CHEM / BCMB 4190/6190/8189

Introductory NMR

Lecture 12

The INEPT Experiment

<u>Sensitivity problem in NMR</u>:

ε = electromagnetic induction force in detection coil

$$\varepsilon \propto N\gamma^3 h^2 B_o^{\,2} I(I+1)/(3k_B T)$$

Small S/N in spectra of insensitive nuclei with low natural abundance (e.g. ^{13}C, ^{15}N) is a main problem in NMR spectroscopy of organic molecules. Example

$$\frac{\varepsilon(^{13}C)}{\varepsilon(^{1}H)} = \frac{1.1\% \;*\; 1}{100\% \;*\; 4^3} = \frac{1}{5818}$$

One would need to record ~33 million (5818^2) more scans in a 1D ^{13}C spectrum to get equal signal intensity than in a 1D ^{1}H spectrum!

Solutions to this problem are:

1) Get more sample
2) Isotope labeling (may be expensive and not practical)
3) Record spectrum at higher field (Bo)
4) Record spectrum at lower temperature (not significant effect)
5) Special NMR experiments

<u>Selective Population Inversion (SPI) Experiment</u>:

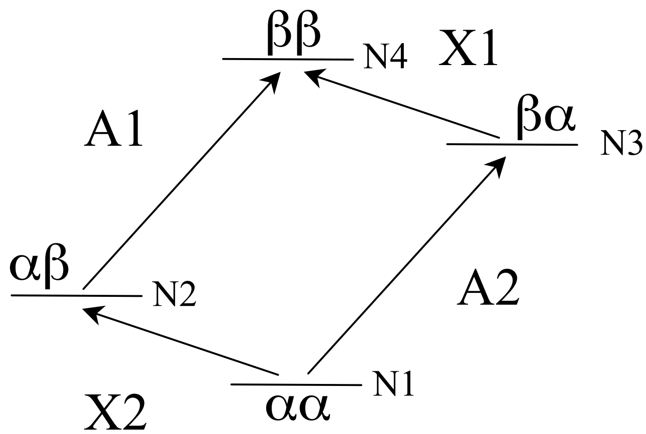
- **<u>Advantage of SPI</u>: Very useful to explain the principle of Selective Population Transfer that provides a means to "recover" one of the γ factor.**

- **<u>Disadvantage of SPI</u>: Not very practical because selective pulses are used.**

Lets consider the two-spin AX system ($^{13}CHCl_3$)
with A=^{1}H = sensitive nuclei
and X=^{13}C = insensitive nuclei

A) At equilibrium:

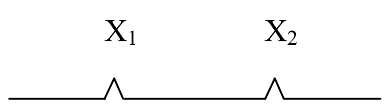
N4 = N
N3 = N + ΔC
N2 = N + ΔH
N1 = N + ΔC + ΔH



N2 - N4 ≈ N1 - N3 = ΔH
N3 - N4 ≈ N1 - N2 = ΔC
ΔH = 4 * ΔC

For ^{13}C spectrum:

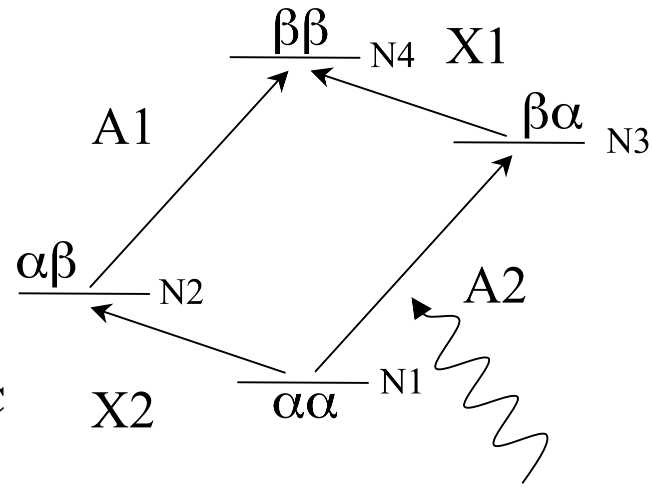
X1 transition: N3 – N4 = ΔC
X2 transition: N1 – N2 = ΔC

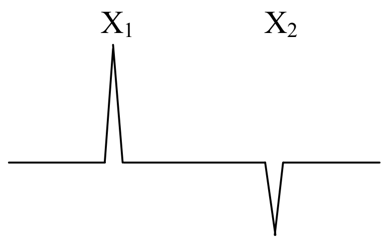


B) After a selective 180° pulse exciting the A2 transition:

The populations of N1 and N3
are inverted:
N4 = N
N3 = N + DC + DH
N2 = N + DH
N1 = N + ΔC

X1 transition: N3 – N4 = ΔC + ΔH = 5ΔC
X2 transition: N1 – N2 = ΔC - ΔH = -3ΔC

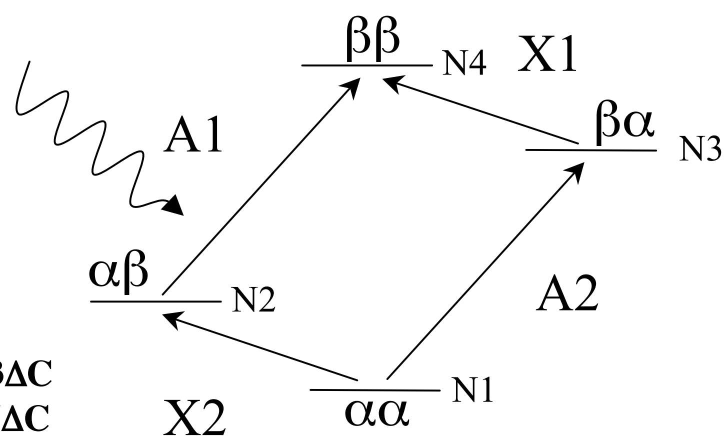


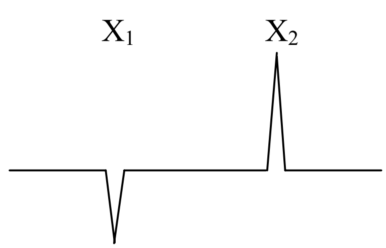


C) After a selective 180° pulse exciting the A1 transition:

The populations of N2 and N4
are inverted:
N4 = N + ΔH
N3 = N + ΔC
N2 = N
N1 = N + ΔC + ΔH

X1 transition: N3 – N4 = ΔC - ΔH = -3ΔC
X2 transition: N1 – N2 = ΔC + ΔH = 5ΔC





After selective inversion of the A1 or A2 transition, the signal amplification factors for the spectra of X are given by:

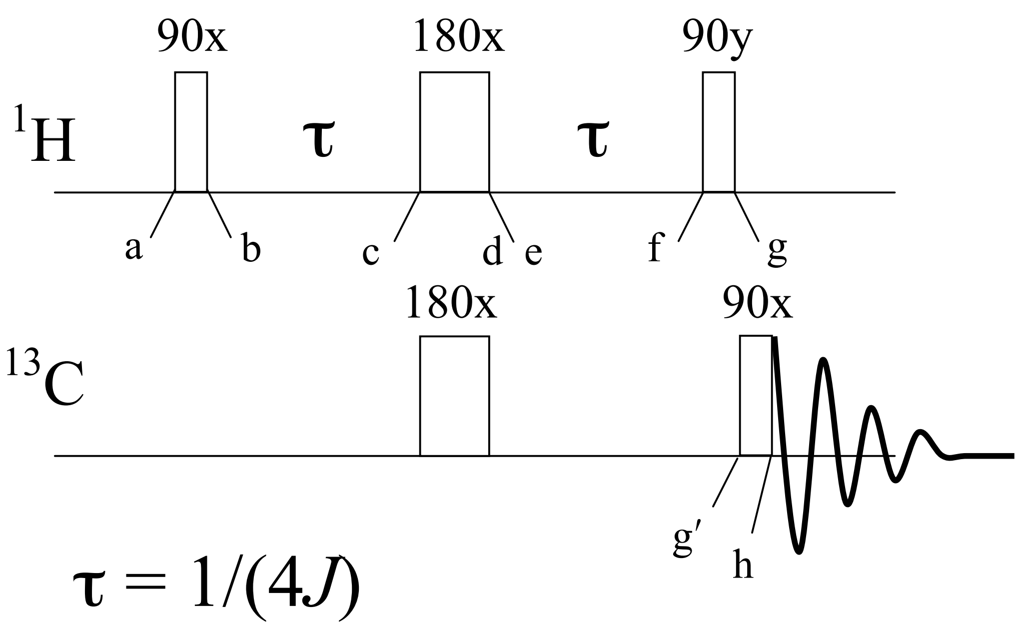
1 + γA / γX and 1 - γA / γX

The INEPT experiment:

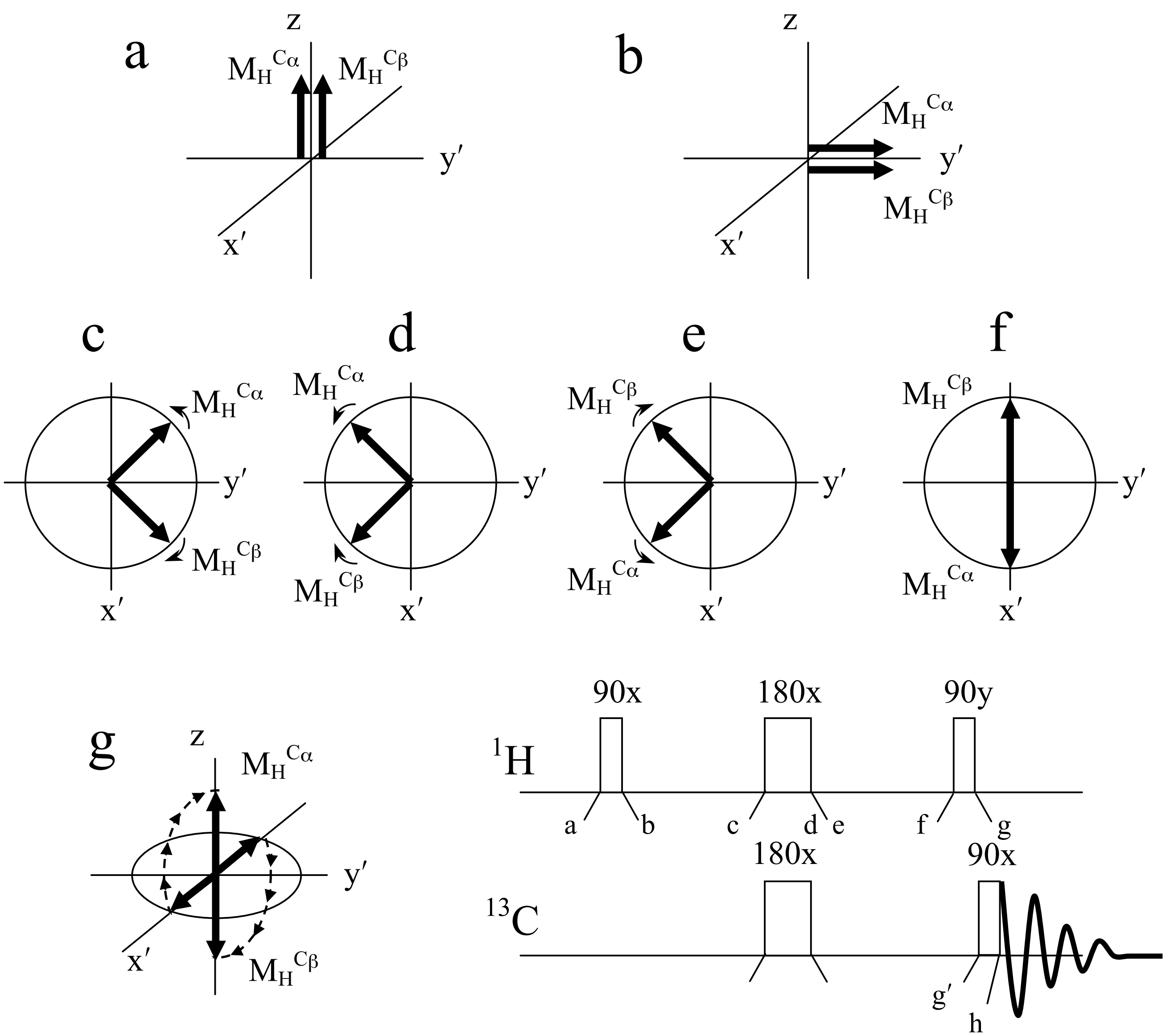
INEPT: Insensitive Nuclei Enhanced by Polarization Transfer
Polarization transfer achieved using non-selective pulses

Example: $^{13}CHCl_3$

A) Pulse sequence in the ^{1}H and ^{13}C channels
(Note: without carbon pulses, this is a spin-echo experiment on ^{1}H)



B) Vector diagrams showing the ^{1}H magnetization vectors



a: $M_H^{C\alpha}$ and $M_H^{C\beta}$ are of approximately equal populations

b: ν ($^{13}C_\alpha$HCl3) = νH – $J_{CH}/2$ and ν ($^{13}C_\beta$HCl3) = νH + $J_{CH}/2$

c- d: until then just like beginning of a spin-echo experiment on ^{1}H

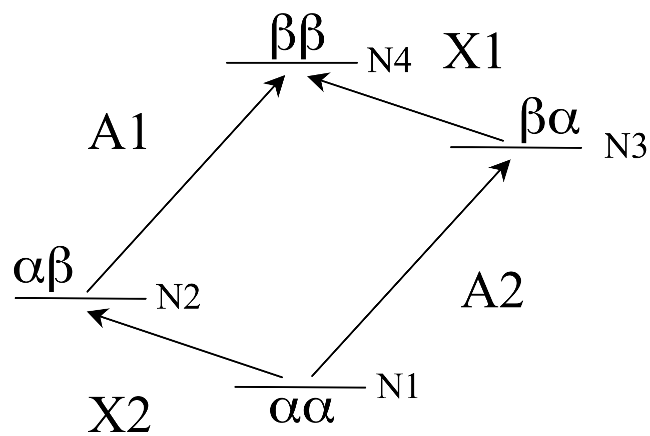
e: Effect of ^{13}C 180˚:

- **phase of 180˚ doesn't matter (x or y), M_C from z to –z**
- **inverts population between N1 and N2 and between N3 and N4**
- **$M_H^{C\alpha}$ becomes $M_H^{C\beta}$ and $M_H^{C\beta}$ becomes $M_H^{C\alpha}$**

f: J_{CH} continue to evolve instead of being refocused during the next τ delay

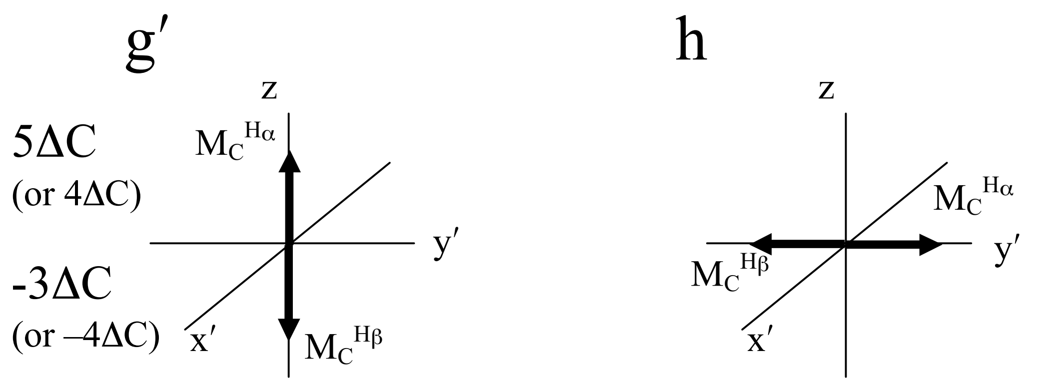
g: ^{1}H 90° pulse rotates $M_H^{C\alpha}$ to +z and $M_H^{C\beta}$ to –z
Same effect as the SPI experiment, but without selective excitation!

The populations of N2 and N4 are inverted:
N4 = N + ΔH
N3 = N + ΔC
N2 = N
N1 = N + ΔC + ΔH



X1 transition: N3 – N4 = ΔC - ΔH = -3ΔC
X2 transition: N1 – N2 = ΔC + ΔH = 5ΔC

C) Vector diagrams showing the ^{13}C magnetization vectors



g': Note that $M_C^{H\alpha}$ is in its original position, but that $M_C^{H\beta}$ is inverted

h: The 90°x pulse on ^{13}C create transverse magnetization components which are observable

The natural I spin magnetization in the INEPT experiment

In many applications of polarization transfer, the contribution from the natural ^{13}C magnetization (ΔC) is unwanted. There are multiple ways to remove it:

1) Presaturate ^{13}C at the start of the pulse sequence

2) Apply a 90° ^{13}C pulse followed by a gradient pulse at the start of the pulse sequence

In cases 1) and 2) the populations at point a are:
$N4 = N + \Delta C/2$
$N3 = N + \Delta C/2$
$N2 = N + \Delta C/2 + \Delta H$
$N1 = N + \Delta C/2 + \Delta H$

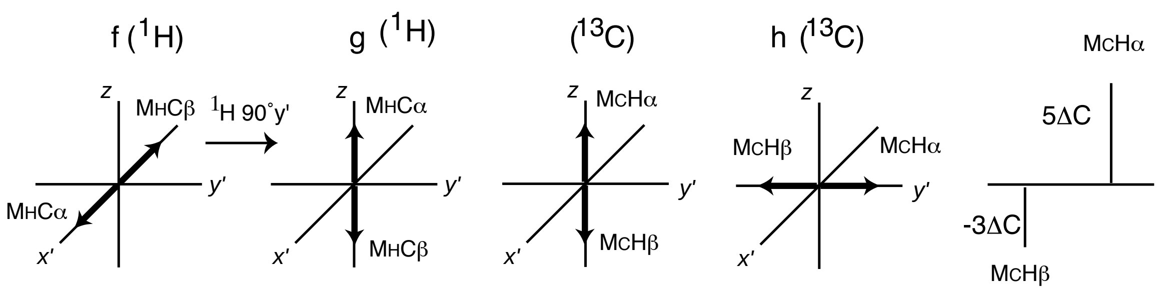
The populations at point g are (N2 and N4 inverted):
$N4 = N + \Delta C/2 + \Delta H$
$N3 = N + \Delta C/2$
$N2 = N + \Delta C/2$
$N1 = N + \Delta C/2 + \Delta H$
X1 transition: $N3 - N4 = -\Delta H = -4\Delta C$
X2 transition: $N1 - N2 = \Delta H = 4\Delta C$

3) By phase cycling

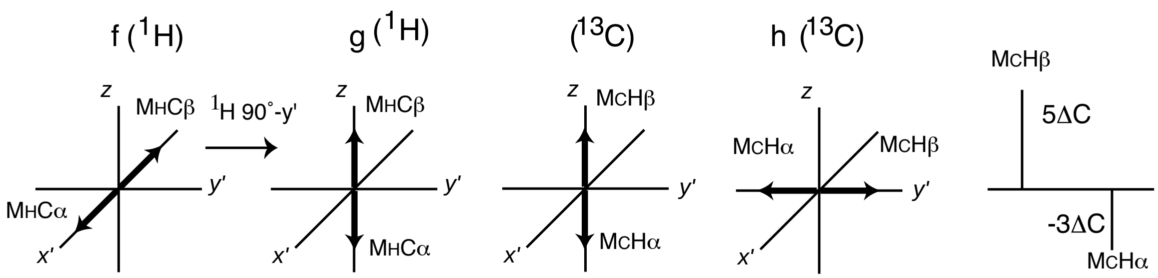
Collect 2 experiments, the phase of the last 90˚ pulse on ^{1}H changes between y and –y.

Lets analyze the effect of the 90˚-y pulse.

□



□



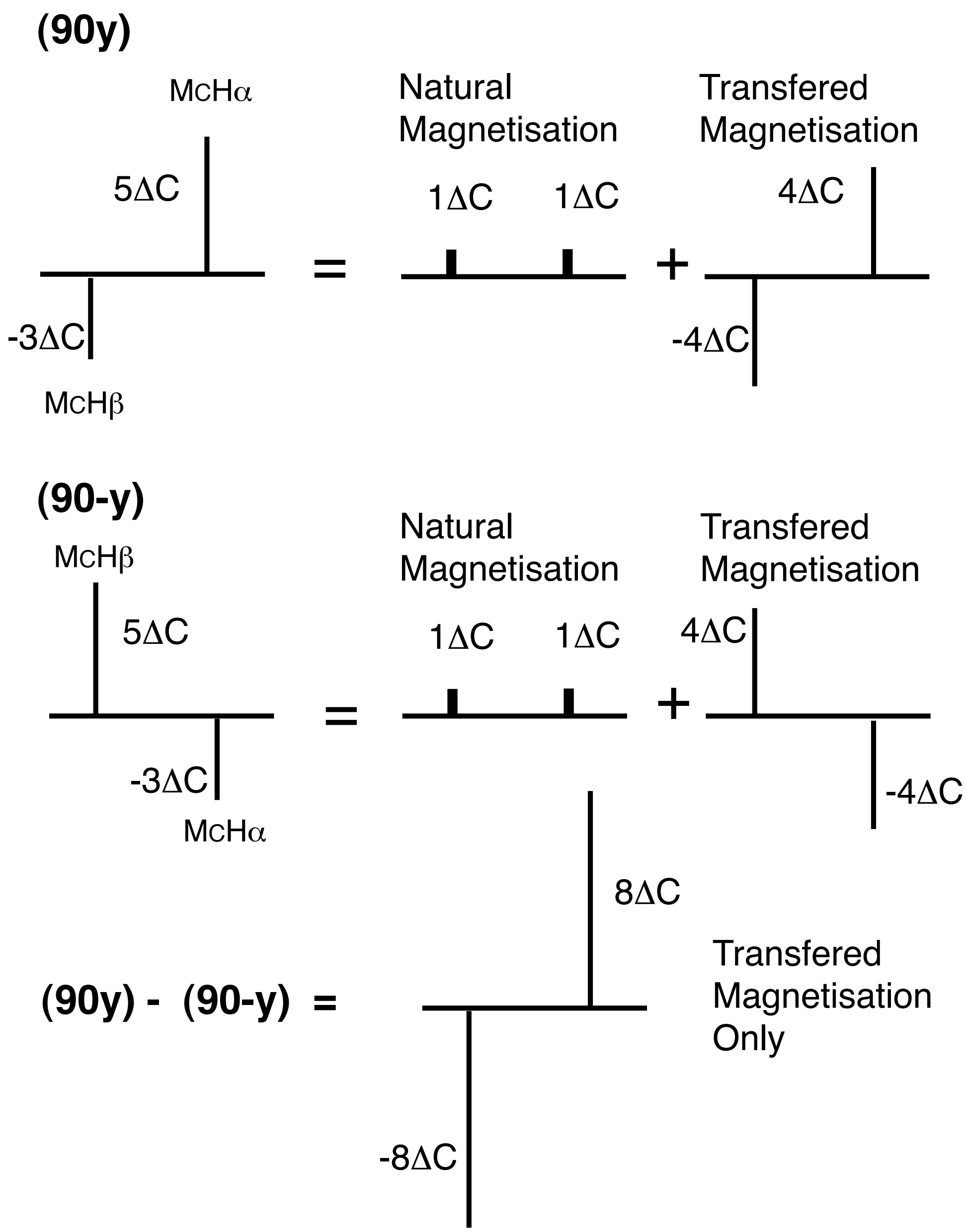
At point g: ^{1}H 90˚-y pulse rotates $M_H^{C\alpha}$ to -z and $M_H^{C\beta}$ to +z
Same effect as the SPI experiment, but without selective excitation!
The populations of N1 and N3 are inverted:
N4 = N
N3 = N + ΔC + ΔH
N2 = N + ΔH
N1 = N + ΔC
X1 transition: N3 – N4 = ΔC + ΔH = 5ΔC
X2 transition: N1 – N2 = ΔC - ΔH = -3ΔC

We have seen the effect of the 90˚y pulse already.
X1 transition: N3 – N4 = ΔC - ΔH = -3ΔC
X2 transition: N1 – N2 = ΔC + ΔH = 5ΔC

The first FID (with 90˚y) and the second FID (with 90˚-y) will be recorded with phases of 0˚ and 180˚ for the receiver. The net effect is substraction of the first spectrum to the second spectrum.

For X1 transition: $-3\Delta C - (5\Delta C) = -8\Delta C$
For X2 transition: $5\Delta C - (-3\Delta C) = +8\Delta C$

The trick here is that in these two experiments, the natural ^{13}C magnetization gives rise to a signal with a constant phase and the change in receiver phase will eliminate it.



INEPT pulse sequence applied to CH_2 and CH_3 groups

Use average value for τ (J_{CH} = 125-150 Hz)

	1D ^{13}C spectrum		INEPT spectrum
CH_2:	1:2:1		$-2\gamma(^1H)/\gamma(^{13}C):0:2\gamma(^1H)/\gamma(^{13}C)$
CH_3:	1:3:3:1	approx.:	$3\gamma(^1H)/\gamma(^{13}C):3\gamma(^1H)/\gamma(^{13}C):$ $-3\gamma(^1H)/\gamma(^{13}C):-3\gamma(^1H)/\gamma(^{13}C)$

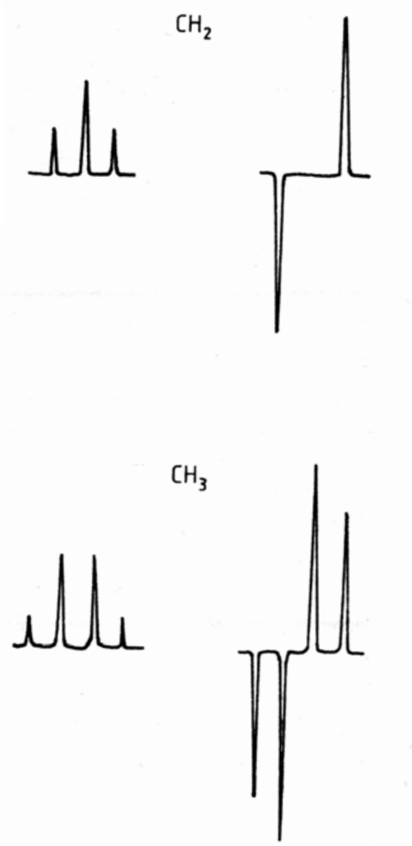
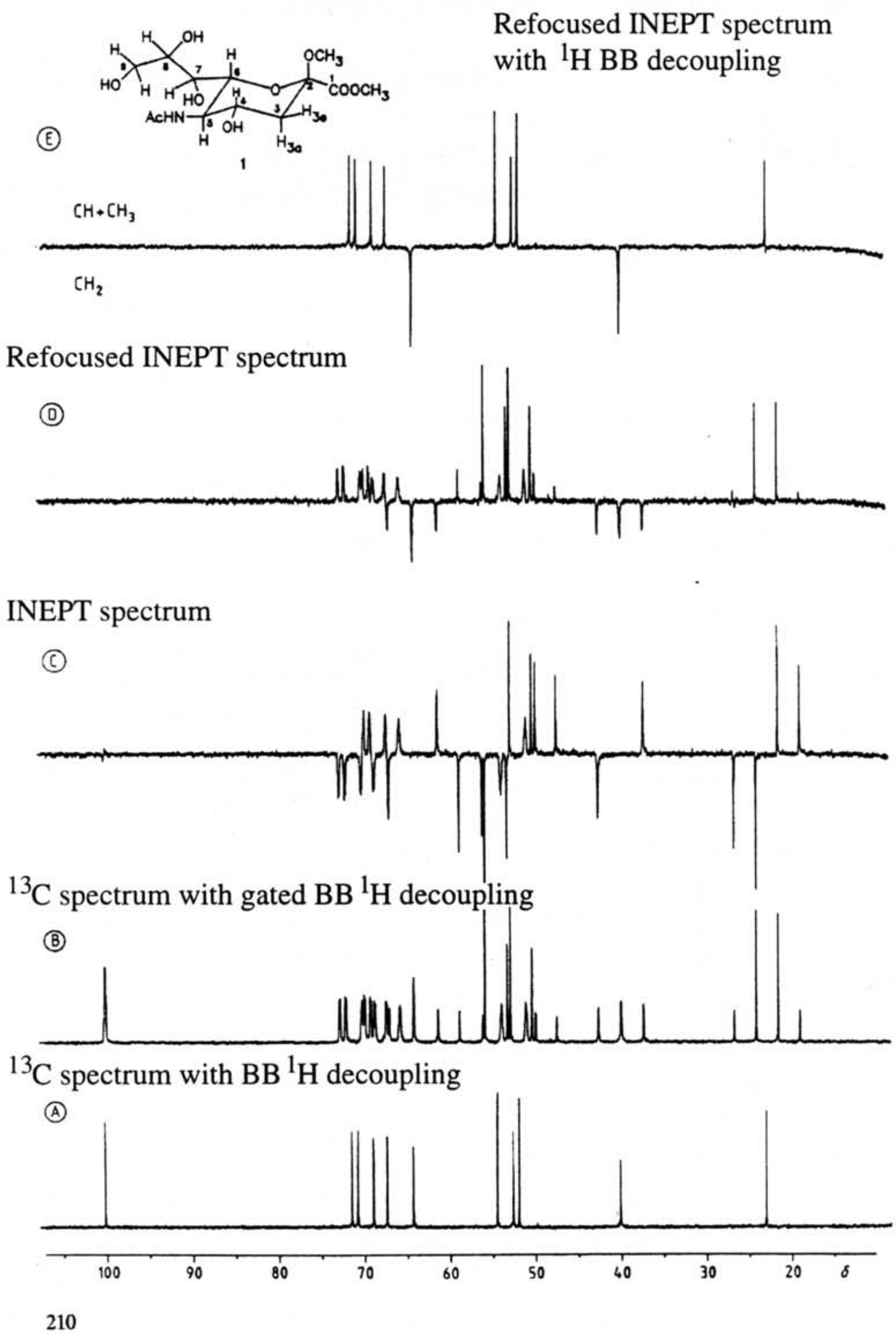


Figure 8-17.
Multiplets observed in the ^{13}C NMR spectrum for CH_2 and CH_3 groups (schematic).
Left: ^{13}C spectra without decoupling.
Right: INEPT spectra.

Examples of INEPT experiments:

(Note: Experiments recorded for different times, S/N should not be compared)



<u>The refocused INEPT experiment</u>:

- **Provides additional delay (2Δ) to refocus J_{CH} coupling.**
- **The additional 180˚ pulse refocuses chemical shift evolution during that delay.**
- **Allows application of ^{1}H BB decoupling during acquisition.**

Optimal delays:

- **For CH groups, the optimal delay Δ is 1 / [4*J_{CH}] (~ 1.79 ms)**
- **For CH2 groups, the optimal delay Δ is 1 / [8*J_{CH}] (~0.89 ms)**
- **For CH3 groups, only two of the four vectors can be refocused, the optimal delay Δ is around 1/[8*J_{CH}]**
- **Need to find a compromise! In practice, a value of 3 / [8*J_{CH}] (2.68 ms) is usually chosen.**

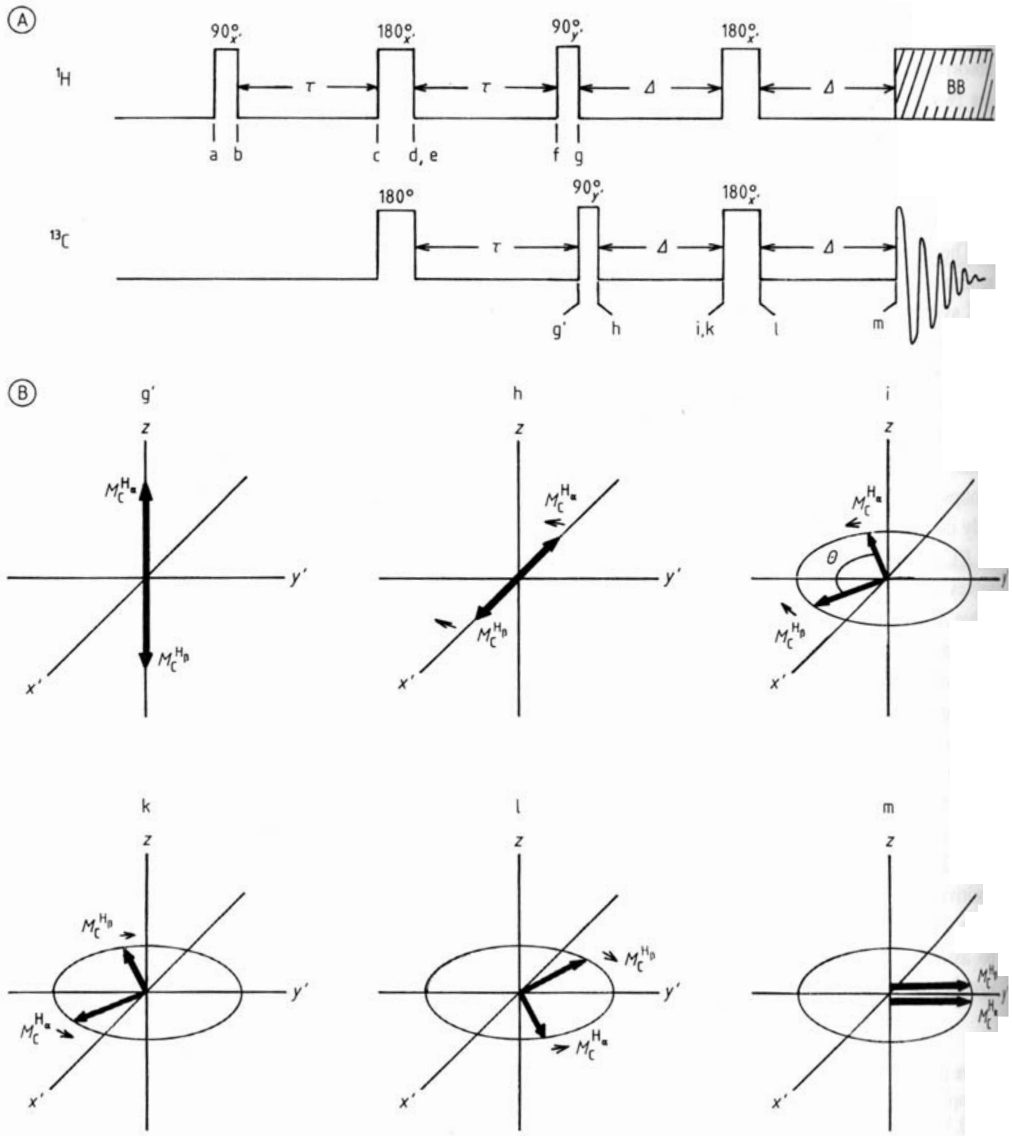


Figure 8-19.
The refocussed INEPT experiment.
A: Pulse sequences in the ^{1}H and ^{13}C channels.
B: Vector diagrams for a two-spin AX system with A = ^{1}H and X = ^{13}C (example: $^{13}CHCl_3$). The evolution of the ^{1}H and ^{13}C magnetization vectors up to the instant g′ is as in Figure 8-15 B, and diagram g′ here is identical to the previous g′. Diagrams h to m show the evolution of the vectors $M_C^{H\alpha}$ and $M_C^{H\beta}$ during the remainder of the pulse sequence A up to the instant m immediately before data acquisition.

Signal intensity enhancement of INEPT spectra

Table 6.1 A comparison of signal strength available by direct observation in the presence of the full nOe from protons, against that resulting from polarisation transfer from protons to the heteronucleus. The figures are *intensities* relative to direct observation of the nucleus without nOe.

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