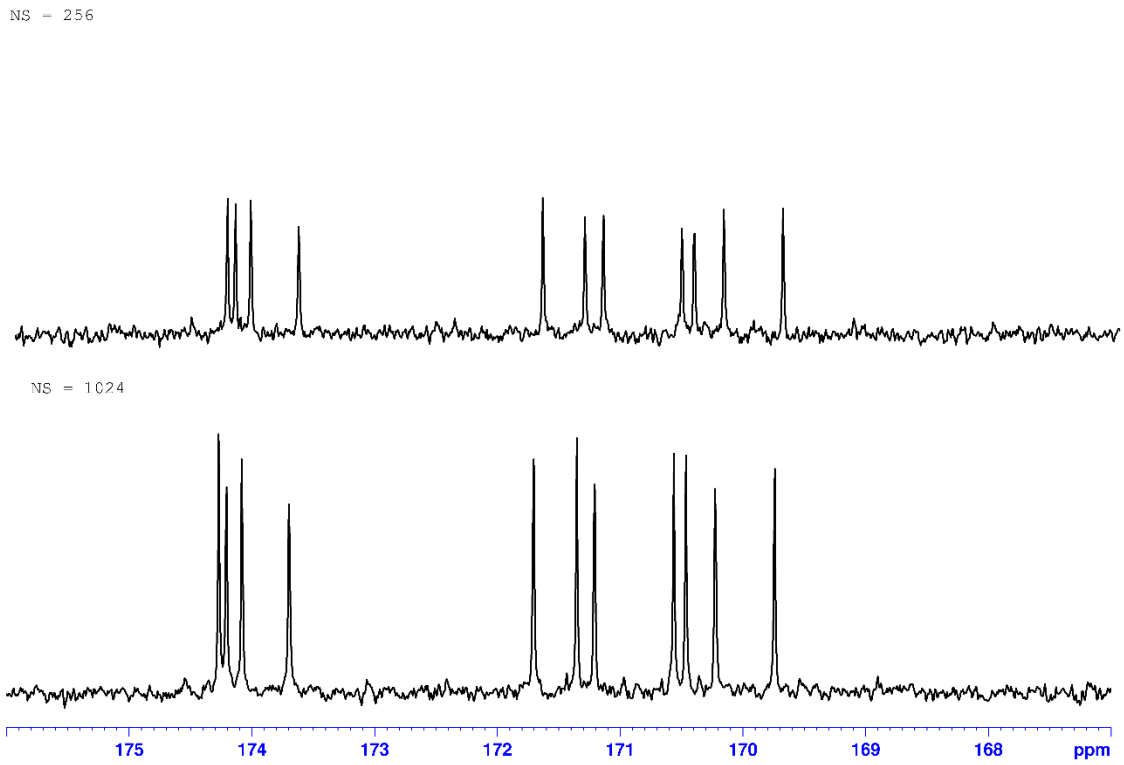
NMR_Service: Tipps&Tricks

I. Basics about sensitivity in NMR Spectroscopy

- The signal-to noise (S/N) of a FT NMR spectrum does only increases with the **square-root of the number of scans (and thus the time)** !

NMR Spectroscopy: $S/N \sim \sqrt{t}$

- So, if we have **2 times less substance**, an identical spectrum (typically a ^{13}C-spectrum) takes **4 times as long**!
- Corollary: A **2-fold** increase in S/N requires **4 times as much time!**



400 MHz ^{13}C spectrum of a 50 mmol sample of Cyclosporine. Bottom: 1024 scans, experiment time: 40 min. Top: 256 scans, experiment time: 10 min.
Clearly, the signal-to noise (S/N) only increases with the **square-root of the number of scans (and thus the time)** !

II. How much substance do I need?

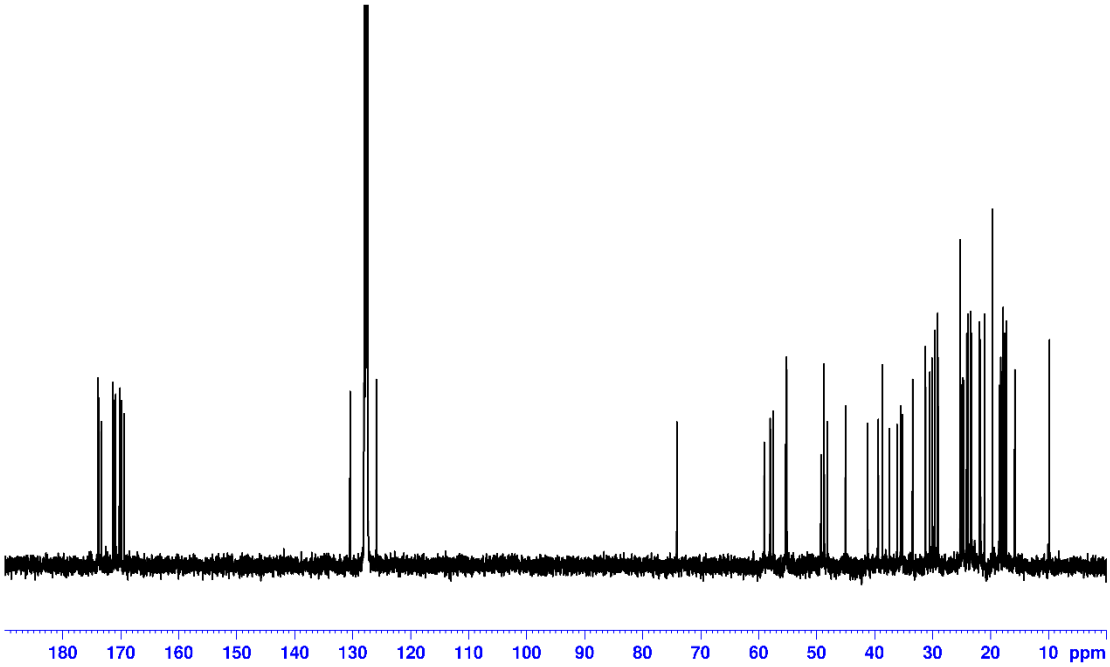
A. Open-Access 300 MHz: characterization incl. ^{13}C-spectrum and/or DEPT135, APT

Since the length of ^{13}C-based experiments is limited to **40 minutes** on both 300 MHz spectrometers, you need **0.6 mL** of a **50 mM solution (at least)** of your molecule to get a good spectrum.

For a typical organic molecule this is about **3-15 mg:**

Molecular mass (g)	100	200	300	400	500
Required quantity (mg) for 0.6 mL for a 100 mM solution	3	6	9	12	15

An example of what we consider a good ^{13}C spectrum is shown below for a 50 mmol cyclosporine sample.



300 MHz ^{13}C spectrum of a 50 mmol sample of Cyclosporine. 1024 scans, experiment time: 40 min.

B. Service: Complete characterization incl. ^{13}C-spectrum and a set of 2D spectra

1. ^{13}C-spectrum

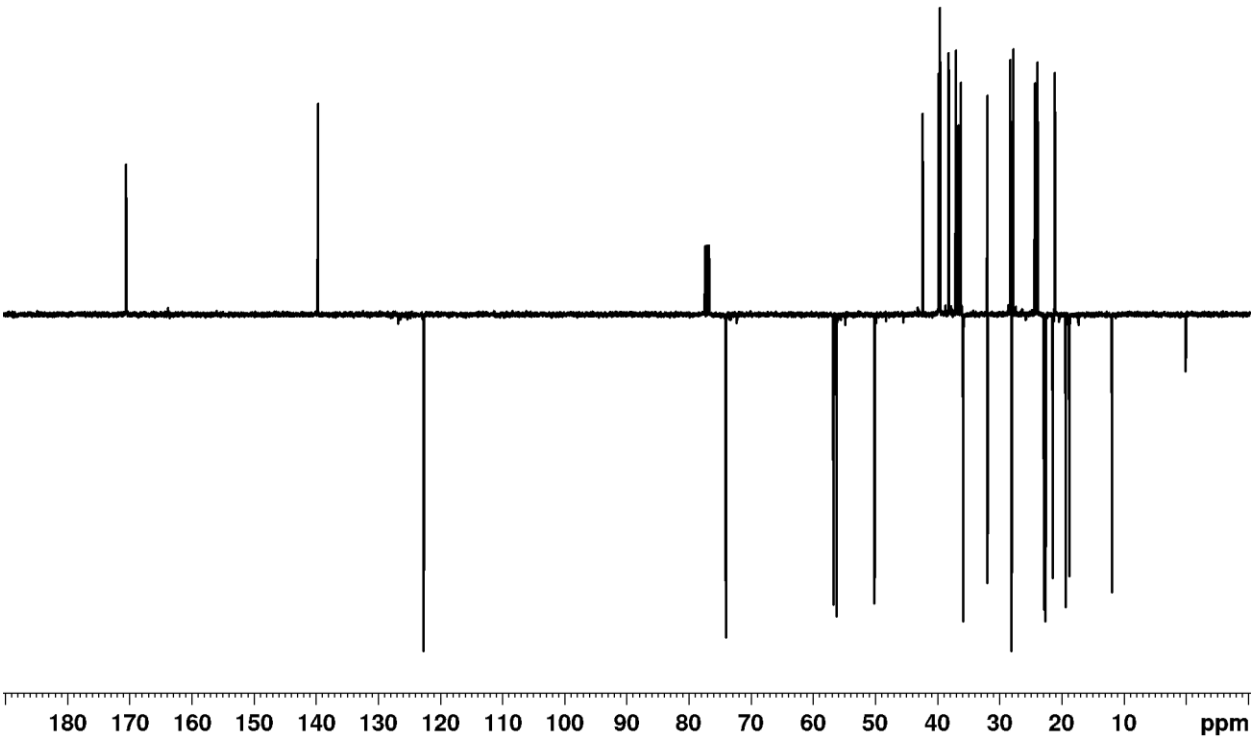
A very important fact: although it may seem counter-intuitive, the ^{13}C spectrum largely determines the length of the NMR-characterization! This characterization will be even longer if a DEPT and/or APT is also requested!

The reason is that the 2D spectra can be kept short because the large signal-to-noise on the ^{1}H channel allows us to use the minimum number of scans, even for relatively low sample concentrations!

Note that very often the ^{13}C-spectrum is not even needed for complete characterization because one can read off the ^{13}C-shifts from the indirect dimension of the HSQC and HMBC spectra.

Important: If you still want a 1D ^{13}C spectrum, we strongly recommend the APT (DEPTQ) spectrum (see below), as it contains everything you need for analyzing ^{13}C spectra.

^{13}C + DEPT or ^{13}C + APT or the completely needless combination ^{13}C + DEPT + APT are just redundant and unnecessarily lengthen the overall measurement time (and increase the bill for your boss...).



400 MHz ^{13}C-APT spectrum of Strychnine. The signals of *CH* and *CH$_3$* are *negative*, while *CH$_2$* and quaternary carbons including the solvent carbon are *positive*.

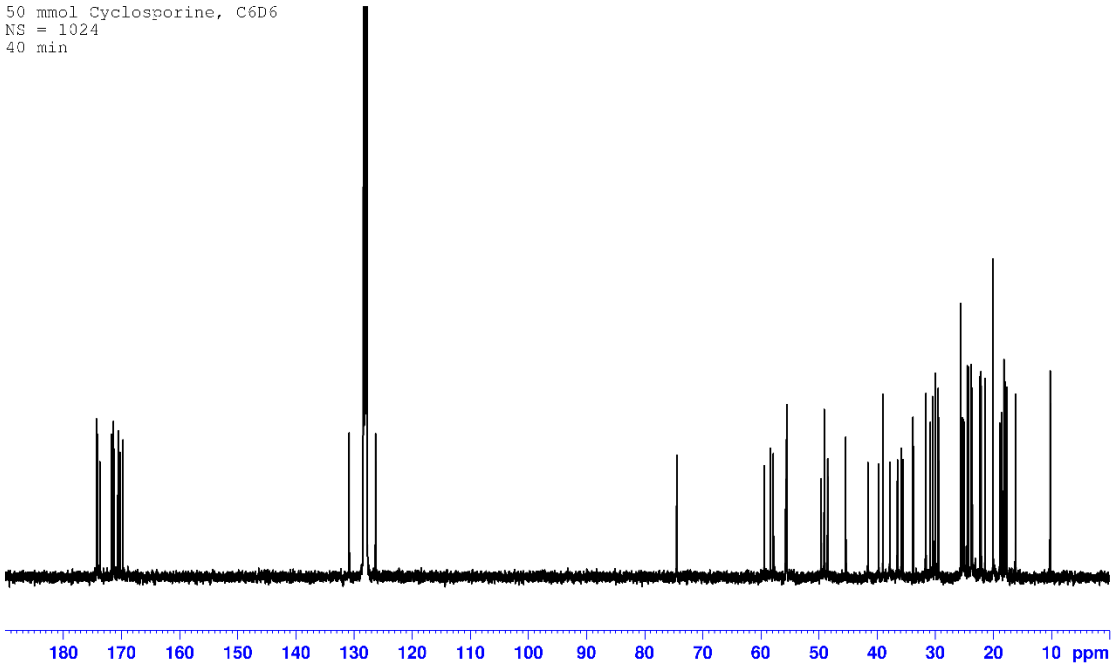
2. Examples

As an example, the following settings provide **good spectra** (see below) for a **50 mM sample** on one of our 400 MHz spectrometers:

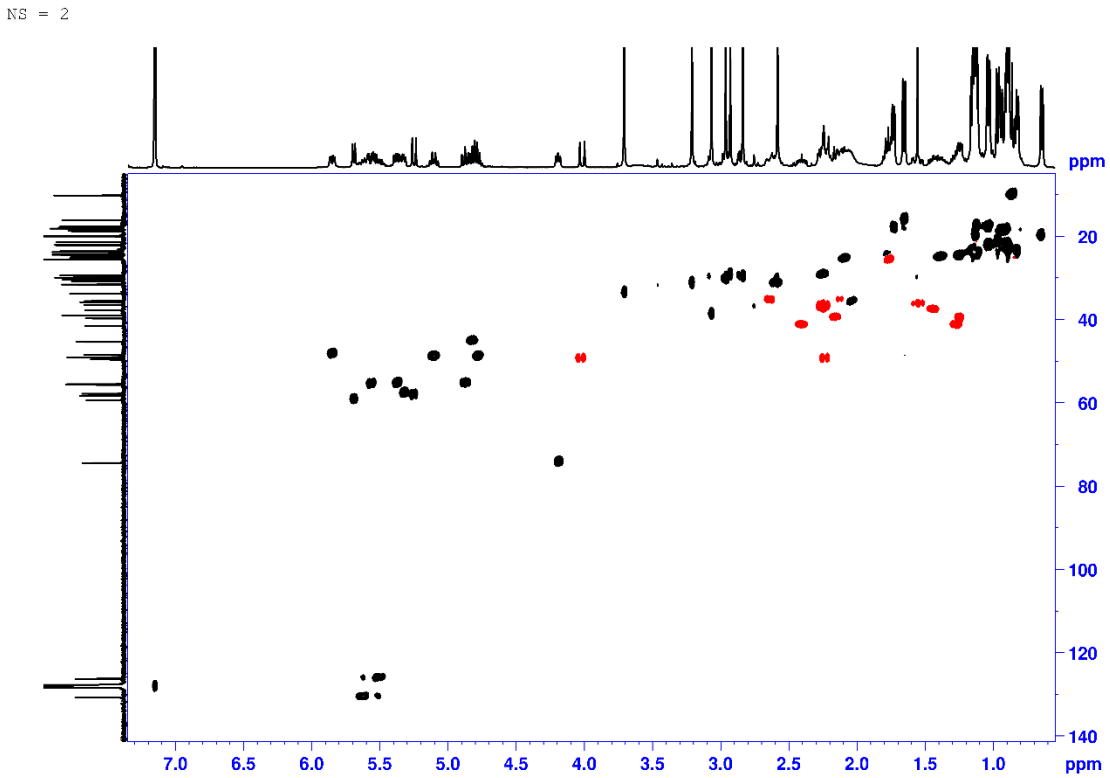
- ^{1}H: 8 scans (1')
- ^{13}C-APT (DEPTQ): 1024 scans (40')
- COSY, 128 increments: 2 scans per increment (4,5')
- HSQC, 128 increments: 2 scans per increment (4,5')
- HMBC, 128 increments: 4 scans per increment (10')

Total experiment time is **60 minutes**
66% of the measurement time is spent for the ^{13}C or APT spectrum…

➢ This percentage increases with decreasing sample concentration...

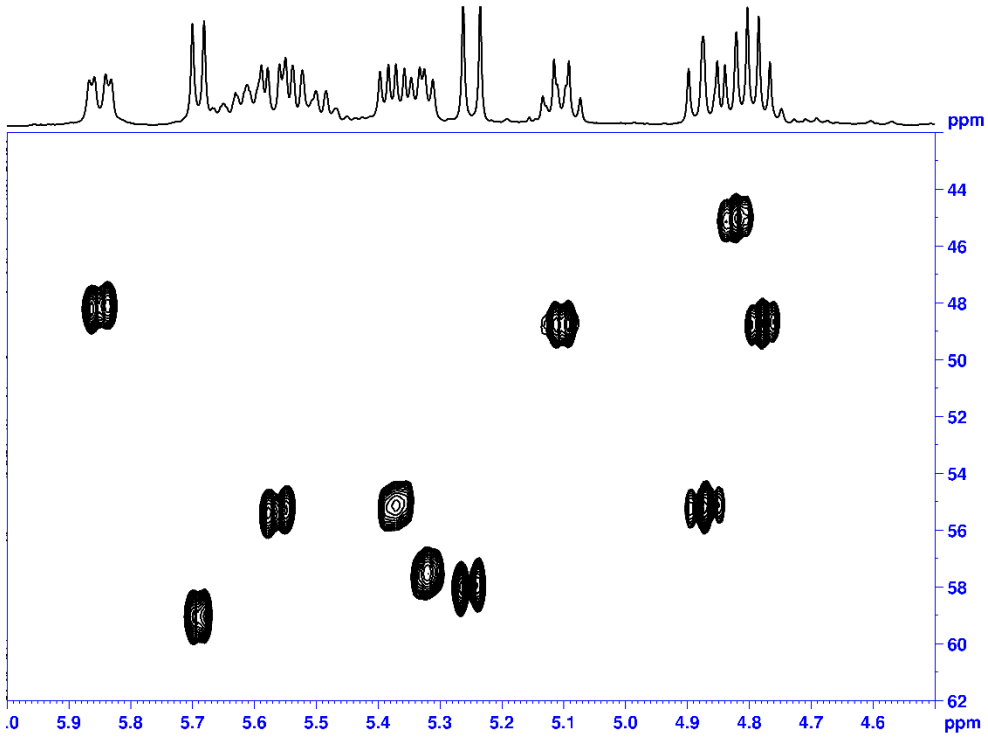


400 MHz ^{13}C spectrum of a 50 mmol sample of Cyclosporine. 1024 scans, experiment time: 40 min.

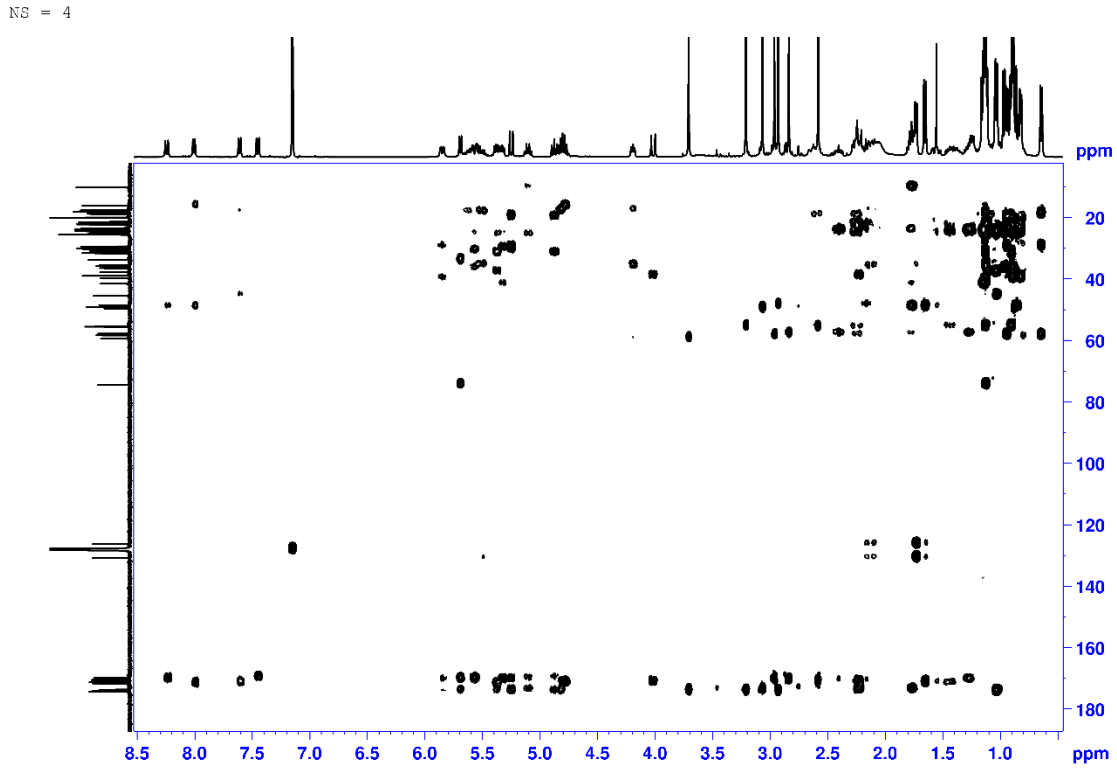


400 MHz 2D ^{1}H-^{13}C edited HSQC spectrum of a 50 mmol sample of Cyclosporine. 128 t_1 points, 2 scans, experiment time: 4,5 min. The signals of *CH* and *CH_3* are *positive (black*

contours), while CH_2 are *negative (red contours)*. *Quaternary carbons* do not provide signals in HSQC spectra!



Zoom of the 400 MHz 2D ^{1}H-^{13}C edited HSQC spectrum of a 50 mmol sample of Cyclosporine. All ^{13}C-shifts can be very easily read off the from the indirect dimension.



400 MHz 2D ^{1}H-^{13}C HMBC spectrum of a 50 mmol sample of Cyclosporine. 128 t_1 points, 4 scans, experiment time: 10 min. *Quaternary carbons* do provide signals in HMBC spectra!

C.I have only enough for a 10 mM sample. Can I still get decent data?

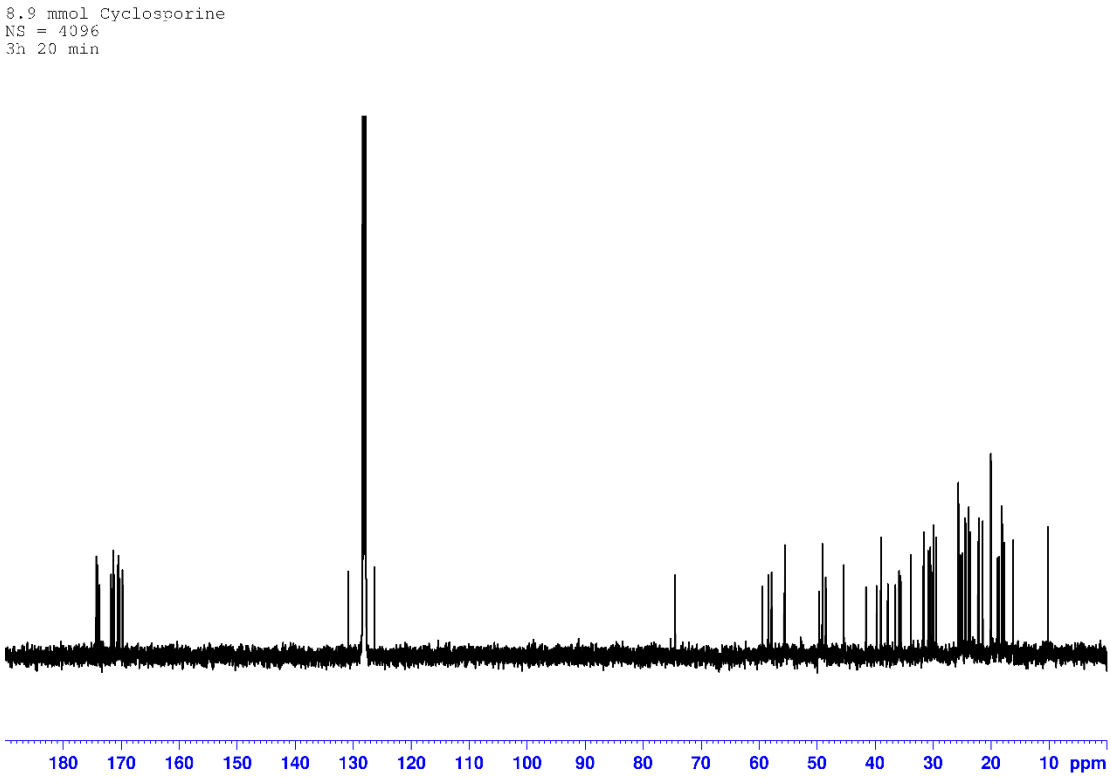
Yes, but the necessary measurement time will be significantly longer (and so the costs for your boss...), especially if you absolutely want the ^{13}C APT(DEPTQ) spectrum.
Remember that compared to the 50 mmol sample, the APT spectrum now needs to be recorded with *25'600 scans* to achieve a comparable result !

- **This takes approximately 17 hours !.**

If you (and your boss...) can make do with a lower-quality ^{13}C-APT spectrum (more noise), a ^{13}C-APT spectrum on a 10 mM sample can of course be recorded in 6 hours. The resulting signal-to-noise (intensity of the resonance, basically) will be ~**half as much** as compared to a 17-hour spectrum.

An example of what we consider a decent ^{13}C spectrum is shown below for a 9 mmol cyclosporine sample.

- decent: all carbons are visible, but the S/N is sometimes poor, and the noise is intense.



400 MHz ^{13}C spectrum of a 9 mmol sample of Cyclosporine. 4096 scans, experiment time: 3h20 min.

At 400 MHz, if you expect a <u>decent</u> ^{13}C-spectrum**,** we recommend a concentration of no less than **<u>10 mM</u>** of your molecule in a deuterated solvent.

For a typical organic molecule this is about **0.6-3 mg**.

Molecular mass (g)	100	200	300	400	500
Required quantity (mg) for 0.6 mL for a 10 mM solution	0.6	1.2	1.8	2.4	3

All proton-detected experiments including COSY, HSQC and HMBC are still possible at relatively low concentrations!

For a 10 mM sample we recommend ns=4, ns=8 and ns=16 for COSY, HSQC and HMBC, respectively.

For a 5 mM sample we recommend ns=8, ns=16 and ns=32 for COSY, HSQC and HMBC, respectively.

At 2.5 mM a good HMBC will take too long, but COSY and HSQC will still be of good quality.

D. I have only a 5 mmol solution of my compound. Can I get a ^{13}C spectrum? 2D spectra?

- For the ^{13}C spectrum, if you consider what we have said above, the answer is **no**, unless:
  You're willing to get a (very)poor carbon spectrum.

- For the 2D spectra, the answer is **yes:**

It means that you should be prepared to characterize your compound only with 2D spectra (COSY, HSQC, HMBC with limitations). Note again that most of the time the ^{13}C-spectrum is superfluous and not even needed for complete characterization because one can read off the ^{13}C-shifts from the indirect dimension of the HSQC and HMBC spectra.