Nuclear Magnetic Resonance
(NMR) Spectroscopy

Definition of NMR Spectroscopy

Nuclear magnetic resonance spectroscopy: commonly referred to as NMR, is a technique which exploits the magnetic properties of certain nuclei to study physical, chemical, and biological properties of matter

Compared to mass spectrometry, larger amounts of sample are needed, but non-destructive

NMR History

- 1937 Rabi’s prediction and observation of nuclear magnetic resonance
- 1945 First NMR of solution (Bloch et al for H2O) and solids (Purcell et al for parafin)!
- 1953 **Overhauser** NOE (nuclear Overhauser effect)
- 1966 Ernst, Anderson Fourier transform NMR
- 1975 Jeener, Ernst 2D NMR
- 1980 NMR protein structure by Wuthrich
- 1990 3D and 1H/15N/13C Triple resonance
- 1997 Ultra high field (~800 MHz) & TROSY(MW 100K)

Continuation of NMR History

Nobel prizes

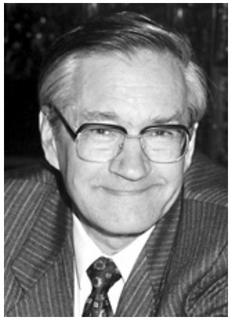
1944 *Physics* Rabi (Columbia)

"for his resonance method for recording the magnetic properties of atomic nuclei"

1952 *Physics* Bloch (Stanford), Purcell (Harvard)

"for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith"

1991 *Chemistry* Ernst (ETH)



"for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy"

Continuation of NMR History

2002 *Chemistry* Wüthrich (ETH)

"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution"

2003 *Medicine* Lauterbur (University of Illinois in Urbana), Mansfield (University of Nottingham)

"for their discoveries concerning magnetic resonance imaging"

Spin of Nuclei

Fermions : Odd mass nuclei with an odd number of nucleons have fractional spins.

I = 1/2 (^{1}H, ^{13}C, ^{19}F, ^{31}P), I = 3/2 (^{11}B, ^{33}S) & I = 5/2 (^{17}O).

Bosons : Even mass nuclei with odd numbers of protons and neutrons have integral spins.

I = 1 (^{2}H, ^{14}N)

Even mass nuclei composed of even numbers of protons and neutrons have zero spin

I = 0 (^{12}C, and ^{16}O, ^{32}S)

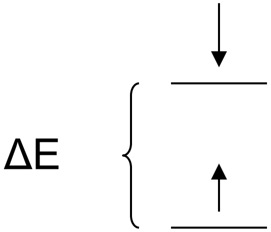
Nuclear Magnetic Resonance (nmr)

-the nuclei of some atoms spin: ^{1}H, ^{13}C, ^{19}F, …

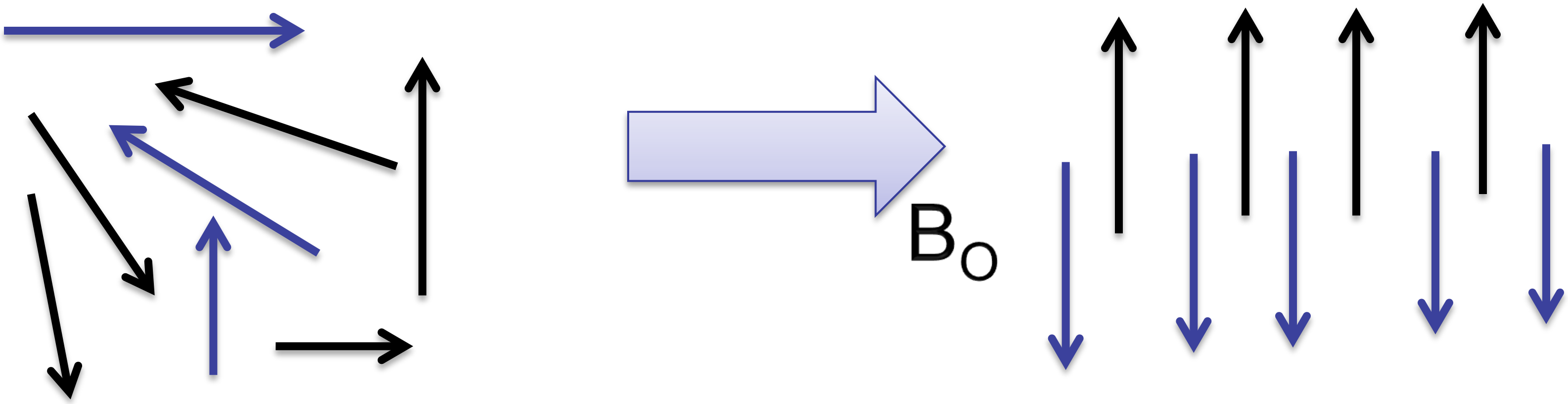
-the nuclei of many atoms do not spin: ^{2}H, ^{12}C, ^{16}O, …

-moving charged particles generate a magnetic field (↗)

-when placed between the poles of a powerful magnet, spinning nuclei will align with or against the applied field creating an energy difference. Using a fixed radio frequency, the magnetic field is changed until the $\Delta E = E_{EM}$. When the energies match, the nuclei can change spin states (resonate) and give off a magnetic signal.

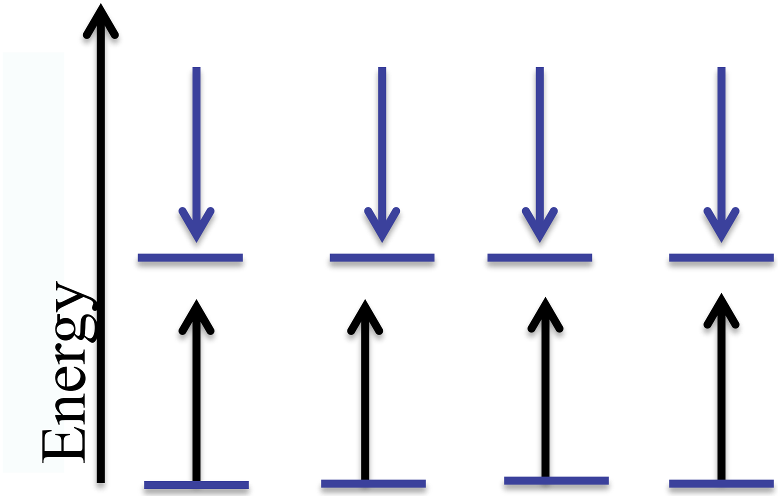


How NMR Works



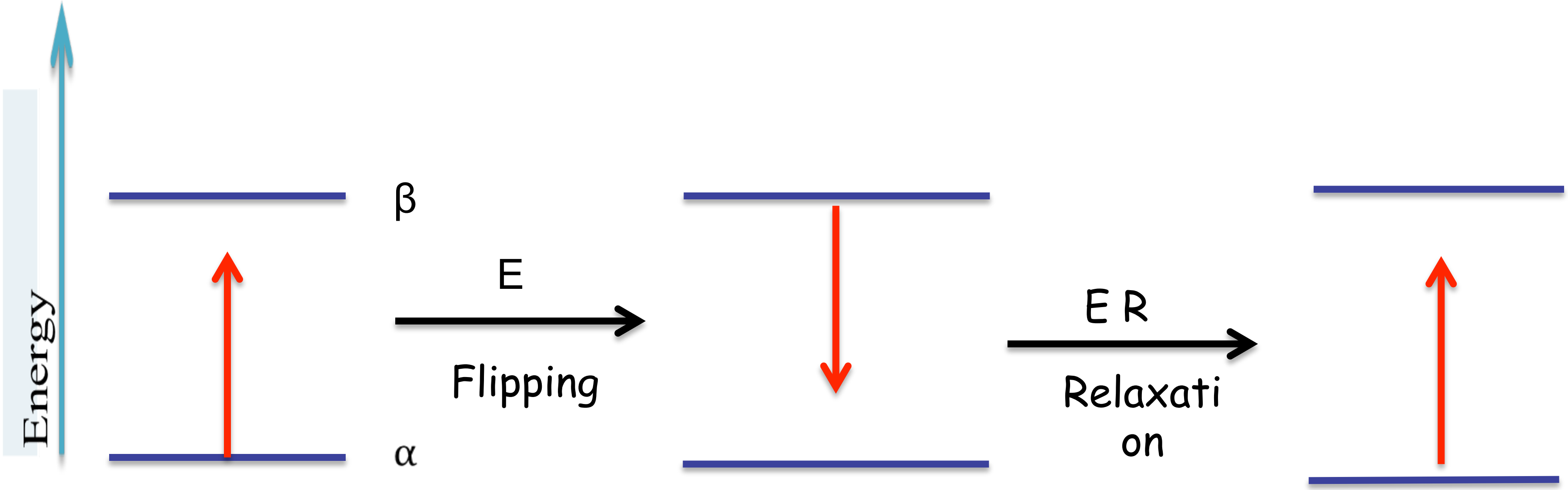
Nuclear spins random

Nuclear spins will aligned or oppose the field



Oppose with B_o (α- spin state)

Aligned the B_o (β-spin state)



Energy

β

α

E

Flipping

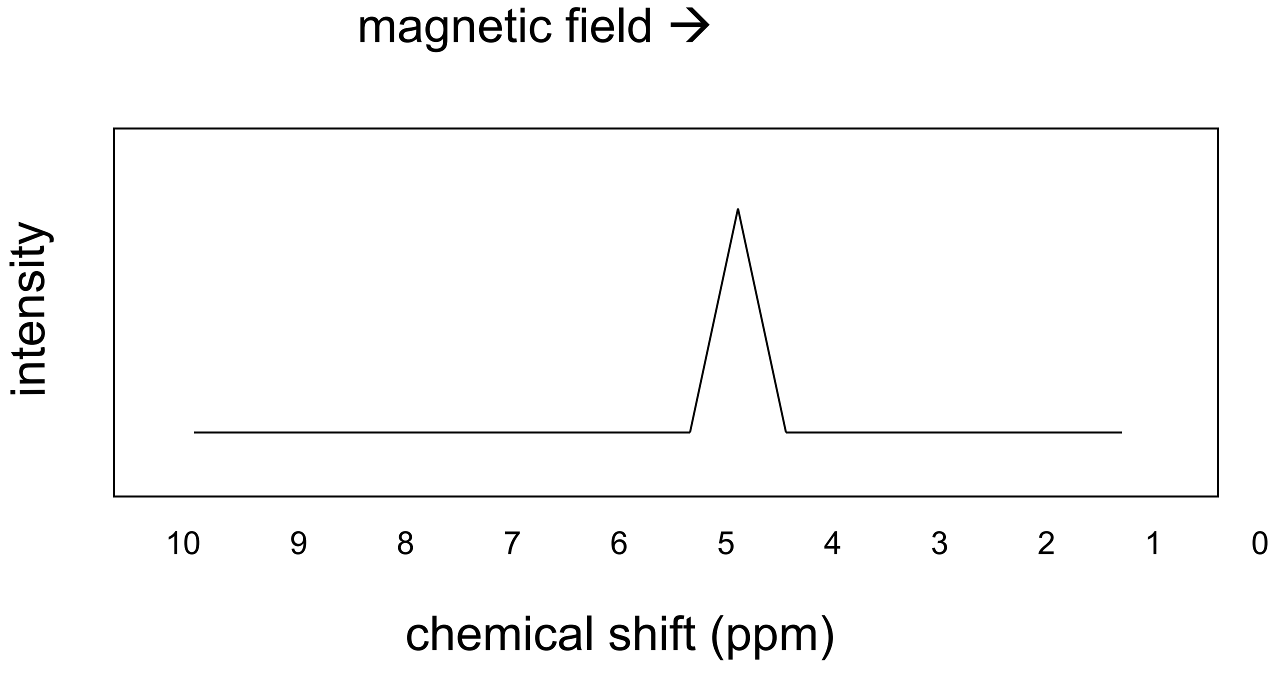
E R

Relaxation

magnetic field = 14,092 gauss

for ^{1}H v = 60,000,000 Hz (60 MHz)

nmr spectrum



^{1}H nuclei are **shielded** by the magnetic field produced by the surrounding electrons. The higher the electron density around the nucleus, the higher the magnetic field required to cause resonance.

CH_3Cl	versus	CH_4
lower electron density		higher electron density
resonate at lower applied field		resonate at higher applied field

$CHCCl_3$??

Information from ^{1}H-nmr spectra:

1. Number of signals: How many different types of hydrogens in the molecule.
2. Position of signals (chemical shift): What types of hydrogens.
3. Relative areas under signals (integration): How many hydrogens of each type.
4. Splitting pattern: How many neighboring hydrogens.

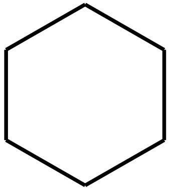
1. Number of signals: How many different types of hydrogens in the molecule.

Magnetically equivalent hydrogens resonate at the same applied field.

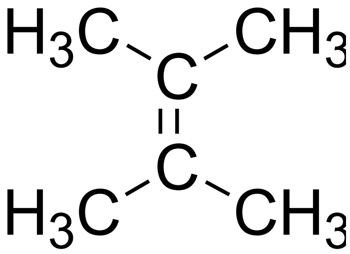
Magnetically equivalent hydrogens are also chemically equivalent.

# of signals? CH_4 CH_3CH_3

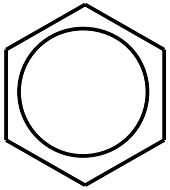
number of signals?



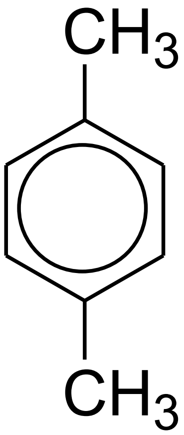
one



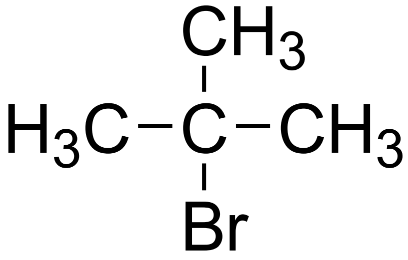
one



one



two



$$H_3C-\overset{\displaystyle CH_3}{\underset{\displaystyle Br}{C}}-CH_3$$

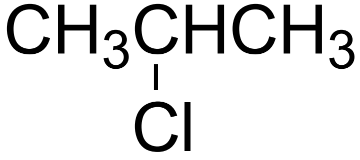
one

$CH_3CH_2\text{-}Br$

two

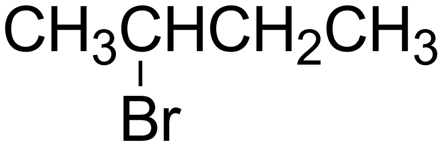
$CH_3CH_2CH_2\text{-}Br$

three



$$CH_3\underset{\displaystyle Cl}{C}HCH_3$$

two

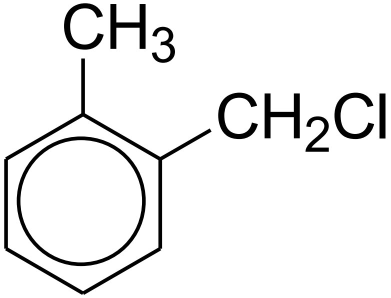


$$\mathrm{CH_3}\underset{\mathrm{Br}}{\underset{|}{\mathrm{C}}}\mathrm{HCH_2CH_3}$$

four

$\mathrm{Cl\text{-}CH_2CH_2CH_2\text{-}Cl}$

two



three

2. Position of signals (chemical shift): what types of hydrogens.

primary	0.9 ppm		
secondary	1.3		
tertiary	1.5		
aromatic	6-8.5		
allyl 1.7			
benzyl	2.2-3		
chlorides	3-4	H-C-Cl	
bromides	2.5-4	H-C-Br	
iodides	2-4	H-C-I	
alcohols	3.4-4	H-C-O	
alcohols	1-5.5	H-O-	(variable)

Note: combinations may greatly influence chemical shifts. For example, the benzyl hydrogens in benzyl chloride are shifted to lower field by the chlorine and resonate at 4.5 ppm.

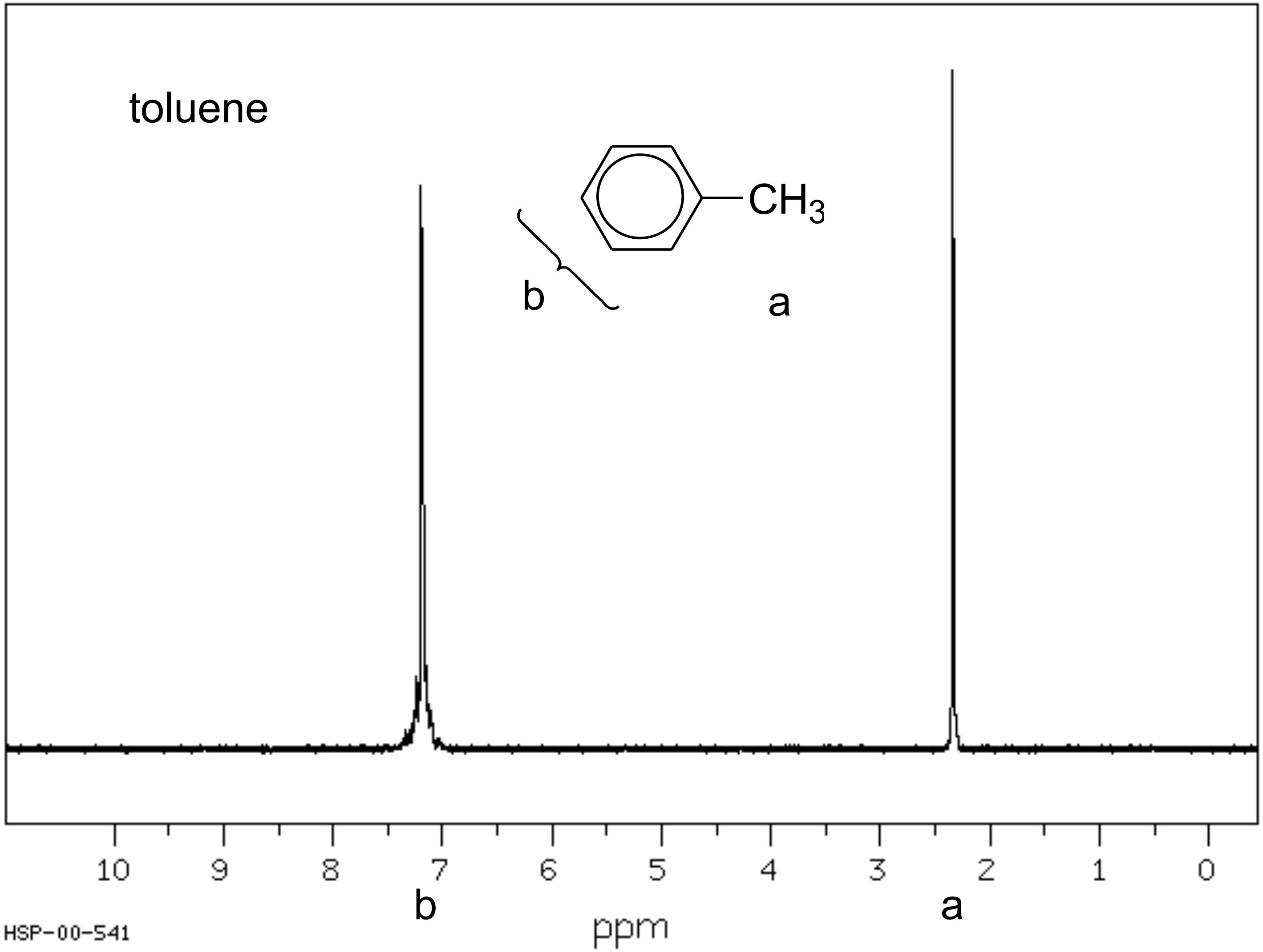
reference compound = tetramethylsilane $(CH_3)_4Si$ @ 0.0 ppm

remember: **magnetic field →**

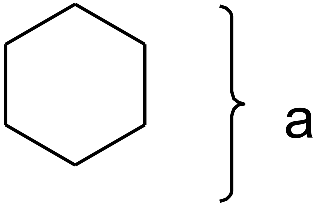
← chemical shift

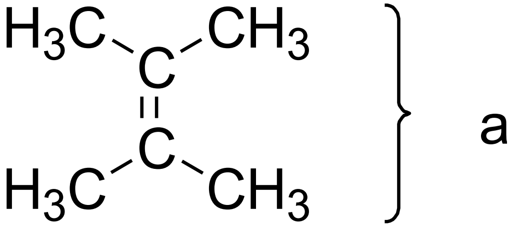
convention: let most upfield signal = a, next most upfield = b, etc.

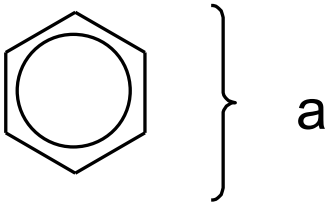
… c b a tms

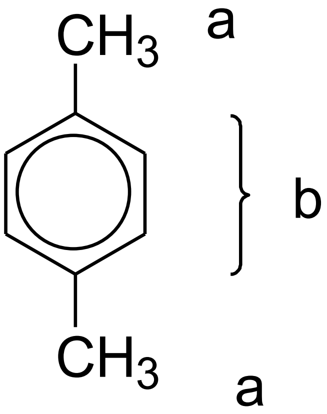

toluene
CH$_3$
b
a
10
9
8
7
6
5
4
3
2
1
0
b
a
ppm
HSP-00-541

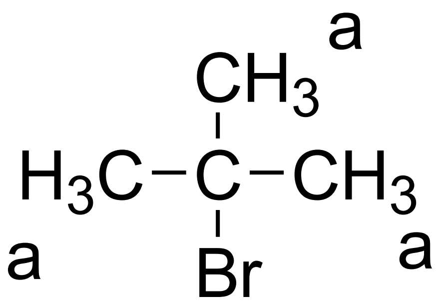
chemical shifts











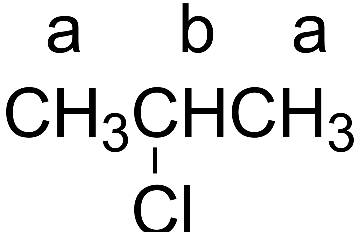
$$H_3C-\overset{CH_3}{\underset{Br}{C}}-CH_3$$

a b

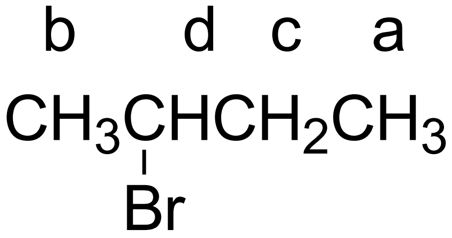
$CH_3CH_2\text{-}Br$

a b c

$CH_3CH_2CH_2\text{-}Br$

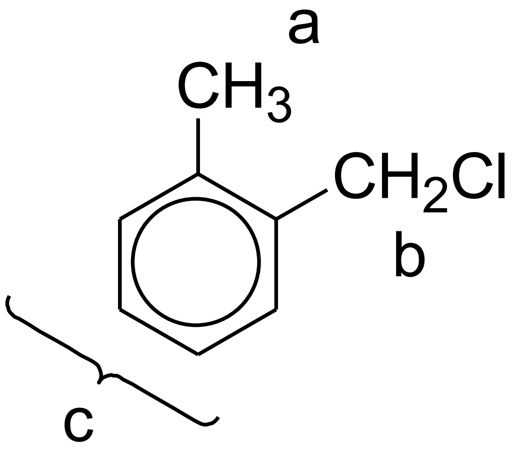


$$CH_3\underset{Cl}{C}HCH_3$$



$\overset{b}{CH_3}\underset{|\atop Br}{\overset{d}{C}}H\overset{c}{CH_2}\overset{a}{CH_3}$

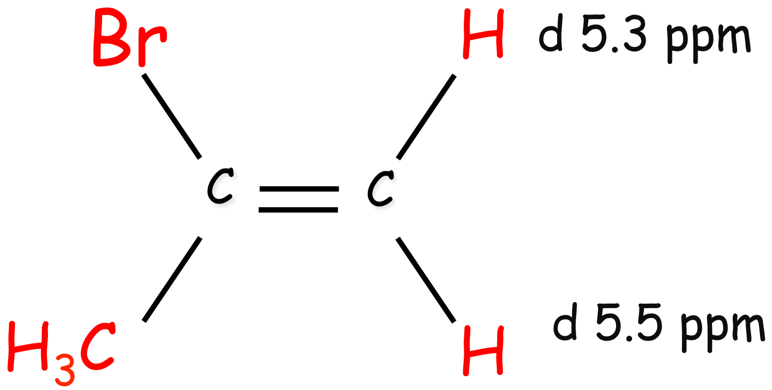
$Cl\text{-}\overset{b}{CH_2}\overset{a}{CH_2}\overset{b}{CH_2}\text{-}Cl$



Chemically Non Equivalent Protons

Diastereotropic protons

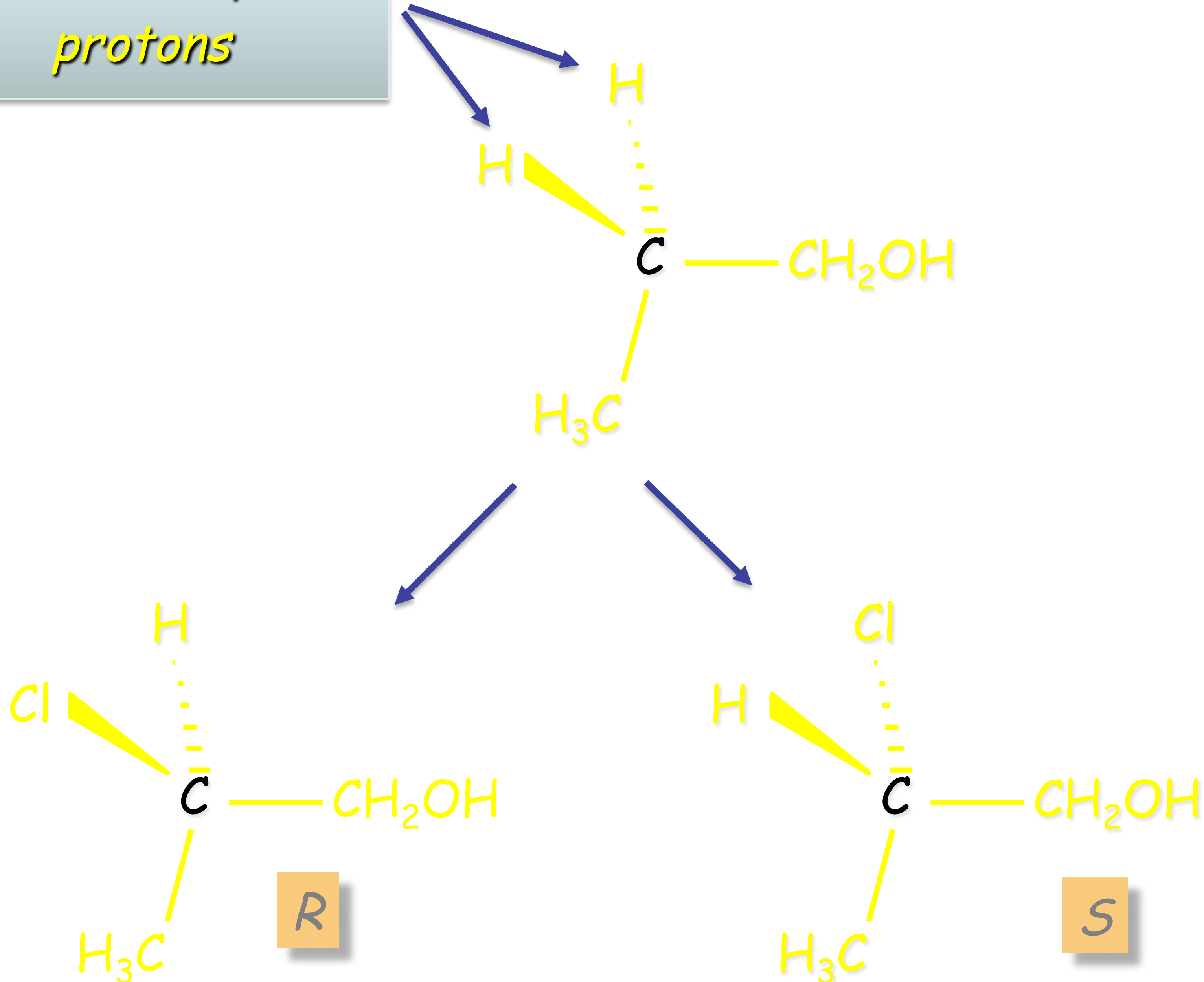
- ✓ Replacement by some arbitrary test group generates Diastereoisomers
- ✓ Diastereotropic protons can have different chemical shifts



Enantiotropic protons

- ✓ Are in mirror-image environments
- ✓ Replacement by some arbitrary test group generates enantiomers
- ✓ Enantiotropic protons have the same chemical shift

Enantiotropic protons



3. Integration (relative areas under each signal): how many hydrogens of each type.

a b c

$CH_3CH_2CH_2Br$

2 : 2

a 3H

b 2H

c 2H

a : b : c = 3 :

a b a

CH_3CHCH_3

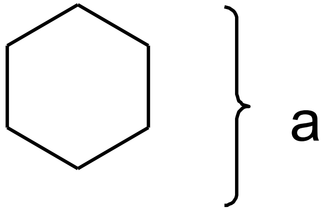
Cl

a 6H

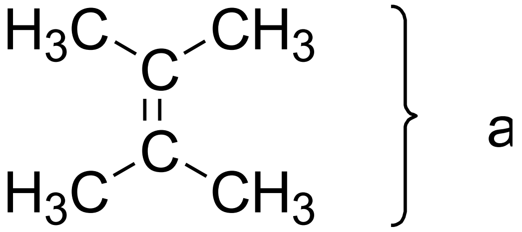
b 1H

a : b = 6 : 1

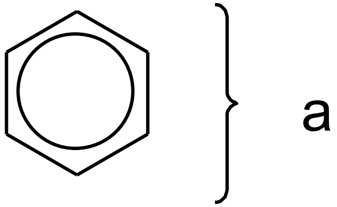
integration



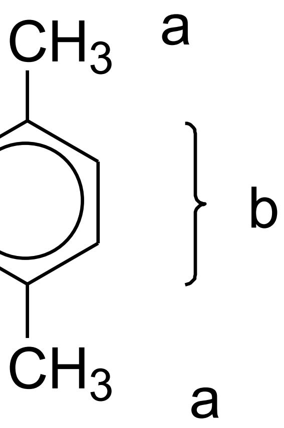
a 12 H



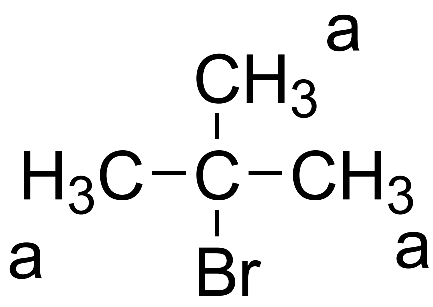
a 12 H



a 6 H



a 6 H
b 4 H



$$\underset{a}{H_3C}-\underset{Br}{\overset{\overset{a}{CH_3}}{C}}-\underset{a}{CH_3}$$

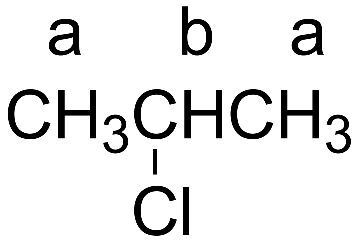
a 9 H

$$\overset{a}{CH_3}\overset{b}{CH_2}\text{-Br}$$

a 3 H
b 2 H

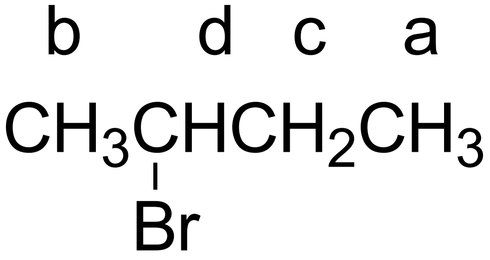
$$\overset{a}{CH_3}\overset{b}{CH_2}\overset{c}{CH_2}\text{-Br}$$

a 3 H
b 2 H
c 2 H



$$\overset{a}{CH_3}\underset{Cl}{\overset{b}{CH}}\overset{a}{CH_3}$$

a 6 H
b 1 H

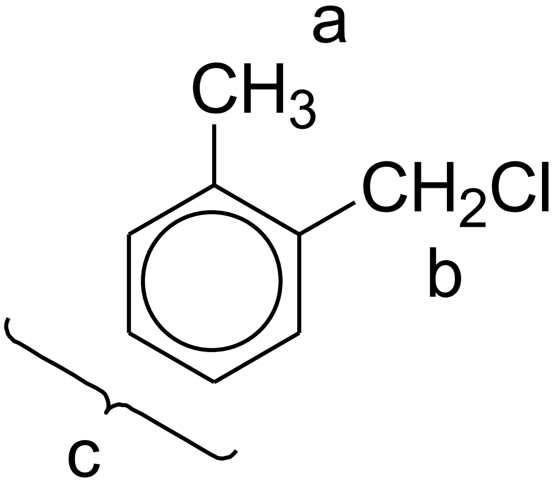


$$\overset{b}{CH_3}\underset{|\atop Br}{\overset{d}{CH}}\overset{c}{CH_2}\overset{a}{CH_3}$$

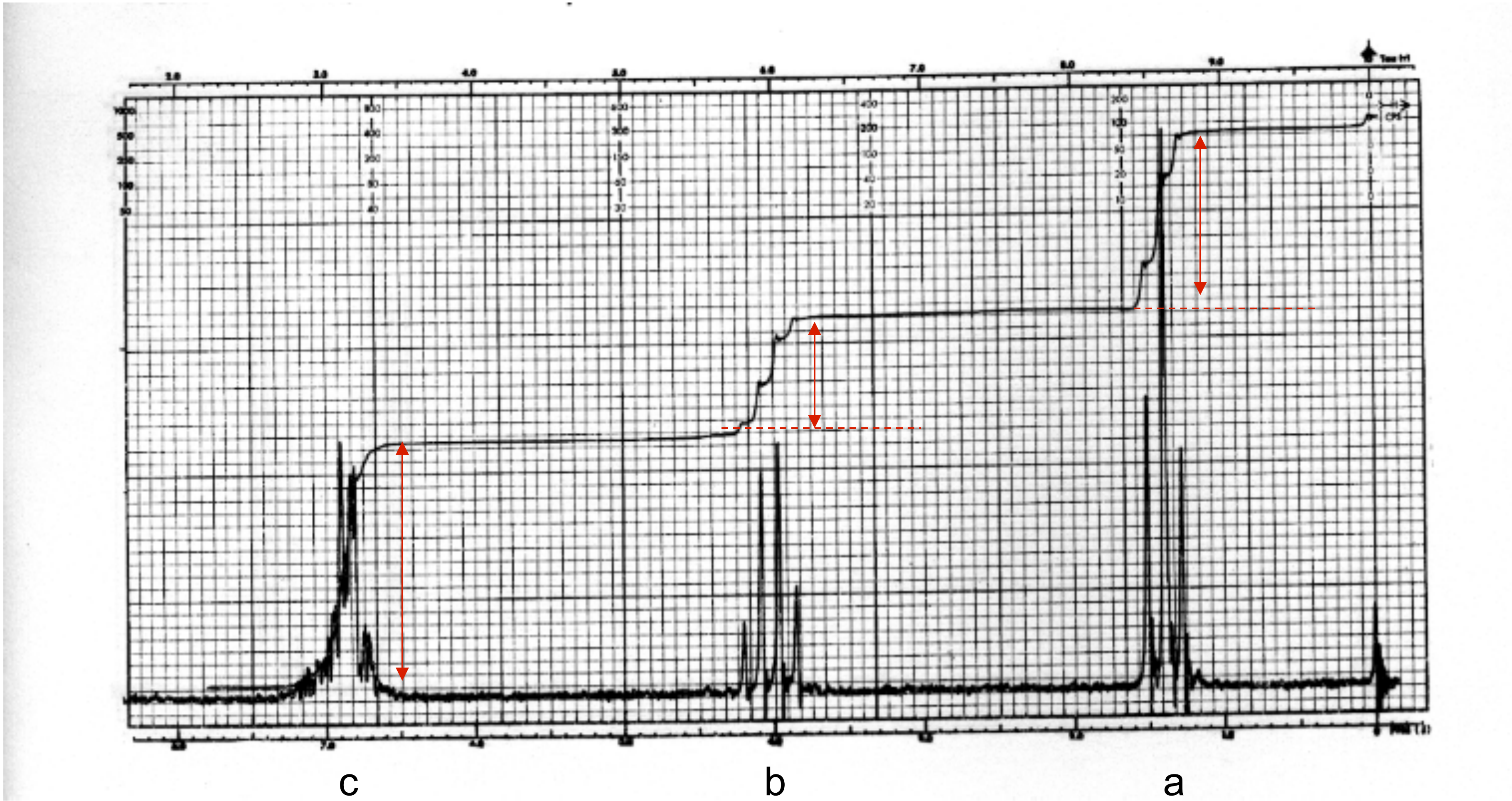
a	3 H
b	3 H
c	2 H
d	1 H

$$Cl\text{-}\overset{b}{CH_2}\overset{a}{CH_2}\overset{b}{CH_2}\text{-}Cl$$

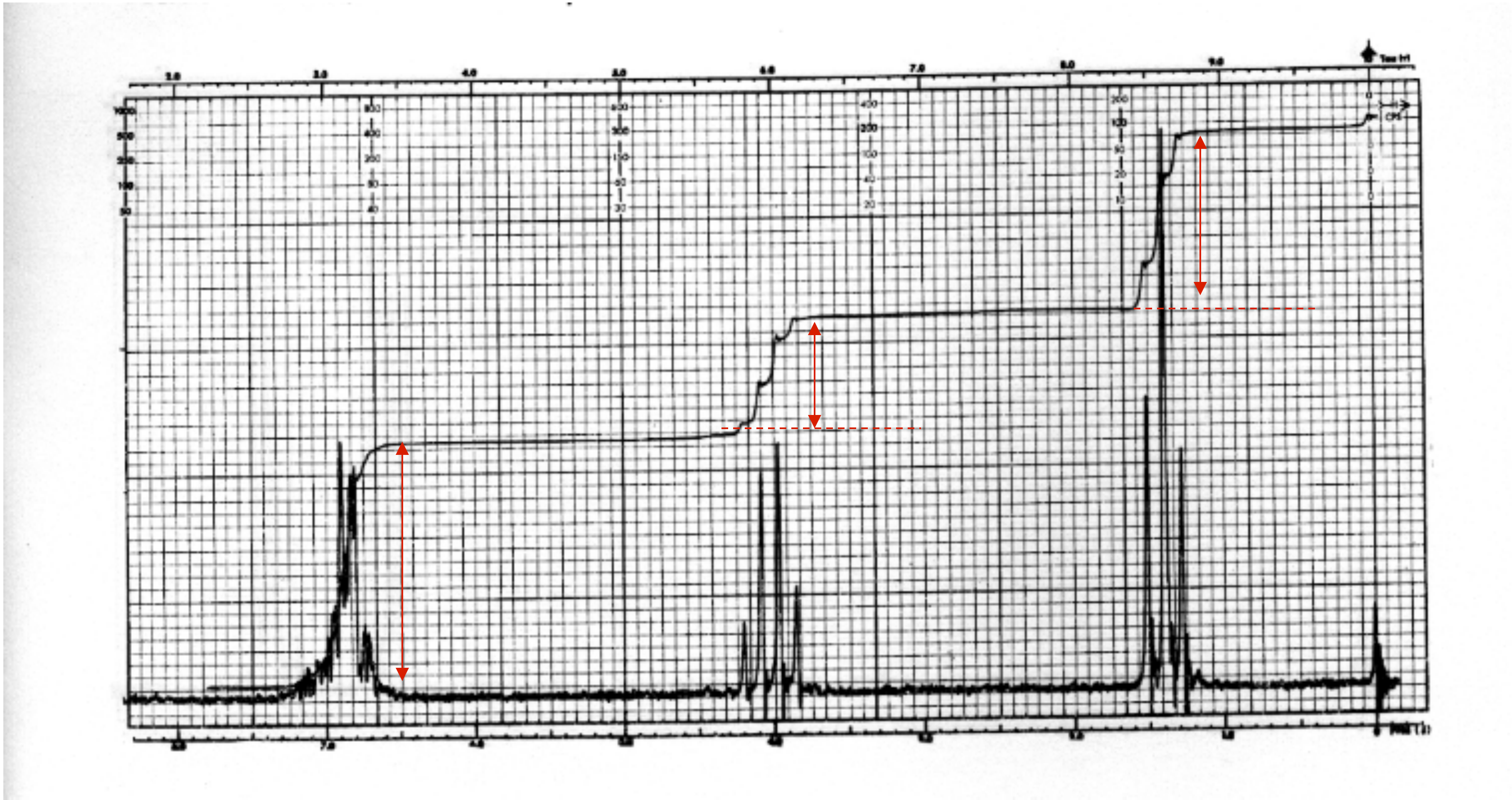
a	2 H
b	4 H



a	3 H
b	2 H
c	4 H



Integration: measure the height of each “step” in the integration and then calculate the lowest whole number ratio: a:b:c = 24 mm : 16 mm : 32 mm = 1.5 : 1.0 : 2.0 → 3H : 2H : 4H



If the formula is known (C_8H_9OF), add up all of the “steps” and divide by the number of hydrogens = (24 + 16 + 32 mm) / 9H = 8.0 mm / Hydrogen. a = 24 mm / 8.0 mm/H → 3 H; b = 16 mm/8.0 mm/H → 2H; c = 32 mm/8.0 mm/H → 4H.

4. Splitting pattern: how many neighboring hydrogens.

In general, n-equivalent neighboring hydrogens will split a ^{1}H signal into an (n + 1) Pascal pattern.

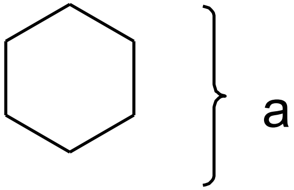
“neighboring” – no more than three bonds away

n	n + 1	Pascal pattern:	
0	1	1	singlet
1	2	1 1	doublet
2	3	1 2 1	triplet
3	4	1 3 3 1	quartet
4	5	1 4 6 4 1	quintet

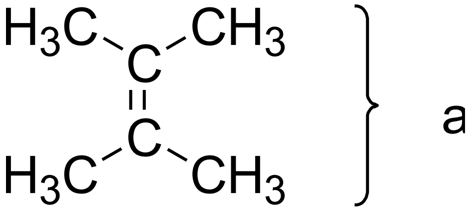
note: n must be equivalent neighboring hydrogens to give rise to a Pascal splitting pattern. If the neighbors are not equivalent, then you will see a complex pattern (aka complex multiplet).

note: the alcohol hydrogen –OH usually does not split neighboring hydrogen signals nor is it split. Normally a singlet of integration 1 between 1 – 5.5 ppm (variable).

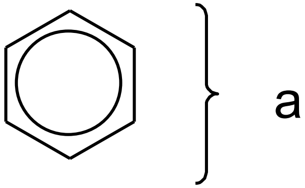
splitting pattern?



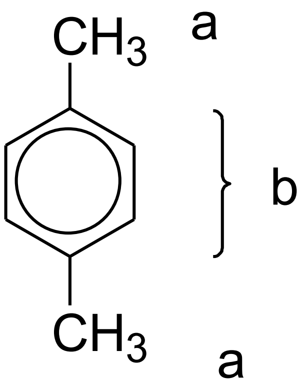
a 12 H singlet



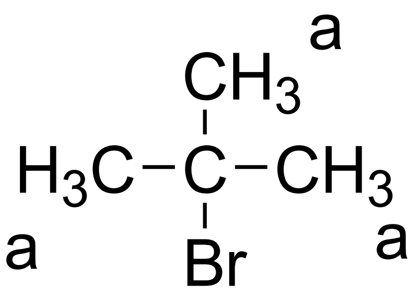
a 12 H singlet



a 6 H singlet



a 6 H singlet
b 4 H singlet



$H_3C-\overset{CH_3}{\underset{Br}{C}}-CH_3$

a	9 H	singlet

a   b

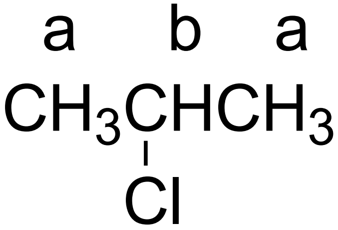
$CH_3CH_2\text{-}Br$

a	3 H	triplet
b	2 H	quartet

a   b   c

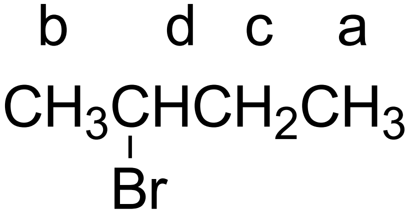
$CH_3CH_2CH_2\text{-}Br$

a	3 H	triplet
b	2 H	complex
c	2 H	triplet



$CH_3\underset{Cl}{CH}CH_3$

a	6 H	doublet
b	1 H	septet

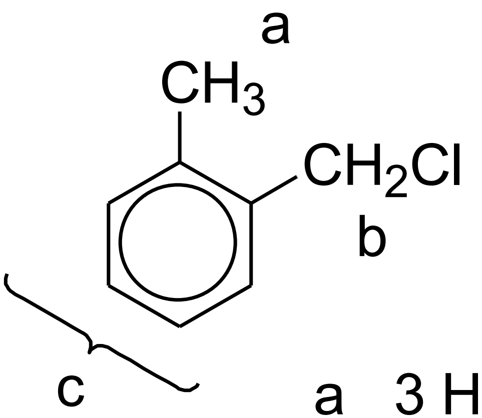


$$\overset{b}{CH_3}\underset{\overset{|}{Br}}{\overset{d}{CH}}\overset{c}{CH_2}\overset{a}{CH_3}$$

a	3 H	triplet
b	3 H	doublet
c	2 H	complex
d	1 H	complex

$$\text{Cl-}\overset{b}{CH_2}\overset{a}{CH_2}\overset{b}{CH_2}\text{-Cl}$$

a	2 H	quintet
b	4 H	triplet



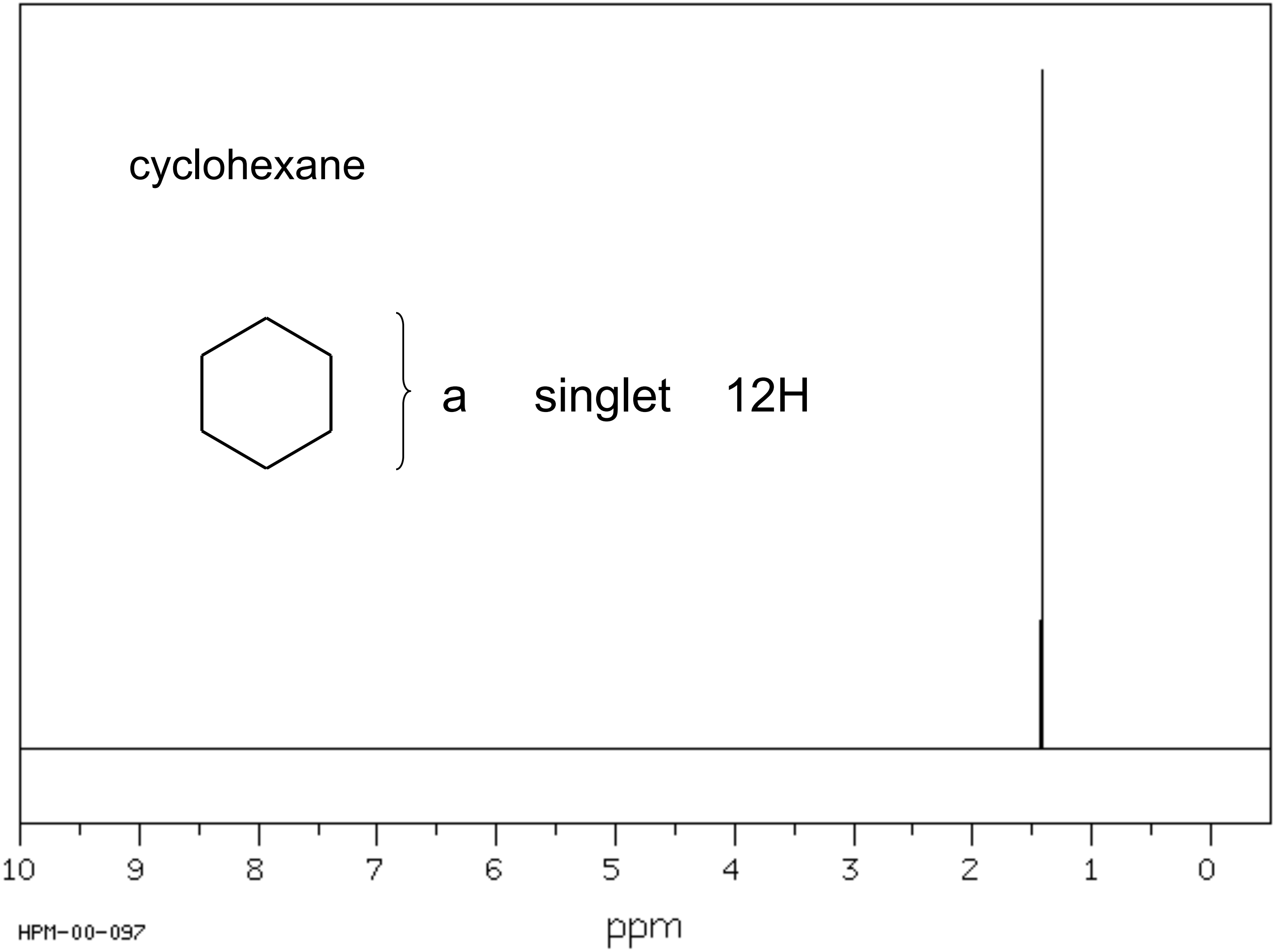
a	3 H	singlet
b	2 H	singlet
c	4 H	~singlet

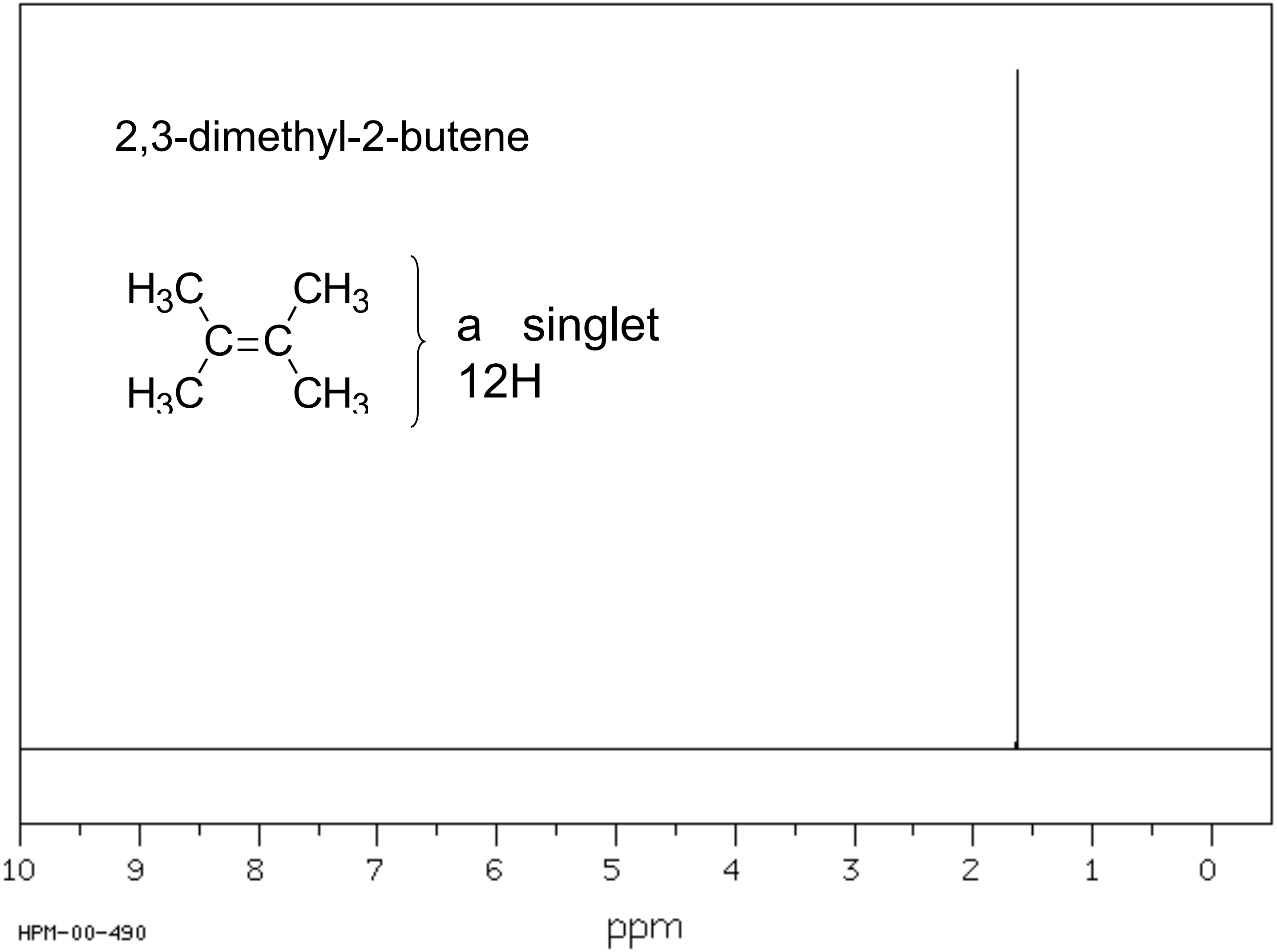
$$\overset{a}{CH_3}\overset{b}{CH_2}\text{-}\overset{c}{OH}$$

a	3 H	triplet
b	2 H	quartet
c	1 H	singlet

Information from ^{1}H-nmr spectra:

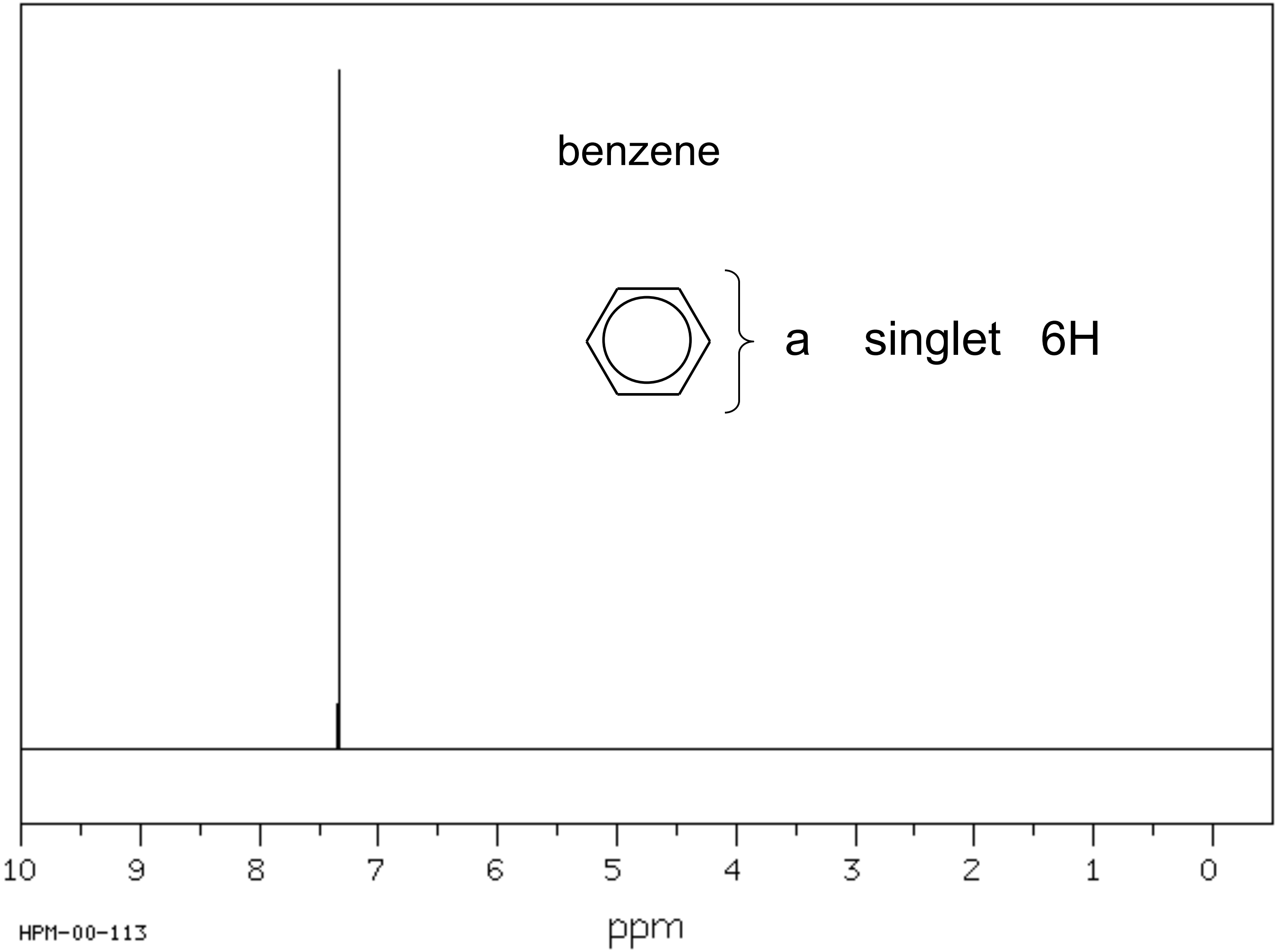
1. Number of signals: How many different types of hydrogens in the molecule.
2. Position of signals (chemical shift): What types of hydrogens.
3. Relative areas under signals (integration): How many hydrogens of each type.
4. Splitting pattern: How many neighboring hydrogens.


cyclohexane
a singlet 12H
10
9
8
7
6
5
4
3
2
1
0
ppm
HPM-00-097

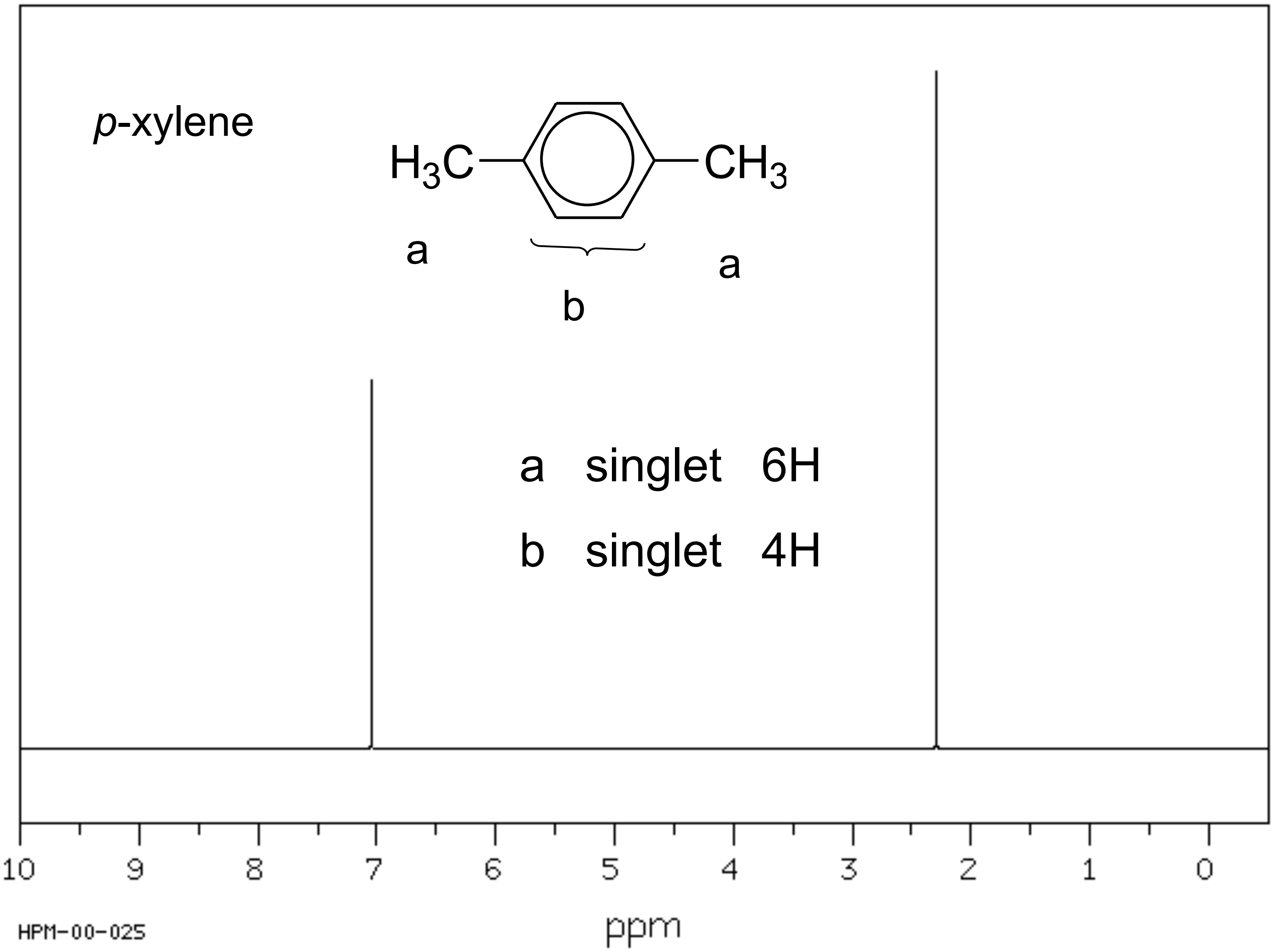


2,3-dimethyl-2-butene

a singlet
12H


benzene
a singlet 6H
10
9
8
7
6
5
4
3
2
1
0
ppm
HPM-00-113

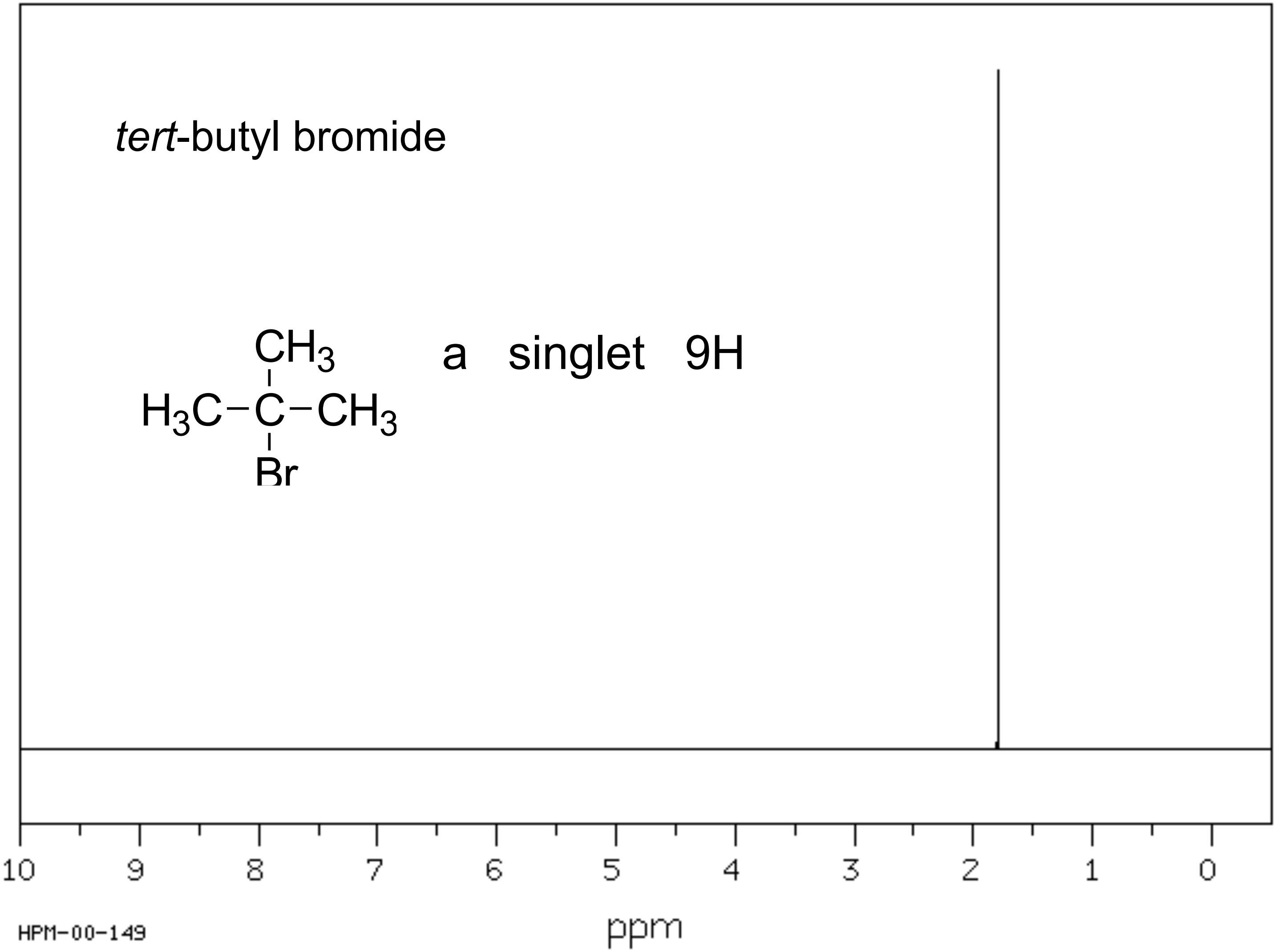
p-xylene



a singlet 6H

b singlet 4H

tert-butyl bromide

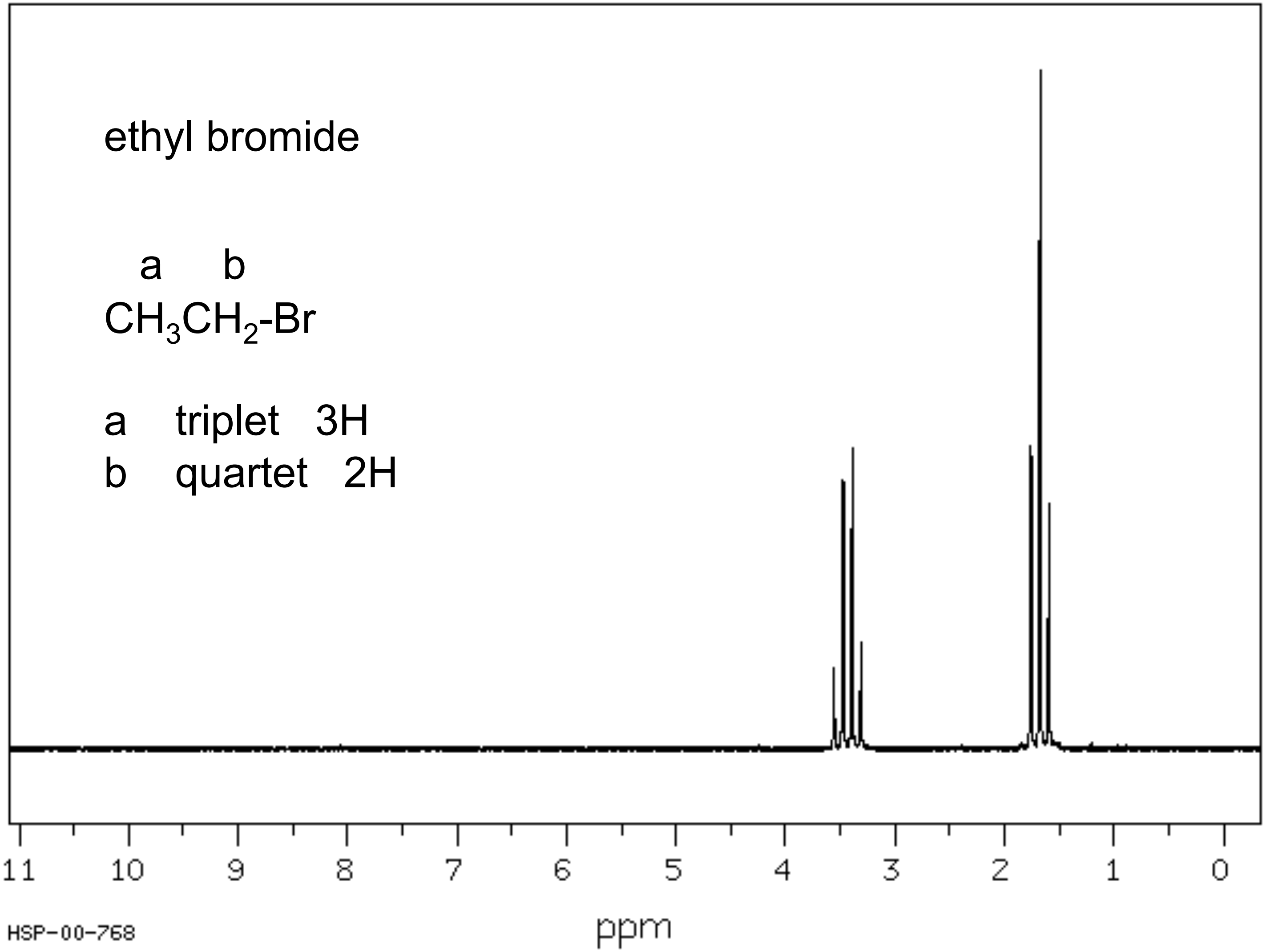


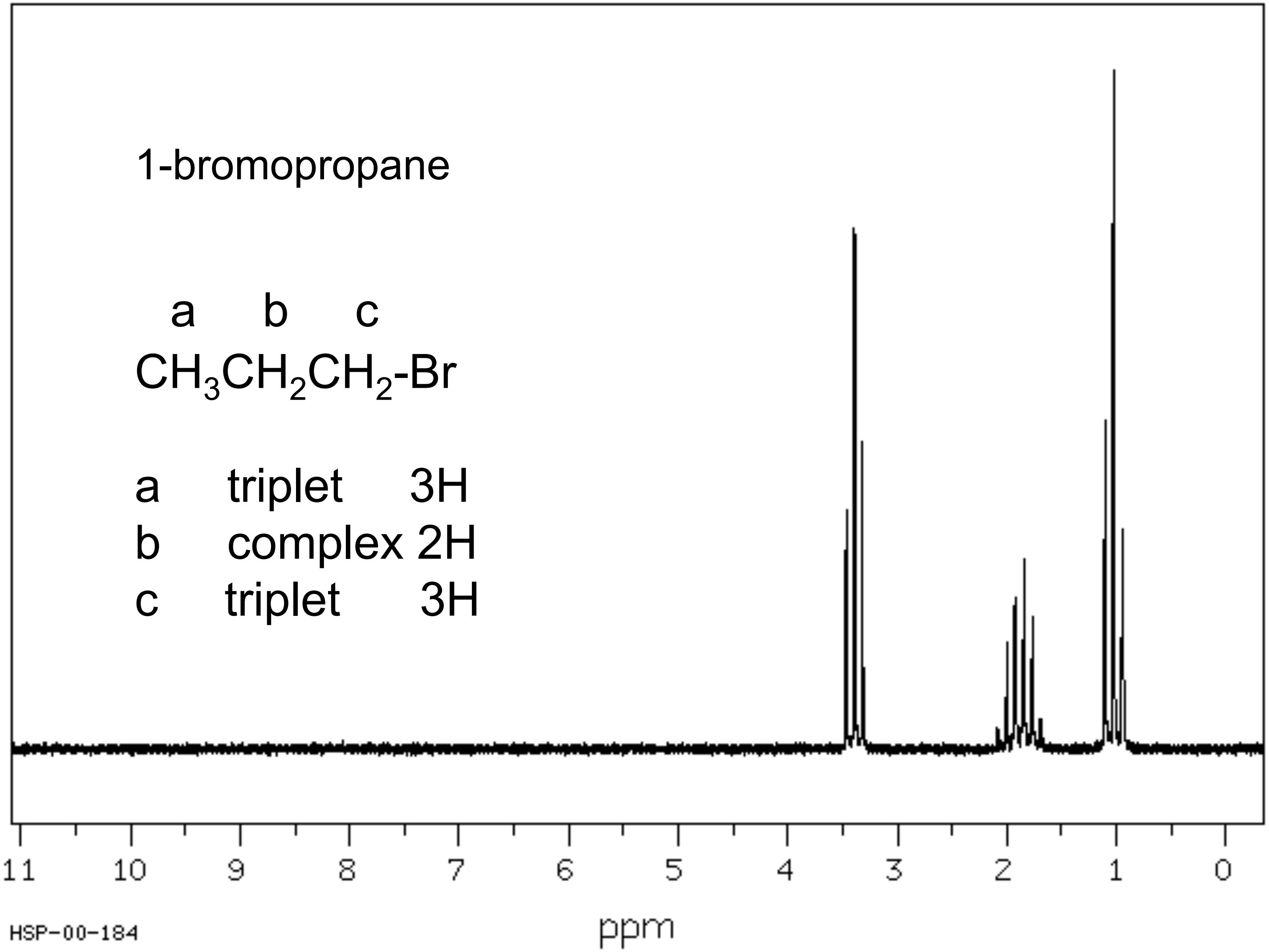
$$H_3C-\overset{\displaystyle CH_3}{\underset{\displaystyle Br}{C}}-CH_3$$

ethyl bromide

$\overset{a}{CH_3}\overset{b}{CH_2}\text{-Br}$

a triplet 3H
b quartet 2H

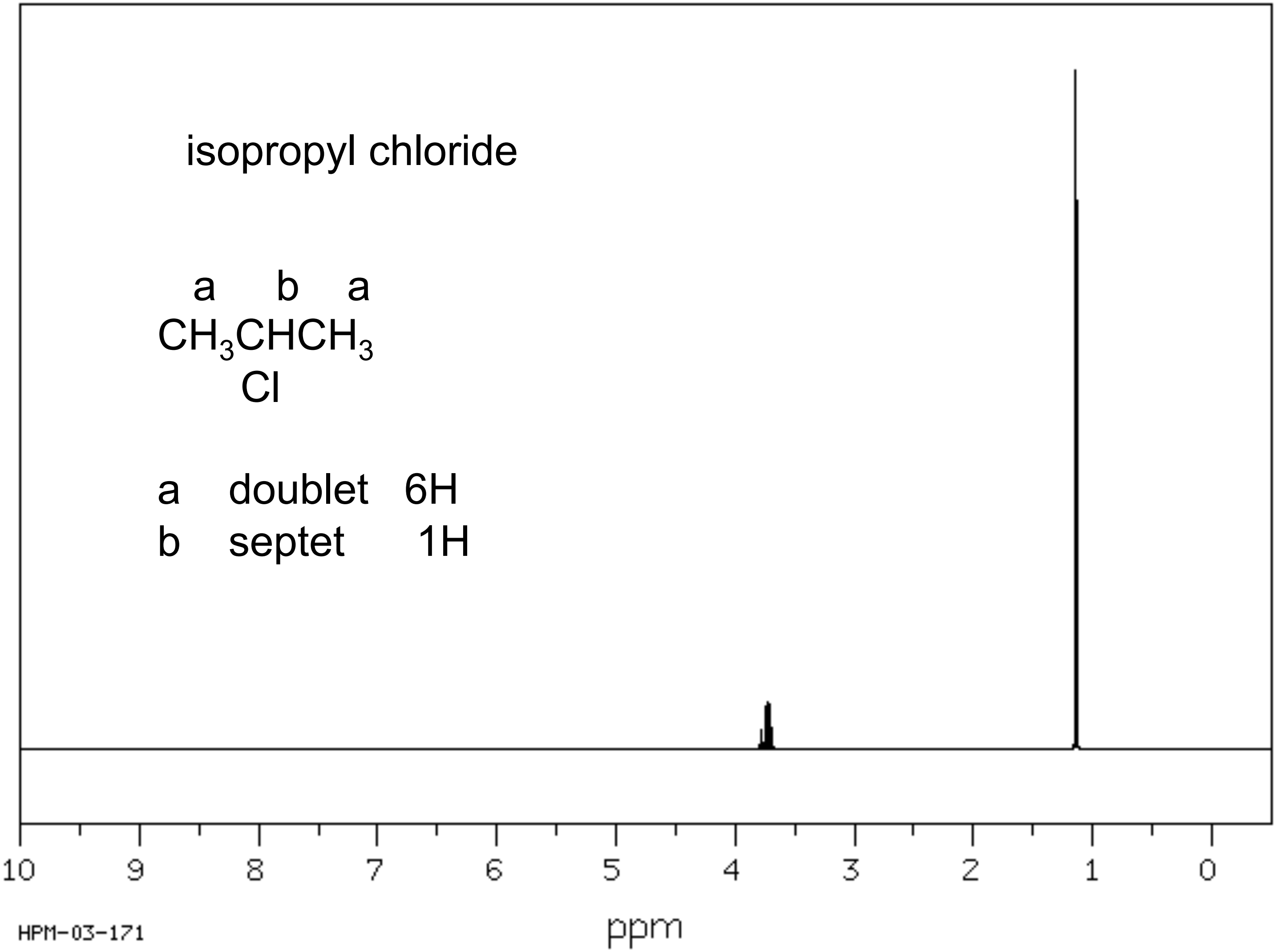




1-bromopropane

$\overset{a}{CH_3}\overset{b}{CH_2}\overset{c}{CH_2}\text{-Br}$

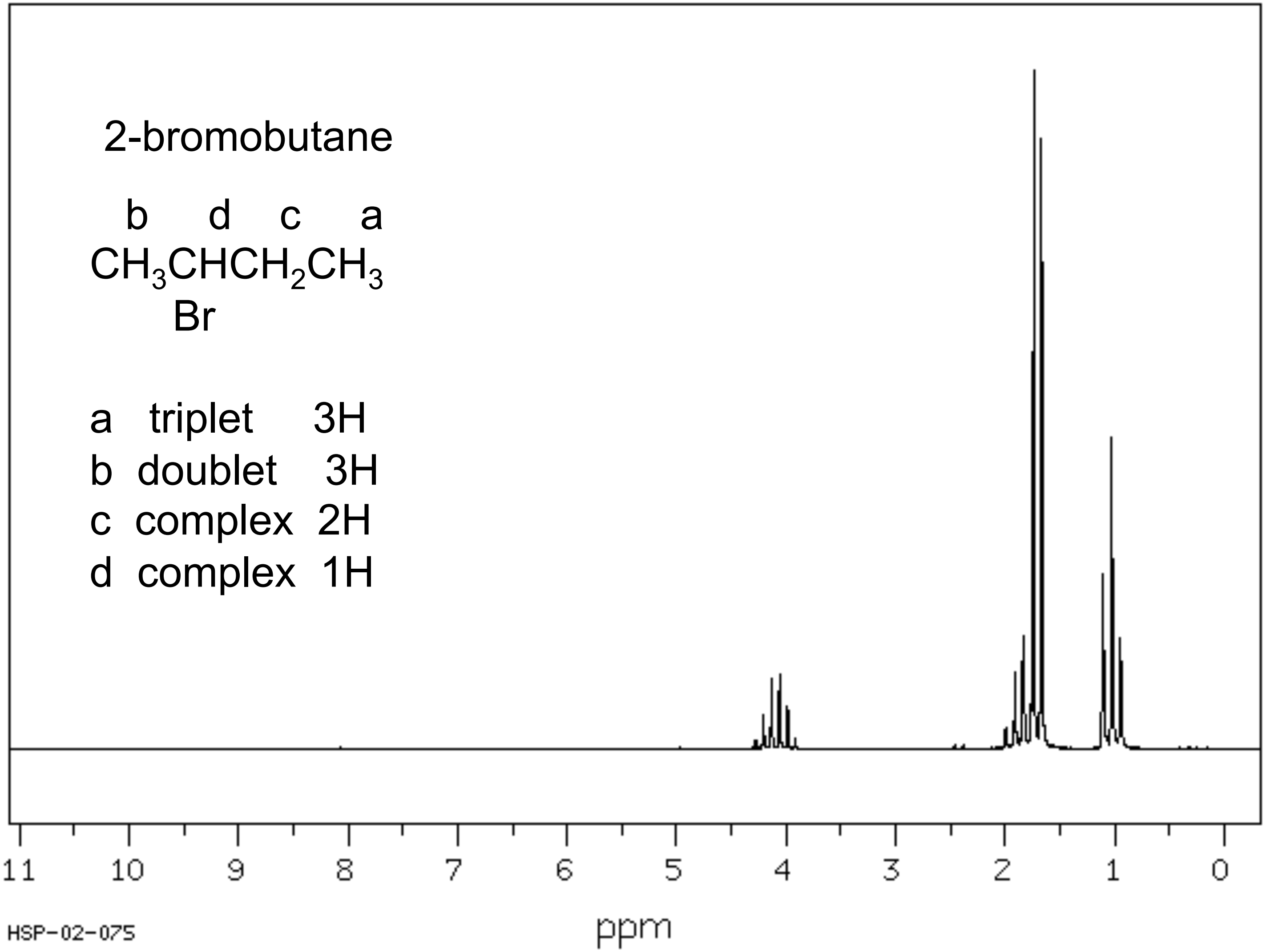
a	triplet	3H
b	complex	2H
c	triplet	3H



isopropyl chloride

$$\begin{array}{c}\overset{a}{CH_3}\overset{b}{\underset{Cl}{CH}}\overset{a}{CH_3}\end{array}$$

a	doublet	6H
b	septet	1H

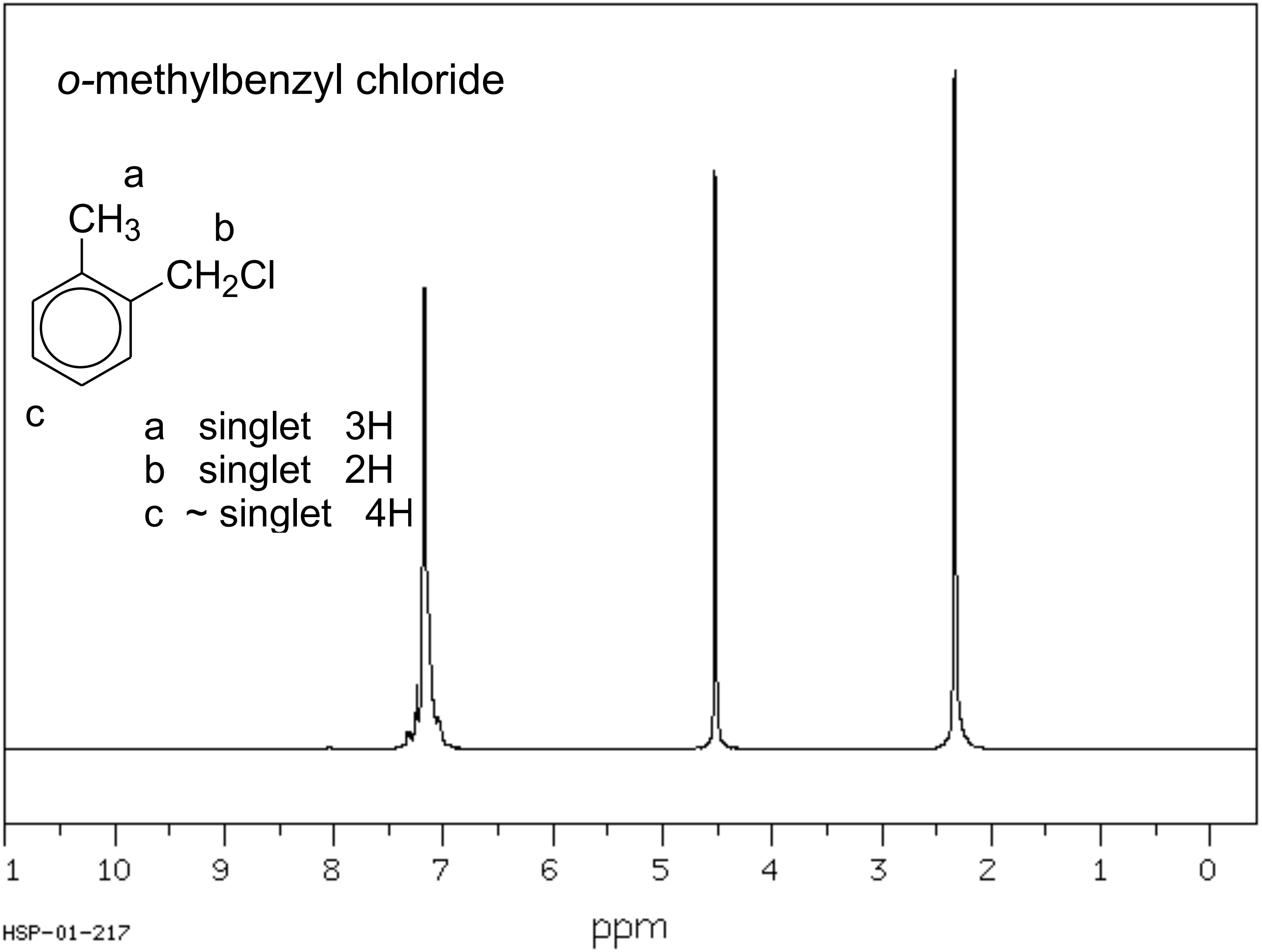


2-bromobutane

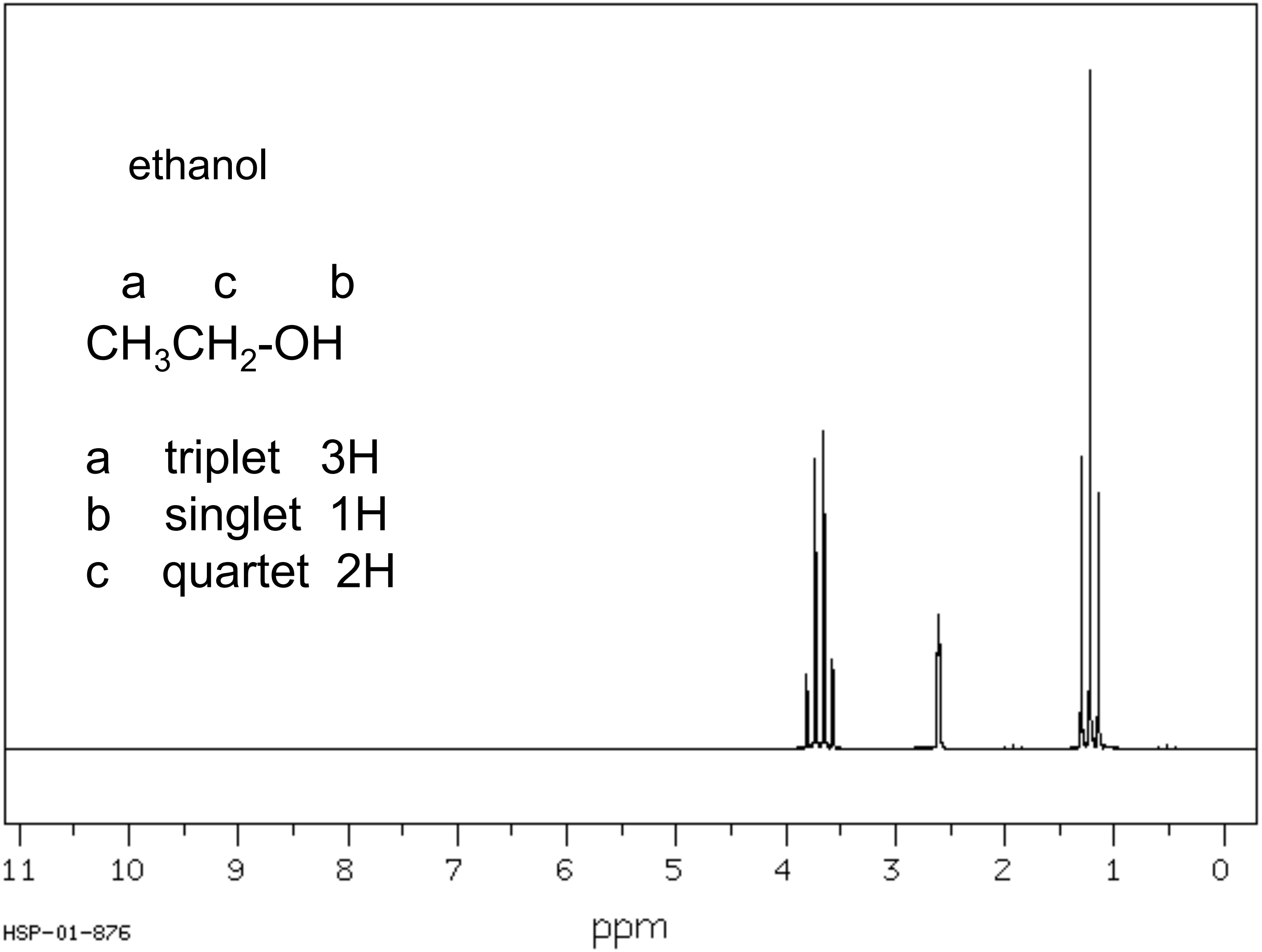
$$\overset{b}{CH_3}\underset{Br}{\overset{d}{CH}}\overset{c}{CH_2}\overset{a}{CH_3}$$

a triplet 3H
b doublet 3H
c complex 2H
d complex 1H

o-methylbenzyl chloride



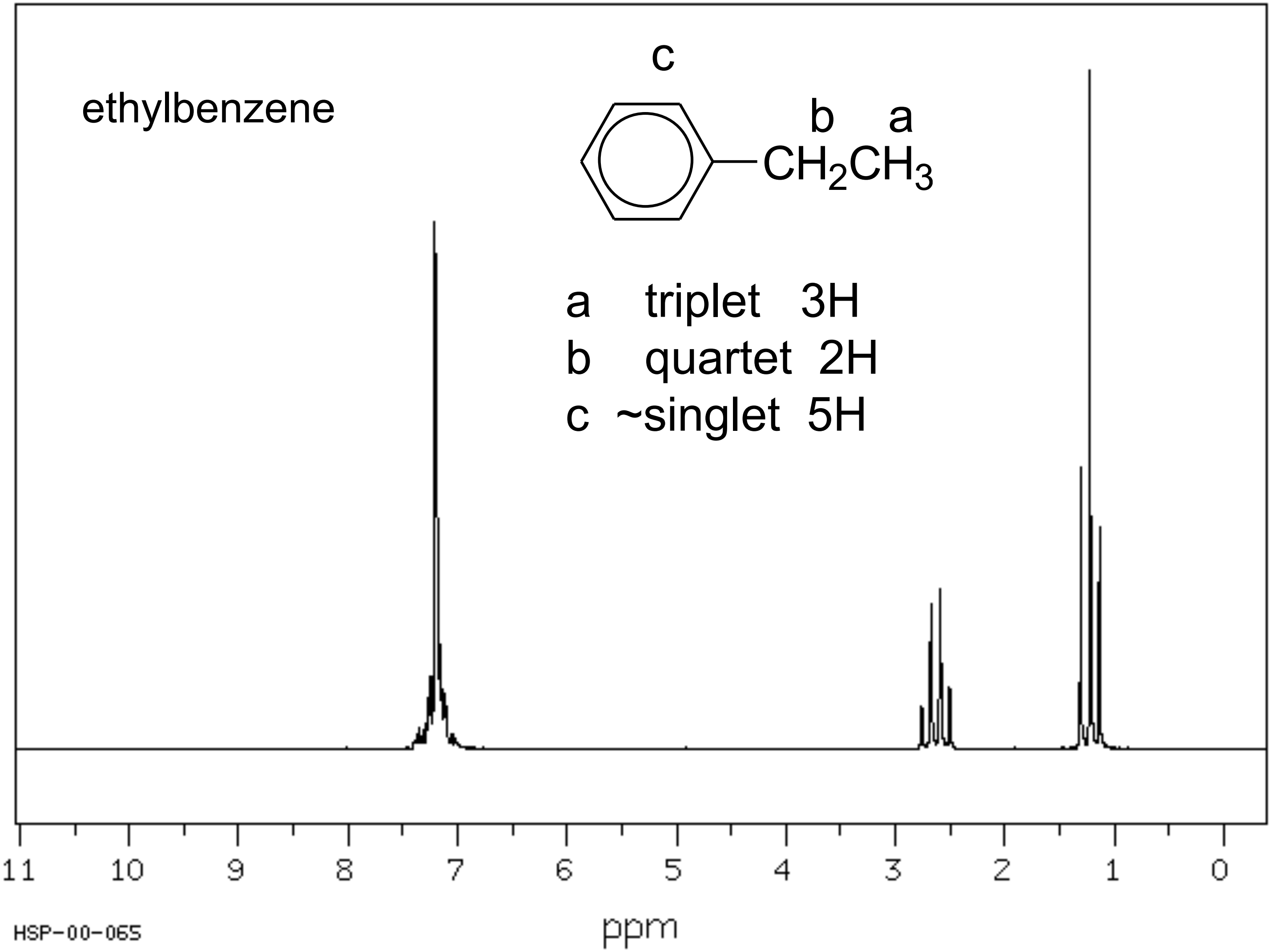
a singlet 3H
b singlet 2H
c ~ singlet 4H



ethanol

a c b
CH_3CH_2-OH

a triplet 3H
b singlet 1H
c quartet 2H



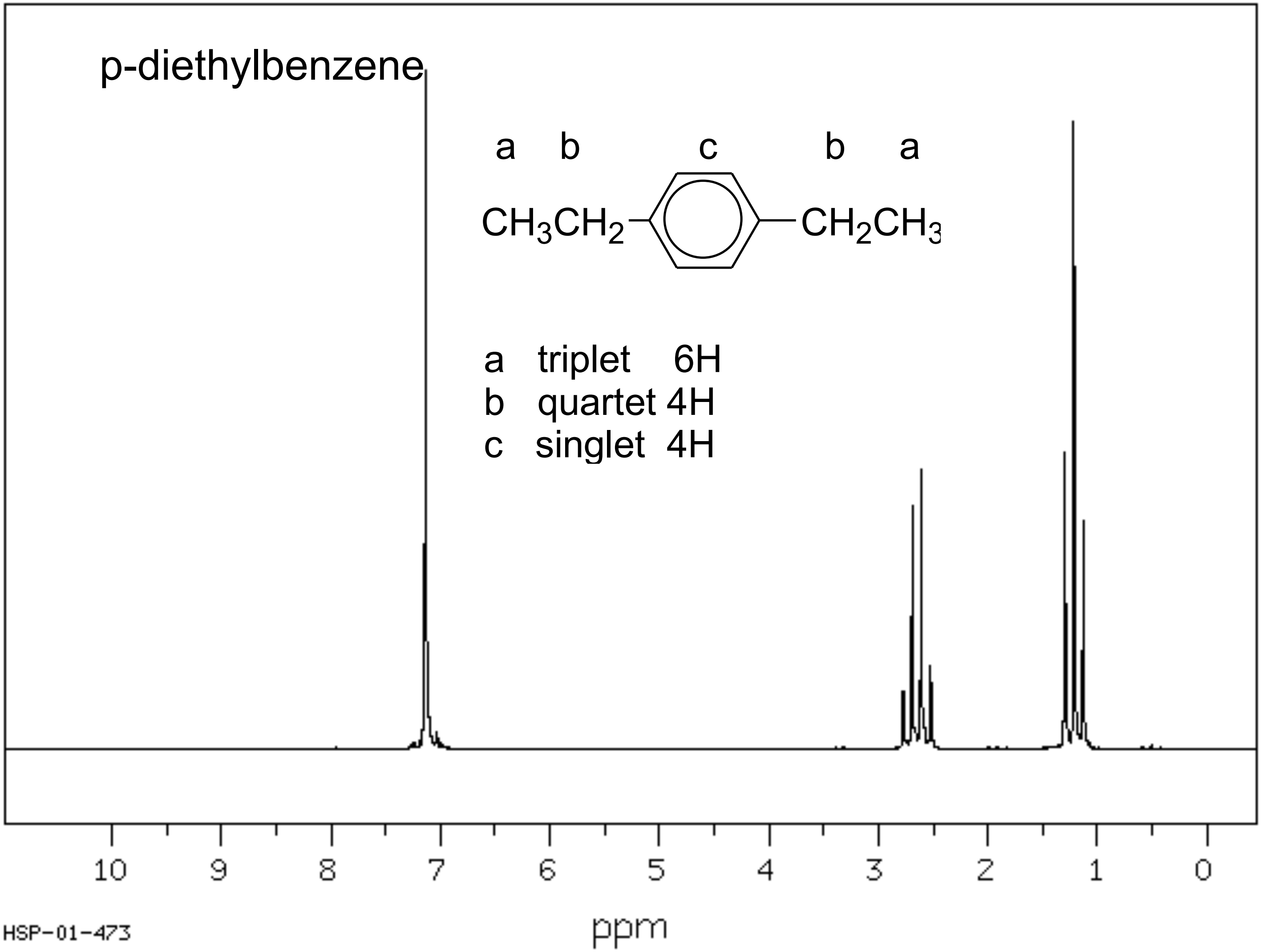
ethylbenzene

a triplet 3H

b quartet 2H

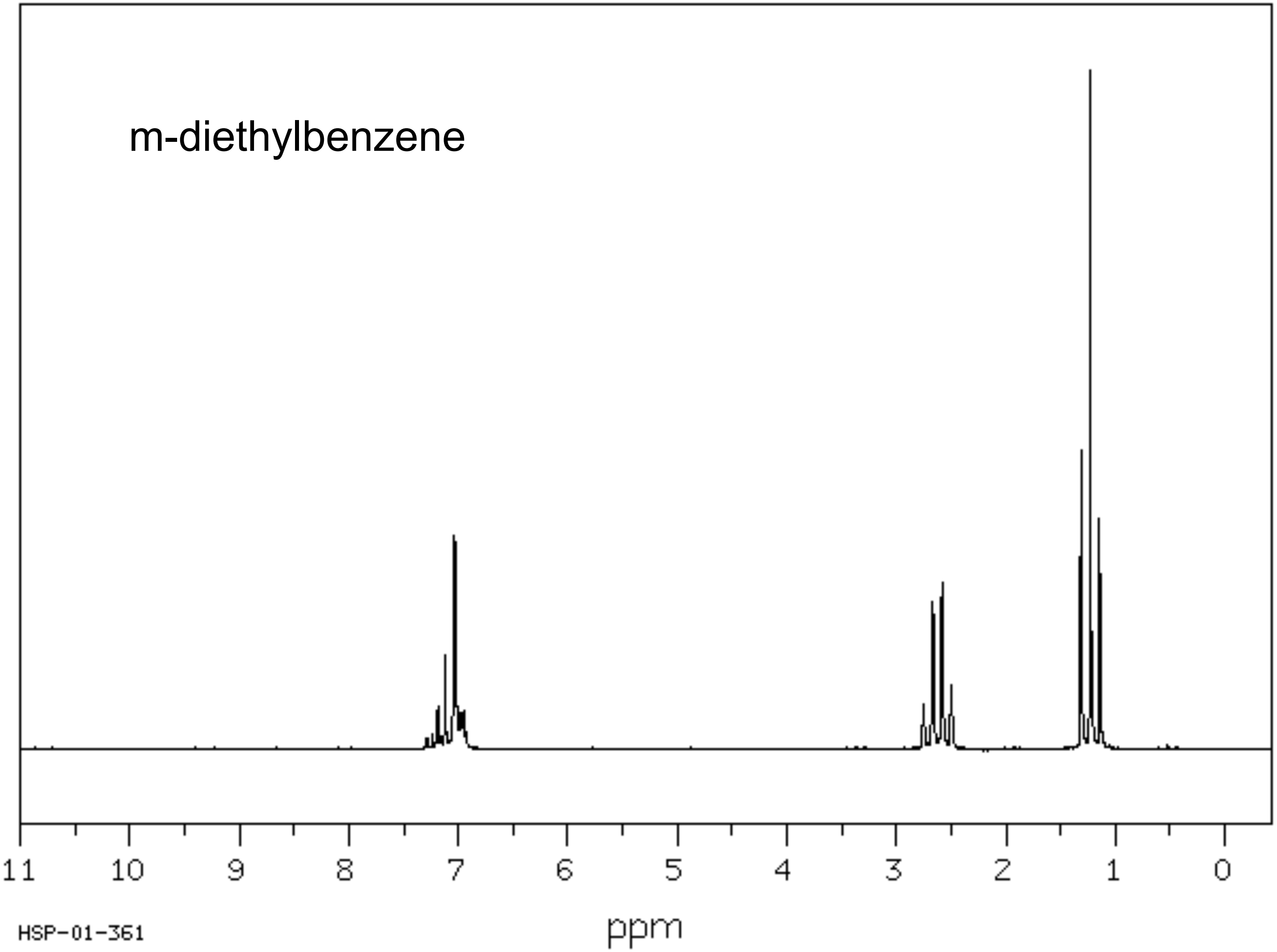
c ~singlet 5H

p-diethylbenzene

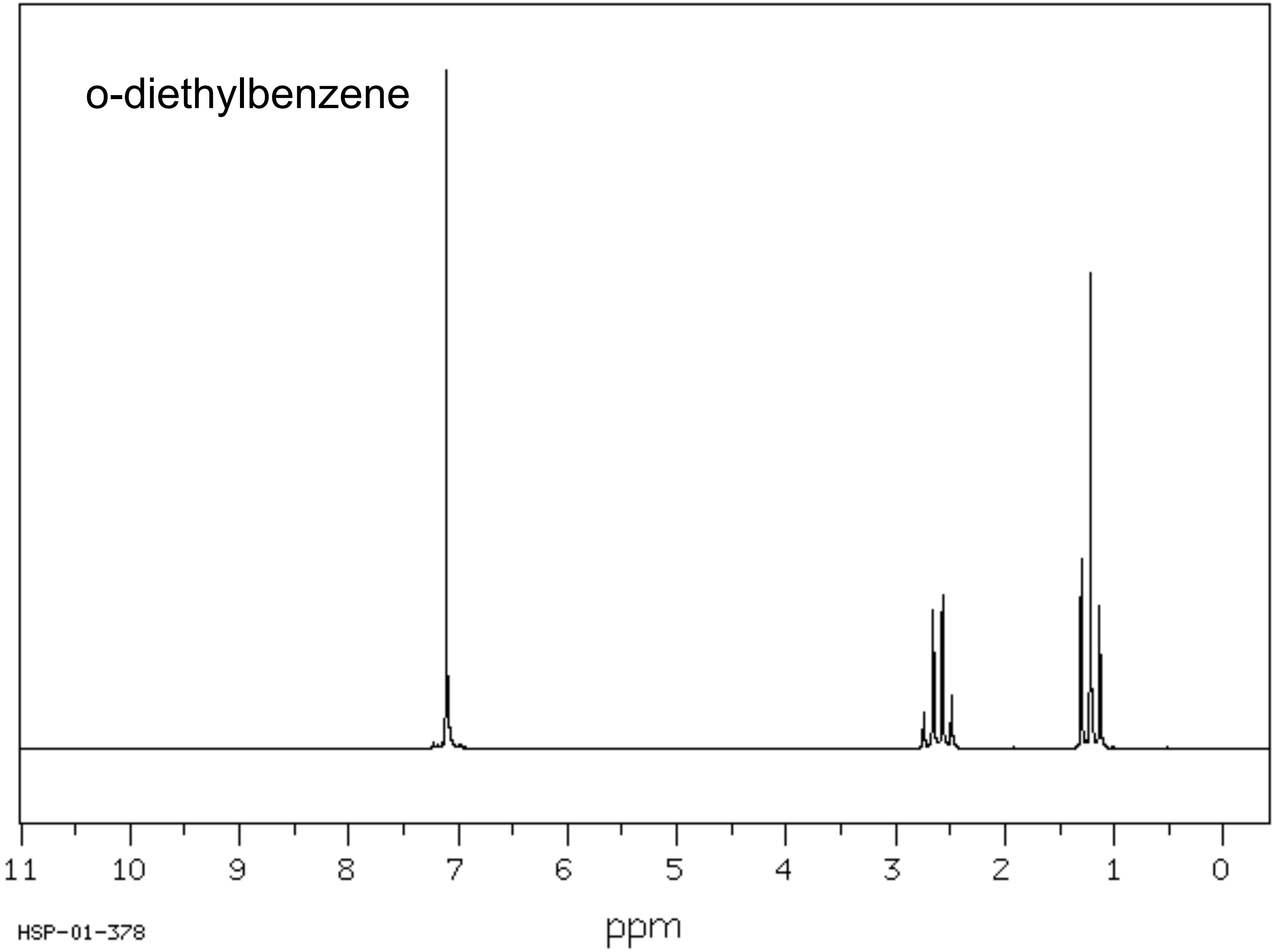


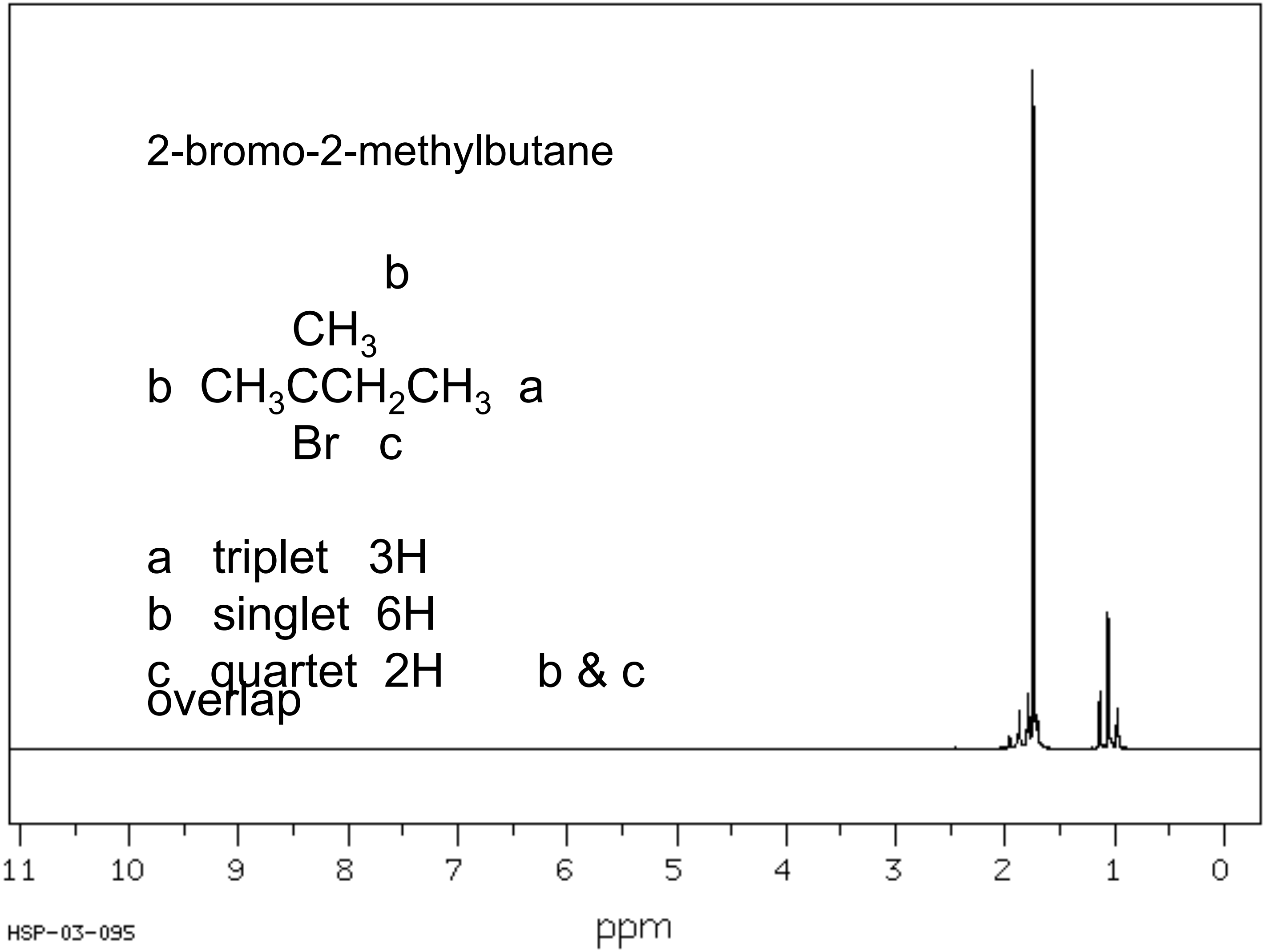
a triplet 6H
b quartet 4H
c singlet 4H

m-diethylbenzene



HSP-01-361


o-diethylbenzene
11
10
9
8
7
6
5
4
3
2
1
0
ppm
HSP-01-378



2-bromo-2-methylbutane

$$\begin{array}{l} \qquad\qquad\quad b \\ \qquad\quad CH_3 \\ b\ \ CH_3CCH_2CH_3\ \ a \\ \qquad\quad Br\ \ \ c \end{array}$$

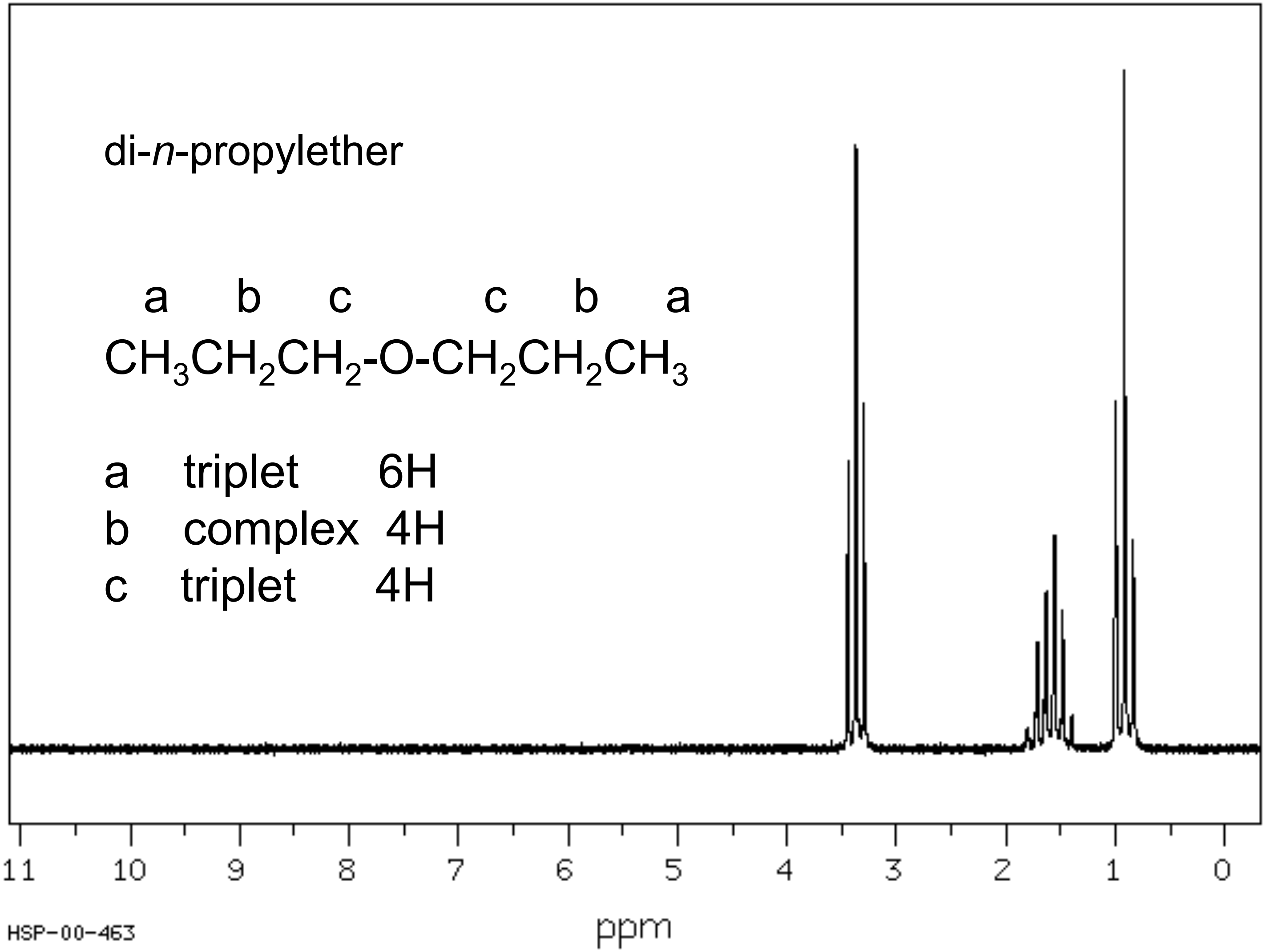
a triplet 3H

b singlet 6H

c quartet 2H b & c

overlap

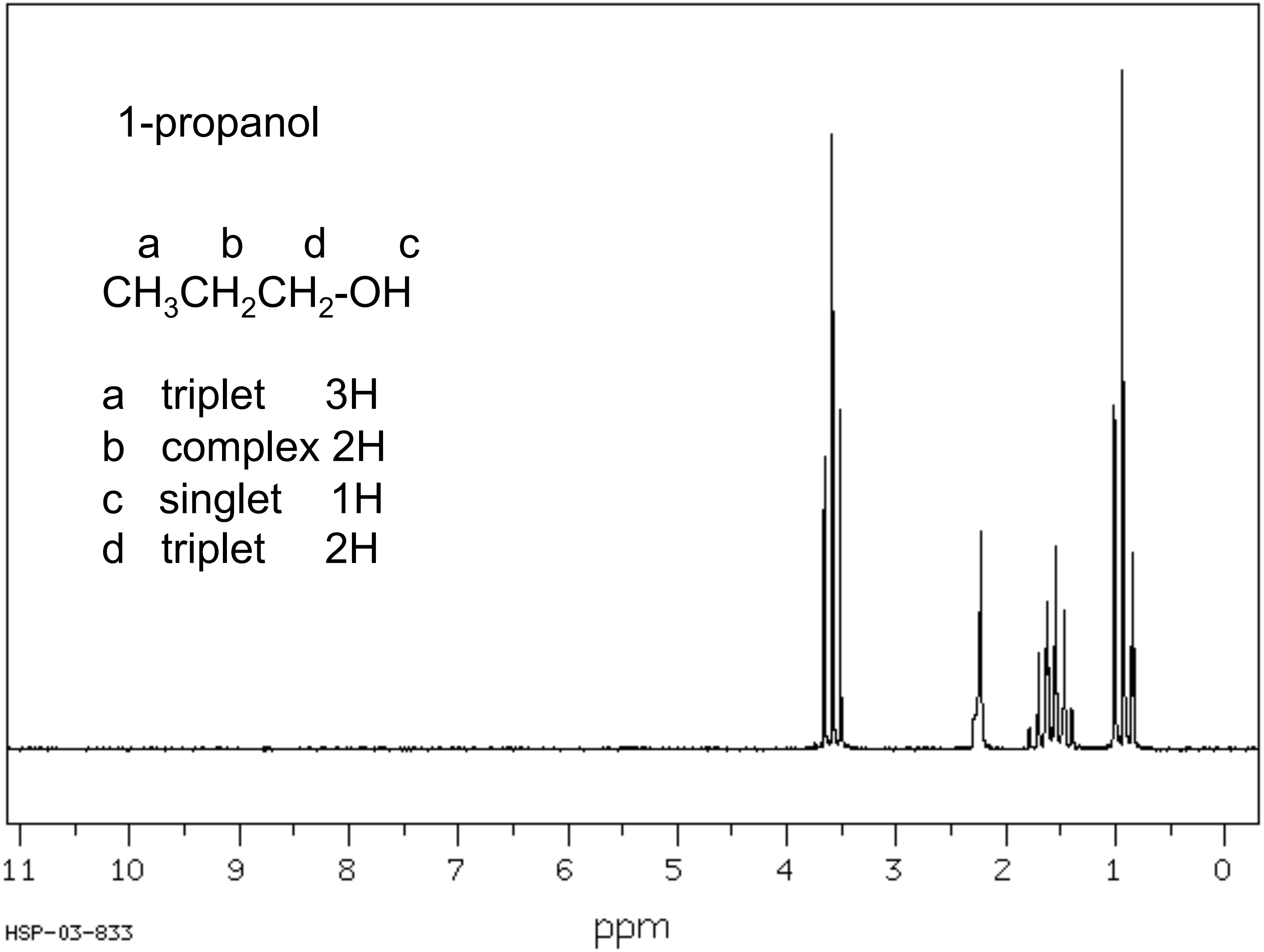
HSP-03-095



di-*n*-propylether

$$\overset{a}{CH_3}\overset{b}{CH_2}\overset{c}{CH_2}\text{-O-}\overset{c}{CH_2}\overset{b}{CH_2}\overset{a}{CH_3}$$

a	triplet	6H
b	complex	4H
c	triplet	4H

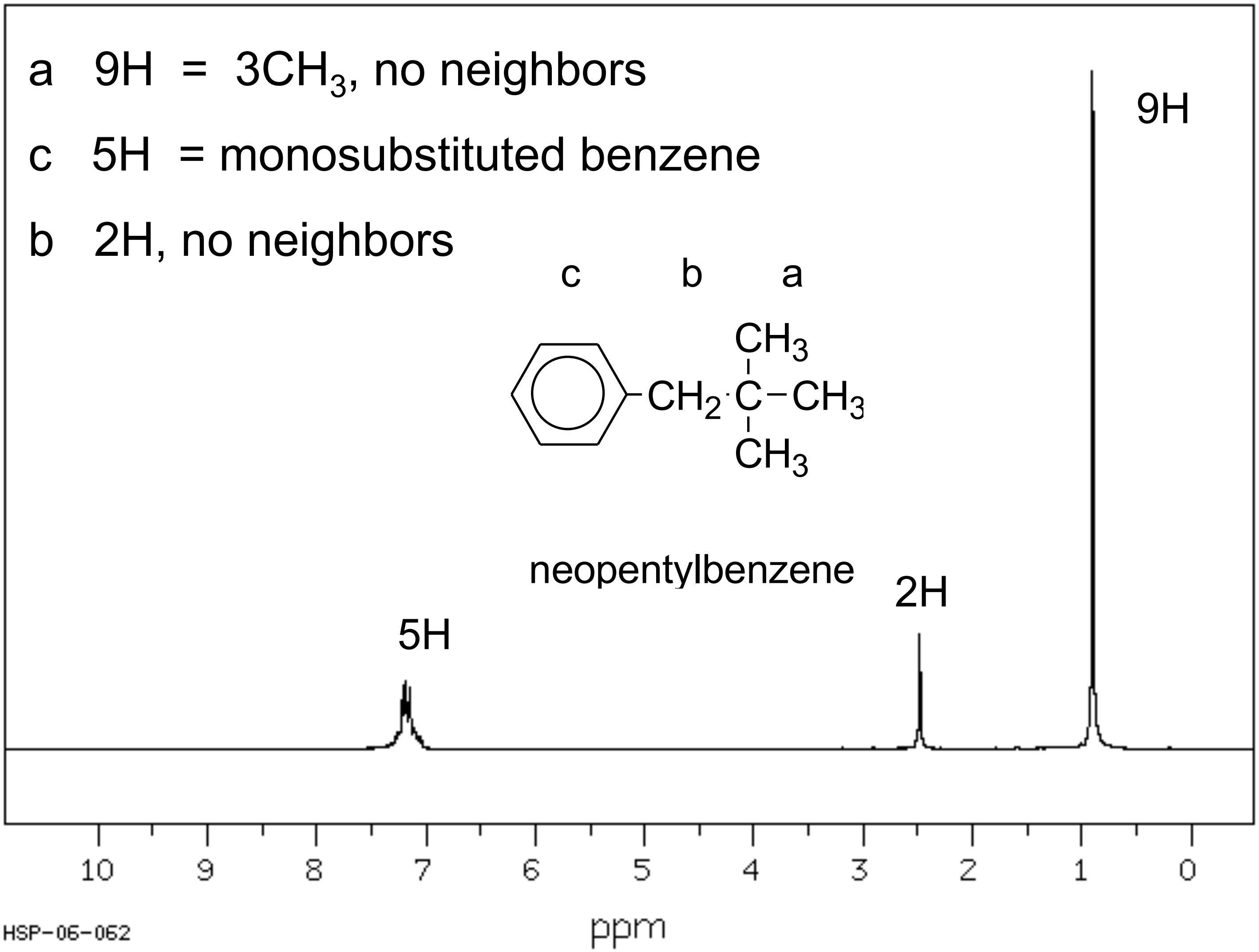


1-propanol

$\overset{a}{CH_3}\overset{b}{CH_2}\overset{d}{CH_2}\text{-}\overset{c}{OH}$

a	triplet	3H
b	complex	2H
c	singlet	1H
d	triplet	2H

$C_{11}H_{16}$



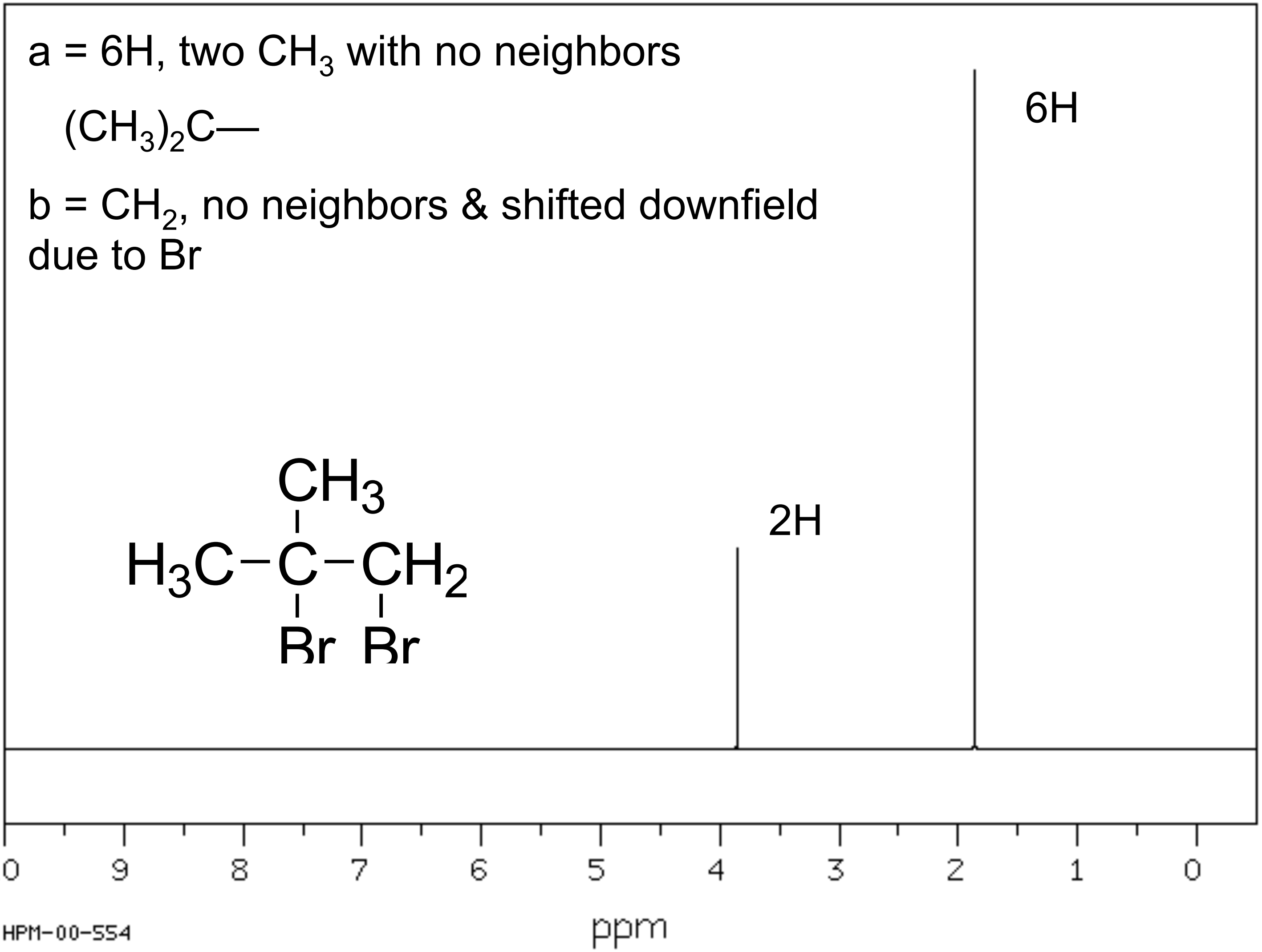
a 9H = $3CH_3$, no neighbors

c 5H = monosubstituted benzene

b 2H, no neighbors

neopentylbenzene

$C_4H_8Br_2$



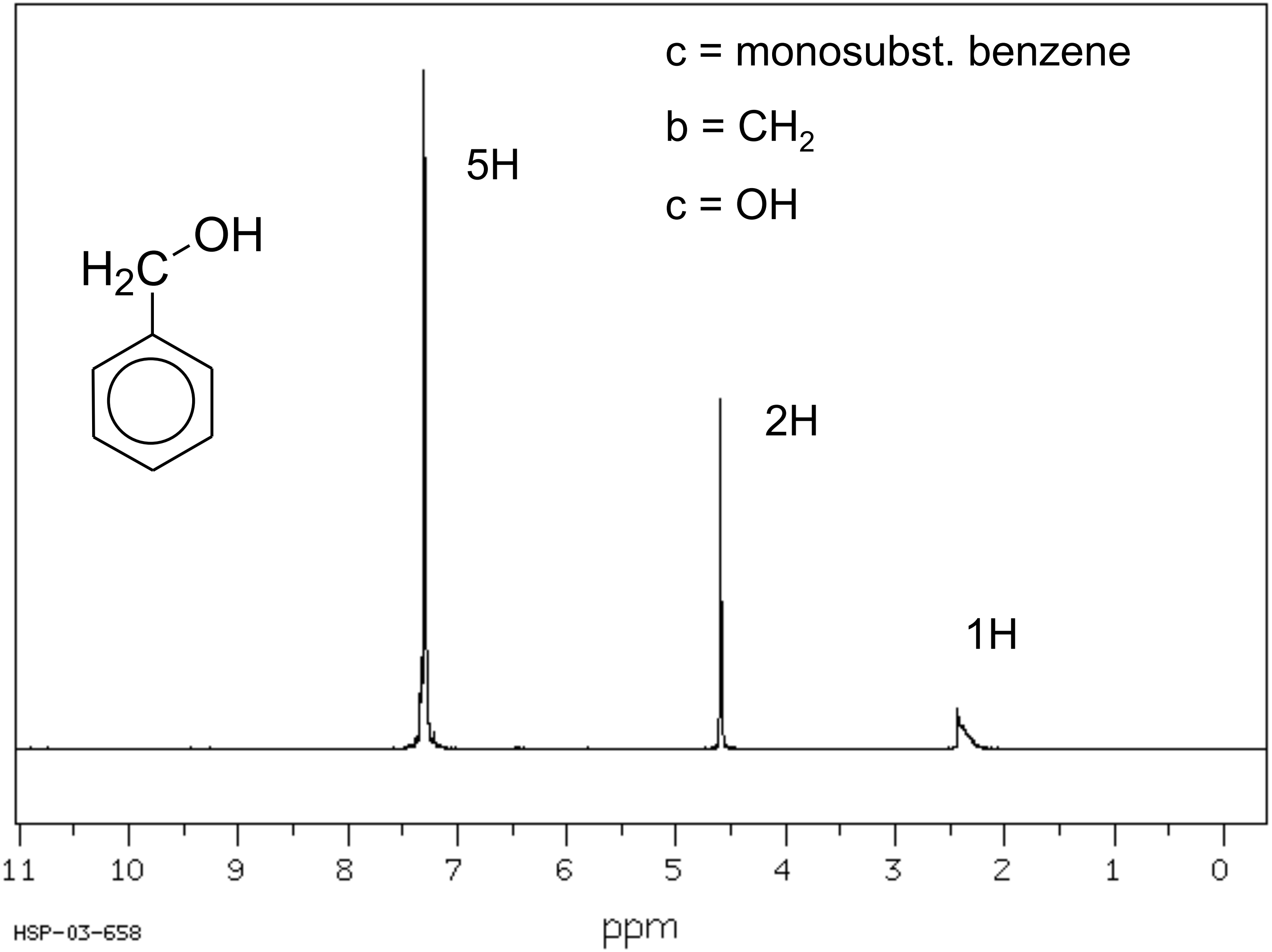
a = 6H, two CH_3 with no neighbors

$(CH_3)_2C$—

b = CH_2, no neighbors & shifted downfield due to Br

$$H_3C-\underset{\displaystyle Br}{\overset{\displaystyle CH_3}{C}}-\underset{\displaystyle Br}{CH_2}$$

C_7H_8O



c = monosubst. benzene

b = CH_2

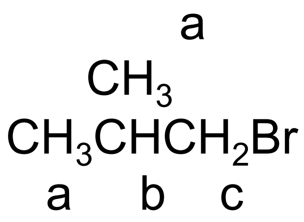
c = OH

C_4H_9Br

a	doublet	1.04 ppm	6H
b	complex	1.95 ppm	1H
c	doublet	3.33 ppm	2H

a = two equivalent CH_3's with one neighboring H (b?)

c = CH_2 with one neighbor H (also b)



$\underset{a}{CH_3}\overset{\overset{a}{CH_3}}{\underset{b}{CH}}\underset{c}{CH_2}Br$

a	6H	doublet
b	1H	complex
c	2H	doublet

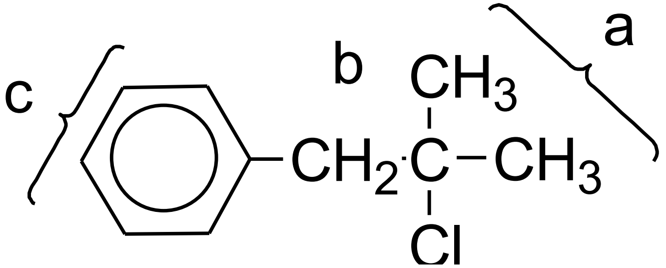
$C_{10}H_{13}Cl$

a	singlet	1.57 ppm	6H
b	singlet	3.07 ppm	2H
c	singlet	7.27 ppm	5H

a = two-equilalent CH_3's with no neighbors

c = monosubstituted benzene ring

b = CH_2



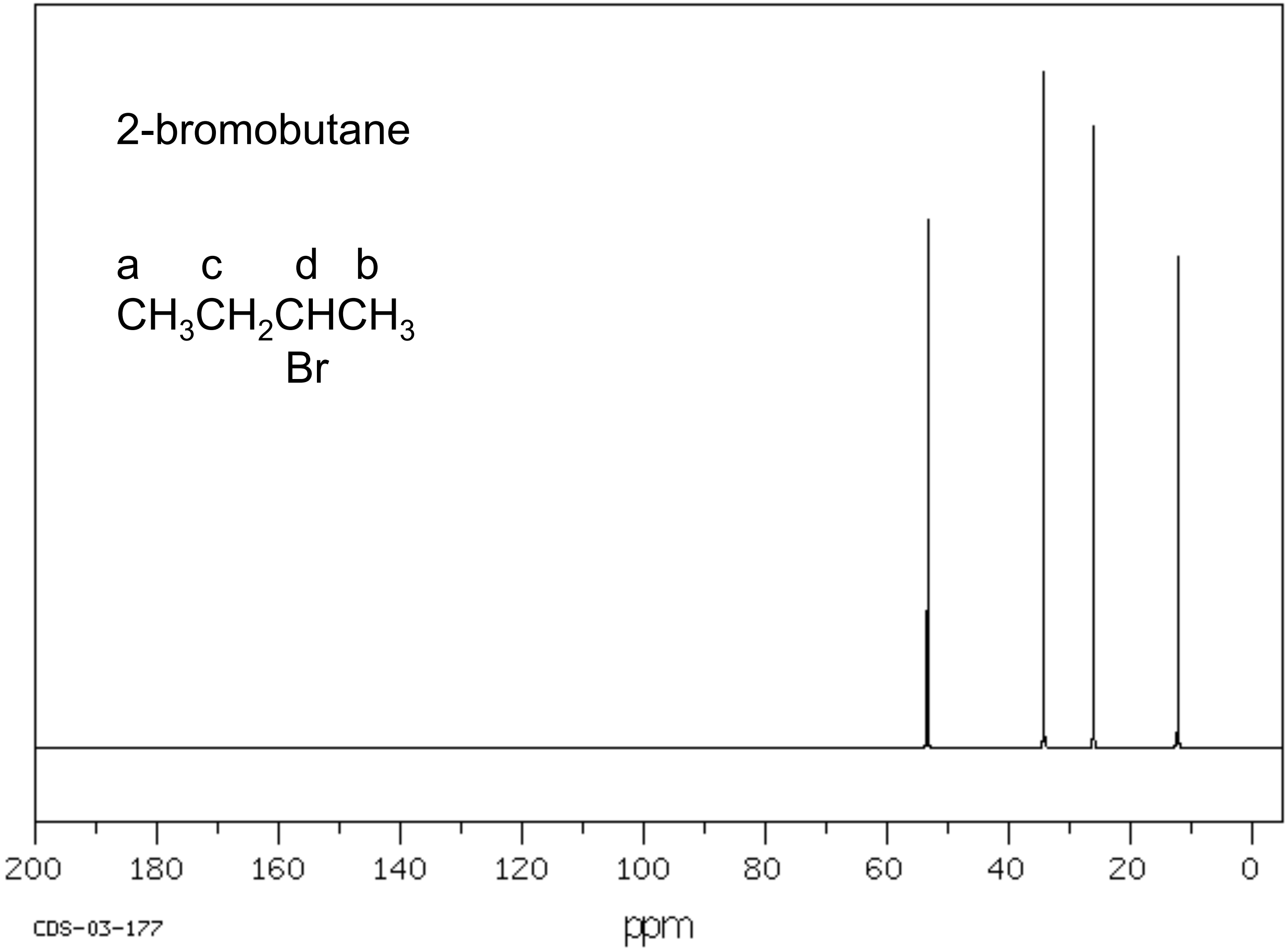
a	singlet	6H
b	singlet	2H
c	singlet	5H

^{13}C – nmr

^{13}C ~ 1.1% of carbons

1) number of signals: how many different types of carbons
2) splitting: number of hydrogens on the carbon
3) chemical shift: hybridization of carbon sp, sp^2, sp^3
4) chemical shift: evironment

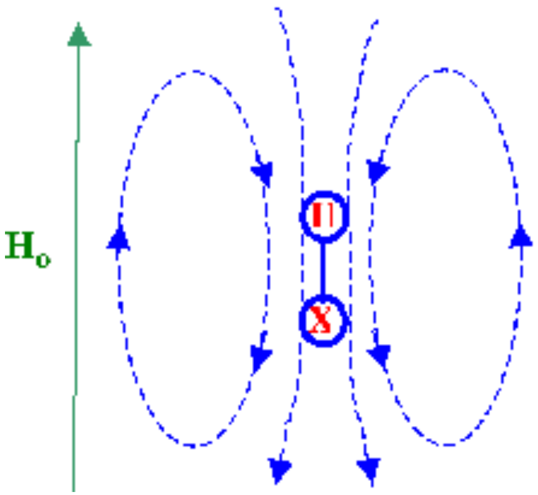
^{13}C-nmr


2-bromobutane
a c d b
$CH_3CH_2CHCH_3$
Br
200
180
160
140
120
100
80
60
40
20
0
ppm
CDS-03-177

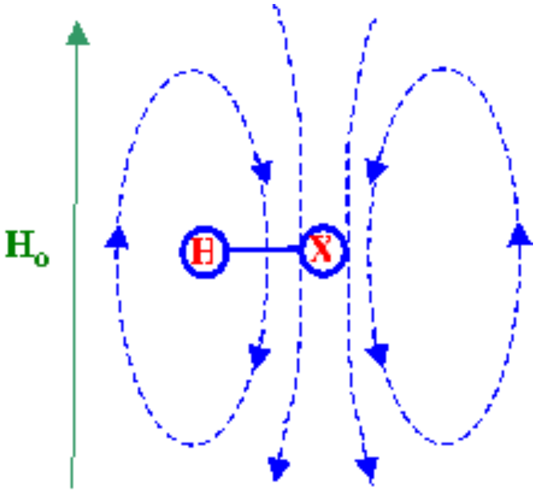
CHEMICAL SHIFT

- The number and position of signals in NMR spectrum signifies the number and nature of protons in the molecule.
- Each of these protons will have different electronic environments and thus they absorb at different applied field strengths.
- When a molecule is placed in a magnetic field, its electrons are caused to circulate and thus they produce secondary magnetic field i.e., induced magnetic field.
- The induced magnetic field can either oppose or reinforce the applied field.

- If the induced magnetic field opposes the applied field, then the nuclei in a molecule exert an external force, which shields the nucleus from the influence of the applied field and the proton is said to be ***shielded.***
- If the induced field reinforces the applied field the proton feels a higher field strength and thus such a proton is said to be ***deshielded.***

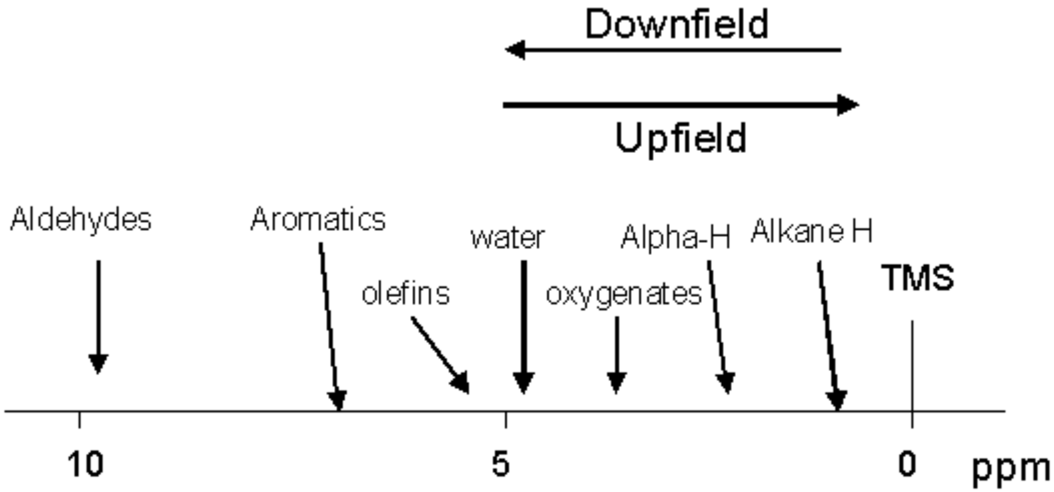


Shielding effect



Deshielding effect

- To overcome the shielding effect and to bring the protons to resonance, greater external field is required i.e., shielding shifts the absorption upfield and deshielding shifts the absorption downfield.
- Such shifts (compared with a standard reference) in the position of NMR absorption which arises due to the shielding or deshielding of protons in a molecule by the electrons are called ***chemical shift***.



Measurement of Chemical Shift

- For the measurement or study of chemical shift ***Tetramethyl silane(TMS)*** is taken as a reference.
- Due to the low electronegativity of silicon the shielding in TMS is greater than most of the organic compounds and the chemical shift for different kinds of protons are measured relative to it.
- **δ** and **τ** scales are commonly used to measure chemical shift.

❑ **δ scale**

- The value of **δ** is expressed in ppm.
- It can be obtained by using the following equations,

$$\delta = \left(\frac{H_S - H_R}{H_R} \right) \times 10^6$$

OR

$$\delta_{sample} = \left(\frac{v_{sample} - v_{reference}}{v_{reference}} \right) \times 10^6$$

where,

υ_S and H_S = the resonance frequency of the sample

υ_R and H_R = resonance frequency of the reference.

❑ τ scale

- The value of **τ** is expressed as 10 ppm.

  i.e., $\boldsymbol{\tau = 10 - \delta}$

- Shielding and deshielding effects **δ** value, i.e., greater the deshielding, larger will be the value of **δ** and vice-versa.
- The shielding parameter **α** can be determined by using the equation,

$$\mathbf{H = H_0\,(1 - \alpha)}$$

Where,

H = field felt by the proton,

H_0 = applied field strength.

- Most of the chemical shift have **δ** value between 0 and 10.

Factors Influencing Chemical Shift

A. Intra-molecular factors

1. Inductive effect.
2. Vander Waal’s deshielding.
3. Anisotropic effects

B. Intermolecular factors

1. Hydrogen bonding.
2. Temperature.
3. Solvents.

❑ Intra-molecular Factors

1. Inductive effect

- The presence of electronegative atoms or groups in a molecule makes the proton deshielded.
- Higher the electronegativity, greater will be the deshielding and thus the **δ** value will also be more.

  i.e., F > Cl > Br > I
- E.g.

CH_3-F	CH_3-Cl	CH_3-Br	CH_3-I
δ = 4.26	**δ** = 3.0	**δ** = 2.82	**δ** = 2.16

2. Vander Waal’s deshielding

- The presence of bulky groups in a molecule can cause deshielding due to the week Vander Waal’s force and give slightly higher value of **δ** than expected.

3. Anisotropic effect (space effect)

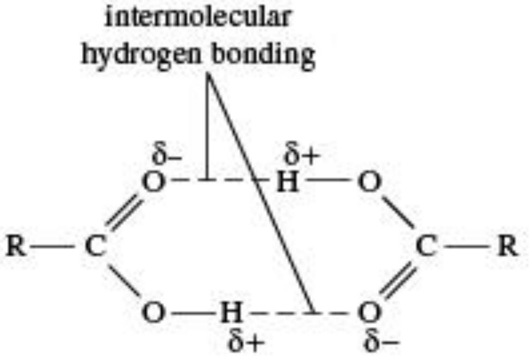
- Anisotropic effect arises due to the orientation of nuclei with respect to the applied magnetic field.
- Chemical bonds can set up magnetic field, the effect of this field on the chemical shift is depend upon the spacial arrangements.
- π – bonds effects the chemical shift and cause downfield shift with higher **δ** value.
- E.g. CH_3H — $\delta_H = 0.23$ $\delta_C = 2.3$

  $CH_2{=}{=}CH_2$ — $\delta_H = 5.25$ $\delta_C = 123.3$

❑ Intermolecular factors

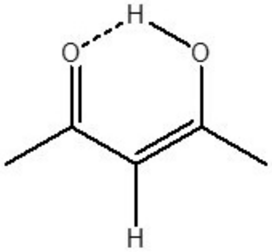
1. Hydrogen bonding

- Intra-molecular hydrogen bonding does not show any change in absorption due to change in concentration.
- While hydrogen atom involved in the intermolecular H-bonding shares its electrons with two electronegative elements and as a result it itself deshielded and get higher **δ** value.
- E.g. Carboxylic acid dimer and β-diketones.



δ = 9-15

Downfield shift



δ = 15.4

No change

2. Temperature

- The resonance position of most signals is little affected by temperature.
- — OH, —NH—, and —SH protons show upfield shift at higher temperature

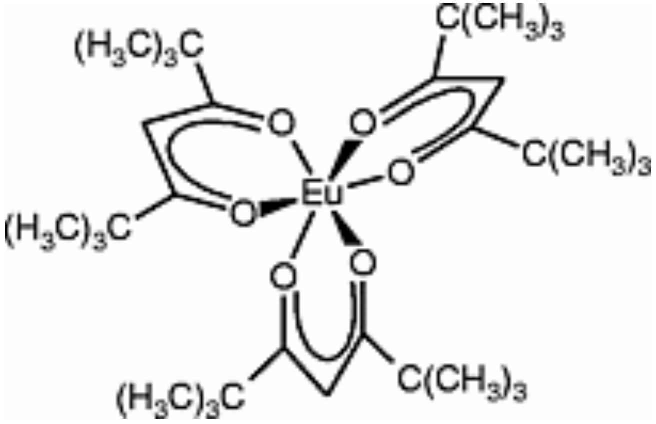
3. Solvents

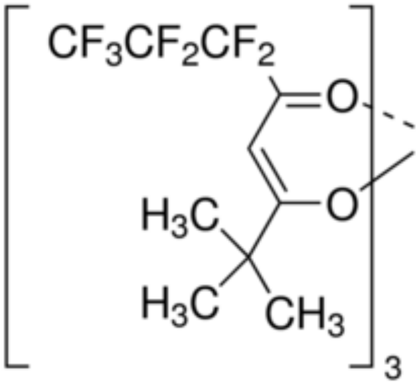
- The solvents used in NMR spectroscopy should be chemically inert, magnetically isotropic, devoid of hydrogen atom and should dissolve the sample to a reasonable extent.
- E.g. CCl_4, CS_2, $CDCl_3$ etc.

Chemical Shift Reagent

- These are the agents used to cause shift in the NMR spectra.
- The amount of shift depends on,
  - Distance between the shift reagent and proton,
  - Concentration of shift reagent.
- The advantages of using shift reagents are,
  - Gives spectra which are much easier to interpret,
  - No chemical manipulation of sample is required,
  - More easily obtained.
- Paramagnetic materials can cause chemical shift, e.g., Lanthanides.
- Complexes of Europium, Erbium, Thallium and Ytterbium shift resonance to lower field.
- Complexes of Cerium, Neodymium and Terbium shift resonance to higher field.

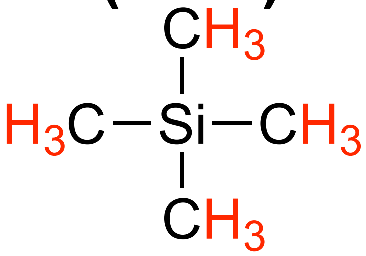
- Europium is probably the most commonly used metal to cause shift in the NMR spectra.
- Two of its widely used complexes are,





REFERENCE STANDARD

1. Tetramethyl Silane (TMS)



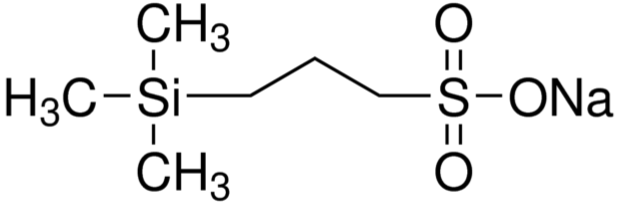
$Si(CH_3)_4$

tetramethylsilane (TMS)

- TMS is the most convenient reference generally employed in NMR for measuring the position of H^1 , C^{13} and Si^{29}.
- The chemical shift of TMS is considered as zero and all the other chemical shifts are determined relative to it.

- It has the following characteristics,
  - It is chemically inert and miscible with large range of organic solvents.
  - It is highly volatile and can be easily removed to get back the sample.
  - It does not take part in intermolecular association with the sample.
  - Its resonance position is far away from absorption due to protons in the molecule.
  - Its 12 protons are all magnetically equivalent.

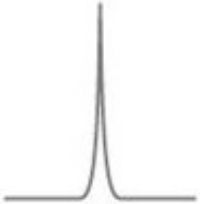
2. 3-(trimethyl silyl) propane sulphonate (sodium salts)



- It is used as internal standard for scanning NMR spectra of water soluble substances in deuterium oxide solvent.
- It has more water solubility than TMS and is commonly used for protein experiments in water.
- The low electro negativity of the silicon shields the nine identical methyl protons and show almost lower chemical shift than naturally occurring organic molecule.

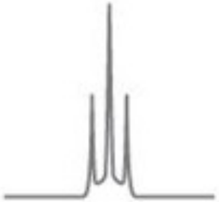
SPLITTING OF THE SIGNALS

- Each signals in NMR spectrum represents one kind or one set of protons in the molecule.
- In certain molecules, instead of a single peak a group of peaks are observed.
- This phenomena of splitting of proton signals into two or more sub-peaks are referred as splitting.
- The splitting pattern of a given nucleus can be predicted by the ***n+1* rule**, where ***n*** is the number of protons on the neighboring carbon.
- The simplest multiplicities are **singlet** (*n* = 0), **doublets** (*n* = 1 or coupling to just one proton), **triplets** (*n* = 2), **quartets** (*n* = 3), **quintets** (*n* = 4), **sextets** (*n* = 5) and **septets** (*n* = 6).

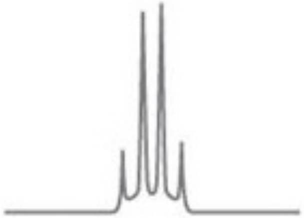


Singlet
(n=0)

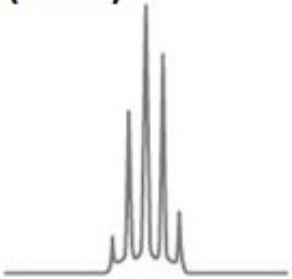
Doublet
(n=1)



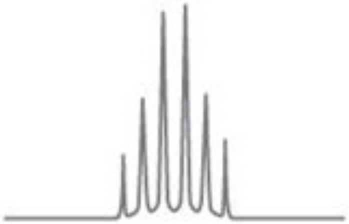
Triplet
(n=2)



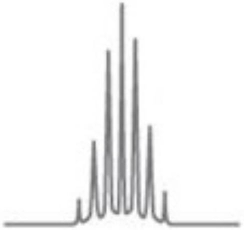
Quartet
(n=3)



Quintet
(n=4)

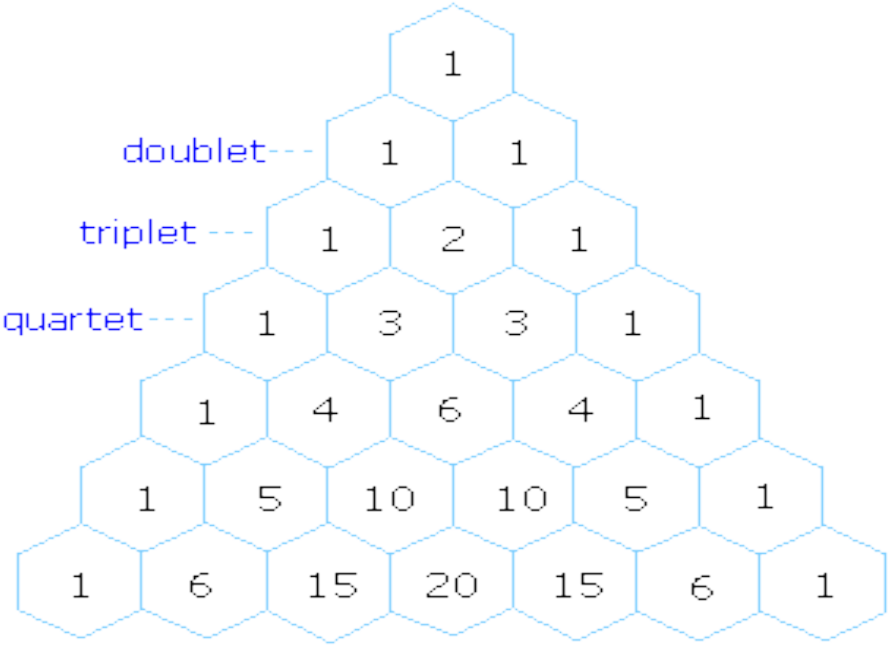


Sextet
(n=5)



Septet
(n=6)

- The theoretical intensity of the individual lines can be derived from Pascal's triangle.

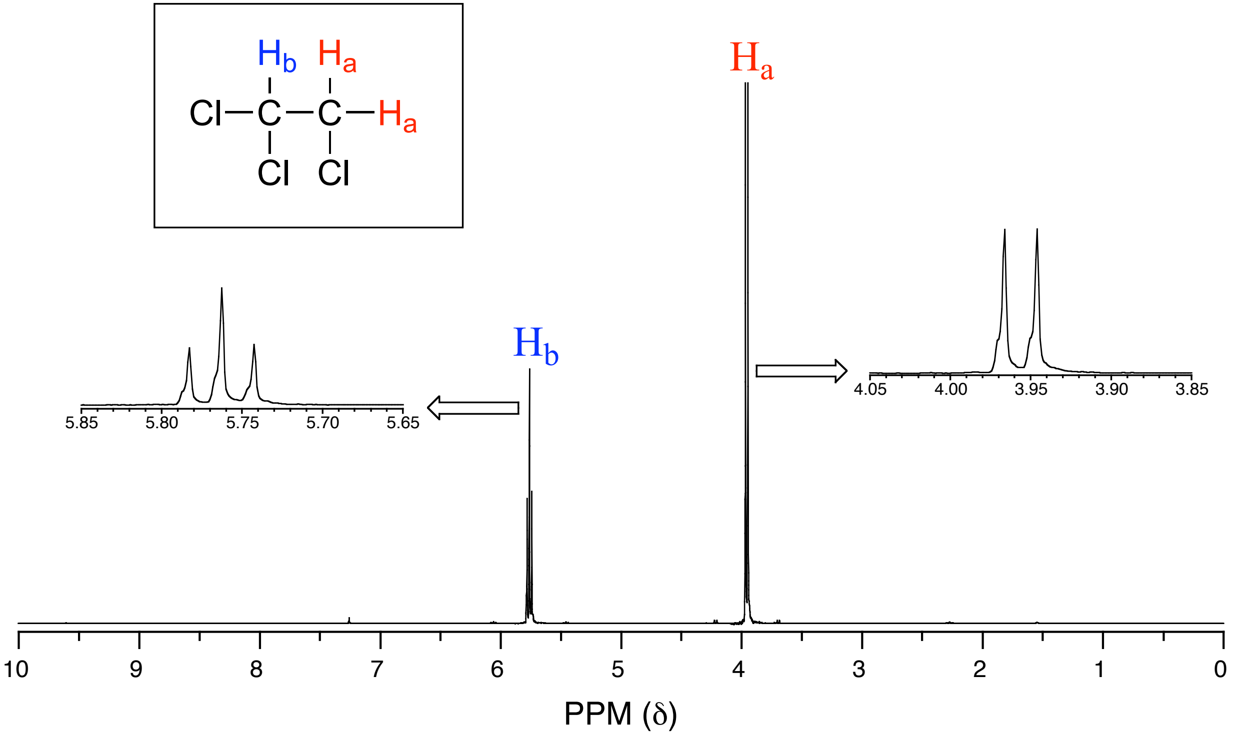


Pascal's Triangle

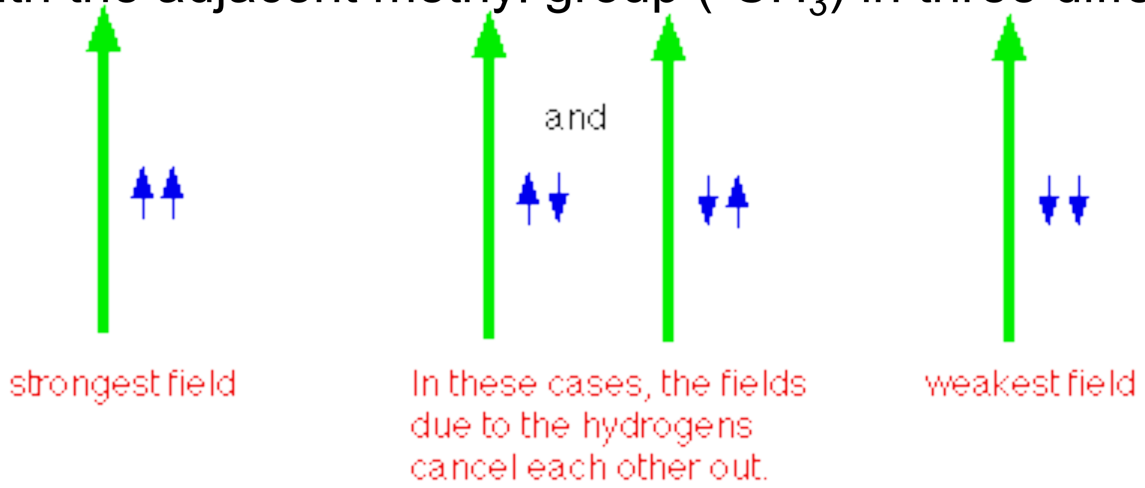
SPIN-SPIN COUPLING

- The source of signal splitting in NMR spectra is a phenomenon called **spin-spin coupling.**
- The interactions between the spins of neighboring magnetic nuclei in a molecule is known as spin-spin coupling.
- The coupling occurs through bonds by means of slight impairing of bonding electrons.
- The complexity of the multiplet depends upon the nature and number of the nearby atoms.
- Chemically equivalent protons do not show spin-spin coupling due to interaction among themselves. i.e., only the nonequivalent protons show the property of coupling.

- E.g., 1,1,2-trichloroethane
- Here the H_a and H_b protons are spin-coupled to each other and therefore it has been observed that for each kind of protons we do not get singlet, but a groups of peaks are observed.



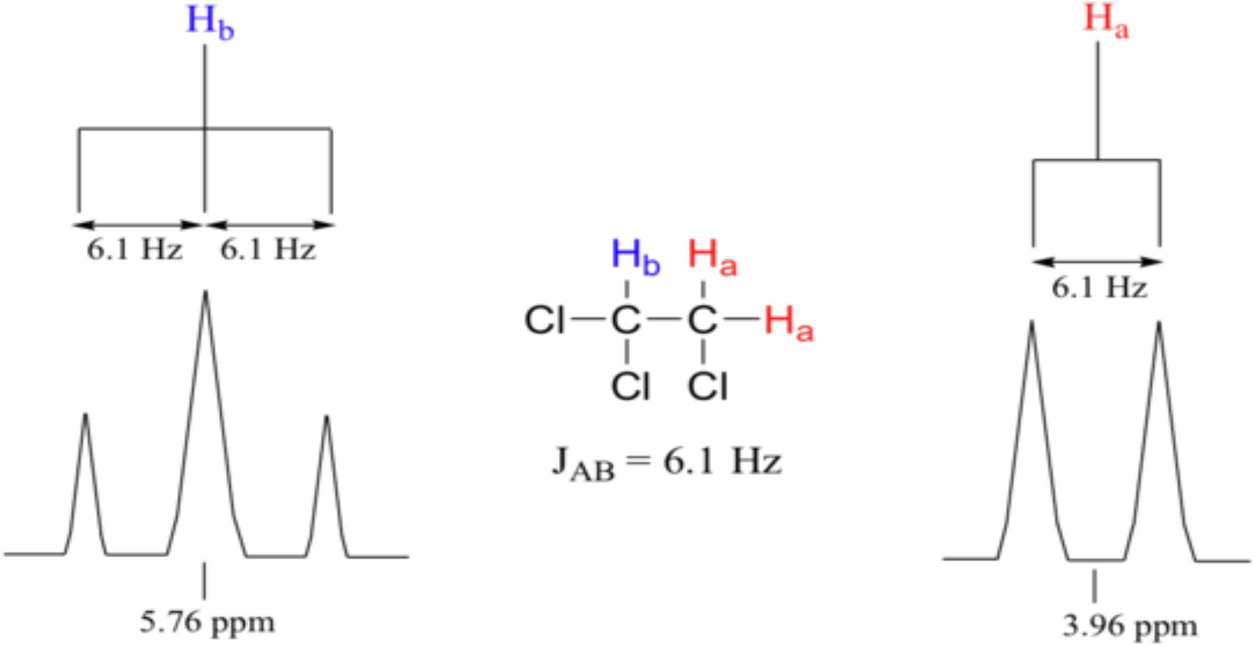
- Coupling may either oppose or reinforce the field felt by the other molecule.
- E.g., Ethyl bromide (CH_3-CH_2Br)
  - In this molecule the spin of two protons (-CH_2-) can couple with the adjacent methyl group (-CH_3) in three different ways,



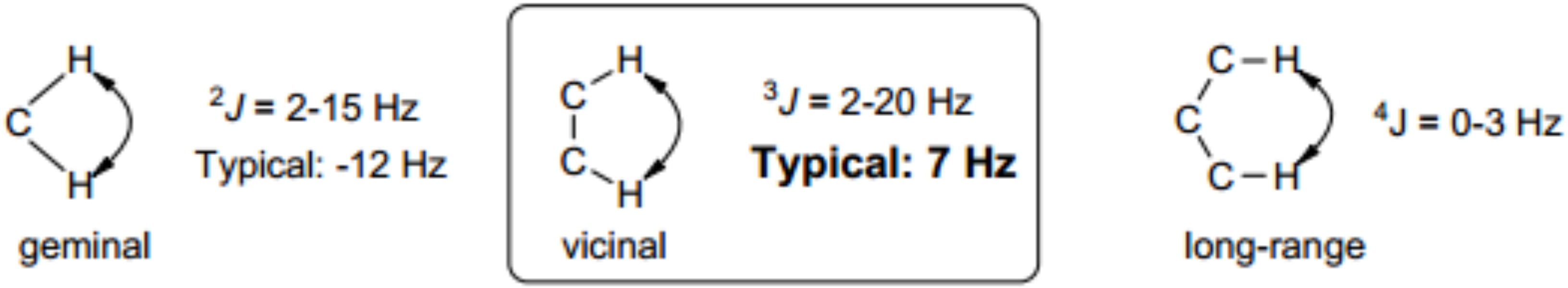
Reinforcing **Not effecting** **Opposing**

COUPLING CONSTANT

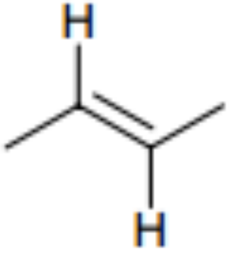
- The spacing of adjacent lines in the multiplet is a direct measure of the spin-spin coupling and is known as ***coupling constant (J).***
- It is the distance between two adjacent sub-peaks in a split signal.
- ***J*** value is expressed in Hertz(Hz) or in cycles per second(cps).



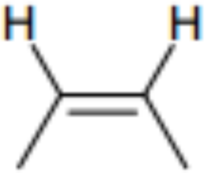
- For nonequivalent hydrogens on the same sp2 carbon, the **J** value is usually very small and are unable to observe.
- But, for nonequivalent hydrogens bonded to adjacent sp2 carbons, the **J** is usually large enough to be observed.
- The leading superscript ($^{X}\mathbf{J}$) indicates the number of bonds between the coupled nuclei.



- **J** value is independent of the external field and it decreases with distance.
- Coupling constants, **J** vary widely in size, but the vicinal couplings in acyclic molecules are usually 7 Hz.
- **J** provides important information in coupling across double bonds, where trans couplings are always substantially larger than cis couplings.



J = 14 - 18 Hz

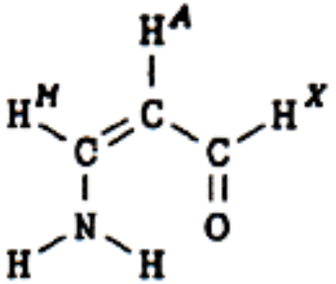


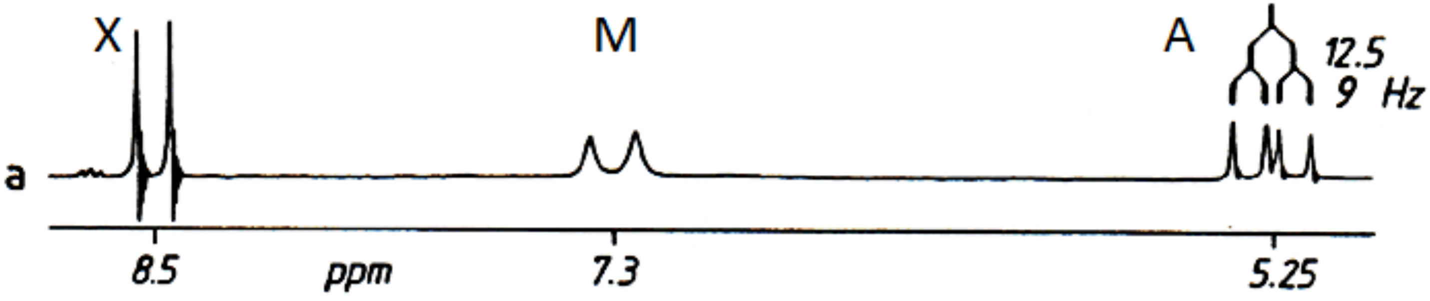
J = 8 - 12 Hz

SPIN DECOUPLING

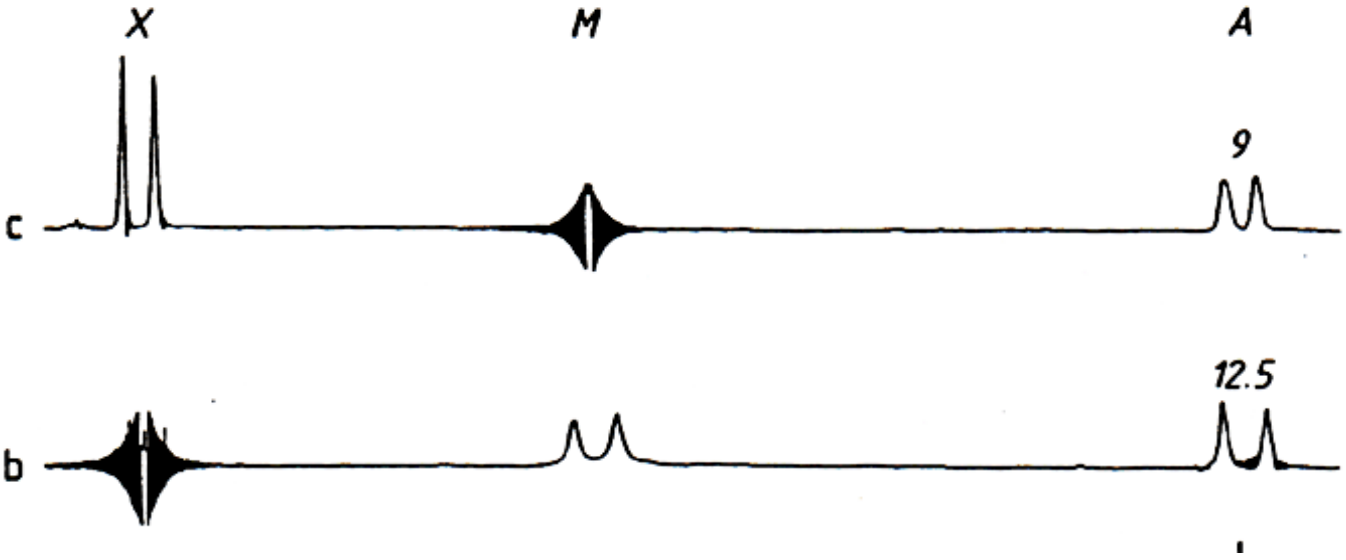
- Coupling causes splitting of signals in NMR spectra.
- Decoupling is a special technique used in NMR spectroscopy to avoid the splitting of the signals by eliminating partially or fully the observed coupling.
- Which involves the irradiation of a proton with sufficiently intense radiofrequency energy, so that it prevents the coupling with the neighboring proton and gives spectral line as a singlet.
- Decoupling can help determine structures of chemical compounds.

- **There are two types of decoupling:**
  - **Homonuclear decoupling -** the nuclei being irradiated are the same isotope as the nuclei being observed in the spectrum.
  - **Heteronuclear decoupling -** the nuclei being irradiated are of a different isotope than the nuclei being observed in the spectrum.
- E.g., 3-amino-acroleine





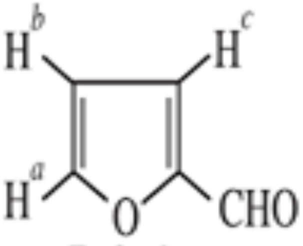
- Selective irradiation of M reduces the AMX spin system to AX : two dublets, A-X coupling constant can be determined



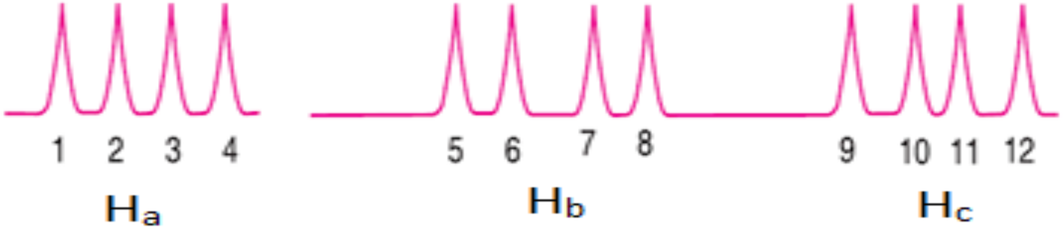
Effects of Coupling and Decoupling in NMR spectra

- The identification of coupled protons in spectra are too complex because they show so many signals.
- Decoupling is used to simplify a complex NMR spectrum.
- It is possible to irradiate (decouple) each coupled protons in the molecule to produce the spectrum with less complexity.
- Decoupling causes the multiplet to collapse to a doublet or singlet and give spectra which are easy to interpret.
- In this way the full coupling relationship can be established and much information can be collected about the connection between alkanes, alkenes and alkynes .

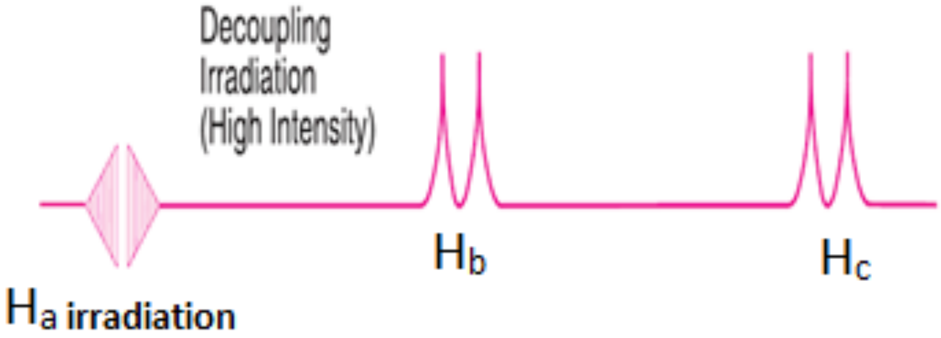
- E.g., Furfural


Furfural

- It is a complex compound because $H_{a,}$ H_b and H_c appear as four lines in the spectrum due to coupling effects.



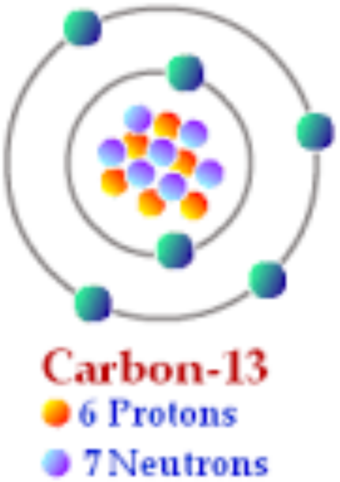
- The effects of strong decoupling eliminates the coupling of H_a with H_b and H_c as a result each multiplet collapse into doublet.



ISOTOPIC NUCLEI

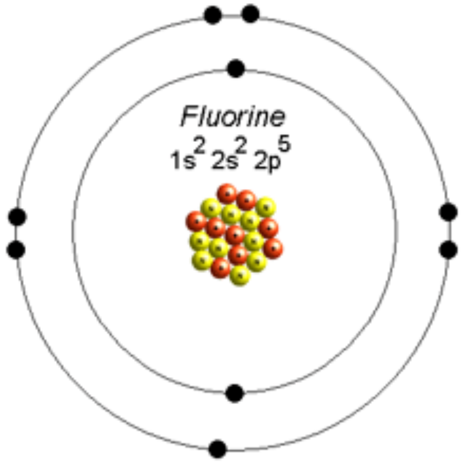
- An isotope can be defined as the atoms with the same number of protons but have a different number of neutrons or the elements with the same atomic number but different mass number.
- Any nucleus with an odd atomic number or odd mass number will have a nuclear magnetic resonance.
- In addition to hydrogen, there are several other nuclei which have magnetic movements and can be studied by NMR spectroscopy.
- E.g., ^{1}H, ^{13}C, ^{19}F, ^{15}N, ^{11}B, ^{31}P, etc.

- **CARBON-13**



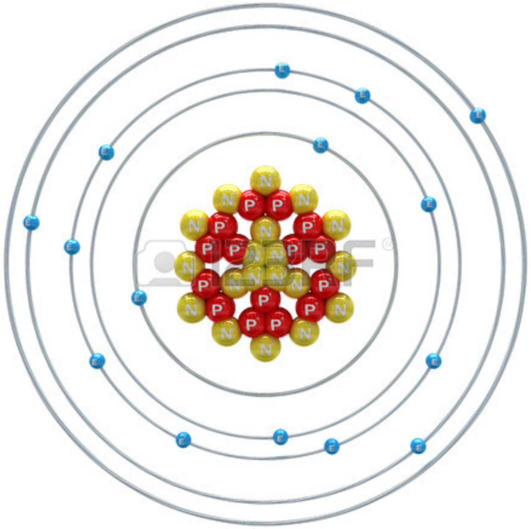
- ^{13}C was difficult to study because it gives rise to extremely weak signals in NMR spectra.
- It can be studied by using Fourier Transform method.
- Principle behind ^{13}C NMR are exactly similar to those of Proton NMR, but the scale of observed chemical shift and coupling is greater for ^{13}C NMR.
- ^{13}C shift range varies from 0-250 ppm.

- **FLUORINE-19**



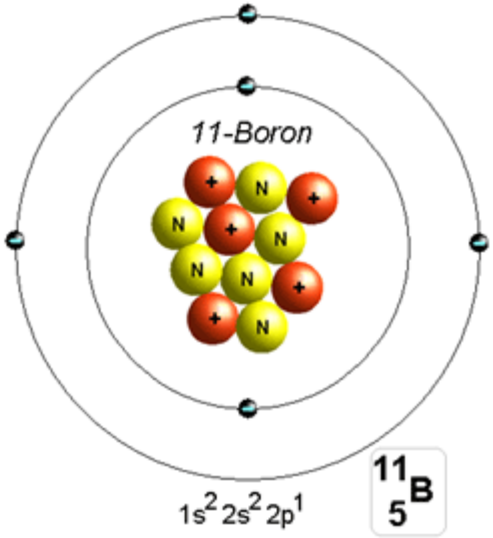
- Fluorine with an atomic number of 9 has a magnetic momentum of 2.6285,can be studied by NMR spectroscopy by the same technique as PMR.
- This technique is commonly used for study of fluorinated aliphatic and aromatic compounds.
- The range of chemical shift for aromatic fluorine atoms are five times greater than the total range of proton shift in PMR.
- The fluorine absorption is sensitive to the environment

- **PHOSPHOROUS -31**



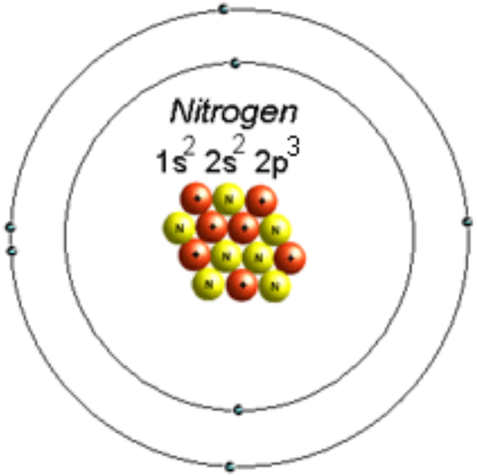
- It shows magnetic property similar to hydrogen and fluorine isotopes.
- It exhibits sharp NMR peaks with chemical shifts extending over range of 700ppm.
- A quantitative analysis of these isotopes are studied out by Colson and Marr, they found out that ^{31}P resonance shift are very large.

- **BORON -11**



- The ^{11}B spectra has been extensively used to analyse the complex boron hydrides
- The chemical shift ranges very large
- It has 2 naturally occuring isotopes (^{11}B and ^{10}B).
- ^{11}B is used mainly in studies of NMR.

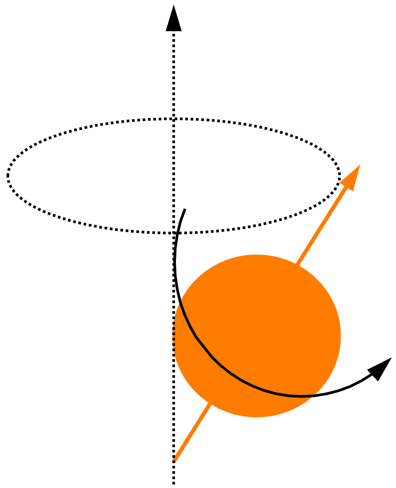
- **NITROGEN -15**



- ^{15}N yields sharp lines in NMR spectra but is very insensitive.
- ^{15}N experiments gives narrow lines and has a larger chemical shift range.
- IUPAC recommends CHNO as the chemical shift standard for nitrogen isotope nucleides.

Angular Momentum

A spinning charge generates a magnetic field, the resulting spin-magnet has a magnetic moment (**μ**) proportional to the spin I



magnetic moment $\boldsymbol{\mu} = \gamma\, \boldsymbol{p}$
where γ is the gyromagnetic ratio,
and it is a constant for a given nucleus

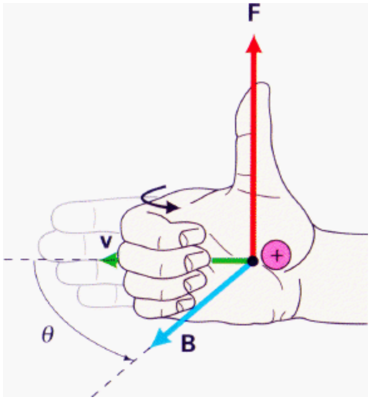
$$\mu = \gamma p = \gamma \sqrt{I(I+1)}\, h / 2\pi$$

When I=0, μ=0

** There is no spin for nuclei with I=0

"Right Hand Rule"

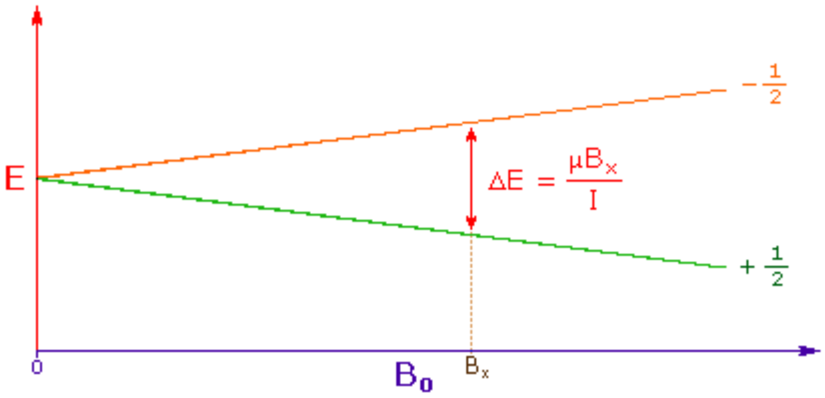
determines the direction of the magnetic field around a current-carrying wire and vice-versa



Energy Differentiation

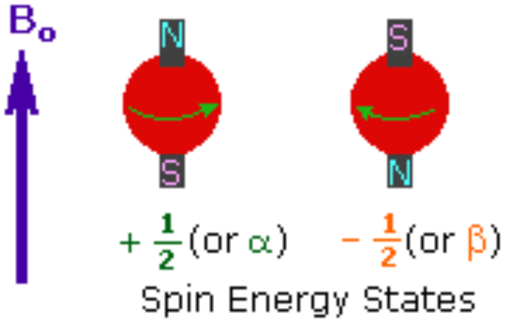
In the presence of an external magnetic field ($\mathbf{B_0}$), two spin states exist, **+1/2** and **-1/2** (For I=1/2).

The magnetic moment of the lower energy +1/2 state is aligned with the external field, and that of the higher energy -1/2 spin state is opposed to the external field.



Aligned against
the applied field

Aligned with
the applied field



Energy Differentiation

Difference in energy between the two states is given by:

$$\Delta E = \gamma\ h\ B_o / 2\pi$$

where:

B_o – external magnetic field
h – Planck's constant
γ – gyromagnetic ratio

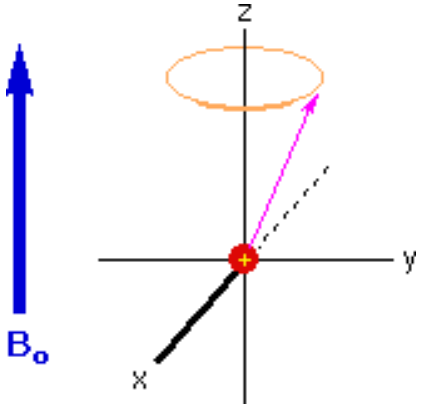
When the energy of the photon matches the energy difference between the two spin states , an absorption of energy occurs. We call that phenomenon *Resonance*

$$\Delta E = h\upsilon = \gamma h B_o / 2\pi$$

So, $\upsilon = \gamma\ B_o / 2\pi$

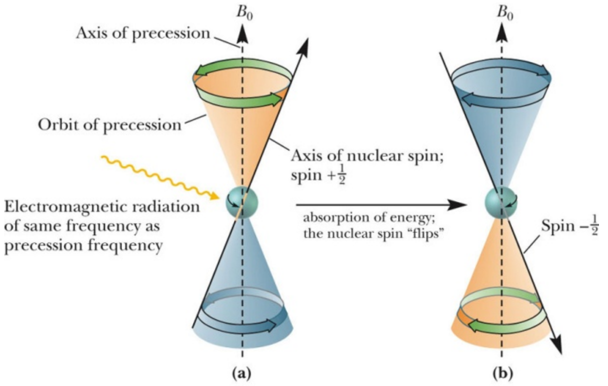
Larmor Precession

Spinning particle precesses about the external field axis with an angular frequency known as the Larmor frequency



$\omega_L = \gamma B_o$

When radio frequency energy matching the Larmor frequency is introduced at a right angle to the external field, it would cause a transition between the two energy levels of the spin. In other world, the precessing nucleus will absorb energy and the magnetic moment will flip to its I = –1/2 state



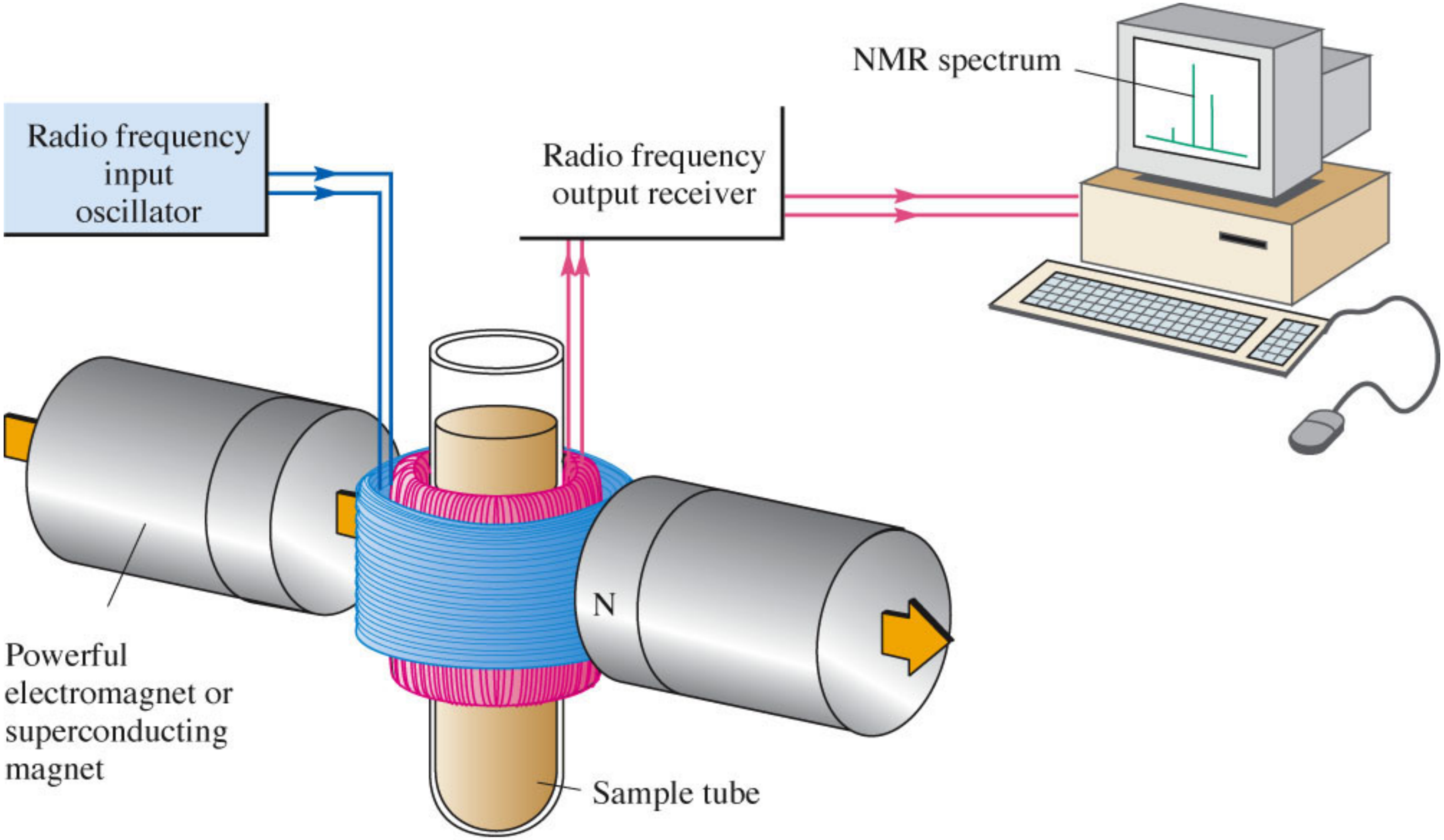
Brown/Foote/Iverson
Organic Chemstry 5e
Figure 13.03

Date_070607

γ- Values for some nuclei

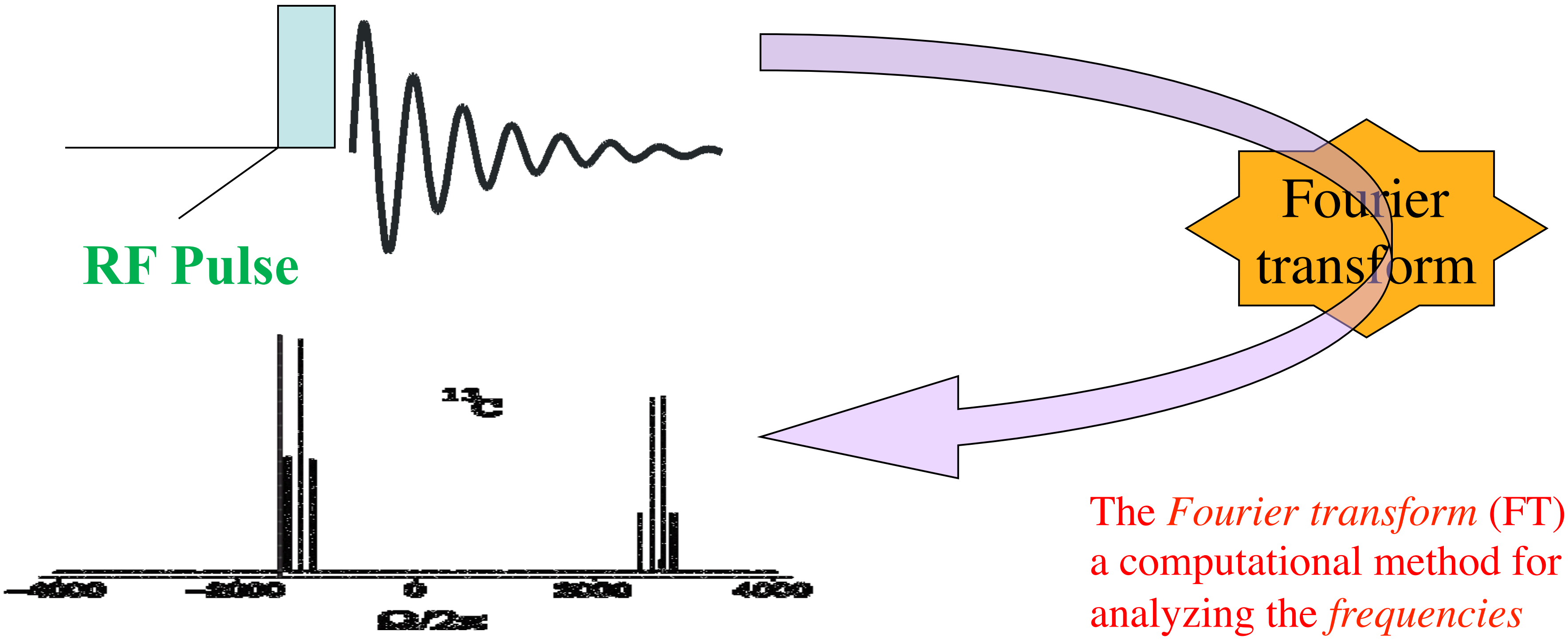
Isotope	**Net Spin**	**γ / MHz T^{-1}**	**Abundance / %**
^{1}H	1/2	42.58	99.98
^{2}H	1	6.54	0.015
^{3}H	1/2	45.41	0.0
^{31}P	1/2	17.25	100.0
^{23}Na	3/2	11.27	100.0
^{14}N	1	3.08	99.63
^{15}N	1/2	4.31	0.37
^{13}C	1/2	10.71	1.108
^{19}F	1/2	40.08	100.0

Schematic NMR Spectrometer



© Brooks/Cole, Cengage Learning

Fourier transformation and the NMR spectrum



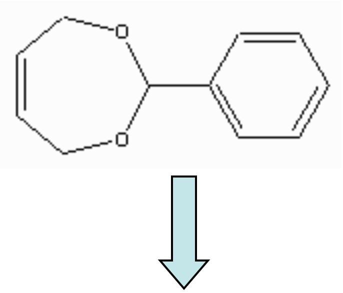
RF Pulse

Fourier transform

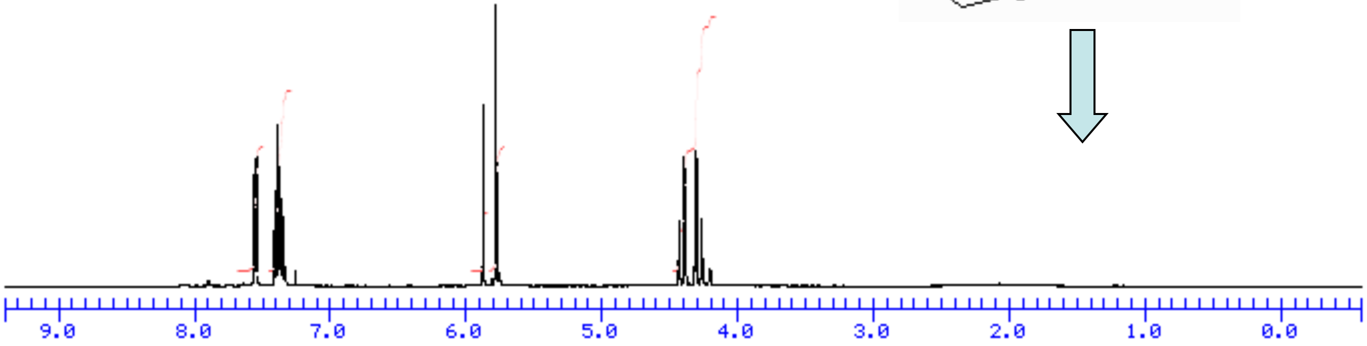
The NMR spectrum

The *Fourier transform* (FT) is a computational method for analyzing the *frequencies* present in an oscillating signal

^{1}H NMR and ^{13}C NMR Spectrum



^{1}H NMR spectra

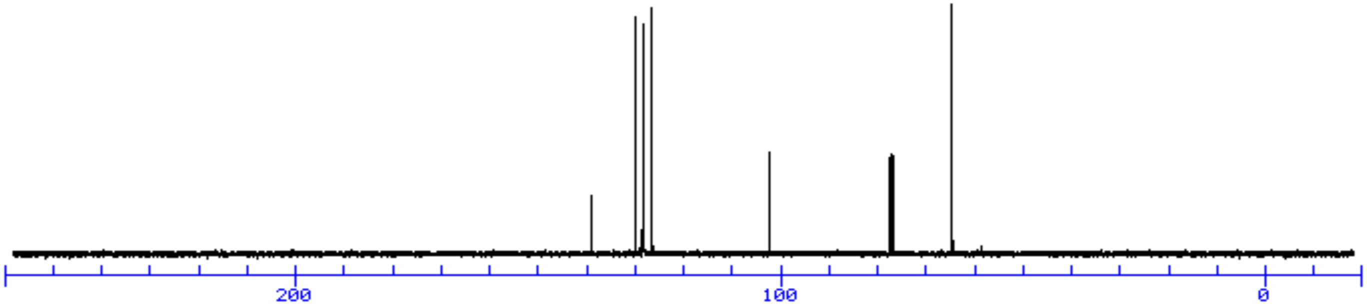


δ ppm

Down field

High field

^{13}C NMR spectra



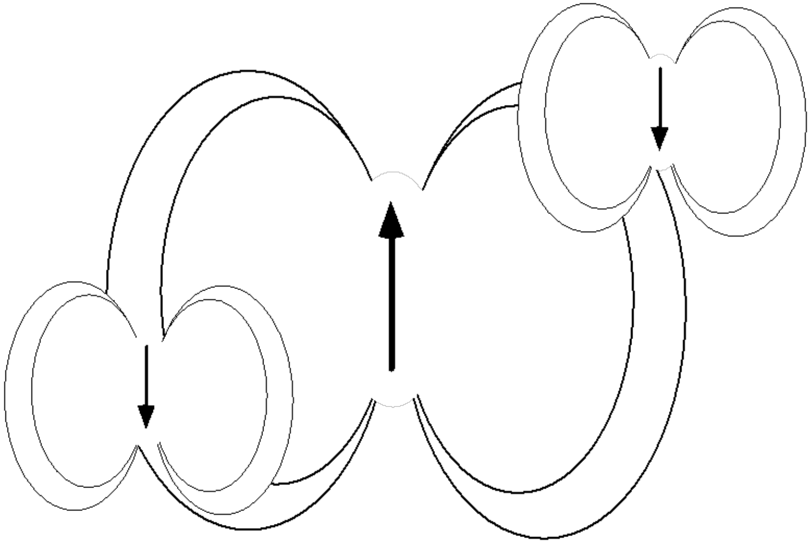
δ ppm

Chemical Shift-δ

When an atom is placed in a magnetic field, its electrons circulate about the direction of the applied magnetic field. This circulation causes a small magnetic field at the nucleus which opposes the externally applied field

The magnetic field at the nucleus (the effective field) is therefore generally less than the applied field by a fraction :

$B = B0\ (1-\sigma)$, So $\upsilon = \gamma B_0 (1-\sigma) / 2\pi$



Chemical Shift-δ

The electron density around each nucleus in a molecule varies according to the types of nuclei and bonds in the molecule. The opposing field and therefore the effective field at each nucleus will vary. This is called the chemical shift phenomenon.

As we can tell from $\nu = \gamma B_0 (1-\sigma) / 2\pi$, the greater the value of B_o, the greater the frequency difference.

This relationship could make it difficult to compare NMR spectra taken on spectrometers operating at different field strengths.

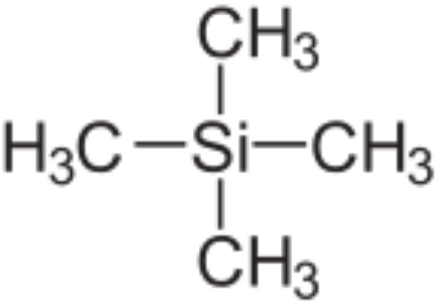
The term chemical shift was developed to avoid this problem. The chemical shift of a nucleus is the difference between the resonance frequency of the nucleus and a standard, relative to the standard. This quantity is reported in ppm and given the symbol delta.

$$\delta = (\nu - \nu_{ref}) \times 10^6 / \nu_{ref}$$

Standard for Chemical Shift

In NMR spectroscopy, the standard is often tetramethylsilane, $Si(CH_3)_4$, abbreviated TMS.

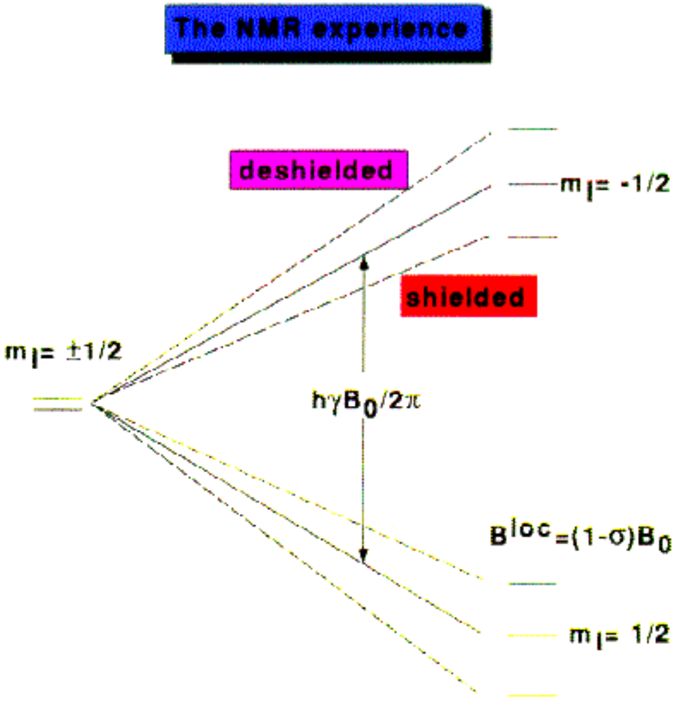
Tetramethyl silane (TMS) is used as reference because it is soluble in most organic solvents, is inert, volatile, and has 12 equivalent 1H and 4 equivalent 13C. TMS signal is set to 0



Shielding and Deshielding

A nucleus is said to be **shielded** when electrons around the nucleus circulates in a magnetic field and create a secondary induced magnetic field which opposes the applied field .

Trends in chemical shift are explained based on the degree of shielding or deshielding , e.g. of deshielding effect



Chemical Shift-δ

Chemical shift depends on :

- Electronegativity of nearby atoms
- Hybridization of adjacent atoms
- diamagnetic effects
- paramagnetic effects
- solvent effect

Spin-Spin Coupling

Spin-spin coupling:

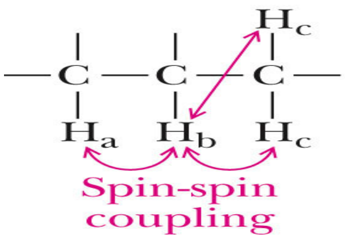
The coupling of the intrinsic angular momentum of different particles. Such coupling between pairs of nuclear spins is an important feature of nuclear magnetic resonance (NMR) spectroscopy as it can provide detailed information about the structure and conformation of molecules. Spin-spin coupling between nuclear spin and electronic spin is responsible for hyperfine structure in atomic spectra.

J-Coupling

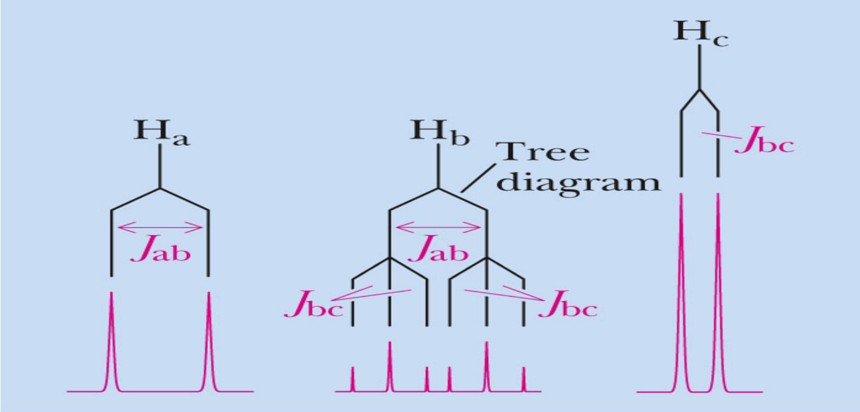
***J*-coupling:**

also called *indirect spin-spin coupling*, is the coupling between two nuclear spins due to the influence of bonding electrons on the magnetic field running between the two nuclei. J-coupling provides information about dihedral angles, which can be estimated using the Karplus equation. It is an important observable effect in 1D NMR spectroscopy.

The coupling constant, *J* (usually in frequency units, Hz) is a measure of the interaction between a pair of nuclei



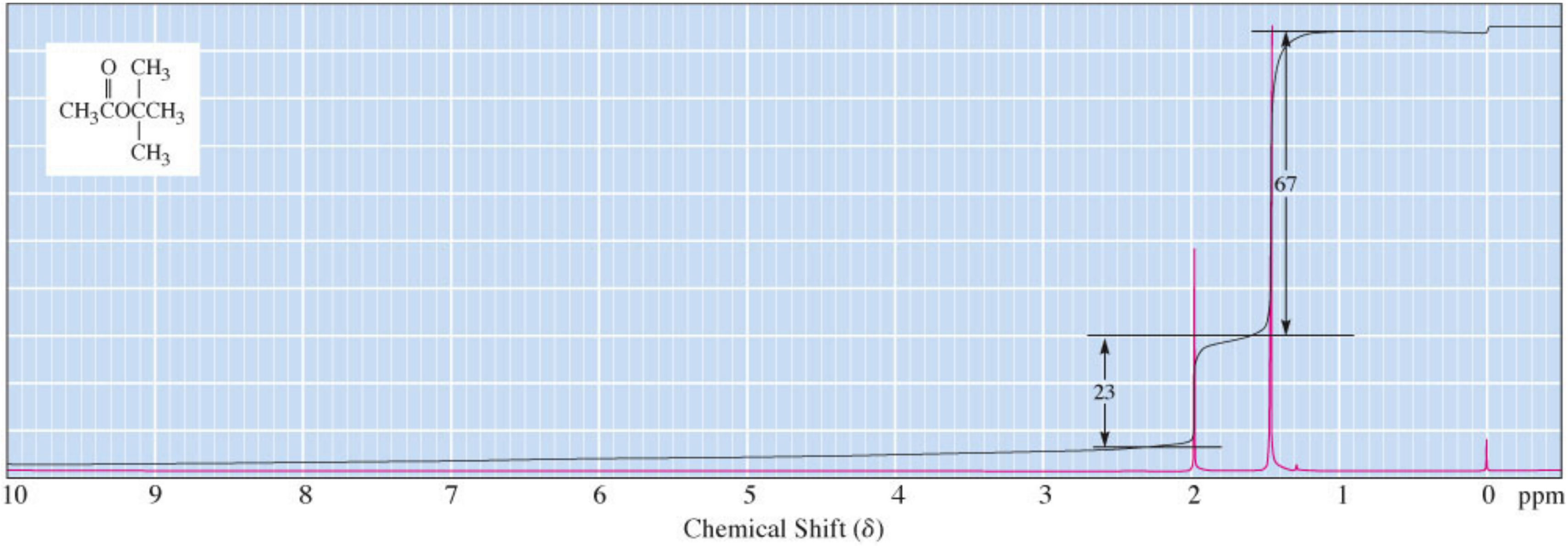
© Brooks/Cole, Cengage Learning



^{1}H-NMR

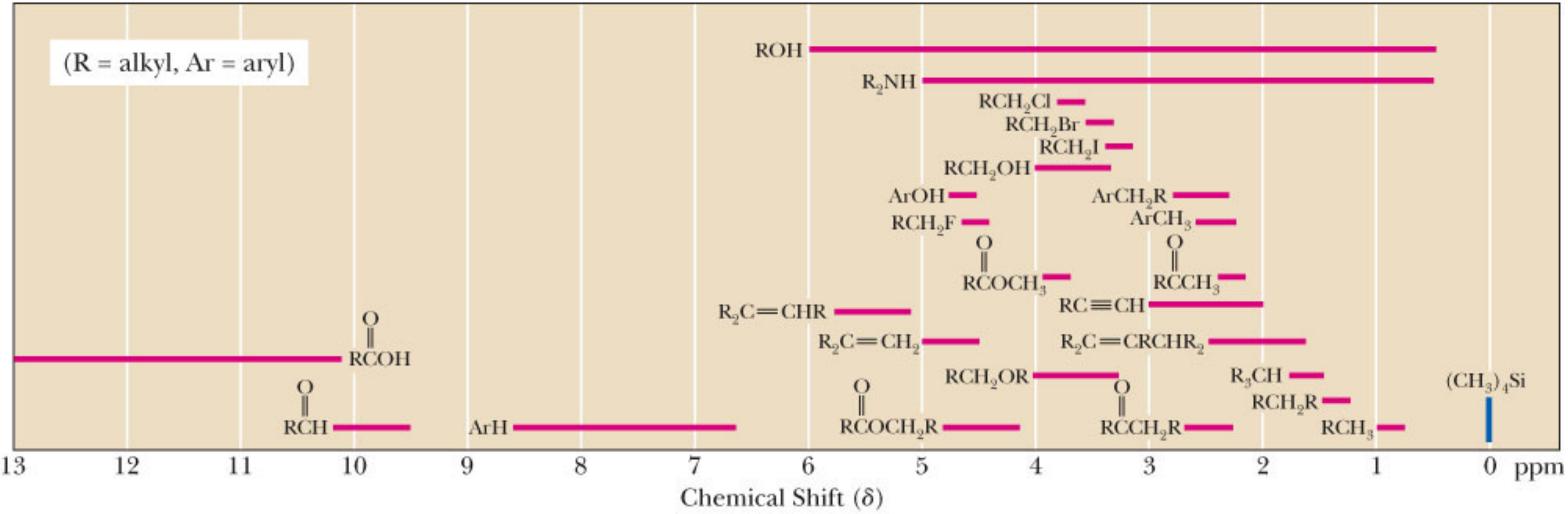
- ^{1}H experiencing the same chemical environment or chemical shift are called equivalent hydrogens.

- ^{1}H experiencing different environment or having different chemical shifts are nonequivalent hydrogens.

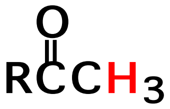
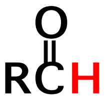
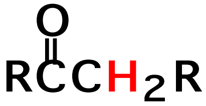
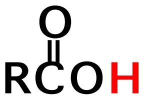


© Brooks/Cole, Cengage Learning

Chemical Shift - ^{1}H-NMR



^{1}H Chemical shifts

Type of Hydrogen	Chemical Shift (δ)	Type of Hydrogen	Chemical Shift (δ)
$(CH_3)_4Si$	0 (by definition)		
RCH_3	0.8-1.0	 $R\overset{\overset{O}{\|}}{C}OCH_3$	3.7-3.9
RCH_2R	1.2-1.4		
R_3CH	1.4-1.7	 $R\overset{\overset{O}{\|}}{C}OCH_2R$	4.1-4.7
$R_2C{=}CRCHR_2$	1.6-2.6	RCH_2I	3.1-3.3
$RC{\equiv}CH$	2.0-3.0	RCH_2Br	3.4-3.6
$ArCH_3$	2.2-2.5	RCH_2Cl	3.6-3.8
$ArCH_2R$	2.3-2.8	RCH_2F	4.4-4.5
ROH	0.5-6.0	$ArOH$	4.5-4.7
RCH_2OH	3.4-4.0	$R_2C{=}CH_2$	4.6-5.0
RCH_2OR	3.3-4.0	$R_2C{=}CHR$	5.0-5.7
R_2NH	0.5-5.0	ArH	6.5-8.5
 $R\overset{\overset{O}{\|}}{C}CH_3$	2.1-2.3	 $R\overset{\overset{O}{\|}}{C}H$	9.5-10.1
 $R\overset{\overset{O}{\|}}{C}CH_2R$	2.2-2.6	 $R\overset{\overset{O}{\|}}{C}OH$	10-13

Factors to Affect ^1H Chemical Shift

Chemical shift : (1) electronegativity of nearby atoms, (2) hybridization of adjacent atoms, and (3) diamagnetic effects

Electronegativity

CH_3-X	Electronegativity of X	Chemical Shift (δ)
CH_3F	4.0	4.26
CH_3OH	3.5	3.47
CH_3Cl	3.1	3.05
CH_3Br	2.8	2.68
CH_3I	2.5	2.16
$(CH_3)_4C$	2.1	0.86
$(CH_3)_4Si$	1.8	0.00

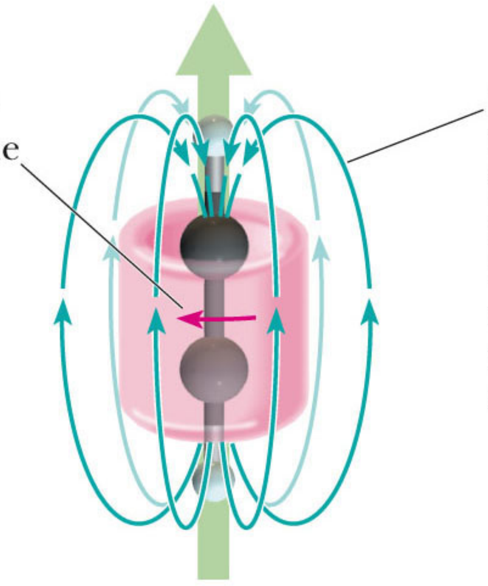
Hybridization of adjacent atoms

Type of Hydrogen (R = alkyl)	Name of Hydrogen	Chemical Shift (δ)
RCH_3, R_2CH_2, R_3CH	Alkyl	0.8 - 1.7
$R_2C{=}C(R)CHR_2$	Allylic	1.6 - 2.6
$RC{\equiv}CH$	Acetylenic	2.0 - 3.0
$R_2C{=}CHR$, $R_2C{=}CH_2$	Vinylic	4.6 - 5.7
$RCHO$	Aldehydic	9.5-10.1

Carbon-Carbon Triple Bond Effect

A carbon-carbon triple bond shields an acetylenic hydrogen and shifts its signal to lower frequency (to the right) to a smaller value

Type of H	Name	Chemical Shift (δ)
RCH_3	Alkyl	0.8- 1.0
$RC{\equiv}CH$	Acetylenic	2.0 - 3.0
$R_2C{=}CH_2$	Vinylic	4.6 - 5.7

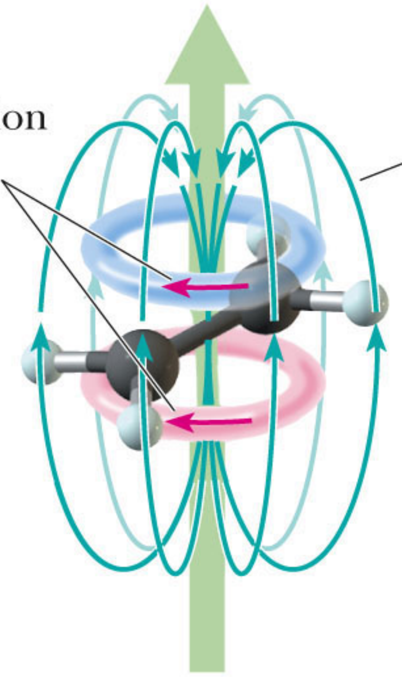


Induced flow of electrons in the π system of an alkyne

Induced local magnetic field of the π electrons is against the applied field at the hydrogen atoms; it requires lower frequency radiation to bring an acetylenic hydrogen nucleus into resonance.

Carbon-Carbon Double Bond Effect

Magnetic induction in the p bond of a carbon-carbon double bond deshields vinylic hydrogens and shifts their signal higher frequency



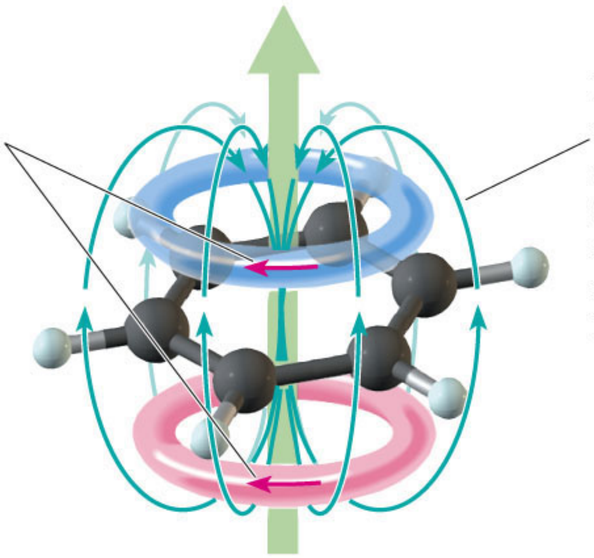
Induced circulation of π electrons in an alkene

Induced local magnetic field of the π electrons reinforces the applied field at the hydrogen atoms; it requires higher frequency radiation to bring a vinylic hydrogen nucleus into resonance.

Applied field, B_0

Aromatic Effect

The magnetic field induced by circulation of p electrons in an aromatic ring <u>deshields</u> the hydrogens on the ring and shifts their signal to higher frequency



Induced circulation of π electrons in a benzene ring

Induced local magnetic field of the circulating π electrons reinforces the applied field at the hydrogen atoms; it requires higher frequency radiation to bring aromatic hydrogen nuclei into resonance

Signal Splitting for ^{1}H

Peak:

The units into which an NMR signal is split; doublet, triplet, quartet, multiplet, etc.

Signal splitting:

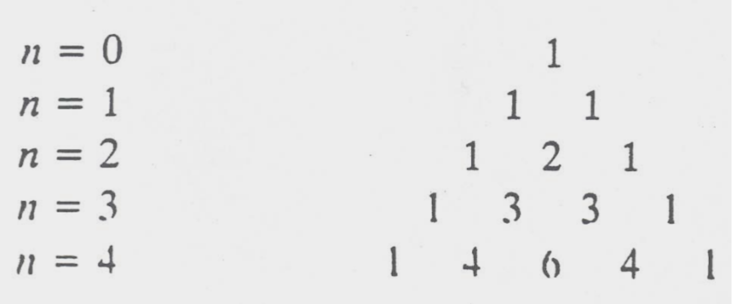
Splitting of an NMR signal into a set of peaks by the influence of neighboring nonequivalent hydrogens.

$(n + 1)$ rule:

If a hydrogen has *n* hydrogens nonequivalent to it but equivalent among themselves on the same or adjacent atom(s), its ^{1}H-NMR signal is split into $(n + 1)$ peaks.

Pascal's triangle

The relative peak intensities for multiplet peaks arising from *J*-coupling of a ^{1}H to N equivalent ^{1}H can be determined using Pascal's triangle:



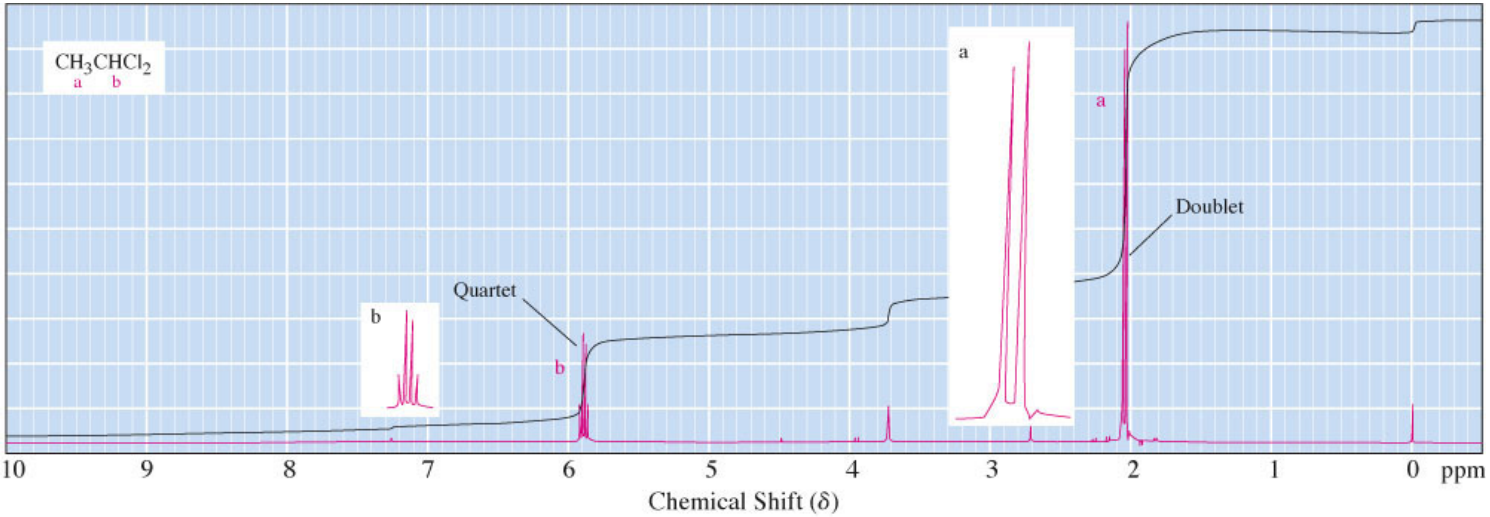
$n = 0$ 1

$n = 1$ 1 1

$n = 2$ 1 2 1

$n = 3$ 1 3 3 1

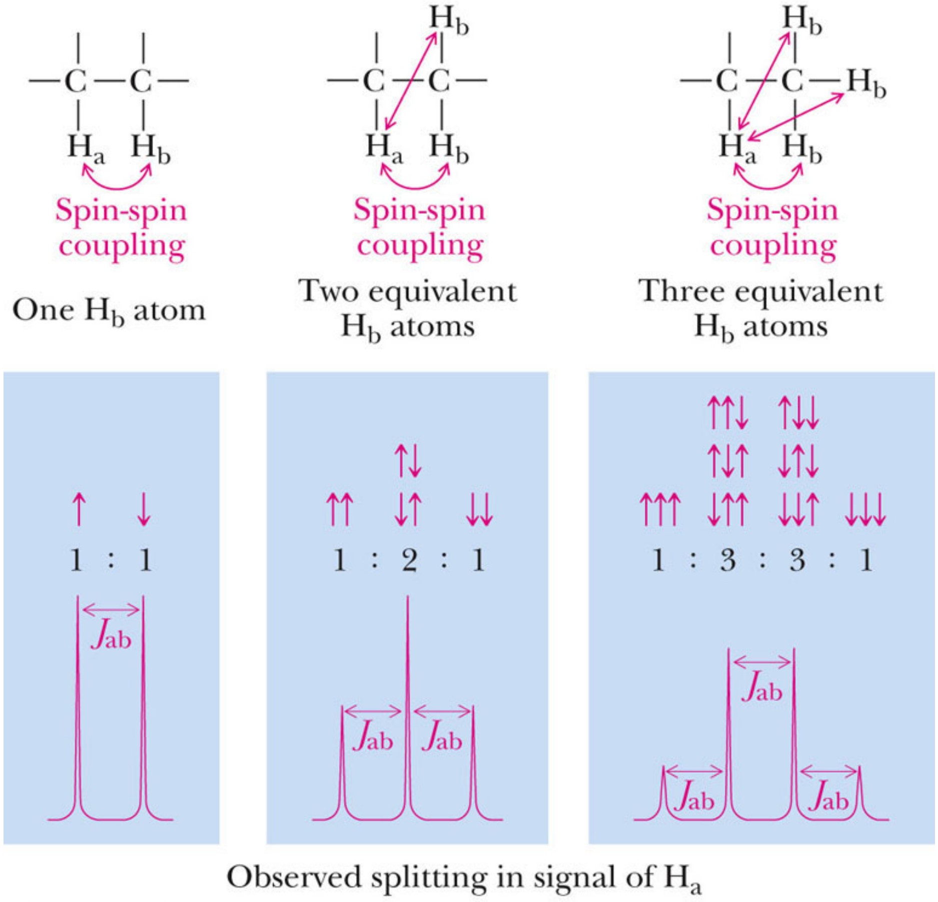
$n = 4$ 1 4 6 4 1



Coupling constant

Coupling constant (*J*):

The separation on an NMR spectrum (in hertz) between adjacent peaks in a multiplet.

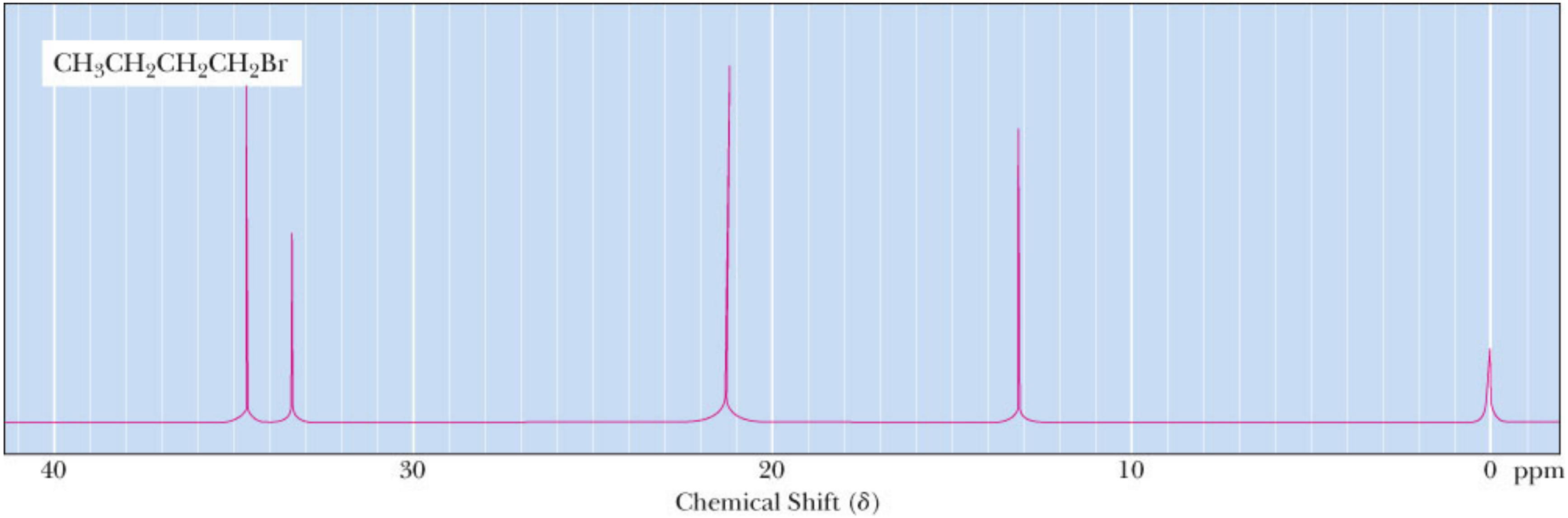


Observed splitting in signal of H_a

© Brooks/Cole, Cengage Learning

^{13}C-NMR Spectroscopy

Organic compounds contain carbon. Unfortunately, the C-12 nucleus does not have a nuclear spin, but the C-13 nucleus does due to the presence of an unpaired neucarbon-1tron. C-13 nuclei make up approximately 1% of the carbon nuclei on earth. Therefore, ^{13}C NMR will be much less sensitive than ^{1}HNMR NMR



© Brooks/Cole, Cengage Learning

^{13}C-NMR Spectroscopy

The presence of spin-spin coupling between a ^{13}C nucleus and the nuclei of ^{1}H atoms bonded to the ^{13}C, splits the carbon-13 peaks and causes an even poorer signal-to-noise ratio

Each nonequivalent ^{13}C gives a different signal

- A ^{13}C signal is split by the ^{1}H bonded to it according to the (n + 1) rule.
- Coupling constants of 100-250 Hz are common, which means that there is often significant overlap between signals, and splitting patterns can be very difficult to determine.

The most common mode of operation of a ^{13}C-NMR spectrometer is a proton-decoupled mode.

Decoupling

proton-decoupled mode,

a sample is irradiated with two different radiofrequencies. One to excite all ^{13}C nuclei, a second to cause all protons in the molecule to undergo rapid transitions between their nuclear spin states.

On the time scale of a ^{13}C-NMR spectrum, each proton is in an average or effectively constant nuclear spin state, with the result that ^{1}H-^{13}C spin-spin interactions are not observed and they are decoupled.

Chemical Shift - ^{13}C-NMR

Characteristic Carbon NMR Chemical Shifts (ppm)

$(CH_3)_4Si$ = TMS = 0.00 ppm (singlet) $CDCl_3$ (solvent) = 77.0 ppm (triplet)

RCH_3	0 – 40	RCH_2Cl	35 – 80	benzene ring	110 – 160
RCH_2R	15 – 55	R_3COH	40 – 80	C=O ester	160 – 180
R_3CH	20 – 60	R_3COR	40 - 80	C=O amide	165 – 180
RCH_2I	0 – 40	$RC\equiv CR$	65 – 85	C=O carboxylic acid	175 – 185
RCH_2Br	25 - 65	$R_2C=CR_2$	100 - 150	C=O aldehyde, ketone	180 – 210

Trends

- $RCH_3 < R_2CH_2 < R_3CH$
- Electronegative atoms cause downfield shift
- Pi bonds cause downfield shift
- C=O 160-210 ppm

^{13}C-NMR: Integration

^{1}H-NMR: Integration reveals relative number of hydrogens per signal

^{13}C-NMR: Integration reveals relative number of carbons per signal

- Rarely useful due to slow relaxation time for ^{13}C

time for nucleus to relax from
excited spin state to ground state

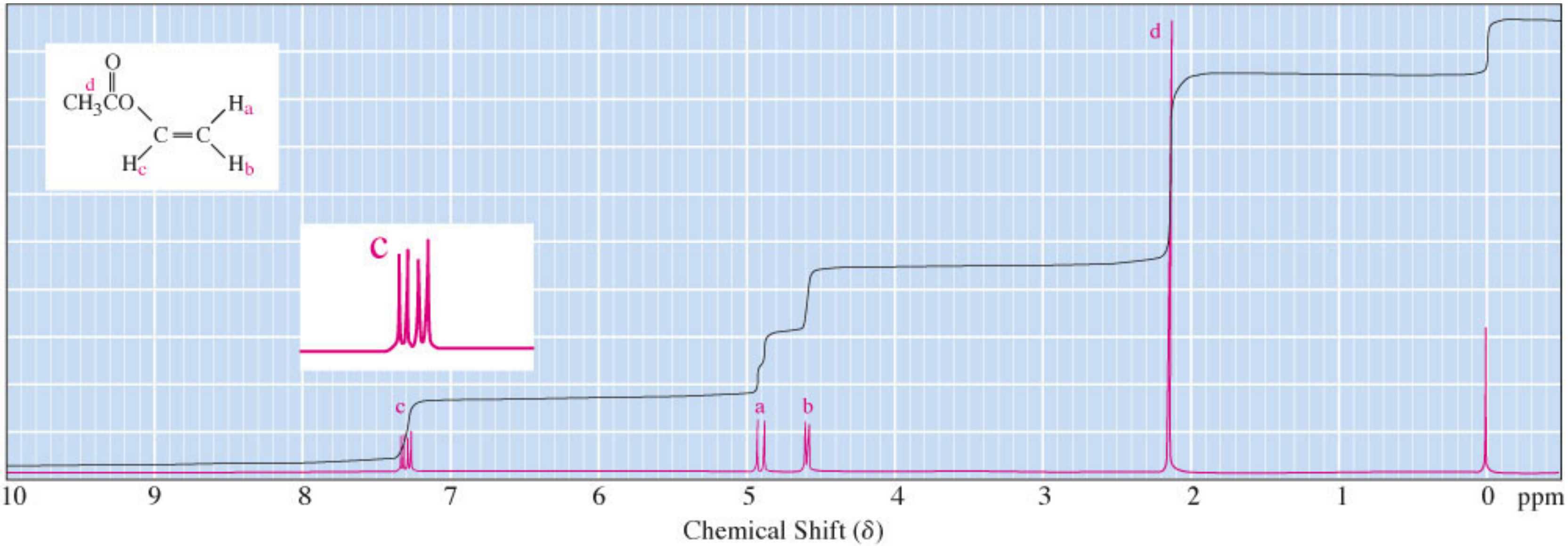
Interpreting NMR Spectra

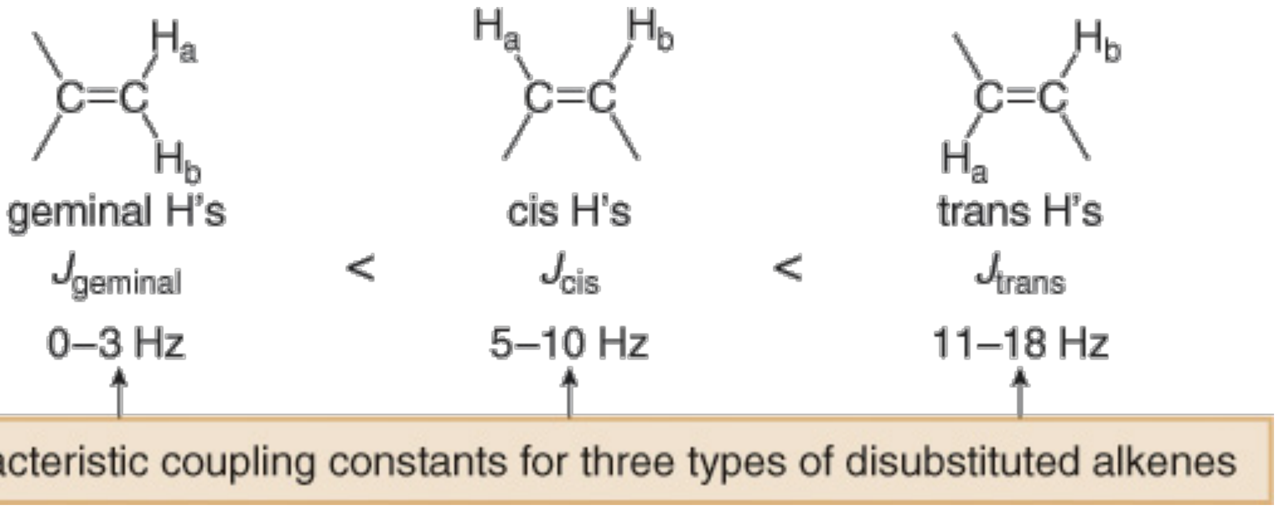
Alkanes

^{1}H-NMR signals appear in the range of 0.8-1.7.
^{13}C-NMR signals appear in the considerably wider range of 10-60.

Alkenes

^{1}H-NMR signals appear in the range 4.6-5.7.
^{1}H-NMR coupling constants are generally larger for *trans*-vinylic hydrogens (J= 11-18 Hz) compared with *cis*-vinylic hydrogens (J= 5-10 Hz).
^{13}C-NMR signals for sp^2 hybridized carbons appear in the range 100-160, which is to higher frequency from the signals of sp^3 hybridized carbons.





geminal H's		cis H's		trans H's
$J_{geminal}$	<	J_{cis}	<	J_{trans}
0–3 Hz		5–10 Hz		11–18 Hz

characteristic coupling constants for three types of disubstituted alkenes

Interpreting NMR Spectra

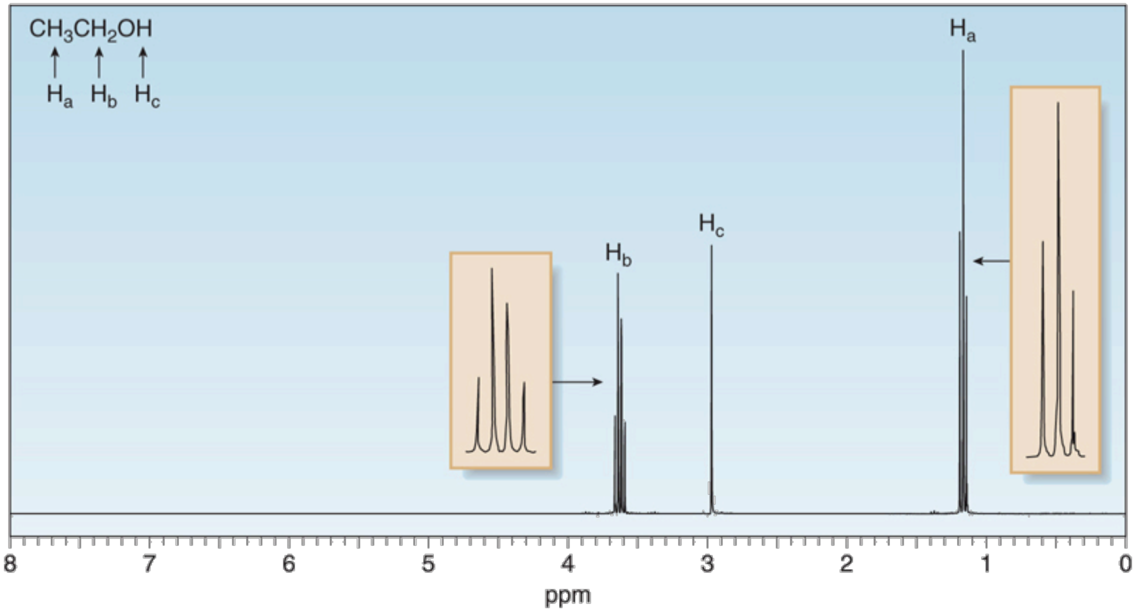
Alcohols

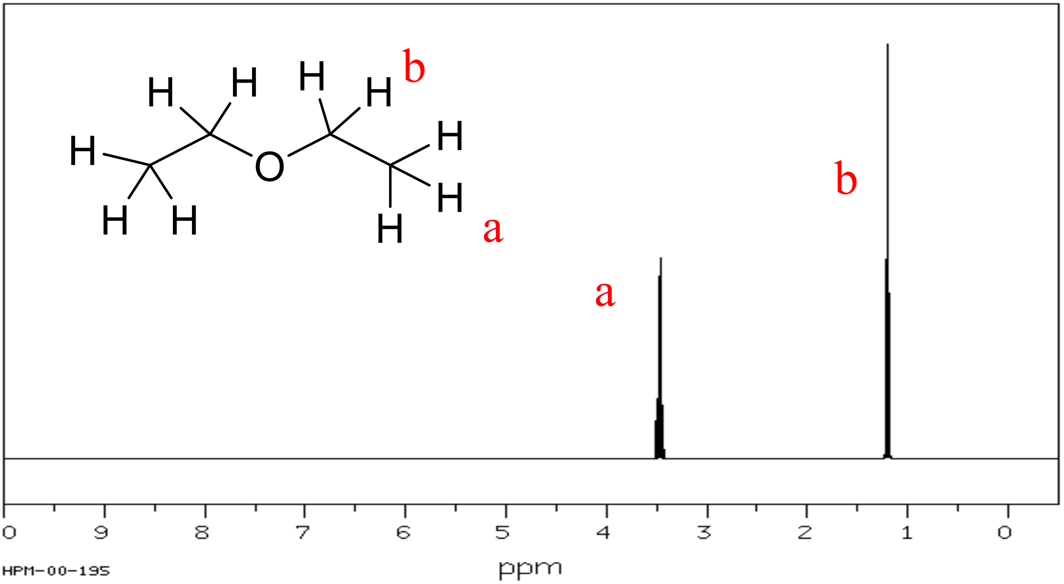
^{1}H-NMR O-H chemical shift often appears in the range 3.0-4.0, but may be as low as 0.5.

^{1}H-NMR chemical shifts of hydrogens on the carbon bearing the -OH group are deshielded by the electron-withdrawing inductive effect of the oxygen and appear in the range 3.0-4.0.

Ethers

A distinctive feature in the ^{1}H-NMR spectra of ethers is the chemical shift, 3.3-4.0, of hydrogens on the carbons bonded to the ether oxygen.


CH3CH2OH
Ha Hb Hc
Ha
Hb
Hc
8 7 6 5 4 3 2 1 0
ppm


H H H H H H H H H H
O
b
a
a
b
10 9 8 7 6 5 4 3 2 1 0
ppm
HPM-00-195

Interpreting NMR Spectra

Aldehydes and ketones

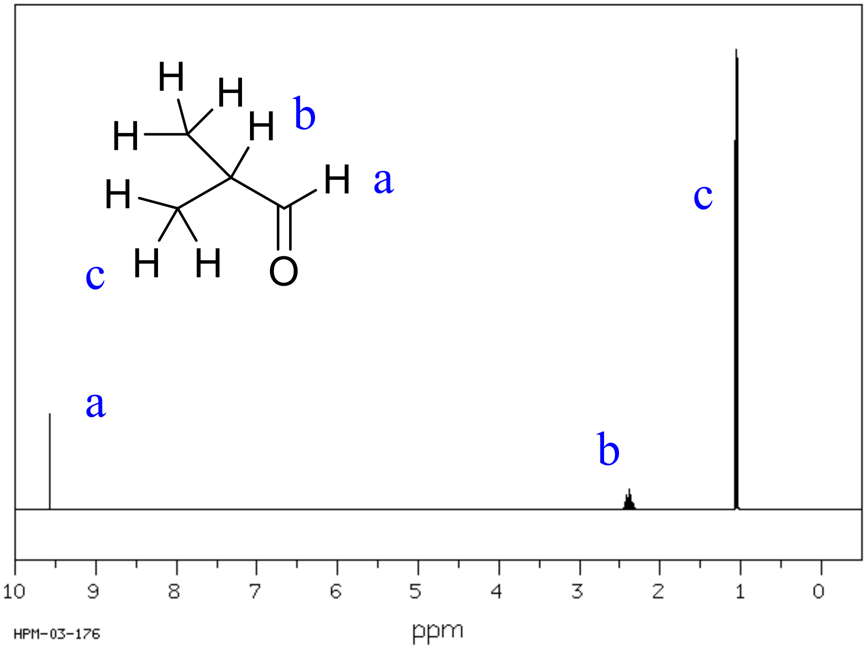
^{1}H-NMR: aldehyde hydrogens appear at 9.5-10.1.

^{1}H-NMR: a-hydrogens of aldehydes and ketones appear at 2.2-2.6.

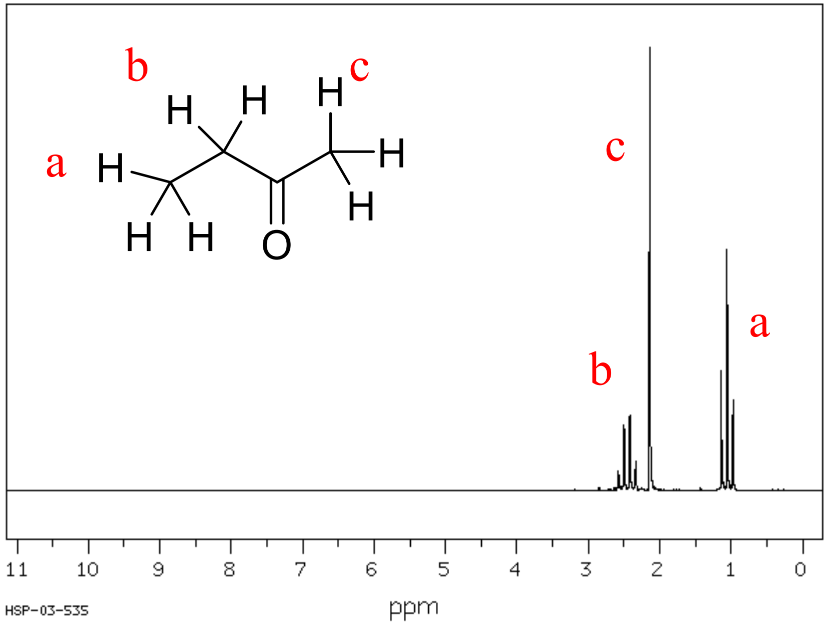
^{13}C-NMR: carbonyl carbons appear at 180-215.

Amines

^{1}H-NMR: amine hydrogens appear at 0.5-5.0 depending on conditions.



^{1}H NMR isobutyraldehyde



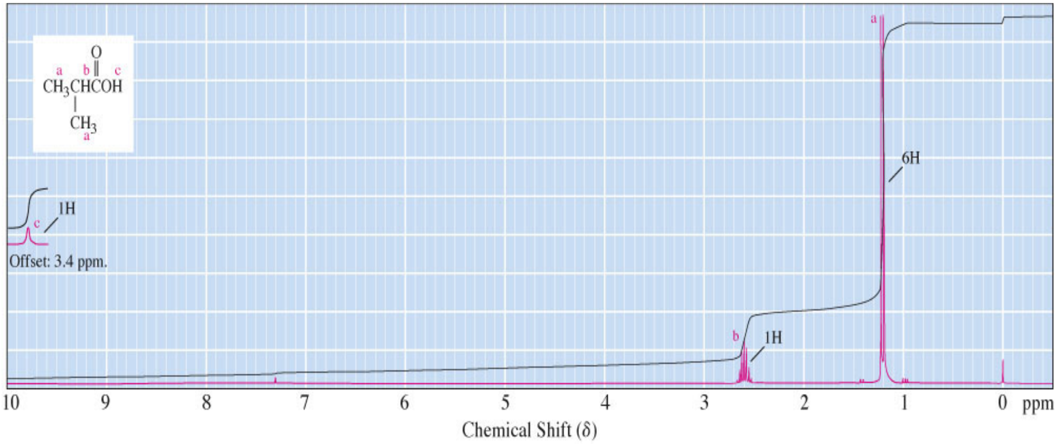
^{1}H NMR Methyl ethyl ketone

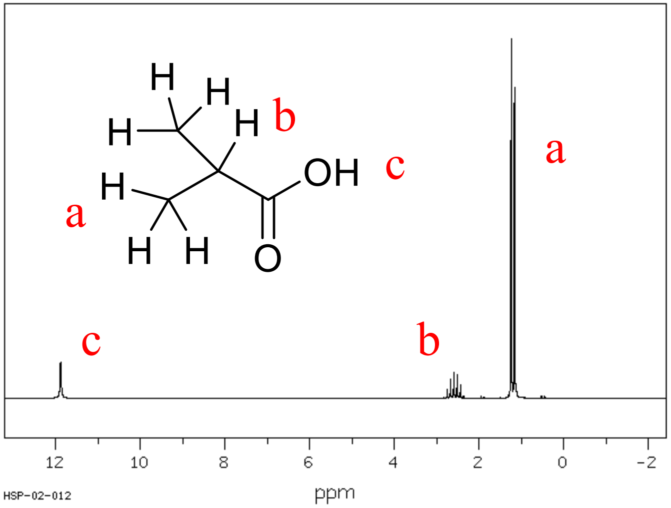
Interpreting NMR Spectra

Carboxylic acids

^{1}H-NMR: carboxyl hydrogens appear at 10-13 ppm, higher than most other types of hydrogens.

^{13}C-NMR: carboxyl carbons in acids and esters appear at 160-180 ppm.

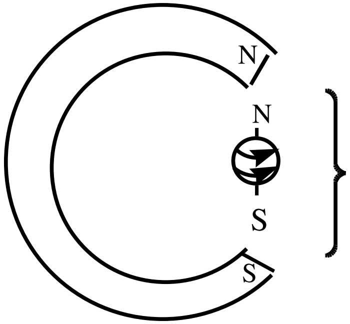




NMR = Nuclear Magnetic Resonance

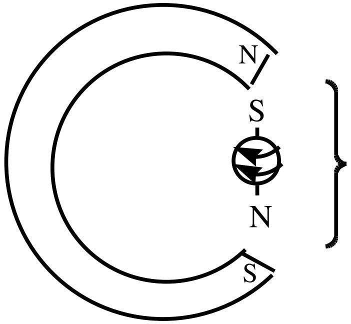
Physical Principles:

Some (but not all) nuclei, such as ^{1}H, ^{13}C, ^{19}F, ^{31}P have nuclear spin.
A spinning charge creates a magnetic moment, so these nuclei can be thought of as tiny magnets.
If we place these nuclei in a magnetic field, they can line up with or against the field by spinning clockwise or counter clockwise.



α- spin state,
favorable,
lower energy

A spinning nucleus with it's magnetic field aligned **with** the magnetic field of a magnet



β- spin state,
unfavorable,
higher energy

A spinning nucleus with it's magnetic field aligned **against** the magnetic field of a magnet

Alignment with the magnetic field (called α) is lower energy than against the magnetic field (called β). How much lower it is depends on the strength of the magnetic field

Note that for nuclei that don’t have spin, such as ^{12}C, there is no difference in energy between alignments in a magnetic field since they are not magnets. As such, we can’t do NMR spectroscopy on ^{12}C.

NMR: Basic Experimental Principles

Imagine placing a molecule, for example, CH_4, in a magnetic field.

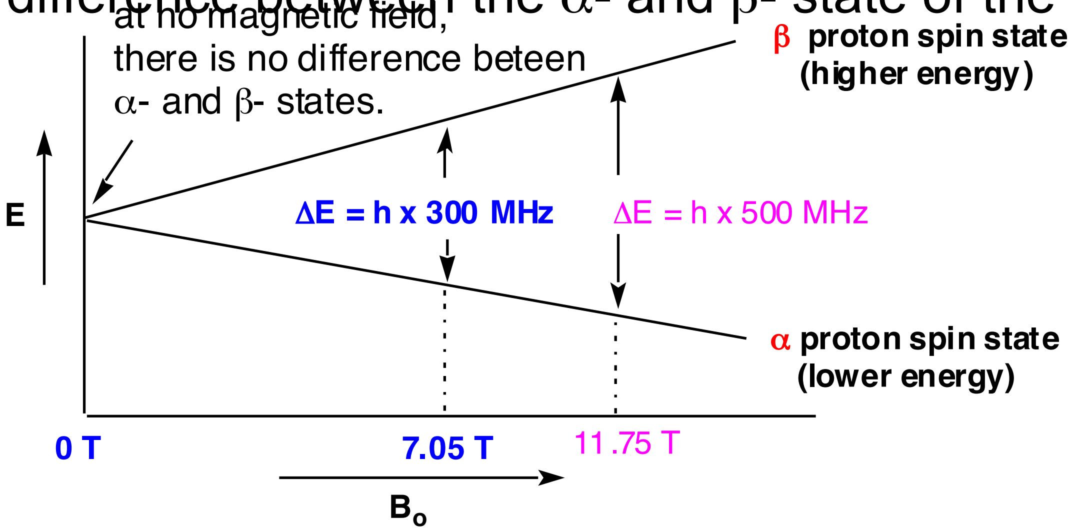
We can probe the energy difference of the α- and β- state of the protons by irradiating them with EM radiation of just the right energy.

In a magnet of 7.05 Tesla, it takes EM radiation of about 300 MHz (radio waves).

So, if we bombard the molecule with 300 MHz radio waves, the protons will absorb that energy and we can measure that absorbance.

In a magnet of 11.75 Tesla, it takes EM radiation of about 500 MHz (stronger magnet means greater energy difference between the α- and β- state of the protons)

Graphical relationship between magnetic field (B_o) and frequency (ν) for ^{1}H NMR absorptions



But there's a problem. If two researchers want to compare their data using magnets of different strengths, they have to adjust for that difference. That's a pain, so, data is instead reported using the "chemical shift" scale as described on the next slide.

The Chemical Shift (Also Called δ) Scale

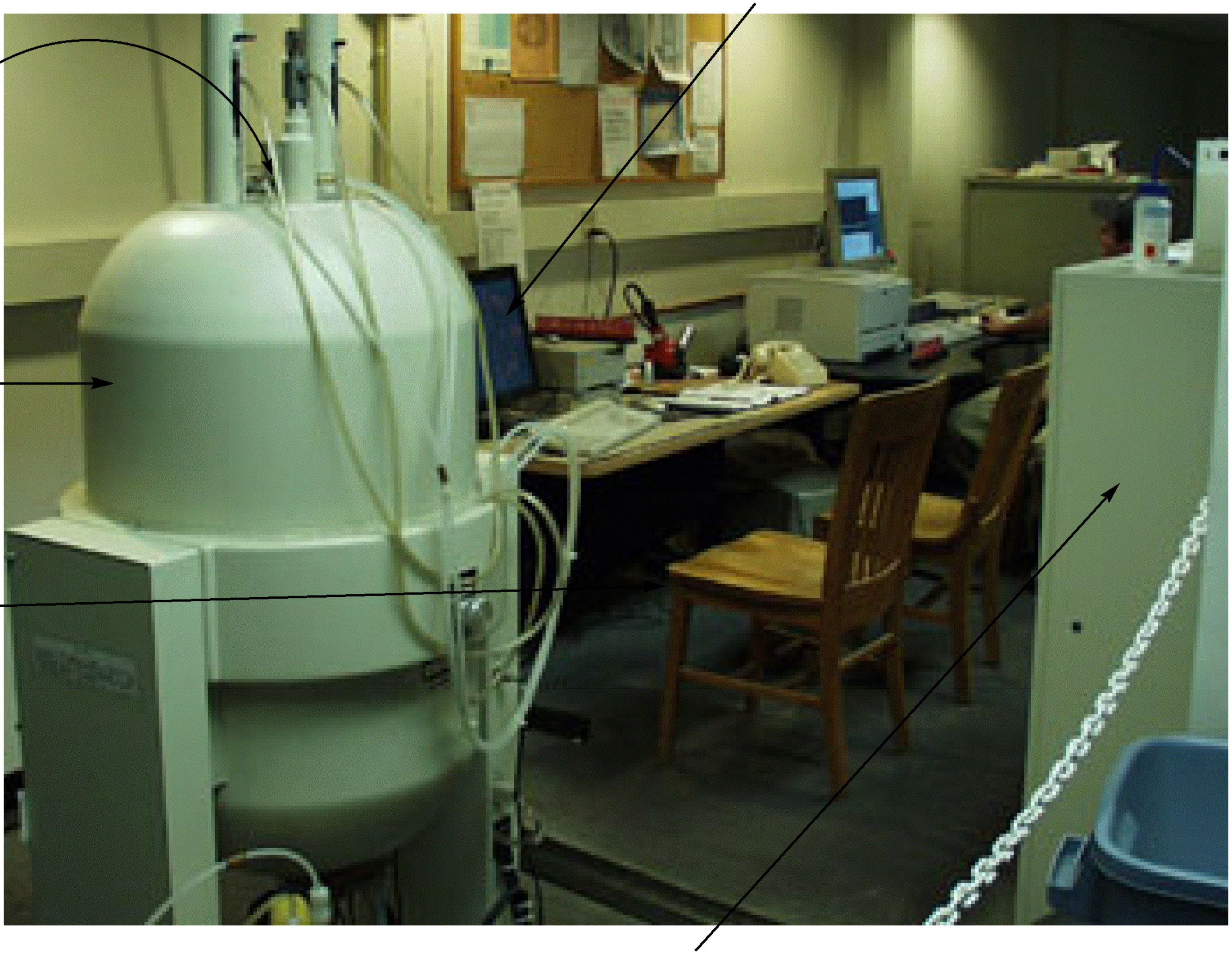
Here's how it works. We decide on a sample we'll use to standardize our instruments. We take an NMR of that standard and measure its absorbance frequency. We then measure the frequency of our sample and subtract its frequency from that of the standard. We then then divide by the frequency of the standard. This gives a number called the "chemical shift," also called δ, which does not depend on the magnetic field strength. Why not? Let's look at two examples.

Imagine that we have a magnet where our standard absorbs at 300,000,000 Hz (300 megahertz), and our sample absorbs at 300,000,300 Hz. The difference is 300 Hz, so we take 300/300,000,000 = 1/1,000,000 and call that 1 part per million (or 1 PPM). Now lets examine the same sample in a stronger magnetic field where the reference comes at 500,000,000 Hz, or 500 megahertz. The frequency of our sample will increase proportionally, and will come at 500,000,500 Hz. The difference is now 500 Hz, but we divide by 500,000,000 (500/500,000,000 = 1/1,000,000, = 1 PPM).
It's brilliant.

Of course, we don't do any of this, it's all done automatically by the NMR machine.
Even more brilliant.

NMR Spectrometer Used by the Undergraduate Organic Labs

Spectrum observed on computer monitor here.



Sample is lowered into the magnetic field here (there's a hole in the center of the magnet).

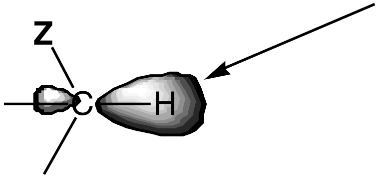
Big superconducting magnet, cooled in liquid helium. Source of the magnetic field (B_o).

Wooden chair, no magnetic metals allowed near the magnet!

Radio transmiter and receiver tuned to 300 MHz

The Chemical Shift of Different Protons

NMR would not be very valuable if all protons absorbed at the same frequency. You'd see a signal that indicates the presence of hydrogens in your sample, but any fool knows there's hydrogen in organic molecules. What makes it useful is that different protons usually appear at different chemical shifts (δ). So, we can distinguish one kind of proton from another. Why do different protons appear at different δ? There are several reasons, one of which is shielding. The electrons in a bond shield the nuclei from the magnetic field. So, if there is more electron density around a proton, it sees a slightly lower magnetic field, less electron density means it sees a higher magnetic field:



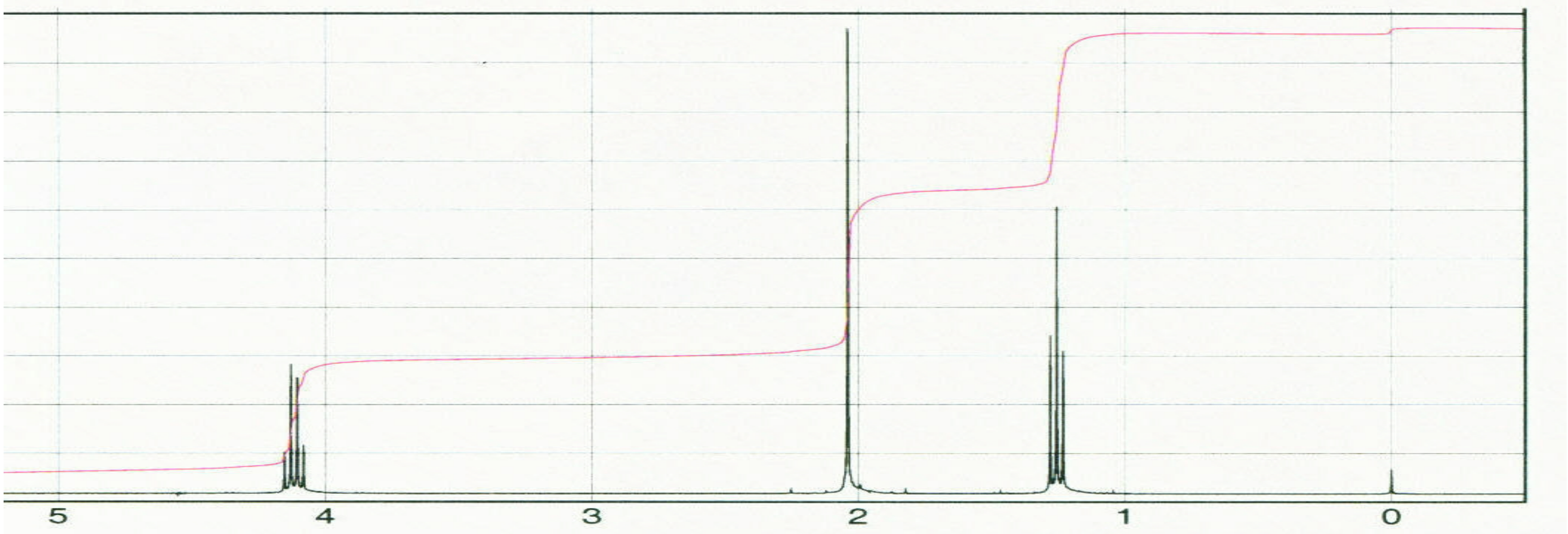
This represents the electron density of a C-H bond. How much electron density is on the proton depends on what else is attached to the carbon. If Z is an elelctronegative atom, the carbon becomes electron deficient and pulls some of the electron density away from the H. if Z is an electron donating group, more electron density ends up on the H.

How do the electrons shield the magnetic field? By moving. A moving charge creates a magnetic field, and the field created by the moving electrons opposes the magnetic field of our NMR machine. It's not a huge effect, but it's enough to enable us to distinguish between different protons in our sample.

The Hard Part - Interpreting Spectra

Learning how an NMR machine works is straightforward. What is less straightforward is learning how to use the data we get from an NMR machine (the spectrum of ethyl acetate is shown below). That's because each NMR spectrum is a puzzle, and there's no single fact that you simply have to memorize to solve these spectra. You have to consider lots of pieces of data and come up with a structure that fits all the data. What kinds of data do we get from NMR spectra? For ^{1}H NMR, there are three kinds each of which we will consider each of these separately:

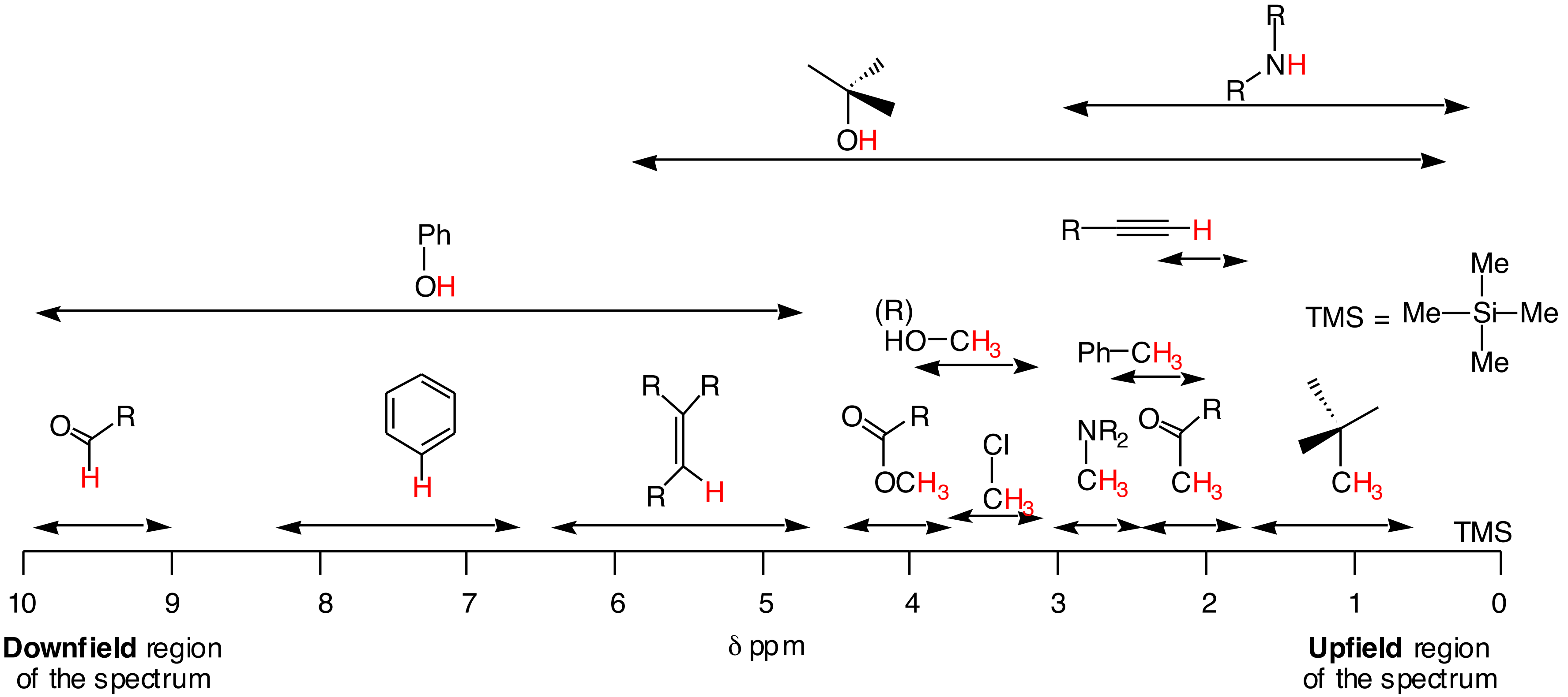
1) Chemical shift data - tells us what kinds of protons we have.
2) Integrals - tells us the ratio of each kind of proton in our sample.
3) ^{1}H - ^{1}H coupling - tells us about protons that are near other protons.



Chemical Shift Data

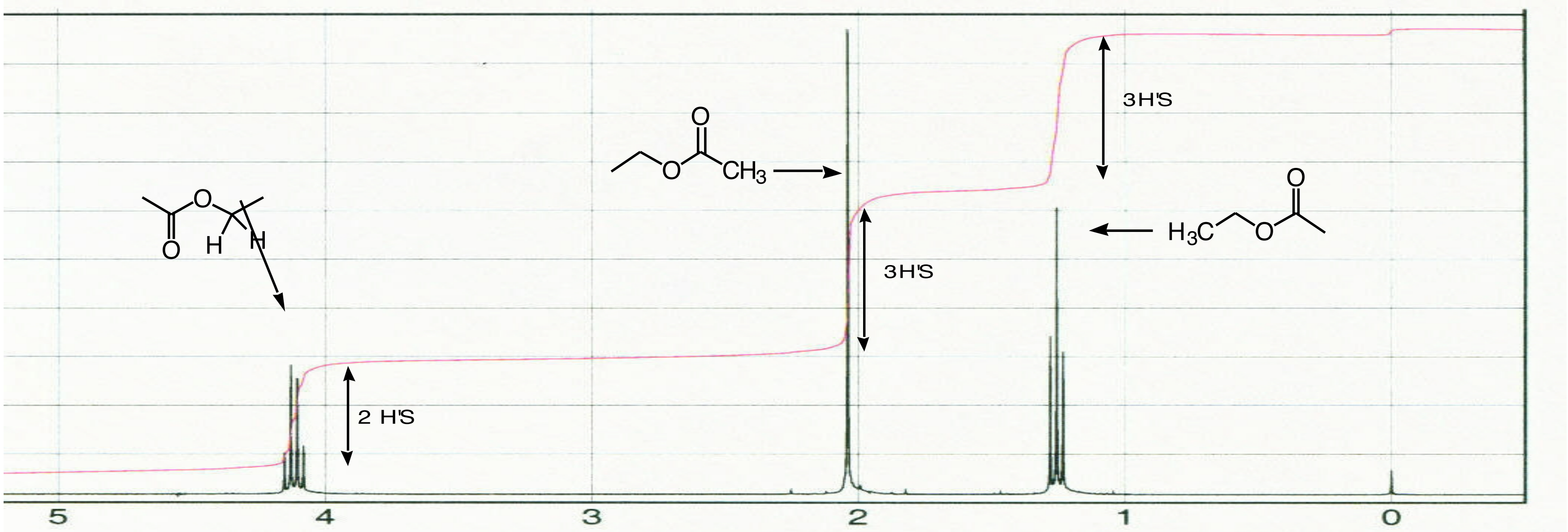
As previously mentioned, different kinds of protons typically come at different chemical shifts. Shown below is a chart of where some common kinds of protons appear in the δ scale. Note that most protons appear between 0 and 10 ppm. The reference, tetramethylsilane (TMS) appears at 0 ppm, and aldehydes appear near 10 ppm. There is a page in your lab handout with more precise values for this chart.

Note that these are typical values and that there are lots of exceptions!



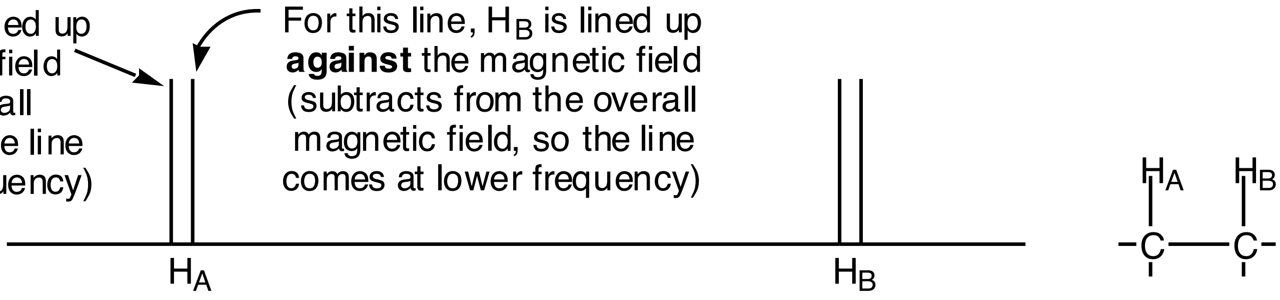
Integrals

Integrals tell us the ratio of each kind of proton. They are lines, the heights of which are proportional to the intensity of the signal. Consider ethyl acetate. There are three kinds of protons in this molecule, the CH_3 next to the carbonyl, the CH_2 next to the O and the CH_3 next to the CH_2. The ratio of the signals arising from each of these kinds of protons should be 3 to 2 to 3, respectively. So, if we look at the height of the integrals they should be 3 to 2 to 3. With this information, we can know which is the CH_2 signal (it’s the smallest one), but to distinguish the other two, we have to be able to predict their chemical shifts. The chart on the previous page allows us to make that assignment (the CH_3 next to the C=O should appear at ~ 2 PPM, while the other CH_3 should be at ~ 1 PPM).



^{1}H - ^{1}H Coupling

You'll notice in the spectra that we've seen that the signals don't appear as single lines, sometimes they appear as multiple lines. This is due to ^{1}H - ^{1}H coupling (also called spin-spin splitting or J-coupling). Here's how it works: Imagine we have a molecule which contains a proton (let's call it H_A) attached to a carbon, and that this carbon is attached to another carbon which also contains a proton (let's call it H_B). It turns out that H_A feels the presence of H_B. Recall that these protons are tiny little magnets, that can be oriented either with or against the magnetic field of the NMR machine. When the field created by H_B reinforces the magnetic field of the NMR machine (B_0) H_A feels a slightly stronger field, but when the field created by H_B opposes B_0, H_A feels a slightly weaker field. So, we see two signals for H_A depending on the alignment of H_B. The same is true for H_B, it can feel either a slightly stronger or weaker field due to H_A's presence. So, rather than see a single line for each of these protons, we see two lines for each.



For this line, H_B is lined up **with** the magnetic field (adds to the overall magnetic field, so the line comes at higher frequency)

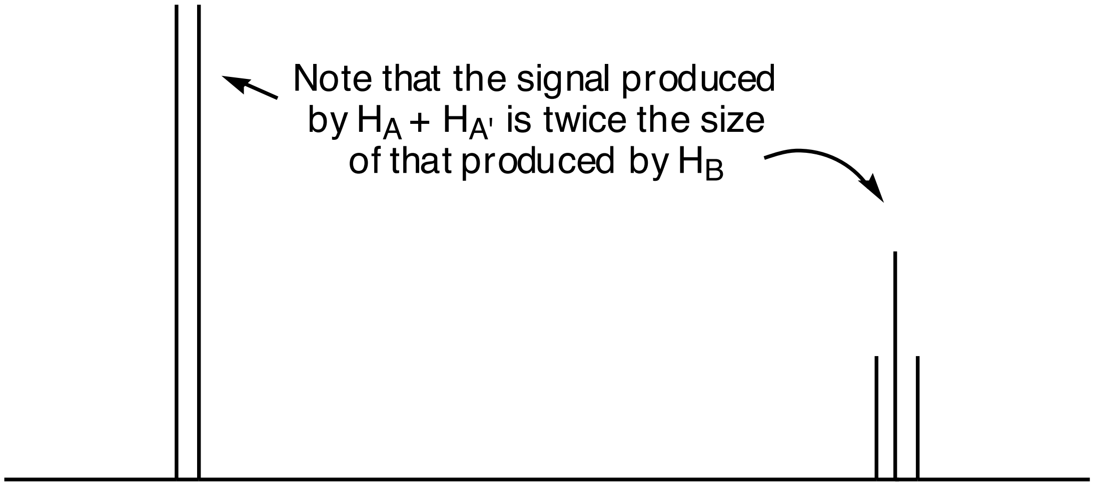
For this line, H_B is lined up **against** the magnetic field (subtracts from the overall magnetic field, so the line comes at lower frequency)

H_A is split into two lines because it feels the magnetic field of H_B.

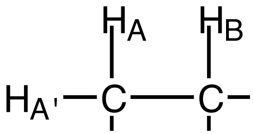
H_B is split into two lines because it feels the magnetic field of H_A.

More ^1H - ^1H Coupling

What happens when there is more than one proton splitting a neighboring proton? We get more lines. Consider the molecule below where we have two protons on one carbon and one proton on another.



Note that the signal produced by $H_A + H_{A'}$ is twice the size of that produced by H_B

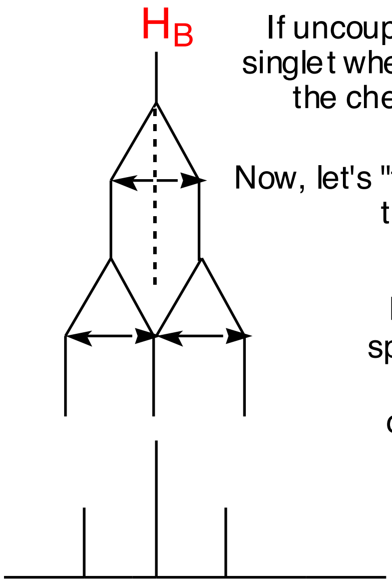


H_A and $H_{A'}$ appear at the same chemical shift because they are in identical environments
They are also split into two lines (called a doublet) because they feel the magnetic field of H_B.

H_B is split into three lines because it feels the magnetic field of H_A and $H_{A'}$

Why are There Three Lines for H_B?

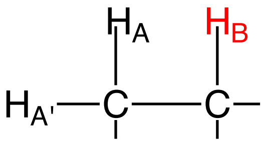
H_B feels the splitting of both H_A and $H_{A'}$. So, let’s imagine starting with H_B as a single line, then let’s “turn on” the coupling from H_A and $H_{A'}$ one at a time:



If uncoupled, H_B would appear as a singlet where the dashed line indicates the chemical shift of the singlet.

Now, let's "turn on" H_B - H_A coupling. This splits the single line into two lines

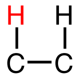
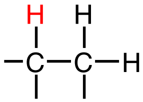
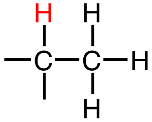
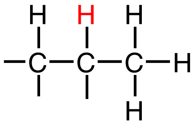
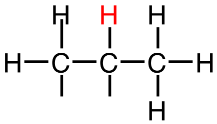
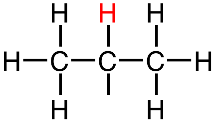
Now, let's "turn on" H_B - $H_{A'}$ coupling. This splits each of the two new lines into two lines, but notice how the two lines in the middle overlap. Overall, we then have three lines.



Because the two lines in the middle overlap, that line is twice as big as the lines on the outside. More neighboring protons leads to more lines as shown on the next slide.

Splitting Patterns with Multiple Neighboring Protons

If a proton has n neighboring protons *that are equivalent,* that proton will be split into n+1 lines. So, if we have four equivalent neighbors, we will have five lines, six equivalent neighbors… well, you can do the math. The lines will not be of equal intensity, rather their intensity will be given by Pascal's triangle as shown below.

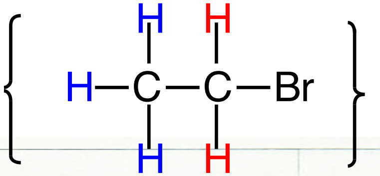
no. of neighbors	relative intensities	pattern	example
0	1	singlet (s)	
1	1 1	doublet (d)	
2	1 2 1	triplet (t)	
3	1 3 3 1	quartet (q)	
4	1 4 6 4 1	pentet	
5	1 5 10 10 5 1	sextet	
6	1 6 15 20 15 6 1	septet	

We keep emphasizing that this pattern only holds for when the neighboring protons are equivalent. Why is that? The answer is two slides away.

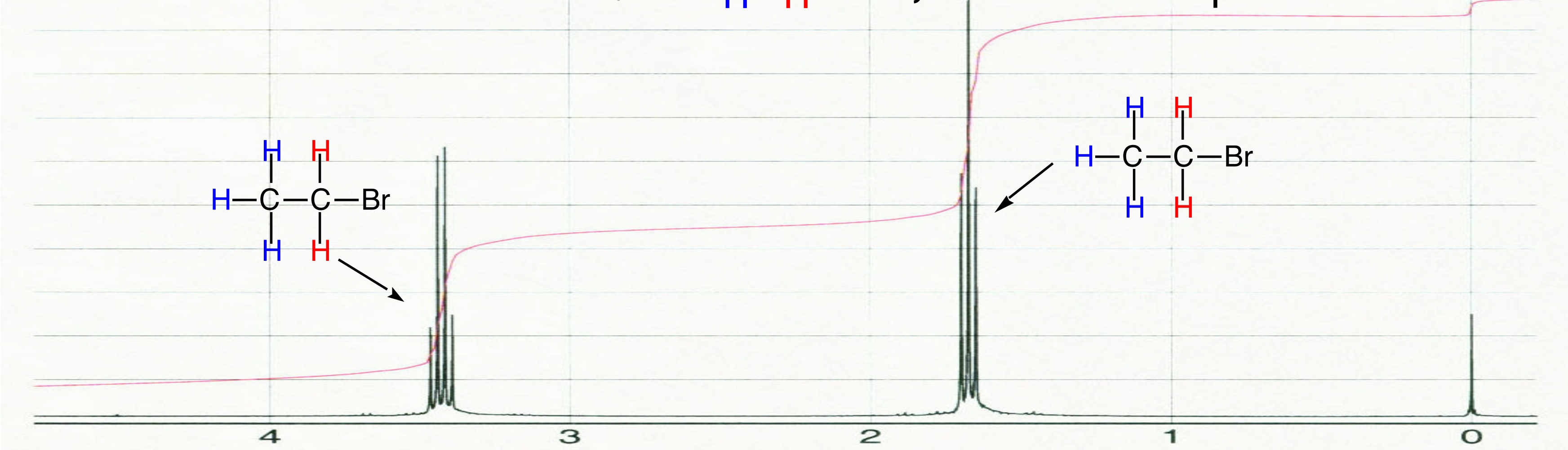
More About Coupling

Earlier we said that protons couple to each other because they feel the magnetic field of the neighboring protons. While this is true, the mechanism by which they feel this field is complicated and is beyond the scope of this class (they don’t just feel it through space, it’s transmitted through the electrons in the bonds). It turns out that when two protons appear at the same chemical shift, they do not split each other. So, in EtBr, we have a CH_3 next to a CH_2, and each proton of the CH_3 group is *only* coupled to the protons of the CH_2 group, not the other CH_3 protons because all the CH_3 protons come at the same chemical shift.

The blue protons all come at the same chemical shift and do not split each other

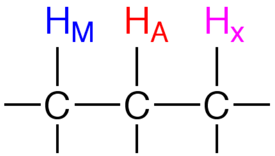


The red protons both come at the same chemical shift and do not split each other

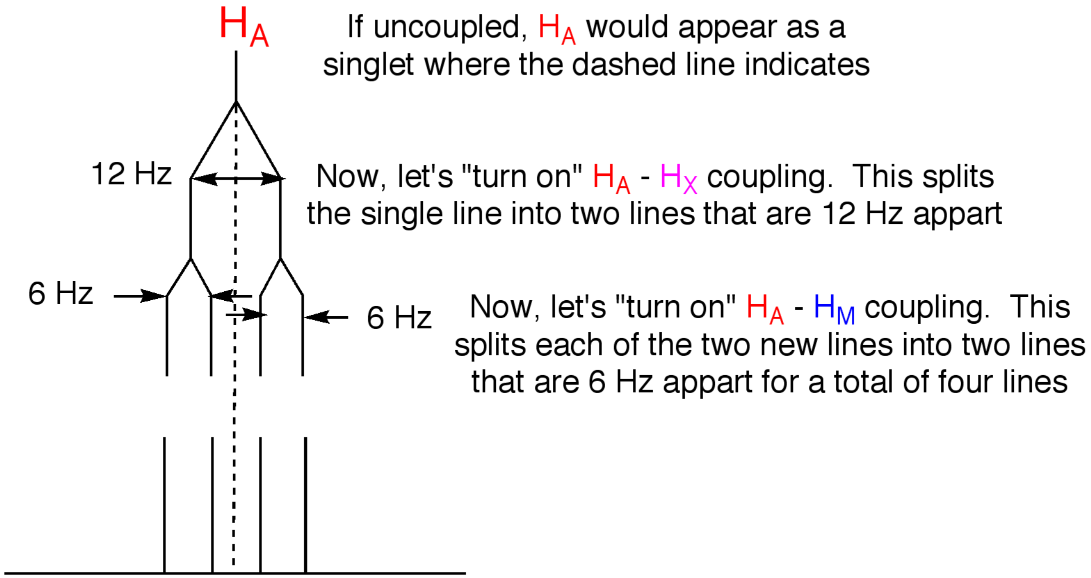


Not all Couplings are Equal

When protons couple to each other, they do so with a certain intensity. This is called the “coupling constant.” Coupling constants can vary from 0 Hz (which means that the protons are not coupled, even though they are neighbors) to 16 Hz. Typically, they are around 7 Hz, but many molecules contain coupling constants that vary significantly from that. So, what happens when a molecule contains a proton which is coupled to two different protons with different coupling constants? We get a different pattern as described in the diagram below.



Coupling constant H_A- H_X = 12
Coupling constant H_A- H_M = 6



If uncoupled, H_A would appear as a singlet where the dashed line indicates

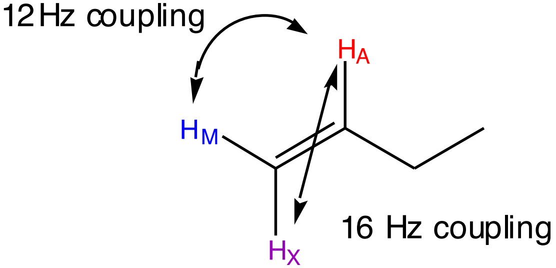
Now, let's "turn on" H_A - H_X coupling. This splits the single line into two lines that are 12 Hz appart

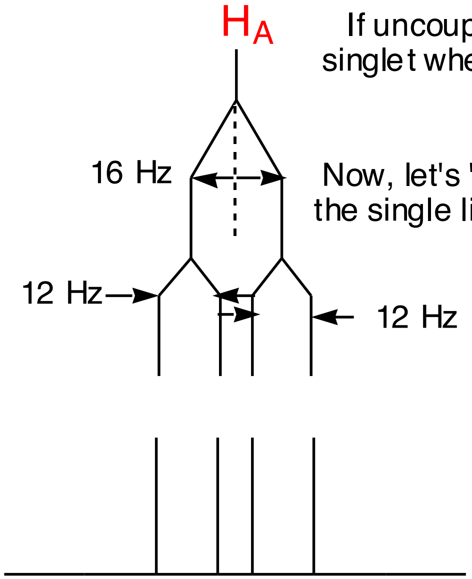
Now, let's "turn on" H_A - H_M coupling. This splits each of the two new lines into two lines that are 6 Hz appart for a total of four lines

So, if the protons are not equivalent, they can have different coupling constants and the resulting pattern will not be a triplet, but a “doublet of doublets.” Sometimes, nonequivalent protons can be on the same carbon as described on the next slide.

Coupling Constants in Alkenes

Coupling constants in alkenes can also differ depending on whether the protons are cis or trans to each other. Note that in a terminal alkene (i.e., an alkene at the end of a carbon chain), the cis and trans protons are NOT equivalent. One is on the same side as the substituent, the other is on the opposite side. The coupling of *trans* protons to each other is typically very large, around 16 Hz, while the coupling of *cis* protons, while still large, is a little smaller, around 12 Hz. This leads to the pattern shown below, and an example of a molecule with this splitting pattern is shown on the next slide.





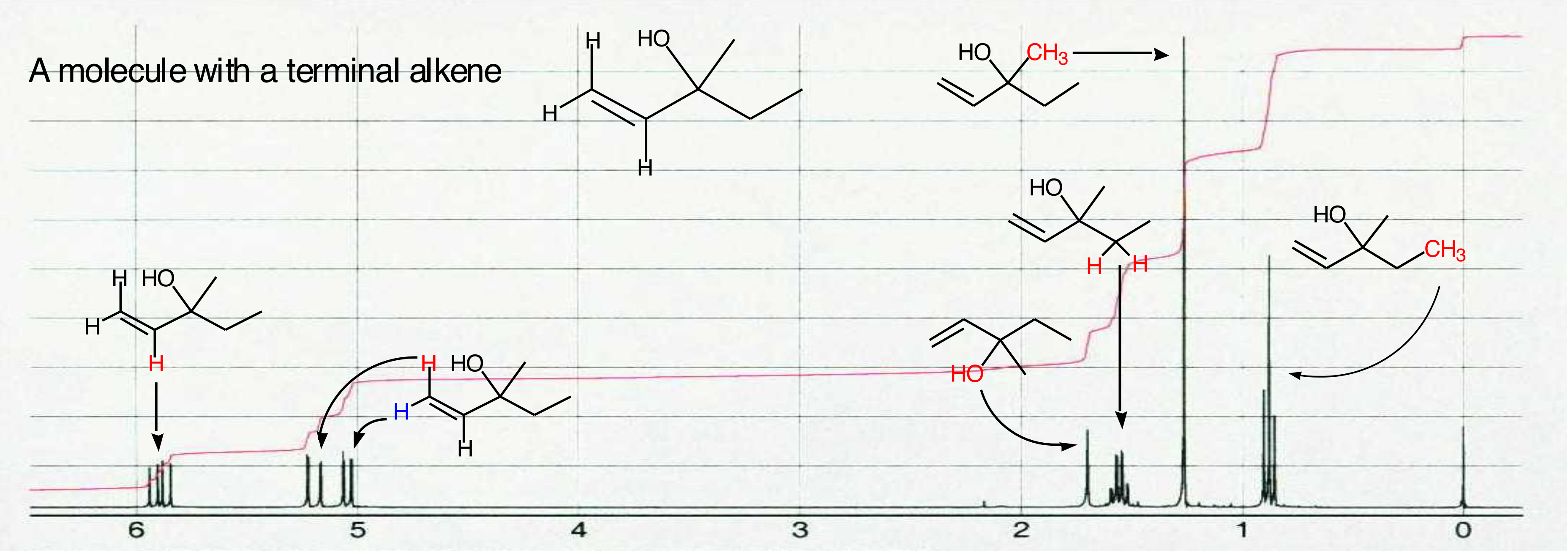
If uncoupled, H_A would appear as a singlet where the dashed line indicates

Now, let's "turn on" H_A - H_X coupling. This splits the single line into two lines that are 16 Hz appart

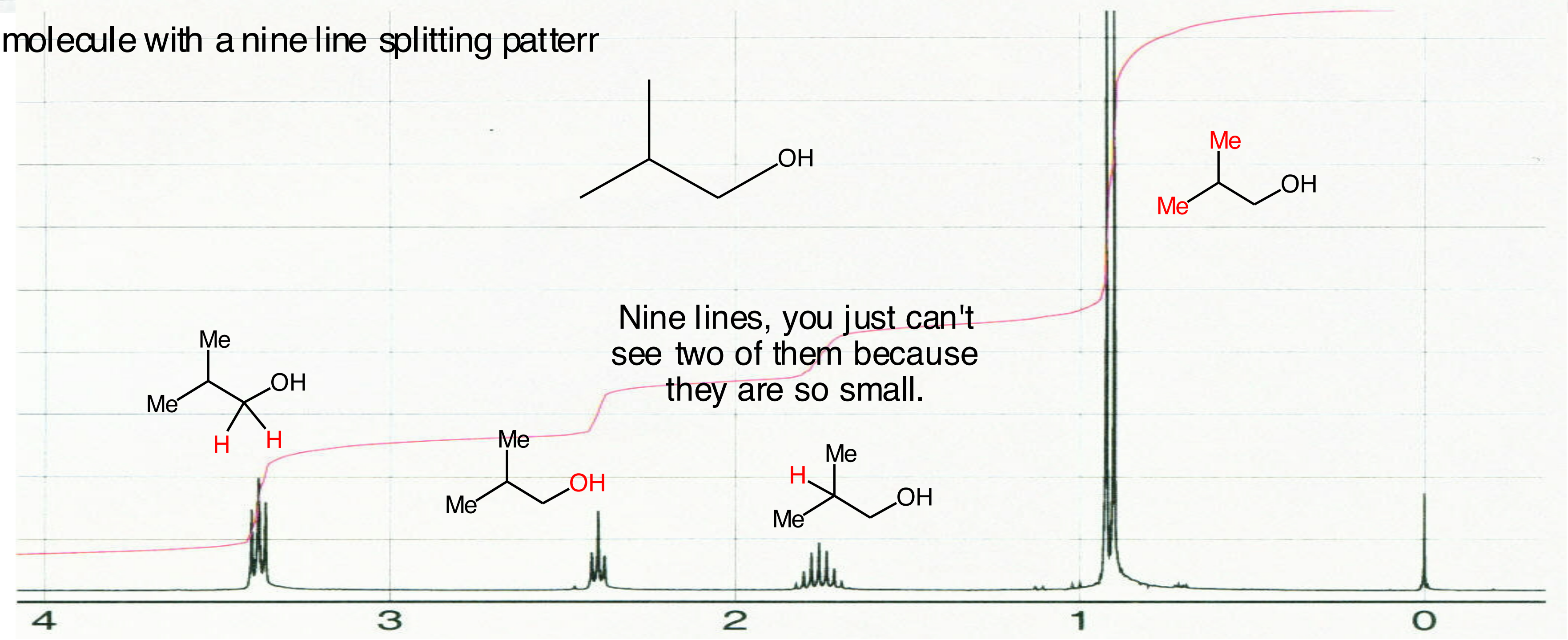
Now, let's "turn on" H_A - H_M coupling. This splits each of the two new lines into two lines that are 12 Hz appart for a total of four lines

There are other times when protons on the same carbon are nonequivalent, which we'll see later.

A molecule with a terminal alkene



A molecule with a nine line splitting patterr



Nine lines, you just can't see two of them because they are so small.

3. Nuclear Magnetic Resonance

- NMR results from resonant absorption of electromagnetic energy by a nucleus (mostly protons) changing its spin orientation

- The resonance frequency depends on the chemical environment of the nucleus giving a specific finger print of particular groups (NMR *spectroscopy)*

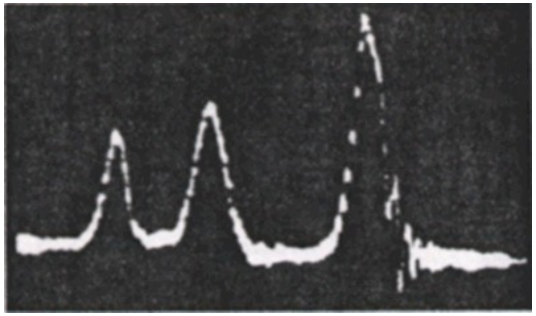
- NMR is nondestructive and contact free

- Modern variants of NMR provide 3D structural resolution of (not too large) proteins in solution

- NMR tomography (Magnetic resonance imaging, MRI) is the most advanced and powerful imaging tool

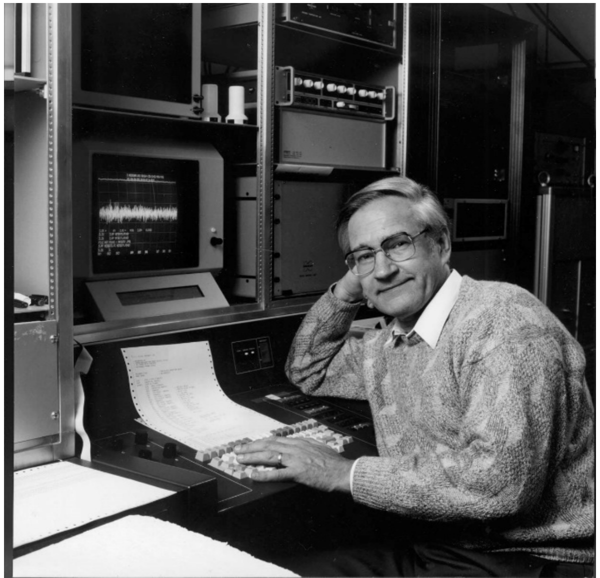
Some history of NMR

1946 Principle of solid state NMR
(Bloch, Purcell)



1950 Resonance frequency depends on chemical environment (Proctor, Yu)

1953 Overhauser effect



1956 First NMR spectra of protein
(Ribonuclease)

1965 Fourier Transform spectroscopy (Ernst)

1973 Imaging tomography (Mansfield)

1985 First protein structure (bovine pancreatic trypsin inhibitor) in solution (Wüthrich)

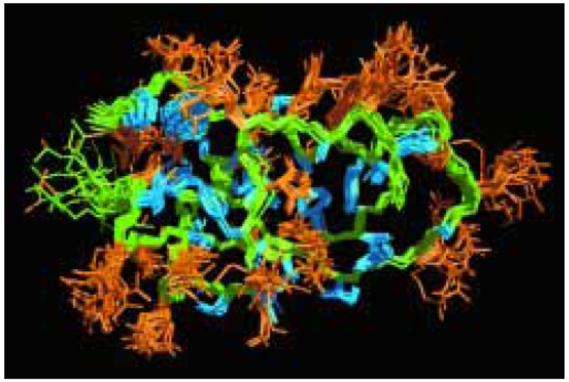


Figure 27. NMR structure of BPTI represented by a bundle of 20 conformers superimposed for best fit of the polypeptide backbone. The polypeptide backbone is green, core side-chains are blue, and solvent-accessible surface side-chains are red.

By now: More than 150 protein structures (M < 60 000)

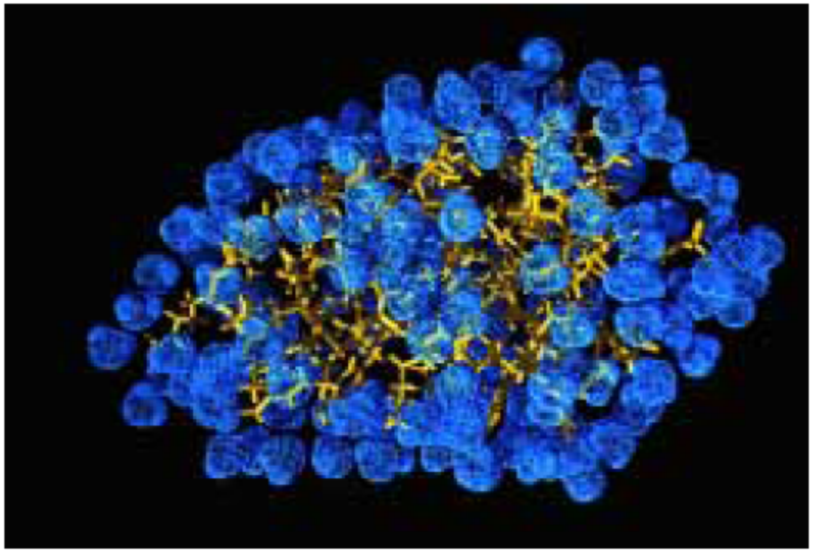
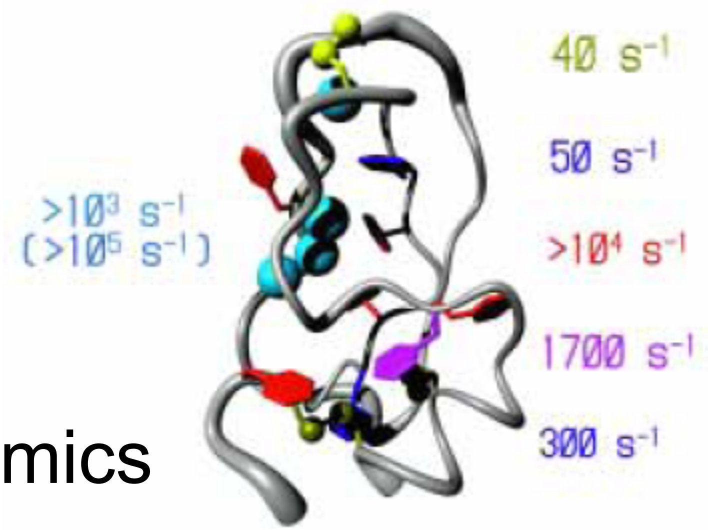


Figure 28. Molecular model of hydrated BPTI in H_2O solution. The drawing shows an all-heavy-atom-presentation of one of the conformers in Figure 27 (yellow) covered with a layer of hydration water molecules (dotted blue spheres).

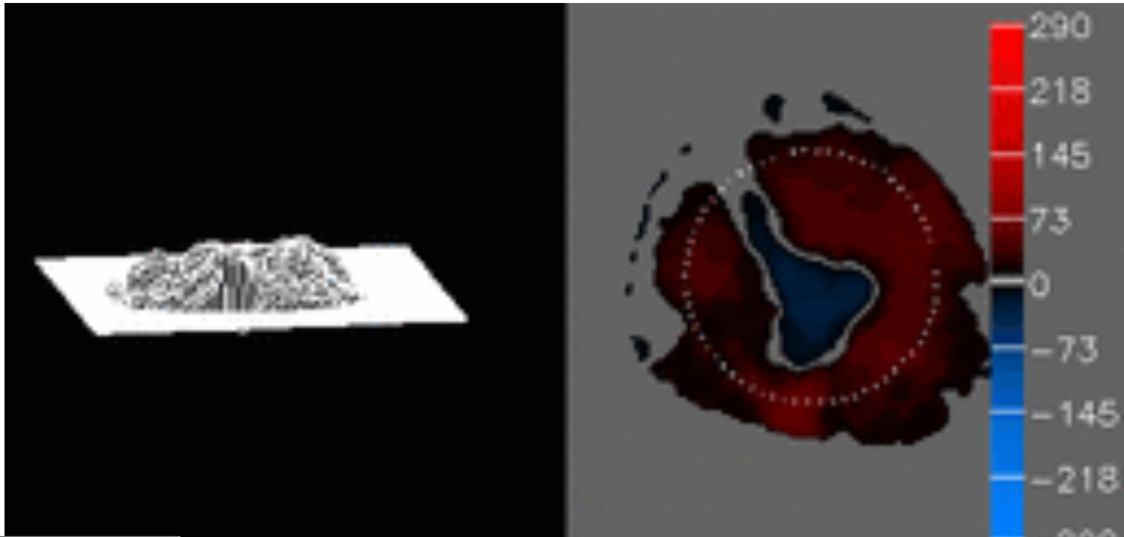
BPTI

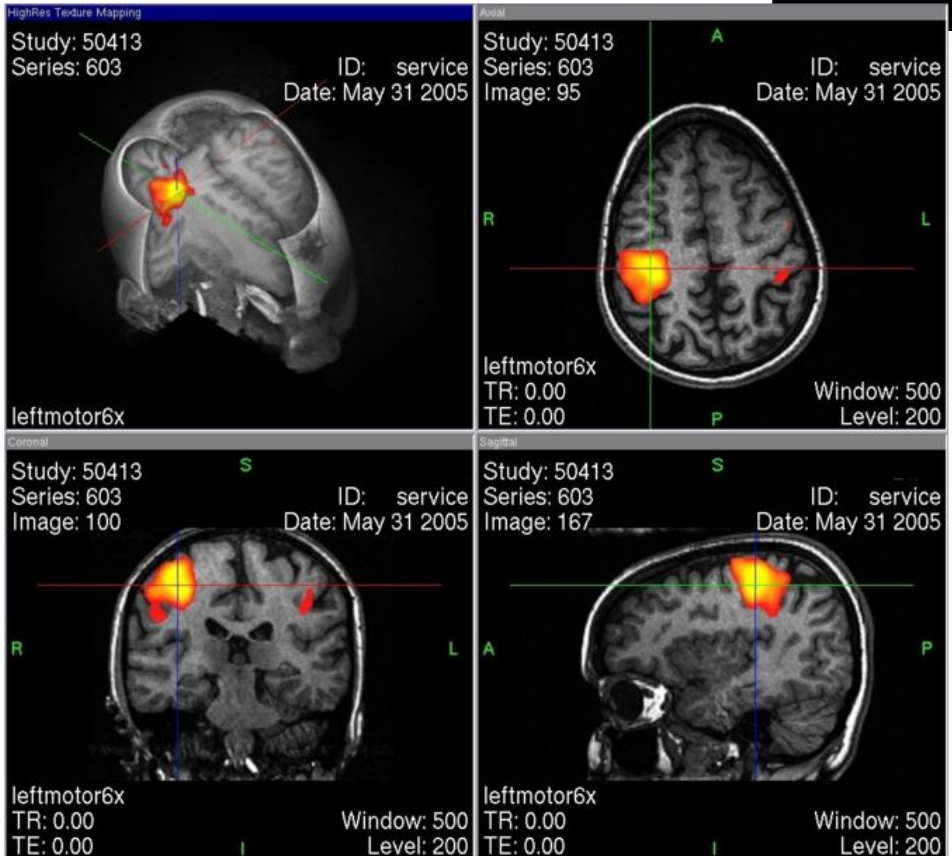
Bound water



Protein dynamics

Functional MRI





3.1 Principle of Nuclear Magnetic Resonance

Many (but not all) nuclei have a spin (**I**). Quantum mechanically **I** can have *2I+1* orientations in an external magnetic field **B**.

$$\boxed{|\mathbf{I}| = \hbar\sqrt{I(I+1)}} \qquad \boxed{I_z = m_I \hbar}$$

$$\boxed{-I \le m_I \le I} \qquad \boxed{\Delta m_I = 1}$$

This spin is associated with a magnetic moment

$$\boxed{\begin{gathered} \mu = \gamma\hbar\sqrt{I(I+1)} \\ \mu_z = \gamma\hbar m_I \end{gathered}} \qquad \gamma = g_I \mu_k / \hbar$$

g_I: nuclear g-factor

$$\mu_k \approx \frac{e\hbar}{2m_p} \qquad \mu_k = 5.05082\ 10^{-27} J/T$$

$$\mu_B \approx \frac{e\hbar}{2m_e} \qquad \mu_B = 9.274015\ 10^{-24} J/T$$

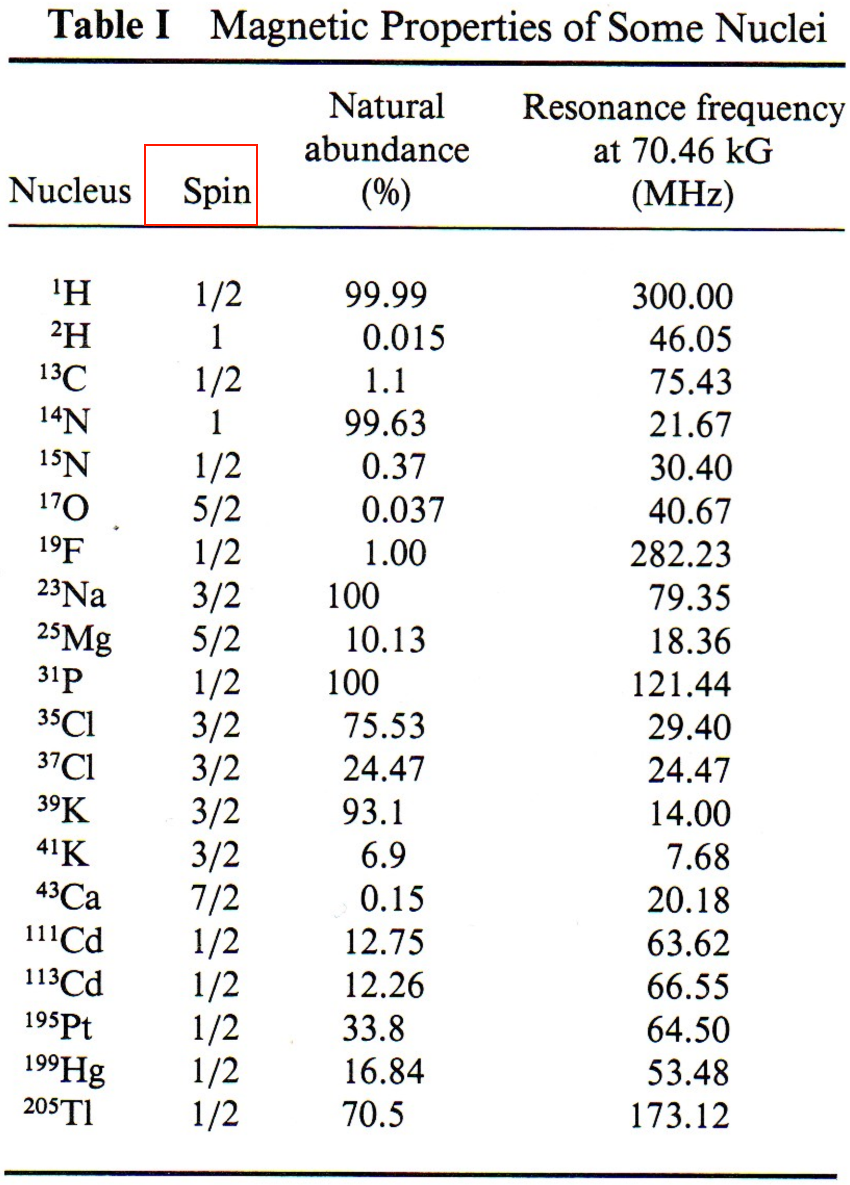
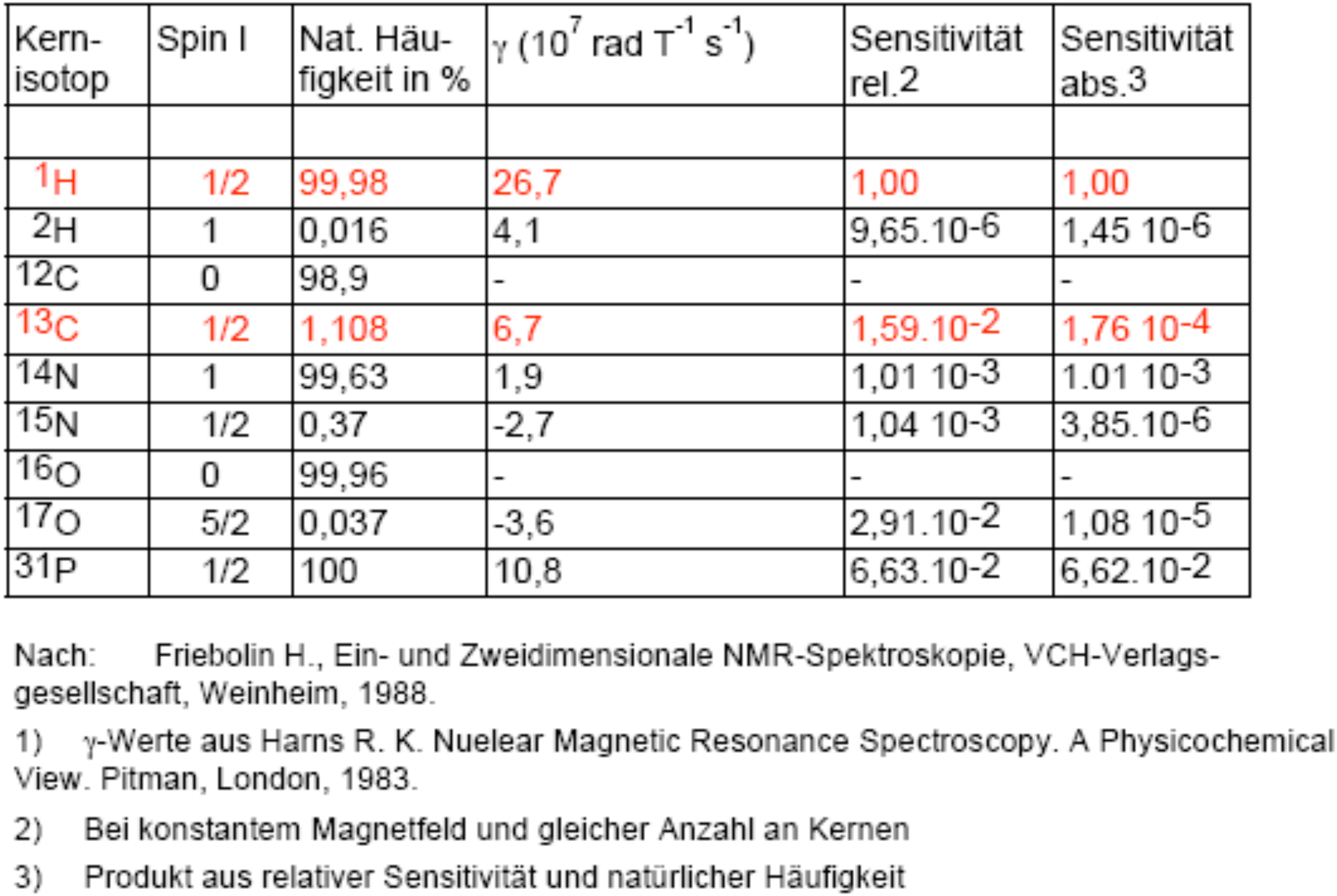


Table I Magnetic Properties of Some Nuclei

Nucleus	Spin	Natural abundance (%)	Resonance frequency at 70.46 kG (MHz)
^{1}H	1/2	99.99	300.00
^{2}H	1	0.015	46.05
^{13}C	1/2	1.1	75.43
^{14}N	1	99.63	21.67
^{15}N	1/2	0.37	30.40
^{17}O	5/2	0.037	40.67
^{19}F	1/2	1.00	282.23
^{23}Na	3/2	100	79.35
^{25}Mg	5/2	10.13	18.36
^{31}P	1/2	100	121.44
^{35}Cl	3/2	75.53	29.40
^{37}Cl	3/2	24.47	24.47
^{39}K	3/2	93.1	14.00
^{41}K	3/2	6.9	7.68
^{43}Ca	7/2	0.15	20.18
^{111}Cd	1/2	12.75	63.62
^{113}Cd	1/2	12.26	66.55
^{195}Pt	1/2	33.8	64.50
^{199}Hg	1/2	16.84	53.48
^{205}Tl	1/2	70.5	173.12

Since biomatter is made of H,C,N and O, these are the most relevant nuclei for biological NMR



Kern-isotop	Spin I	Nat. Häu-figkeit in %	γ (10^{7} rad T^{-1} s^{-1})	Sensitivität rel.[2]	Sensitivität abs.[3]
^{1}H	1/2	99,98	26,7	1,00	1,00
^{2}H	1	0,016	4,1	$9,65.10^{-6}$	$1,45\ 10^{-6}$
^{12}C	0	98,9	-	-	-
^{13}C	1/2	1,108	6,7	$1,59.10^{-2}$	$1,76\ 10^{-4}$
^{14}N	1	99,63	1,9	$1,01\ 10^{-3}$	$1.01\ 10^{-3}$
^{15}N	1/2	0,37	-2,7	$1,04\ 10^{-3}$	$3,85.10^{-6}$
^{16}O	0	99,96	-	-	-
^{17}O	5/2	0,037	-3,6	$2,91.10^{-2}$	$1,08\ 10^{-5}$
^{31}P	1/2	100	10,8	$6,63.10^{-2}$	$6,62.10^{-2}$

Nach: Friebolin H., Ein- und Zweidimensionale NMR-Spektroskopie, VCH-Verlagsgesellschaft, Weinheim, 1988.

1) γ-Werte aus Harns R. K. Nuclear Magnetic Resonance Spectroscopy. A Physicochemical View. Pitman, London, 1983.

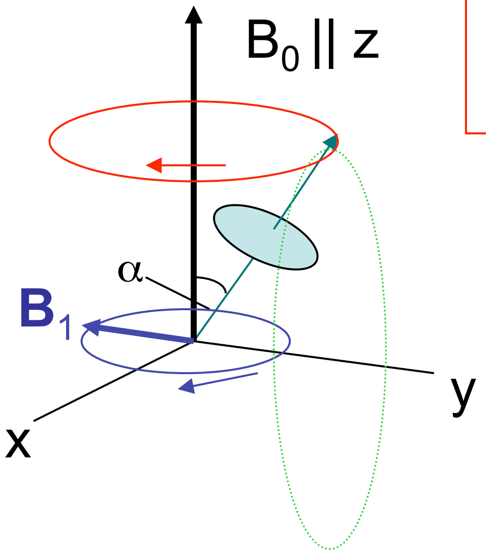
2) Bei konstantem Magnetfeld und gleicher Anzahl an Kernen

3) Produkt aus relativer Sensitivität und natürlicher Häufigkeit

Mechanical (classical) model

Spinning top with magnetic moment μ_L and angular momentum **I** precesses with frequency ω_L under torque **D**

$$\omega_L = \frac{|\mathbf{D}|}{|\mathbf{I}| \cdot sin\alpha}$$



$$\boxed{\omega_L = \gamma B_0}$$

Larmor precession of μ_L around B_0

Larmor precession around B_1

Torque on magnetic moment μ_L in B_0

$$\mathbf{D} = \mu \times \mathbf{B_0} \longrightarrow \omega_L = \frac{\mu B_o sin\alpha}{I\ sin\alpha} = \frac{\mu}{I} B_0 = \gamma B_0$$

The precession frequency is independent of α and equals the Larmor frequency

Application of a horizontal magnetic field $\mathbf{B}_1$ which rotates at ω_L:

$$B_{1x} = B_{10}\ sin(\omega_L t)$$

$$B_{1y} = B_{10}\ cos(\omega_L t)$$

In the frame rotating with μ_L the orientation of $\mathbf{B}_1$ relative to μ_L is constant

Additional precession of μ_L around $\mathbf{B}_1$ at frequency $2\pi\dot{\alpha} = \omega_1 = \gamma B_{10}$

Quantum mechanical description

The magnetic moment orients in a magnetic field B_0. Different orientations correspond to different energies

$$\Delta E = g_I \mu_k B_0 = \hbar \omega_L$$

$I = 1/2$ ^{1}H, ^{13}C, ^{31}P

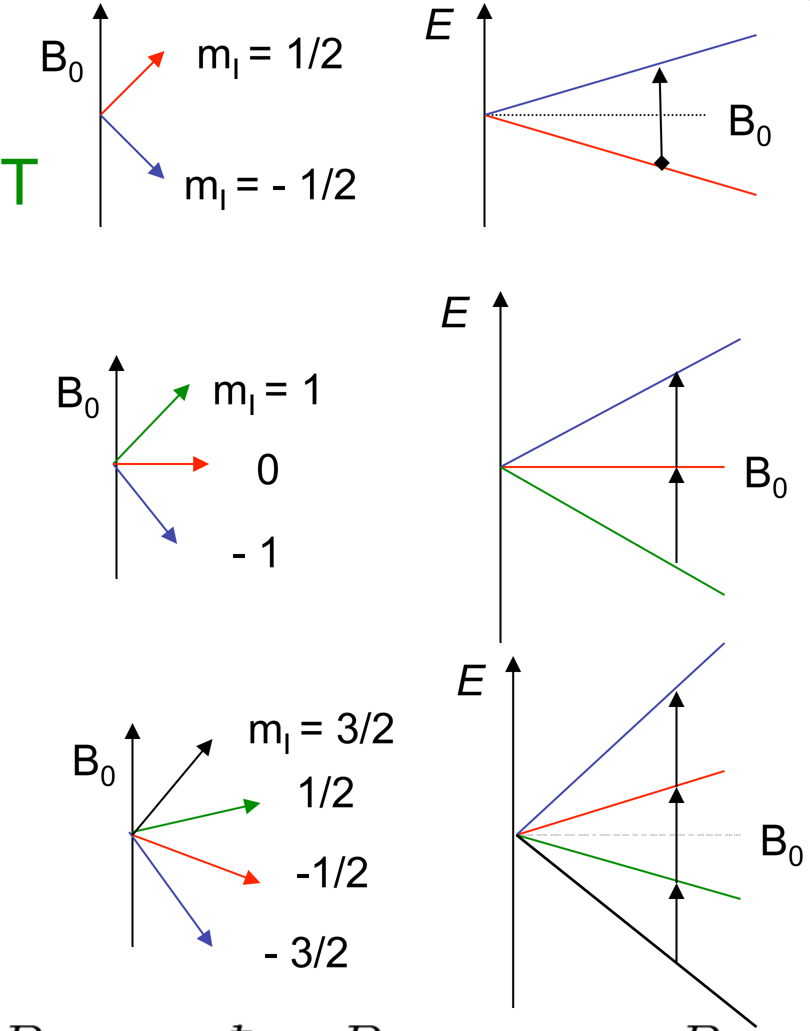
$g_I = 5.58$

$\gamma = 42.576$ MHz/T

$$\boxed{\omega_L = \gamma B_0}$$

$I = 1$ ^{2}H, ^{14}N,

$I = 3/2$ ^{23}Na,



When photons with frequency ω_L are absorbed a transition from the lower to the upper level occurs. Selection rule $\Delta m_I = 1$

$$E = -\mu \cdot \mathbf{B_0} = -\mu_z B_0 = -\gamma \hbar m_I B_0 = g_I \mu_k m_I B_0$$

Bulk magnetization

A sample contains many nuclei (typically N ~ 10^{17} or higher). In zero field all spin orientations are equivalent. The bulk magnetization (I.e. is the sum of all m's) is very small and fluctuates around M=0.

At finite fields B_0 (and finite temperature) the occupation of states at different energies E obeys Boltzmann statistics $\exp(-E/k_BT)$ – thermal equilibrium is assumed. For I=1/2 the spin state "parallel" to B_0 has lower energy E_1 than the " antiparallel" state with energy E_2.

Therefore there is a net magnetization along the z-axis. However since $\Delta E = E_2 - E_1$ is much smaller than k_BT the magnetization is far from saturation.

The number of spins in state 1,2 is

$$N_{1,2} = N e^{-E_{1,2}/k_B T}$$

Thus the population imbalance is

$$N_2 - N_1 \approx N \frac{(E_1 - E_2)}{k_B T}$$

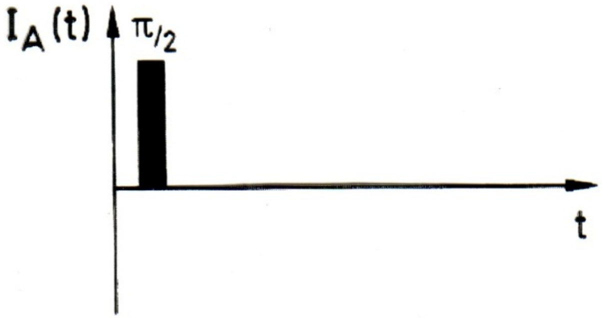
Which yields a bulk magnetization

$$M_z \approx N\mu \cdot \frac{\mu B_0}{3 k_B T} \qquad \text{with} \qquad \mu = \gamma\hbar\sqrt{I(I+1)}$$

The average magnetization in x,y vanishes because the precessions of individual spins are uncorrelated.

The application of a pulse of duration t changes the average angle of the magnetization by a certain angle (c.f. the mechanical model or a change in population densities), given by:

$$t(\vartheta) = \frac{\vartheta}{\gamma B_1}$$



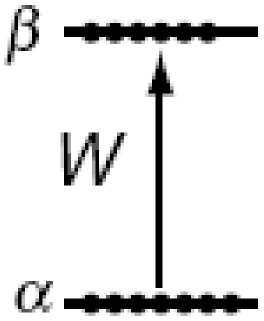
Thus a pulse of duration $\tau = 2\pi/4\ \omega_1$ gives a change in angle of $\pi/2$ – pulse I.e. the magnetization is flipped into the xy plane. M_x and M_y now oscillate with ω_L.

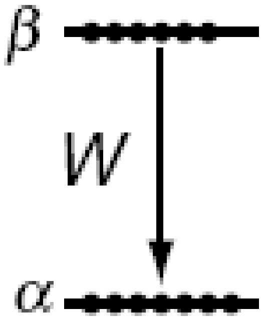
If *M* is flipped out of equilibrium (out of the z-direction) by a B_1- pulse, it will relax back to M_z into thermal equilibrium. This occurs because of magnetic interaction of μ with the environment (atoms, eventually in crystalline lattice) and is characterized by the so–called longitudinal (or spin-lattice) relaxation time T_1.

This relaxation is described by a set of rate equations for the transitions between the states

$$\frac{dn_\alpha}{dt} = W(n_\beta - n_\beta^0) - W(n_\alpha - n_\alpha^0)$$

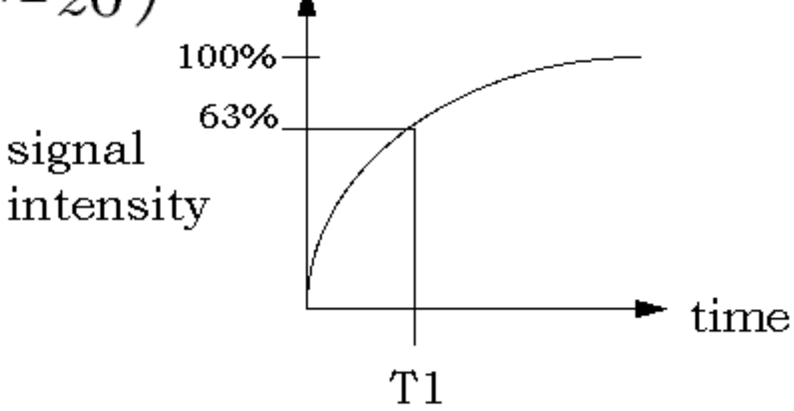
$$\frac{dn_\beta}{dt} = W(n_\alpha - n_\alpha^0) - W(n_\beta - n_\beta^0)$$





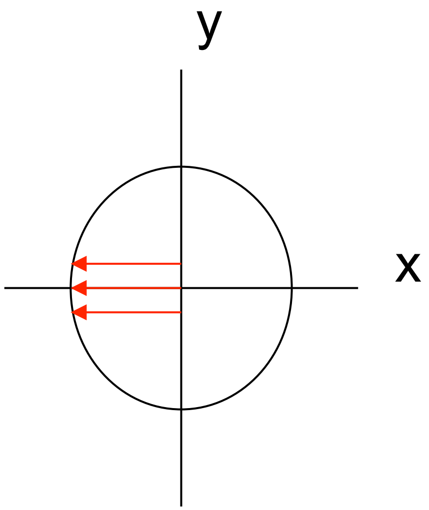
Which yields a simple exponential relaxation of the magnetization in the z-direction

$$dM_z/dt = -T_1^{-1}(M_z - M_{z0})$$

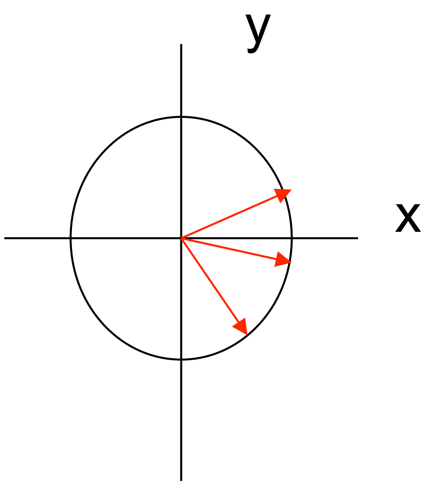


The amplitudes of M_x and M_y decay with another relaxation time T_2 called spin-spin relaxation time. This relaxation originates from inhomogeneity of B_0 . It is described by another phenomenological equation

$$dM_i/dt = -T_2^{-1} M_i \qquad i = x, y$$



Immediately after π/2 pulse



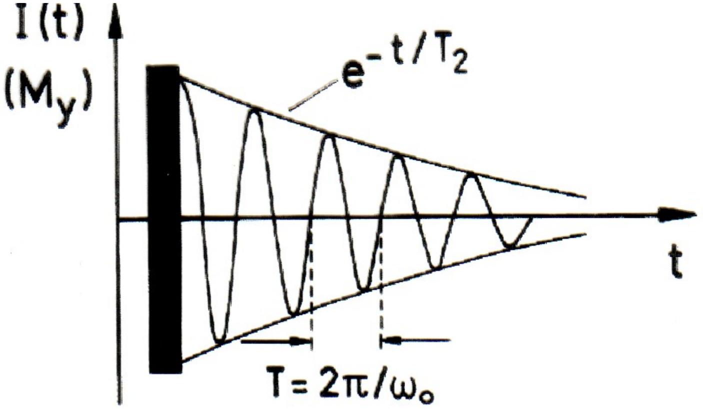
later

To be complete, the precession in the static field has to be taken into account as well, which is described by the Bloch equations

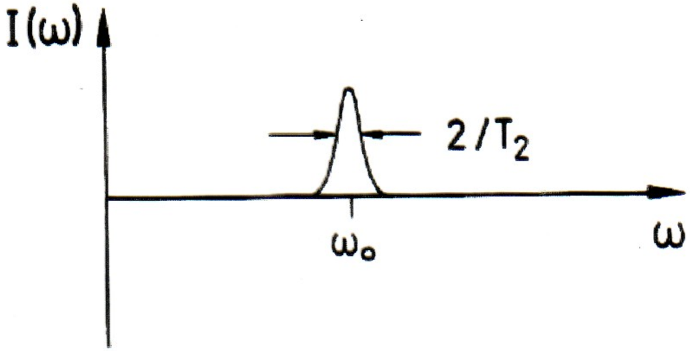
$$dM_z/dt = -(M_z - M_z^0)/T_1 + \gamma(\mathbf{M} \times \mathbf{H})_z,$$

$$dM_x/dt = -M_x/T_2 + \gamma(\mathbf{M} \times \mathbf{H})_x,$$

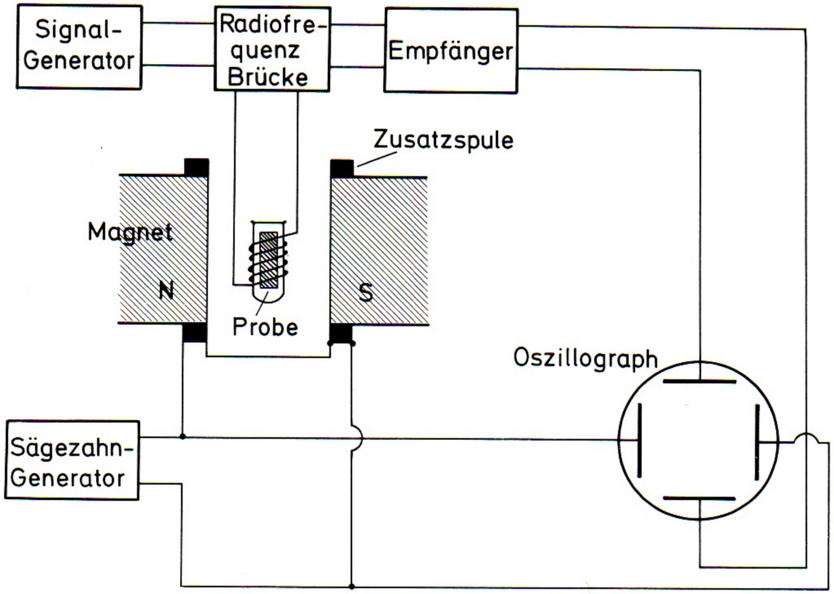
$$dM_y/dt = -M_y/T_2 + \gamma(\mathbf{M} \times \mathbf{H})_y.$$



One can detect the transverse magnetization M_x or M_y by a pick up coil where a current I(t) is induced by the oscillating transverse magnetization. The width of the FT of I(t) provides a measurement of T_2 (Method of free induction decay)



3.2 Classical NMR experiments



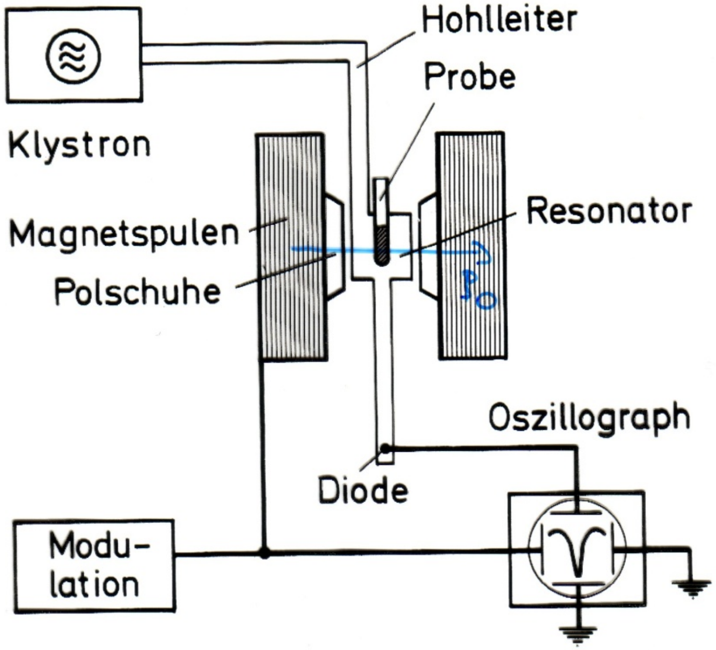
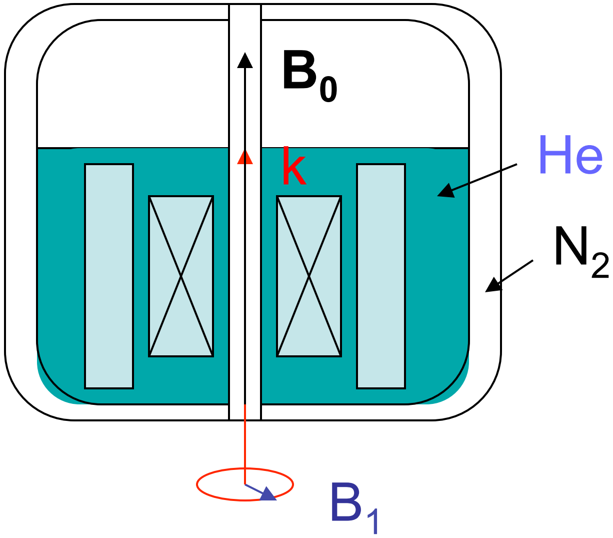


Abb. 20.18. Schema einer einfachen Kernspin-Resonanz-Apparatur. Die Probe befindet sich im Reagenzglas zwischen den Polschuhen eines homogenen Magneten. Das hochfrequente B_1-Feld wird über eine Brücke und eine Induktionsspule eingestrahlt. Zum besseren Nachweis der Resonanz kann das B_0-Feld durch eine Zusatzspule moduliert werden

Absorption signal

600 MHz Proton NMR Spectrometer

High frequency NMR spectrometers require very strong magnetic fields, which are produced using super-cooled coils (T = 4.2K, liquid He). The superconducting coils are surrounded by a giant vessel containing liquid N_2.



3.3 Chemical shift

The external field B_0 is changed (reduced in amplitude) due to local field $-\sigma B_0$ generated by the diamagnetic currents induced by B_0 in the electron system near the nucleus. s is the shielding constant (diamagnetic susceptibility)

$$\boxed{\begin{aligned} B_{loc} &= B_0 - \sigma B_0 \\ \omega_L &= \gamma B_0 (1 - \sigma) \end{aligned}}$$

The shielding depends on the orientation of B_0 with respect to the molecules (e.g. benzene ring) near the nucleus. σ is a tensor. If the rotational motion of the molecules is fast compared to $1/\omega_L$ the precessing spin I sees an effective (time averaged) field B_{loc}. If the rotation is free (like in most simple liquids) the anisotropy of the shielding is averaged out, σ becomes a number. The NMR lines are very narrow.

NB. In solids or large proteins in viscous environment where motions are strongly hindered or slowed down, the NMR lines are significantly broader.

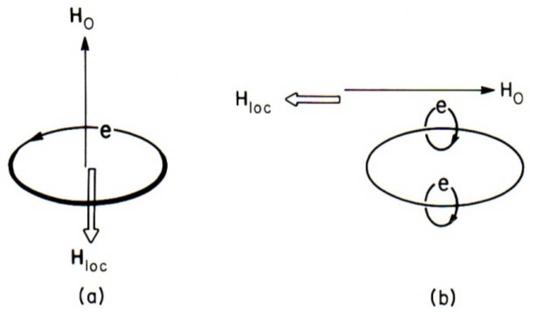


Fig. 9. Differing response of the π electrons in a benzene molecule at two orientations of the molecule relative to H_0: when the external field is (a) perpendicular to the molecular plane and (b) parallel to the molecular field.

Motional narrowing!

^{13}C NMR spectrum of liquid benzene

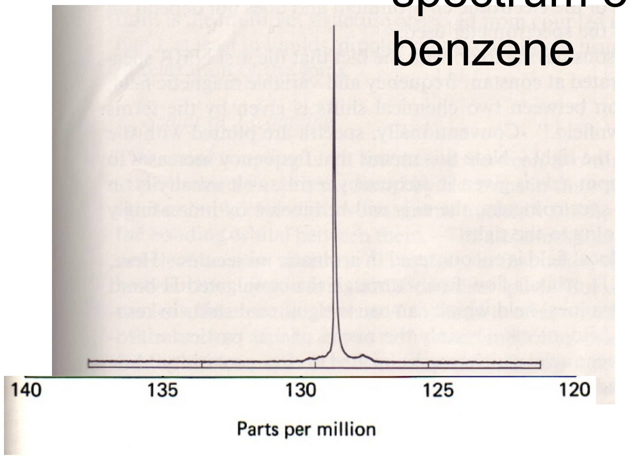
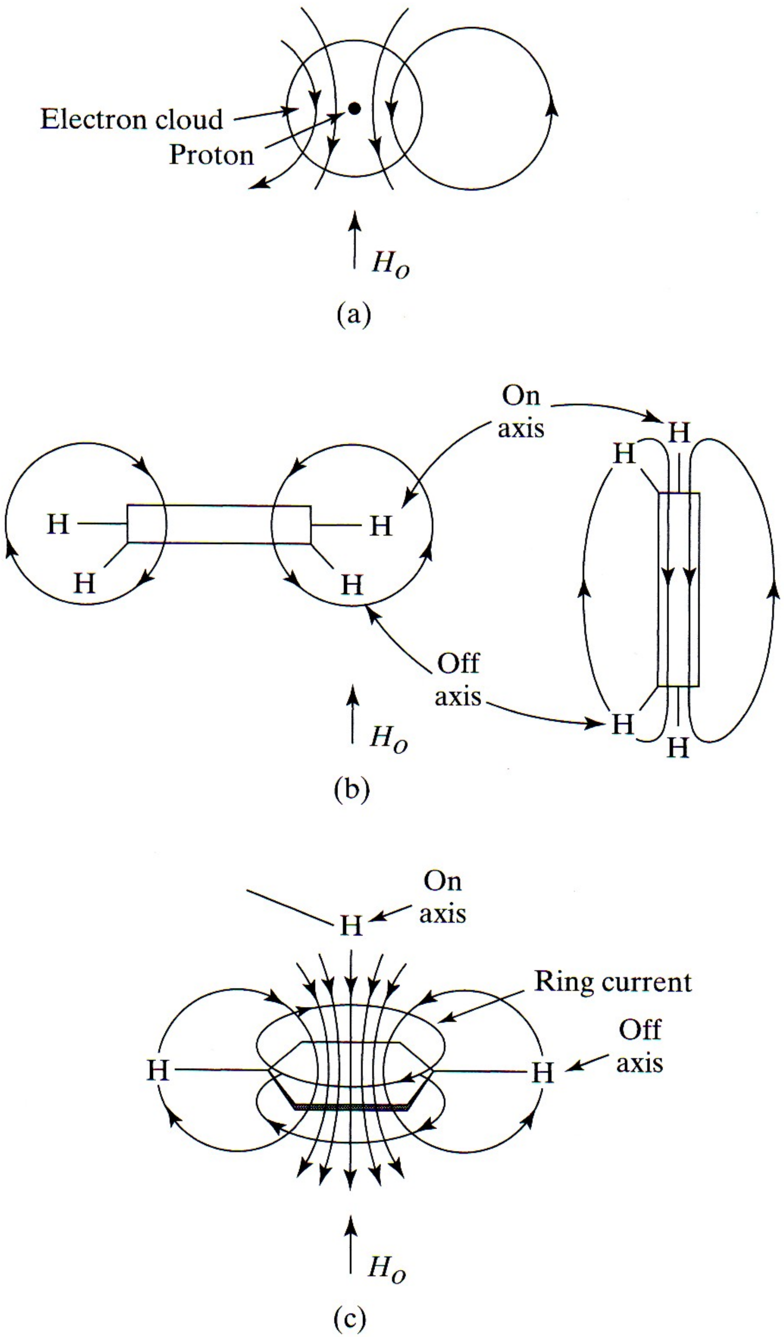


Figure 12.3 The field induced in some electron clouds for specific orientations in the magnetic field. (a) Local positive shielding for the proton in its electron cloud. (b) Interatomic shielding from a bond depends on whether the proton is on axis or off axis. (c) Interatomic shielding from a ring current depends on the position of the proton relative to the ring. This specific orientation gives the same effect as the net effect from averaging over all orientations.



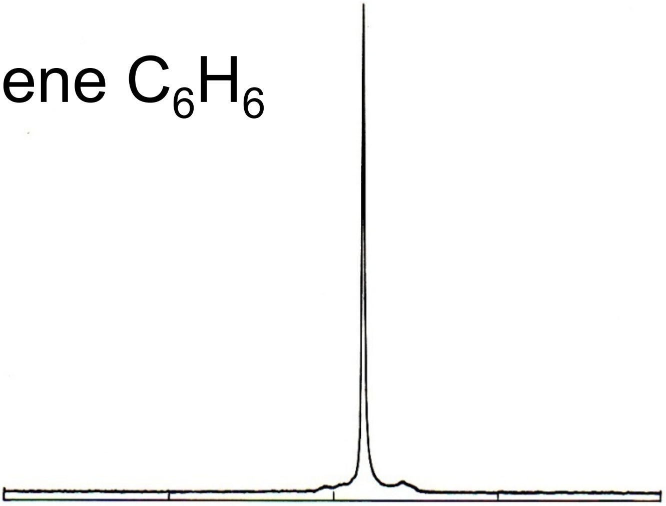
Usual measure: Frequency shift of sample (1) relative to some reference sample (2); unit: ppm

$$(\sigma_1 - \sigma_2)\gamma B_0/\gamma B_0$$

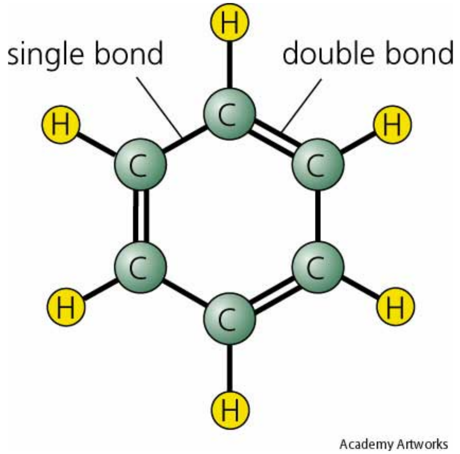
Origin of chemical shift: = shielding of B_0

Examples: ^{13}C NMR

Benzene C_6H_6

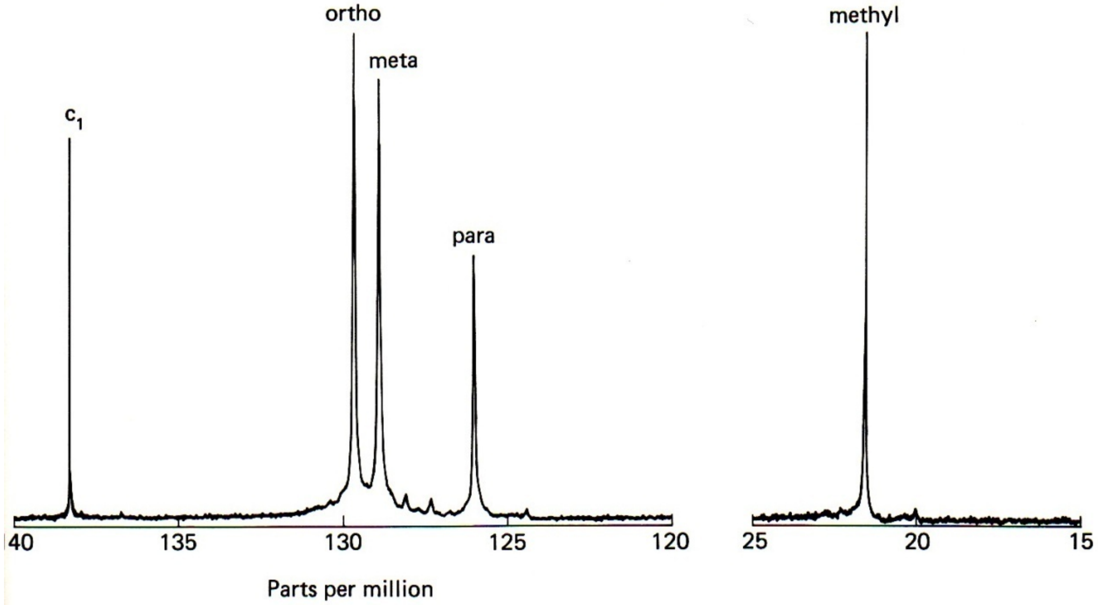


(a)

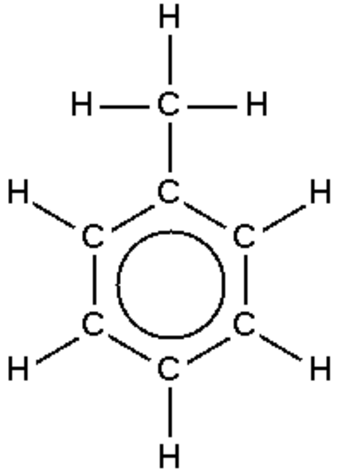


All 6 carbons are identical
same chemical shift, one line

Toluene C_6H_5-CH_3



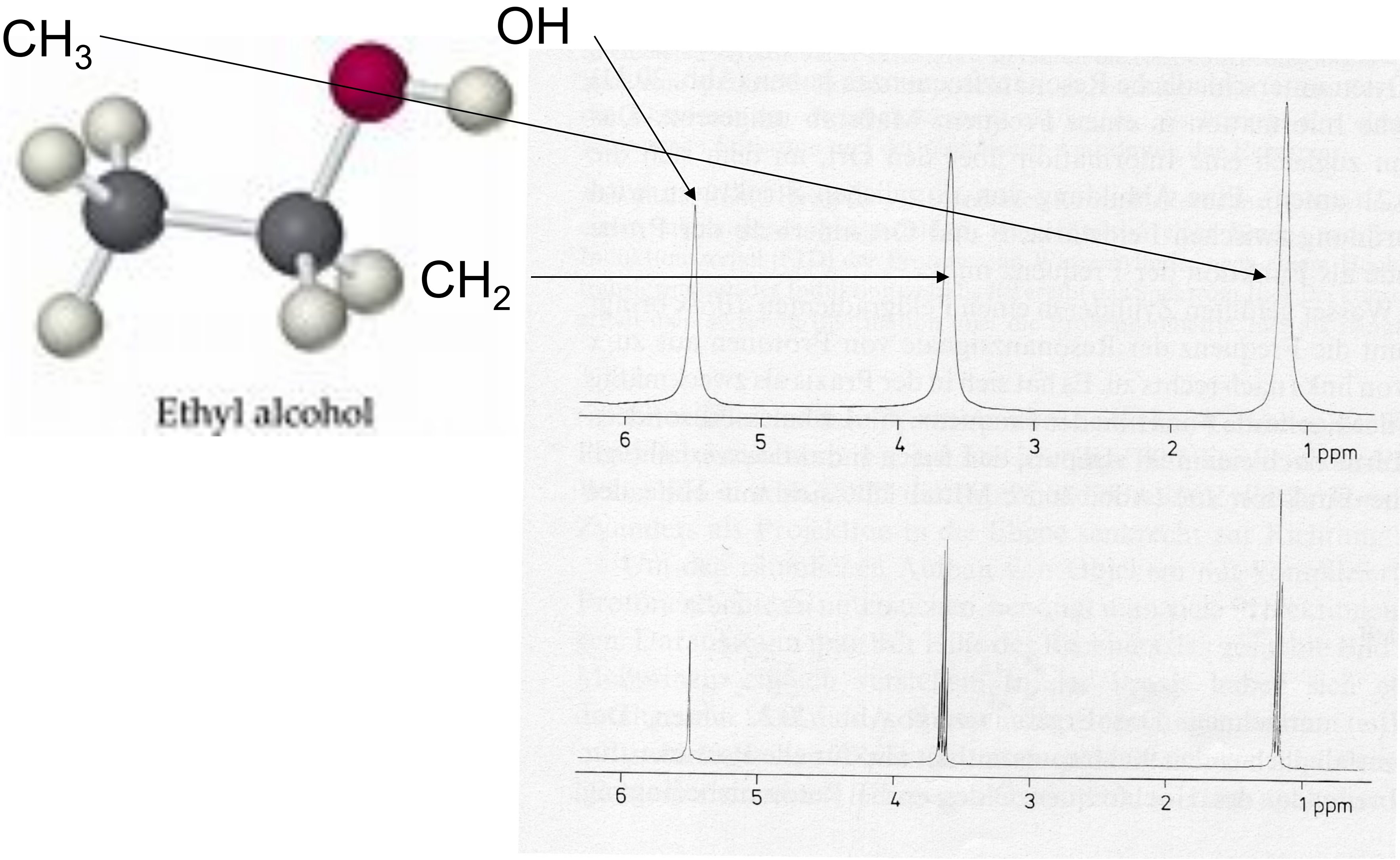
(b)



5 different types of
C-atoms, 5 lines

^{1}H-NMR of ethyl alcohol, CH_3CH_2OH

Three types of protons



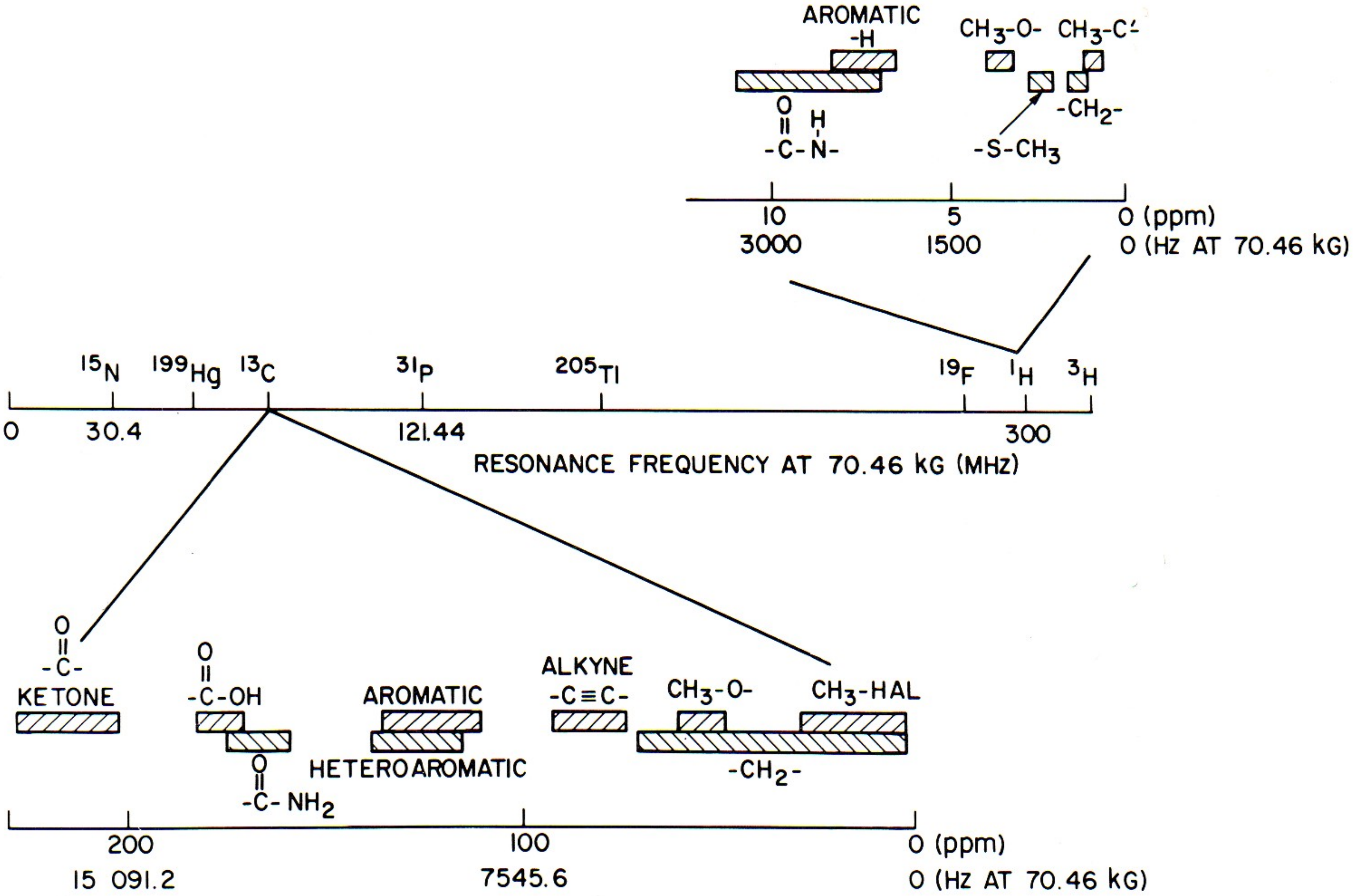
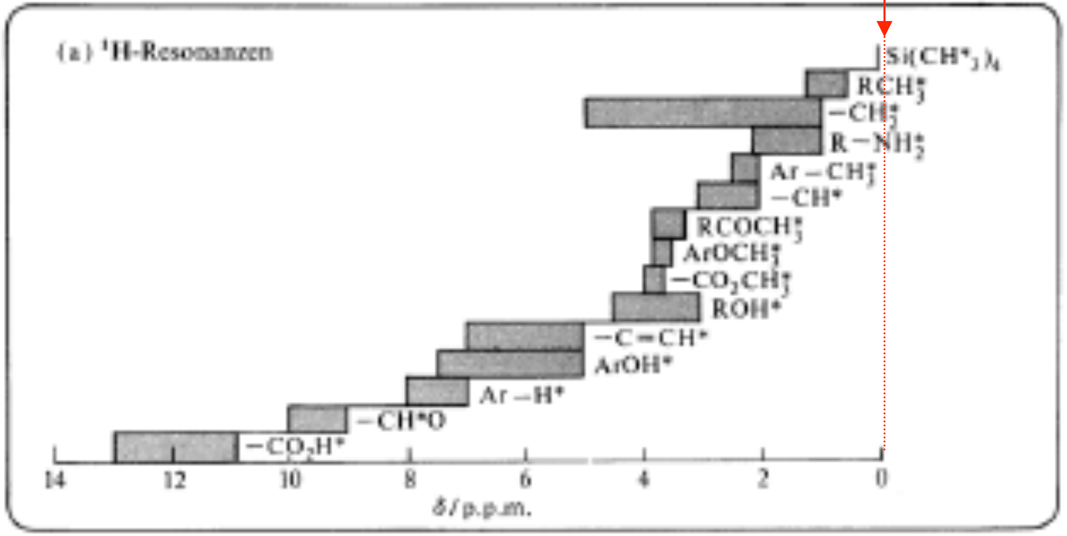


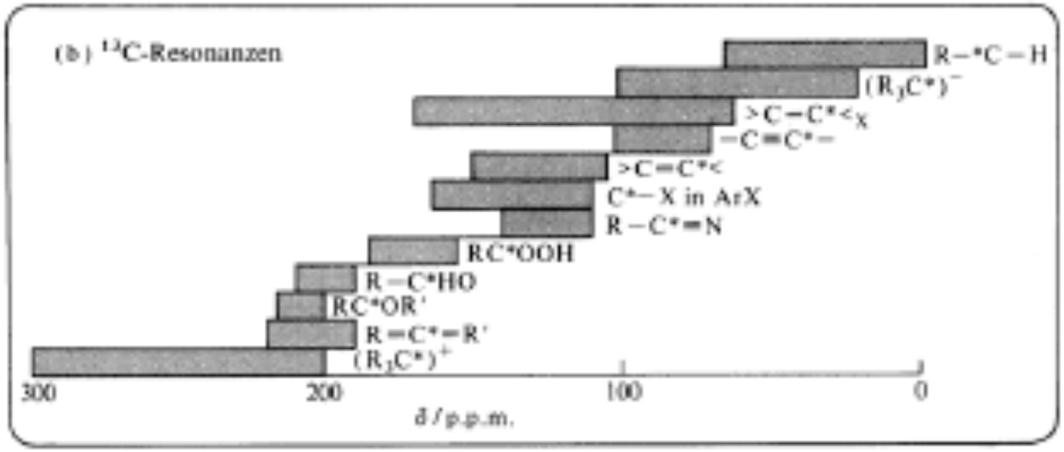
Fig. 2. Nuclear resonance frequencies of several magnetic nuclei at 70.46 kG and the chemical shift range ^{13}C and ^{1}H nuclei.

Typical chemical shifts

Reference Tetramethylsilane Si $(CH_3)_4$

Has very narrow line





Chemical shifts are frequently used in chemistry and biology to determine amount of specific groups in sample (quantitative spectroscopy)

Table 12.3 ^{1}H Chemical Shifts for the 20 Common Amino Acids (in ppm)

Residue	NH	$C_\alpha H$	$C_\beta H$	Others	
Gly	8.39	3.97			
Ala	8.25	4.35	1.39		
Val	8.44	4.18	2.13	$C_\gamma H_3$	0.97, 0.94
Ile	8.19	4.23	1.90	$C_\gamma H_2$	1.48, 1.19
				$C_\gamma H_3$	0.95
				$C_\delta H_3$	0.89
Leu	8.42	4.38	1.65, 1.65	$C_\gamma H$	1.64
				$C_\delta H_3$	0.94, 0.90
Pro(*trans*)		4.44	2.28, 2.02	$C_\gamma H_2$	2.03, 2.03
				$C_\delta H_2$	3.68, 3.65
Ser	8.38	4.50	3.88, 3.88		
Thr	8.24	4.35	4.22	$C_\gamma H_3$	1.23
Met	8.42	4.52	2.15, 2.01	$C_\gamma H_2$	2.64, 2.64
				$C_\varepsilon H_3$	2.13
Cys	8.31	4.69	3.28, 2.96		
Asp	8.41	4.76	2.84, 2.75		
Asn	8.75	4.75	2.83, 2.75	$N_\gamma H_2$	7.59, 6.91
Glu	8.37	4.29	2.09, 1.97	$C_\gamma H_2$	2.31, 2.28
Gln	8.41	4.37	2.13, 2.01	$C_\gamma H_2$	2.38, 2.38
				$N_\delta H_2$	6.87, 7.59
Lys	8.41	4.36	1.85, 1.76	$C_\gamma H_2$	1.45, 1.45
				$C_\delta H_2$	1.70, 1.70
				$C_\varepsilon H_2$	3.02, 3.02
				$N_\varepsilon H_3$	7.52
Arg	8.27	4.38	1.89, 1.79	$C_\gamma H_2$	1.70, 1.70
				$C_\delta H_2$	3.32, 3.32
				$N_\delta H$	7.17, 6.62
His	8.41	4.63	3.26, 3.20	C_2H	8.12
				C_4H	7.14
Phe	8.23	4.66	3.22, 2.99	C_2H, C_6H	7.30
				C_3H, C_5H	7.39
				C_4H	7.34
Tyr	8.18	4.60	3.13, 2.92	C_2H, C_6H	7.15
				C_3H, C_5H	6.86
Trp	8.09	4.70	3.32, 3.19	C_2H	7.24
				C_4H	7.65
				C_5H	7.17
				C_6H	7.24
				C_7H	7.50
				NH	10.22