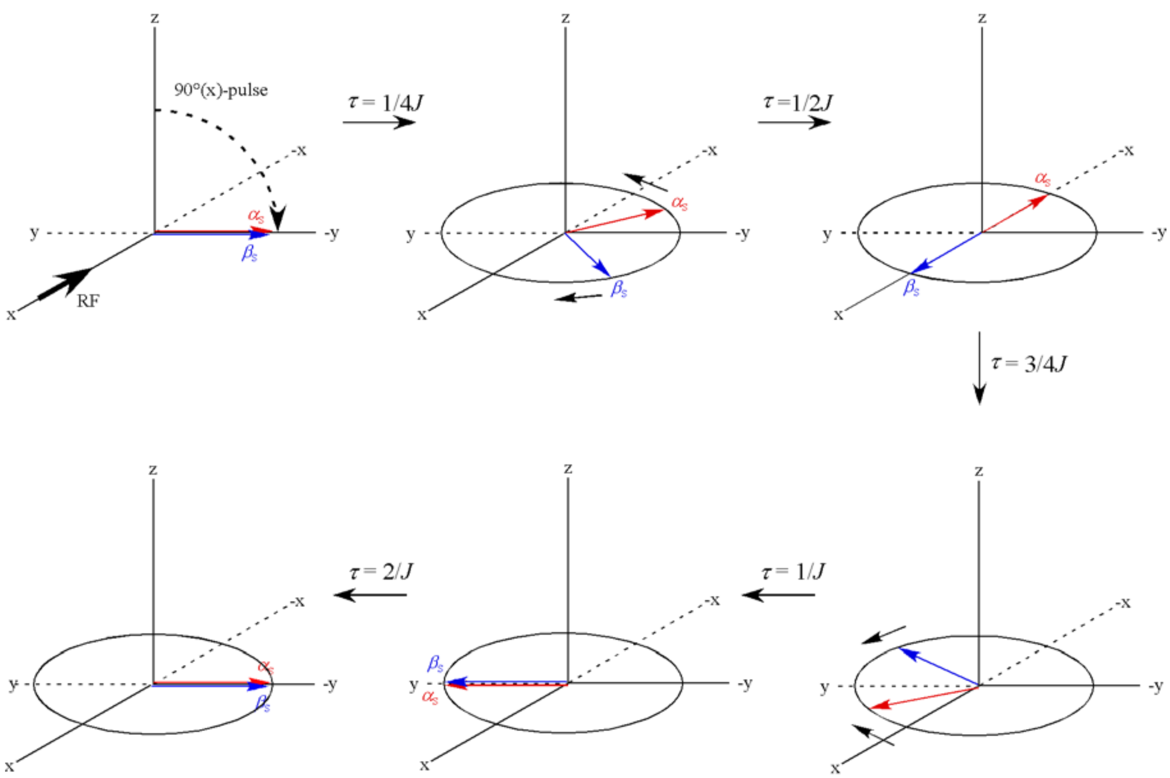
Polarization Transfer

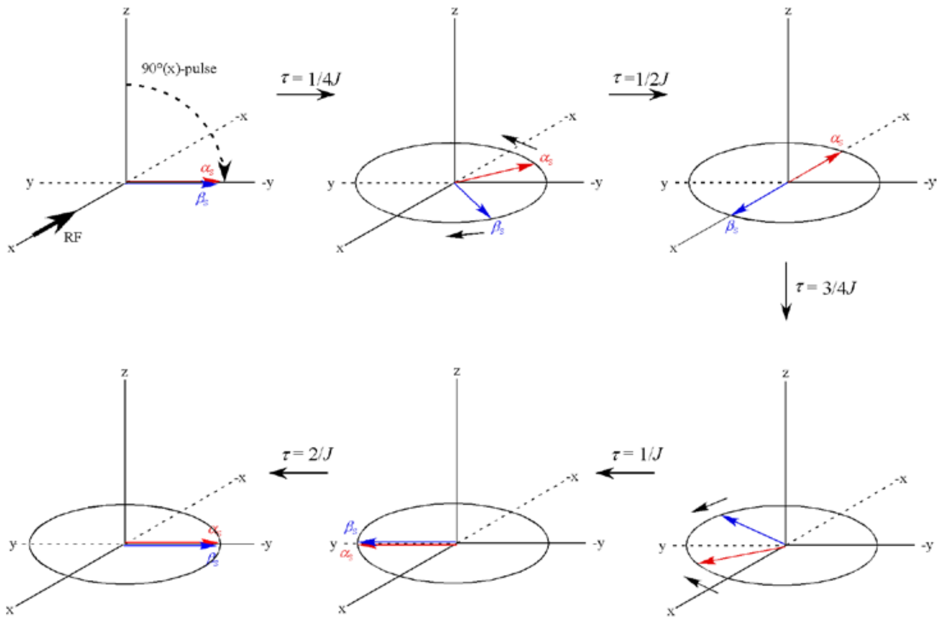
Now something really interesting happens…

From the perspective of our instrument (lock-in receiver) spins that have been rotated onto the xy-plane by a 90°-pulse and will either appear to remain on the –y-axis or precess about the z-axis depending on whether they resonate at the carrier frequency.

If we consider *J*-coupled spins, *I* and *S*, things get even more complicated. Since *J*-coupling causes a single resonance to split into two peaks at $\pm J/2$, even resonances that would otherwise resonate at the carrier frequency will be offset from the carrier frequency and precess about the z-axis.

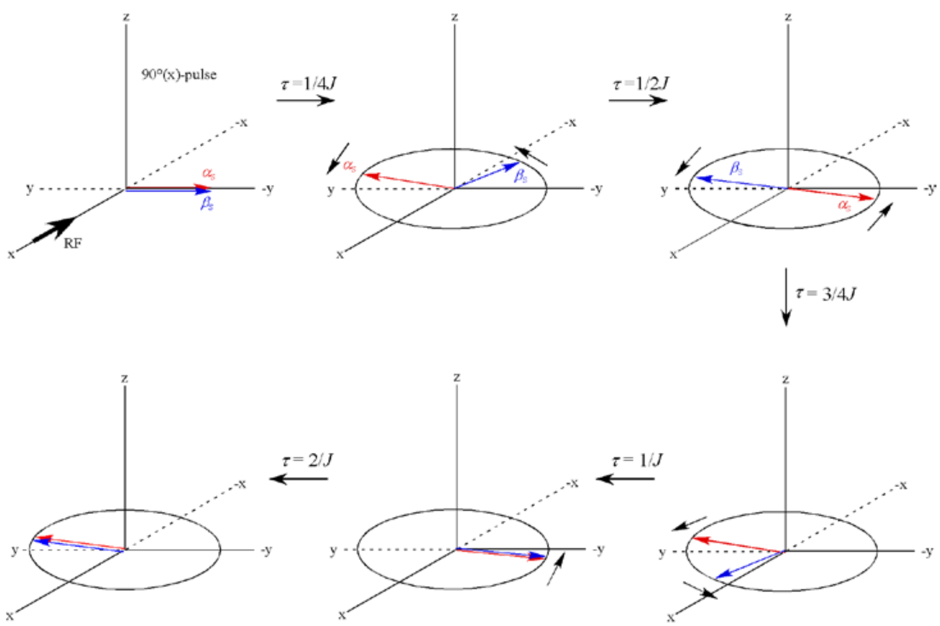
The figure below demonstrates how this effect would manifest itself if we applied a 90°-pulse to the *I*-spins and observed the subsequent time evolution:





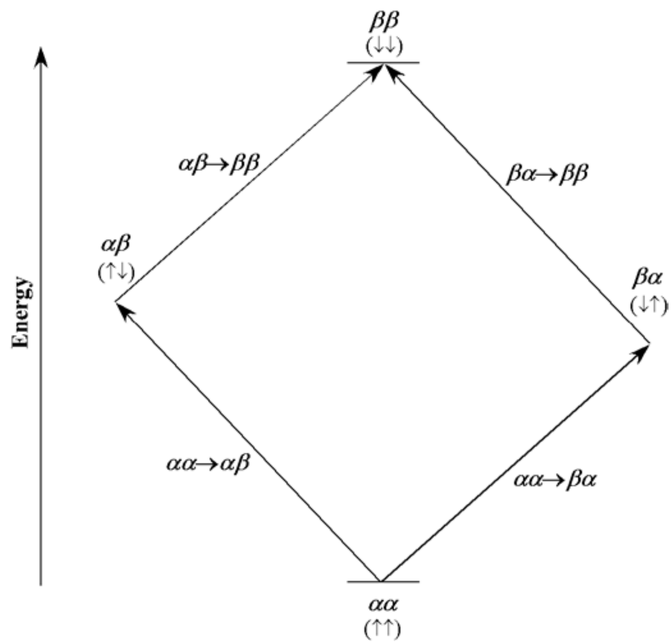
The α_S and β_S designate components of the *I*-spin (aka ^{1}H) that are coupled to the $(\uparrow)$ and $(\downarrow)$ states of the *S*-spin (aka ^{13}C).

If the resonance frequency of the *S*-spin is different from the carrier frequency, we would see the following:



Note that the precession of the vectors in the offset-case depends both on the frequency offset and the magnitude of the *J*-coupling.

Energy Levels and Populations



The quantum mechanical representation of the energy of a spin in a magnetic field is given by the following:

$$E = -\hbar \gamma m_s B_0$$

wherein $m_s = \pm 1/2$.

In explicit terms then, for a spin-1/2 particle in a magnetic field there are 2*I*+1 or 2 energy levels:

$$E_\alpha = -\hbar \gamma B_0 / 2 \text{ and } E_\beta = \hbar \gamma B_0 / 2$$

The energy separation of these levels is linearly dependent on the strength of the applied magnetic field

$$\Delta E = \hbar \gamma B_0$$

The populations of the energy levels conform to a Boltzmann distribution:

$$\frac{N_\beta}{N_\alpha} = e^{-\Delta E / k_b T}$$

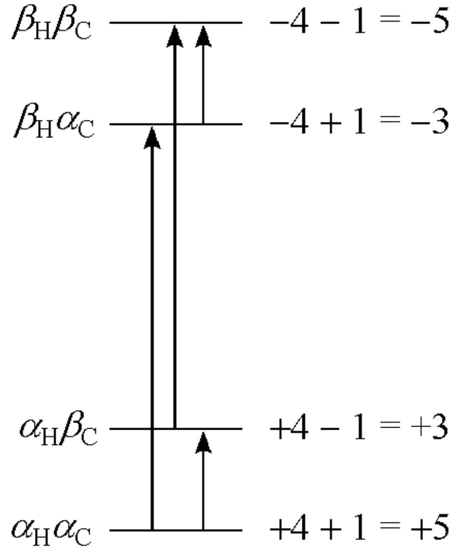
As shown above, the energy depends upon the strength of the applied static magnetic field and the gyromagnetic ratio, γ, of the nuclei.

Selective Population Transfer

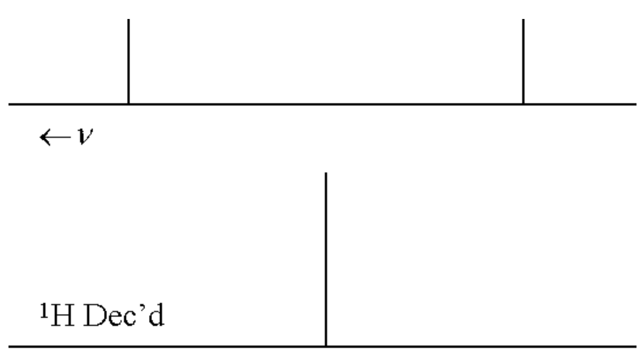
Pachler, K.G.R. and Wessels, P.L. (1973) Selective Population Inversion (SPI). Pulsed Double Resonance Methods in FT (Fourier Transform) NMR Spectroscopy Equivalent to INDOR (Internuclear Double Resonance). *J. Magn. Reson.* **12**, 337-339.

If we consider a coupled ^{1}H-^{13}C spin-pair, then energy separation for the ^{1}H transition is 4 times greater than the energy of the ^{13}C transition, i.e., $\gamma_{\mathrm{H}} = 4\gamma_{\mathrm{C}}$

We can specify the relative populations of the energy levels for the ^{1}H-^{13}C pair at equilibrium in the figure shown below:

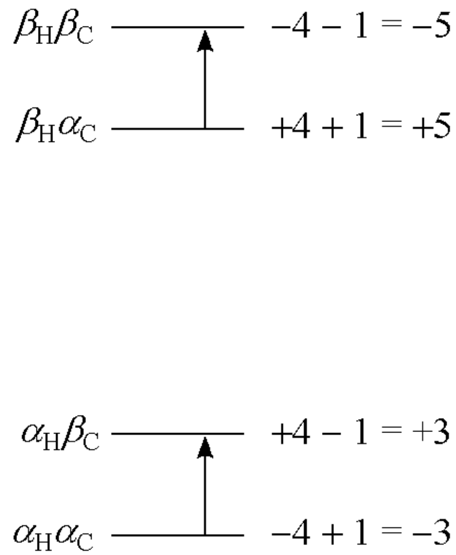


The ^{13}C NMR spectrum in this case would schematically look like that shown below:

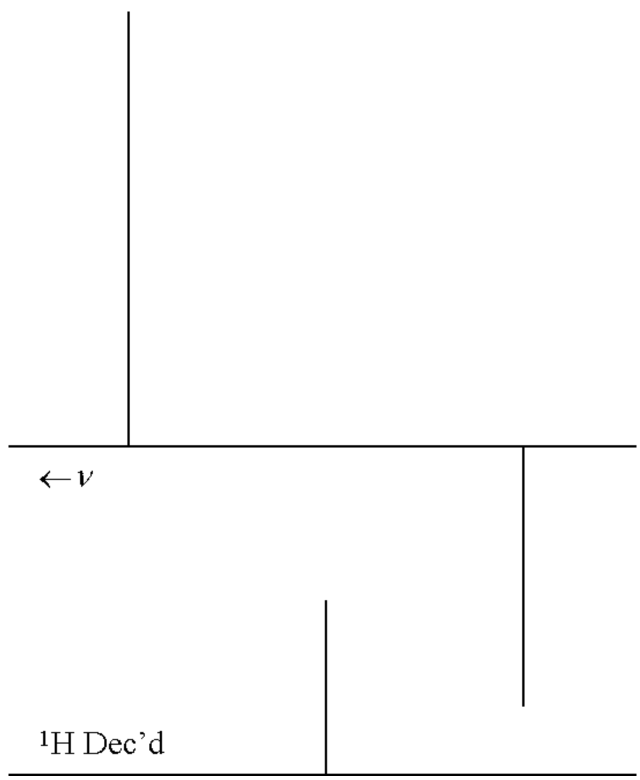


Remember that the peak intensity depends upon the population difference, i.e, $5-3=2$ and $-3-(-5)=2$.

If we apply a [1]H inversion pulse to the $\alpha_{\mathrm{H}}\alpha_{\mathrm{C}} \rightarrow \beta_{\mathrm{H}}\alpha_{\mathrm{C}}$ transition, we will convert the relative populations as shown below:



In this case the relative population differences have changed to $-3-3=-6$ and $5-(-5)=10$, and the ^{13}C NMR spectrum in this case would change as indicated in the following figure:



In this example, the population inversion of the ^1H spins causes a significant change in the relative intensity of the two ^{13}C peaks. The original ratio of 1:1 has been changed to 5:-3.

Note that the polarization change depends upon the relative magnitude of the gyromagnetic ratio of the interacting spins.

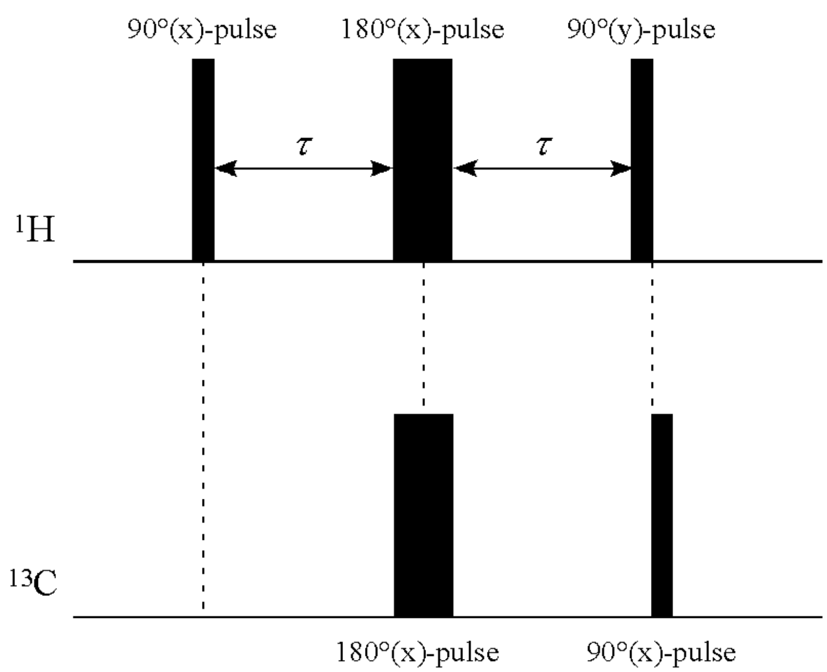
Note also that the decoupled spectrum has the same intensity as the case in which ^1H were left unperturbed.

Of course such selective inversion of a particular ^1H transition will be possible only in the simplest cases and as usual, we seek a general solution.

INEPT: Insensitive Nuclei Enhanced by Polarization Transfer.

Morris, G.A. and Freeman, R. (1979) Enhancement of Nuclear Magnetic Resonance by Polarization Transfer. *J. Am. Chem. Soc.* **101**, 760-762.

Consider the following pulse sequence:



The sequence may be written in our shorthand notation as:

$$
\begin{array}{ll}
{}^{1}\text{H: } & 90^{\circ}_{x} - \tau - 180^{\circ}_{x} - \tau - 90^{\circ}_{y} \\
{}^{13}\text{C: } & \qquad\qquad\quad 180^{\circ}_{x} - \tau - 90^{\circ}_{x}
\end{array}
$$

The pulse sequence consists of a few more pulses than we have previously encountered, so let's look at the sequence in detail.

The initial ^{1}H 90°(x)-pulse, generates transverse magnetization that evolves for an amount of time given by $\tau = 1/4J$.

Next a pair of 180°-pulses simultaneously at ^{1}H and ^{13}C frequencies if applied. These pulses should be expected to invert the magnetization of the ^{1}H and ^{13}C nuclei simultaneously.

The magnetization continues to evolve for another interval, $\tau = 1/4J$.

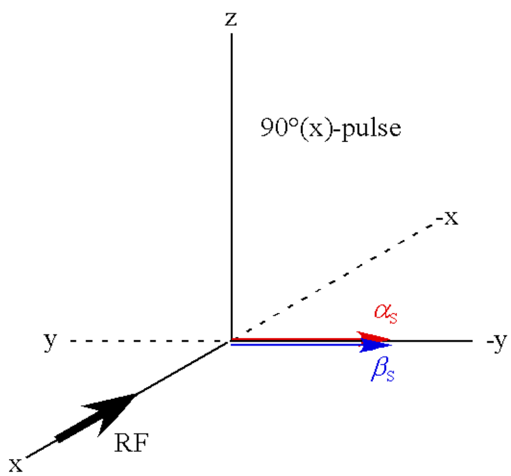
Finally, a ^{1}H 90°(y/□y)-pulse is applied followed by a ^{13}C 90°(x)-pulse.

We have already seen that we can control the evolution (precession) of magnetization in the xy-plane using 180°-pulses, i.e., spin echoes. This sequence makes use of this concept, albeit in a more complex way.

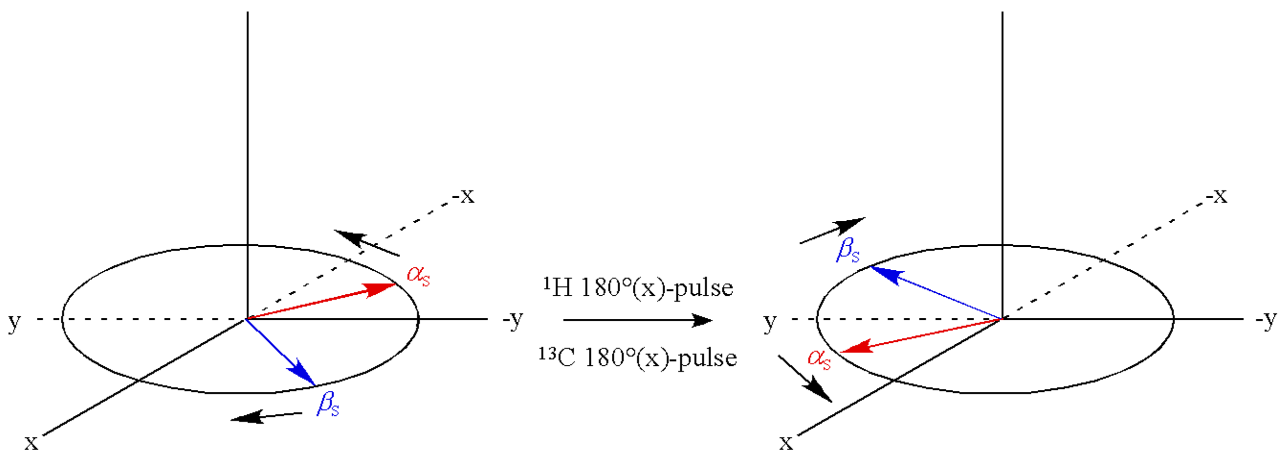
The INEPT sequence allow us to invert the ^{1}H spins that are coupled to the β ^{13}C spins will leaving the state of the ^{1}H spins that are coupled to the α ^{13}C spins unchanged.

We'll now consider this process in a step-by-step fashion…

The initial step in the process involves generation of transverse ^1H magnetization using a 90°(x)-pulse:

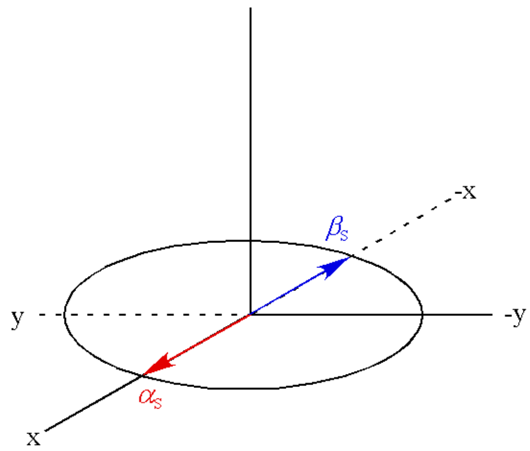


The spins evolve in the xy-plane and at precisely $\tau = 1/4J$, the pair of 180°(x)-pulses is applied:



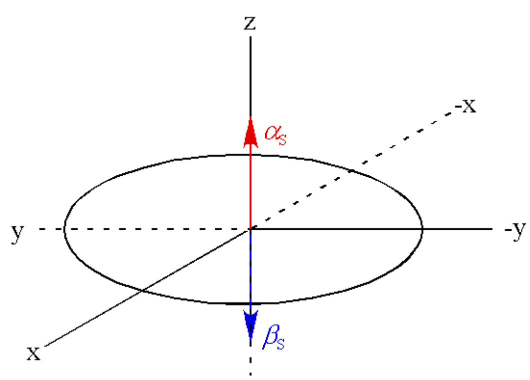
This causes the ^1H vectors to interchange, but also causes the ^{13}C spin-states to invert, which causes the vectors to continue to precess in the same direction. Contrast this behavior with what we see for the spin echo experiment.

After another $\tau = 1/4J$ interval – total evolution for time equal to $1/2J$, the two ^{1}H vectors will be aligned in opposite directions:



Thus although frequency offsets will be refocused in a spin-echo fashion, the components of the ^{1}H spins coupled to the α and β ^{13}C states have ended up in opposite directions, i.e., were not refocused.

The ^{1}H 90°(y)-pulse converts this +/– transverse condition into a longitudinal condition in which one state (α or β depending on whether a +y or –y pulse is used) is inverted while the other is unchanged.



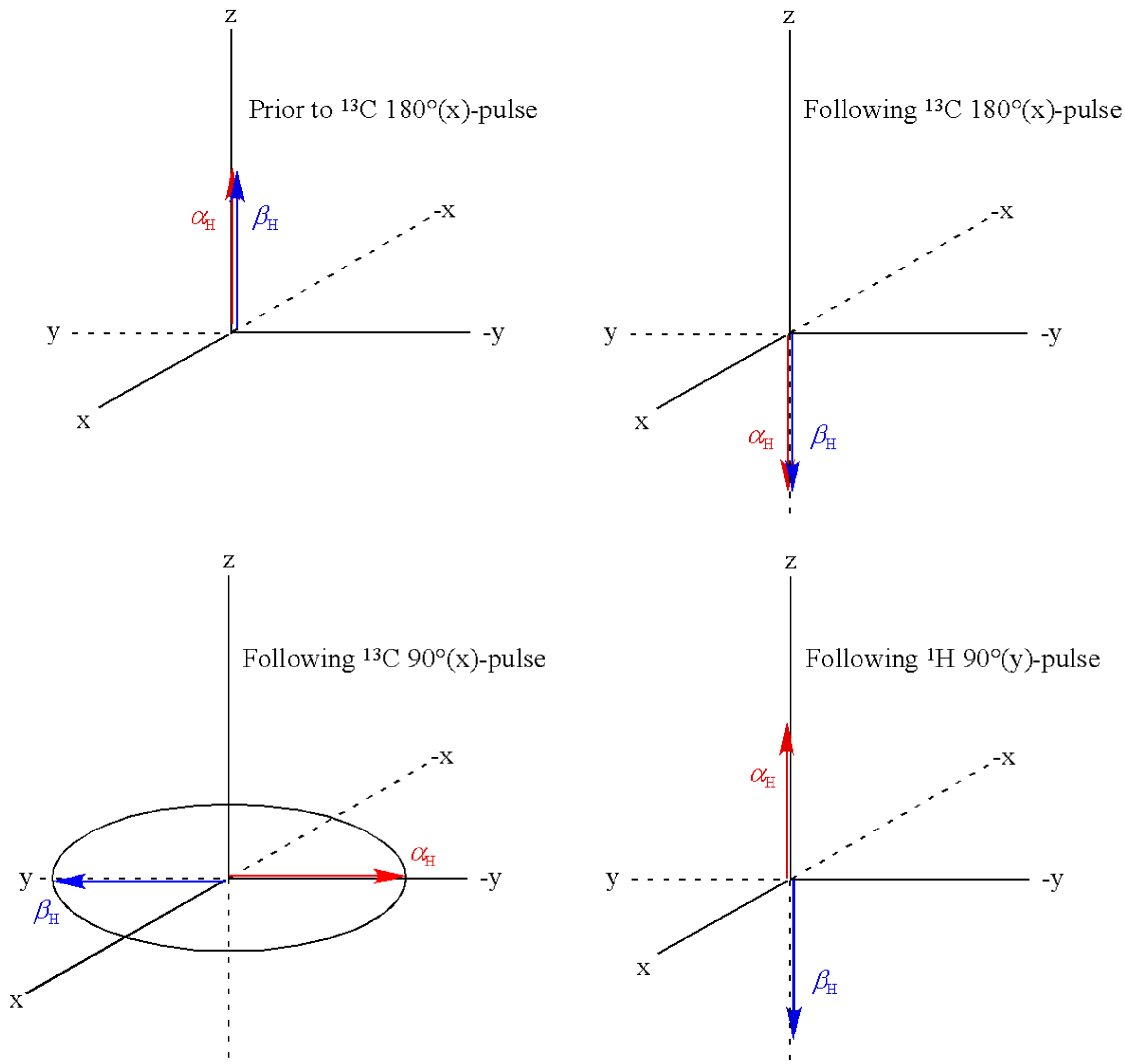
The INEPT experiment is thus a broadband version of the SPT experiment.

The delays in the pulse sequence we have outlined serve to build a phase (angular) relationship between the α and β components of the evolving *I*-spin magnetization.

The 180°-pulses on ^{1}H and ^{13}C resonances insure that frequency offsets are refocused while the phase relationship the develops due to the *J* coupling continues to evolve.

The final pair 90°-pulses, first on ^{1}H and then on ^{13}C resonances, transfers the phase relationship and the polarization from ^{1}H nuclei to the ^{13}C nuclei.

This transfer is demonstrated in the following figure:



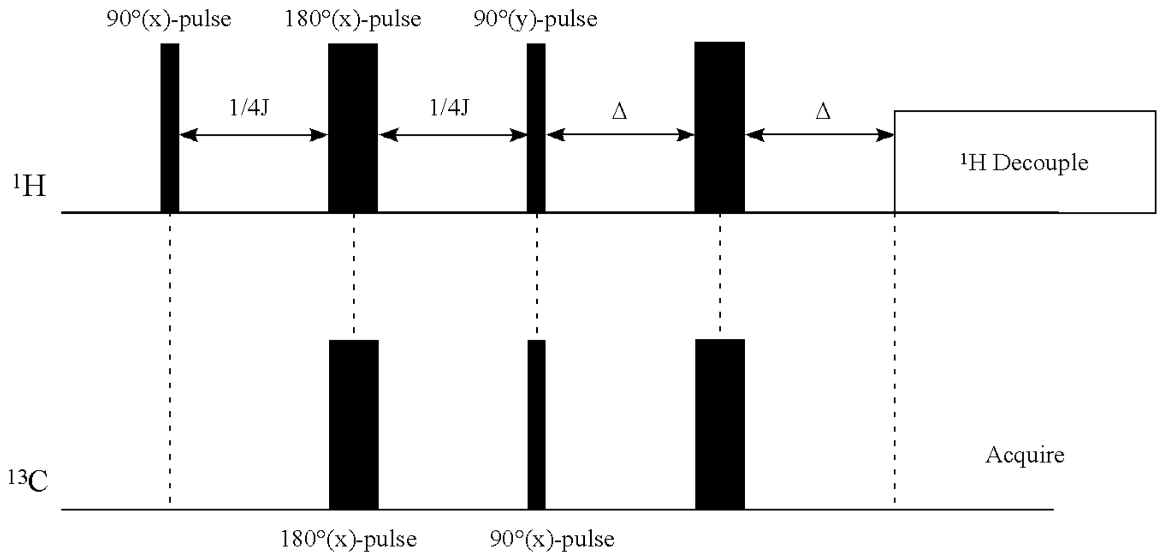
Although the basic INEPT sequence produces a substantial enhancement of the ^{13}C polarization, the inability to use ^{1}H decoupling effectively poses a serious limitation.

This drawback was immediately recognized by the inventors of the method and an improved version of the sequence was soon produced.

In this improved sequence, the two ^{13}C vectors are allowed to refocus, using the same techniques we have just described, producing a greater level of *sensitivity enhancement*.

Refocused INEPT

The timing diagram for the new sequence is shown below:



The optimal value for the Δ delay depends on the multiplet pattern, $1/4J$ is optimal for doublets and $1/6J$ is the accepted consensus value.

A direct comparison between the sensitivity enhancement possible using INEPT to that obtained from the NOE is given below – note that since ^{1}H nuclei are decoupled during ^{13}C acquisition, the INEPT experiment benefits from NOE enhancement as well:

$$\text{NOE: } I = I_0\left(1+\frac{\gamma_I}{2\gamma_S}\right) \approx 3I_0$$

$$\text{INEPT: } I = I_0\left|\frac{\gamma_I}{\gamma_S}\right| \approx 4I_0$$