

C8953
NMR strukturní analýza
seminář

Introduction to heteronuclear correlations

Jan Novotný

novotnyjan@mail.muni.cz

March 25, 2020

Polarization transfer

- ▶ bigger population difference of ^1H nucleus is transferred via J-coupling to less sensitive nucleus X (^{13}C , ^{15}N)
- ▶ fundamental building block of heteronuclear correlation experiments: in 2D-HX experiment each crosspeak manifests interaction of H and X nucleus coupled through bonds
- ▶ **Task: Draw the evolution of magnetization during basic INEPT pulse sequence. Consider C-H interacting pair.**

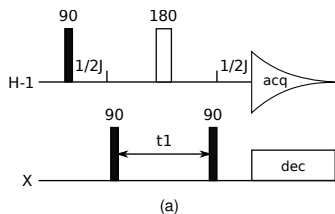
HMQC (Heteronuclear Multiple Quantum Correlation)

HSQC (Heteronuclear Single Quantum Correlation)

correlate $^1\text{H-X}$ ($\text{X} = ^{13}\text{C}, ^{15}\text{N}, \dots$) based on $^1J_{\text{HX}}$

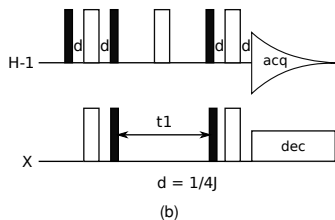
HMQC (a)

- + more robust experiment
- + change of parameters - HMBC
- lower sensitivity and worse resolution



HSQC (b)

- + better resolution, sensitivity
- + part of more complex multidimensional experiments
- less robust



Practical notes $^1\text{H-X HSQC}$

- ▶ 2D spectrum with $X = ^{13}\text{C}$ or ^{15}N chemical shift in indirect dimension (f1) and ^1H in direct dimension (f2)
- ▶ heteronuclear correlation \Rightarrow no diagonal crosspeak, no symmetry
- ▶ X decoupled during acquisition \Rightarrow singlet crosspeak
- ▶ resolution of overlapped ^1H signals
- ▶ routine experiments to control biomolecular sample (in proteins each aminoacid is represented by N-H crosspeak)
- ▶ easy identification of geminal diastereotopic protons (CH_2)
- ▶ indirect determination of protons bonded to NMR inactive heteroatom

HMBC(Heteronuclear Multiple-Bond Correlation)

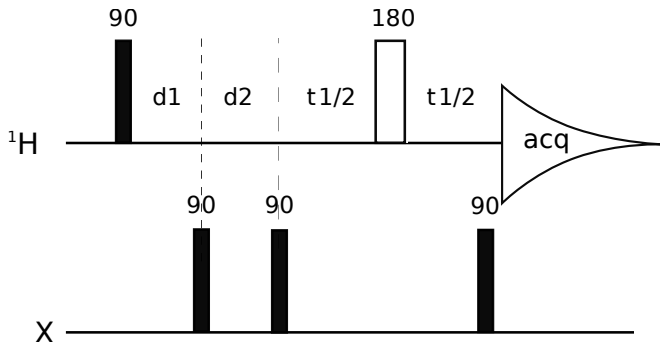
heteronuclear correlation based on long-range H-X spin-spin interaction(${}^nJ_{HX}$, $n>1$)

- ▶ utilizes polarization transfer from H through 2-5 bonds on heteroatom (${}^{13}\text{C}$, ${}^{15}\text{N}$)
- ▶ allows to detect quaternary heteroatoms (Cq) or connect signals among isolated spin systems
- ▶ small ${}^nJ_{HX}$ means longer evolution of polarisation transfer - sensitivity drops because of relaxation

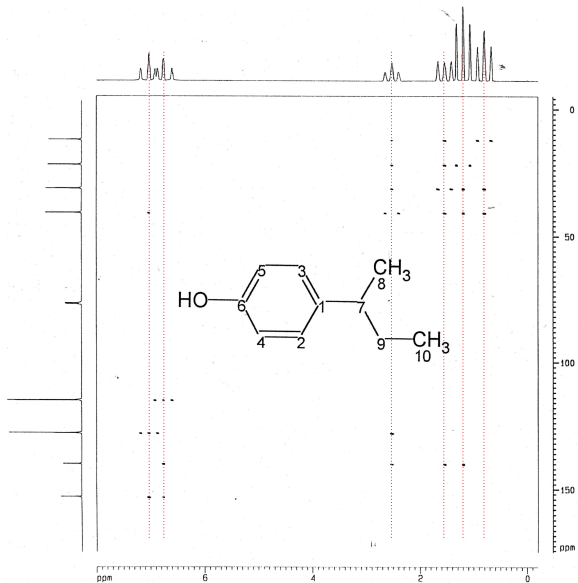
HMBC

correct settings of $d1$, $d2$ for evolution of J -coupling necessary

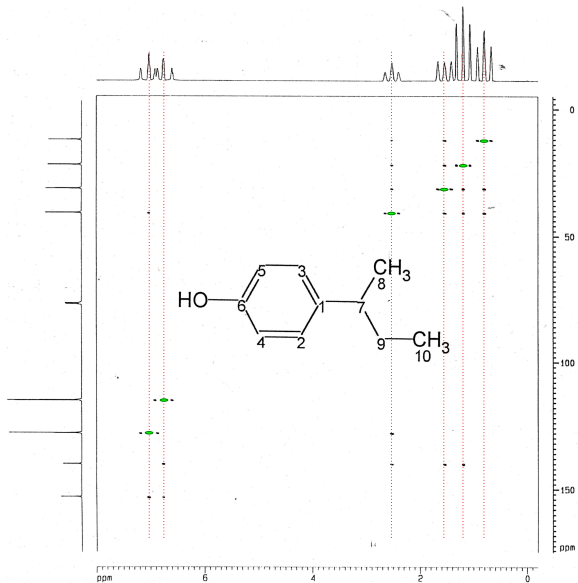
- ▶ $d1 = 1/2 * {}^1J_{C-H}$ - (120-180 Hz)
- ▶ $d2 = 1/2 * {}^{2-5}J_{C-H}$ - (3-12 Hz)
- ▶ in real HMBC spectrum, single bond (HSQC-like) correlations might be partially visible as doublets



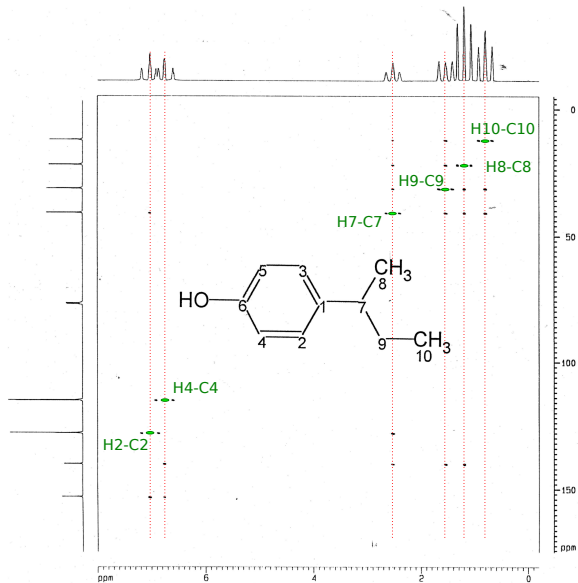
^1H - ^{13}C HMBC + ^1H - ^{13}C HSQC



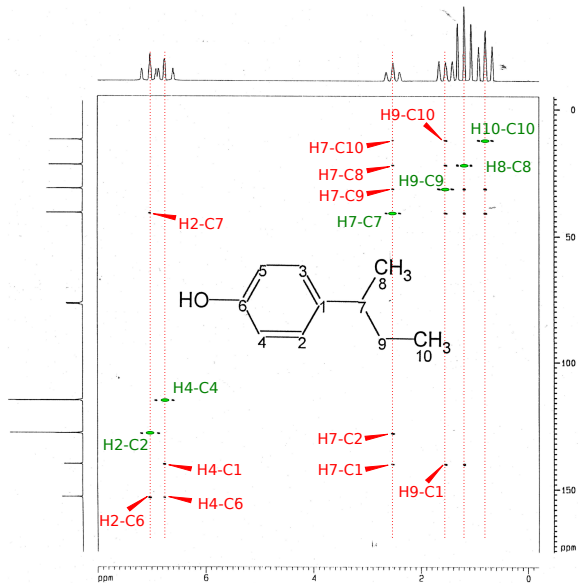
^1H - ^{13}C HMBC + ^1H - ^{13}C HSQC



^1H - ^{13}C HMBC + ^1H - ^{13}C HSQC

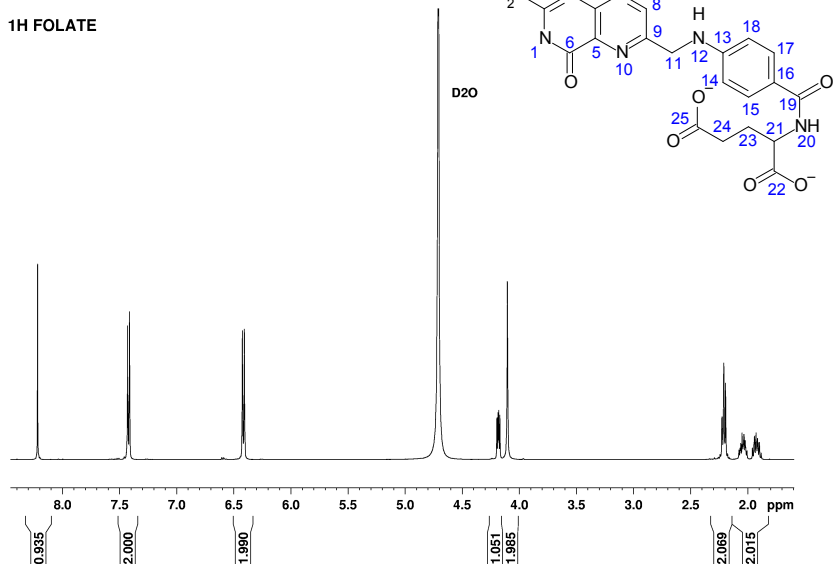


^1H - ^{13}C HMBC + ^1H - ^{13}C HSQC



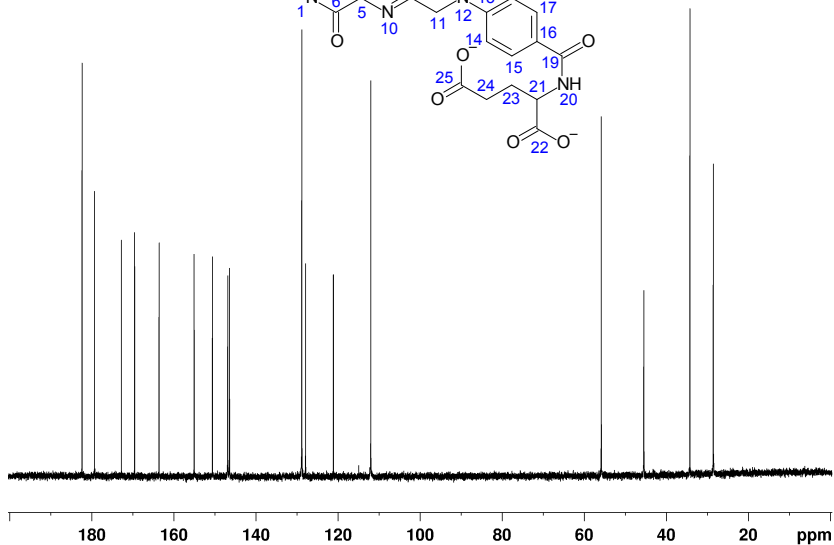
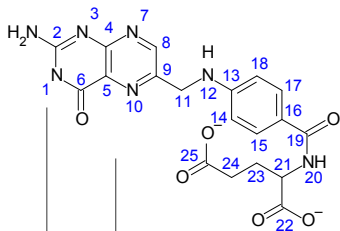
Folic Acid: ^1H 1D

^1H FOLATE

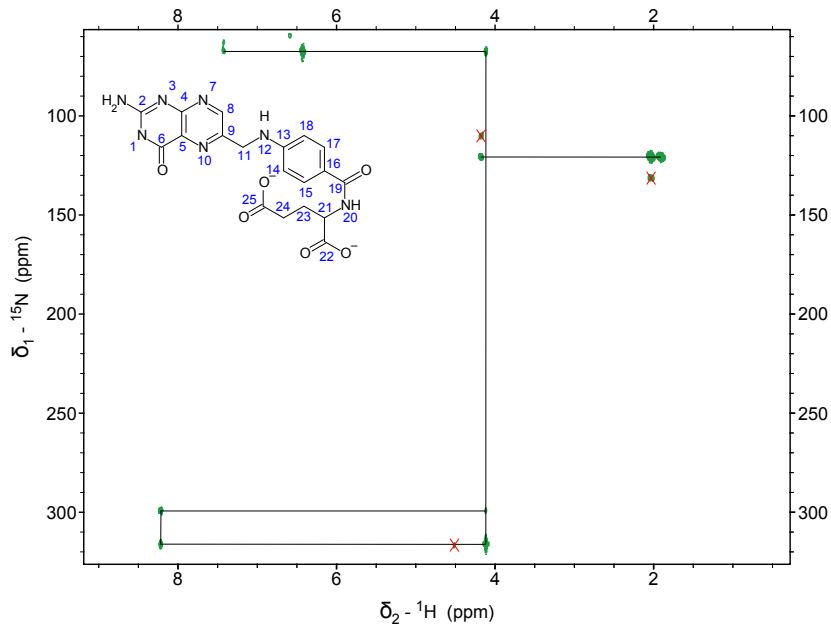


Folic Acid: ^{13}C 1D

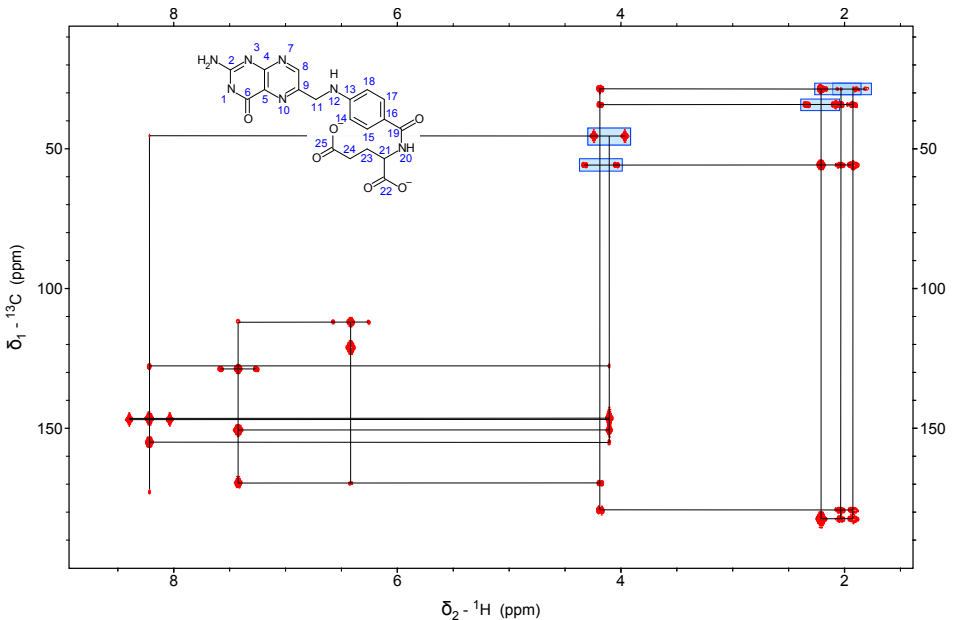
^{13}C FOLATE



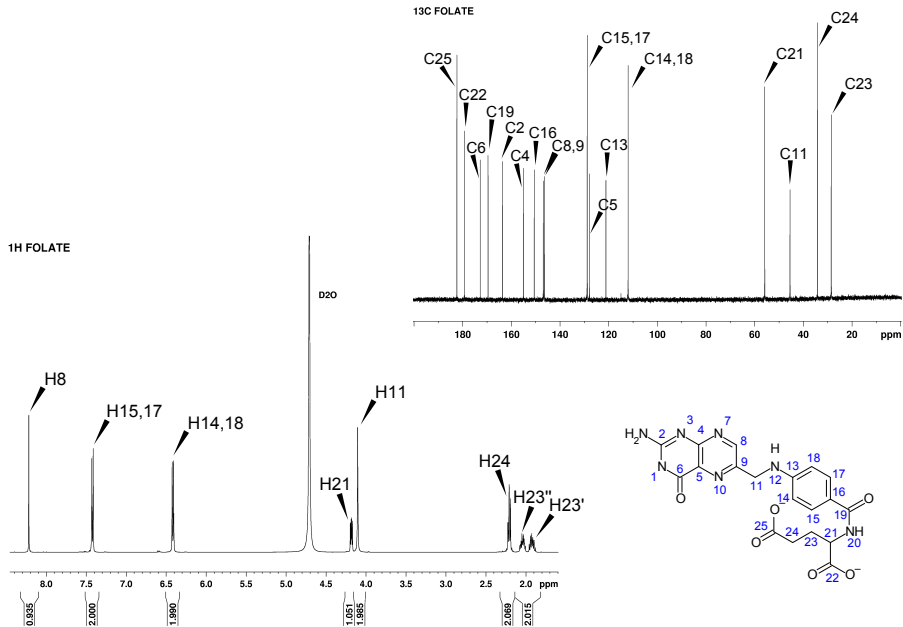
Folic Acid: ^1H - ^{15}N HMBC



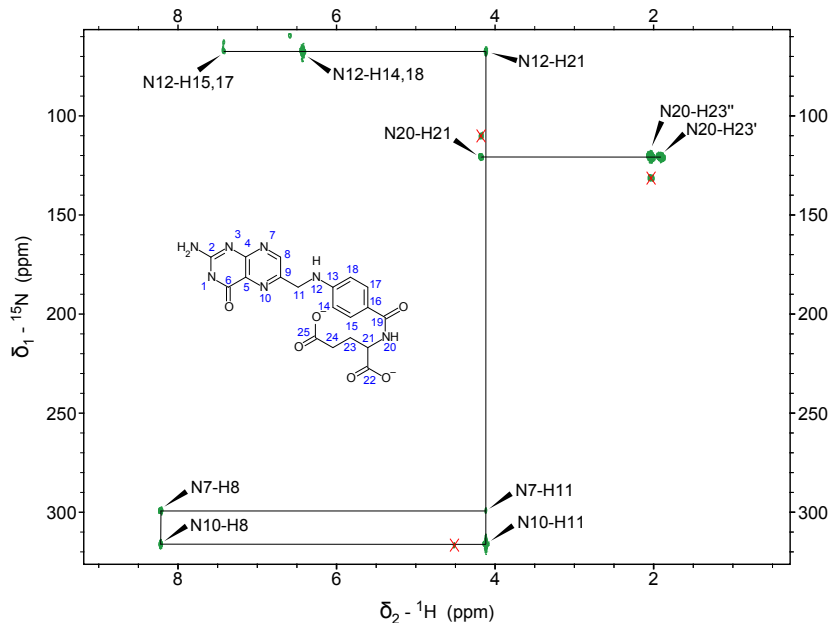
Folic Acid: ^1H - ^{13}C HMBC



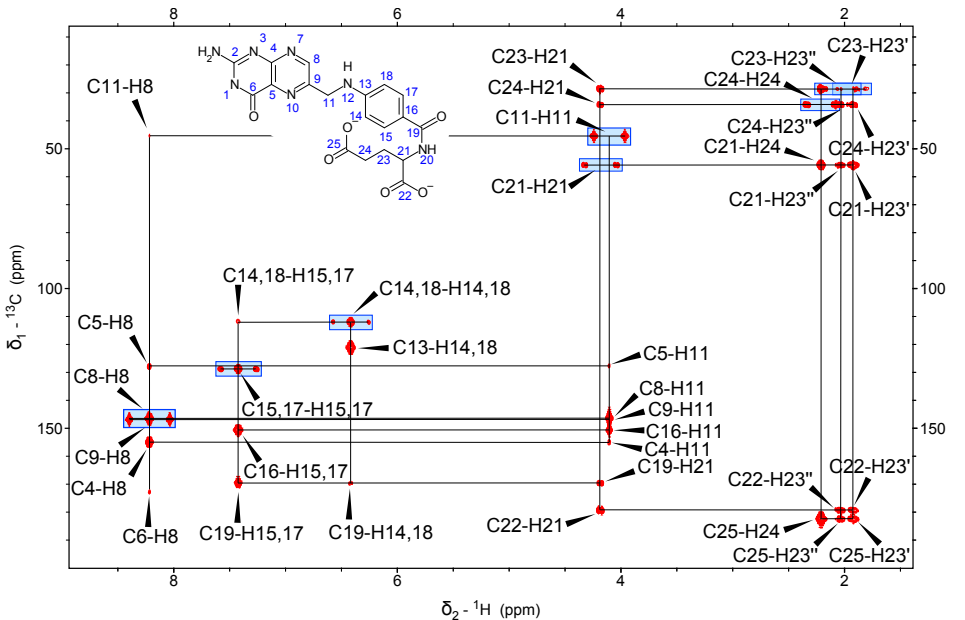
Folic Acid: ^1H and ^{13}C 1D



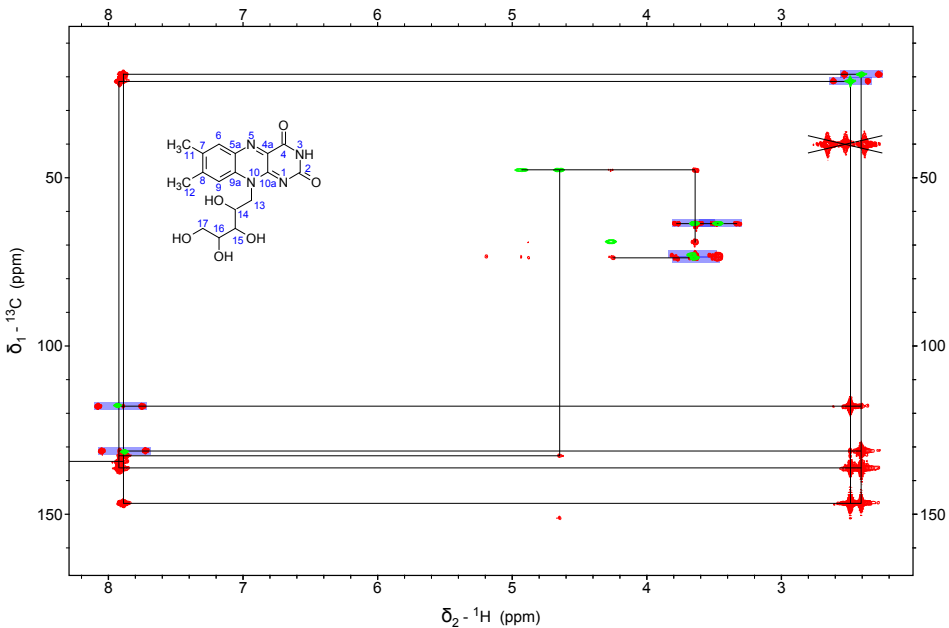
Folic Acid: ^1H - ^{15}N HMBC



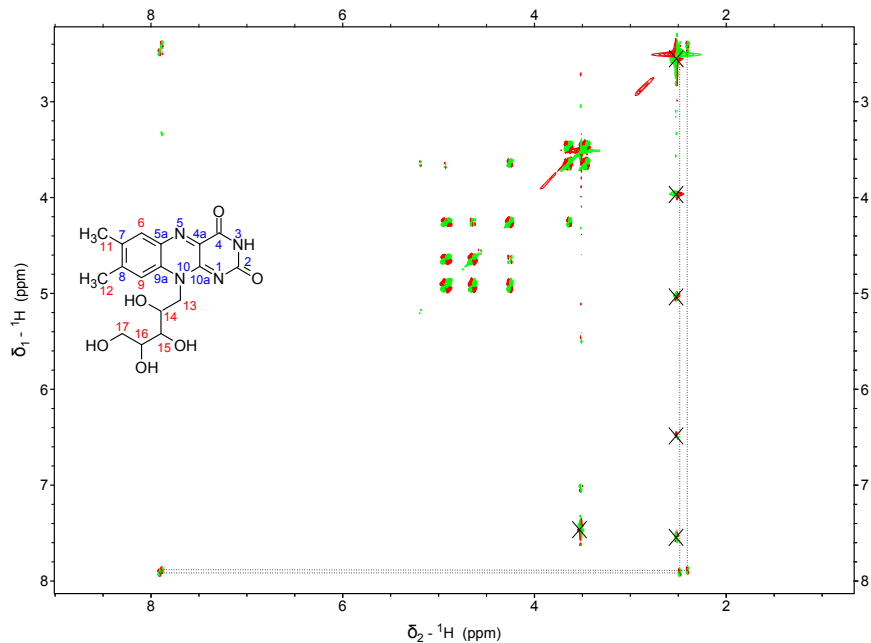
Folic Acid: ^1H - ^{13}C HMBC



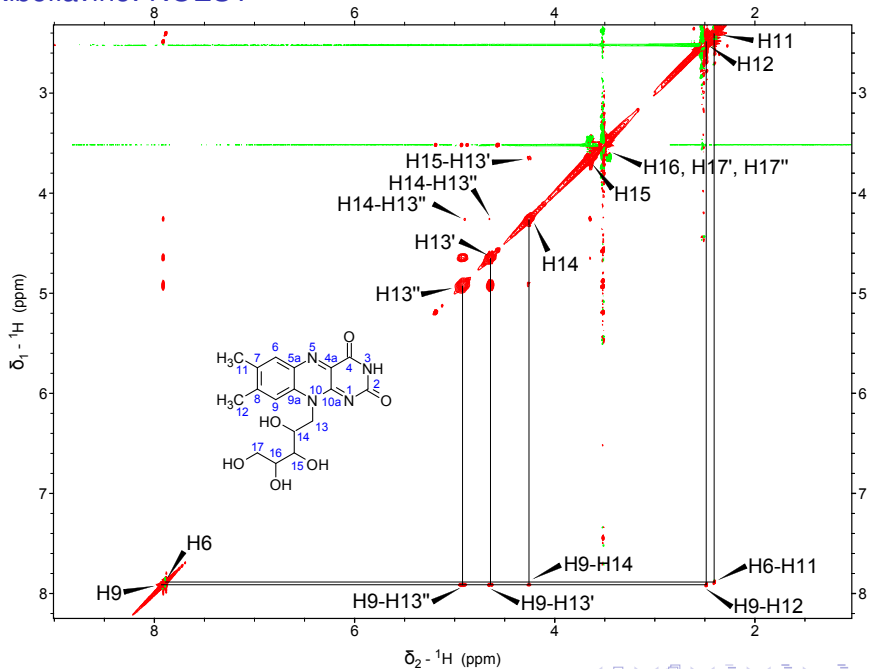
Riboflavine: ^1H - ^{13}C HMBC + HSQC



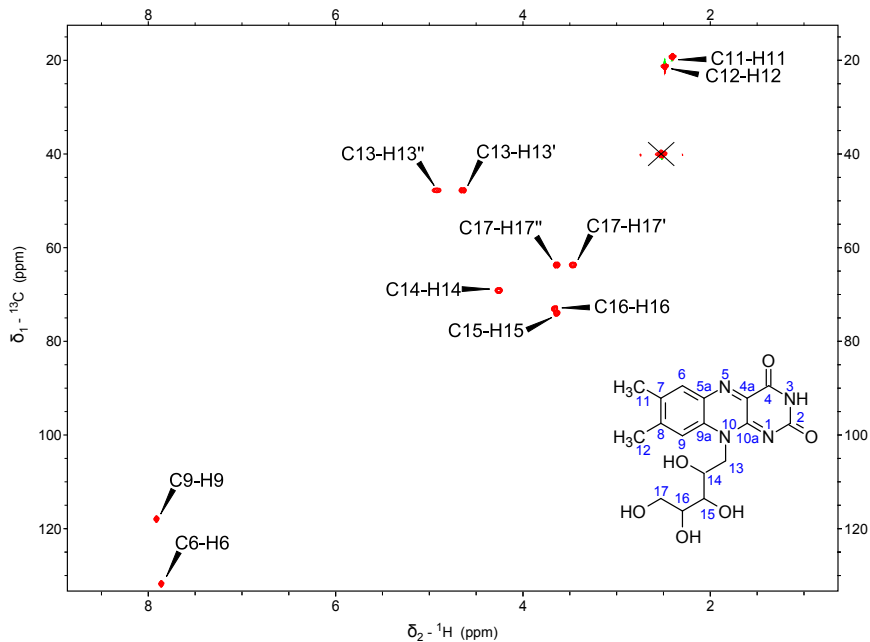
Riboflavine: DQF-COSY



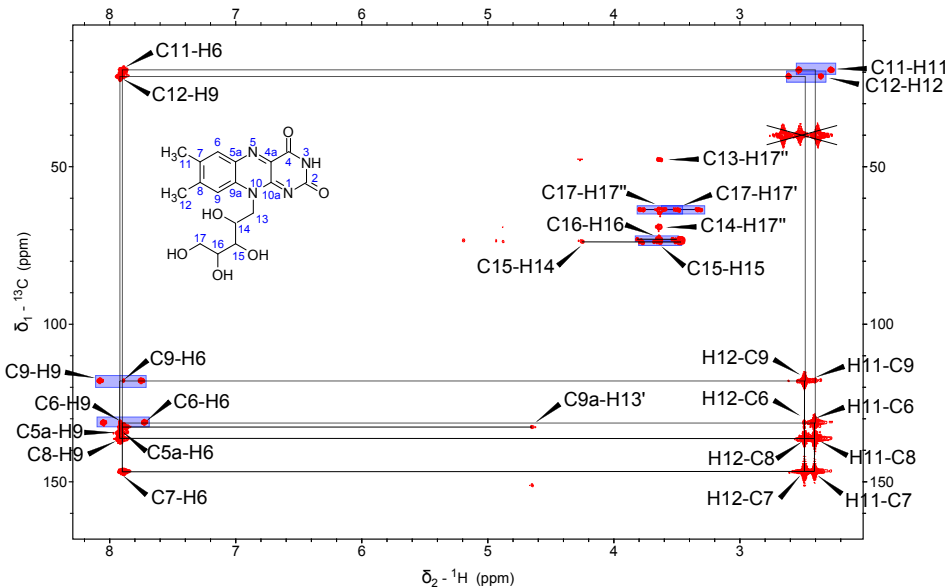
Riboflavine: NOESY



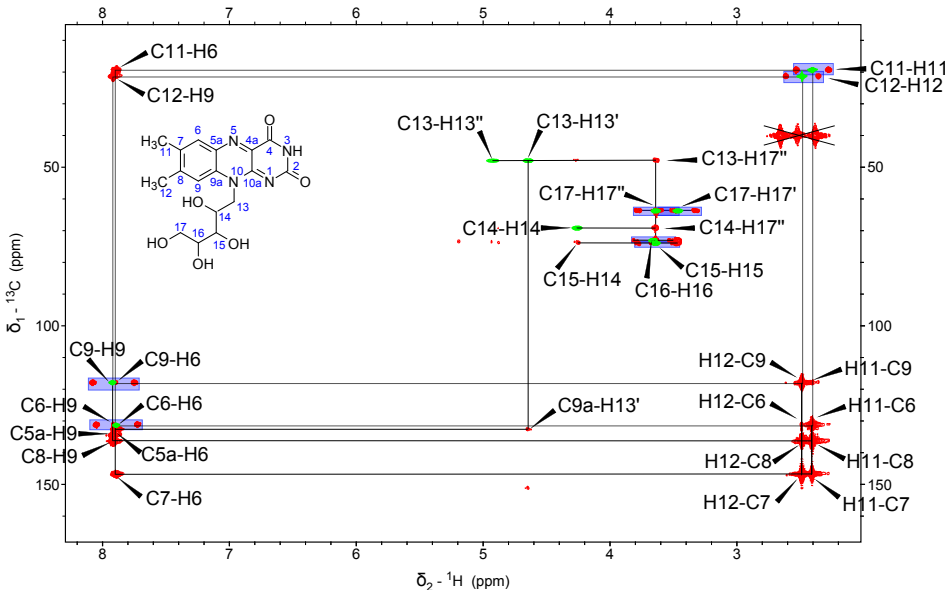
Riboflavine: ^1H - ^{13}C HSQC



Riboflavine: ^1H - ^{13}C HMBC



Riboflavine: ^1H - ^{13}C HMBC + HSQC



Next session:

Complex exercises