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SUPPORTING INFORMATION

Title: Structural Elucidation with NMR Spectroscopy: Practical Strategies for Organic Chemists
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Menthol: Sample Experimental Procedures

Introduction

The following gives detailed instructions on how to acquire and process a full set of NMR experiments under realistic conditions using menthol as a test sample (sample spectra are included below). These procedures can be carried out using any modern NMR spectrometer and should be applicable to most unknowns likely to be encountered on a routine basis. The expected experimental times are indicated for each experiment; the experiments can be performed sequentially overnight, or individually, time permitting.

General Experimental

Data were acquired using a Varian INOVA 500 MHz spectrometer and a standard 5 mm Z-gradient-capable inverse-detection probe (any comparable inverse-detection, or proton-optimized probe setup should be sufficient). Spectra were processed using ACD/NMR Processor (version 9.0). (–)-Menthol was used as received from Aldrich. Abbreviations: at, acquisition time (s); d1, delay between scans (s); ni, number of time increments; nt, number of transients or scans; j1xh, estimated one-bond carbon-proton coupling constant (Hz); jnxh, estimated multiple-bond carbon-proton coupling constant (Hz); mix, mixing time (s); F1, the indirectly detected dimension (carbon for HSQC and HMBC and proton for COSY-45 and HETLOC); and F2, the directly detected dimension (proton); FT, Fourier transform; and LP, linear prediction.

Initial Setup **(30 min)**

An 18 mM solution of (-)-menthol (2.0 mg, 13 μmol) in 700 μL $CDCl_3$ was prepared in a standard 5 mm NMR tube. Standard solvent parameters were selected and the spectrometer was locked and shimmed. The spectrometer was tuned (this operation varies between spectrometers; proper training should be obtained before attempting this simple, but delicate procedure). The lock level was noted and a one-scan 1D ^1H spectrum was recorded while spinning. The spinning was then stopped. If the lock level dropped more than 10%, the shims Z1, X, Y, XZ, and YZ were adjusted followed by Z1, Z2, and the lock phase. The lock power was slowly increased until the lock level stopped increasing, and then decreased slightly.

The proton and carbon 90° pulses were adjusted using to the following procedure. A one-scan 1D spectrum was obtained at the estimated 90° pulse width and phased appropriately. A similar spectrum was then obtained with a pulse width four times as large. The pulse width was increased slightly if negative peaks were obtained and decreased if positive peaks were obtained, and another

spectrum was acquired. This process was repeated until the spectrum was mainly noise. The corrected 90° pulse width was set to one quarter of this final pulse width.

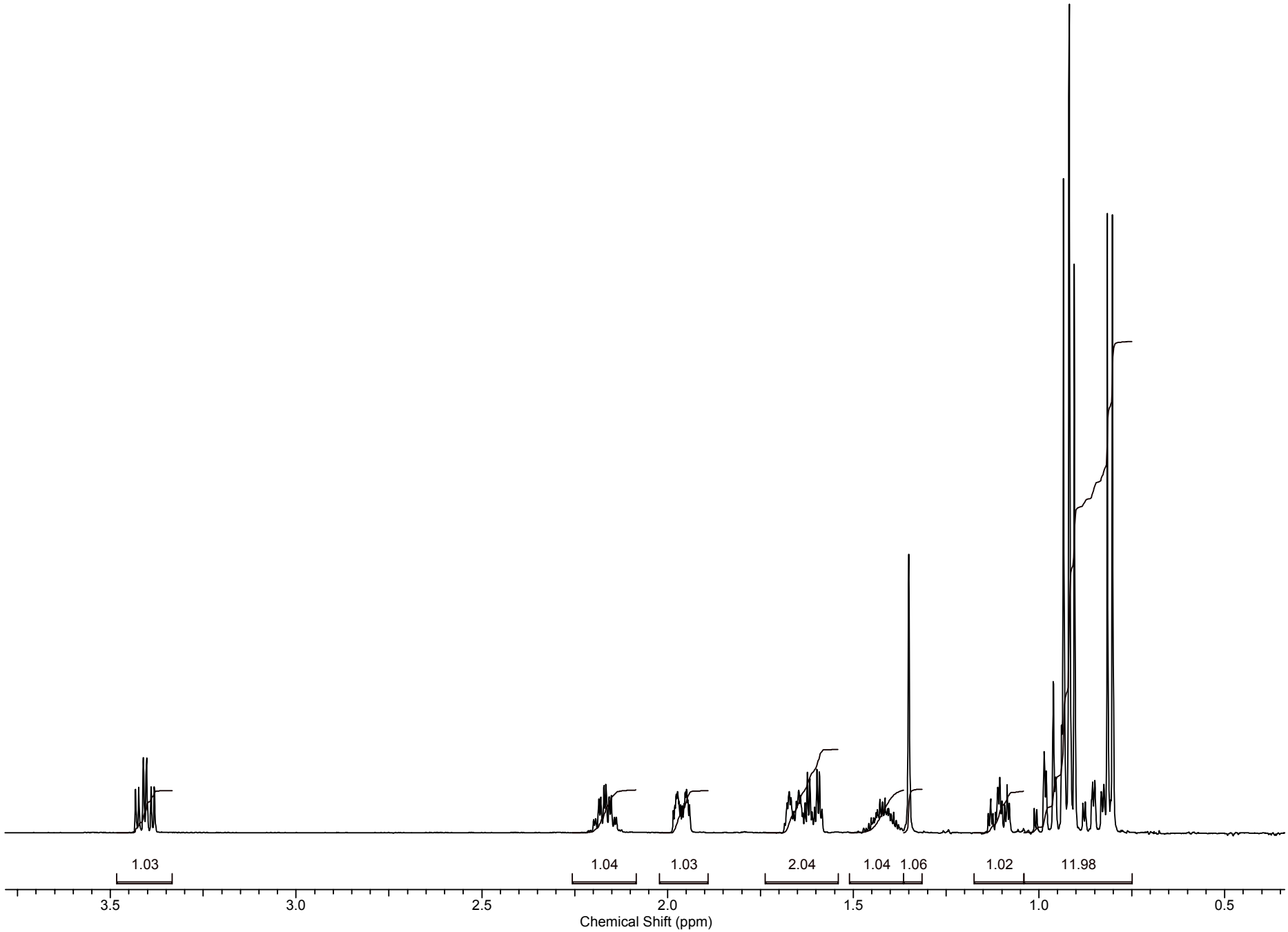
Acquisition

The following experiments were run using a proton spectral window from 0 to 4.5 ppm and a carbon spectral window from 0 to 85 ppm without sample spinning (spinning causes artifacts through Q-modulation; Hodgson, C.M., Comina, P.J. *Tet. Lett.* **1996**, *37*, 5613-5614.). In general, the spectral window should enclose the entire spectrum, leaving at least a 0.5 ppm margin on either side; however, omitting the chloroform peak usually does not cause a problem. **HSQC (110 min):** A gradient-selected, phase-sensitive HSQC spectrum was obtained in hypercomplex mode with the following parameters: at=0.2, d1=1.0, ni=64, nt=40, and j1xh=125. **HMBC (176 min)**. A gradient-selected absolute-value HMBC spectrum was obtained with the following parameters: at=0.2, d1=1.0, ni=64, nt=16, j1xh=125, and jnxh=8. **COSY-45 (13 min)**. A gradient-selected, absolute-value COSY spectrum was obtained with the following parameters: at=0.3, d1=1.2, ni=128, and nt=4. **1D DPFGSE-NOESY (4 min).** A 1D DPFGSE-NOESY spectrum was obtained by selectively irradiating the region between 3.35 and 3.45 ppm with the following parameters: at=2.0, d1=1.0, nt=64, and mix=0.5. **HETLOC (261 min).** A gradient-selected sensitivity-enhanced HETLOC spectrum was obtained using the following parameters: at=0.3, d1=1.1, ni=128, j1xh=130, mix=0.06; G-BIRD^{r} pulse, on; $^{1}J_{CH}$ scaling factor, off; and reverse-tilting, off. **1D DPFGSE-TOCSY (4 min).** A 1D DPFGSE-TOCSY spectrum was obtained by selectively irradiating the region between 3.35 and 3.45 ppm with the following parameters: at=2.0, d1=2.0, nt=64, mix=0.06. The use of 1D-TOCSY to measure $^{n}J_{CH}$ is given below.

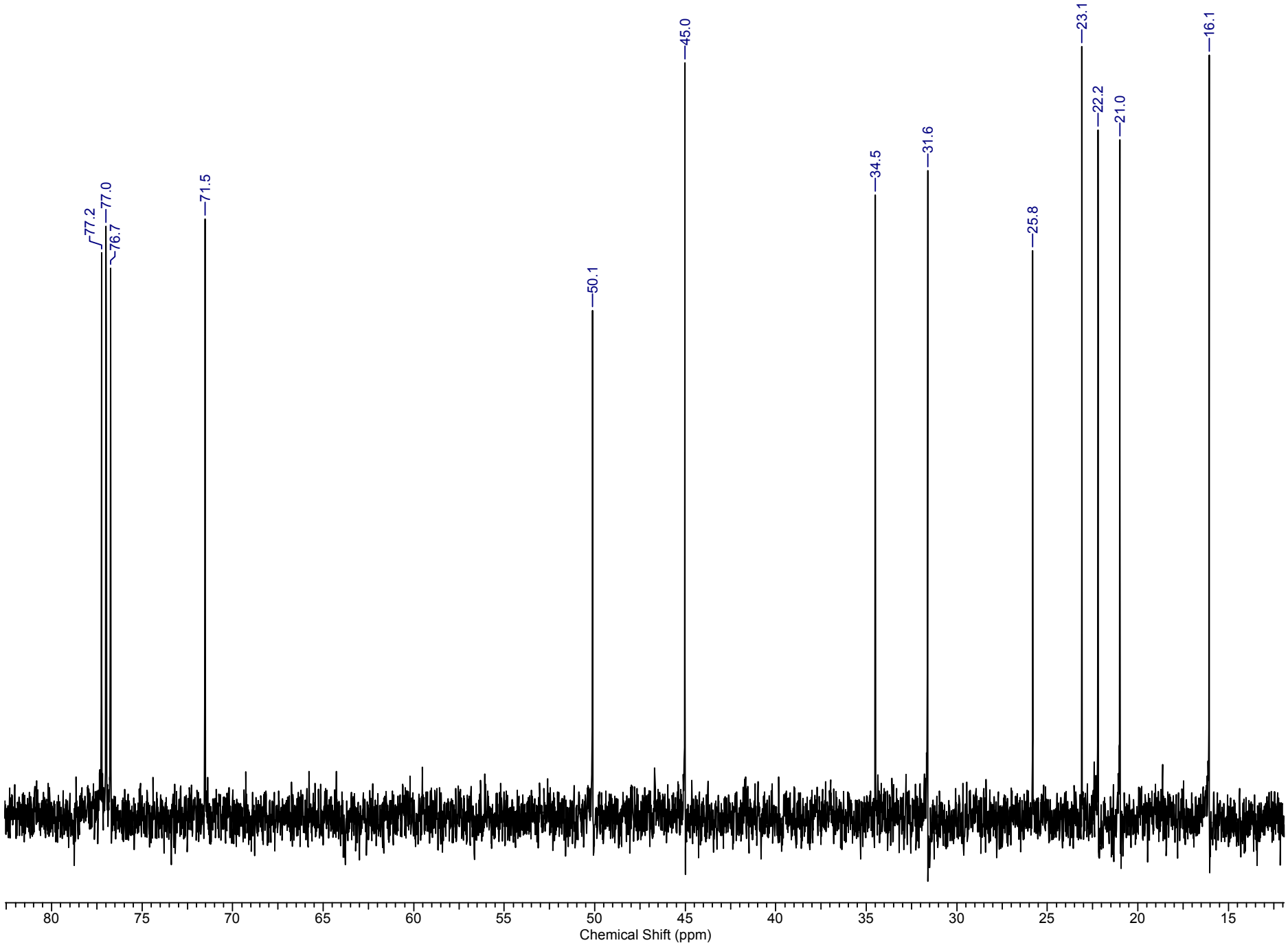
Processing

The spectra were processed with the following parameters: **HSQC**, 1024×1024 FT with LP in F1 from 32 to 512 and Gaussian apodization in F1 and F2; **HMBC**, 1024×1024 FT with LP in F1 from 64 to 256 and Gaussian apodization in F1 and F2; **COSY-45**, 1024×1024 FT, with LP in F1 from 128 to 256 and sine-bell squared apodization in F1 and F2; **1D DPFGSE-NOESY**, 65 536 point FT with exponential line-broadening apodization; **HETLOC**, 2048×2048 FT with LP in F1 from 128 to 1024 and Gaussian apodization in F1 and F2; and **1D DPFGSE-TOCSY**, 65 536 point FT with exponential line-broadening apodization. The weights of the apodization functions were interactively adjusted to match the decay of the FID. With 8 coefficients for LP in all cases, the 2D FTs took less than one minute. The HSQC and HETLOC spectra required minor phase adjustments. The appropriate coefficients must be used to FT HETLOC data (consult the pulse sequence instructions for details; typically, -1, 0, 1, 0, 0, -1, 0, -1).

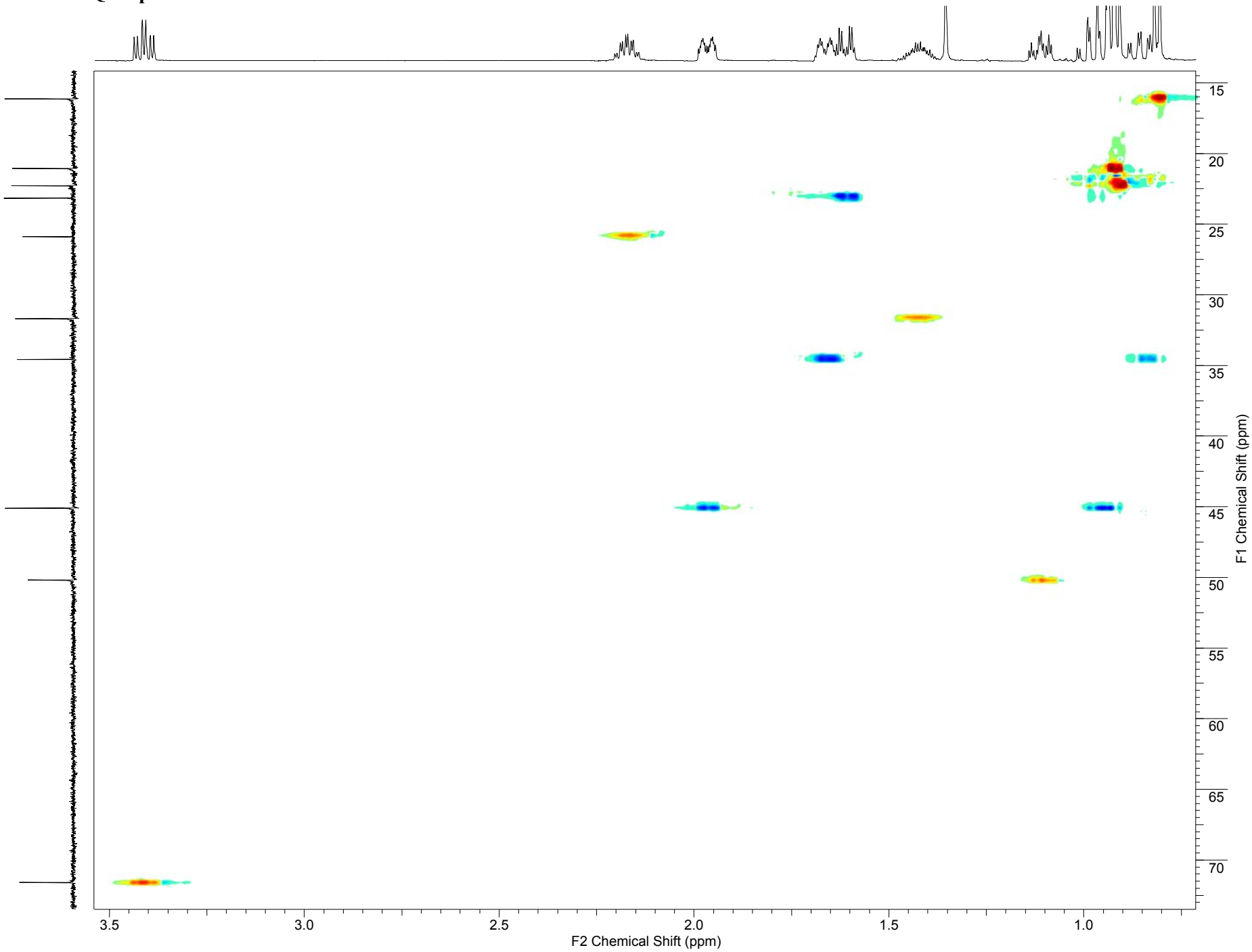
Menthol: 1D ^{1}H Spectrum



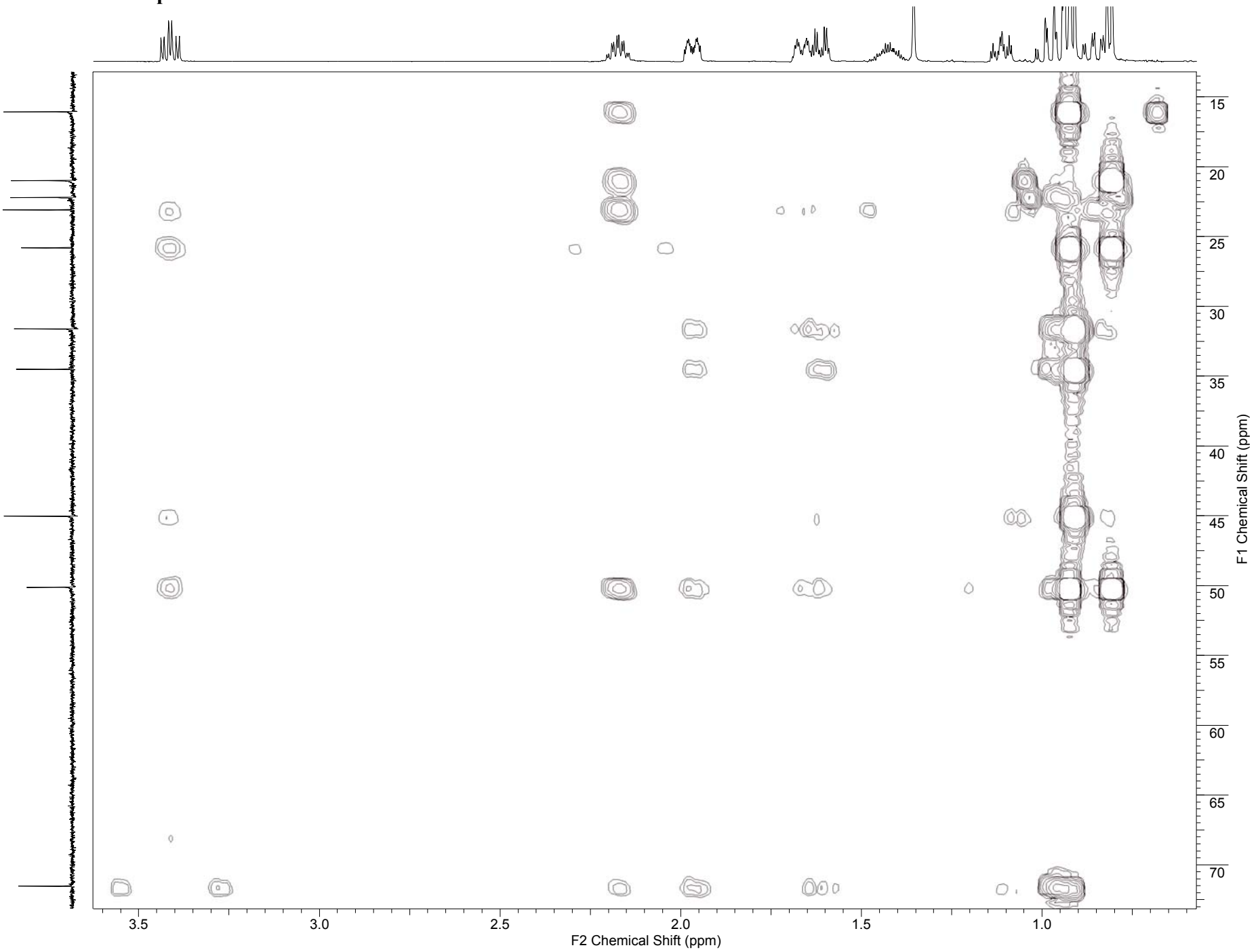
Menthol: 1D ^{13}C Spectrum



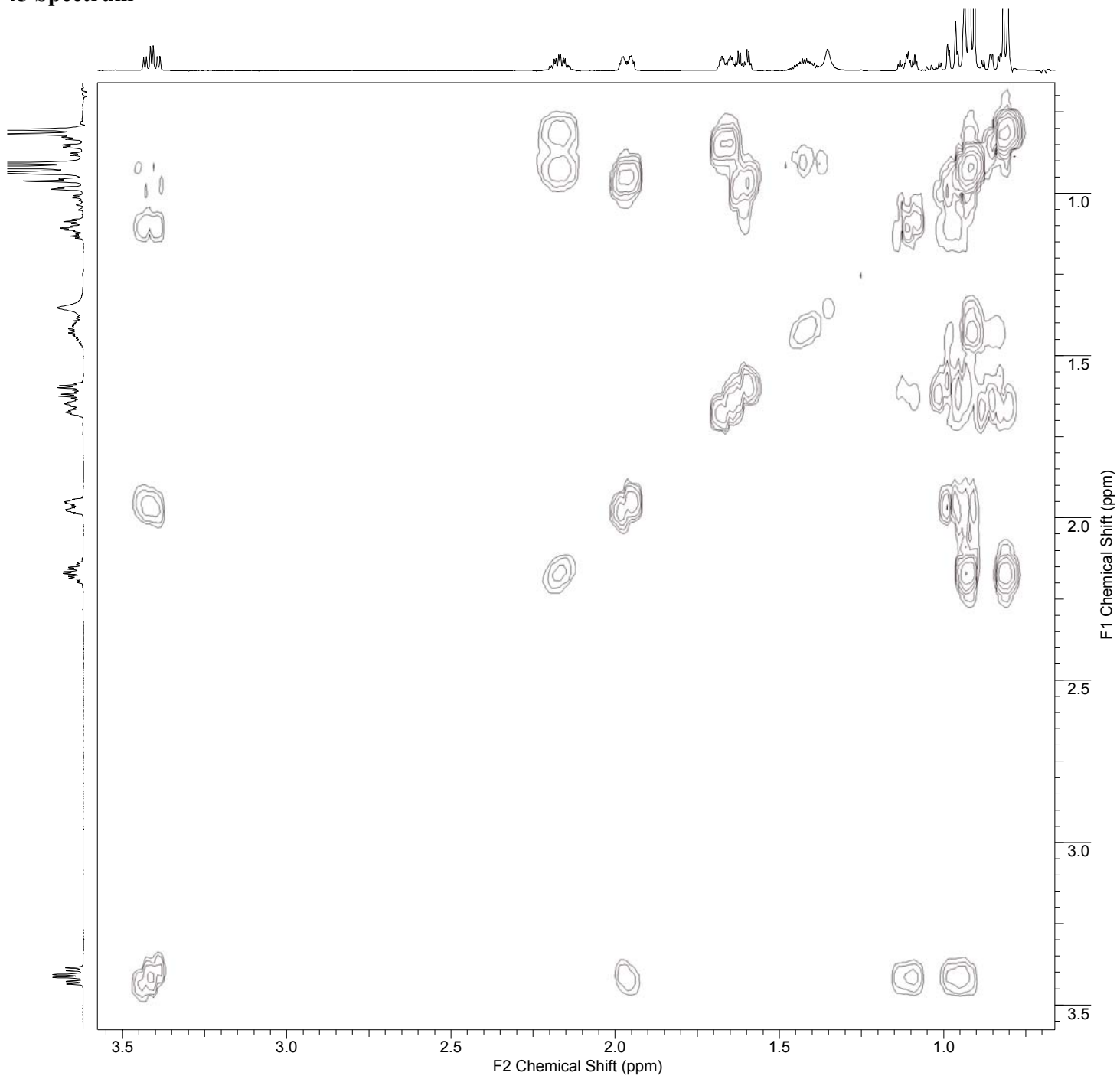
Menthol: HSQC Spectrum



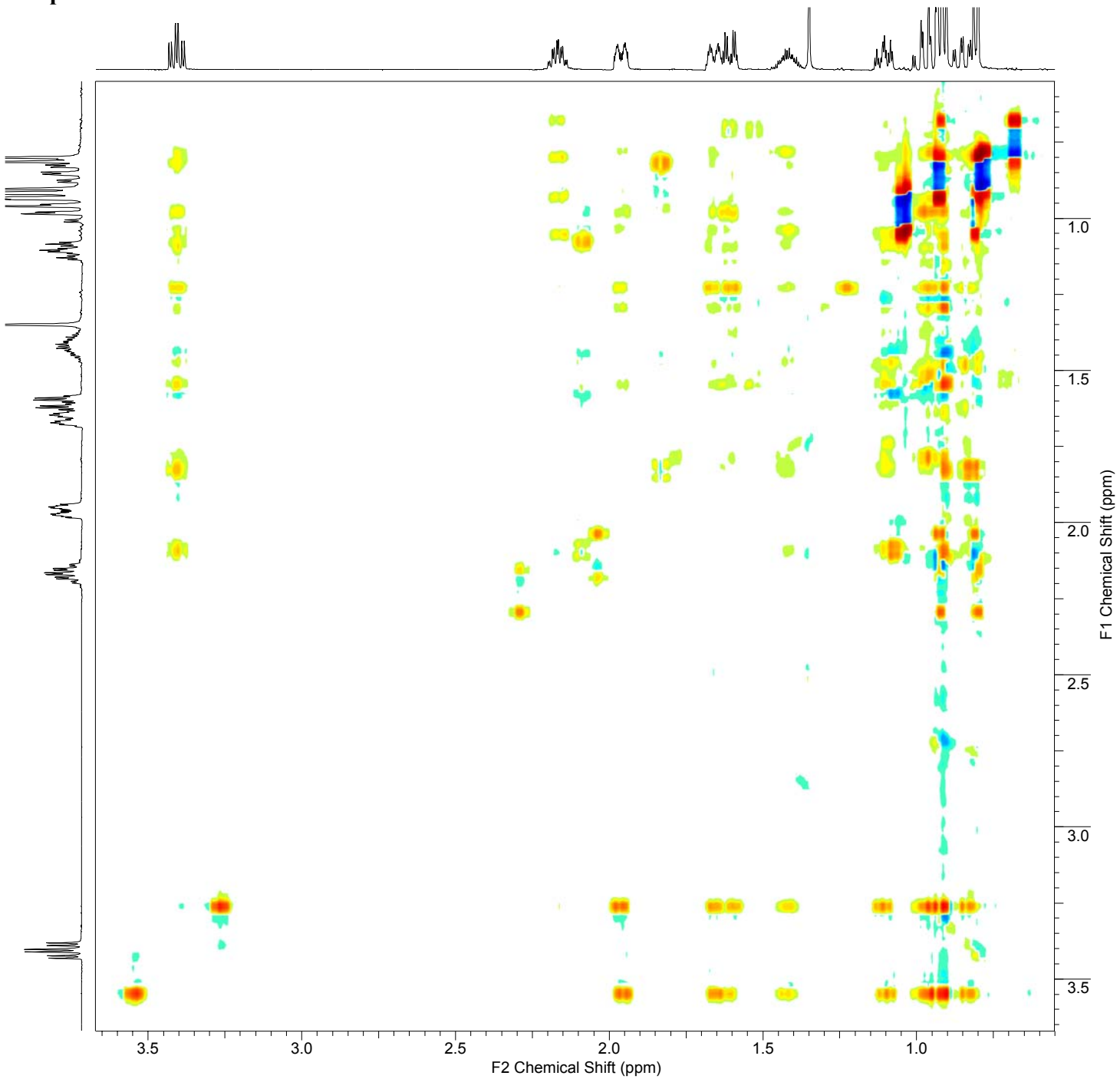
Menthol: HMBC Spectrum



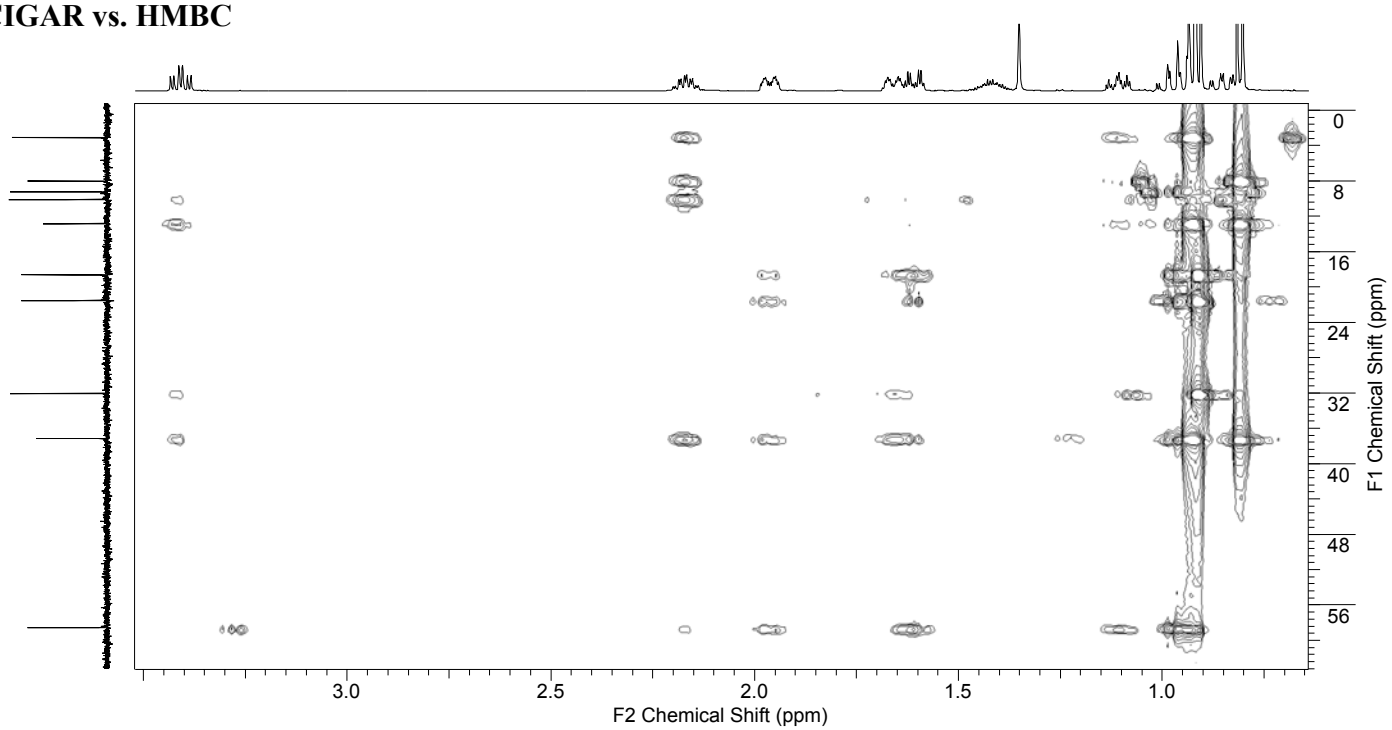
Menthol: COSY-45 Spectrum



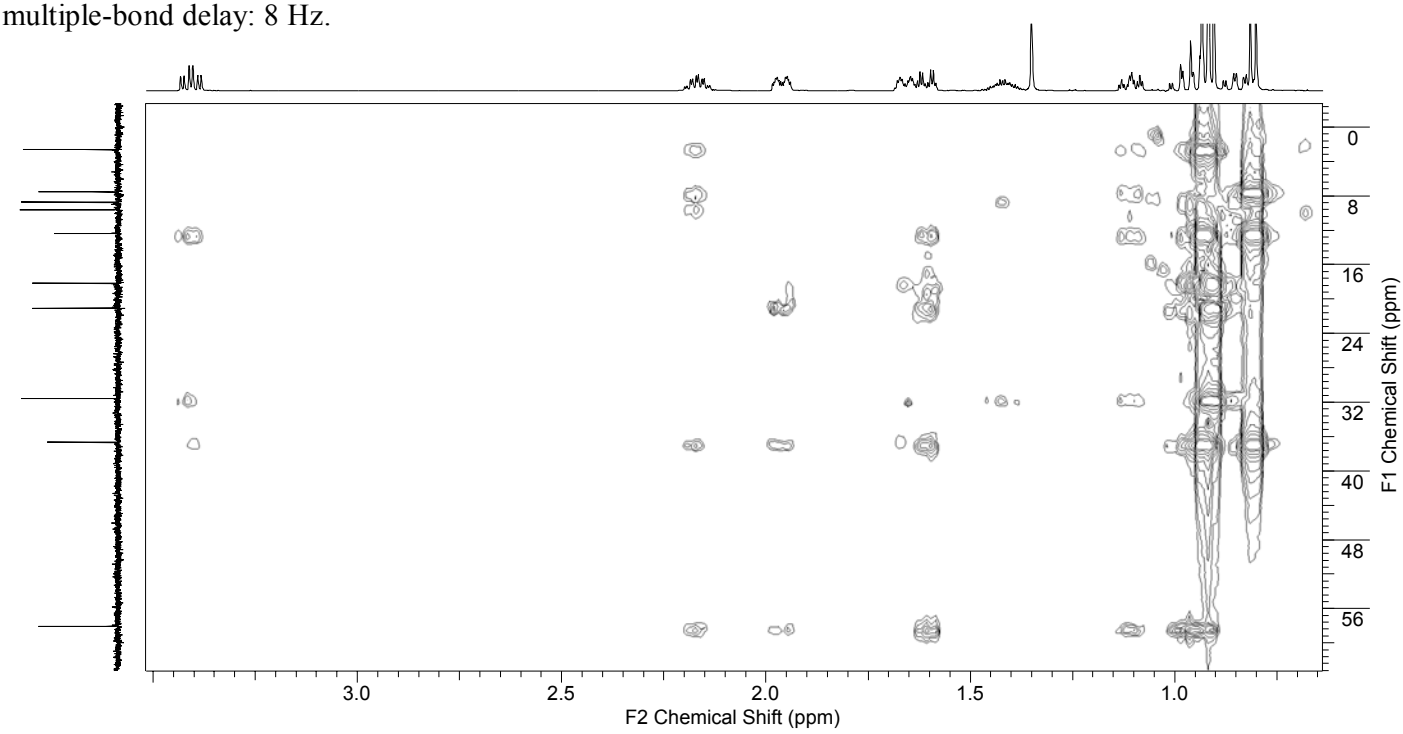
Menthol: HETLOC Spectrum



CIGAR vs. HMBC



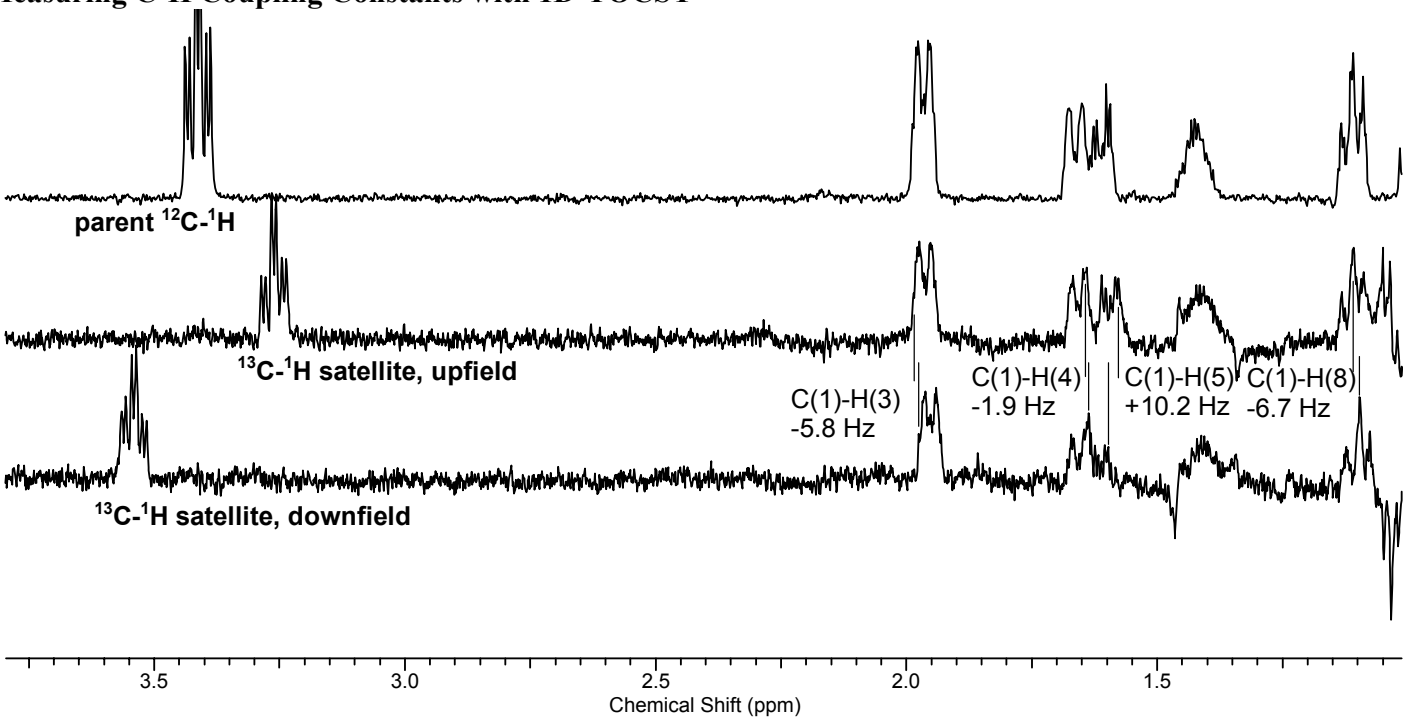
A. HMBC spectrm of menthol. 64 transients (176 min), 128 increments. One-bond supression: 125 Hz; multiple-bond delay: 8 Hz.



B. CIGAR spectrm of menthol. 232 transients (302 min), 64 increments. One-bond supression range: 120 to 145 Hz; multiple-bond delay: 4-10 Hz. One-bond correlations are better supressed while several more HMBC correlations are visible.

Comments. The spectra have similar S/N and resolutions. Based on the increased number of scans, and assuming that halving the number of increments increases S/N by ~41%, the CIGAR spectrum should have a S/N advantage of 2.7 times; therefore, CIGAR is much less sensitive than HMBC. Based on the number of increments, the CIGAR spectrum should have half the resolution of the HMBC spectrum; therefore, CIGAR resolves correlations much better than HMBC.

Measuring C-H Coupling Constants with 1D-TOCSY



For full details, see: Vidal, P., Esturau, N., Parella, T., Espinosa, J.F. *J. Org. Chem.* **2007**, *72*, 3166-3170.

Experimental Procedure:

A one-scan 1D ^{1}H spectrum of (-)-menthol (25 mM, $CDCl_3$) was obtained (500 MHz, 5 mm inverse-detected probe). The 1D-DPFGSE-TOCSY sequence was loaded (DPFGSE-TOCSY provided better results than the reported sequence). H(1) was chosen for selective irradiation (a window approximately 50 Hz wide, corresponding to a 80 ms pulse; g3 pulse shape). A 1D-TOCSY spectrum was obtained with the following parameters: acquisition time, 2.048 s; repetition delay, 2.0 s; transients, 16; mixing time, 0.06 s (advanced parameters: Z-filter, on; 90-homospoil gradient-90, on; spin-lock, MLEV-17; trim pulse, 2 ms; gradient levels, 3 and 4.5 G/cm; gradient times, 1.5 ms; trim pulse, gradient recovery time, 0.5 ms; and steady state scans, 4). This gave the top spectrum in the above figure. The upfield and downfield satellites were then successively irradiated using the same parameters and 128 transients to give the middle and bottom spectra.

Coupling constants were calculated by measuring the displacement of the multiplets in the satellite-irradiated spectra. The signs of the coupling constants are indicated by whether the upfield-irradiated correlation is shifted upfield (positive coupling) or downfield (negative coupling) relative to the downfield-irradiated correlation. As a check, notice that one-bond coupling constants are always positive.

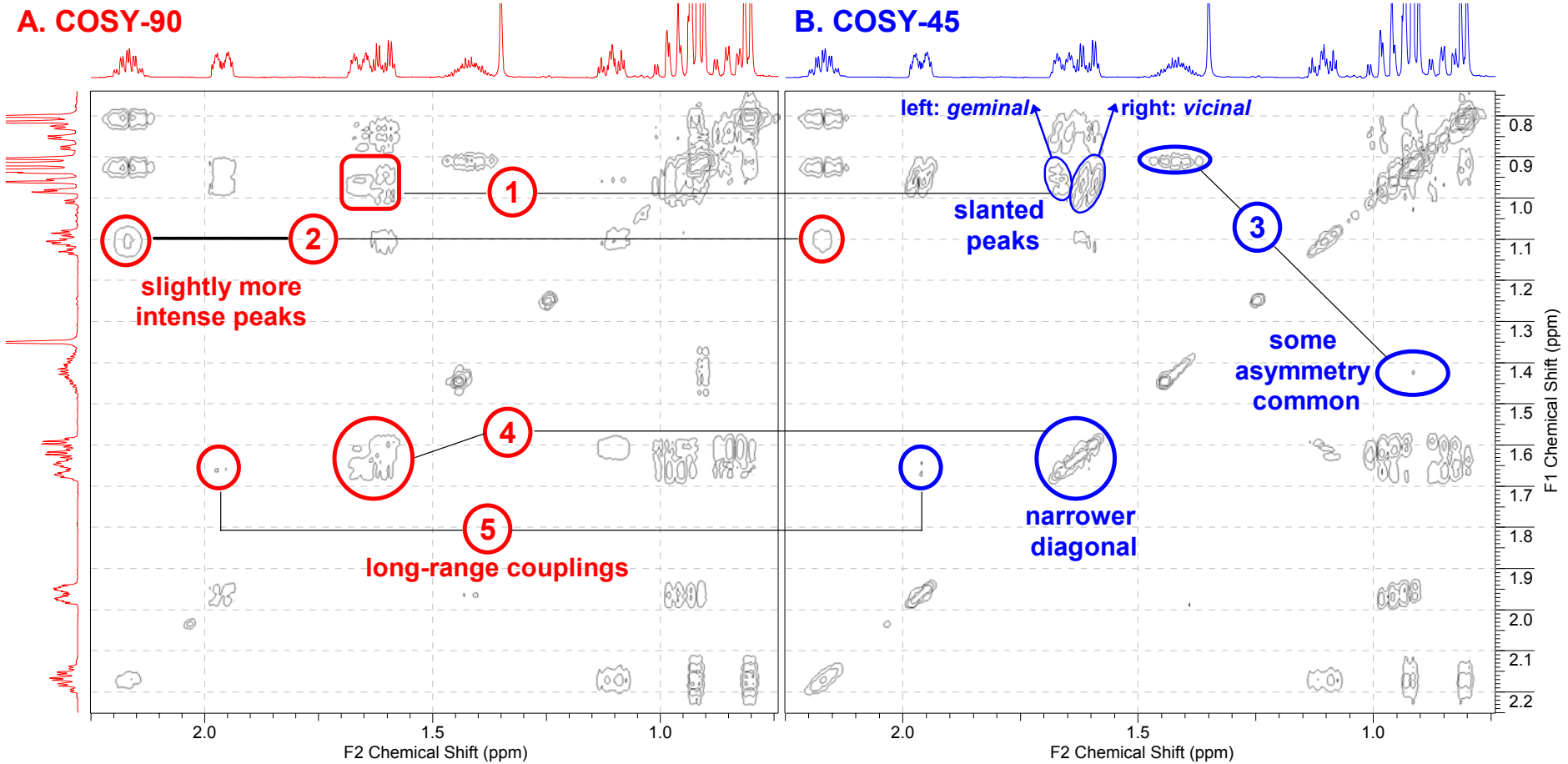
The results seem to be in qualitative agreement with literature data:

Coupling (Hz)/Method	C(1)-H(3)	C(1)-H(4)	C(1)-H(5)	C(1)-H(6)	C(1)-H(8)
1D-TOCSY (reported)	-4.8	-0.5	+9.7	+1.5	-6.3
1D-TOCSY (this report)	-5.8	-1.9	+10.2	+2.7	-6.7
HETLOC (this report)	-5.9	-0.8	+7.5	+2.0	-6.3
Independent Method[1]	-4.7	-0.7	+9.7	+1.4	-6.3

[1] Parella, T.; Belloc, J., Sánchez-Ferrando, F. *Magn. Reson. Chem.* **2004**, *42*, 852-862.

Note: The success of this experiment appears to depend on the delay time. Delay times below 2.0 s gave unsatisfactory results. Success also depends on suppressing resonances arising from the ^{12}C-^{1}H isotopomer. This can be checked by running the satellite-excitation experiments with no mixing period. Such an experiment should display only the irradiated satellite.

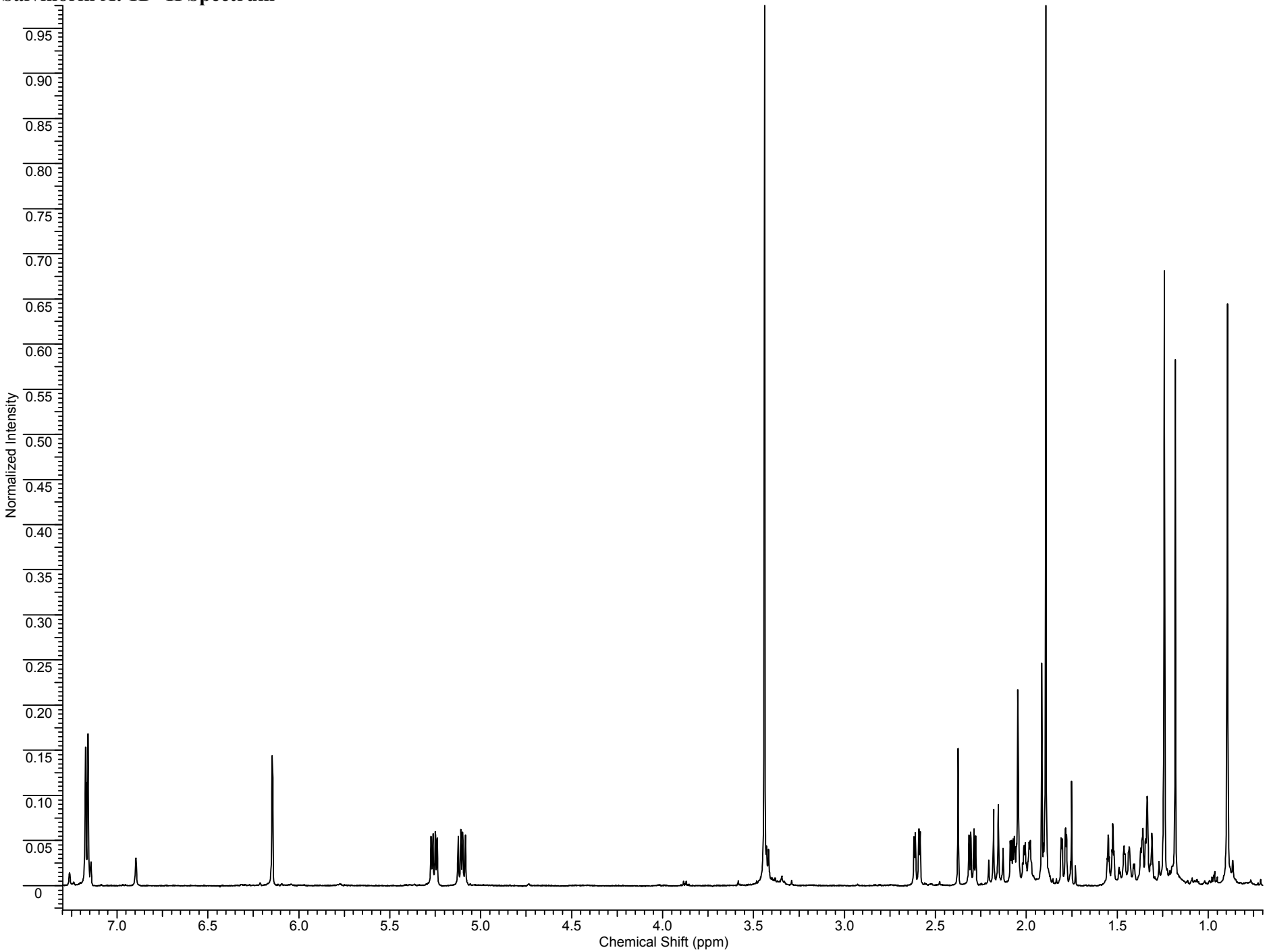
COSY-90 vs. COSY-45



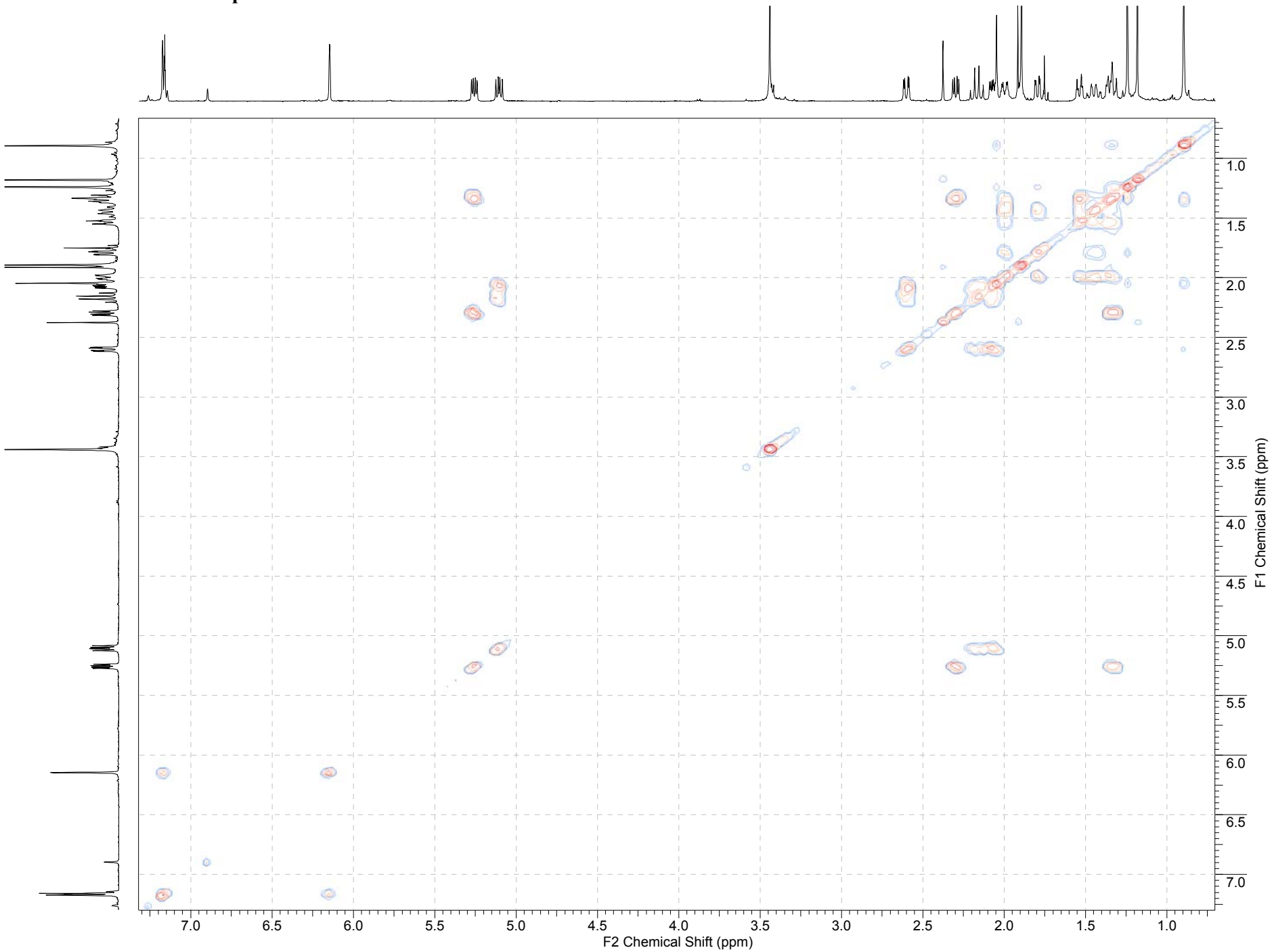
1. **Slant:** COSY-90 peaks are rectangular while COSY-45 peaks allow positive and negative couplings to be distinguished. Positive couplings (right) are usually vicinal while negative couplings (left) are usually geminal. This effect is more apparent with increasing resolution in F1.
2. **Diagonal:** The diagonal in the COSY-45 is narrower.
3. **Asymmetry:** Some minor asymmetry is common in COSY-45 and COSY-90 spectra. Avoid using symmetrization: the S/N in such spectra is usually satisfactory and symmetrization can introduce artifacts. Asymmetry often occurs because the spectrum is much better digitized in F2 than F1. For example, the labeled crosspeak arises from a correlation between H(6) and H(12).
4. **S/N:** COSY-90 peaks are slightly more intense.
5. **Long-Range Couplings:** The indicated crosspeak is a 5-bond W coupling. In general, crosspeaks arising from a coupling constant of *n* Hz or greater will be visible in a spectrum with a digital resolution of 5*n* Hz/point (digital resolution = spectral window (Hz) / number of points). For details, please see: Allman, T.; Bain, A.D. *J. Magn, Reson.* **1986**, *68*, 533-539.

Spectrum parameters: at=0.3, d1=0.8, ni=256, nt=4 (20 minutes). (absolute value gradient-selected mode; homospoil purging gradient on; both 1839x1839 Hz spectral window; 3.3 and 7.2 Hz/point in F2 and F1, respectively; sine-bell squared in F1 and F2, 2xLP in F1; 2048x2048 FT)

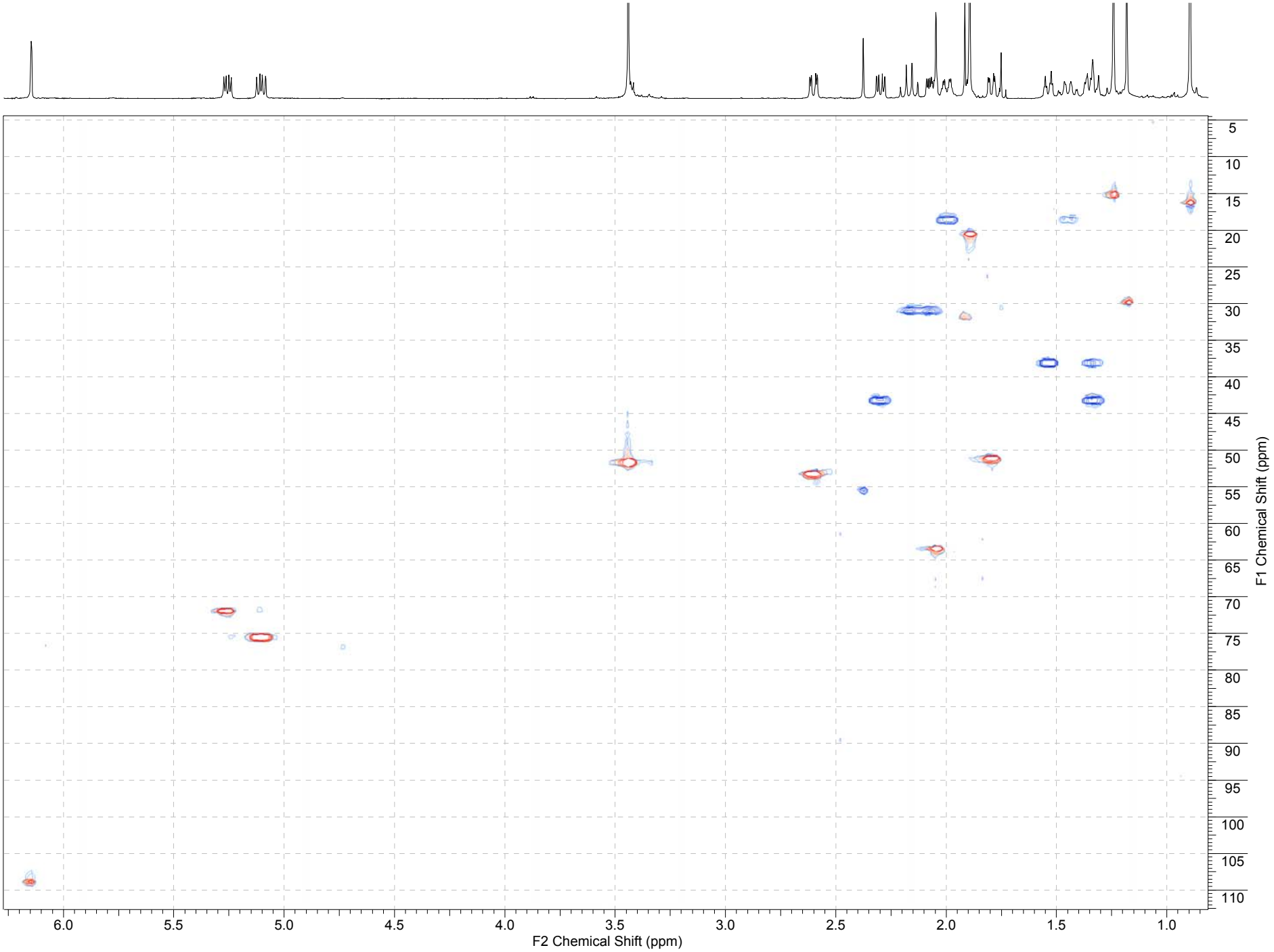
Salvinorin A: 1D ^{1}H Spectrum



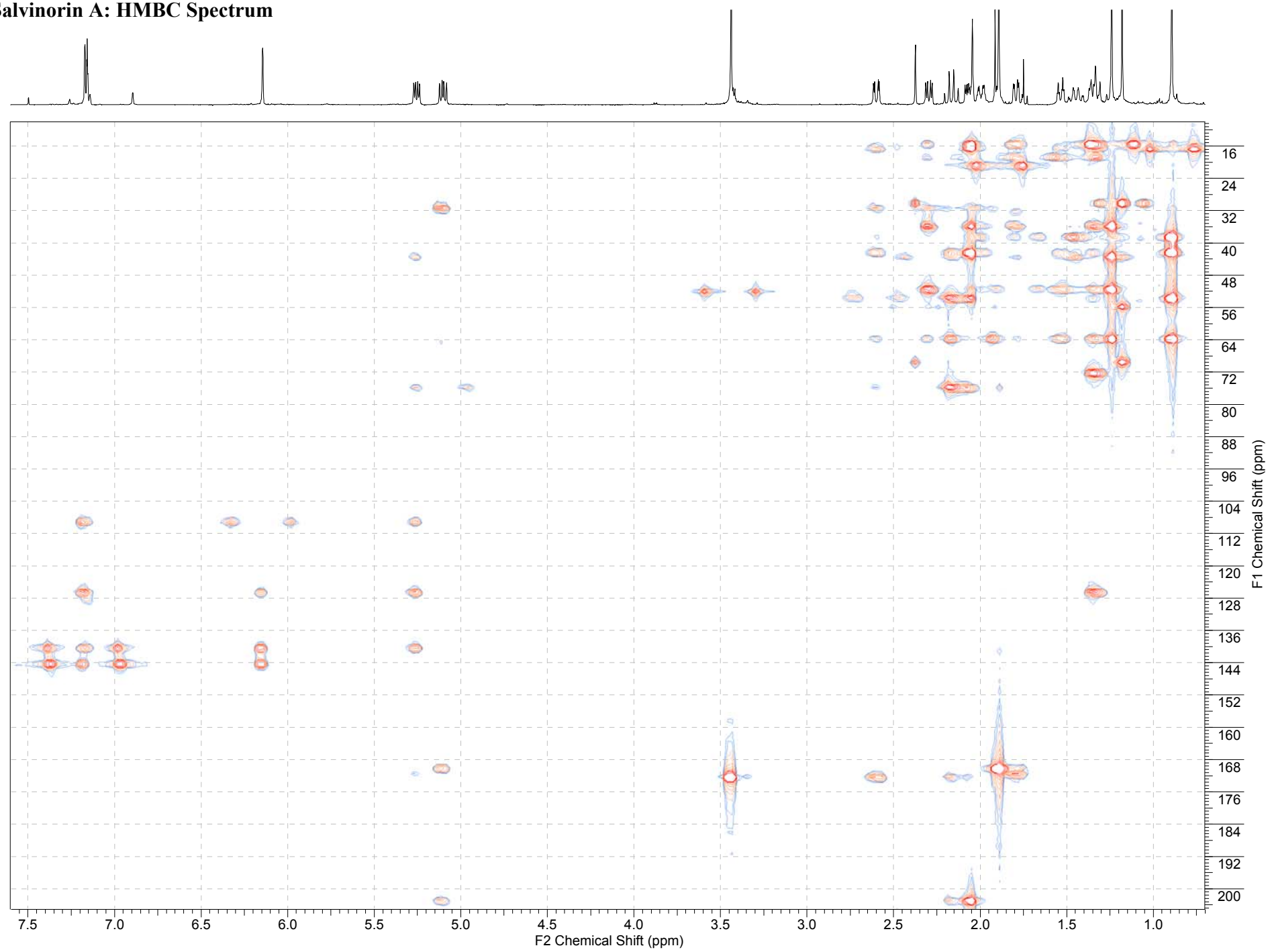
Salvinorin A: COSY-45 Spectrum



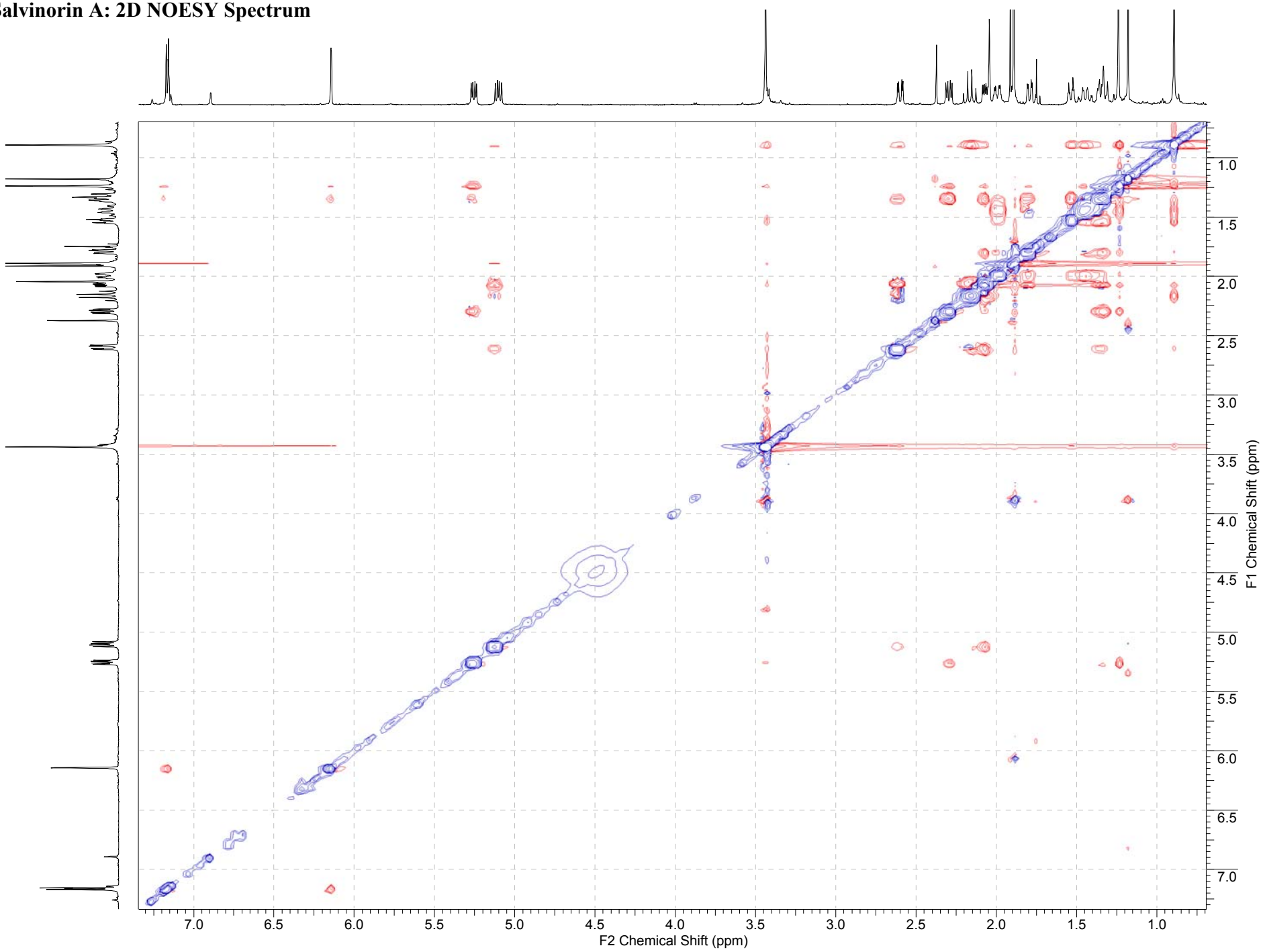
Salvinorin A: HSQC Spectrum



Salvinorin A: HMBC Spectrum



Salvinorin A: 2D NOESY Spectrum



Salvinorin A: HETLOC Spectrum