C8953
NMR strukturní analýza
seminář

TOCSY & NOESY

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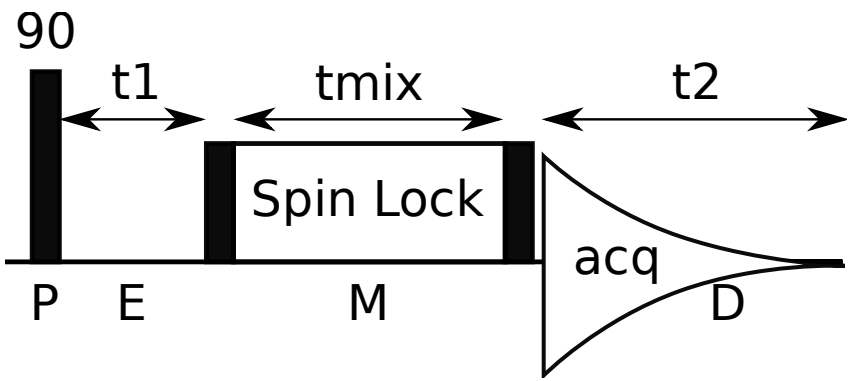
TOCSY (TOtal Correlation SpectroscopY)

spin lock - isotropic mixing

- series of short 180° pulses
- ”lock-in” of spins in transversal plane
- higher power in case of TOCSY, offset set into the middle (on resonance)
- smaller power in case of ROESY, offset set into the edge (off resonance)
- crosstalk (ROE transfer in TOCSY, *J*-coupling in ROESY)



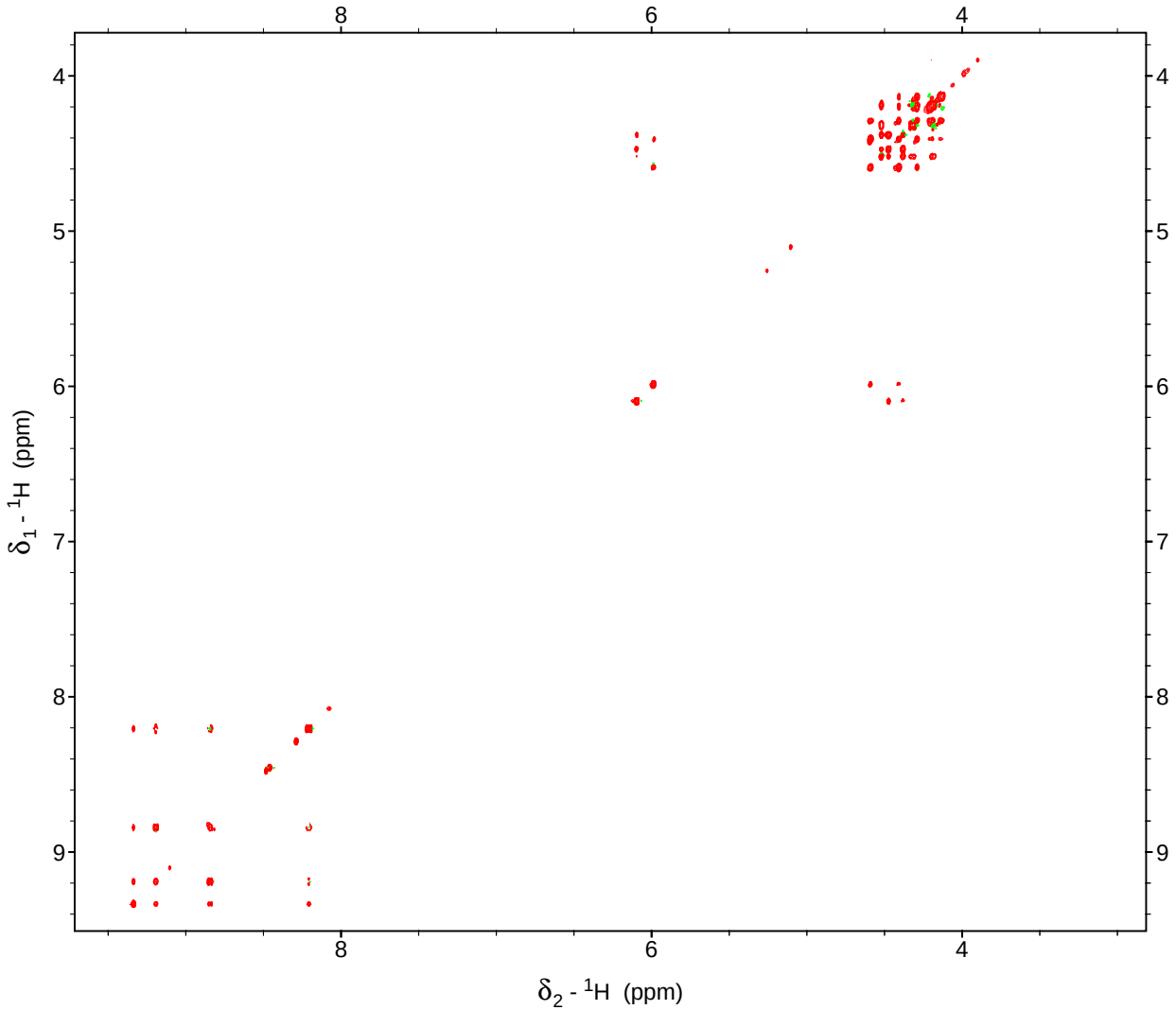
TOCSY (TOtal Correlation SpectroscopY)

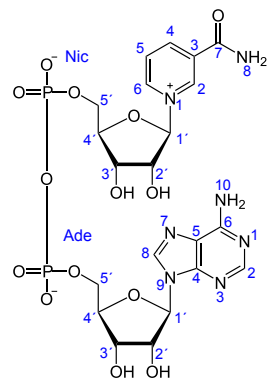
correlation based on J

- mutual correlation of all protons in one spin system
- $\tau_{mix} \approx$ 20 - 120ms
- crosspeak intensity depends on τ_{mix} a J value

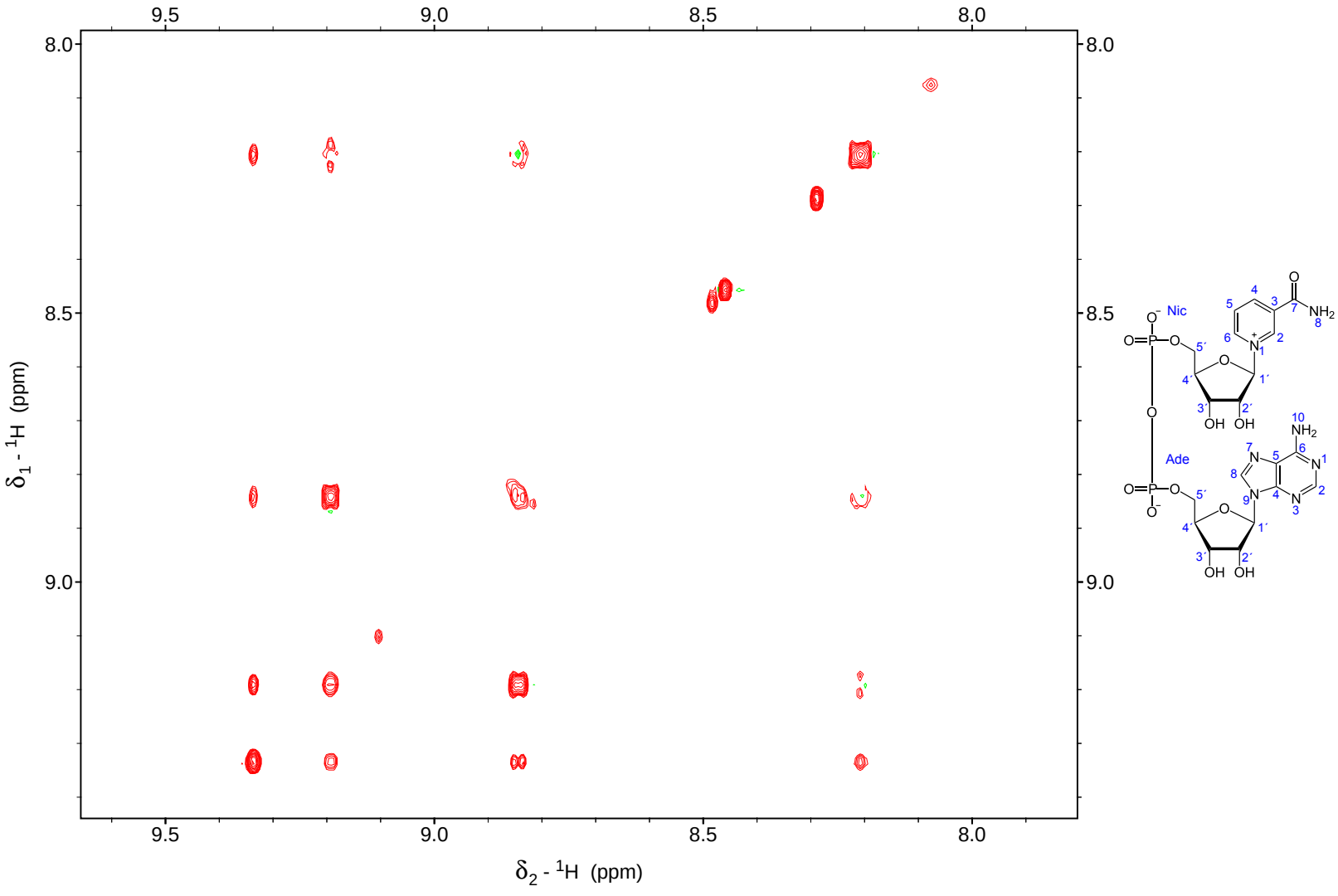
$H_A \overset{J_{AB}}{\Longleftrightarrow} H_B \overset{J_{BC}}{\Longleftrightarrow} H_C \overset{J_{CD}}{\Longleftrightarrow} H_D$

NAD^+: TOCSY (40ms)





NAD$^+$: TOCSY (40ms), detail of aromatics



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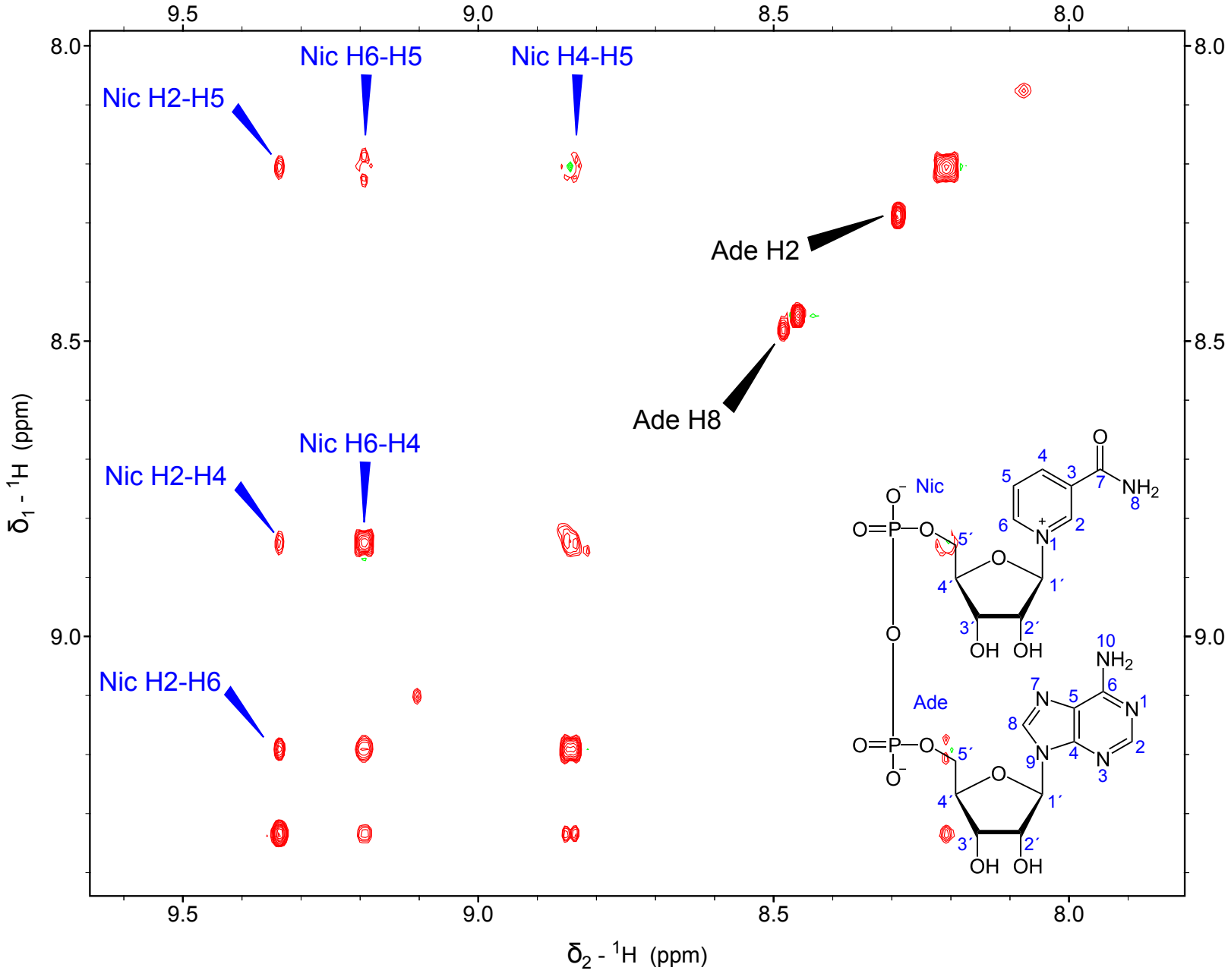
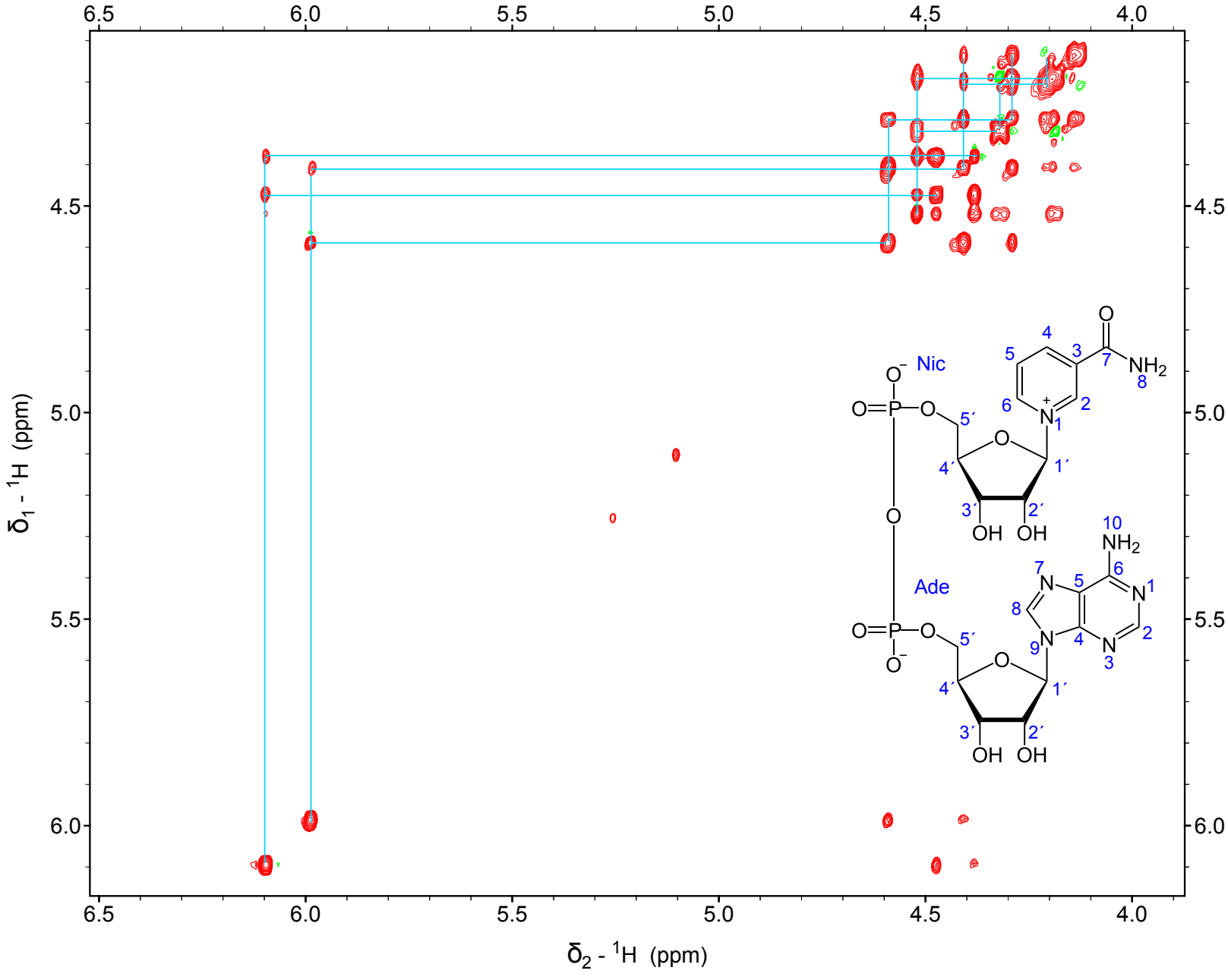


NAD$^+$: TOCSY (40ms), detail of aliphatics



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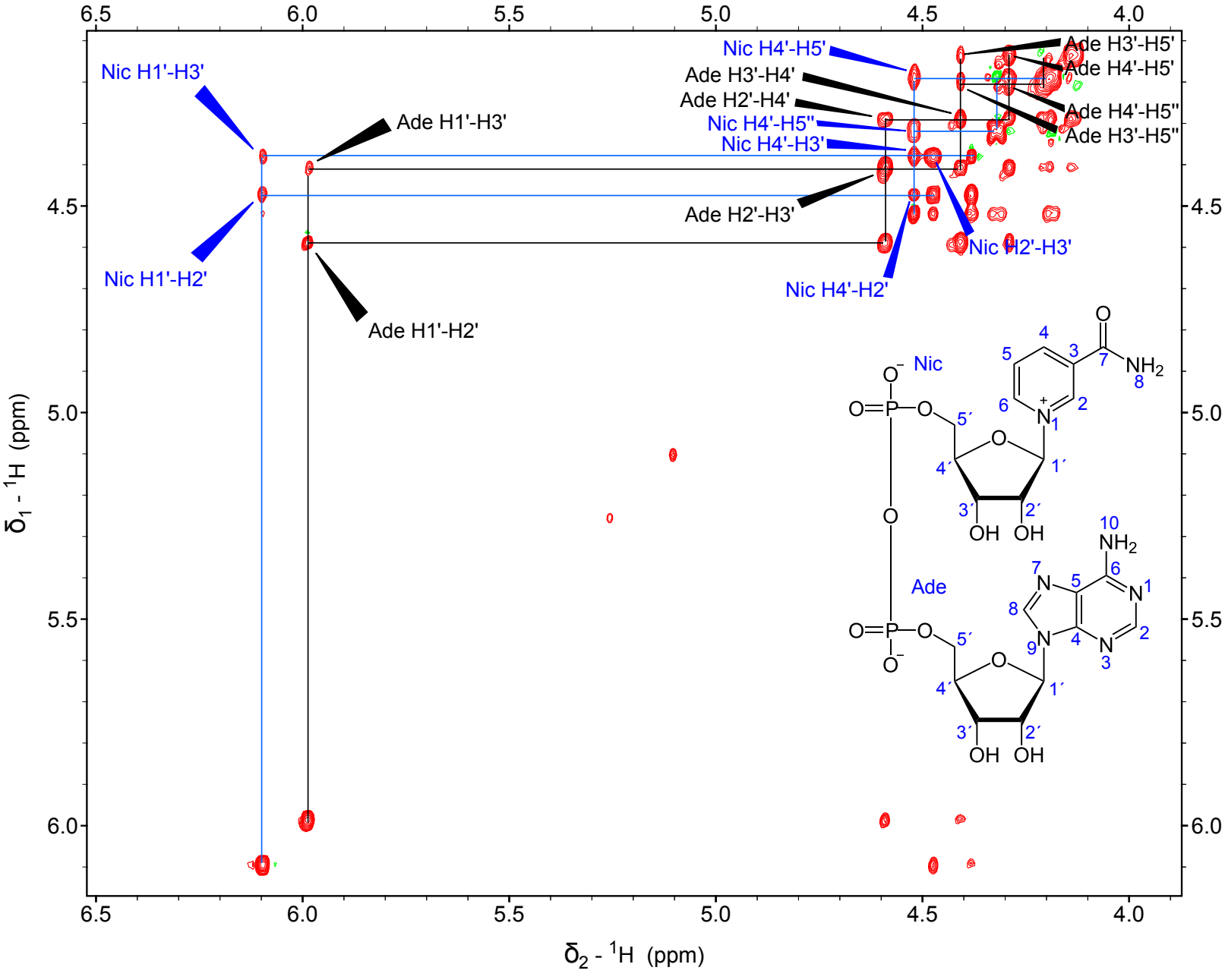


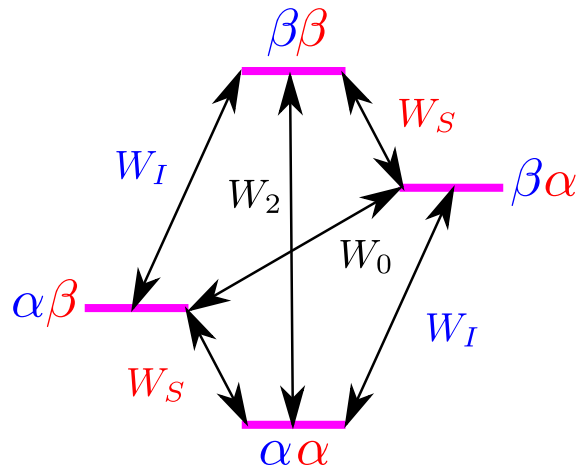
NOESY - introduction

Nuclear Overhauser effect

- dipol-dipol interaction
- magnetisation transfer TROUGH SPACE as a consequence of cross-relaxation

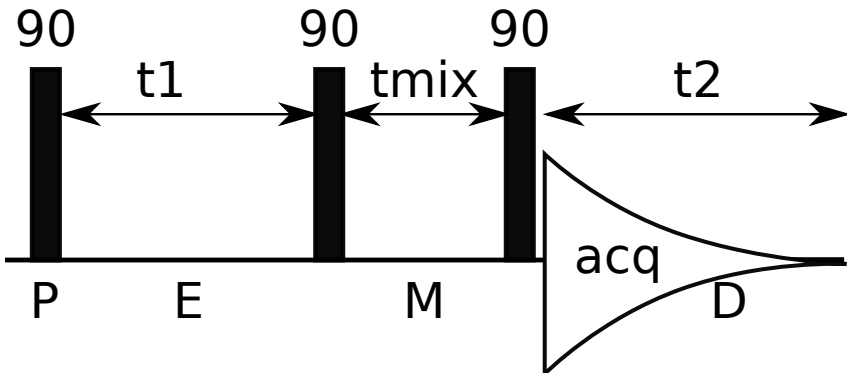
NOESY

- correlates nuclei if their distance is **smaller than 5 Å**



$$\frac{\mathrm{d}\Delta I_z}{\mathrm{d}t} = -\rho_I(I_z - I_z^0) - \sigma_{IS}(S_z - S_z^0)$$

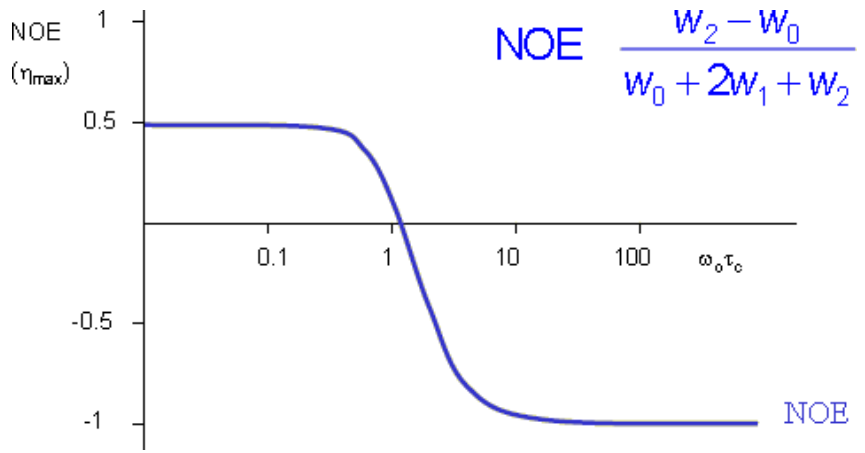
$$\sigma_{IS} = W_2 - W_0$$



NOE vs. size of a molecule

Correlation time τ_c

- $\omega_0\tau_c < 1 \Leftrightarrow \omega_0 \frac{1}{f} < 1 \Leftrightarrow \omega_0 < f$ (small molecules $\ll$ 1 kDa)
  - **fast molecular motion**, $\beta\beta \rightarrow \alpha\alpha$ dominates $\Rightarrow W_2 > W_0$
  - positive NOE
  - crosspeaks have opposite phase relative to diagonal
- $\omega_0\tau_c > 1$ (large molecules $\gg$ 1 kDa)
  - **slow molecular motion**, $\alpha\beta \rightarrow \beta\alpha$ dominates $\Rightarrow W_0 > W_2$
  - negative NOE
  - crosspeaks have the same phase
- $\omega_0\tau_c \approx 1$ (cca 1 kDa)
  - NOE$\approx$0 - no crosspeaks
  - ROESY



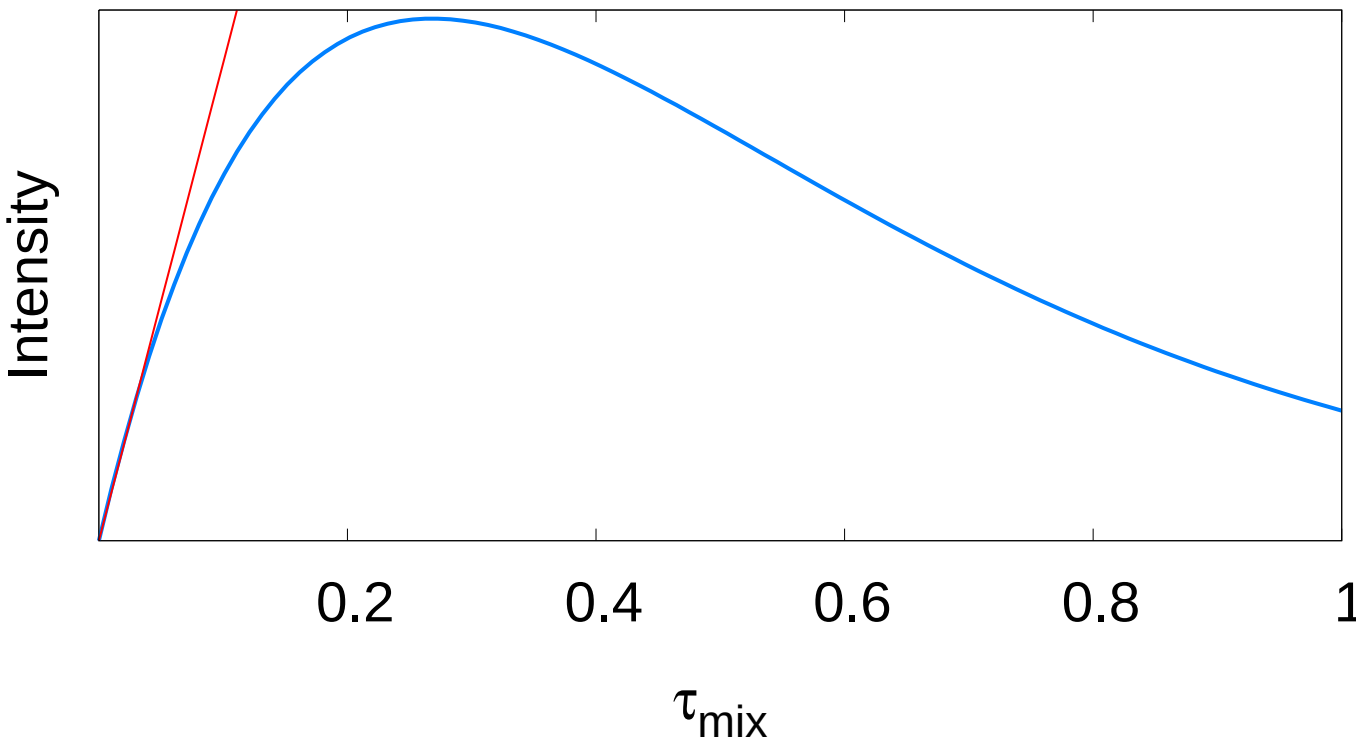
Application of NOESY

Mixing time τ_{mix}

- small molecules $\tau_{mix} \approx 500 - 800\,ms$
- biomolecules $\tau_{mix} \approx 50 - 300\,ms$

approximative determination of interatomic distatces ($< 5\,Å$)

- at short τ_{mix}
- $r_{ij} \approx A \times I_{ij}$

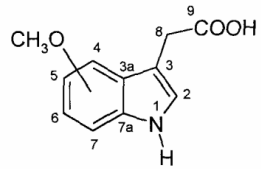


NOE differential experiment

PROBLEM 4

NOE- Difference Spectroscopy

Figure 4.1 shows the ^{1}H NMR and a ^{1}H NOE difference spectrum of a 3-indolylacetic acid derivative **13** bearing a methoxy group at the benzenoic ring.



What is the position of the methoxy group? **(400 MHz ^{1}H)**

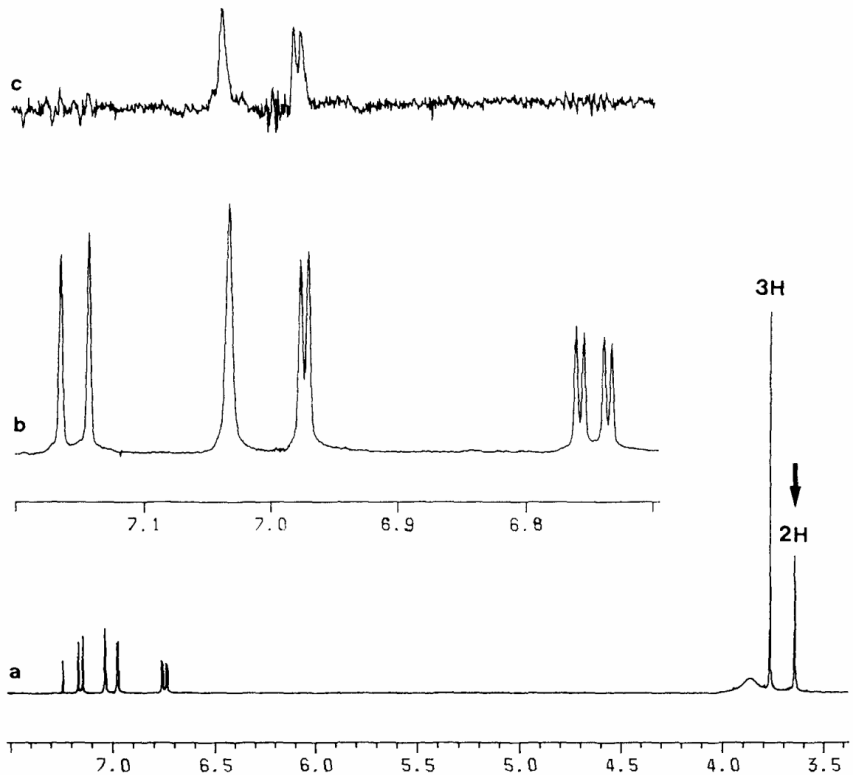


Fig. 4.1. 400 MHz ^{1}H NMR spectrum of **13** in a mixture of $CDCl_3$ and CD_3OD. **a** Full spectrum; **b** expanded section of the aromatic proton signals; **c** ^{1}H NOE difference spectrum, same section as in **b**, irradiation position at $\delta = 3.64$.

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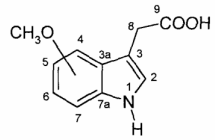

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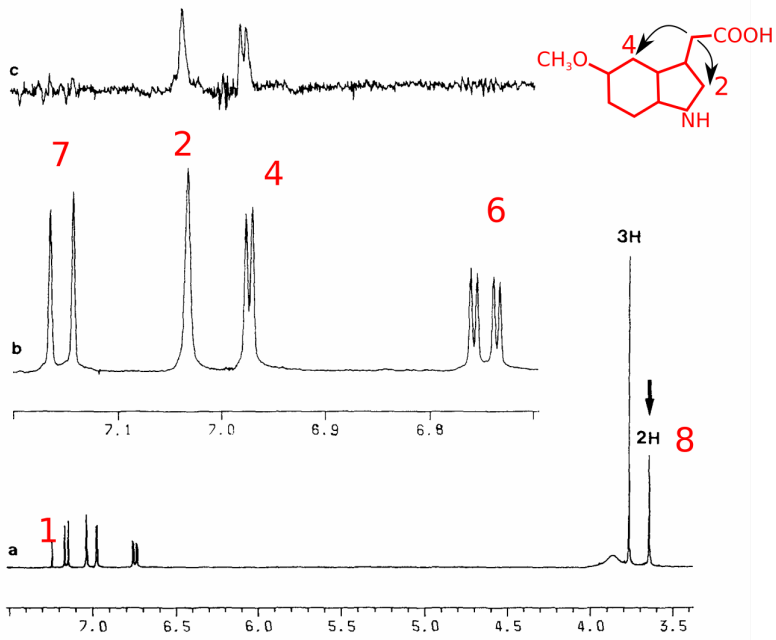

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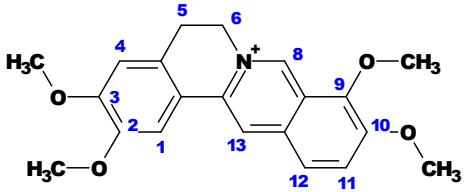
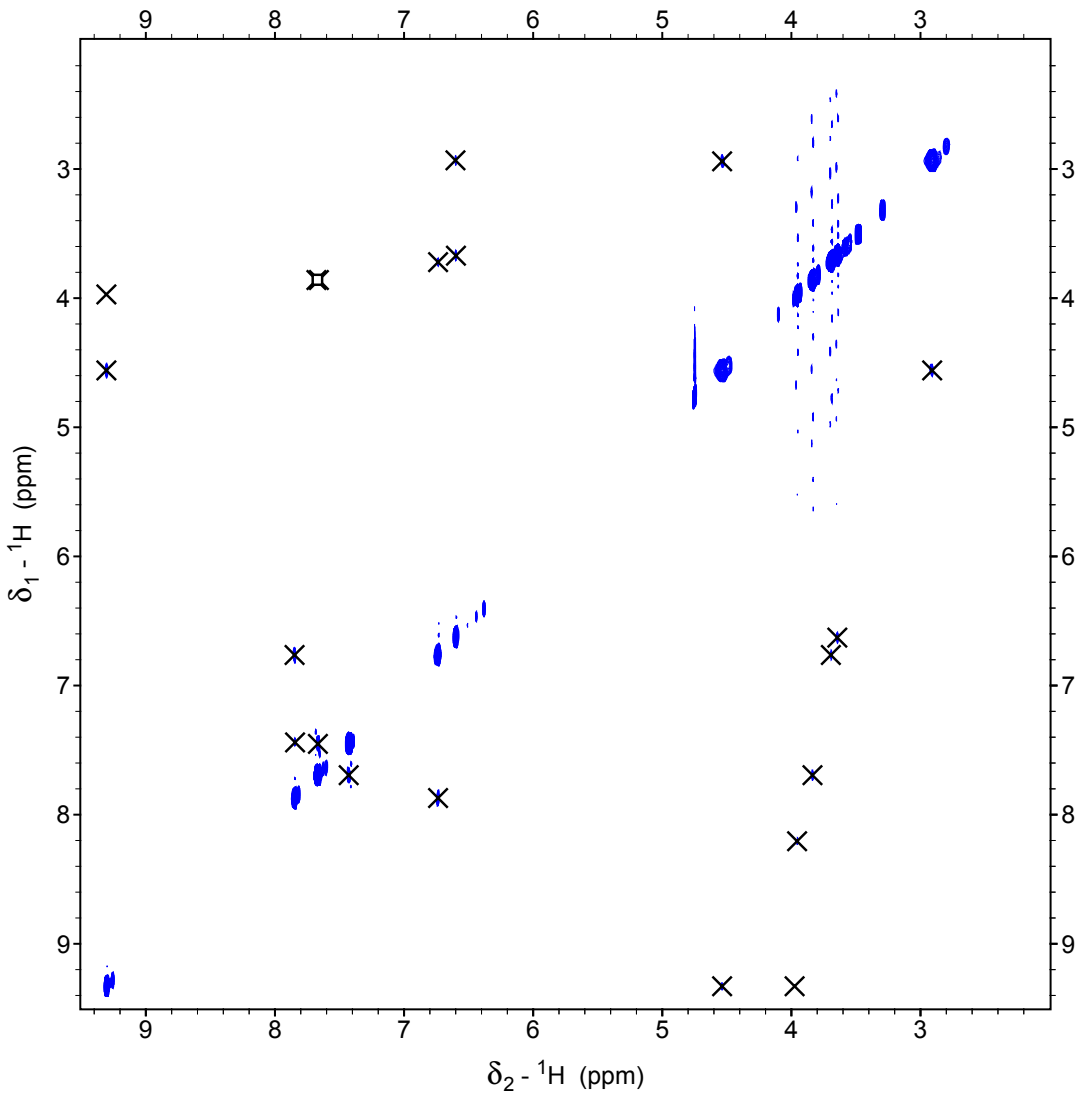
NOESY - Palmatine





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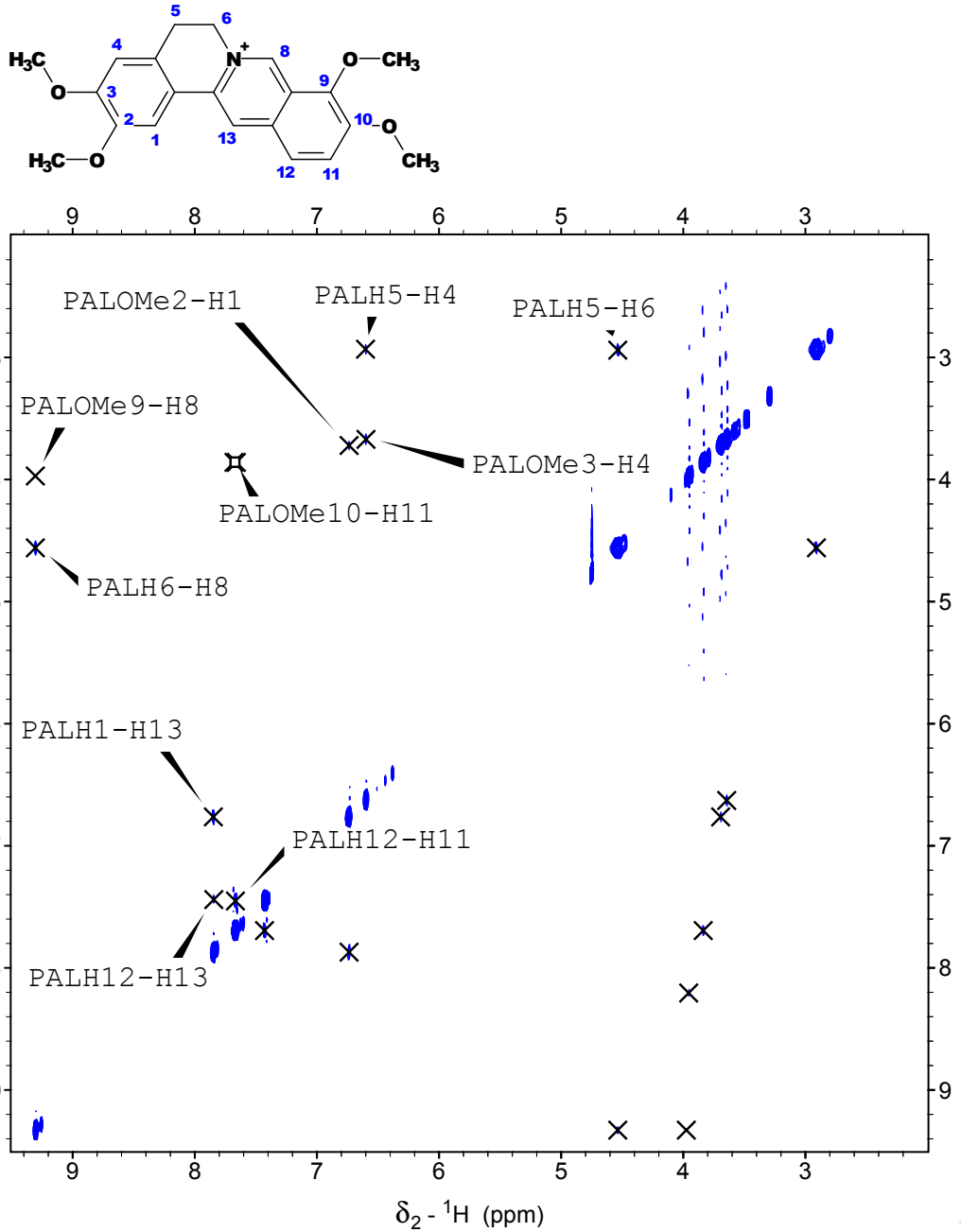
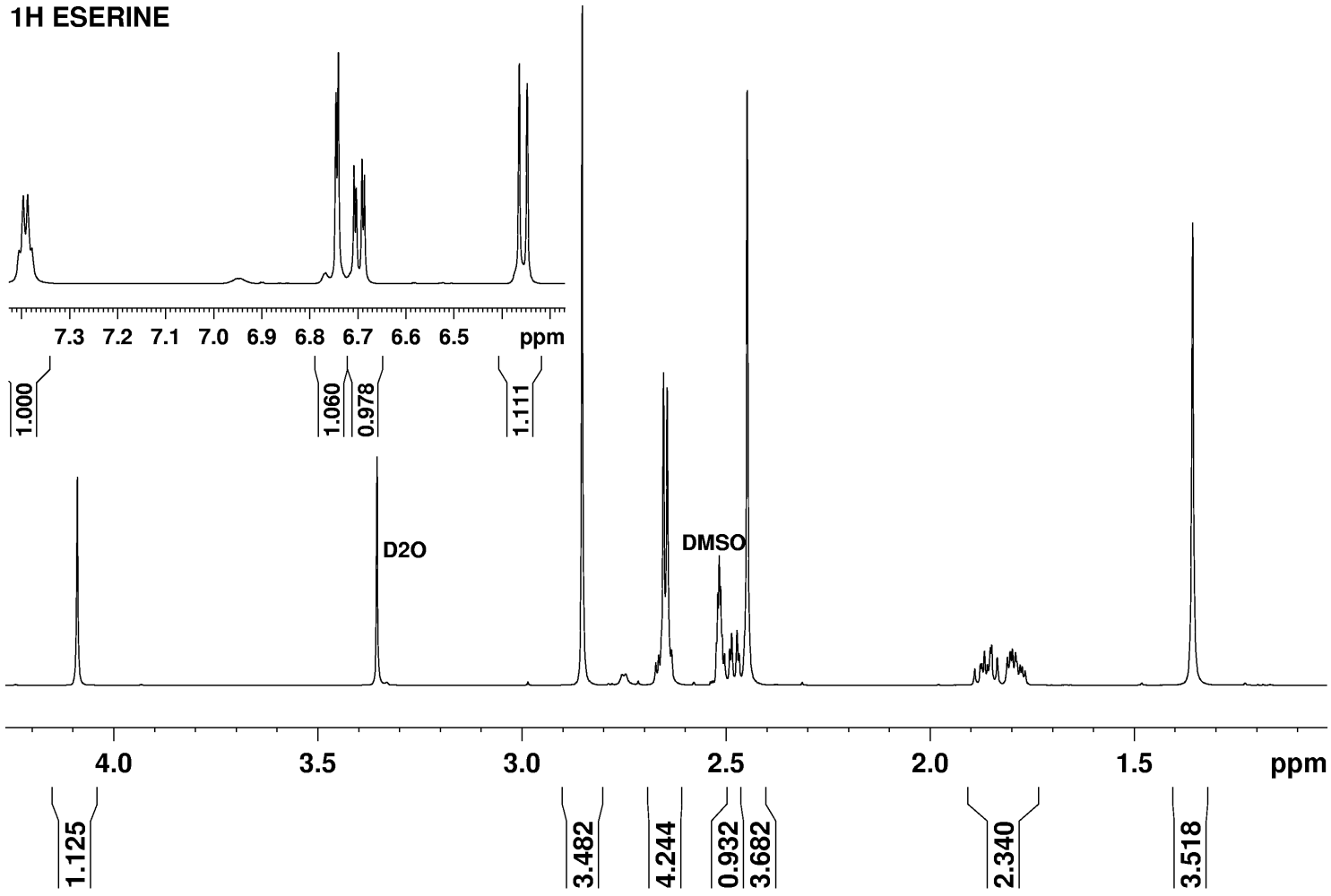
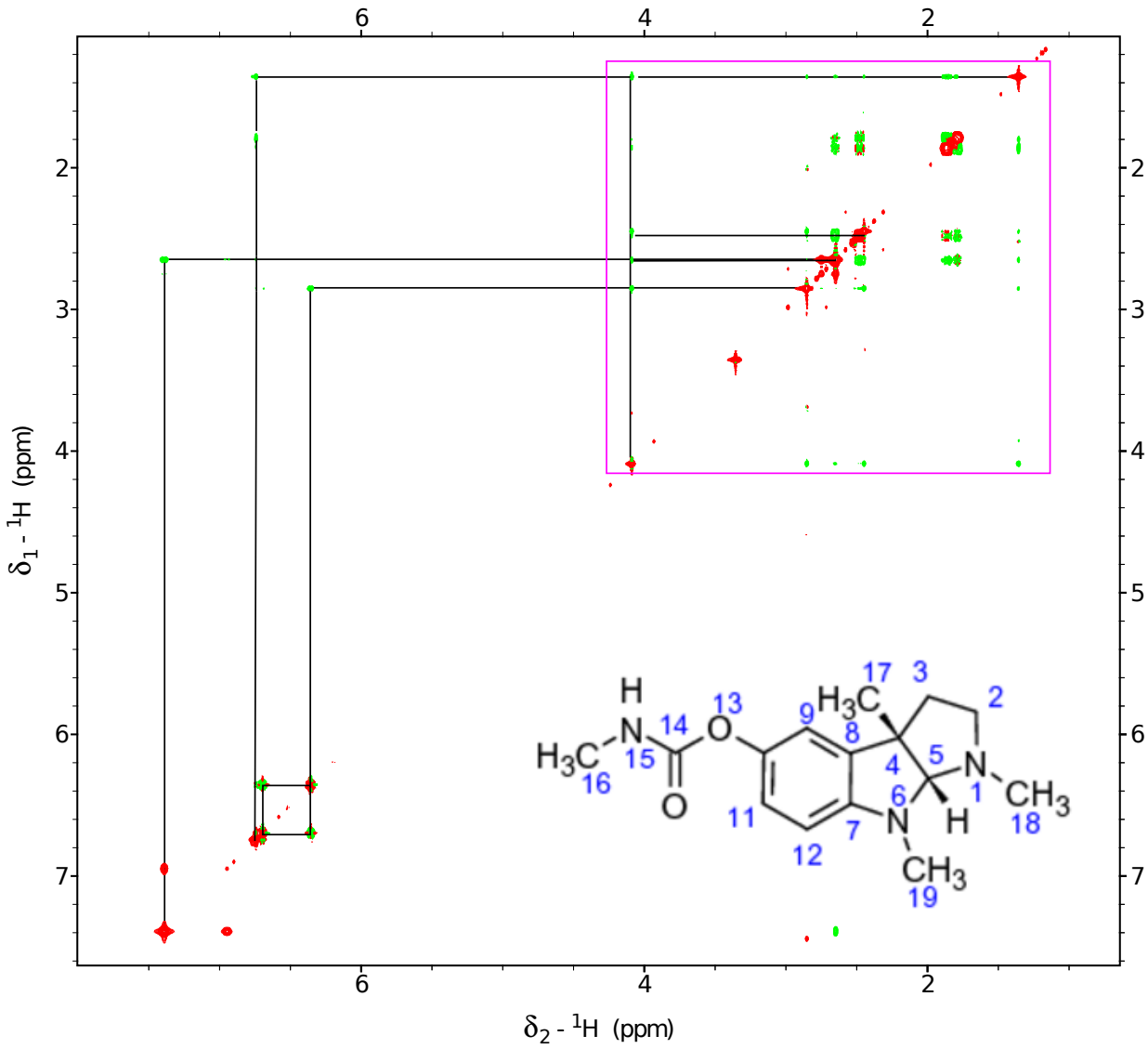


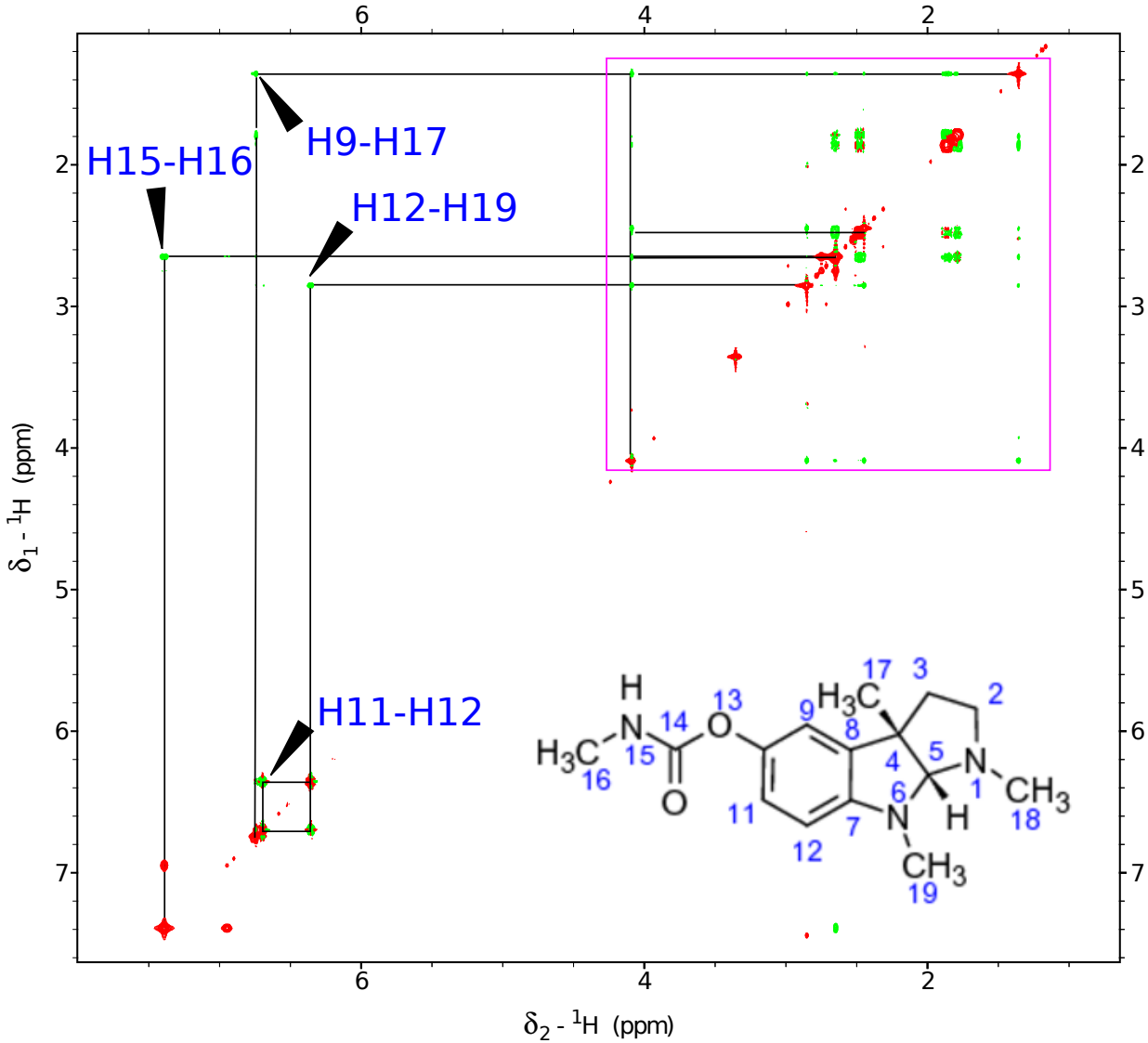
Eserine ^{1}H


1H ESERINE
7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 ppm
1.000
1.060
0.978
1.111
D2O
DMSO
4.0 3.5 3.0 2.5 2.0 1.5 ppm
1.125
3.482
4.244
0.932
3.682
2.340
3.518

NOESY - Eserine in DMSO



NOESY - Eserine in DMSO



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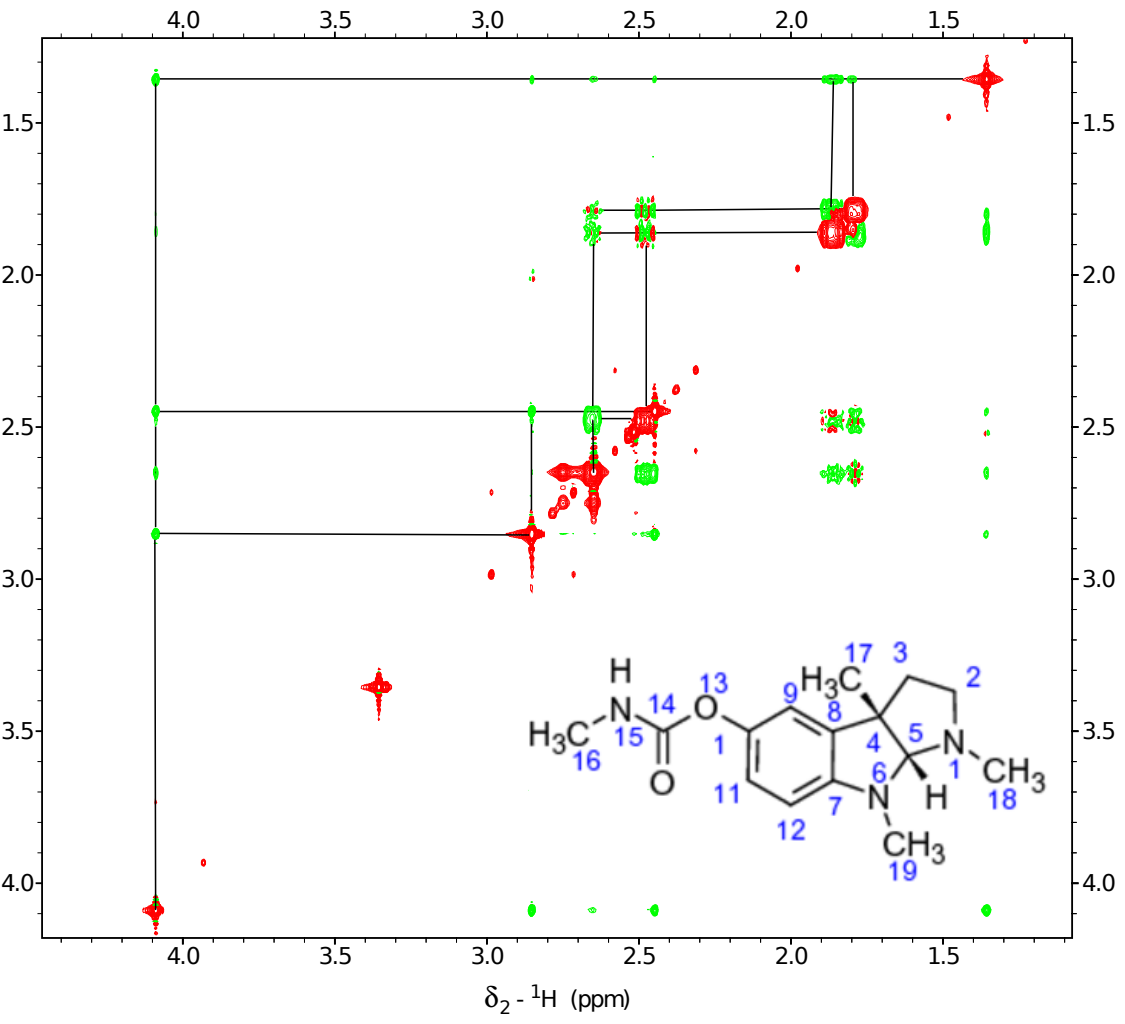
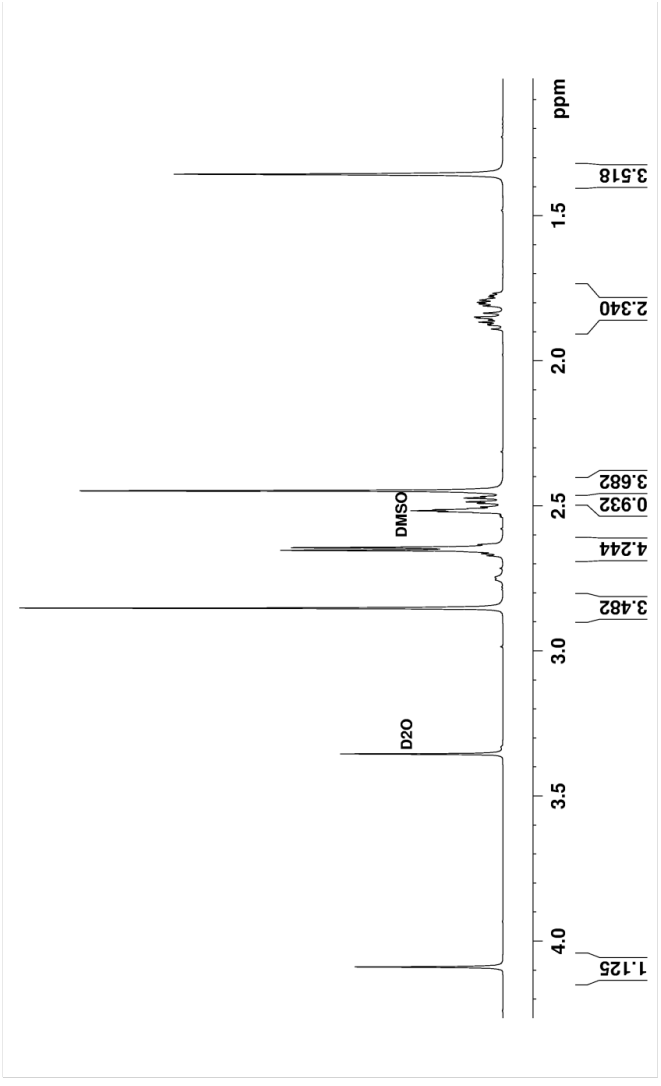
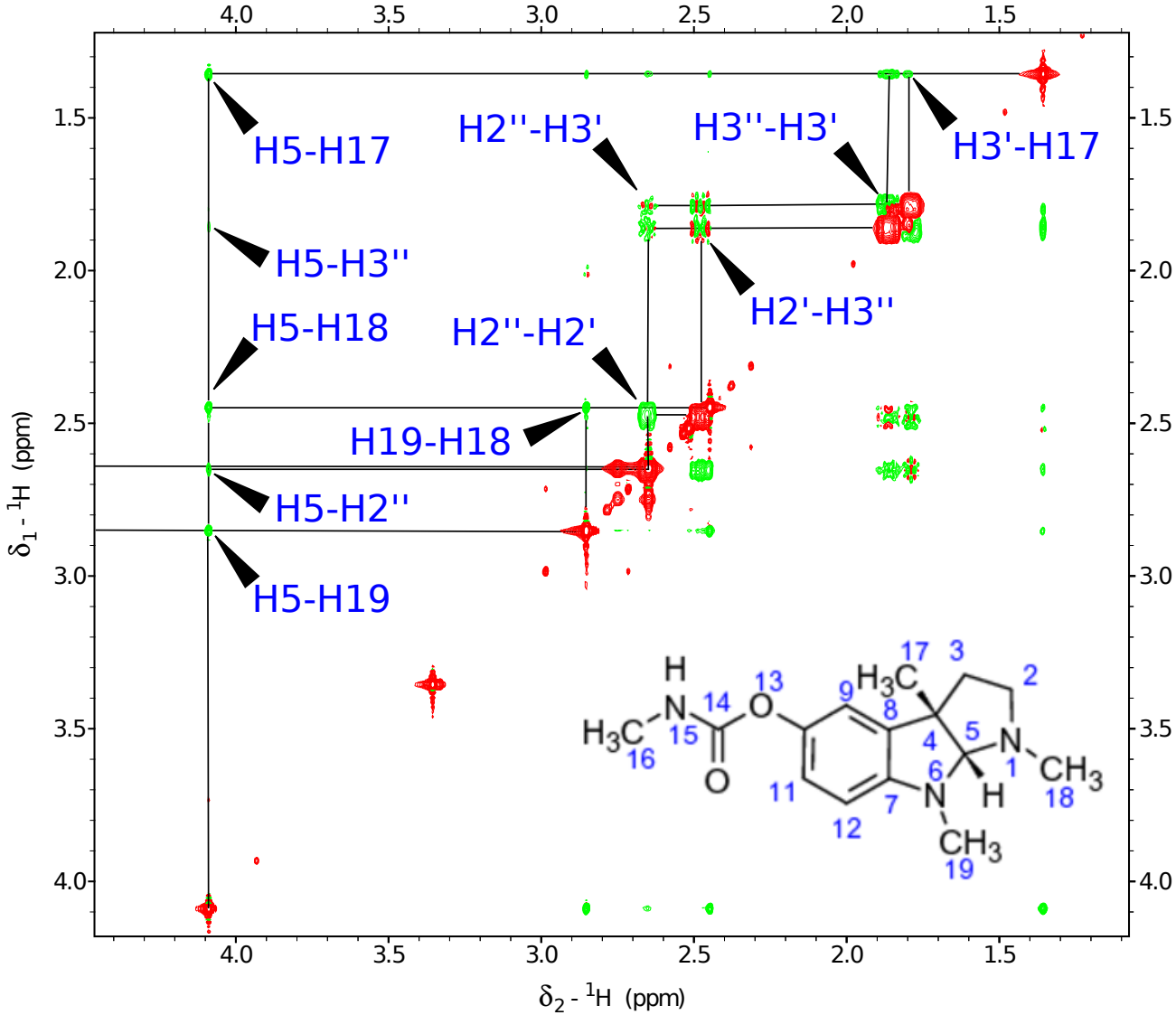
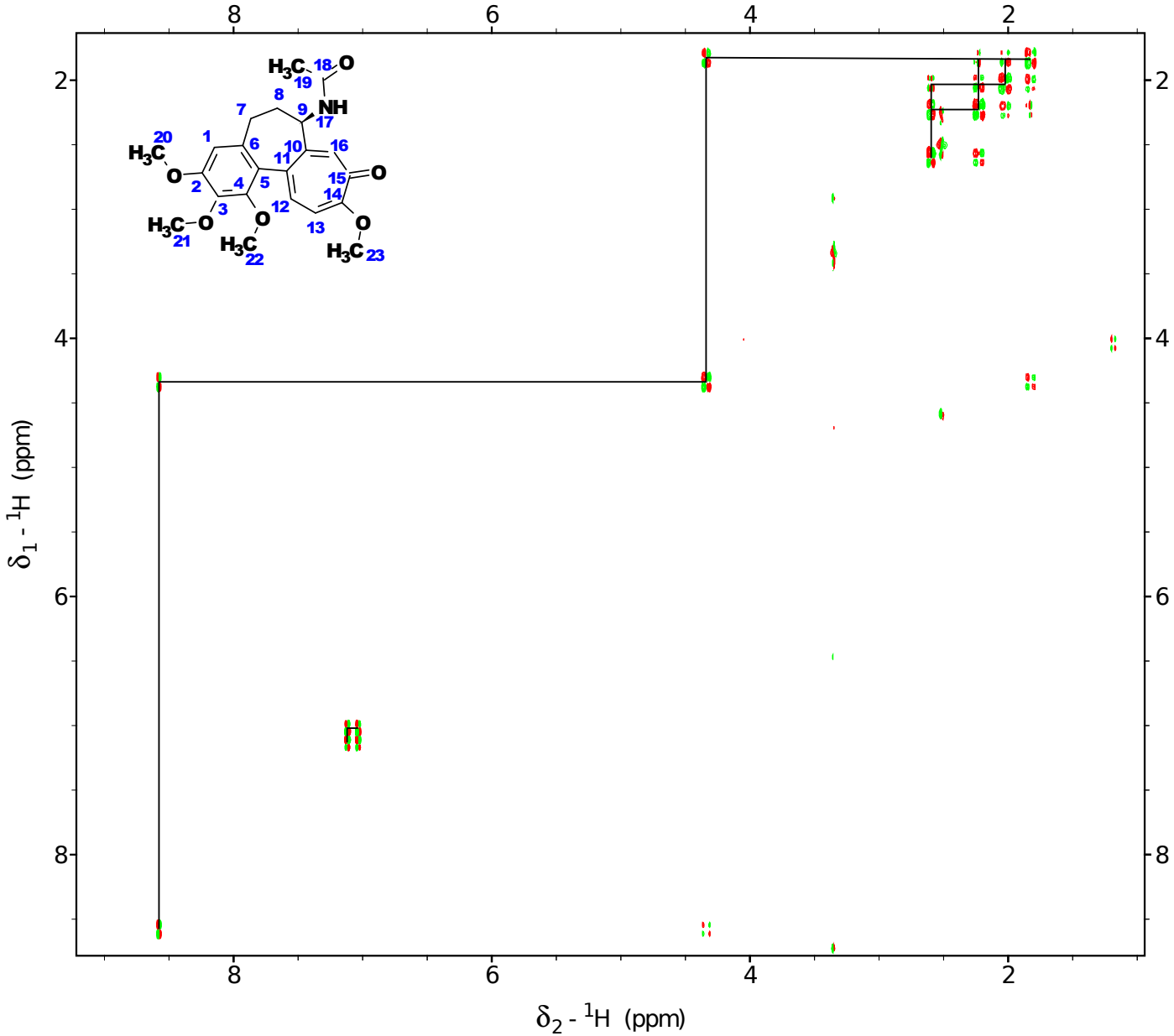




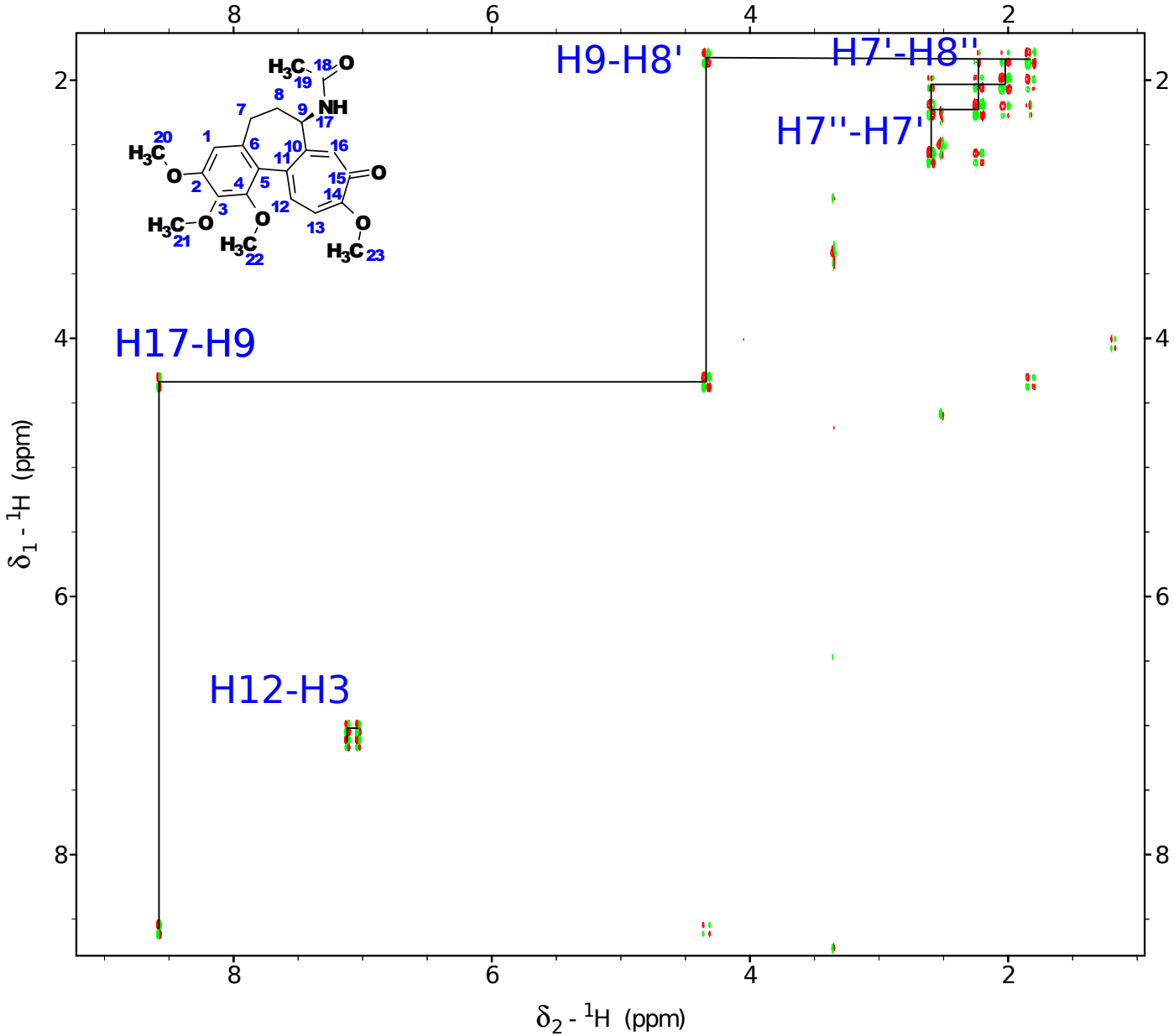
NOESY - Eserine



Colchicine - DQF-COSY



Colchicine - DQF-COSY



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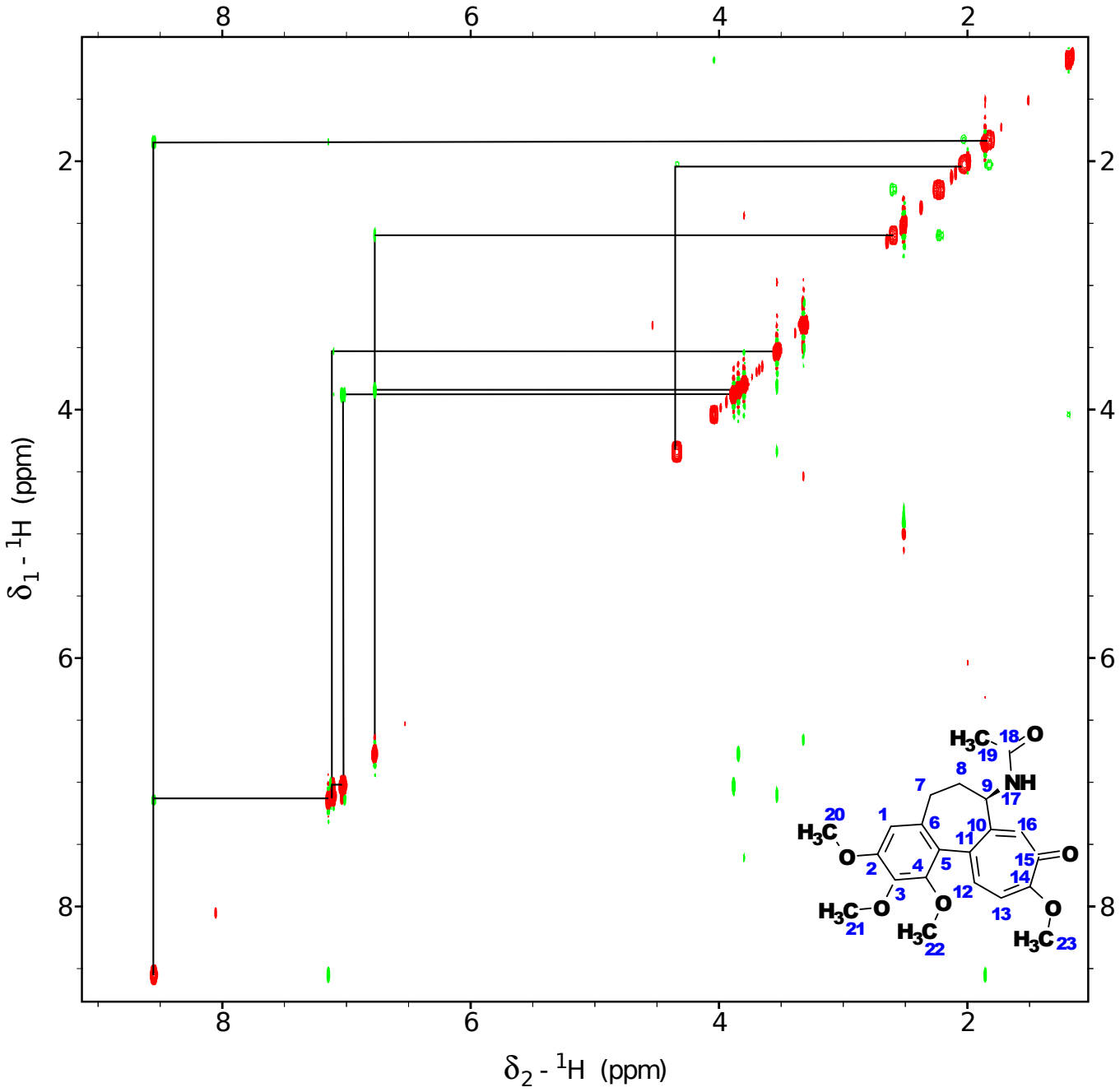

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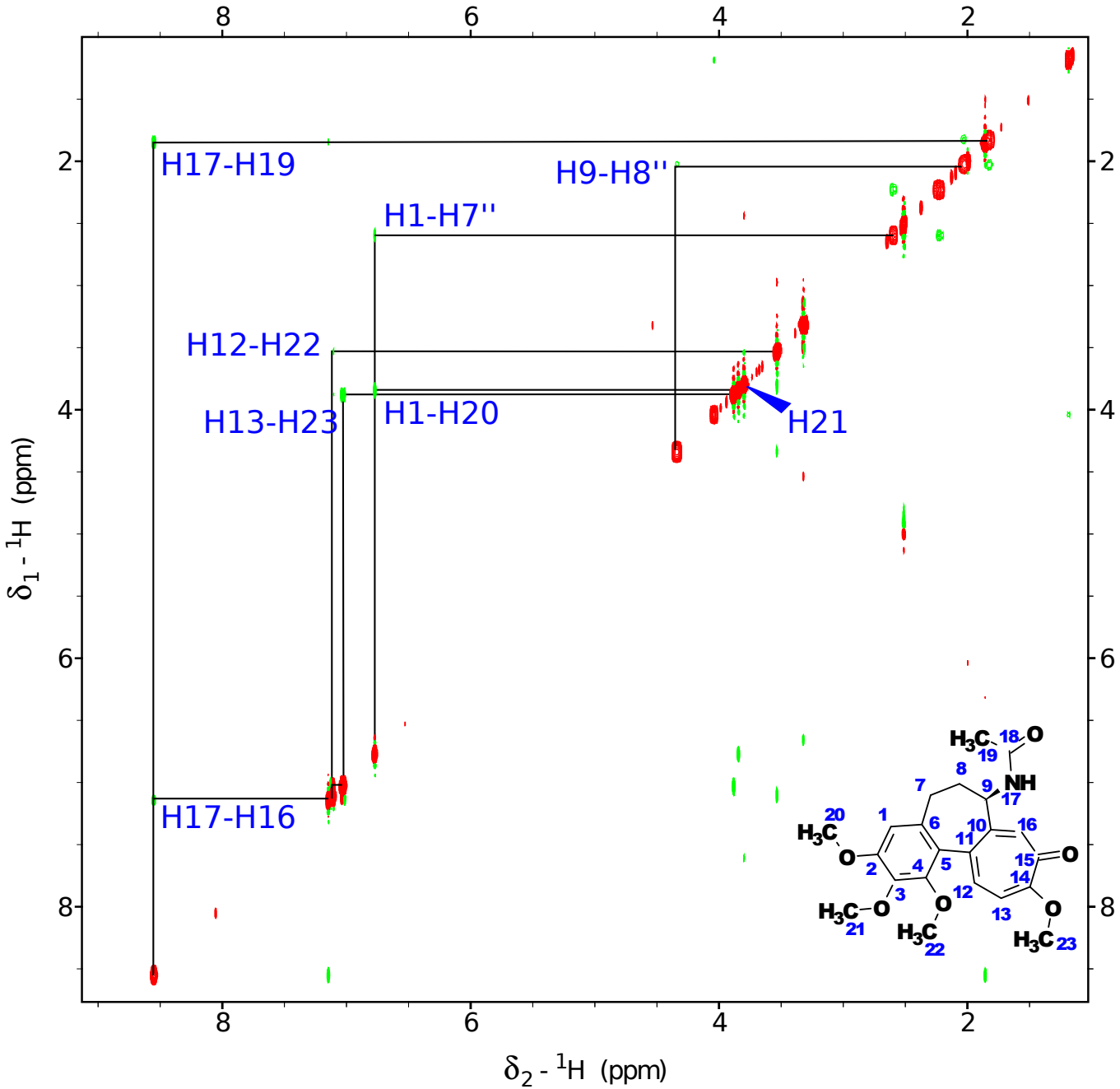

Next session:

Midterm Test, Heteronuclear correlations