

Name: Key

BCMB/CHEM 8190, BIOMOLECULAR NMR
MIDTERM – 03/03/06

Instructions: This is an open book, limited time, exam. You may use notes you have from class and any text book you find useful, but you must bring materials you plan to use with you to the exam; you cannot leave the room to get other materials. You may also use a calculator. You will have 50 minutes to complete the exam. Write your answers and name on the exam; turn it in at the end of the period.

Part I (50 pts). Give short answers to the following:

- 1) Based on the shell model of the nucleus what fundamental particle would you expect to determine the magnetic properties of ^{17}O (atomic number 8)? What is the spin of ^{17}O ? What is the sign of the magnetogyric ratio?

2 neutrons, spin $5/2$, negative. (particle neg, adds to l)

- 2) We have a strong signal -100 Hz from the center of a quadrature spectrum collected with a total spectral width of 1000 Hz. If the two channels in our quadrature receiver amplify to slightly different extents, at what frequency would you expect to see an artifact?

+ 100 Hz

- 3) Assuming the one bond scalar coupling between ^{13}C and ^{15}N in ethylamine ($\text{CH}_3\text{CH}_2\text{-NH}_2$) is approximately 10 Hz. What do you expect one bond scalar coupling between ^{13}C and ^{15}N in acetonitrile ($\text{CH}_3\text{C}\equiv\text{N}$) to be?

$$sp^3 \cdot sp^3 = \frac{1}{4} \cdot \frac{1}{4} = \frac{1}{16}, \quad sp \cdot sp = \frac{1}{2} \cdot \frac{1}{2} = \frac{1}{4}$$

~ 40 Hz. 2

- 4) When using exponential multiplication to improve signal to noise ratios, what is the best line broadening value (π times the reciprocal of the exponential decay constant) to use when natural line widths are about 2 Hz?

2 Hz

- 5) Which do you expect to be more shielded, a proton 3Å above the plane of a carbonyl group in an amide bond or a proton in the plane but on a line perpendicular to the C=O bond and 3Å away?

above the plane

- 6) A 10kDa protein forms a dimer at 1mM concentration and 10°C, but is mostly monomer at 0.2mM concentration. If linewidths are approximately 15 Hz at 1mM, what would they be at 0.2mM? If viscosity of the solution used decreases a factor of 2 for every 20°, what line width would you expect for the 0.2mM solution at 30°?

3
7.5 Hz

$$\tau_c \propto \frac{\eta}{T}$$

decrease by $2 \cdot \frac{303}{283} \approx 2$
 ≈ 3.5 Hz

- 7) In a 2D homonuclear J-resolved spectrum (90-t1/2-180-t1/2-observe) which of the following do you expect to be removed from the indirect dimension, chemical shift offsets, line broadening from a heterogeneous magnetic field, homonuclear scalar couplings, heteronuclear scalar couplings?

all but homonuclear scalar couplings

- 8) We achieve a signal to noise ratio for a peak of interest of 3:1 after collecting 8 scans. What do you expect for the signal to noise ratio after 72 scans?

$$\sqrt{\frac{72}{8}} \cdot 3 : 1 = 9 : 1$$

- 9) We are interested in ^{15}N chemical shifts of amide nitrogens in a protein that has most resonances within a 30 ppm range and want to measure shifts to approximately 0.5 ppm accuracy. We decide to do this with indirect detection using a ^1H - ^{15}N HSQC experiment. How many t1 points (indirect dimension) would you collect?

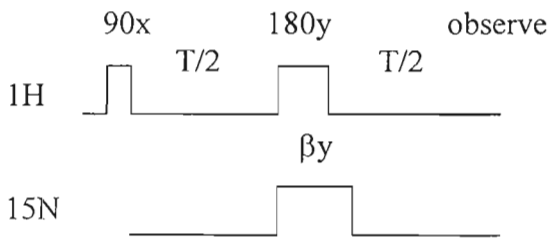
≈ 64 pts (quadrature, complex)

10) In the above experiment, assuming a repetition rate of one per second and a phase cycle length of 8, what is the minimum time for data acquisition? How long would it take to collect a 1D spectrum of similar signal to noise ratio by direct detection of ^{15}N assuming the same repetition rate and complete magnetization recovery between pulses?

3 for quadrature
 $8 \cdot 1 \cdot 64 \cdot 2$
 17 min
 $^{15}\text{N} \approx 1000 \times$
 less sensitive
 assuming 8 scans/FID
 $\approx 8000 \text{ sec}$
 133 min

Part II (25 points) Answer the following showing your work.

The following pulse sequence is frequently used to determine the appropriate pulse width for a 90° pulse on a heteronucleus such as ^{15}N . The two spins are coupled and the time period T is fixed at $1/J$. The ^{15}N pulse length is incremented in successive observations.



a) Using product operators starting with equilibrium proton magnetization and neglecting nitrogen magnetization, what is the product operator representation of the density matrix at observation (the end of period T) when the ^{15}N pulse length is short ($\beta=0$)? Sketch what the ^1H magnetization would look like.

$I_z \xrightarrow{90^\circ} -I_y \xrightarrow{\text{evolve}} \cos(\omega T/2) I_x S_z - \sin(\omega T/2) I_y S_z$
 Sketch:

b) As the ^{15}N pulse length approaches one appropriate for $\beta=90$ what would the product operator representation of the density matrix be on observation? What would the observed spectrum look like?

$I_z \xrightarrow{90^\circ} -I_y \xrightarrow{\text{evolve}} \cos(\omega T/2) I_x S_x - \sin(\omega T/2) I_y S_x$
 - 2+0 quantum coherence unobservable

Part III (25 points) Answer the following showing your work.

The attached spectra are COSY and ^{13}C - ^1H HSQC spectra for simple peracetylated monosaccharide.

a) Show connectivities on the COSY spectrum and list the chemical shifts for the anomeric proton (H1) and protons 2-5.

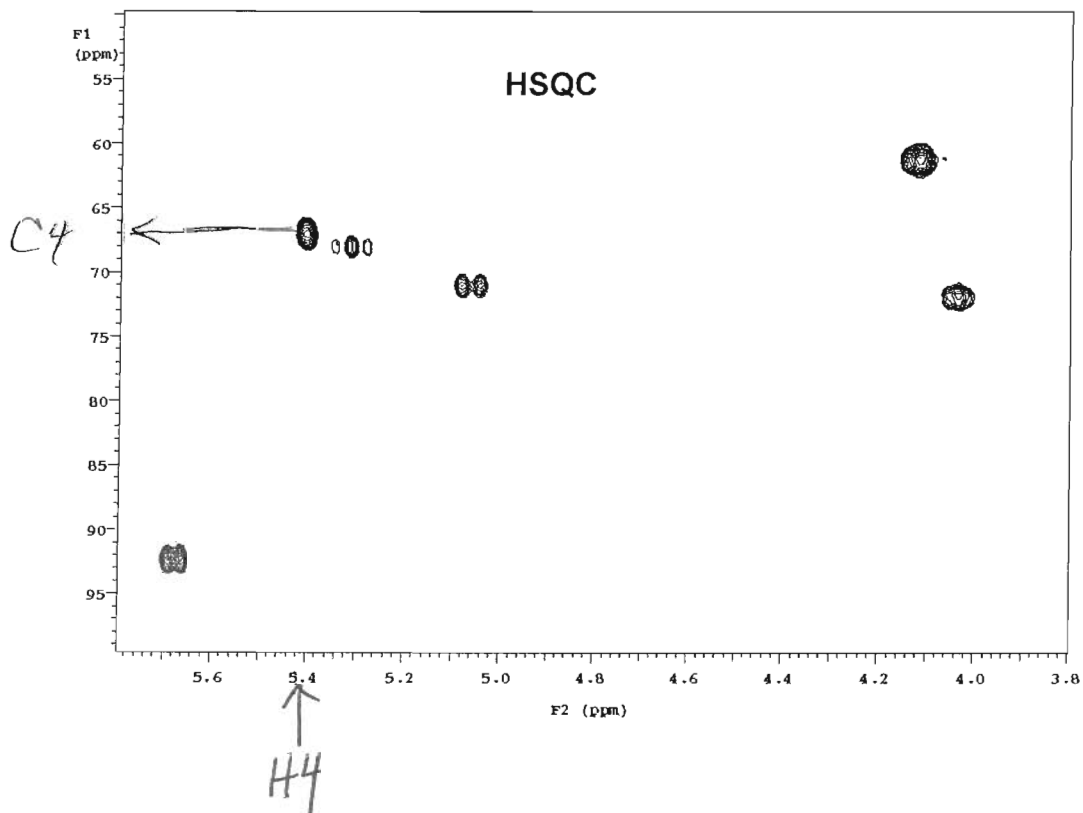
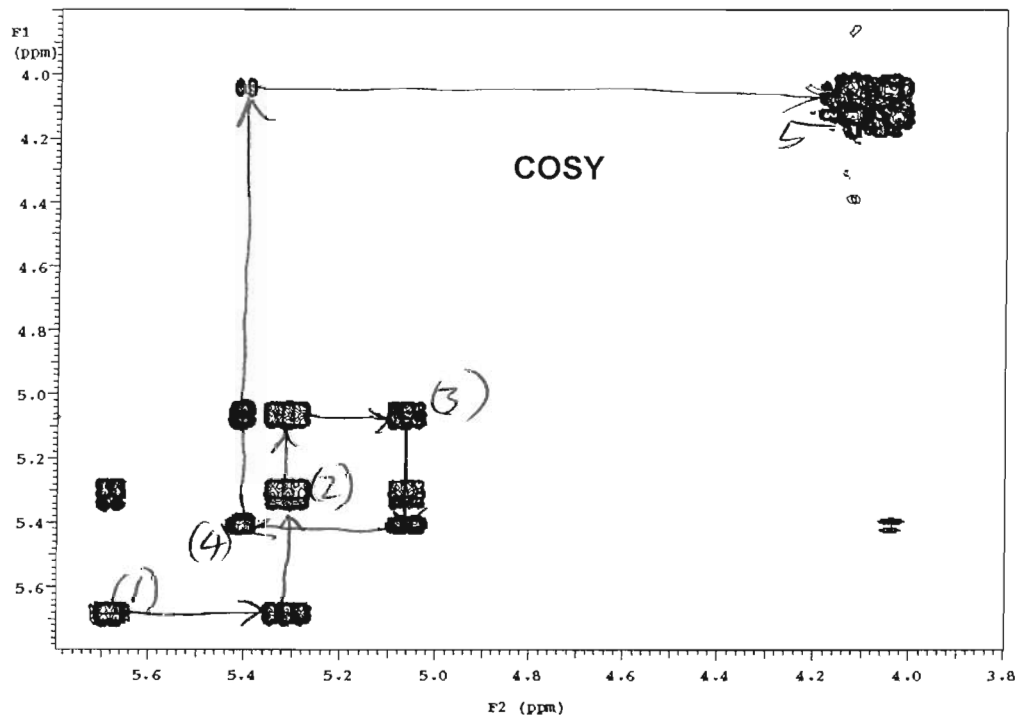
1	5.68
2	5.32
3	5.06
4	5.40
5	4.04

b) What can you say about the relative sizes of the scalar couplings between 2-3 and 4-5 and give a rationale.

4-5 cross peak is weak, coupling much smaller than 2-3 coupling

c) Using the HSQC spectrum, what is the chemical shift of the C4 carbon?

67 ppm



Some additional useful material:

Transformations Caused by Various Evolution Operators

Product Oper.	$I1x + I2x$	$I1y + I2y$	$I1z + I2z$	$2 I1zI2z$
$\frac{1}{2} E$	$\frac{1}{2} E$	$\frac{1}{2} E$	$\frac{1}{2} E$	$\frac{1}{2} E$
$I1z$	$-I1y$	$I1x$	$I1z$	$I1z$
$I2z$	$-I2y$	$I2x$	$I2z$	$I2z$
$2 I1zI2z$	$(2 I1yI2y)$	$(2 I1xI2x)$	$2 I1zI2z$	$2 I1zI2x$
$I1x$	$I1x$	$-I1x$	$I1y$	$2 I1yI2z$
$I1y$	$I1z$	$I1y$	$-I1x$	$-2 I1xI2z$
$I2x$	$I2x$	$-I2x$	$I2y$	$2 I1zI2x$
$I2y$	$I2z$	$I2y$	$-I2x$	$-2 I1zI2x$
$2 I1xI2z$	$-2 I1xI2y$	$(-2 I1zI2x)$	$2 I1yI2z$	$I1y$
$2 I1yI2z$	$(-2 I1zI2y)$	$2 I1yI2x$	$-2 I1xI2z$	$-I1x$
$2 I1zI2x$	$-2 I1yI2x$	$(-2 I1xI2z)$	$2 I1zI2y$	$I2y$
$2 I1zI2y$	$(-2 I1yI2z)$	$2 I1xI2y$	$-2 I1zI2x$	$-I2x$
$2 I1xI2x$	$2 I1xI2x$	$(2 I1zI2z)$	---	$2 I1xI2x$
$2 I1yI2x$	$2 I1zI2x$	$-2 I1yI2z$	---	$2 I1yI2x$
$2 I1xI2y$	$2 I1xI2z$	$-2 I1zI2y$	---	$2 I1xI2y$
$2 I1yI2y$	$(2 I1zI2z)$	$2 I1yI2y$	---	$2 I1yI2y$

Evolution is to 2, 4(), or more ---. Coefficient is sin of β , $\frac{1}{2} Jt$, or ωt .

Energy Level Diagram

$n+1$	j	degeneracy	total
2s	1/2	2	20
1d	3/2	4	6
	5/2	6	
1p	1/2	2	8
	3/2	4	
1s	1/2	2	2