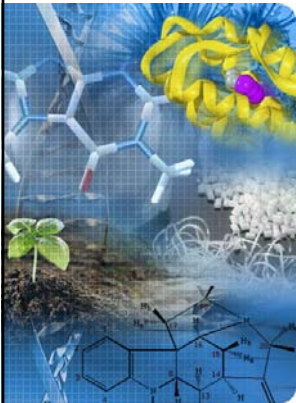


BRUKER



Structural elucidation in natural product research

Dr. Till Kühn

Bruker BioSpin

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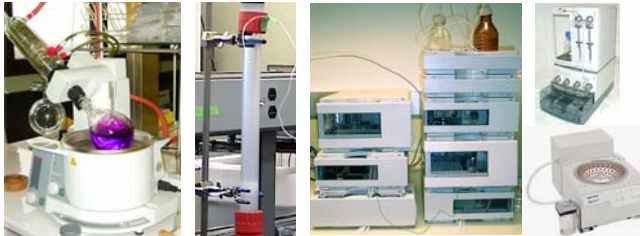
NMR in natural product research

Extract

Flash chromatography

HPLC

Concentration



SPE

Evaporation

Fate of a plant / animal / cell in NP research

⇒ a fraction of mg of a (hopefully) pure compound

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High Res. MS, Elemental Analysis (MW and DBE)

NMR Standard parameter-set:
rpar ...
PROTON

¹H NMR
(¹H shifts + integrals: some functional groups)

¹³C NMR, DEPT
(¹³C shifts, multiplicity: some functional groups and features)

(DQF)COSY + (ed)HSQC
(fragments, ¹³C multiplicity)

HMBC + TOCSY (+ HSQC-TOCSY + H2BC)
(connecting the dots, ¹³C info)

ROESY / NOESY
(stereochemistry)

CI3CPD, CI3DEPT135

HSQCEDETGPSI (TS)
INVIEDETGPSISW (XW)
COSYGPSW, COSYDFGPPHSW

HMBCGP (TS)
INV4GPLEPLRNDISW (XW)
MLEVPHSW
HSQC GP + h2bcctet13

ROESYPHSW + roesyph.2
for up to 400MHz
NOESYPHSW
for 500MHz and up

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General setup for all Bruker NMR experiments

- put the sample into magnet
 - Make sure you use the correct filling height:
 - 5mm probe: > 4cm (> 550ul in a 5mm tube)
 - 1.7mm probe: > 2cm (> 30ul in a 1.7mm tube)
 - 1mm probe: > 1cm (> 5ul in a 1mm tube)
 - for 5mm tubes: use depth gauge to position sample tube in spinner

Bruker BioSpin



General setup for all Bruker NMR experiments

- read the desired parameter set: `rpar PARAMETERSET all`
 - possibly adjust desired parameters (`ns`, `td`, `o1p`, `o2p`, `sw`)
- read the probe dependant parameters: `getprosol`
- lock on the solvent: `lock`
- match and tune the probe: `wobb` or `atma`
- shim: `topshim`, `gradshim` or shim manually
- start the experiment: `xaua` or `rga` (except for DQF COSY) and `zg`
- process the data:
 - automatically: `xaup` or
 - manually: 1D: `efp`, `apk`, `abs`
2D: `xfb`, correct the phase and `abs1`, `abs2`

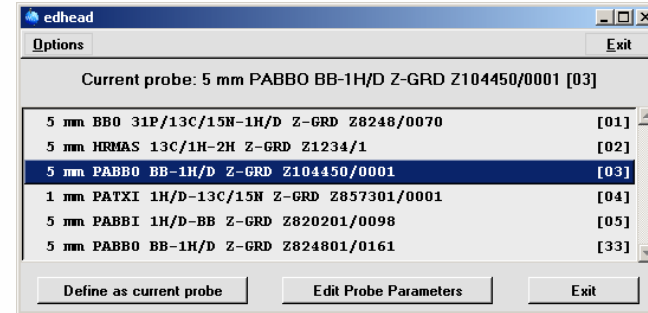
5

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Important-1: define the current probe (edhead)

Type `edhead` after each probe change, or try it out at any time



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Bruker BioSpin



Important-2: have a complete edprosol table!

PROTON Channel, standard hard pulses:

here the probe appears, which is defined as the "current probe" by `edhead`!

⇒ Important: always run `edhead` after a probe change!

Description	Parameter	Value	Action
90 deg. transmitter	P90	7 - 10	determine
cpd	PCPDP	80	calculate
tocsy spin lock	PTOC	0.06	calculate
roesy spin lock	PROE	0.2	calculate
cw irradiation	PLCW	50	calculate

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Important-2: have a complete edprosol table!

CARBON Channel, standard hard pulses:

Description	Parameter	Value	Action
90 deg. transmitter	P90	8 - 18	determine
cpd	PCPDP	80	calculate
tocsy spin lock	PTOC	0.06	calculate
roesy spin lock	PROE	0.2	calculate
cw irradiation	PLCW	120	calculate

8

Important-2: have a complete edprosol table!

CARBON Channel, standard soft pulses (=shaped pulses):

Standard soft pulses for ^{13}C on channel F2 routed to amplifier A1:

Description:	Pulses:	P.Level:	Alignm.:	Wave:	
90 excitation	PSH1	0	120 calc.	0.5	Gaus1.1000
180 adia refocuss	PSH2	2000	calculate calc.	0.5	Crp60comp.4
180 adia inversio	PSH3	500	calculate calc.	0.5	Crp60,0.5,20.1

Standard soft pulses

Important-2: have a complete edprosol table!

Global parameters: Gradients, trim pulses:

Special parameters: values used for all nuclei

Description:

grad. recovery delay	D_grad	0.0002	sec
grad. pulse 1	P_grad1	1000	usec
grad. pulse 2	P_grad2	600	usec
trim pulse mlev	P_mlev	2500	usec
trim pulse hsqc	P_hsqc	0.5	usec

Even setup a list of experiments in IconNMR

Modus	Name	Experimentkommentar
N	PROTON	1H experiment 16 scans
N	C13CPD	C13 exp. comp. pulse dec. 1024 scans
N	C13DEPT45	C13 dept all positive
N	C13DEPT135	C13 dept CH,CH3 pos. CH2 neg.
C	COSYGPDPHWSW	sw opt. COSY with gradients and dq filter (States-TFPI)
C	MLEVPHSW	sw opt. TOCSY (States-TFPI)
C	HSQCDETGTP	sw opt. edited HSQC with gradients (s/a TFPI)
C	HMBGCP	sw opt. HMBC with gradients, low pass 3-filter, no decoupling
C	h2bc	H2BC 2 bond C H correlation
C	ROESYPHWSW	sw opt. ROESY (States-TFPI)
C	COSYGPBWSW	sw opt. COSY with gradients (magn. mode)
C	HSQCETGPM	sw opt. HSQC-TOCSY with gradients (s/a TFPI)
C	HSQCQP	sw opt. HSQC sens. improved with gradients (s/a TFPI)

Dr. Vatcharin's compound: Instrumentation

- ca. **0.6mg** (NMR quantification) HPLC purified compound
- dissolved in **30ul** MeOD
- all experiments in a **1.7mm MicroProbe**
- on a **400MHz** instrument
 - 1D Proton (**PROTON**)
 - 1D ^{13}C (**C13CPD**) and 1D ^{13}C -Dept-90 (**C13DEPT90**)
 - 2D edited-HSQC (**HSQCDETGTP**)
 - 2D COSY (**cosygpSW**) and DQF-COSY (**cosygpDPDPHWSW**)
 - 2D HMBC (**HMBGCP**) and 2D H2BC (**HSQCETGTP** and type: **pulprog h2bcctea13**)
 - 2D ROESY (**ROESYPHWSW**)
- Total experiment time: **less than a weekend**



Advantages of small volume NMR

- High mass-sensitivity (4 x sensitivity = 16 times faster):
 - 1.7mm: ¹H sensitivity = 2 x 5mm BBI, ¹³C sensitivity = 2 x BBO
 - 1mm: ¹H sensitivity = 4 x 5mm BBI
 - 1.7mm MicroCryoProbe: ¹H sens. = 14 x BBI, ¹³C sens. = 4 x BBO
- No problems with solvent impurities (high concentration even with very small sample amounts)
- No problems with solvent suppression (no radiation damping etc.)
- Advantage of working with small sample amounts: cleaner samples!
 - It's often much easier to get **CLEAN** samples in small amounts
 - No danger to overload flash chromatography or HPLC columns
 - You might even use analytical HPLC columns and fraction collect (better separation on with small sample amounts)

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High Res. MS, Elemental Analysis (MW and DBE)

NMR Standard parameter-set:rpar ...
PROTON**¹H NMR**

(1H shifts + integrals: some functional groups)

C13CPD, C13DEPT135

¹³C NMR, DEPT

(13C shifts, multiplicity: some functional groups and features)

HSQCDETGPSI (TS)
INV4DETGPSISW (XW)
COSYGPSW, COSYDFGPPHSW**(DQF)COSY + (ed)HSQC**

(fragments, 13C multiplicity)

HMBCGP (TS)
INV4GPLRLRNSW (XW)
MLEVPHSW
HSQCGP + h2bcctet13**HMBC + TOCSY (+ HSQC-TOCSY + H2BC)**

(connecting the dots, 13C info)

ROESYPHSW + roesyph.2
for up to 400MHz
NOESYPHSW
for 500MHz and up**ROESY / NOESY**

(stereochemistry)

Bruker BioSpin



Dr. Vatcharin's compound: HR-MS: C₂₀H₂₈O₄

- Application of the rule for double-bond equivalents (DBE):

$$C_a H_b O_c N_d \quad DBE = \frac{2a + 2 + d - b}{2} = \frac{2 \cdot 20 + 2 - 28}{2} = 7$$

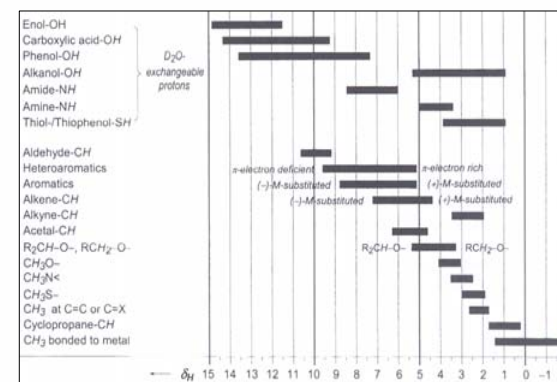
- 7 double bond equivalents (DBE):
 - 1 double bond or 1 ring closure = 1 DBE
 - 1 triple bond = 2 DBE's
- ⇒ 7 double bonds or 6 rings and 1 db or 5 rings and 2 db...

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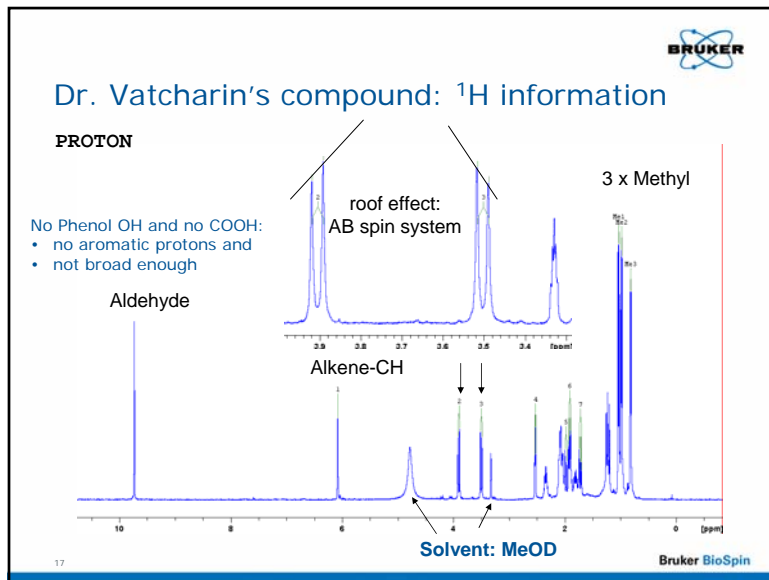


1st. thing in NMR: ¹H shifts + integrals

Source: E. Breitmaier:
Structure Elucidation by NMR in Organic Chemistry, John Wiley & Sons

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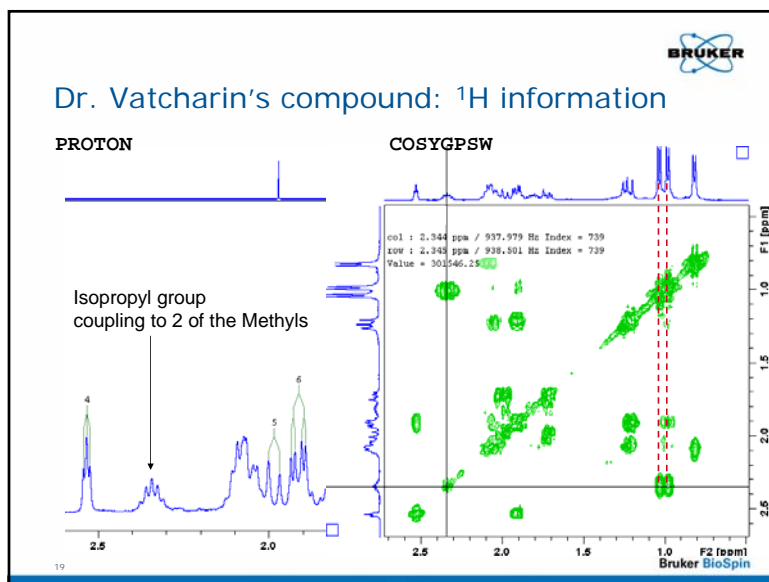


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Setup a list of identified groups

- Molecular formula: C₂₀H₂₈O₄
- Identified groups:
 - Alkene C=C-H 6.08 ppm
 - Aldehyde CHO 9.73 ppm
 - 3 Methyl groups CH₃ 1.04 ppm
CH₃ 0.98 ppm
CH₃ 0.82 ppm
- Additional information:
 - AB spin sytem
 - No aromatics

18 Bruker BioSpin



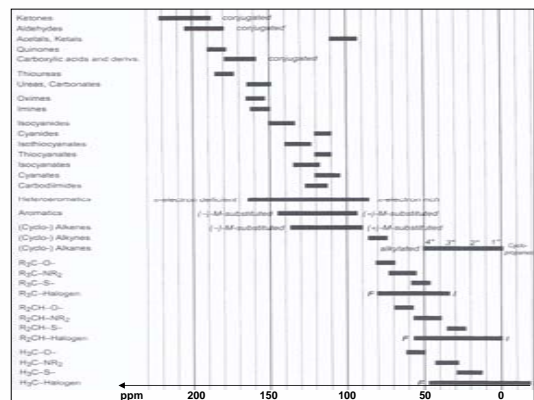
BRUKER

Setup a list of identified groups

- Molecular formula: C₂₀H₂₈O₄
- Identified groups:
 - Alkene C=C-H 6.08 ppm
 - Aldehyde CHO 9.73 ppm
 - 3 Methyl groups CH₃ 1.04 ppm
CH₃ 0.98 ppm
CH₃ 0.82 ppm
- isopropyl group (2.34 ppm) coupling to the first 2 Me's
- Additional information:
 - AB spin sytem
 - No aromatics

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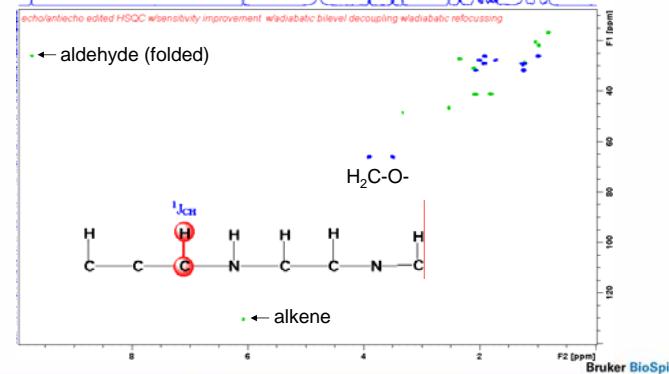
^{13}C information: chemical shift + multiplicity



Source: E. Breitmaier: Structure Elucidation by NMR in Organic Chemistry, John Wiley & Sons

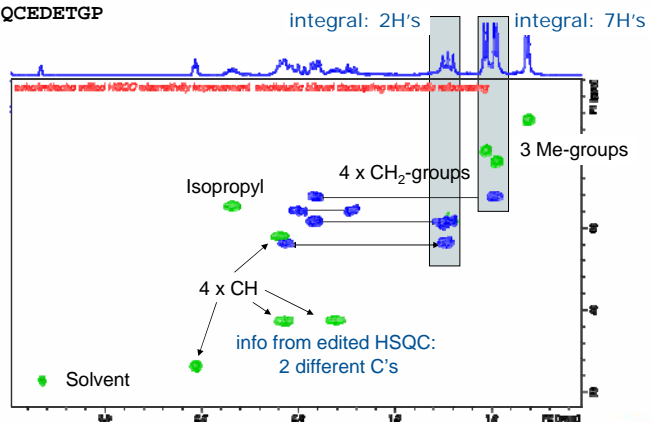
$^1\text{J}_{\text{CH}}$ correlation: multiplicity edited HSQC

HSQCEDETGPD DEPT-135 type info in an inverse experiment



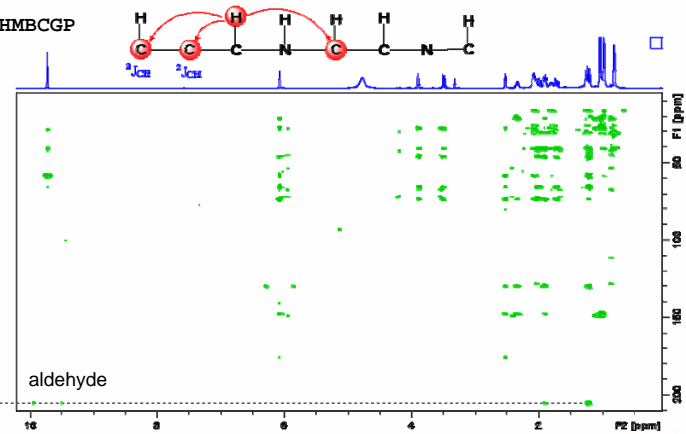
$^1\text{J}_{\text{CH}}$ correlation: multiplicity edited HSQC

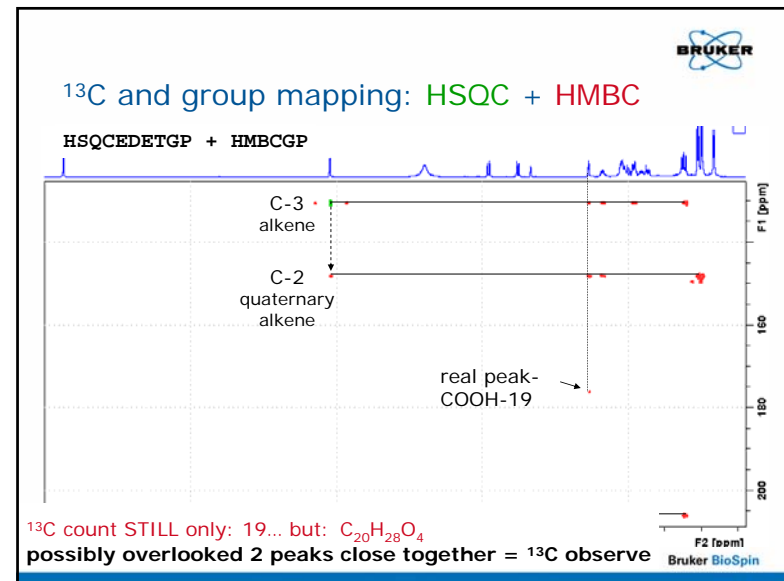
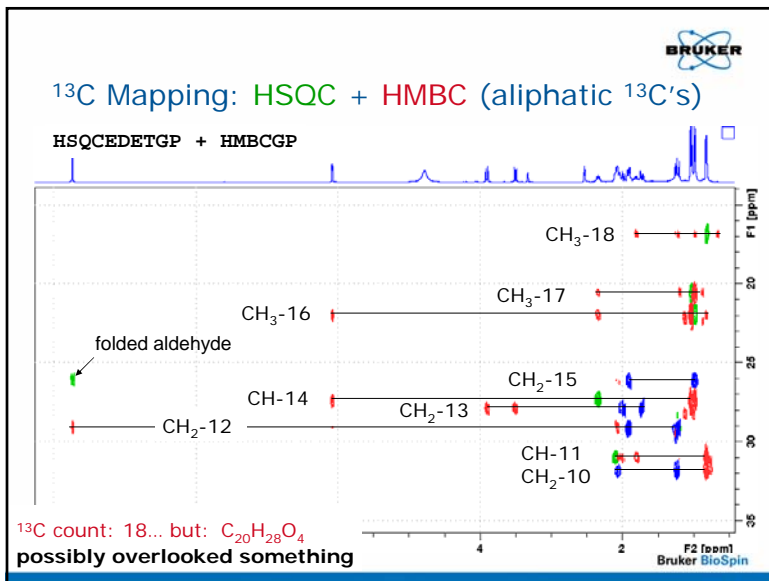
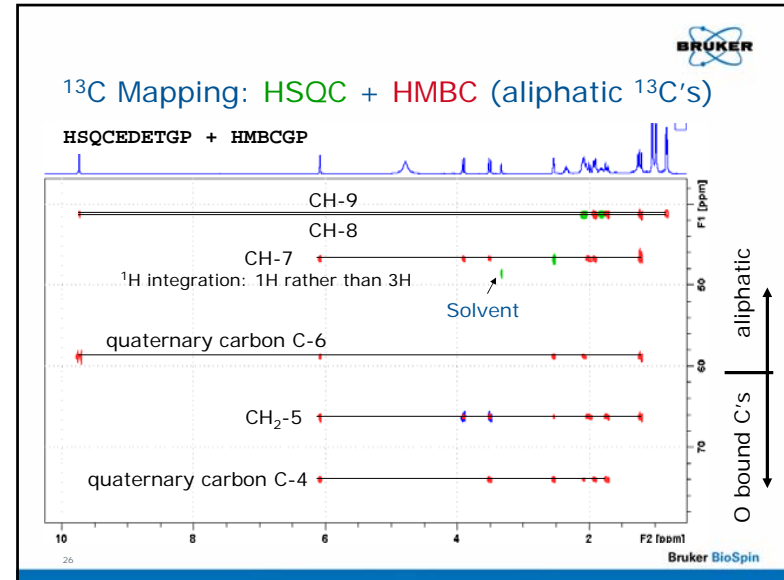
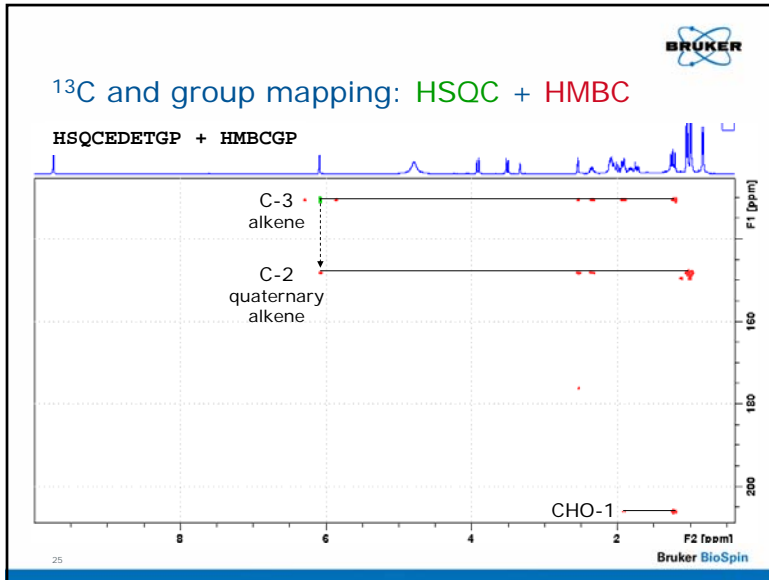
HSQCEDETGPD

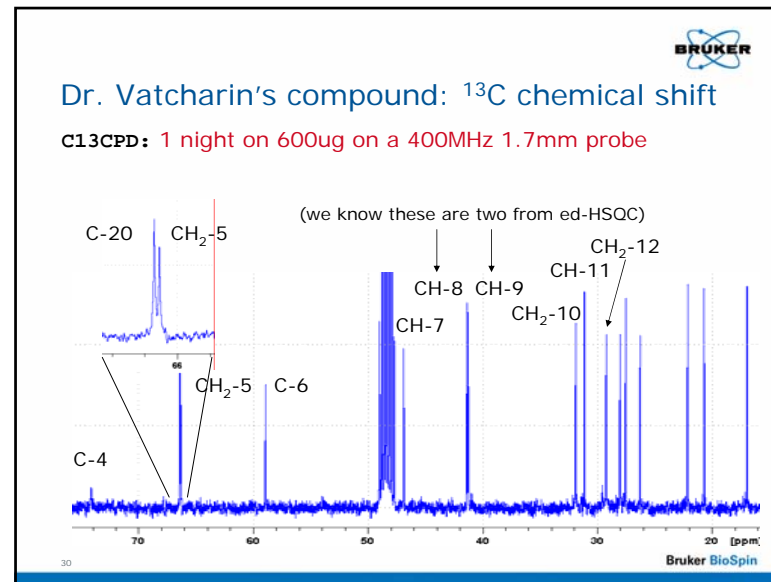
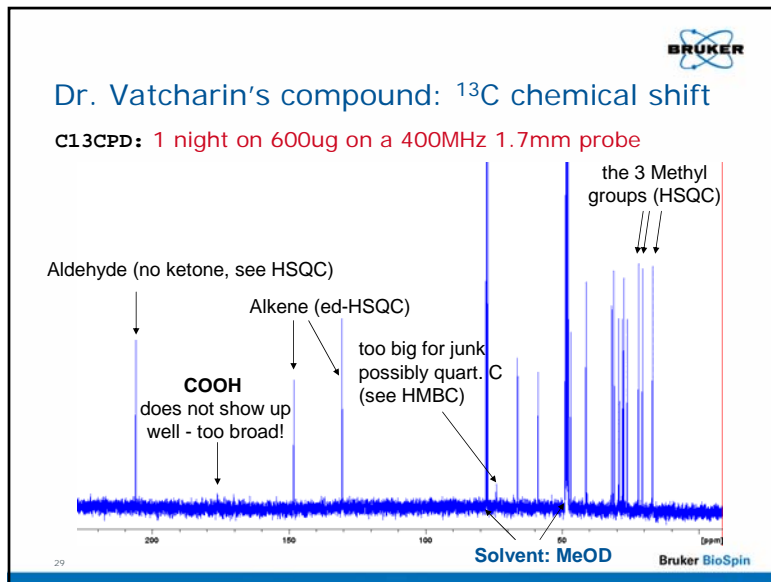


Long range correlation: HMBC

HMBCGP







List of identified groups = $\text{C}_{20}\text{H}_{28}\text{O}_4$

Number (arbitrary)	^{13}C shift	$^{13}\text{C}_q$ Attached ^1H shift	Group type
1	206.0	9.75	-CHO (Aldehyde)
2	148.0	--	Alkene Cq=C
3	130.5	6.08	Alkene C=C-H
4	74.0	--	Cq
5	66.5	3.90, 3.50	-H ₂ C-O (geminal AB)
6	59.0	--	Cq
7	46.9	2.53	CH
8	41.3	2.07	CH
9	41.2	1.81	CH
10	31.8	2.05, 1.24	CH ₂
11	31.1	2.10	CH
12	29.2	1.91, 1.23	CH ₂
13	28.0	2.00, 1.72	CH ₂
14	27.5	2.35	CH (isopropyl)
15	26.2	1.92, 0.98	CH ₂
16	22.1	0.97	CH ₃ (isopropyl)
17	20.6	1.04	CH ₃ (isopropyl)
18	16.9	0.82	CH ₃
19	176.5	--	COOH
20	66.5	--	Cq

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List of identified groups = $\text{C}_{20}\text{H}_{28}\text{O}_4$

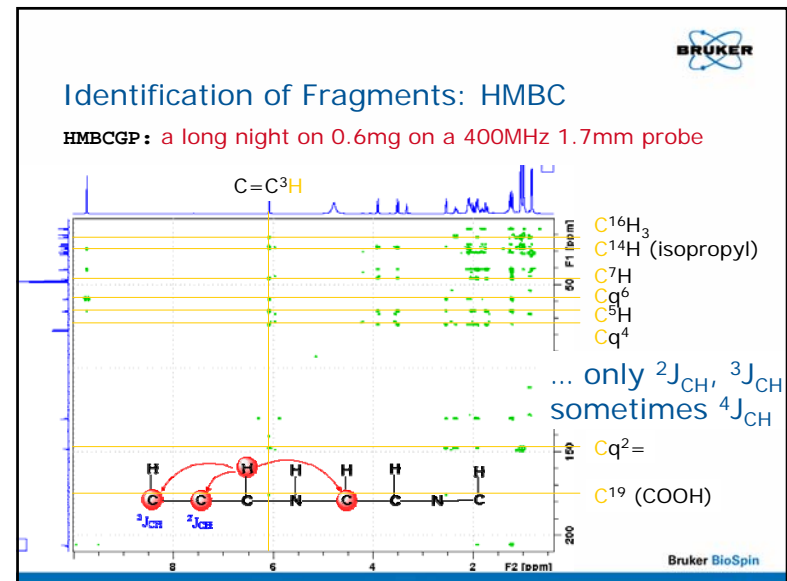
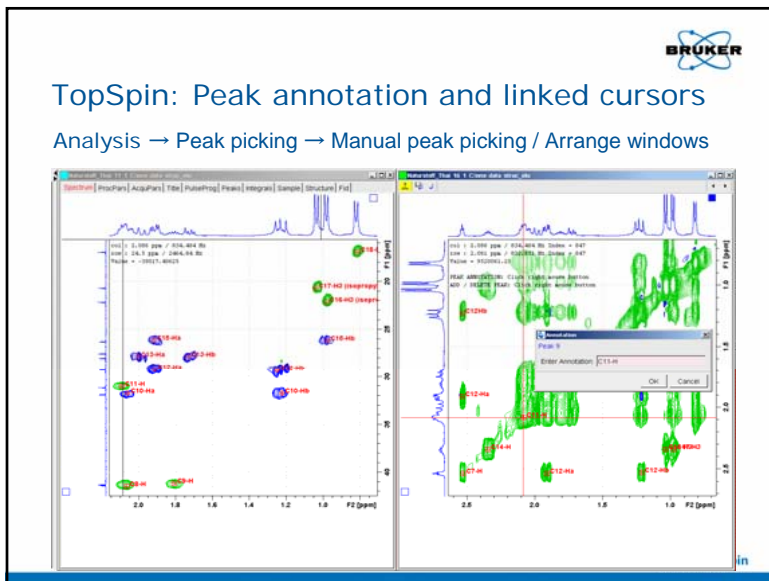
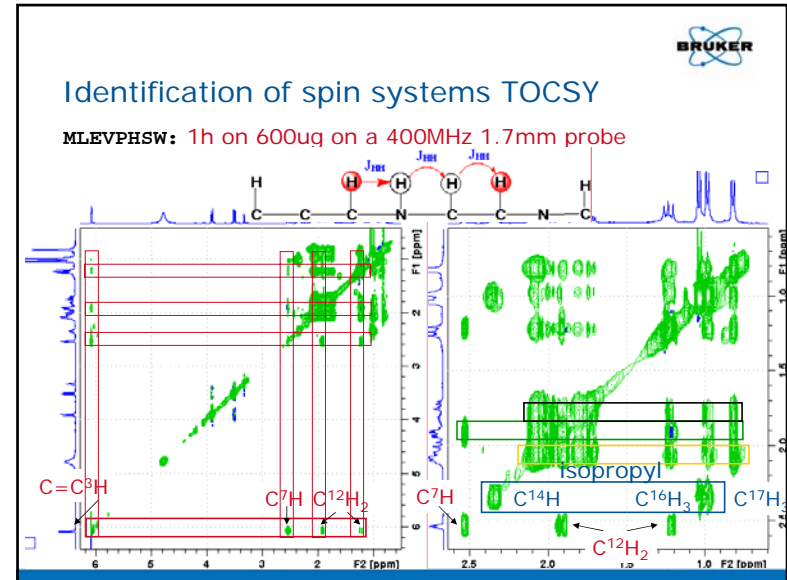
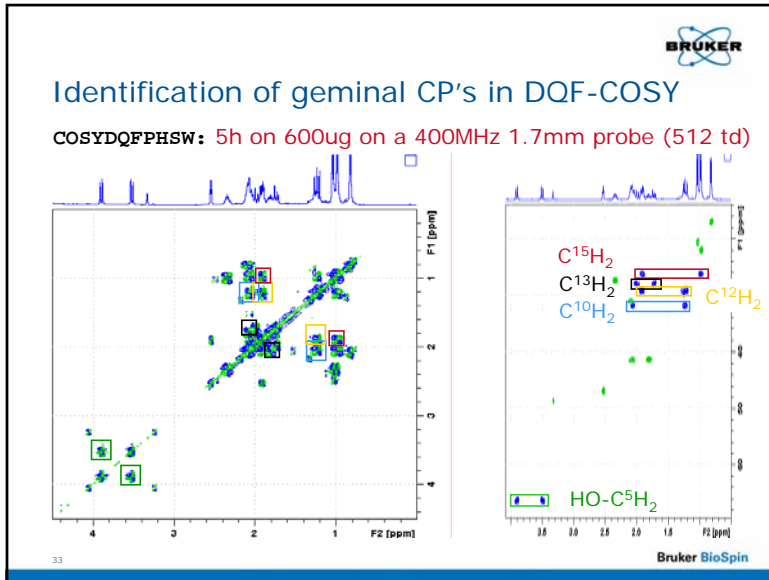
Number (arbitrary)	^{13}C shift	$^{13}\text{C}_q$ Attached ^1H shift	Group type
1	206.0	9.75	-CHO (Aldehyde)
2	148.0	--	Alkene Cq=C
3	130.5	6.08	Alkene C=C-H
4	74.0	--	Cq
5	66.5	3.90, 3.50	-H ₂ C-O (geminal AB)
6	59.0	--	Cq
7	46.9	2.53	CH
8	41.3	2.07	CH
9			CH
10			CH ₂
11			CH
12			CH ₂
13			CH ₂
14			CH (isopropyl)
15			CH ₂
16			CH ₃ (isopropyl)
17	20.6	1.04	CH ₃ (isopropyl)
18	16.9	0.82	CH ₃
19	176.5	--	COOH
20	66.5	--	Cq

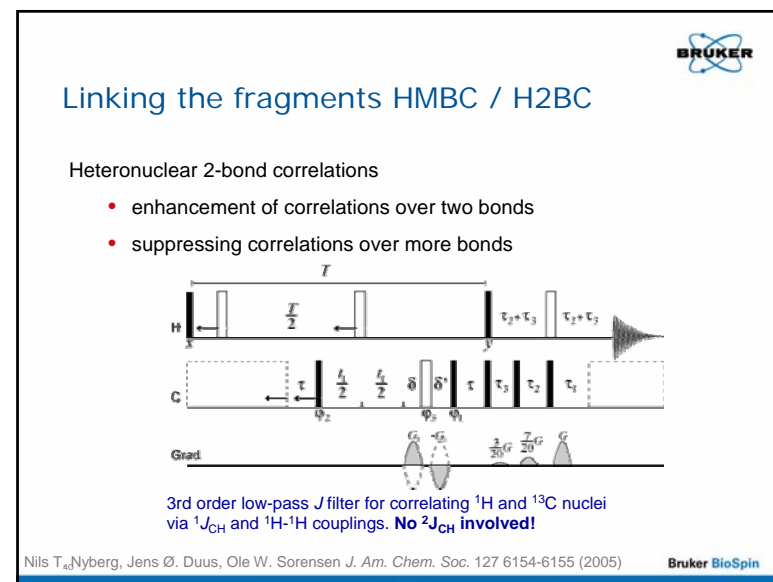
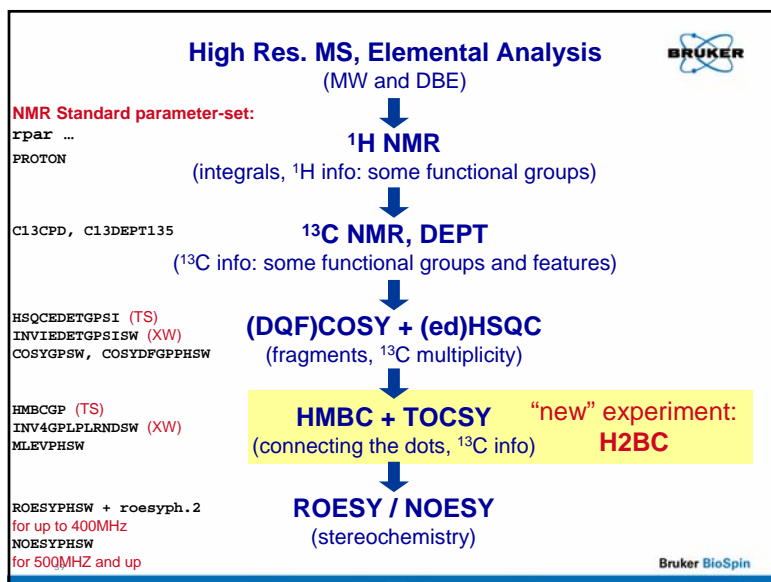
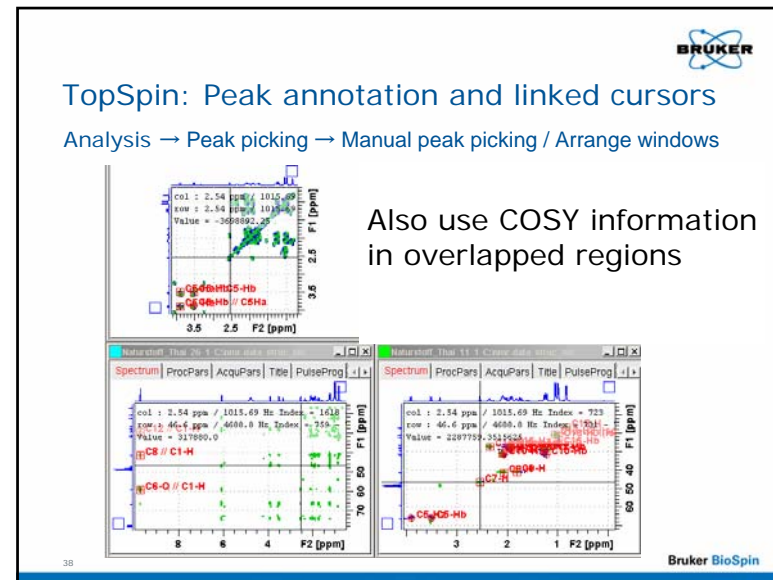
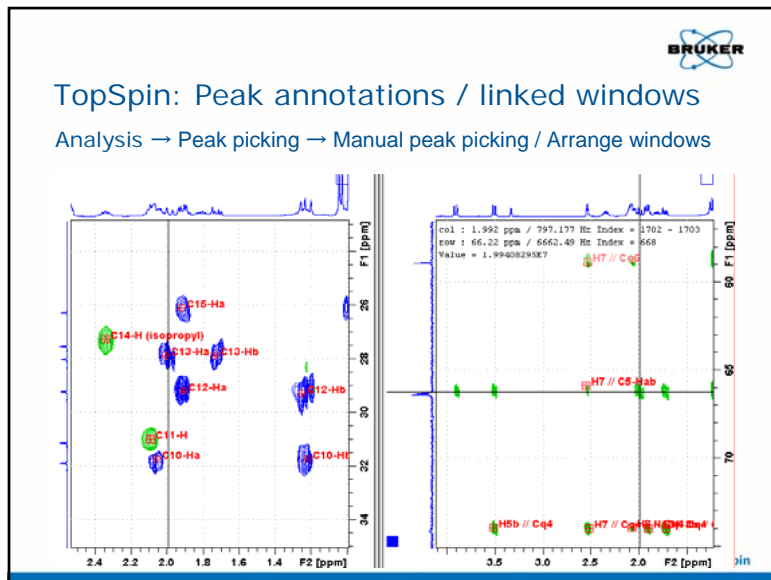
Σ Oxygens = 4, OK!

Σ Protons = 27...

\Rightarrow 1H possibly OH

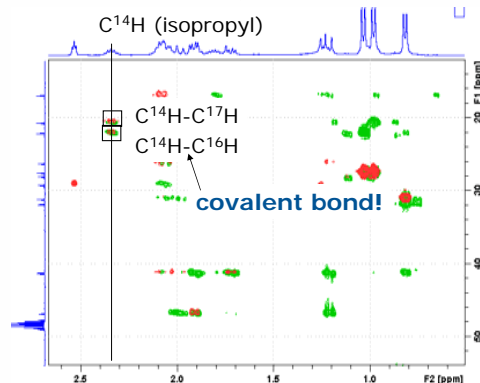
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2-Bond correlation – so what?

HSQC/GP: and change pulseprogram to: h2bcctea13



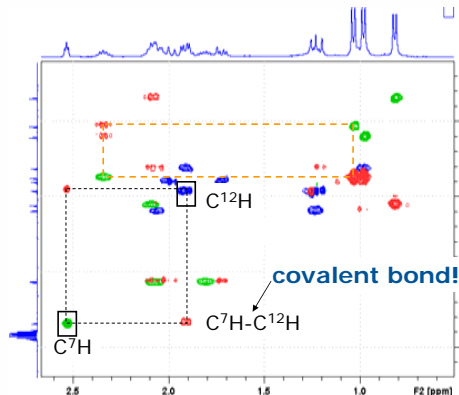
- Overlay **HMBC** / **H2BC**
- Distinguish between 2 bond and 3 bond CP
- Observe 2 bond CP even when they are missing in HMBC (vanishing $^2J_{CH}$)

Niils T_aNyberg, Jens Ø. Duus, Ole W. Sorensen *J. Am. Chem. Soc.* 127 6154-6155 (2005)

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2-Bond correlation – so what?

HSQC/GP: and change pulseprogram to: h2bcctea13

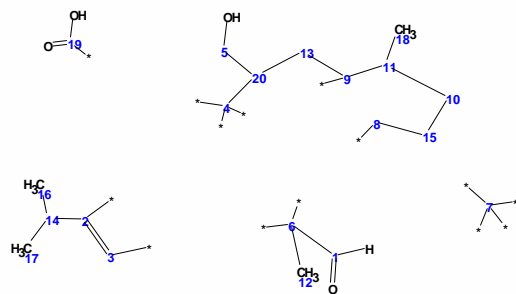


- Step through **HSQC** and **H2BC** overlaid like through a COSY
- Start from HSQC CP and use H2BC CP as relay points
- No correlation to quaternary carbons

Niils T_aNyberg, Jens Ø. Duus, Ole W. Sorensen *J. Am. Chem. Soc.* 127 6154-6155 (2005)

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Some fragments of C₂₀H₂₈O₄



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Putting together the fragments of C₂₀H₂₈O₄

- Application of the rule for double-bond equivalents (DBE):

$$C_a H_b O_c N_d \quad DBE = \frac{2a + 2 + d - b}{2} = \frac{2 \cdot 20 + 2 - 28}{2} = 7$$

- 3 DBE's are used by: CHO, COOH and C=C
- ⇒ 4 ring closures are left for C₂₀H₂₈O₄

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The structure of C₂₀H₂₈O₄:

... after quite some detective work ...

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High Res. MS, Elemental Analysis (MW and DBE)

NMR Standard parameter-set:

rpar ...
PROTON

(integrals, ¹H info: some functional groups)

C13CPD, C13DEPT135

¹³C NMR, DEPT
(¹³C info: some functional groups and features)

HSQCDETGPSI (TS)
INVIEDETGPSISW (XW)
COSYGPSW, COSYDFGPPHSW

(DQF)COSY + (ed)HSQC
(fragments, ¹³C multiplicity)

HMBCGP (TS)
INV4GPLELRNDSW (XW)
MLEVPHSW

HMBC + TOCSY "new" experiment:
(connecting the dots, ¹³C info) **H2BC**

ROESY / NOESY
(stereochemistry)

ROESYPHSW + roesyph.2
for up to 400MHz
NOESYPHSW
for 500MHz and up

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ROESY / NOESY for stereochemistry

NOESY

cross peak intensity with respect to diagonal peak

ROESY

positive
negative

1.0 $\omega_0 \tau_c$

molecule size: ← smaller larger → ← smaller larger →

temperature: ← higher lower → ← higher lower →

ω_0 = spectrometer frequency
 τ_c = rotational correlation time (proportional to molecular size, temperature dependent)

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ROESY spectrum for stereochemistry

ROESYPHSW: 4h on ca. 0.6mg on a 400MHz 1.7mm MicroProbe

the CH₃ group C¹⁸ and Ha on C¹⁰ are on the same side

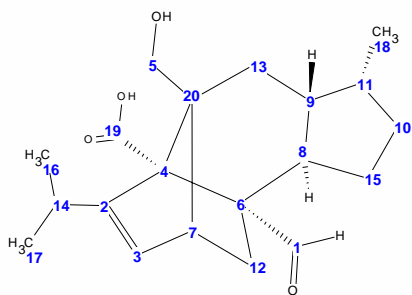
C¹⁸H₃ --- C¹⁰Ha

C¹⁸H₃ --- C¹⁰Ha

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The absolute Stereochemistry of $C_{20}H_{28}O_4$:

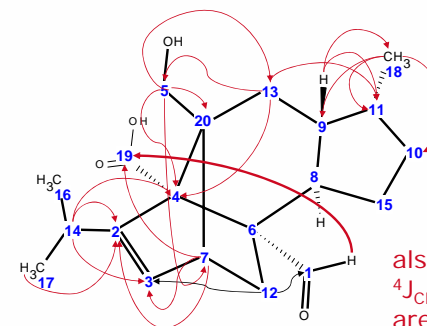


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Some HMBC correlations in $C_{20}H_{28}O_4$:



also ${}^4J_{CH}$ couplings are observable!

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Thank you very much ...

Dr. Vatcharin



Lukas Oberer



Sandra Loss, Matthias Pelzing



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