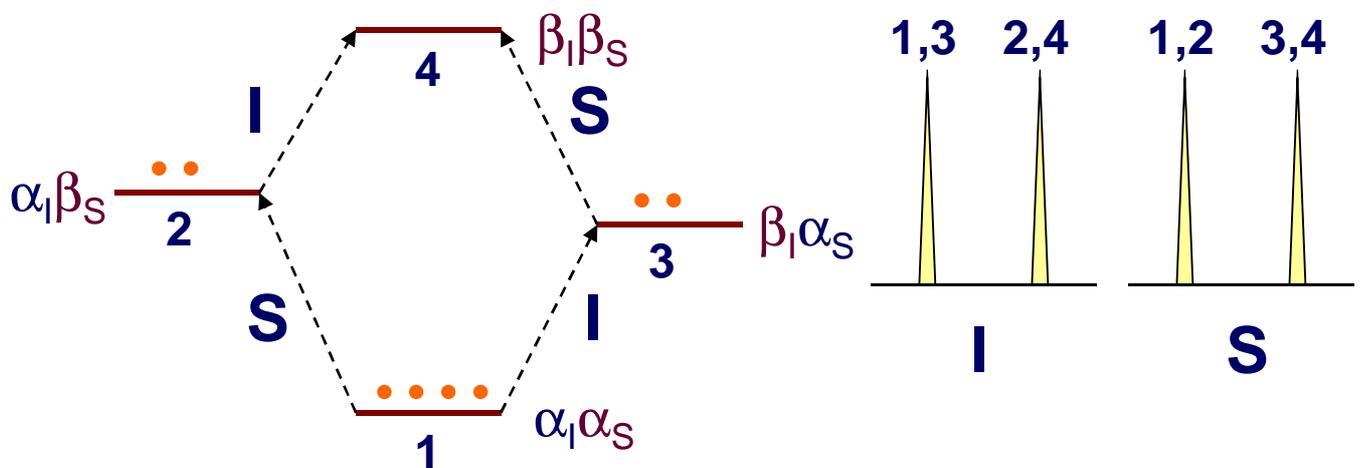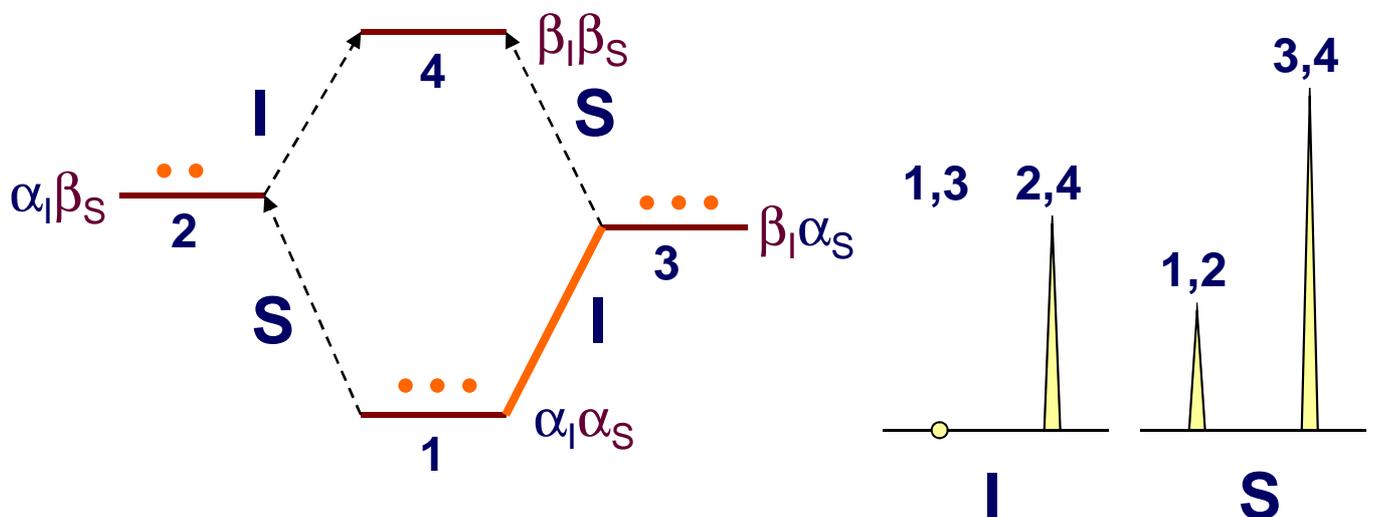# Polarization transfer

- So far we have dealt vectors (magnetizations) that are proportional to the sensitivity of the nuclei we are studying.

- In multiple pulse experiments, were we are doing many things to a group of spins in order to get information. If the nuclei we are acting on are insensitive, anything they do on other nuclei (NOE, coupling, etc.) will be hard to see.

- In the case of APT, we were looking at ^{13}C and decoupling ^{1}H during the second delay. If we consider that we have NOE effects, we have at the most a 4-fold enhancement ($\gamma H / \gamma C$) of the carbon signal. As there are many other relaxation pathways, this is rarely the case…

- What if we could use the bigger population difference from a sensitive nuclei (^{1}H) and pass it on to the insensitive nuclei (^{13}C, ^{15}N), all in a predictable manner?

- The method is called ***polarization transfer***, and basically it involves passing the large excess population (polarization) of the ^{1}H to the insensitive nuclei before we perturb it.

- To understand how it works we use the weakly coupled two spin system energy diagram for an homonuclear system...

Polarization transfer (continued)

- For this diagram, we'll use two protons that are **J**-coupled weakly and have a large δ difference. We name them **I** and **S** to maintain I don't know which convention, and we indicate with a • the excess population from one state to the other:


- Now we irradiate and saturate only one of the lines of one of the nuclei **selectively** (with CW…). After a certain time, the population differences for that transition become equalized.
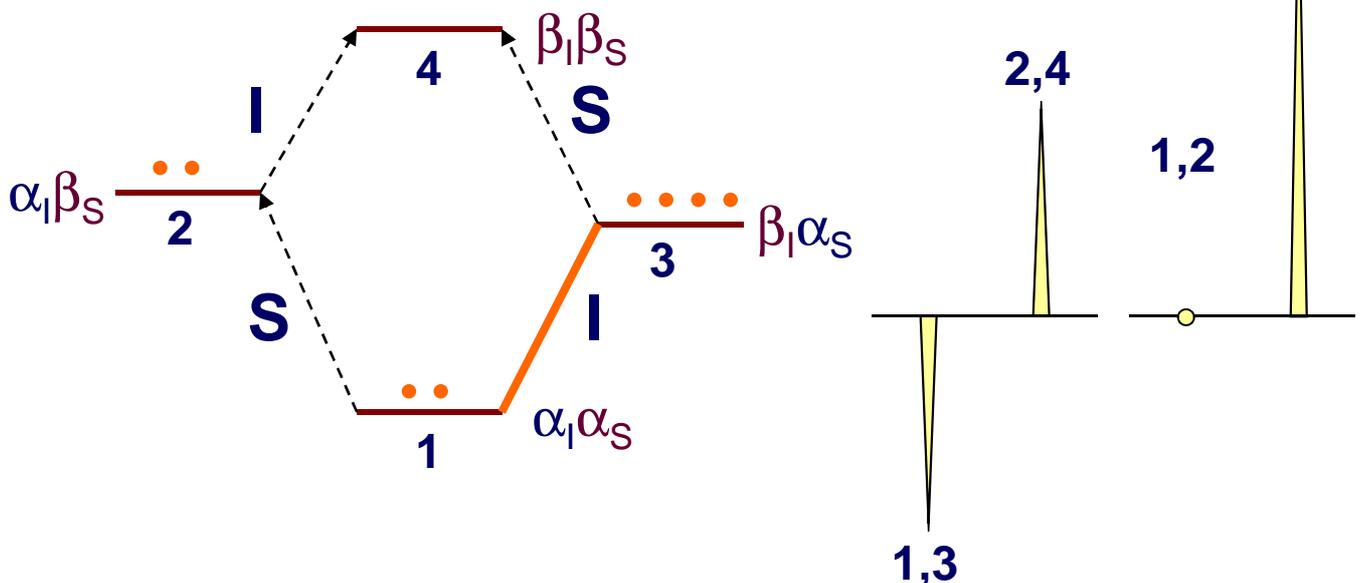
Polarization transfer. SPT and SPI

- Since we changed the populations of the spin system, the lines in the spectrum change intensity accordingly. What we did is transfer polarization from one nuclei to the other. This is called **selective polarization transfer**, or **SPT**.

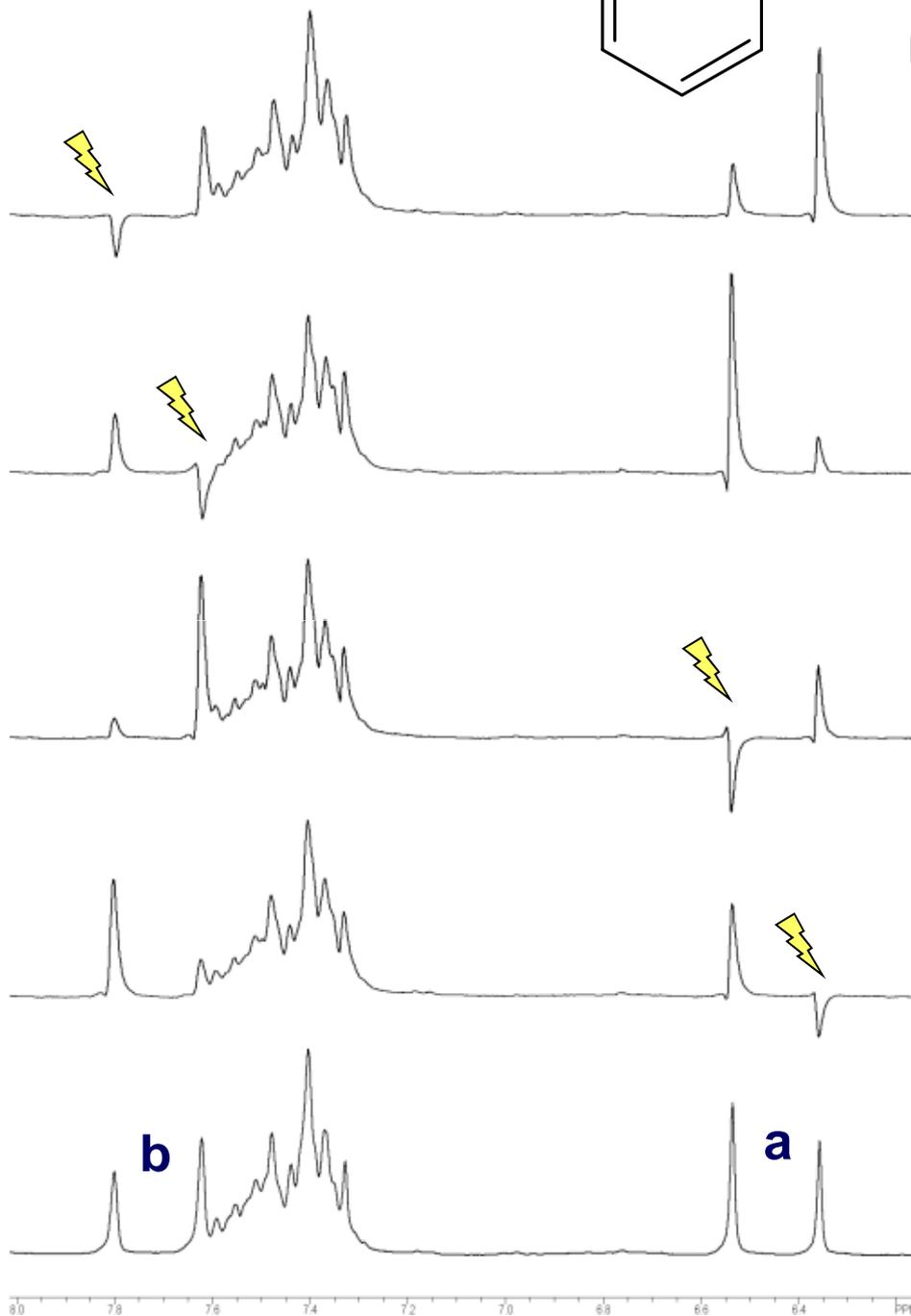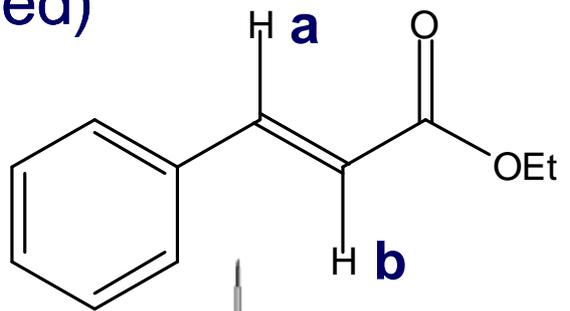- There is one variation of this technique. Think of the following pulse sequence:
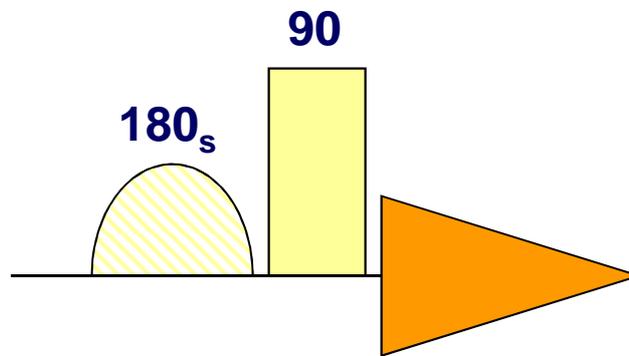
- The chubby pulse is a low power, selective π pulse. It inverts the populations of only one of the transitions in the spin system.

PT - SPT and SPI (continued)

- A practical example using ethylcinnamate:

- In this case we invert selectively each line of the **a** and **b** olefinic ^{1}H's and see how the intensities of the others vary.

Heteronuclear polarization transfer

- In this case, we call the experiment *selective population inversion*, or *SPI*. Again, the intensities of the lines reflect what we've done to the populations of the spin system.

- Despite that we can use SPT and SPI to identify coupled spin systems in very crowded regions of the spectra, homonuclear PT is not as useful as heteronuclear PT. Lets think of the two experiments in a heteronuclear system:
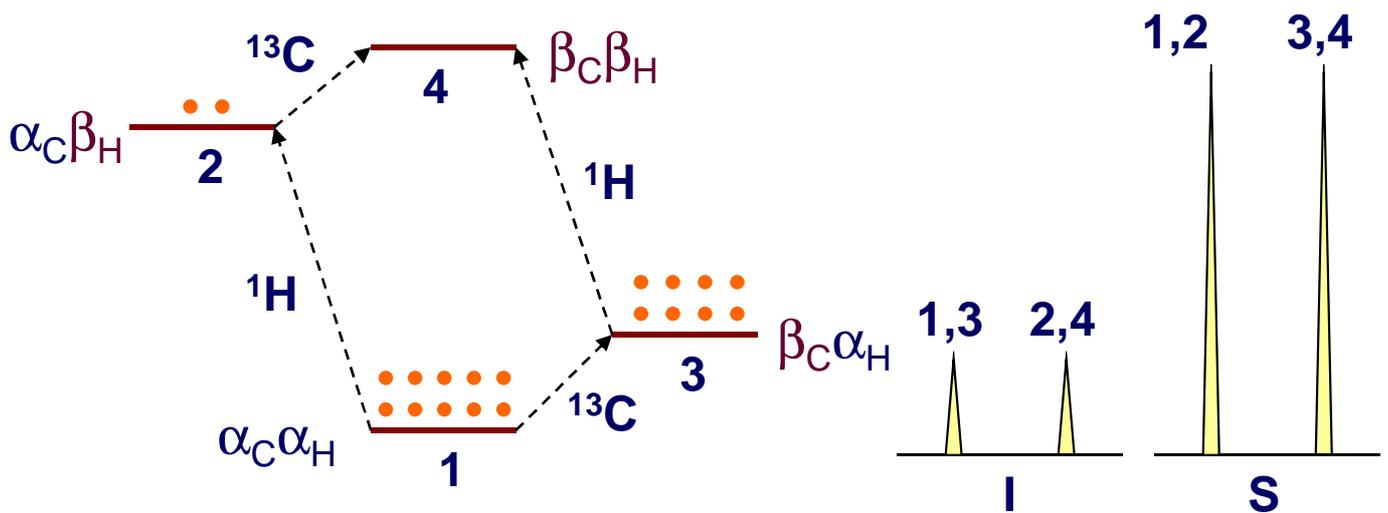
- Here the population differences between the energy levels reflect that we have a **1** to **4** ratio between ^{13}C and ^{1}H due to the differences in the gyromagnetic rations. Here is were we start seeing why it may be useful…

- One thing that we have wrong in the drawing is the relative intensities. Here we are drawing using only the ratios of the gyromagnetic ratio, not the abundance...

Heteronuclear polarization transfer - SPT

- Now we'll apply SPT and SPI on this spin system, and see what happens. First SPT…

- After we saturate, say, the **1,2** transition we get the following populations in the energy diagram:
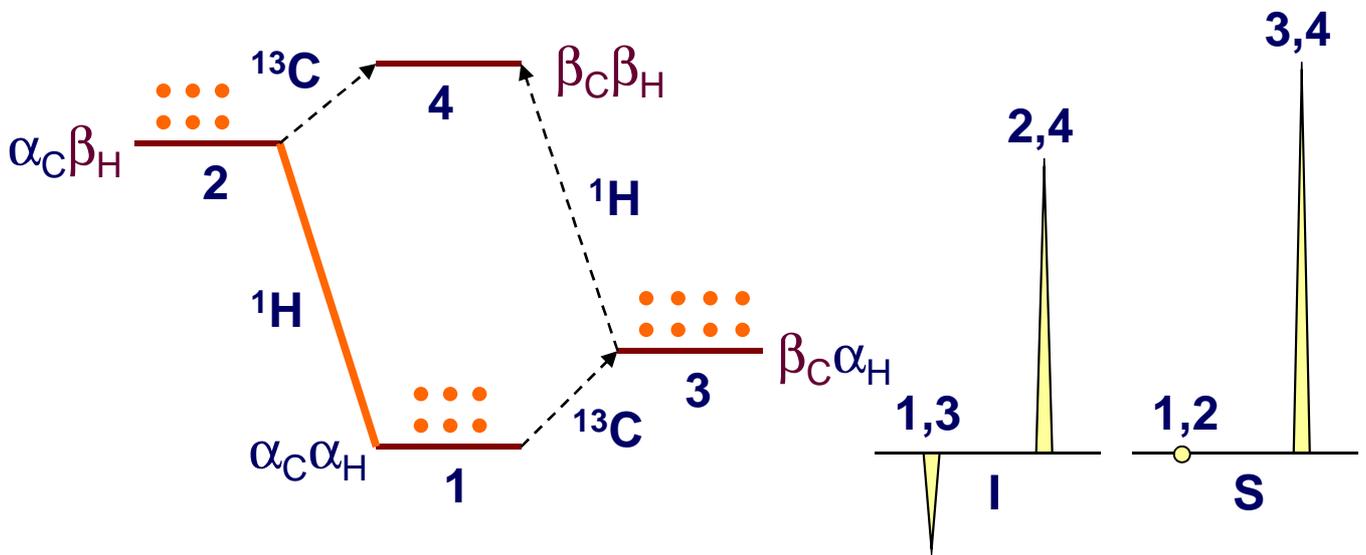

- The signals of both spins change accordingly, but now we have a **3-fold** increase for one of the ^{13}C transitions. Now we are talking

- If we consider the absolute values of the signal, we have twice as much signal as in the original spectrum...

Heteronuclear polarization transfer - SPI

- Now we do the same analysis for SPI. If we invert selectively the populations of **1**,**2**, we get the following:
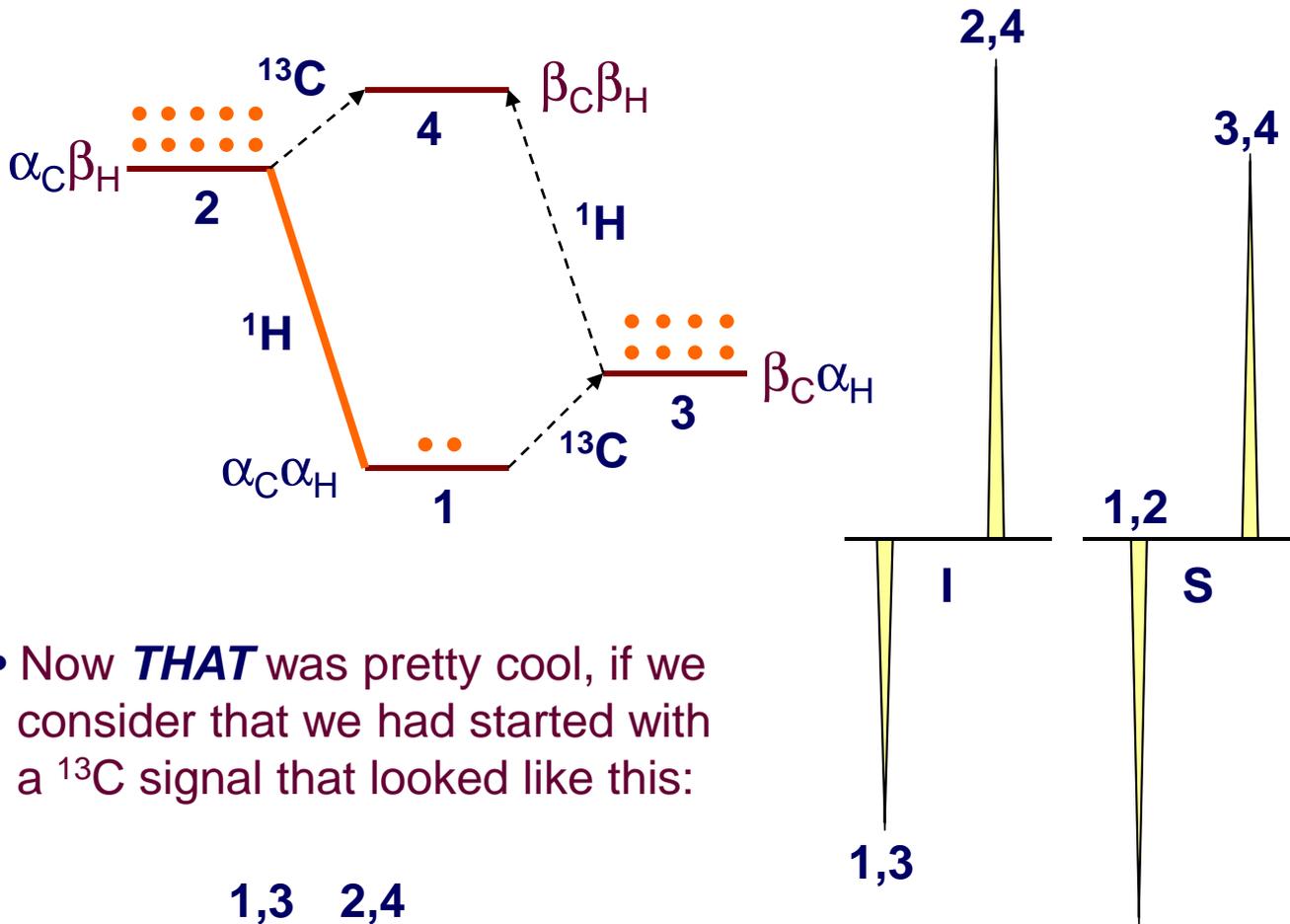

- Now **THAT** was pretty cool, if we consider that we had started with a ^{13}C signal that looked like this:
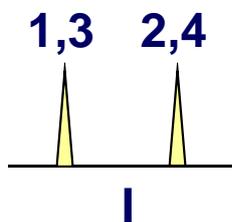
- By manipulating the polarization of the protons, we obtain an enhancement of **4** in the ^{13}C signal (considering positive and negative signals).
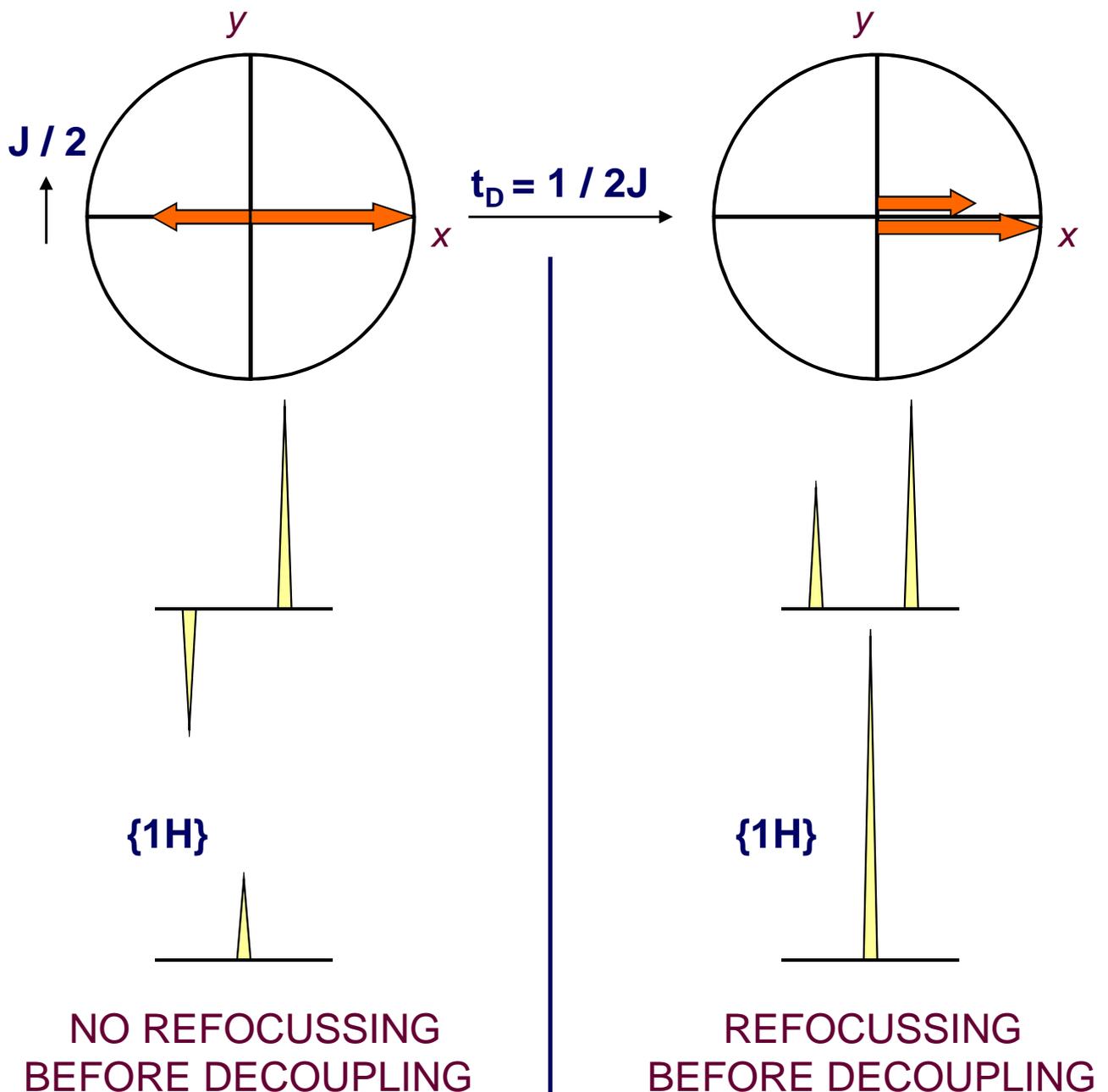
J-modulation and polarization transfer

• The increase of the ^{13}C signal is good and all that, but we still have to deal with a spectrum that is proton-coupled and has up and down peaks. We cannot decouple to do this, because the enhancement is there due the ^1H levels, which would be gone if we decouple…

• What we do is combine it with ***J-modulation***. Consider that we use the following pulse sequence:
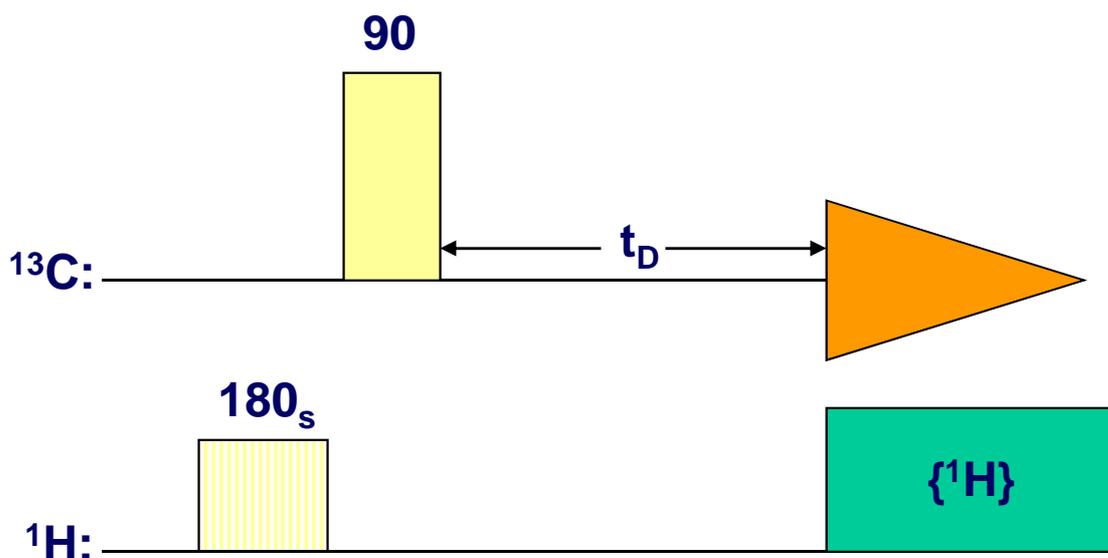
• We set t_D to **1 / 2J**. This means that after the $\pi / 2$ pulse on ^{13}C and the t_D, the ^{13}C magnetization will have ***refocussing*** couplings. We'll see it with vectors…

J-modulation and polarization transfer (…)

• We will only consider the ^{13}C magnetization, because for the ^{1}H we only inverted selectively the populations (the chubby π pulse). After the $\pi / 2$ ^{13}C pulse, we have the **+5** and **-3** components of the magnetization in the **<xy>** plane:

NO REFOCUSSING BEFORE DECOUPLING

REFOCUSSING BEFORE DECOUPLING

Selective polarization transfer with hard pulses

- So far, so good. One of the drawbacks of SPI and SPT is that we use selective pulses, which many times are hard to come by. It would be good if we could use hard pulses to do the same thing. The following ^{1}H pulse sequences do this.

- The first one is selective for ^{1}H lines that are on-resonance with both $\pi / 2$ pulses. Note that the pulses are applied on the same axis:

**90**   **90**

$t_D = 1 / 2J_{CH}$

t_D
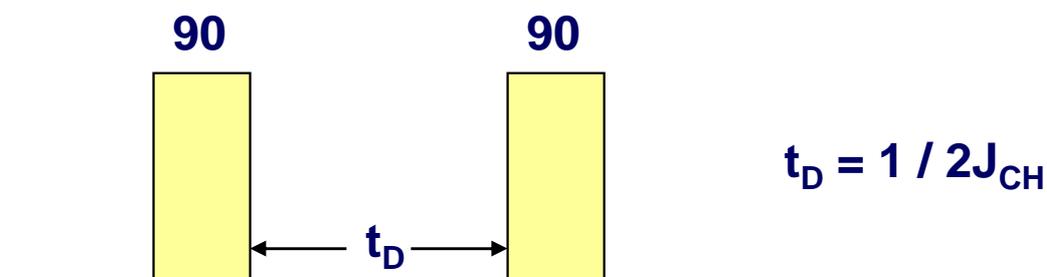
- The other one will invert the population of a single proton if the pulse is on resonance with the chemical shift of the doublet - It is at the center of the doublet:

90$_x$ **90$_y$**

$t_D = 1 / 2J_{CH}$

t_D
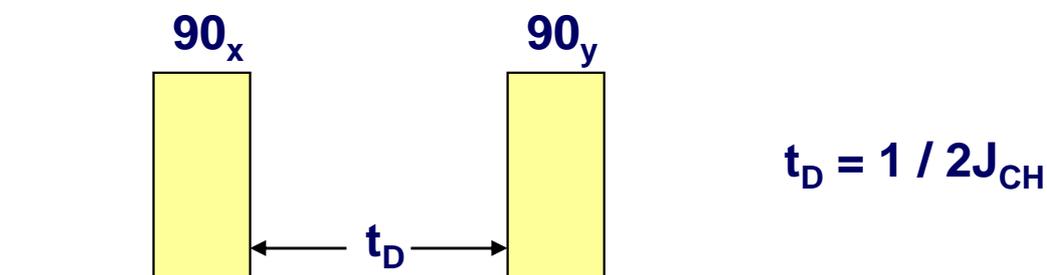
- In both cases, $t_D = 1 / 2J_{CH}$. We'll analyze the first one, and the other one will be part of homework or something...

SPT with hard pulses (continued)

- After the $\pi / 2$ pulse, both α and β vectors lie in the **+x** axis:
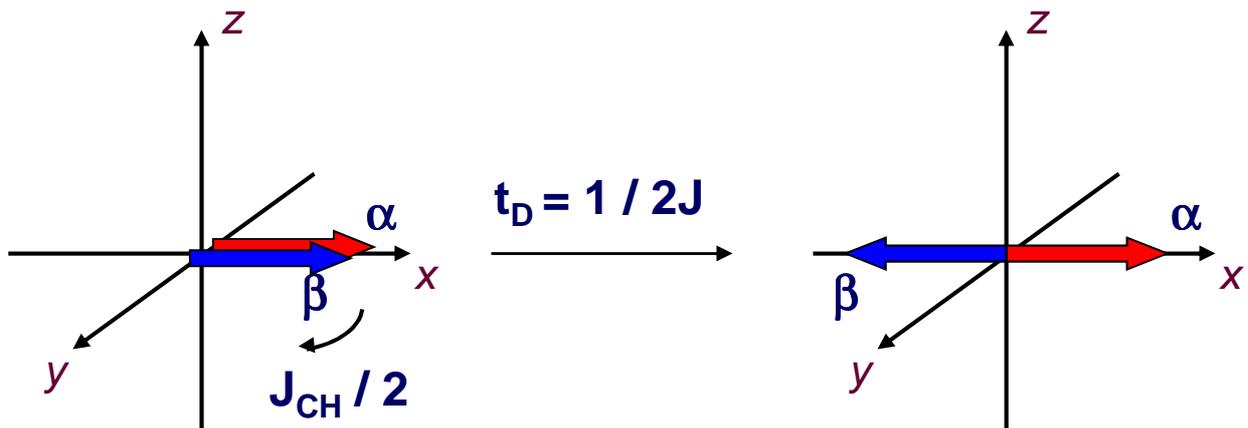
- If we wait $1 / 2J_{CH}$. seconds, we have that the faster vector (α) moves away from β by π radians. If at this point we apply the second $\pi / 2$ pulse, we invert the populations (α and β states will change location).
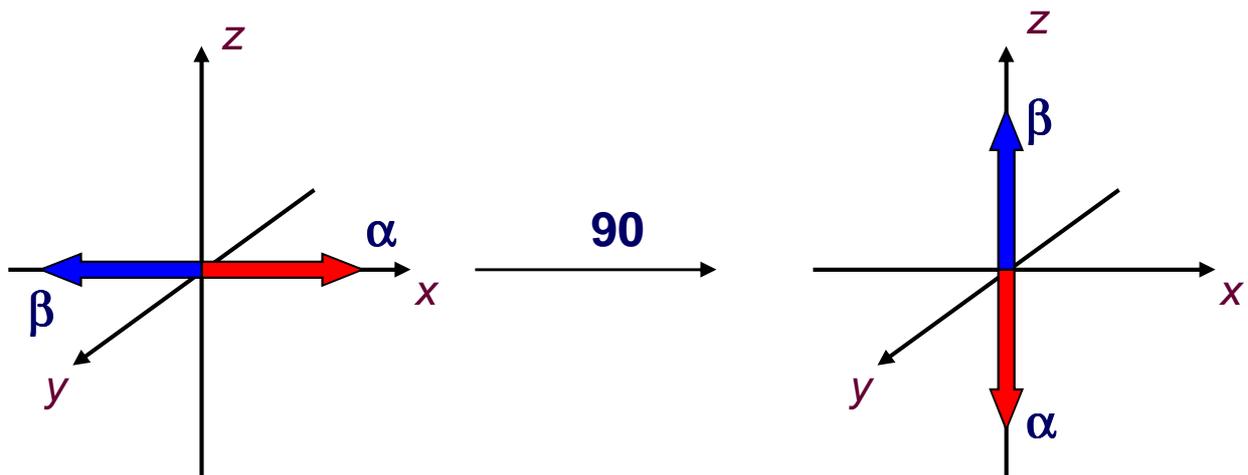

- This sequence can now be used together with ^{13}C excitation to see enhancement of ^{13}C nuclei attached to this proton.

Non-selective polarization transfer

- Another big pain of SPT and SPI is that it is selective, and we have to go one proton at a time. It would be nice if we could do all at once, so we transfer polarization from all protons to all the insensitive nuclei attached to them (^{13}C or ^{15}N)

- One way of doing this is combining the last pulse sequence with a spin-echo with a $t_D = 1 / 4J_{CH}$

90   **180$_{^1H}$ & 180$_{^{13}C}$**   **90**

$$t_D = 1 / 4J_{CH}$$

t_D t_D
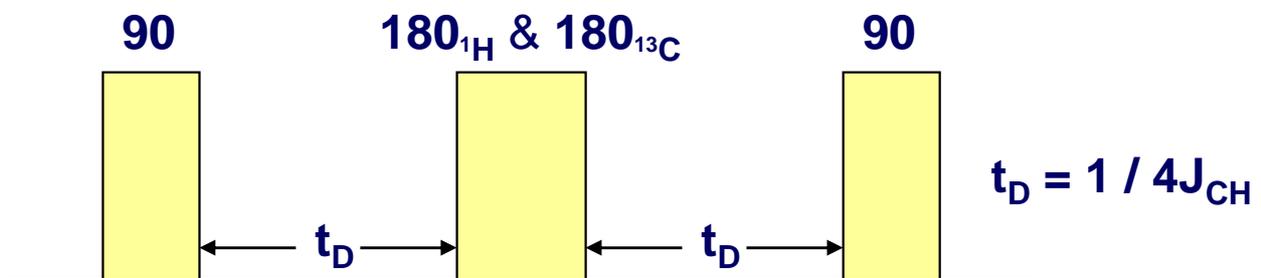
- The π pulse and the 2 t_D delays refocus chemical shift, so the populations of all protons in the molecule will be inverted The π pulse on the **X** nucleus flips the α and β labels:

180$_{^1H}$
180$_{^{13}C}$

t_D
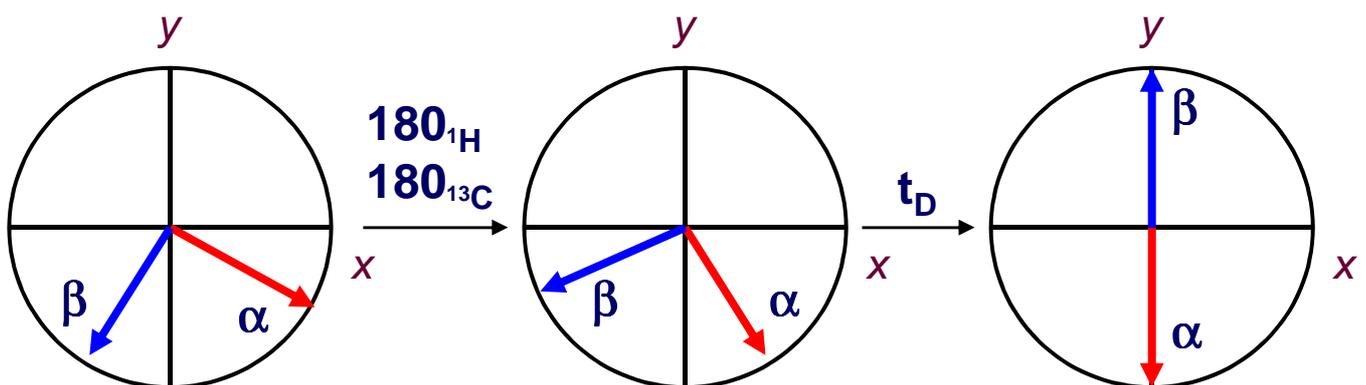
- Now the $\pi / 2$ will flip the α and β vectors back to the **z** axis, and we have inversion of the ^1H populations.

Non-selective polarization transfer - INEPT

- If we expand this last sequence a little bit more we get **INEPT** (*Insensitive Nuclei Enhancement by Polarization Transfer*). It is an important pulse sequence building block found throughout multiple pulse sequences.

- It is used to increase the sensitivity (polarization) of nuclei such as ^{13}C and ^{15}N. It looks like this:
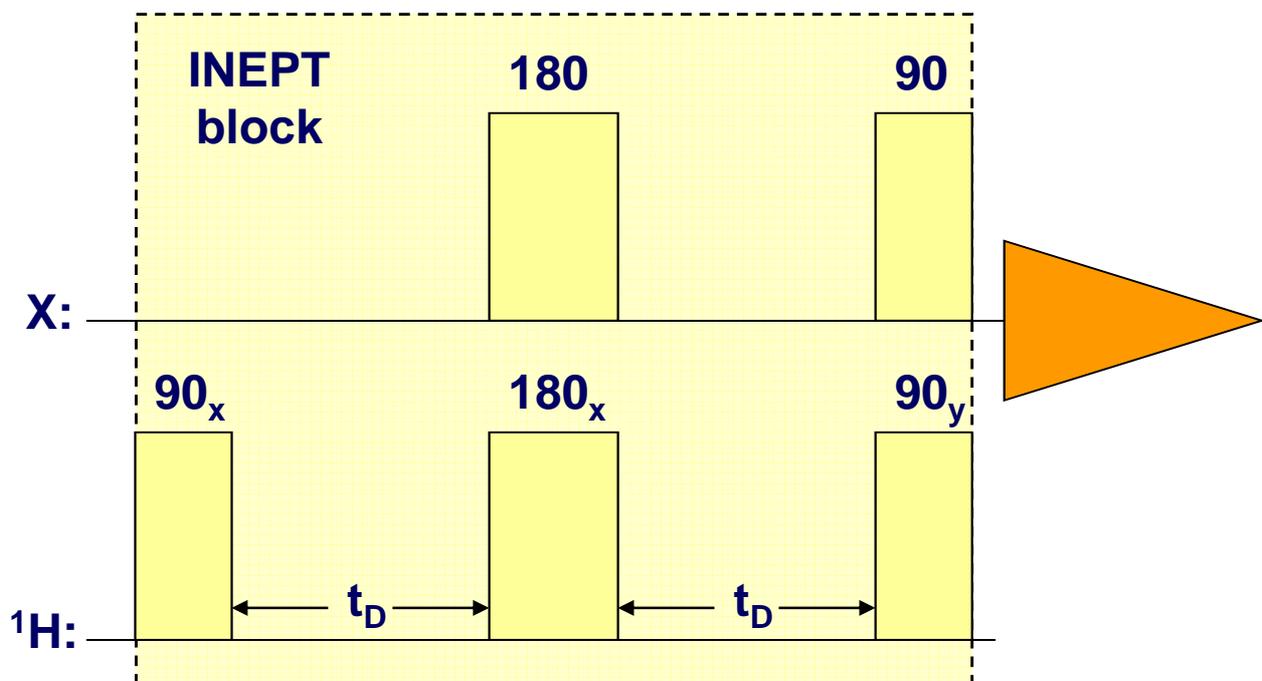
- Here **X** is either ^{13}C or ^{15}N. The analysis is the same as what we saw for the protons, plus the 'read' $\pi / 2$ pulse for the **X** nuclei to create (and be able to detect) transverse magnetization.

Refocused INEPT

- With the regular INEPT we still have the '**+5** up' and '**-3** down' problem. We would like to have the two lines refocused into a single line, and we already know normal decoupling is not and option.

- We simply combine the INEPT sequence with a refocusing chunk at the end, and detect in the **-y** axis:
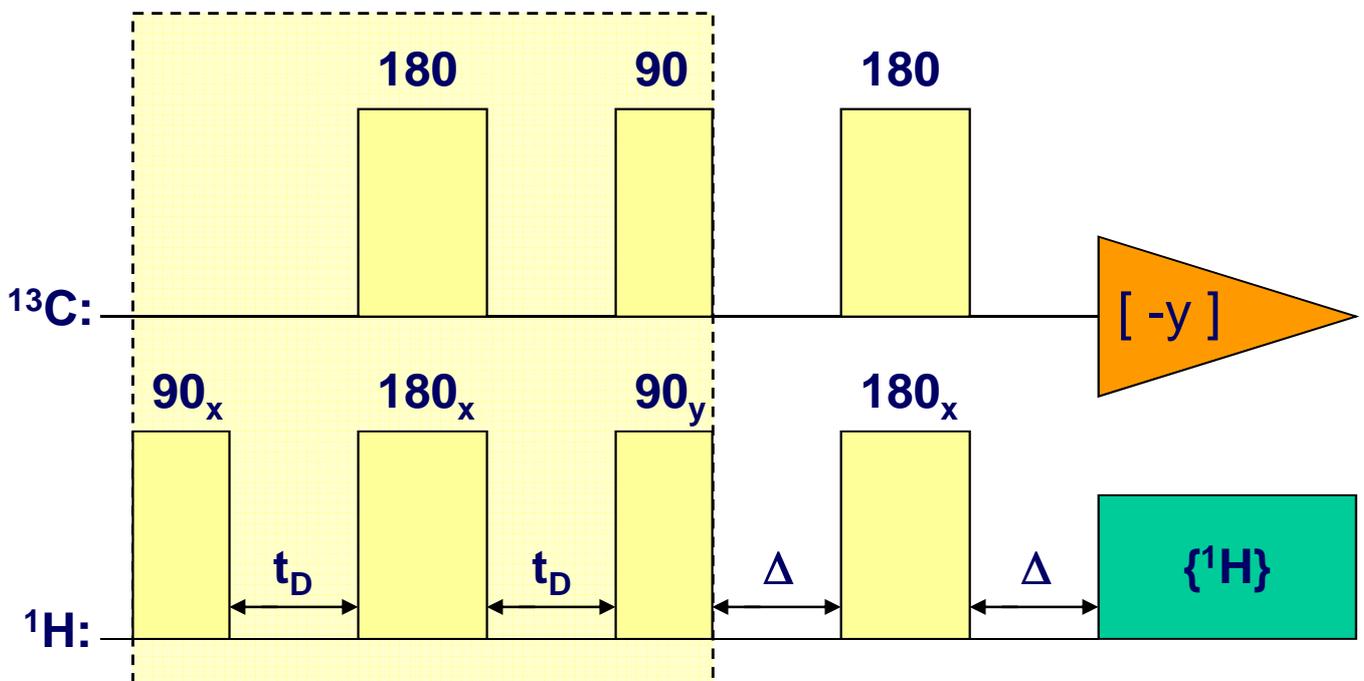
- Depending on the type of carbon we use different Δ delays:

    - CH - $\Delta = 1 / 4J$
    - CH$_2$ - $\Delta = 1 / 8J$

- For all types of carbons to have more or less the same enhancement, we use $\Delta \approx 1 / 7J$.

Refocused INEPT (continued)

- After the $\pi/2$ ^{13}C pulse, we have the enhanced (**+5** & **-3**) ^{13}C magnetization on the **<xy>** plane.
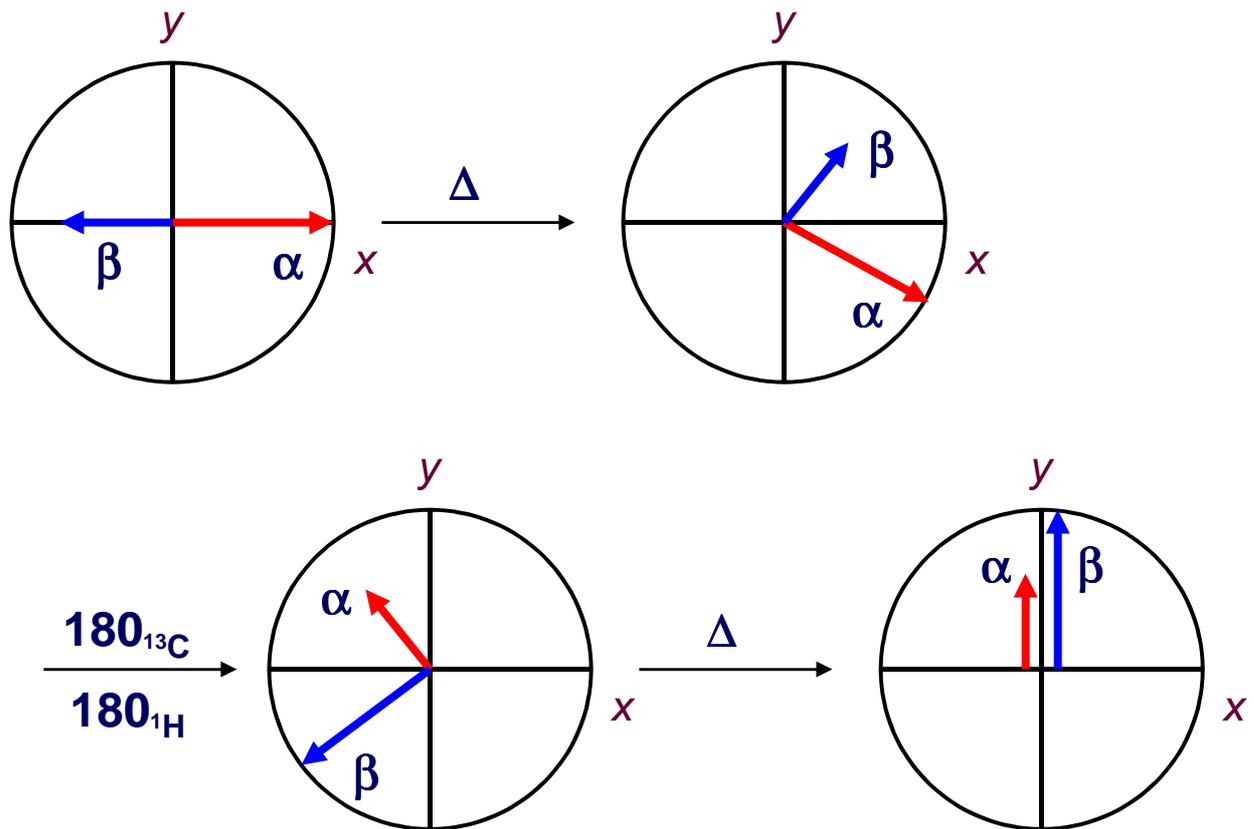

- Variations of this sequence is all over the place. With it we can transfer polarization to and from insensitive nuclei (^{13}C, ^{15}N, ^{29}Si, etc., etc.). We can also use it to *edit* spectra as well as to *label* a certain type of nuclei in the sample with information from others (δ's, **J**'s).

- The block is used in almost all protein NMR pulse sequences involving ^{1}H, ^{15}N, and ^{13}C.
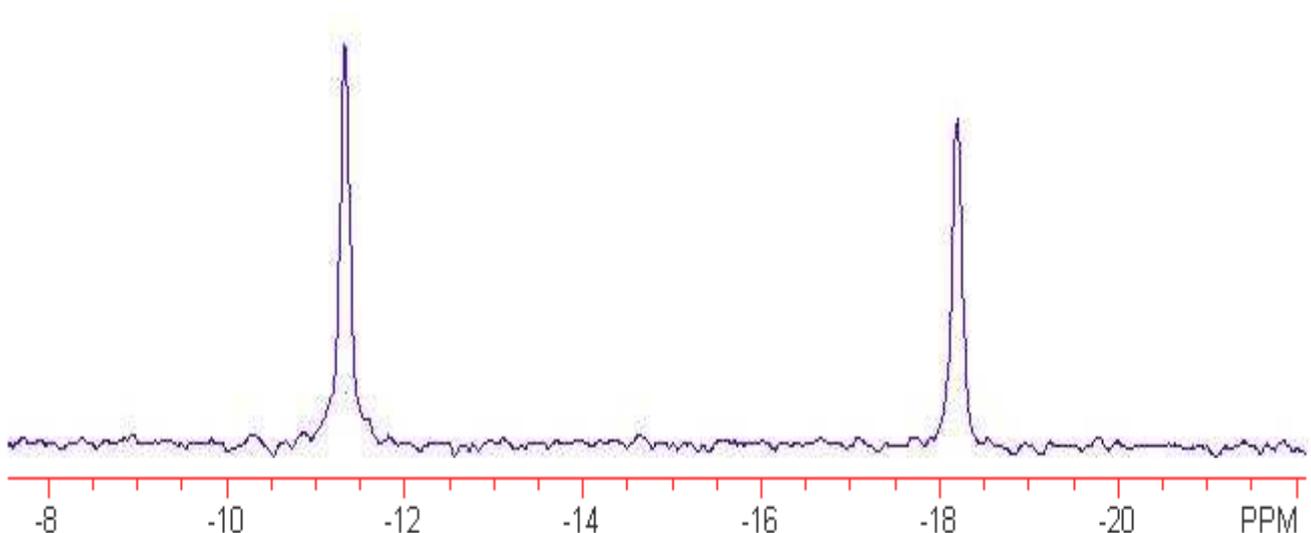
INEPT reenfocado (continuado)

- Example of INEPT from ^{1}H to ^{29}Si. Dow 709 difussion pump oil (courtesy of Anasazi Instruments, Inc.):

- Normal ^{29}Si 1D spectrum:
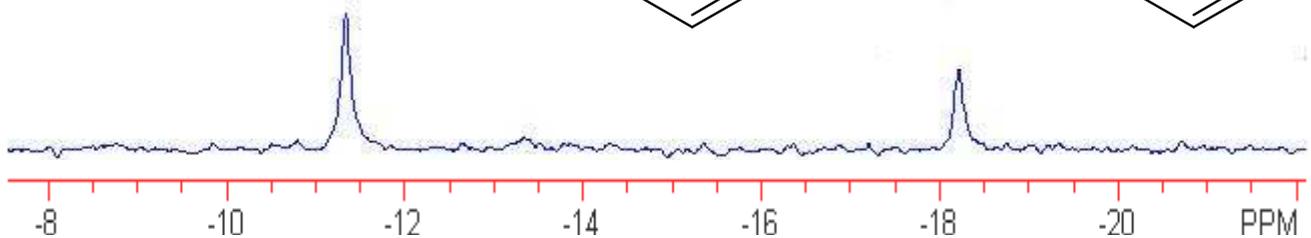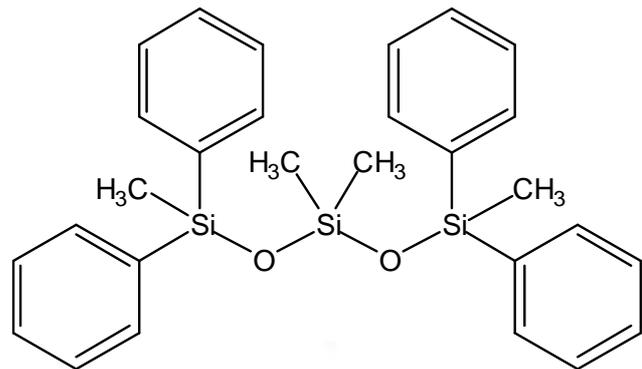
- refocused ^{29}Si INEPT spectum:

- The $^2J_{1H-29Si}$ coupling is ~7 Hz, and the $\gamma_{1H} / \gamma_{29Si}$ ratio is 5.

More polarization transfer - DEPT

- **DEPT** (**Distortionles Enhancement by Polarization Transfer**) is another sequence that takes advantage of the surplus ^1H population to see ^{13}C signals. Furthermore, it can **edit** the signals in order to obtain response from CH, CH_2, and CH_3 according to the settings of the sequence:
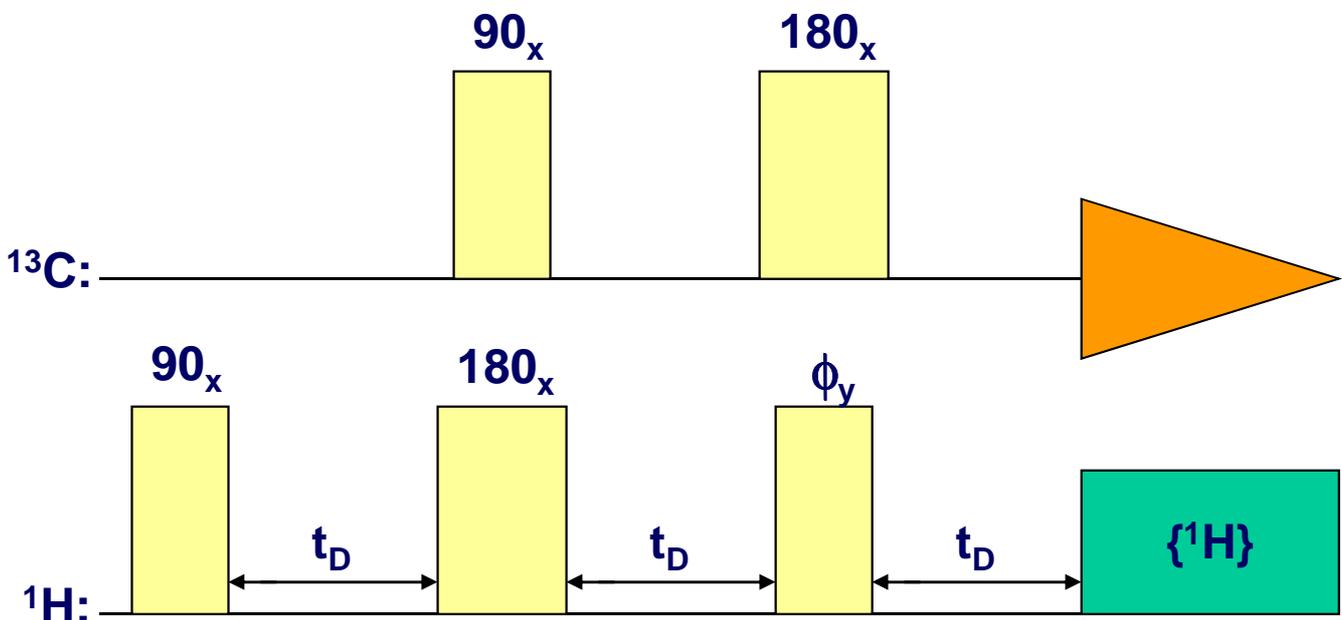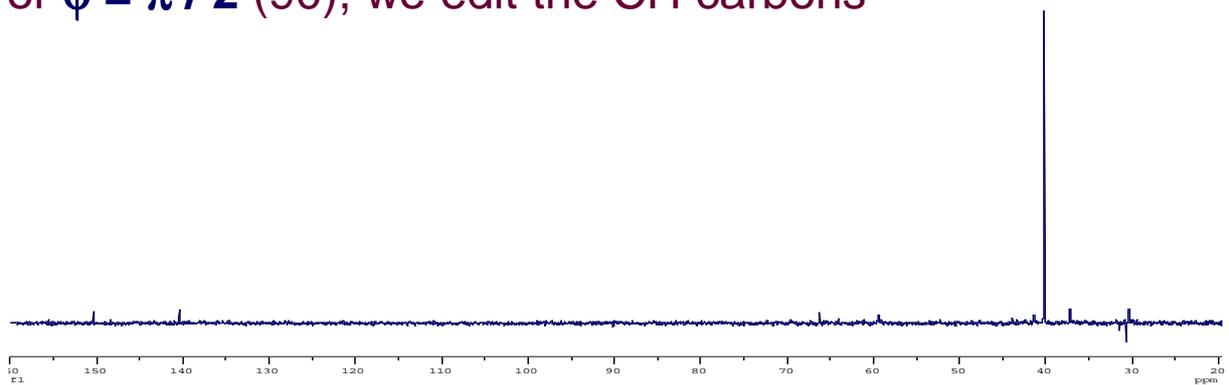

- Unfortunately, it relies on the creation and manipulation of **multiple quantum** magnetization (the ^{13}C $\pi / 2$ pulse) which we cannot see or represent with vectors.

- However, we'll describe the results for different phases of ϕ.
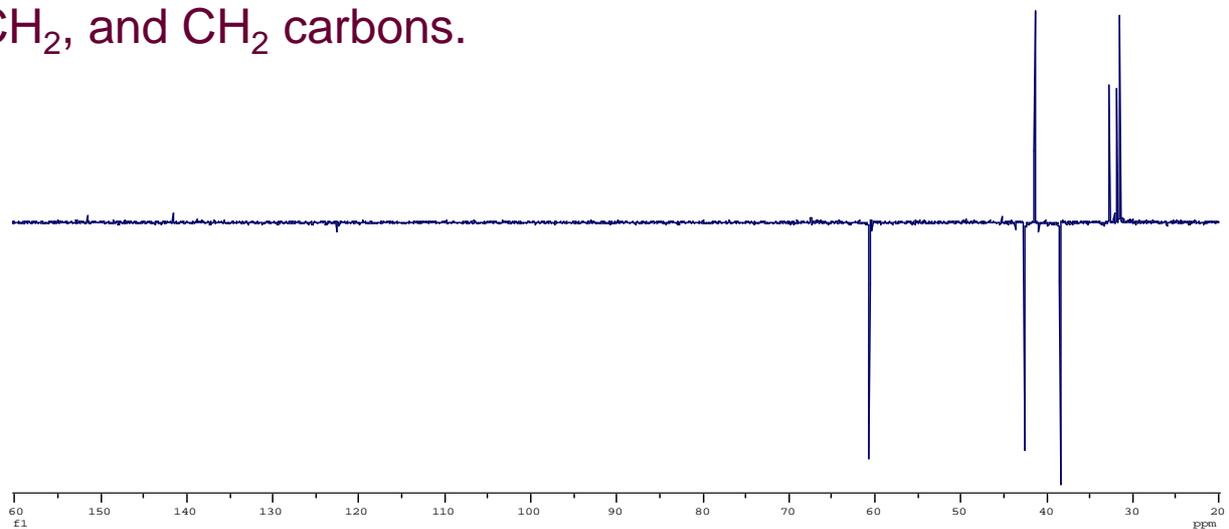
DEPT results for different φ values

- Using pulegone as an example (real data…)
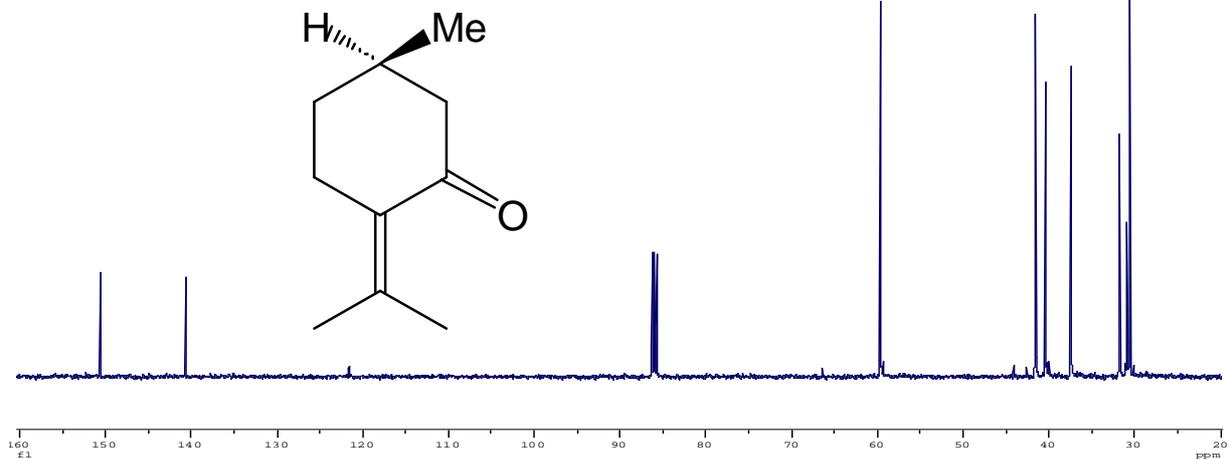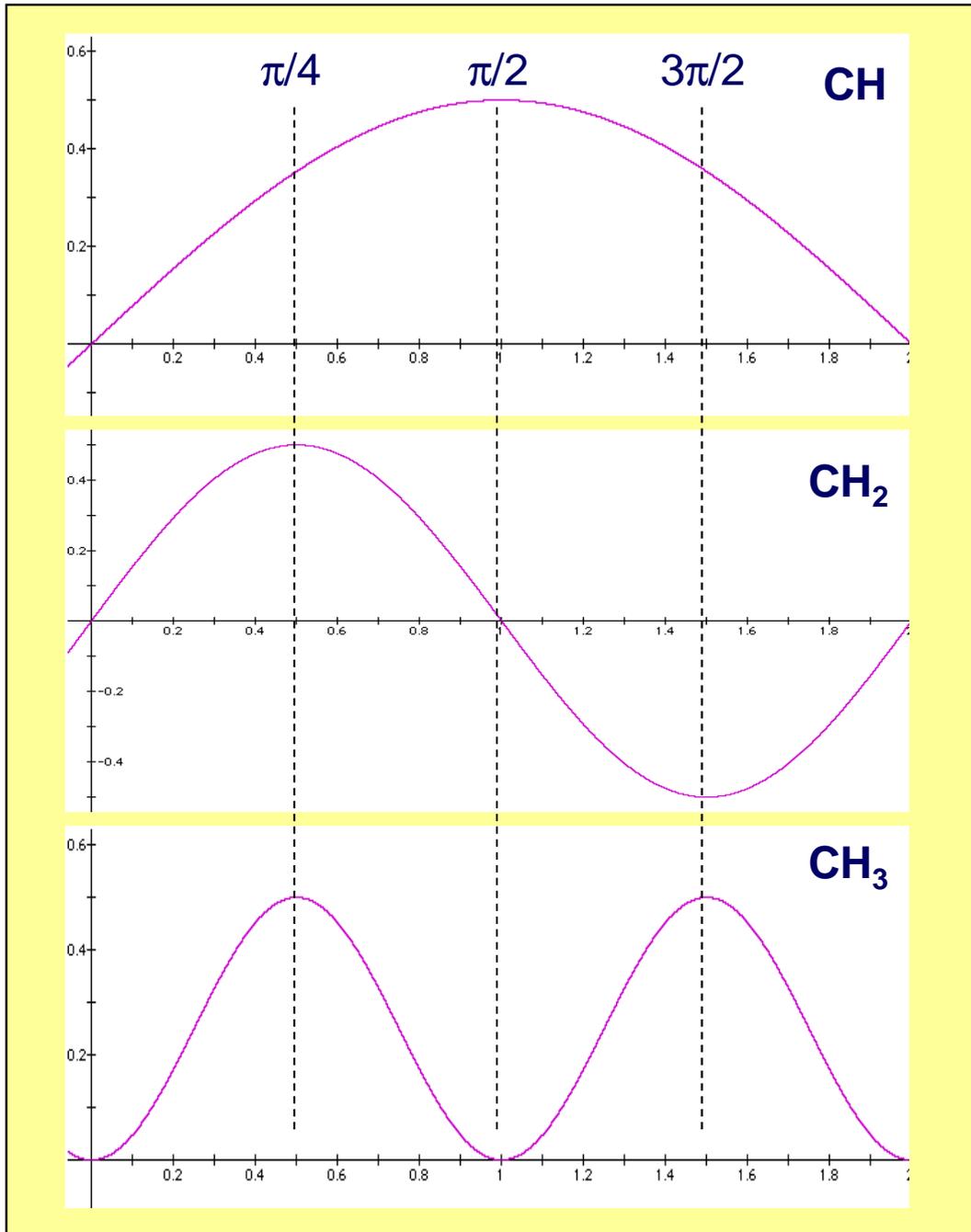

- For φ = π / 2 (90), we edit the CH carbons

- For φ = 3π / 4 (135), we can distinguish CH, CH_2, and CH_2 carbons.

DEPT (continued)

- If we plot the responses for different carbons versus the tip angle ϕ of the ^1H pulse, we get:

- By adding/substractig different DEPT's we can get four independent spectra for C, CH, CH_2, and CH_3…