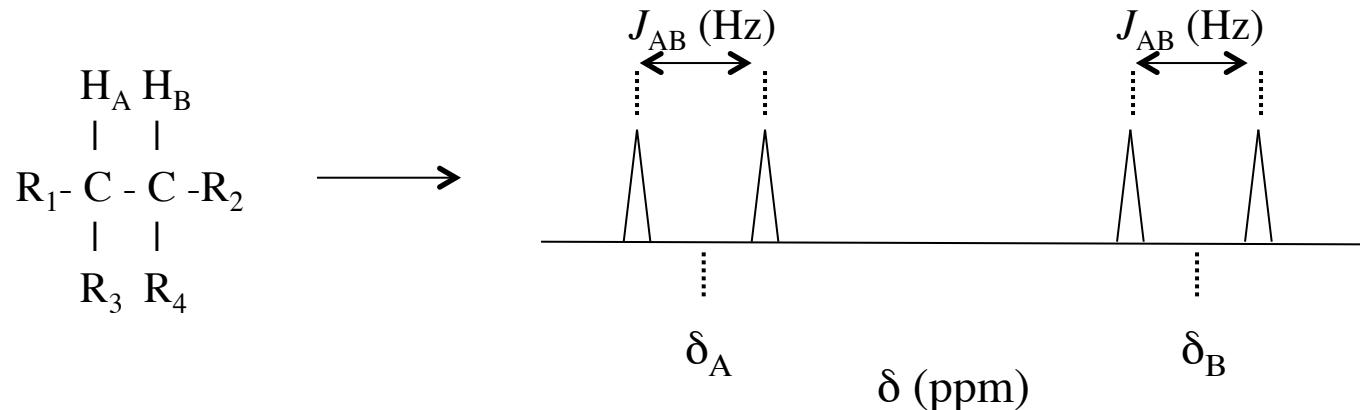


# NMR Spectroscopy:

## 3

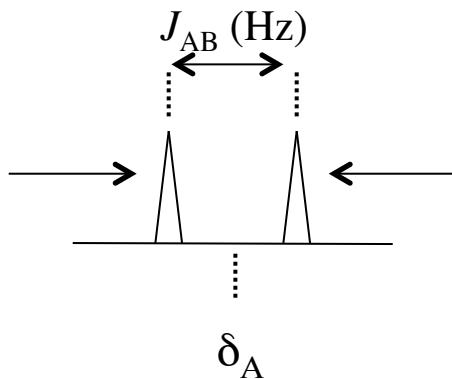
# Through-Bond Coupling

- Frequency-domain NMR signals are often split into two or more component peaks due to the effect of directly bonded nuclei or neighboring nuclei 2 or 3 bonds away



- This is due to *through-bond coupling*, also known as *scalar coupling*, *indirect coupling*, *spin-spin coupling*, or *J coupling*.....
- In the example above, if we consider spin A (for instance) in an ensemble of molecules, in some of the molecules spin B will be in the  $\alpha$  ( $m = +1/2$ ) state, and in others spin B will be in the  $\beta$  ( $m = -1/2$ ) state

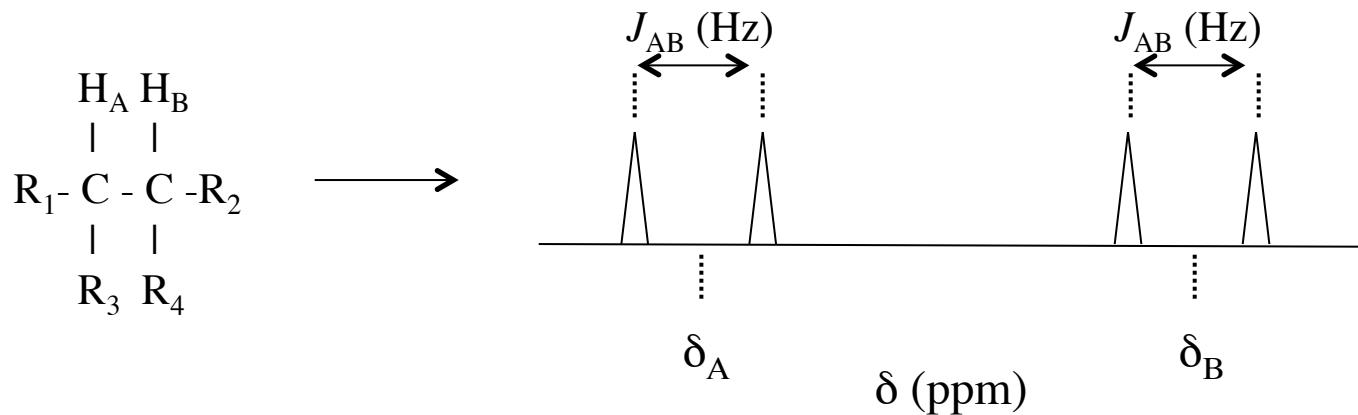
for spin B,  $m = +1/2$ , which augments  $B_0$ , and *deshields* spin A



for spin B,  $m = -1/2$ , which opposes  $B_0$ , and *shields* spin A

# Through-Bond Coupling

- The splitting between the components of the signal is called the *coupling constant*, or  $J$



- $J$  is usually given a superscript and subscript, the former describing the number of bonds separating the coupled nuclei, the latter designating the two atoms involved  
-for the example above,  $^3J_{AB}$  or  $^3J_{H_A H_B}$
- $J$  is *always* measured in Hz
- $J$  (in Hz) is *independent* of the magnitude of  $B_0$
- The value of  $J$  measured from the splitting of the signal from one of the coupled nuclei is always the same as the value measured at the signal from the other coupled nucleus
- The chemical shift of the nucleus is the center of the multiplet

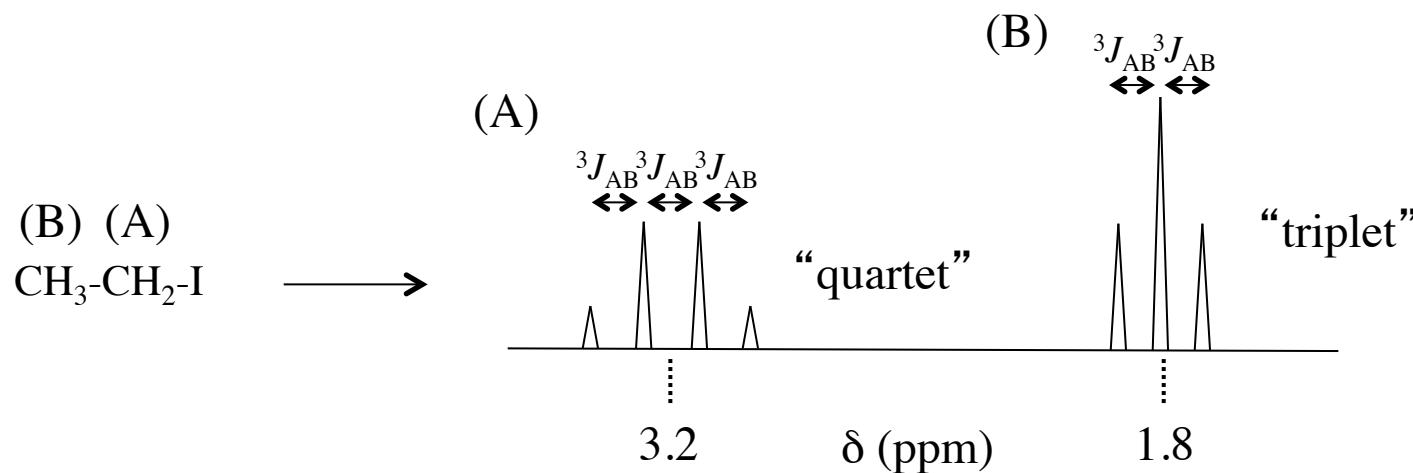
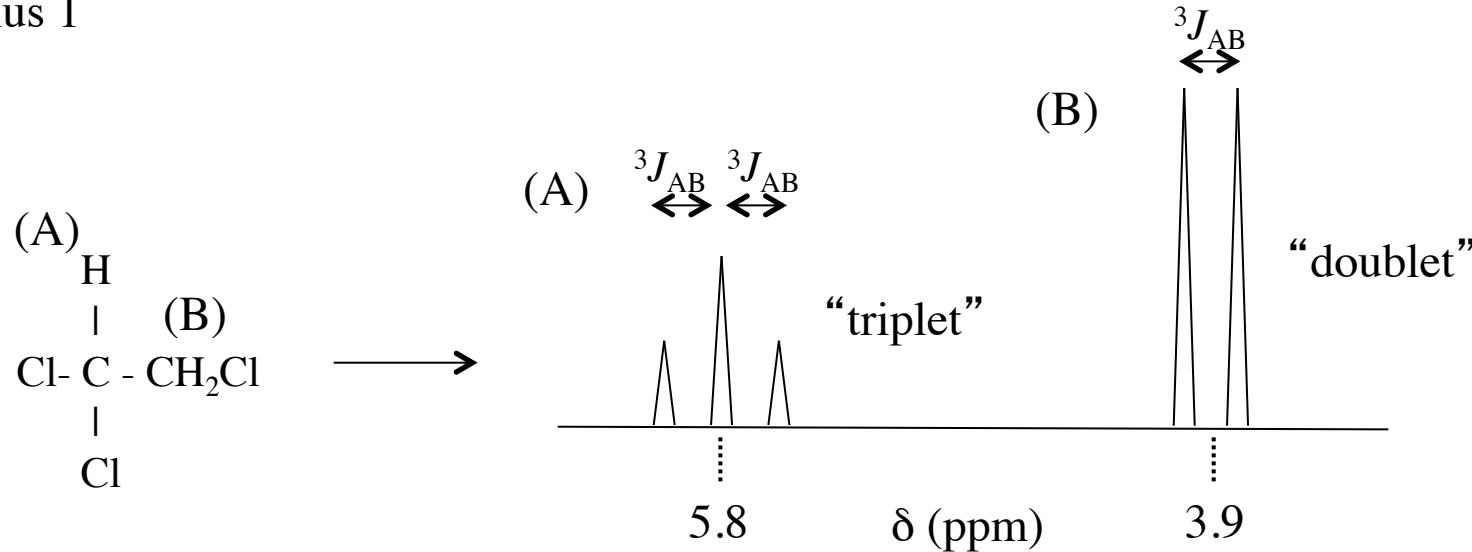
# Mechanism of Through-Bond Coupling

- Fermi contact: interaction of nuclear spins via bonding electrons
  - depends on electron density at the pair of nuclei
  - depends on the “*s*” electrons, or *s* character of the bond(s) between the nuclei
  - electron spins must be correlated, i.e. there must be a bond
- Dependence on “*s*” character
  - consider one bond  $^{13}\text{C}$ - $^1\text{H}$  couplings:

$\text{CH}_3\text{-CH}_3$	$s, sp^3$	$1 \times 1/4 = 1/4$	125 Hz
$\text{CH}_2=\text{CH}_2$	$s, sp^2$	$1 \times 1/3 = 1/3$	156 Hz
$\text{C}_6\text{H}_6$	$s, sp^2$	$1 \times 1/3 = 1/3$	158 Hz
$\text{HC}\equiv\text{CH}$	$s, sp$	$1 \times 1/2 = 1/2$	249 Hz

# Spin-Spin Splitting: $n+1$ Rule

- Multiplet splitting patterns are determined by the number of nuclei ( $n$ ) doing the splitting plus 1



# Spin-Spin Splitting: Intensities and Pascal's Triangle

- The relative intensities of the multiplet components in a signal split by spin-spin coupling can be obtained from Pascal's triangle

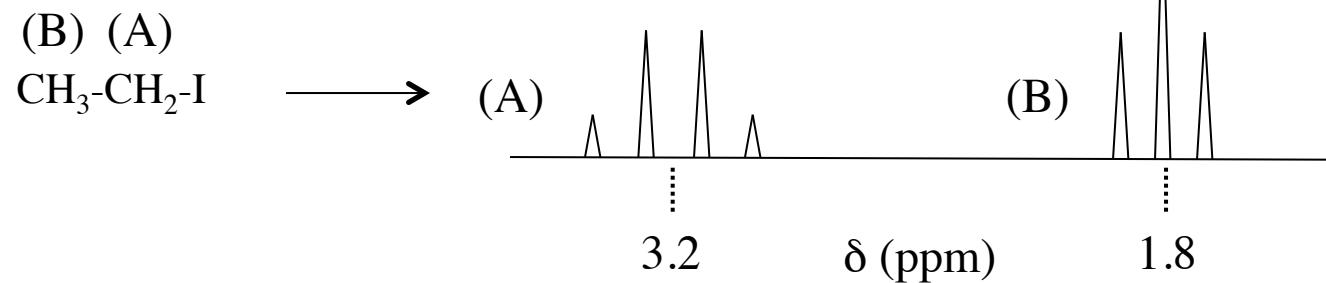
singlet				1
doublet			1	1
triplet		1	2	1
quartet		1	3	3
quintet	1	4	6	4
sextet	1	5	10	10
septet	1	6	15	20

relative intensities (in the spectrum) 1 3 3 1

relative intensities (in the multiplet) 1 3 3 1

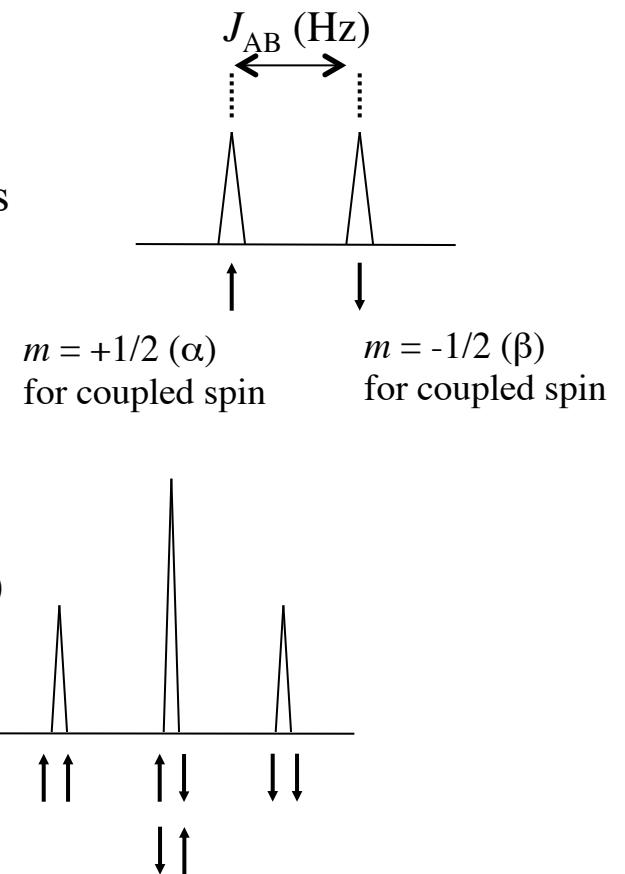
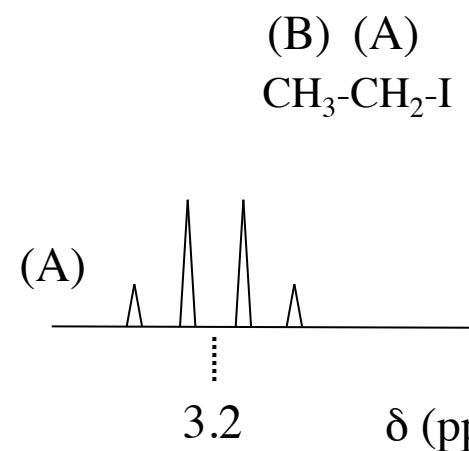
3 6 3

1 2 1



# Spin-Spin Splitting: Intensities and Pascal's Triangle

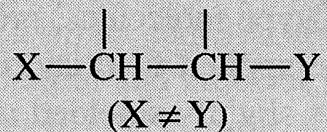
- For a doublet, we saw that there are two peaks, because the coupled spin can be in either the  $\alpha$  or  $\beta$  state
- The peak heights are equal because, for the coupled spin, there are essentially equal numbers of spins in  $\alpha$  and  $\beta$  states



- For a triplet, there are three peaks, because the two coupled spins can both be in the  $\alpha$  state, they can both be in the  $\beta$  state, or one can be  $\alpha$  and the other  $\beta$
- The relative peak heights are 1:2:1 because there are 2 ways that one spin can be  $\alpha$  and the other  $\beta$ , and only one way both can be  $\alpha$  and only one way both can be  $\beta$

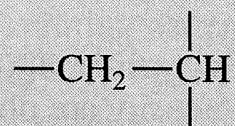
# Common Splitting Patterns

II



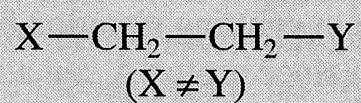
II

II



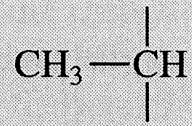
III

III



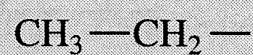
III

II



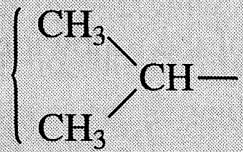
III

III



III

II

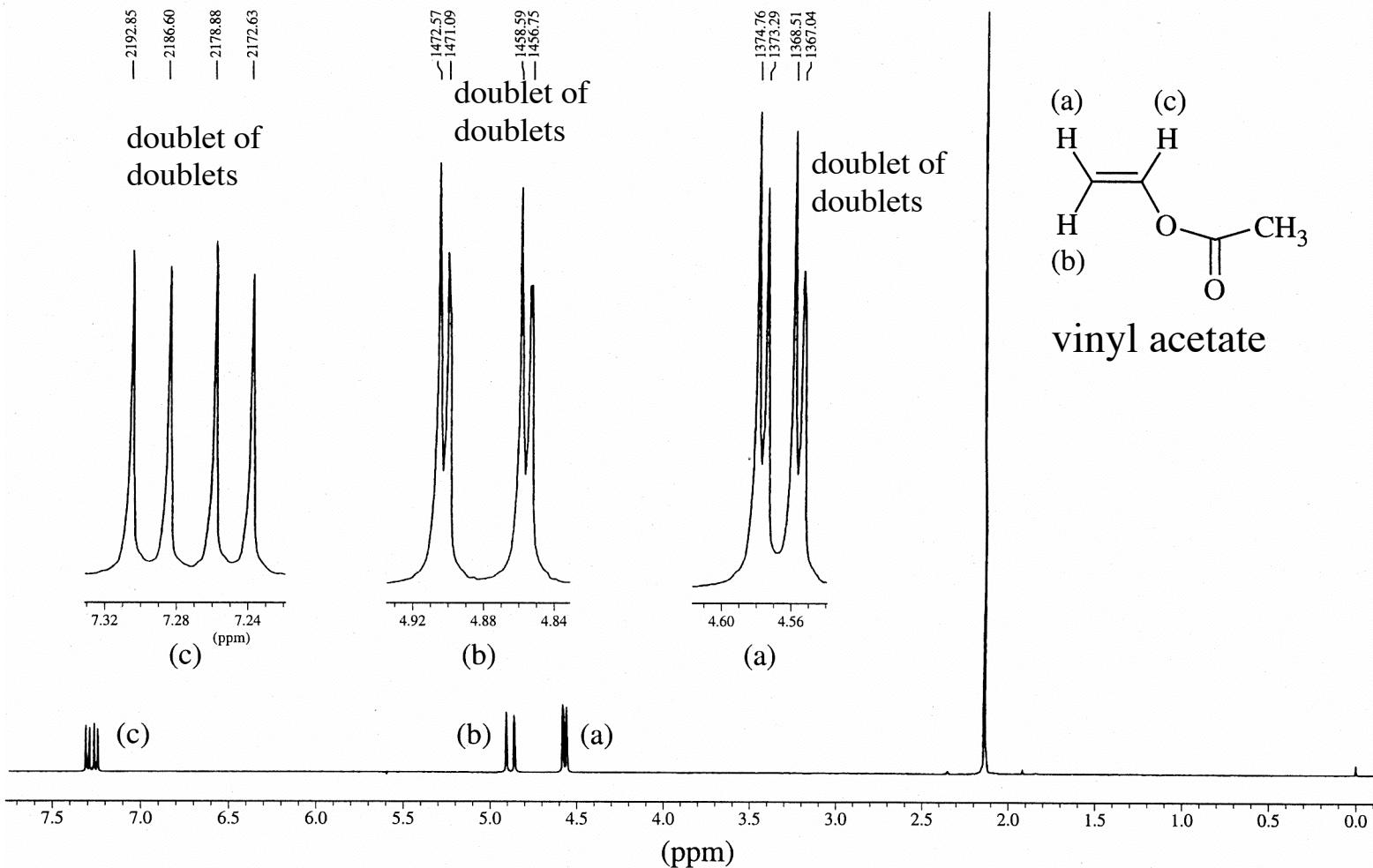


III

- Some commonly observed splitting patterns

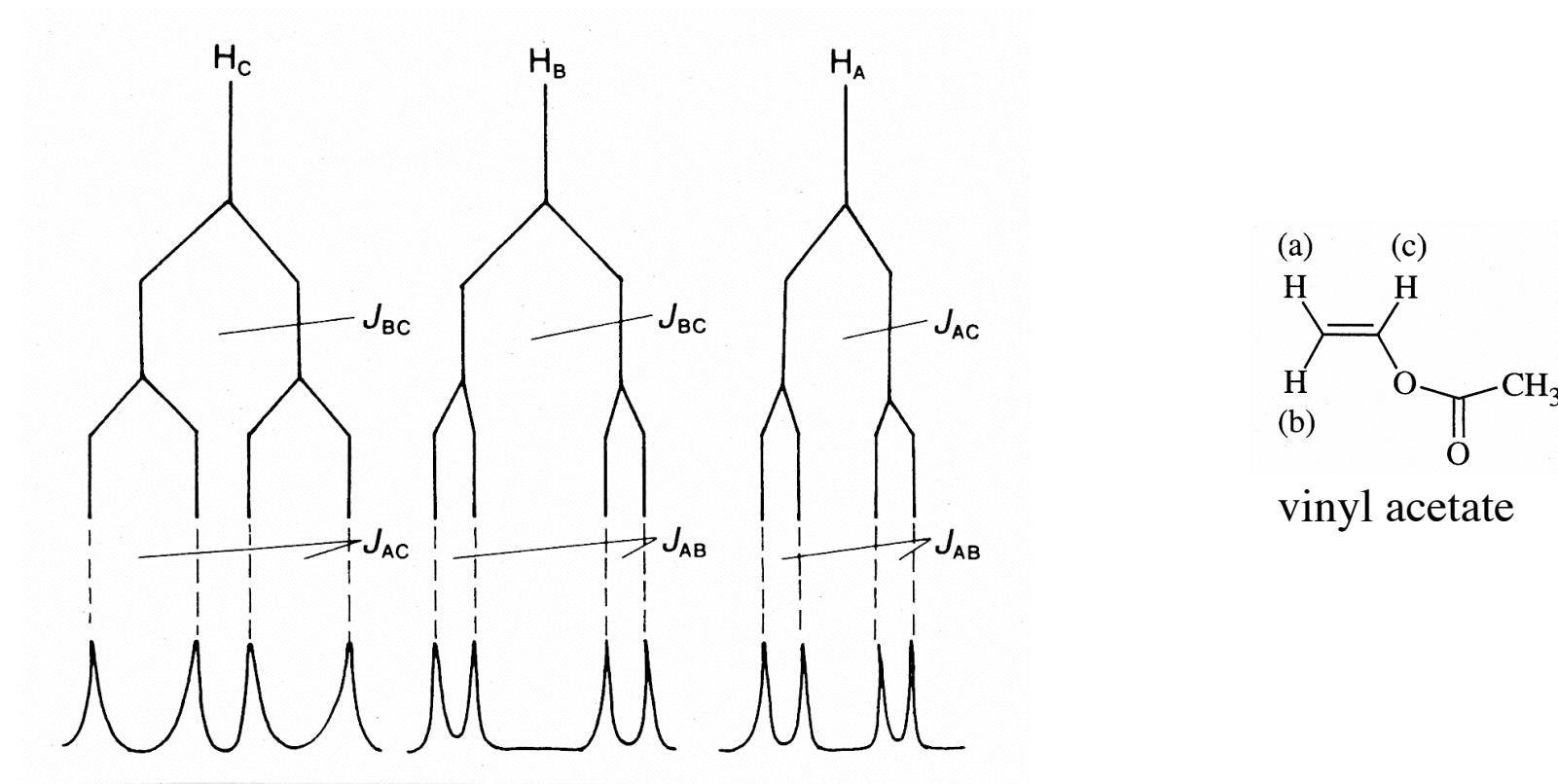
# Multiple/Complex Splittings

- Split signals can be split again by other spins
  - in vinyl acetate, for instance, the signal from “c” is split into a doublet by “b”, and this doublet is split into a doublet of doublets by “a”
  - likewise, “b” is split by “a” and “c”, and “a” is split by “b” and “c”



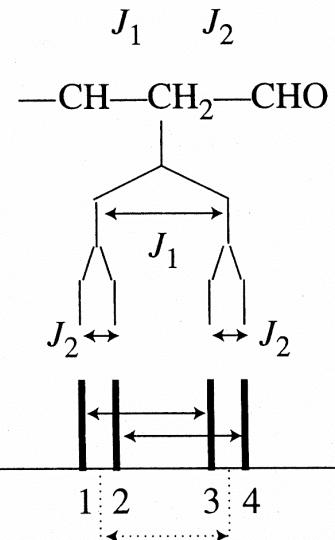
# Multiple/Complex Splittings

- The coupling constant between any two spins will be observed in the signal from each of the spins
- Observation of a coupling in more than one signal identifies coupled spins



# Extracting the Coupling Constants

- Analysis and determination of coupling constants

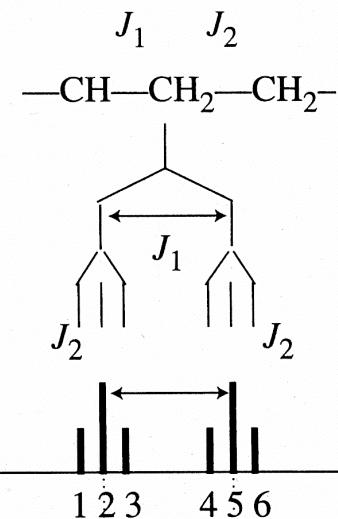


## Doublet of Doublets (dd)

To obtain  $J_1$  measure the difference between lines 1 and 3, or 2 and 4, in Hz.\*

\*Do not try to find the centers of the doublets!

$J_2$  is the spacing between lines 1 and 2, or 3 and 4



## Doublet of Triplets (dt)

To obtain  $J_1$  measure the difference between the most intense lines (2 and 5) in Hz

$J_2$  is the spacing between lines 1 and 2, or 2 and 3, or those in the other triplet.

# Spin Decoupling

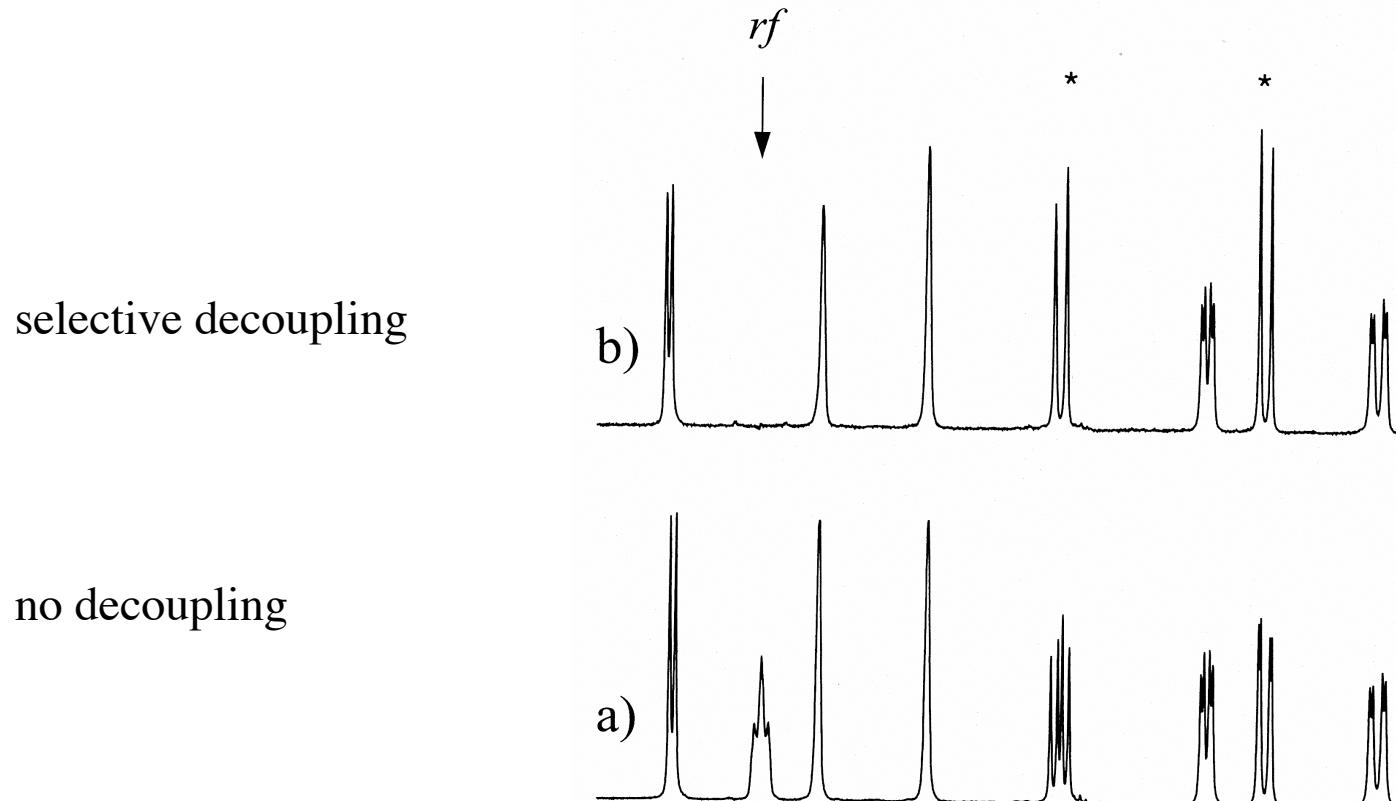
- It is often advantageous to reverse the splitting caused by spin-spin coupling by *spin decoupling*
- Spin decoupling (or just “decoupling) can be used for several reasons
  - to simplify spectra
  - to assist in identification of coupling between nuclei
  - to improve signal-to-noise
- How do we decouple spins from one another?
- Remember, coupling occurs because the transitions of one spin ( $\alpha$  to  $\beta$  or  $\beta$  to  $\alpha$ ) occurs when the other (coupled) spin is in either the  $\alpha$  or  $\beta$  state
  - thus, for one spin to couple to another, the lifetime of the coupled spin in a given state must be long enough for the transitions of the other to occur
  - this lifetime is proportional to  $1/J$

$$\tau_1 = 1/J$$

- if this lifetime is significantly shortened, the coupling will not occur, and the splitting of the signal will not occur

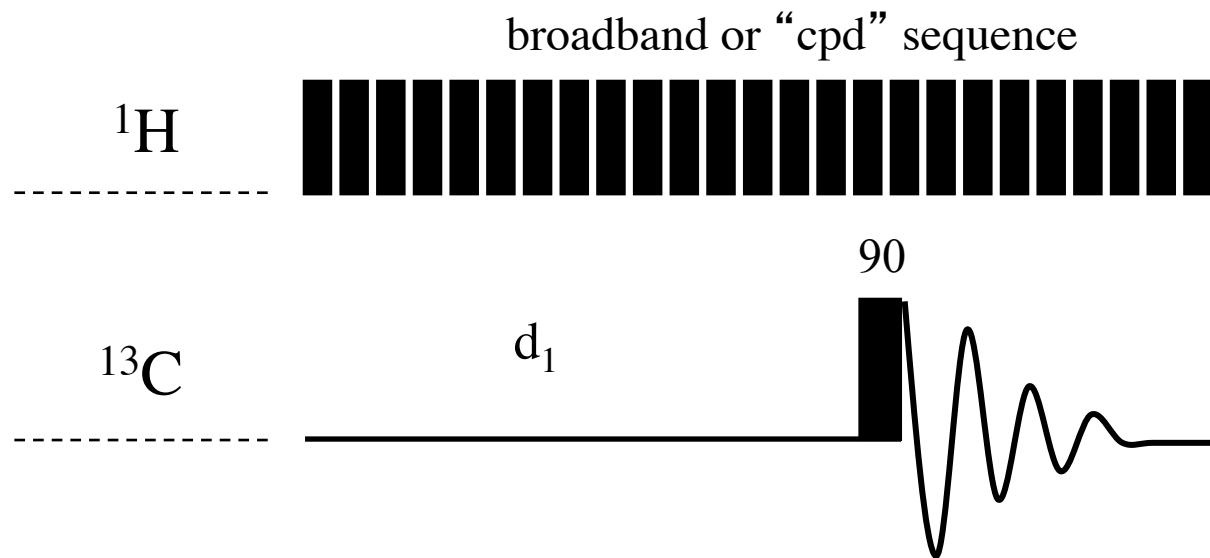
# Selective Spin Decoupling

- Selective excitation of one signal (spin) in an NMR spectrum leads to simplification of the spin-spin splitting patterns to which the spin is coupled
  - selective excitation is accomplished by a very long pulse centered at the Larmor frequency of the signal of interest
  - the long pulse causes rapid  $\alpha \rightarrow \beta$  and  $\beta \rightarrow \alpha$  transitions such that the lifetime of any particular spin in any given state is short (i.e.  $\tau_1 < J$ )



# Broad-Band Decoupling

- Often it is useful to remove *all* coupling of a given type
- For instance, it is often useful in  $^{13}\text{C}$  NMR/spectra to remove all coupling from  $^1\text{H}$
- This is accomplished by *broad-band decoupling* methods (also called *composite pulse decoupling* or “cpd”)
- In this case, a large series of pulses is rapidly applied to the nuclei to be decoupled so that all spins undergo rapid  $\alpha \rightarrow \beta$  and  $\beta \rightarrow \alpha$  transitions



# Broad-Band Decoupling

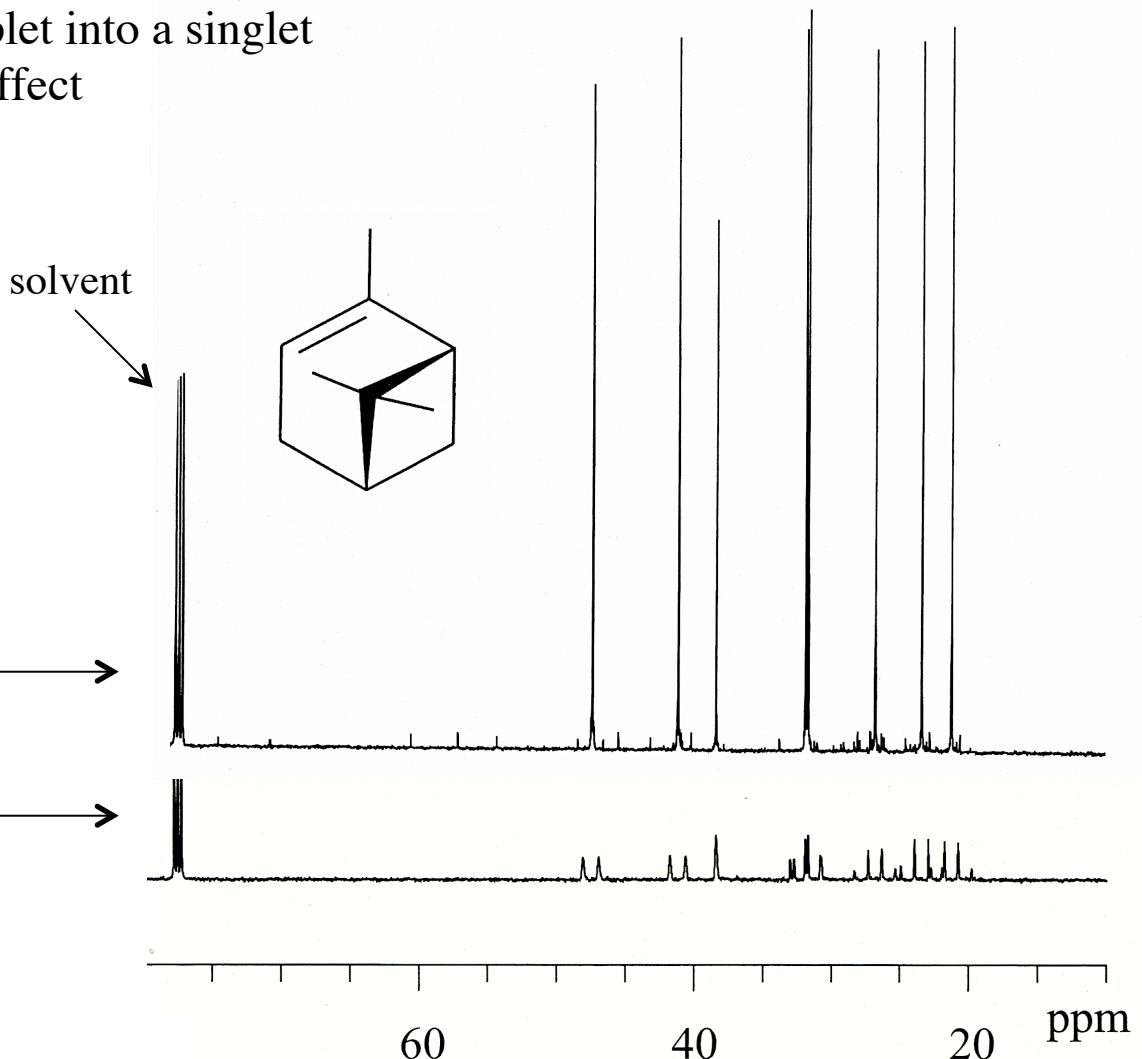
- Substantial signal-to-noise gains can accompany broad-band decoupling

- collapse of the multiplet into a singlet
- nuclear Overhauser effect

- $^{13}\text{C}$  spectra of alpha pinene

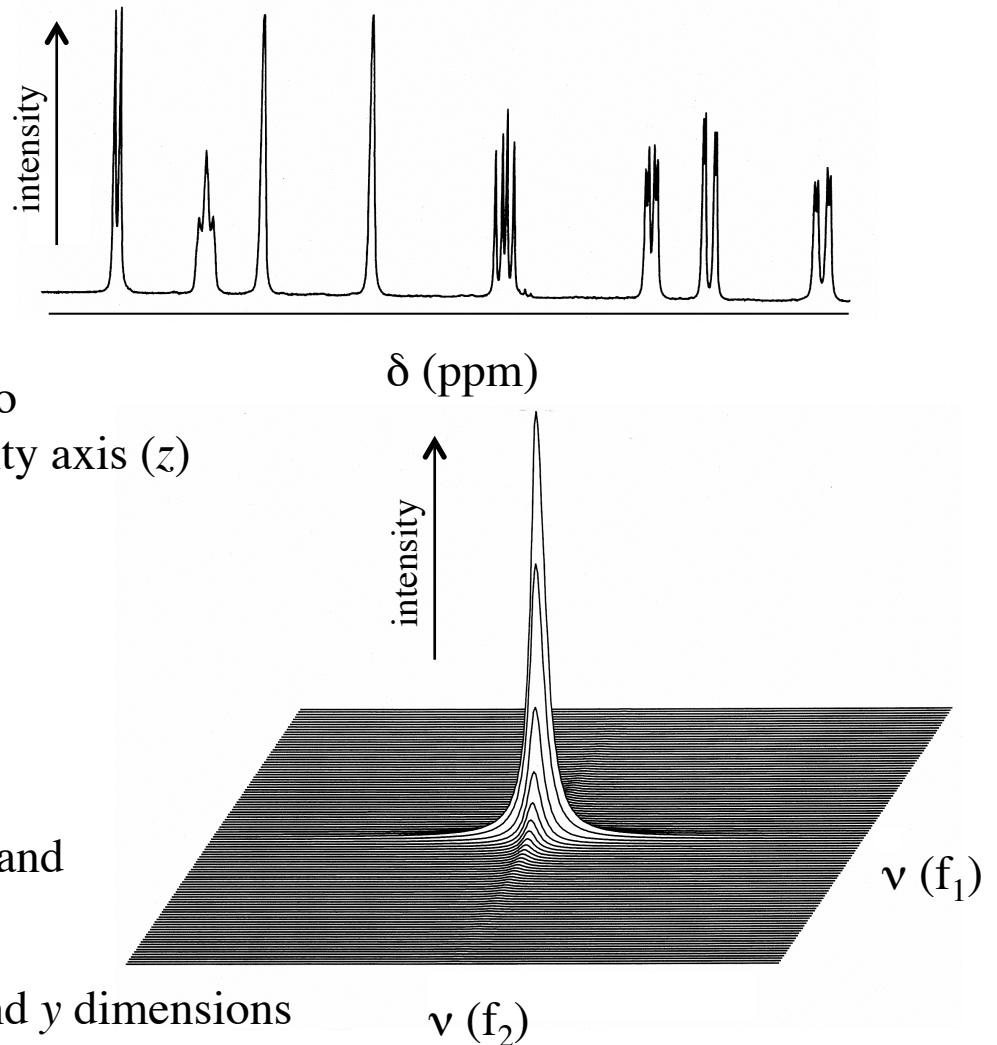
- broad-band  
 $^1\text{H}$  decoupling

- no decoupling



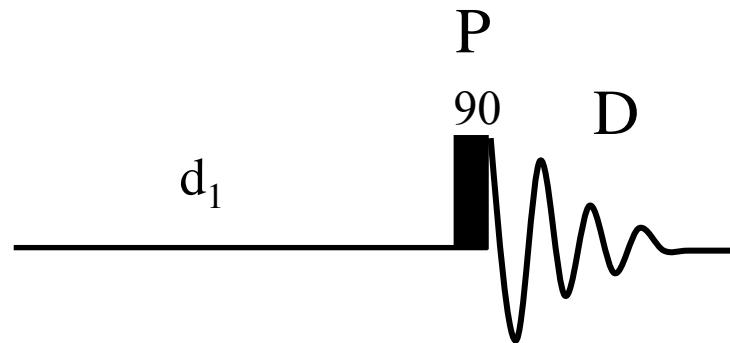
# Two-Dimensional NMR

- One-dimensional (1D) NMR: one frequency axis ( $x$ ), and one intensity axis ( $y$ )
- Two-dimensional (2D) NMR: usually two frequency axes ( $x$  and  $y$ ), and one intensity axis ( $z$ )
  - often, both frequency axes are chemical shifts (chemical shift correlation experiments)
  - one frequency axis, however, might be, for instance, a coupling constant (in Hz)
  - the directly detected dimension is  $f_2$ , and the indirectly detected dimension  $f_1$
- In two dimensional experiments, the  $x$  and  $y$  dimensions are related or *correlated* based on:
  - through bond coupling (COSY, TOCSY, HSQC)
  - through space interactions (NOESY)
  - chemical exchange processes (EXSY)

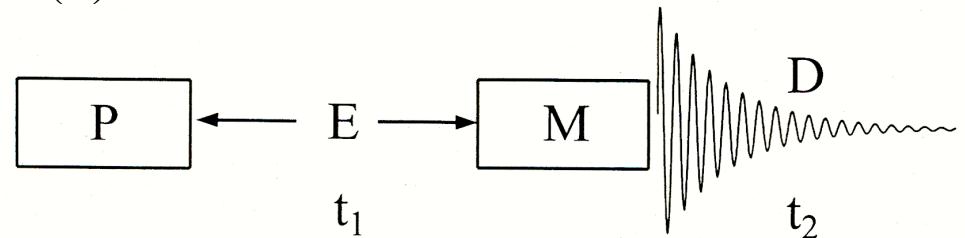


# Two-Dimensional NMR

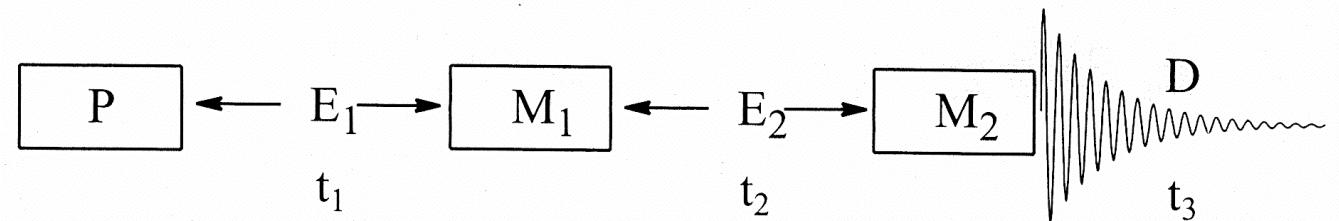
- One-dimensional experiments can be described as consisting of a spin “preparation” (P) phase (often just a single pulse) and a “detection” (D) phase, when the magnetization is detected



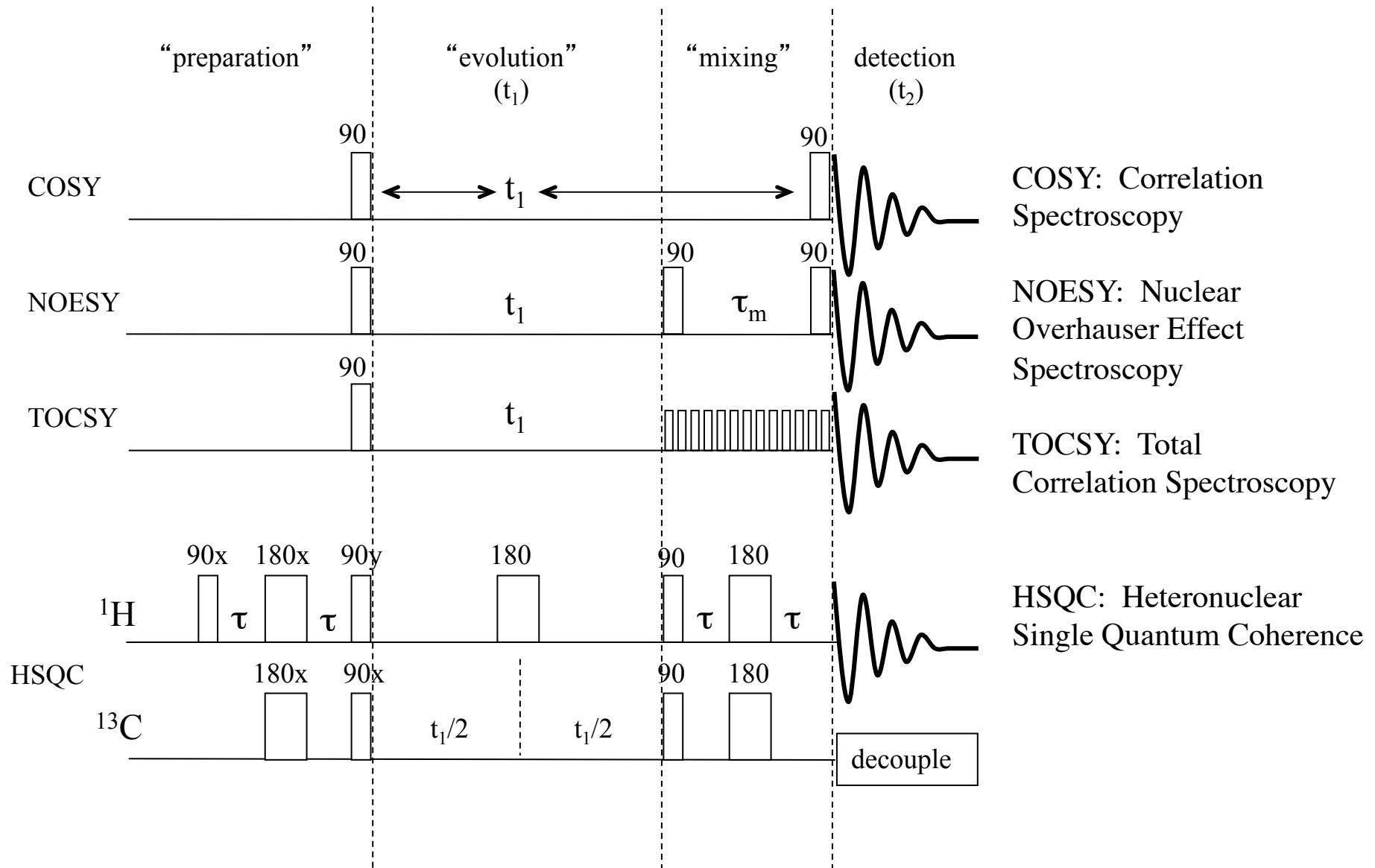
- Two-dimensional experiments include, in addition, a magnetization “evolution” period (E) and a “mixing” (M) period
  - during the evolution period, the modulation of the signal for the second dimension occurs
  - during the mixing period, magnetization or “coherence” transfer between spins occurs



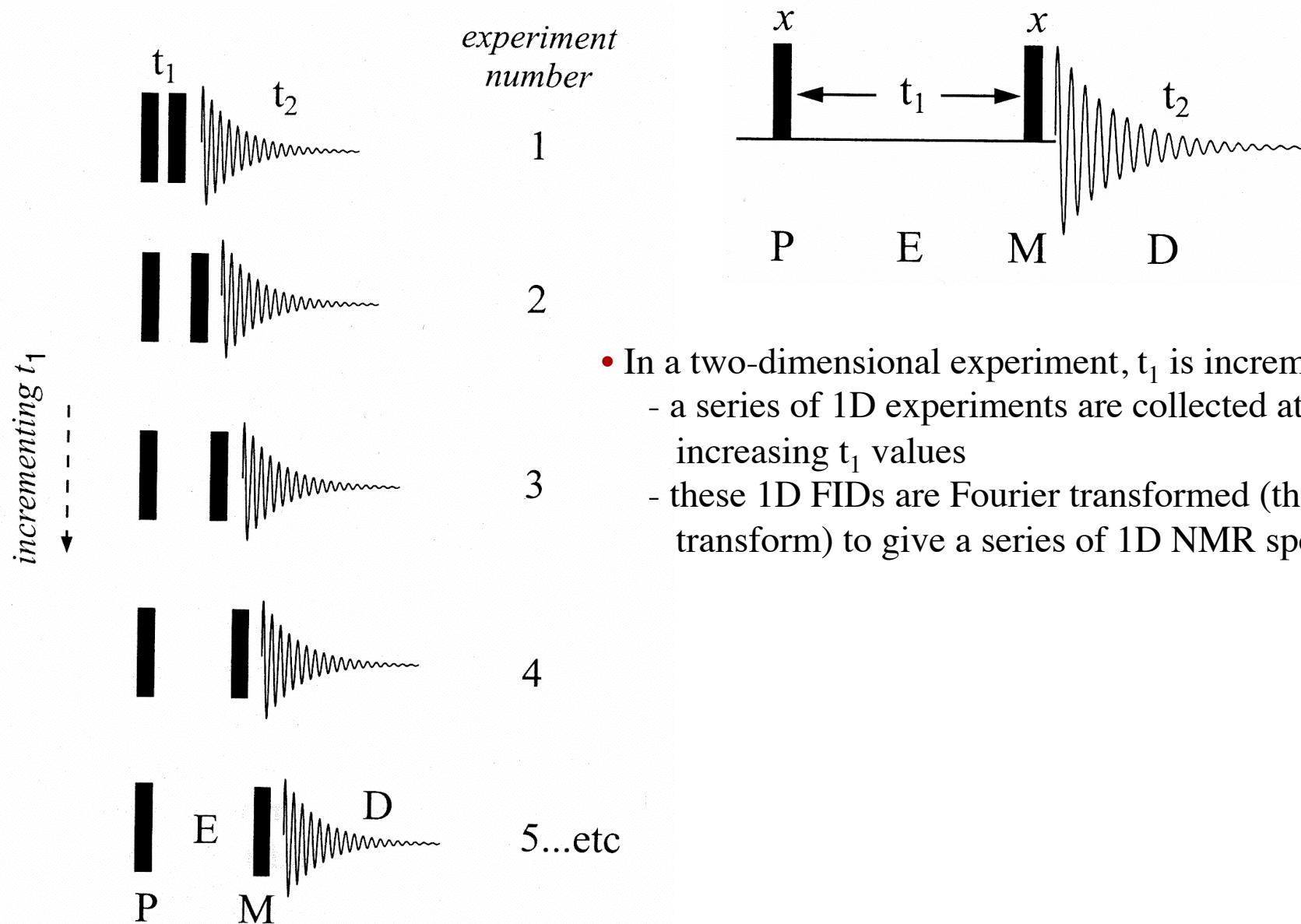
- Additional E and M periods can be added to create 3D and 4D experiments



# Some Two-Dimensional NMR Experiments



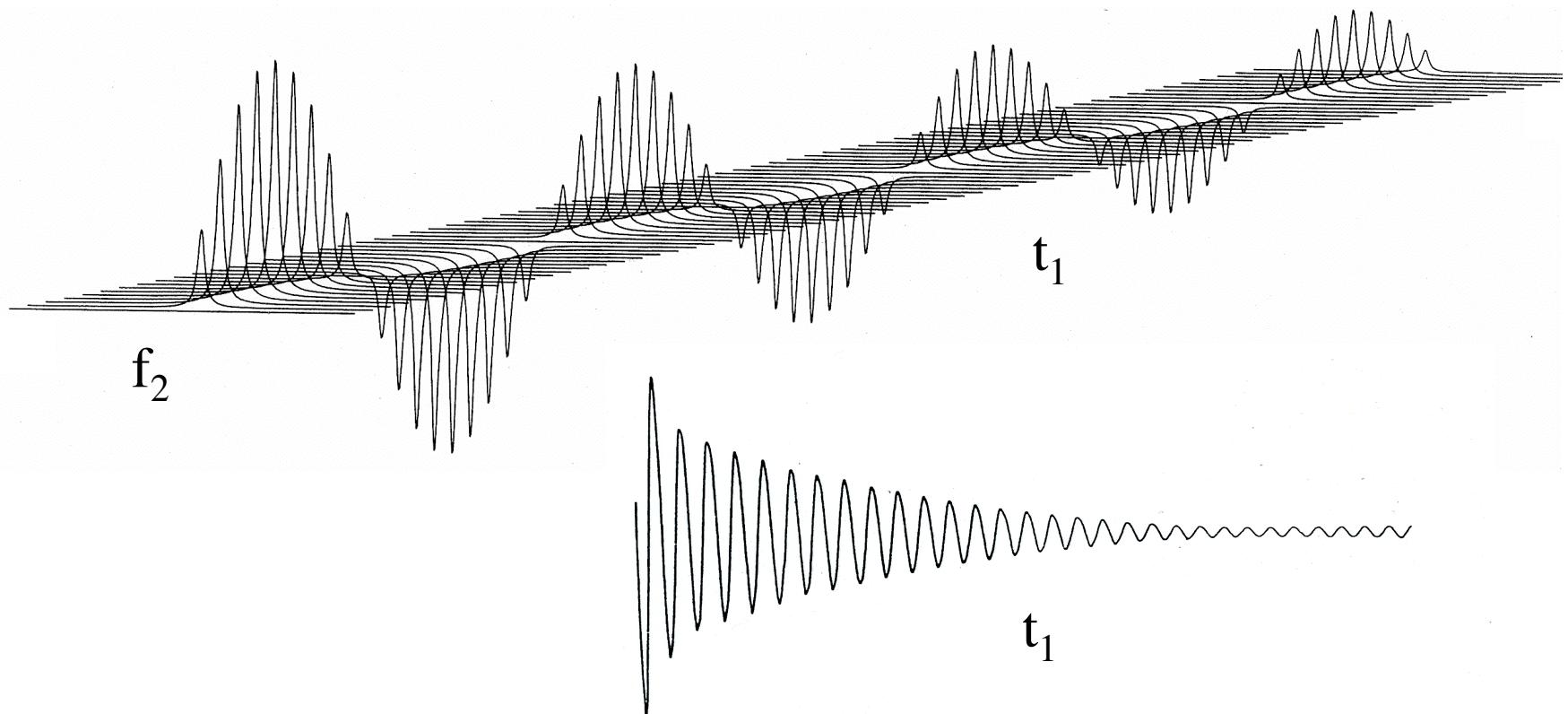
# Two-Dimensional NMR Data Collection and Manipulation



- In a two-dimensional experiment,  $t_1$  is incremented
  - a series of 1D experiments are collected at increasing  $t_1$  values
  - these 1D FIDs are Fourier transformed (the “ $t_2$ ” transform) to give a series of 1D NMR spectra

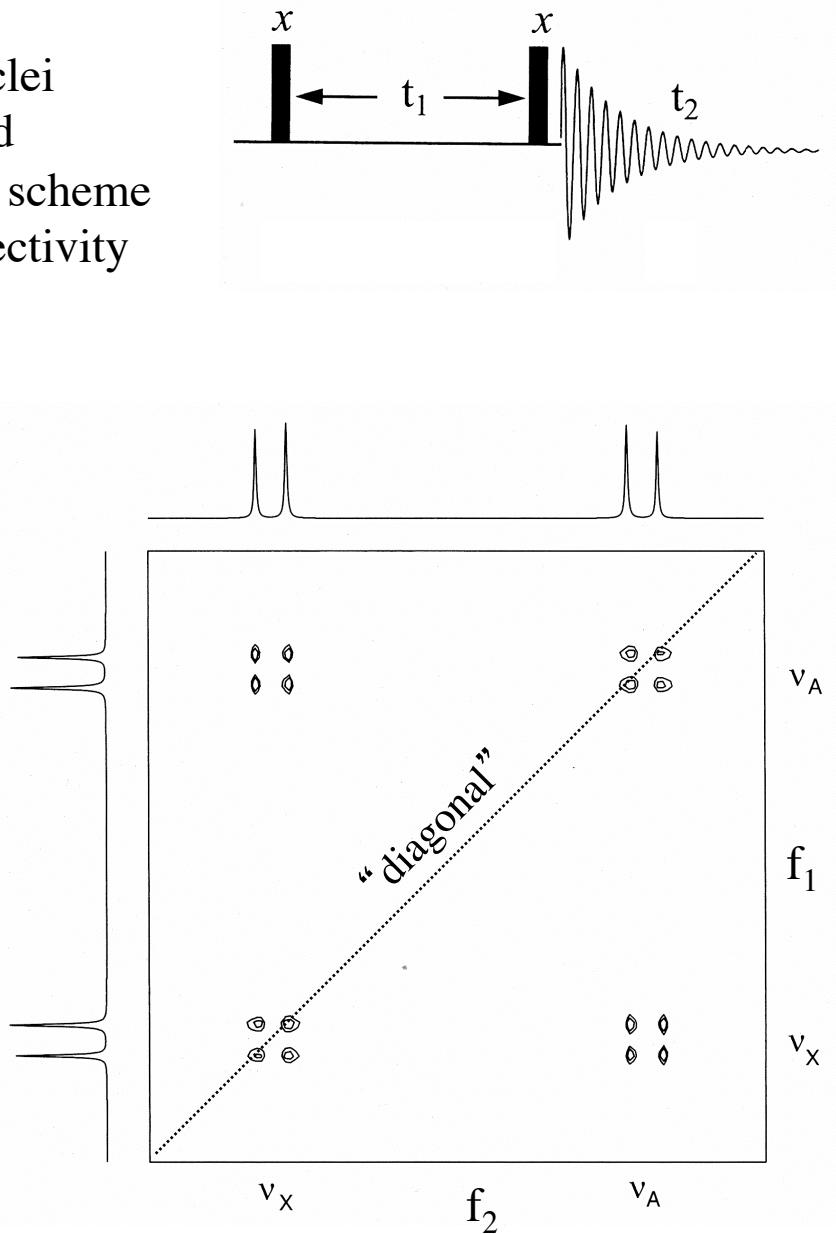
# Two-Dimensional NMR Data Collection and Manipulation

- Peaks (signals) in the series of 1D spectra are modulated by  $t_1$  and decay with  $t_1$ 
  - thus, the signals in the 1D spectra form a FID in the  $t_1$  (second) dimension
  - so, a Fourier transform (the “ $t_1$ ” transform) can be performed on these signals to give the 2D plot
  - different signals in  $f_2$  may be modulated differently in  $t_1$ , resulting in different  $f_1$  frequencies for different  $f_2$  signals



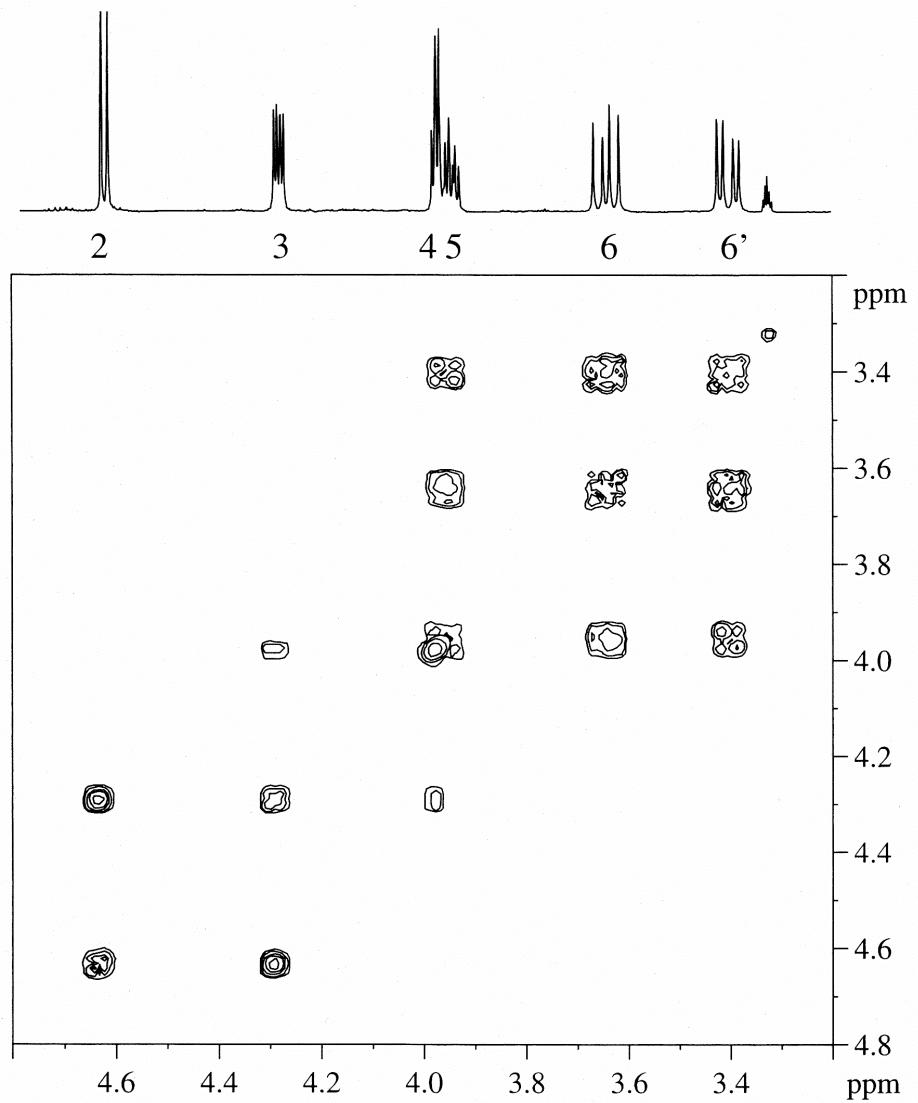
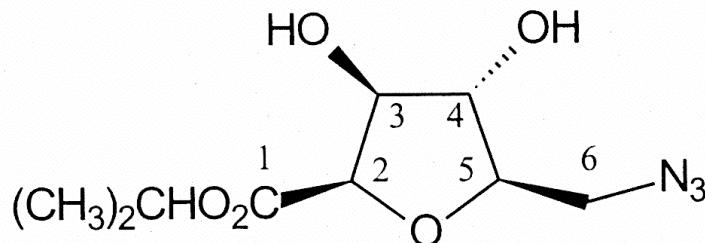
# COSY: 2D Chemical Shift Correlation

- COSY correlates chemical shifts of coupled nuclei
  - magnetization is transferred between coupled nuclei as a result of the COSY experimental scheme
  - provides information on through-bond connectivity
- consider two coupled spins, A and X:
  - X magnetization will precess at  $\nu_X$  during  $t_1$
  - X magnetization that is *not* subsequently transferred to A will also precess at  $\nu_X$  during  $t_2$
  - however, X magnetization subsequently transferred to A will precess during  $t_2$  at  $\nu_A$
  - thus, off diagonal signals (with different chemical shifts in  $f_2$  and  $f_1$ ) indicate that X and A are coupled
  - diagonal elements are, in general, not interesting



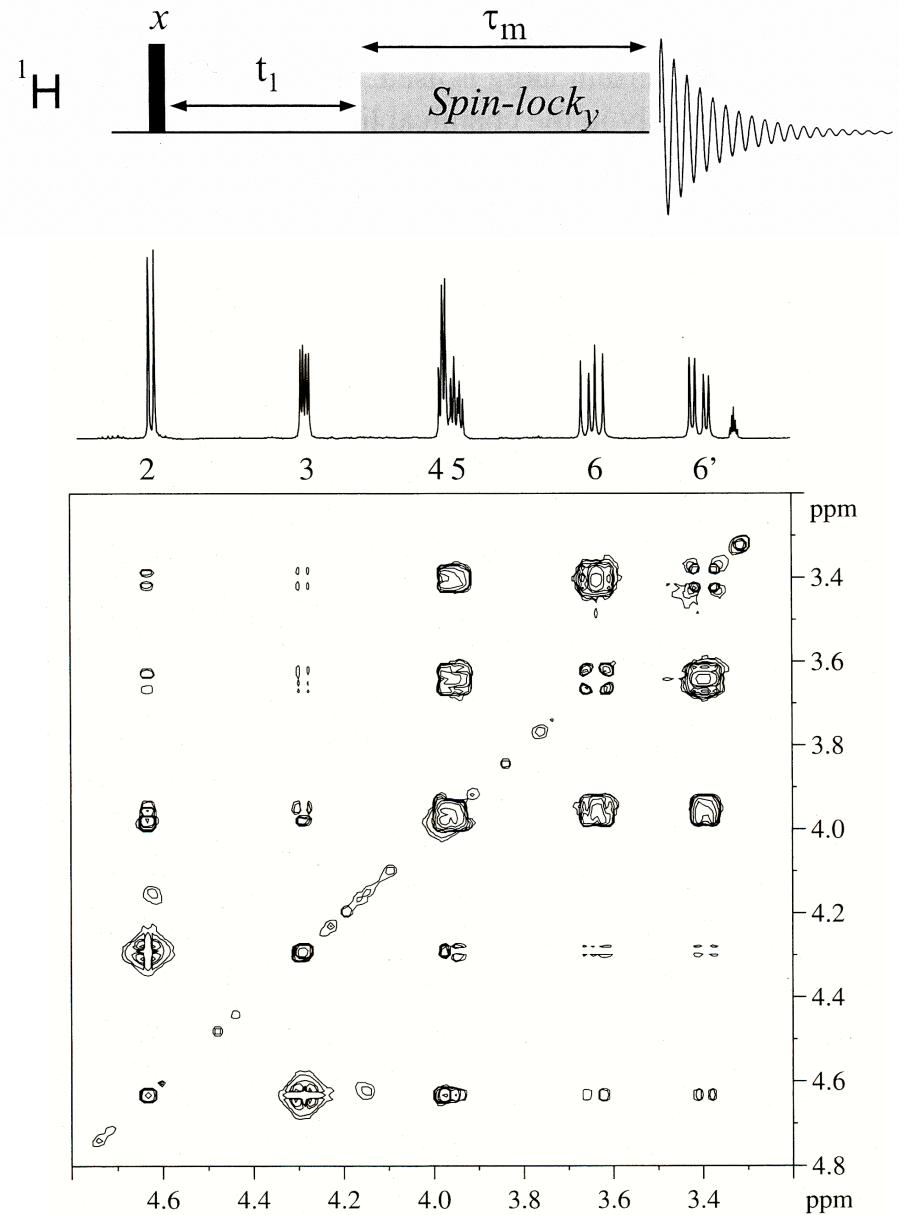
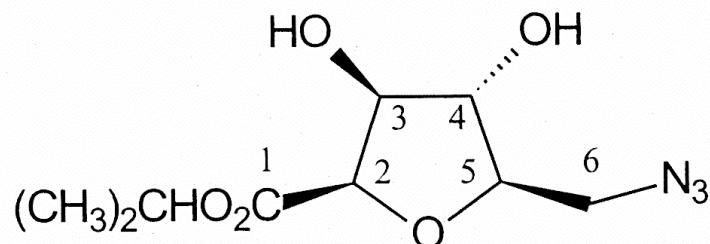
# COSY: Example

- COSY spectrum of an azo-sugar
- crosspeaks appear at positions corresponding to the chemical shifts of  $^1\text{H}$  nuclei on adjacent carbon atoms
  - crosspeaks indicate significant coupling between the  $^1\text{H}$  nuclei
  - *usually*, therefore, crosspeaks are not observed for  $^1\text{H}$  nuclei separated by more than 3 bonds



# TOCSY: “Total” Correlation Spectroscopy

- TOCSY spectrum of an azo-sugar
- The TOCSY “spin-lock” sequence enables transfer of magnetization between nuclei even if the coupling between them is very weak
  - thus, for the example shown, crosspeaks between most of the nuclei are observed



# Heteronuclear Correlation Spectroscopy

- HSQC spectrum of menthol
- No diagonal peaks
- The chemical shifts of directly bonded  $^1\text{H}$ - $^{13}\text{C}$  pairs are correlated
  - one axis is  $^{13}\text{C}$  chemical shift
  - one axis is  $^1\text{H}$  chemical shift

