/6190/8189
5. (30 pts) Consider the normal spin-echo pulse sequence (right) and the effect of this pulse sequence on heteronuclear $J$ coupling, ignoring the effects of chemical shift evolution and magnetic field inhomogeneity. We will consider a single $^{13}$C nucleus with three attached protons (i.e. $^{13}$CH$_3$Cl) with a Larmor frequency equal to our reference frequency, $\nu_c=\nu_{rf}$.

a. Sketch the normal (coupled) $^{13}$C signal (multiplet) for this nucleus.

b. What are the theoretical relative intensities of the individual components of the multiplet?

$1:3:3:1$

c. In terms of the Larmor frequency, $\nu_c$, and the coupling constant, $J_{CH}$, what will be the frequencies of each of the components of the multiplet?

$\nu_c - 3J_{CH}/2, \nu_c - J_{CH}/2, \nu_c + J_{CH}/2, \nu_c + 3J_{CH}/2$

d. What is the frequency difference between the outermost components of the multiplet? What is the frequency difference between the innermost components of the multiplet?

From ‘c’, the frequency difference between the outermost components is $3J_{CH}$, and the frequency difference between the innermost components is $J_{CH}$.

e. How large is the phase angle (degrees) between the two vectors corresponding to the two outermost components of the multiplet after a time $\tau=1/4(J_{CH})$? How large is the phase angle between the two vectors corresponding to the two innermost components of the multiplet after a time $\tau=1/4(J_{CH})$? Please show your work.

outermost: phase angle, $\Theta = 2\pi(3J_{CH})\tau = 2\pi(3J_{CH})/4(J_{CH}) = 3\pi/2$ radians = 270°
innermost: phase angle, $\Theta = 2\pi(J_{CH})\tau = 2\pi(J_{CH})/4(J_{CH}) = \pi/2$ radians = 90°
f. Using vector diagrams, show the effect of the spin echo pulse sequence on this spin system with $\tau = 1/(4J_{\text{CH}})$. Show diagrams corresponding to points ‘a’-‘e’ in the pulse sequence. Label the vectors corresponding to the individual multiplet components with the frequencies from your answer to ‘c’. Be sure to label your axes. Be sure to show the direction of precession of each component of the multiplet relative to the reference (axes).
6. (20 pts) Repeat part ‘f’ of the previous question for \( \tau = 1/(2J_{\text{CH}}) \), \( \tau = 3/(4J_{\text{CH}}) \), and \( \tau = 1/(J_{\text{CH}}) \). In addition, show the Fourier transformation of the signal that you would get (for \( \tau = 1/(4J_{\text{CH}}) \), \( \tau = 1/(2J_{\text{CH}}) \), \( \tau = 3/(4J_{\text{CH}}) \), and \( \tau = 1/(J_{\text{CH}}) \)) after the second \( \tau \) period if you applied broadband \(^1\text{H}\) decoupling during acquisition only (as shown in the pulse sequence to the upper right). Finally, also show the Fourier transformation of the signal that you would get if instead you used the second pulse sequence (lower right) where the broadband decoupling is applied immediately after the 180° pulse.

\[ \Theta = \text{angle between } \nu_{c+J_{\text{CH}}/2} \text{ and } \nu_{c-J_{\text{CH}}/2} \]

\[ \Omega = \text{angle between } \nu_{c+3J_{\text{CH}}/2} \text{ and } \nu_{c-3J_{\text{CH}}/2} \]
7). (10 pts) In the Friebolin text (page 95), he discusses H, 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 and derivatives is small, please explain *in detail* the highlighted (bold) sentence.

*If the frequency difference, \( \Delta \nu \), between coupled nuclei is large compared with the coupling constants, (i.e. if \( \Delta \nu >> J \)), 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, \( B_0 \) (\( \nu = \gamma B_0/(2\pi) \)), 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.*
8).  (20 pts) The COSY spectrum for menthol is shown (next page), along with the 1D $^1$H spectrum of menthol, the structure of menthol and the correct $^1$H assignments (next page). Circled in the COSY spectrum is a crosspeak (and the symmetry related crosspeak) that remains unassigned. Scheme IV (next page) shows coupling constants for the indicated $^1$H pairs in compounds 15-18.

a. Please give a concise explanation of Scheme IV. What is the point that this figure/scheme is trying to make? What does this have to do with COSY?

$^1$H-$^1$H couplings through more than 3 bonds ($H$-$C$-$C$-$H$) are normally very small. This is the reason that crosspeaks for protons more than 3 bonds away from one another normally do not appear in COSY spectra. However, as shown in Scheme IV, in some cases, couplings through 4 bonds are substantial (1Hz or more). For instance, when the four bonds linking the nuclei form a fixed “W” conformation, the couplings can be pretty large (up to 4 Hz). In these cases, crosspeaks for coupled protons can be observed in COSY spectra.

b. Assign the circled crosspeak(s) and justify your assignment(s).

The chemical shifts of the crosspeaks indicate that one of the ‘8’ and one of the ‘7’ protons are involved. Given Scheme IV, which suggests that a substantial coupling should be observed between the equatorial positions across the ring, the best assignment for the crosspeak would be that it arises from the equatorial protons at “8” and “7”.
9. (30 pts) The diagram for the INEPT pulse sequence is shown below.

Consider the effect of this pulse sequence on heteronuclear ($^{13}$C-$^1$H) $J$ coupling, ignoring the effects of chemical shift evolution and magnetic field inhomogeneity. We will consider a simple $^1$H-$^{13}$C- spin system (i.e. $^{13}$CHCl$_3$) with a Larmor frequency equal to our reference frequency, $\nu_H = \nu_{rf}$. We will assume that the delay $\tau$ is equal to $1/(4J_{CH})$.

a. What does INEPT stand for?

**Insensitive Nuclei Enhanced by Polarization Transfer**

b. Complete the vector diagrams below for points ‘c’, ‘d’, ‘e’, and ‘f’ in the pulse sequence. Be sure to label the vectors ($M_{H\alpha}, M_{H\beta}$), to include arrows indicating the direction of precession for the vectors in the rotating frame, and to indicate the angle ($\Theta$) between the vectors at each point.
c. Explain the consequences of changing the phase of the $^{13}$C 180°x pulse to y.

The 180° pulse on $^{13}$C serves to exchange the $^{13}$C α and β spin populations. In this case, the axis (x or y) about which it is applied is (for the most part) irrelevant.

d. Explain the consequences of changing $\tau$ from $1/(4J_{CH})$ to $1/(2J_{CH})$ (for the simple $^1$H-$^{13}$C- spin system). Your answer should include vector diagrams for points ‘c’ through ‘f’ for $\tau = 1/(2J_{CH})$ and an explanation of the results.

For the simple $^1$H-$^{13}$C-system, the phase angle ($\Theta$) between the two vector components $M_{H,\alpha}$ and $M_{H,\beta}$ is equal to $2\pi J_{CH} \tau$ since the frequency difference between the two components is $J_{CH}$. When $\tau = 1/(4J_{CH})$, $\Theta = 2\pi J_{CH}/(4J_{CH}) = \pi/2$ radians or 90°. If $\tau = 1/(2J_{CH})$, $\Theta = 2\pi J_{CH}/(2J_{CH}) = \pi$ radians or 180°. Thus from ‘b’ to ‘c’ ($\tau$), the vectors rotate 180° apart from one another rather than 90°. The $^1$H 180°x pulse has no effect on the vectors, and the $^{13}$C pulse exchanges $^{13}$Cα and $^{13}$Cβ spin populations and the direction of rotation. During the last $\tau$ period, the vectors thus move 180° towards each other and are refocused at ‘f’.
10. (50 pts) Again consider the INEPT pulse sequence, and the simple \(^1\)H-\(^{13}\)C spin system (i.e. CHCl\(_3\)). At point ‘g’ in the pulse sequence, the \(M_{\text{H}}^\alpha\) and \(M_{\text{H}}^\beta\) components appear as shown below. The energy diagram for this system is depicted (far right), where \(A_1\) and \(A_2\) are the \(^1\)H transitions, and \(X_1\) and \(X_2\) are the \(^{13}\)C transitions. We define \(\Delta H\) as the difference in the number of spins in \(\alpha\) and \(\beta\) states for \(^1\)H, and \(\Delta X\) as the difference in the number of spins in \(\alpha\) and \(\beta\) states for \(^{13}\)C.

a. How is \(\Delta H\) related to \(\Delta X\)? Quantitatively, what is their ratio, and how is this ratio derived?

\[ \text{The ratio } \frac{\Delta H}{\Delta X} \text{ is proportional to the ratio of the gyromagnetic ratios for } ^1\text{H and } ^{13}\text{C.} \]
\[ \gamma^\text{H}/\gamma^{13}\text{C} = 26.7519/6.7283 = 3.98 \approx 4, \text{ so } \Delta H = 4\Delta X. \]

b. Which transition corresponds to the \(M_{\text{H}}^\alpha\) vector: \(A_1\), \(A_2\), \(X_1\) or \(X_2\)?

As indicated in the above diagram (above, right), the first spin in the pairs corresponds to the \(^1\)H spin and the second corresponds to the \(^{13}\)C spin. Thus, as shown in the diagram, the \(^1\)H transition (\(M_{\text{H}}\)) with the \(^{13}\)C spin in the \(\alpha\) state (\(C\alpha\)) is the \(A_2\) transition (\(\alpha\alpha \rightarrow \beta\alpha\)).

c. Complete the table below where \(N_1\)-\(N_4\) are the populations of the spin states in the above diagram, and \(A_1\), \(A_2\), \(X_1\) and \(X_2\) are the population differences for the \(A_1\), \(A_2\), \(X_1\) and \(X_2\) transitions respectively. Assume that \(N_4 = N\)

<table>
<thead>
<tr>
<th>at equilibrium</th>
<th>at ‘g’</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N_4 = N)</td>
<td>(N_4 = N + \Delta H)</td>
</tr>
<tr>
<td>(N_3 = N + \Delta X)</td>
<td>(N_3 = N + \Delta X)</td>
</tr>
<tr>
<td>(N_2 = N + \Delta H)</td>
<td>(N_2 = N)</td>
</tr>
<tr>
<td>(N_1 = N + \Delta H + \Delta X)</td>
<td>(N_1 = N + \Delta H + \Delta X)</td>
</tr>
<tr>
<td>(A_1 = N_2 - N_4 = \Delta H)</td>
<td>(A_1 = N_2 - N_4 = -\Delta H)</td>
</tr>
<tr>
<td>(A_2 = N_1 - N_4 = \Delta H)</td>
<td>(A_2 = N_1 - N_4 = \Delta H)</td>
</tr>
<tr>
<td>(X_1 = N_3 - N_4 = \Delta X)</td>
<td>(X_1 = N_3 - N_4 = \Delta X - \Delta H = -3\Delta X)</td>
</tr>
<tr>
<td>(X_2 = N_1 - N_2 = \Delta X)</td>
<td>(X_2 = N_1 - N_2 = \Delta X + \Delta H = 5\Delta X)</td>
</tr>
</tbody>
</table>
d. The vector diagram corresponding to point g' in the INEPT pulse sequence (see question 4) is shown below (right). The magnitudes of the $^{13}$C vectors are not drawn to scale, and the correct magnitudes should be available to you from your table above. Label each vector properly with the following information: $M_{C}^{H_a}$ or $M_{C}^{H_b}$, $X_1$ or $X_2$ (transition) and magnitude (in terms of $\Delta X$).

\[ \begin{align*}
M_{C}^{H_a}, X_2, & \quad 5\Delta X \\
M_{C}^{H_b}, X_1, & \quad -3\Delta X
\end{align*} \]


g'

\[ \begin{align*}
M_{C}^{H_a}, X_2, & \quad 5\Delta X \\
M_{C}^{H_b}, X_1, & \quad -3\Delta X
\end{align*} \]

e. At point g' in the INEPT pulse sequence, a $^{13}$C 90°x pulse is applied to create transverse $^{13}$C magnetization that subsequently is recorded (the FID, which we will call FID ‘A’) and Fourier transformed. Two signals are observed, corresponding to $M_{C}^{H_a}$ and $M_{C}^{H_b}$, of opposite phase, whose magnitudes are indicated in your table in ‘c’ and in your answer to ‘d’ above. Now, if the phase of the last $^1H$ 90°y pulse (point ‘f’) is changed to –y, and the FID (which we will call FID ‘B’) is added to the FID from the first experiment (FID ‘A’ + FID ‘B’), after Fourier transformation of the sum of the two FIDs, what will the signal look like (what will be the magnitude and phase of each component, how does this compare to the normal $^{13}$C spectrum/signal)? Please be sure to show your work. What happens if we instead subtract FID ‘B’ and FID ‘A’?

Changing the phase of the last $^1H$ pulse to –y will result in opposite phases for the vectors at ‘g’ with respect to the case where the pulse is y (see figure at right), and will result in the populations and population differences shown below:

\[
\begin{align*}
N_4 &= N \\
N_3 &= N + \Delta H + \Delta X \\
N_2 &= N + \Delta H \\
N_1 &= N + \Delta X \\
A_1 &= N_2 - N_4 = \Delta H \\
A_2 &= N_3 - N_2 = -\Delta H \\
X_1 &= N_3 - N_4 = \Delta X + \Delta H = 5\Delta X \\
X_2 &= N_4 - N_2 = \Delta X - \Delta H = -3\Delta X
\end{align*}
\]

Thus, if we add the components:

\[
\begin{align*}
M_{H}^{Ca}(X_2) \ 'y' & + M_{H}^{Ca}(X_2) \ '-y' = 5\Delta X + (-3\Delta X) = 2\Delta X \\
M_{H}^{CP}(X_1) \ 'y' & + M_{H}^{CP}(X_1) \ '-y' = (-3\Delta X) + 5\Delta X = 2\Delta X
\end{align*}
\]

The signal will look like the normal $^{13}$C signal: a normal signal would show $M_{H}^{Ca}$ and $M_{H}^{CP}$ to be of the same phase and each of magnitude $\Delta X$. In this case, each signal is $2\Delta X$, but we added two signals together. Thus, we have achieved absolutely nothing by adding the signals.

However, if we subtract the components:

\[
\begin{align*}
M_{H}^{Ca}(X_2) \ 'y' & - M_{H}^{Ca}(X_2) \ '-y' = 5\Delta X - (-3\Delta X) = 8\Delta X \\
M_{H}^{CP}(X_1) \ 'y' & - M_{H}^{CP}(X_1) \ '-y' = (-3\Delta X) - 5\Delta X = -8\Delta X
\end{align*}
\]

The signal will show $M_{H}^{Ca}$ and $M_{H}^{CP}$ to be of opposite phase but each is eight times as large as the normal $^{13}$C signal. Since we have used 2 FIDs, we have essentially enhanced the signals each equally by a factor of 4.
11. **(30 pts)** 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 $^1$H-$^1$H 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 $^1$H nuclei, and the X’s represent the transitions of the other $^1$H nucleus. We will define $\Delta H$ as the difference in the number of spins in $\alpha$ and $\beta$ states for A, and $\Delta H$ will also represent the difference in the number of spins in $\alpha$ and $\beta$ states for X.

**a.** Write down the equilibrium values for $N_1$-$N_4$ and the equilibrium population differences for the A and X transitions (please show how you calculate the A and X transition population differences). Assume that $N_4 = N$.

\[
\begin{align*}
N_4 & = N \\
N_2 & = N + \Delta H \\
N_1 & = N + 2\Delta H \\
N_3 & = N + \Delta H \\
A & = N_2 - N_4 = N_1 - N_3 = \Delta H \\
X & = N_3 - N_4 = N_1 - N_2 = \Delta H 
\end{align*}
\]

**b.** On the energy diagram above, draw dashed lines showing the $W_0$ (zero quantum) and $W_2$ (double quantum) relaxation pathways. Make sure to clearly, correctly and unambiguously label each pathway.

*The $W_2$ pathway connects the $\beta\beta$ and $\alpha\alpha$ states, whereas the $W_0$ pathway connects the $\alpha\beta$ and $\beta\alpha$ states.

**c.** (fill in the blanks) The $W_0$ and $W_2$ pathways do not operate with equal efficiencies for all molecules. For small molecules, the _____ pathway is more efficient, whereas for large molecules the _____ pathway is more efficient.

*The $W_0$ and $W_2$ pathways do not operate with equal efficiencies with all sizes of molecules. For small molecules, the $W_2$ pathway is more efficient, whereas for large molecules the $W_0$ pathway is more efficient.*
d. For a particular medium-sized molecule, the efficiencies of the $W_0$ and $W_2$ pathways are identical. If we perform a simple 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? What are the resulting values for $N_1$-$N_4$ and the population differences for the X transitions after relaxation via $W_0$ and $W_2$ take place (assume that the number of spins ($\delta$) relaxing via the $W_0$ pathway is equal to the number relaxing through the $W_2$ pathway)?

Before any relaxation takes place, the values for $N_1$-$N_4$ and the population differences for the A and X transitions are:

\[
\begin{align*}
N_4 &= N + \Delta H/2 \\
N_3 &= N + 3\Delta H/2 \\
N_2 &= N + \Delta H/2 \\
N_1 &= N + 3\Delta H/2
\end{align*}
\]

\[
A = N_2 - N_4 = N_1 - N_3 = 0, \\
X = N_3 - N_4 = N_1 - N_2 = \Delta H
\]

If we assume that $\delta$ spins relax via $W_2$ (add $\delta$ to $N_1$, subtract $\delta$ from $N_4$) and $W_0$ (add $\delta$ to $N_2$, subtract $\delta$ from $N_3$) then the new values for $N_1$-$N_4$ and new population differences for X become:

\[
\begin{align*}
N_4 &= N + \Delta H/2 - \delta \\
N_3 &= N + 3\Delta H/2 - \delta \\
N_2 &= N + \Delta H/2 + \delta \\
N_1 &= N + 3\Delta H/2 + \delta
\end{align*}
\]

\[
X = N_3 - N_4 = N_1 - N_2 = \Delta H
\]

e. Explain your result to question ‘d’ in terms of medium-sized molecules in general.

The result in ‘d’ suggests that when both $W_0$ and $W_2$ are operating with equal efficiencies, the population differences for the X transitions following saturation do not differ from those at equilibrium, i.e. there is no NOE enhancement. Thus, in general, for medium-sized molecules, neither the $W_0$ nor the $W_2$ pathway predominates, but both are operative, and the balance of $W_0$ and $W_2$ prevents observation of measurable NOE effects.
12. (30 pts) The HSQC pulse sequence is shown below.

![HSQC Pulse Sequence Diagram]

a. The magnetization vectors present at point ‘h’ in the pulse sequence are shown, as are the vectors at point ‘i’ (following the first t₁/2 period), for a simple spin system. The vector $M_C^{Hα}$ precesses slower than the reference frequency, and $M_C^{Hβ}$ precesses faster than the reference frequency, such that after t₁/2, the vectors have moved $\psi$ and $\phi$ degrees, respectively, away from the y axis. Complete the vector diagrams for points ‘j’ and ‘k’ below. Be complete.

![Vector Diagrams ‘h’, ‘i’, ‘j’, ‘k’]

b. Based on your knowledge of the HSQC pulse sequence and method, and based on your answer to ‘a’ above, what is the purpose of the $^1H$ 180° pulse in the center of the t₁ evolution period? How does your answer to ‘a’ support your contention?

*The 180° pulse in the middle of the t₁ evolution period prevents net $^1H$-$^{13}C$ coupling evolution. As shown in the answer to ‘a’, both at the beginning (‘h’) and at the end (‘k’) of t₁, the $^{13}C$ magnetization components are 180° out of phase with one another, with no net phase change between them occurring during t₁. Thus, the 180° pulse decouples $^1H$ from $^{13}C$ during t₁.*
c. Let’s compare the sensitivity of INEPT and HSQC. If, at point ‘h’ in HSQC, we simply detect the $^{13}$C magnetization, this is essentially the INEPT experiment, and, as we know, the signals from $^{13}$C nuclei are enhanced substantially by INEPT. On the other hand, if the magnetization from ‘h’ is subjected to a reverse INEPT (this is HSQC) and the $^1$H magnetization is detected, the signal is much more intense compared to INEPT. Assuming no additional magnetization losses associated with the reverse INEPT period, calculate the ratio of the intensity of HSQC to INEPT.

Sensitivity is proportional to the electromotive force ($\varepsilon$) induced in the receiver coil by the bulk magnetic moment. The magnitude of $\varepsilon$ is proportional to the rate of change in the magnetic moment:

$$\varepsilon \propto \frac{dM}{dt} = \gamma M_0 B$$

Remember, $M_0 = \frac{N\gamma^2 h^2 B_0 I(I+1)}{3k_BT}$ (“h” here is “h-bar”, Planck’s constant divided by $2\pi$), so

$$\varepsilon \propto \gamma M_0 B = \frac{N\gamma h^2 B_0^2 I(I+1)}{3k_BT}.$$ 

So, whereas the magnitude of the bulk magnetization is dependent on $\gamma^2$, the sensitivity is dependent on $\gamma^3$.

The main difference between HSQC and HETCOR in this respect is that the magnetization is detected on $^1$H in HSQC and on $^{13}$C in HETCOR.

Thus,

$$\frac{\varepsilon_{^{1}H}}{\varepsilon_{^{13}C}} = \frac{\frac{N\gamma^{3}h^{3}B_{0}^{2}I(I+1)}{3k_{B}T}}{\frac{N\gamma^{3}h^{3}B_{0}^{2}I(I+1)}{3k_{B}T}} = \frac{\gamma_{^{1}H}^{3}}{\gamma_{^{13}C}^{3}} = \left(\frac{26.7519 \times 10^{7} \text{ rad/Ts}}{6.7283 \times 10^{7} \text{ rad/Ts}}\right)^{3} = \frac{(26.7519 \times 10^{7} \text{ rad/Ts})^{3}}{(6.7283 \times 10^{7} \text{ rad/Ts})^{3}} = 62.856$$

So, all else being equal, HSQC is about 63 times more sensitive than HETCOR.
You may find some of the following information or equations useful:

\[
\begin{array}{|c|c|c|}
\hline
B_0 \\
\text{(Tesla, T)} & \text{Resonance frequencies} & \text{MHz} \\
\hline
\hline
9.4 & 400 & 100.6 \\
11.74 & 500 & 125.7 \\
14.09 & 600 & 150.9 \\
18.79 & 800 & 201.2 \\
\hline
\end{array}
\]

\(\gamma_{^1H} = 26.7519 \times 10^7 \text{ rad/Ts, } I = 1/2\)

\(\gamma_{^{15}N} = -2.7126 \times 10^7 \text{ rad/Ts, } I = 1/2\)

\(\gamma_{^{10}B} = 2.8747 \times 10^7 \text{ rad/Ts, } I = 3\)

\(\gamma_{^{17}O} = -3.6280 \times 10^7 \text{ rad/Ts, } I = 5/2\)

\(\gamma_{^{13}C} = 6.7283 \times 10^7 \text{ rad/Ts, } I = 1/2\)

\[
M_0 = \frac{N\gamma^2h^2B_0I(I+1)}{3k_BT} \quad ("h" \text{ here is "h-bar", Planck's constant divided by } 2\pi)
\]

\[
e \propto \frac{\gamma M_0 B}{\gamma^2h^2B_0^2I(I+1)} \quad ("h" \text{ here is "h-bar", Planck's constant divided by } 2\pi)
\]

\[
S/N \propto N/N^{1/2} = N^{1/2} \quad (\text{signal-to-noise improves with (number of scans)}^{1/2})
\]

\[
m = (-I, -I+1, \ldots, I-1, I)
\]

\[
E = \mu_zB_0 = -n\gamma hB_0
\]

\[
\Delta E = \mu_zB_0 = \gamma hB_0 
\]

\[
I = (1 + \eta) I_0 
\]

\[
\nu = \frac{\gamma B_0}{(2\pi)} 
\]

\[
f \propto 1/r^6
\]

\[
\Delta \delta = \frac{\Delta \nu}{\text{observe frequency}} \times 10^6
\]

\[
I \propto 1/r^6
\]

\[
M_z = M_0(1 - e^{-t/T_1})
\]

\[
M_z = M_0(1 - 2e^{-t/T_1})
\]

\[
t_{zero} = T_1\ln(2)
\]