# CHEM / BCMB 4190/6190/8189

# Introductory NMR

Lecture 16 supplement

**Lets consider the two-spin AX system ($^{13}CHCl_3$)**

**with A=$^1H$ = sensitive nuclei**

**and X=$^{13}C$ = insensitive nuclei**

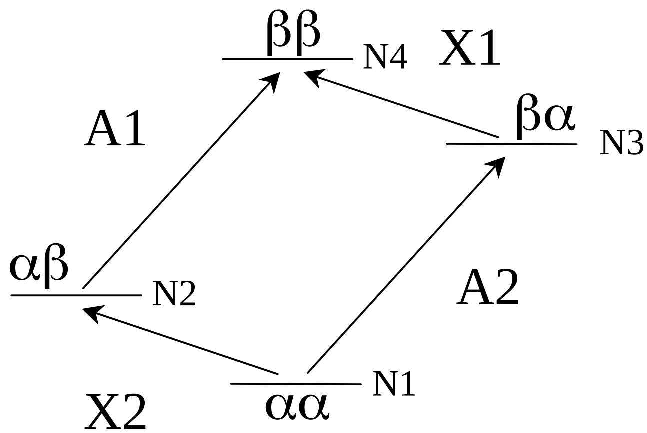
**A) At equilibrium:**

**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:**

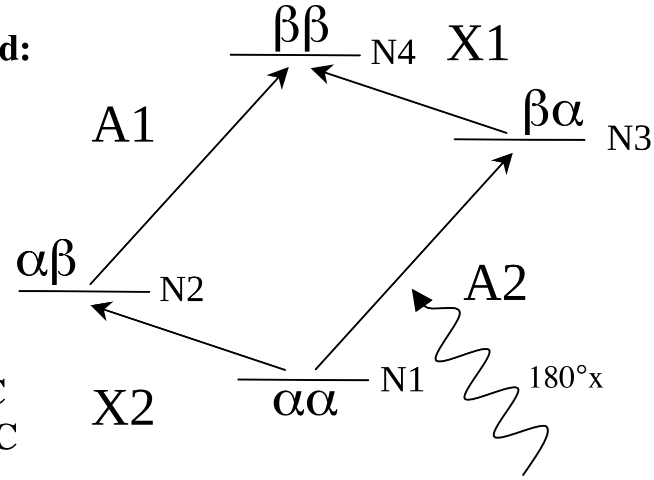
**X1 transition: N3 – N4 = ΔC**

**X2 transition: N1 – N2 = ΔC**

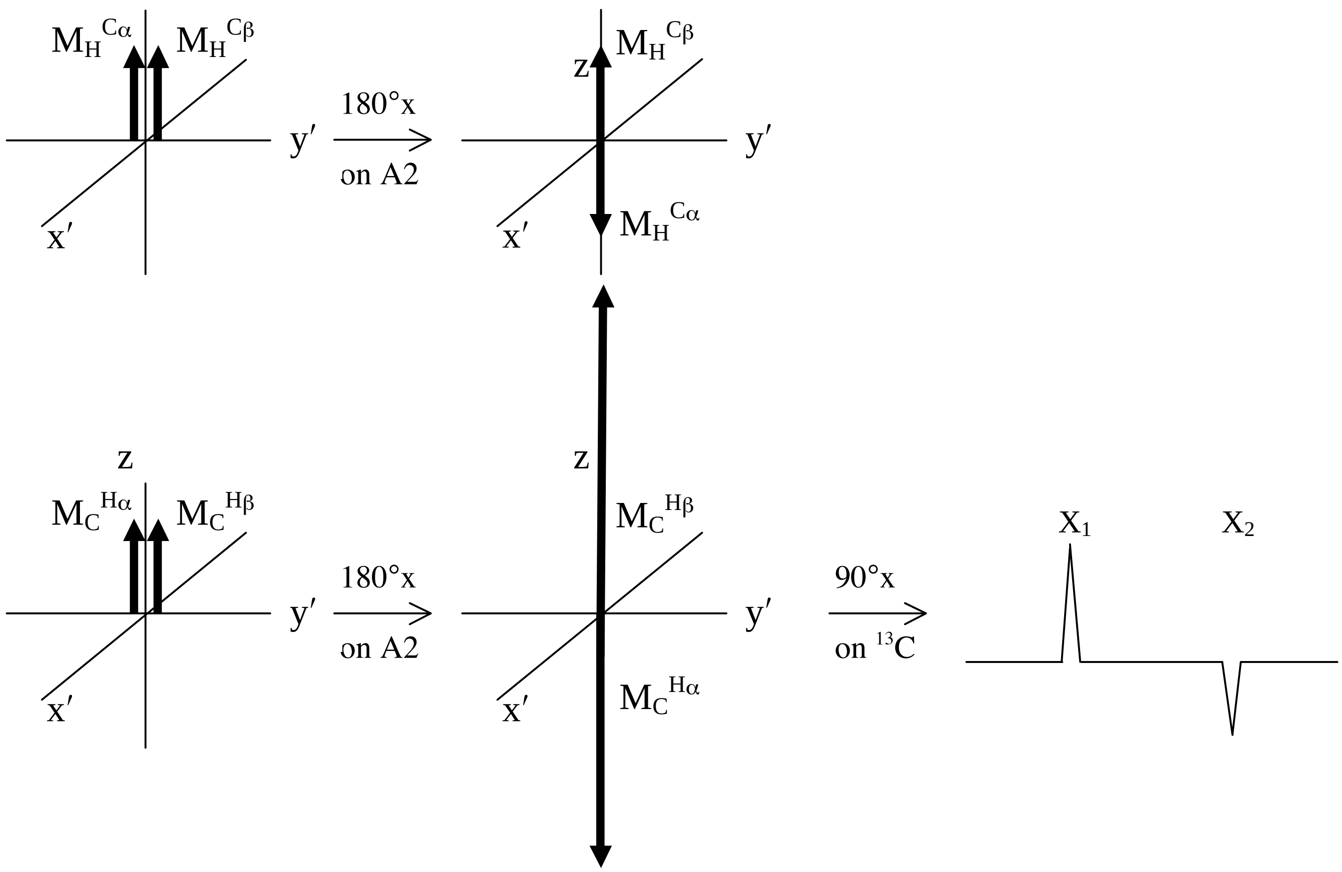
**A) After a selective 180° pulse exciting the A2 transition:**

**The populations of N1 and N3 are inverted:**

| | before 180 A2 pulse | after 180 A2 pulse |
|---|---|---|
| **N4 =** | **N** | **N** |
| **N3 =** | **N + ΔC** | **N + ΔC + ΔH** |
| **N2 =** | **N + ΔH** | **N + ΔH** |
| **N1 =** | **N + ΔC + ΔH** | **N + ΔC** |




**After 180° A2 pulse:**
**X1 transition: N3 – N4 = ΔC + ΔH = 5ΔC**
**X2 transition: N1 – N2 = ΔC - ΔH = -3ΔC**




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

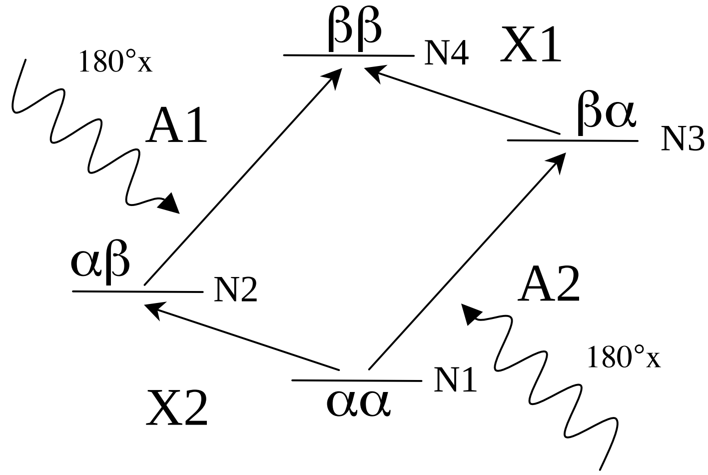
$$1 + \gamma A / \gamma X \quad \text{and} \quad 1 - \gamma A / \gamma X$$

**B) How about if we "selectively" apply a 180° pulse to both the A1 and A2 transitions:** this is essentially, therefore, just a 180 pulse on $^1$H

**The populations of N1 and N3, and N2 and N4 are inverted:**

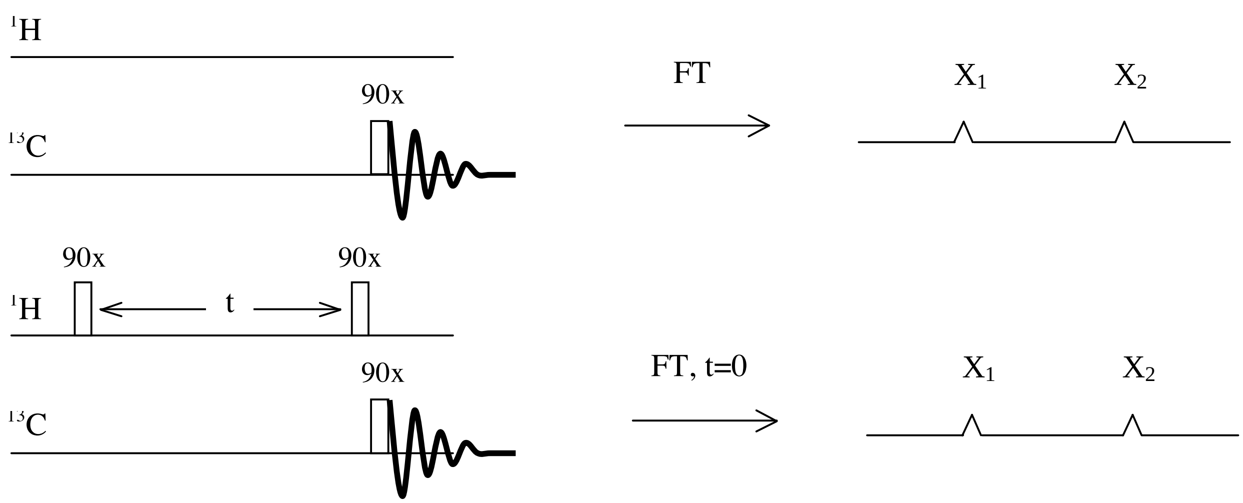
| | before 180° pulse | after 180° pulse |
|---|---|---|
| **N4 =** | **N** | **N + ΔH** |
| **N3 =** | **N + ΔC** | **N + ΔC + ΔH** |
| **N2 =** | **N + ΔH** | **N** |
| **N1 =** | **N + ΔC + ΔH** | **N + ΔC** |

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





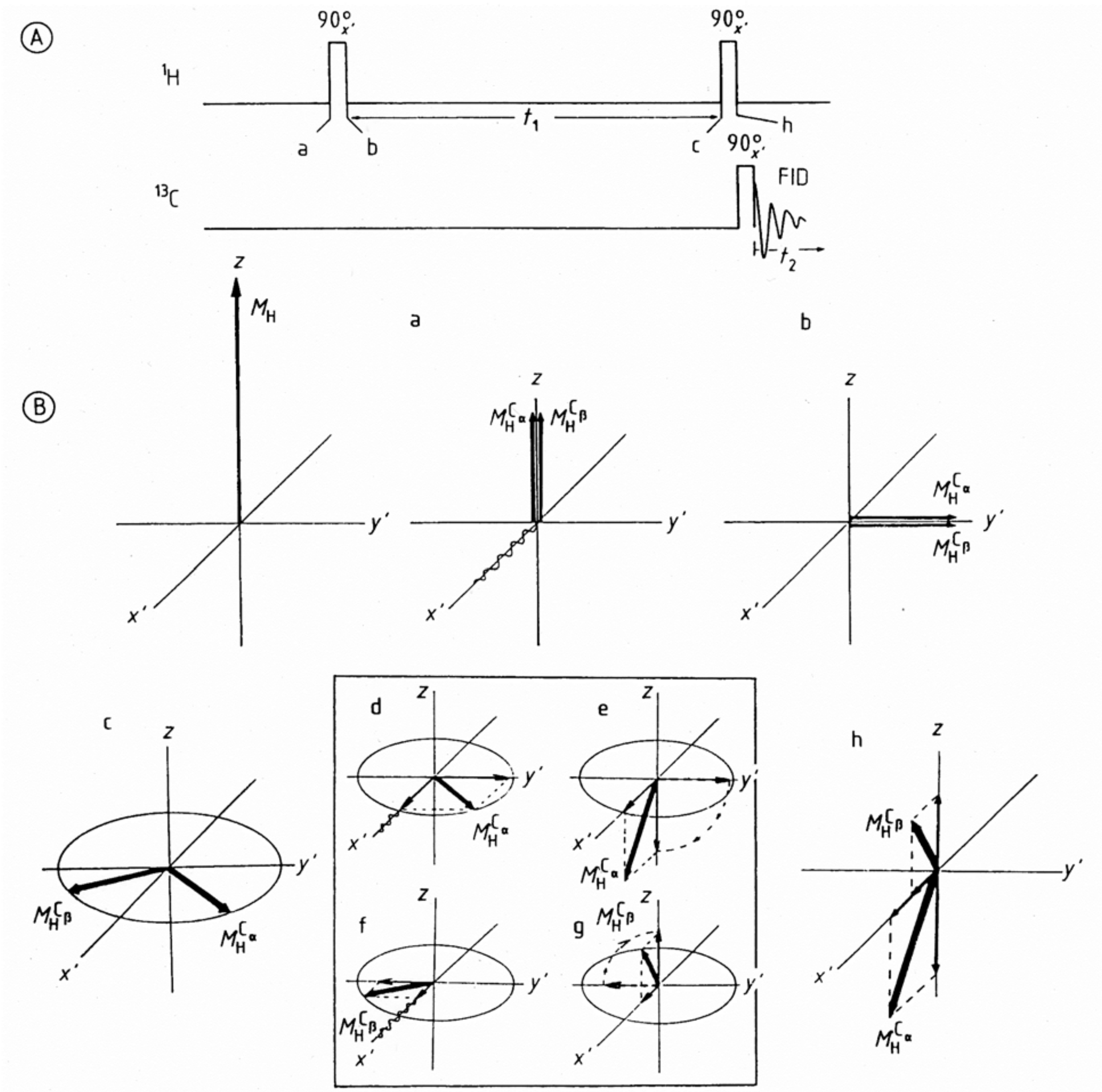
**As you would expect, a non-selective 180° $^1$H pulse does not change the sensitivity of the $^{13}$C spectrum:**




**What if t ≠ 0?**

**HETCOR or C, H-COSY: correlates $^1H$ and $^{13}C$ chemical shifts**

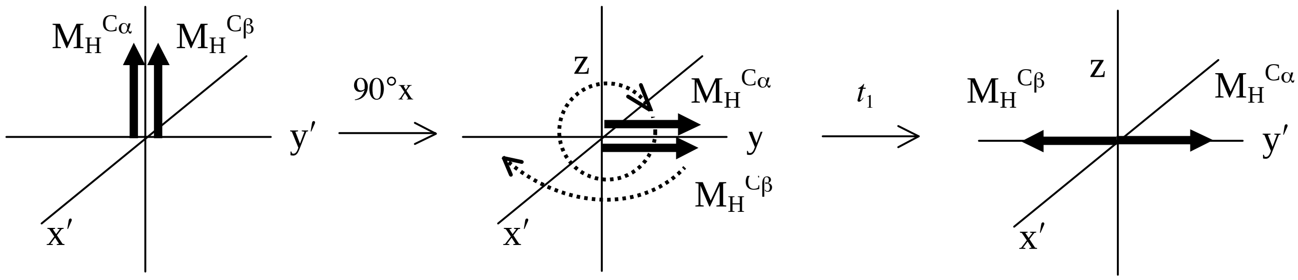
**-in this case $t_1$ varies (increasing $t_1$ allows for $^1H$ chemical shift evolution)**

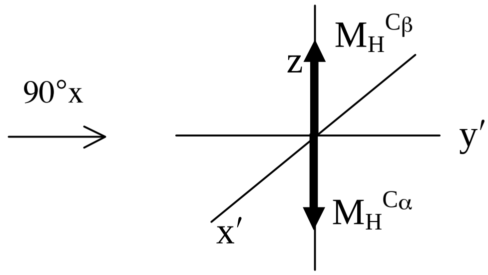



**-we will not ignore chemical shift evolution: $M_H^{C\beta} \neq M_H^{C\alpha} \neq \nu_H$**

**-the vectors $M_H^{C\beta}$ and $M_H^{C\alpha}$ precess and diverge according to $\nu_H$, $J_{CH}$ and $t_1$**

**Let's look at a simple case: assume that during t1, $M_H^{C\alpha}$ rotates 360° (so that it ends up on y) and $M_H^{C\beta}$ rotates 180° (so that it ends up on –y)**




**!! This is exactly what we get if we selectively excite the A2 transition !!**
**-so, depending on $J_{CH}$, $\nu_H$, and $t_1$, we can selectively alter the N1 and N3 populations and alter the signal intensity**

**-When t1 ≠ 0, the population differences between levels 1 and 3 (the $^1H^{C\alpha}$ transitions) change, and those between levels 2 and 4 (the $^1H^{C\beta}$ transitions) change, leading to changes in the differences between levels 1 and 2 and between levels 3 and 4 (the $^{13}C$ transitions).**

**-Thus, as $t_1$ is varied, the intensity of the $^{13}C$ signal will vary, depending on $\nu_H$ and $J_{CH}$**

**Now, we'll look at the more complicated case:**

# CHEM / BCMB 4190/6190/8189

# Introductory NMR

Lecture 16

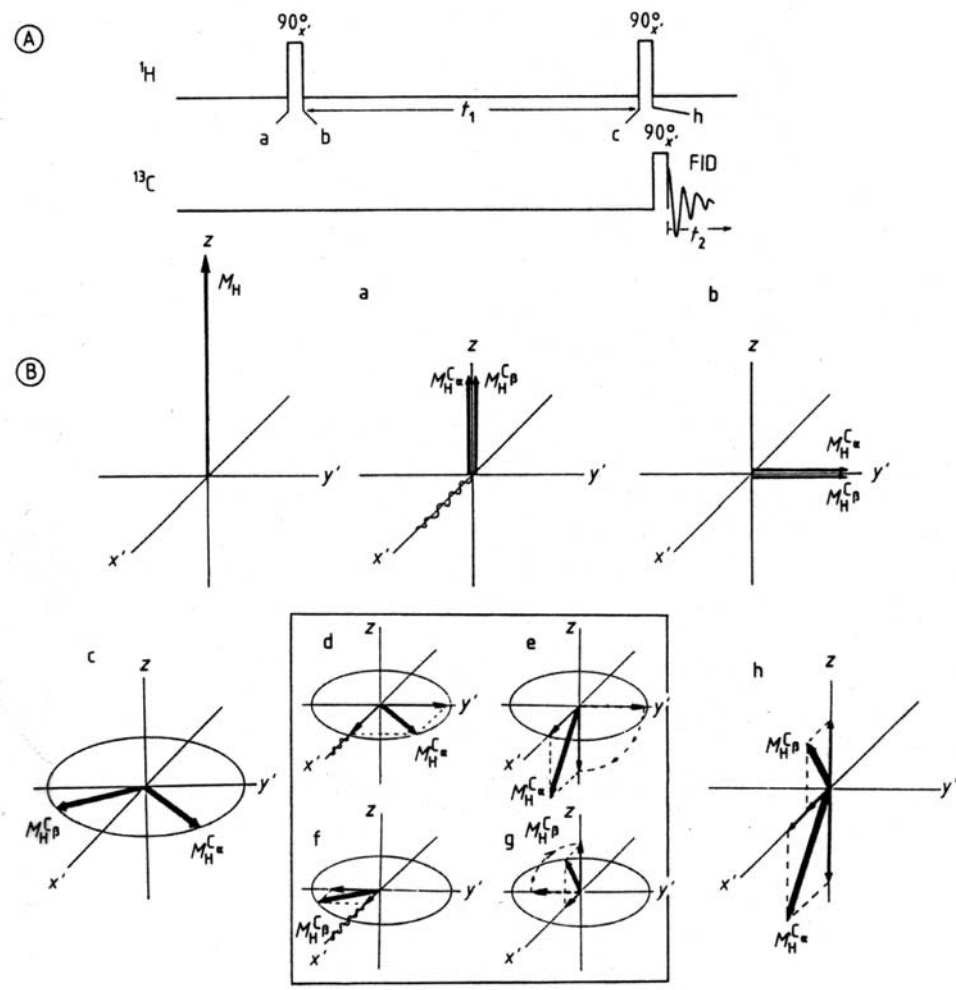
**Two-Dimensional Correlated NMR Spectroscopy**

## 1. <u>Two-Dimensional Heteronuclear (C,H)-Correlated NMR Spectroscopy (HETCOR or C,H-COSY)</u>:

**HETCOR:** **<u>HET</u>eronuclear <u>COR</u>relation**

**C,H-COSY:** **<u>C</u>orrelated Spectroscop<u>Y</u> (Observed nuclei is first)**

**A) Pulse sequence and vector diagram**



**Lets consider a two-spin AX system with A= $^{1}H$ and X = $^{13}C$ ( $^{13}CHCl_3$)**

**During $t_1$:**

- $\nu( MH\{C\alpha\} ) = \nu_H - 1/2*J_{CH}$; $\nu( MH\{C\beta\} ) = \nu_H + 1/2*J_{CH}$
- $\nu_H$ = Larmor frequency in the absence of coupling
  (Here $\nu_H$ > frequency of the rotating frame)
- Ignore effect of relaxation and field inhomogeneity
- $\varphi\alpha = 2\pi (\nu_H - 1/2*J_{CH})\, t_1$; $\varphi\beta = 2\pi (\nu_H + 1/2*J_{CH})\, t_1$
  $\Theta = \varphi\alpha - \varphi\beta = 2\pi J_{CH}*\, t_1$

**After the second $^{1}H$ 90˚x' pulse:**

- $^{1}H$ magnetization is transferred to the x'-z plane
- The z-magnetization components are proportional to the population differences:
  N1 and N3 for $MH\{C\alpha\}$
  N2 and N4 for $MH\{C\beta\}$
- In Figure 9-10 the populations of N1 and N3 are partially inverted and the population difference between N2 and N4 is modified:
- In general, the population differences depend on $t_1$, $\nu_H$, and $J_{CH}$.
- Population transfer from $^{1}H$ to $^{13}C$ as in SPI and INEPT, although here the transfer depends on $t_1$.

**After the $^{13}C$ 90˚x' pulse:**

- Turns the two longitudinal $^{13}C$ vectors +z and -z into the +y' and -y' directions, respectively.
- Two frequencies are detected by the receiver:
  $\nu_C - 1/2*J_{CH}$
  $\nu_C + 1/2*J_{CH}$

## B) Spectrum

- **FT with respect to t2 gives two $^{13}C$ signals ( $\nu_C - 1/2*J_{CH}$ and $\nu_C + 1/2*J_{CH}$) along the F2 axis (one positive and one negative);these signals are modulated in t1 by $\nu_H$ and $J_{CH}$.**
- **FT with respect to t1 gives two $^{1}H$ signals ( $\nu_H - 1/2*J_{CH}$ and $\nu_H + 1/2*J_{CH}$) for each $^{13}C$ signal along the F1 axis.**
- **2D NMR spectrum has therefore 4 signals, 2 with negative amplitude.**
- **$^{1}H$ decoupling during acquisition would remove the signal**

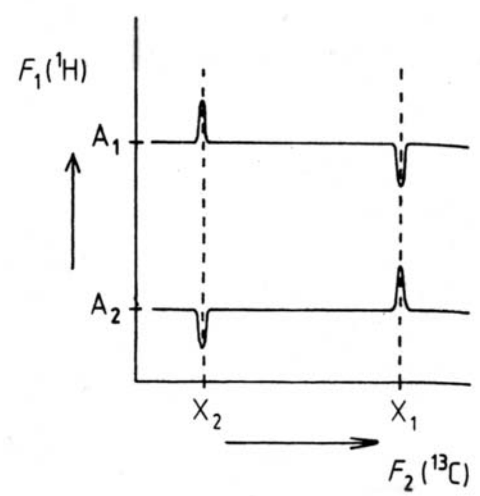
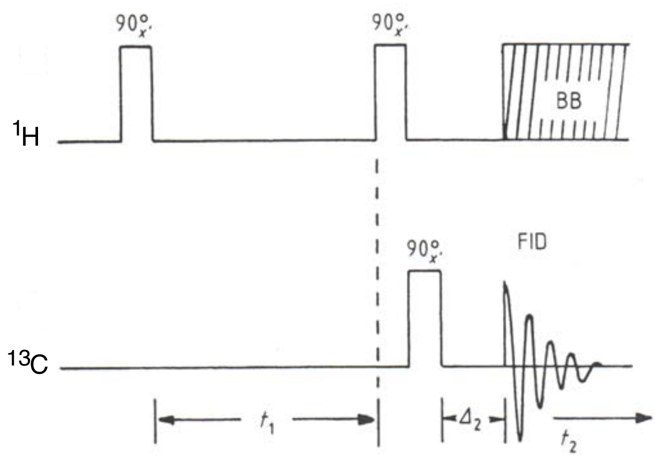



**Figure 9-11.**
Schematic two-dimensional C,H-correlated NMR spectrum of a two-spin AX system (for pulse sequence see Fig. 9-10). The two signals along the $F_2$-direction correspond to the one-dimensional $^{13}C$ NMR spectrum without decoupling, except that the signals have opposite signs. Along the $F_1$-direction is seen the doublet of the $^{1}H$ NMR spectrum with the C,H coupling (the $^{13}C$ satellites, also with opposite signal amplitudes).

## 2. Modified HETCOR Pulse Sequence to Remove Splitting in F2

### A) Pulse sequence

- **Insertion of a delay 1/(2JCH) between the $^{13}C$ 90˚x pulse and the acquisition of the FID, which allows refocusing of MC{Hα} and MC{Hβ}.**
- **BB decoupling during acquisition cause MC{Hα} and MC{Hβ} to precess at the same rate during that time.**




$\Delta_2 = 1/(2J_{CH})$

### B) Spectrum

- **After FT with respect to t2, only one signal is observed in F2 with a frequency $\nu_C$, and this signal is modulated in t1 by $\nu_H$ and $J_{CH}$.**
- **FT with respect to t1 gives two $^1H$ signals ( $\nu_H$ - 1/2*$J_{CH}$ and $\nu_H$ + 1/2*$J_{CH}$) along the F1 axis.**

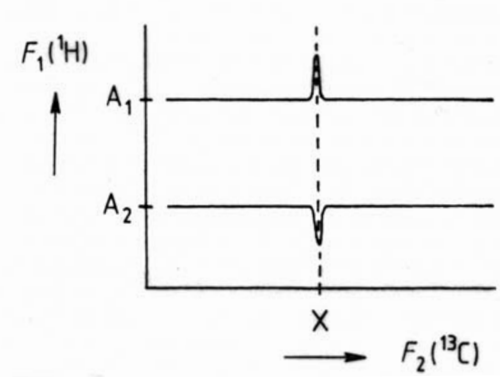
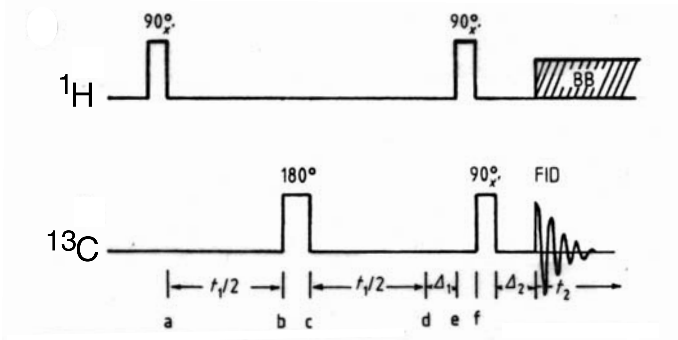



**Figure 9-13.**
Schematic two-dimensional C,H-correlated NMR spectrum of a two-spin AX system (pulse sequence as in Fig. 9-12). The 2D spectrum is reduced to two signals with opposite signs; their separation along the $F_1$ frequency axis is equal to $J$ (C,H).

## 3. Modified HETCOR Pulse Sequence to Remove Splitting in F1 and F2

### A) Pulse sequence




### B) Vector Diagram

- **Insertion of a $^{13}$C 180°x pulse in the middle of t1, which allows refocusing of MH{Cα} and MH{Cβ}.**
- **Insertion of a delay 1/(2JCH) after t1 and before the second $^1$H 90°x pulse. This constant delay is needed for optimal population transfer. After a delay of 1/(2JCH) MH{Cα} and MH{Cβ} have a 180° phase difference.**
- **The magnitude of the polarization transfer depends only on φ, which is independent of JCH. φα = φβ = 2π (νH) t1**

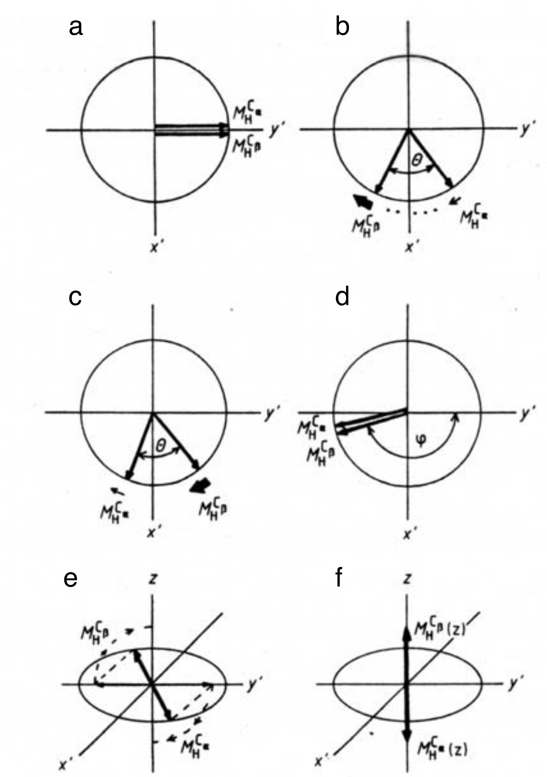
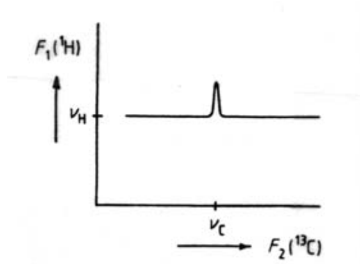



**Figure 9-14.**
A: Pulse sequence for a two-dimensional C,H-correlated NMR experiment which reduces the 2D spectrum of a two-spin AX system to only one peak.
B: The vector diagrams a to f show the positions of the $^1$H magnetization vectors $M_H^{C\alpha}$ and $M_H^{C\beta}$ or their $z$-components (f) at the instants indicated in A; in diagrams a to d only the $x'$, $y'$-plane is shown.

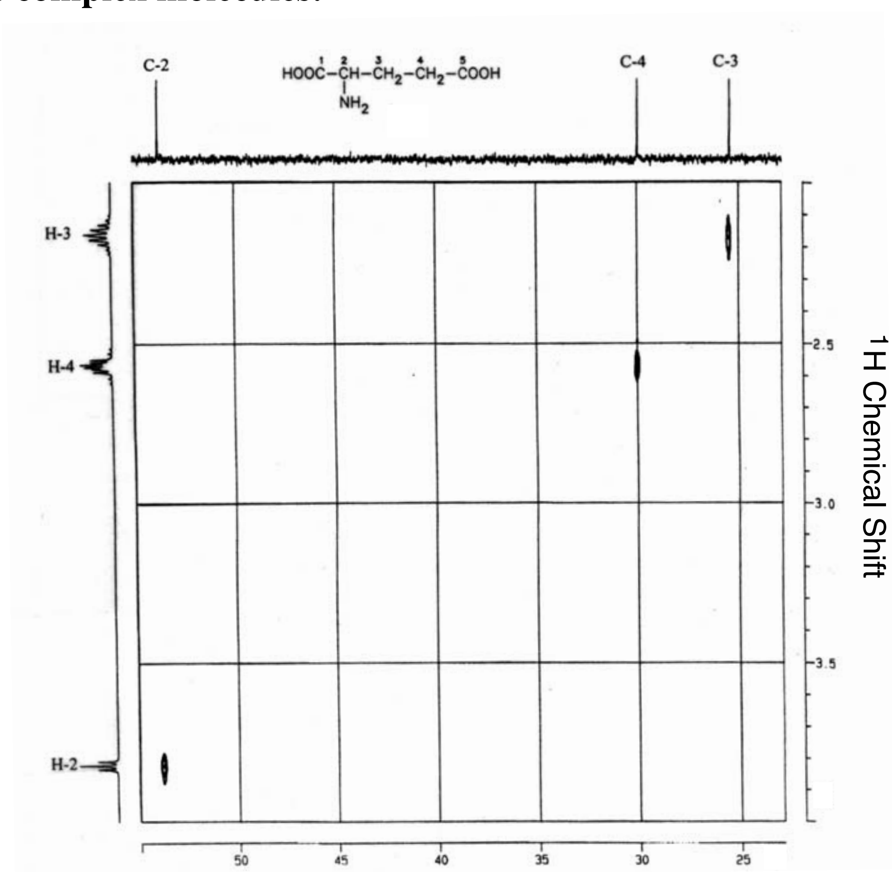
## C) Spectra

- **FT with respect to t2 gives one $^{13}C$ signal along the F2 axis with a frequency $\nu_C$, and this signal is modulated in t1 by $\nu_H$ only.**
- **FT with respect to t1 gives one $^{1}H$ signal along the F1 axis with a frequency $\nu_H$.**

**For $^{13}CHCl_3$:**




**For more complex molecules:**




$^{13}C$ Chemical Shift

**NOTE: 1) Easy to assign $^{13}C$ signals if $^{1}H$ signals are assigned, or vice versa. 2) Little overlap of the correlation peak.**

## 4. <u>Two-Dimensional Homonuclear (H,H)-Correlated NMR Spectroscopy.</u> <u>H,H-COSY</u>:

### A) Pulse sequence

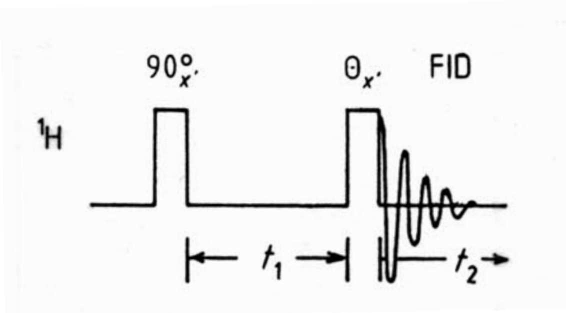



**Figure 9-17.** Pulse sequence for the two-dimensional homonuclear H,H-correlated NMR experiment COSY. The variable is $t_1$. The pulse angle $\Theta$ is usually 90° or 45°, or occasionally 60°.

**Lets consider the case where $\Theta$x' = 90˚x' and lets consider a homonuclear two-spin AX system.**

**First 90˚x' pulse:**

- **Tilt both vectors $M_A$ and $M_X$ along y'**
- **Due to $J_{AX}$, $M_A$ has two components $M_A(X\alpha)$ and $M_A(X\beta)$, and $M_X$ has two components $M_X(A\alpha)$ and $M_X(A\beta)$.**

**During t1:**

- **$\nu_A\ (X\alpha) = \nu_A - 1/2*J_{AX}$; $\nu_A\ (X\beta) = \nu_A + 1/2*J_{AX}$**
  **$\nu_X\ (A\alpha) = \nu_X - 1/2*J_{AX}$; $\nu_X\ (A\beta) = \nu_X + 1/2*J_{AX}$**

- **$\varphi(M_A(X\alpha))=2\pi\ (\nu_A - 1/2*J_{AX})\ t1$; $\varphi(M_A(X\beta))=2\pi\ (\nu_A + 1/2*J_{AX})\ t1$**
  **$\varphi(M_X(A\alpha))=2\pi\ (\nu_X - 1/2*J_{AX})\ t1$; $\varphi(M_X(A\beta))=2\pi\ (\nu_X + 1/2*J_{AX})\ t1$**

- **At the end of t1, the vectors have components along x' and y'**

**After the second 90°x' pulse:**

- **The y and -y vector components are tilted along z and -z, which results in polarization transfer. The transfer depends on t1, $\nu$, and $J_{AX}$.**
- **Four frequencies are detected by the receiver:**

$$A2 = \nu_A\ (X\alpha)$$
$$A1 = \nu_A\ (X\beta)$$
$$X2 = \nu_X\ (A\alpha)$$
$$X1 = \nu_X\ (A\beta)$$

**B) Magnitude Spectrum**

- **FT with respect to t2 yields four signals at A1, A2, X1, and X2. These signals are modulated in t1 with these same four frequencies.**

- **FT with respect to t1 gives a 2D NMR spectrum with four groups of signals, each containing four signals.**
  - ♦ **Groups centered at ($\nu_A$, $\nu_A$) and ($\nu_X$, $\nu_X$) are diagonal peaks.**
  - ♦ **Groups centered at ($\nu_A$, $\nu_X$) and ($\nu_X$, $\nu_A$) are cross peaks.**
  - ♦ **Within each group, separation in F1 and F2 is $J_{AX}$.**

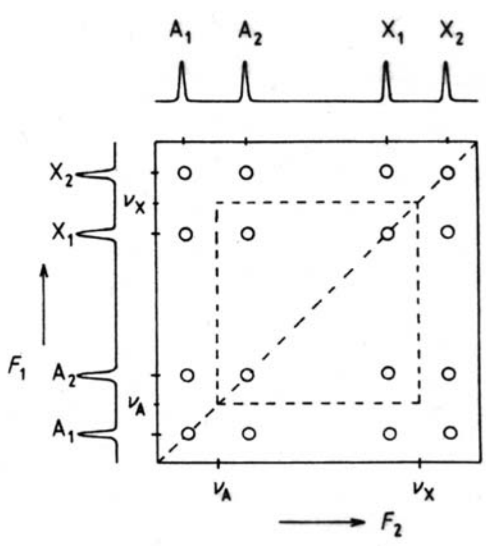
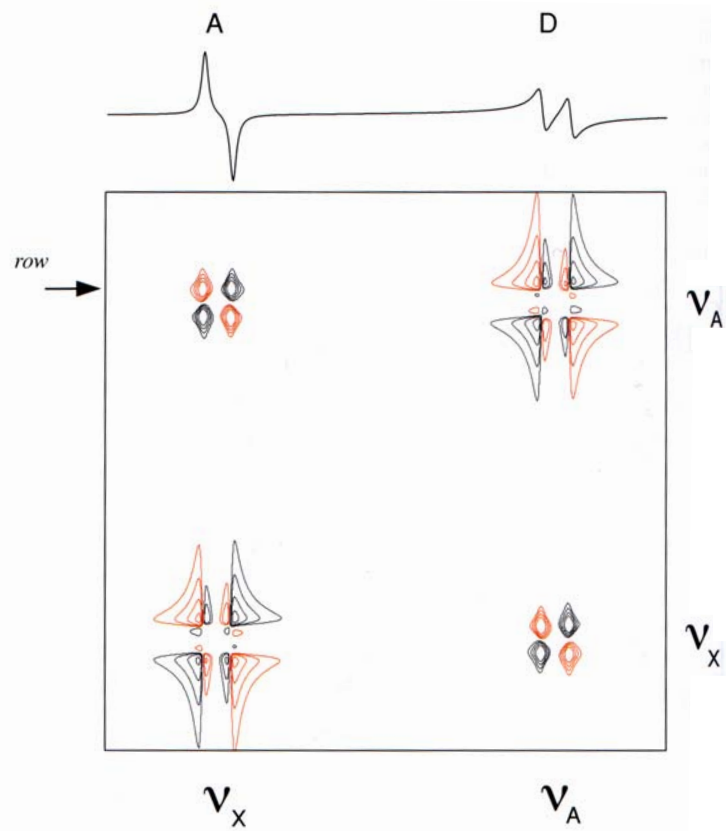



**Figure 9-18.**
Schematic representation of a COSY experiment on a two-spin AX system in which A and X are protons. The signal amplitudes are shown here as absolute values. In an actual spectrum the peaks on the diagonal are dispersion signals, while the correlation peaks (cross peaks) are absorption signals with alternating signs. The diagonal peaks of a pair of mutually coupled nuclei and their cross peaks form the corners of a square.

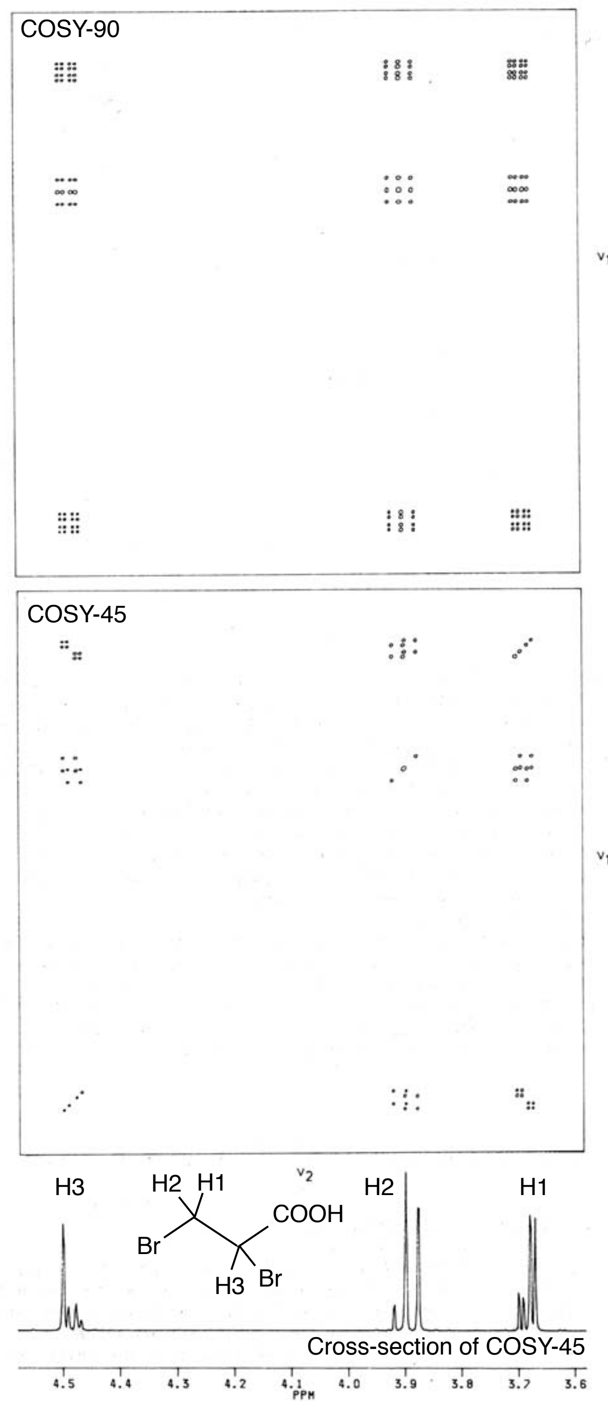
## C) Phase-Sensitive Spectrum

**Figure 5.19.** The phase-sensitive COSY for a coupled two-spin AX system. Diagonal peaks have broad, in-phase double-*dispersion* lineshapes (**D**) whereas crosspeaks have narrow, antiphase double-*absorption* lineshapes (**A**), as further illustrated in the row extracted from the spectrum.



**5. <u>Two-Dimensional Homonuclear (H,H)-Correlated NMR Spectroscopy.</u> <u>COSY-45:</u>**

**Same as COSY-90, but second $^1$H pulse is 45°x' instead of 90°x'. Reduces the intensity of the signal, but simplifies the spectrum.**



## 6. Two-Dimensional Homonuclear (H,H)-Correlated NMR Spectroscopy. Long-range COSY:

**A fixed delay Δ (0.1 to 0.4 s) is added before and after the second $^1$H 90˚x' pulse:**

$$90^\circ_{x'} - t_1 - \Delta - 90^\circ_{x'} - \Delta - \mathrm{FID}$$

**Allows development of correlation effects for very weakly coupled $^1$H.**

**h**