

**Exam 3: CHEM/BCMB 4190/6190/8189 (184 points) Tuesday, 26 October, 2021**

1). In a magnetic field (500 MHz), the energy difference between the lowest and highest energy states for a particular nucleus ( $\gamma = 3.00 \times 10^7 \text{ rad T}^{-1} \text{ s}^{-1}$ ) is  $3.501 \times 10^{-25} \text{ J}$ . What is the spin angular momentum quantum number,  $I$ , for this nucleus? You will have to show your work and explain your answer for credit. (6 points)

*The energy between any two adjacent energy levels is:*

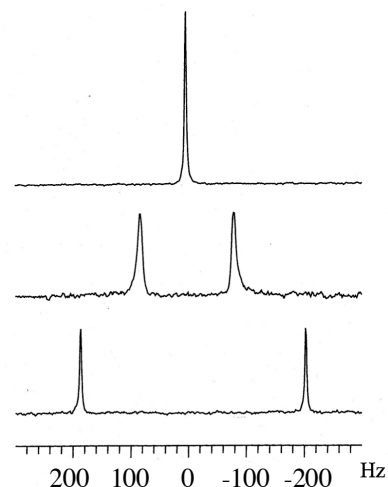
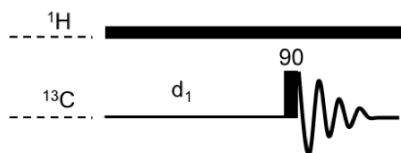
$$E = \gamma \hbar B_0$$

*There are  $(2I+1)$  levels, ranging from  $I$  to  $-I$  ( $I, I-1, I-2, \dots, -I$ ). So, the highest and lowest values of  $I$  are separated by  $2I$  ( $I - (-I) = 2I$ ). So, the energy between the highest and lowest energy levels is then:*

$$E = (2I)\gamma \hbar B_0$$

$$E = (2I)\gamma \hbar B_0 \quad I = \frac{E}{2\gamma \hbar B_0} = \frac{3.501 \times 10^{-25} \text{ J}}{3.00 \times 10^7 \text{ rad T}^{-1} \text{ s}^{-1} \frac{6.626 \times 10^{-34} \text{ J s}}{\pi} 11.74 \text{ T}} = 1.5 = \frac{3}{2}$$

2). The  $^{13}\text{C}$  NMR spectrum (right, bottom) shows the  $^{13}\text{C}$ - $^1\text{H}$  doublet from a methine group in the molecule  $\text{CHX}_3$ , where 'X' are halides (i.e., chloroform). The spectrum at the top shows the results of selective, on-resonance, decoupling of  $^1\text{H}$  from  $^{13}\text{C}$  in this molecule. This is done with the pulse sequence below with the decoupler frequency matching the Larmor frequency of the  $^1\text{H}$  in the molecule. As a result, the  $^{13}\text{C}$  signal is decoupled from the  $^1\text{H}$  and appears as a singlet. The spectrum in the center (right) is the result of off-resonance decoupling of  $^1\text{H}$ , where the decoupler frequency is not at the Larmor frequency of the  $^1\text{H}$ . The multiplet has partially collapsed (the apparent coupling constant is smaller than the actual value).



a. What determines the difference between the actual and observed coupling constants (in Hz) in an off-resonance decoupling experiment such as the one described for the spectrum above? (4 points)

*The difference between the actual and observed coupling constant, or the decrease in the coupling constant as a result of the off-resonance decoupling, is dependent on the strength of the decoupler field ( $B_2$ ), and the frequency difference between the Larmor frequency of the  $^1\text{H}$  nucleus of interest and the frequency at which the decoupling field is applied ( $\Delta\nu$ ). The equation below describes the relationship, where  $J$  is the actual  $^{13}\text{C}$ - $^1\text{H}$  coupling constant and  $J_r$  is the reduced (observed) coupling constant resulting from the off-resonance decoupling.*

$$B_2 = \frac{\Delta\nu \sqrt{J^2 - J_r^2}}{J_r} \approx \frac{J \Delta\nu}{J_r}$$

b. Calculate the difference between the Larmor frequency of the  $^1\text{H}$  in the molecule and the frequency at which the decoupling field is applied for the off-resonance experiment shown above if the decoupler field strength ( $B_2$ ) used is 10 kHz. (4 points)

*From the spectra above, we can estimate the actual coupling ( $J$ ) as 400 Hz, and we can estimate the reduced coupling ( $J_r$ , observed coupling in the off-resonance experiment) to be 160 Hz.*

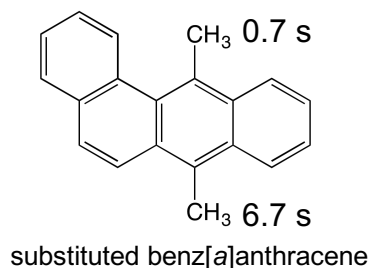
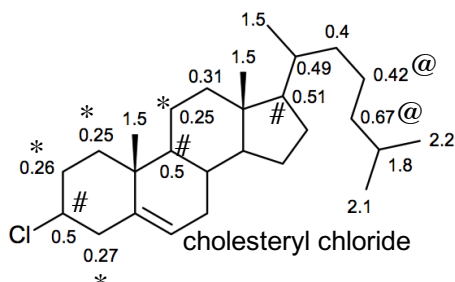
$$\Delta\nu = \frac{B_2 \times J_r}{\sqrt{J^2 - J_r^2}} = \frac{10,000 \text{ Hz} \times 160 \text{ Hz}}{\sqrt{400^2 - 160^2} \text{ Hz}} = \frac{1.6 \times 10^6 \text{ Hz}^2}{366.6 \text{ Hz}} = 4364 \text{ Hz}$$

$$\Delta\nu \approx \frac{B_2 \times J_r}{J} = \frac{10,000 \text{ Hz} \times 160 \text{ Hz}}{400 \text{ Hz}} = \frac{1.6 \times 10^6 \text{ Hz}^2}{400 \text{ Hz}} = 4000 \text{ Hz}$$

3). For each statement below, circle the option that correctly completes the statement.

- $T_1$  relaxation is sometimes called ( spin-lattice : spin-spin ) relaxation. (2 points)
- The molecular correlation time,  $\tau_c$ , is inversely proportional to (  $T_1$  :  $1/T_1$  ). (2 points)
- $T_1$  values ( increase : decrease ) as molecular size decreases. (2 points)
- For  $^{13}\text{C}$  relaxation, decreasing the number of hydrogens attached to a carbon ( increases : decreases ) the  $^{13}\text{C}$   $T_1$ . (2 points)
- $T_2$  can be (longer than : shorter than)  $T_1$ . (2 points)
- If  $T_2^*$  times are short, NMR signals are ( narrow : broad ). (2 points)

4). The values of the  $^{13}\text{C}$   $T_1$  relaxation time constants (seconds) are shown for nuclei in the compounds shown below.



- For the molecule on the left (cholesteryl chloride), explain why the  $T_1$  relaxation time constants for the  $^{13}\text{C}$  nuclei labeled with asterisks (\*) are about  $\frac{1}{2}$  as large as those marked with pound signs (#). (4 points)

*The  $^{13}\text{C}$  nuclei in the methylene ( $-\text{CH}_2$ ) groups (“\*”) have two attached hydrogens, whereas those in the methine ( $-\text{CH}$ ) groups (“#”) each have a single attached hydrogen. They all are in the same rigid ring frame in the molecule, so their mobilities are the same. Because the dipolar interaction with attached hydrogens is the main mechanism for  $^{13}\text{C}$  relaxation, the methylene carbons relax faster (shorter  $T_1$  time constants) because of the additional attached hydrogen.*

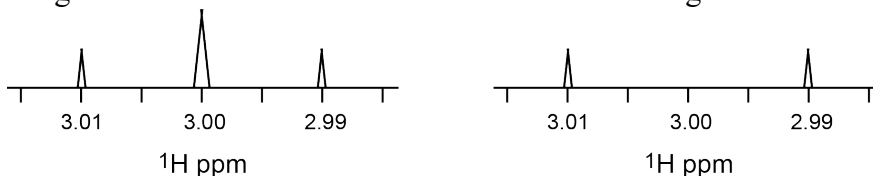
- For the molecule on the left (cholesteryl chloride), explain why the  $T_1$  relaxation time constants for the  $^{13}\text{C}$  nuclei labeled with asterisks (\*) are about  $\frac{1}{2}$  as large (on average, approximately) as those marked with “at” signs (@). (4 points)

*These are all methylene groups (same number of attached hydrogens), so the differences must result from mobility differences. The nuclei labeled “@” are in a flexible hydrocarbon chain with many degrees of freedom and few steric barriers to motion, relative to those (“\*”) in the rigid ring system. So, these relax more slowly (shorter correlation time, longer  $T_1$  time constant).*

- For the molecule on the right (substituted benz[a]anthracene), explain why the  $^{13}\text{C}$   $T_1$  relaxation time constants for the methyl groups are so different from one another. (4 points)

*In methyl groups,  $^{13}\text{C}$  nuclei often exhibit longer relaxation time constants due to the high mobility resulting from relatively unrestricted rotation about the C-C bond. The shorter  $T_1$  constant for the top methyl group must result from restriction of that rotation from the neighboring ring (the ring to the left in the structure above).*

5). A  $^1\text{H}$  nucleus (bolded, in the molecule shown, right) in a molecule is coupled to three other  $^1\text{H}$  nuclei. One coupling is a three-bond coupling to a neighboring methine hydrogen, the other is a three-bond coupling to a pair of neighboring equivalent hydrogens in a methylene group.  $^1\text{H}$  NMR spectra of the molecule were acquired using a 500 MHz instrument. When the methine hydrogen is selectively decoupled, the signal from the bolded hydrogen is shown below on the left, and when the two methylene hydrogens are selectively decoupled, the signal from the bolded  $^1\text{H}$  is shown below on the right. The chemical shift of the bolded hydrogen is 3.00 ppm.



a). Why is the signal on the left an apparent triplet, whereas the signal on the right is an apparent doublet. You will have to explain for credit. **(4 points)**

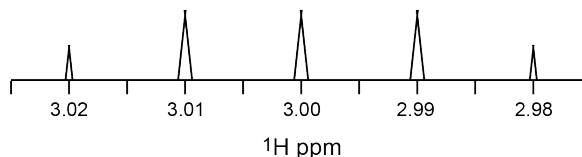
*$^1\text{H}$  nuclei are spin  $\frac{1}{2}$ , so the “ $n+1$ ” rule applies. For coupling to the single methine hydrogen,  $n+1=2$ , hence a doublet. For coupling to the equivalent methylene hydrogens,  $n+1=3$ , so a triplet.*

b). What are the magnitudes of the coupling constants for coupling of the bolded hydrogen to the methine hydrogen and to the methylene hydrogens? You will have to explain your reasoning for credit. You will have to report the coupling constants properly for credit. **(4 points)**

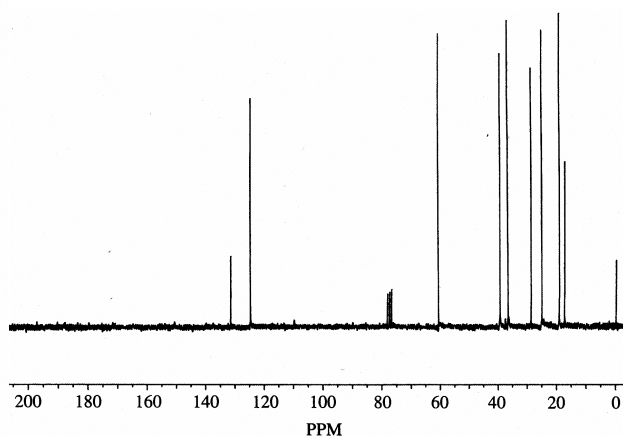
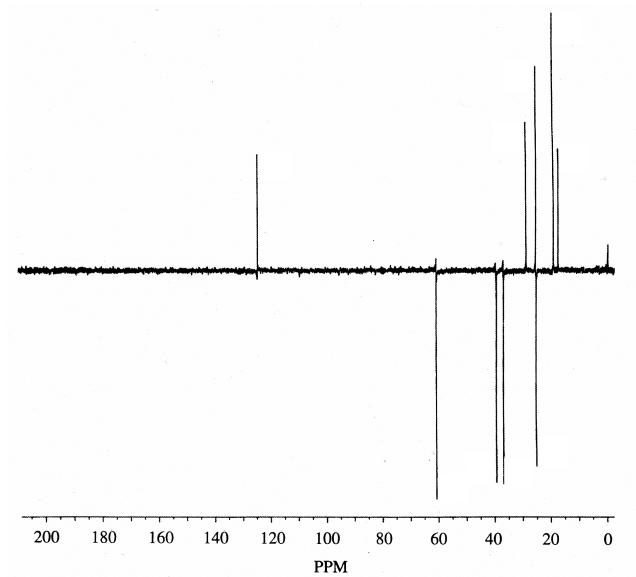
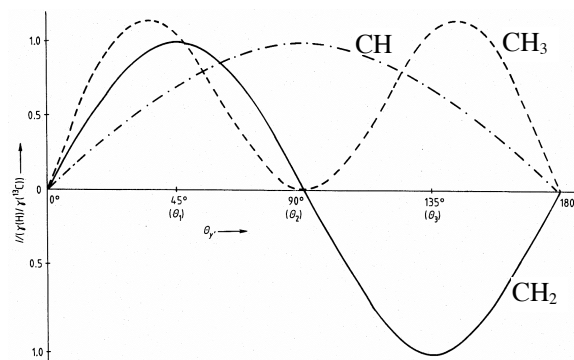
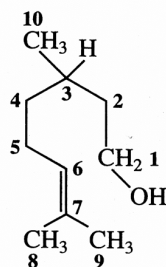
*Using a 500 MHz instrument, for  $^1\text{H}$ , there are 500 Hz/ppm. For the spectrum on the left (coupling to methylene hydrogens), the distance in ppm between each adjacent peak is 0.01 ppm, or 5 Hz. So, this three-bond coupling constant is 5 Hz. For the spectrum on the right (coupling to the methine hydrogen), the distance in ppm between the two peaks is 0.02 ppm, or 10 Hz. So, this three-bond coupling constant is 10 Hz.*

c). Sketch the signal from the bolded hydrogen without any decoupling. Clearly indicate the chemical shift of each peak in the signal. You will have to provide a detailed description of the multiplet structure and how it arises for credit. **(6 points)**

*The multiplet will be a doublet of triplets. The methine hydrogen splits the signal into a doublet, then each component of the doublet is split into a triplet by the methylene hydrogens. The doublet peaks were at 3.01 and 2.99 ppm, so the triplets will be centered there. The outer components of the triplets will be 5 Hz outside of this, or at 3.02 and 3.00 for one and 2.98 and 3.00 for the other. Because the inner components overlap, they will add together, so that the final signal looks like two small peaks (2.98 and 3.02 ppm) flanking three large peaks (3.01, 3.00 and 2.99 ppm).*



6). The DEPT spectrum and the  $^{13}\text{C}$  spectrum with broadband  $^1\text{H}$  decoupling of the compound citronellol (right) are shown below. Also shown (far right) is a plot of the phase angle of the third  $^1\text{H}$  pulse ( $\Theta_y$ ), applied along the 'y' axis, versus the expected intensities of the signals for the various groups in DEPT spectra.



a). What is  $\Theta_y$  for the DEPT spectrum shown? Please explain how you came to this conclusion based on the spectrum of the compound shown. (4 points)

*The value of  $\Theta_y$  is  $135^\circ$ . As shown in the plot of intensities versus  $\Theta_y$ , when  $\Theta_y$  is  $135^\circ$  we expect the signals from the  $-\text{CH}_2$  groups to have the opposite phase (down in the spectrum) to the  $-\text{CH}$  and  $-\text{CH}_3$  groups (up in the spectra). This is apparently what we see in the DEPT spectrum. There are 5 signals phased up, corresponding to the  $-\text{CH}$  and  $-\text{CH}_3$  groups, and 4 signals down, corresponding to the four  $-\text{CH}_2$  groups. The small peak at  $\sim 0.0$  ppm is most likely due to added TMS (see 'd' below).*

- b). The signal at ~132 ppm in the  $^{13}\text{C}$  spectrum is not observed in the DEPT spectrum. What carbon atom gives rise to this signal? Why does the signal not appear in the DEPT spectrum? Please explain your answers for credit. **(4 points)**

*The peak at 132 ppm is due to carbon atom 7. This quaternary carbon will not be observed in the DEPT spectrum. This is due to the fact that in DEPT, polarization is transferred from  $^1\text{H}$  to  $^{13}\text{C}$ , and ultimately we observe the  $^{13}\text{C}$  magnetization created. For quaternary carbons, there are no attached  $^1\text{H}$  spins from which to transfer the polarization.*

- c). Describe and discuss the two reasons that could explain why the signal at 132 ppm is small compared to the other signals in the  $^{13}\text{C}$  spectrum of citronellol? **(6 points)**

*The  $^{13}\text{C}$  spectrum is clearly broadband decoupled (high intensity, no signal splitting), with the high signal intensity resulting from the nuclear overhauser effect (NOE). The enhancement is largest when the density of hydrogen atoms close to the  $^{13}\text{C}$  nucleus is large, and usually is largest when these are hydrogens attached directly to the  $^{13}\text{C}$  nucleus. Because this signal is from a quaternary carbon, the nuclear overhauser effect is probably small, so the intensity is small. Secondly, quaternary  $^{13}\text{C}$  nuclei relax very slowly. Often, the relaxation delay between scans is long enough to allow good recovery of alpha and beta equilibrium spin state populations for other nuclei, but not for very slowly relaxing quaternary carbons, thus, lower apparent intensities for quaternary carbons.*

- d). Which carbon atom gives rise to the signal at ~125 ppm? Please explain in detail how you arrived at your answer. **(4 points)**

*There are two  $^{13}\text{C}$  nuclei that we would suspect would have chemical shifts that are far downfield. These would be carbons 6 and 7, the ethylenic carbons. Also, the signal is phased up (positive intensity) in the DEPT spectrum, so is due to either a  $-\text{CH}$  or  $-\text{CH}_3$  group, and the signals for  $-\text{CH}_3$  groups would be expected to be far upfield. Because we know that carbon 7 is quaternary and does not give a signal in the DEPT spectrum (see 'b' above), we then know that the signal at 125 ppm is due to carbon 6.*

- e). What gives rise to the signal at 0 ppm? How do you know this based on the DEPT spectrum? Why is this signal small compared to most of the other signals in these spectra. You will have to provide explanations for credit. **(4 points)**

*Most likely this is due to the four equivalent  $^{13}\text{C}$  atoms of tetramethylsilane (TMS) added to the sample as a chemical shift reference (the  $^{13}\text{C}$  chemical shift of the carbon atoms in TMS is defined to be 0 ppm). There are ten carbon atoms in citronellol, one of which is a quaternary carbon, so there will only be nine signals in the DEPT spectrum. So, this small, tenth signal at 0 ppm strongly suggests it is from TMS. Usually, only a small amount of TMS is added to the sample for chemical shift referencing, and the signal is small for this reason.*

- f). Compare the signals in the two spectra at 24 ppm. Explain what is occurring and why the signals appear as they do in the context of the particular molecule being examined and the NMR spectra themselves. **(4 points)**

*In the  $^{13}\text{C}$  spectrum, there is apparently only a single signal at 24 ppm, but in the DEPT spectrum it is clear that there are two nuclei that have the same chemical shift (24 ppm), one is a  $-\text{CH}_2$  group (phased down, negative intensity) whereas the other is either a  $-\text{CH}$  or  $-\text{CH}_3$  group (phased up, positive intensity). In the molecule, there are 10 carbon atoms (none of which are equivalent), and one of these is a quaternary carbon. So, there should be nine signals in the DEPT spectrum, and there are (ignoring the small signal from TMS at 0.0 ppm). Likewise, there appears to be a signal missing from the  $^{13}\text{C}$  spectrum (all ten signals should be observed), but there are only nine signals, apparently from the molecule (ignoring the  $\text{CDCl}_3$  signal and the TMS signal).*

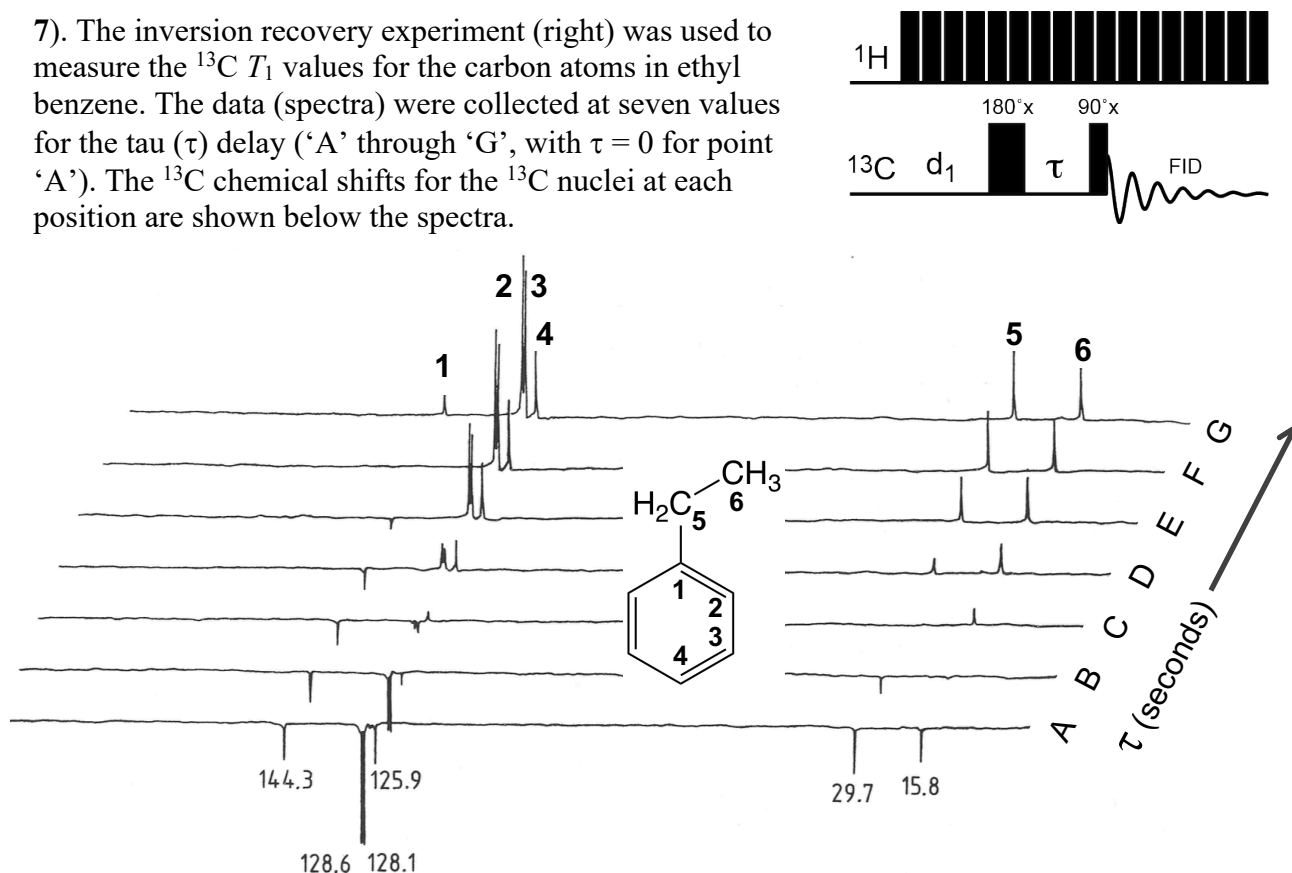
- f). The signal at  $\sim 77$  ppm in the  $^{13}\text{C}$  spectrum is due to the natural abundance  $^{13}\text{C}$  in  $\text{CDCl}_3$ . It appears as a triplet, where all of the peaks are of equal intensity. Explain why the signal appears in the  $^{13}\text{C}$  spectrum but not in the DEPT spectrum. **(4 points)**

*The DEPT experiment relies on polarization transfer from hydrogen nuclei to  $^{13}\text{C}$ . There are no hydrogen nuclei (only deuterium nuclei) attached to the  $^{13}\text{C}$  atom in  $\text{CDCl}_3$ . Therefore, there cannot be a signal corresponding to this  $^{13}\text{C}$  nucleus in the DEPT spectrum.*

- g). The acronym DEPT in NMR stands for Distortionless Enhancement by Polarization Transfer. What is meant by “Distortionless”? You will have to explain for credit. **(2 points)**

*“Distortionless” is in comparison to INEPT where there is a distortion of peak intensities and phases in signals (compared to simple  $^{13}\text{C}$  experiments) as well as differences in signal enhancements for  $-\text{CH}$ ,  $-\text{CH}_2$  and  $-\text{CH}_3$  groups.*

7). The inversion recovery experiment (right) was used to measure the  $^{13}\text{C}$   $T_1$  values for the carbon atoms in ethyl benzene. The data (spectra) were collected at seven values for the tau ( $\tau$ ) delay ('A' through 'G', with  $\tau = 0$  for point 'A'). The  $^{13}\text{C}$  chemical shifts for the  $^{13}\text{C}$  nuclei at each position are shown below the spectra.



a). Order the nuclei (1 through 6) from smallest to largest value of the longitudinal relaxation time constant  $T_1$ . You will have to provide a detailed explanation, based on the data shown, for credit. **(6 points)**

*Smaller values of  $T_1$  indicate nuclei that relax faster. In the inversion recovery experiment, with  $\tau = 0$ , all signals are phased down (negative intensity), so, as  $\tau$  is increased, the negative intensity decreases to zero, then as  $\tau$  continues to increase, positive intensity is observed, and the maximum intensity is approached as  $\tau$  becomes very large. So, from the data, it is observed that the nucleus at position 6 is relaxing the fastest (smallest  $T_1$  value). At time point 'B', the signal (negative intensity) has already decreased to zero, whereas all other signals still maintain some negative intensity. At time point 'C', the signal for position 6 is positive, as is the signal for position 4, whereas the signals from all other nuclei still have negative or zero intensity. The nucleus at position 4 is therefore relaxing the second fastest. Also, at point 'C', the signal from the nucleus at position 5 has reached zero intensity, whereas those from the nuclei at positions 1, 2, and 3 are still negative. Therefore, the nucleus at position 5 is the third fastest relaxing. The nucleus at position 1 is the slowest relaxing, with positive intensity only occurring at the final time point ('G'). The nuclei at 2 and 3 relax faster than the nucleus at position 1, and they appear to relax at about the same rate. Therefore, the order from smallest to largest  $T_1$  values is:  $6 < 4 < 5 < 2,3 < 1$ .*



b). Provide explanations/rationalizations for the relaxation times for each of the individual  $^{13}\text{C}$  nuclei in ethyl benzene, and the order of relaxation times you determined in part 'a', based on the molecular structure and the relationship(s) between  $T_1$  and molecular structure. **(6 points)**

*For small organic molecules, the predominant mechanism for relaxation of  $^{13}\text{C}$  nuclei is the dipolar interaction with attached hydrogens. Nuclei with more attached hydrogens tend to relax faster than those with fewer, with quaternary carbon nuclei relaxing very slowly. Here, the methyl group (position 6) is relaxing fastest, and the quaternary carbon (position 1) the slowest, consistent with this explanation. Accordingly, the  $-\text{CH}_2$  group (position 5) should then relax the second fastest, but it does not. Instead, the carbon at position 4, with only a single attached hydrogen, relaxes faster than that at position 5. One explanation would be that the molecule does not tumble isotropically in solution, but instead predominantly rotates about an axis through carbons at positions 4 and 1, with motion describing a cone, with the small end at position 4. This would increase the correlation time for position 4 more than position 5, which would decrease the relaxation time. Also, this would suggest that positions 2 and 3 would have relaxation times slower than position 4 because their correlation times would be shorter than position 4, which is the case.*

c). For the nucleus at position 5, the  $T_1$  value has been measured to be 15 s. What is the value of the  $\tau$  delay time (in seconds) at time point 'C'? You must do a relevant calculation to determine this value and provide an explanation for credit. **(4 points)**

*In the inversion-recovery sequence, a  $180^\circ$  pulse on magnetization initially at equilibrium (+z) inverts the spin populations to give -z magnetization, with  $M_z = -M_0$ . The  $180^\circ$  pulse is followed by a variable delay ( $\tau$ ), which is then followed by a  $90^\circ$  pulse to create the observable transverse magnetization. We know that, following a  $180^\circ$  pulse, the return of bulk magnetization along z to equilibrium ( $M_z = M_0$ ) is described by the following first order equation:*

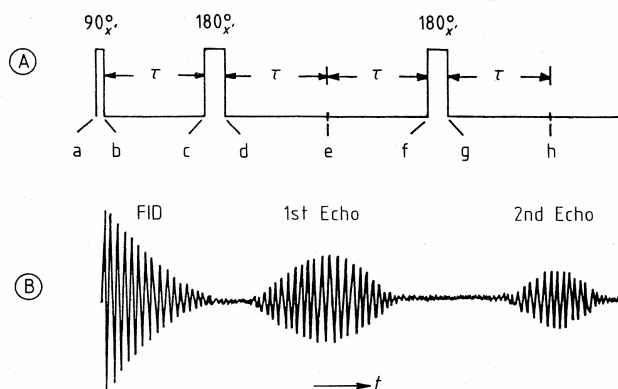
$$M_z = M_0(1 - 2e^{-t/T_1})$$

*For the relaxation data shown, the magnitudes of the signals at the various delay times are not shown. However, for the nucleus at position 5, the value of the magnitude of the signal at time point 'C' is approximately zero. So, at time point 'C',  $M_z = 0$ . We'll call the value of the delay  $\tau$  that produces no observable signal  $\tau_{\text{zero}}$ . Knowing  $T_1$  (15 s) we then can easily calculate  $\tau_{\text{zero}}$ :*

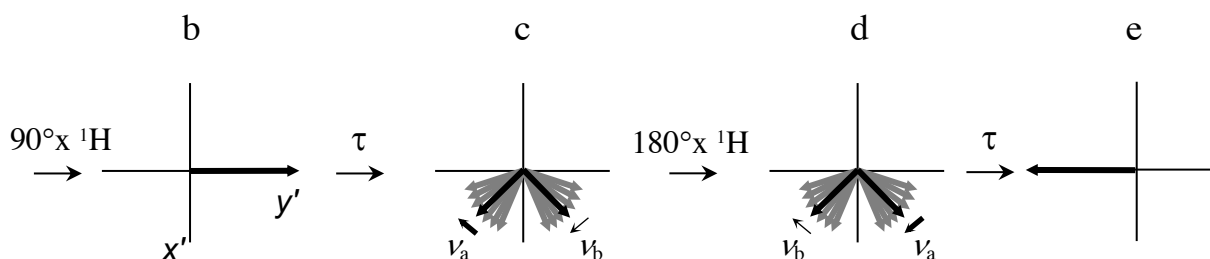
$$M_z = M_0(1 - 2e^{-\tau/T_1}) \quad 0 = M_0(1 - 2e^{-\tau_{\text{zero}}/T_1}) \quad -M_0 = -M_0 2e^{-\tau_{\text{zero}}/T_1}$$

$$\left(\frac{1}{2}\right) = e^{-\tau_{\text{zero}}/T_1} \quad \ln\left(\frac{1}{2}\right) = -\frac{\tau_{\text{zero}}}{T_1} \quad -\ln\left(\frac{1}{2}\right)T_1 = \tau_{\text{zero}} = 10.4 \text{ s}$$

8). The pulse sequence shown (A, right), called the Carr-Purcell spin-echo experiment can be used to measure the transverse relaxation time constant  $T_2$ . Also shown (B, right) is raw data from an experiment to determine  $T_2$ . Following the initial  $90^\circ$  pulse, at point 'b' transverse magnetization along the y axis is generated. In this case, two nuclei are present in the molecule, with rotating frame frequencies  $\nu_a$  and  $\nu_b$  ( $\nu_a$  faster than  $\nu_b$ , both faster than the reference



frequency). During the first  $\tau$  period, phase coherence is gradually lost, as is indicated by the vector diagram below representing point 'c'. Here the dark arrows indicate the average precession frequencies of the nuclei, with the light colored arrows indicating coherence loss. The subsequent  $180^\circ$  pulse reflects the magnetization vectors through the  $x$ - $z$  plane to the positions shown in the vector diagram corresponding to point 'd'. During the second  $\tau$  period, for each of the nuclei, the component vectors move together by the same amount they moved apart during the first  $\tau$  period and are refocused. So, at point 'e', they are all refocused along the  $-y$  axis.



a). The  $\tau$ - $180^\circ$ - $\tau$  element, the spin-echo element, is a key element in Carr-Purcell experiment. Please explain in detail why this is, based on the vector diagrams in 'c', 'd', and 'e'. (6 points)

*The light colored vectors in the vector diagrams demonstrate that some types of phase coherence losses can be refocused using the spin-echo. These include the effects of magnetic field inhomogeneities. Thus, intensity losses due to magnetic field inhomogeneities can be removed by the spin-echo. The example also demonstrates that chemical shift evolution can be refocused, and that this is independent of the magnitude of the chemical shift. Finally, if  $\nu_a$  and  $\nu_b$  are considered to be the two component vectors of a heteronuclear coupling, it is clear that the coupling is also refocused, regardless of the magnitude of the coupling. Thus, the spin-echo element eliminates any magnetization/intensity losses due to magnetic field inhomogeneities, chemical shift, and heteronuclear coupling during the experiment. This allows separation of magnetization losses due to random processes (spin-spin relaxation, or "true  $T_2$ ").*

b). How does the Carr-Purcell experiment permit the measurement of the spin-spin component of  $T_2^*$ ? Please explain. Your answer must include a description of the nature of transverse relaxation. (4 points).

*Transverse relaxation,  $T_2^*$ , includes contributions from both magnetic field inhomogeneity and the “true  $T_2$ ” (spin-spin interactions). As detailed in the answer to part ‘a’, coherence losses due to magnetic field inhomogeneity are refocused in the Carr-Purcell sequence repeatedly by the action of multiple, back-to-back  $\tau$ -180- $\tau$  spin-echo elements, so, intensity losses at the maxima of the echos do not include losses due to magnetic field inhomogeneity. Importantly, magnetization/intensity losses due to random spin-spin (“true  $T_2$ ”) processes cannot be refocused. So, the decrease in the magnitudes of the echo maxima with time are due only to the spin-spin component of transverse relaxation, and the decrease in the amplitudes of the echo maxima with time describes the exponential decay of magnetization due to the spin-spin component.*

$$\frac{1}{T_2^*} = \frac{\gamma \Delta B_0}{2} + \frac{1}{T_2}$$

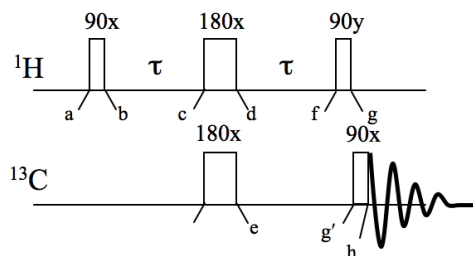
c). In highly viscous fluids, the spin-spin contribution to  $T_2$  (i.e. the “true  $T_2$ ”) can be approximated directly from linewidths in NMR spectra. Please explain why. (4 points)

*The linewidths of signals in typical NMR spectra of small molecules in normal, low-viscosity solvents are dependent on  $T_2^*$ . As shown below (equation 1), the width of signals at half of their maximum heights ( $\Delta\nu_{1/2}$ ) is proportional to  $1/T_2^*$ .  $1/T_2^*$  is the sum of two components, the first contributed by magnetic field inhomogeneity, and the second by the “true  $T_2$ ”, or the spin-spin contribution (equation 2).*

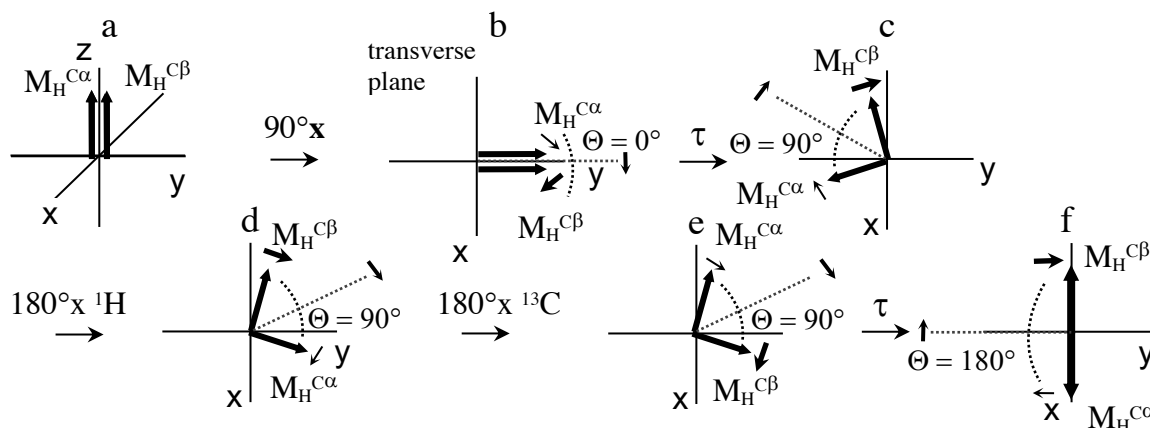
$$1). \quad \Delta\nu_{1/2} = \frac{1}{\pi T_2^*} \qquad 2). \quad \frac{1}{T_2^*} = \frac{\gamma \Delta B_0}{2} + \frac{1}{T_2}$$

*In highly viscous fluids, molecules will tumble VERY slowly, so their correlation times ( $\tau_c$ ) will be VERY long. Thus, relaxation (“true  $T_2$ ”) will be very fast. In this case,  $1/T_2$  will become very large, and will dominate the right hand side of equation 2, so that  $1/T_2^* \approx 1/T_2$ , so that  $\Delta\nu_{1/2}$  is then approximately proportional to  $1/T_2$ .*

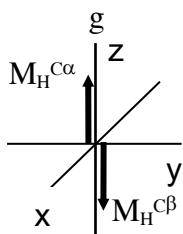
9). The diagram (right) shows the INEPT pulse sequence. Consider the effect of this pulse sequence on chemical shift evolution and heteronuclear ( $^{13}\text{C}$ - $^1\text{H}$ )  $J$  coupling, *ignoring the effects of magnetic field inhomogeneity*. We will consider a simple  $^1\text{H}$ - $^{13}\text{C}$ - spin system (i.e.  $^{13}\text{CHCl}_3$ ). The Larmor frequency is faster than reference frequency,  $\nu_{\text{H}} > \nu_{\text{rf}}$ , as indicated in 'c' below. We will assume that the delay  $\tau$  is equal to  $1/(4J_{\text{CH}})$ .



a). Complete the vector diagrams below for points 'c', 'd', 'e', and 'f' in the pulse sequence. The dashed line in 'b' indicates the Larmor frequency, and the position of the Larmor frequency following the first  $\tau$  period is indicated by the dashed line in 'c'. The  $M_{\text{H}}^{\text{C}\beta}$  component vector moves faster than the Larmor frequency, and the  $M_{\text{H}}^{\text{C}\alpha}$  vector moves slower, as indicated by the sizes of the arrows in 'b'. Be sure to label the vectors ( $M_{\text{H}}^{\text{C}\alpha}$ ,  $M_{\text{H}}^{\text{C}\beta}$ ), 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. **(8 points)**



b). At point 'f' in the pulse sequence, a  $^1\text{H}$   $90^\circ$  pulse along the  $y$ -axis is applied. Show the result of this on the vector diagram below (i.e. point 'g'). Be sure to label the vectors ( $M_{\text{H}}^{\text{C}\alpha}$ ,  $M_{\text{H}}^{\text{C}\beta}$ ). Then, discuss the significance of this result for the INEPT method. **(6 points)**

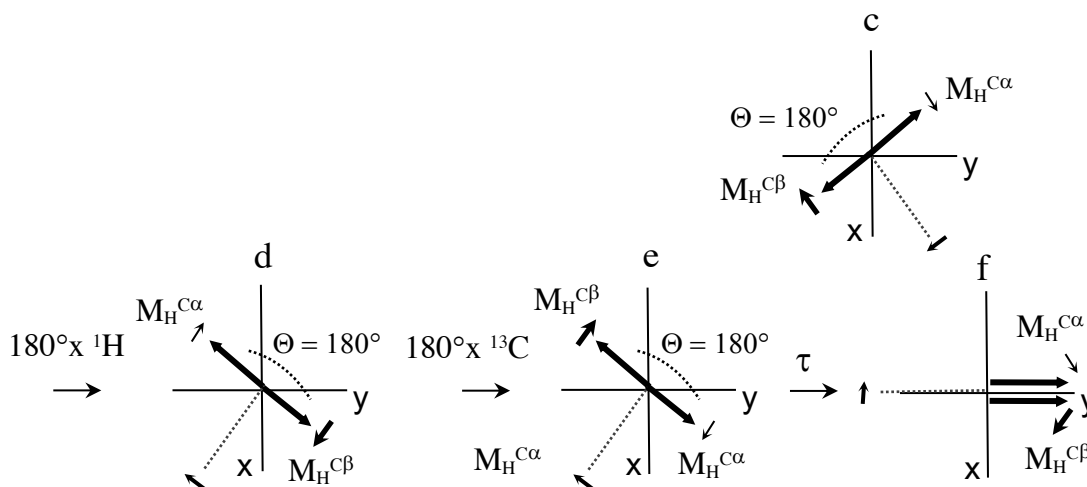


*In order to transfer polarization from  $^1\text{H}$  to  $^{13}\text{C}$ , selective inversion of one of the two component vectors, either  $M_{\text{H}}^{\text{C}\alpha}$  or  $M_{\text{H}}^{\text{C}\beta}$ , must occur. When this happens, the population transfers increase the magnitudes of the corresponding  $^{13}\text{C}$  vectors ( $M_{\text{C}}^{\text{H}\alpha}$  and  $M_{\text{C}}^{\text{H}\beta}$ ) by an average of four-fold. So, it is essential for this selective inversion to be produced at this point in the pulse sequence.*

c). Which of the hydrogen transitions,  $H_1$  ( $N_2-N_4$ ) or  $H_2$  ( $N_1-N_3$ ) corresponds to  $M_H^{C\alpha}$ ? (2 points)

$H_2$

d). For the INEPT pulse sequence, the delay  $\tau$  is usually set to be equal to  $1/(4J_{CH})$ . However, it has been suggested that any multiple of  $1/(4J_{CH})$  will suffice. Complete the diagrams for points 'c', 'd', 'e', and 'f', as in your answer to part 'a' of this question, with the value of delay  $\tau$  is to  $2/(4J_{CH})$ . Then discuss whether this value of  $\tau$  is suitable for INEPT. (8 points)



In part 'a' of the question, with the delay  $\tau$  is set to  $1/(4J_{CH})$ , during the first  $\tau$  period the Larmor vector moved approximately 210 degrees (clockwise). The vector for  $M_H^{C\beta}$ , which is moving faster than the Larmor vector (by  $J/2$  Hz) moves about 255 degrees, with  $M_H^{C\alpha}$  moving about 165 degrees so, they move apart by 90 degrees ( $255-165=90$ ) in  $1/(4J_{CH})$ . So, if the delay  $\tau$  is twice as long, the Larmor vector will move approximately 420 degrees, as shown for the vectors at point 'c',  $M_H^{C\beta}$  will move about 510 degrees, and the vector  $M_H^{C\alpha}$  will move about 330 degrees. So, the angle between the two vectors ends up to be 360 degrees (or zero degrees). The subsequent 90 degree pulse will NOT give the result necessary for INEPT (there will not be selective inversion of  $M_H^{C\alpha}$  or  $M_H^{C\beta}$ ). So, this value of  $\tau$  will not work for INEPT.

e). Given the results above, are there any multiples of  $1/(4J)$  that  $\tau$  could be set to that would work properly for INEPT? You will have to explain for credit. **(4 points)**

*Yes. The results above indicate that for each successive multiple of  $1/(4J)$ , the component vectors move apart an additional 180 degrees from points 'b' to 'f' in the pulse sequence. For  $1/(4J)$ , at the end of the pulse sequence, the vectors are 180 degrees apart, which is necessary for INEPT to work properly. For  $2/(4J)$ , the vectors are 360 (0) degrees apart, which is unacceptable for INEPT. However, for  $3/(4J)$ , the vectors will be 540 (180) degrees apart again, and this will again work for INEPT. So, odd multiples of  $1/(4J)$  should all work for INEPT.*

f). If there are multiples of  $1/(4J)$  that  $\tau$  can be set to that will work properly for the INEPT pulse sequence, why is it advantageous to always use  $1/(4J)$ ? You will have to explain for credit. **(4 points)**

*The shorter values are better because signal intensity losses due to relaxation during the  $\tau$  delay period are minimized. In other words, the best signal-to-noise is obtained with the shortest values of  $\tau$  that will work.*

g). In this question we considered a simple  $^1\text{H}$ - $^{13}\text{C}$ - spin (-CH) system (i.e.  $^{13}\text{CHCl}_3$ ). If  $\tau$  is set to  $1/(4J)$ , the INEPT sequence functions properly for -CH groups. What should the value of  $\tau$  be set to for -CH<sub>2</sub> groups? You will have to explain for credit. You can use any diagrams that you may need for the explanation. **(6 points)**

*The value for  $\tau$  should also be  $1/(4J)$  for -CH<sub>2</sub> groups (and -CH<sub>3</sub> groups as well). In the analysis, we are considering the  $^1\text{H}$  vectors ( $M_H^{C\alpha}$  and  $M_H^{C\beta}$ ). The relevant coupling constant in the INEPT  $\tau$  delay is the  $^1\text{H}$ - $^{13}\text{C}$  coupling only, and each  $^1\text{H}$  is split by the bound  $^{13}\text{C}$  into a doublet only. The  $^1\text{H}$ - $^1\text{H}$  couplings are not relevant. Furthermore, the  $^1\text{H}$ - $^{13}\text{C}$  coupling constants for hydrogens in -CH, -CH<sub>2</sub>, and -CH<sub>3</sub> are very similar in most molecules (120-140 Hz).*

h). Ignoring the effects of relaxation and magnetic field inhomogeneity, write down the populations of the states  $N_1$ ,  $N_2$ ,  $N_3$  and  $N_4$  at each point to 'g/g'' in the sequence. The values at equilibrium (at point 'a') are given below (note: point 'd' is following the  $^1\text{H}$   $180^\circ$  pulse, and 'e/f' is, subsequently, following the  $^{13}\text{C}$   $180^\circ$  pulse). **(8 points)**

'a'	'b/c'	'd'
$N_4 = N$	$N_4 = N + \Delta H/2$	$N_4 = N + \Delta H/2$
$N_3 = N + \Delta C$	$N_3 = N + \Delta C + \Delta H/2$	$N_3 = N + \Delta C + \Delta H/2$
$N_2 = N + \Delta H$	$N_2 = N + \Delta H/2$	$N_2 = N + \Delta H/2$
$N_1 = N + \Delta C + \Delta H$	$N_1 = N + \Delta C + \Delta H/2$	$N_1 = N + \Delta C + \Delta H/2$

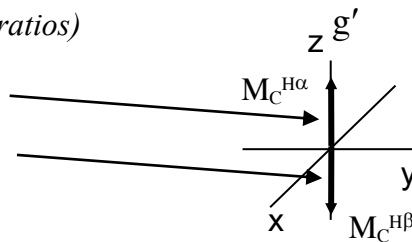
'e/f'	'g/g''
$N_4 = N + \Delta H/2$	$N_4 = N + \Delta H$
$N_3 = N + \Delta C + \Delta H/2$	$N_3 = N + \Delta C$
$N_2 = N + \Delta H/2$	$N_2 = N$
$N_1 = N + \Delta C + \Delta H/2$	$N_1 = N + \Delta C + \Delta H$

i). Based on your answer to part 'e', show the vector diagram at g' (just before the  $^{13}\text{C}$   $90^\circ$  pulse) for the  $^{13}\text{C}$  vectors ( $M_C^{H\alpha}$  and  $M_C^{H\beta}$ ). Also, express final populations for  $M_C^{H\alpha}$  and  $M_C^{H\beta}$ , and do so in terms of  $\Delta C$  and not  $\Delta H$ . You will have to define the relationship between  $\Delta H$  and  $\Delta C$ . **(4 points)**

$$\Delta H = 4 \times \Delta C \text{ (based on gyromagnetic ratios)}$$

$$N_1 - N_2 \text{ is } M_C^{H\alpha} = \Delta C + \Delta H = 5\Delta C$$

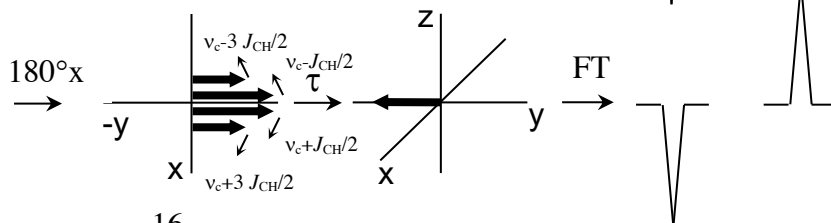
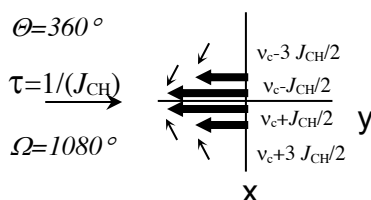
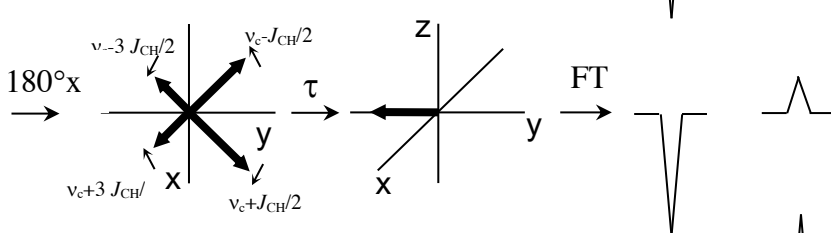
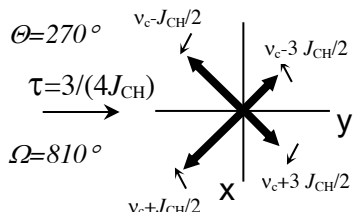
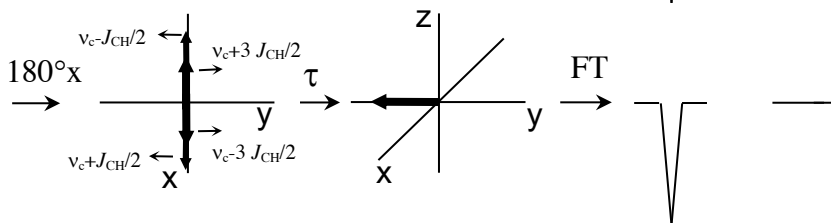
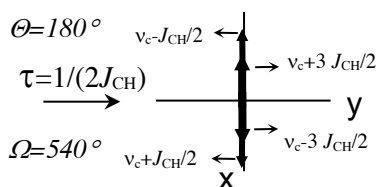
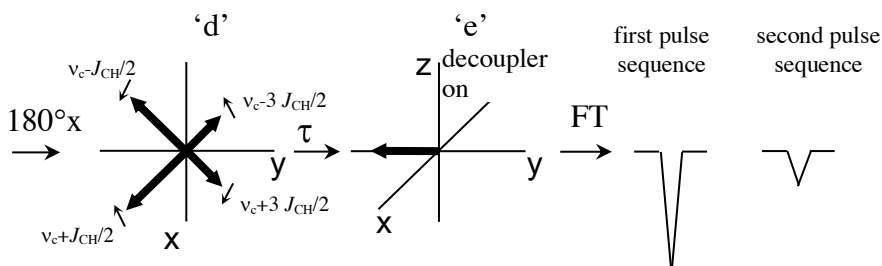
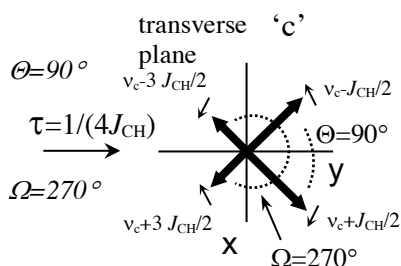
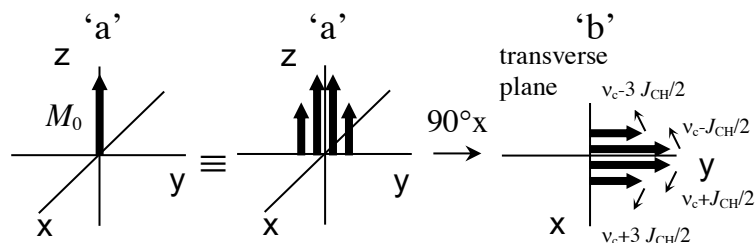
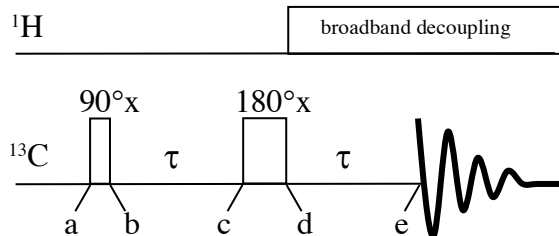
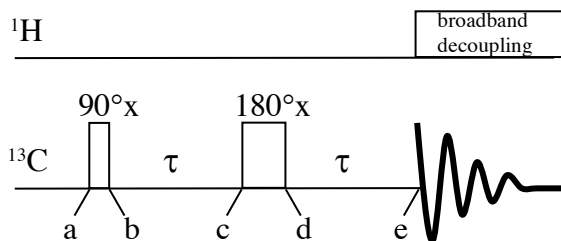
$$N_3 - N_4 \text{ is } M_C^{H\beta} = \Delta C - \Delta H = -3\Delta C$$



10). Consider the spin-echo pulse sequences (right) and their effects on heteronuclear  $J$  coupling (ignoring chemical shift evolution and magnetic field inhomogeneity.). Consider a  $^{13}\text{C}$  nucleus with three attached protons (i.e.  $^{13}\text{CH}_3\text{Cl}$ ) with the Larmor frequency equal to the reference frequency,  $\nu_c = \nu_{\text{rf}}$ . Complete the vector diagrams for points 'c'-'e' to show the effect of the first (top) sequence on this spin system with  $\tau = 1/(4J_{\text{CH}})$ ,  $\tau = 1/(2J_{\text{CH}})$ ,  $\tau = 3/(4J_{\text{CH}})$ , and  $\tau = 1/(J_{\text{CH}})$ . Assume point 'd' is just after the  $180^\circ$  pulse but just before the decoupler is turned on. Label the vectors corresponding to the individual multiplet components with the frequencies as shown below (right). Be sure to show the direction of precession of each component of the multiplet relative to the reference (axes). Also, show the Fourier transformed signal observed for both the top (first) and bottom (second) sequences. (20 points)

$\Theta$  = angle between  $\nu_c + J_{\text{CH}}/2$  and  $\nu_c - J_{\text{CH}}/2$

$\Omega$  = angle between  $\nu_c + 3J_{\text{CH}}/2$  and  $\nu_c - 3J_{\text{CH}}/2$





Name \_\_\_\_\_

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You may find some of the information below useful:

**Table 1-1.**

Properties of some nuclides of importance in NMR spectroscopy.

Nuclide	Spin $I$	Electric quadrupole moment <sup>a)</sup> [ $eQ$ ] [ $10^{-28} \text{ m}^2$ ]	Natural abundance) [%]	Relative sensitivity <sup>b)</sup>	Gyromagnetic ratio $\gamma^a)$ [ $10^7 \text{ rad T}^{-1} \text{ s}^{-1}$ ]	NMR frequency [MHz] <sup>b)</sup> ( $B_0 = 2.3488 \text{ T}$ )
$^1\text{H}$	1/2	—	99.985	1.00	26.7519	100.0
$^2\text{H}$	1	$2.87 \times 10^{-3}$	0.015	$9.65 \times 10^3$	4.1066	15.351
$^3\text{H}^c)$	1/2	—	—	1.21	28.5350	106.664
$^6\text{Li}$	1	$-6.4 \times 10^{-4}$	7.42	$8.5 \times 10^{-3}$	3.9371	14.716
$^{10}\text{B}$	3	$8.5 \times 10^{-2}$	19.58	$1.99 \times 10^{-2}$	2.8747	10.746
$^{11}\text{B}$	3/2	$4.1 \times 10^{-2}$	80.42	0.17	8.5847	32.084
$^{12}\text{C}$	0	—	98.9	—	—	—
$^{13}\text{C}$	1/2	—	1.108	$1.59 \times 10^{-2}$	6.7283	25.144
$^{14}\text{N}$	1	$1.67 \times 10^{-2}$	99.63	$1.01 \times 10^{-3}$	1.9338	7.224
$^{15}\text{N}$	1/2	—	0.37	$1.04 \times 10^{-3}$	-2.7126	10.133
$^{16}\text{O}$	0	—	99.96	—	—	—
$^{17}\text{O}$	5/2	$-2.6 \times 10^{-2}$	0.037	$2.91 \times 10^{-2}$	-3.6280	13.557
$^{19}\text{F}$	1/2	—	100	0.83	25.1815	94.077
$^{23}\text{Na}$	3/2	0.1	100	$9.25 \times 10^{-2}$	7.0704	26.451
$^{25}\text{Mg}$	5/2	0.22	10.13	$2.67 \times 10^{-3}$	-1.6389	6.1195
$^{29}\text{Si}$	1/2	—	4.70	$7.84 \times 10^{-3}$	-5.3190	19.865
$^{31}\text{P}$	1/2	—	100	$6.63 \times 10^{-2}$	10.8394	40.481
$^{39}\text{K}$	3/2	$5.5 \times 10^{-2}$	93.1	$5.08 \times 10^{-4}$	1.2499	4.667
$^{43}\text{Ca}$	7/2	$-5.0 \times 10^{-2}$	0.145	$6.40 \times 10^{-3}$	-1.8028	6.728
$^{57}\text{Fe}$	1/2	—	2.19	$3.37 \times 10^{-5}$	0.8687	3.231
$^{59}\text{Co}$	7/2	0.42	100	0.28	6.3015	23.614
$^{119}\text{Sn}$	1/2	—	8.58	$5.18 \times 10^{-2}$	-10.0318	37.272
$^{133}\text{Cs}$	7/2	$-3.0 \times 10^{-3}$	100	$4.74 \times 10^{-2}$	3.5339	13.117
$^{195}\text{Pt}$	1/2	—	33.8	$9.94 \times 10^{-3}$	5.8383	21.499

$B_0$ (Tesla, T)	Resonance frequencies (MHz)	
	$^1\text{H}$	$^{13}\text{C}$
9.4	400	100.6
11.74	500	125.7
14.09	600	150.9
18.79	800	201.2

$$\gamma_{^1\text{H}} = 26.7519 \times 10^7 \text{ rad/T/s}, I = 1/2$$

$$\gamma_{^{10}\text{B}} = 2.8747 \times 10^7 \text{ rad/T/s}, I = 3$$

$$\gamma_{^{11}\text{B}} = 8.5847 \times 10^7 \text{ rad/T/s}, I = 3/2$$

$$\gamma_{^{13}\text{C}} = 6.7283 \times 10^7 \text{ rad/T/s}, I = 1/2$$

$$\gamma_{^{15}\text{N}} = -2.7126 \times 10^7 \text{ rad/T/s}, I = 1/2$$

$$\gamma_{^{17}\text{O}} = -3.6280 \times 10^7 \text{ rad/T/s}, I = 5/2$$

**You may find some of the following information or equations useful:**

$$k_B = 1.381 \times 10^{-23} \text{ J/K}$$

$$\text{Avagadro's number} = 6.02214179 \times 10^{23} \text{ mol}^{-1}$$

$$h = 6.626 \times 10^{-34} \text{ Js}$$

$$\hbar = h/(2\pi)$$

$$P = \hbar\sqrt{I(I+1)}$$

$$P_z = m\hbar$$

$$\mu = \gamma P = \hbar\gamma\sqrt{I(I+1)}$$

$$\text{for } m = \frac{1}{2}, \cos(\theta) = \frac{m\hbar}{\hbar\sqrt{I(I+1)}} = \frac{m}{\sqrt{I(I+1)}}$$

$$\pi/2 \text{ radians} = 90^\circ$$

$$M_0 = \frac{N\gamma^2\hbar^2 B_0 I(I+1)}{3k_B T}$$

$$B_2 = \frac{\Delta\nu\sqrt{f^2 - f_r^2}}{J_r} = \frac{J\Delta\nu}{J_r}$$

$$\varepsilon = \frac{dM}{dt} = \gamma M_0 B_0 = \frac{N\gamma^3\hbar^2 B_0^2 I(I+1)}{3k_B T}$$

$$\Delta\nu = \nu_{BS} - \nu_0 = \frac{B_2^2}{2(\Delta B)} = \frac{B_2^2}{2(\nu_0 - \nu_i)}$$

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

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

$$\Theta = \gamma B_1 \tau_p$$

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

$$\frac{N_\beta}{N_\alpha} \approx 1 - \left( \frac{\gamma\hbar B_0}{k_B T} \right)$$

$$\Delta E = \mu_z B_0 = \gamma\hbar B_0 = h\nu_L = h\nu_1$$

$$B_{eff} = B_0(1 - \sigma)$$

$$\nu_L = |\gamma/(2\pi)| B_0 = \omega_0/(2\pi)$$

$$\nu_L = \frac{\gamma}{2\pi} (1 - \sigma) B_0$$

$$\omega_0 = \gamma B_0$$

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

$$SW = 1/(2DW) = \text{Nyquist frequency } (\nu_{NQ})/2$$

$$M_y = M_0 e^{-t/T_2^*}$$

$$AQ = DW * TD$$

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

$$DR = 2SW/TD = 1/AQ$$

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

$$(TD \equiv NP)$$

$$\Delta\nu_{1/2} = \frac{1}{\pi T_2^*}$$

$$\cos\alpha_{Ernst} = e^{-((d_1 + AQ)/T_1)}$$

$$\frac{1}{T_2^*} = \frac{\gamma\Delta B_0}{2} + \frac{1}{T_2} \quad \frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2(B_0)}$$

$$\pi/2 \text{ radians} = 90^\circ$$

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

$$\Delta\nu \gg J$$

$$\eta = \gamma_a / (2\gamma_x)$$

$$1 + \gamma_A/\gamma_X \quad 1 - \gamma_A/\gamma_X$$

$$I = (1 + \eta) I_0$$

$$\text{multiplicity} = 2nI + 1$$

$$I \propto 1/r^6$$

$$\Theta = 2\pi J\tau$$