

Knowing More & More About Less & Less Until You Know Almost Everything About Almost Nothing

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Protasis CapNMR Probe & Robot Sample Injection System

A) Probe Characteristics:

- 10 μL flow cell
- Double-tuned $^1\text{H}/^{13}\text{C}$ coil ($^1\text{H}/^{13}\text{C}/^{15}\text{N}$ available as an option) plus z-axis gradient
- Sample injection via robot, direct injection or from HPLC

B) Robot Characteristics:

- full computer control
- Automated sample injection & rinsing of flow cell & capillary
- Option to have up to 3 different solvents
- Option to recover sample, if desired
- ACD software to check sample structures

Advantages

- 1) Comparable sensitivity to a 5 mm cryoprobe on a mass basis
- 2) Significantly lower capital & operating costs than cryoprobe
- 3) Changing probes is comparable to normal probes & much faster and easier than cryoprobes
- 4) Minimal reshimming is required when changing samples
- 5) Very short 90° pulse widths at relatively low power (i.e. ^{13}C 90° pulse width = $3.0 \mu\text{s}$)
- 6) Robot hardware seems rugged & reliable
- 7) Double-tuned coil means no compromise on ^{13}C sensitivity

Disadvantages

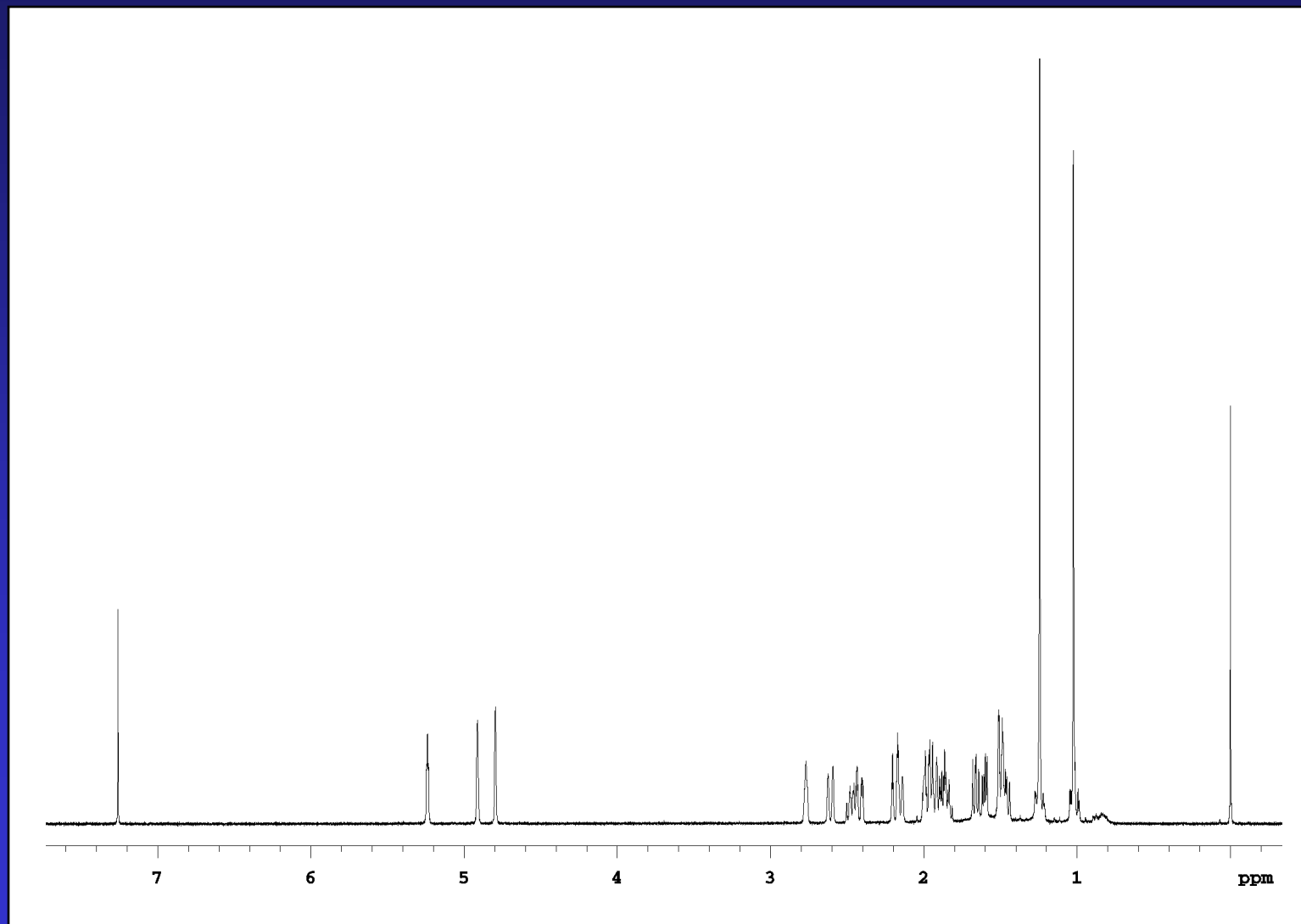
- 1) Solubility may be a problem in some cases
- 2) Samples must always be prefiltered to avoid blockage of capillary lines
- 3) Software for robot injection system is tricky to learn & could still be improved
- 4) Initial probe shimming is tricky
- 5) The probe has a ^{13}C (^{19}F) background which must be removed by subtracting a blank spectrum

Test of the ability to fully characterize small natural product samples in under 24 hours

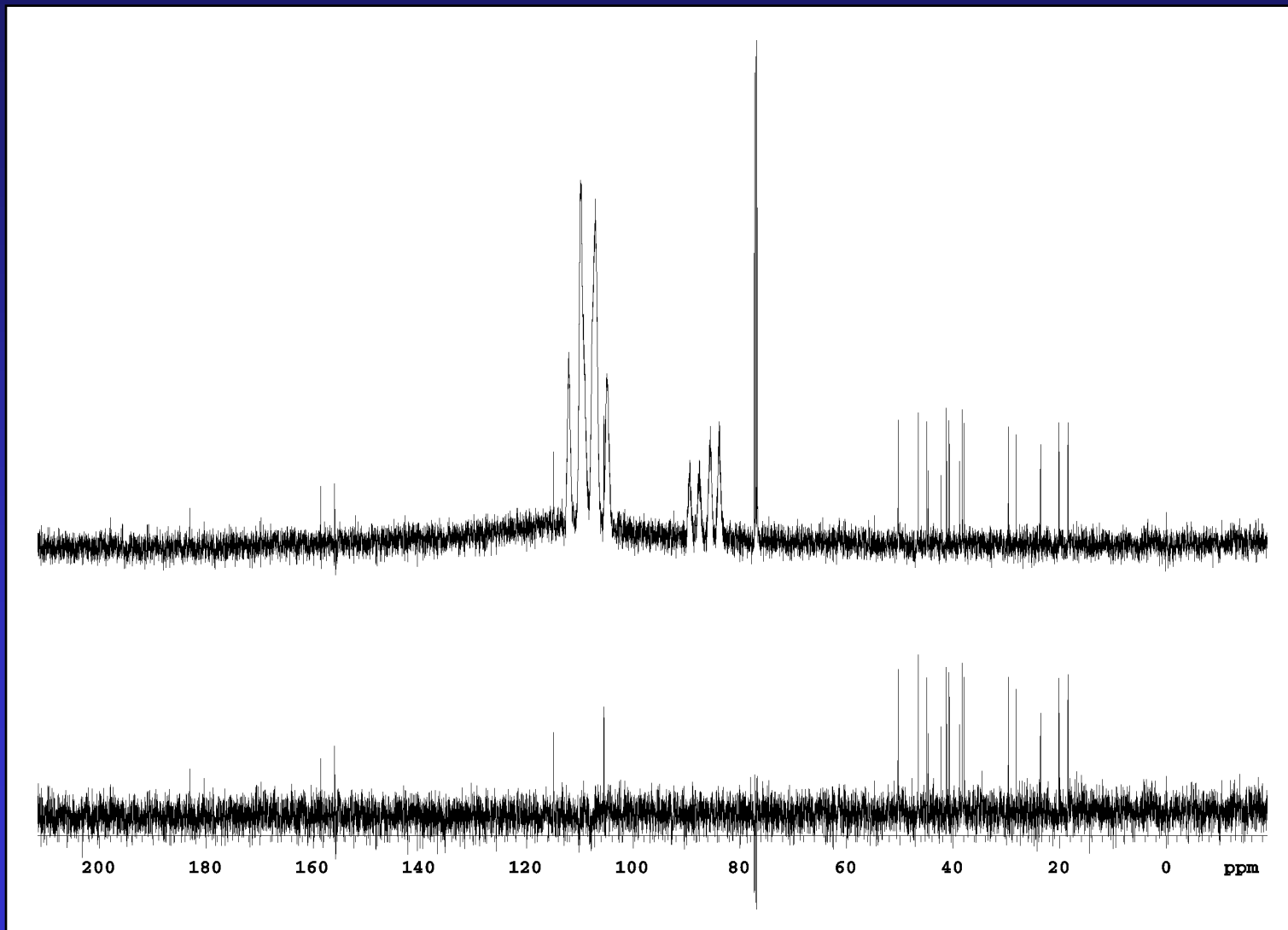
Sample: 120 μG of kauradienoic acid (diterpene of molecular weight 300) in 20 μL of CDCl_3

Experiment	(Time)
1) ^1H	(2 minutes)
2) ^{13}C	(10 hours, 25 minutes)
3) COSY	(29 minutes)
4) ROESY	(1 hour, 43 minutes)
5) TOCSY	(1 hour, 50 minutes)
6) HSQC	(1 hour, 19 minutes)
7) HMBC	(8 hours, 52 minutes)
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Total	23 hours, 20 minutes

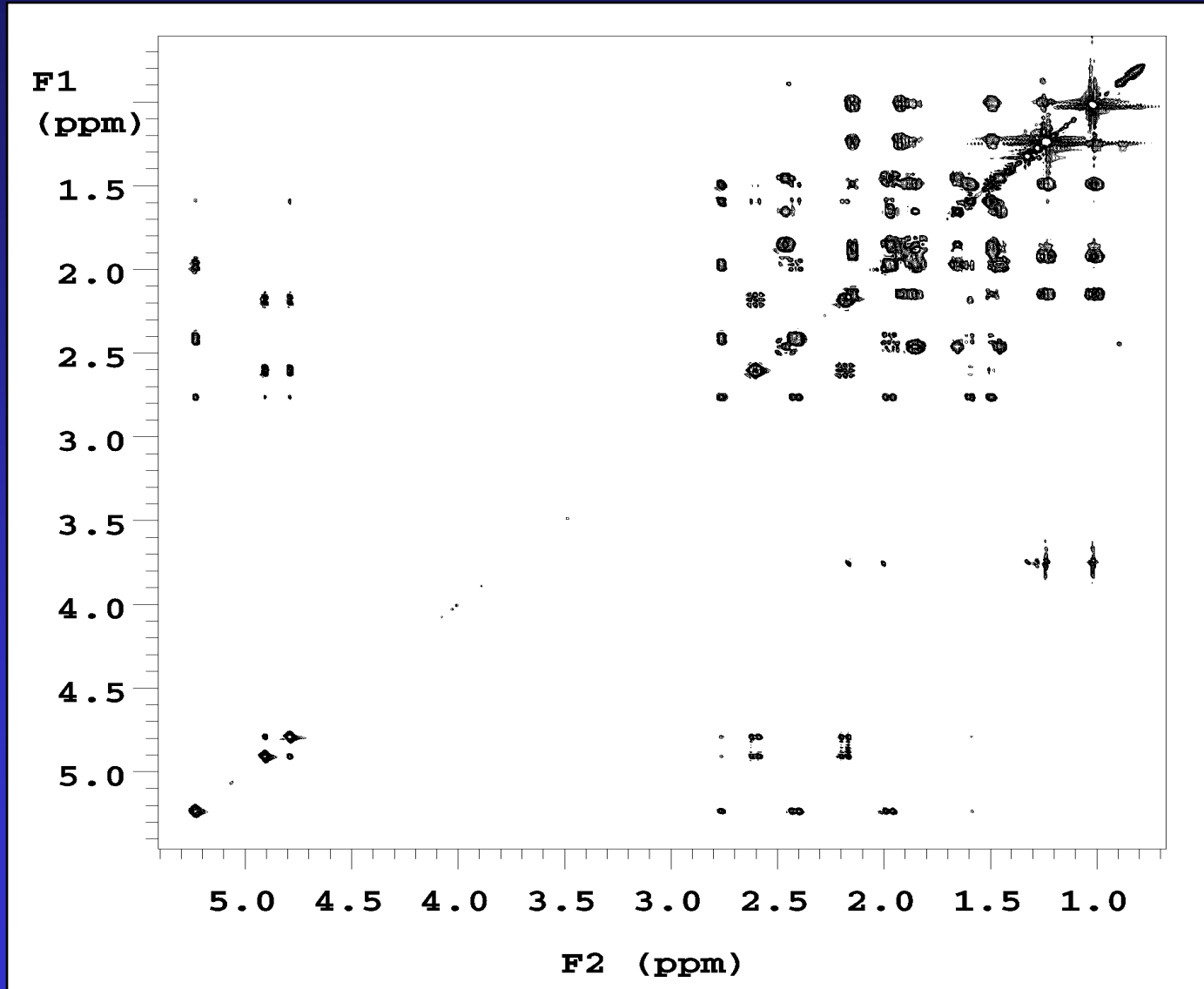
Proton Spectrum



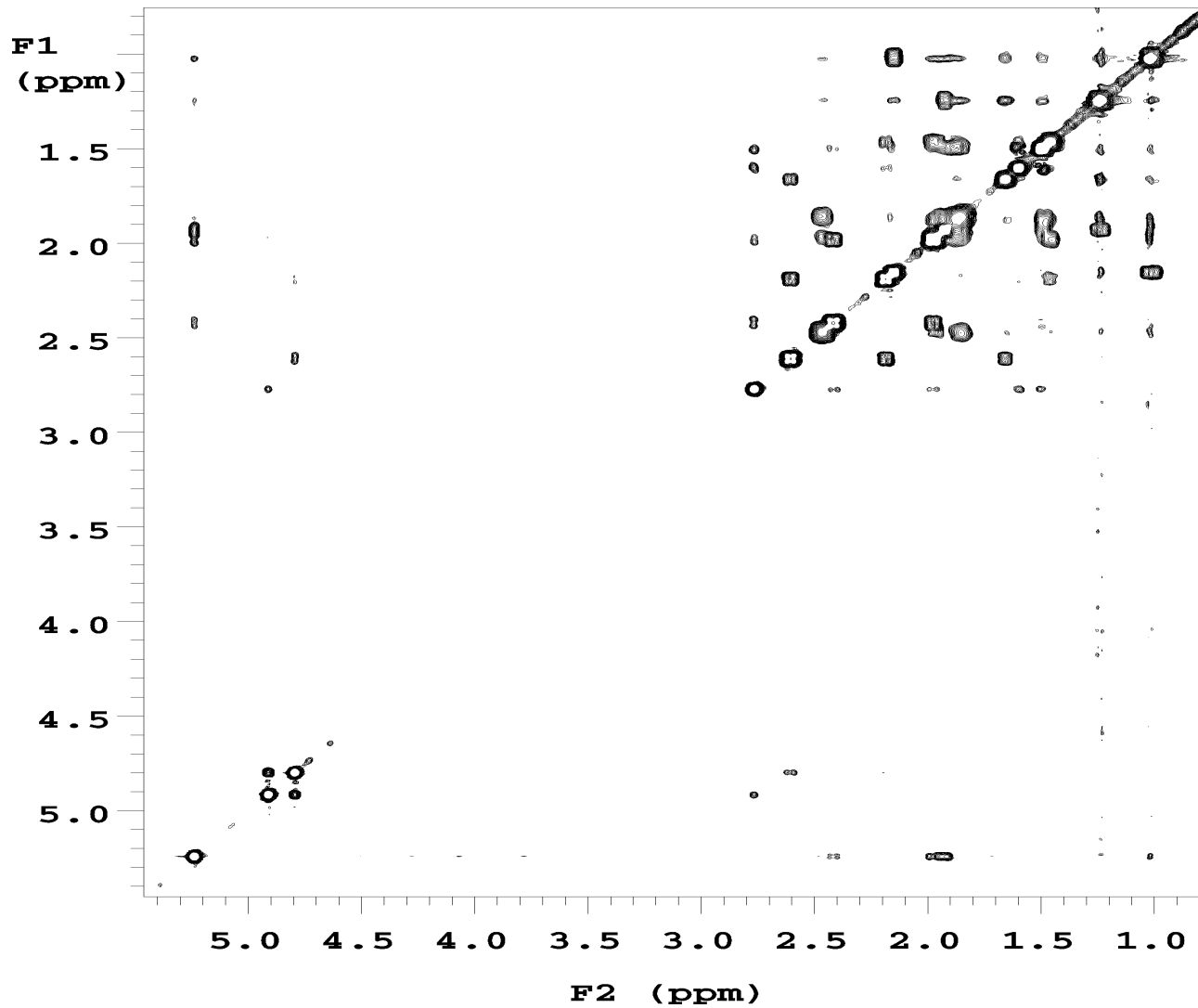
Carbon Spectra



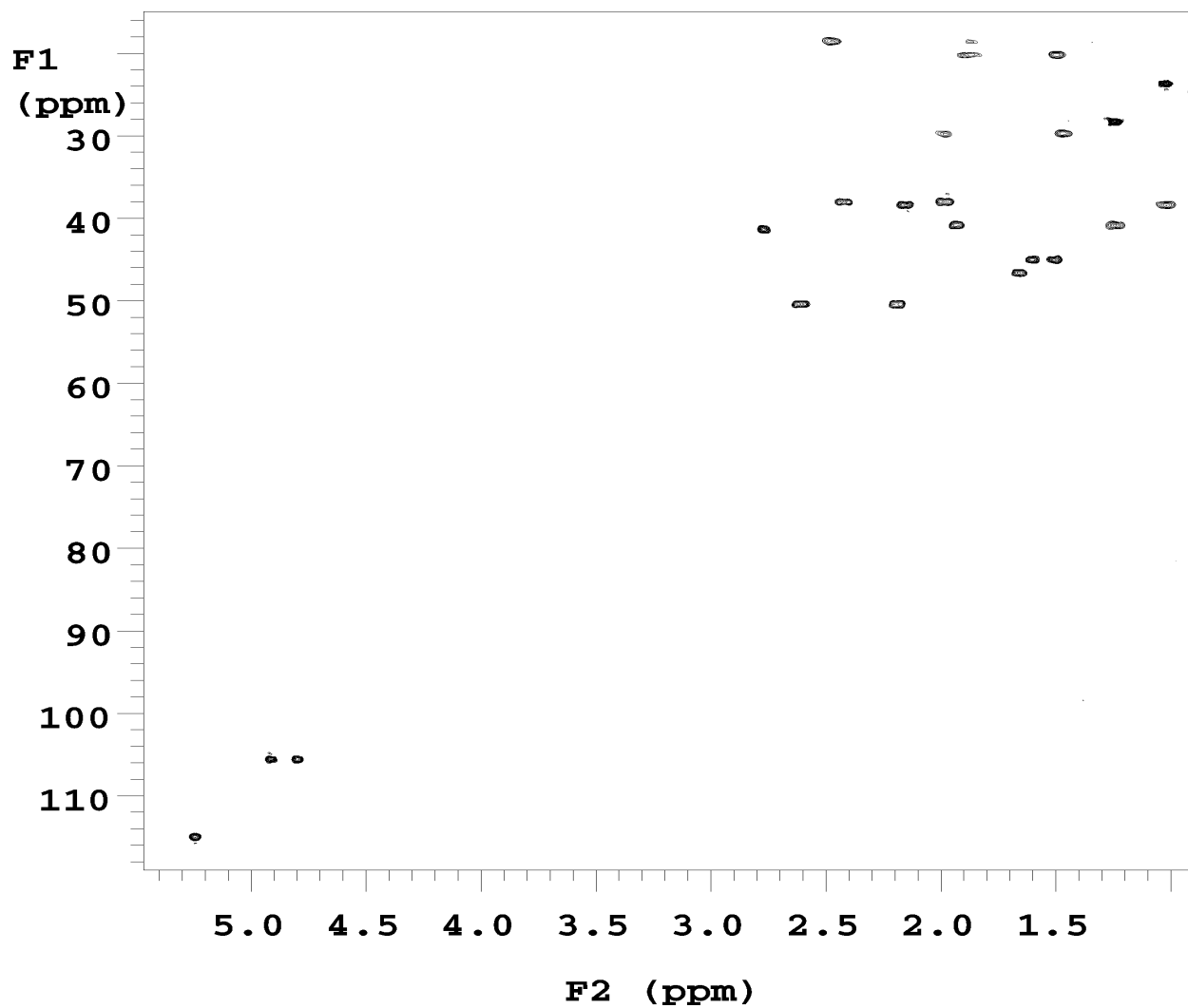
TOCSY Spectrum



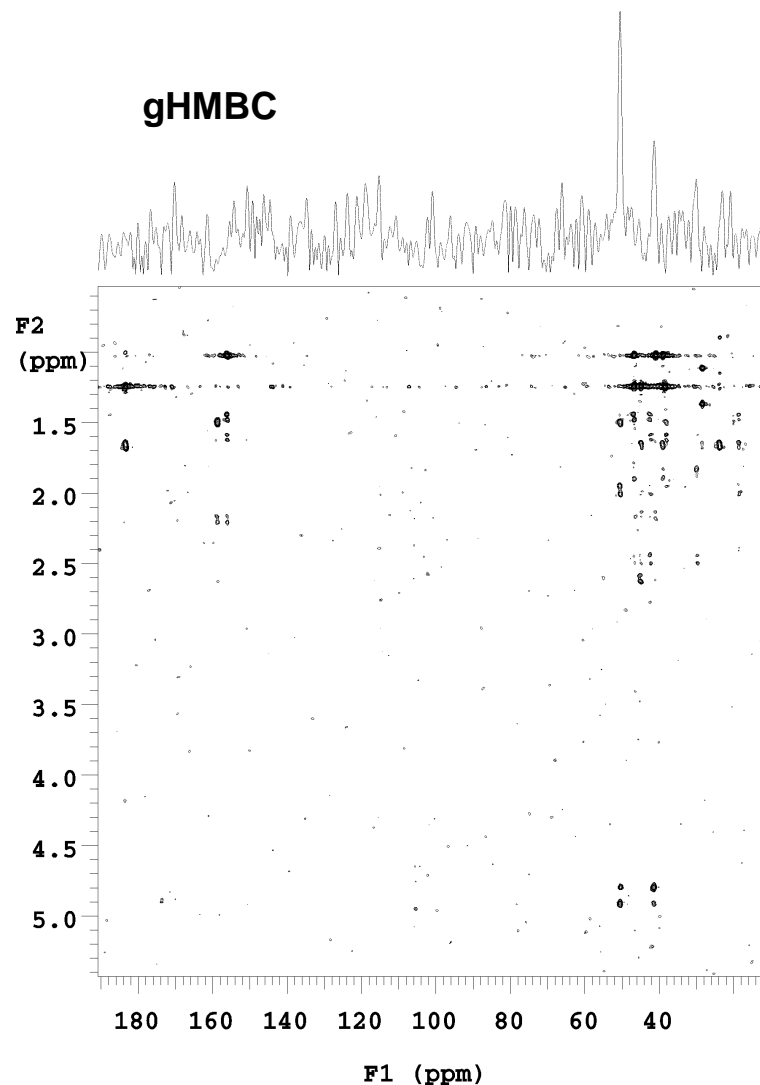
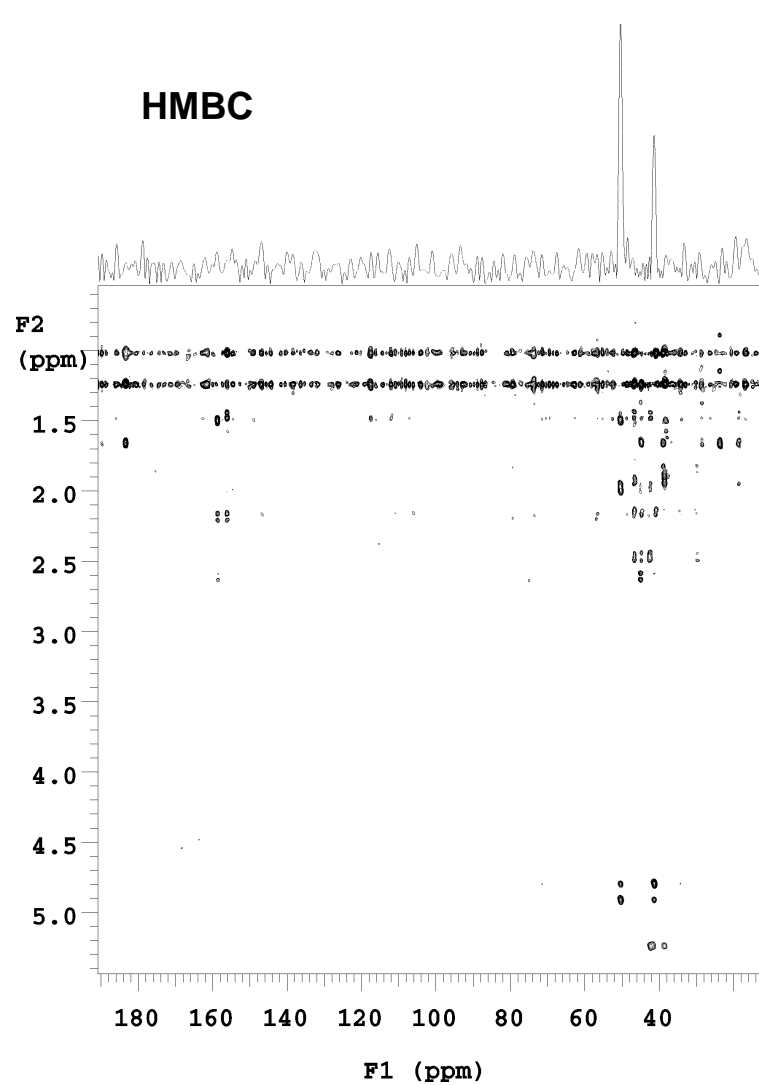
ROESY Spectrum



gHSQC Spectrum



HMBC & gHMBC Spectra



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Results:

- 1) Very good high resolution ^1H , COSY, ROESY, TOCSY & HSQC spectra
- 2) Adequate ^{13}C & HMBC spectra

Comments:

- 1) Comparable ^{13}C & HMBC spectra can be obtained in similar times
- 2) Significantly better spectra with phase cycled rather than gradient-selected HMBC (W.F. Reynolds & R.G. Enriquez, *Magn. Reson. Chem.* 39, 531 (2001))

Where is there room for improvement ?

- 1) Direct sample injection would allow dissolving the same sample in 12 μL of solvent in place of 20 μL , almost doubling the sensitivity
- 2) Replacing the current console by a state-of-the-art console should give a minimum of 40% increase in sensitivity
- 3) In some cases, it may be possible to save time by sacrificing 2D resolution
- 4) It may often not be necessary to obtain both ^{13}C & HMBC spectra. Since these two types of spectra can be obtained in comparable time, one approach would be to obtain a ^{13}C spectrum plus a selective pulse Hadamard HMBC spectrum for only quaternary carbons.

Estimated lower limit: 30-40 μG of sample in 24 hours or ca 20 μG over a weekend (64 hours)