Midterm Exam: CHEM/BCMB 4190/6190/8189 (155 pts)  
Tuesday, 7 October, 2003

1). In the example (right), the effect of a 90° (π/2) pulse applied along the “x” axis (90°x) is shown. For a-f below, show the effect of the indicated pulses on the indicated magnetization vectors by drawing the resulting vectors on the coordinate axes. (12 pts)

(a)  

(b)  

(c)  

(d)  

(e)  

(f)  

2). A diagram of the Zeeman levels/states for $^{13}$C is shown to the right. Draw the Zeeman diagram for $^{10}$B ($I = 3$). (4 pts)

\[
\begin{align*}
E &= +3\gamma H B_0 \\
E &= +2\gamma H B_0 \\
E &= +1\gamma H B_0 \\
E &= 0\gamma H B_0 = 0 \\
E &= -1\gamma H B_0 \\
E &= -2\gamma H B_0 \\
E &= -3\gamma H B_0 \\
E &= +1/2\gamma H B_0 \\
E &= -1/2\gamma H B_0
\end{align*}
\]
3). If we directly compare $^{10}$B spins and $^{13}$C spins:
   a. What is the ratio of the bulk/macroscopic magnetization ($M_{0\,^{10}B}/M_{0\,^{13}C}$) produced for
equal numbers of nuclei? (6 pts)
b. How does the sensitivity of the NMR signal compare for $^{10}$B and $^{13}$C spins? (6 pts)

   a. 
   \[
   \frac{M_{0\,^{10}B}}{M_{0\,^{13}C}} = \frac{N\gamma_{^{10}B^2h^2B_0I_{^{10}B}(I_{^{10}B} + 1)}}{3k_BT} \times \frac{3k_BT}{N\gamma_{^{13}C^2h^2B_0I_{^{13}C}(I_{^{13}C} + 1)}} = \frac{\gamma_{^{10}B^2} \times 3(3 + 1)}{\gamma_{^{13}C^2} \times \frac{1}{2}(\frac{1}{2} + 1)} = \frac{(2.8747 \times 10^7 \text{ rad/Ts})^2 \times 12}{(6.7283 \times 10^7 \text{ rad/Ts})^2 \times \frac{3}{4}} = 2.9207
   \]
   So, the bulk magnetization is about 2.9 times as large for $^{10}$B as for $^{13}$C.

   b. 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 ($\varepsilon \propto dM/dt = \gamma M_0 B$).

   Remember, $M_0 = \frac{N\gamma^2h^2B_0I(I+1)}{3k_BT}$ (“$h$” here is “h-bar”, Planck’s constant divided by $2\pi$),
   so $\varepsilon \propto \gamma M_0 B = \frac{N\gamma^2h^2B_0^2I(I+1)}{3k_BT}$. So, whereas the magnitude of the bulk magnetization was
   dependent on $\gamma^2$, the sensitivity is dependent on $\gamma^3$.

   Thus, 
   \[
   \frac{\varepsilon_{^{10}B}}{\varepsilon_{^{13}C}} = \frac{N\gamma_{^{10}B}^3h^2B_0^2I_{^{10}B}(I_{^{10}B} + 1)}{3k_BT} \times \frac{3k_BT}{N\gamma_{^{13}C}^3h^2B_0^2I_{^{13}C}(I_{^{13}C} + 1)} = \frac{\gamma_{^{10}B}^3I_{^{10}B}(I_{^{10}B} + 1)}{\gamma_{^{13}C}^3I_{^{13}C}(I_{^{13}C} + 1)}\left(\frac{2.8747 \times 10^7 \text{ rad/Ts})^3 \times 12}{(6.7283 \times 10^7 \text{ rad/Ts})^3 \times \frac{3}{4}}\right) = 1.248
   \]
   So, $^{10}$B nuclei are about 1.25 times more sensitive than $^{13}$C nuclei.
4). The frequency difference ($\Delta \nu$) between two $^1$H signals in a $^1$H spectrum is 4320 Hz when the spectrum is acquired with a magnetic field strength of 9.4 T (corresponding to a $^1$H resonance/observe frequency of 400 MHz).

a. What would be the frequency difference between the two $^1$H signals if the spectrum was acquired with a 18.79 T magnet? (5 pts)

b. What is the chemical shift difference ($\Delta \delta$) between the two signals? (5 pts)

\[ \text{a. We know that } 18.79 \text{ T} = 800 \text{ MHz, } \Delta \delta_{400} = \frac{\Delta \nu_{400}}{400 \text{ MHz}} \times 10^6 \text{ and } \Delta \delta_{800} = \frac{\Delta \nu_{800}}{800 \text{ MHz}} \times 10^6. \text{ We also know that, by definition, } \Delta \delta_{400} = \Delta \delta_{800} \text{ (chemical shift is independent of } B_0). \text{ So, the ratio of the frequencies is equal to the ratio of the field strengths:} \]
\[
\frac{\Delta \nu_{400}}{\Delta \nu_{800}} = \frac{400 \text{ MHz}}{800 \text{ MHz}}, \text{ therefore, } \Delta \nu_{800} = 4320 \text{ Hz} \times \frac{800 \text{ MHz}}{400 \text{ MHz}} = 8640 \text{ Hz}.
\]

\[ \text{b. } \Delta \delta = \frac{\Delta \nu}{\text{observe frequency}} \times 10^6 = \frac{4320 \text{ Hz}}{400 \text{ MHz}} \times 10^6 = \frac{4320 \text{ Hz}}{400 \text{ Hz}} = 10.8 \text{ "ppm"} \]

5). a. For the hydrocarbon below, the $T_1$ values for the $^{13}$C nuclei (in seconds, shown below the respective carbon atoms) increase towards the ends of the hydrocarbon chain. Please explain this observation. (4 pts)

\[
\begin{array}{cccccccc}
\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3 \\
6.6 & 5.7 & 5.0 & 4.4 & 4.4 & 5.0 & 5.7 & 6.6
\end{array}
\]

The data would indicate that the ends of the long hydrocarbon chain are probably somewhat more flexible than the center, or are able to move somewhat more independently of the rest of the molecule than the center of the chain. This has the effect of increasing the $T_1$ times.

b. You measure $T_2$ values for the same $^{13}$C nuclei (seconds, below). You notice the same trend (increasing values towards the ends of the hydrocarbon chain). Your measurements are, however, unequivocally incorrect. Why? (4 pts)

\[
\begin{array}{cccccccc}
\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3 \\
6.4 & 5.9 & 4.8 & 4.6 & 4.6 & 4.8 & 5.9 & 6.4
\end{array}
\]

In two cases, the measured values for $T_2$ are larger than the measured values for $T_1$. $T_2$ for a given nucleus cannot be larger than $T_1$. Therefore, the measurements must be in error.
6). For monosubstituted benzene molecules of the type shown at the right, the chemical shifts at the ortho, meta, and para positions can be calculated by the following simple empirical relationship: \( \delta^1H = 7.27 + \delta_{corr} \), where \( \delta_{corr} \) is an empirical correction factor from a table, and is specific for the ortho (\( \delta_{ortho} \)), meta (\( \delta_{meta} \)), or para (\( \delta_{para} \)) position. For instance, when R = -OCOCH₃, \( \delta^1H_{para} = 7.27 + (\delta_{para}) = 7.27 + (-0.13) = 7.14 \) ppm. For R = -COR and R = -NH₂, the entries for \( \delta_{ortho} \), \( \delta_{meta} \), and \( \delta_{para} \) are missing from the table below. The numerical values for these entries are, from smallest to largest, -0.75, -0.65, -0.25, 0.14, 0.21, and 0.62. Your job is to put these numbers in their correct places in the table. In order to receive credit, you will have to a). draw appropriate resonance structures to justify your table entries, and b). discuss why certain positions are more/less shielded relative to others. (16 pts)

<table>
<thead>
<tr>
<th>R</th>
<th>( \delta_{ortho} )</th>
<th>( \delta_{meta} )</th>
<th>( \delta_{para} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>-COR</td>
<td>0.62</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>-NH₂</td>
<td>-0.75</td>
<td>-0.25</td>
<td>-0.65</td>
</tr>
<tr>
<td>-OCOCH₃</td>
<td>-0.25</td>
<td>0.03</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

For R = -COR:
\( \delta^1H_{ortho} = 7.27 + (\delta_{ortho}) = 7.27 + (0.62) = 7.89 \) ppm  
\( \delta^1H_{meta} = 7.27 + (\delta_{meta}) = 7.27 + (0.14) = 7.41 \) ppm  
\( \delta^1H_{para} = 7.27 + (\delta_{para}) = 7.27 + (0.21) = 7.48 \) ppm  
Resonance structures show that ortho and para positions will be deshielded, thus the chemical shifts will be highest in these positions. The chemical shift will be highest at ortho due to proximity to the electron withdrawing substituent.

For R = -NH₂:
\( \delta^1H_{ortho} = 7.27 + (\delta_{ortho}) = 7.27 + (-0.75) = 6.52 \) ppm  
\( \delta^1H_{meta} = 7.27 + (\delta_{meta}) = 7.27 + (-0.25) = 7.02 \) ppm  
\( \delta^1H_{para} = 7.27 + (\delta_{para}) = 7.27 + (-0.65) = 6.62 \) ppm  
Resonance structures show that ortho and para positions will be shielded, thus the chemical shifts will be smallest in these positions. The chemical shift will be smallest at ortho due to proximity to the electron donating substituent.
7). You record an $^{15}$N NMR spectrum of your compound using 100 scans. You realize that you need to improve the sensitivity (signal-to-noise) by 5-fold. How many scans will be required to record a spectrum with 5 times the signal-to-noise? (5 pts)

Signal-to-noise (S/N) increases as the square root of the number of scans ($S/N \propto N^{1/2}$). In order to improve S/N by 5×, one would have to acquire $5^2$ or $25 \times$ the number of scans. So, if 100 scans were used initially, $25 \times 100 = 2500$ scans would be required for a 5× S/N improvement.

8). Fill in the blanks:

a. At equilibrium, for a given nucleus in a sample, the number of alpha spins ($N_\alpha$) = X, and the number of beta spins ($N_\beta$) = Y. Immediately after a 180° pulse, the number of alpha spins = ______ Y _____ and the number of beta spins = _______ X _____. (2 pts)

b. At equilibrium, for a given nucleus in a sample, the number of alpha spins ($N_\alpha$) = X, and the number of beta spins ($N_\beta$) = Y. Immediately after a 90° pulse, the number of alpha spins = __ (X+Y)/2 ____ and the number of beta spins = ____ (X+Y)/2 ___. (4 pts)

For “a”, the answers are “Y” and “X”. A 180 degree pulse simply interconverts the number of alpha and beta spins. For “b”, the answers are “(X+Y)/2” and “(X+Y)/2”. A 90 degree pulse makes the number of alpha and beta spins equal to one another.

9). The signal (large doublet, 6.37 ppm) in the $^1$H NMR spectrum from one of the aliphatic protons in 4′-hydroxy cinnamic acid is shown at the right. Two small signals (both doublets) on either side of the large doublet are highlighted (*), and are shown more clearly in the magnified region (far right). Please explain precisely what these peaks are (no jargon, please). Also, the distance between the centers of these two small doublets (frequency difference) is a measure of what? (6 pts)

The large doublet, of course, results from the $^1$H bound to $^{12}$C (at natural abundance, about 99% of all carbon is $^{12}$C). The splitting is due to coupling to a neighboring $^1$H ($^3J_{H,H}$). The small doublets result from the $^1$H bound to $^{13}$C (about 1%). Since $^{13}$C is also spin 1/2 ($I=1/2$), the signal is split into a doublet by $^1J_{C,H}$, about 110 Hz in the example shown.
10). For the following compound (where the R groups contain no $^1$H, and R$'$≠R$''$);

$$(R')_2$-CH$_1$-CH$_2$-(R$''$)$_2$$

a. draw the expected $^1$H NMR spectrum if the spectrum is strictly first order. (2 pts)
b. draw the expected $^1$H NMR spectrum if the spectrum is higher order. (2 pts)
c. You record the spectrum of this compound and it looks to be higher order. Your friend records the spectrum and it looks to be first order. Who is correct? Is it possible that both of you are correct? If so, why? If not, why not? (4 pts)

$a$ and $b$. 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. For both ‘a’ and ‘b’, we would expect to see two signals, each a doublet (being split by the neighboring $^1$H). In the case of ‘a’, the intensities of the multiplet components would be equivalent, in the case of ‘b’ they would not be. In the case of ‘a’, the multiplet components would be symmetrically disposed about the absolute frequency of the nucleus. Again, not so in the case of b:

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{spectrum.png}
\end{figure}

\begin{align*}
&\text{a.} & \nu_1 & \nu_2 \\
&\text{b.} & \nu_1 & \nu_2
\end{align*}

c. Both of you could of course be correct. If you increase the field strength at which you make your measurements, spectra tend to become more first order, because the frequency difference between signals (which, of course, is dependent on field strength) becomes larger. If your friend recorded the spectrum at a very high field strength, such that $\Delta \nu >> J$, and if you recorded your spectrum at a very low field strength such that $\Delta \nu = J$, then the observations that both of you made could of course both be correct.

11). You have a sample for NMR analysis that contains a single proton. How would you determine what the $180^\circ$ pulse width is for the single $^1$H resonance in your sample? (4 pts)

We know that only transverse magnetization is detected by the receiver of our NMR instrumentation. A perfect $180^\circ$ pulse creates no transverse magnetization, so should give no detectible signal. Normally, one simple slowly increases the pulse width from zero through a maximum ($90^\circ$ pulse) until no detectible signal remains. This should be the $180^\circ$ pulse width, and should be $2 \times$ the $90^\circ$ pulse width.
12). a. Explain, in words (no equations or pictures) the Karplus relationship. (3 pts)

The Karplus relationship is the quantitative dependence of the magnitude of the three-bond coupling constant ($^3J$) on the magnitude of the dihedral (torsional) angle between the nuclei.

b. For most substituted ethane derivatives, the vicinal $^3J$ $^1$H coupling constants are usually about 7 Hz. Explain this observation. (3 pts)

In ethane derivatives, the different gauche and trans conformers display $^3J$ $^1$H coupling constants varying from 3-16 Hz. Fast conformational averaging results in an arithmetic average value of about 7 Hz. The observation that most ethane derivatives display this average value for this coupling indicates rapid exchange between the conformers with no energetic preference for one distinct conformation over another.

c. For a particular substituted ethane, you measure the vicinal $^3J$ $^1$H coupling constant and find it to be 11 Hz. How would you interpret this finding? (3 pts)

Probably, in this case, this would indicate that one conformer is energetically preferred over the others. If this is a disubstituted ethane, the large coupling would indicate that the dihedral angle is large, and the orientation probably trans.

13). In the $^1$H NMR spectrum of menthol, the peak at ~2.2 ppm is a doublet of septets. In general, for a septet in a first order spectrum, what are the relative intensities of the individual peaks that comprise a septet? (5 pts)

The intensities of the individual peaks in first order multiplets are given by the coefficients of the binomial series (Pascal’s Triangle):

<table>
<thead>
<tr>
<th>intensities</th>
<th>multiplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>singlet</td>
</tr>
<tr>
<td>1:1</td>
<td>doublet</td>
</tr>
<tr>
<td>1:2:1</td>
<td>triplet</td>
</tr>
<tr>
<td>1:3:3:1</td>
<td>quartet</td>
</tr>
<tr>
<td>1:4:6:4:1</td>
<td>quintet</td>
</tr>
<tr>
<td>1:5:10:10:5:1</td>
<td>sextet</td>
</tr>
</tbody>
</table>
14). The $^1$H NMR signal ($B_0=9.4$ Tesla, 400.00 MHz) of a single $^1$H nucleus of a complicated organic molecule is shown below. The $^1$H chemical shifts of three of the multiplet components are shown in the table. There are two couplings giving rise to the multiplet, characterized by the coupling constants $J_s$ (small) and $J_b$ (big). Figure out the chemical shifts for the remaining multiplet components, and the values for the coupling constants. Guesses will not be rewarded: show your work and/or describe how you determine the missing values. Fill in the remaining chemical shifts in the table and the values for the coupling constants. (15 pts)

The smaller coupling is responsible for the a/b, c/d and e/f pairs. Thus, calculating $\delta_e$ is easy:

$$\delta_a - \delta_b = 0.01 = \delta_c - \delta_d = \delta_e - \delta_f$$

therefore $\delta_e = 3.380 + 0.01 = 3.390$ ppm.

We therefore also know that:

$$\frac{\delta_a - \delta_b}{2} = \frac{\delta_c - \delta_d}{2} = \frac{\delta_e - \delta_f}{2} = \frac{0.01}{2} = 0.05$$

There are lots of different ways to find $\delta_c$ and $\delta_d$. Probably the easiest is to calculate the center of the signal, and then add and subtract $\frac{\delta_c - \delta_d}{2} = 0.05$:

$$\delta_{\text{center}} = \frac{\delta_a + \delta_f}{2} = \frac{3.440 + 3.380}{2} = 3.410$$

$$\delta_{\text{center}} = \frac{\delta_b + \delta_e}{2} = \frac{3.430 + 3.390}{2} = 3.410$$

$$\delta_c = \delta_{\text{center}} + \frac{\delta_c - \delta_d}{2} = 3.410 + 0.05 = 3.415$$

and

$$\delta_d = \delta_{\text{center}} - \frac{\delta_c - \delta_d}{2} = 3.410 - 0.05 = 3.405$$

We know that the small coupling constant is $J_s = 0.01$ ppm $\times \frac{400 \text{ Hz}}{\text{ppm}} = 4$ Hz.

We can calculate the large coupling any number of ways (we don’t need to know $\delta_c$ or $\delta_d$);

$$J_b = \frac{(\delta_a + \delta_b)}{2} - \frac{(\delta_c + \delta_d)}{2} \times \frac{400 \text{ Hz}}{\text{ppm}} = \frac{(3.440 + 3.430)}{2} - \frac{(3.390 + 3.380)}{2} \times \frac{400 \text{ Hz}}{\text{ppm}} = 0.025 \text{ ppm} \times \frac{400 \text{ Hz}}{\text{ppm}} = 10 \text{ Hz}$$
15). Heteronuclear decoupling (note: in figures, bb = broadband)

a. In the broadband heteronuclear decoupling experiment (right), all $^1$H-X couplings are removed. When X=$^{13}$C, substantial signal-to-noise (S/N) gains (relative to the experiment without decoupling) can be achieved. What are the two mechanisms by which the S/N is increased in this case? Discuss how each contributes to increased S/N. Is the S/N of all $^{13}$C nuclei equally affected? Why or why not? (5 pts)

Decoupling causes collapse of the multiplet into a singlet. The addition of the multiplet components into the singlet increases the S/N. During the relaxation delay, the broadband decoupling establishes the heteronuclear ($^1$H-$^{13}$C) NOE, which can substantially increase the S/N. The contribution to the S/N increase from the NOE is generally larger for carbon nuclei with more bound protons (-CH$_3$ > -CH$_2$ > etc.).

b. In the gated decoupling experiment (right), are $^1$H-X couplings observed in the spectra? If X=$^{13}$C, is the S/N increased in this experiment? Why or why not? (5 pts)

$^1$H-X couplings ARE observed….the couplings are active immediately once decoupling is ceased. If X=$^{13}$C, the S/N is still increased, however. Even though the S/N increase from multiplet collapse is not achieved in this experiment (in fact, this experiment is often performed because the coupling information is sought), the S/N enhancement from the $^1$H-$^{13}$C NOE is still attained (the NOE is established by the broadband decoupling during the relaxation delay and is usually only slightly diminished during acquisition).

c. You are studying a small organic molecule containing $^{15}$N and you would like to decouple $^1$H nuclei from $^{15}$N (X=$^{15}$N). You wisely choose to use the inverse gated decoupling scheme (right) as opposed to the scheme in ‘a’. Why? What would be the consequence of using the scheme in ‘a’ as opposed to the inverse gated scheme, and what is the reason? (5 pts)

This scheme is chosen, firstly, because it enables the decoupling (multiplet structure eliminated), as the broadband decoupling is performed during acquisition. Also, decoupling is NOT performed during the relaxation delay. This is important since X=$^{15}$N, and the gyromagnetic ration for $^{15}$N is negative. In such a case, the heteronuclear NOE is negative, and any NOE would lead to a DECREASE in S/N rather than an NOE enhancement of S/N.
16). The following diagram (below, left) represents the inversion-recovery pulse sequence (experiment) used to measure $T_1$ for $^{13}$C nuclei. Initially (point “a”), the bulk magnetization is aligned along $+z$, and is inverted by the $180^\circ x$ pulse ($-z$, point “b”). For a-e, for the indicated values of $\tau$, indicate the orientation and magnitude of the bulk magnetization vector on the coordinates axes. Also, sketch the signal obtained after Fourier transform of the FID. Assume the molecule we are studying has a single carbon atom and $T_1$ is 10 s. (20 pts)

f. If $T_1 = 10$ s, how long does $\tau$ have to be before 90% of the bulk magnetization at point “b” is returned to $+z$ at point “c”? 

$$M_z = M_0(1 - 2e^{-\tau/T_1}) \quad \frac{M_z}{M_0} = 0.9 = 1 - 2e^{-\tau/T_1} \quad 0.1 = 2e^{-\tau/T_1} \quad 0.05 = e^{-\tau/T_1} \quad 2.9957 = \tau/T_1 \quad \tau = 29.957 \text{s}$$
You may find some of the following information or equations useful:

<table>
<thead>
<tr>
<th>$B_0$ (Tesla, T)</th>
<th>Resonance frequencies (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^1H$</td>
</tr>
<tr>
<td>9.4</td>
<td>400</td>
</tr>
<tr>
<td>11.74</td>
<td>500</td>
</tr>
<tr>
<td>14.09</td>
<td>600</td>
</tr>
<tr>
<td>18.79</td>
<td>800</td>
</tr>
</tbody>
</table>

$\gamma_{^1H} = 26.7519 \times 10^7$ rad/Ts, $I = 1/2$
$\gamma_{^{15}N} = -2.7126 \times 10^7$ rad/Ts, $I = 1/2$
$\gamma_{^{10}B} = 2.8747 \times 10^7$ rad/Ts, $I = 3$
$\gamma_{^{17}O} = -3.6280 \times 10^7$ rad/Ts, $I = 5/2$
$\gamma_{^{13}C} = 6.7283 \times 10^7$ rad/Ts, $I = 1/2$

$M_0 = \frac{N\gamma^2\hbar^2B_0I(I+1)}{3k_BT}$ (“h” here is “h-bar”, Planck’s constant divided by 2π)

$\varepsilon \propto \frac{\Delta M}{\Delta t} = \gamma M_0 B = \frac{N\gamma^2\hbar^2B_0^2I(I+1)}{3k_BT}$ (“h” here is “h-bar”, Planck’s constant divided by 2π)

$S/N \propto \frac{N}{N^{1/2}} = N^{1/2}$ (signal-to-noise improves with (number of scans)$^{1/2}$)

$m = (-I, -I+1, \ldots, I-1, I)$

$E = \mu_zB_0 = -m\gamma\hbar B_0$

$\Delta E = \mu_z B_0 = \gamma\hbar B_0$

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

$M_z = M_0(1 - e^{-t/T_1})$

$M_z = M_0(1 - 2e^{-t/T_1})$

$t_{\text{zero}} = T_1 \ln(2)$