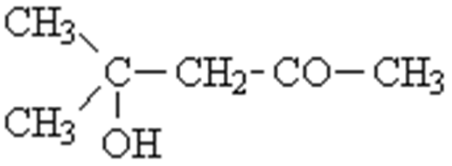
Signal intensities

Next to the *number* of signals in an NMR spectrum and their *chemical shifts*, additional information about the compound in question can also be derived from the intensities of the signals.

1. The intensity is measured as the ***integral*** below the resonance signal. To determine the area, the spectrum is integrated automatically, and the cumulative integral is plotted on the spectrum.
2. The intensities are proportional to the ***number of protons***, that contribute to the signal.

Signal intensities

The molecule

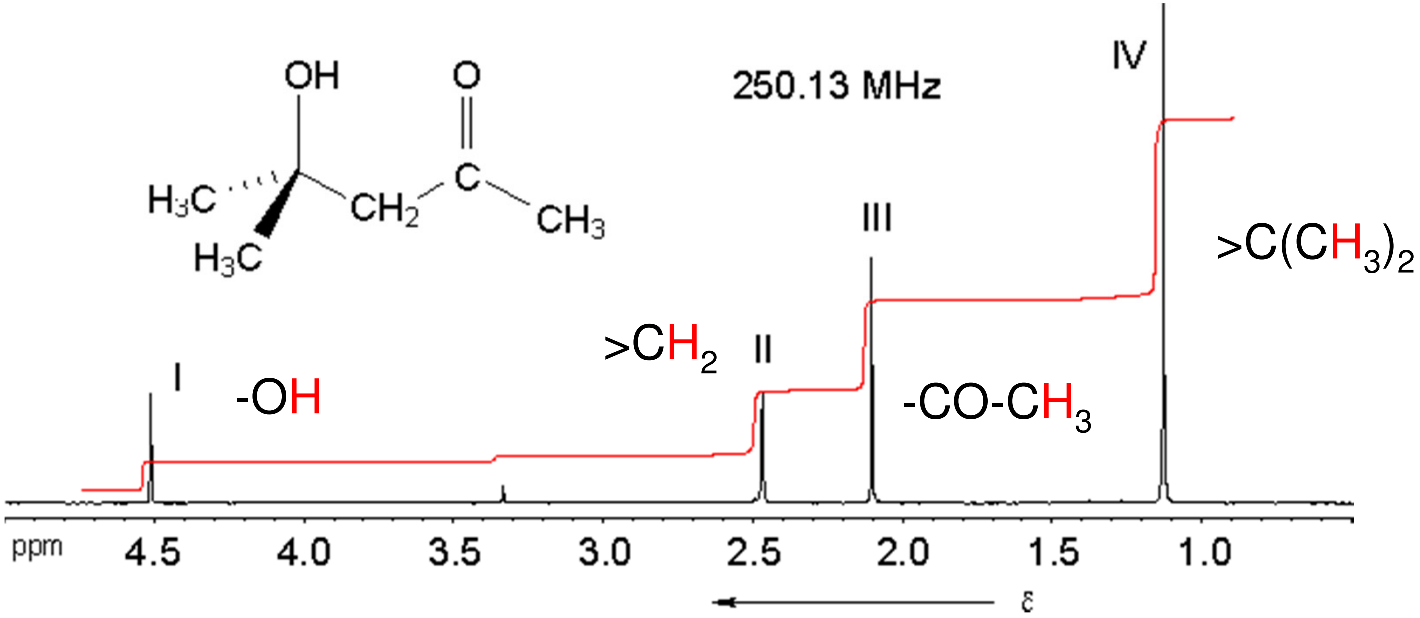


can be characterized as follows, based on the number of signals and their integrals:

	equivalent protons	**number of protons**
a)	**$(CH_3)_2$-C<**	**6**
b)	**-OH**	**1**
c)	**-CH_2-**	**2**
d)	**-CO-CH_3**	**3**

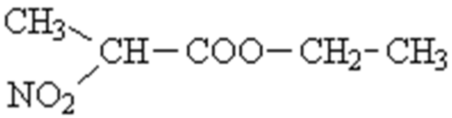
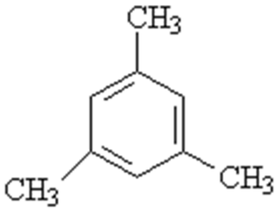
$$I_{(CH3)2} : I_{CH3} : I_{CH2} : I_{OH} = 6 : 3 : 2 : 1$$

Signal intensities



Signal intensities

For the following set of molecules, determine the groups of equivalent protons and the relative intensities of their signals in an NMR spectrum!

Substance	**Equivalent protons**	**Relative intensities**
	a) CH_3-CH<	3
	b) >CH-	1
	c) -CH_2-	2
	d) -CH_3	3
$(CH_3)_2CHOCH(CH_3)_2$	a) 2 * $(CH_3)_2C<$	6(12)
	b) 2 * >CH-O	1 (2)
	a) 3 * -CH_3	3 (9)
	b) 3 * Phenyl-H	1 (3)

Through-bond spin-spin coupling

If you want to use NMR spectroscopy in the investigation of the structure of molecules, there are additional fine structure features of the spectra that can help you to determine the conformation of the molecule in question.

Certain signals do not appear as simple lines, but as *multiplets* of closely spaced lines with characteristic patterns of intensities.

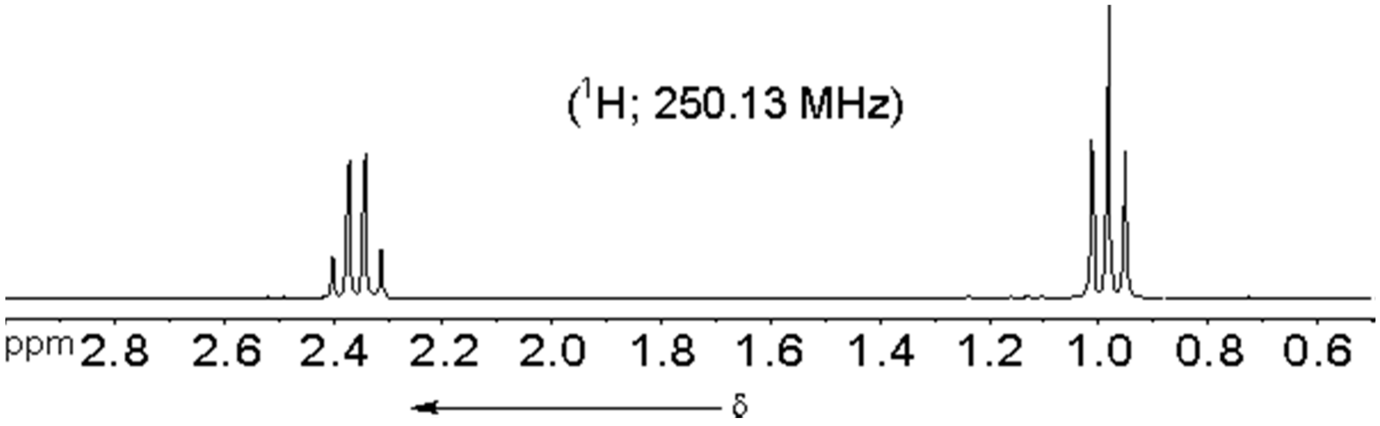
Here is an example:

- The molecule CH_3-CH_2-CO-CH_2-CH_3 should have only two signals in its proton NMR spectrum:

a) 2 -CH_3 $\delta = 0.6 ... 1.9$ ppm
b) 2 -CH_2-CO $\delta = 1.9 ... 3.2$ ppm.

The actual spectrum does confirm these predictions:

Through-bond spin-spin coupling

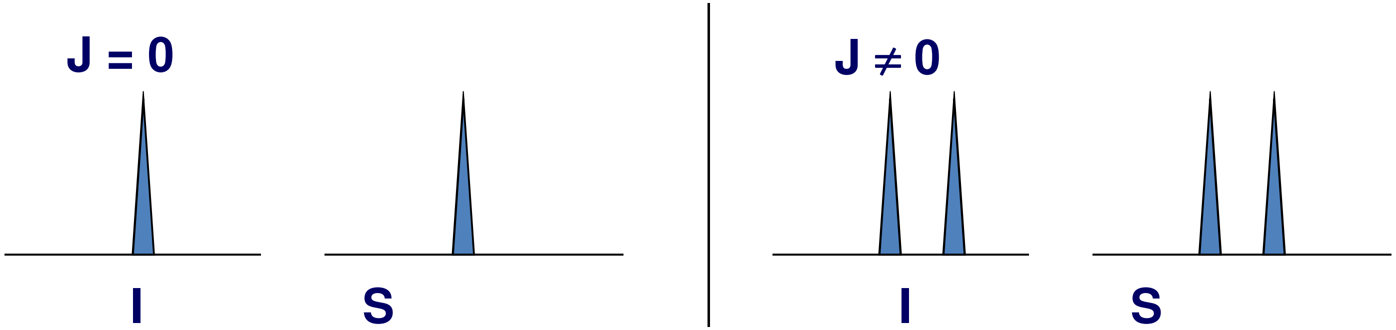


However, at closer inspection the signal of the *methyl* protons turns out to be composed of three lines (a *triplet*) with relative intensities of approximately 1 : 2 : 1, while the signal of the *methylene* protons is composed of four lines (a *quartet*) with relative intensities of approximately 1 : 3 : 3 : 1

Spin-spin coupling analysis

- The last parameter that we will discuss concerning the interpretation of NMR spectra are ^1H spin-spin couplings. Couplings are perhaps the most important parameter in NMR, as they allow us to elucidate chemical structure.

- Scalar spin-spin coupling shows up as a splitting, or fine structure, in our spectrum. It will occur between two magnetically active nuclei that are connected through chemical bonds. We can see it for two atoms directly connected, or for atoms that 'see' one another across several bonds.

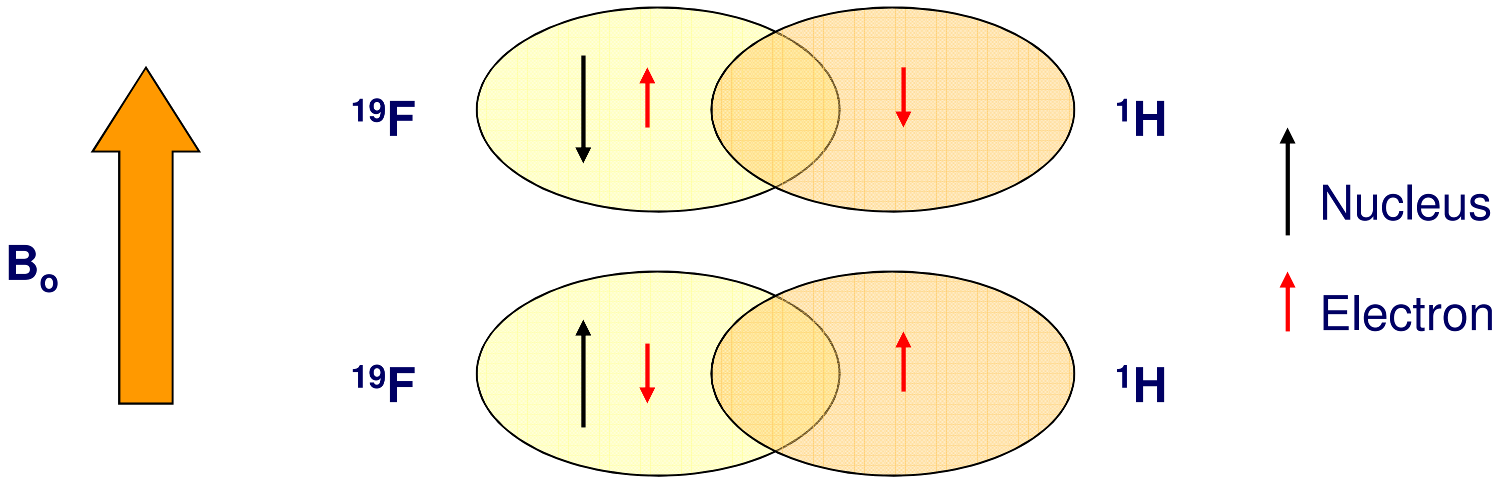


- A splitting of a signal means that we have more energies involved in the transition of a certain nuclei. So why do we have more energies?

- The reason is the bonding electrons and their magnetic moments. The magnetic moment of the nuclei produces a small ***polarization*** (orientation…) of the bonding electron, and this is transmitted by overlapping orbitals to the other nuclei.

Spin-spin coupling (continued)

- We can explain this better by looking at HF:



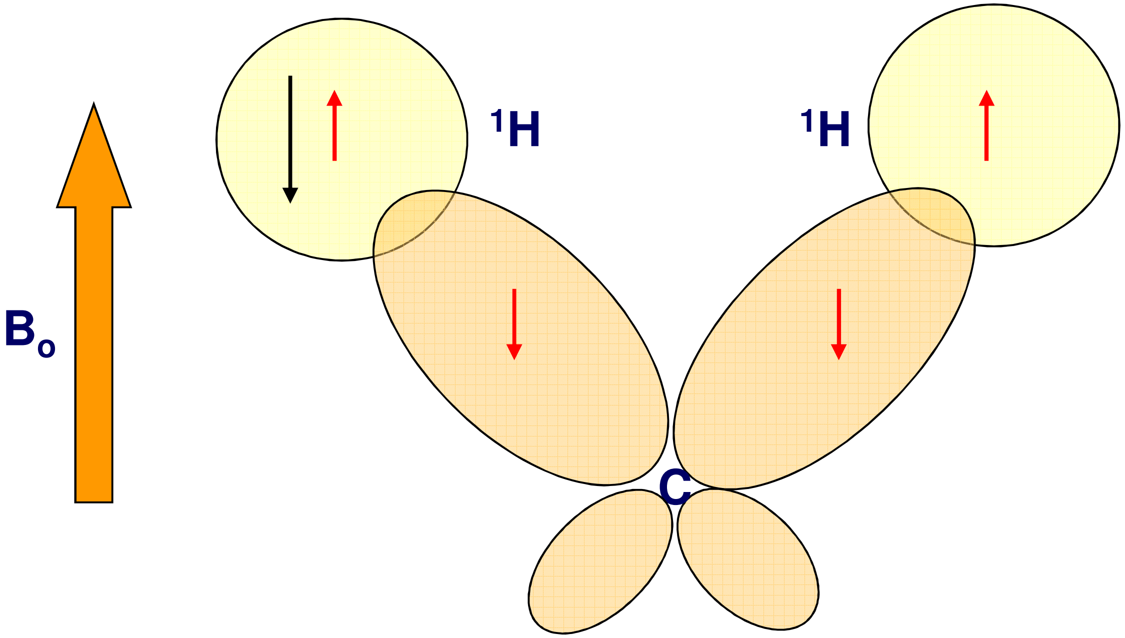
- The nuclear magnetic moment of ^{19}F polarizes the F bonding electron (up), which, since we are following quantum mechanics rules, makes the other electron point down (the electron spins have to be antiparallel).

- Now, the since we have different states for the ^{1}H electrons depending on the state of the ^{19}F nucleus, we will have slightly different energies for the ^{1}H nuclear magnetic moment (remember that the ***1s*** electron of the ^{1}H generates an induced field…).

- This difference in energies for the ^{1}H result in a splitting of the ^{1}H resonance line.

Spin-spin coupling (continued)

- We can do a similar analysis for a CH_2 group:



- The only difference here is that the C bonds are hybrid bonds (***sp^3***), and therefore the ***Pauli principle*** and ***Hundi's rules*** predict that the two electrons will be parallel.

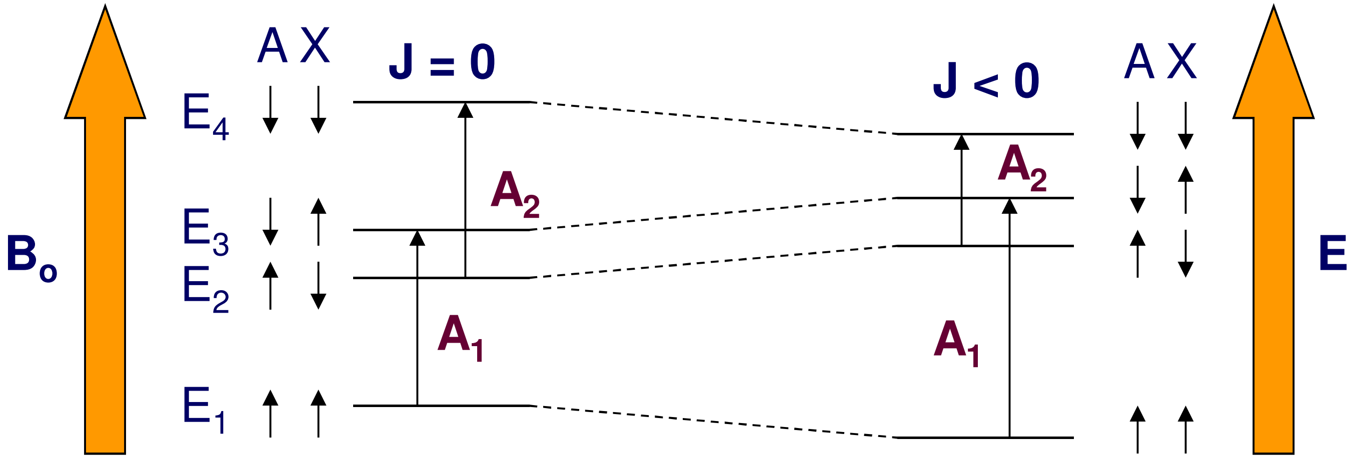
- Irrespective of this, the state of one of the ^{1}H nuclei is transmitted along the bonds to the other ^{1}H, and we get a splitting (a doublet in this case…). The energy of the interactions between two spins **A** and **B** can be found by the relationship:

$$\boxed{E = J_{AB} * I_A * I_B}$$

Spin-spin coupling (…)

- $\mathbf{I}_A$ and $\mathbf{I}_B$ are the nuclear spin vectors, and are proportional to μ_A and μ_B, the magnetic moments of the two nuclei. $\mathbf{J}_{AB}$ is the ***scalar coupling constant***. So we see a very important feature of couplings. It does not matter if we have a 60, a 400, or an 800 MHz magnet, **THE COUPLING CONSTANTS ARE ALWAYS THE SAME!!!**

- Now lets do a more detailed analysis in term of the energies. Lets think a two energy level system, and the transitions for nuclei **A**. When we have no coupling (**J = 0**), the energy involved in either transition (**A_1** or **A_2**) are the same, because we have no spin-spin interaction.



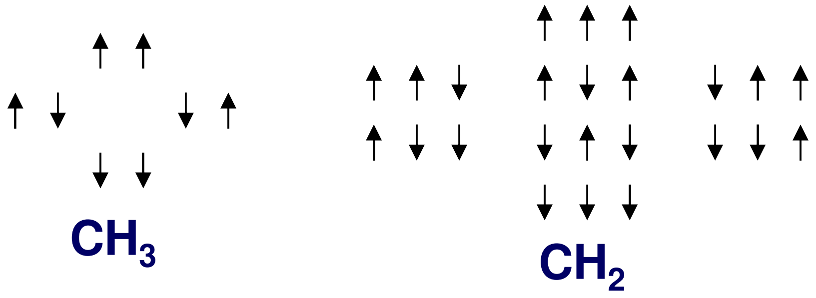
- Therefore, the relative orientations of the nuclear moments does not matter - We see a single line (two with equal frequency). When **J > 0**, the energy levels of the spin system will be either stabilized or destabilized, depending on the relative orientations of the nuclear moments, and the energies for the **A_1** and **A_2** transition change, and we have two different frequencies (two peaks for **A**).

1st order systems

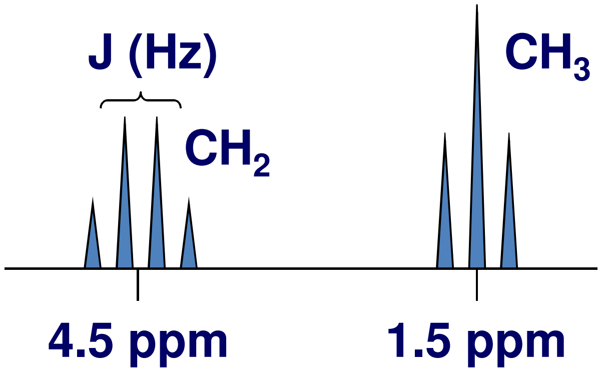
- If a certain nuclei **A** is coupled to ***n*** identical nuclei **X** (of spin $^1/_2$), **A** will show up as ***n + 1*** lines in the spectrum. Therefore, the CH_2 in EtOAc will show up as four lines, or a ***quartet***. Analogously, the CH_3 in EtOAc will show up as three lines, or a ***triplet***.

- The separation of the lines will be equal to the coupling constant between the two types of nuclei (CH_2's and CH_3's in EtOAc, approximately 7 Hz).

- If we consider the diagram of the possible states of each nuclei, we can also see what will be the intensities of the lines:



- Since we have the same probability of finding the system in any of the states, and states in the same rows have equal energy, the intensity will have a ratio **1:2:1** for the CH_3, and a ratio of **1:3:3:1** for the CH_2:

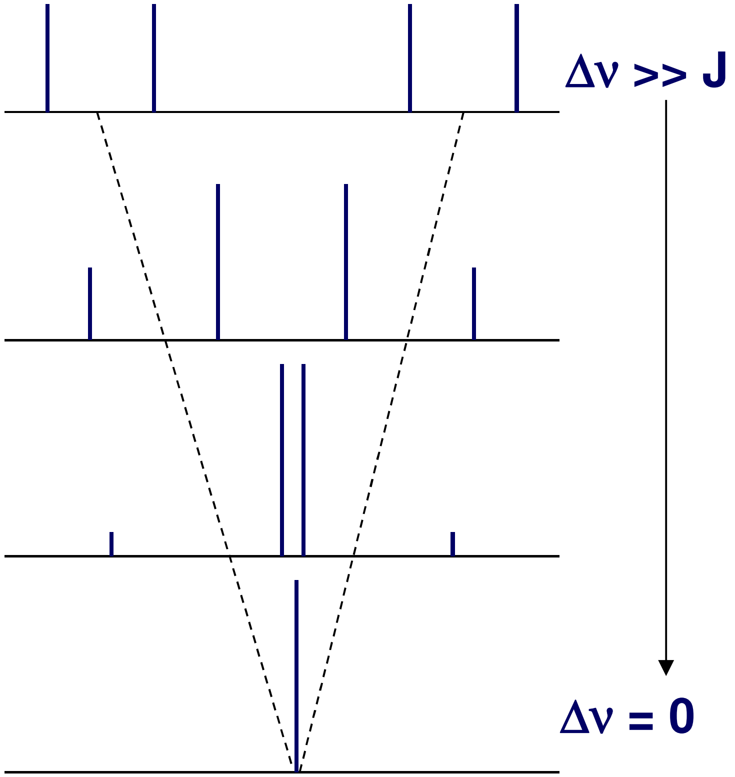


2nd order systems. The AB system

- What we have been describing so far is a spin system in which **$\Delta\nu$ >> J**, and as we said, we are analyzing one of the limiting cases that QM predict.

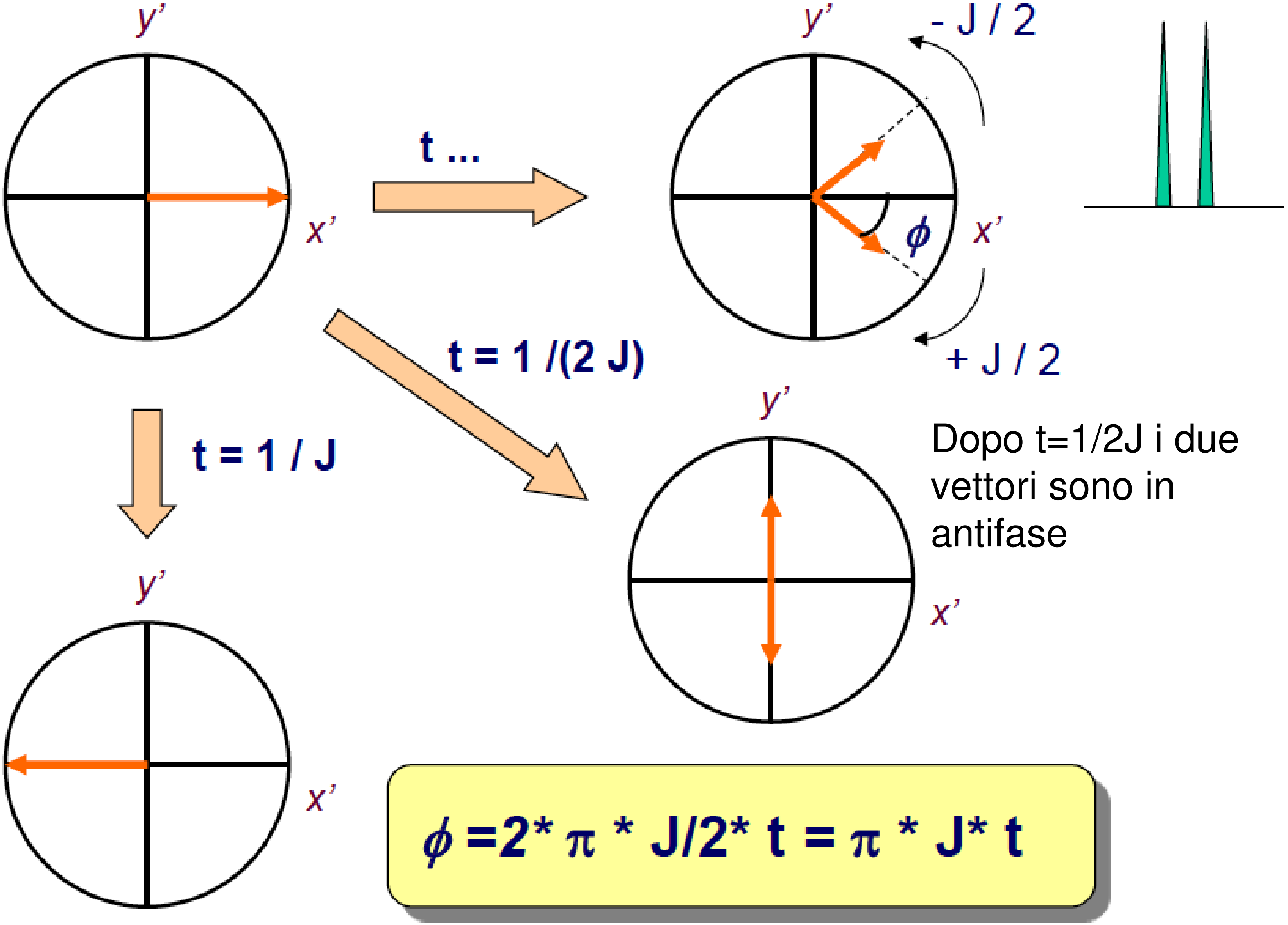
- As **$\Delta\nu$** approaches **J**, there will be more transitions of similar energy and thus our spectrum will start showing more signals than our simple analysis predicted. Furthermore, the intensities and positions of the lines of the multiplets will be different from what we saw so far.

- Lets say that we have two coupled nuclei, **A** and **B**, and we start decreasing our **B_o**. **$\Delta\nu$** will get smaller with **J** staying the same. After a while, **$\Delta\nu$ ~ J**. What we see is the following:



- What we did here is to start with an **AX** system (the chemical shifts of **A** and **X** are very different) and finish with an **AB** system, in which **$\Delta\nu$ ~ J**.

COUPLING CONSTANTS IN THE ROTATING FRAME

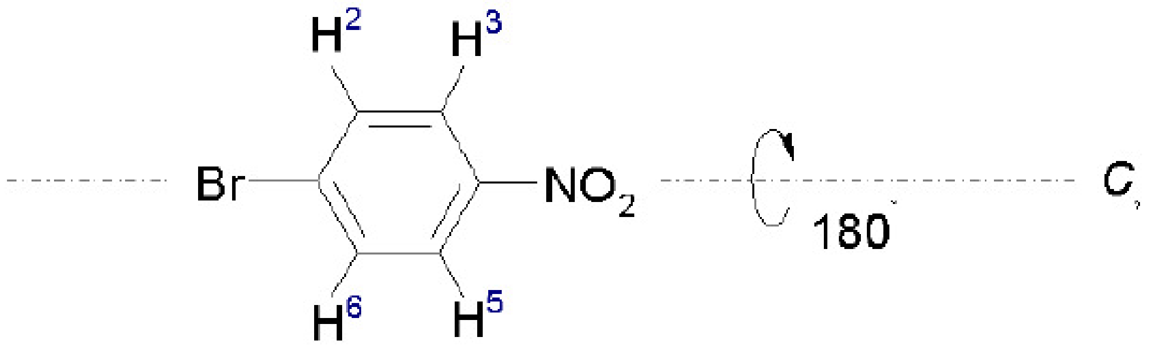


Dopo t=1/2J i due vettori sono in antifase

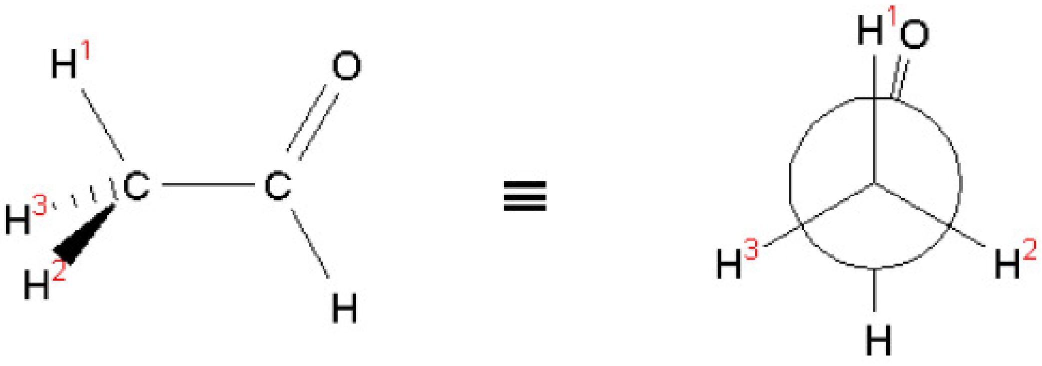
$$\phi = 2 * \pi * J/2 * t = \pi * J * t$$

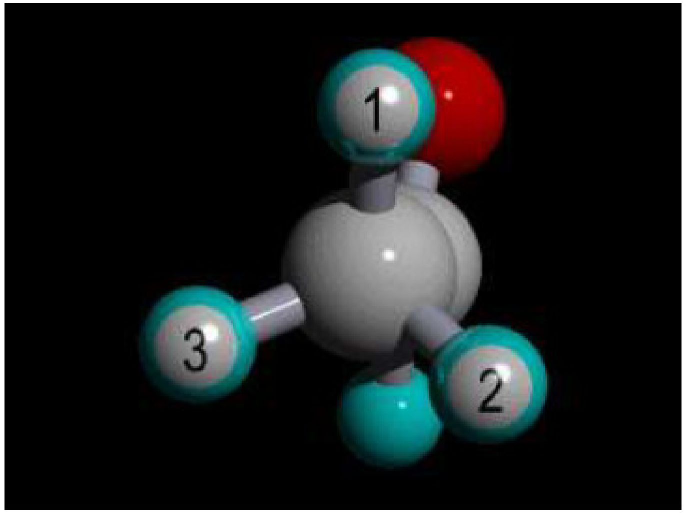
Equivalent spins

a) Hydrogens showing identical chemical environment

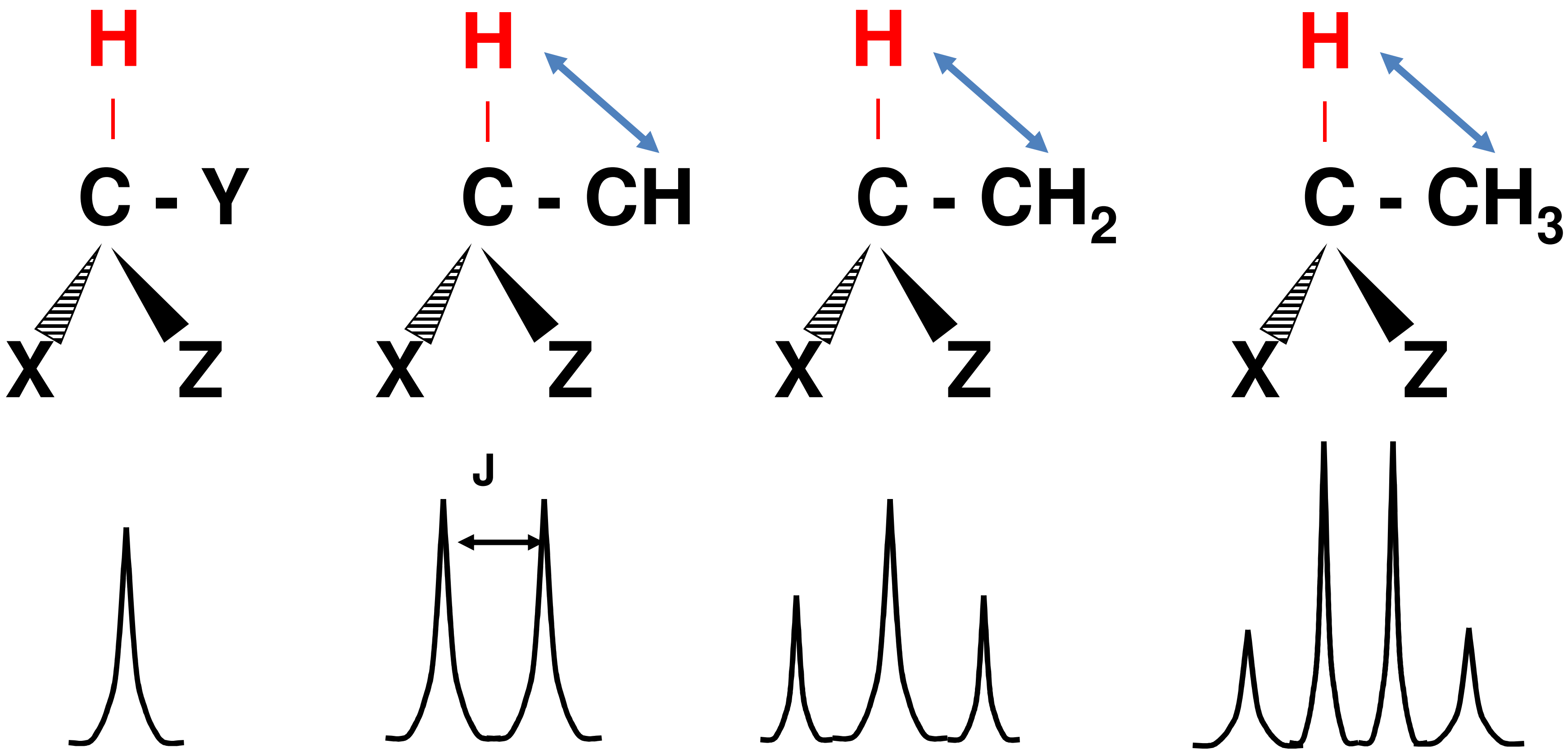


b) Hydrogens that average very similar environment with rotation





Spin-Spin Coupling



singlet **doublet** **triplet** **quartet**

a multiplicity of $M = n + 1$

Through-bond spin-spin coupling

Multiplicity	Relative intensities
2	1 : 1
3	1 : 2 : 1
4	1 : 3 : 3 : 1
5	1 : 4 : 6 : 4 : 1

The general rule therefore is:

The relative intensities of the individual multiplet lines follow the same patterns as the *n*th binominal coefficients!

Prediction of ^{1}H NMR spectra

Molecule

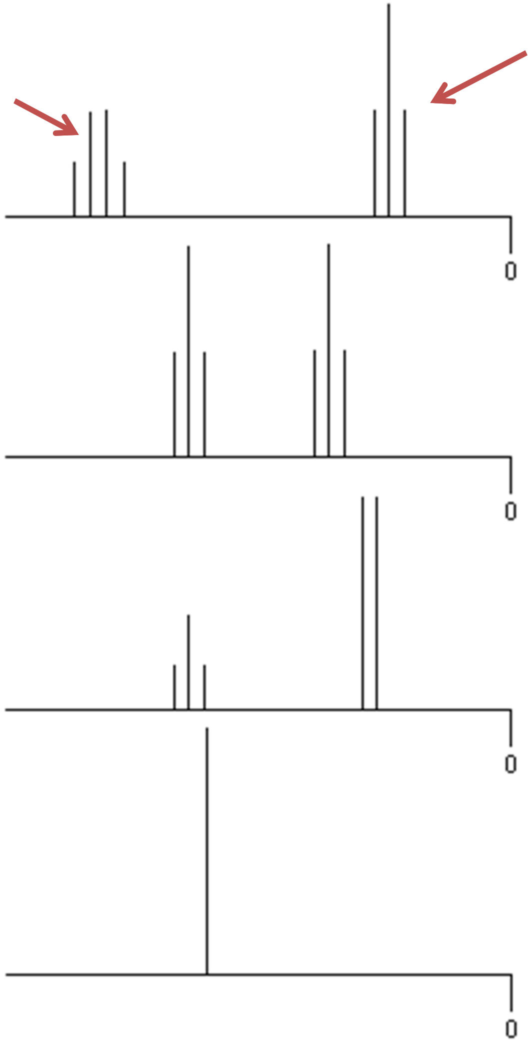
Idealized spectrum

RCH_2-CH_3

RCH_2-CH_2R'

RCH_2-CHR_2'

RCH_2-CR_3'



CH_2 che vede i 3 H equivalenti di CH_3

CH_3 che vede i 2 H equivalenti di CH_2

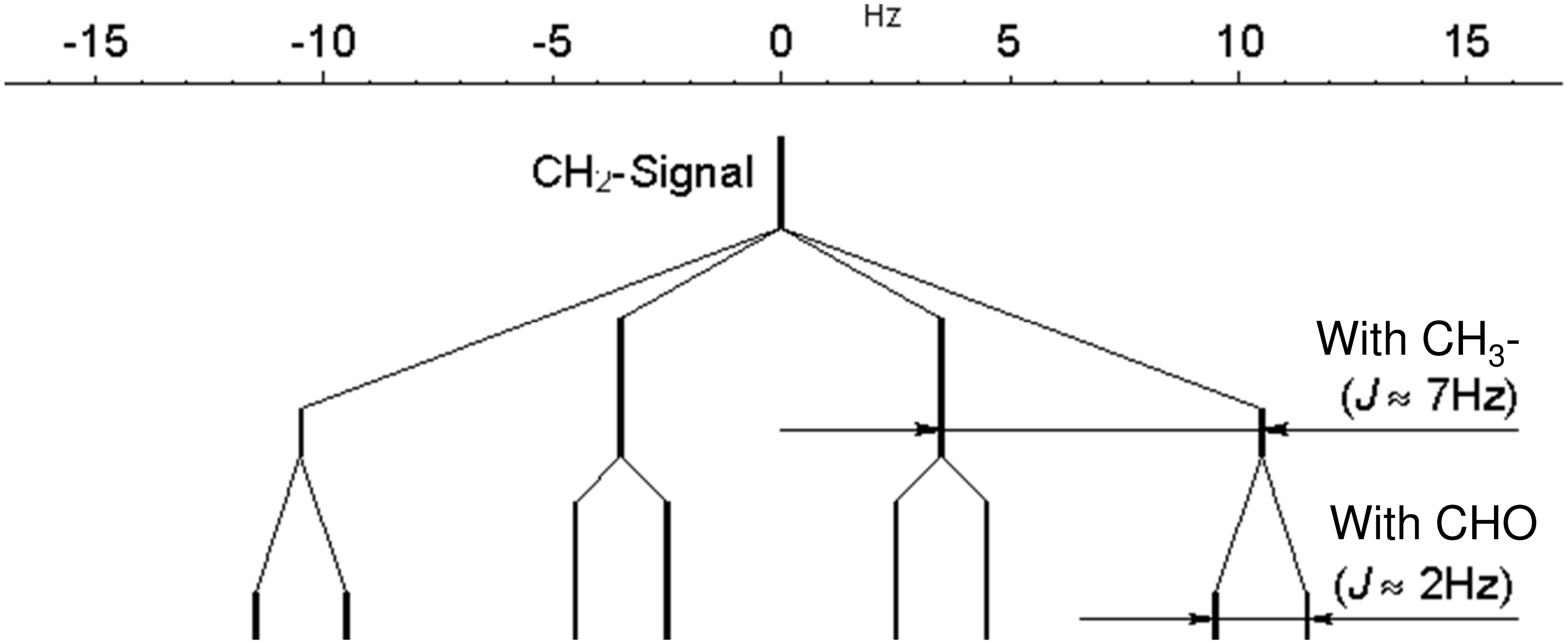
Coupling constants

Try to predict the spectrum of CH_3-CH_2-CHO

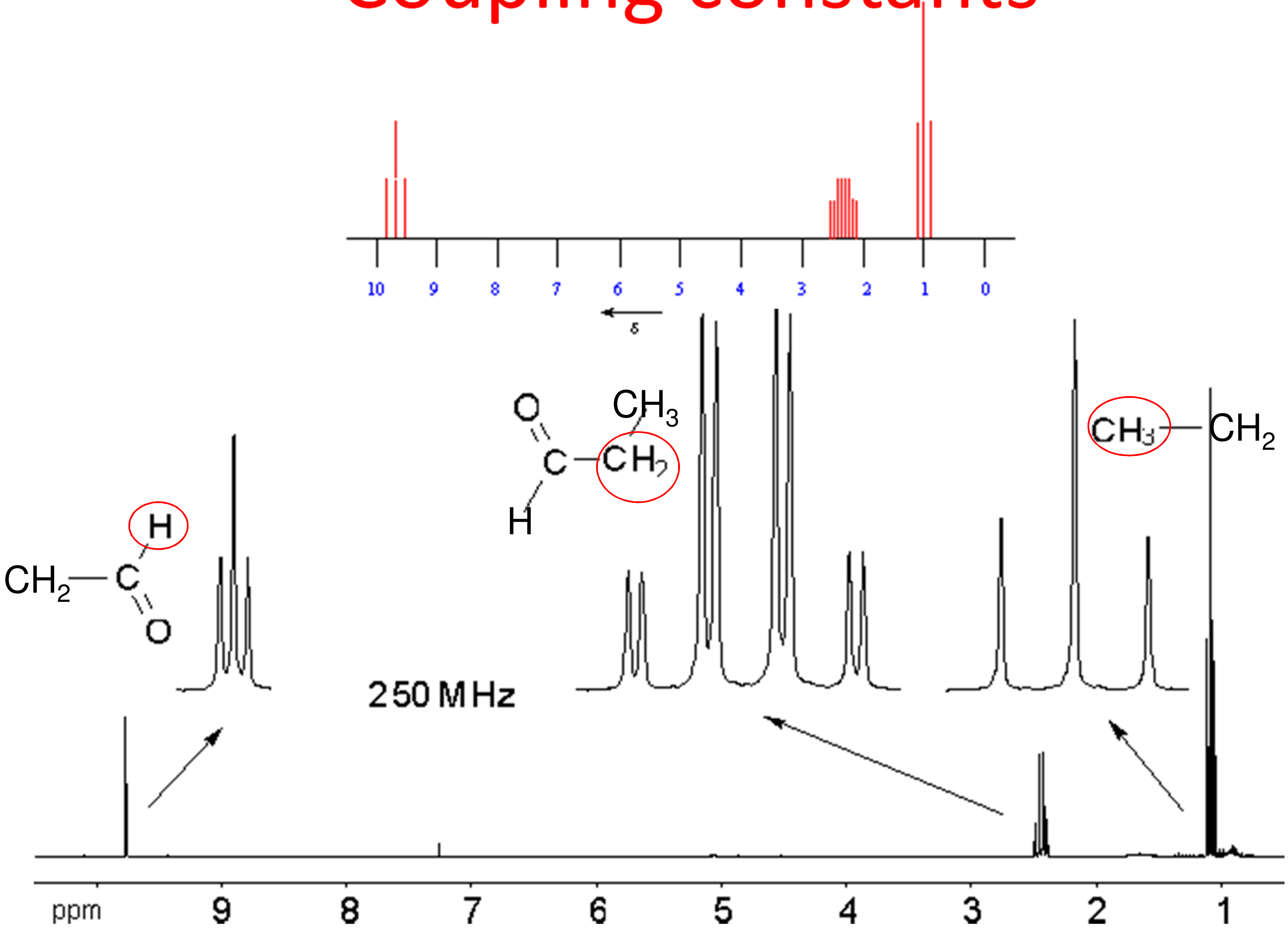
2. Determine the multiplicity of the signals and the relative intensities of the lines in a multiplet.

Group	**Coupling with**	***J* [Hz]**	Multiplicity	Intensity
CH_3-CH_2-CHO	CH_3-CH_2-CHO	**≈ 7**	3	1:2:1
CH_3-CH_2-CHO	CH_3-CH_2-CHO + CH_3-CH_2-CHO	**≈ 7** ≈ 2	4*2	see below
CH_3-CH_2-CHO	CH_3-CH_2-CHO	≈ 2	3	1:2:1

Coupling constants

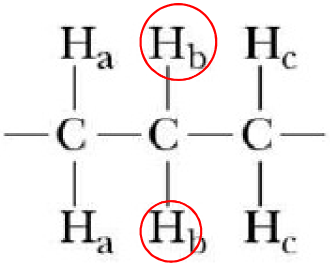


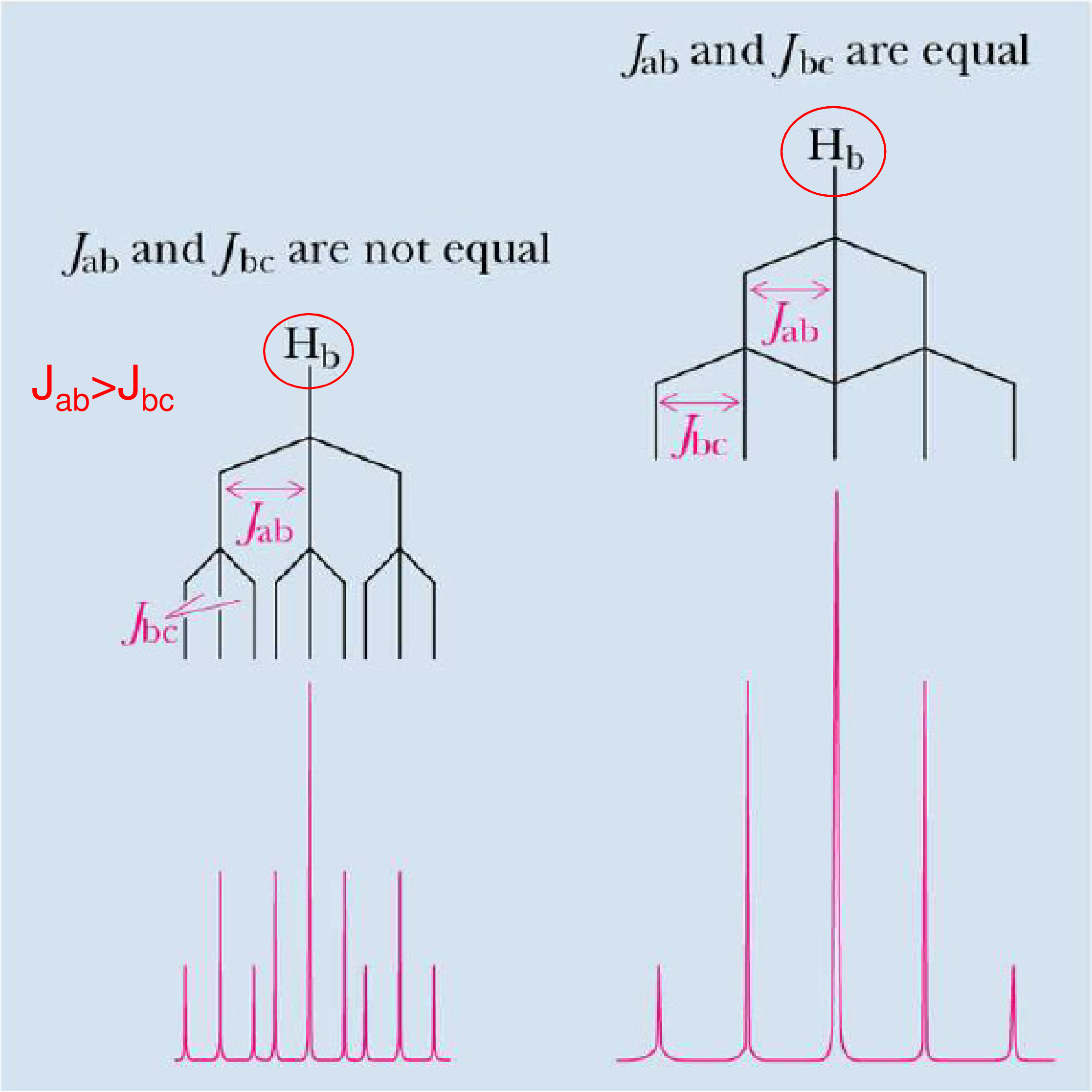
Coupling constants



Nuclear Magnetic Resonance Spectroscopy

Complex Splitting Patterns:Non-equivalent coupling




$J_{ab}>J_{bc}$

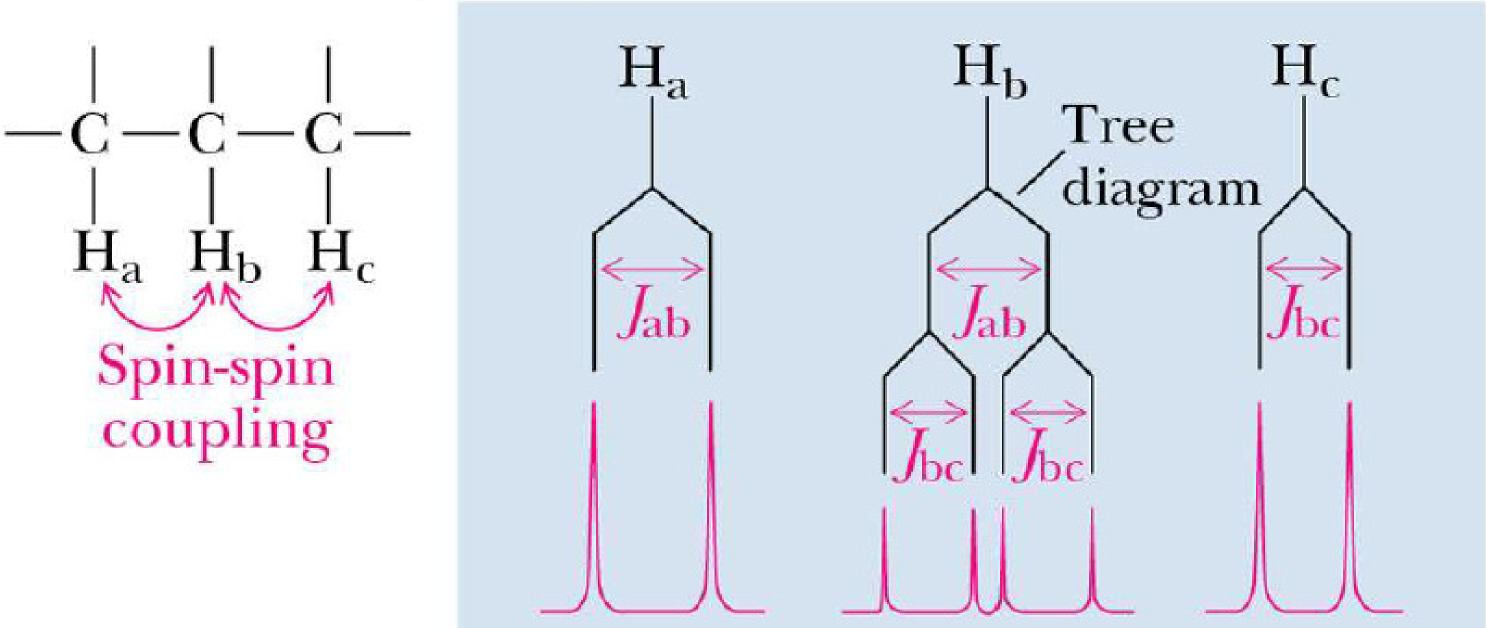
Nuclear Magnetic Resonance Spectroscopy

Complex Splitting Patterns:Non-equivalent coupling

thus far, we have concentrated on spin-spin coupling with only one other nonequivalent set of H atoms

more complex splittings arise when a set of H atoms couples to more than one set H atoms

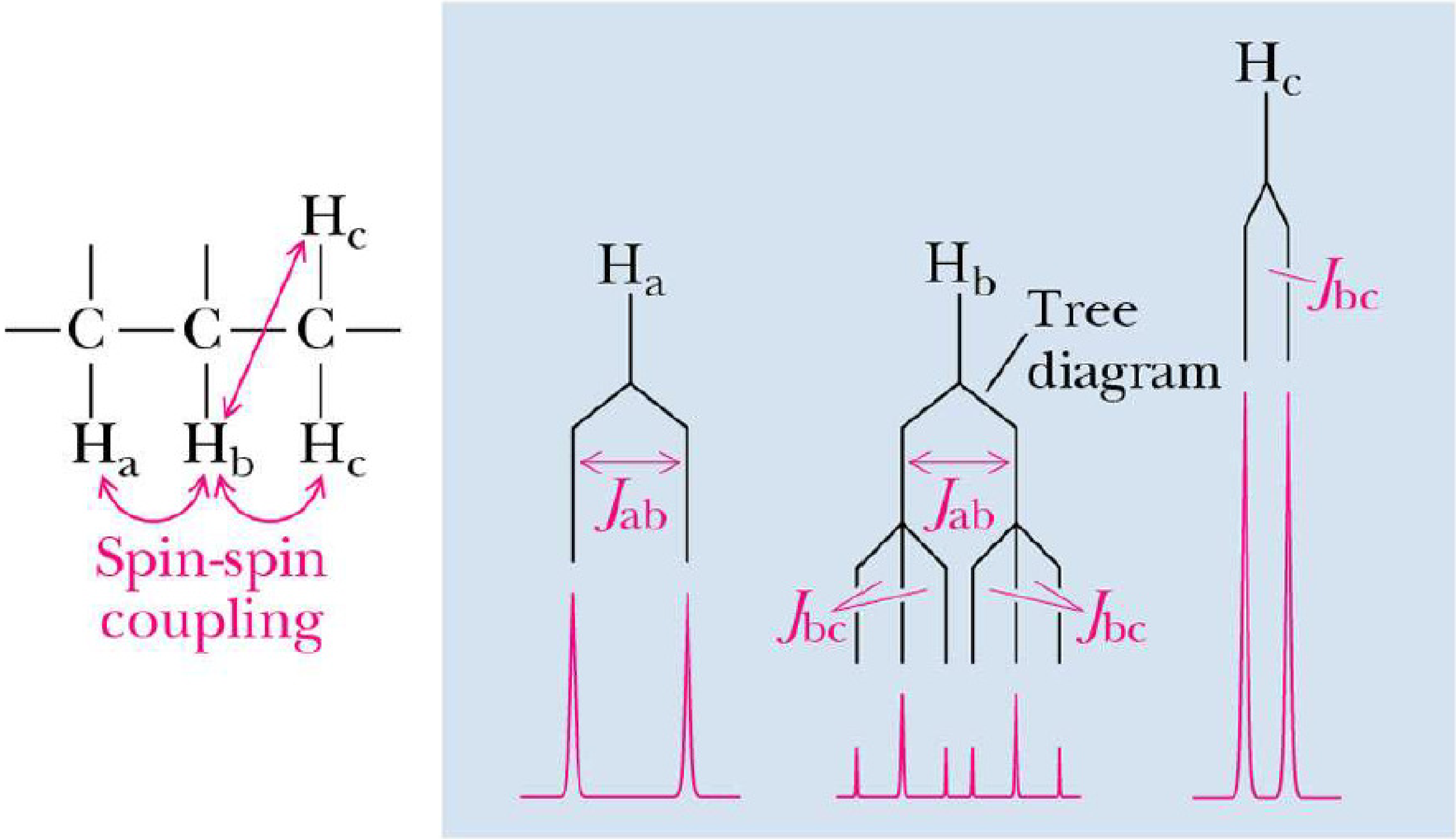
a tree diagram shows that when H_b is adjacent to nonequivalent H_a on one side and H_c on the other, the resulting coupling gives rise to a doublet of doublets ($J_{ab} > J_{bc}$)



Nuclear Magnetic Resonance Spectroscopy

Complex Splitting Patterns:Non-equivalent coupling

if H_c is a set of two equivalent H, then the observed splitting is a doublet of triplets

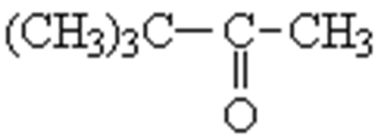


Pick the molecule that gives rise to the following ^{1}H NMR spectrum !

a)

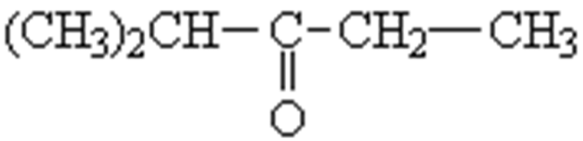
$(CH_3)_3C-CH_2-CHO$

b)

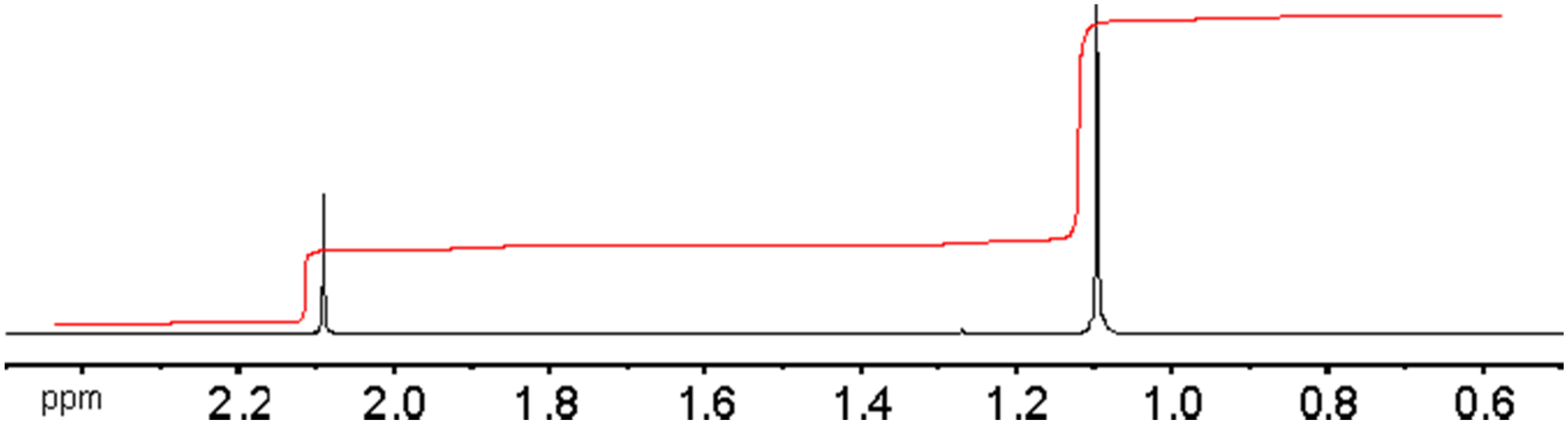


$(CH_3)_3C-\underset{\underset{O}{\|}}{C}-CH_3$

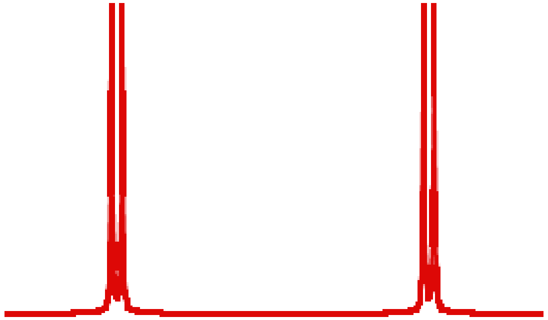
c)



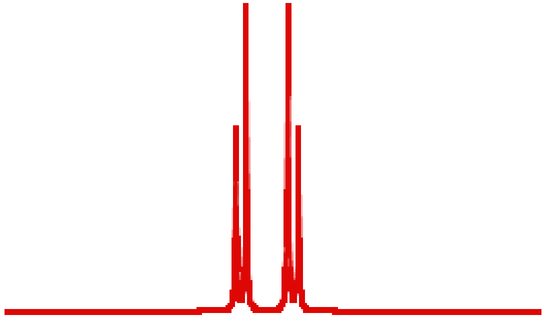
$(CH_3)_2CH-\underset{\underset{O}{\|}}{C}-CH_2-CH_3$



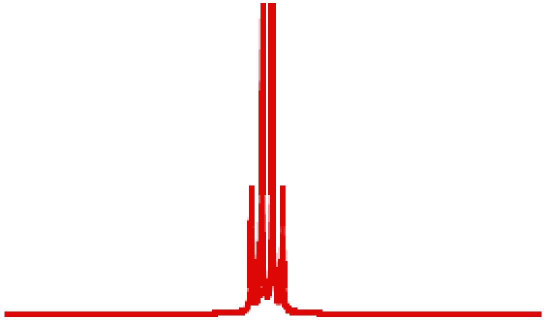
Strong coupling



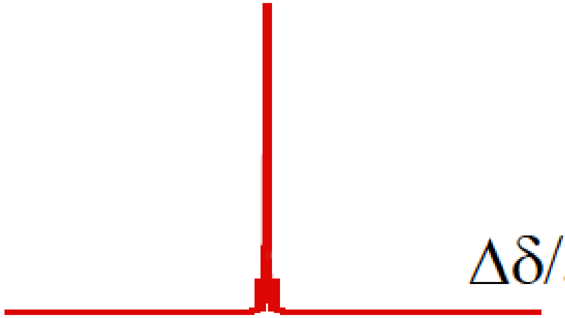
$\Delta\delta/J = 40$



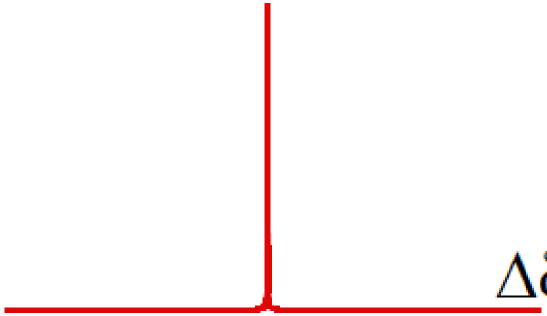
$\Delta\delta/J = 5$



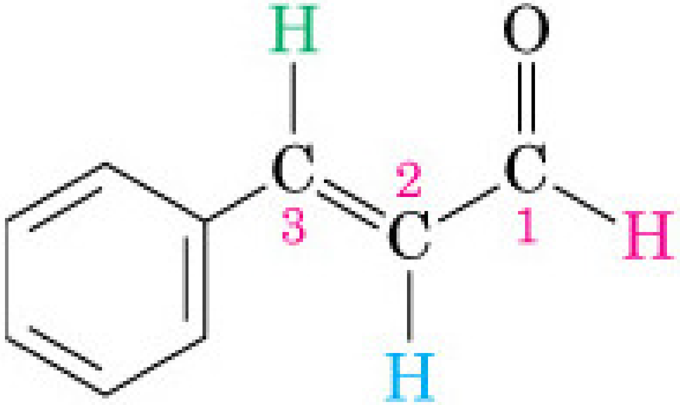
$\Delta\delta/J = 1.7$

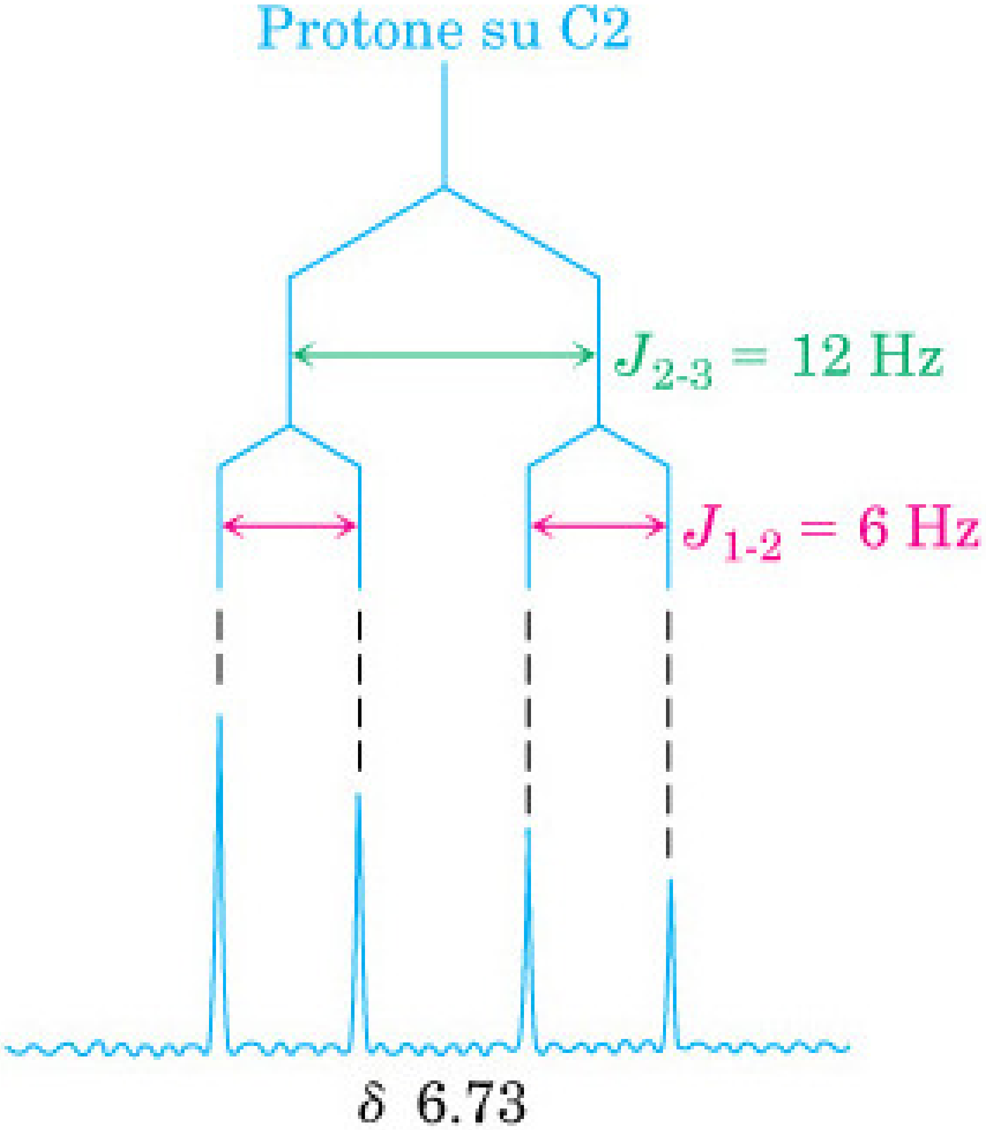


$\Delta\delta/J = 0.3$



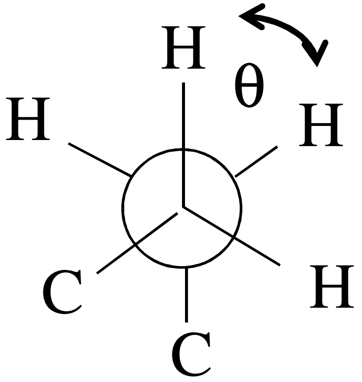
$\Delta\delta/J = 0$


H
O
C
2
C
3
C
1
H
H


Protone su C2
$J_{2\text{-}3} = 12$ Hz
$J_{1\text{-}2} = 6$ Hz
δ 6.73

Scalar Coupling

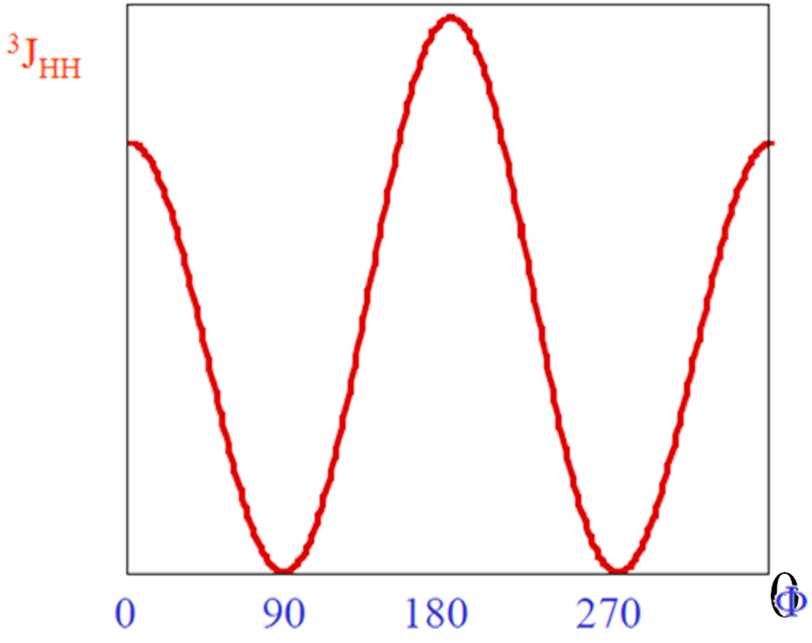
J depends from the dihedral angle between the two coupled nuclei, according to the Karplus equation



Karplus equation

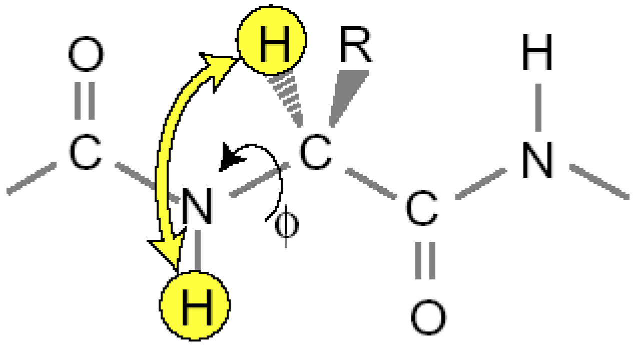
$$J = A + B\cos(\theta) + C\cos^2(\theta)$$

$$A = 1.9,\ B = -1.4,\ X = 6.4$$

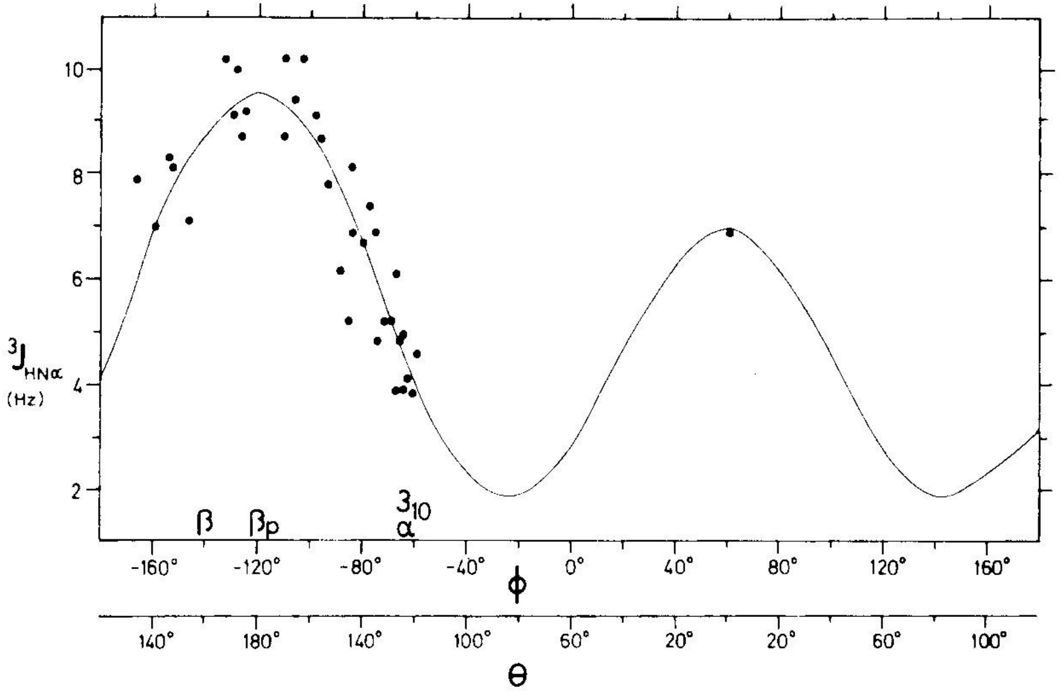


A, B e C empirical constants

Karplus equation

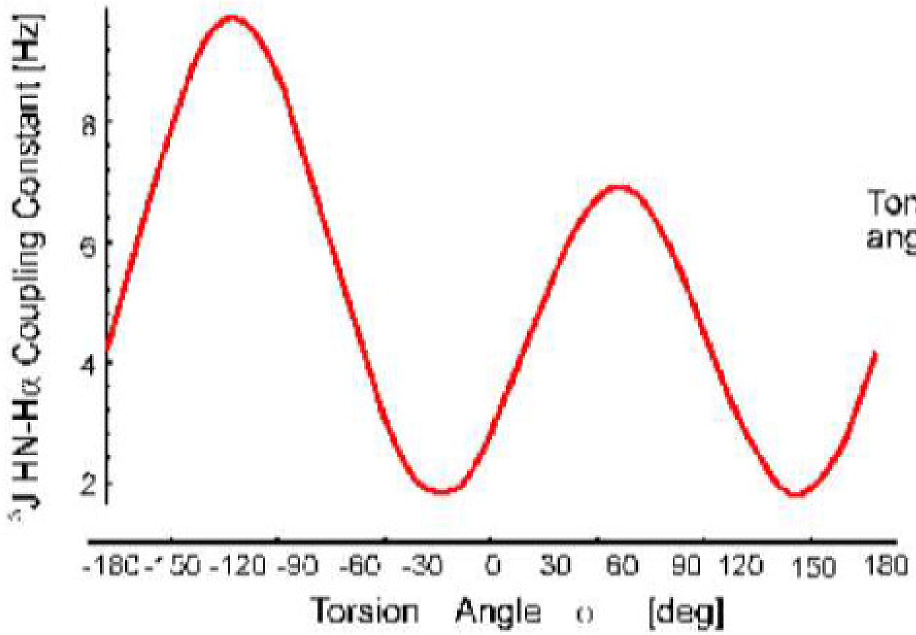


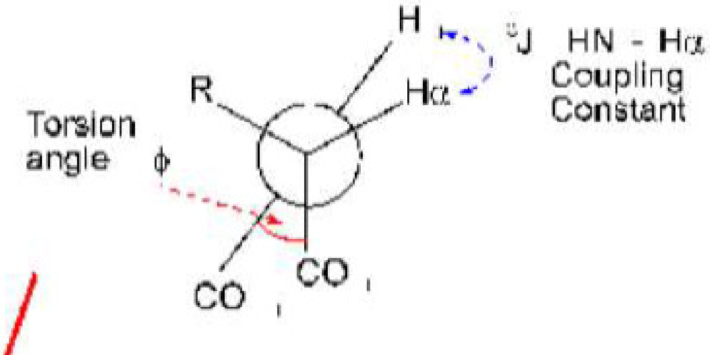
- comparison of ^3J values measured in solution with dihedral angles observed in crystal structures of the same protein allows one to derive *empirical* Karplus relations that give a good fit between the coupling constants and the angles



coupling constants in solution vs. φ angles from crystal structure for BPTI

Scalar Couplings





Newman Projection of the Cα - N Bond of a Protein

^3J(HN-HA) = 4 – 11 Hz depends on secondary structure.

< 6 Hz → α-helix

> 8 Hz → β-stand

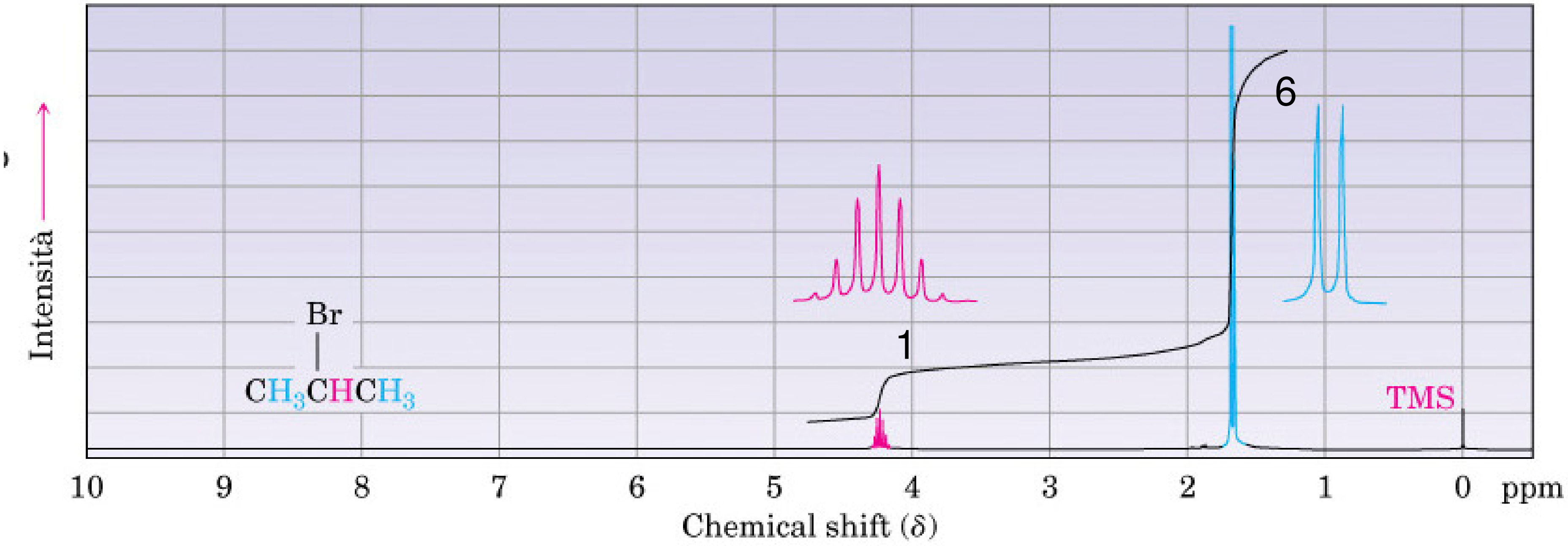
Determination of coupling constants

- The value of the coupling constant *J* depends on the *distance* between the two coupling nuclei, as well as their *orientation* towards each other and *character of the bonds* between the two coupling nucleii

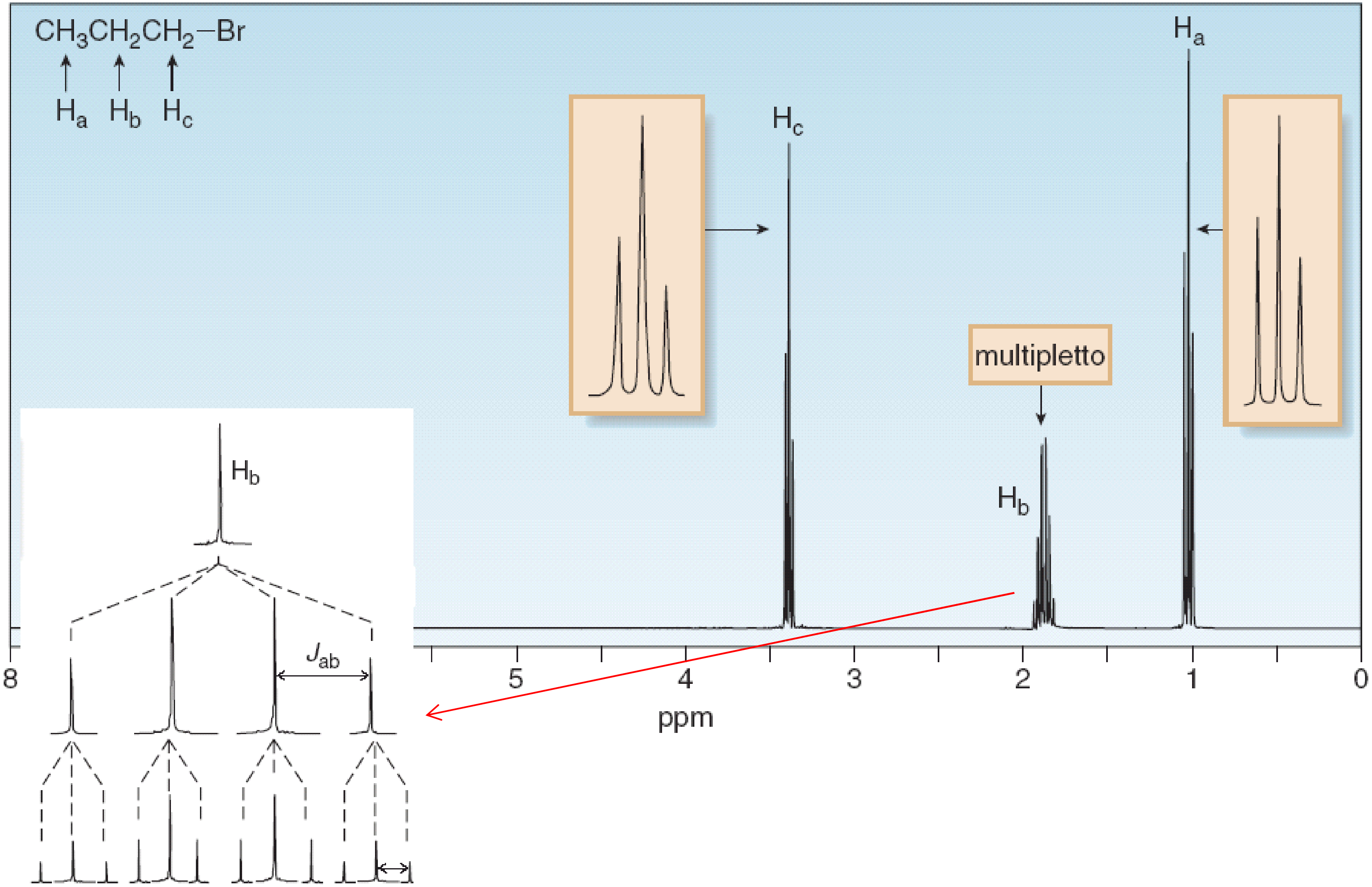
- In the case of σ bonds spin-spin couplings typically can only be observed *across three bonds or fewer* (*e.g.,* H-C-C-H). If there are four bonds between the two coupling nucleii, the coupling constant typically already drops towards zero.

- So-called *long range couplings* across a larger number of bonds typically only occurs if more polarizable *π-bond systems* are involved. (*e.g.,* H-C=C-C-H).

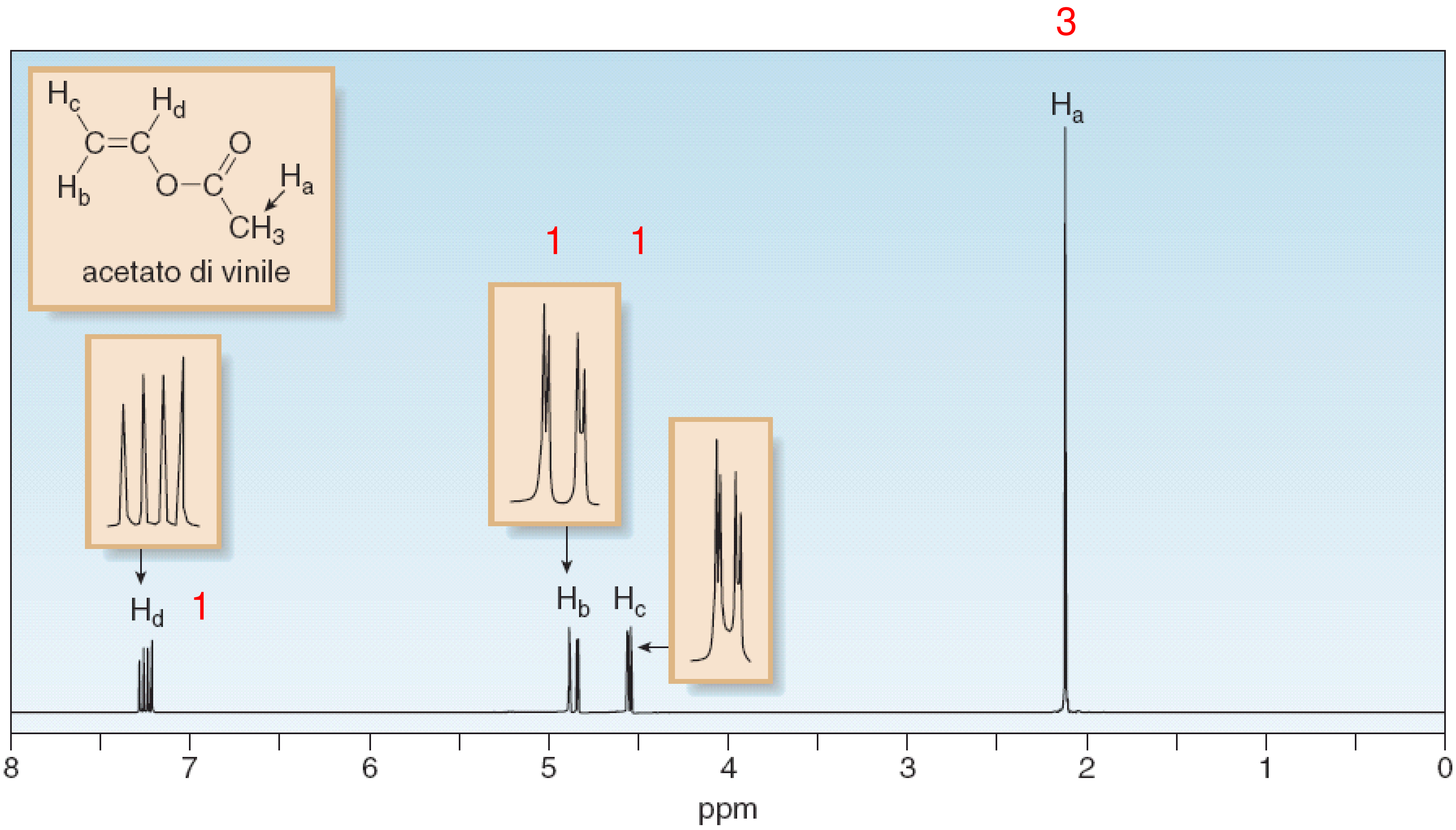
^{1}H NMR of C_3H_7Br



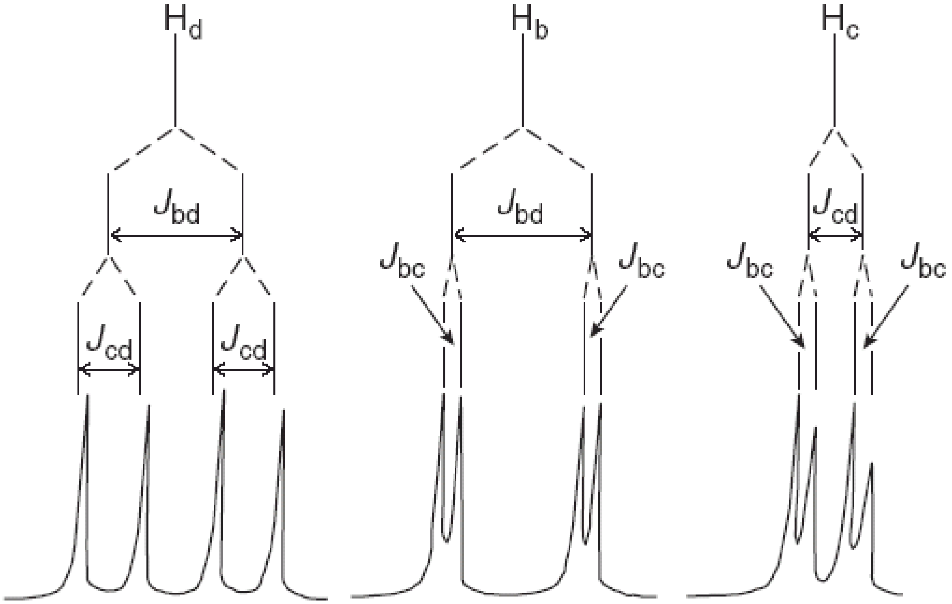
C_3H_7Br



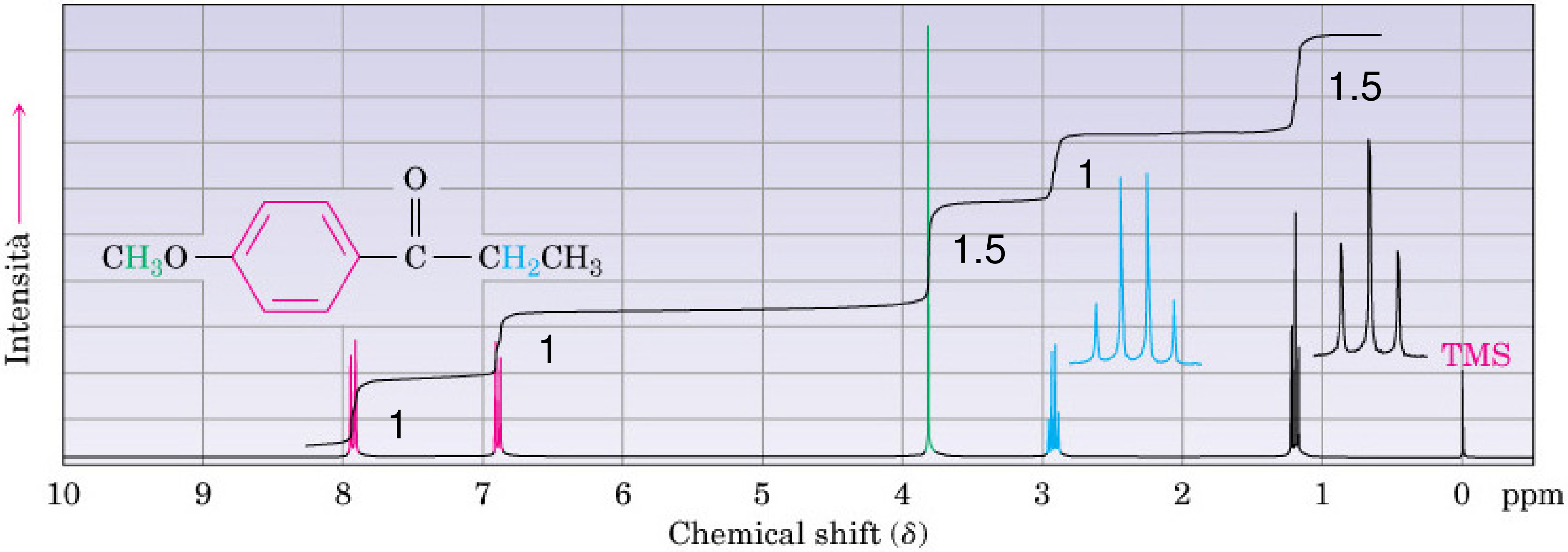
$C_4H_6O_2$


3
H$_c$
H$_d$
C=C
O
H$_b$
O–C
H$_a$
CH$_3$
acetato di vinile
1
1
H$_a$
H$_d$
1
H$_b$
H$_c$
8
7
6
5
4
3
2
1
0
ppm

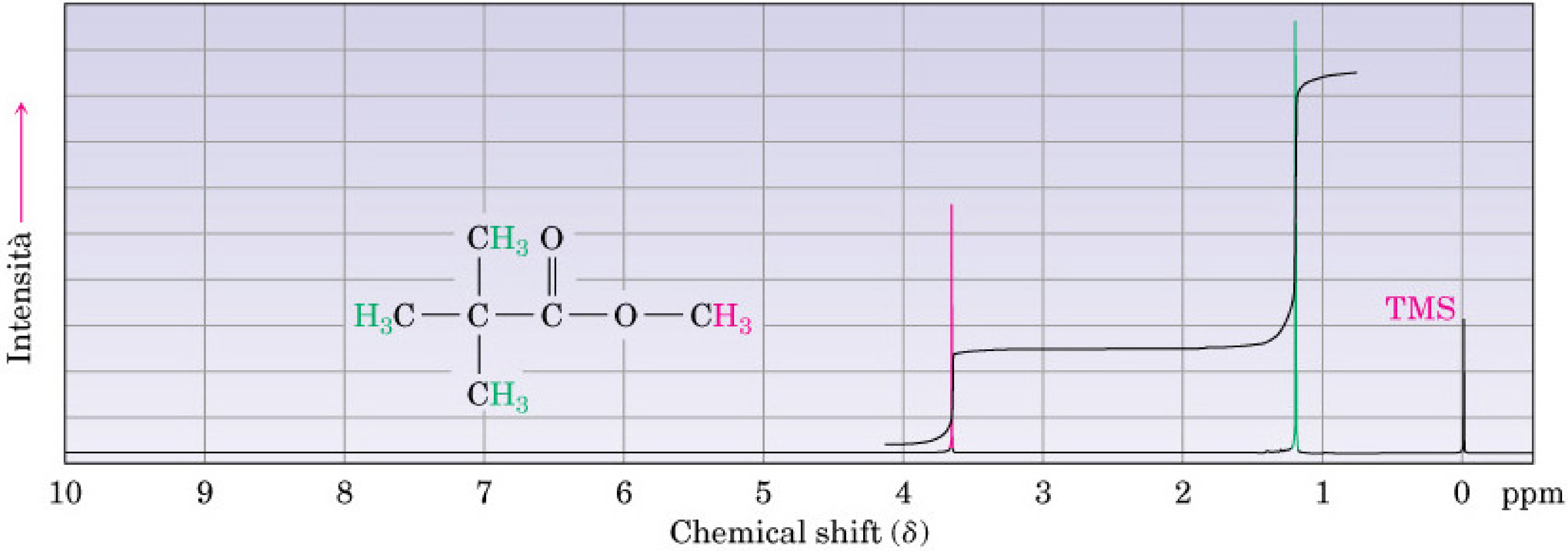
J_{bc} = 1.2 Hz (germinale)
J_{cd} = 6.5 Hz (cis)
J_{bd} = 14 Hz (trans)

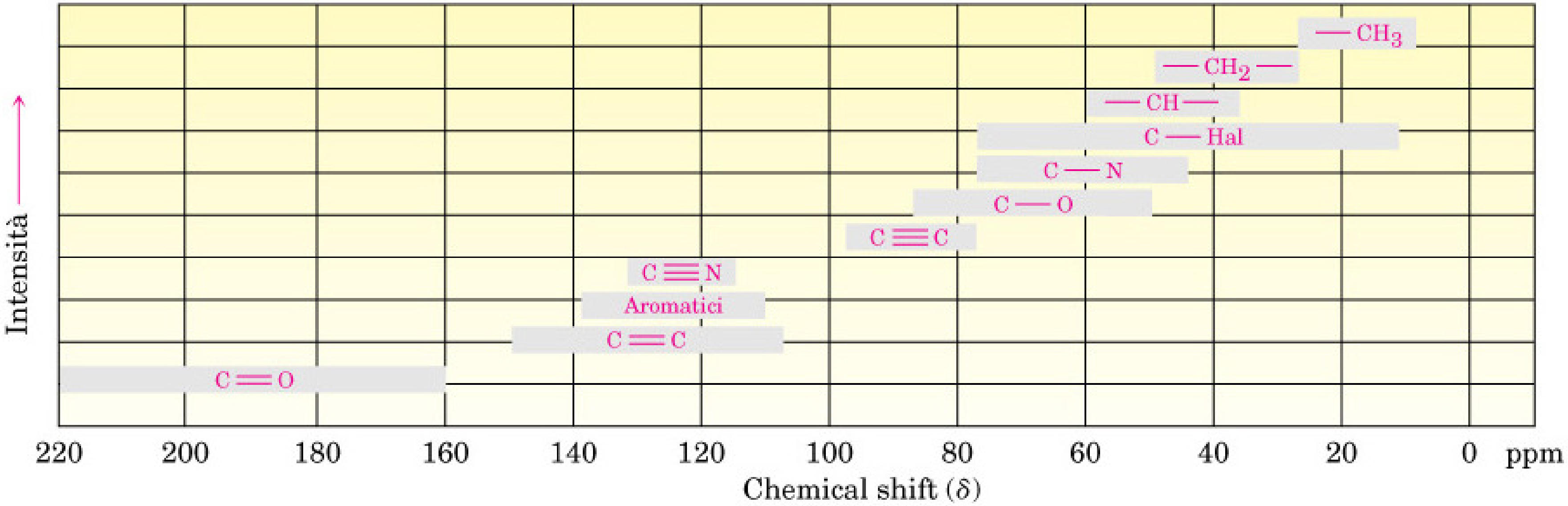


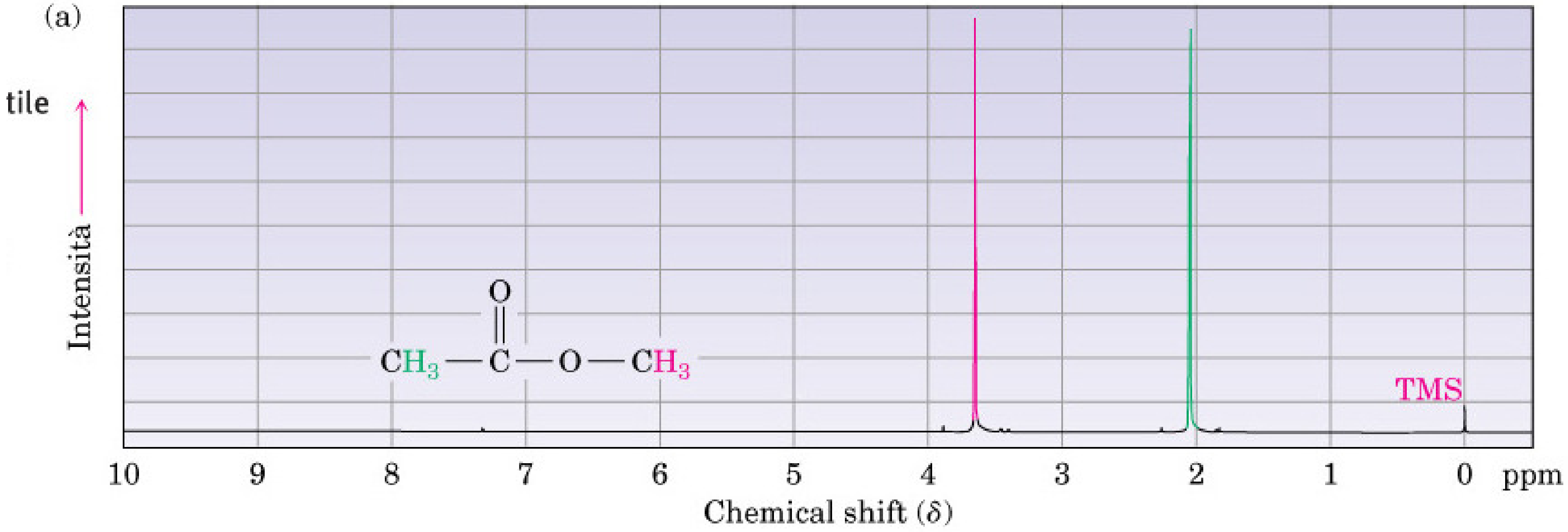
Spettro ^1H di $C_{10}H_{12}O_2$

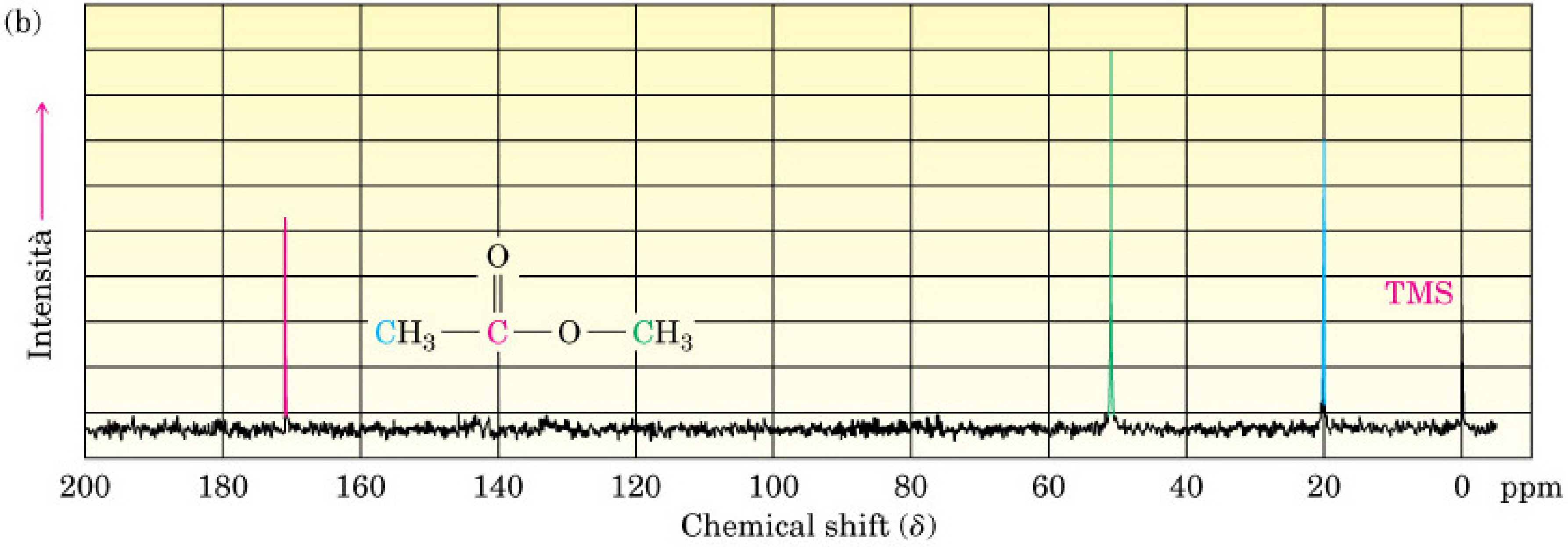


Spettro ^1H di $C_6H_{12}O_2$

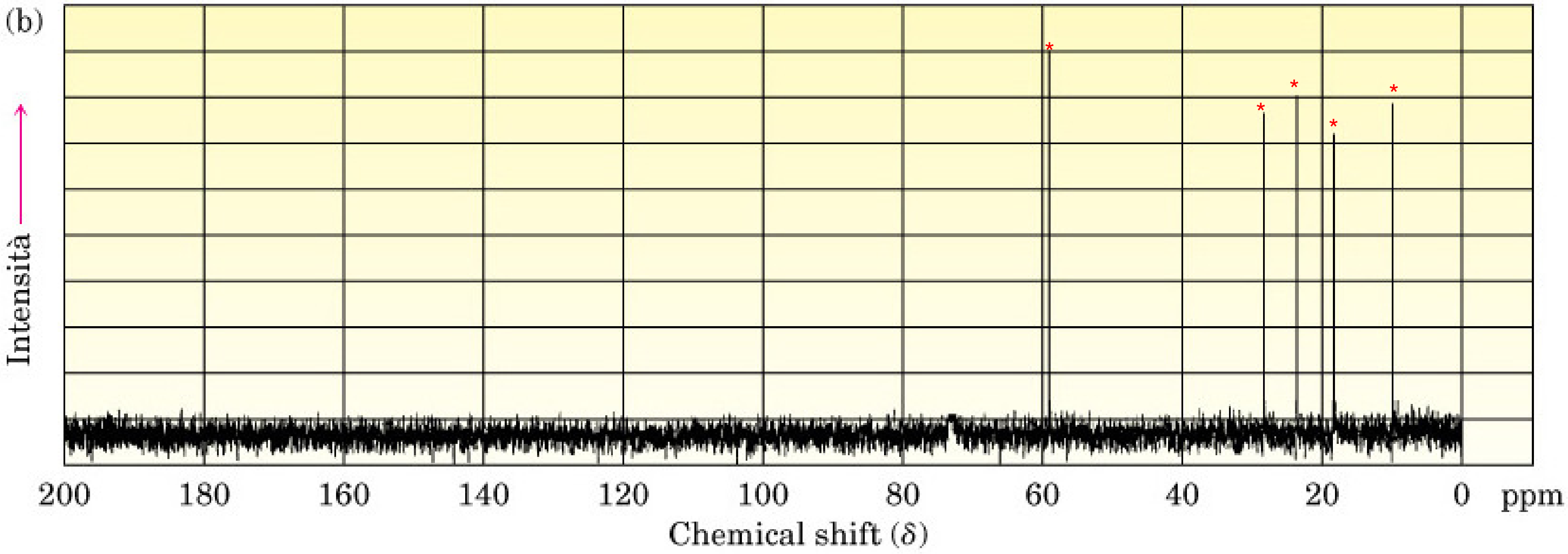


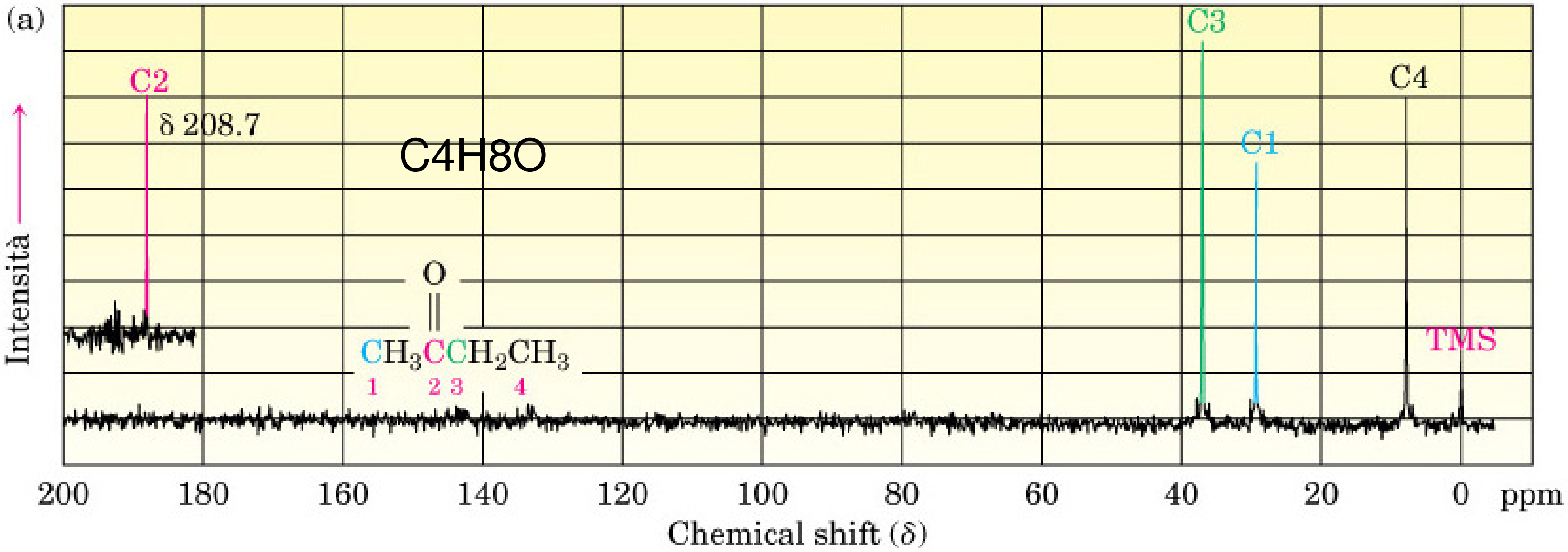

— CH$_3$
— CH$_2$ —
— CH —
C — Hal
C — N
C — O
C ≡ C
C ≡ N
Aromatici
C = C
C = O
Intensità
220
200
180
160
140
120
100
80
60
40
20
0
ppm
Chemical shift (δ)

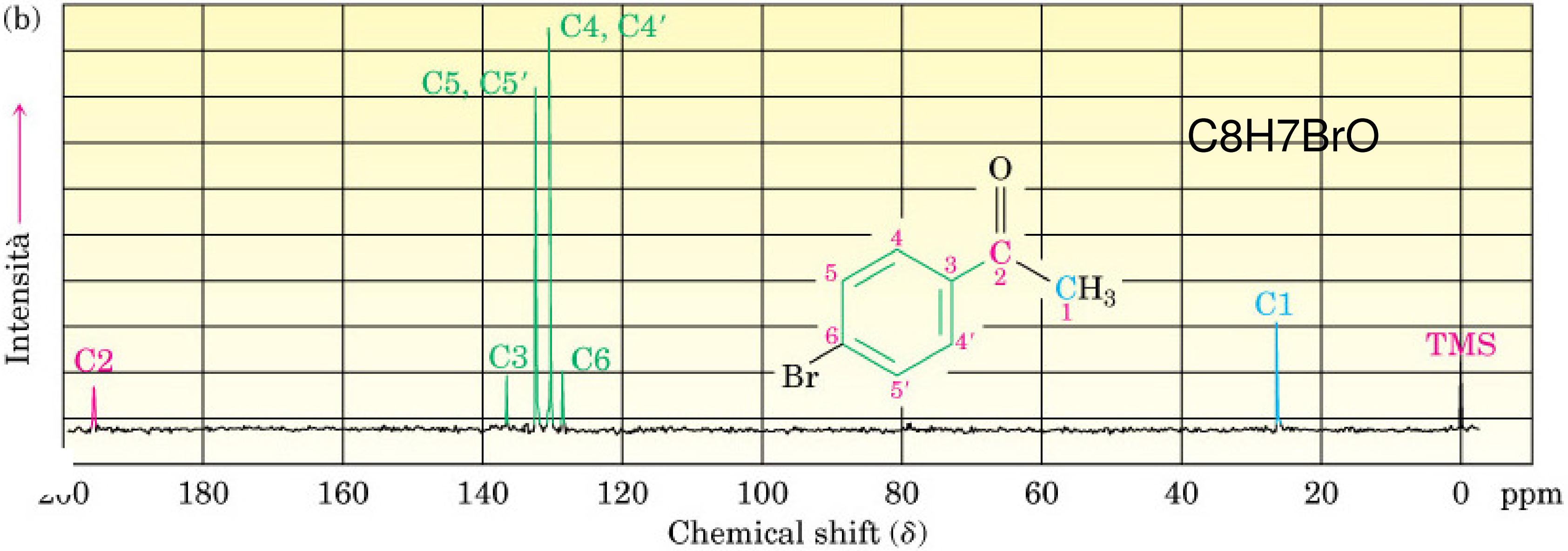

(a)
tile
Intensità
O
CH3 — C — O — CH3
TMS
10
9
8
7
6
5
4
3
2
1
0
ppm
Chemical shift (δ)


(b)
Intensità
O
CH3 — C — O — CH3
TMS
200
180
160
140
120
100
80
60
40
20
0
ppm
Chemical shift (δ)

$CH_3CH_2CH_2CH_2CH_2OH$

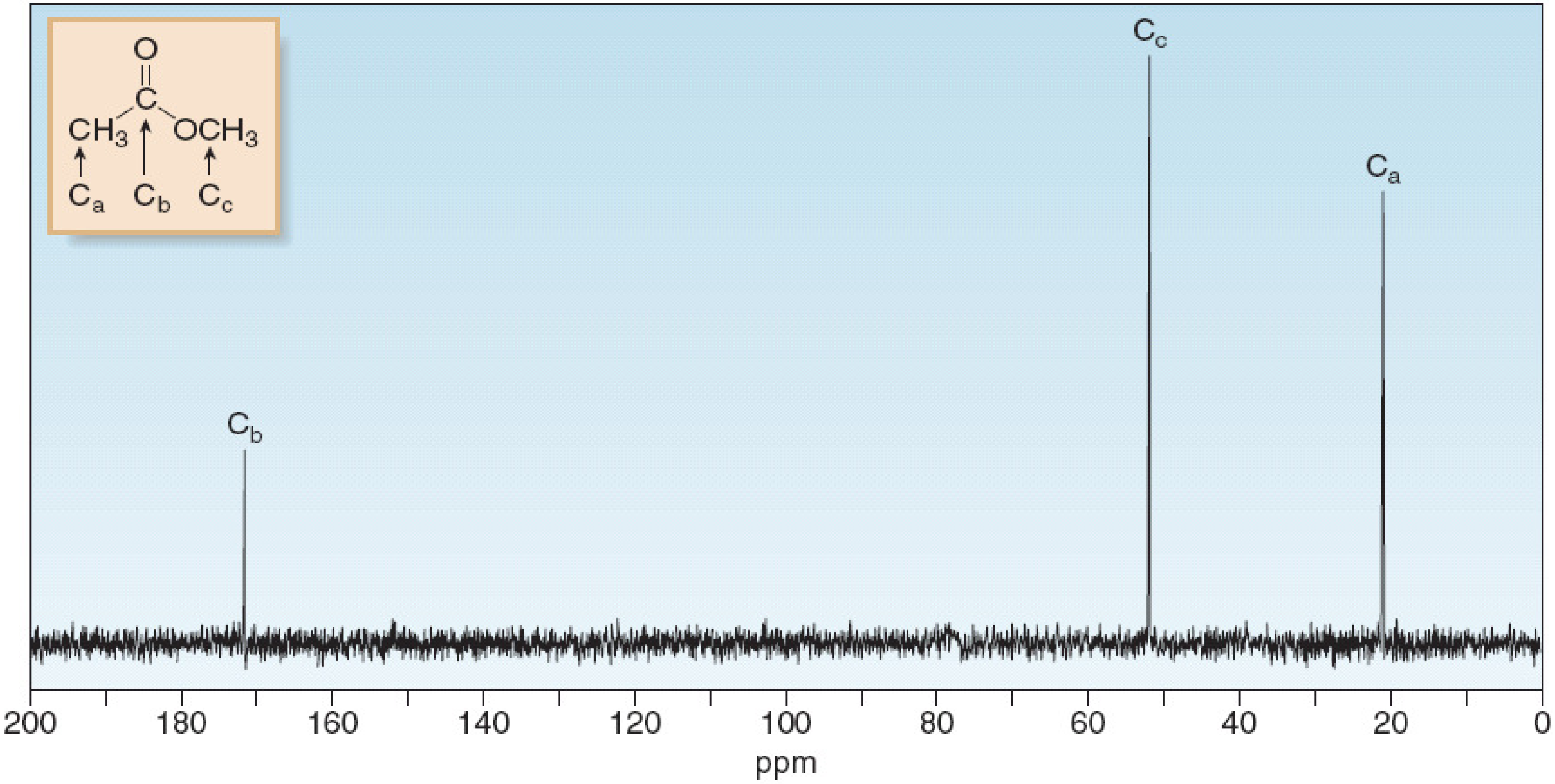



(a)
C2
δ 208.7
C4H8O
C3
C4
C1
TMS
O
CH3CCH2CH3
1 2 3 4
Intensità
200 180 160 140 120 100 80 60 40 20 0 ppm
Chemical shift (δ)


(b)
C4, C4′
C5, C5′
C8H7BrO
O
4
3
C
2
5
CH3
1
6
4′
Br
5′
C1
TMS
C2
C3
C6
Intensità
200 180 160 140 120 100 80 60 40 20 0 ppm
Chemical shift (δ)

$C_3H_6O_2$

[b] Metil acetato



Scalar Couplings

- Coupling constant values are field independent (Hz)
- They are classified according to the number of chemical bonds mediating the two nuclei:

H-C	^1J	120-250 Hz
H-N	^1J	50-140 Hz
H-C-H	^2J	2-15 Hz
H-C-C-H	^3J	1-12 Hz

- ^nJ couplings with $5>n>3$ are only observable for certain systems (aromatics). For $n>6$ they are not observable.

J-couplings in peptides