NMR Spectroscopy – Fundamental & Applications

TIGP Academia Sinica 2020

Instructor: Der-Lii M. Tzou

(02) 5572-8524

e-mail: tzougate@gate.sinica.edu.tw

Date: 2020-03-05 09:00 ~ 12:00

- ***NMR basics and principle***
  - (a) Nuclear spin and Larmor frequency
  - (b) Rotation spectroscopy
  - (c) Pulse NMR and Fourier transfer
  - (d) Chemical shielding effect (Chemical shift)
  - (e) J-coupling
- ***NMR applications***
  - (a) T1 and T2 relaxation
  - (b) 1D NMR spectroscopy: ^{1}H, ^{13}C, DEPT
  - (c) 2D NMR spectroscopy: COSY, NOESY, HSQC, HMBC
  - (d) ^{1}H, ^{13}C, ^{15}N NMR spectroscopy and high resolution multi-dimensional NMR

NMR 中央研究院化學研究所 核磁共振實驗室

中央研究院化學研究所
Institute of Chemistry, Academia Sinica

Nobel Laureates related to NMR

2013 M. Karplus in Chemistry

2003 P. C. Lauterbur and P. Mansfield in Medicine

2002 Kurt Wüthrich in Chemistry

1991 Richard R. Ernst in Chemistry

1952 Felix Block and E. M. Purcell in Physics

What is NMR ?

Nuclear Magnetic Resonance

N : Nuclei

M: Magnet

R: Resonance

Nuclear magnetic resonance; magnetic resonance

中央研究院化學研究所 核磁共振實驗室

中央研究院化學研究所
Institute of Chemistry, Academia Sinica

What NMR can do ?

NMR results from the *absorption of energy* by a nucleus changing its spin orientation in a *magnetic field*.

^{1}*H NMR* is the most commonly studied and a valuable analytical technique.

Two types of NMR interactions can be used to determine the 3d structure and dynamic state in solution phase. The molecular structure are determined via through-bond spin-spin & through- space *NOE.*

NMR resolution & Sensitivity

The method exploits the magnetic nuclei in molecules and the sensitivity of their NMR transitions to

(a). ***the electronic environments***

(b). ***geometric dispositon*****, in terms of the number of bonds and the distance directly through spaces**

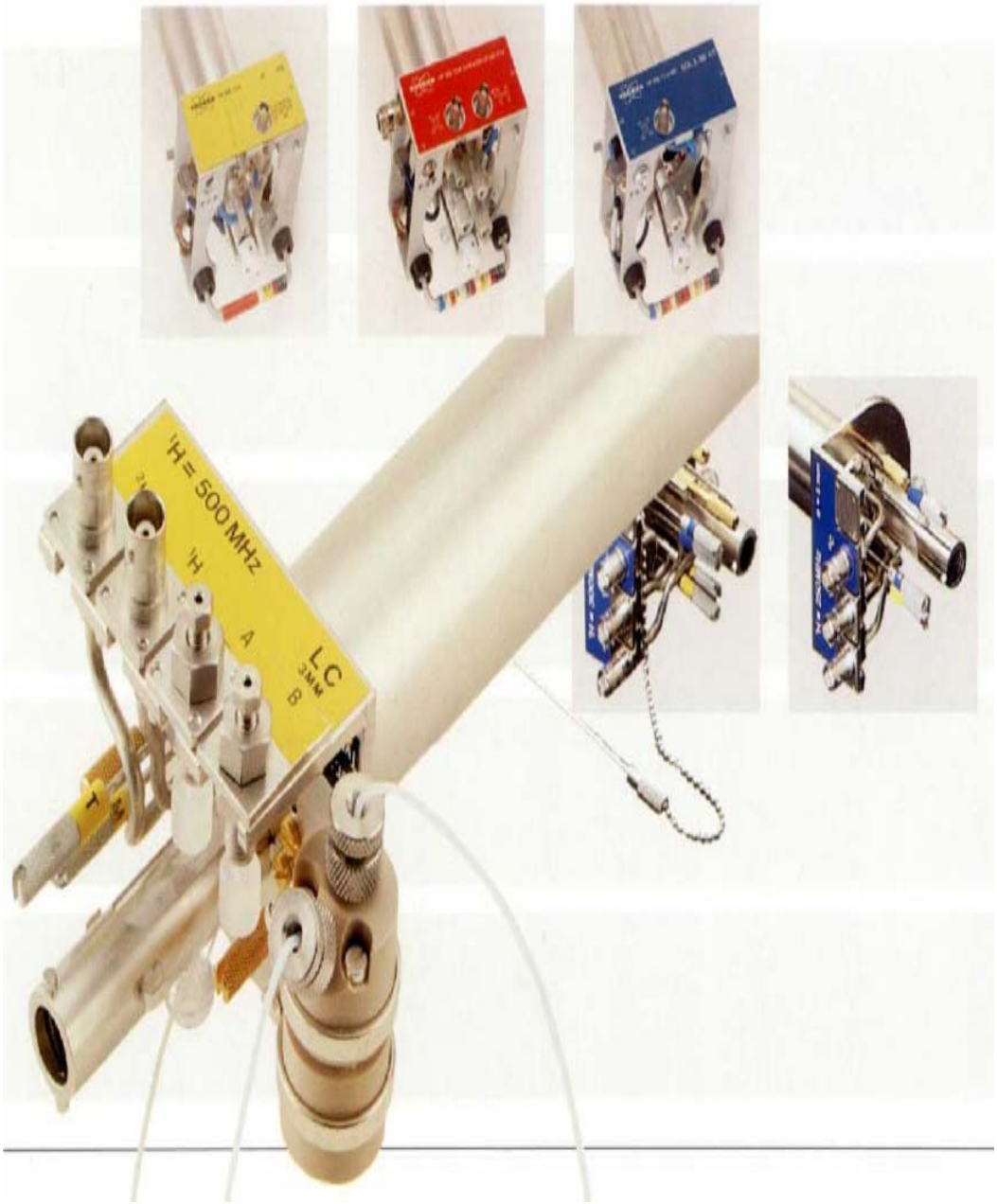
(c). ***molecular motion*** **or** ***dynamic state***

NMR Instrument

中央研究院化學研究所
核磁共振實驗室

中央研究院化學研究所
Institute of Chemistry, Academia Sinica

Probe-head



Nuclear spin in the absence of magnetic field

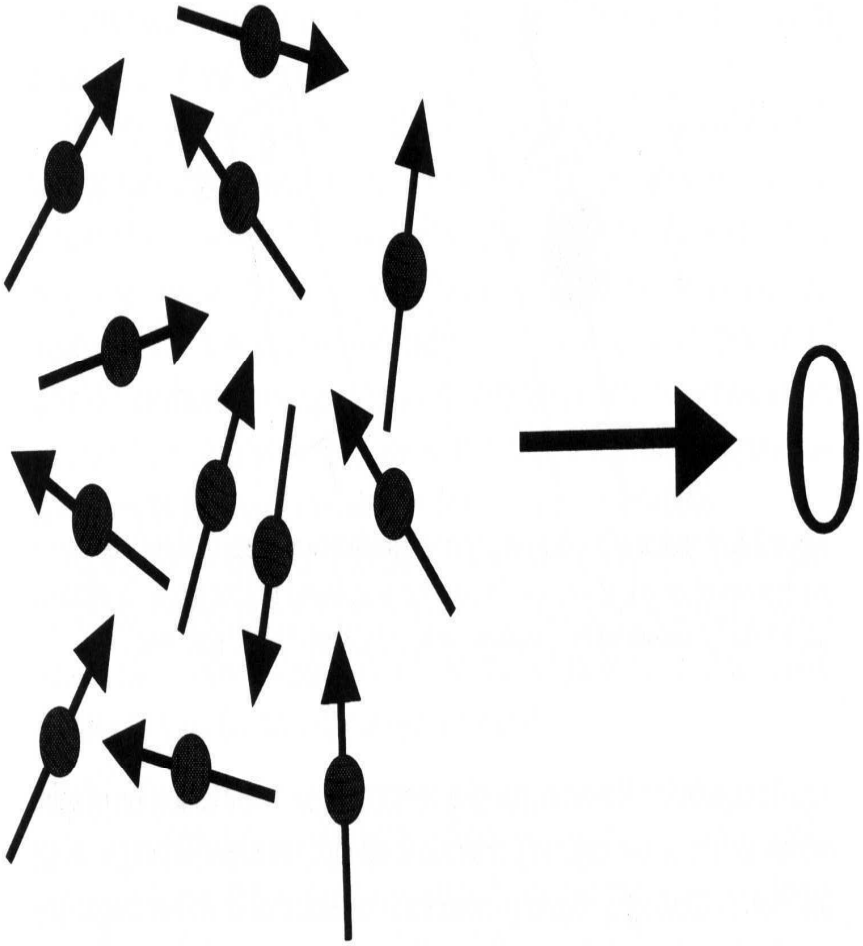
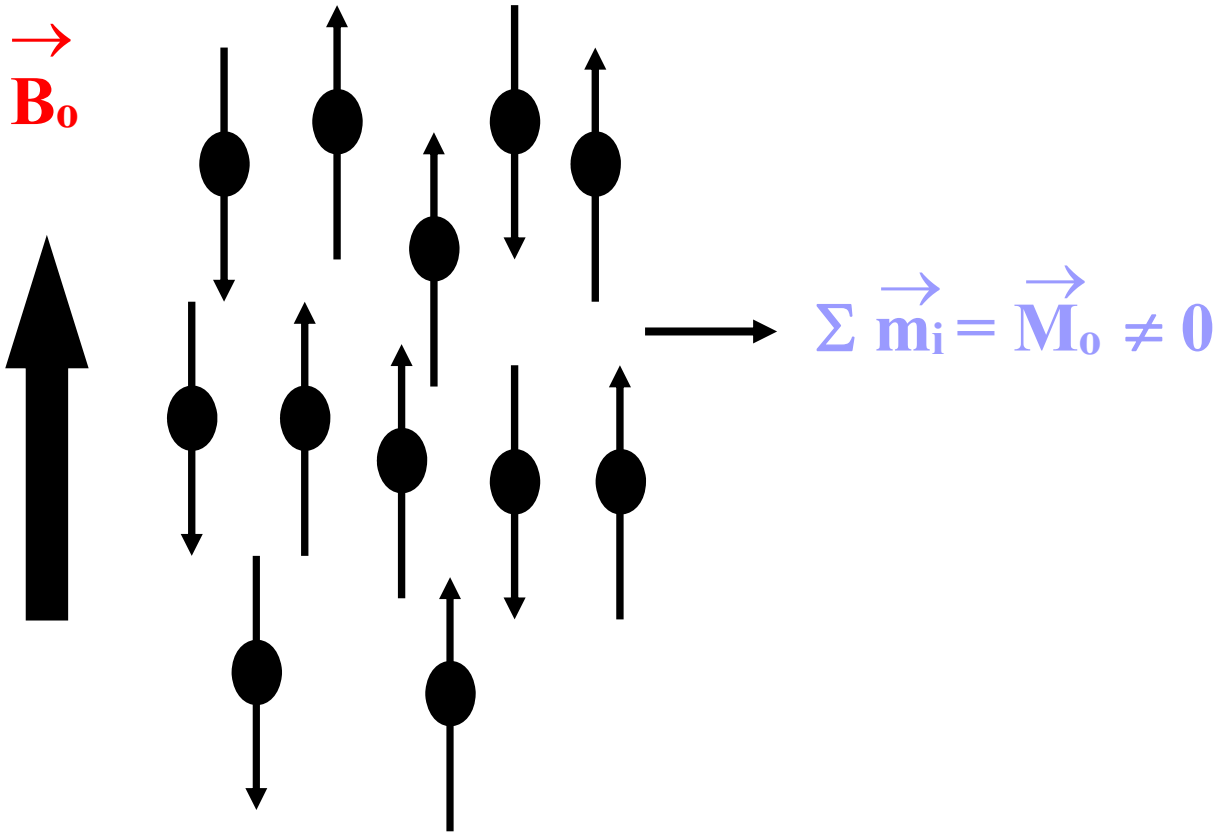


Figure 2.2. Microscopic picture and macroscopic picture of a collection of spins in the absence of an external magnetic field. In the absence of a magnetic field, the spins will have their spin vectors oriented randomly (microscopic picture). The vector sum of these spin vectors will be zero (macroscopic picture).

Nuclear spin in the presence of magnetic field

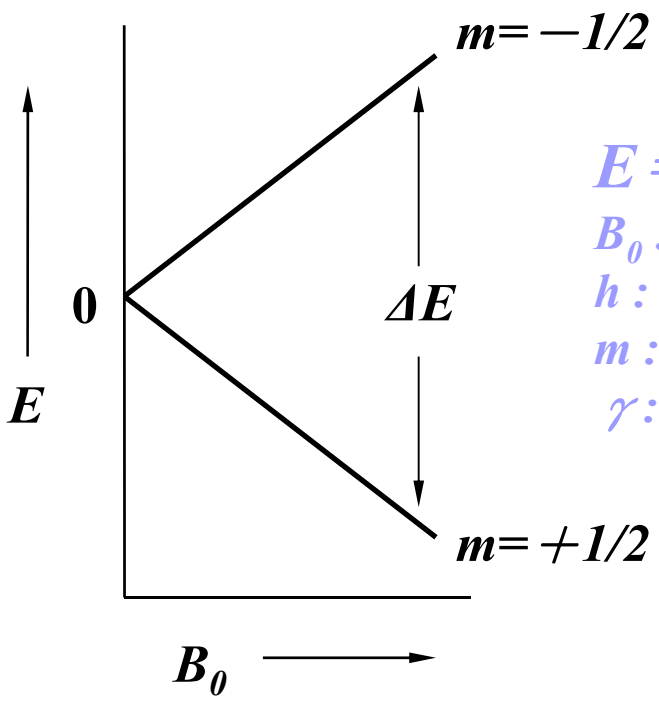


$\vec{B}_o \gg \vec{M}_o$

Number of spin states (2I＋1)

A nucleus with spin I can have 2I＋1 spin states. Each of these states has its own spin quantum number m (m＝－I,－I＋1,…, I－1, I). For nuclei with I＝1/2, only two states are possible : m＝＋1/2 and m＝－1/2.

Nuclear Zeeman effect :



$$E = -mB_0(\gamma h/2\pi)$$

B_0 : magnetic field strength
h : Planck's constant
m : spin quantum number
γ : magnetogyric ratio

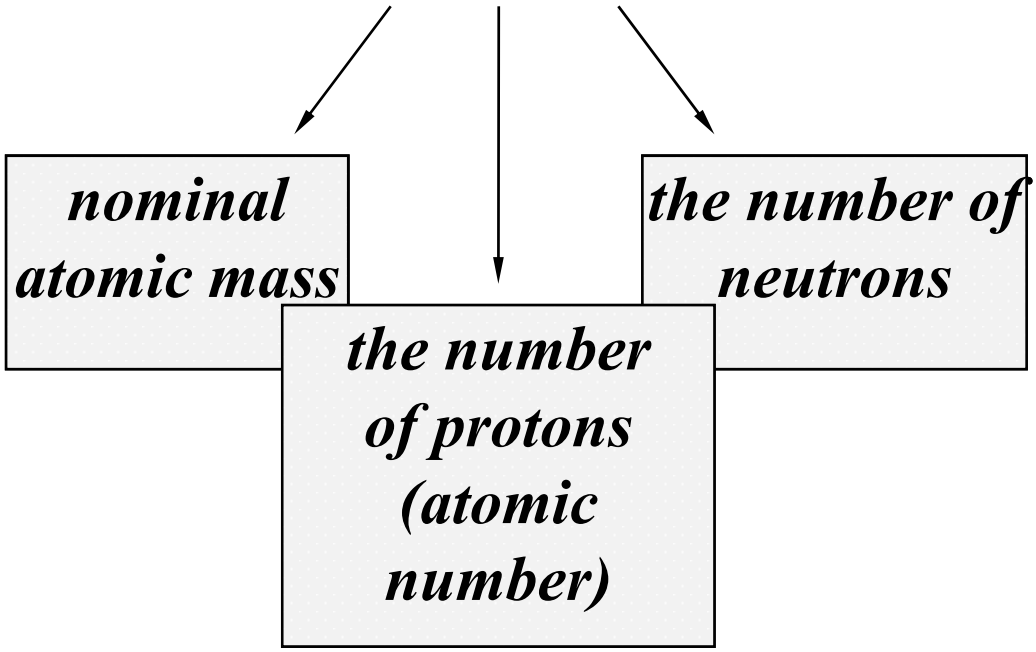
Larmor Frequency

$$\Delta E = \gamma \hbar B_0 = h\nu$$

$$\nu = \frac{\gamma}{2\pi} B_0$$

Nuclear spin

$$A = Z + N$$



Nuclear spin

A nucleus with an even mass A and even charge Z, and therefore also an even N, will have a nuclear spin I of zero. (^{12}C, ^{16}O, and ^{18}O).

$$N + Z\,(P) = A$$

^{12}C : 6 + 6 (6) = 12
^{16}O : 8 + 8 (8) = 16
} I = 0

A nucleus with an even mass and odd charge (both Z and N odd) will exhibit an integer value of I. (^{2}H(I=1), ^{14}N(I=1), and ^{10}B(I=3))

^{14}N : 7 + 7 (7) = 14
^{2}H : 1 + 1 (1) = 2
} I = 1

Nuclear spin

A nucleus with odd mass (Z odd and N even, or Z even and N odd) will have nuclear spin with an I value that we can express as n/2, where n is an odd integer. ($^{1}H(I=1/2)$, $^{13}C(I=1/2)$, and $^{17}O(I=5/2)$)

^{1}H : 0 + 1 (1) = 1
^{13}C: 7 + 6 (6) = 13
} I = 1/2

❖**Nuclei with I = 0 cannot be detected by NMR.**

❖**Nuclei with I≠ 0 can be detected by NMR.**

❖**Different isotopes of the same element have different nuclear spins, some are detectable by NMR, some are not.**

Frequently used nuclei in NMR applications

Isotope	Spin	Frequency (MHz) at 11.74 T	Nature abundance(%)	Relative sensitivity
^{1}H	1/2	500.0	99.98	1.00
^{2}H	1	76.7	1.5×10^{-2}	9.65×10^{-3}
^{3}H	1/2	533.3	0	1.21
^{12}C	0	------	98.89	------
^{13}C	1/2	125.7	1.108	1.59×10^{-2}
^{14}N	1	36.1	99.63	1.01×10^{-3}
^{15}N	1/2	50.7	0.37	1.04×10^{-3}
^{16}O	0	------	~100	------
^{17}O	5/2	67.8	3.7×10^{-2}	2.91×10^{-2}
^{19}F	1/2	470.4	100	0.83
^{31}P	1/2	202.4	100	6.63×10^{-2}

Free Induction Decay (FID)

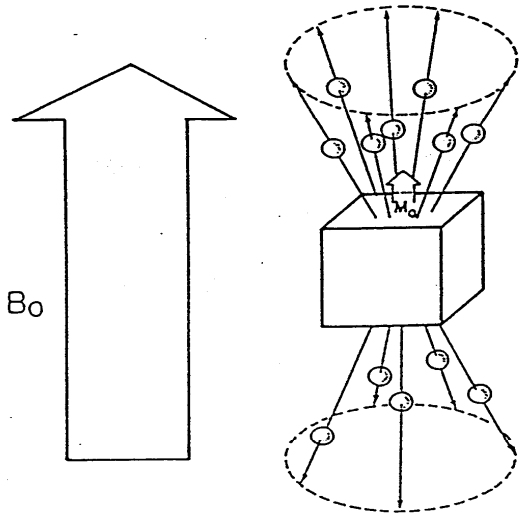


Figure 5. The net magnetization (M_0) resulting from the imbalance of hydrogen nuclear dipoles points along B_0 but is small compared with B_0.

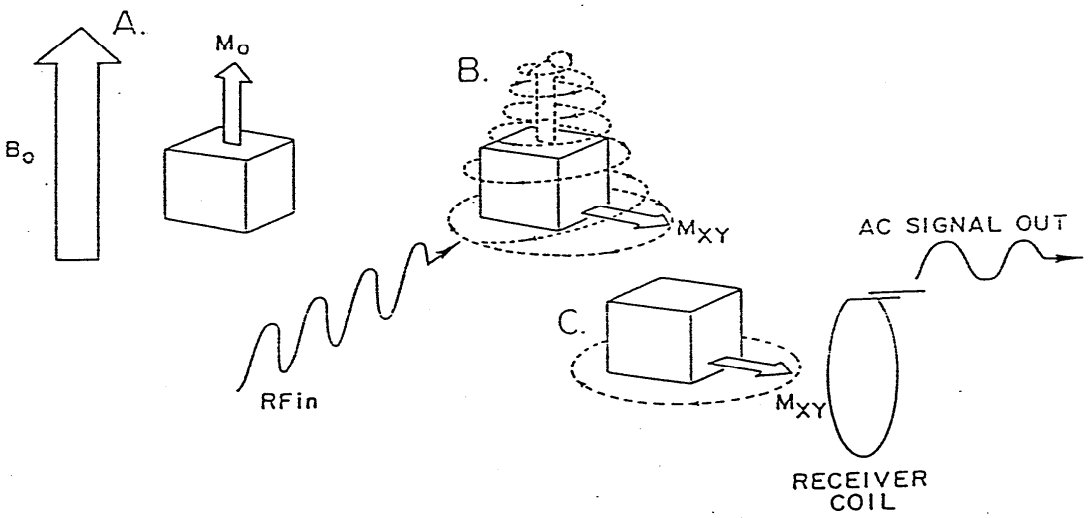
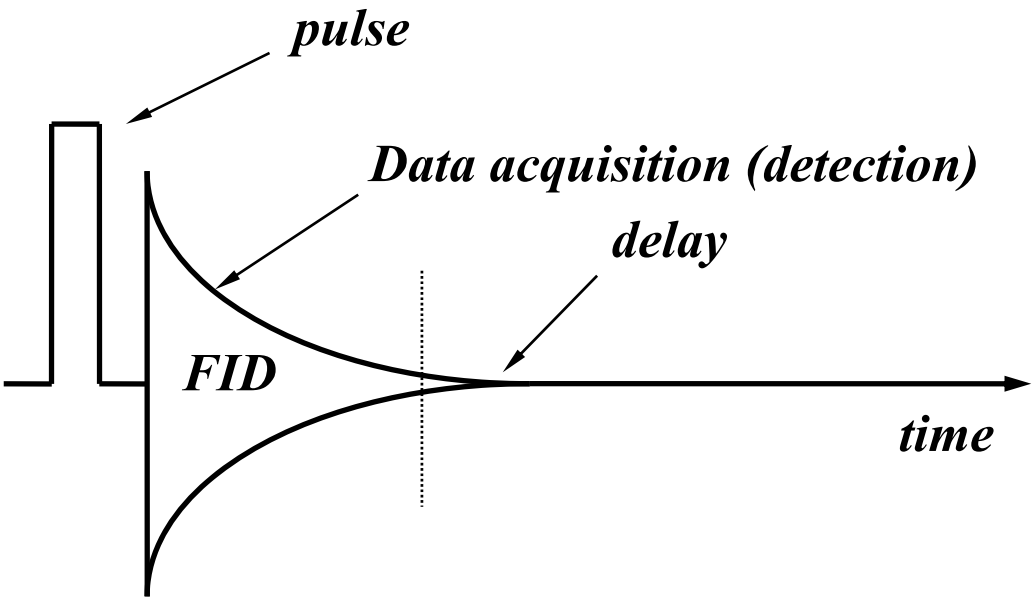
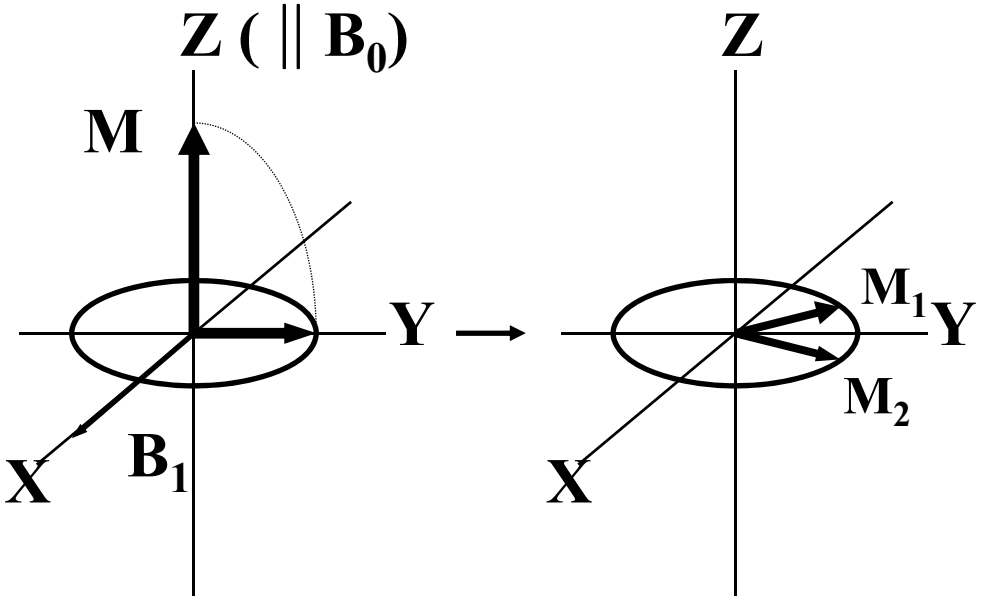


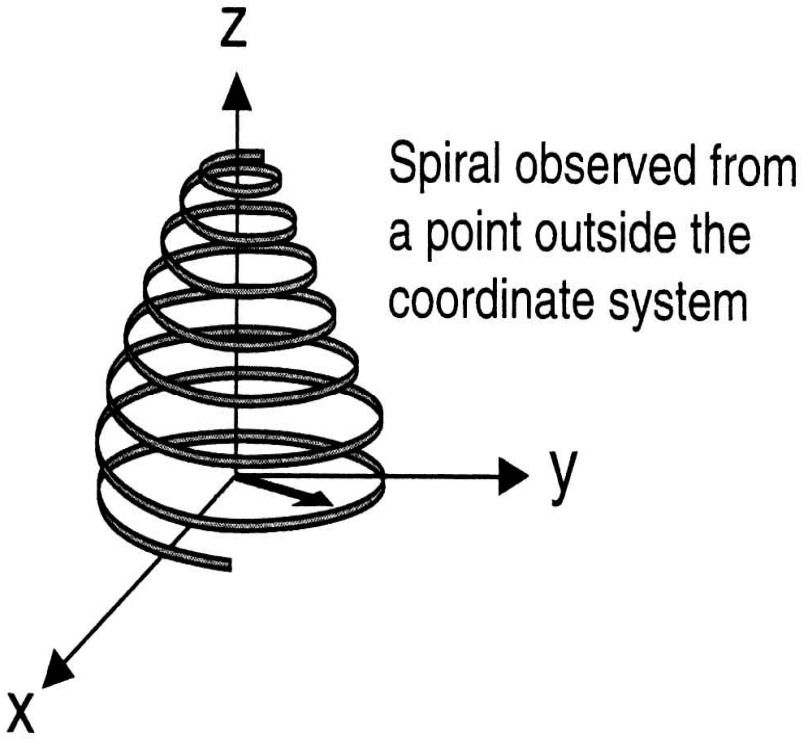
Figure 6. *A.* Net magnetization of the sample M_0 initially is aligned with the main magnetic field B_0, but it is so small in comparison to B_0 that it is undetectable. *B.* A radio-frequency (RF) field applied at the Larmor frequency tips tissue magnetization into the transverse plane, rendering it measurable as transverse magnetization, M_{xy}. *C.* Measurement of M_{xy} is possible because of its precession, which produces a changing magnetic flux linking a properly oriented loop receiver coil. The changing magnetic flux linking the coil induces an alternating current (AC) (alternating at the Larmor frequency) in the receiver coil. This alternating current, when amplified and digitized, becomes the signal from which the MR image is reconstructed.

Pulse & Free Induction Decay (FID)



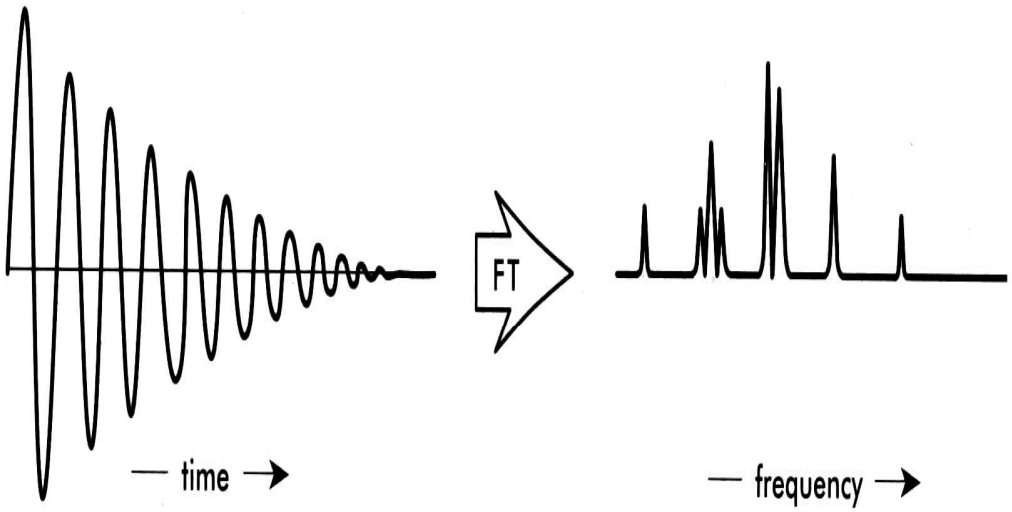


A 3D view of FID



The outside observer sees a spiral motion of the magnetization vector towards the x-y plane.

Fourier Transform of FID



Pulse NMR against C

In the CW experiment, requiring 1 Hz resolution over a 1000 Hz spectral width led to a 1000 s experiment time.

Supposing it proves possible to analyze the response of the sample to an impulse instead, we can evidently complete this alternative experiment in just 1 s, because while the requirement for spending 1 s on the measurement of each frequency remains, we are measuring all frequencies simultaneously instead of one after another.

Pulse NMR against C

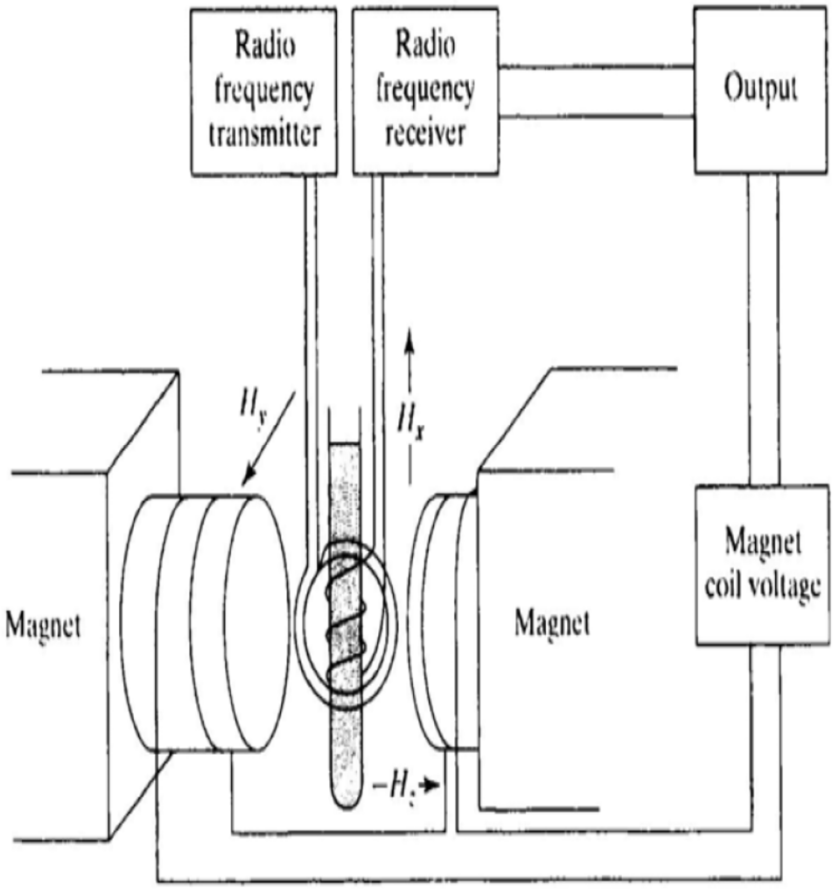
Older CW (continuous wave) NMR:

a single frequency of light
+
varied the magnetic field

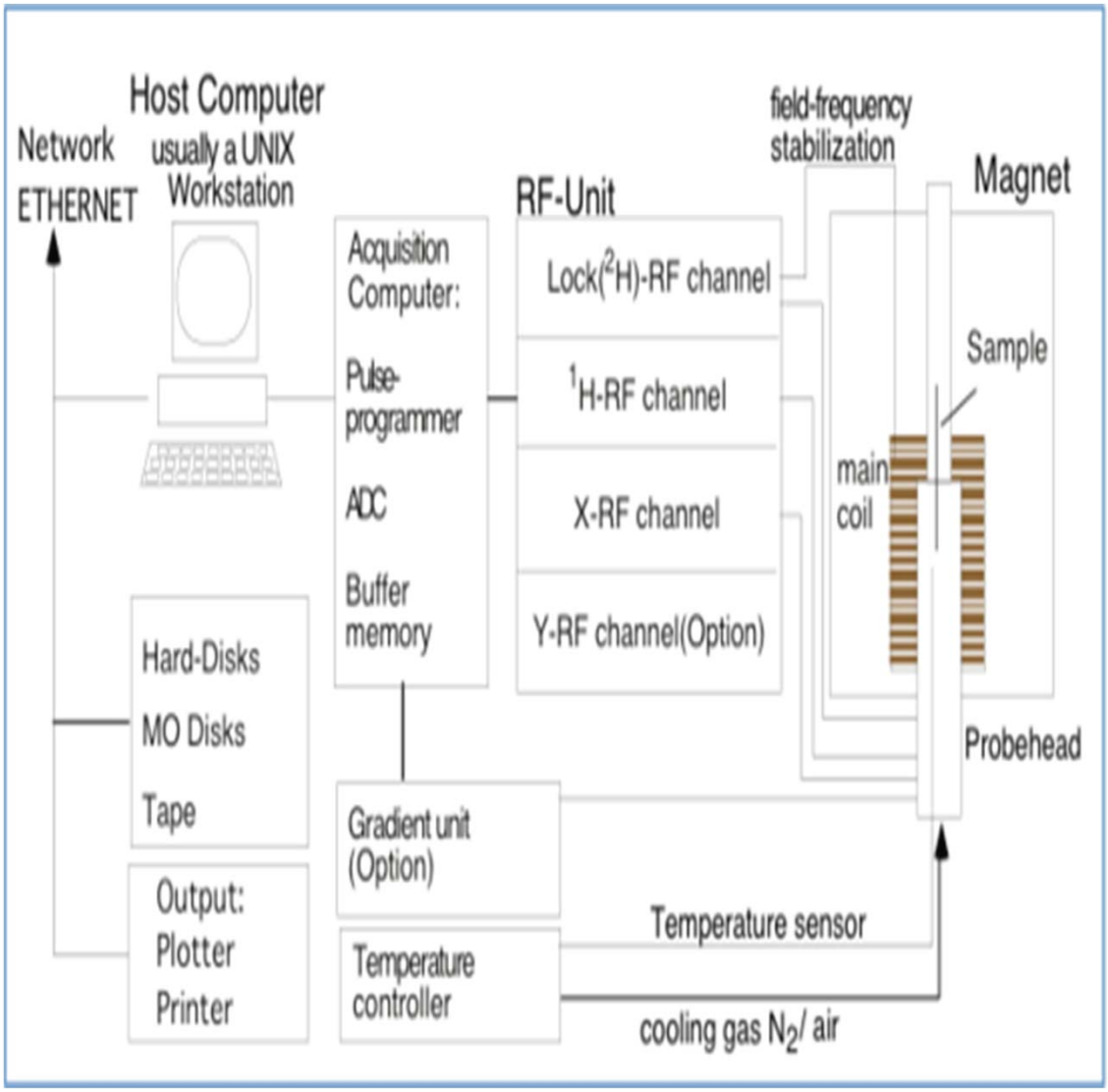
Modern FT (Fourier Transform) NMR:

a pulse contains many frequencies
+
a constant field

Older **CW** (*continuous*



Modern FT (*Fourier Transform*) NMR:

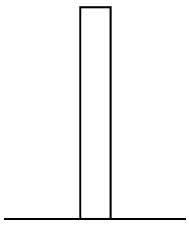


Fourier transform

The Fourier transform allows us to interconvert the amplitude evolving in the time domain into the frequency domain :

$$F(\omega) = \int f(t) \exp(i\omega t)\, dt$$

The integral can be approximated as a sum of signals at different frequencies.



$$\sum_n A_n \exp(-i\omega_n t)$$

A pulse can be expanded in terms of infinite plane waves

What is chemical shift ?

Consider an ***indirect coupling*** of the nuclei to external static ***magnetic field*** by interacting with the ***surrounding electrons***.

This interaction leads to magnetic shielding in resonance frequencies that are a reflection of the ***chemical environment*** of a nucleus in an atom or molecule and are therefore important in the analytical application of NMR, as well as in testing theoretical descriptions of molecules.

$$B=Bo(1-\sigma),$$

The dimensionless number σ is a small fraction, describing the electron shielding in the external magnetic field.

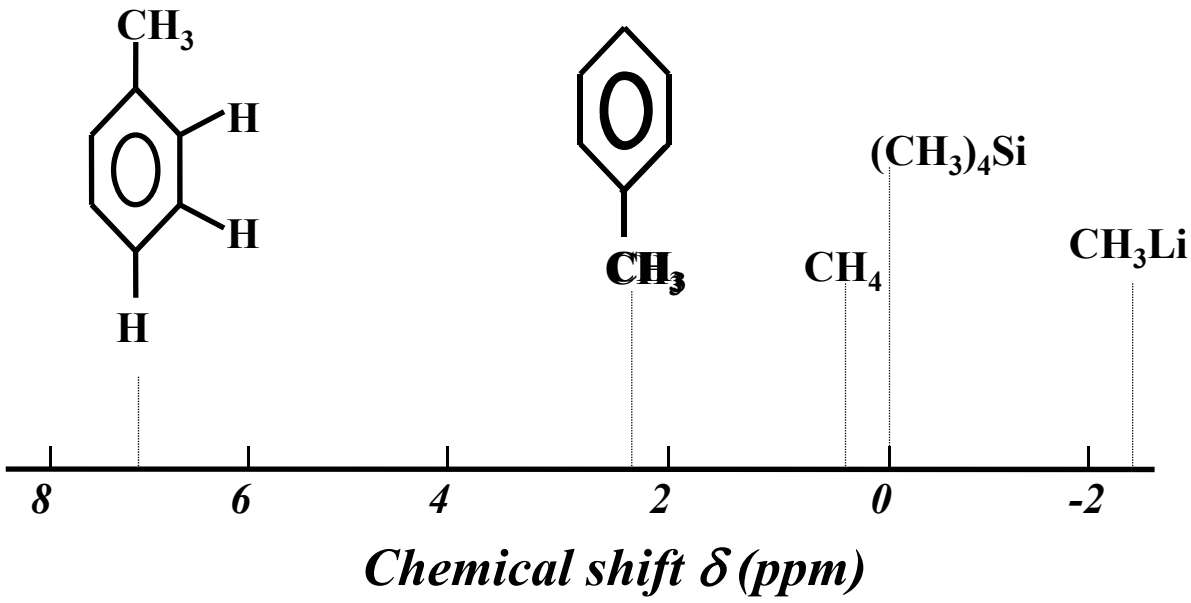
Chemical shift is measured relative to a reference

Chemical shift defines the location of a NMR line along the rf axis. Normally it is measured relative to a reference compound. In frequency units the chemical shift is proportional to the applied static magnetic field, and therefore chemical shifts are customarily quoted in parts per million (ppm) units.

$$\Delta\nu = \nu_{signal} - \nu_{reference}$$

$$\delta\ (ppm) = \Delta\nu/\nu_0\ [MHz]$$

Examples :



downfield
high frequency

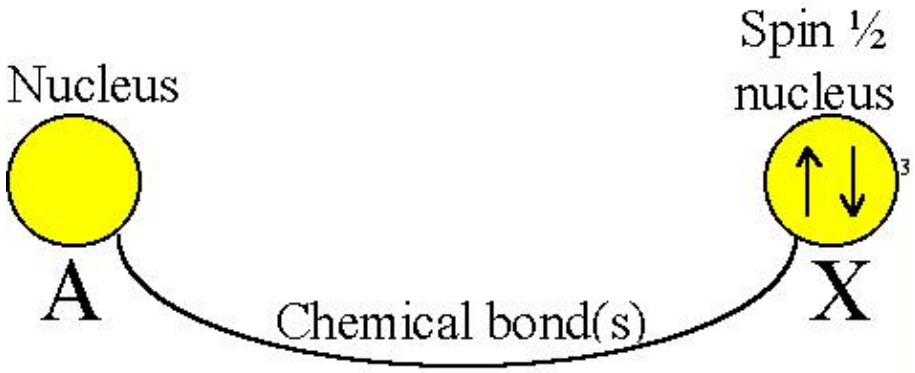
Increased shielding

Increased deshielding

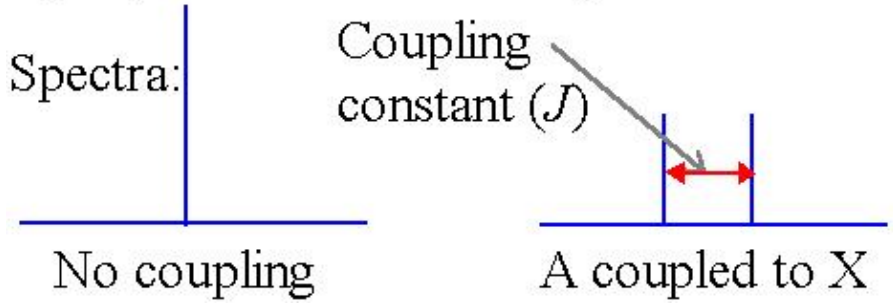
upfield
low frequency

http://www.google.com.tw/imgres?imgurl=http://chem.ch.huji.ac.il/nmr/whatisnmr/whatisnmr_files/ethylbenzene.jpg&imgrefurl=http://chem.ch.huji.ac.il/nmr/whatisnmr/whatisnmr.html&usg=__7O-ywEKn3KfNRWGz_8xZXnv883E=&h=284&w=451&sz=17&hl=zh-TW&start=1&zoom=1&tbnid=iW-dgHjwqwrGFM:&tbnh=80&tbnw=127&ei=oFuIUdC8HIvxlAWth4H4Bw&prev=/images%3Fq%3Dethylbenzene%2BNMR%2Bsignals%26sa%3DX%26hl%3Dzh-TW%26gbv%3D2%26tbm%3Disch&itbs=1&sa=X&ved=0CCsQrQMwAA

J-coupling splitting



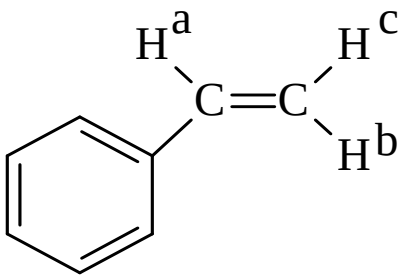
The resonant frequency of A is dependent on the alignment of X. A's signal is split into two equal parts because X is about equally divided between alignments.

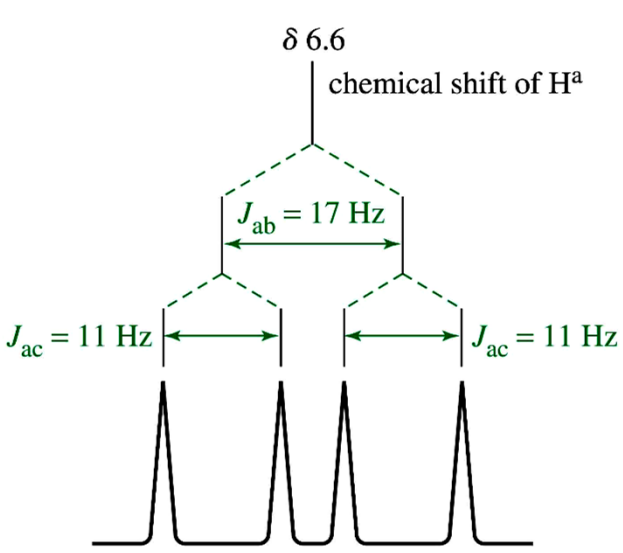


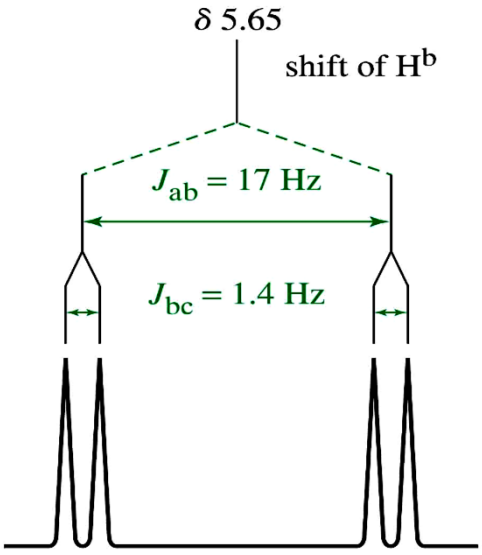
The effective magnetic field is affected by the orientation of neighboring nuclei. This effect is known as spin-spin coupling which can cause splitting of the signal for each type of nucleus into two or more lines.

1. No. of j-splitting (2jn+1)
2. J-coupling constant is measured in Hz

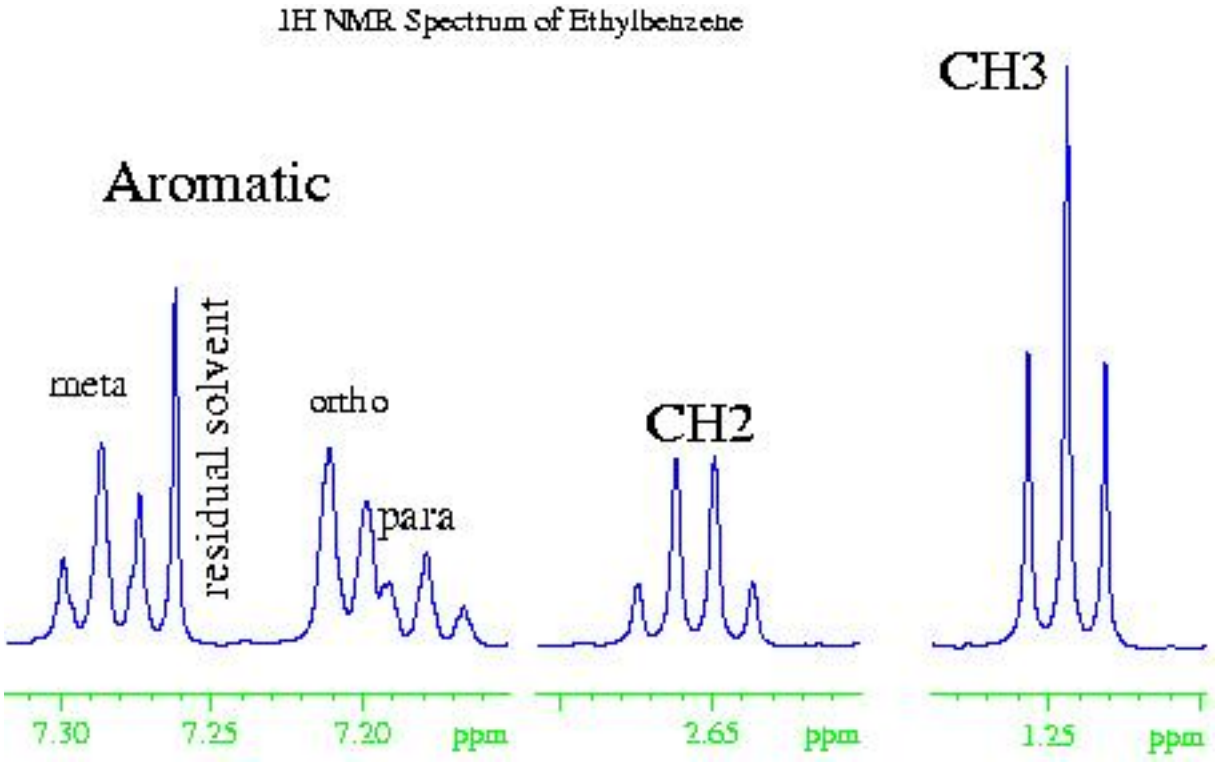
J-coupling splitting: $J_{H\text{-}H}$



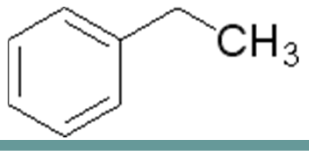




J-coupling splitting: J_{H-H}

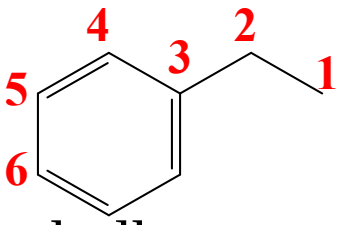


Returning to the example of ethylbenzene, the methyl (CH3) group has a coupling pattern in the form of A3X2 which to a first order approximation looks like an AX2 multiplet. Likewise, the methylene (CH2) group has the form A2X3 that is equivalent to AX3. The first order approximation works because the groups are widely separated in the spectrum. The aromatic signals are close together and display second order effects. The *ortho* signal is a doublet AX while the *meta* and *para* signals are triplets

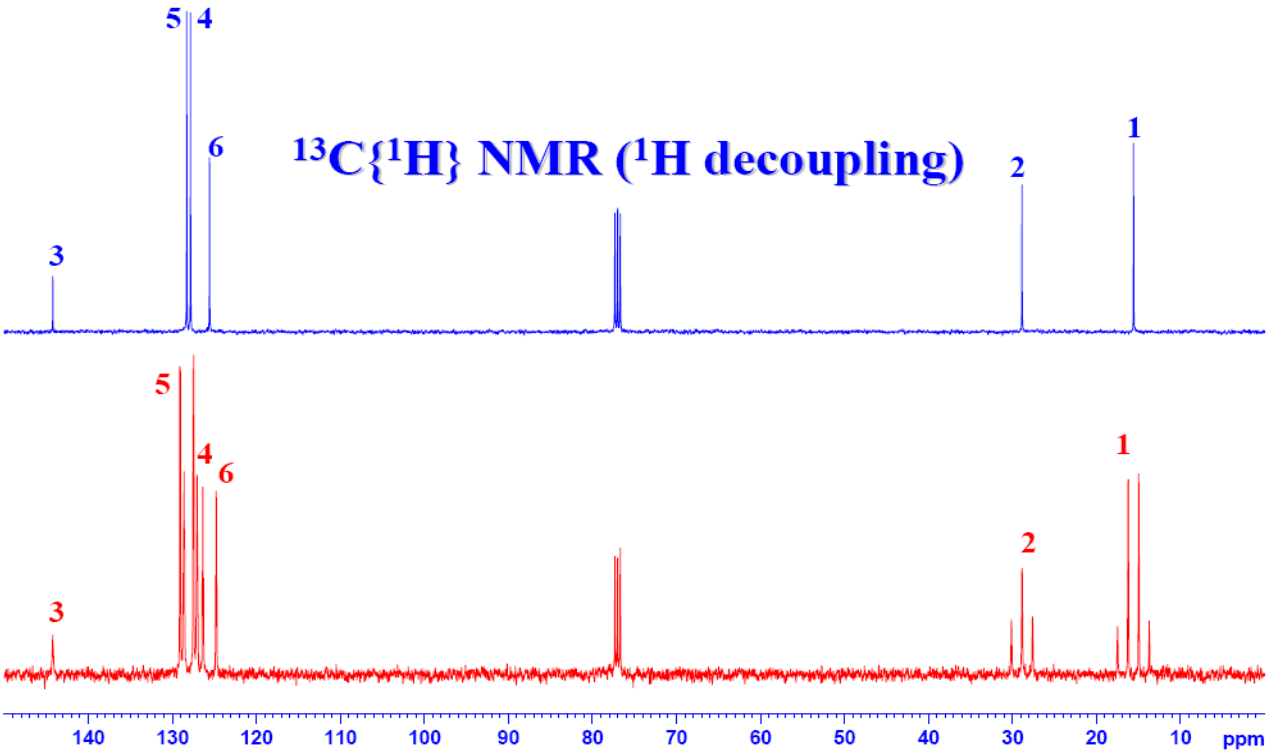


ethylbenzene

J-coupling splitting: J_{C-H}



ethylbenzene



Summary

Isolated spins
(Zeeman effect, Larmor Frequency)

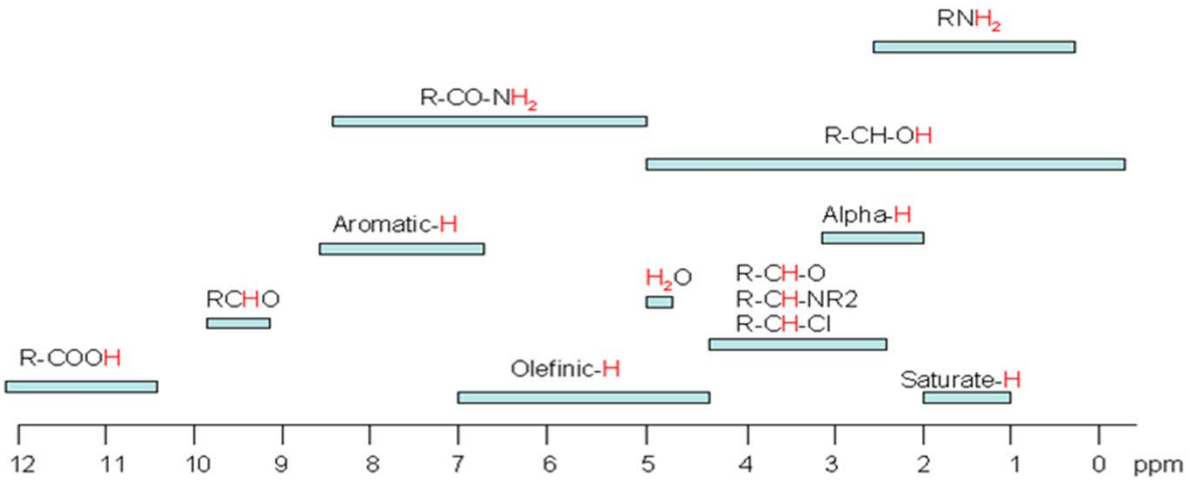
Electron cloud
(chemical shift)

Scalar coupling
(J-coupling through chemical bonds)

Dipolar interaction
(NOE through space)

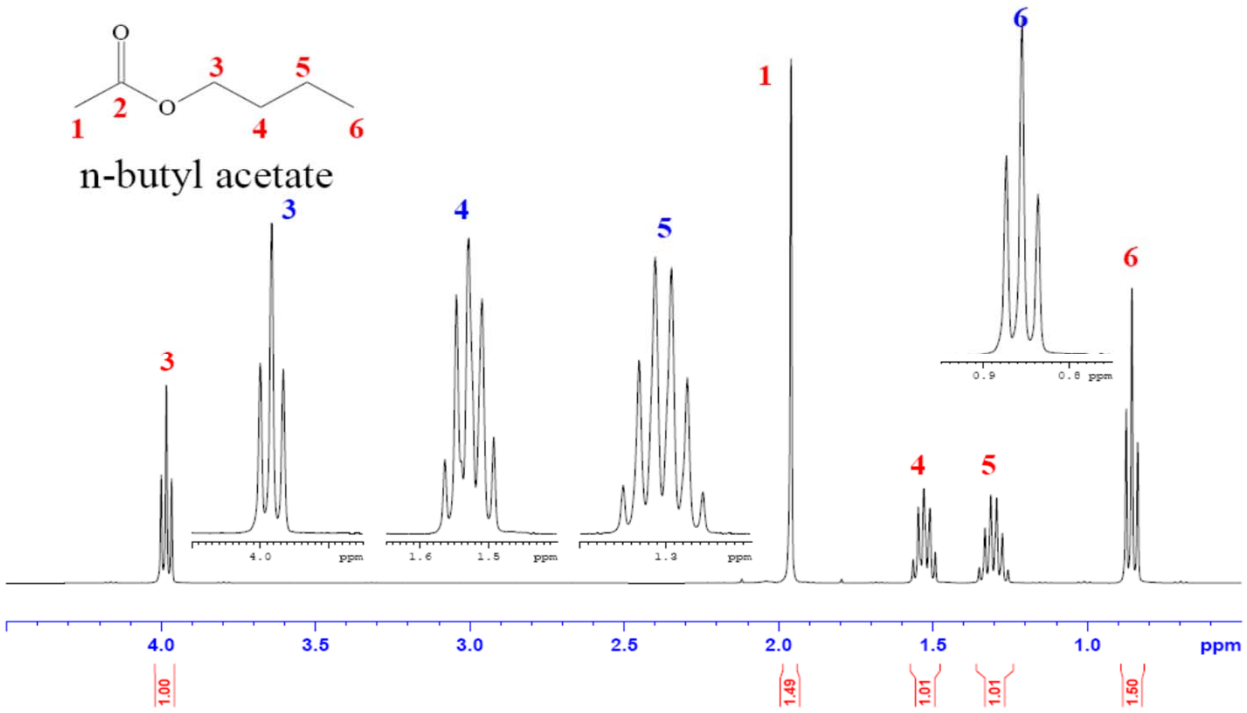
Spin dynamics
(T1, T2… relaxations)

^{1}H NMR

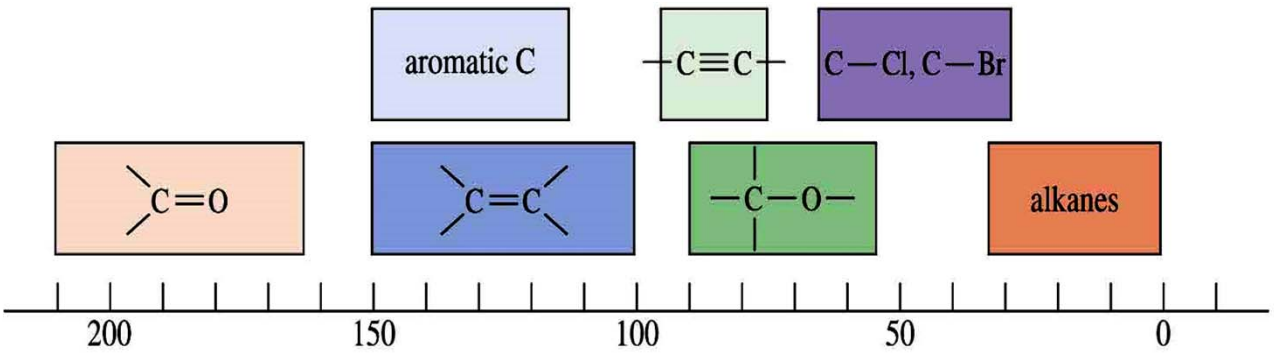


1. Number vs. different kinds of protons
2. Location vs. chemical envornments, shielding or deshielding
3. Intensity vs. the type number of protons
4. Splitting vs. adjacent atoms

^{1}H NMR


O
O
1
2
3
4
5
6
n-butyl acetate
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
1.49
1.01
1.01
1.50

^{13}C NMR

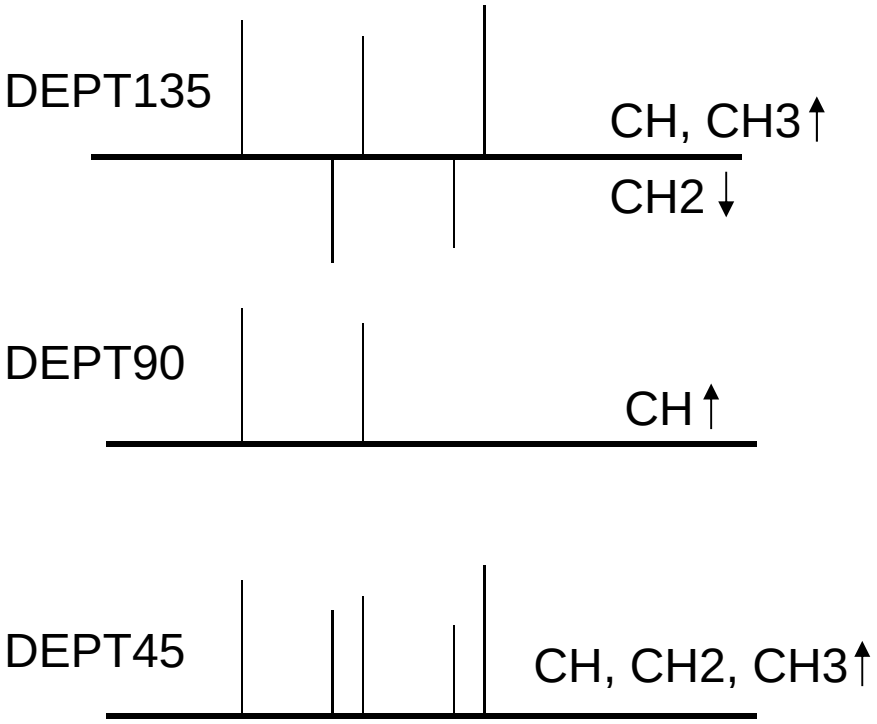


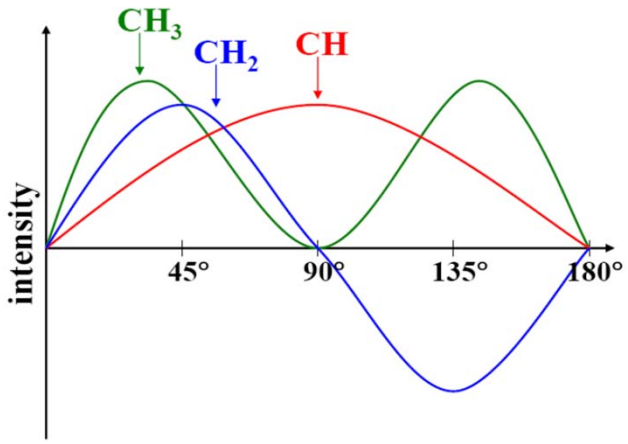
1. Ignored 13C-13C coupling for low natural aboundance

2. Common 13C-expts are acquired by decoupling 1H. the splitting pattern follows 2N+1 rule

3. There is no expts to enhance the sensitivity of quaternary 13C: Time is the only solution. Long T1 in quaternary 13C

^{13}C-DEPT NMR


DEPT135
CH, CH3
CH2
DEPT90
CH
DEPT45
CH, CH2, CH3


CH3
CH2
CH
intensity
45°
90°
135°
180°

NMR applications

(a) *T1 and T2 relaxation*

(b) *1D and 2D NMR spectroscopy*

(c) *NOE and 2D NOE spectroscopy*

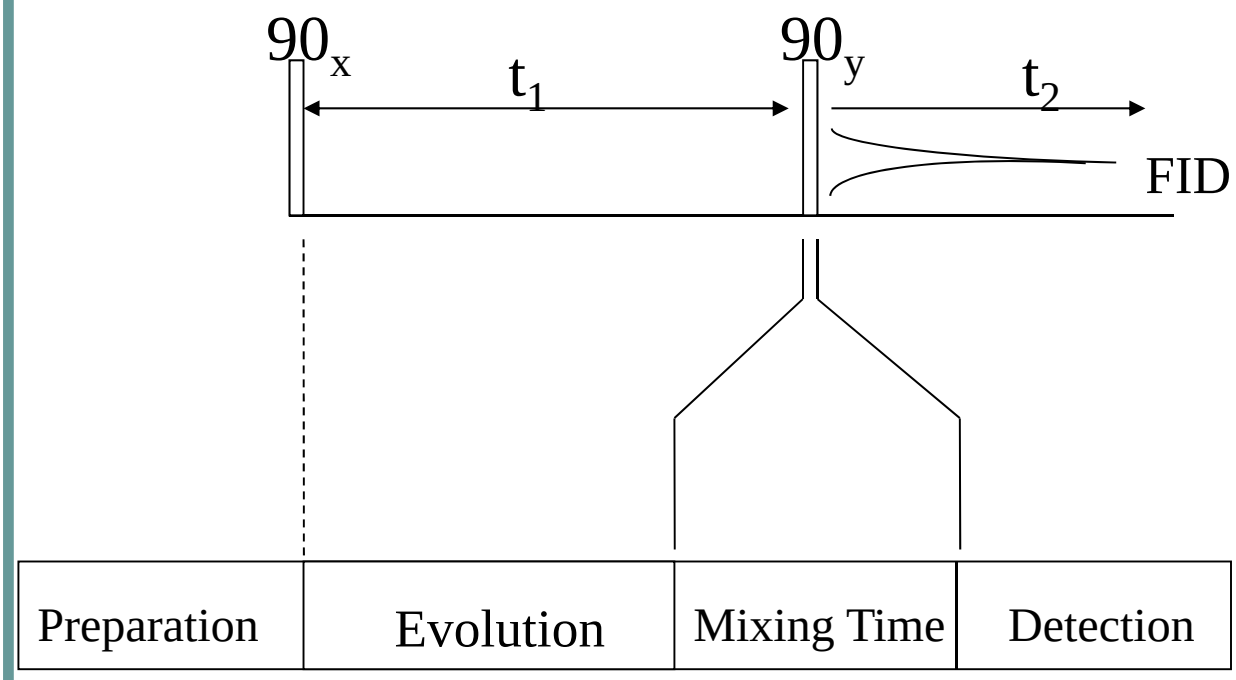
(d) *2D TOCSY spectroscopy*

(e) *2D COSY spectroscopy*

(f) *1H, 13C, 15N NMR spectroscopy and high resolution multi-dimensional NMR*

(g) *Other nuclear spin interactions*

Anatomy of a 2D experiment



The construction of a 2D experiment is simple: in addition to preparation and detection which are already known from 1D experiments the 2D experiment has indirect evolution time t1 and a mixing sequence. The scheme can be viewed as :

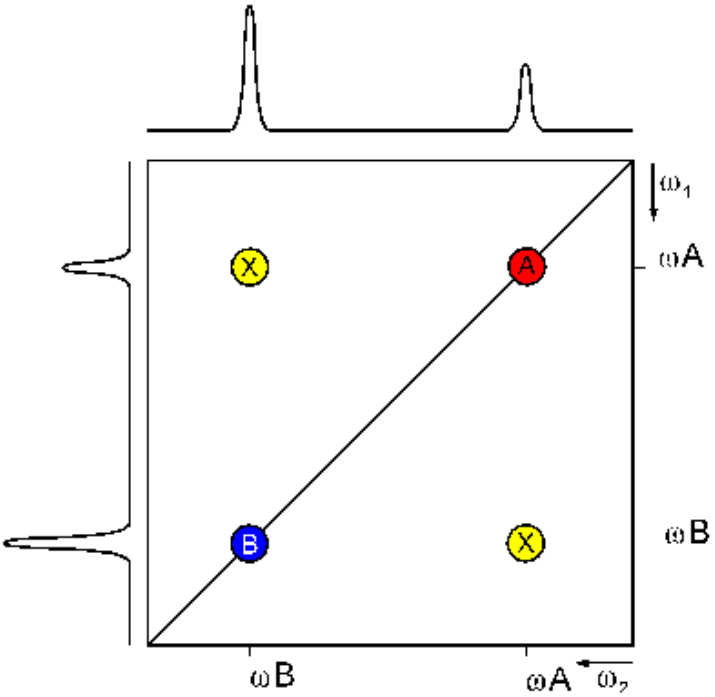
Do something with the nuclei (preparation),

let them precess freely (evolution),

do something else (mixing),

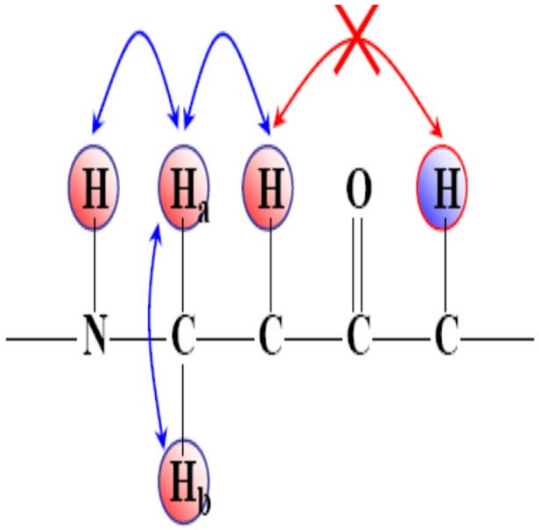
and detect the result (detection, of course)

Anatomy of a 2D experiment - continued



After preparation the spins can precess freely for a given time t1. During this time the magnetization is labeled with the chemical shift of the first nucleus. During the mixing time magnetization is then transferred from the first nucleus to a second one. Mixing sequence utilize two mechanisms for magnetization transfer: scalar coupling (J-coupling) or dipolar interaction (NOE). Data are acquired at the end of the experiment (detection, often called direct evolution time); during this time the magnetization is labeled with the chemical shift of the second nucleus.

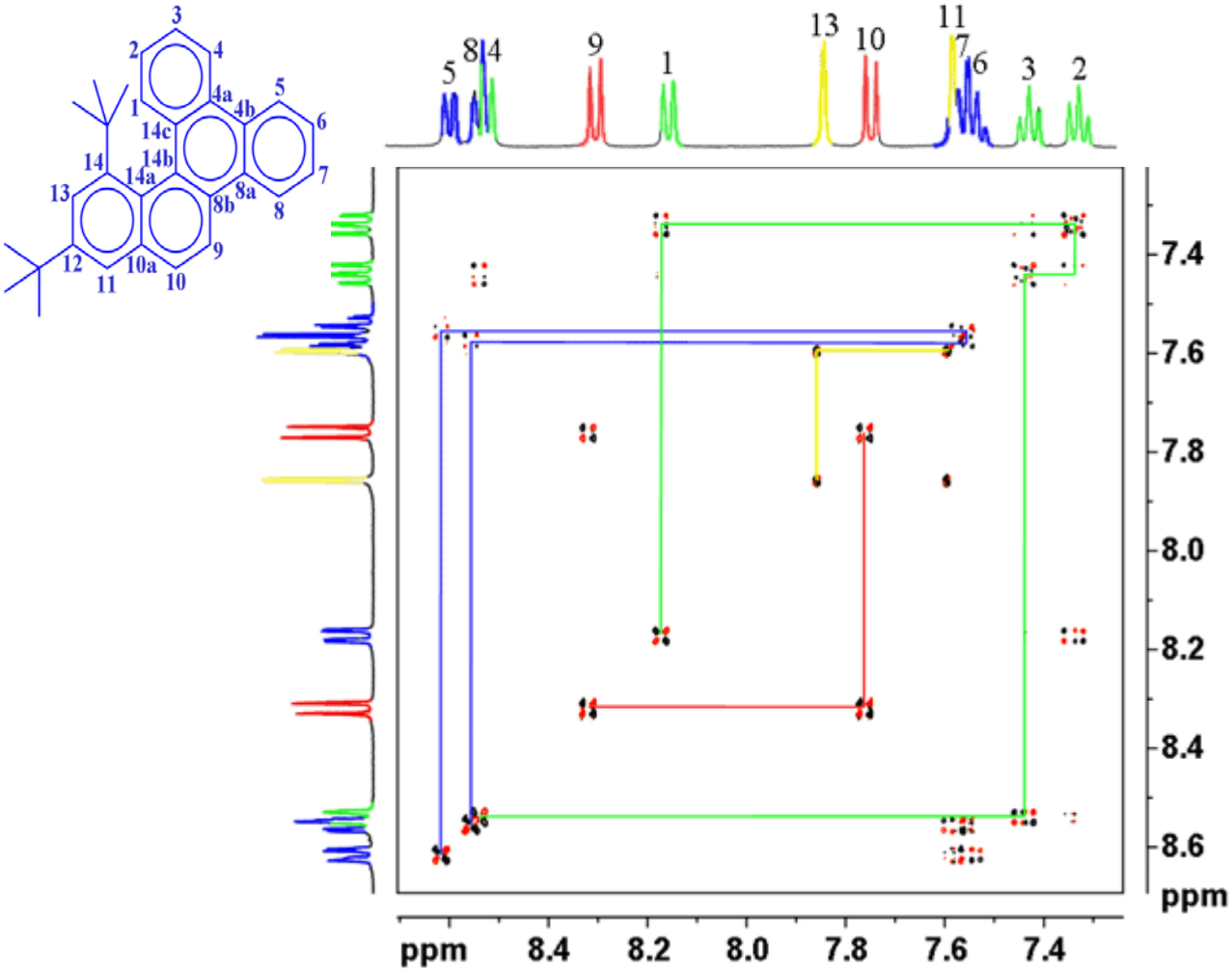
2D-COSY (Correlation Spectroscopy)



$H_a \neq H_b$

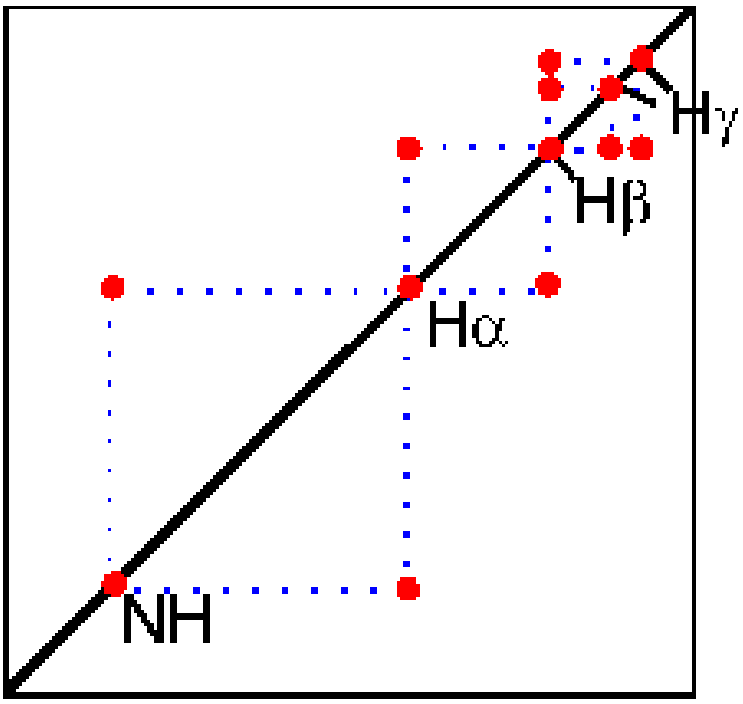
Get information from ^{2}J and ^{3}J

2D COSY spectrum of aromatic region of 12,14-ditbutylbenzo[g]chrysene



There are separated into four color-coded proton groups showing connectivity. Using horizontal and vertical lines, it is possible to separate each group and follow its connectivity. The **blue** group of four protons is connected in the order 8.62 ppm to 7.55 to 7.59 to 8.56, the **green** group of four protons in the order 8.15 to 7.34 to 7.44 to 8.56 and the **red** group or two protons, that correspond to H9 and H10 because they are the only group of two protons expected to have a three-bond coupling constant (8.9 Hz), are at 7.76 and 8.32 ppm. The **yellow** group of two protons correspond to H11 and H13 because the coupling constant is small (1.9 Hz) and consistent with a four bond correlation.

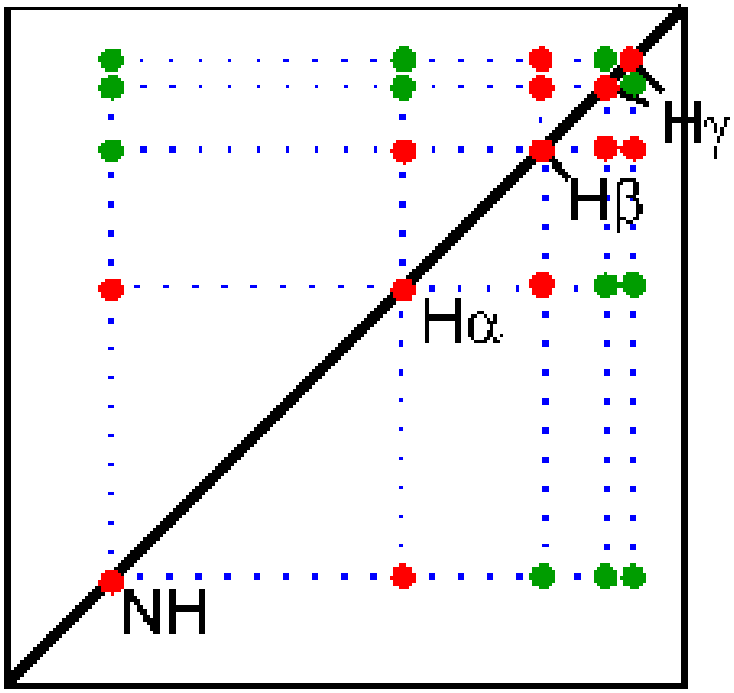
2D COSY (COrrelated SpectroscopY



In the COSY experiment, magnetization is transferred by scalar coupling (J-coupling). Protons that are more than three chemical bonds apart give no cross signal because the ^{4}J coupling constants are close to 0. Therefore, only signals of protons which are two or three bonds apart are visible in a COSY spectrum (red signals). The cross signals between HN and Hα protons are of special importance because the *phi* torsion angle of the protein backbone can be derived from the ^{3}J coupling constant between them.

2D TOCSY

(TOtal Correlated SpectroscopY)

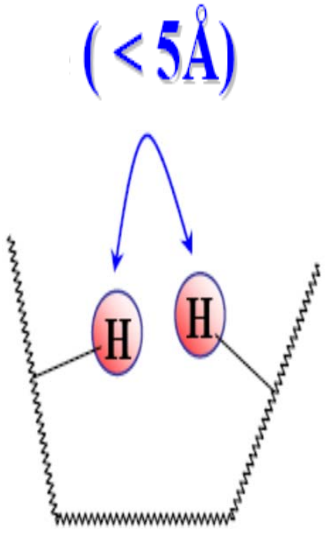


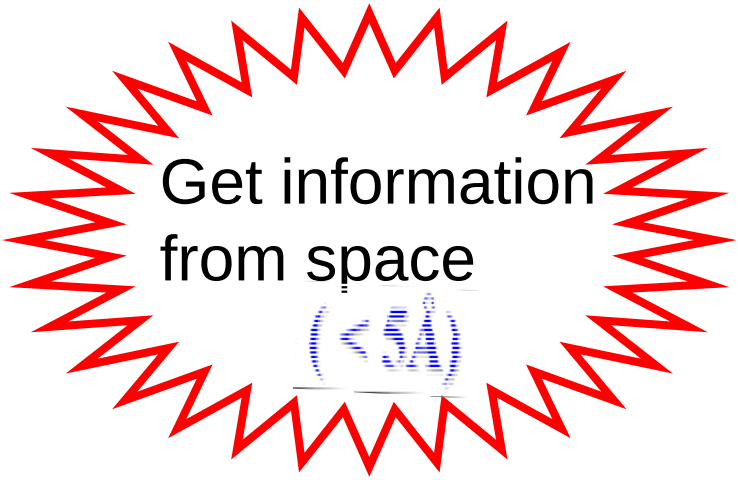
In the TOCSY experiment, magnetization is dispersed over a complete spin system of an amino acid by successive scalar coupling (J-coupling). The TOCSY experiment correlates all protons of a spin system. Therefore, not only the red signals are visible (which also appear in a COSY spectrum) but also additional signals (green) which originate from the interaction of all protons of a spin system that are not directly connected via three chemical bonds. Thus a characteristic pattern of signals results for each amino acid from which the amino acid can be identified. However, some amino acids have identical spin systems and therefore identical signal patterns. They are: cysteine, aspartic acid, phenylalanine, histidine, asparagine, tryptophane and tyrosine ('AMX systems') on the one hand and glutamic acid, glutamine and methionine ('AM(PT)X systems') on the other hand.

2D-NOESY/ROESY Spectroscopy

(Nuclear Overhauser Effect SpectroscopY)

(Rotating-frame Overhauser Effect SpectroscopY)

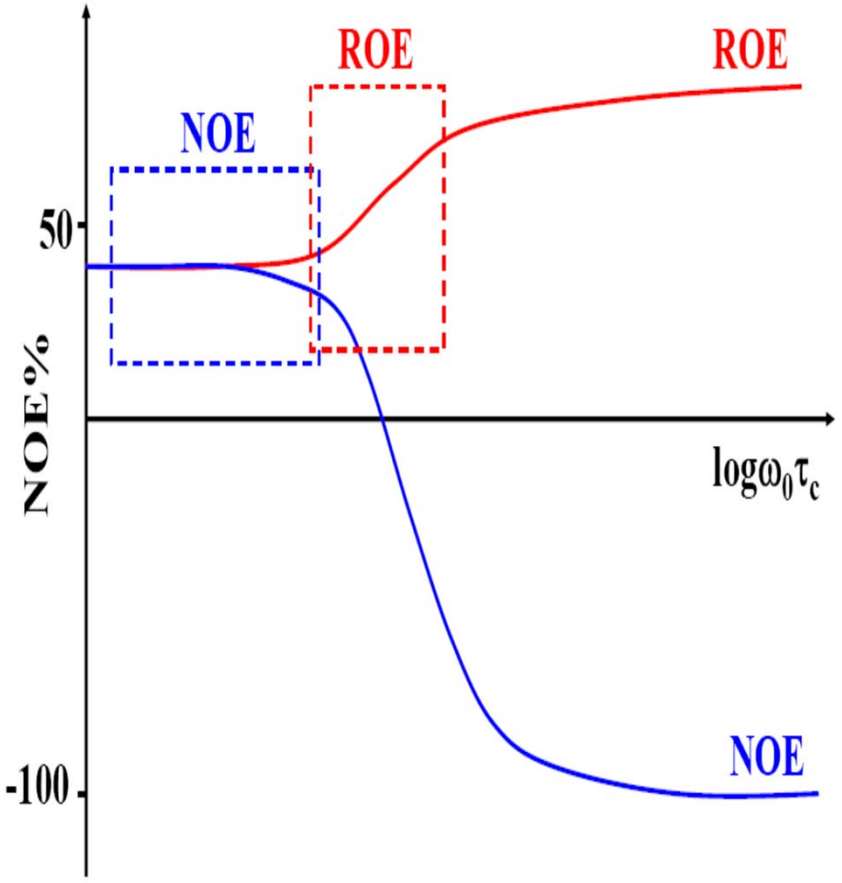




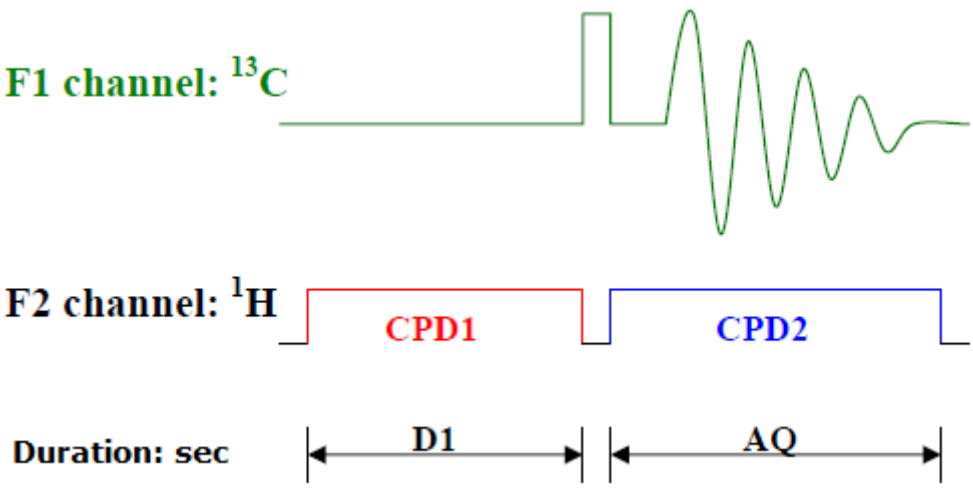
中央研究院化學研究所
核磁共振實驗室
NMR

中央研究院化學研究所
Institute of Chemistry, Academia Sinica

2D-NOESY /ROESY



^{13}C NMR



NOE decoupling

Pulse program:	CPD1	CPD2	advantage
zg/zg30	X	X	w/o decp.
zgcw/zgcw30	selective pl26& O2p		
zgdc/zgdc30	V	V	heating, s.t. d.
zggd/zggd30	V	X	w/o decp.
zgig/zgig30	X	V	D1≧10s, interg.
zgpg/zppg30	V(low)	V	Xheating, l.t. d.

^{13}C NMR S/N

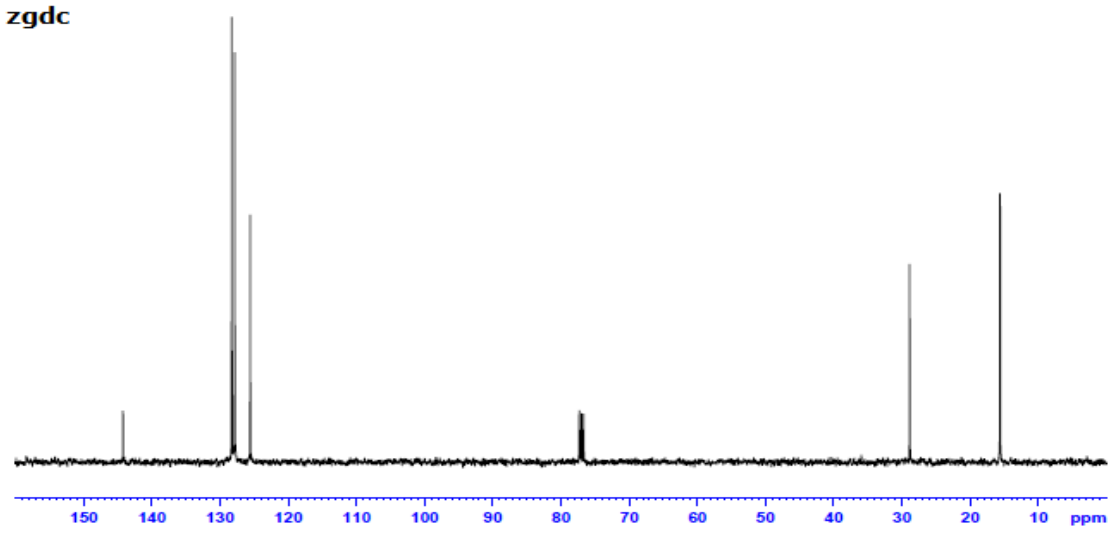
sample: 10% ethylbenzene in CDCl3

range : signal = 20 ~ 35 ppm

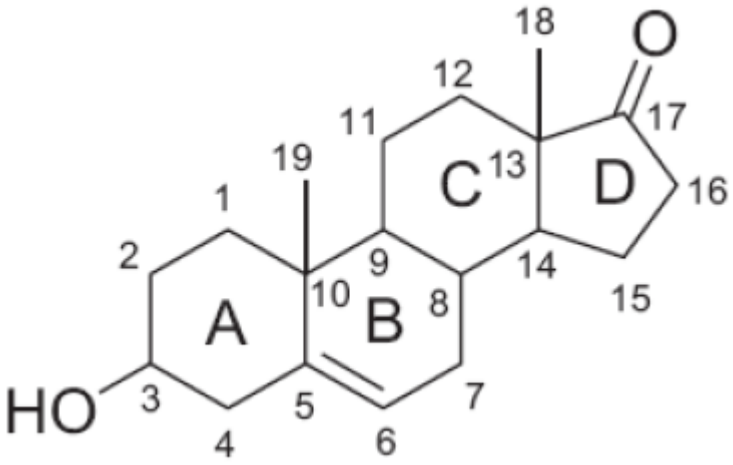
noise = 40 ~ 70 ppm

S/N value

6.4	zg
29.2	zggd
58.1	zgpg
63.3	zgdc



ADR, *trans*-dehydroandrosterone



$C_{19}H_{28}O_2$

CH: 3, 6, 8, 9, 14

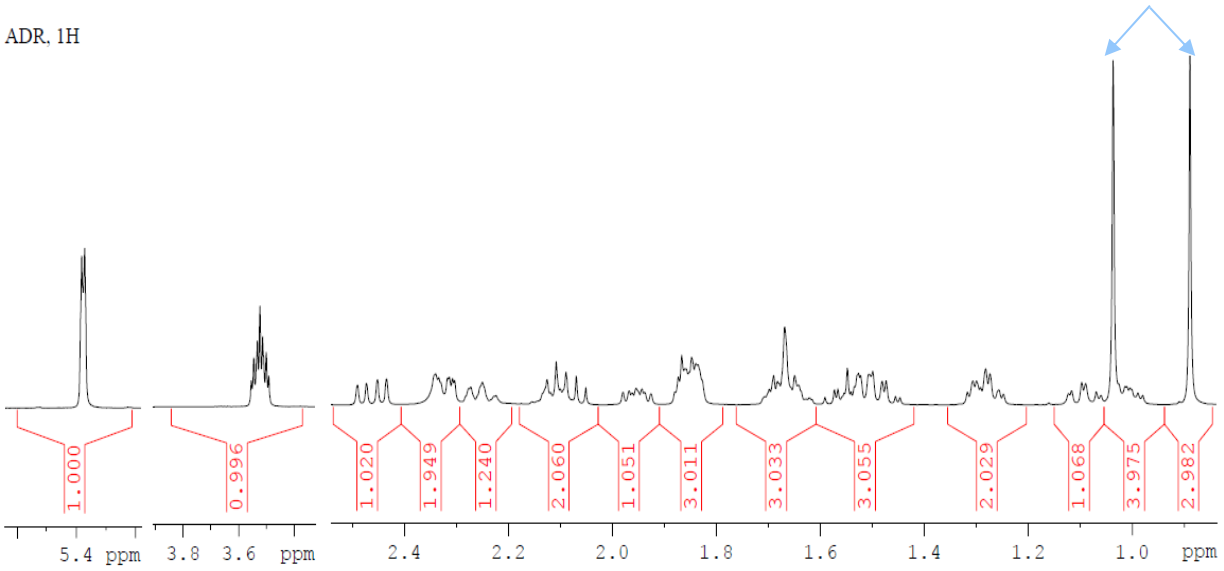
CH2: 1, 2, 4, 7 ,11 , 12, 15, 16

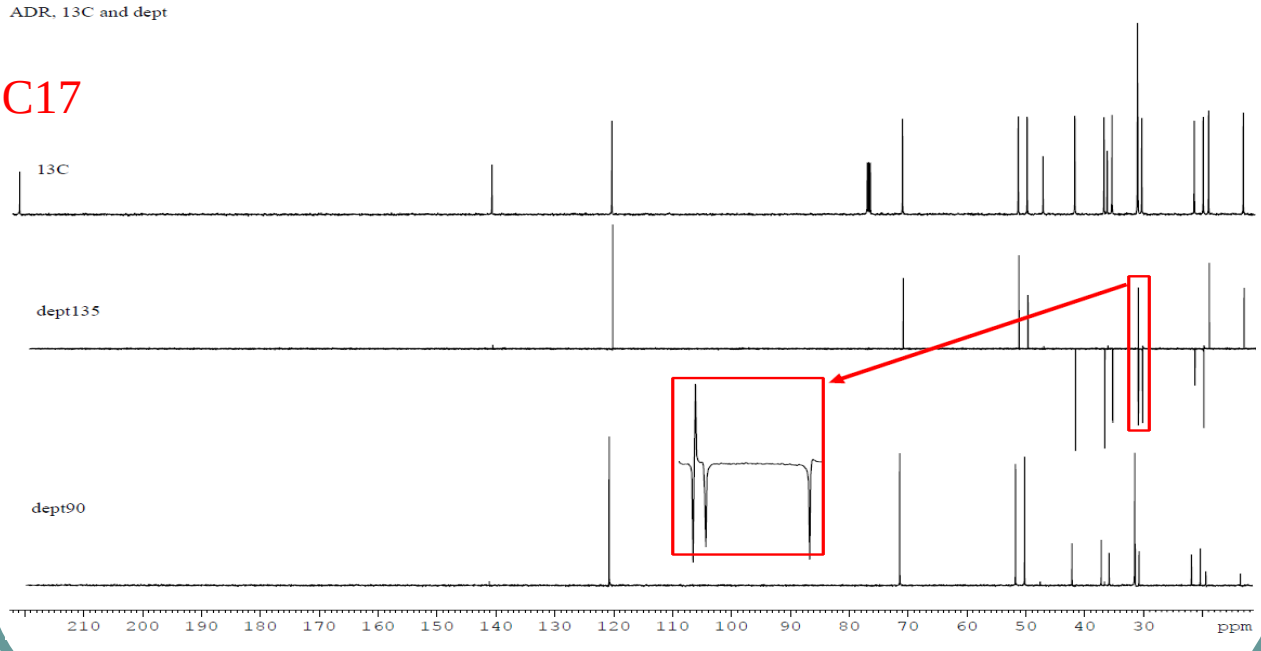
CH3: 18, 19

C: 5, 10, 13, 17

ADR, ^{1}H, ^{13}C and DEPT

H18,19

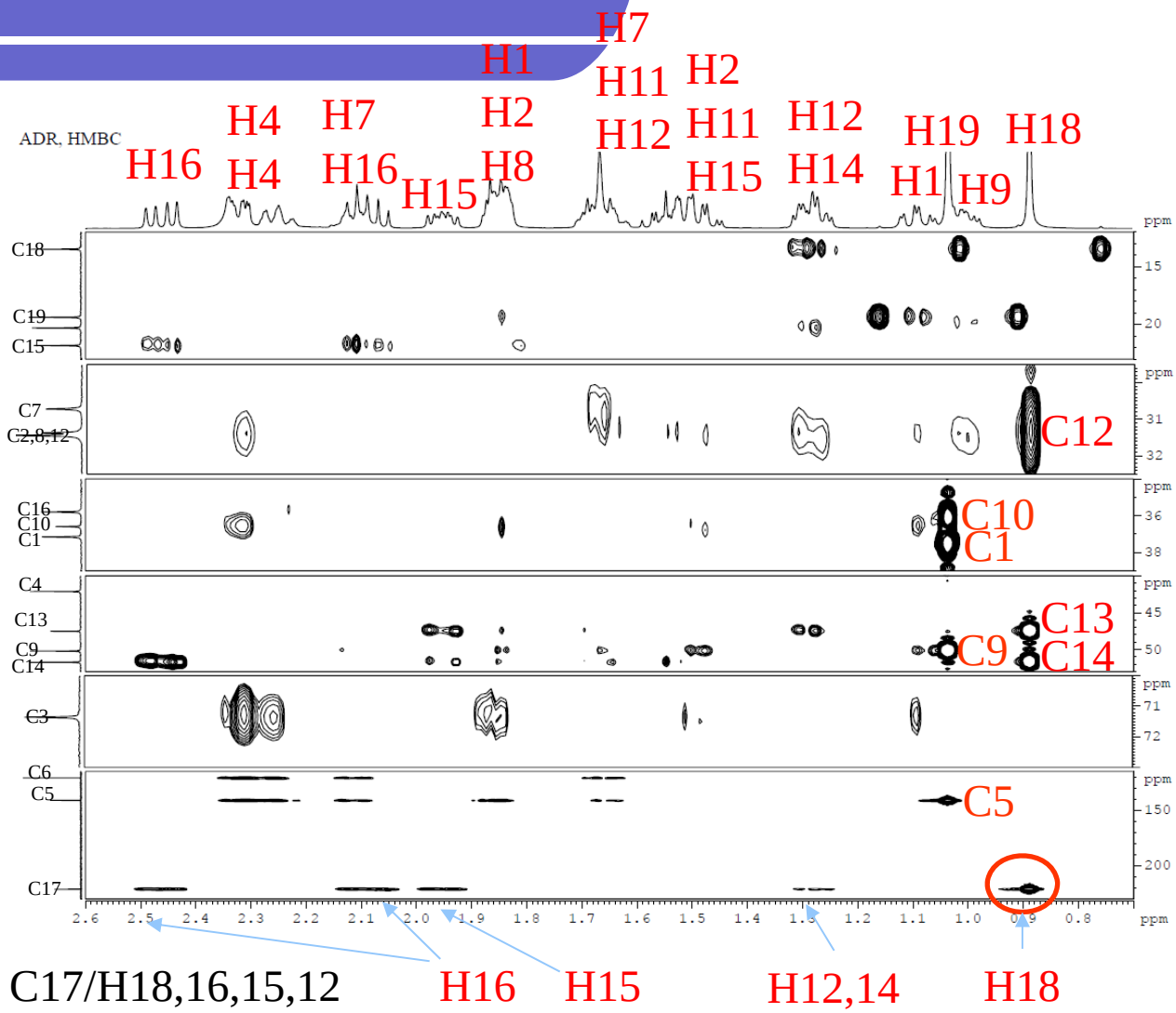




中央研究院化學研究所 核磁共振實驗室

中央研究院化學研究所
Institute of Chemistry, Academia Sinica

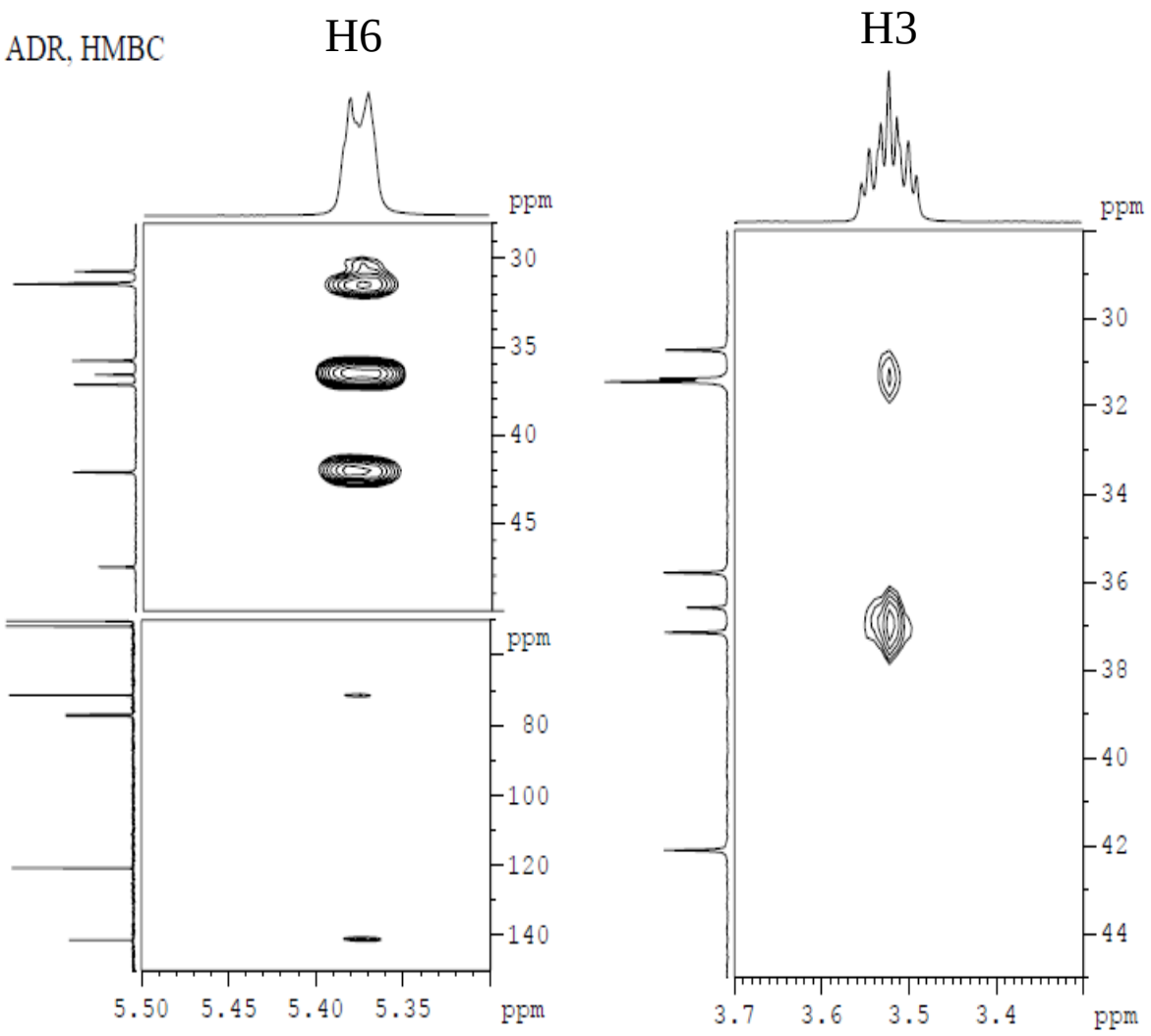
ADR, *HMBC*



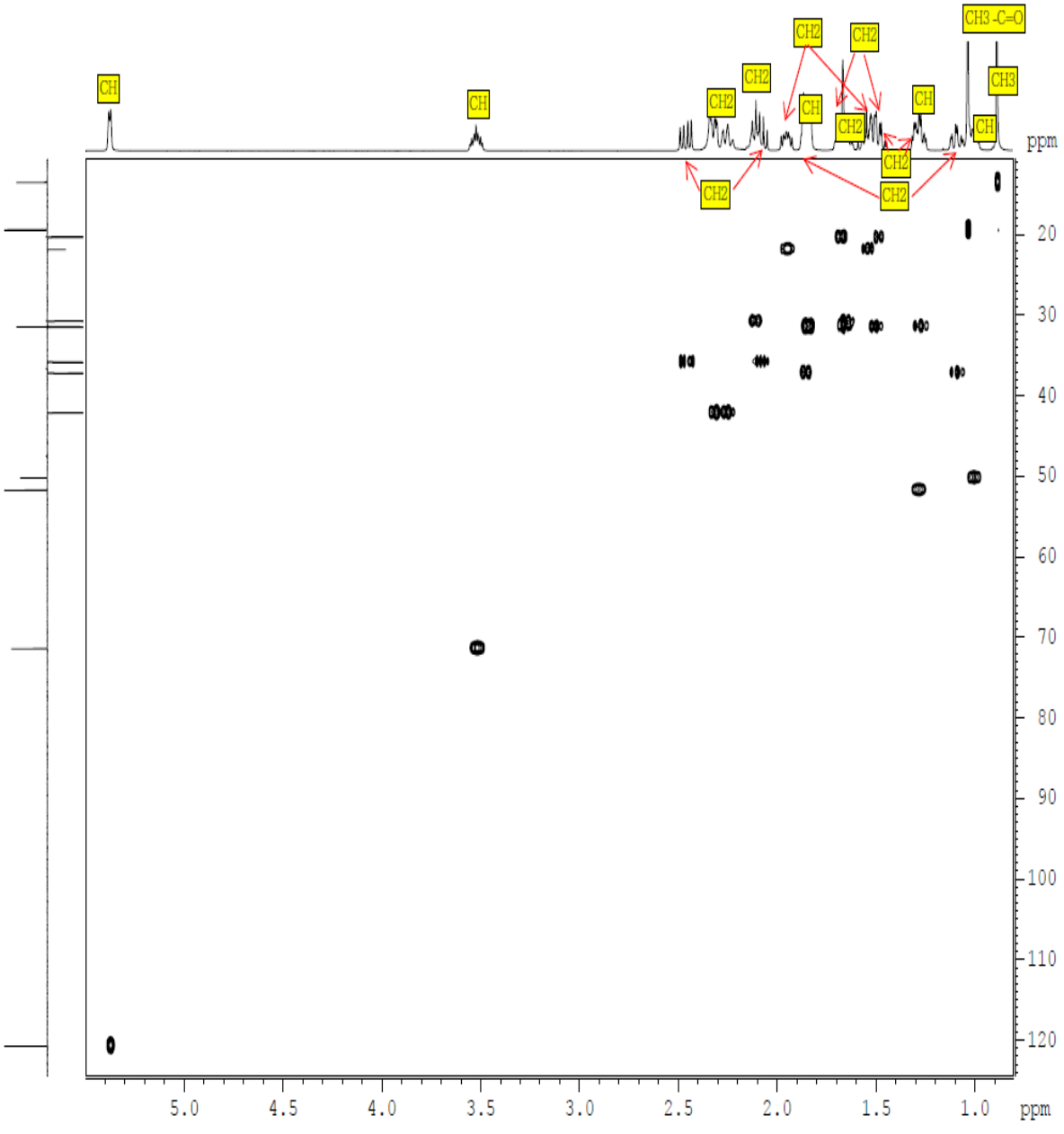
H18/C13,17,14(CH),12(CH2)

H19/C10,5,9(CH),1(CH2)

ADR, *HMBC*


ADR, HMBC
H6
H3
ppm
30
35
40
45
80
100
120
140
5.50
5.45
5.40
5.35
32
34
36
38
42
44
3.7
3.6
3.5
3.4

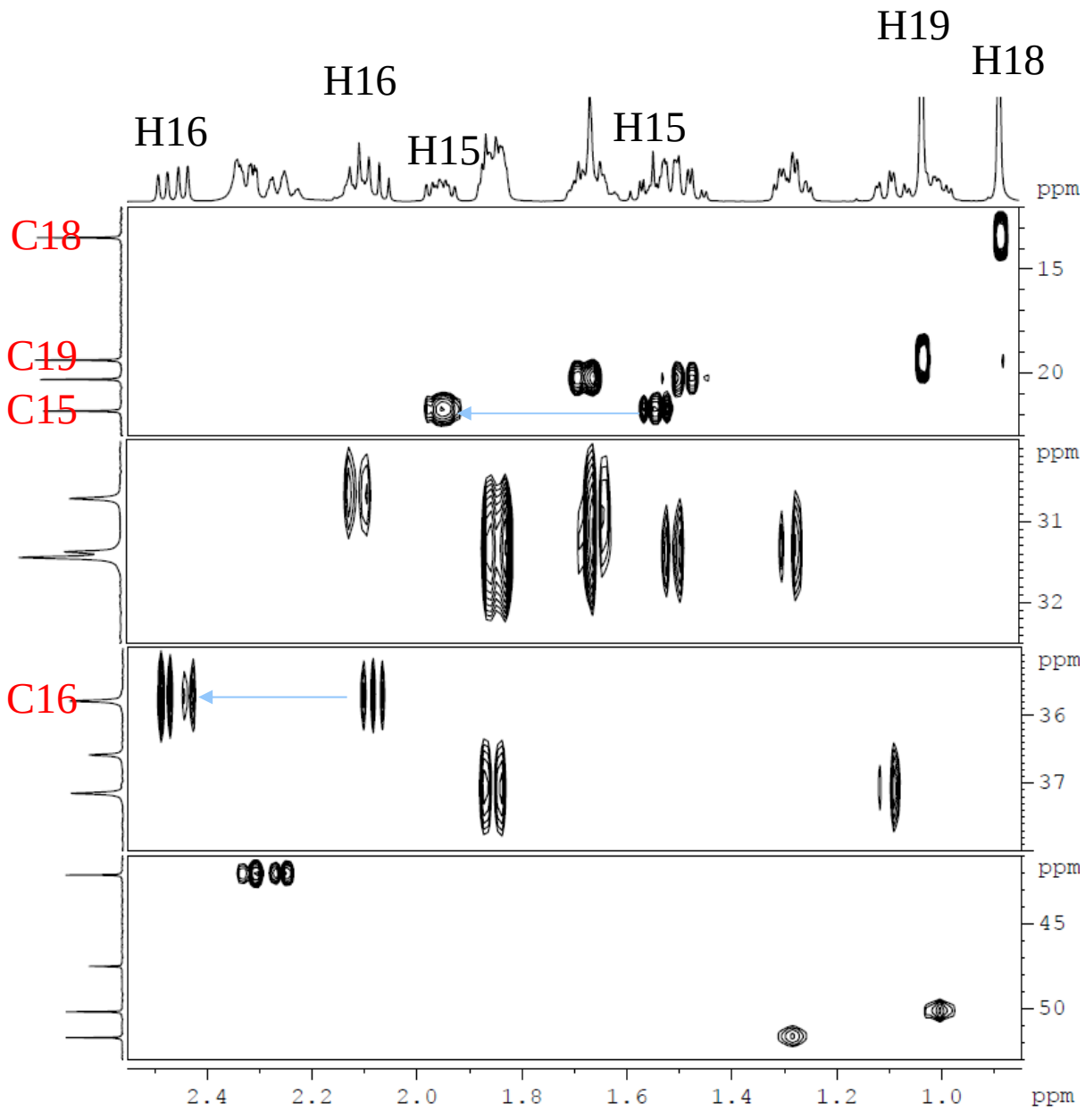
ADR, *HSQC*



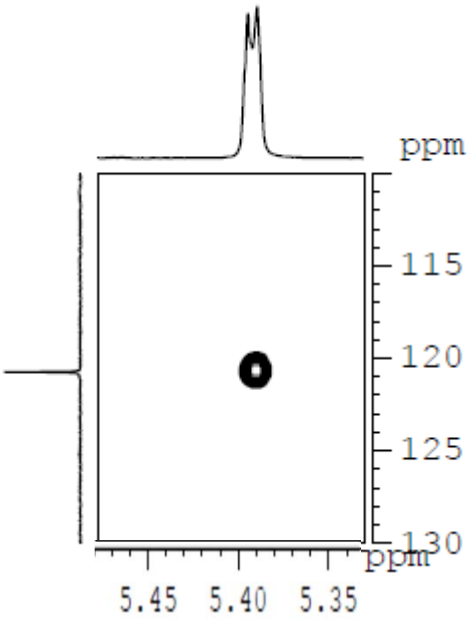
中央研究院化學研究所
核磁共振實驗室

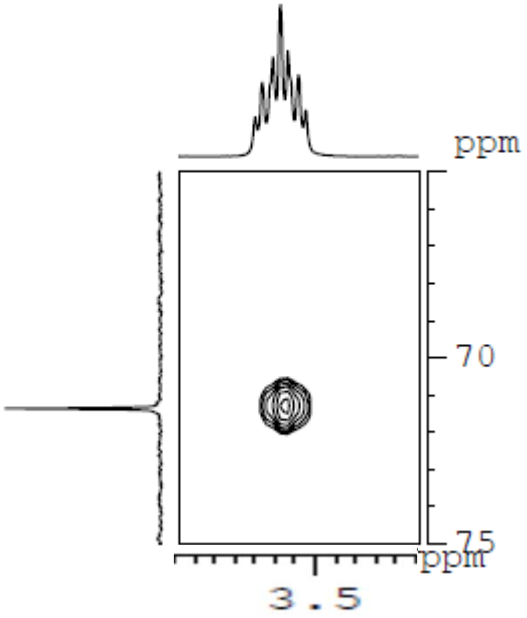
中央研究院化學研究所
Institute of Chemistry, Academia Sinica

ADR, *HSQC*

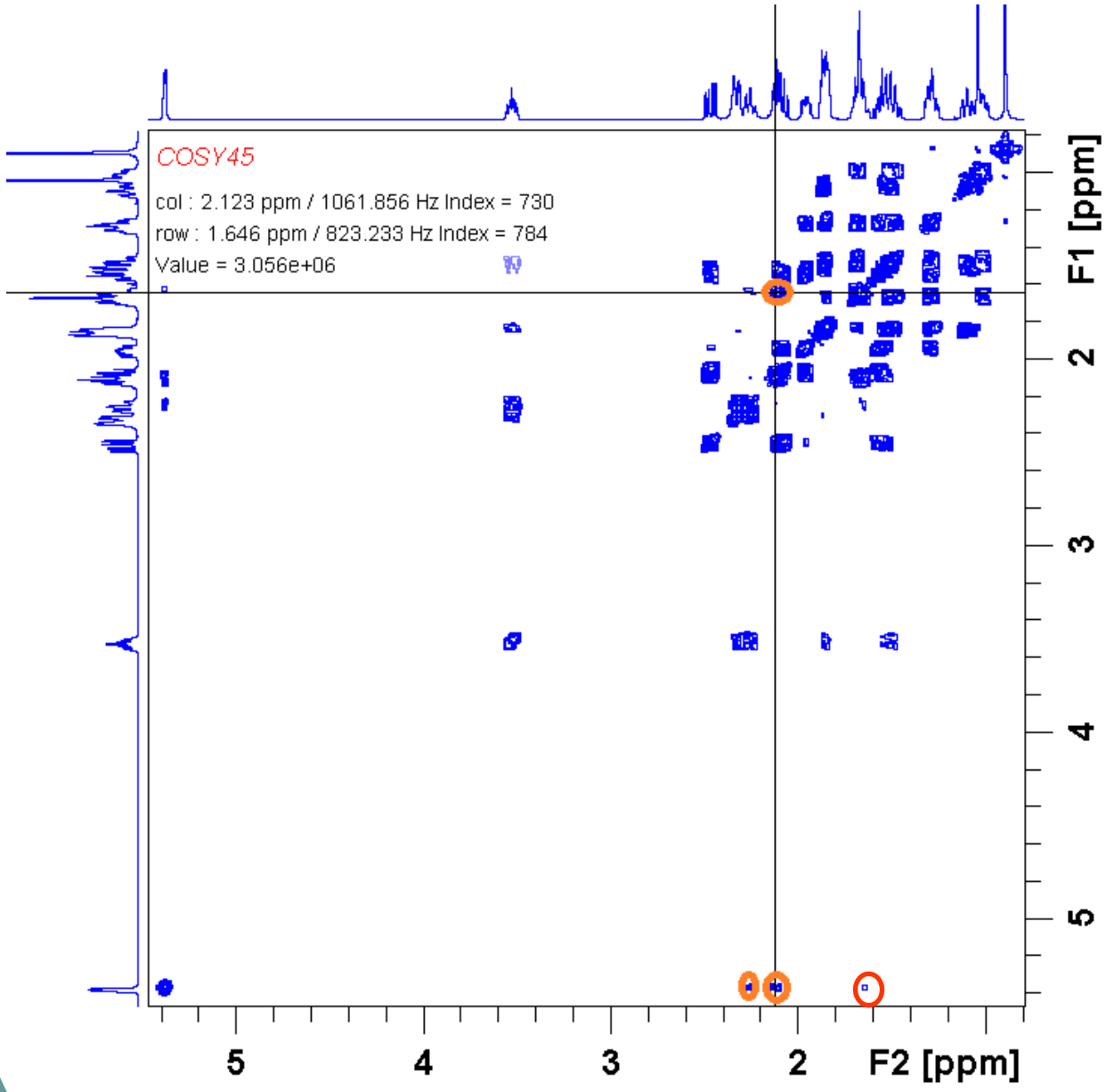

H19
H18
H16
H16
H15
H15
ppm
C18
C19
C15
C16
15
20
31
32
36
37
45
50
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0

ADR, *HSQC*

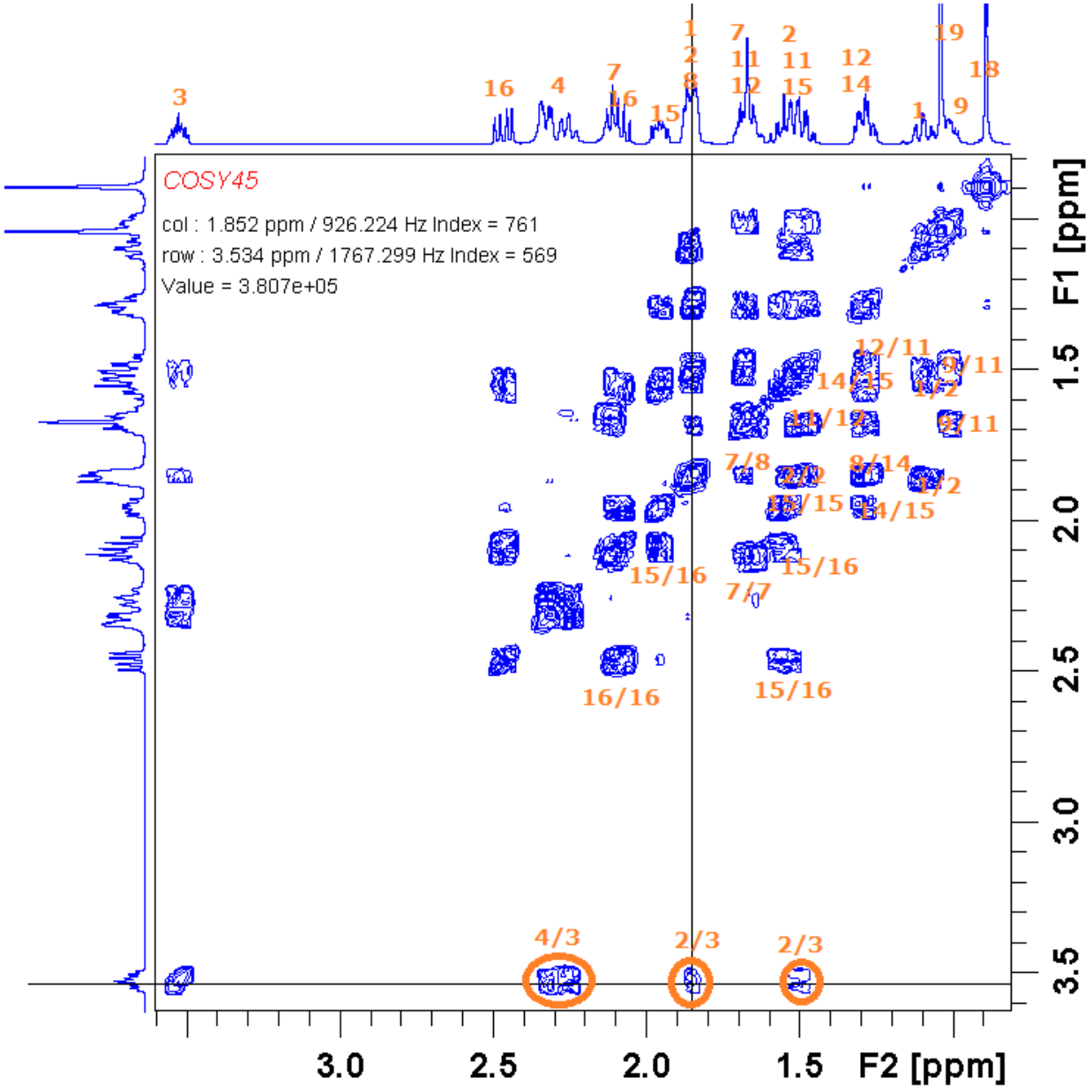




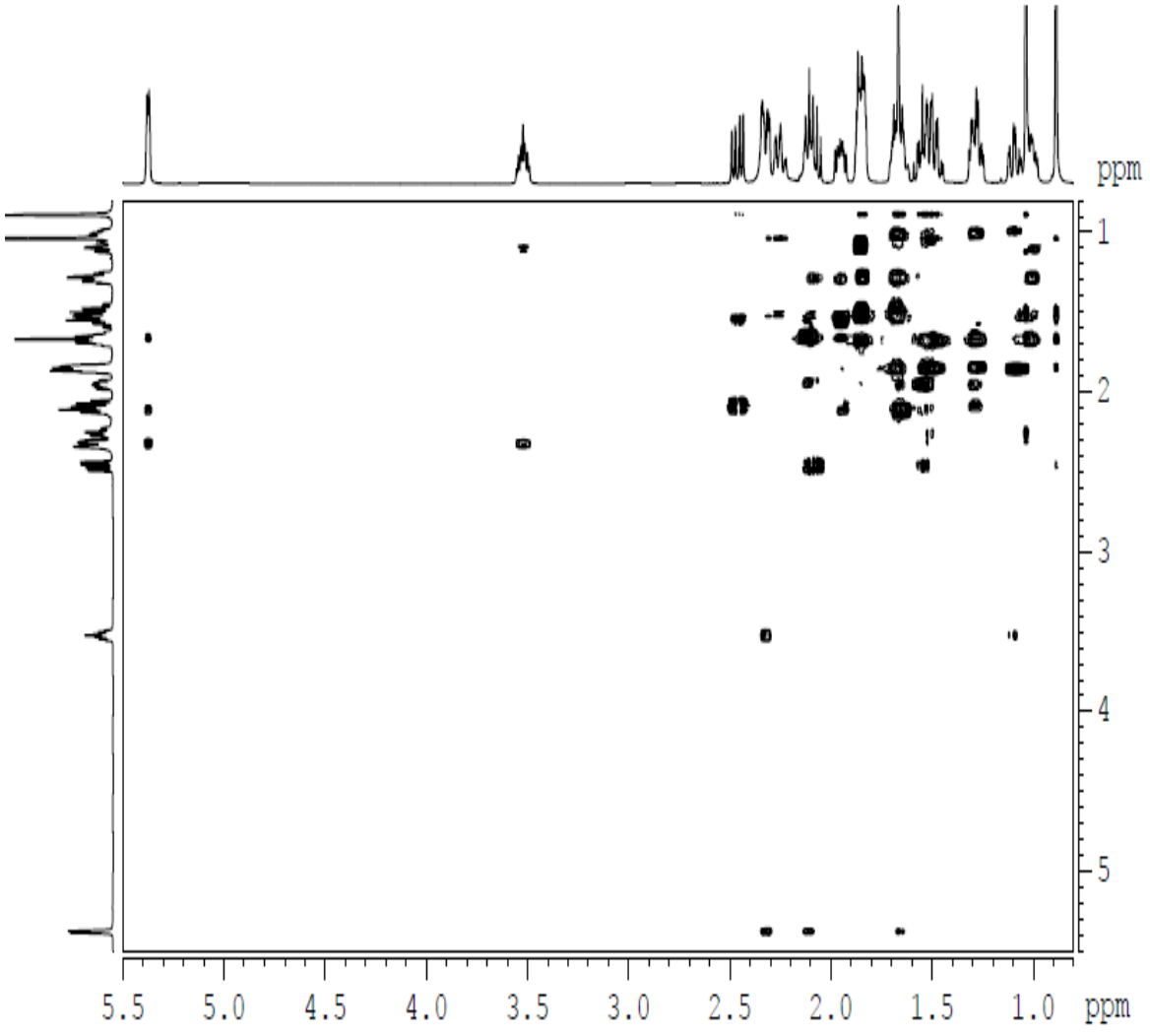
ADR, COSY


COSY45
col : 2.123 ppm / 1061.856 Hz Index = 730
row : 1.646 ppm / 823.233 Hz Index = 784
Value = 3.056e+06
F1 [ppm]
F2 [ppm]
5
4
3
2

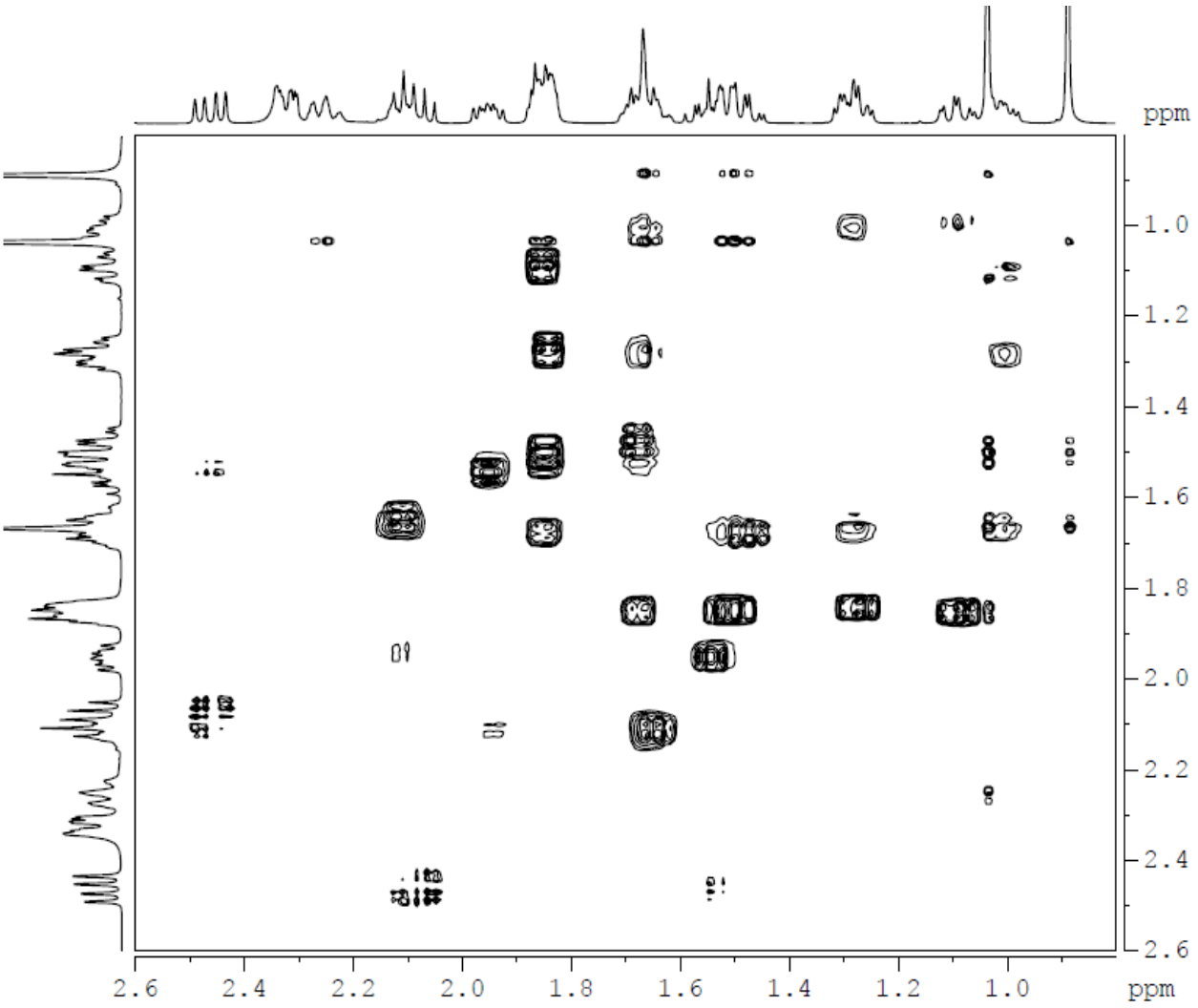
ADR, COSY


COSY45
col : 1.852 ppm / 926.224 Hz Index = 761
row : 3.534 ppm / 1767.299 Hz Index = 569
Value = 3.807e+05
F1 [ppm]
F2 [ppm]
3.0
2.5
2.0
1.5
3.5
4/3
2/3
2/3
15/16
15/16
16/16
15/16
7/7
7/8
12/11

ADR, *NOESY*



ADR, *NOESY*



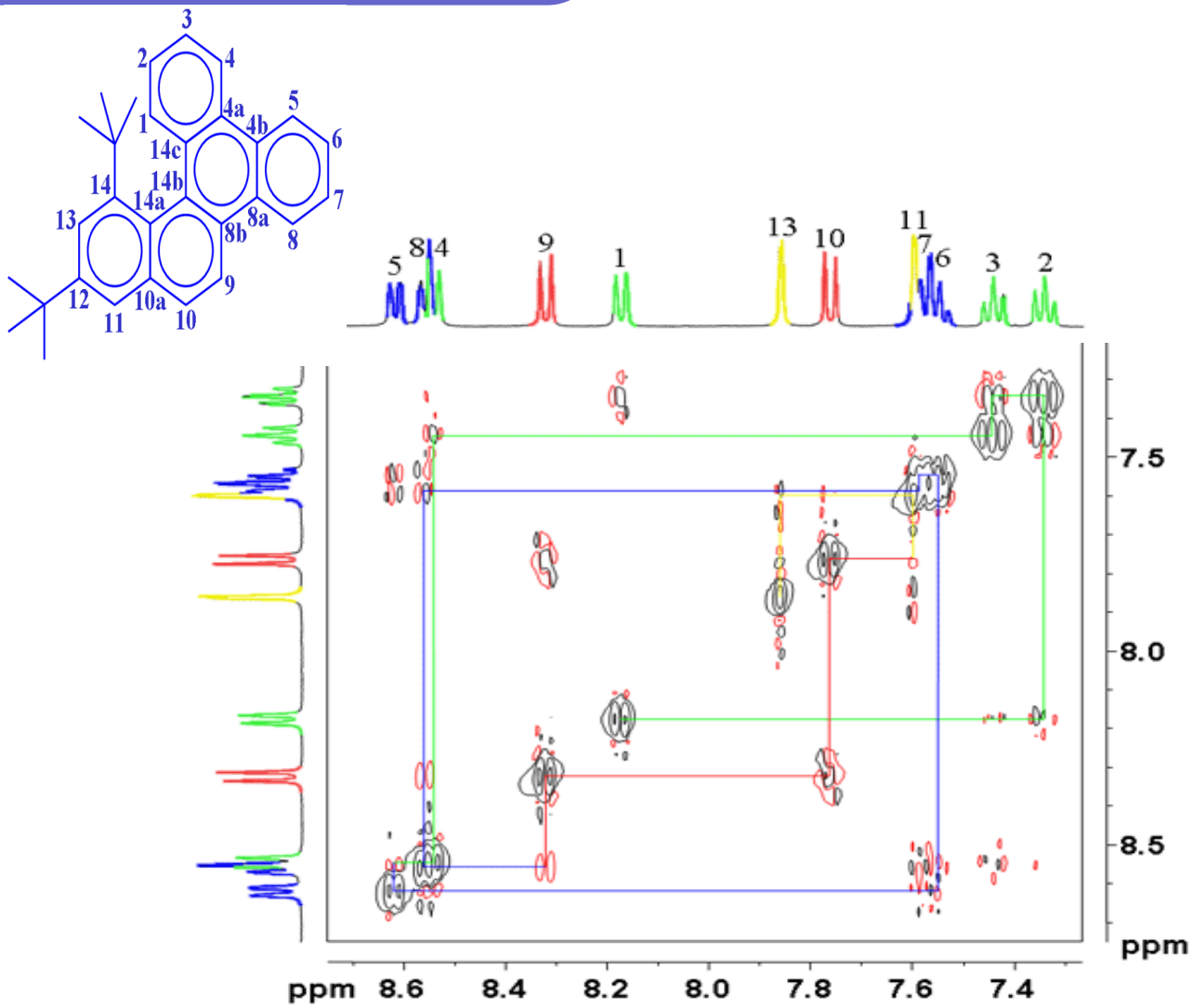
ADR

C17	hmbc	H18	hsqc	C18							
			hmbc	C12	hsqc	H12					
				C13	hsqc	H13					
				C14	hsqc	H14					
		H16	hqsc	C16							
		H15	hqsc	C15							
		H14	hqsc	C14							
		H12	hqsc	C12							
C19	hsqc	H19	hmbc	C10							
				C1	hsqc	H1	cosy	H2			
				C9	hsqc	H9	cosy	H11	cosy	H12	
				C5					hsqc	C11	
C6	hsqc	H6	cosy	H4	hsqc	C4					
			cosy	H7	hsqc	C7					
					cosy	H8					
			hmbc	C10							
				C4	hsqc	H4					
C3	hsqc	H3	hmbc	C2							
				C1							
			cosy	H4	hsqc	C4					
				H2	hsqc	C2					

ADR

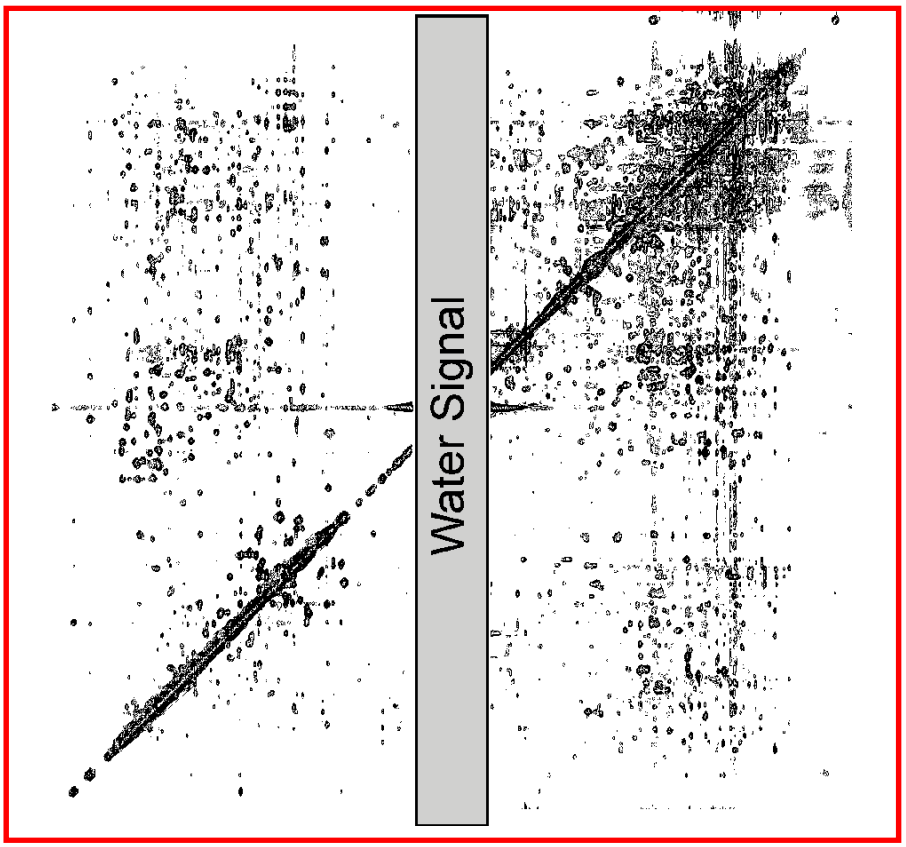
trans-dehydroandrosterone		
item	13C	1H
1	37.20	1.86, 1.10
2	31.42	1.66, 1.27
3	71.41	3.53
4	42.14	2.33, 2.25
5	141.09	NA
6	120.77	5.38
7	30.77	2.11, 1.67
8	31.48	1.843
9	50.22	1.01
10	36.61	NA
11	20.35	1.69, 1.48
12	31.50	1.66, 1.27
13	47.53	NA
14	51.74	1.29
15	21.86	1.96, 1.55
16	35.84	2.47, 2.08
17	221.27	NA
18	13.53	0.89
19	19.42	1.04

2D NOESY spectrum of aromatic region of 12,14-ditbutylbenzo[g]chrysene



Continuing the connectivity, we can assign H10 as 7.76 ppm H11 as 7.60 ppm and H13 as 7.86 ppm. In the opposite direction, H7 is at 7.59 ppm , H6 at 7.55 ppm, H5 at 8.62 ppm, H4 at 8.54 ppm, H3 at 7.44 ppm, H2 at 7.34 ppm and H1 at 8.17 ppm. Aromaric region shows connectivity and separation into four color-coded proton groups

2D NOESY (Nuclear Overhauser Effect Spectroscopy)



The NOESY experiment is crucial for the determination of protein structure. It uses the dipolar interaction of spins (the nuclear Overhauser effect, NOE) for correlation of protons. The intensity of the NOE is in first approximation proportional to $1/r^6$, with r being the distance between the protons: The correlation between two protons depends on the distance between them, but normally a signal is only observed if their distance is smaller than 5 Å. The NOESY experiment correlates all protons which are close enough. It also correlates protons which are distant in the amino acid sequence but close in space due to tertiary structure. This is the most important information for the determination of protein structures