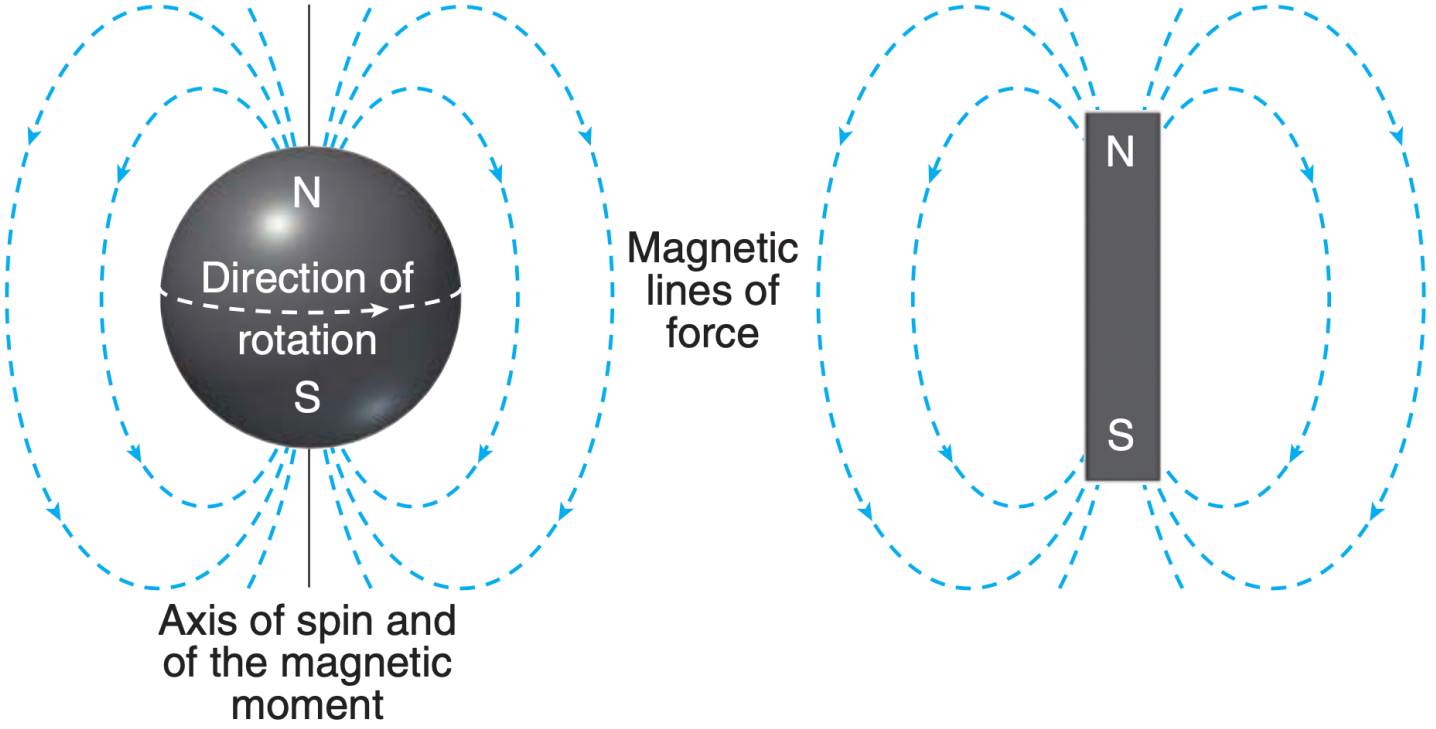
Inter-Lecture D

Nuclear Magnetic Resonance Spectroscopy

А.И.Соч

2022/3/31

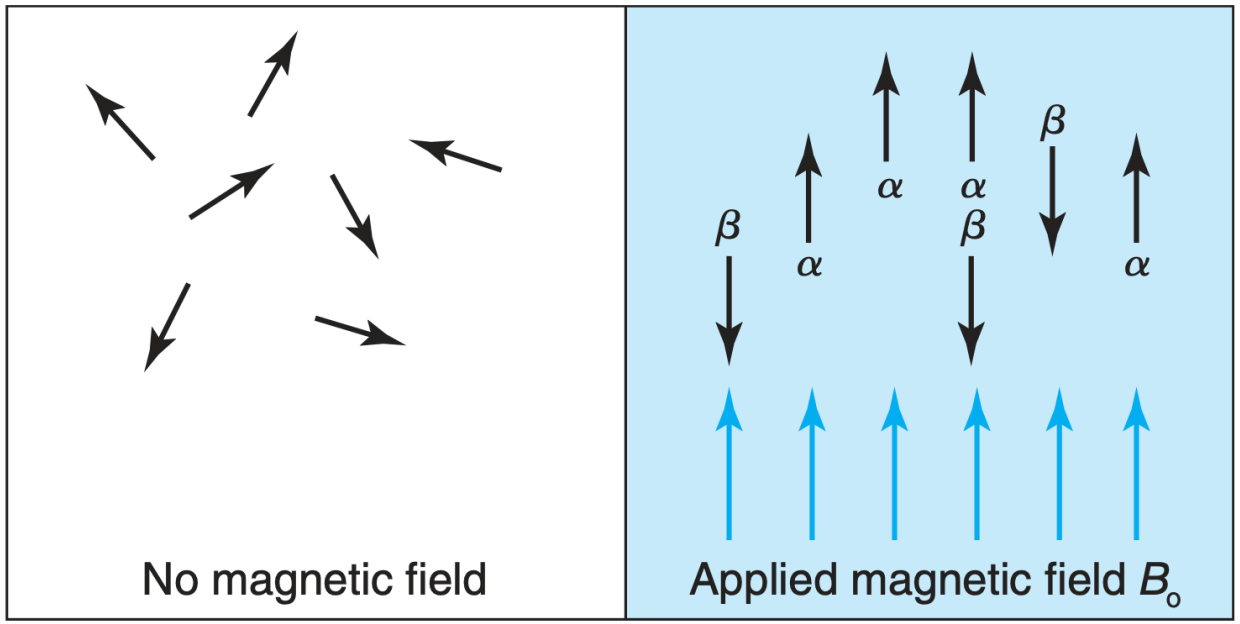
- Nuclear spin and magnetic moment

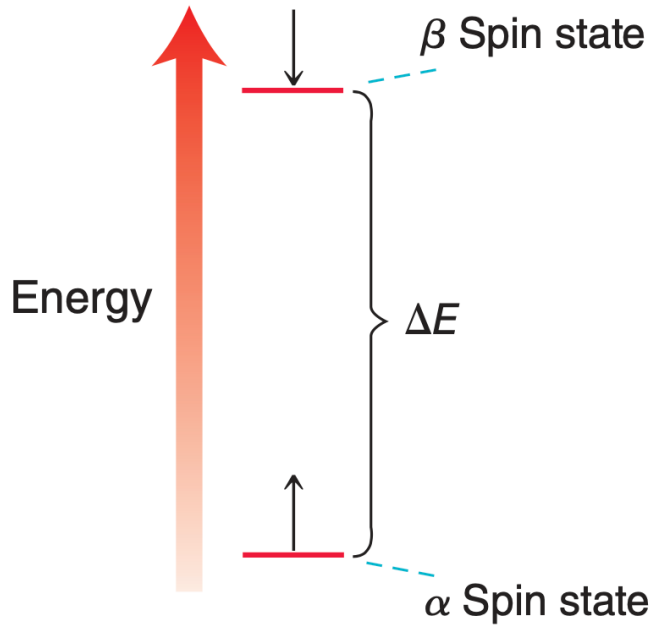


- Nuclear spin and nuclear numbers

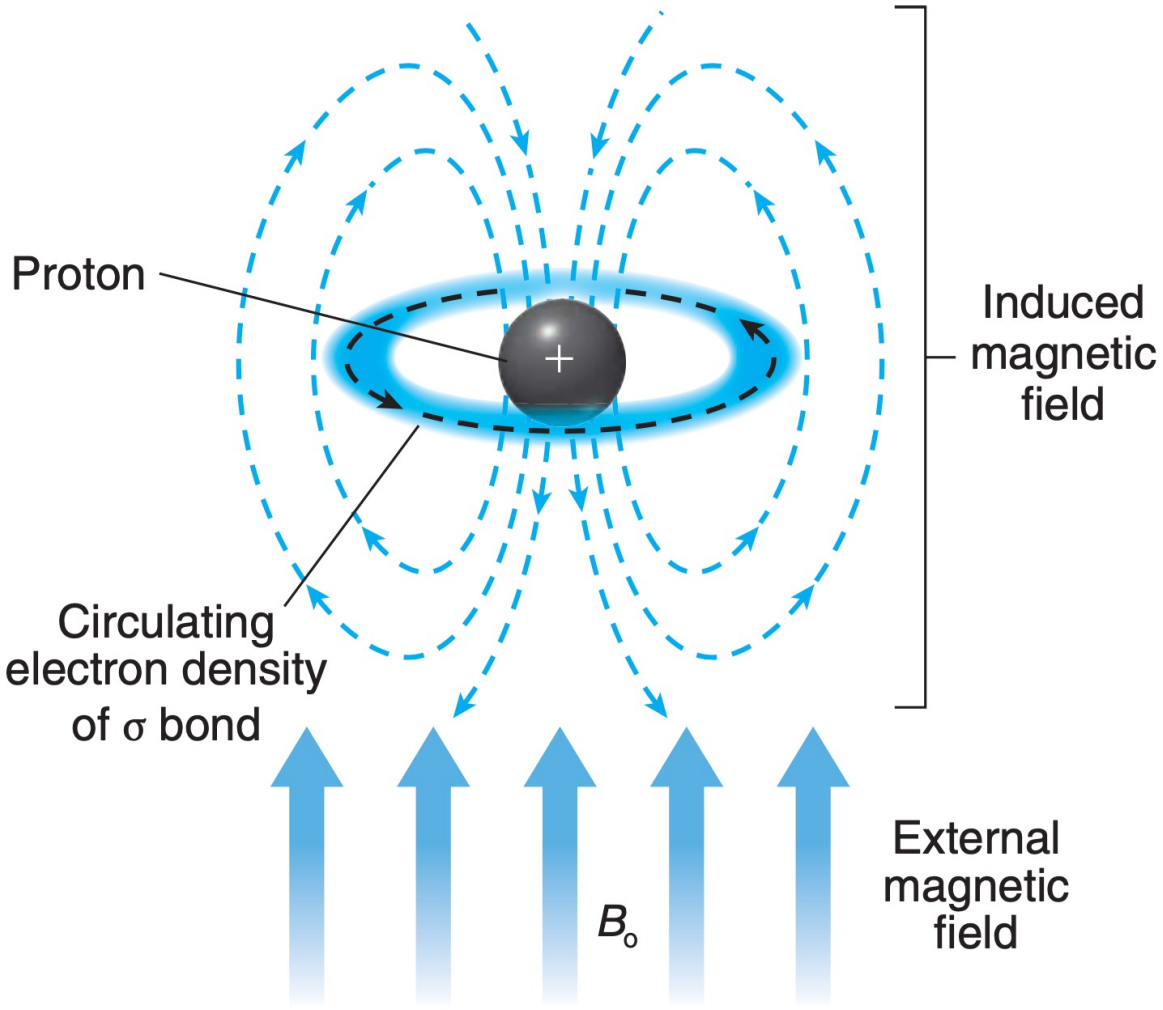
Atomic Weight	Proton Number	Spin Quantum Number (I)	NMR Signal
even	even	0	no
even	odd	$1,\ 2,\ 3, \ldots\ (I \in \mathbb{Z})$	yes
odd	odd / even	$\frac{1}{2},\ \frac{3}{2},\ \frac{5}{2}, \ldots\ (I = \frac{k}{2},\ k \in \mathbb{Z})$	yes

- External magnetic field and energy absorption

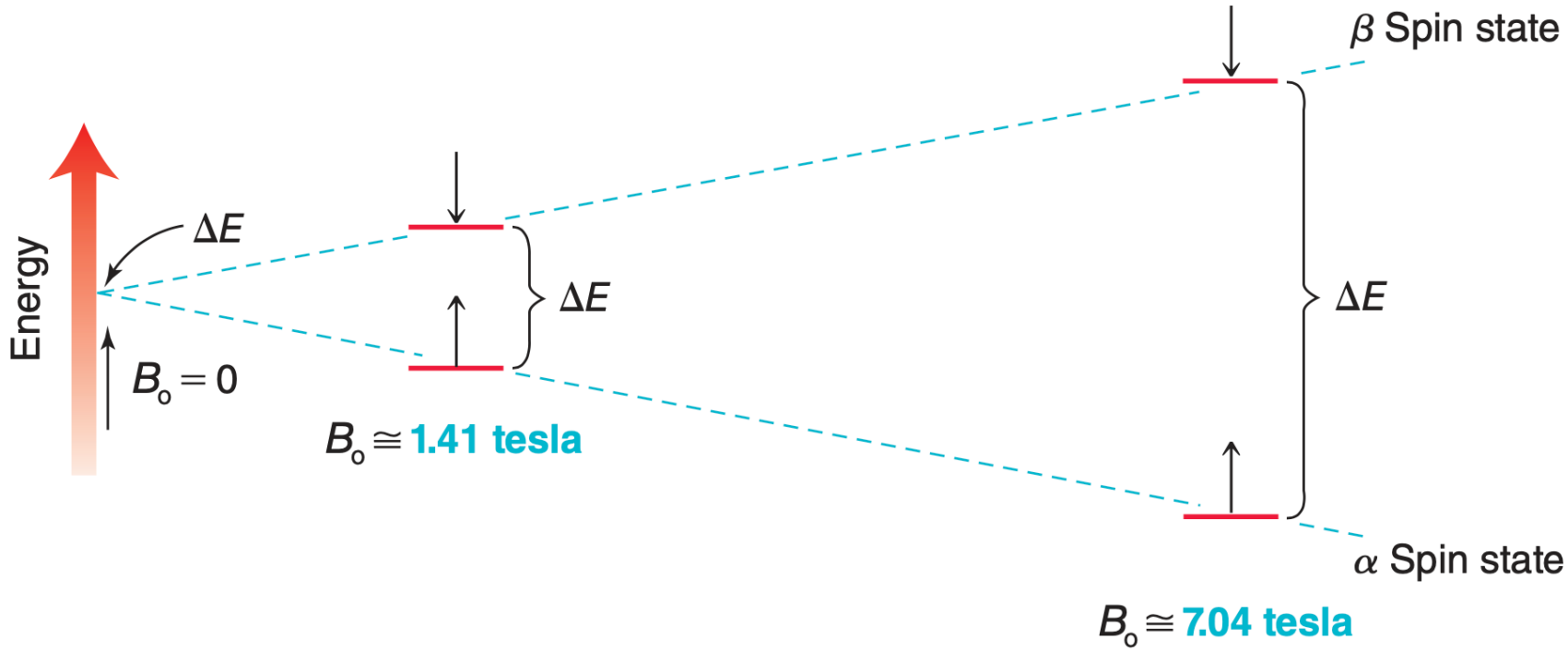




- Induced magnetic field – diamagnetism

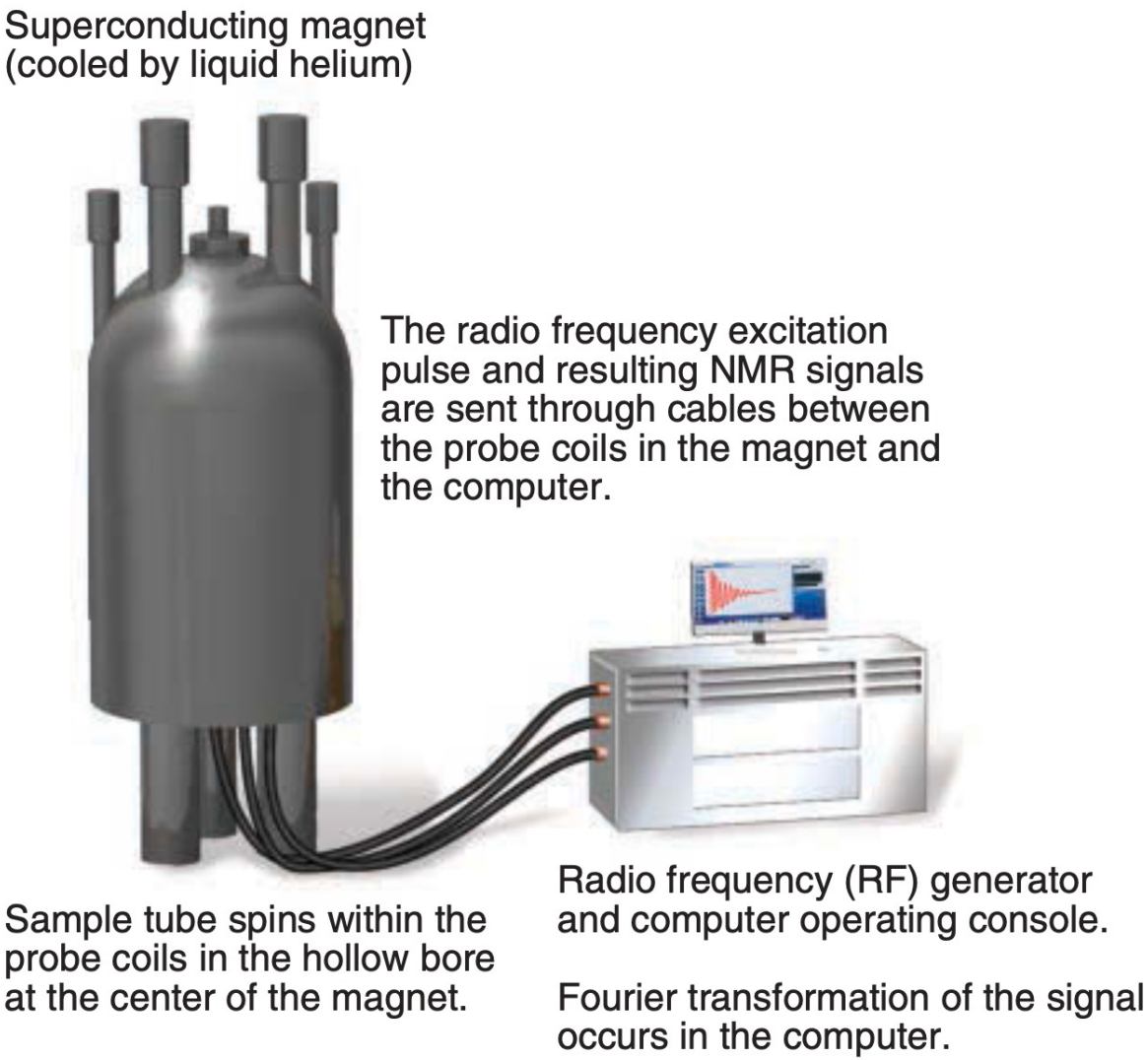


- Magnetic field strength

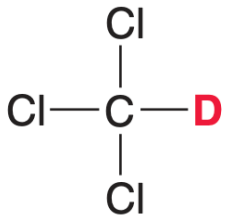


- NMR Spectrometers
  - **Continues-wave (CW) spectrometers**: holding the frequency of rf radiation constant and slowly increasing the magnetic field strength
  - **Fourier-transform NMR (FT-NMR):** holding the magnetic field constant and irradiating the sample with a short pulse that covers the entire range of relevant rf frequencies

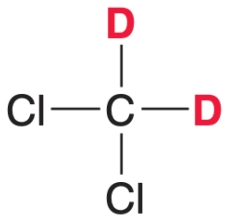
- FT-NMR
  - Detecting the free induction decay (FID)
  - Using Fourier transform to acquire frequency signal
  - Acquiring FID for hundreds of trials, then averaging



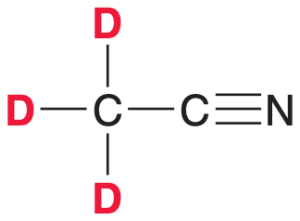
- Sample preparation



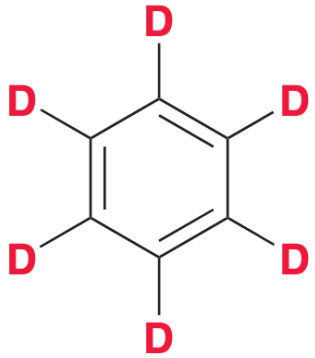
Chloroform-d



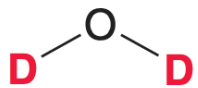
Methylene chloride-d_2



Acetonitrile-d_3



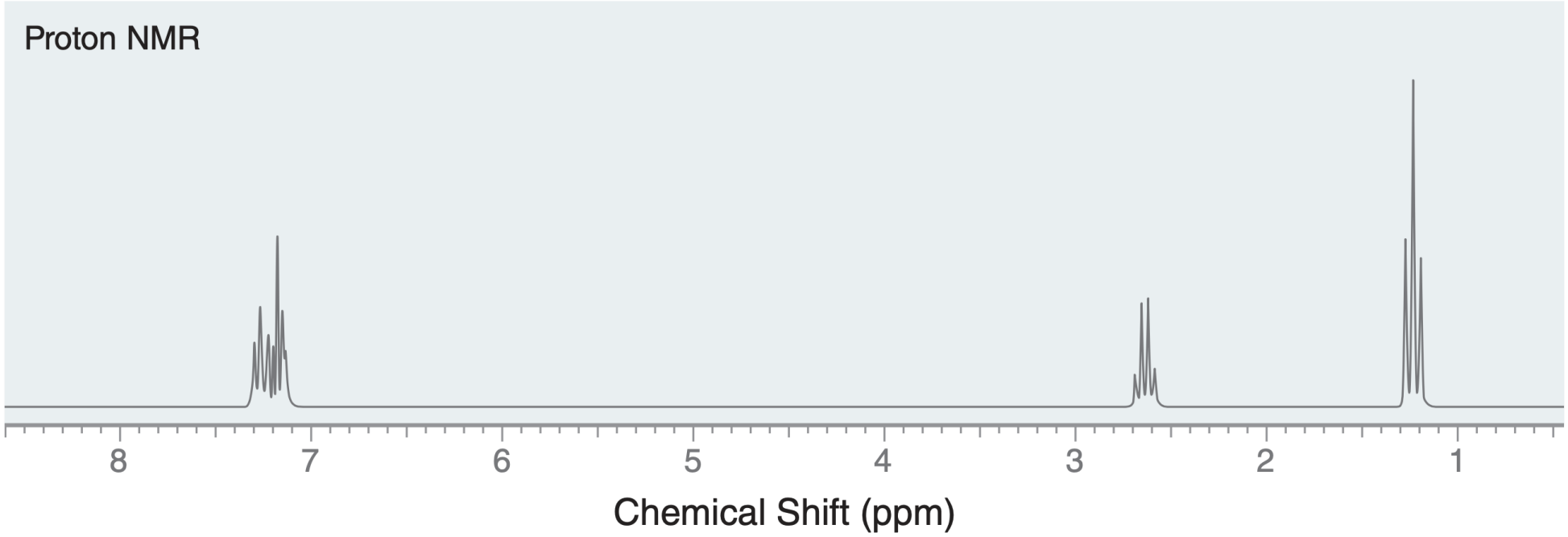
Benzene-d_6



Deuterium oxide

using deuterated solvents to prevent solvent signal

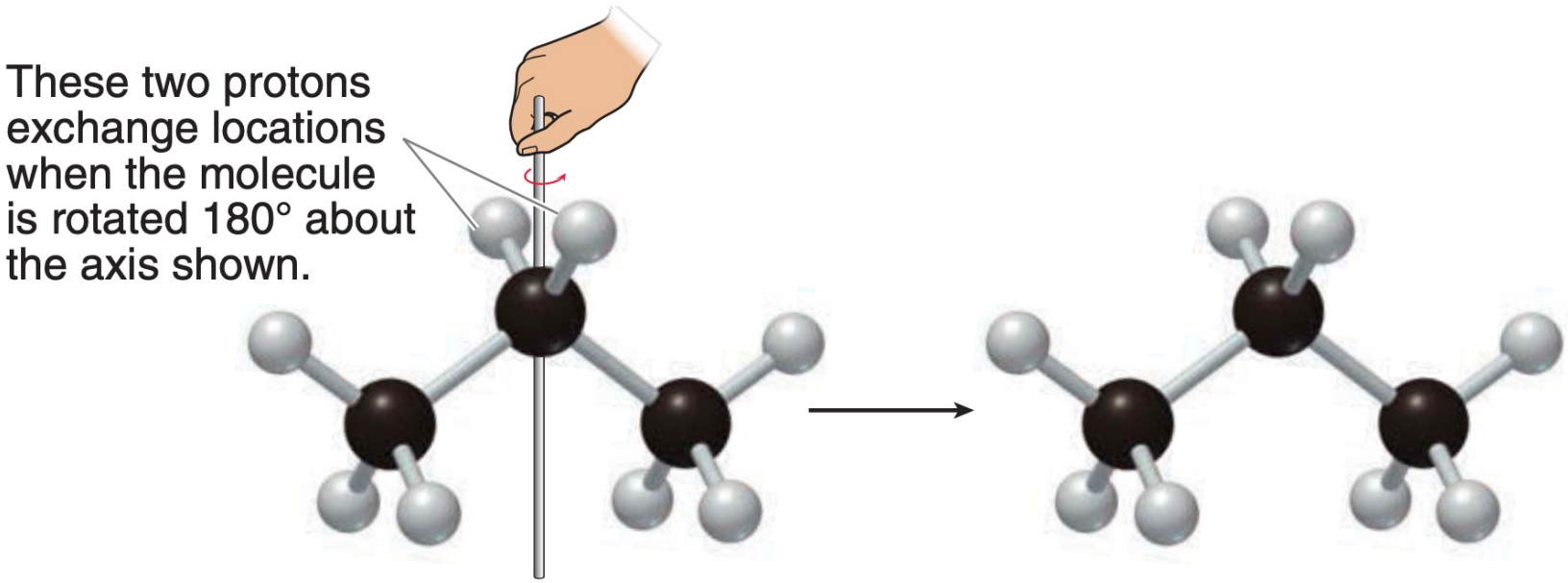
- A typical ^{1}H NMR spectrum



- Characteristics of a ^{1}H NMR spectrum
  - **Location of each signal** – indicating the electronic environment of the protons giving rise to the signal
  - **Area under each signal** – indicating the number of protons giving rise to the signal
  - **Shape of the signal** – indicating the number of neighboring protons

- Chemical equivalence
  - The number of signals in a ^{1}H NMR spectrum indicates the number of different kinds of protons (protons in different electronic environments)
  - Protons that occupy identical electronic environments are called **chemically equivalent**
  - Chemically equivalent protons produce only one signal in ^{1}H NMR
  - Symmetry operation (rotation / reflection) can be used to determine chemical equivalence

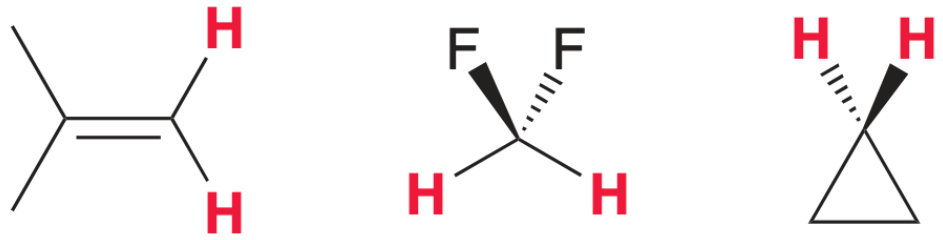
- Homotopic protons – *interchangeable by rotational symmetry*



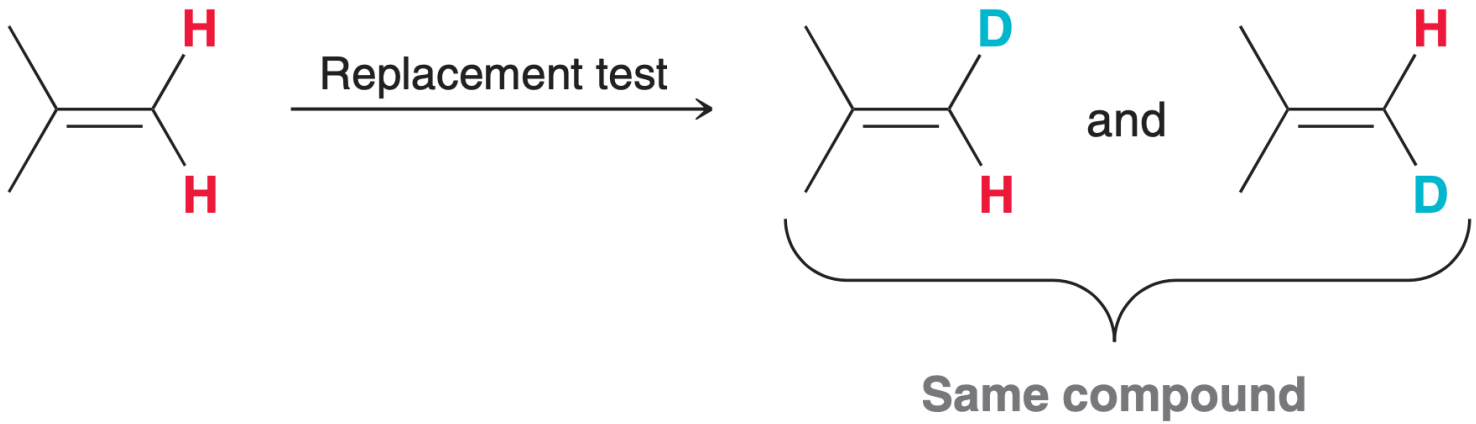
close your eyes…

you cannot determine whether this compound was rotated or not!

- Examples of homotopic protons

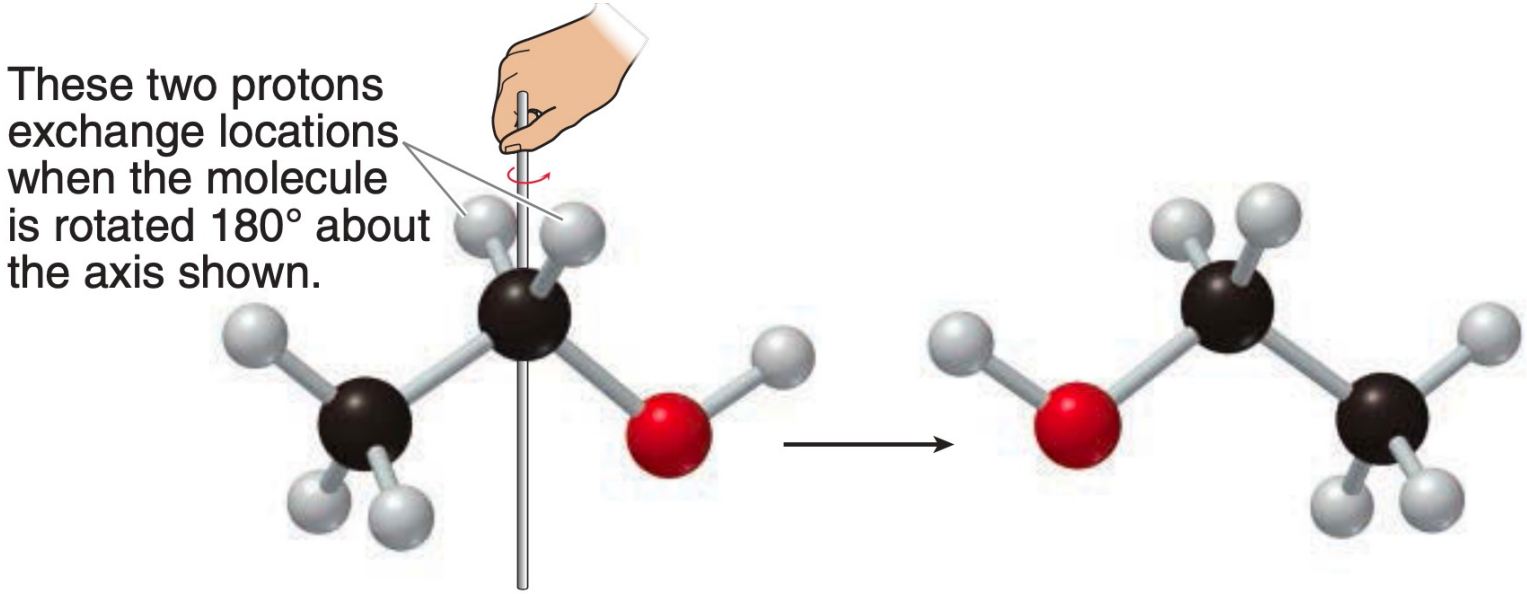


- Replacement test for rotational symmetry

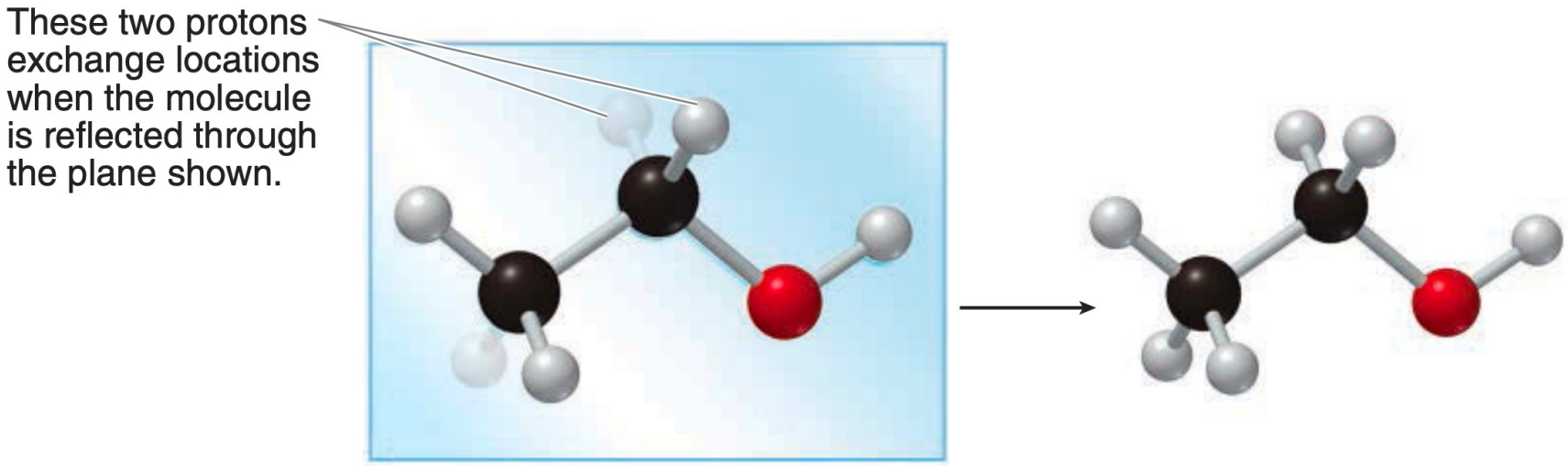


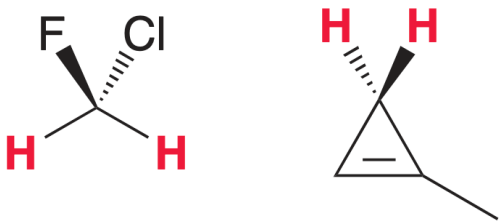
if the results are identical compounds, the tested protons are homotopic

- For ethanol… the same?

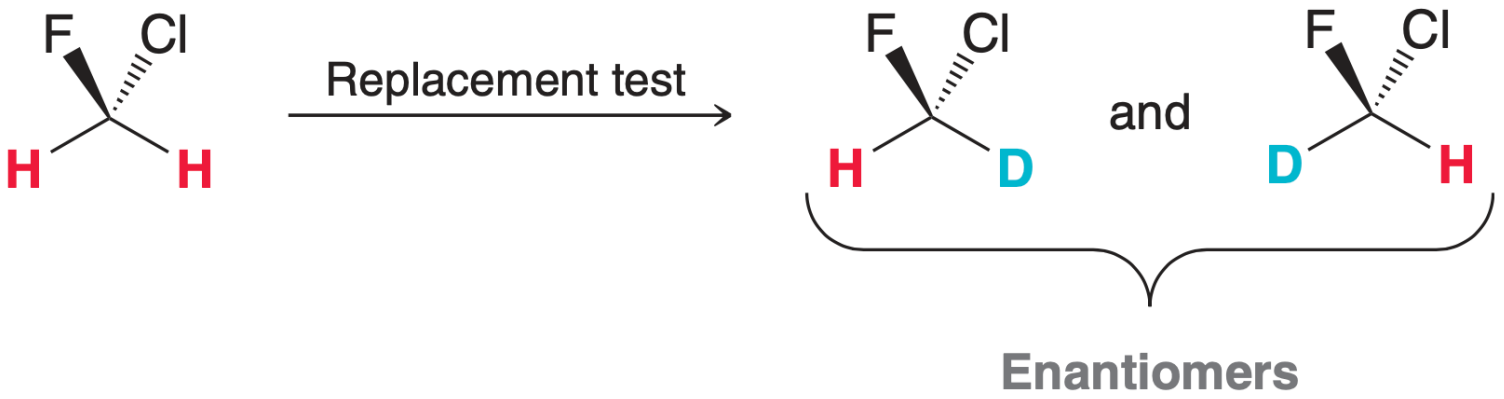


- Enantiotopic protons – *interchangeable by reflectional symmetry*



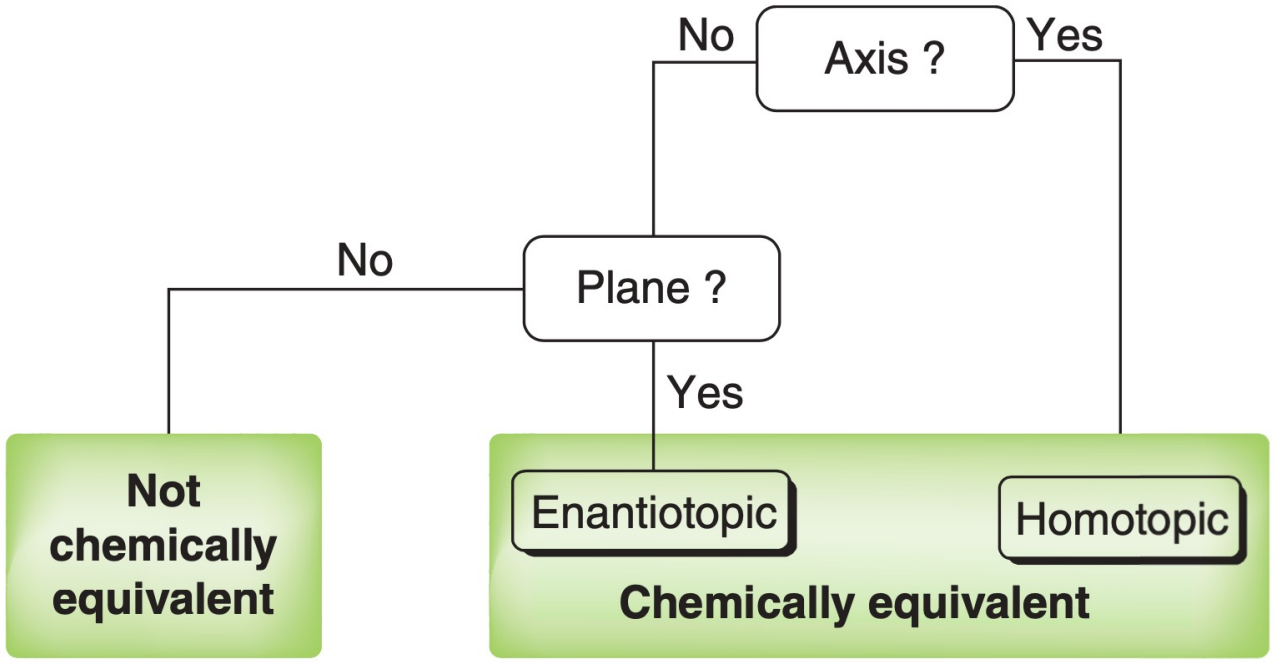


- Replacement test for reflectional symmetry

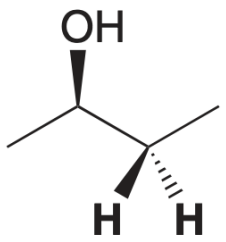


if the results are enantiomers, the tested protons are enantiotopic

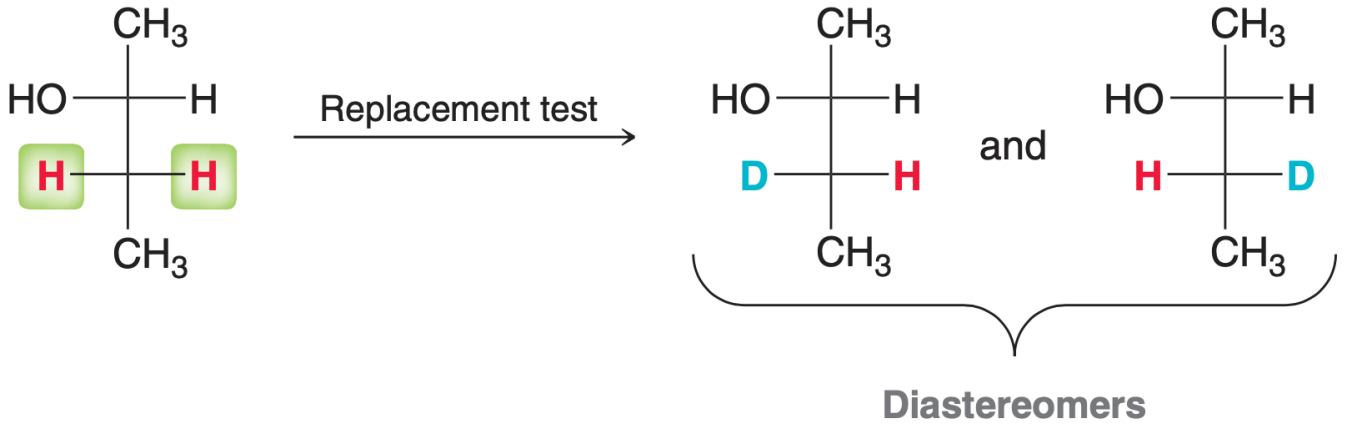
- Determine whether two protons are chemically equivalent



- Non-chemically equivalent protons

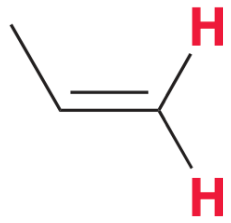


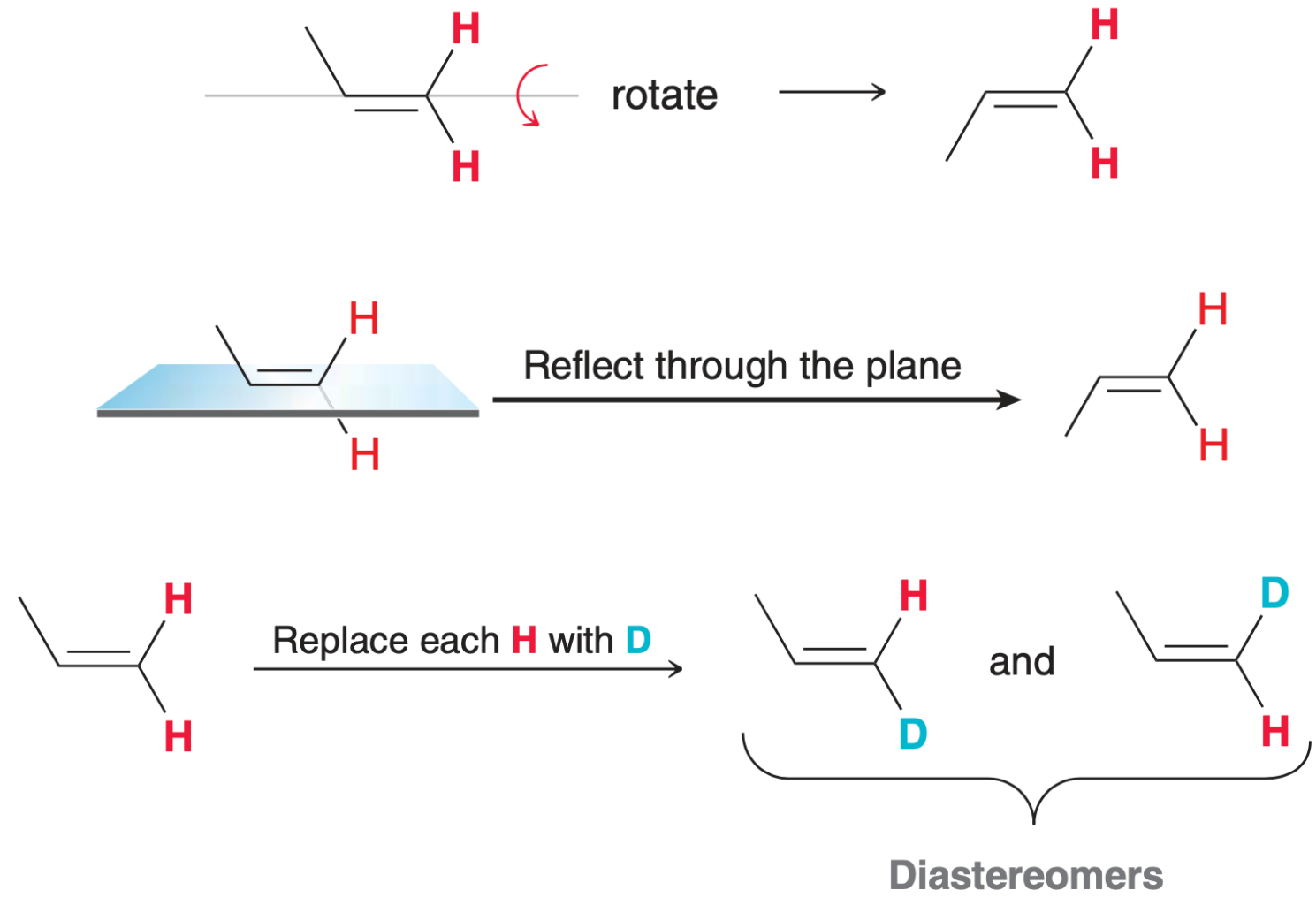
protons on C3 of (*R*)-2-butanol



the replacement test produces diastereomers – diastereotopic

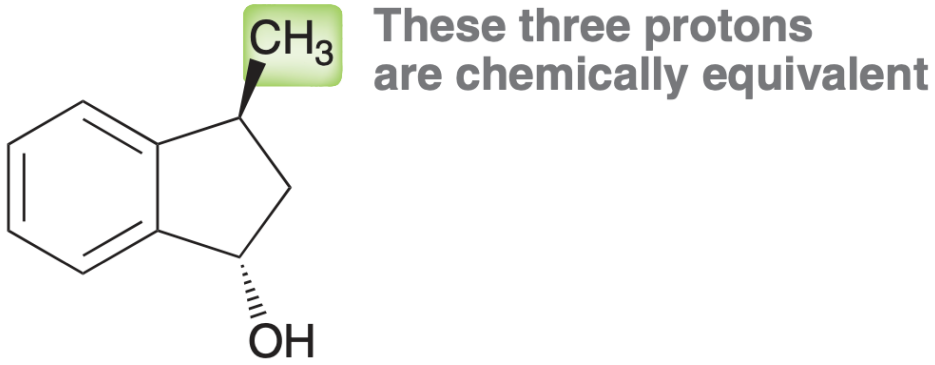
- Practice: determine whether the two protons shown in red are homotopic, enantiotopic, diastereotopic, or simply not related at all.



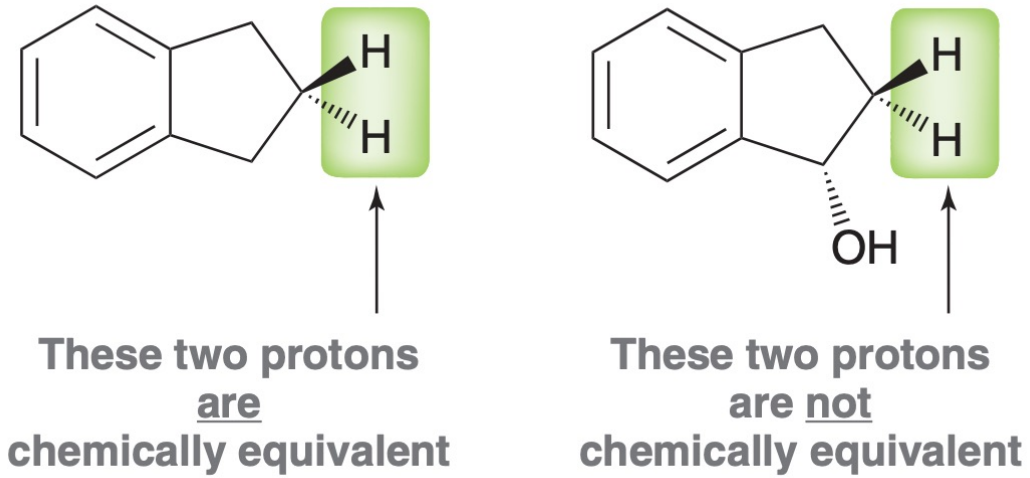

H
H
rotate
H
H
H
H
Reflect through the plane
H
H
H
H
Replace each H with D
H
D
and
D
H
Diastereomers

- Rules that can be used to determine the number of expected signals
  - The three protons of a CH_3 group are always chemically equivalent
  - The two protons of a CH_2 group will generally be chemically equivalent if the compound has no chiral centers; if the compound has a chiral center, then the protons of a CH_2 group will generally not be chemically equivalent
  - Two CH_2 groups will be equivalent to each other (giving four equivalent protons) if the CH_2 groups can be interchanged by either rotation or reflection

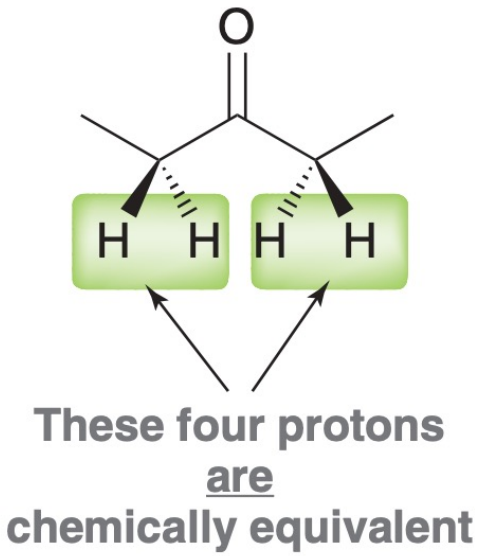
- The CH_3 protons – always chemically equivalent



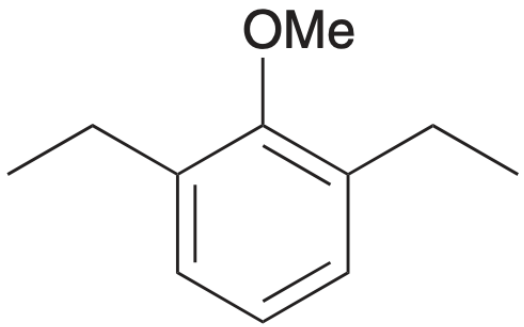
- The CH_2 protons – generally chemically equivalent (for achiral CH_2)

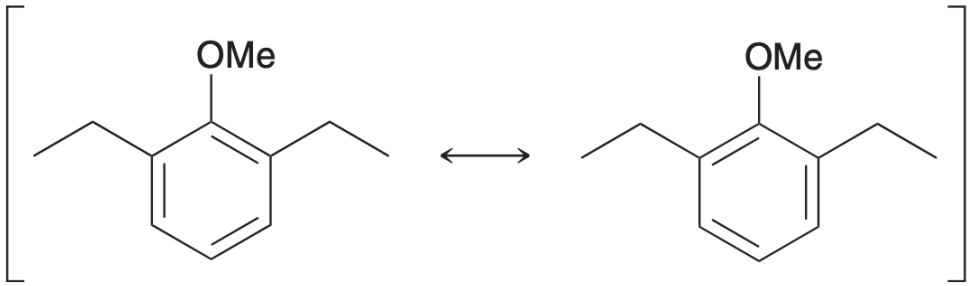


- Symmetrically interchangeable CH_2 groups – chemically equivalent

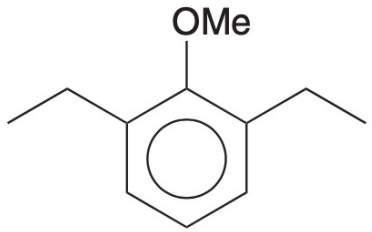


- Practice: identify the number of signals expected in the ^{1}H NMR spectrum of the following compound:

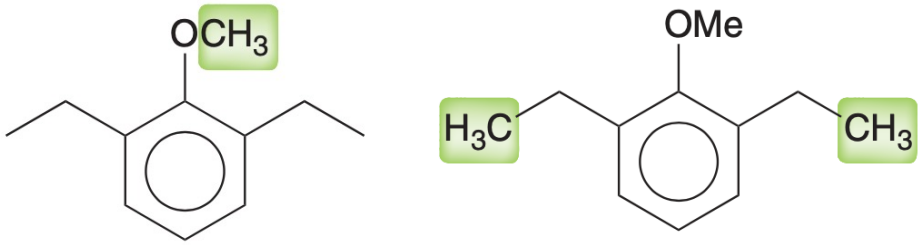




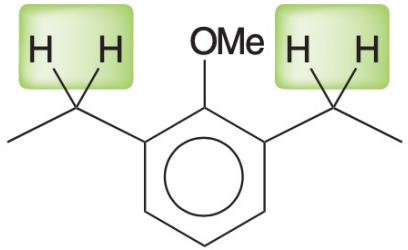
resonance – identical



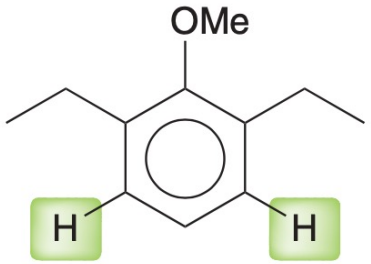
avoid confusing



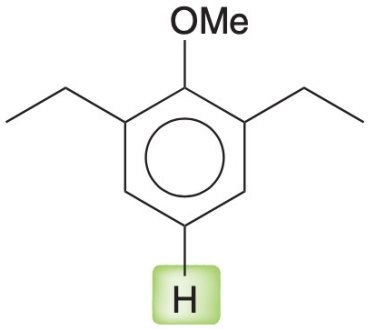
CH_3 protons – equivalent



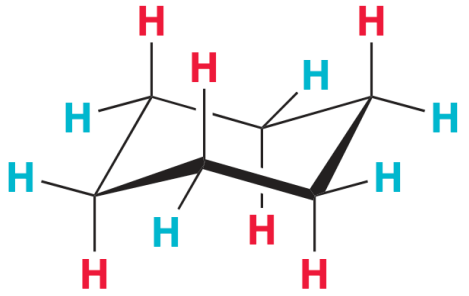
achiral CH_2 protons – equivalent



homotopic protons – equivalent



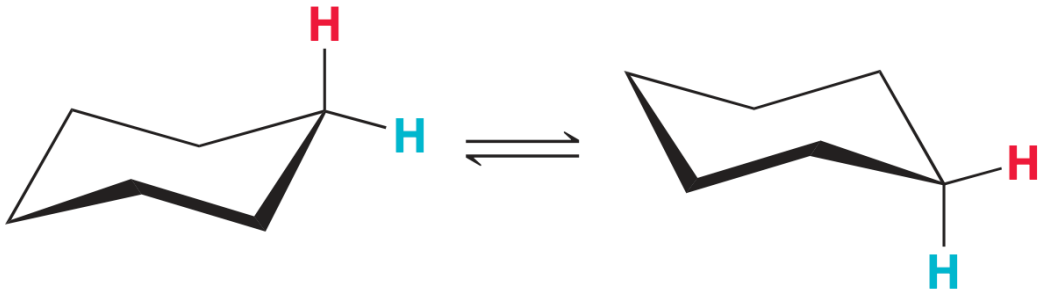
last one – one more signal – totally five signals



H = axial

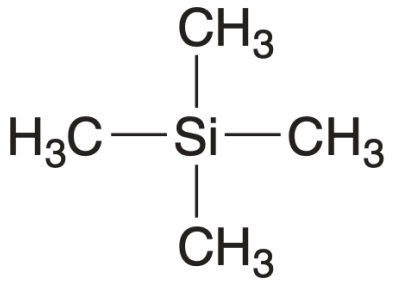
H = equatorial

- Variable-temperature NMR



- room temperature – rapidly flipped – one signal
- low temperature (–100 ℃) – slowly flipped – two signals

- Chemical shift (δ) – referencing to tetramethylsilane (TMS)



TMS

$$\delta = \frac{\text{observed shift from TMS in hertz}}{\text{operating frequency of the instrument in hertz}}$$

- Chemical shift – a constant regardless of the operating frequency

$$\delta = \frac{\text{observed shift from TMS in hertz}}{\text{operating frequency of the instrument in hertz}}$$

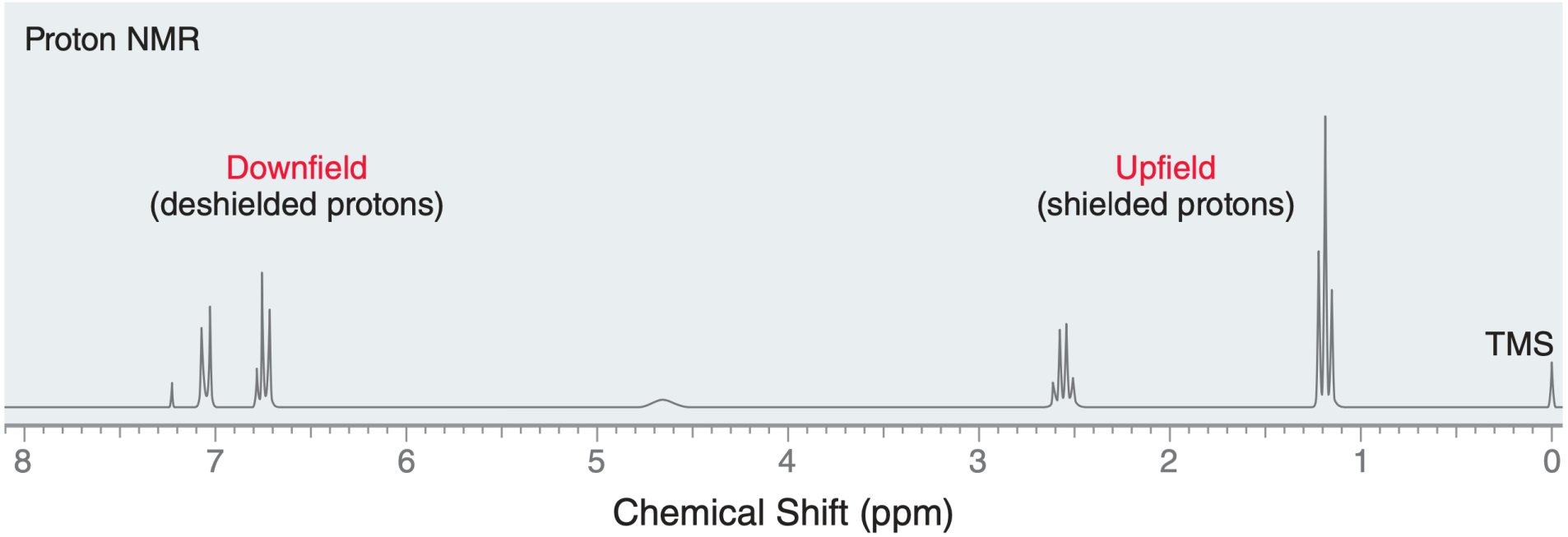
$$\delta = \frac{2181\ \text{Hz}}{300 \times 10^6\ \text{Hz}} = 7.27 \times 10^{-6}$$

300 MHz NMR
(absorbing at 2181 Hz)

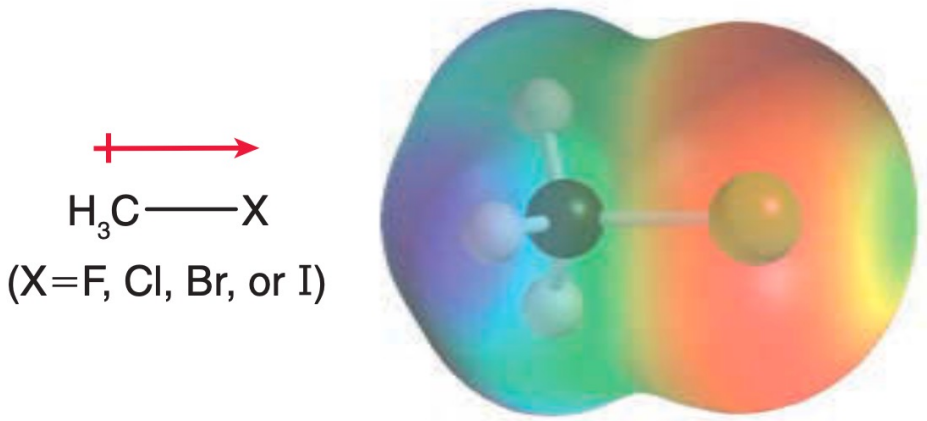
$$\delta = \frac{436\ \text{Hz}}{60 \times 10^6\ \text{Hz}} = 7.27 \times 10^{-6}$$

60 MHz NMR
(absorbing at 436 Hz)

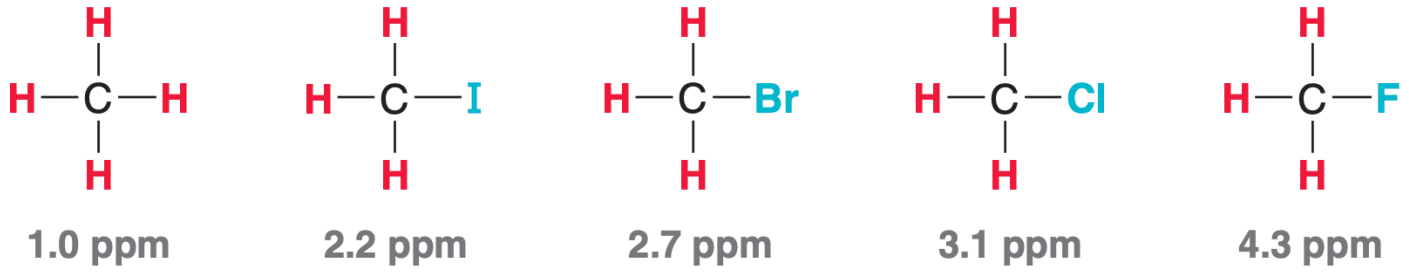
- Upfield and downfield

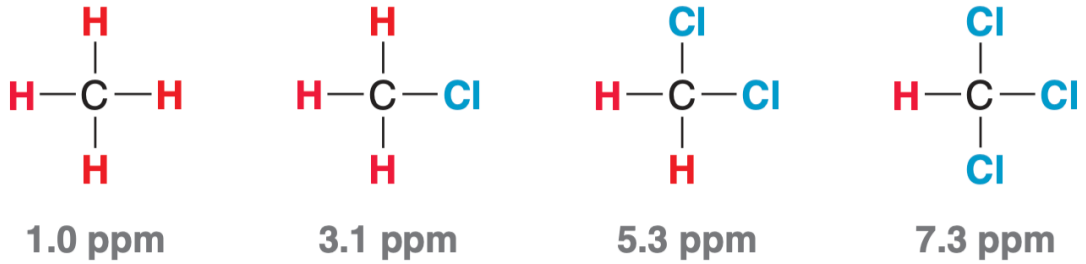


- Factors that affect chemical shift: inductive effects

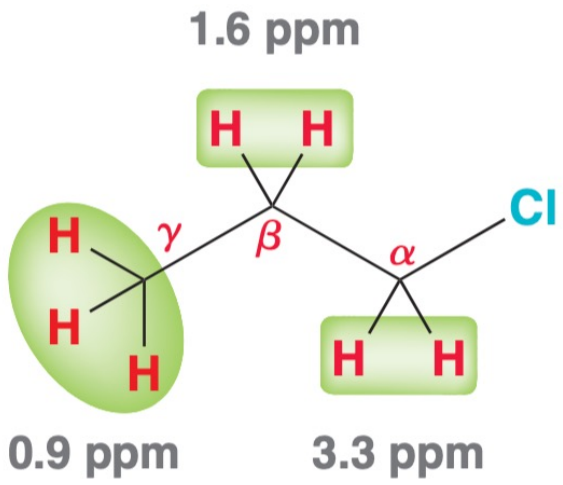


- Electronegativity and deshielding effect

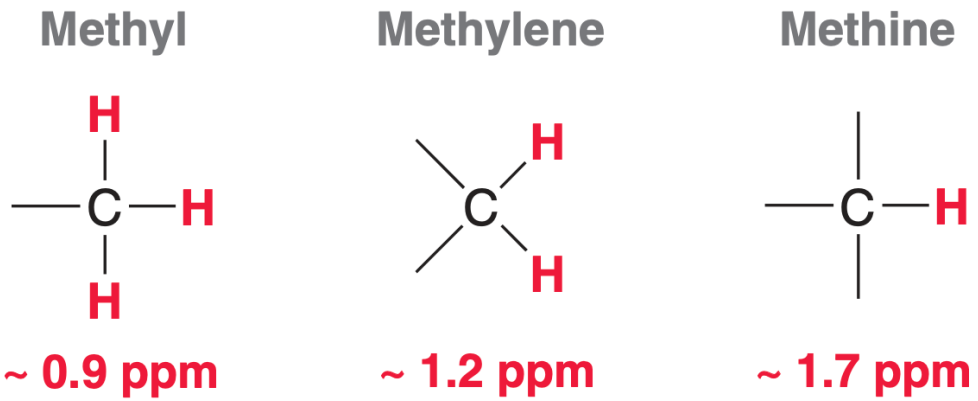




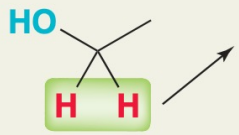
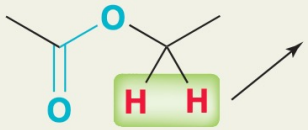
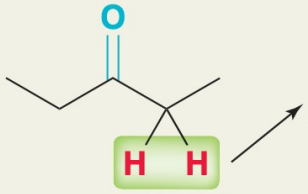
- The inductive effect tapers off drastically with distance



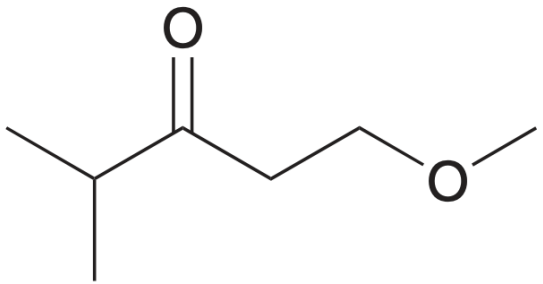
- Benchmark values for common protons

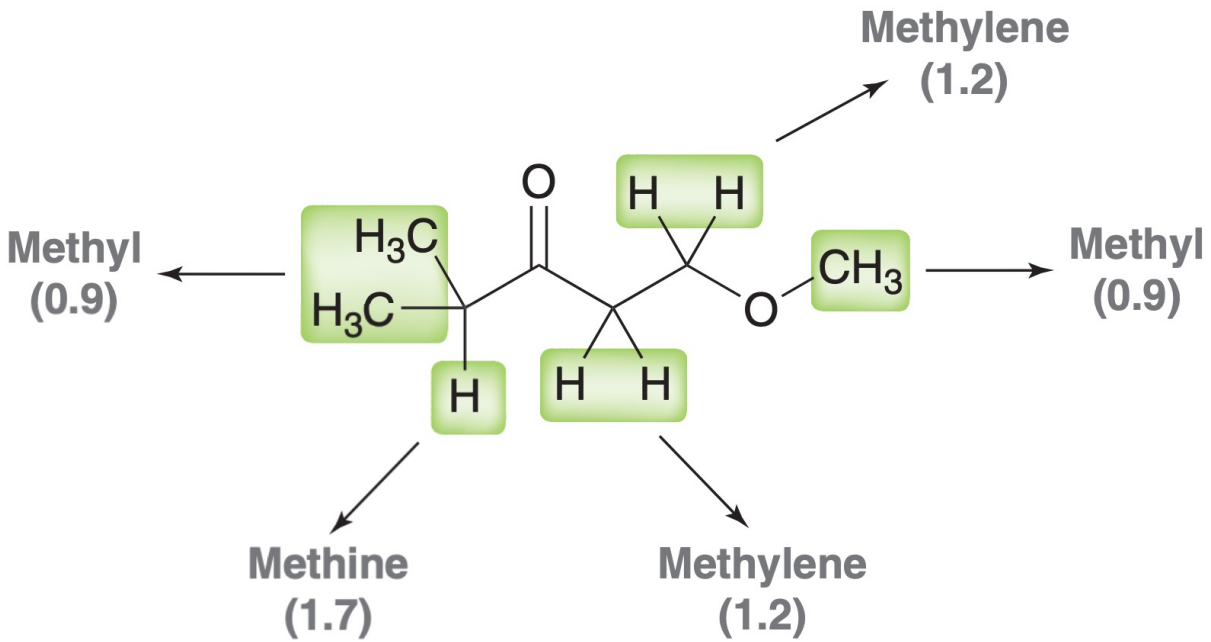


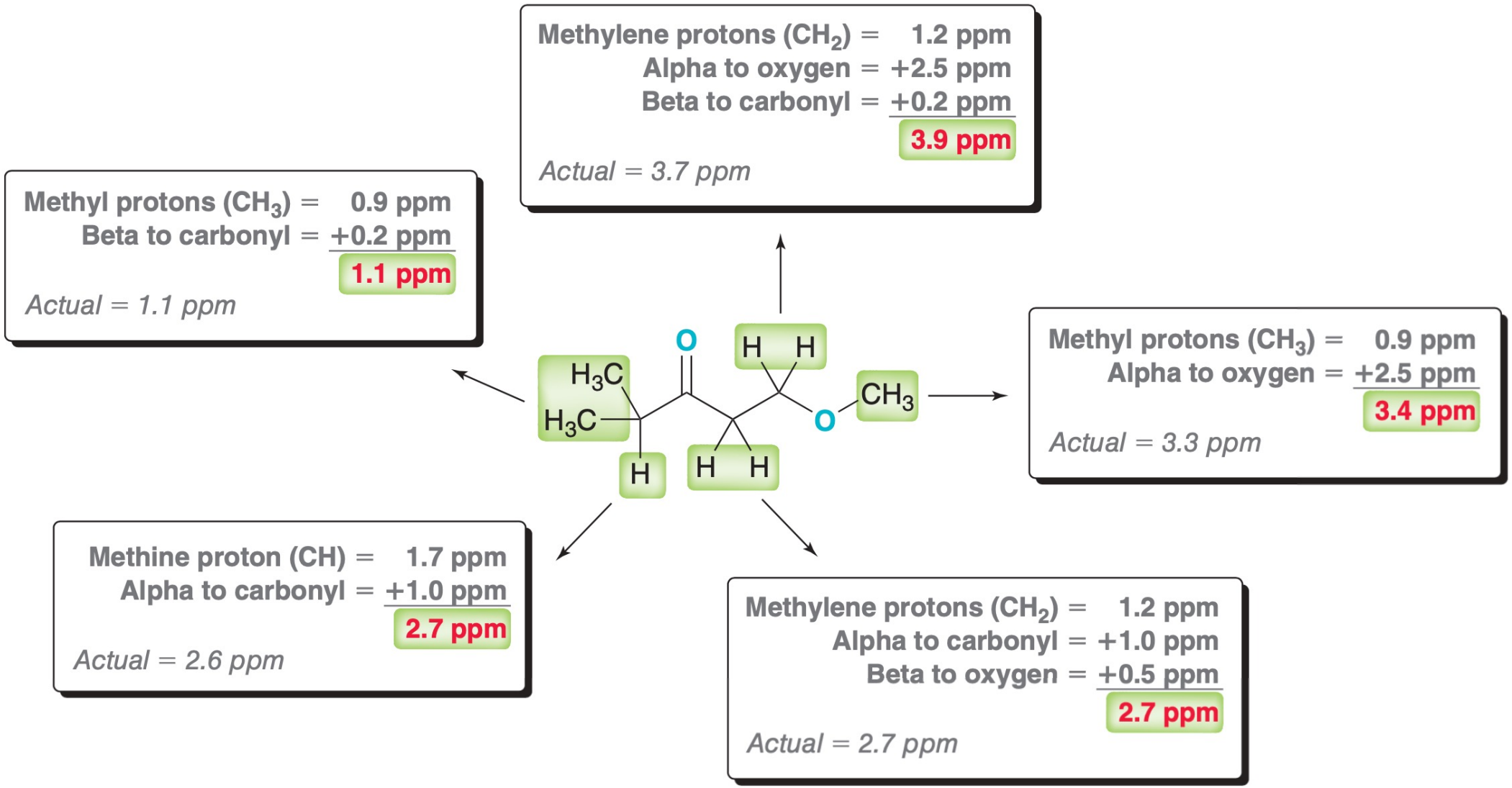
- Predicting chemical shift from neighboring functional groups

FUNCTIONAL GROUP	EFFECT ON ALPHA PROTONS	EXAMPLE
Oxygen of an alcohol or ether	+2.5	 Methylene group (CH_2) = 1.2 ppm Next to oxygen = +2.5 ppm **3.7 ppm** Actual chemical shift = 3.7 ppm
Oxygen of an ester	+3	 Methylene group (CH_2) = 1.2 ppm Next to oxygen = +3.0 ppm **4.2 ppm** Actual chemical shift = 4.1 ppm
Carbonyl group (C═O) All carbonyl groups, including ketones, aldehydes, esters, etc.	+1	 Methylene group (CH_2) = 1.2 ppm Next to carbonyl group = +1.0 ppm **2.2 ppm** Actual chemical shift = 2.4 ppm

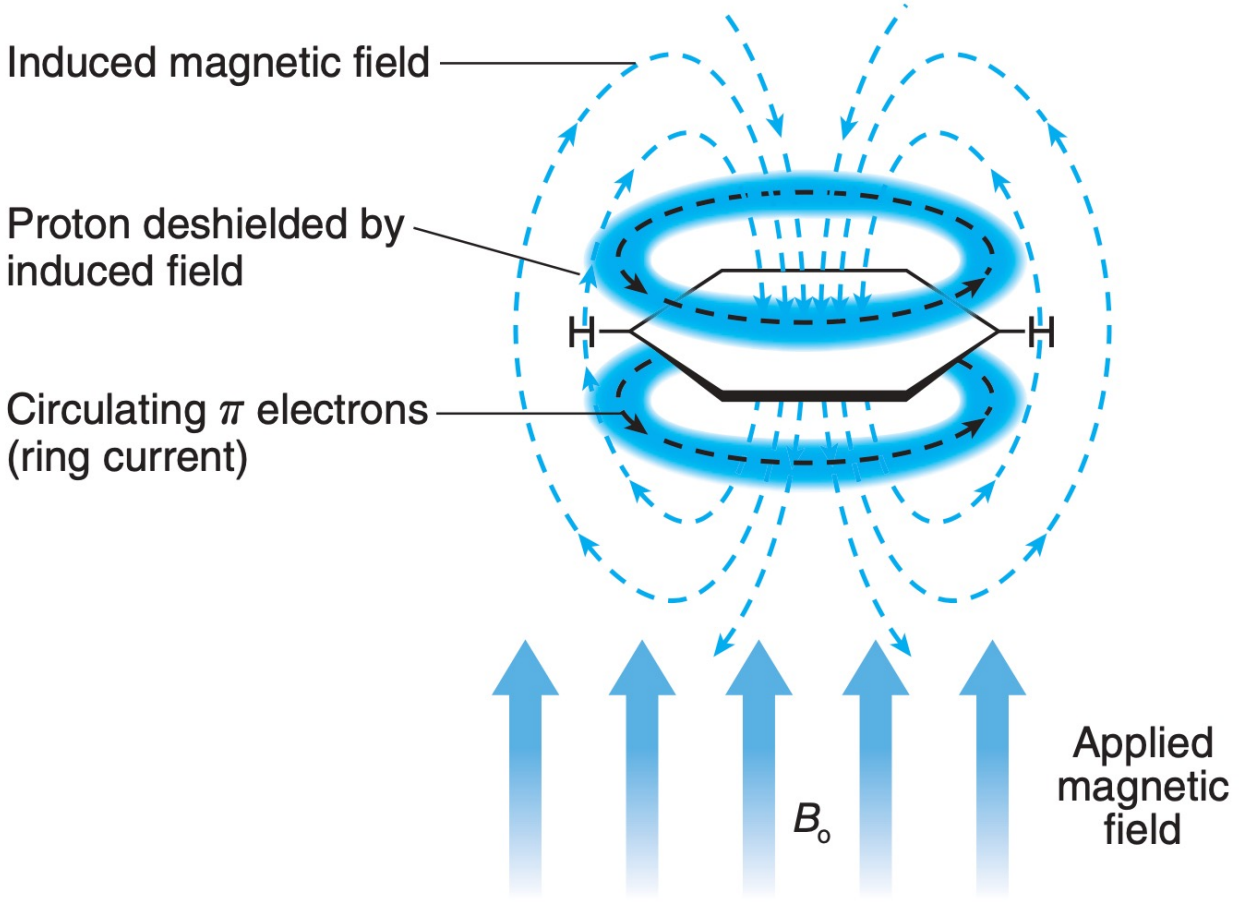
- Practice: predict the chemical shifts for the signals in the ^{1}H NMR spectrum of the following compound:




Methylene
(1.2)
Methyl
(0.9)
Methyl
(0.9)
Methine
(1.7)
Methylene
(1.2)
O
H H
H3C
H3C
CH3
O
H
H H

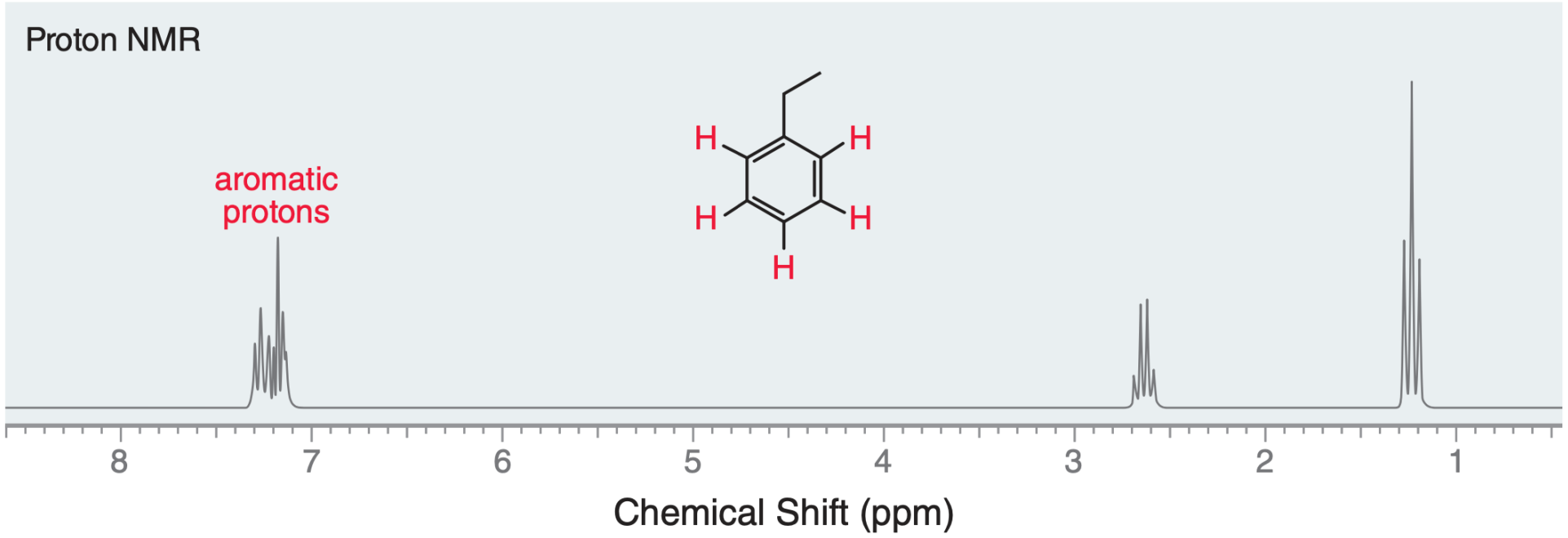

Methylene protons (CH_2) = 1.2 ppm
Alpha to oxygen = +2.5 ppm
Beta to carbonyl = +0.2 ppm
3.9 ppm
Actual = 3.7 ppm
Methyl protons (CH_3) = 0.9 ppm
Beta to carbonyl = +0.2 ppm
1.1 ppm
Actual = 1.1 ppm
Methyl protons (CH_3) = 0.9 ppm
Alpha to oxygen = +2.5 ppm
3.4 ppm
Actual = 3.3 ppm
Methine proton (CH) = 1.7 ppm
Alpha to carbonyl = +1.0 ppm
2.7 ppm
Actual = 2.6 ppm
Methylene protons (CH_2) = 1.2 ppm
Alpha to carbonyl = +1.0 ppm
Beta to oxygen = +0.5 ppm
2.7 ppm
Actual = 2.7 ppm
H_3C
H_3C
O
H H
O
CH_3
H
H H

- Factors that affect chemical shift: anisotropic effects

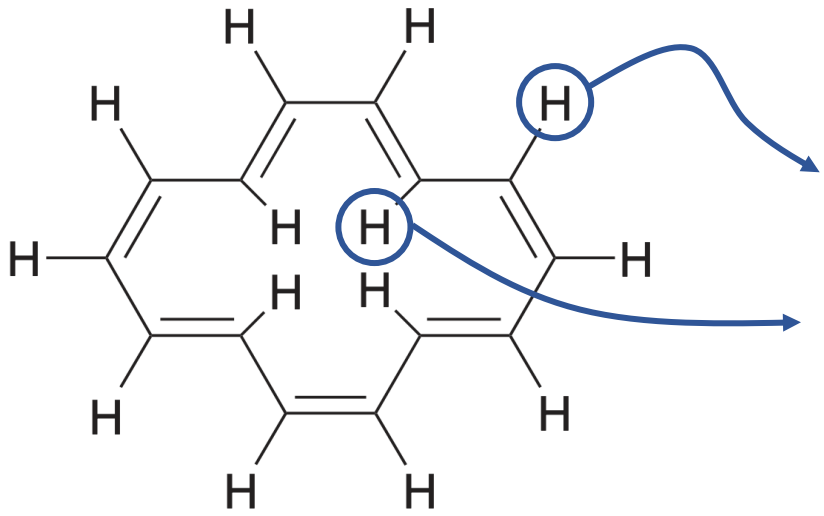


diamagnetic anisotropy

- Aromatic protons and anisotropic effects



- Anisotropic effects of [14] annulene

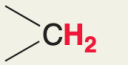
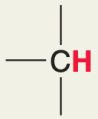
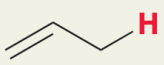
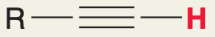
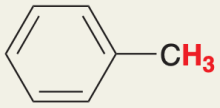


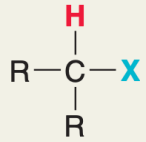
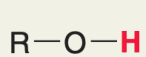
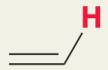
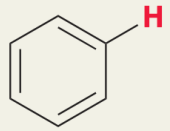
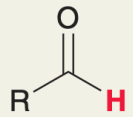
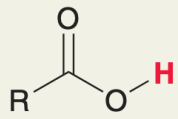
[14] Annulene

outside the ring: δ ≈ 8 ppm

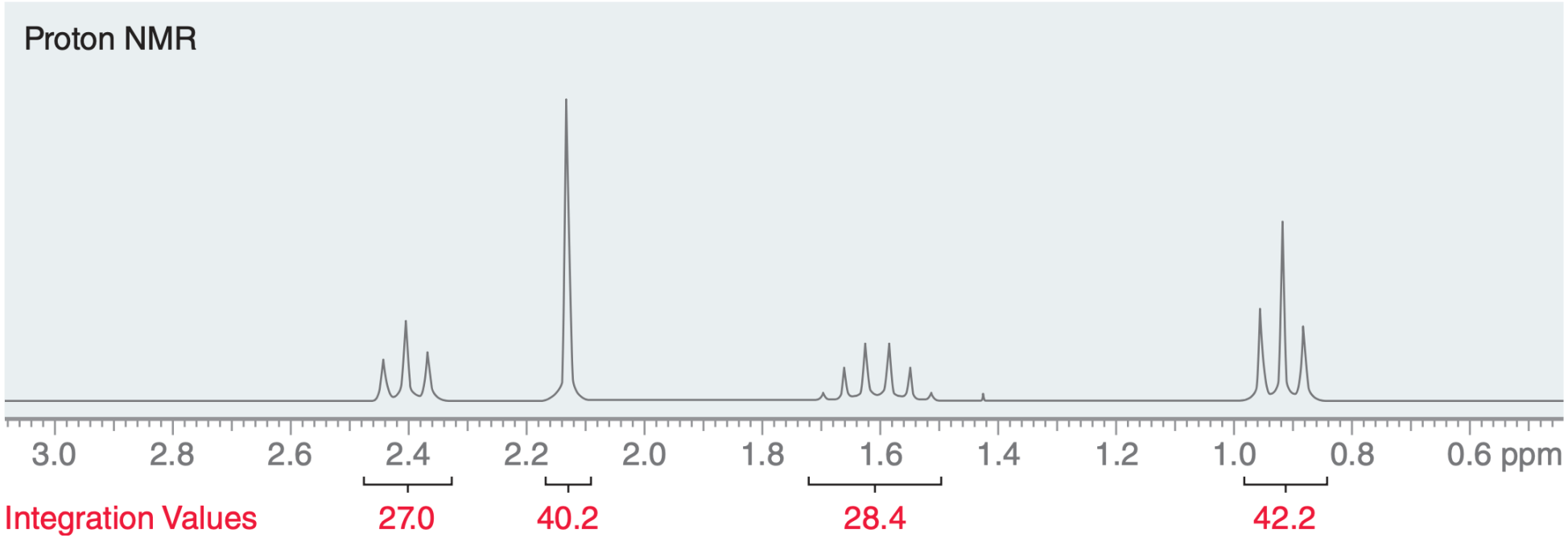
inside the ring: δ ≈ –1 ppm

- Chemical shifts for protons in different electronic environments

TYPE OF PROTON		CHEMICAL SHIFT (δ)
Methyl		~0.9
Methylene		~1.2
Methine		~1.7
Allylic		~2
Alkynyl		~2.5
Aromatic methyl		~2.5

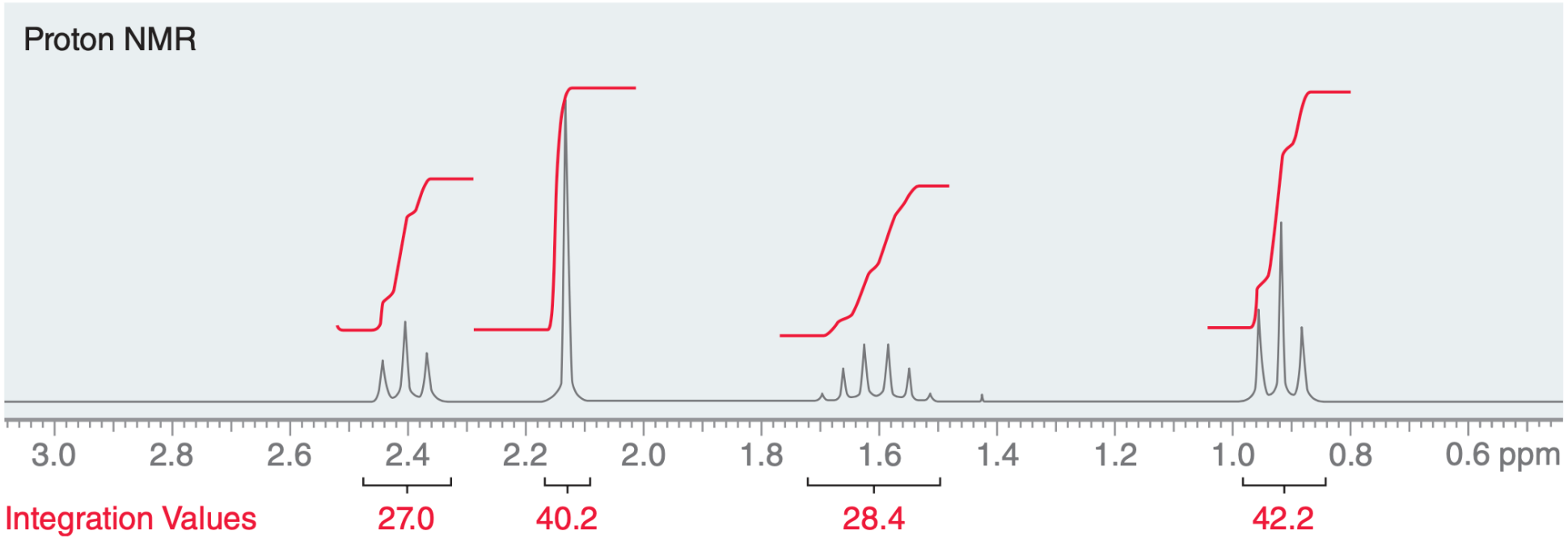
TYPE OF PROTON		CHEMICAL SHIFT (δ)
Alkyl halide		2–4
Alcohol		2–5
Vinylic		4.5–6.5
Aryl		6.5–8
Aldehyde		~10
Carboxylic acid		~12

- Integration: relative number of protons

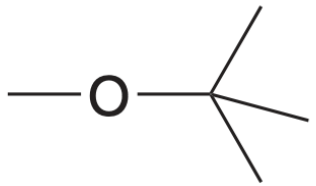


$$\frac{27.0}{27.0} = 1 \qquad \frac{40.2}{27.0} = 1.49 \qquad \frac{28.4}{27.0} = 1.05 \qquad \frac{42.2}{27.0} = 1.56$$

- Step curve representation



- Integration **ONLY** shows relative numbers!

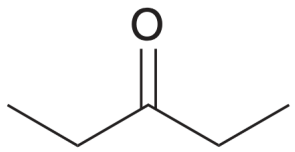


MTBE

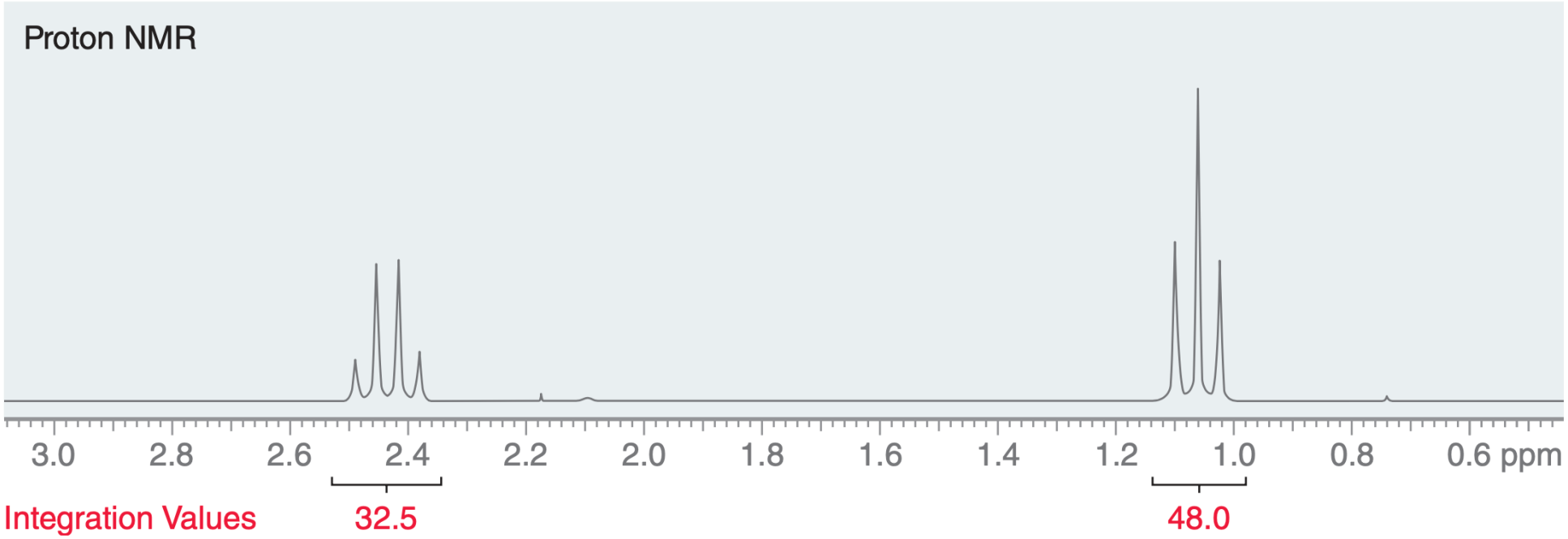
two signals, ratio of 1 : 3

(12 protons in total)

- The impact of symmetry on integration

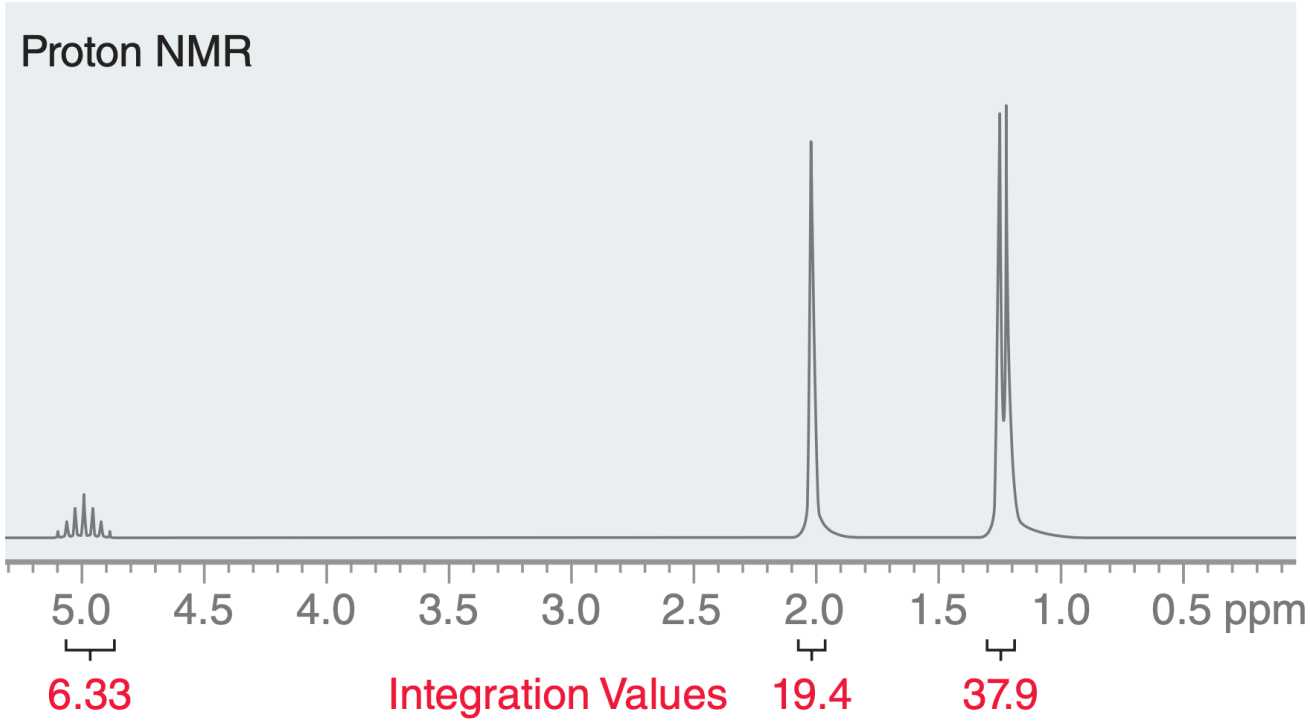


3-Pentanone

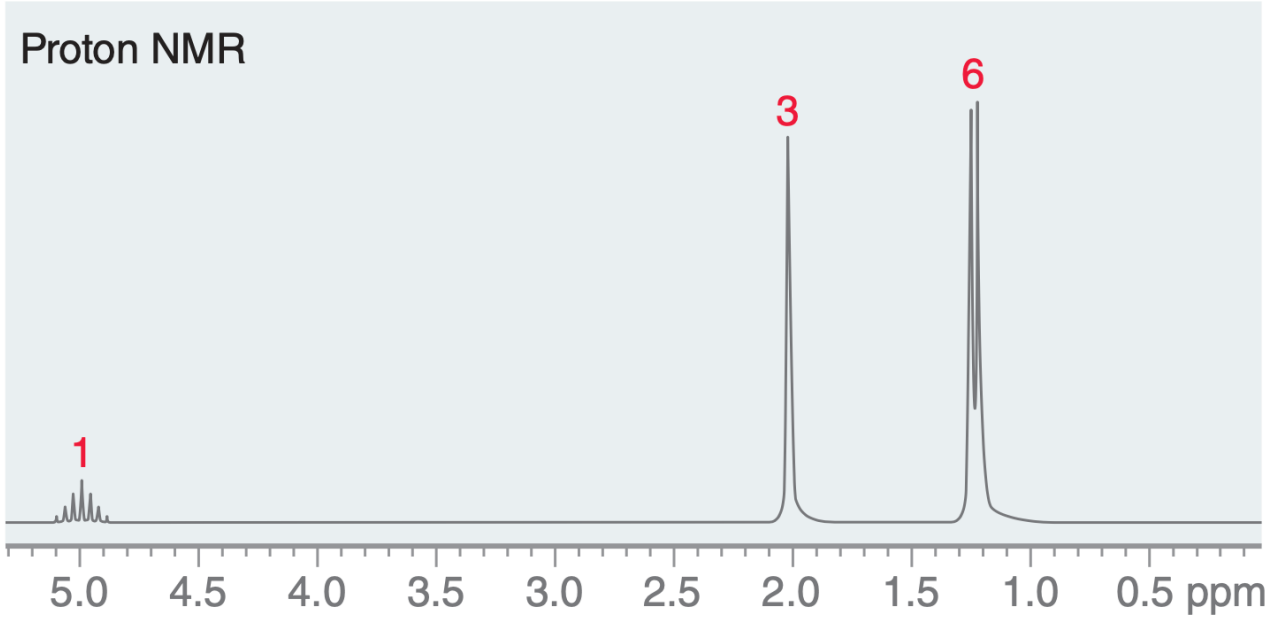


ratio of 2 : 3 (actually 4 protons and 6 protons)

- Practice: a compound with the molecular formula $C_5H_{10}O_2$ has the ^1H NMR spectrum show below. Determine the number of protons giving rise to each signal.

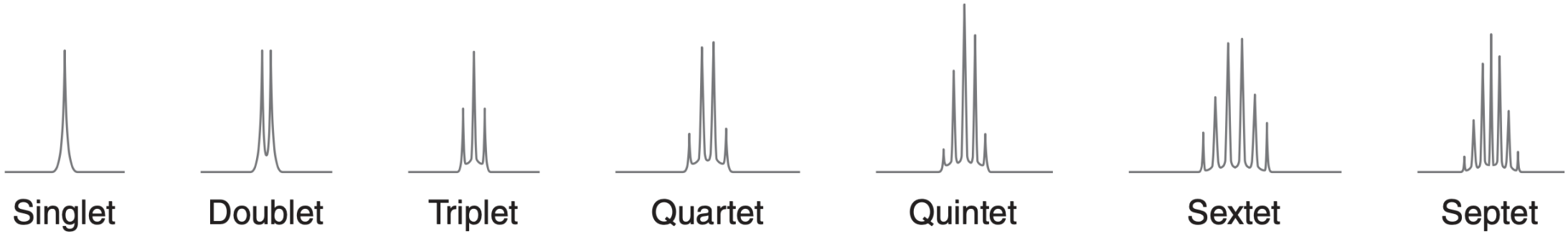


$$\frac{6.33}{6.33} = 1 \qquad \frac{19.4}{6.33} = 3.06 \qquad \frac{37.9}{6.33} = 5.99$$

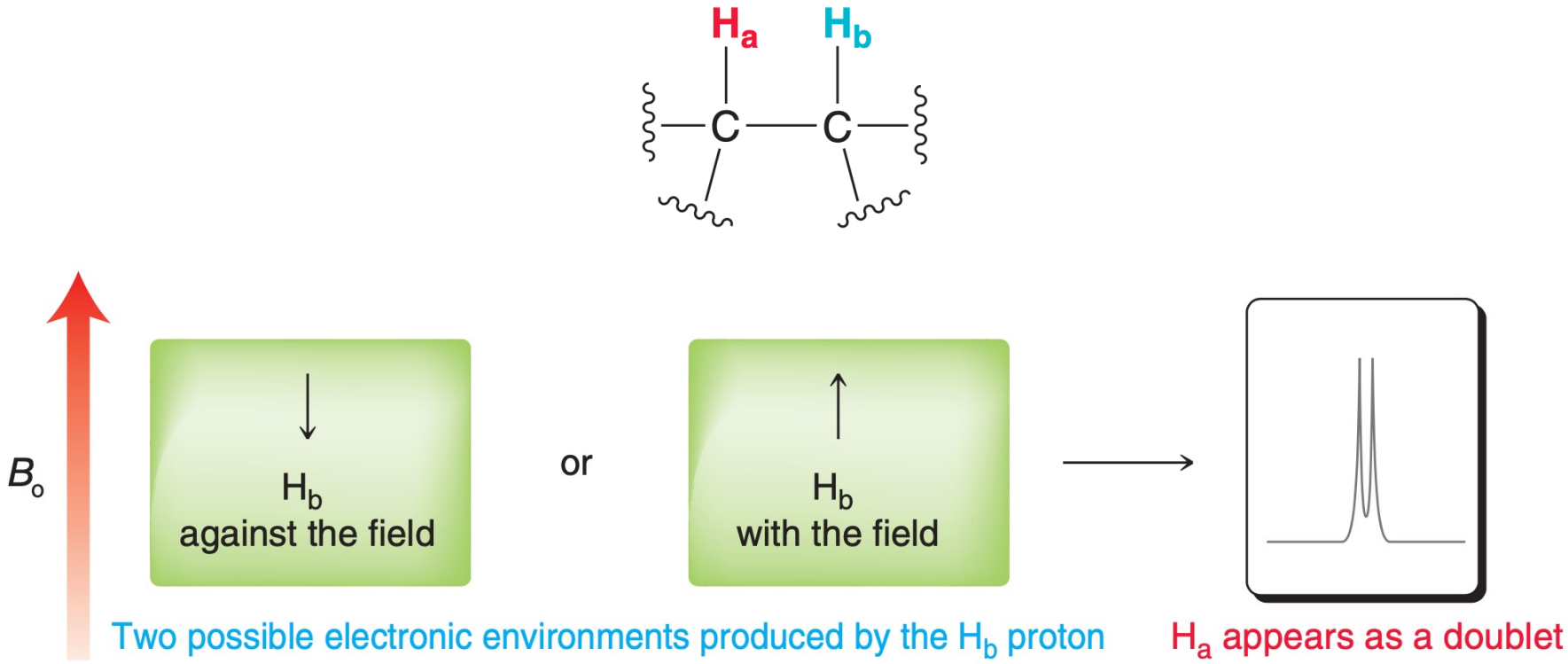


$C_5H_{10}O_2$ – 10 protons – 1, 3, 6 for each peak (exact values)

- Multiplicity

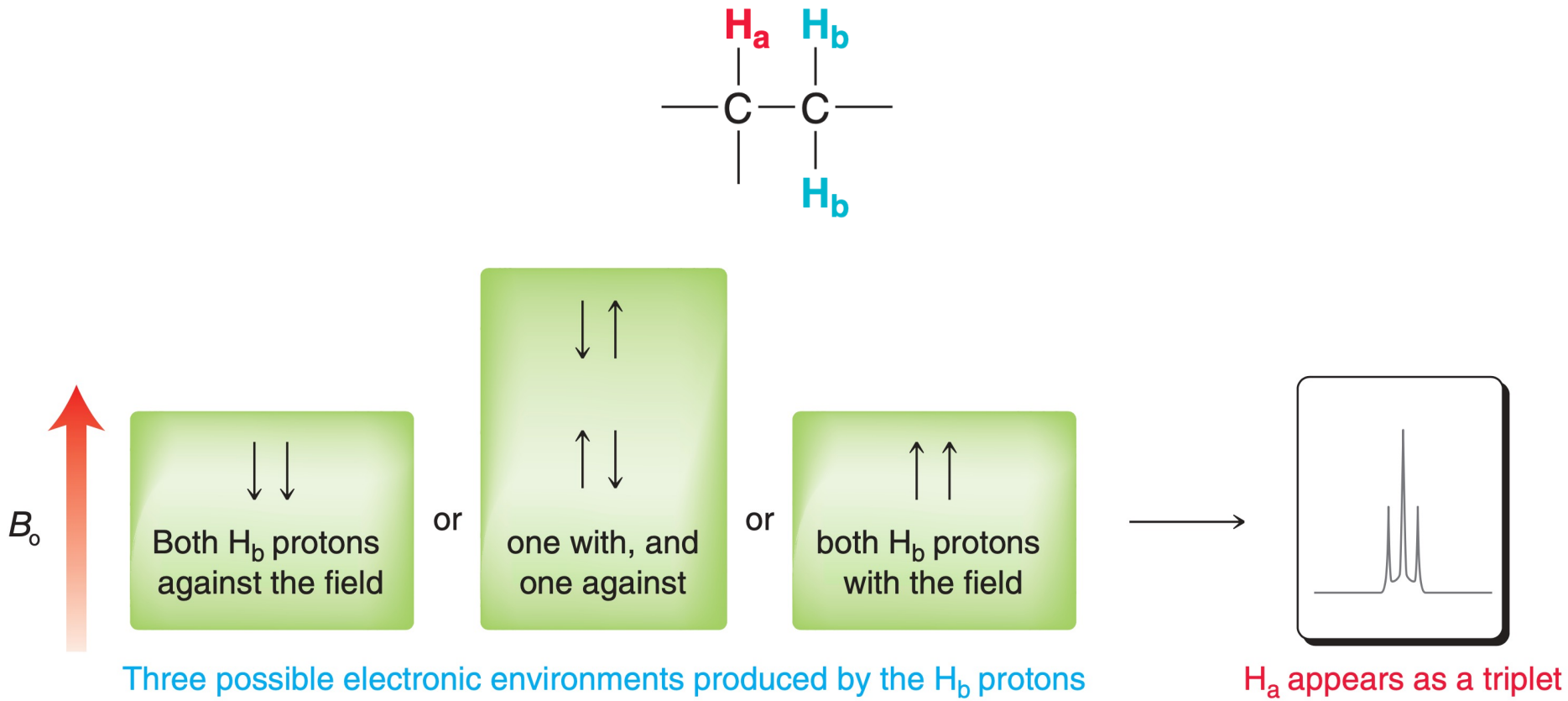


- Coupling: magnetic effects of neighboring protons

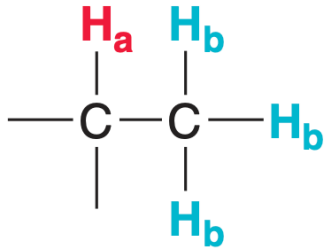


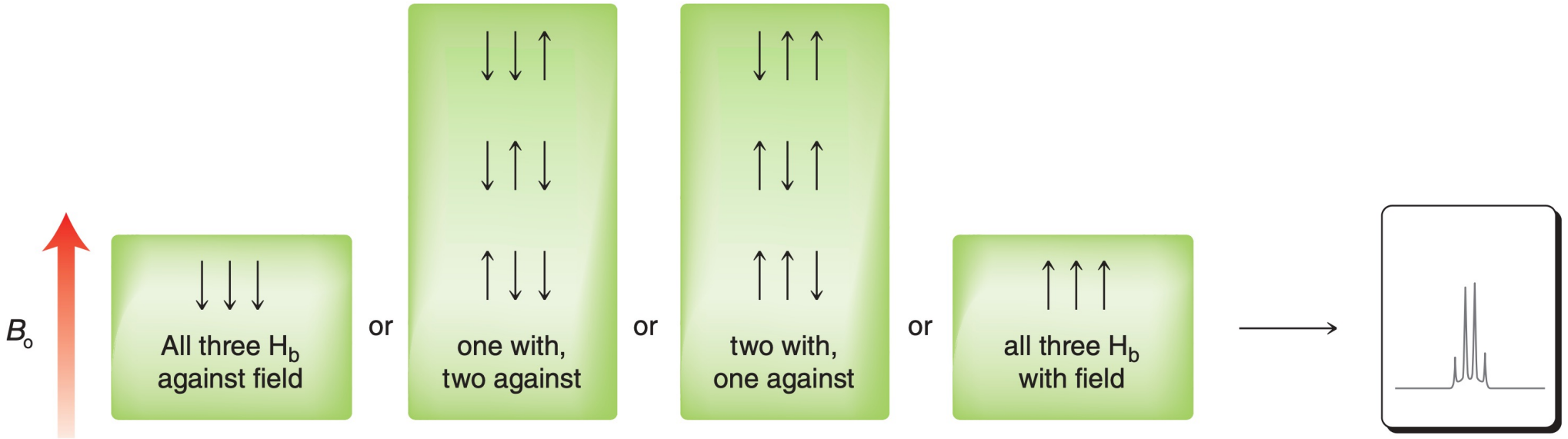
spin-spin splitting / coupling

- Two neighboring protons for H_a



- Three neighboring protons for H_a





Four possible electronic environments produced by the H_b protons

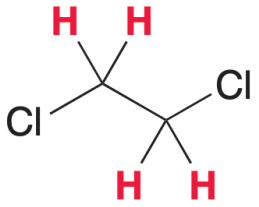
H_a appears as a quartet

- **The *n* + 1 rule: one more multiplicity for *n* protons**

NUMBER OF NEIGHBORS	MULTIPLICITY	RELATIVE INTENSITIES OF INDIVIDUAL PEAKS
0	Singlet	1
1	Doublet	1 : 1
2	Triplet	1 : 2 : 1
3	Quartet	1 : 3 : 3 : 1
4	Quintet	1 : 4 : 6 : 4 : 1
5	Sextet	1 : 5 : 10 : 10 : 5 : 1
6	Septet	1 : 6 : 15 : 20 : 15 : 6 : 1

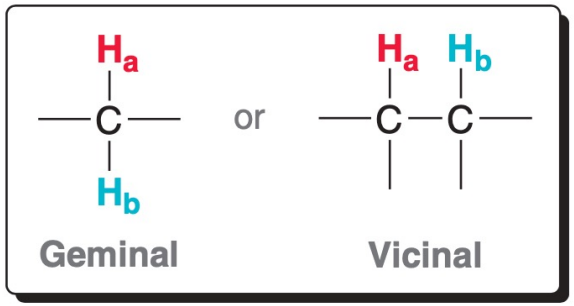
(Pascal's Triangle)

- Whether splitting occurs…?
  - Equivalent protons do not split each other

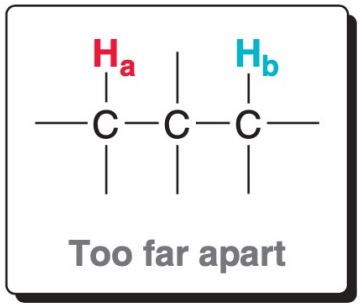


Four equivalent protons
No splitting

  - Splitting is most commonly observed on geminal and vicinal protons

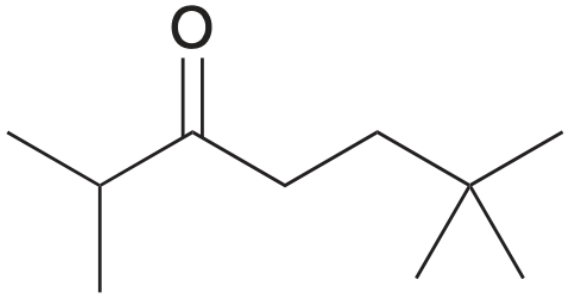


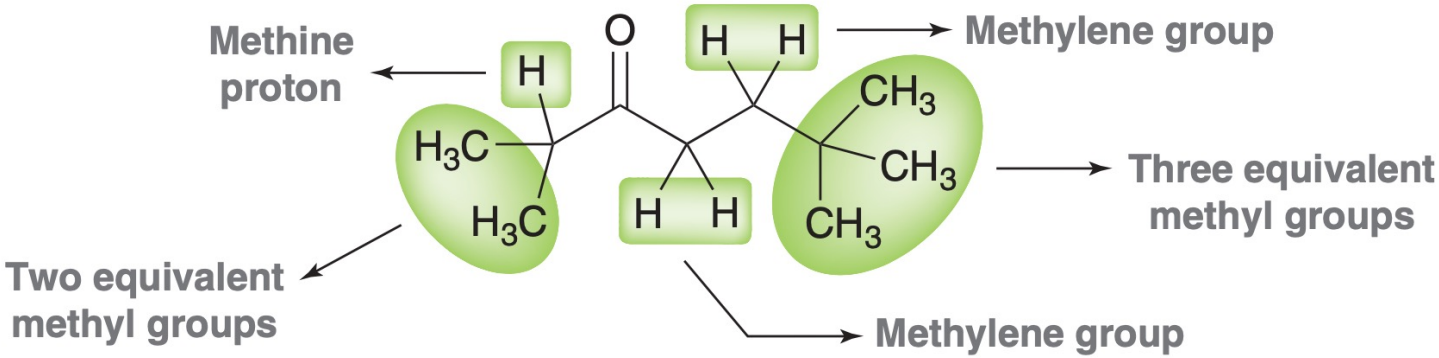
Splitting is observed

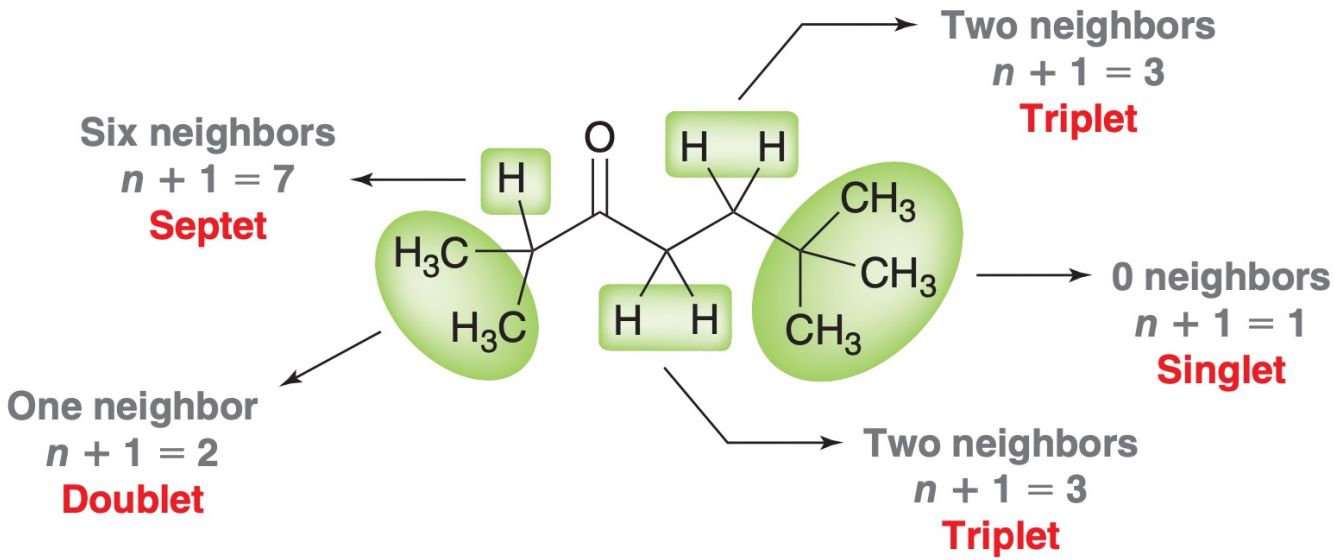


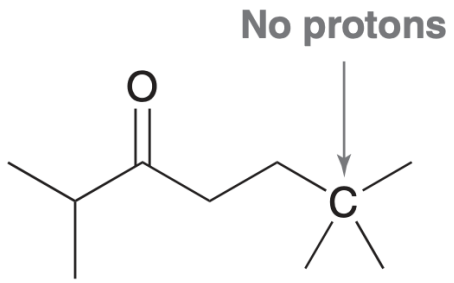
Splitting is generally not observed

- Practice: determine the multiplicity of each signal in the expected ^{1}H NMR spectrum of the following compound:

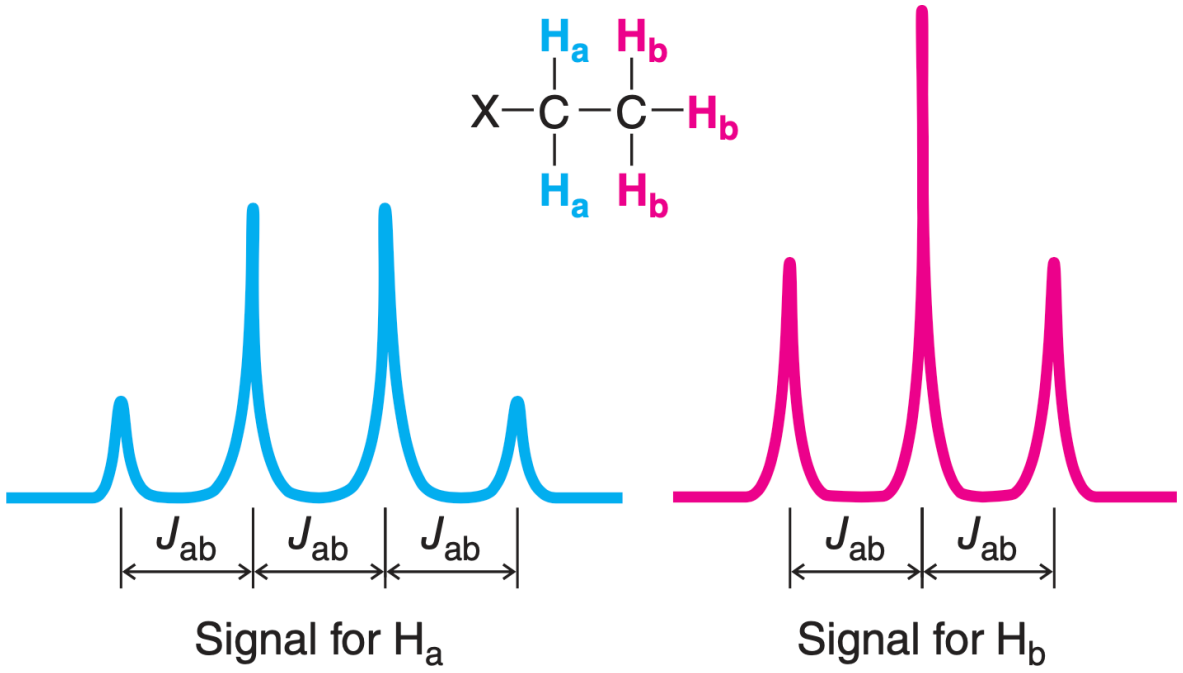



Methine proton
Methylene group
Three equivalent methyl groups
Two equivalent methyl groups
Methylene group


Two neighbors
n + 1 = 3
Triplet
Six neighbors
n + 1 = 7
Septet
0 neighbors
n + 1 = 1
Singlet
One neighbor
n + 1 = 2
Doublet
Two neighbors
n + 1 = 3
Triplet


No protons

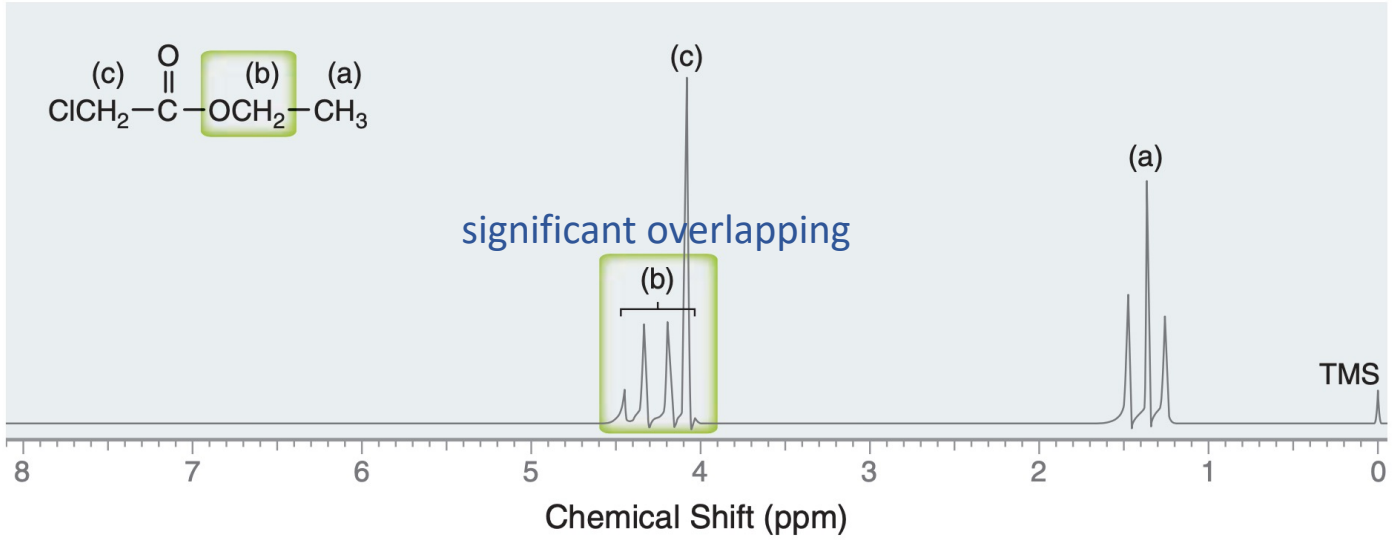
- Coupling constant (*J* value)



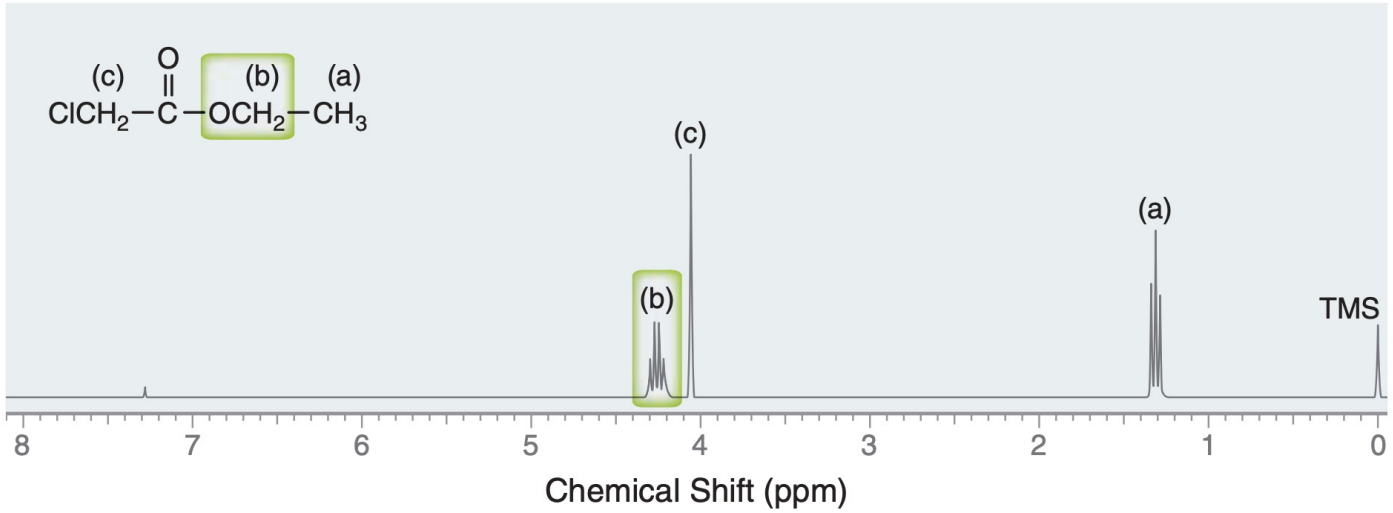
J value is ranged 0 to 20 Hz

it is independent of the operating frequency of NMR

- Using stronger magnetic field (higher operating frequency) to avoid overlapping

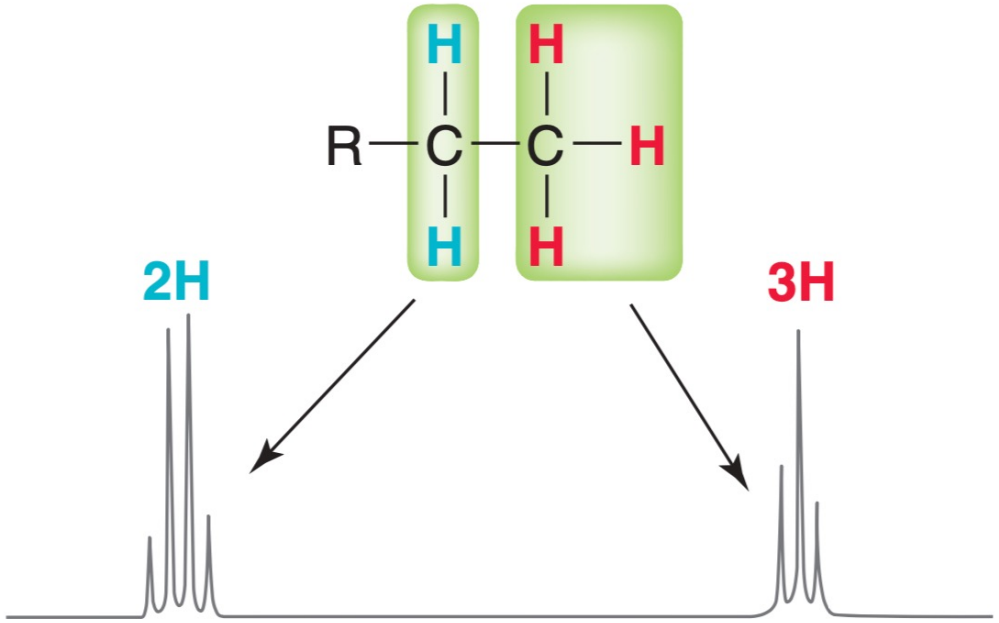


60-MHz NMR

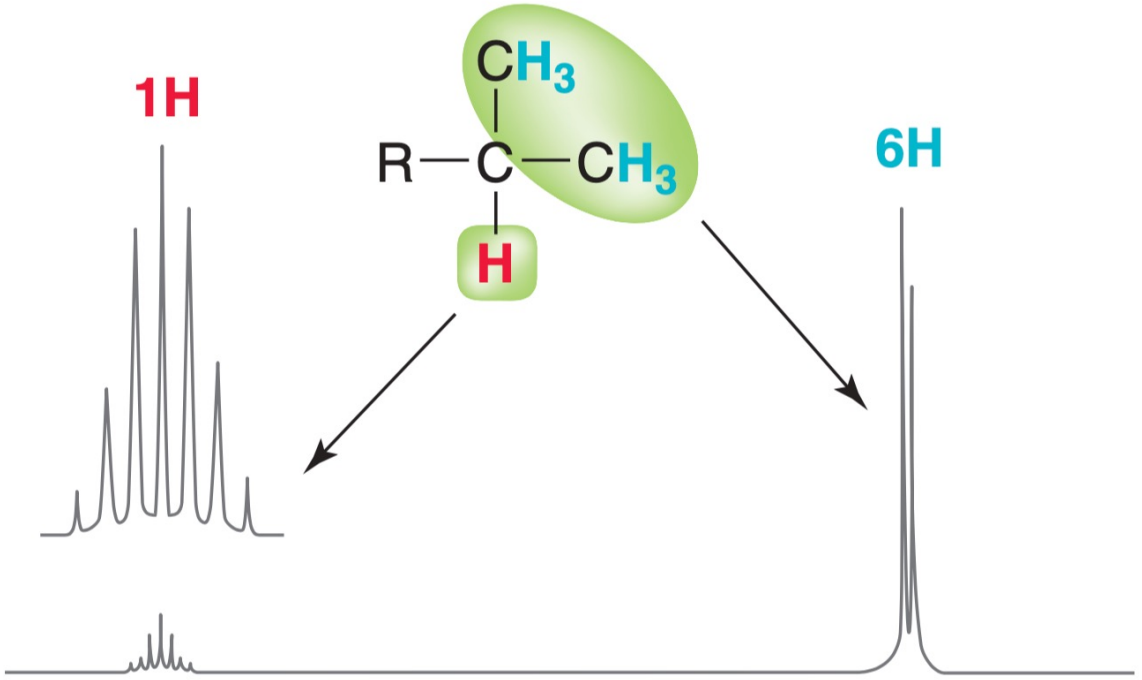


300-MHz NMR

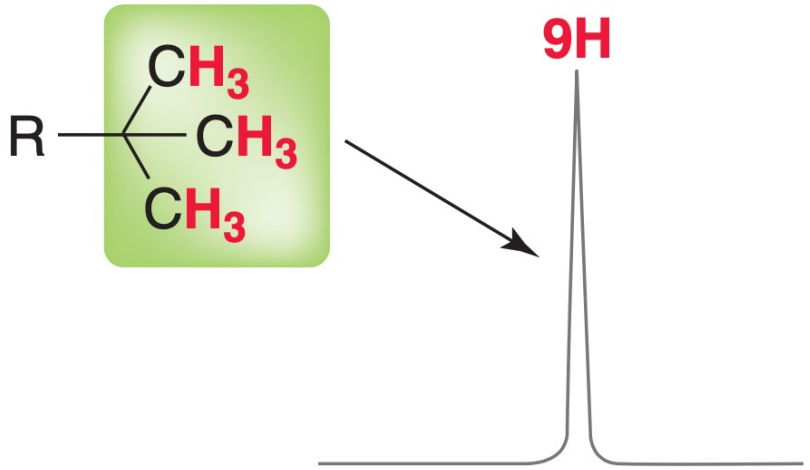
- Pattern recognition: ethyl group



- Pattern recognition: isopropyl group

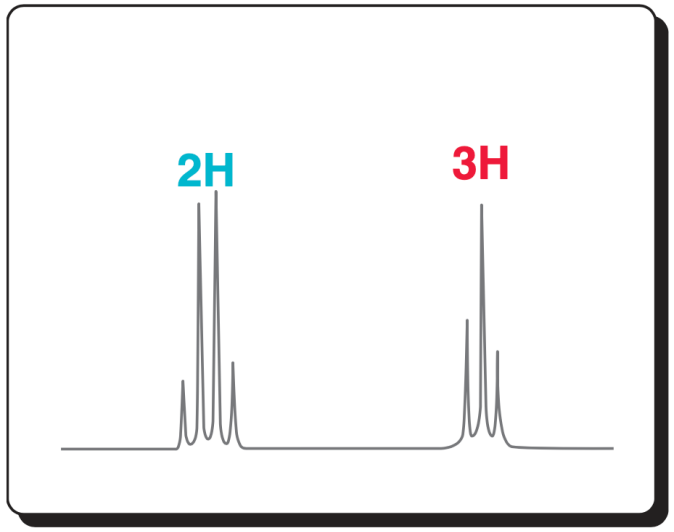


- Pattern recognition: *tert*-butyl group

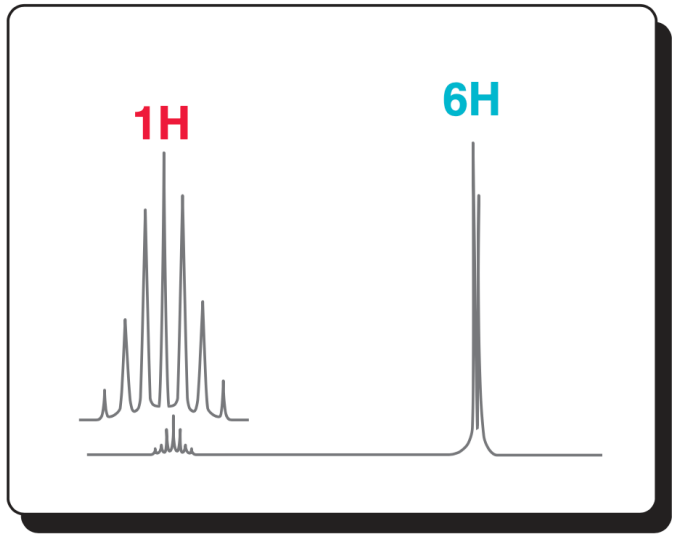


- Summary for common patterns

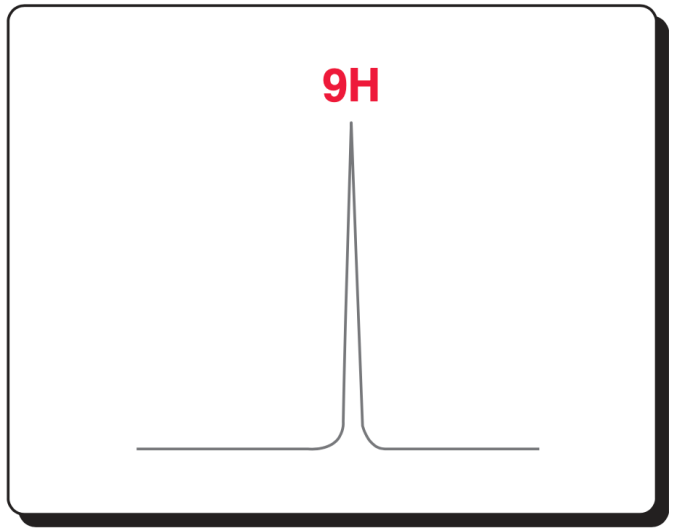
Ethyl



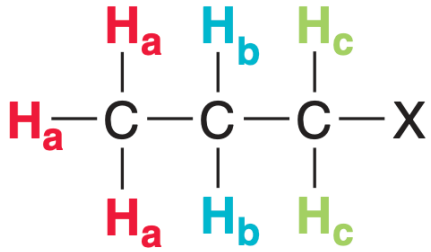
Isopropyl



***tert*-Butyl**



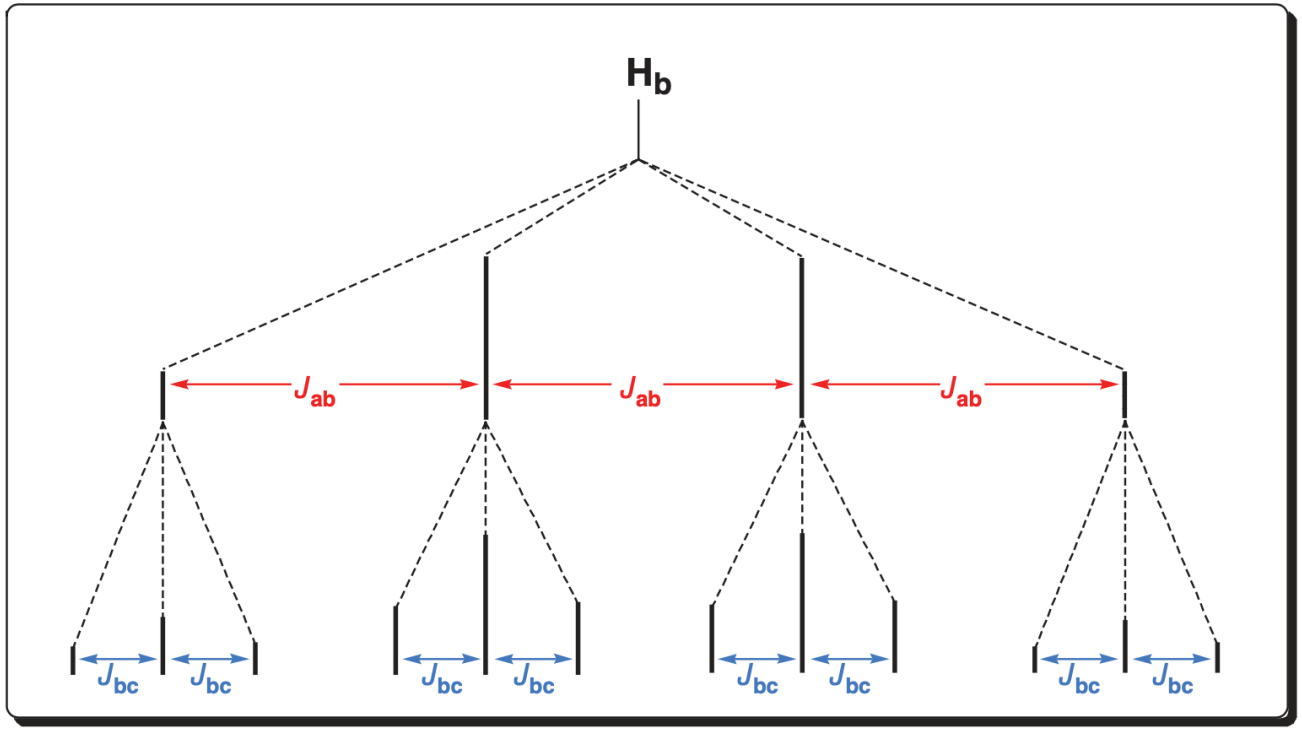
- Complex splitting

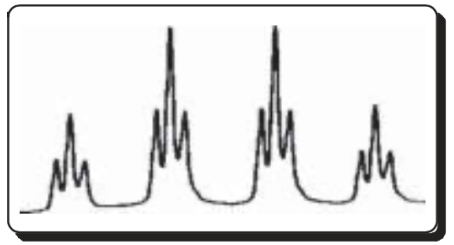


more than one kind of different neighboring protons

the signal for H_b is comprised of 12 peaks (4×3)

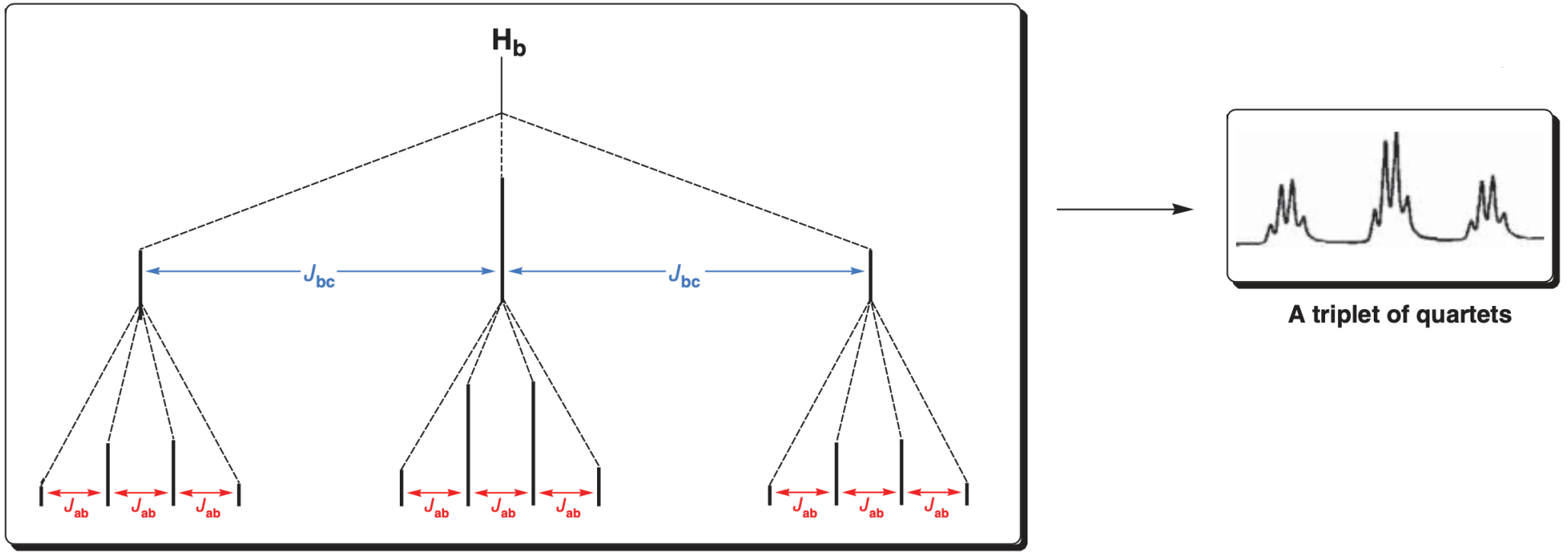
- $J_{ab} >> J_{bc}$ – a quartet of triplets



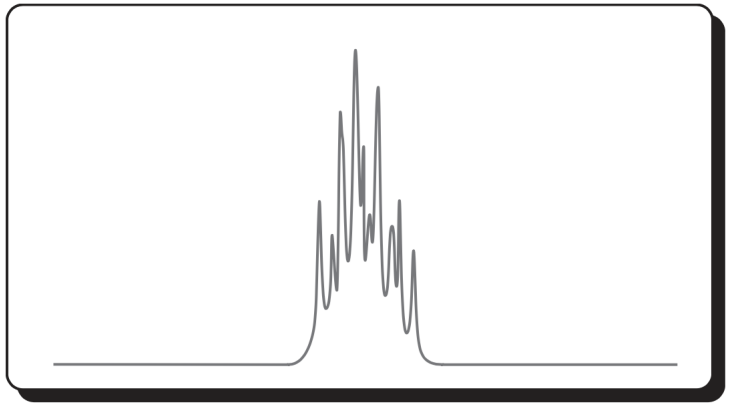


A quartet of triplets

- $J_{bc} >> J_{ab}$ – a triplet of quartets



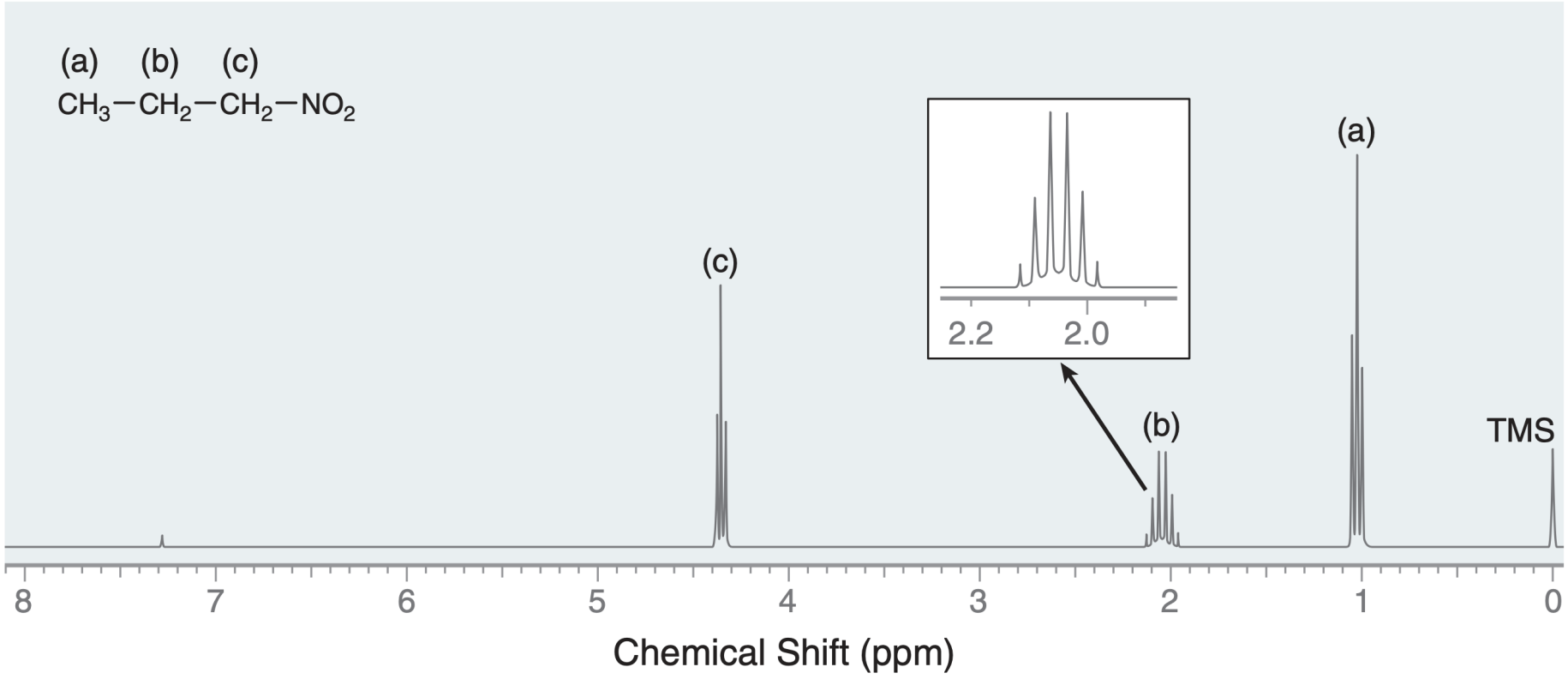
- Fairly similar values of J_{ab} and J_{bc} – a multiplet



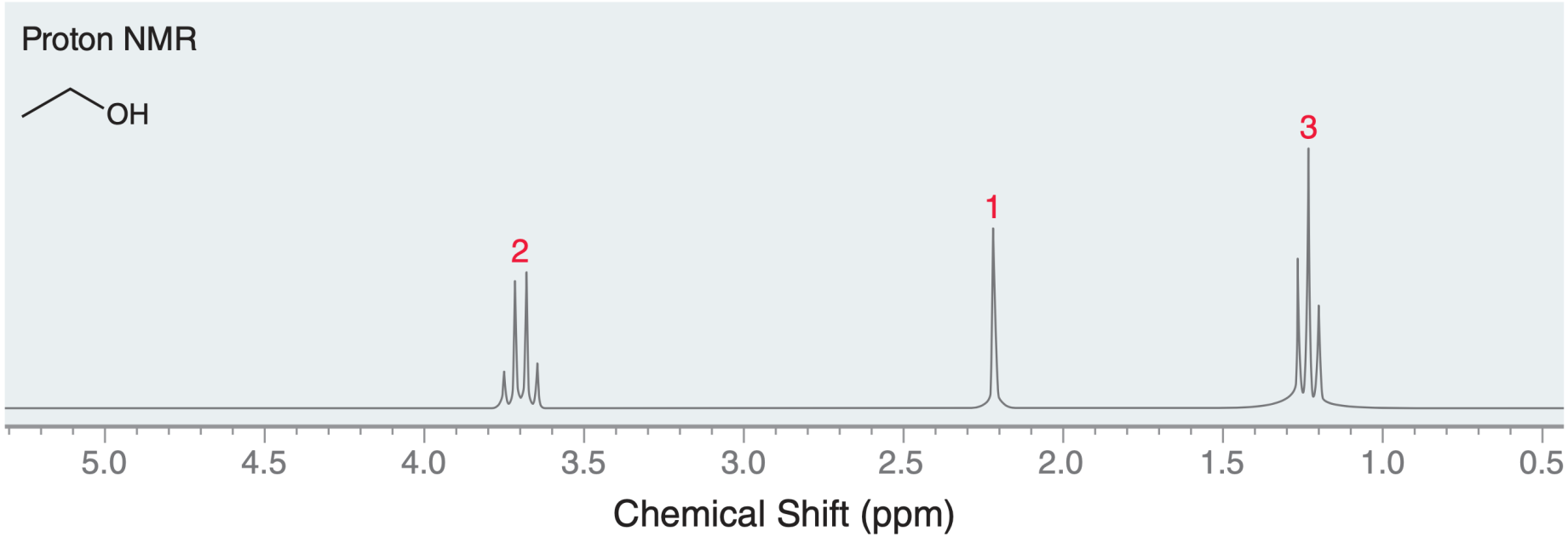
A multiplet

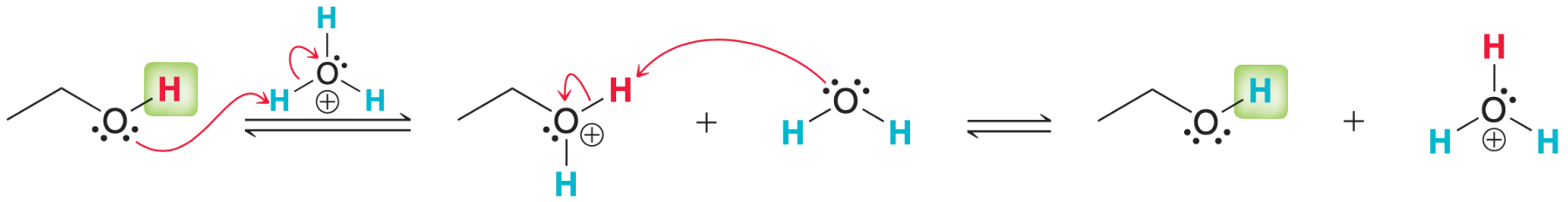
multiplet requires a more detailed analysis

- Almost identical J_{ab} and J_{ac} values – e.g. 1-nitropropane



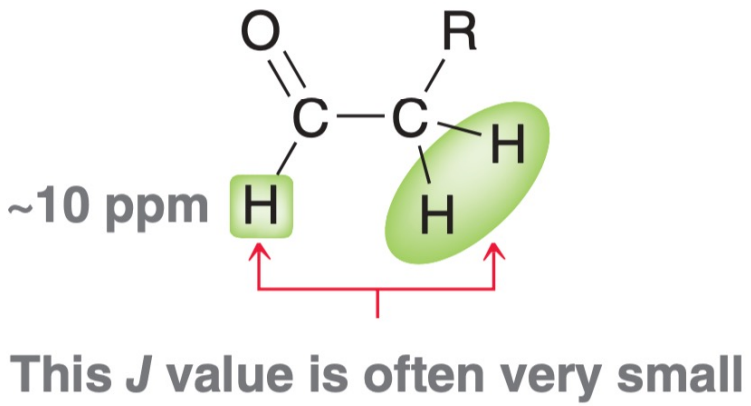
- Protons that lack observable coupling constants – producing a singlet





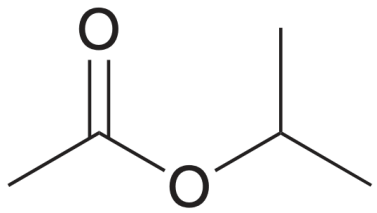
hydroxyl protons are **labile**

- No splitting for aldehydic protons (very small *J* value)

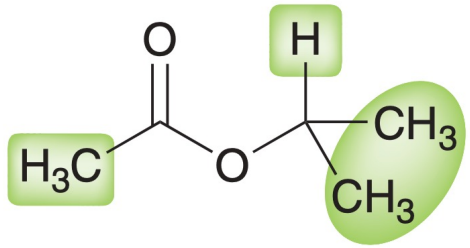


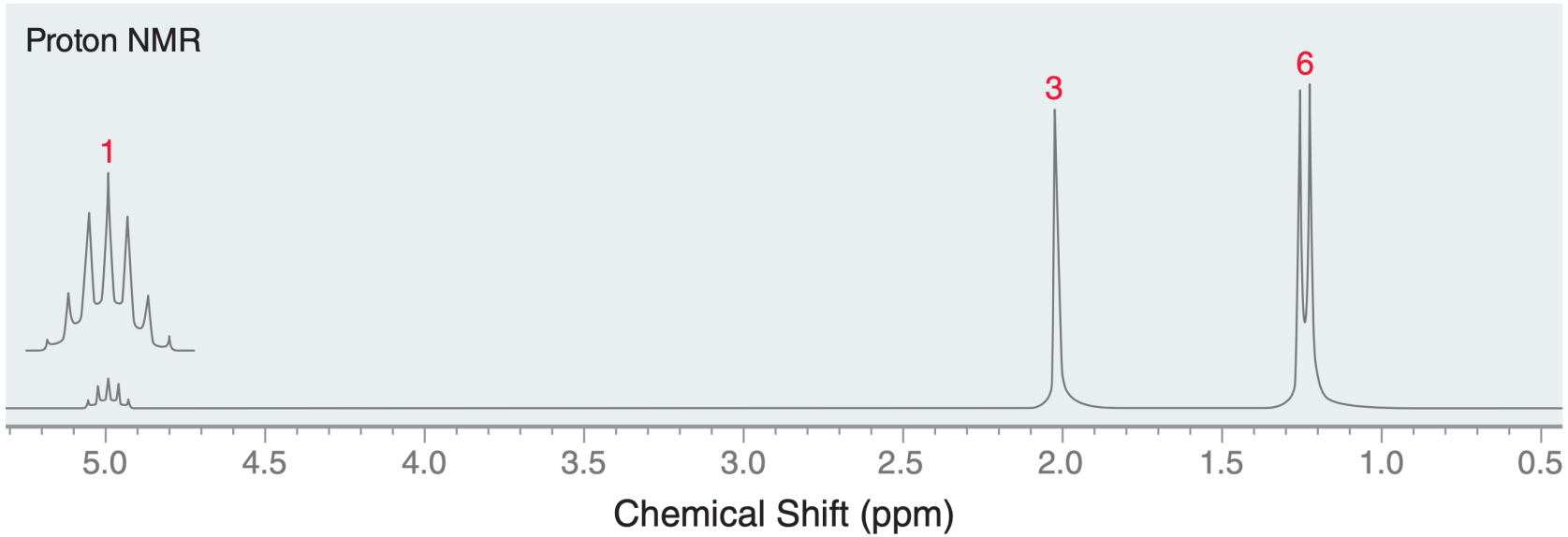
- Drawing the expected ^{1}H NMR spectrum of a compound
  - Determining the number of signals
  - Predicting chemical shifts
  - Determining the integration values
  - Predicting the multiplicity

- Practice: draw the expected ^{1}H NMR spectrum of isopropyl acetate.

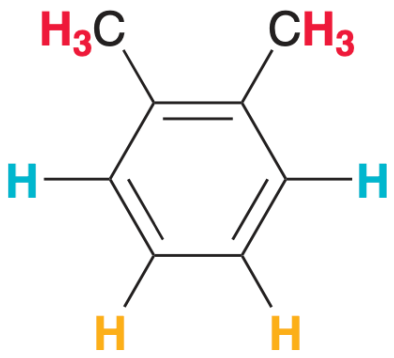


Analyzing a ^1H NMR Spectrum

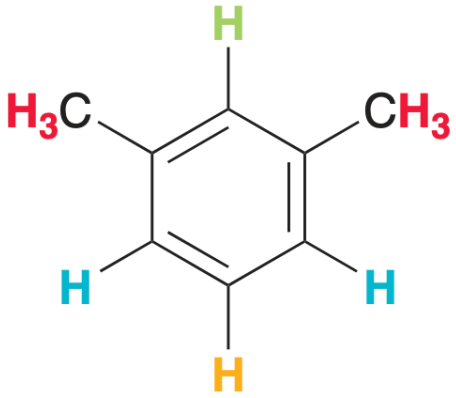




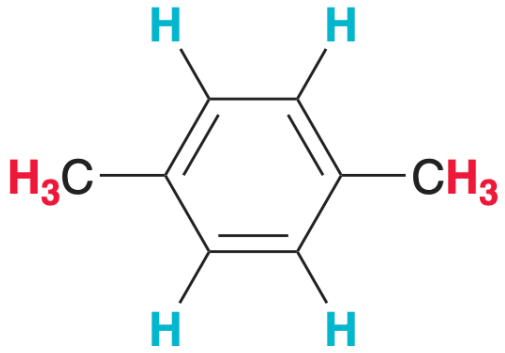
- Using ^{1}H NMR spectroscopy to distinguish between compounds



Three signals

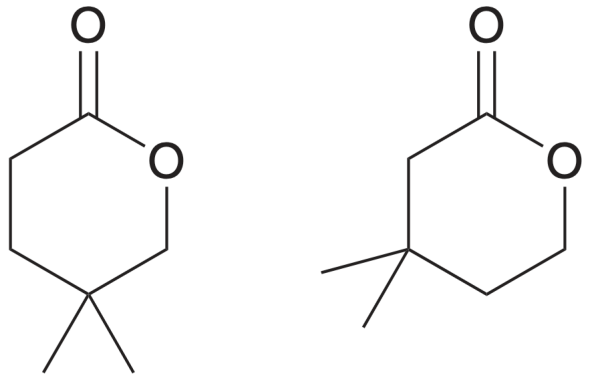


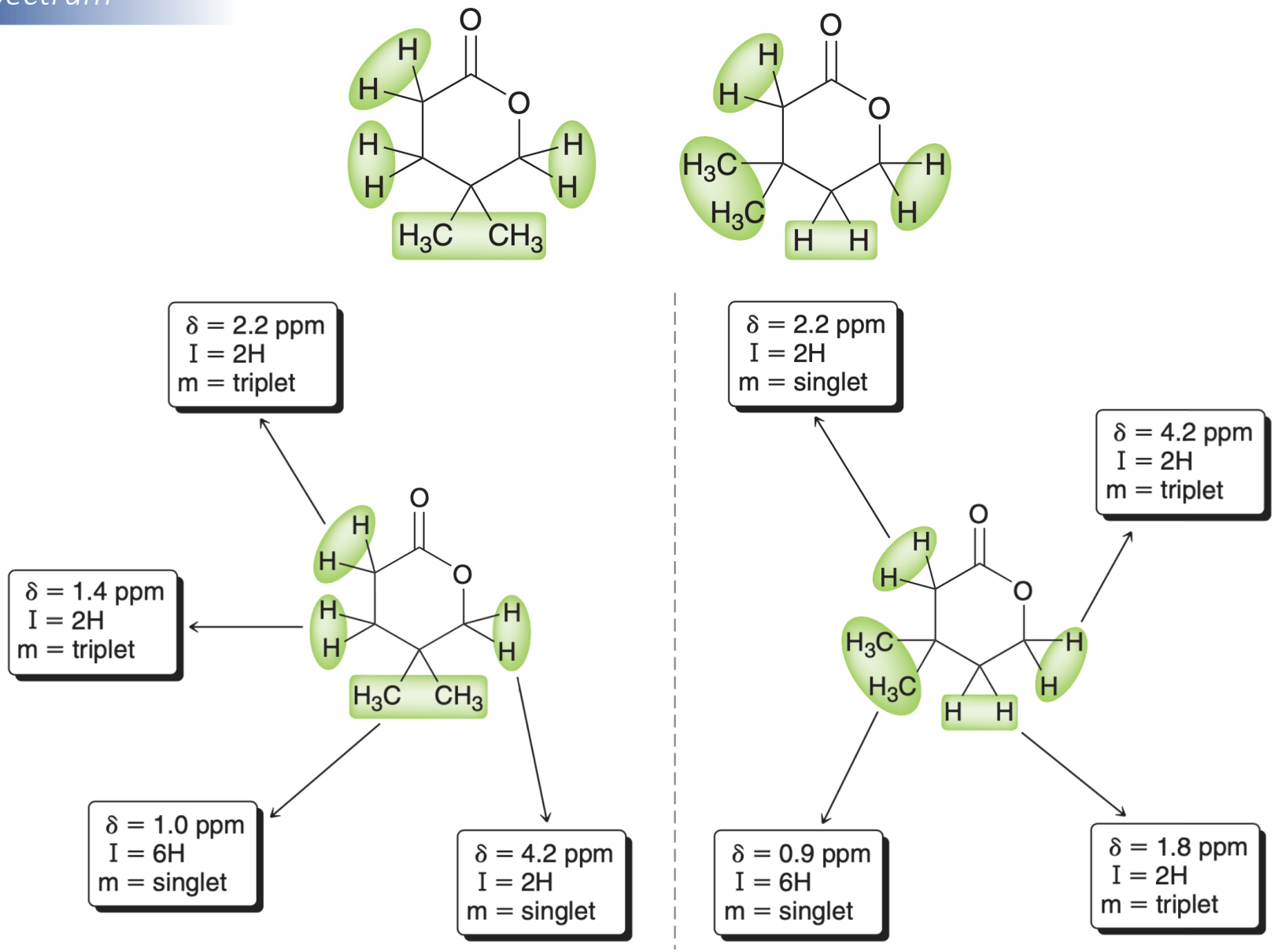
Four signals



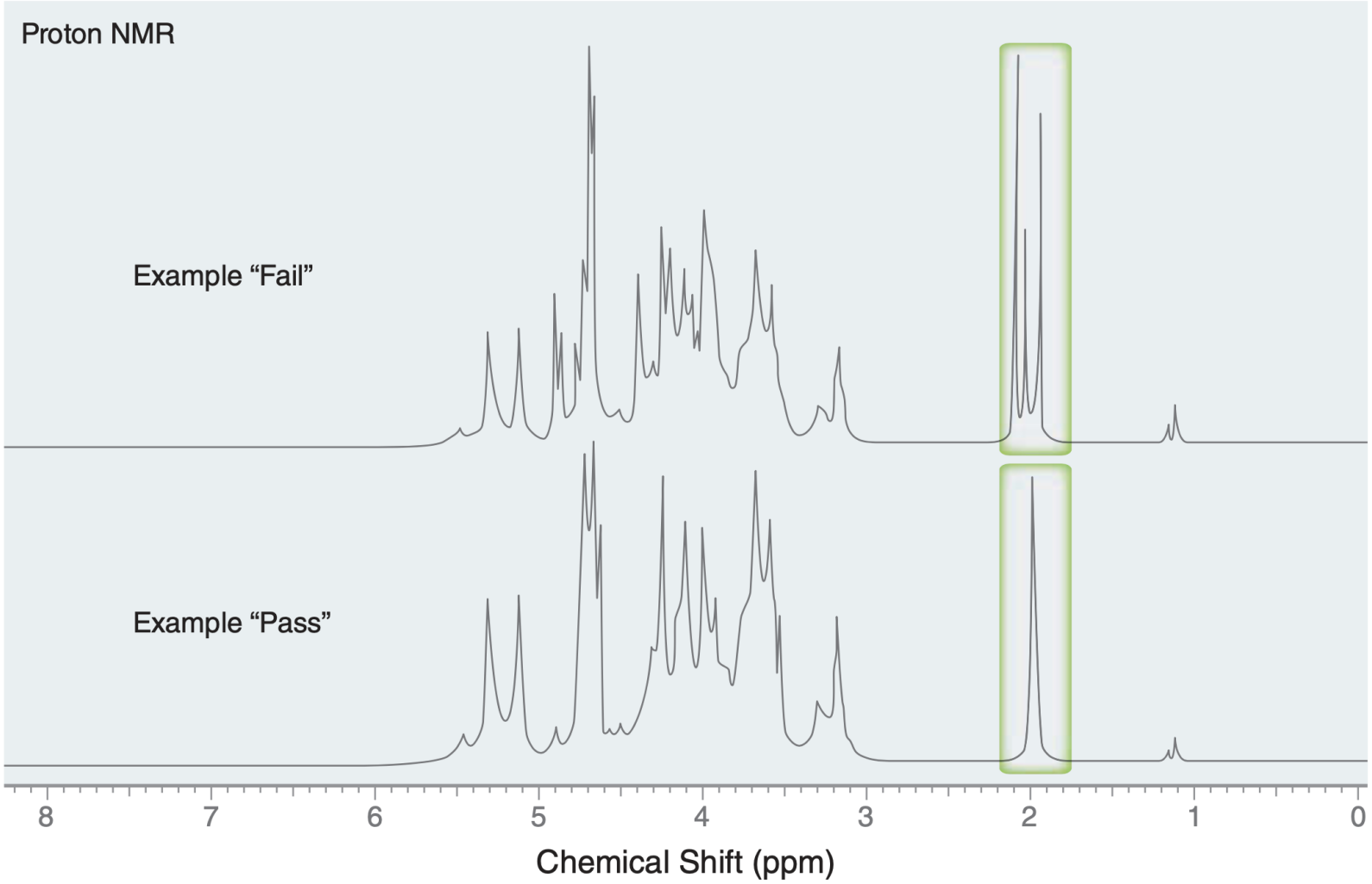
Two signals

- Practice: how would you use ^{1}H NMR spectroscopy to distinguish between the following compounds?



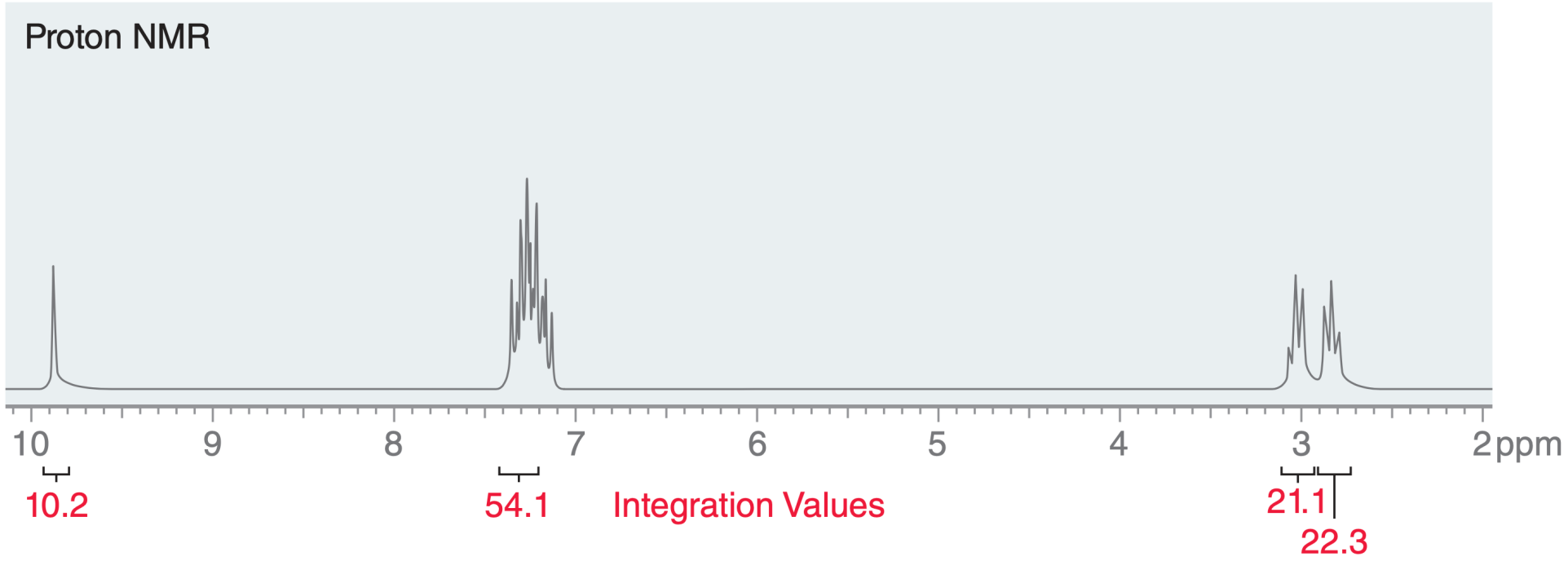

δ = 2.2 ppm
I = 2H
m = triplet
δ = 1.4 ppm
I = 2H
m = triplet
δ = 1.0 ppm
I = 6H
m = singlet
δ = 4.2 ppm
I = 2H
m = singlet
δ = 2.2 ppm
I = 2H
m = singlet
δ = 4.2 ppm
I = 2H
m = triplet
δ = 0.9 ppm
I = 6H
m = singlet
δ = 1.8 ppm
I = 2H
m = triplet

- Detection of impurities in heparin sodium by ^{1}H NMR



- Analyzing a ^{1}H NMR Spectrum
  - Inspecting the molecular formula (if it is given) – and acquiring HDI
  - Consider the number of signals and integration of each signal (gives clues about the symmetry of the compound)
  - Analyze each signal (chemical shift, integration, and multiplicity) and then draw fragments consistent with each signal
  - Assemble the fragments into a molecular structure
  - Verify that the proposed structure is consistent with all of the spectral data

- Practice: identify the structure of a compound with the molecular formula $C_9H_{10}O$ that exhibits the following ^{1}H NMR spectrum:

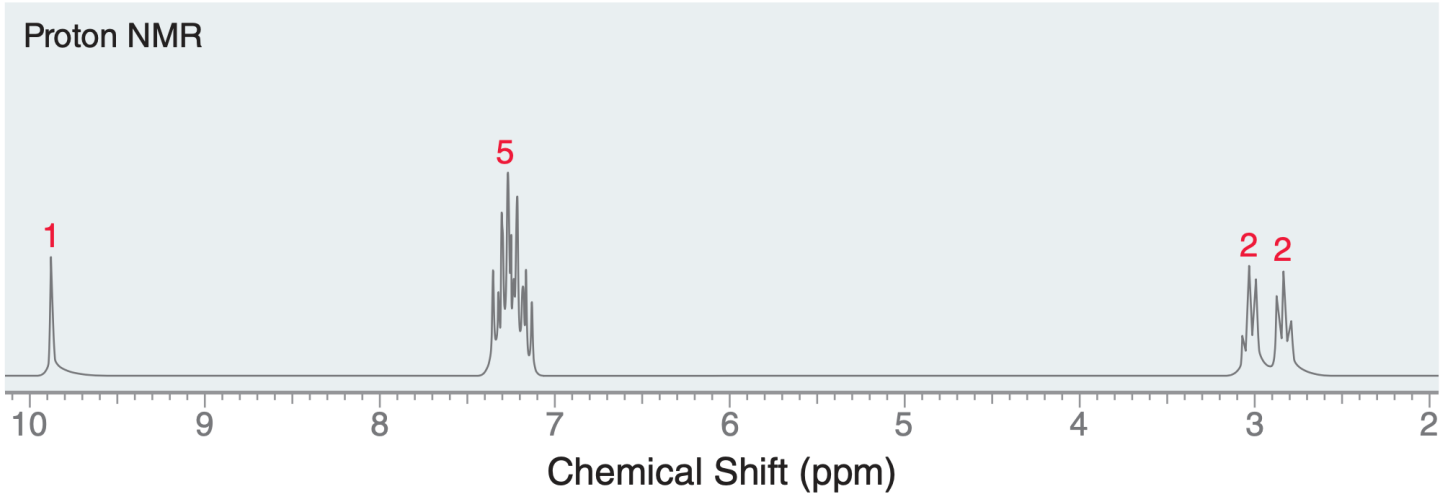


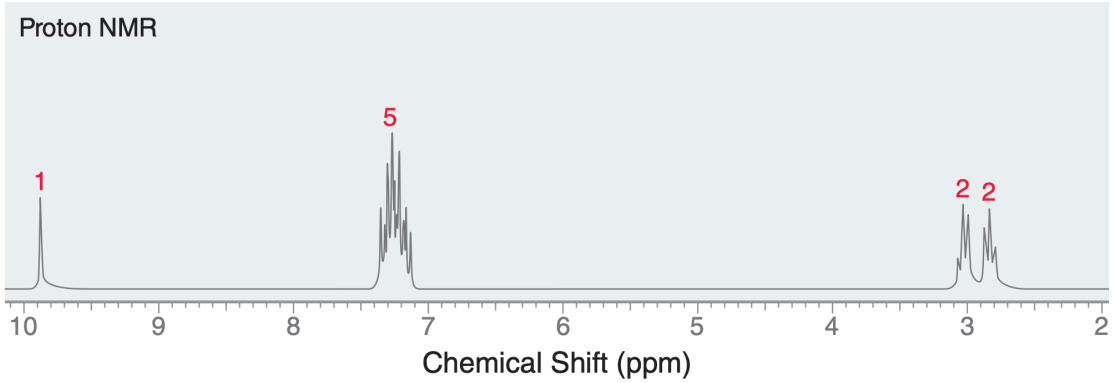
step.1 calculate the HDI

HDI = 5 (usually an aromatic ring + one other unsaturation degree)

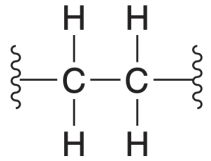
step.2 consider the relative number of H

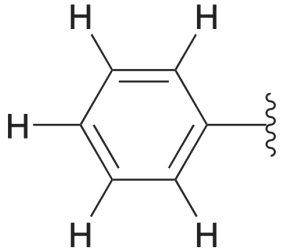
$$\frac{10.2}{10.2} = 1 \qquad \frac{54.1}{10.2} = 5.30 \qquad \frac{21.1}{10.2} = 2.07 \qquad \frac{22.3}{10.2} = 2.19$$



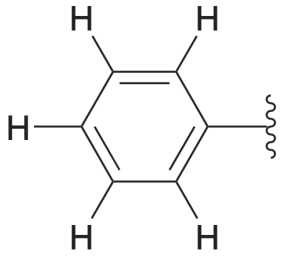


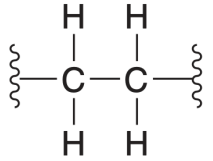
step.3 analyze each signals (fragments)

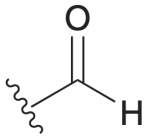




Five aromatic protons

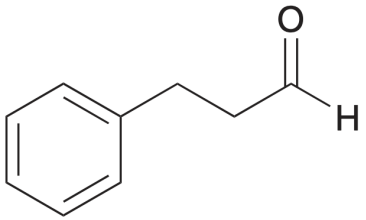




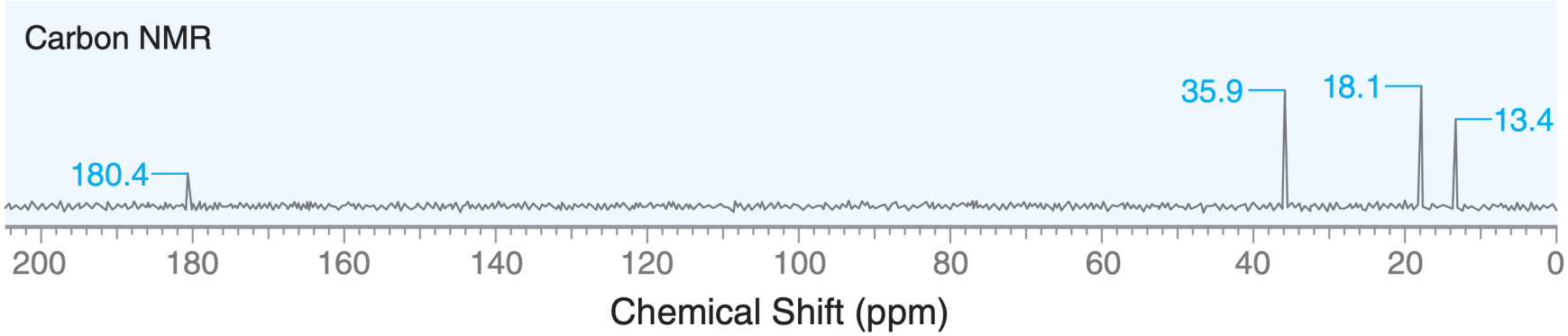


totally

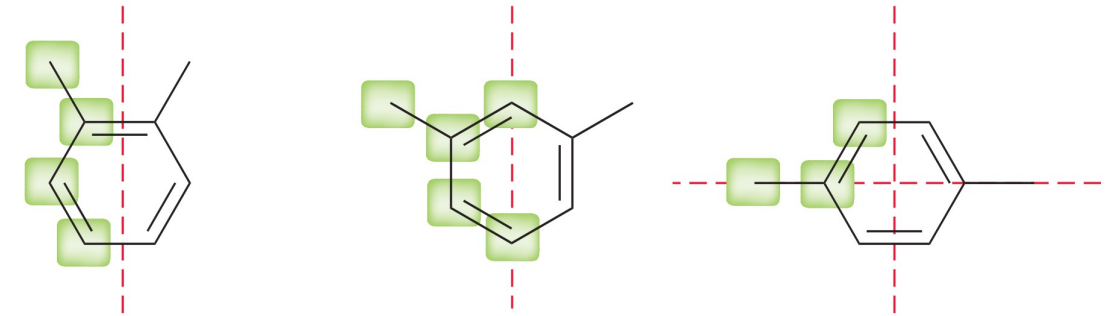
step.4 assemble the fragments



- ^{13}C NMR Spectrum
  - Similar principle as ^{1}H NMR
  - Low abundance & low gyromagnetic ratio – low detectability – low SNR
  - No integration – no relationship between integration & relative ^{13}C numbers
  - **Broadband decoupling** is used to suppress ^{13}C – ^{1}H splitting – avoiding complex splitting & signal overlapping
  - **Off-resonance decoupling** can be used to retrieve one-bond couplings



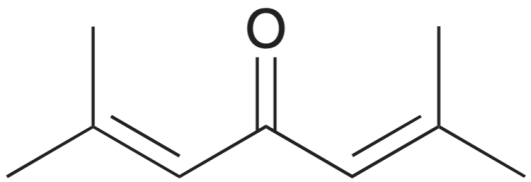
- Chemical Shifts in ^{13}C NMR Spectroscopy



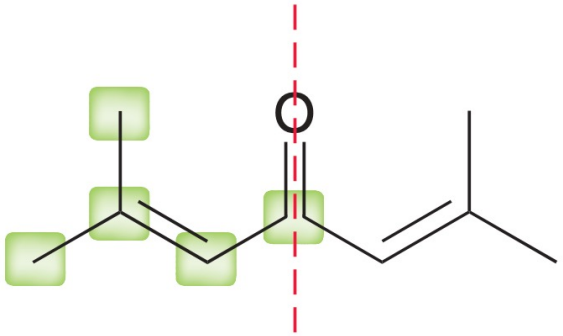
Carbon atoms of carbonyl groups. These carbon atoms are highly deshielded.	sp^2-hybridized carbon atoms.	sp-hybridized carbon atoms as well as sp^3-hybridized carbon atoms that are deshielded by electronegative atoms.	sp^3-hybridized carbon atoms (methyl, methylene, and methine groups).

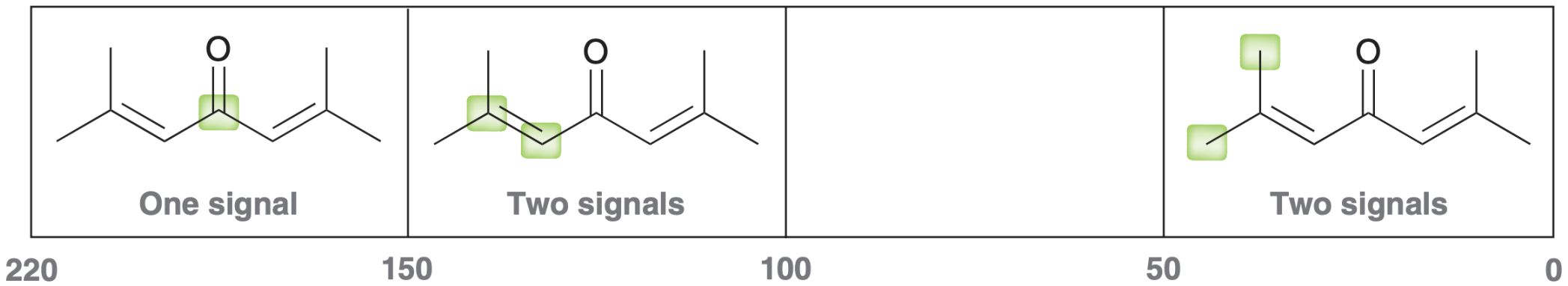
220 150 100 50 0 ppm

- Practice: predict the number of signals and the location of each signal in a ^{13}C NMR spectrum in the following compound:

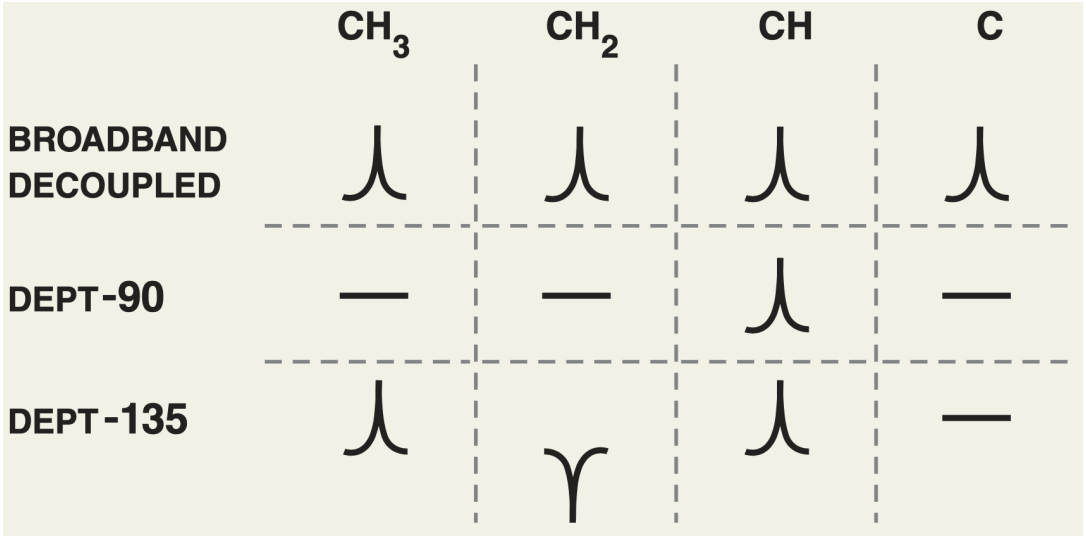


^{13}C NMR Spectrum

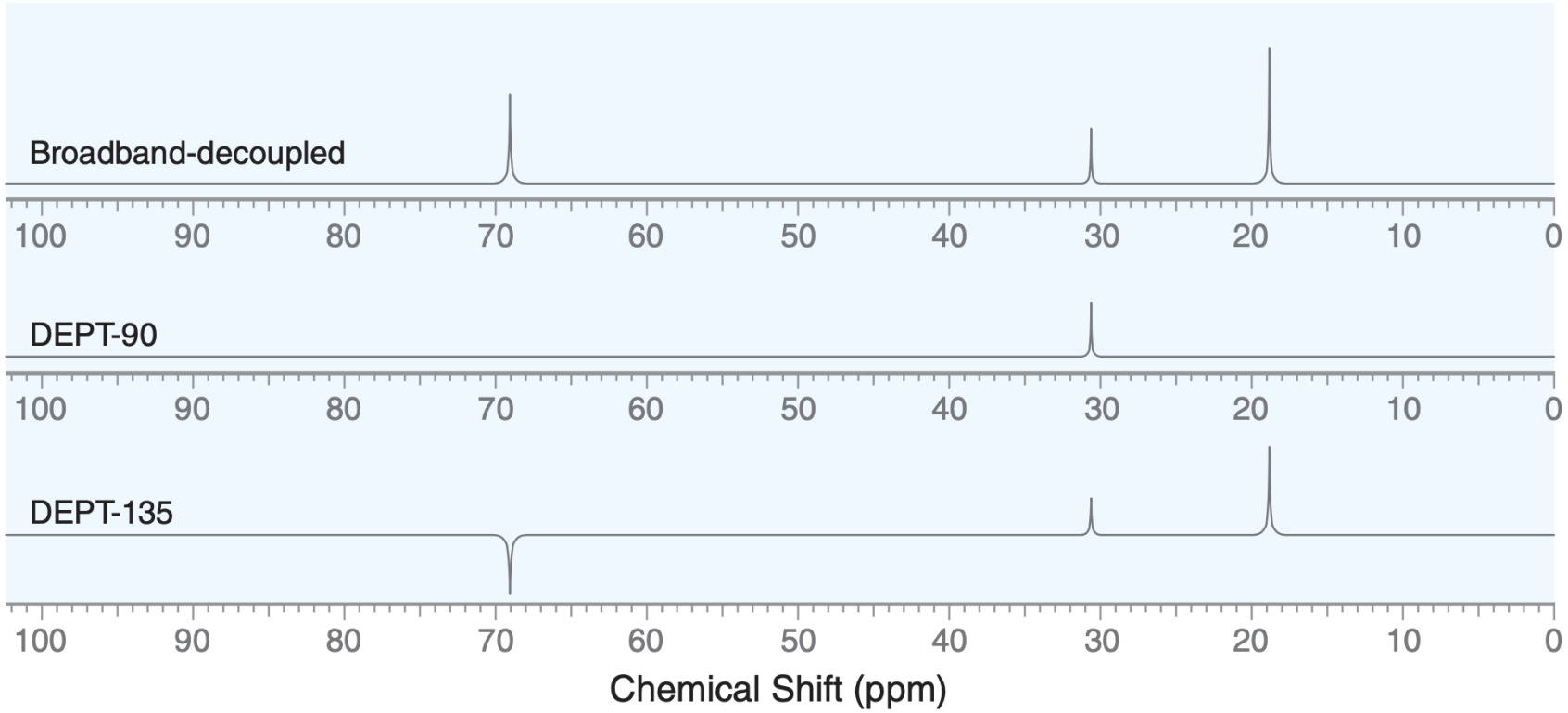




- **D**istortionless **E**nhancement by **P**olarization **T**ransfer **(DEPT) ^{13}C NMR**
  - Regular broadband-decoupled ^{13}C NMR: all signals
  - DEPT-90: only CH group signals appear
  - DEPT-135: CH_3 and CH groups appear as positive signals, CH_2 groups appear as negative signals (pointing down), quaternary carbon atoms do not appear



- Practice: determine the structure of an alcohol with the molecular formula $C_4H_{10}O$ that exhibits the following ^{13}C NMR spectra:



- HDI = 0, which indicates not rings or π bonds
- Three signals in broadband-decoupled spectrum
- The signal at approximately 69 ppm is a CH_2 group (signal is negative in DEPT-135)
- The signal at approximately 30 ppm is a CH group (signal is positive in all spectra)
- The signal at approximately 19 ppm is a CH_3 group (signal is positive in the broadband-decoupled spectrum, absent in DEPT-90, and positive in DEPT-135)