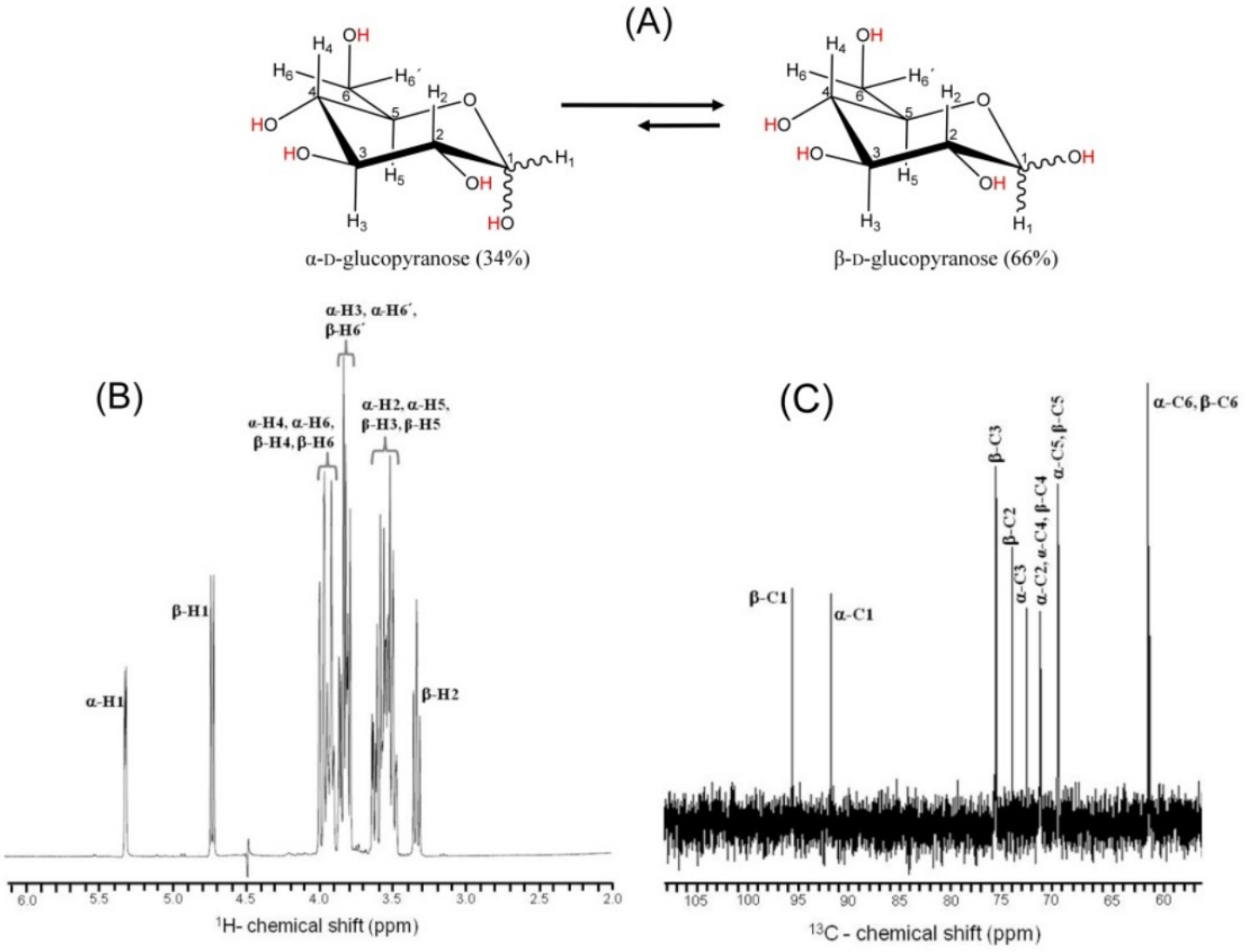
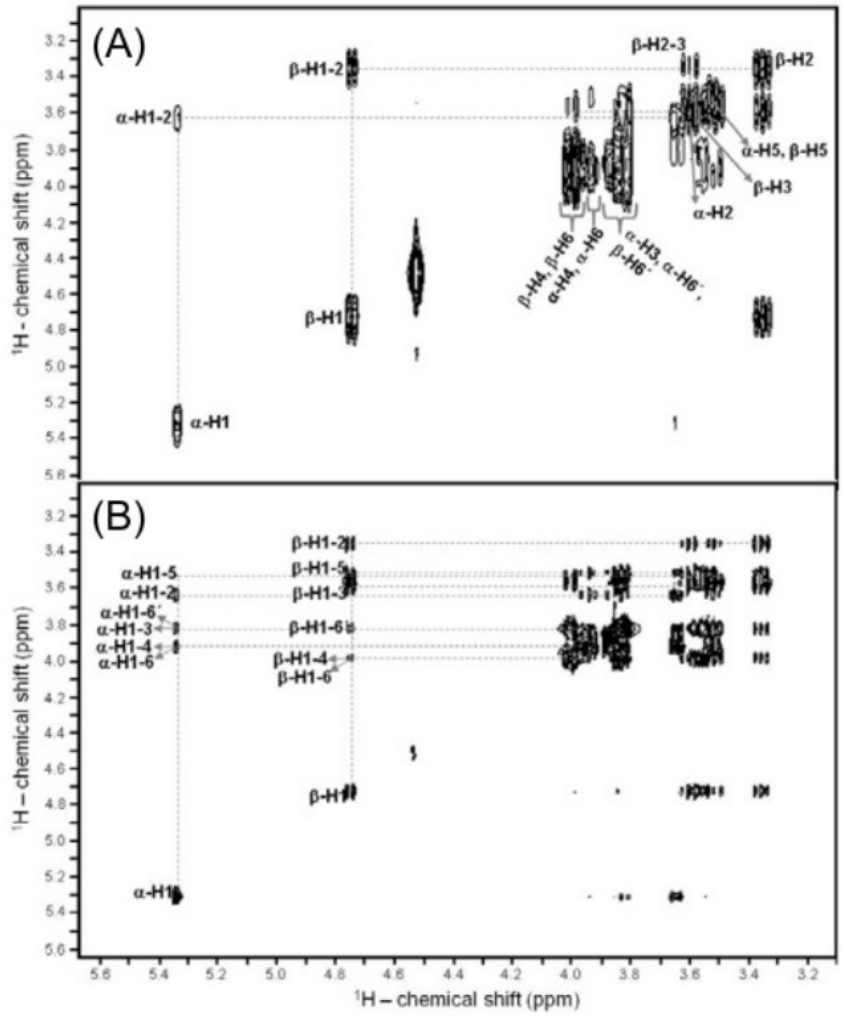
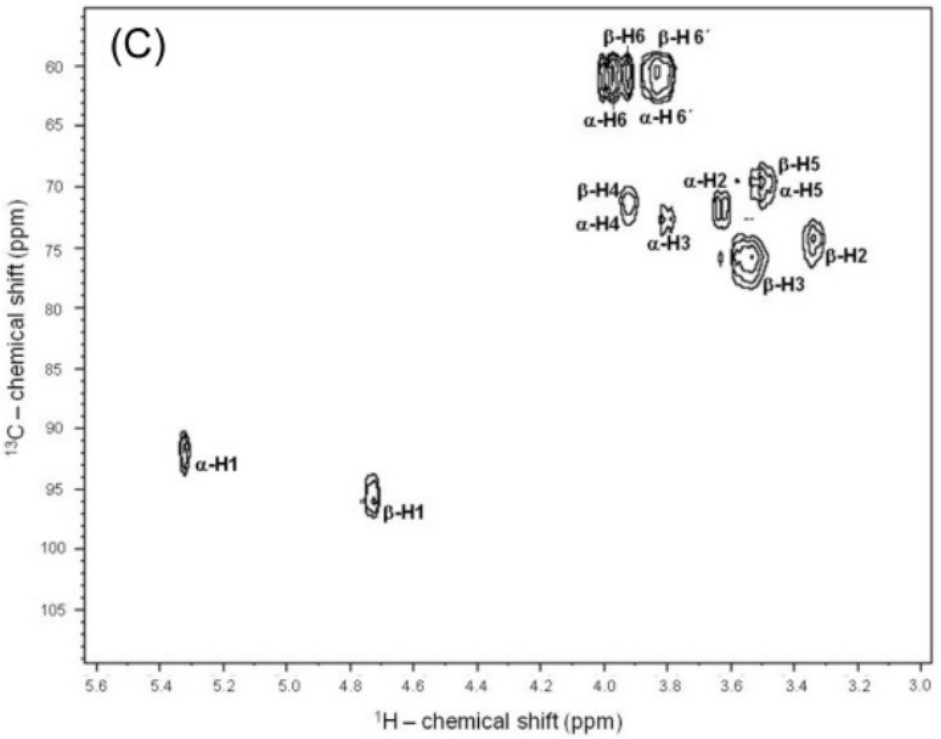
Structure elucidation (1D spectra)



Structure elucidation (2D spectra)





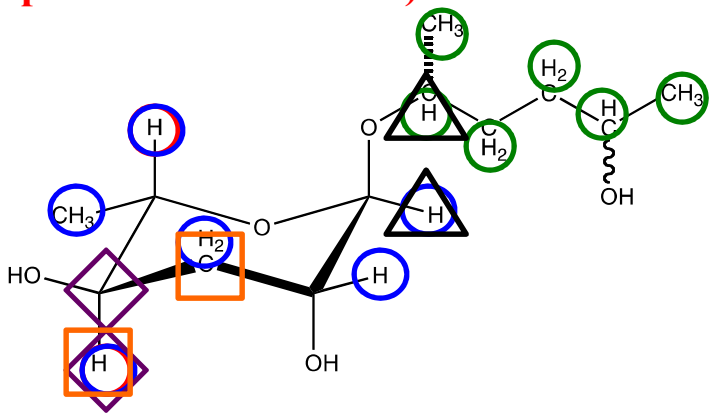
α-D-Glc*p*	β-D-Glc*p*
α-H1 – 5.32	β-H1 – 4.74
α-H2 – 3.63	β-H2 – 3.37
α-H3 – 3.83	β-H3 – 3.60
α-H4 – 3.92	β-H4 – 3.92
α-H5 – 3.50	β-H5 – 3.5
H6/6´ - 3.91/3.82	H6/6´ - 3.91/3.82
α-C1 – 91.4	β-C1 – 95.9
α-C2 – 71.8	β-C2 – 74.1
α-C3 – 72.4	β-C3 – 75.8
α-C4 – 71.2	β-C4 – 71.2
α-C5 – 69.8	β-C5 – 69.8
α-C6 – 60.3	β-C6 – 60.3

Information Content of Common 2D NMR Experiments

COSY (Correlation SpectroscopY): J coupling (generally up to 3 covalent bonds)

TOCSY (Total Correlation SpectroscopY):
J coupling along coupled networks

Blue is one TOCSY network, green is another

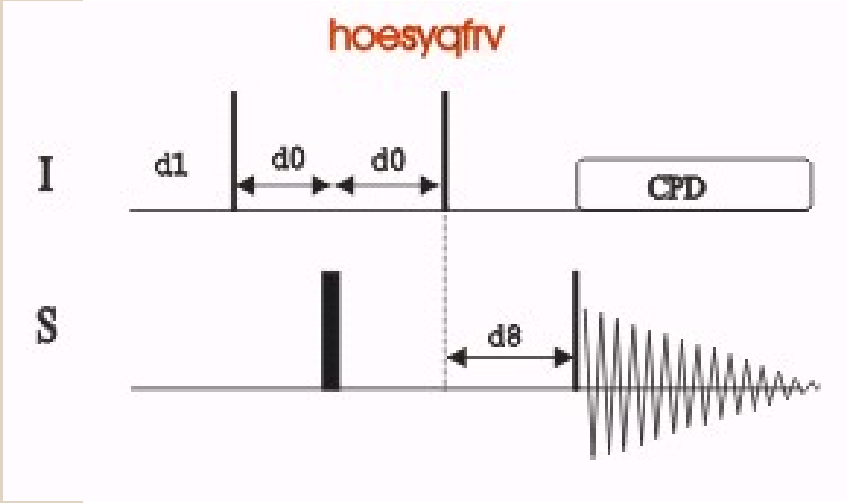


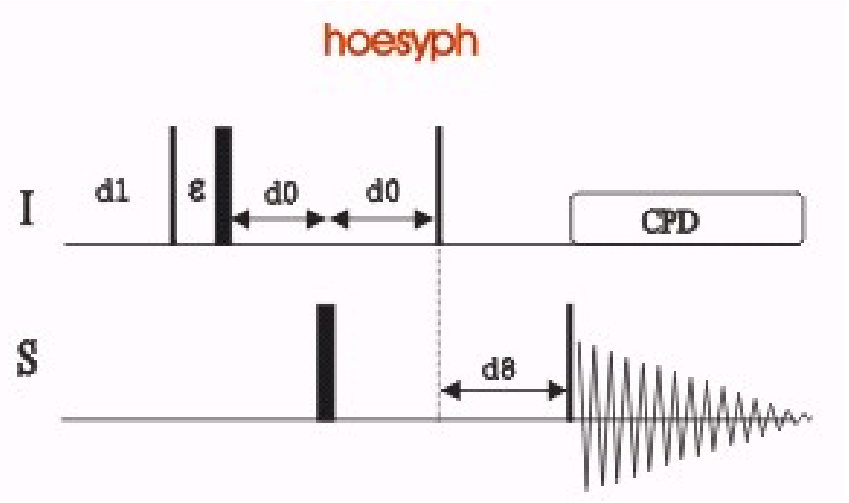
HSQC (Heteronuclear Single Quantum Correlation):
Directly bonded ^{13}C-^{1}H or ^{15}N-^{1}H

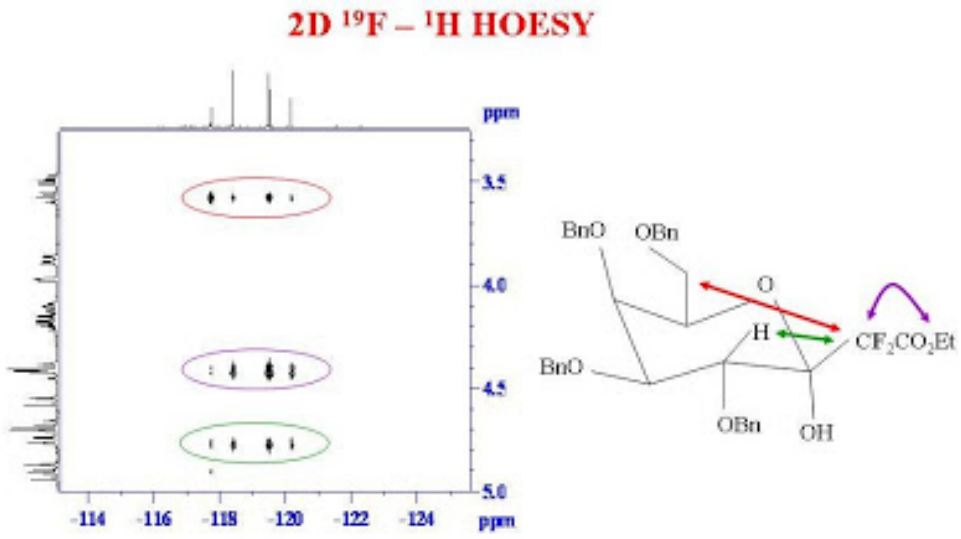
HMBC (Heteronuclear Multiple Bond Correlation):
2 or 3 bond ^{13}C---^{1}H or ^{15}N----^{1}H

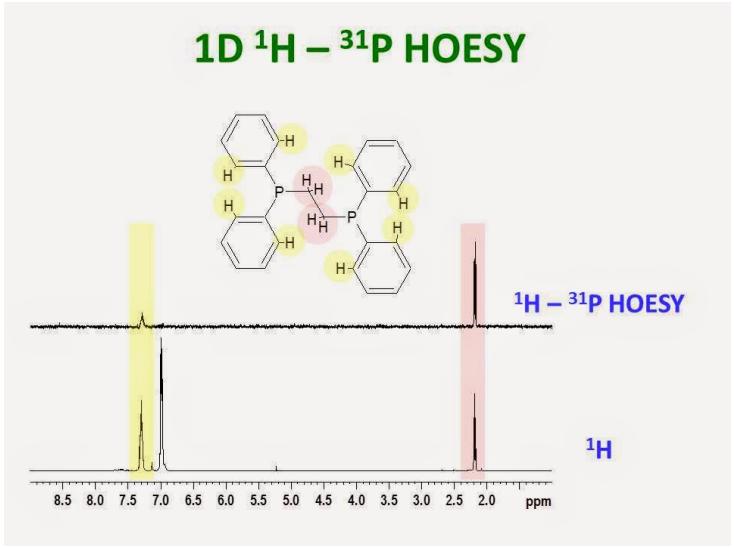
NOESY (Nuclear Overhauser Effect Spectroscopy)
Or ROESY (Rotating Frame Overhauser Effect Spectroscopy):
^{1}H to ^{1}H distances up to 5-6 Å

Heteronuclear Overhauser Enhancement/Effect SpectroscopY (HOESY)

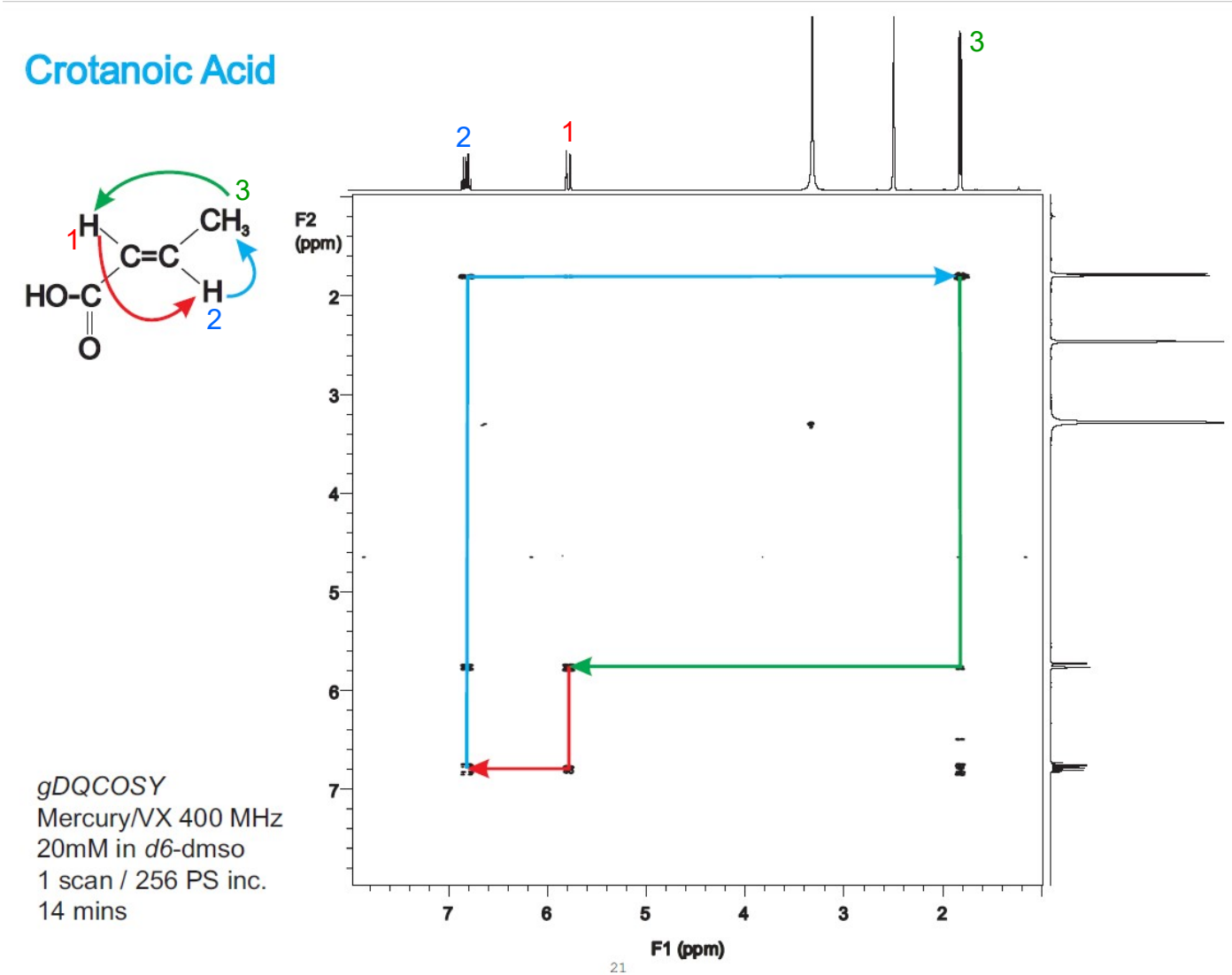








^{1}H assignment of crotanoic acid through COSY

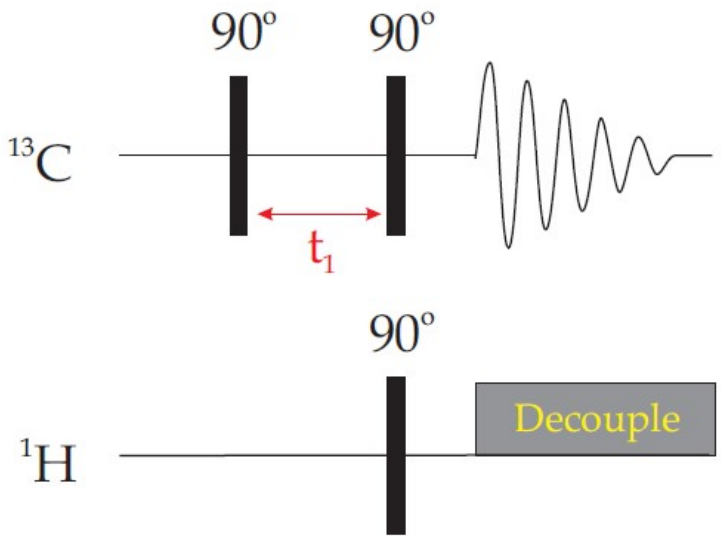


HETeronuclear Shift CORrelation (HETCOR)

Single bond correlations (J-coupling) observed ***directly*** from the low γ nuclei (usually ^{13}C). The directly observed nucleus axis usually has higher resolution, and the ^{1}H axis poorly resolved.

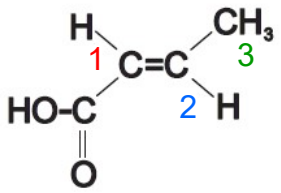
Since observation is on the low γ nucleus, sensitivity is a problem. These type of experiments have been mostly replaced with variants that detect low γ information through the more sensitive ^{1}H (***indirect detection***).

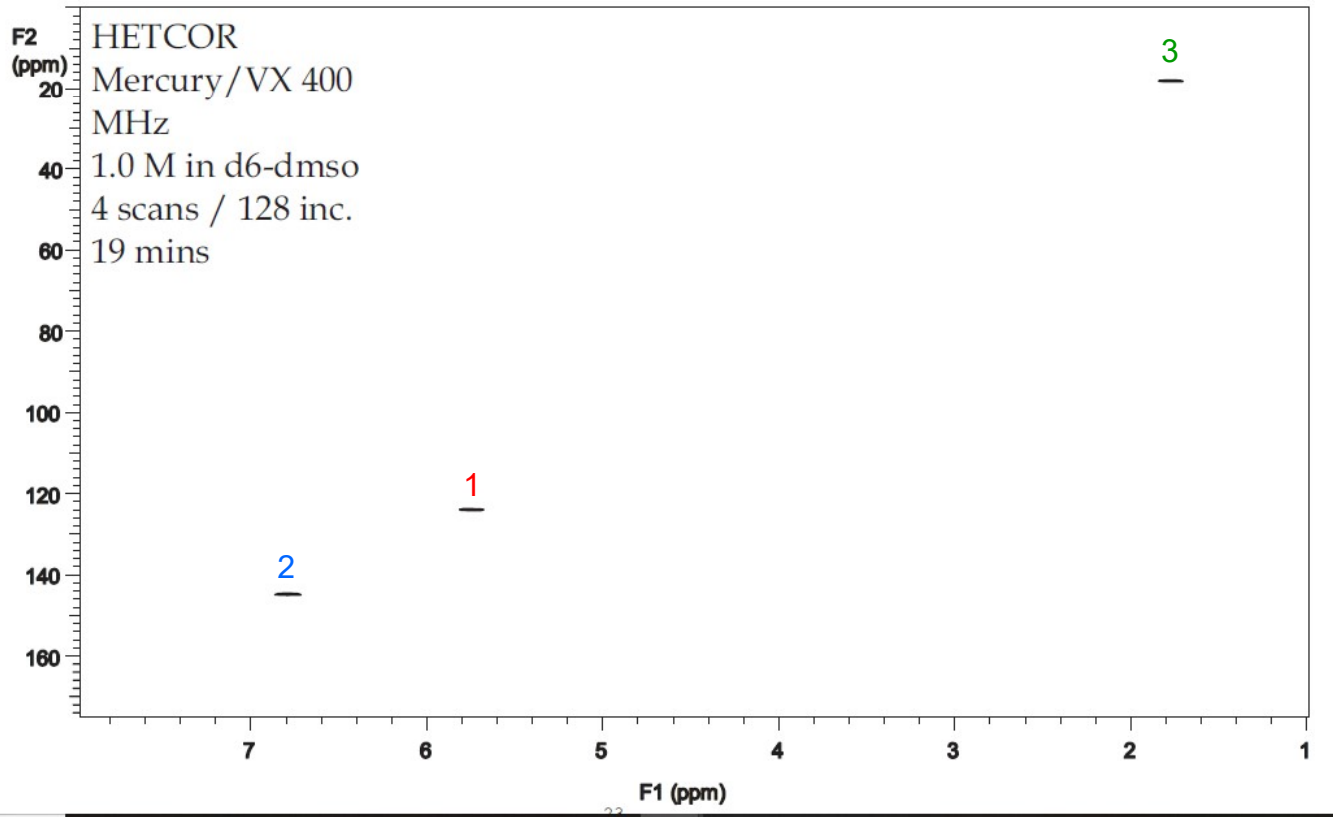
HETCOR:



^{1}H-^{13}C correlation of crotanoic acid by HETCOR

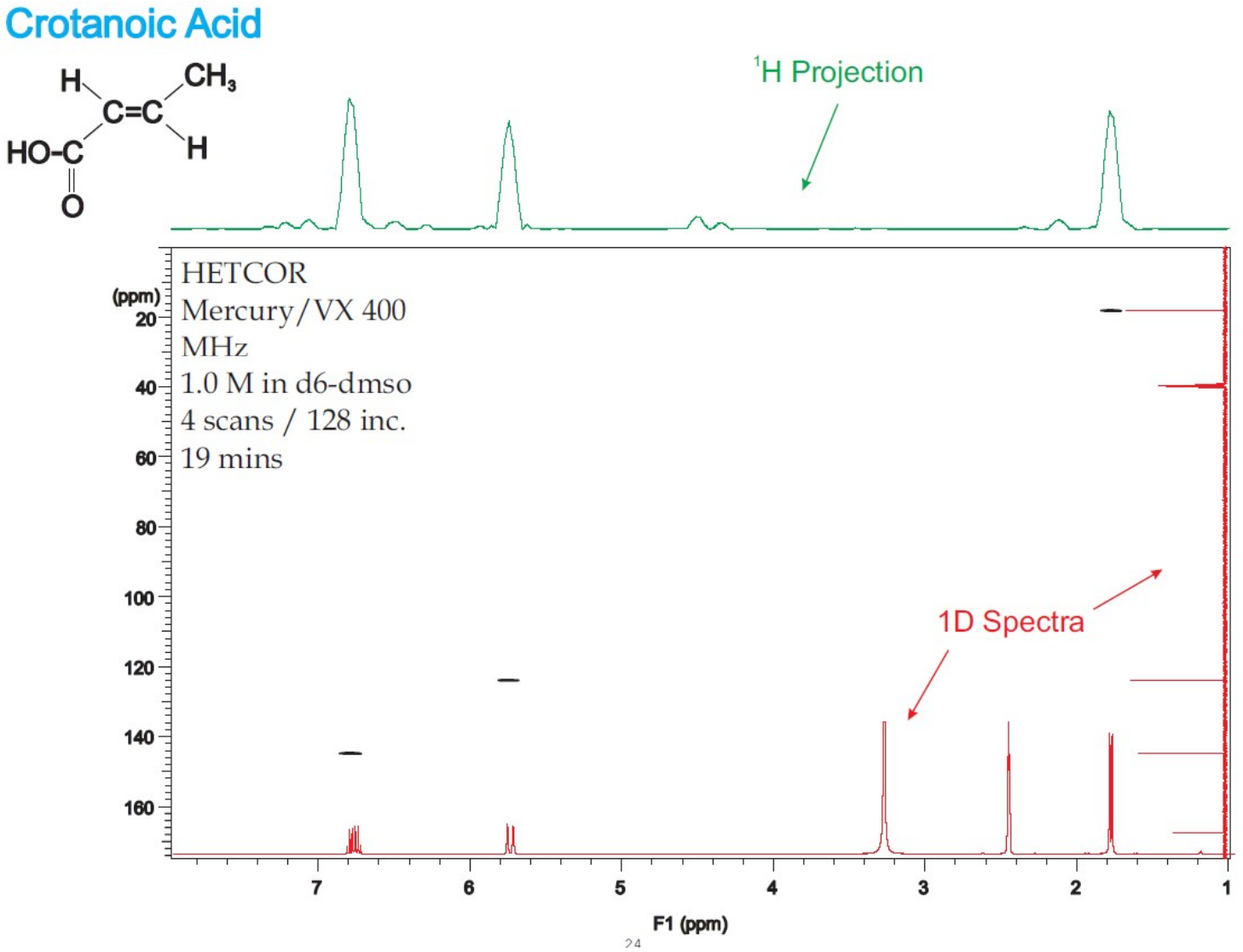
Crotanoic Acid





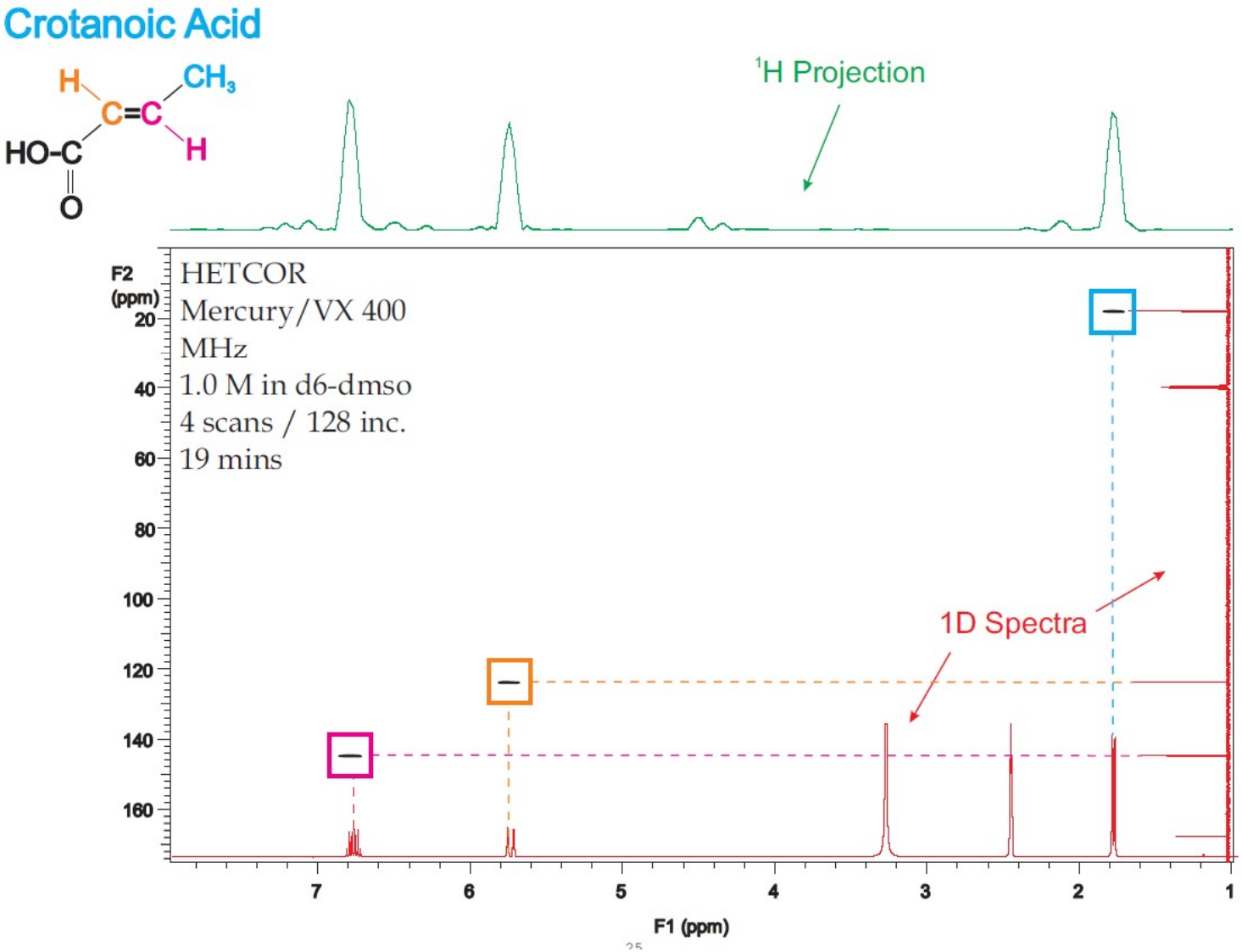
^{1}H-^{13}C correlation of crotanoic acid by HETCOR

1D projections



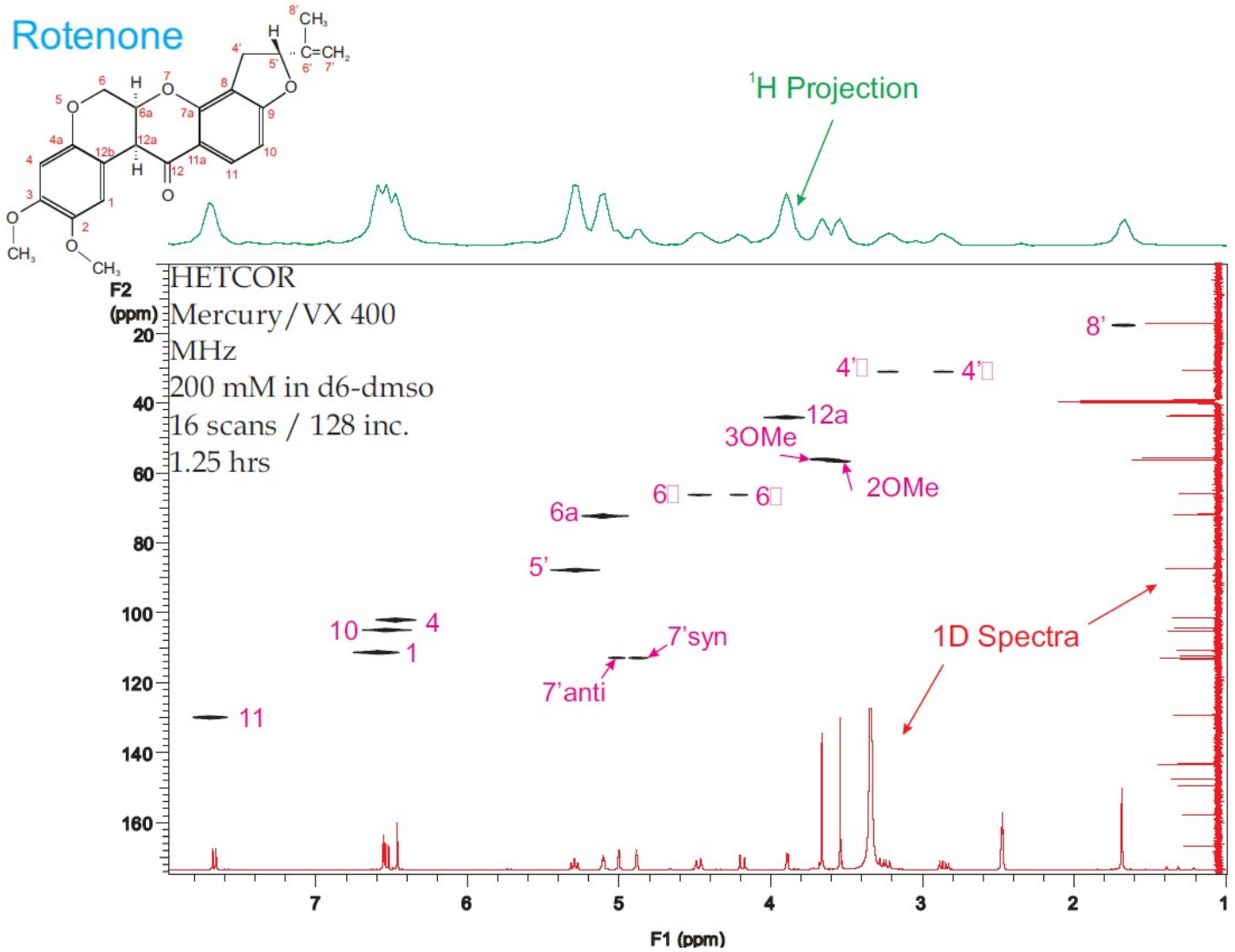
^{1}H-^{13}C correlation of crotanoic acid by HETCOR

1D projections and assignments



^{1}H-^{13}C correlation of Rotenone by HETCOR

1D projections and assignments



Direct detection (HETCOR) *vs* Indirect Detection (HSQC, HMQC, HMBC)

Indirect Detection

HETCOR uses J-coupling between ^{13}C and ^{1}H to allow polarization of the attached ^{1}H to transfer to the observed ^{13}C signal. This produces the correlations in the 2D experiment.

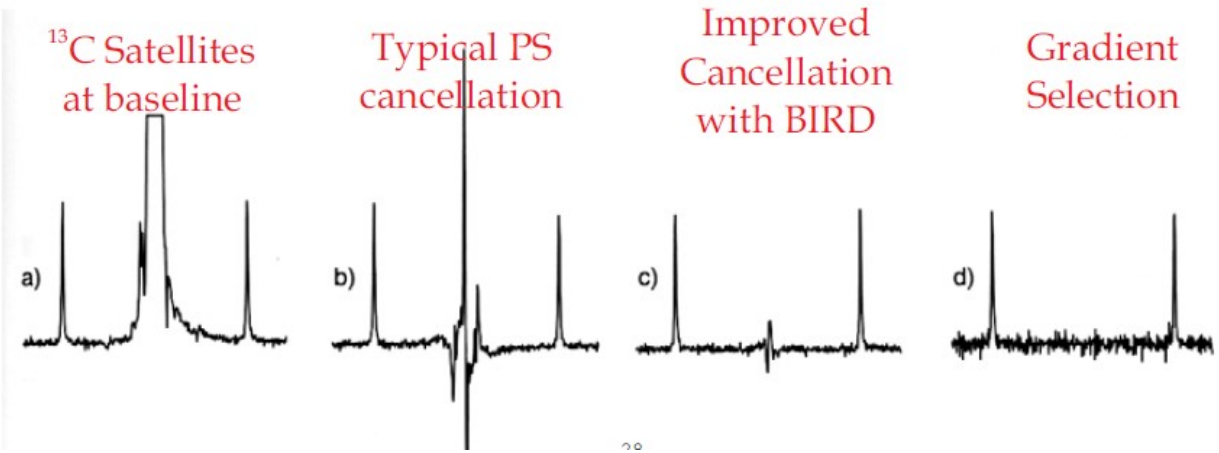
However, heteronuclear J-coupling is bidirectional (i.e., both nuclei are split to the same extent). So we could reverse the concept and observe ^{1}H with the transferred ^{13}C chemical shifts. This method is called ***Indirect Detection*** (**ID**).

The main advantage of ID is that the higher frequency ^{1}H is observed and this gives an significant sensitive advantage. The ^{1}H axis will have higher resolution than the ^{13}C axis, but this is usually limited by hardware considerations.

Challenges of ID experiments

For ^{13}C and other low natural abundance isotopes, the actual component of the sample that is observed is a minor fraction. Coupling between these nuclei shows up as ***satellite*** splitting that are on the baseline of ^{1}H resonances. Removal of the resonance of the more abundant signal (which does not lead to correlations) is very important.

Phase cycling schemes are far from perfect. Additional suppression by saturation methods i.e., bilinear rotation decoupling (BIRD) can improve the suppresion. However, gradient coherence selection is the preferred method.



Challenges of ID experiments

The resulting peaks for single bond correlation ID experiments are doublets. Sensitivity is enhanced by decoupling the low γ (^{13}C) during data acquisition. However, the spectral dispersion of carbon and other low γ nuclei are quite large and the power needed to cover the full window can be quite high. Probe design and the use of modern broadband modulation to cover the ^{13}C have helped reduce the amount of power required for effective decoupling.

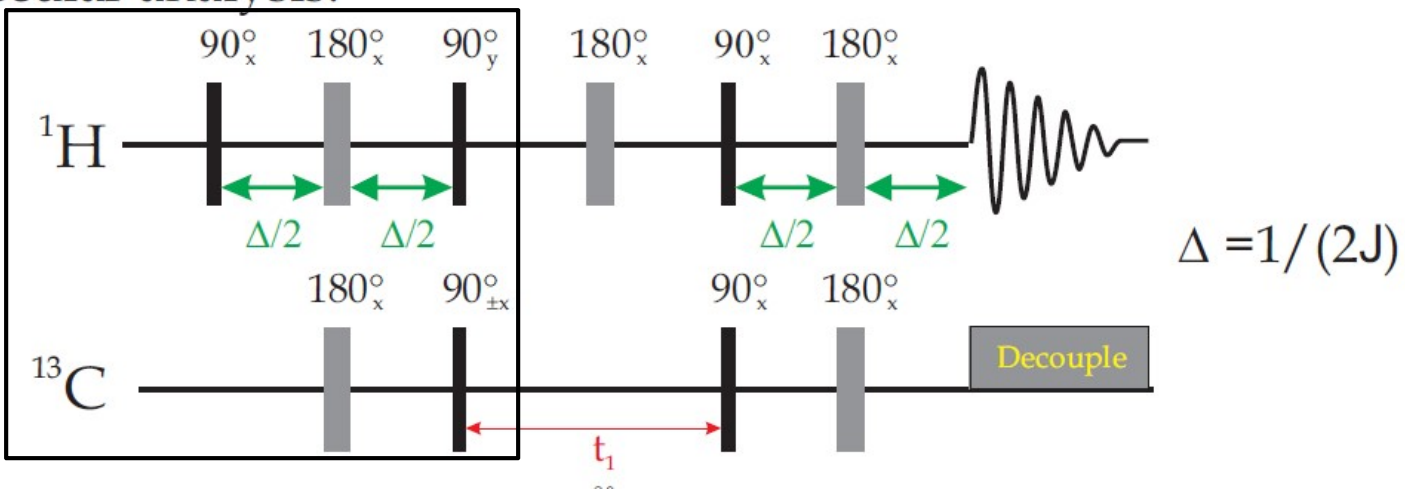
However, sample heating and hardware failure issues (arching) still limit the duty cycle and the length of the FID (t_2). While the limit of pulse repetition is usually controlled by relaxation, the ^{1}H resolution is limited by these power handling factors.

Heteronuclear Single Quantum Corehence/Correlation (HSQC)

One of the more common ID experiments that yields ***single bond correlations*** (J-coupling). The sequence has a gradient coherence selection version (not shown) that greatly improves the selectivity and reduces artifacts.

Polarization of the ^{1}H is passed to ^{13}C, (via an INEPT sequence) where it is allowed to evolve (chemical shift). After the evolution period (t_1), it is passed back to the ^{1}H (reverse-INEPT) for detection.

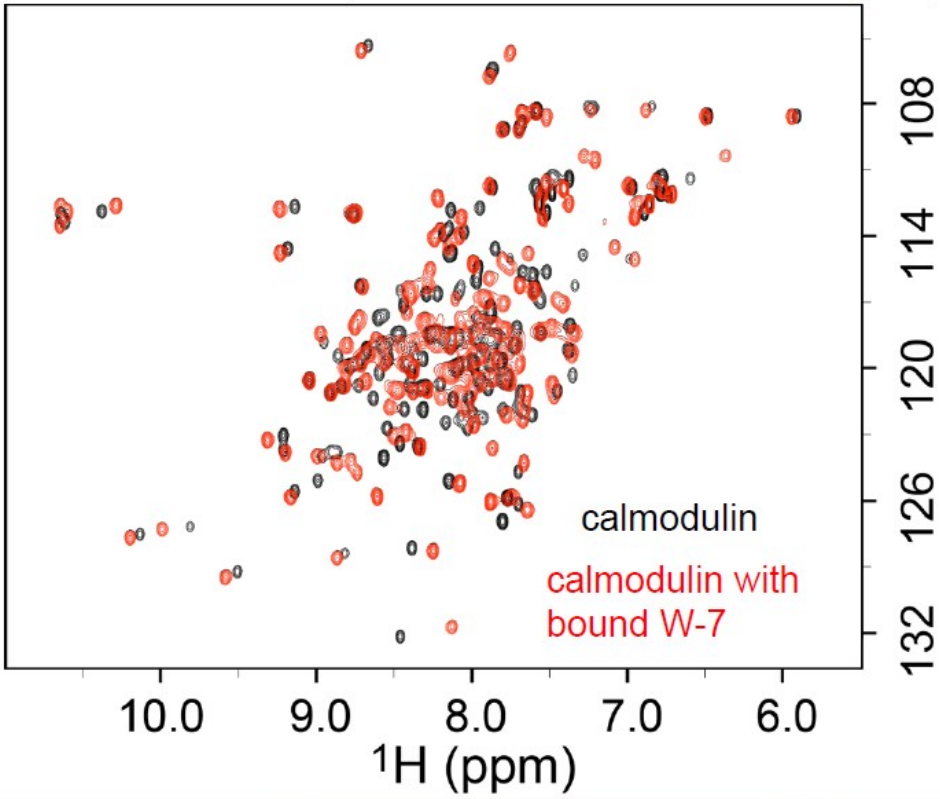
The HSQC is the basis for many sequences that were developed for bio-molecular analysis.

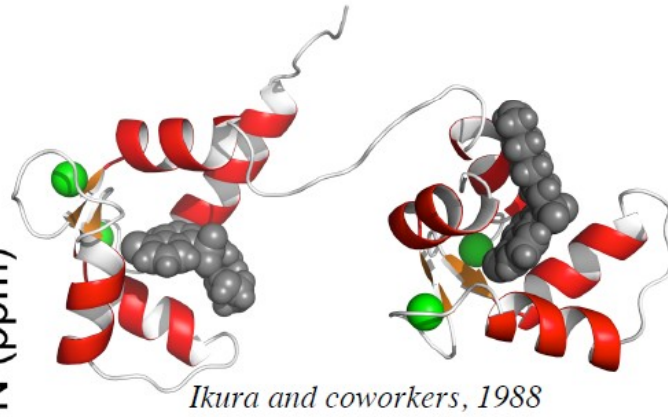


Insensitive nuclei enhanced by polarization transfer (INEPT)

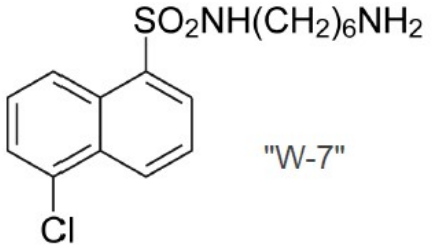
^{1}H-^{15}N HSQC spectrum of proteins at bound and unbound states

- HSQC spectrum of a protein (calmodulin) in the unbound state and bound to a drug ("W-7")
  - the HSQC experiment is one of the fundamental building blocks of scores of multidimensional, heteronuclear and triple resonance NMR experiments
  - chemical shift of amide ^{1}H correlated to directly bonded ^{15}N, for each amino acid (notice chemical shift ranges)





Ikura and coworkers, 1988



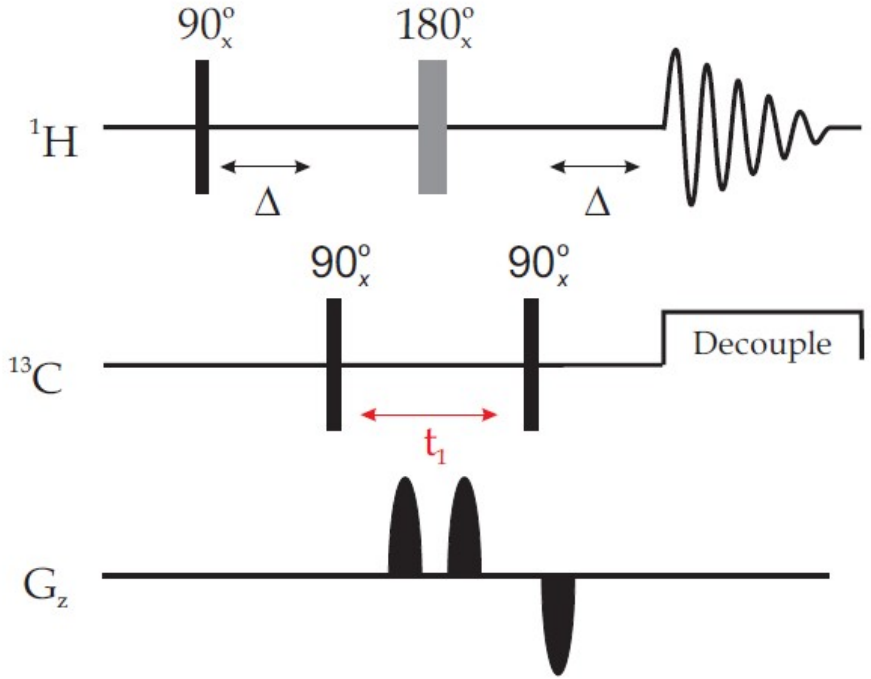
"W-7"

N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

Heteronuclear Multiple Quantum Corehence/Correlation (HMQC)

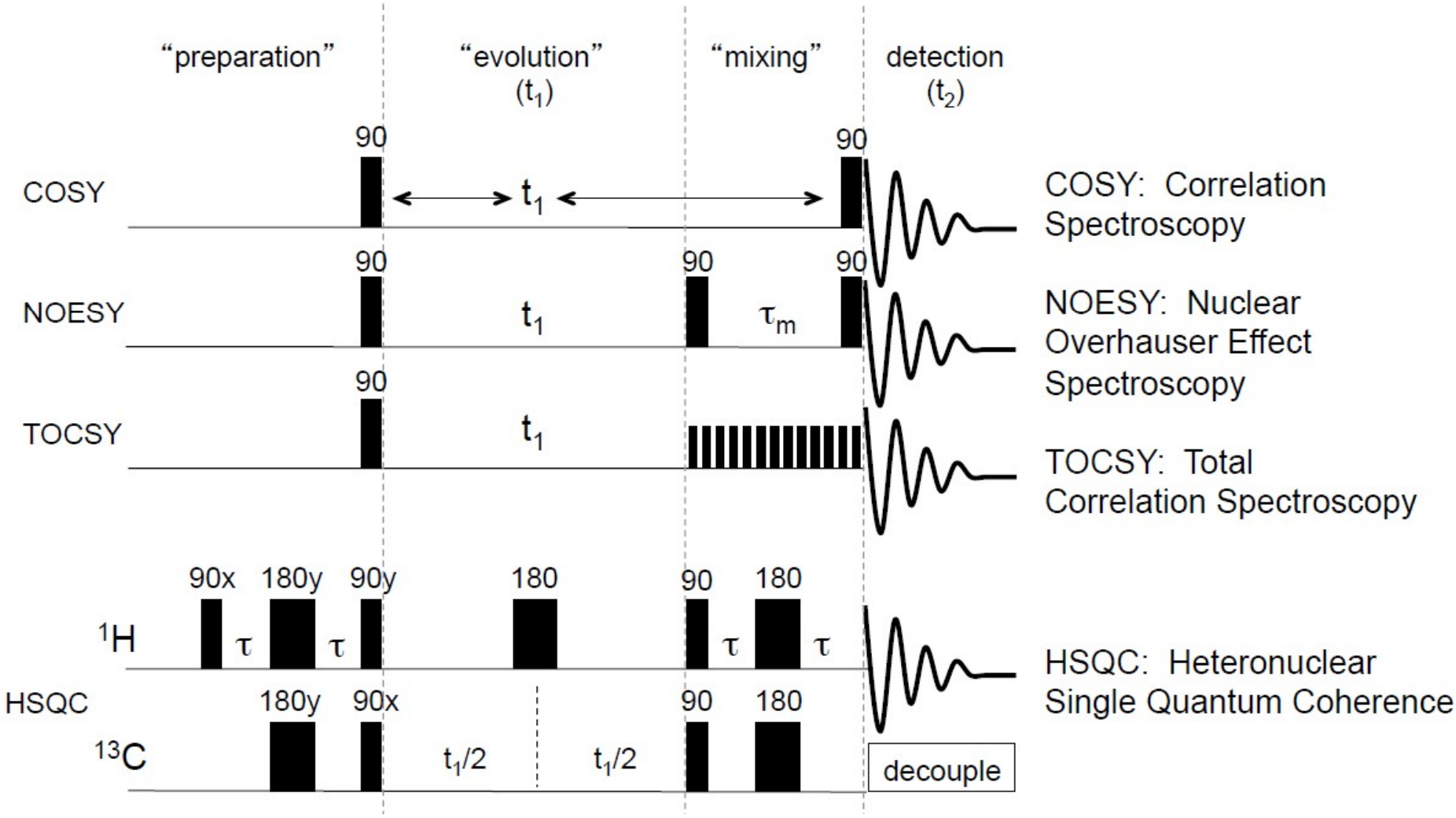
Another common ID experiment that yields ***single bond correlations***. The main benefit of HMQC over HSQC is that HMQC is more robust against mis-calibrations in pulse widths and delay times. Also, HMQC has a simple modification that allows for long range correlations. There are both gradient and non-gradient versions of the sequence.

gHMQC:



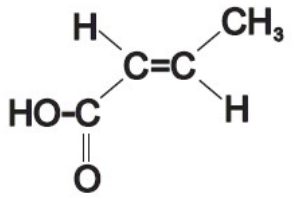
$\Delta = 1/(2J)$

Pulse sequences of common 2D NMR expts

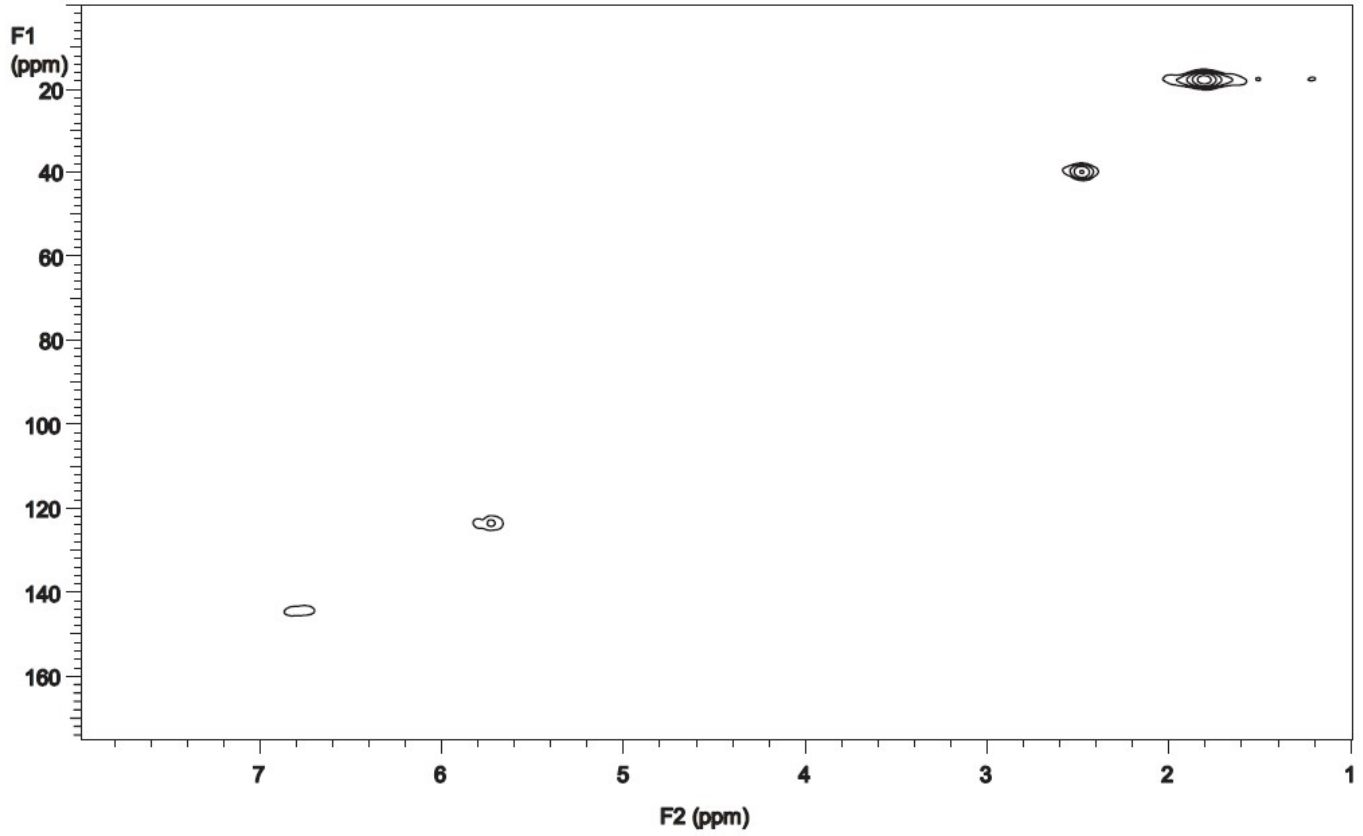


^{1}H-^{13}C correlation of crotanoic acid by gHMQC

Crotanoic Acid

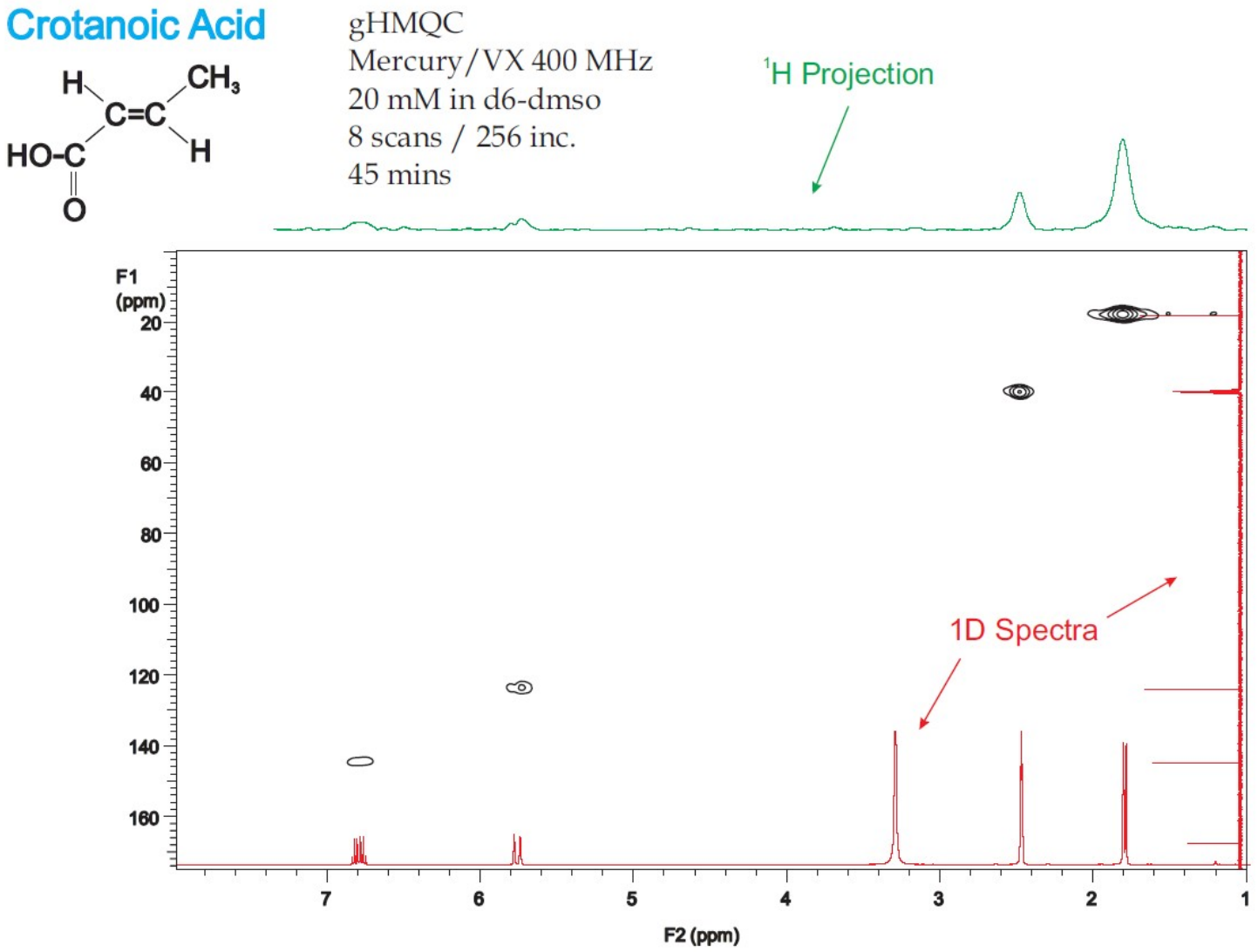


gHMQC
Mercury/VX 400 MHz
20 mM in d6-dmso
8 scans / 256 inc.
45 mins



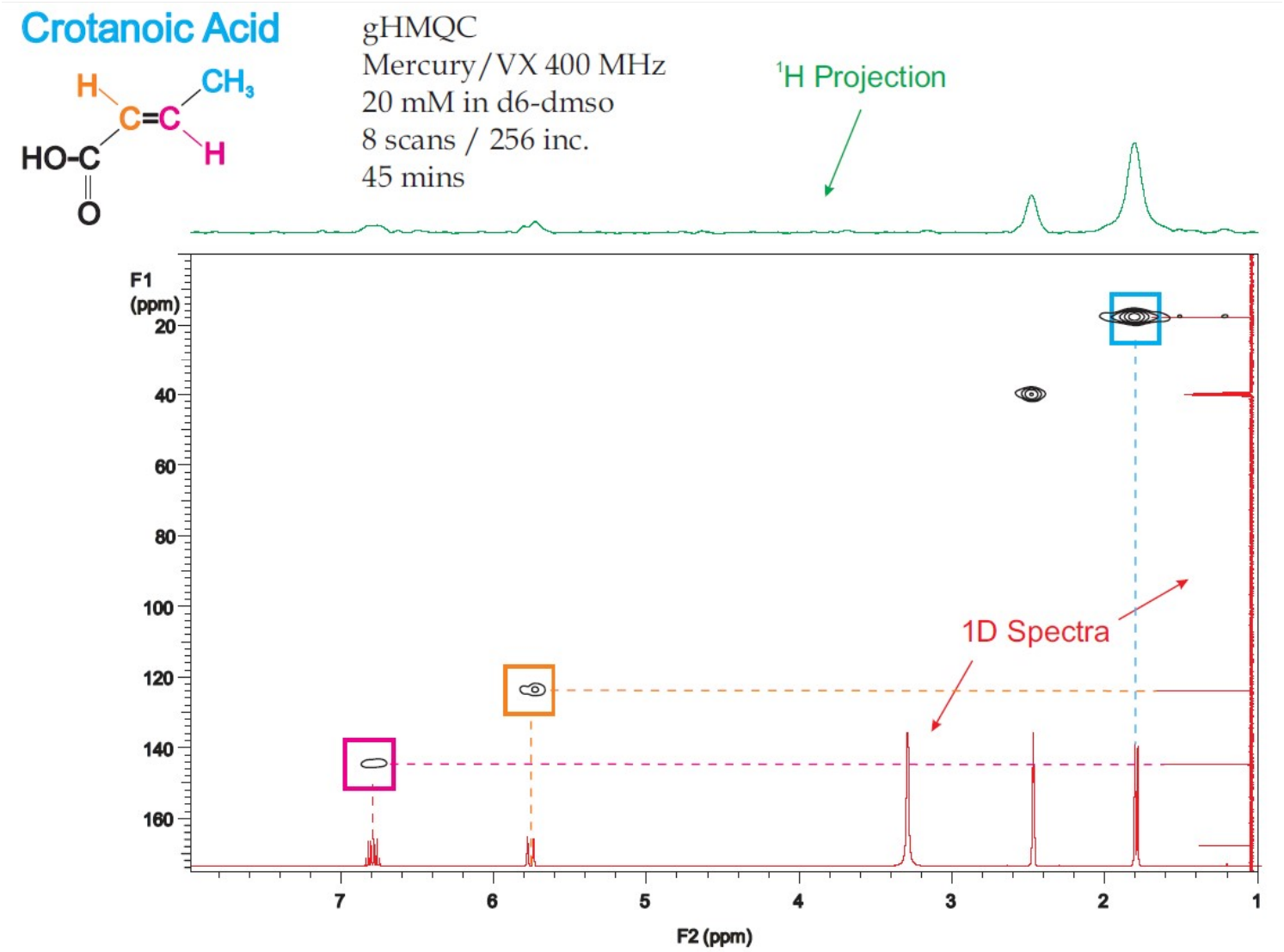
^{1}H-^{13}C assignment of crotanoic acid by gHMQC

1D projections



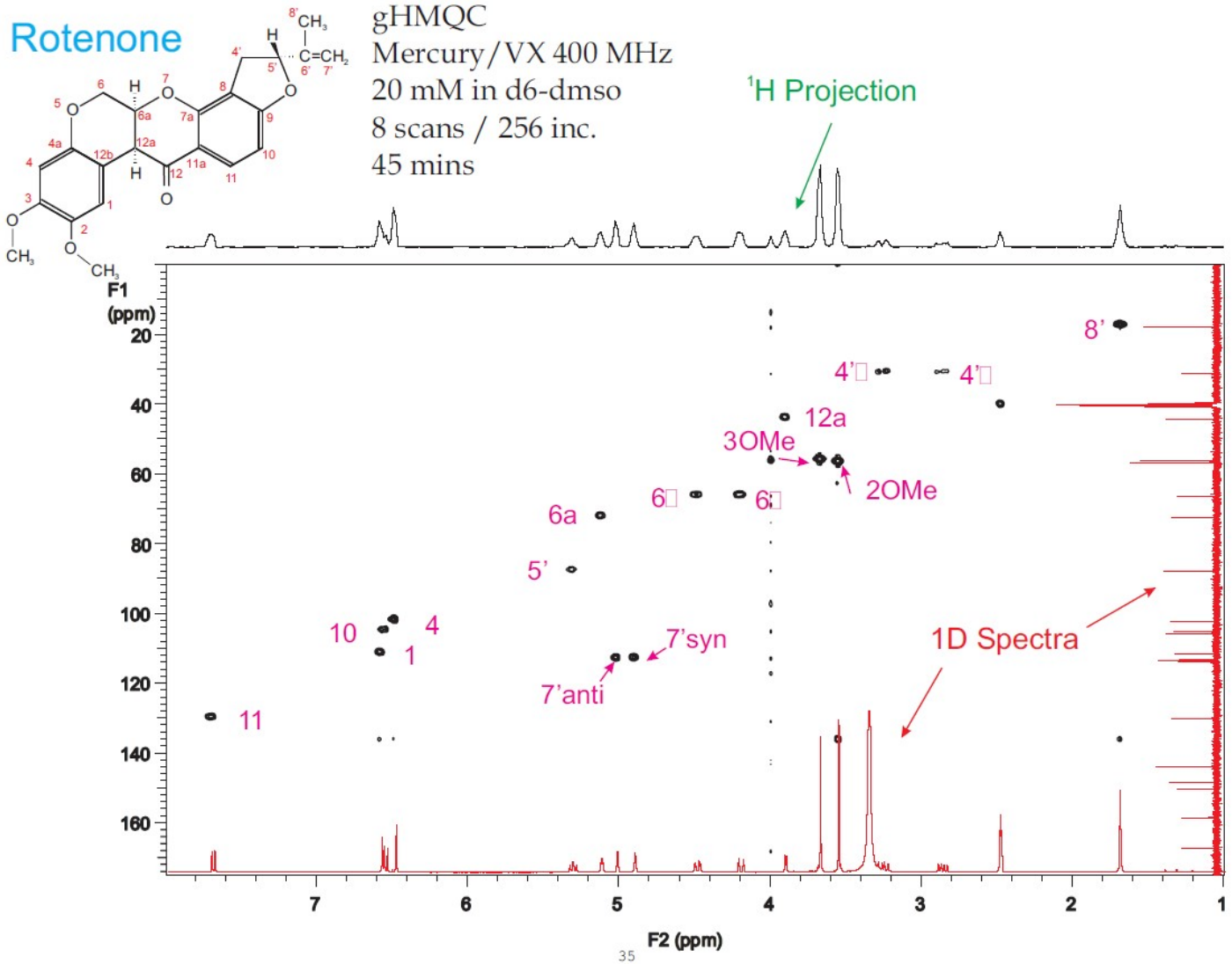
^{1}H-^{13}C correlation of crotanoic acid by gHMQC

1D projections and assignments



^{1}H-^{13}C correlation of Rotenone by gHMQC

1D projections and assignments



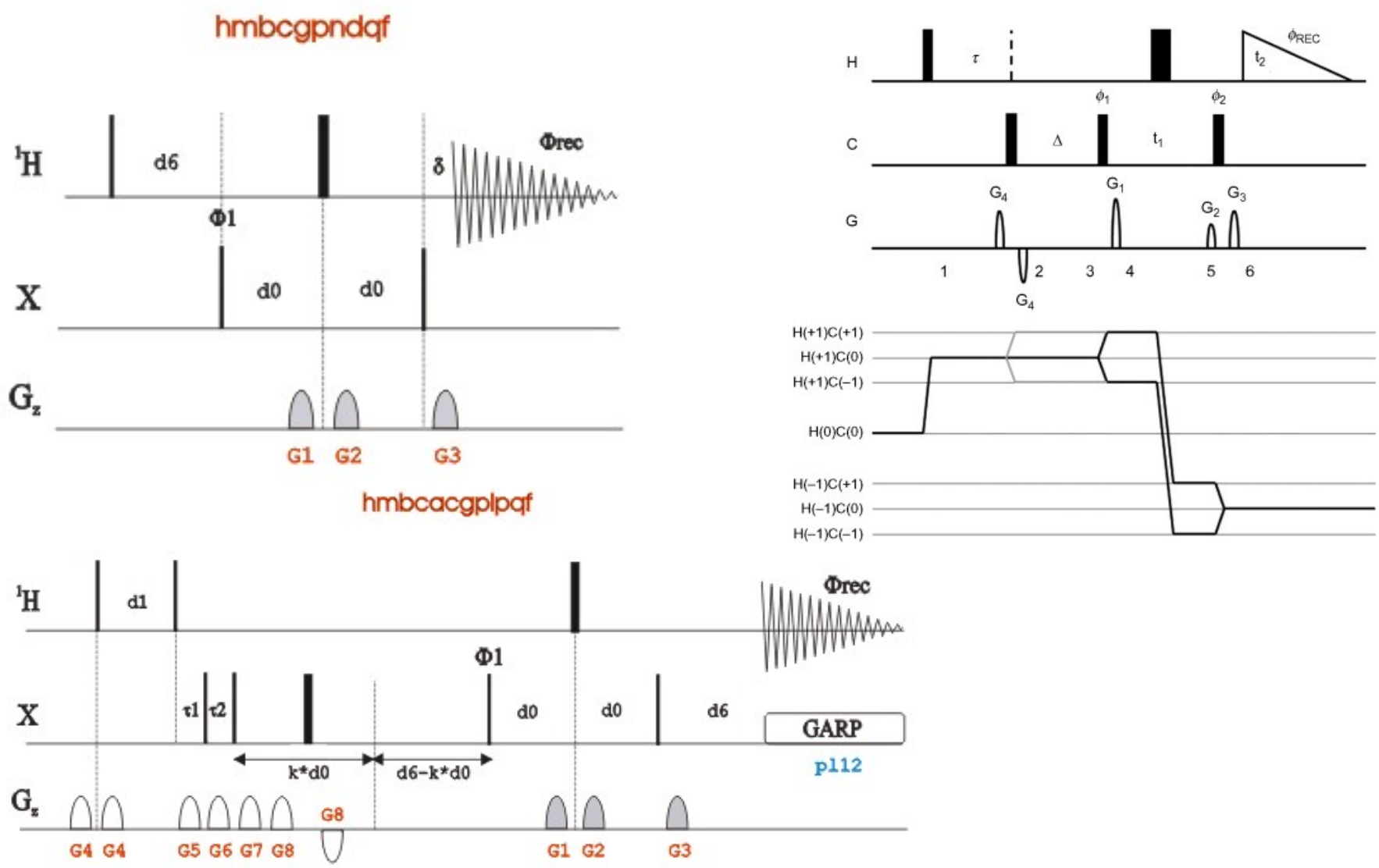
Heteronuclear Multiple Bond Corehence/Correlation (HBQC)

The HMBC sequence is a variation on the HMQC experiment. The gradient HMBC has perhaps the greatest improvement over its non-gradient sequence than any other experiment. The suppression of artifacts using the gradient selection has made these experiments much more useful. It was very difficult to get successful data using the non-gradient sequence.

The sequence utilizes fixed delays that are longer to match the smaller multiple bond coupling constants. The gradients are set to a different coherence pathway.

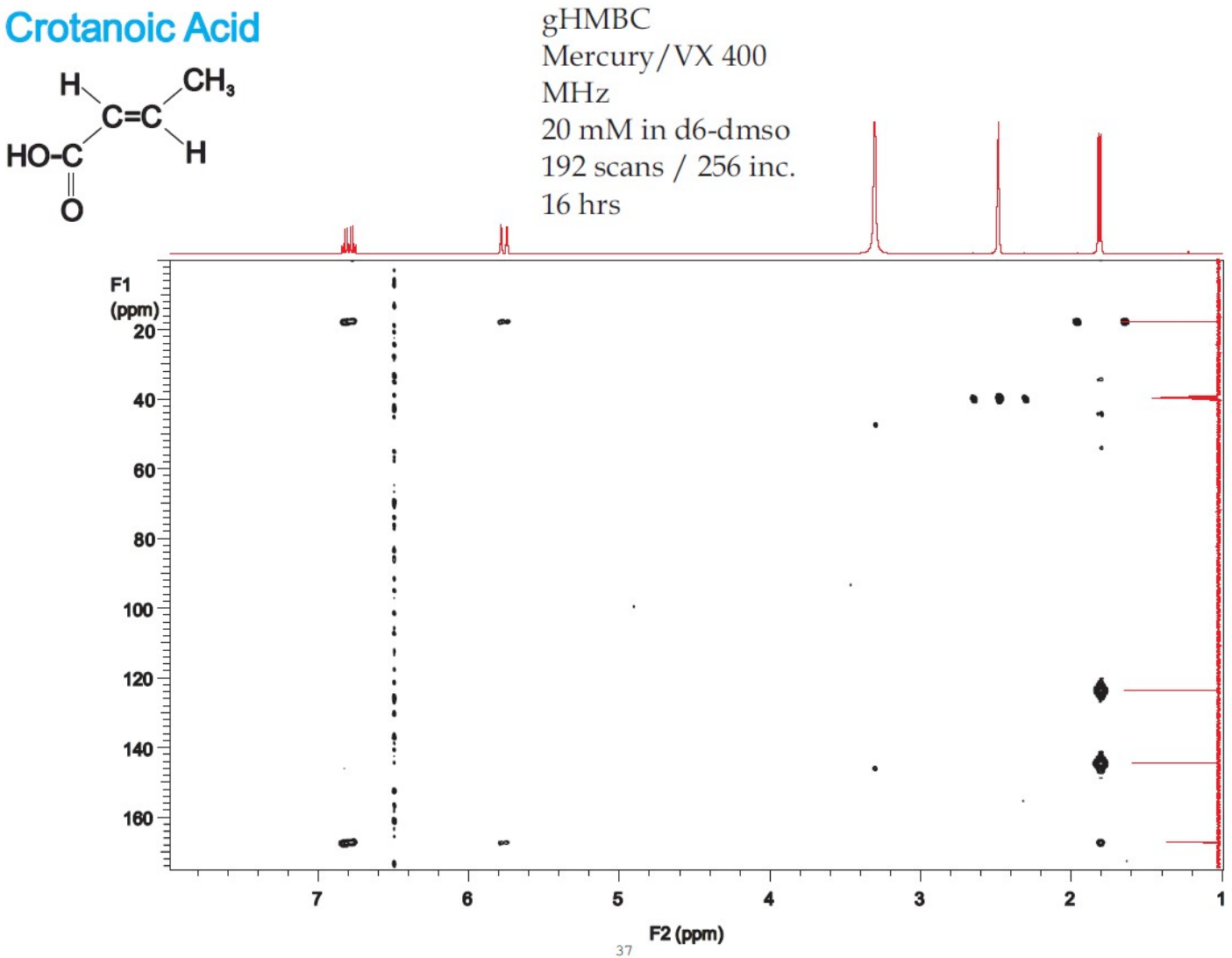
Long range coupling allows resonance/ structural assignments through small and moderately sized molecules.

HMBC pulse sequence



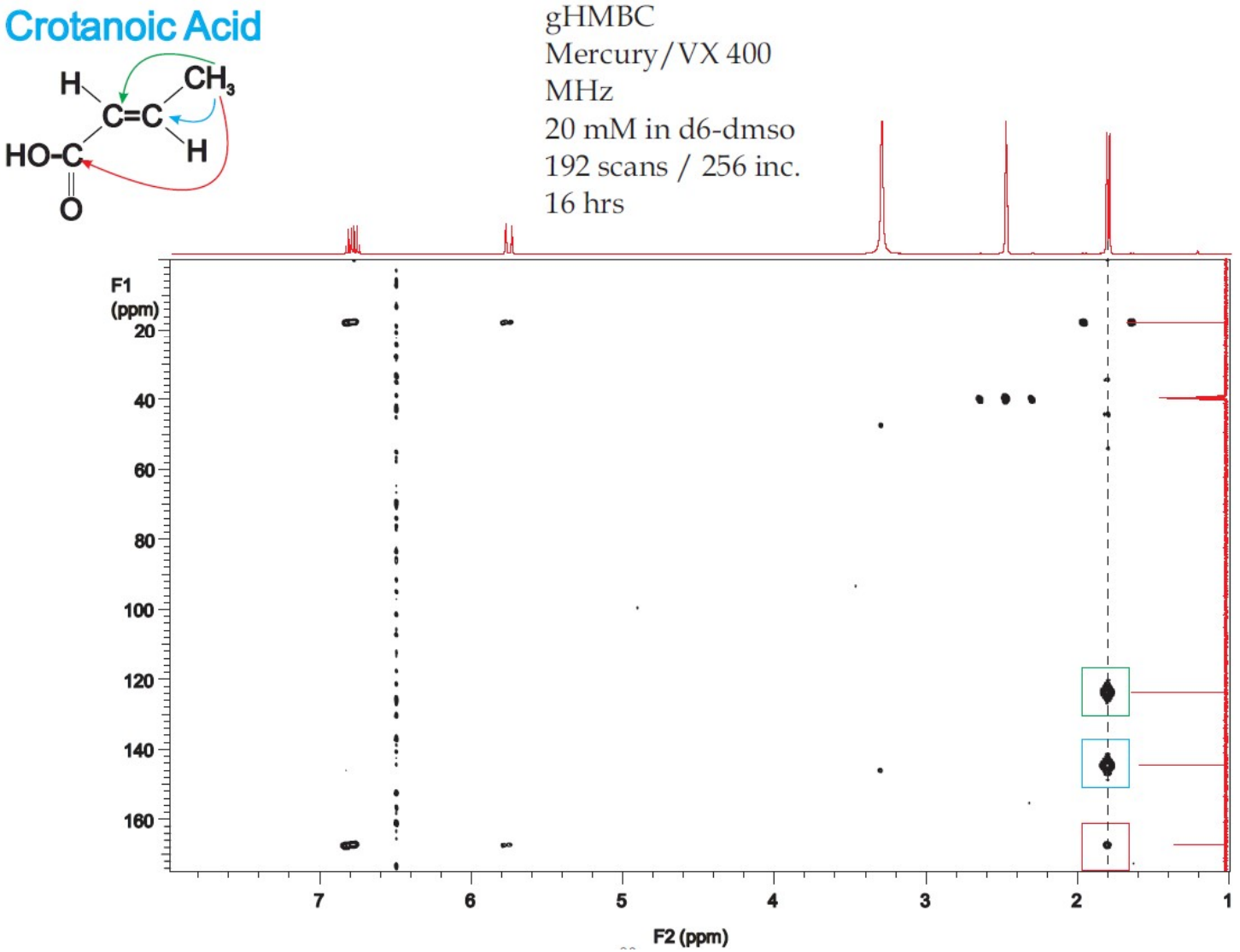
^{1}H-^{13}C assignment of crotanoic acid by gHMBC

1D projections and assignments



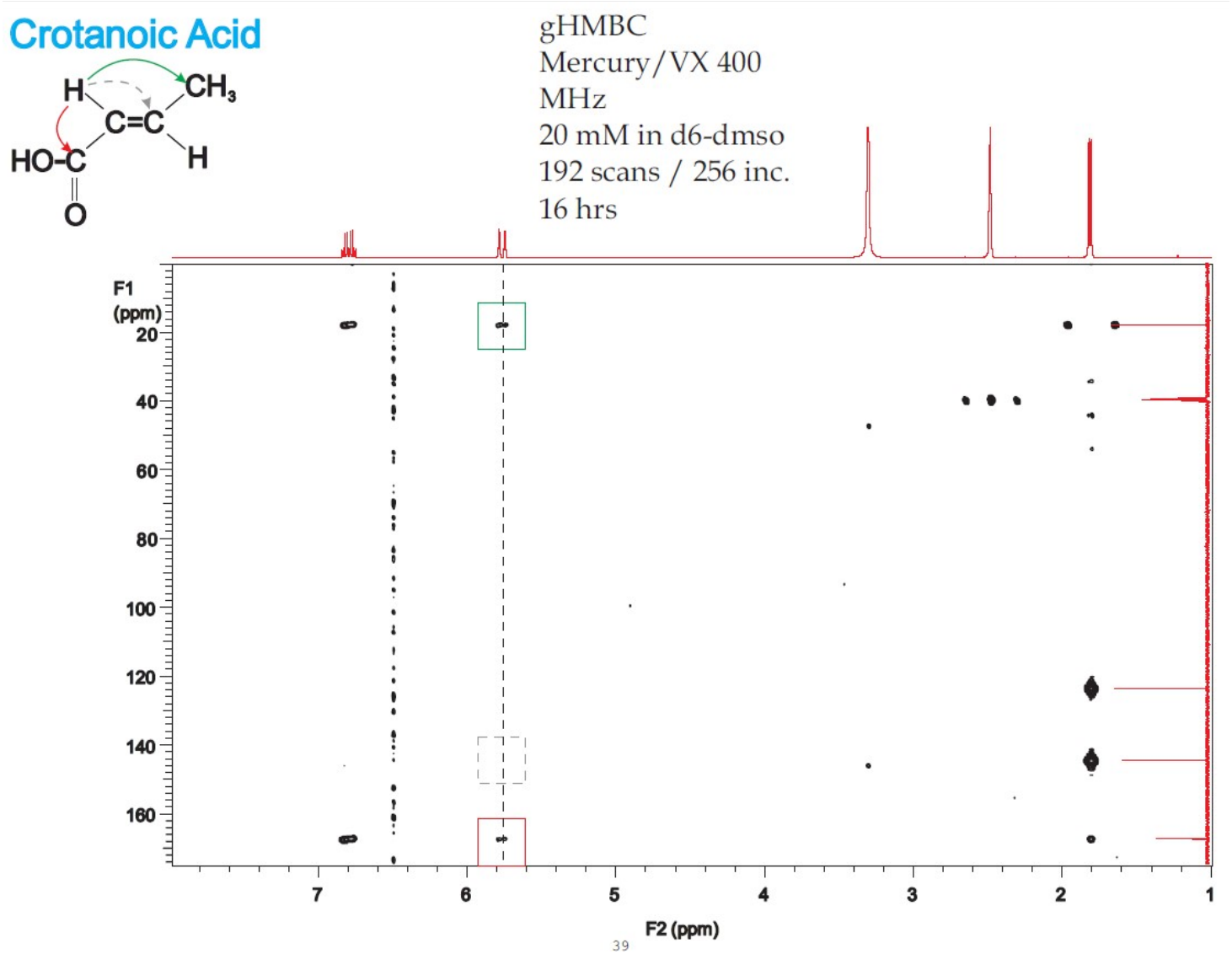
^{1}H-^{13}C assignment of crotanoic acid by gHMBC

1D projections



^{1}H-^{13}C assignment of crotanoic acid by gHMBC

1D projections and assignments



^{1}H-^{13}C assignment of crotanoic acid by gHMBC

1D projections and assignments