

BCMB/CHEM 8190, BIOMOLECULAR NMR
MIDTERM – 3/5/04

Instructions: This is an open book, limited time, exam. You may use notes you have taken in 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 1. (20 points) Give short answers to the following practical questions and questions related to the lab portion of the course.

1. Understanding fold over in 1D ^1H NMR: In nucleic acids, imino protons are usually detected between 10 ppm and 15 ppm.
 - a) Which value of spectral width (in Hz) would you use to detect these signals given that the carrier is positioned on water (4.773 ppm), you have quadrature detection, you want to minimize the spectral width as much as possible, and you are working with a 600 MHz NMR spectrometer ?

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- b) Rarely, protonated adenines and protonated cytidines give rise to imino proton signals that resonate more downfield than 15 ppm. Let's suppose that in the spectrum that you set up in a), an imino proton signal which would normally be observed at 16 ppm is aliased in the spectrum. Given the carrier frequency and the spectral width chosen in a), at which frequency would you see the aliased signal?

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2. Why would an unshifted sinebell function ($f(t)=\sin(\theta+(180-\theta)t/T)$, $\theta=0^\circ$, T =acquisition time) not normally be used to apodize the FID acquired for a standard one-dimensional ^1H spectrum?

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3. Again using the sinebell function described above, what value of theta would you chose to optimize the signal to noise ratio of your spectrum?
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4. Bulk z magnetization (I_z) can be inverted ($-I_z$) using a simple π (180°) pulse or using the sequence $\pi/2_x, \pi_y, \pi/2_x$ ($90^\circ_x 180^\circ_y 90^\circ_x$). The product operator treatment for each is shown below ($\theta \approx 90^\circ$):

$$I_z \xrightarrow{2\theta_x} -I_y \sin 2\theta + I_z \cos 2\theta = -I_y \sin 2\theta - I_z (1 - 2 \cos^2\theta)$$

$$I_z \xrightarrow{\theta_x, 2\theta_y, \theta_x} I_x \sin 2\theta \cos \theta - I_y \sin \theta \cos \theta (1 + \cos 2\theta) - I_z (\sin^2\theta - \cos^2\theta \cos 2\theta) \\ = 2I_x \cos^2\theta \sin \theta - 2I_y \cos^3\theta \sin \theta - I_z (1 - 2 \cos^4\theta)$$

In cases of pulse imperfections or improper pulse calibrations, these two methods for spin inversion do not perform identically. Which will perform better and why?

Part II (20 pts). Give short answers to the following questions related to the lectures:

- 1) We manage to adjust the phase of the reference frequency in our spectrometer so that a resonance at exactly the reference frequency is pure absorption. However, we must delay collecting our first FID point by 100 microsec in order to avoid ring-down of our receiver coil. What will the phase error in a resonance 1kHz off the reference be?

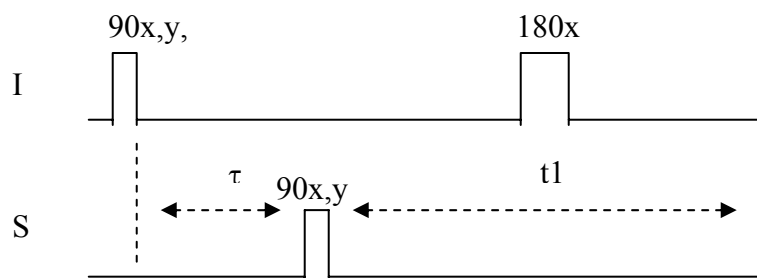
- 2) The decay constant (1/e reduction in amplitude) for an FID from a single peak is 20msec. What is the line width at half height for the peak resulting from Fourier transformation of the FID?

- 3) A NOESY spectrum of an alpha helix shows cross peaks connecting an amide proton to both the amide protons of the sequential residues ($i - 1$ and $i + 1$) and the alpha proton of a residue 3 amino acids removed ($i - 3$). The distances to the $i - 3$ alpha proton is 3.5 Angstroms and the intensity ratios for the connectivity peaks are 1.0 : 1.0 : 0.3. What are the distances to the other protons? _

- 4) Assuming that the time we allow between acquisitions is adequate for full recovery of all types of magnetization, compare the sensitivity of the following two experiments taking into account the difference in magnetogyric ratios of observed nuclei and the increased number of individual FIDs that must be collected for a 2D experiment: A 1D ^{13}C spectrum (1 FID); An indirectly detected (through ^1H) HSQC spectrum (requiring 64 indirect time points)?

Part 3 (36 pts).

The following pulse sequence, applied to a J coupled two spin ^1H - ^{15}N pair (symbolized I and S), is closely related to an HSQC experiment. The system starts at equilibrium, the proton resonance is assumed to be at the transmitter frequency (no chemical shift evolution), and the time τ in the period after the first 90° pulse is $1/(2J)$.



- a) What product operators represent the density matrix at the beginning of the t_1 period when a pair of x and y pulses are used for the I and S spin 90° pulses?
- b) What types of coherence (number of quanta involved in transitions) evolve during the first half of t_1 when the results of two sequences (with the 90° x and 90° y pairs) are added?

- c) What is the effect of the 180° pulse in the middle of t_1 and what is the nature of the evolution after the 180° pulse? (you need only look near $t_1 = 0$ to see this). What is the net effect of evolution for equal times before and after the pulse?

Part 4 (24 pts)

The attached spectra are proton COSY and TOCSY spectra of acetylated lactose (a glucose sugar ring connected to a galactose sugar ring). The anomeric proton (H1) of glucose is at 5.68 ppm.

- a) What are the chemical shifts of the H2, H3, H4, and H5 protons on the glucose ring?
- b) Why might connections to the H6 protons be missing in H1 column of the TOCSY spectrum?
- c) In the projection at the top there is a doublet of doublets at 4.96 ppm. Why are the doublets of unequal intensity? Can scalar couplings be measured accurately as simple separations of peak positions in this case?