Natural-abundance ^{13}C Connectivity Experiments in Cryogenically-cooled Probes

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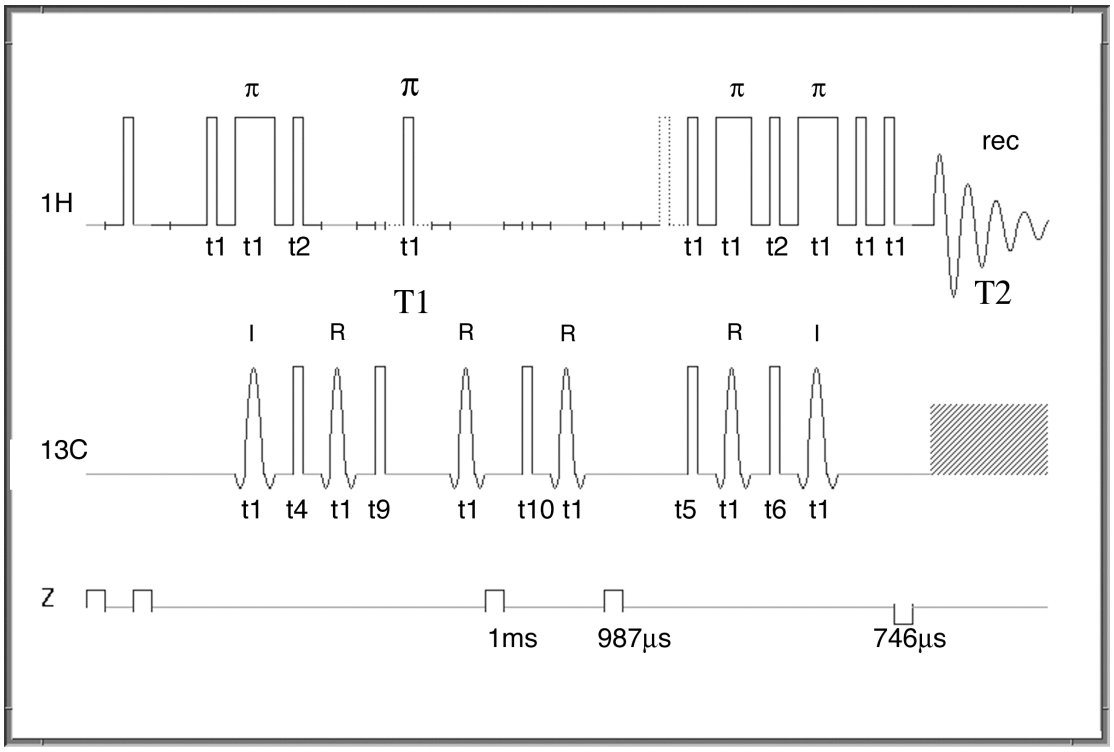
At natural abundance, ^{1}H detected experiments designed to provide ^{13}C – ^{13}C connectivity information require very high sensitivity and excellent stability. To date, the most robust experiment for this purpose is the ADEQUATE[1,2] pulse sequence that affords ^{13}C – ^{13}C connectivity data via ^{1}H detection. Cryogenically cooled NMR sample probes provide ~ 3 to 4 fold gains in sensitivity compared to conventional sample probes and this added performance affords significant advantages that are of special significance with ^{1}H detected ^{13}C – ^{13}C pulse sequences such as ADEQUATE. Inspection of the pulse sequence (Figure 1) reveals two ^{13}C inversion pulses and four ^{13}C refocusing pulses. The requirement for uniform ^{13}C refocusing pulses encompassing the entire carbon spectrum means that as the spectrometer field strength is increased the refocusing requirement becomes more demanding. Poor refocusing effects are cumulative so ideal pulses for this purpose are critical for good NMR performance in a pulse sequence with so many refocusing pulses. The refocusing requirements in 2D ADEQUATE are so demanding that even at 500 MHz, simple square ^{13}C refocusing pulses can not the used in a practical manner with acceptable sensitivity in experiments with typical ^{13}C bandwidth requirements. The lower power requirement for ^{13}C field strength in ColdProbes relative to conventional HCN probes provides another real advantage with ADEQUATE in such cooled probes. Experiment code available in user library as adequate_AD.tar.Z

This document shows representative 1,1-ADEQUATE results obtained quickly with relatively small samples because of the overall increased performance afforded by ColdProbe technology. We find that < 10 μM of solid sample is sufficient for good results in ~ 7 hours in a 500 MHz ColdProbe. A robust pulse sequence utilizing both adiabatic inversion pulses and shaped composite refocusing pulses tuned for optimal performance at 500 MHz is presented.

1,1-ADEQUATE[1,2] pulse sequence used in these Experiments



Phases:

t1 = 0
t2 = 1
t4 = 0 2
t5 = 0 0 2 2
t6 = 1 1 3 3
t9 = 0 0 0 0 2 2 2 2
t10 = 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
rec = 0 2 2 0 2 0 0 2 2 0 0 2 0 2 2 0

I = adiabatic "cawurst" inversion pulse 420µs
R = "av180b" refocusing pulse 324µs
13C power @ level for 14 µs 90 for all pulses

1H pw90 ~ 6.5 µs

Figure 1.
In this pulse sequence, all six of the ^{13}C 180 pulses have been replaced by either adiabatic inversion, or broadband refocusing pulses, as required. The near-ideal inversion/refocusing affords a dramatic increase in sensitivity. Whereas the adiabatic CAWURST[3,4] inversion pulses are self-compensating and insensitive to Rf homogeneity or calibrations, the av180b[5] refocusing pulses require accurate power calibration, and good phase and amplitude linearity from both the probe and amplifier to give the best performance.

The Problem – Simple ^{13}C 180's do not have sufficient bandwidth to cover required bandwidth.

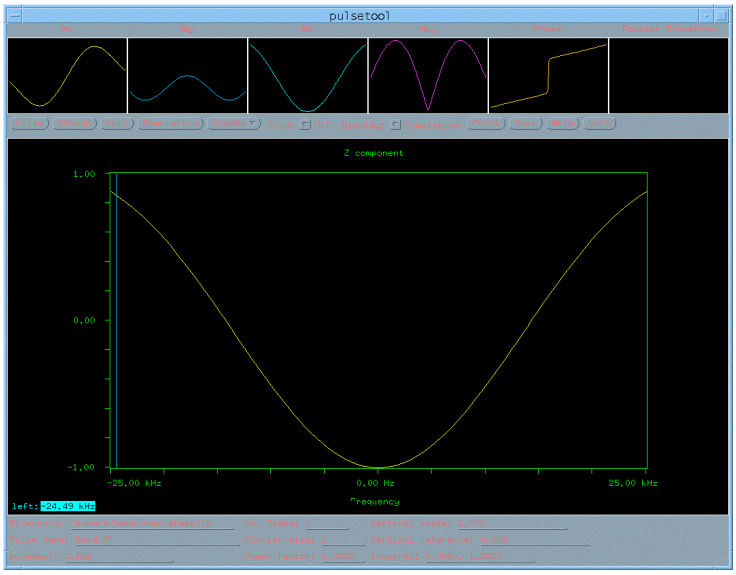


Figure 2a. Inversion profile obtained with a 24 μs square pulse B1 =20.8 KHz.

The Solution – Use shaped pulses to achieve much greater bandwidth at the same power level

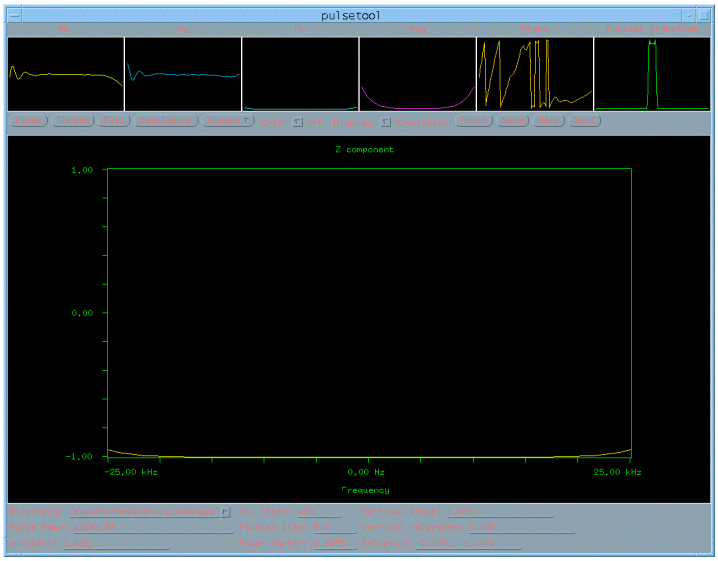


Figure 2b. Inversion profile for a CAWURST[3,4] adiabatic 180 with B1 = 17.8 KHz.

Refocusing is even more demanding!

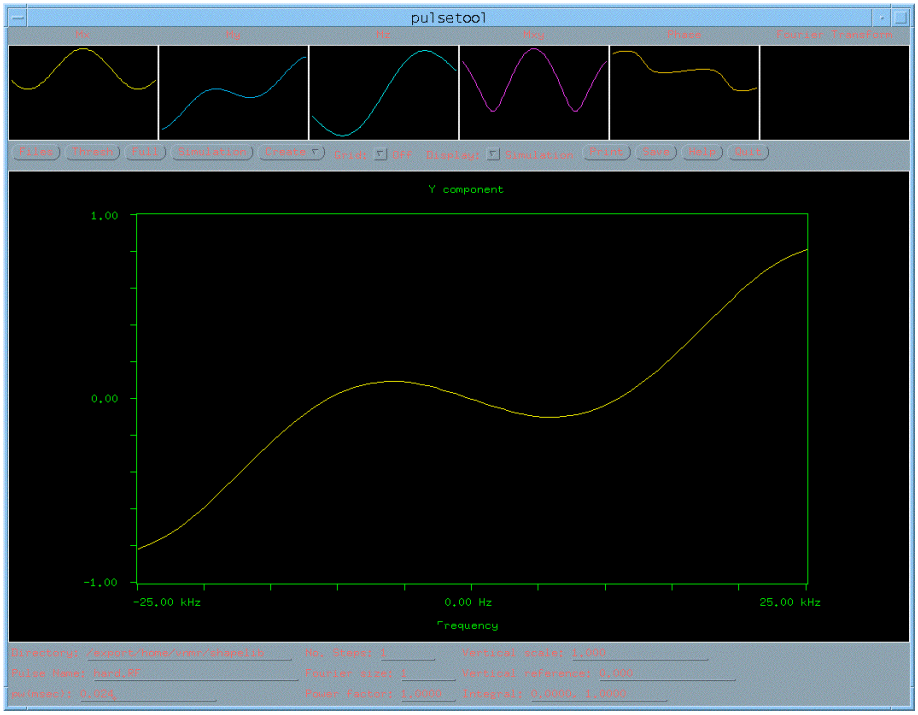


Figure 3a. Refocusing profile for a square 20.8 KHz pulse.

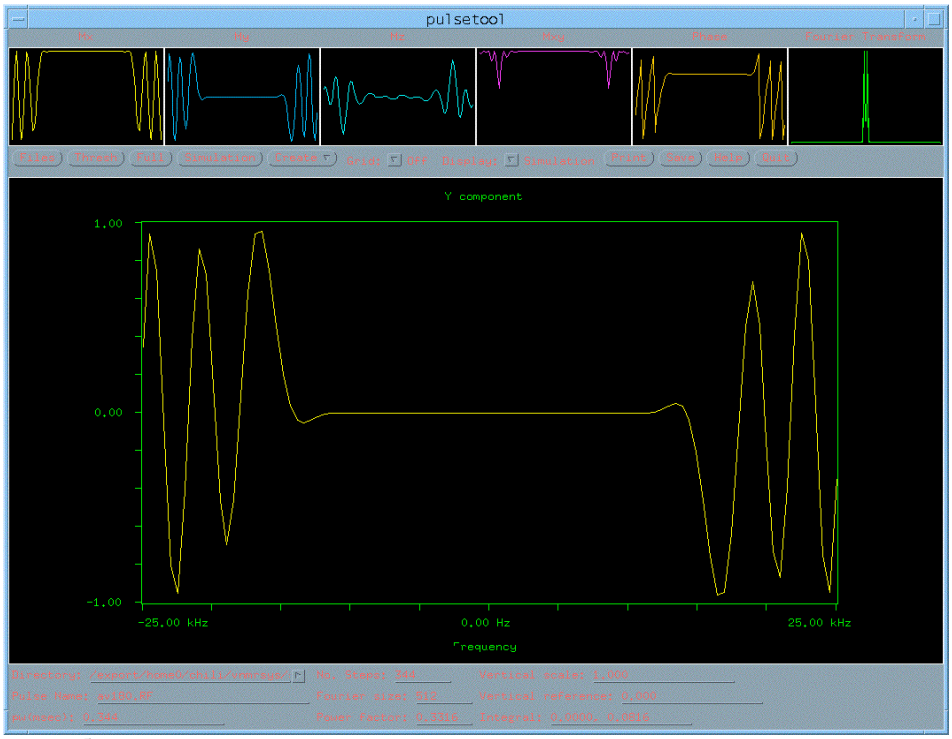


Figure 3b. av180b[5] refocusing profile for a 17.8 KHz pulse.

1D Spectra of Strychnine MW = 334; 3mg = ~9 μMoles

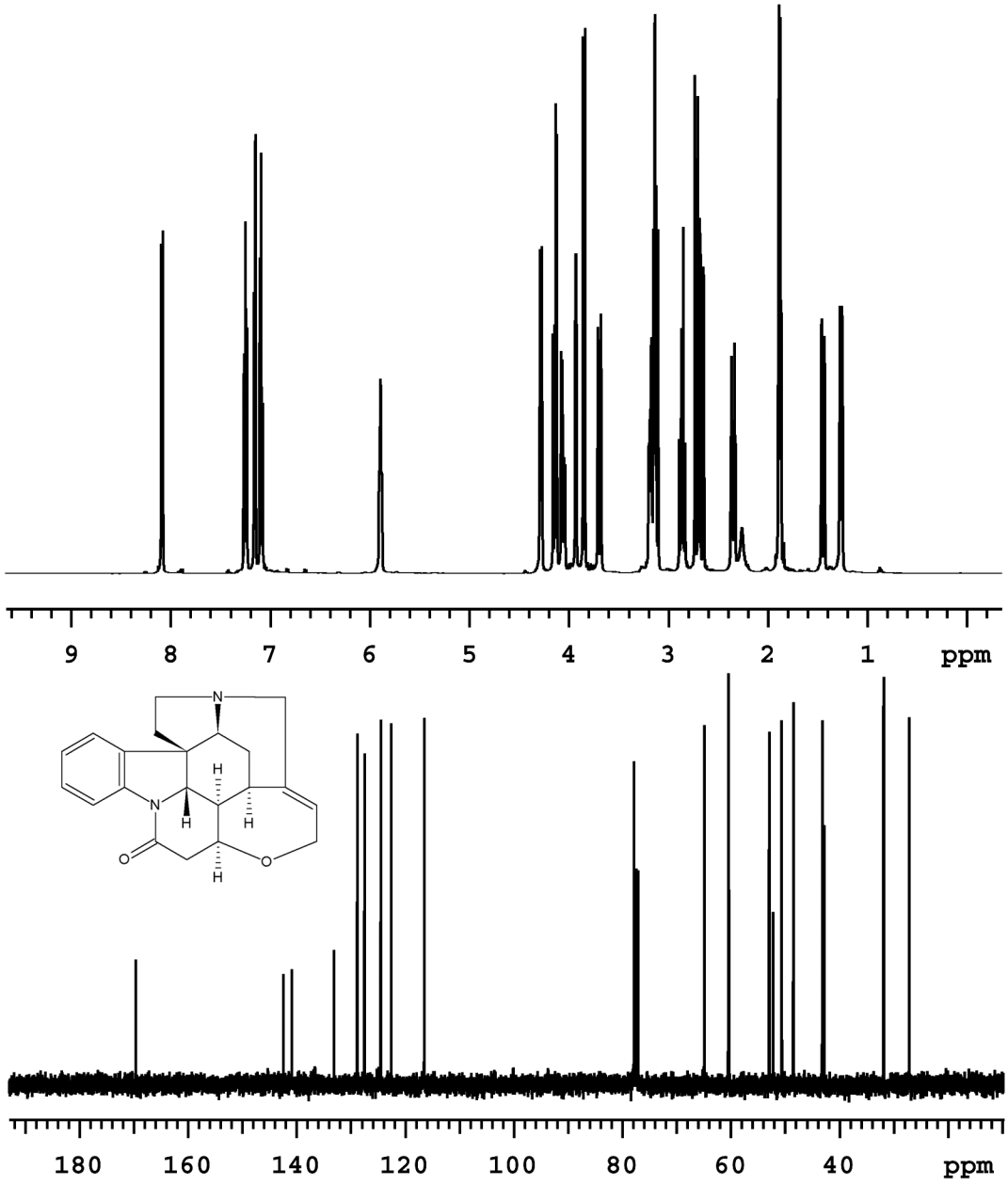


Figure 4.

Top – Four scan ^{1}H spectrum with 3 mg sample of strychnine.
Bottom – 1000 scan ^{13}C spectrum on 3 mg sample of strychnine.

Data acquired on 500 MHz HCN ColdProbe. ^{1}H pw90 = 6.5μs @ ~10 watts, ^{13}C pw90 set at 14 μs @ ~60watts.

2D 1,1-ADEQUATE results – 3 mg Strychnine @ 14 hours

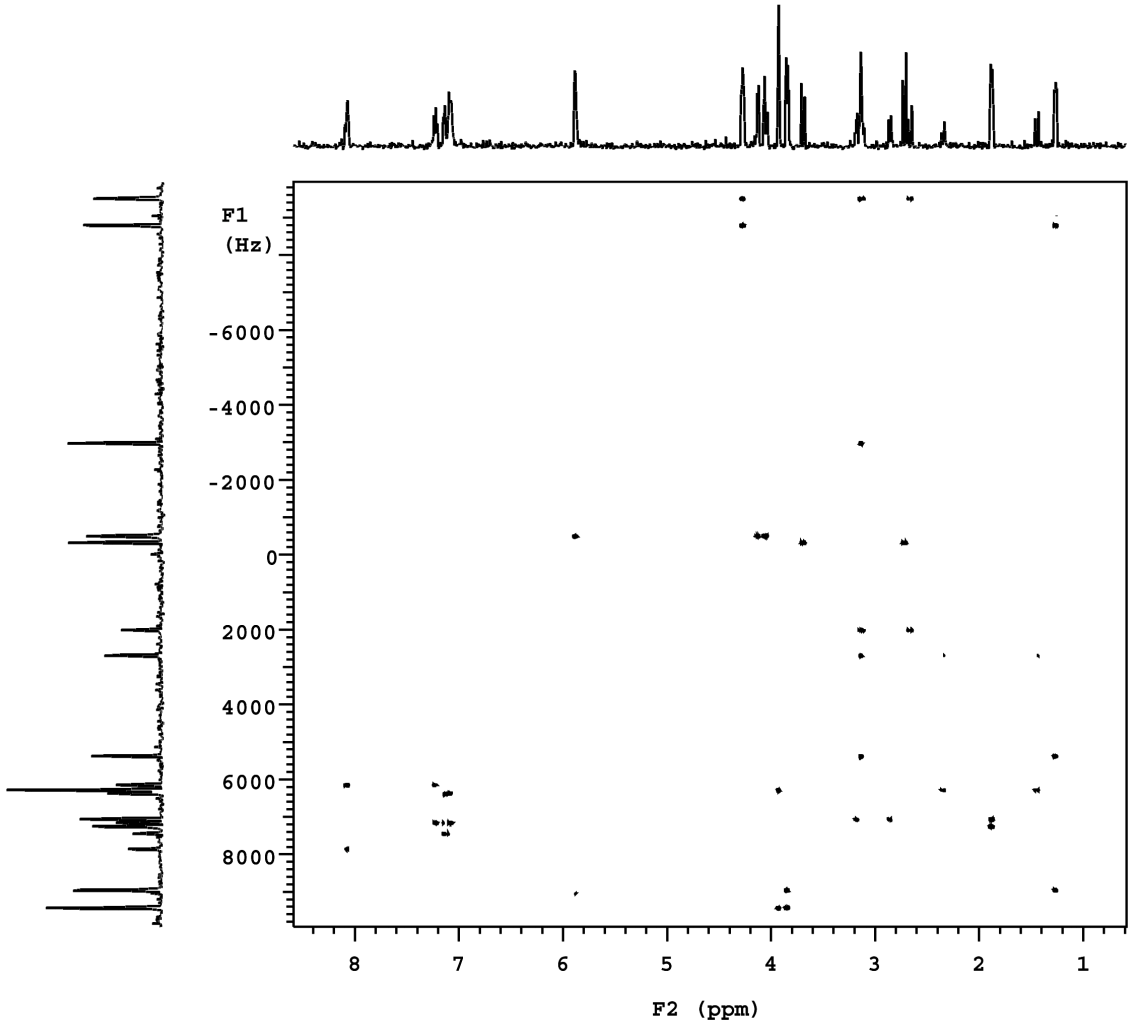


Figure 5. Data acquired with 48 transients for 256 hypercomplex F1 pairs. 14 hours total acquisition time. Plot shown with projections of 2D dataset. ^{1}H-^{13}C polarization during the INEPT transfers set at 1.6 ms; ^{13}C-^{13}C transfer delays set at 6.2 ms. Data acquired with a 500 MHz ColdProbe.

2D 1,1-ADEQUATE results – 3 mg (9 μMoles) Strychnine @ 14 hours - Plotted near noise floor

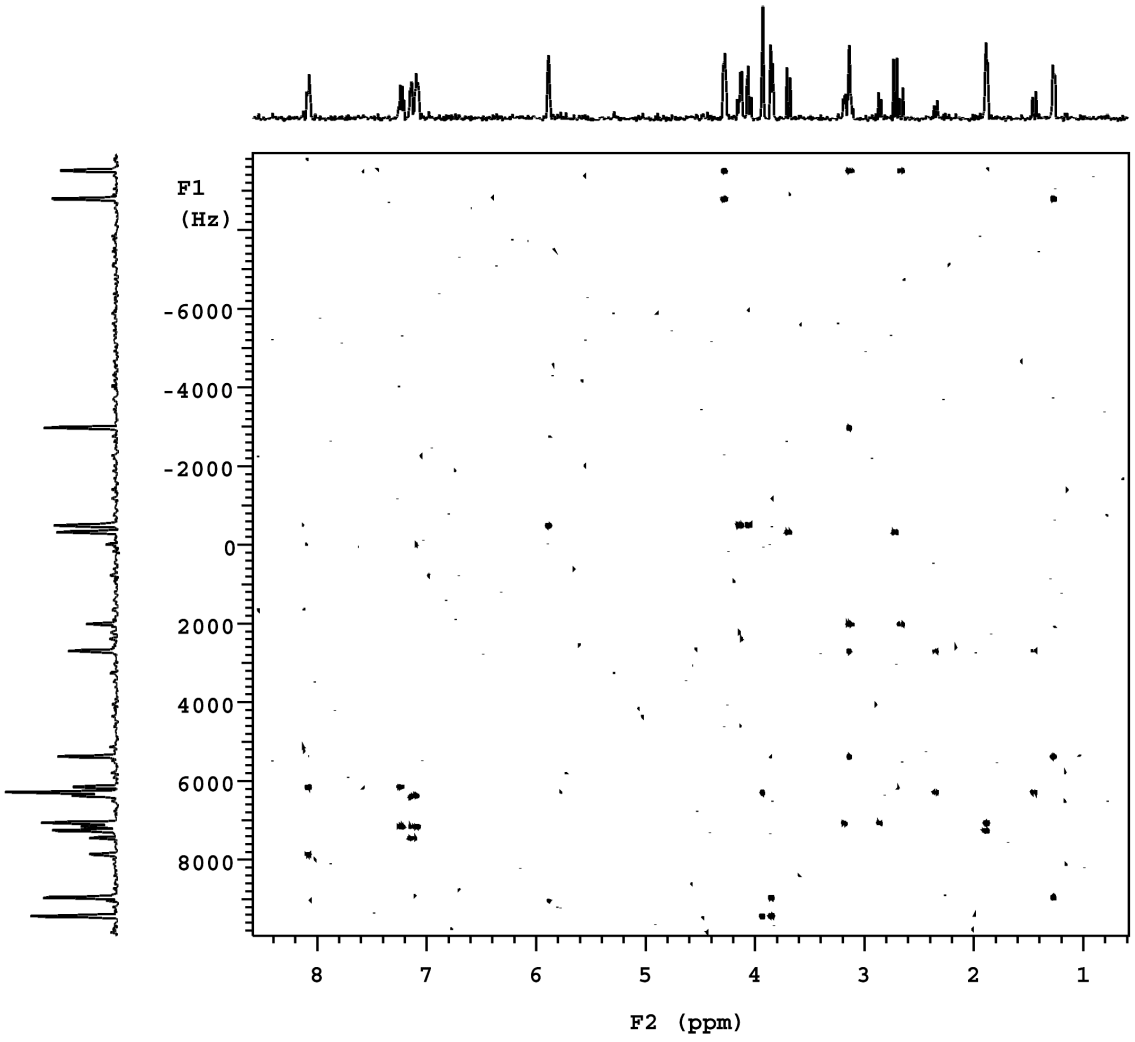


Figure 6. This data is the same experiment in the previous figure but has been plotted to show the noise floor. All expected responses are observed in both figure 4 & 5.

2D 1,1-ADEQUATE on 3mg strychnine in 7 hours

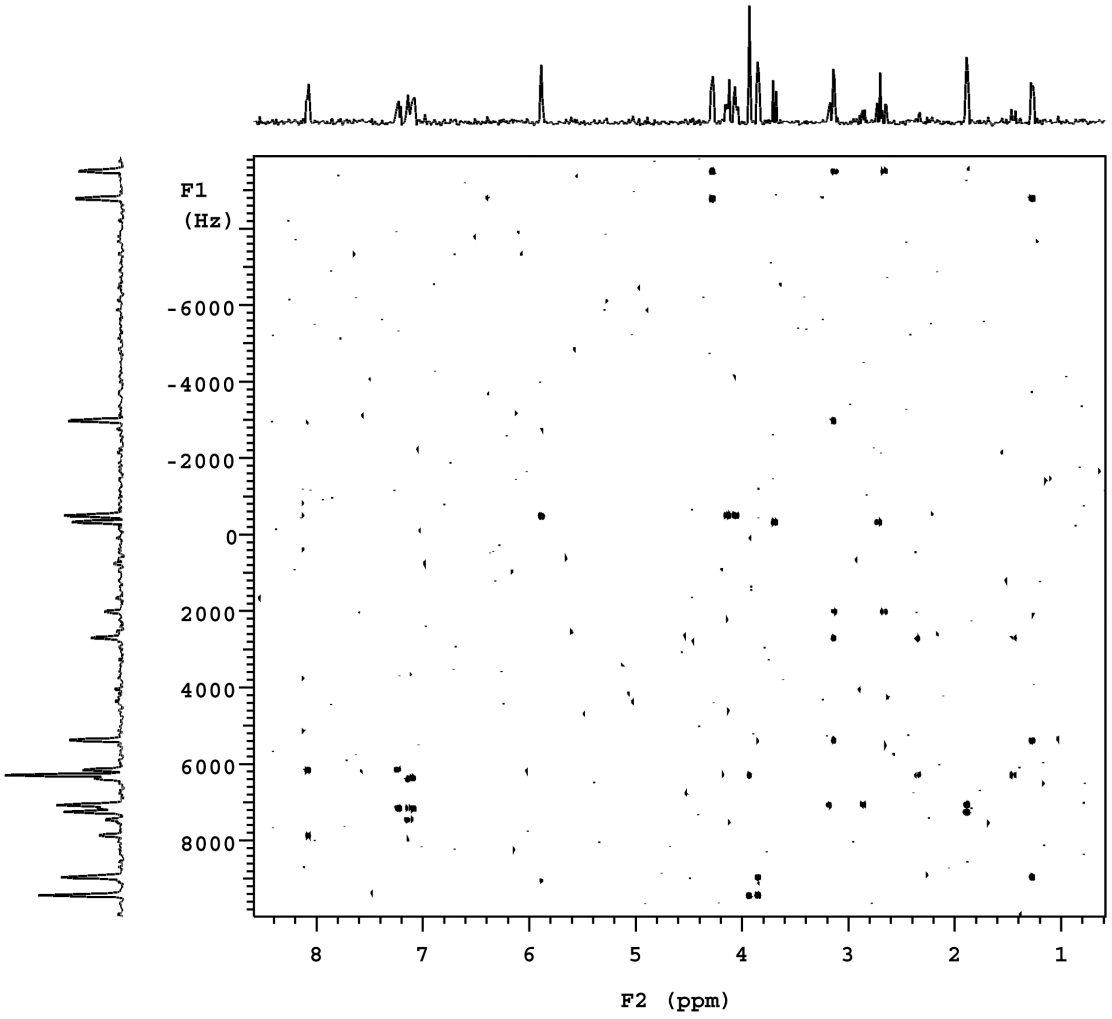


Figure 7. 2D 1,1-ADEQUATE data acquired in 7 hours with the same 3mg sample of strychnine used in previous figures. Data was acquired with 48 transients but with 128 t1 increments rather than the 256 increments shown in the data acquired in 14 hours. Data acquired using a 500 MHz HCN ColdProbe. As in Figure 5, the plot is presented showing the noise floor. All expected responses are observed.

Comparisons of projections – 14 versus 7 hour 2D 1,1-ADEQUATE Data

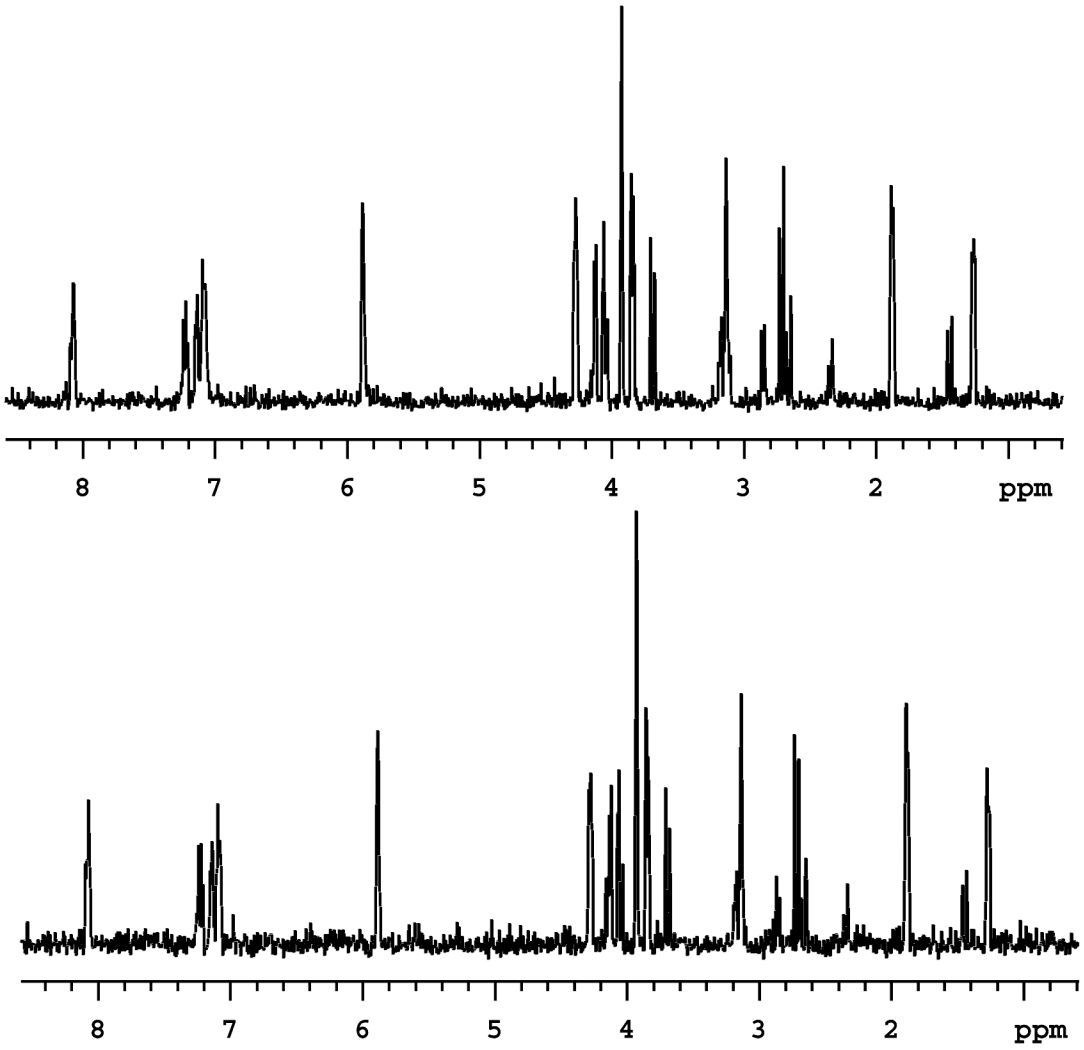


Figure 8. Plots showing the effect of F1 digital resolution on S/N in 2D 1,1-ADEQUATE acquired on a 3mg sample of strychnine in a 500 MHz HCN ColdProbe. **Top** – 14 hours (256 increments); **Bottom** – 7 hours (128 increments).

^{1}H & ^{13}C Spectra for 8.3 mg of Taxol, MW = 865; 9.5 μMoles

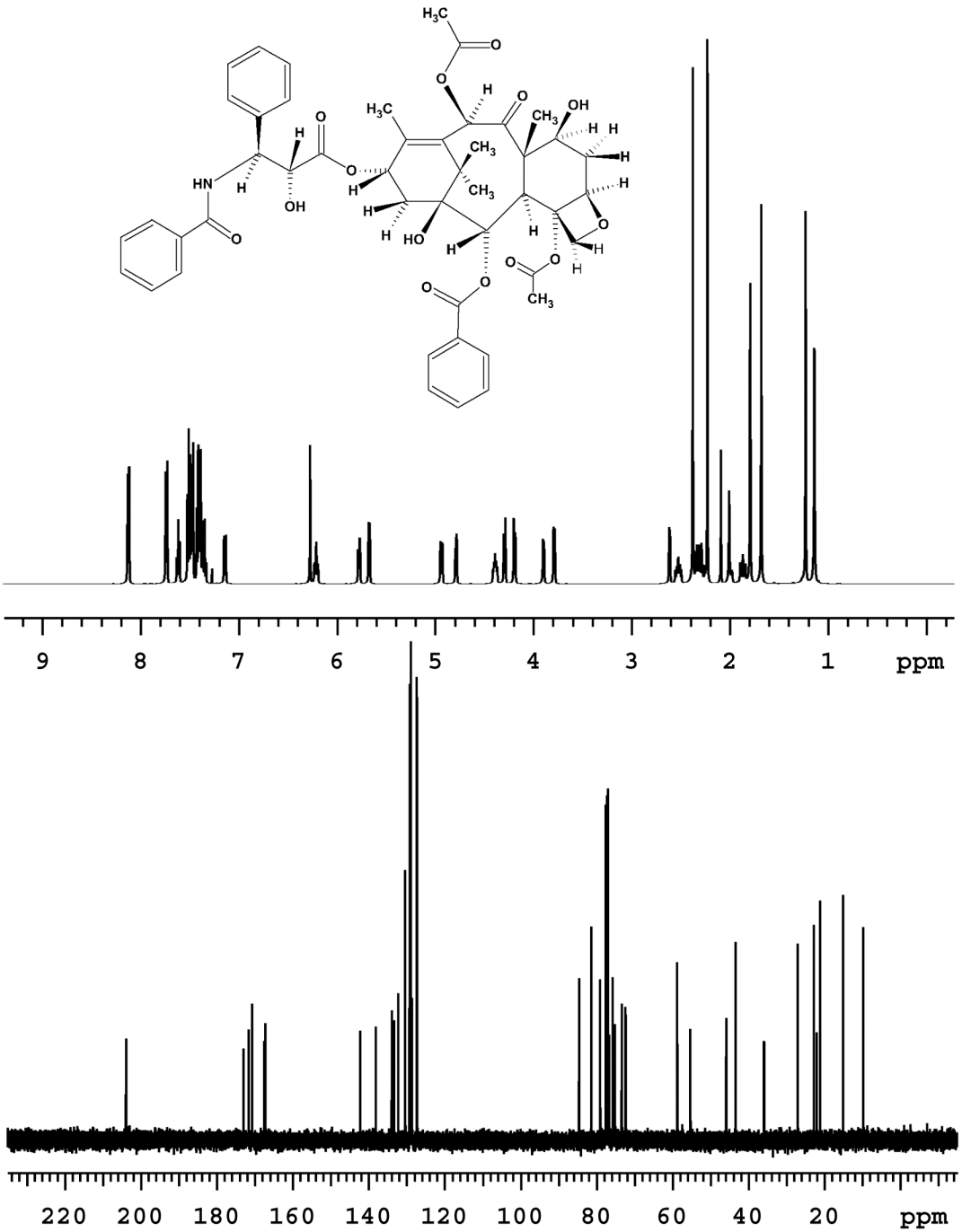


Figure 9. Top – ^{1}H spectrum for 8.3 mg Taxol, 4 scans, 500 Mhz ColdProbe
Bottom –^{13}C spectrum acquired in same HCN ColdProbe in 1 hour.

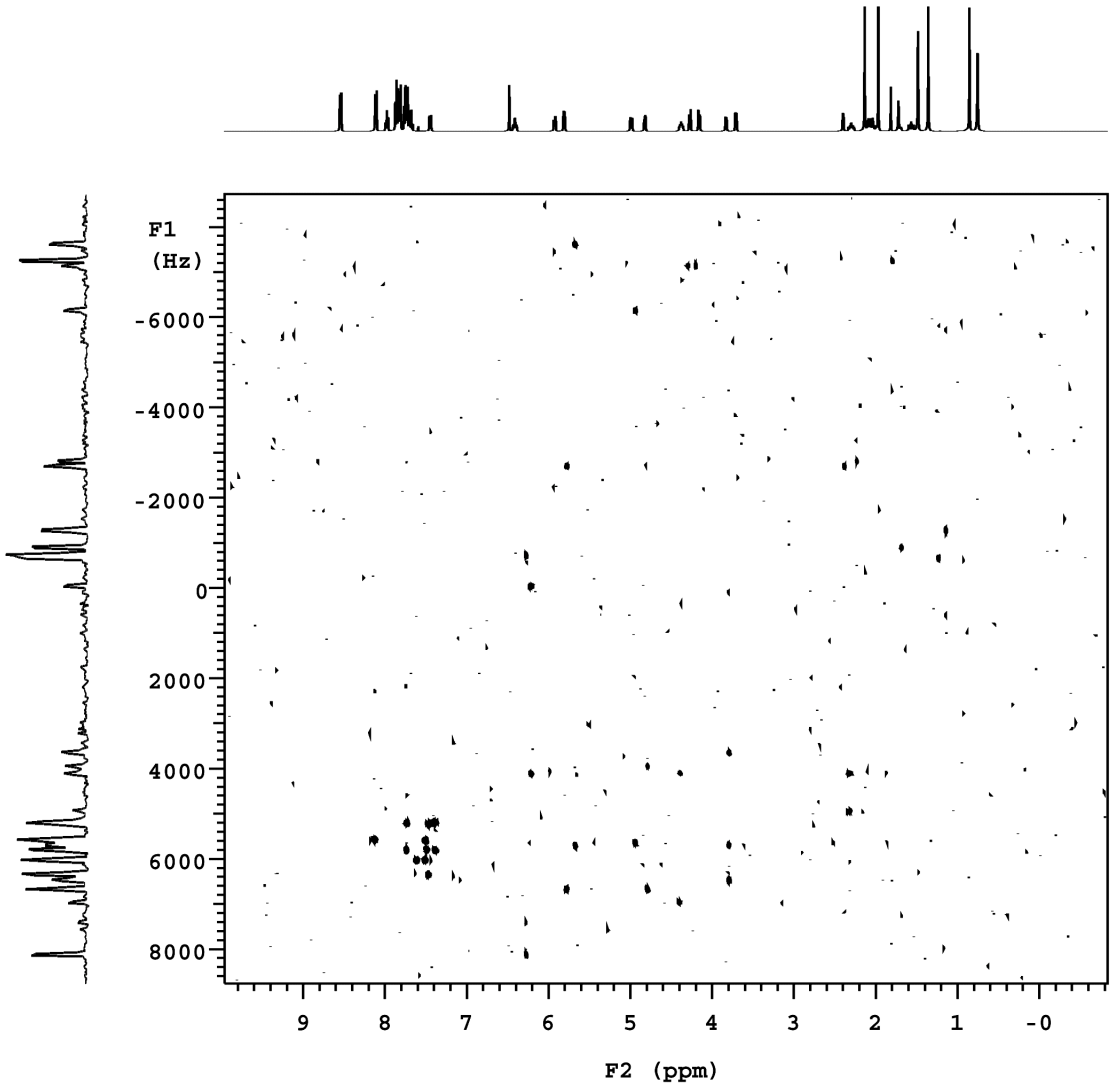


Figure 10. Top – 64 Scan 2D 1,1-ADEQUATE acquired on 8.3 mg Taxol in 7.5 hours using a 500 MHz ColdProbe. Data plotted with normal ^{1}H reference spectrum on top and F1 projection on the side. Data are presented at the noise floor to show the complete lack of T1 noise.

2D ADEQUATE - 8.3 mg (9.5 μMoles) Taxol - 7.5 hours.

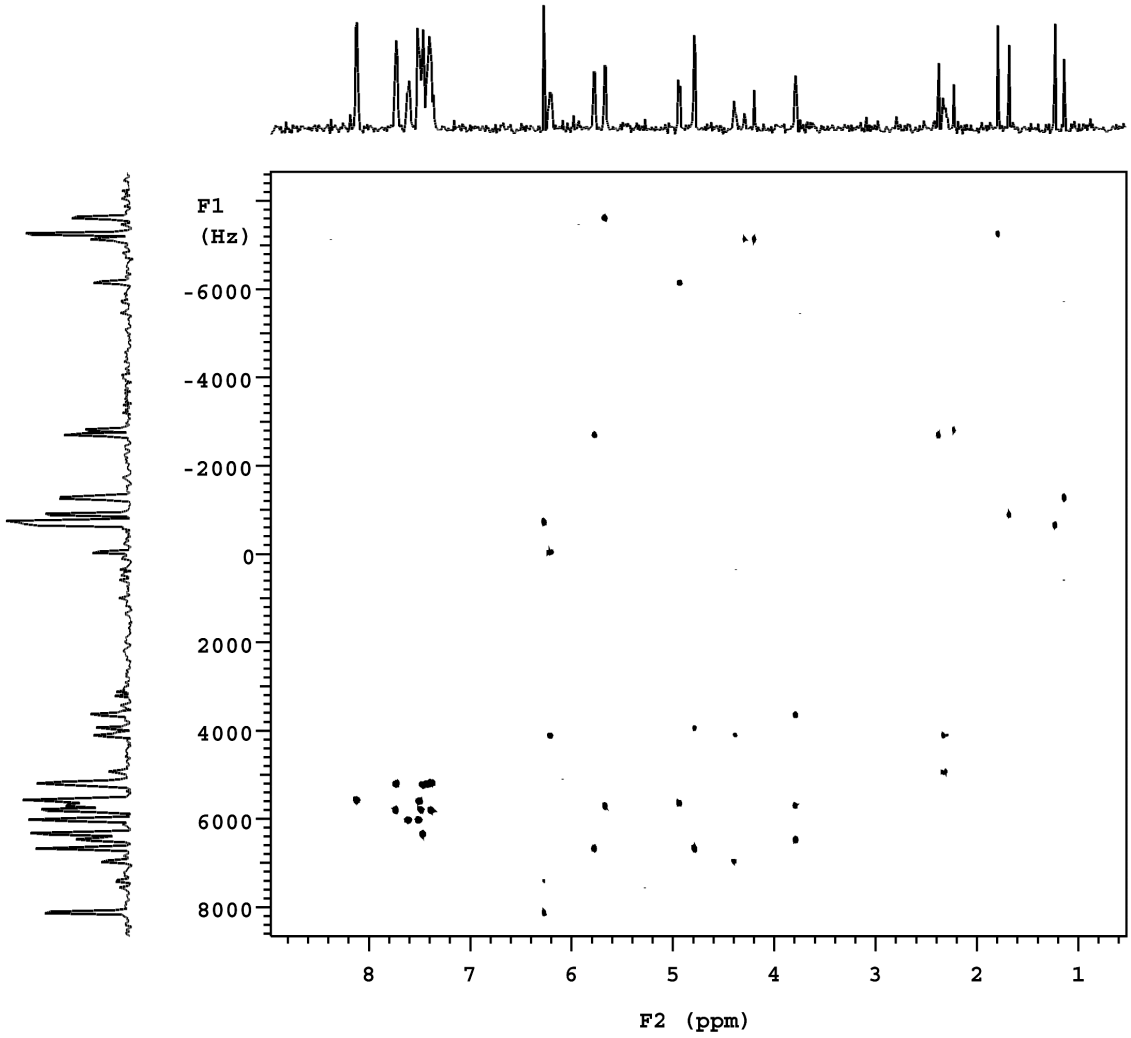


Figure 11. 2D 1,1-ADEQUATE acquired in 7.5 hours on an 8.3 mg sample of Taxol dissolved in $CDCl_3$. Data acquired in a 500 MHz ColdProbe in 64 transients with 128 hyper-complex pairs in F1. All expected responses are observed. Same data as Figure 10 but plotted above the noise floor.

References

1.) B. Rief, M. Koeck, R. Kerssebaum, H. Kang, W. Fenical and C. Griesinger, *J. Magn. Reson. A* **118**, 282 (1996).

2.) M. Koeck, B. Rief, W. Fenical and C. Griesinger : *Tetrahedron Lett.* **37**(3), 363 (1996).

3.) E. Kupce and R. Freeman, *J. Magn. Reson. A* **118**, 199 (1996).

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5.) Abramovich and Vega, *J. Magn. Reson. A* **105**, 30 (1993).