

ESPERIMENTI 1D NMR CON SEQUENZE COMPLESSE

ESPERIMENTI 1D NMR CON SEQUENZE COMPLESSE

VEDREMO ALCUNE SEQUENZE COMPLESSE NEL ^{13}C NMR

1. **J-MOD** J-MODULATED **SPIN ECHO**
2. **APT** ATTACHED PROTON TEST (SPECTRUM EDITING)
3. **SPT** SELECTIVE POLARIZATION TRANSFER (SPI SELECTIVE POLARIZZAZION INVERSION)
4. **INEPT** INSENSITIVE NUCLEI ENHANCED BY POLARIZATION TRANSFER
5. **DEPT** DISTORTIONLESS ENHANCEMENT BY POLARIZATION TRANSFER (SPECTRUM EDITING)

Servono a:

RECUPERARE L'INFORMAZIONE DELLA MOLTEPLICITA (EDITING)
MIGLIORARE ULTERIORMENTE LA SENSIBILITA' RISPETTO AL NOE

6. **INADEQUATE** INCREDIBLE NATURAL ABUNDANCE DOUBLE QUANTUM TRANSFER EXPERIMENT

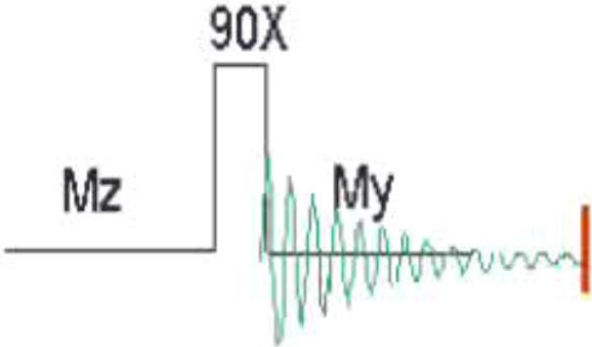
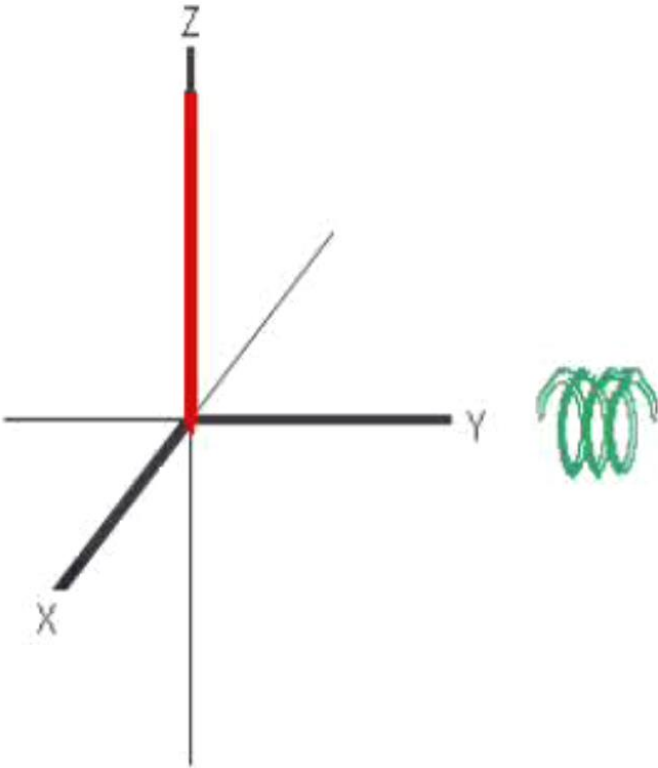
Serve a RILEVARE GLI ACCOPPIAMENTI C-C e DETERMINARE le $J_{\text{C-C}}$

NEL ^1H NMR

7. **TOCSY 1D** TOTAL CORRELATION SPECTROSCOPY

Serve a DISTINGUERE SISTEMI DI SPIN IN MOLECOLE COMPLESSE

EFFETTI DEGLI IMPULSI DEL VETTORE MAGNETIZZAZIONE



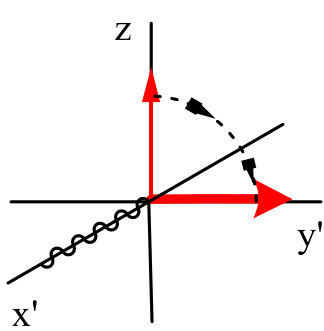
90 x

90 y

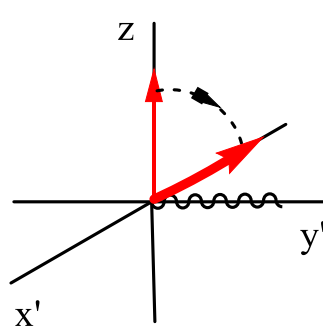
90 -x

90 -y

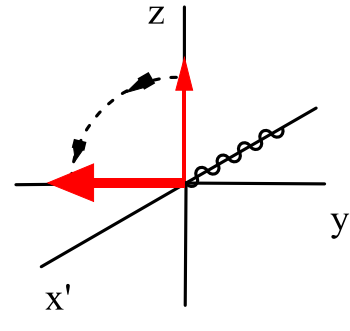
Effetto su M_z



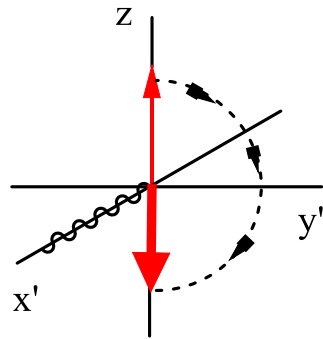
$90^\circ_{x'}$



$90^\circ_{y'}$

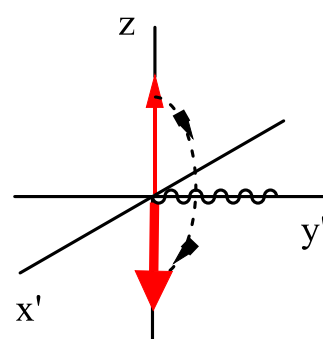


$90^\circ_{-x'}$



$180^\circ_{x'}$

Ruota sul piano yz

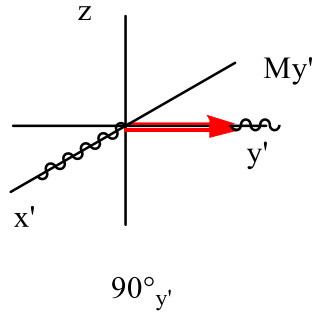
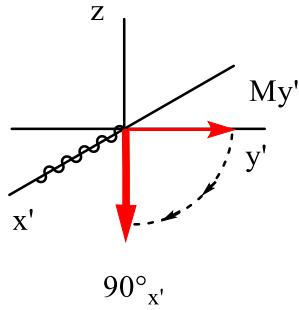


$180^\circ_{y'}$

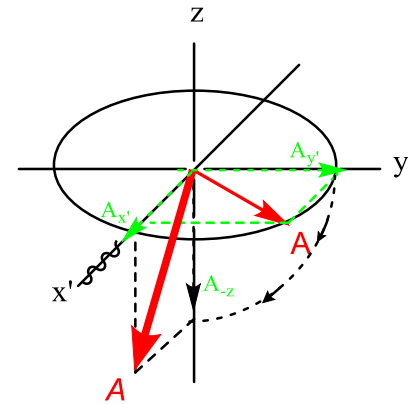
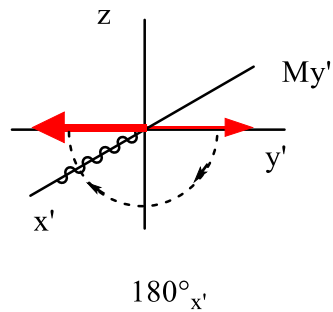
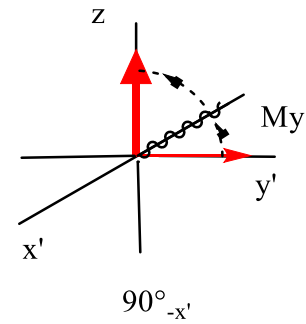
Ruota sul piano xz

Effetto su M_y , M_x

$180^\circ y$

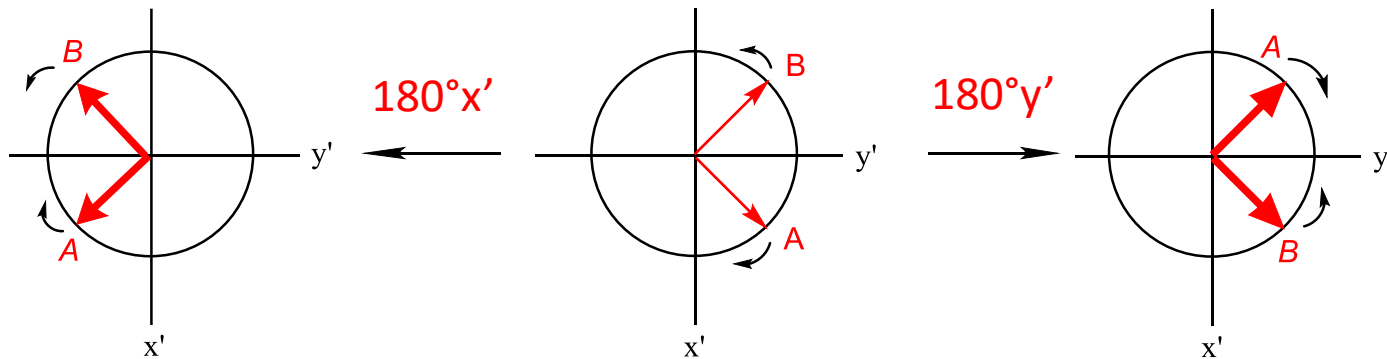
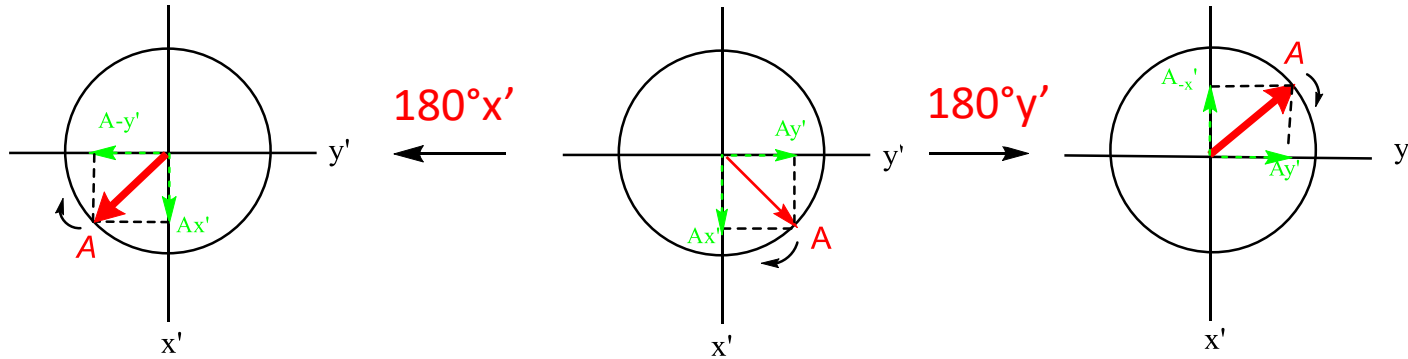


Nessun effetto!



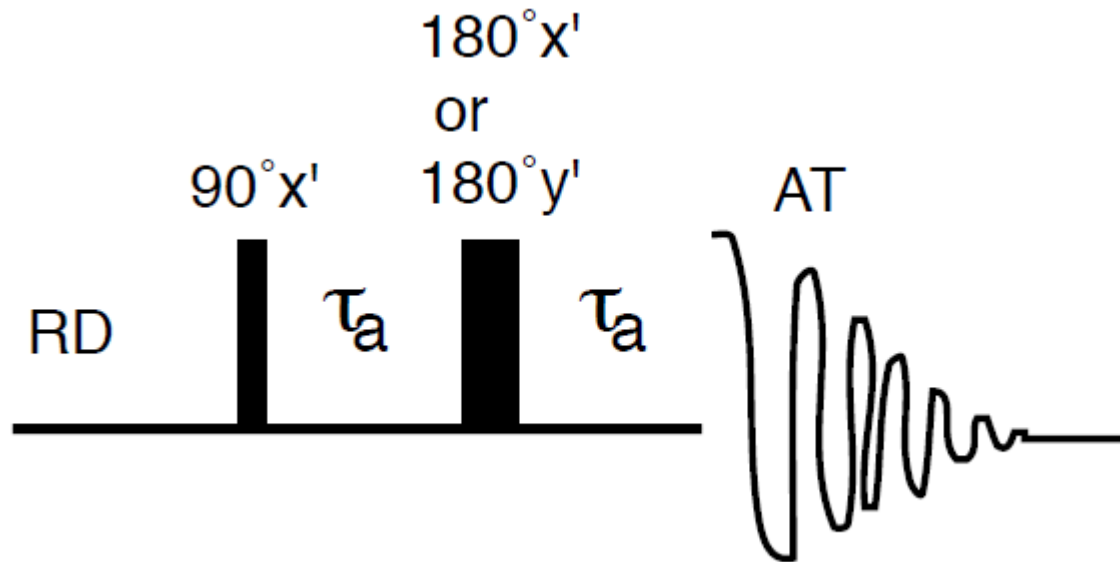
Effetto su M_y , M_x

180°_x



SPIN ECHO

$90^\circ x - \tau - 180^\circ x - \tau$ (echo)

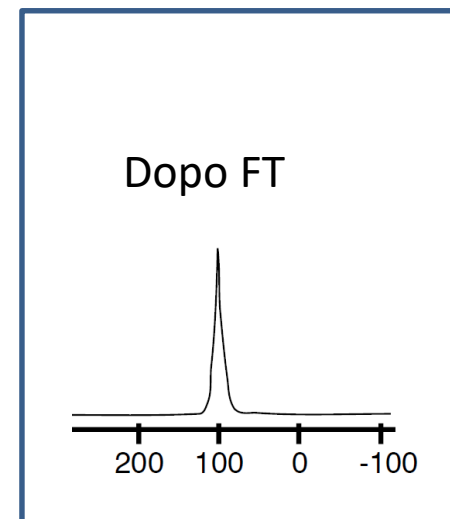
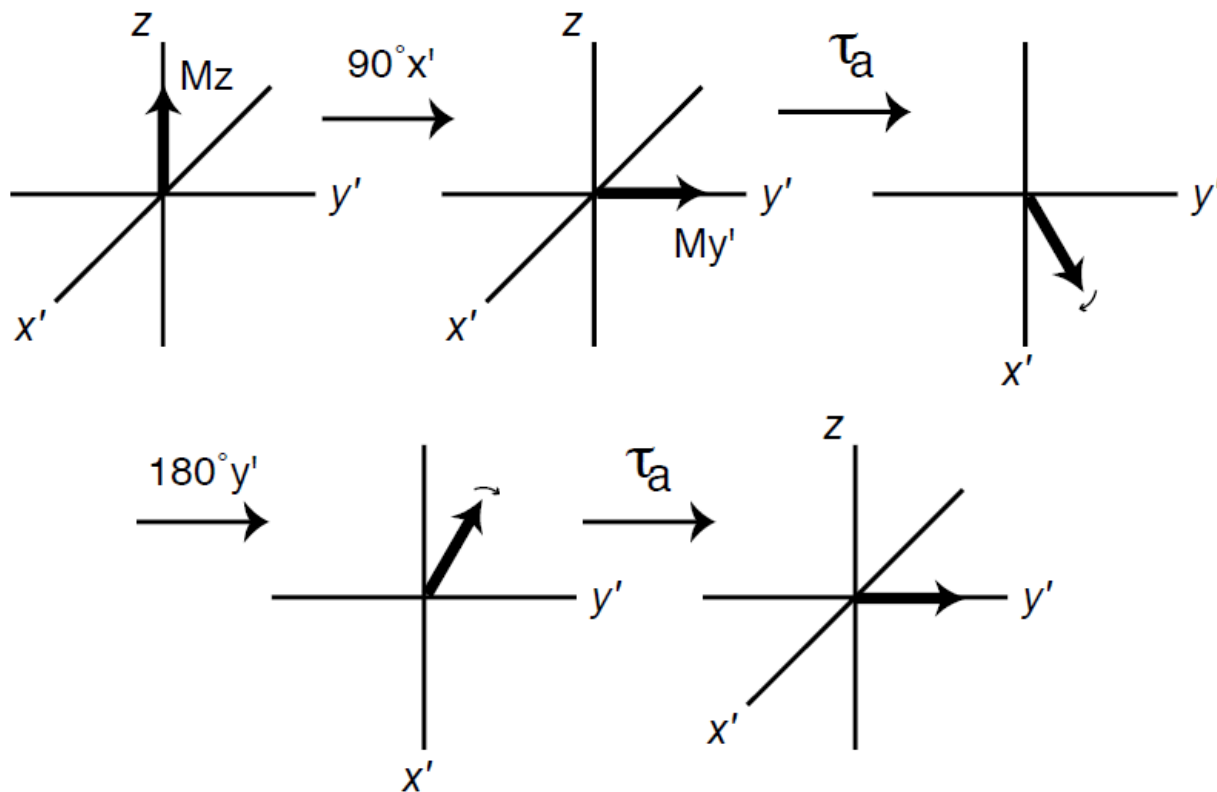


EFFETTO DELLA SEQUENZA SPIN ECHO SU UN'UNICA MAGNETIZZAZIONE

$$\nu_H = 100\text{Hz}$$

^1H : $90^\circ_x - \tau_a - 180^\circ_y - \tau_a$ (echo)

Impulso di 180° lungo y

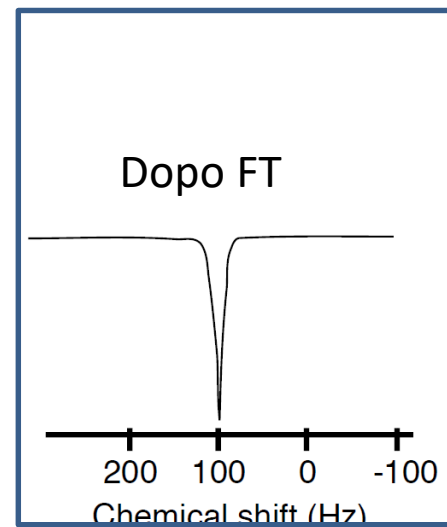
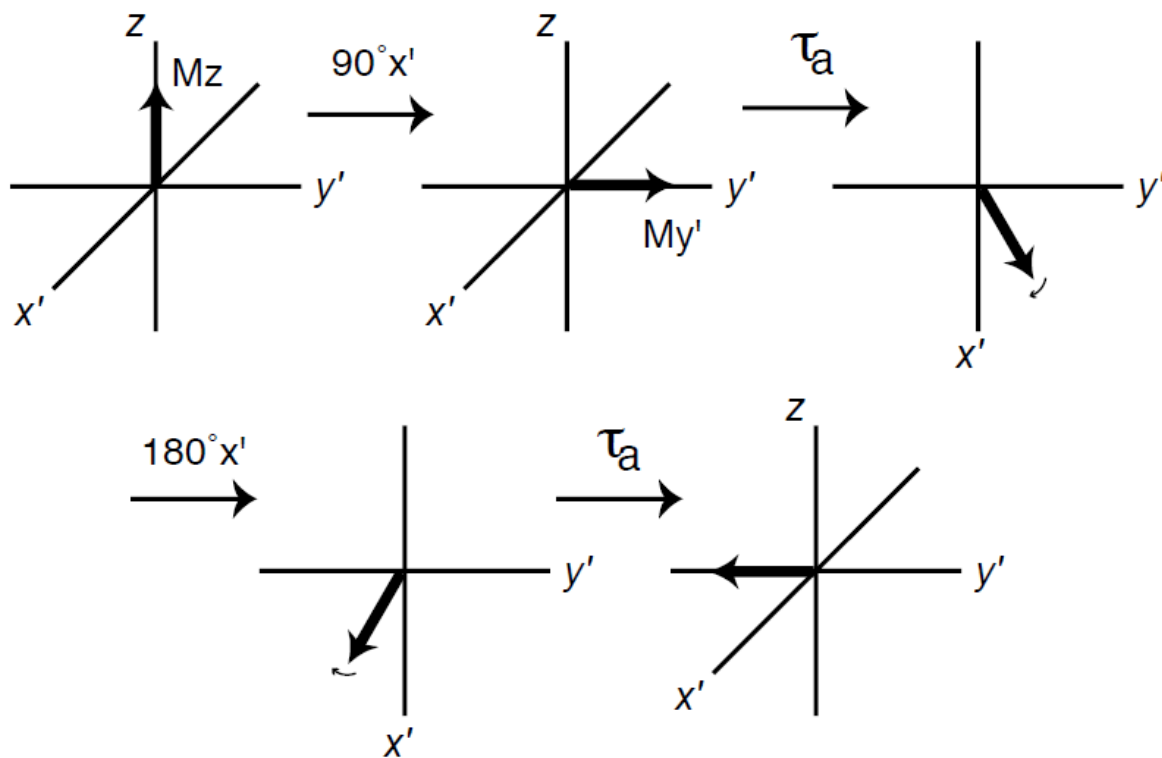


EFFETTO DELLA SEQUENZA SPIN ECHO SU UN'UNICA MAGNETIZZAZIONE

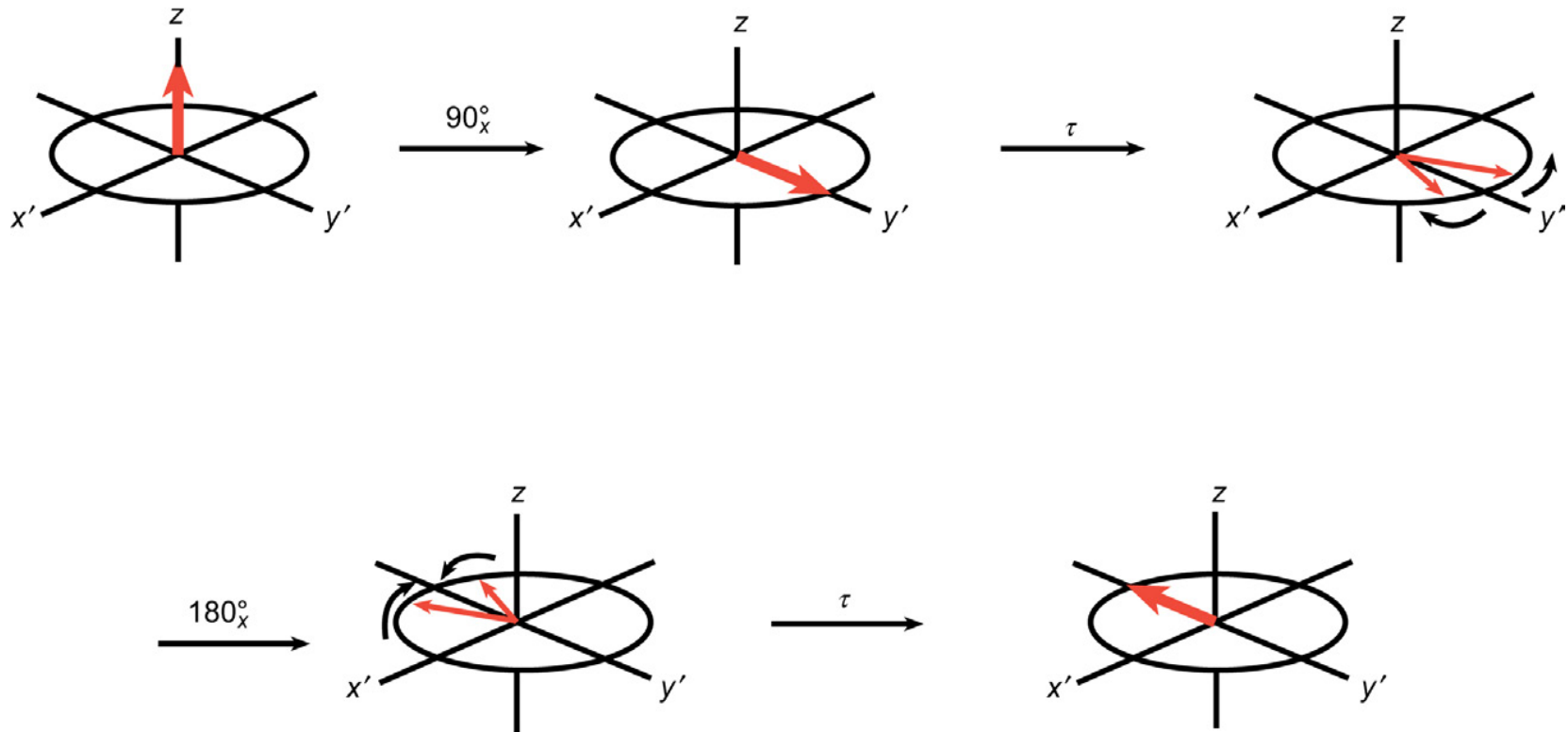
^1H : $90^\circ_x - \tau_a - 180^\circ_x - \tau_a$ (echo)

Impulso di 180° lungo x

Cambia solo la fase



EFFETTO DELLO SPIN ECHO SU UN SISTEMA DI PIU' MAGNETIZZAZIONI



Rifocalizza i vettori magnetizzazione che si sono sfasati sul piano xy o per effetto di disomogeneità di campo o per effetto di diverso chemical shift o anche per effetto dell'accoppiamento.

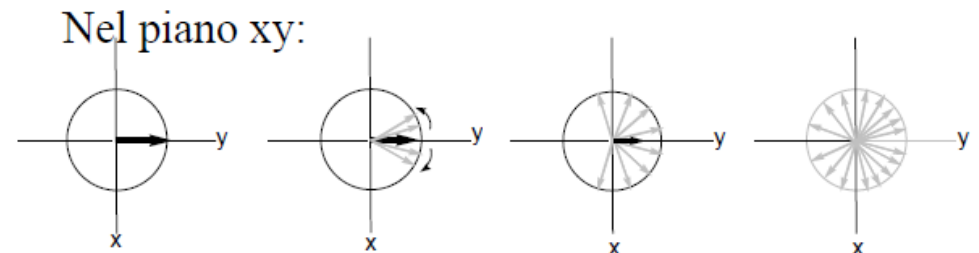
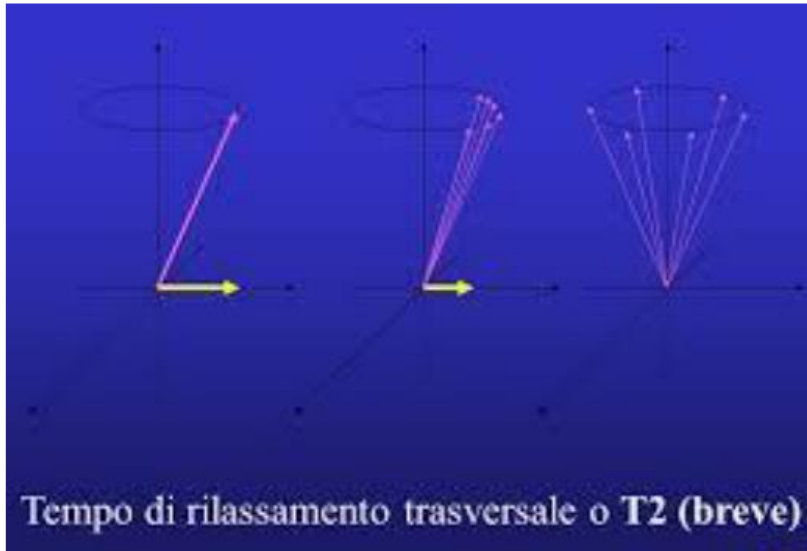
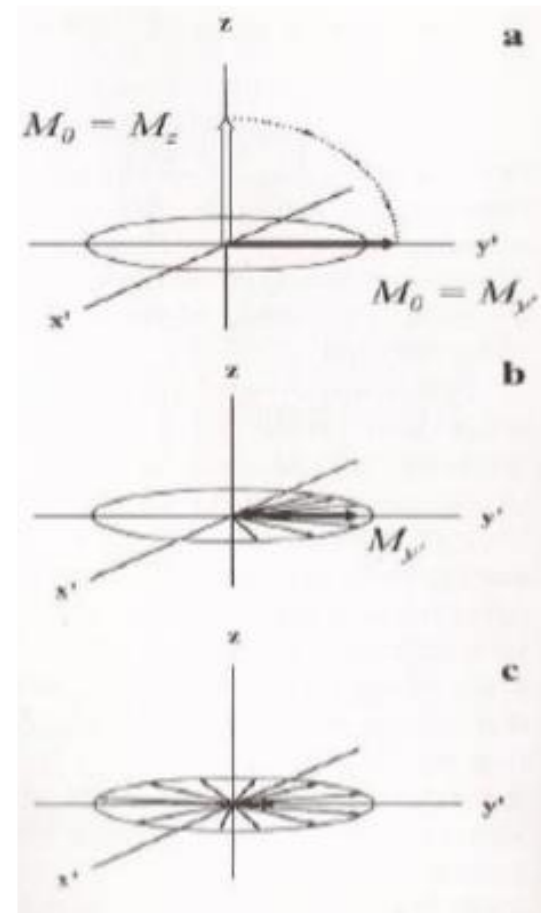
Determinazione di T_2 con una sequenza spin-echo

Rilassamento trasversale

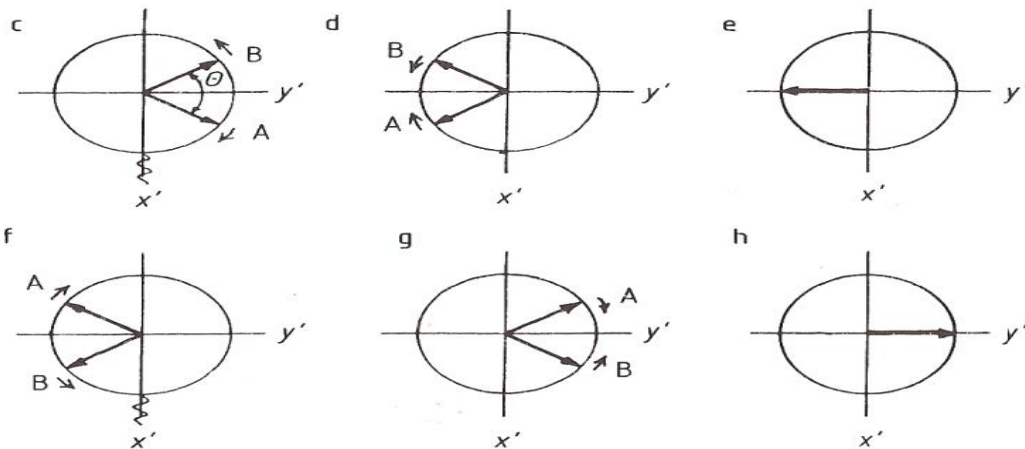
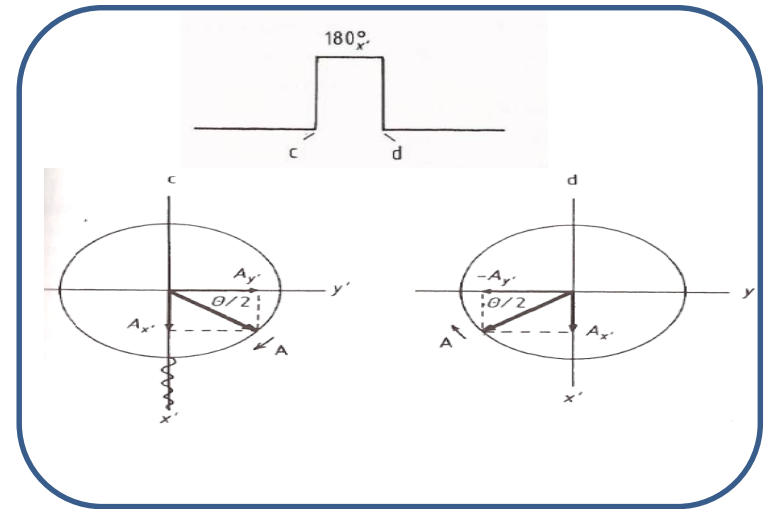
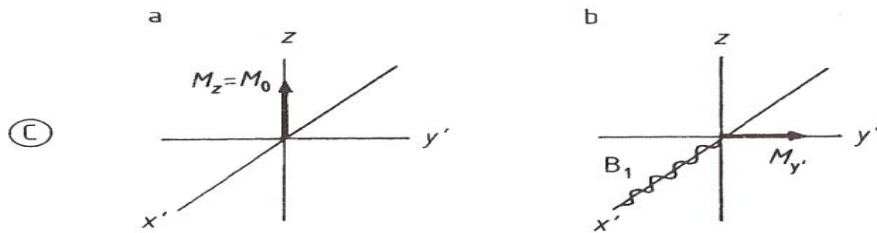
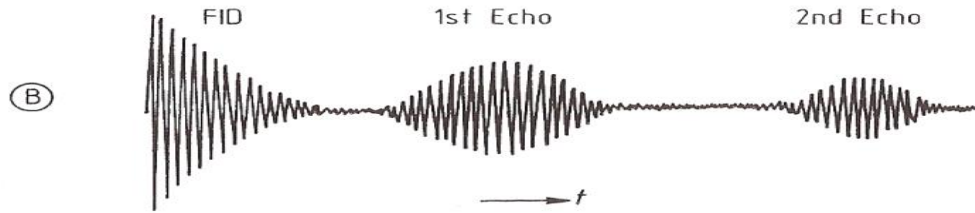
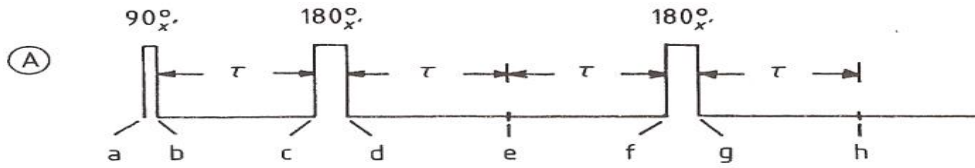
$$T_2^* = T_2 + T_2(\Delta B_0)$$

T_2 costante di tempo di rilassamento trasversale spin – spin (intrinseco)

$T_2(\Delta B_0)$ costante di tempo di rilassamento dovuto alle disomogeneità di campo



Determinazione di T_2 con una sequenza spin-echo



Determinazione di T_2 con una sequenza spin-echo

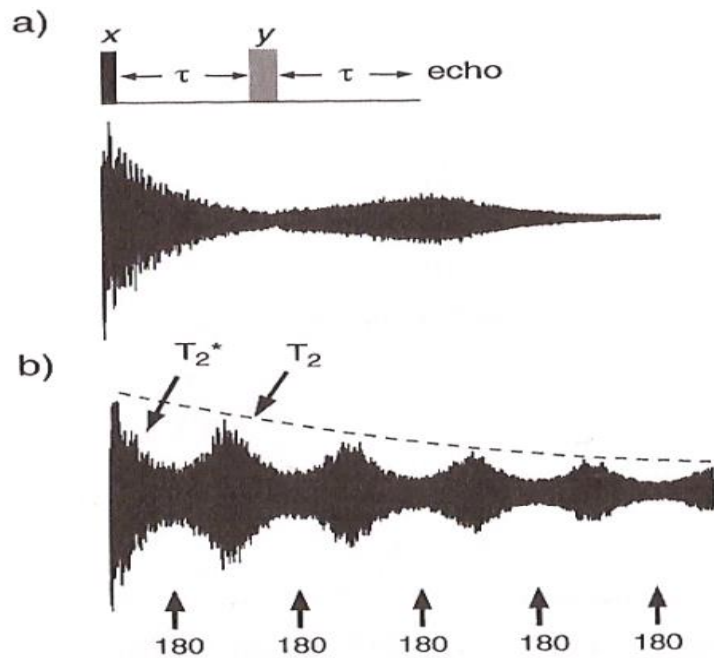
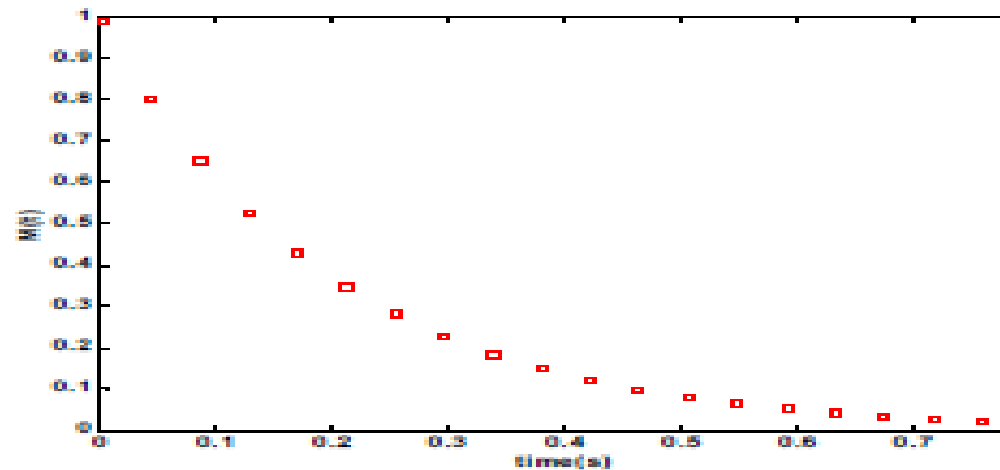
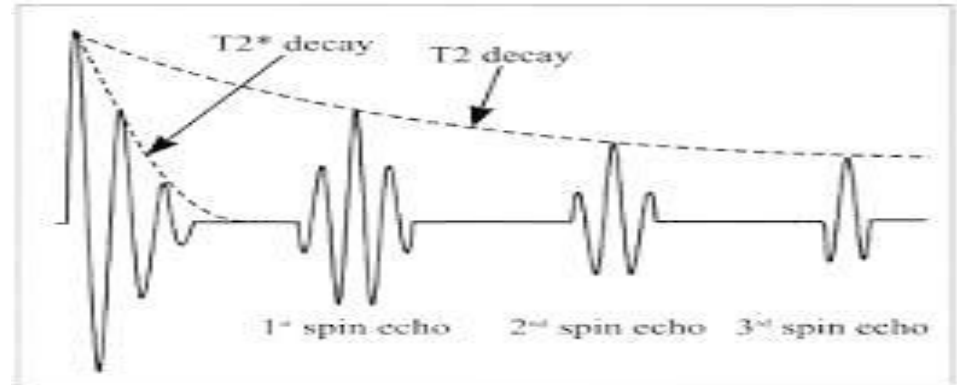


Figure 2.29. Experimental observation of spin-echoes. (a) Signal acquisition was started immediately after a 90° excitation pulse and a 180° pulse applied to refocus field inhomogeneity losses and produce the observed echo. (b) A train of spin-echoes reveals the true T_2 relaxation of magnetisation (dashed line).



Determinazione di T_2 con una sequenza spin-echo

$$My_{(t)} = My_0 \exp^{-t/T_2}$$

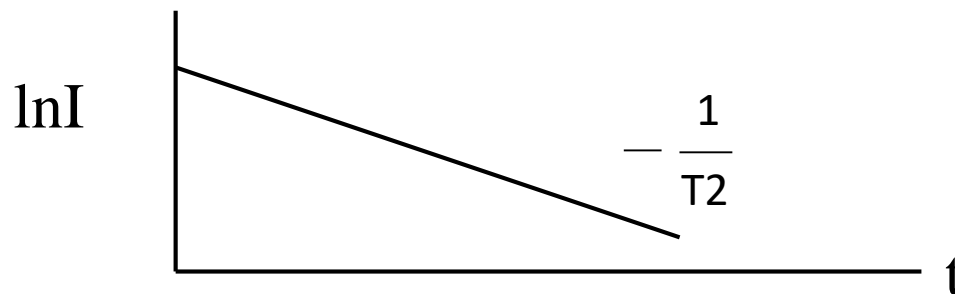
$$I \text{ (intensità)} \equiv My$$

 quindi

$$I = I_0 \exp^{-t/T_2}$$

 quindi

$$\ln I = \ln I_0 - t/T_2$$



EFFETTO DELLO SPIN ECHO SU UN SISTEMA IN ACCOPPIAMENTO SCALARE ETERONUCLEARE: **J-MODULATED SPIN ECHO**

ES: ACCOPPIAMENTO $^{13}\text{C} - ^1\text{H}$

SISTEMA AX CON $A = ^1\text{H}$, $X = ^{13}\text{C}$

ES $^{13}\text{CHCl}_3$

Nucleo in osservazione = ^{13}C

$^1J_{\text{CH}} = 209 \text{ Hz}$, doppietto Nello spettro **accoppiato** del ^{13}C

Ch.sh. $^{13}\text{C} = 77.7 \text{ ppm}$

Due frequenze di Larmor

$$M_{\text{C}}^{\text{H}\alpha} : \nu(^{13}\text{CH}\alpha\text{Cl}_3) = \nu_{\text{C}} - 1/2J_{\text{CH}} \text{ (Hz)}$$

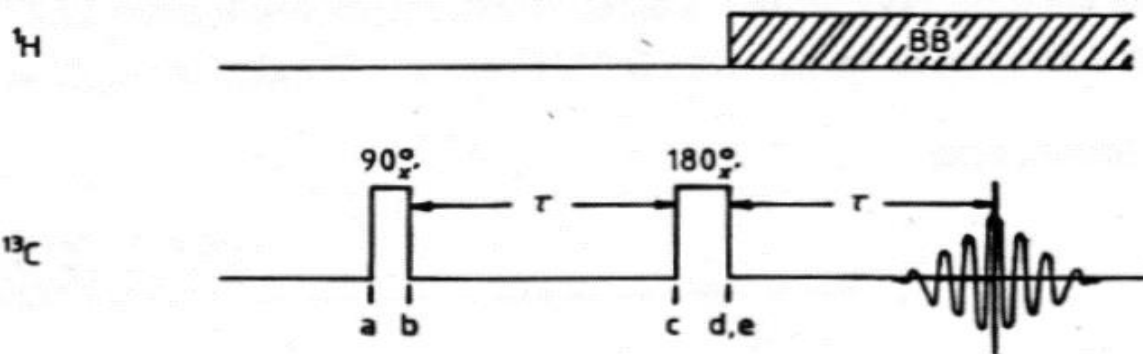
$$M_{\text{C}}^{\text{H}\beta} : \nu(^{13}\text{CH}\beta\text{Cl}_3) = \nu_{\text{C}} + 1/2J_{\text{CH}} \text{ (Hz)}$$

$$\Delta\nu = J$$

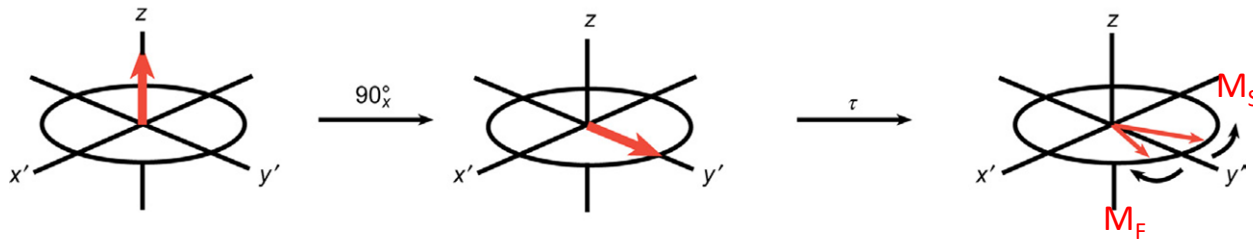
Siccome $J_{\text{CH}} > 0$
allora $\nu(^{13}\text{CH}\beta\text{Cl}_3) > \nu(^{13}\text{CH}\alpha\text{Cl}_3)$

L' ESPERIMENTO J – MODULATED SPIN ECHO

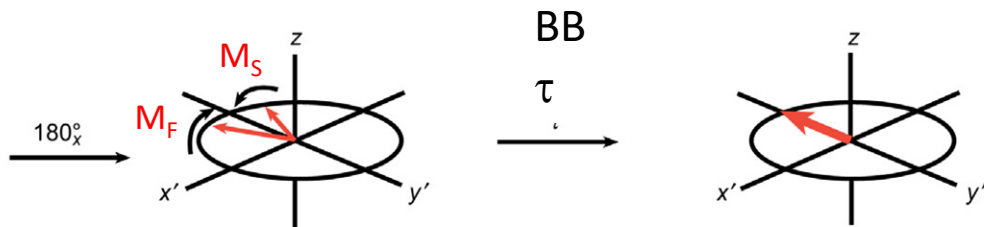
Si applica la sequenza spin-echo al nucleo in osservazione (^{13}C) e il disaccoppiamento broad-band dopo l'impulso a 180° durante l'acquisizione



$$\theta(t) = 2\pi^1 J_{CH} \tau$$



θ è lo sfasamento (angolo) fra i due vettori al tempo τ



S = Slow
F = Fast

τ	Θ
0	0°
$\frac{1}{4J(\text{C,H})}$	90°
$\frac{1}{2J(\text{C,H})}$	180°
$\frac{3}{4J(\text{C,H})}$	270°
$\frac{1}{J(\text{C,H})}$	360°

EFFETTO DELLO SPIN ECHO SU UN SISTEMA IN ACCOPPIAMENTO SCALARE

2 magnetizzazioni per i nuclei ^{13}C : $M_C^{\text{H}\alpha}$ (M_S) e $M_C^{\text{H}\beta}$ (M_F)

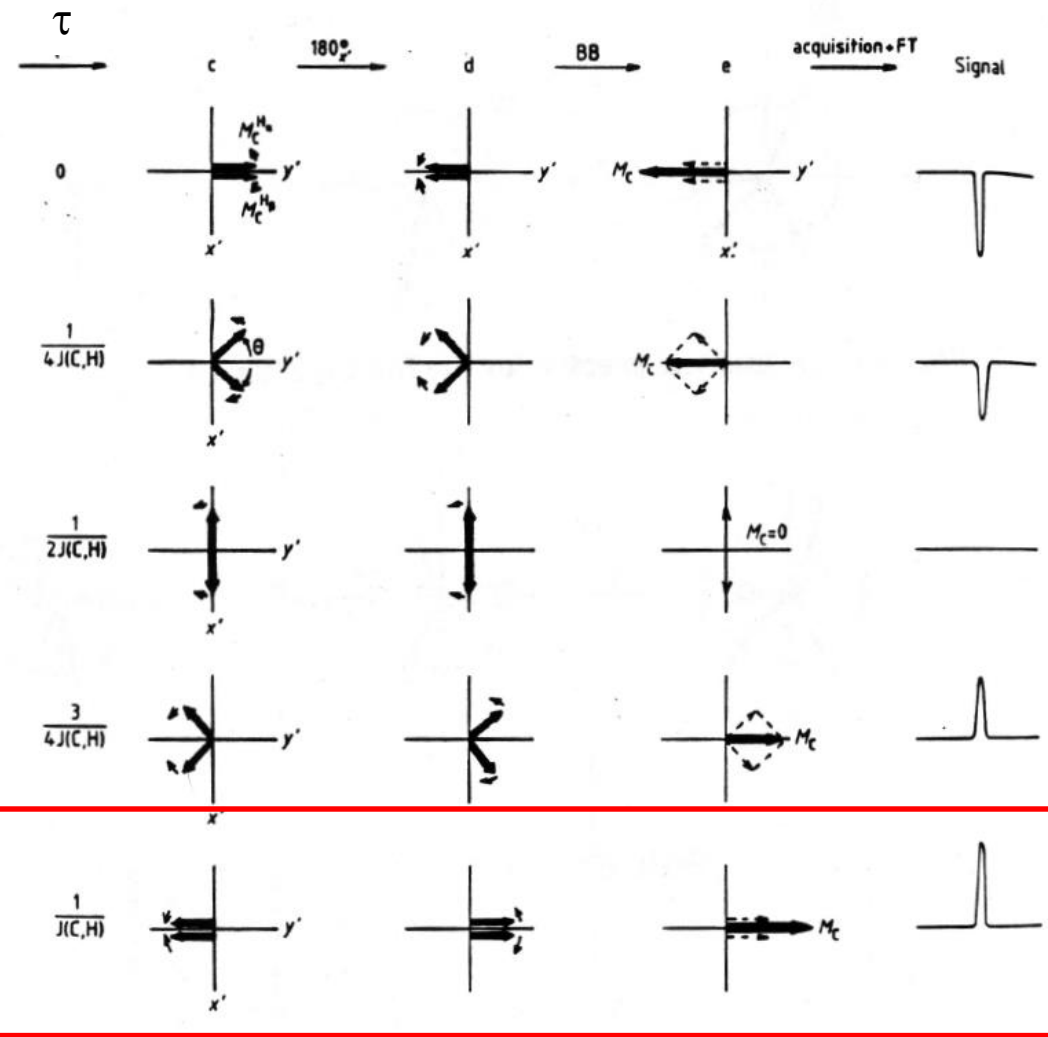
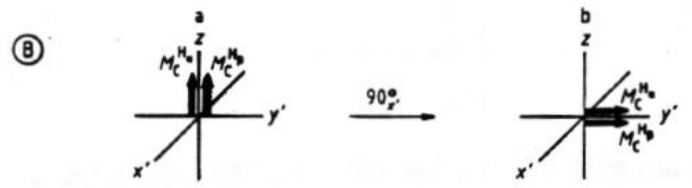
S= Slow

F= fast

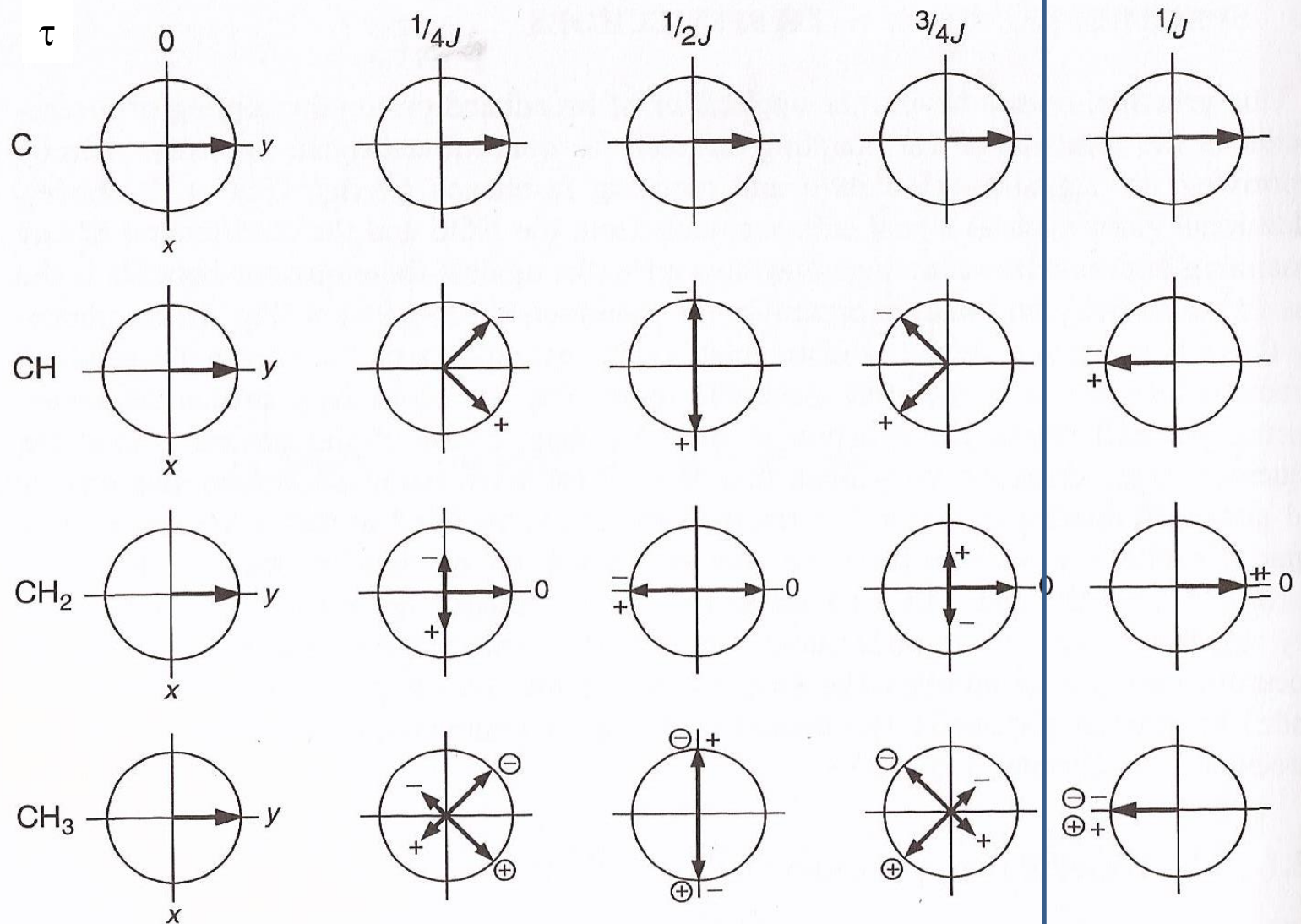
Effetto dell'esperimento spin echo:

- L'impulso a 90° porta entrambe le magnetizzazioni sul piano xy, lungo y
- $M_C^{\text{H}\alpha}$ (M_S , S= slow) ruota più lentamente di $M_C^{\text{H}\beta}$
- La loro differenza di frequenza è esattamente $^1J_{\text{CH}}$
- Dopo un tempo t la fase θ fra i due vettori è data da : $\theta = 2\pi^1J_{\text{CH}} \tau$ $\Delta\nu = J$
- Impulso 180°
- Disaccoppiamento broad band durante il secondo delay

ACCOPPIAMENTO $^{13}\text{C} - ^1\text{H}$

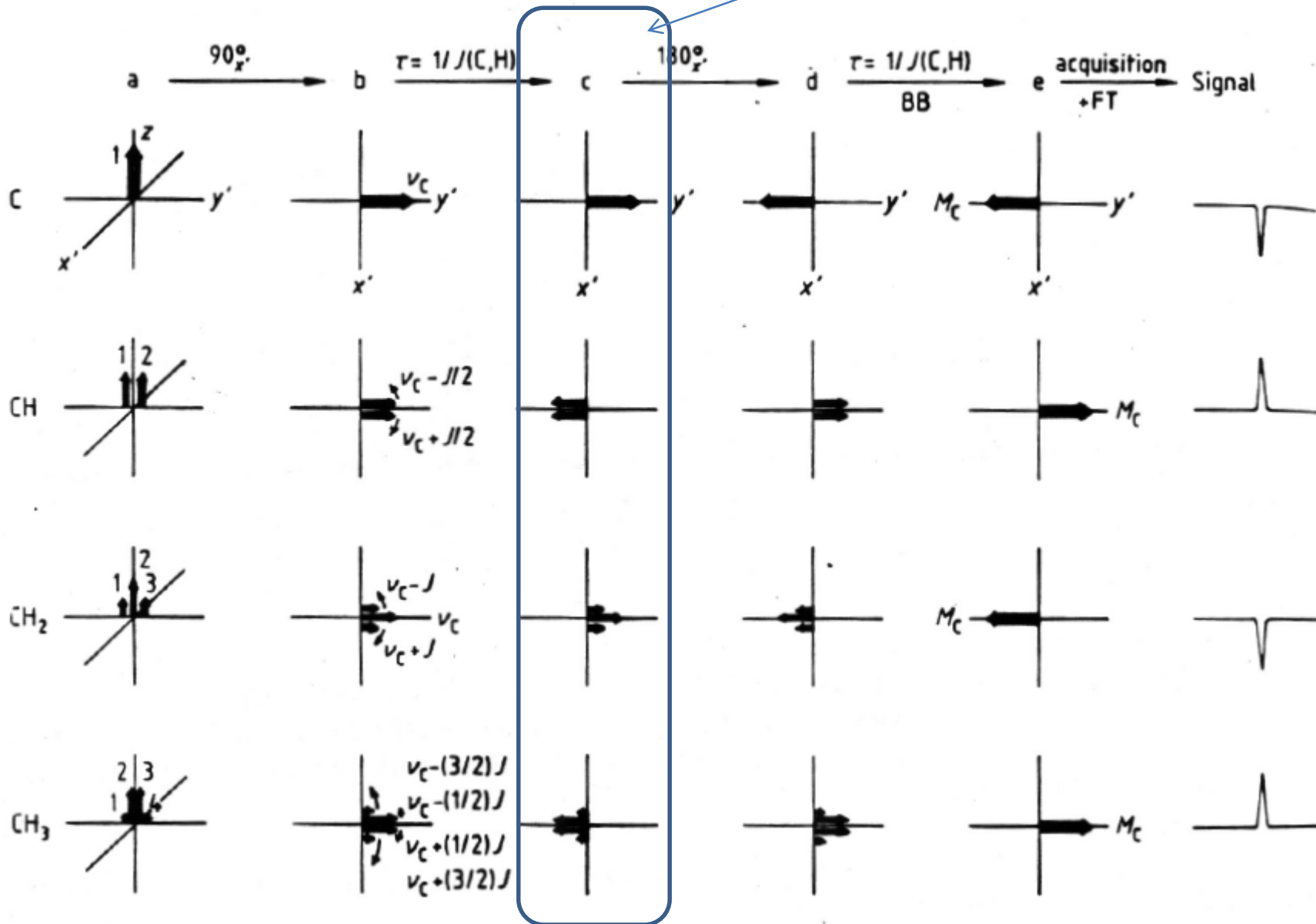


Evoluzione con t della magnetizzazione per C, CH, CH₂ e CH₃

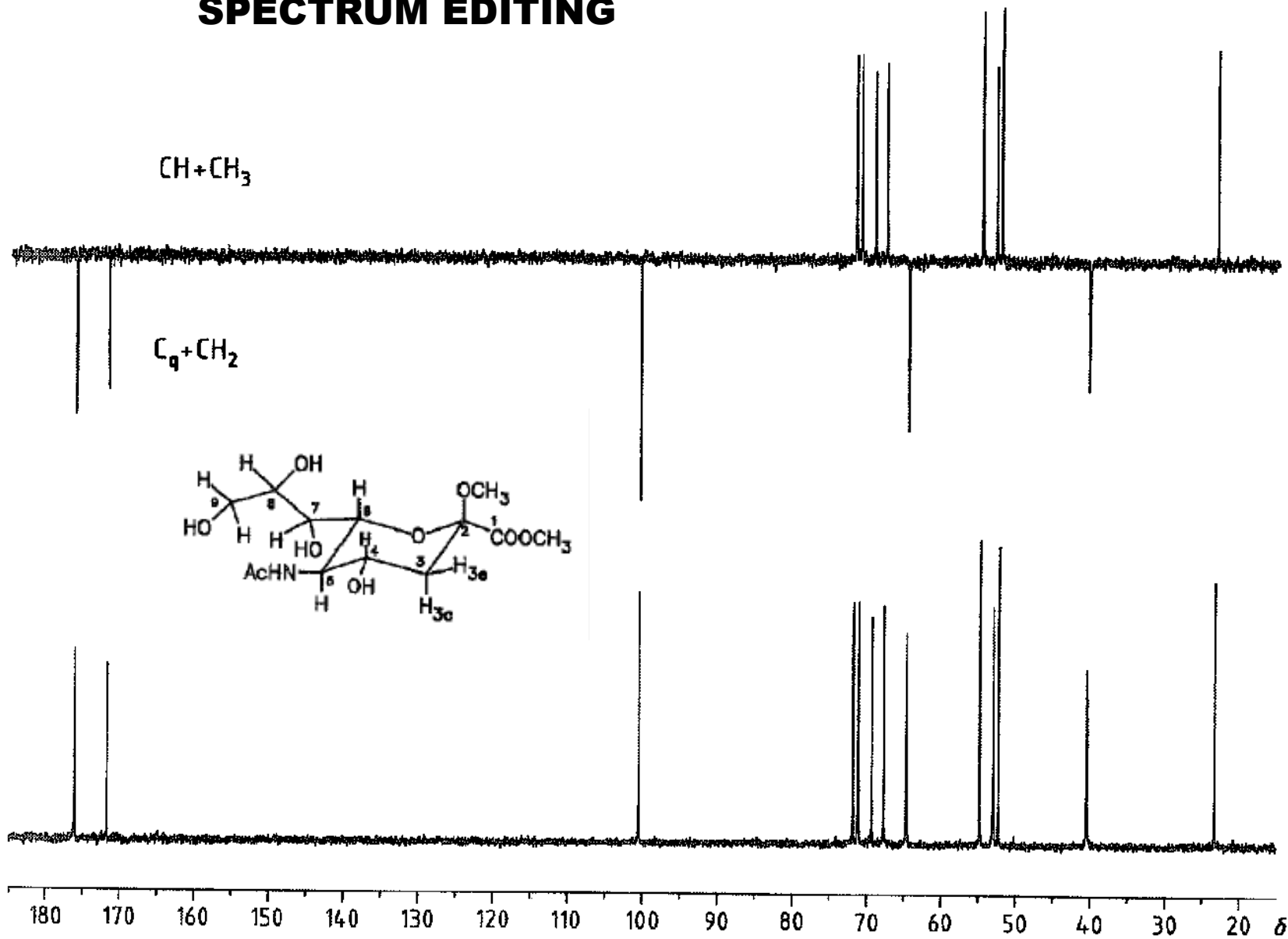


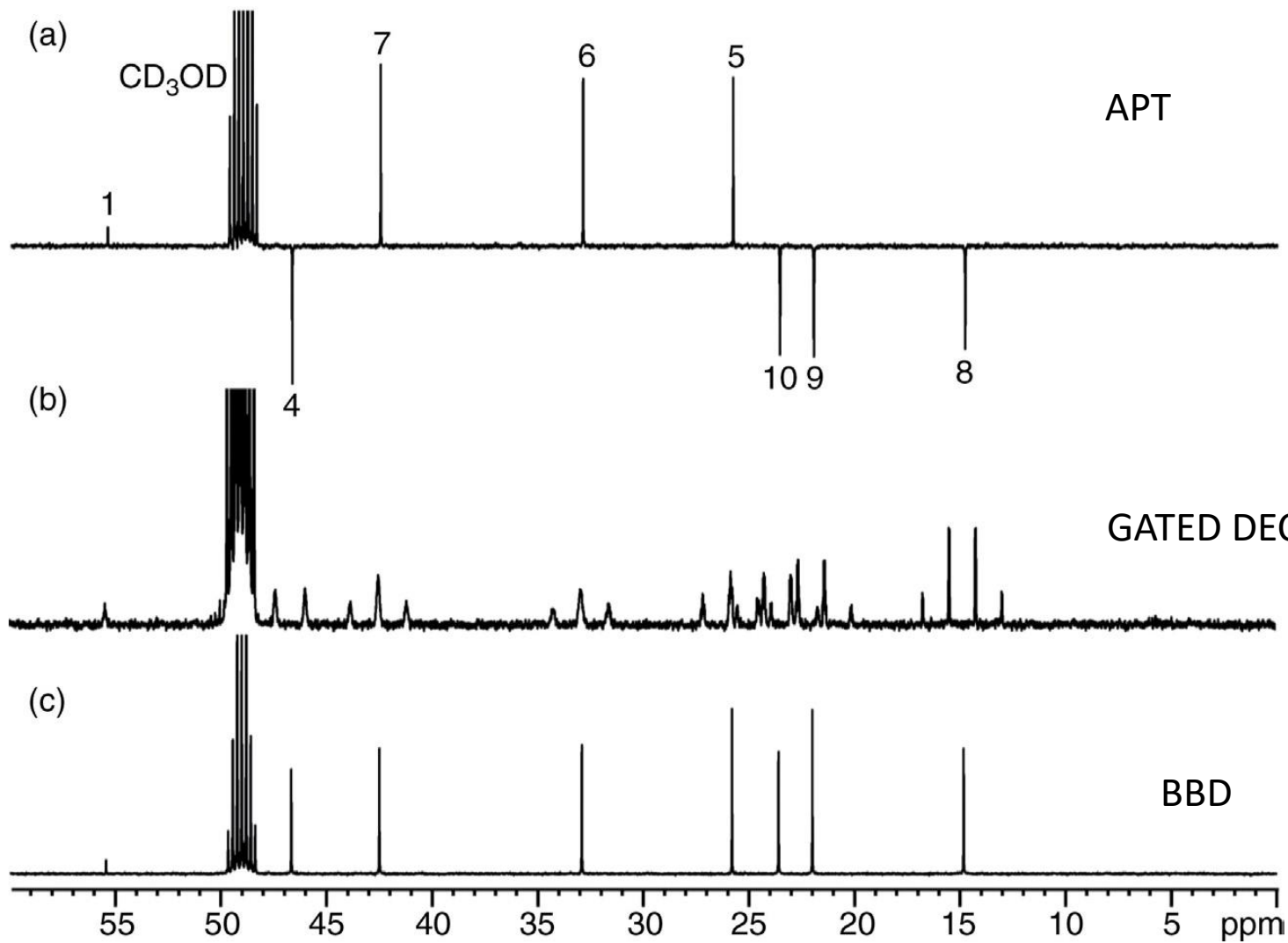
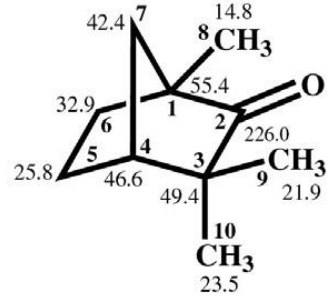
APT ATTACHED PROTON TEST

Evoluzione della magnetizzazione per C, CH, CH₂ e CH₃ per il caso $\tau = 1/J$
 Vedi 2 pagine precedenti



APT ATTACHED PROTON TEST SPECTRUM EDITING





TRASFERIMENTO DI POLARIZZAZIONE

SPT SELECTIVE POLARIZATION TRANSFER (INVERSION)

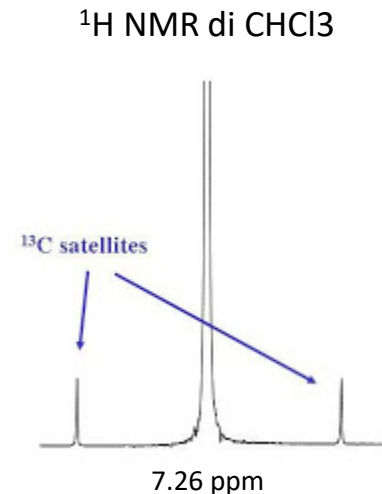
- Problema generale in NMR: bassa sensibilità per alcuni nuclei (^{13}C , ^{15}N)
 - Piccolo valore di rapporto giromagnetico
 - Piccola differenza di popolazione fra stati di spin a e b
 - Scarsa abbondanza naturale
- SPT esperimento che ha lo scopo di aumentare la intensità di segnale di nuclei insensibili accoppiati con nuclei sensibili attraverso un trasferimento di polarizzazione



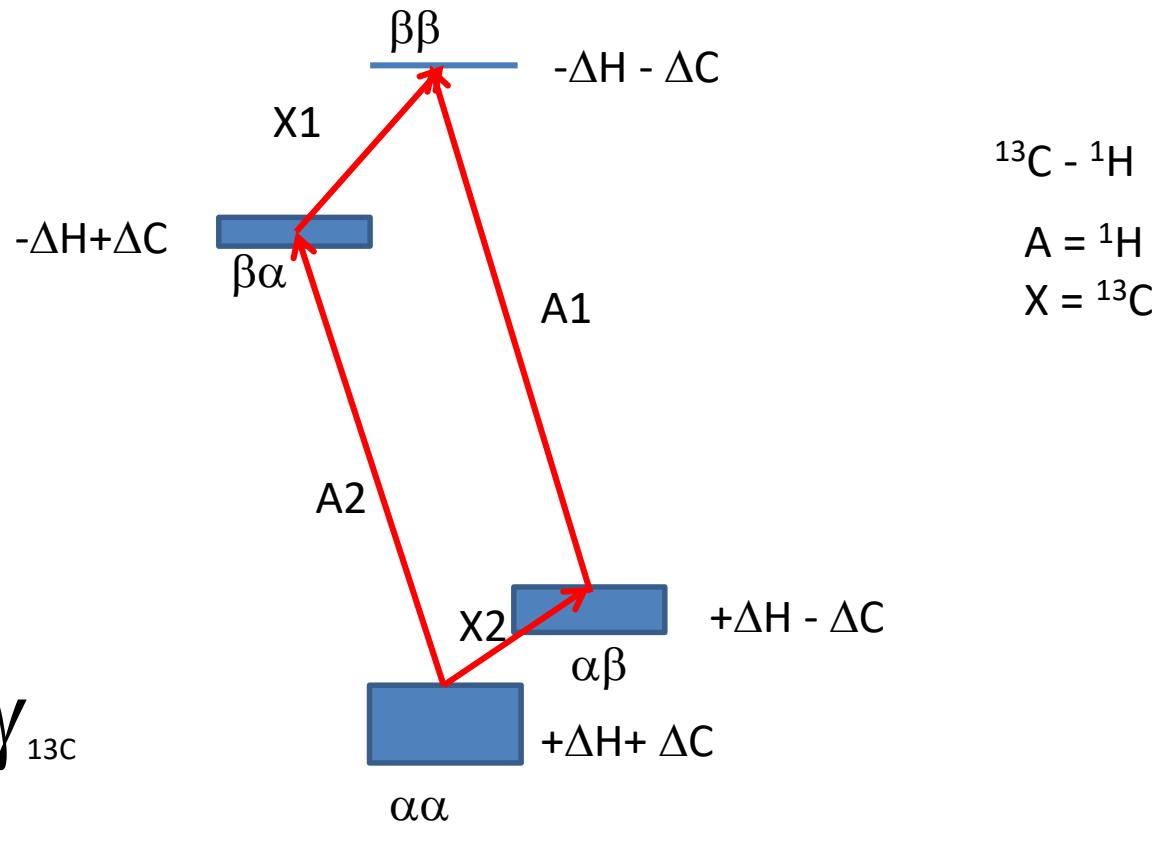
$$\gamma_{^1\text{H}} = 4 \gamma_{^{13}\text{C}}$$

Spettro ^{13}C : doppietto, $J = 209 \text{ Hz}$ $\delta = 77.7 \text{ ppm}$

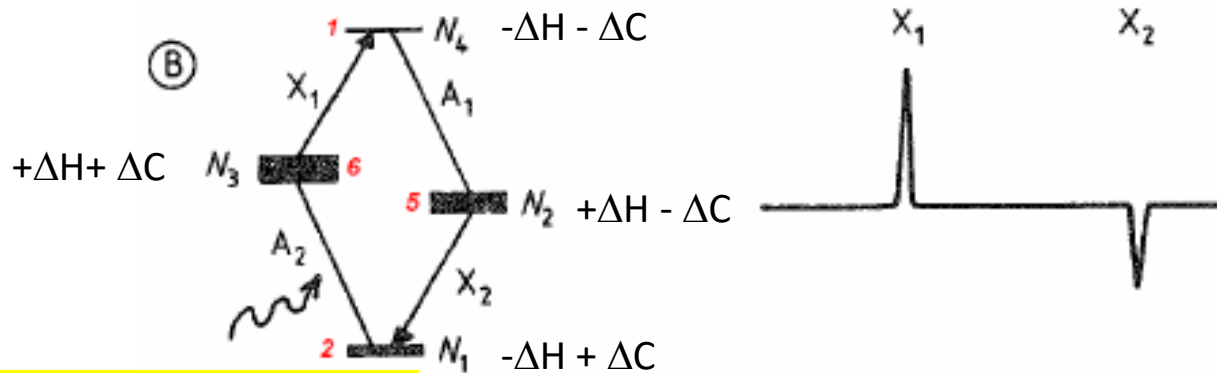
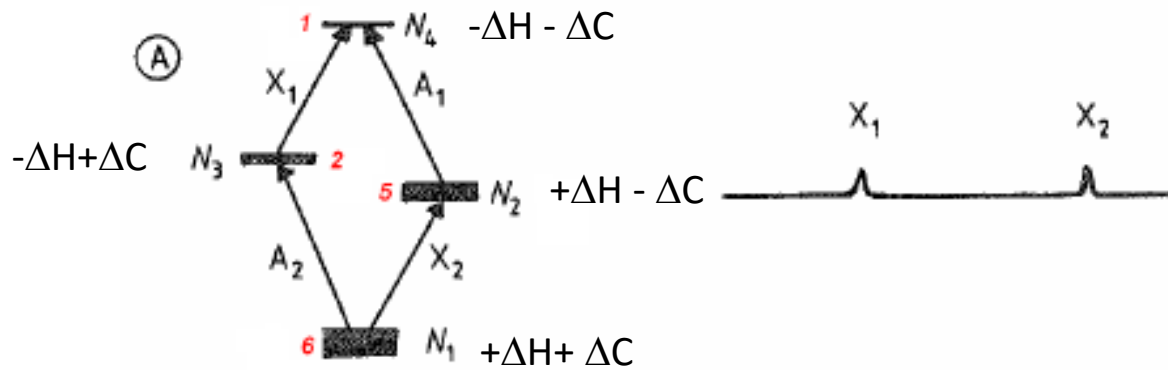
Spettro ^1H : satelliti del ^{13}C intorno al segnale principale



SPI SELECTIVE POPULATION INVERSION



SCHEMA DI LIVELLI ENERGETICI PER UN SISTEMA AX ($^{13}\text{C} - ^1\text{H}$) ACCOPPIATO E DIFFERENZE DI POPOLAZIONE

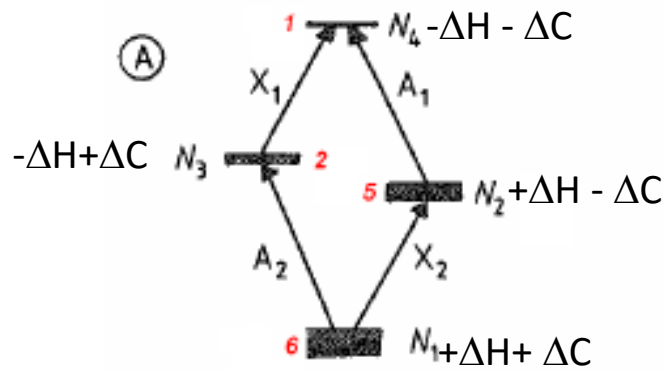


Irraggiamento selettivo
Con impulso 180° soft
Inversione della popolazione
della transizione A2

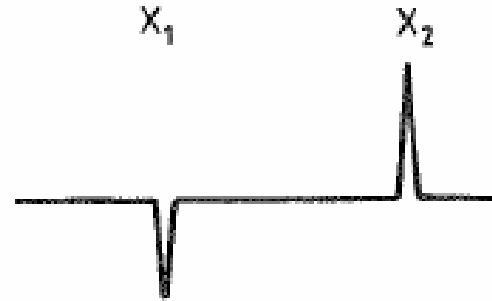
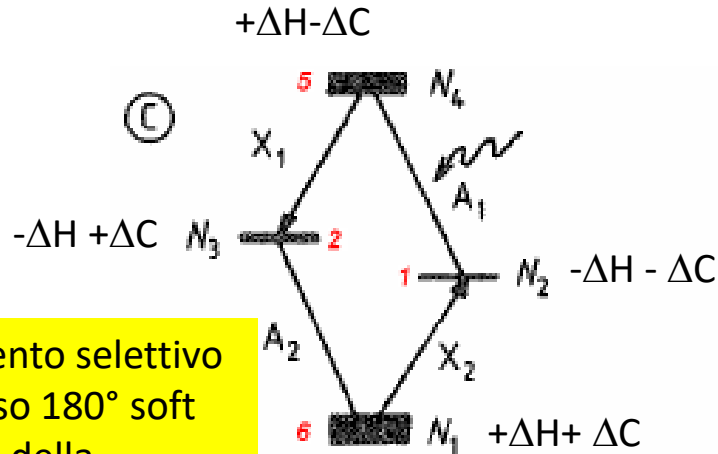
Nel diagramma A: $N_3 - N_4 (X_1) = -\Delta H + \Delta C - (-\Delta H - \Delta C) = 2\Delta C$
 $N_1 - N_2 (X_2) = +\Delta H + \Delta C - (+\Delta H - \Delta C) = 2\Delta C$

$\Delta H = 4 \Delta C$

Nel diagramma B: $N_3 - N_4 (X_1) = \Delta H + \Delta C - (-\Delta H - \Delta C) = 2\Delta H + 2\Delta C = 10 \Delta C$
 $N_1 - N_2 (X_2) = -\Delta H + \Delta C - (+\Delta H - \Delta C) = -2\Delta H + 2\Delta C = -6 \Delta C$



Spettro della parte X del sistema AX



X2 aumenta la sua intensità 5 volte
X1 aumenta la sua intensità 3 volte (negativo)

Irraggiamento selettivo con impulso 180° soft
Inversione della popolazione della transizione A1

Nel diagramma A: $N_3 - N_4 (X_1) = -\Delta H + \Delta C - (-\Delta H - \Delta C) = 2\Delta C$
 $N_1 - N_2 (X_2) = +\Delta H + \Delta C - (+\Delta H - \Delta C) = 2\Delta C$

$\Delta H = 4 \Delta C$

Nel diagramma C: $N_3 - N_4 (X_1) = -\Delta H + \Delta C - (\Delta H - \Delta C) = -2\Delta H + 2\Delta C = -6\Delta C$
 $N_1 - N_2 (X_2) = +\Delta H + \Delta C - (-\Delta H - \Delta C) = 2\Delta H + 2\Delta C = 10\Delta C$

AMPLIFICAZIONE DEL SEGNALE

L'amplificazione del segnale dipende dal fattore $\frac{\gamma_{\text{HIGH}}}{\gamma_{\text{LOW}}}$

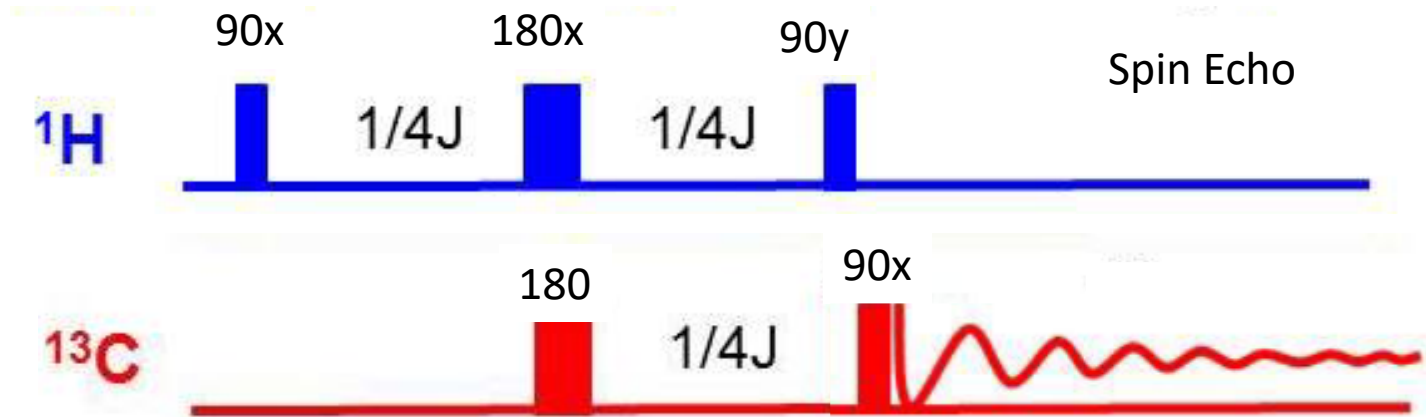
Nel caso del segnale C-H (sistema AX) l'amplificazione è data da

$$\left(1 + \frac{\gamma_{\text{H}}}{\gamma_{13\text{C}}} \right) \text{ e } \left(1 - \frac{\gamma_{\text{H}}}{\gamma_{13\text{C}}} \right)$$

5 -3

Per $^{15}\text{N} - \text{H}$ è +11 e -9 $\frac{\gamma_{\text{H}}}{\gamma_{15\text{N}}} = 10$

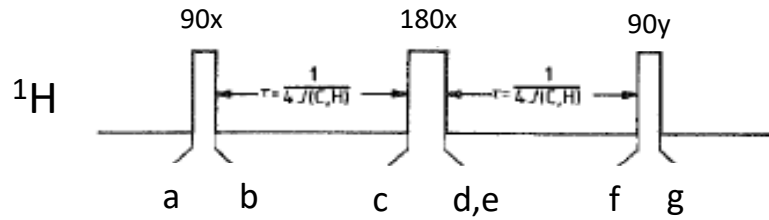
INEPT INSENSITIVE NUCLEI ENHANCED BY POLARIZATION TRANSFER



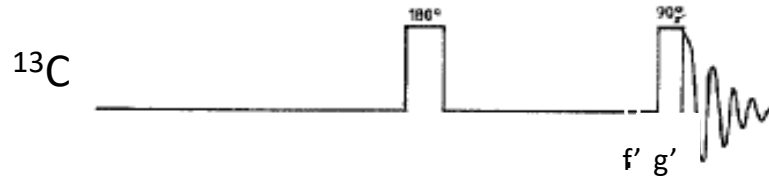
Sistema a due spin $^{13}\text{CHCl}_3$
accoppiato con J_{CH}

INEPT

$^{13}\text{CHCl}_3$
Sistema AX

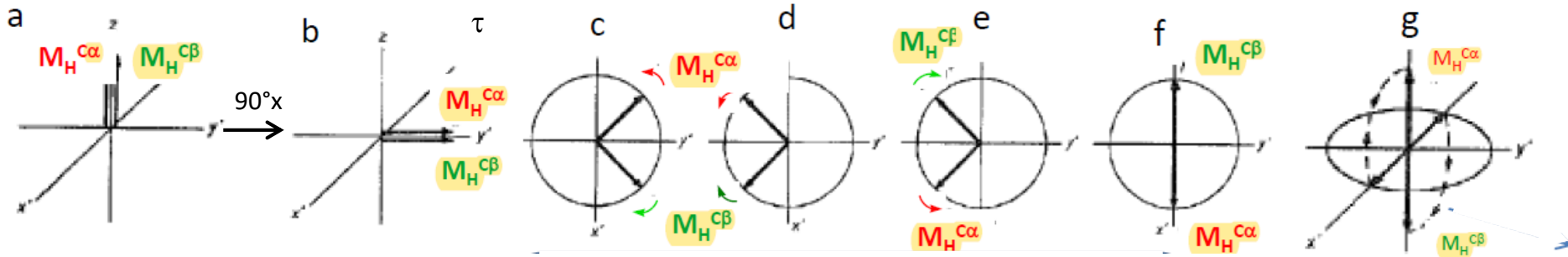


$M_H^{\text{C}\alpha}$ e $M_H^{\text{C}\beta}$

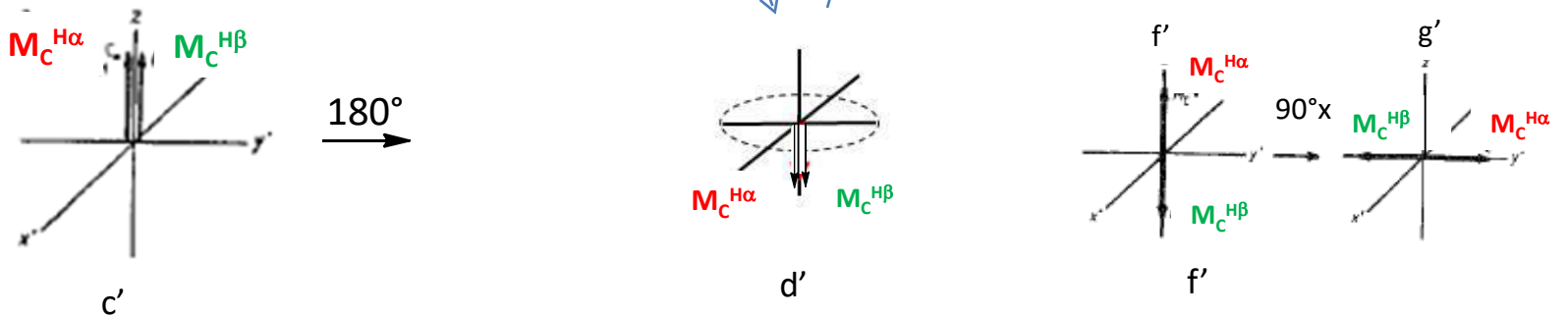


$M_C^{\text{H}\alpha}$ e $M_C^{\text{H}\beta}$

Guardo M_H



Guardo M_C



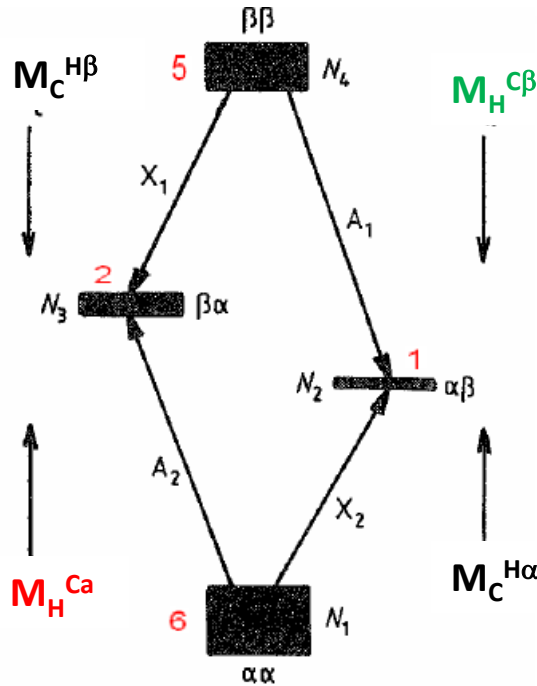
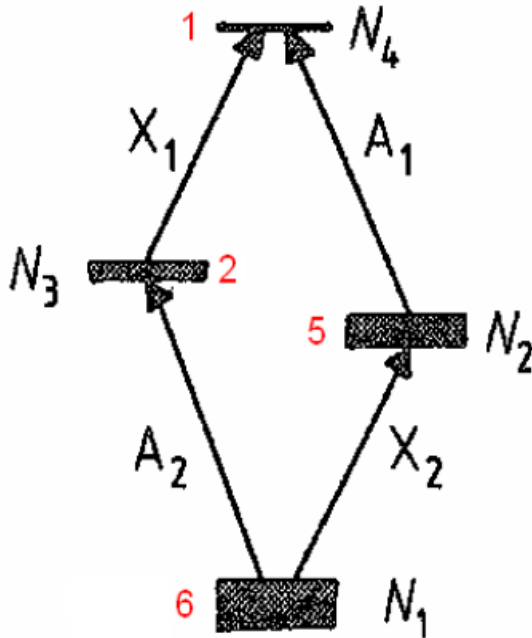
Rispetto ad a si è invertita la popolazione di $M_H^{\text{C}\beta}$ TRASFERIMENTO DI POLARIZZAZIONE

Si inverte $M_C^{\text{H}\beta}$ (rispetto all'inizio) a causa dell'inversione di $M_H^{\text{C}\beta}$ (slide successiva) TRASFERIMENTO DI POLARIZZAZIONE

INEPT

Trasferimento di polarizzazione da H a C

a

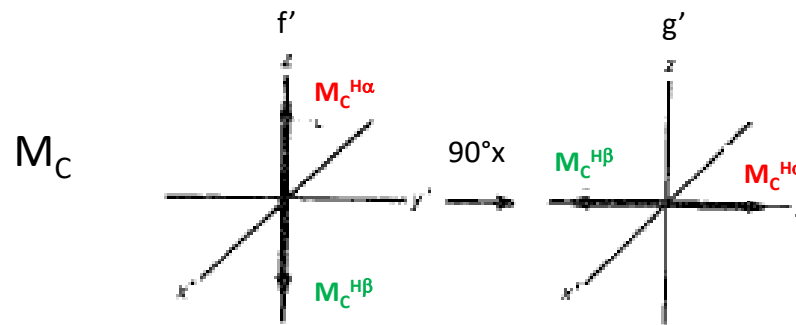


f, f'

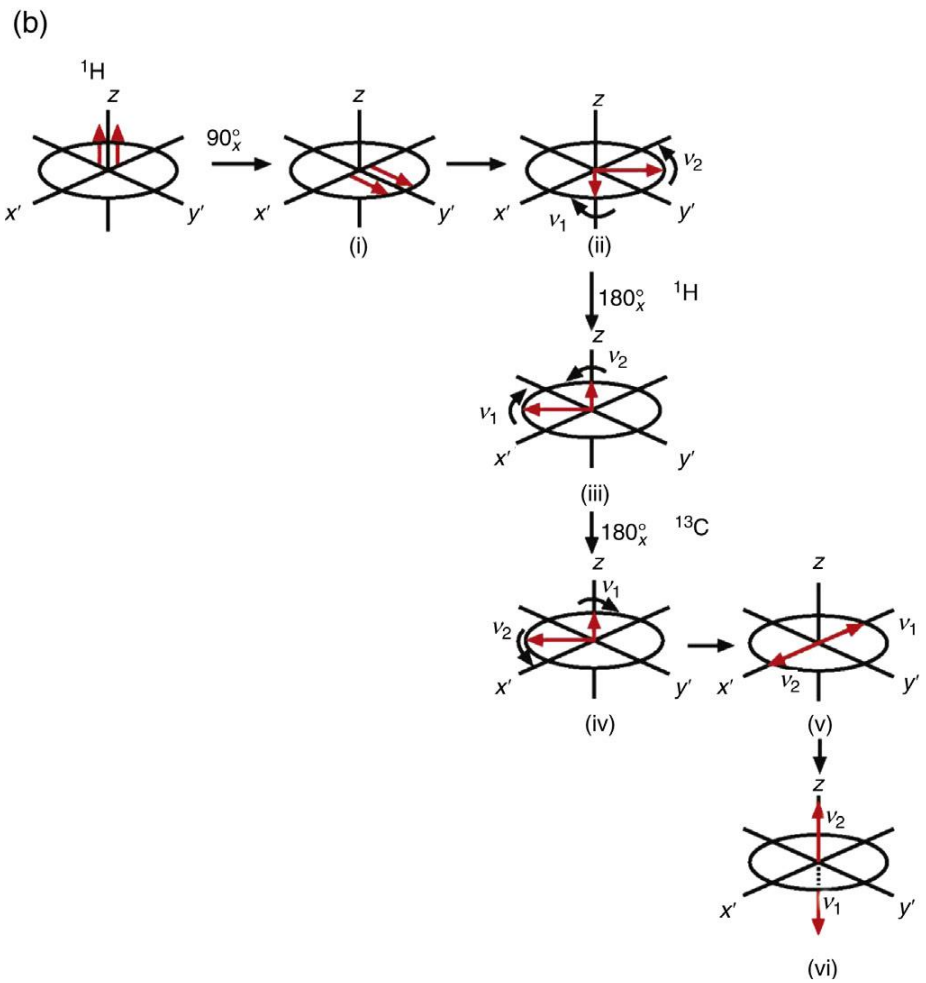
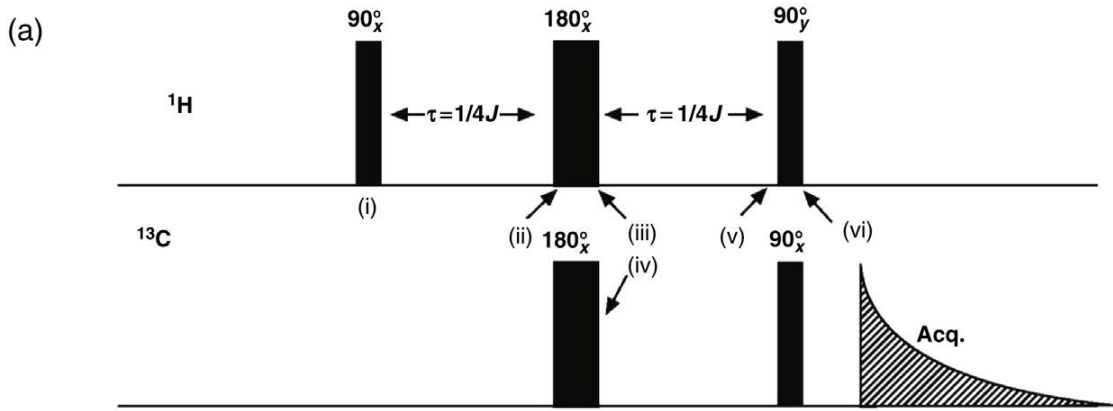
Si è invertita anche la $M_C^{H\beta}$ perché $N_4 > N_3$

E' aumentata la differenza di popolazione per X2 e X1

Il diagramma energetico è lo stesso di SPI quindi è lo stesso anche lo spettro ma è ottenuto più facilmente



Ultimo impulso a 90°_x serve a creare magnetizzazione osservabile su x,y (Impulso di osservazione sul ^{13}C : doppietto con le righe in antifase)

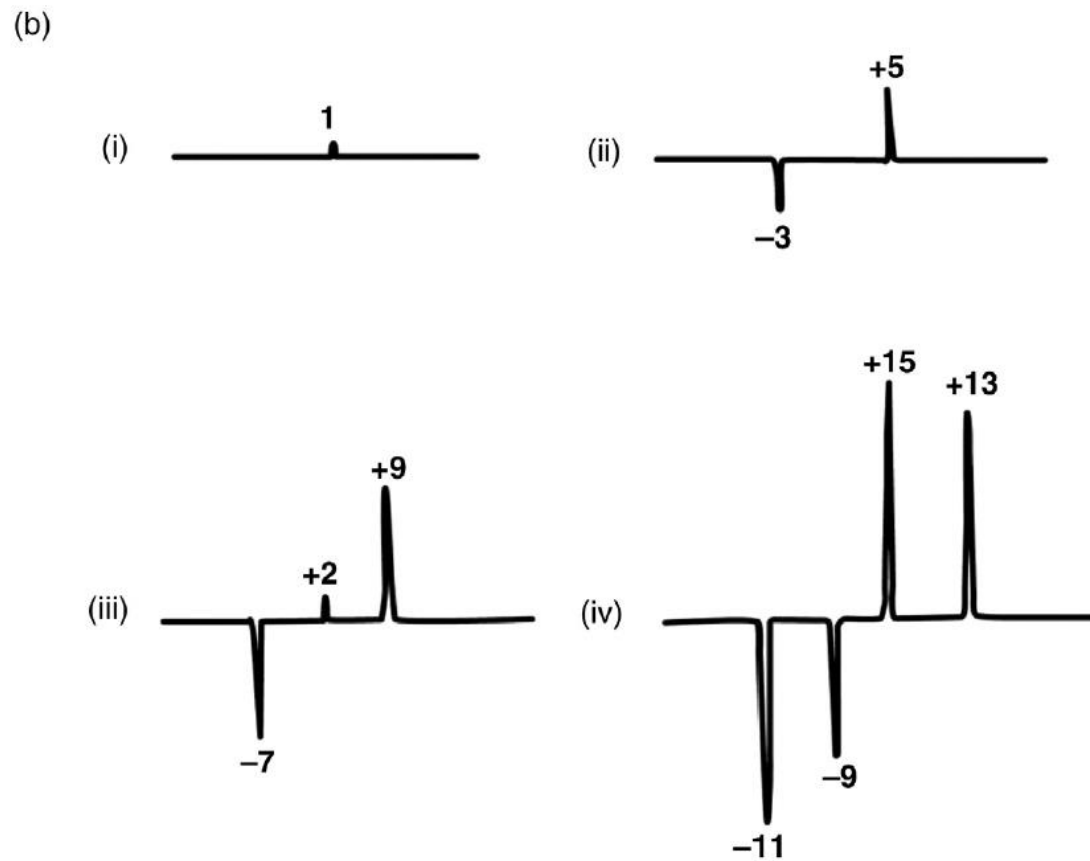


M_H

TRASFERIMENTO DI POLARIZZAZIONE

FATTORI DI INNALZAMENTO NELL'INEPT

(a)		1		(i) C	
		-3	5	(ii) CH	
	-7	2	9	(iii) CH ₂	
	-11	-9	15	13	(iv) CH ₃



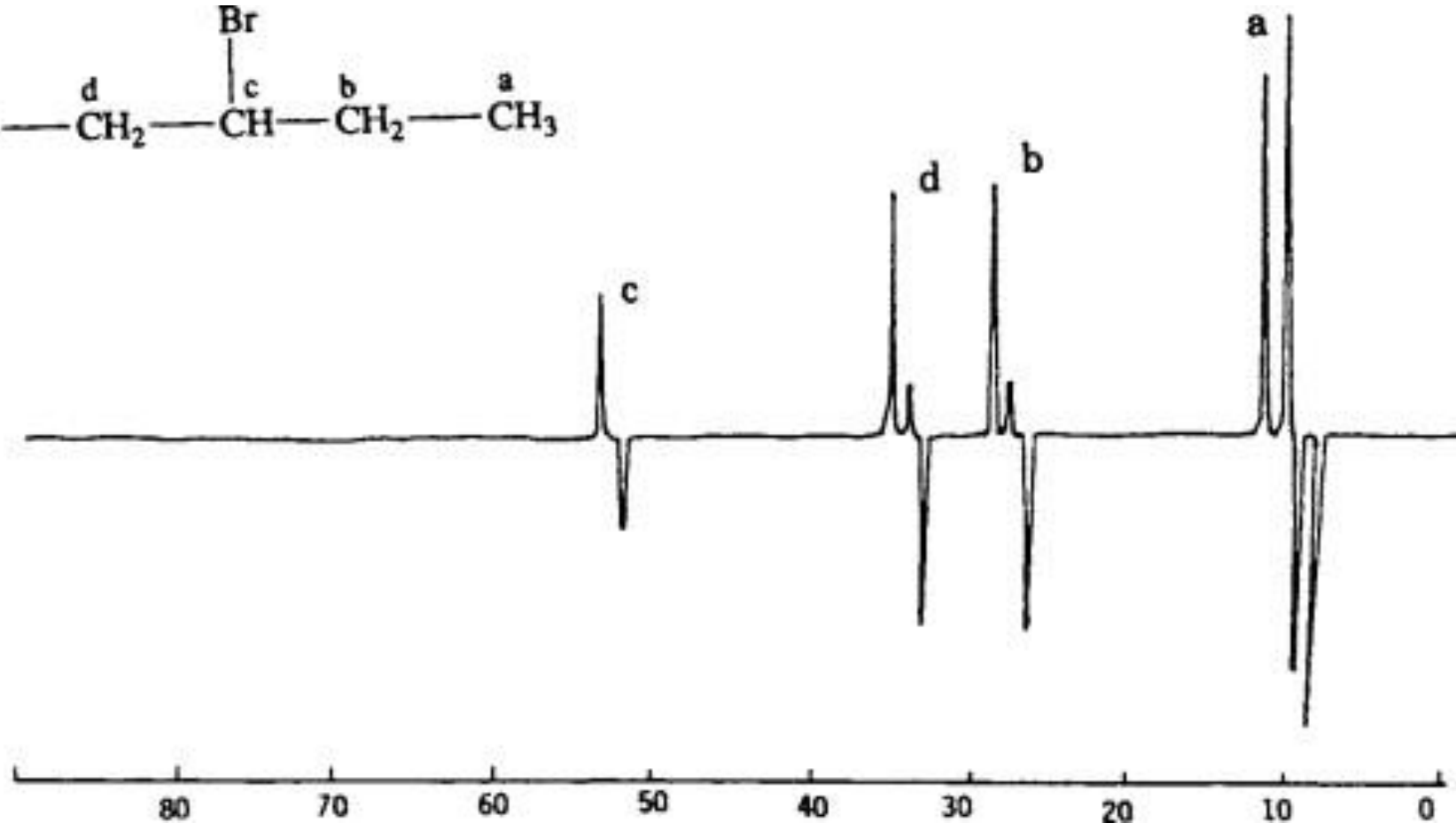
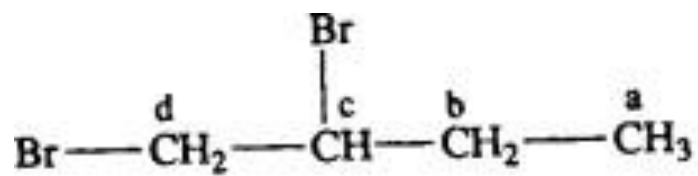
CONFRONTO CON NOE

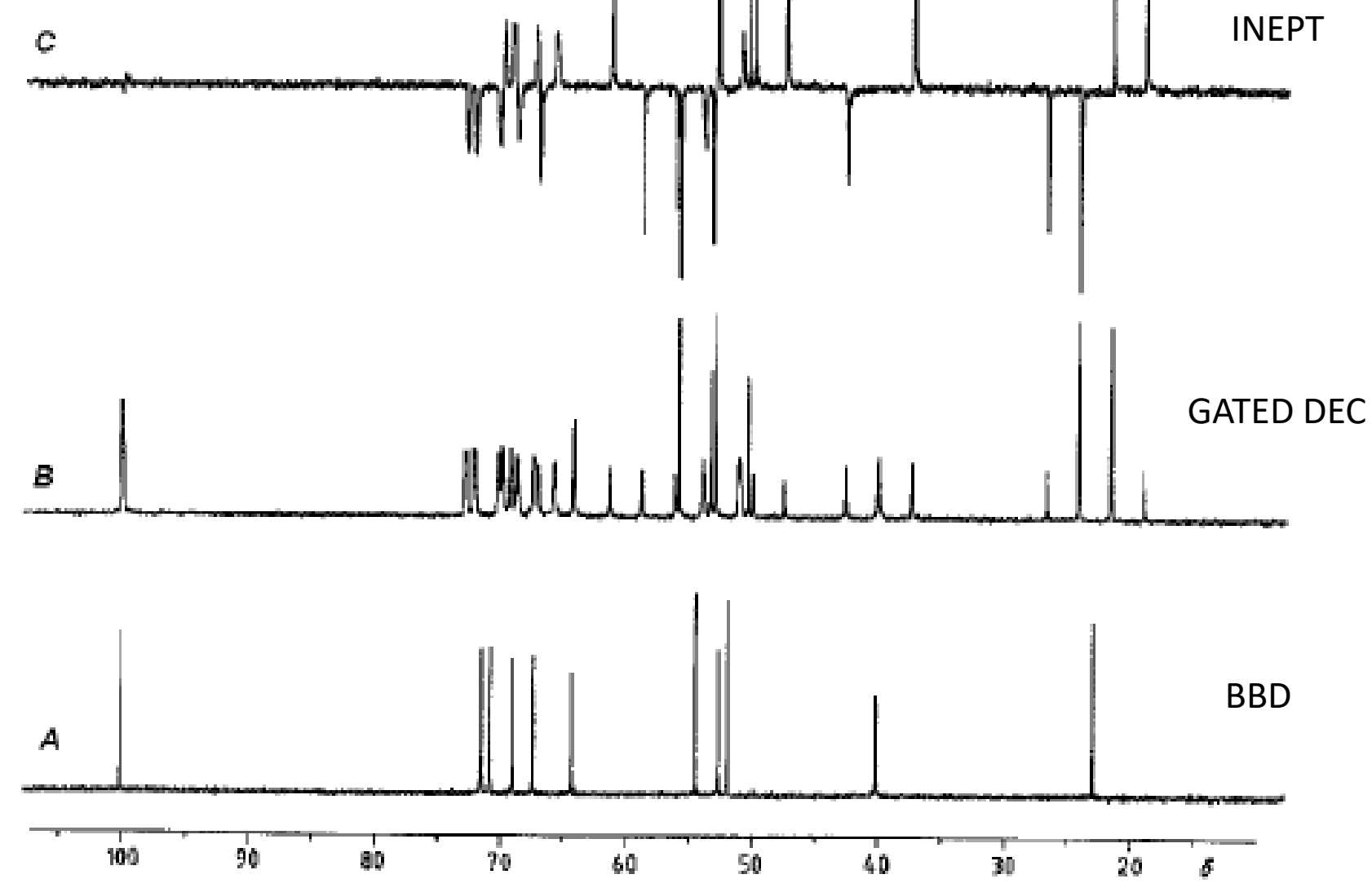
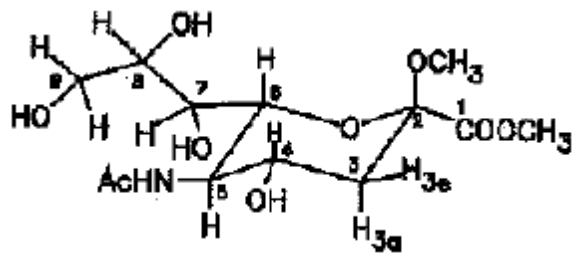
TABLE 4.1 A Comparison of Signal Intensities Obtained by Polarization Transfer against Those Obtained by Full nOe from Protons to the Heteronucleus*

Nucleus	Polarization Transfer	Maximum nOe
^{13}C	3.98	2.99
^{15}N	9.87	-3.94
^{31}P	2.47	2.24
^{29}Si	5.03	-1.52
^{57}Fe	30.95	16.48
^{103}Rh	31.78	-14.98

*The intensities given are relative to those observed without nOe.

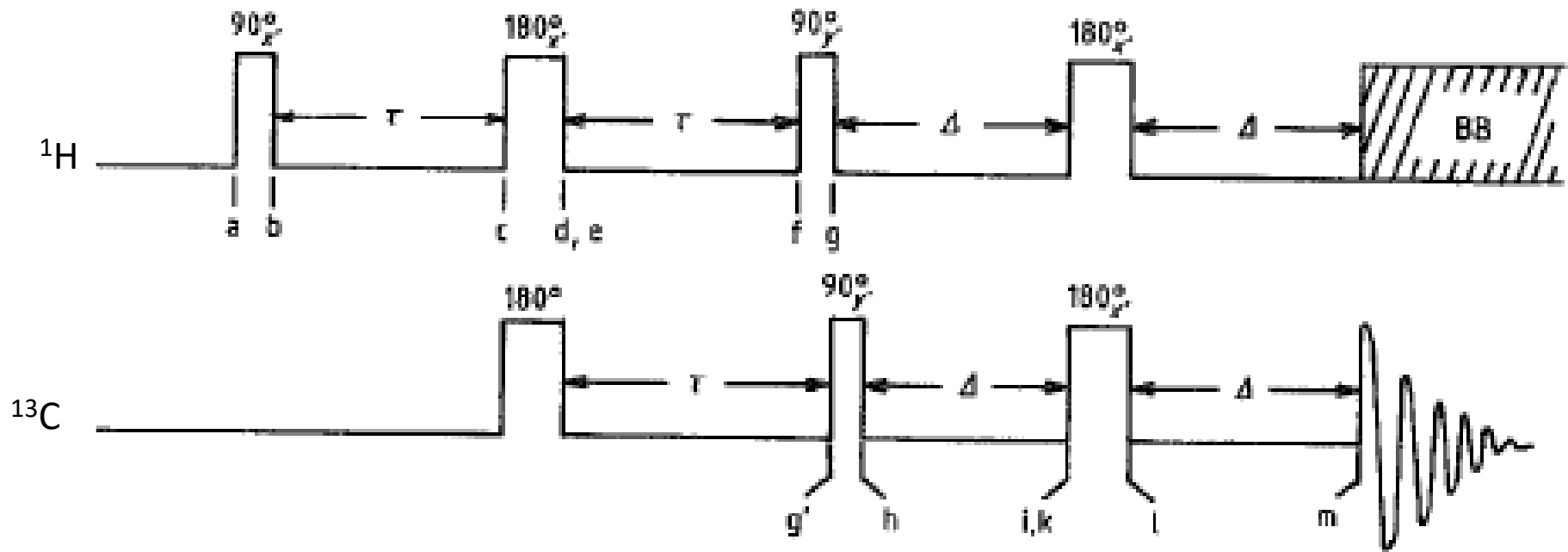
INEPT





REFOCUSSED INEPT

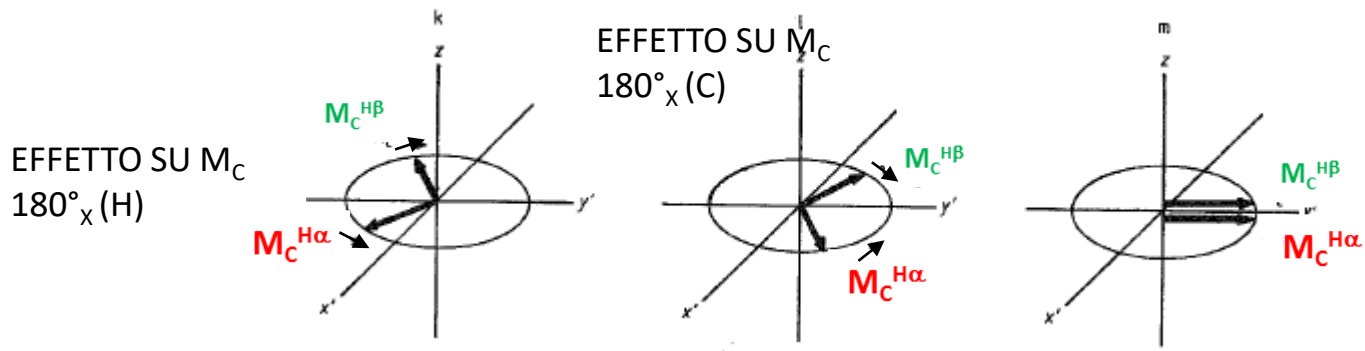
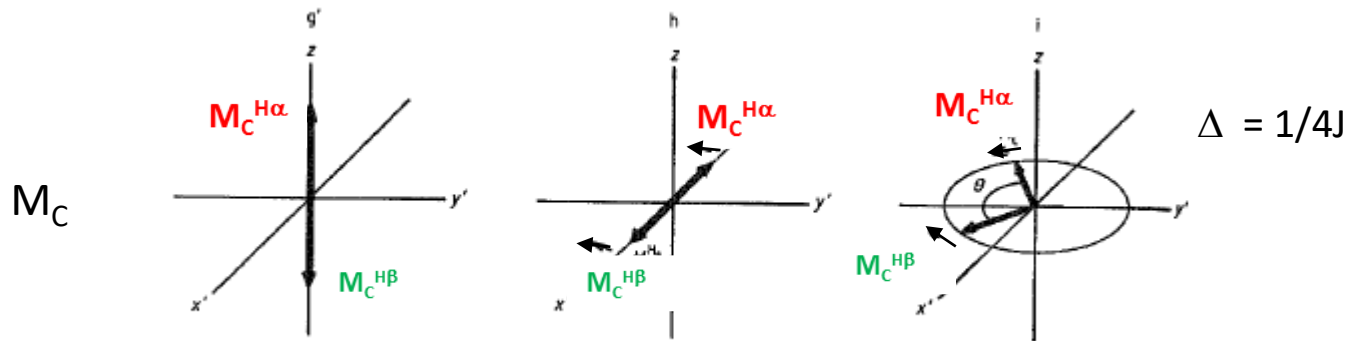
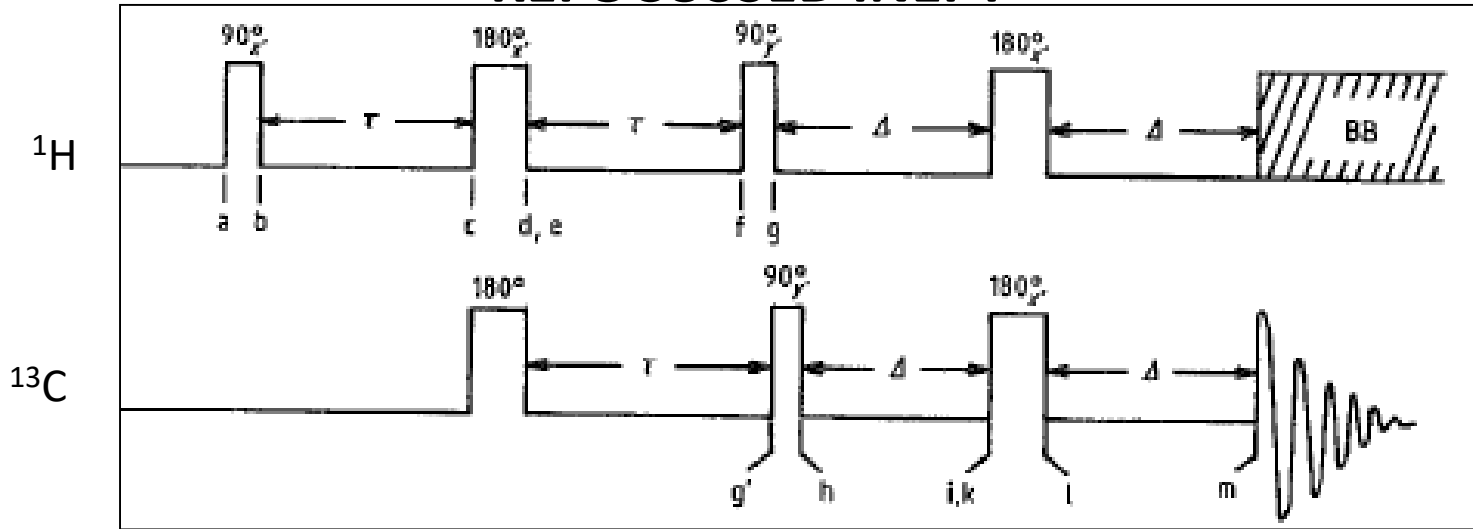
Svantaggio dell'INEPT: non si può disaccoppiare, i segnali di segno opposto collasrebbero in un segnale nullo



Fino a g,g' è come l'INEPT

$$\Delta = 1/4J$$

REFOCUSSED INEPT

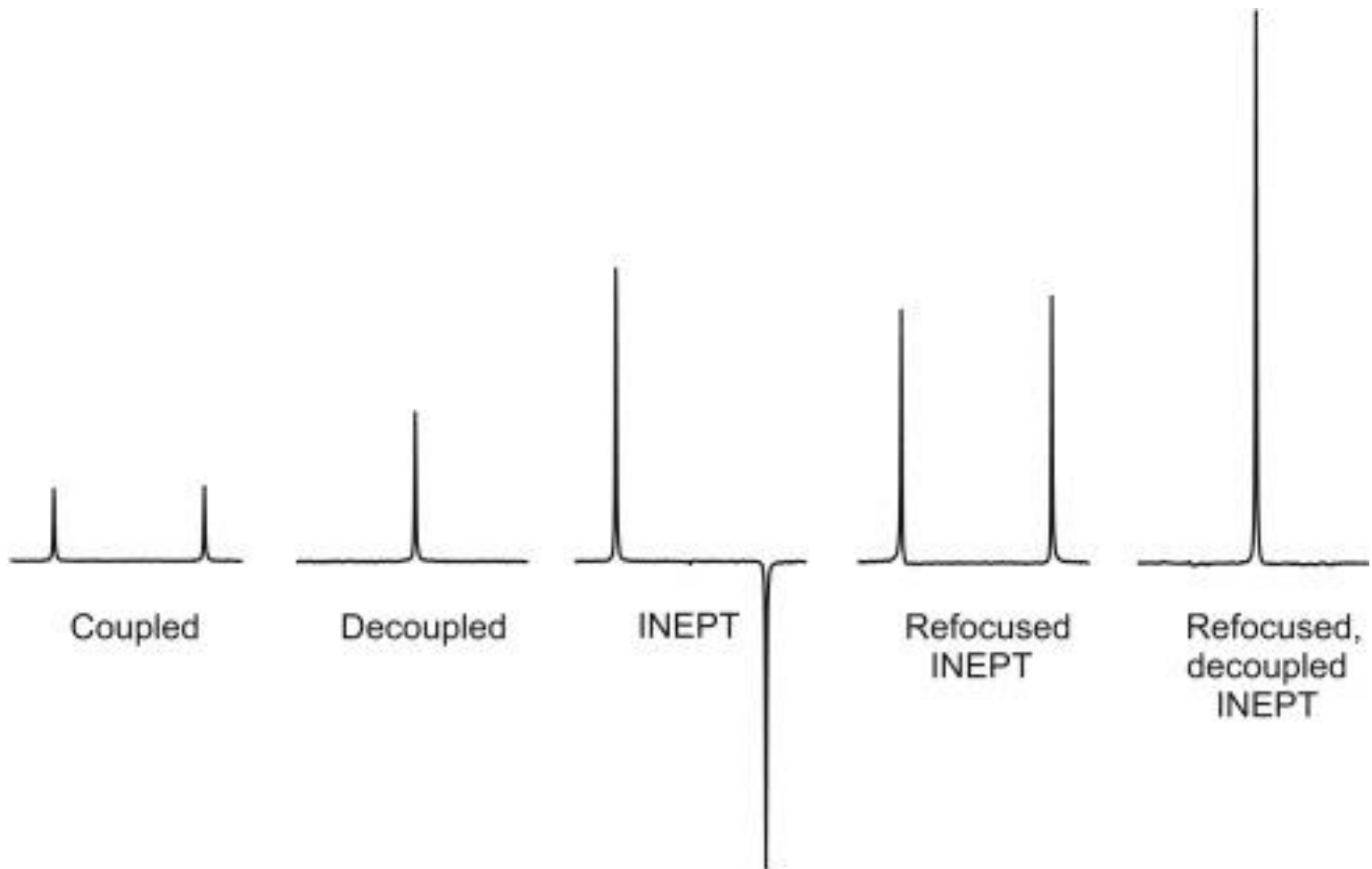


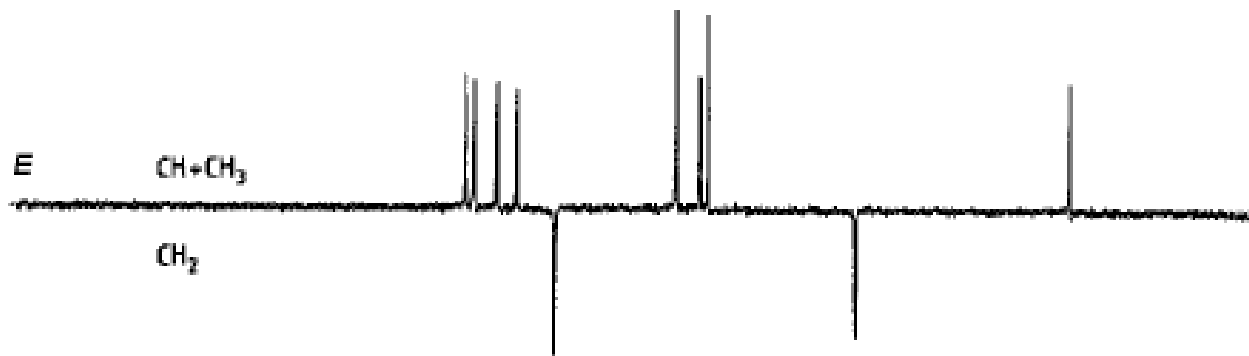
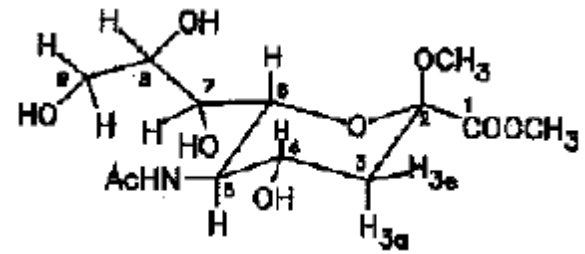
REFOCUSED INEPT

Il delay con cui si ha massima amplificazione

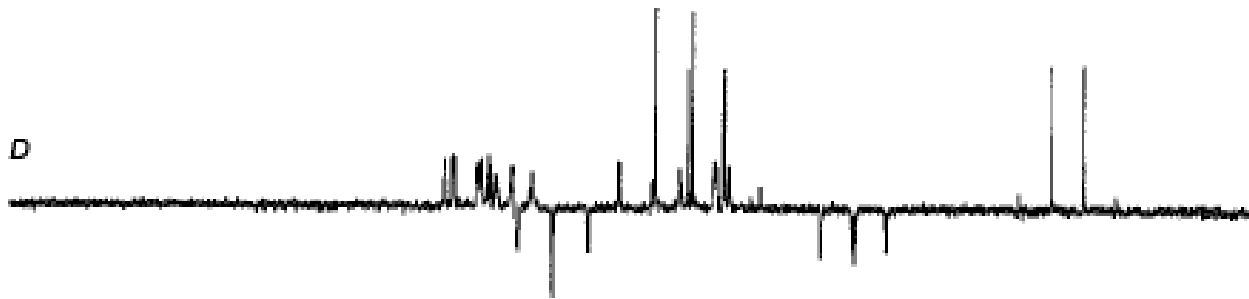
- Per gruppi CH: $\frac{1}{4} J(C,H)$
 - per gruppi CH₂ : $\frac{1}{8} J(C,H)$
 - per gruppi CH₃ : circa lo stesso ma non si ha mai esatta rifocalizzazione
-
- Un valore in pratica: $\frac{3}{8} J(C,H)$
 - CH, CH₃ positivi, CH₂ negativi EDITING DELLO SPETTRO

CONFRONTO FRA LE TECNICHE

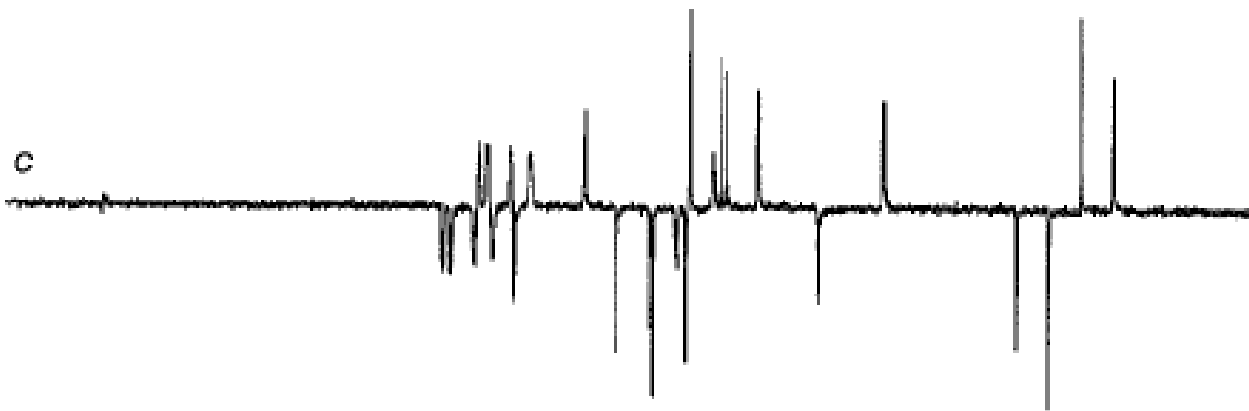




REFOCUSED con BBD



REFOCUSED senza BBD



INEPT

DEPT

DISTORTIONLESS **E**NHANCEMENT BY **P**OLARIZATION **T**RANSFER

Precedenti esperimenti :

J-modulated spin echo (APT) : non differenzia C(q) da CH₂, e CH da CH₃

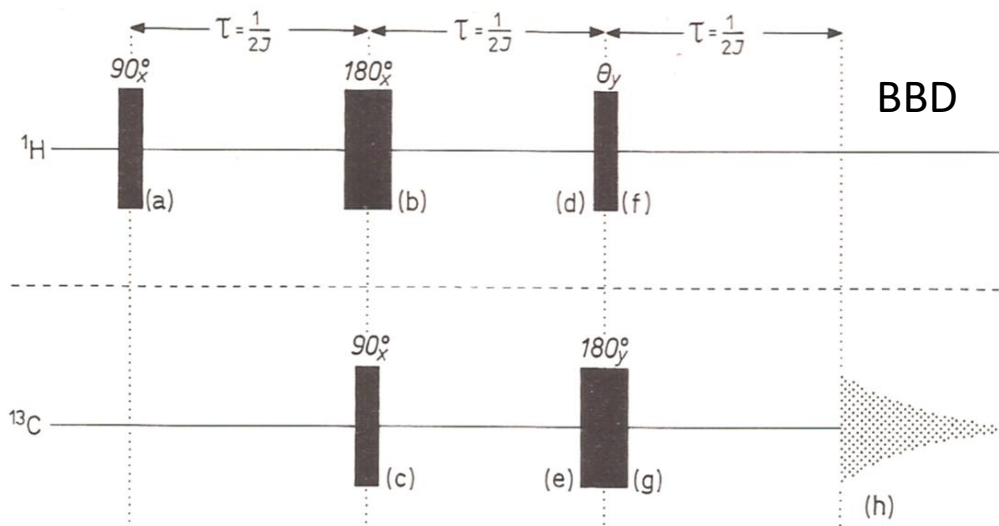
INEPT: sovrapposizione di segnali, non si può disaccoppiare

INEPT refocussed: non differenzia CH da CH₃

DEPT supera queste limitazioni, dando multipletti non distorti (versione accoppiata)

e completo editing nella versione disaccoppiata (più usata)

Sistema C-H

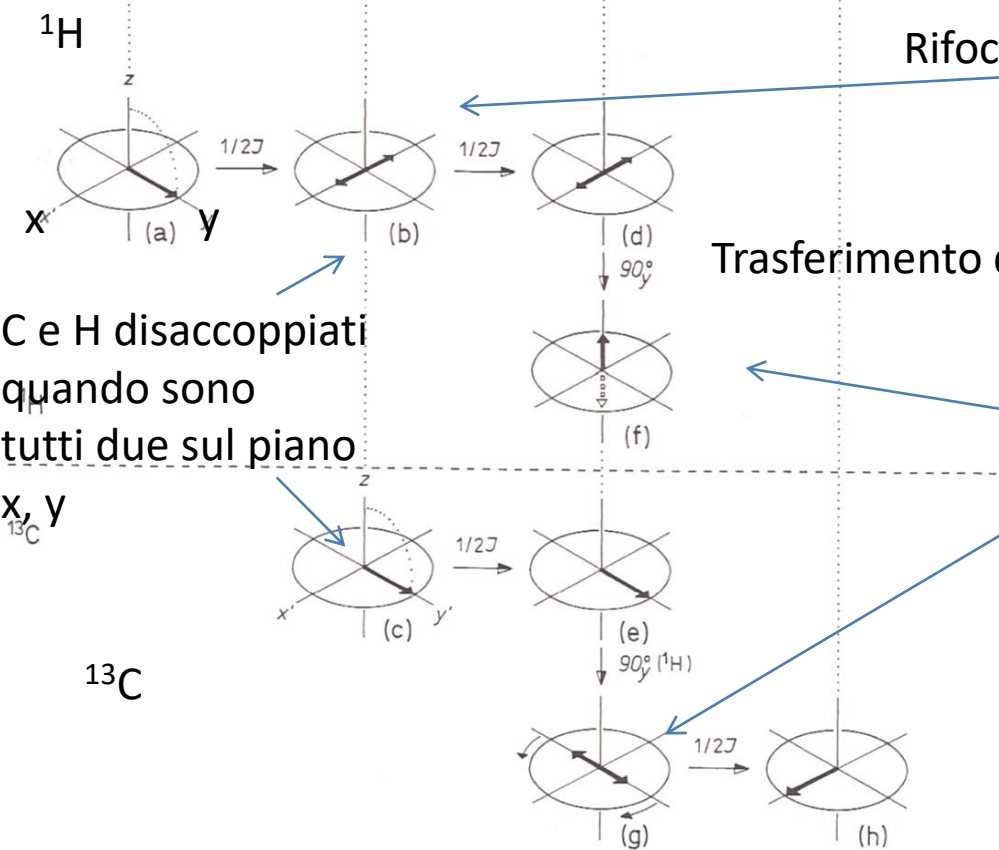


L'impulso θ sull' ^1H determina il PT

$$\theta = 45^\circ, 90^\circ, 135^\circ$$

Disaccoppiatore acceso durante AT

Rifocalizzazione delle disomogeneità di campo



Trasferimento di polarizzazione

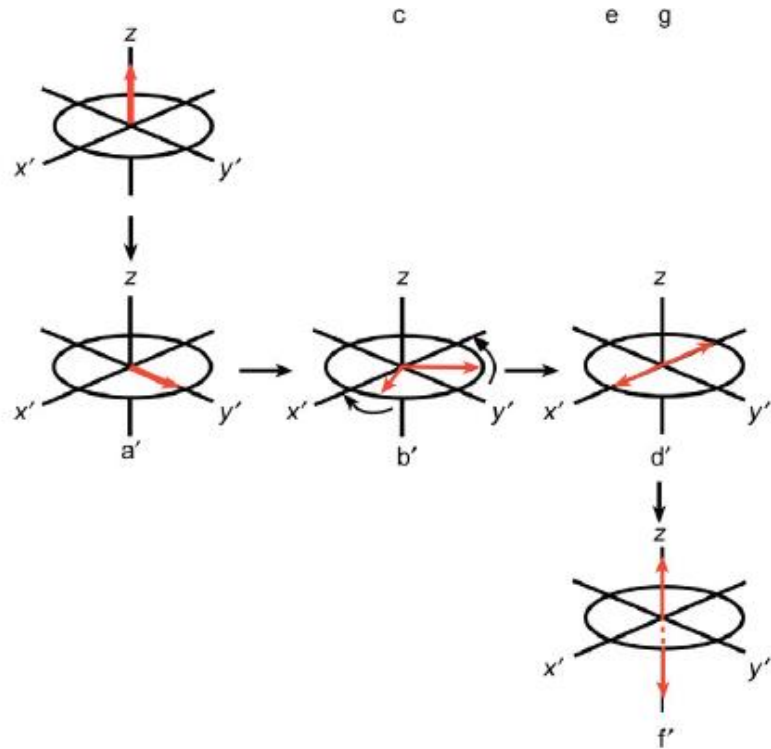
C e H disaccoppiati quando sono tutti due sul piano

C e H di nuovo accoppiati

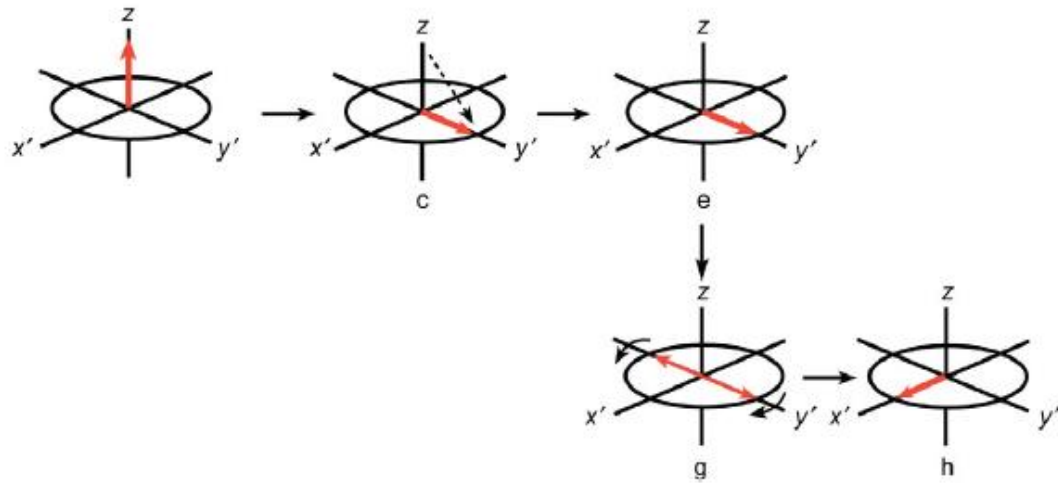
Per CH si ha il massimo PT per $\theta = 90^\circ$

(b)

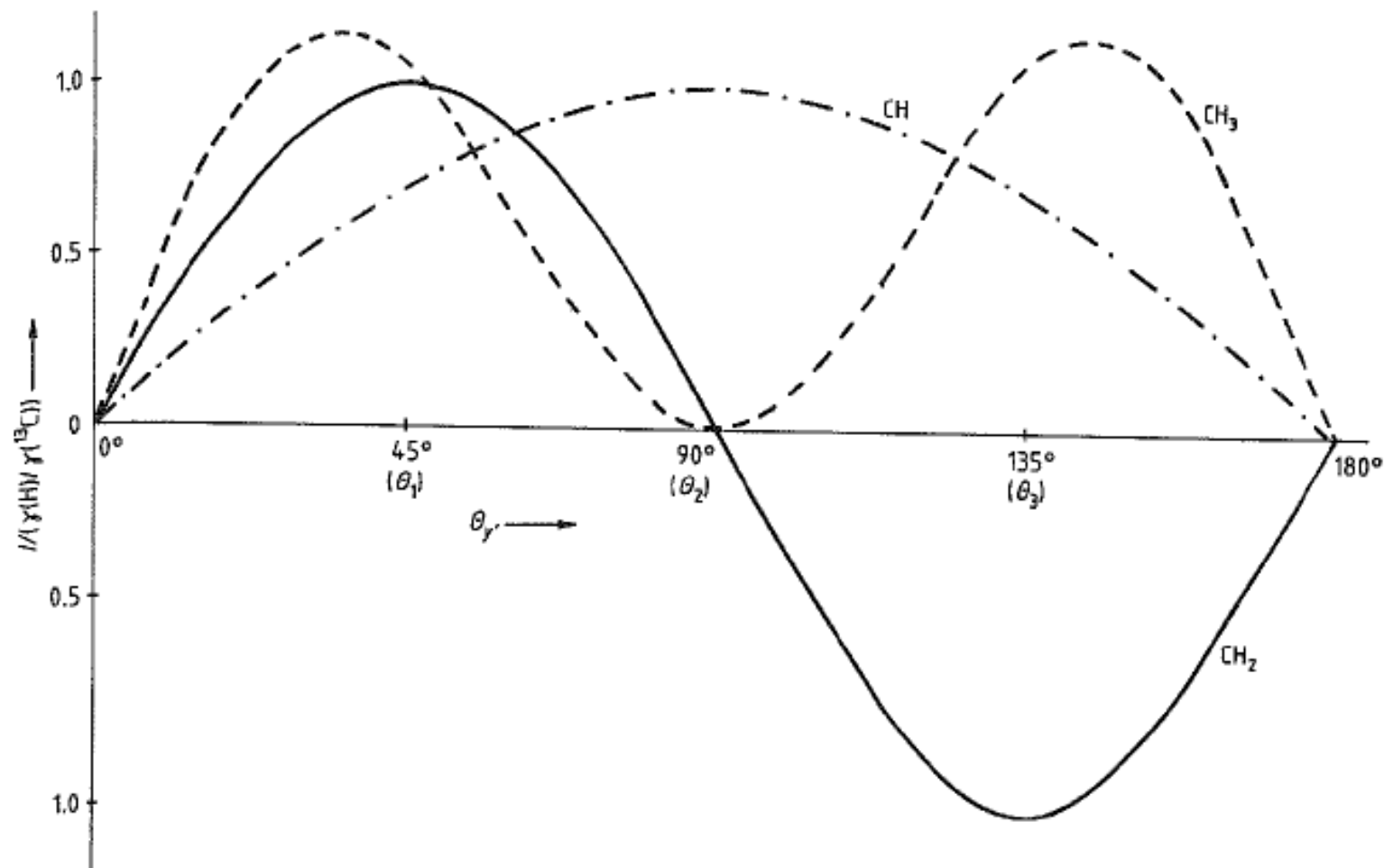
^1H



^{13}C

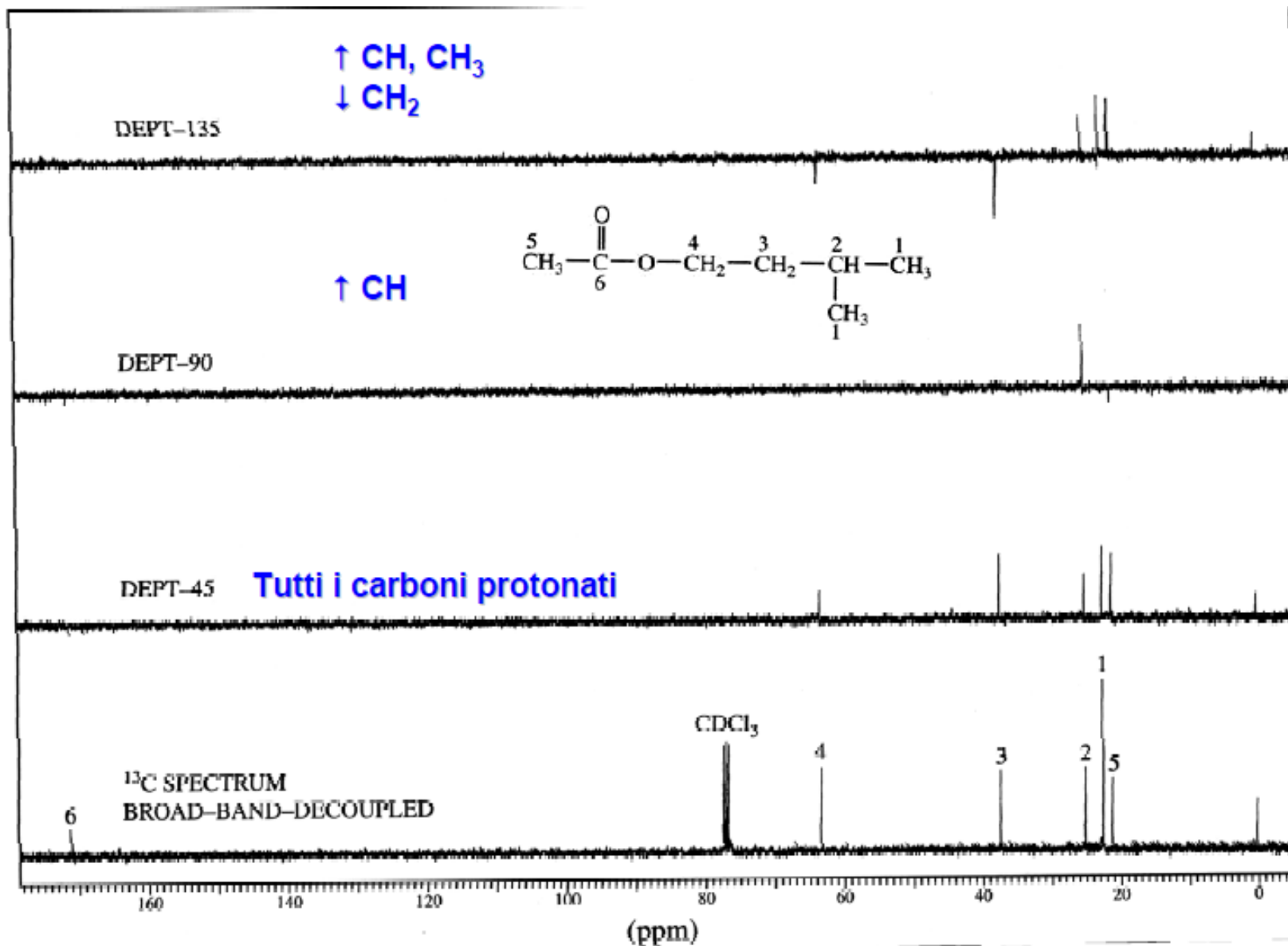


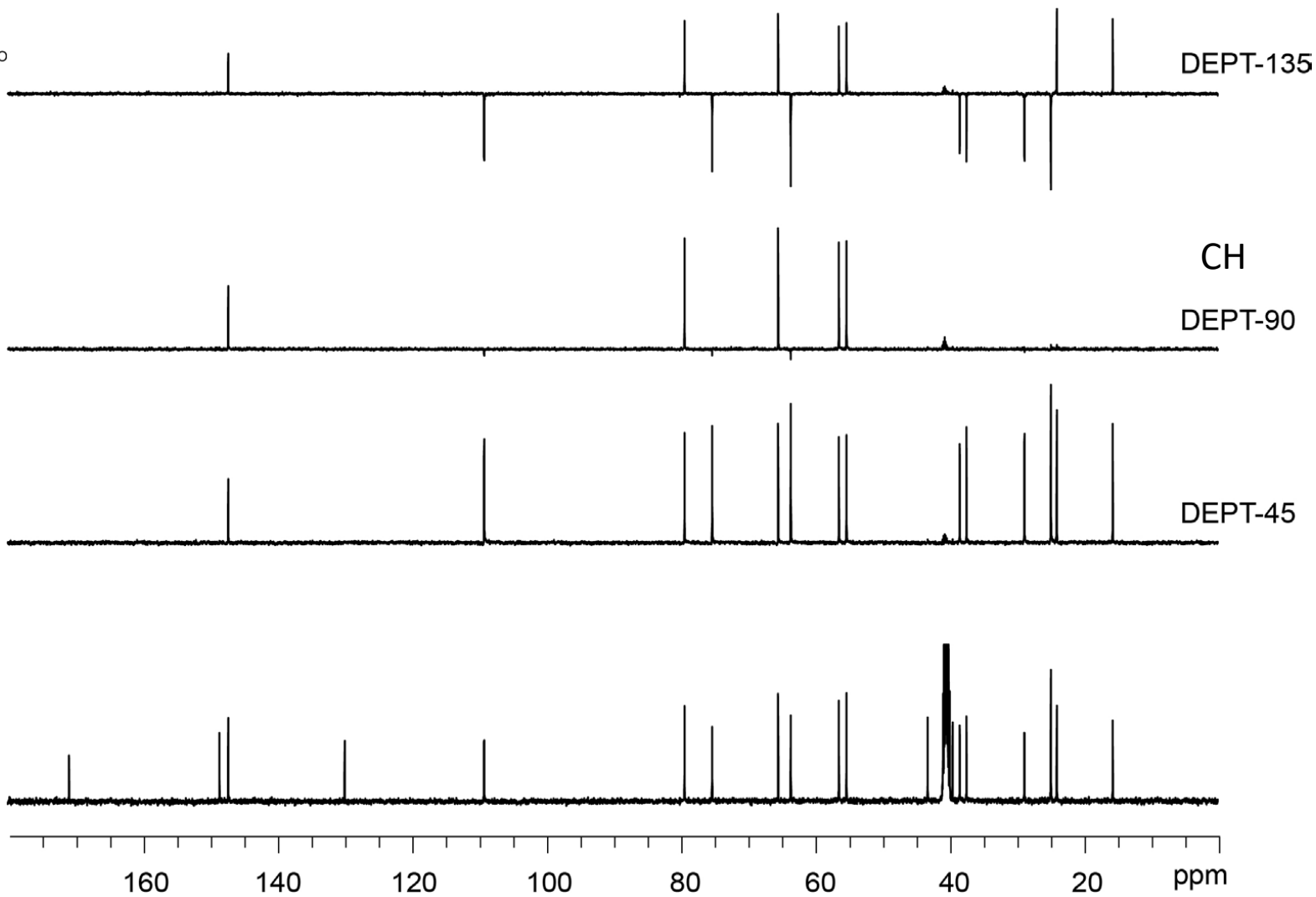
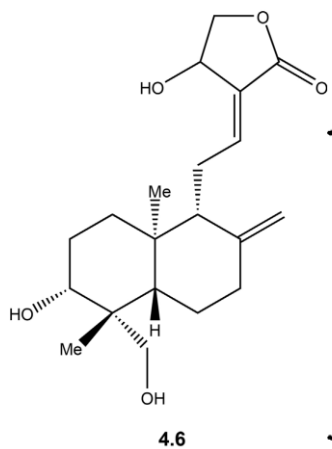
DEPT



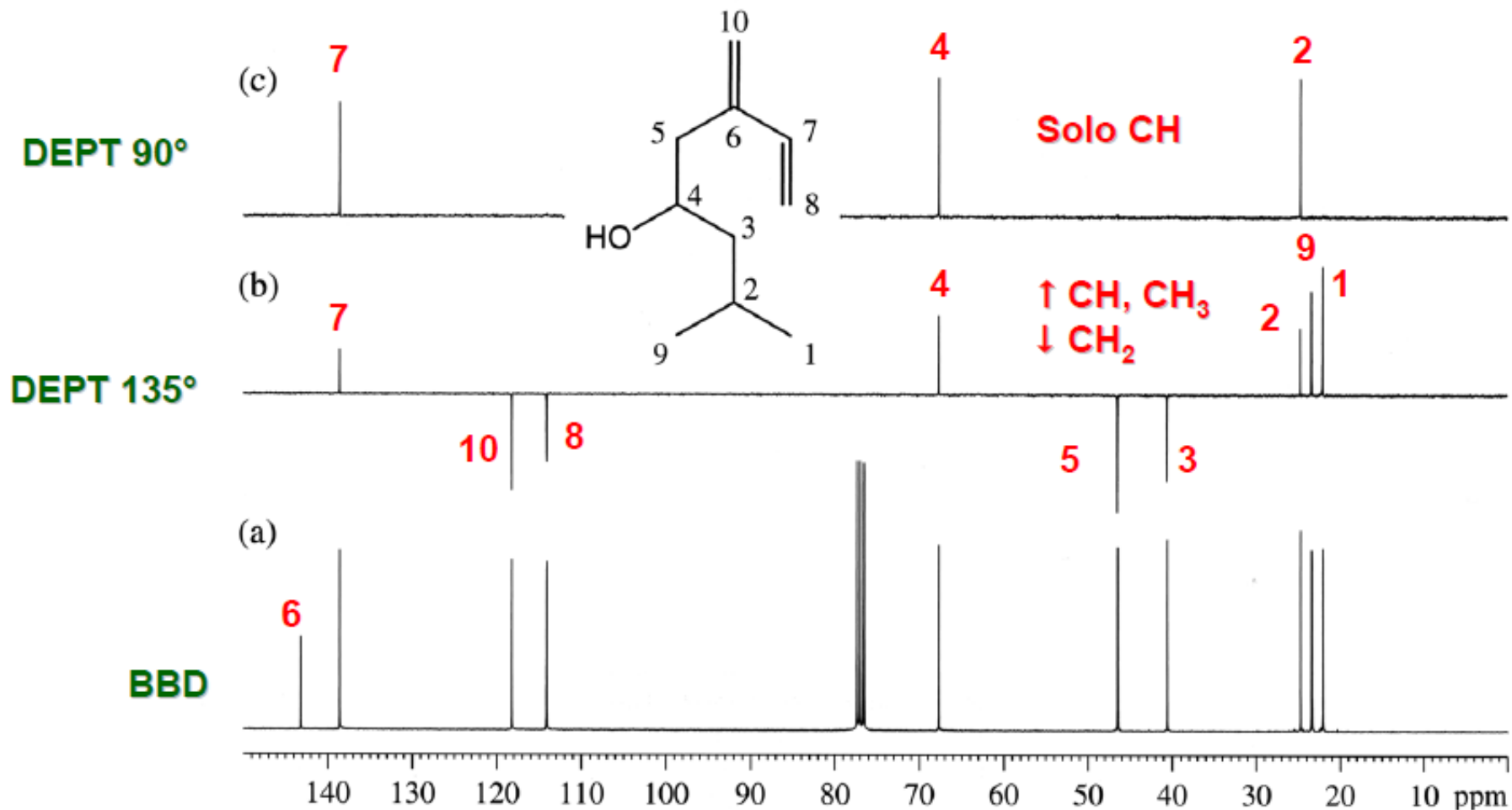
- CH: $I = [\gamma(^1\text{H})/\gamma(^{13}\text{C})]\sin\theta$
- CH₂: $I = [\gamma(^1\text{H})/\gamma(^{13}\text{C})]\sin 2\theta$
- CH₃: $I = [3\gamma(^1\text{H})/4\gamma(^{13}\text{C})] (\sin\theta + \sin 3\theta)$

EDITING





EDITING

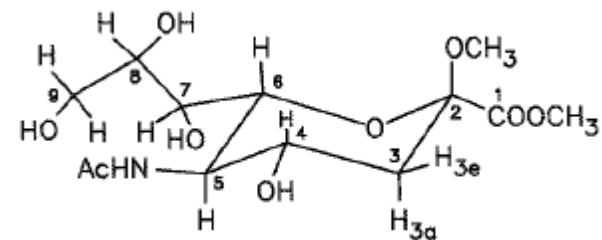
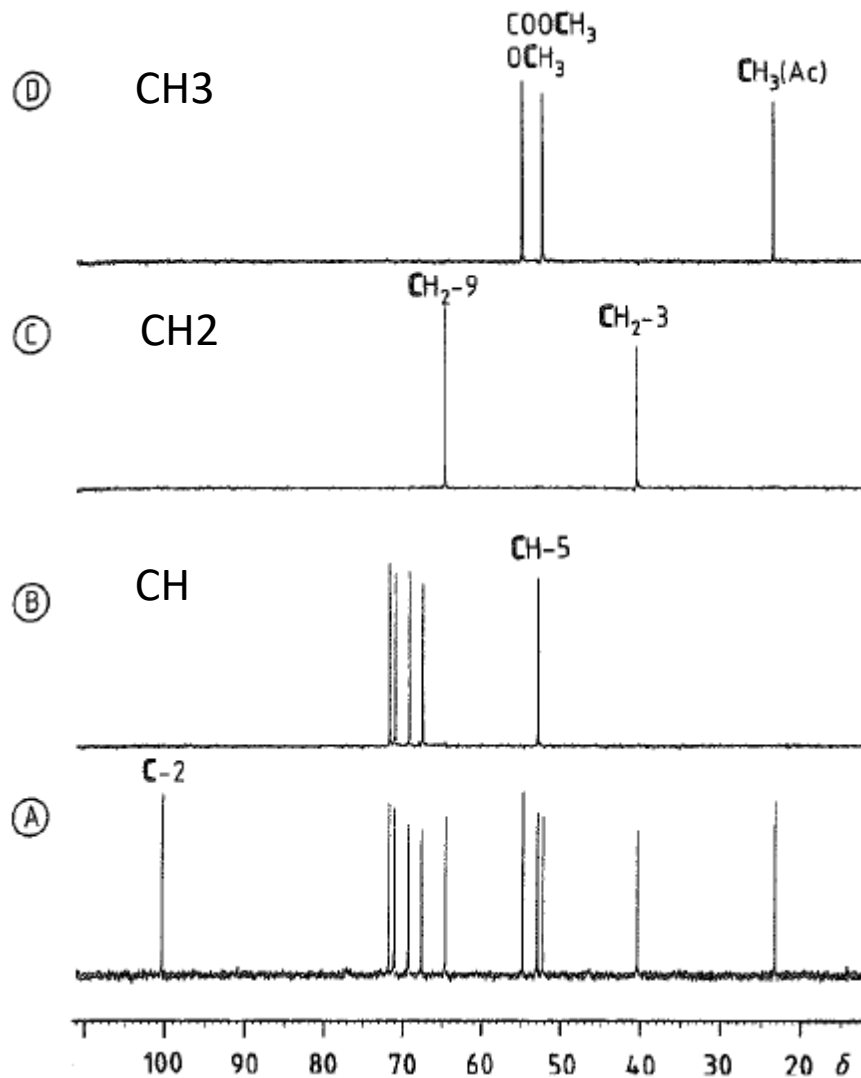


EDITING

CH subspetro: DEPT(90)

CH2 subspetro (DEPT(45) –DEPT (135))

CH3 subspetro DEPT(45)-DEPT(135) – 0.707 DEPT(90)



BBD

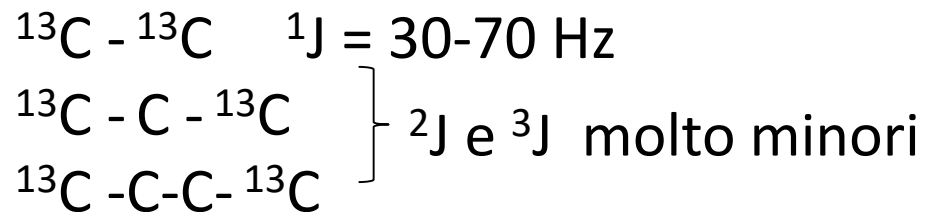
INADEQUATE 1D

Incredible Natural Abundance Double QUantum Transfer Experiment

Abbondanza naturale ^{13}C 1.1% (10^{-2})

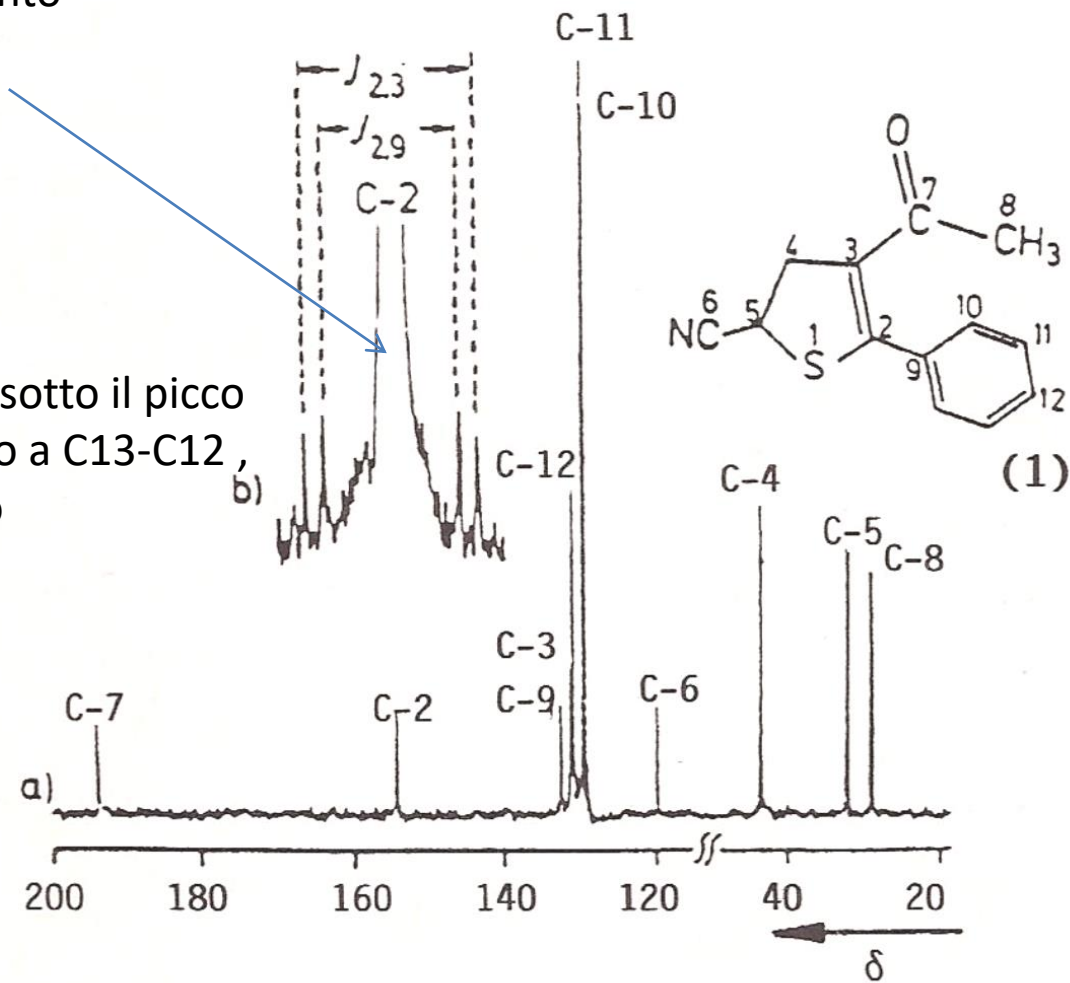
Accoppiamento $^{13}\text{C} - ^{13}\text{C}$ probabilità 10^{-4}
si vede come picchi satelliti ai lati di $^{12}\text{C}-^{13}\text{C}$
con intensità 0.5% per ciascun picco

Accoppiamento $^{13}\text{C} - ^{13}\text{C} - ^{13}\text{C}$ 100 volte ancora minore
impossibile da vedere



INADEQUATE 1D

Accoppiamento
 ^{13}C con ^{12}C



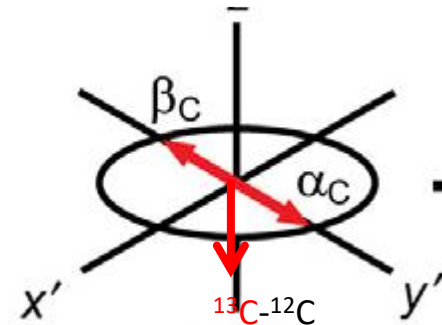
^2J e ^3J cadono sotto il picco
intenso dovuto a C-12,
Non si vedono

INADEQUATE 1D

Incredible Natural Abundance Double Quantum Transfer Experiment

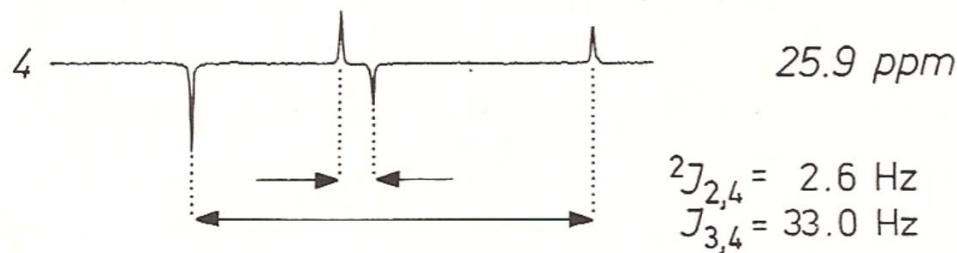
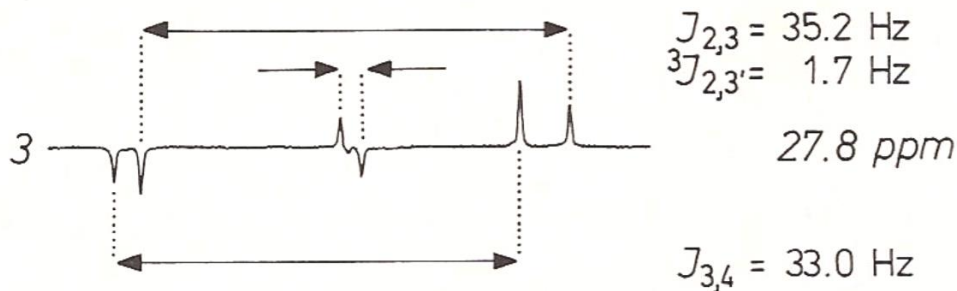
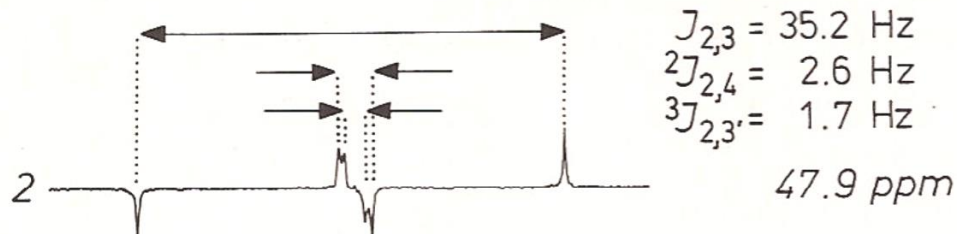
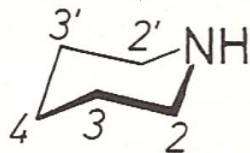
INADEQUATE è una sequenza che sopprime il picco centrale

$90^\circ x - \tau - 180^\circ y - \tau - 90^\circ x - \Delta - 90^\circ x - \text{FID}$



La sequenza: $90^\circ x - 2\tau - 90^\circ x$ (Il $180^\circ y$ serve per rifocalizzare le inhomogeneità) induce coerenza a doppio quanto, cioè una transizione a doppio quanto, non rappresentabile con la rappresentazione vettoriale e che non induce segnale in quanto proibita. Essa è convertita dall'ultimo impulso $90^\circ x$ in transizioni a singolo quanto, rendendole osservabili.

INADEQUATE 1D



INADEQUATE 1D

Vantaggi:

Informazioni sulla connettività identificando i legami C-C attraverso le $J(C,C)$

Svantaggi: tempi molto lunghi, grandi quantità di campione, e nessun intensificazione di segnale (no trasferimento di popolazione)



INADEQUATE 2D

TOCSY 1D (TOTAL CORRELATION SPECTROSCOPY) [HOHAHA (HOMONUCLEAR HARTMAN HAHN)]

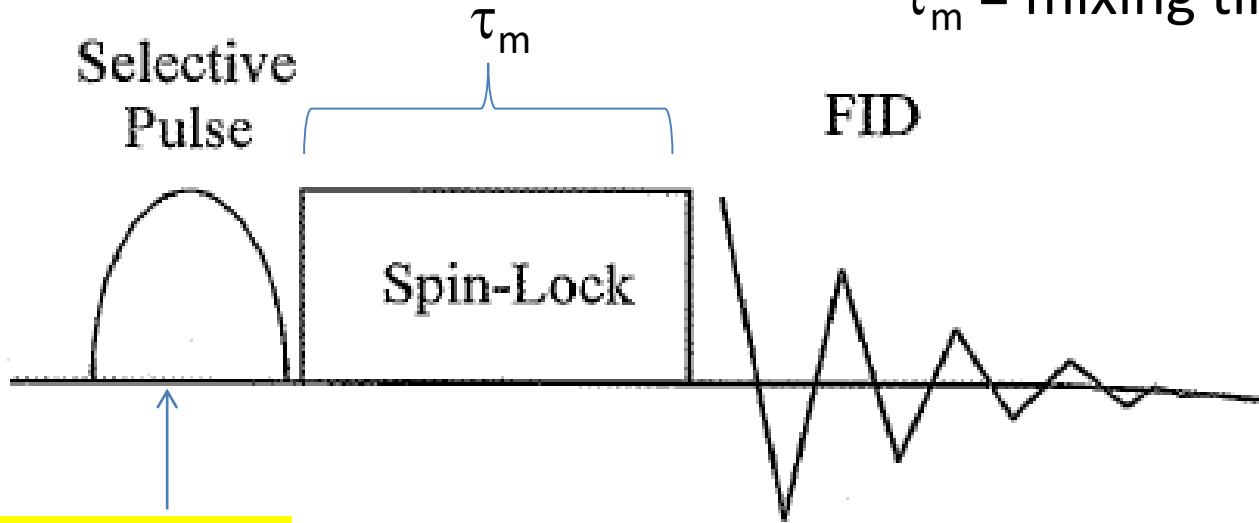
Utile nell'analisi di sistemi complessi con segnali sovrapposti, formati da sistemi di spin separati (Polisaccaridi, proteine, etc)

Divide uno spettro in sottospettri in cui sono presenti tutti i nuclei appartenenti allo stesso sistema di spin.

Sia TOCSY-1D che 2D

Impulso soft

τ_m = mixing time

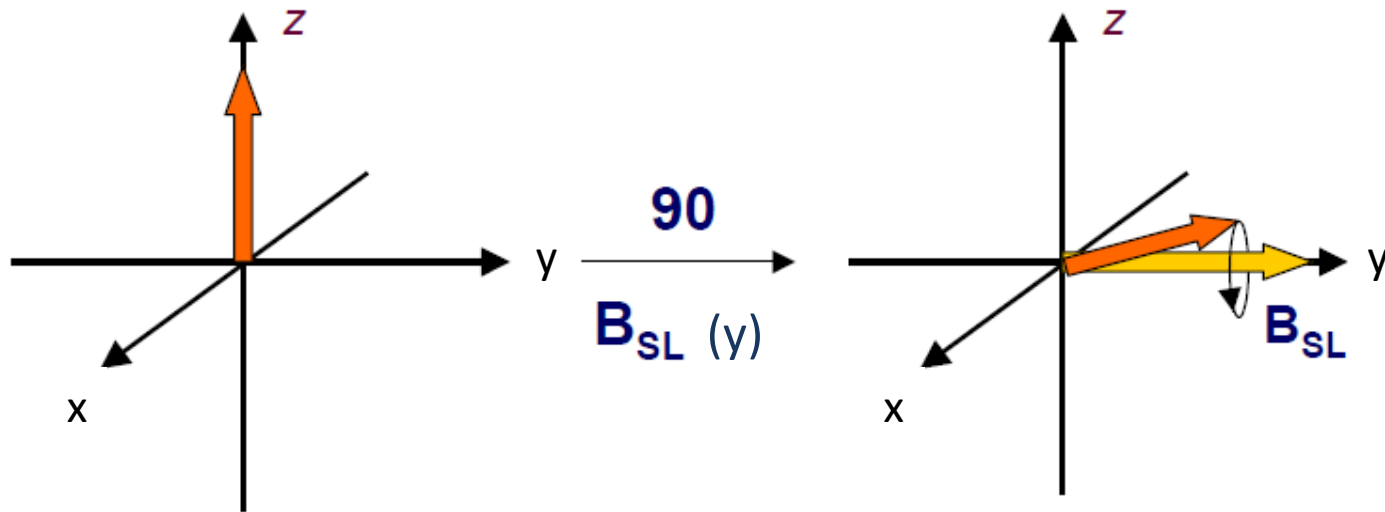


Impulso soft $90^\circ x$

Impulso soft: bassa intensità e tempo lungo

$$\theta = \gamma B_1 \tau_p = 90^\circ$$

Spin lock



Spin lock: serie continua di impulsi 180°_y separati da un tempo δ infinitesimo .
Corrisponde ad un impulso debole e continuo applicato per un tempo lungo (decine di ms)
Rifocalizza continuamente la magnetizzazione intorno all'asse y

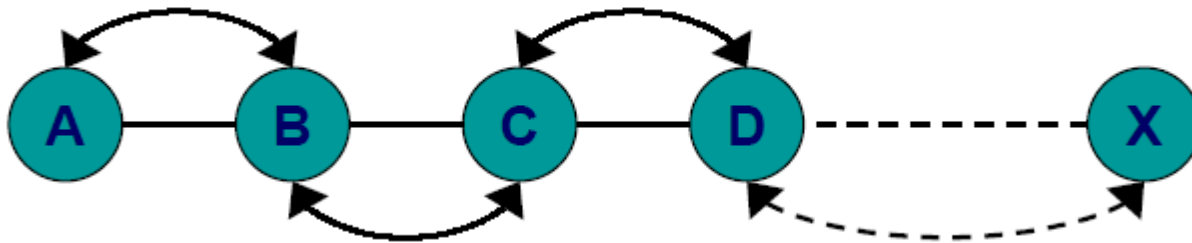
$$\text{Prima di SL: } \nu_0 = \gamma B_0$$

$$\text{Dopo SL: } \nu_{SL} = B_{SL}$$

$$B_{SL} \ll B_0$$

B_{SL} è il campo che serve per far risuonare il nostro spin

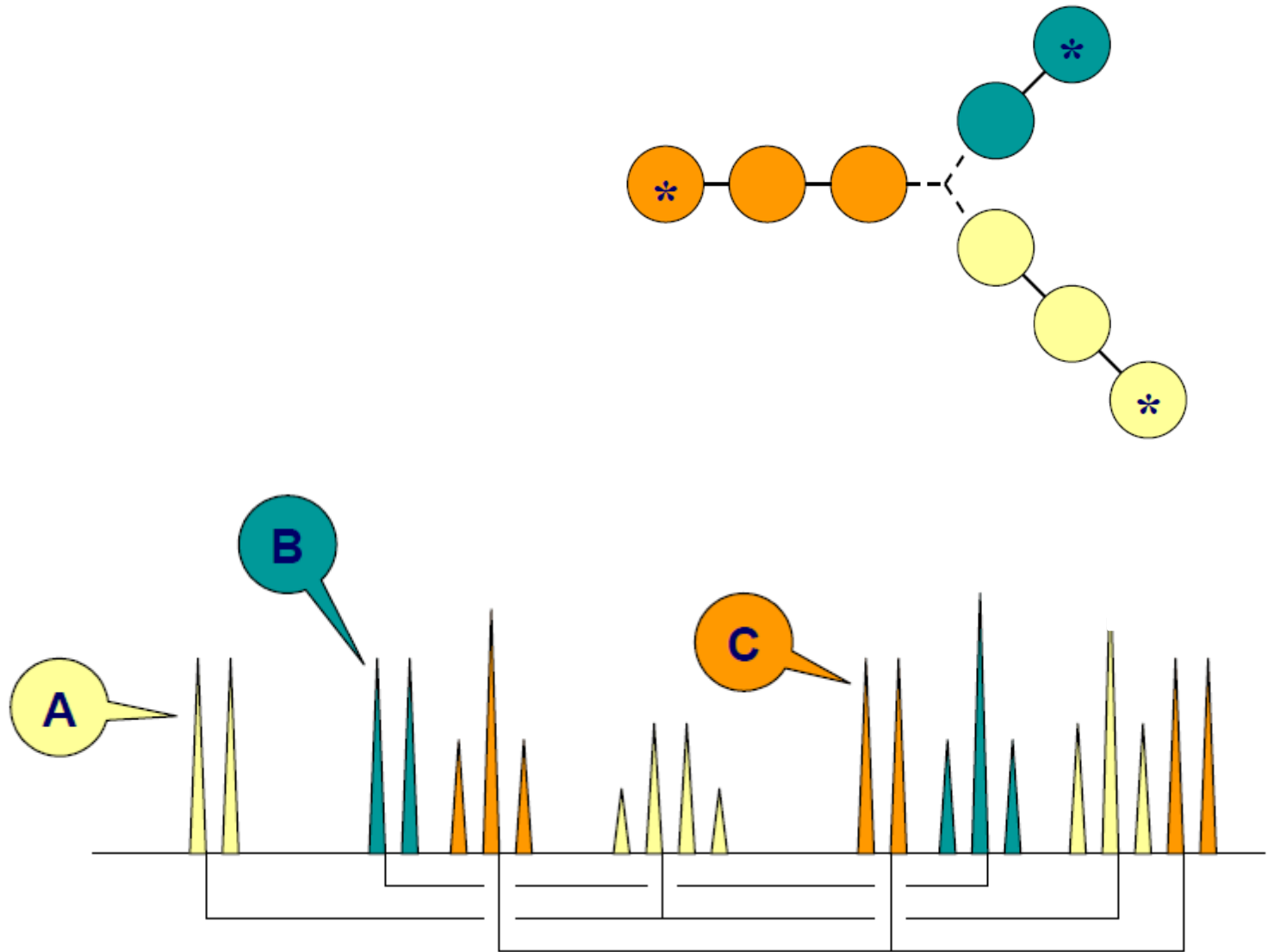
Spin lock



L'applicazione dello spin lock causa un trasferimento di polarizzazione dal nucleo irraggiato agli altri nuclei dello stesso sistema di spin

Si ottengono segnali di tutti gli spin accoppiati del sistema

TOCSY 1D



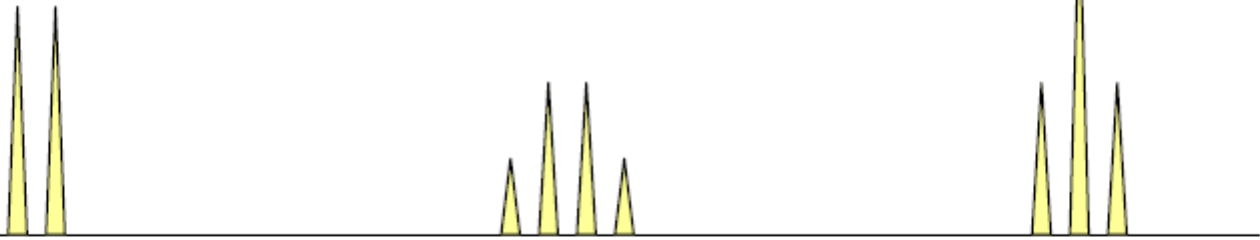
TOCSY 1D

Locked spin:

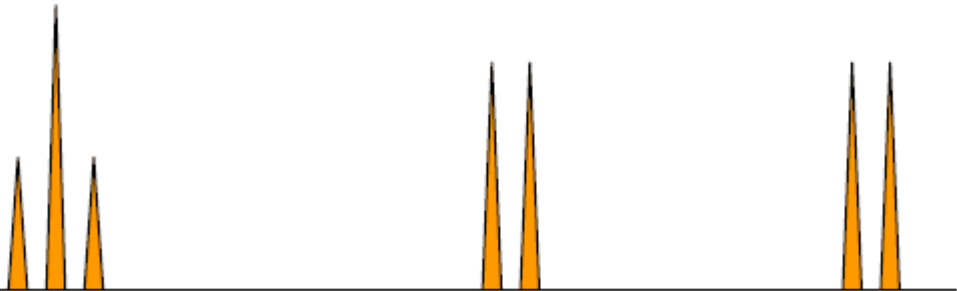
B



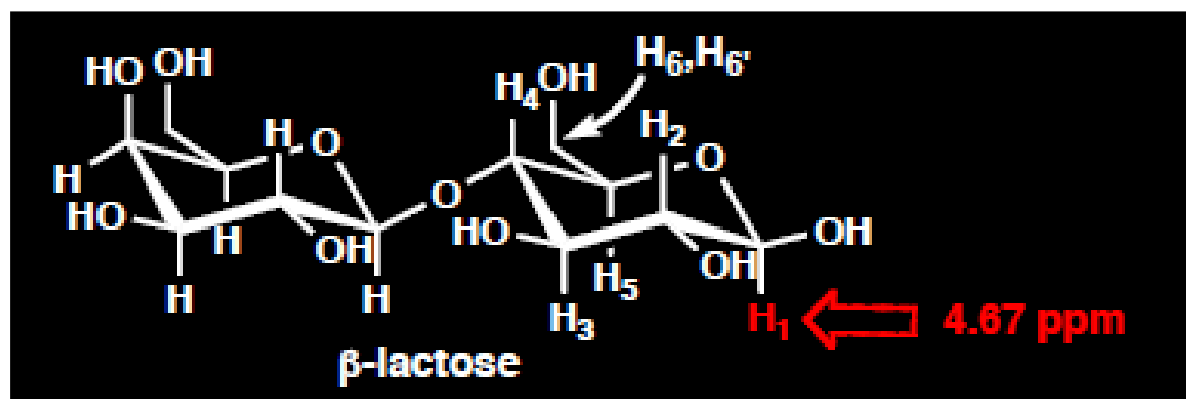
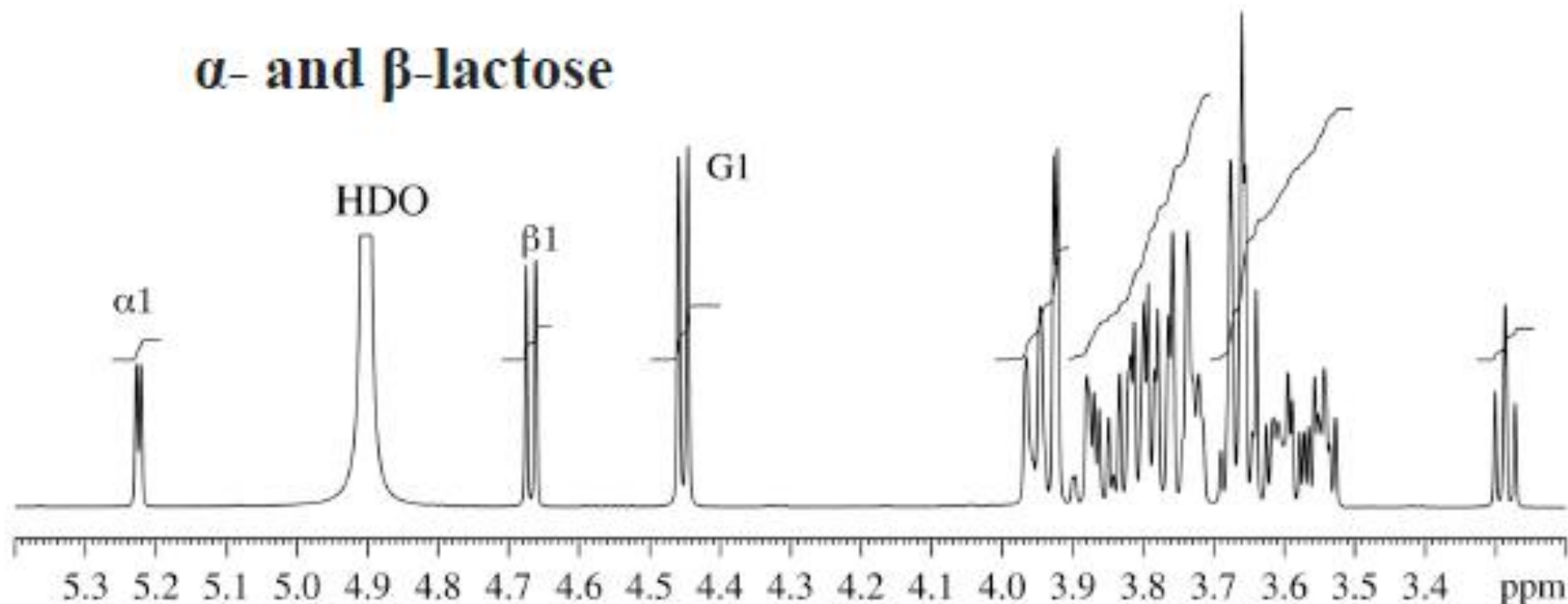
A

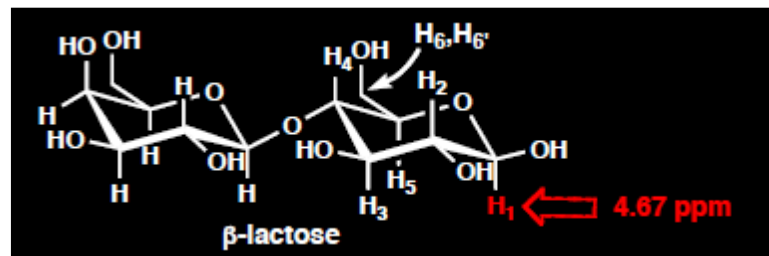


C

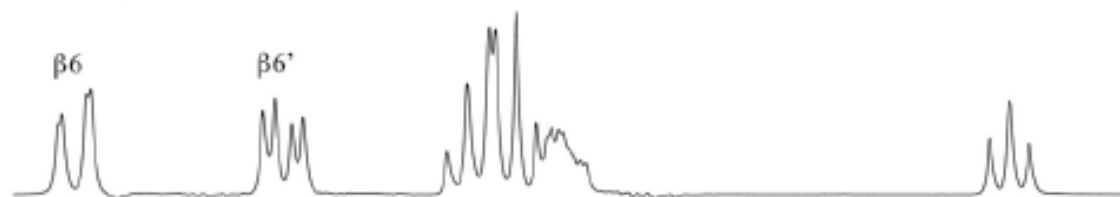


α - and β -lactose

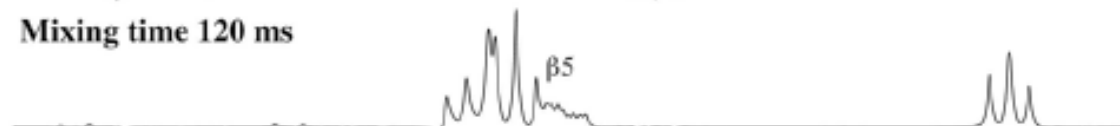




Mixing time 400 ms β 1 irradiated



Mixing time 120 ms



Mixing time 80 ms



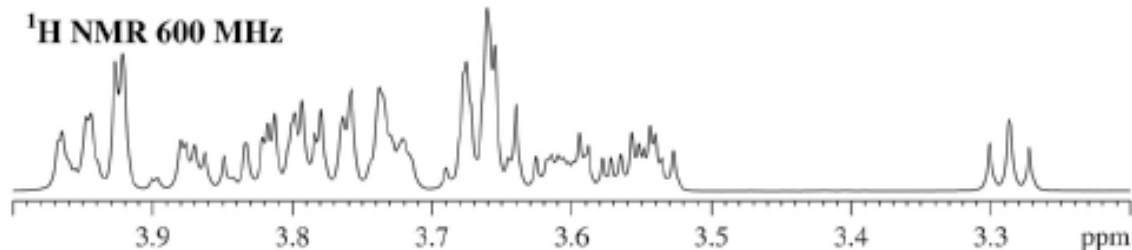
Mixing time 40 ms



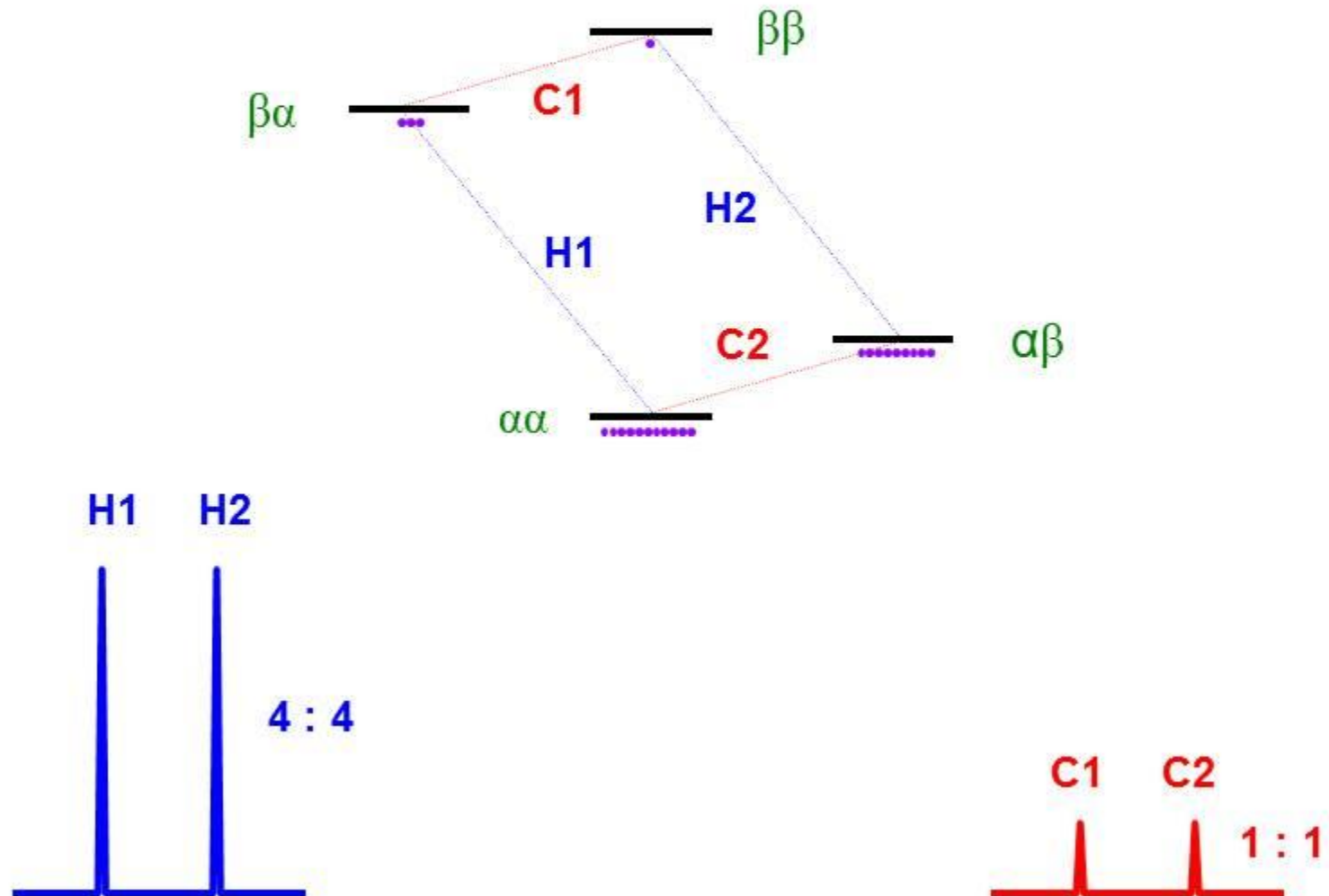
Mixing time 20 ms



^1H NMR 600 MHz



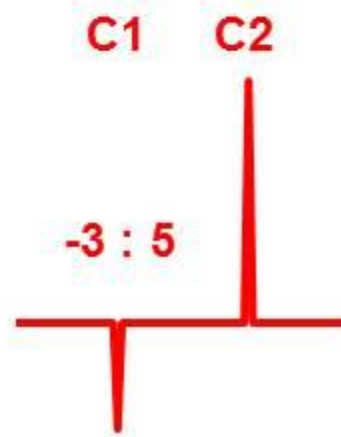
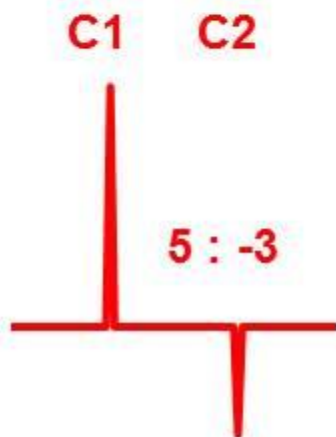
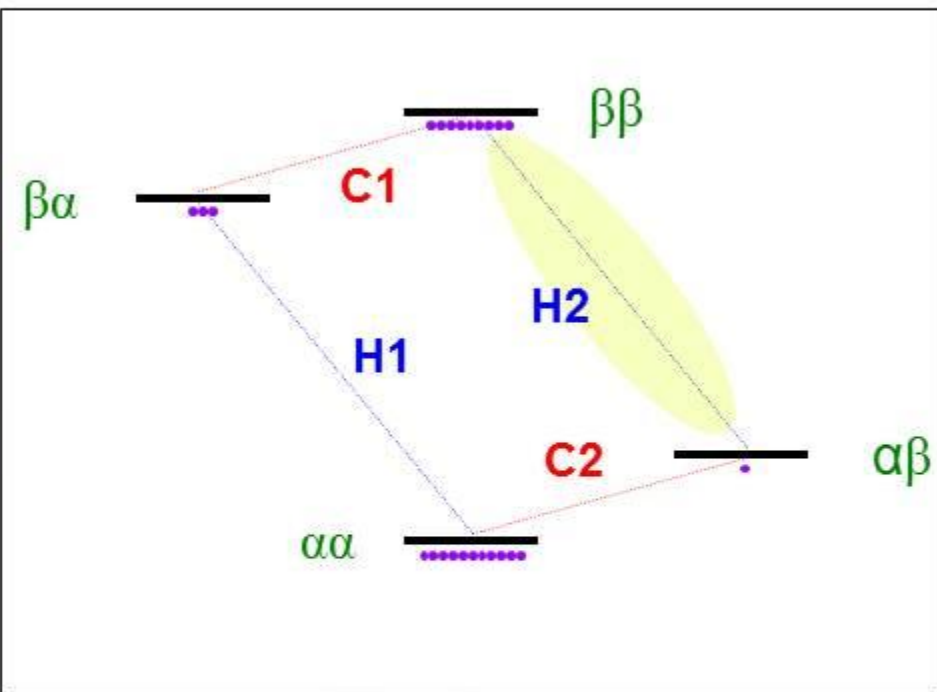
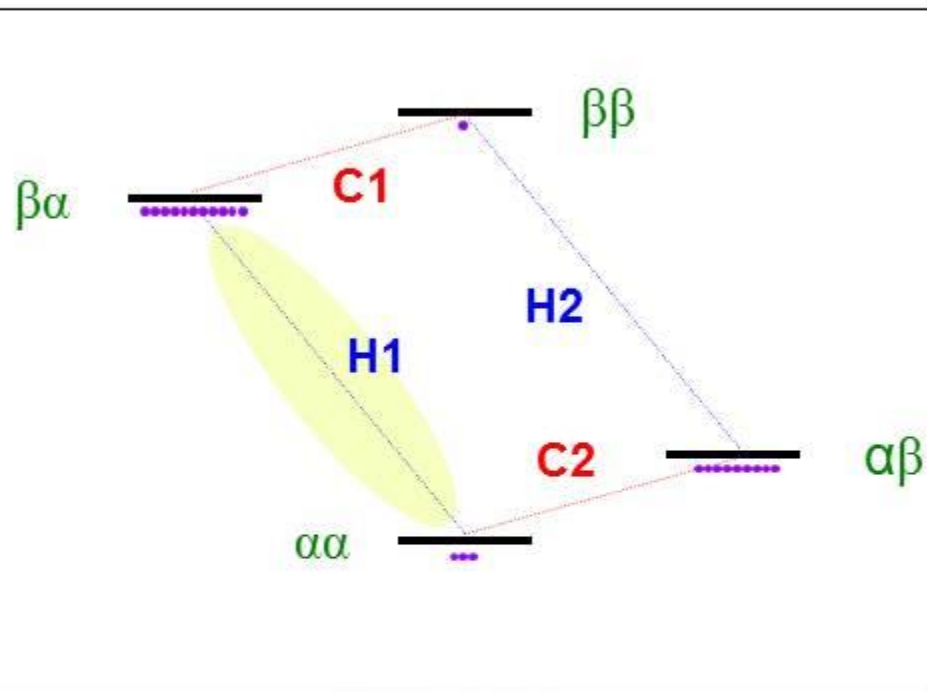
Equilibrium Energy Level Diagram for a ^{13}C - ^1H Spin Pair



Polarization Transfer and Signal Enhancement

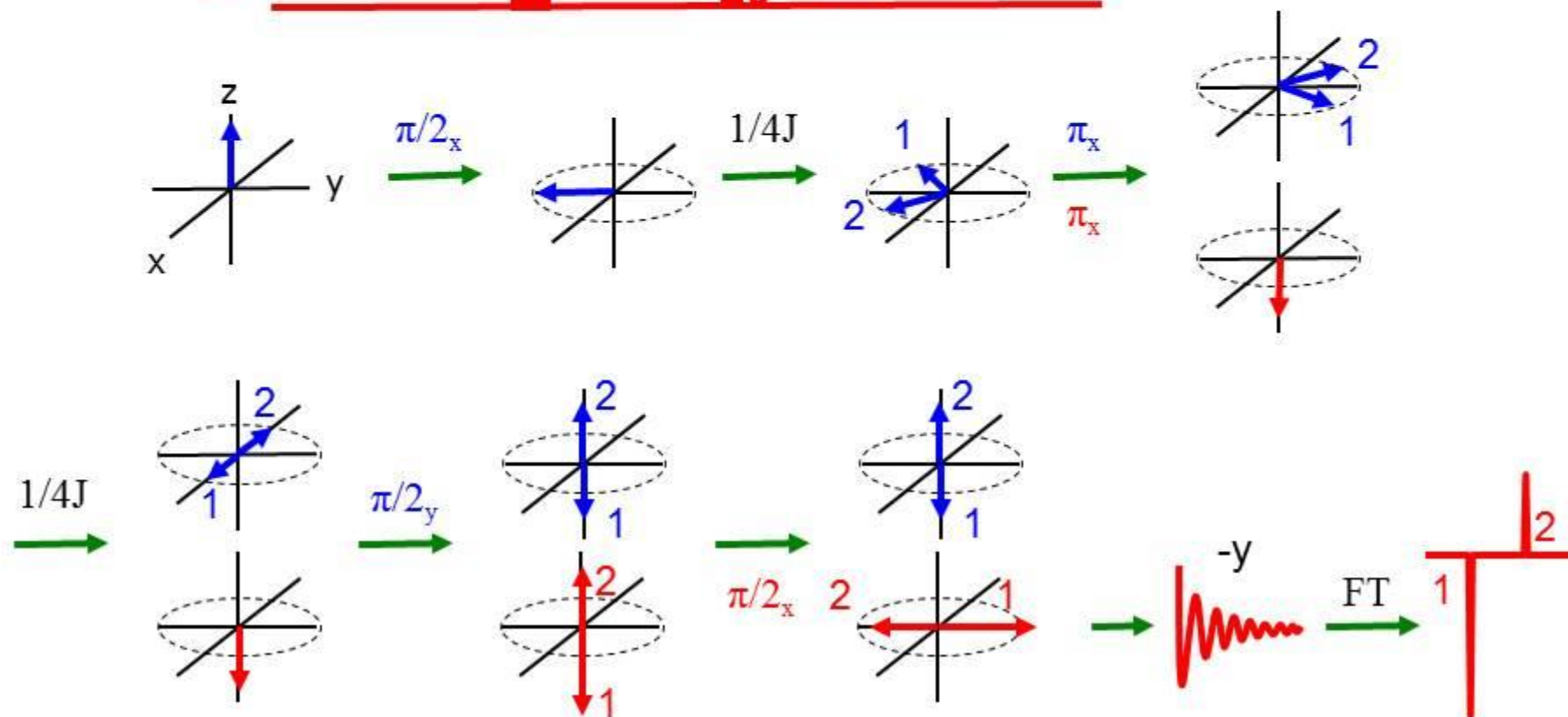
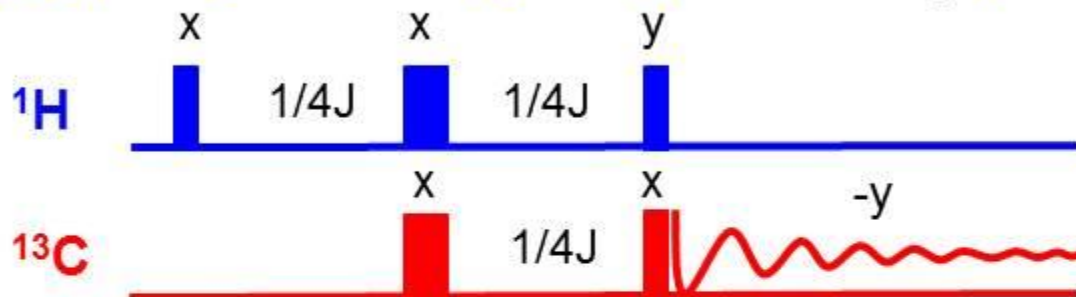
H1 Transition Inverted

H2 Transition Inverted



How Its Done – The "INEPT" Experiment

INEPT Insensitive **N**uclei **E**nhanced by **P**olarization **T**ransfer

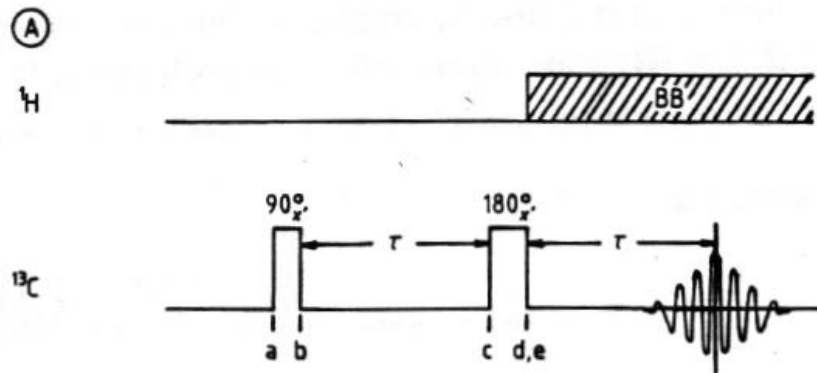


RIFOCALIZZAZIONE DEI CHEMICAL SHIFT NELL'ESPERIMENTO J-MODULATED SPIN ECO

ESEMPIO:

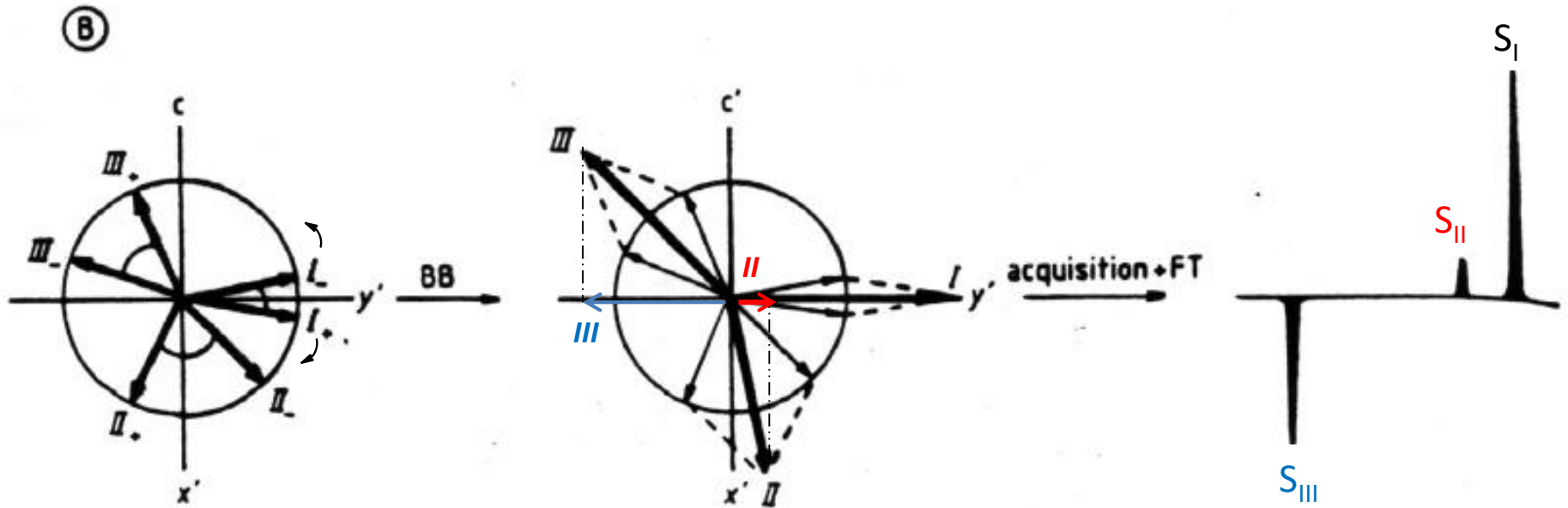
3 DIVERSI GRUPPI di CH (I, II E III)
CON FREQUENZE DI LARMOR E J DIVERSE

$$\nu_I < \nu_{II} < \nu_{III} \text{ e } J_I \neq J_{II} \neq J_{III}$$



RIFOCAZZIAZIONE DEI CHEMICAL SHIFT NELL'ESPERIMENTO J-MODULATED SPIN ECO

$90^\circ x'$ - τ (BB)-acquisition

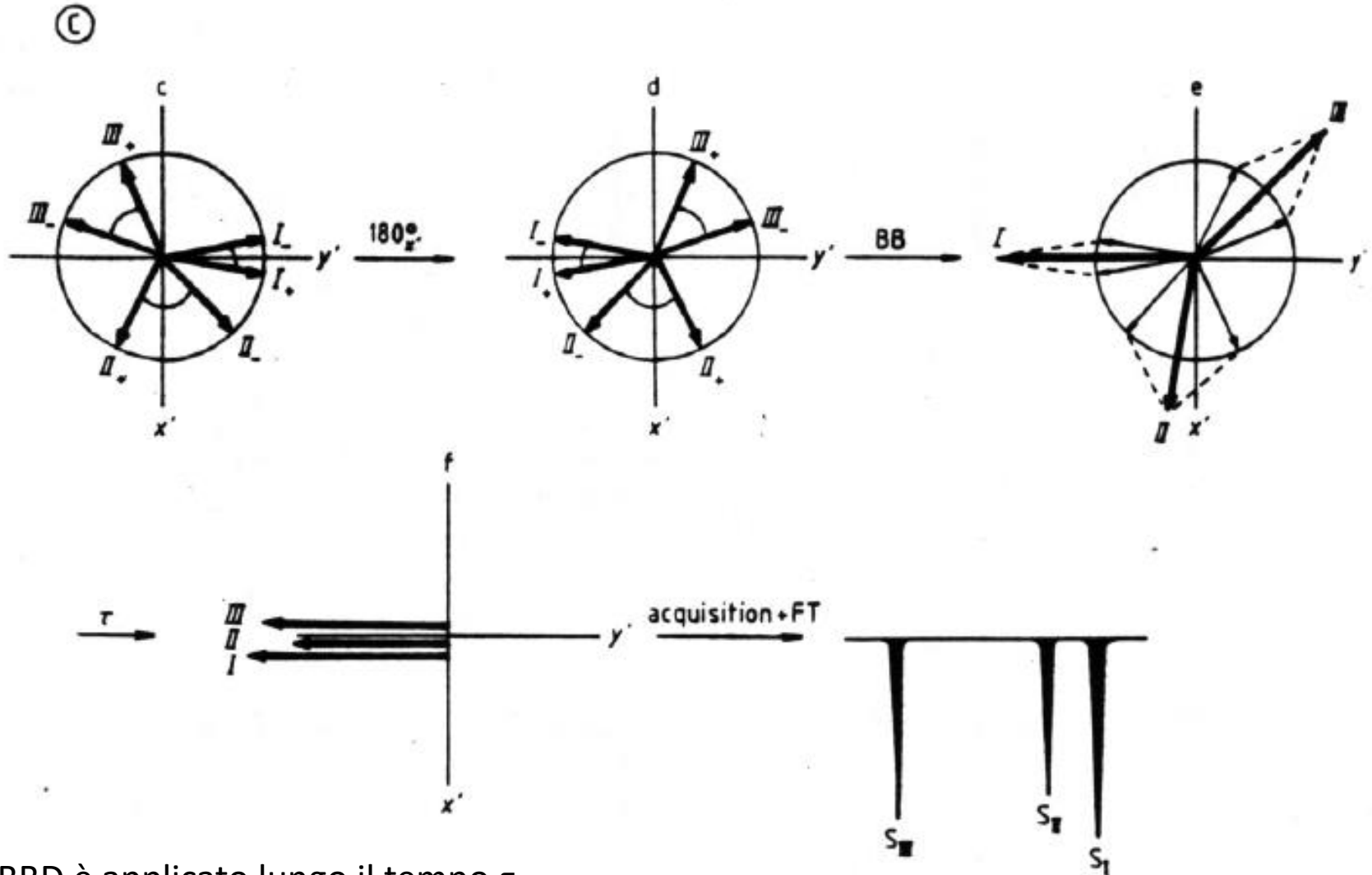


$$V_I = V_{REF}$$

$$V_I < V_{II} < V_{III}$$

RIFOCALIZZAZIONE DEI CHEMICAL SHIFT NELL'ESPERIMENTO J-MODULATED SPIN ECO

Esperimento completo



Il BBD è applicato lungo il tempo τ

EFFETTO DELL'IMPULSO A 180°_x

