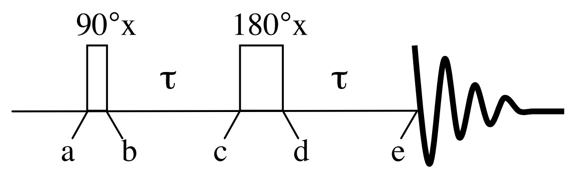
Problem Set #3, CHEM/BCMB 4190/6190/8189

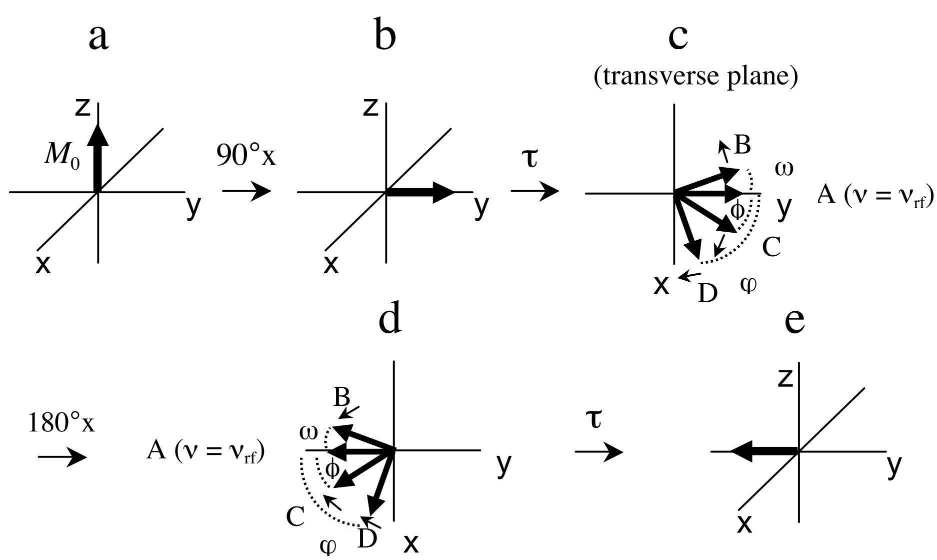
1). In Lab #3, we collected 1D ^{1}H-decoupled ^{13}C (natural abundance) spectra of menthol. We discussed the fact that the NOE enhancements were in general larger for carbon atoms with more attached protons ($-CH_3 > -CH_2 > -CH$). What is the other major contributor to differences in intensities of specific nuclei in ^{13}C spectra and how does it affect the intensities of the signals from the different types of carbon center? If you wanted to set up your 1D ^{13}C experiment to alleviate the affects of this contributor, how would you do it?

The other contributor is of course the longitudinal relaxation time, T_1. As we learned in lecture, dipole-dipole interactions are the major contributors to T_1 relaxation. For ^{13}C, the dipole-dipole interactions with the directly bonded hydrogen atoms are the largest contributors to T_1. Thus, for normal organic compounds, T_1 values for ^{13}C decrease as hydrogens are added (quaternary > $-CH$ > $-CH_2$ > $-CH_3$).

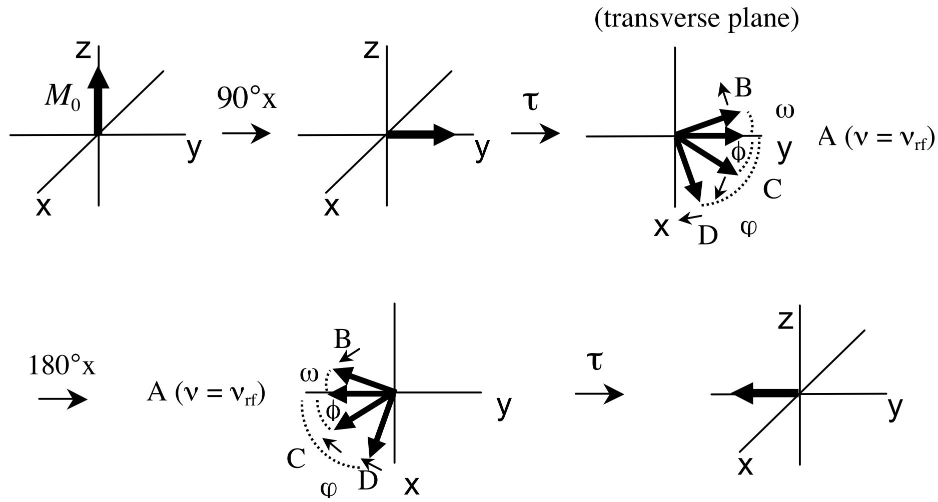
So, if the recycle delay in our experiment was short, and the T_1 values for some of the ^{13}C nuclei in our compound (sample) were long, the signals from the nuclei with the long T_1 values would be attenuated. If we wanted to alleviate this affect, we could measure the T_1 values, and then set the recycle delay to be substantially larger than the longest T_1 value (i.e. 5 times the longest T_1).

2). A normal spin-echo pulse sequence is shown (right). Let's consider the elimination (by refocusing) of magnetic field inhomogeneity effects by this pulse sequence, *ignoring the effects of J coupling and chemical shift evolution*. Our nucleus precesses with a Larmour frequence ν_{rf}. During the first τ delay, magnetic field inhomogeneity results in some nuclei precessing slower (vector B) and some faster (vectors C and D) than ν_{rf} (vector A is rotating at ν_{rf}), such that at point 'c', each vector has rotated through a specific angle (vector B, angle=ω; vector C, angle=ϕ; vector D, angle=φ). Using vector diagrams, show the effect of the 180°x pulse on the vectors (what does the vector diagram look like at point 'd') and the effect of the final τ delay (what does the vector diagram look like at point 'e').





3). Consider again the normal spin-echo pulse (problem 2, above). Let's *now* consider the effect of this pulse sequence on chemical shift evolution, *ignoring the effects of J coupling and magnetic field inhomogeneity*. We will consider four nuclei (A, B, C, and D) each precessing with a different Larmor frequency (A precesses with a frequency equal to our reference frequency, $\nu_A = \nu_{rf;}$ B precesses slower and C and D faster than the reference), such that at point 'c' in the pulse sequence each vector has rotated through a specific angle (vector B, angle=ω; vector C, angle=ϕ; vector D, angle=φ) during τ. Using vector diagrams, show the effect of the 180°x pulse on the vectors (what does the vector diagram look like at point 'd') and the effect of the final τ delay (what does the vector diagram look like at point 'e').



4). Once again, consider again the normal spin-echo pulse (problem 2, above). Let's *now* consider the effect of this pulse sequence on heteronuclear (-^{13}C-^{1}H) *J* coupling, *ignoring the effects of chemical shift evolution and magnetic field inhomogeneity.* We will consider a single ^{13}C nucleus (with a single attached proton, i.e. $^{13}CHCl_3$) with a Larmor frequency equal to our reference frequency, $\nu_c = \nu_{rf}$.

a. What will be the precession frequencies for the two vectors, $M_C^{H\alpha}$ and $M_C^{H\beta}$, that result from the coupling, J_{CH}?

b. What is the difference between these frequencies?

c. What is the phase angle between the two vectors described by $M_C^{H\alpha}$ and $M_C^{H\beta}$ after a time τ?

d. How large is the phase angle after a time $\tau = 1/(4J_{CH})$?

e. Using vector diagrams, show the effect of the spin echo pulse sequence on this spin system with $\tau = 1/(4J_{CH})$.

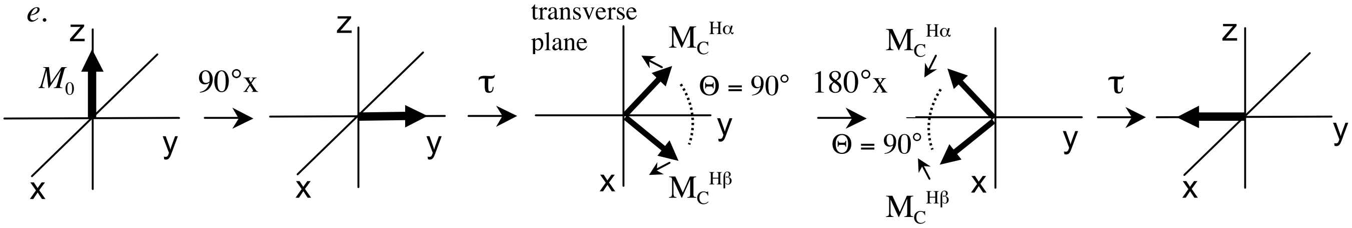
a. $\nu(M_C^{H\alpha}) = \nu_c - J_{CH}/2,\ \nu(M_C^{H\beta}) = \nu_c + J_{CH}/2$

b. $(\nu_c - J_{CH}/2) - (\nu_c + J_{CH}/2) = J_{CH}$

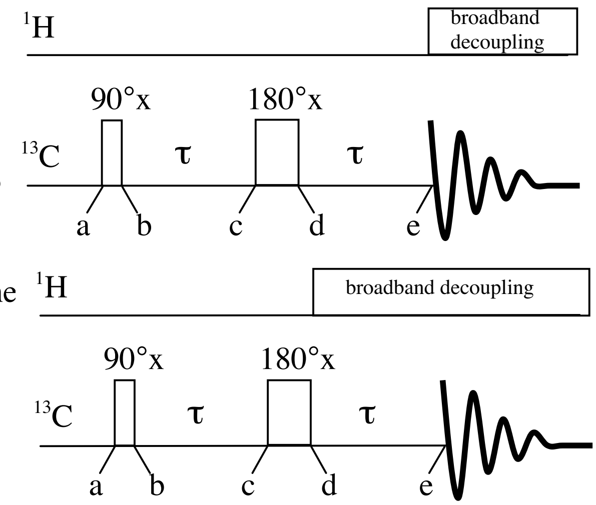
c. *phase angle* $= \Theta = 2\pi J_{CH}\tau$

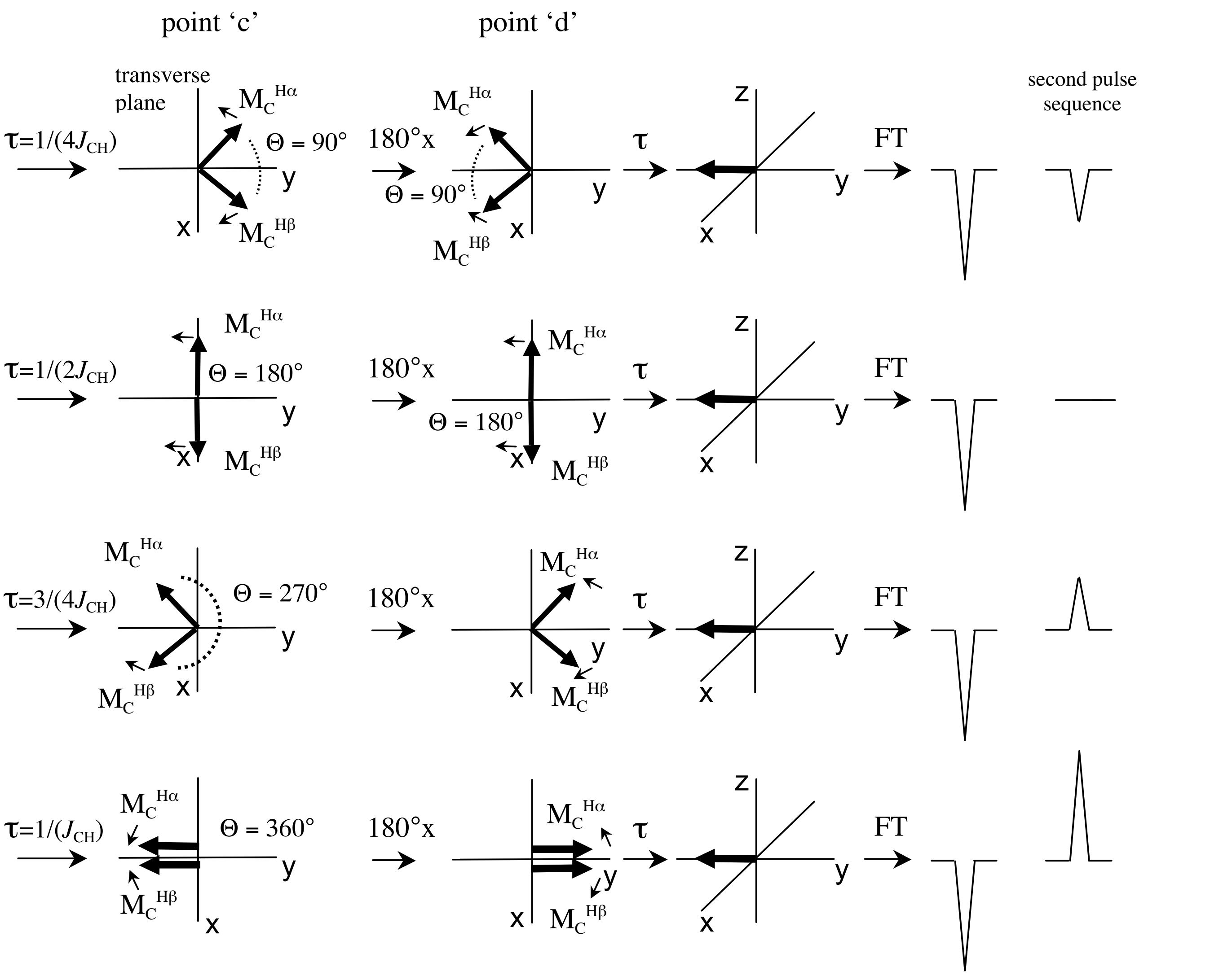
d. $\Theta = 2\pi J_{CH}\tau = \Theta = 2\pi J_{CH}(1/(4J_{CH})) = \pi/2$ *radians* $= 90°$

e.



5). Repeat part 'e' of question 4 for $\tau = 1/(2J_{CH})$, $\tau = 3/(4J_{CH})$, and $\tau = 1/(J_{CH})$. Also, show the Fourier transformation of the signal that you would get after the second τ period if you applied broadband ^1H decoupling during acquisition only (as shown in the pulse sequence to the upper right). Finally, also show the Fourier transformation of the signal that you would get if instead you used the second pulse sequence (lower right) where the broadband decoupling is applied immediatedly after the 180° pulse.





For the second pulse sequence, the decoupler is turned on at point'd'. At this point, the two vectors rotate with the same frequency (the coupling is removed), and the signal becomes the vector average of the two.

6). Once again, consider the normal spin-echo pulse sequence (problem 2, above). Let's once again consider the effect of this pulse sequence on heteronuclear J coupling, *ignoring the effects of chemical shift evolution and magnetic field inhomogeneity*. This time we will consider a single ^{13}C nucleus with two attached protons (i.e. $^{13}CH_2Cl_2$) with a Larmor frequency equal to our reference frequency, $\nu_c = \nu_{rf}$.

a. What will be the frequencies of the three components of the signal (triplet) from the ^{13}C nucleus?

b. What is the difference between the frequencies of these components?

c. What is the phase angle between the two vectors corresponding to the two outer components of the triplet after a time τ?

d. How large is the phase angle between the two vectors corresponding to the two outer components of the triplet after a time $\tau = 1/(4J_{CH})$?

e. Using vector diagrams, show the effect of the spin echo pulse sequence on this spin system with $\tau = 1/(4J_{CH})$.

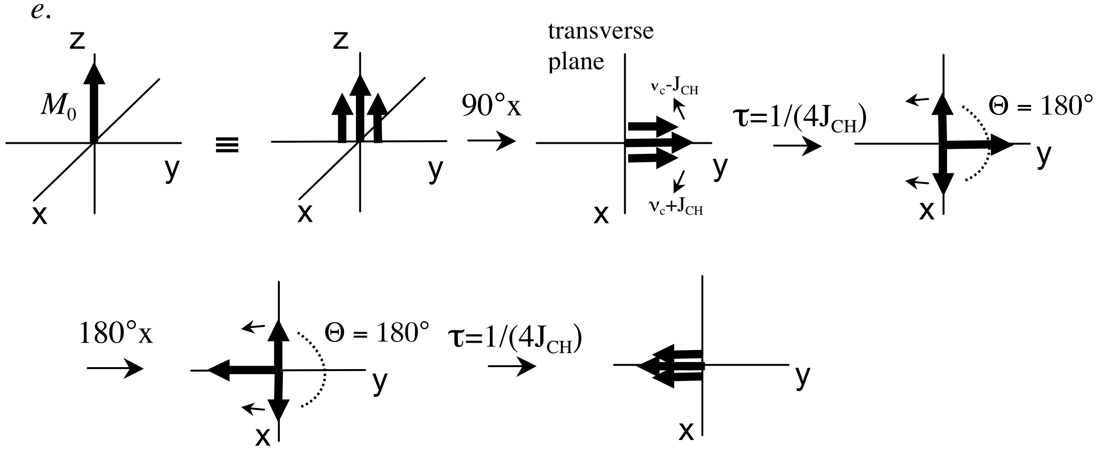
a. the three frequencies are ν_c, $\nu_c - J_{CH}$ *and* $\nu_c + J_{CH}$

b. The frequency difference between the center component of the triplet and the outer components is $\pm J_{CH}$. *The frequency difference between the two outer components of the triplet is* $2J_{CH}$.

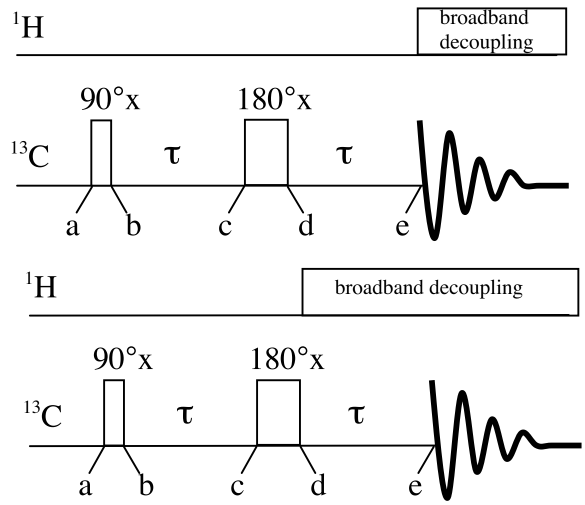
c. phase angle = $\Theta = 2\pi(2J_{CH})\tau$

d. $\Theta = 2\pi(2J_{CH})\tau = \Theta = 2\pi J_{CH}(2/(4J_{CH})) = \pi$ *radians = 180°*

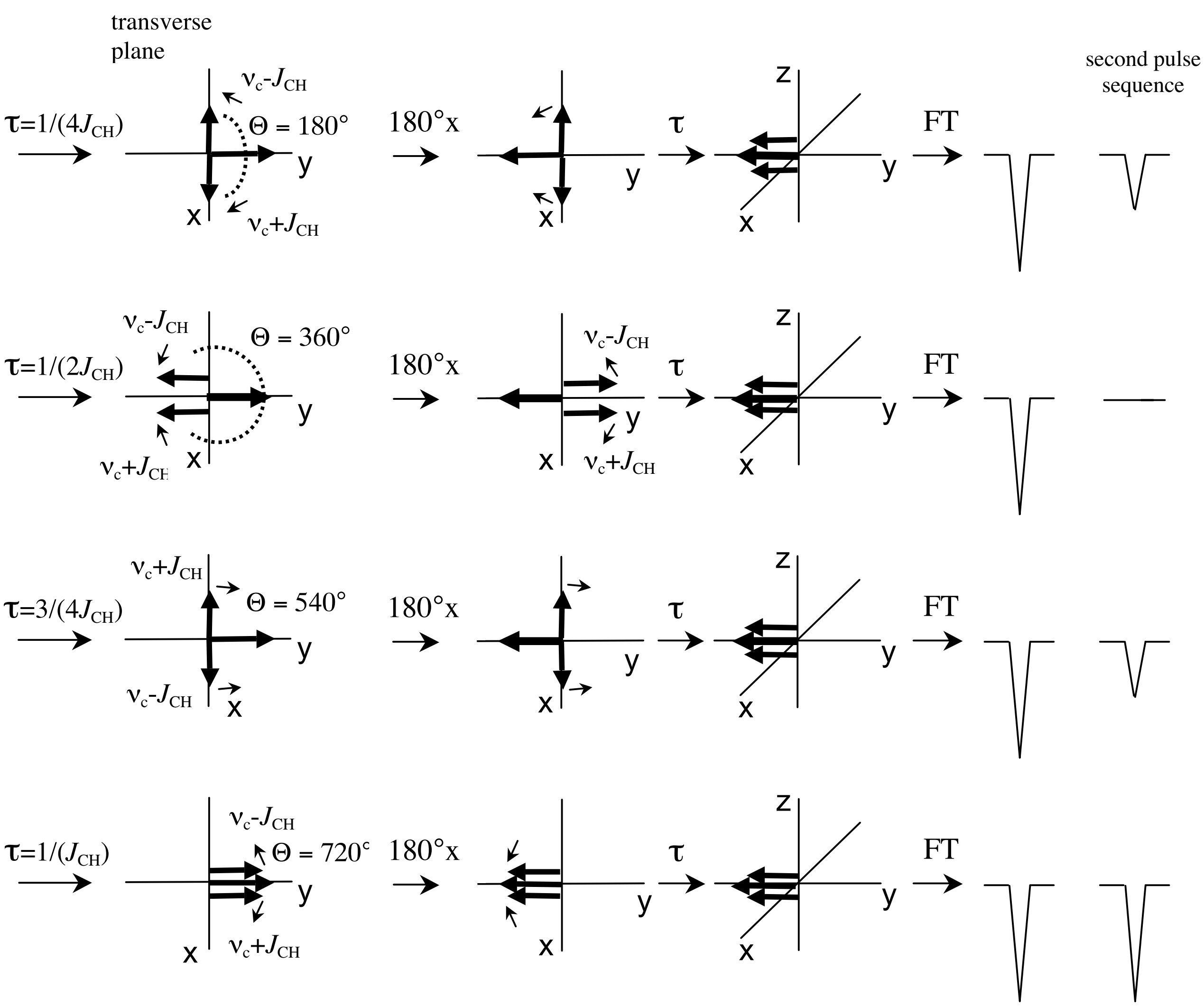
e.



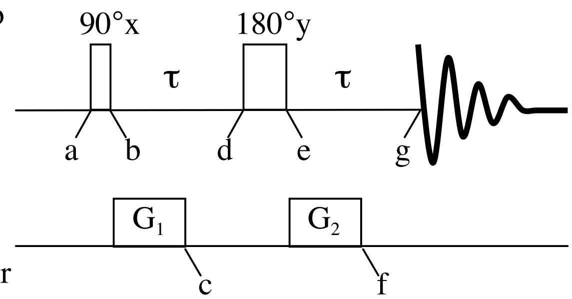
7). Repeat part ‘e’ of question 6 for $\tau = 1/(2J_{CH})$, $\tau = 3/(4J_{CH})$, and $\tau = 1/(J_{CH})$ for the $^{13}CH_2Cl_2$ sample. Also, show the Fourier transformation of the signal that you would get after the second τ period if you applied broadband ^1H decoupling during acquisition only (as shown in the pulse sequence to the upper right). Finally, also show the Fourier transformation of the signal that you would get if instead you used the second pulse sequence (lower right) where the broadband decoupling is applied immediatedly after the 180° pulse.



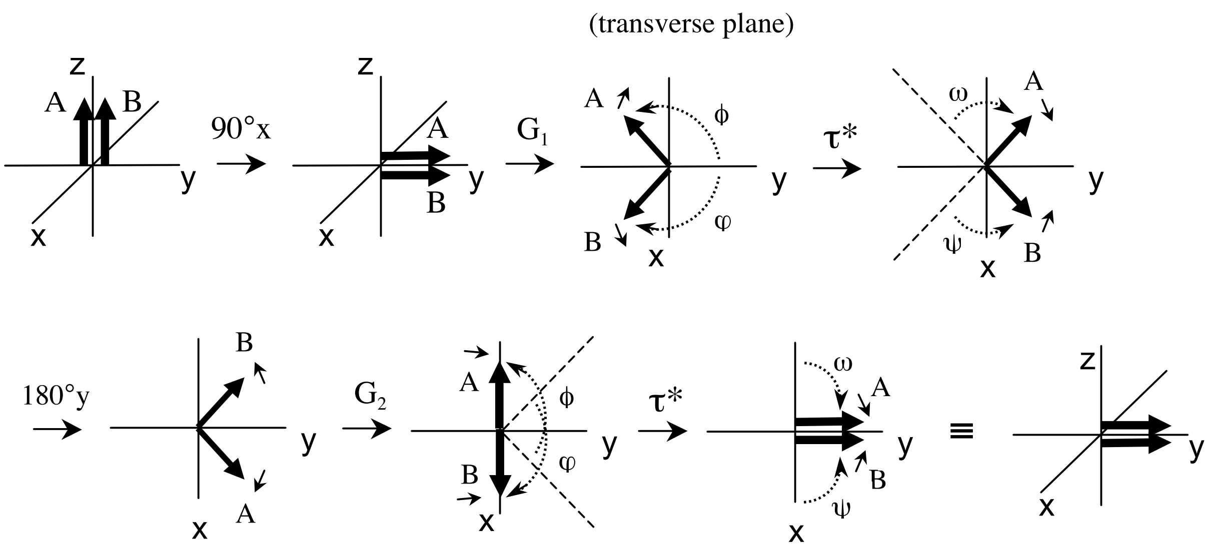
point ‘c’ point ‘d’



For the second pulse sequence, the decoupler is turned on at point’d’. At this point, the three vectors rotate with the same frequency (the coupling is removed), and the signal becomes the vector average of the three.

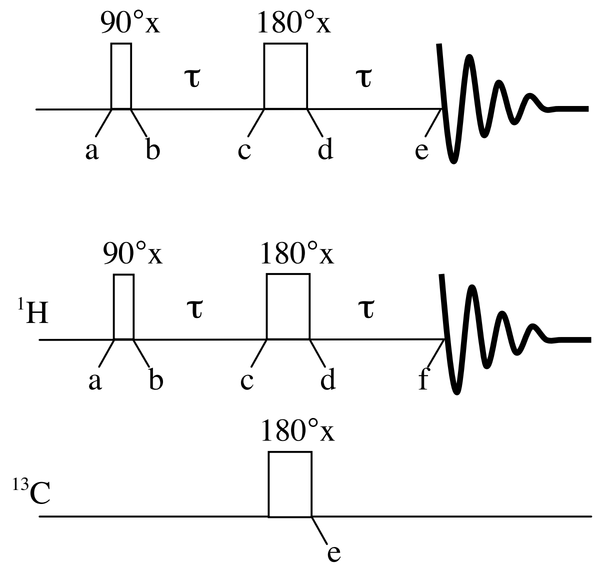


8). Now let's consider the pulsed field gradient spin-echo experiment. We will consider two [1]H nuclei at different vertical planes in the sample (plane 'A' and plane 'B'), and we will consider chemical shift evolution of nuclei in these planes but we will *ignore the effects of magnetic field inhomogeneity and J coupling*. The Larmor frequency of nucleus 'A', which is in plane 'A', is higher than our reference frequency ($\nu_A > \nu_{rf}$). The Larmor frequency of nucleus 'B', which is in plane 'B', is lower than our reference frequency ($\nu_B < \nu_{rf}$). Nuclei in plane 'A' experience a *reduced* field by gradient pulses G_1 and G_2 such that during these gradient pulses the precession frequency is *decreased* ($\nu = \frac{\gamma}{2\pi}(B_0 - g_n)$), whereas nuclei in plane 'B' experience an augmented field during the gradient pulses that *increase* the precession frequencies ($\nu = \frac{\gamma}{2\pi}(B_0 + g_n)$). Draw vector diagrams describing the effect of pulsed field gradient spin-echo experiment on the two nuclei 'A' and 'B'. (Note: the 180° pulse is on 'y' in this pulse sequence. Also note that the gradient pulses G_1 and G_2 are applied with identical power and for identical lengths of time)

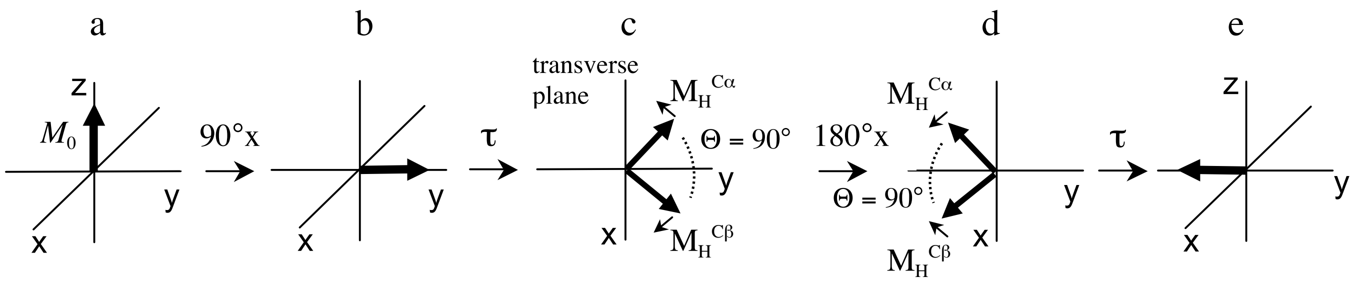


τ* is the period of time equal to τ minus the time for the gradient pulse G_1 or G_2

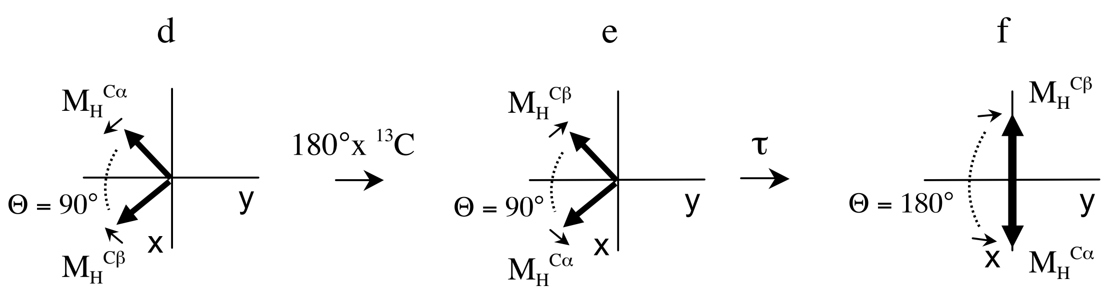
9). In problem 4e, using vector diagrams you considered the effect of the normal spin-echo pulse sequence (right, top) on heteronuclear ($-^{13}C-^{1}H$) J coupling. Using vector diagrams, compare that result with the result that you would get from the modified pulse sequence (right, bottom) where a 180°x ^{13}C pulse has been added. For this comparison, consider the effect of the pulse sequences on a single ^{1}H nucleus, with a Larmor frequency equal to our reference frequency ($\nu_c=\nu_{rf}$) attached to a single ^{13}C nucleus (i.e. $^{13}CHCl_3$).



The normal spin-echo pulse sequence (top) produces the following result:



The effect of the additional pulse in the modified sequence (bottom) exchanges $^{13}C_\alpha$ and $^{13}C_\beta$ spins: nuclei that were in the α state are now in the β state and vice versa. This effectively exchanges the positions of the two vectors:



So, whereas the spin-echo pulse sequence refocuses the two components $M_H^{C\alpha}$ and $M_H^{C\beta}$, the modified sequence serves to position them 180° out of phase. In other words, **J coupling is not refocused** *by this modified sequence. This modified sequence is the basis of the INEPT pulse sequence, and related pulse sequences.*