FT-NMR

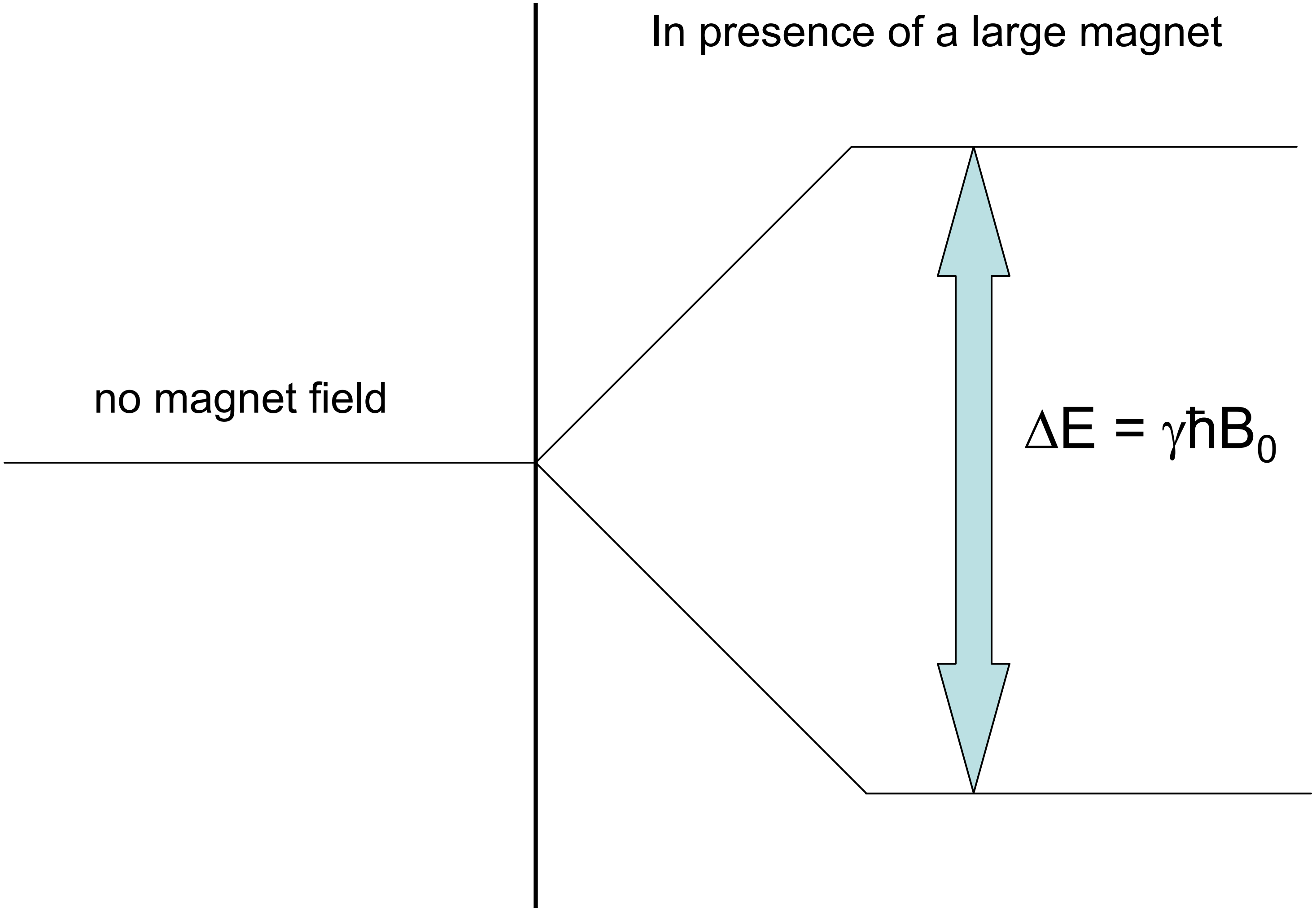
What can NMR do for you?

- Structural analysis of compounds
- Biochemical methods
  - Small molecule characterization (hormones, cross-linking agents, ect.)
  - Protein secondary structure
  - metabolomics
- Medicinal
  - Imaging

Fundamentals

- Nuclear spin
- Spin quantum number – ½
- Nuclei with spin state ½ are like little bar magnets and align with a magnetic field (B) field.
- Aligns with (++) or against (+-) B
- Small energy gap between + and – spin alignment (NMR insensitive/Boltzman dist)
- Probe the difference with RW

Lose Degeneracy in the presence of a magnetic field



In presence of a large magnet

no magnet field

$\Delta E = \gamma \hbar B_0$

(NMR insensitive/Boltzman dist)

- Small population difference between +1/2 and -1/2 state
- It is the small excess of nuclei in the -1/2 that produce NMR signal
- 1 in 10^6
  - 1000001 nuclei in +1/2 state vs 1000000 nuclei in -1/2 state

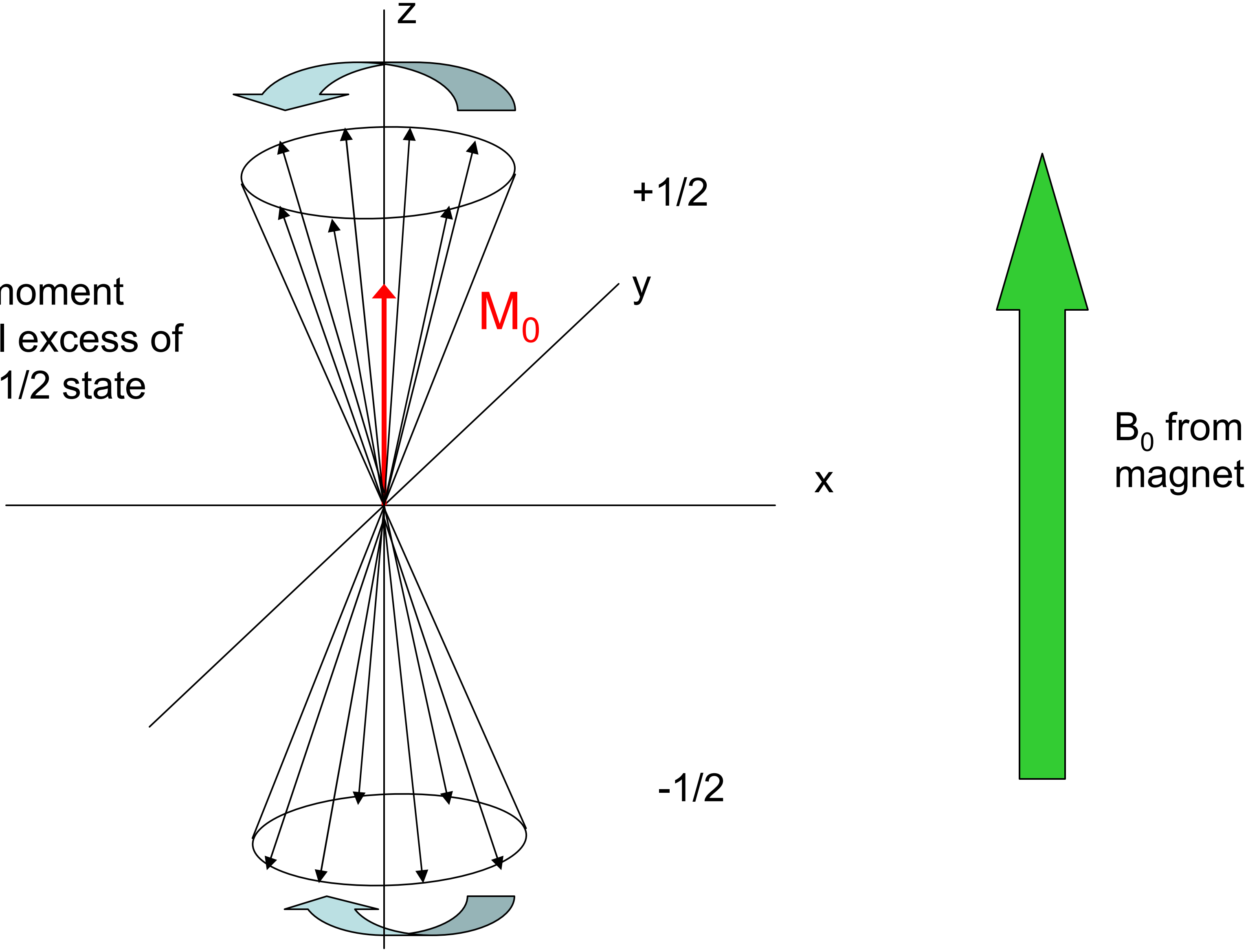
Common NMR nuclei

- Protons, ^1H
- ^{13}C
- ^{15}N
- ^{19}F
- ^{31}P
- Sensitivity depends on natural isotopic abundance and γ

  $\Delta E = \gamma \hbar B_0$, bigger magnet, greater sensitivity

Precession of nuclear dipoles

M_0; net
magnetic moment
From small excess of
Nuclei in +1/2 state



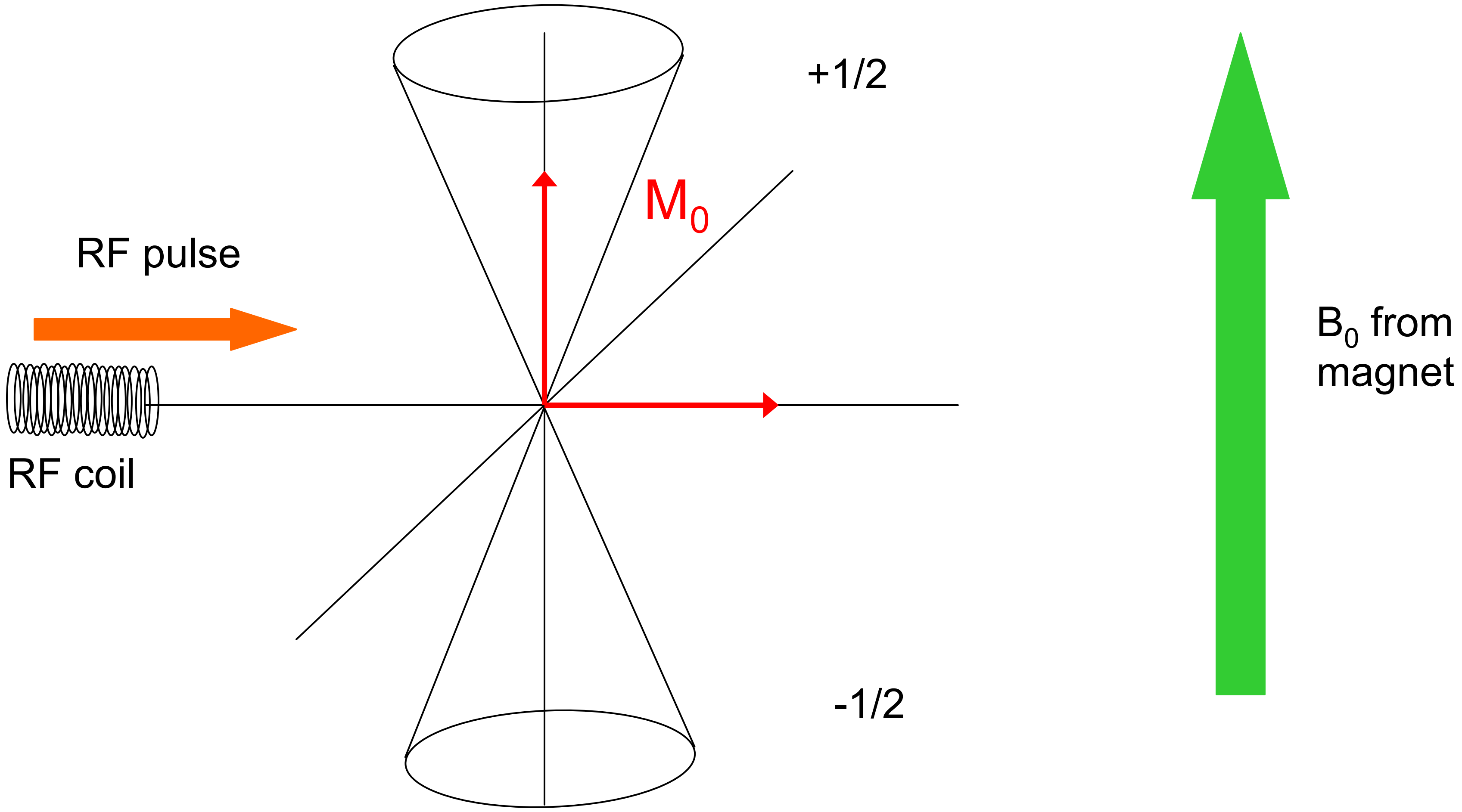
FT pulse

- Radiofrequency generator
  - – A short, intense pulse generates a magnetic field in the x-y plane (excites all nuclei)
  - – M_0 of the nuclei interacts with the magnetic field produced by the pulse.
  - – Tips M_0 off axis

$\Theta = \gamma B1\tau_p$

τ_p – length of pulse, 90° pulse

Vector Illustration of the pulse



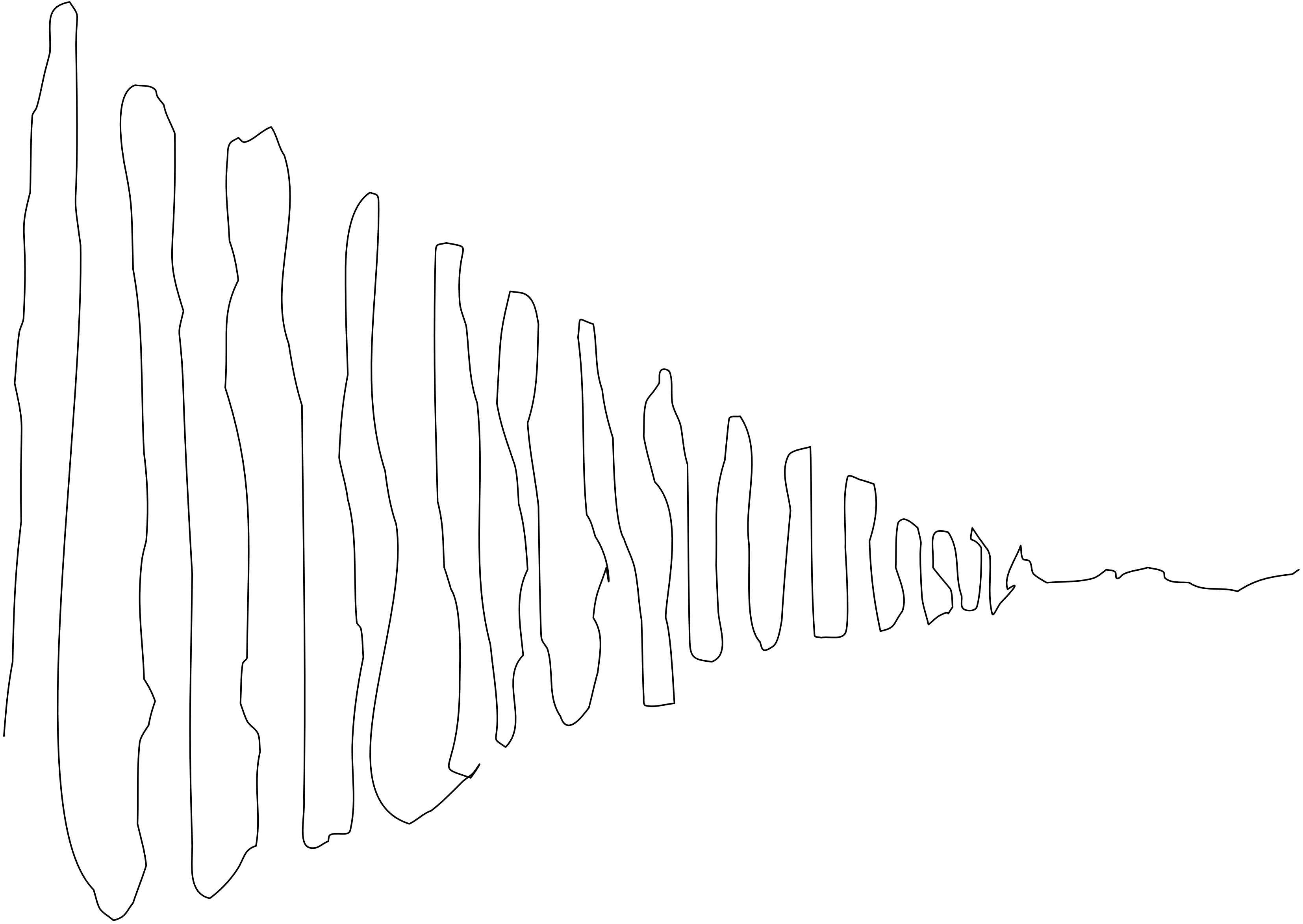
Relaxation

- T_1 spin-lattice (relaxing back to precessing about the z axis)
- T_2 spin-spin (fanning out)

Induced current in coil

- After pulse, nuclei begin to precess in phase in the x-y plane
- Packet of nuclei induce current in RF coil
- Relaxation is measured by monitoring the induced coil
- → FID (→ FT) NMR spectrum

FID



Noise reduction and increasing resolution

- Apodization: Multiply the ***free-induction decay (FID)*** by a decreasing exponential function which mathematically suppresses the noise at long times. Other forms of apodization functions can be used to improve ***resolution*** or ***lineshape***.
- Zero filling

Chemical Shift

- Shielding
  - Electrons have spin, produce local B environments
  - Protons in different electronic environments experience different B, different precessing frequencies, $\Delta E = h\nu$
  - Chemical shift (σ), $\nu = k(1-\sigma)]$
  - Large σ corresponds to high level of shielding
  - Chemical shift parameter is independent of the size of the magnet
  - $\delta = (\sigma-\sigma_{ref})*10^6$ ppm (the x axis of the NMR spectrum)

Spin-Spin Coupling

- Adjacent nuclei have a 50/50 chance of being spin up (+1/2) or spin down (-1/2)
- Each produce a small magnetic field that is either with or against B_0
- 1 adjacent proton CHOCH3
  - CH_3 is a doublet at frequencies

    υ_0-υ_a, υ_0+υ_a (equal intensity), 1:1
  - CH is a quadruplet

    1:3:3:1

Splitting Patterns

- J values
- Quadruplet ↾↾↾ ↾↾↿ ↿↾↾ ↾↿↾ ↿↿↾ ↿↾↿ ↾↿↿ ↿↿↿
- Triplet ↾↾ ↾↿ ↿↾ ↿↿
- Multiplets

1 3 3 1

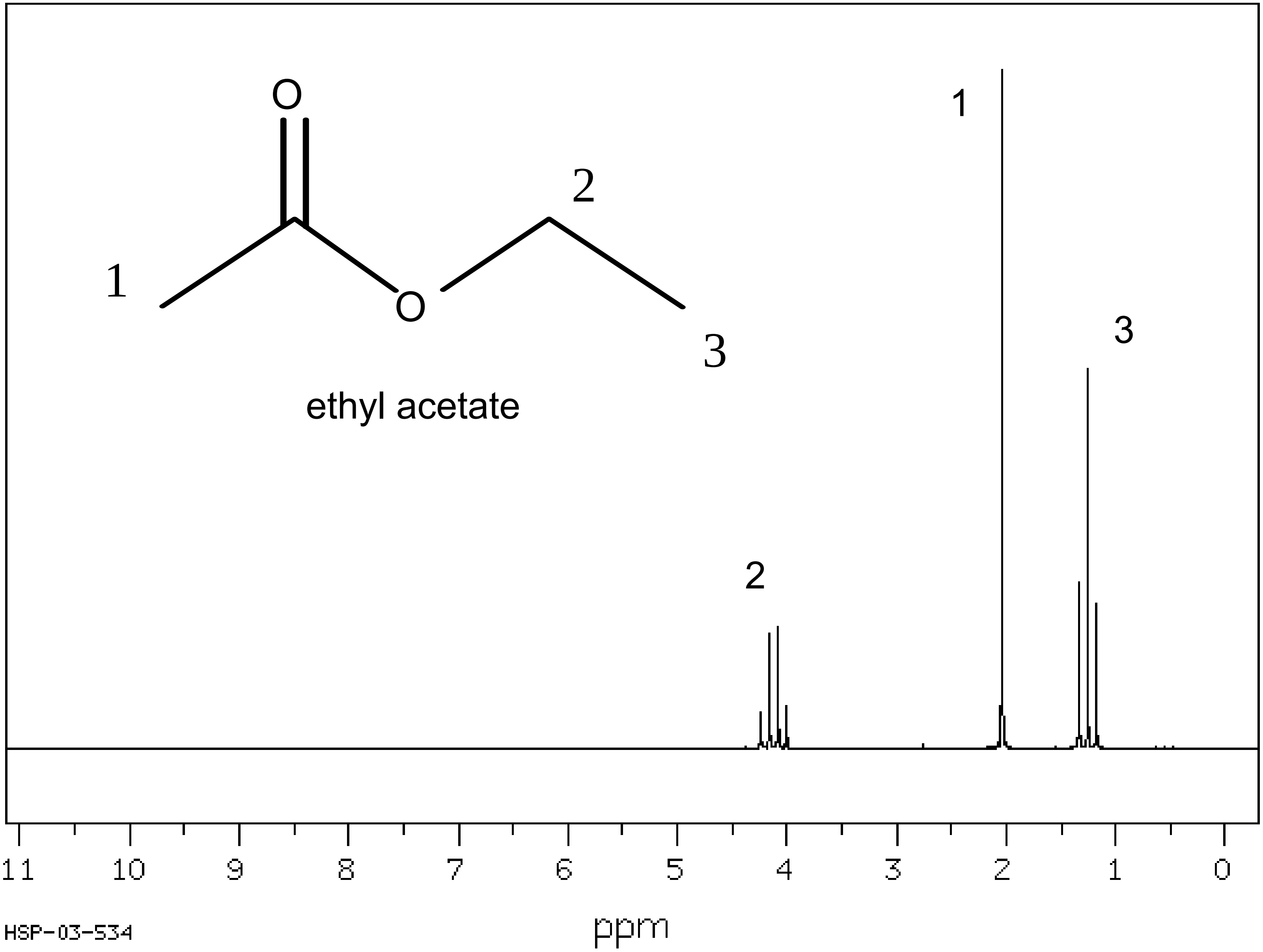
1 2 1

¼ ½ ¼ ¾ 1 ½ ¾ ¾ 1 ½ ¾ ¼ ½ ¼

1 2 1 3 6 3 3 6 3 1 2 1

J Values

- Coupling constants between coupled nuclei
- The coupling constants are in units of frequency (Hz)
- Coupling constants are independent of magnetic field strength
- Provide valuable information about the nature of the bonding
- For example, cis vs. trans conformation have very different J values



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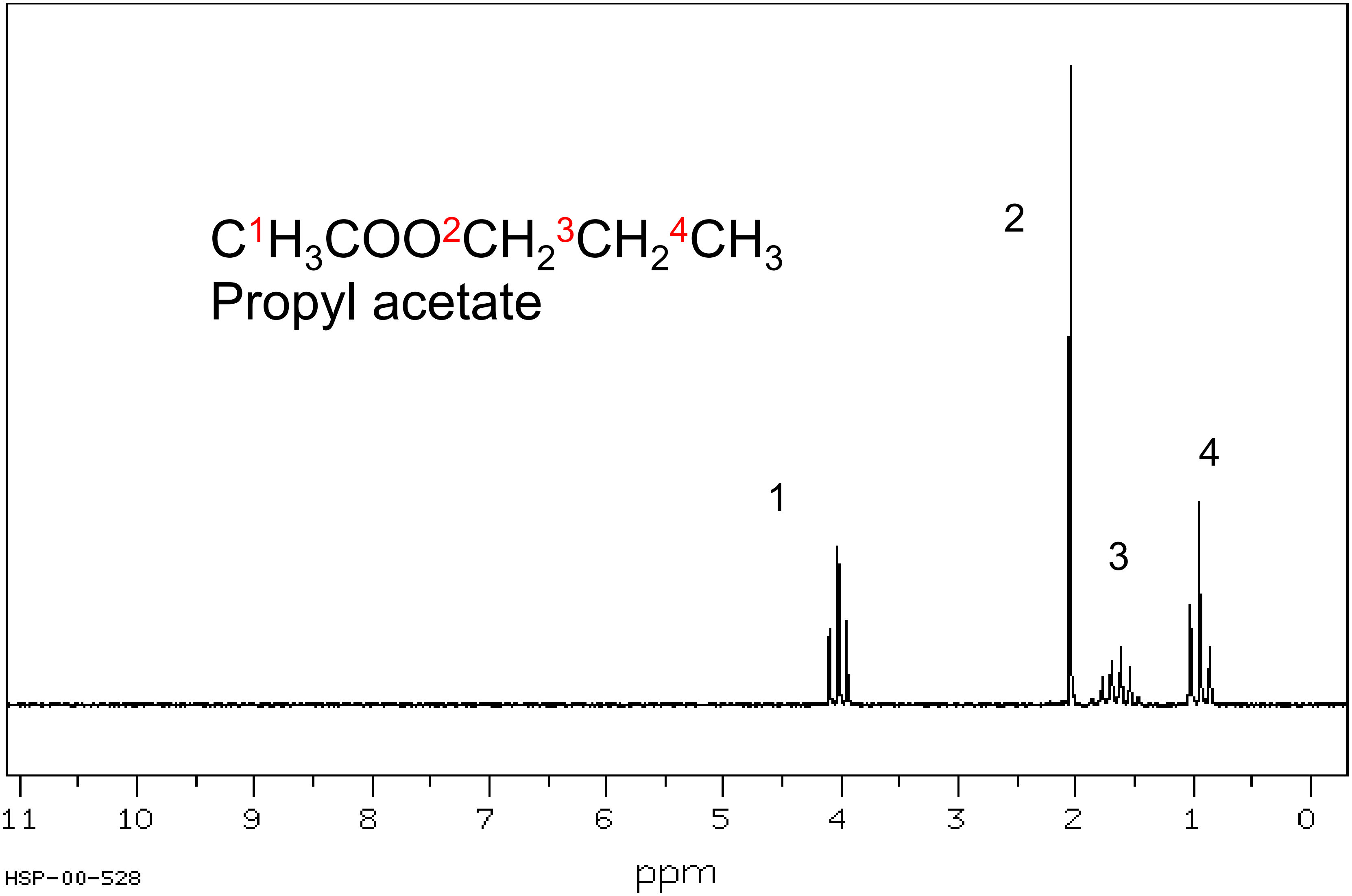
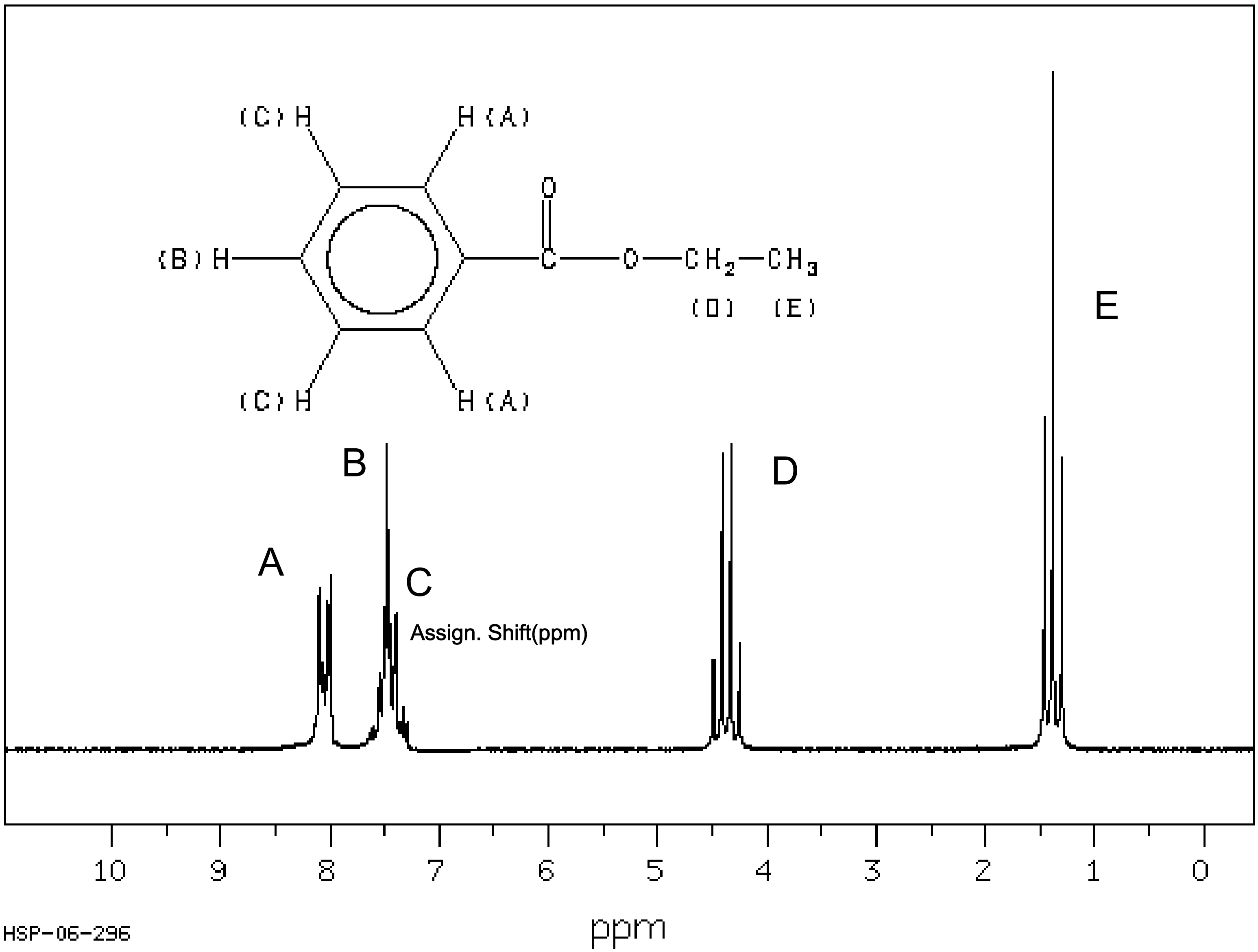


$C^{1}H_3COO^{2}CH_2{}^{3}CH_2{}^{4}CH_3$

Propyl acetate


(C) H
H (A)
O
(B) H
C
O
CH2
CH3
(D)
(E)
(C) H
H (A)
A
B
C
D
E
Assign. Shift(ppm)
10
9
8
7
6
5
4
3
2
1
0
ppm
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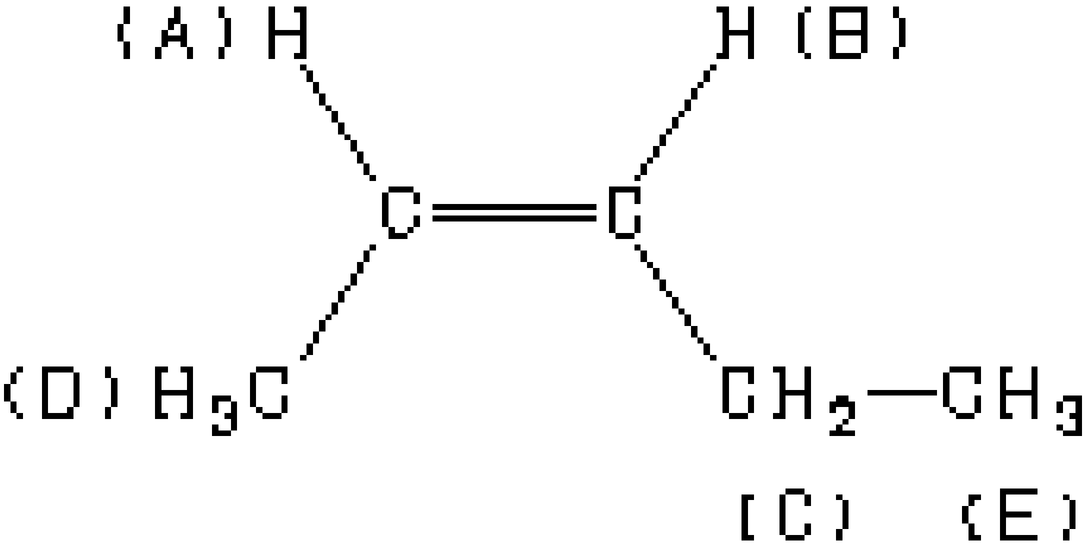
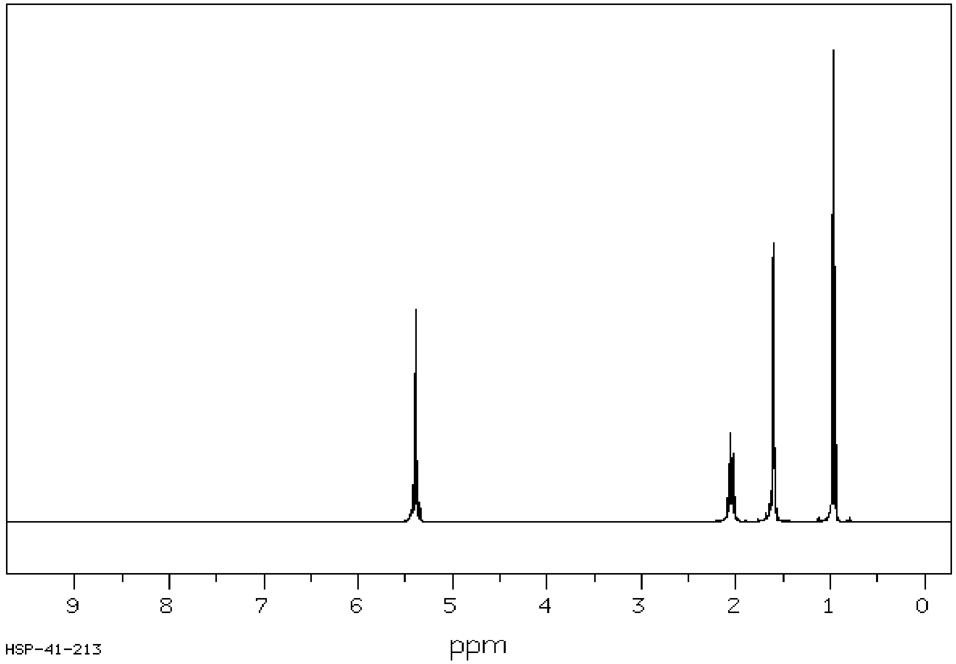
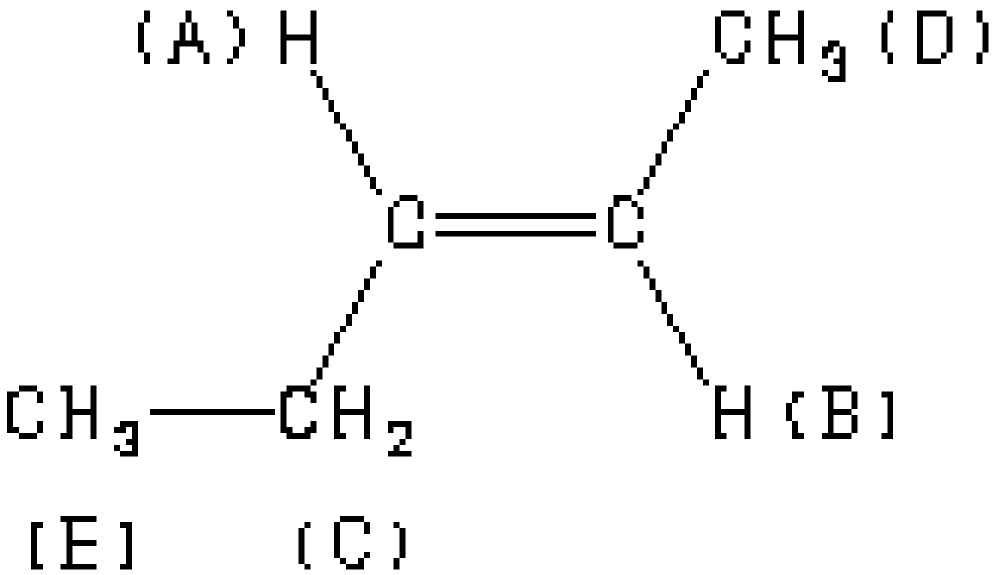
2-cis pentene



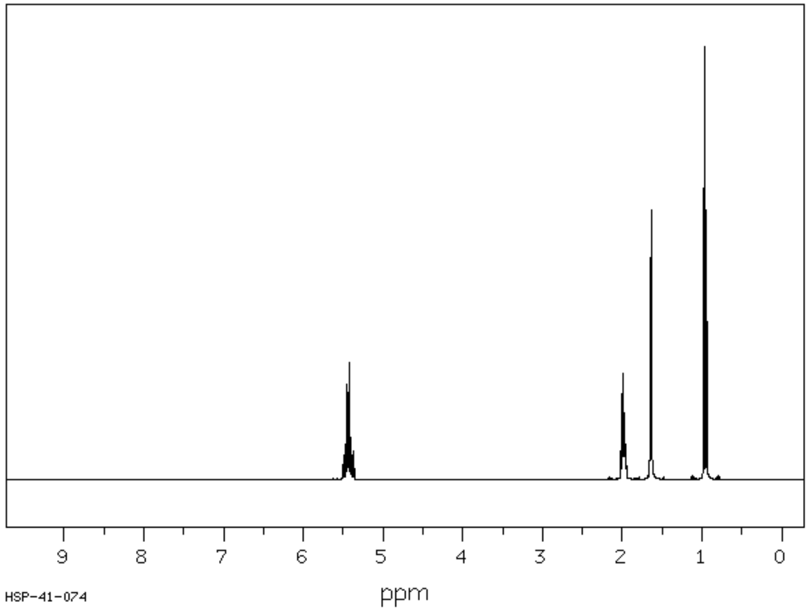
Assign.
Shift(ppm)
A 5.40
B 5.38
C 2.050
D 1.599
E 0.960

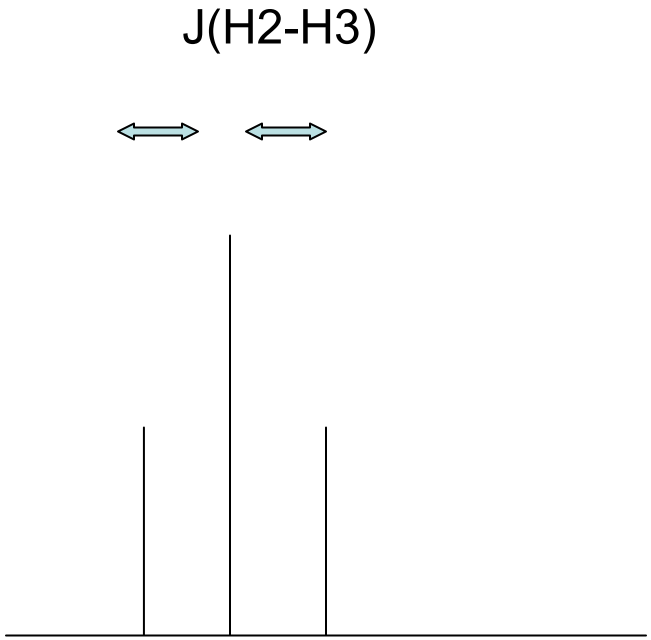


2-trans pentene



Assign.	Shift(ppm)
A	5.45
B	5.41
C	1.985
D	1.637
E	0.958





Bigger magnets provide better spectral resolution. This is especially key For biological applications. There are larger differences between different Precessing nuclei. This spreads the signals out across a larger range of Frequencies.

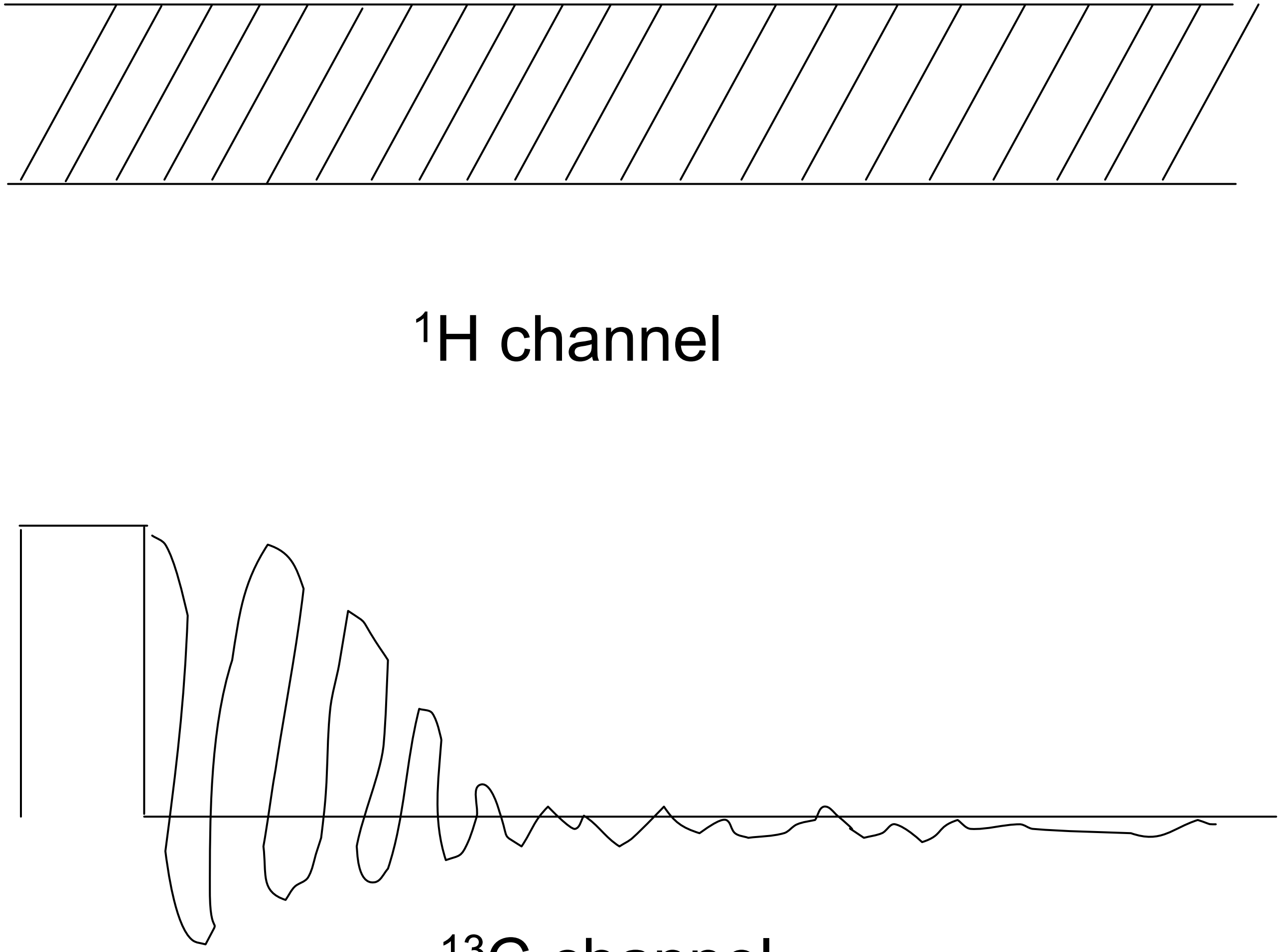
^{13}C NMR

- ^{13}C frequency
- Different tuning folk
- Broadband Decoupling of ^{1}H
- No spin-spin coupling
- NOE effect
- Assignments based on chemical shift
- Wider frequency range

Obtaining a ^{13}C NMR Spectrum

- ^{1}H Broadband decoupling
  - Gives singlet ^{13}C peaks, provided no F, P, or D present in the molecule)
  - Continuous sequence of pulses at the ^{1}H frequency causes a rapid reversal of spin orientation relative to the B_0, causing coupling to ^{13}C to disappear

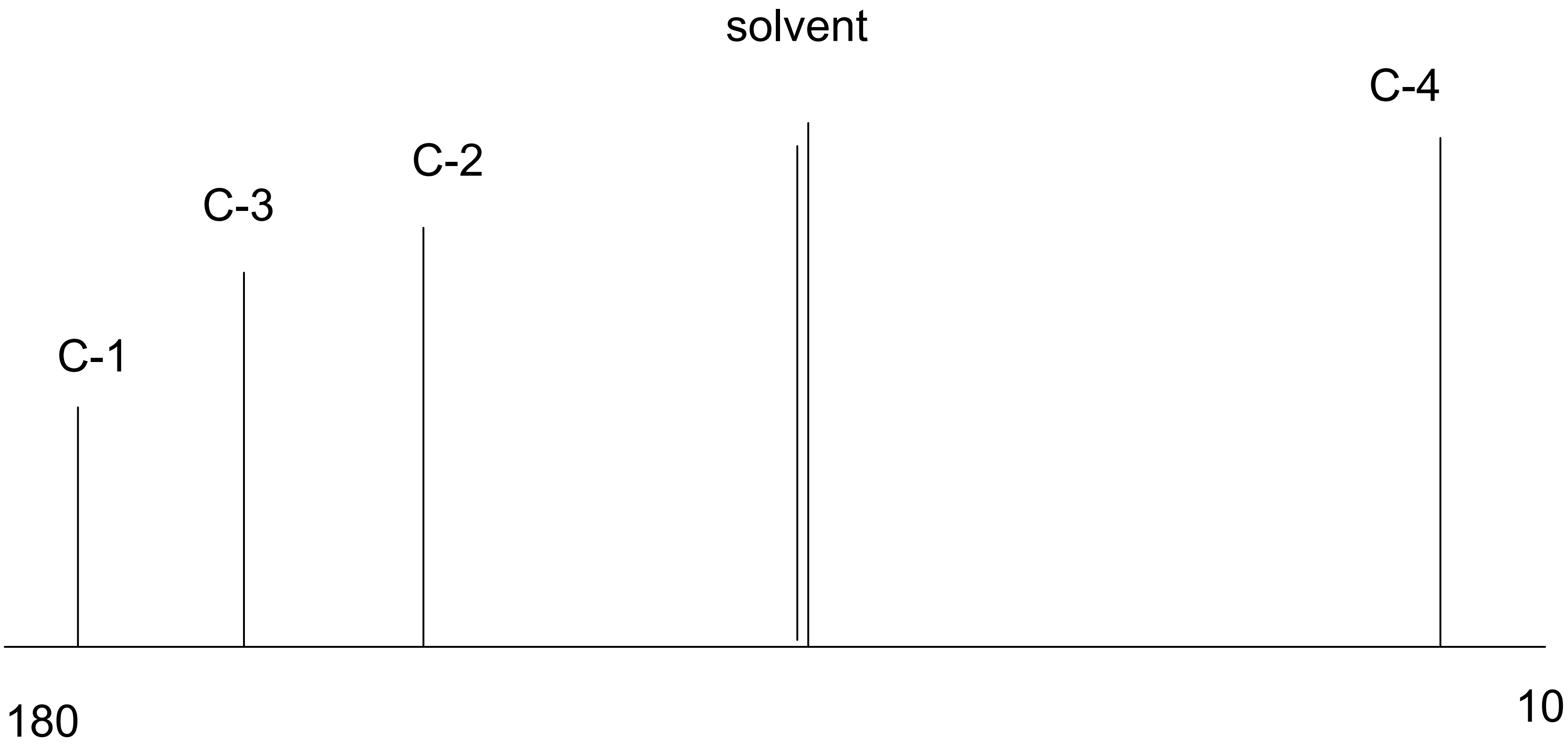
Broadband Decoupling



^{1}H channel

^{13}C channel

$$H_3C^4\text{-}C^3H{=}C^2H\text{-}C^1OOH$$

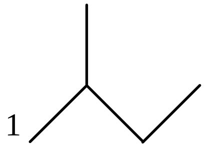


^{13}C Chemical Shifts

- Reference is TMS, sets 0 ppm
- A range of 200 ppm
- Chemical shifts can be predicted
  - Empirical correlations
  - Ex. Alkanes

$$\delta i = -2.3 + 9.1n\alpha + 9.4n\beta - 2.5n\gamma + 0.3n\delta + 0.1n\varepsilon + Sij$$

2-methylbutane



$$\delta i = -2.3 + 9.1*1 + 9.4*2 - 2.5*1 - 1.1 = 22.0\ (22.3)$$

Other notes of ^{13}C NMR

- ^{13}C experiment generally take longer than ^{1}H experiments because many more FIDs need to be acquired and averaged to obtain adequate sensitivity.
- Integrated signals have little meaning (NOE)