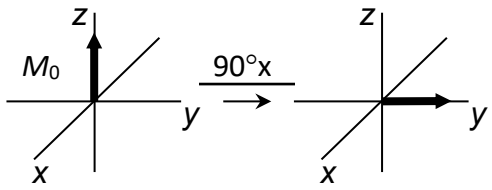
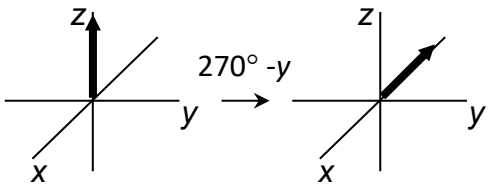
Name _____

Exam 3: CHEM/BCMB 4190/6190/8189 (206 points) Tuesday, 25 October, 2022

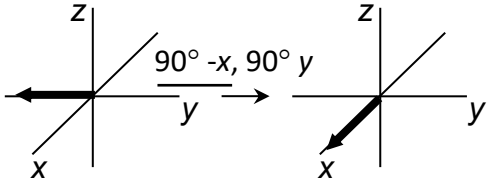
1). In the example (right), the effect of a 90° (π/2) pulse applied along the "x" axis (90°x) is shown for a bulk magnetization vector (M_0) at equilibrium (on the '*z*' axis). For 'a', 'c', 'd' and 'f' below, show the effects of the indicated pulses by drawing the missing (originating or resulting) vectors on the coordinate axes. For 'b' and 'e', fill in the blank with the correct pulse that will promote the indicated movement of the bulk magnetization vector (there may be more than one correct answer for some of these). Also, pulses along +*z* or -*z* are not permitted. **(12 points)**



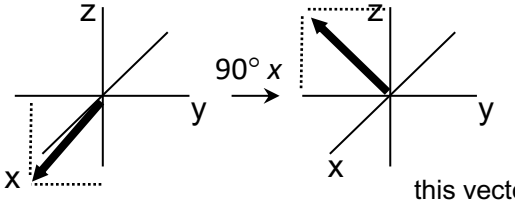
a.



b.



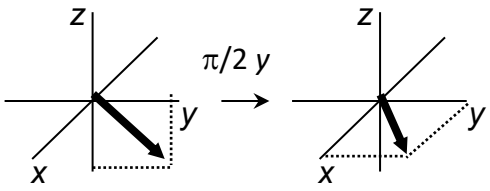
c.



this vector is in the *y*-*z* plane

this vector is in the *y*-*z* plane (NOT along the *x*-axis)

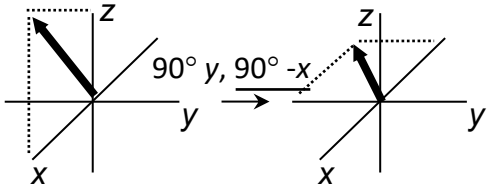
d.



this vector is in the *y*-*z* plane

this vector is in the *x*-*y* plane

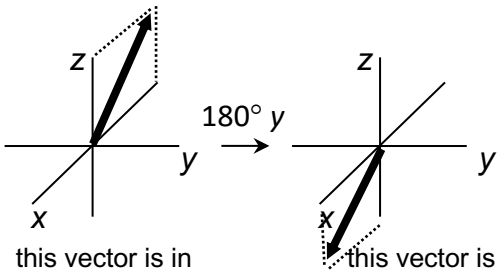
e.



this vector is in the *x*-*z* plane

this vector is in the *x*-*y* plane

f.



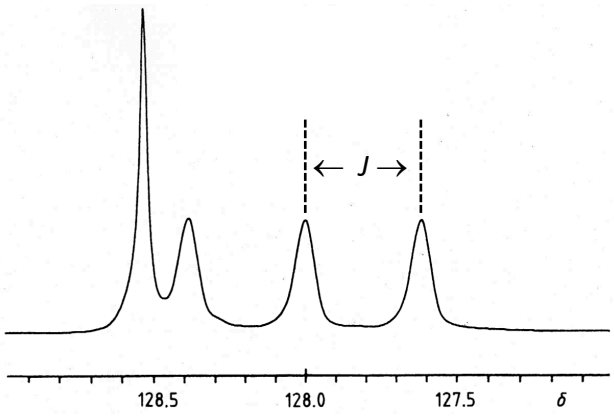
this vector is in the *x*-*z* plane

this vector is in the *x*-*z* plane

Note: as stated in the question, for some of these there may be more than one correct answer. For instance, for 'b', 270°x would be a correct answer.

Name _____

2). The ^{13}C NMR spectrum shown (right) is from a mixture of C_6H_6 (benzene) and C_6D_6 (deuterated benzene). The B_0 field was 5.87 T.



a. What are the chemical shifts of the two ^{13}C signals present? Which is from C_6H_6 and which is from C_6D_6? You will have to explain your answer for credit. This will include a detailed analysis of multiplet structures, including intensities of peaks in multiplets. (**6 points**)

When a ^{13}C nucleus is attached to a ^{1}H nucleus it is split into a doublet, where the individual peaks in the doublet are of equal intensity. This is because the ^{1}H (I=½) nucleus can be in either the α (low energy) or β (high energy) state. Because the α and β states are nearly equally populated, the intensities of the doublet components in the ^{13}C spectrum are nearly identical. We know also that for a ^{2}H (D) nucleus, where I=1, there will be <u>three</u> possible energy states, $E = -1\gamma\hbar B_0$, $E = 0\gamma\hbar B_0$, and, $E = 0\gamma\hbar B_0$, and that they will be nearly equally populated. Thus, these will split the attached ^{13}C nucleus into a triplet with nearly equal intensities for the three peaks.

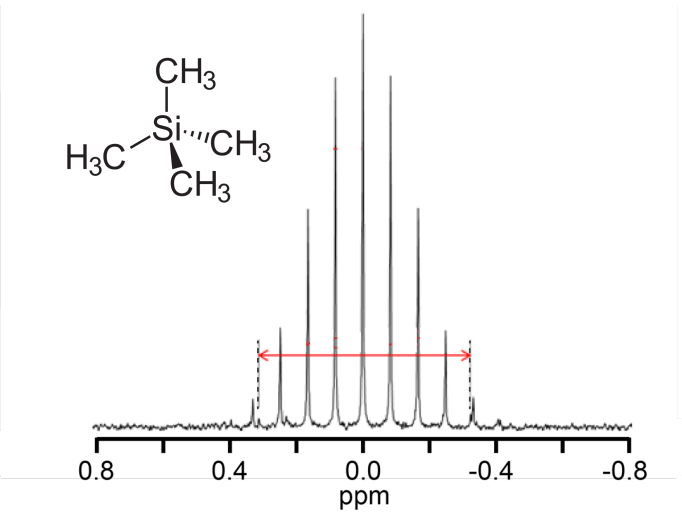
Therefore, the singlet at 128.53 is due to C_6H_6. There is only one signal because the six carbons are chemically equivalent. Each ^{13}C nucleus is coupled to a directly bonded ^{1}H nucleus, so the ^{13}C signal should appear as a doublet. The fact that it is a singlet indicates that there was ^{1}H decoupling during the experiment (most likely broadband ^{1}H decoupling). The ^{13}C signal from the likewise chemically equivalent carbon atoms of C_6D_6 is split by coupling to the attached (quadrupolar) ^{2}H (D) nucleus. This coupling/splitting would not be removed by broadband ^{1}H decoupling (to remove this splitting would require broadband ^{2}H decoupling). The chemical shift is the center of the signal, at 128.0 ppm.

b. What is the correct nomenclature for the indicated coupling constant? (**4 points**)

$^{1}J_{C,D}$ or $^{1}J_{13C,D}$ or $^{1}J_{13C,\,2H}$, etc.

Name _____

3). The compound TMS (tetramethylsilane, right) is a symmetrical molecule where all -CH_3 groups are in identical chemical environments. The chemical shift of the hydrogen atoms is used as a universal chemical shift standard in organic chemistry for ^{1}H (0.00 ppm), as is the chemical shift of the ^{13}C atoms (0.00 ppm). Likewise, the chemical shift of the Si atom is often used as a chemical shift standard for ^{29}Si (0.00 ppm). The ^{29}Si NMR spectrum of TMS is shown (right).



a. Please explain why the spectrum looks as it does. Your explanation should include an explanation of how many signals/peaks should be observed, are observed, and why, and the relative intensities of the peaks. (**8 points**)

^{29}Si is spin ½. In TMS, the Si atom is coupled (^{3}J) to the equivalent hydrogen atoms of the methyl groups. The 12 hydrogens will split the Si signal into 13 peaks (n=12, n+1=13). Only 9 of the peaks are observed in the spectrum shown because the signal-to-noise is not high enough to observe the smaller peaks. The relative intensities of the 13 peaks should be as shown in the last (bottom) line in the "Pascal's Triangle" shown here.

1
1 1
1 2 1
1 3 3 1
1 4 6 4 1
1 5 10 10 5 1
1 6 15 20 15 6 1
1 7 21 35 35 21 7 1
1 8 28 56 70 56 28 8 1
1 9 36 84 126 126 84 36 9 1
1 10 45 120 210 252 210 120 45 10 1
1 11 55 165 330 462 462 330 165 55 11 1
1 12 66 220 495 792 924 792 495 220 66 12 1

b. In the spectrum is an arrow pointing to two dashed vertical lines. These dashed lines point to two very small peaks that clearly are not part of the larger multiplet. The lines are 50.8 Hz apart (length of the arrow in Hz). Please explain what gives rise to these two small peaks, why they are small, what their approximate intensities should be, and why they are 50.8 Hz apart. (**4 points**)

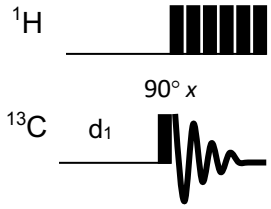
The ^{29}Si atom is also coupled to ^{13}C in the carbon atoms. Because only 1% of the carbon is ^{13}C, these peaks are very small, and each of them should therefore be 0.5% as large as the center component of the ^{29}Si multiplet. They are 50.8 Hz apart because that is the magnitude of the one-bond ^{29}Si-^{13}C coupling constant.

c. What field strength, in Tesla, was the magnet that was used to acquire this ^{29}Si spectrum? You will have to perform a calculation and show your work for credit. (**6 points**)

The arrow shows two small peaks that are 50.8 Hz apart. If we look at the ppm scale, these peaks are located at approximately 0.32 and -0.32 ppm, or 0.64 ppm apart. So, that's 79.375 Hz/ppm. From the table at the end of this exam, we see that, for ^{29}Si, 19.865 Hz/ppm corresponds to a magnetic field strength of 2.36885 Tesla, so 79.375 corresponds to a field strength of 9.465 Tesla (or a "400 MHz" magnet approximately).

Name _____

4). The inverse-gated ^{1}H broadband decoupling pulse sequence for recording ^{13}C spectra is shown. Please explain for what specific purpose you would use this particular broadband decoupling scheme, and why you would want to keep the acquisition time short. (**6 points**)



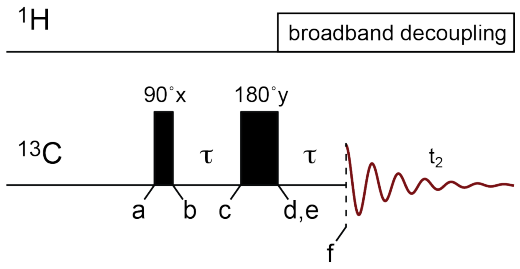
The inverse-gated broadband ^{1}H decoupling scheme can be used when it becomes important or necessary to integrate signals in ^{13}C spectra. When full broadband decoupling is employed, with decoupling during both the interscan delay (d1) and during acquisition, the nuclear Overhauser effect (NOE) is maximized. Unfortunately, the intensities of individual ^{13}C signals are not necessarily enhance equally, so the integrals of the ^{13}C signals are not necessarily representative of the number of nuclei giving rise to the signal. In order to be able to integrate signals, the NOE has to be minimized. For the inverse-gated broadband ^{1}H decoupling scheme, there is no NOE buildup during d_1 because there is no decoupling during d_1. Decoupling during acquisition removes signal splitting, making individual signal identification straightforward. Thus, the only contribution to the intensities from the NOE would be due to decoupling during acquisition. This can be minimized by making the acquisition time as short as possible.

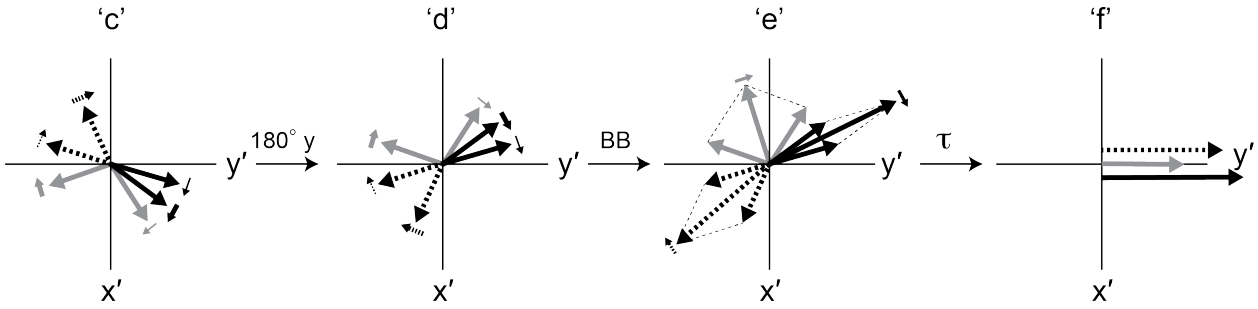
5). The longitudinal relaxation time constants (T_1) for ^{1}H relaxation in uniformly (100%) ^{13}C-labeled chloroform ($^{13}CHCl_3$) and uniformly (100%) ^{12}C-labeled chloroform ($^{12}CHCl_3$) were measured under identical conditions using the inversion-recovery method. The measured value of T_1 for the ^{1}H nucleus in $^{13}CHCl_3$ was 13 seconds, or about half the value measured for the ^{1}H nucleus in $^{12}CHCl_3$ (28 seconds). Please explain the physical basis for this result. (**8 points**)

For ^{13}C T_1 relaxation, the dominant mechanism promoting relaxation is the dipolar coupling interaction with directly bonded ^{1}H nuclei. Likewise, for ^{1}H nuclei directly bonded to ^{13}C nuclei, the dipolar coupling interaction with the ^{13}C nuclei strongly promotes T_1 relaxation, so relaxation is relatively fast. For ^{1}H nuclei bonded directly to ^{12}C carbon atoms, given that ^{12}C is not NMR active (I = 0), there is no dipolar coupling interaction between ^{1}H and ^{12}C, so the dipolar coupling mechanism for promoting relaxation is absent. So, without this mechanism, relaxation is much less efficient and much slower for ^{1}H bound to ^{12}C.

Name _____

6). The pulse sequence for the *J*-modulated spin-echo experiment is shown. Point 'c' in the pulse sequence is immediately before the 180° pulse. Point 'd' in the sequence is immediately after the 180° pulse but before broadband ^{1}H decoupling has begun, and point 'e' is also immediately after the 180° pulse but just after beginning broadband ^{1}H decoupling. Point 'f' is at the beginning of the acquisition period. This experiment was performed on a molecule with three -CH groups, all in different chemical environments. The vector diagram below (transverse plane) for point 'c' for this molecule for a given value of τ shows M_CH^{α} and M_CH^{β} for each of the three of the -CH groups. The Larmor frequencies of all of the nuclei are faster than the reference frequency. The Larmor frequency for the nucleus corresponding to the dark arrows is the slowest (arrows move the least during τ), followed by the nucleus corresponding to the light arrows, and the nucleus corresponding to the dashed arrows has the fastest Larmor frequency (arrows move the most during τ).





a. Complete the vector diagrams for points 'd', 'e', and 'f'. Please provide any explanation you feel necessary to justify your answers. (**6 points**)

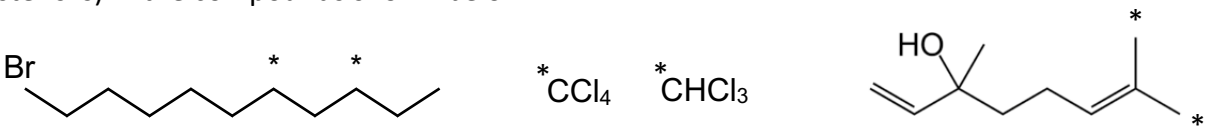
The 180° y pulse reflects all vectors through the y-z plane, to give the result at 'd'. Then the broadband ^{1}H decoupling is turned on. This removes the splitting. For each pair of vectors (dark colored pair, light colored pair, dashed pair), the two component vectors are then added together to give a resultant vector. These are then refocused by the second τ period along the 'y' axis. The lengths of the resultant vectors depend on the coupling constants. The dark colored pair has the smallest coupling constant as indicated by the fact that the dark vectors move apart only a small amount during the first τ period. When these nearly parallel vectors are added together, they give a large resultant. Conversely, the light colored pair has the largest coupling constant as indicated by the fact that the light vectors move apart the largest amount during the first τ period. When these nearly orthogonal vectors are added together, they give a small resultant vector. The dashed vector is somewhere in between.

b. What are the relative intensities of the three signals observed for this molecule with this value of τ? You will have to explain your reasoning for credit. (**8 points**)

In this J-modulated experiment, with a fixed value of τ, the intensities are dependent on the coupling constants ($^1J_{CH}$). For this molecule, the -CH group corresponding to the dark colored vectors clearly has the smallest coupling constant because the vectors move away from each other only a very small amount (approximately 20°) during the first τ period. Conversely, the -CH group corresponding to the light colored vectors has the largest coupling constant because the vectors move away from each other considerably (approximately 100°) during the first τ period. The -CH group corresponding to the dashed vectors has a coupling constant whose magnitude is somewhere between the other two (the vectors move away from each other approximately 35° during the first τ period). Following the 180° y pulse, all the vectors are reflected through the y-z plane, and the angles between the vector pairs remain the same. For the -CH group with the smallest coupling constant (dark vectors), the vectors are nearly parallel to each other (only 20° apart) so, when the broadband decoupling is turned on (point 'e') and they are added together, the resultant is nearly twice as long as each of the two component vectors. On the other hand, for the -CH group with the largest coupling constant (light vectors), the vectors are nearly 180° apart, so when broadband decoupling is turned on, they add together to give a resultant vector much smaller than the resultant from the dark vectors. The -CH group corresponding to the dashed vectors is somewhere in between. So, when the vectors are refocused by the second τ period, as acquisition starts the largest vector is the dark colored one, the smallest is from the light colored one, and the dashed one is in between. So, the largest signal is from the dark colored one, the smallest from the light colored one, and the signal from the dashed one is somewhere in between.

Name _____

7). The values of the ^{13}C T_1 relaxation times (seconds) are known for the indicated nuclei (asterisks) in the compounds shown below.



a. In 1-bromodecane (above, left), the T_1 value for one of the indicated (asterisks) ^{13}C nuclei is 3.1 s, whereas the T_1 value for the other is 2.1 s. Please explain which nucleus gives rise to each T_1 value. Your explanation will have to address the principles underlying the differences to receive credit. (**4 points**)

Molecular mobility dictates T_1 relaxation. The T_1 relaxation time constant is inversely proportional to the molecular correlation time, τ_c ($\tau_c \propto T_1^{-1}$), and the molecular correlation time is proportional to molecular size. For highly flexible molecules such as straight-chain polymers, the ends of the polymers exhibit increased mobility (i.e. decreased effective size) compared to internal segments of the polymers. Thus, the ends of polymers exhibit decreased molecular correlation times (τ_c), and larger T_1 values compared to internal segments. So, the ^{13}C nucleus in the -CH_2- group nearer the end of the polymer chain is the one with the largest (3.1 s) T_1 value.

b. The ^{13}C atoms in carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$) have much different T_1 values. The T_1 value for one of them is 160 s and for the other is 32.4 s. Please explain which nucleus gives rise to each T_1 value. Your explanation will have to address the principles underlying the differences to receive credit. (**4 points**)

The principle contributors to T_1 relaxation are dipole-dipole interactions. For ^{13}C nuclei, dipole-dipole interactions with hydrogen nuclei, in particular directly bonded hydrogens, have large influences on T_1 relaxation. The effect scales with the number of directly attached hydrogens, so ^{13}C nuclei in methene groups (-CH_2), with two attached hydrogens, typically relax faster than ^{13}C nuclei in methine groups (-CH), with only a single attached hydrogen. ^{13}C nuclei in quaternary carbons, with no attached hydrogens, relax slowest. Thus, the ^{13}C nucleus in CCl_4 would relax most slowly (160 s).

Name _____

c. In linalool (above, right), the ^{13}C T_1 times for the two methyl carbons indicated (asterisks) are quite different, with one being 8 s and one 3.5 s. Please explain which nucleus gives rise to each T_1 value. Your explanation will have to address the principles underlying the differences to receive credit. (**4 points**)

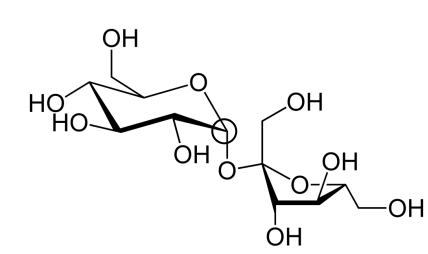
As discussed in 'b', T_1 is dictated by molecular motion. For methyl groups, there is additional rotational motion about the C-CH_3 bond, leading to shorter effective correlation times (τ_c) and longer T_1 values. In linalool, one of the methyl groups shows a significantly decreased T_1 (3.5 s). This must mean that rotational motion about the C-CH_3 bond is restricted. This would most likely be the upper methyl group (the one pointed up in the model shown above), as rotation could be sterically hindered by interaction with the neighboring methylene hydrogens. Also, the H density is somewhat higher near this -CH_3 group, which also has some effect on shortening T_1 via dipolar interactions.

8). Explain in words what the Karplus relationship is. Why is it important and useful for structural analysis of small organic molecules. (**6 points**)

The Karplus relationship is the relationship between the magnitude of 3-bond (vicinal) scalar coupling constants and the dihedral (torsion) angle between the relevant bond vectors. According to this relationship, the magnitude of the coupling constant can reveal the magnitude of the dihedral angle. Thus, these coupling constants can be used to define the conformations of various parts of molecules, and therefore are useful for defining these aspects of molecular structure.

Name _____

9). An inversion recovery NMR experiment (pulse diagram shown below) was used to measure the T_1 for the anomeric proton of sucrose (hydrogen on the carbon circled in the sucrose structure below). The sucrose sample consisted of 1 mM sucrose in D_2O. A signal from residual water in the D_2O (HOD) is also observed, and the T_1 for the HOD hydrogen was also determined. The ^1H spectra for each value of τ used are shown. The signals of the anomeric proton and the hydrogen in HOD are indicated for the first spectrum (τ = 0.1 s). The intensity values (arbitrary units) for the anomeric hydrogen and HOD signals are shown in the table (below) for each τ.



Anomeric proton	HDO	τ (s)
–32.9	–132	0.1
–25.9	–126	0.2
–13.9	–117	0.4
3.72	–101	0.8
23.9	–70.7	1.6
38.1	–21.4	3.2
42.1	46.7	6.4
42.1	114	12.8
42.1	149	25.6

a. Which hydrogen (the anomeric hydrogen or the hydrogen in HOD) has the shortest T_1? Please explain. (**4 points**)

The data show that as τ increases, the return of the signal from the anomeric hydrogen to its equilibrium intensity is much faster than that for the HOD indicating T_1 is shorter for the anomeric hydrogen.

b. For the HOD signal in the inversion recovery experiment, the ratio of the intensity of the observed signal at τ = 3.2 s to the signal at τ = 0 s is 0.14, what is T_1? You must do a calculation based on these times and intensities and get an answer for credit. (**4 points**)

$$M_z = M_0\left(1 - \mathbf{2}e^{-\frac{\tau}{T_1}}\right) \quad \frac{M_z}{M_0} = 0.14 = \left(1 - \mathbf{2}e^{-\frac{\tau}{T_1}}\right) \quad 0.86 = \mathbf{2}e^{-\left(\frac{3.2}{T_1}\right)} \quad 0.43 = e^{-\left(\frac{3.2}{T_1}\right)}$$

$$-0.84 = -3.2/T_1 \qquad T_1 = 3.81\ s$$

c. If a sample of 1 mM sucrose was prepared in H_2O rather than D_2O, the large ^1H signal from the H_2O would dominate the spectrum, and the small signals from the sucrose would not be observed. Based on the results of the inversion recovery experiment shown above, please explain how the H_2O signal could be attenuated (without saturating the H_2O signal) to allow observation of the sucrose signals. (**4 points**)

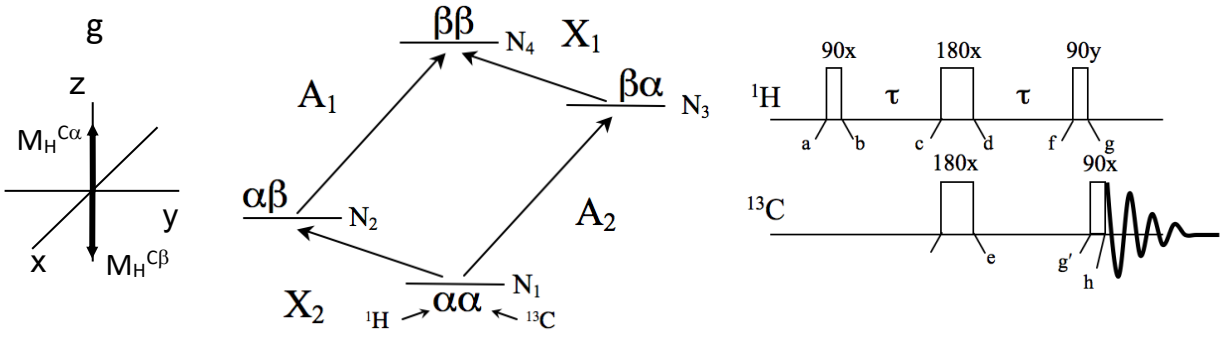
Relaxation of the sucrose signals is much faster than relaxation of the HOD signal, as shown in the inversion recovery experiment results. When $\tau \approx$ 4-5 s, the results indicate that the signal from HOD is nearly completely attenuated, whereas the signals from sucrose are nearly returned to their maximum intensities. Thus, if the relaxation time of H_2O is similar to HOD, the inversion recovery sequence, with $\tau \approx$ 4-5 s, can be used to attenuate the H_2O signal and allow observation of the sucrose signals.

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

a. T_2 relaxation is sometimes called (spin-lattice : (spin-spin)) relaxation. (**2 points**)
b. The molecular correlation time, τ_C, is directly proportional to (T_1 : ($1/T_1$)). (**2 points**)
c. T_1 values (increase : (decrease)) with molecular size. (**2 points**)
d. For ^{13}C relaxation, increasing the number of hydrogens attached to a carbon (increases : (decreases)) the ^{13}C T_1. (**2 points**)
e. T_2 can never be ((longer than) : shorter than) T_1. (**2 points**)
f. If T_2* times are long, NMR signals are ((narrow) : broad). (**2 points**)

Name _____

11). Consider the INEPT pulse sequence (below, right), and the simple ^{1}H-^{13}C spin system (i.e. $CHCl_3$). At point 'g' in the pulse sequence, the $M_H^{C\alpha}$ and $M_H^{C\beta}$ components appear as shown here (below, left). The energy diagram for this system is depicted (below, center), where A_1 and A_2 are the ^{1}H transitions, and X_1 and X_2 are the ^{13}C transitions. We define ΔH as the difference in the number of spins in α and β states for ^{1}H, and ΔX as the difference in the number of spins in α and β states for ^{13}C (i.e. ΔC).



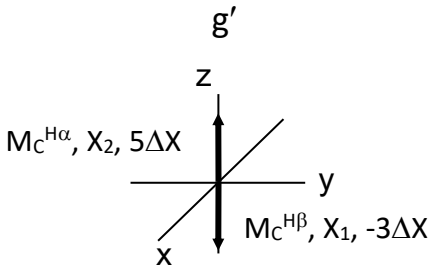
a. How is ΔH related to ΔX (i.e., ΔC)? Quantitatively, what is their ratio, and how is this ratio derived? (**2 points**)

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

b. 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 $N_4 = N$. (**14 points**)

at equilibrium	at 'g'
$N_4 = N$	$N_4 = N + \Delta H$
$N_3 = N + \Delta X$	$N_3 = N + \Delta X$
$N_2 = N + \Delta H$	$N_2 = N$
$N_1 = N + \Delta H + \Delta X$	$N_1 = N + \Delta H + \Delta X$
$A_1 = N_2 - N_4 = \Delta H$	$A_1 = N_2 - N_4 = -\Delta H$
$A_2 = N_1 - N_3 = \Delta H$	$A_2 = N_1 - N_3 = \Delta H$
$X_1 = N_3 - N_4 = \Delta X$	$X_1 = N_3 - N_4 = \Delta X - \Delta H = -3\Delta X$
$X_2 = N_1 - N_2 = \Delta X$	$X_2 = N_1 - N_2 = \Delta X + \Delta H = 5\Delta X$

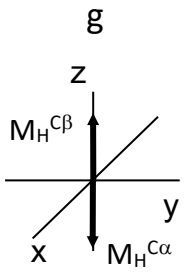
c. The vector diagram corresponding to point g′ in the INEPT pulse sequence (see above) is shown here (right). The magnitudes of the ^{13}C vectors are not drawn to scale, and the correct magnitudes should be available to you from your table above. Label each vector properly with the following information: $M_C^{H\alpha}$ or $M_C^{H\beta}$, X_1 or X_2 (transition) and magnitude (in terms of ΔX). (**6 points**)



Name _____

d. 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 (we will call this FID 'A') and Fourier transformed. Two signals are observed, corresponding to $M_C^{H\alpha}$ and $M_C^{H\beta}$, of opposite phase, whose magnitudes are indicated in your table in 'b' and in your answer to 'c' above. Now, if the phase of the last ^1H 90°y pulse (point 'f') is changed to -y, and the FID (we will call this 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' (again, you must show your work or otherwise provide a complete justification)? (**10 points**)

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:



$N_4 = N$ $\quad A_1 = N_2 - N_4 = \Delta H$

$N_3 = N + \Delta H + \Delta X$ $\quad A_2 = N_1 - N_3 = -\Delta H$

$N_2 = N + \Delta H$ $\quad X_1 = N_3 - N_4 = \Delta X + \Delta H = 5\Delta X$

$N_1 = N + \Delta X$ $\quad X_2 = N_1 - N_2 = \Delta X - \Delta H = -3\Delta X$

*Thus, if we **add** the components:*

$M_H^{C\alpha}$ (X_2) 'y' + $M_H^{C\alpha}$ (X_2) '-y' = 5ΔX + (-3ΔX) = 2ΔX

$M_H^{C\beta}$ (X_1) 'y' + $M_H^{C\beta}$ (X_1) '-y' = (-3ΔX) + 5ΔX = 2ΔX

The signal will look like the normal ^{13}C signal: a normal signal would show $M_H^{C\alpha}$ and $M_H^{C\beta}$ to be of the same phase and each of magnitude ΔX. 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:*

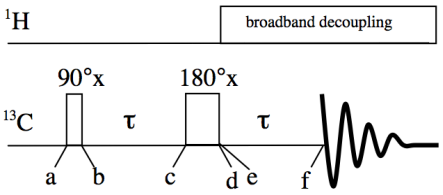
$M_H^{C\alpha}$ (X_2) 'y' - $M_H^{C\alpha}$ (X_2) '-y' = 5ΔX - (-3ΔX) = 8ΔX

$M_H^{C\beta}$ (X_1) 'y' - $M_H^{C\beta}$ (X_1) '-y' = (-3ΔX) - 5ΔX = -8ΔX

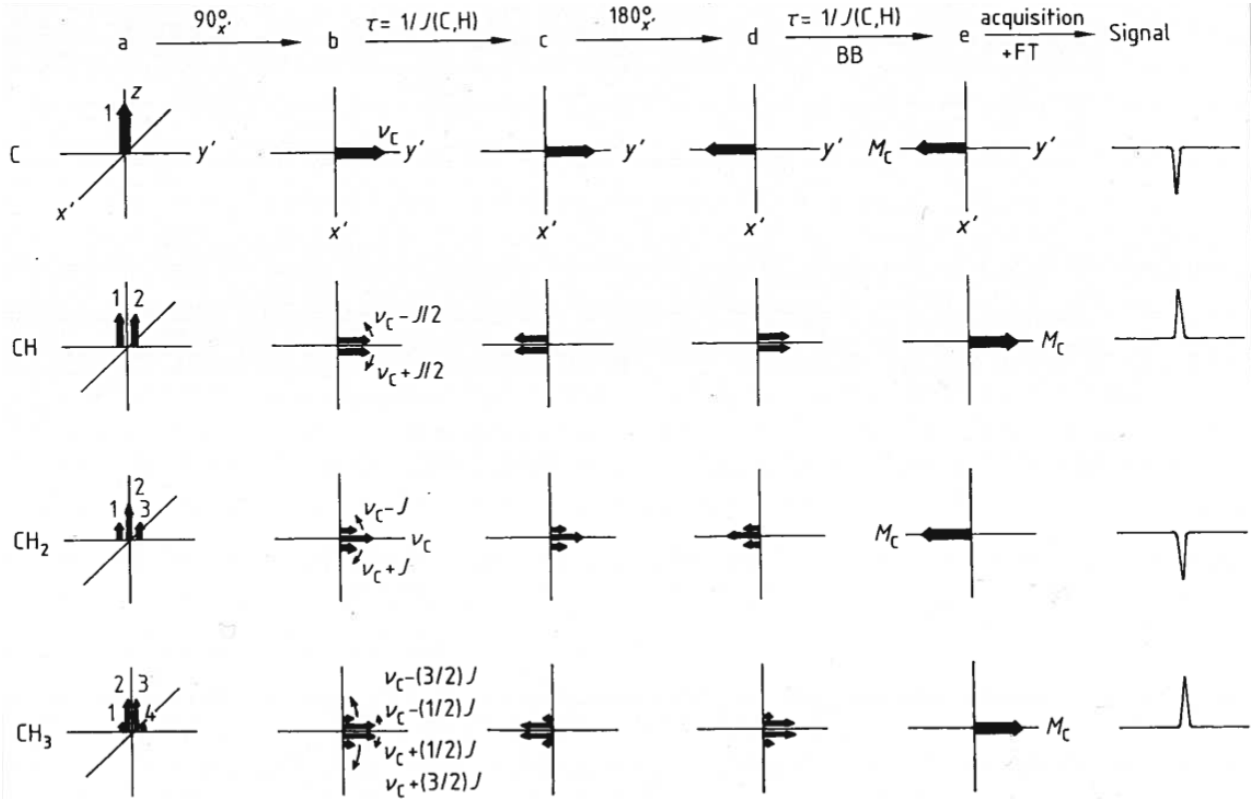
The signal will show *$M_H^{C\alpha}$ and $M_H^{C\beta}$ to be of opposite phase but each is eight times as large as the normal ^{13}C signal. Since we have used 2 FIDs, we have essentially enhanced the signals each equally by a factor of 4.*

Name _____

12). The spin-echo Attached Proton Test experiment (right) can be used to determine the number of protons attached to a given carbon atom with τ set to $1/(J_{CH})$. Shown below are vector diagrams for each point in the spin echo pulse sequence for -C, -CH, -CH_2, and -CH_3 groups using the spin-echo pulse sequence with $\tau = 1/(J_{CH})$ (in each case, the reference frequency is chosen to be equal to the Larmor frequency). Also shown is the Fourier transformation of the signal collected at point 'f' in each case.



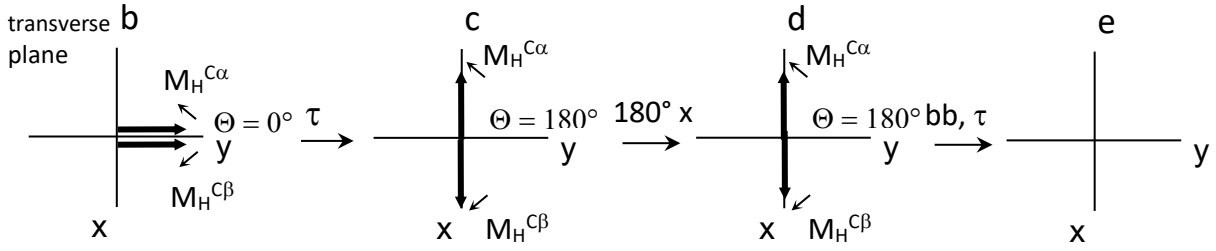
Could the APT also be a useful experiment if τ is set to $1/(2J_{CH})$? Draw vector diagrams for C, CH, CH_2, and CH_3 vectors with $\tau = 1/(2J_{CH})$ for each point in the spin-echo/APT pulse sequence. Indicate on your diagrams the angle(s) between vector components and the direction of rotation of vector components. Also sketch the Fourier transform of the signal that you will obtain at point 'f'. Then, discuss the differences between the results of the two experiments ($1/(J_{CH})$ vs $1/(2J_{CH})$) and how the experiment with $\tau = 1/(2J_{CH})$ might be useful (<u>you can use the next page to show your work</u>). (**12 points**)



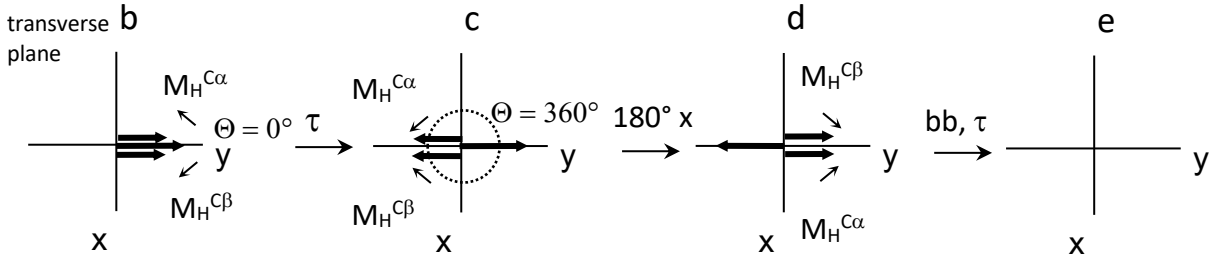
Name _____

C : The result for quaternary carbons is the same as shown above for $\tau = 1/J_{CH}$. *Because there are no attached protons, there are no couplings and thus no changes as tau changes*

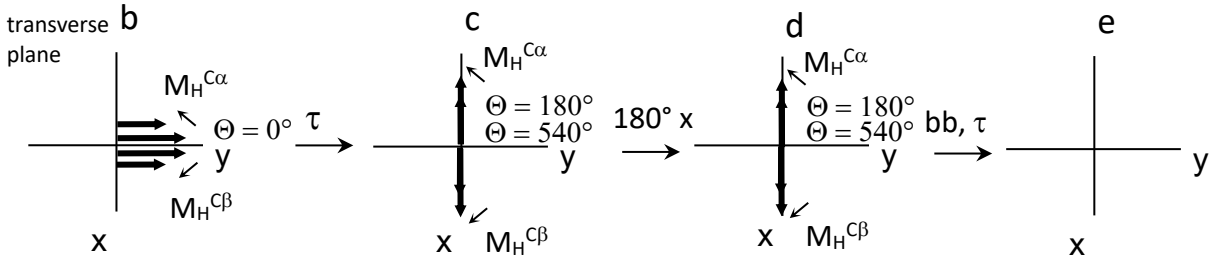
CH : When $\tau = 1/(2J_{CH})$, $M_H^{C\alpha}$ *and* $M_H^{C\beta}$ *move apart by 180° during* τ, *as opposed to 360° when* τ *is* $1/(J_{CH})$. *The 180° pulse has no effect, and the broadband decoupling results in cancellation of the signals. Thus, no signal would be observed*



CH₂ : When $\tau = 1/(2J_{CH})$, $M_H^{C\alpha}$ *and* $M_H^{C\beta}$ *move apart by 360° during* τ, *as opposed to 720° when* τ *is* $1/(J_{CH})$. *Thus,* $M_H^{C\alpha}$ *and* $M_H^{C\beta}$ *end up opposite in phase to the center component. The 180° pulse reflects all vectors about 'x', so that the phases of the outer and inner components are still opposite. Once decoupling is turned on at 'd', the broadband decoupling results in cancellation of the signals. Thus, no signal would be observed*



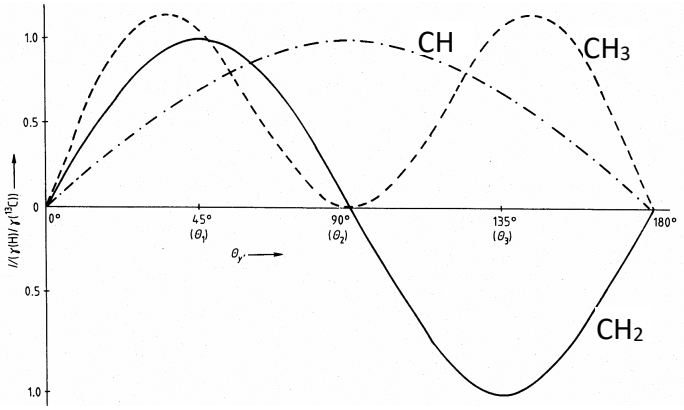
CH₃ : When $\tau = 1/(2J_{CH})$, *the inner components move apart by 180° (same as for CH), and the outer components move apart by 540°, as opposed to 1080° when* $\tau = 1/(J_{CH})$. *The 180° pulse has no effect, and the broadband decoupling results in cancellation of the signals. Thus, no signal would be observed*



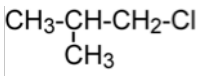
So, this experiment, with $\tau = 1/(2J_{CH})$, *would allow unambiguous identification of quaternary carbons.*

Name _____

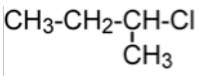
13). In the DEPT experiment, the pulse angle (Θy, pulse width) of the third ^{1}H pulse (applied along the '*y*' axis) can be set to any value in order to achieve the desired result. Shown in the diagram (right) are the intensities of signals from –CH, -CH_2, -CH_3 groups as a function of the pulse angle Θy. Broadband ^{1}H-decoupled ^{13}C spectra (spectra A, B, C, and D) in $CDCl_3$ solvent were acquired for samples of each of the molecules (1, 2, 3, and 4) shown. Expansions of these spectra around 0 ppm and 75-80 ppm are shown (below, right). Further below are shown DEPT spectra (E and F) for two of the molecules/samples.



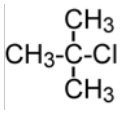
1



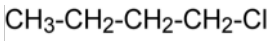
2

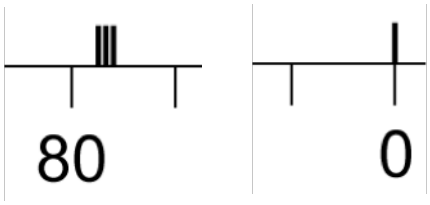


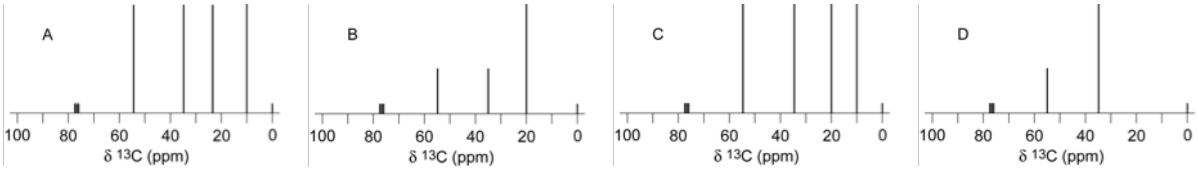
3

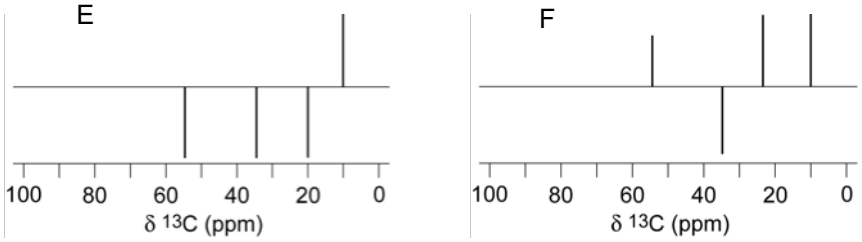


4









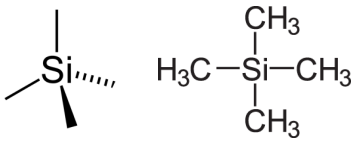
a. What does the acronym 'DEPT' stand for? (**2 points**)

***D**istortionless **E**nhancement by **P**olarization **T**ransfer*

Name _____

b. Explain what gives rise to the small signals centered at 0 ppm and 77 ppm. Explain the multiplicities of the signals. You may need to provide a structural description to do so. Explain the multiplet structures and relative heights of the individual peaks in the signals. (**8 points**).

The signal at 0 ppm is most likely due to tetramethylsilane (TMS) added as a chemical shift standard. In TMS, the methyl groups are arranged at the four corners of a perfect tetrahedron, and therefore are chemically equivalent and give identical chemical shifts (singlets for both ^{13}C and ^{1}H). The ^{13}C and ^{1}H shifts typically define 0 ppm in ^{13}C and ^{1}H spectra, respectively.



The signal at 77 ppm is most likely due to the ^{13}C in $CDCl_3$. The ^{13}C signal is split by coupling to the attached (quadrupolar) ^{2}H (D) nucleus. When a ^{13}C nucleus is attached to a ^{1}H nucleus it is split into a doublet, where the individual peaks in the doublet are of equal intensity. This is because the attached ^{1}H nucleus (I=½) can be in either the alpha (α, low energy) or beta (β, higher energy) state. Because the α and β states are nearly equally populated, the intensities of the doublet components in the ^{13}C spectrum are nearly identical. We know also that for a ^{2}H nucleus, where I=1, there will be three possible energy states, $E = -1\gamma\hbar B_0$, $E = 0\gamma\hbar B_0$, $E = 1\gamma\hbar B_0$, and that these will be very nearly equally populated. Thus, these will split the attached ^{13}C nucleus into a triplet with nearly equal intensities for the three peaks. This of course is consistent with the general rule that multiplicity is equal to 2nI+1 where n is the number of coupled nuclei and I is the spin quantum number. This is also consistent with the downfield shift of the signal due to deshielding by the three Cl atoms.

c. Why is the intensity of the signal at ~77 ppm so small compared to the other signals in the spectrum? (**6 points**)

The dominant mechanism for ^{13}C relaxation in organic compounds is via the dipolar interaction with directly attached hydrogen atoms. In $CDCl_3$ (which gives rise to the ^{13}C signal at ~77 ppm), there are no hydrogen atoms, but deuterium atoms. The relaxation mechanism is much less efficient with attached deuterium, so the ^{13}C relaxation rate is much slower. Thus, like ^{13}C signals from quaternary carbon atoms (which also have no attached hydrogen atoms and relax very slowly), the signal intensity is small because the relaxation delay in the experiment is too short to allow for relaxation, so transverse magnetization from these nuclei is small. If the relaxation delay were lengthened dramatically, the signal at 77 ppm would be relatively much larger.

It should be noted the compounds in question are all liquids, and given that there is TMS in the sample there is no need necessarily to use a solvent at all. However, as the question states that these samples are all in $CDCl_3$ solvent, the small magnitude of the signal is not simply due to the fact that the $CDCl_3$ concentration is low.

d. Two of the molecules can be matched unambiguously with their respective ^{13}C spectra. Please match these two molecules with their respective ^{13}C spectra (do NOT consider the DEPT spectra here). Please consider all of the information available in the spectra, and explain why all of the information available in the spectra are consistent with your matches. Thoroughly justify (explain) your answer. (**8 points**)

All four molecules have the same chemical formula (C_4H_9Cl). Molecule 3 would be expected to show only two signals in the ^{13}C spectrum because rotation about the C-Cl bound renders the three methyl groups chemically equivalent resulting in only one signal from them. The remaining signal will be from the quaternary carbon atom. The intensity of the ^{13}C signal from the methyl groups should be much larger than the other signal, first because three carbons give rise to it, second because the NOE effect will be maximized (depends on the number of directly attached hydrogens), and third because the other signal is from a quaternary carbon (smaller NOE effect, and the relaxation delay is most likely too short for the signal from the quaternary carbon to be maximized). Finally, the large signal from the methyl groups should be further upfield because the carbon atom bound to the electronegative Cl atom will be deshielded and downfield. These characteristics are only consistent with spectrum D, thus spectrum D is of molecule 3.

Molecule 1 would be expected to show only three signals in the ^{13}C spectrum because there is a clear plane of symmetry rendering the two methyl groups equivalent. Thus, there will be one signal from the methyl groups, one from the methine and one from the methylene. The intensity of the ^{13}C signal from the methyl groups should be larger than the signal from the other carbons because two carbons give rise to it (rather than 1 for the other signals) and because the NOE effect will be maximized. The intensities of the signals from the other carbons should be somewhat comparable to one another, and smaller than the signal from the methyl groups. The signal from the methyl groups should be the furthest upfield, because it is furthest away from the electronegative Cl atom and, thus, will be most shielded. The signal from the methylene should be quite far downfield because the methylene carbon is attached to the Cl atom. The other signal (methine) should be between the others. These characteristics are only consistent with spectrum B, thus, spectrum B is of molecule 1.

e. Two of the molecules can be matched unambiguously with the two DEPT spectra. Please match these two molecules with their respective DEPT spectra. Please consider all of the information available in the spectra. Thoroughly justify (explain) your answer. As part of your explanation, you will have to decide what the phase angle Θy is for the DEPT spectra shown and clearly explain your reasoning for this. (**8 points**)

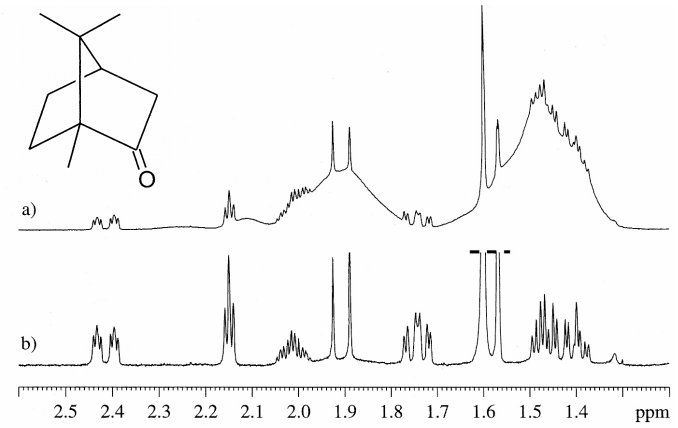
There are 4 signals in each of the DEPT spectra. Regardless of the phase angle Θy, this means there are at least 4 different (not chemically equivalent) ^{13}C nuclei (with attached hydrogens) in the molecules that give rise to these DEPT spectra. Both molecules 2 and 4 meet this criterion, whereas molecules 1 and 3 (as described in part 'd', above) do not. Therefore, the DEPT spectra are of molecules 2 and 4.

Molecule 4 is comprised of three methylene groups and one methyl. None of the ^{13}C nuclei are equivalent. A DEPT-135 spectrum of molecule 4 should show one signal phased "up" and three signals phased "down" corresponding to the methyl and methylene groups, respectively. The "up" signal, corresponding to the methyl group, should be the furthest upfield, as the methyl carbon is the most shielded (furthest from the electronegative Cl). These attributes are seen in DEPT spectrum E, indicating that this is the DEPT-135 spectrum of molecule 4.

Molecule 2 is comprised of two methyl groups, one methine and one methylene. None of the ^{13}C nuclei are equivalent. A DEPT-135 spectrum of molecule 2 should show one signal phased "down", corresponding to the single methylene group, and three signals phased "up" corresponding to the two methyl and one methine groups. The methine group is bonded to the electronegative Cl and so would be highly deshielded, so the "up" signal corresponding to the methine would be expected to be the furthest downfield. The methyl groups would be most shielded and would be expected to give the most upfield signals. Also, as shown in the plot of intensity versus the phase angle Θy, for a DEPT-135, the "up" signals from methine groups would be expected to be less intense than the "up" signals of methyl groups. These attributes are seen in DEPT spectrum F, indicating that this is the DEPT-135 spectrum of molecule 2.

Name _____

14). The ^{1}H NMR spectrum of a sample consisting of a mixture of the small molecule camphor (right, Mr=152) and the very large polymer polystyrene (Mr=50,000) is shown (right, spectrum 'a'). The ^{1}H NMR spectrum shown in 'b' results when a 'T_2 filter' experiment is used. The T_2 filter experiment uses the Carr-Purcell spin-echo pulse sequence shown above (question 10). For the spectrum in 'b', the τ delay was 1.5 ms and the echo was repeated 150 times to produce a total relaxation delay period of 450 ms.



a. Describe the presumed relative NMR T_2 relaxation properties of polystyrene and camphor. Why does the spectrum in 'a' look as it does. (**6 points**)

For very large molecules, like the polystyrene molecule, the correlation time is long and the T_2 relaxation time is very short. For small molecules, like camphor, the correlation time is much shorter and the T_2 relaxation time is much longer. The NMR line width is dependent inversely on T_2, so when T_2 is very short, the lines/signals are very broad. In spectrum 'a', we see the relatively sharp signals from the small molecule camphor, and the very broad signals (due to the short T2) of the polystyrene molecule. The latter signals are so broad that much of the spectrum from the camphor is somewhat 'hidden' under these broad polystyrene signals.

b. Given your answer to part 'a', describe why/how the result shown in spectrum 'b' is obtained. (**8 points**)

In the T_2 filter experiment, the contribution to relaxation from magnetic field inhomogeneity is removed by the Carr-Purcell spin-echo sequence. Thus, during the experiment, the amplitudes of the echos from the polystyrene decay according to the fast T_2 of polystyrene, whereas the amplitudes of the echos from the camphor decay much more slowly due to the much longer T_2 of the camphor. After 150 echos, or 450 ms, the signal (echo amplitudes) from the polystyrene are very small (too small to be observed), whereas the amplitudes of the echos from the camphor signals are still observable. Then, acquisition and Fourier transformation will yield the spectrum of camphor only, and this is what is observed in spectrum 'b'. So, the T_2 filter is useful for selectively observing signals from smaller molecules in the presence of larger molecules.

c. Can you describe an experiment that would give a result similar to (or better than) the result shown in spectrum 'b', but is much simpler to implement? (**6 points**)

It may be possible simply to apply a 90° pulse and wait for an appropriate time period following the pulse before acquiring the data. In this case, the signal from the polystyrene will decay quickly, leaving only signal from the more slowly relaxation camphor. As it turns out, there will be a very large and complex phase correction to the spectrum.

Name _____

THIS PAGE IS BLANK. FEEL FREE TO USE IT AS “SCRATCH” IF YOU LIKE

Name _____

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

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

$\gamma_{1H} = 26.7519 \times 10^7$ rad/T/s, $I = 1/2$
$\gamma_{10B} = 2.8747 \times 10^7$ rad/T/s, $I = 3$
$\gamma_{11B} = 8.5847 \times 10^7$ rad/T/s, $I = 3/2$

$\gamma_{13C} = 6.7283 \times 10^7$ rad/T/s, $I = 1/2$
$\gamma_{15N} = -2.7126 \times 10^7$ rad/T/s, $I = 1/2$
$\gamma_{17O} = -3.6280 \times 10^7$ rad/T/s, $I = 5/2$

Name _____

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

k_B = 1.381 x 10^{-23} J/K Avagadro's number = 6.02214179 x 10^{23} mol^{-1}

h = 6.626 x 10^{-34} Js $\hbar = h/(2\pi)$

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

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

$\pi/2$ radians = 90°

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

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

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

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

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

m = ($-I$, $-I$+1, $-I$+2,....., I) ($2I$+1)

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

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

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

$$\omega_0 = \gamma B_0$$

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

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

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

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

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

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

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2(B_0)}$$

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

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

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

$I \propto 1/r^6$

$\Theta = \gamma B_1 \tau_p$

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

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

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

SW=1/(2DW)=Nyquist frequency (ν_{NQ})/2

AQ=DW*TD

DR=2SW/TD=1/AQ

(TD≡NP)

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

$\pi/2$ radians = 90°

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

multiplicity=$2nI + 1$

$\Theta = 2\pi J\tau$