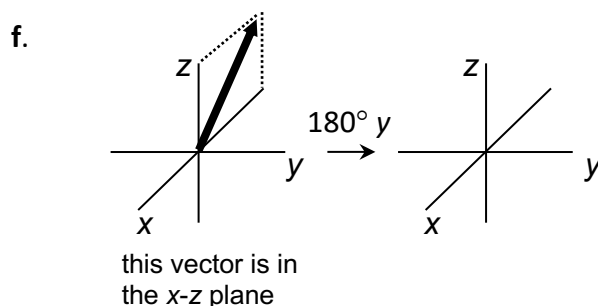
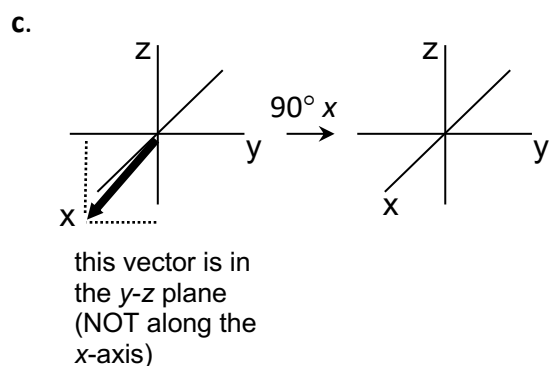
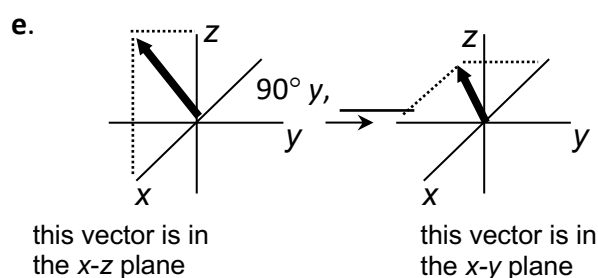
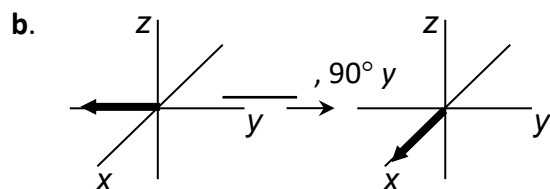
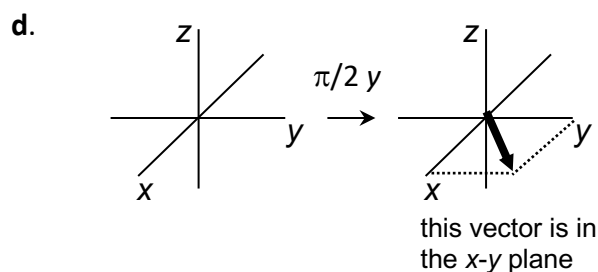
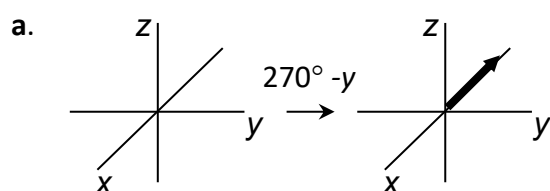
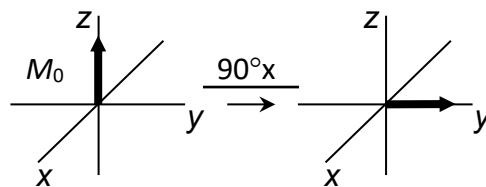


Exam 3: CHEM/BCMB 4190/6190/8189 (206 points) Tuesday, 25 October, 2022

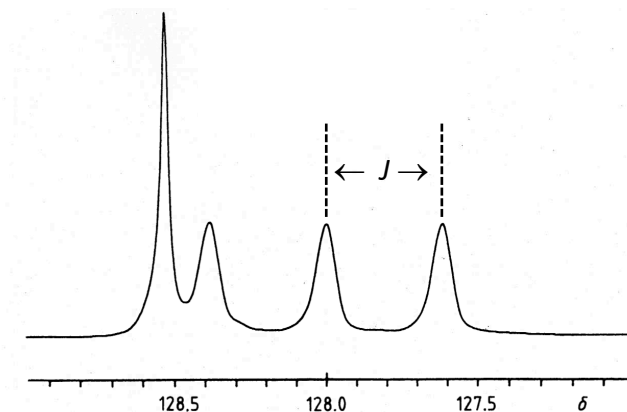
1). In the example (right), the effect of a 90° ($\pi/2$) pulse applied along the "x" axis (90°_x) is shown for a bulk magnetization vector (M_0) at equilibrium (on the 'z' axis).

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)**



Name _____

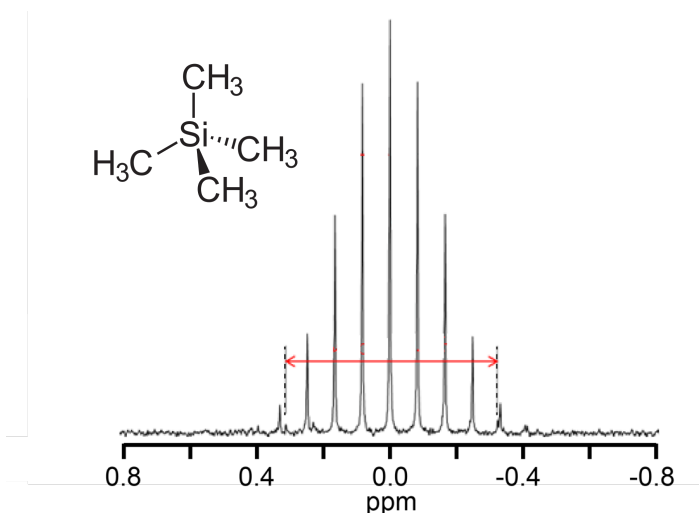
2). The ^{13}C NMR spectrum shown (right) is from a mixture of C_6H_6 (benzene) and C_6D_6 (deuterated benzene). The B_0 field was 5.87 T.



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)**

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

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 ^1H (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).

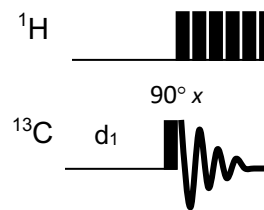


a. Please explain why the spectrum looks as it does. Your explanation should include an explanation of how many signals/peaks should be observed, are observed, and why, and the relative intensities of the peaks. **(8 points)**

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)**

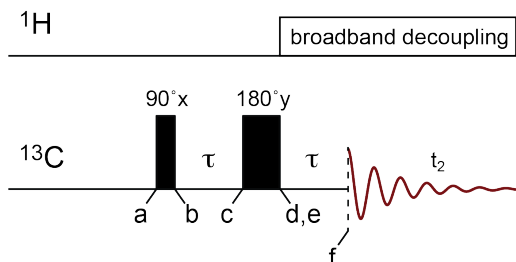
c. What field strength, in Tesla, was the magnet that was used to acquire this ^{29}Si spectrum? You will have to perform a calculation and show your work for credit. **(6 points)**

4). The inverse-gated ^1H 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)

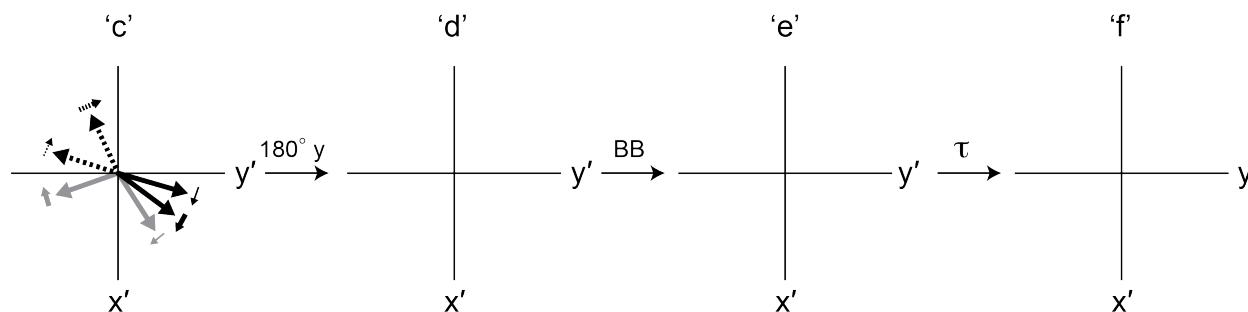


5). The longitudinal relaxation time constants (T_1) for ^1H relaxation in uniformly (100%) ^{13}C -labeled chloroform ($^{13}\text{CHCl}_3$) and uniformly (100%) ^{12}C -labeled chloroform ($^{12}\text{CHCl}_3$) were measured under identical conditions using the inversion-recovery method. The measured value of T_1 for the ^1H nucleus in $^{13}\text{CHCl}_3$ was 13 seconds, or about half the value measured for the ^1H nucleus in $^{12}\text{CHCl}_3$ (28 seconds). Please explain the physical basis for this result. (8 points)

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 ^1H decoupling has begun, and point 'e' is also immediately after the 180° pulse but just after beginning broadband ^1H decoupling. Point 'f' is at the beginning of the acquisition period. This experiment was performed on a molecule with three $-\text{CH}$ 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 $-\text{CH}$ 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).

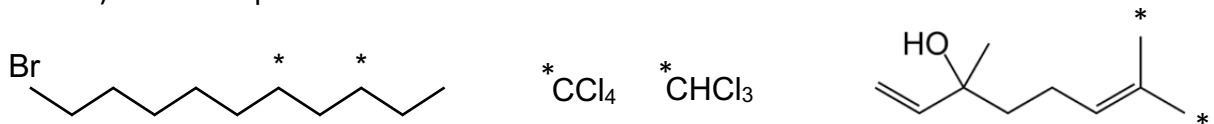


a. Complete the vector diagrams for points 'd', 'e', and 'f'. Please provide any explanation you feel necessary to justify your answers. **(6 points)**

Name _____

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)**

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



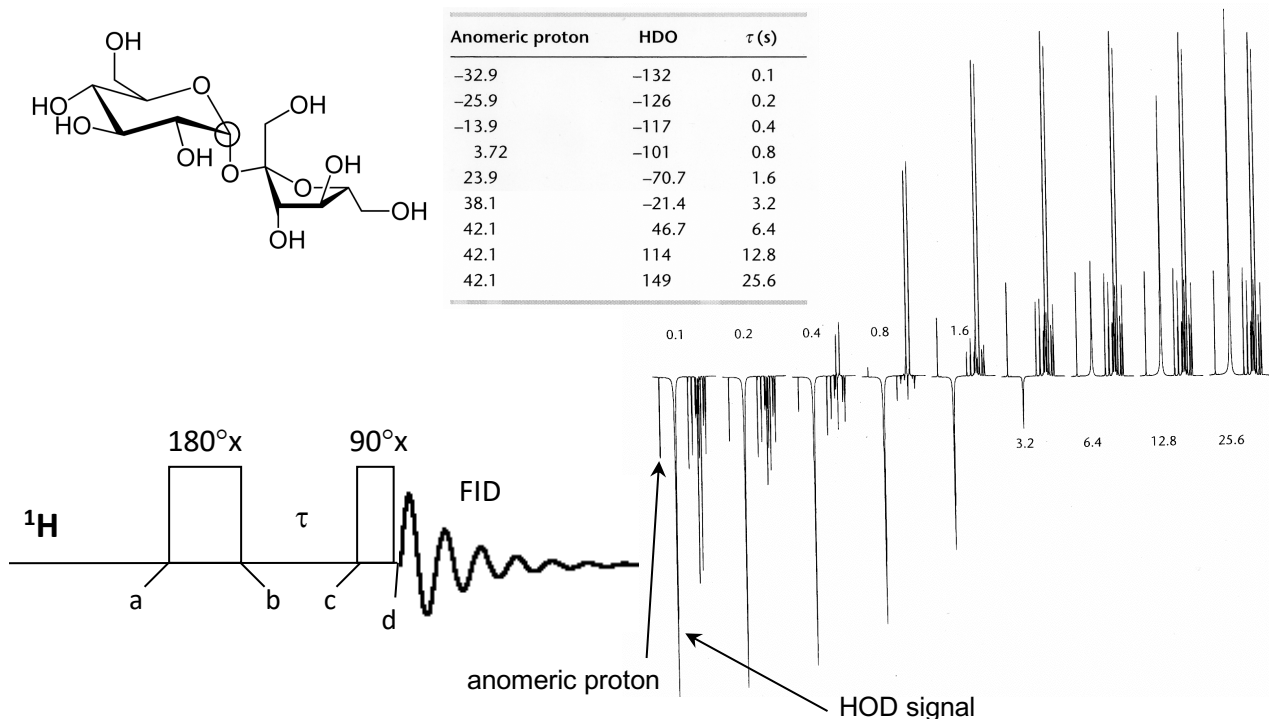
a. In 1-bromodecane (above, left), the T_1 value for one of the indicated (asterisks) ^{13}C nuclei is 3.1 s, whereas the T_1 value for the other is 2.1 s. Please explain which nucleus gives rise to each T_1 value. Your explanation will have to address the principles underlying the differences to receive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

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)

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)**

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

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 ($\tau = 0.1$ s). The intensity values (arbitrary units) for the anomeric hydrogen and HOD signals are shown in the table (below) for each τ .



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

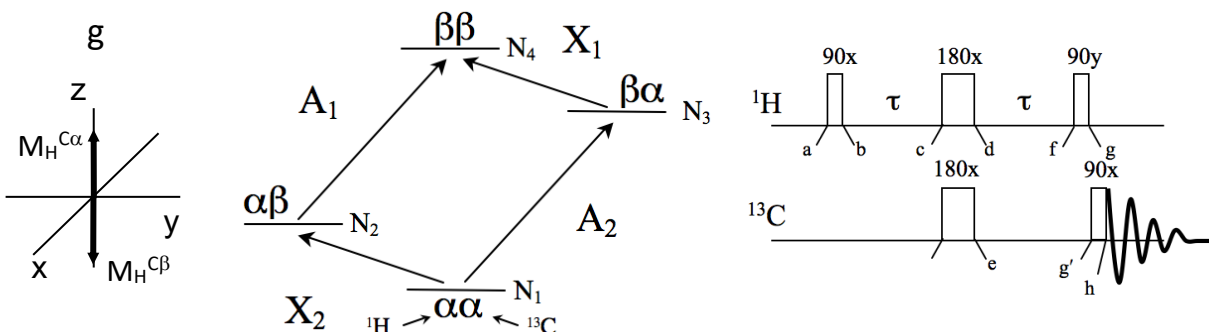
b. For the HOD signal in the inversion recovery experiment, the ratio of the intensity of the observed signal at $\tau = 3.2$ s to the signal at $\tau = 0$ s is 0.14, what is T_1 ? You must do a calculation based on these times and intensities and get an answer for credit. **(4 points)**

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)**

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)**

11). Consider the INEPT pulse sequence (below, right), and the simple ^1H - ^{13}C spin system (i.e. CHCl_3). At point 'g' in the pulse sequence, the $M_{\text{H}}^{\text{C}\alpha}$ and $M_{\text{H}}^{\text{C}\beta}$ 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 ^1H 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 ^1H , and ΔX as the difference in the number of spins in α and β states for ^{13}C (i.e. ΔC).

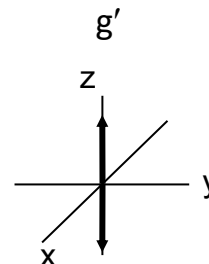


a. How is ΔH related to ΔX (i.e., ΔC)? Quantitatively, what is their ratio, and how is this ratio derived? (2 points)

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)

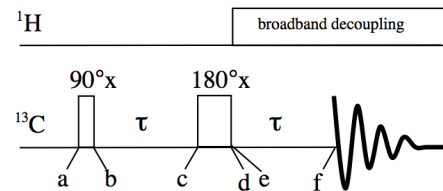
at equilibrium	at 'g'
$N_4 = N$	$N_4 =$
$N_3 =$	$N_3 =$
$N_2 =$	$N_2 =$
$N_1 = N + \Delta H + \Delta X$	$N_1 =$
$A_1 =$	$A_1 =$
$A_2 =$	$A_2 =$
$X_1 =$	$X_1 =$
$X_2 =$	$X_2 =$

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: $M_{\text{C}}^{\text{H}\alpha}$ or $M_{\text{C}}^{\text{H}\beta}$, X_1 or X_2 (transition) and magnitude (in terms of ΔX). (6 points)

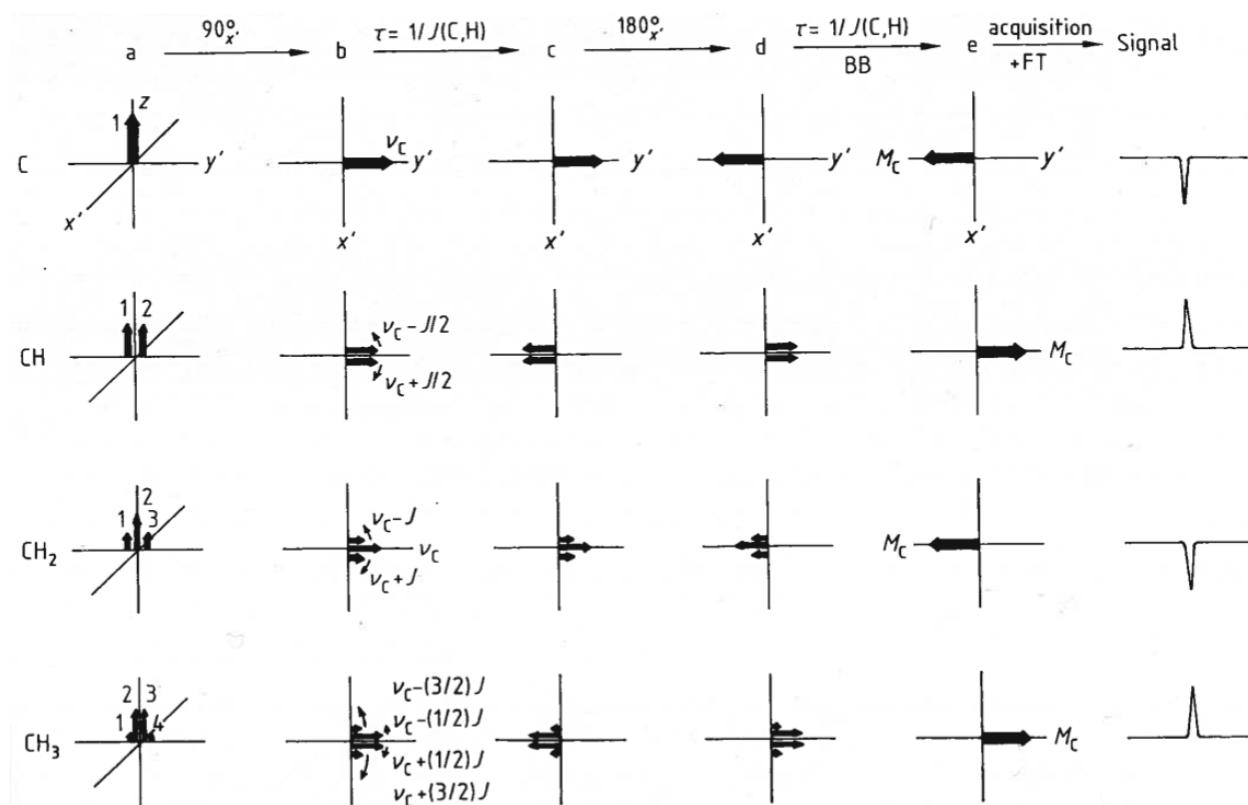


d. At point g' in the INEPT pulse sequence, a ^{13}C 90°_x pulse is applied to create transverse ^{13}C magnetization that subsequently is recorded (we will call this FID 'A') and Fourier transformed. Two signals are observed, corresponding to $M_{\text{C}}^{\text{H}\alpha}$ and $M_{\text{C}}^{\text{H}\beta}$, 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)**

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, -CH₂, and -CH₃ 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.



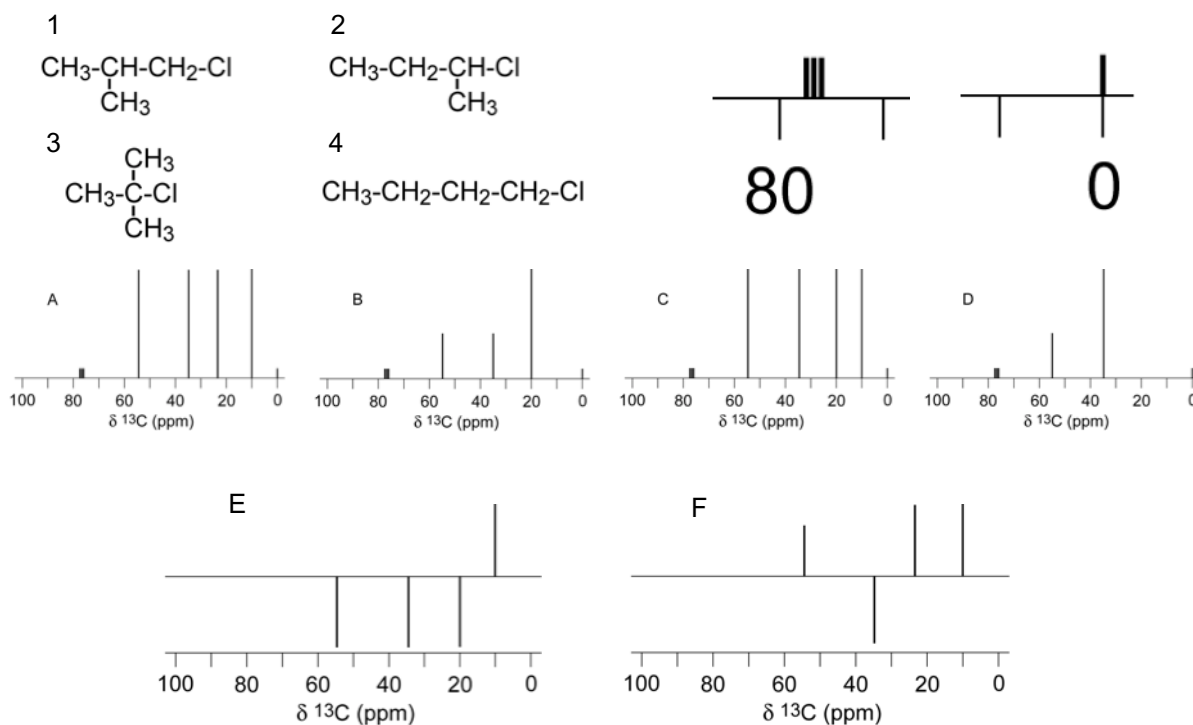
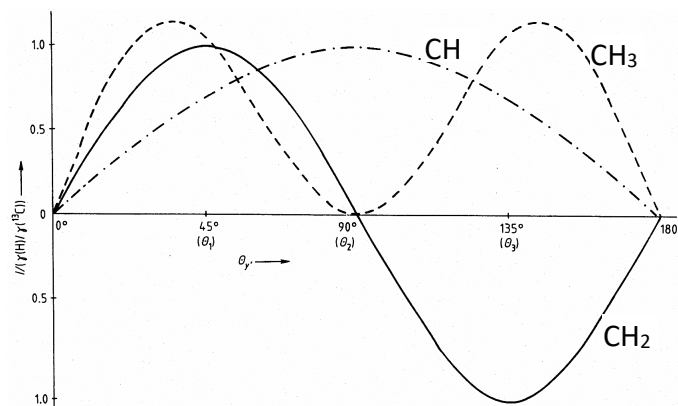
Could the APT also be a useful experiment if τ is set to $1/(2J_{CH})$? Draw vector diagrams for C, CH, CH₂, and CH₃ vectors with $\tau = 1/(2J_{CH})$ for each point in the spin-echo/APT pulse sequence. Indicate on your diagrams the angle(s) between vector components and the direction of rotation of 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 (you can use the next page to show your work). (12 points)



Name _____

Please answer question #12 on this page:

13). In the DEPT experiment, the pulse angle (θ_y) of the third ^1H 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 $-\text{CH}$, $-\text{CH}_2$, $-\text{CH}_3$ groups as a function of the pulse angle θ_y . Broadband ^1H -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.



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

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)**.

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

Name _____

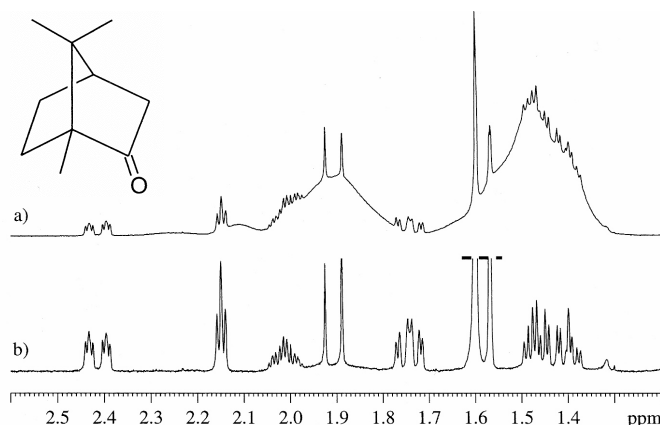
d. Two of the molecules can be matched unambiguously with their respective ^{13}C spectra. Please match these two molecules with their respective ^{13}C spectra (do NOT consider the DEPT spectra here). Please consider all of the information available in the spectra, and explain why all of the information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

Name _____

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 ϕ is for the DEPT spectra shown and clearly explain your reasoning for this. **(8 points)**

Name _____

14). The ^1H NMR spectrum of a sample consisting of a mixture of the small molecule camphor (right, $M_r=152$) and the very large polymer polystyrene ($M_r=50,000$) is shown (right, spectrum 'a'). The ^1H 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)**

Name _____

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

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)**

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 ^{a)} [eQ] [10^{-28} m^2]	Natural abundance) [%]	Relative sensitivity ^{b)}	Gyromagnetic ratio $\gamma^a)$ [$10^7 \text{ rad T}^{-1} \text{ s}^{-1}$]	NMR frequency [MHz] ^{b)} ($B_0 = 2.3488 \text{ T}$)
^1H	1/2	–	99.985	1.00	26.7519	100.0
^2H	1	2.87×10^{-3}	0.015	9.65×10^3	4.1066	15.351
$^3\text{H}^c)$	1/2	–	–	1.21	28.5350	106.664
^6Li	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)	
	^1H	^{13}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:

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

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

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

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

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

$$P_Z = m\hbar$$

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

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

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

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

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

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

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

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

$$m = (-I, -I+1, -I+2, \dots, I) \quad (2I+1)$$

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

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

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

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

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

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

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

$$\omega_0 = \gamma B_0$$

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

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

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

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

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

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

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

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

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

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

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

$$AQ = DW * TD$$

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

$$(TD \equiv NP)$$

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

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

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

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

$$\Theta = 2\pi/\tau$$