**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 ($\gamma = 3.00 \times 10^7$ rad T^{-1} s^{-1}) is 3.501×10^{-25} 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**)

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

^1H

90

^{13}C d$_1$

200 100   0 -100 -200   Hz
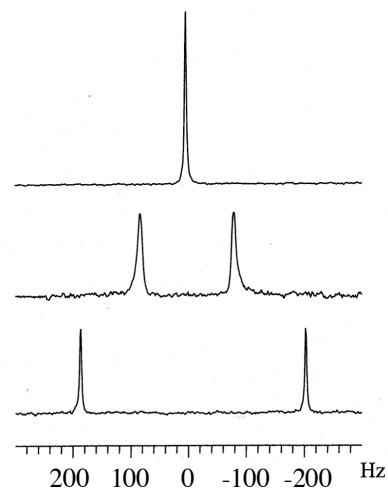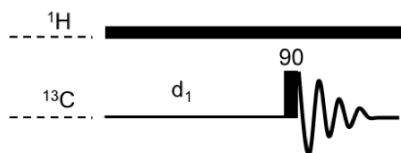
a. What determines the difference between the actual and observed coupling constants (in Hz) in
an off-resonance decoupling experiment such as the one described for the spectrum above? (**4
points**)

b. Calculate the difference between the Larmor frequency of the ^1H 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**)

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

a. T_1 relaxation is sometimes called (spin-lattice : spin-spin) relaxation. (**2 points**)

b. The molecular correlation time, τ_C, is inversely proportional to (T_1 : $1/T_1$). (**2 points**)

c. T_1 values (increase : decrease) as molecular size decreases. (**2 points**)

d. For ^{13}C relaxation, decreasing the number of hydrogens attached to a carbon (increases : decreases) the ^{13}C T_1. (**2 points**)

e. T_2 can be (longer than : shorter than) T_1. (**2 points**)

f. If T_2* times are short, NMR signals are (narrow : broad). (**2 points**)

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

cholesteryl chloride

substituted benz[a]anthracene
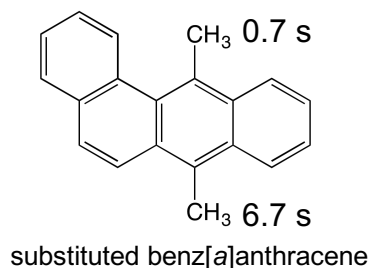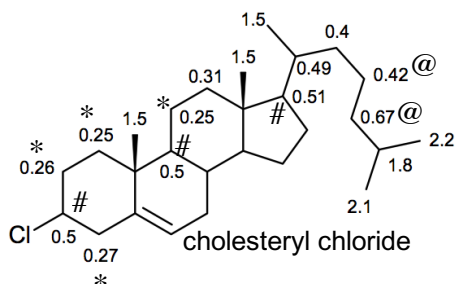
a. For the molecule on the left (cholesteryl chloride), explain why the T_1 relaxation time constants for the ^{13}C nuclei labeled with asterisks (*) are about ½ as large as those marked with pound signs (#). (**4 points**)

b. For the molecule on the left (cholesteryl chloride), explain why the T_1 relaxation time constants for the ^{13}C nuclei labeled with asterisks (*) are about ½ as large (on average, approximately) as those marked with "at" signs (@). (**4 points**)

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

5). A ^1H nucleus (bolded, in the molecule shown, right) in a molecule is coupled to three other ^1H 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. ^1H 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 ^1H is shown below on the right. The chemical shift of the bolded hydrogen is 3.00 ppm.

–CH-C**H**-CH$_2$–
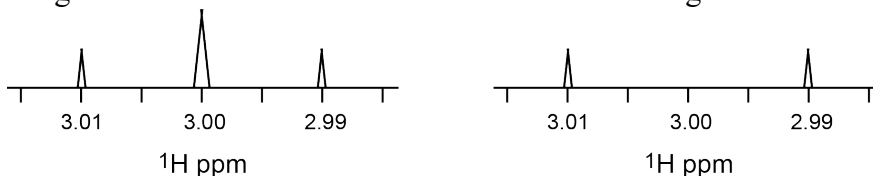
a). Why is the signal on the left an apparent triplet, whereas the signal on the right is an apparent doublet. You will have to explain for credit. (**4 points**)

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**)

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**)

6). The DEPT spectrum and the ^{13}C spectrum with broadband ^1H decoupling of the compound citronellol (right) are shown below. Also shown (far right) is a plot of the phase angle of the third ^1H pulse (Θy), applied along the 'y' axis, versus the expected intensities of the signals for the various groups in DEPT spectra.
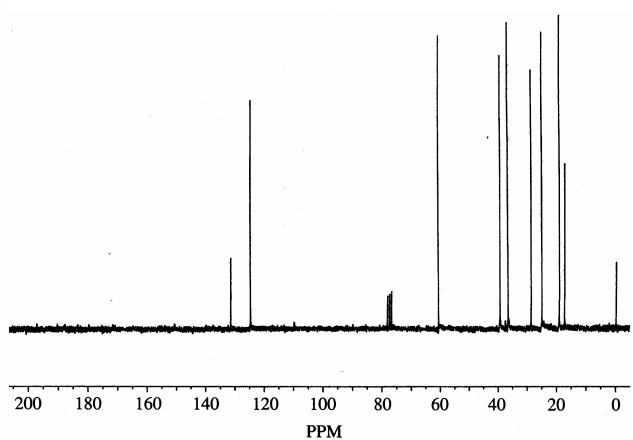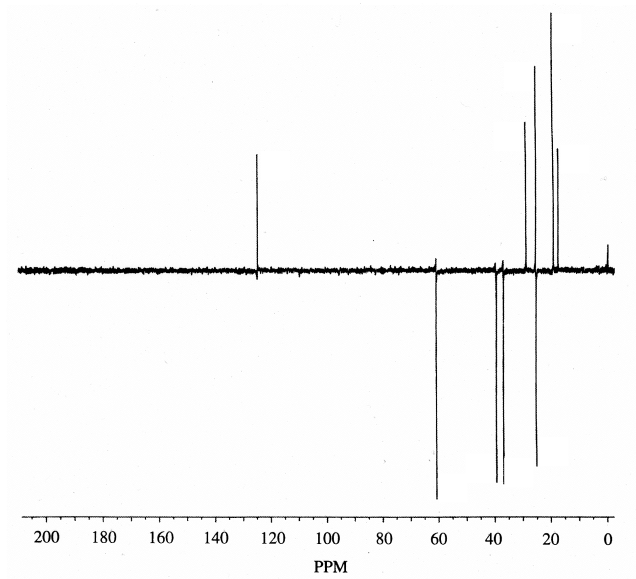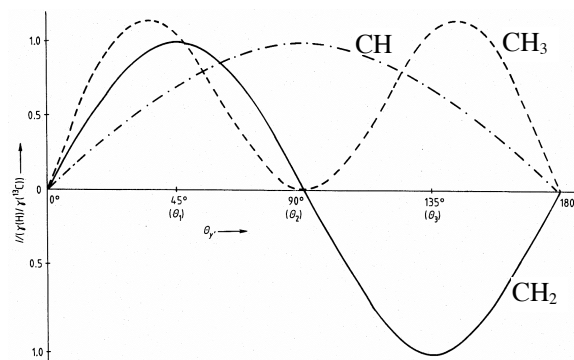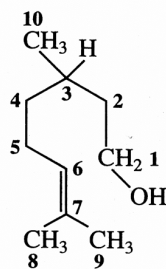
a). What is Θy for the DEPT spectrum shown? Please explain how you came to this conclusion based on the spectrum of the compound shown. (**4 points**)

b).   The signal at ~132 ppm in the ^{13}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**)

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}C spectrum of citronellol? (**6 points**)

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

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**)

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**)

f). The signal at ~77 ppm in the ^{13}C spectrum is due to the natural abundance ^{13}C in $CDCl_3$. It appears as a triplet, where all of the peaks are of equal intensity. Explain why the signal appears in the ^{13}C spectrum but not in the DEPT spectrum. (**4 points**)

g). The acronym DEPT in NMR stands for <u>D</u>istortionless <u>E</u>nhancement by <u>P</u>olarization <u>T</u>ransfer. What is meant by "Distortionless"? You will have to explain for credit. (**2 points**)

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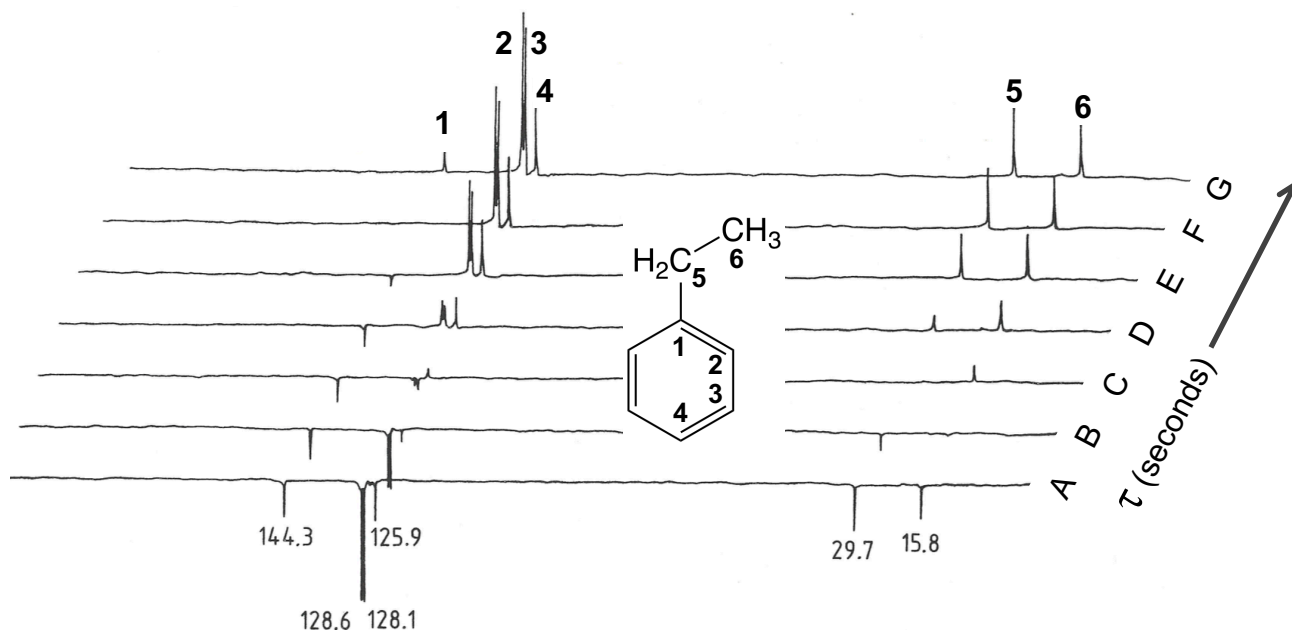

a). Order the nuclei (1 through 6) from smallest to largest value of the longitudinal relaxation time constant T_1. You will have to provide a detailed explanation, based on the data shown, for credit. **(6 points)**

b). Provide explanations/rationalizations for the relaxation times for each of the individual ^{13}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**)

c). For the nucleus at position 5, the T_1 value has been measured to be 15 s. What is the value of the τ 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**)

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° 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 ν_a and ν_b (ν_a faster than ν_b, both faster than the reference frequency). During the first τ 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° x 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 τ period, for each of the nuclei, the component vectors move together by the same amount they moved apart during the first τ period and are refocused. So, at point 'e', they are all refocused along the –y axis.
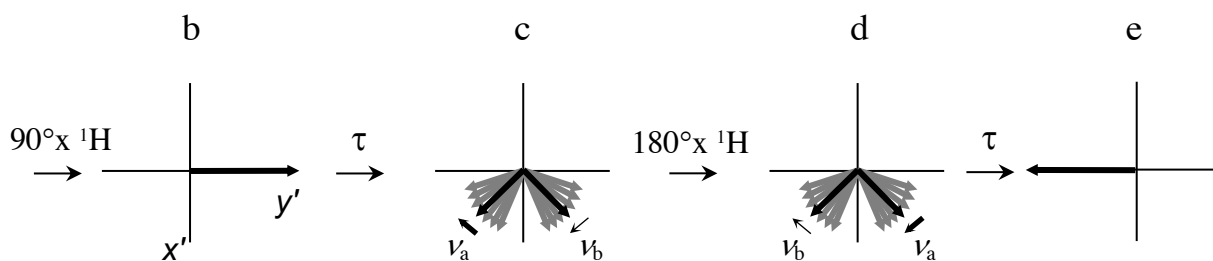
a). The τ–180–τ element, the spin-echo element, is a key element in Carr-Purcell experiment. Please explain in detail why this is, based on the vector diagrams in 'c', 'd', and 'e'. (**6 points**)

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**).

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**)

9). The diagram (right) shows the INEPT pulse sequence. Consider the effect of this pulse sequence on chemical shift evolution and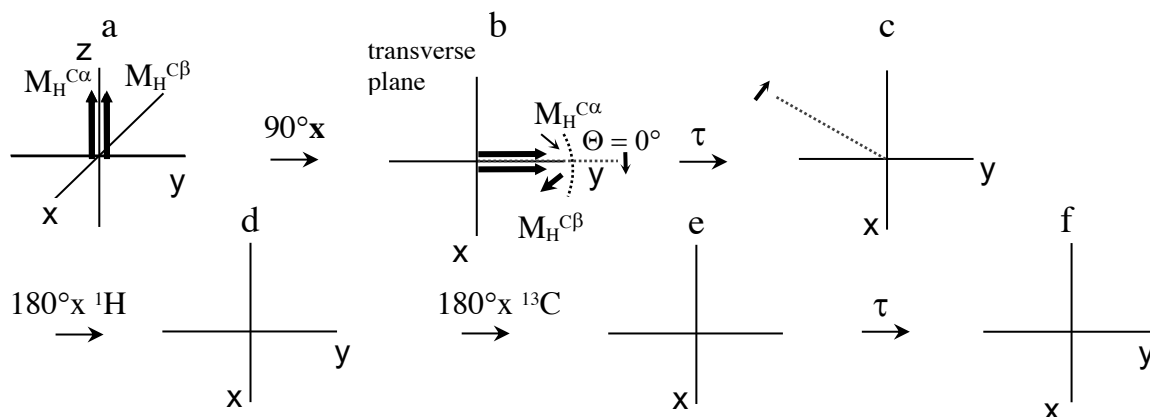 heteronuclear ($-^{13}C-^{1}H$) J coupling, *ignoring the effects of magnetic field inhomogeneity*. We will consider a simple $^{1}H-^{13}C-$ spin system (i.e. $^{13}CHCl_3$). The Larmor frequency is faster than reference frequency, $\nu_H > \nu_{rf}$, as indicated in 'c' below. We will assume that the delay τ is equal to $1/(4J_{CH})$.

90x   180x   90y

^{1}H τ   τ

a b   c d   f g
       180x   90x

^{13}C 180x

e   g'   h
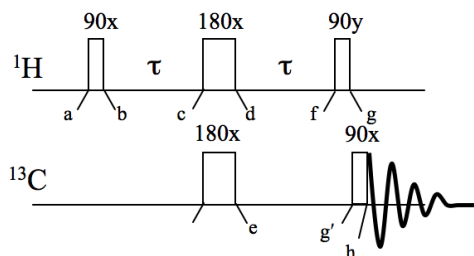
a). Complete the vector diagrams below for points 'c', 'd', 'e', and 'f' in the pulse sequence. The dashed line in 'b' indicates the Larmor frequency, and the position of the Larmor frequency following the first τ period is indicated by the dashed line in 'c'. The $M_H^{C\beta}$ component vector moves faster than the Larmor frequency, and the $M_H^{C\alpha}$ vector moves slower, as indicated by the sizes of the arrows in 'b'. Be sure to label the vectors ($M_H^{C\alpha}$, $M_H^{C\beta}$), to include arrows indicating the direction of precession for the vectors in the rotating frame, and to indicate the angle (Θ) between the vectors at each point. (**8 points**)

a

z

$M_H^{C\alpha}$ $M_H^{C\beta}$

y

x

90°**x**

b

transverse plane

$M_H^{C\alpha}$

$\Theta = 0°$ τ

y

$M_H^{C\beta}$

x

c

y

x

d

180°x ^{1}H

y

x

e

180°x ^{13}C

y

x

f

τ

y

x

b). At point 'f' in the pulse sequence, a ^{1}H 90° 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 ($M_H^{C\alpha}$, $M_H^{C\beta}$). Then, discuss the significance of this result for the INEPT method. (**6 points**)

g

z

y

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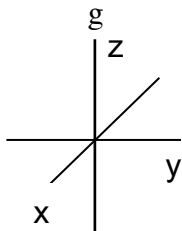
c). Which of the hydrogen transitions, H_1 (N_2-N_4) or H_2 (N_1-N_3) corresponds to $M_H{}^{C\alpha}$? (**2 points**)

d). For the INEPT pulse sequence, the delay τ 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 τ is to $2/(4J_{CH})$. Then discuss whether this value of τ is suitable for INEPT. (**8 points**)

c

y

x

d

180°x ^{1}H

y

x

e

180°x ^{13}C

y

x

τ

f

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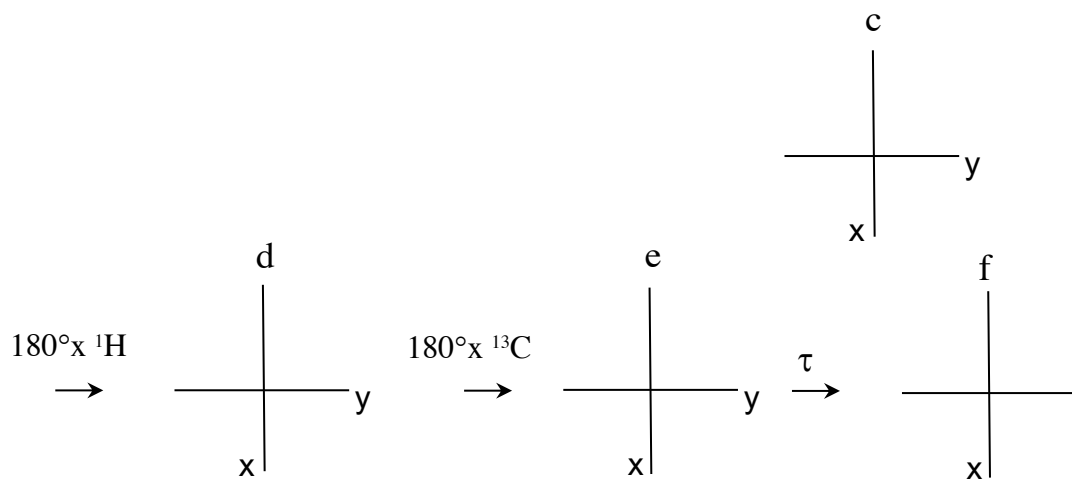
e). Given the results above, are there any multiples of $1/(4J)$ that τ could be set to that would work properly for INEPT? You will have to explain for credit. (**4 points**)

f). If there are multiples of $1/(4J)$ that τ 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**)

g). In this question we considered a simple ^1H-^{13}C- spin (-CH) system (i.e. $^{13}CHCl_3$). If τ is set to $1/(4J)$, the INEPT sequence functions properly for -CH groups. What should the value of τ be set to for -CH$_2$ groups? You will have to explain for credit. You can use any diagrams that you may need for the explanation. (**6 points**)

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 ^1H 180°x pulse, and 'e/f' is, subsequently, following the ^{13}C 180°x pulse). (**8 points**)

i). Based on your answer to part 'e', show the vector diagram at g' (just before the ^{13}C 90°x pulse) for the ^{13}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 ΔC and not ΔH. You will have to define the relationship between ΔH and ΔC. (**4 points**)

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}C nucleus with three attached protons (i.e. $^{13}CH_3Cl$) with the Larmor frequency equal to the reference frequency, $\nu_c = \nu_{rf}$. Complete the vector diagrams for points 'c'-'e' to show the effect of the first (top) sequence on this spin system with $\tau = 1/(4J_{CH})$, $\tau = 1/(2J_{CH})$, $\tau = 3/(4J_{CH})$, and $\tau = 1/(J_{CH})$. Assume point 'd' is just after the 180° 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)**

^1H — broadband decoupling

^{13}C : 90°x — τ — 180°x — τ
a b   c   d   e

^1H — broadband decoupling

^{13}C : 90°x — τ — 180°x — τ
a b   c   d   e

'a'

M_0 (z axis) $\equiv$ 'a' (multiple vectors along z)

'b' transverse plane

90°x →

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

transverse plane 'c'   'd'   'e'   first pulse sequence   second pulse sequence

$\tau = 1/(4J_{CH})$ →   180°x → τ →   FT →

$\tau = 1/(2J_{CH})$ →   180°x → τ →   FT →

$\tau = 3/(4J_{CH})$ →   180°x → τ →   FT →

$\tau = 1/(J_{CH})$ →   180°x → τ →   FT →
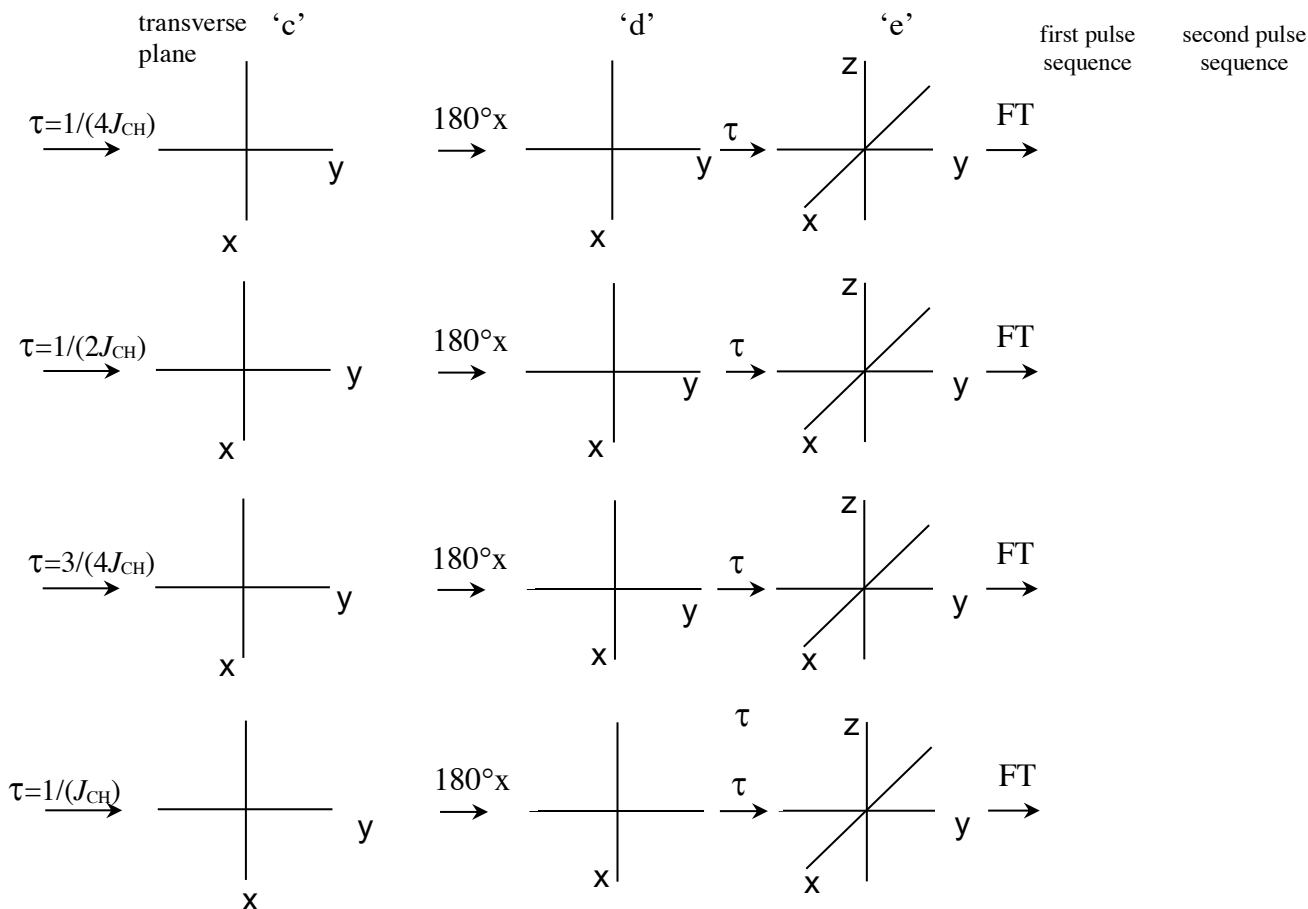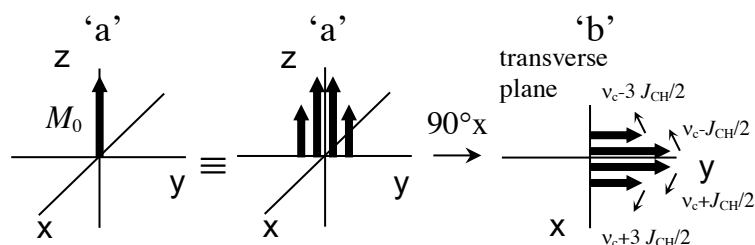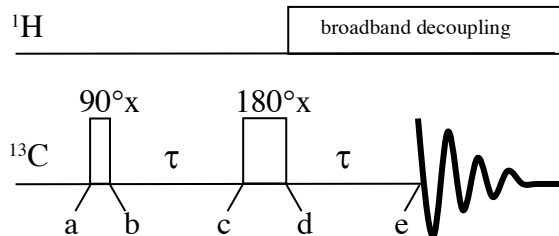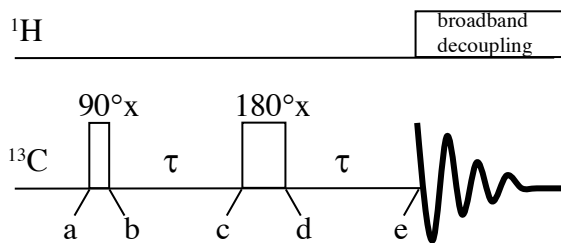
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You may find some of the information below useful:

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 γ[a] $[10^7$ rad $T^{-1} s^{-1}]$	NMR frequency [MHz][b] ($B_0 = 2.3488$ T)
^1H	1/2	–	99.985	1.00	26.7519	100.0
^2H	1	2.87 x 10^{-3}	0.015	9.65 x 10^3	4.1066	15.351
^3H[c]	1/2	–	–	1.21	28.5350	106.664
^6Li	1	–6.4 x 10^{-4}	7.42	8.5 x 10^{-3}	3.9371	14.716
^{10}B	3	8.5 x 10^{-2}	19.58	1.99 x 10^{-2}	2.8747	10.746
^{11}B	3/2	4.1 x 10^{-2}	80.42	0.17	8.5847	32.084
^{12}C	0	–	98.9	–	–	–
^{13}C	1/2	–	1.108	1.59 x 10^{-2}	6.7283	25.144
^{14}N	1	1.67 x 10^{-2}	99.63	1.01 x 10^{-3}	1.9338	7.224
^{15}N	1/2	–	0.37	1.04 x 10^{-3}	–2.7126	10.133
^{16}O	0	–	99.96	–	–	–
^{17}O	5/2	–2.6 x 10^{-2}	0.037	2.91 x 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 x 10^{-2}	7.0704	26.451
^{25}Mg	5/2	0.22	10.13	2.67 x 10^{-3}	–1.6389	6.1195
^{29}Si	1/2	–	4.70	7.84 x 10^{-3}	–5.3190	19.865
^{31}P	1/2	–	100	6.63 x 10^{-2}	10.8394	40.481
^{39}K	3/2	5.5 x 10^{-2}	93.1	5.08 x 10^{-4}	1.2499	4.667
^{43}Ca	7/2	–5.0 x 10^{-2}	0.145	6.40 x 10^{-3}	–1.8028	6.728
^{57}Fe	1/2	–	2.19	3.37 x 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 x 10^{-2}	–10.0318	37.272
^{133}Cs	7/2	–3.0 x 10^{-3}	100	4.74 x 10^{-2}	3.5339	13.117
^{195}Pt	1/2	–	33.8	9.94 x 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

$\gamma_{1H} = 26.7519$ x 10^7 rad/T/s, $I = 1/2$ $\gamma_{13C} = 6.7283$ x 10^7 rad/T/s, $I = 1/2$
$\gamma_{10B} = 2.8747$ x 10^7 rad/T/s, $I = 3$ $\gamma_{15N} = -2.7126$ x 10^7 rad/T/s, $I = 1/2$
$\gamma_{11B} = 8.5847$ x 10^7 rad/T/s, $I = 3/2$ $\gamma_{17O} = -3.6280$ x 10^7 rad/T/s, $I = 5/2$

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

$\pi/2$ radians = 90° $M_0 = \dfrac{N\gamma^2\hbar^2 B_0 I(I+1)}{3k_B T}$

$B_2 = \dfrac{\Delta v\sqrt{J^2 - J_r^2}}{J_r} = \dfrac{J\Delta v}{J_r}$

$\varepsilon = \dfrac{dM}{dt} = \gamma M_0 B_0 = \dfrac{N\gamma^3\hbar^2 B_0^2 I(I+1)}{3k_B T}$ $\Delta v = v_{BS} - v_0 = \dfrac{B_2^2}{2(\Delta B)} = \dfrac{B_2^2}{2(v_0 - v_i)}$

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

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

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

$\Delta E = \mu_z B_0 = \gamma\hbar B_0 = hv_L = hv_1$

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

$\omega_0 = \gamma B_0$

$\Delta\delta = \dfrac{\Delta v}{\text{observe frequency}} \times 10^6$

$M_y = M_0 e^{-t/T_2^*}$
$M_z = M_0(1 - e^{-t/T_1})$
$M_z = M_0(1 - 2e^{-t/T_1})$

$\Delta v_{1/2} = \dfrac{1}{\pi T_2^*}$

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

$t_{zero} = T_1\ln(2)$
$\eta = \gamma_a/(2\gamma_x)$
$I = (1 + \eta) I_0$
$I \propto 1/r^6$

$\Theta = \gamma B_1 \tau_p$
$\dfrac{N_\beta}{N_\alpha} \approx 1 - \left(\dfrac{\gamma\hbar B_0}{k_B T}\right)$
$B_{eff} = B_0(1-\sigma)$

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

SW=1/(2DW)=Nyquist frequency (v_{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°

$\Delta v \gg J$
1+ γ_A/γ_X 1-γ_A/γ_X
multiplicity=$2nI + 1$
$\Theta = 2\pi J\tau$