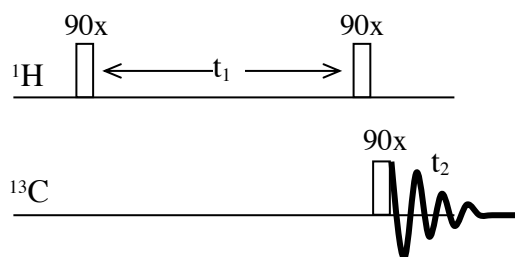
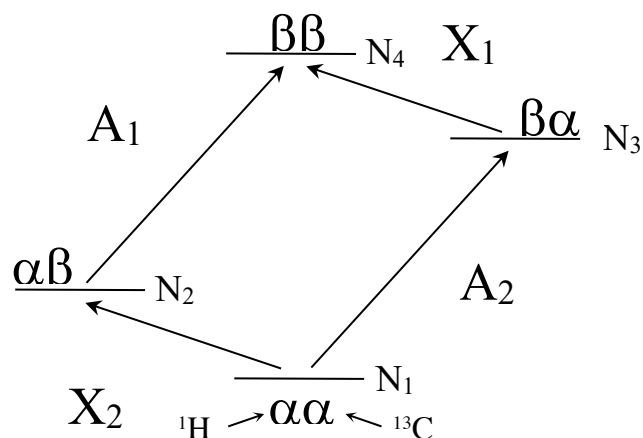
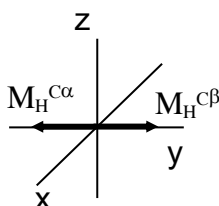


Exam 4: CHEM/BCMB 4190/6190/8189 (154 points) Tuesday, 24 November, 2020

1). Consider the HETCOR pulse sequence, and the energy diagram for the simple ^1H - ^{13}C spin system (i.e. CHCl_3).



Just *before* the second ^1H 90° pulse, for a particular t_1 value, the $M_{\text{H}}^{\text{C}\alpha}$ and $M_{\text{H}}^{\text{C}\beta}$ vectors happen to be positioned along the $-y$ and y axes, respectively.



a. Define the populations for the individual states N_1 - N_4 and the population differences for the A_1 , A_2 , X_1 and X_2 transitions *both before* the beginning of the pulse sequence and *after* the second ^1H 90° pulse (assuming the particular value of t_1 mentioned above, and no relaxation losses during t_1). Assume that $N_4 = N$ at equilibrium, and ΔH is the difference in the number of spins in α and β states for ^1H , and ΔX is the difference in the number of spins in α and β states for ^{13}C . Also, define the relationship between ΔH and ΔX . **(8 points)**

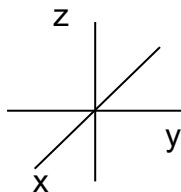
before pulse sequence

$N_4 = N$
 $N_3 =$
 $N_2 =$
 $N_1 =$
 $A_1 =$
 $A_2 =$
 $X_1 =$
 $X_2 =$

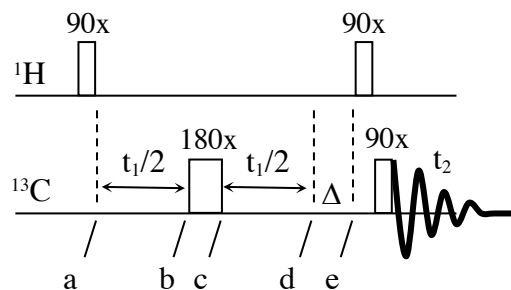
after second ^1H pulse

$N_4 =$
 $N_3 =$
 $N_2 =$
 $N_1 =$
 $A_1 =$
 $A_2 =$
 $X_1 =$
 $X_2 =$

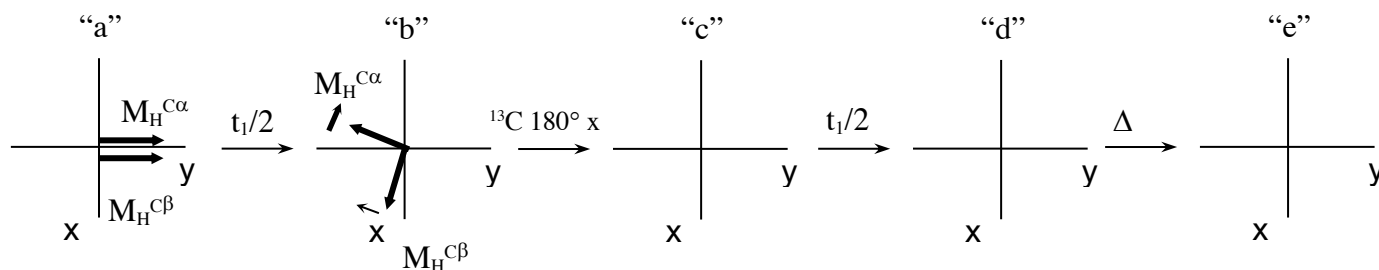
b. Draw the vectors $M_C^{H\alpha}$ and $M_C^{H\beta}$ that are present just before the ^{13}C pulse. Make sure to label each with the correct name ($M_C^{H\alpha}$ or $M_C^{H\beta}$) and with the proper magnitude (expressed in terms of ΔX). (4 points)



c. In the modified HETCOR experiment (right) an additional pulse (^{13}C 180° x) and an additional delay (Δ) have been added in order to remove the $^1\text{J}_{\text{CH}}$ splitting in the t_1 dimension (decouple). For the simple ^1H - ^{13}C spin system (i.e. CHCl_3), at point “a” in the sequence, the $M_H^{C\alpha}$ and $M_H^{C\beta}$ vectors are positioned as shown below (in the transverse plane). For an arbitrary value of $t_1/2$, the vectors have moved into the positions shown in “b”.

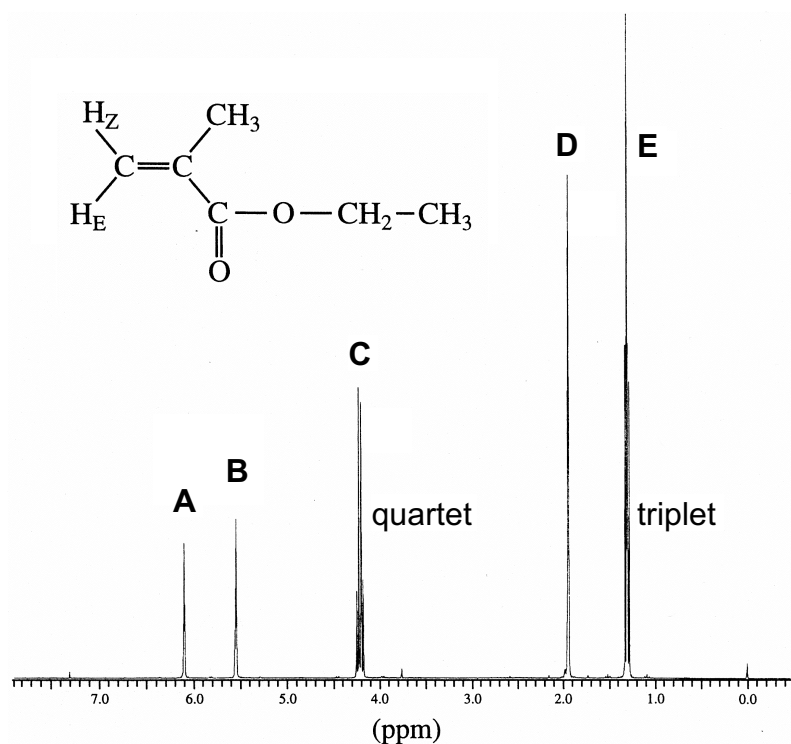


Show the results at points “c” and “d”. Then explain why decoupling is achieved. (8 points)



d. The delay Δ has to be set appropriately in order for the pulse sequence to operate properly. What value should Δ be set to? Please explain your answer both by showing the results at “e” and by explaining why the value of Δ that you selected is appropriate. (6 points)

2). Shown (below, right) are the chemical structure and 1D, ^1H NMR spectrum of ethylmethacrylate. The five individual signals in the spectrum are labeled **A-E**.



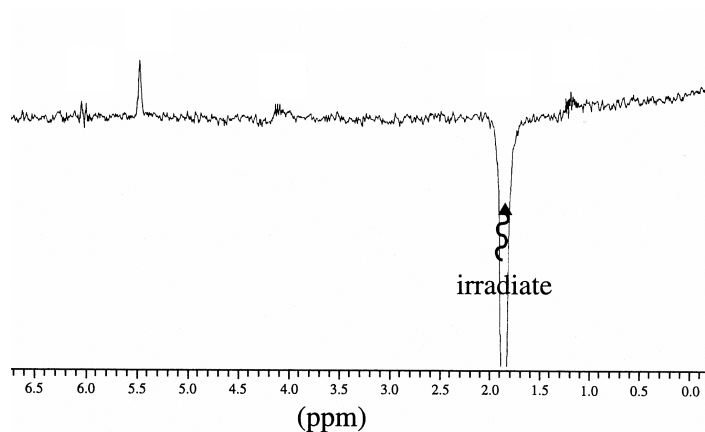
a. Assign each of the five signals (A, B, C, D, and E) in the 1D, ^1H NMR spectrum of ethylmethacrylate to a hydrogen or hydrogens in the molecule. Justify (explain) each of your assignments. In your justifications make sure to include signal multiplicity. (6 points)

b. Shown (right) is the result of a 1D selective difference NOE experiment on ethylmethacrylate. There are five observable signals in this spectrum (6.1, 5.55, 4.2, 1.95, and 1.3 ppm).

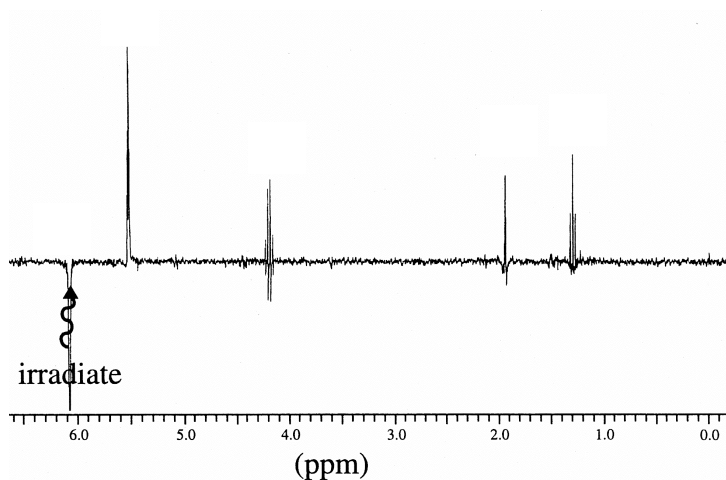
-Explain how a 1D selective difference NOE experiment works.

-Explain in detail the relative magnitudes and signs of these signals in the spectrum shown.

-Explain in detail why the results of this experiment are consistent with the assignments that you made (part 'a'), or why they are not. **(10 points)**



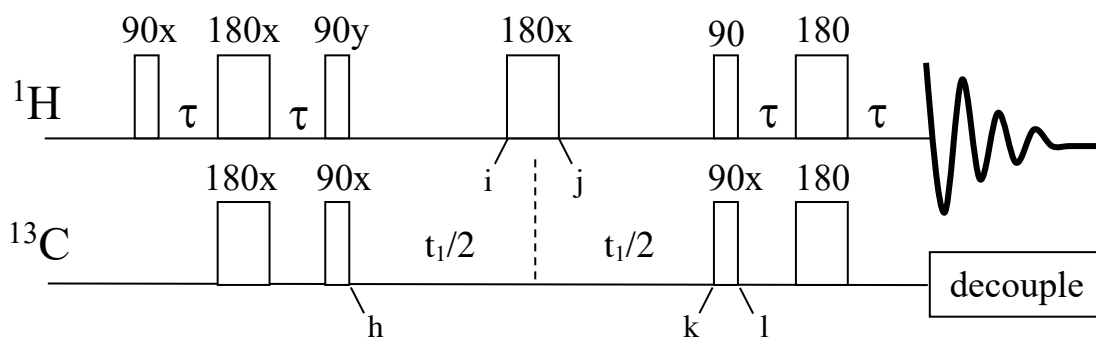
c. Shown (right) is the result of a second selective difference NOE experiment on ethylmethacrylate. Are the results consistent with the results of the experiment presented in 'b' above? Are they consistent with your assignments from 'a'? Please explain. **(8 points)**



3). In a particular molecule, the distance between H_a and H_b is known to be 3.92 angstroms. In a NOESY spectrum, the intensity of the crosspeak between these hydrogens is 2827 (arbitrary units). In the same NOESY spectrum, the intensity of the crosspeak between H_c and H_d in the same molecule is 13627. What is the distance between H_c and H_d ? **(6 points)**

4). In a simple 1D, 1H experiment, the intensity of the signal from H_E is 7.53 (arbitrary units). If we now saturate the signal from H_Z , what would be the signal intensity of H_E if we assume the maximum possible (distance independent) NOE enhancement is produced? **(6 points)**

5). The pulse sequence for the HSQC experiment is shown below.

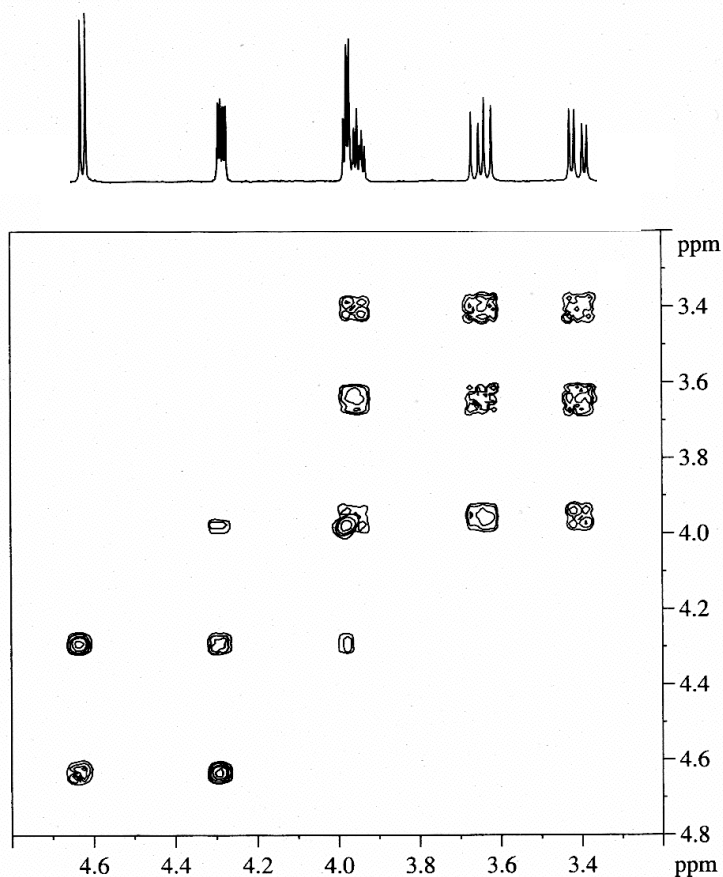
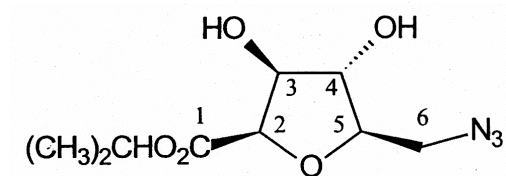


a. At the end of the final τ period (just before acquisition and decoupling begin), sketch what the vectors $M_H^{C\alpha}$ and $M_H^{C\beta}$ look like for a simple $-CH$ group. Use an appropriate system of axes, and label the vectors. Also sketch the vectors for a different $-CH$ group that has a Larmor frequency that is different than the Larmor frequency of the first. Provide any necessary explanation/justification. **(4 points)**

b. Explain why it is necessary for the vectors that you have drawn in 'a' to be positioned the way that you have drawn them. **(4 points)**

6). Below is a region of the 2D ^1H , ^1H -COSY spectrum for the azo-sugar whose structure is also shown below. This region shows diagonal and cross peaks for hydrogens on carbons 2-6. Above the COSY spectrum is shown the 1D ^1H spectrum.

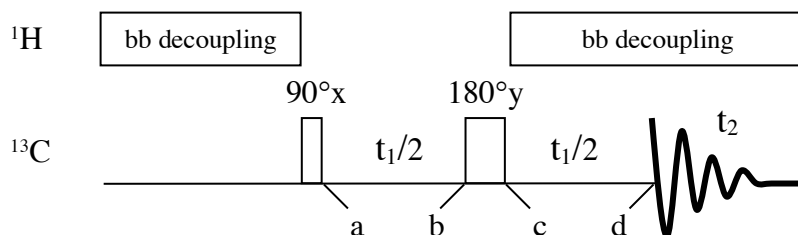
a. Label correctly the diagonal peaks (for instance “3,3” to indicate the diagonal peak for the proton on carbon 3), and label correctly the crosspeaks on one side of the diagonal (for instance, “5,2” for a crosspeak resulting from protons on carbons 5 and 2). Note that the methylene hydrogens at 6 are NOT equivalent. The 1D spectrum is shown above the COSY spectrum. Use the numbering as shown on the molecular structure below. (10 points).



b. On the other side of the diagonal, draw additional crosspeaks that you might expect to be observed in a TOCSY spectrum. Label these additional peaks appropriately. Also, what does TOCSY stand for? (8 points)

7). Shown (right) is the pulse sequence for ^1H heteronuclear two-dimensional J -resolved experiment.

a). What parameter is measured in the directly detected dimension? (**2 points**)

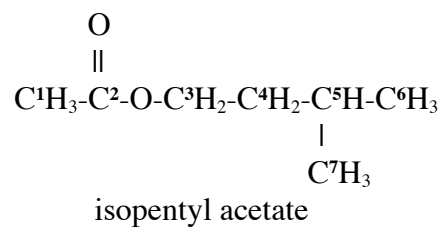


b). What parameter is measured in the indirectly detected dimension? (**2 points**)

c). What is the role of the ^{13}C 180°Y pulse? Why is it necessary (or is it?) to include this pulse? For full credit you will have to use vector diagrams to illustrate your point(s) and provide a complete but concise explanation. (**10 points**)

8). The ^{13}C and ^1H chemical shifts and $^1J_{\text{CH}}$ coupling constants for isopentyl acetate are shown below:

carbon	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm)	$^1J_{\text{CH}}$ (Hz)
$-\text{C}^1\text{H}_3$	21	2.1	120
$-\text{C}^2$	171	-	-
$-\text{C}^3\text{H}_2$	63	4.1	130
$-\text{C}^4\text{H}_2$	37	1.5	130
$-\text{C}^5\text{H}$	25	1.7	140
$-\text{C}^6\text{H}_3$	23	0.9	120
$-\text{C}^7\text{H}_3$	23	0.9	120



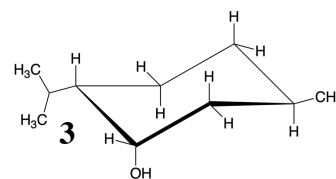
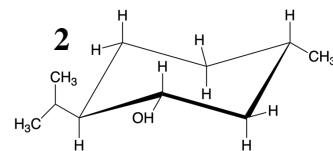
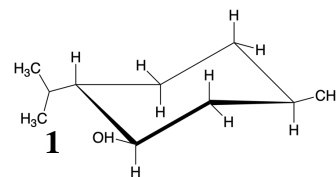
Draw the heteronuclear two-dimensional J -resolved spectrum of isopentyl acetate. Label the axes properly, and indicate which is F_1 (indirectly detected dimension) and which is F_2 (directly detected dimension). **(10 points)**

9). Draw the heteronuclear two-dimensional ^1H , ^{13}C -HSQC spectrum of isopentyl acetate (see question 8). Label the axes properly, and indicate which is F_1 (indirectly detected dimension) and which is F_2 (directly detected dimension). **(10 points)**

10). Draw the two-dimensional ^1H , ^1H -COSY spectrum of isopentyl acetate (see question 8). Label the axes properly, and indicate which is F_1 (indirectly detected dimension) and which is F_2 (directly detected dimension). State clearly any assumptions you make regarding which cross peaks appear. **(10 points)**

10). Shown (right) are three isomers of menthol ('1' is (-)-menthol, '2' is (+)-menthol, '3' is (+)-neomenthol). For separate samples of each isomer, simple one-dimensional ^1H NMR spectra are collected under identical conditions.

a. Using only the simple one-dimensional ^1H NMR spectra, describe how you would unambiguously distinguish between the sample containing (-)-menthol ('1') and the sample containing (+)-menthol ('2'). (6 points)



b. If you compare the simple one-dimensional ^1H NMR spectrum of the sample containing (-)-menthol and the spectrum of the sample containing (+)-neomenthol, which signals would you expect would show the most significant changes. You will have to explain for credit, and your explanation must include the origins of the changes. **(8 points)**

c. Sketch the ^1H NMR signal you would observe in a simple one-dimensional ^1H NMR spectrum for the hydrogen on the carbon bearing the hydroxyl group in (+)-neomenthol. For credit you will have to explain the multiplet structure, justify why it appears as it does, and you will have to indicate distances between peaks in the signal and coupling constants. **(8 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:

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

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

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

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

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

$$P_z = m\hbar$$

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

$$\omega_0 = \gamma B_0$$

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

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

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

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

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

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

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

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

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

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

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

$$AQ = DW * TD$$

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

$$(TD \equiv NP)$$

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

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

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

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

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