Nuclear Magnetic Resonance (NMR) Spectroscopy

By_ Saurav k. Rawat
M.Sc. Chem.
(Physical special)

School of Chemical Science,
St. John's College, Agra

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)

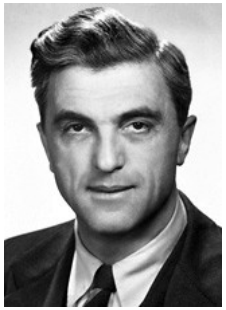
Continuation of NMR History

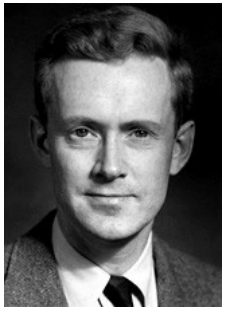
Nobel prizes

1944 *Physics* Rabi (Columbia)

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

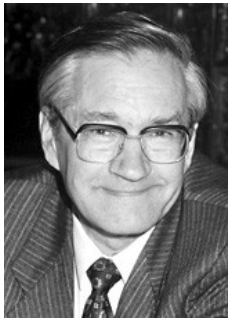
1952 *Physics* Bloch (Stanford), Purcell (Harvard)





"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)

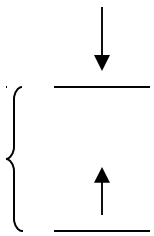
-the nuclei of some atoms spin: ^{1}H, ^{13}C, ^{19}F, …

-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 $\Delta E = E_{EM}$.

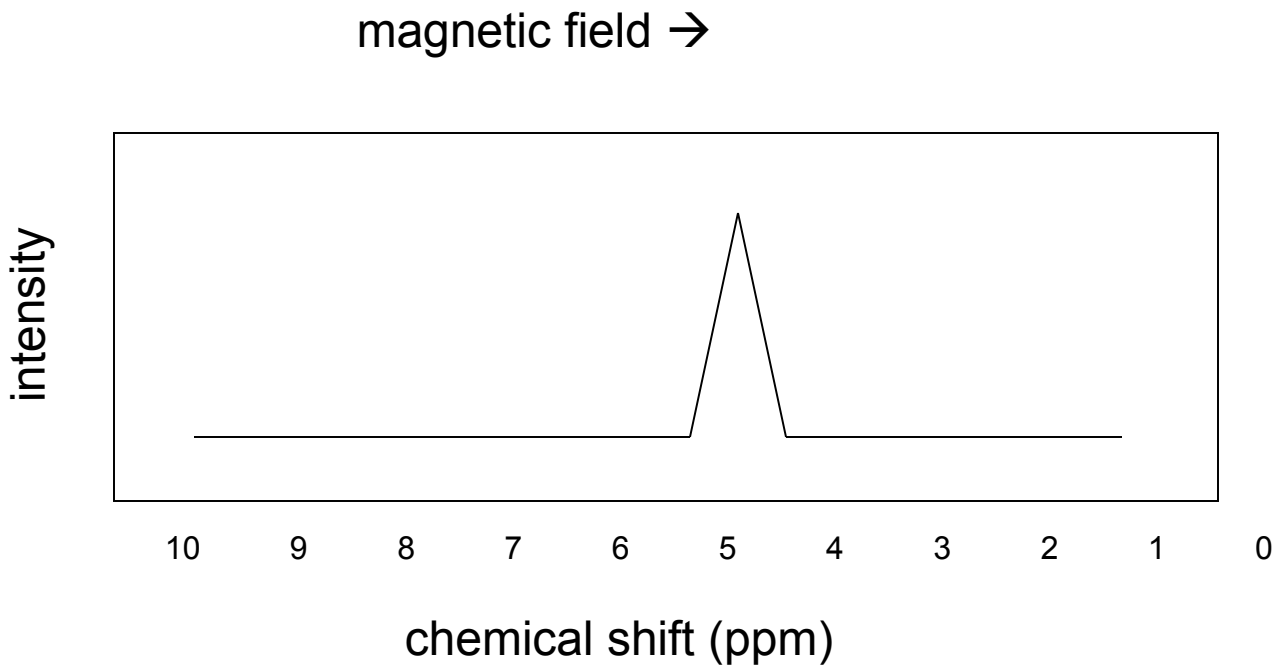
When the energies match, the nuclei can change spin states (resonate) and give off a magnetic signal.



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.

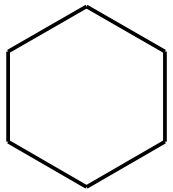
1. Number of signals: How many different types of hydrogens in the molecule.

Magnetically equivalent hydrogens resonate at the same applied field.

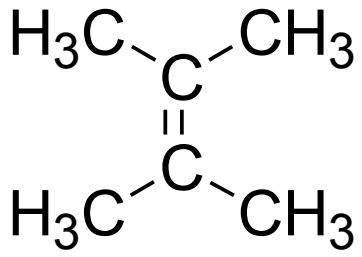
Magnetically equivalent hydrogens are also chemically equivalent.

# of signals? CH_4 CH_3CH_3

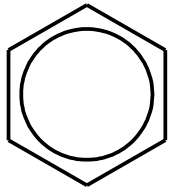
number of signals?



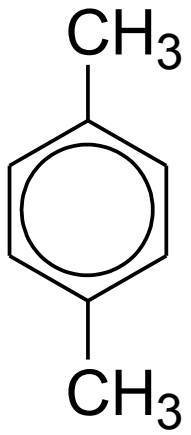
one



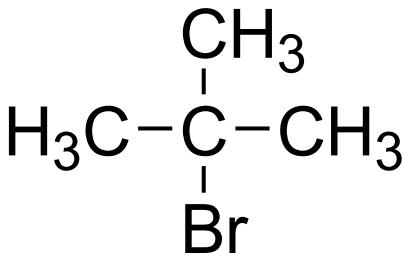
one



one



two



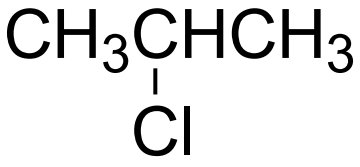
one

CH_3CH_2-Br

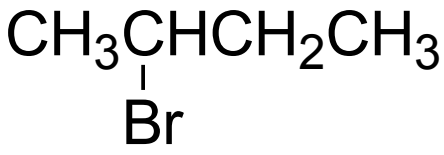
two

$CH_3CH_2CH_2$-Br

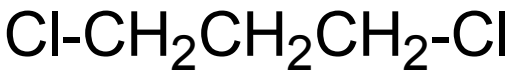
three



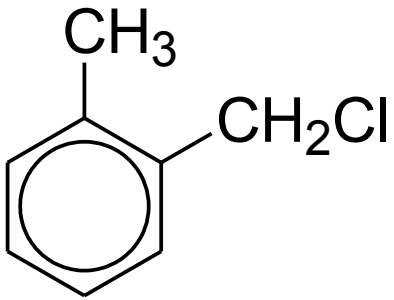
two



four



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.

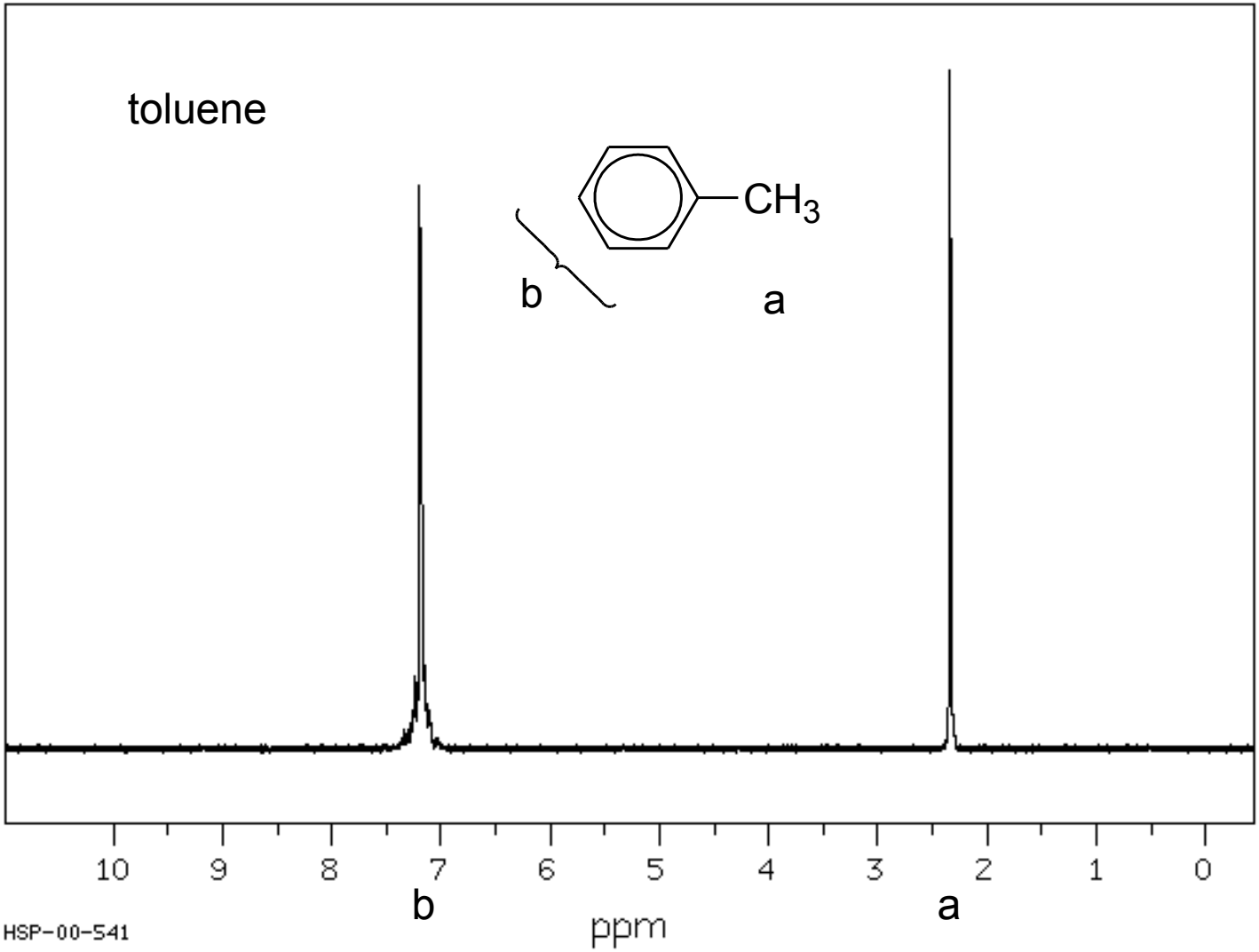
reference compound = tetramethylsilane $(CH_3)_4Si$ @ 0.0 ppm

remember: **magnetic field →**

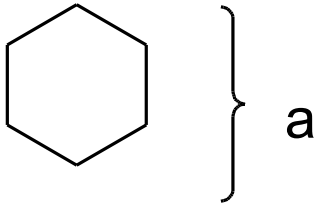
← chemical shift

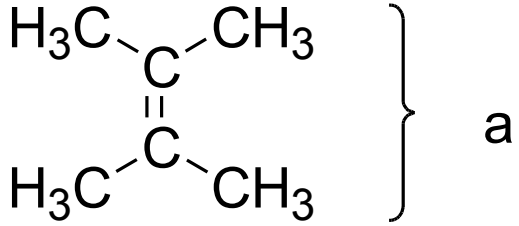
convention: let most upfield signal = a, next most upfield = b, etc.

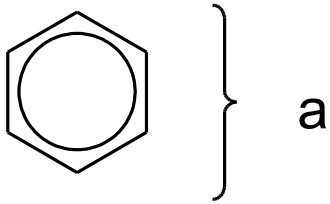
… c b a tms

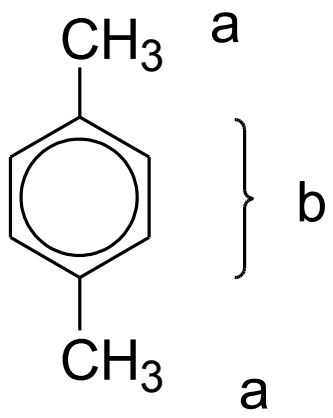

toluene
CH3
b
a
10
9
8
7
6
5
4
3
2
1
0
b
ppm
a
HSP-00-541

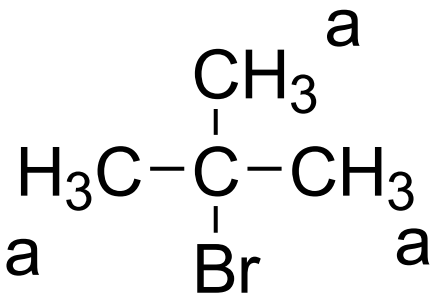
chemical shifts

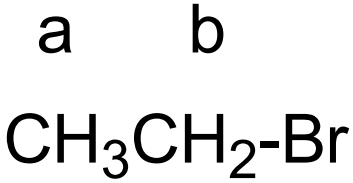


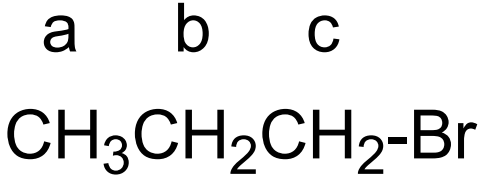


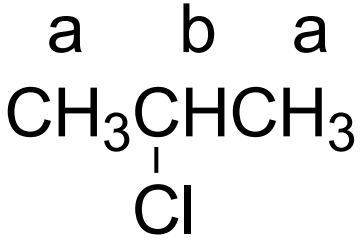


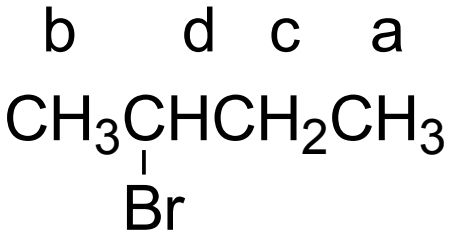


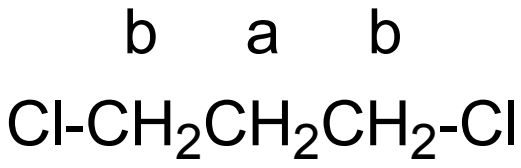

a
CH3
H3C
C
CH3
a
Br
a

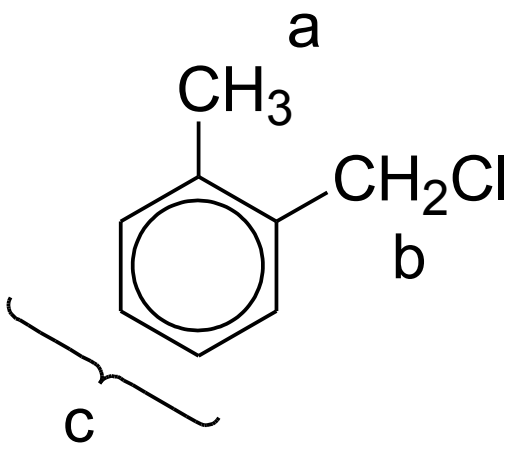

a b
CH3CH2-Br


a b c
CH3CH2CH2-Br


a b a
CH3CHCH3
Cl


b d c a
CH3CHCH2CH3
Br


b a b
Cl-CH2CH2CH2-Cl

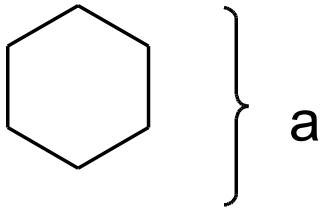

a
CH3
CH2Cl
b
c

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

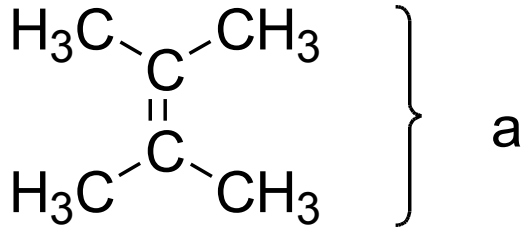
a b c
$CH_3CH_2CH_2Br$ a 3H a : b : c = 3 : 2 : 2
b 2H
c 2H

a b a
CH_3CHCH_3 a 6H a : b = 6 : 1
Cl b 1H

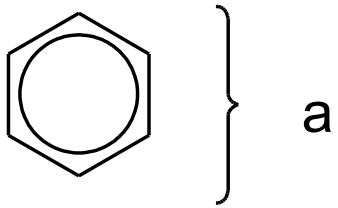
integration



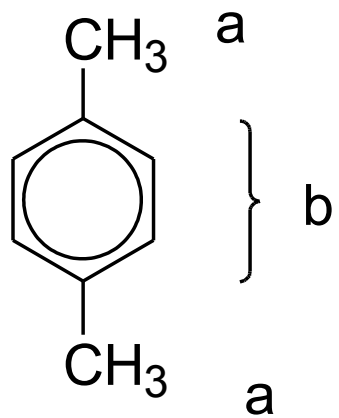
a 12 H



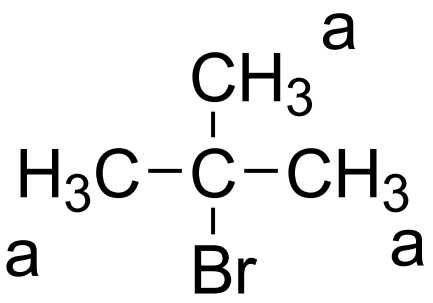
a 12 H



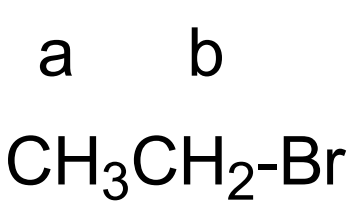
a 6 H



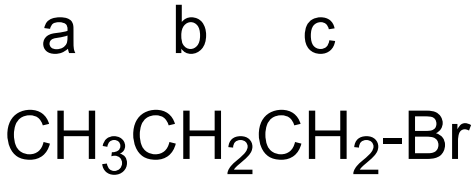
a 6 H
b 4 H



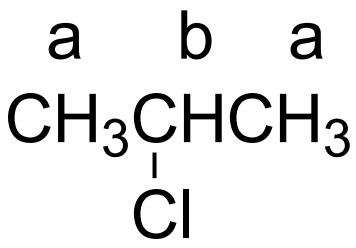
a 9 H



a 3 H
b 2 H

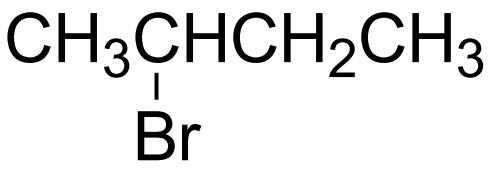


a 3 H
b 2 H
c 2 H



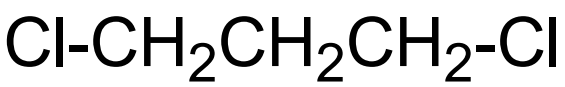
a 6 H
b 1 H

b d c a

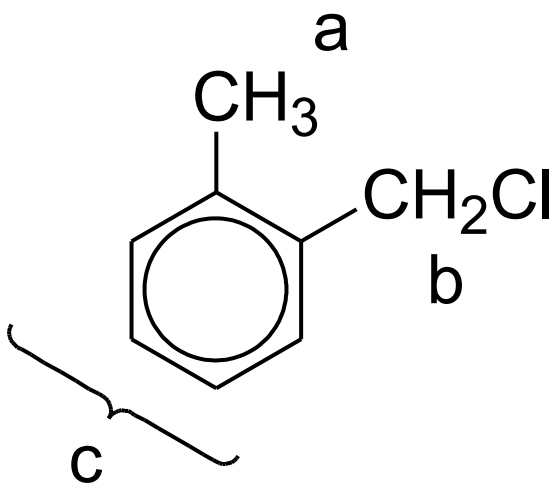


a 3 H
b 3 H
c 2 H
d 1 H

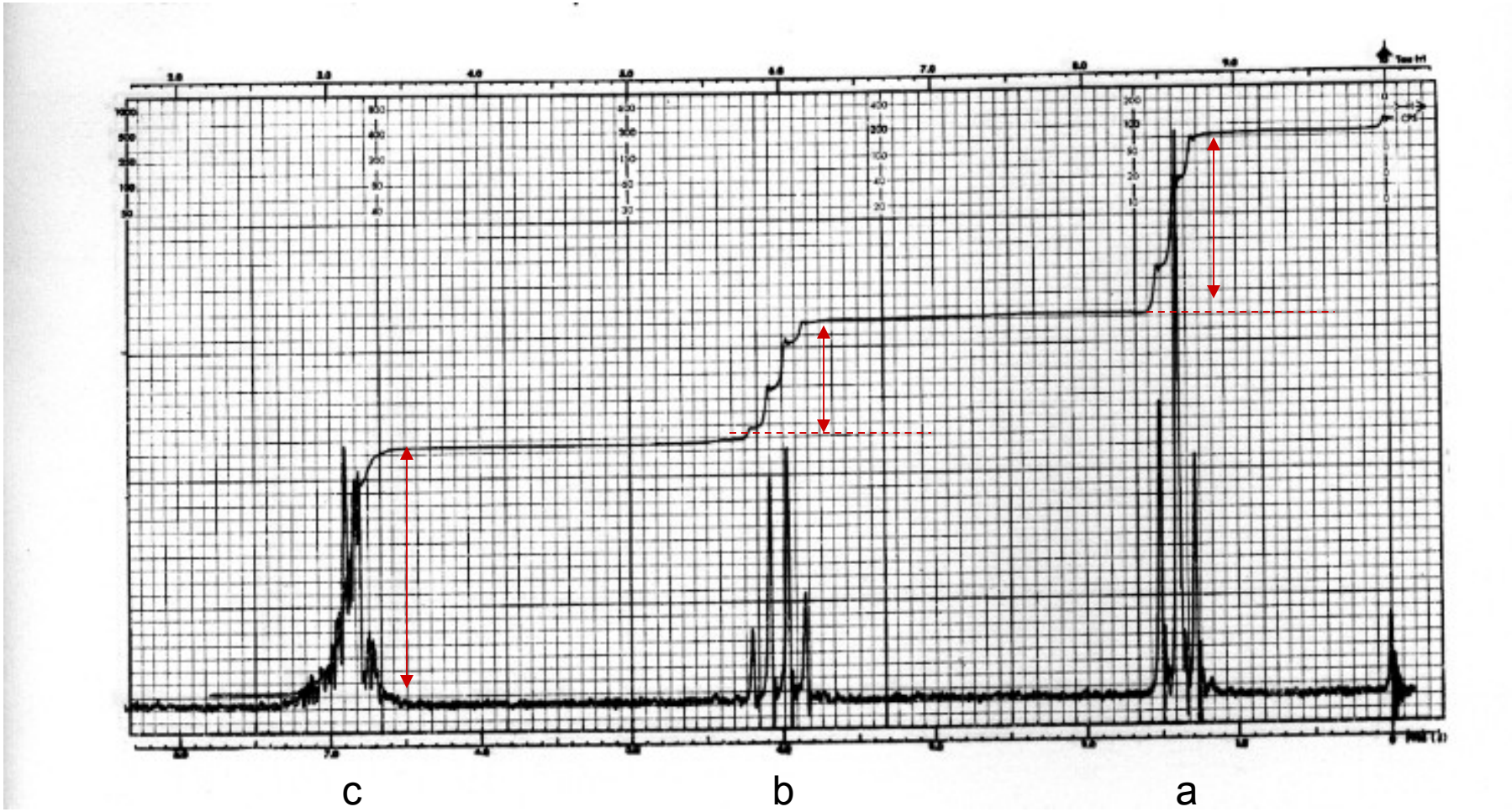
b a b



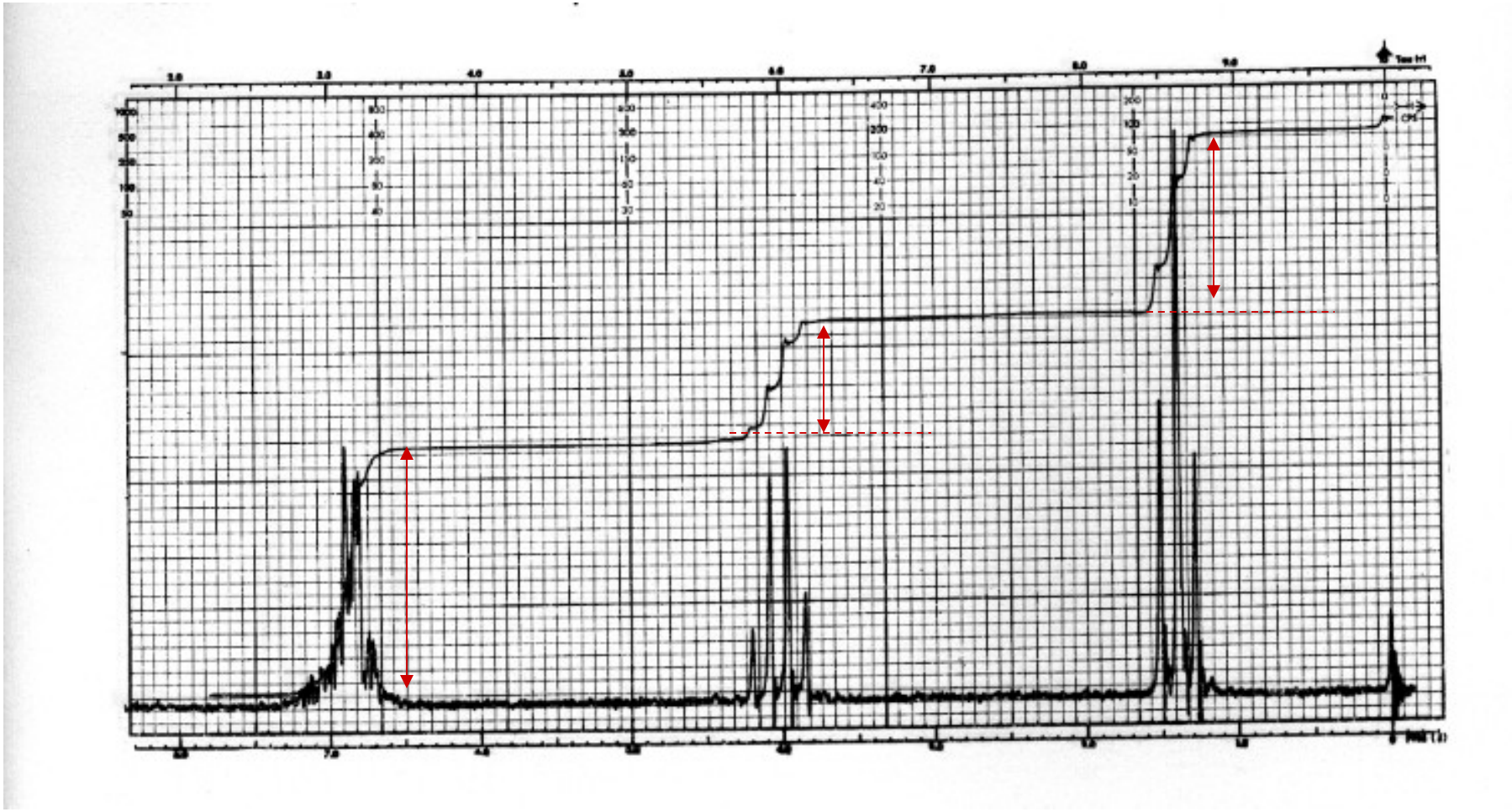
a 2 H
b 4 H



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.

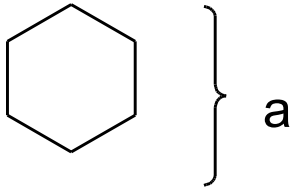
“neighboring” – no more than three bonds away

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

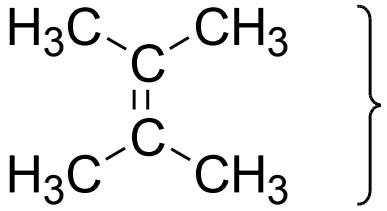
note: n must be equivalent neighboring hydrogens to give rise to a Pascal splitting pattern. If the neighbors are not equivalent, then you will see a complex pattern (aka complex multiplet).

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).

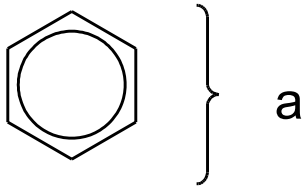
splitting pattern?



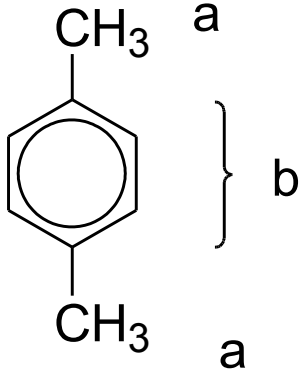
a 12 H singlet



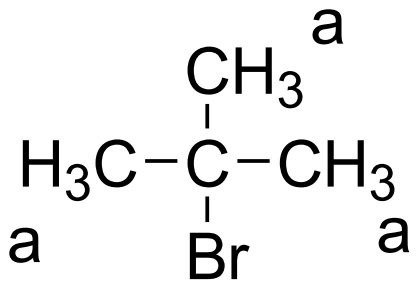
a 12 H singlet



a 6 H singlet



a 6 H singlet
b 4 H singlet



a 9 H singlet

a b

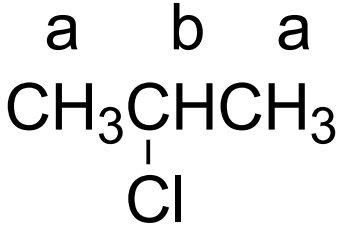
CH_3CH_2-Br

a 3 H triplet
b 2 H quartet

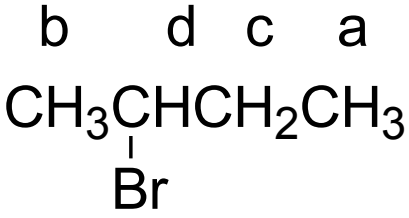
a b c

$CH_3CH_2CH_2$-Br

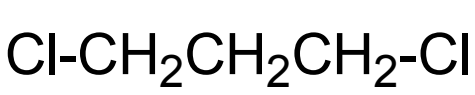
a 3 H triplet
b 2 H complex
c 2 H triplet



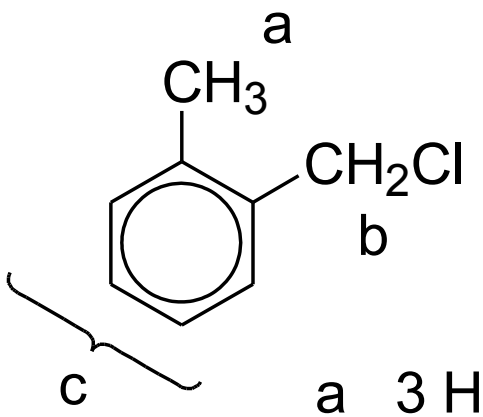
a 6 H doublet
b 1 H septet



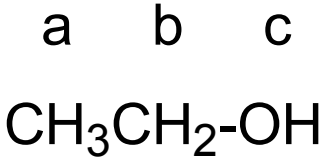
a 3 H triplet
b 3 H doublet
c 2 H complex
d 1 H complex



a 2 H quintet
b 4 H triplet



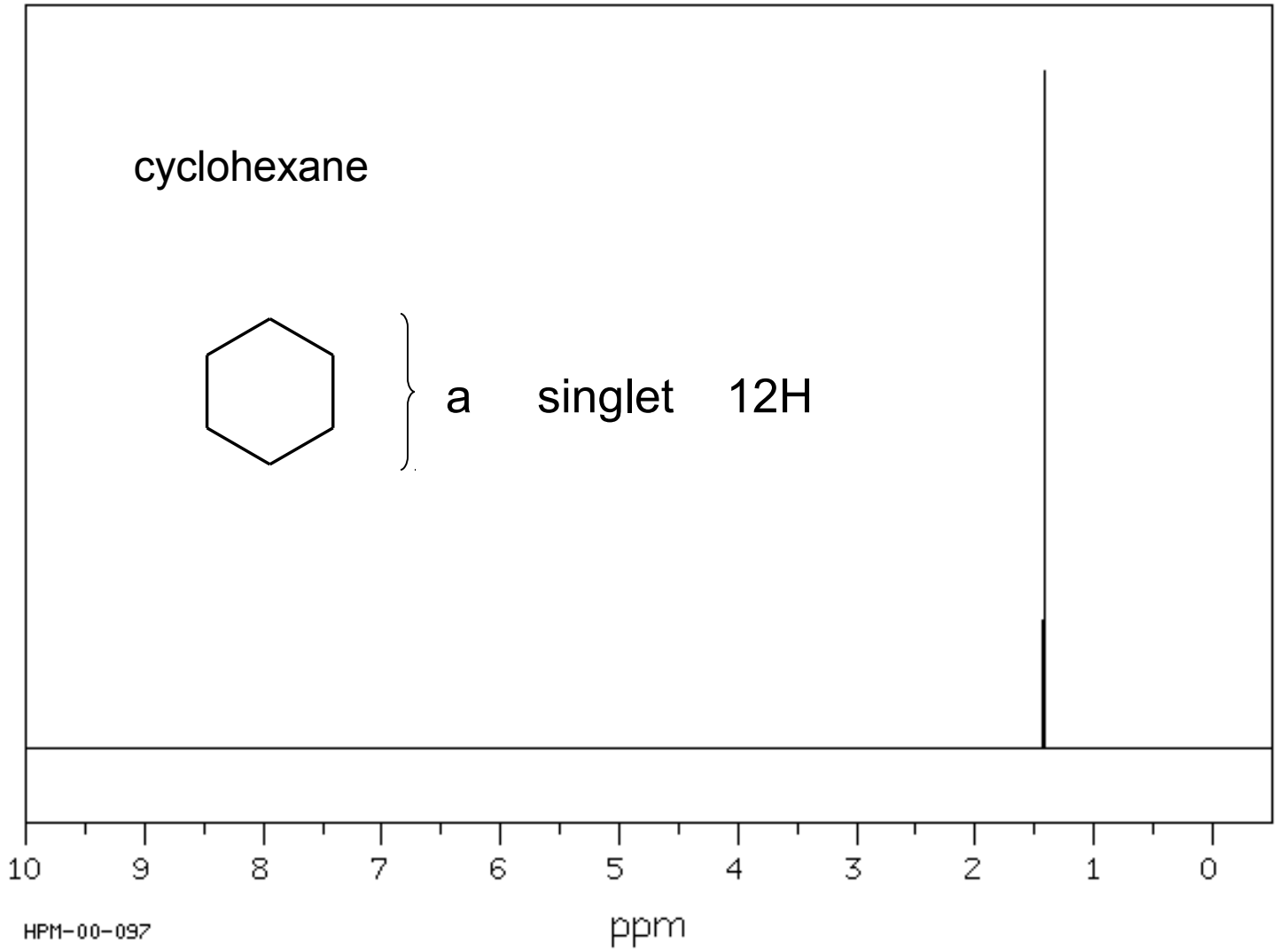
a 3 H singlet
b 2 H singlet
c 4 H ~singlet

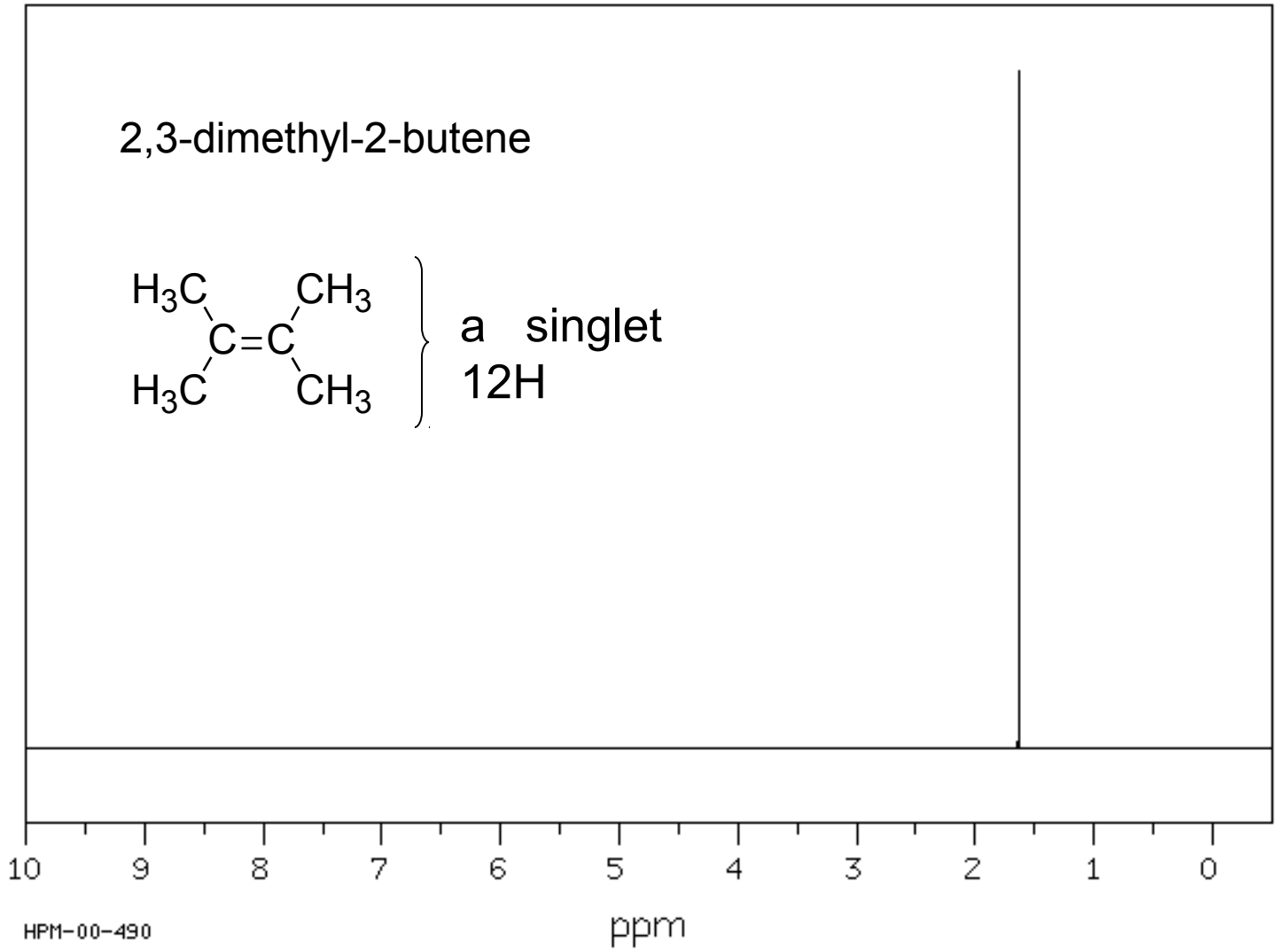


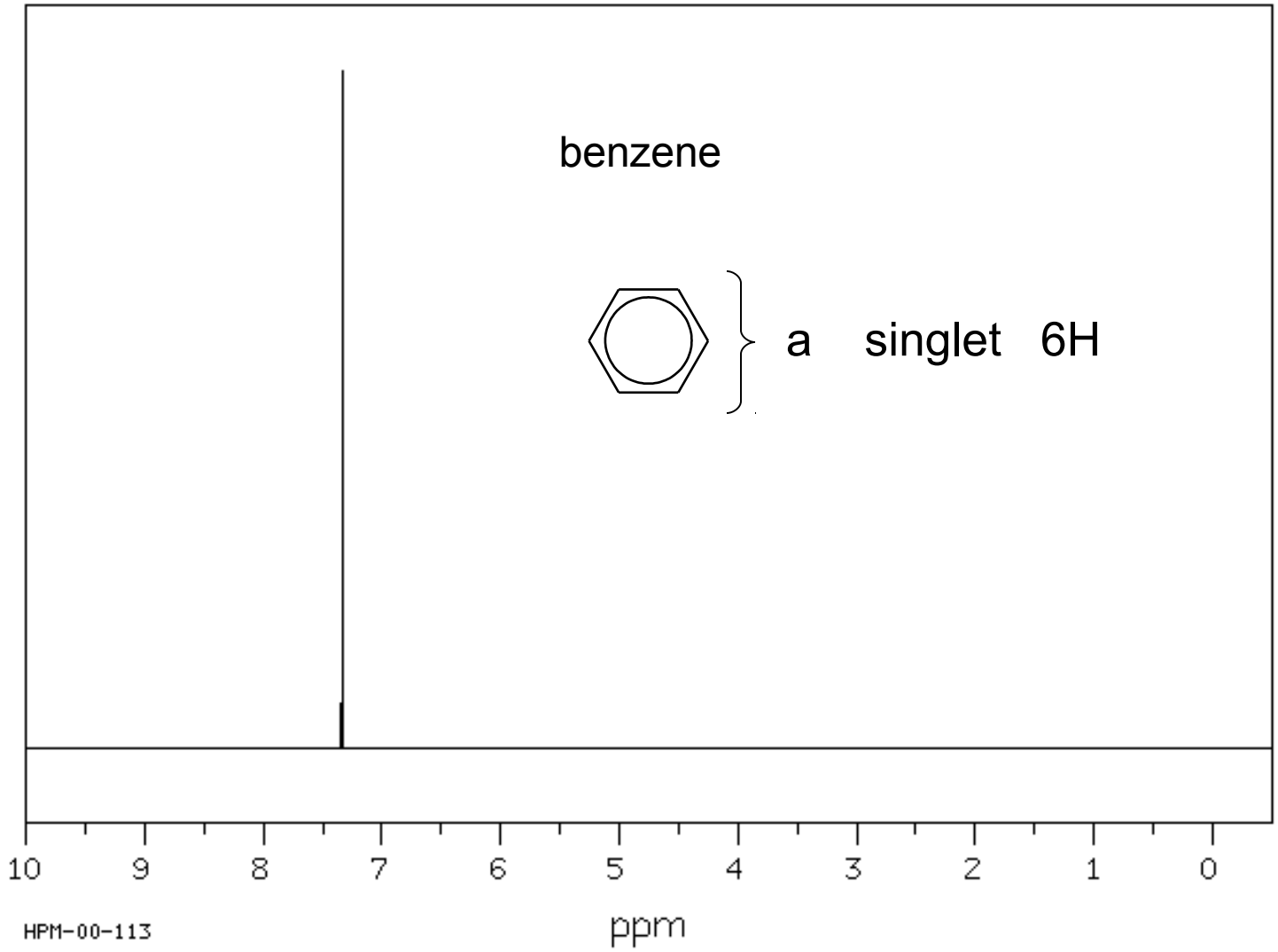
a 3 H triplet
b 2 H quartet
c 1 H singlet

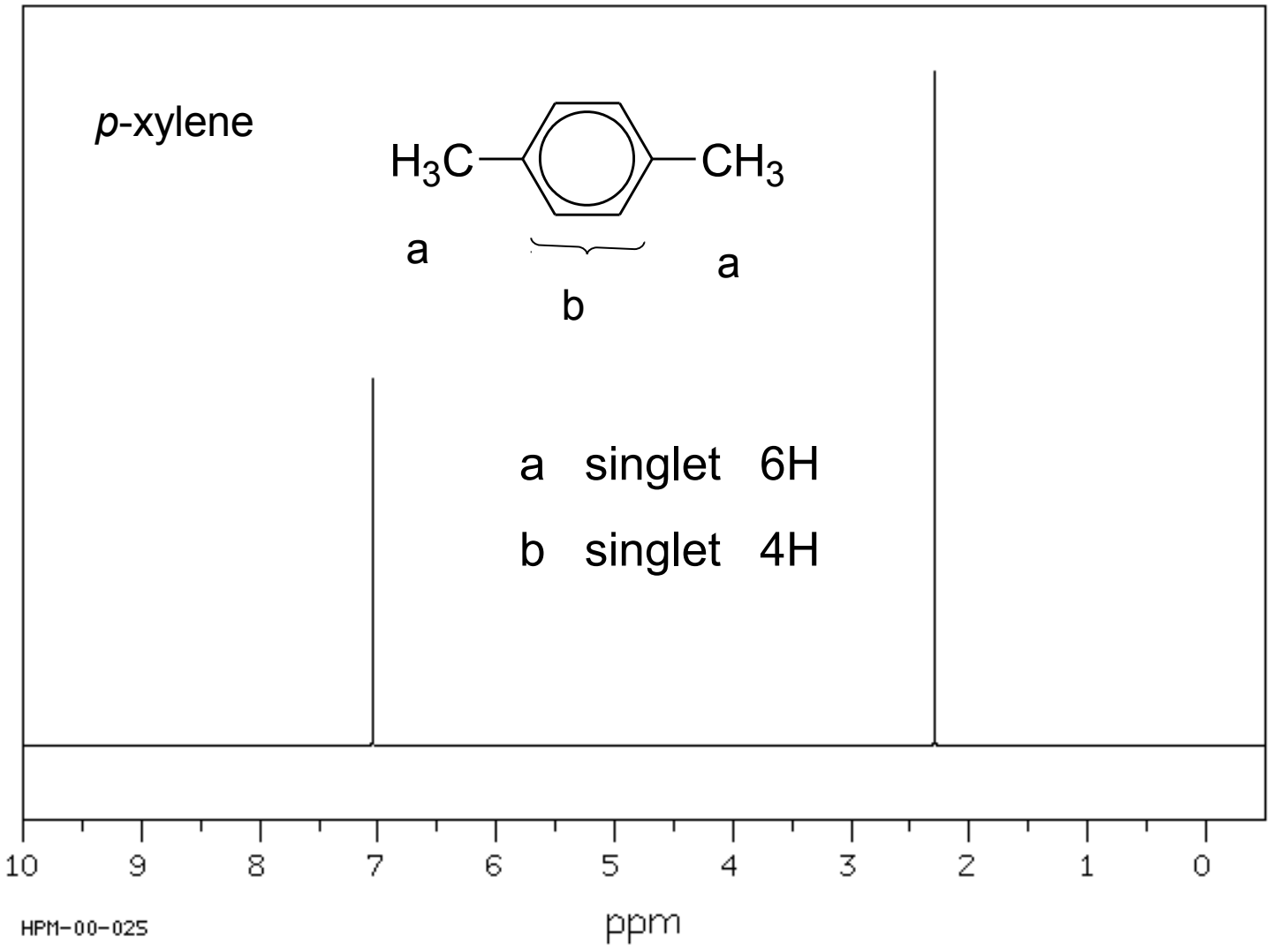
Information from ^{1}H-nmr spectra:

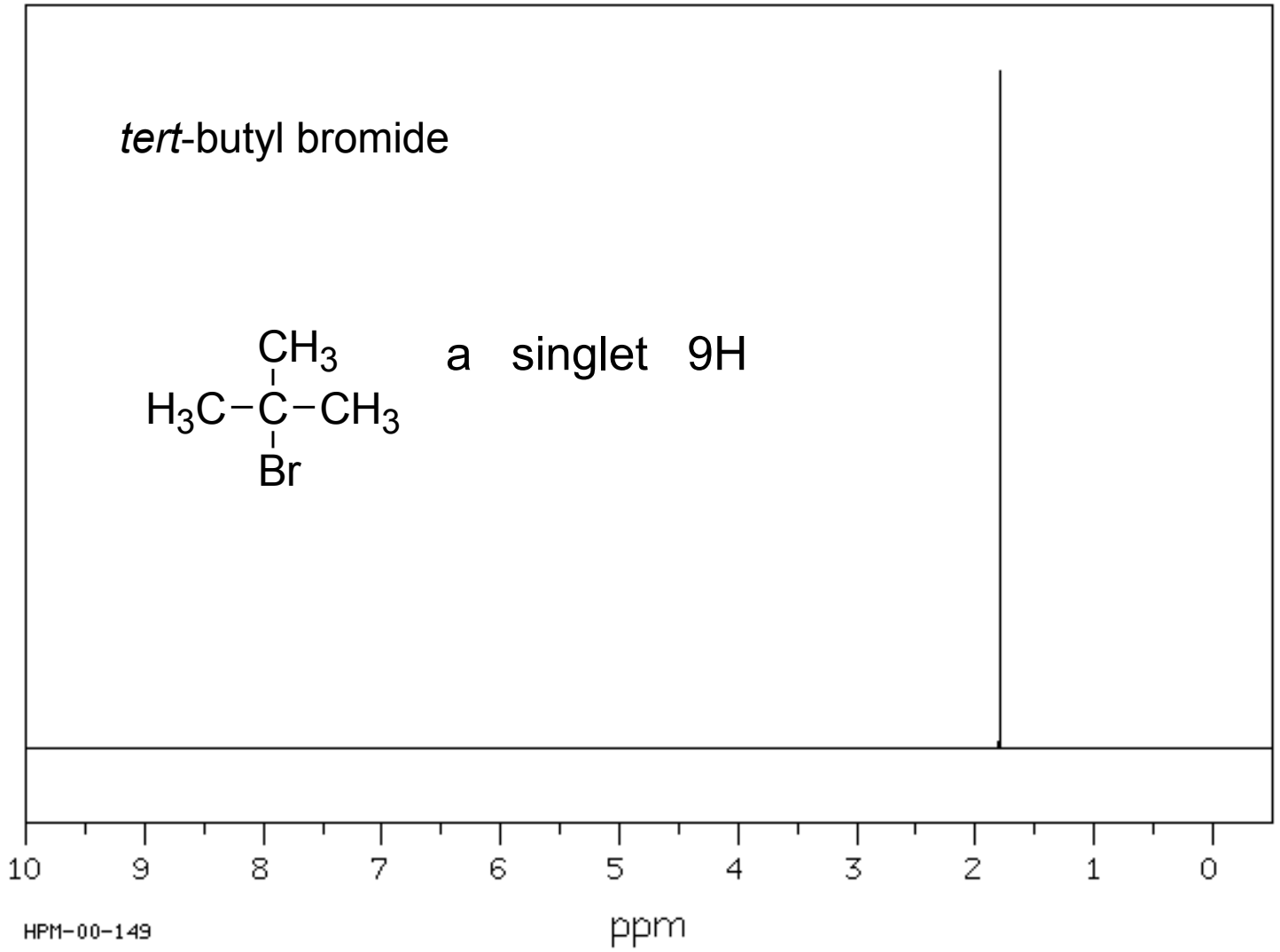
1. Number of signals: How many different types of hydrogens in the molecule.
2. Position of signals (chemical shift): What types of hydrogens.
3. Relative areas under signals (integration): How many hydrogens of each type.
4. Splitting pattern: How many neighboring hydrogens.

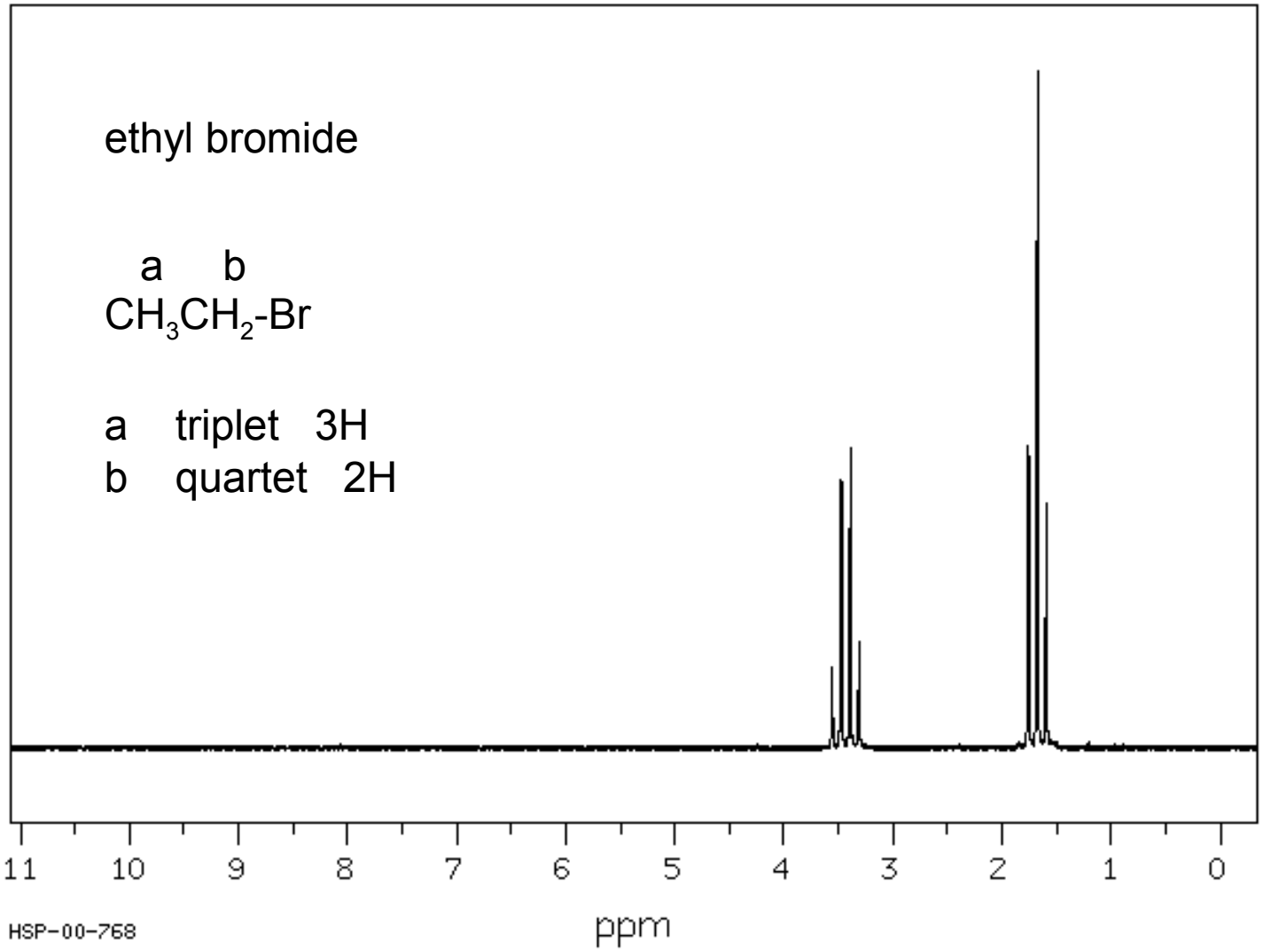

cyclohexane
a singlet 12H
10
9
8
7
6
5
4
3
2
1
0
ppm
HPM-00-097

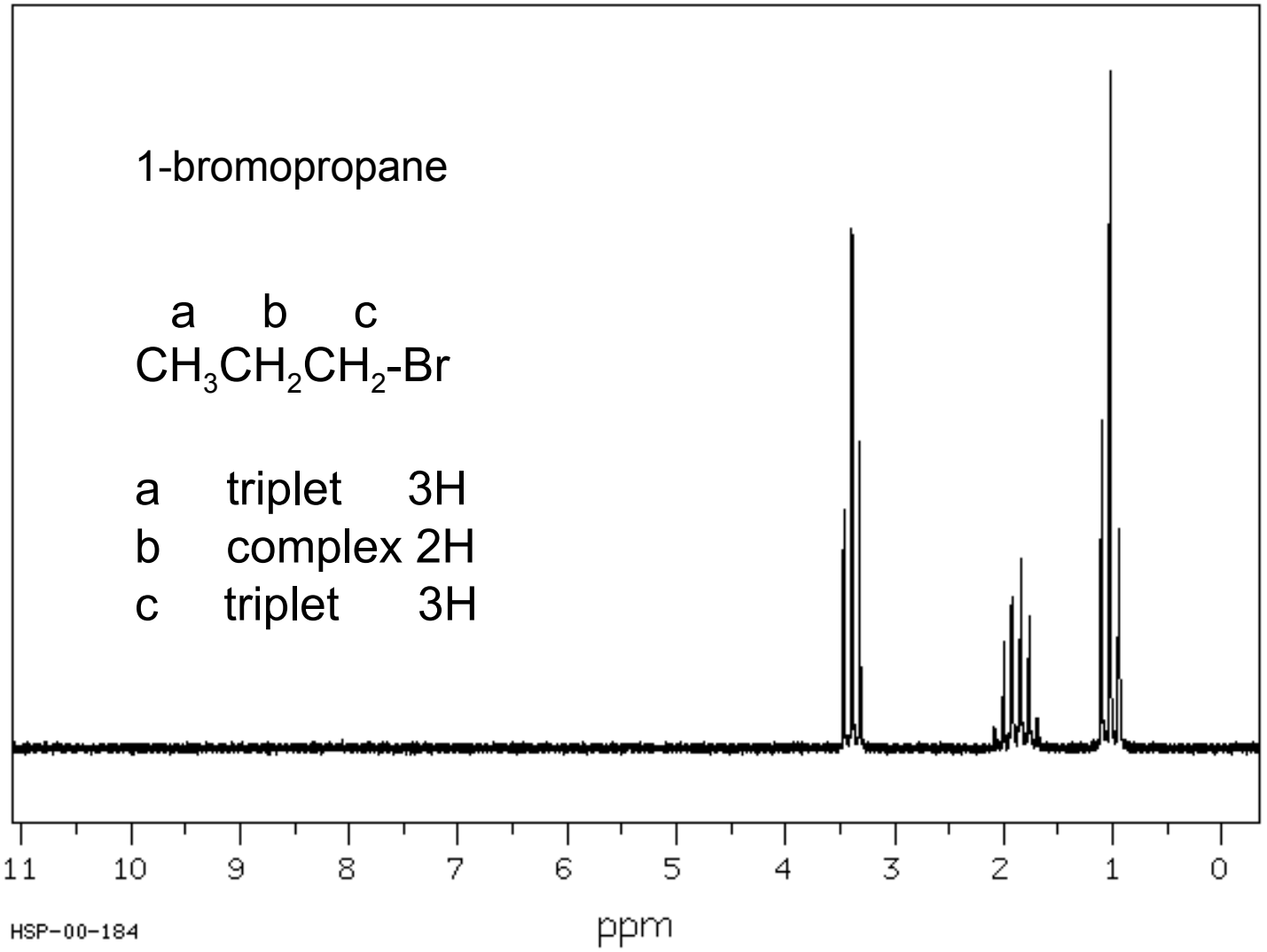

2,3-dimethyl-2-butene
H3C
CH3
C=C
H3C
CH3
a singlet
12H
10
9
8
7
6
5
4
3
2
1
0
HPM-00-490
ppm

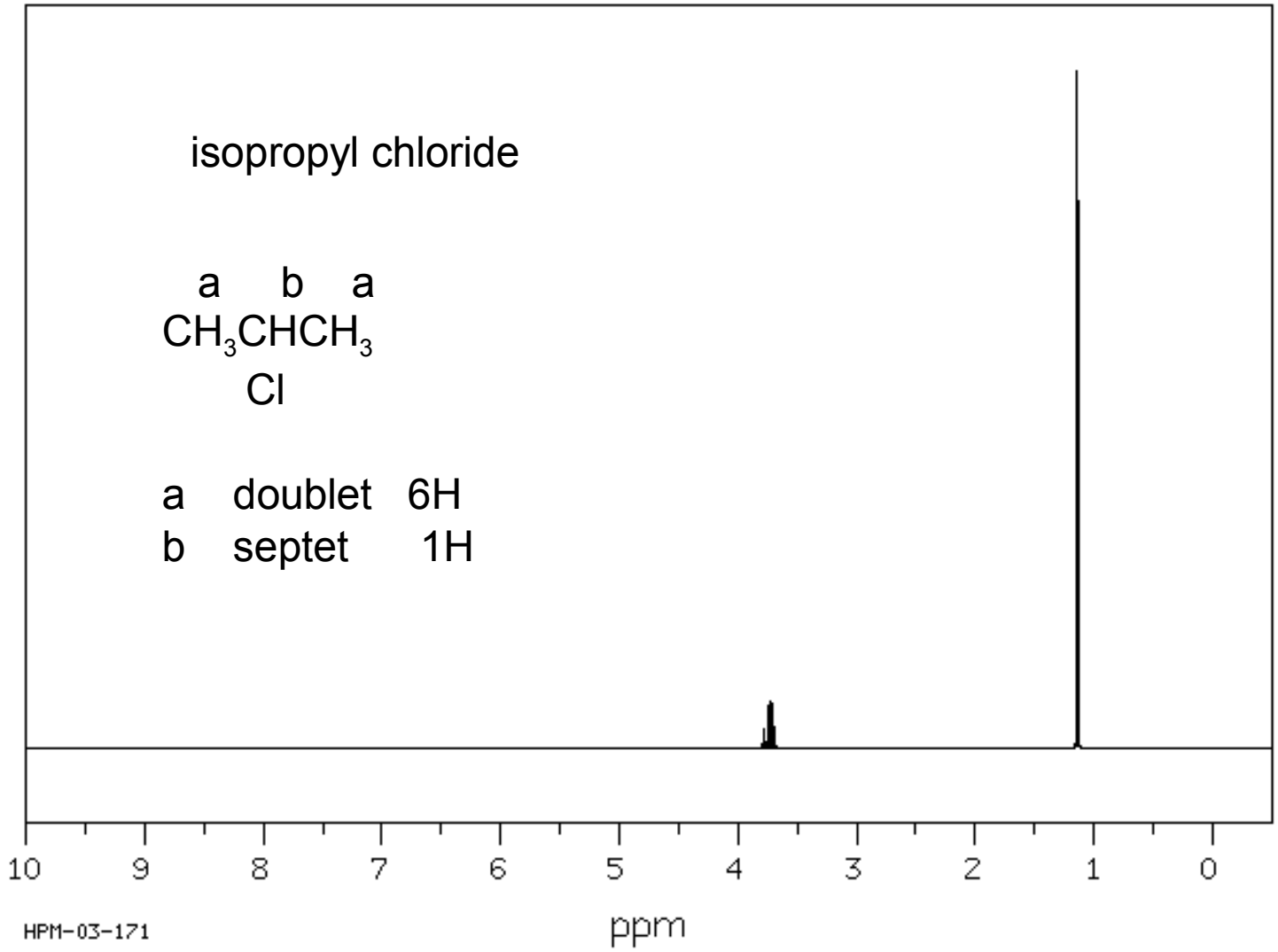

benzene
a singlet 6H
10
9
8
7
6
5
4
3
2
1
0
ppm
HPM-00-113

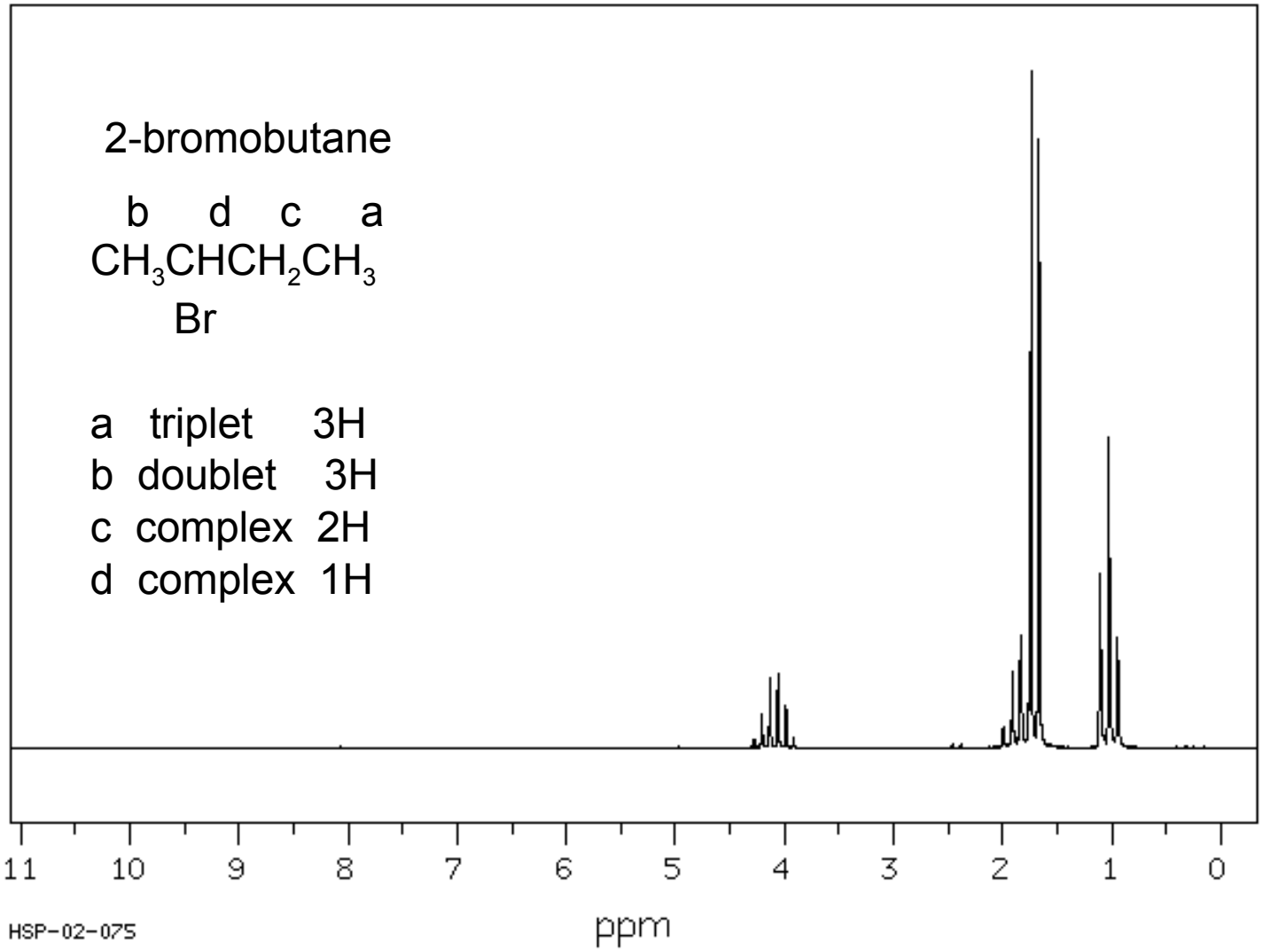

p-xylene
H_3C
CH_3
a
a
b
a singlet 6H
b singlet 4H
10
9
8
7
6
5
4
3
2
1
0
HPM-00-025
ppm

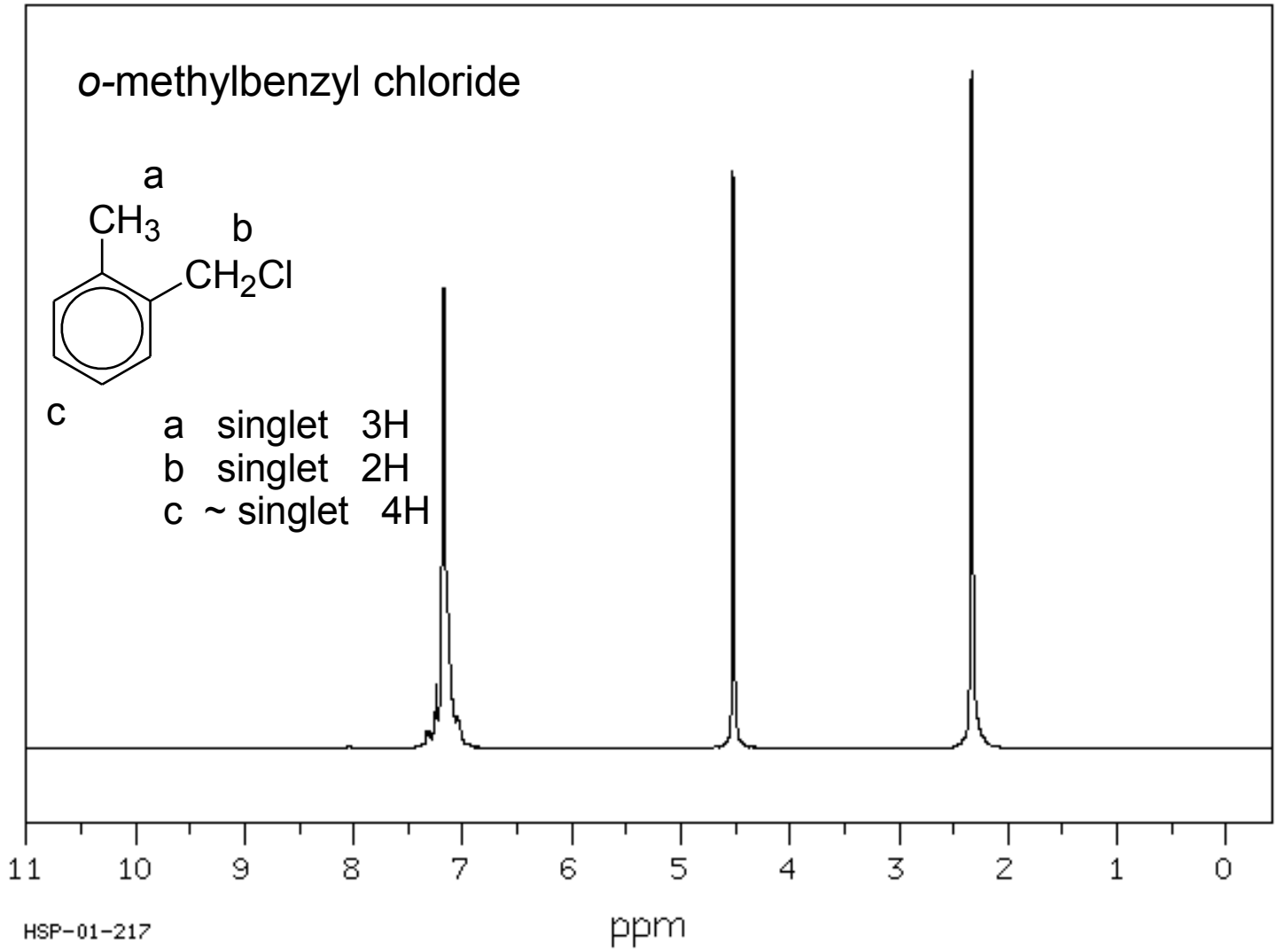

tert-butyl bromide
CH3
H3C–C–CH3
Br
a singlet 9H
10
9
8
7
6
5
4
3
2
1
0
HPM-00-149
ppm

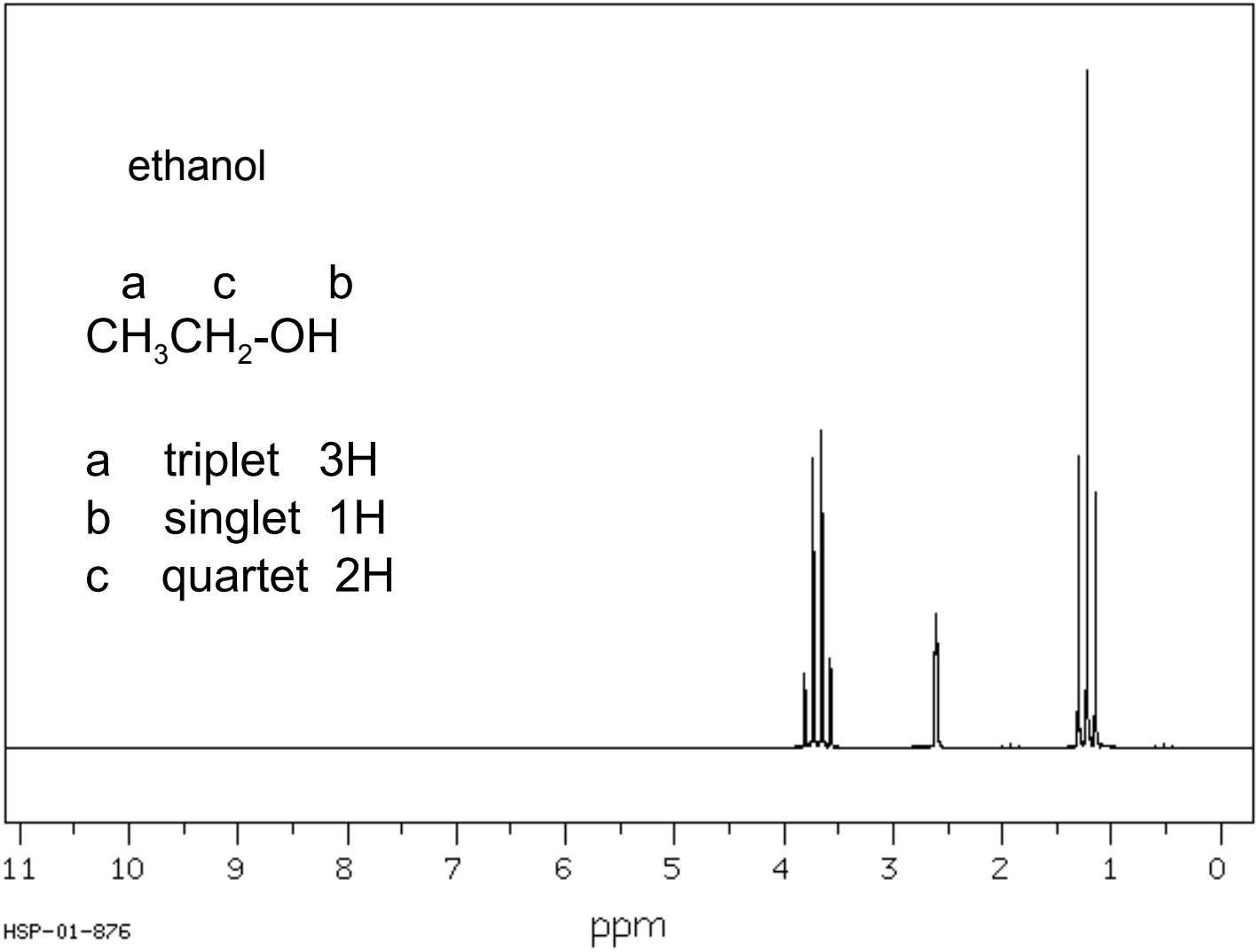

ethyl bromide
a b
CH3CH2-Br
a triplet 3H
b quartet 2H
11
10
9
8
7
6
5
4
3
2
1
0
HSP-00-768
ppm

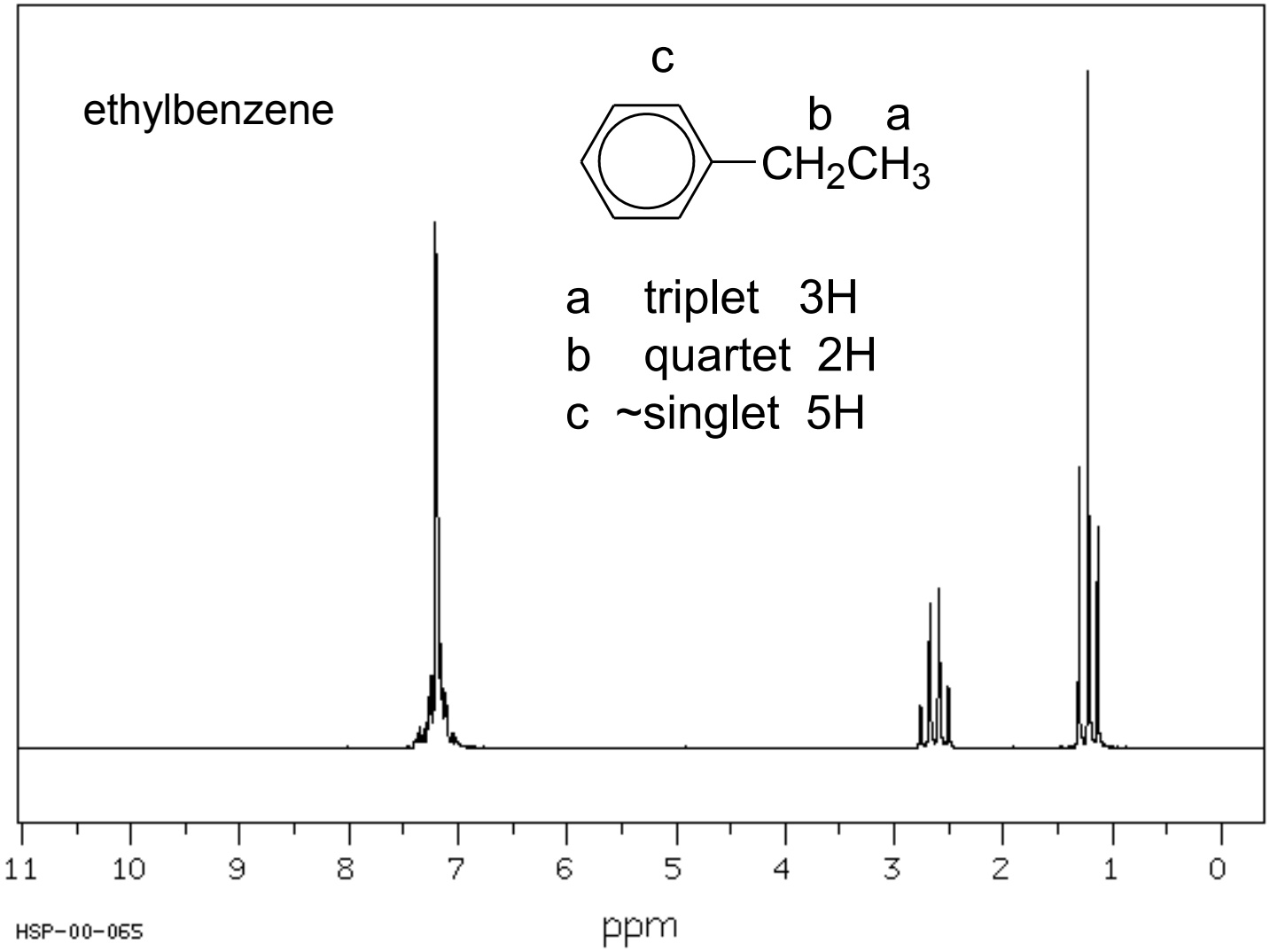

1-bromopropane
a b c
CH3CH2CH2-Br
a triplet 3H
b complex 2H
c triplet 3H
11
10
9
8
7
6
5
4
3
2
1
0
HSP-00-184
ppm

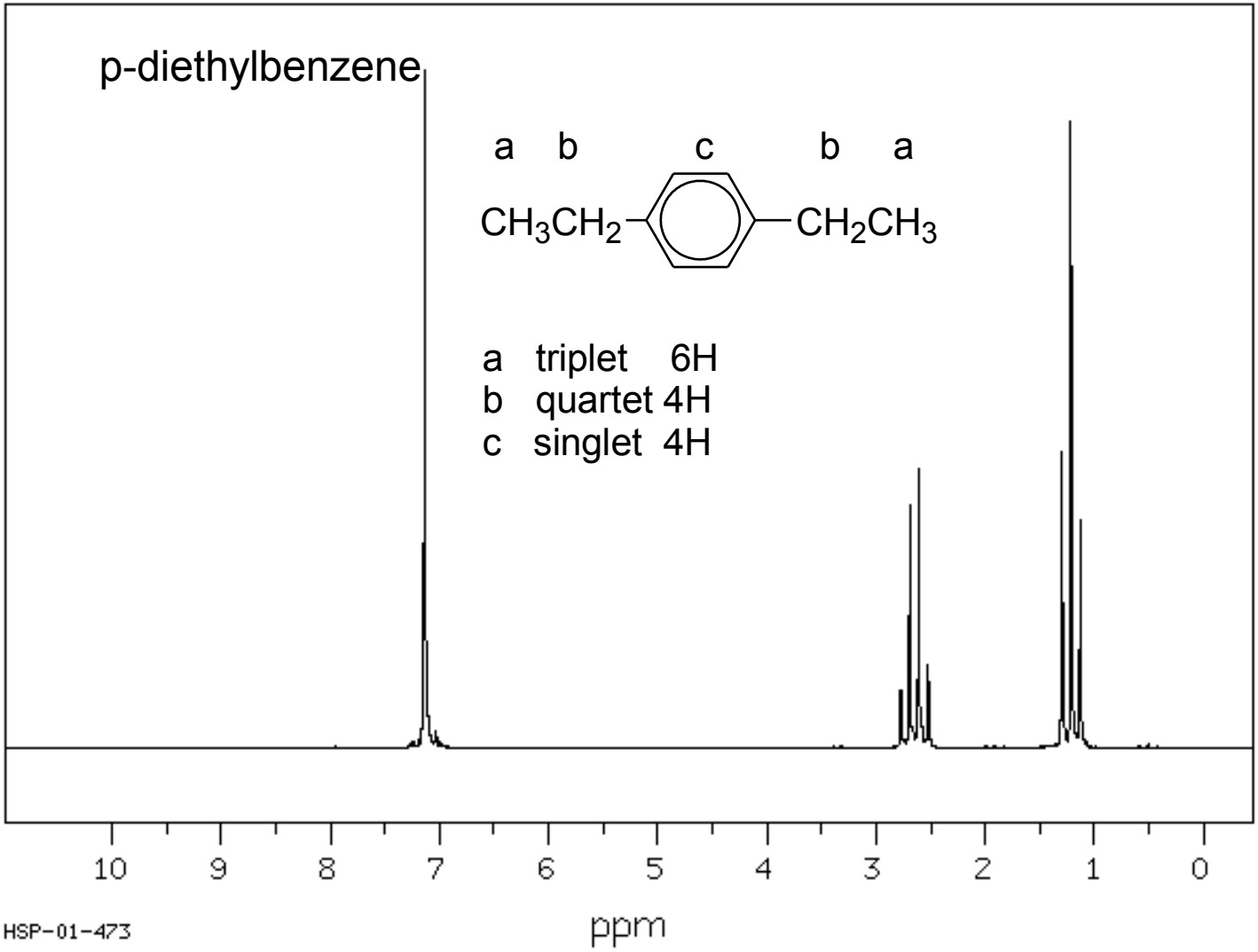

isopropyl chloride
a b a
CH3CHCH3
Cl
a doublet 6H
b septet 1H
10
9
8
7
6
5
4
3
2
1
0
ppm
HPM-03-171

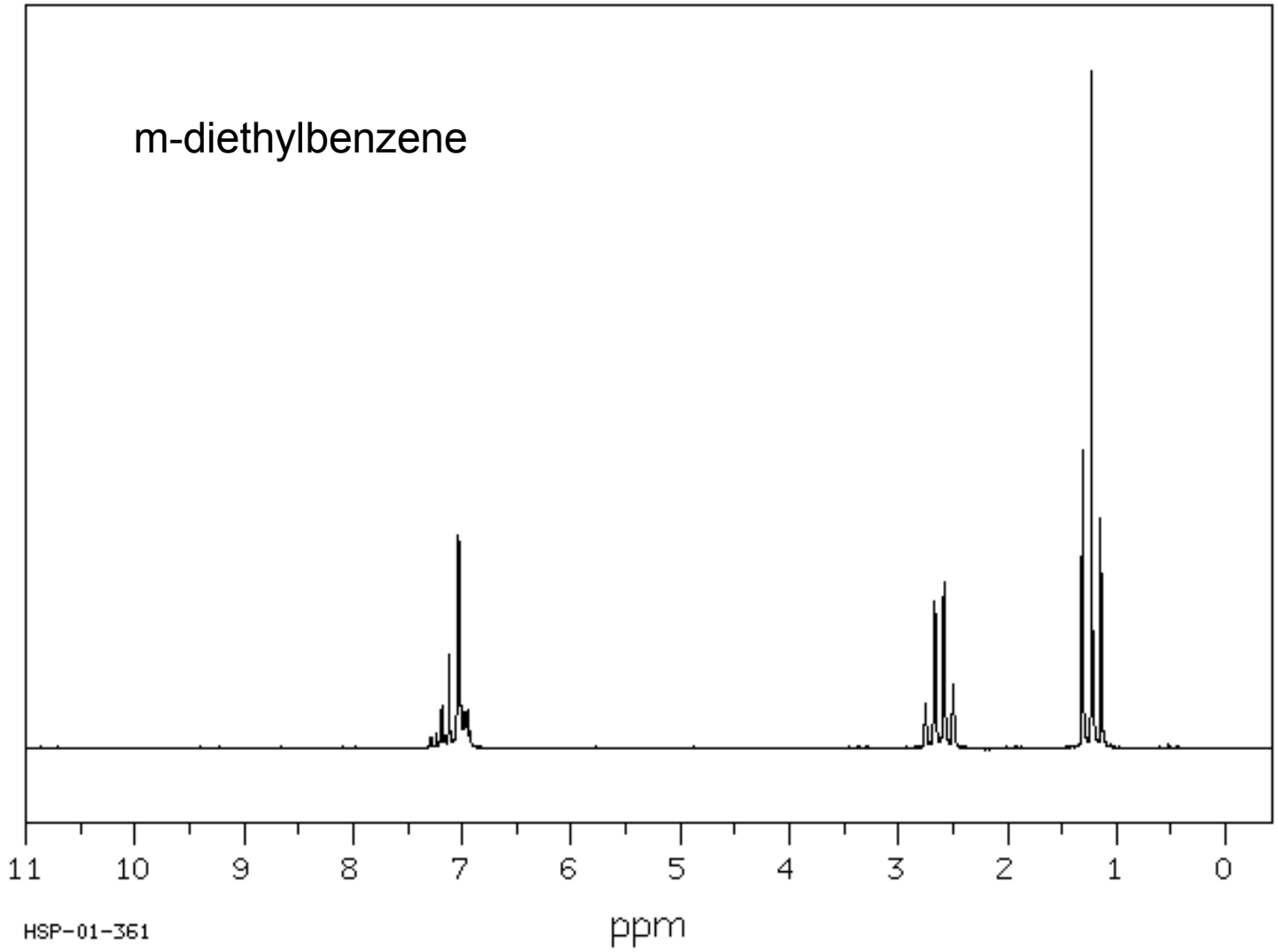

2-bromobutane
b d c a
CH3CHCH2CH3
Br
a triplet 3H
b doublet 3H
c complex 2H
d complex 1H
11 10 9 8 7 6 5 4 3 2 1 0
ppm
HSP-02-075

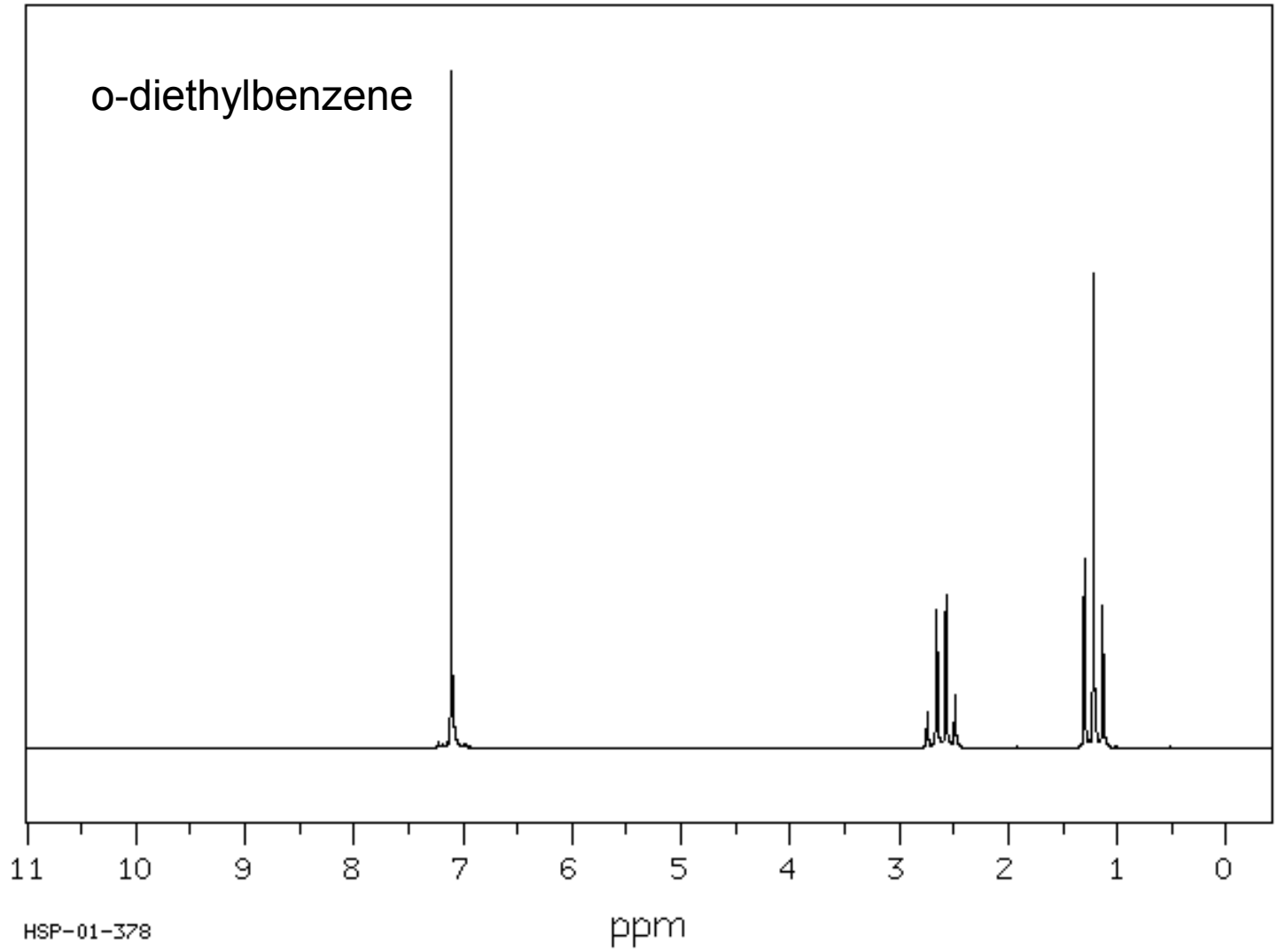

o-methylbenzyl chloride
a
CH3
b
CH2Cl
c
a singlet 3H
b singlet 2H
c ~ singlet 4H
11
10
9
8
7
6
5
4
3
2
1
0
ppm
HSP-01-217

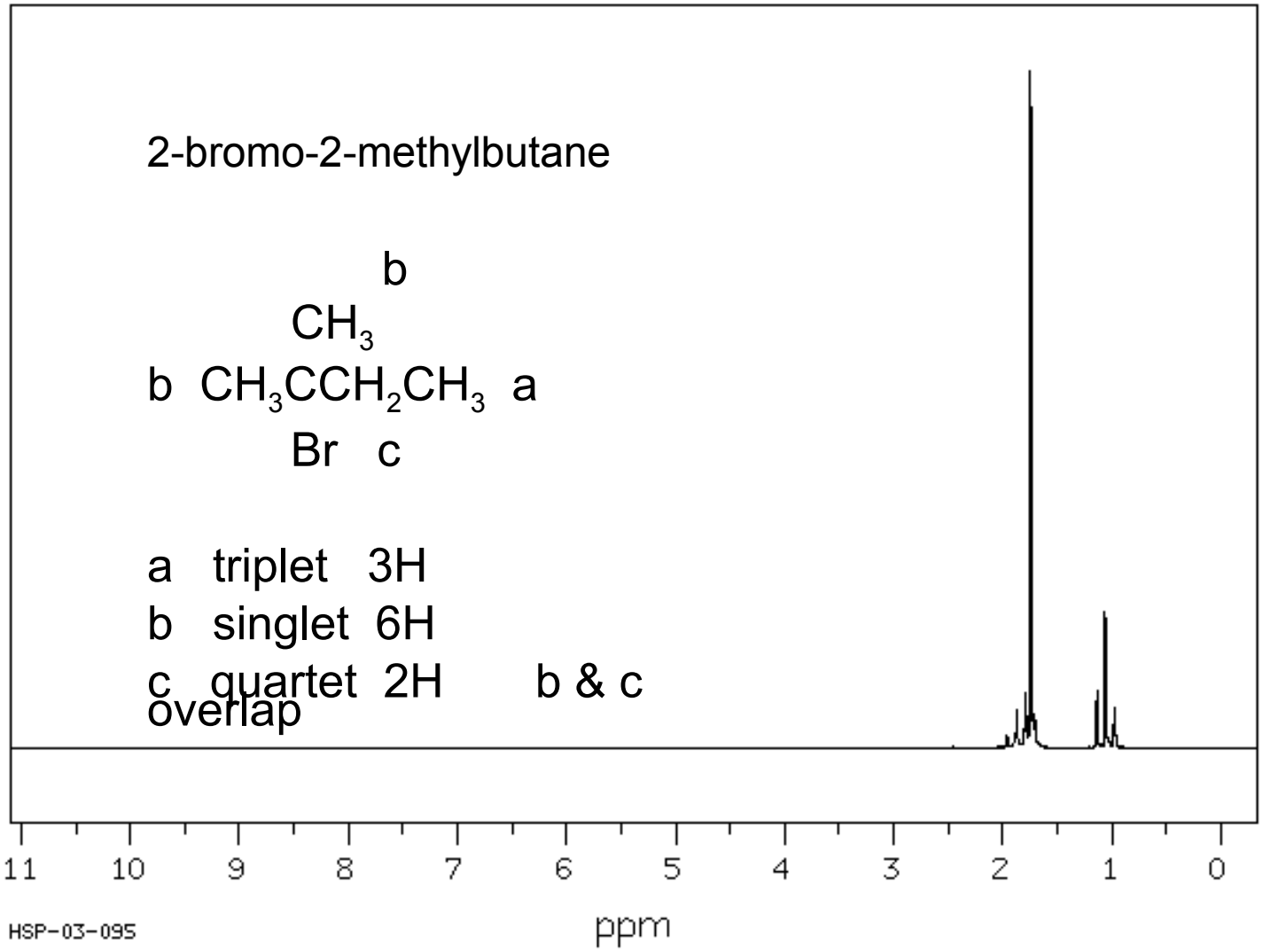

ethanol
a c b
CH3CH2-OH
a triplet 3H
b singlet 1H
c quartet 2H
11 10 9 8 7 6 5 4 3 2 1 0
HSP-01-876
ppm

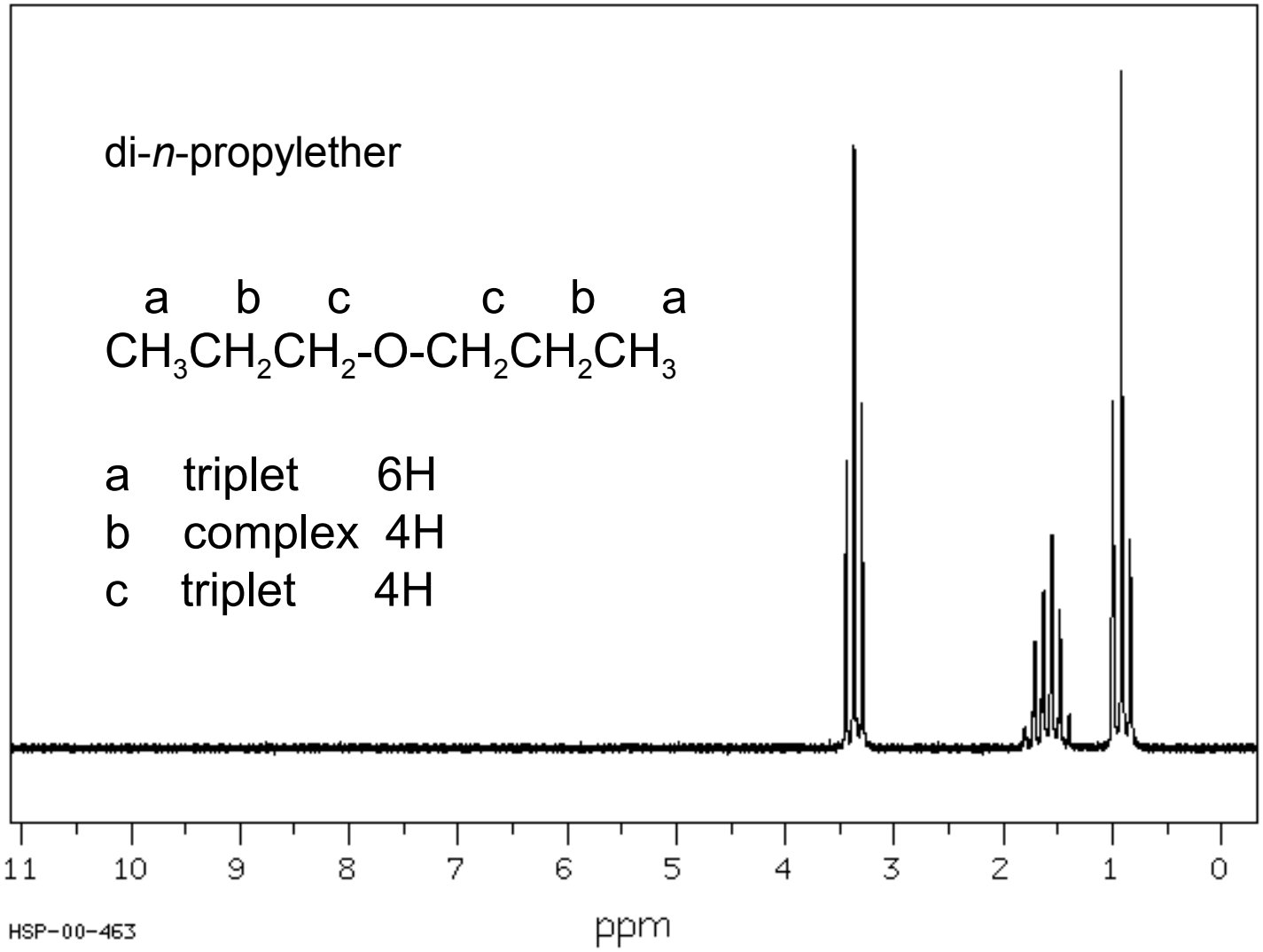

ethylbenzene
c
b a
CH_2CH_3
a triplet 3H
b quartet 2H
c ~singlet 5H
11 10 9 8 7 6 5 4 3 2 1 0
ppm
HSP-00-065

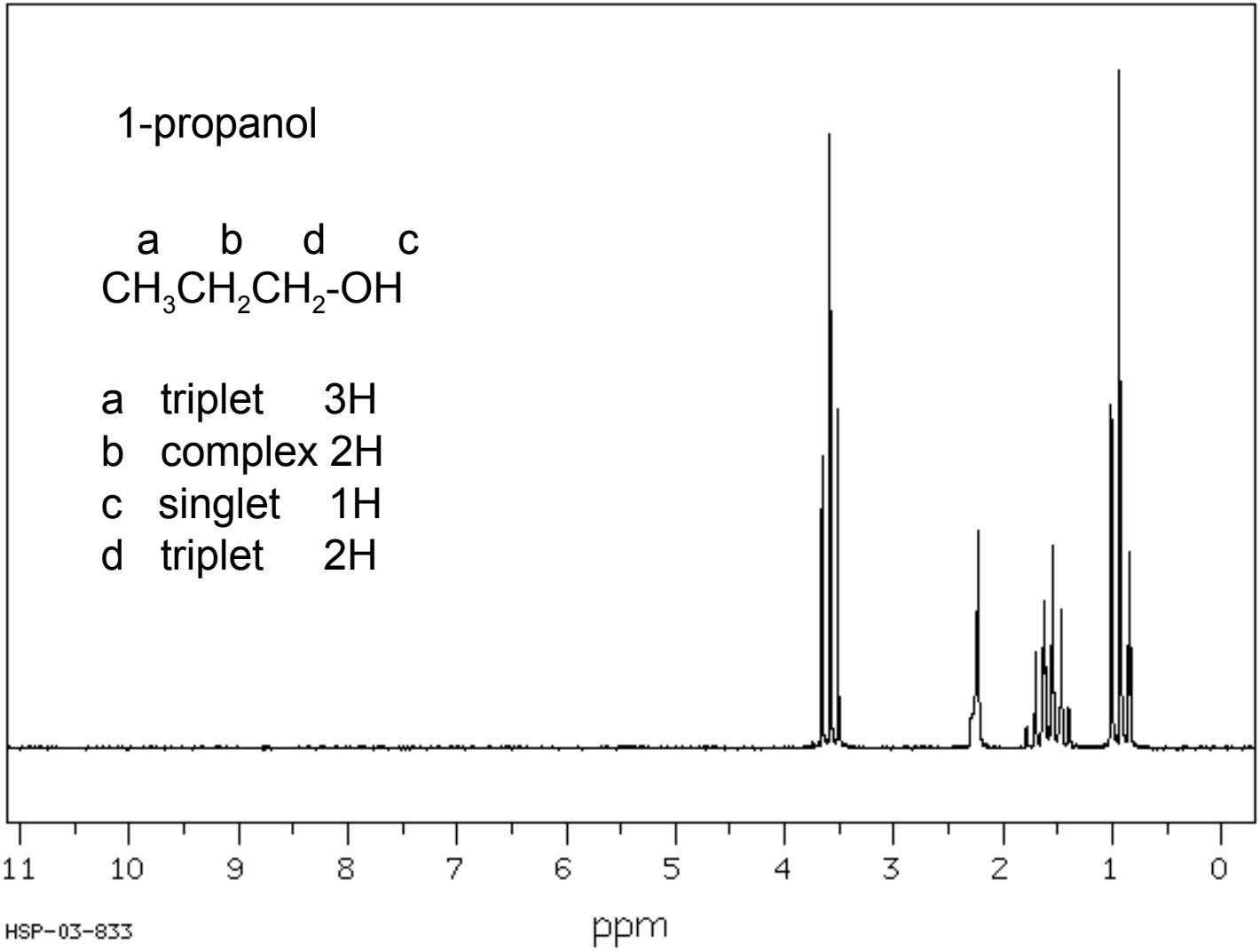

p-diethylbenzene
a b c b a
CH3CH2
CH2CH3
a triplet 6H
b quartet 4H
c singlet 4H
10 9 8 7 6 5 4 3 2 1 0
ppm
HSP-01-473


m-diethylbenzene
11
10
9
8
7
6
5
4
3
2
1
0
HSP-01-361
ppm

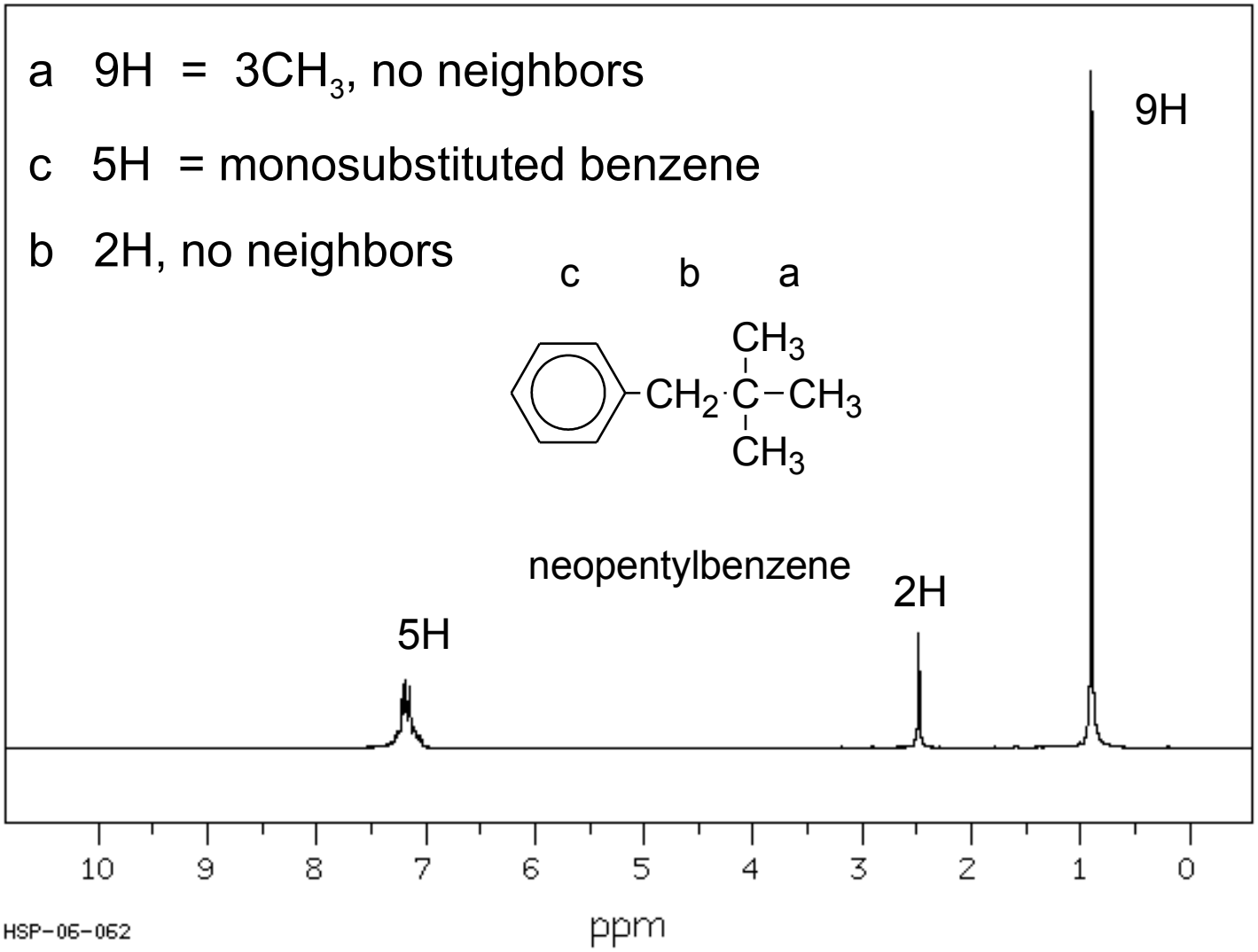

o-diethylbenzene
11
10
9
8
7
6
5
4
3
2
1
0
HSP-01-378
ppm


2-bromo-2-methylbutane
b
CH3
b CH3CCH2CH3 a
Br c
a triplet 3H
b singlet 6H
c quartet 2H b & c
overlap
11 10 9 8 7 6 5 4 3 2 1 0
HSP-03-095
ppm

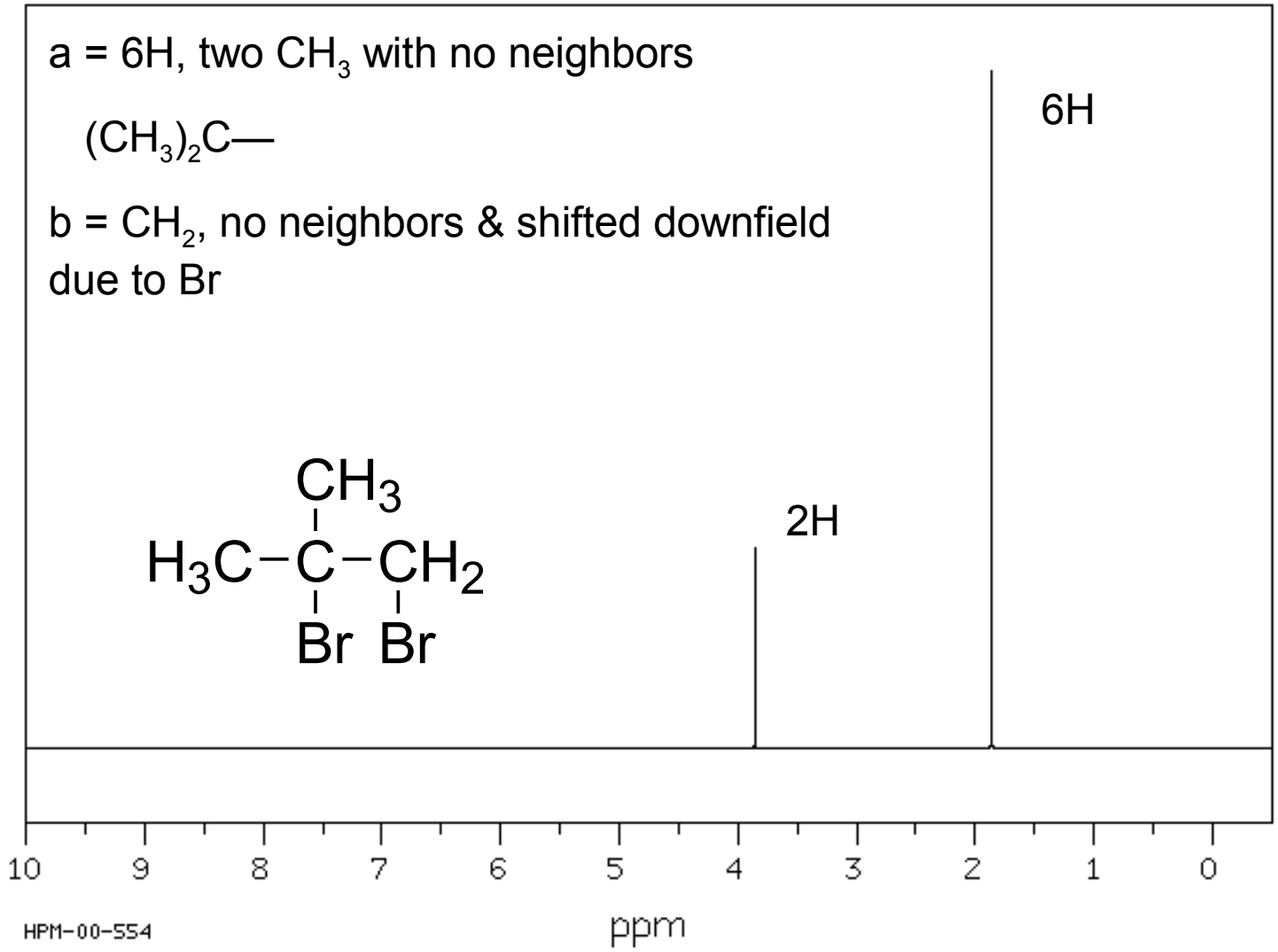

di-*n*-propylether
a b c c b a
$CH_3CH_2CH_2$-O-$CH_2CH_2CH_3$
a triplet 6H
b complex 4H
c triplet 4H
11 10 9 8 7 6 5 4 3 2 1 0
ppm
HSP-00-463


1-propanol
a b d c
CH3CH2CH2-OH
a triplet 3H
b complex 2H
c singlet 1H
d triplet 2H
11
10
9
8
7
6
5
4
3
2
1
0
HSP-03-833
ppm

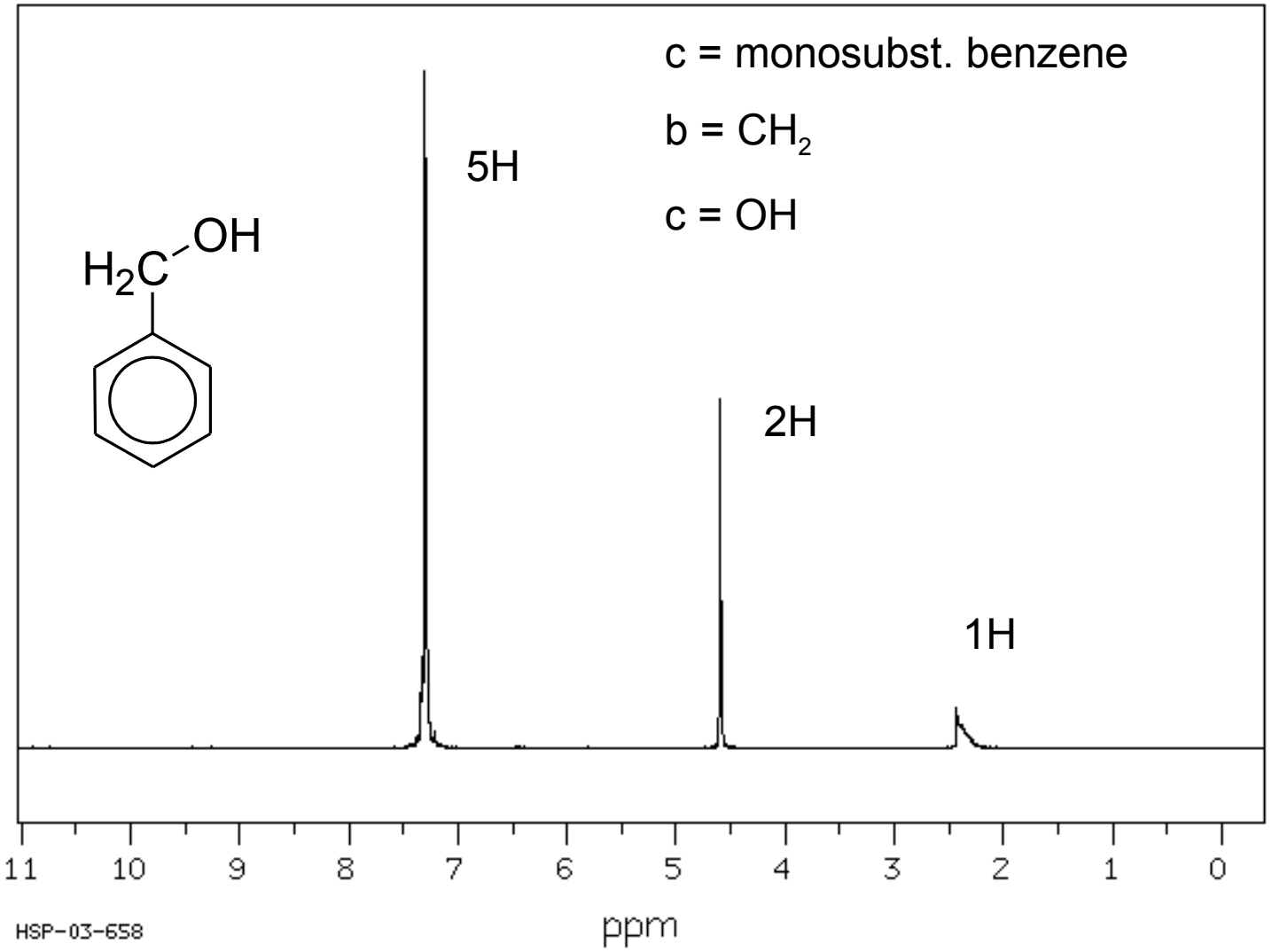
$C_{11}H_{16}$


a 9H = 3CH3, no neighbors
c 5H = monosubstituted benzene
b 2H, no neighbors
c b a
CH3
CH2 C CH3
CH3
neopentylbenzene
9H
2H
5H
10 9 8 7 6 5 4 3 2 1 0
ppm
HSP-06-062

$C_4H_8Br_2$


a = 6H, two CH3 with no neighbors
(CH3)2C—
b = CH2, no neighbors & shifted downfield due to Br
6H
2H
CH3
H3C–C–CH2
Br Br
10 9 8 7 6 5 4 3 2 1 0
HPM-00-554
ppm

C_7H_8O

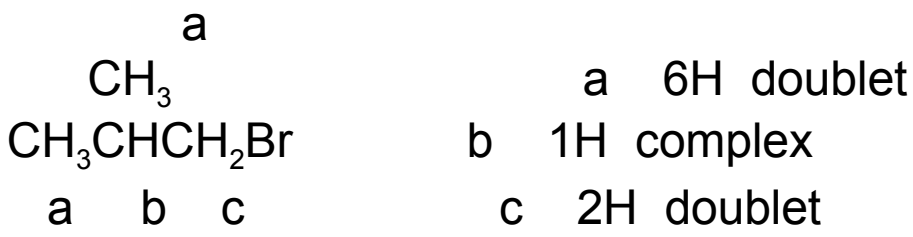

c = monosubst. benzene
b = CH2
c = OH
5H
2H
1H
H2C
OH
11
10
9
8
7
6
5
4
3
2
1
0
ppm
HSP-03-658

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)



a 6H doublet

b 1H complex

c 2H doublet

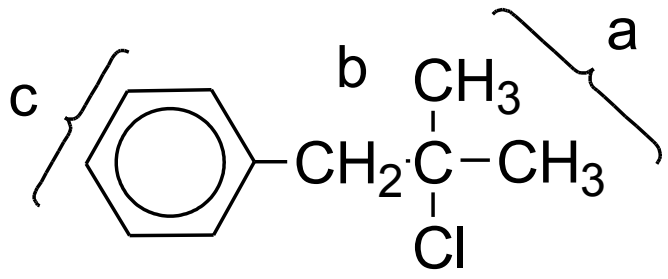
$C_{10}H_{13}Cl$

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



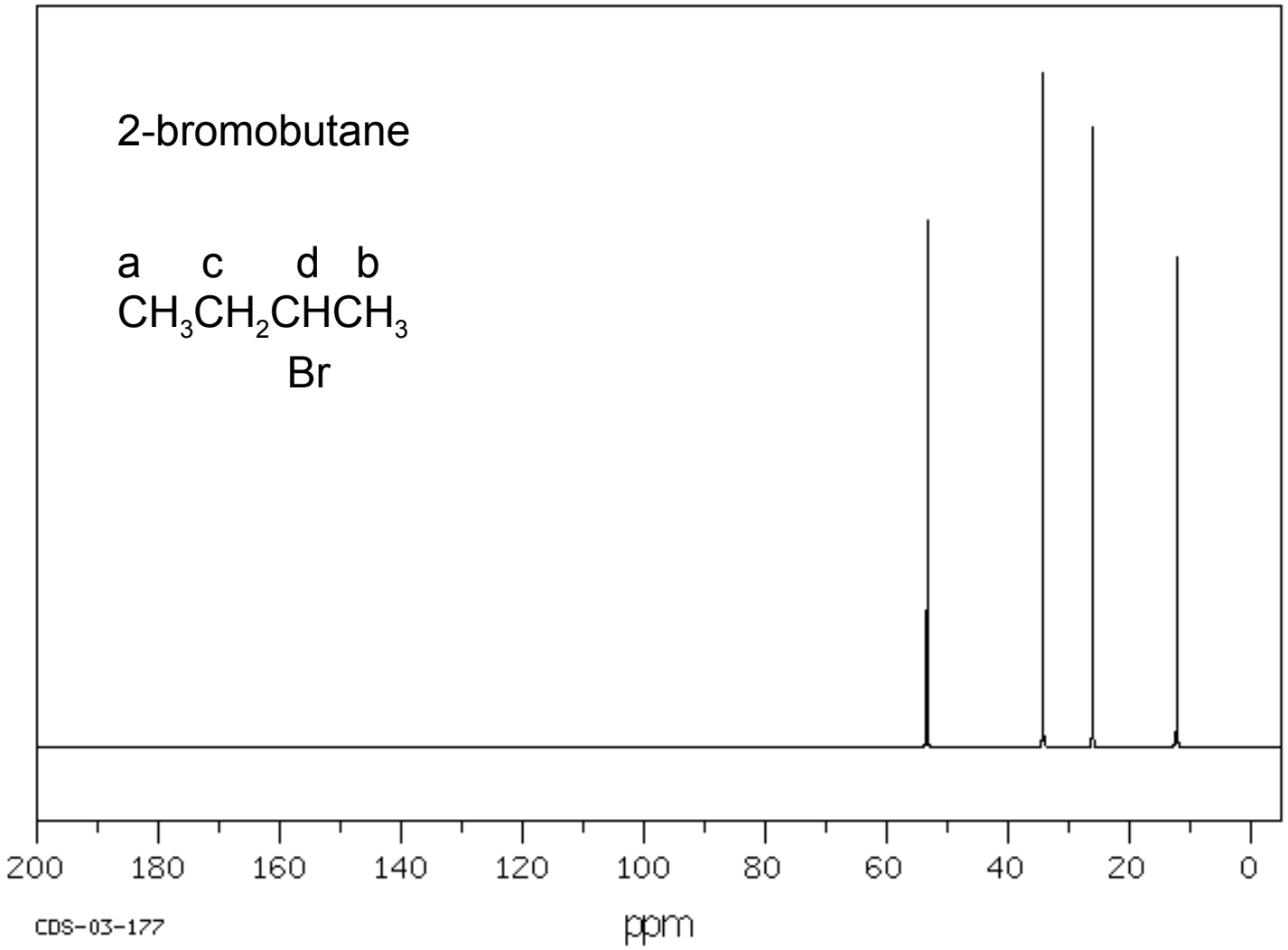
a singlet 6H
b singlet 2H
c singlet 5H

^{13}C – nmr

^{13}C ~ 1.1% of carbons

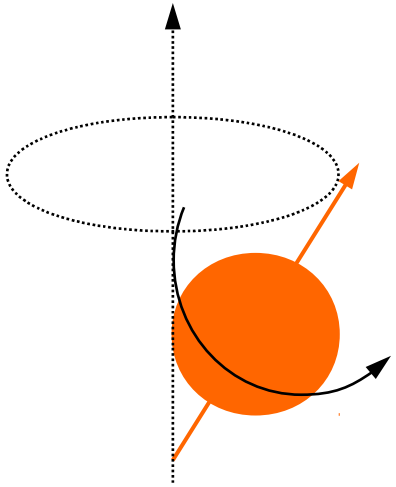
1) number of signals: how many different types of carbons
2) splitting: number of hydrogens on the carbon
3) chemical shift: hybridization of carbon sp, sp^2, sp^3
4) chemical shift: evironment

^{13}C-nmr


2-bromobutane
a c d b
CH3CH2CHCH3
Br
200
180
160
140
120
100
80
60
40
20
0
CDS-03-177
ppm

Angular Momentum

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



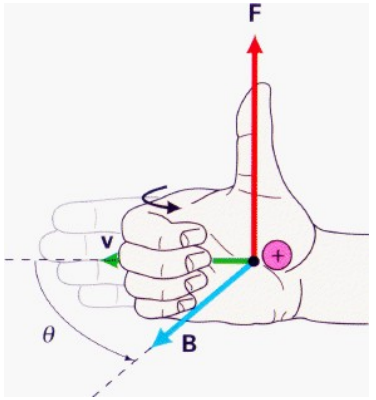
magnetic moment $\boldsymbol{\mu} = \gamma \boldsymbol{p}$
where γ is the gyromagnetic ratio,
and it is a constant for a given nucleus

$$\mu = \gamma p = \gamma \sqrt{I(I+1)}h/2\pi$$ When I=0, μ=0

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

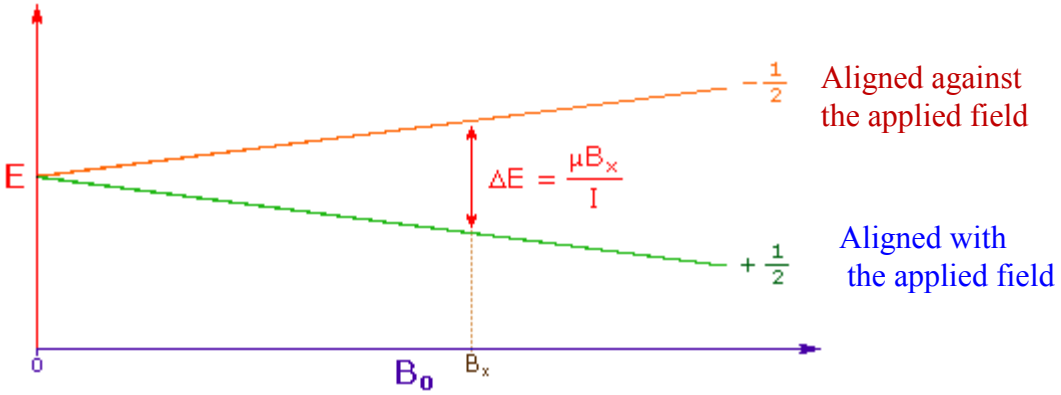
"Right Hand Rule"

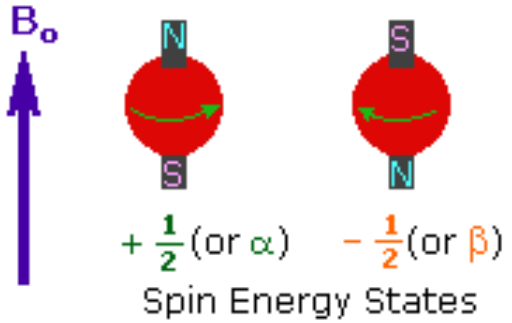
determines the direction of the magnetic field around a current-carrying wire and vice-versa



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.





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

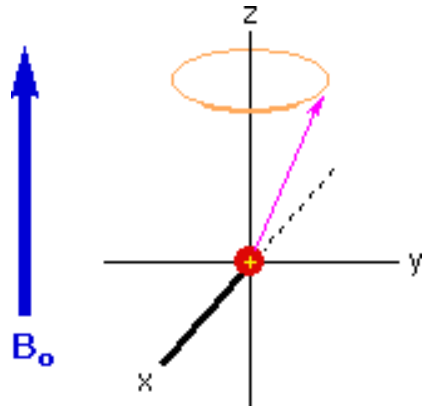
When the energy of the photon matches the energy difference between the two spin states , an absorption of energy occurs. We call that phenomenon *Resonance*

$$\Delta E = h\upsilon = \gamma h B_o / 2\pi \qquad \text{So, } \upsilon = \gamma B_o / 2\pi$$

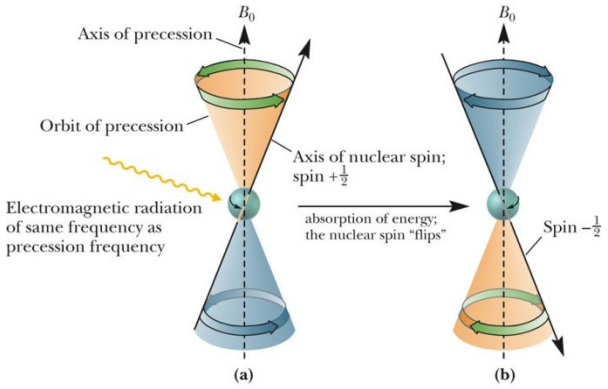
Larmor Precession

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



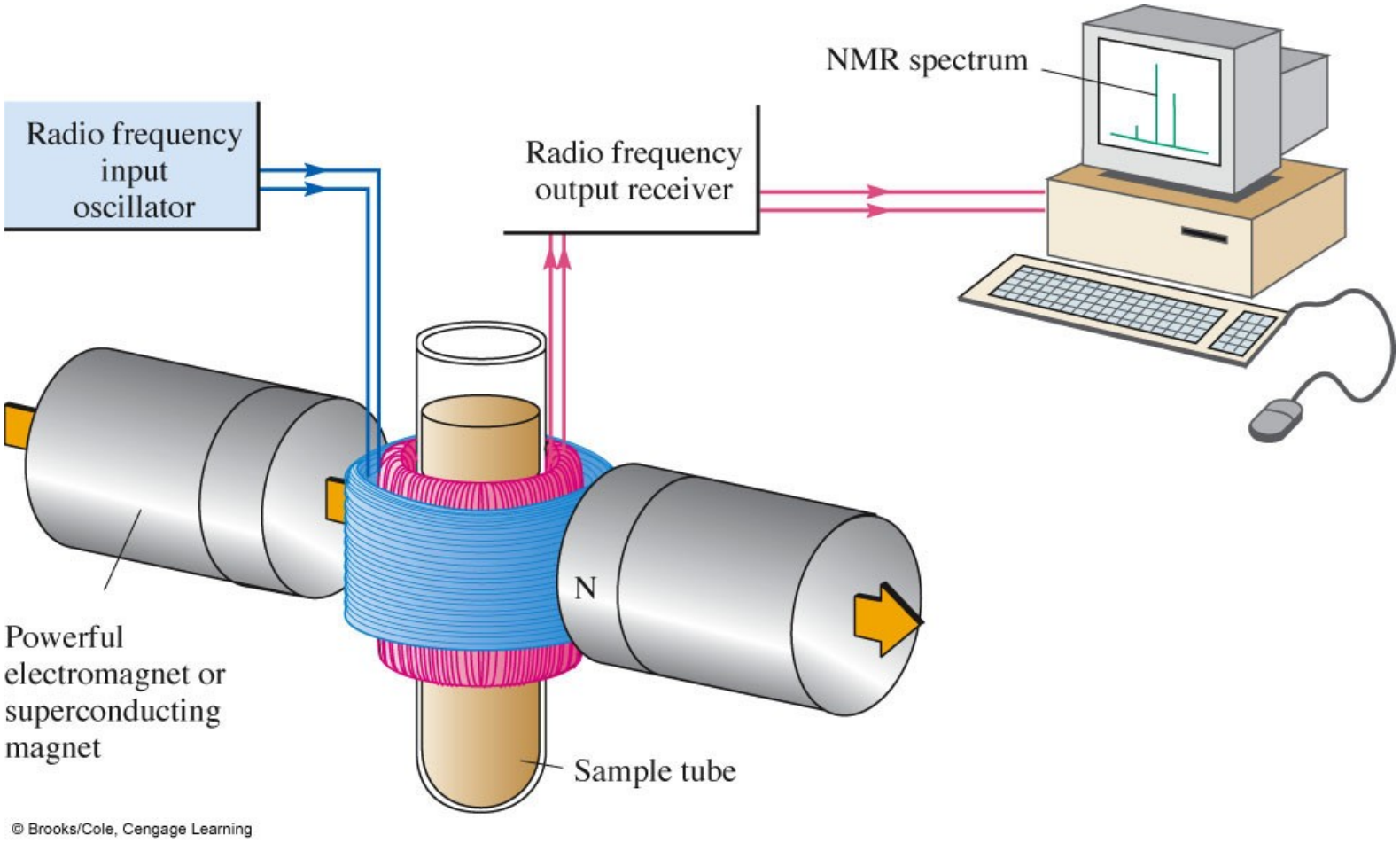
Brown/Foote/Iverson
Organic Chemstry 5e
Figure 13.03

Date_070607

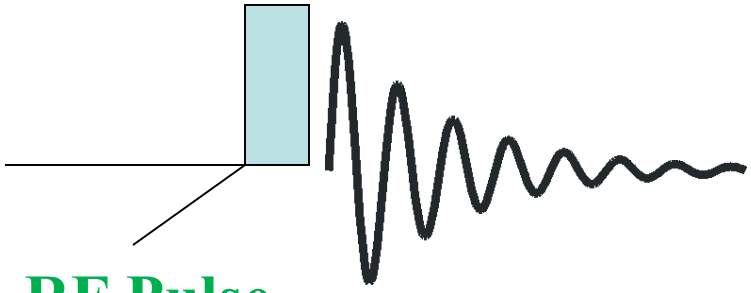
γ- Values for some nuclei

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

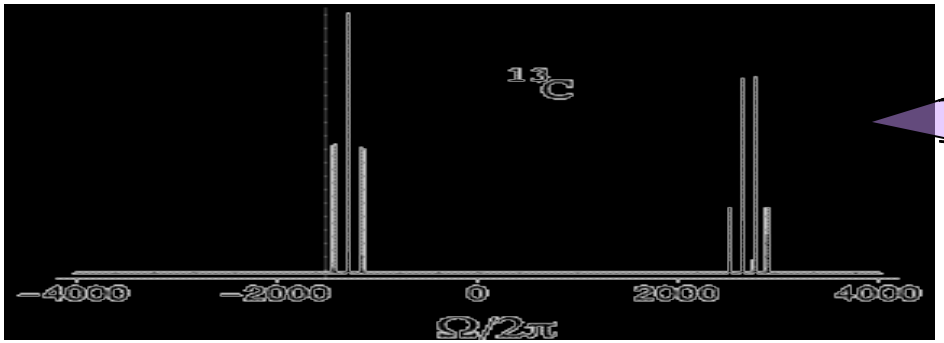


Fourier transformation and the NMR spectrum



RF Pulse

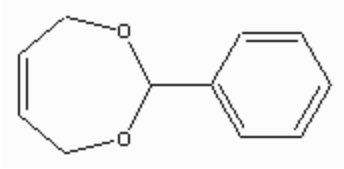
Fourier transform



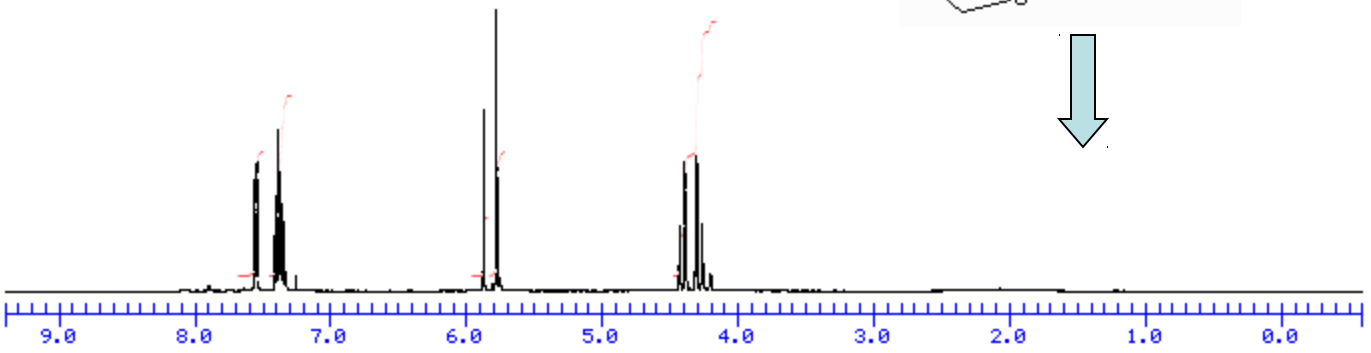
The NMR spectrum

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

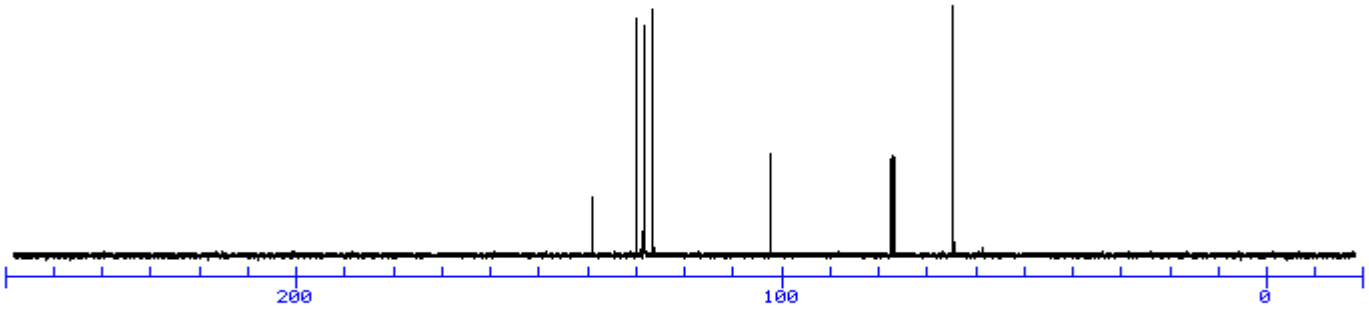


δ ppm

Down field

High field

^{13}C NMR spectra



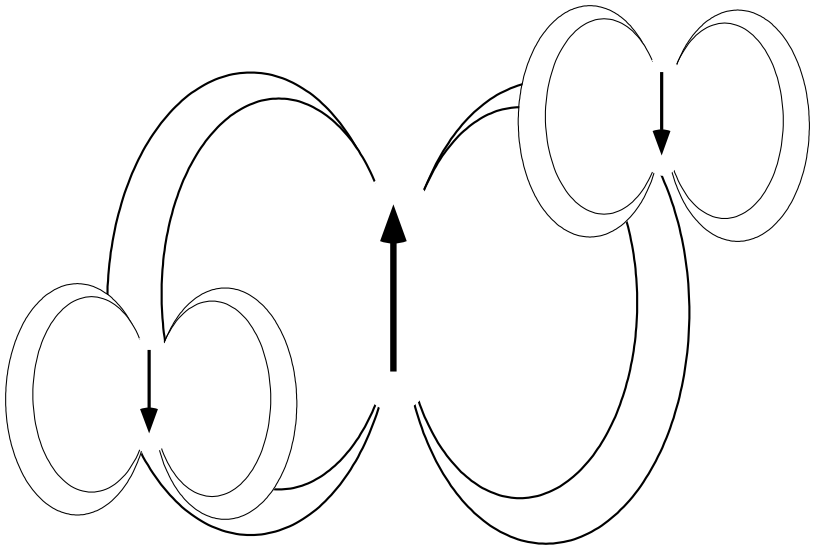
δ ppm

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.

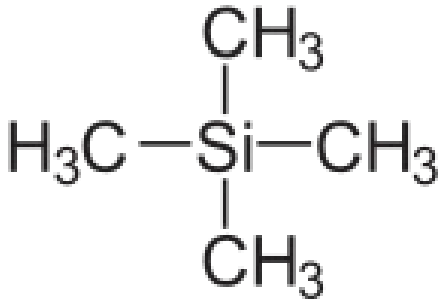
The term chemical shift was developed to avoid this problem. The chemical shift of a nucleus is the difference between the resonance frequency of the nucleus and a standard, relative to the standard. This quantity is reported in ppm and given the symbol delta.

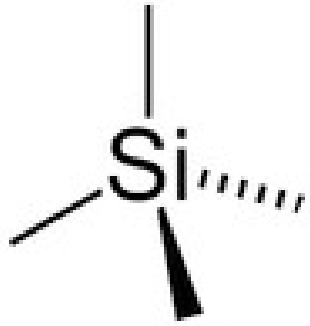
$$\delta = (\nu - \nu_{ref}) \times 10^6 / \nu_{ref}$$

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

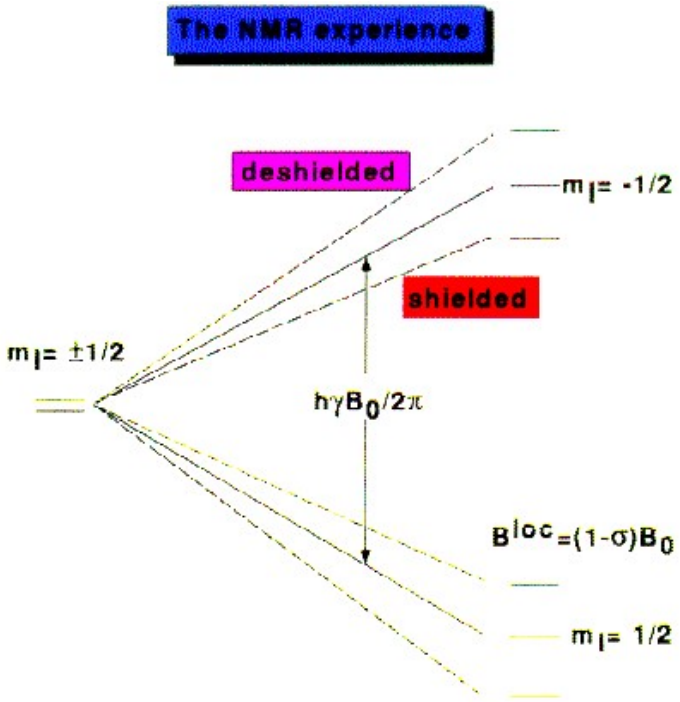




Shielding and Deshielding

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:

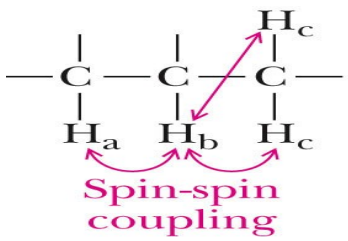
The coupling of the intrinsic angular momentum of different particles. Such coupling between pairs of nuclear spins is an important feature of nuclear magnetic resonance (NMR) spectroscopy as it can provide detailed information about the structure and conformation of molecules. Spin-spin coupling between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

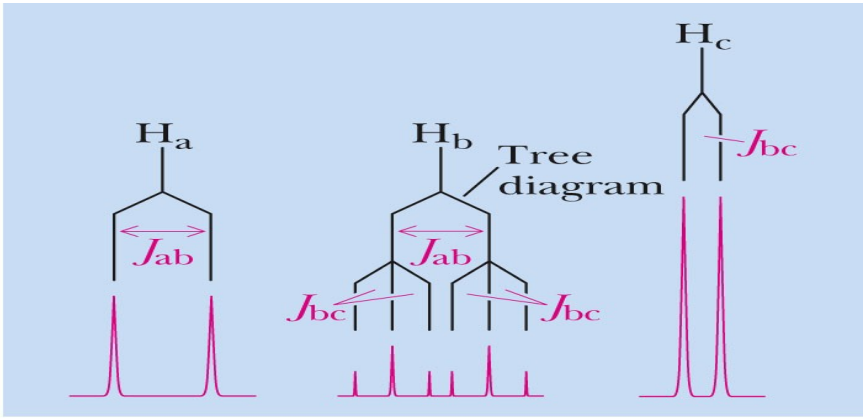
J-Coupling

***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

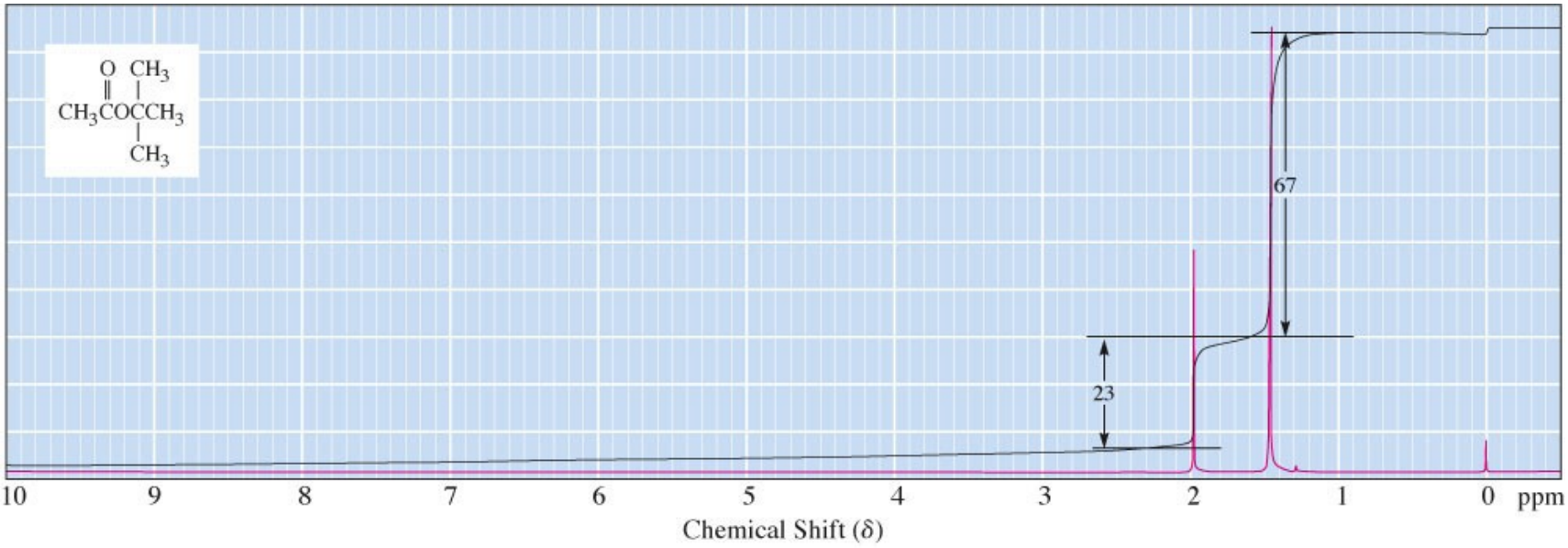




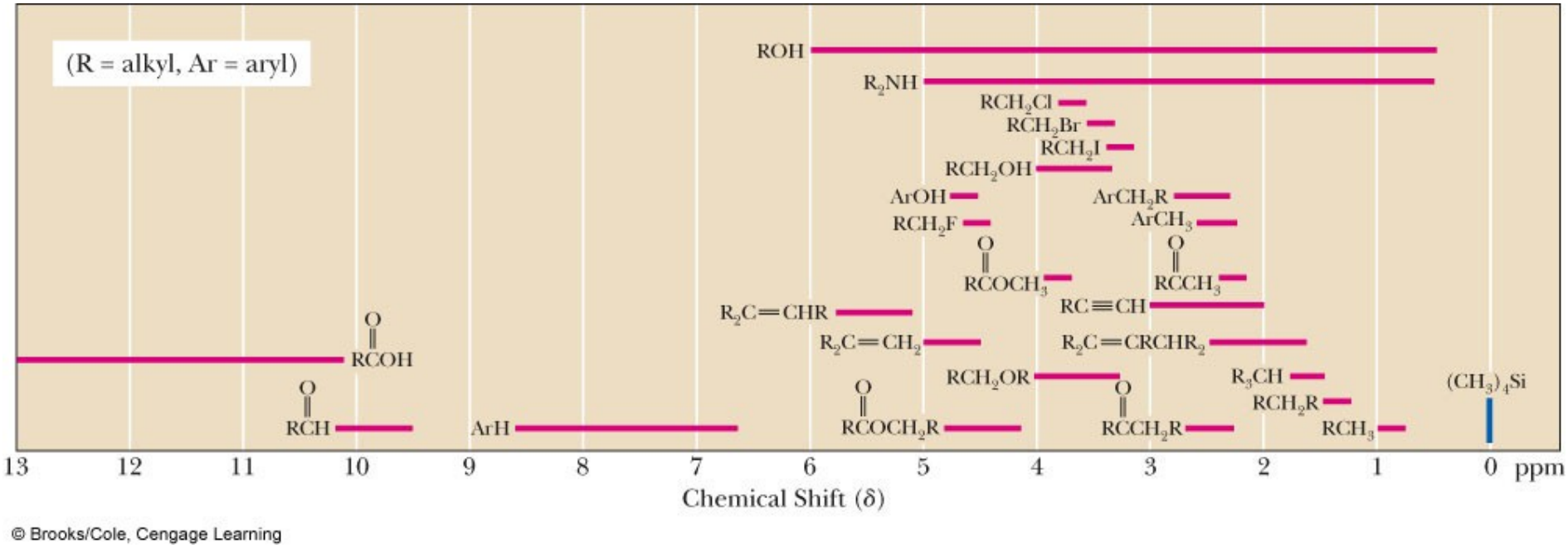
^{1}H-NMR

- ^{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.



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)	$RCOCH_3$	3.7-3.9
RCH_3	0.8-1.0	$RCOCH_2R$	4.1-4.7
RCH_2R	1.2-1.4	RCH_2I	3.1-3.3
R_3CH	1.4-1.7	RCH_2Br	3.4-3.6
$R_2C=CRCHR_2$	1.6-2.6	RCH_2Cl	3.6-3.8
$RC\equiv CH$	2.0-3.0	RCH_2F	4.4-4.5
$ArCH_3$	2.2-2.5	$ArOH$	4.5-4.7
$ArCH_2R$	2.3-2.8	$R_2C=CH_2$	4.6-5.0
ROH	0.5-6.0	$R_2C=CHR$	5.0-5.7
RCH_2OH	3.4-4.0	ArH	6.5-8.5
RCH_2OR	3.3-4.0	$RCHO$	9.5-10.1
R_2NH	0.5-5.0	$RCOH$	10-13
$RCCH_3$	2.1-2.3		
$RCCH_2R$	2.2-2.6		

Factors to Affect ^{1}H Chemical Shift

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

Electronegativity

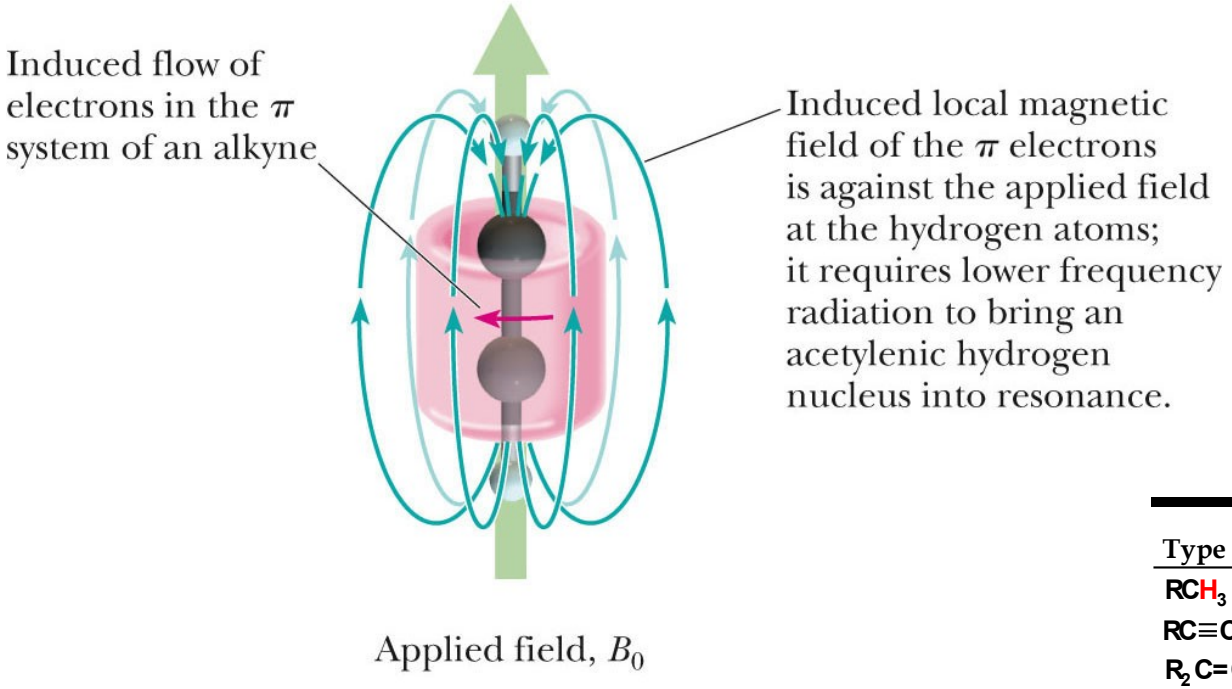
CH_3-X	Electroneg-ativity of X	Chemical Shift (δ)
CH_3F	4.0	4.26
CH_3OH	3.5	3.47
CH_3Cl	3.1	3.05
CH_3Br	2.8	2.68
CH_3I	2.5	2.16
$(CH_3)_4C$	2.1	0.86
$(CH_3)_4Si$	1.8	0.00

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

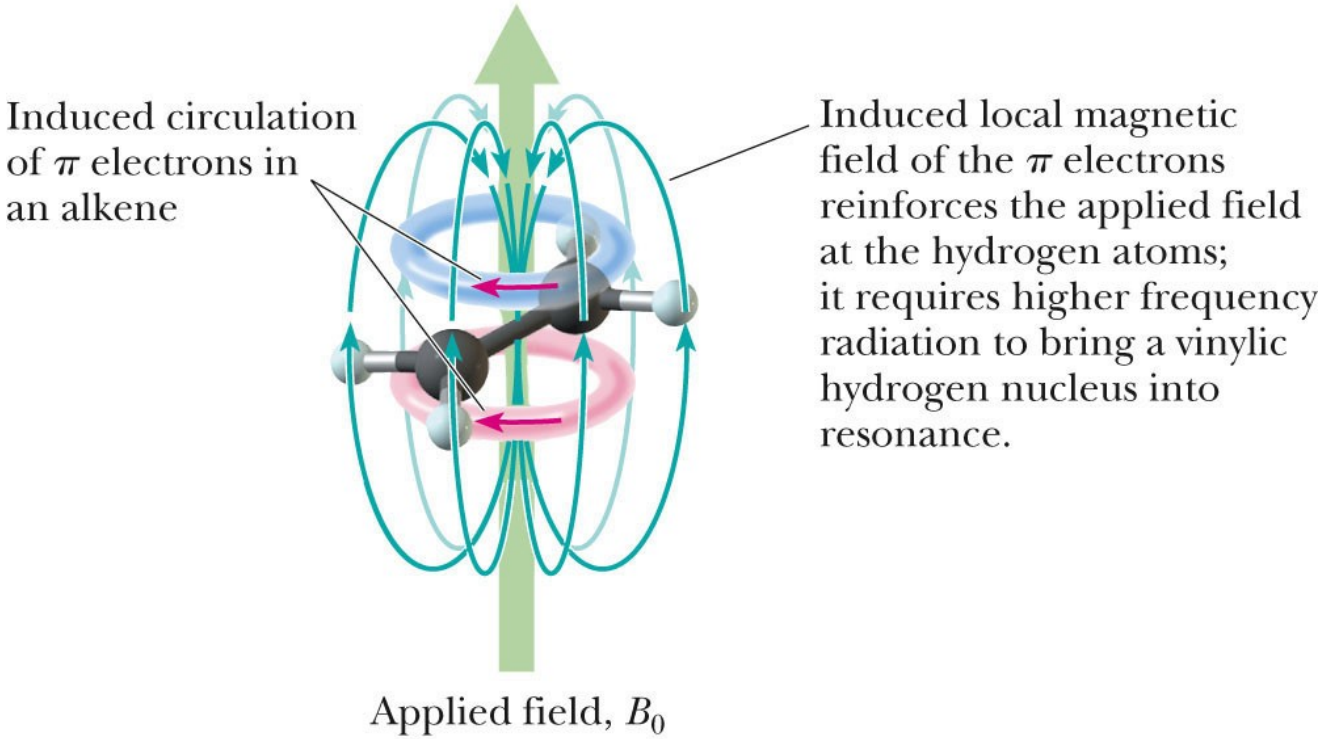
A carbon-carbon triple bond shields an acetylenic hydrogen and shifts its signal to lower frequency (to the right) to a smaller value



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

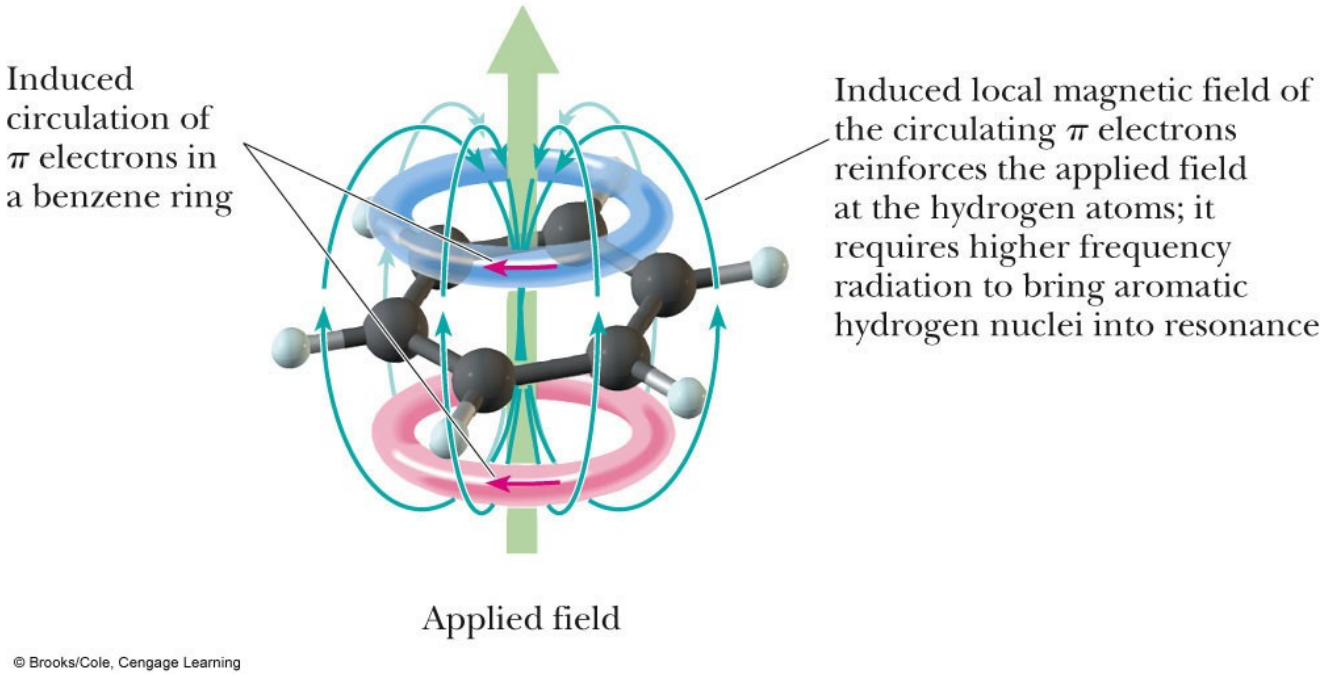
Carbon-Carbon Double Bond Effect

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



Aromatic Effect

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



Signal Splitting for ^1H

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.

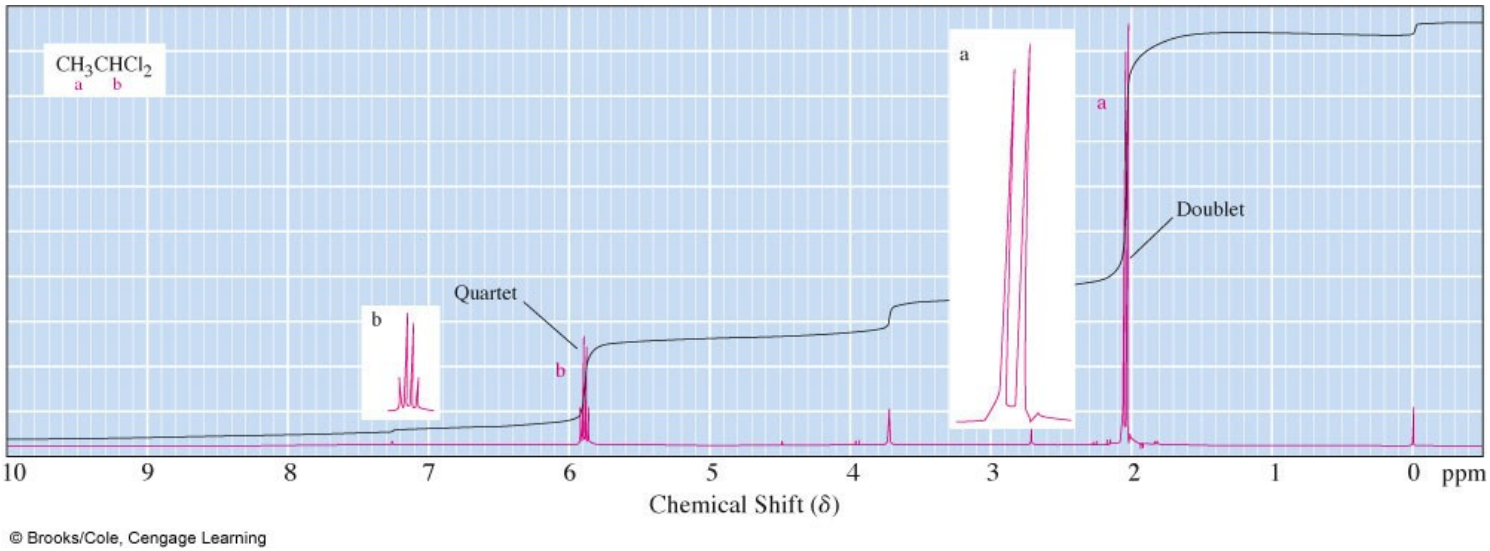
$(n + 1)$ rule:

If a hydrogen has n hydrogens nonequivalent to it but equivalent among themselves on the same or adjacent atom(s), its ^1H-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:

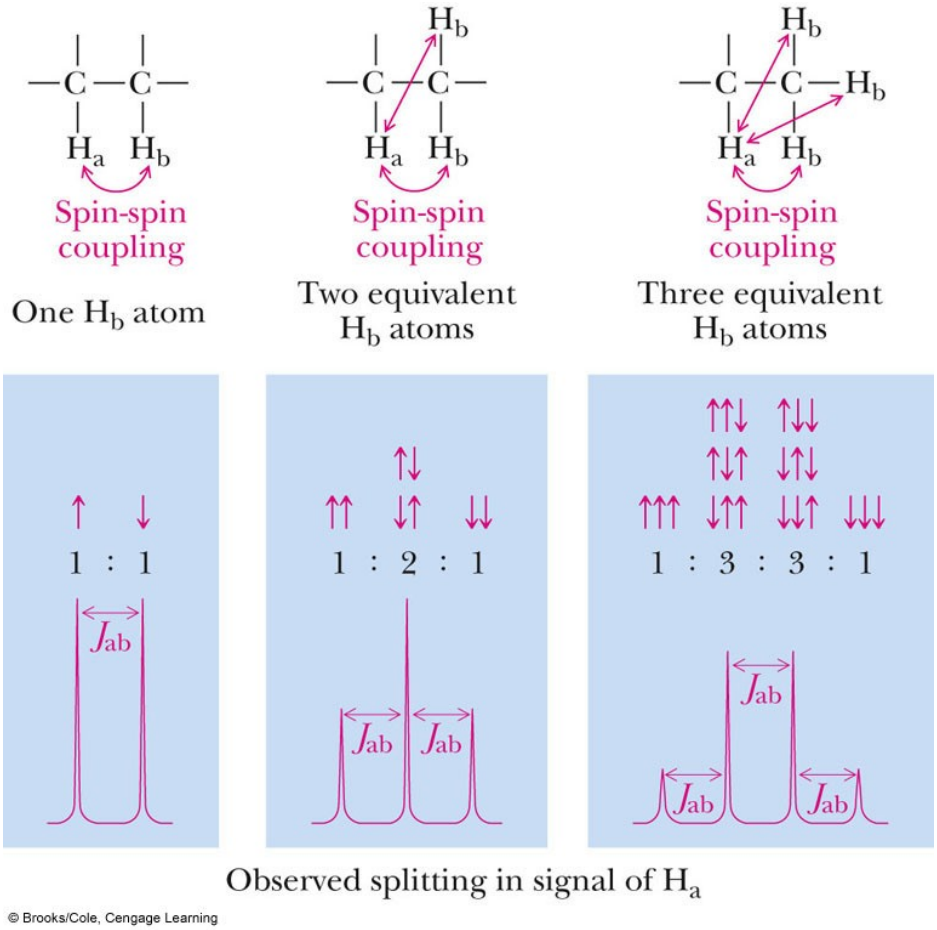
$n = 0$			1			
$n = 1$		1		1		
$n = 2$		1	2	1		
$n = 3$	1	3		3	1	
$n = 4$	1	4	6	4	1	



Coupling constant

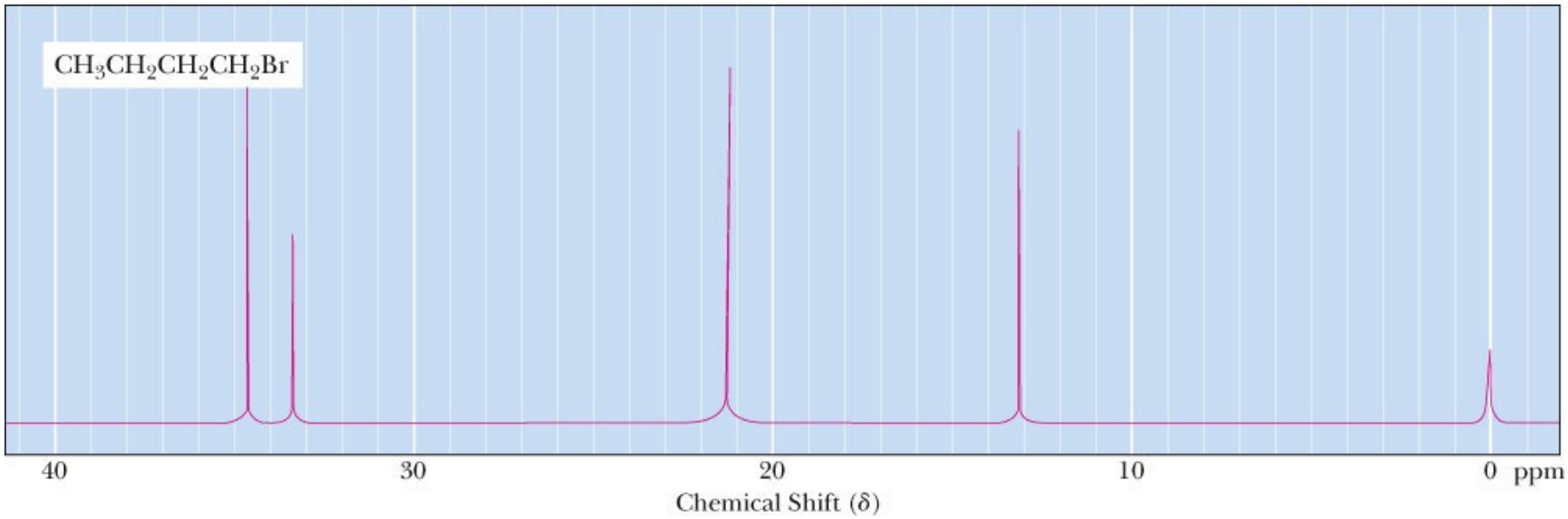
Coupling constant (*J*):

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



^{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



^{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

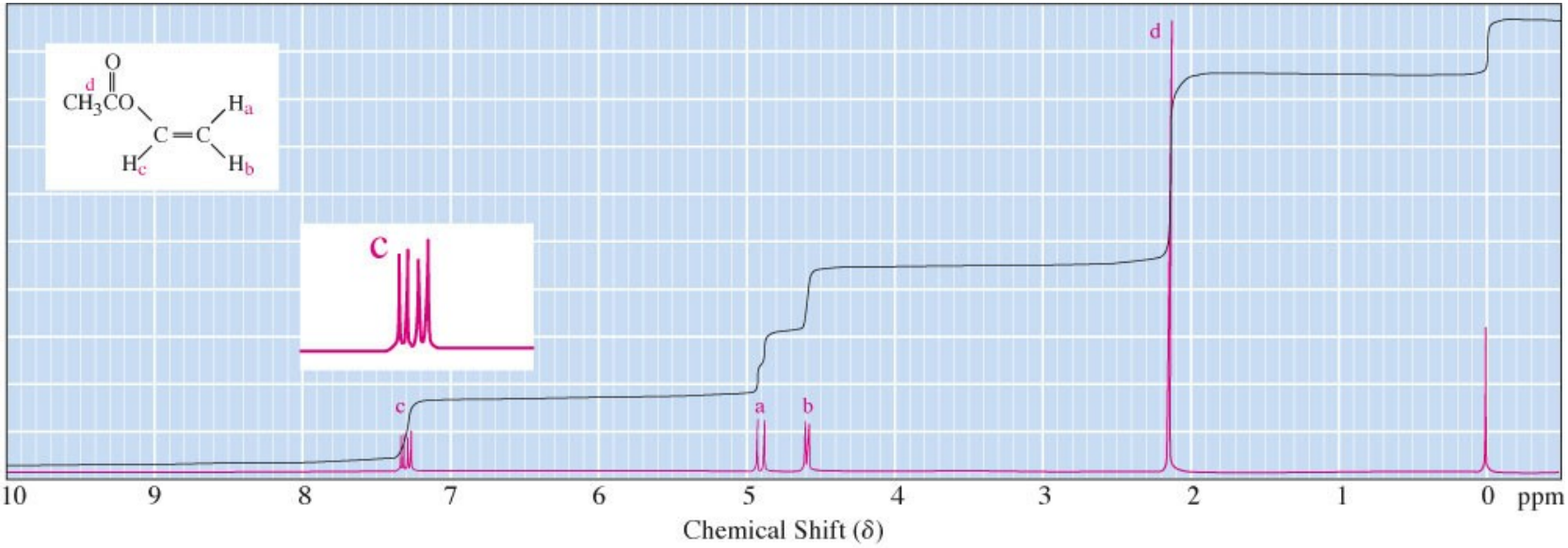
Interpreting NMR Spectra

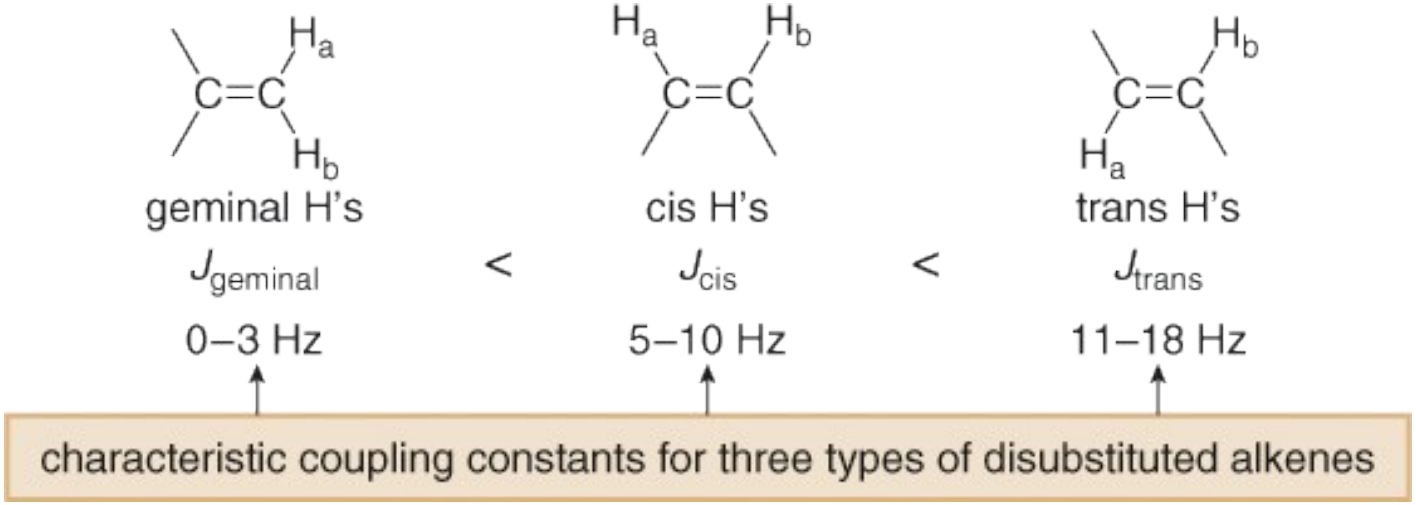
Alkanes

^{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.





Interpreting NMR Spectra

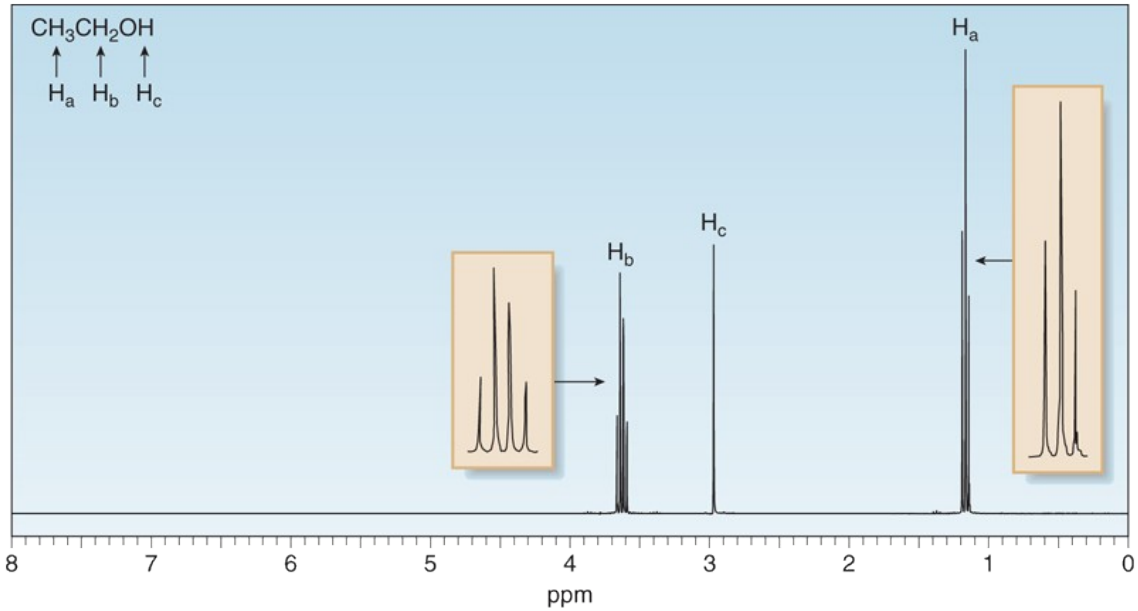
Alcohols

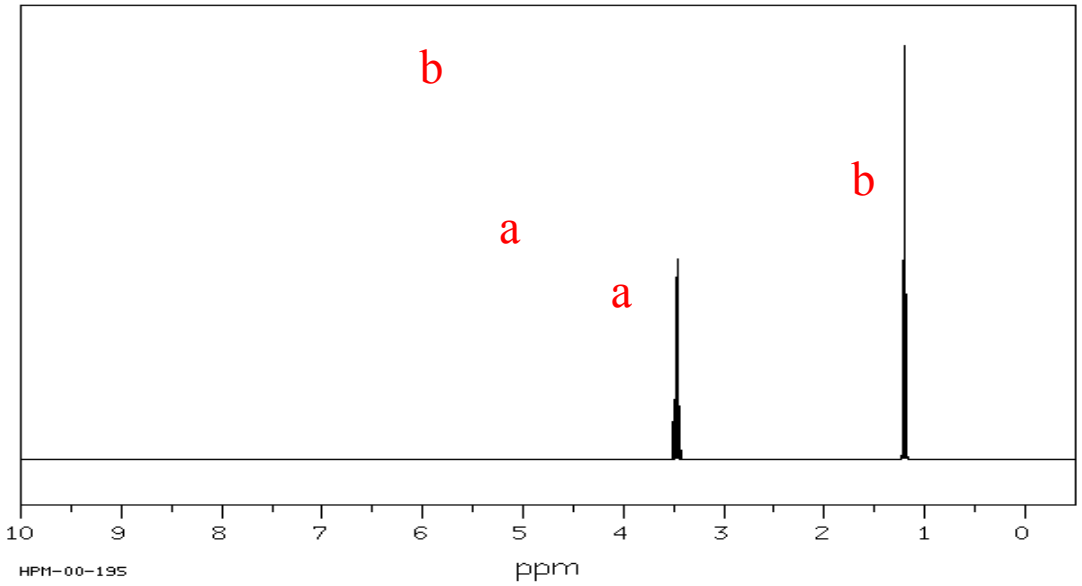
^{1}H-NMR O-H chemical shift often appears in the range 3.0-4.0, but may be as low as 0.5.

^{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


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

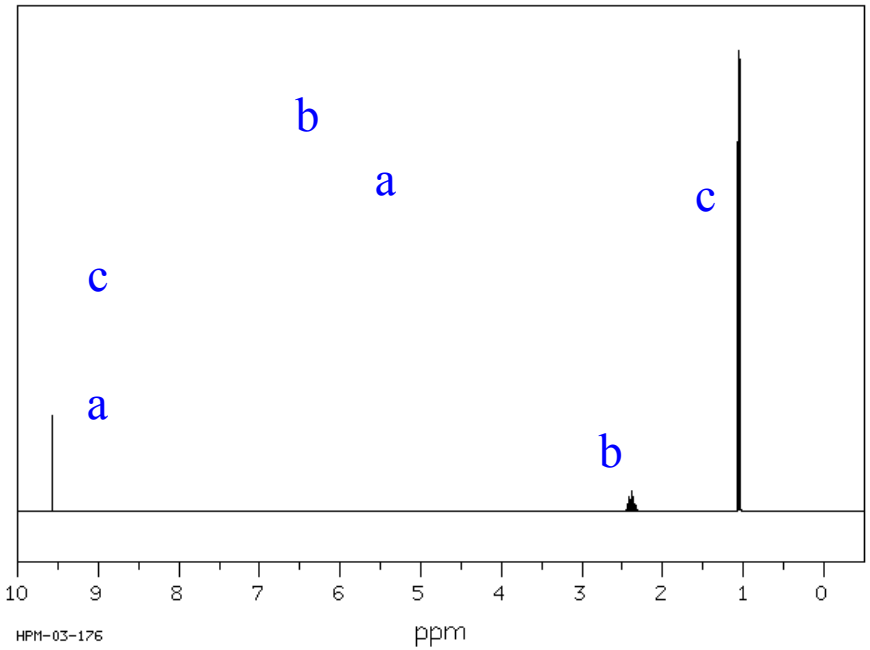
^{1}H-NMR: aldehyde hydrogens appear at 9.5-10.1.

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

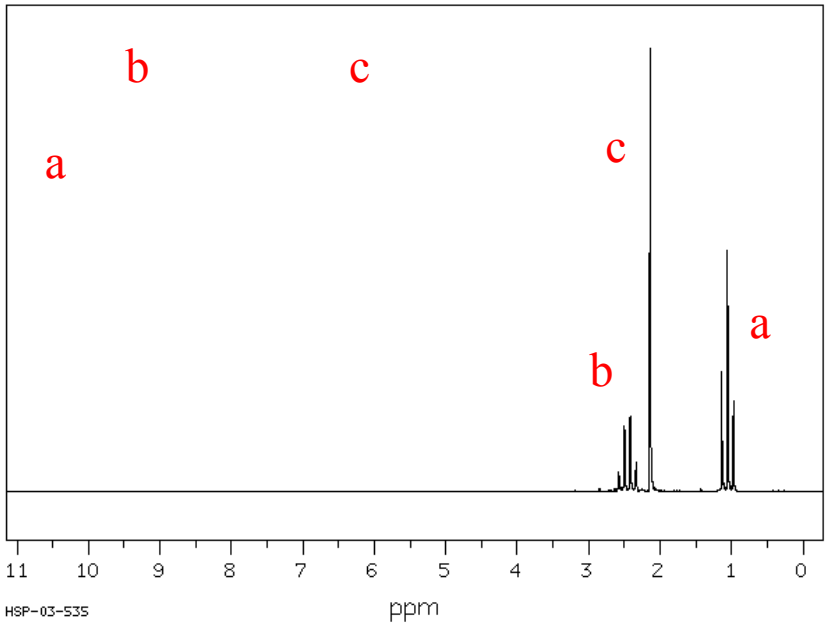
^{13}C-NMR: carbonyl carbons appear at 180-215.

Amines

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



^{1}H NMR isobutyraldehyde



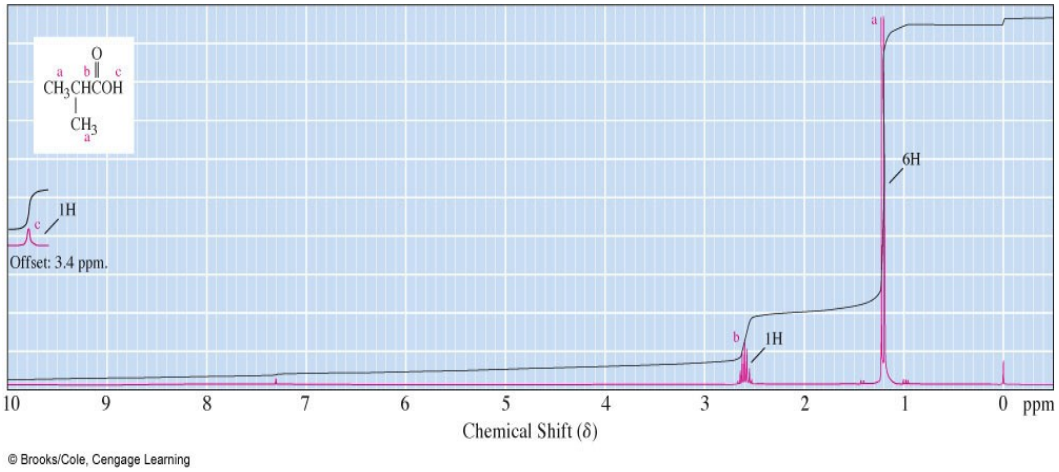
^{1}H NMR Methyl ethyl ketone

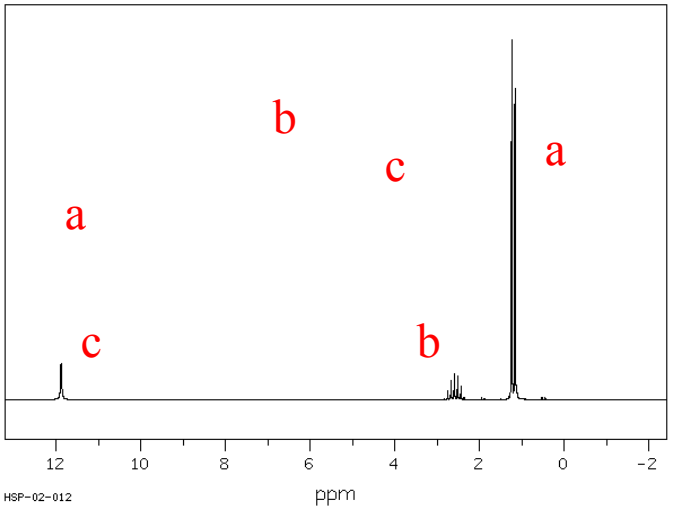
Interpreting NMR Spectra

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.

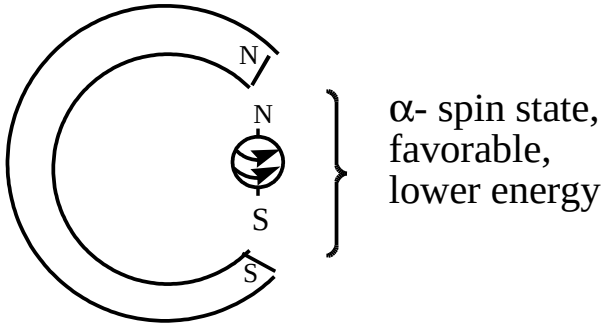




NMR = Nuclear Magnetic Resonance

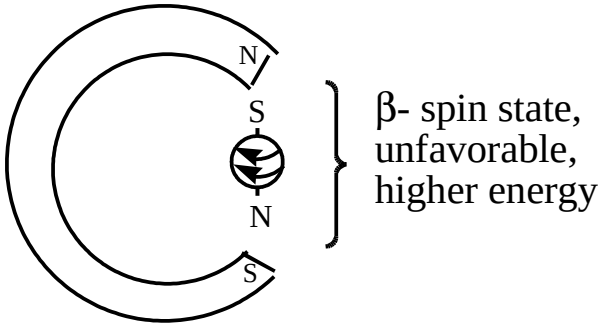
Physical Principles:

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.

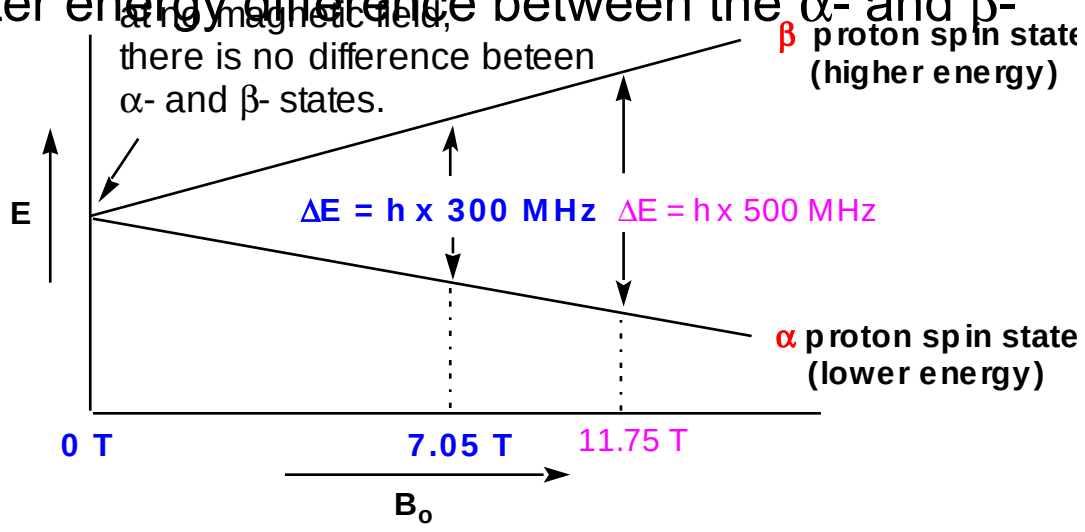
We can probe the energy difference of the α- and β- state of the protons by irradiating them with EM radiation of just the right energy.

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

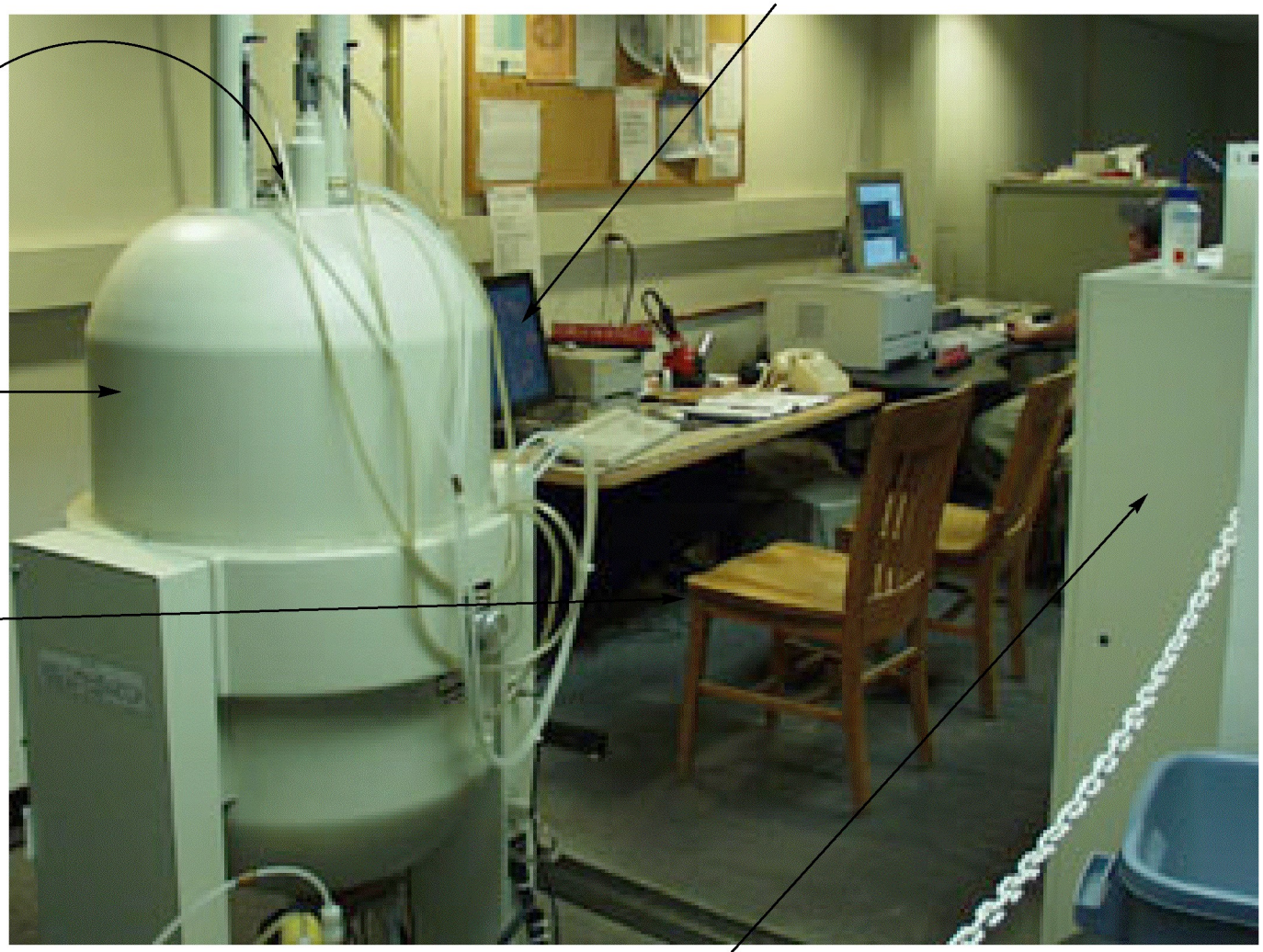
The Chemical Shift (Also Called δ) Scale

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.

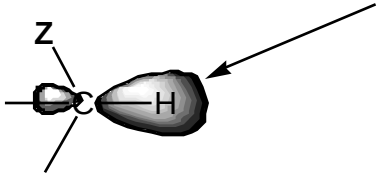
Of course, we don't do any of this, it's all done automatically by the NMR machine.
Even more brilliant.

NMR Spectrometer Used by the Undergraduate Organic Labs



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:



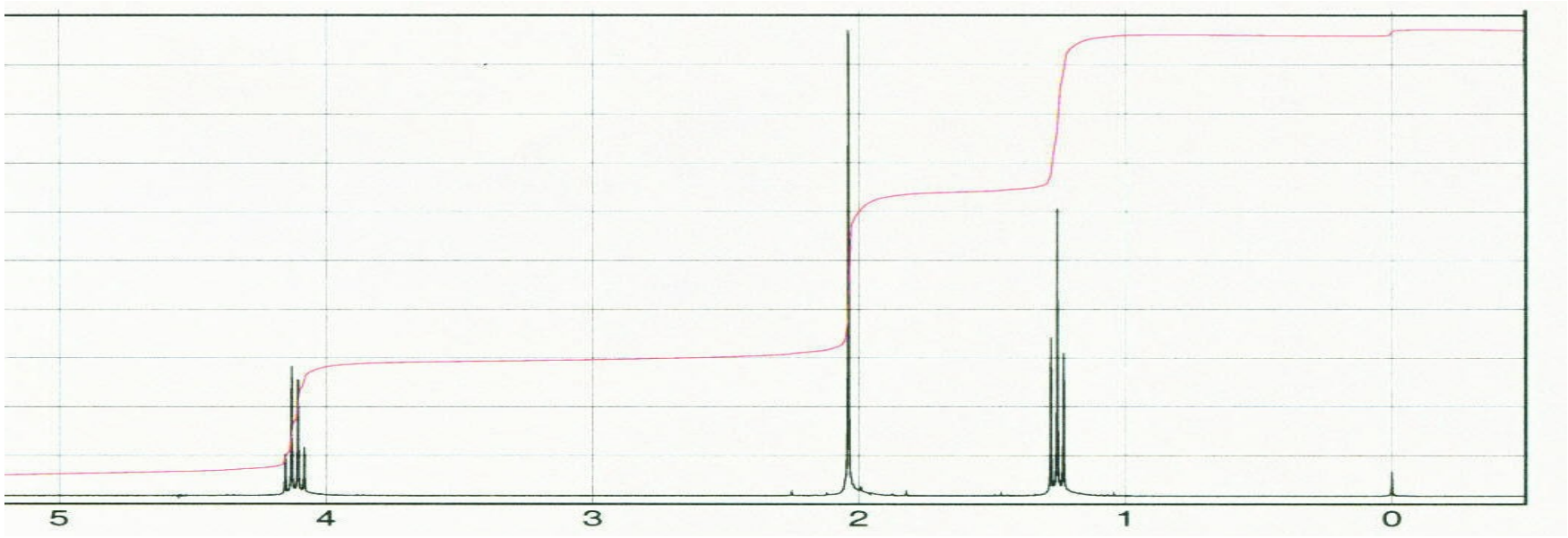
This represents the electron density of a C-H bond. How much electron density is on the proton depends on what else is attached to the carbon. If is an elelctronegative atom, the carbon becomes electron deficient and pull some of the electron density away from the H. if Z is an electron donating group,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

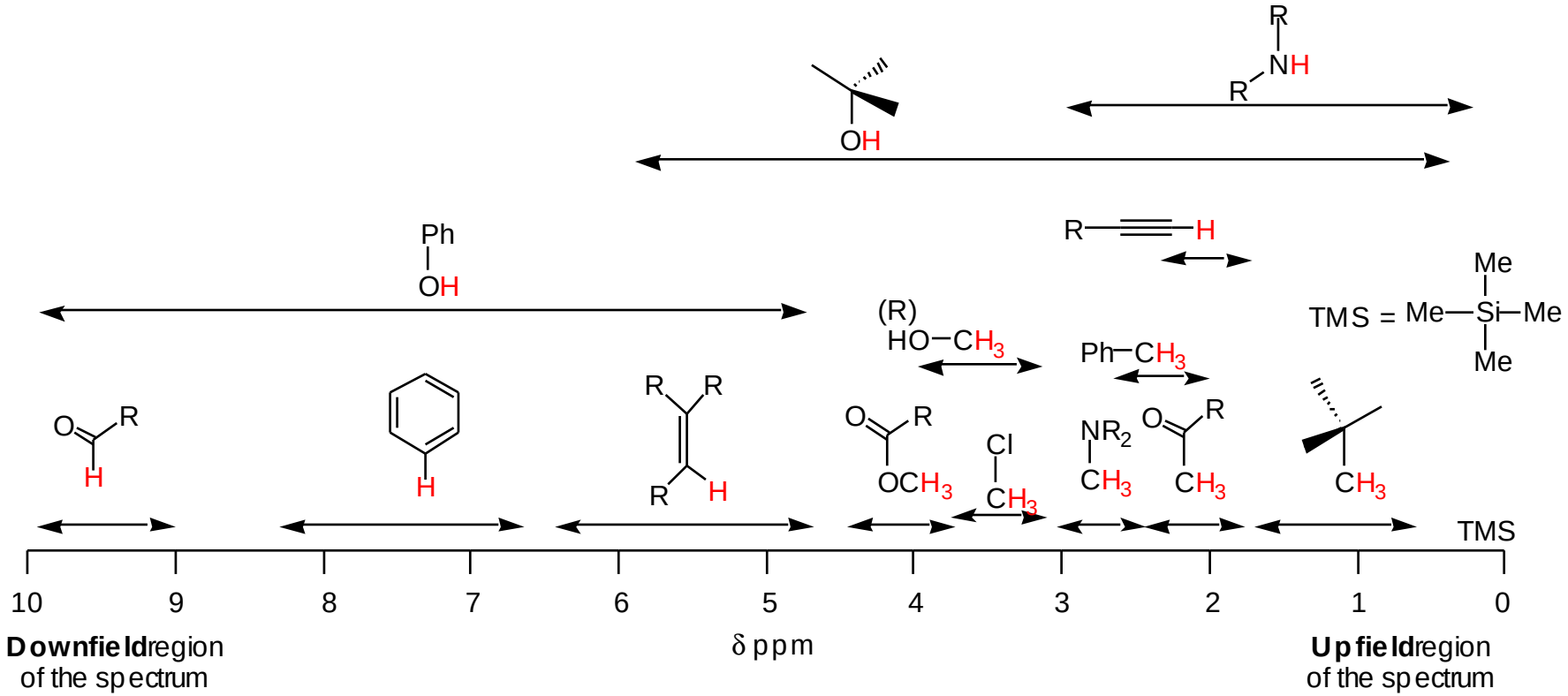
1) Chemical shift data - tells us what kinds of protons we have.
2) Integrals - tells us the ratio of each kind of proton in our sample.
3) ^{1}H - ^{1}H coupling - tells us about protons that are near other protons.



Chemical Shift Data

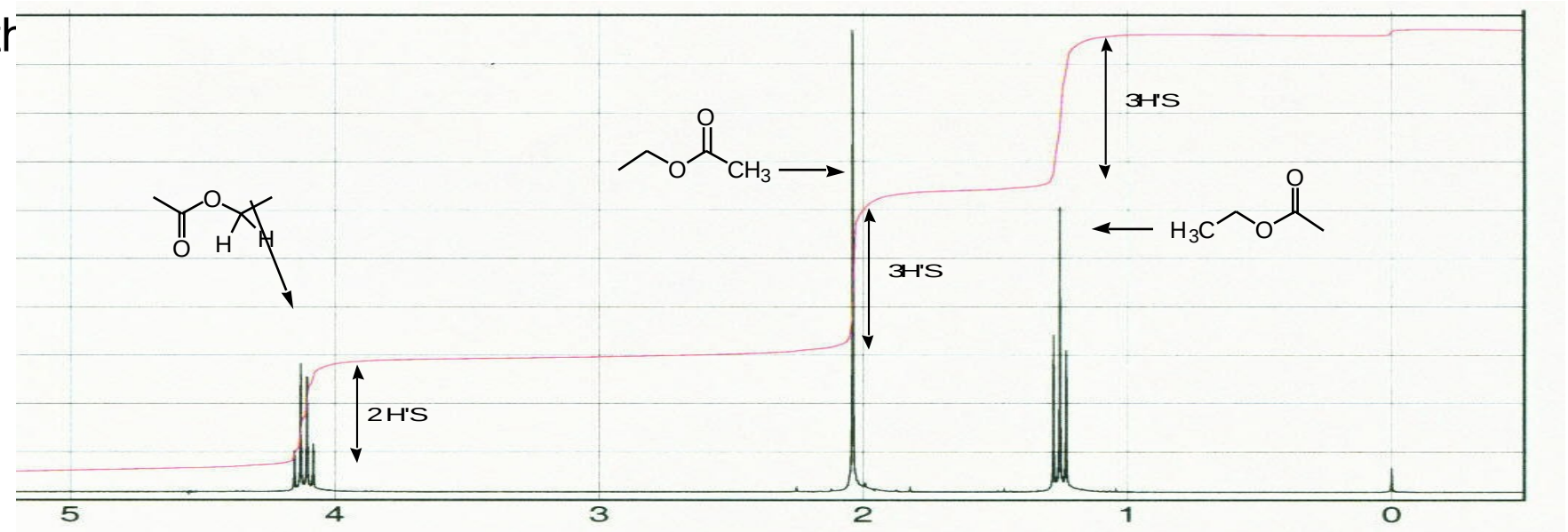
As previously mentioned, different kinds of protons typically come at different chemical shifts. Shown below is a chart of where some common kinds of protons appear in the δ scale. Note that most protons appear between 0 and 10 ppm. The reference, tetramethylsilane (TMS) appears at 0 ppm, and aldehydes 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!



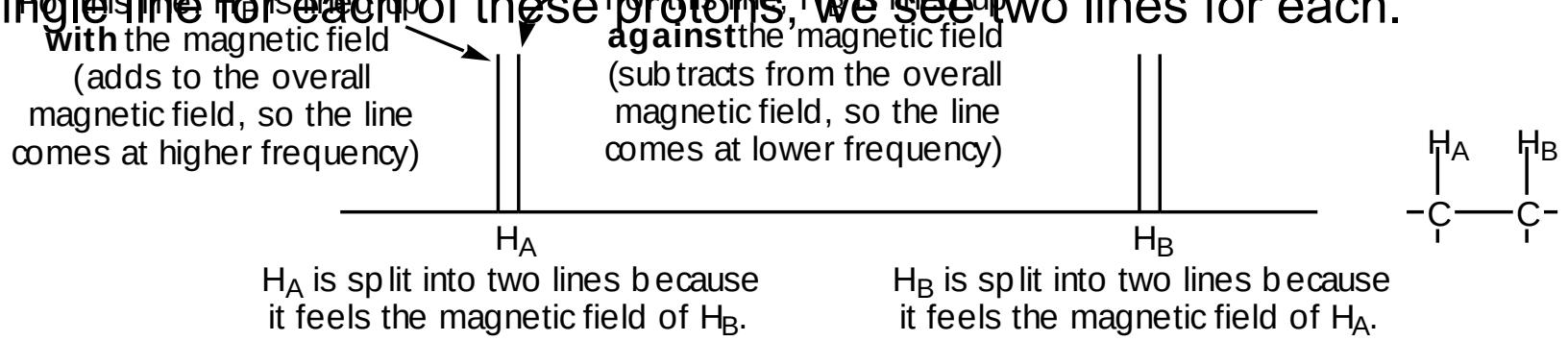
Integrals

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 oth



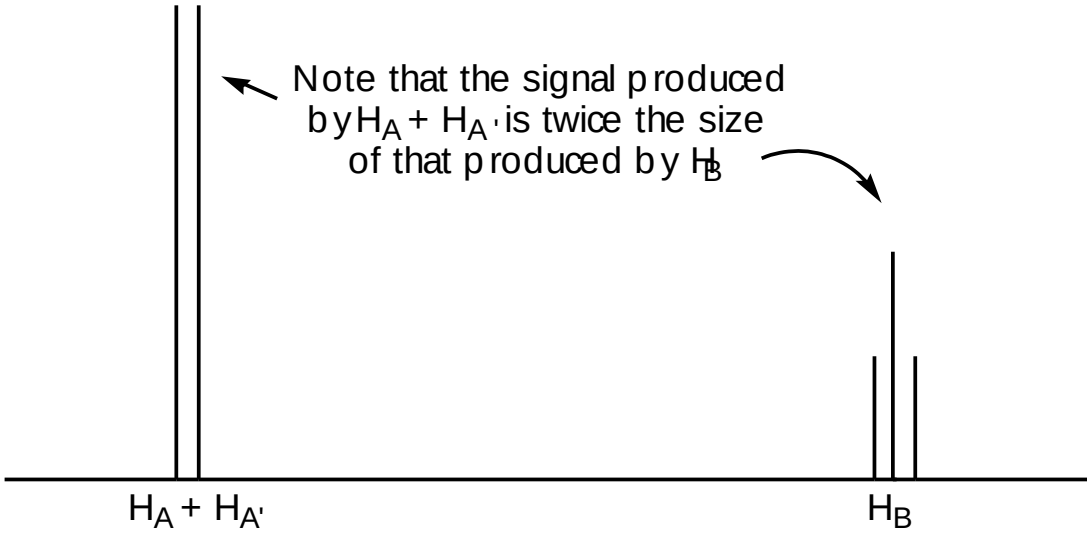
^1H - ^1H Coupling

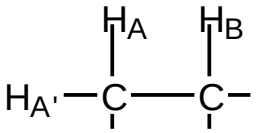
You'll notice in the spectra that we've seen that the signals don't appear as single lines, sometimes they appear as multiple lines. This is due to ^1H - ^1H coupling (also called spin-spin splitting or J-coupling). Here's how it works: Imagine we have a molecule which contains a proton (let's call it 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.



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.



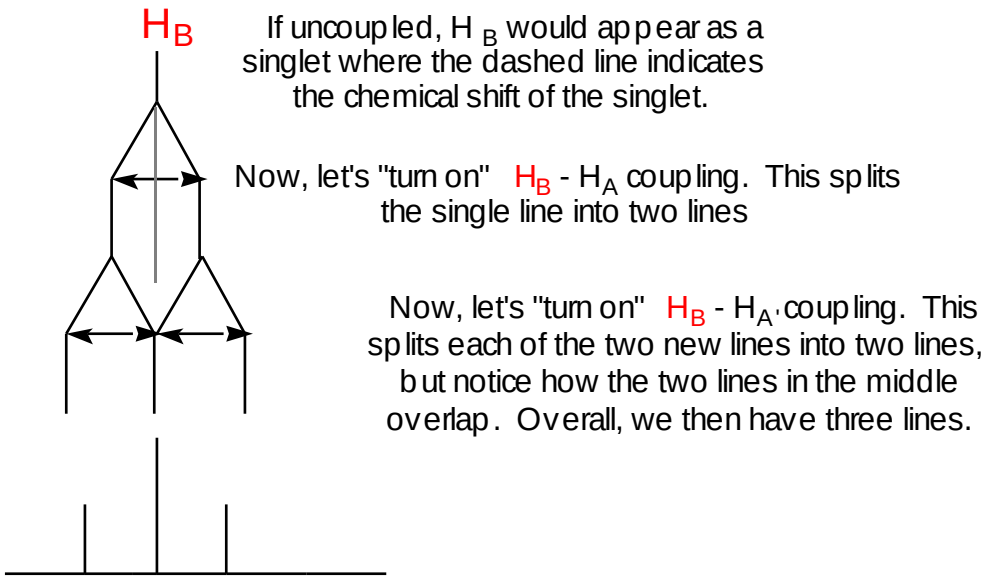


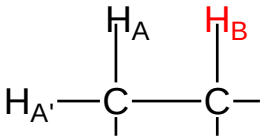
H_A and $H_{A'}$ appear at the same chemical shift because they are in identical environments
They are also split into two lines (called a doublet) because they feel the magnetic field of H_B.

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:

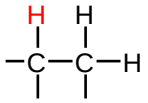
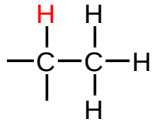
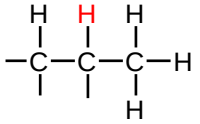
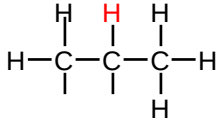
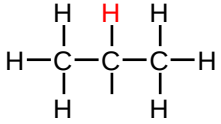




Because the two lines in the middle overlap, that line is twice as big as the lines on the outside. More neighboring protons leads to more lines as shown on the next slide.

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.

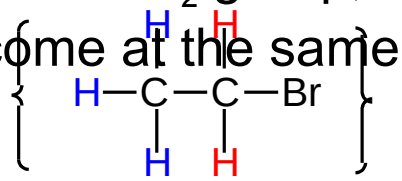
No. of neighbors	relative intensities	pattern	example
0	1	singlet (s)	
1	1 1	doublet (d)	
2	1 2 1	triplet (t)	
3	1 3 3 1	quartet (q)	
4	1 4 6 4 1	pentet	
5	1 5 10 10 5 1	sextet	
6	1 6 15 20 15 6 1	septet	

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

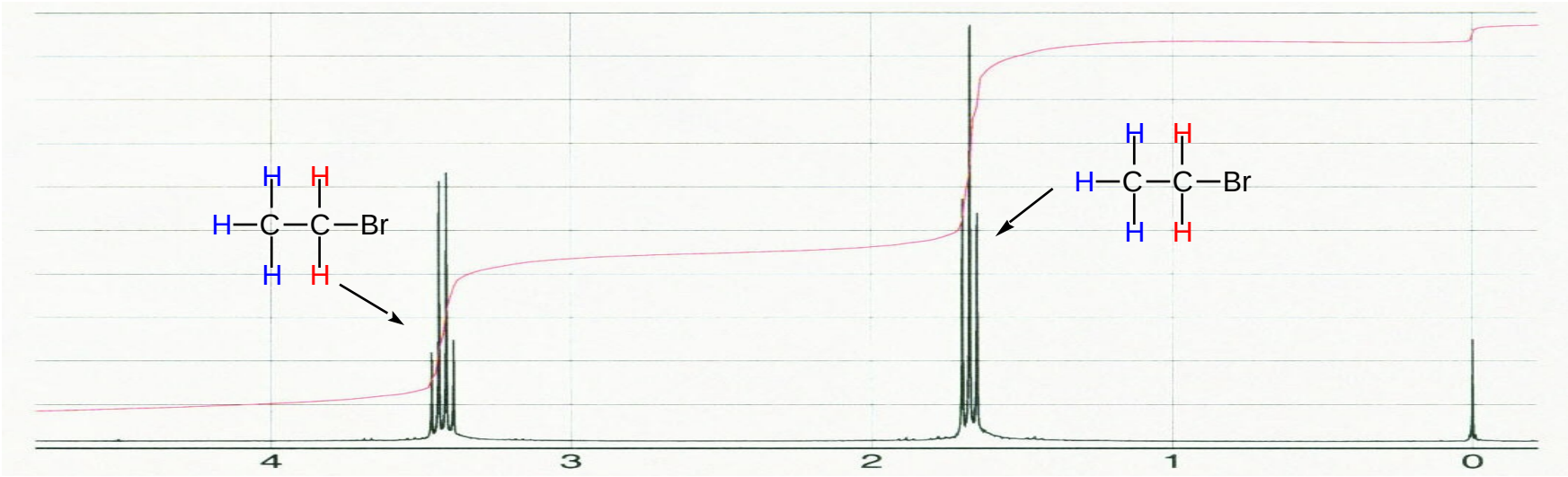
More About Coupling

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

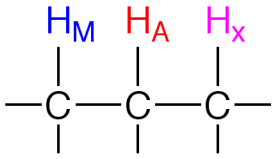


The red protons both come at the same chemical shift and do not split each other

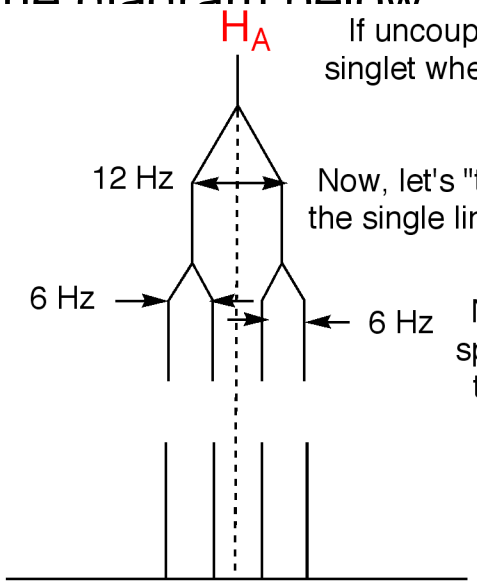


Not all Couplings are Equal

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

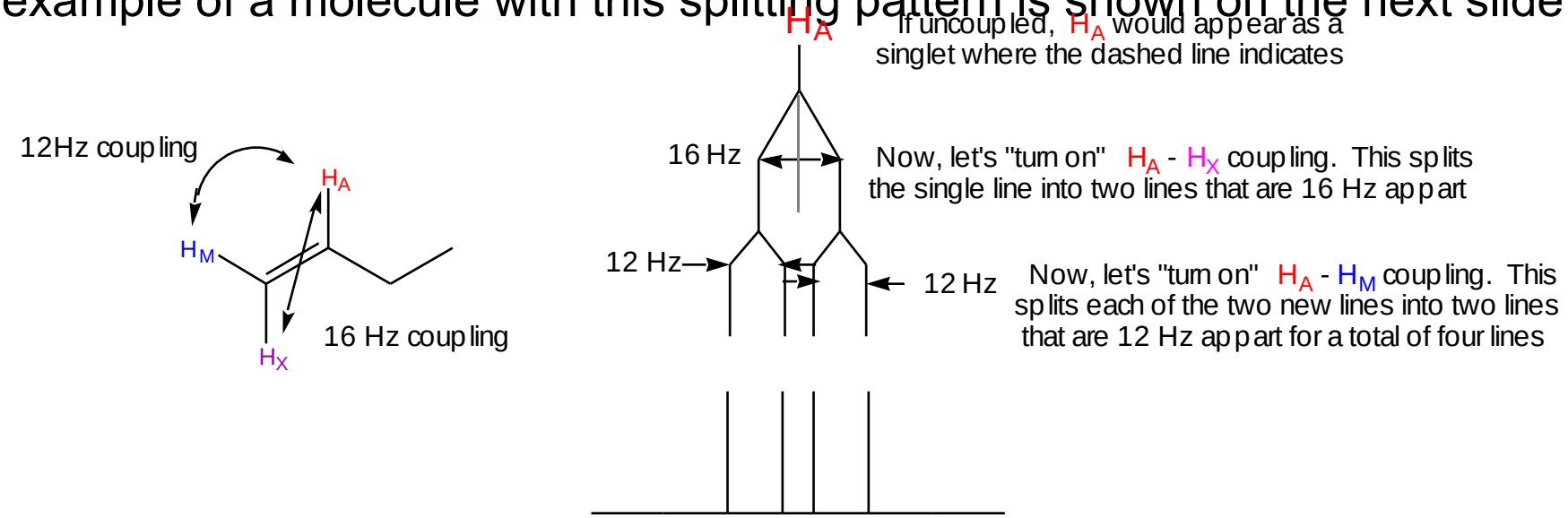
Now, let's "turn on" H_A - H_X coupling. This splits the single line into two lines that are 12 Hz appart

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.

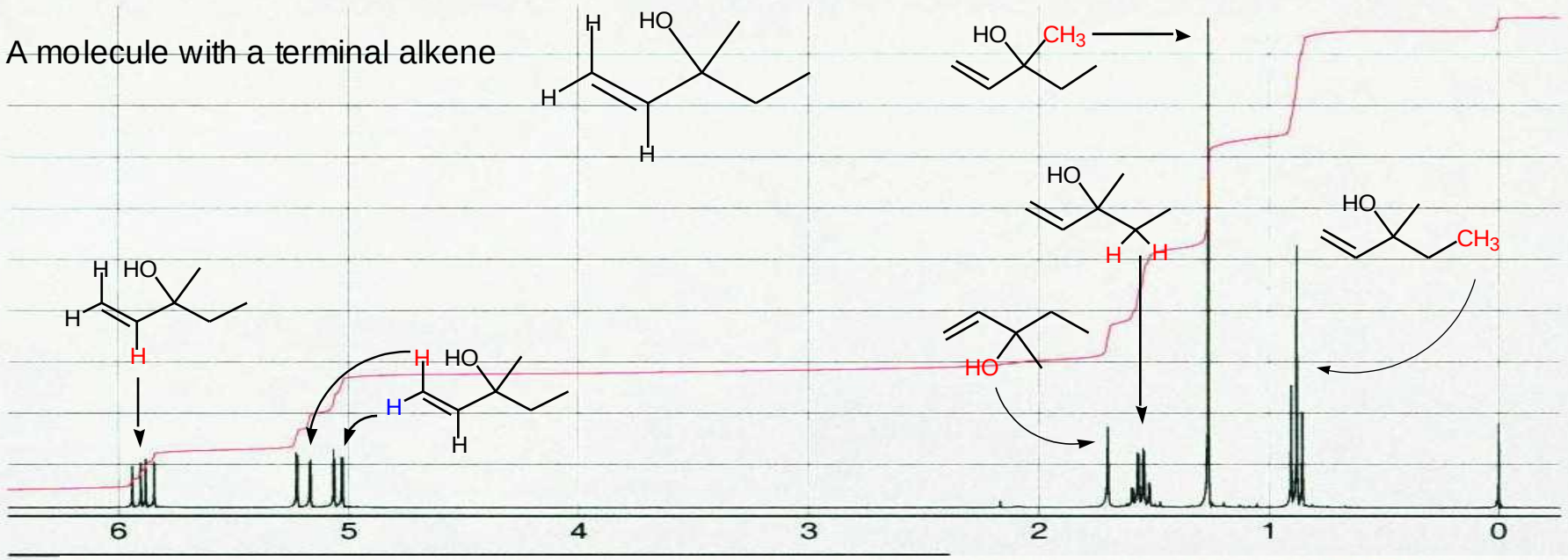
Coupling Constants in Alkenes

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.

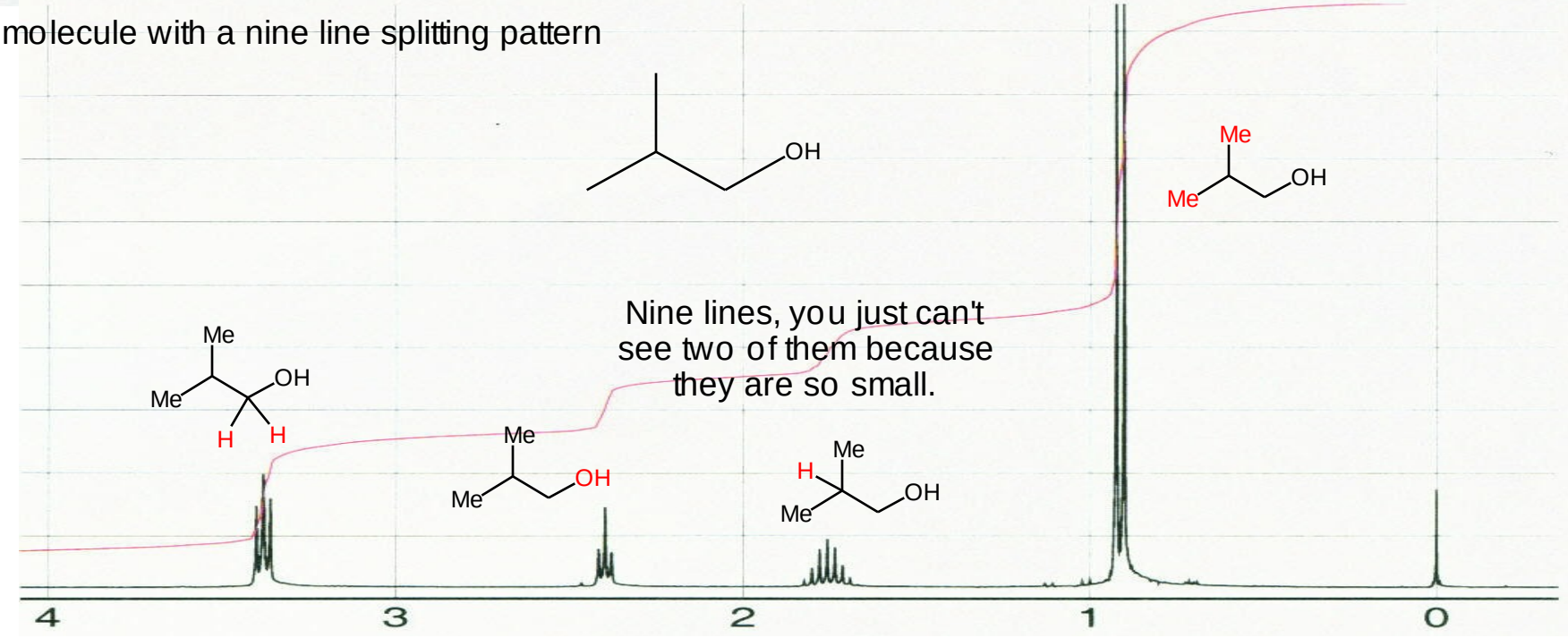


There are other times when protons on the same carbon are nonequivalent, which we'll see later.

A molecule with a terminal alkene



A molecule with a nine line splitting pattern



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

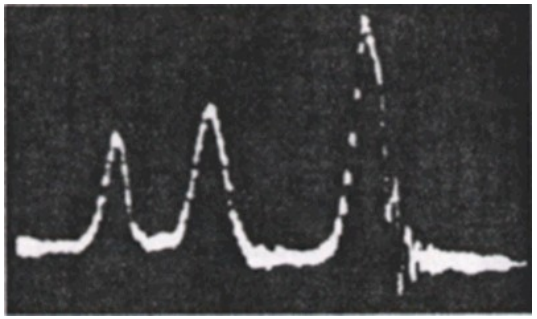
- Modern variants of NMR provide 3D structural resolution of (not too large) proteins in solution

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

Some history of NMR

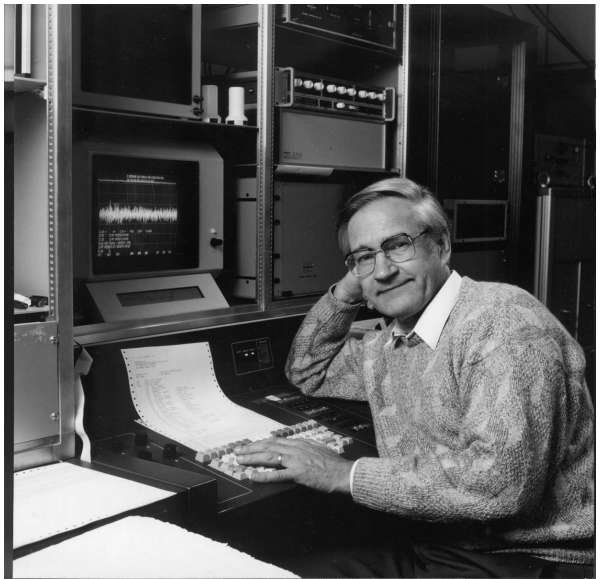
1946 Principle of solid state NMR (Bloch, Purcell)

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



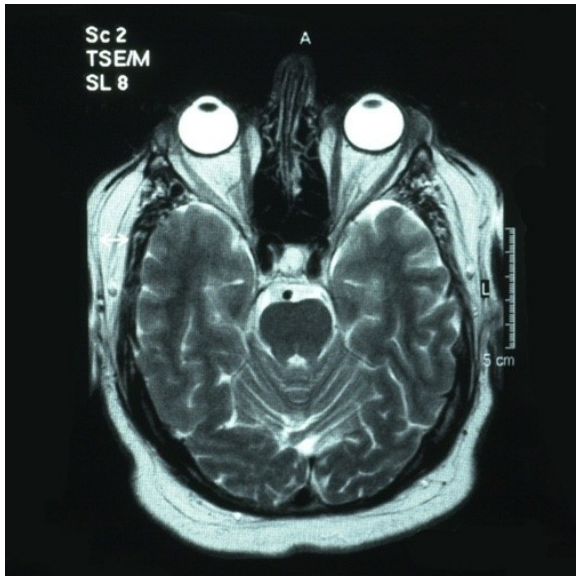
1953 Overhauser effect

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)

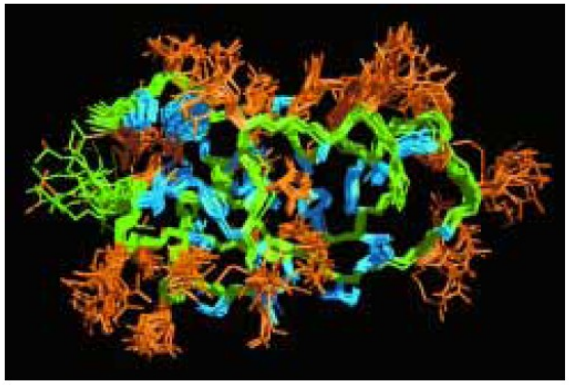


Figure 27. NMR structure of BPTI represented by a bundle of 20 conformers superimposed for best fit of the polypeptide backbone. The polypeptide backbone is green, core side-chains are blue, and solvent-accessible surface side-chains are red.

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

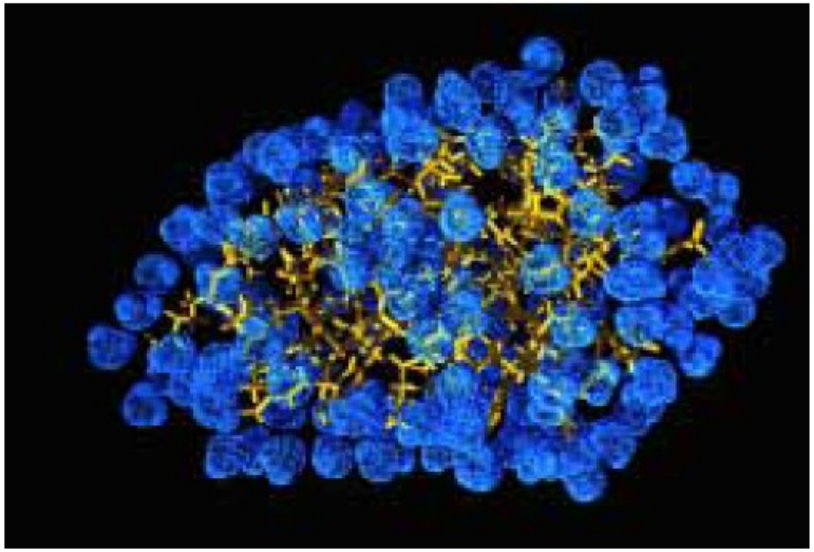
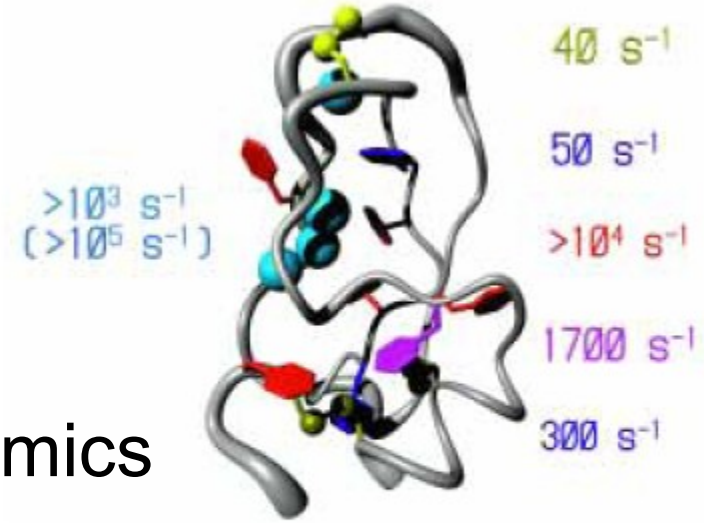


Figure 28. Molecular model of hydrated BPTI in H_2O solution. The drawing shows an all-heavy-atom-presentation of one of the conformers in Figure 27 (yellow) covered with a layer of hydration water molecules (dotted blue spheres).

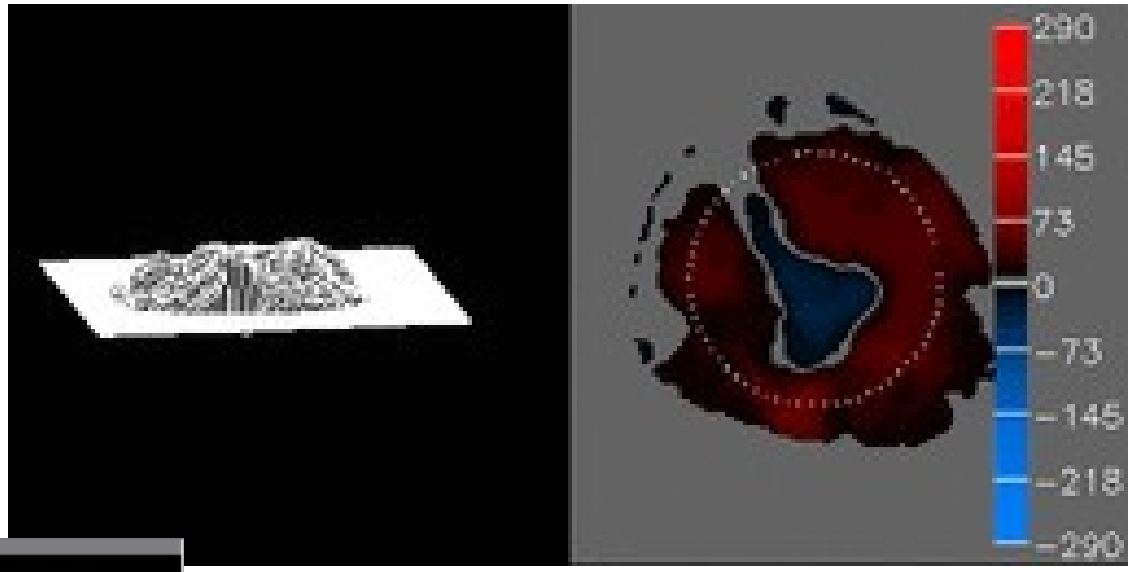
BPTI

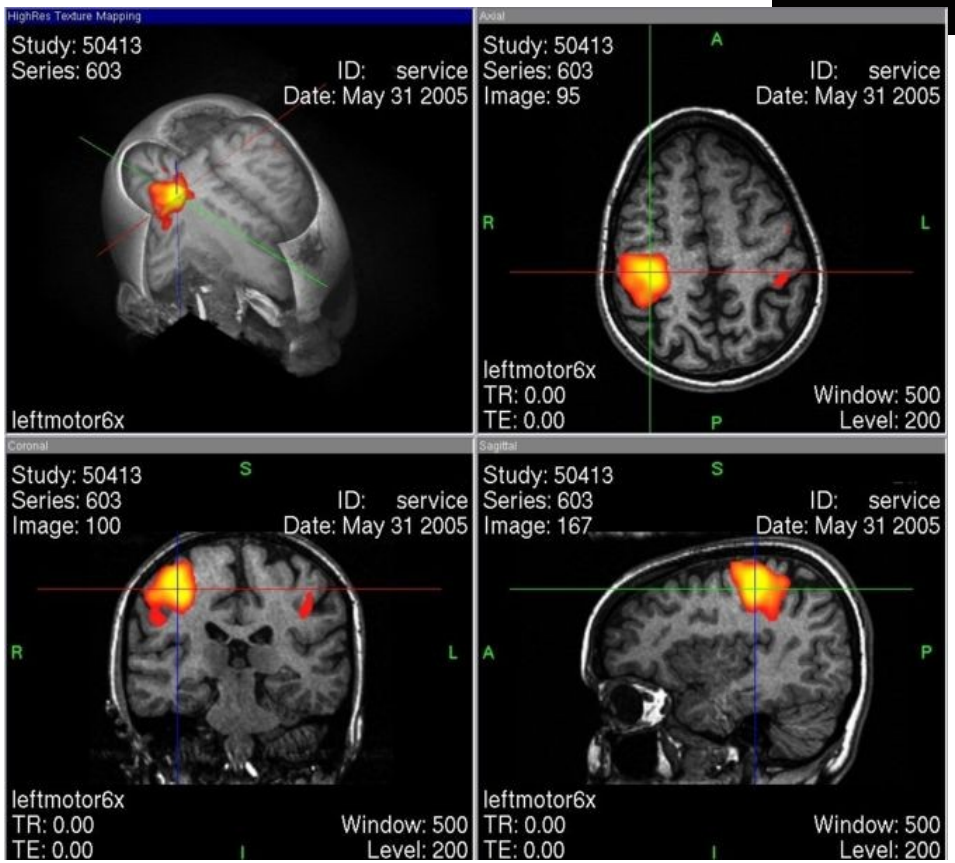
Bound water



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**.

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

$$-I \leq m_I \leq I \qquad \Delta m_I = 1$$

This spin is associated with a magnetic moment

$$\mu = \gamma\hbar\sqrt{I(I+1)} \qquad \gamma = g_I\mu_k/\hbar$$

$$\mu_z = \gamma\hbar m_I$$

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-Verlags-gesellschaft, Weinheim, 1988.

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

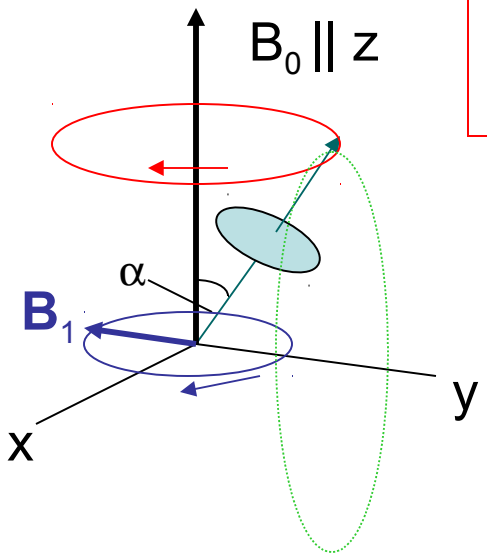
2) Bei konstantem Magnetfeld und gleicher Anzahl an Kernen

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**

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



$$\omega_L = \gamma B_0$$

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 $2\pi\dot{\alpha} = \omega_1 = \gamma B_{10}$

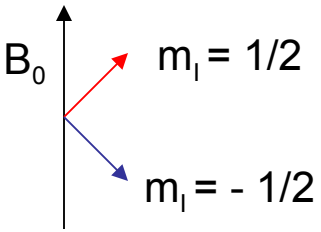
Quantum mechanical description

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

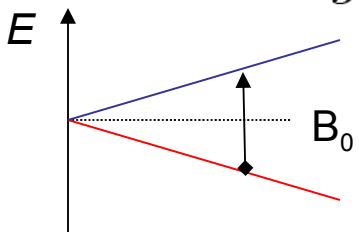
I = 1/2 ^{1}H, ^{13}C, ^{31}P

$g_I = 5.58$

$\gamma = 42.576$ MHz/T

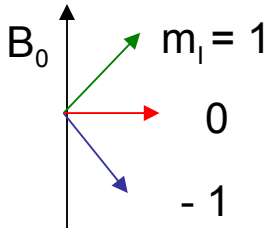


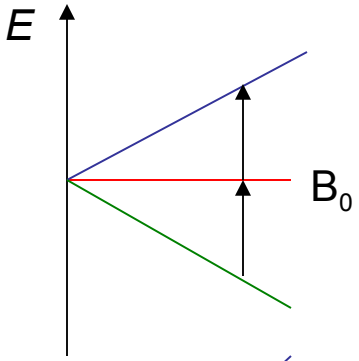
$$\Delta E = g_I \mu_k B_0 = \hbar \omega_L$$



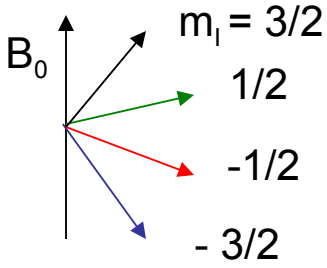
$$\omega_L = \gamma B_0$$

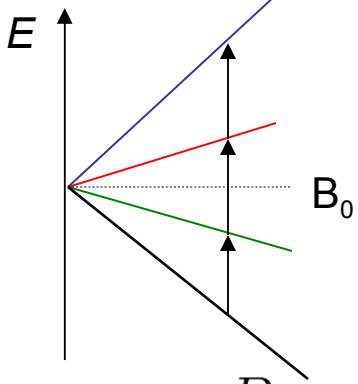
I = 1 ^{2}H, ^{14}N,





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

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

Thus the population imbalance is

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

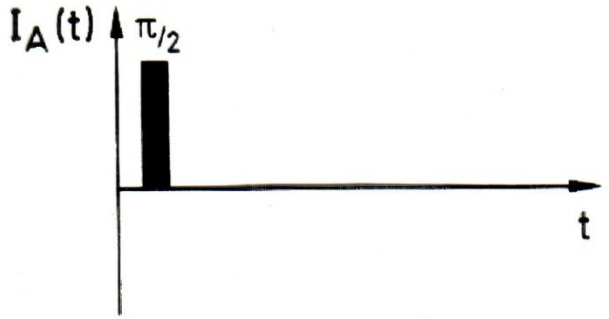
Which yields a bulk magnetization

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

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:

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



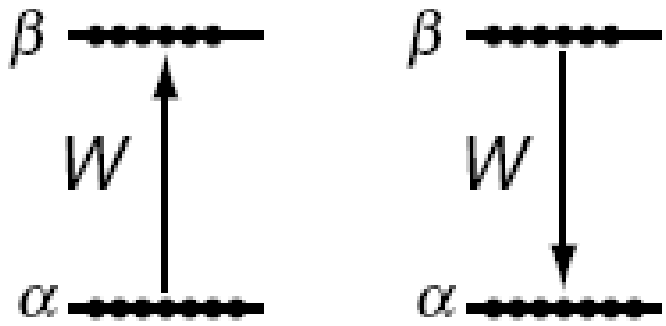
Thus a pulse of duration $\tau = 2\pi/4\ \omega_1$ gives a change in angle of $\pi/2$ – pulse I.e. the magnetization is flipped into the xy plane. M_x and M_y now oscillate with ω_L.

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

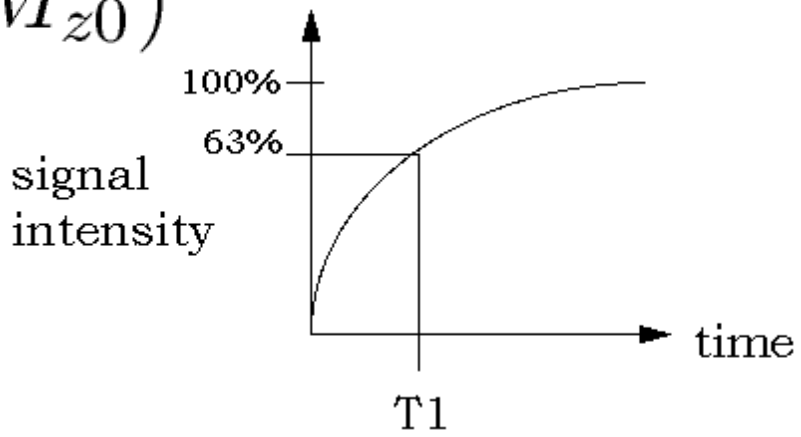
$$\frac{dn_\alpha}{dt} = W(n_\beta - n_\beta^0) - W(n_\alpha - n_\alpha^0)$$

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



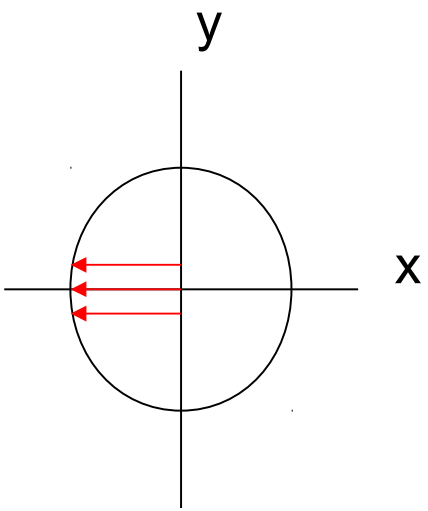
Which yields a simple exponential relaxation of the magnetization in the z-direction

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

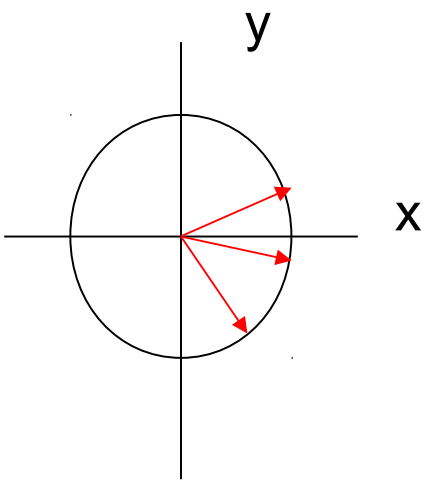


The amplitudes of M_x and M_y decay with another relaxation time T_2 called spin-spin relaxation time. This relaxation originates from inhomogeneity of B_0. It is described by another phenomenological equation

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



Immediately after $\pi/2$ pulse

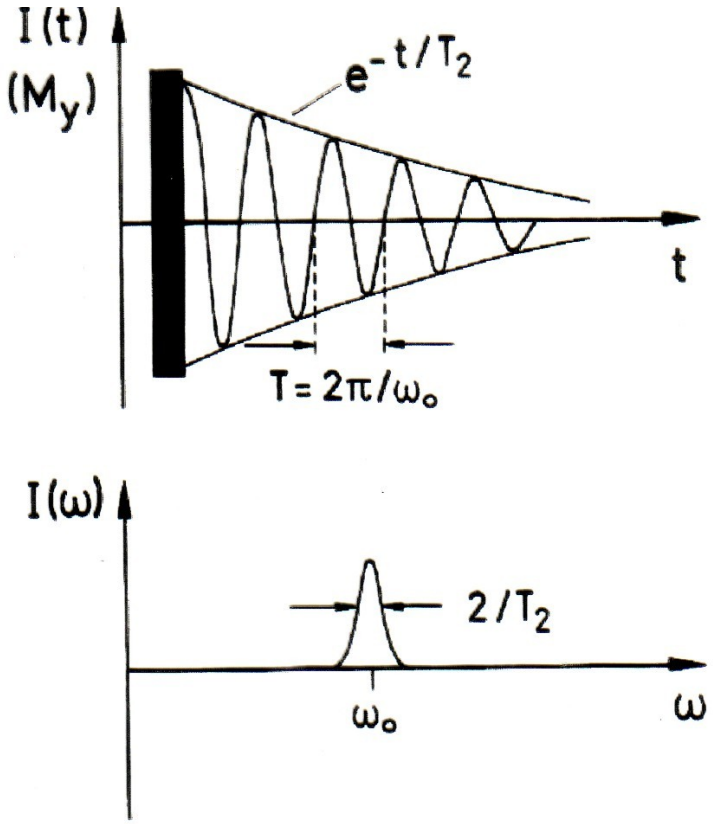


later

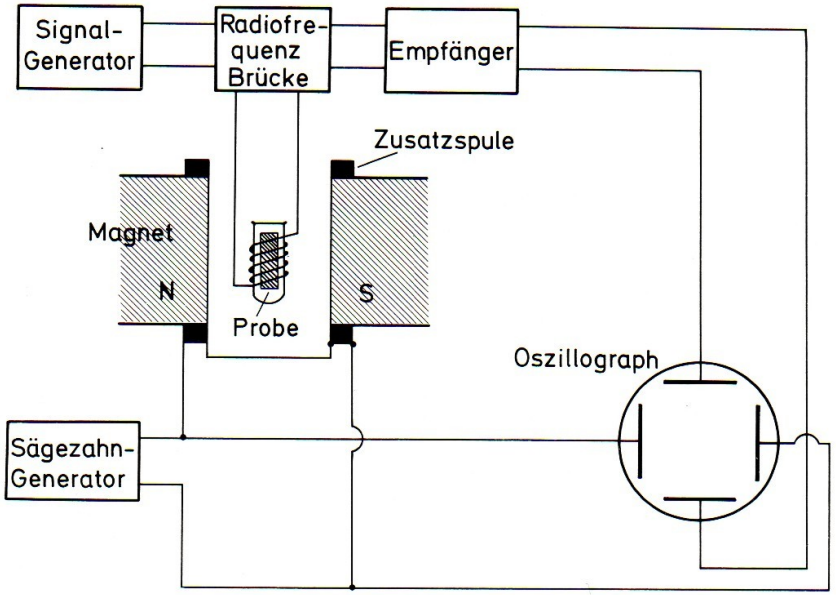
To be complete, the precession in the static field has to be taken into account as well, which is described by the Bloch equations

$$dM_z/dt = -(M_z - M_z^0)/T_1 + \gamma(\mathbf{M} \times \mathbf{H})_z,$$
$$dM_x/dt = -M_x/T_2 + \gamma(\mathbf{M} \times \mathbf{H})_x,$$
$$dM_y/dt = -M_y/T_2 + \gamma(\mathbf{M} \times \mathbf{H})_y.$$

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



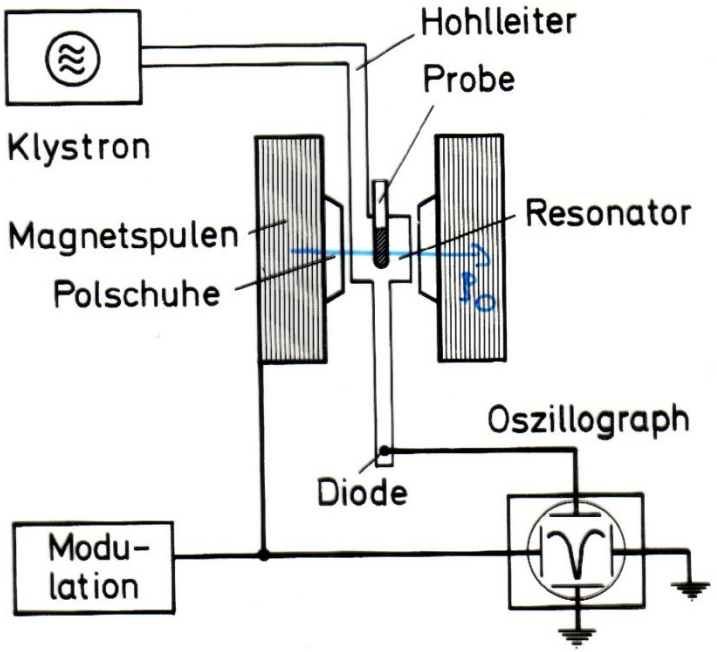
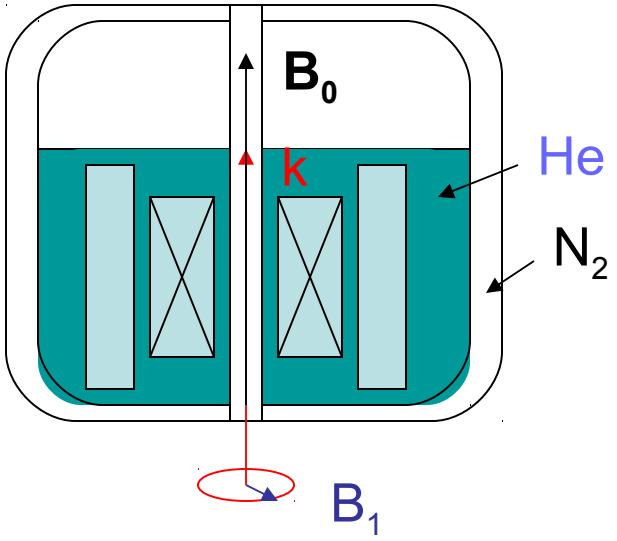


Abb. 20.18. Schema einer einfachen Kernspin-Resonanz-Apparatur. Die Probe befindet sich im Reagenzglas zwischen den Polschuhen eines homogenen Magneten. Das hochfrequente B_1-Feld wird über eine Brücke und eine Induktionsspule eingestrahlt. Zum besseren Nachweis der Resonanz kann das B_0-Feld durch eine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

$$B_{loc} = B_0 - \sigma B_0$$

$$\omega_L = \gamma B_0 (1 - \sigma)$$

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.

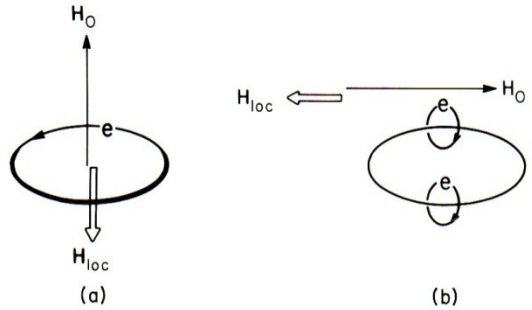


Fig. 9. Differing response of the π electrons in a benzene molecule at two orientations of the molecule relative to H_0: when the external field is (a) perpendicular to the molecular plane and (b) parallel to the molecular field.

Motional narrowing!

^{13}C NMR spectrum of liquid benzene

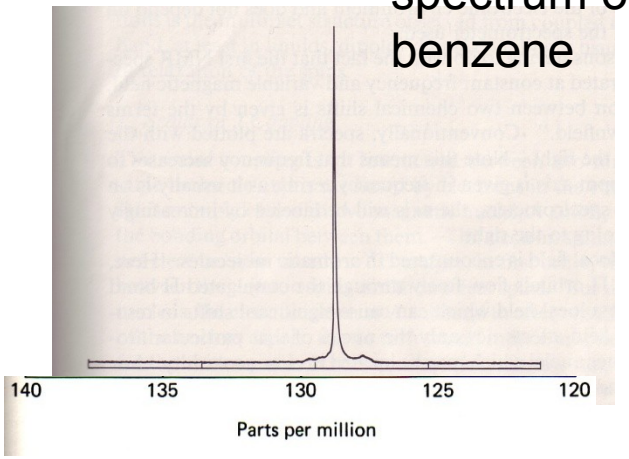
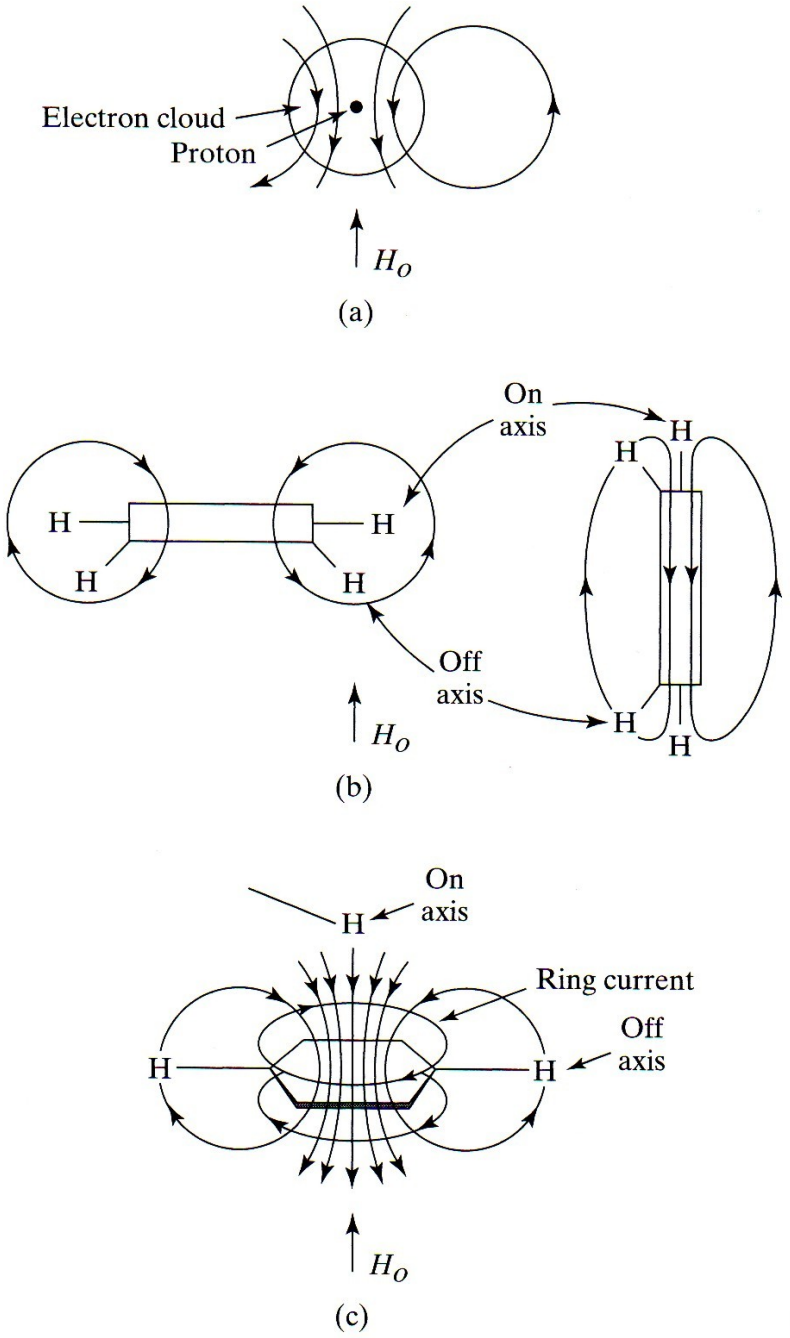


Figure 12.3 The field induced in some electron clouds for specific orientations in the magnetic field. (a) Local positive shielding for the proton in its electron cloud. (b) Interatomic shielding from a bond depends on whether the proton is on axis or off axis. (c) Interatomic shielding from a ring current 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.



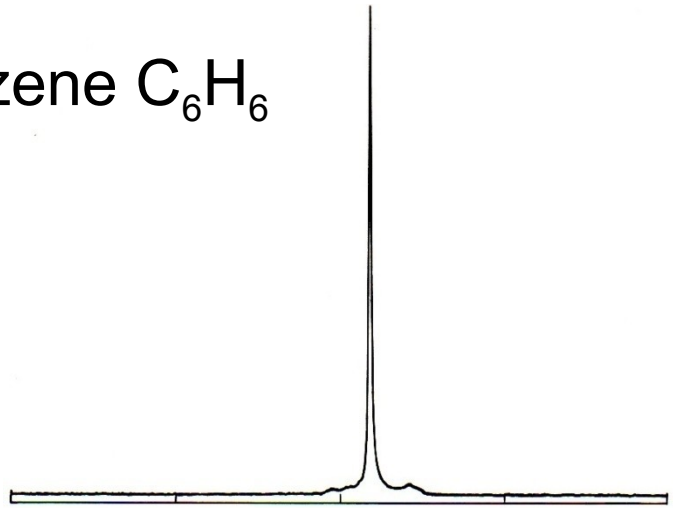
Usual measure: Frequency shift of sample (1) relative to some reference sample (2); unit: ppm

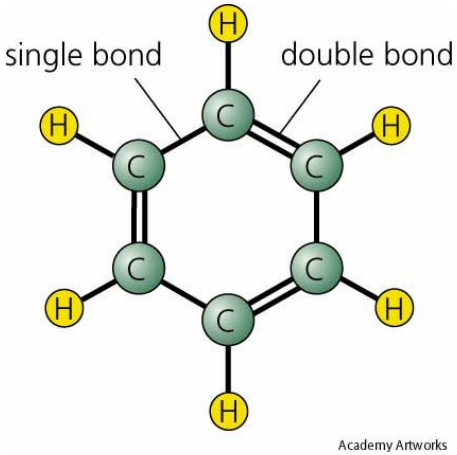
$$(\sigma_1 - \sigma_2)\gamma B_0 / \gamma B_0$$

Origin of chemical shift: = shielding of B_0

Examples: ^{13}C NMR

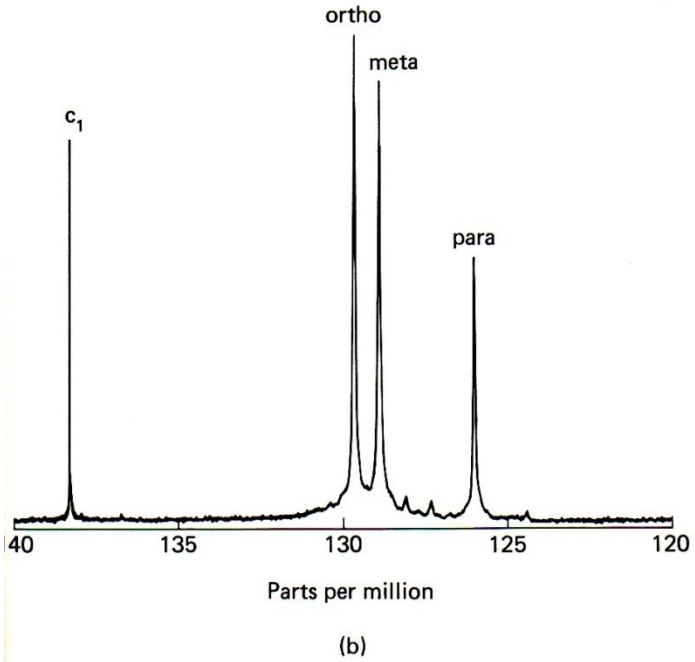
Benzene C_6H_6

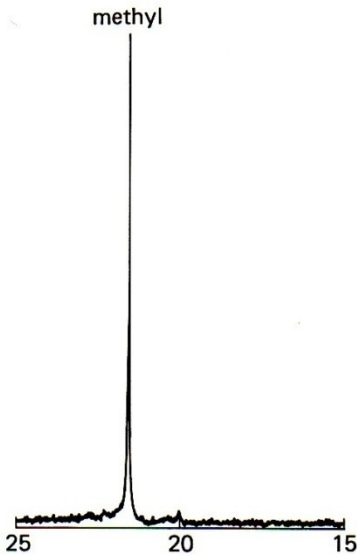


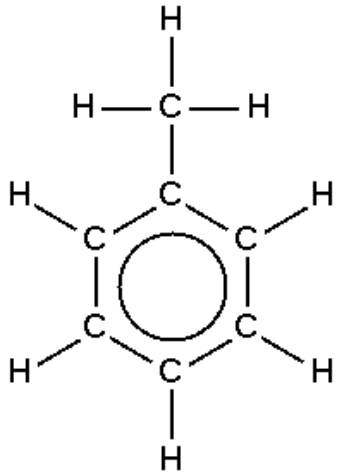


All 6 carbons are identical
same chemical shift, one line

Toluene C_6H_5-CH_3



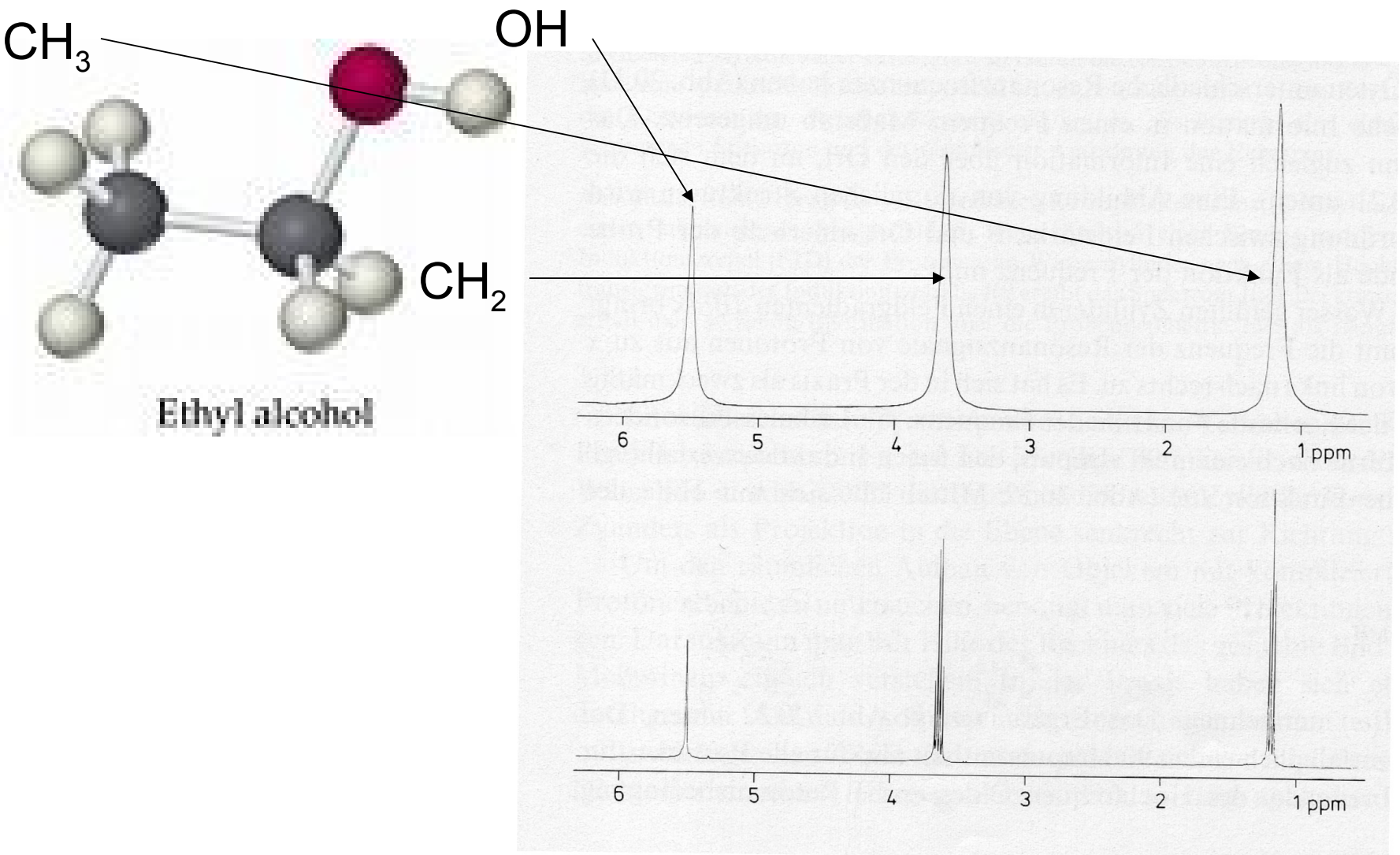




5 different types of
C-atoms, 5 lines

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

Three types of protons



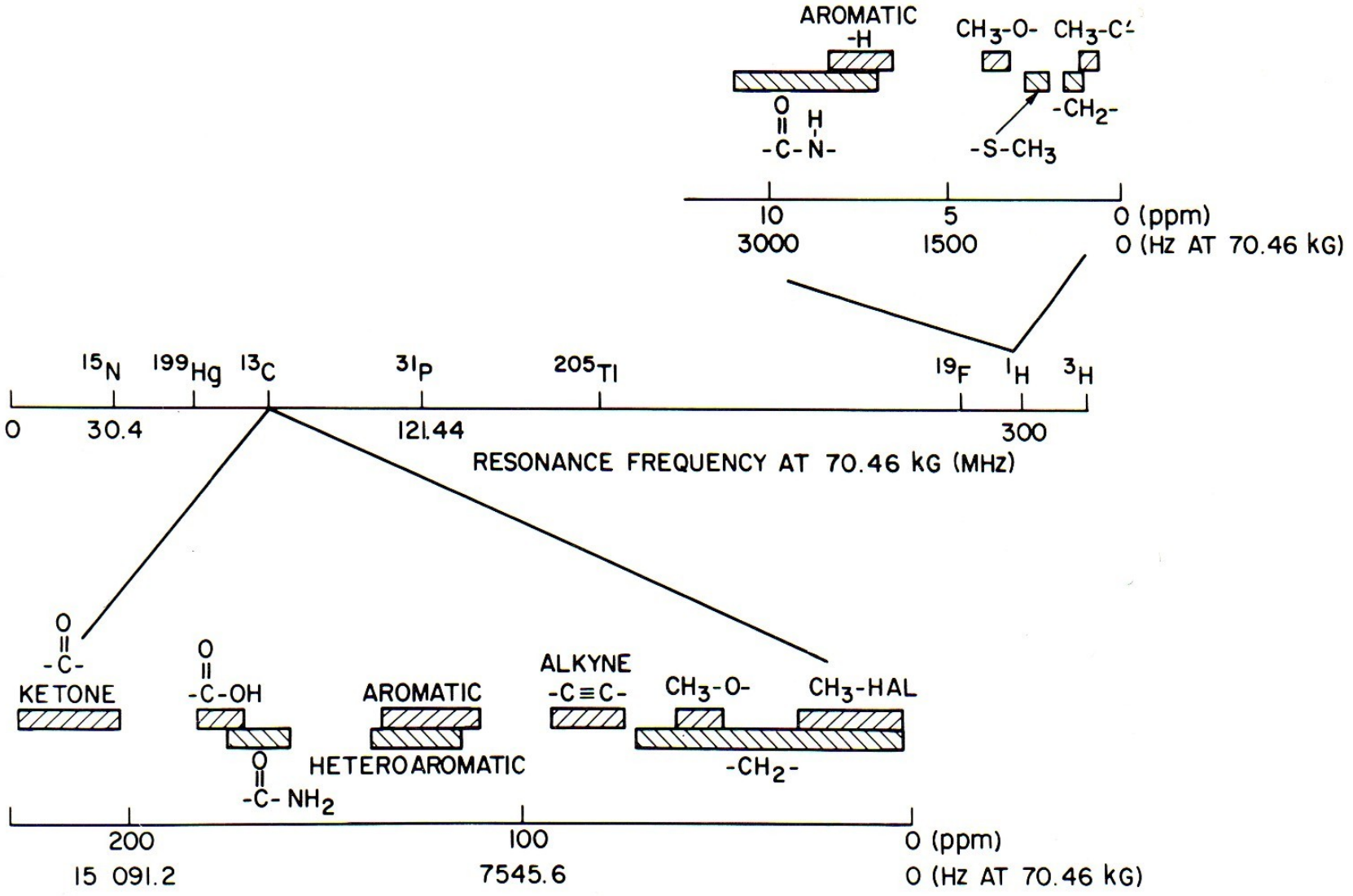
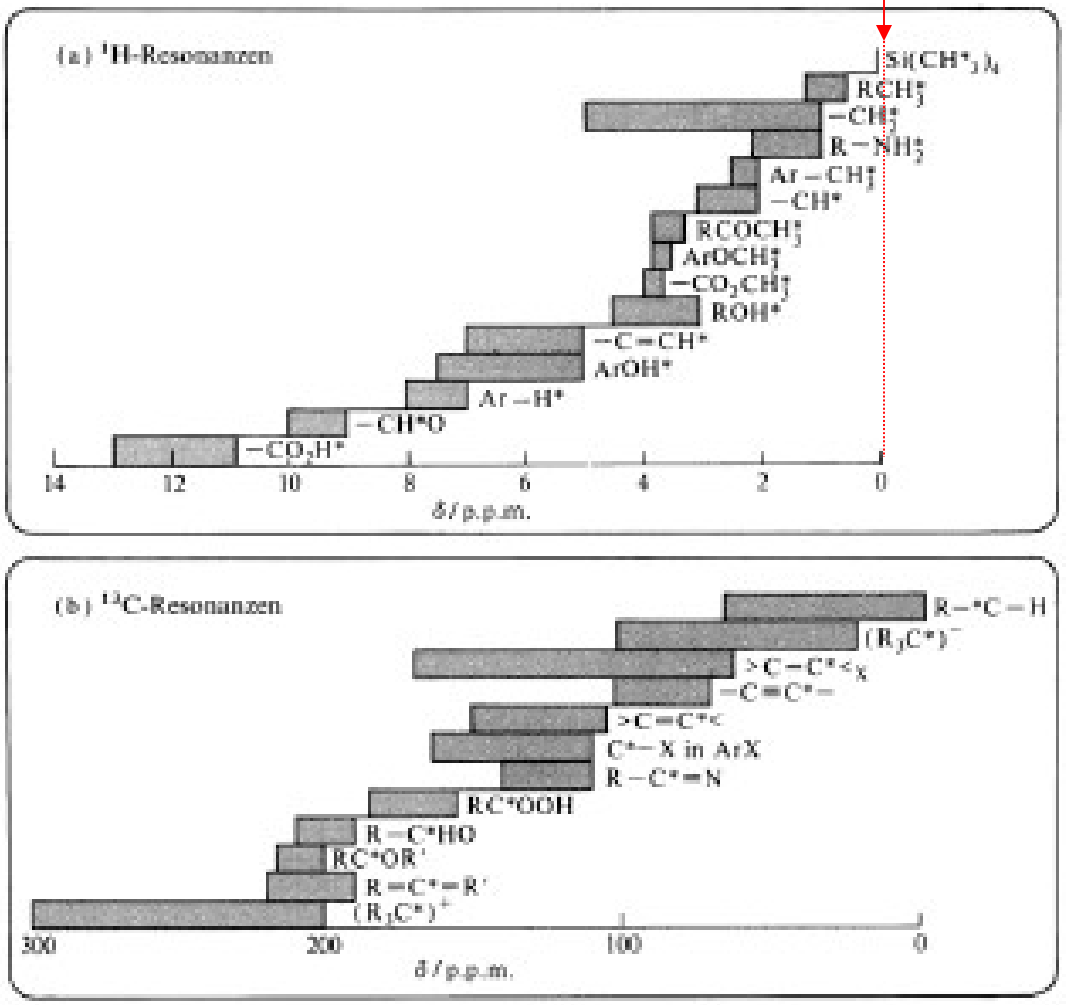


Fig. 2. Nuclear resonance frequencies of several magnetic nuclei at 70.46 kG and the chemical shift range ^{13}C and ^{1}H nuclei.

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	$C_2 H$	8.12
				$C_4 H$	7.14
Phe	8.23	4.66	3.22, 2.99	$C_2 H$, $C_6 H$	7.30
				$C_3 H$, $C_5 H$	7.39
				$C_4 H$	7.34
Tyr	8.18	4.60	3.13, 2.92	$C_2 H$, $C_6 H$	7.15
				$C_3 H$, $C_5 H$	6.86
Trp	8.09	4.70	3.32, 3.19	$C_2 H$	7.24
				$C_4 H$	7.65
				$C_5 H$	7.17
				$C_6 H$	7.24
				$C_7 H$	7.50
				NH	10.22

3.4 Pulsed NMR

More efficient than classical (frequency or B) scans

Study the free induction decay (FID)

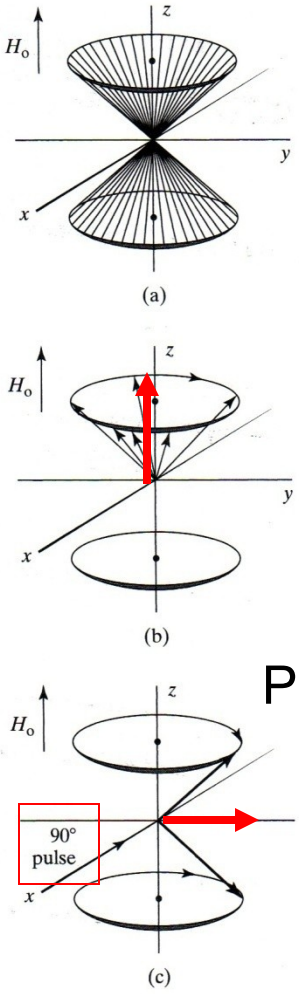
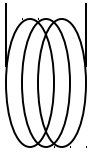


Figure 12.10 (a) A sample of molecules will have the spins for a certain equivalent type of proton in both ground and excited states precessing in random phase around the z-axis. (b) The excess of spins in the ground state sum to a net magnetic dipole along the z-axis, but the x and y components cancel because their phases are random. (c) A 90° pulse excites half of the excess protons to the excited state and causes all of the excess protons to precess in phase. This gives a net magnetization precessing in the xy-plane.

"Ideal" FID = one precession frequency

Pick up coil



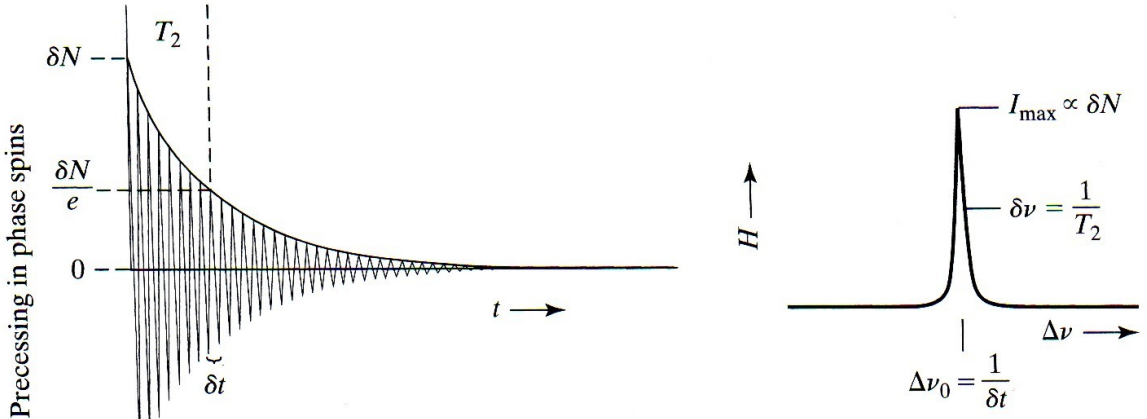


Figure 12.11 A FID with a single frequency $\Delta\nu_0 = 1/\delta t$ and spin-spin relaxation T_2 is easily transformed to the frequency domain.

“Real” FID = several precession frequencies because of several nuclei with different chemical shifts

^{31}P NMR

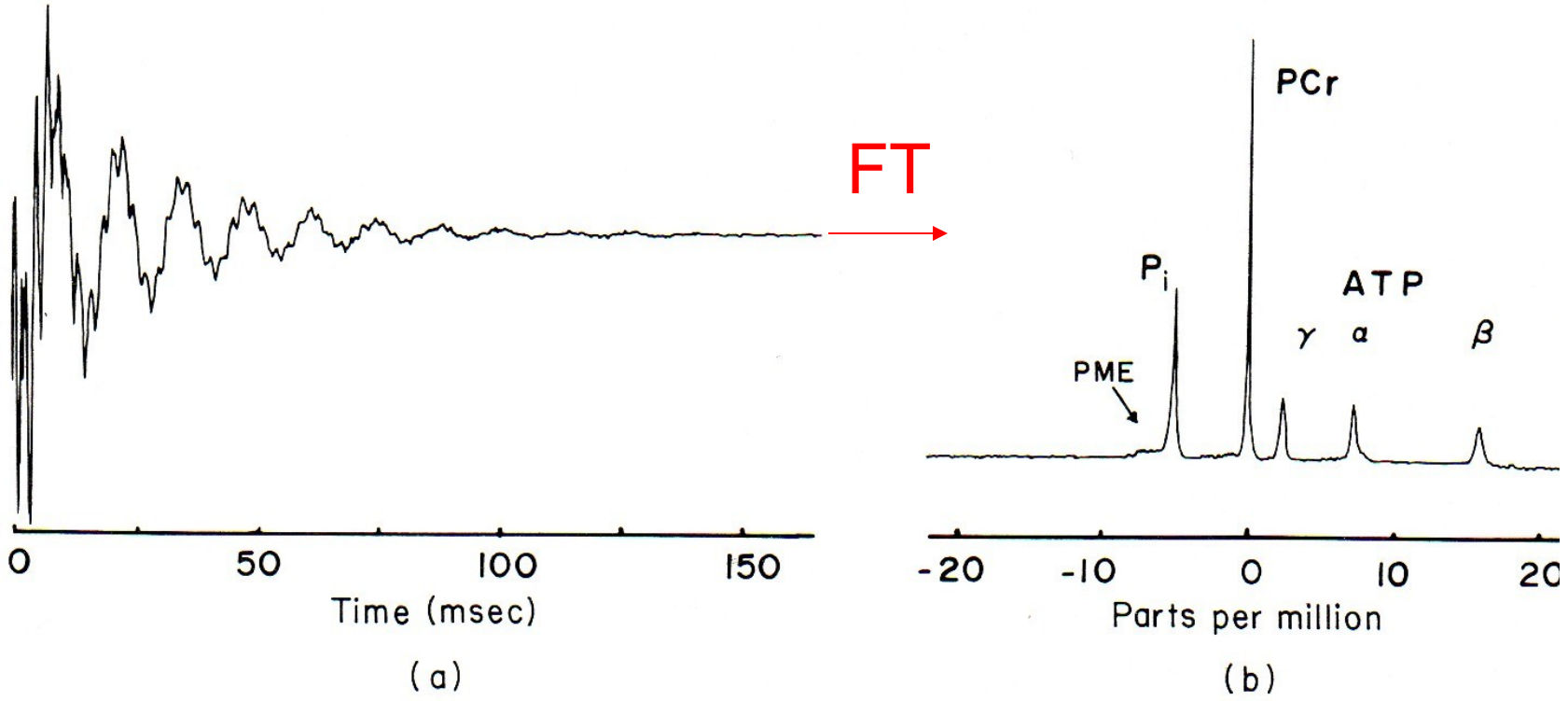
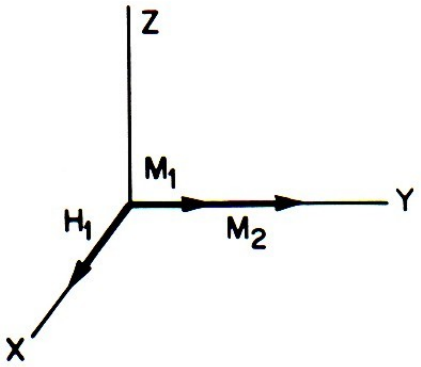


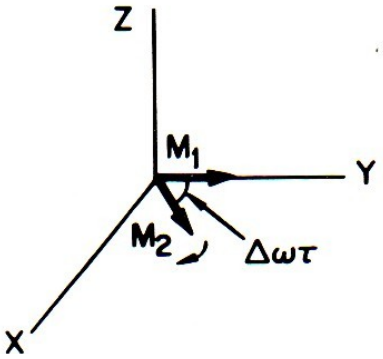
Fig. 18. Free induction decay (FID) signal (a) and its Fourier transform (b) from an isolated cat biceps muscle. Twenty FIDs were summed to increase S/N. The absorption peaks are identified as the three phosphates of ATP (α, β, and γ), phosphocreatine (PCr), inorganic phosphate (P_i), and a small amount of phosphomonoesters (PME).

Spin echo

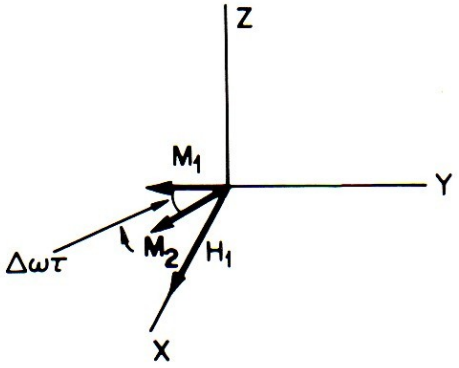
90 degree flip



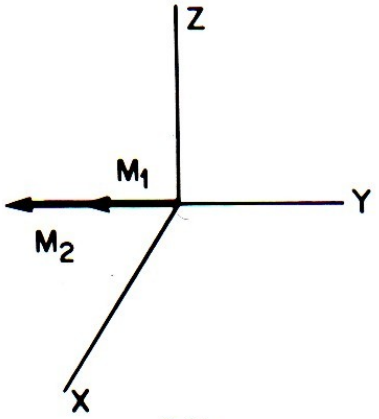
Evolution = spreading (dephasing) in x,y plane

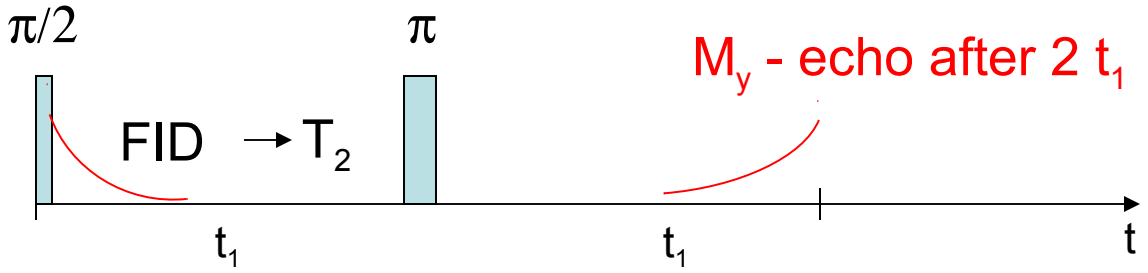


180 degree flip = mirror image relative to x



Refocusing = spin echo





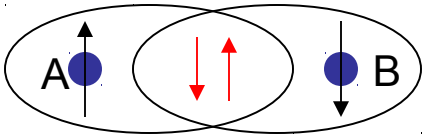
Spin-Spin Interactions

give rise to relaxation of the magnetization

Scalar or *J* – coupling (through bond)

Most bonds are characterized by antiparallel orientation of electron spins (bonding orbital) The nuclear spins are oriented antiparallel to " their " bond electron

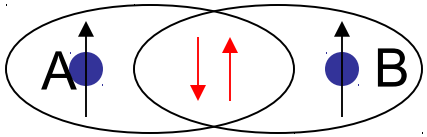
eg H_2



The nuclear spins μ_A and μ_B are coupled, independent of the direction of the external field; Interaction energy: $\Delta E = \alpha\, \mu_A \cdot \mu_B$

$$\frac{\Delta E}{2\pi\hbar} = \Delta\nu = J[Hz]\ \frac{\mu_A \cdot \mu_B}{\gamma^2\hbar^2} \qquad J = \alpha\gamma^2\hbar/2\pi.$$

Energy to flip eg spin B



NB: In polyatomic molecules the J-coupling can also be promoted by -C- bonds or other bonds (A – C – B). It is short ranged (max. 2 or 3 bond lengths)

J- coupling results in additional splitting of (chemically shifted) lines

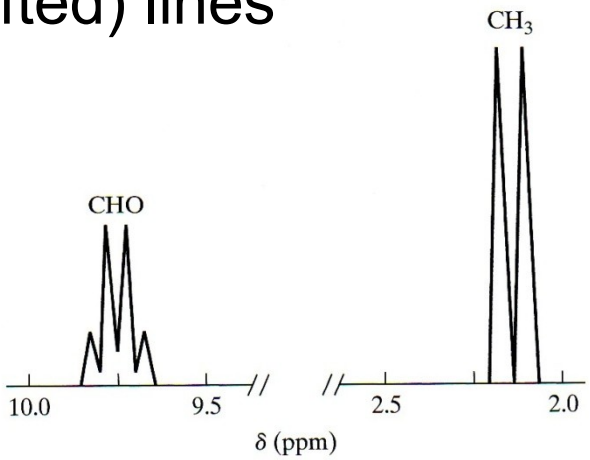
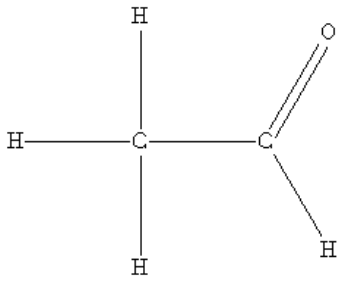
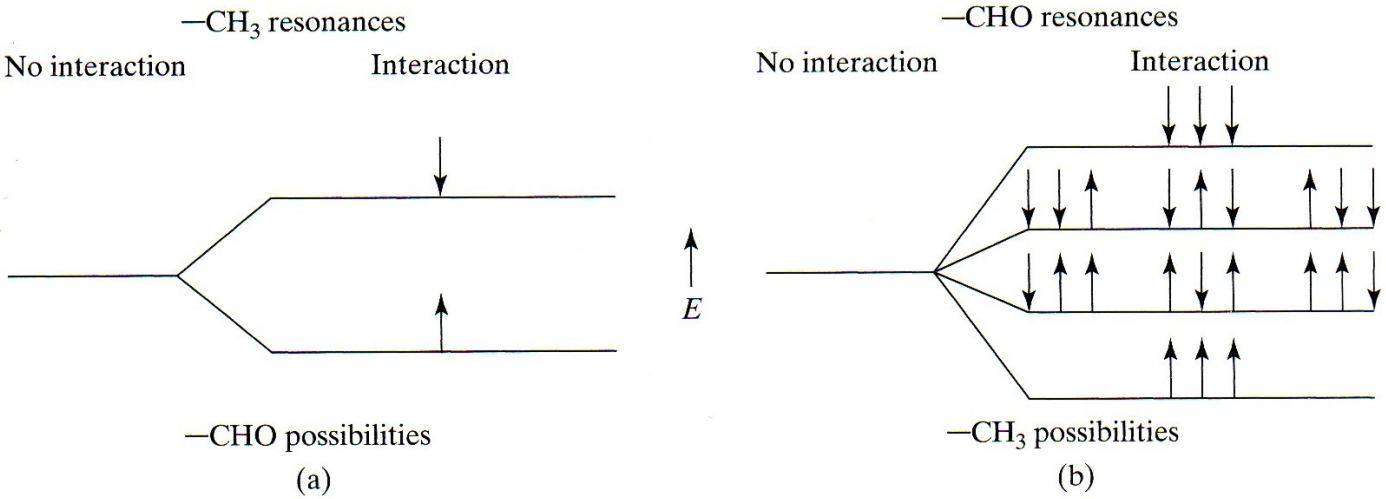


Figure 12.4 Spin-spin interaction in the ^1H NMR spectrum of acetaldehyde.



The magnetic dipoles of the CH_3 group protons interact with the aldehyde proton spin and vice versa. Parallel orientations have higher energies.



NB: the spin-spin coupling constant J also depends on the bond angle
-> info on conformation

1D NMR of macromolecules

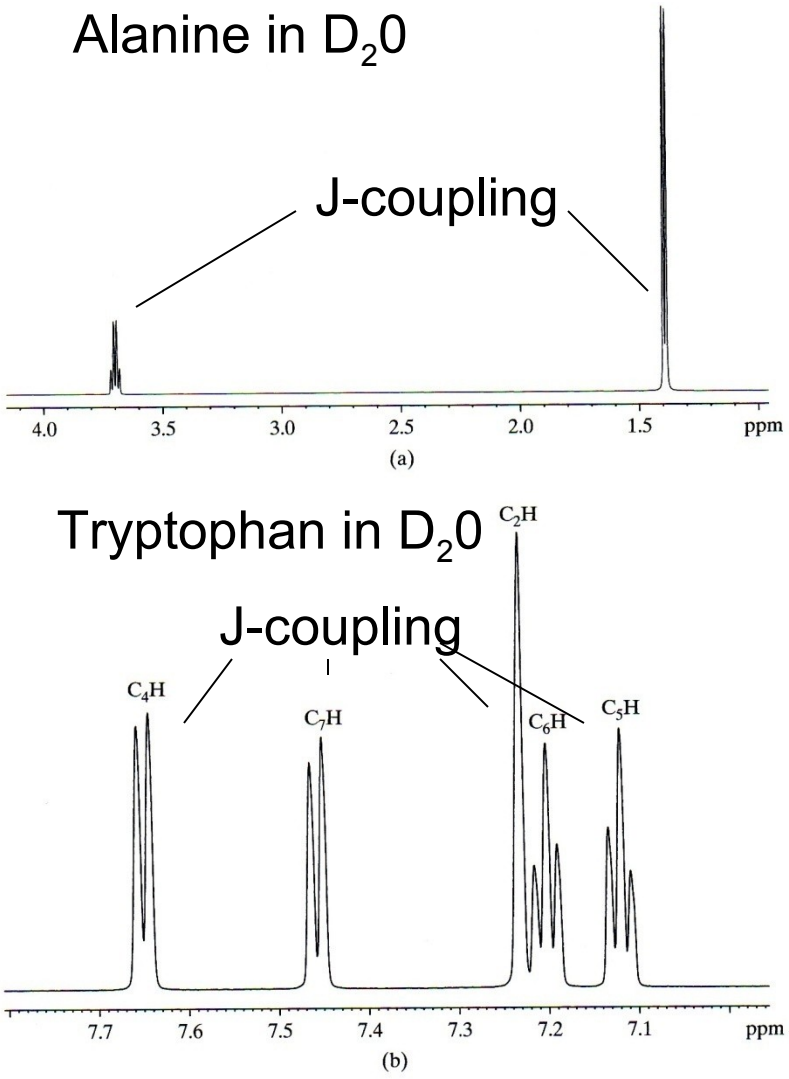


Figure 12.13 (a) The ^1H resonances for the CH and CH_3 of alanine. (b) The ^1H resonances for the nonexchangeable protons on the indole side chain of tryptophan. [Courtesy of V. L. Hsu.]

Assignment of lines ok → structure

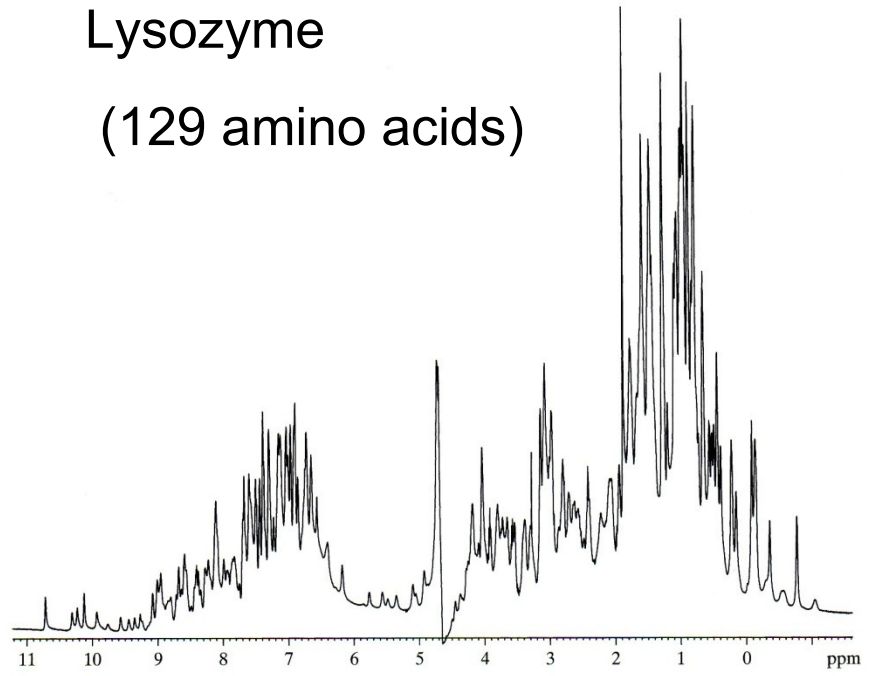
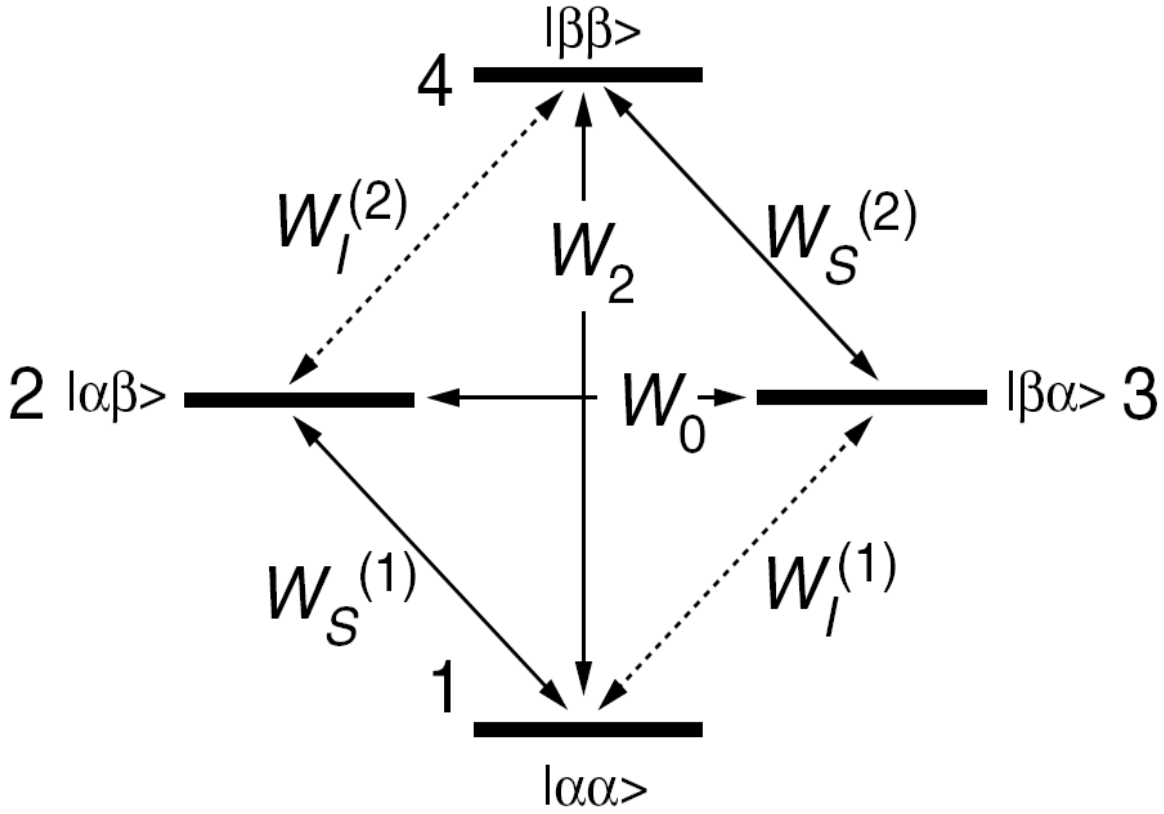


Figure 12.14 The ^1H 1D NMR spectrum of lysozyme at pH 5.3 on a 600 MHz instrument. [Courtesy of V. L. Hsu.]

Assignment too complicated

NB: VERY high field NMR, in principle could solve resolution problem

Interactions between different spin-states



Selection rule demands

$$\Delta m = \pm 1$$

Gives rate equations of the type:

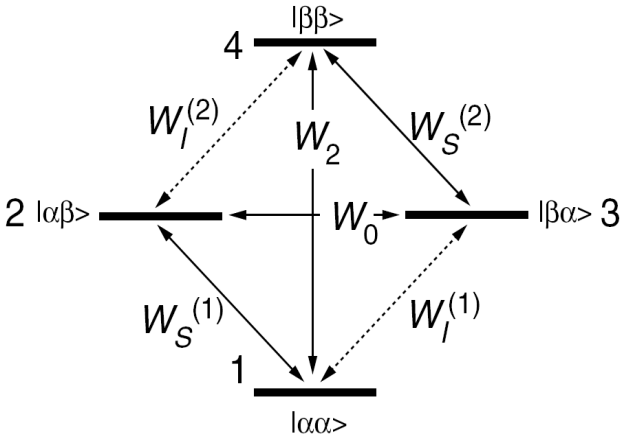
$$\frac{dn_1}{dt} = W_s^{(1)}\left(\Delta n_2 - \Delta n_1\right) + W_I^{(1)}\left(\Delta n_3 - \Delta n_1\right) + W_2\left(\Delta n_4 - \Delta n_1\right)$$

Generalizing from before, we obtain the magnetizations of the two spin states and the population difference:

$$\Delta I_z = \Delta n_1 - \Delta n_3 + \Delta n_2 - \Delta n_4$$

$$\Delta S_z = \Delta n_1 - \Delta n_2 + \Delta n_3 - \Delta n_4$$

$$\Delta 2 I_z S_z = \Delta n_1 - \Delta n_3 - \Delta n_2 + \Delta n_4$$



Thus one obtains a rate equation for the magnetization:

$$\frac{d\Delta I_z}{dt} = \frac{d\Delta n_1}{dt} - \frac{d\Delta n_3}{dt} + \frac{d\Delta n_2}{dt} - \frac{d\Delta n_4}{dt}$$

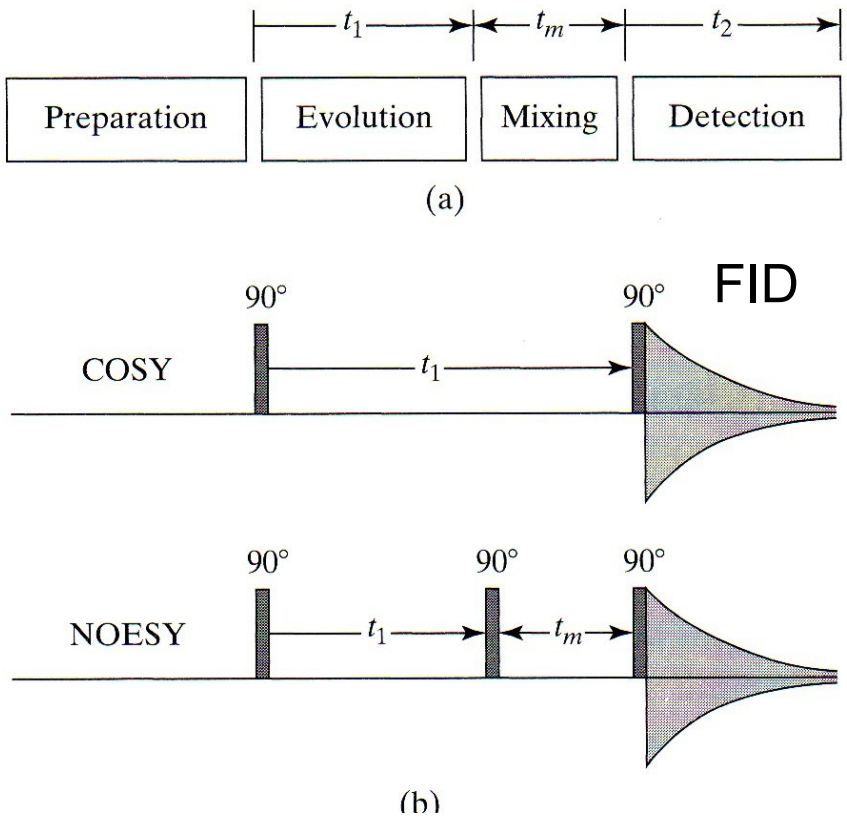
Which is more useful written in terms of magnetizations:

$$\frac{d\Delta I_z}{dt} = -\left(W_I^{(1)} + W_I^{(2)} + W_2 + W_0 \right) \Delta I_z - \left(W_2 - W_0 \right) \Delta S_z - \left(W_I^{(1)} - W_I^{(2)} \right) 2\Delta I_z S_z$$

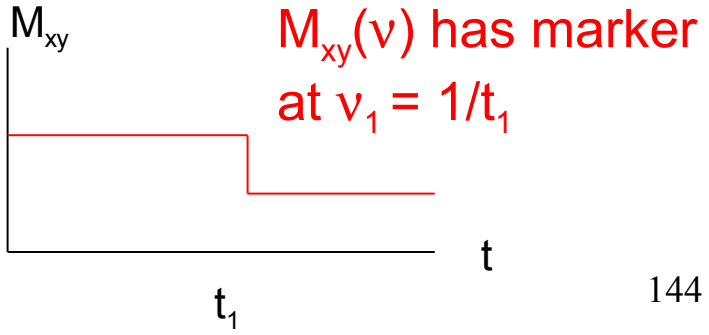
Note selection rules demand $W_2 = W_0 = 0$

The same game can be played for the other magnetization, giving an analogue equation, which cross correlate the different spins.

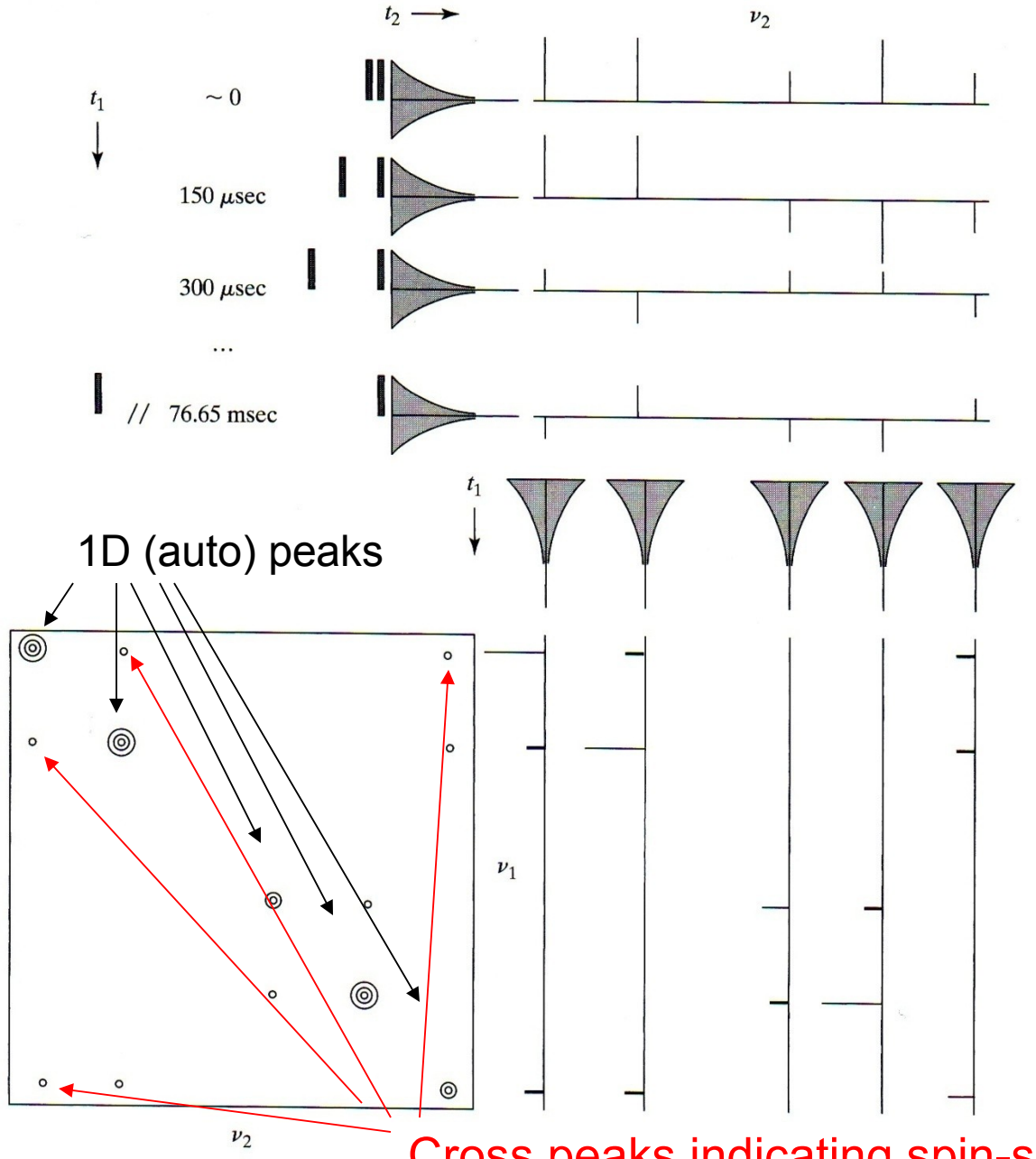
2D NMR of macromolecules makes use of these cross correlations



A second 90° pulse in the same (x) direction as the first one flips all spins pointing into y back to z. The instant M_x stays unaffected.



Protocol: Take FID's at variable values of t_1



Cross peaks indicating spin-spin coupling

2D COSY spectrum of isoleucine

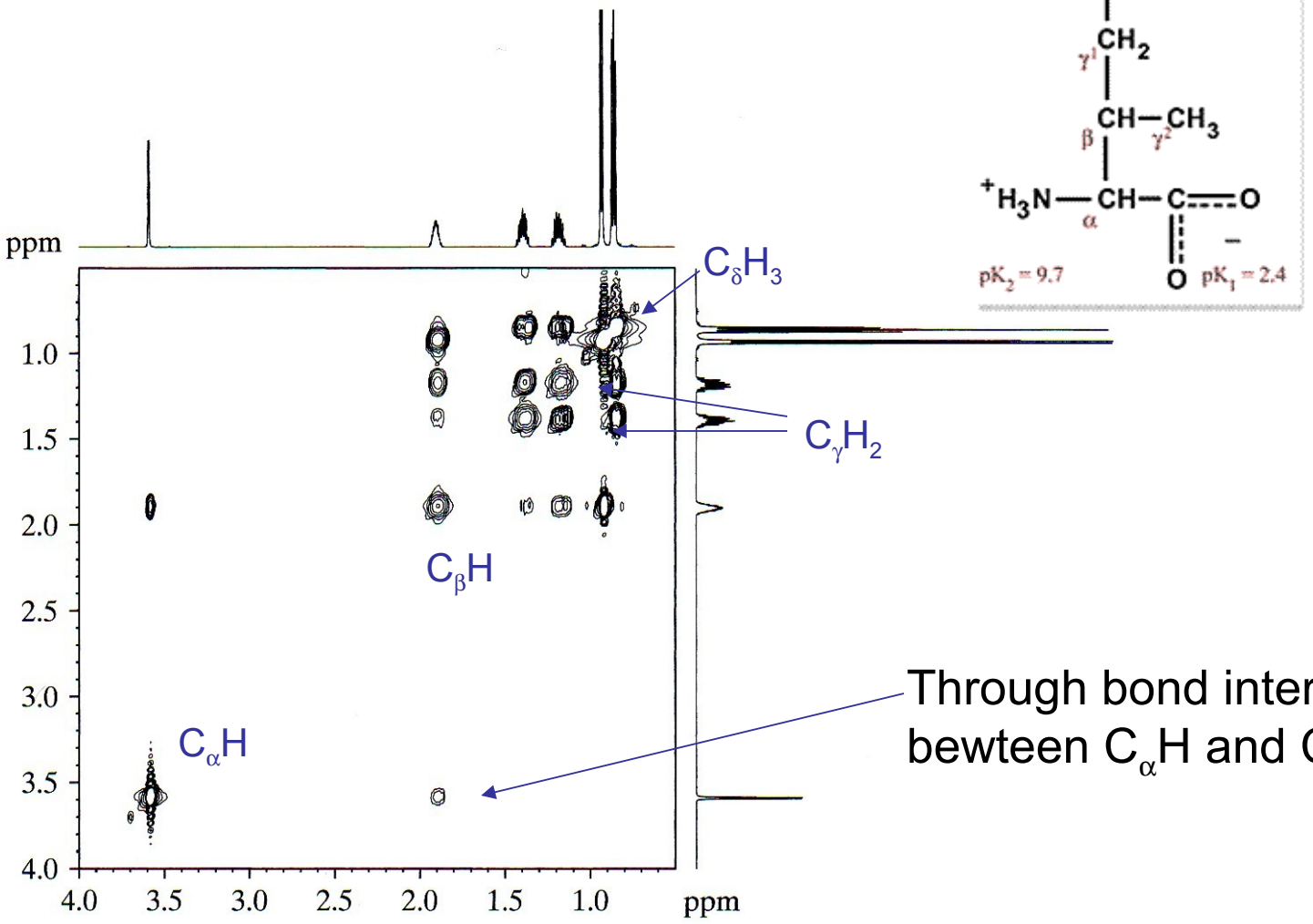
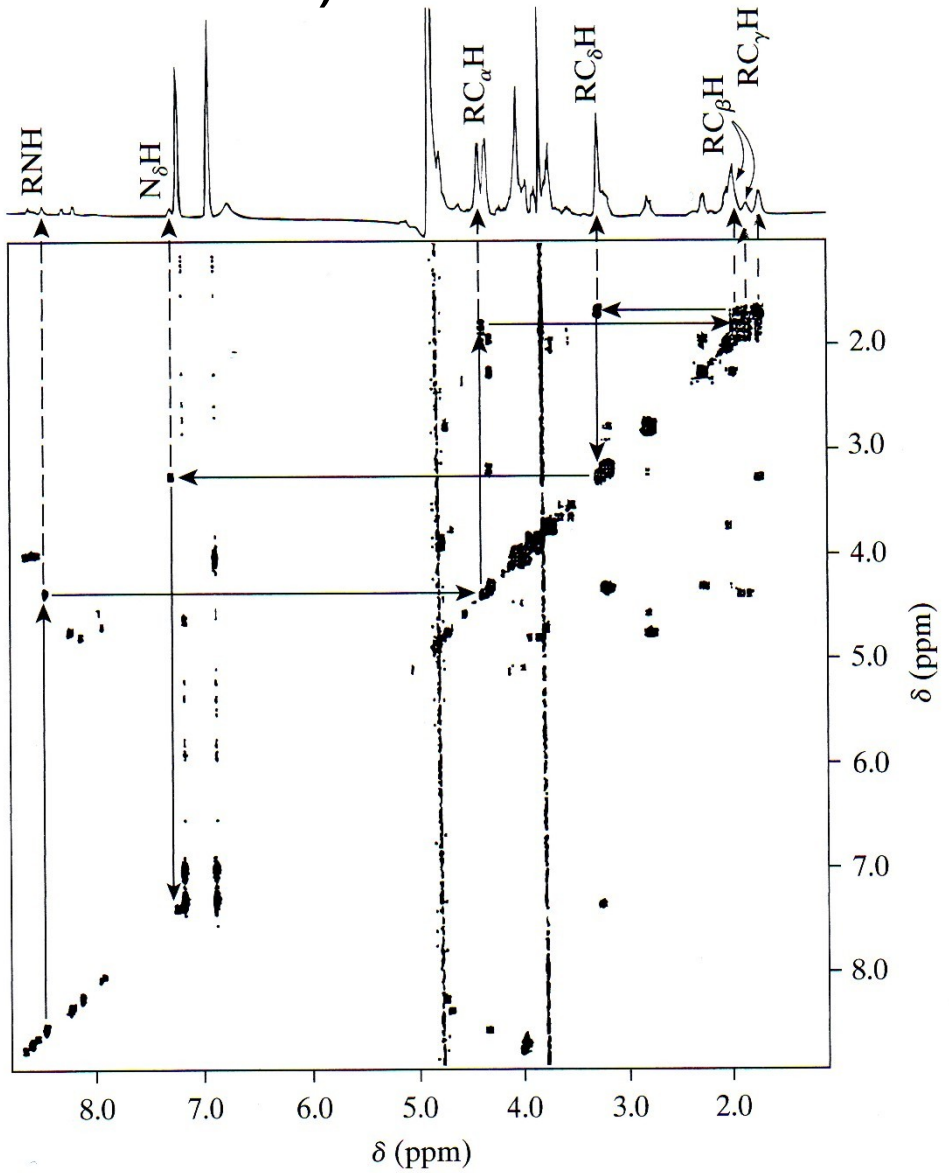


Figure 12.21 The 2D COSY spectrum of isoleucine with the corresponding 1D spectrum displayed along the axes. [Courtesy of V. L. Hsu.]

→ Cross peaks give information on distance along the bond

2D COSY spectrum of a heptapeptide Tyr-Glu-Arg-Gly-Asp-Ser-Pro (YGRGDSP)



Direct dipole-dipole interaction (through space) can take up a change of $\Delta m = +/- 1$, I.e. relax the selection rules.

B-field generated by dipole μ $$\mathbf{B}_{dipole} = [3(\mu \cdot \mathbf{e}_r)\mathbf{e}_r - \mu]/r^3$$

Transition rates go with the square of the interaction $$V_{IS} : \frac{\gamma^2}{r_{IS}^3} \quad , \quad W_{0,2} : \frac{\gamma^4}{r_{IS}^6}$$

Related to the energy changes of A and B due to the induced fields at A and B: $-\mu_A \mathbf{B}_B$ and $-\mu_B \mathbf{B}_A$

Strong dependence on distance between the different spin sites (r^{-6} due to dipole interaction) gives very sensitive spatial information about distances between spins down to 0.5 nm

Now take along the cross terms of the magnetizations gives the Solomon equation:

$$\begin{pmatrix} \dot{\Delta I}_z \\ \dot{\Delta S}_z \end{pmatrix} = \begin{pmatrix} -R_I & -\sigma \\ -\sigma & -R_S \end{pmatrix} \begin{pmatrix} \Delta I_z \\ \Delta S_z \end{pmatrix}$$

Solved by:

$$\begin{pmatrix} \Delta I_z(t) \\ \Delta S_z(t) \end{pmatrix} = \exp(Lt) \begin{pmatrix} \Delta I_z(t=0) \\ \Delta S_z(t=0) \end{pmatrix}$$

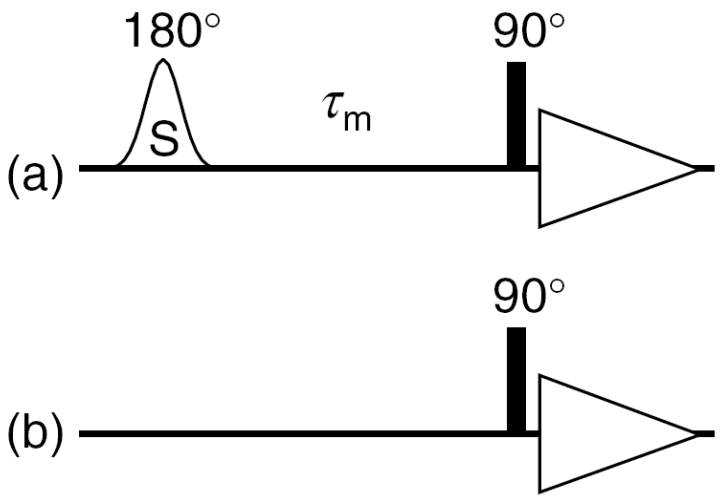
$$\exp(Lt) = \begin{pmatrix} \left(\frac{R_S - R_I}{2R} + \frac{1}{2}\right)\exp(-\lambda_1 t) + \left(\frac{R_I - R_S}{2R} + \frac{1}{2}\right)\exp(-\lambda_2 t) & \frac{\sigma}{R}\left(\exp(-\lambda_1 t) - \exp(-\lambda_2 t)\right) \\ \frac{\sigma}{R}\left(\exp(-\lambda_1 t) - \exp(-\lambda_2 t)\right) & \left(\frac{R_I - R_S}{2R} + \frac{1}{2}\right)\exp(-\lambda_1 t) + \left(\frac{R_S - R_I}{2R} + \frac{1}{2}\right)\exp(-\lambda_2 t) \end{pmatrix}$$

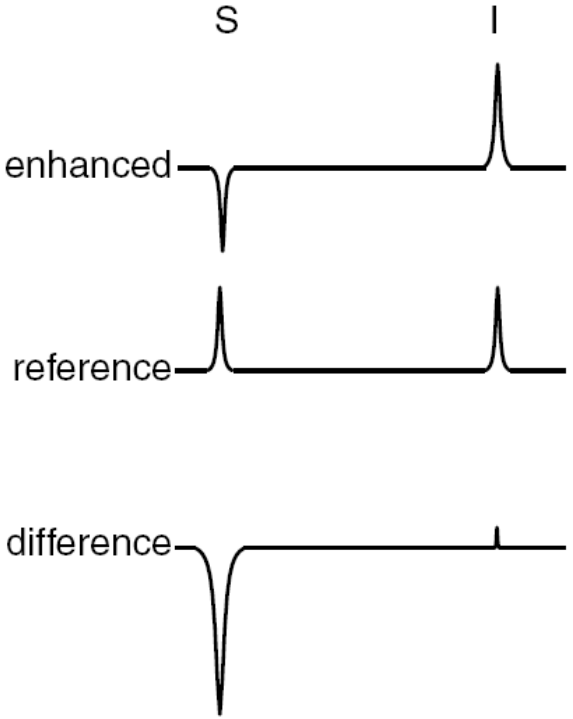
$$R = \sqrt{(R_I - R_S)^2 + 4\sigma^2} \qquad \lambda_{1,2} = \frac{1}{2}(R_I + R_S \pm R)$$

Simplify by assuming $R_I = R_S$:

$$\exp(Lt) = \begin{pmatrix} \frac{1}{2}\left(\exp(-\lambda_1 t) + \exp(-\lambda_2 t)\right) & \frac{\sigma}{R}\left(\exp(-\lambda_1 t) - \exp(-\lambda_2 t)\right) \\ \frac{\sigma}{R}\left(\exp(-\lambda_1 t) - \exp(-\lambda_2 t)\right) & \frac{1}{2}\left(\exp(-\lambda_1 t) + \exp(-\lambda_2 t)\right) \end{pmatrix}$$

This implies maximum mixing after a time scale τ_m



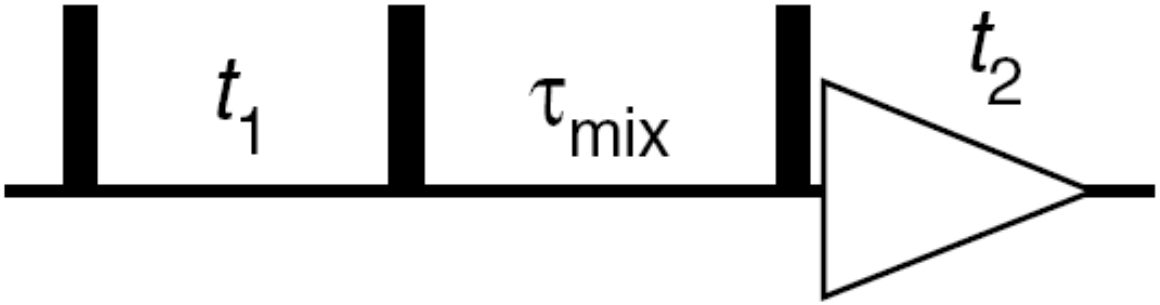


Flip the spins S at that time to enhance contrast

For macromolecules, there are many interacting spins, thus a much more complicated set of equations would have to be solved

$$\frac{\bullet}{\Delta I} = \begin{pmatrix} R_1 & \sigma_{1j} & \sigma_{1n} \\ \sigma_{i1} & \ddots & \sigma_{in} \\ \sigma_{n1} & \sigma_{nj} & R_n \end{pmatrix} \Delta I$$

Combine this (Nuclear Overhauser) enhancement with the technique of 2D spectroscopy gives NOESY:



The appearance of correlation peaks as a function of τ_{mix} gives information about the spatial properties (σ) of the atoms

Part of 2D NOESY spectrum of a YGRGDSP

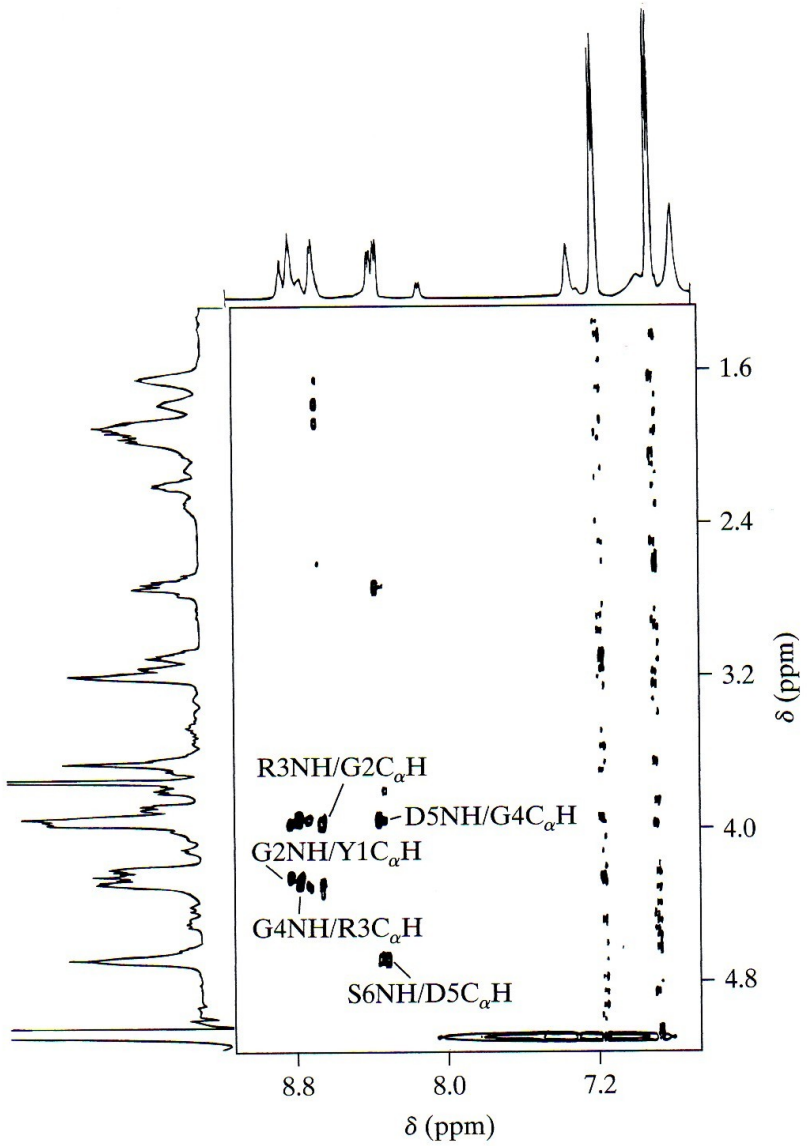
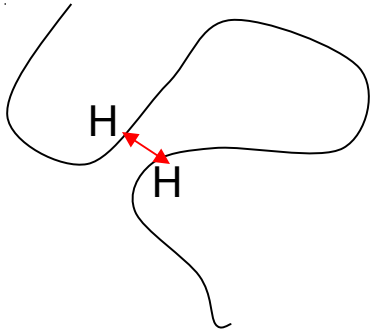
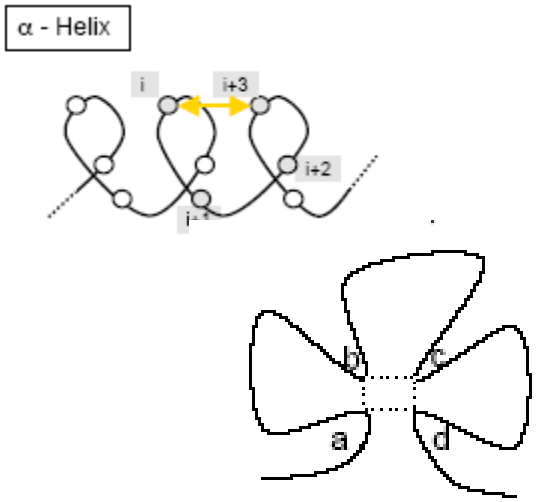


Figure 12.27 A portion of the NOESY spectrum for YGRGDSP.



NOESY correlates all protons near in real space even if the are chemically distant

Typical NOESY signatures



Determination of protein structure from multi-dimensional NMR - data

Starting structure (from chemical sequence)

Random folding at start of simulation

Heating to overcome local energy barriers

Cooling under distance constraints from NMR

Repeating for many starting structures

→ Family of structures

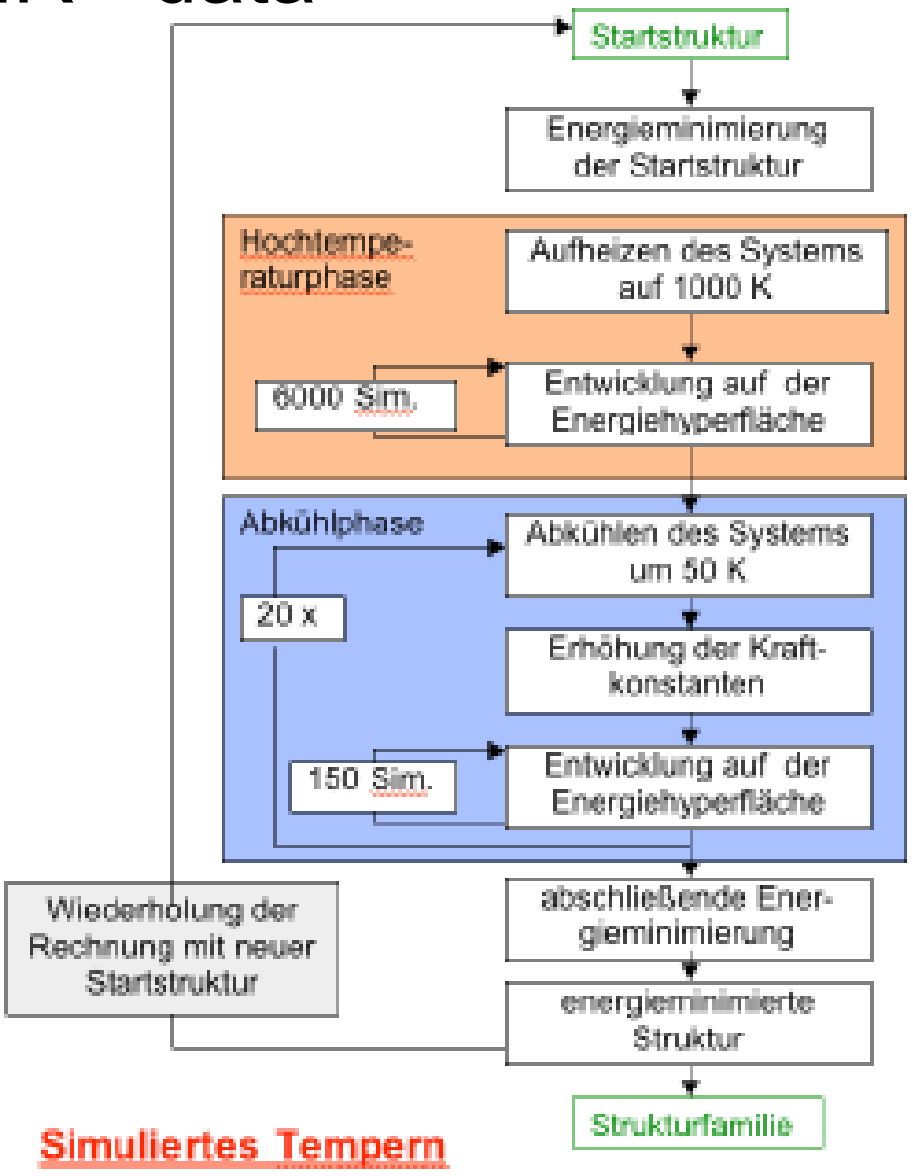


Table 1. Standard protocol for NMR structure determination of proteins

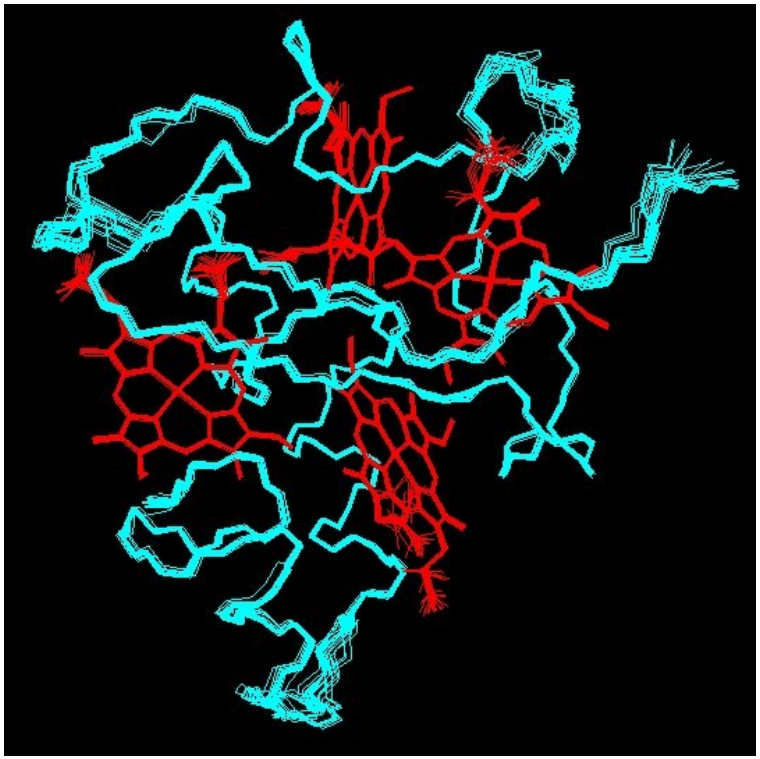
Step [a]		BUSI IIA [b]
I	Sample preparation	Protein isolated from natural source; natural isotope distribution; 16 mM solutions in H_2O and in ^2H_2O, respectively
II	NMR spectroscopy	2D ^1H NMR
IIIa	Resonance assignments	Sequential NOEs
IIIb	Conformational constraints	[^1H,^1H]-NOEs, $^3J_{HN\alpha}$, $^3J_{\alpha\beta}$
IIIc	Structure calculation	Metric matrix distance geometry
IIId	Structure refinement	Restrained energy minimization

[a] The structural interpretation of the NMR data, III, is somewhat arbitrarily divided up into four steps; in practice, one goes though multiple cycles of collection of conformational constraints (IIIb) and structure calculation (IIIc), and the completion of the sequence-specific assignments (IIIa) as well as the structure refinement (IIId) may also be part of this iterative approach.

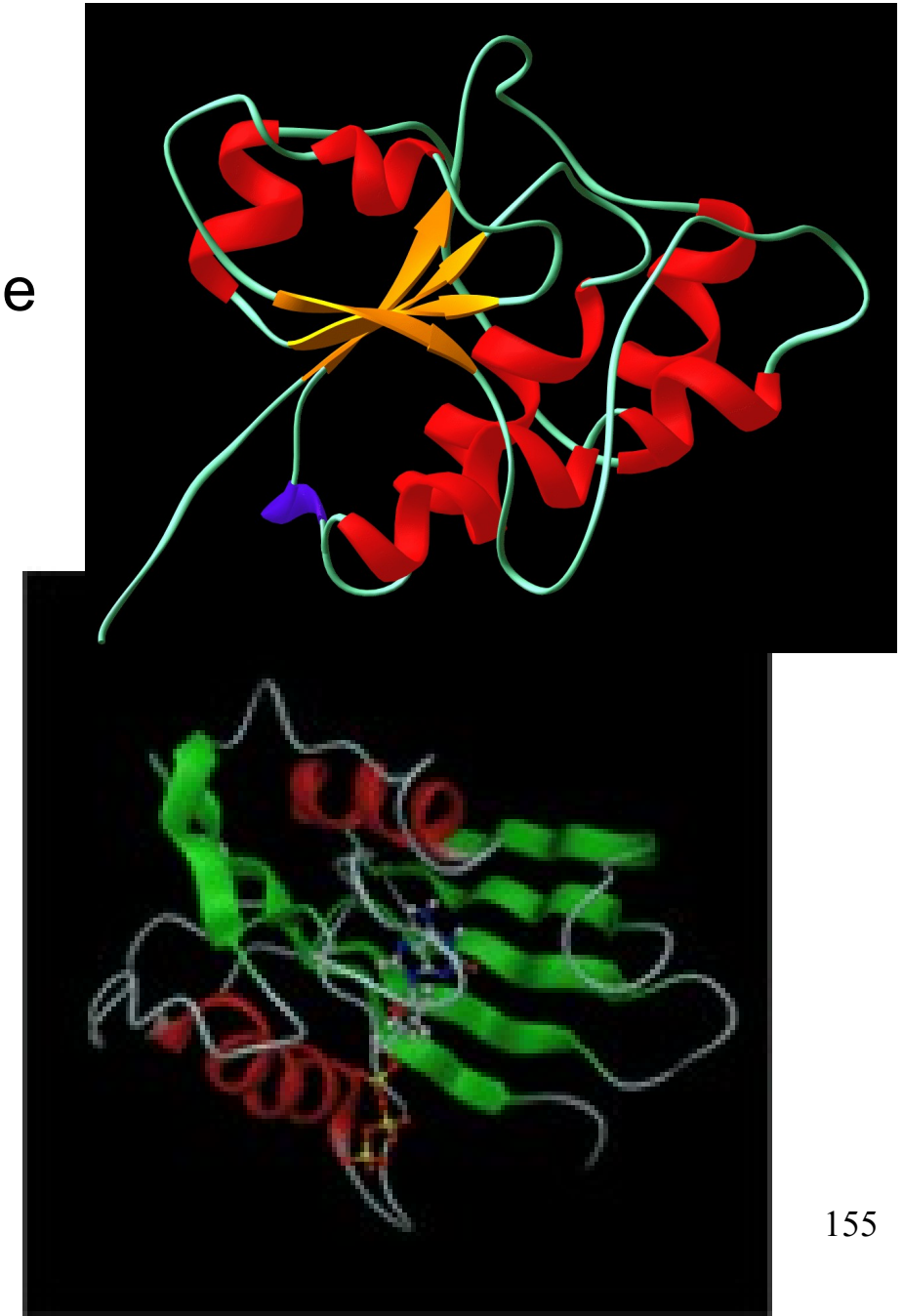
[b] This column lists the techniques used in the first structure determination of a globular protein in 1984 (43).

NMR solution structures of proteins

Tyrosine Phosphatase

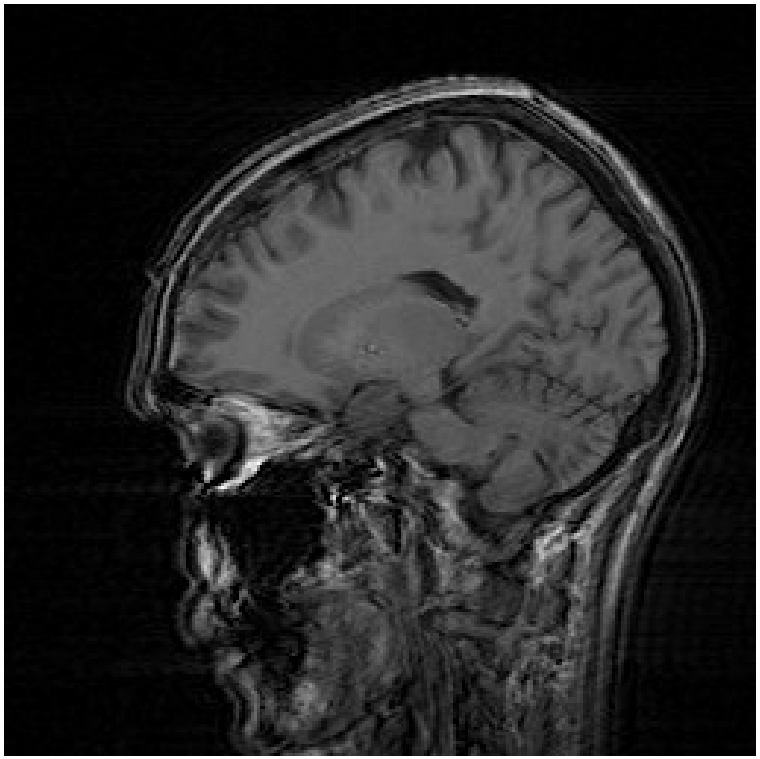


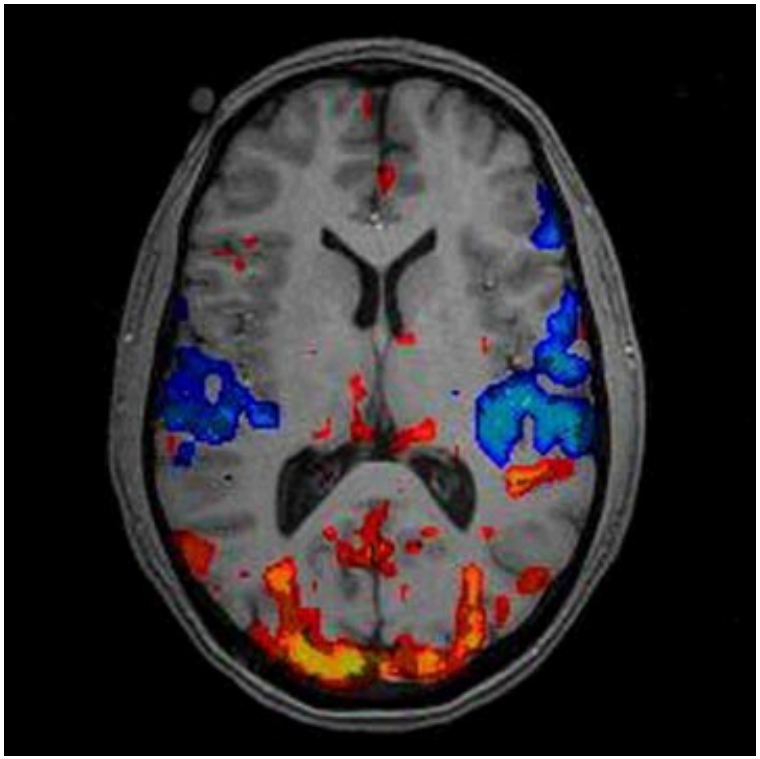
Cytochrome 3



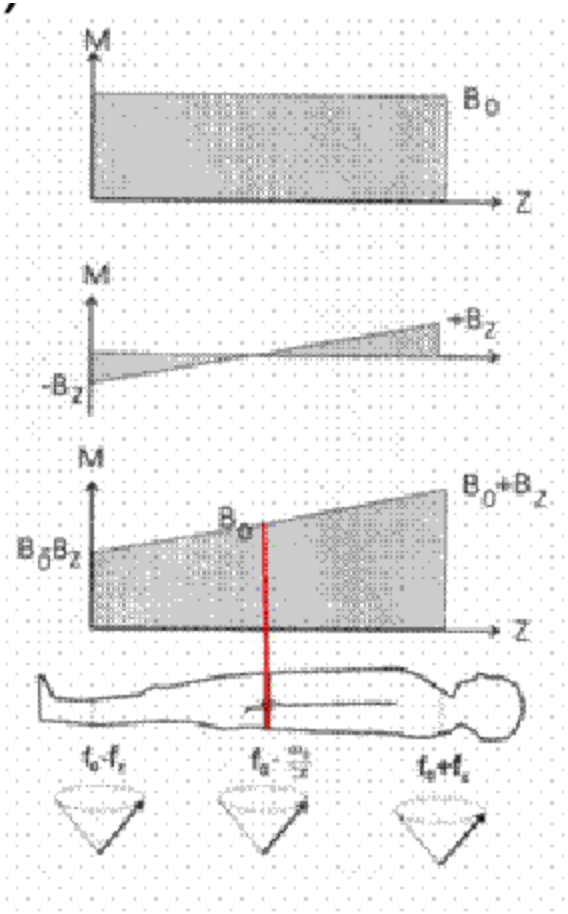
3.5 MRI

At much reduced spatial resolution, NMR can also be used as an imaging tool, where the spatial resolution is obtained by encoding space by a frequency (i.e. a field gradient)



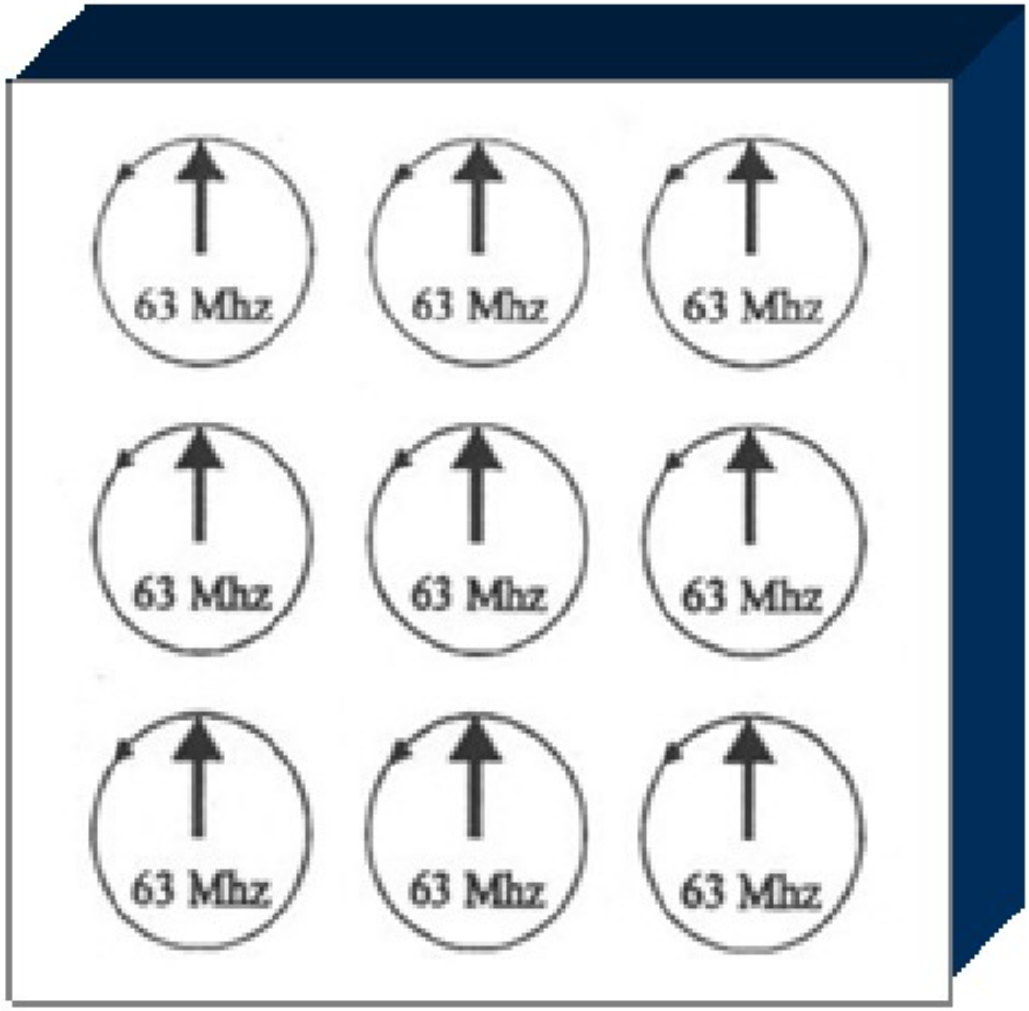


Mostly driven by T_2 relaxations, apply a gradient field across the sample, which gives different Larmor frequencies for different positions (all done at H frequencies)

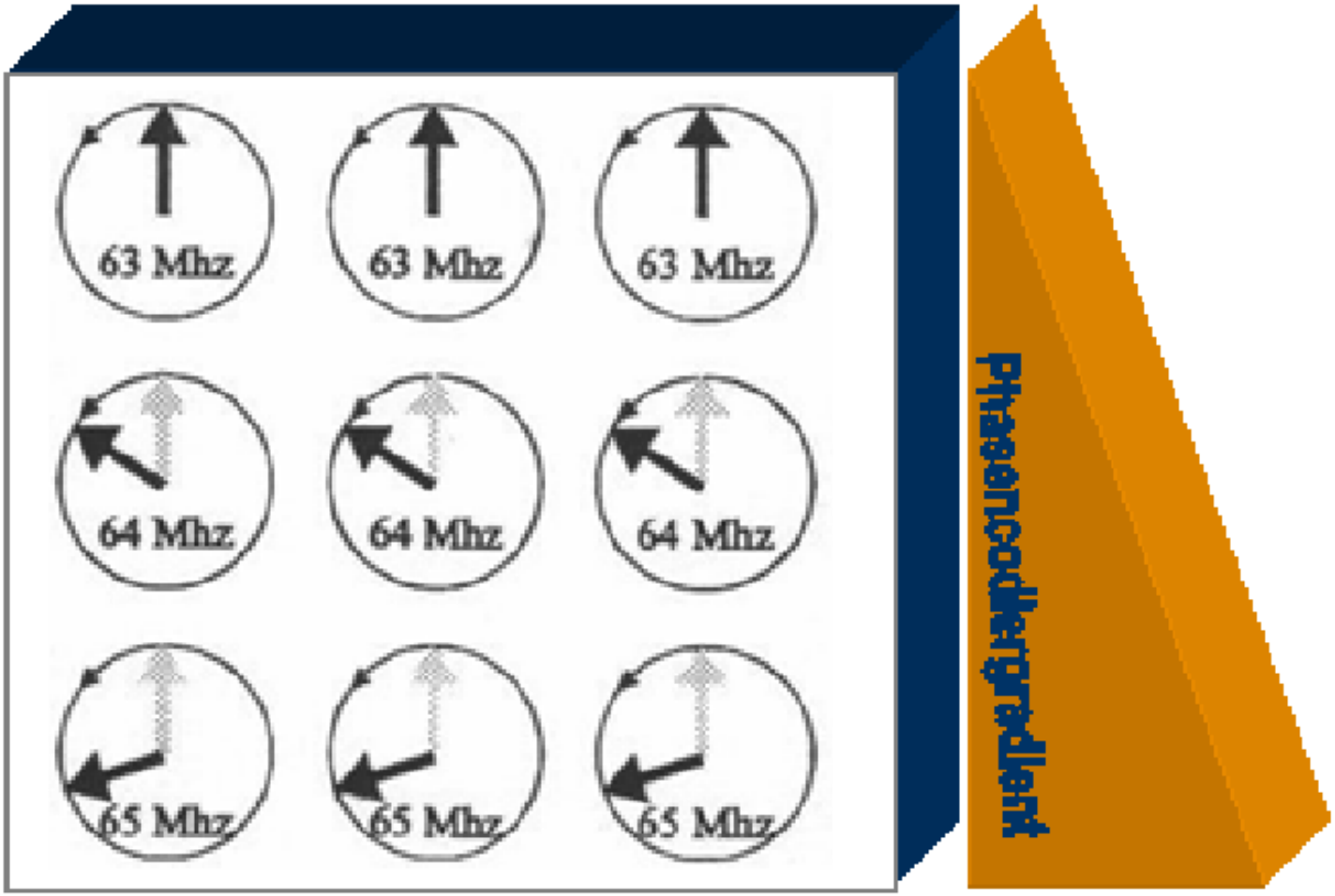


Resonance condition only fulfilled at one specific position

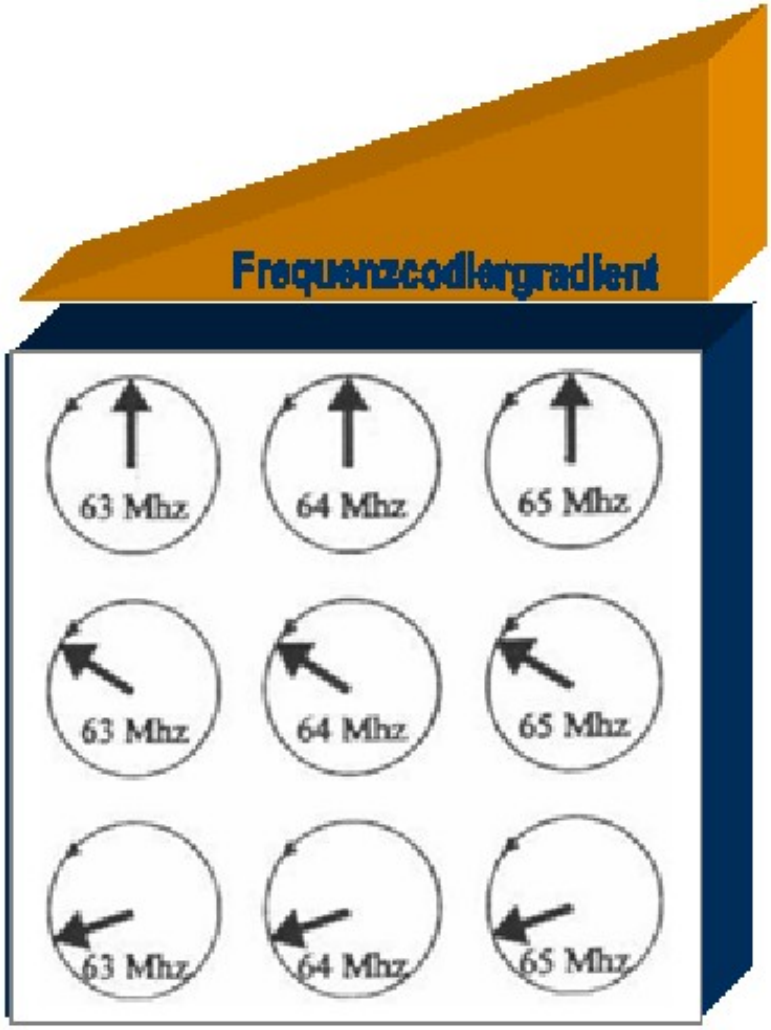
Now we have to also encode position in the x-y direction



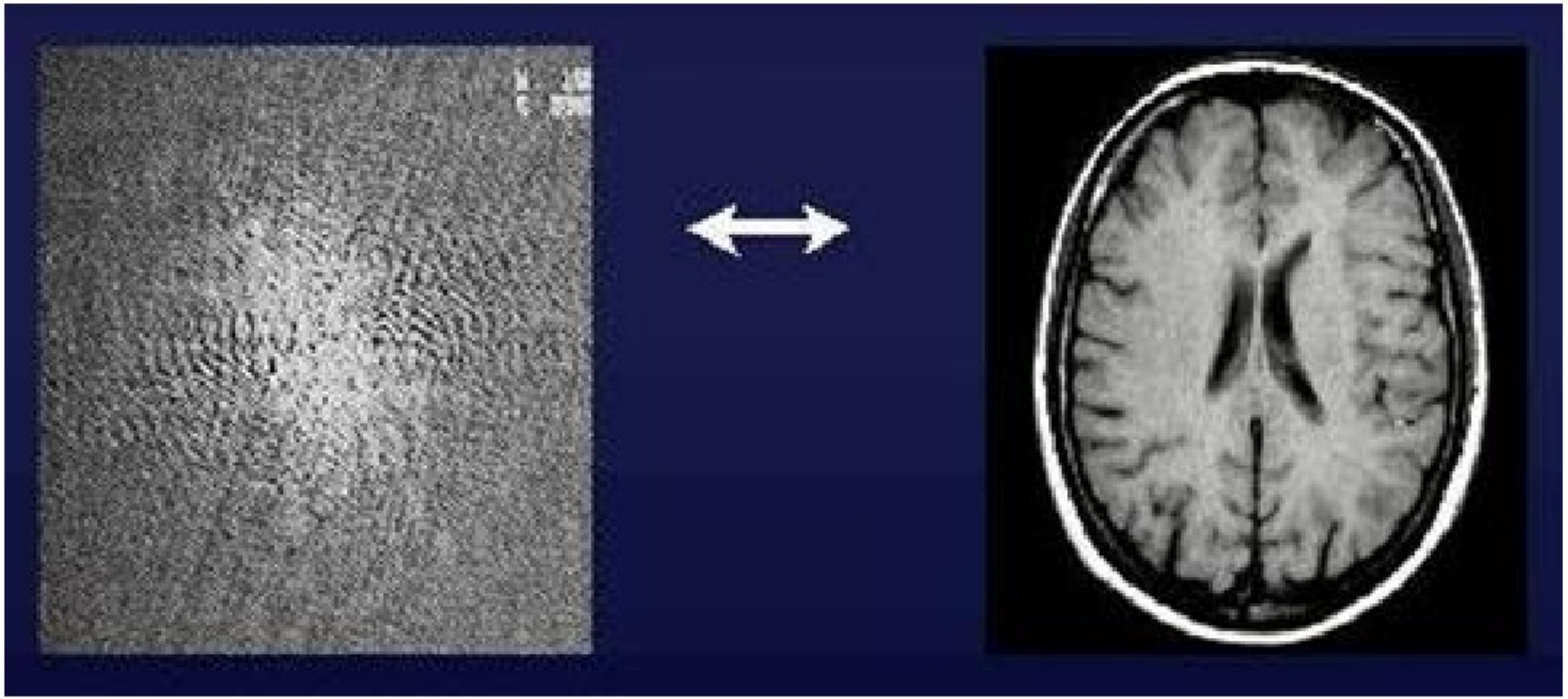
Apply a field gradient along the y-direction for a short time, which gives a phase shift to the different nuclei as a function of depth



Finally apply a field gradient along the x-direction during readout, which gives a frequency shift of the FID precession

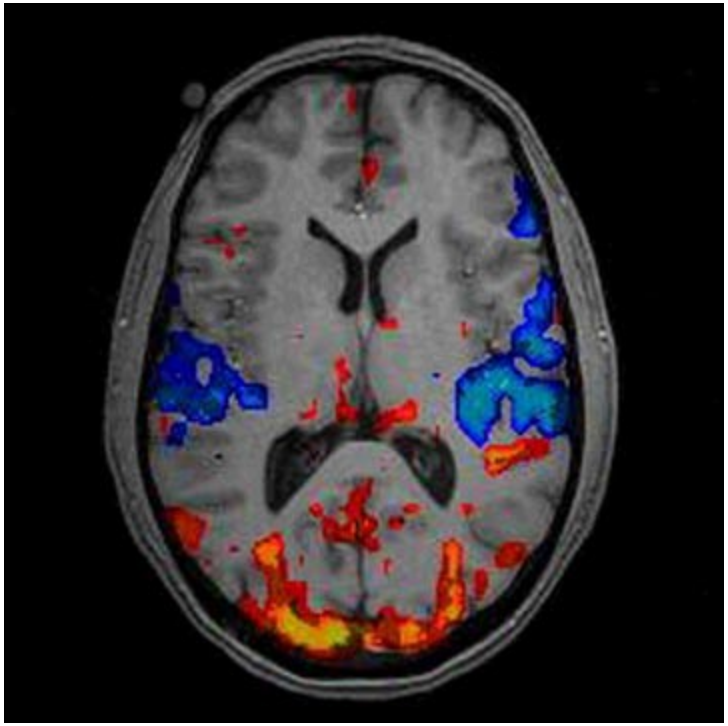


Then you take a signal with a pickup coil as a function of FID time and time duration of the phase coding pulse, which you Fourier transform to obtain a proper image



Since you have turned a spatial measurement into a spectroscopic one, the resolution is spectroscopically limited (or limited by the gradients you apply)

Therefore fast scans (needed for functional studies have less resolution)



Recap Sec. 3

NMR is a spectroscopic method given by the absorption of em radiation by nuclei

The signals depend on the nuclei, the applied field and the chemical environment

Using Fourier-transform methods, a fast characterization of different freqeuncy spectra is possible

Sensitivity is enhanced by using cross correlations in 2D NMR

More recap

Dipole-Dipole interactions can be used to characterize spatial relationships

Spin-Spin interactions are used to determine chemical bonds

Gives atomic resolution for macromolecules including dynamics

Using magnetic field gradients, spatially resolved measurements are possible resulting in MRI

Rawat's Creation-
rwtdgreat@gmail.com
rwtdgreat@yahoo.co.uk

www.slideshare.net/

@RawatDAgreatt

+919808050301
+919958249693