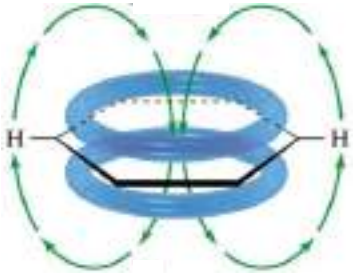

^{1}H Nuclear Magnetic Resonance Spectroscopy

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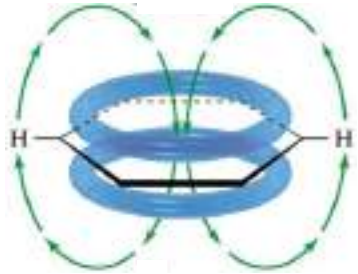
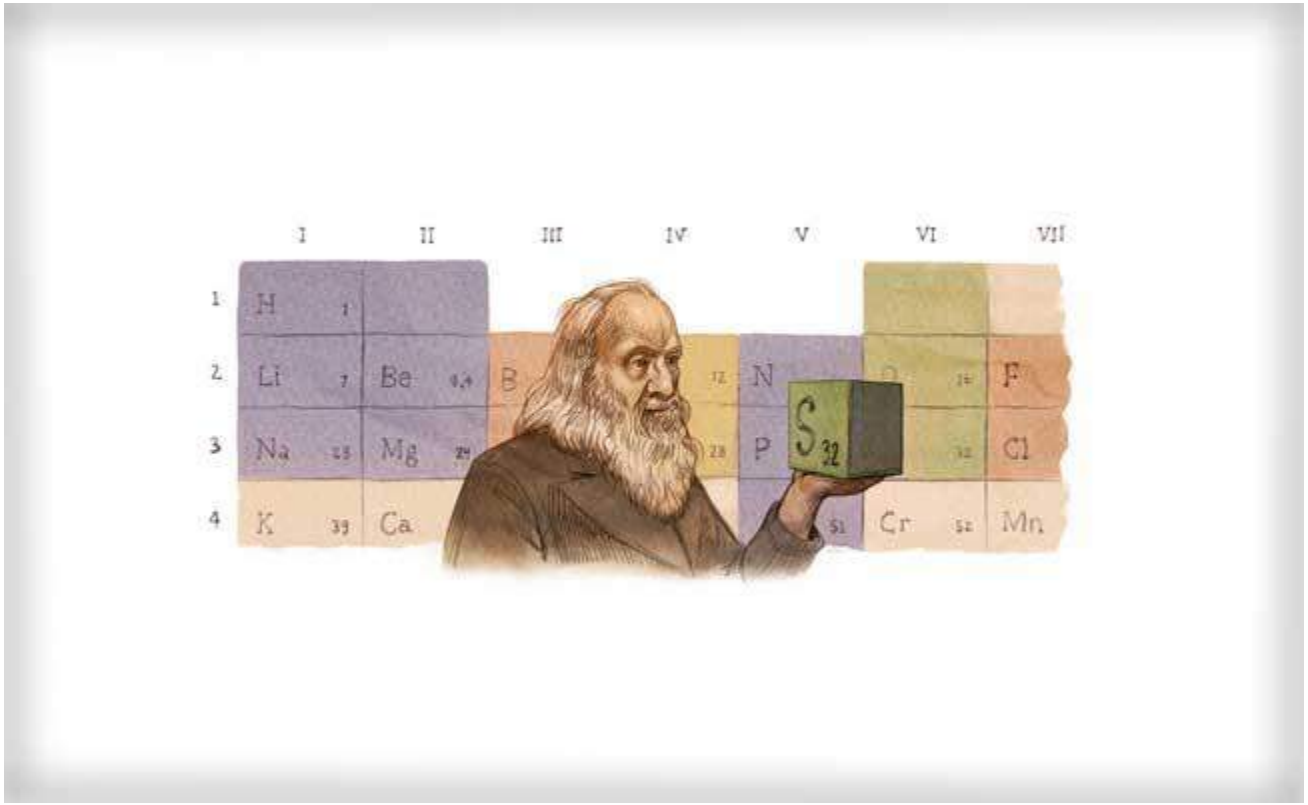
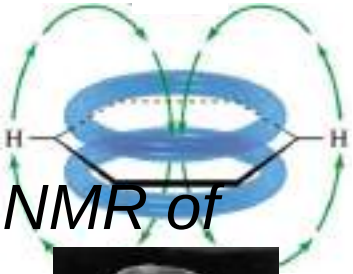

H
H

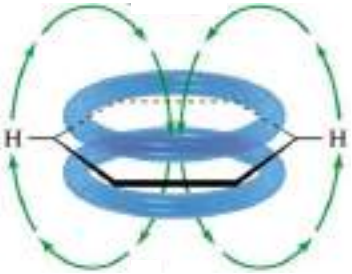

I
II
III
IV
V
VI
VII
1
H
2
Li
Be
B
N
F
3
Na
Mg
P
S 32
Cl
4
K
Ca
Cr
Mn

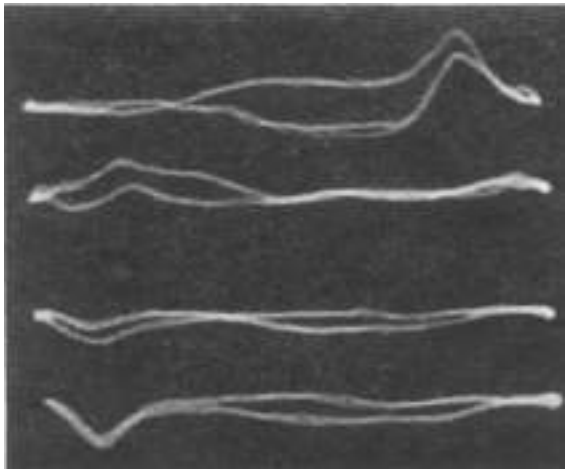
Historical development



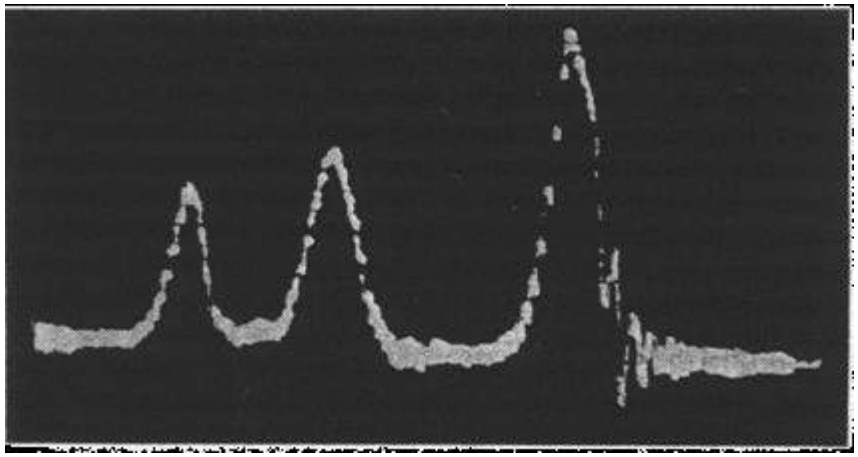
1937 *Rabi predicted NMR 1946 Bloch, Purcell first NMR of bulk sample*
1953 *Overhauser NOE (nuclear Overhauser effect)*
1966 *Ernst, Anderson Fourier transform NMR*
1975 *Jeener, Ernst 2D NMR*
1985 *Wüthrich first solution structure of a small protein (BPTI)* from NOE derived distance restraints
1987 3D 13C, 15N isotope labeling of recombinant prote
(resolution)NMR +
1990 pulsed field gradients (artifact suppression)
1996/7 new *long range structural parameters:*
- *residual dipolar couplings from partial alignment in liquid crystalline media*
- projection angle restraints from *cross-correlated relaxation TROSY (molecular weight > 100 kDa)*

NMR HISTORY



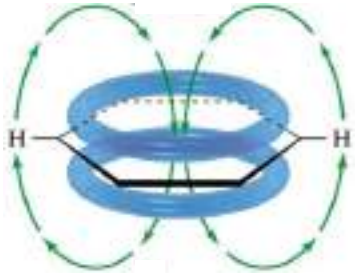


1H H NMR spectra of water (*First NMR Spectra on Water, 1946)*



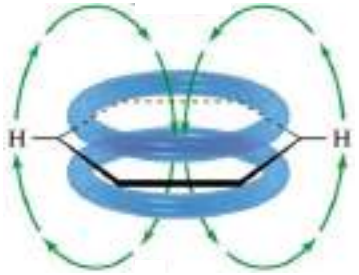
1H (*First Observation of the Chemical Shift, 1951) H NMR spectra ethanol*

Introduction

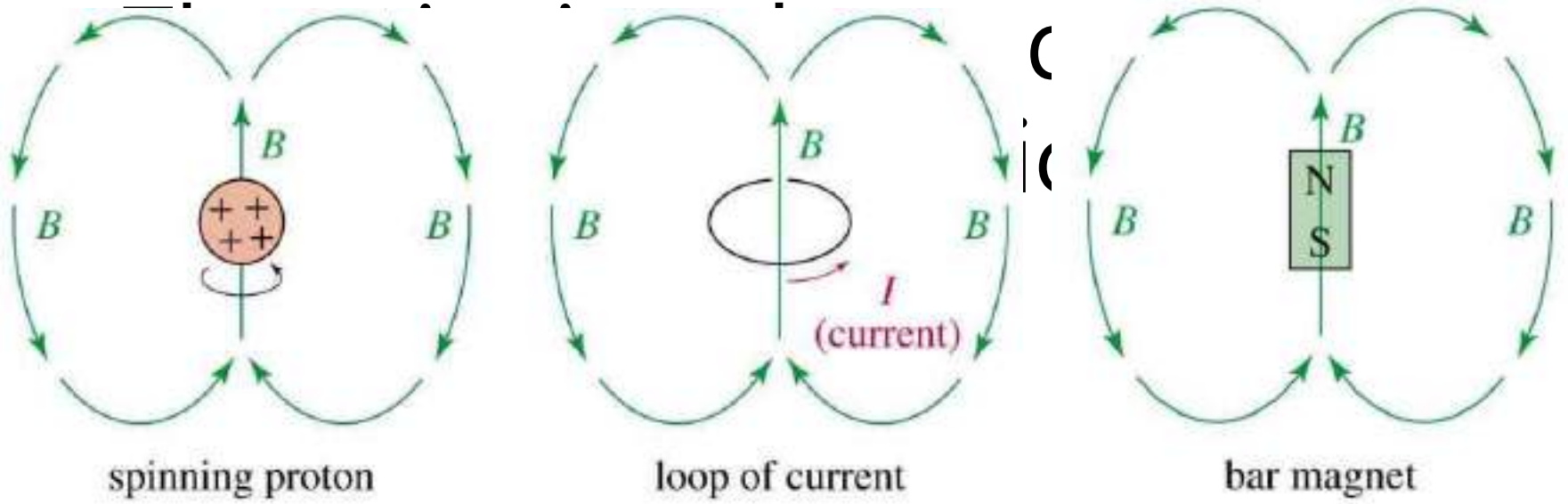


- NMR is the most powerful tool available for organic structure determination.
- It is used to study a wide variety of nuclei:
  - ^{1}H
  - ^{13}C
  - ^{15}N
  - ^{19}F

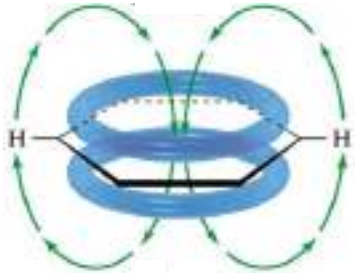
Nuclear Spin



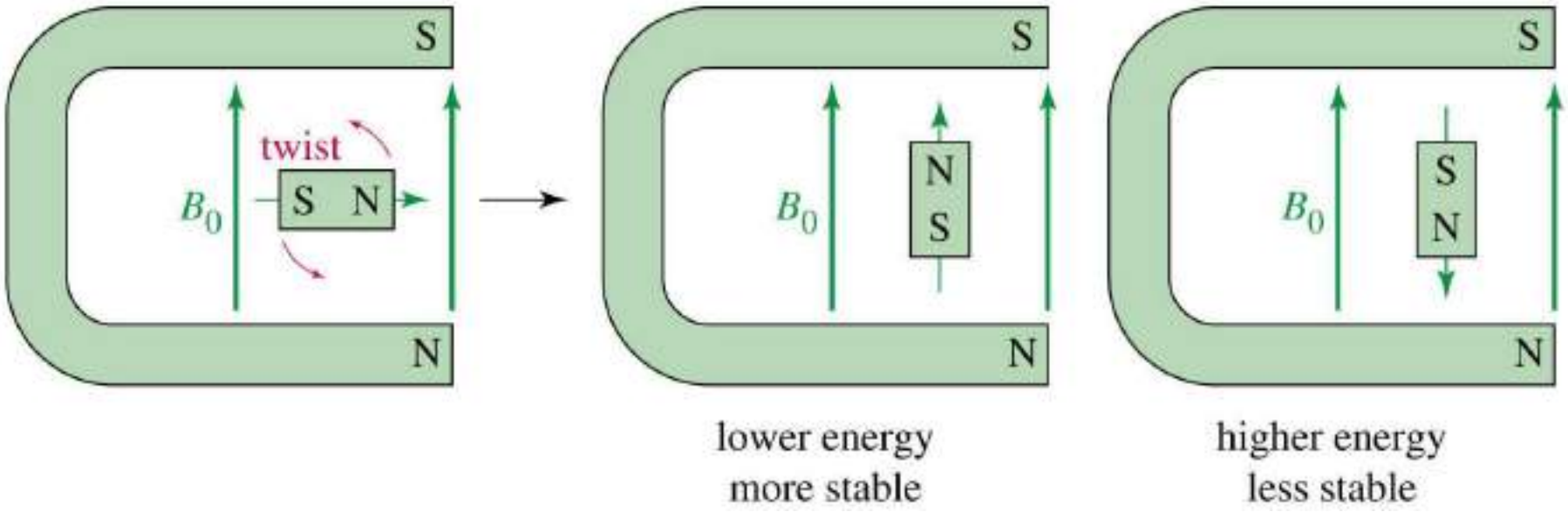
- A nucleus with an odd atomic number or an odd mass number has a nuclear spin.



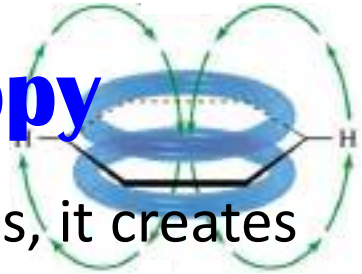
External Magnetic Field



When placed in an external field, spinning protons act like bar magnets.



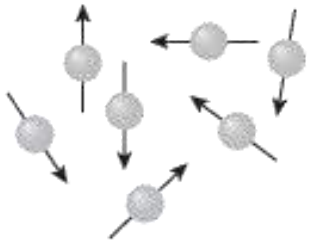
Introduction to NMR Spectroscopy



- When a charged particle such as a proton spins on its axis, it creates a magnetic field. Thus, the nucleus can be considered to be a tiny bar magnet.
- Normally, these tiny bar magnets are randomly oriented in space. However, in the presence of a magnetic field B_0, they are oriented with or against this applied field. More nuclei are oriented with the applied field because this arrangement is lower in energy.
- The energy difference between these two states is very small (<0.1 cal).

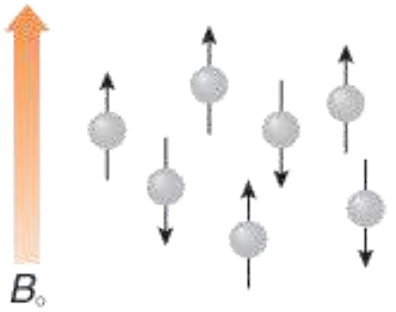
A spinning proton creates a magnetic field.

With no external magnetic field...



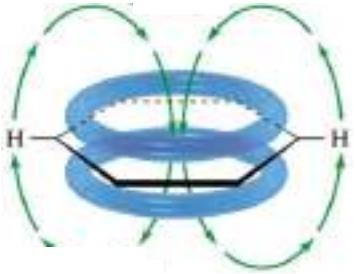
The nuclear magnets are randomly oriented.

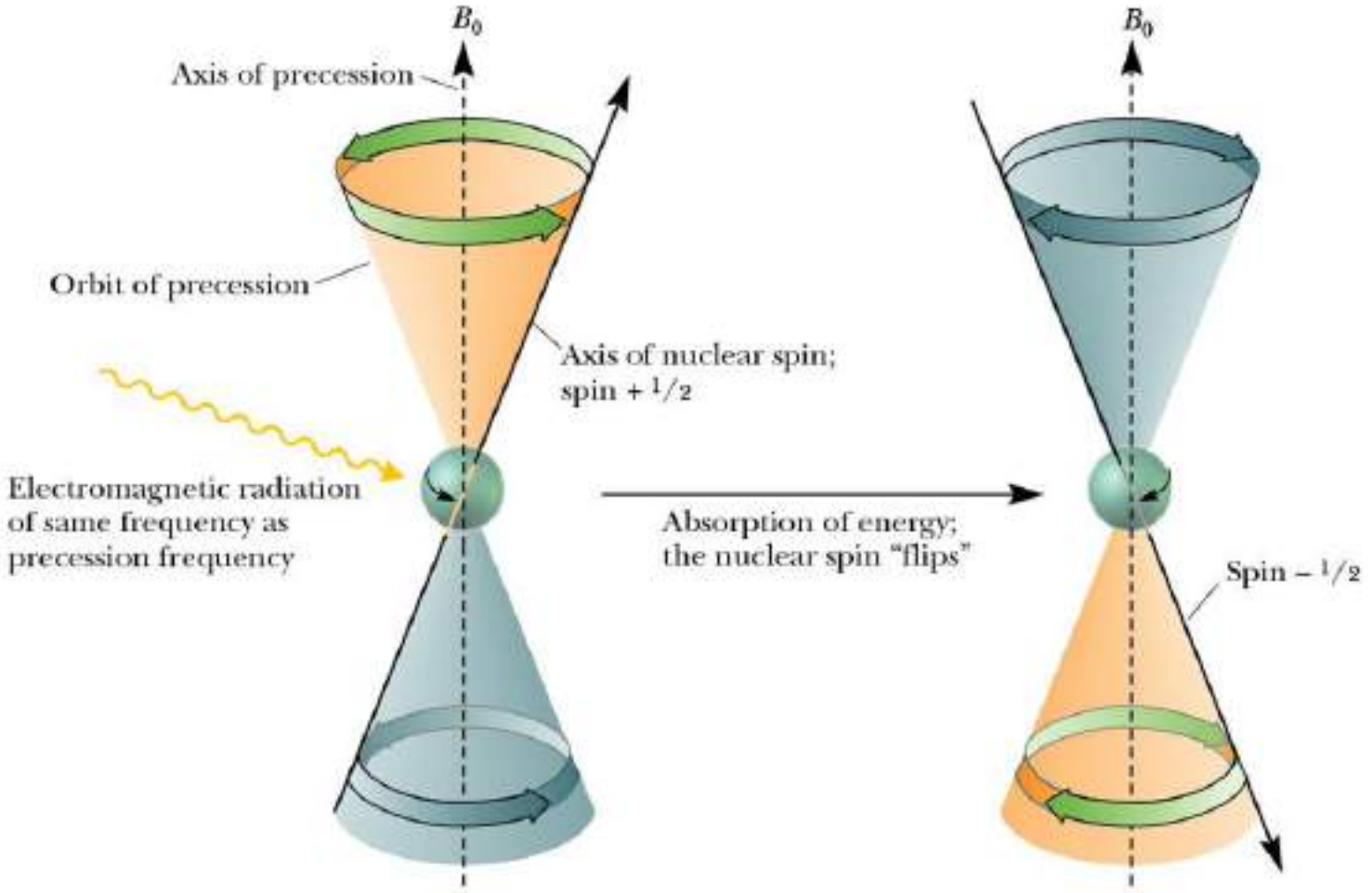
In a magnetic field...



The nuclear magnets are oriented **with or against B_0.**

Processional motion





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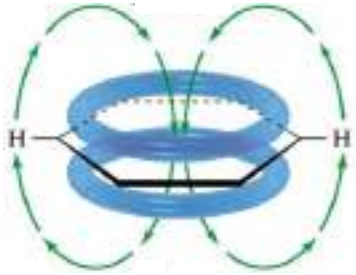
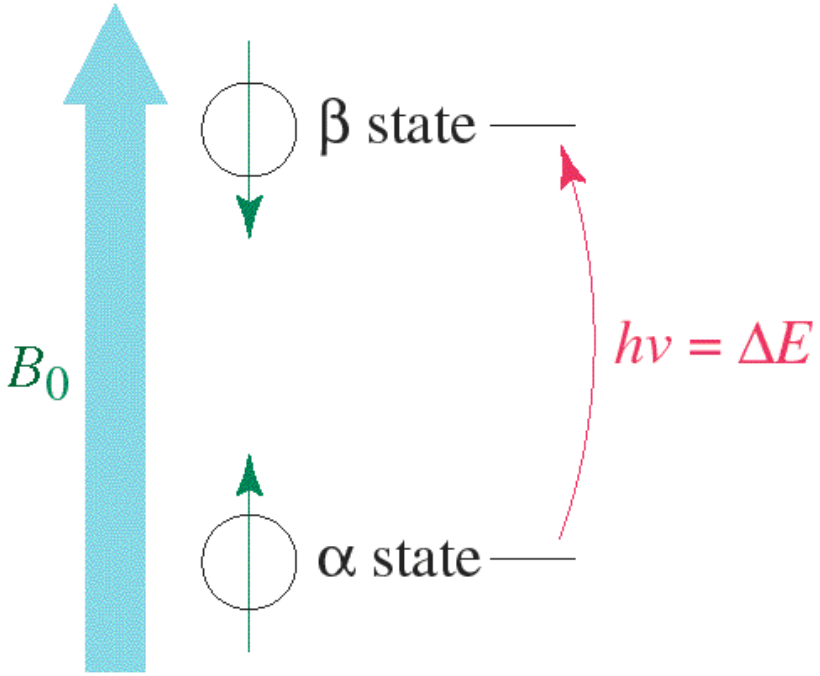
Two Energy States

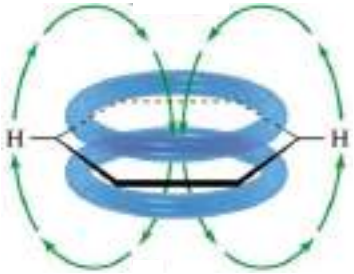


The magnetic fields of the spinning nuclei will align either *with* the external field, or *against* the field.

A photon with the right amount of energy can be absorbed and cause the spinning proton to flip

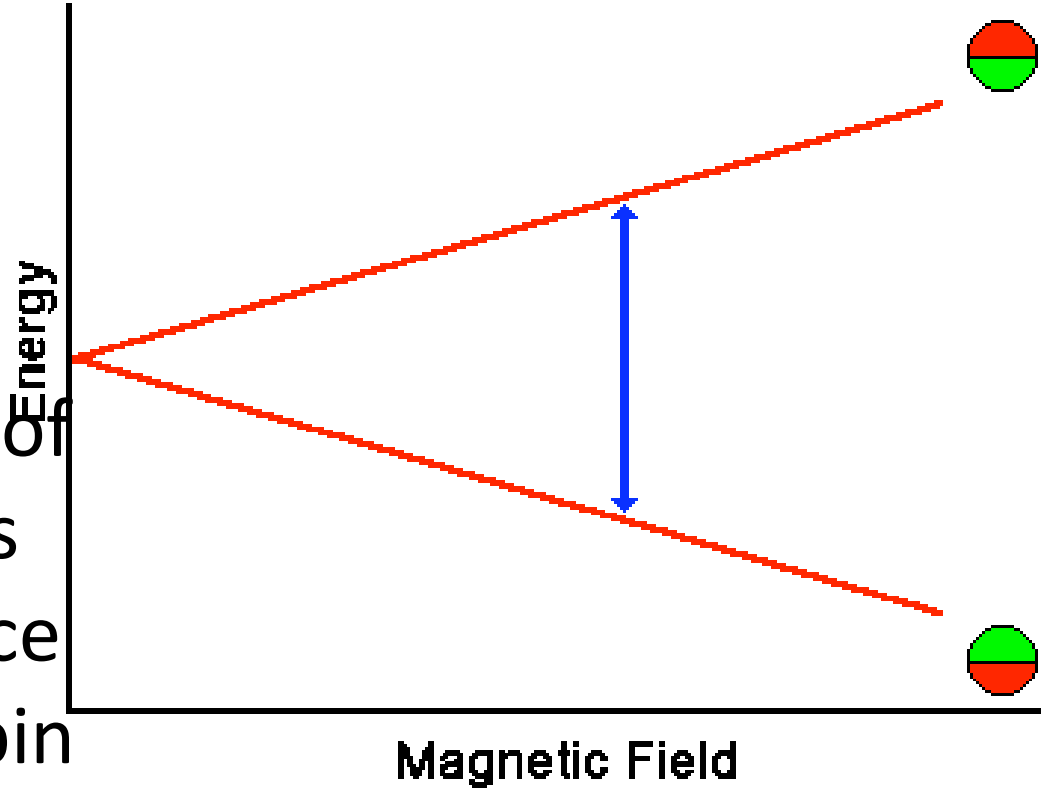


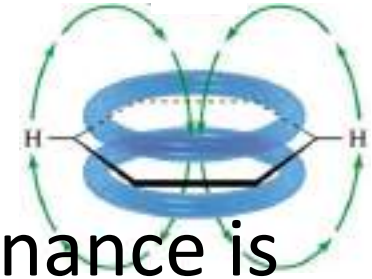
RESONANCE



Two spin states in the magnetic field!

- Energy needed to cause a transition E=hn
- When the energy of the photon matches the energy difference between the two spin states an absorption of energy occurs



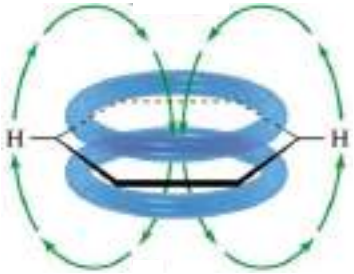


- Resonance: In NMR spectroscopy, resonance is the absorption of electromagnetic radiation by a precessing nucleus and the resulting “flip” of its nuclear spin from a lower energy state to a higher energy state

- The instrument used to detect this coupling of precession frequency and electromagnetic radiation records it as a signal.

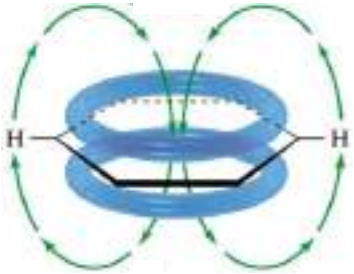
signal: A recording in an NMR spectrum of a nuclear

ΔE and Magnet Strength

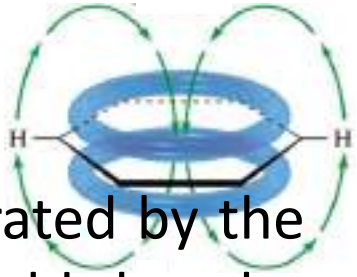


- Energy difference is proportional to the magnetic field strength.
- $\Delta E = h\nu = \gamma \frac{h}{2\pi} B_0$
- Gyromagnetic ratio, γ, is a constant for each nucleus (26,753 $s^{-1}gauss^{-1}$ for H).
- In a 14,092 gauss field, a 60 MHz photon is required to flip a proton.
- Low energy, radio frequency.

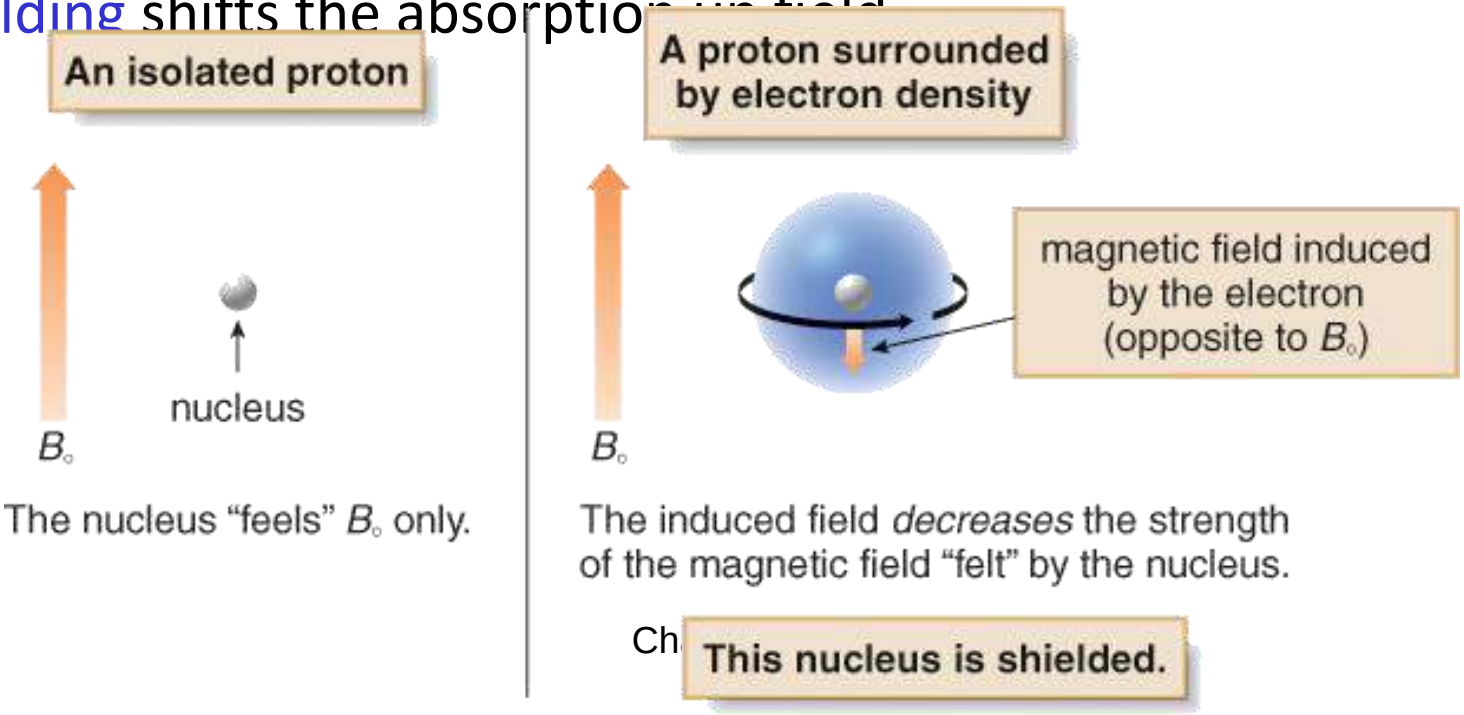
Magnetic Shielding



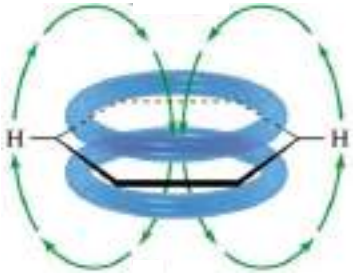
- If all protons absorbed the same amount of energy in a given magnetic field, not much information could be obtained.
- But protons are surrounded by electrons that shield them from the external field.
- Circulating electrons create an



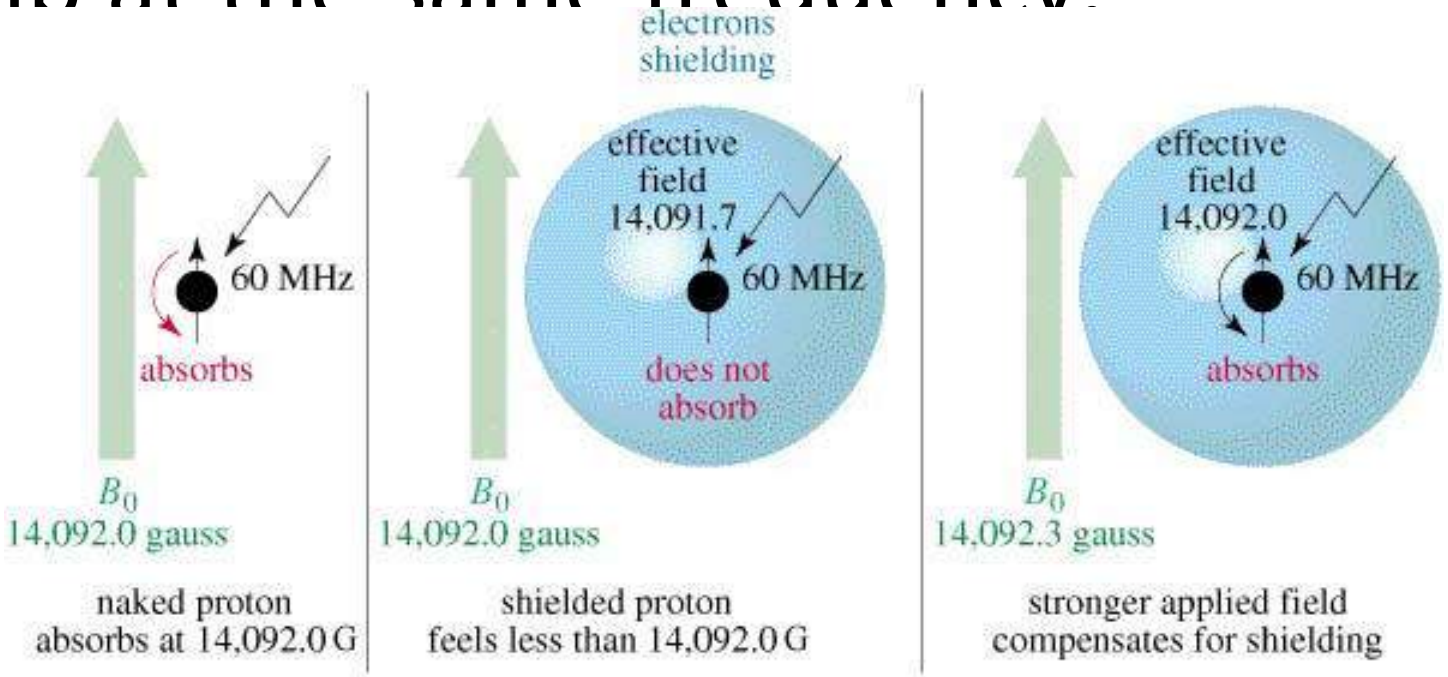
- In the vicinity of the nucleus, the magnetic field generated by the circulating electron decreases the external magnetic field that the proton “feels”.
- Since the electron experiences a lower magnetic field strength, it needs a lower frequency to achieve resonance. Lower frequency is to the right in an NMR spectrum, toward a lower chemical shift, so shielding shifts the absorption up field.

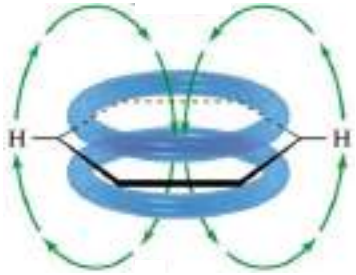


Shielded Protons



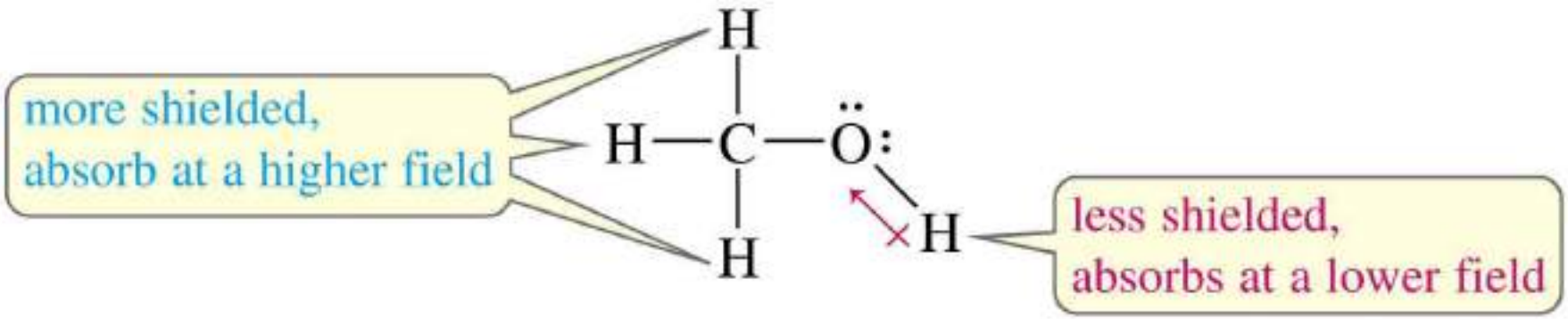
Magnetic field strength must be increased for a shielded proton to flip at the same frequency.



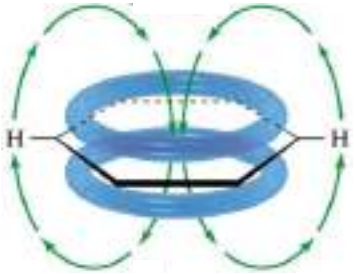


Protons in a Molecule

Depending on their chemical environment, protons in a molecule are shielded by different amounts.

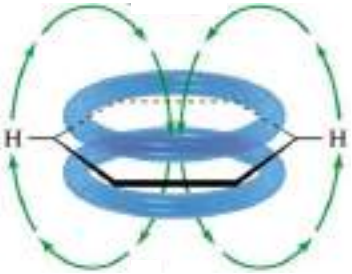


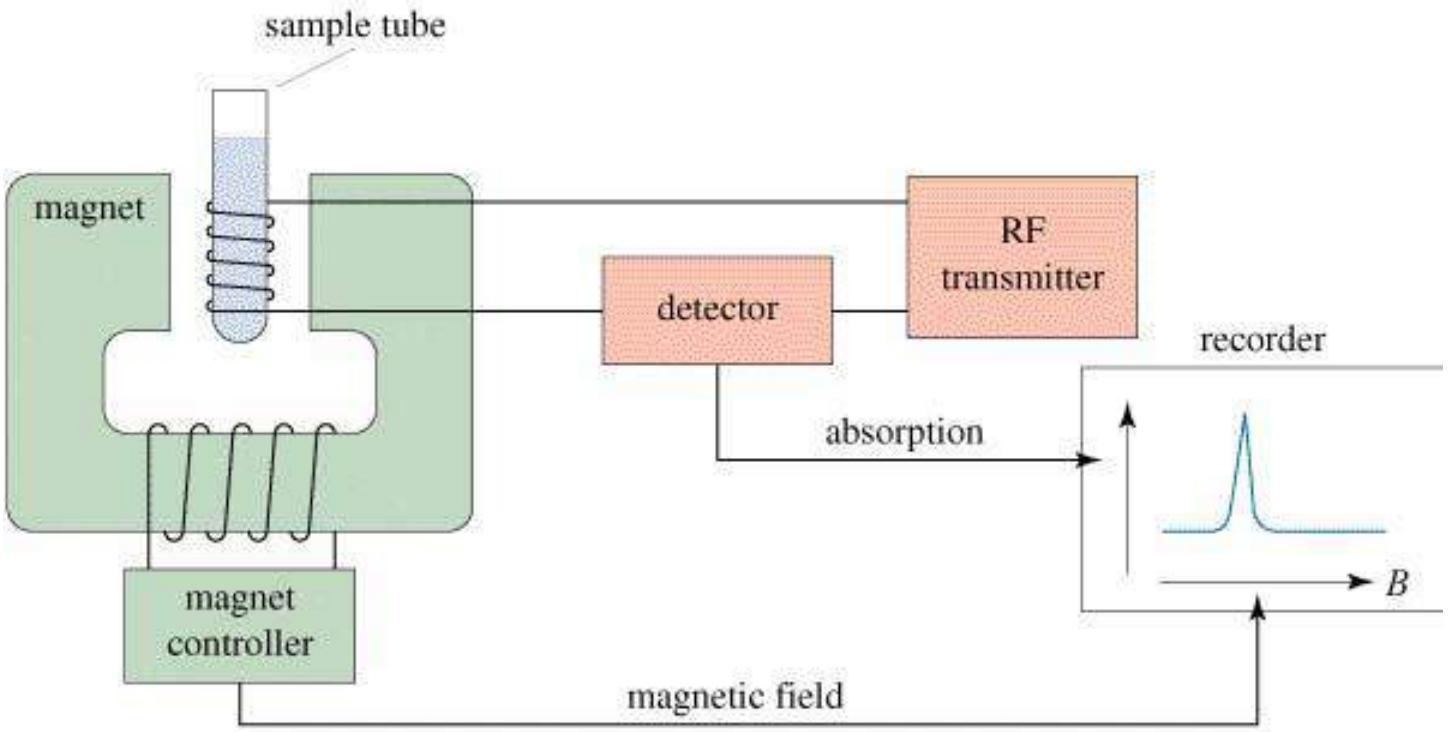
NMR Signals

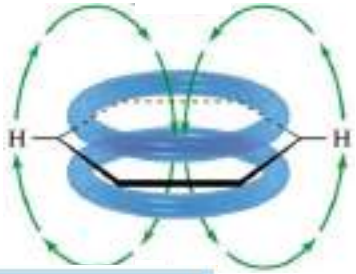


- The **number** of signals shows how many different kinds of protons are present.
- The **location** of the signals shows how shielded or deshielded the proton is.
- The **intensity** of the signal shows the number of protons of that type.
- Signal **splitting** shows the number of protons on adjacent atoms.

The NMR Spectrometer







The sample is dissolved in solvent in a thin NMR tube, and placed in a magnetic field.

NMR spectrum

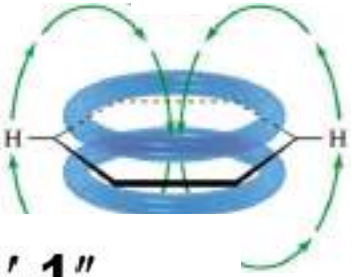
sample tube

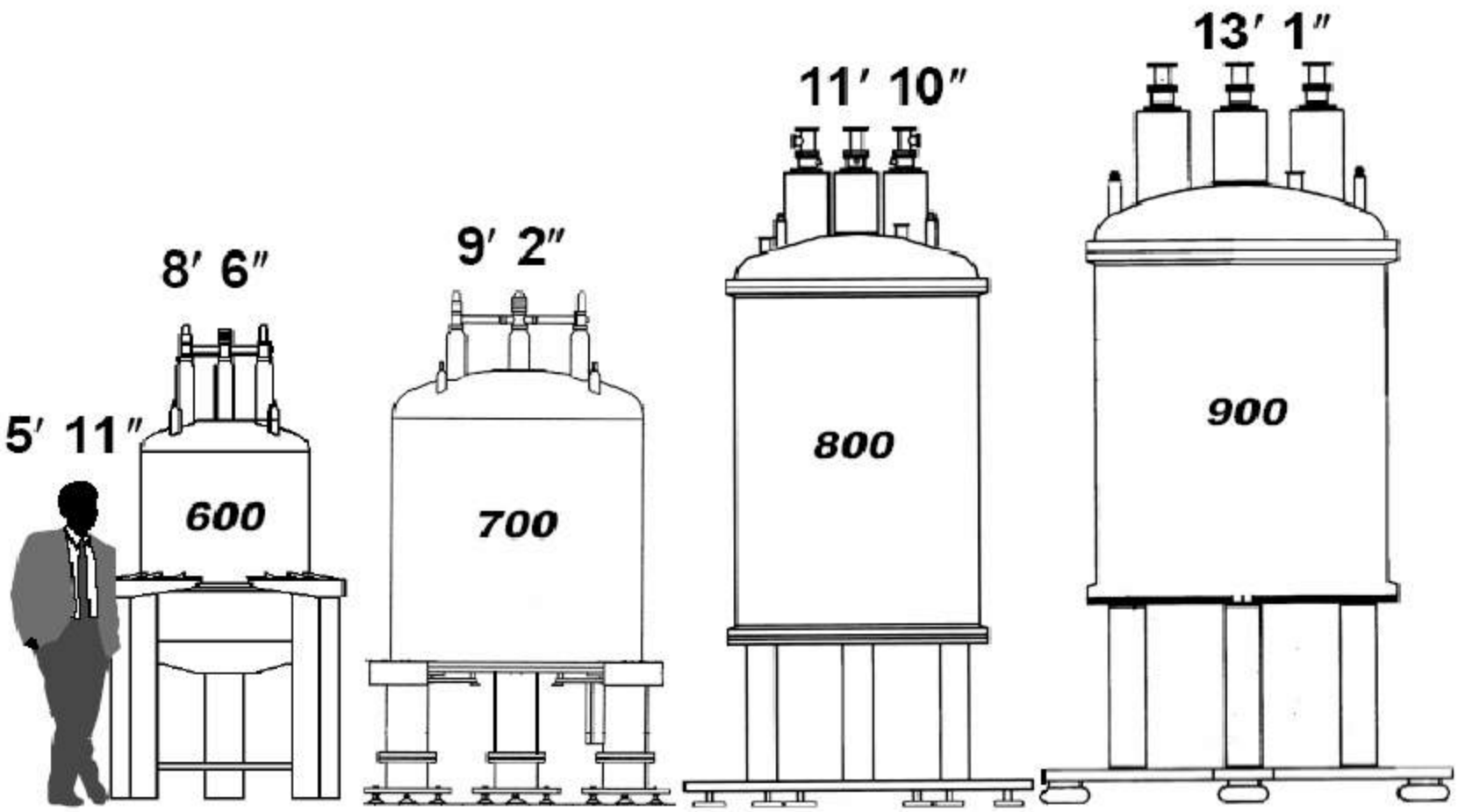
superconducting magnet

NMR console

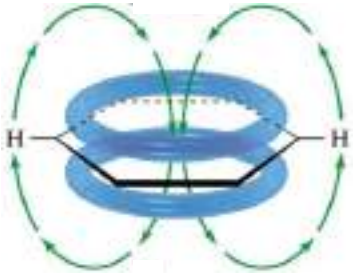
workstation

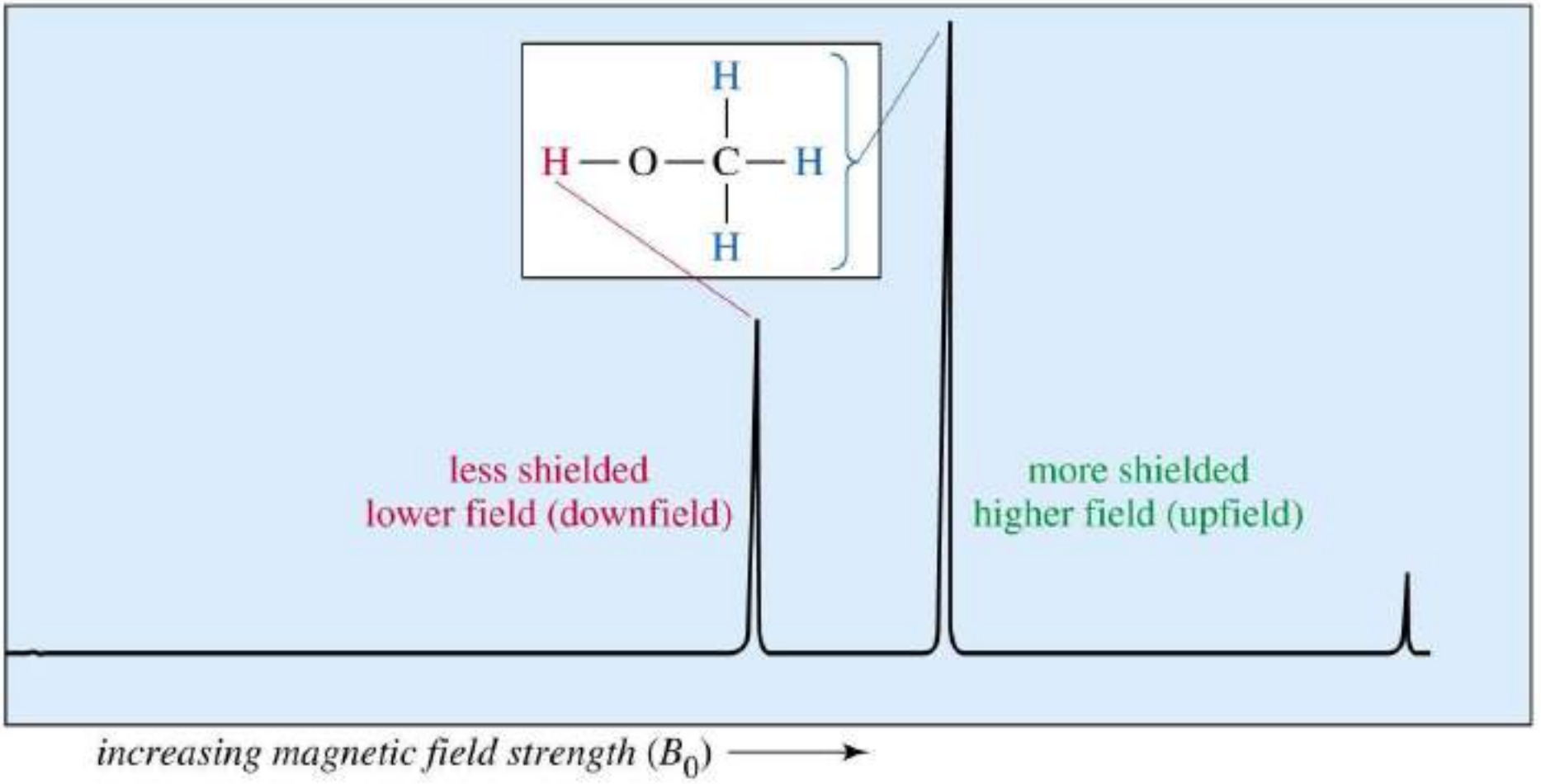
In the NMR probe, the sample is rotated in a magnetic field and irradiated with a short pulse of RF radiation.


H
H

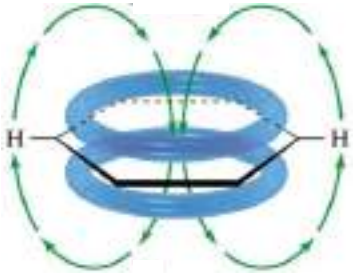

5′ 11″
8′ 6″
600
9′ 2″
700
11′ 10″
800
13′ 1″
900

The NMR Graph

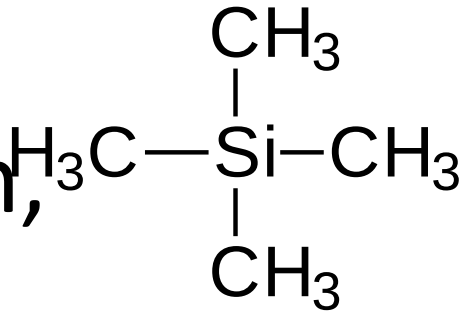




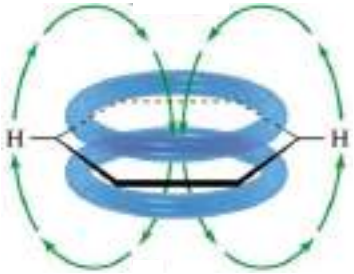
Tetramethyl silane



- TMS is added to the sample.
- Since silicon is less electronegative than carbon, TMS protons are highly shielded. Signal defined as zero.
- Organic protons absorb downfield (to the left) of the TMS signal



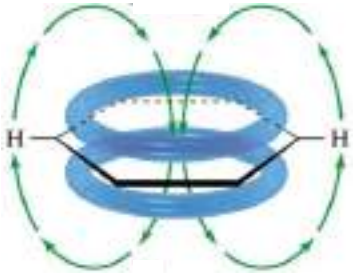
Chemical Shift



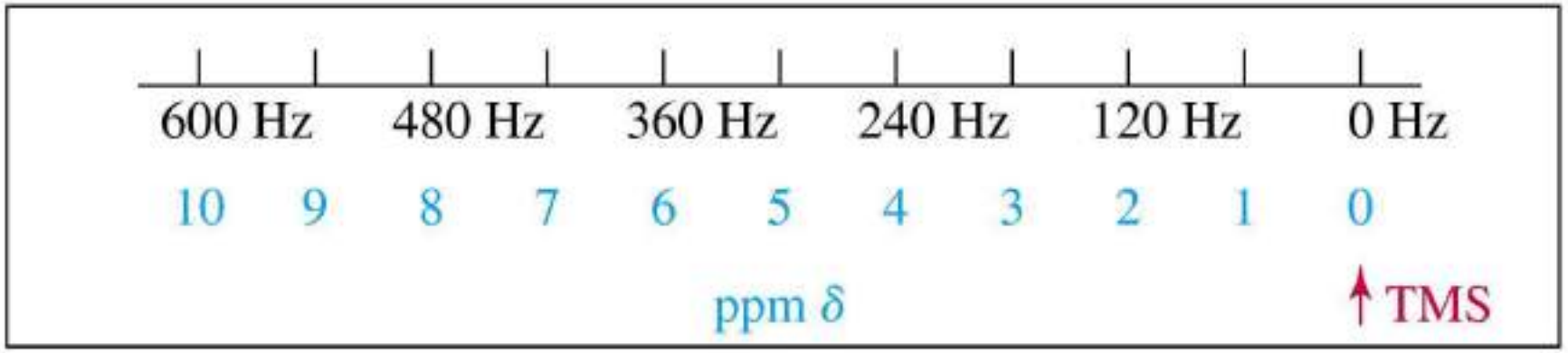
- Measured in parts per million.
- Ratio of shift downfield from TMS (Hz) to total spectrometer frequency (Hz).
- Same value for 60, 100, or 300

$$\textbf{chemical shift} \text{ (in ppm on the } \delta \text{ scale)} = \frac{\text{observed chemical shift (in Hz) downfield from TMS}}{\nu \text{ of the NMR spectrometer (in MHz)}}$$

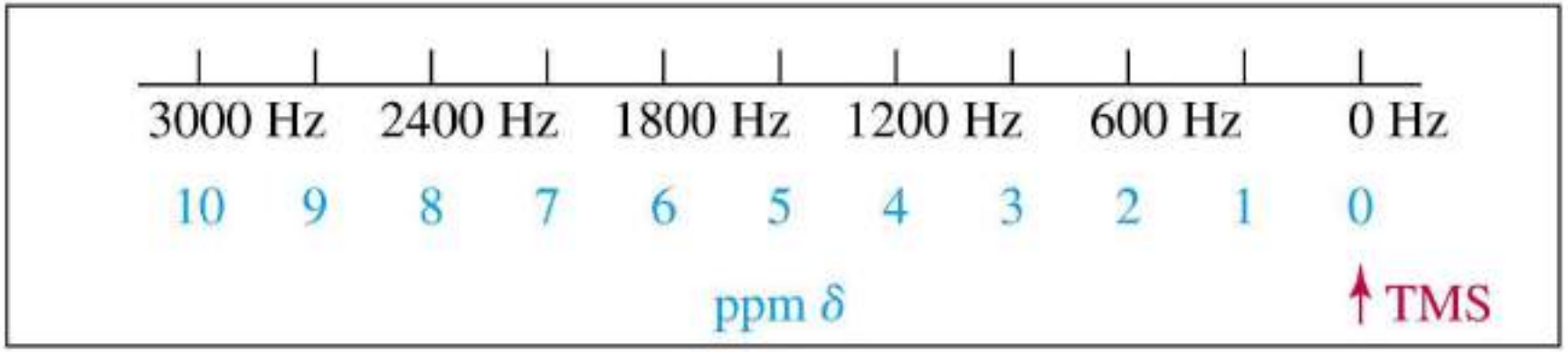
Delta Scale



$$\text{chemical shift, ppm } \delta = \frac{\text{shift downfield from TMS (in Hz)}}{\text{spectrometer frequency (in MHz)}}$$

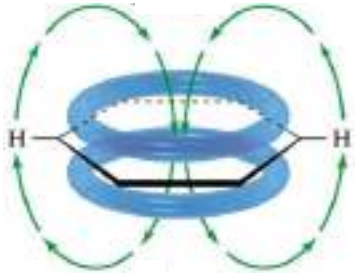


60 MHz



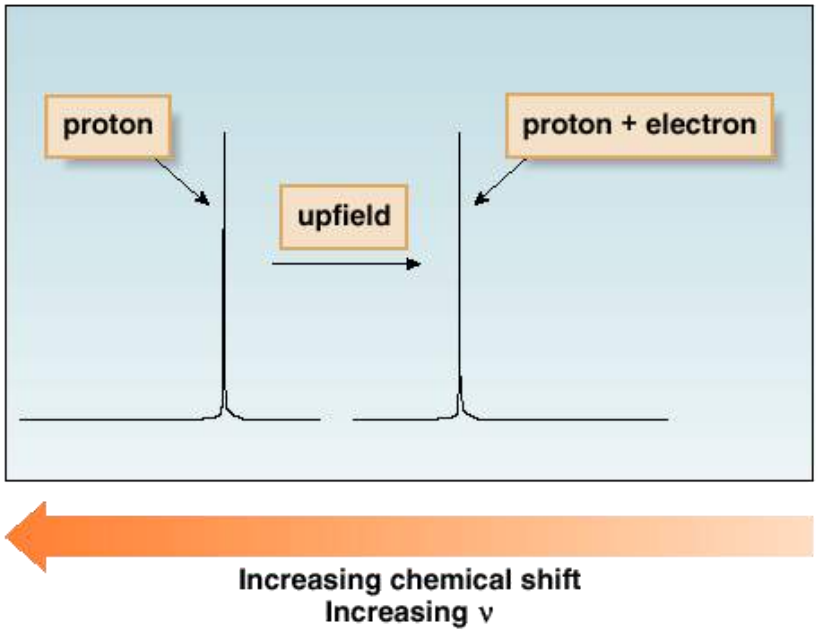
300 MHz

^{1}H NMR—Position of Signals



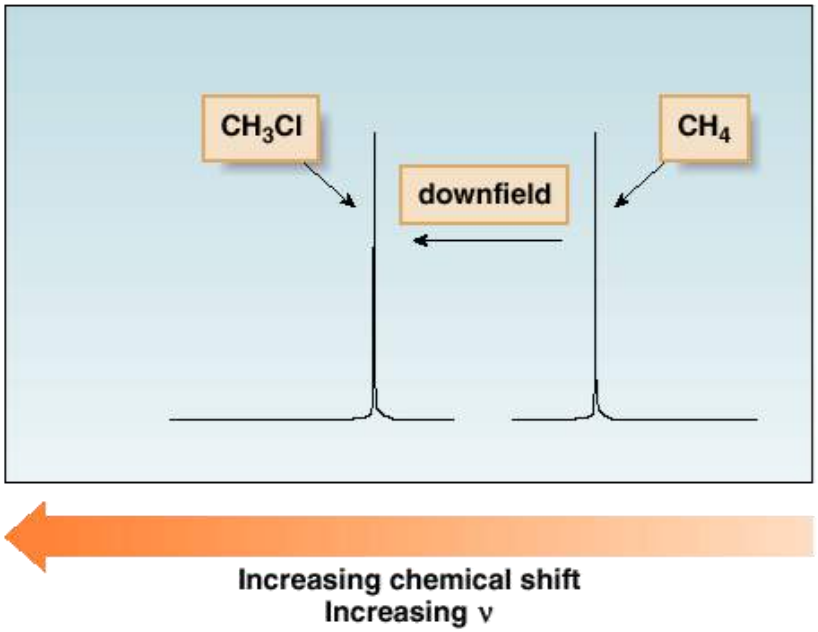
a. Shielding effects

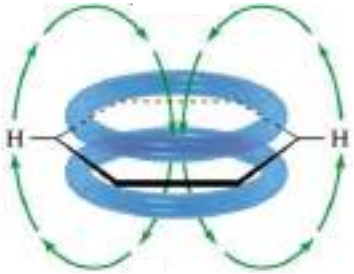
- An electron shields the nucleus.
- The absorption shifts *upfield*.



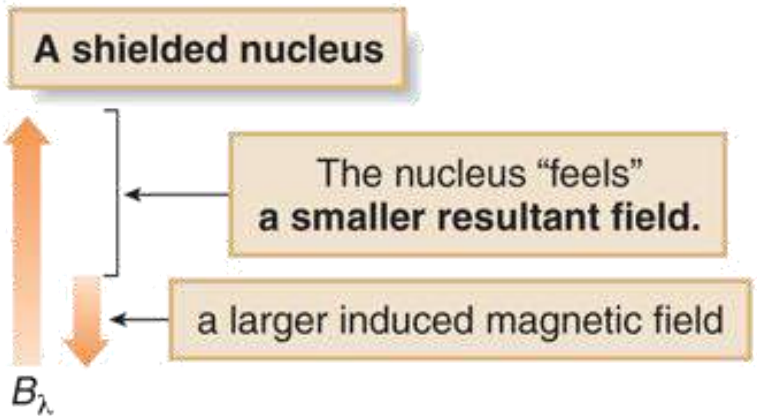
b. Deshielding effects

- Decreased electron density deshields a nucleus.
- The absorption shifts *downfield*.

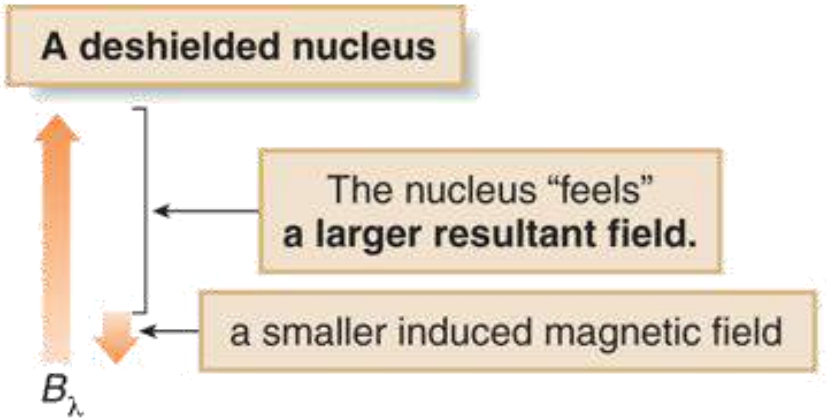




^{1}H NMR—Position of Signals



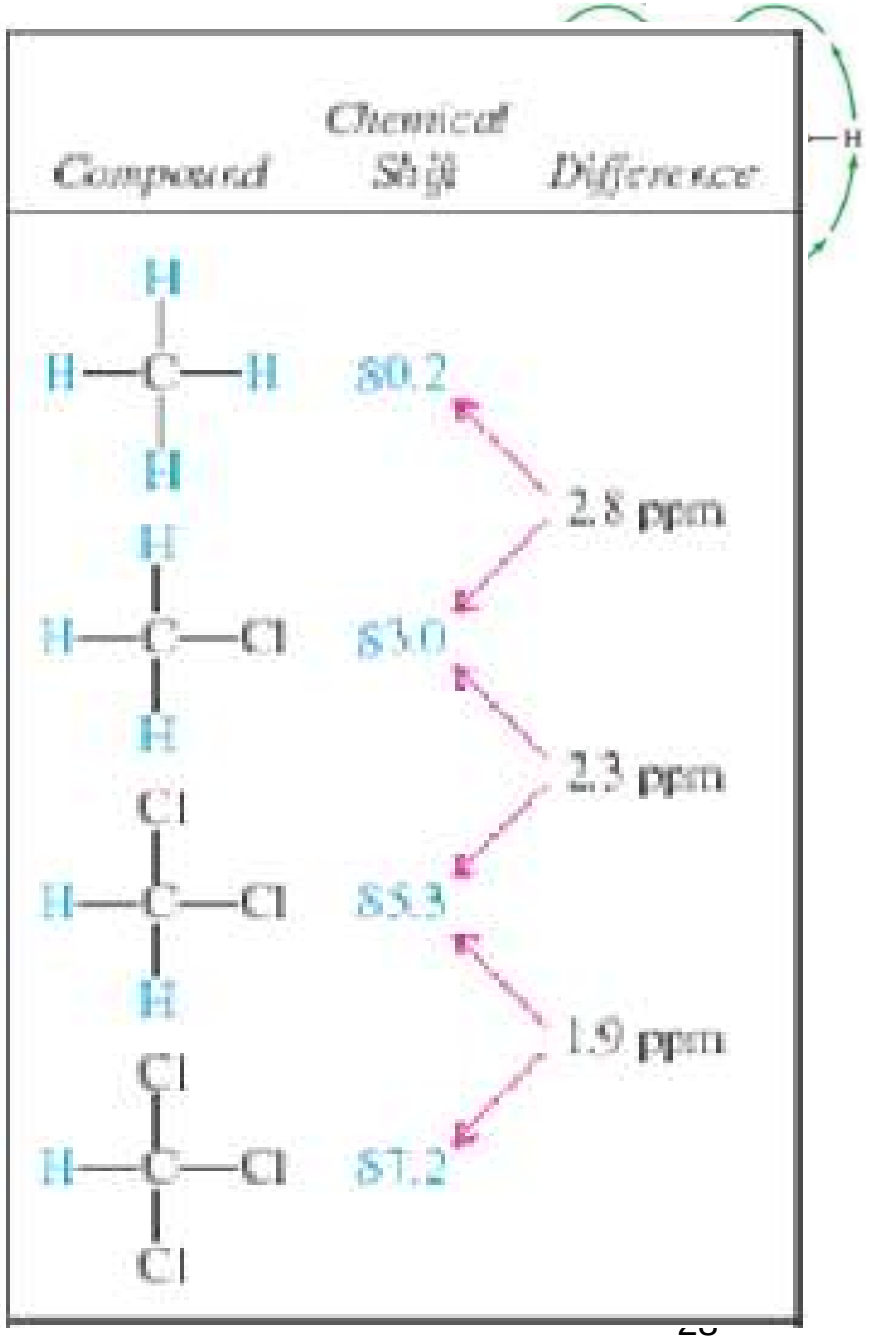
- As the electron density around the nucleus increases, the nucleus feels a smaller resultant magnetic field, so a lower frequency is needed to achieve resonance.
- **The absorption shifts upfield.**



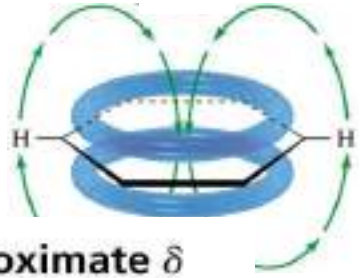
- As the electron density around the nucleus decreases, the nucleus feels a larger resultant magnetic field, so a higher frequency is needed to achieve resonance.
- **The absorption shifts downfield.**

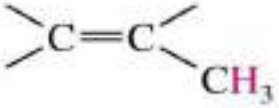
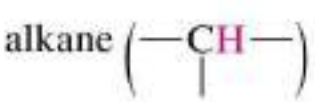
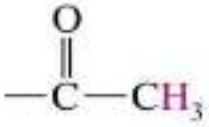
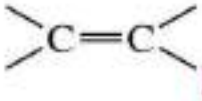
Location of Signals

- More electronegative atoms deshield more and give larger shift values.
- Effect decreases with distance.
- Additional electronegative atoms cause increase in



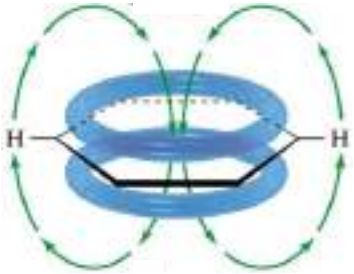
Typical δ Values

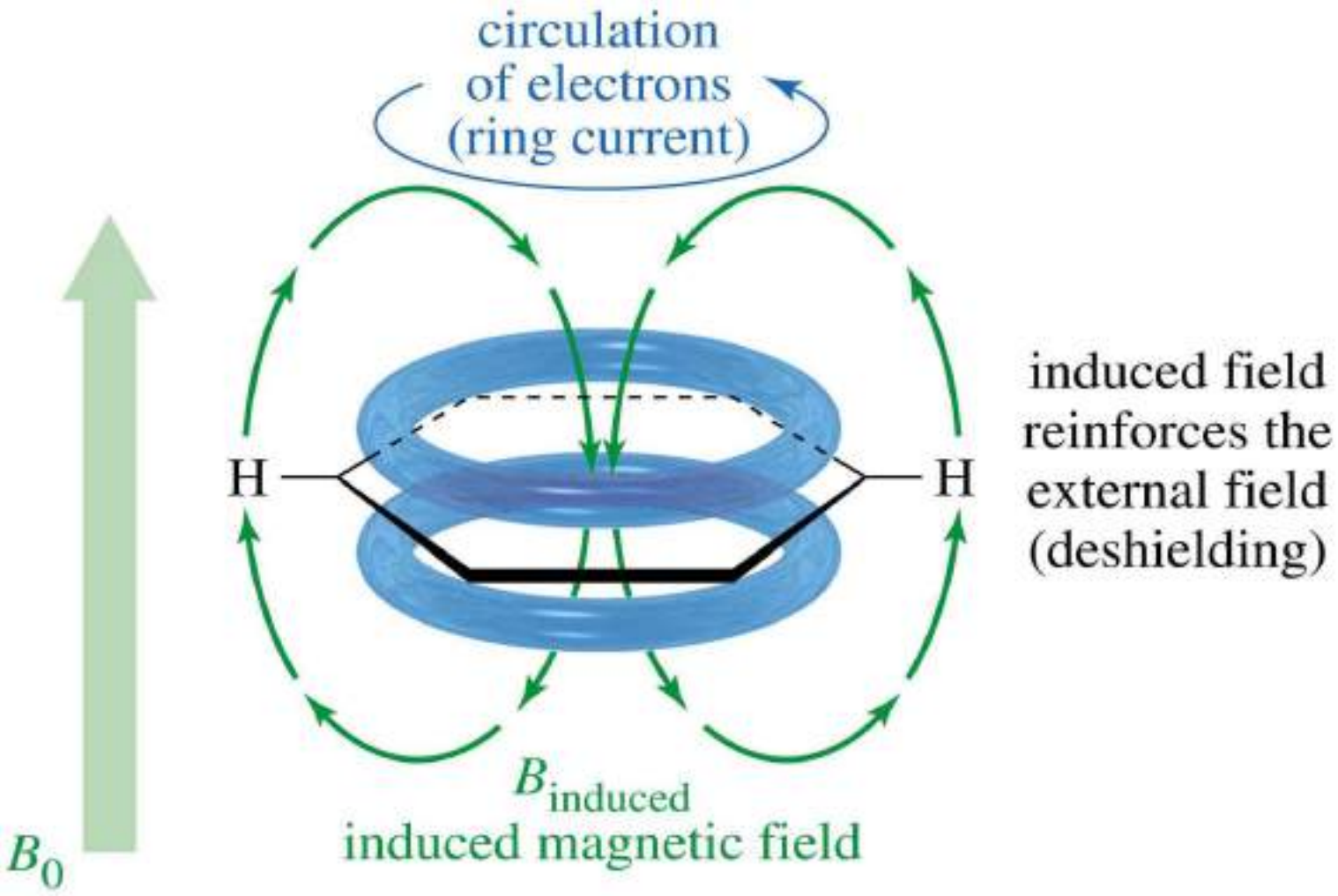


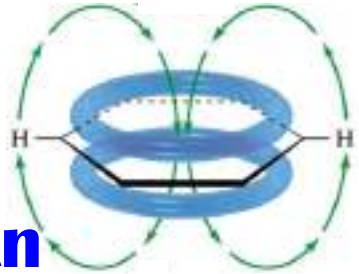
Type of Proton	Approximate δ	Type of Proton	Approximate δ
alkane (—CH_3)	0.9		1.7
alkane (—CH_2—)	1.3		
alkane 	1.4	Ph—H	7.2
		Ph—CH_3	2.3
	2.1	R—CHO	9–10
		R—COOH	10–12
—C≡C—H	2.5	R—OH	variable, about 2–5
R—CH_2—X	3–4	Ar—OH	variable, about 4–7
(X = halogen, O)		R—NH_2	variable, about 1.5–4
	5–6		

Note: These values are approximate, as all chemical shifts are affected by neighboring substituents. The numbers given here assume that alkyl groups are the only other substituents present. A more complete table of chemical shifts appears in Appendix 1.

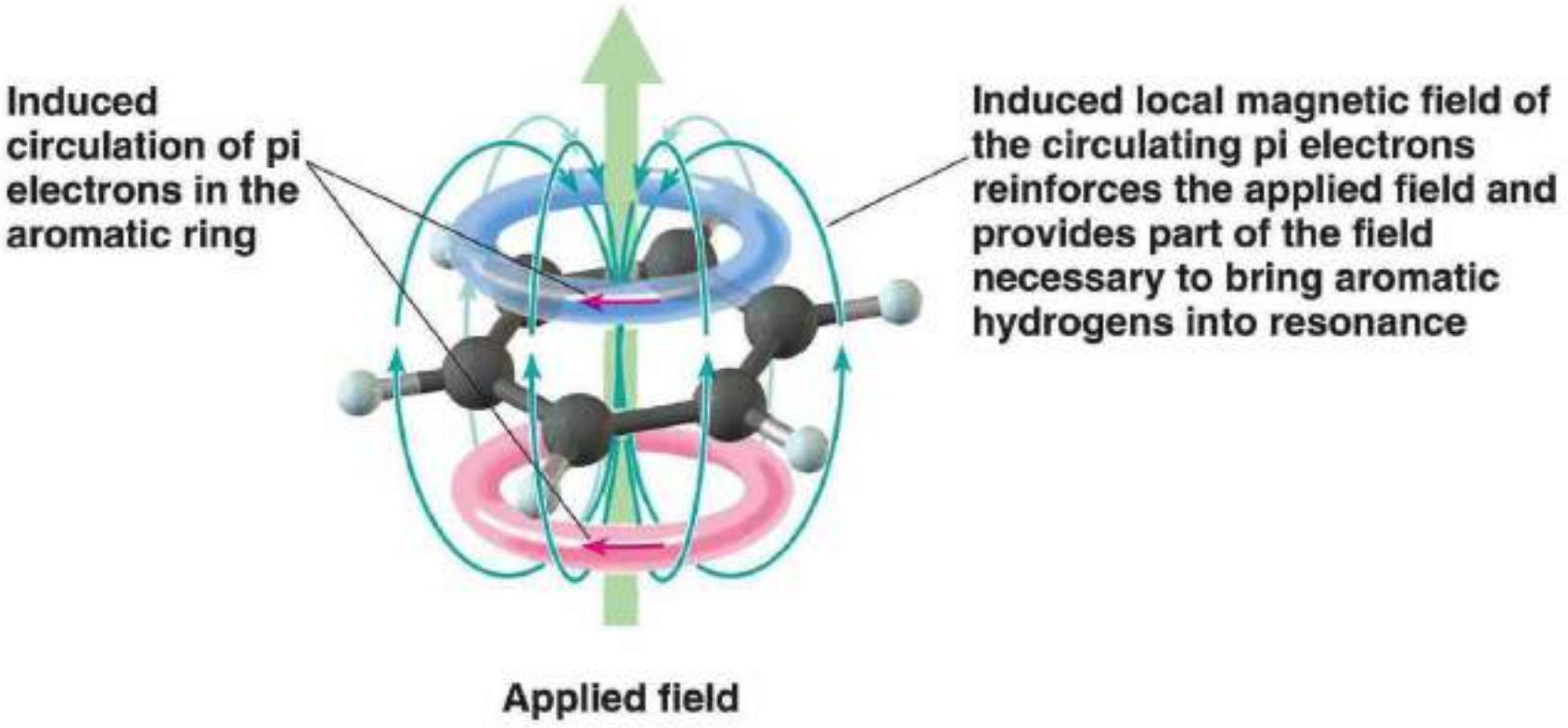
Aromatic Protons δ 7-8



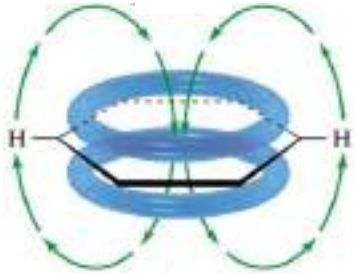


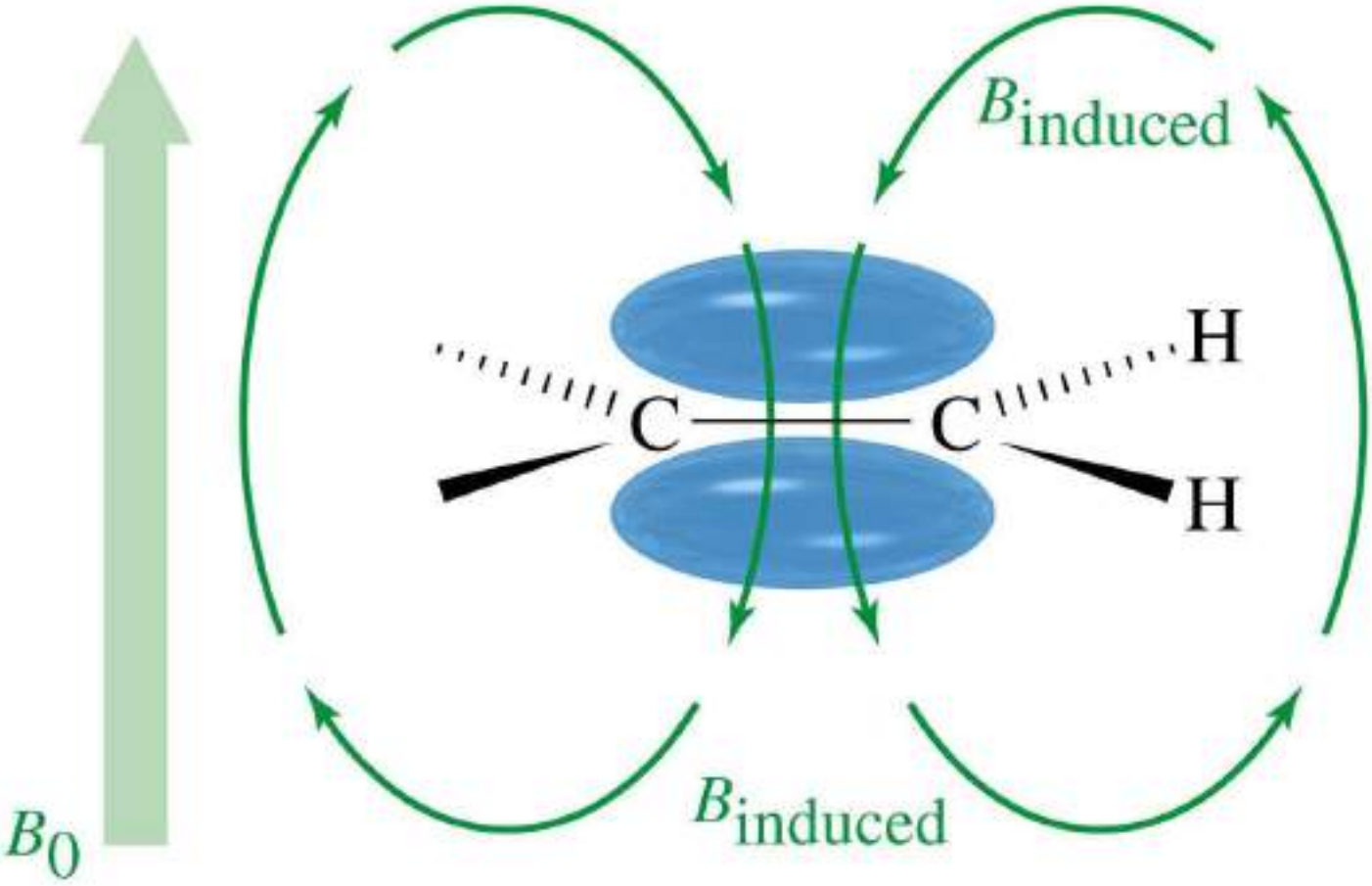


Magnetic induction of the pi electrons in an aromatic ring

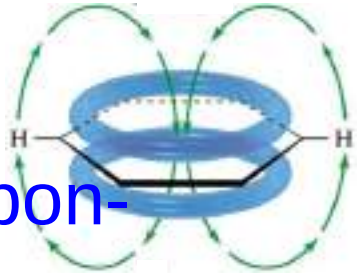


Vinyl Protons, δ 5-6

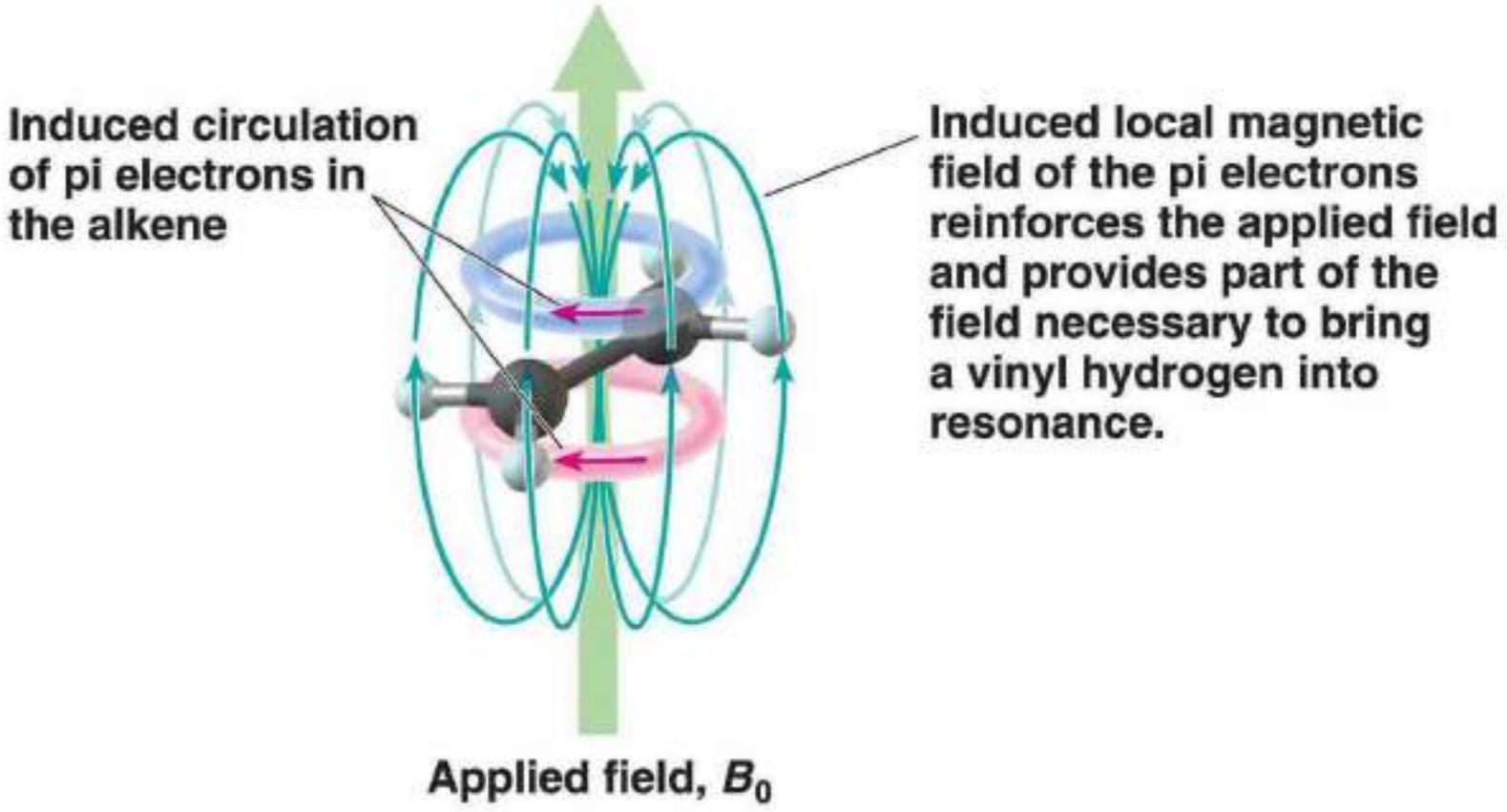




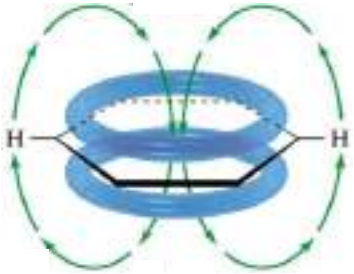
induced field reinforces the external field (deshielding)

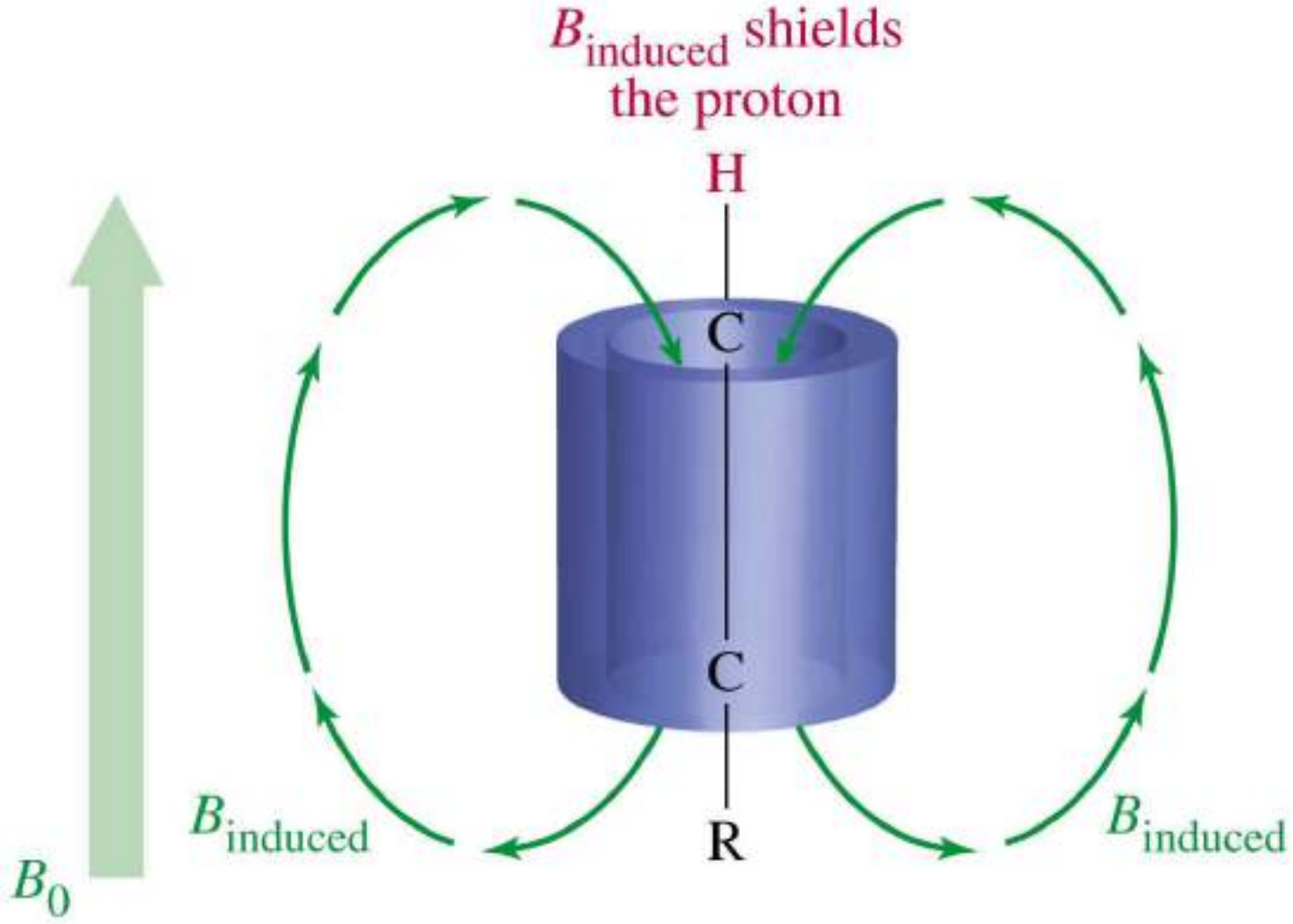


Magnetic induction in the pi bond of a carbon-carbon double bond

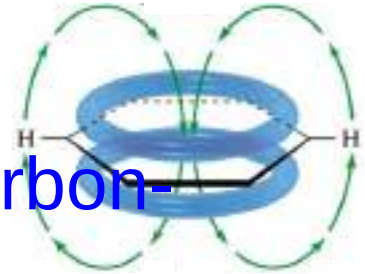


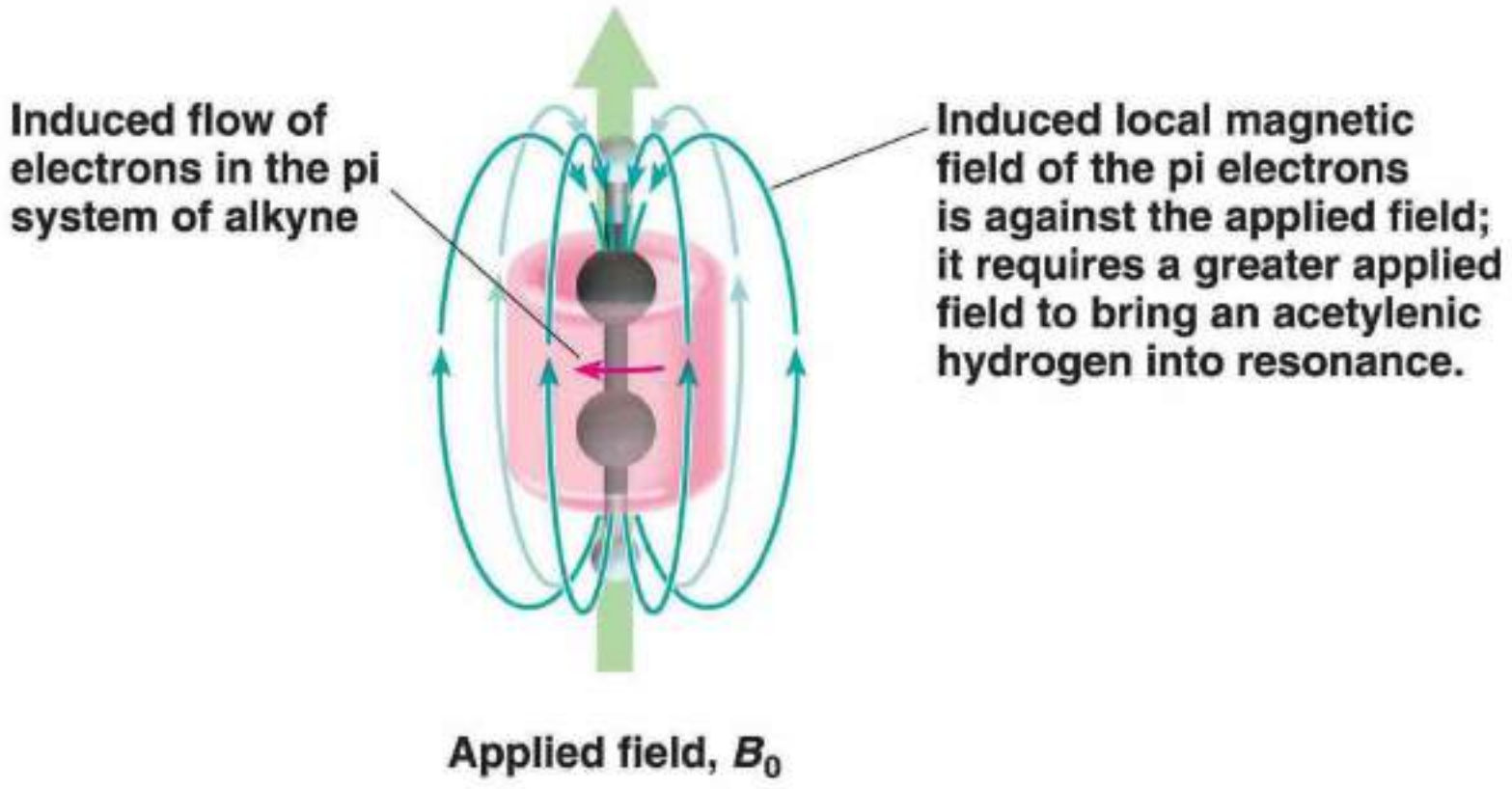
Acetylenic Protons, δ 2.5



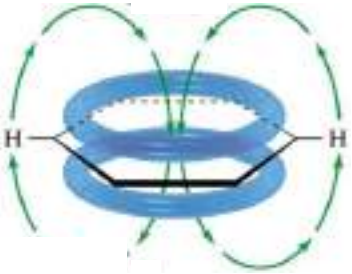


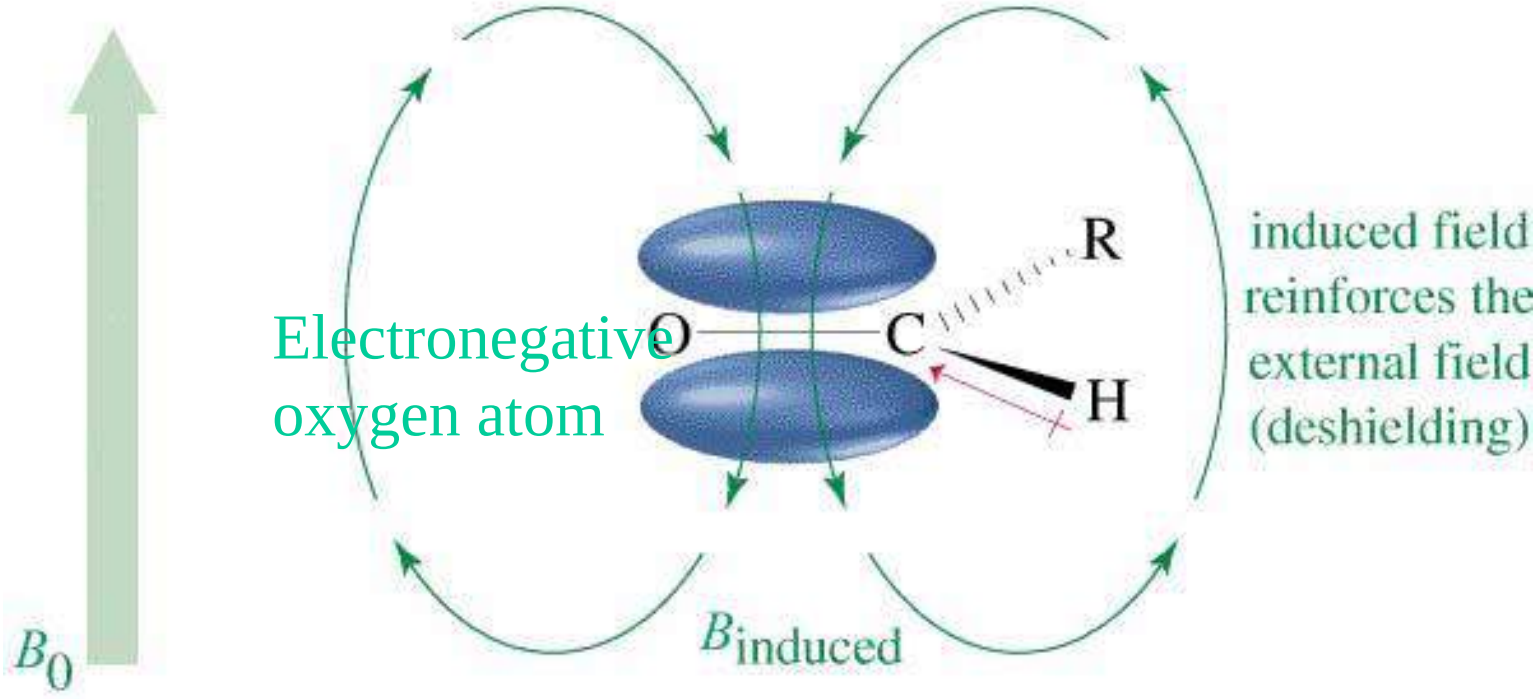
Magnetic induction in the pi bonds of a carbon-carbon triple bond



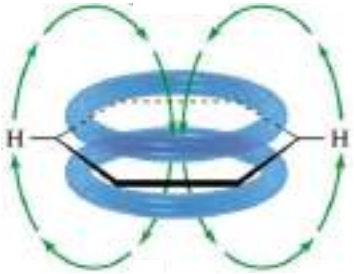


Aldehydic Proton, δ 9-10



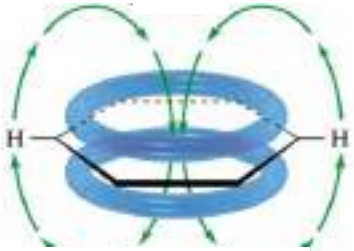


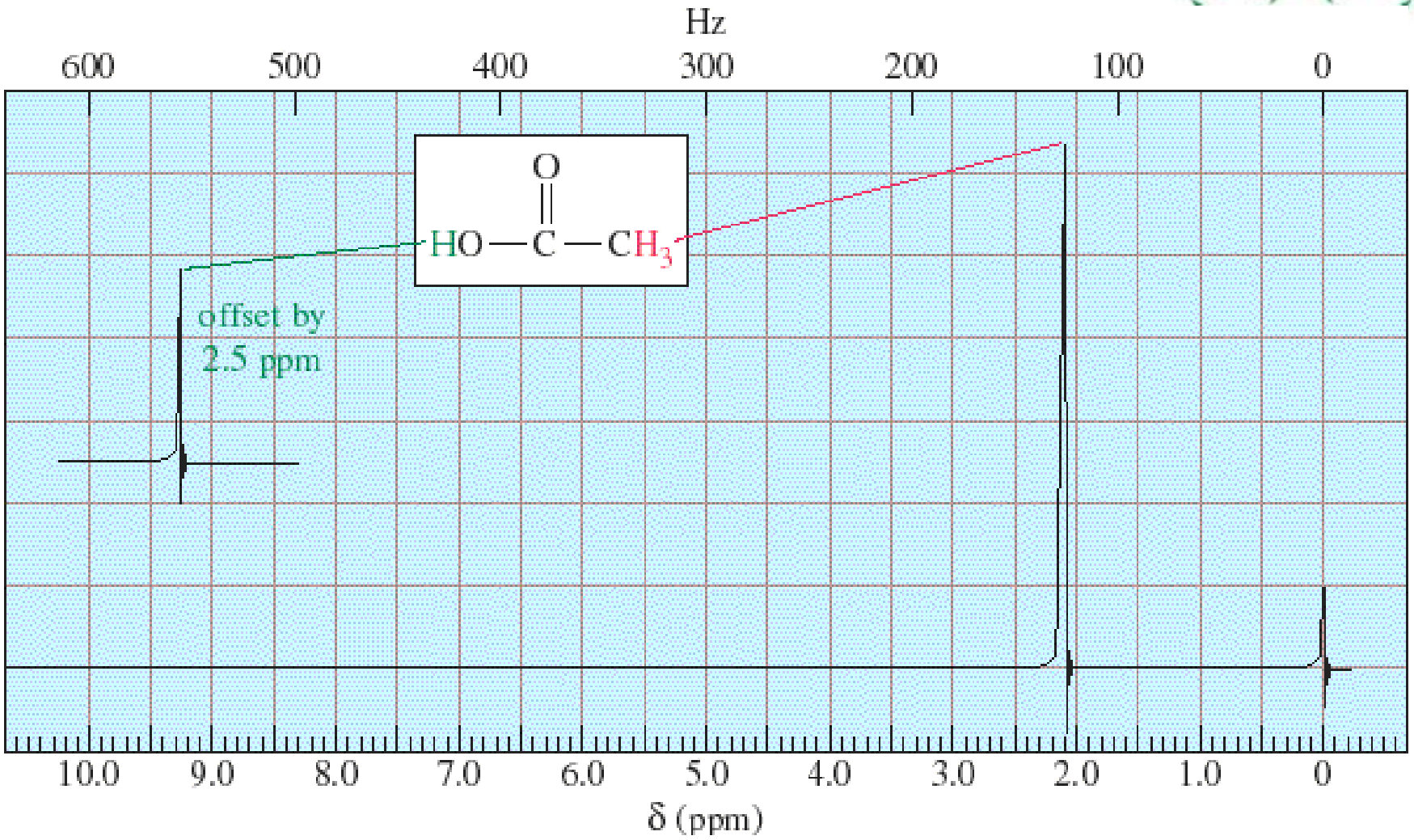
O-H and N-H Signals



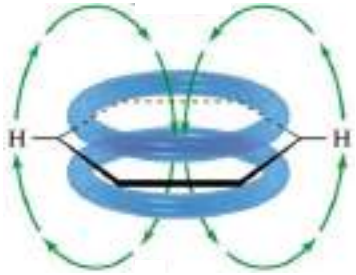
- Chemical shift depends on concentration.
- Hydrogen bonding in concentrated solutions deshield the protons, so signal is around δ3.5 for N-H and δ4.5 for O-H.
- Proton exchanges between the molecules broaden the peak.

Carboxylic Acid Proton, δ 10+

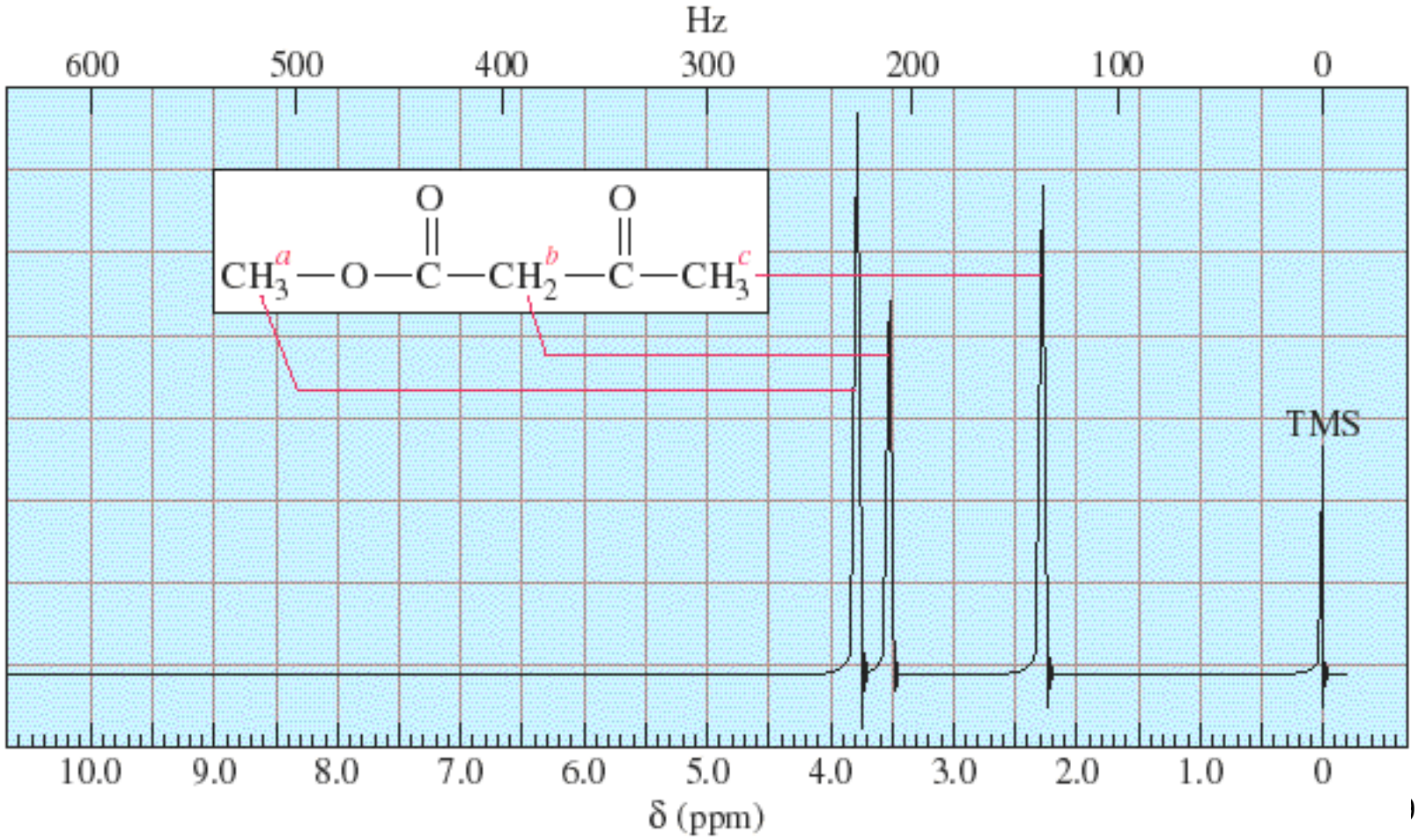




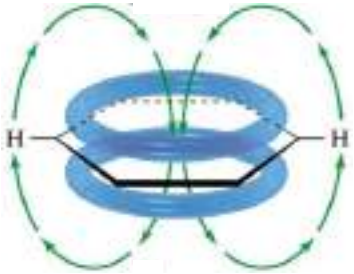
Number of Signals



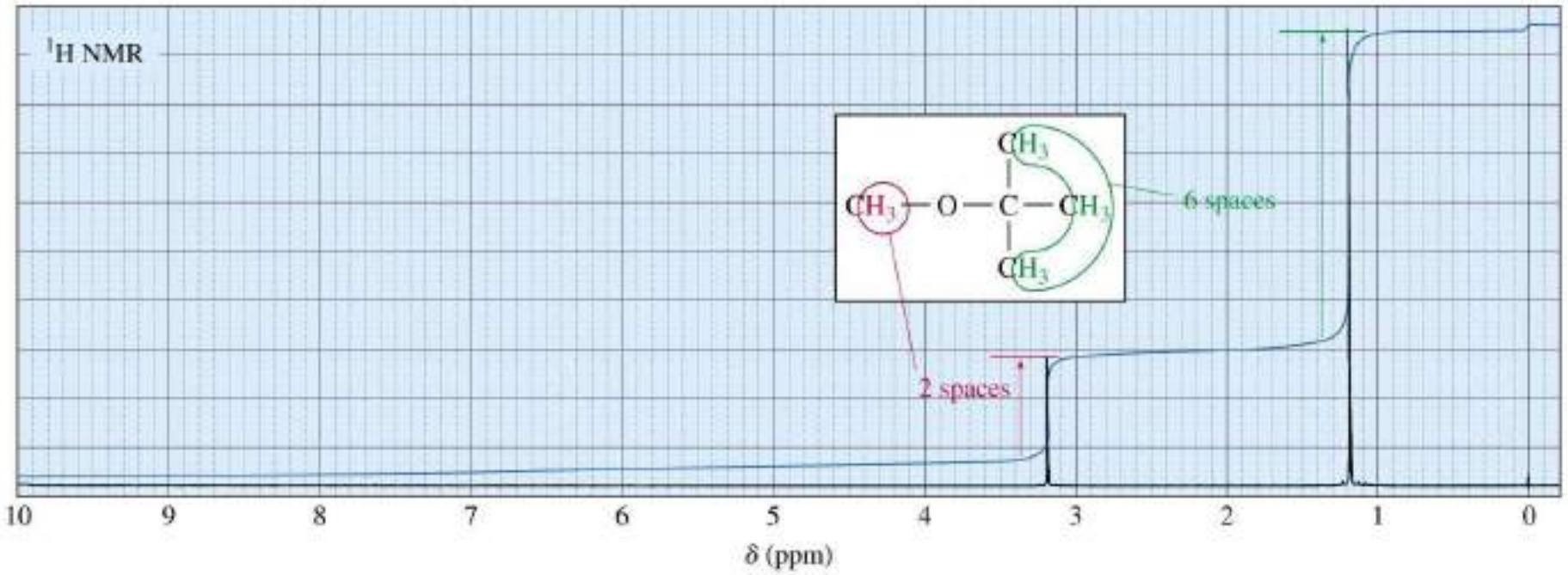
Equivalent hydrogens have the same



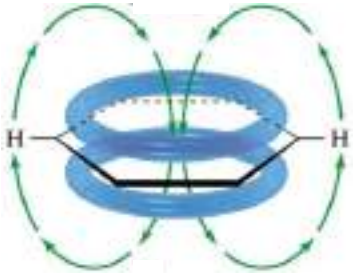
Intensity of Signals



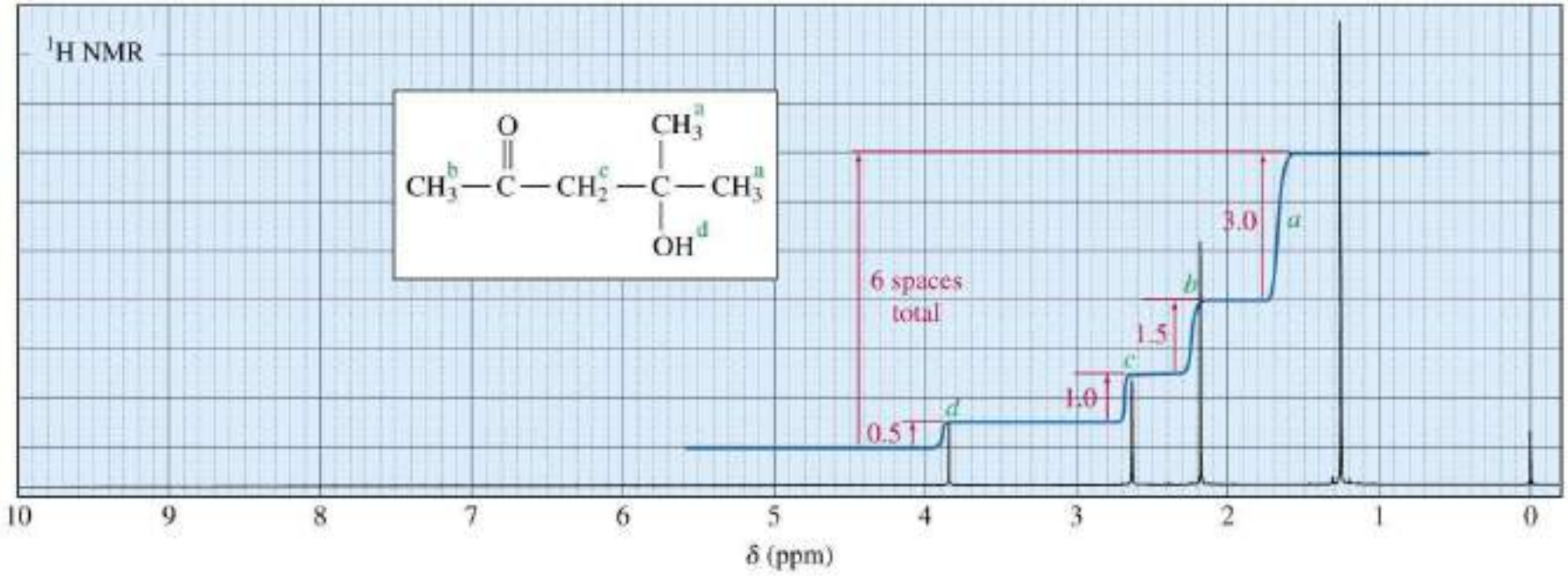
- The area under each peak is proportional to the number of protons in that group.



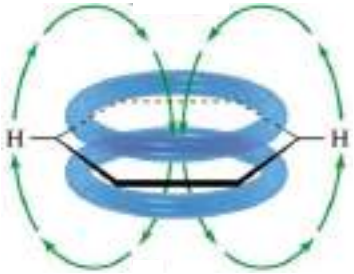
How Many Hydrogens?



When the molecular formula is known, each integral rise can be assigned to a particular number of hydrogens.

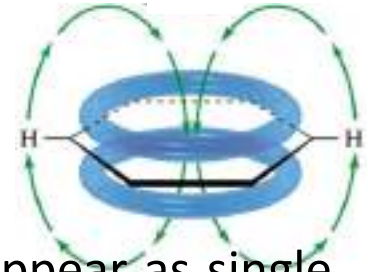


Spin-Spin Splitting

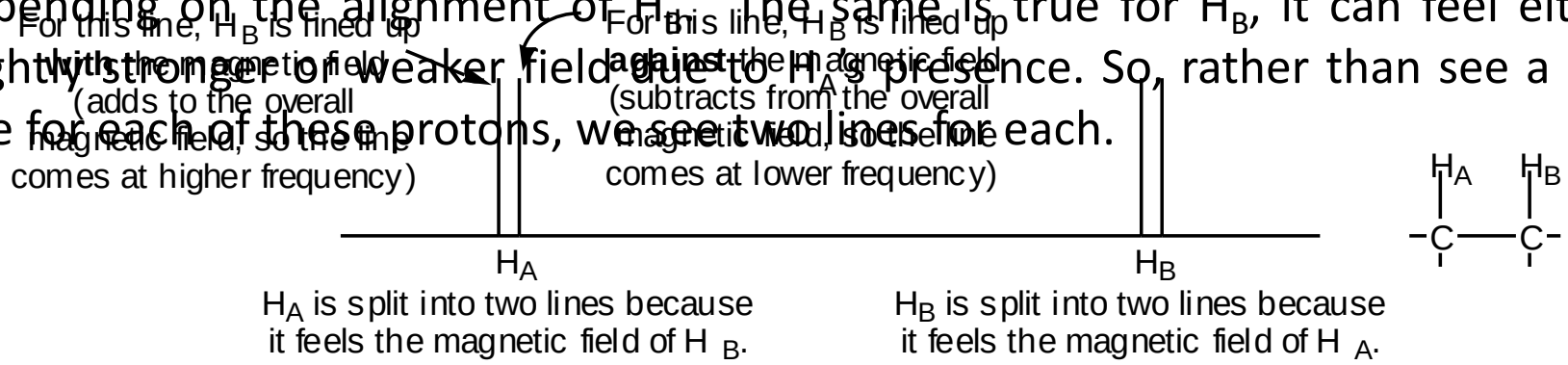


- Nonequivalent protons on adjacent carbons have magnetic fields that may align with or oppose the external field.
- This magnetic coupling causes the proton to absorb slightly downfield when the external field is reinforced and slightly upfield when the external field is opposed.
- All possibilities exist, so signal is split.

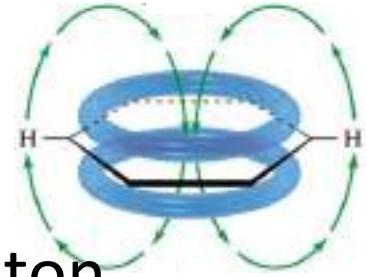
^1H - ^1H Coupling



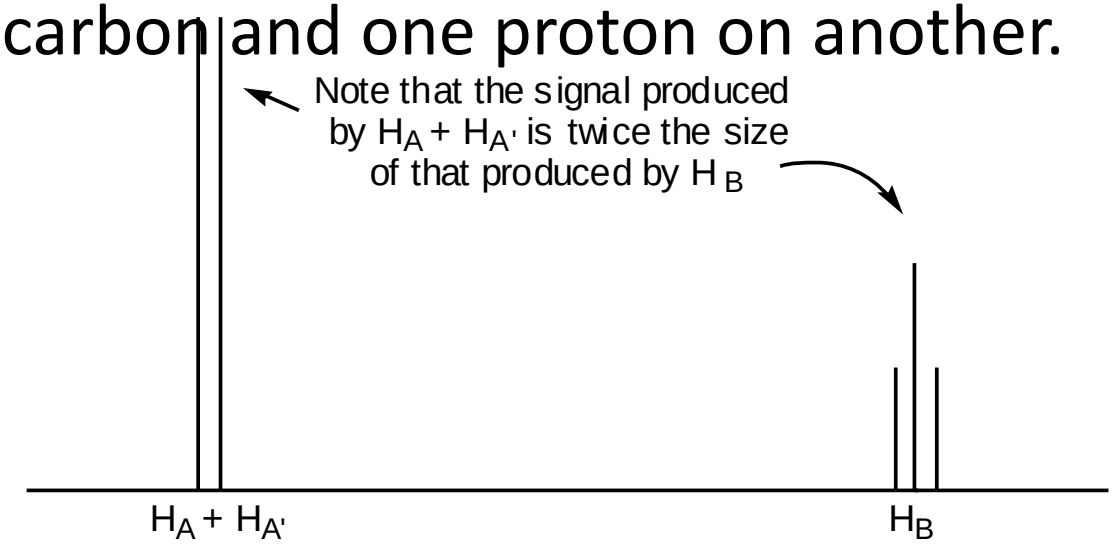
You'll notice in the spectra that we've seen that the signals don't appear as single lines, sometimes they appear as multiple lines. This is due to ^1H - ^1H coupling (also called spin-spin splitting or J-coupling). Here's how it works: Imagine we have a molecule which contains a proton (let's call it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



^1H - ^1H Coupling

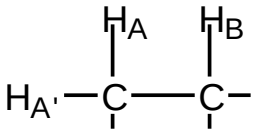


What happens when there is more than one proton splitting a neighboring proton? We get more lines. Consider the molecule below where we have two protons on one carbon and one proton on another.



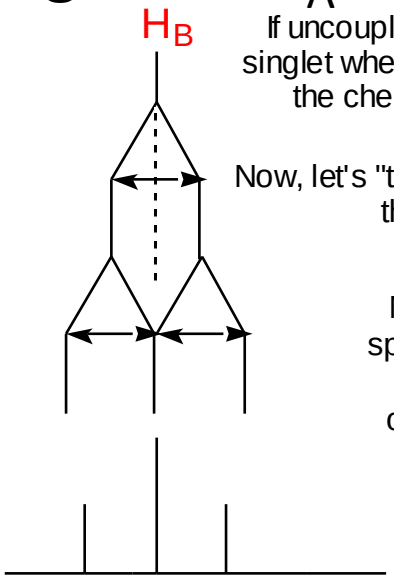
H_A and $H_{A'}$ appear at the same chemical shift because they are in identical environments
They are also split into two lines (called a doublet) because they feel the magnetic field of H_B.

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?

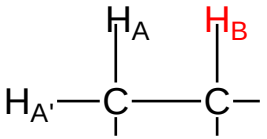
H_B feels the splitting of both H_A and $H_{A'}$. So, let's imagine starting with H_B as a single line, then let's "turn on" the coupling from H_A and $H_{A'}$ one at a time:



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

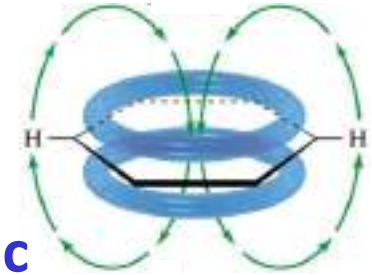
Now, let's "turn on" H_B - H_A coupling. This splits the single line into two lines

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

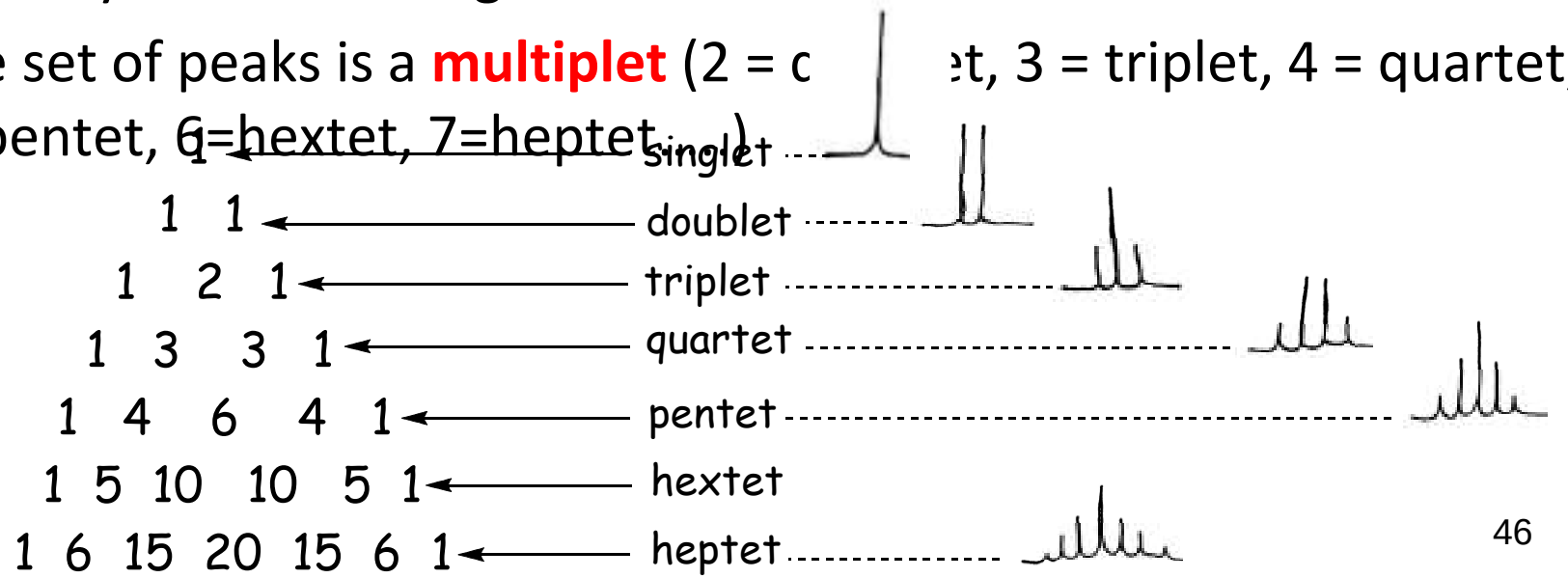


Because the two lines in the middle overlap, that line is twice as big as the lines on the outside. More neighboring protons leads to more lines as shown on the next slide.

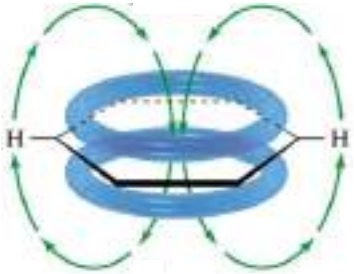
Spin-Spin Splitting in ^{1}H NMR



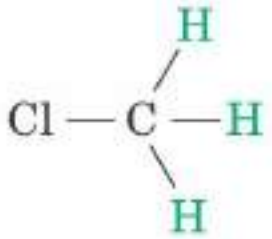
- Peaks are often split into multiple peaks due to **magnetic interactions** between nonequivalent protons on adjacent carbons, The process is called **spin-spin splitting**
- The splitting is into one more peak than the number of H's on the adjacent carbon(s), This is the **"n+1 rule"**
- The relative intensities are in proportion of a binomial distribution given by Pascal's Triangle
- The set of peaks is a **multiplet** (2 = d et, 3 = triplet, 4 = quartet, 5=pentet, 6=hextet, 7=heptet,)



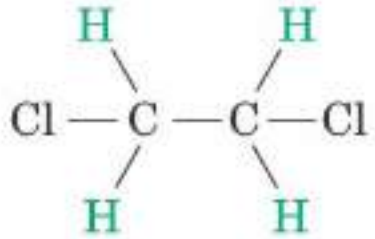
Rules for Spin-Spin Splitting



- **Equivalent** protons **do not** split each other

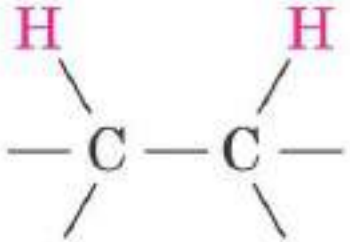


Three C–H protons are chemically equivalent; no splitting occurs.

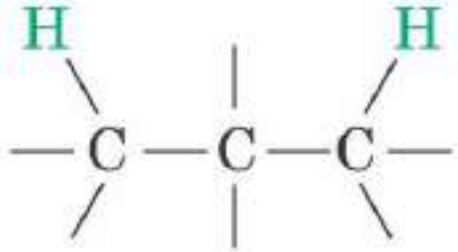


Four C–H protons are chemically equivalent; no splitting occurs.

- Protons that are **farther than two carbon atoms apart do not** split each other



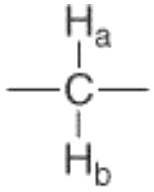
Splitting observed

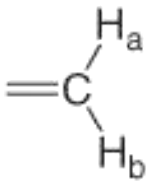


Splitting not usually observed

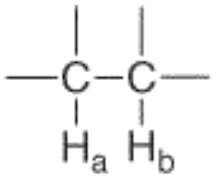
^{1}H NMR—Spin-Spin Splitting

If H_a and H_b are not equivalent, splitting is observed w



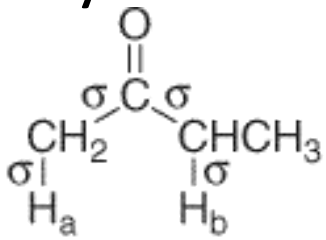


H_a and H_b are on the **same** carbon.



H_a and H_b are on **adjacent** carbons.

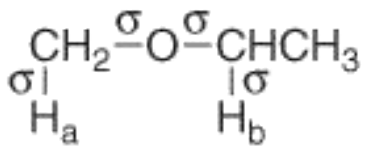
Splitting is not generally observed between protons separated by more than three σ bonds.



2-butanone

H_a and H_b are separated by four σ bonds.

no splitting between H_a and H_b

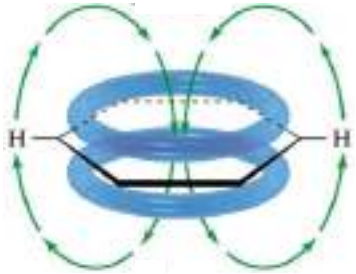


ethyl methyl ether

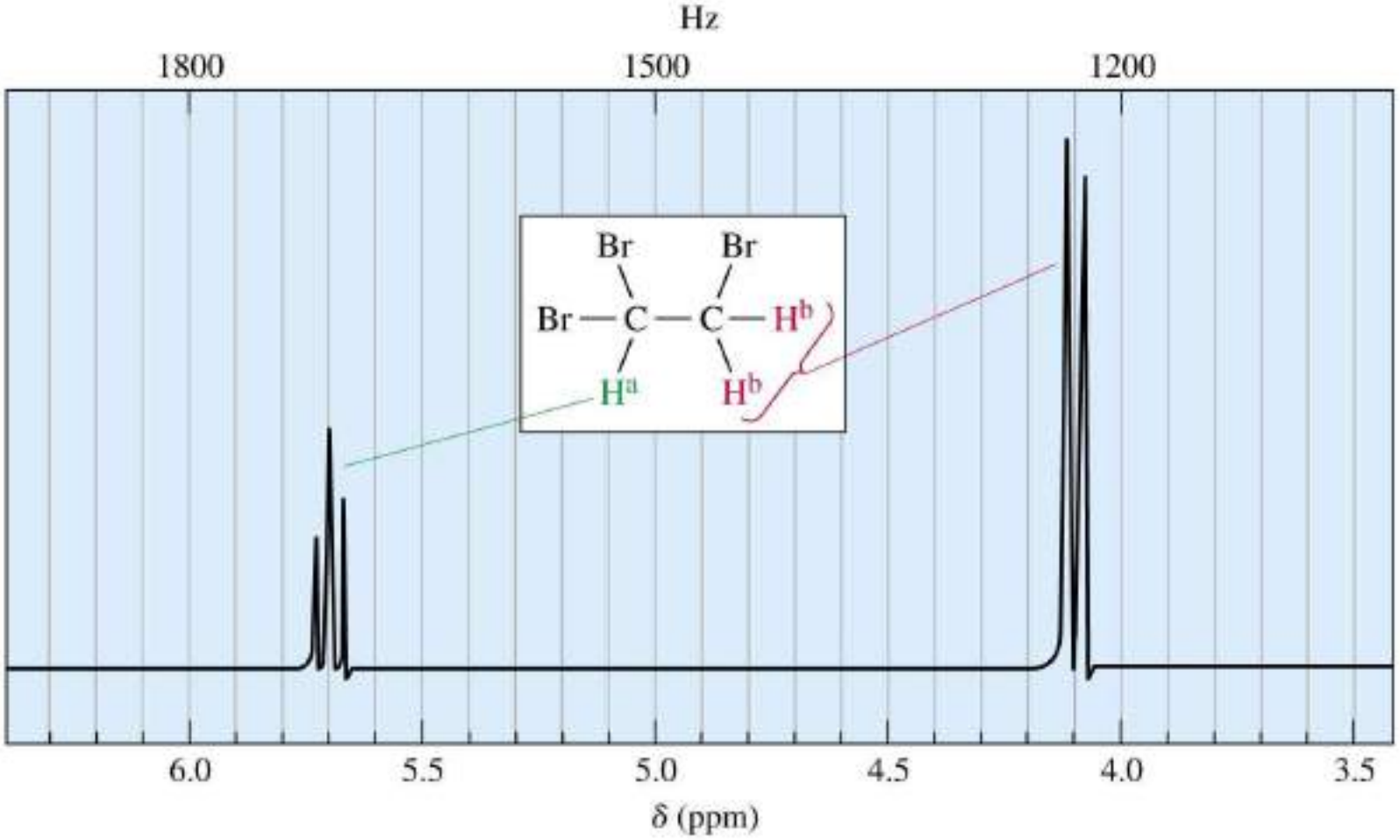
H_a and H_b are separated by four σ bonds.

no splitting between H_a and H_b

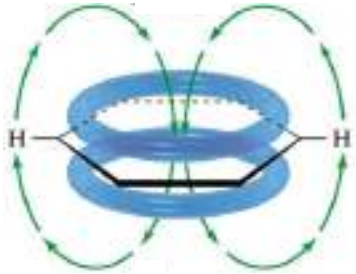
1,1,2-Tribromoethane



Nonequivalent protons on adjacent carbons.



Doublet: 1 Adjacent Proton



chemical shift of H^b

Br Br
Br—C—C—H^b
H^b

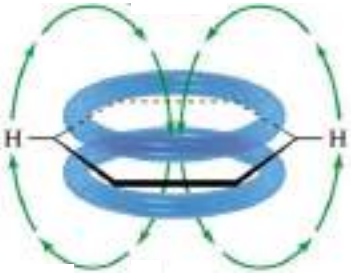
H^a reinforces field

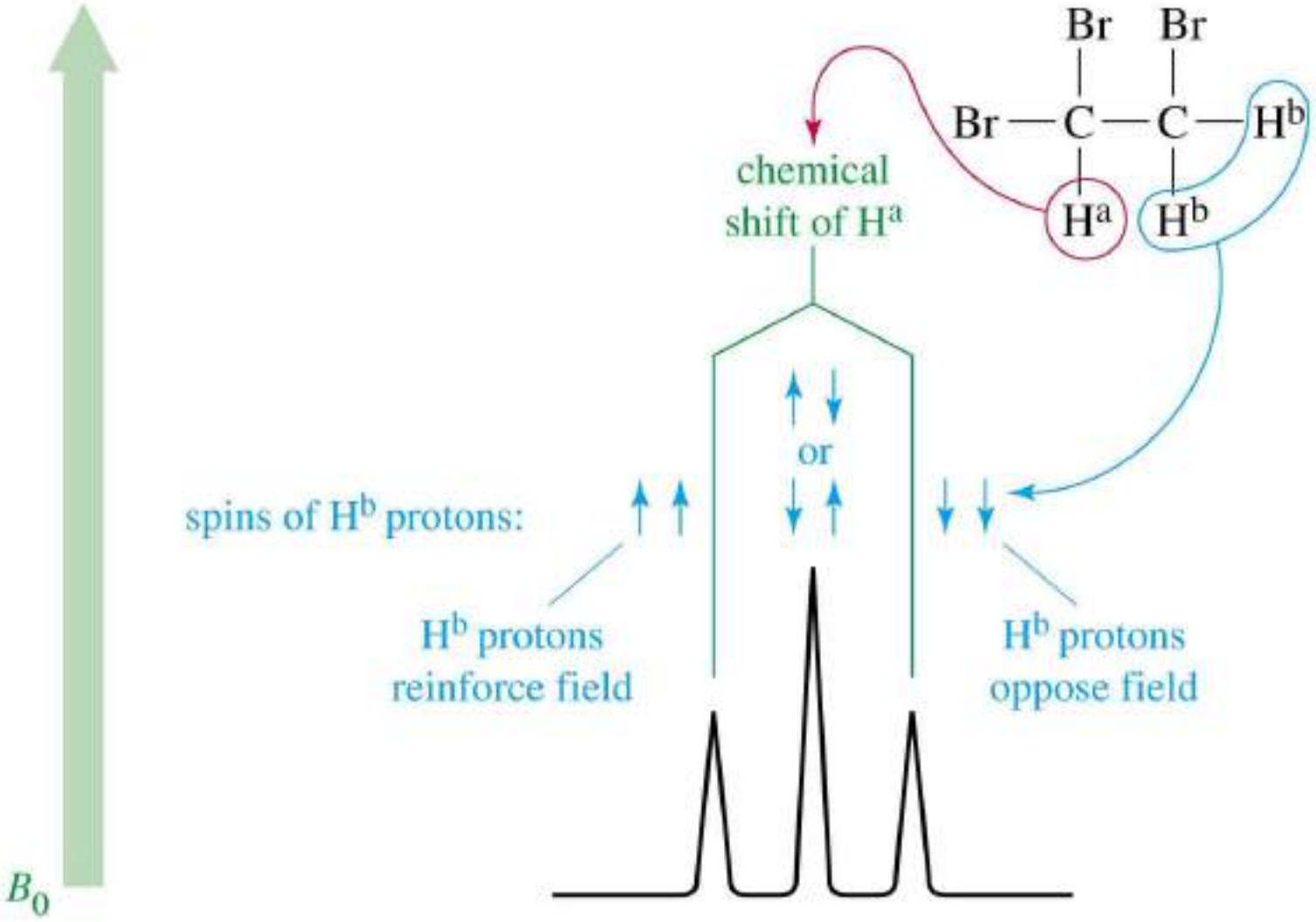
Br Br
Br—C—C—H^b
H^b

H^a opposes field

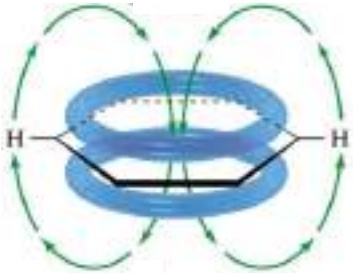
B_0

Triplet: 2 Adjacent Protons



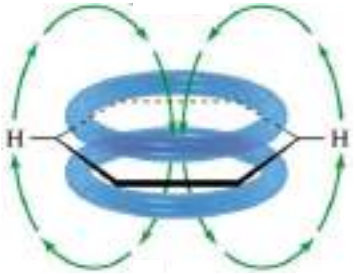


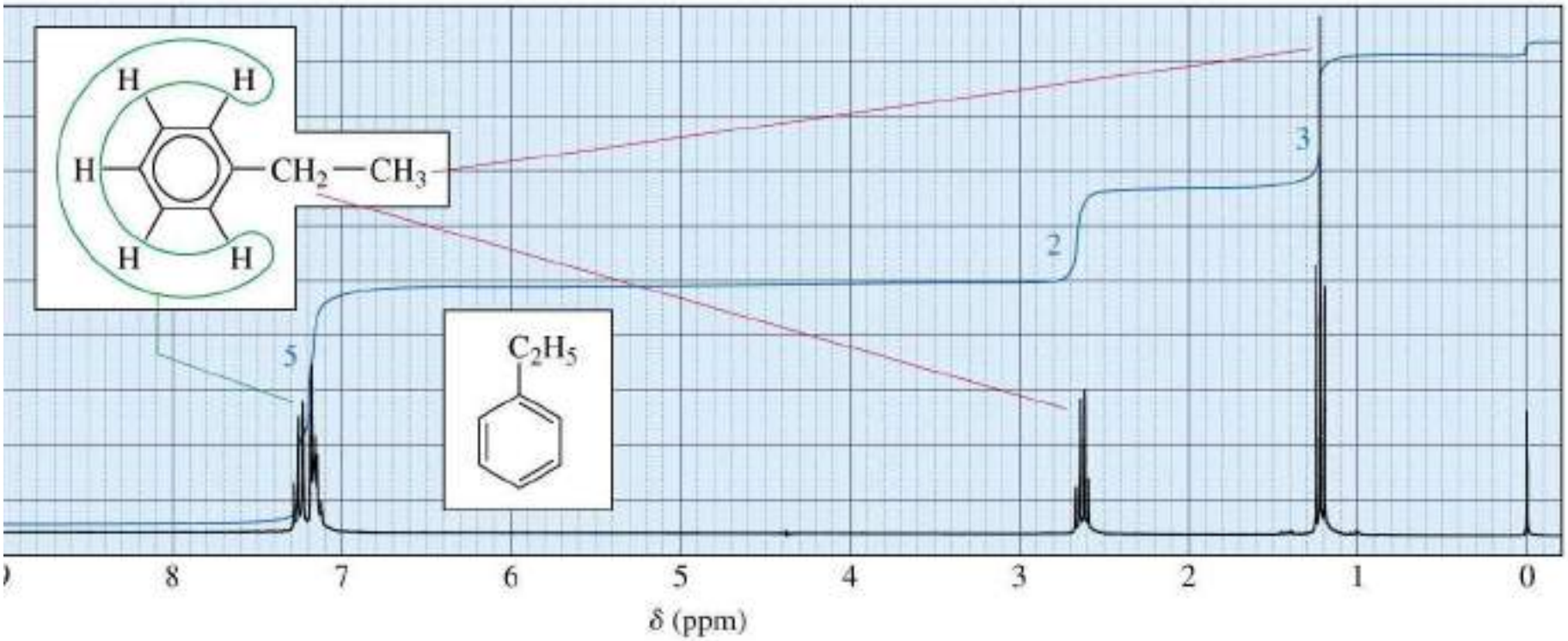
Range of Magnetic Coupling

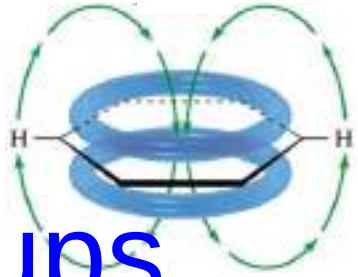


- Equivalent protons do not split each other.
- Protons bonded to the same carbon will split each other only if they are not equivalent.
- Protons on adjacent carbons normally will couple.
- Protons separated by four or more bonds will not couple.

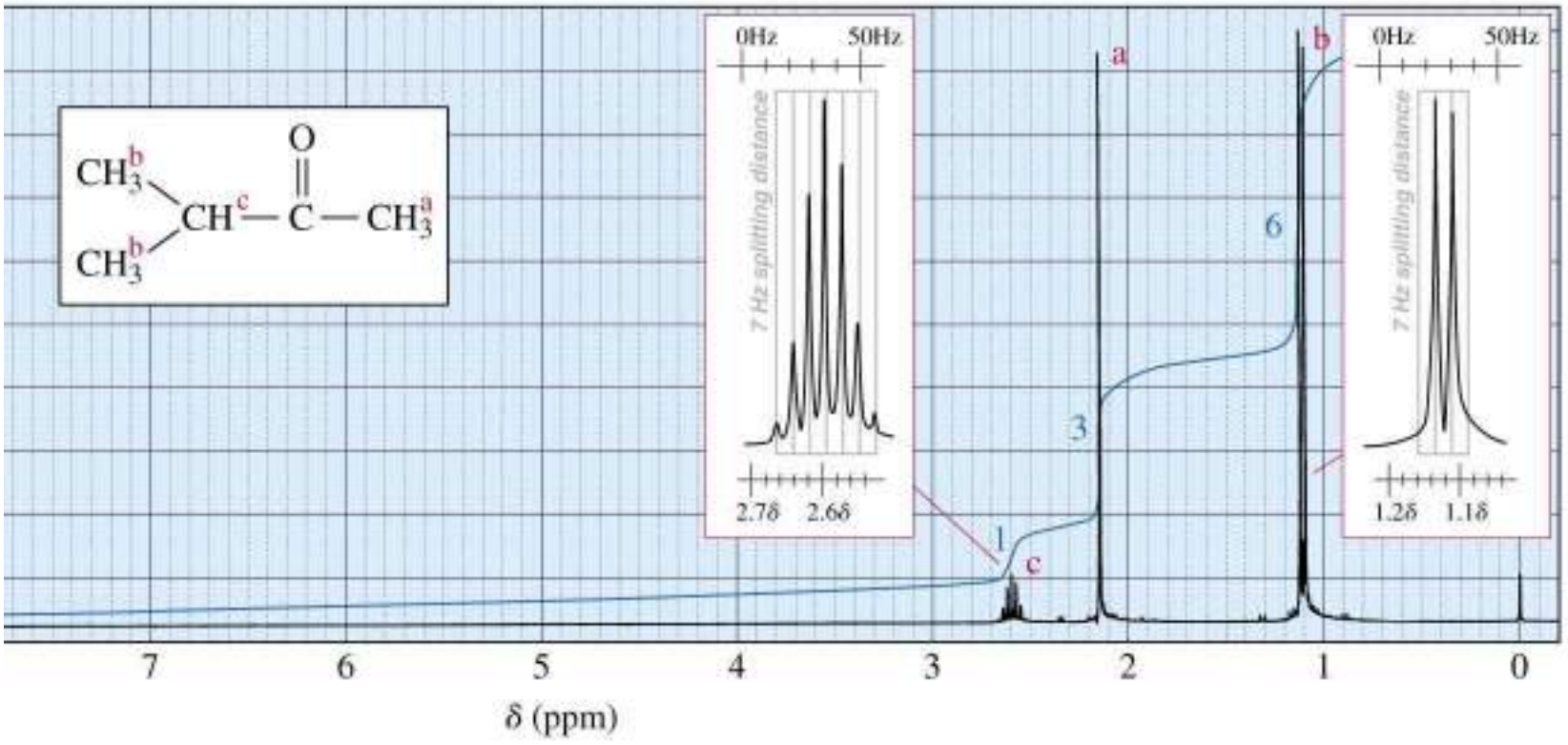
Splitting for Ethyl Groups



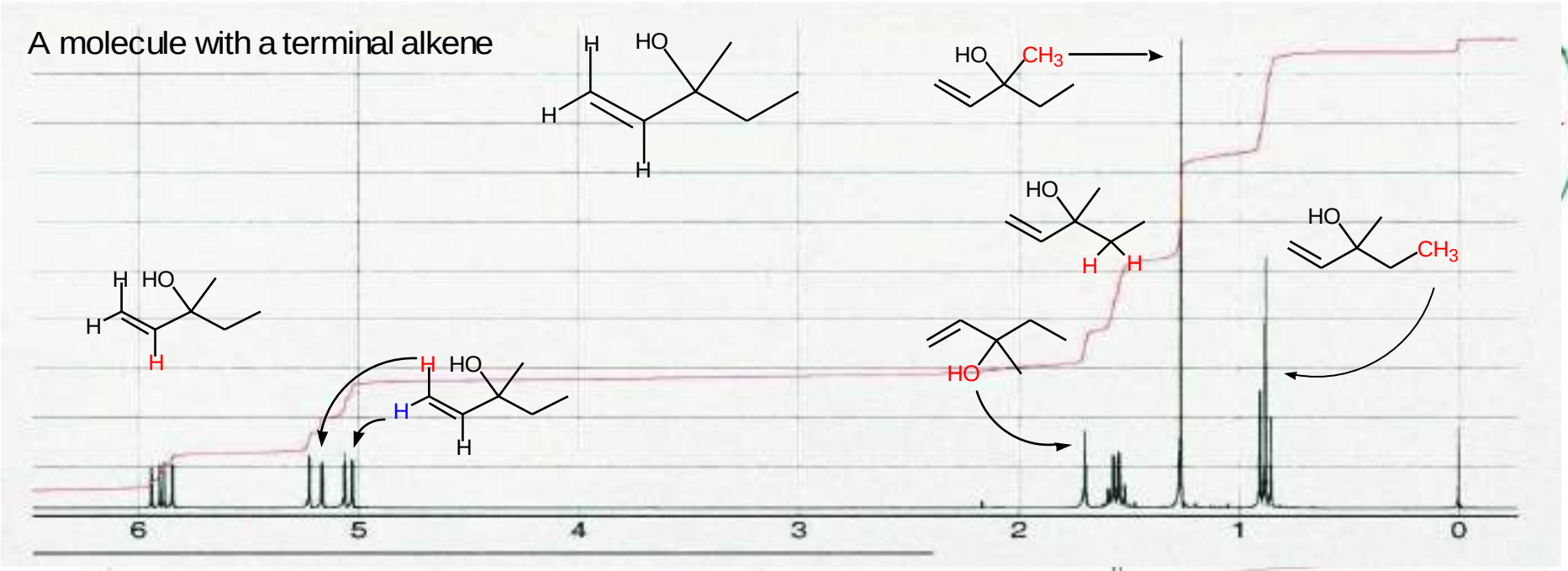




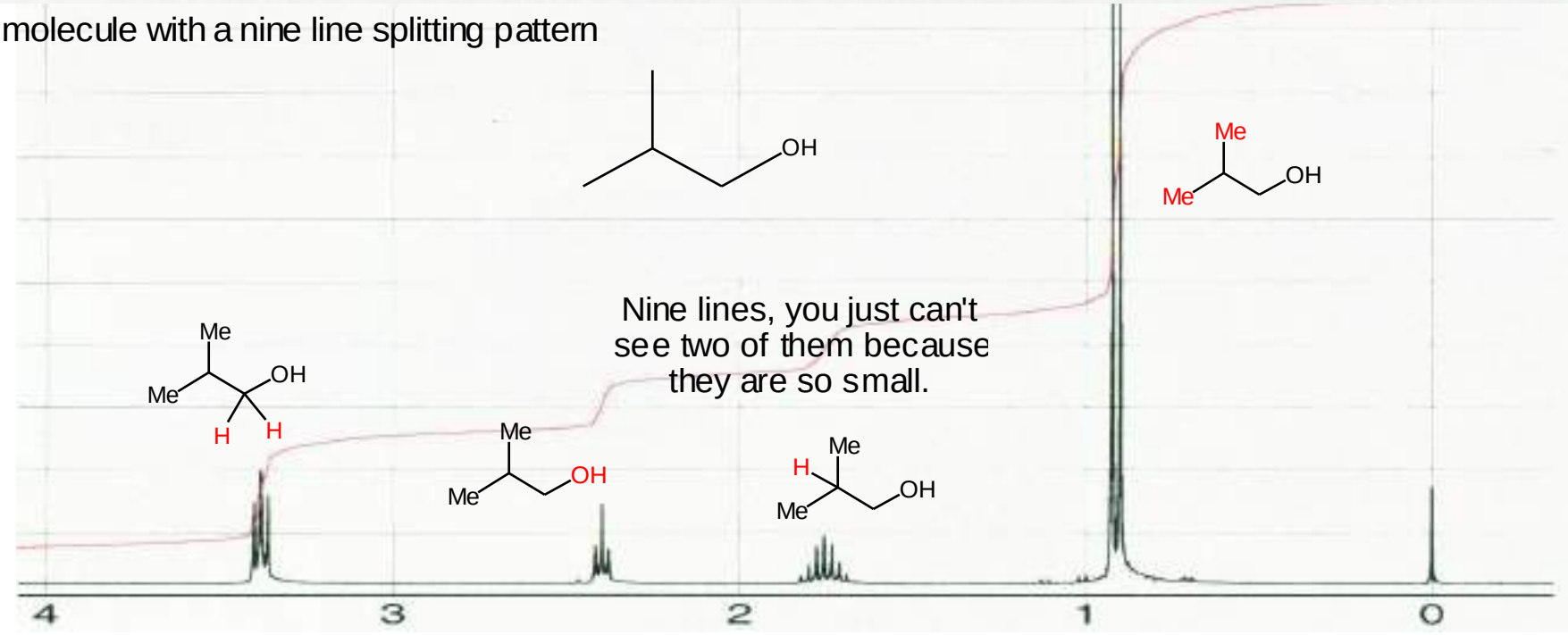
Splitting for Isopropyl Groups



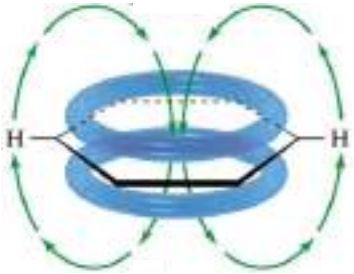
A molecule with a terminal alkene



A molecule with a nine line splitting pattern

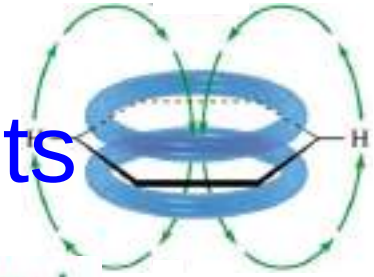


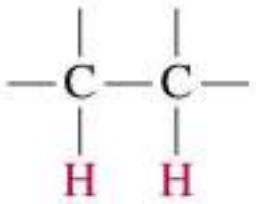
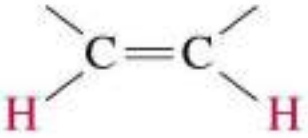
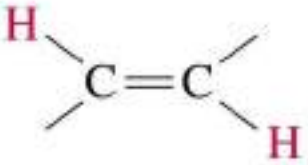
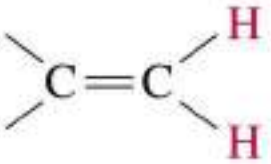
Coupling Constants

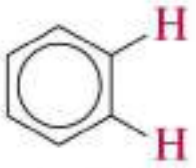
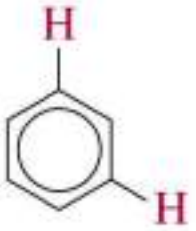
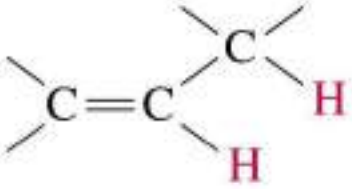


- Distance between the peaks of multiplet
- Measured in Hz
- Not dependent on strength of the external field
- Multiplets with the same coupling constants may come from adjacent groups of protons that split each other.

Values for Coupling Constants

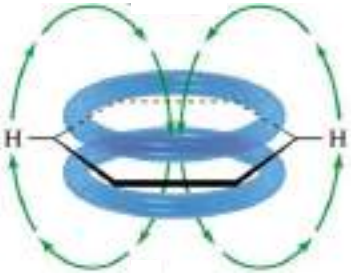


		Approx. J
	(free rotation)	7 Hz[a]
	(cis)	10 Hz
	(trans)	15 Hz
	(geminal)	2 Hz

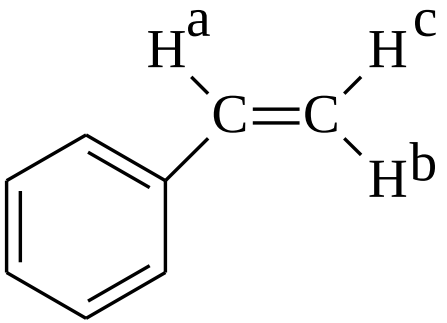
		Approx. J
	(ortho)	8 Hz
	(meta)	2 Hz
	(allylic)	6 Hz

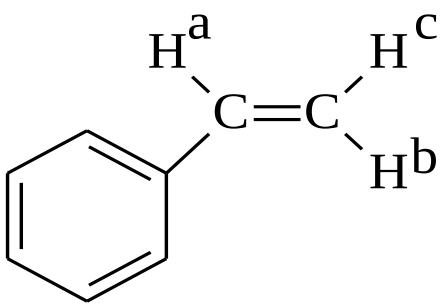
[a]The value of 7 Hz in an alkyl group is averaged for rapid rotation about the carbon–carbon bond. If rotation is hindered by a ring or bulky groups, other splitting constants may be observed.

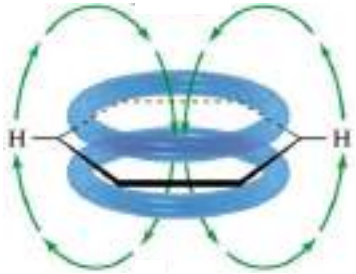
Complex Splitting

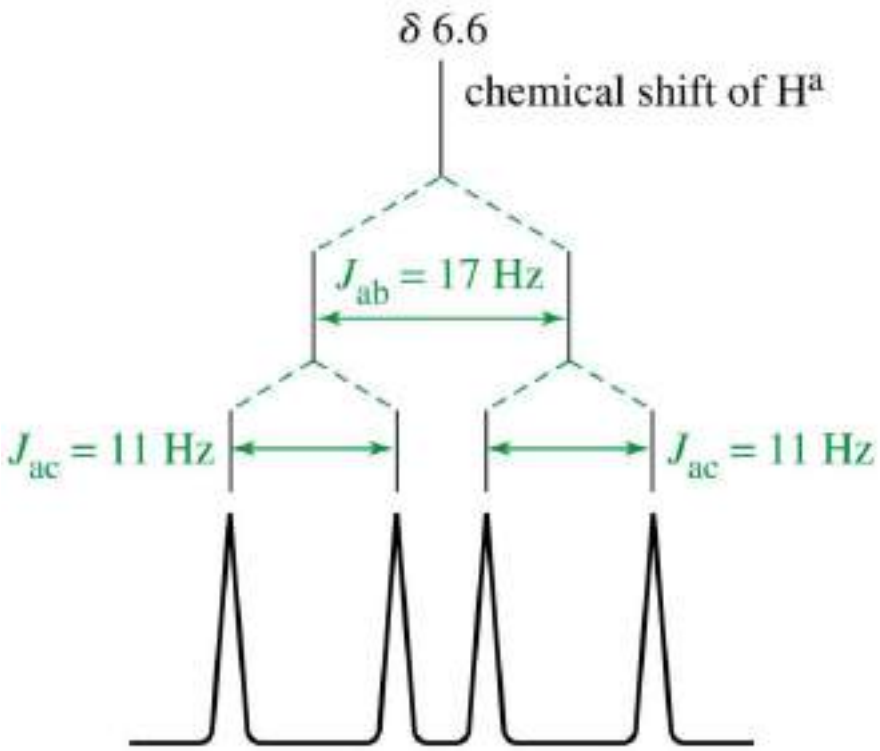


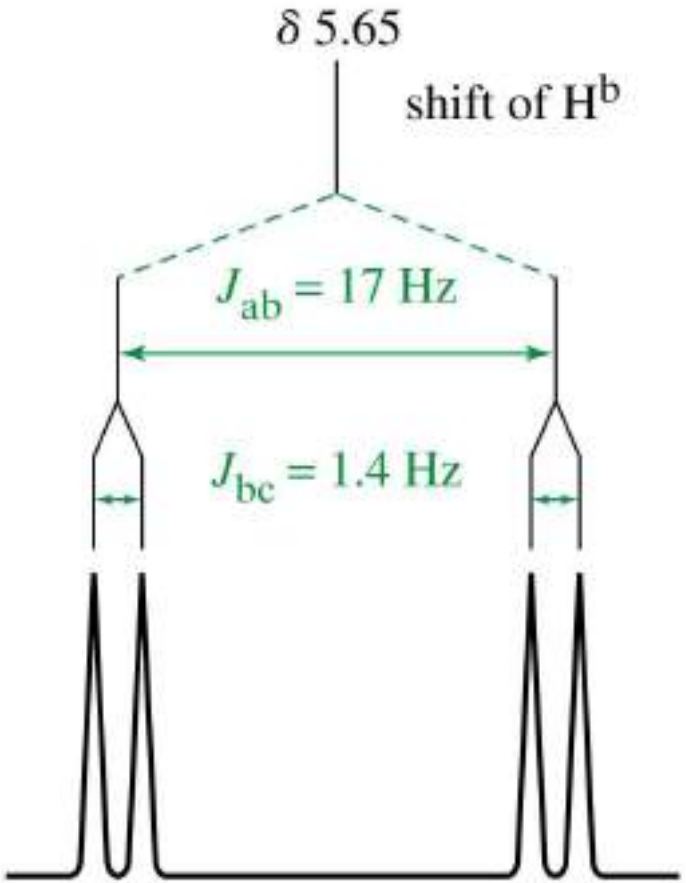
- Signals may be split by adjacent protons, different from each other, with different coupling constants.
- Example: H^a of styrene which is split by an adjacent H *trans* to it (J = 17 Hz) and an adjacent H *cis* to it (J = 11 Hz)



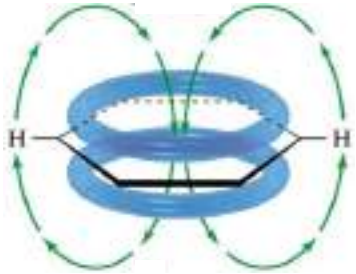

H^a
H^c
C=C
H^b

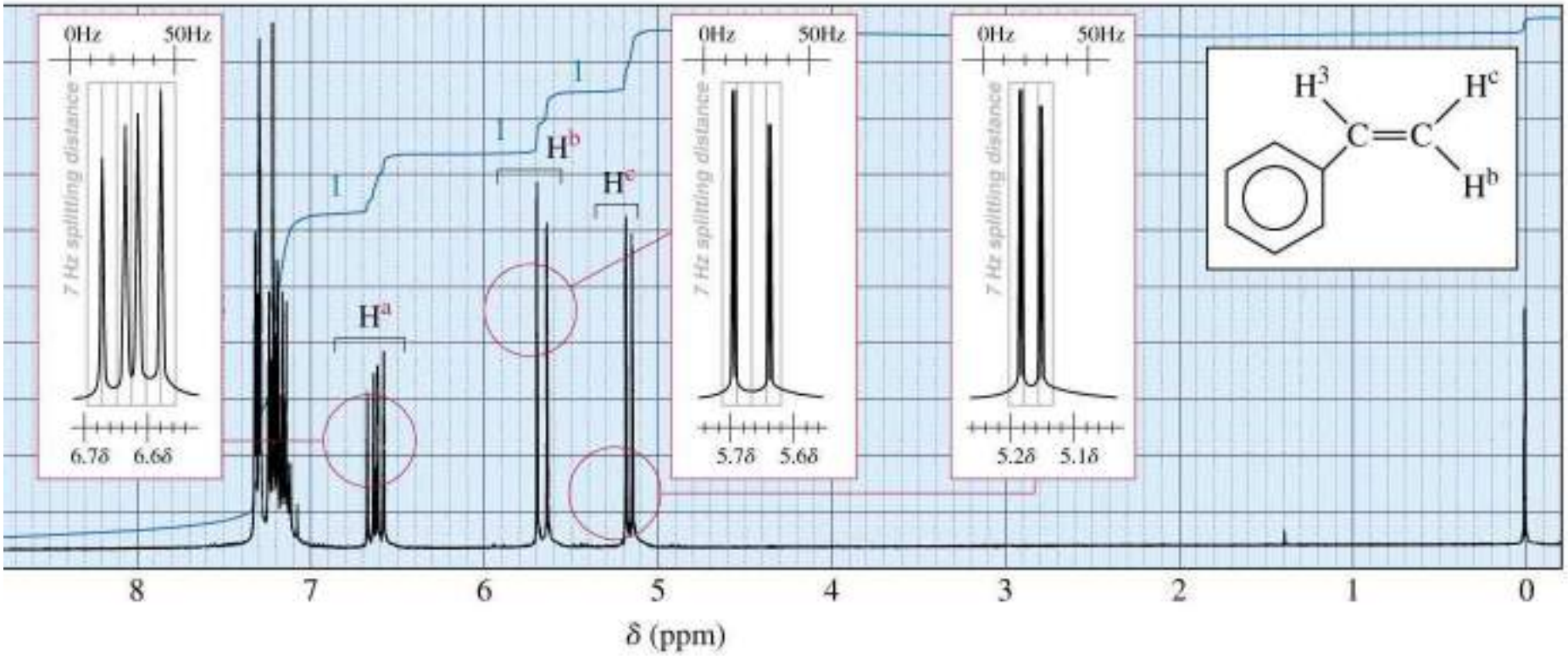

H
H


δ 6.6
chemical shift of H^a
J_ab = 17 Hz
J_ac = 11 Hz
J_ac = 11 Hz


δ 5.65
shift of H^b
J_ab = 17 Hz
J_bc = 1.4 Hz

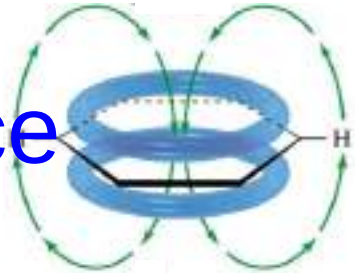
Spectrum for Styrene





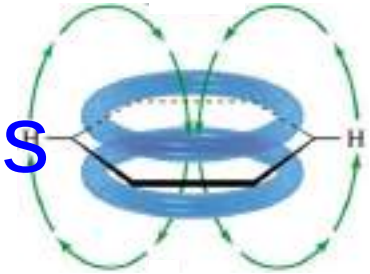
=>

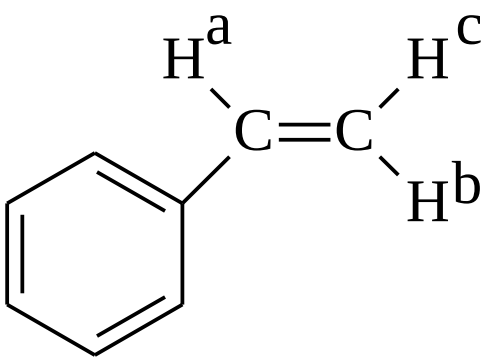
Stereochemical Nonequivalence

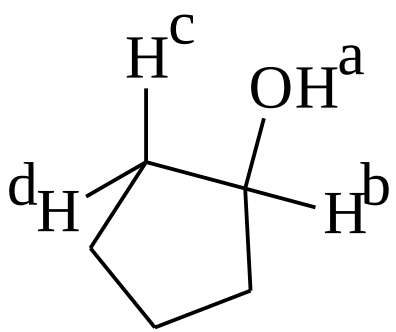


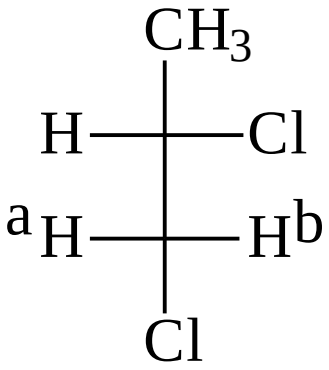
- Usually, two protons on the same C are equivalent and do not split each other.
- If the replacement of each of the protons of a -CH_2 group with an imaginary "Z" gives stereoisomers, then the protons are non-equivalent and will split each other.

Some Nonequivalent Protons

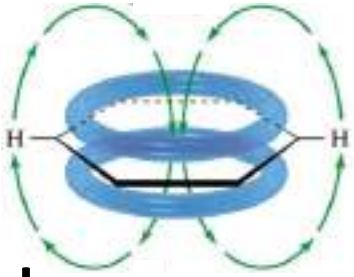






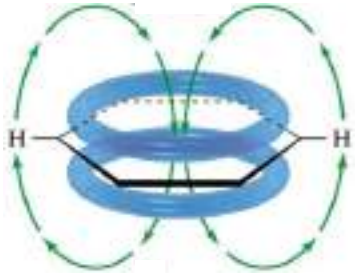


Dependence of Time

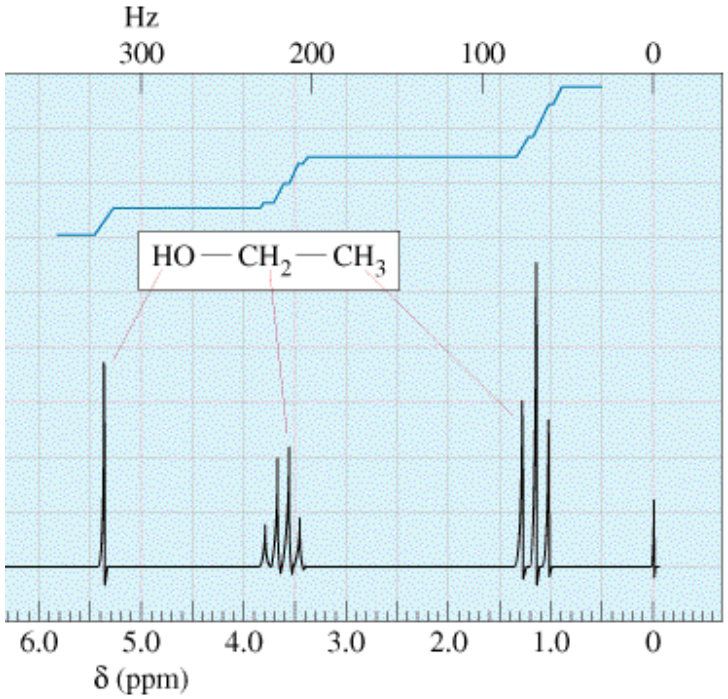


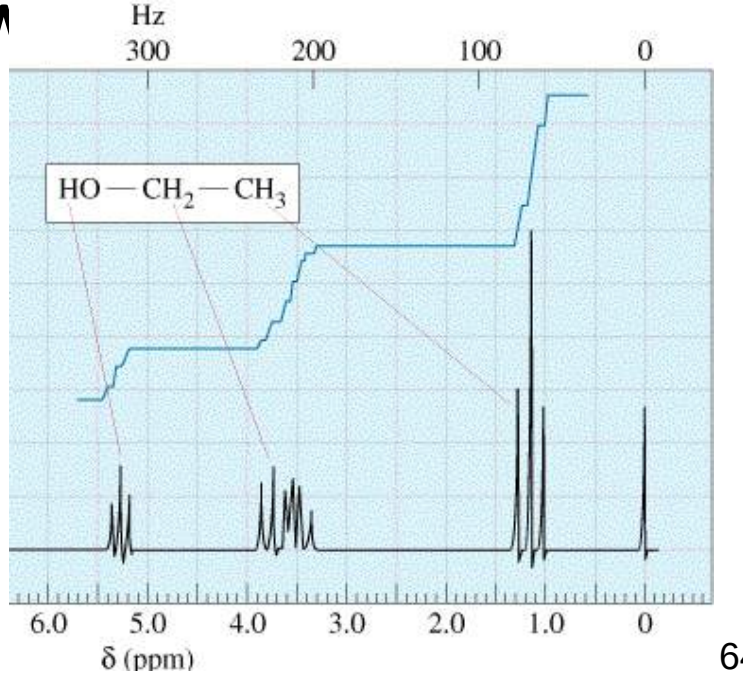
- Molecules are tumbling relative to the magnetic field, so NMR is an averaged spectrum of all the orientations.
- Axial and equatorial protons on cyclohexane interconvert so rapidly that they give a single signal.
- Proton transfers for OH and NH may occur so quickly that the proton is not split by adjacent protons in the molecule.

Hydroxyl Proton

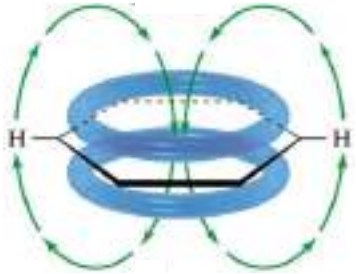


- Ultrapure samples of ethanol show splitting.
- Ethanol with a small amount of acidic impurities will show splitting.

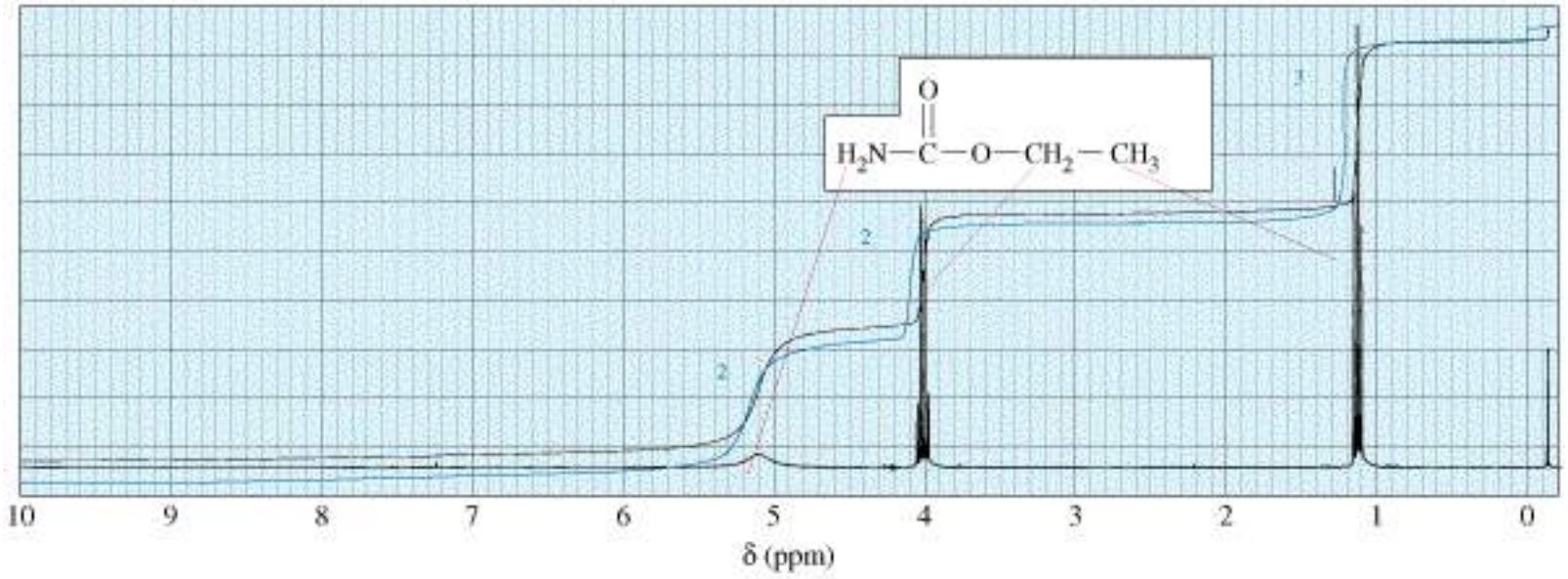




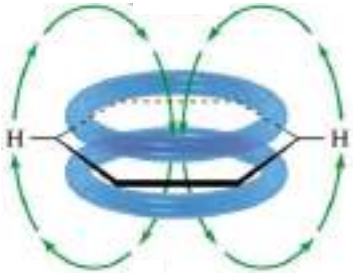
N-H Proton



- Moderate rate of exchange.
- Peak may be broad.

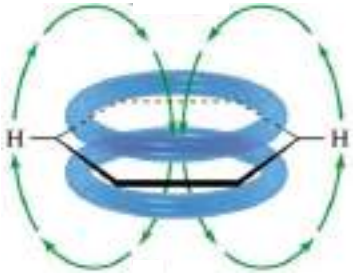


Identifying the O-H or N-H Peak

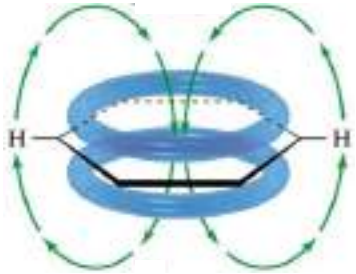


- Chemical shift will depend on concentration and solvent.
- To verify that a particular peak is due to O-H or N-H, shake the sample with D_2O
- Deuterium will exchange with the O-H or N-H protons.
- On a second NMR spectrum the peak will be absent, or much less intense.

Fourier Transform NMR



- Nuclei in a magnetic field are given a radio-frequency pulse close to their resonance frequency.
- The nuclei absorb energy and precess (spin) like little tops.
- A complex signal is produced, then decays as the nuclei lose energy.
- Free induction decay is converted to spectrum.



THANK YOU

Acknowledgement to those all for the very good images