بِسْمِ اللهِ الرَّحْمَنِ الرَّحِيمِ

Advanced Analysis
for Pharmacy Students

By

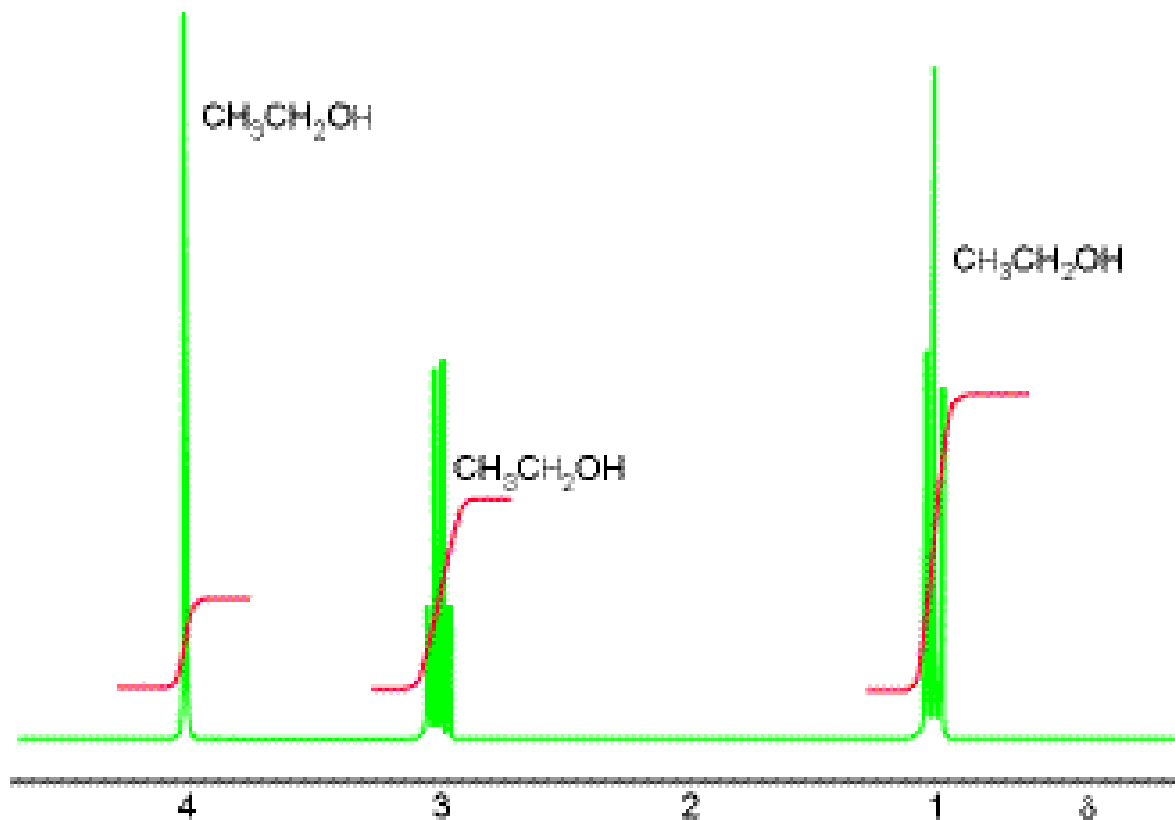## Professor Dr. Mohie Sharaf El Din

NMR-Spectroscopy

H^1-Nuclear Magnetic Resonance

C^{13}-Nuclear Magnetic Resonance

Nuclear Magnetic Resonance Spectroscopy Structure Determination:

Introduction to NMR Spectroscopy

- Nuclear magnetic resonance spectroscopy is a powerful analytical technique used to characterize organic molecules by <u>identifying carbon-hydrogen frameworks</u> within molecules.

- Two common types of NMR spectroscopy are used to characterize organic structure:

- ^{1}H NMR is used to determine the type and number of H atoms in a molecule;

- ^{13}C NMR is used to determine the type of carbon atoms in the molecule.

- The source of energy in NMR is radio waves which have long wavelengths, and thus low energy and frequency.

- When low-energy radio waves interact with a molecule, they can change the nuclear spins of some elements, including ^1H and ^{13}C.

- 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.
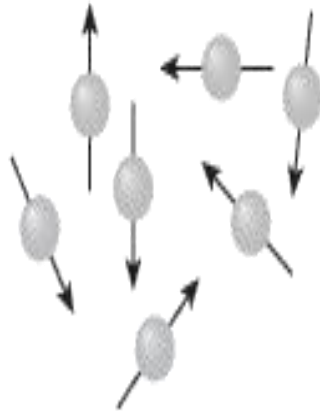
Introduction to NMR Spectroscopy

•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).
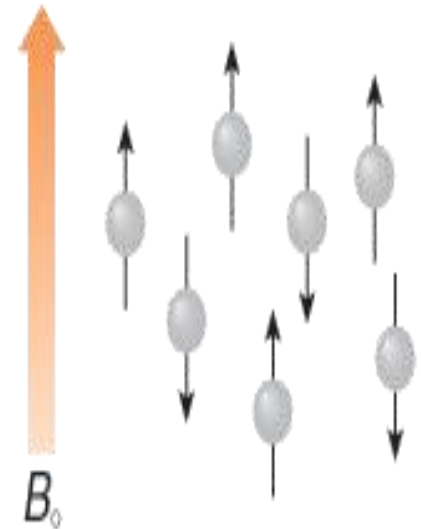
A spinning proton creates a magnetic field.

With no external magnetic field...

The nuclear magnets are randomly oriented.

In a magnetic field...

B_0

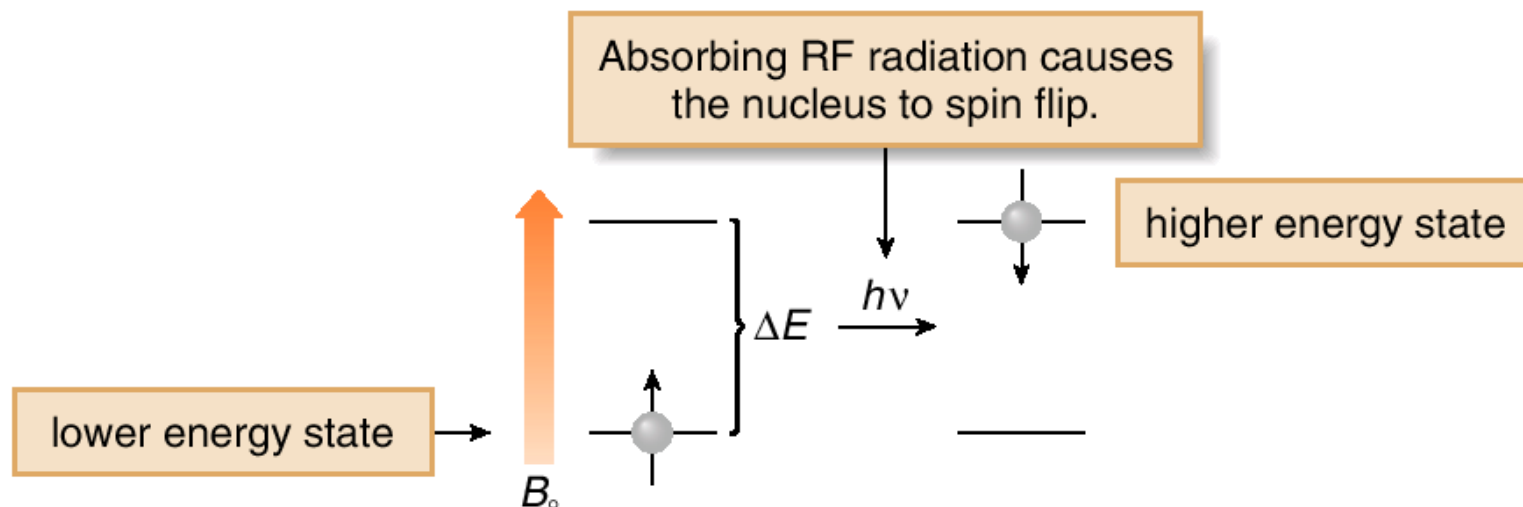The nuclear magnets are oriented **with or against** B_0.

Nuclear Magnetic Resonance Spectroscopy

Introduction to NMR Spectroscopy

- **In a magnetic field, there are now two energy states for a proton: a lower energy state with the nucleus aligned in the same direction as B0, and a higher energy state in which the nucleus aligned against B0.**

- **When an external energy source (hv) that matches the energy difference (ΔE) between these two states is applied, energy is absorbed, causing the nucleus to "spin flip" from one orientation to another.**

- **The energy difference between these two nuclear spin states corresponds to the low frequency RF region of the electromagnetic spectrum.**

Introduction to NMR Spectroscopy

- **Thus, two variables characterize NMR: an applied magnetic field B0, the strength of which is measured in tesla (T), and the frequency n of radiation used for resonance, measured in hertz (Hz), or megahertz (MHz)—(1 MHz = 10^6 Hz).**

Absorbing RF radiation causes the nucleus to spin flip.

higher energy state

$\Delta E \xrightarrow{h\nu}$

lower energy state

B_o

- A nucleus is in *resonance* when it absorbs RF radiation and "spin flips" to a higher energy state.

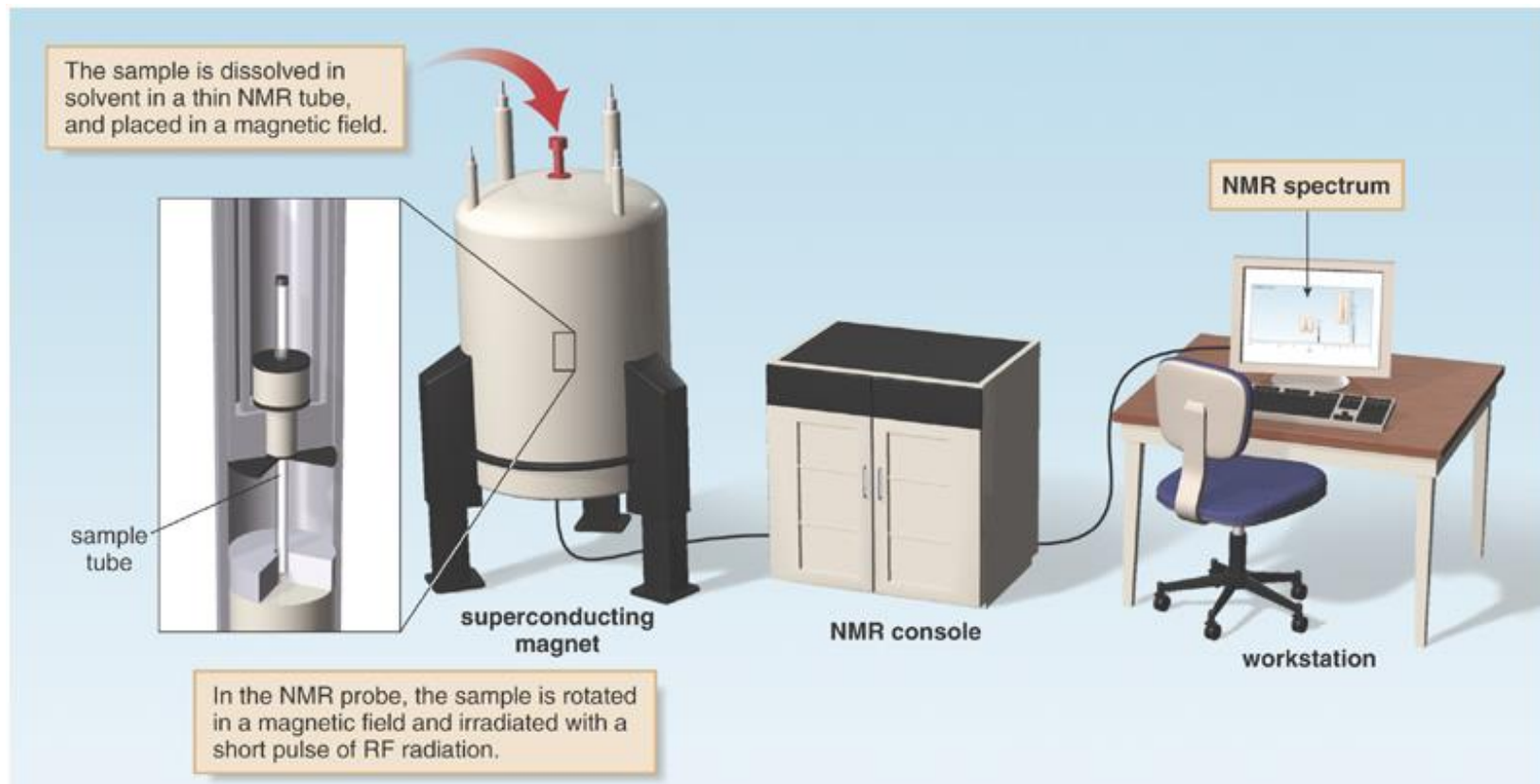# Nuclear Magnetic Resonance Spectroscopy

Introduction to NMR Spectroscopy

- The frequency needed for resonance and the applied magnetic field strength are proportionally related:

$$\nu \quad \propto \quad B_{o}$$

- The stronger the magnetic field, the larger the energy difference between the two nuclear spin states, and the higher the ν needed for resonance.

- NMR spectrometers are referred to as 300 MHz instruments, 500 MHz instruments, and so forth, depending on the frequency of the RF radiation used for resonance.

- These spectrometers use very powerful magnets to create a small but measurable energy difference between two possible spin states.

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Nuclear Magnetic Resonance Spectroscopy

Introduction to NMR Spectroscopy

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

sample tube

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

superconducting magnet

NMR console

NMR spectrum

workstation

An NMR spectrometer. The sample is dissolved in a solvent, usually $CDCl_3$ (deuterochloroform), and placed in a magnetic field. A radiofrequency generator then irradiates the sample with a short pulse of radiation, causing resonance. When the nuclei fall back to their lower energy state, the detector measures the energy released, and a spectrum is recorded. The superconducting magnets in modern NMR spectrometers have coils that are cooled in liquid helium and conduct electricity with essentially no resistance.

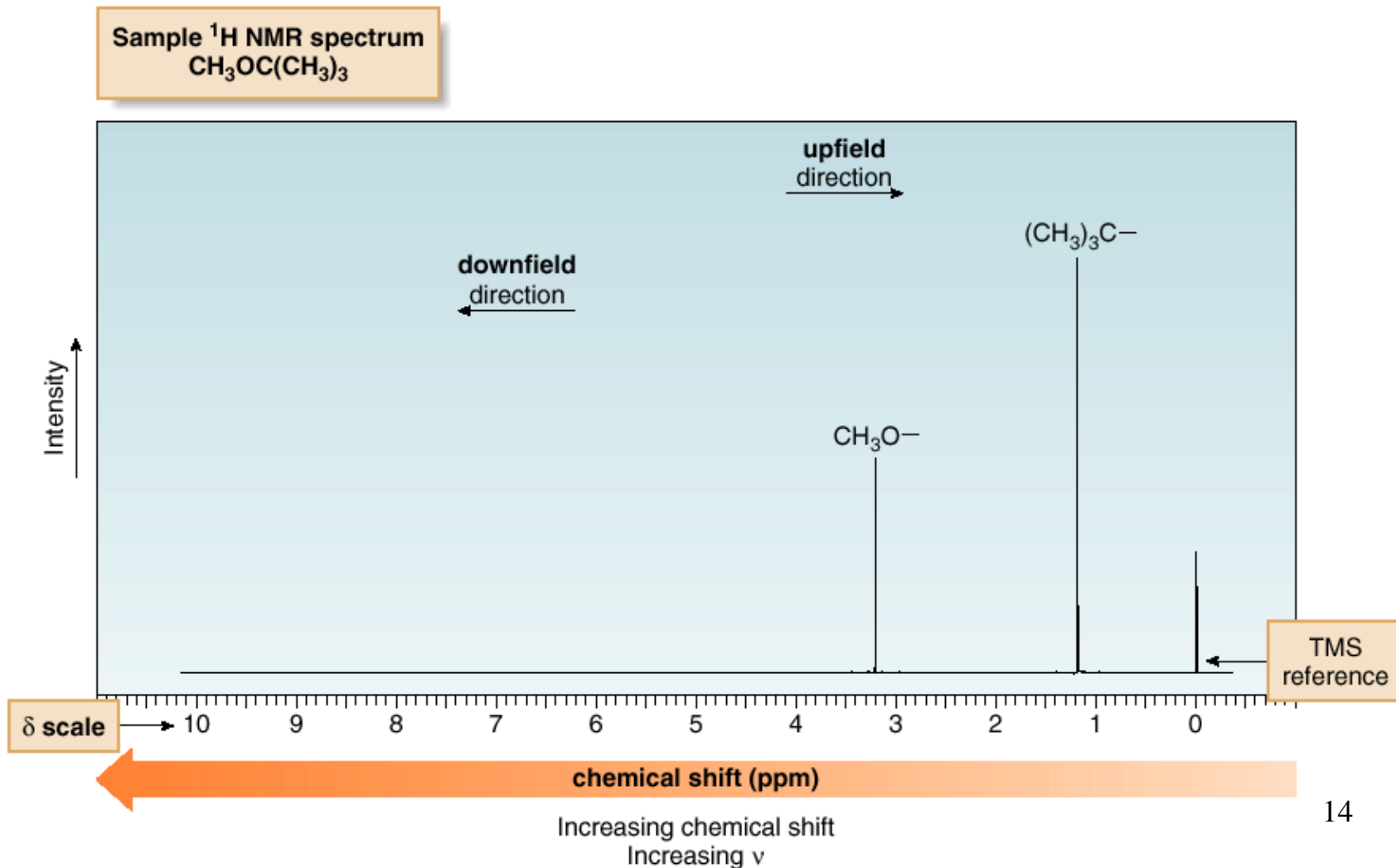# Nuclear Magnetic Resonance Spectroscopy

Introduction to NMR Spectroscopy

- Protons in different environments absorb at slightly different frequencies, so they are distinguishable by NMR.

- The frequency at which a particular proton absorbs is determined by its electronic environment.

- The size of the magnetic field generated by the electrons around a proton determines where it absorbs.

- Modern NMR spectrometers use a constant magnetic field strength B_0, and then a narrow range of frequencies is applied to achieve the resonance of all protons.

- Only nuclei that contain odd mass numbers (such as ^1H, ^{13}C, ^{19}F and ^{31}P) or odd atomic numbers (such as ^2H and ^{14}N) give rise to NMR signals.

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—The Spectrum

- An NMR spectrum is a plot of the intensity of a peak against its chemical shift, measured in **parts per million (ppm)**.

Sample ^{1}H NMR spectrum
$CH_3OC(CH_3)_3$

upfield direction

$(CH_3)_3C-$

downfield direction

CH$_3$O—

Intensity

TMS reference

δ scale → 10 9 8 7 6 5 4 3 2 1 0

chemical shift (ppm)

Increasing chemical shift
Increasing ν

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—The Spectrum

- NMR absorptions generally appear as sharp peaks.
- Increasing chemical shift is plotted from left to right.
- Most protons absorb between 0-10 ppm.
- The terms "upfield" and "downfield" describe the relative location of peaks. Upfield means to the right. Downfield means to the left.
- NMR absorptions are measured relative to the position of a reference peak at 0 ppm on the d scale due to tetramethylsilane (TMS). TMS is a volatile inert compound that gives a single peak upfield from typical NMR absorptions.

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—The Spectrum

- The chemical shift of the *x* axis gives the position of an NMR signal, measured in ppm, according to the following equation:

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

- By reporting the NMR absorption as a fraction of the NMR operating frequency, we get units, ppm, that are independent of the spectrometer.

- Four different features of a ^{1}H NMR spectrum provide information about a compound's structure:
  a. Number of signals
  b. Position of signals
  c. Intensity of signals.
  d. Spin-spin splitting of signals.

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Nuclear Magnetic Resonance Spectroscopy

a- ^{1}H NMR—Number of Signals

- The number of NMR signals equals the number of different types of protons in a compound.
- Protons in different environments give different NMR signals.
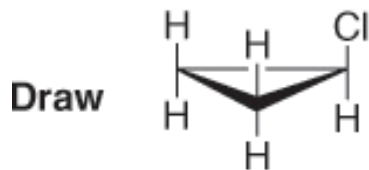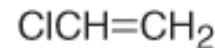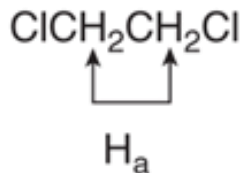- Equivalent protons give the same NMR signal.

CH_3-O-CH_3 CH_3CH_2-Cl $CH_3-O-CH_2CH_3$

H$_a$ H$_a$ H$_a$ H$_b$ H$_a$ H$_b$ H$_c$
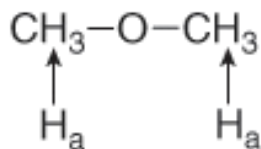
| All equivalent H's 1 NMR signal | 2 types of H's 2 NMR signals | 3 types of H's 3 NMR signals |
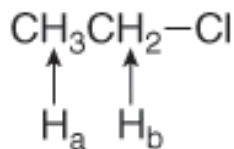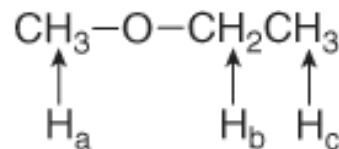
- To determine equivalent protons in cycloalkanes and alkenes, always draw all bonds to hydrogen.

Draw   **NOT**   **Draw**   **NOT** $ClCH=CH_2$

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Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Number of Signals

ClCH$_2$CH$_2$Cl
H$_a$

| 1 type of H |
| 1 NMR signal |

ClCH$_2$CH$_2$CH$_2$Br
H$_a$ H$_b$ H$_c$

| 3 types of H's |
| 3 NMR signals |

CH$_3$—C(=O)—OCH$_3$
H$_a$ H$_b$

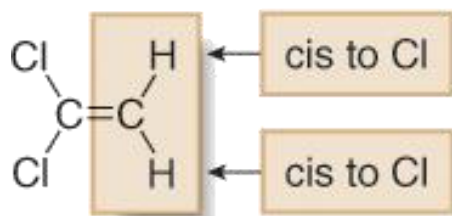| 2 types of H's |
| 2 NMR signals |

CH$_3$CH$_2$OH
H$_a$ H$_b$ H$_c$

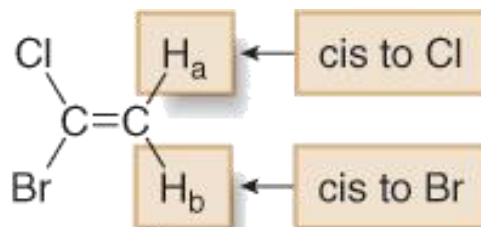| 3 types of H's |
| 3 NMR signals |

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Number of Signals

- **In comparing two H atoms on a ring or double bond, two protons are equivalent only if they are cis (or trans) to the same groups.**
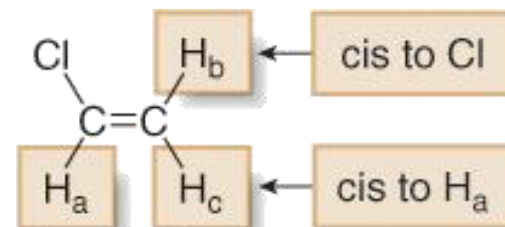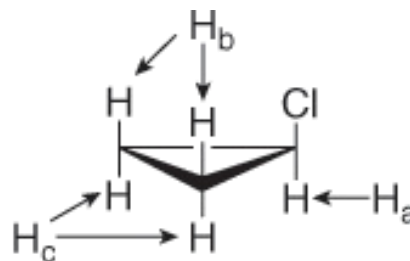
Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Number of Signals

• **Proton equivalency in cycloalkanes can be determined similarly.**
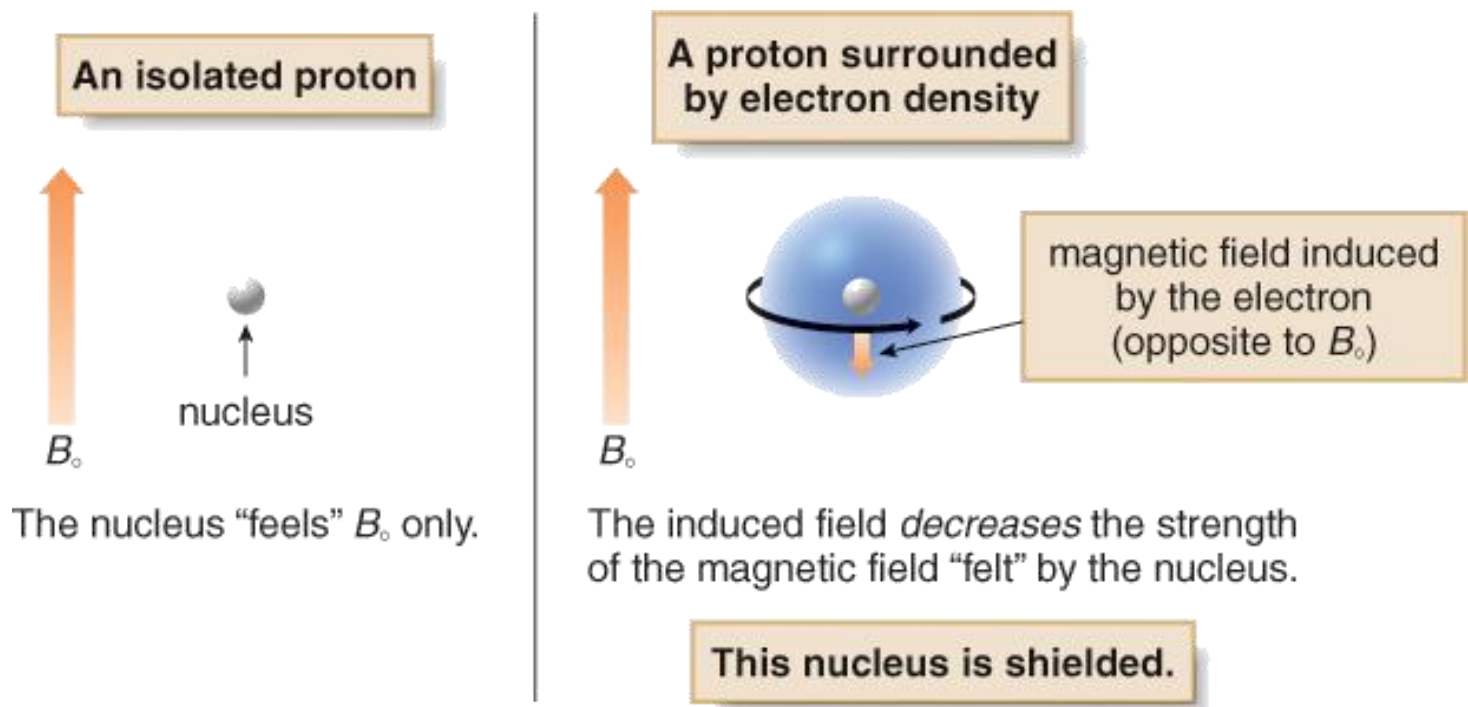

cyclopropane

All H's are equivalent.
1 NMR signal

chlorocyclopropane

3 types of H's
3 NMR signals

Nuclear Magnetic Resonance Spectroscopy

b- ^{1}H NMR—Position of Signals

- 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 upfield.

An isolated proton

nucleus

B_o

The nucleus "feels" B_o only.

A proton surrounded by electron density

magnetic field induced by the electron (opposite to B_o)

B_o

The induced field *decreases* the strength of the magnetic field "felt" by the nucleus.

This nucleus is shielded.

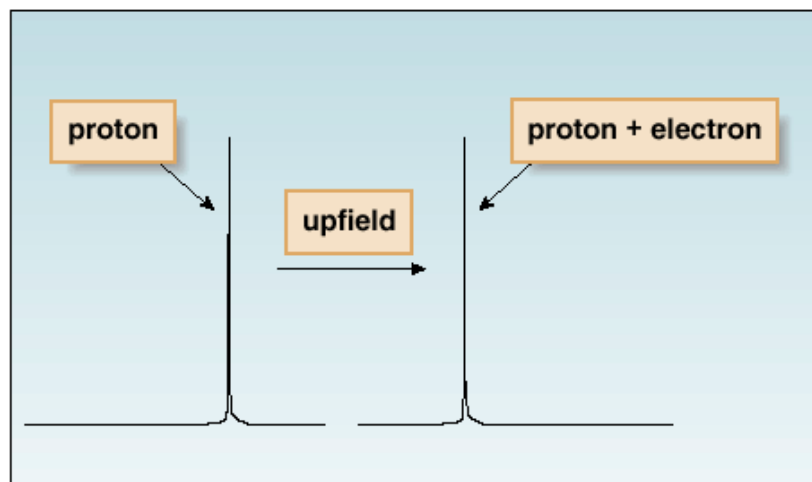# Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Position of Signals

- The less shielded the nucleus becomes, the more of the applied magnetic field (B_0) it feels.

- This deshielded nucleus experiences a higher magnetic field strength, to it needs a higher frequency to achieve resonance.

- Higher frequency is to the left in an NMR spectrum, toward higher chemical shift—so deshielding shifts an absorption downfield.

- Protons near electronegative atoms are deshielded, so they absorb downfield.

Nuclear Magnetic Resonance Spectroscopy

¹H NMR—Position of Signals

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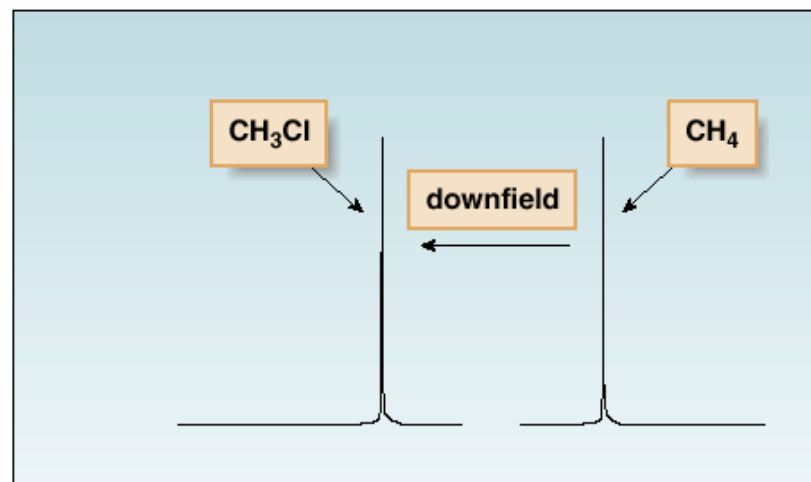- An electron shields the nucleus.
- The absorption shifts *upfield*.

proton proton + electron

upfield

Increasing chemical shift
Increasing ν

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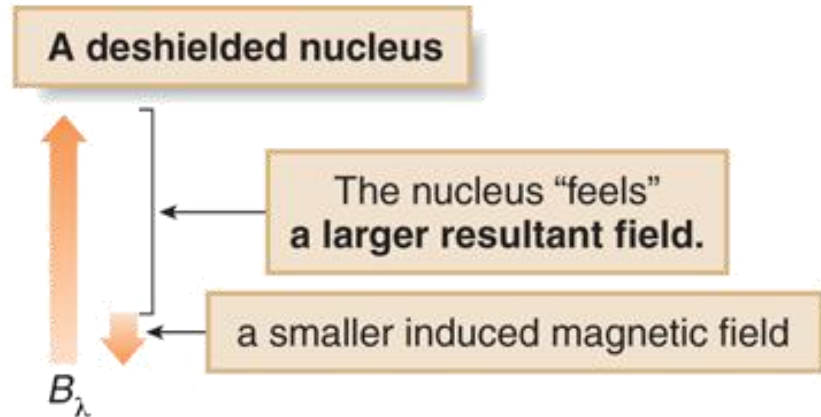- The absorption shifts *downfield*.

CH_3Cl CH_4

downfield

Increasing chemical shift
Increasing ν

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Position of Signals

A shielded nucleus

The nucleus "feels" **a smaller resultant field.**

a larger induced magnetic field

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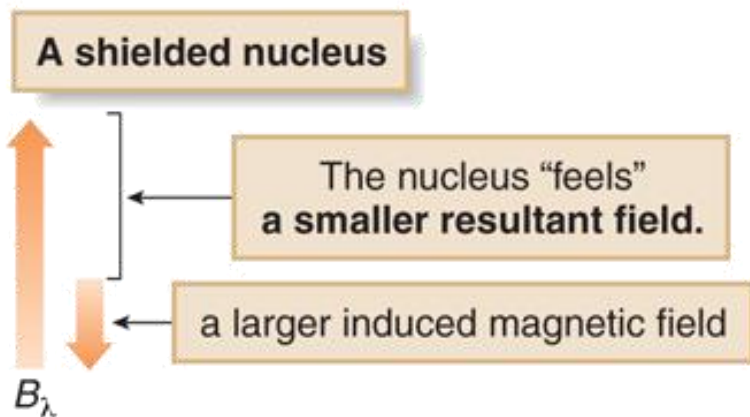
- As the electron density around the nucleus increases, the nucleus feels a smaller resultant magnetic field, so a lower frequency is needed to achieve resonance.
- **The absorption shifts upfield.**

A deshielded nucleus

The nucleus "feels" **a larger resultant field.**

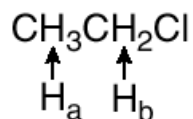a smaller induced magnetic field

B_λ

- 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.
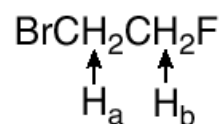- **The absorption shifts downfield.**
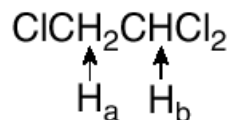
Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Position of Signals

CH_3CH_2Cl
H_a H_b

- The H_b protons are **deshielded** because they are closer to the electronegative Cl atom, so they absorb **downfield** from H_a.

$BrCH_2CH_2F$
H_a H_b

- Because F is more electronegative than Br, the H_b protons are more **deshielded** than the H_a protons and absorb farther **downfield.**

$ClCH_2CHCl_2$
H_a H_b

- The larger number of electronegative Cl atoms (two versus one) **deshields** H_b more than H_a, so it absorbs **downfield** from H_a.