

Part V: Multiple Pulse NMR and double resonance

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1. Heteronuclear Decoupling

In the ^{13}C spectra of $^{13}\text{C}-^1\text{H}_n$ groups the signal will be split by large $^1J_{\text{CH}}$. This splitting not only reduces the sensitivity of signals which often are already naturally weak, but also can cause severe overlap in complex spectra. Therefore, in routine C-13 NMR spectra this coupling is removed by applying decoupling.

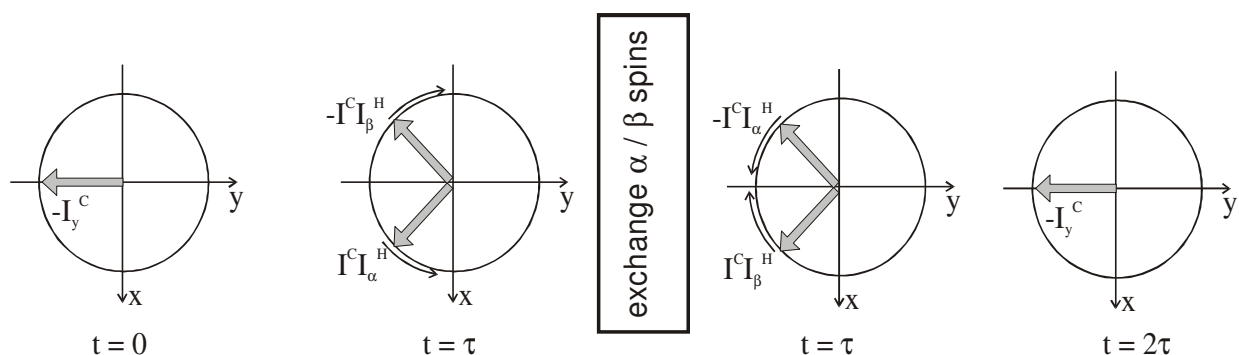
1.1 Basic idea of decoupling

From the vector picture one can see that the dephasing of the doublet components can be reversed by interchanging the α and β states of the coupling partner (here proton):

Such an exchange of α/β states can be achieved by application of a 180° pulse on the protons (remember that an 180° pulse inverts all spins). In the product operator formalism, that is equivalent to reversing the sign of the $I_x^{\text{C}}I_z^{\text{H}}$ term:

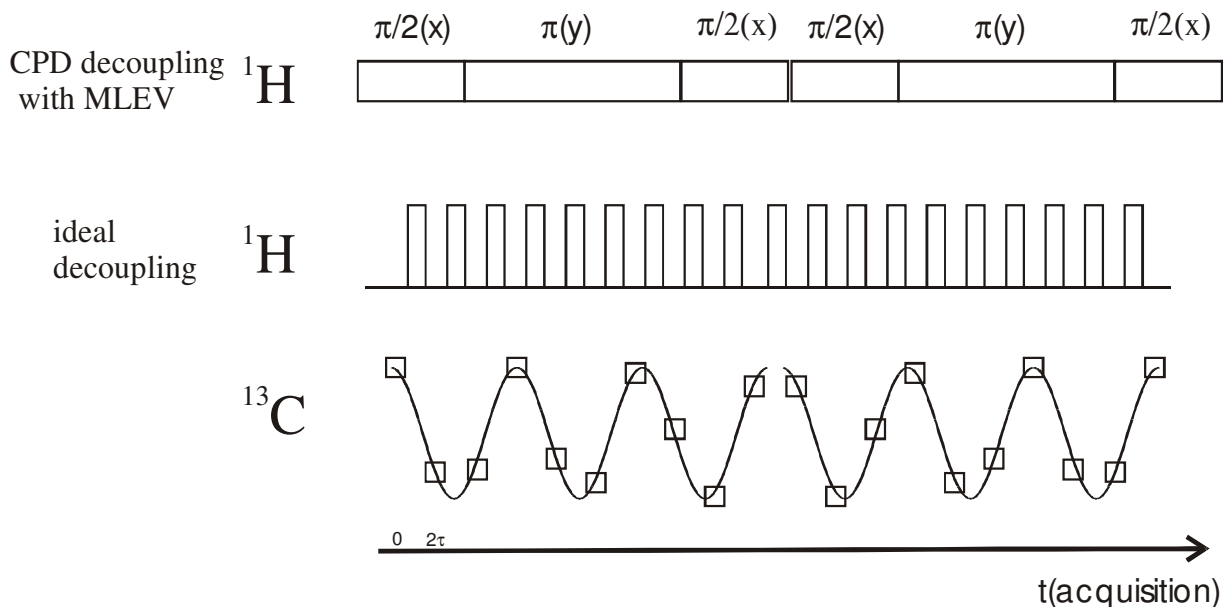
$$-I_y^{\text{C}} \cos(J_{\text{CH}}\pi\tau) + I_x^{\text{C}}I_z^{\text{H}} \sin(\pi J_{\text{CH}}\tau) \xrightarrow{\pi_x} -I_y^{\text{C}} \cos(J_{\text{CH}}\pi\tau) - I_x^{\text{C}}I_z^{\text{H}} \sin(\pi J_{\text{CH}}\tau)$$

The ideal solution would therefore be to apply a 180° proton pulse between each sampled data point



Interchanging α and β states of the coupling partner will reverse the sense of rotation for the two doublet components

during acquisition. In order to achieve 180° pulse between every sampled data point many (32000) strong pulses ($\tau_\pi \ll \text{DW}$) would have to be applied in rapid succession. In a typical ^{13}C spectrum (220 ppm at 75 MHz) $\text{DW} \approx 30 \mu\text{s}$ or less. That is usually not achievable experimentally:



1.1.1 Problems with ideal decoupling requirements

- duty cycle of amplifier
- damage to probe
- sample heating (too much energy absorbed)

1.2 Real life Decoupling sequences

In practice the requirement for efficient decoupling is less demanding. A more realistic requirement is that the dephasing from J coupling during/between the 180° pulses is small, that is if $\gamma B_1/2\pi \gg J$ or $\tau_p^{180} \ll 1/J$. As $DW \ll 1/J$ ($J_{\text{CH}} \approx 150 \text{ Hz}$, $1/J = 6.7 \text{ ms}$) dephasing from J coupling is small during DW, so inversion does not have to occur between every sampled point.

Typically, decoupler pulses with $\tau_{90} = 100 \mu\text{s}$ are used ($\gamma B_1/2\pi = 2.5 \text{ kHz}$).

Most basic: continuous irradiation with that power (cw decoupling)

1.2.1 Bandwidth and Composite pulse decoupling

At the same time proton signals over a shift range of 10 ppm (3 kHz at 300 MHz, 5 kHz at 500 MHz) have to be decoupled, so $\gamma B_1/2\pi$ needs to be strong enough to accomplish that.

CW decoupling can often not be performed with the power required to achieve the required bandwidth.

It is more efficient to use a series of pulses with variable phases (composite pulses):

=> **composite pulse decoupling (CPD)**

MLEV (**Malcom Levitts** CPD-sequence): $(90^\circ_x 180^\circ_y 90^\circ_x)_n$

WALTZ (1x $\pi/2$, 2x $\pi/2$, 3x $\pi/2 = 1,2,3$) $(90^\circ_x 180^\circ_{-x} 270^\circ_x)_n$

other more complex schemes are also in use:

GARP (**Globally Optimized Alternating Phase Rectangular Pulse**)

2. Nuclear Overhauser effect (NOE):

In addition to removing couplings from the spectrum, decoupling will also affect the intensities of the signals.

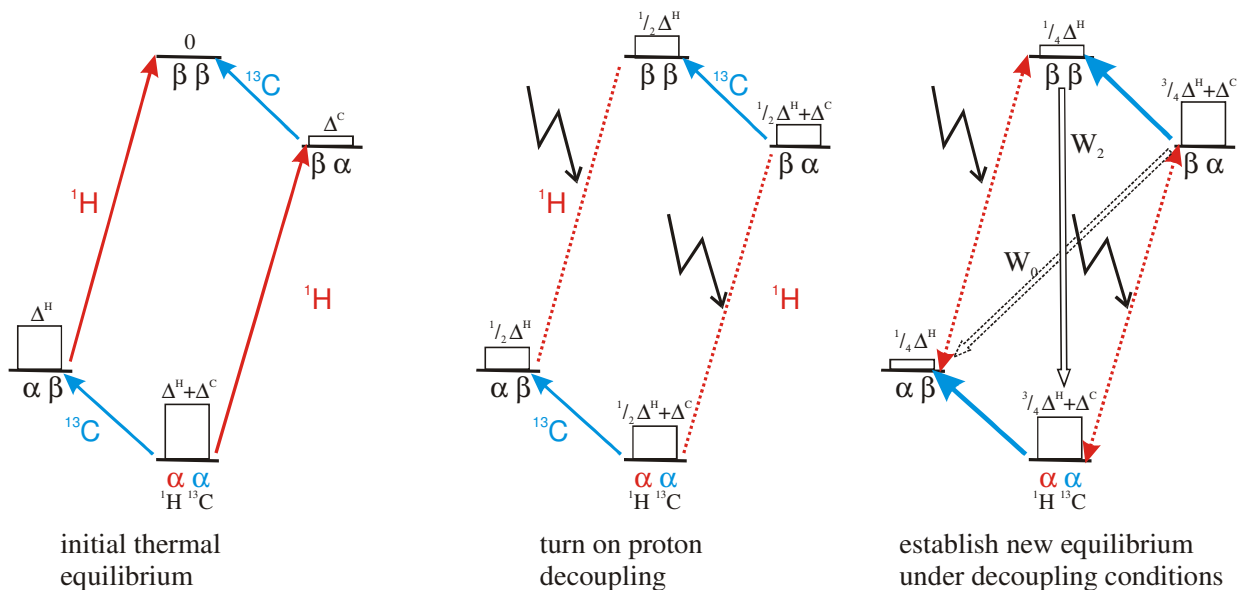
2.1 Basic theory

The observation of NOE enhancement is a special relaxation effect caused by the change in population of the proton levels during the decoupling. The details of how relaxation is induced will be discussed later. For now we accept that in the presence of **through space** (dipolar) spin-spin couplings, molecular motion induces random transitions between levels related by flipping one or both coupled spins.

For an isolated ^{13}C - ^1H pair these transitions can occur with the rates W_1^C , W_2^H , W_2^{HC} and W_0^{HC} . W_1^C are the observable NMR transitions of C and H with frequency ν_0^H and ν_0^C , and W_0 and W_2 the two simultaneous flip transitions not observed in the NMR spectrum (cross relaxation), with the frequencies $\nu_0^H - \nu_0^C$ and $\nu_0^H + \nu_0^C$.

In general, the NOE enhancement η is given by relative relaxation rates:

$$\eta = \frac{\overbrace{W_2 - W_0}^{\sigma}}{\underbrace{W_1^C + W_1^H + W_0 + W_2}_{\frac{1}{T_1}}} = \frac{\text{cross relaxation}}{\text{spin - lattice relaxation}}$$



Population differences:

$^1\text{H}: \alpha\alpha \rightarrow \beta\alpha$	} Δ^{H}	0	0
$\alpha\beta \rightarrow \beta\beta$			
$^{13}\text{C}: \alpha\alpha \rightarrow \alpha\beta$	} Δ^{C}	Δ^{C}	if $W_2 \gg W_1: \frac{1}{2}\Delta^{\text{H}} + \Delta^{\text{C}}$ $= \Delta^{\text{C}}[1 + \gamma_{\text{H}}/(2\gamma_{\text{C}})]$
$\beta\alpha \rightarrow \beta\beta$			
$\Delta m_s = 2: \alpha\alpha \rightarrow \beta\beta$	$\Delta^{\text{H}} + \Delta^{\text{C}}$	Δ^{C} (not longer	
$\Delta m_s = 0: \alpha\beta \rightarrow \beta\alpha$	$\Delta^{\text{H}} - \Delta^{\text{C}}$	Δ^{C} at equilibrium)	

Figure 3: Effect of ^1H decoupling on ^{13}C population differences. The initial excess populations are denoted Δ^{H} and Δ^{C} , the spin function for $m_s = +1/2$ and $-1/2$ are α and β , respectively.

For small molecules W_2 is dominant over W_0 (tumbling rate of molecules $\gg \omega_0$) and under optimal conditions the enhancement will be $\eta^{\text{max}}_{\text{C(H)}} = \gamma_{\text{H}}/(2\gamma_{\text{C}}) \approx 2$

Thus the carbon signal will increase up to a factor of three in intensity upon decoupling of protons:

$$M_0^{\text{C}} \sim \Delta^{\text{C}} \quad M_{\text{NOE}}^{\text{C}} \sim (\Delta^{\text{C}} + \Delta^{\text{H}}/2) \quad \text{or} \quad M_{\text{NOE}}^{\text{C}} = M_0^{\text{C}} + \eta M_0^{\text{C}}$$

W_0 and W_2 depend *only* on dipolar interaction between ^1H and ^{13}C , whereas W_1^{H} and W_1^{C} may also include other contributions to the relaxation (Chemical shift anisotropy, paramagnetic impurities like oxygen). Note that dipolar coupling does not require a bond, but directly bound protons are also close in space.

2.2 Practical considerations

- distance dependence: Tertiary carbons like C=O and >C< show only very little enhancement and show up weaker than protonated carbons=> intensities will not be proportional to number of C-atoms
- additional mechanisms of relaxation will only contribute to W_1 and may reduce NOE (Chemical shift anisotropy, unpaired electrons from oxygen)
- nuclei with negative γ will exhibit a *negative* NOE, which in some cases may decrease signal (^{15}N : $\gamma_{\text{N-15}} \approx -1/10 \gamma_{\text{H}}$, ^{113}Cd) large molecules ($M > 3000$): $W_2^{\text{CH}} \approx W_0^{\text{CH}}$ and $\eta^{\text{max}} \approx 0.25$ (small NOE)
- NOE will depend on molecular size and will be close to zero for macromolecules.
- As NOE is a relaxation phenomenon it will take time to build up after turning the decoupler on

3. Basic multiple pulse experiments

3.1 Quantitative ^{13}C NMR

Goal: get decoupled spectrum without (or with minimum) NOE:

NOE takes time to build up (up to a few seconds). On the other hand decoupling occurs instantaneously after turning on decoupling:

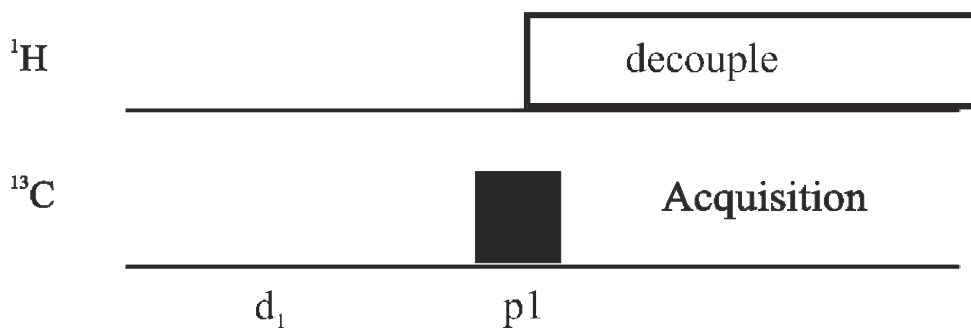
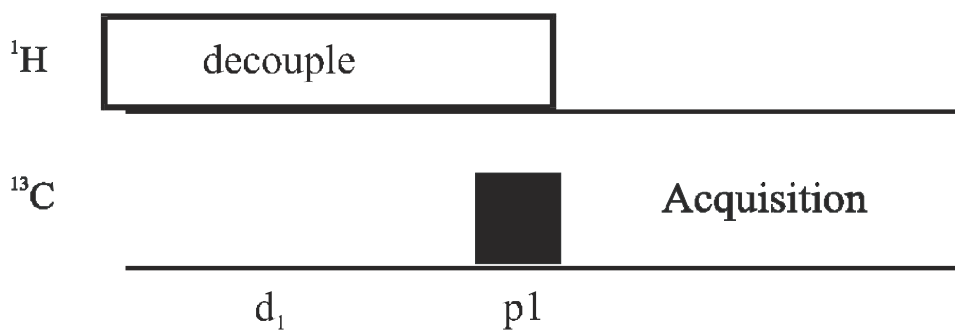
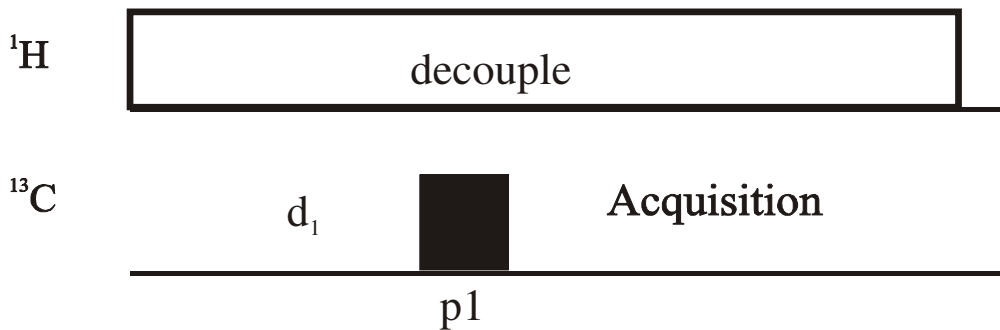
=> decouple only during **aq**, not during **d1**: “inverse gated decoupling” (zgif)

- **aq** needs to be kept to minimum to avoid NOE during acquisition
- **d1** needs to be long enough to maintain M_0^{H} before each scan and ensure sufficient relaxation of M_z^{C}
- This method is also used for 1D ^{15}N , ^{113}Cd NMR where NOE is undesired

3.2 Get spectrum with coupling and with NOE:

Decouple during **d1**, but not during **aq**: “gated decoupling” (zggd)

This will not solve the problem of spreading signal intensity over many multiplet lines and potential overlap in crowded spectra, but it gives improved sensitivity over simple one pulse experiment.

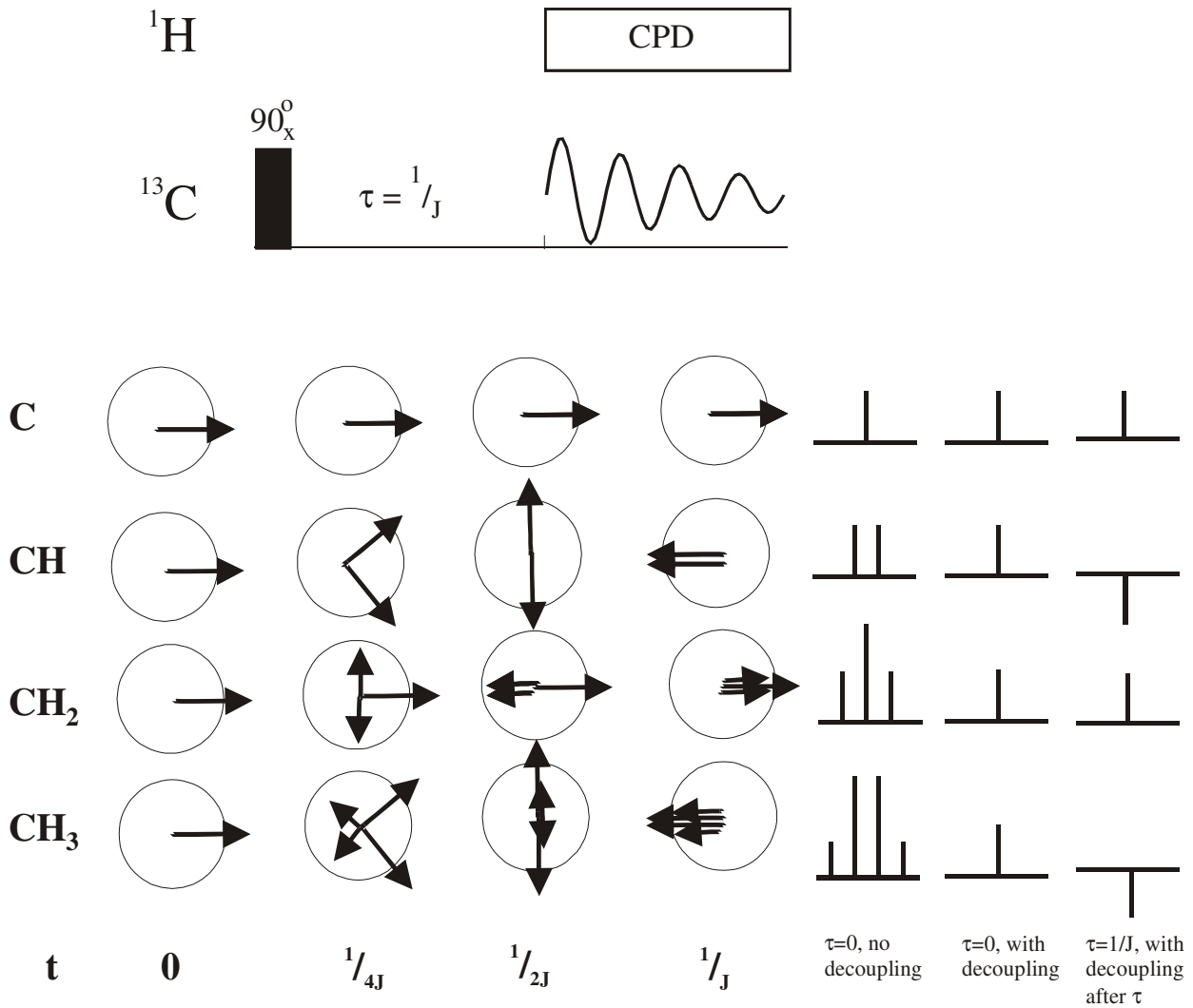


Observing carbon spectra with both proton decoupling and NOE (zgpg30) (*top*), with NOE but no decoupling (zgpg30) (*middle*) and with decoupling but no NOE (zgpg30) (*bottom*)

3.3 Simple Spectrum editing - APT (Attached Proton Test) experiment

During decoupling information about coupling partners is lost. How to reintroduce this information and still get decoupled spectrum ? Or: can we get the advantage of a decoupled spectrum and still retain some coupling information ?

Consider an experiment where we add a time $\tau = 1/J_{CH}$ between the ^{13}C excitation pulse and the start of acquisition with decoupling:



Regard a $C-H_x$ fragment: Evolution of ^{13}C x,y -magnetization with 1H coupling after time t:

After $\tau = 1/J$ all spins will again be aligned, but their relative phases will depend on multiplicity.

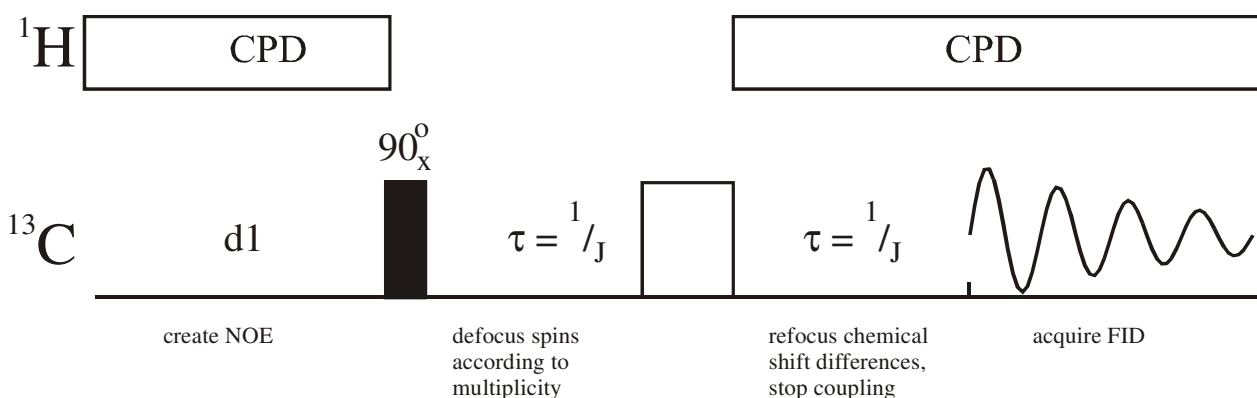
On first sight one could use such an experiment to get a multiplicity edited spectrum.

Why will this not work in a real experiment ?

Such an experiment does not take into consideration rotation of spins due to chemical shift, so it would only work for one spin exactly on resonance. For many different spins, dephasing will occur during τ also for different Ω values as $\Omega \gg \pi \cdot J$.

=> removal of chemical shift necessary during τ :

Solution: apply 180° pulse to refocus shift. This is called a spin echo (remember transparency with runners on track):



APT experiment (**A**ttached **P**roton **T**est): In the resulting spectrum a phase difference of 180° results in peaks of opposite sign in spectrum \approx partial recovery of coupling information

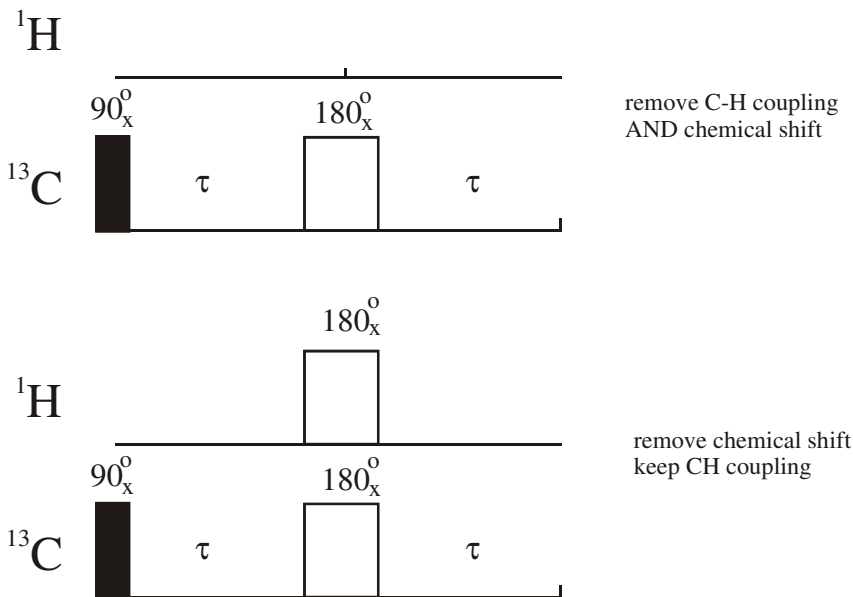
It is important to turn the decoupler on during the second τ period. With decoupling on the coupling will be "frozen" after the first τ and only chemical shift will refocus. With decoupling off during second τ also coupling will be refocused (it will also act as a decoupling pulse).

Note that the condition $\tau = 1/J_{\text{CH}}$ can not perfectly achieved for all carbons as the coupling constants can vary (typically 125 - 160 Hz) and non-perfect editing will occur.

${}^1\text{H}$

Note: Spectra can be phased arbitrarily, so it can be either CH, CH₃ “up”, C, CH₂ “down” or CH, CH₃ “down”, C, CH₂ “up”. The command **apk** can give you this either result as the computer itself does not know or care whether a signal is CH or CH₂.

180_x^o



Common building blocks of NMR experiments allowing manipulation of evolution of coupling or shift only. In each case, the effect on ${}^{13}\text{C}$ magnetization is considered.

3.4 Typical use of 180° pulses

Combinations of 180° pulses are frequently used in NMR experiments to remove one or more interactions for a period of time from the spectrum and allow for selective manipulation of magnetization. Three common building blocks are shown below:

Top: $-I_y^{\text{C}} \longrightarrow -I_y^{\text{C}} \cos(\Omega \cdot 2\tau) + I_x^{\text{C}} \sin(\Omega \cdot 2\tau)$

middle: $-I_y^{\text{C}} \longrightarrow +I_y^{\text{C}}$

bottom: $-I_y^{\text{C}} \longrightarrow -I_y^{\text{C}} \cos(\pi J \cdot 2\tau) + I_x^{\text{C}} I_z^{\text{H}} \sin(\pi J \cdot 2\tau)$

Note that the last sequence offers an alternate way of doing APT.

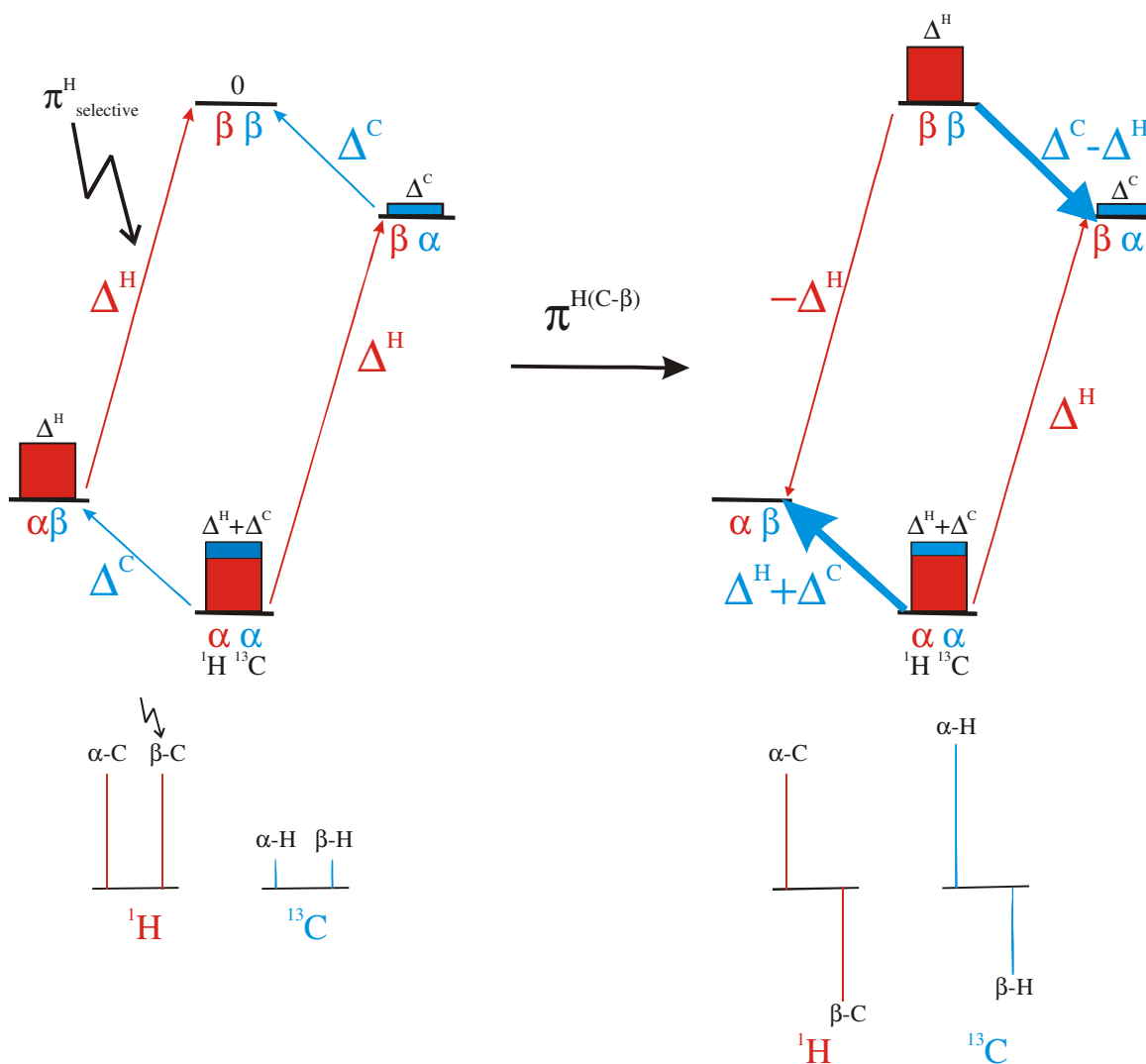
4. Magnetization transfer through scalar coupling:

4.1 Selective Polarization Transfer (SPT)

$^1\text{H}/^{13}\text{C}$ -NOE transfers a maximum of *half* of proton polarization to ^{13}C . How can we do better ?

Consider ^{13}C - ^1H fragment: doublet split by $^1J_{\text{C-H}}$

Applying a selective 180° proton pulse on one of the two lines of the doublet will invert the population along that transition. As a result the population difference of the corresponding ^{13}C lines will also change



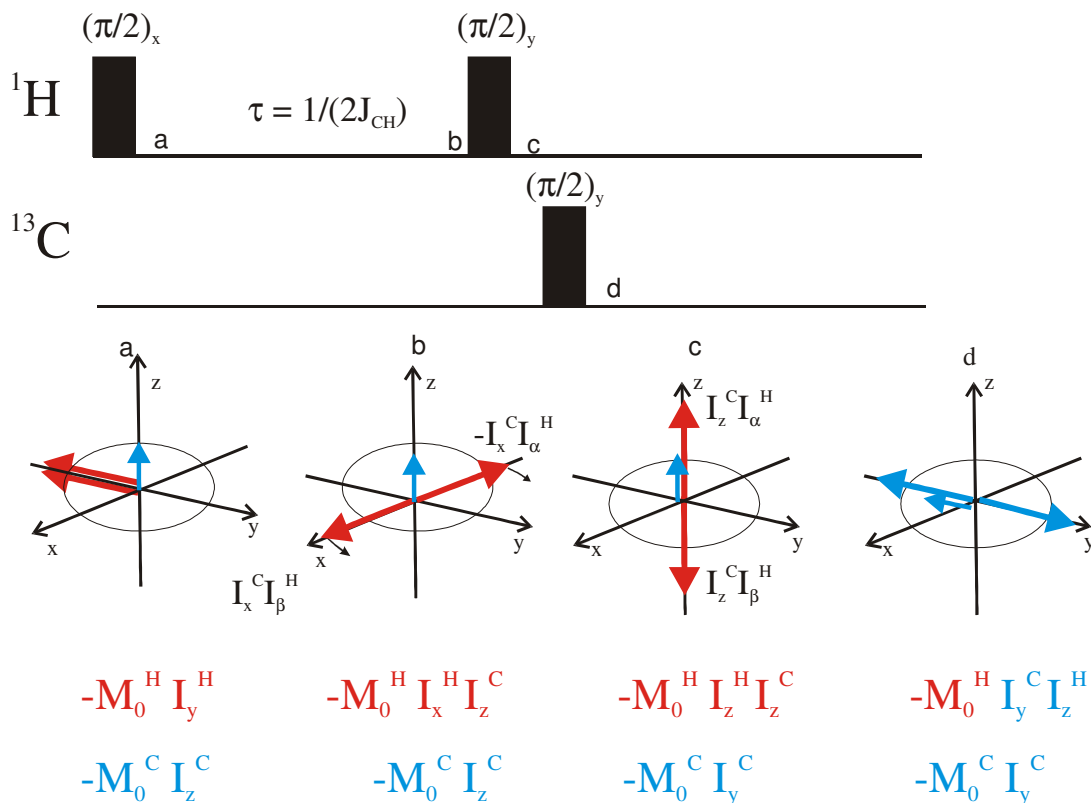
Magnetization transfer using selective inversion: Populations at thermal equilibrium (*left*) and after application of a selective $(180^\circ)^{\text{H}}$ pulse on the C- β line in the proton spectrum. The spectra obtained after an applying a regular $(90^\circ)^{\text{H}}$ or $(90^\circ)^{\text{C}}$ pulse after the selective pulse are shown at the bottom.

- The result is an enhancement of the carbon signal by $|\gamma_H/\gamma_C|$
- Transfer is instantaneous, no need to wait for relaxation to take place
- The experiment works only in presence of a scalar coupling: otherwise we can not select one line without selecting the other, and inversion of both proton lines will have no effect on ^{13}C population. Also, overlap of a positive and negative line will cancel the enhancement.

4.2 Transfer using non selective pulses (INEPT)

SPT will work only one signal at a time. Also selective 180° pulse ($\gamma B_1/2\pi \ll J_{\text{CH}}$) not easily implemented.

However, the effect of selective 180° pulse can be achieved for all signals by applying two 90° pulses separated by $\tau = 1/2J_{\text{CH}}$:



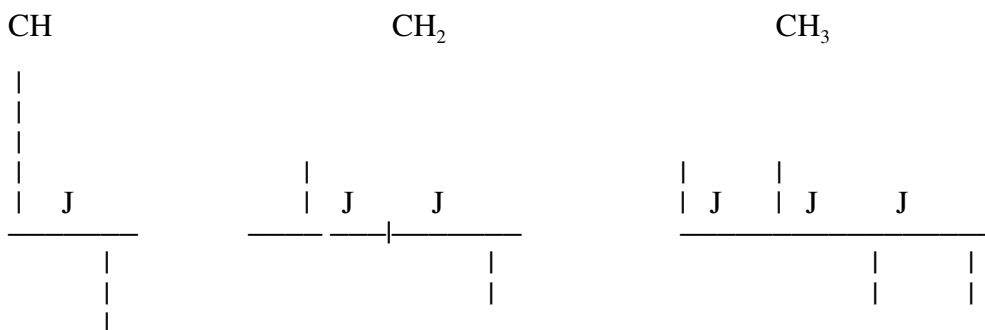
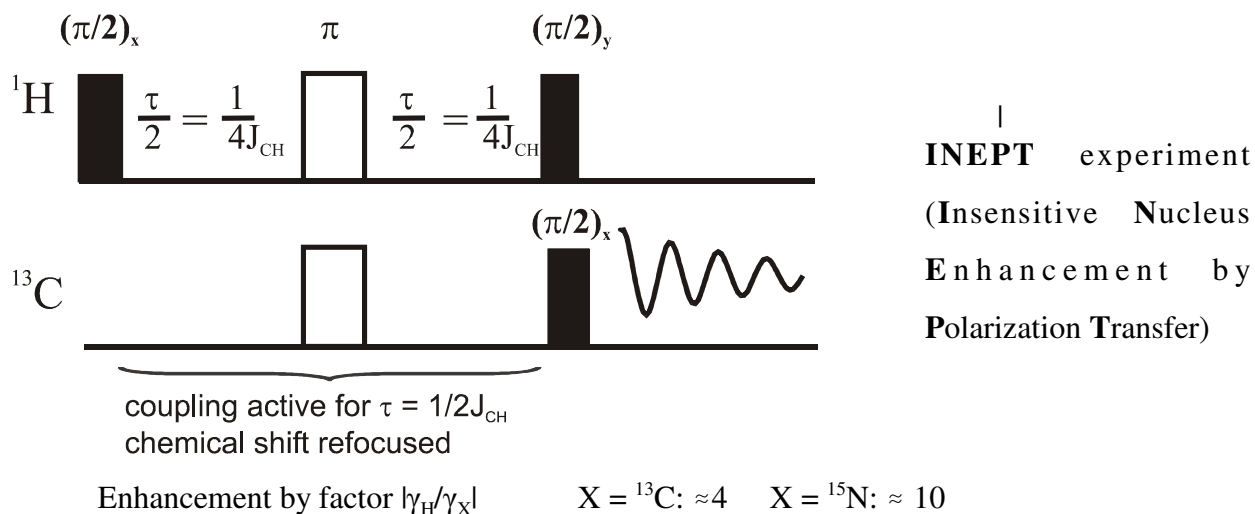
Note that the classical vector picture is very limited in describing the transfer of magnetization

through coupling. The $I_z^H I_z^C$ term is called z spin order and describes the right side of the energy level diagram above.

This sequence will work for one C-H if pulses are applied *on resonance* (Ω^C and $\Omega^H = 0$).

In real life however many peaks will be present and additional rotation by Ω^H will occur during $\tau = 1/2J$. Thus the vectors will not be aligned along x at point *b*.

However, insertion of two 180° pulses in the center of τ will remove the chemical shift dephasing but keep the coupling evolution:



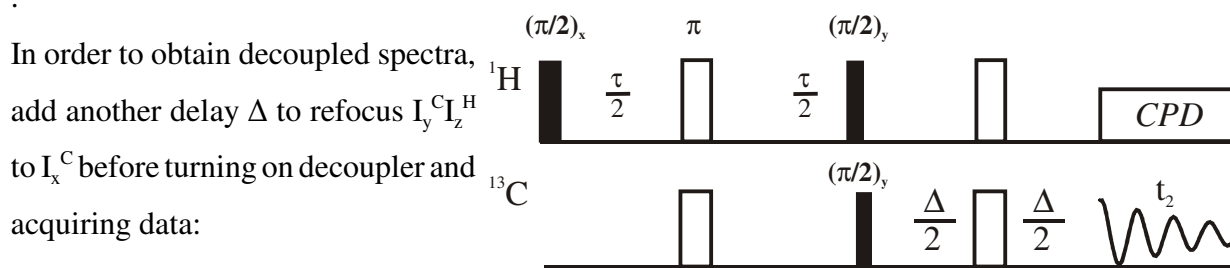
4.3 Refocused INEPT

One disadvantage of the basic INEPT is that the multiplets appear in anti phase

- No decoupling possible: positive and negative component will cancel. In vector picture, the magnetization will remain anti phase and will not develop x,y

magnetization ($I_x^C I_z^H$ is not observable)

- The multiplets are asymmetric due to natural C-13 magnetization



Note that during τ one considers a proton coupled to *one* carbon, while during Δ one has to consider a carbon coupled to *one, two* or *three* protons. The situation will be similar, but not identical to APT (one starts with anti phase magnetization at the beginning of Δ)

APT: $I_y^C \Rightarrow \pm I_y^C$ refocused INEPT: $I_y^C I_z^H \Rightarrow \pm I_x^C$
 \Rightarrow the length of Δ can be used for multiplicity editing

$\Delta = 1/2J$: CH only, optimum value for C-H

$\Delta = 3/4J$: CH, CH₃ positive, CH₂ negative (or vice versa), but not with optimum sensitivity

$\Delta = 1/4J$: CH, CH₂ and CH₃ all positive

note that non-protonated carbons will not show up in that experiment as all magnetization arises from initial proton magnetization

4.4 Editing using DEPT

Disadvantage of editing with refocussed INEPT: As in APT it works perfect only if all coupling constants $^1J_{CH}$ are the same. Typical values vary from 125 Hz - 150 Hz (up to 200 Hz in some cases).

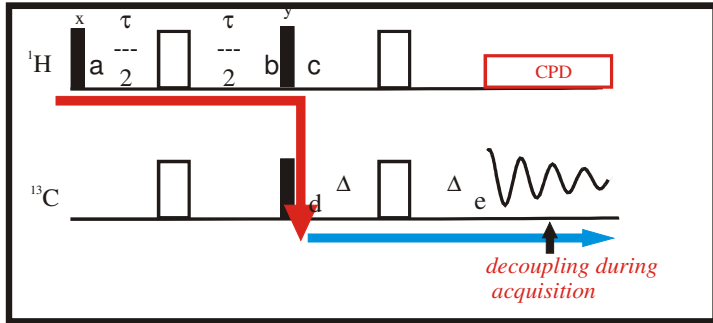
Better way: **DEPT** experiment (**D**istortionless **E**nhancement by **P**olarization **T**ransfer):

Pulse angle θ takes role of Δ :

$\theta = \pi/2$ (90°): DEPT-90 CH only

$\theta = 3\pi/4$ (135°): DEPT-135 CH, CH₃ up, CH₂ down (or vice versa)

$\theta = \pi/4$ (45°): DEPT-45 CH, CH₂, CH₃ positive

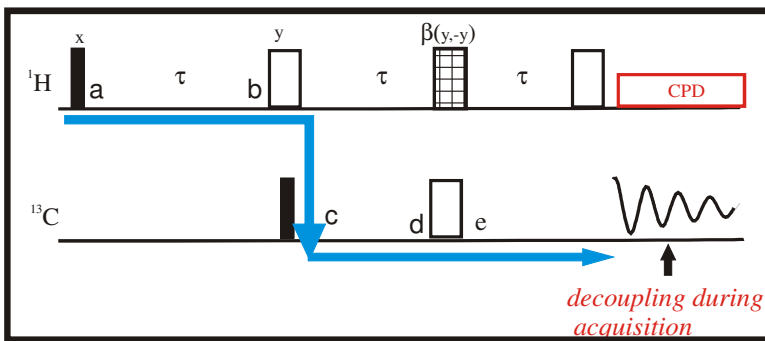


a) $-M_0^H I_y^H$ b) $-M_0^H I_x^H I_z^C$ c) $-M_0^H I_z^H I_z^C$ d) $-M_0^H I_y^C I_z^H$ e) $M_0^H I_x^C \sin(\pi J_{CH}\Delta)$ (CH)
 $\sin(\pi J_{CH}\Delta) \cos(\pi J_{CH}\Delta)$ (CH₂)
 $\sin(\pi J_{CH}\Delta) \cos^2(\pi J_{CH}\Delta)$ (CH₃)

refocused INEPT:

editing with delay Δ
 $\Delta = 1/2J$: CH only
 $\Delta = 3/(4J)$: CH, CH₃ up, CH₂ down
 $\Delta = 1/(4J)$: CH, CH₂, CH₃ positive

differences in J values effect phases of signals



a) $-M_0^H I_y^H$ b) $-M_0^H I_x^H I_z^C$ c) $-M_0^H I_y^C I_x^H$ d) $-M_0^H I_y^C I_x^H$
 $I_x^C I_x^{H1} I_z^{H2}$
 $I_y^C I_x^{H1} I_z^{H2} I_z^{H3}$ e) $-M_0^H I_y^C I_z^H \sin\beta$ (CH)
 $I_y^C I_z^H I_z^{H2} \sin\beta \cos\beta$ (CH₂)
 $I_y^C I_z^H I_z^{H2} I_z^{H3} \sin\beta \cos^2\beta$ (CH₃)

DEPT (Distortionless Editing by Polarization Transfer)

editing with pulse β
 $\beta = \pi/2$ (90°): CH only (DEPT-90)
 $\beta = 3\pi/4$ (135°): CH, CH₃ up, CH₂ down (DEPT-135)
 $\beta = \pi/4$ (45°) CH, CH₂, CH₃ positive (DEPT-45)

differences in J values effect intensities of signals

The **DEPT** experiment can not easily visualized by using the vector model but requires quantum mechanical operator description. The problem is the effect of the first ¹³C $\pi/2$ pulse which converts $I_x^H I_z^C$ into $-I_x^H I_y^C$. This is called a double/zero quantum coherence. The quality of editing in the **DEPT** does **not** depend on the accuracy of matching the delay τ to the coupling constant but is determined by the pulse width β and depends on the different operators generated by different numbers of protons coupled to the ¹³C.

Table 6.3 Relative signal intensities during spectrum editing.

$\theta (= \pi J \Delta)$	CH	CH ₂	CH ₃
$\pi/4$	$1/\sqrt{2}$	1	$3/2\sqrt{2}$
$\pi/2$	1	0	0
$3\pi/4$	$1/\sqrt{2}$	-1	$3/2\sqrt{2}$

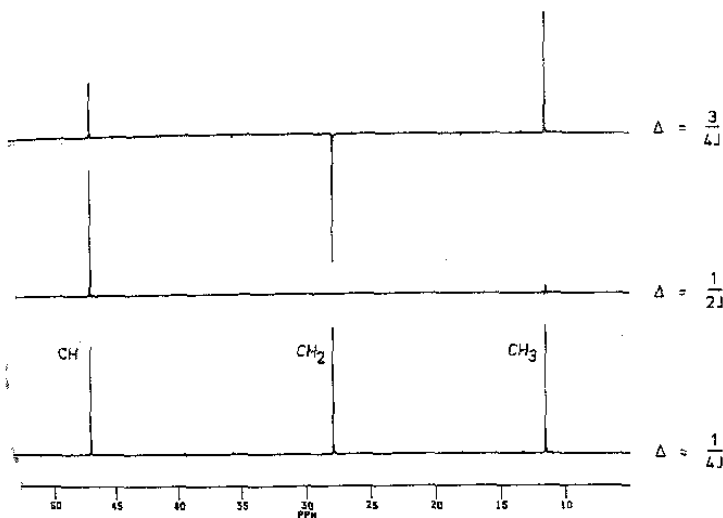


Figure 6.15 The behaviour of CH, CH₂ and CH₃ groups during editing.

$$\begin{aligned} \text{CH} &: \sin(\pi J \tau) \sin(\pi J \Delta) \\ \text{CH}_2 &: 2 \sin(\pi J \tau) \sin(\pi J \Delta) \cos(\pi J \Delta) \\ \text{CH}_3 &: 3 \sin(\pi J \tau) \sin(\pi J \Delta) \cos^2(\pi J \Delta), \\ \text{CH}_n &: n \sin(\pi J \tau) \sin(\pi J \Delta) \cos^{n-1}(\pi J \Delta) \end{aligned}$$

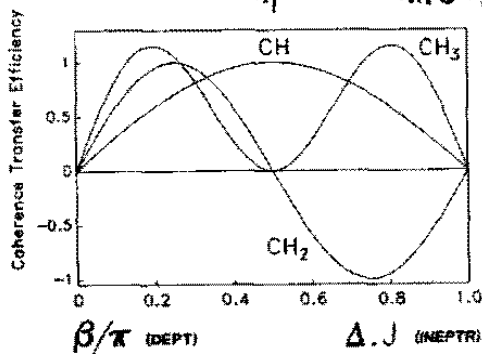
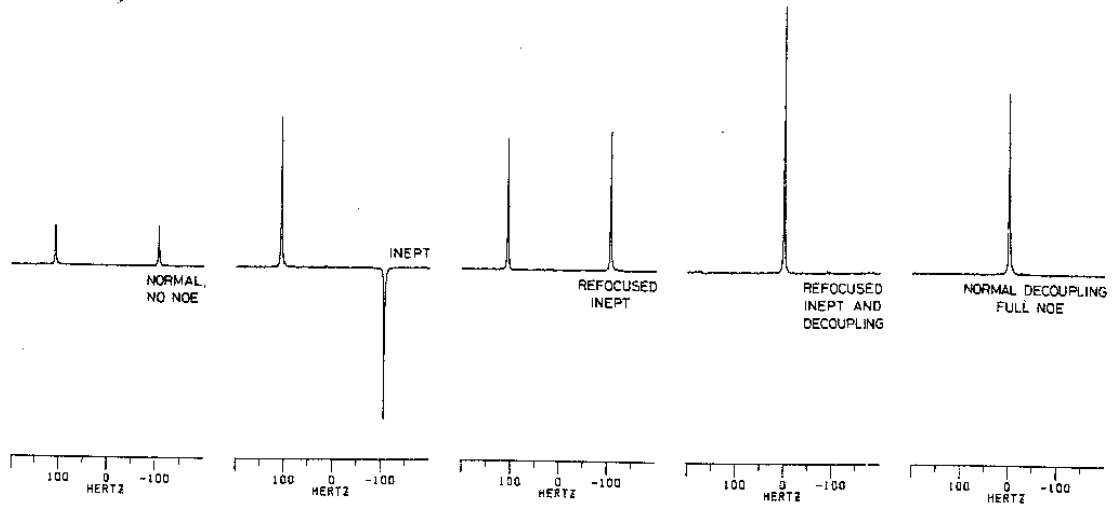


Figure 3.18. Coherence transfer efficiency as a function of Δ in the INEPT and of β for the DEPT experiment.

π_x

Figure 6.8 Refocusing for an AX system; this allows the use of broadband decoupling during acquisition.



Nucleus	Maximum nOe	Polarisation Transfer
³¹ P	2.24	2.47
¹³ C	2.99	3.98
²⁹ Si	-1.52	5.03
¹⁵ N	-3.94	9.87
⁵⁷ Fe	16.48	30.95
¹⁰³ Rh	-14.89	31.78

INEPT†: $I = I_0 \left| \frac{\gamma_S}{\gamma_I} \right|$

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

† This is the enhancement obtained when transfer is from one S nucleus only. With more nuclei the enhancement factor is slightly different - see Table 6.2

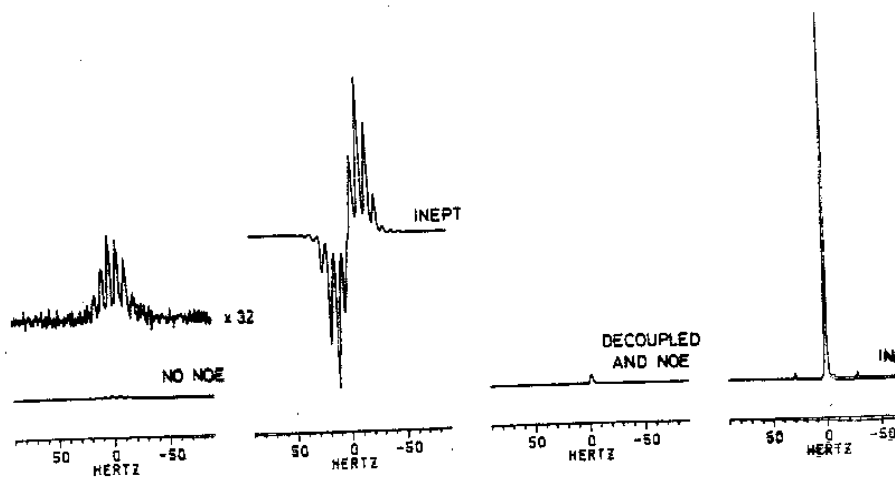


Figure 6.14 ^{29}Si via INEPT from ^1H , showing spectacular enhancements (all spectra acquired with optimum repetition and equal total accumulation time).

Table 6.2 Optimum Δ values (in units of $1/J$), and the corresponding enhancements (in units of γ_s/γ_I) for refocused polarisation transfer from n spins- $\frac{1}{2}$.

n	1	2	3	4	5	6
$\Delta (\times 1/J)$	0.5	0.25	0.196	0.167	0.148	0.134
$E (\times \gamma_s/\gamma_I)$	1.0	1.0	1.15	1.30	1.43	1.55

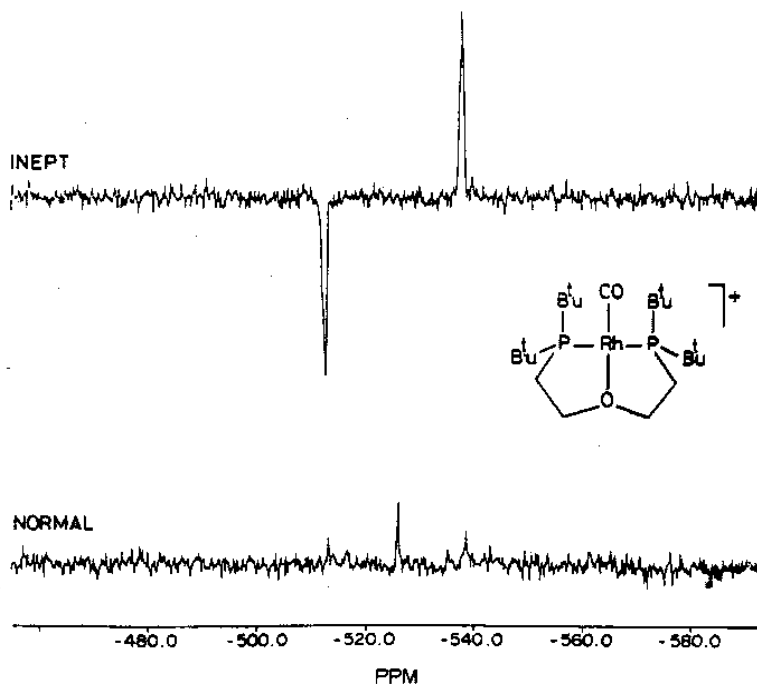


Figure 6.11 INEPT enhancement of ^{103}Rh by polarisation transfer from ^{31}P (courtesy of Dr. C. Brevard, Bruker Spectrospin, and Dr. D. H. M. W. Thewissen *et al.*, ITC-TNO, Utrecht, The Netherlands).