# CHEM / BCMB 4190/6190/8189

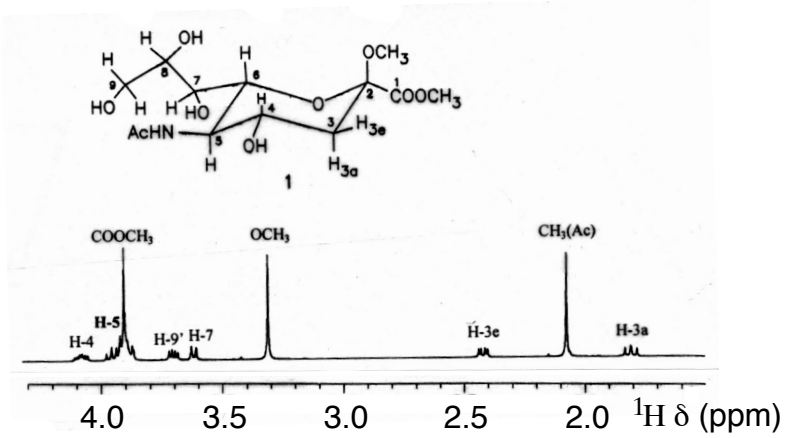
# Introductory NMR

Lecture 15

## Introduction to 2D NMR

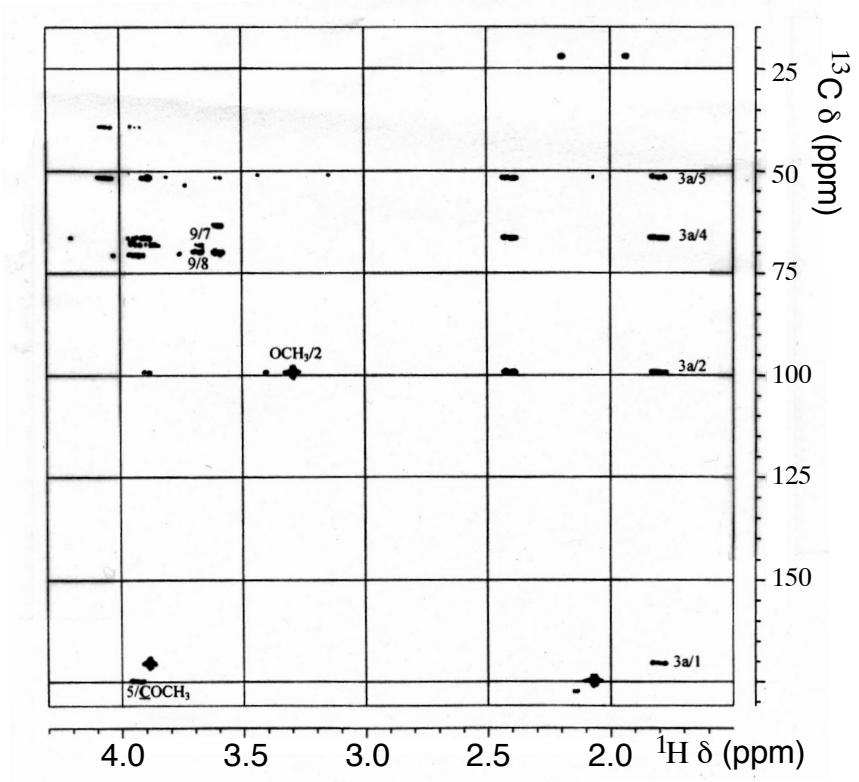
**1D NMR spectrum:**

- **x axis is the directly detected frequency and y axis gives the intensity**
- **problem of overlap of the detected frequencies; defining connections between spins using selective excitation may be impossible**




**2D NMR spectrum:**

- **x and y are frequency axes and the intensity constitutes the z axis**



**2D J-resolved spectrum:**

- **x axis is the directly detected frequency**
- **y axis is the coupling constant**
- **resolves problems of overlap in 1D spectrum**

**2D (shift) correlated NMR spectrum:**

- **x and y are frequency axes ($^{1}H$ - $^{1}H$, $^{1}H$ - $^{13}C$, $^{1}H$ - $^{15}N$, etc.)**
- **defines connections between nuclear spins**

**3D NMR spectrum:**

- **2D NMR is good for M.W. up to 10-12 kD in studies of proteins: more dimensions increased the resolution for studies of larger systems.**
- **x , y, and z are frequency axes**
- **the intensity constitutes the fourth dimension**
- **for sensitivity reason, $^{1}H$ is usually the detected frequency ($\varepsilon \propto \gamma^{3}$)**

**<u>Connections between two dimensions</u>:**

**1. Through-bond correlation:**

- **based of J coupling between nuclear dipoles**
- **gives information on the covalent structure (torsion angle constraint)**
- **Example: COSY, HETCOR, TOCSY, HSQC**

**2. Through-space correlation:**

- **based on dipolar interactions (NOE; nuclear Overhauser effect)**
- **gives information on the geometrical structure (distance constraint)**
- **Example: NOESY**

**3. Chemical exchange correlation:**

- **based on dynamic processes**
- **gives info on these dynamic processes**
- **Example: EXSY**

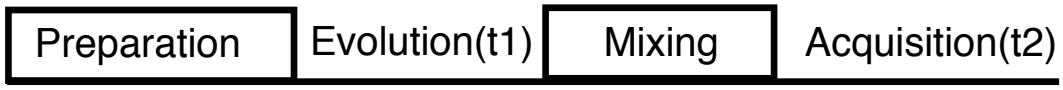
**General Experimental Scheme:**

**In 1D NMR:**


**PREPARATION period: a single pulse in the most simple case and multiple pulses and delays in more complex pulse sequences (Example: J-modulated spin-echo experiment, INEPT, DEPT)**

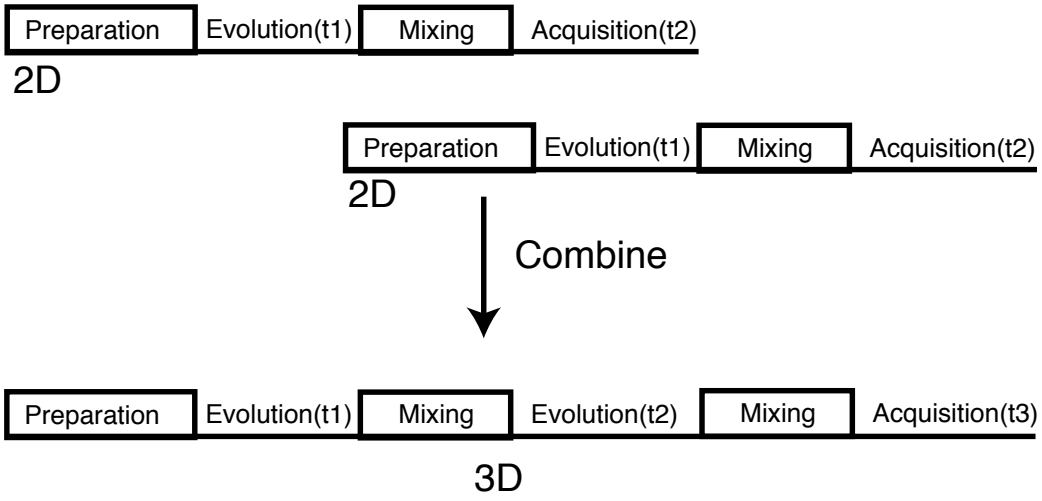
**In 2D NMR:**

**Two new elements, the EVOLUTION and MIXING periods are introduced between the preparation and the acquisition period. The EVOLUTION period introduces an indirectly-detected frequency dimension. During the MIXING period coherence is transferred from one spin to another.**

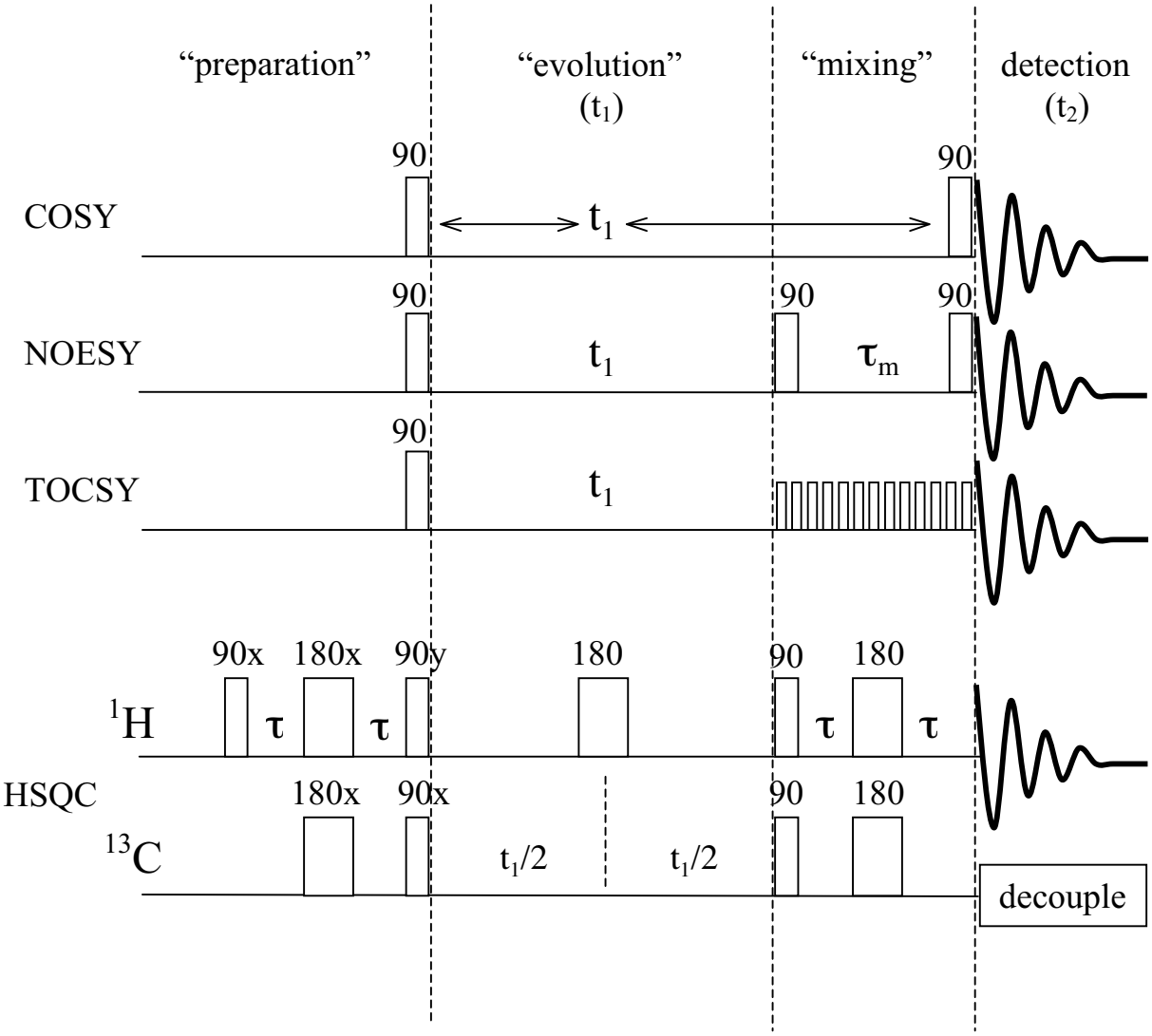



**In 3D NMR:**

**Combination of 2D NMR experiments.**



**General Scheme for Two-Dimensional NMR: selected 2D experiments**

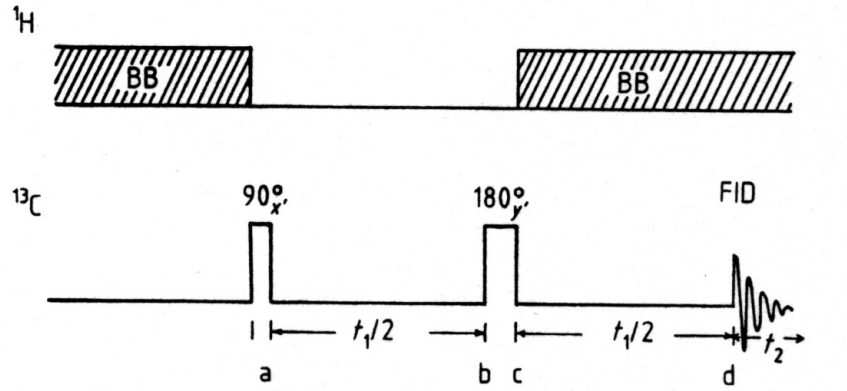



**COSY: Correlated Spectroscopy**
**NOESY: Nuclear Overhauser Effect Spectroscopy**
**TOCSY: Total Correlation Spectroscopy**
**HSQC: Heteronuclear Single Quantum Correlation**

## Heteronuclear Two-Dimensional J-Resolved NMR Spectroscopy::

### A) Pulse sequence

- **Experiment similar to the J-modulated spin-echo pulse-sequence: The fixed delay τ is now a variable delay t1/2**



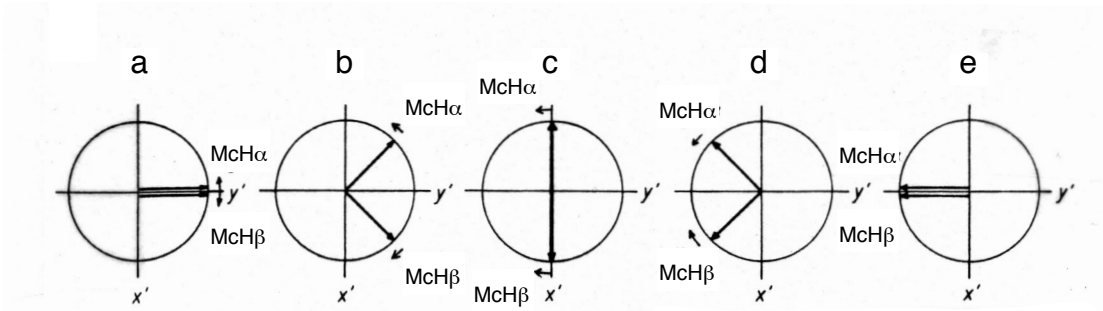

### B) Vector diagram

**Lets consider a two-spin AX system with A= $^{1}$H and X = $^{13}$C as in $^{13}$CHCl3**

$$\nu\ (^{13}CH\alpha Cl_3) = \nu c - 1/2 * J_{CH}$$

$$\nu\ (^{13}CH\beta Cl_3) = \nu c + 1/2 * J_{CH}$$

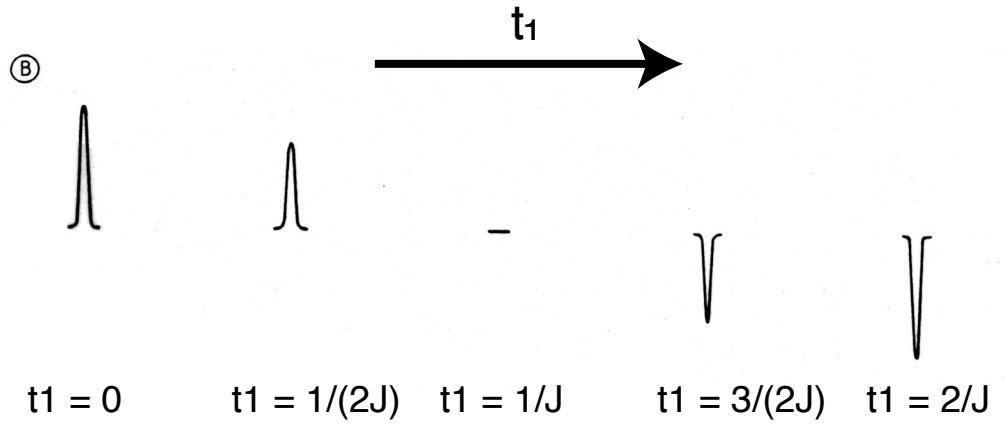
$$\Theta = 2\pi J_{CH} * (t1/2)$$



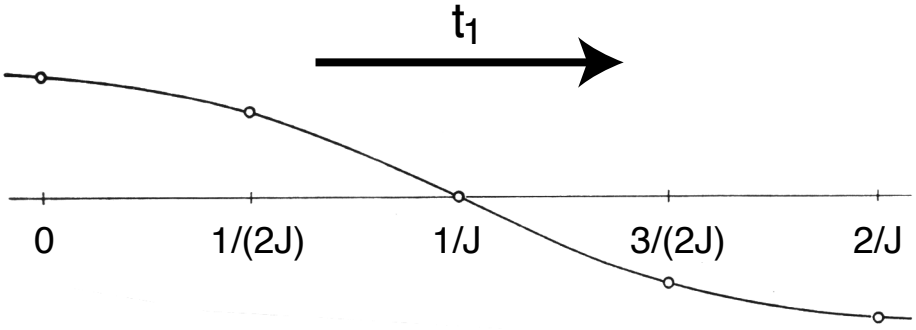

- **J coupling evolves during the first t1/2 delay only**
- **Field inhomogeneity is refocused by the 180˚y pulse**
- **Chemical shift evolution in t1 is refocused by the 180˚y' pulse**
- **For simplicity, ignore relaxation**

**C) Fourier transform in t2**

**We perform "n" experiments with various values of t1 and Fourier transform all "n" experiments**

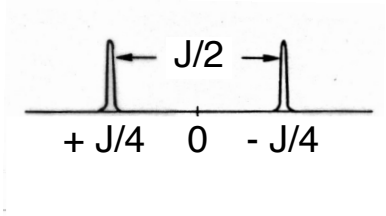



**D) Amplitude modulation in t1**




**The amplitude is modulated in t1 at a frequency related to $J_{CH}$**

**E) Second Fourier transform in t1**




**A second Fourier transform with respect to t1 yields two frequencies with a separation of $J_{CH}/2$**

**F) Summary**

$$S(t_1, t_2) \xrightarrow{FT} S(t_1, F_2) \xrightarrow{FT} S(F_1, F_2)$$

**$F_2$ dimension contains the $^{13}C$ chemical shift**
**$F_1$ dimension contains the coupling constant**

**<u>For $^{13}CHCl_3$</u>:**

- **The signal induced in the receiver depends on the sum of the two vectors, which depends on $J_{CH}$. After FT of the FID with respect to $t_2$, a single $^{13}C$ signal is observed in the frequency spectrum $F_2$ (no splitting).**

- **The $^{13}C$ signal is modulated by the value of $J_{CH}$ and the value of $t_1$. After FT of the FID with respect to $t_1$, a doublet centered at zero frequency is observed in the frequency spectrum $F_1$ with a splitting of $J_{CH}$ / 2.**

**<u>For more complex molecules</u>:**

- **In $F_2$: $C_q$, CH, $CH_2$, and $CH_3$ are all singlets**

- **In $F_1$: $C_q$: singlet $CH_2$: triplet**
  **CH: doublet $CH_3$: quartet**

**G) Choice of acquisition time in $t_1$**

- **Should be long enough to give satisfactory resolution in $F_1$**

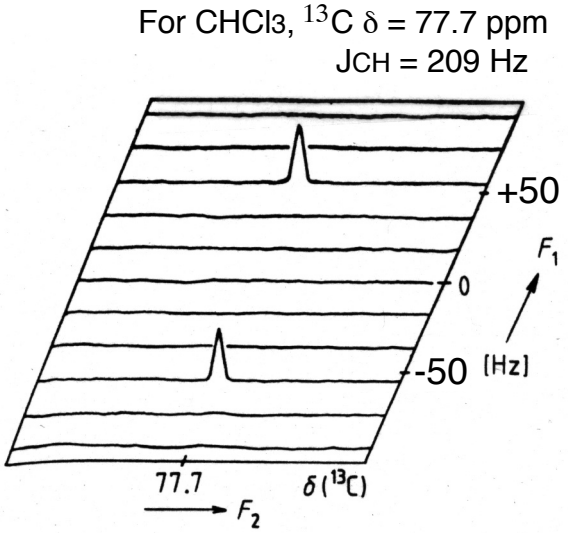
  **Digital resolution = 1 / (acquisition time in $t_1$)**

- **Also limited by relaxation**

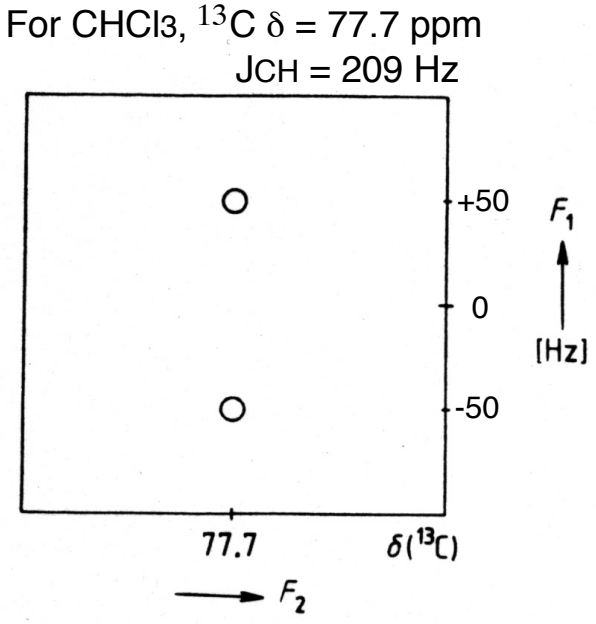
**Graphical Representation:**

**Two forms of display are generally used:**

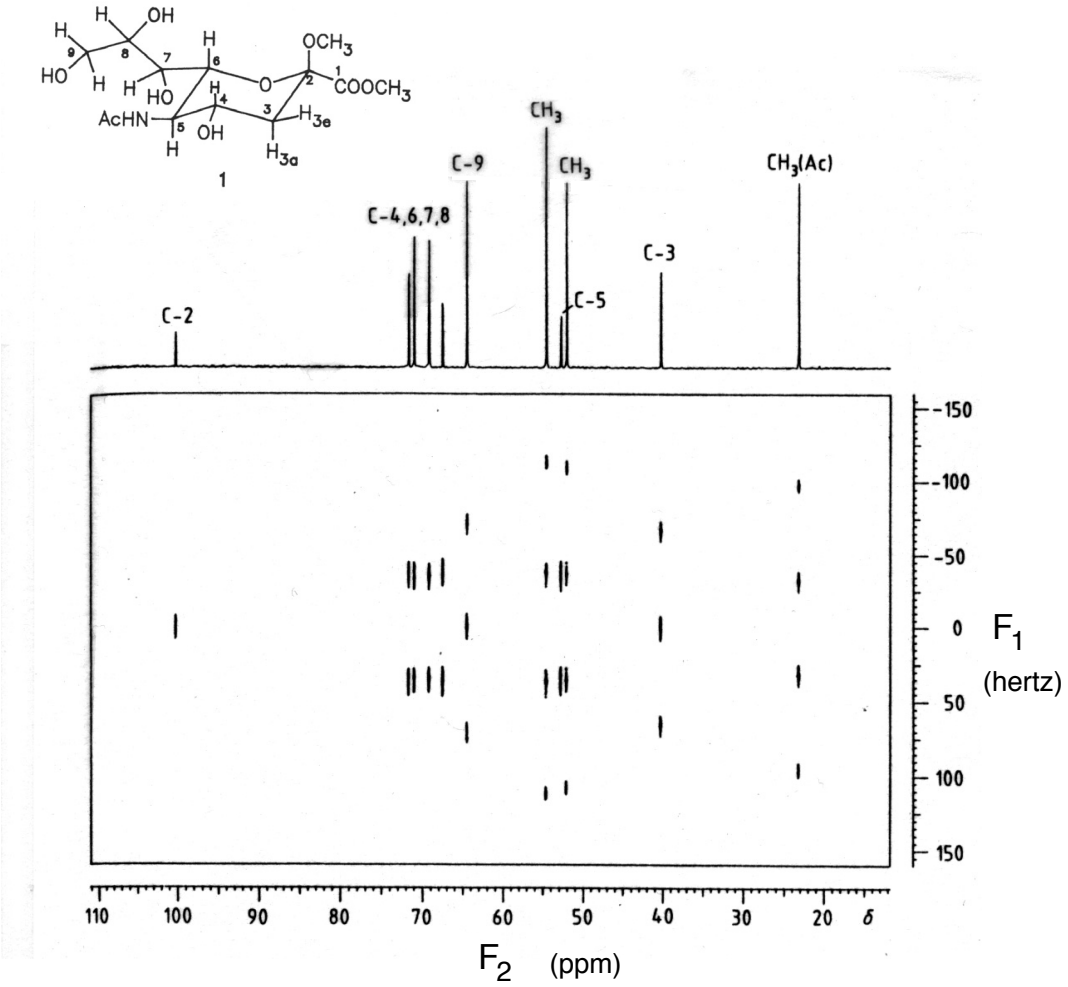
**Stacked plot: a series of F2 spectra for different F1 values are plotted above one another. Each trace is shifted by a constant amount relative to the preceding one.**



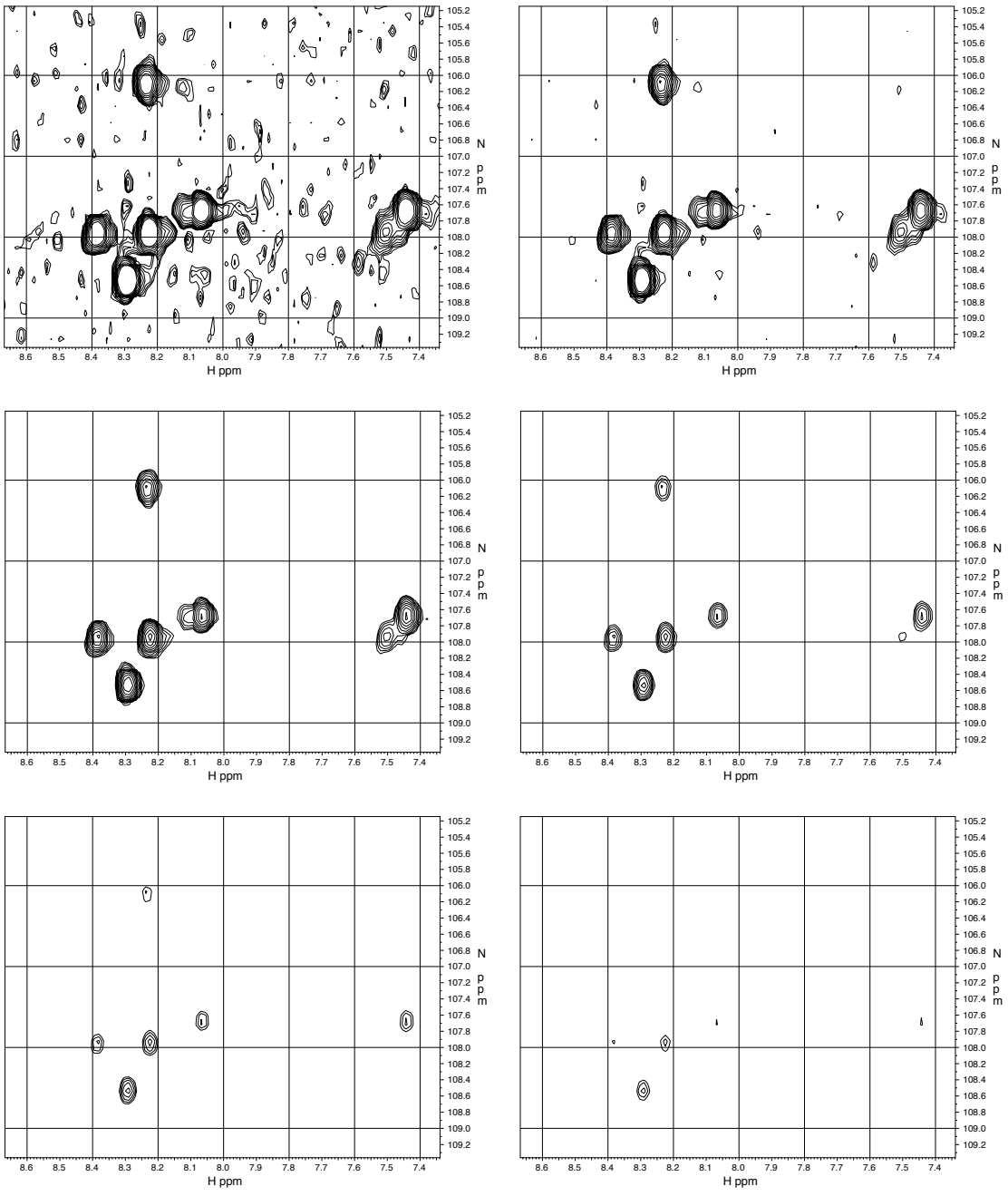

**Contour plot: the peaks are seen from above as in a topographic map. The section is taken at certain height above the plane of the F1 and F2 axes, and the contours at that level are plotted**



For more complex molecules



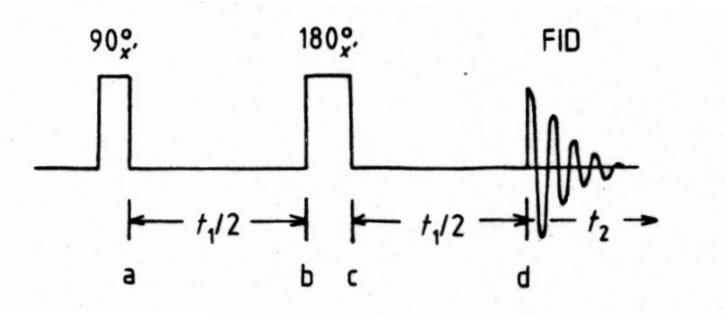
## Other example of contour plots: 2D $^{1}H$-$^{15}N$ correlation spectrum (HSQC) plotted at various levels



## Homonuclear Two-Dimensional J-Resolved NMR Spectroscopy:

### A) Pulse sequence

- **Experiment similar to the J-modulated spin-echo pulse sequence: The fixed delay τ is now a variable delay t1**
- **In homonuclear case, not possible to use BB decoupling**
- **The 180x' pulse is applied to both spins (A and X)**



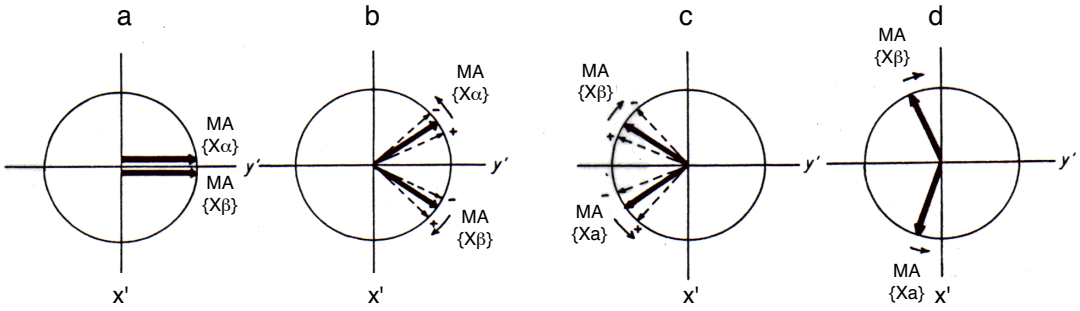

### B) Vector diagram

**Lets consider a homonuclear two-spin AX system:**

$$\nu_A(X\alpha) = \nu_A - 1/2 * J_{AX}$$

$$\nu_A(X\beta) = \nu_A + 1/2 * J_{AX}$$

$$\Theta = 2\pi J_{AX} * t1$$



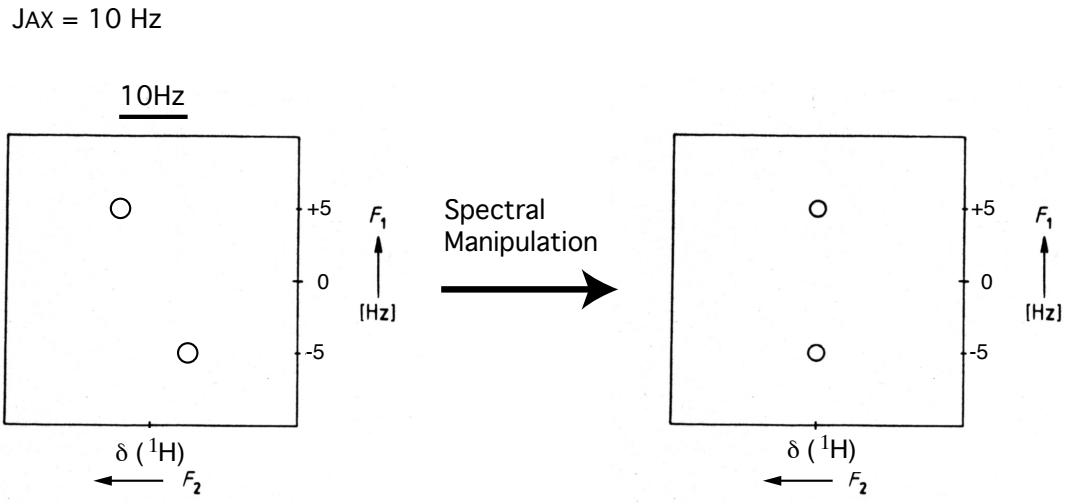

- **J coupling evolves during both t1/2 delays**
- **Field inhomogeneity is refocused by the 180˚x' pulse**
- **Chemical shift evolution in t1 is refocused by the 180˚x' pulse**
- **For simplicity, ignore relaxation**

## B) Contour plot

**<u>For a $^1$H AX system</u>:**

- **The signal induced in the receiver depends on the sum of the two vectors, which depends on JAX. After FT of the FID with respect to t2, two $^1$H peaks are observed. These two peaks are tilted relative to F2, but the spectrum can be manipulated to bring all the component of the multiplet at the same F2 frequency (no splitting).**

- **The $^1$H signals are modulated by the value of JAX and the value of t1. After FT of the FID with respect to t1, a doublet centered at zero frequency is observed in the frequency spectrum F1 with a splitting of JAX.**



## For more complex molecules:

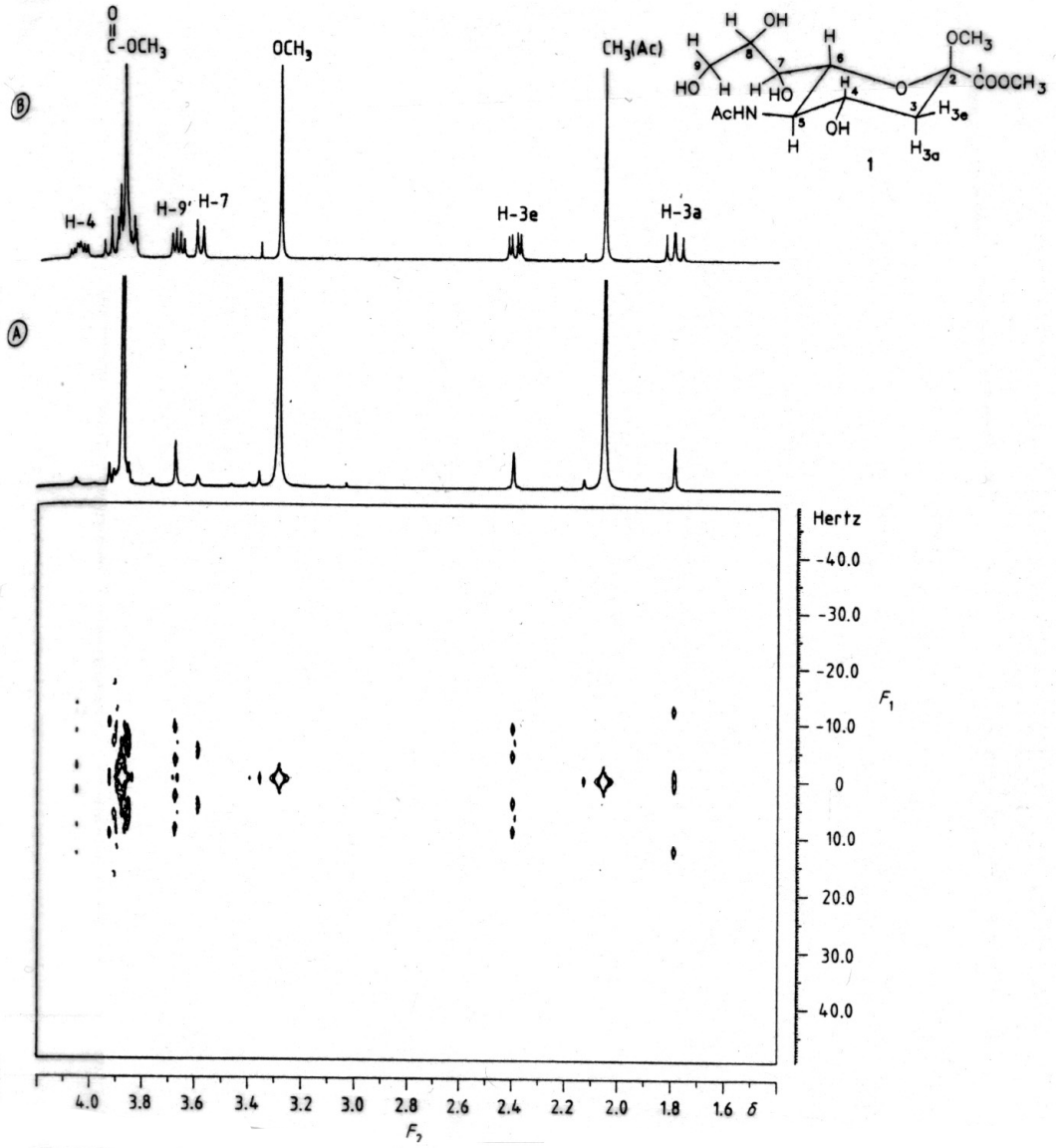



**Figure 9-8.**
Contour plot of the two-dimensional homonuclear *J*-resolved 400 MHz $^1$H NMR spectrum of **1**.
A: Projection of the 2D spectrum onto the $F_2$-axis. This is, in effect, a "decoupled" $^1$H NMR spectrum.
B: Normal 400 MHz $^1$H NMR spectrum of **1**.
(*Experimental conditions:*
20 mg of the compound in 0.5 ml $D_2O$; 5 mm sample tube; 128 measurements with $t_1$ altered in 5.06 ms increments; each measurement with 48 FIDs and 4 K data points; total time 4.2 h.)