BCMB/CHEM 8190
ANSWERS TO PROBLEM SET 5

1) Following the second pulse, multiple quantum coherences are created ($I_{1x}I_{2y}$, $I_{1y}I_{2x}$). These are then converted back to single quantum magnetization following the third pulse, for detection. The overall goal of the double quantum filter is to select for the two-quantum magnetization for detection following conversion back to single quantum. So, the first gradient pulse is applied when the coherences of interest are two-quantum. These coherences evolve as the sum of the precession frequencies of the coupled spins. So, the effect of a gradient pulse on two-quantum magnetization is twice that of single quantum magnetization. Thus, in order to refocus the magnetization, the second gradient pulse has to be applied at twice the strength of the first.

2) The carbon atoms at position 1 in both rings are directly bonded to two oxygen atoms each, so, for the ring carbons, these would be expected to have the highest ^{13}C chemical shifts (most deshielded, most downfield, typically ~ 100 ppm). In the HMQC spectrum, there are two signals with high ^{13}C chemical shifts (^1H=5.60, ^{13}C=93 ppm, and ^1H=4.55, ^{13}C=101 ppm). It cannot yet be determined which corresponds to ring A and which to ring B.

In the HMBC spectrum, the hydrogen at position 1 in ring B should show a crosspeak to the carbonyl carbon of the acetyl group attached at position 1. Carbonyl carbons appear far downfield (~170 ppm, highly deshielded). In the HMBC spectrum, there are signals at both ^1H=5.60 and 4.55 ppm, but in only the former case, ^1H=5.60 ppm, is there a peak with a far downfield (~172 ppm) ^{13}C chemical shift. Therefore, ^1H=5.60 ppm corresponds to the ^1H chemical shift of the hydrogen at position 1 in ring B, and 4.55 is the ^1H chemical shift of the hydrogen at position 1 in ring A.

To begin to assign the ^1H signals in ring B, the TOCSY spectrum shows that there are 5 crosspeaks (10 if you count the symmetrically disposed peaks) that are correlated to ^1H=5.60 (hydrogen at position 1), with ^1H chemical shifts of 5.20, 4.95, 4.05, 3.85, and 3.50 ppm. These must be the hydrogens at positions 2, 3, 4 and 5 (two hydrogens at 5, axial and equatorial) in ring B. In the COSY spectrum, the hydrogen at position 1 is correlated with the hydrogen with the ^1H chemical shift of 4.95 (crosspeak at 5.6/4.95). So, the signal at 4.95 is from the hydrogen at position 2. This hydrogen also is correlated with the hydrogen with the ^1H chemical shift of 5.20 (crosspeak at 4.95/5.20). Thus, the signal at 5.20 is from the hydrogen at position 3. Likewise, the crosspeak at 5.20/3.85 indicates that the signal at 3.85 is from the hydrogen at position 4. Finally, there are two additional crosspeaks, corresponding to the hydrogens at position 5, correlated to the chemical shift of the hydrogen at position 4 (crosspeaks at 3.85/3.50, and 3.85/4.05). So, the hydrogens at position 5 have chemical shifts of 3.50 and 4.05. It is not known at this point which corresponds to the axial, and which to the equatorial, hydrogen.

For ring B, the ^{13}C chemical shifts are now easily assigned from the HMQC spectrum, knowing the ^1H chemical shifts. In the HMQC spectrum, the two peaks at ^1H chemical shifts of 3.50 and 4.05 have identical ^{13}C chemical shifts (64.0 ppm), indicating the hydrogens are bound to the same carbon nucleus, i.e. these are the hydrogens at position 5. The ^{13}C chemical shift of the carbon at position 5 is then 64.0 ppm. In the HMQC spectrum, the signal at ^1H=4.95 is at ^{13}C=70.5 ppm. Thus, this is the ^{13}C chemical shift of the carbon nucleus at position 2. Likewise, the two signals that appear at ^1H=5.20/^{13}C=72.5 ppm, and ^1H=3.85 ppm/^{13}C=75.0 ppm correspond to positions 3 and 4.

In ring A, the ^1H chemical shift of the hydrogen at position 1 is 4.55 ppm (see above). In the TOCSY spectrum, there are 5 crosspeaks that are correlated to ^1H=4.55, with ^1H chemical shifts of 4.80, ppm, so these are the ^1H chemical shifts of the hydrogens at positions 2, 3, 4, and 5. The crosspeak at 4.55/4.80 indicates that the ^1H chemical shift of the hydrogen at position 2 is 4.80. The crosspeak at 4.80/5.10 indicates that the ^1H chemical shift of the hydrogen at position 3 is 5.10. The crosspeak at 5.10/4.90 indicates that the ^1H chemical shift of the hydrogen at position 4 is 4.90. Finally, The crosspeaks at 4.90/3.40 and 4.90/4.10 indicate that the ^1H chemical shifts of the hydrogens at position 5 are 3.40 and 4.10. Knowing the ^1H chemical shifts, the ^{13}C chemical shifts are simply read from the HMQC spectrum (see table).
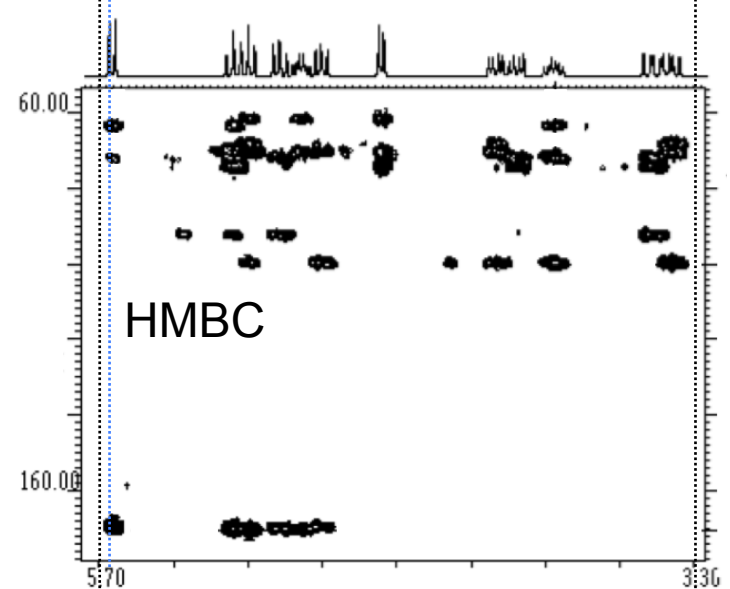
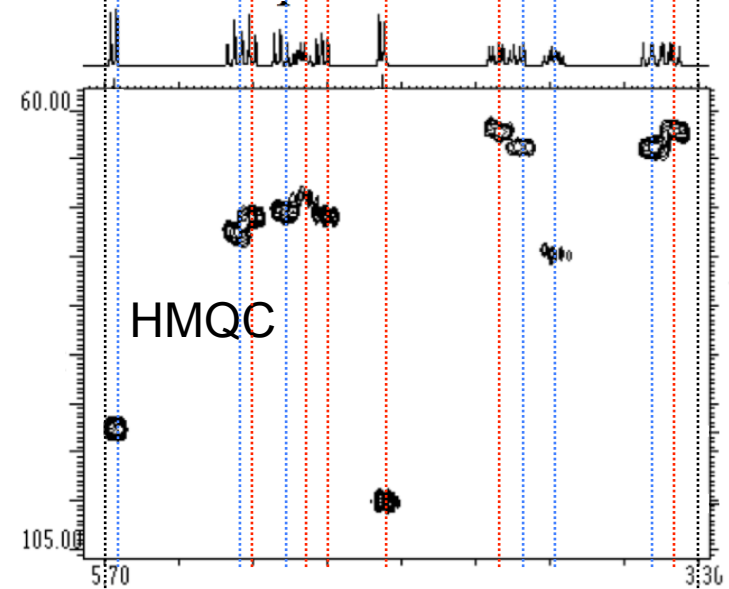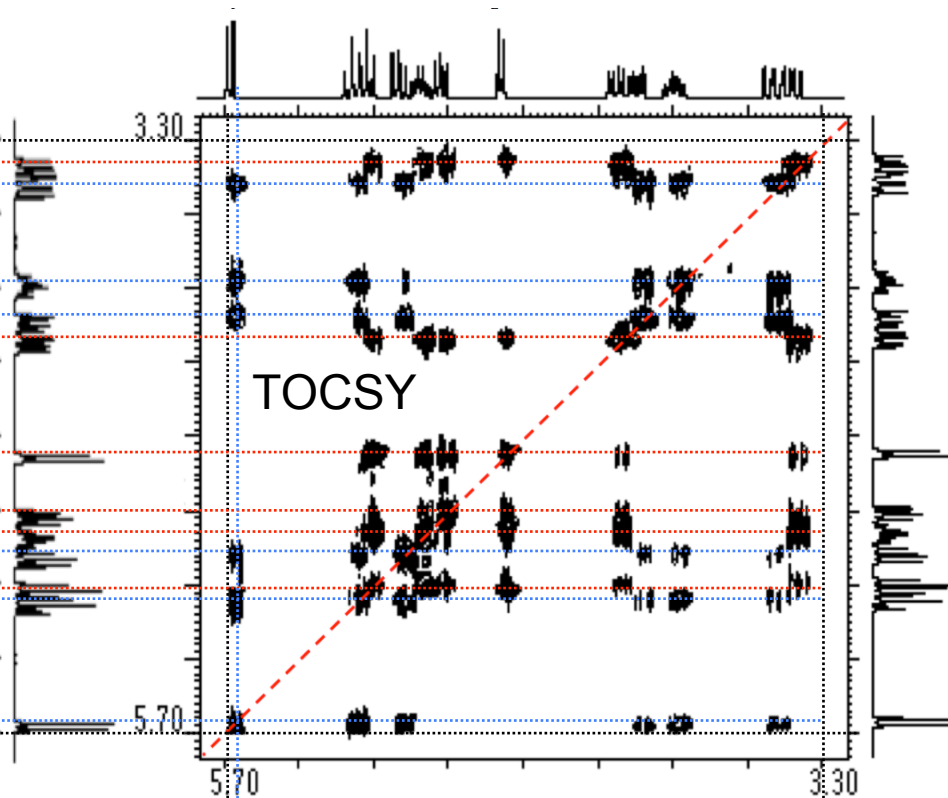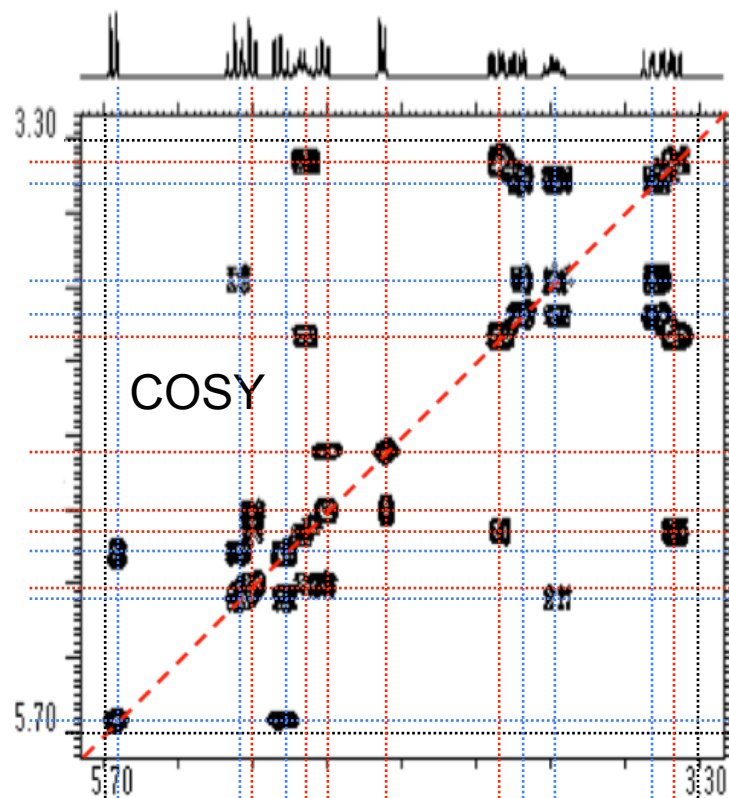|	A ring						B ring					
	1	2	3	4	5 ax	5 eq	1	2	3	4	5 ax	5 eq
^1H	4.55	4.80	5.10	4.90	3.40	4.10	5.60	4.95	5.20	3.85	3.50	4.05
^{13}C	101	71.0	71.5	69.0	62.5	62.5	93	70.5	72.5	75.0	64.0	64.0

In the HMBC spectrum, in the region from ~4.7-5.3 ppm (corresponding to ^1H chemical shifts for hydrogens 2, 3, and 4 in ring A and 2 and 3 in ring B) there are signals at ~170 ppm, indicating correlations to the carbonyl carbons of the acetyl groups, as expected.

In the 1D ^1H spectrum, the signals at ~3.4 and 4.1, corresponding to the hydrogens at position 5 of ring A, are each a doublet of doublets (germinal coupling between 5 axial and 5 equatorial hydrogens, and 3 bond coupling to axial hydrogen at position 4). The largest splitting in each signal is common to both, indicating this is due to the germinal coupling. The smaller splitting in each signal is smallest in the signal at 4.1 ppm, indicating a coupling constant between the axial position at 4 and the equatorial position at 5 (about 60 degree dihedral angle, small coupling constant according to the Karplus relationship). The splitting is larger in the signal at 3.4, indicating an axial-axial coupling (180 degrees). So, the equatorial hydrogen is the downfield signal (4.10), and the upfield signal (3.40) is the axial hydrogen. Similar arguments apply for the B ring.

There is a nice animation of this assignment (you have to have Adobe Shockwave installed on your computer) at the Queen's University website:

https://qshare.queensu.ca/Users01/sauriolf/www/webcourse/2d_nmr.htm

COSY

TOCSY

HMQC

HMBC

$^2J_{5ax,5eq}$ →| |←   →| |← $^2J_{5ax,5eq}$

$^3J_{4ax,5eq}$ →| |←   →| |← $^3J_{4ax,5ax}$