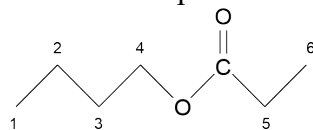**Exam 4: CHEM/BCMB 4190/6190/8189 (152 points) Tuesday, 23 November, 2021**

1). The ^{1}H-^{1}H COSY spectrum for butyl propionate is shown. The spectrum shows six diagonal signals (3.8, 2.1, 1.3, 1.1, 0.9 and 0.7 ppm) and a number of crosspeaks.


a. Please provide chemical shift assignments for the hydrogens in butyl propionate. You will have to provide a detailed justification for credit. You should begin by defining how many different ^{1}H signals you would expect to see in the spectrum, with an explanation of your rationale. You must detail your justification for your assignments for credit. (**8 points**)
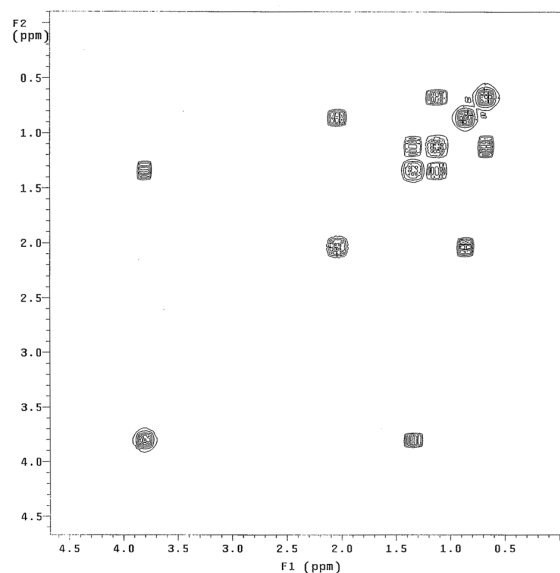
b. On the COSY spectrum of butyl propionate shown (below, right), draw additional signals/crosspeaks that you would expect to observe in a TOCSY spectrum of butyl propionate. You will have to explain your reasoning to receive credit. (**6 points**)
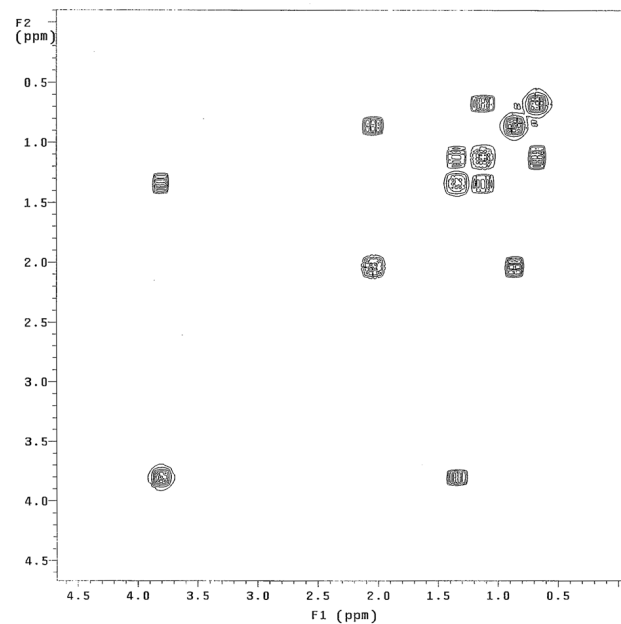
2). Draw the ^{1}H NMR signal you would observe in a simple one-dimensional ^{1}H NMR spectrum for the hydrogen on the carbon bearing the hydroxyl group in the molecule shown. For credit you will have to explain the multiplet structure, justify why it appears as it does, and you will have to indicate and justify distances between peaks in the signal, estimated coupling constants, and relative peak intensities. Assume that no 4-bond, 5-bond, or more-bond couplings contribute to the splitting of the signal. (**8 points**)
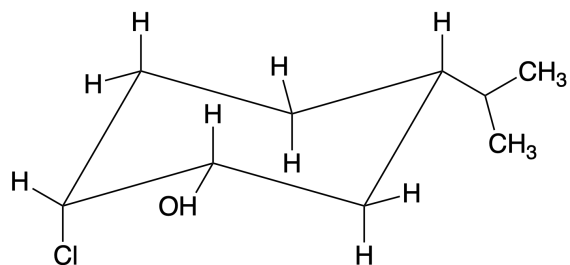
3). The pulse sequence for a modified HETCOR pulse sequence is shown (right). Delays Δ_1 and Δ_2 have been added, as has a 180° pulse.
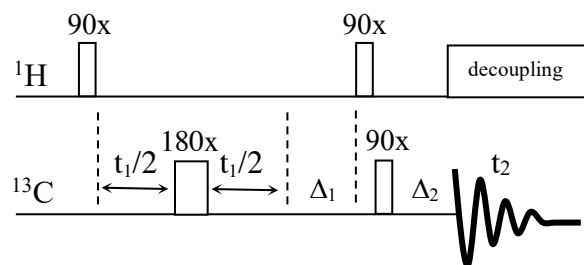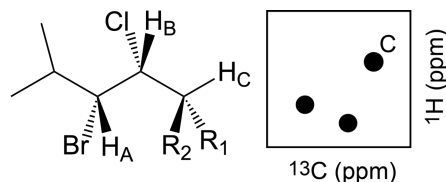

a). Explain the purpose of the 180° pulse and how it accomplishes this purpose. (**4 points**)

b). Explain the purpose of the Δ_1 delay and how it accomplishes this purpose. Your explanation must include what the proper value of Δ_1 should be. You may want to include vector diagrams to assist in your explanation. (**4 points**)

c). Explain the purpose of the Δ_2 delay and how it accomplishes this purpose. Your explanation must include what the proper value of Δ_2 should be. You may want to include vector diagrams to assist in your explanation. (**4 points**)

d). The HETCOR experiment shown above was used to acquire the HETCOR spectrum of the molecule shown (right). The region of the spectrum showing the correlations from H_A, H_B, and H_C is shown. It is known which signal belongs to H_C, but it is not known which of the remaining two signals belongs to H_A and which belongs to H_B. If the delays Δ_1 and Δ_2 in the HETCOR experiment are set to 50 ms instead of the usual value, it is possible to show which signal is from H_A, and which is from H_B. Please explain in detail how this is accomplished. (**10 points**)

4). The COSY spectrum for menthol is shown (next page), along with the 1D ^1H spectrum of menthol, the structure of menthol and the correct ^1H assignments. Circled in the COSY spectrum is a crosspeak (and the symmetry related crosspeak) that remains unassigned. Scheme IV (next page) shows coupling constants for the indicated ^1H pairs in compounds 15-18.

a). Please give a concise explanation of Scheme IV. You must explain what point this figure/scheme is trying to make, AND, you must explain what this has to do with COSY. (**6 points**)

b. Assign the circled crosspeak(s). For credit you must clearly and completely justify your assignment(s). (**6 points**)

Scheme IV

+3 to +4
15

+1.1
16

+0.9
17

+1.2
18

solvent

7
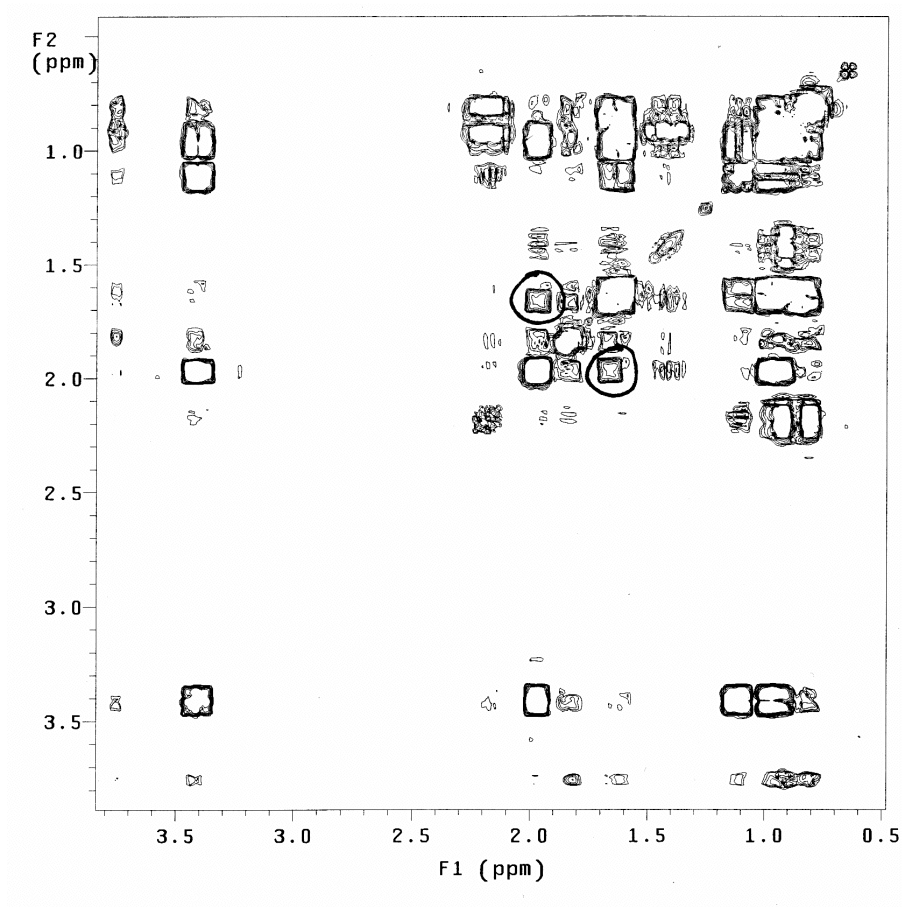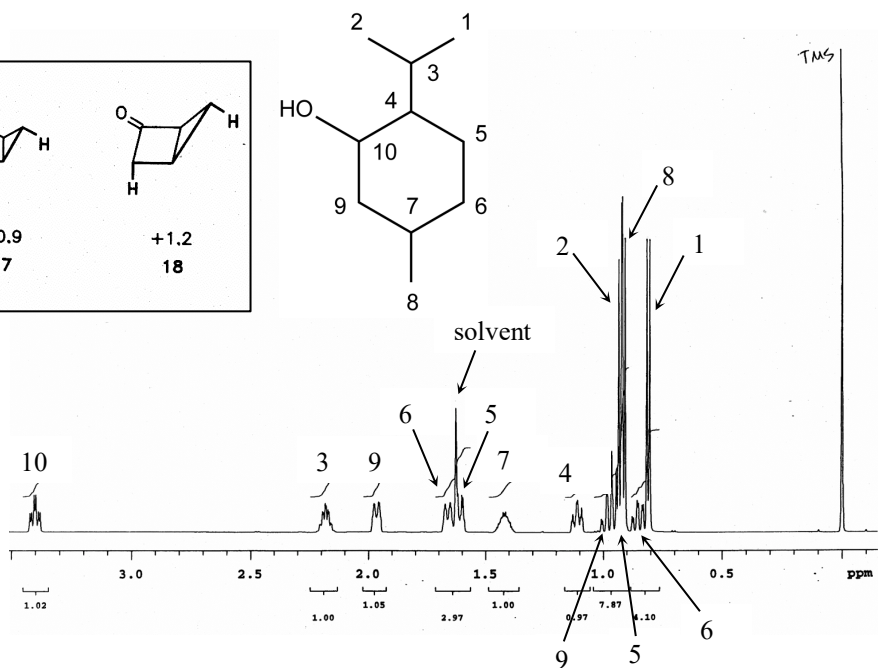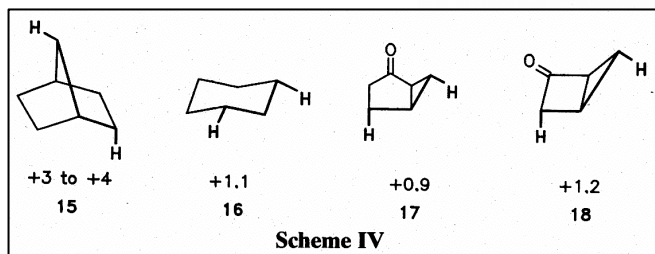
5). In menthol, the distance between proton 10 and proton 5a (axial) is 2.48 Å, whereas the distance between proton 10 and proton 5e (equatorial) is 3.65 Å. In a NOESY experiment, if the intensity of the crosspeak between 10 and 5a is 100 (arbitrary units), what would be the expected intensity for the crosspeak between 10 and 5e? You will have to show your work for credit. (**6 points**)
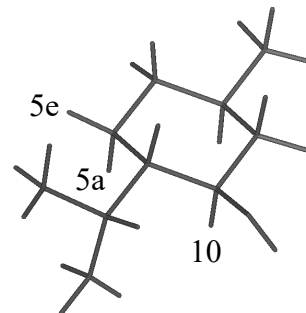
6). You synthesized the phosphonate compound (right) in your laboratory using routine methods. You are collecting ^{13}C NMR spectra on this compound and would like to remove the ^{13}C-^{31}P coupling (splitting) by performing broadband ^{31}P decoupling. You also realize that there will be an enhancement in S/N (signal-to-noise) due to the ^{13}C-^{31}P NOE.
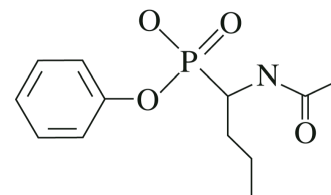
a. Calculate the percentage increase in S/N due to the ^{13}C-^{31}P NOE (for the ^{13}C nucleus bound to the phosphorous atom) when applying broadband ^{31}P decoupling versus no decoupling. You will have to show your work for credit (**4 points**)

b. Without any decoupling, what will the signal from the carbon bonded to the phosphorous atom look like in a normal 1D, ^{13}C spectrum? Draw the signal and explain the multiplet structure (assume there is no splitting due to the attached nitrogen nucleus). (**4 points**)

c. In a normal, 1D, ^{31}P spectrum (no decoupling), what will the signal from the ^{31}P nucleus look like? Draw the signal and explain the multiplet structure. (**6 points**)

d. Is ^{31}P a 'good' NMR nucleus? Explain in detail. (**4 points**)

7). Consider the populations N_1, N_2, N_3 and N_4 of the $\alpha\alpha$, $\alpha\beta$, $\beta\alpha$ and $\beta\beta$ states, respectively, for a ^{1}H-^{1}H spin system (spins 'A' and 'X') without J coupling (no coupling between A and X). The energy diagram for this system is depicted (right), where the A's represent the transitions of one of the ^{1}H nuclei, and the X's represent the transitions of the other ^{1}H nucleus. We will define ΔH as the difference in the number of spins in α and β states for A, and ΔH will also represent the difference in the number of spins in α and β states for X.

$$\underline{\beta\beta}\ N_4 \quad X$$

$$A \qquad \underline{\beta\alpha}\ N_3$$

$$\underline{\alpha\beta}\ \overline{}\ N_2 \qquad\qquad A$$

$$X \qquad A \nearrow \underline{\alpha\alpha} \nwarrow X \quad N_1$$
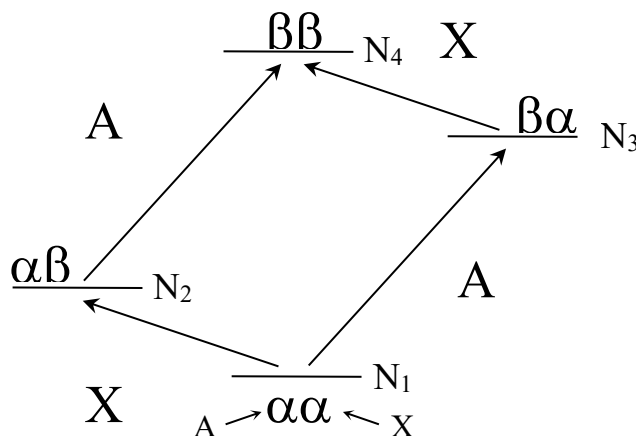
a. Write down the equilibrium values for N_1-N_4 and the equilibrium population differences for the A and X transitions (please show how you calculate the A and X transition population differences). Assume that $N_4 = N$. **(6 points)**

$N_4 = N$
$N_3 =$ $A =$
$N_2 =$ $X =$
$N_1 =$

b. On the energy diagram above, draw dashed lines showing the W_0 (zero quantum) and W_2 (double quantum) relaxation pathways. Make sure to clearly, correctly and unambiguously label each pathway. **(4 points)**

c. (fill in the blanks) The W_0 and W_2 pathways do not operate with equal efficiencies for all molecules. For large molecules, the _____ pathway is more efficient, whereas for small molecules the __-__ pathway is more efficient. **(2 points)**

d. For a particular medium-sized molecule, the efficiencies of the W_0 and W_2 pathways for relaxation are identical. If we perform a simple NOE experiment by selective saturation of both of the A transitions, what are the new values for N_1-N_4 and the population differences for the A and X transitions after saturation but without considering any relaxation? Then, what are the resulting values for N_1-N_4 and the population differences for the X transitions including relaxation via W_0 and W_2 pathways (assume that the number of spins (δ) relaxing via the W_0 pathway is equal to the number relaxing through the W_2 pathway)? (**12 points**)

e. Explain your result to question 'd' in terms of medium-sized molecules in general. (**6 points**)

8). The HSQC pulse sequence is shown below.
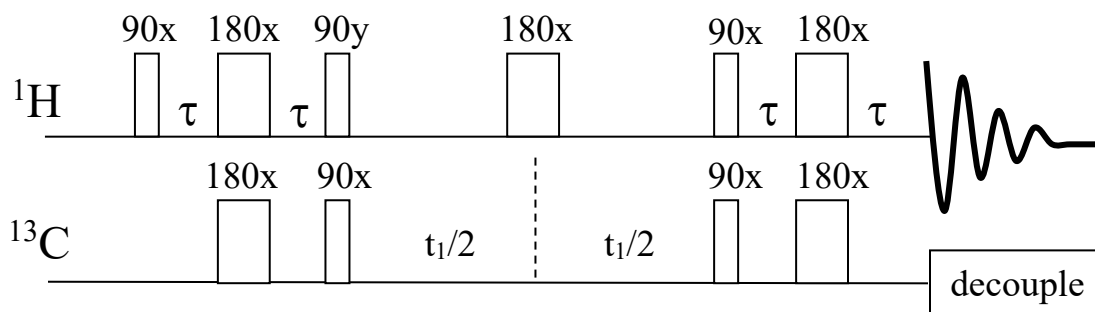
a. The magnetization vectors just after the second 90° ^{13}C pulse are shown below. Complete the vector diagrams for the remaining steps in the HSQC sequence (assume a simple ^{1}H-^{13}CCl$_3$ system and a τ of $1/(4J_{CH})$). Make sure to label properly the vectors, indicate the directions that the vectors are moving, and indicate important angles between the vectors or the vectors and the axes. (**6 points**)
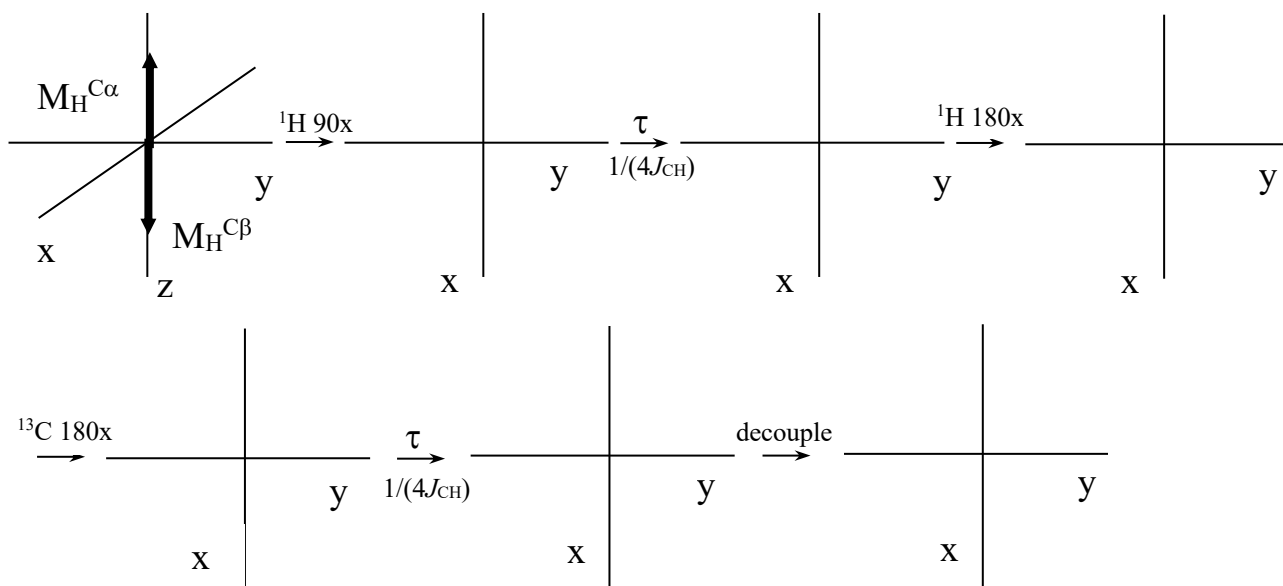
b. For the previous example, a simple ^{1}H-^{13}CCl$_3$ system, a value of $1/(4J_{CH})$ is appropriate for τ in the HSQC experiment. If, instead, you were considering a methylene group (^{13}CH$_2$-Cl$_2$), what would be the appropriate value of τ? Please provide a detailed explanation. (**6 points**)

9). The HSQC pulse sequence is shown below.
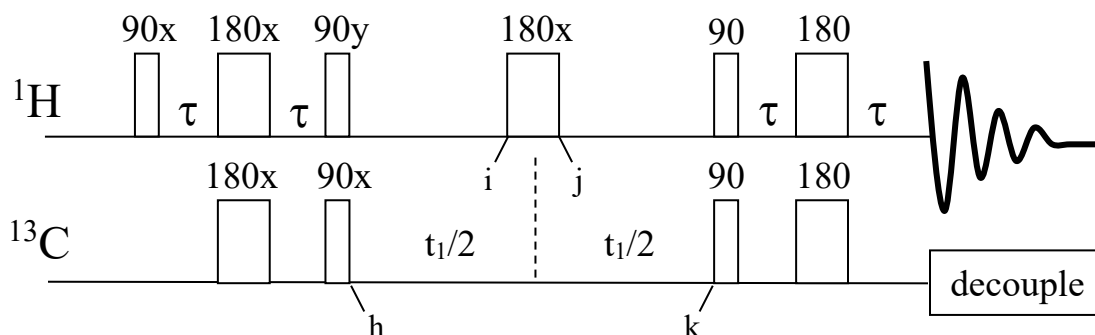
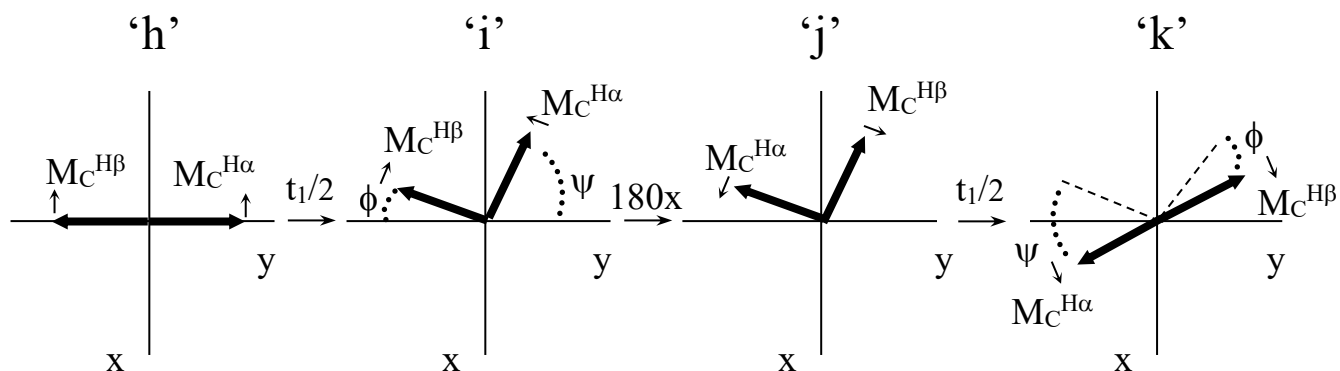The magnetization vectors present at point 'h' in the pulse sequence are shown, as are the vectors at points 'i', 'j', and 'k' for a simple spin system. The vector $M_C^{H\alpha}$ precesses slower than the reference frequency, and $M_C^{H\beta}$ precesses faster than the reference frequency, such that after $t_1/2$, the vectors have moved ψ and ϕ degrees, respectively, away from the y axis.

a. What is the role of the ^1H 180° pulse in the middle of the t_1 evolution period? You should state exactly why the pulse is there, and how it accomplishes its intended task. (**4 points**)

b. As shown <u>above,</u> at point 'k' in the sequence, the two vectors ($M_C{}^{H\alpha}$ and $M_C{}^{H\beta}$) are antiphase with respect to one another (180° out of phase with one another), and this is an essential condition for the pulse sequence to function properly. If we replace the ^1H 180°x pulse in the center of the t_1 evolution period with a ^{13}C 180°x pulse, we get the same result, as shown in the vector diagrams for points 'j' and 'k' below: the ^{13}C magnetization components are 180° out of phase with one another at point 'k'. So, the question is, why can't we replace the ^1H 180°x pulse with a ^{13}C 180°x pulse in the pulse sequence? (**8 points**)

'j'   'k'

$M_C{}^{H\alpha}$

$M_C{}^{H\beta}$

^{13}C 180x $t_1/2$ $M_C{}^{H\beta}$

y $M_C{}^{H\alpha}$ y

x   x
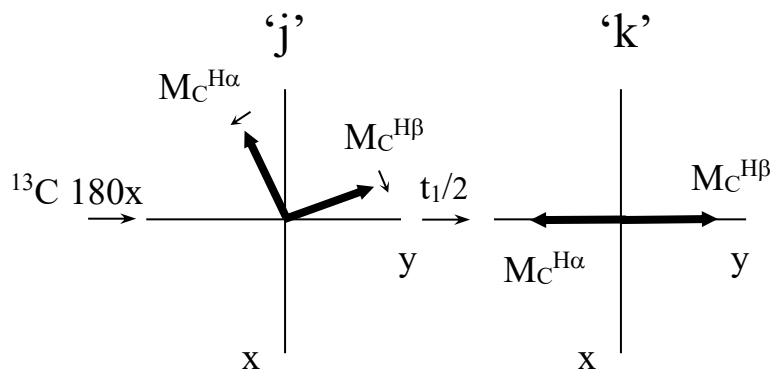
10). The "jump-return" NMR experiment (right) is designed to permit suppression of large signals in 1D spectra, such as those from solvent. It can be used, for instance, to suppress the large signal from H_2O in ^1H NMR spectra of samples prepared in aqueous solutions. This experiment does not provide equal excitation of all regions of the spectrum, so the region of interest is chosen, and then the parameters of the experiment are adjusted to both suppress the solvent signal and excite the signals in the region of interest. First, the carrier or reference frequency is set at the Larmor frequency of the signal (solvent) that is to be suppressed. Then, the delay τ is adjusted to $1/(4\Delta v)$, where Δv is the frequency difference between the center of the region of interest (v_H) and the solvent signal (v_{H2O}). So, $\tau = 1/(4\Delta v) = 1/(4(v_H - v_{H2O}))$.
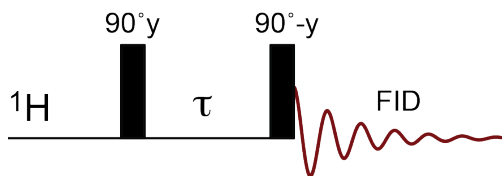
Consider the use of the jump-return experiment for a sample in H_2O using a 600 MHz instrument. The goal is to suppress the H_2O signal at 4.75 ppm with maximal excitation of a ^1H signal at 8.0 ppm.

a). How long, <u>in seconds</u>, should the delay τ be? You must do the appropriate calculations, report an answer, and show your work for credit. (**4 points**)

b). Using vector diagrams, describe what happens during the pulse sequence. Then, provide a detailed explanation of how the pulse sequence suppresses the solvent signal while maximizing the signal of interest. (**8 points**)

c). Assume there is also another ^{1}H nucleus in the sample with a chemical shift of 1.5 ppm. What will the signal of this ^{1}H nucleus look like if the experiment above is performed as described above (with the τ period optimized for the signal at 8.0 ppm and the carrier/referency set at the Larmor frequency of the H_2O signal)? You will have to provide a detailed explanation/justification for credit. (**6 points**)

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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)
^{1}H	1/2	–	99.985	1.00	26.7519	100.0
^{2}H	1	2.87 x 10^{-3}	0.015	9.65 x 10^3	4.1066	15.351
^{3}H[c]	1/2	–	–	1.21	28.5350	106.664
^{6}Li	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)	
	^{1}H	^{13}C
9.4	400	100.6
11.74	500	125.7
14.09	600	150.9
18.79	800	201.2

$\gamma_{1H} = 26.7519$ 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:

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

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

$h = 6.626 \times 10^{-34}$ 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)$^{1/2}$)

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

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

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

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

$\omega_0 = \gamma B_0$

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

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

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

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

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

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

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

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

$I = (1+\eta)I_0$

$I \propto 1/r^6$

$\Theta = \gamma B_1 \tau_p$

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

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

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

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

AQ=DW*TD

DR=2SW/TD=1/AQ

(TD$\equiv$NP)

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

$\pi/2$ radians $= 90°$

$\Delta v \gg J$

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

multiplicity=$2nI + 1$

$\Theta = 2\pi J\tau$