

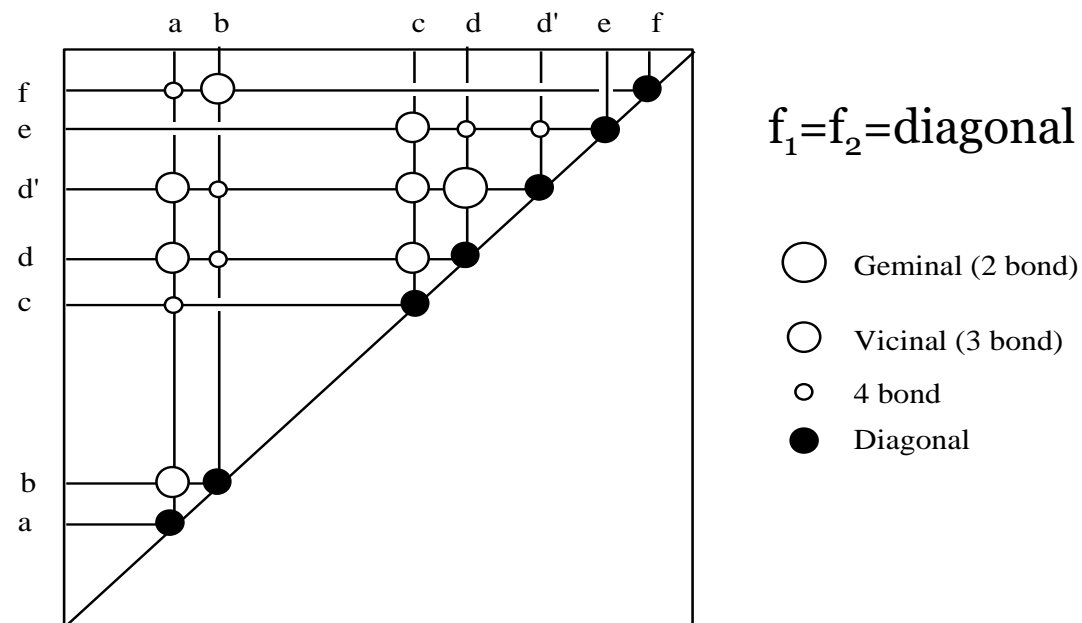
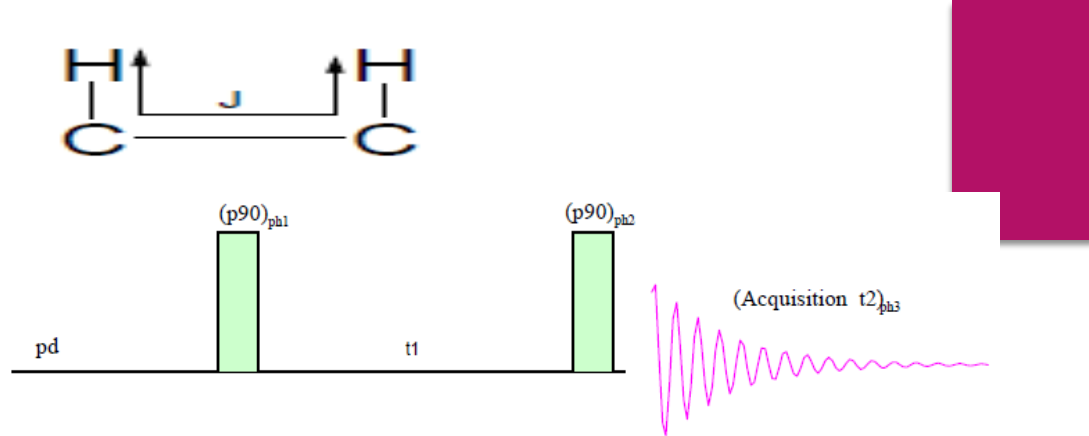
# Two Dimensional NMR-Part II

Dr. Darshana Mehta

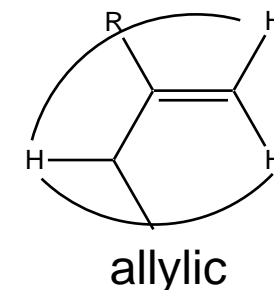
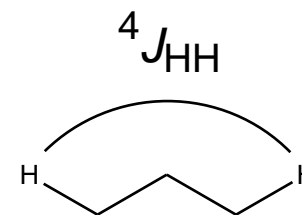
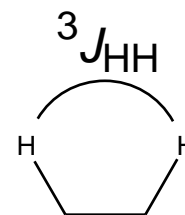
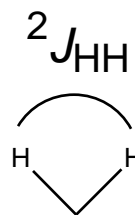
School of Studies in Chemistry and Biochemistry, Vikram University, Ujjain – 456 010, Madhya Pradesh, India

# $^1\text{H}$ - $^1\text{H}$ COSY (CORrelated SpectroscopY)

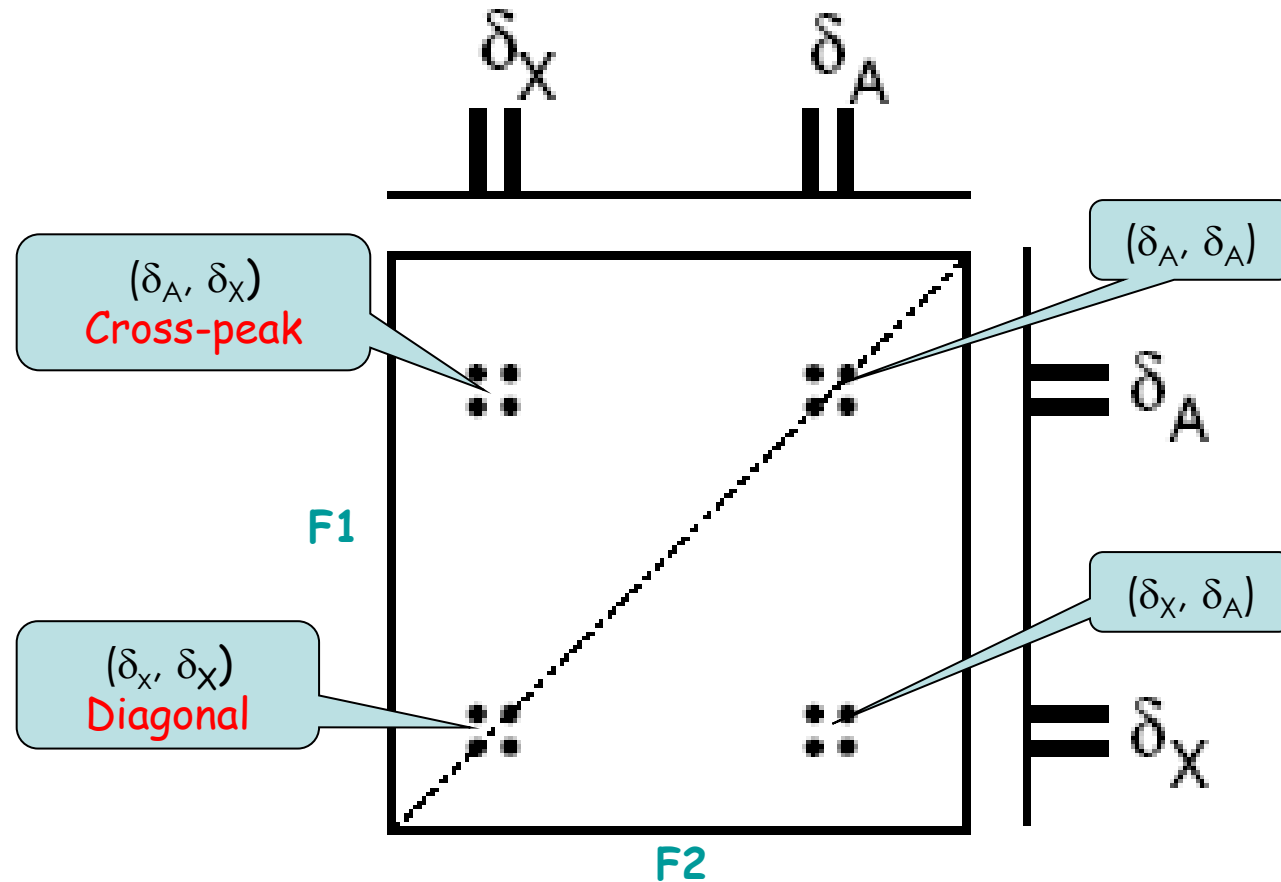
- ❖ The pulse sequence includes two  $90^\circ$  pulses separated by an evolution time  $t_1$ .
- ❖  $t_1$  is varied to obtain a data surface, then a two dimensional FT is applied to give a spectrum that shows correlations based on  $J$ -coupling.
- ❖ The diagonal peaks of COSY are **dispersive**, and the cross peaks are **antiphase**. The long tails of dispersive peaks can make it difficult to resolve cross peaks near the diagonal.



Gives:



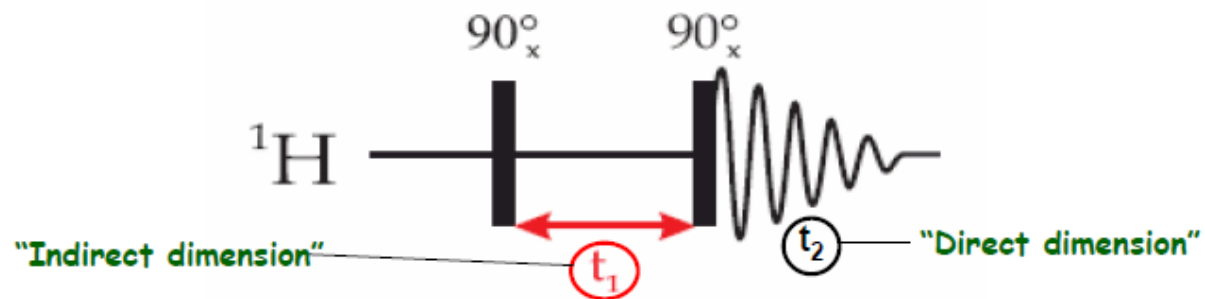
# $^1\text{H}$ - $^1\text{H}$ COSY (CORrelated SpectroscopY)



Schematic COSY spectrum for two coupled spins, A and X

## **$^1\text{H}$ - $^1\text{H}$ COSY (COrrrelated SpectroscopY)**

- ❖ **Homonuclear correlation spectroscopy is a most useful experiment to find coupling information.**
- ❖ **In the COSY spectrum, both vertical and horizontal axes provide proton chemical shift.**
- ❖ **The diagonal peaks correspond to the normal 1D spectrum.**
- ❖ **There are also cross peaks that appear as a result of spin-spin coupling.**
- ❖ **Cross peaks must always appear as a pair in matched positions in the diagonal peaks.**



Generates a 2d map which has cross peaks due to geminal and vicinal coupling  
**ONLY**

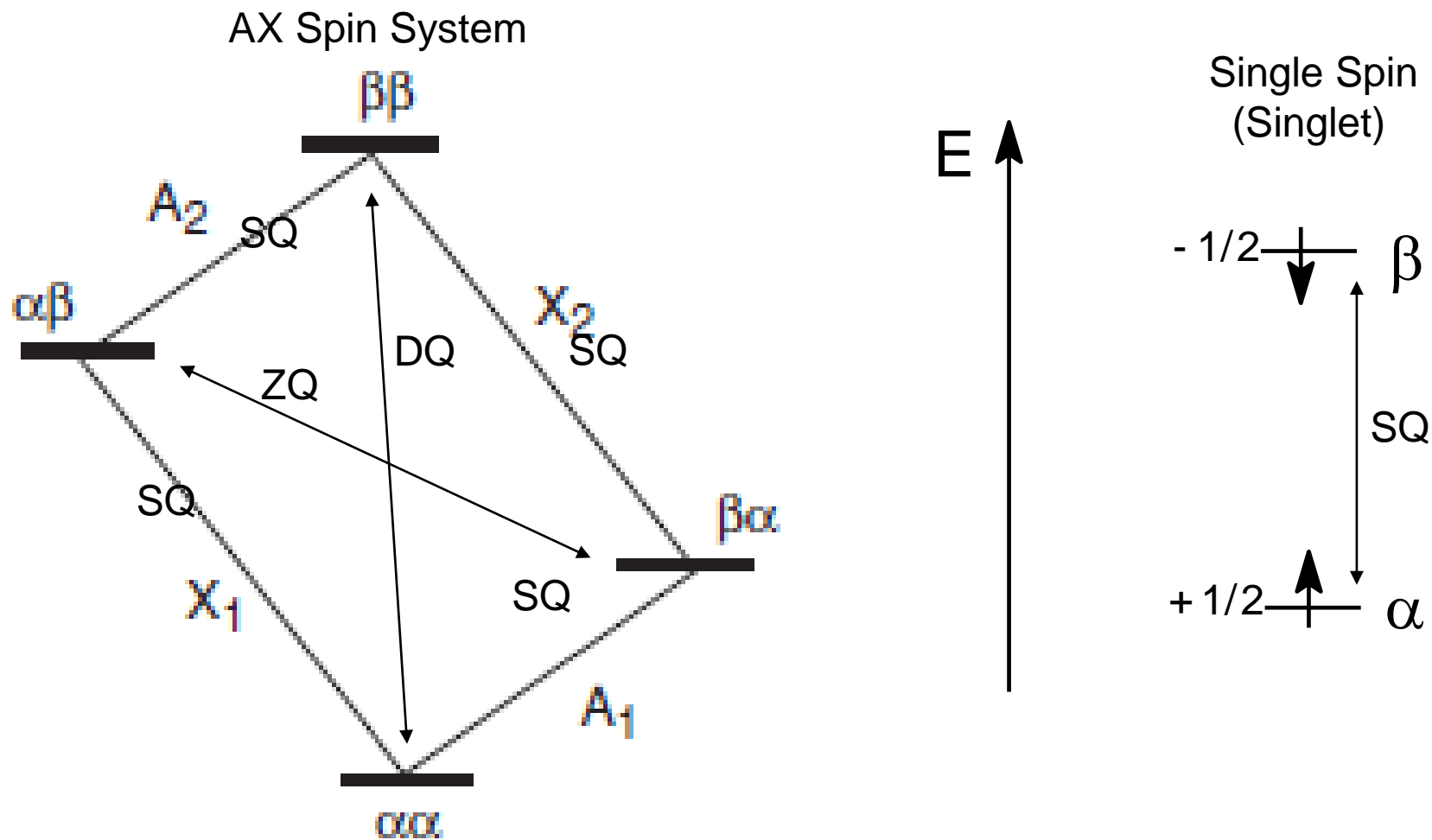
### Advantages

- ❖ Simplest type of 2D experiment
- ❖ Easiest to set up
- ❖ Forgiving of pulse width errors

### Disadvantages

- ❖ Has inherently low resolution and relatively low sensitivity compared to other types of proton-proton 2D's
- ❖ Contains the least amount of information of proton-proton 2D experiments
- ❖ Should be used only for routine assignment of low molecular weight compounds that have little resonance overlap

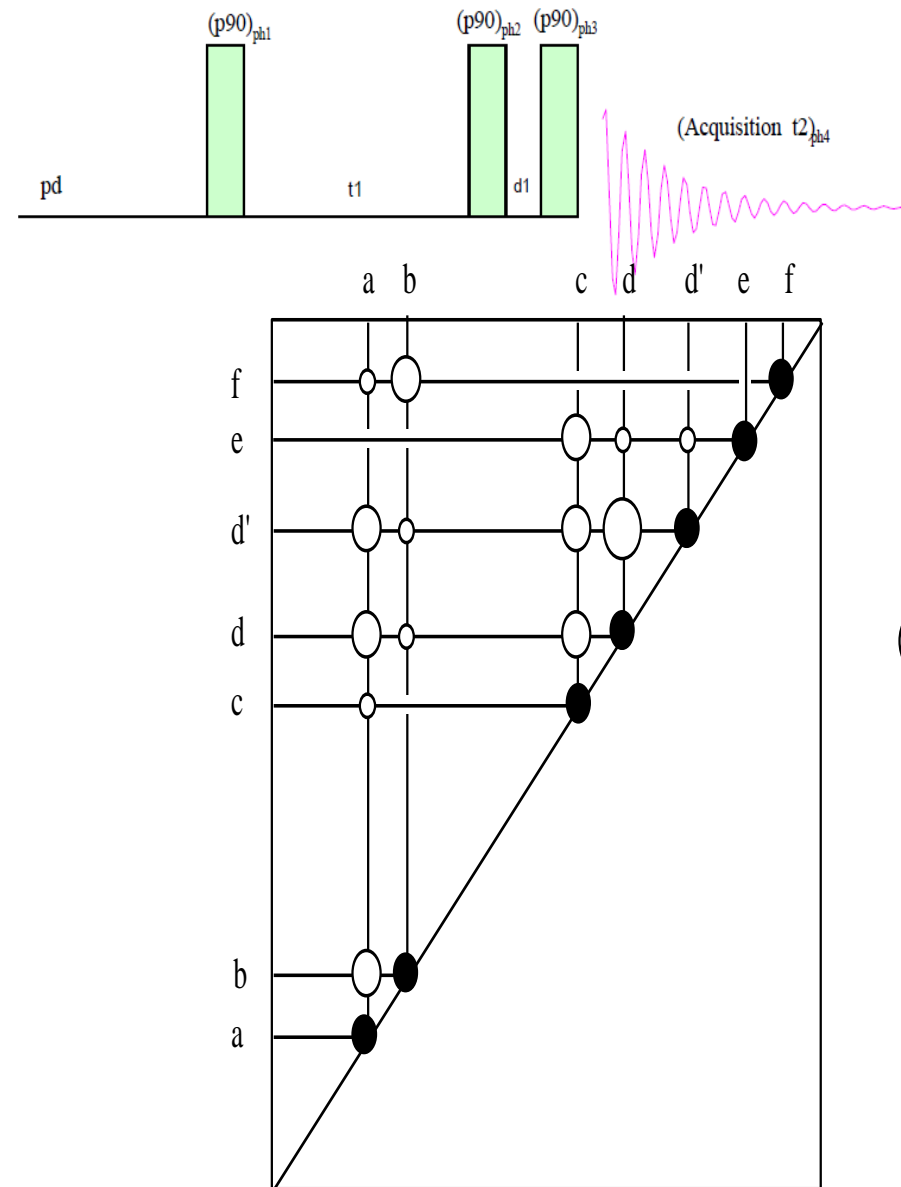
# The Multiple Quantum Filter (MQF)



A1, A2, X1 and X2 are Single Quantum Transitions (SQ). Total Spin Change is 1  
 DQ: Double Quantum. Total Spin Change is 2  
 ZQ: Zero Quantum. Total Spin Change is 0 (zero)

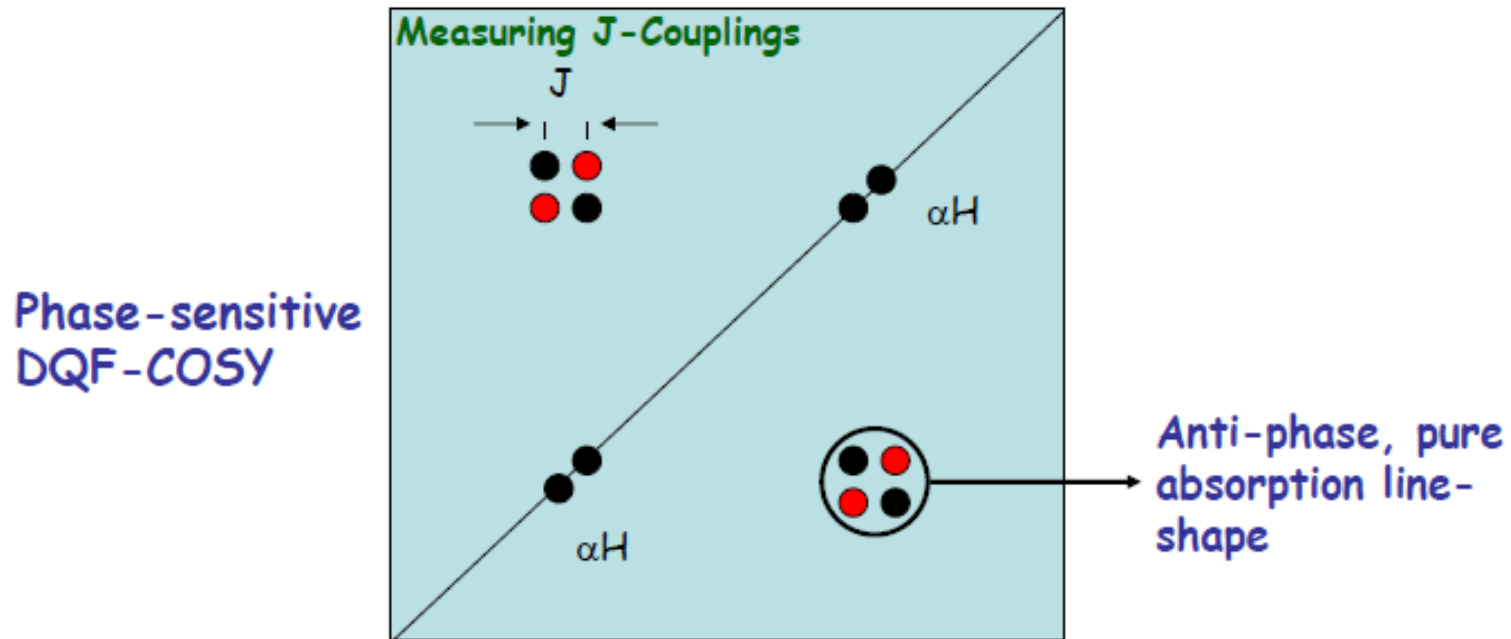
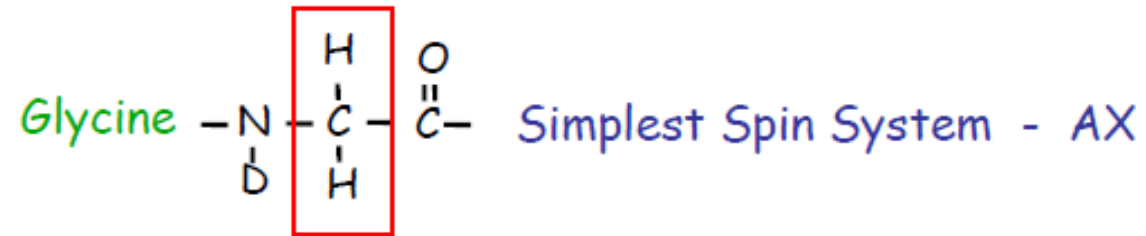
# Double Quantum Filtered COSY (DQF-COSY)

- ❖ Same information as COSY but removes single quantum transitions (large singlet peaks from Me groups), meaning we can see things closer to the diagonal.
- ❖ Improved lineshapes and resolution
- ❖ It is phase sensitive, we acquire 2 x number of increments (real and imaginary). Get coupling information from phases of correlation peaks.
- ❖ Coupled diagonal will be absorptive rather than dispersive, and uncoupled peaks are strongly attenuated.



# Phase Sensitive COSY (DQFCOSY)

(Most often used for assignment in small molecules)

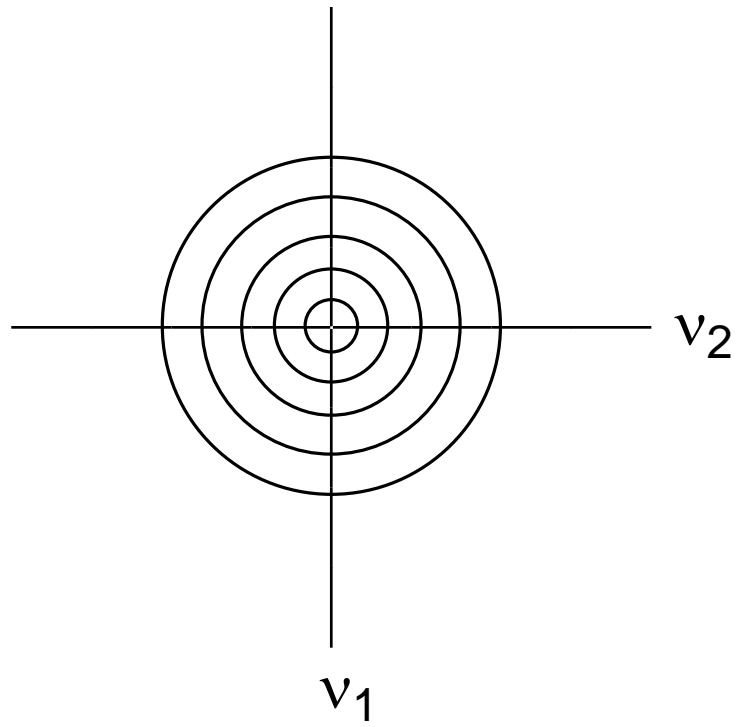




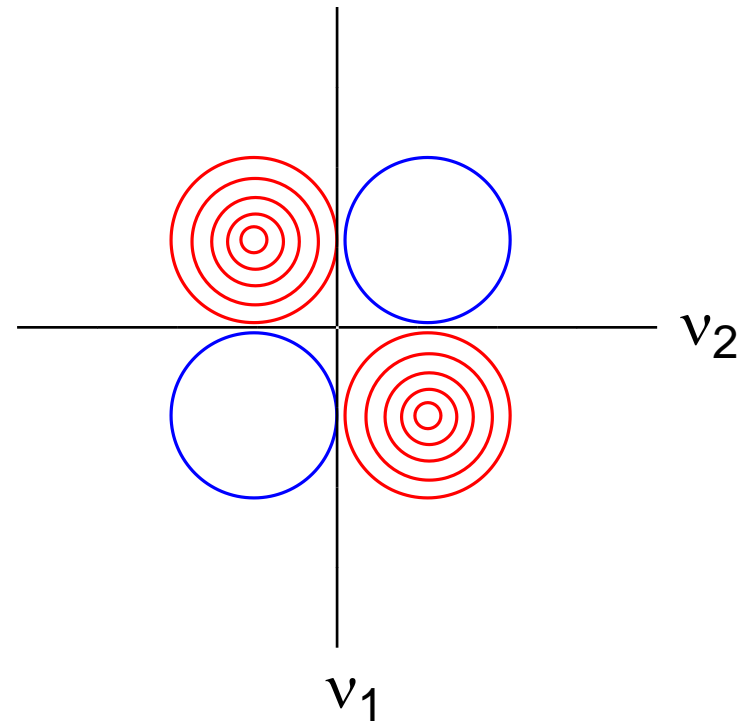
## Double Quantum Filtered COSY (DQF-COSY)

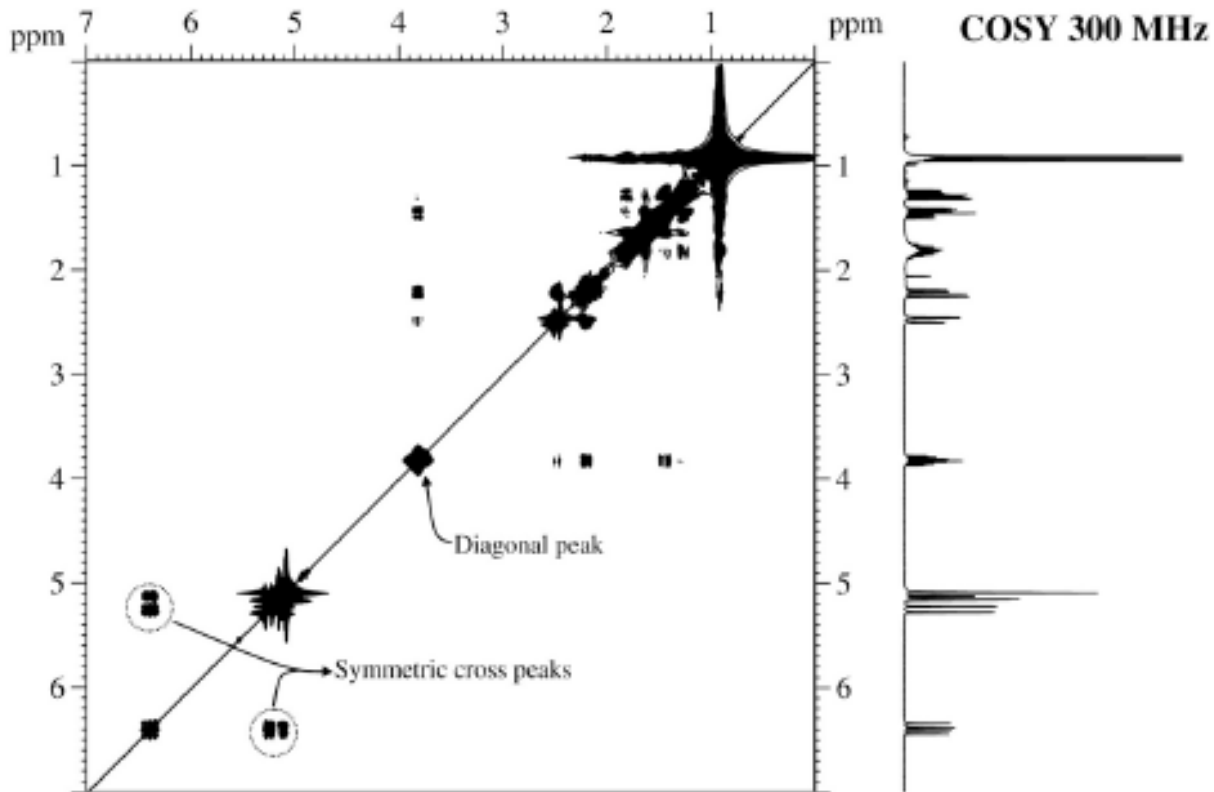
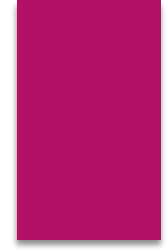
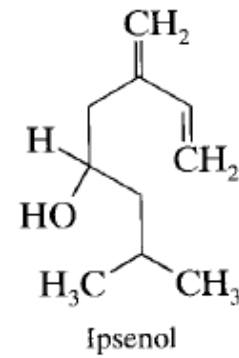
- ❖ The DQF-COSY sequence differs from the basic COSY experiment by the addition of a third pulse and the use of a modified phase cycle or gradient sequence to provide the desired selection.
- ❖ Thus, following  $t_1$  frequency labelling, the second 90 pulse generates multiple-quantum coherence, which is not observed in the COSY-90 sequence since it remains invisible to the detector.
- ❖ This may, however, be reconverted into SQC by the application of the third pulse and hence subsequently detected.
- ❖ cleans up the spectrum by reducing noncoupled systems (e.g. CH<sub>3</sub> singlets)
- ❖ The required phase cycle or gradient combination selects only signals that existed as DQC between the last two pulses, whilst all other routes are cancelled, hence the term DQF-COSY.

# COSY

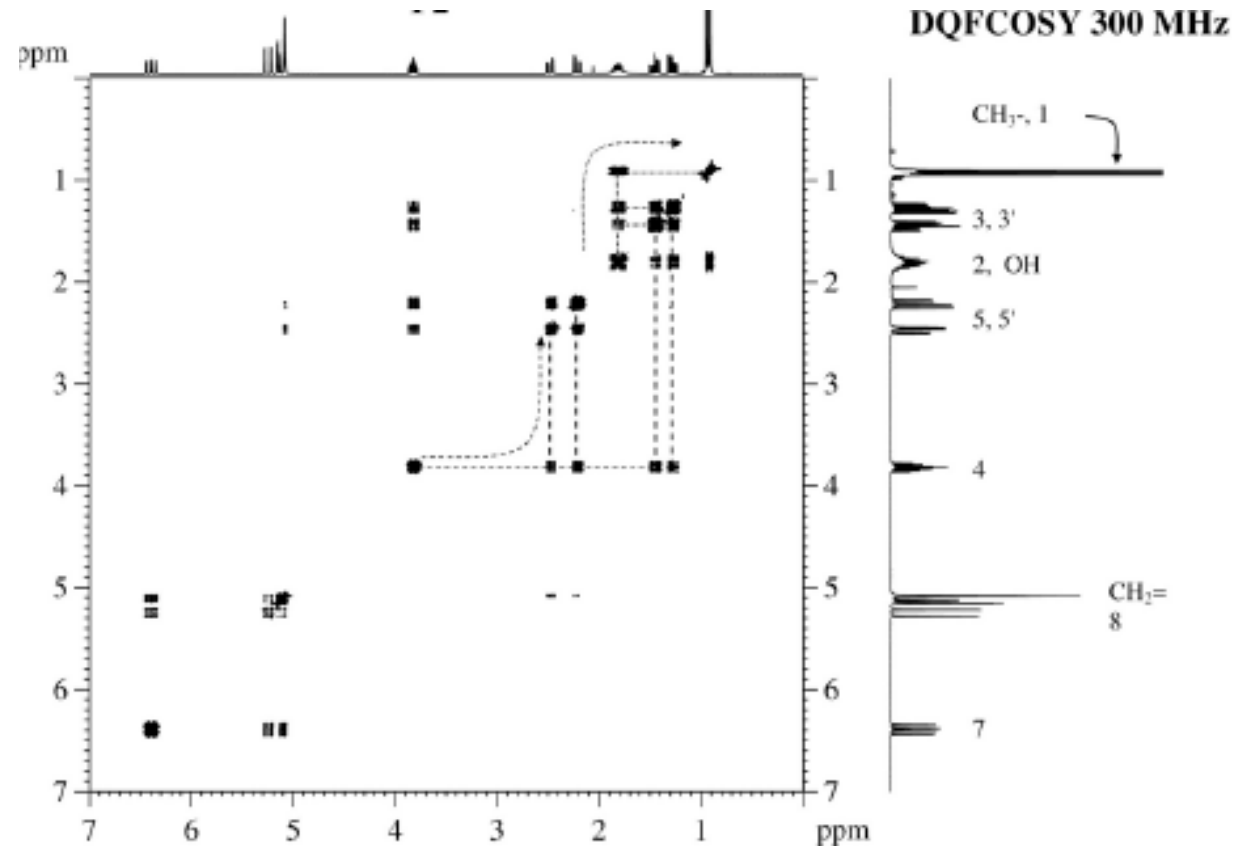


# DQF-COSY





**COSY**



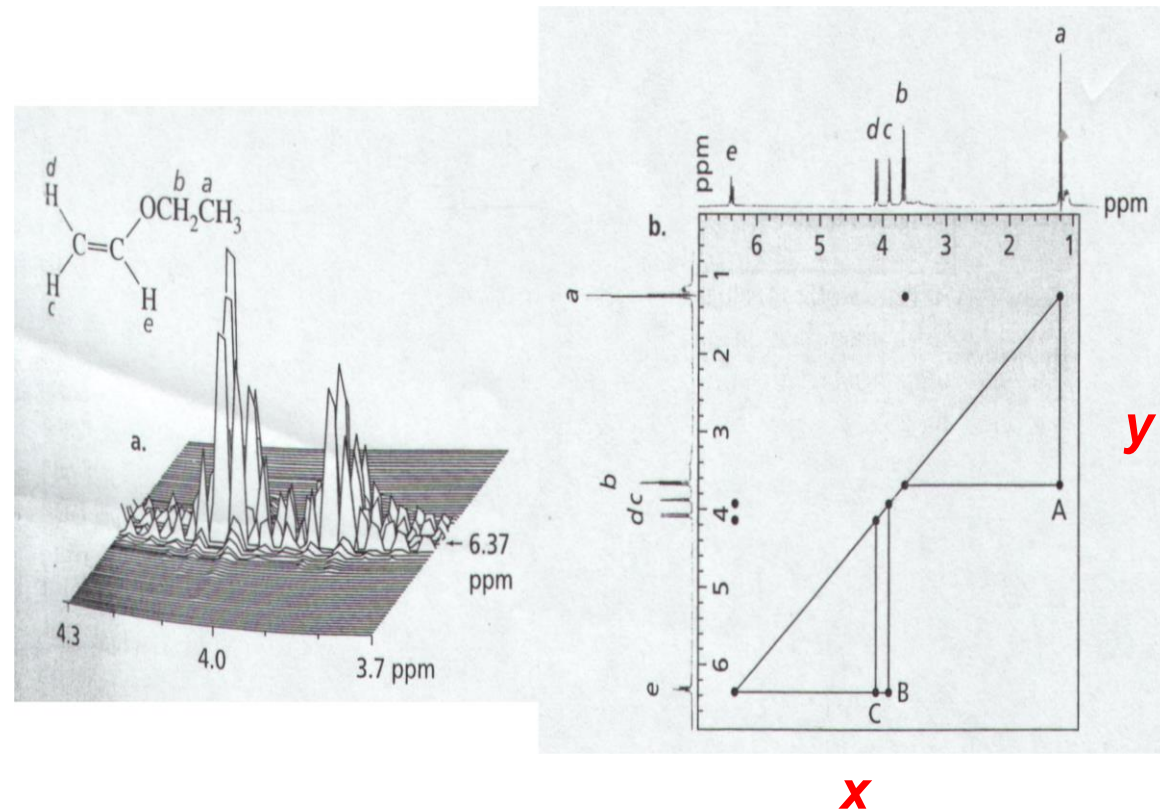
**DQF-COSY**

# COSY spectrum of Ethyl Vinyl Ether

Fig:1. It looks like a mountain range viewed from the air because intensity is the third axis. These “mountain-like” spectra (known as *stack plots*) are not the spectra actually used to identify a compound.

Instead, the compound is identified using a contour plot Fig:2, where each mountain in Fig:1 is represented by a large dot (as if its top had been cut off). The two mountains shown in Fig:1 correspond to the dots labelled B and C in Fig: 2

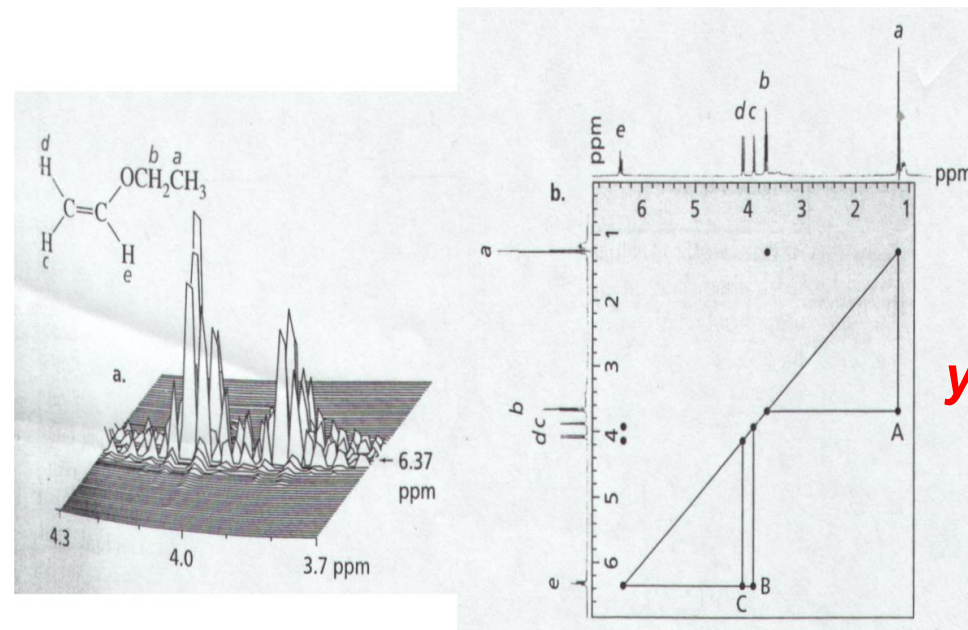
Fig:2, the usual one-dimensional  $^1\text{H}$  NMR spectrum is plotted on both the x- and y- axes.

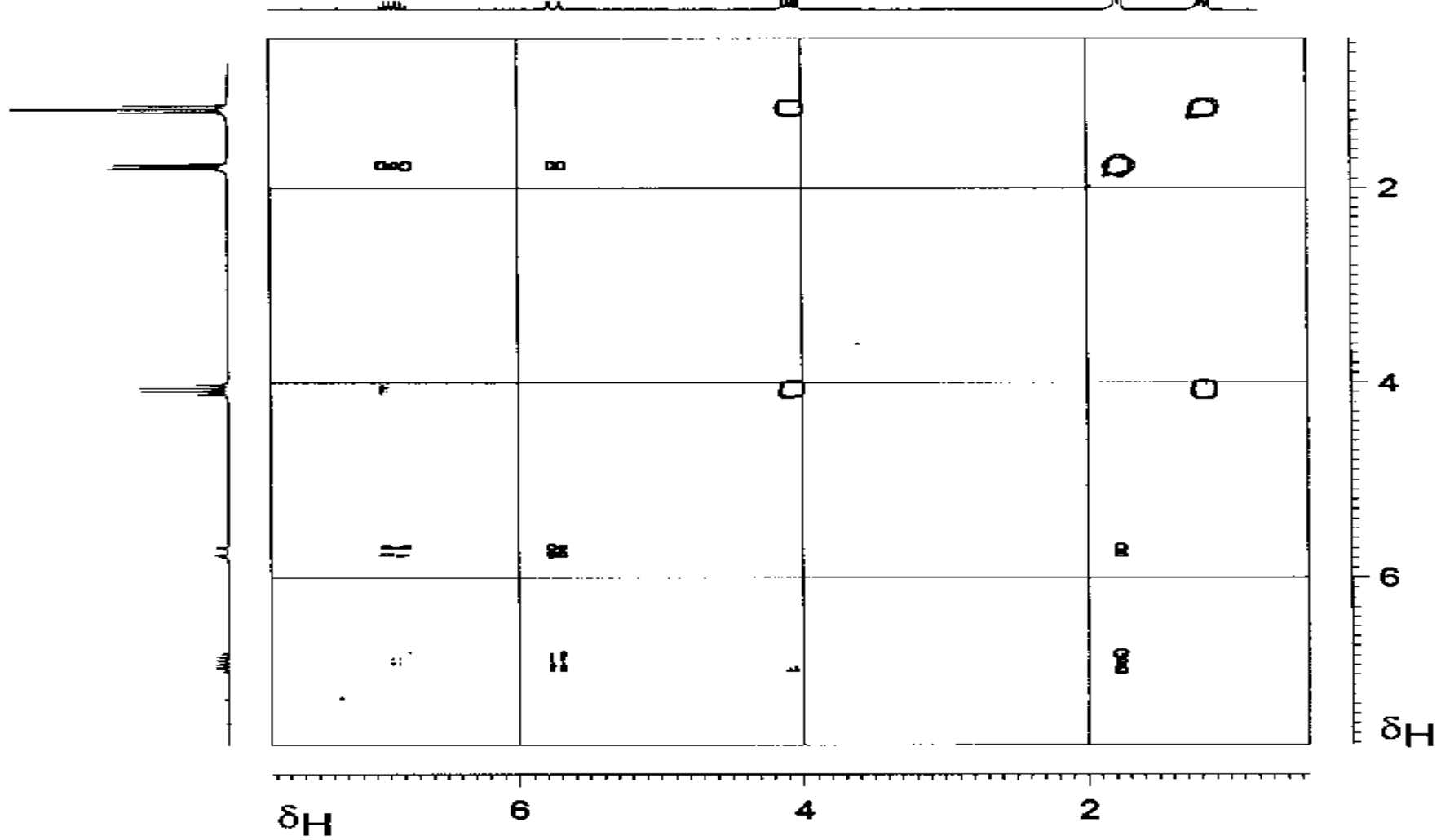
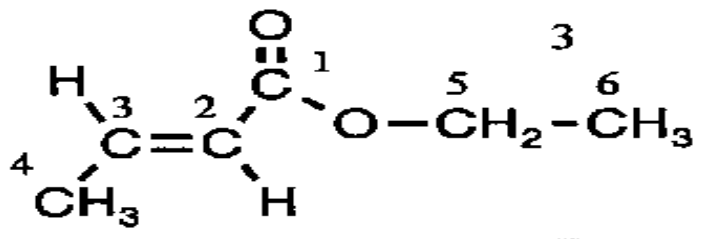
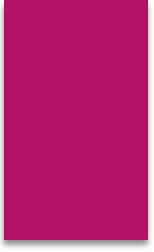


# COSY spectrum of Ethyl Vinyl Ether

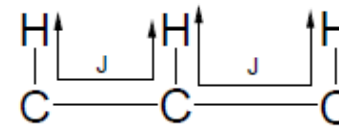
To analyze the spectrum, a diagonal line is drawn through the dots that bisect the spectrum. The dots that are *not* on the diagonal (A, B, C) are called *cross peaks*. Cross peaks indicate pairs of protons that are coupled.

For example, if we start at the cross peak labeled A and draw a straight line parallel to the y-axis back to the diagonal, we hit the dot on the diagonal at  $\sim 1.1$  ppm produced by the  $H_a$  protons. If we next go back to A and draw a straight line parallel to the x-axis back to the diagonal, we hit the dot on the diagonal at  $\sim 3.8$  ppm produced by the  $H_b$  protons. This means that the  $H_a$  and  $H_b$  protons are coupled.

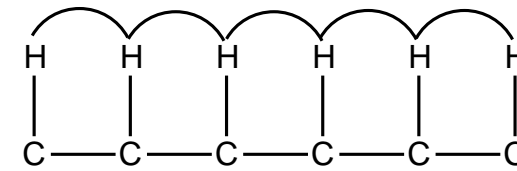
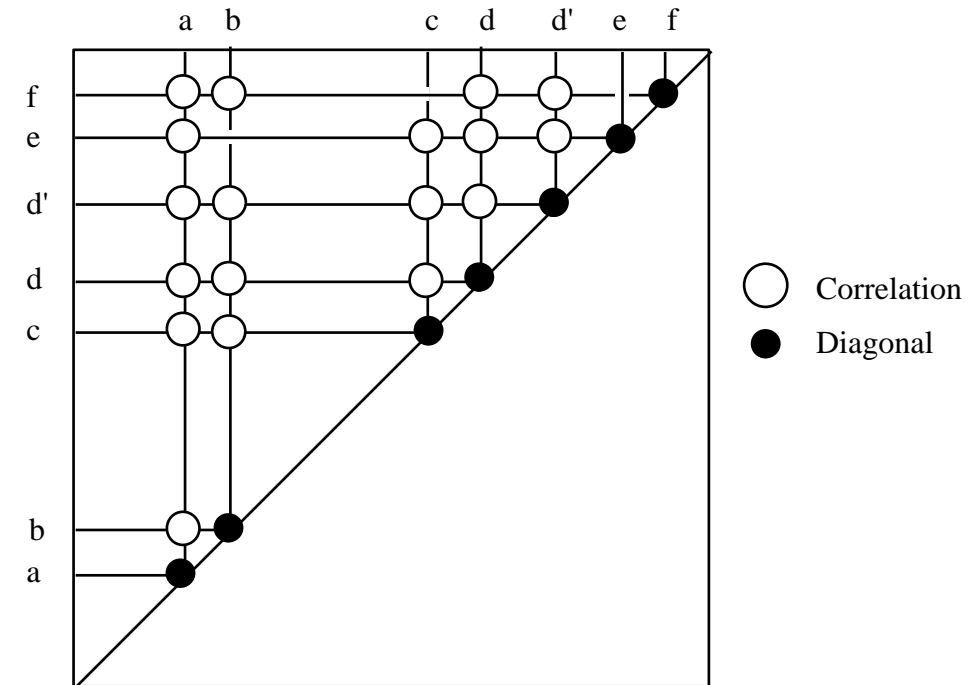
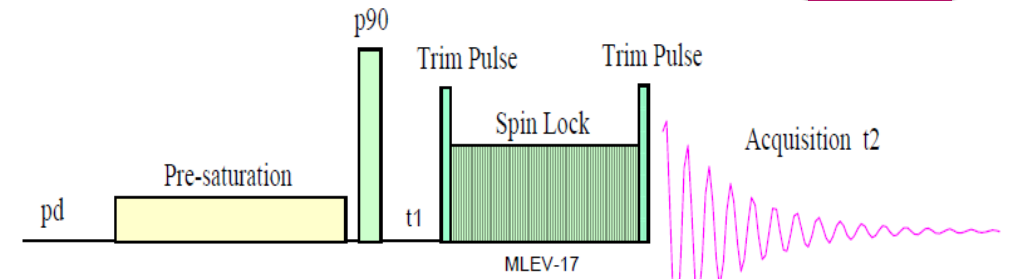




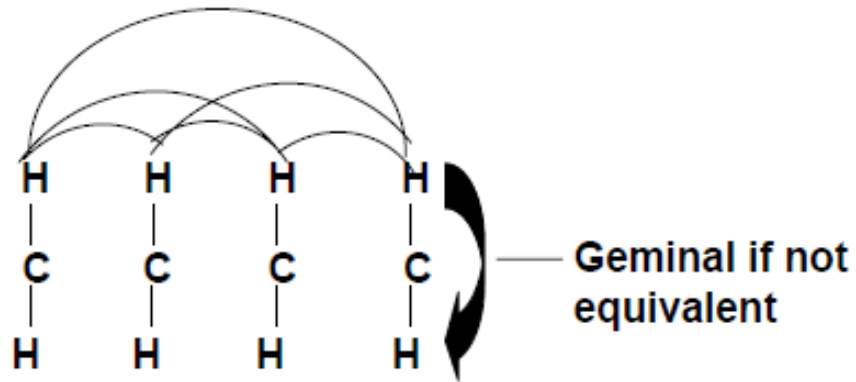
# Total Correlation Spectroscopy (TOCSY) Homonuclear Hartman-Hahn spectroscopy (HOHAHA)



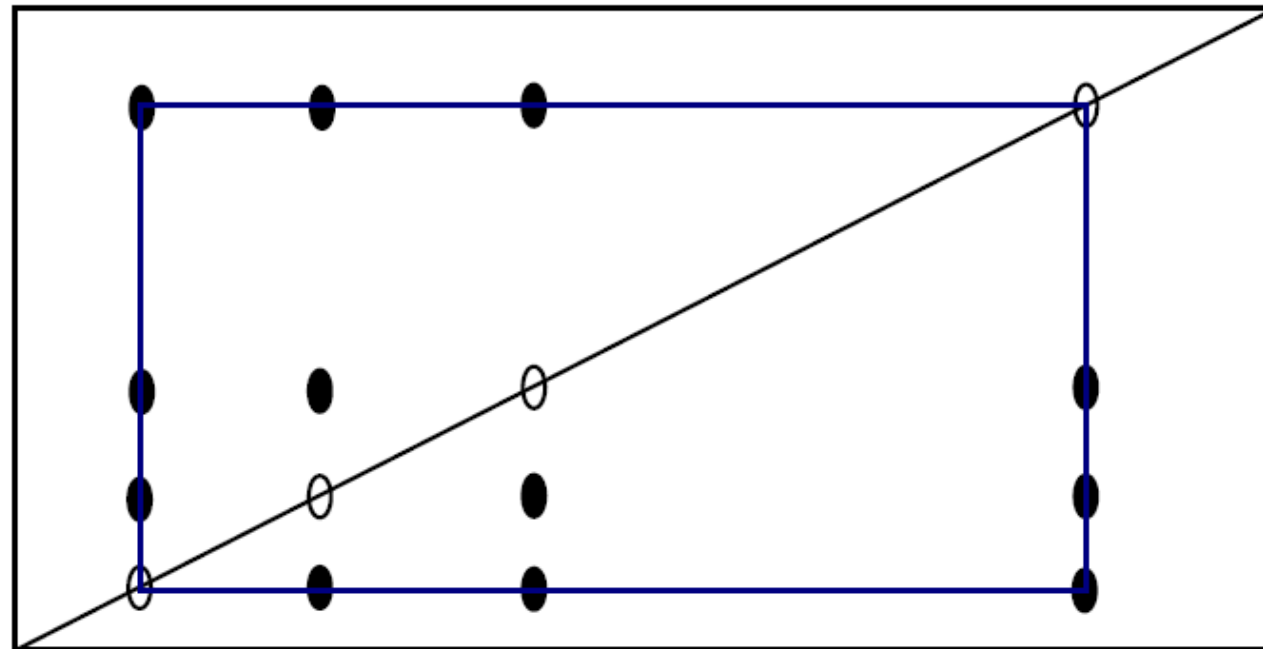
- ❖ Like COSY in appearance
- ❖ Relies on relayed coherence during spin-lock mixing time (magnetization exchange through scalar coupling)
- ❖ The longer  $t_{mix}$ , the longer the correlations (30 – 180 ms gives 3 - 7 bonds)
- ❖ Relays can occur only across protonated carbons – not across quaternary carbons (spin systems)
- ❖ Very useful for systems containing discrete units eg proteins and polysaccharides



# Total Correlation Spectroscopy (TOCSY)



In general, the TOCSY mixing time determines the number of bonds over which signal can be transferred, assuming that none of the coupling constants = 0

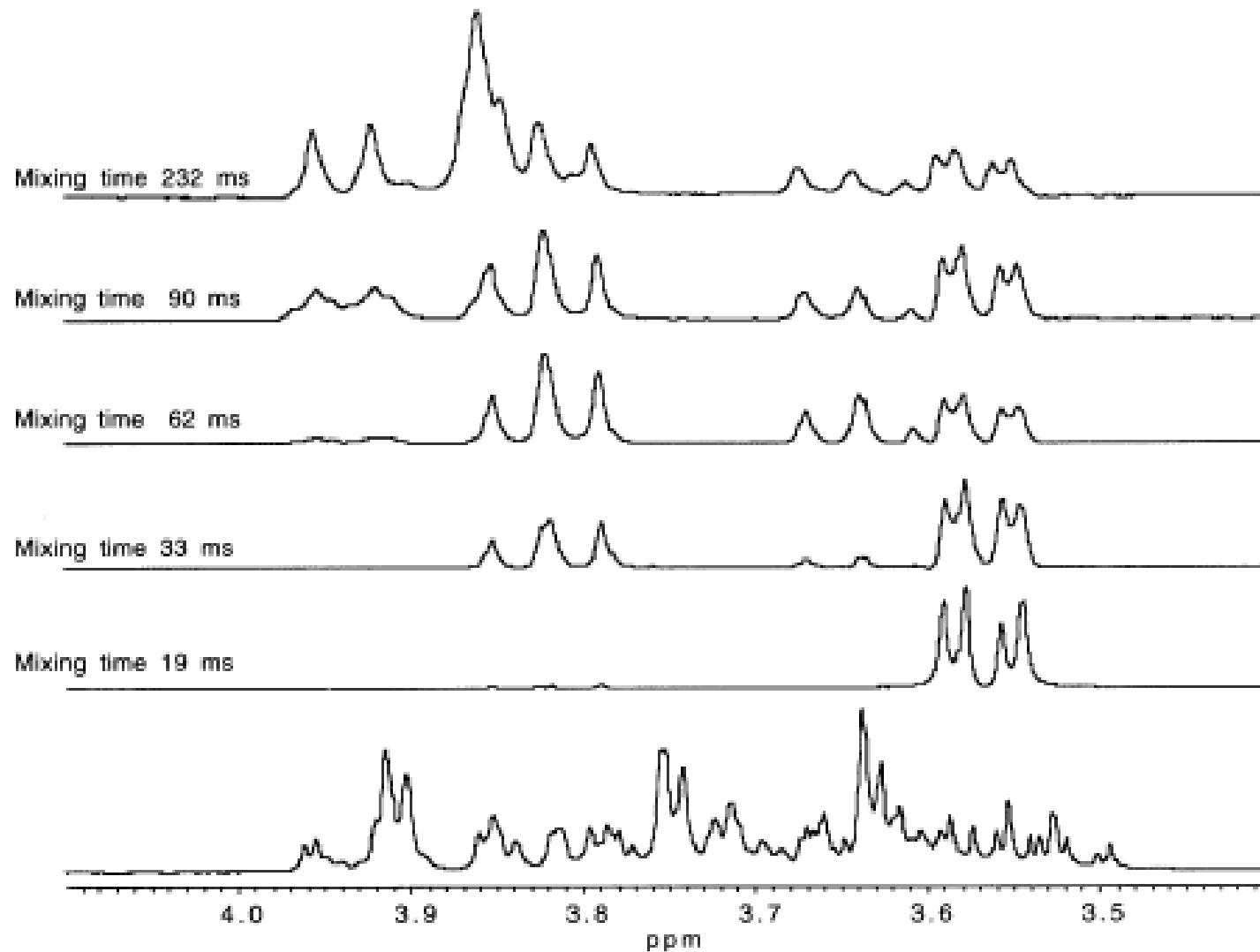




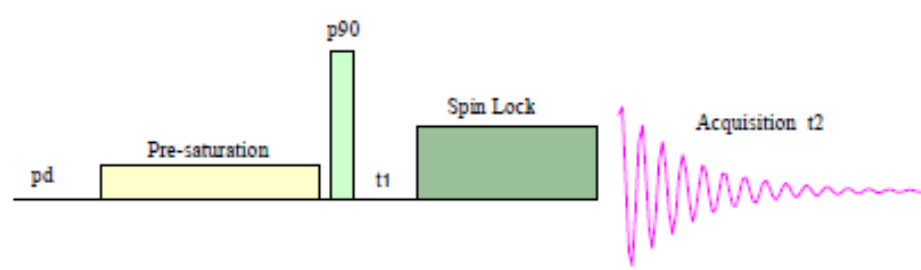
# TOtal Correlation SpectroscopY (TOCSY)

- ❖ Powerful variant of the COSY experiment
- ❖ Transfers magnetization throughout a spin system, provided that no coupling = 0
- ❖ Length of the mixing time determines how far the magnetization is transferred (i.e. how many bonds)
- ❖ Longer mixing = greater transfer, but < signal
- ❖ Typical mixing times are 30-200 msec
- ❖ Magnitude of mixing time related to  $1/2J$  for smallest coupling

# Total Correlation Spectroscopy (TOCSY)

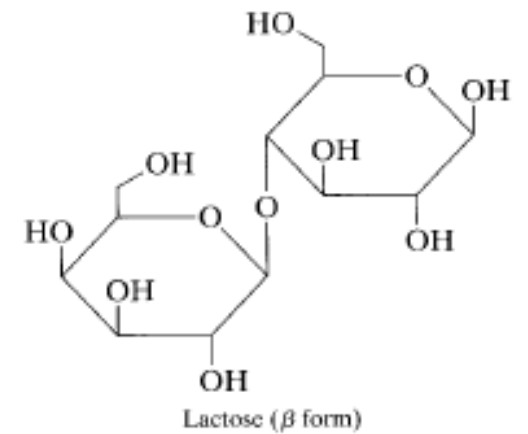
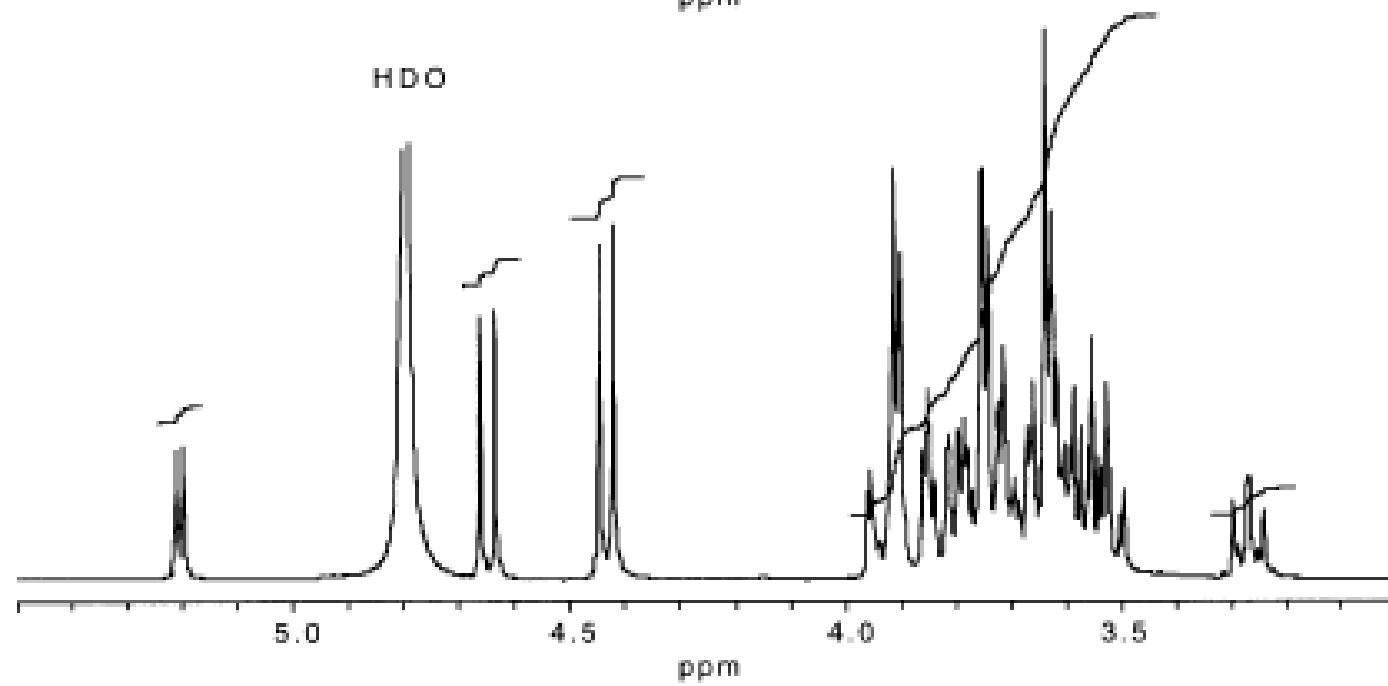
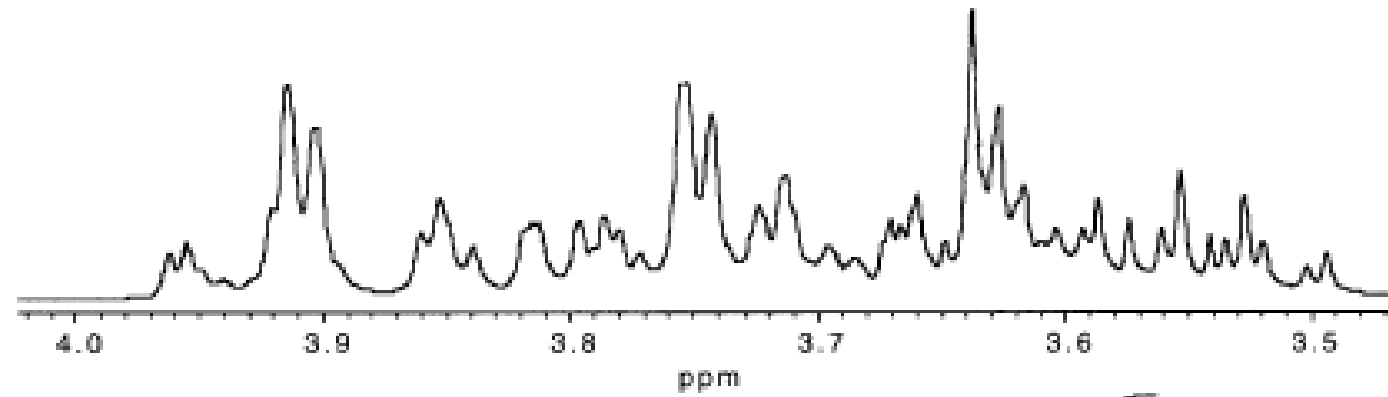


## Total Correlation Spectroscopy (TOCSY)

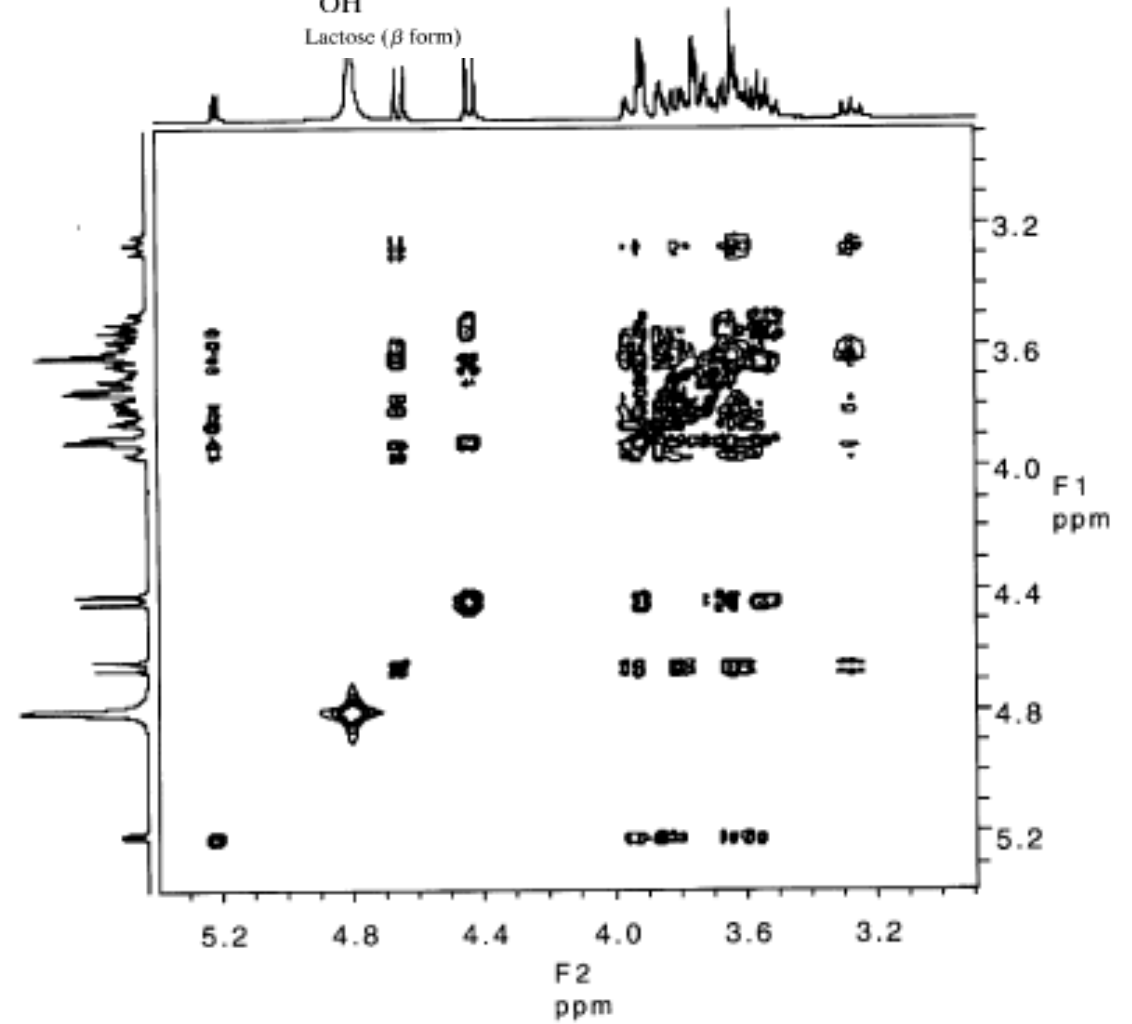
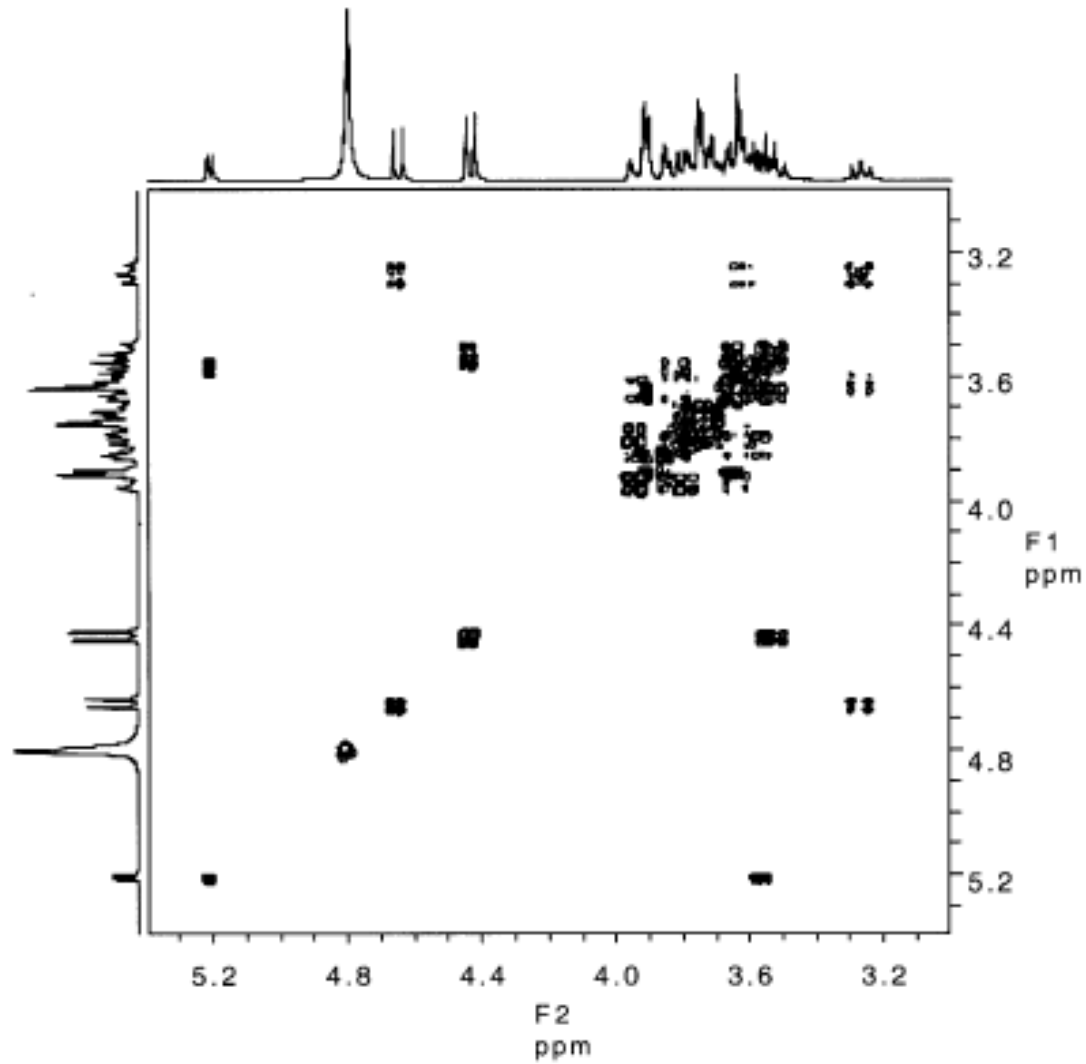
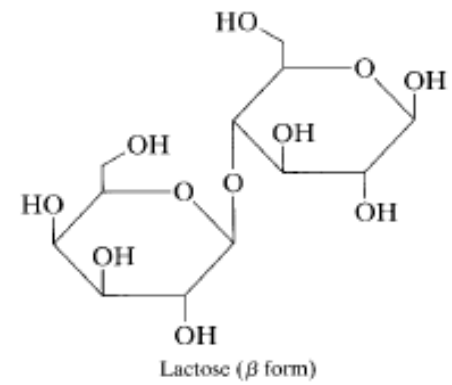


- ❖ Total Correlation Spectroscopy (TOCSY) is one of the principal experiments used in establishing connectivity between nuclei of scalar coupled spin systems.
- ❖ The experiment is designed to eliminate Zeeman contributions from the interactions of isotropically coupled spin systems.
- ❖ The method is based on the homonuclear cross polarization technique, i.e. by using an MLEV-17 pulse sequence to spin lock the magnetization to obtain polarization transfer.
- ❖ Under this condition, coherence migrates in an oscillatory manner throughout the entire spin system. This technique is therefore mostly used for peptides or oligosaccharides, since it could identify a single residue.
- ❖ For complex molecules, the resulting TOCSY spectrum provides a reliable identification of spin systems that cannot be resolved by other methods.
- ❖ A major advantage of TOCSY is that net magnetization transfer occurs and a phase sensitive 2D spectrum with all peaks in the absorption mode can be obtained.

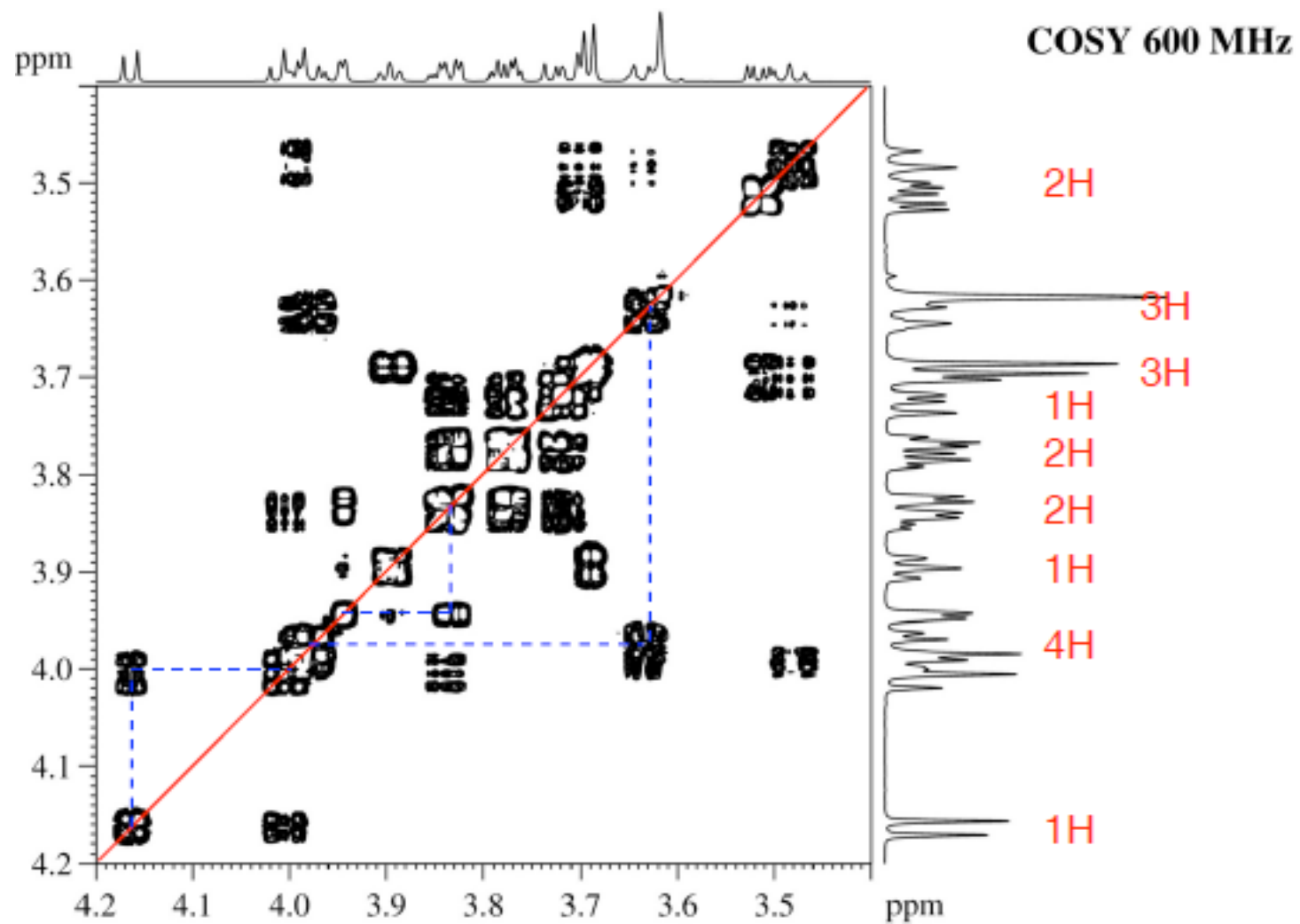
# Total Correlation Spectroscopy (TOCSY)



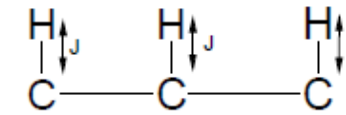
# Total Correlation Spectroscopy (TOCSY)



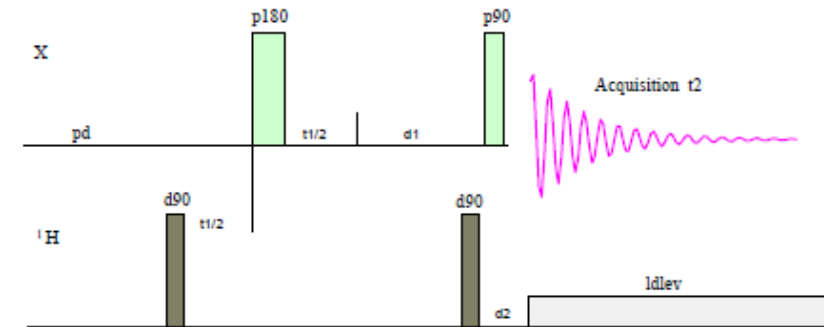
# Total Correlation Spectroscopy (TOCSY)



# Heteronuclear Correlation Spectroscopy (HETCOR)

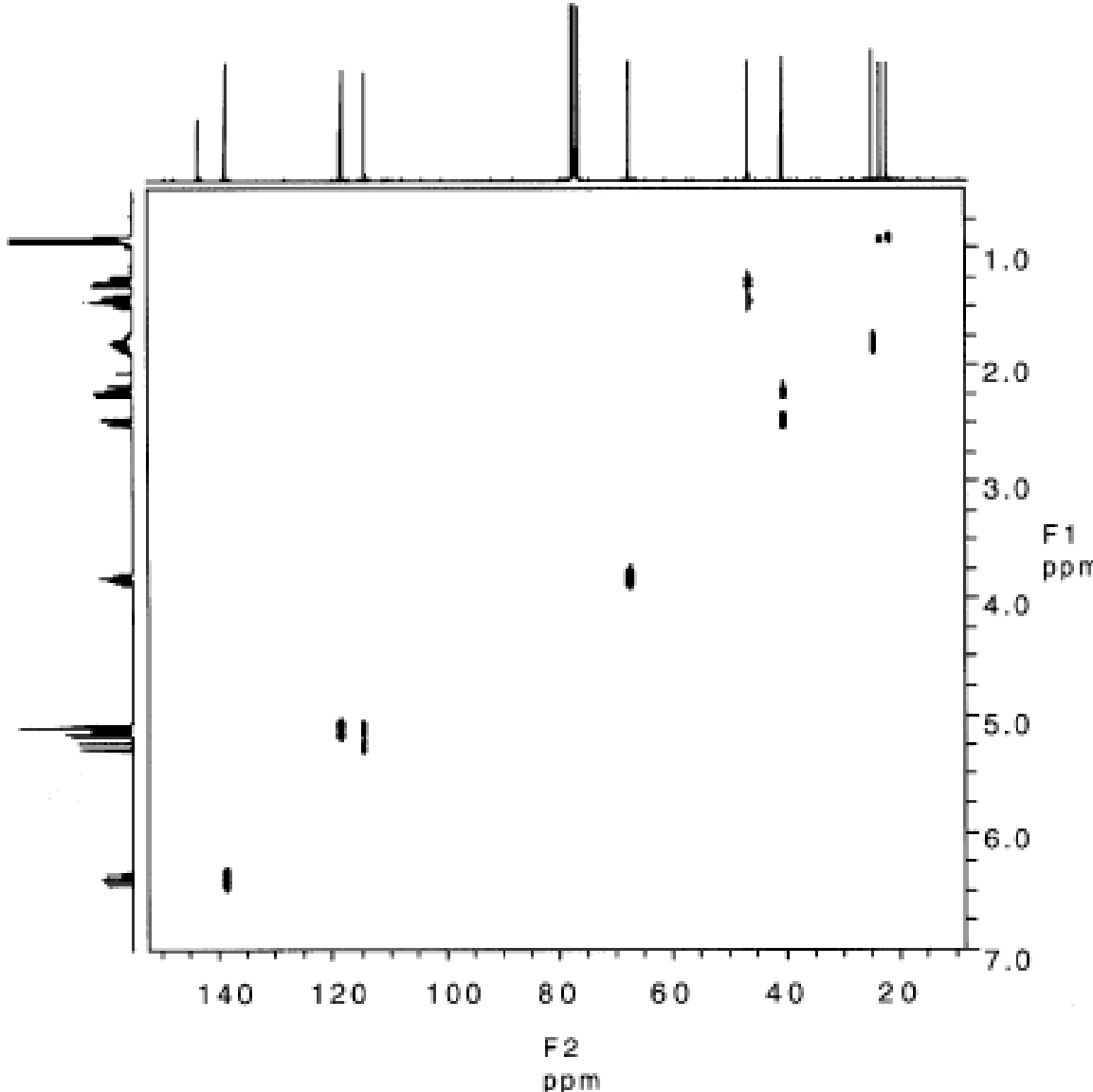


- ❖ The principles of HETCOR are precisely analogous to COSY. A different experimental regime however is required since two observing nuclei with different Larmor frequencies are involved. That is why this technique is referred to H, X-COSY, where X could be  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{31}\text{P}$ ,  $^{29}\text{Si}$  etc.
- ❖ The experiment is used to correlate the chemical shifts of X-nuclei with the chemical shifts of protons coupled with the X-nuclei.
- ❖ The assignment of one member of a spin-coupled pair leads immediately to the assignment of the other.
- ❖ Most NMR instruments with two channels can perform the experiment. The 90 degree pulses for X nucleus and proton need to be calibrated.



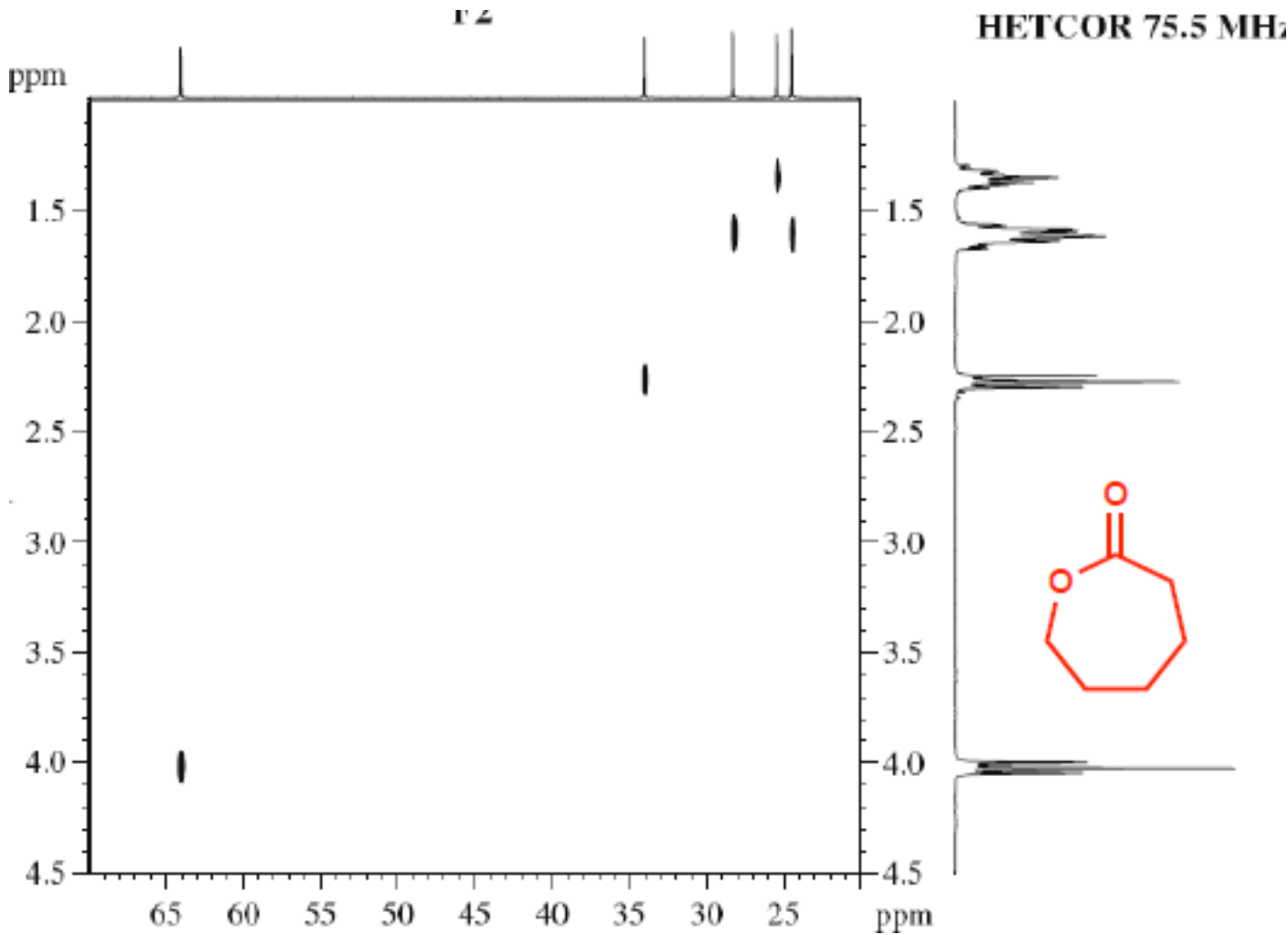
# Heteronuclear Correlation Spectroscopy (HETCOR)

IPSENOL





# Heteronuclear Correlation Spectroscopy (HETCOR)



# DEPT $^{13}\text{C}$ NMR Spectroscopy

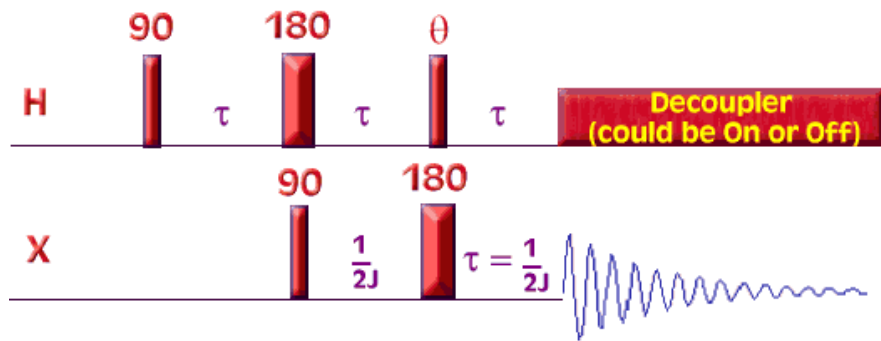
## *Distortionless Enhancement by Polarization Transfer (DEPT-NMR) experiment*

- Run in three stages
  1. Ordinary *broadband-decoupled spectrum*
    - Locates chemical shifts of all carbons
  2. DEPT-90
    - Only signals due to CH carbons appear
  3. DEPT-135
    - $\text{CH}_3$  and CH resonances appear positive
    - $\text{CH}_2$  signals appear as *negative* signals (below the baseline)
- Used to determine number of hydrogens attached to each carbon

# DEPT $^{13}\text{C}$ NMR Spectroscopy

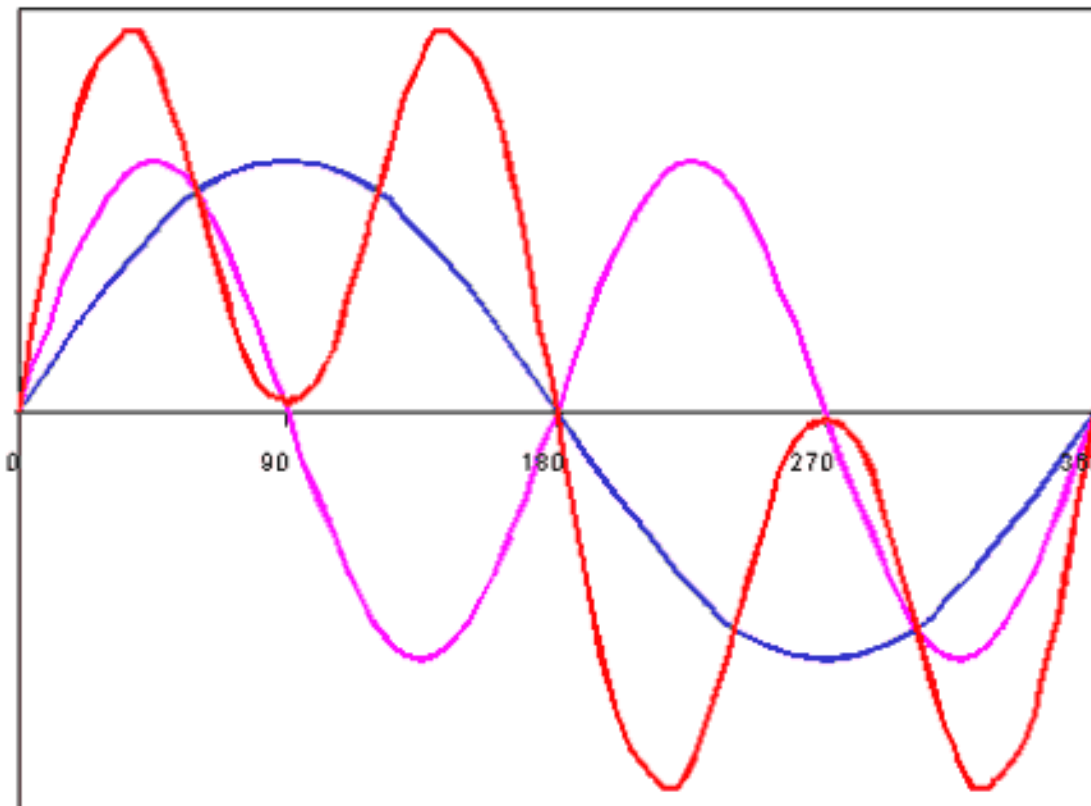
- ❖ For reasons of sensitivity carbon spectra are usually recorded in the fully proton decoupled mode. The disadvantage is that information about the number of attached protons is lost.
- ❖ DEPT spectra are a very valuable source of such information, which is very useful for signal assignment. In addition, the polarization transfer in DEPT spectra leads to considerably higher signal intensities.
- ❖ In the DEPT experiment the proton polarization (the population difference between  $\alpha$  and  $\beta$  levels) is transferred via the large  $^1J_{\text{C,H}}$ -couplings onto the carbons. In addition, DEPT experiments allow us to edit signal phases according to the number of directly attached protons.
- ❖ In the DEPT-135 experiments, methyl and methine signals have positive and methylene protons negative phases (or vice versa), whereas the quaternary carbons are completely missing.

# DEPT $^{13}\text{C}$ NMR Spectroscopy



$^{13}\text{C}$  spectra is perturbed based  
On the number of attached  $^1\text{H}$

Takes advantage of different  
patterns of polarization transfer  
 $^1\text{H}$ - $^{13}\text{C}$  NOE



- CH  $\Rightarrow \sin(\theta)$
- CH<sub>2</sub>  $\Rightarrow 2 \cdot \sin(\theta) \cdot \cos(\theta)$
- CH<sub>3</sub>  $\Rightarrow 4 \cdot \sin(\theta) \cdot \cos^2(\theta)$

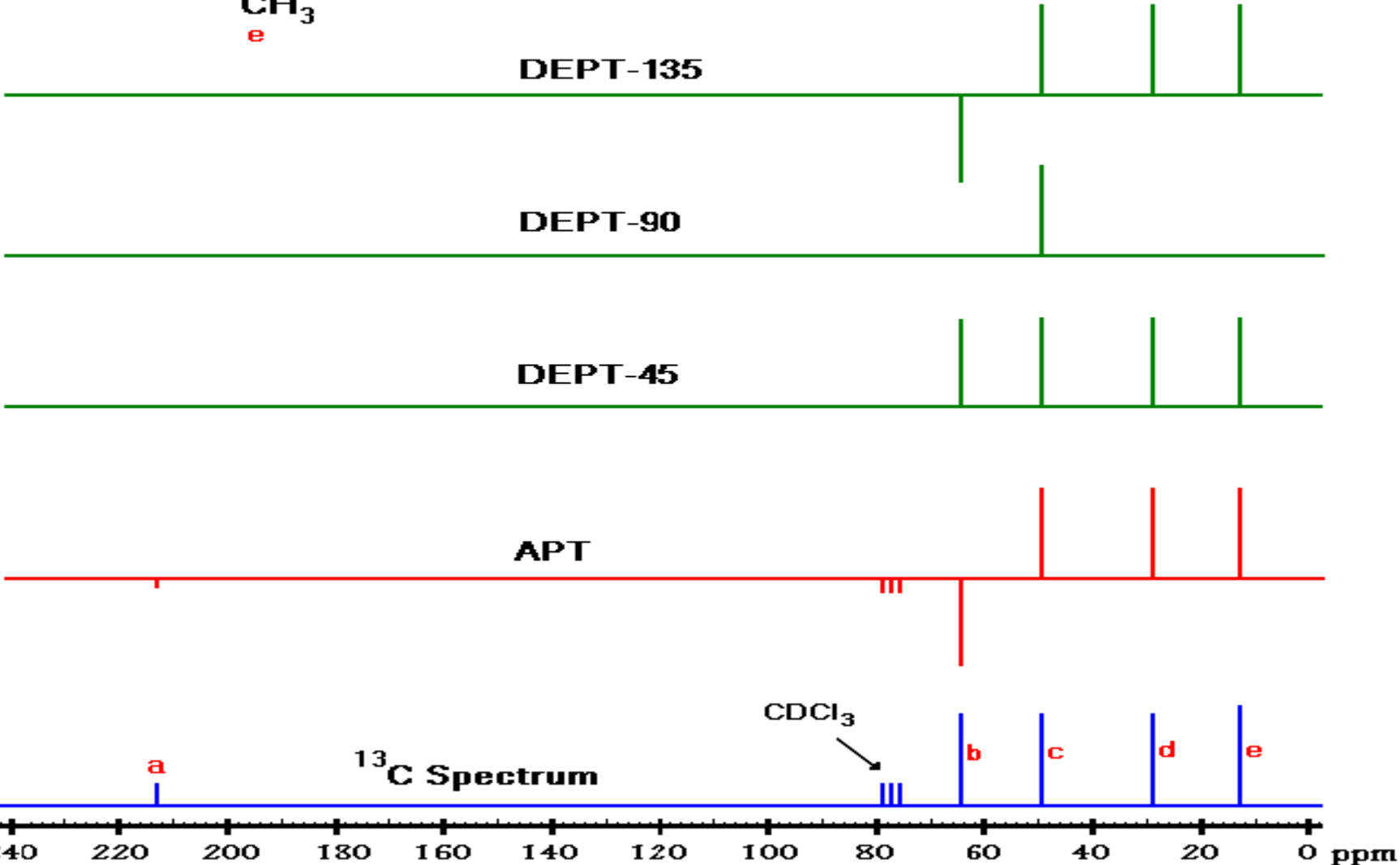
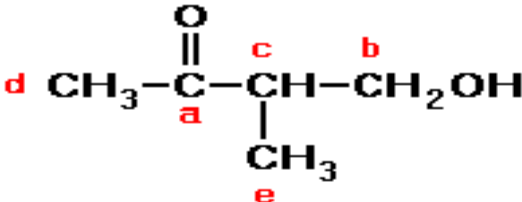
# DEPT $^{13}\text{C}$ NMR Spectroscopy

The DEPT spectrum is much more sensitive than a normal  $^{13}\text{C}$  spectrum because the observed carbon signal originates from an attached proton and is transferred to the carbon ("polarization transfer"). The down-side to this is that carbons without attached protons (quaternary carbons) cannot be observed.

APT gives all of the information of a normal carbon spectrum with somewhat reduced sensitivity, and it tells you if the number of attached protons is odd ( $\text{CH}_3$  or  $\text{CH}$ ) or even ( $\text{CH}_2$  or quaternary). DEPT is much more sensitive than a normal carbon spectrum, and it can unambiguously identify the  $\text{CH}_3$ ,  $\text{CH}_2$  and  $\text{CH}$  carbon peaks. This requires acquiring and processing three separate spectra, however, and does not detect the quaternary carbons or solvent at all.

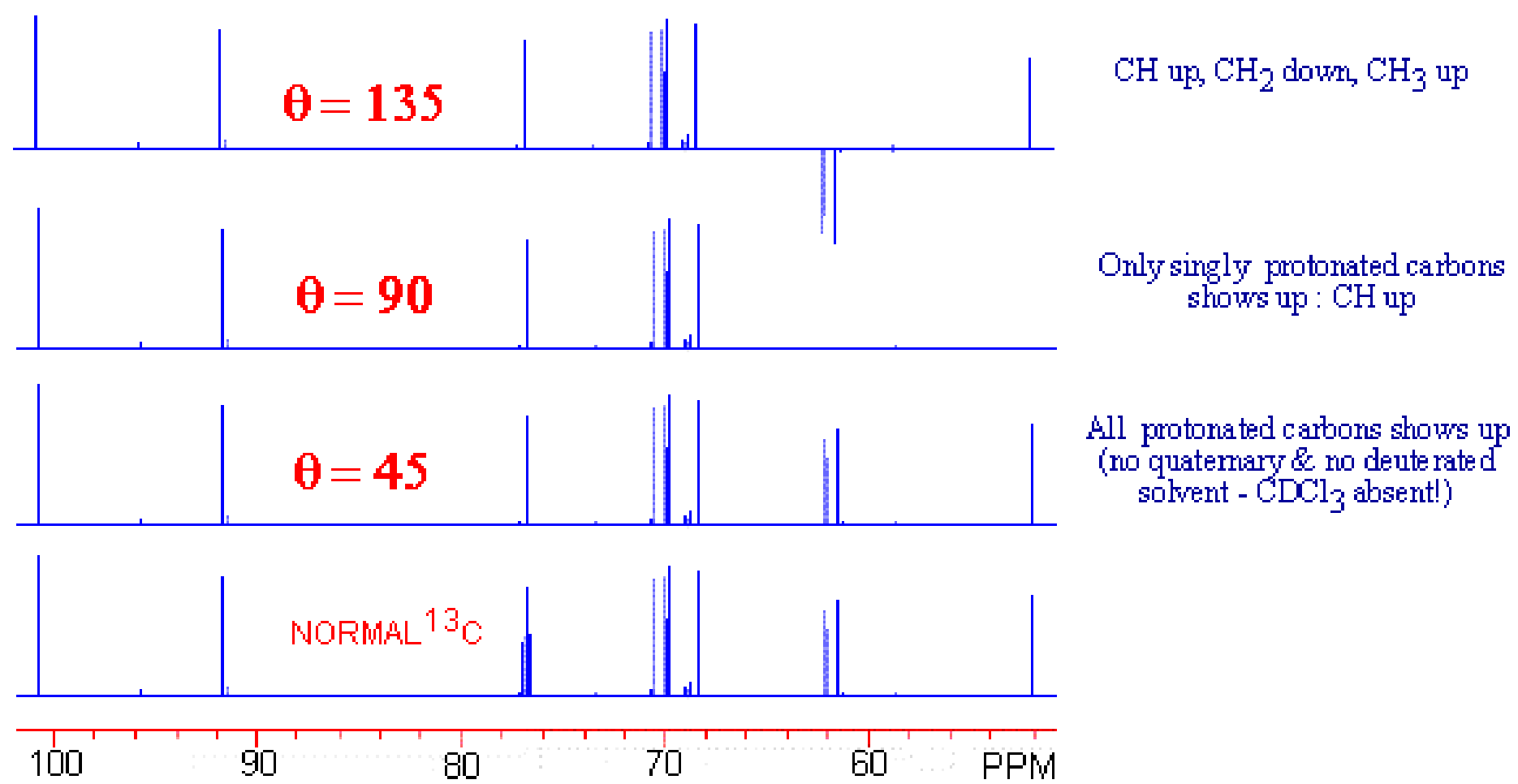
# DEPT <sup>13</sup>C NMR Spectroscopy

4-Hydroxy-3-Methyl-2-Butanone

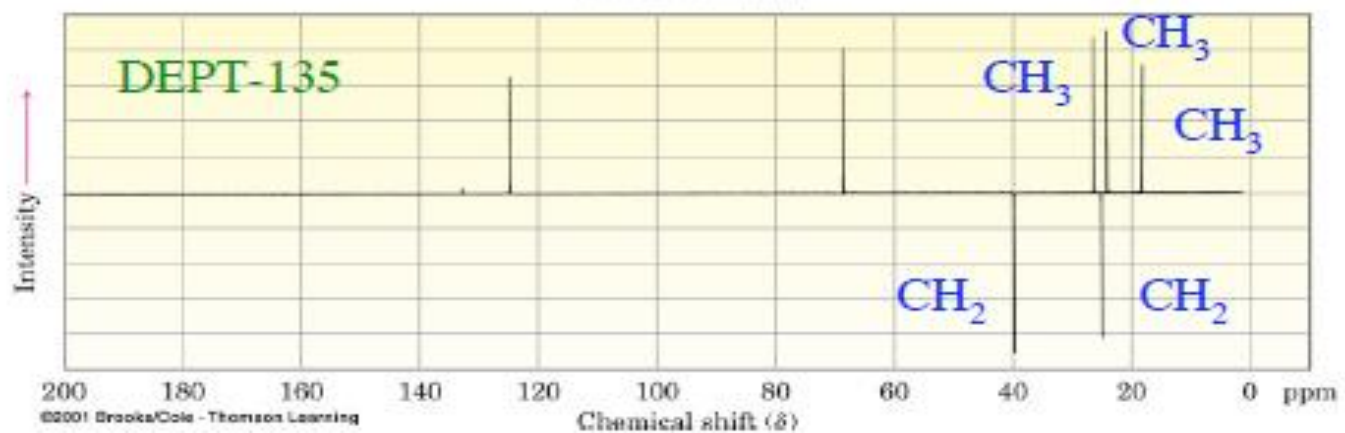
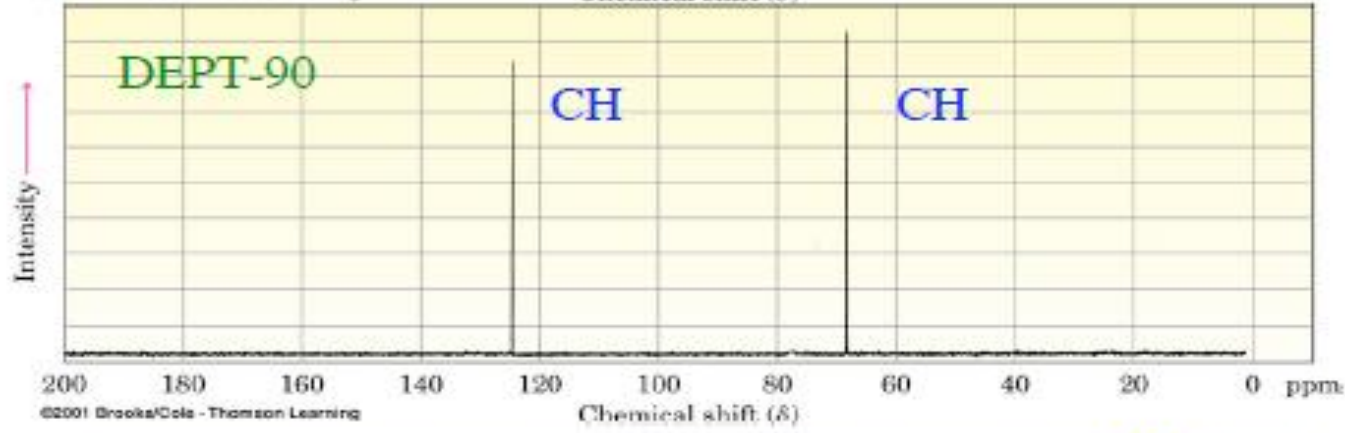
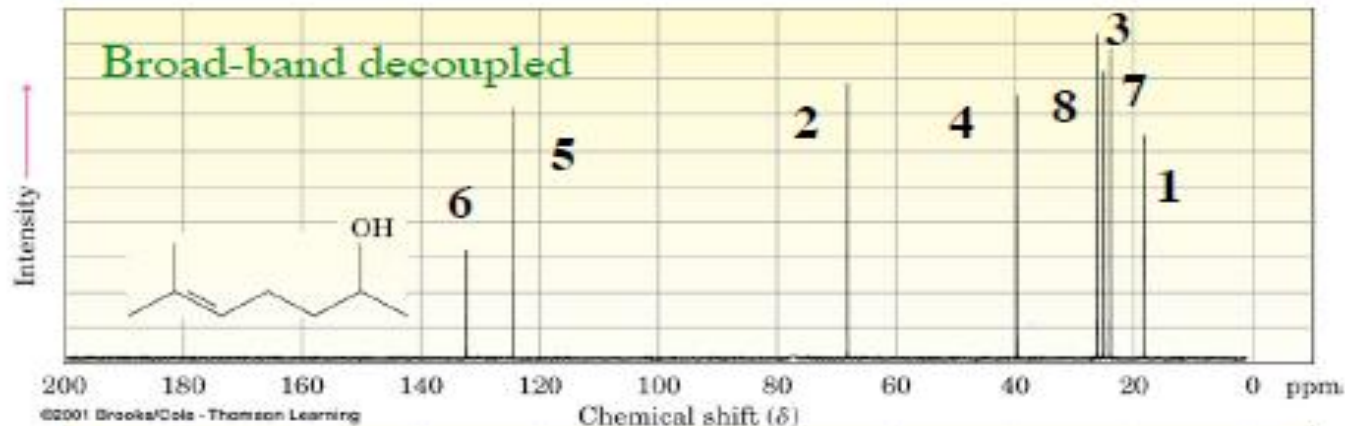
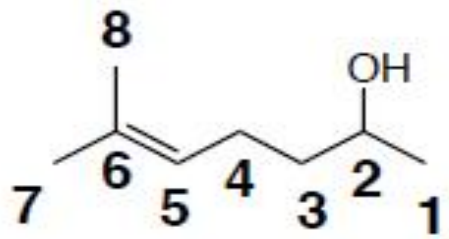


# DEPT $^{13}\text{C}$ NMR Spectroscopy

## DEPT experiment



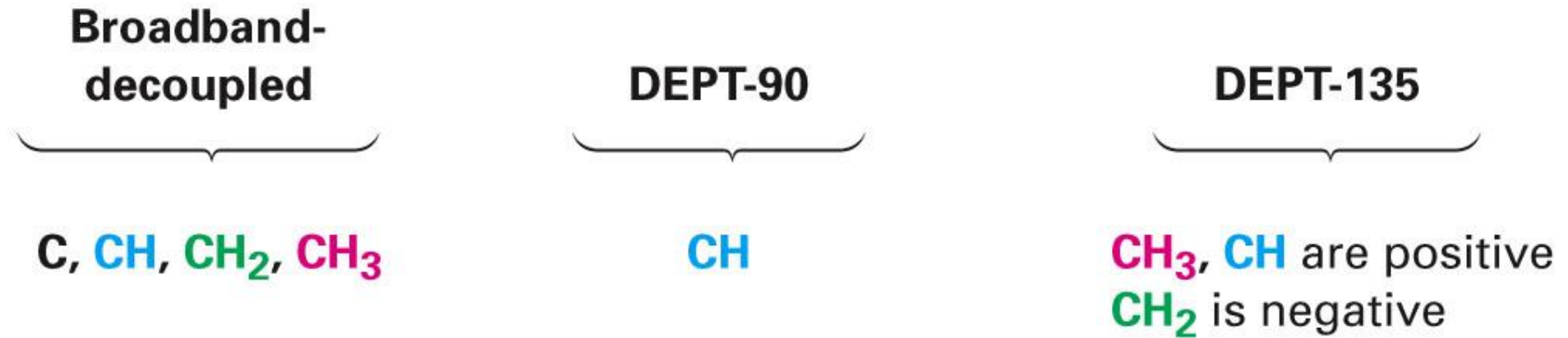
# DEPT <sup>13</sup>C NMR Spectroscopy





# DEPT $^{13}\text{C}$ NMR Spectroscopy

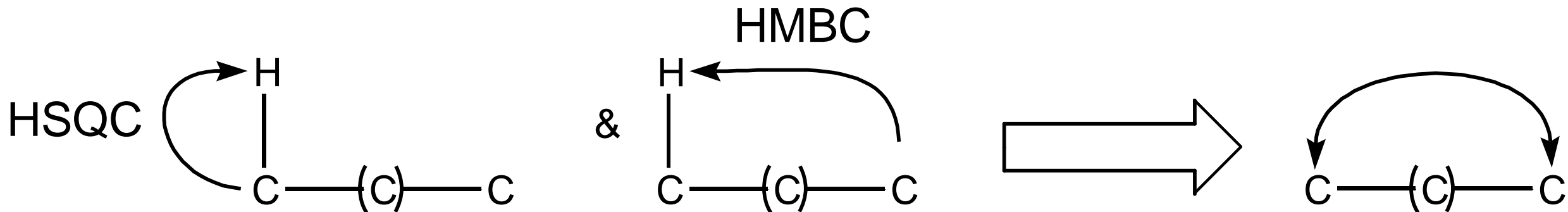
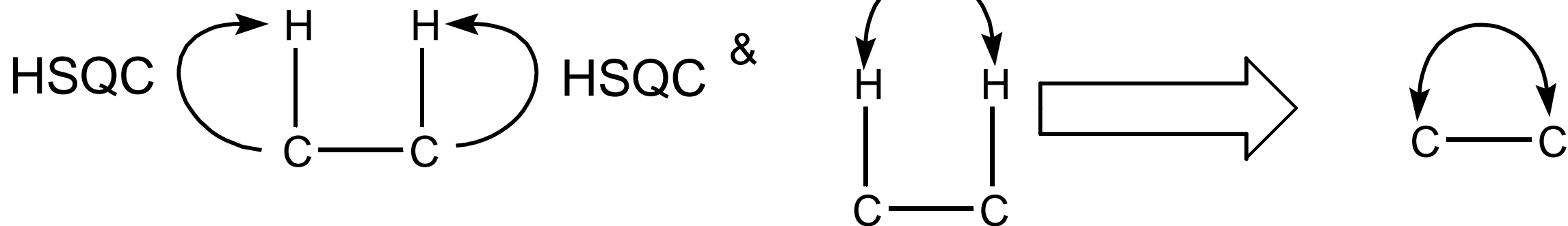
Summary of signals in the three stage DEPT experiment



- C** Subtract DEPT-135 from broadband-decoupled spectrum
- CH** DEPT-90
- CH<sub>2</sub>** Negative DEPT-135
- CH<sub>3</sub>** Subtract DEPT-90 from positive DEPT-135

# STRATEGY BASED ON C-H CONNECTIVITY

$^1\text{H}$ - $^1\text{H}$  COSY



HMBC data is ambiguous  
(2 or 3 bond correlations – impossible to tell which)