Rapid Emerging Drug Deployment Characterization Results

Item identifier: PSS0146

Date of report: February 24, 2022

Analysts: Edward Sisco & Aaron Urbas

Summary Results

Qualitative identity of compound: N-Methyl-N-Isopropyltryptamine fumarate

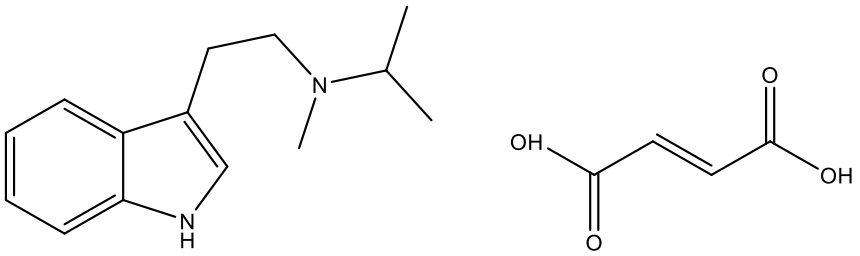
Synonyms (if known): MiPT

Chemical formula: $C_{14}H_{20}N_2$

Monoisotopic Molecular Mass: 216.1621 Da

InChiKey (Neutral Molecule): KTQJVAJLJZIKKD-UHFFFAOYSA-N

Structure:



Purity (if measured): Not measured

Sample characteristics: Tan powder

Sample origin: Seized substance provided by collaborating laboratory

Analytical techniques used: Nuclear magnetic resonance spectroscopy (NMR), direct analysis in real time mass spectrometry (DART-MS), gas chromatography mass spectrometry (GC-MS), gas chromatography flame ionization detection (GC-FID), and Raman spectroscopy

Note: Supporting data and supplementary information can be found at the following link: https://doi.org/10.18434/mds2-2527.

Disclaimer: Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.

Analytical Results - NMR

Instrument and method used: Measurements were made using a Bruker Avance II 600 MHz NMR equipped with a broadband-inverse (BBI) probe. A single aliquot (approximately 10 mg) of the sample was used for all NMR analysis. Multiple 1D and 2D spectra were collected to characterize the sample including ^{1}H and ^{13}C, ^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, and ^{1}H-^{15}N HMBC. Acquisition parameters for the experiments are given in Table 1. TMS was used as the 0 ppm reference for ^{1}H with the chemical shift axis scale of the remaining nuclei established according to the IUPAC unified scale.

Table 1. Acquisition parameters for 1D and 2D NMR spectral data.

Parameter	^{1}H 1D	^{13}C 1D	HSQC-EDITED (^{1}H, ^{13}C)	HMBC (^{1}H, ^{13}C)	COSY (^{1}H, ^{1}H)	HMBC (^{1}H, ^{15}N)
Pulse Sequence	zg (90 deg pulse)	zgpg (90 deg pulse)	hsqcedetgpsisp2.3	hmbcgplpndqf	cosygpppqf	hmbcgpndqf
Number of Scans	32	4096	4	8	1	16
Relaxation Delay (s)	45	2	2	1.4427	1.9394	1.9427
Acquisition Time (s)	5.4526	0.9088	0.142	0.1884	0.1884	0.1884
Spectrometer Frequency (MHz)	600.13	150.92	(600.13, 150.92)	(600.13, 150.92)	(600.13, 600.13)	(600.13, 60.82)
Spectral Width (Hz)	12019.2	36057.7	(7211.5, 24875.6)	(5434.8, 33557.0)	(5434.8, 5434.8)	(5434.8, 24271.8)
Lowest Frequency (Hz)	-2316.6	-2942.6	(-798.2, -1877.9)	(157.1, -1716.3)	(157.1, 157.1)	(157.1, -26159.8)
Spectral Width (ppm)	20.03	238.92	(12.02, 164.83)	(9.06, 222.35)	(9.06, 9.06)	(9.06, 399.08)
Acquired Size	65536	32768	(1024, 256)	(1024, 256)	(1024, 256)	(1024, 128)

Form sample was analyzed in: A $CDCl_3$ (D, 99.96%, with 0.05% v/v TMS) solution with an approximate concentration of 15 mg/mL.

Controls used: A sample of N-methyl-N-isopropyltryptamine was obtained from Cayman Chemical (17296) and used for comparison.

Results: The sample aliquot was first dissolved in ~700 uL of MeOD and found to be readily soluble. The NMR data from this sample were used to confirm the presence of fumarate but exhibited significant differences in most ^{1}H and ^{13}C peak locations when compared with the reference material, N-methyl-N-isopropyltryptamine (17296, Cayman Chemical), available only in neutral form. While the MeOD data set was suitable for analysis, a chloroform extraction was preferred to facilitate comparison with the reference material. After drying to remove MeOD, the sample solution was basified and the free molecule was extracted into $CDCl_3$ by adding 700 µL $CDCl_3$ and 300 µL of a saturated sodium carbonate D_2O solution, mixed and vortexed, and allowed to separate. The sample readily dissolved upon addition of the D_2O solution but was not appreciably soluble in

$CDCl_3$ prior to this. The full set of 1D and 2D NMR spectra were acquired from the resulting $CDCl_3$ layer and used for structure confirmation.

The confirmed structure, atom numbering used for NMR assignments and observed ^1H COSY and ^1H-^{13}C HMBC correlations are shown in Figure 1. The ^1H spectrum shown in Figure 2 exhibits 10 distinct proton signals, two of which are overlapping, across the nine signal regions shown in Figure 2. These were attributed to 19 hydrogens including 9 methyl, 4 methylene and 6 methine protons. The ^{13}C spectrum, shown in Figure 3, exhibited 14 distinct carbon peaks that were attributed to 15 carbon atoms. The 2D NMR data indicated 5 methine and 4 methylene protons on a tryptamine core structure with the terminal amine substituted with both methyl and isopropyl groups. Connectivity across the structure was established largely through the ^1H-^{13}C HMBC spectrum and additional ^1H-^{15}N HMBC correlations. Table 2 is a summary of the NMR peak assignment data and observed 2D correlations. The complete collection of 1D and 2D NMR associated with the structure elucidation are included in the appendix (Figures A1-A6).

Comparison of the ^1H and ^{13}C spectra of this sample with a sample of N-methyl-N-isopropyltryptamine (Cayman, 17296) prepared in $CDCl_3$ were consistent. Table 3 compares the ^{13}C peak locations between the two samples. Additional, lower intensity, satellite peaks were observed in the reference material for several carbons on the indole moiety, which were attributed to a form of the molecule with the indole nitrogen protonated. This proton was observed as a broad peak in the ^1H spectrum (not shown) of the reference material at 8.02 ppm with an integrated area of approximately 1/3 of the other protons on the molecule and also showed a ^1H COSY correlation with H8. This proton was not observed in the PSS0146 sample in either MeOD or $CDCl_3$, presumably due to deuterium exchange when dissolved in MeOD or $CDCl_3$ extraction with D_2O.

The presence of fumarate was confirmed in the initial MeOD preparation of the PSS0146 sample based on a singlet at 6.71 ppm in the ^1H spectrum with HSQC and HMBC correlations observed with two carbon peaks at 134.8 ppm and 169.7 ppm, which correspond to the alkene and carbonyl carbons, respectively, on fumarate (see Figure 4). A separate sample of fumaric acid in MeOD (data not shown) exhibited a singlet ^1H peak at 6.75 ppm and ^{13}C peaks at 133.8 and 166.7 ppm.

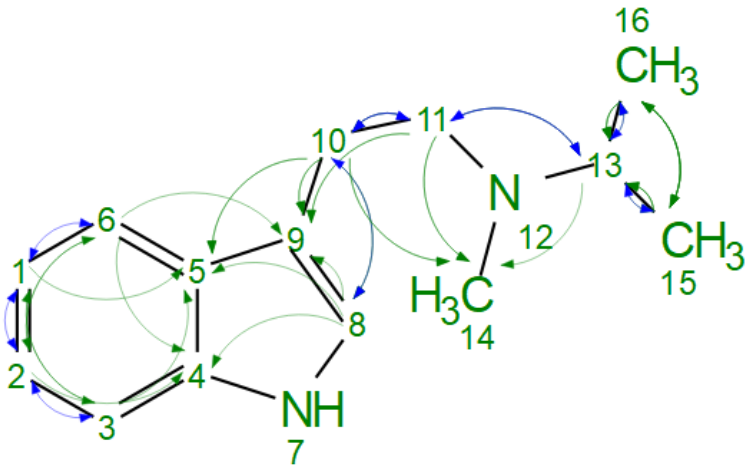


Figure 1. Confirmed structure with atom numbering used for NMR data peak assignments and observed ^1H COSY (blue) and ^1H-^{13}C HMBC (green) correlations labeled as arrows.

Table 2. Summary of NMR peak locations, assignments and observed 2D correlations.

Atom	δ (ppm)	Multiplicity	COSY	HSQC	HMBC	Equivalent Atom(s)
1 C	119.17	1		1	3	
1 H	7.12	1	2, 6	1	3, 5	
2 C	121.91	1		2	6	
2 H	7.19	1	1, 3	2	4, 6	
3 C	111.03	1		3	1	
3 H	7.35	1	2	3	1, 5	
4 C	136.10	1			2, 6, 8	
5 C	127.53	1			1, 3, 8, 10	
6 C	118.87	1		6	2, 6	
6 H	7.62	1	1	6	2, 4, 6, 9	
7 N	-260.15	1			8	
7 H	NA	1				
8 C	121.22	1		8	10	
8 H	7.03	1	10	8	4, 5, 9	
9 C	114.76	1			6, 8, 10, 11	
10 C	24.11	1		10	11	
10 H	2.94	2	8, 11	10	5, 8, 9, 14	
11 C	54.20	1		11		
11 H	2.73	2	10, 13	11	9, 10, 13, 14	
12 N	-338.07	1			13,14,15,16	
13 C	53.49	1		13	11, 13, 15, 16	
13 H	2.93	1	11, 15, 16	13	13, 14, 15, 16	
14 C	37.15	1		14	10, 11, 13	
14 H	2.35	3		14		
15 C	18.01	1		15	13, 16	16 C
15 H	1.05	3	13	15	13, 16	16 H
16 C	18.01	1		16	13, 15	15 C
16 H	1.05	3	13	16	13, 15	15 H

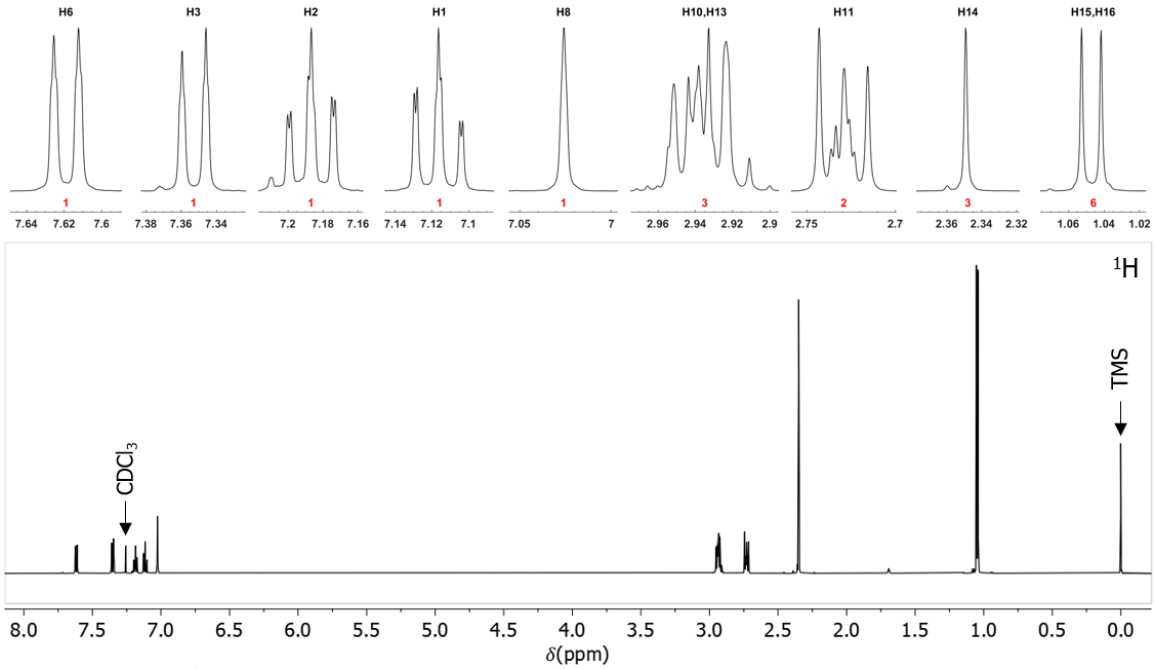


Figure 2. The ^{1}H NMR spectrum of PSS0146 sample from the $CDCl_3$ extraction is shown in the bottom panel. The top panel shows expanded views of each proton signal with proton counts and assignments correspond to the structure of N-methyl-N-isopropyltryptamine in Figure 1.

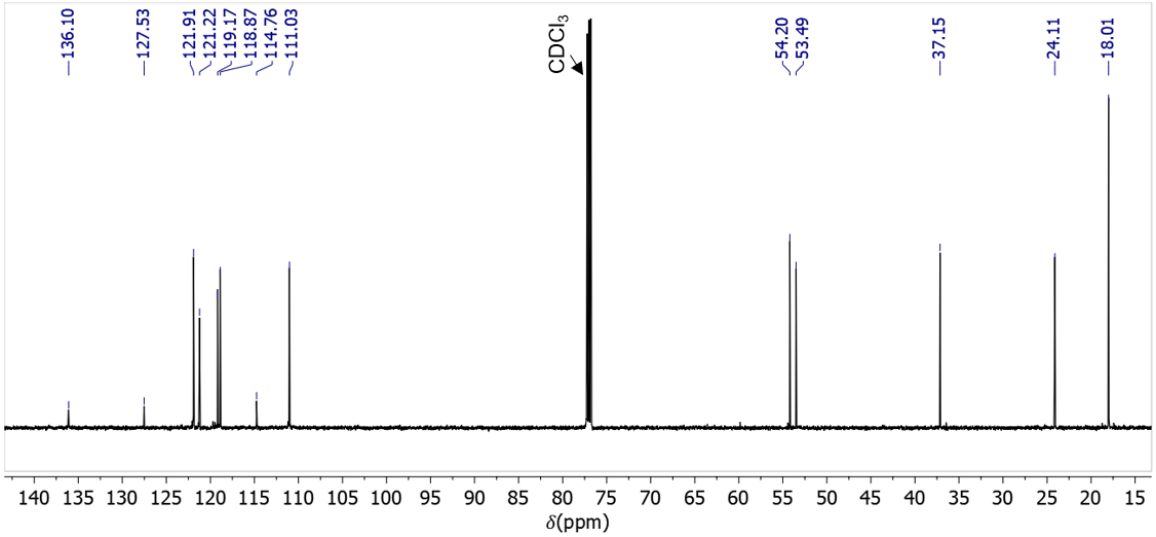


Figure 3. The ^{13}C NMR spectrum of PSS0146 sample from the $CDCl_3$ extraction.

Table 3. ^{13}C peak locations of the PSS0146 $CDCl_3$ extract compared to a N-methyl-N-isopropyltryptamine (MiPT) reference material. Assignments are according to the structure in Figure 1. See text for discussion of satellite peaks observed in reference sample.

Peak	Chemical Shift (ppm)			Atom
	PSS-0146	MiPT Ref	Difference	
1	-	136.25		C4
2	136.10	136.09	0.01	C4
3	-	127.57		C5
4	127.53	127.54	-0.01	C5
5	121.91	121.90	0.01	C2
6	-	121.37		C8
7	121.22	121.21	0.01	C8
8	119.17	119.18	-0.01	C1
9	-	119.16		C6
10	118.87	118.88	-0.01	C6
11	114.76	114.81	-0.05	C9
12	-	111.07		C3
13	111.03	111.01	0.02	C3
14	54.20	54.22	-0.02	C11
15	53.49	53.52	-0.03	C13
16	37.15	37.21	-0.06	C14
17	24.11	24.18	-0.07	C10
18	18.01	18.05	-0.04	C15,C16

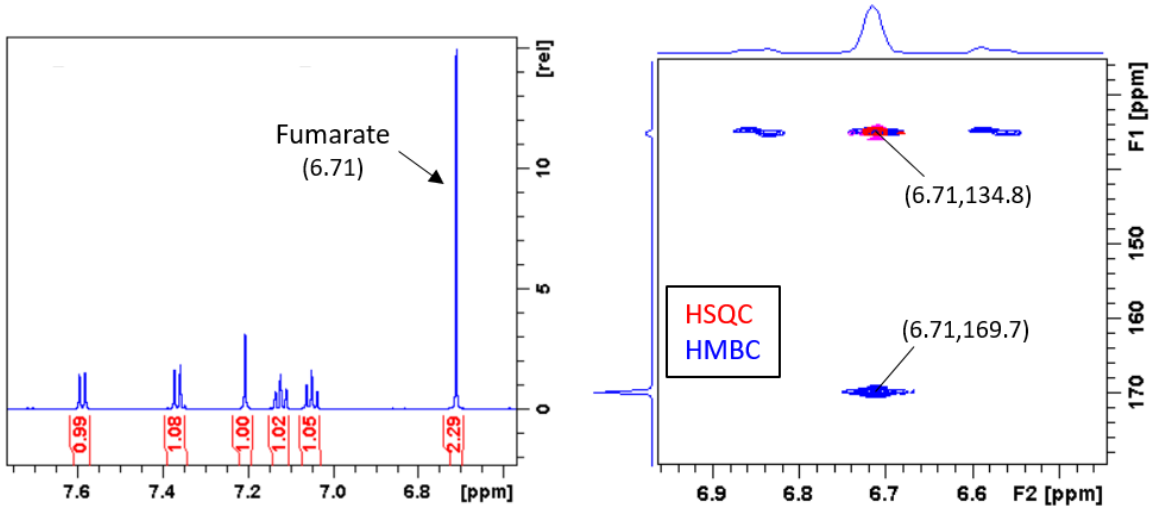


Figure 4. ^{1}H and ^{1}H-^{13}C HSQC/HMBC NMR data from PSS0146 sample dissolved in MeOD with peaks identified that correspond to fumarate.

Analytical Results – DART-MS

Instrument and method used: Measurements were made using an IonSense DART-SVP ion source coupled to a JEOL AccuTOF 4G LC-plus mass spectrometer. The sample was analyzed in both positive and negative ionization modes. For both analyses, helium (99.999 % purity) was used as the source gas with a gas stream temperature of 400 °C and a grid voltage of ±150 V. For the positive mode analysis, a scan range of *m/z* 80 to *m/z* 800 was used along with an RF Guide voltage of +700 V, a ring lens voltage of +5 V, and an orifice 2 voltage of +5 V. The orifice 1 voltage was cycled (+30 V, +60 V, and +90 V) at 0.2 s $cycle^{-1}$. For negative mode analysis a scan range of *m/z* 30 to *m/z* 550 was used, at 0.2 s $scan^{-1}$ along with an RF Guide voltage of -250 V, an orifice 1 voltage of -30 V, a ring lens voltage of -5 V, and an orifice 2 voltage of -5 V.

Form sample was analyzed in: A methanolic solution with an approximate concentration of 1 mg mL^{-1}. Additionally, an aqueous solution with an approximate concentration of 1 mg mL^{-1} was analyzed in negative ionization mode for salt form determination.

Controls used: Polyethylene glycol 600 was used an *m/z* calibration compound in both positive and negative ionization modes. A ~0.1 mg/mL methanolic solution of cocaine was used a positive control in positive ionization mode. A ~0.1 mg/mL methanolic solution of AB-FUBINACA was used as a positive control in negative ionization mode. Methanol was run as a negative control in both positive and negative ionization modes.

Results: In the low fragmentation orifice 1 voltage (+30 V) spectrum of the sample a dominant peak at *m/z* 217.171 was observed (Figure 5). The observed peak had a corresponding formula of $[C_{14}H_{21}N_2]^+$ (Δ_{mmu} = -1.02 mmu) resulting in a presumed molecular formula of $C_{14}H_{20}N_2$, assuming the ion was the protonated molecule. The result was supported by the observance of fragment ions in all three orifice 1 voltage spectra at *m/z* 86.097 ($[C_5H_{12}N]^+$, Δ_{mmu} = -0.13 mmu) and 144.082 ($[C_{10}H_{10}N]^+$, Δ_{mmu} = -0.78 mmu). The +60 V and +90 V orifice 1 spectra are shown in Figures 6 and 7. No other major peaks were observed in the +30 V spectrum. The negative mode spectrum had a peak corresponding to the fumarate ion $[C_4H_3O]^-$ at *m/z* 115.004 (Δ_{mmu} = -1.09 mmu) as shown in Figure 8. The deprotonated molecule of the main compound was also observed at *m/z* 215.156 ($C_{14}H_{19}N_2$, Δ_{mmu} = -1.18 mmu).

The positive ionization mode DART-MS spectra were searched against the NIST DART-MS Forensics Database (version Dragonfly) using the NIST/NIJ Data Interpretation Tool (DIT) (version 1.0). The DIT produced two potential matches – diethyltryptamine (FPIE score: 0.836, RevMF score: 0.916) and N,N-methylisopropyltryptamine (FPIE score: 0.829, RevMF score: 0.863).

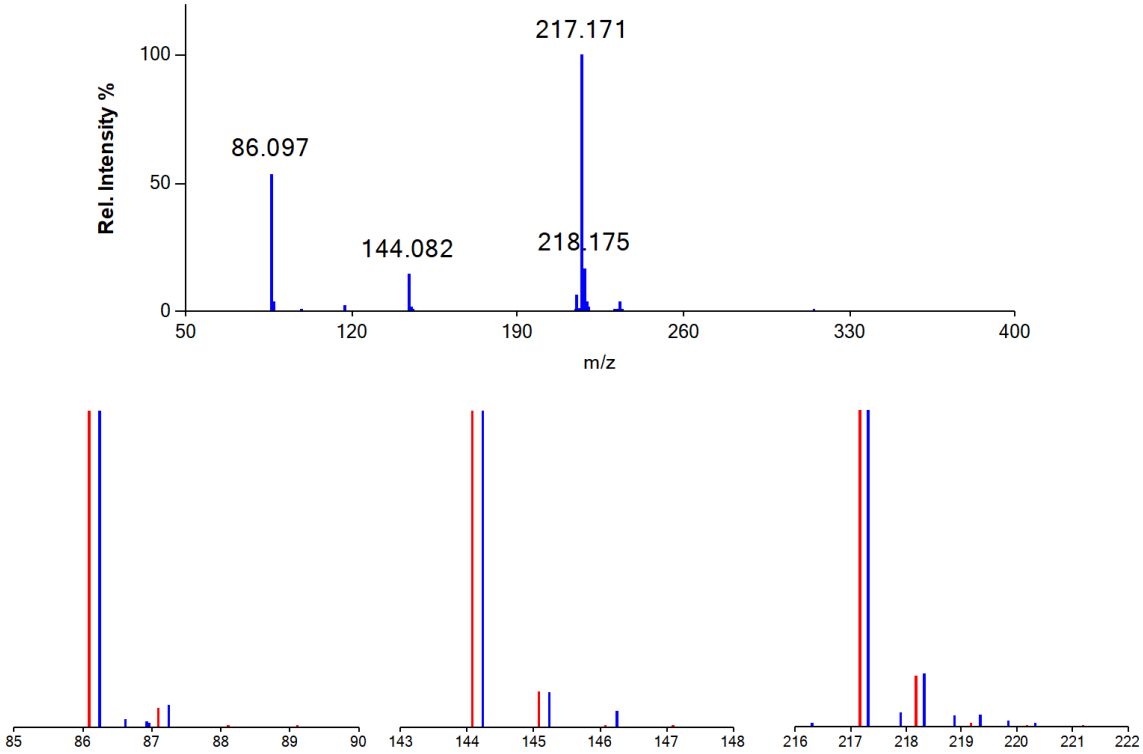


Figure 5. Low fragmentation orifice 1 voltage (+30 V) positive mode spectrum of the sample (top). Isotope matches (red is theoretical, blue is measured) for the *m/z* 86.097 ion to $[C_5H_{12}N]^+$ (botton left), the *m/z* 114.082 ion to $[C_{10}H_{10}N]^+$ (botton center) and the *m/z* 217.171 ion to $[C_{14}H_{21}N_2]^+$ (bottom right) are also shown.

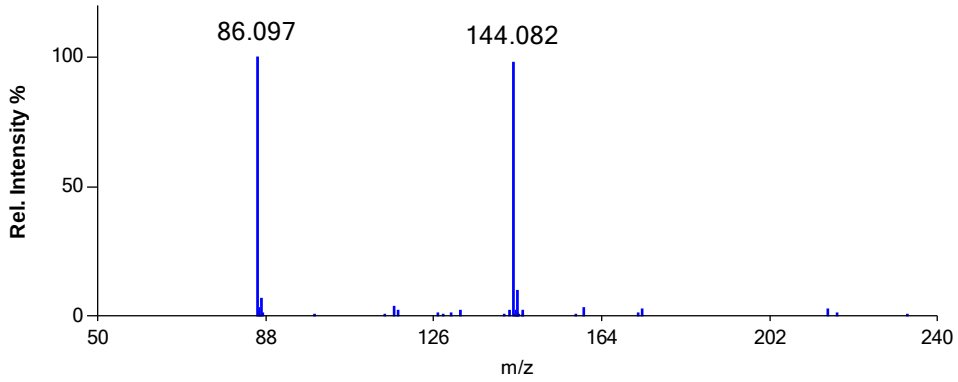


Figure 6. Mid-range fragmentation orifice 1 voltage (+60 V) positive mode spectrum of the sample.

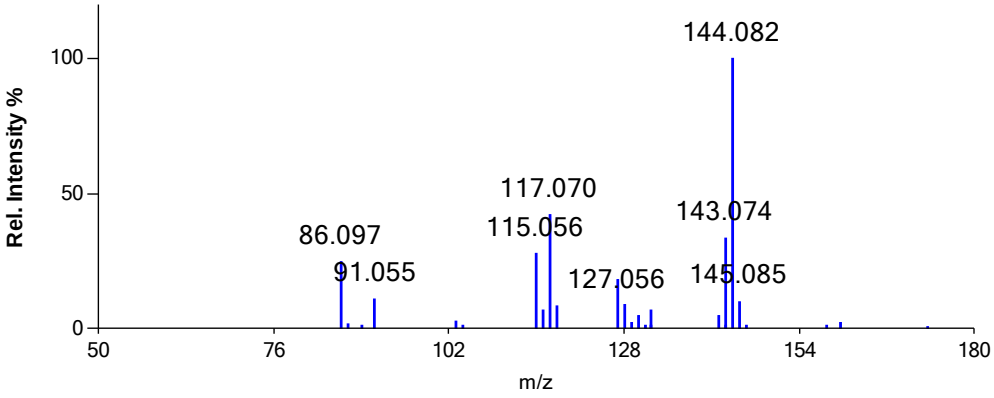


Figure 7. High fragmentation orifice 1 voltage (+90 V) posititive mode spectrum of the sample.

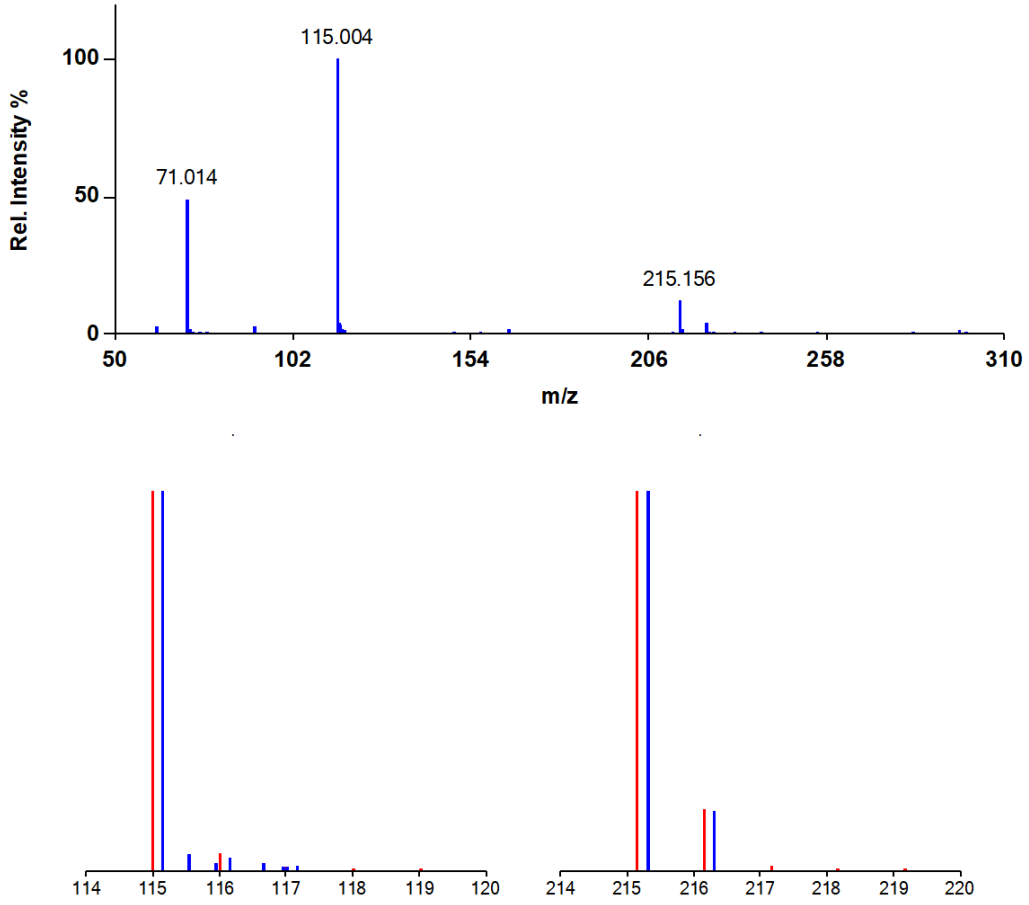


Figure 8. Negative mode spectrum of the sample (top). Isotope matches (red is theoretical, blue is measured) for the *m/z* 115.004 ion to $[C_4H_3O_4]^-$ (botton left) and the *m/z* 215.156 ion to $[C_{14}H_{19}N_2]^-$ (bottom right) are also shown.

Analytical Results- GC-MS

Instrument and method used: A Thermo Trace 1310 gas chromatograph coupled with a TSQ8000evo mass spectrometer was used for this analysis. Helium (99.999 %) was used as the carrier gas along with a Restek RSI-35Sil ms column (30 m x 0.25 mm x 0.25 µm). Relevant method parameters are provided in Table 4.

Table 4. GC-MS method parameters.

Temperature Program	1) 80 °C for 0.5 min 2) Ramp 15 °C min^{-1} to 290 °C 3) Hold 10 min
Flow Rate	1.5 mL min^{-1}
Injection Volume	1.0 µL
Inlet Temperature	250 °C
Split Ratio	30:1
Transfer Line	300 °C
Quad Temperature	150 °C
Source Temperature	280 °C
Tune Mode	EI Standard Tune
Solvent Delay	1.5 min
Mass Scan Range	*m/z* 40 – *m/z* 600
Threshold	150
Scan Speed	0.2 s $scan^{-1}$

Form sample was analyzed in: A methanolic solution with an approximate concentration of 0.25 mg/mL.

Controls used: A ~0.1 mg/mL methanolic solution of cocaine was used as a positive control. Methanol was used as a negative control. An alkane ladder (C_7-C_{40}) was used for retention index calculations.

Results: The compound was found to have a retention time of 12.06 min (12 min 3.6 s) using the method specified, which was the only peak above background (Figure 9, left). The corresponding mass spectrum (Figure 9, right) was dominated by the *m/z* 86 and *m/z* 44 ions. A presumed molecular ion at *m/z* 216 was observed. Using an alkane ladder, a retention index of 2255 a.u. was obtained.

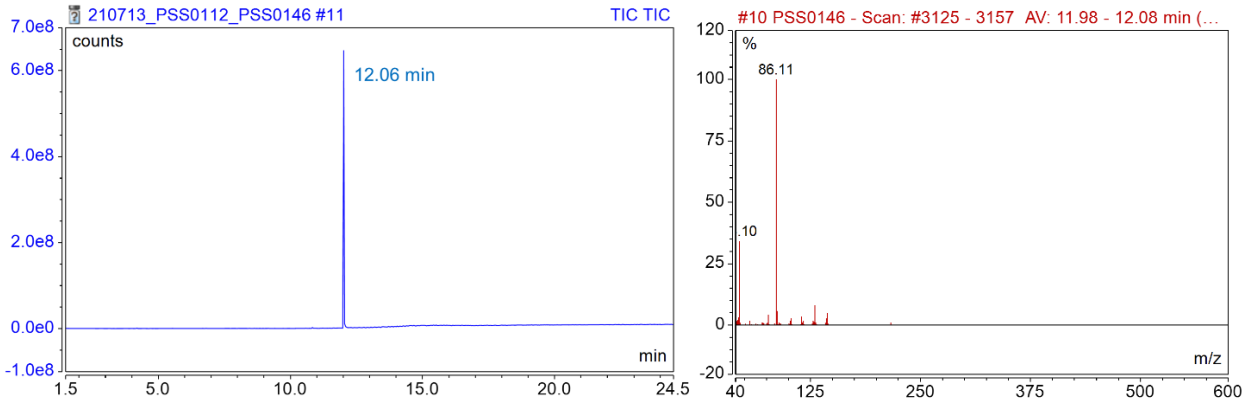


Figure 9. Representative chromatogram (left) and mass spectrum (right) of the sample.

Comparison of the measured spectrum to the SWGDRUG 3.8 spectral library (Figure 10) showed an excellent match to N-Methyl-N-isopropyltryptamine. A standard of the N-Methyl-N-isopropyltryptamine was obtained from Cayman Chemical and run on the identical method. Identical retention times 12.06 min (12 min 3.6 s) were obtained for the sample and the standard.

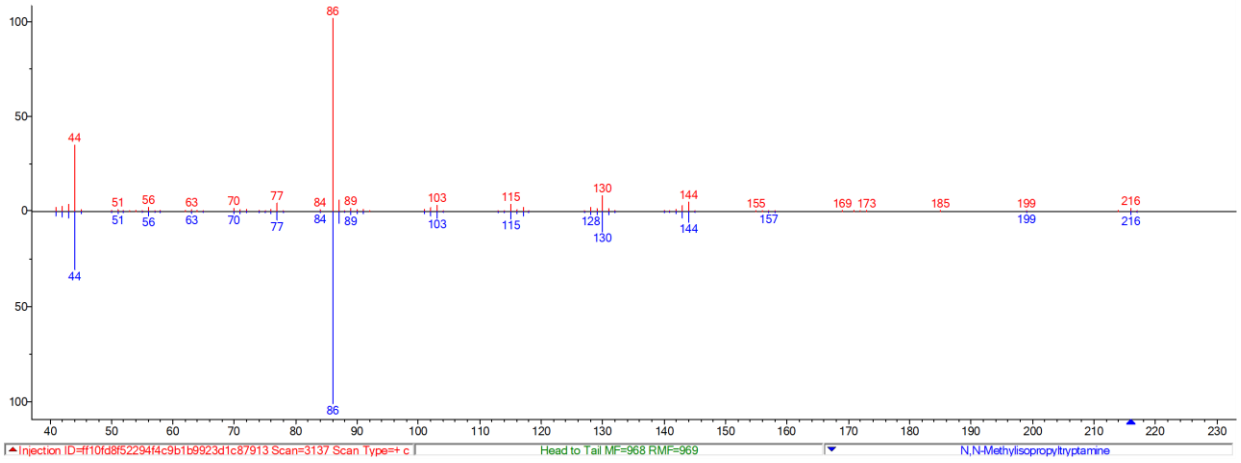


Figure 10. Comparison of the mass spectrum of the sample (top, red) to the mass spectrum N-Methyl-N-isopropyltryptamine (bottom, blue) from the SWDRUG library (v3.8) using NIST MS Search

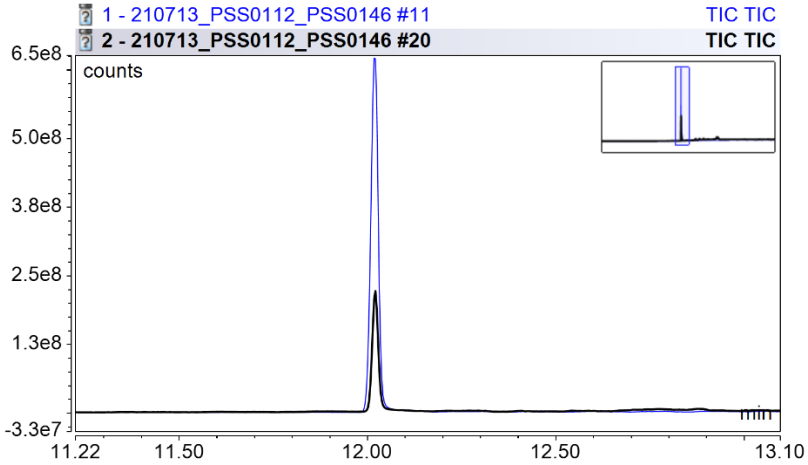


Figure 11. Comparison of the chromatograms of the sample (blue) to the standard of N-methyl-N-isopropyltryptamine (black) obtained from Cayman Chemical.

Analytical Results – GC-FID

Instrument and method used: An Agilent 8890 gas chromatograph was used for analysis. Helium (99.999 %) was used as the carrier gas along with an Agilent DB-5 column (30 m x 0.25 mm x 0.25 µm). Relevant method parameters are provided in Table 5.

Table 5. GC-FID method parameters.

Temperature Program	1) 80 °C for 0.5 min 2) Ramp 30 °C min^{-1} to 290 °C 3) Hold 8 min
Flow Rate	1.8 mL min^{-1}
Injection Volume	1.0 µL
Inlet Temperature	200 °C
Split Ratio	5:1
Detector Temperature	300 °C
H_2 Flow	30 mL min^{-1}
Air Flow	400 mL min^{-1}
Makeup Flow	25 mL min^{-1}
Data Collection Rate	20 Hz

Form sample was analyzed in: A methanolic solution with an approximate concentration of 1.0 mg/mL.

Controls used: A 0.1 mg/mL methanolic solution of cocaine was used as a positive control. Methanol was used as a negative control. An alkane chain (C_7-C_{40}) was used for retention index calculations.

Results: The compound was found to have a retention time of 9.602 min (9 min 36.12 s) using the method specified, which was the only peak above background that was observed. The N-Methyl-N-isopropyltryptamine standard from Cayman Chemicals was also run and had a retention time of 9.610 min (9 min 36.6 s). Using an even-numbered alkane ladder, a retention index of 2795 a.u. was obtained.

Analytical Results – Raman (1064 nm)

Instrument and method used: A Bruker Vertex-70 FTIR equipped with the Ram-II 1064 nm Raman module was used for analysis. Relevant method parameters are provided in Table 6.

Table 6. Raman method parameters.

Laser Power (setting)	300-500 mW
Number of Scans	32
Resolution	2 cm^{-1}
Aperture	5 mm
Beamsplitter	CaF_2
Detector	LN-cooled Ge Diode

Form sample was analyzed in: The sample was analyzed in solid form as received in clear glass vials or deposited in a glass vial by solvent evaporation.

Controls used: A sample of N-methyl-N-isopropyltryptamine was obtained from Cayman Chemical (17296) and used for comparison. Neat benzonitrile (HPLC grade) was used to periodically check the accuracy of the Raman shift axis as described in ASTM E1840-96(2014). The positions of 11 peaks were measured to verify the accuracy of the Raman shift axis to within 0.5 cm^{-1}.

Results: The PSS0146 sample was analyzed as received through the wall of a clear glass vial. The vial was rotated several times to collect spectra from different portions of the sample within the vial. There was no indication of multiple components in the collection of 14 acquired spectra from the sample and they were subsequently averaged. The spectrum was baseline corrected to remove a broad background signal attributed to fluorescence that was approximately equal in intensity to the strongest Raman band at 1550 cm^{-1}. A reference sample of N-methyl-N-isopropyltryptamine was obtained and analyzed similarly in the clear glass vial it was packaged in. The spectrum of this reference sample and PSS0146 are overlaid for comparison in Panel A of Figure 12. Panels B and C of Figure 12 show the Raman spectra of PSS0146 and N-methyl-N-isopropyltryptamine, respectively, with peaks identified. While there were a few similarities between these spectra, the differences were numerous and stark and preclude identification.

The sample resulting from the $CDCl_3$ extraction previously described for the NMR analysis to isolate the neutral compound from fumarate was transferred to a glass vial and the solvent was evaporated. Raman spectra were collected from the dried sample, averaged and baseline corrected. The resulting spectrum from this sample is compared to the N-methyl-N-isopropyltryptamine reference spectrum in Figure 13. A high degree of correspondence was observed for nearly all Raman bands in these samples with some minor discrepancies in relative peak heights. The dried PSS0146 sample exhibited several small peaks not seen in the reference compound. The origin of these was not determined but may be due to an impurity or possible polymorph.

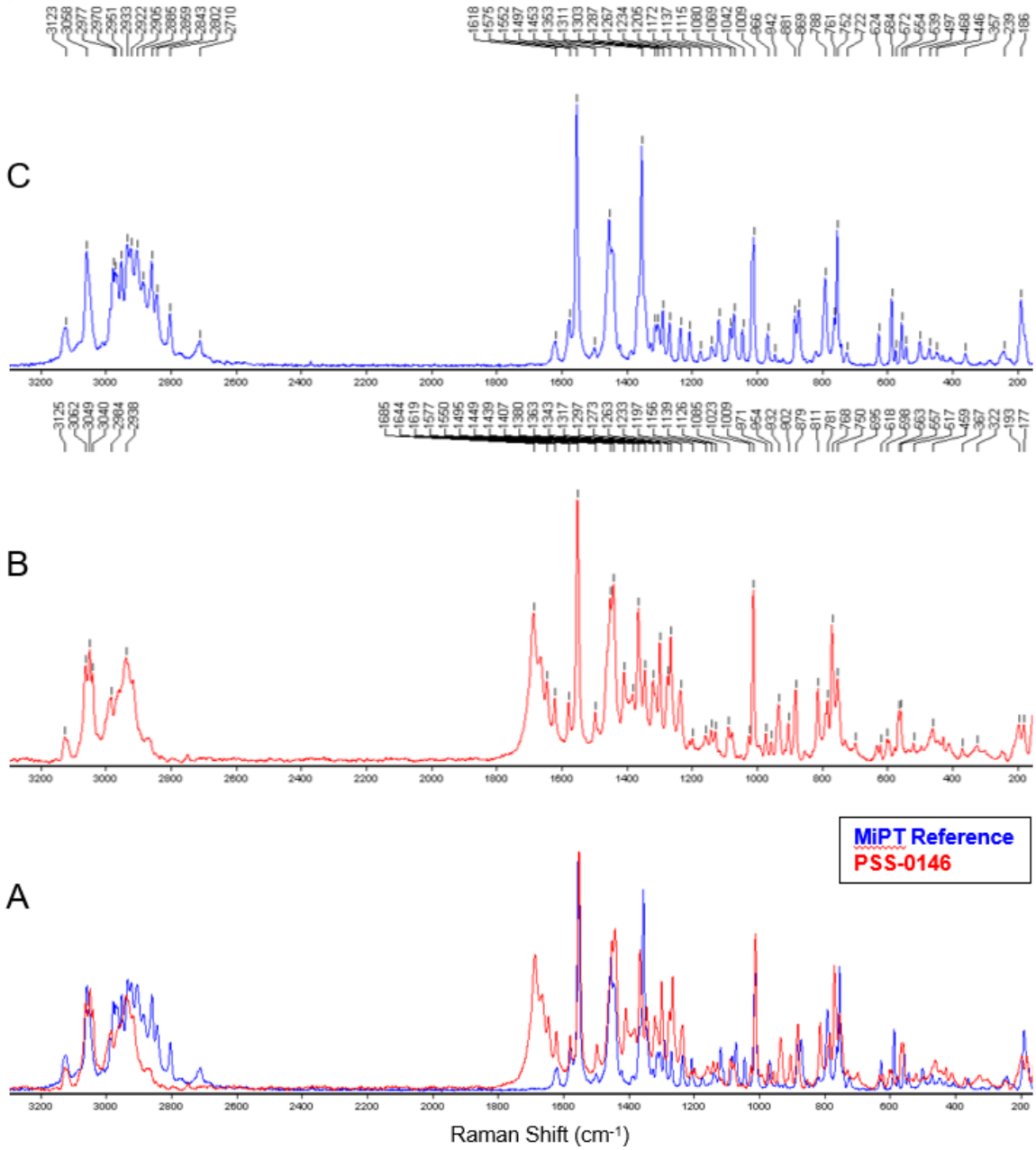


Figure 12. Panel A) Overlaid 1064 nm Raman spectra of PSS0146 as received and N-methyl-N-isopropyltryptamine (MiPT). Panel B) 1064 nm Raman spectrum of PSS-0146 as received with peaks identified. Panel C) 1064 nm Raman spectrum of N-methyl-N-isopropyltryptamine (MiPT) with peaks identified.

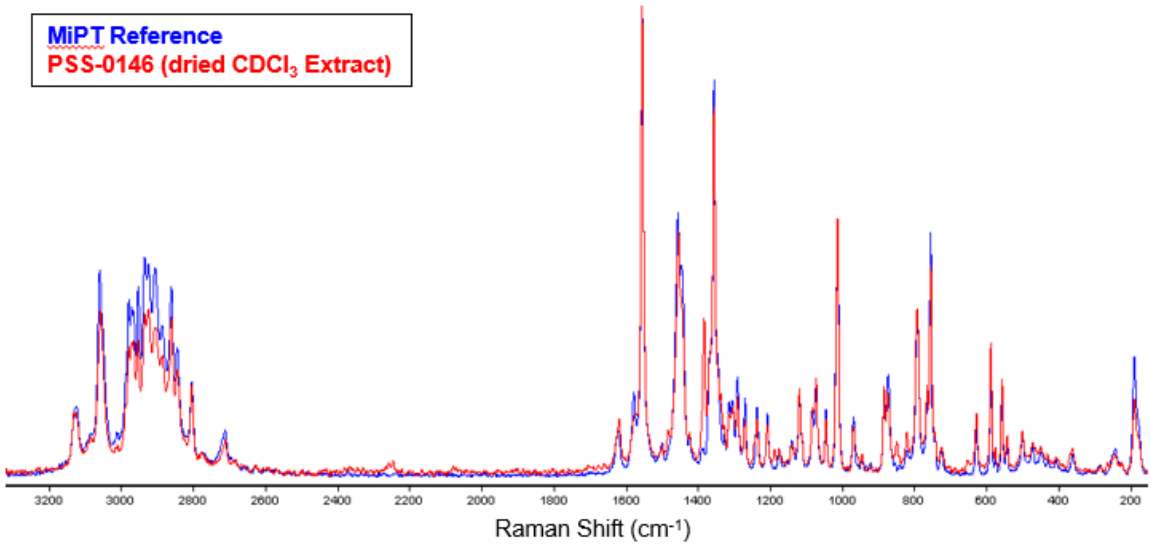


Figure 13. Overlaid 1064 nm Raman spectra of the N-methyl-N-isopropyltryptamine (MiPT) reference standard and the PSS0146 sample deposited after $CDCl_3$ extraction used in the NMR analysis to isolate the neutral compound from the fumarate counter ion.

Supplemental NMR Data

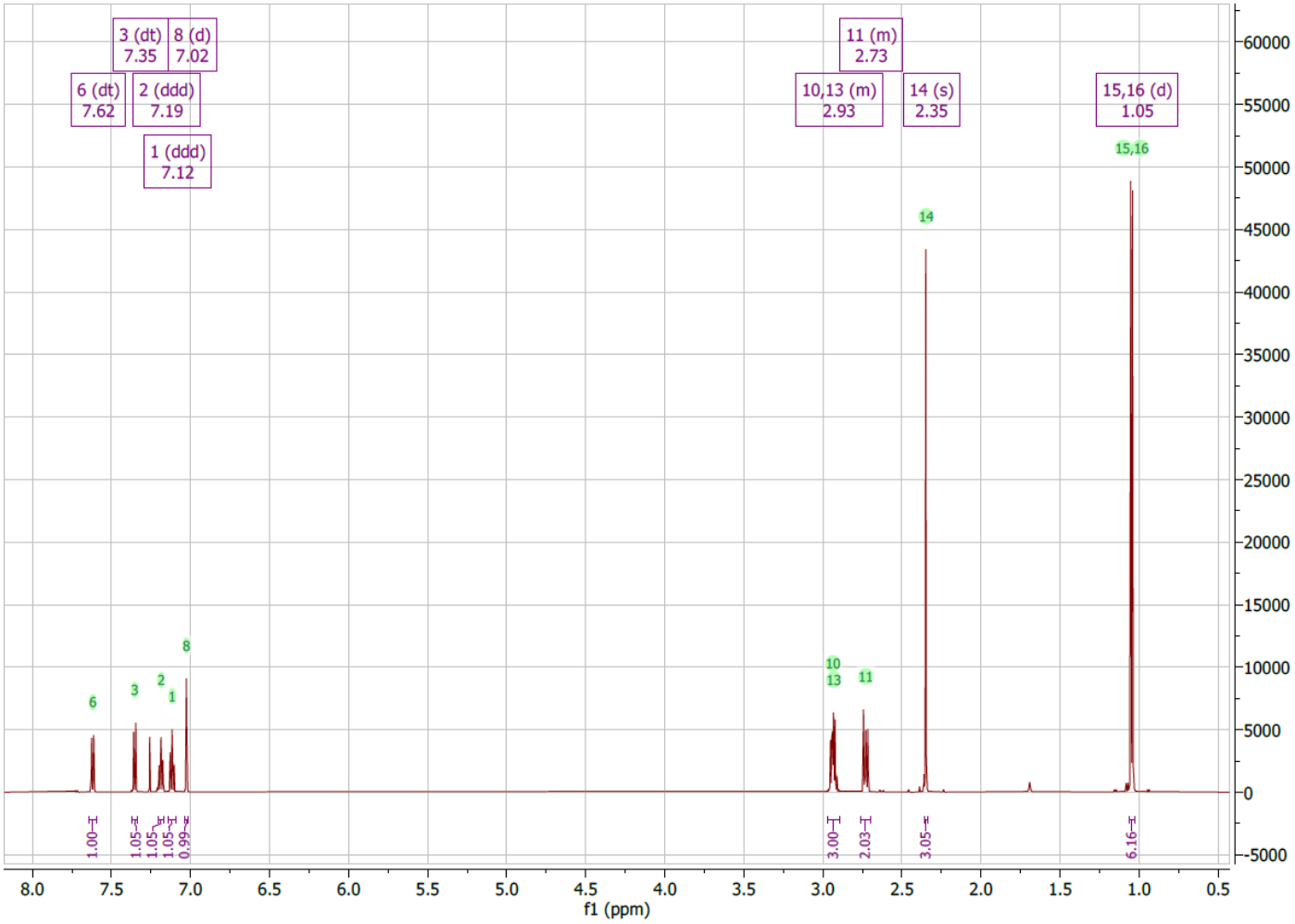


Figure A1. Assigned ^{1}H spectrum. Assignments based on structure in Figure 1.

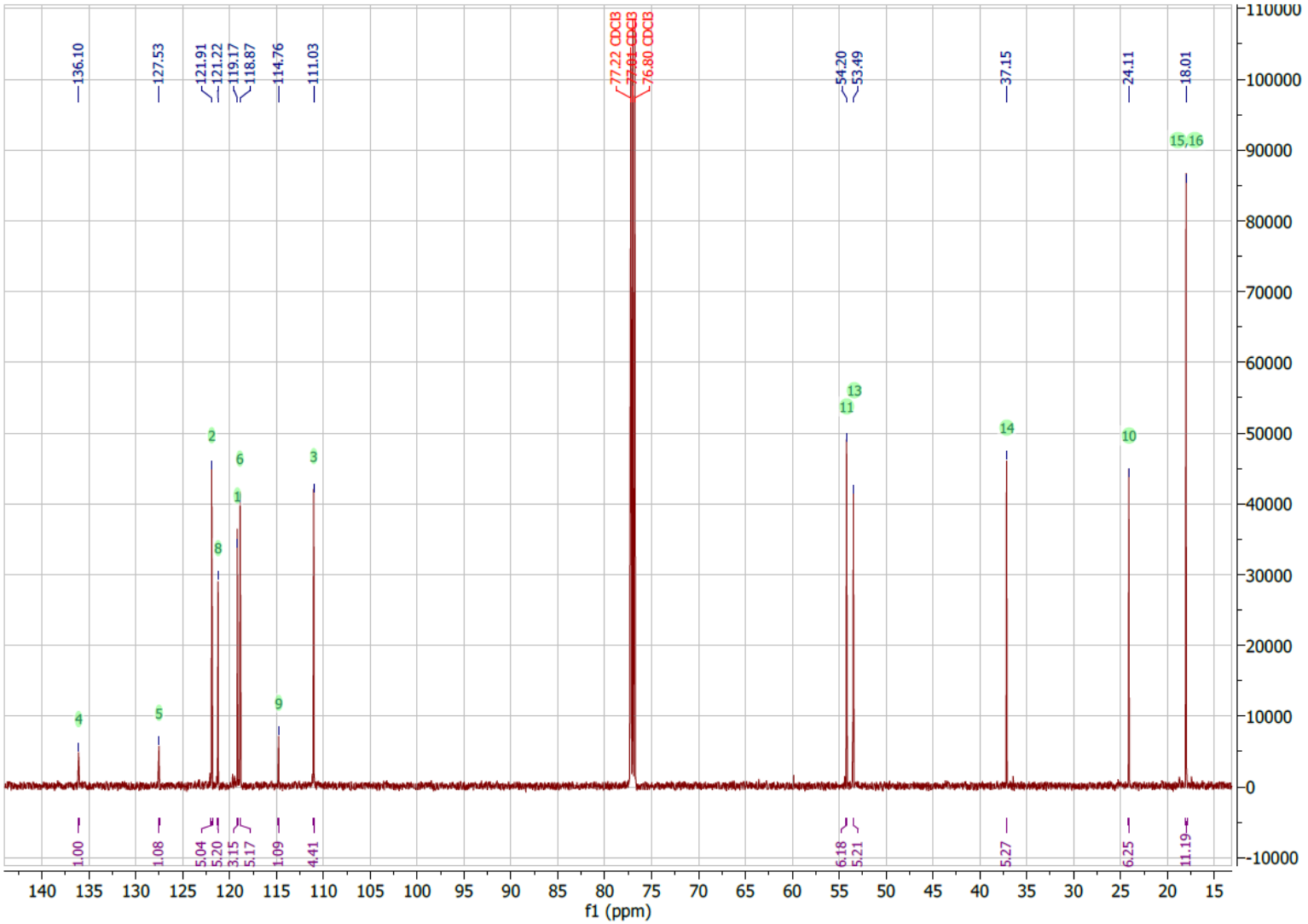


Figure A2. Assigned ^{13}C spectrum. Assignments based on structure in Figure 1.

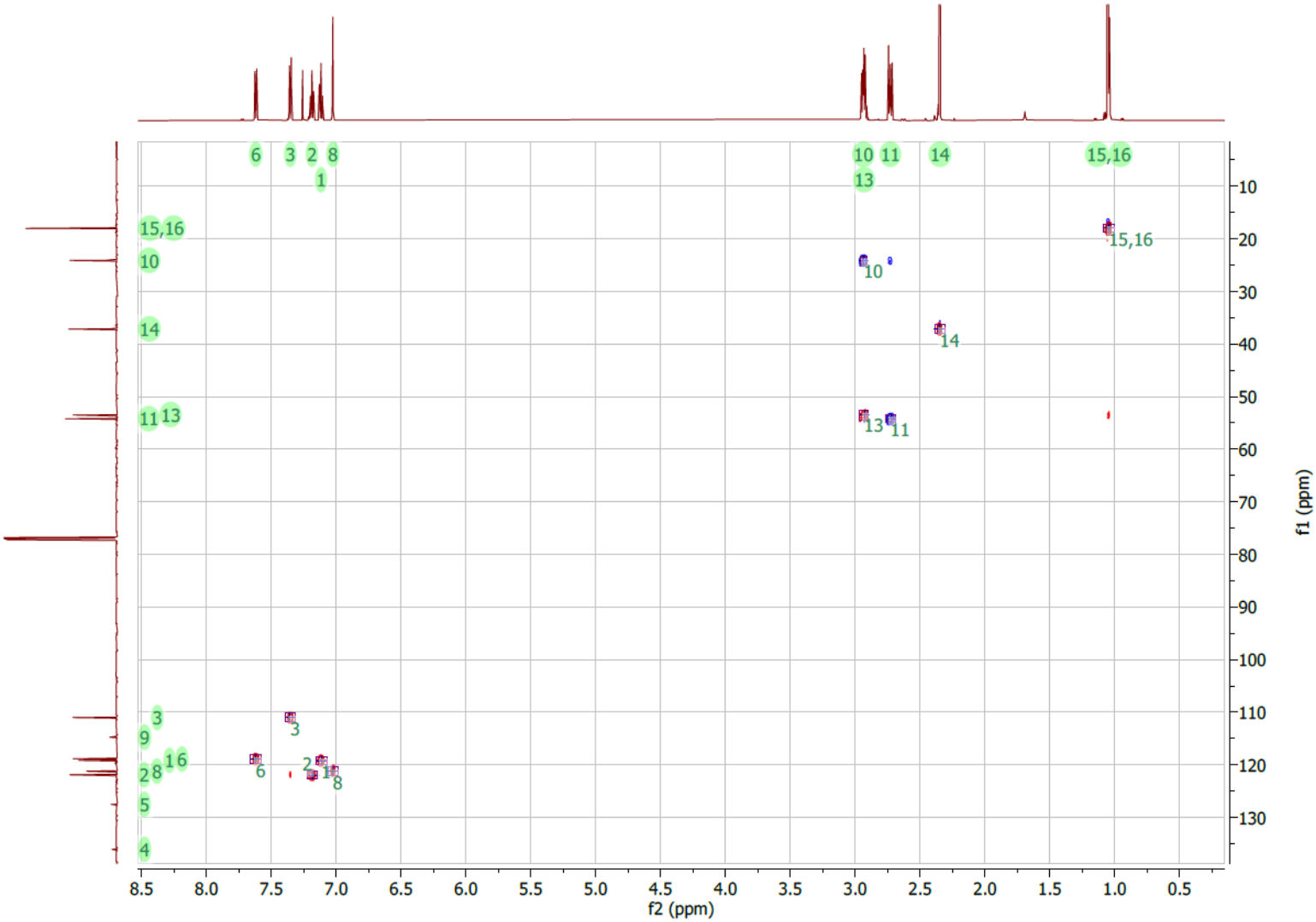


Figure A3. Assigned ^{1}H-^{13}C HSQC spectrum. Assignments based on structure in Figure 1.

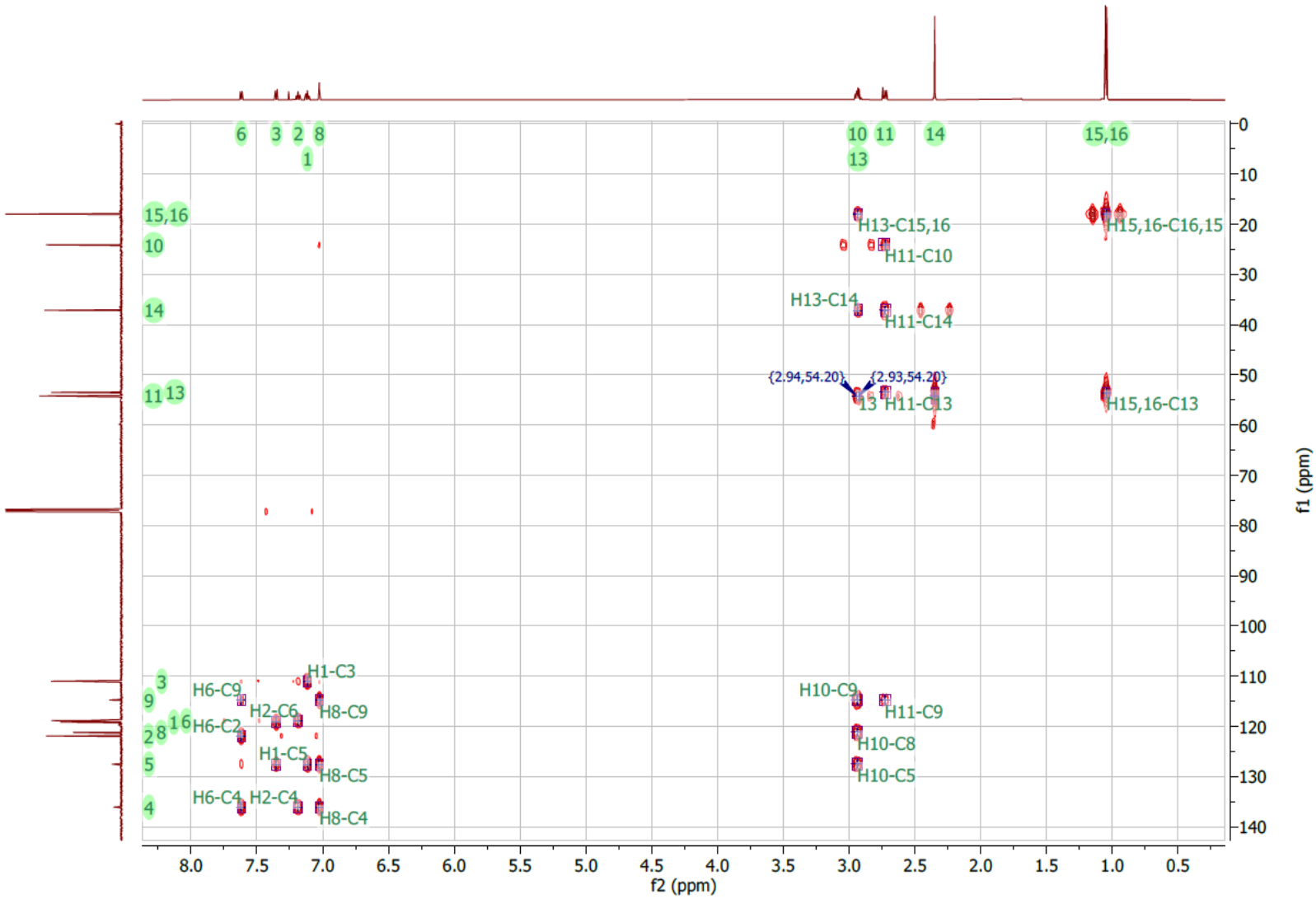


Figure A4. Assigned ^{1}H-^{13}C HMBC spectrum. Assignments based on structure in Figure 1.

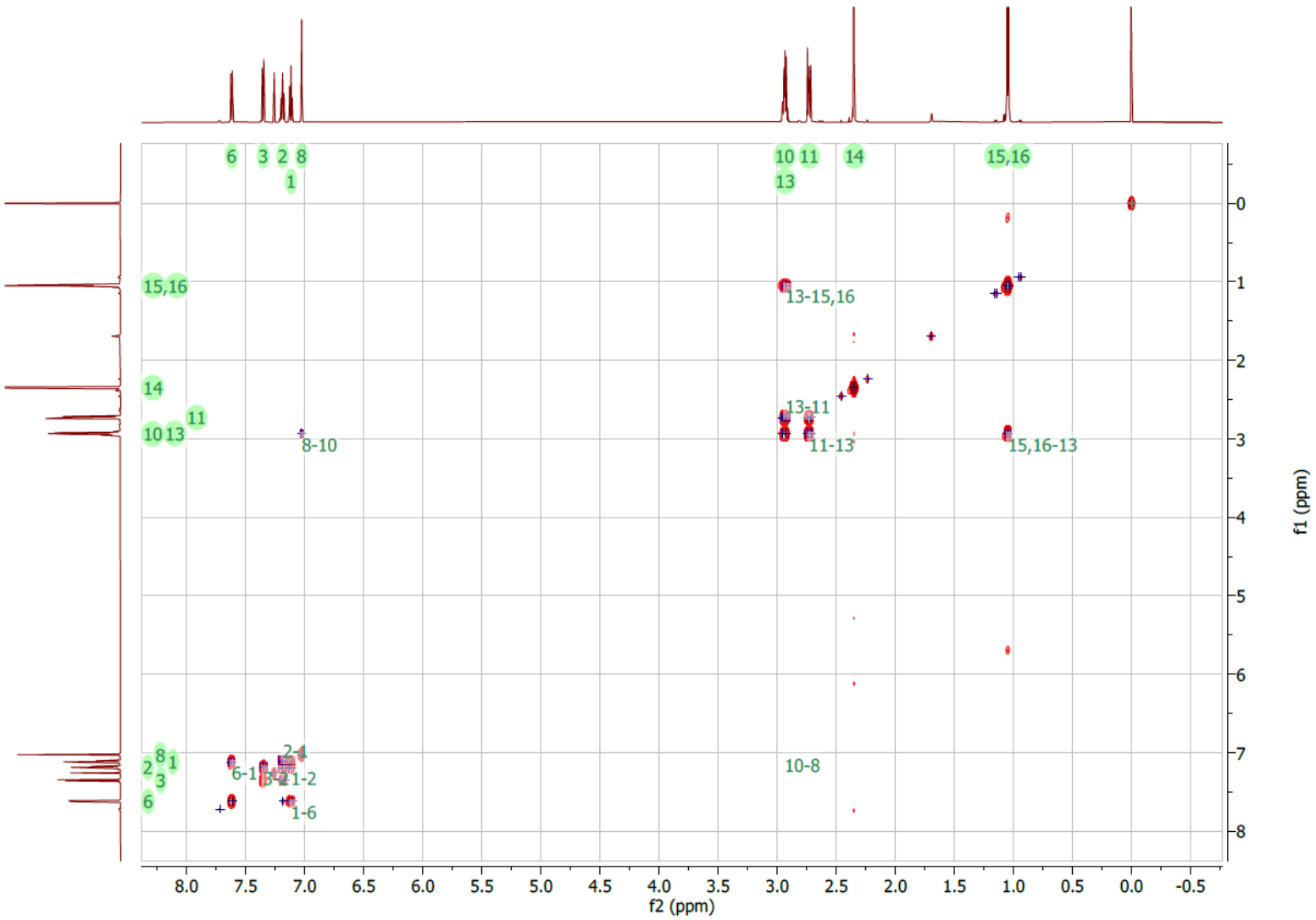


Figure A5. Assigned ^{1}H-^{1}H COSY spectrum. Assignments based on structure in Figure 1.

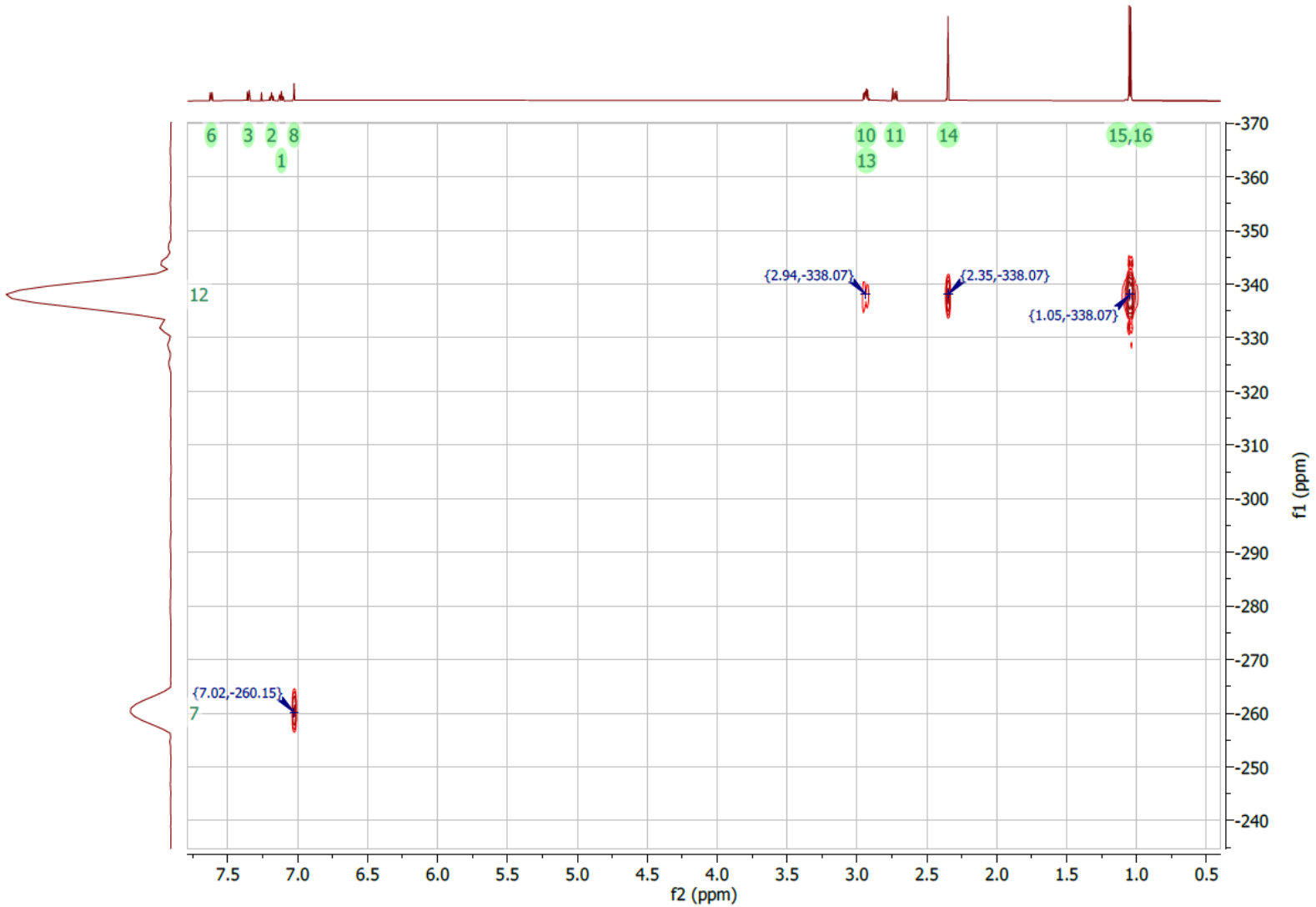


Figure A6. Assigned ^{1}H-^{15}N HMBC spectrum. Assignments based on structure in Figure 1.