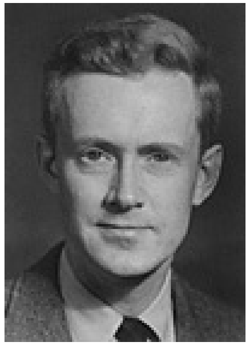
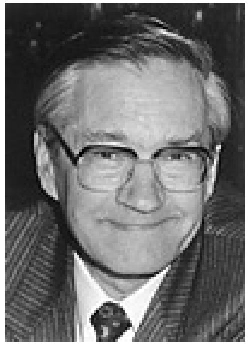
1952


Felix Bloch


Edward Mills Purcell

1991

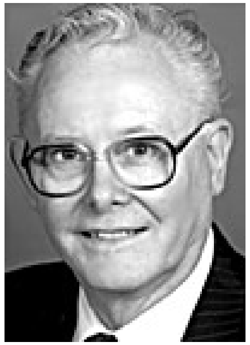

Richard R. Ernst

2002

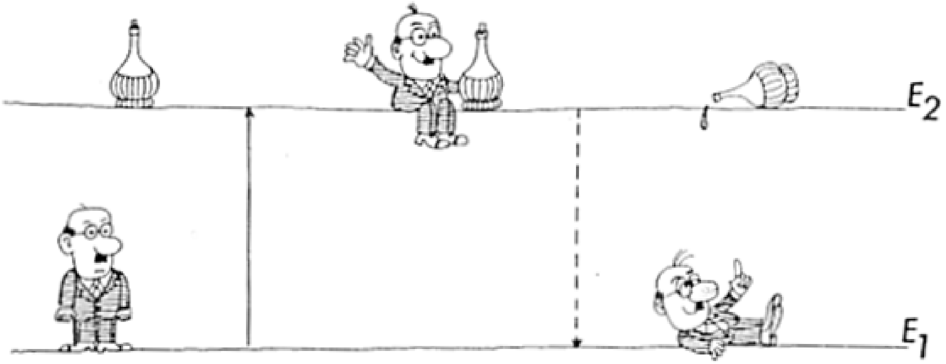

Kurt Wüthrich

2003


Paul C. Lauterbur

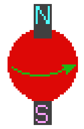

Sir Peter Mansfield

Nuclear Magnetic Resonance

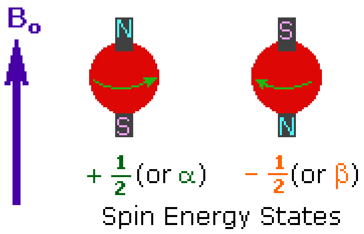


Background

- **1.** A spinning charge generates a magnetic field, The resulting spin-magnet has a magnetic moment (**μ**).



- **2.** In the presence of an external magnetic field (**B$_o$**), two spin states exist, **+1/2** and **-1/2**.

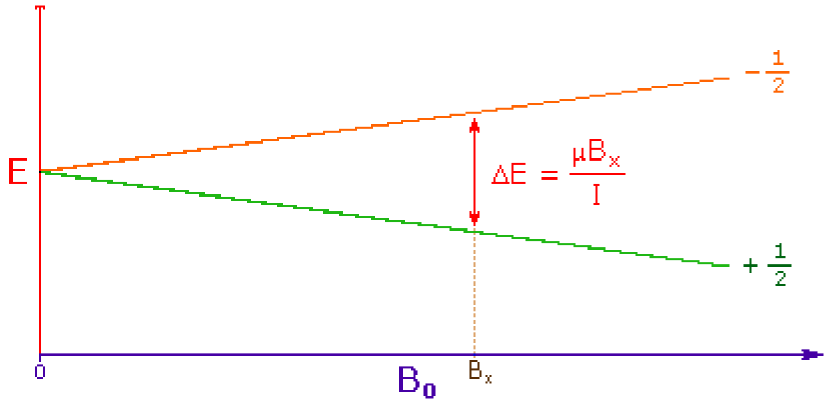


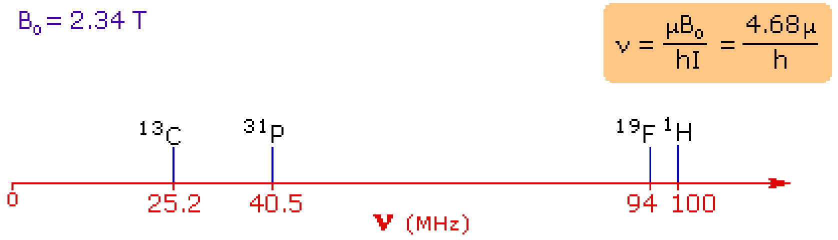
Nuclei	**Unpaired Protons**	**Unpaired Neutrons**	**Net Spin**	**(MHz/T)**
^{1}H	1	0	1/2	42.58
^{2}H	1	1	1	6.54
^{31}P	1	0	1/2	17.25
^{23}Na	1	2	3/2	11.27
^{14}N	1	1	1	3.08
^{13}C	0	1	1/2	10.71
^{19}F	1	0	1/2	40.08

Background

- **3.** The difference in energy between the two spin states is dependent on the external magnetic field strength

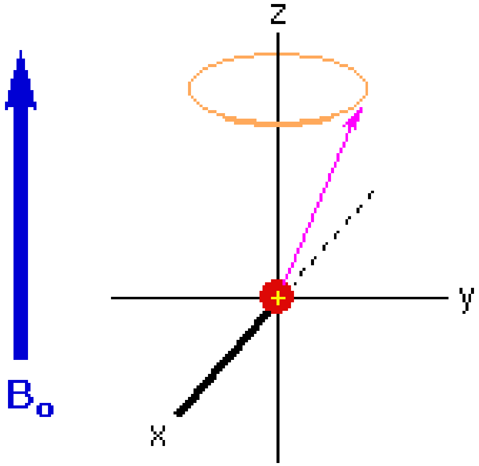
- **4.** For the four common nuclei the magnetic moments are: 1H μ = 2.7927, 19F μ = 2.6273, 31P μ = 1.1305 & 13C μ = 0.7022. The approximate frequencies that correspond to the spin state energy separations for each of these nuclei in an external magnetic field of 2.34 T.





A Model for NMR Spectroscopy

- Just as a spinning mass will precess in a gravitational field (a gyroscope), the magnetic moment **μ** associated with a spinning spherical charge will precess in an external magnetic field.
- The frequency of precession : $\boldsymbol{\omega_o = \gamma B_o}$.
- The frequency $\boldsymbol{\omega_o}$ is called the **Larmor frequency**
- The proportionality constant **γ** is known as the **gyromagnetic ratio** (proportional to the magnetic moment $\boldsymbol{\gamma = 2pm/hI}$).



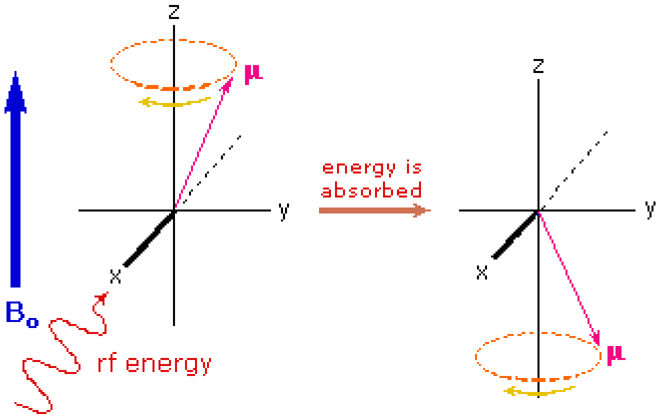
A Spinning Charge in a Magnetic Field

A Spinning Gyroscope in a Gravity Field

A Model for NMR Spectroscopy

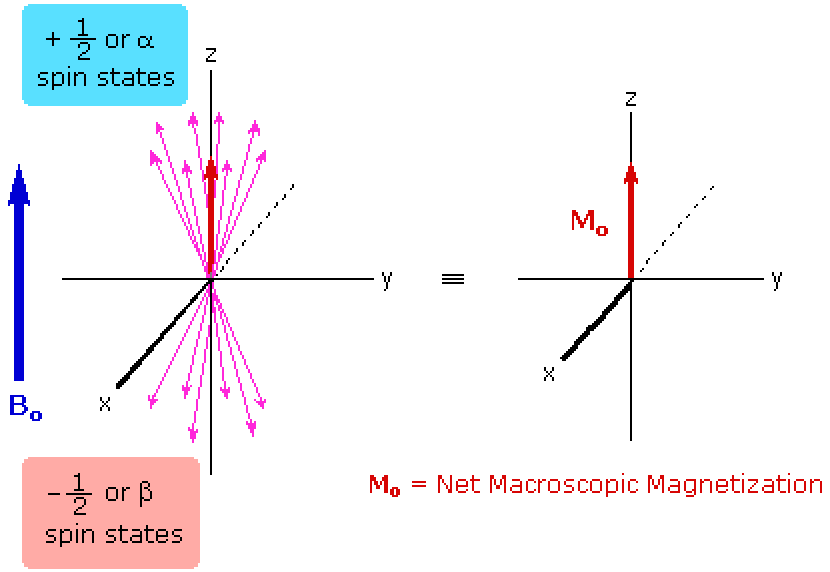
- If rf energy having a frequency matching the Larmor frequency is introduced at a right angle to the external field (e.g. along the x-axis), the precessing nucleus will absorb energy and the magnetic moment will flip

  to its I = -1/2 state.



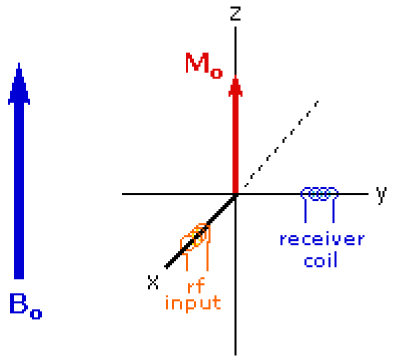
A Model for NMR Spectroscopy

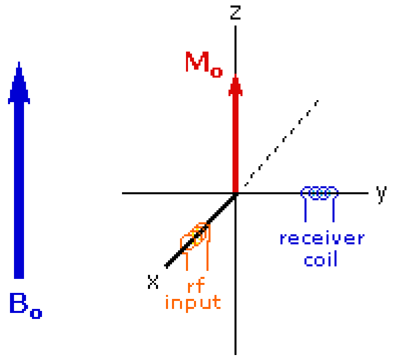
- The energy difference between nuclear spin states is small compared with the average kinetic energy of room temperature samples, and the +1/2 and -1/2 states are nearly equally populated. Indeed, in a field of 2.34 T the excess population of the lower energy state is only six nuclei per million.
- The macroscopic magnetization of a sample containing large numbers of spin 1/2 nuclei at equilibrium in a strong external magnetic field (Bo). A slight excess of +1/2 spin states precess randomly in alignment with the external field and a smaller population of -1/2 spin states precess randomly in an opposite alignment. An overall net magnetization therefore lies along the z-axis.

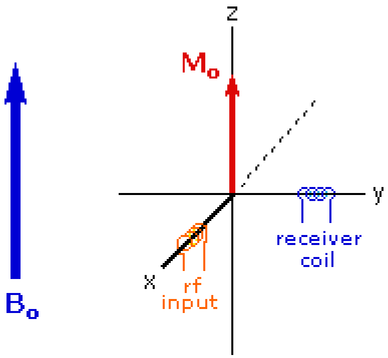


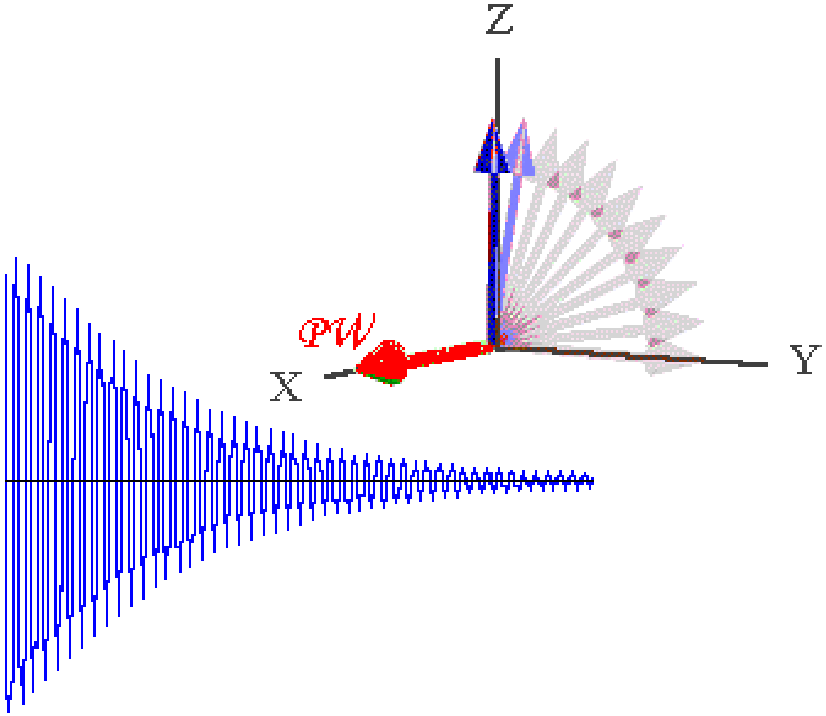
A Model for NMR Spectroscopy

- First, the net magnetization shifts away from the z-axis and toward the y-axis. This occurs because some of the +1/2 nuclei are excited to the -1/2 state, generating a significant y component to the net magnetization (**M**).
- After irradiation the nuclear spins return to equilibrium in a process called **relaxation**. As the xy coherence disappears and the population of the +1/2 state increases, energy is released and detected by the receiver. The net magnetization spirals back, and eventually the equilibrium state is reestablished

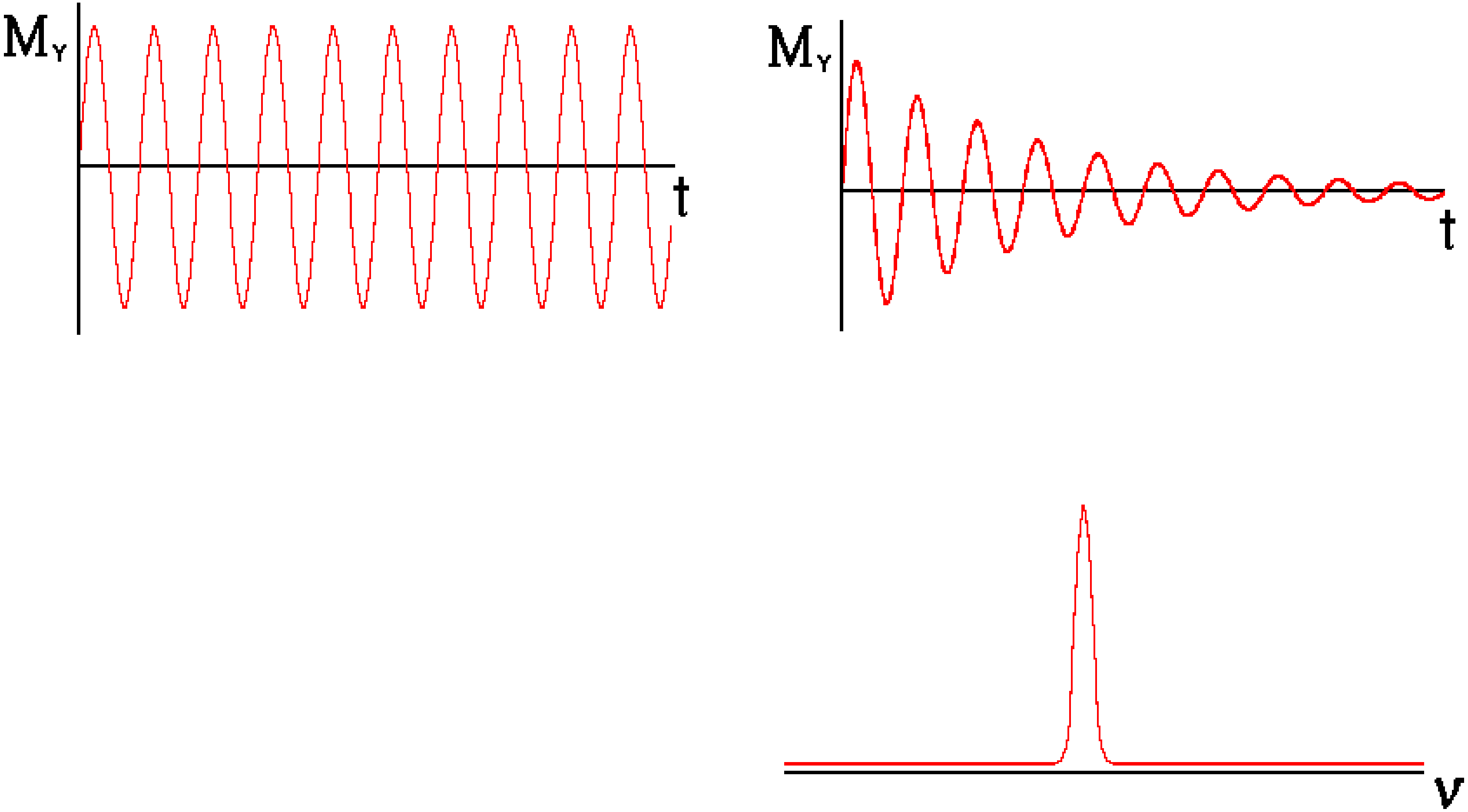




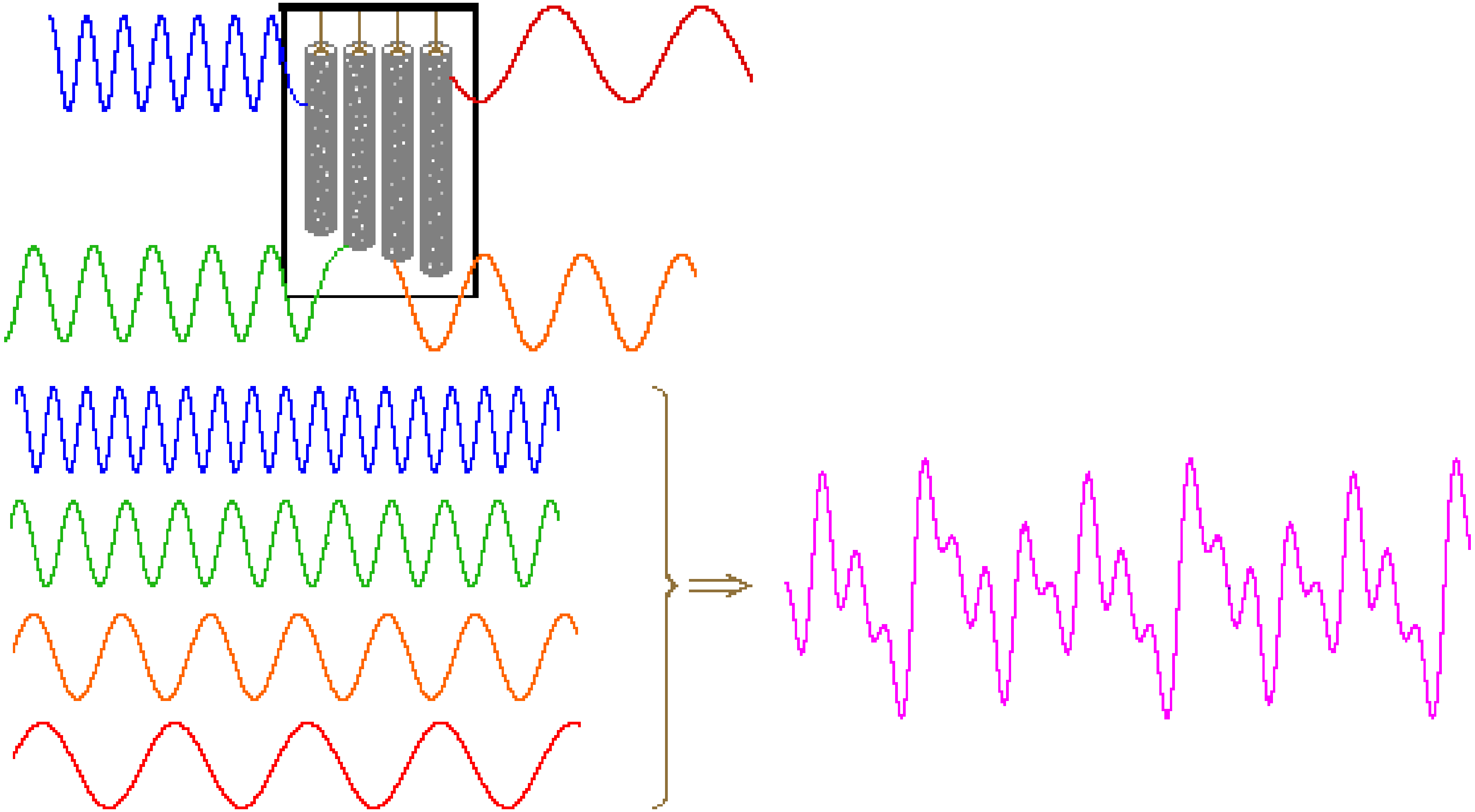

B_o
z
M_o
y
receiver coil
x
rf input


Z
PW
X
Y

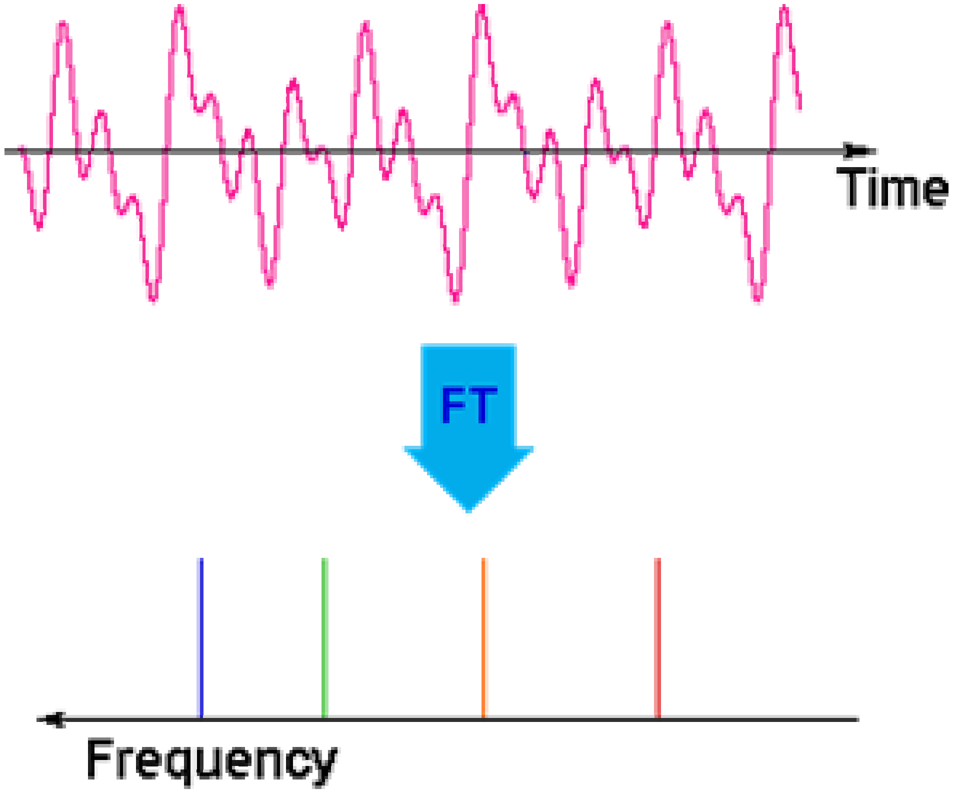
Fourier Transform



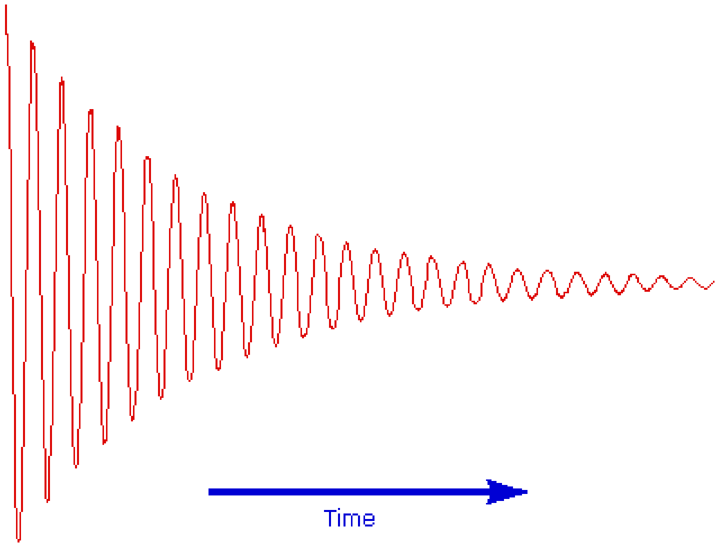
Pulsed Fourier Transform Spectroscopy

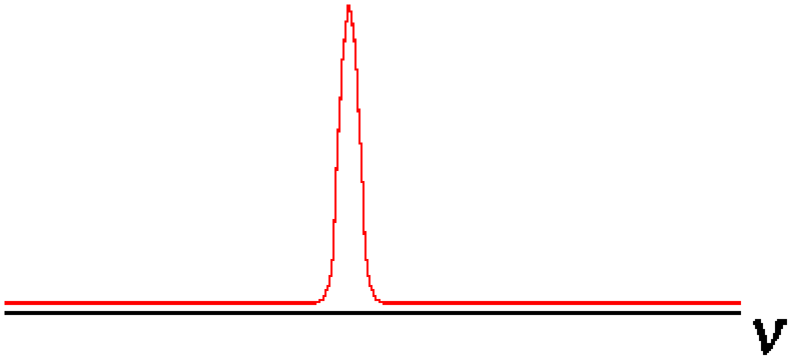


Pulsed Fourier Transform Spectroscopy

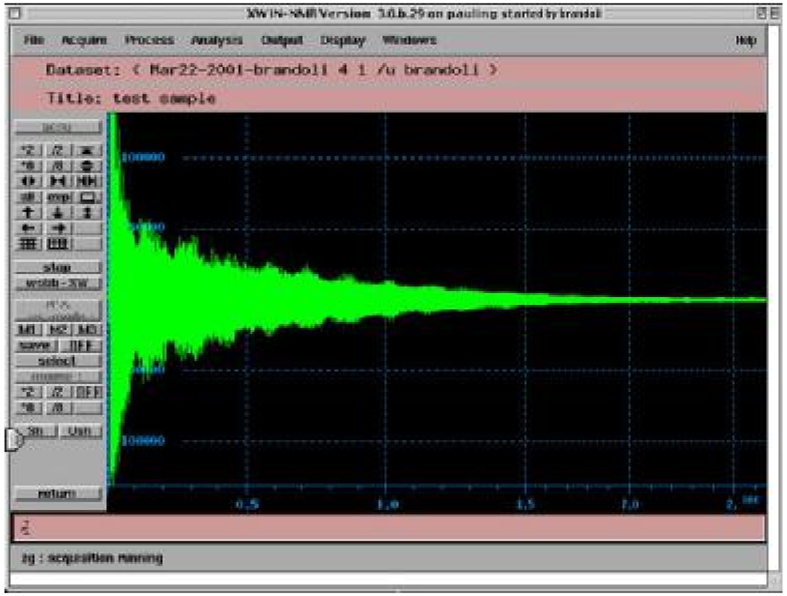


Pulsed Fourier Transform Spectroscopy





Pulsed Fourier Transform Spectroscopy



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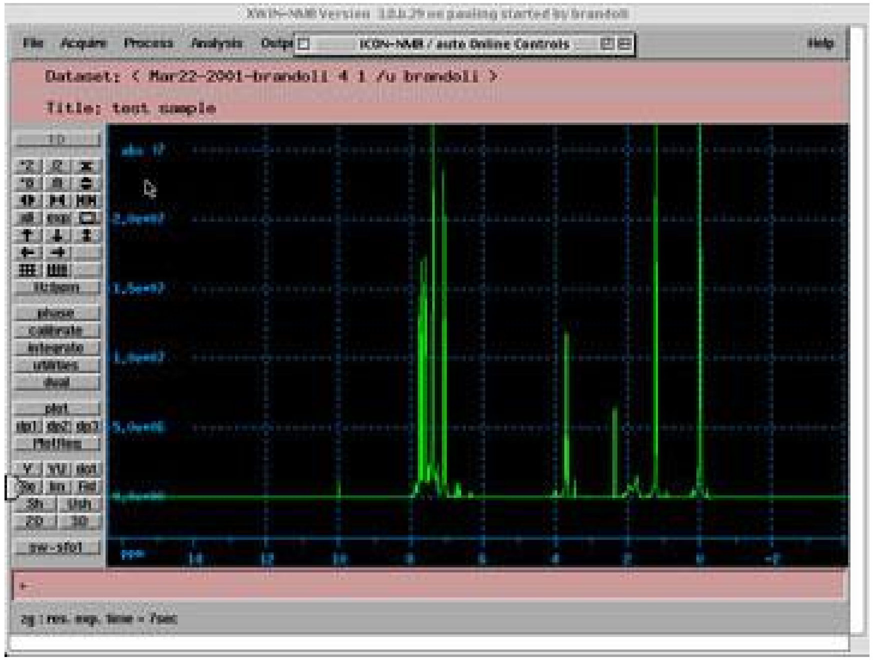
Title: test sample
zg : acquisition running
```



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Title: test sample
zg : res. exp. time = 7sec
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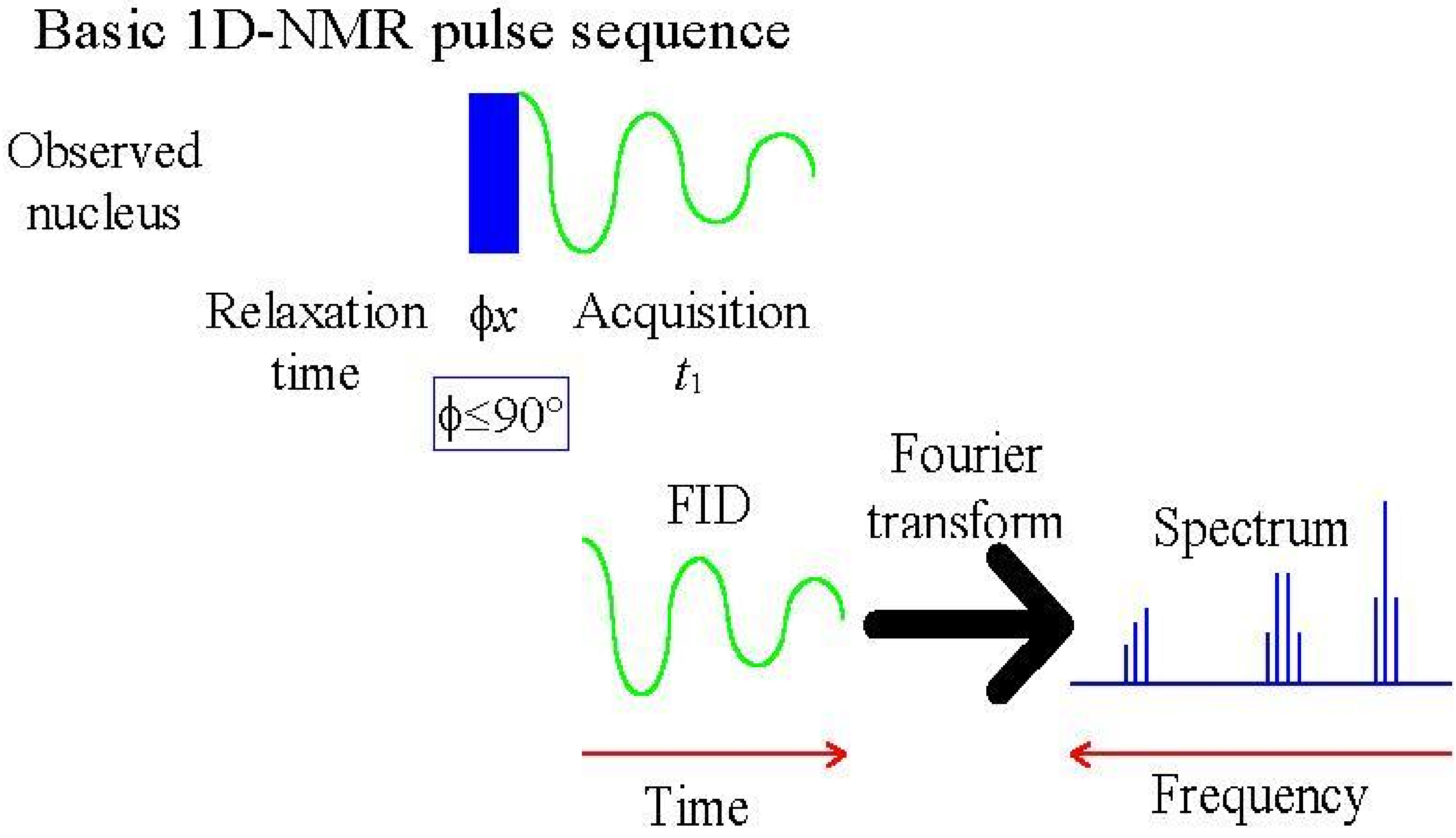
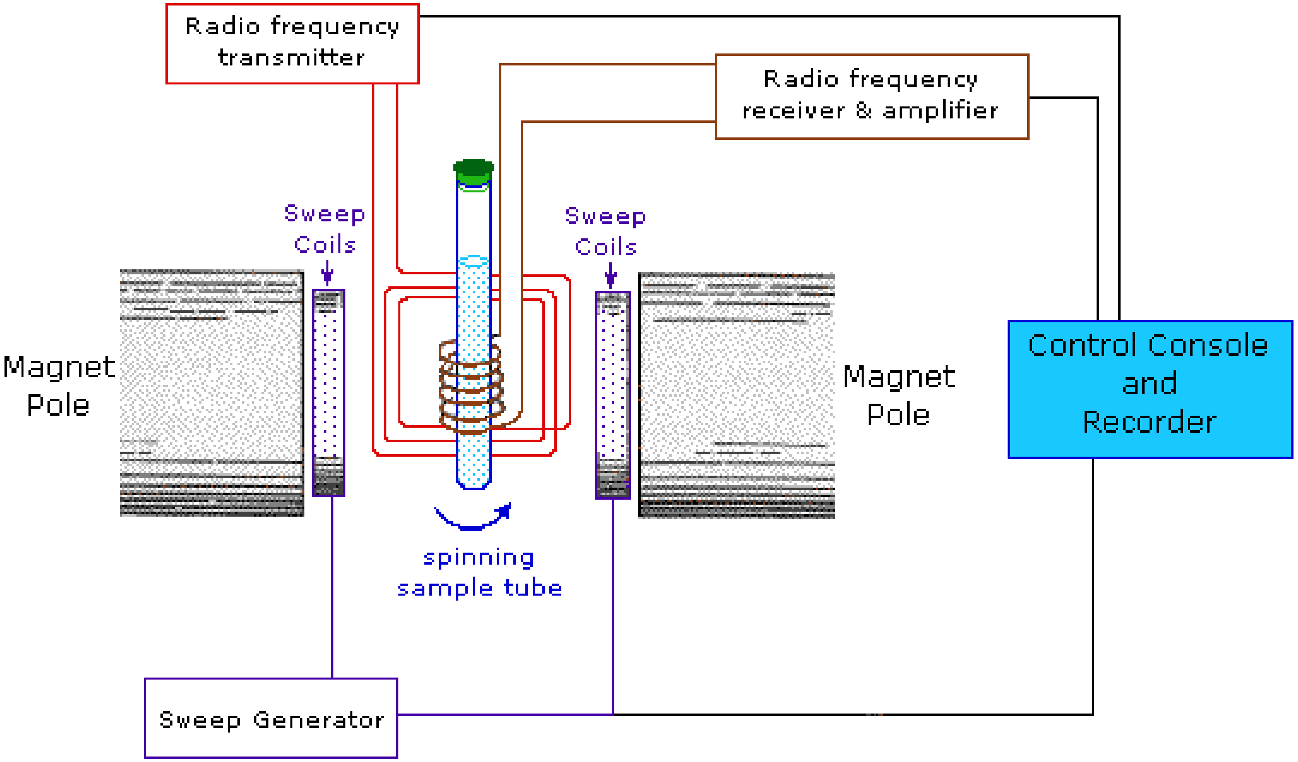
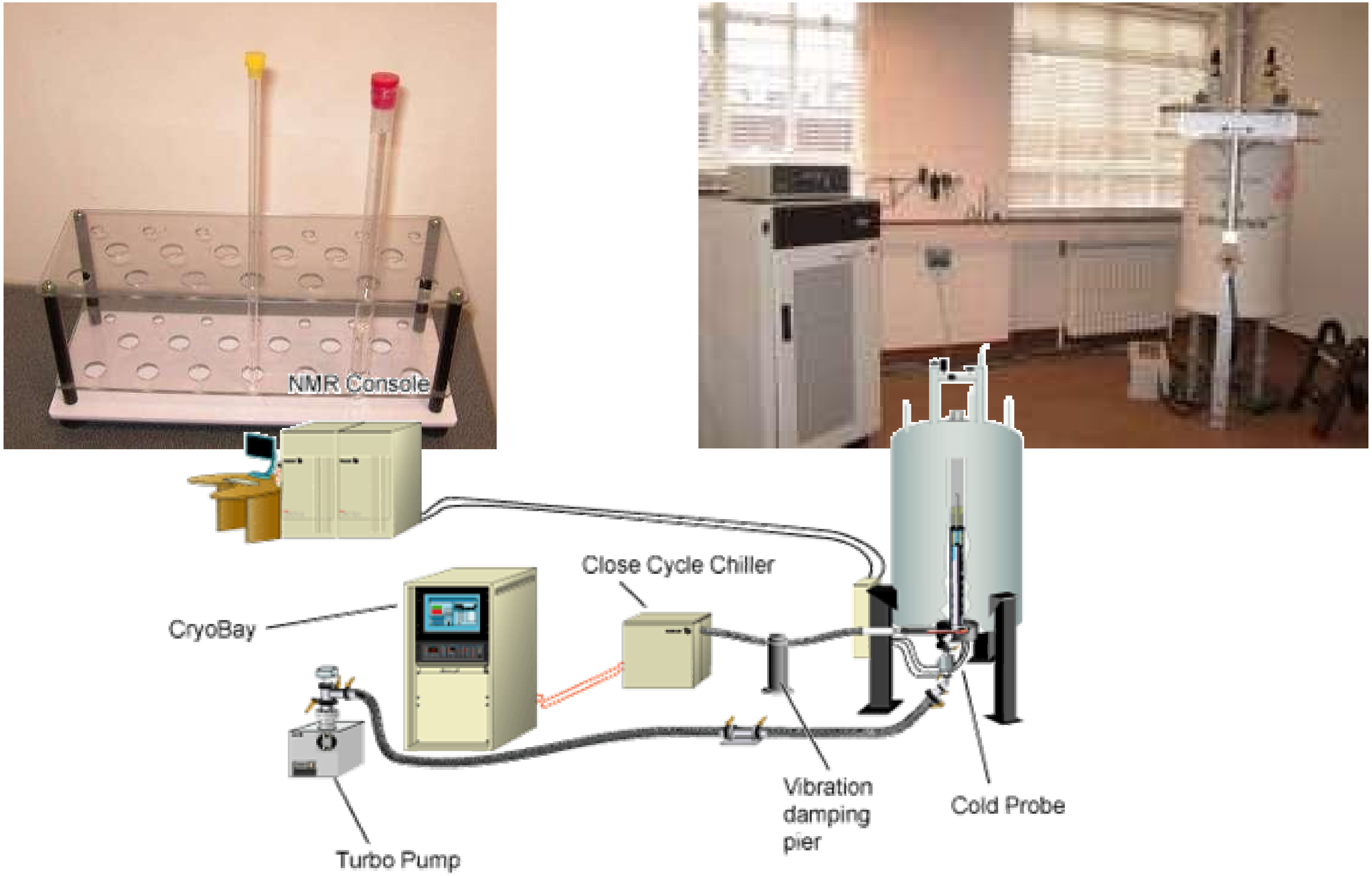
Simple 1D Spectrum



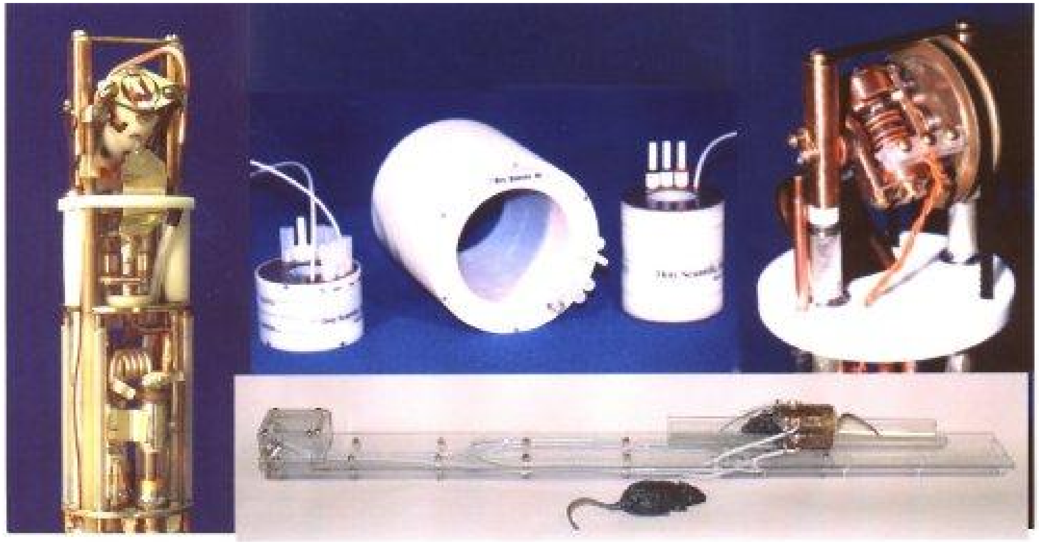
NMR spectrometer



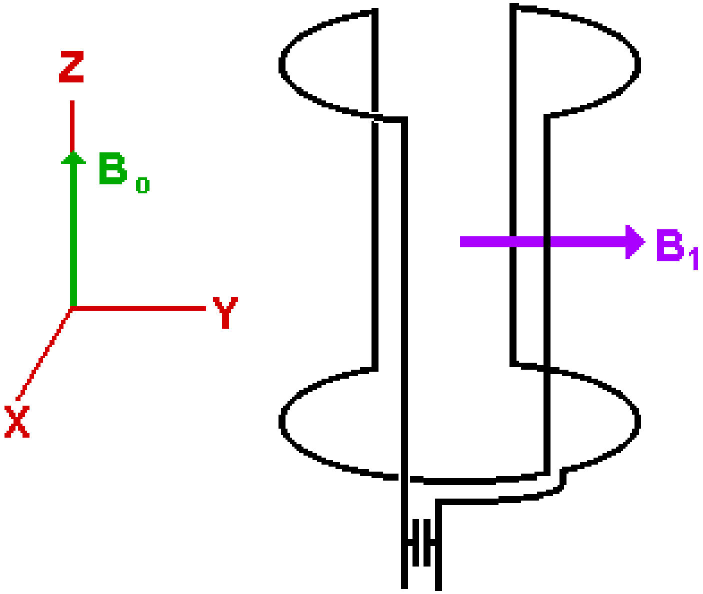
NMR Facility



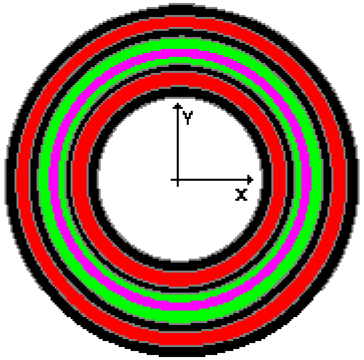
NMR Probes



Saddle Coil



NMR Magnet



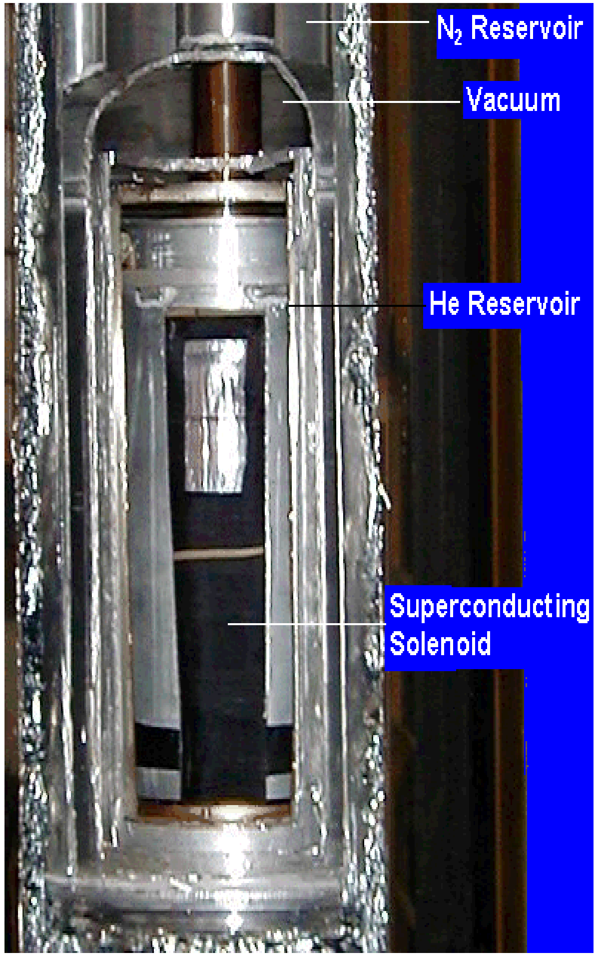
⬛ **Vacuum**

🟩 **Liquid Helium**

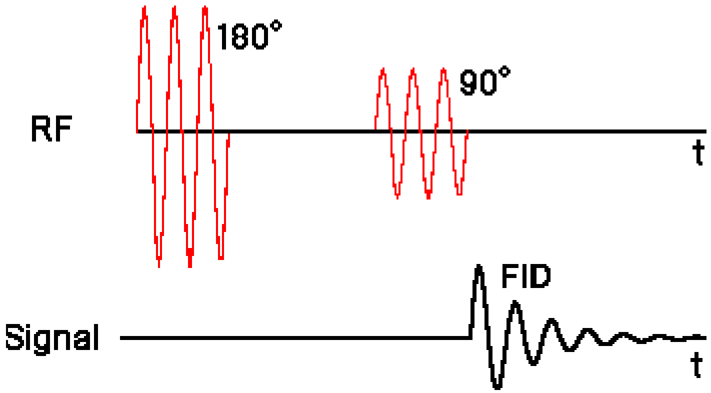
🟥 **Liquid Nitrogen**

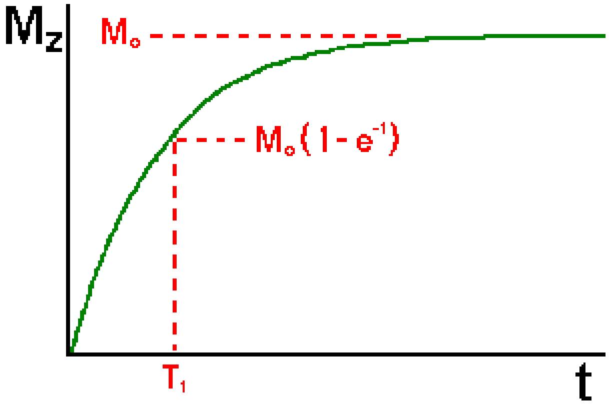
▩ **Container & Support**

🟪 **Superconducting Coil**

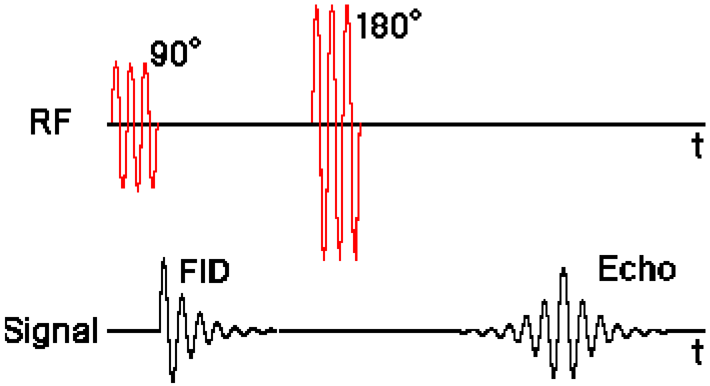


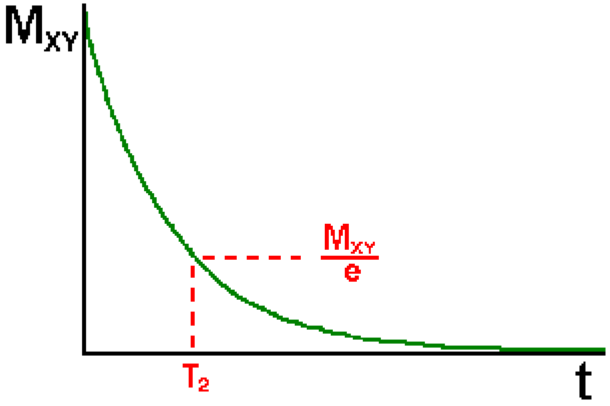
Relaxation





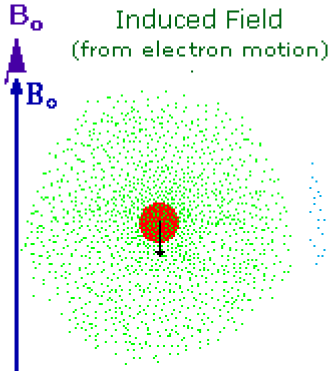
Relaxation



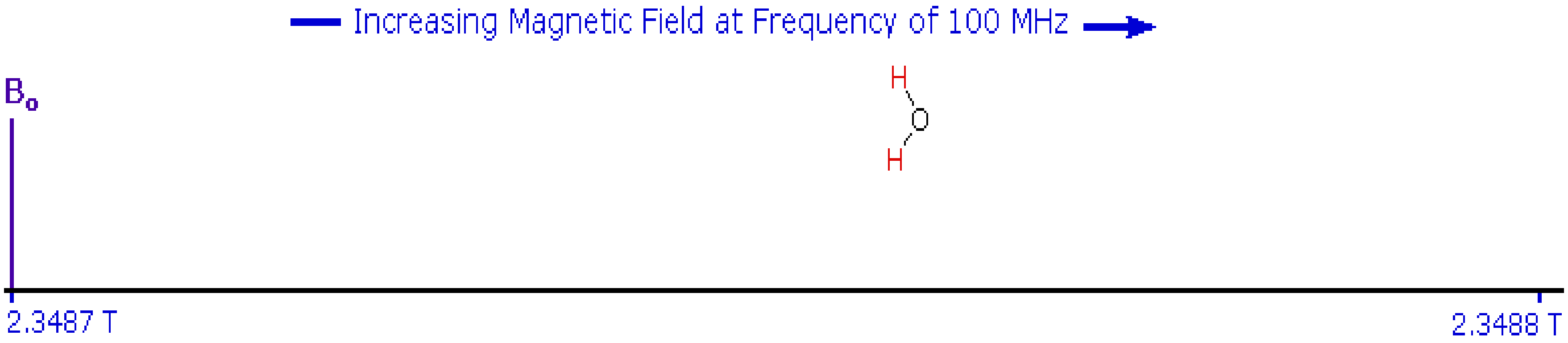


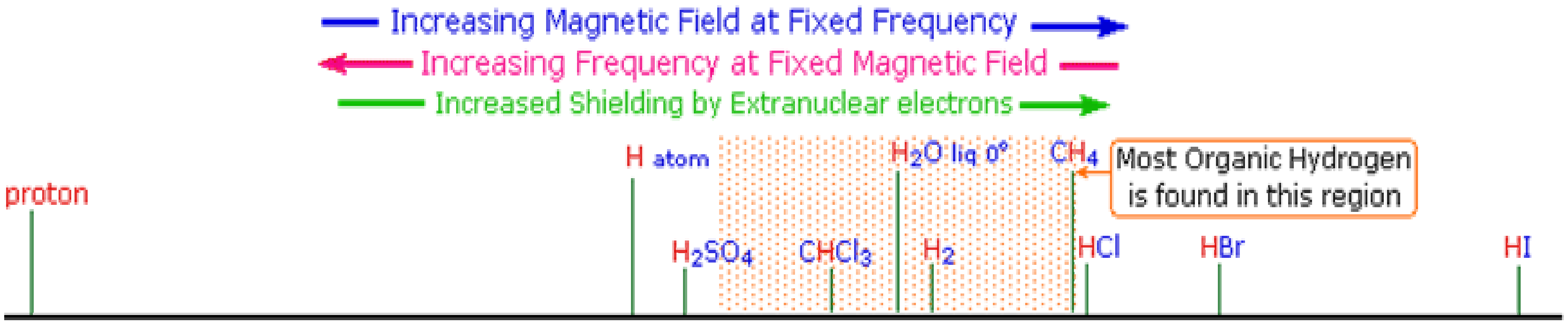
Chemical Shift

- **Why should the proton nuclei in different compounds behave differently in the nmr experiment?**
  - – Since electrons are charged particles, they move in response to the external magnetic field (Bo) so as to generate a secondary field that opposes the much stronger applied field. This secondary field **shields** the nucleus from the applied field, so Bo must be increased in order to achieve resonance (absorption of rf energy).

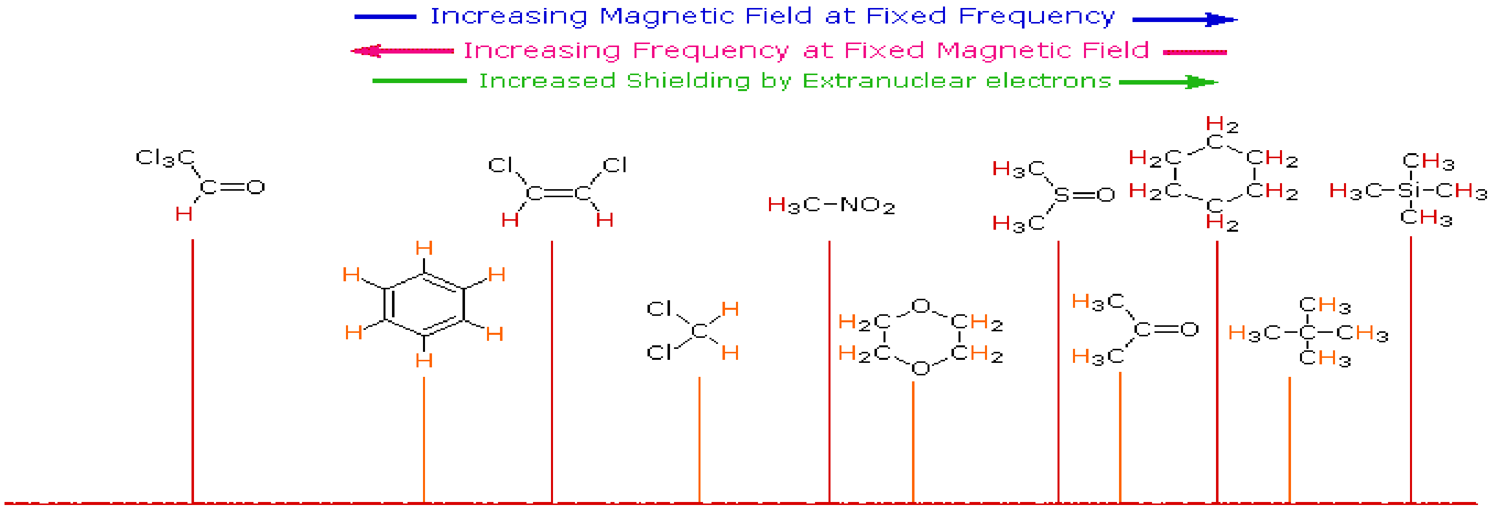


Chemical Shift





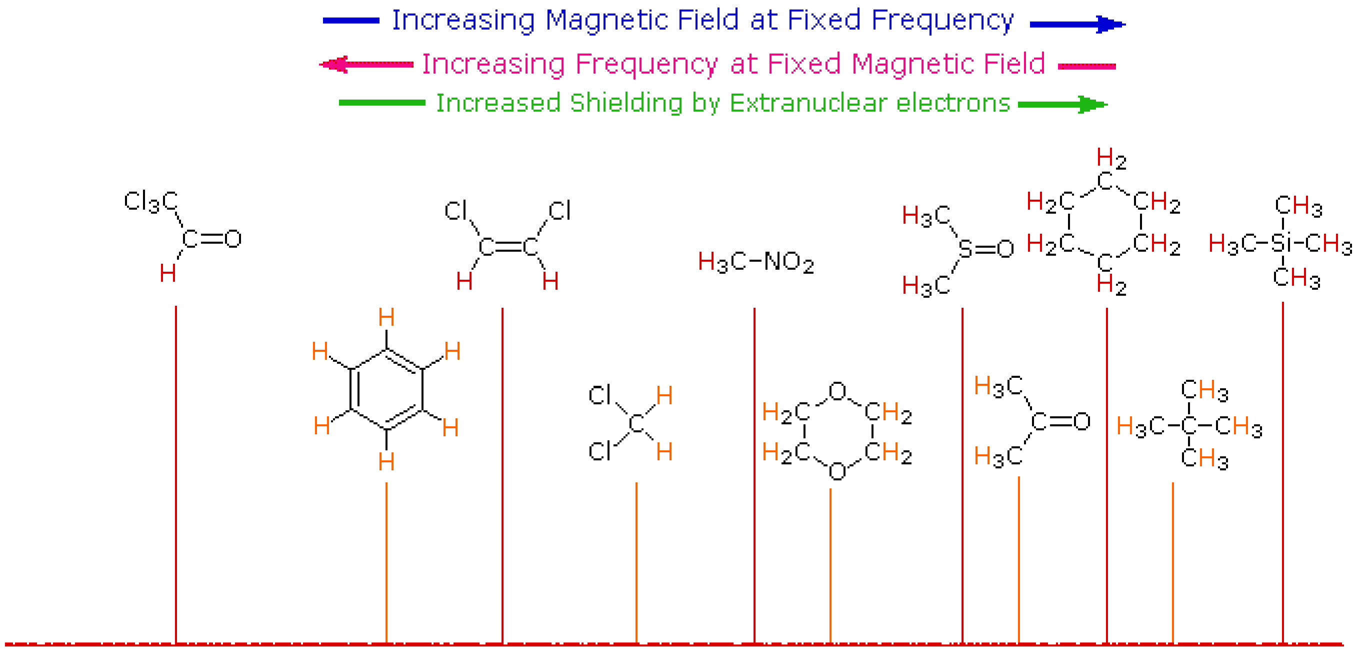
Chemical Shift



^{1}H NMR Resonance Signals for some Different Compounds

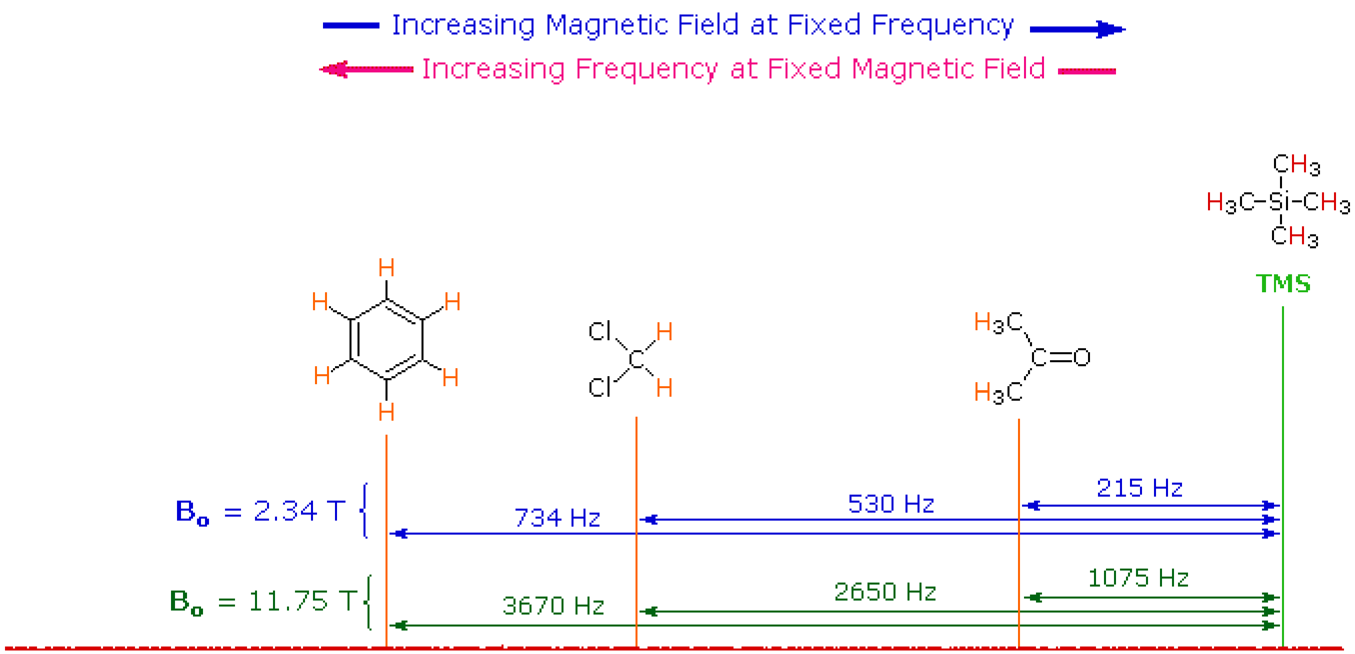
- the location of different nmr resonance signals is dependent on both the external magnetic field strength and the rf frequency
- an alternative method for characterising and specifying the location of nmr signals is needed
- One method of solving this problem is to report the location of an nmr signal in a spectrum relative to a reference signal from a standard compound added to the sample

Chemical Shift



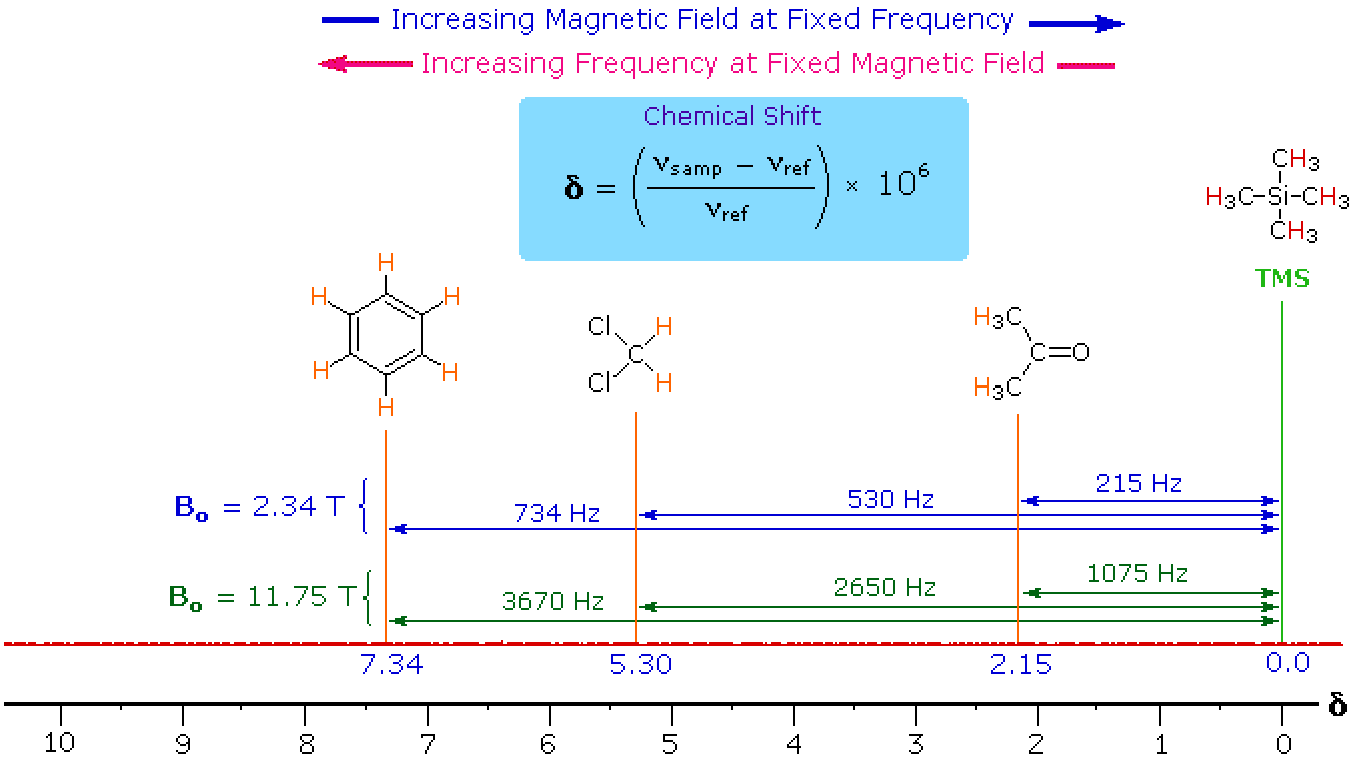
^{1}H NMR Resonance Signals for some Different Compounds

Chemical Shift

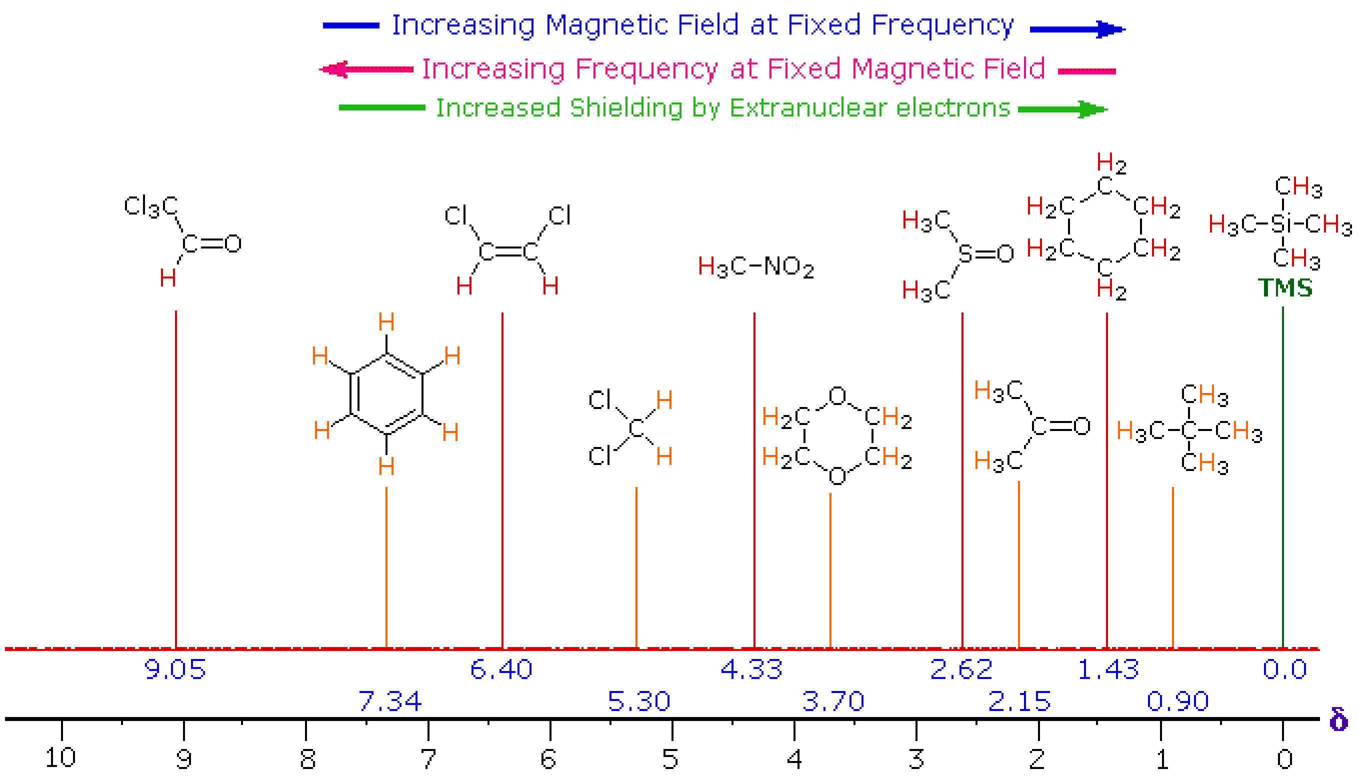


The Separation of Resonance Signals (in Hz) Increases with Increasing Field Strength

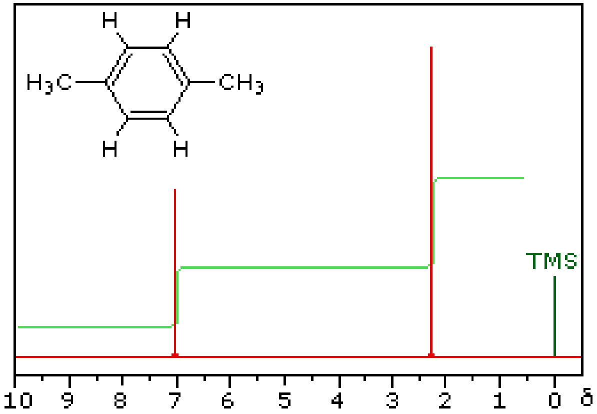
Chemical Shift

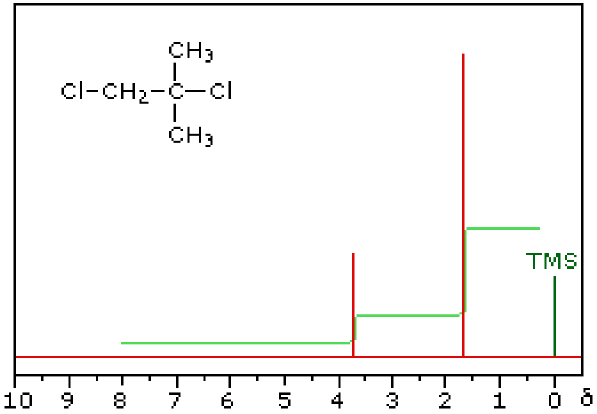


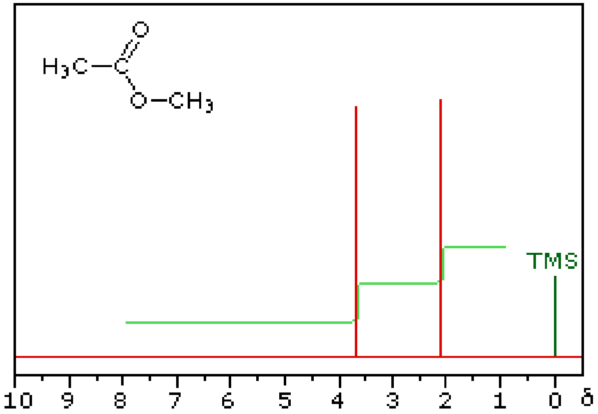
Chemical Shift



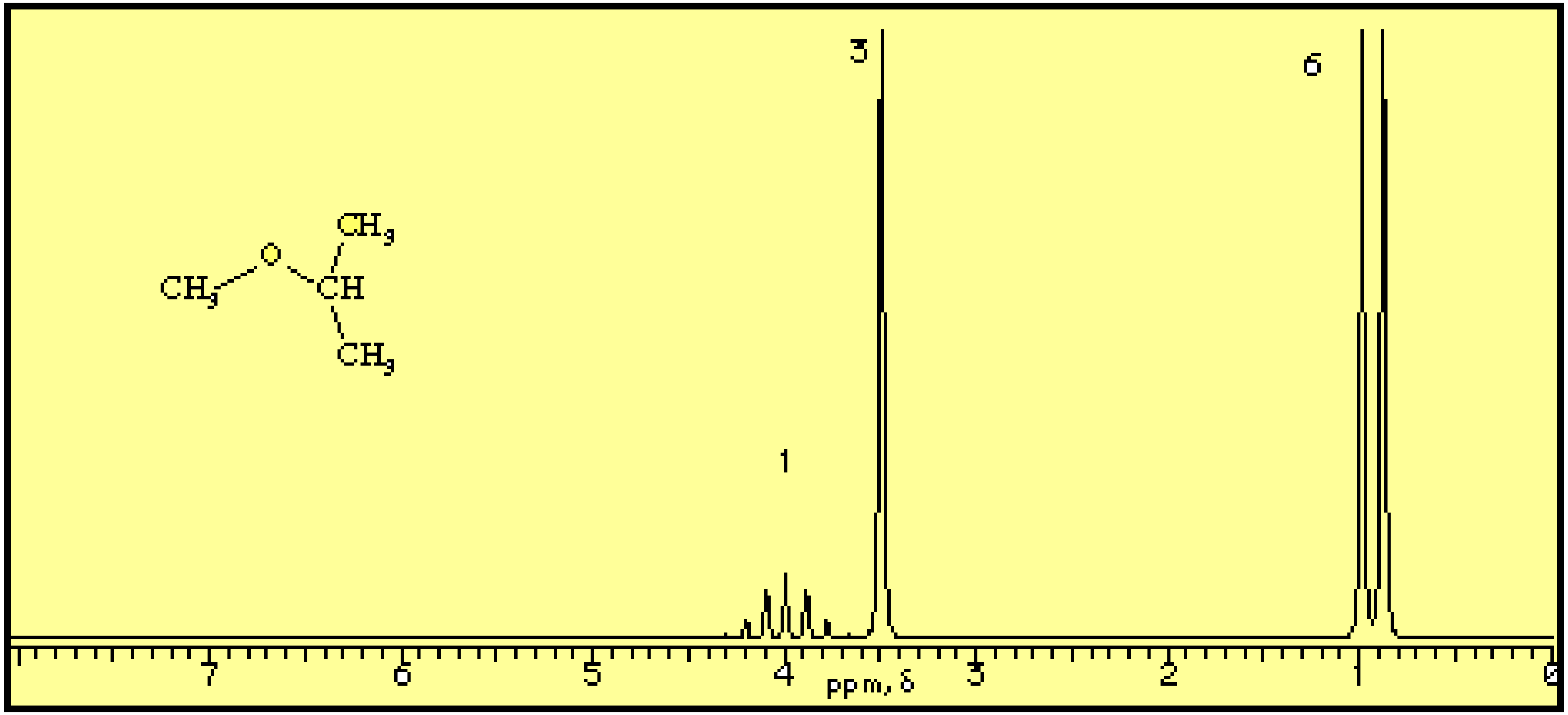
Integration



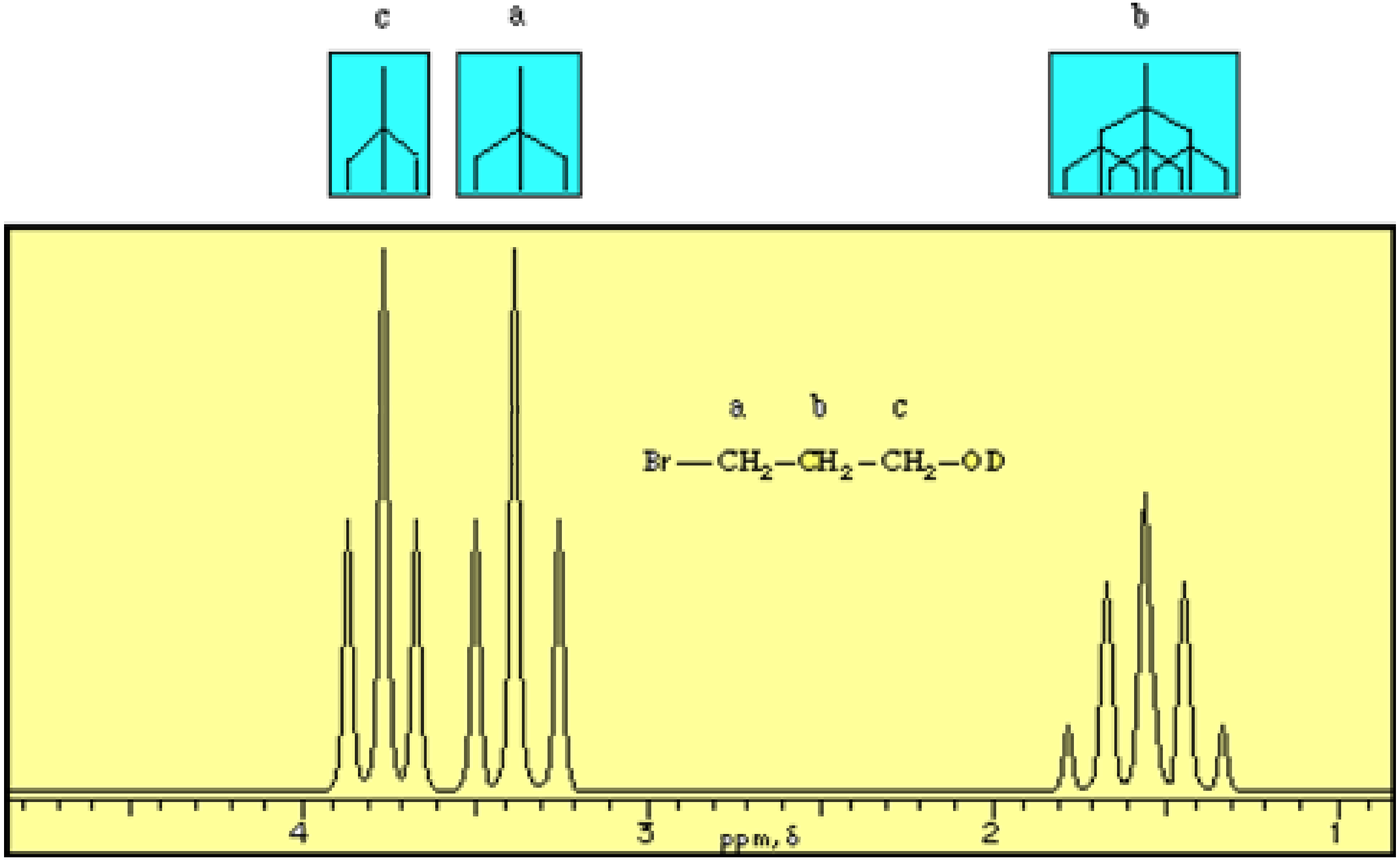




Spin-Coupling/Splitting



Spin-Coupling/Splitting



Spin-Coupling/Splitting

Multiplicity **Relative Line Intensity**

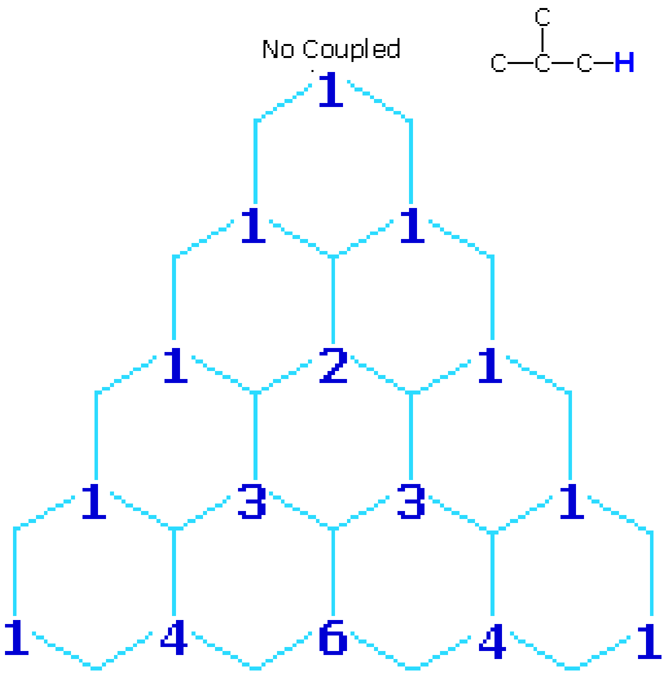
singlet

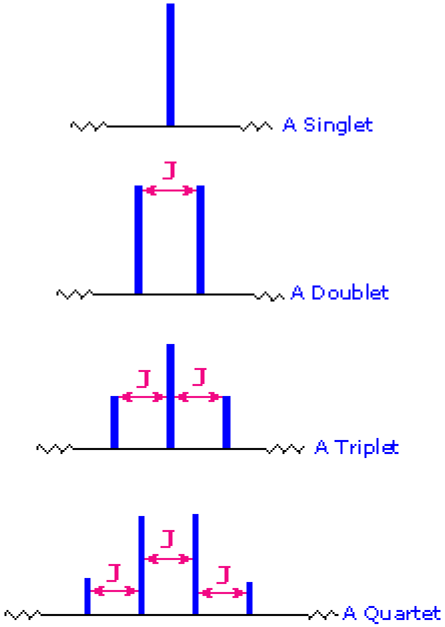
doublet

triplet

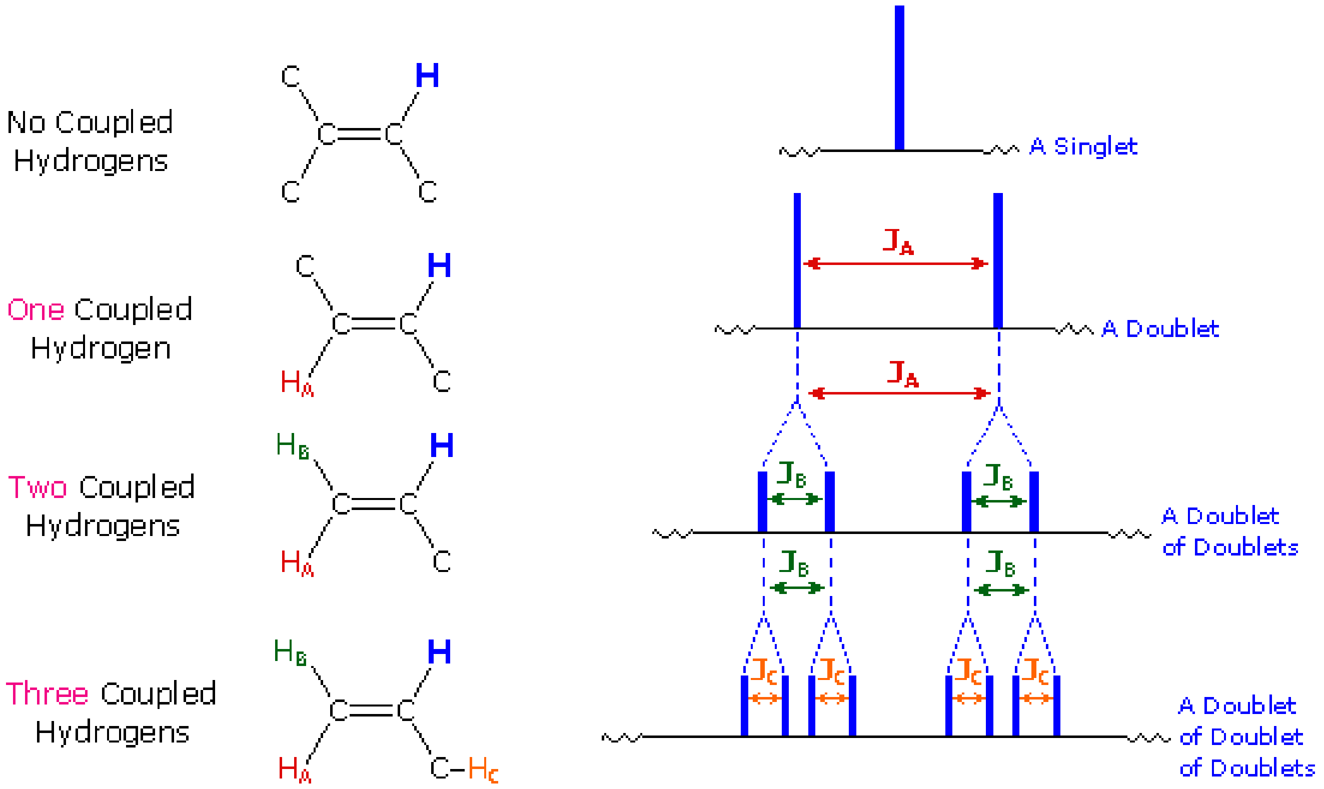
quartet

quintet



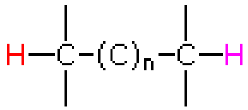
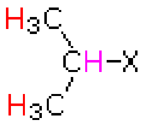
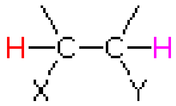
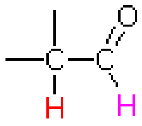
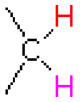


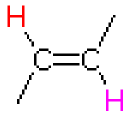
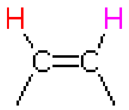
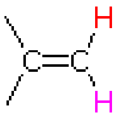
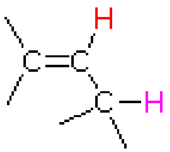
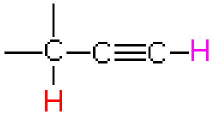
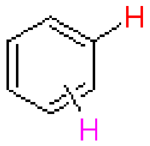
Spin-Coupling/Splitting



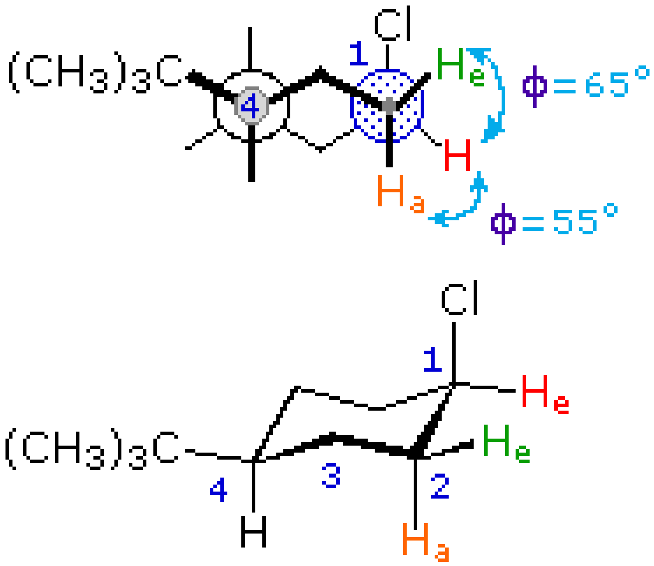
Spin-Coupling/Splitting

Magnitude of Some Typical Coupling Constants

Structural Type	J (Hz)
	0 (unless in a rigid ideal orientation)
H_3C-CH_2-X	6 to 8
	5 to 7
	2 to 12 (depends on dihedral angle and the nature of X and Y)
	0.5 to 3
	12 to 15 (must be diastereotopic)

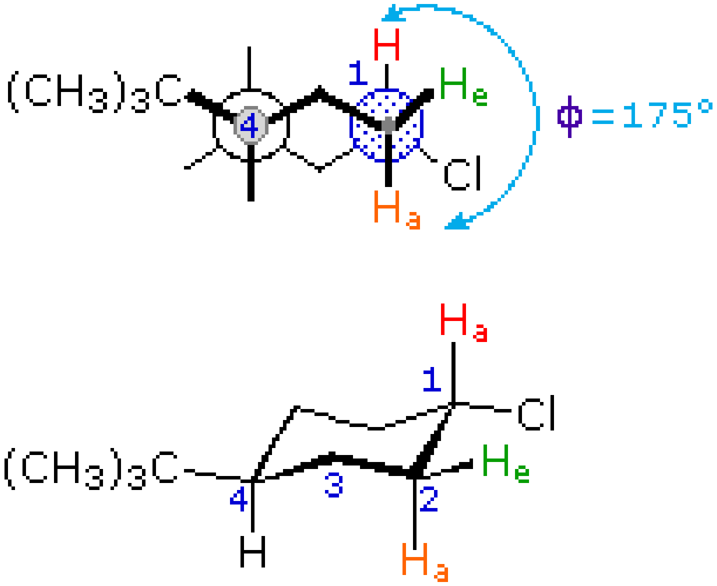
Structural Type	J (Hz)
	12 to 18
	7 to 12
	0.5 to 3
	3 to 11 (depends on dihedral angle)
	2 to 3
	o 6 to 9 m 1 to 3 p 0 to 1

Spin-Coupling/Splitting



J_{ee} = 3.5 Hz J_{ea} = 4.0 Hz

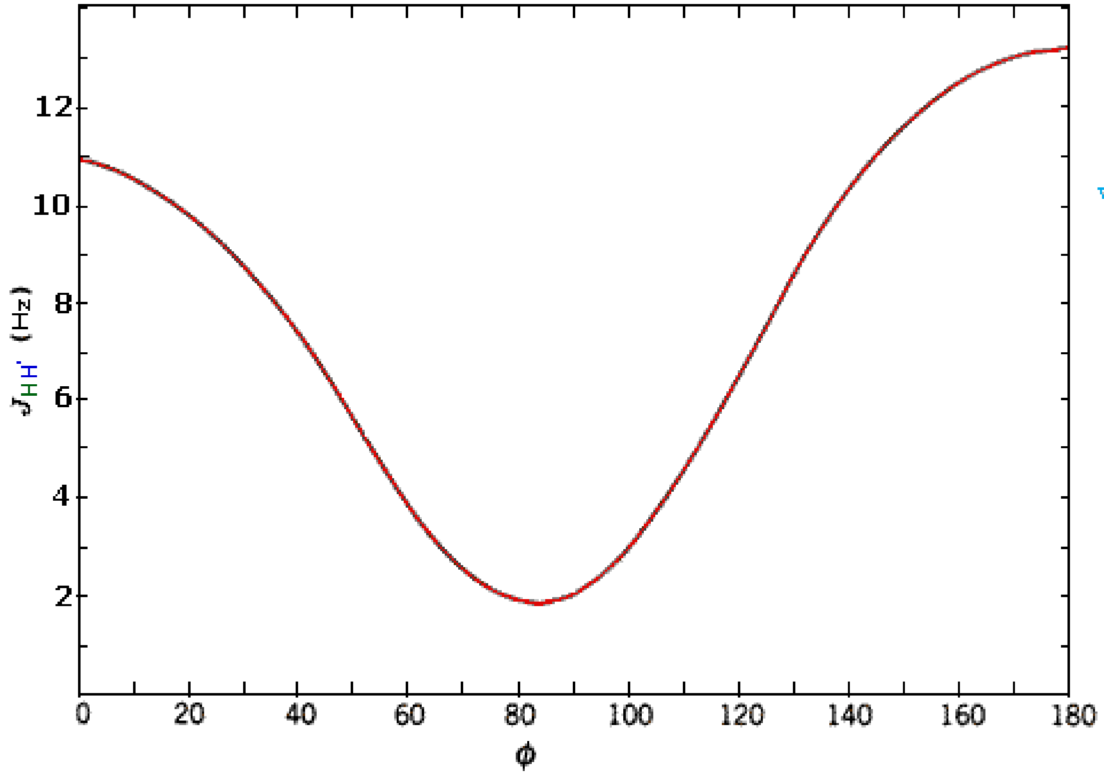
cis-4-tert-butyl-1-chlorocyclohexane



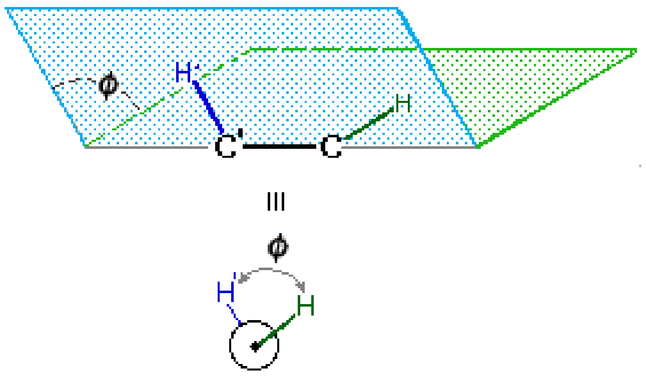
J_{aa} = 12.0 Hz

trans-4-tert-butyl-1-chlorocyclohexane

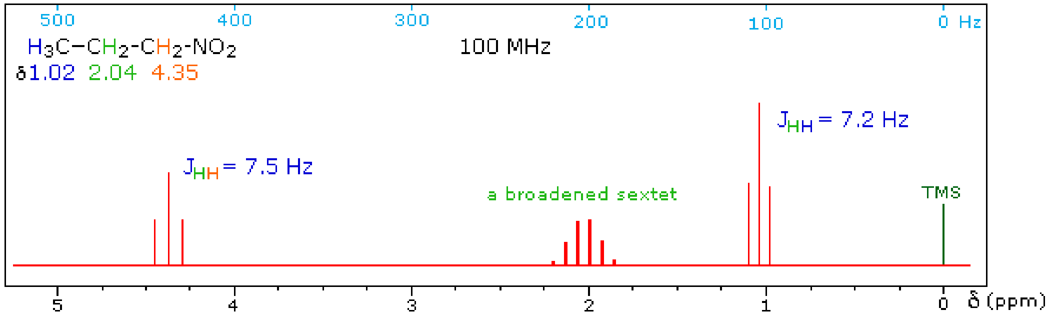
Spin-Coupling/Splitting

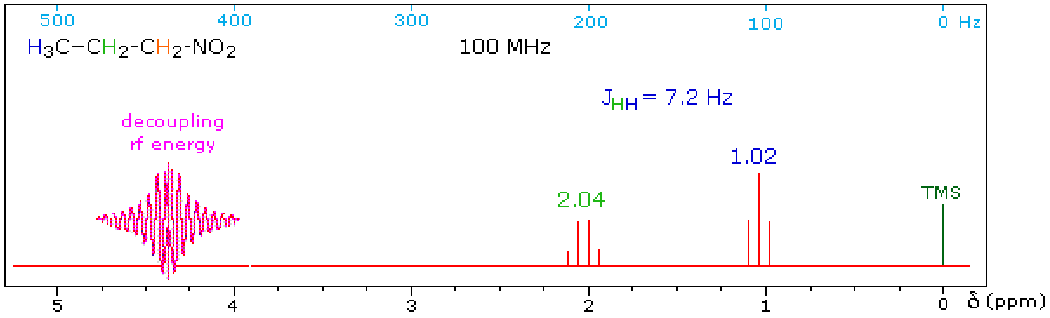


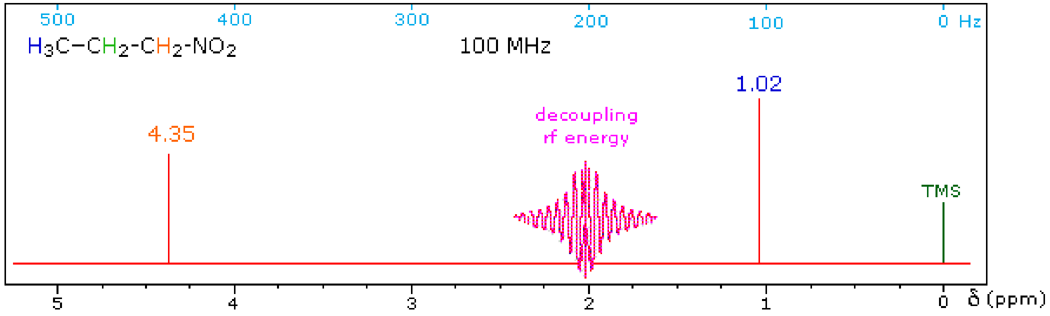
$$J = A - B\cos\phi + C\cos 2\phi$$

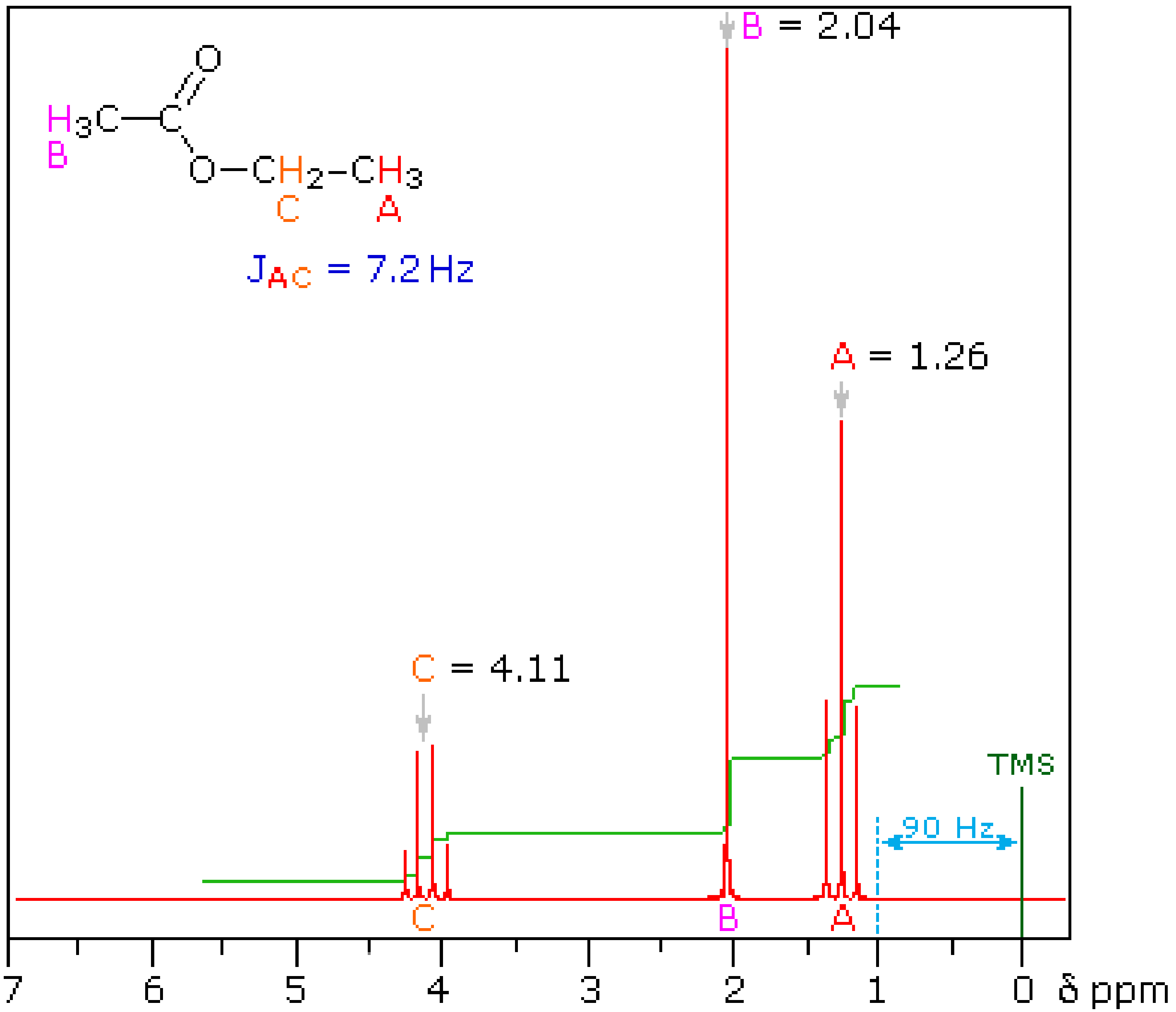


Spin-Coupling/Splitting



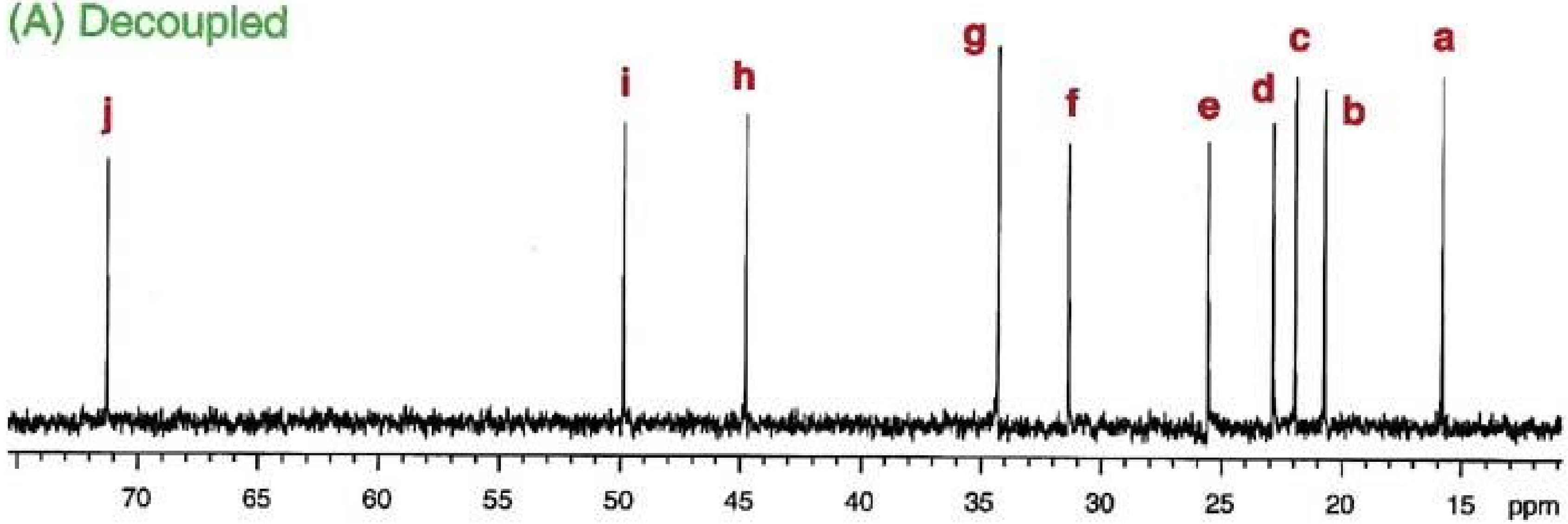




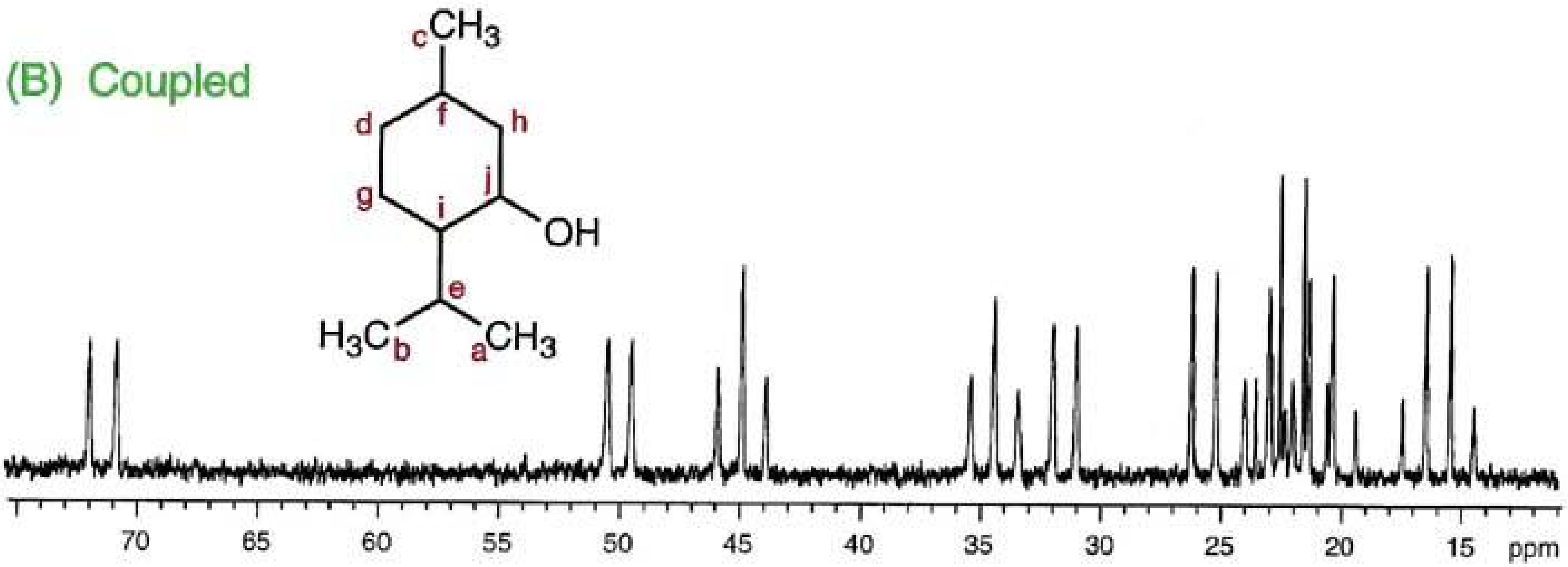

O
H3C
C
B
O
CH2
CH3
C
A
JAC = 7.2 Hz
B = 2.04
A = 1.26
C = 4.11
TMS
90 Hz
C
B
A
7
6
5
4
3
2
1
0
δ ppm

1D CARBON SPECTRA

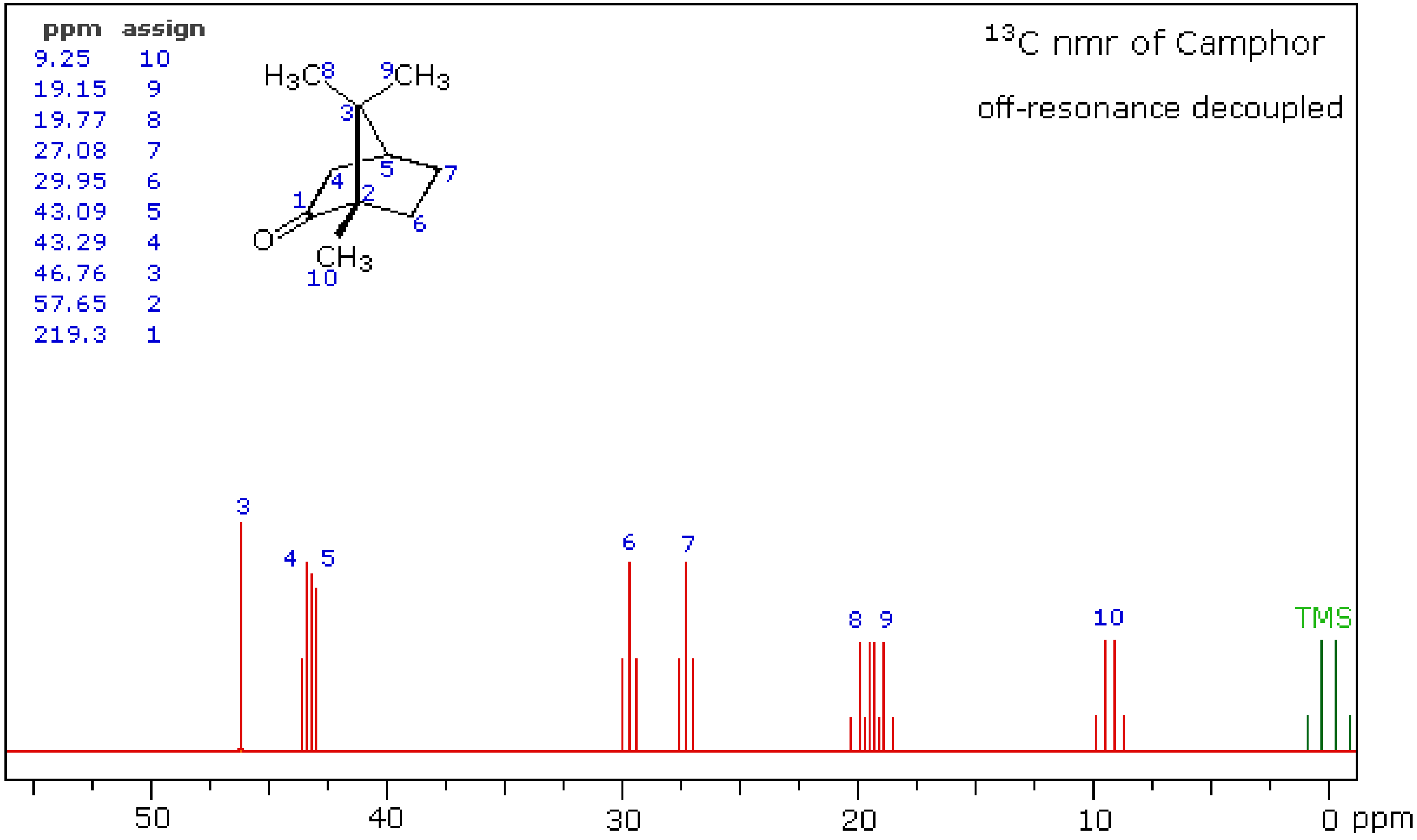
(A) Decoupled



(B) Coupled



^{13}C spectroscopy

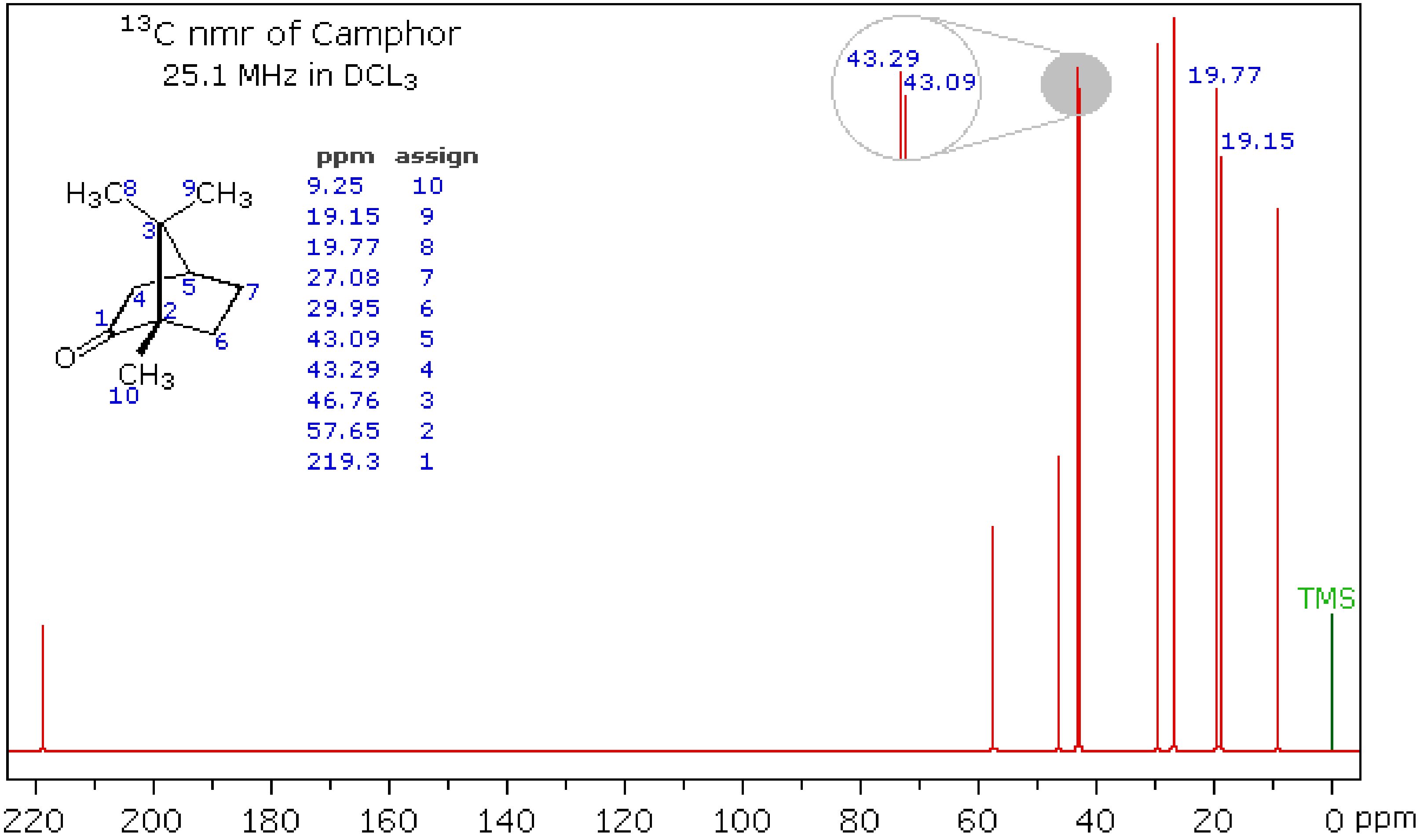


^{13}C nmr of Camphor

off-resonance decoupled

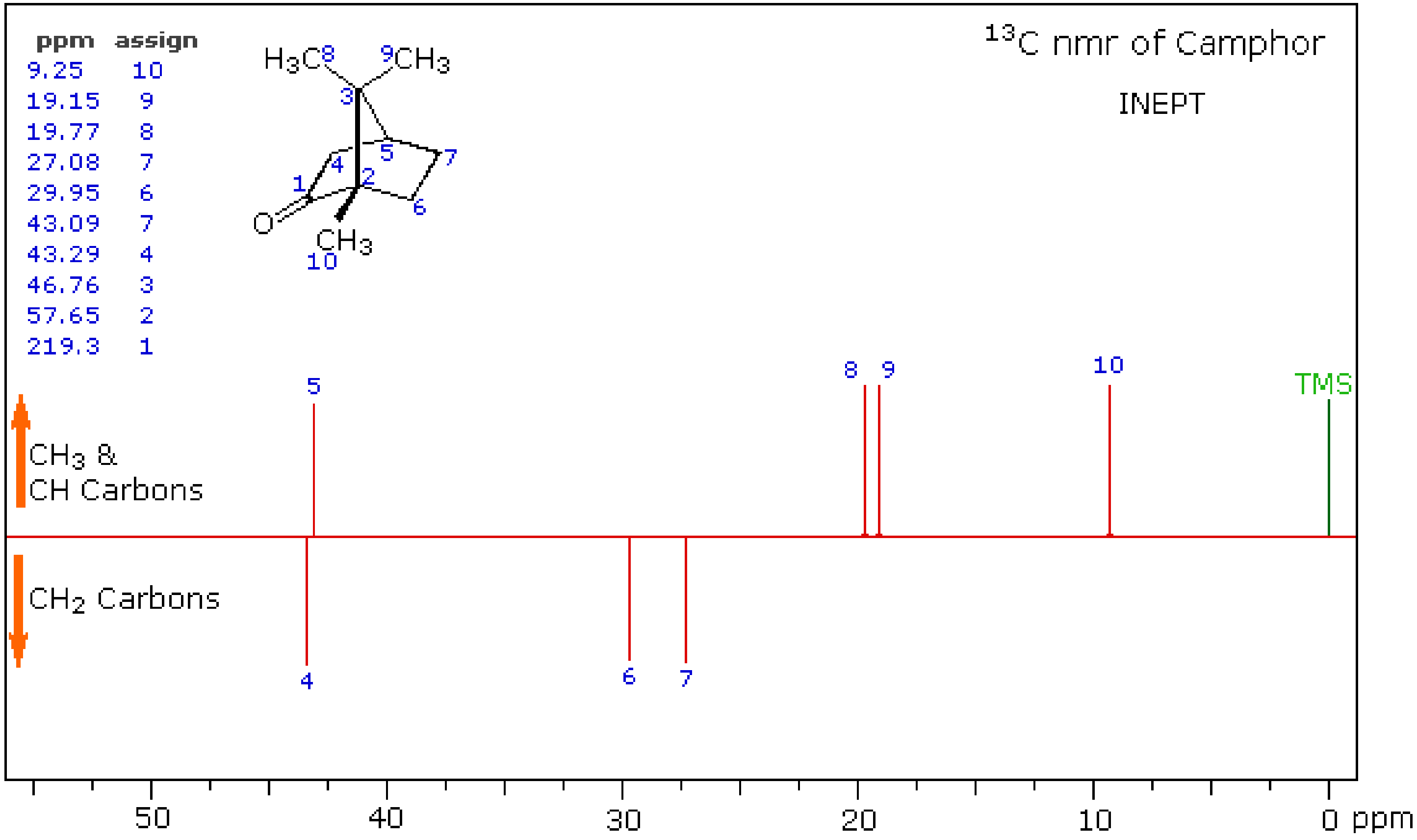
ppm	assign
9.25	10
19.15	9
19.77	8
27.08	7
29.95	6
43.09	5
43.29	4
46.76	3
57.65	2
219.3	1

^{13}C spectroscopy



ppm	assign
9.25	10
19.15	9
19.77	8
27.08	7
29.95	6
43.09	5
43.29	4
46.76	3
57.65	2
219.3	1

^{13}C spectroscopy

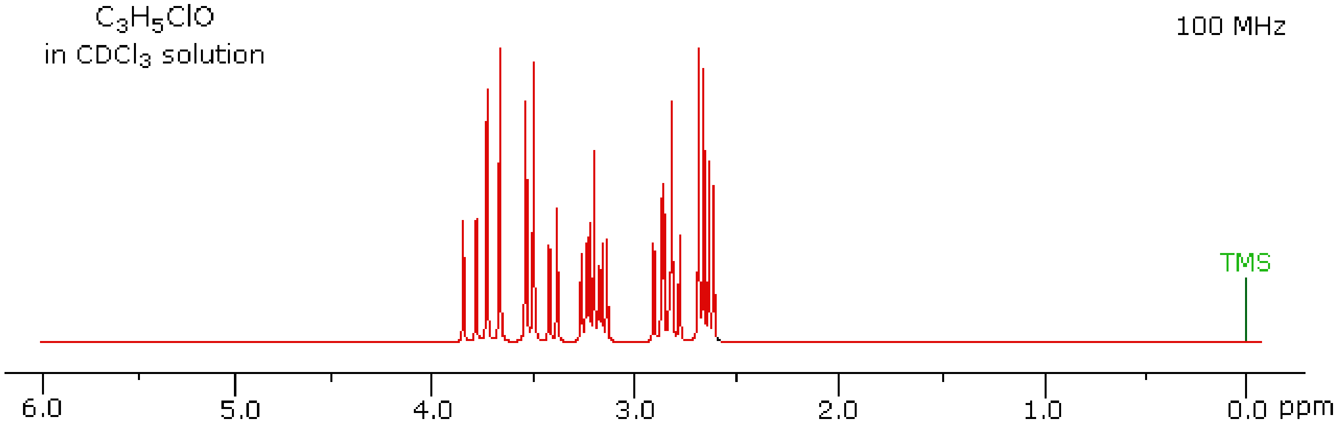


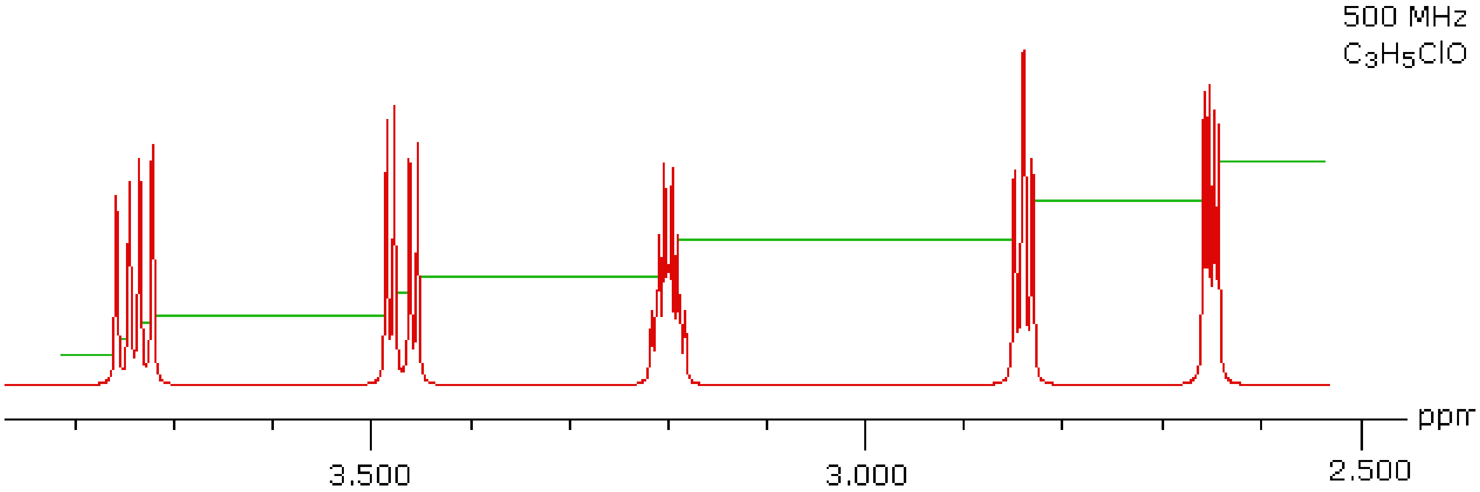
ppm	assign
9.25	10
19.15	9
19.77	8
27.08	7
29.95	6
43.09	7
43.29	4
46.76	3
57.65	2
219.3	1

<

>

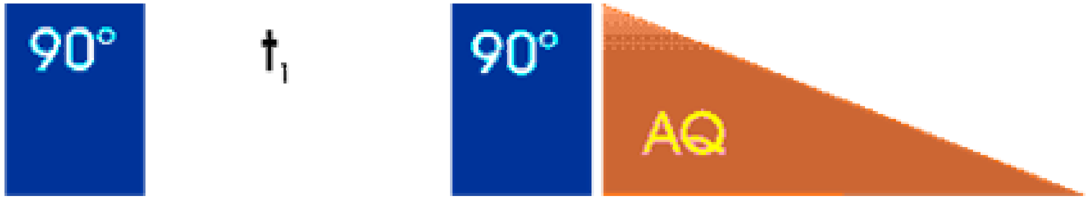
The Influence of Magnetic Field Strength

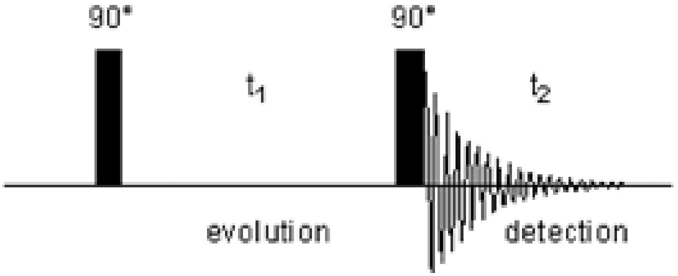




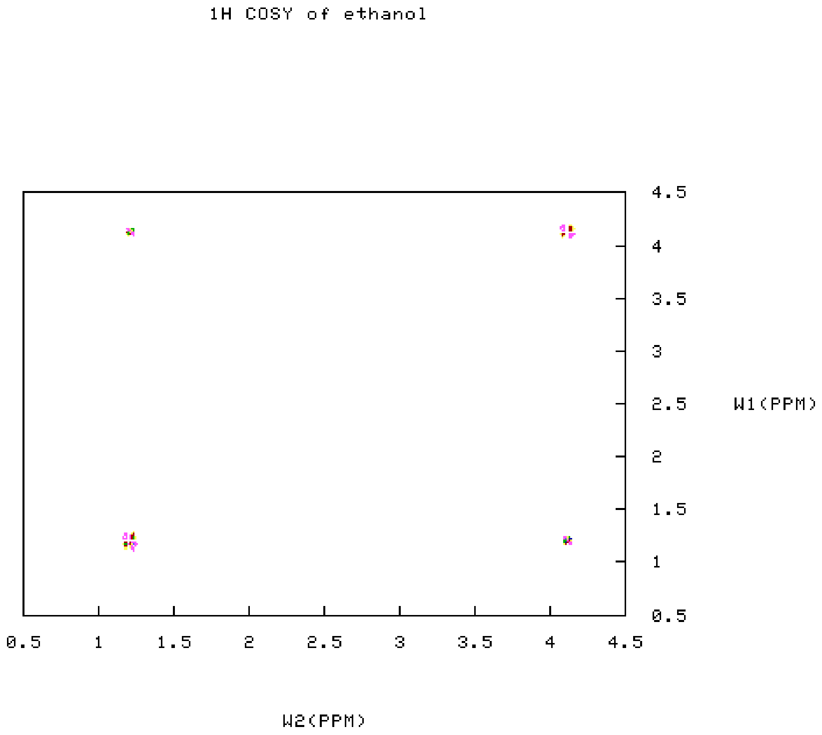
2D Spectroscopy

COSY pulse sequence

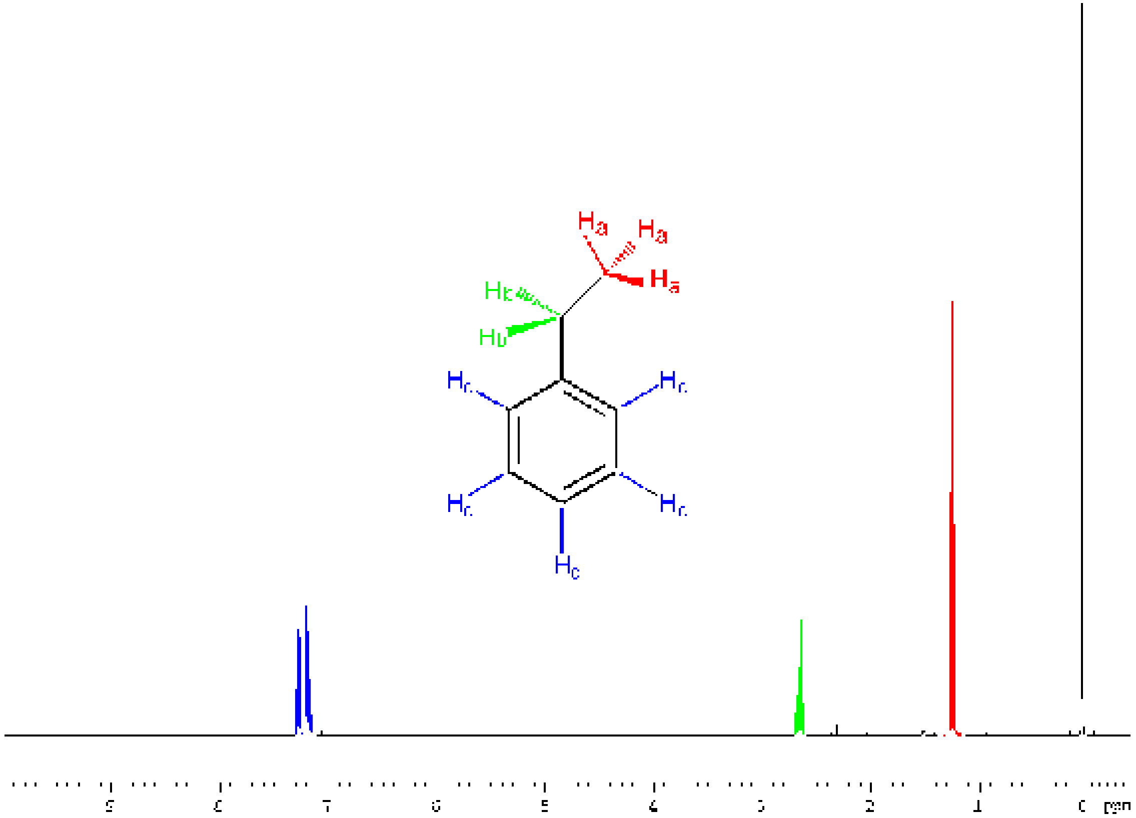




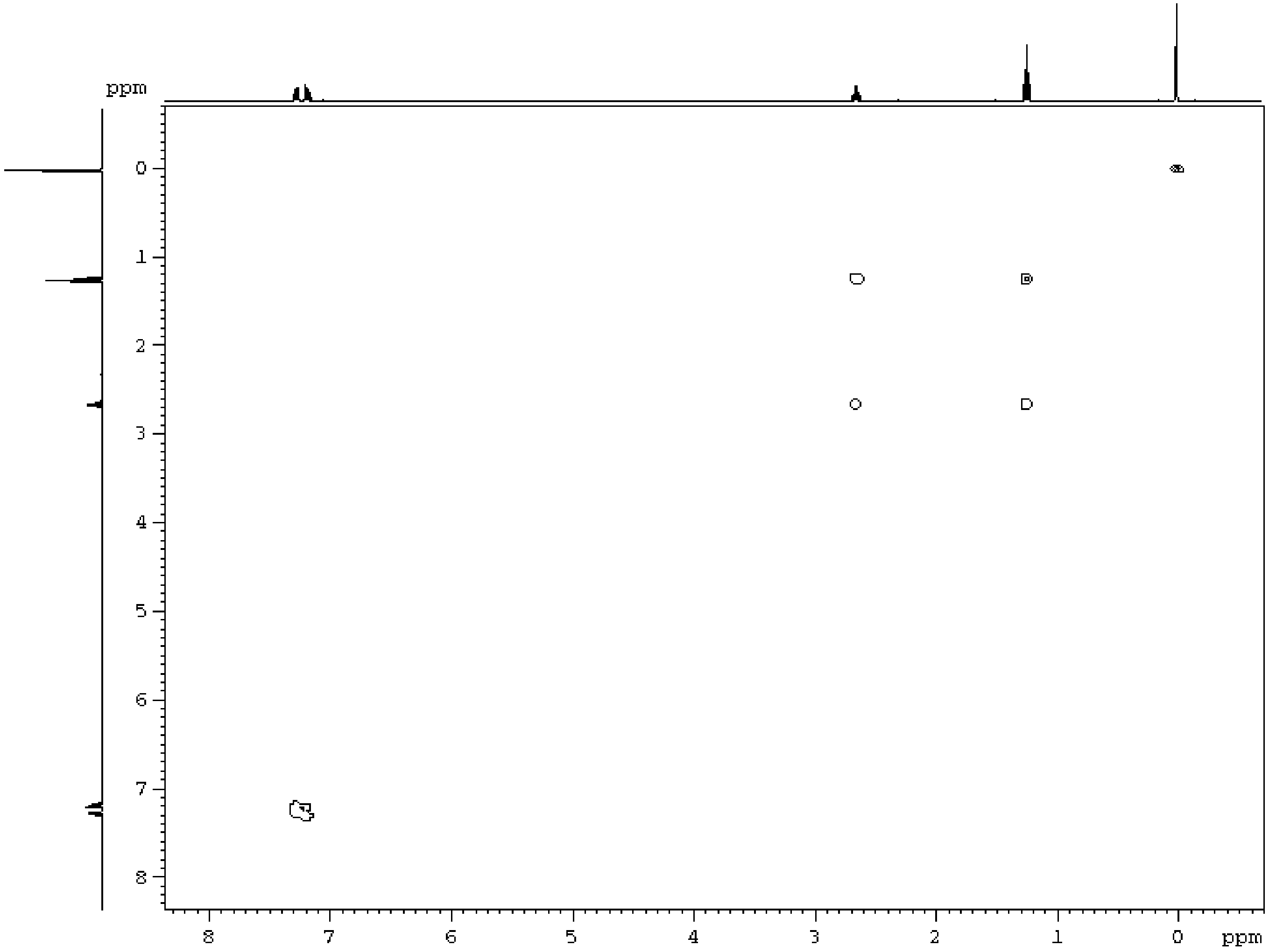
2D Spectroscopy



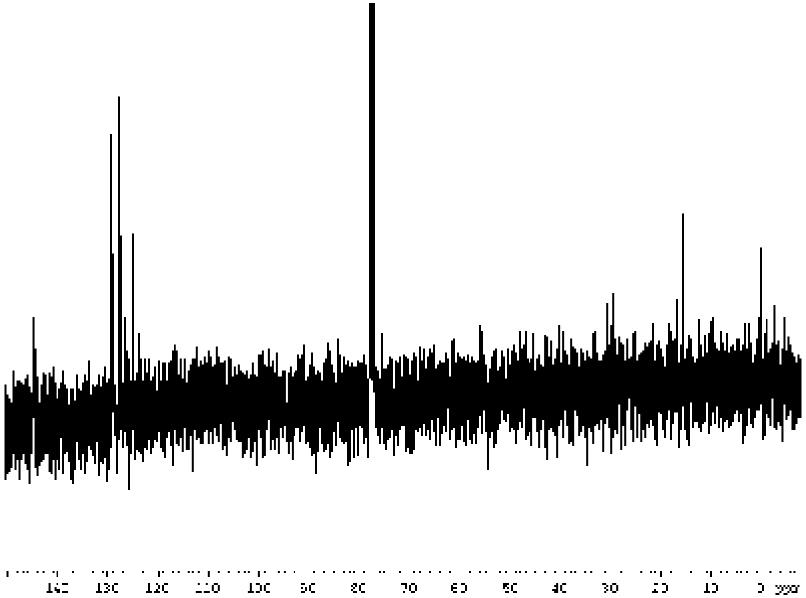
2D Spectroscopy

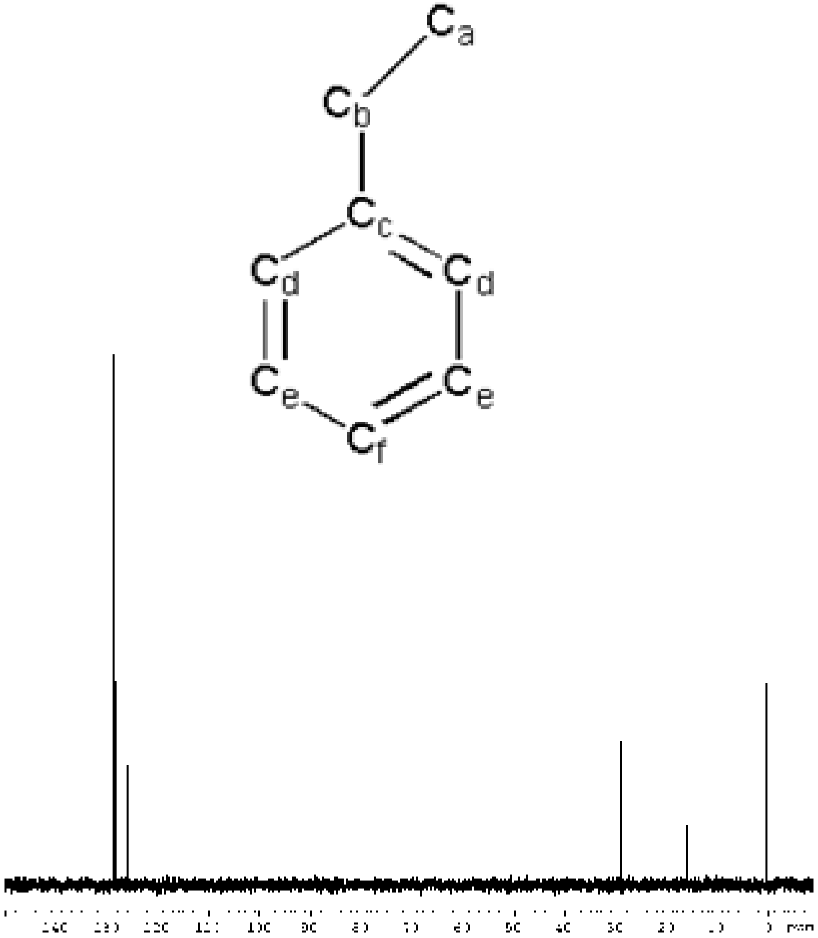


2D Spectroscopy

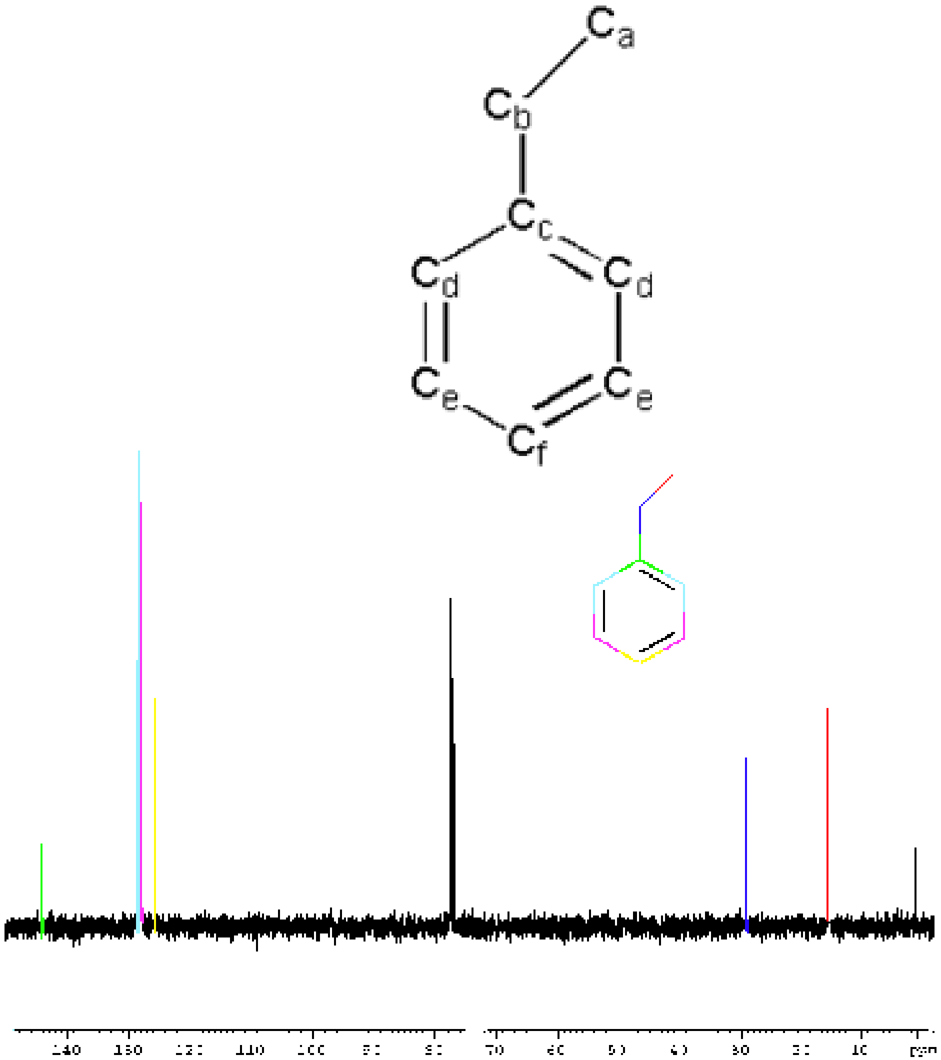


^{13}C resonances assignment

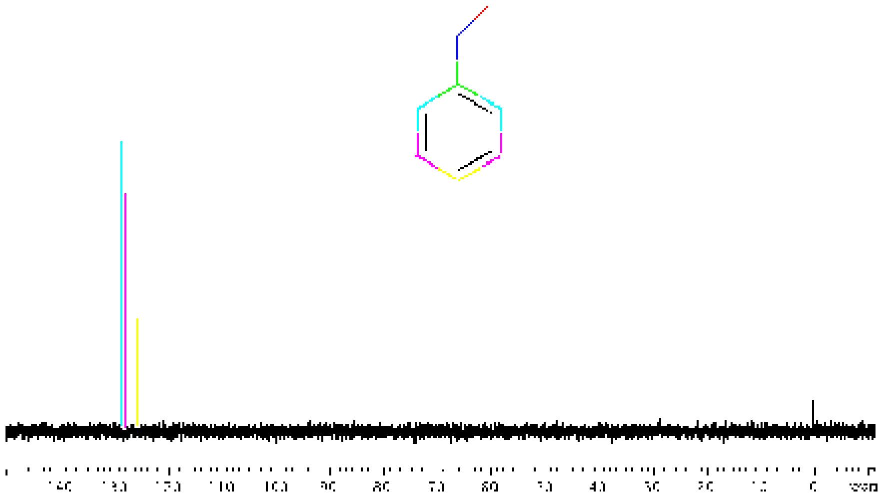




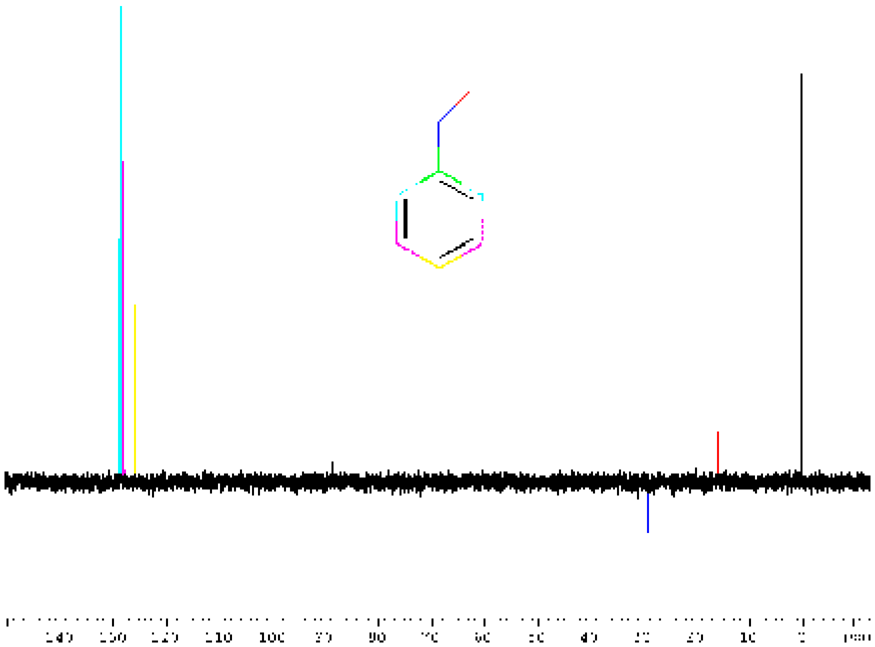
DEPT



C_a
C_b
C_c
C_d
C_d
C_e
C_e
C_f
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

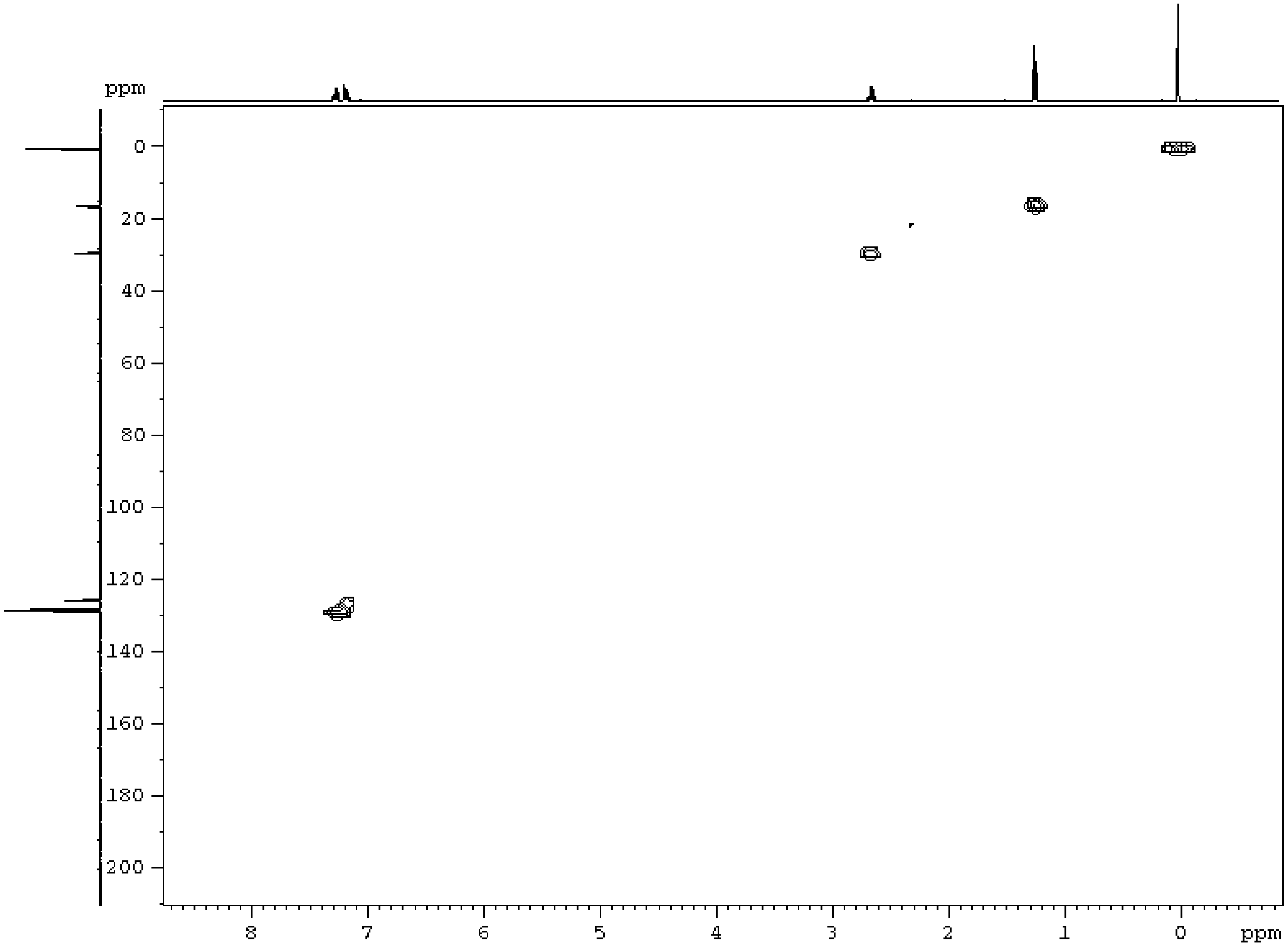


140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

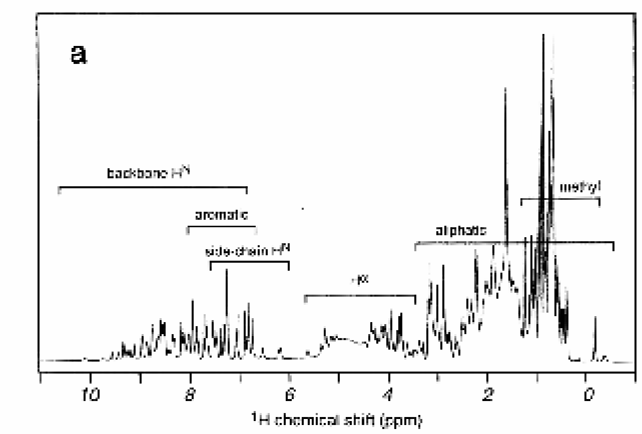


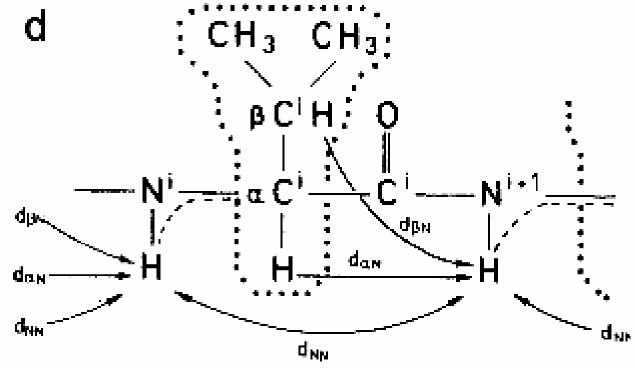
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

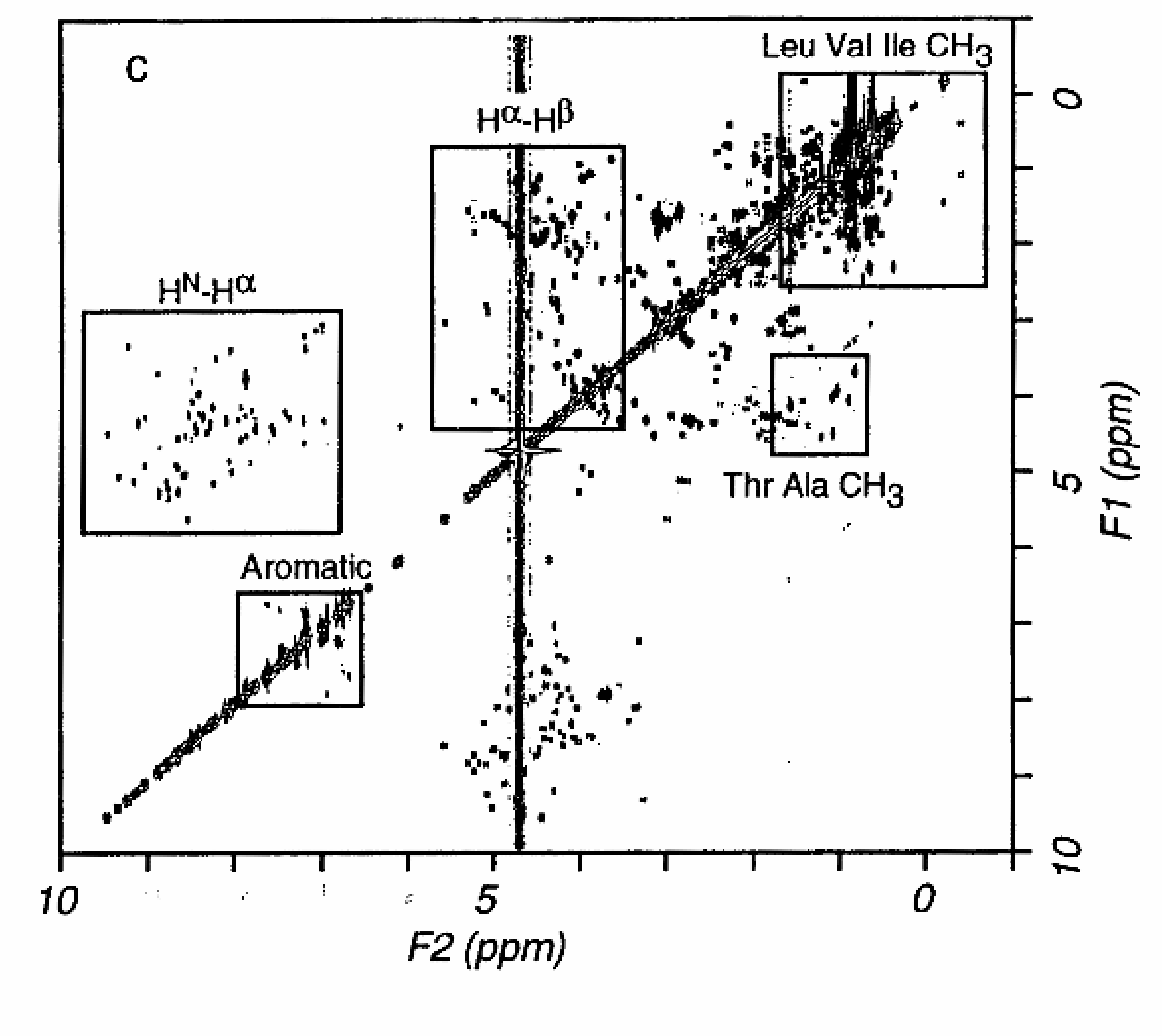
C-H correlation

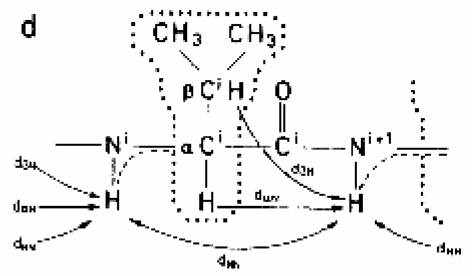


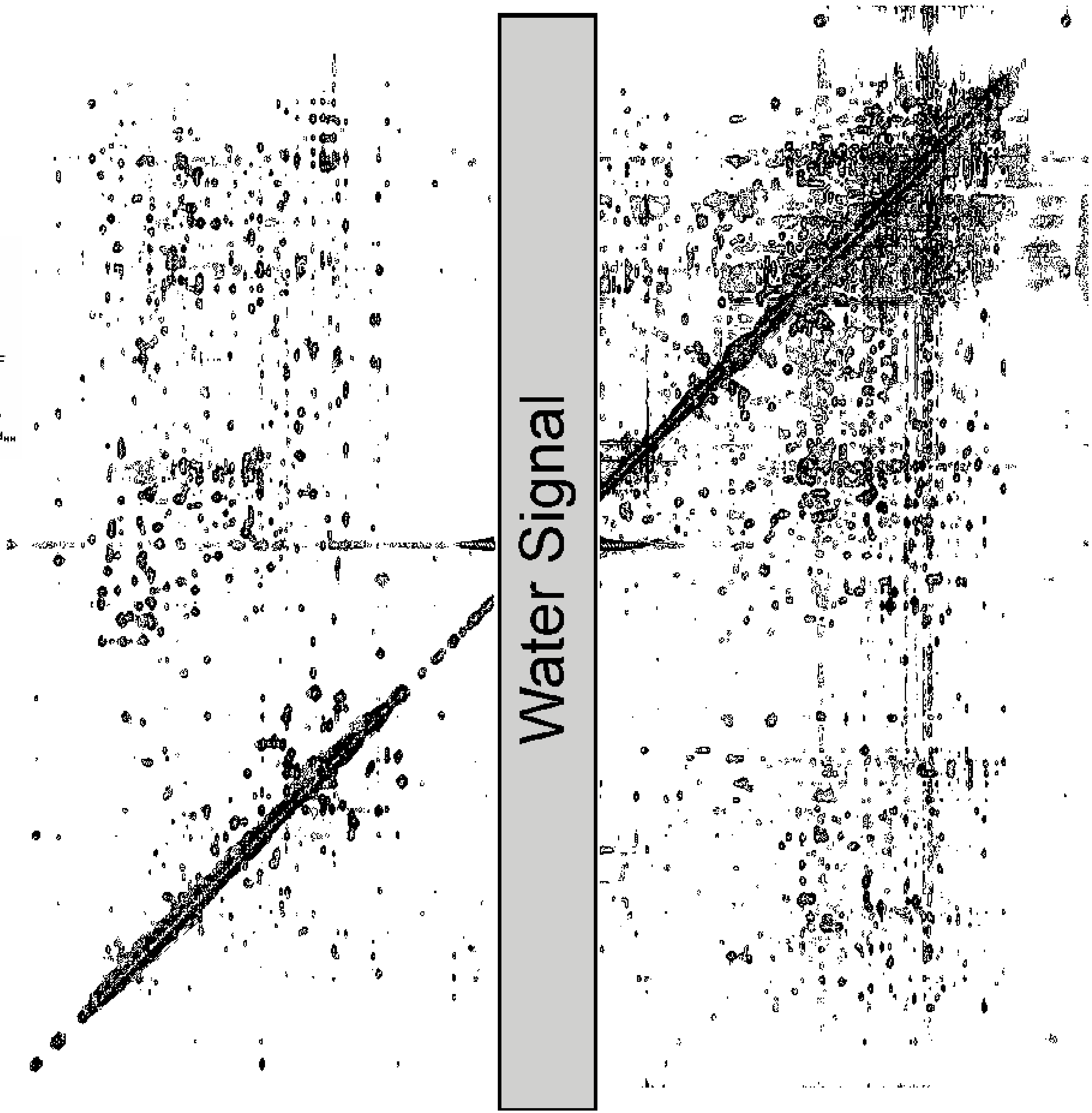
Protein NMR


a
backbone H^N
aromatic
side-chain H^N
Hα
aliphatic
methyl
10
8
6
4
2
0
¹H chemical shift (ppm)


d
CH3
CH3
β Cⁱ H
O
Nⁱ
α Cⁱ
Cⁱ
Nⁱ⁺¹
H
H
H
dβN
dαN
dNN
dβN
dαN
dNN
dNN

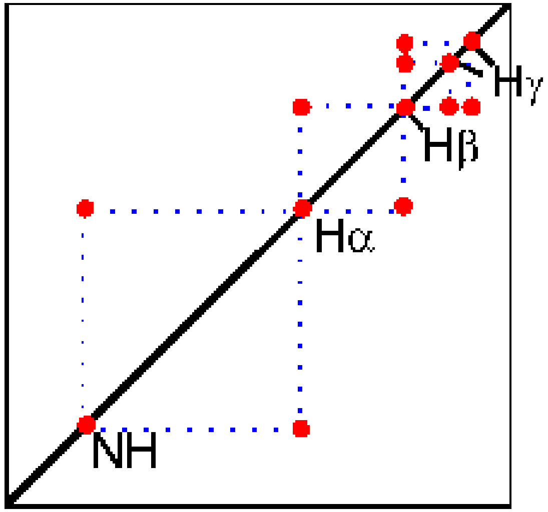

c
Leu Val Ile CH3
Hα-Hβ
HN-Hα
Thr Ala CH3
Aromatic
0
5
10
F1 (ppm)
10
5
0
F2 (ppm)

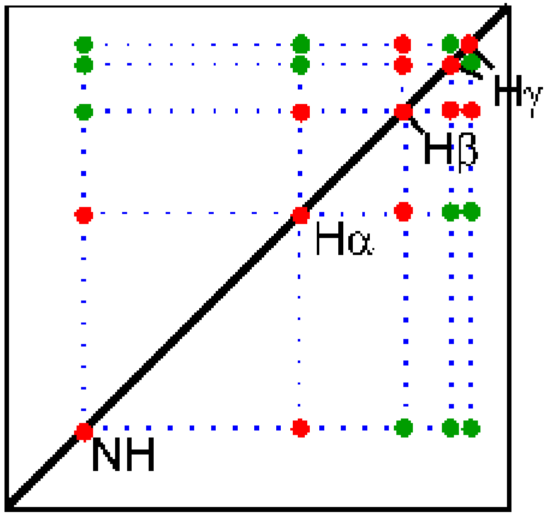

d
CH3
CH3
βC^iH
O
N^i
αC^i
C^i
N^{i+1}
H
H
H
d_βN
d_αN
d_NN
d_βN
d_αN
d_NN
d_NN


Water Signal

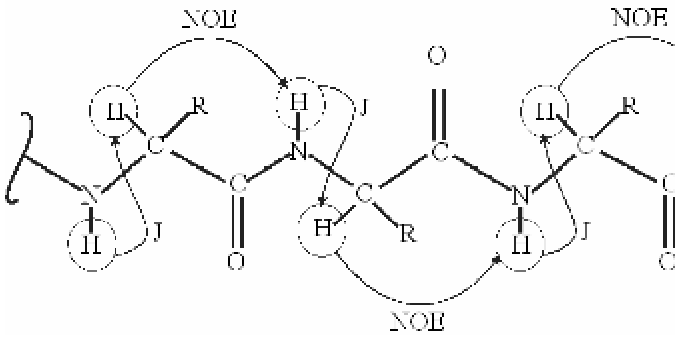
Protein NMR

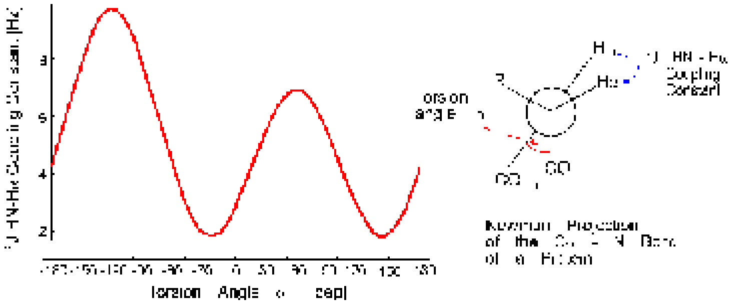
-

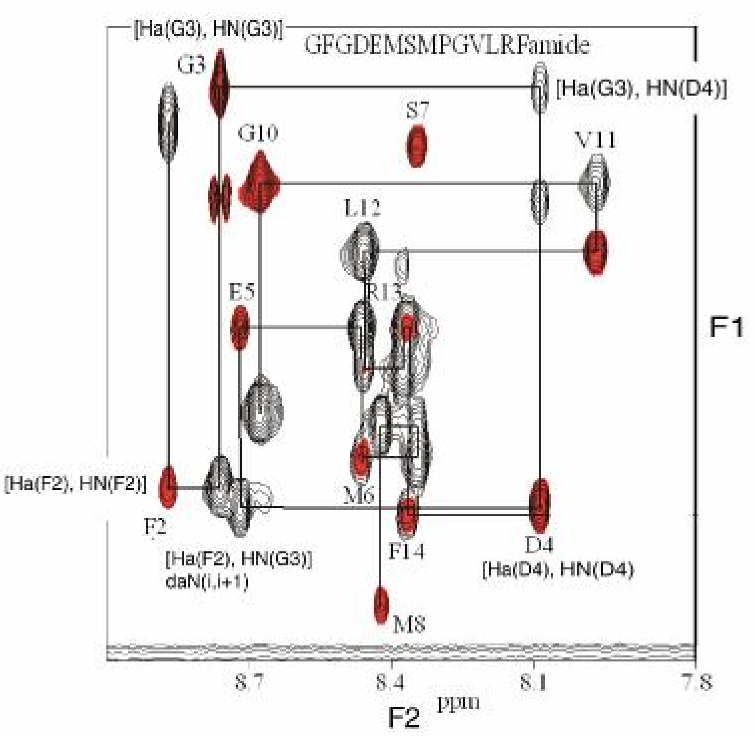


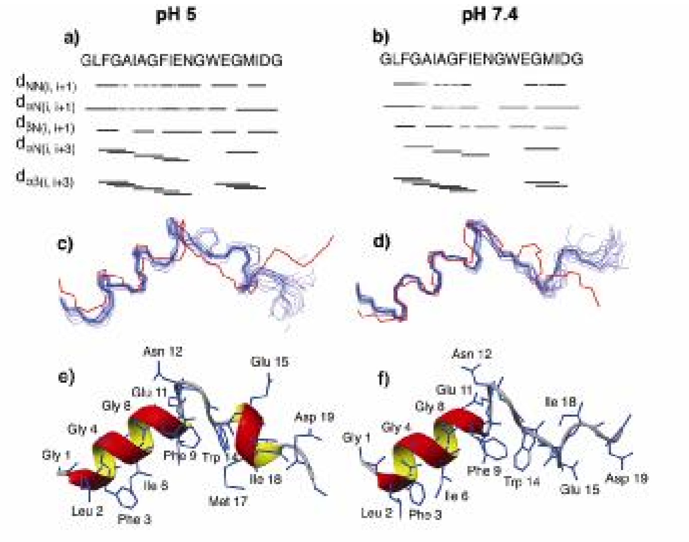


Protein NMR

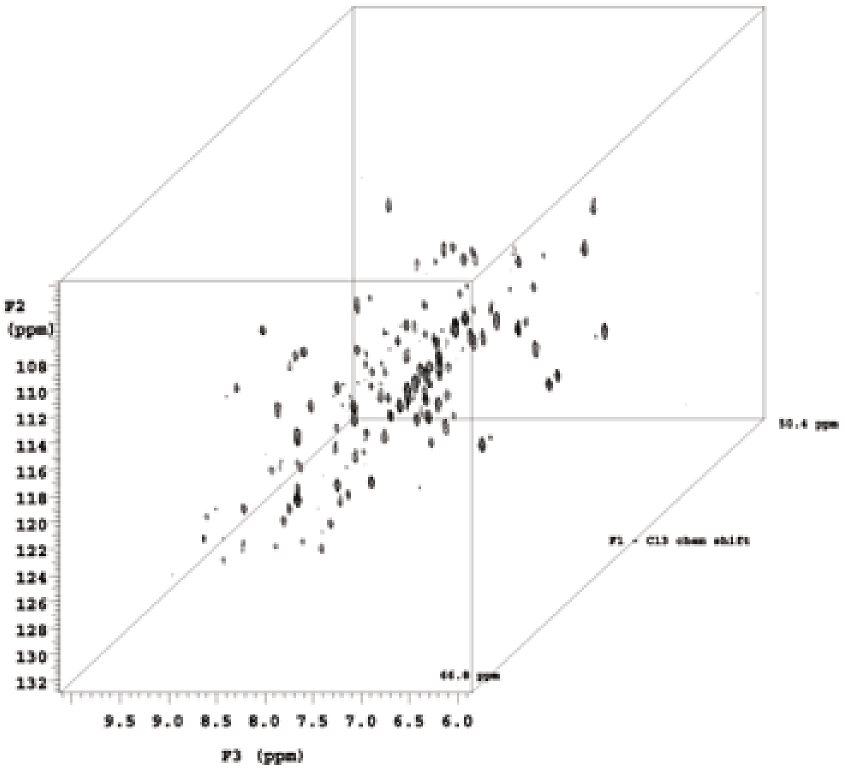

NOE
NOE
H
R
H
J
O
H
R
C
C
N
C
N
C
N
H
J
H
R
H
J
O
C
NOE

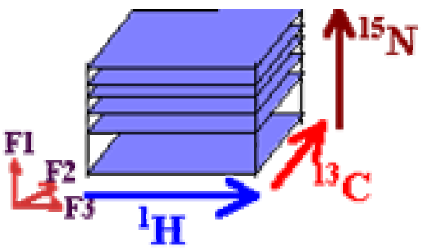

³J HN-Hα Coupling Constant [Hz]
2
4
6
8
-180 -150 -120 -90 -60 -30 0 30 60 90 120 150 180
Torsion Angle (deg)
Torsion angle
H
Hα
R
CO
CO
³J HN - Hα Coupling Constant
Newman Projection of the Cα - N Bond of a Protein


[Ha(G3), HN(G3)]
GFGDEMSMPGVLRFamide
G3
[Ha(G3), HN(D4)]
S7
G10
V11
L12
R13
E5
[Ha(F2), HN(F2)]
F2
M6
F14
D4
[Ha(F2), HN(G3)]
daN(i,i+1)
[Ha(D4), HN(D4)]
M8
F1
8.7
8.4
8.1
7.8
F2
ppm

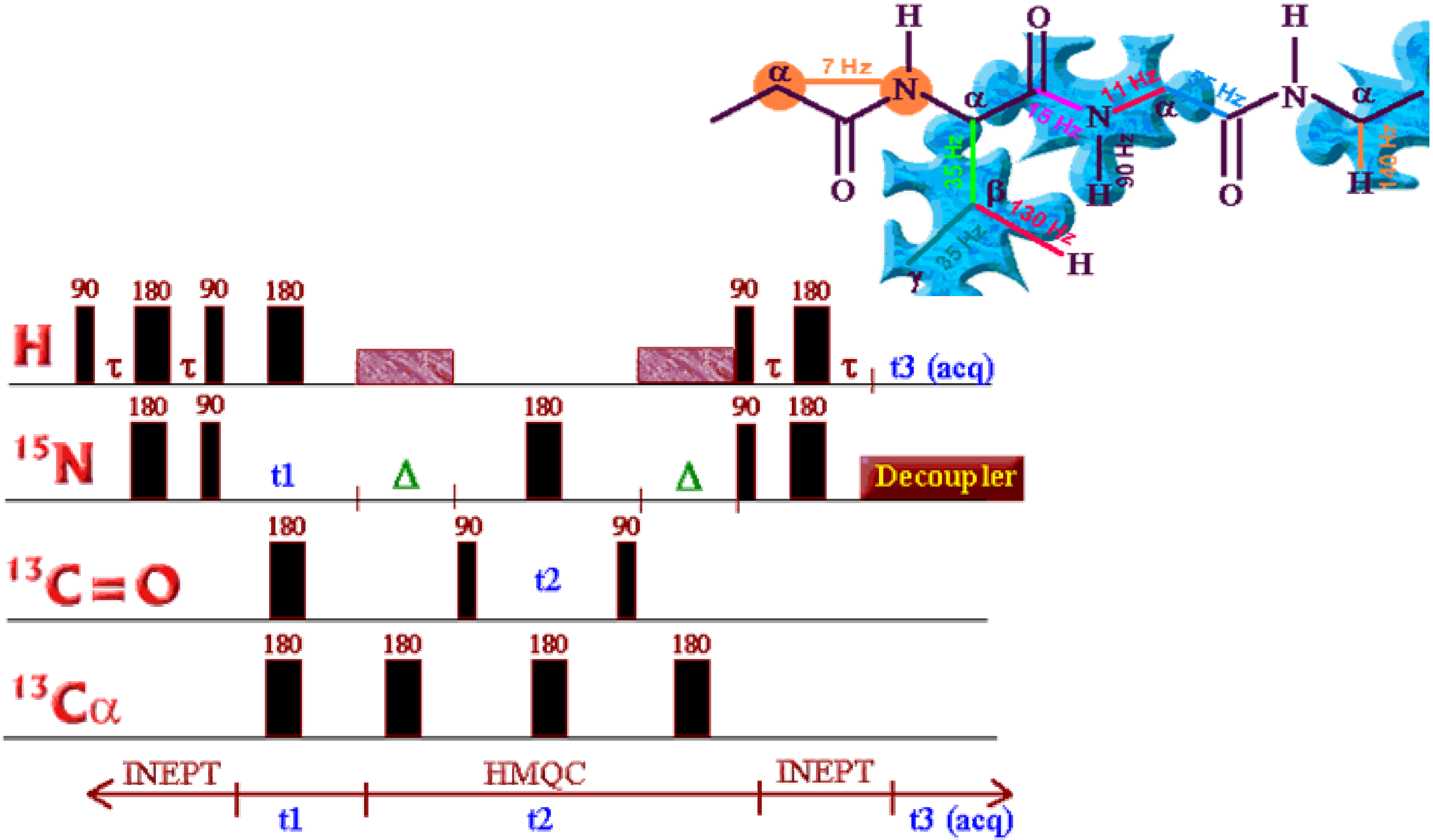

pH 5
pH 7.4
a)
b)
GLFGAIAGFIENGWEGMIDG
GLFGAIAGFIENGWEGMIDG
$d_{NN(i,i+1)}$
$d_{\alpha N(i,i+1)}$
$d_{\beta N(i,i+1)}$
$d_{\alpha N(i,i+3)}$
$d_{\alpha\beta(i,i+3)}$
c)
d)
e)
f)
Asn 12
Glu 15
Glu 11
Gly 8
Gly 4
Asp 19
Gly 1
Phe 9
Trp 14
Ile 18
Ile 6
Met 17
Leu 2
Phe 3
Asn 12
Glu 11
Gly 8
Ile 18
Gly 4
Gly 1
Phe 9
Trp 14
Asp 19
Glu 15
Ile 6
Leu 2
Phe 3

Protein NMR

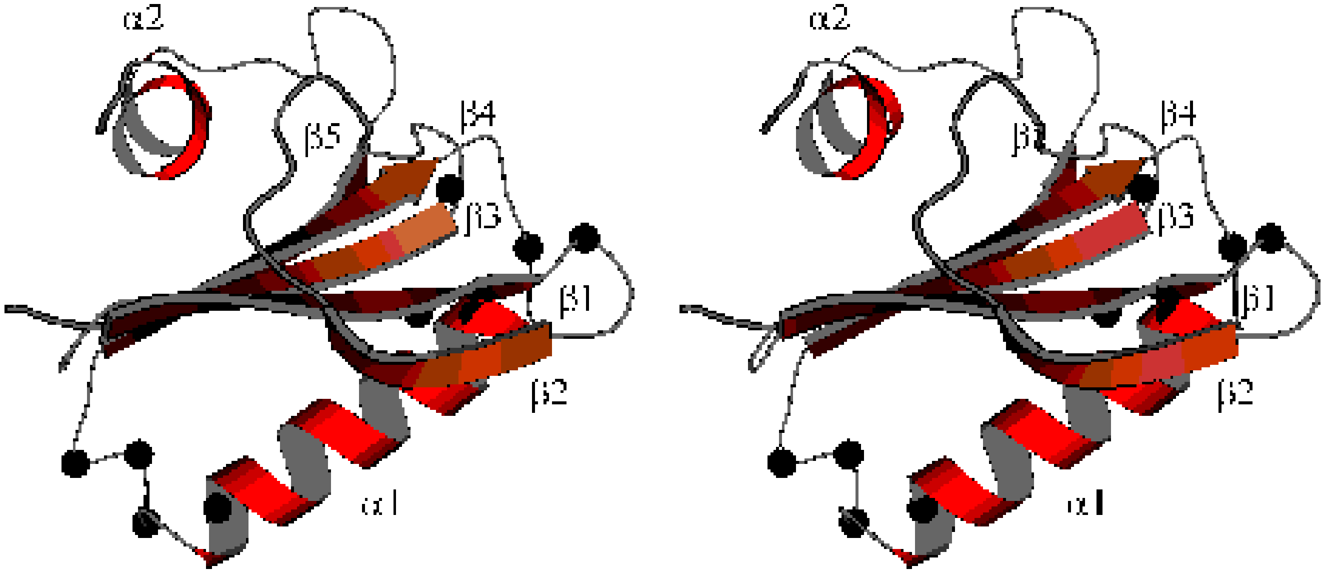

F2
(ppm)
108
110
112
114
116
118
120
122
124
126
128
130
132
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0
F3 (ppm)
50.4 ppm
F1 - C13 chem shift
66.8 ppm

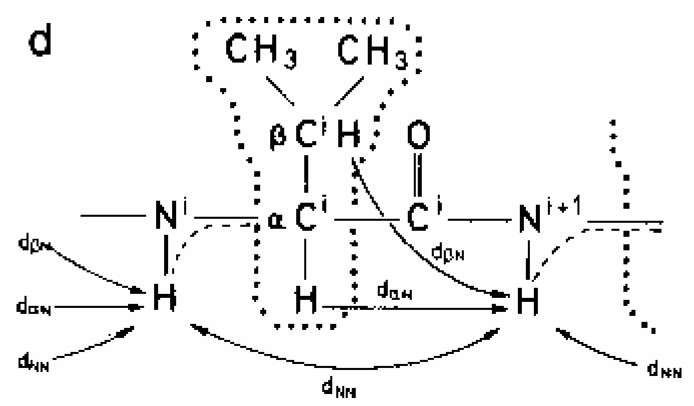

F1
F2
F3
^{1}H
^{13}C
^{15}N

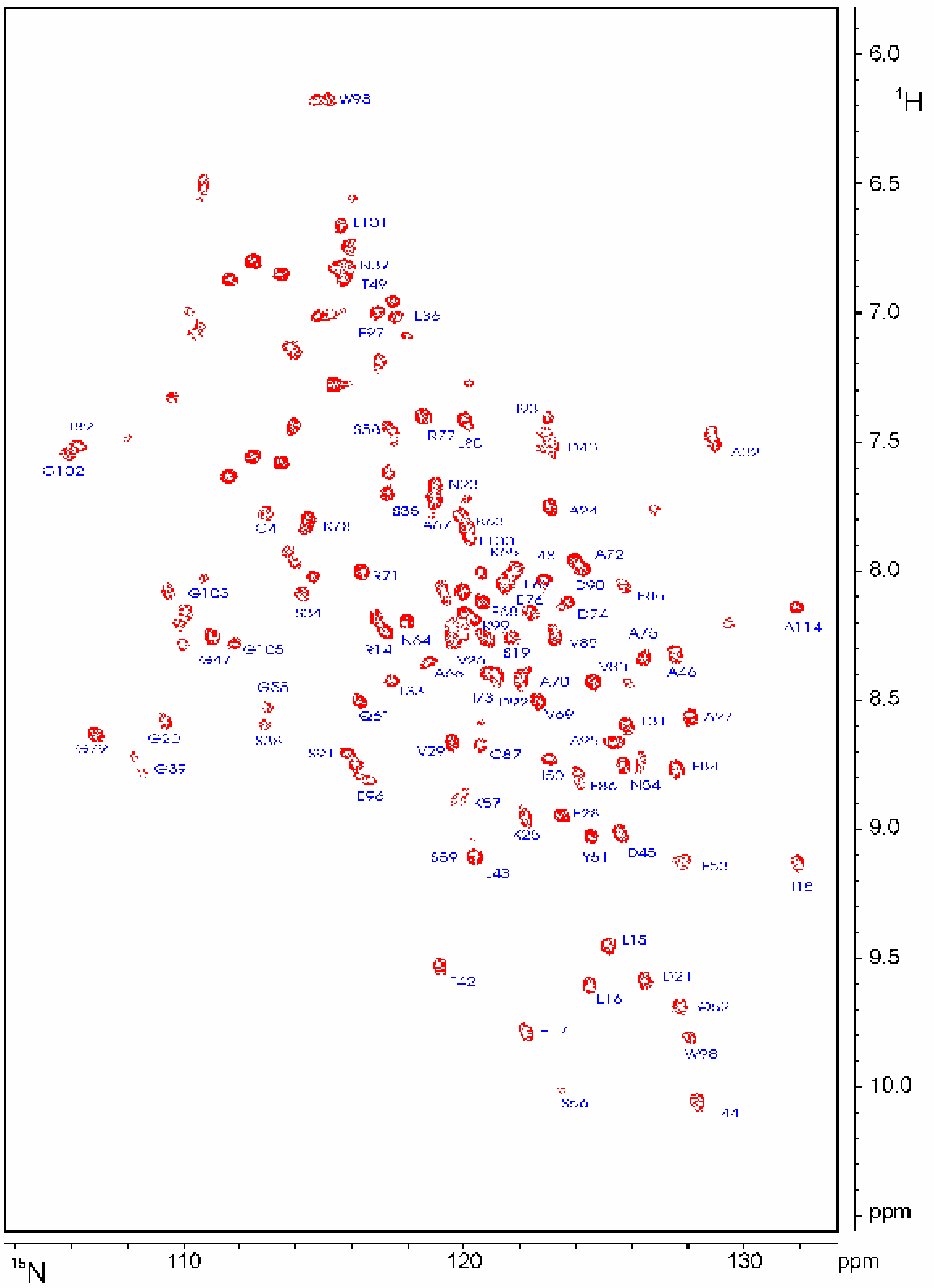
Protein NMR


H
O
H
α
7 Hz
N
α
11 Hz
15 Hz
N
α
N
α
35 Hz
90 Hz
140 Hz
O
β
130 Hz
H
O
H
35 Hz
γ
H
H
90
180
90
180
τ
τ
90
180
τ
τ
t3 (acq)
^{15}N
180
90
t1
Δ
180
Δ
90
180
Decoupler
^{13}C=O
180
90
t2
90
^{13}Cα
180
180
180
180
INEPT
HMQC
INEPT
t1
t2
t3 (acq)

Protein NMR




d
CH3
CH3
β C^i H
O
N^i
α C^i
C^i
N^(i+1)
H
H
H
dβN
dαN
dNN
dβN
dαN
dNN
dNN

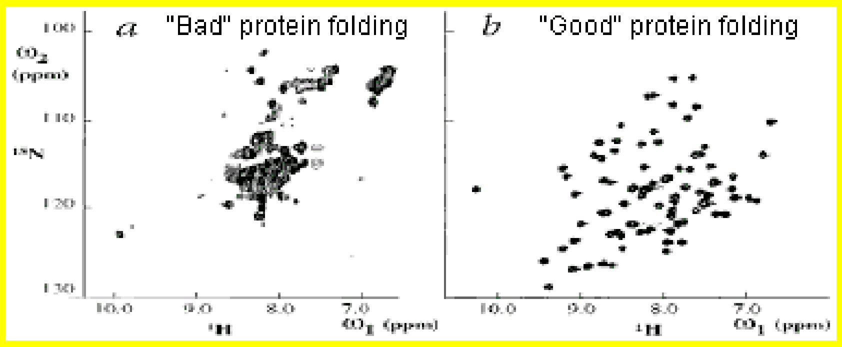

W98
L101
N37
T49
L36
F97
S58
R77
L80
D45
A39
G102
N29
S36
A94
C4
K78
A72
R71
D90
G103
E76
D74
A114
S34
R14
K64
S19
V85
A75
G105
A46
G55
A70
V69
Q51
A77
G20
V29
C87
G79
S91
G37
H84
N54
E96
K57
F28
K26
S59
Y51
D45
L43
I18
L15
D21
L16
W98
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
ppm
1H
15N
110
120
130
ppm

Drug discovery

Taget gene

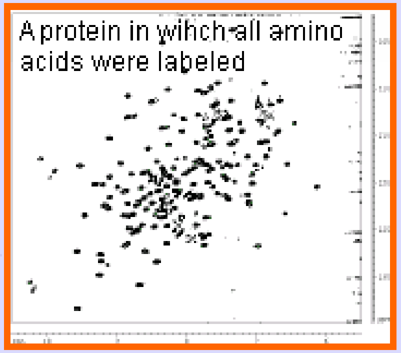
High throughput search of soluble functional domain

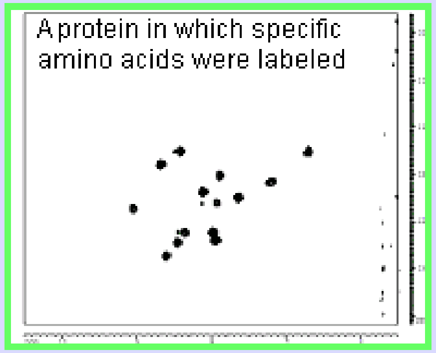
Determination of a domain with "Good" folding



Amino acid labeled protein sysnthesis

NMR

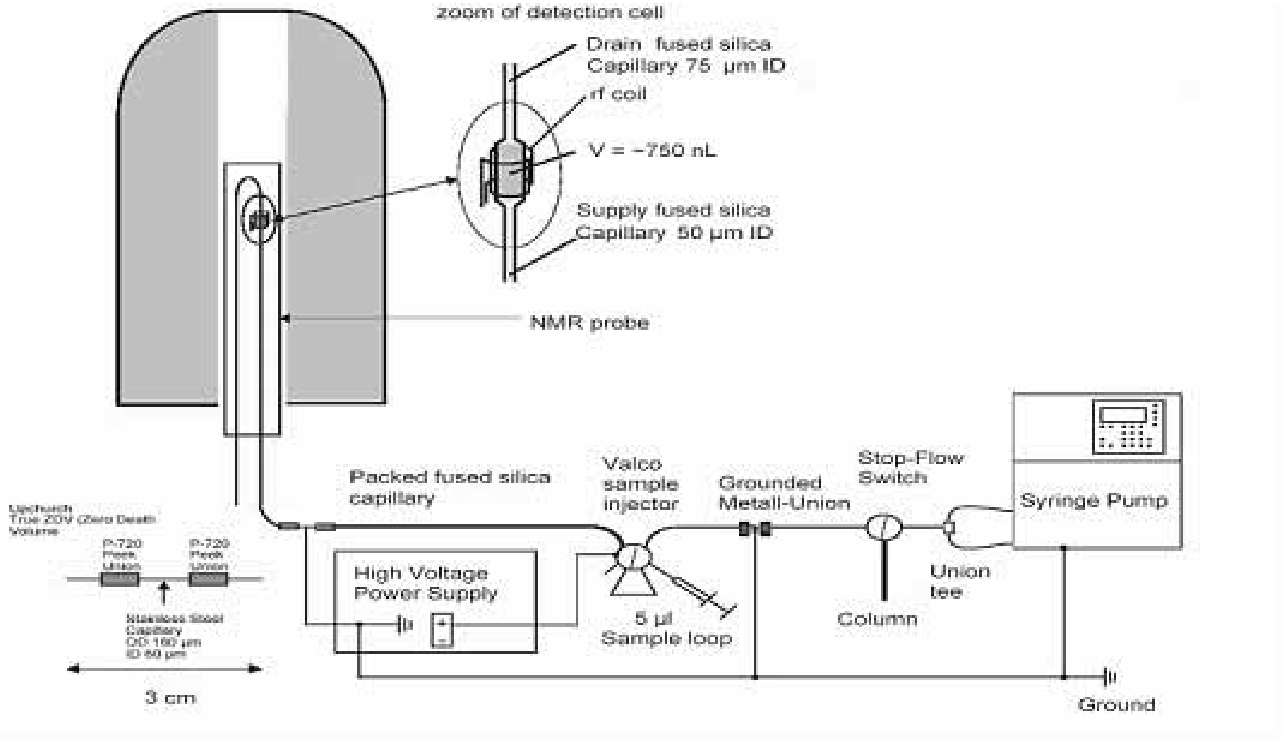




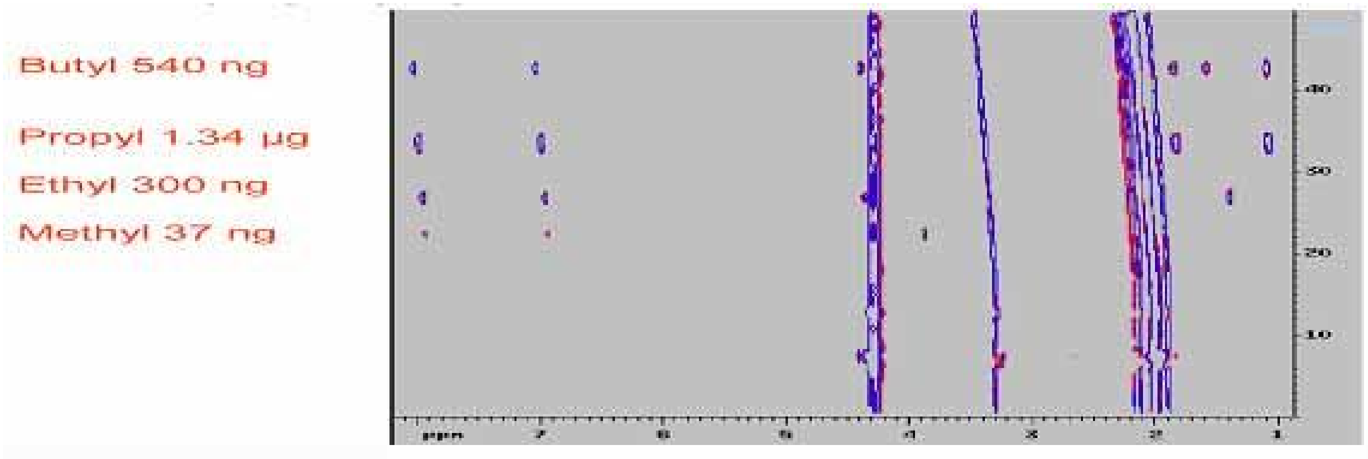
Analysis of folding, spetrum assignment, and interaction

for drug design

LC-NMR

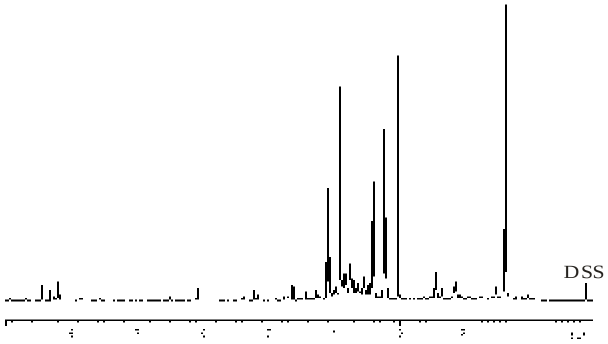

zoom of detection cell
Drain fused silica Capillary 75 µm ID
rf coil
V = ~750 nL
Supply fused silica Capillary 50 µm ID
NMR probe
Upchurch True ZDV (Zero Death Volume
P-720 Peek Union
P-720 Peek Union
Stainless Steel Capillary OD 160 µm ID 60 µm
3 cm
Packed fused silica capillary
High Voltage Power Supply
+
−
Valco sample injector
5 µl Sample loop
Grounded Metall-Union
Stop-Flow Switch
Column
Union tee
Syringe Pump
Ground

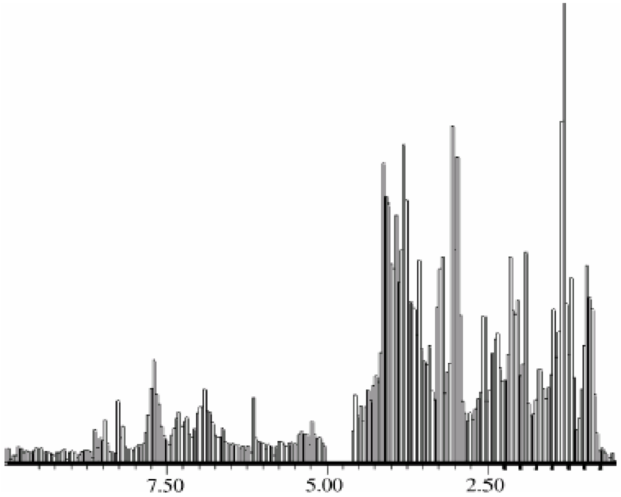
LC-NMR


Butyl 540 ng
Propyl 1.34 µg
Ethyl 300 ng
Methyl 37 ng
ppm
7
6
5
4
3
2
1
40
30
20
10

A procedure for Metabonomics

Tissue or biofluid sample

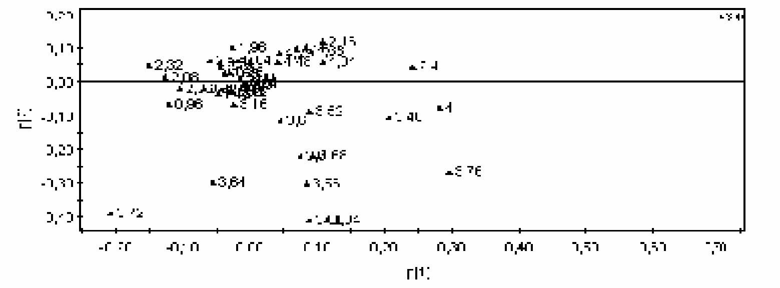




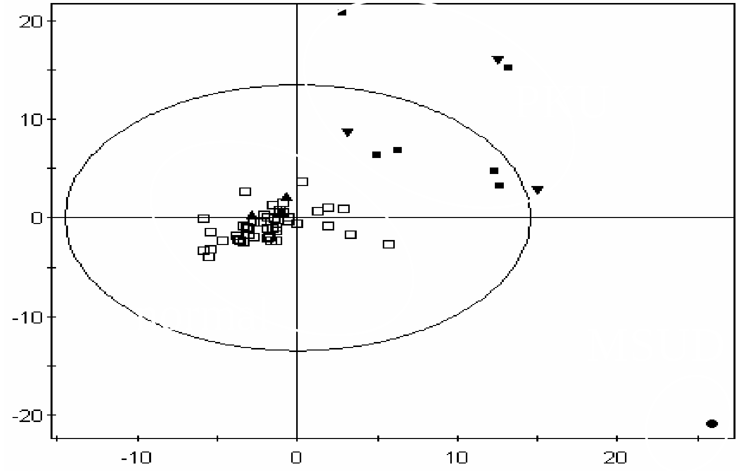
Measure the metabolite profile

Explore metabolite profile to gain mechanistic insight

Treat metabolite profile as
statistical ‘object’ for

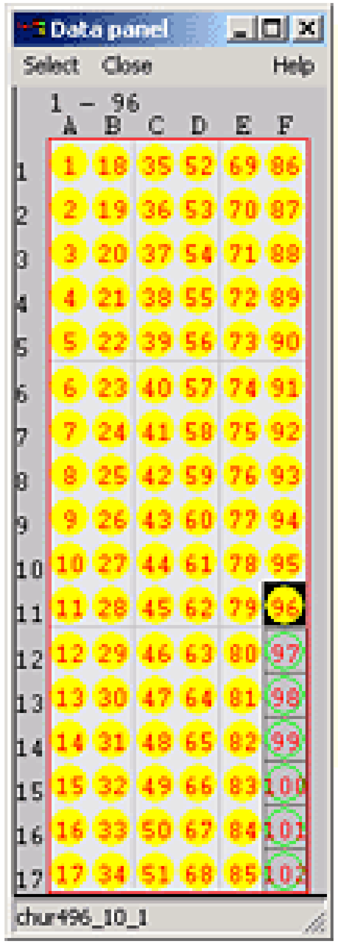


PCA

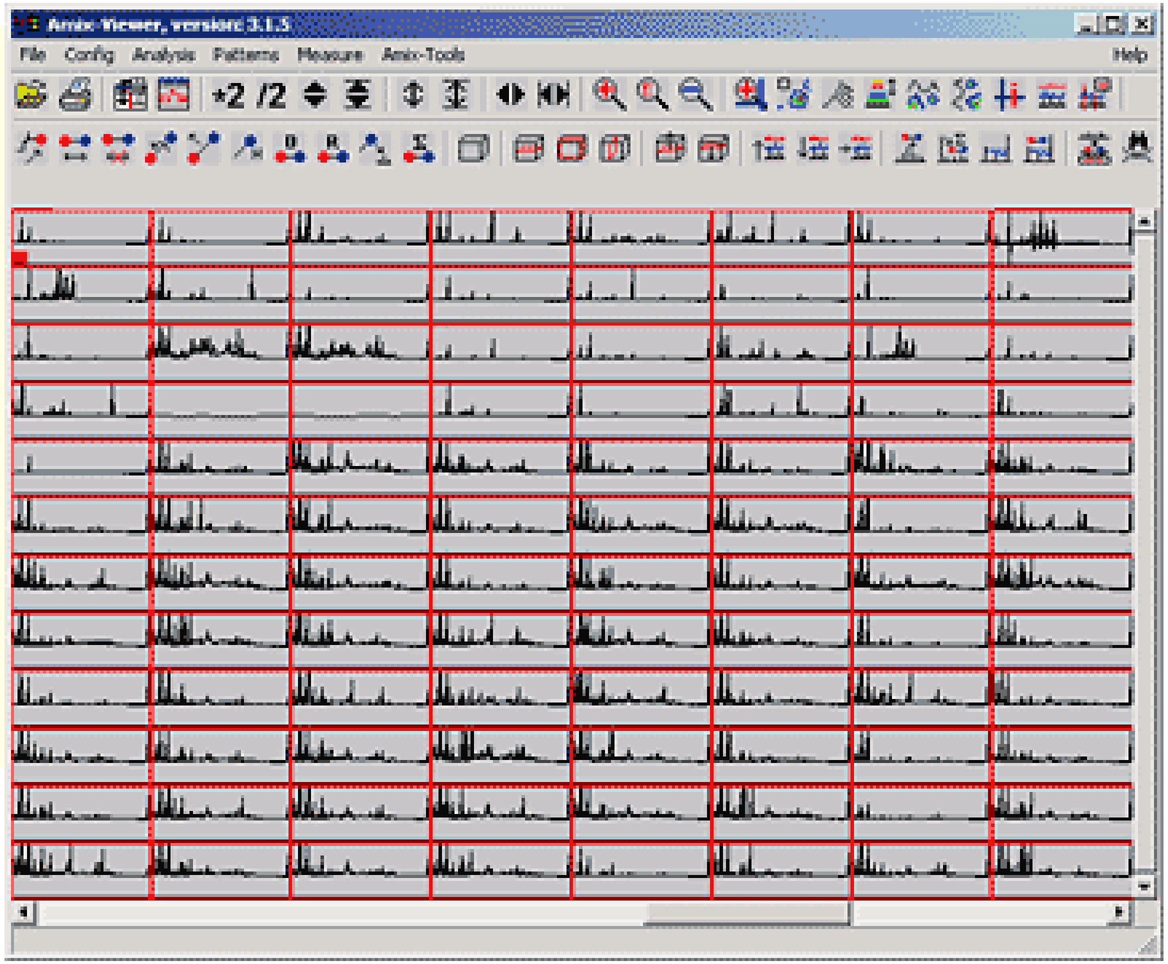


Application of NMR spectroscopy combined with principal component analysis in detecting inborn errors of metabolism using blood spots. A metabonomic approach
M.A. Constantinou, E. Papakonstantinou, M. Spraul, K. Shulpis, M.A. Koupparis, E. Mikros *Analytica Chimica Acta*, 511, 303-312, 2004

Metabonomics



	A	B	C	D	E	F
1	1	18	35	52	69	86
2	2	19	36	53	70	87
3	3	20	37	54	71	88
4	4	21	38	55	72	89
5	5	22	39	56	73	90
6	6	23	40	57	74	91
7	7	24	41	58	75	92
8	8	25	42	59	76	93
9	9	26	43	60	77	94
10	10	27	44	61	78	95
11	11	28	45	62	79	96
12	12	29	46	63	80	97
13	13	30	47	64	81	98
14	14	31	48	65	82	99
15	15	32	49	66	83	100
16	16	33	50	67	84	101
17	17	34	51	68	85	102



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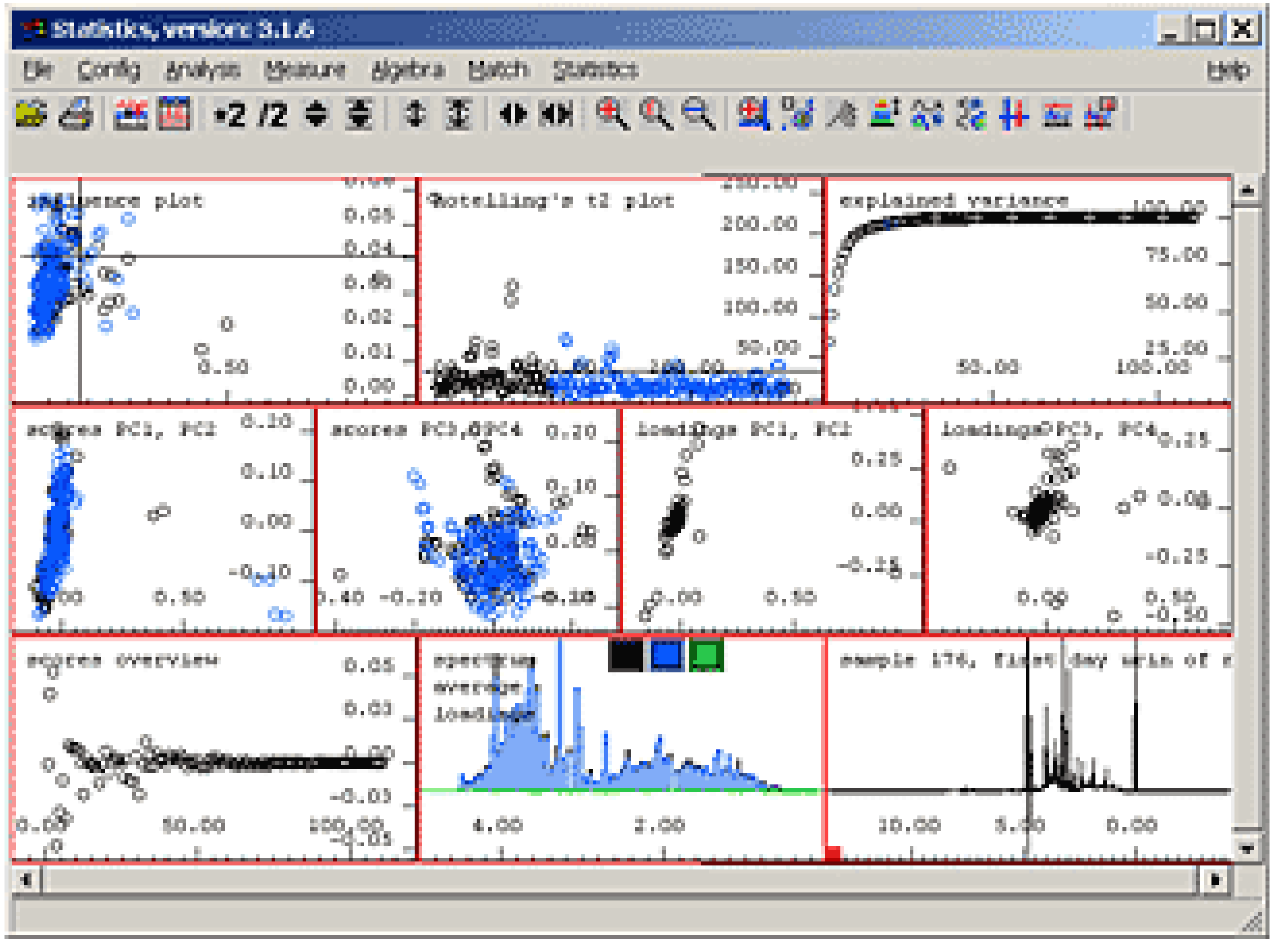

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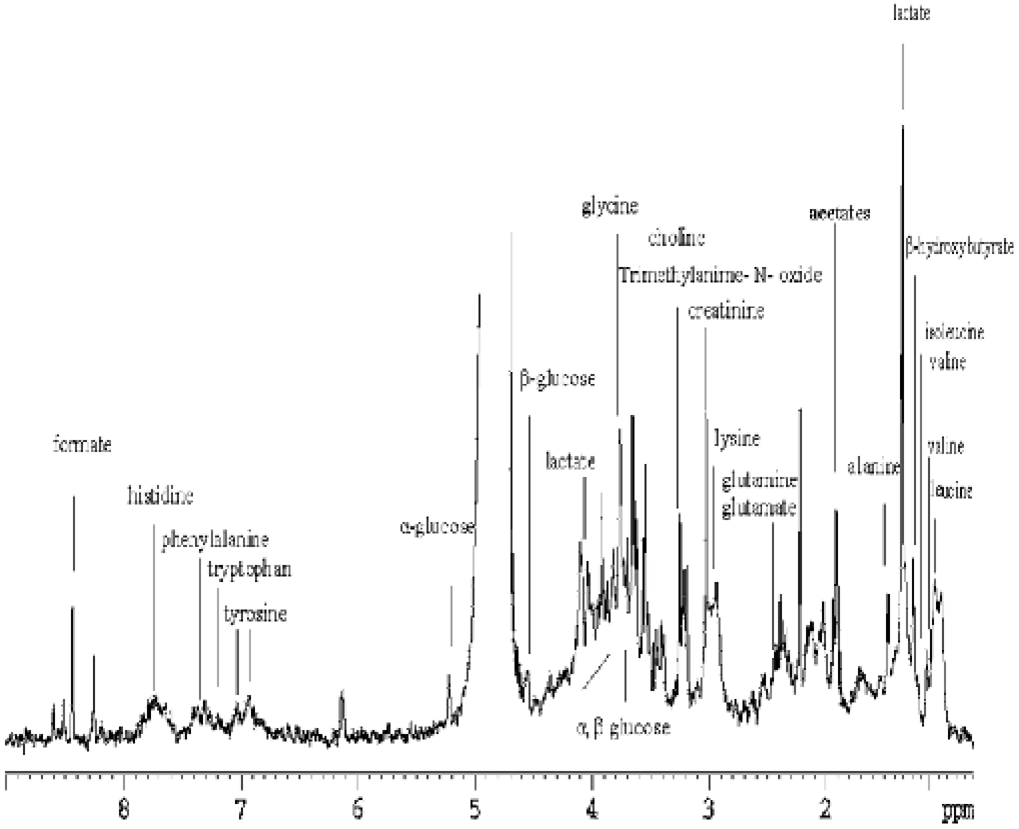
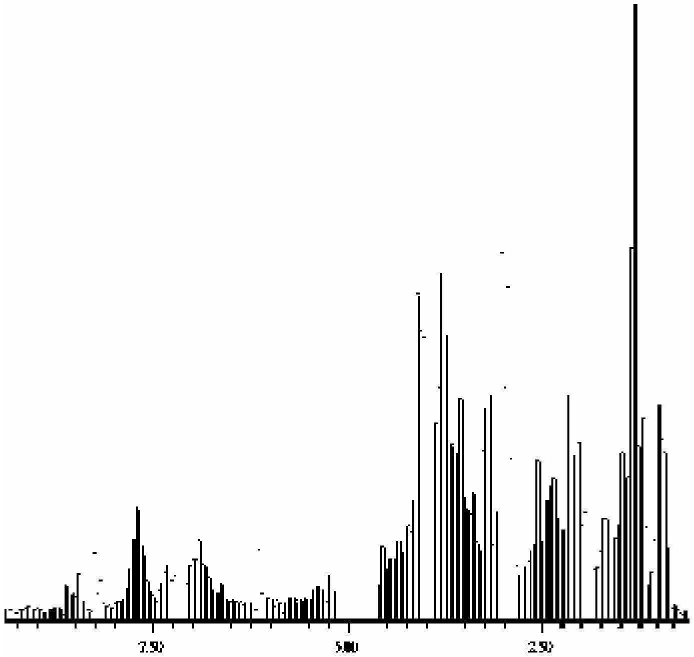


Metabonomics



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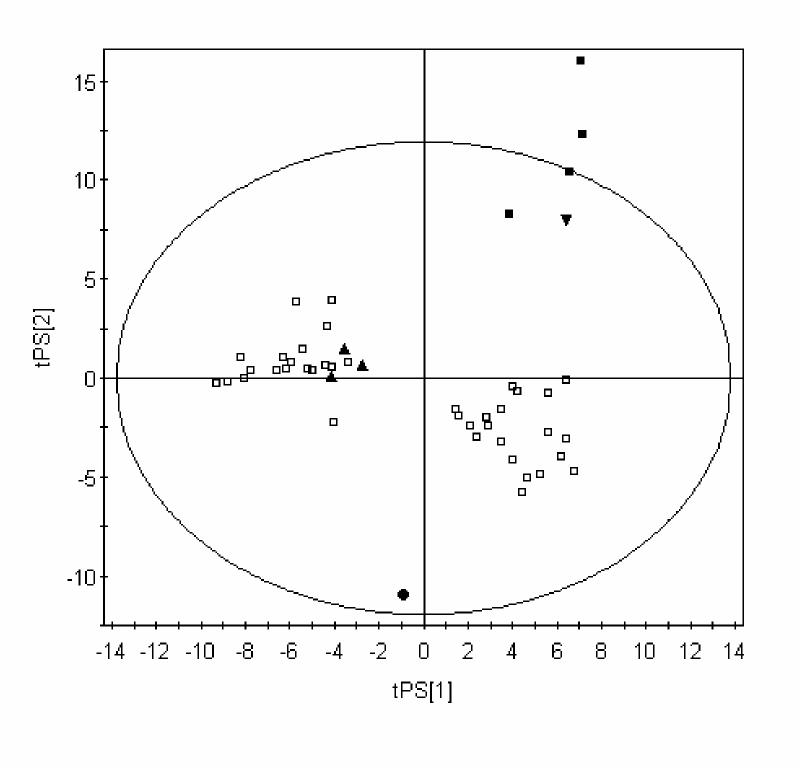
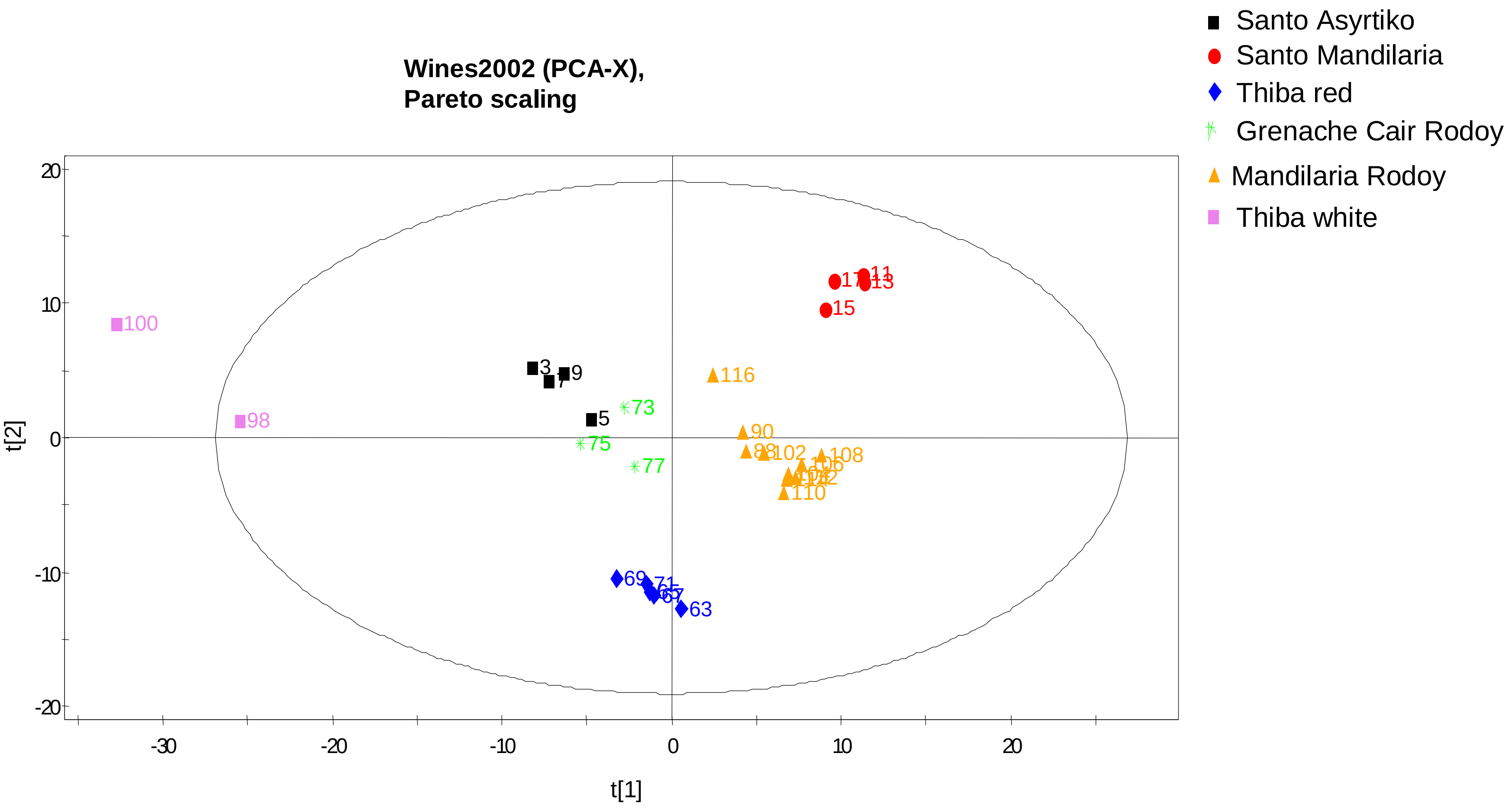
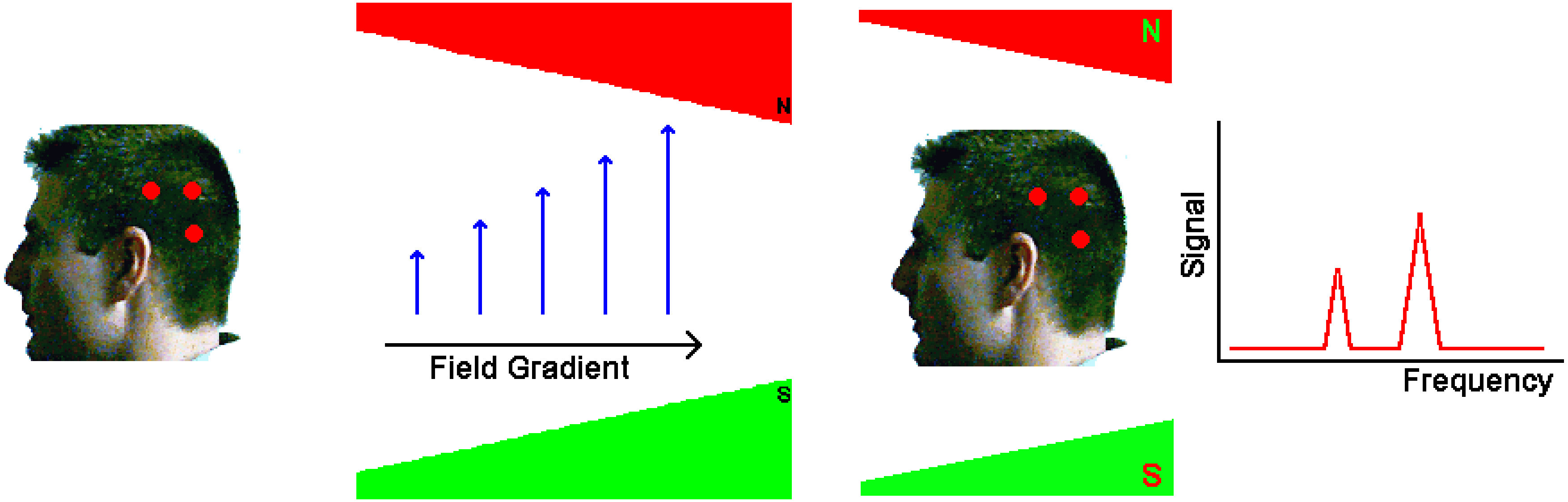


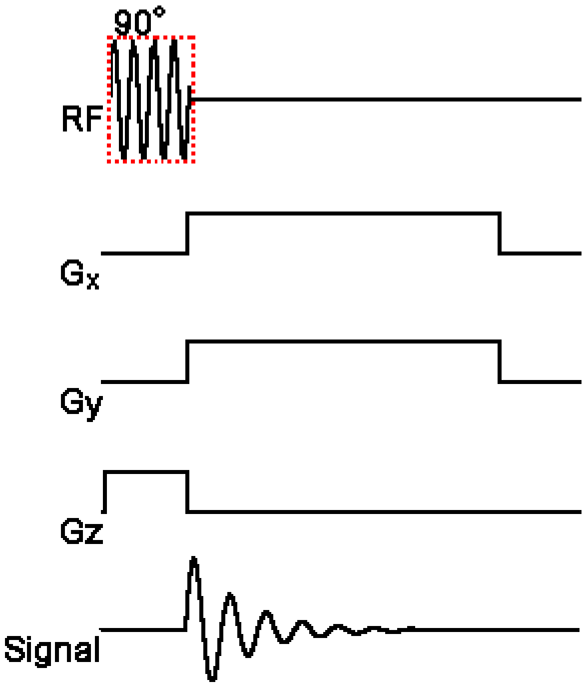
Metabonomics

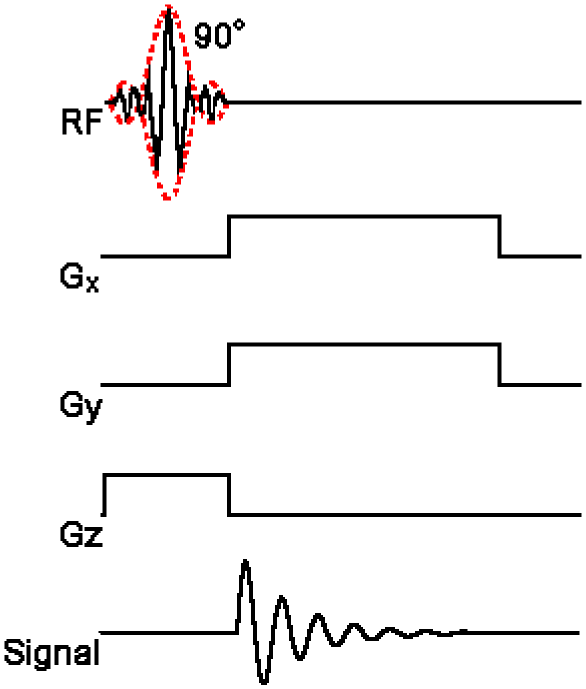


Imaging MRI

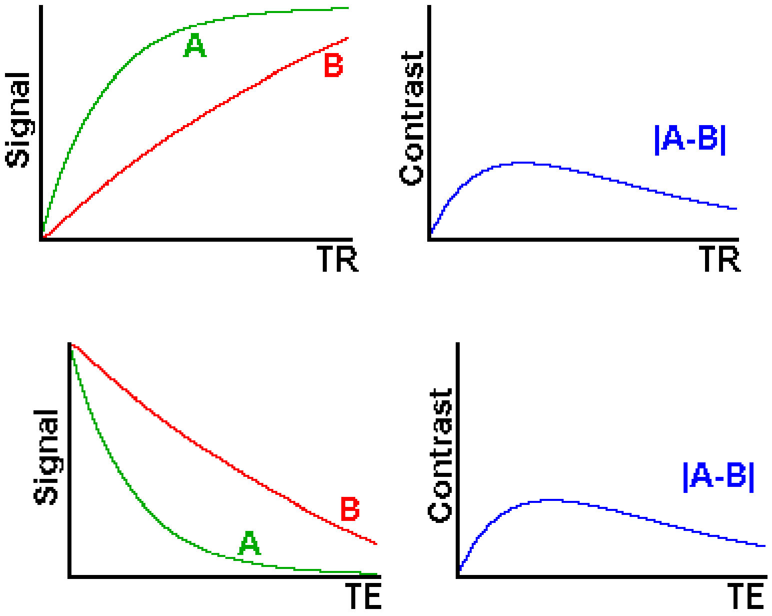


Imaging MRI

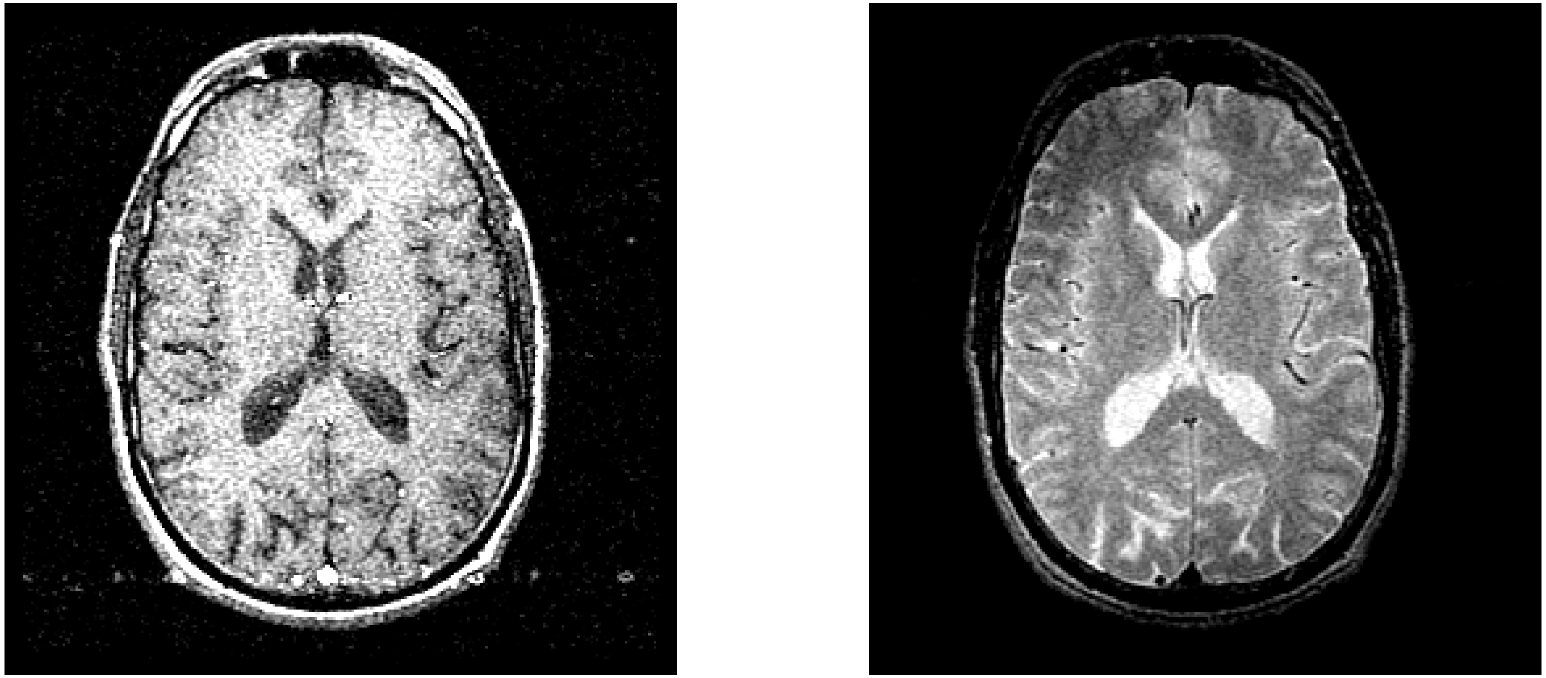


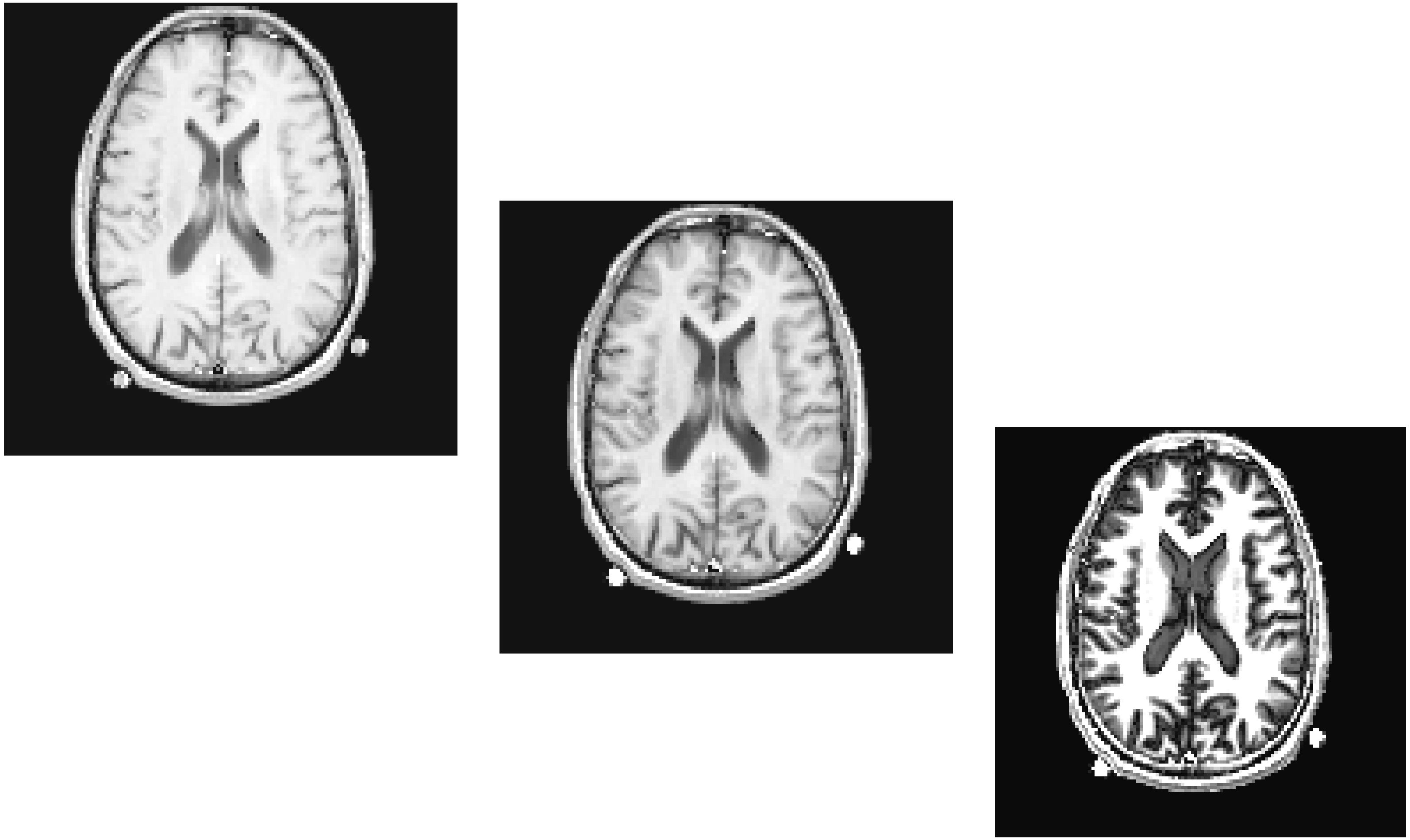


Relaxation contrast

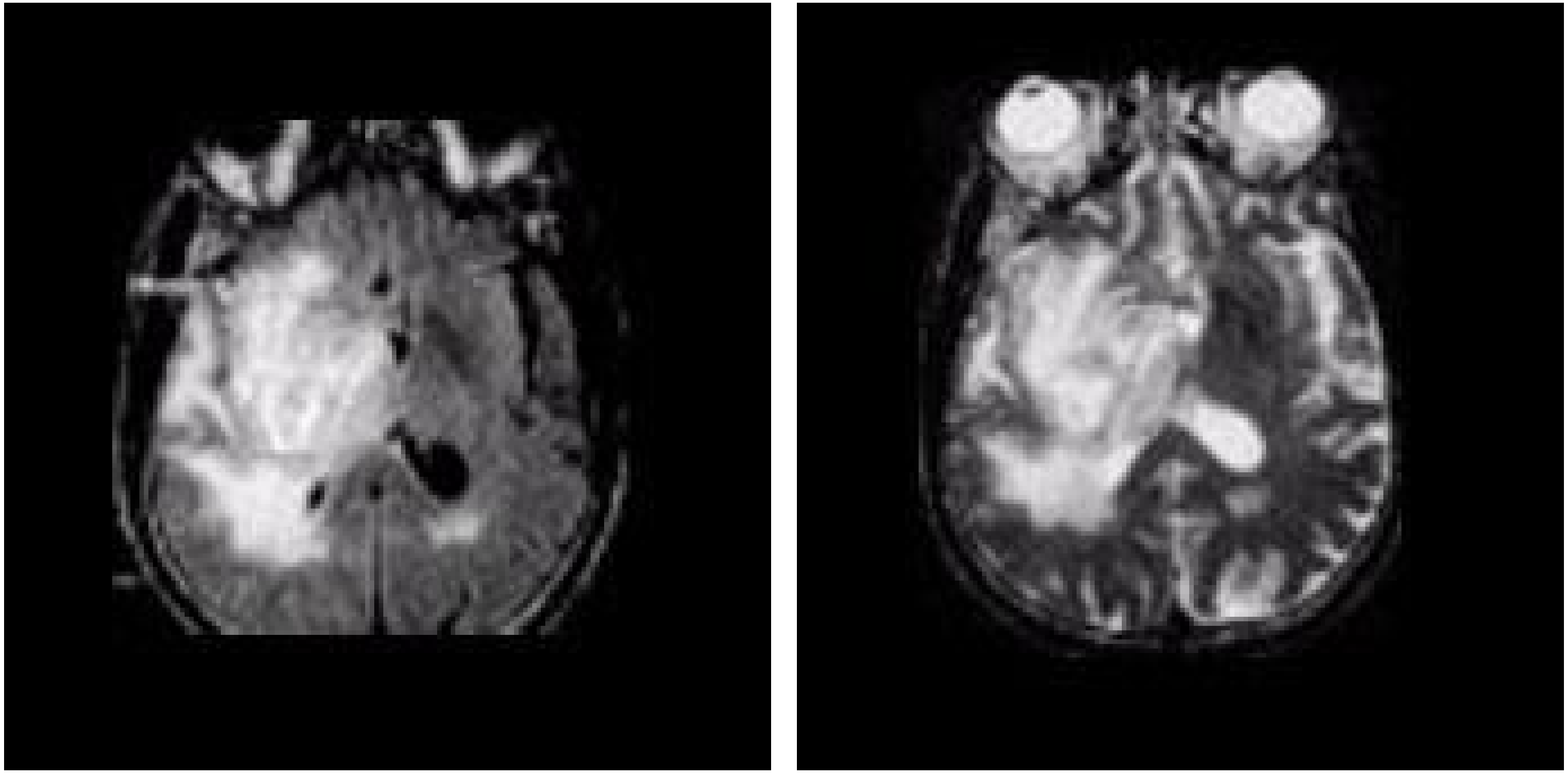


Imaging MRI



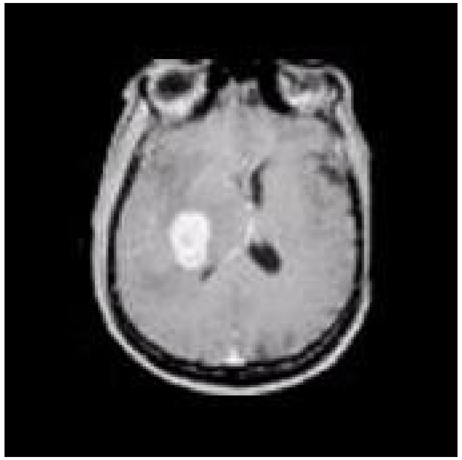


Imaging MRI



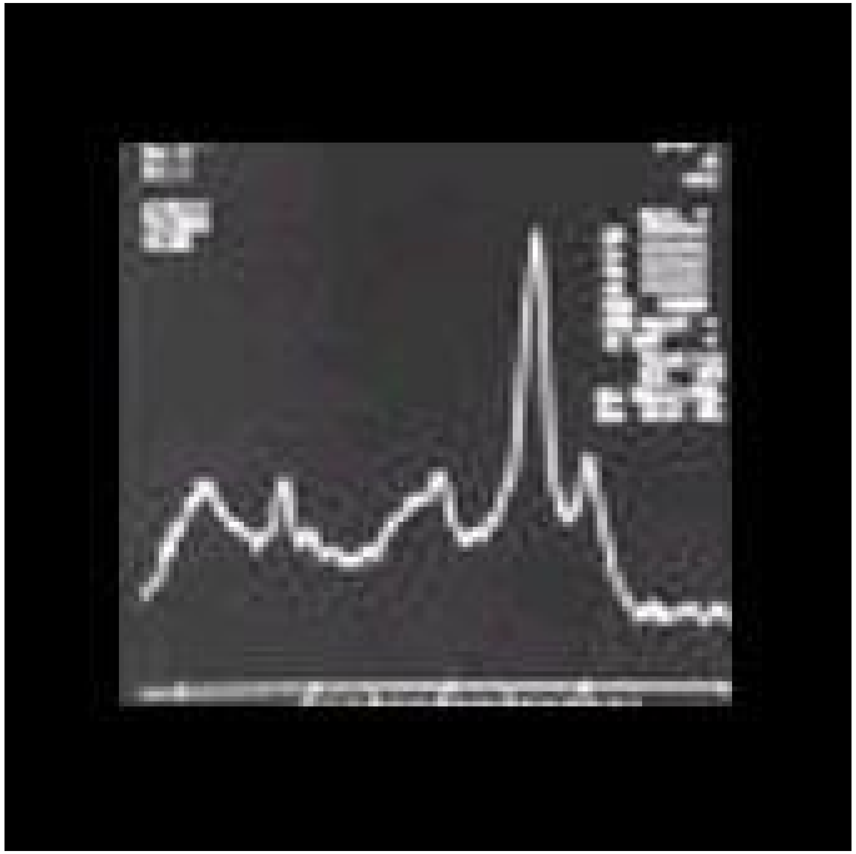
In vivo Spectroscopy

- A 57 year old male presents with left hemisensory deficit and increasing somnolence. MRI demonstrates an enhancing right ganglionic mass (Figure A) with central hyperintensity and surrounding edema on the T2-weighted and FLAIR images (Figures B & C).



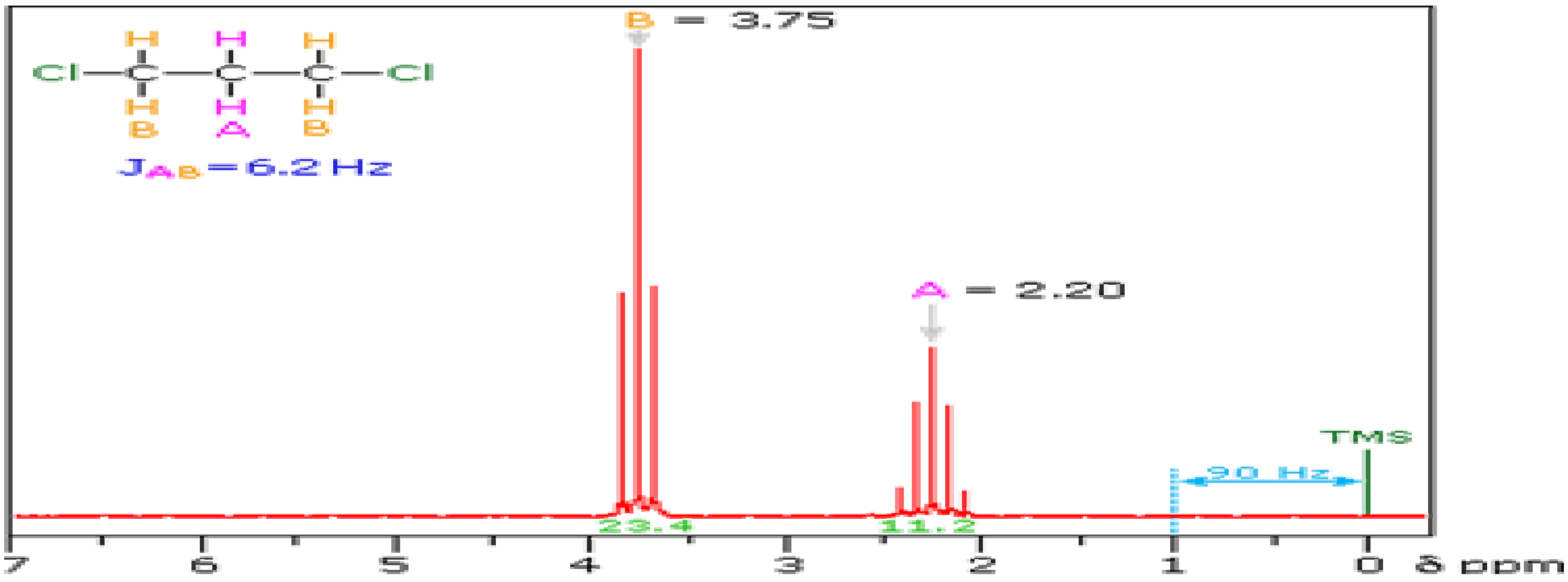
In vivo Spectroscopy

- Differential diagnosis favored a tumor, however, an abscess could not be excluded. Proton spectroscopy (PROBE-SV) (Figure D) demonstrated a surprising lack of choline (which would have indicated a tumor) with high lipid and lactate indicating membrane breakdown, necrosis, and anaerobic metabolism more suggestive of an abscess.

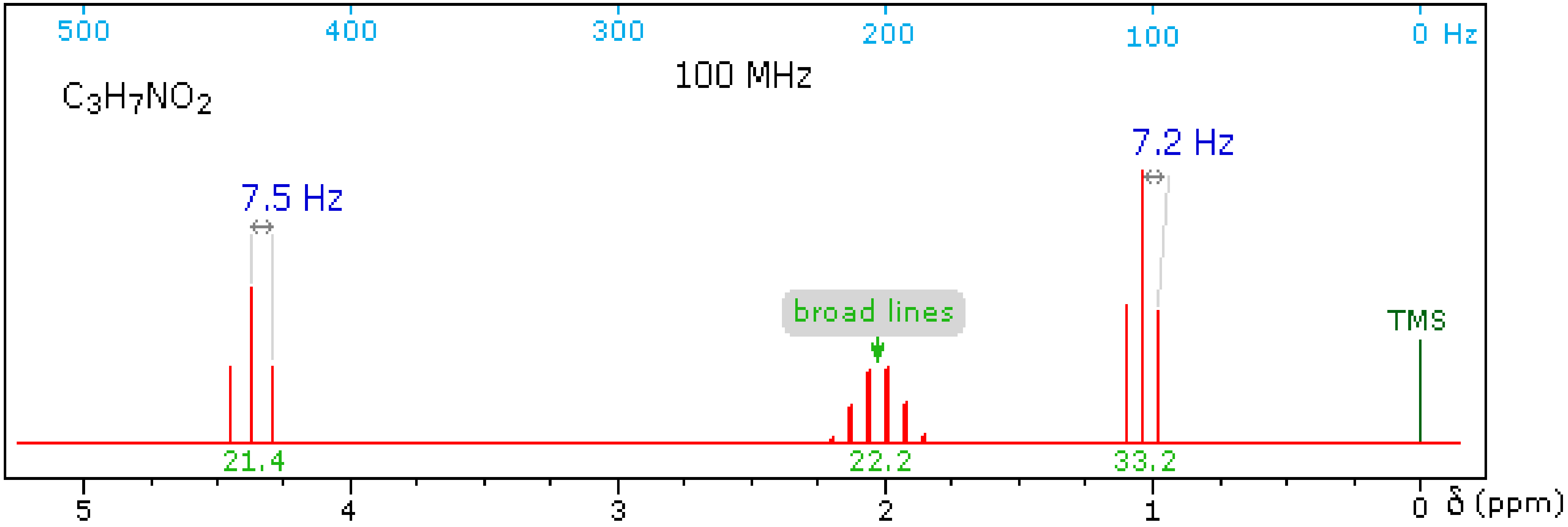


In vivo Spectroscopy

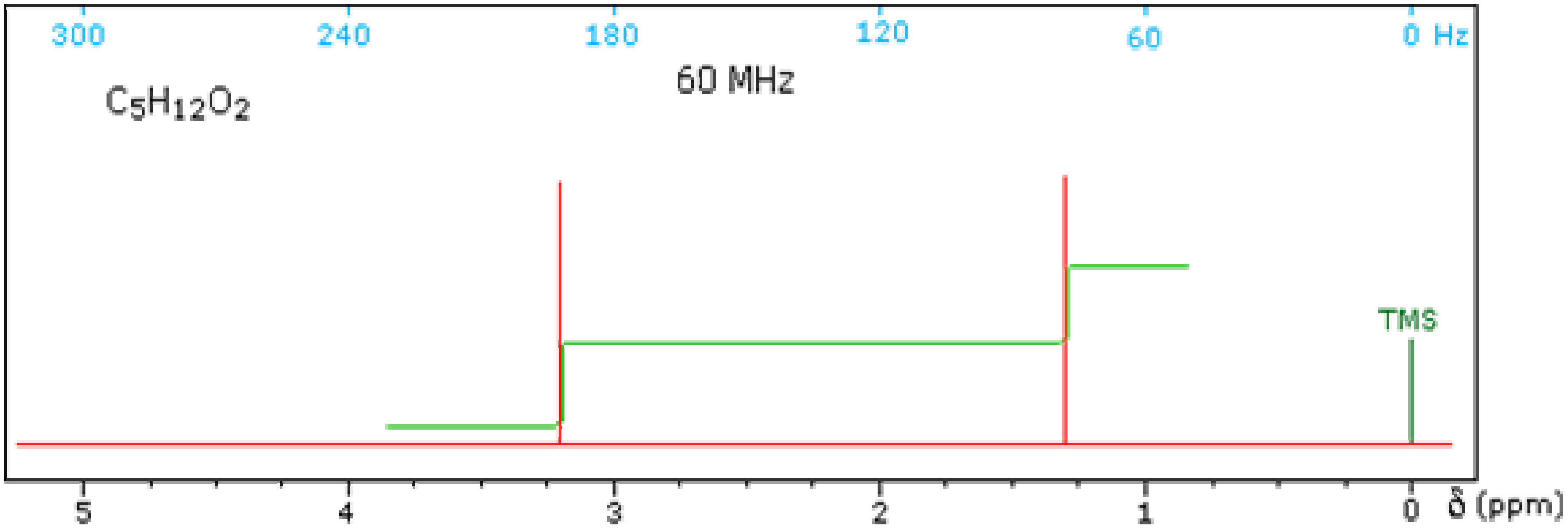
- As this was early in our experience, the neurosurgeon insisted on an MR-guided brain biopsy which yielded toxoplasmosis (Figure E).
- In AIDS patients, proton spectroscopy can often make the difference between biopsy and no biopsy for necrotic lymphoma vs. toxoplasmosis.

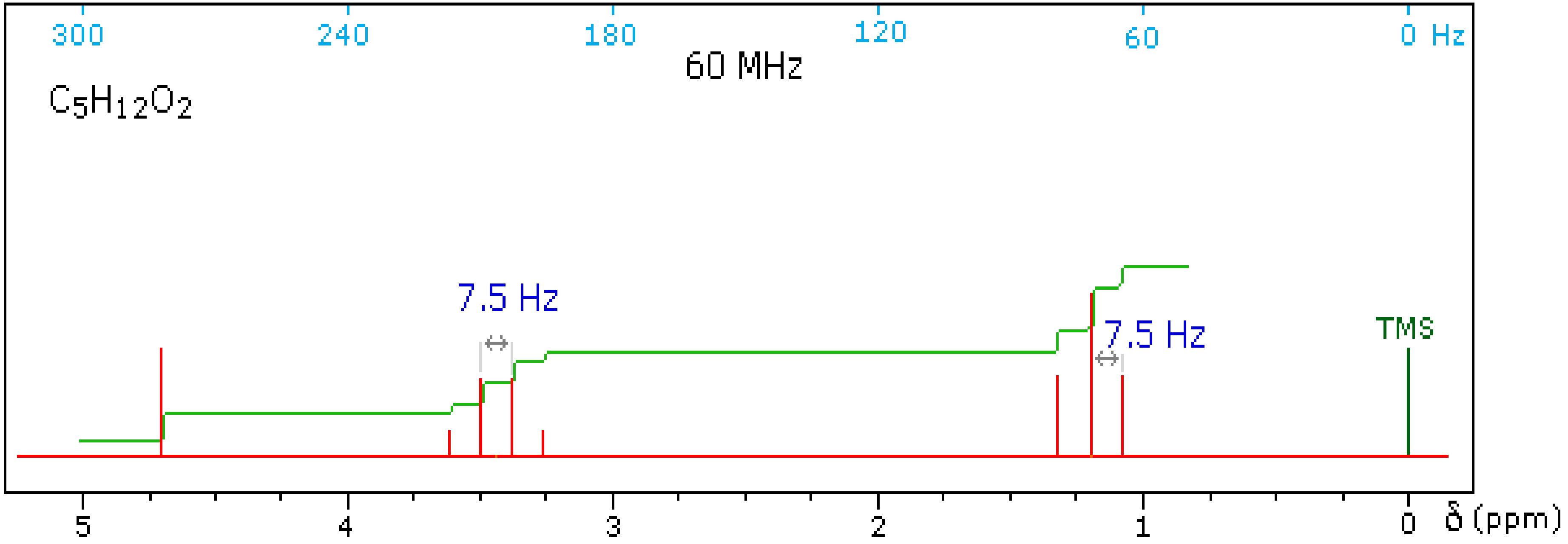


Cl–CH₂–CH₂–CH₂–Cl
H H H
H H H
B A B
J_AB = 6.2 Hz
B = 3.75
A = 2.20
TMS
90 Hz
23.4
11.2
7 6 5 4 3 2 1 0 δ ppm

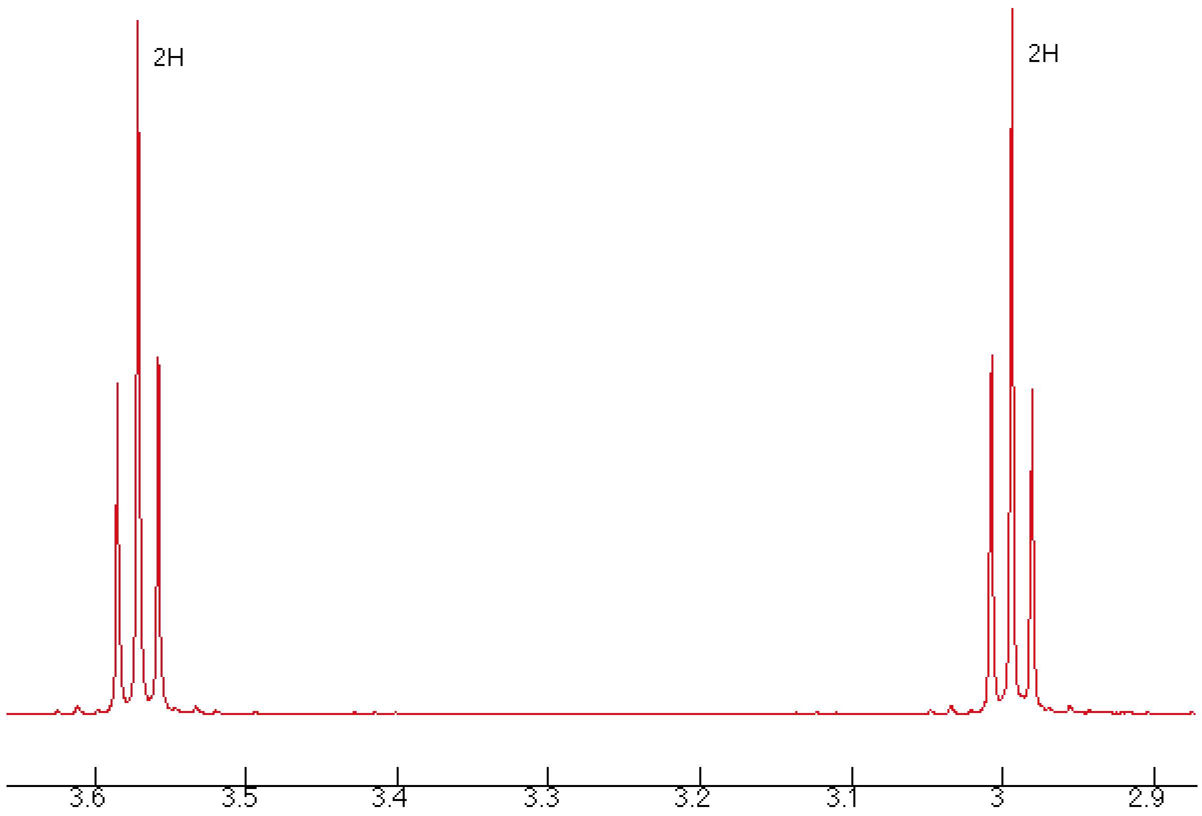


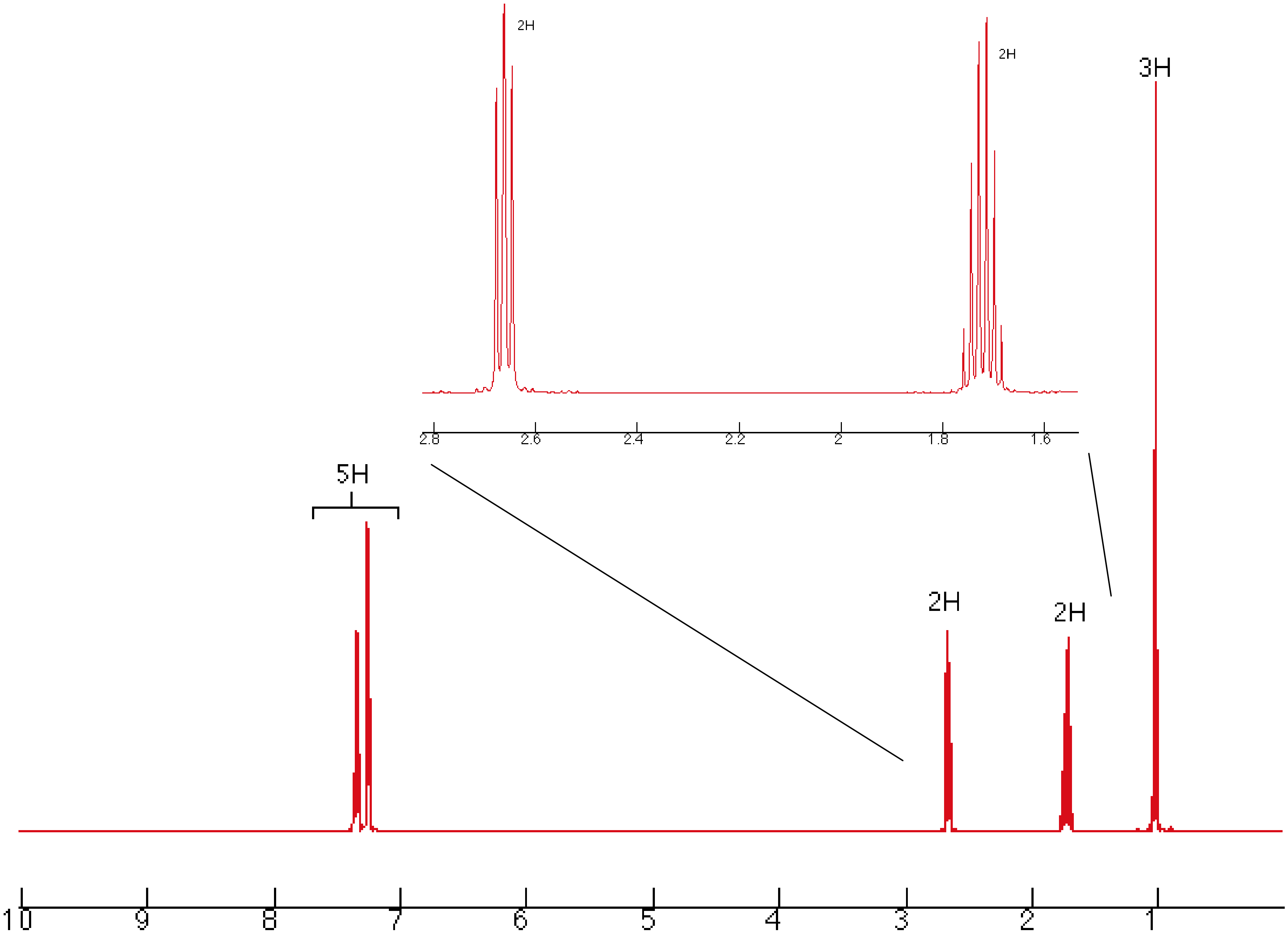
500 400 300 200 100 0 Hz
100 MHz
C3H7NO2
7.5 Hz
7.2 Hz
broad lines
TMS
21.4
22.2
33.2
5 4 3 2 1 0 δ (ppm)

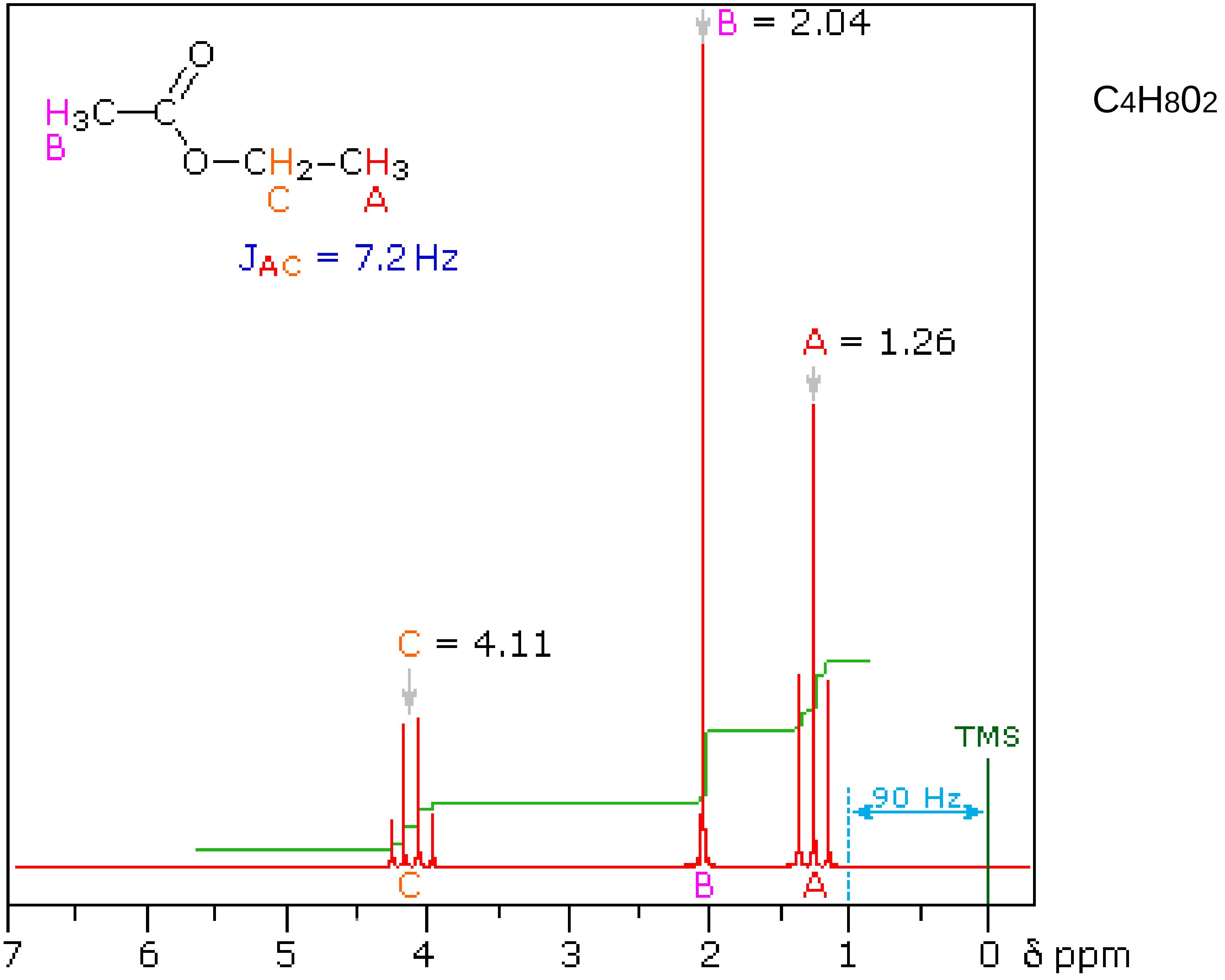

300
240
180
120
60
0 Hz
$C_5H_{12}O_2$
60 MHz
TMS
5
4
3
2
1
0
δ (ppm)

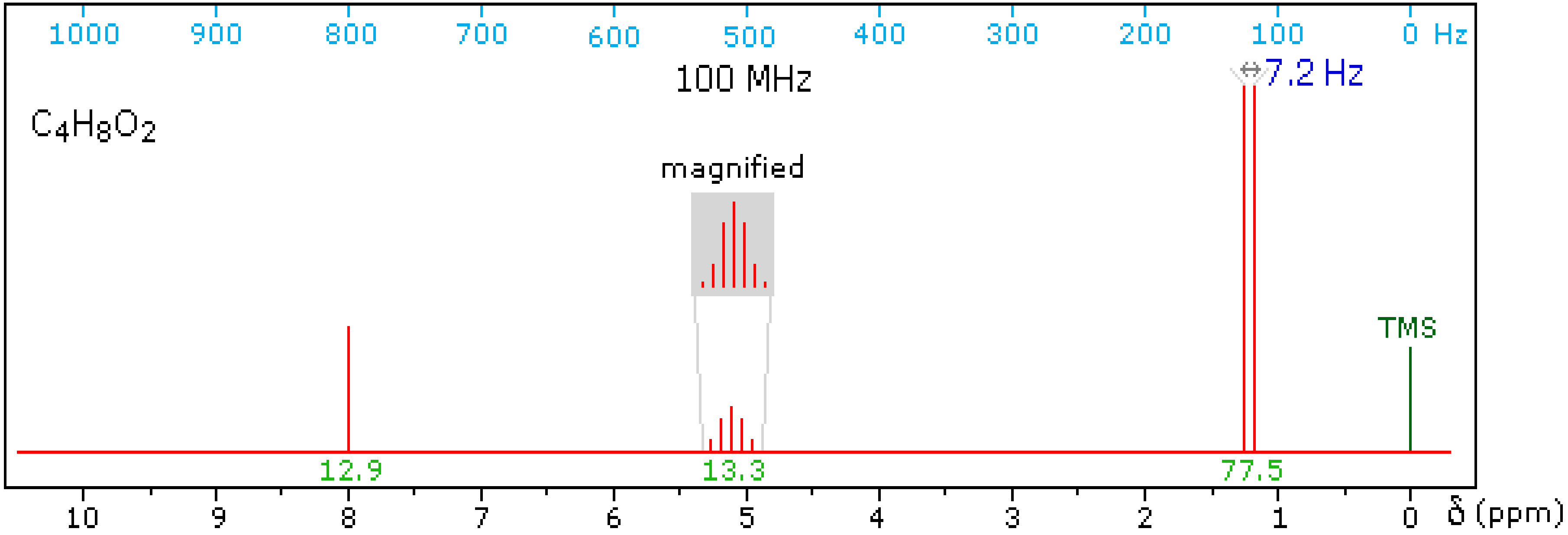

300
240
180
120
60
0 Hz
$C_5H_{12}O_2$
60 MHz
7.5 Hz
7.5 Hz
TMS
5
4
3
2
1
0
δ (ppm)

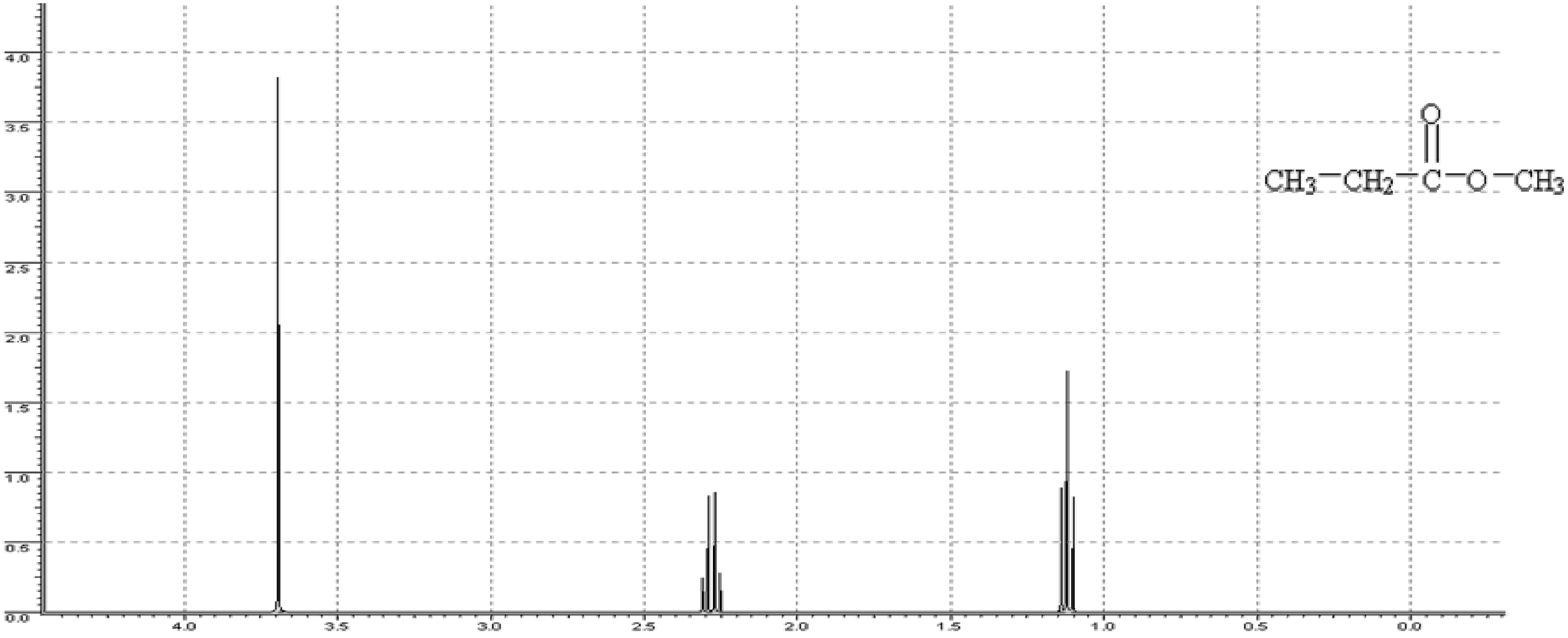
$C_3H_5BrO_2$

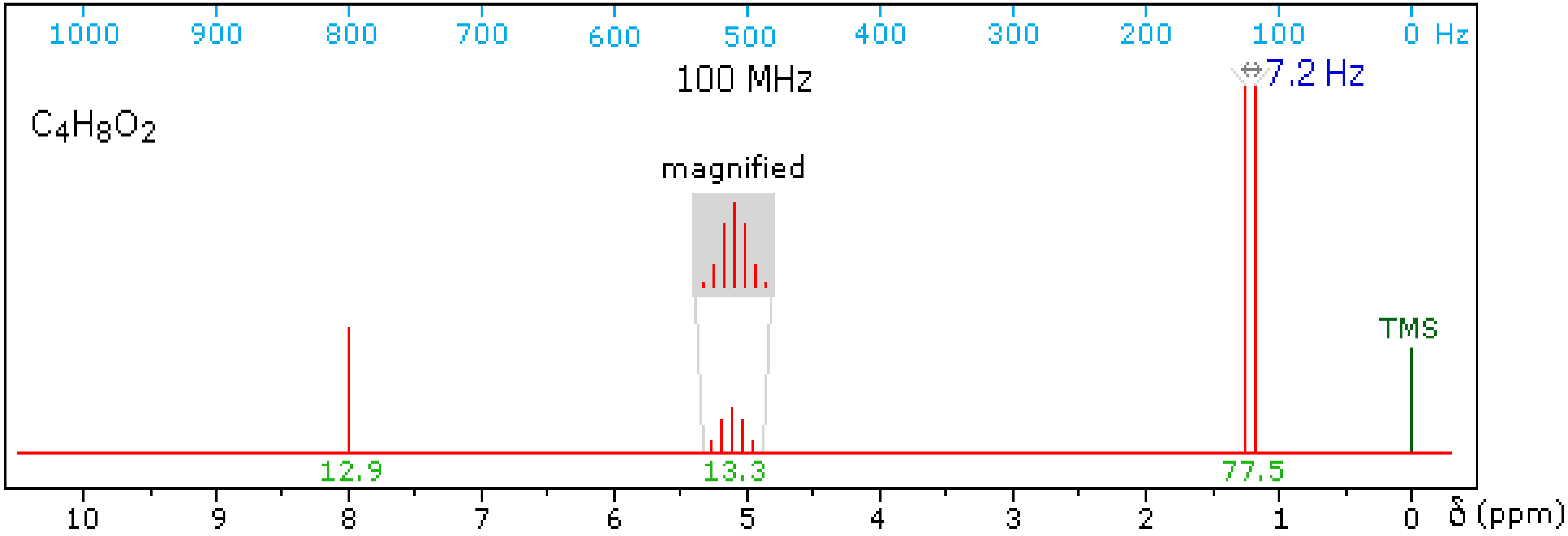



2H
2H
3H
2.8
2.6
2.4
2.2
2
1.8
1.6
5H
2H
2H
10
9
8
7
6
5
4
3
2
1

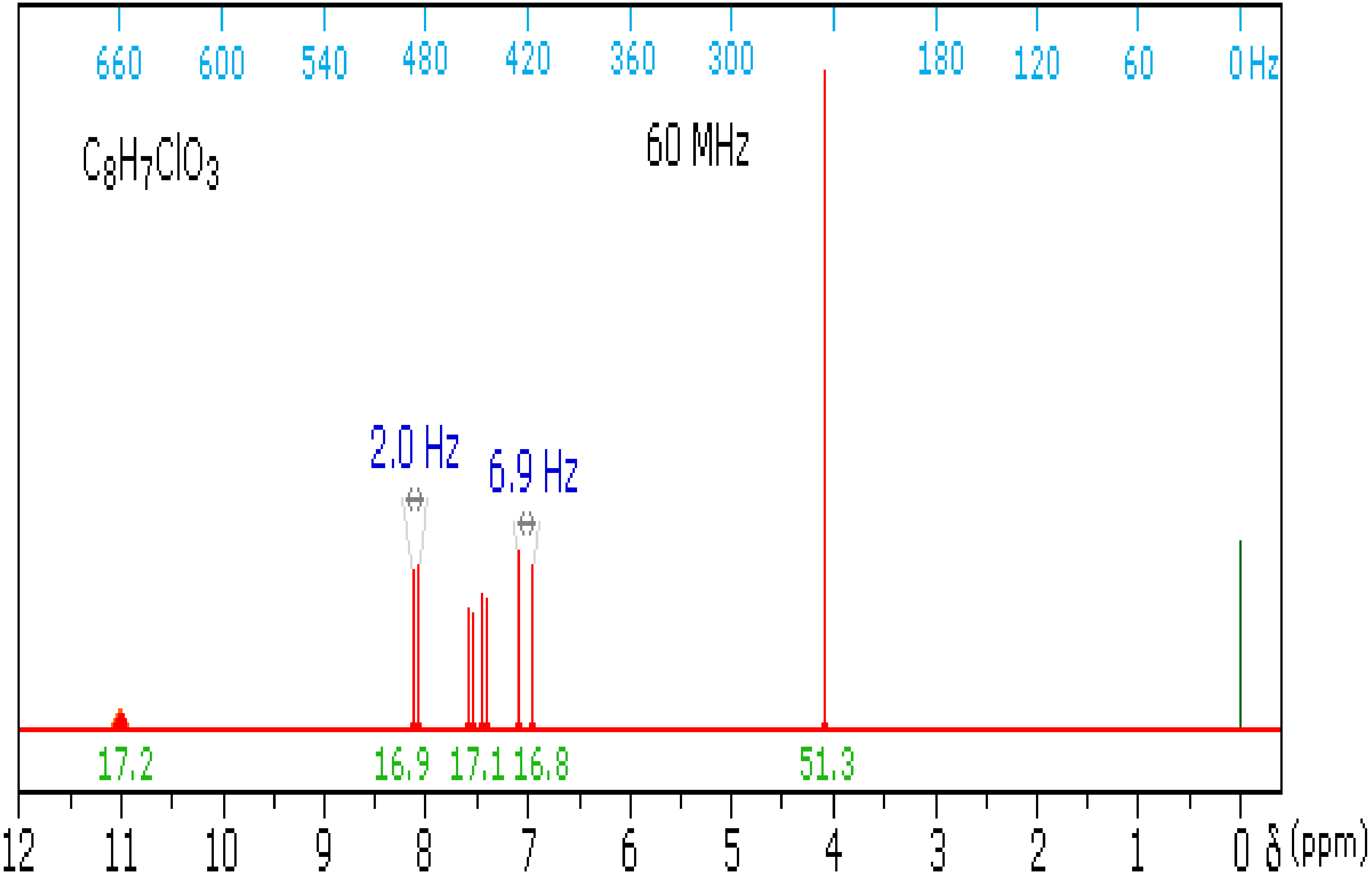

O
H3C—C
B
O—CH2—CH3
C
A
JAC = 7.2 Hz
B = 2.04
A = 1.26
C = 4.11
TMS
90 Hz
C
B
A
7
6
5
4
3
2
1
0
δ ppm
C4H8O2


1000
900
800
700
600
500
400
300
200
100
0 Hz
100 MHz
$C_4H_8O_2$
magnified
7.2 Hz
TMS
12.9
13.3
77.5
10
9
8
7
6
5
4
3
2
1
0
δ (ppm)

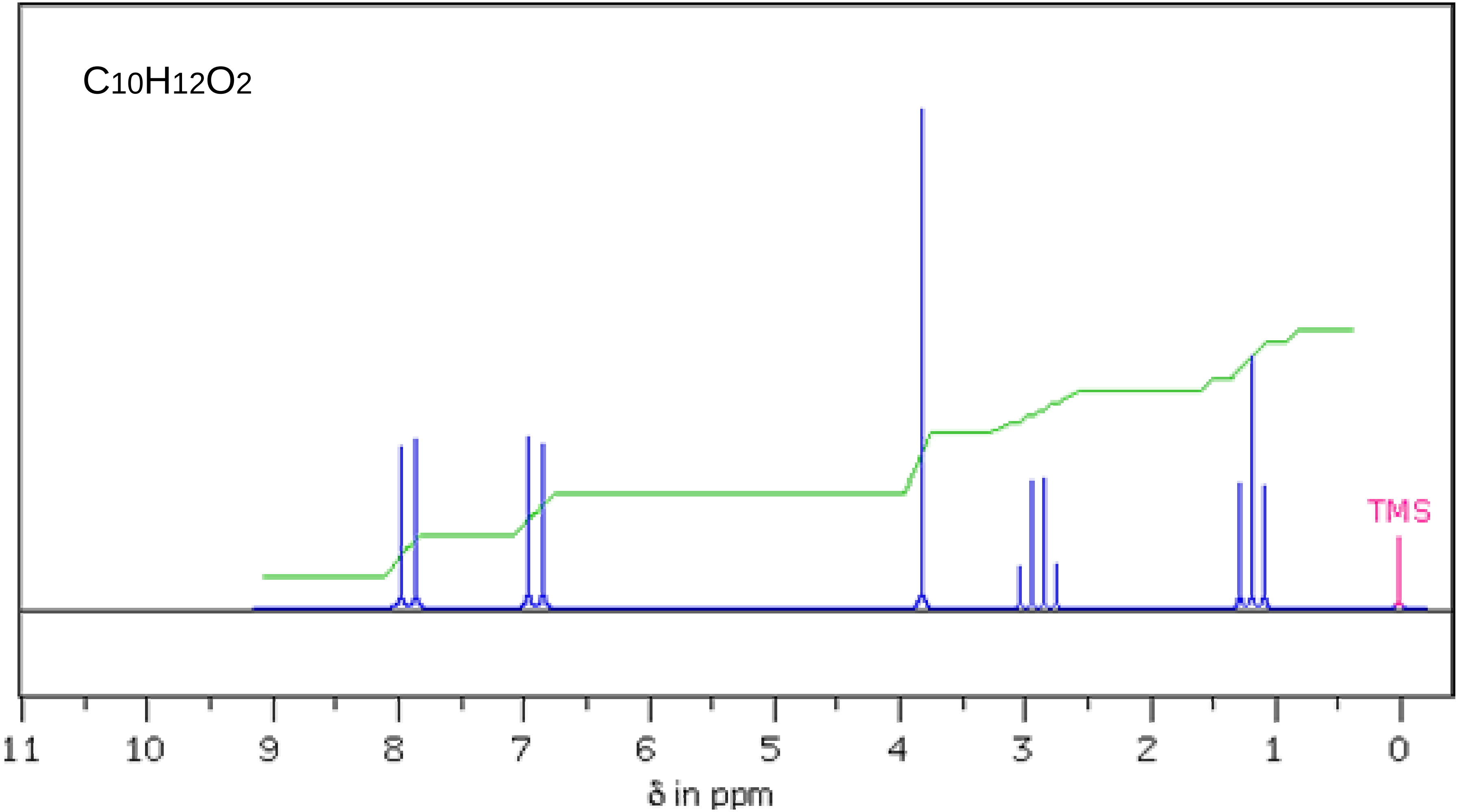

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
$CH_3-CH_2-C(=O)-O-CH_3$

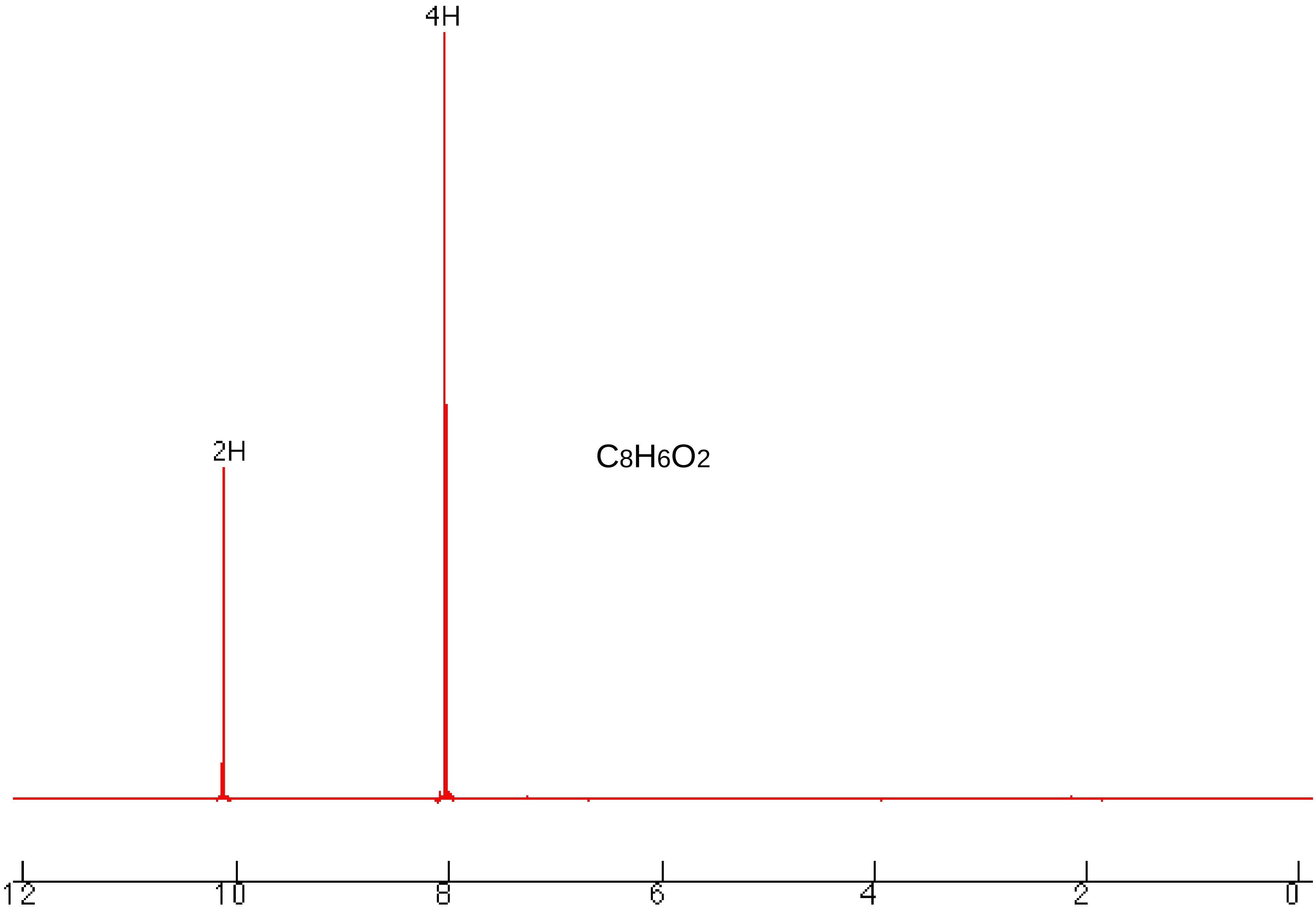


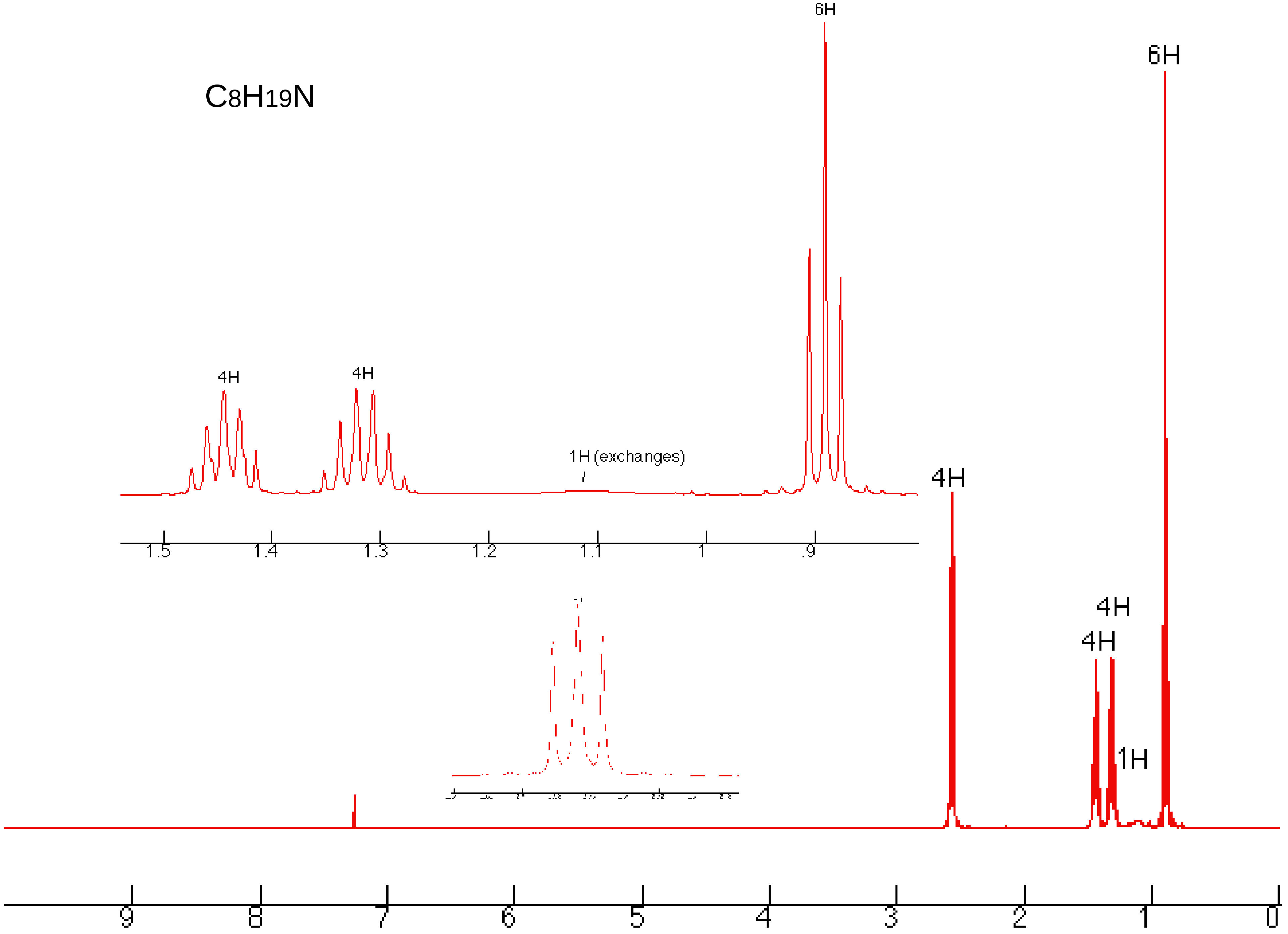
A $C_4H_8O_2$ compound has a strong infrared absorption at 1150 cm-1, but no absorption at 3300 to 3400 cm-1. It's 1H NMR spectrum shows a singlet at δ 3.55 ppm. The 13C NMR spectrum shows one signal at δ 66.5 ppm. Suggest a structure for this compound.

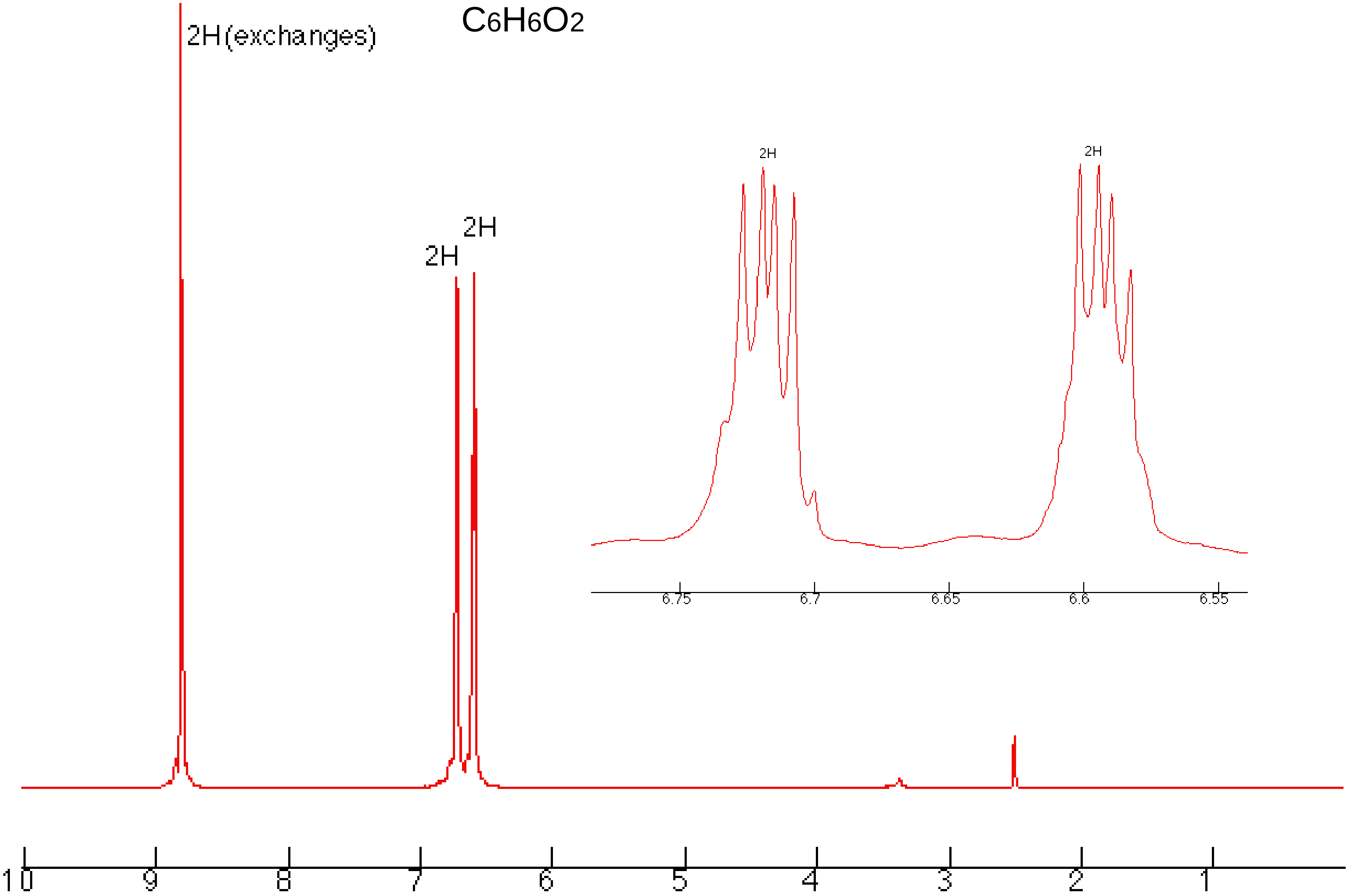


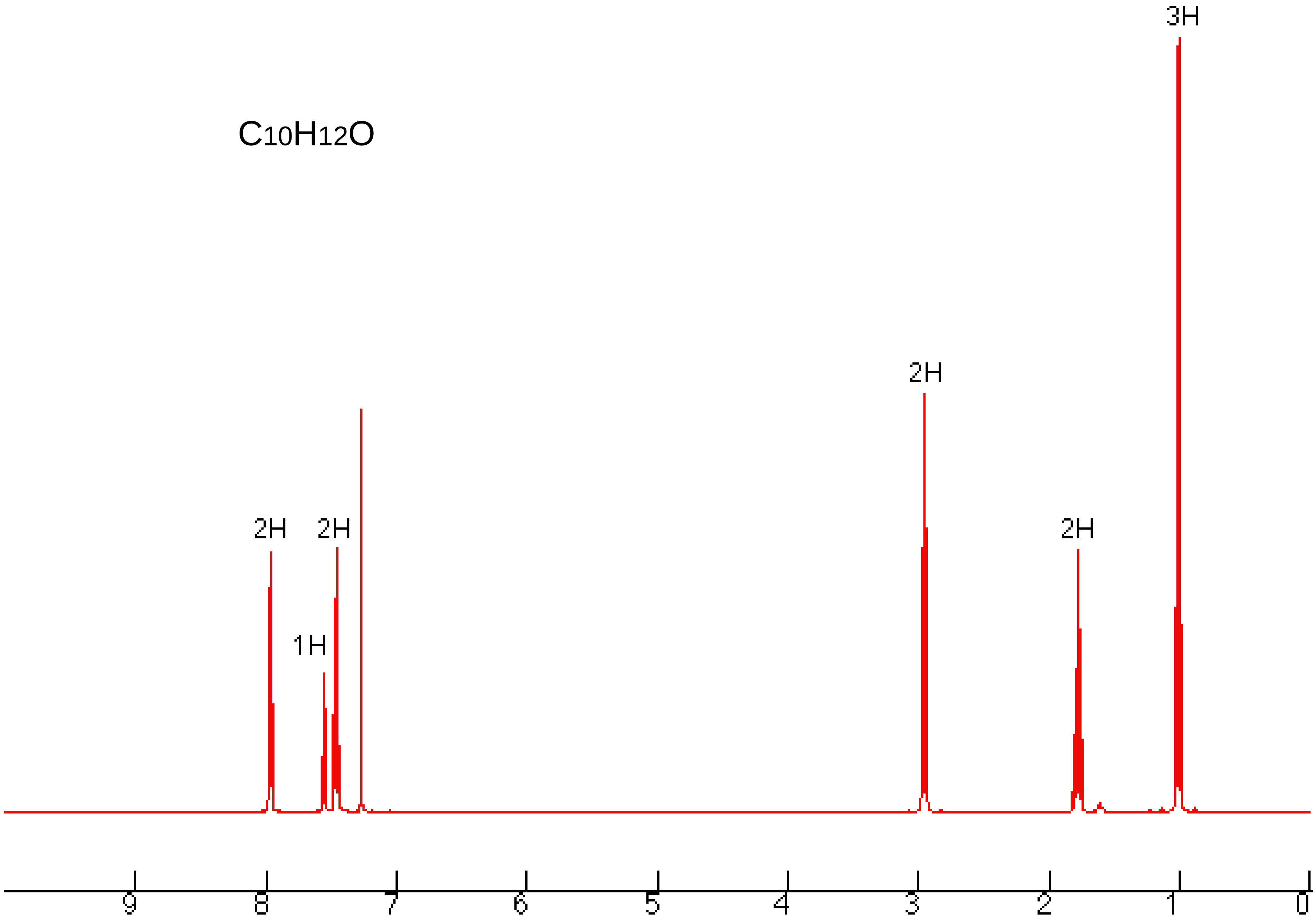
660
600
540
480
420
360
300
180
120
60
0 Hz
$C_8H_7ClO_3$
60 MHz
2.0 Hz
6.9 Hz
17.2
16.9
17.1
16.8
51.3
12
11
10
9
8
7
6
5
4
3
2
1
0
δ (ppm)


$C_{10}H_{12}O_2$
TMS
11
10
9
8
7
6
5
4
3
2
1
0
δ in ppm


4H
2H
$C_8H_6O_2$
12
10
8
6
4
2
0


C8H19N
4H
4H
1H (exchanges)
6H
1.5
1.4
1.3
1.2
1.1
1
.9
4H
4H
4H
1H
6H
9
8
7
6
5
4
3
2
1
0


$C_6H_6O_2$
2H(exchanges)
2H
2H
2H
2H
6.75
6.7
6.65
6.6
6.55
10
9
8
7
6
5
4
3
2
1



$C_{10}H_{12}O$