

C8953  
NMR strukturní analýza  
seminář  
NOESY

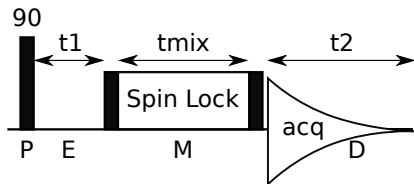
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March 29, 2022

# TOCSY (Total Correlation SpectroscopY)

spin lock - isotropic mixing

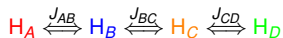
- ▶ series of short  $180^\circ$  pulses
- ▶ "lock-in" of spins in transversal plane
- ▶ higher power in case of TOCSY, offset set into the middle (on resonance)
- ▶ smaller power in case of ROESY, offset set into the edge (off resonance)
- ▶ crosstalk (ROE transfer in TOCSY,  $J$ -coupling in ROESY)



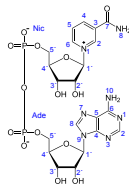
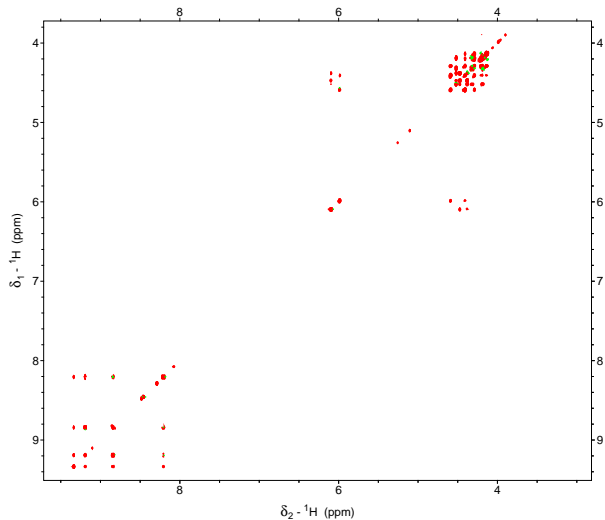
# TOCSY (Total Correlation Spectroscopy)

correlation based on  $J$

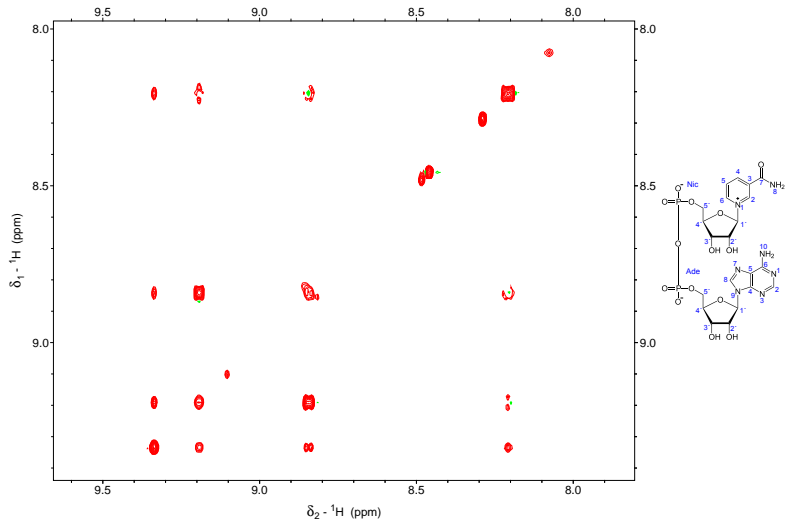
- ▶ mutual correlation of **all protons** in one spin system
- ▶  $\tau_{mix} \approx 20 - 120\text{ms}$
- ▶ crosspeak intensity depends on  $\tau_{mix}$  and  $J$  value



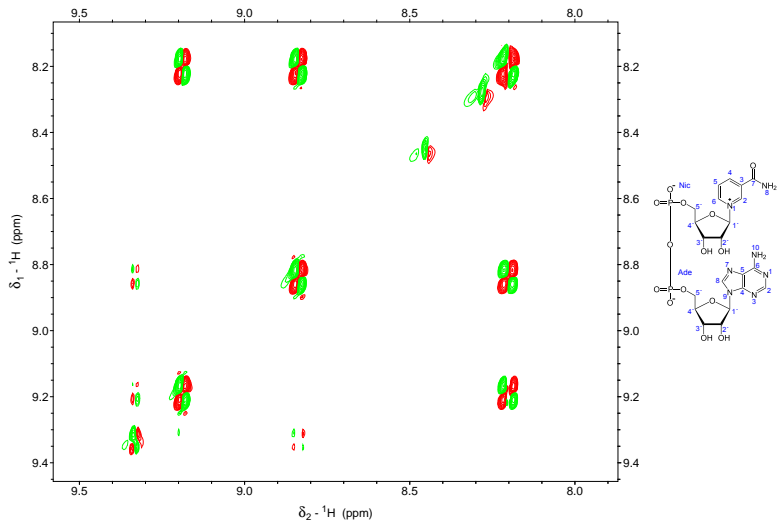
# NAD<sup>+</sup>: TOCSY (40ms)



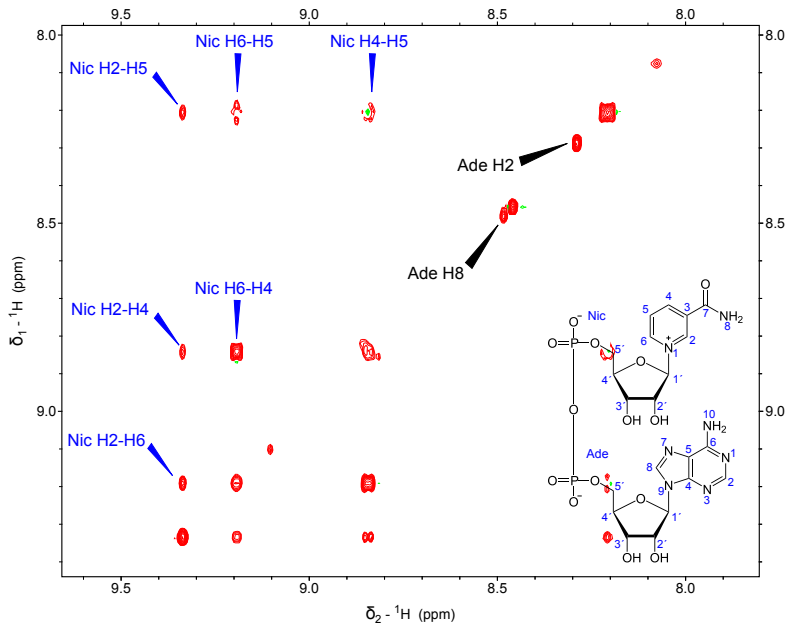
# NAD<sup>+</sup>: TOCSY (40ms), detail of aromatics



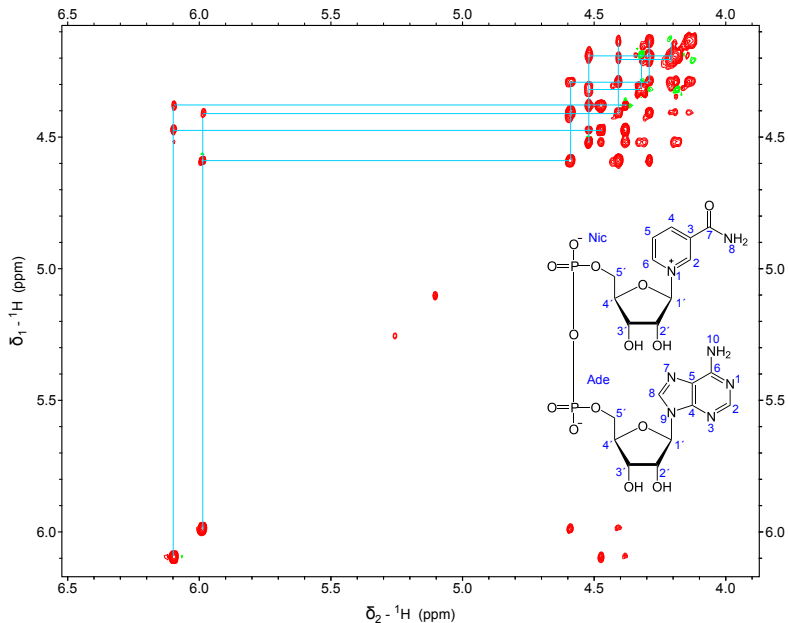
# NAD<sup>+</sup>: DQF-COSY, detail of aromatics



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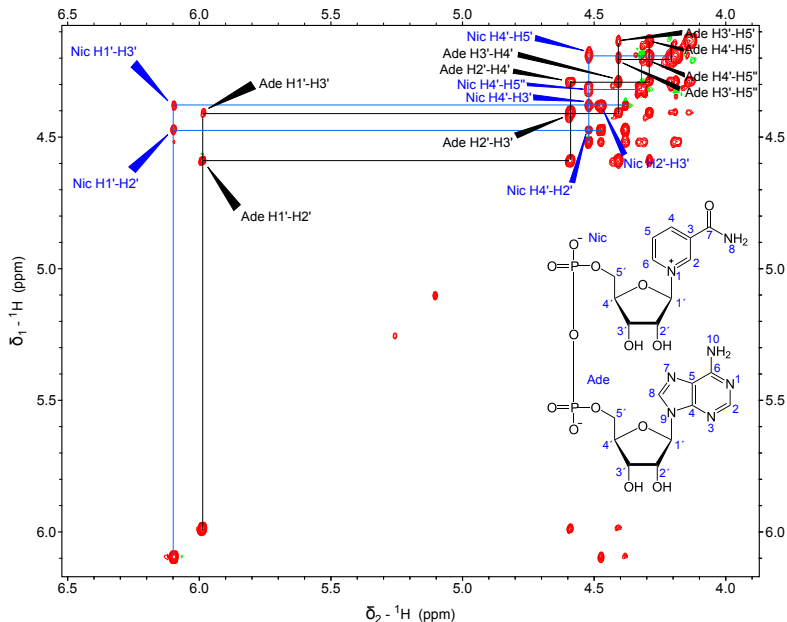


# NAD<sup>+</sup>: TOCSY (40ms), detail of aliphatics





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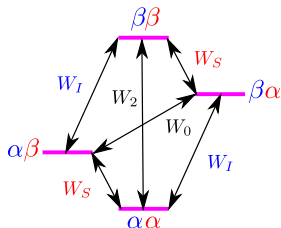
# NOESY - introduction

## Nuclear Overhauser effect

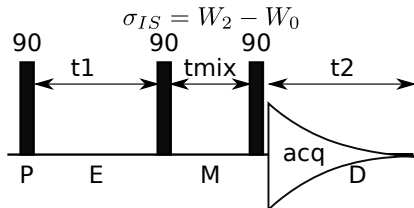
- ▶ dipol-dipol interaction
- ▶ magnetisation transfer THROUGH SPACE as a consequence of cross-relaxation

## NOESY

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



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



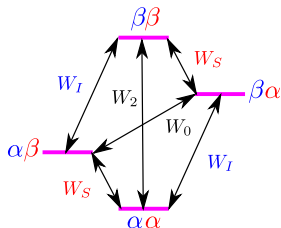
# NOE vs. size of a molecule

NOE enhancement

$\frac{M_I^S - M_{0I}}{M_{0I}} \approx \frac{\sigma_{IS}}{\rho_I} = \frac{W_2 - W_0}{W_0 + 2W_1 + W_2}$ , where  $M_I^S$  is magnetisation of I perturbed by saturation of S

Correlation time  $\tau_c$

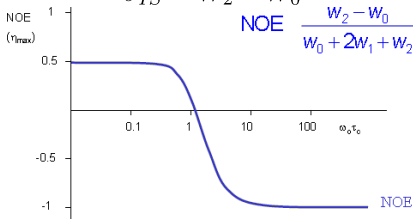
- ▶  $\omega_0 \tau_c < 1 \Leftrightarrow \omega_0 \frac{1}{f} < 1 \Leftrightarrow \omega_0 < f$  (small molecules  $\ll 1$  kDa)
  - ▶ **fast molecular motion**,  $\beta\beta \rightarrow \alpha\alpha$  dominates  $\Rightarrow W_2 > W_0$
  - ▶ positive NOE
  - ▶ crosspeaks have opposite phase relative 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 is an alternative



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

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

$$\text{NOE} = \frac{W_2 - W_0}{W_0 + 2W_1 + W_2}$$



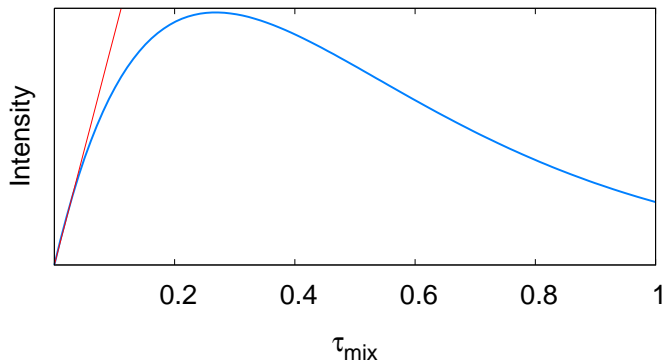
# Application of NOESY

## Mixing time $\tau_{\text{mix}}$

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

## approximative determination of interatomic distances ( $< 5 \text{ \AA}$ )

- ▶ at short  $\tau_{\text{mix}}$
- ▶  $r_{ij} \approx A \times I_{ij}$

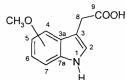


# NOE differential experiment

## PROBLEM 4

### NOE- Difference Spectroscopy

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



What is the position of the methoxy group?

(400 MHz  $^1\text{H}$ )

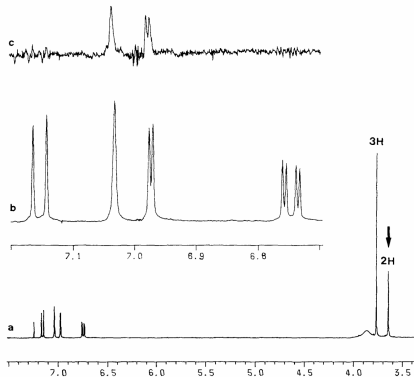


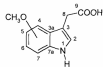
Fig. 4.1. 400 MHz  $^1\text{H}$  NMR spectrum of **13** in a mixture of  $\text{CDCl}_3$  and  $\text{CD}_3\text{OD}$ . a Full spectrum; b expanded section of the aromatic proton signals; c  $^1\text{H}$  NOE difference spectrum, same section as in b, irradiation position at  $\delta = 3.64$ .

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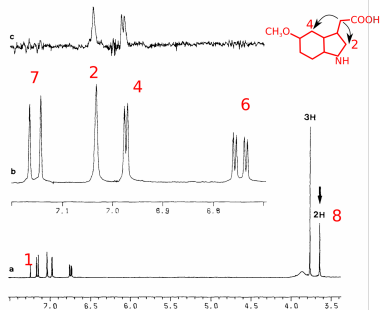
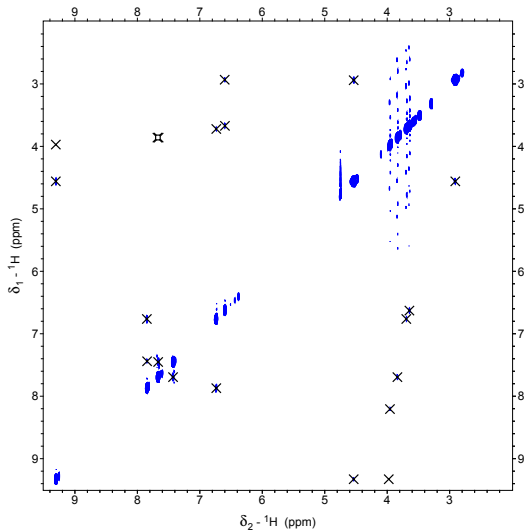
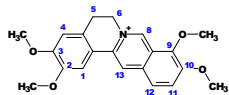
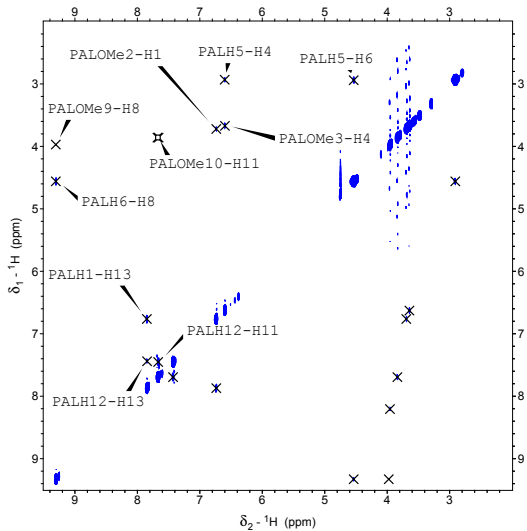
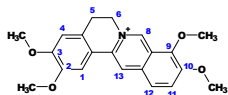


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# NOESY - Palmatine

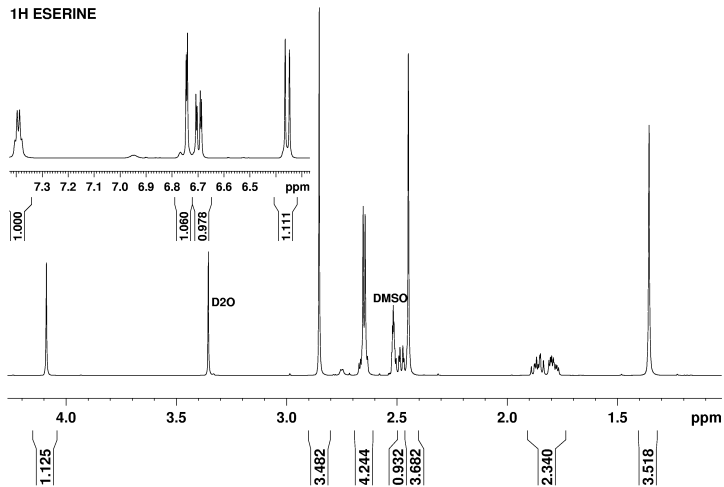


# NOESY - Palmatine

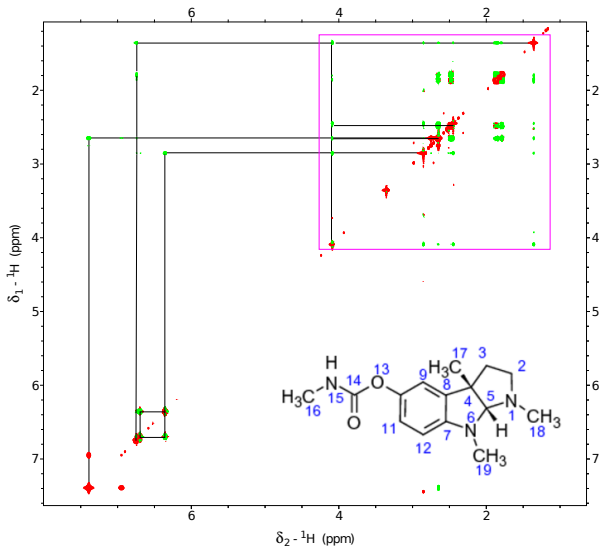




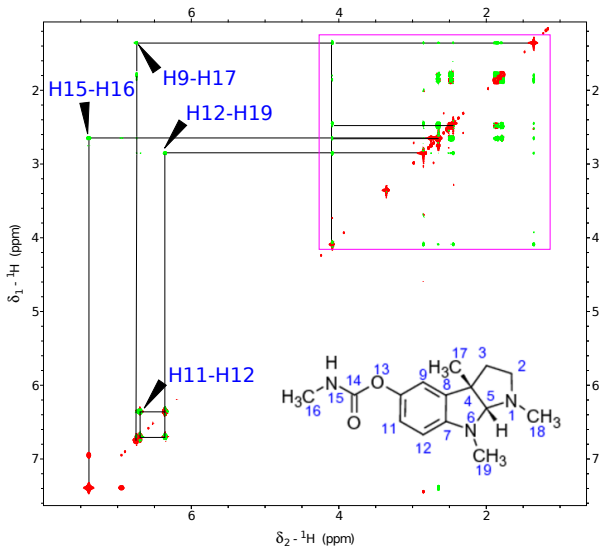
# Eserine $^1\text{H}$



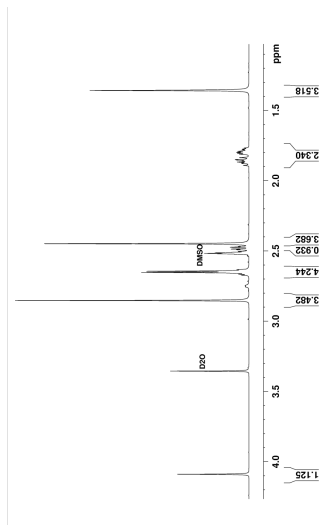
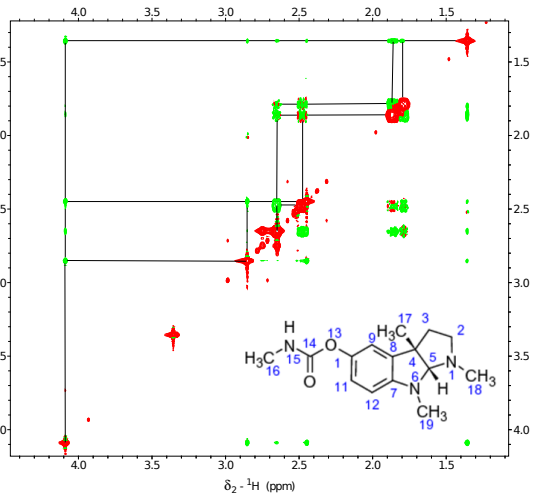
# NOESY - Eserine in DMSO



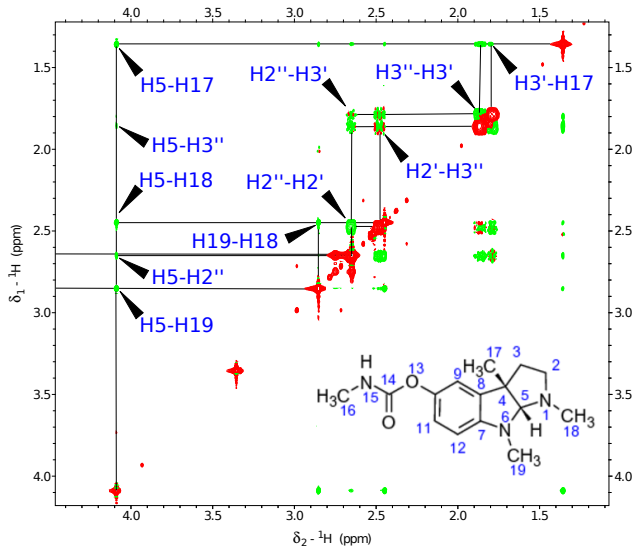
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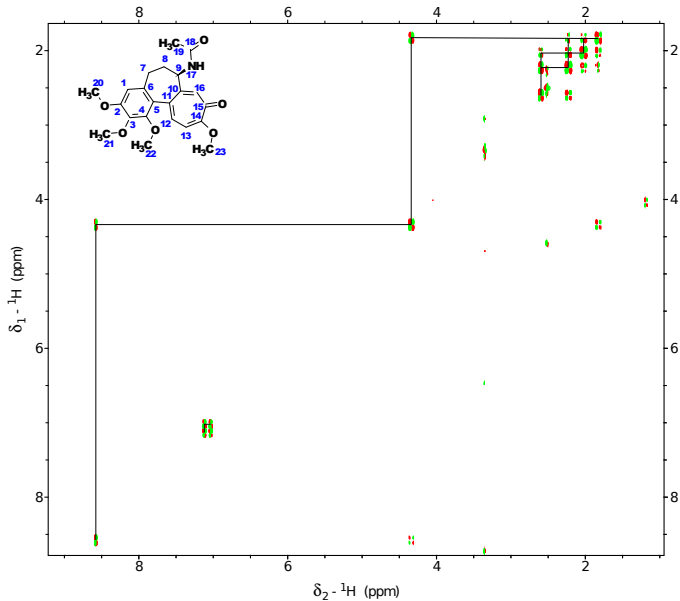
# NOESY - Eserine



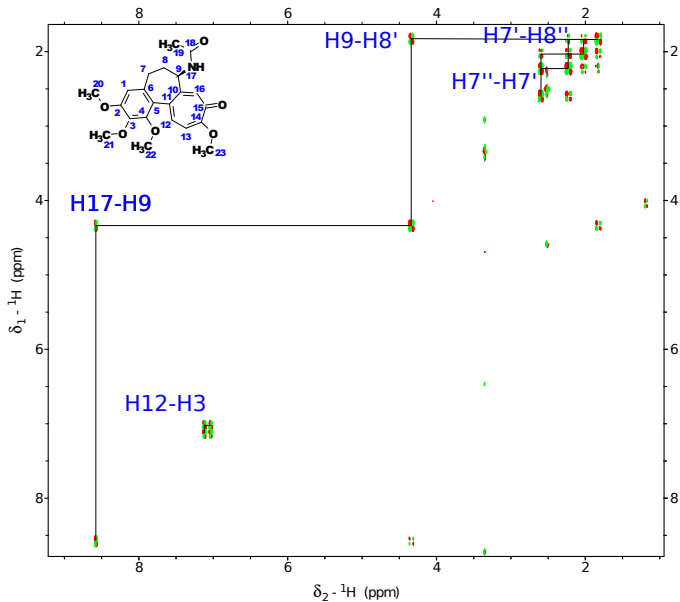
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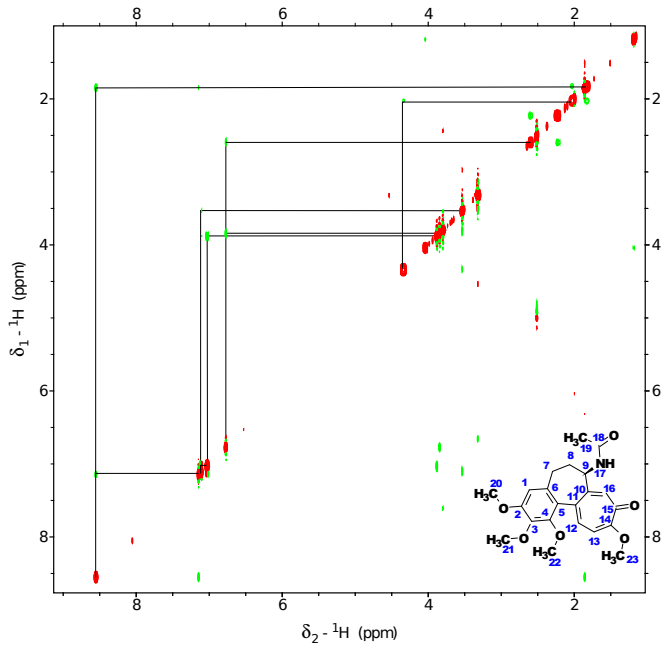
# Colchicine - DQF-COSY



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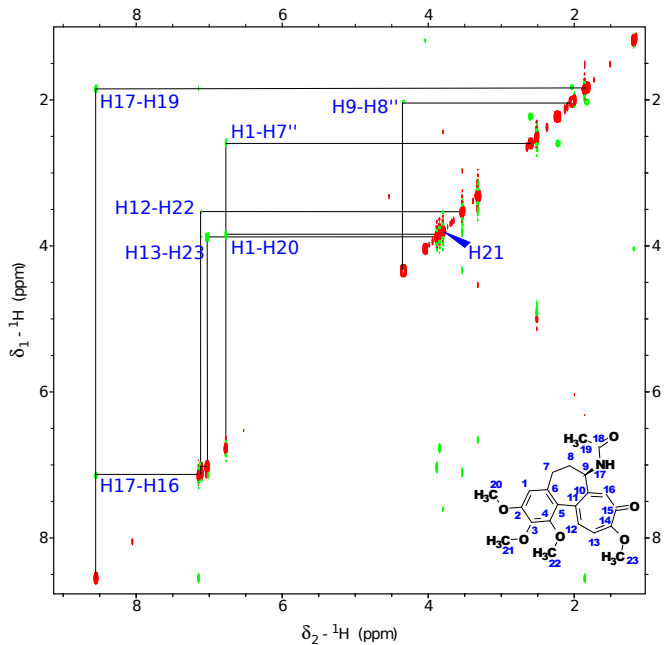


# Colchicine - NOESY





# Colchicine - NOESY



Next session:

Heteronuclear correlations