Assignment of PROMs

and new tricks for an old dog

Peter Schmieder

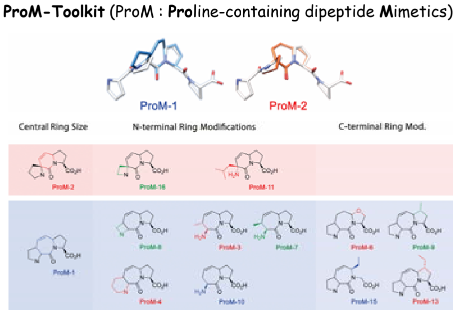
Structural Biology seminar, 19.10.2016

The ProMs: Control of Poly-Proline binding to EVH1 domains

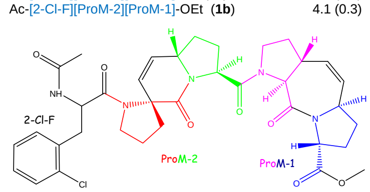
(AG Kühne, FMP, AG Schmalz, Univ. Köln)

(D/E)F(L/W)PxxPX(D/E)(D/E) | Profilin | G- and F-actin | Tetramerization

ProM-Toolkit (ProM : **Pro**line-containing dipeptide **M**imetics)

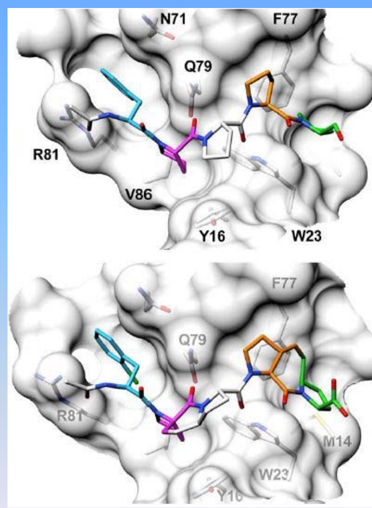


Ligand	$K_{d,\ FT}$ [µM]
Ac-SFEFPPPPTEDEL-NH_2	13.0 (0.6)
Ac-SFE[2-Cl-F][ProM-2][ProM-1]TEDEL-NH_2	0.15 (0.04)
Ac-FPPPP-OH	460 (70)
Ac-[2-Cl-F][ProM-2][ProM-1]-OH (**1a**)	2.3 (0.2)
Ac-[2-Cl-F][ProM-2][ProM-1]-OEt (**1b**)	4.1 (0.3)



Opitz et al. *PNAS (2015)*

The ProM-based EVH1 ligands are developed by rational design

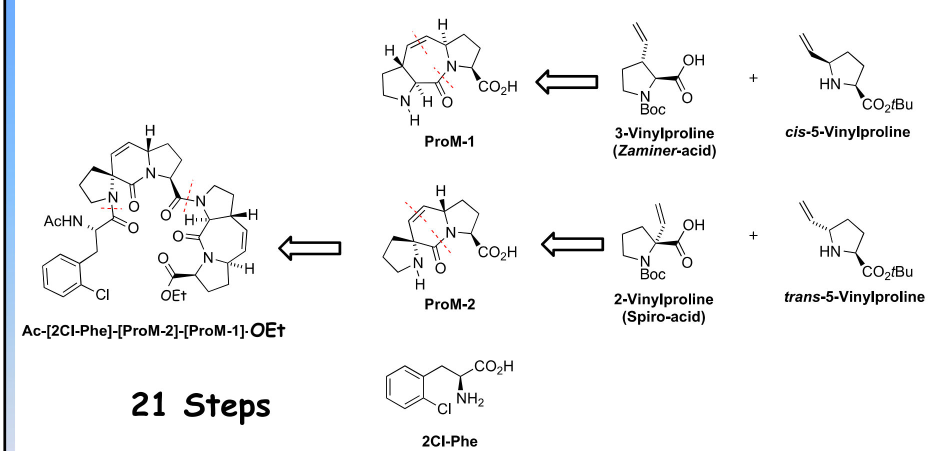


X-ray structure of Enah in complex with ...FPPPPT...(1EVH)

X-ray structure of Enah in complex with Ac-[2-Cl-F][ProM-2][ProM-1]-OH (4MY6)

(AG Kühne, FMP)

Synthesis of the ProM-based EVH1 ligands is challenging



AG Schmalz, Köln

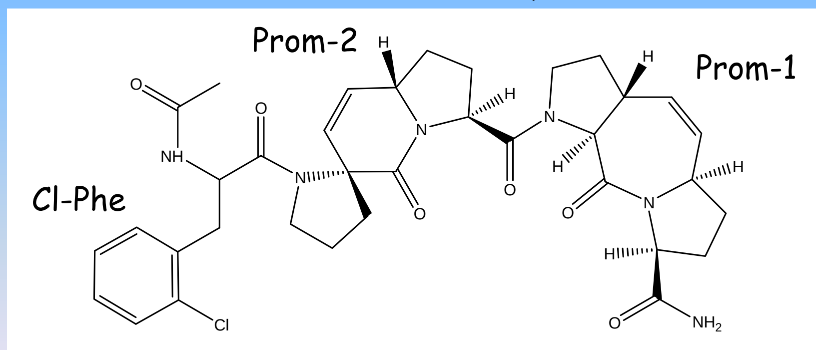
The molecule: "4c"

Potential difficulties:

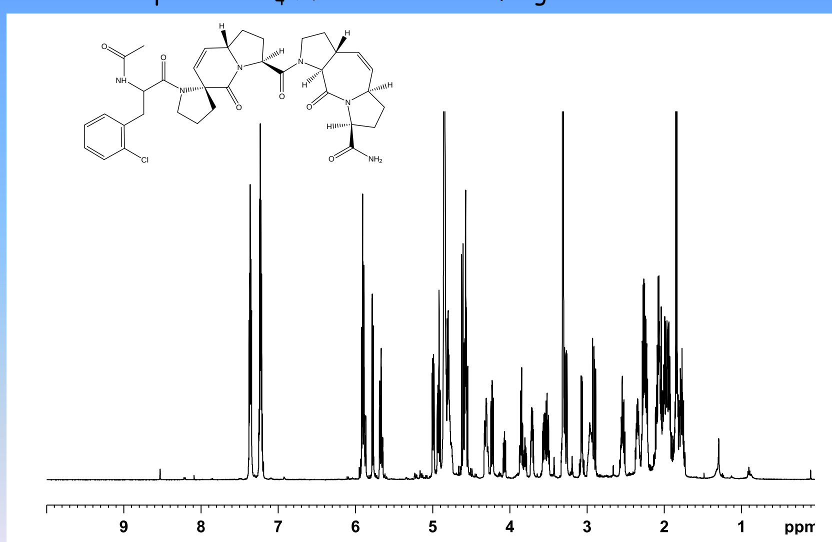
similarity between Prom-1 and Prom-2

"long" spin systems

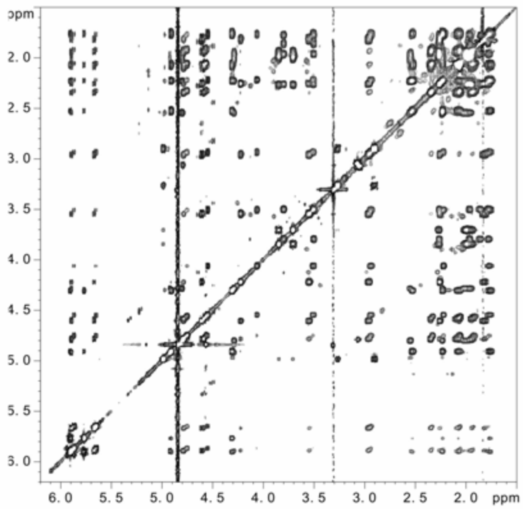
cis/trans isomers can be expected

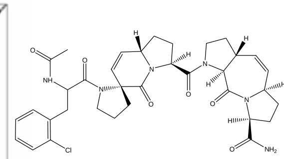


Spectra in d_4-MeOH: two sets of signals but not 1:1



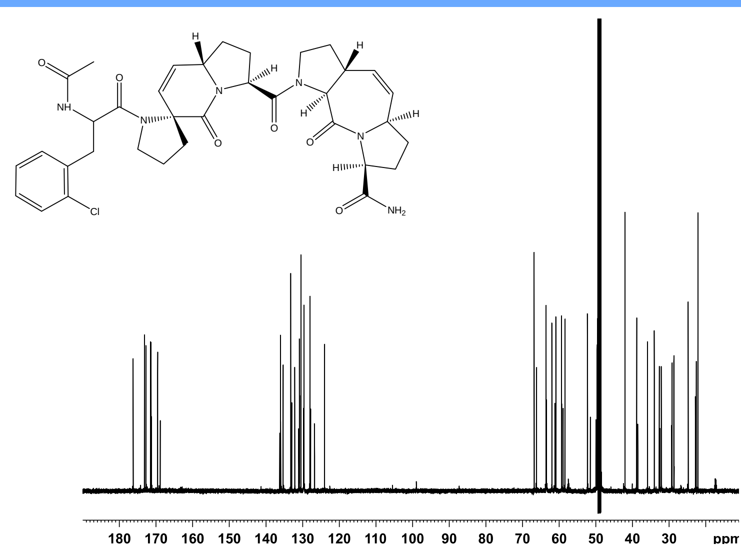
Spectra in d_4-MeOH: two sets of signals



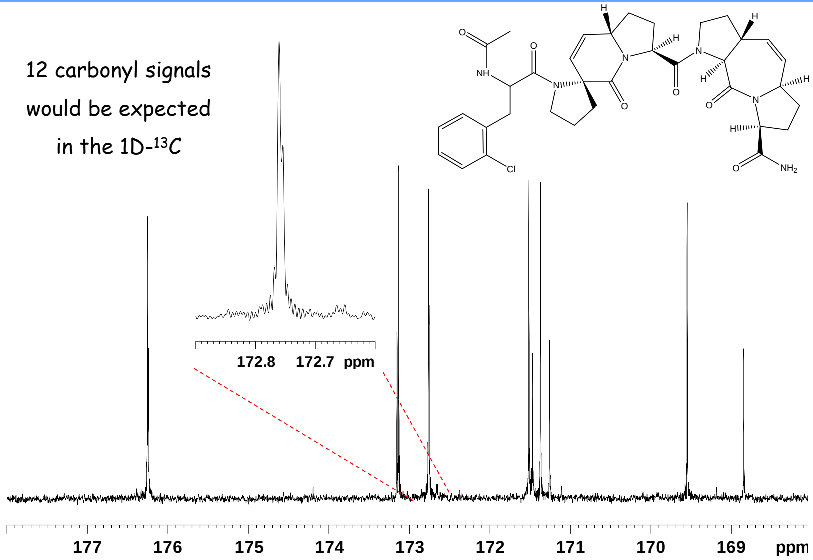


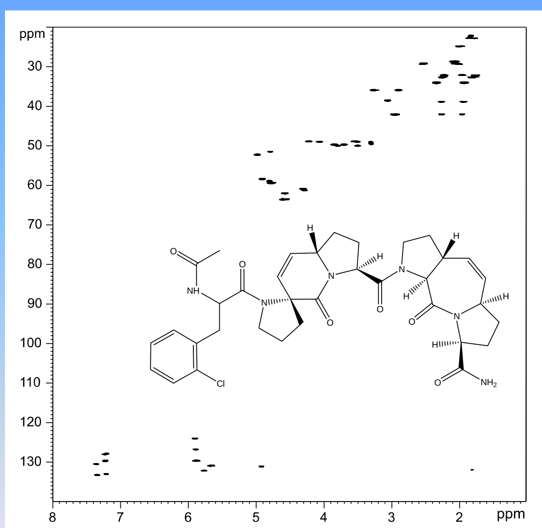
"long" spin systems in combination with overlap make an assignment tedious

Spectra in d_4-MeOH: two sets of signals



12 carbonyl signals
would be expected
in the 1D-^{13}C





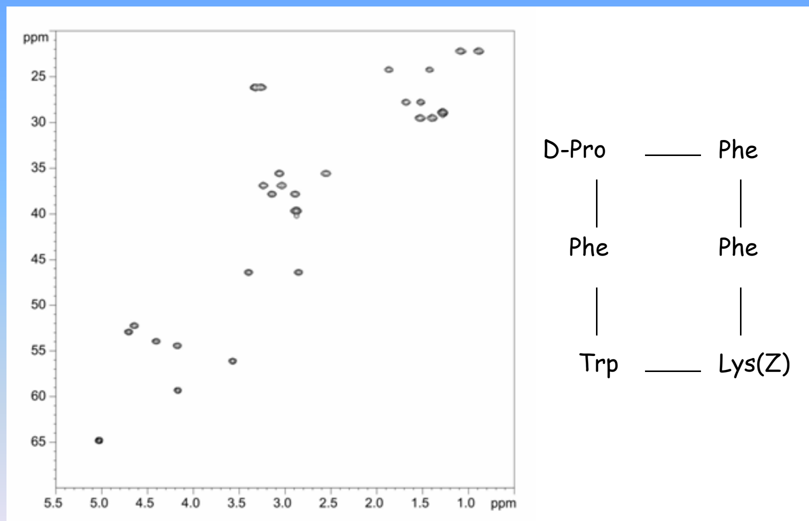
Assignment strategy:

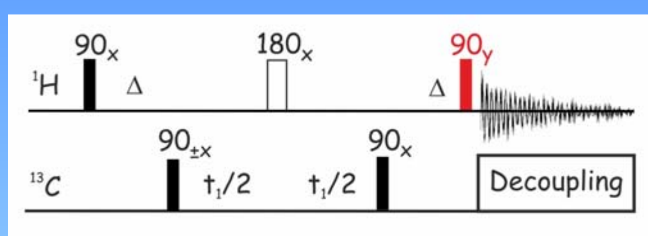
High-resolution, heteronuclear 2D spectra

HMQC or HSQC with COSY transfer

HMBC

F3-008, a peptide to play: *cyc*-(dP-F-F-K(Z)-W-F)





Introducing a COSY step in an HMQC is not difficult. Coupling is evolving during 2Δ and the carbon evolution time. A simple 90° pulse at the end will transfer magnetization based on scalar coupling:

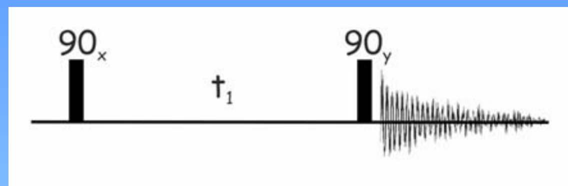
$$H_{1y} \xrightarrow{2\Delta+t_1} H_{1y}\cos\delta_c t_1 \cos\pi J(2\Delta+t_1) - H_{1x}H_{2z}\cos\delta_c t_1 \sin\pi J(2\Delta+t_1)$$

$$\xrightarrow{90_y} H_{1y}\cos\delta_c t_1 \cos\pi J(2\Delta+t_1) + H_{1z}H_{2x}\cos\delta_c t_1 \sin\pi J(2\Delta+t_1)$$

$$\xrightarrow{t_2}$$

$$H_{1y}\cos\delta_{c1}t_1 \cos\pi J(2\Delta+t_1)\cos\delta_{H1}t_2 \cos\pi J t_2 + H_{2y}\cos\delta_{c1}t_1 \sin\pi J(2\Delta+t_1)\cos\delta_{H2}t_2 \sin\pi J t_2$$

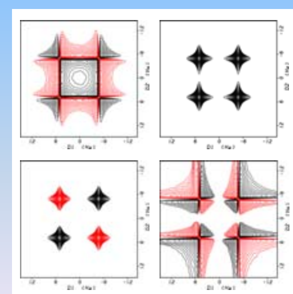
L. Lerner, A. Bax *J. Magn. Reson.* **69**,375 -380 (1986)

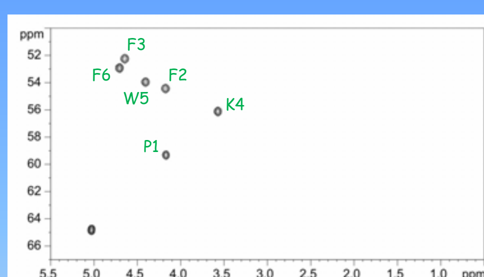


$- H_{1y} \cos 2\pi\delta_{H1}t_1 \cos \pi J_{HH}t_1 \cos 2\pi\delta_{H1}t_2 \cos \pi J_{HH}t_2$

$- H_{2y} \cos 2\pi\delta_{H1}t_1 \sin \pi J_{HH}t_1 \cos 2\pi\delta_{H2}t_2 \sin \pi J_{HH}t_2$

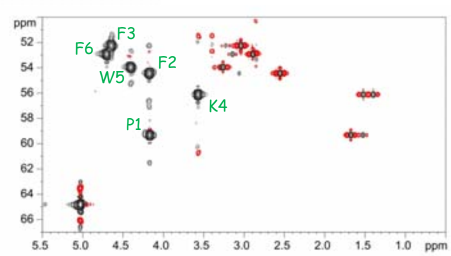
But there is the well known problem of this simple COSY-scheme: the diagonal peaks and the cross peaks are 90° out of phase. That's why usually a magnitude calculation is performed.

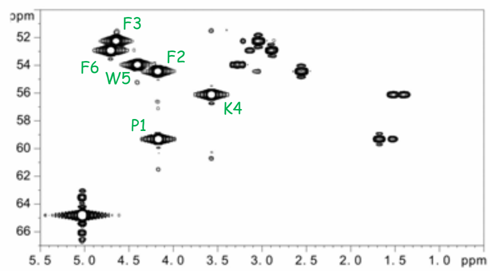




HMQC of F3-008

HMQC-COSY of F3-008

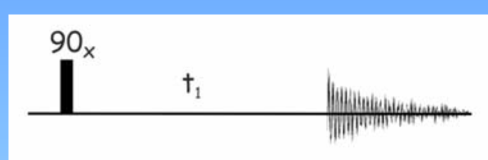




HMQC-COSY of F3-008 after a magnitude calculation in F2

The magnitude calculation broadens the lines and should be avoided. In the COSY the DQF-COSY or the P.COSY were introduced to avoid a magnitude calculation. The DQF-COSY solves the problem by removing the in-phase terms but at the cost of sensitivity.

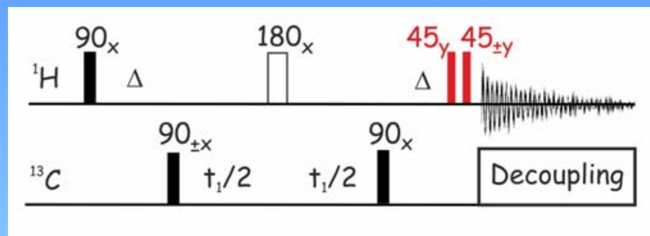
In the P.COSY a second two-dimensional spectrum is subtracted to alleviate this problem



It is recorded without the mixing pulse and will thus only contain a diagonal. To avoid a waste of spectrometer time it can be created from a one-dimensional spectrum recorded in the right way by shifting time points to create a 2D-FID.

We can use the same trick and create a spectrum that contains the diagonal. It can, however, not be created from a 1D spectrum but by subtracting another 2D without a pulse.

L. Mueller *J. Magn. Reson.* **72**, 191-196 (1987)
D. Marion, A. Bax *J. Magn. Reson.* **80**, 528-533 (1988)



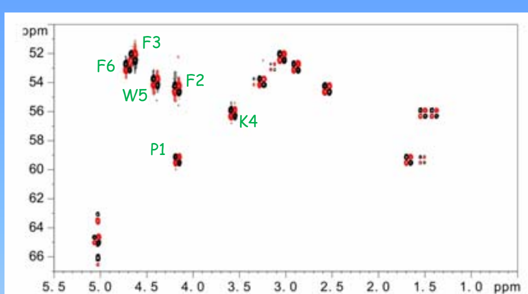
$$H_{1y} \xrightarrow{2\Delta+t_1} H_{1y}\cos\delta_c t_1 \cos\pi J(2\Delta+t_1) - H_{1x}H_{2z}\cos\delta_c t_1 \sin\pi J(2\Delta+t_1)$$

$$\xrightarrow{90_y} H_{1y}\cos\delta_c t_1 \cos\pi J(2\Delta+t_1) + H_{1z}H_{2x}\cos\delta_c t_1 \sin\pi J(2\Delta+t_1)$$

$$\xrightarrow{0_y} H_{1y}\cos\delta_c t_1 \cos\pi J(2\Delta+t_1) - H_{1x}H_{2z}\cos\delta_c t_1 \sin\pi J(2\Delta+t_1)$$

$$\text{Difference} = H_{1x}H_{2z}\cos\delta_c t_1 \sin\pi J(2\Delta+t_1) + H_{1z}H_{2x}\cos\delta_c t_1 \sin\pi J(2\Delta+t_1)$$

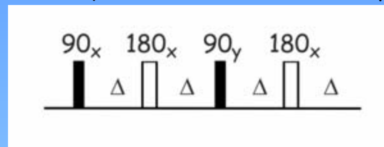
$$H_{1y}\cos\delta_{c1} t_1 \sin\pi J(2\Delta+t_1)\cos\delta_{H1} t_2 \sin\pi J t_2 + H_{2y}\cos\delta_{c1} t_1 \sin\pi J(2\Delta+t_1)\cos\delta_{H2} t_2 \sin\pi J t_2$$



HMQC-COSY of F3-008 subtraction of the "diagonal peaks"

The signals get sharper, the fine structure can be analyzed but there is the potential problem of cancellation. And weak peaks (due to small coupling constants) are hardly visible. It would be better to have the peaks with the coupling in phase.

This leads to the sequence that is called "the perfect echo"



Let us assume we have to protons H_1 and H_2 exhibiting a J-coupling

$$I_{1z} \xrightarrow{90_x} -I_{1y} \xrightarrow{\pi J I_{1z} I_{2z} 2\Delta} -I_{1y}\cos\pi J2\Delta + 2I_{1x}I_{2z}\sin\pi J2\Delta$$

$$\xrightarrow{90_y} -I_{1y}\cos\pi J2\Delta - 2I_{1z}I_{2x}\sin\pi J2\Delta \xrightarrow{\pi J I_{1z} I_{2z} 2\Delta}$$

$$-I_{1y}\cos\pi J2\Delta\cos\pi J2\Delta + 2I_{1x}I_{2z}\cos\pi J2\Delta\sin\pi J2\Delta$$

$$-2I_{1z}I_{2x}\sin\pi J2\Delta\cos\pi J2\Delta - I_{2y}\sin\pi J2\Delta\sin\pi J2\Delta$$

We get the same for the other proton H_2

$$-I_{2y}\cos\pi J2\Delta\cos\pi J2\Delta + 2I_{2x}I_{1z}\cos\pi J2\Delta\sin\pi J2\Delta$$

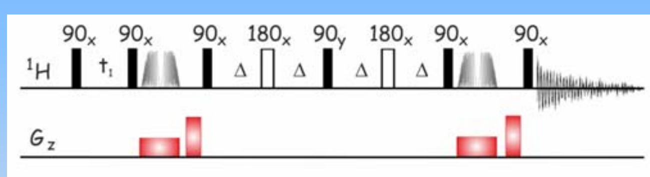
$$-2I_{2z}I_{1x}\sin\pi J2\Delta\cos\pi J2\Delta - I_{1y}\sin\pi J2\Delta\sin\pi J2\Delta$$

If we add all those terms up we get

$$-I_{1y} \; -I_{2y}$$

independent of J or Δ and it works also for more protons in the coupling network.

This leads to the idea of the "clean in-phase" or CLIP-COSY



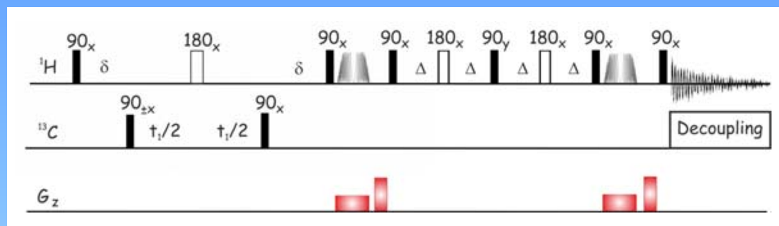
If we repeat the calculation keeping in mind the origin of the magnetization we get

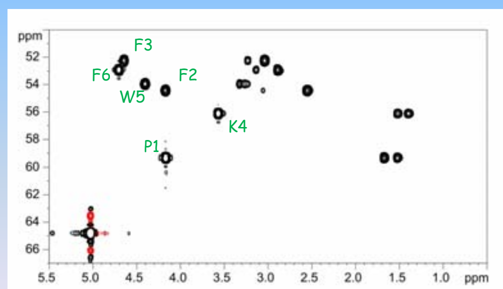
$$^{①}I_{1y} + {}^{②}I_{2y} \longrightarrow -{}^{①}I_{1y}\cos^2\pi J\Delta/2 - {}^{①}I_{2y}\sin^2\pi J\Delta/2 - {}^{②}I_{2y}\cos^2\pi J\Delta/2 - {}^{②}I_{1y}\sin^2\pi J\Delta/2$$

and for Δ = 1/2J we get equal intensity for all four terms

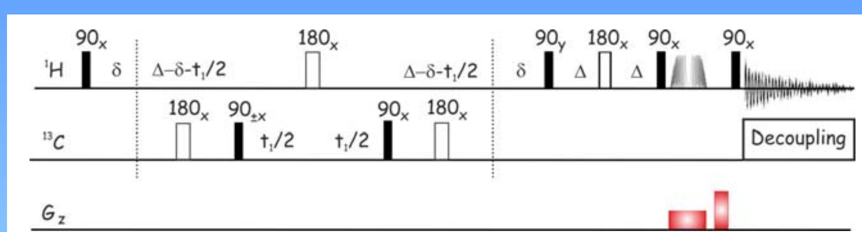
M.R.M. Koos et al. *Angew. Chem. Int. Ed.* **55**, 7655 -7659 (2016)

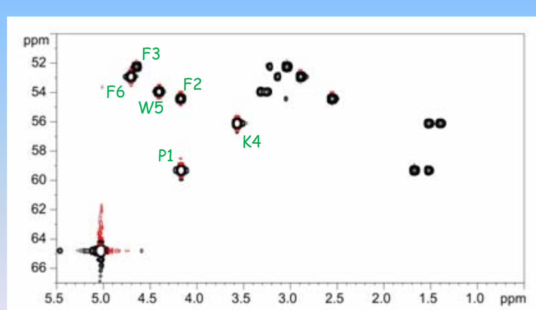
We can fuse that to the HMQC-Sequence





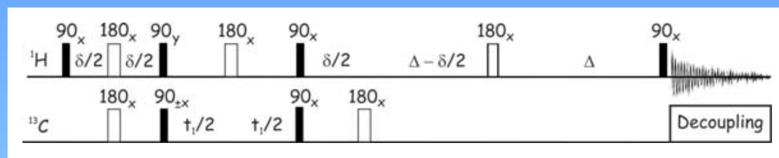
It works and the smaller peaks are more prominent as well. But we can make the sequence shorter and avoid proton-coupling during t_1



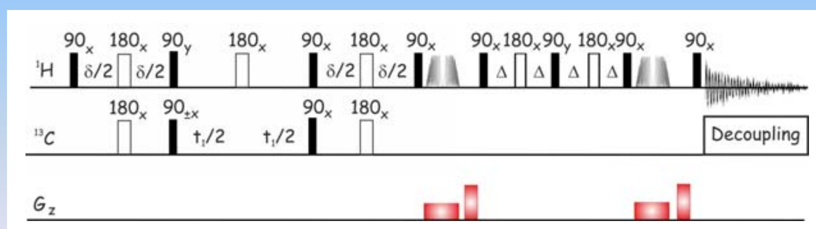


We can do that by converting the sequence to constant time, since the HMQC is an echo as well. But that limits the maximum number of t_1-increments

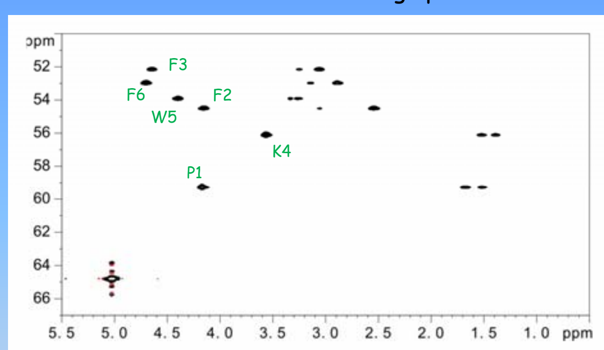
So to get maximum resolution in t_1 we need to use the HSQC-Sequence



The simple HSQC-COSY has all the problems the HMQC-COSY has as well, so we can fuse the HSQC with the CLIP-COSY

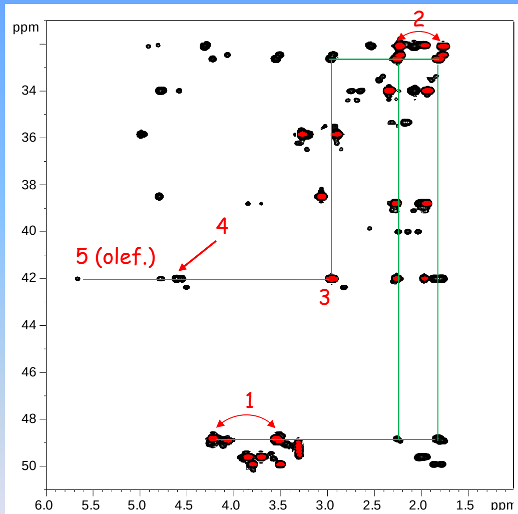


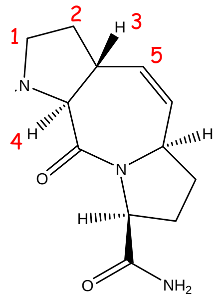
This leads to the following spectrum



There is one "caveat": The fact that COSY yields only correlation to the next neighbor results from the cancellation of smaller couplings in anti-phase-peaks. Now we have in-phase and might get signals for ^{4}J scalar couplings, i.e. not only the next neighbor.

Application to the ProMs made the assignment more straightforward





HSQC
HSQC-CLIP-COSY

acknowledgement

FMP
Monika Beerbaum
AG Kühne
Kathi Francke

Universität Köln
AG Schmalz

Bruker
W. Bermel
R. Kerssebaum