Steps for NMR Experiments small molecule

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OUTLINE

1. 樣品配製
2. 儀器選擇
3. NMR探頭選擇
4. 勻場
5. 1D ^{1}H NMR
6. 1D ^{13}C NMR
7. 2D homonuclear NMR
8. 2D heteronuclear NMR

1.1 樣品配製

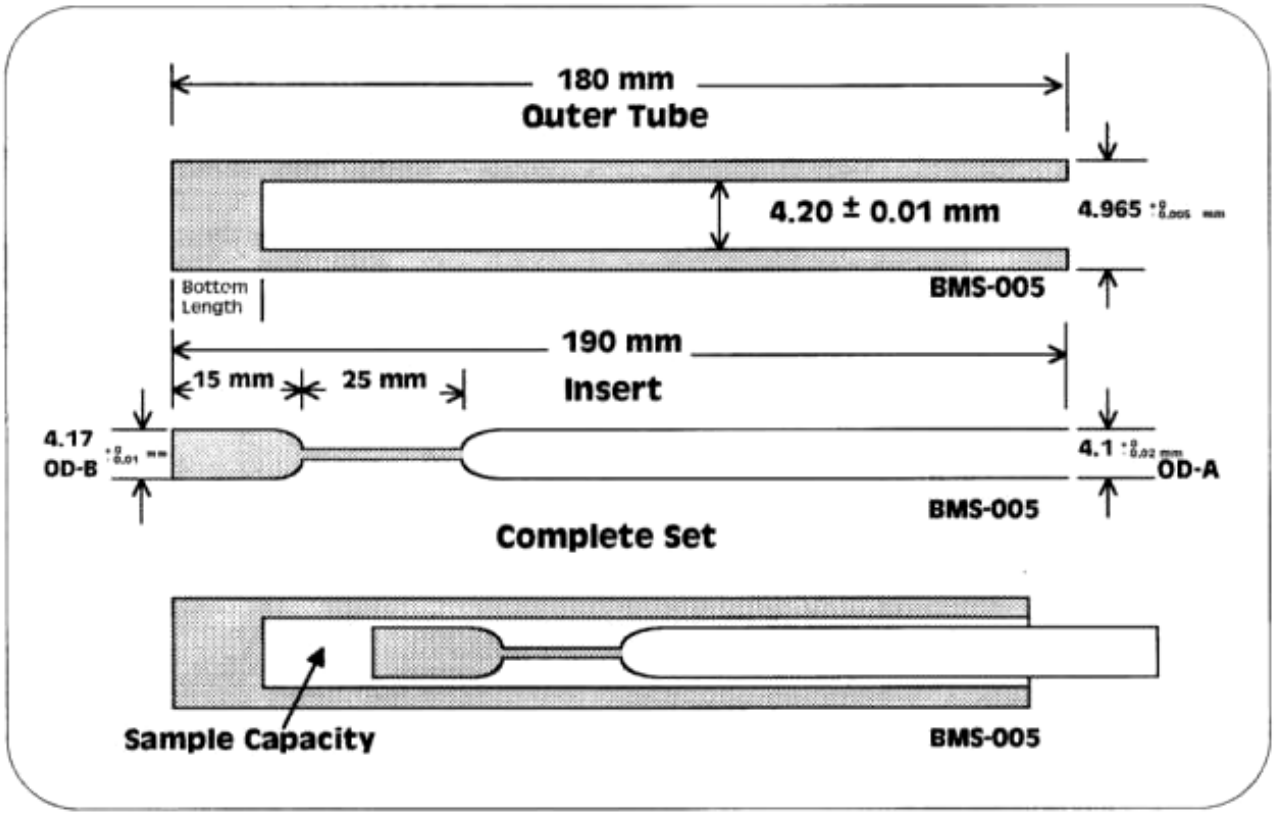
- 取用適當的樣品量(10 mg)
- 選擇合適之D-solvent
- 取用合適的NMR sample tube
- 配製適當的量(5mm)

  ⇒一般樣品管: 體積 ≈ 0.5 mL, 3 cm

  ⇒SHIGEMI樣品管: 體積 ≤ 0.3 mL

1.2 樣品配製

- SHIGEMI樣品管



2.1 儀器選擇

	低磁場	高磁場
化學位移(ppm)	相同	
偶合常數(J, Hz)	相同	
靈敏度(sensitively)	低	高
peak鑑別度	低	高

2.2 儀器選擇(靈敏度)

For ^{1}H: $\gamma = 26753\ (s^{-1}Gauss^{-1})$

$= 2.6753\ (s^{-1}Tesla^{-1})$

(1Tesla = 10^4 Gauss)

when B_o = 4.69T $\Rightarrow \Delta E = h\nu = (\gamma h B_o)/2\pi$

$\Rightarrow \nu = \gamma B_o/2\pi = 2\times10^8\ (s^{-1})$ = 200 MHz

2.3 儀器選擇(靈敏度)

- **Boltzmann equation:**

  $N_\beta/N_\alpha = \exp(-\Delta E/kT) = \exp[(\gamma h B_o)/(2\pi kT)]$

- **when B_o = 4.69T (200MHz) $\Rightarrow$ N_β/N_α = 0.999967**

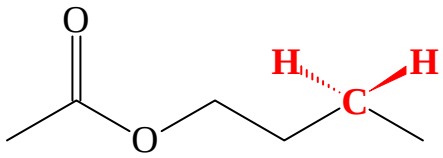
  i.e. $N_\beta = 10^6 \Rightarrow N_\alpha$ = 1000033

- **when B_o = 9.38T (400MHz)**

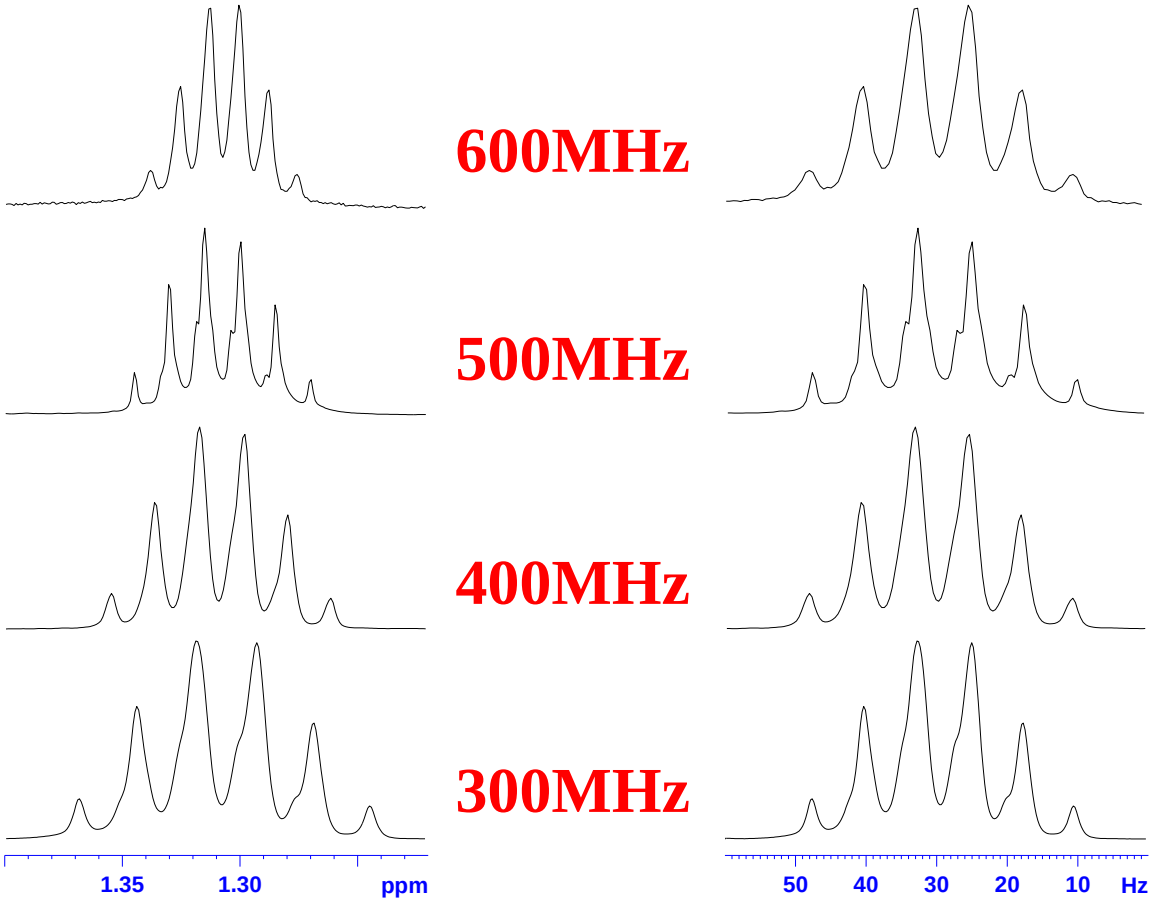
  $N_\beta = 10^6 \Rightarrow N_\alpha$ = 1000066

$\therefore$ field $\times$ 2 $\Rightarrow$ sensitively $\times$ 2

2.4 儀器選擇(鑑別度)

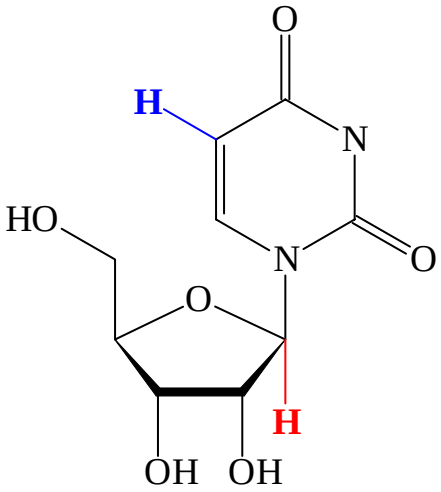


n-butyl acetate

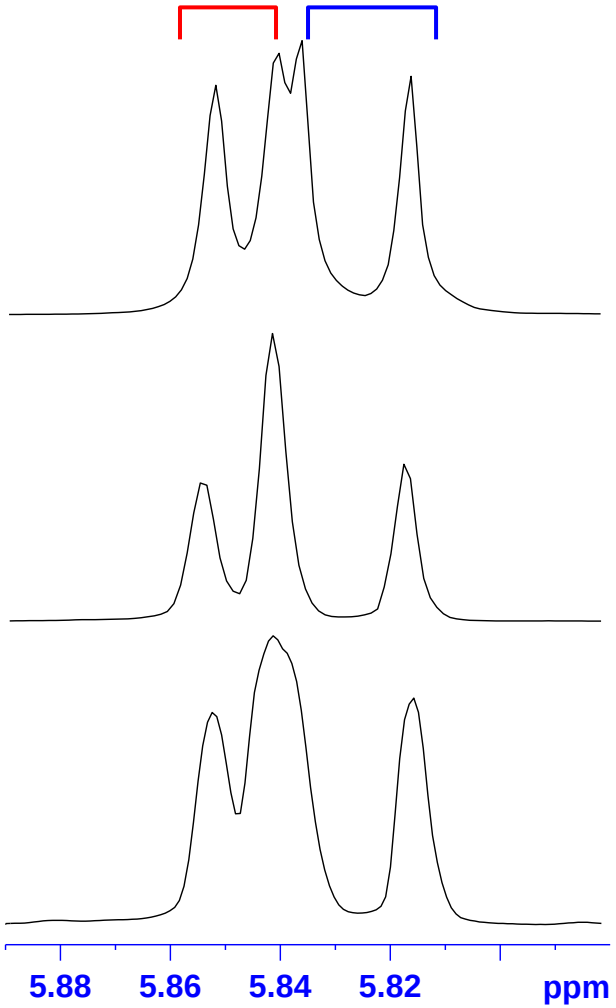


ppm **Hz**

2.5 儀器選擇(鑑別度)



uridine



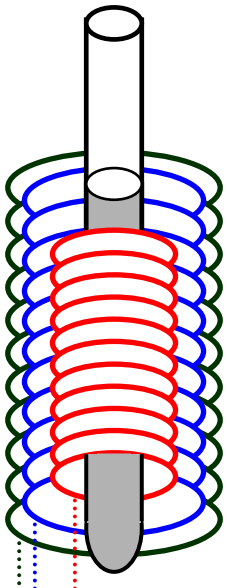
500MHz

400MHz

300MHz

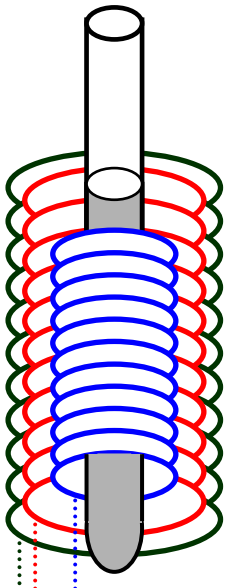
3.1 NMR探頭選擇

- **Inverse** **and** **Observe**



$^{1}H/^{2}D$
X
gradient

^{1}H sensitivity × 50%



X
$^{1}H/^{2}D$
gradient

3.2 NMR探頭選擇

- **Fixed-channel probe**
  - **SEL**: ^{1}H selective
  - **DUL**: ^{13}C and ^{1}H (inner ^{13}C, outer ^{1}H)
  - **SEI**: selective inverse (inner ^{1}H, outer X)
  - **TXO**: triple resonance observe
  - **TXI**: triple resonance inverse
  - **QXI**: quattro resonance inverce

3.3 NMR探頭選擇

- **Broad-band probe**
  - **BBO**: broad band observe
  - **BBI**: broad band inverse
  - **TBO**: tripe resonance broad band observe
  - **TBI**: tripe resonance broad band inverse
  - **QNP**: quattro nucleus probe
    ($^{1}H/^{13}C/^{19}F/^{31}P$, $^{1}H/^{13}C/^{15}N/^{31}P$)

3.4 NMR探頭選擇

- **CryoProbe**

  樣品熱絕緣式冷卻核磁共振探頭

  cryoprobe{^{1}H}, {^{1}H, X}

  ➢ 將探頭內之線圈系統

  冷卻到液氦溫度 (4 K)

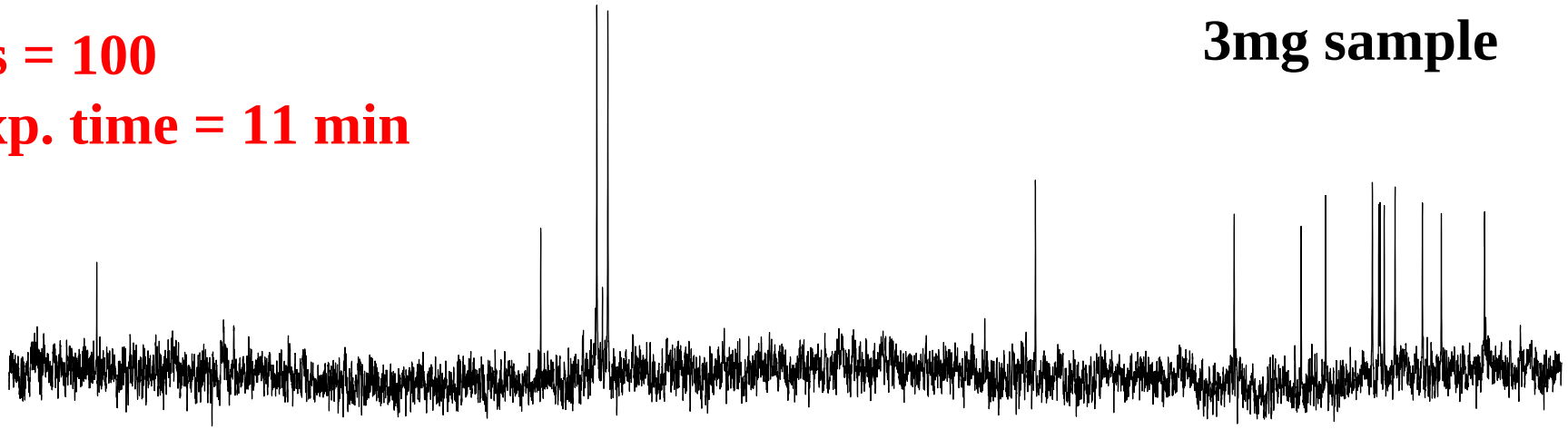
  ➢ 降低電子線路所產生之雜訊

  提高signal-to-noise ratio (S/N)

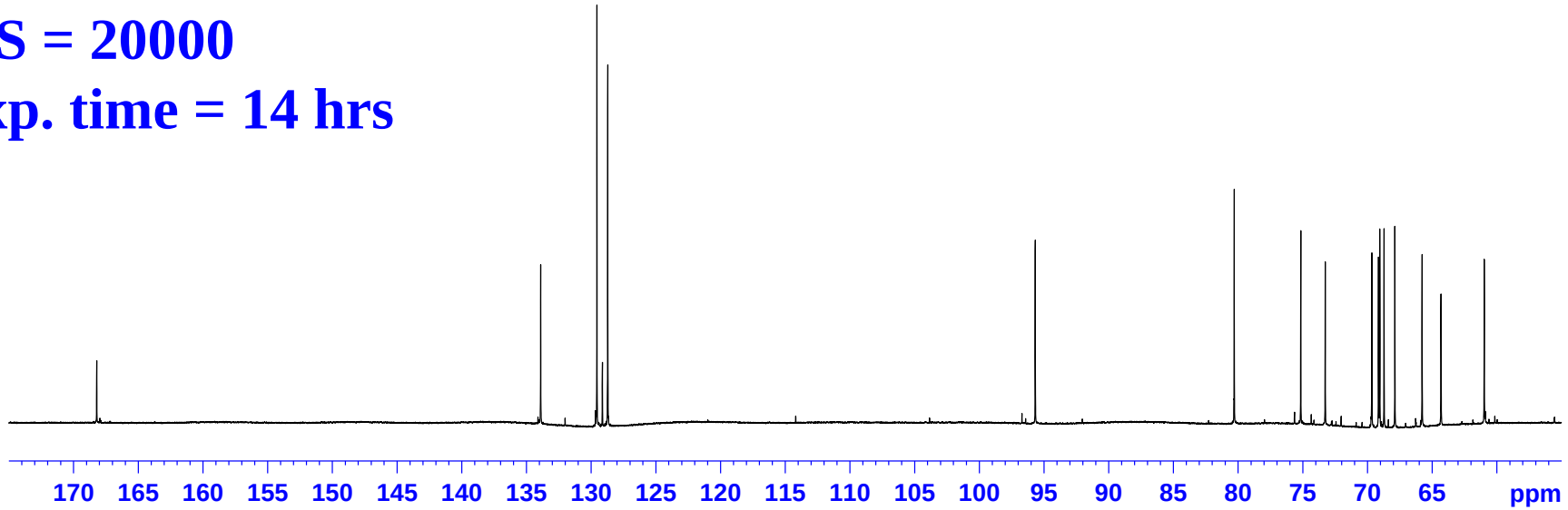
3.5 NMR探頭選擇

ns = 100
exp. time = 11 min

3mg sample



NS = 20000
exp. time = 14 hrs



3.6 NMR探頭選擇

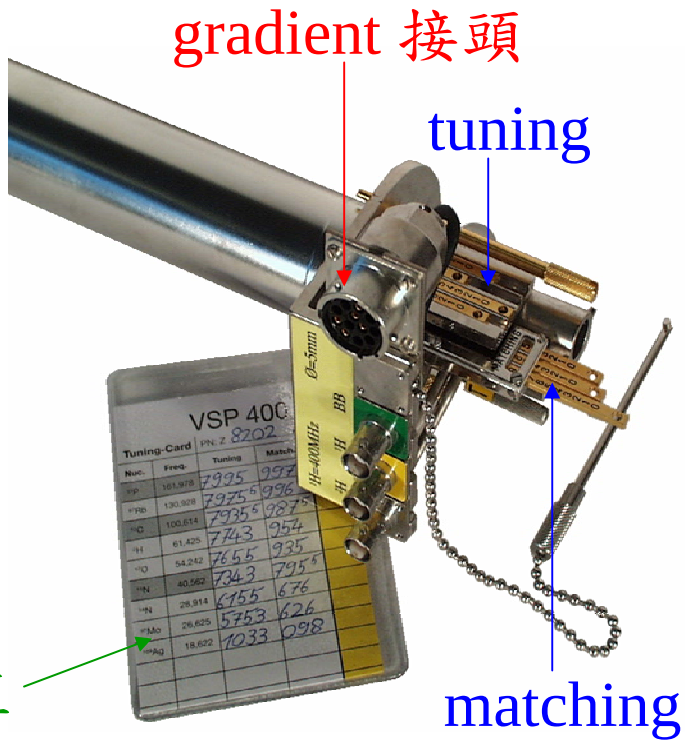
- **Gradient (磁場梯度)**

  Z-GRAD: z gradient

  XYZ-G: x, y, z gradient

➢ 節省 5 倍以上的實驗時間

➢ 執行自動化的勻場動作



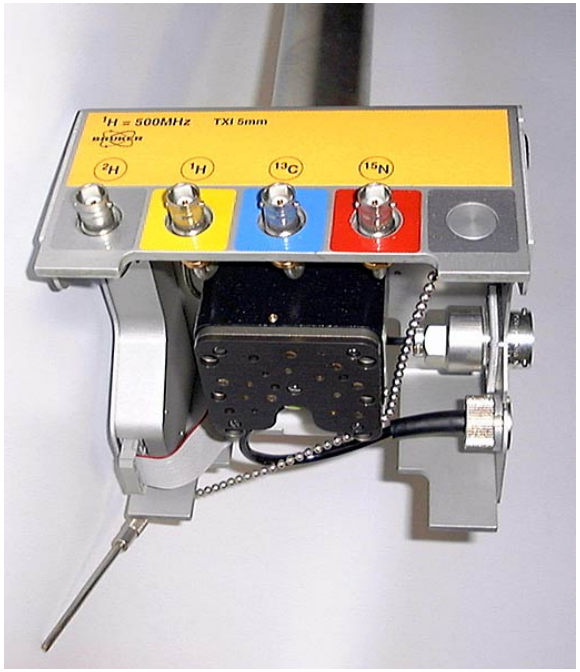
BBO z-grad

3.7 NMR探頭選擇

- **ATM Probe**

  Automatic Tuning and Matching

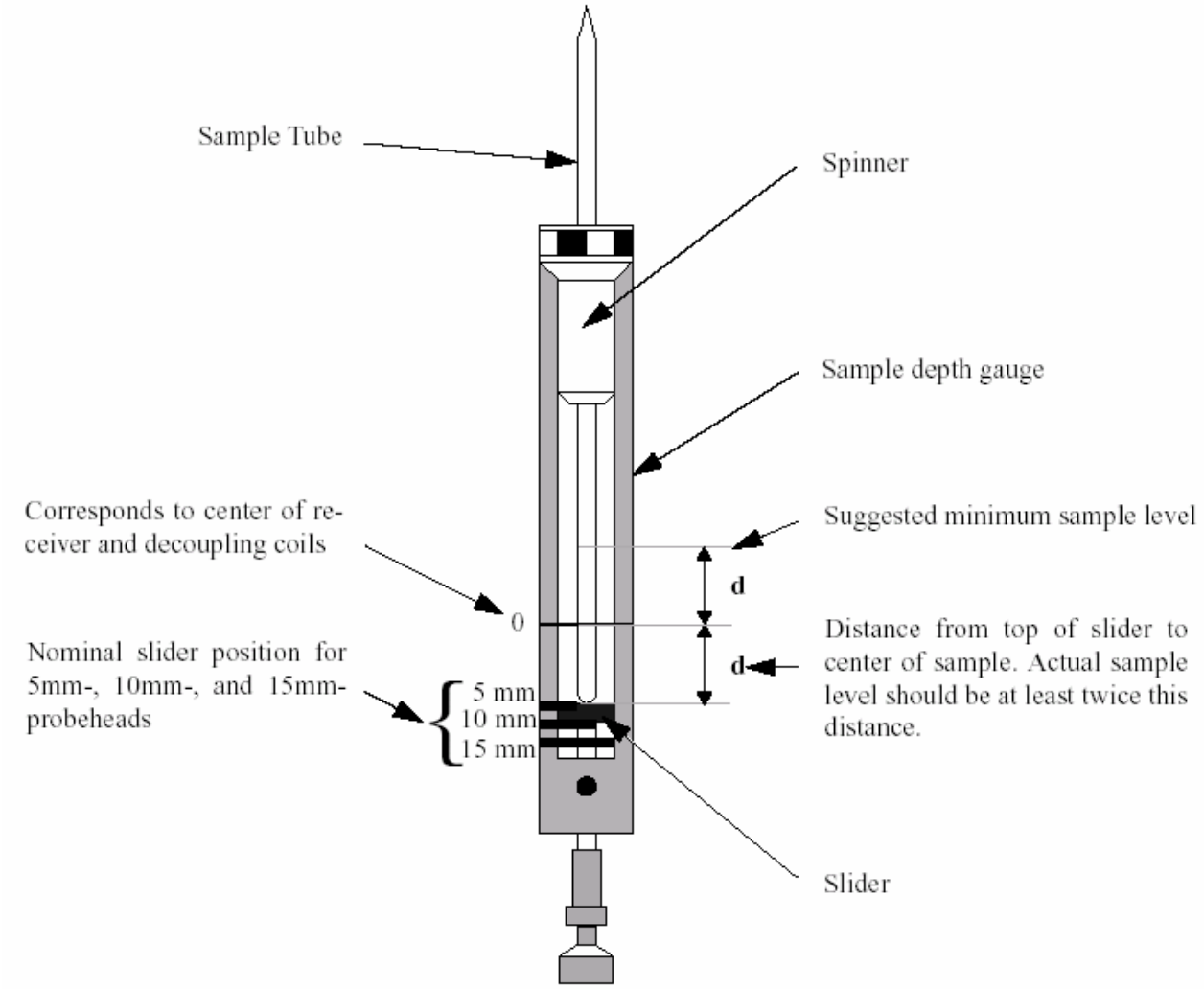
➢ 以簡單的方式使機器自動調整樣品於探頭中頻率(tune)與阻抗(match)



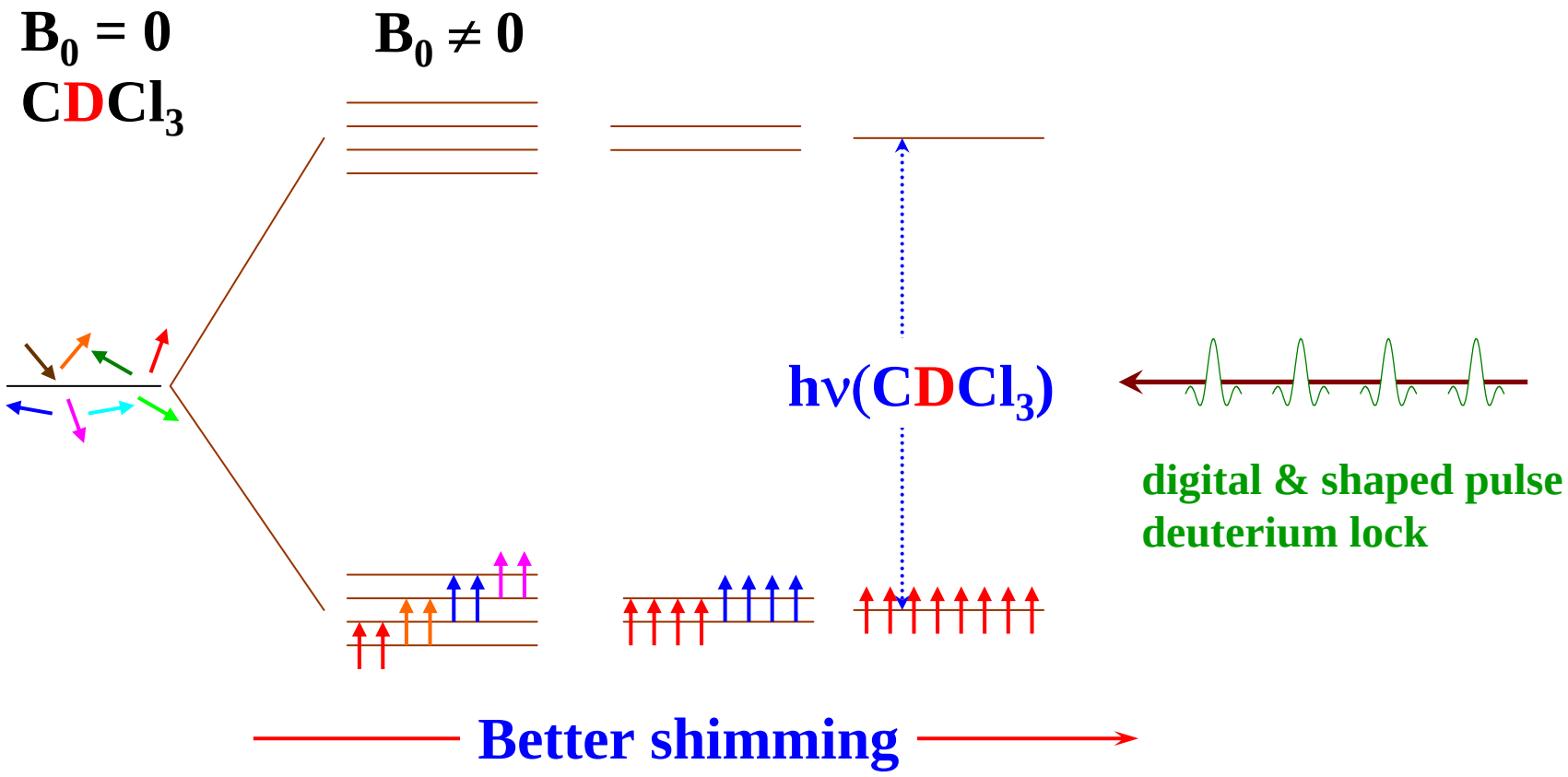
tune: 輸出正確的共振頻率

match: 使系統的阻抗值為 50Ω

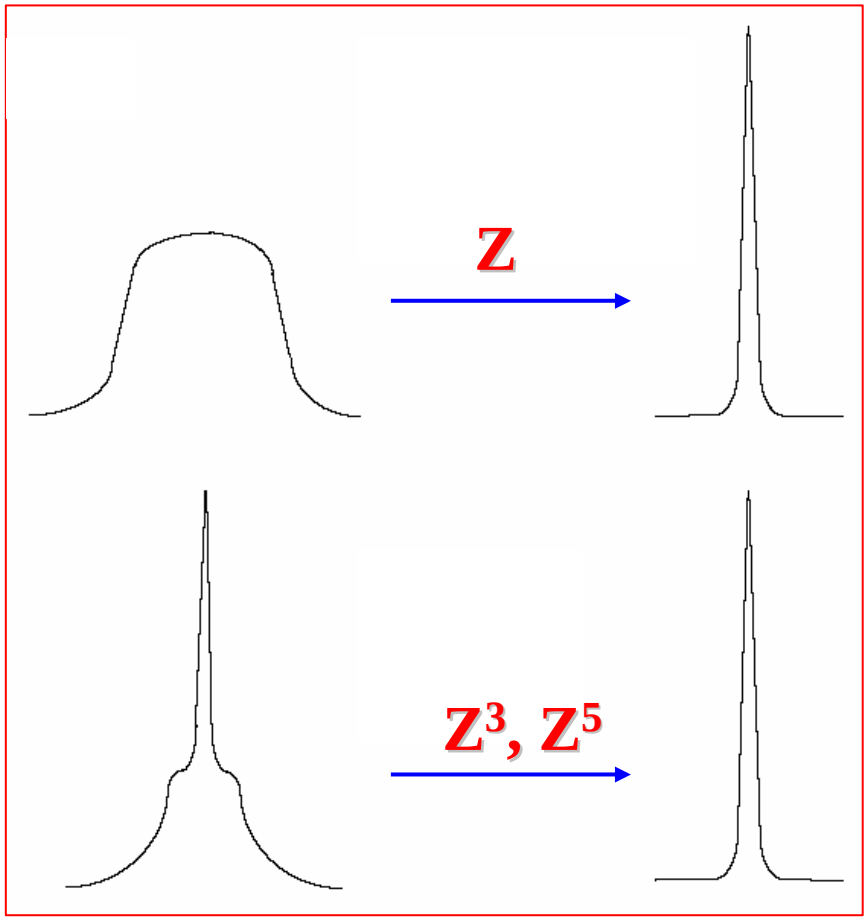
4.1 (Shim)

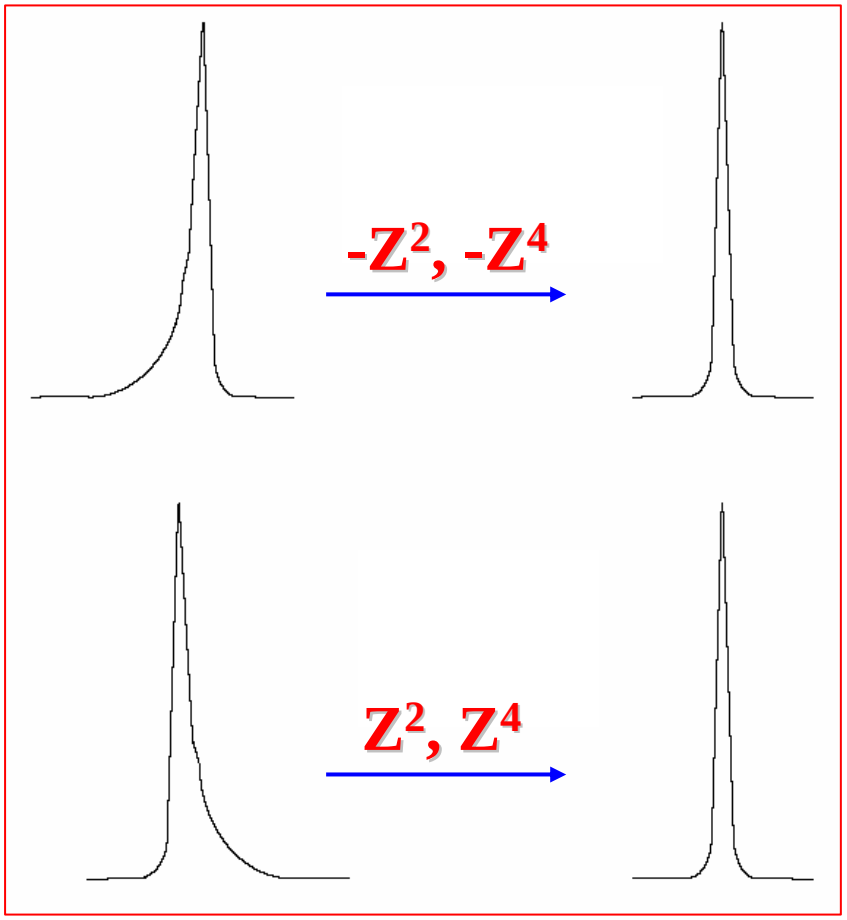

Sample Tube
Spinner
Sample depth gauge
Corresponds to center of receiver and decoupling coils
Suggested minimum sample level
d
0
Distance from top of slider to center of sample. Actual sample level should be at least twice this distance.
d
Nominal slider position for 5mm-, 10mm-, and 15mm-probeheads
5 mm
10 mm
15 mm
Slider

4.2 氘鎖定 (Deuterium Lock)
勻場(Shim)

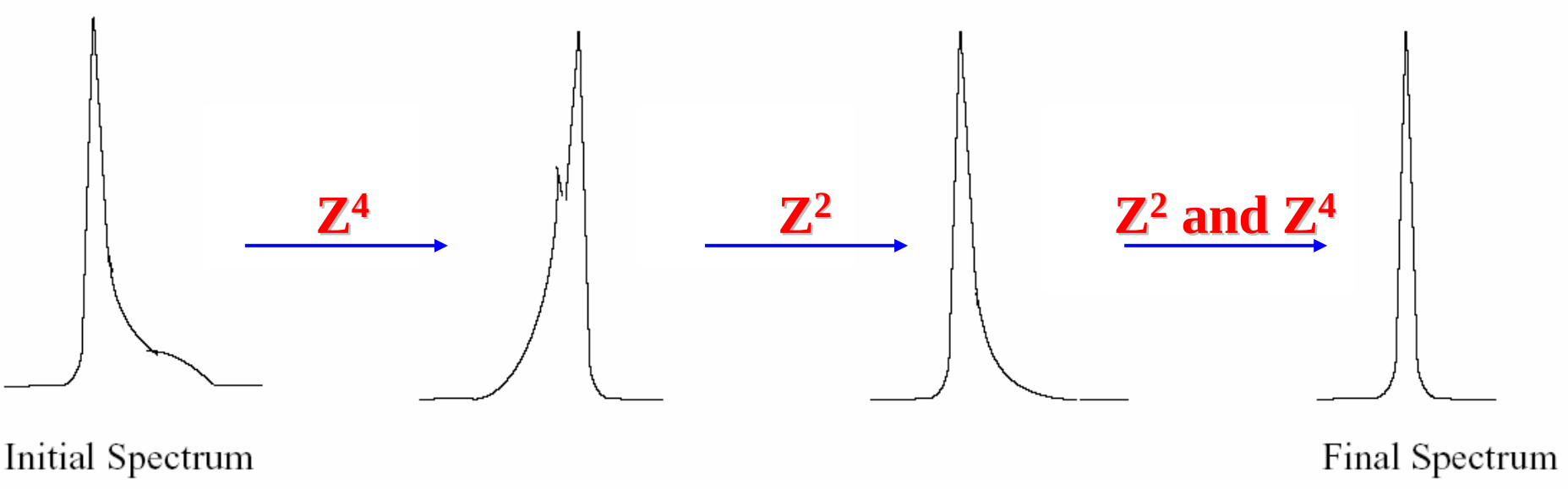


4.3 勻場(Shim)





4.4 勻場(Shim)



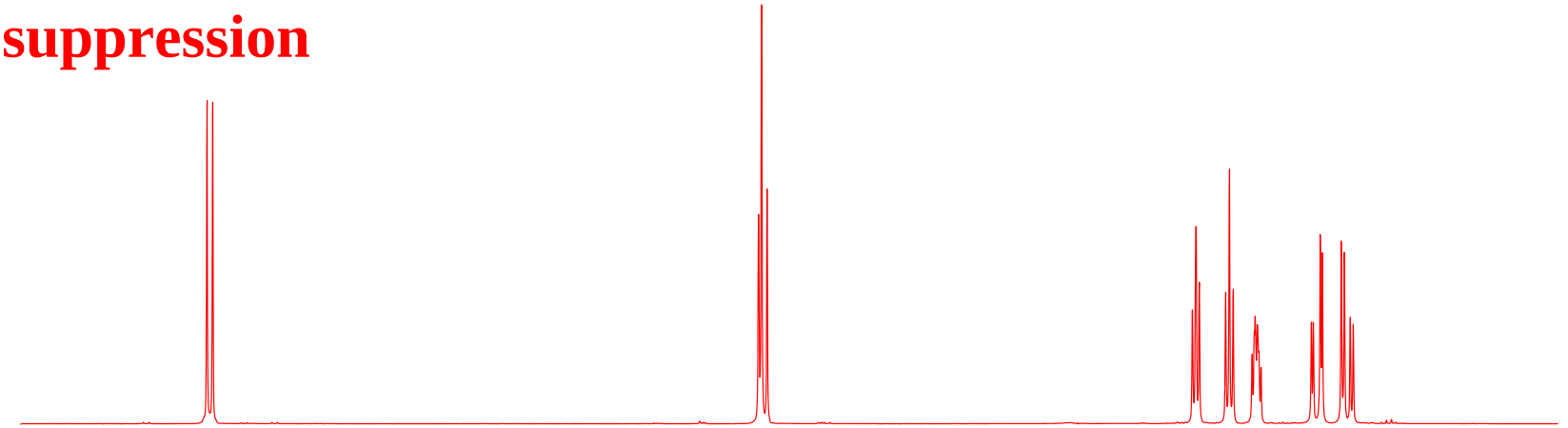
5.1 ^{1}H NMR

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

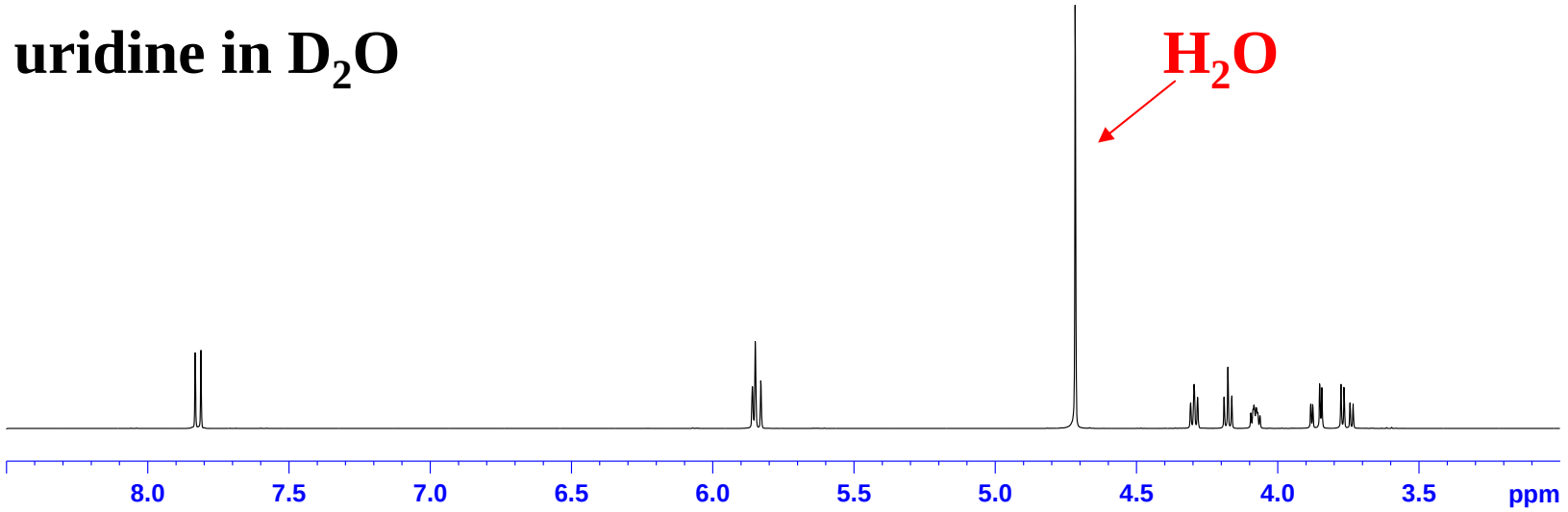
5.2 Solvent Suppression

提高濃度較稀或高含水量之樣品的靈敏度

H_2O suppression

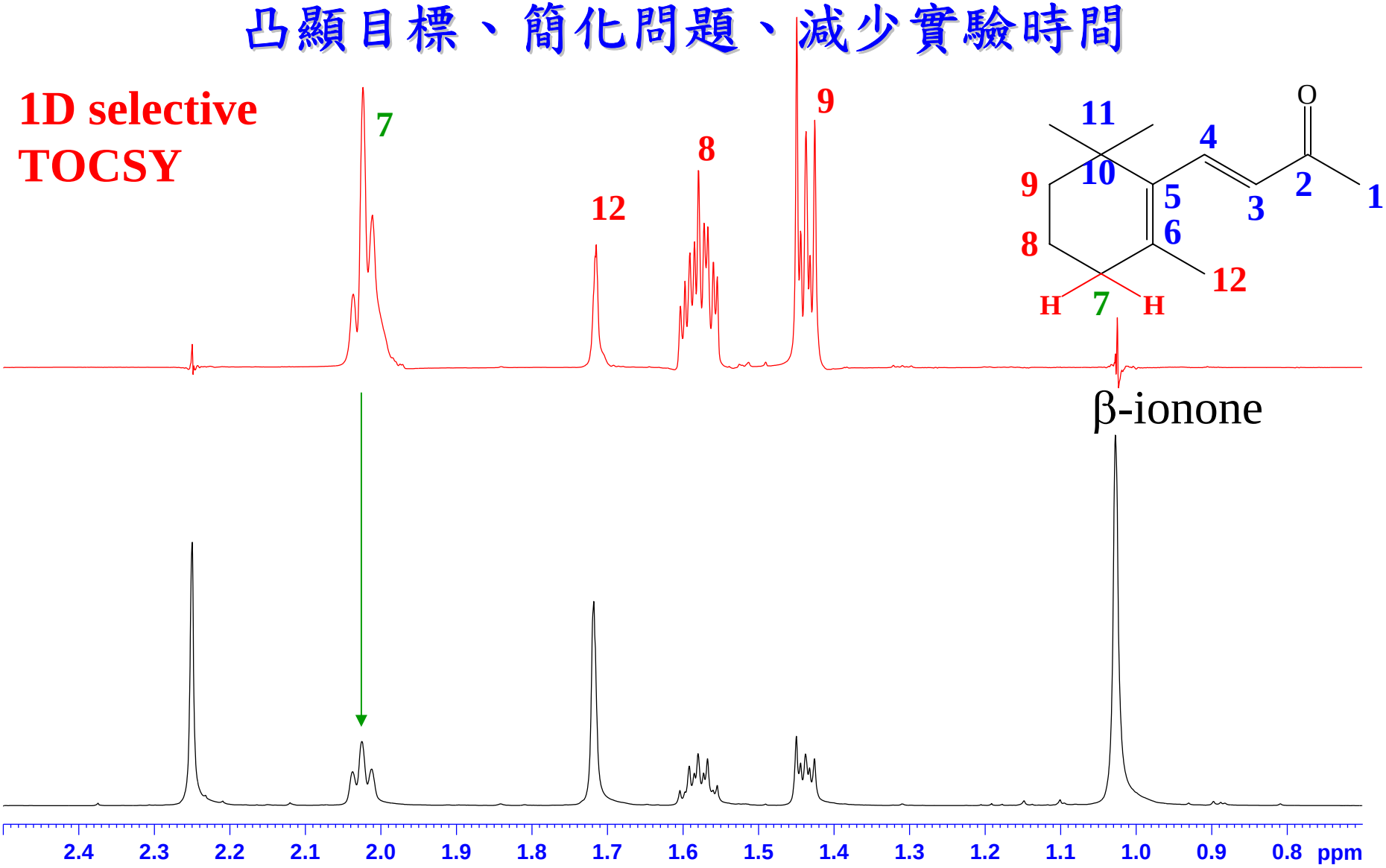


25mg uridine in D_2O



5.4.1 1D Selective Excitation

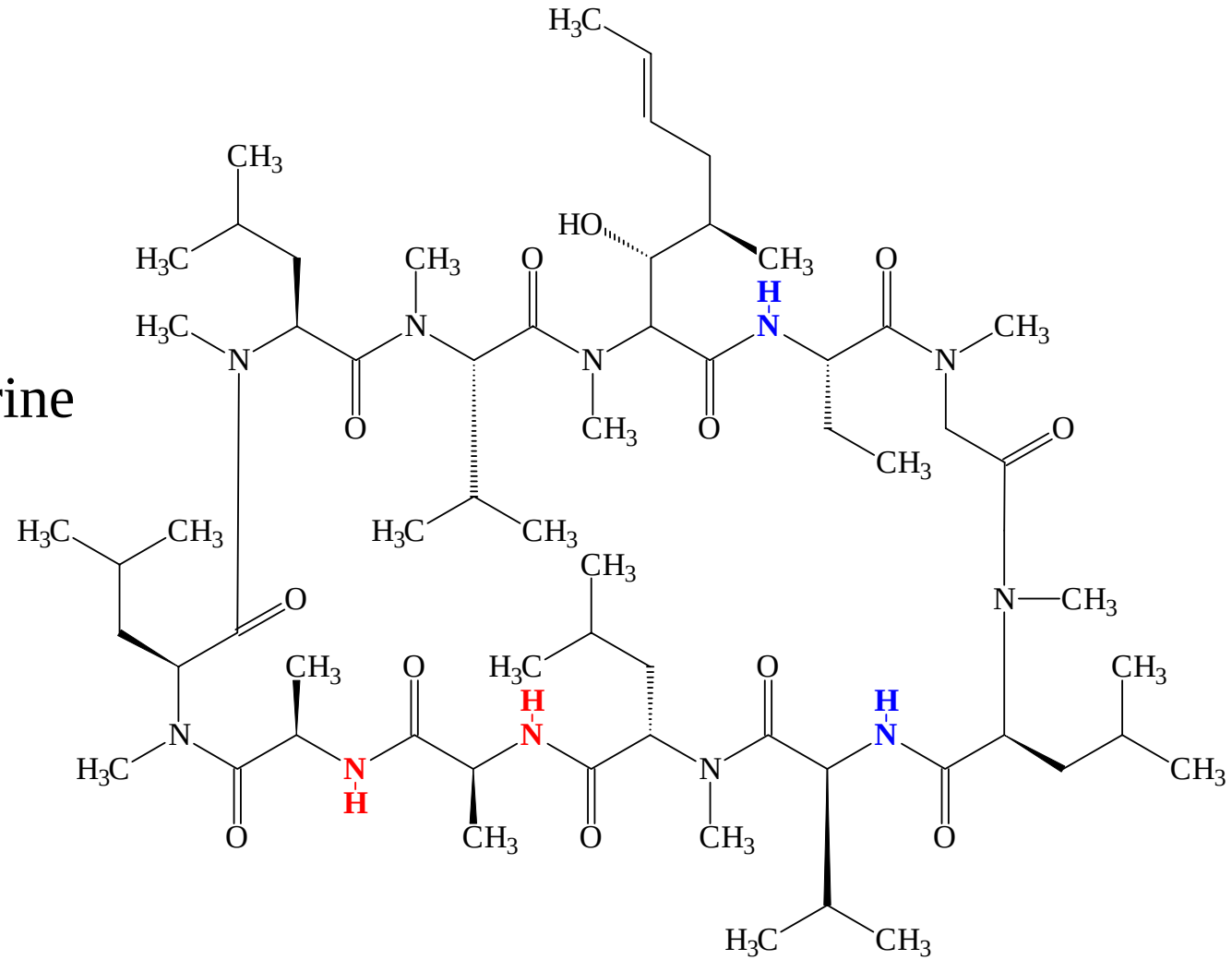
凸顯目標、簡化問題、減少實驗時間



5.4.2 1D Selective Excitation

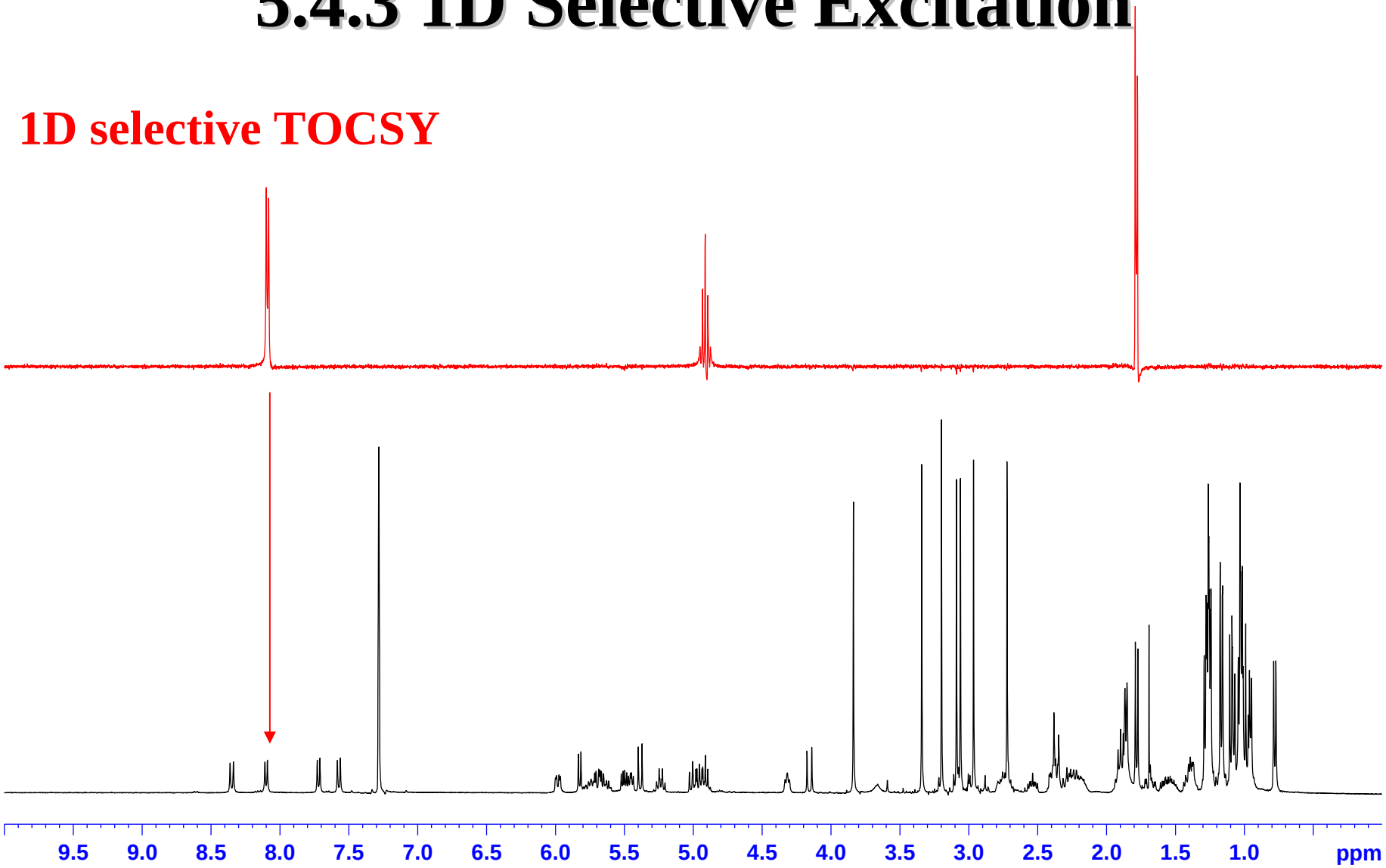
COSY, TOCSY, NOESY, ROESY

Cyclosporine



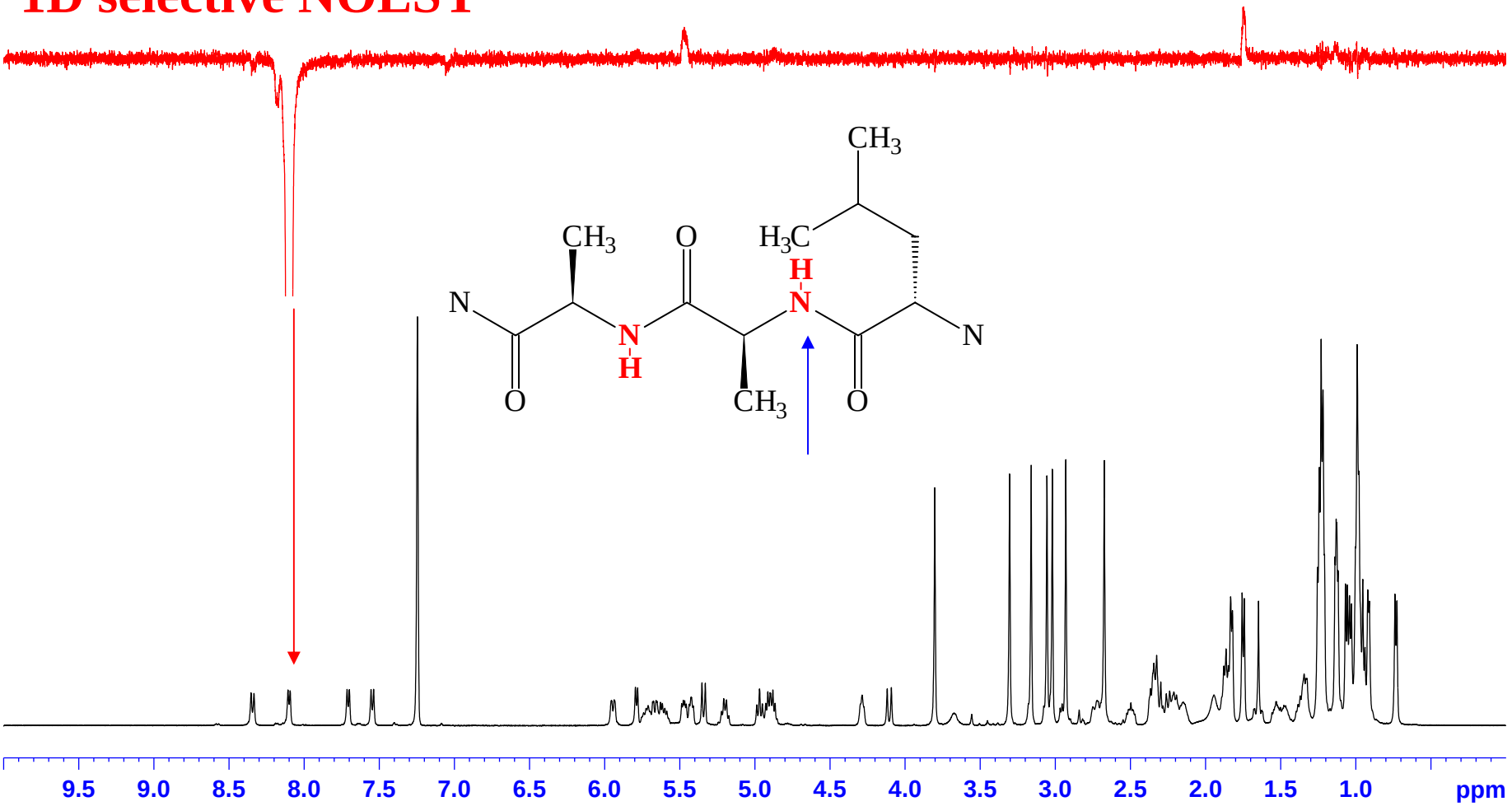
5.4.3 1D Selective Excitation

1D selective TOCSY



5.4.4 1D Selective Excitation

1D selective NOESY



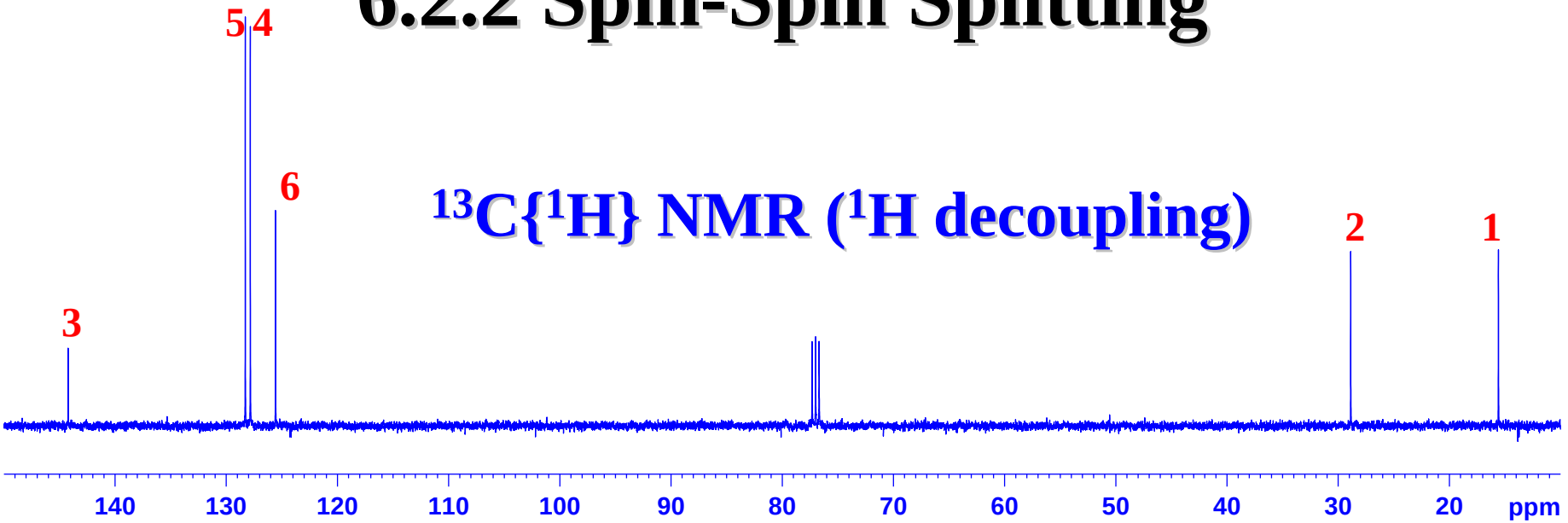
6.1 ^{13}C NMR

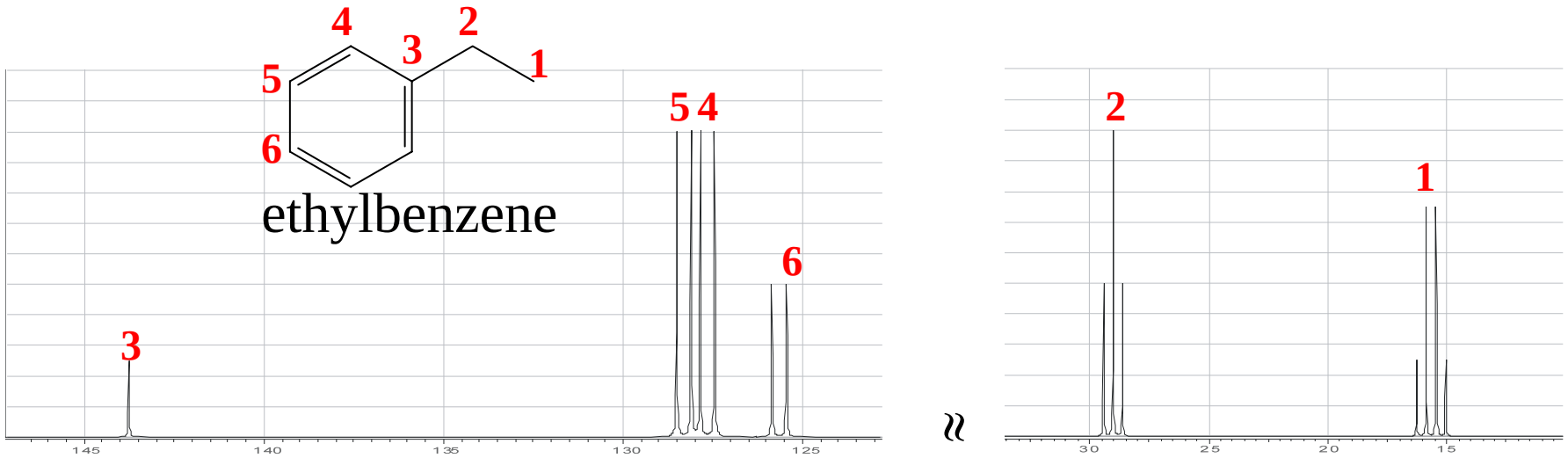
- ^{12}C has no magnetic spin.
- ^{13}C has a magnetic spin, but is only 1.1% of the carbon in a sample.
- The gyromagnetic ratio of ^{13}C is one-fourth of that of ^{1}H.
- Signals are weak, getting lost in noise.

6.2.1 Spin-Spin Splitting

- It is unlikely that a ^{13}C would be adjacent to another ^{13}C, so splitting by carbon is negligible.
- ^{13}C will magnetically couple with attached protons and adjacent protons.
- These complex splitting patterns are difficult to interpret.

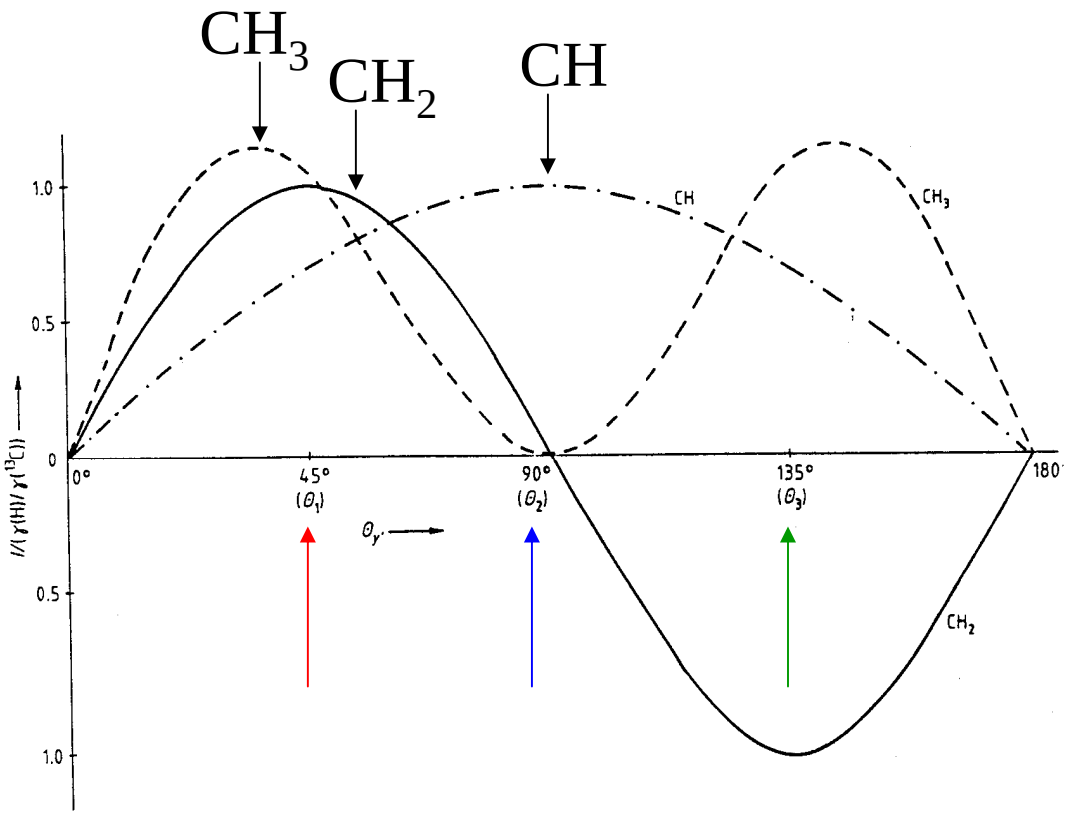
6.2.2 Spin-Spin Splitting





6.3.1 DEPT

Distortionless Enhancement by Polarization Transfer

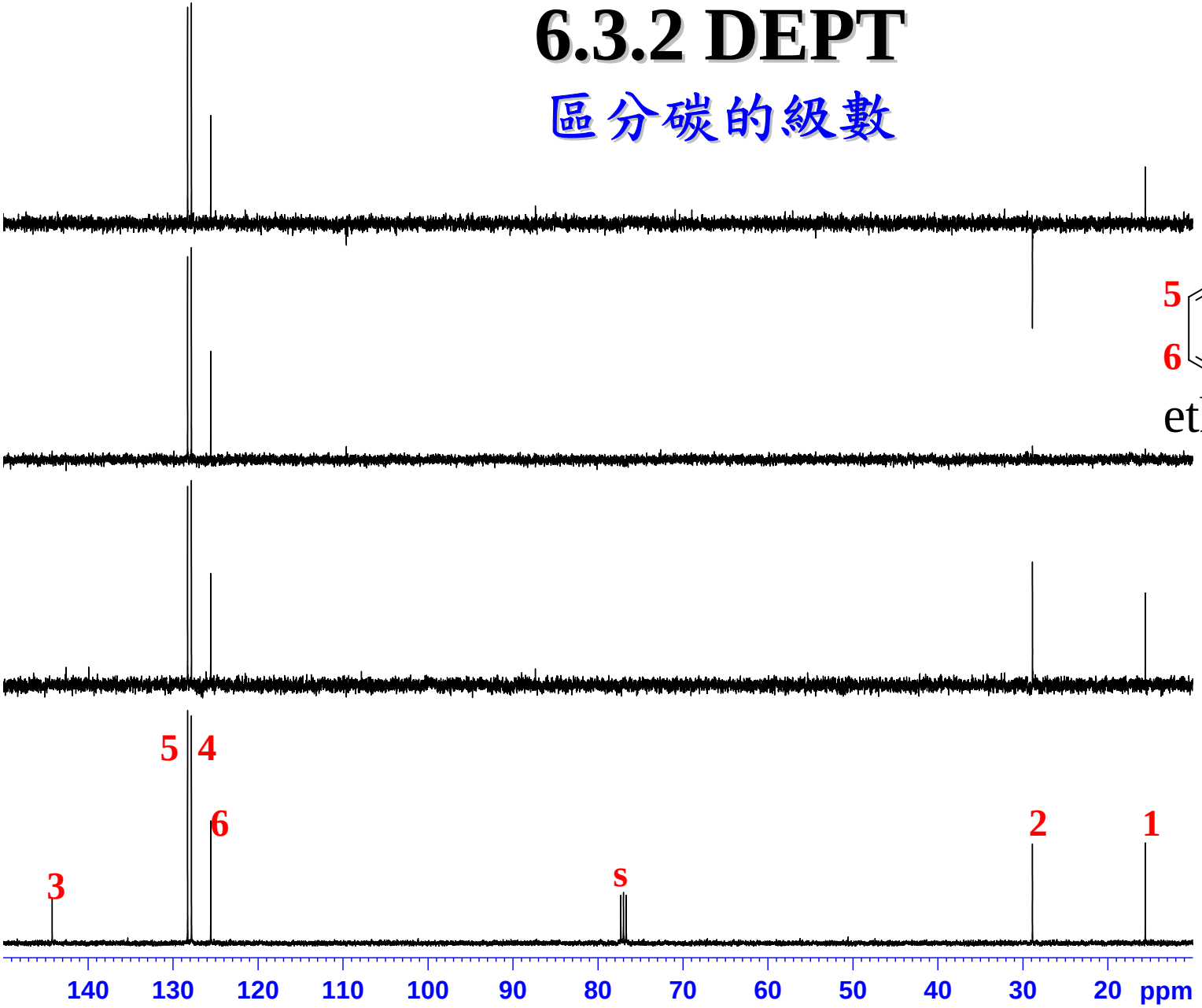


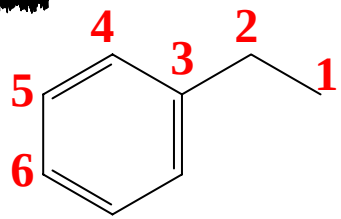
	45°	90 °	135 °
CH	+	+	+
CH_2	+	×	-
CH_3	+	×	+

CH

6.3.2 DEPT

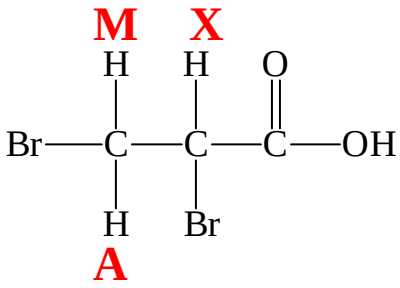
區分碳的級數



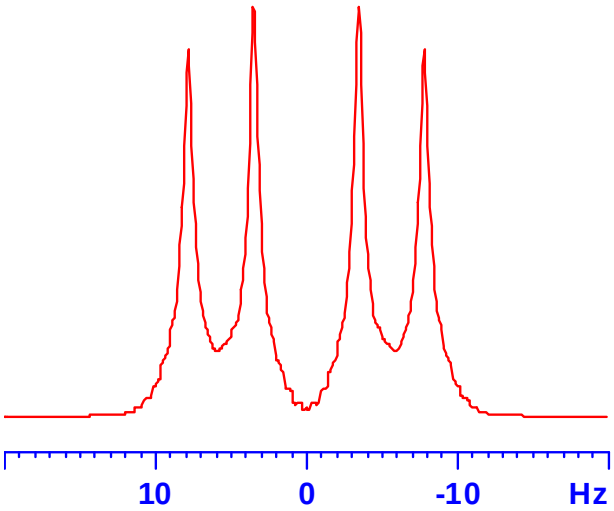


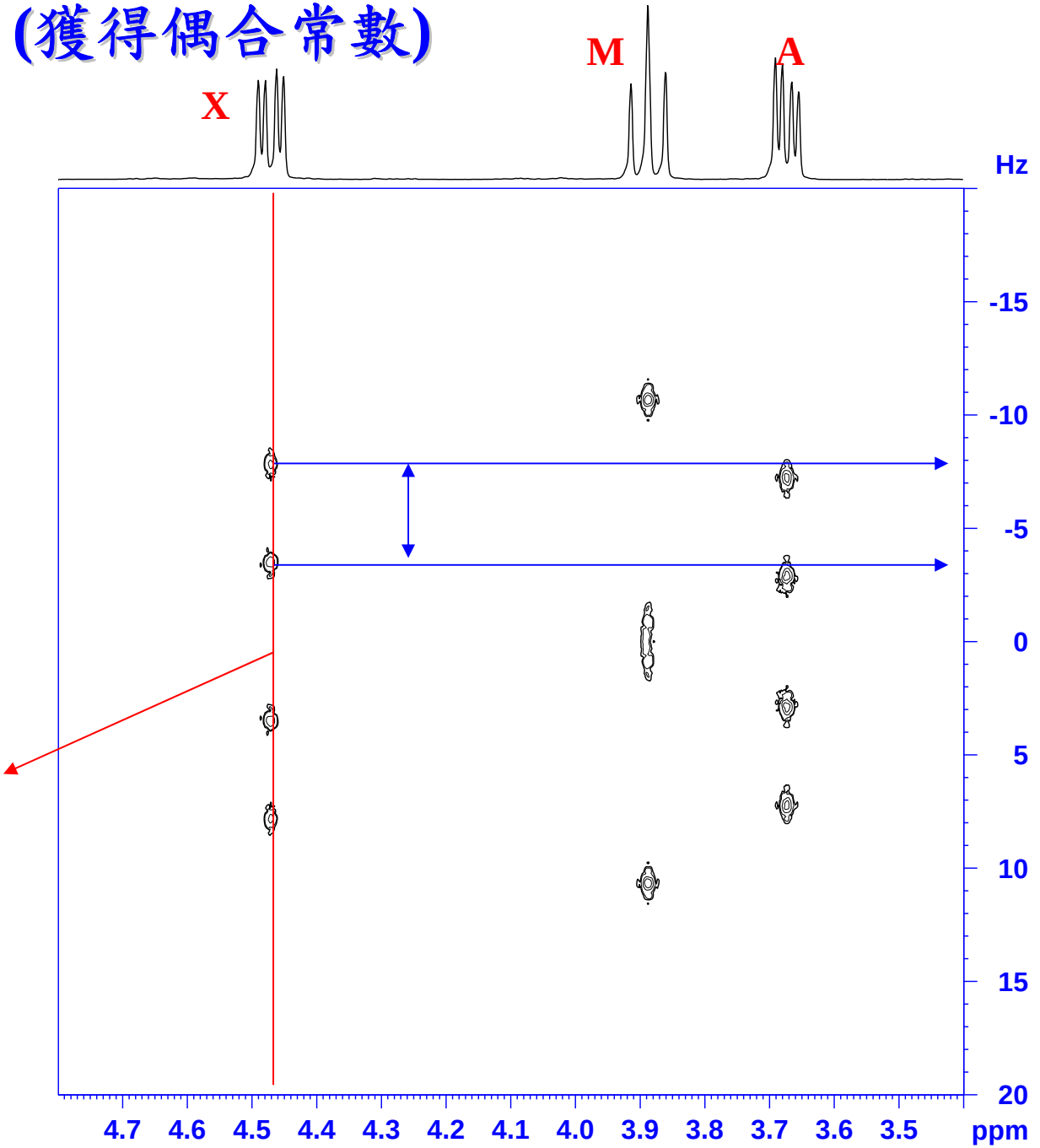
ethylbenzene

7.1.1 同核J-resolved (獲得偶合常數)

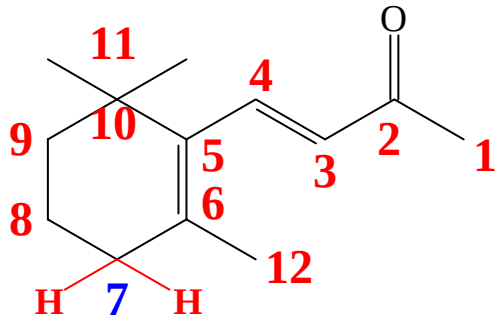


2,3-dibromo-
propionic acid

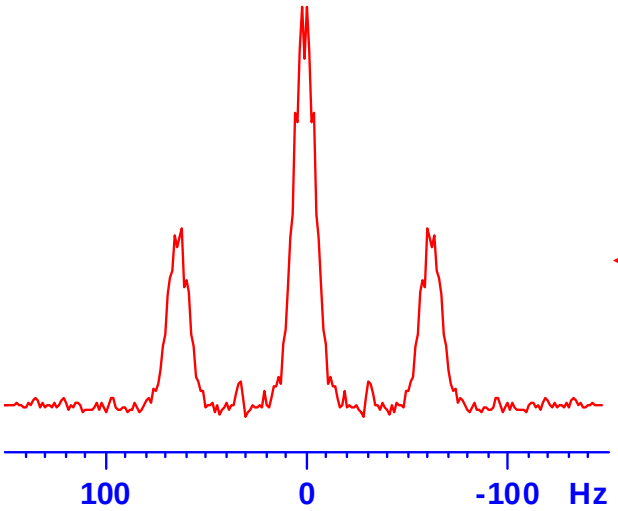


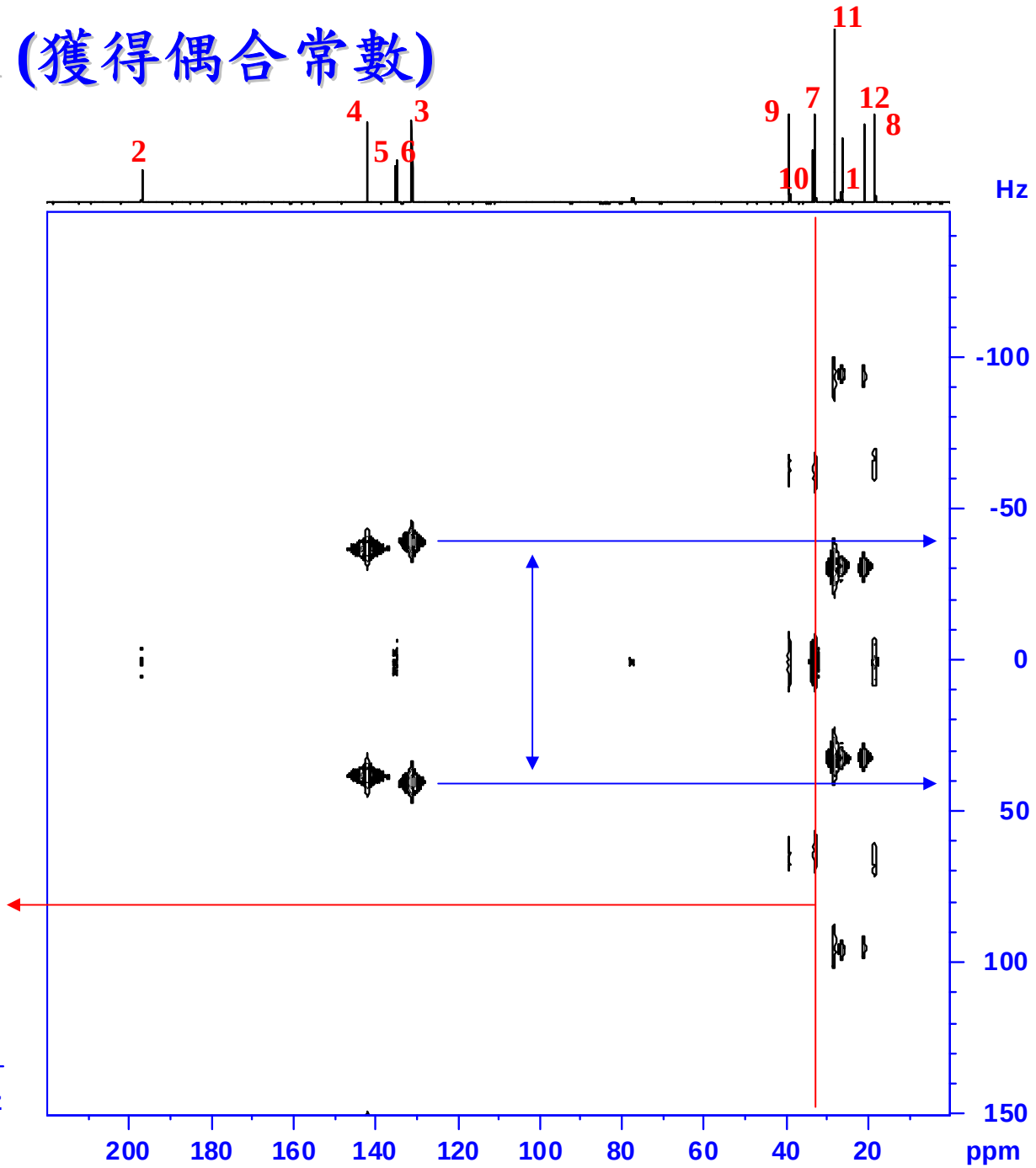


7.1.2 異核J-resolved (獲得偶合常數)

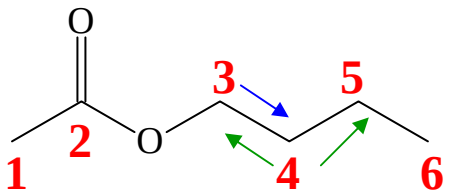


β-ionone

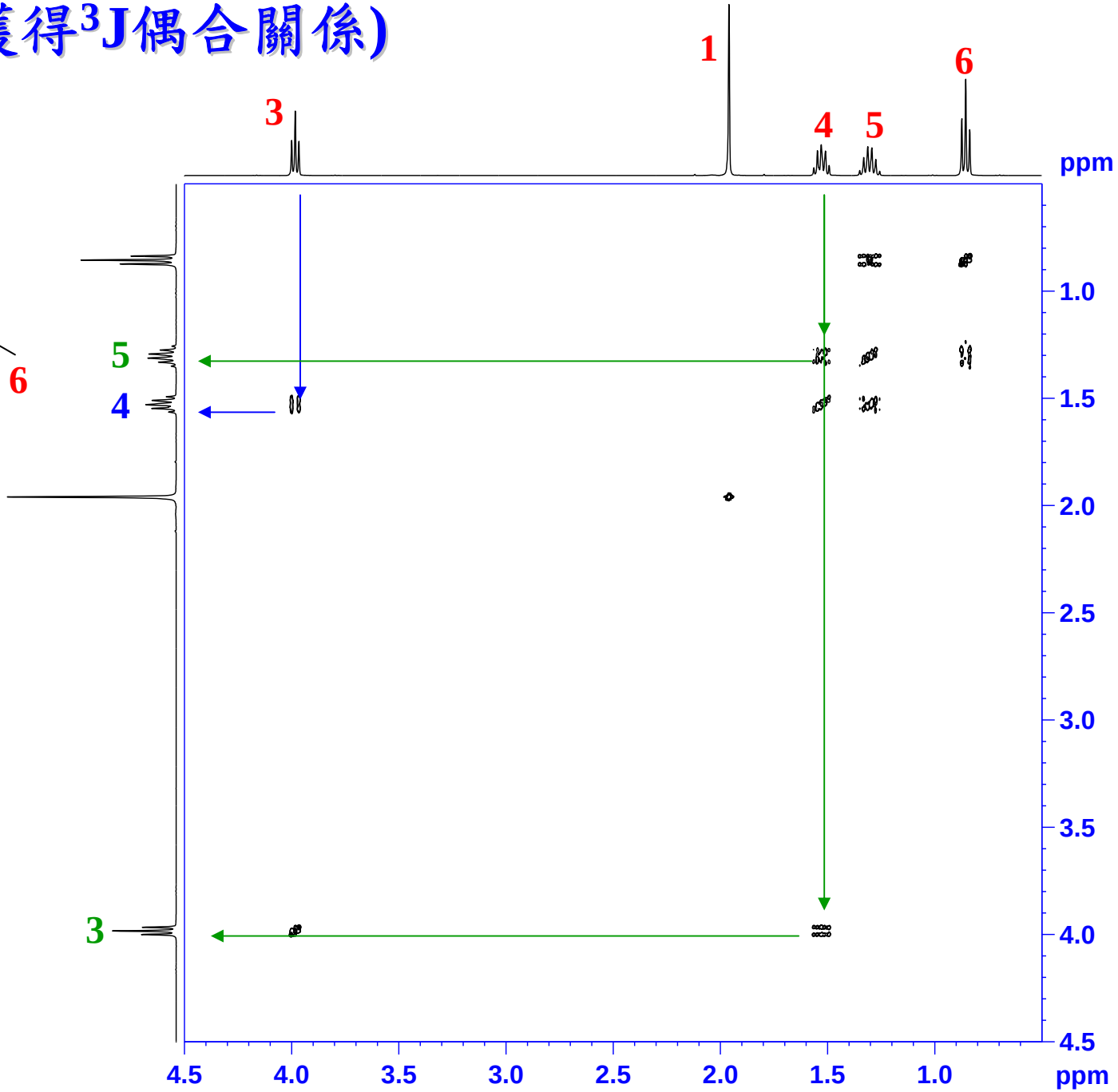




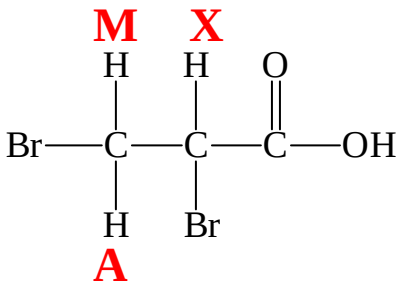
7.2.2 COSY (獲得^{3}J偶合關係)



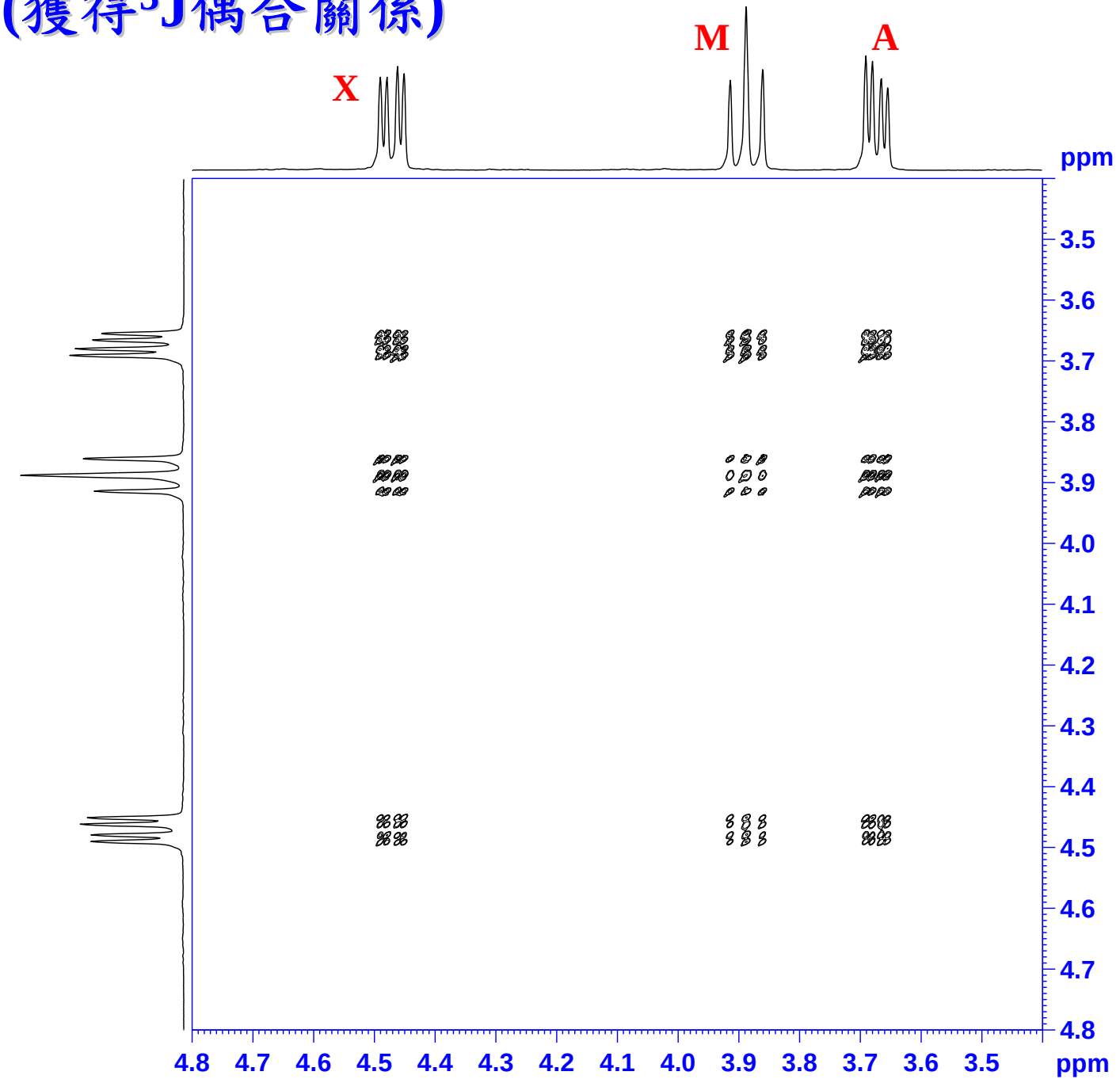
n-butyl acetate



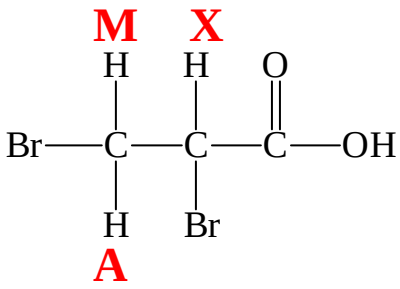
7.2.2 COSY90 (獲得^{3}J偶合關係)



2,3-dibromo-
propionic acid



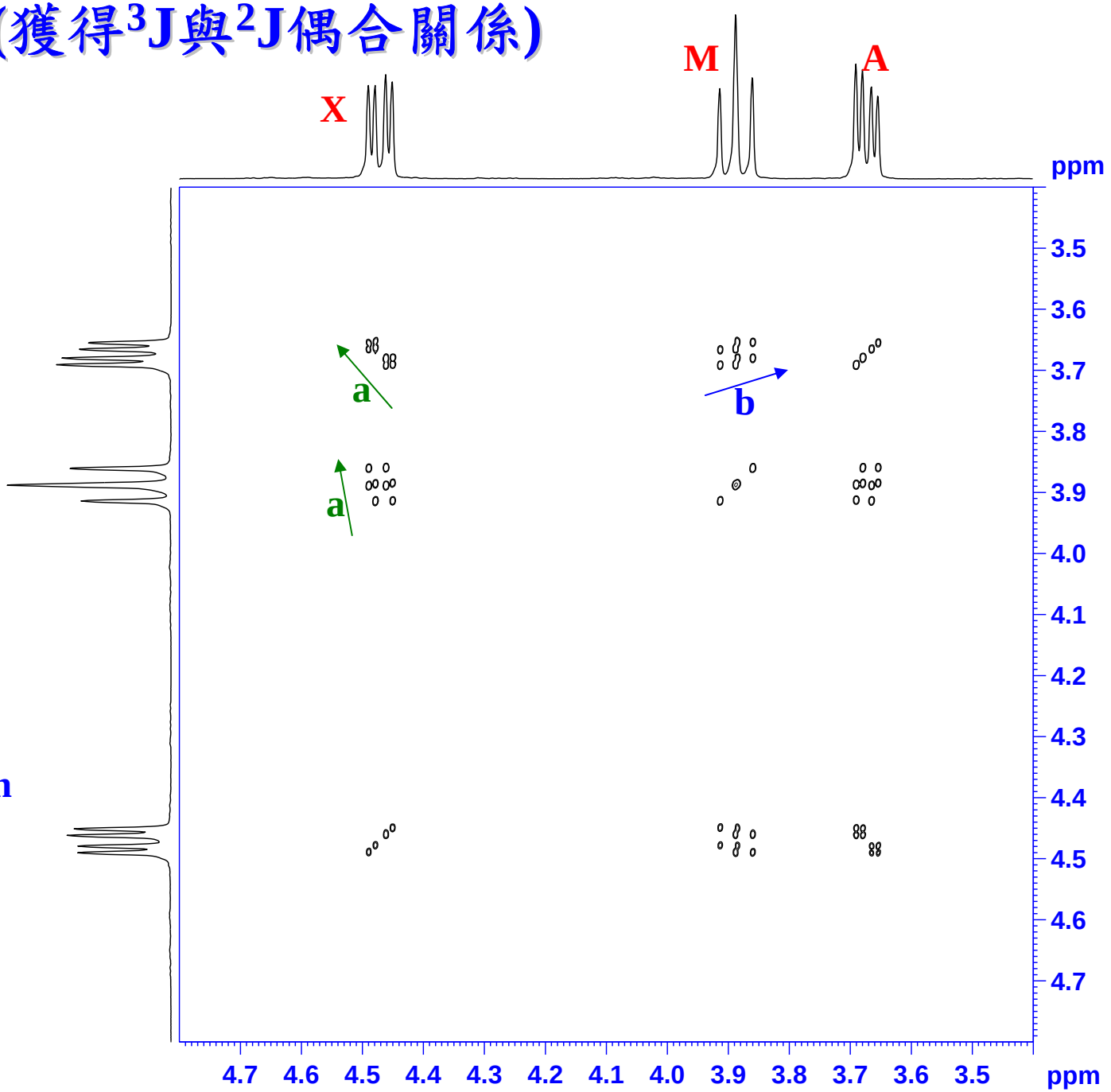
7.2.3 COSY45 (獲得^{3}J與^{2}J偶合關係)



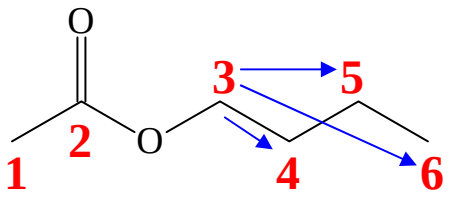
2,3-dibromo-
propionic acid

(a)vicinal spin-spin coupling (^{3}J)

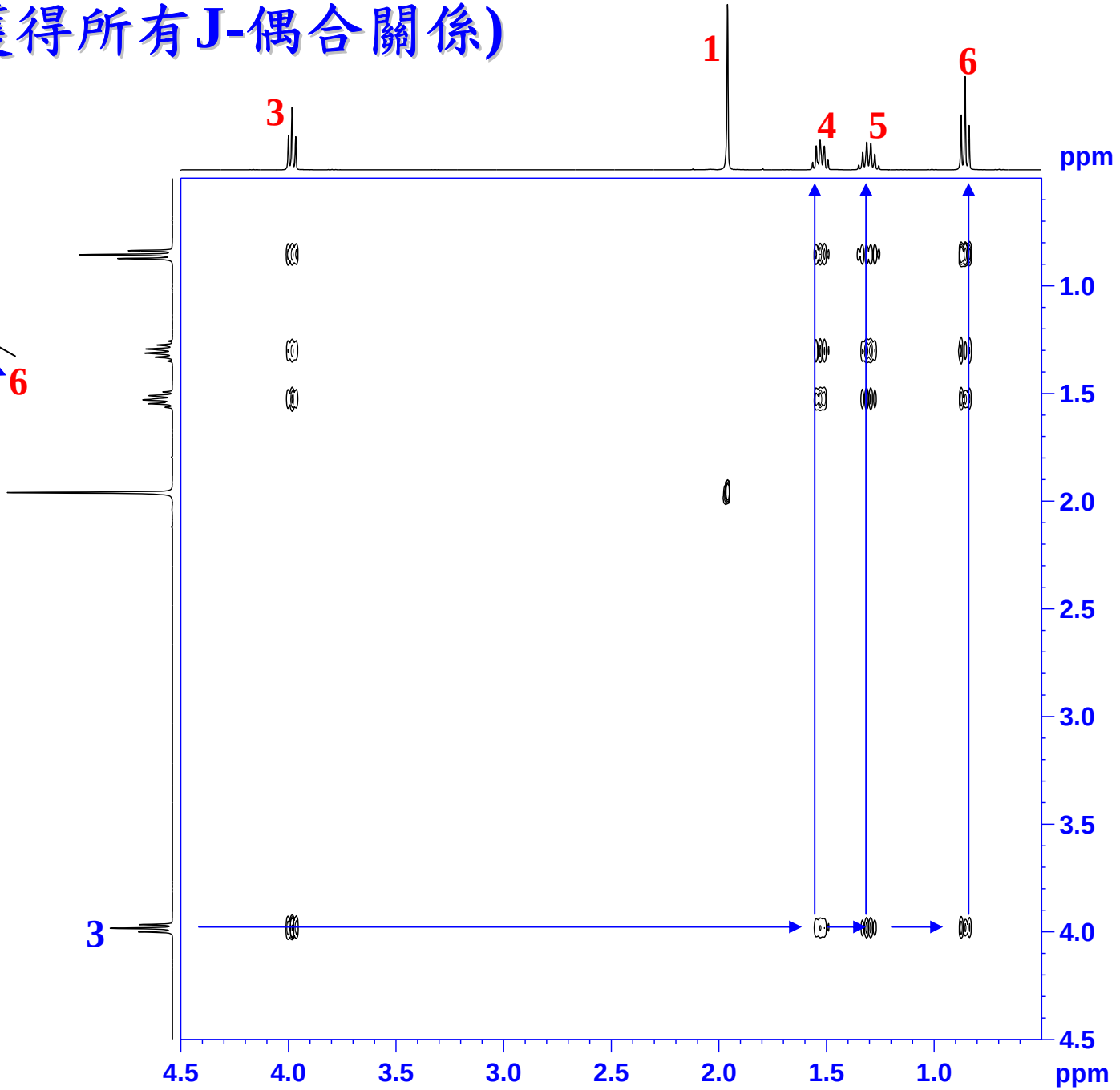
(b)geminal spin-spin coupling (^{2}J)



7.3 TOCSY (獲得所有J-偶合關係)

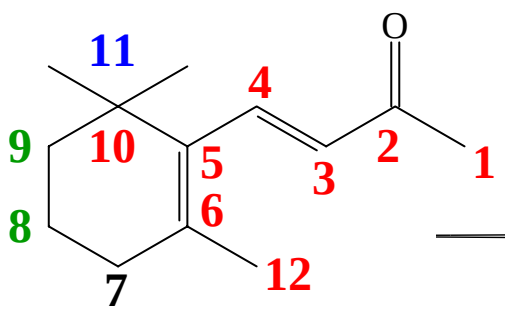


n-butyl acetate

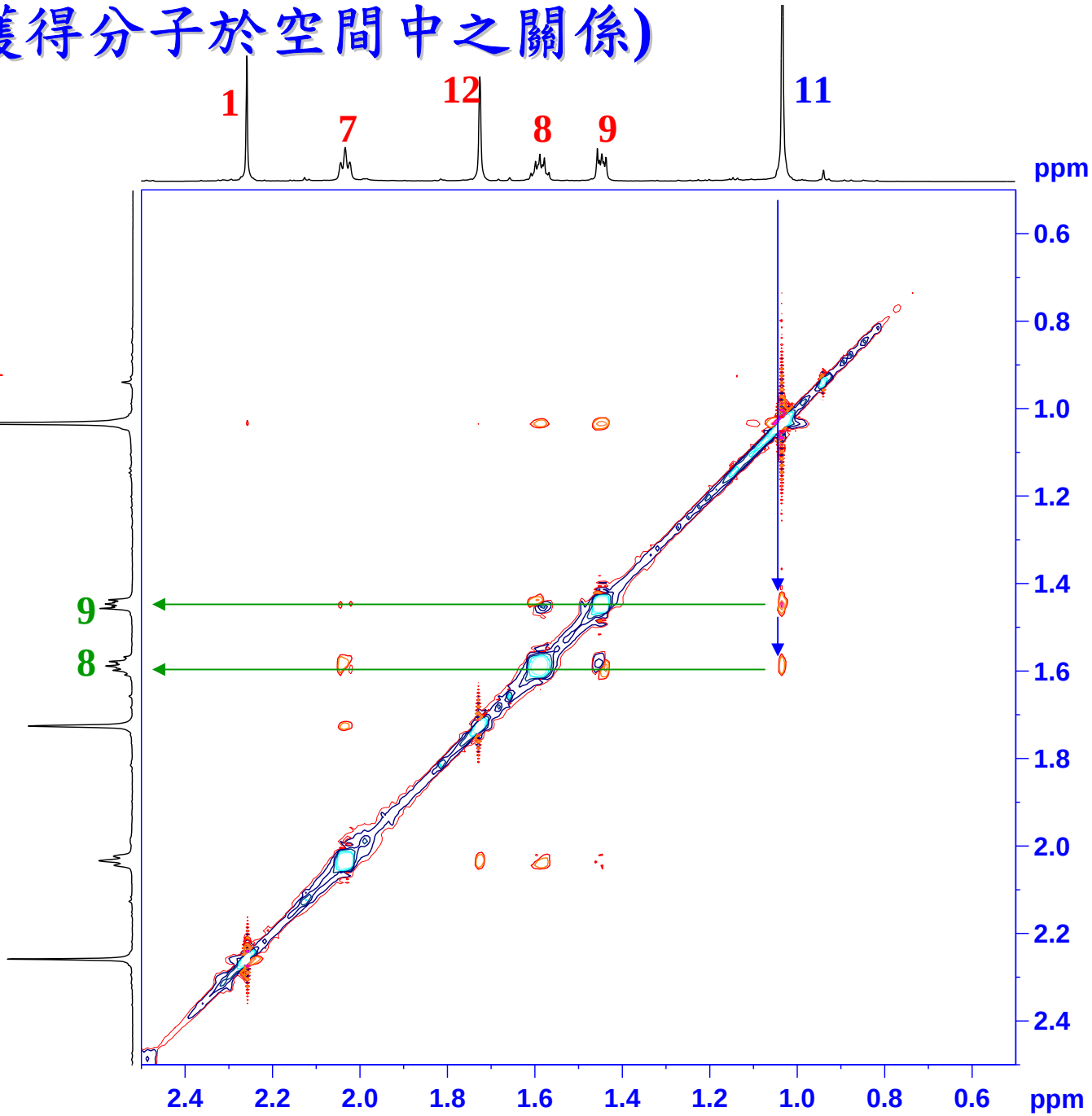


7.4.1 NOESY (獲得分子於空間中之關係)

(<5Å)

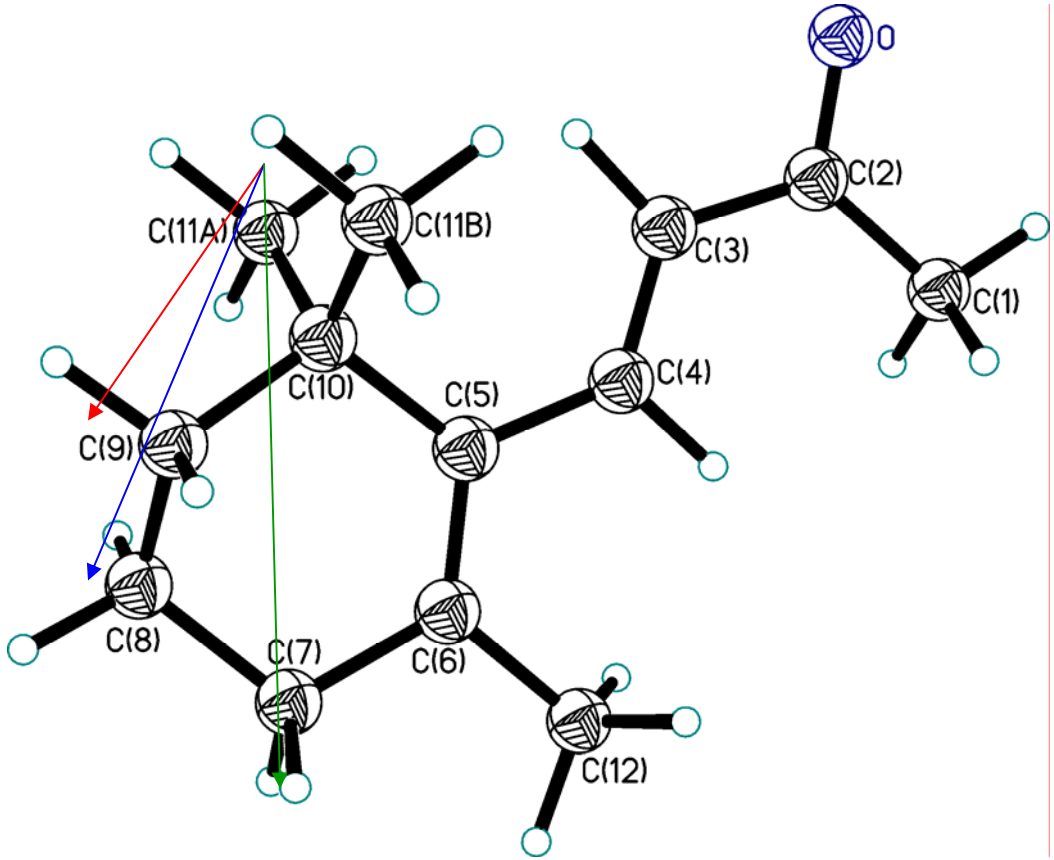


β-ionone

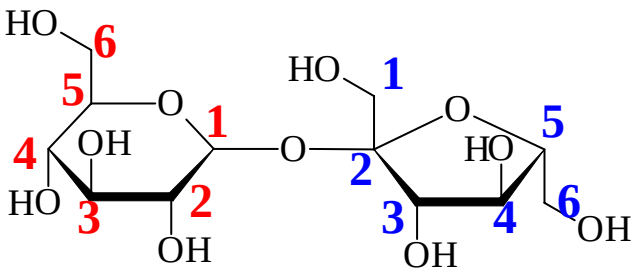


7.4.2 NOESY (獲得分子於空間中之關係)
(<5Å)

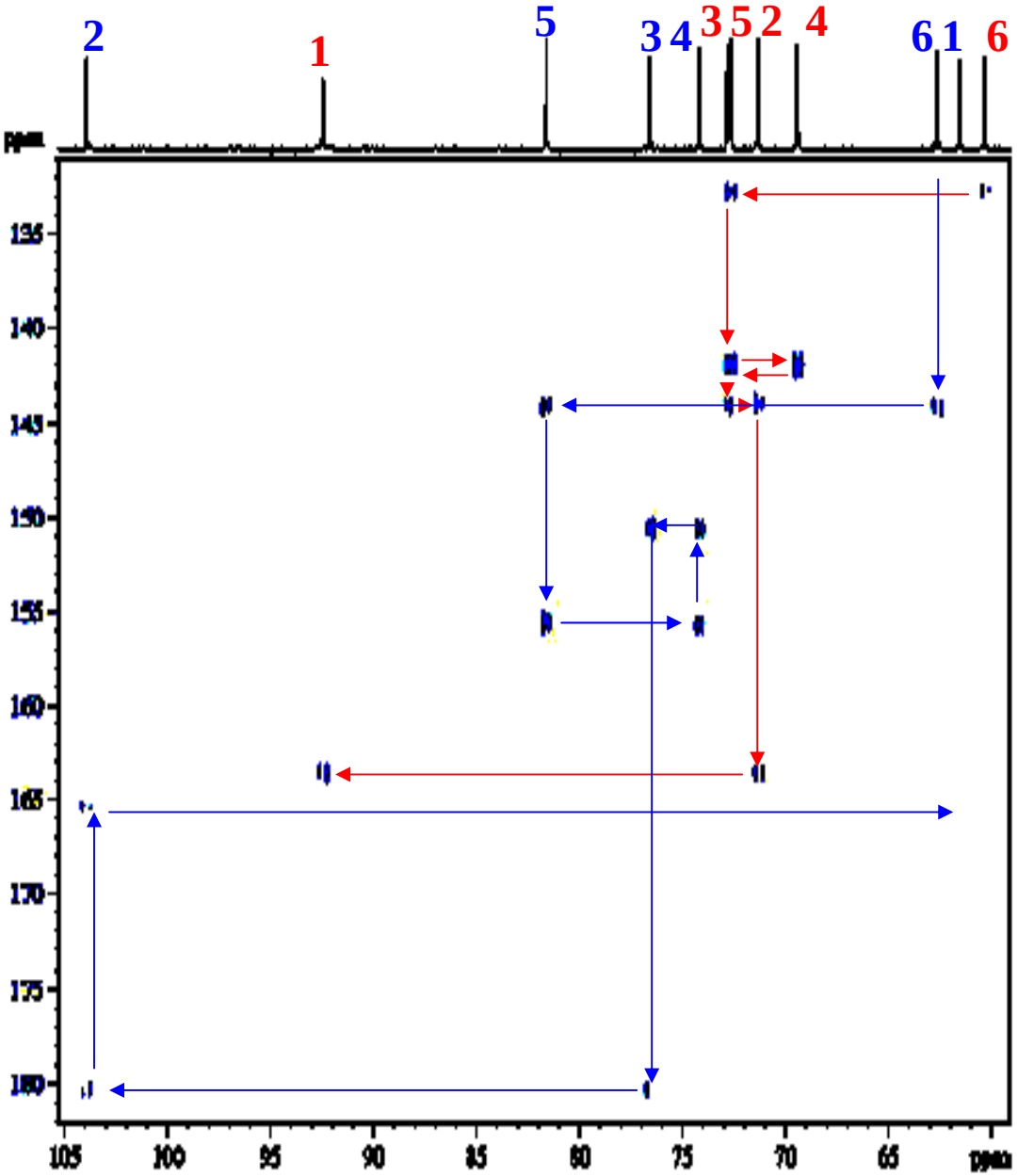
- C(11)上的H --- C(9)上的H
  ≈ 2.5Å
- C(11)上的H --- C(8)上的H
  ≈ 3.8Å
- C(11)上的H --- C(7)上的H
  ≈ 4.7Å



7.5 INADEQUATE (獲得$^{1}J_{C\text{-}C}$之關係)

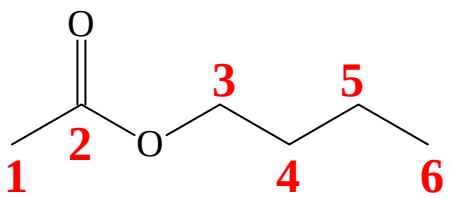


Sucrose

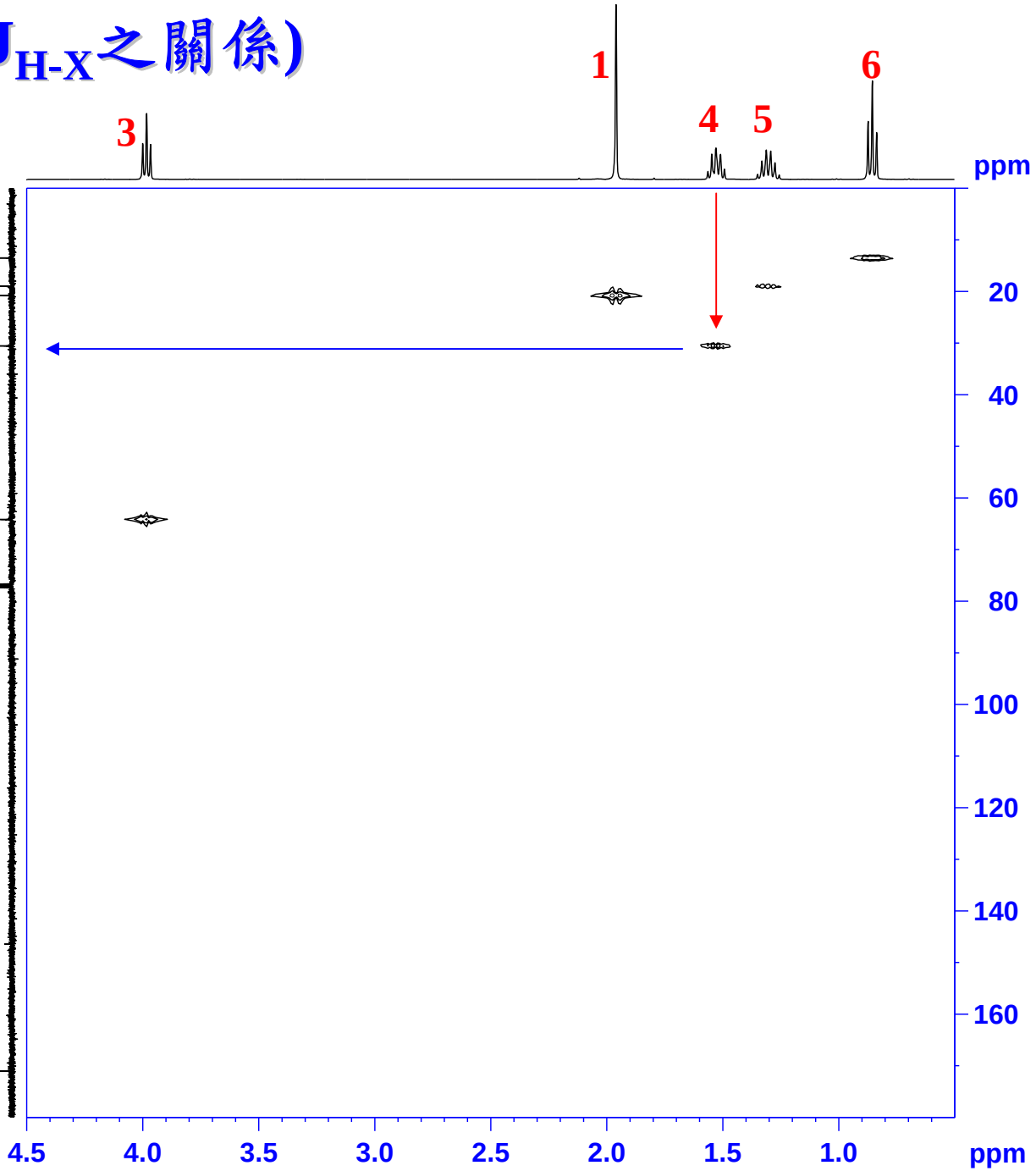


8.1.1 HMQC (獲得$^{1}J_{H-X}$之關係)

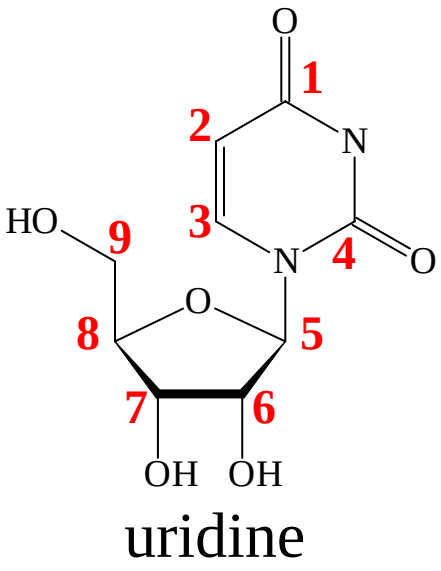
(X = ^{13}C, γ > 0)



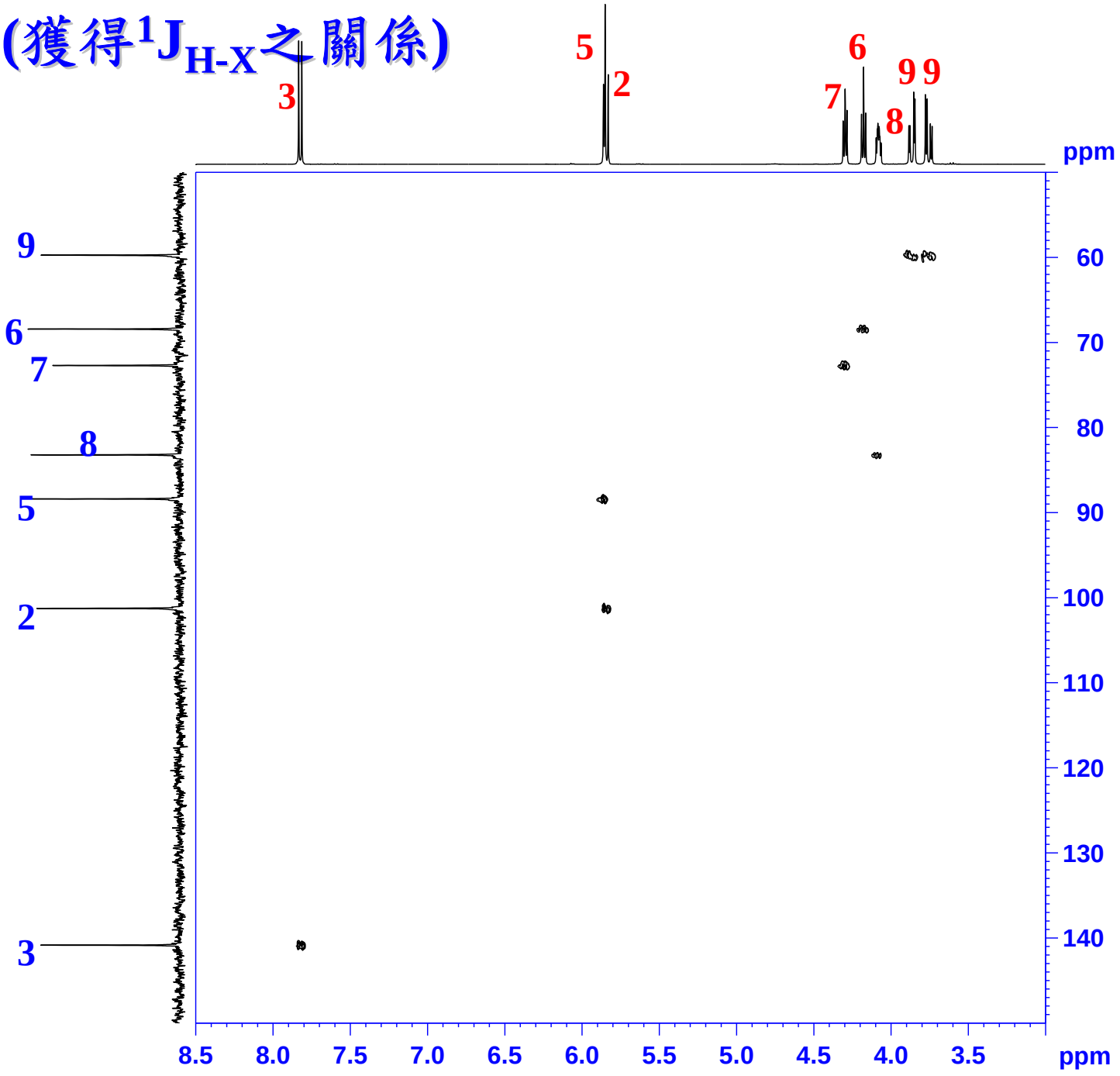
n-butyl acetate



8.1.2 HMQC (獲得$^{1}J_{H\text{-}X}$之關係)

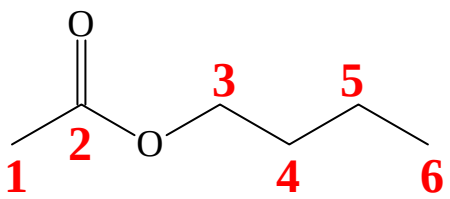


uridine

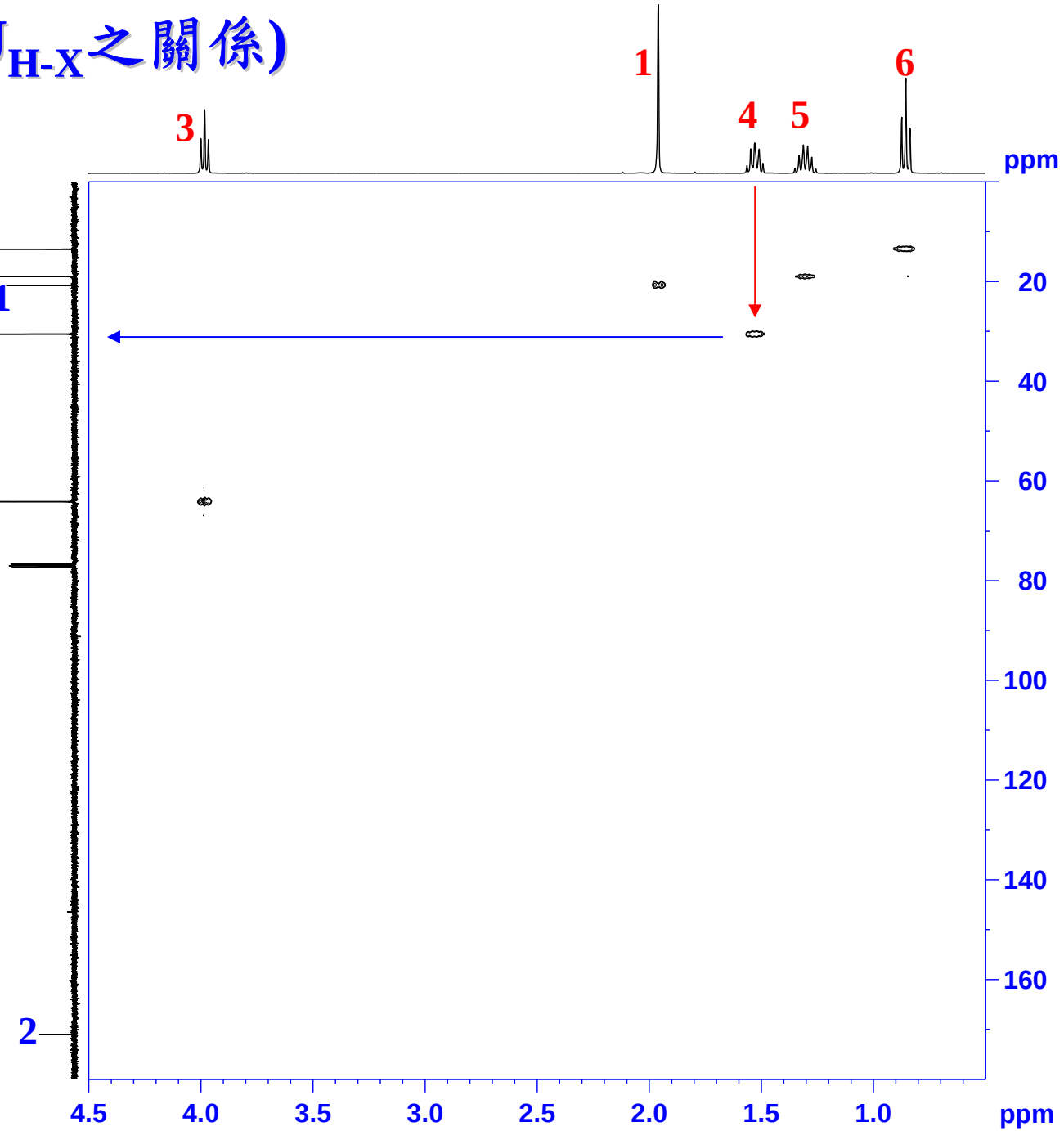


8.2 HSQC (獲得$^{1}J_{H-X}$之關係)

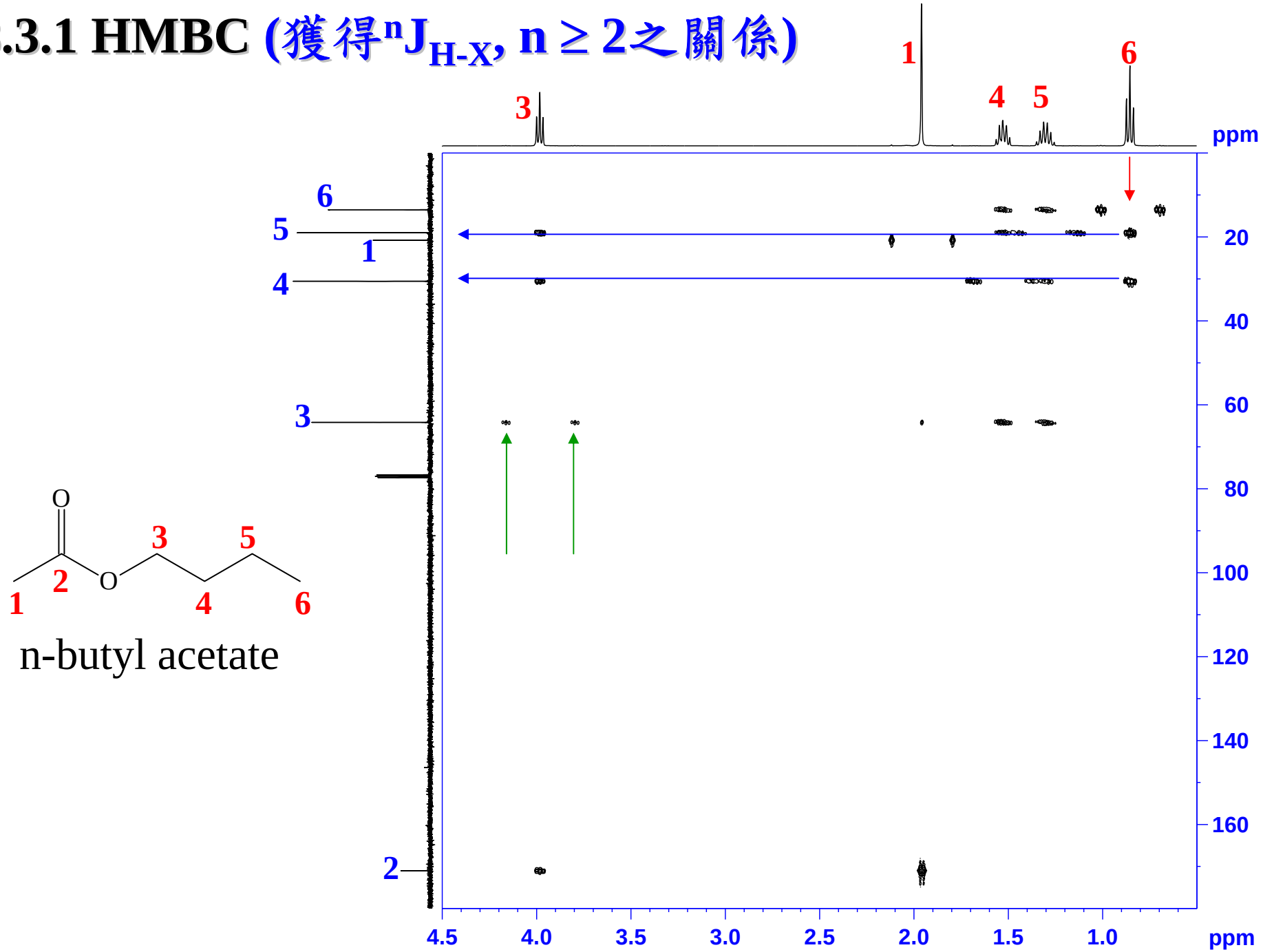
(X = ^{15}N, γ < 0)



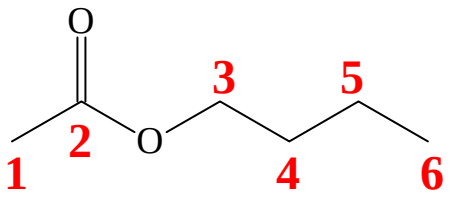
n-butyl acetate



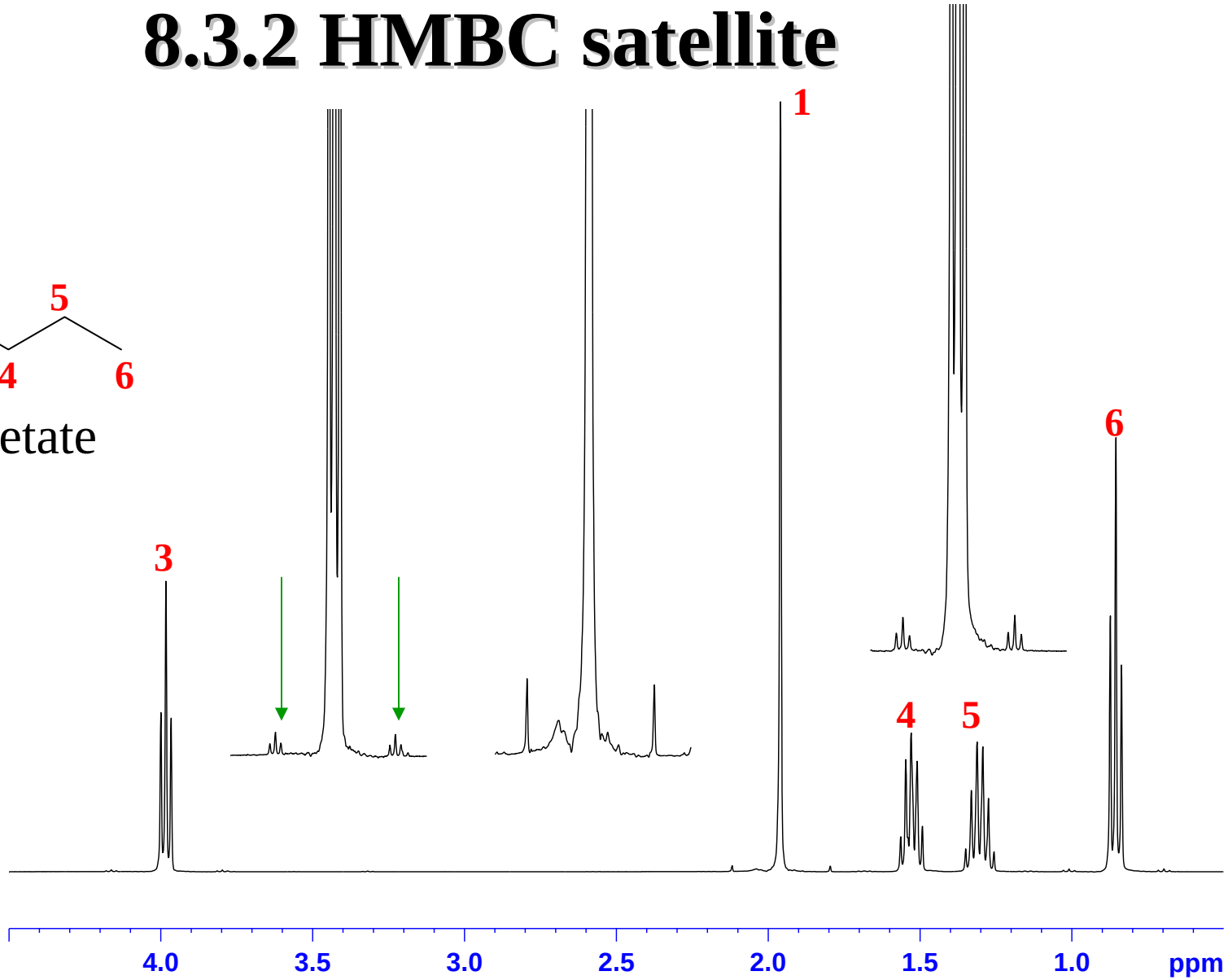
8.3.1 HMBC (獲得$^{n}J_{H-X}$, $n \geq 2$之關係)



8.3.2 HMBC satellite



n-butyl acetate



Thank You

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