Characterization and Synthesis of Eudistidine C, a Bioactive Marine Alkaloid with an Intriguing Molecular Scaffold

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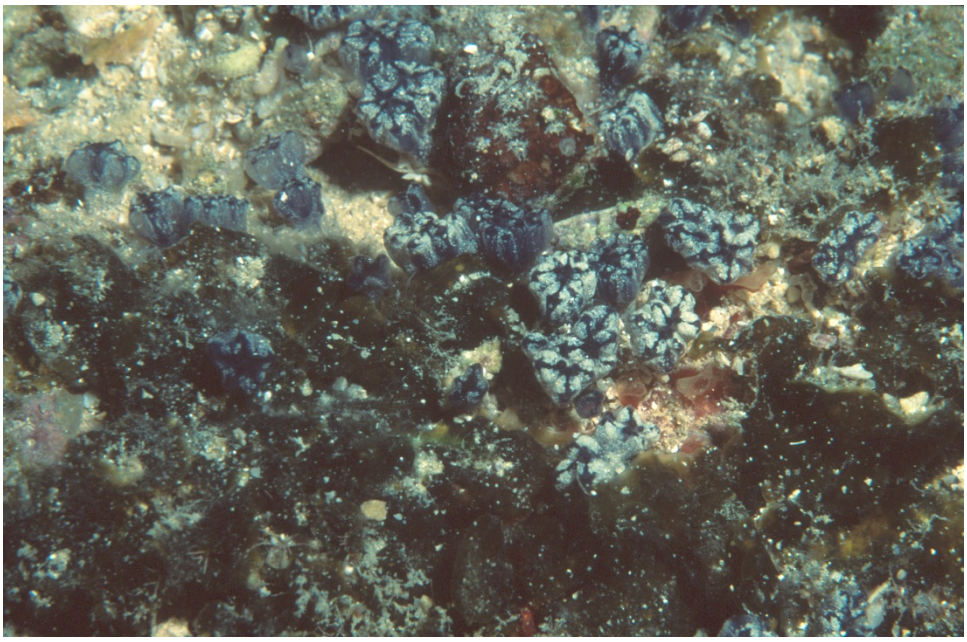
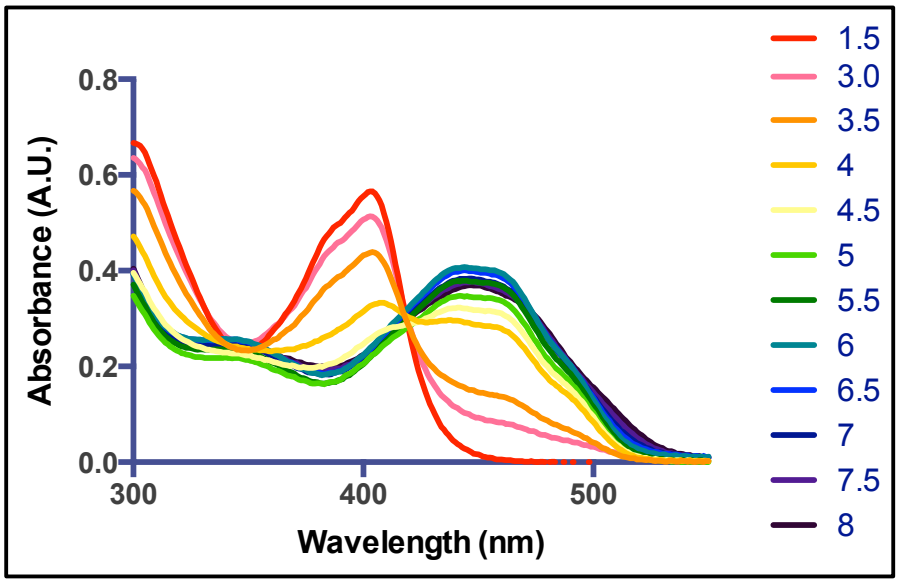
Supporting Information:

Color *in situ* photograph of *Eudistoma* sp.



UV/Vis Absorption Profile of Eudistidine C (1)



pH effects on the UV/Vis absorption spectra of eudistidine C (**1**). Absorbance maxima were observed at 446 and 404 nm with the isobestic point at 420 nm.

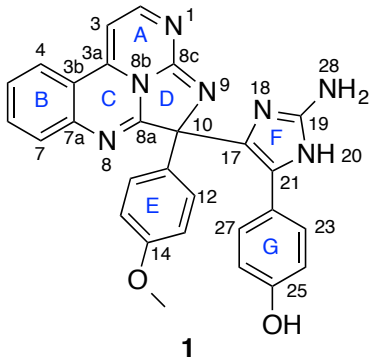
Experimental and DFT Calculated ^{13}C NMR Data for Eudistidine C (1) in CD_3OD

Position	Experimentally measured	DFT calculated
	δ_C	δ_C
2	167.5	166.1
3	94.3	93.0
3a	147.4	146.5
3b	117.6	117.1
4	126.1	126.2
5	130.2	128.7
6	136.5	136.3
7	129.7	129.2
7a	147.0	146.8
8a	159.5	160.0
8c	157.3	156.2
10	73.7	75.5
11	130.9	134.7
12 & 16	129.8	128.9
13 & 15	115.2	113.3
14	161.7	160.3
14-OMe	55.8	53.5
17	126.1	125.9
19	148.0	143.5
21	127.5	124.9
22	120.0	122.1
23 & 27	131.9	132.8
24 & 26	115.7	113.9
25	159.3	157.5

DFT Calculated 4- and 5-Bond Carbon-Proton Coupling Constants for Eudistidine C (1)[a]

	DFT calculated $^4J_{CH}$ and $^5J_{CH}$ (Hz)									
	Proton									
Carbon	2	3	4	5	6	7	15	16	23	24
3a				0.79		0.95				
3b	1.33				-1.46					
4						-1.19				
7			-1.54							
7a				-1.51						
8a		0.84					0.34	0.87		
8c										
10	0.47						0.44			
17								0.41	0.47	0.40
21										0.37

[a]four-bond couplings highlighted in green and five-bond couplings in pink



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Summary of the Spectroscopic Data Used by ACD/Structure Elucidator

The following input was used in ACD/Structure Elucidator: molecular formula $C_{28}H_{21}O_2N_7$, two *para*-disubstituted benzene rings, a 1,2-disubstituted benzene ring, and the chemical shift data below. The software was run on a PC with Windows 7, Dual Core CPU 2.9 GHz and 16 GB RAM.

1H			13C			COSY		
#	Shift (ppm)	Atoms	#	Shift (ppm)	XHn	#	F2(ppm)	F1(ppm)
1	3.75	3	1	55.86	CH3(q)	1	6.29	6.84
2	6.29	2	2	94.31	CH(d)	2	6.84	6.29
3	6.76	1	3	115.22	CH(d)	3	7.57	7.80
4	6.84	2	4	115.66	CH(d)	4	7.57	8.05
5	6.90	2	5	117.64	C(s)	5	7.64	7.80
6	7.57	1	6	120.01	C(s)	6	7.67	6.90
7	7.64	1	7	126.14	CH(d)	7	7.80	7.64
8	7.67	2	8	127.51	C(s)	8	8.05	7.57
9	7.80	1	9	129.75	CH(d)	9	8.43	6.76
10	8.05	1	10	129.78	CH(d)			
11	8.43	1	11	130.15	CH(d)			
			12	130.94	C(s)			
			13	131.89	CH(d)			
			14	136.46	CH(d)			
			15	146.97	C(s)			
			16	147.39	C(s)			
			17	148.04	C(s)			
			18	157.24	C(s)			
			19	159.28	C(s)			
			20	159.47	C(s)			
			21	161.68	C(s)			
			22	167.50	CH(d)			

LR-HSQMBC

#	F2(ppm)	F1(ppm)
1	3.69	128.28
2	3.69	114.00
3	3.69	54.49
4	3.69	160.90
5	6.32	120.55
6	6.32	117.20
7	6.32	158.44
8	6.32	128.08
9	6.32	114.61
10	6.80	130.66
11	6.80	114.61
12	6.80	128.08
13	6.80	114.00
14	6.80	67.84
15	6.80	160.90
16	6.80	120.55
17	6.80	158.44
18	7.58	160.90
19	7.58	128.28
20	7.58	114.00
21	7.58	120.55
22	7.58	125.98
23	7.58	151.72
24	7.89	137.18
25	7.89	146.60
26	7.89	145.22
27	7.89	125.78
28	7.89	116.29
29	7.89	129.32
30	8.02	125.78
31	8.02	116.29
32	8.02	146.60
33	8.02	145.22
34	8.02	137.18
35	8.11	129.32
36	8.11	145.22
37	8.11	116.29
38	8.56	116.29
39	8.56	129.32
40	8.56	137.18

HSQC

#	F2(ppm)	F1(ppm)
1	7.80	136.46
2	7.67	129.78
3	7.64	129.75
4	7.57	130.15
5	6.29	115.66
6	6.84	131.89
7	6.76	94.31
8	8.43	167.50
9	8.05	126.14
10	6.90	115.22
11	3.75	55.86

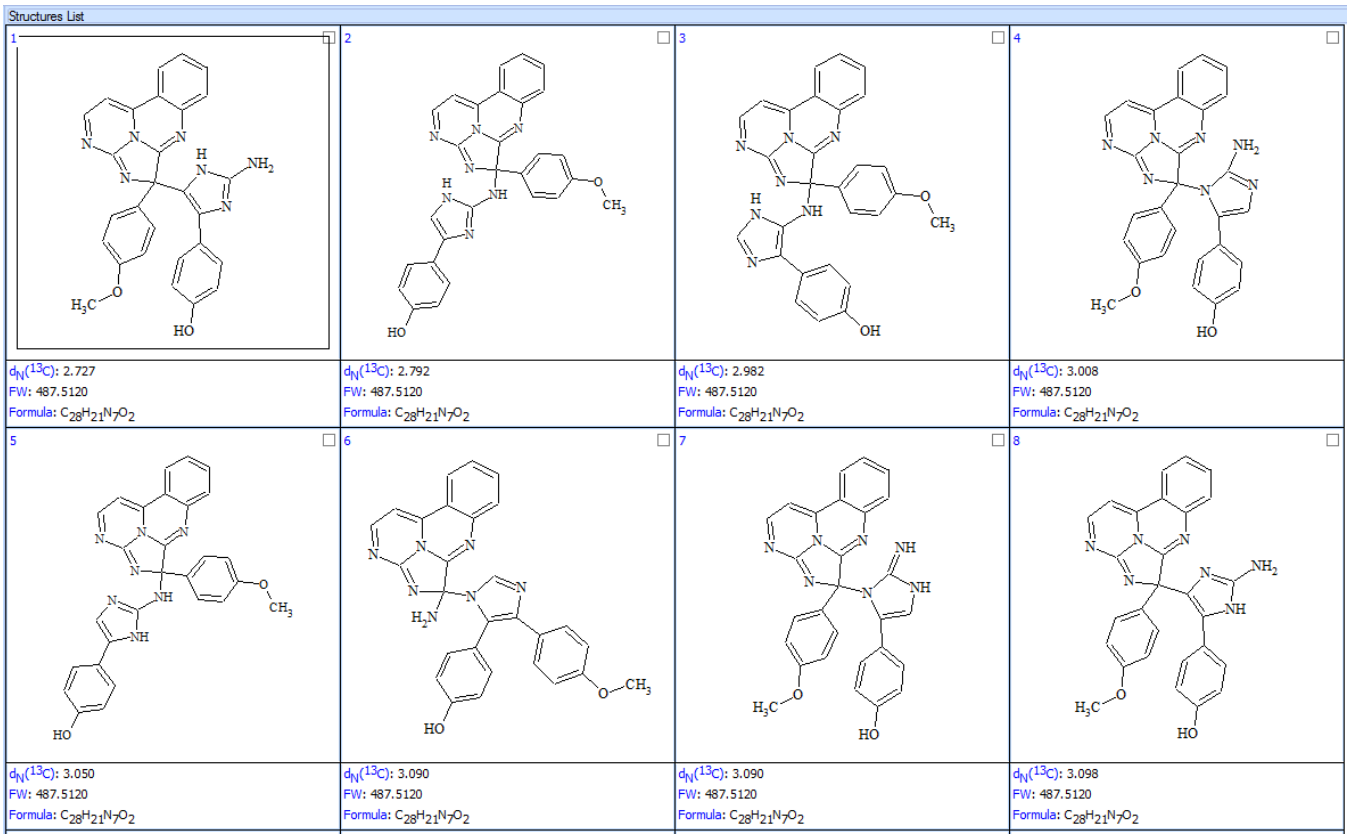
1H-15C HMBC

#	F2(ppm)	F1(ppm)
1	7.87	169.83
2	8.89	236.56
3	7.87	236.56
4	8.02	267.63

HMBC

#	F2(ppm)	F1(ppm)
1	6.29	115.66
2	6.84	131.89
3	8.43	94.31
4	7.64	130.15
5	7.64	117.64
6	7.57	117.64
7	6.76	117.64
8	6.29	120.01
9	6.84	127.51
10	7.80	146.97
11	8.43	147.39
12	6.76	147.39
13	6.84	159.28
14	6.29	159.28
15	7.67	161.68
16	8.43	157.24
17	8.05	146.97
18	8.05	136.46
19	6.90	130.94
20	6.90	115.22
21	3.75	161.68
22	6.90	161.68
23	8.43	117.64
24	6.84	115.66
25	7.80	126.14
26	8.05	117.64
27	6.29	127.51
28	6.29	131.89
29	7.57	136.46
30	7.64	136.46
31	6.76	167.50

Top 8 Structural Candidates Generated by ACD/Structure Elucidator Program



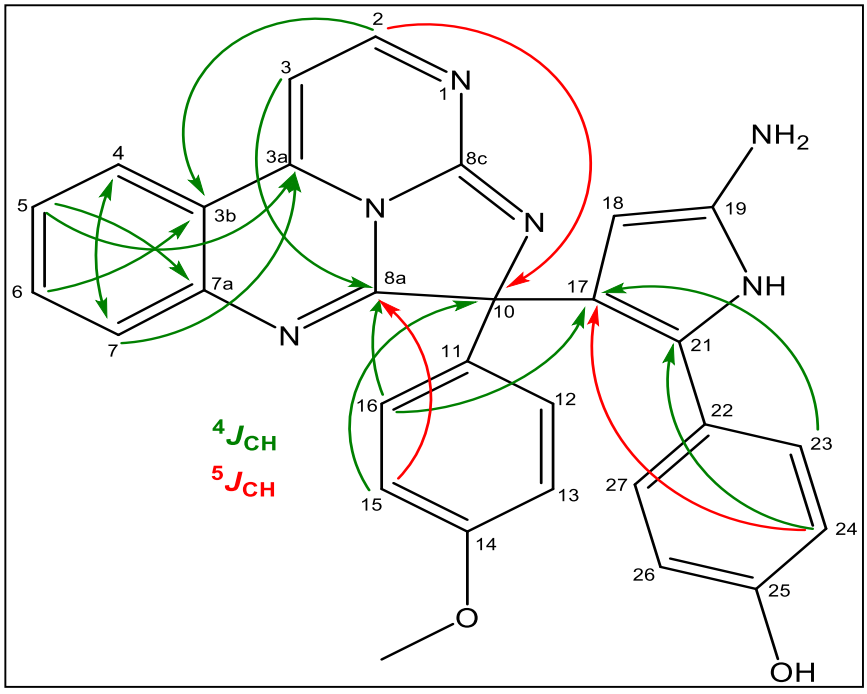
Top 8 candidates generated by ACD/Structure Elucidator. Structure ranking is based on the overall differences between experimental and predicted ^{13}C chemical shifts. The lower the $d_N(^{13}C)$ value, the better the ranking.

Isomers Generated and Time Difference for ACD/Structure Elucidator With and Without the LR-HSQMBC data.

	With LR-HSQMBC*	W/out LR-HSQMBC*
Isomers Generated	35,928	267,468
Post-filter	335	419
Time (seconds)	35	247
Top Candidate	1	1

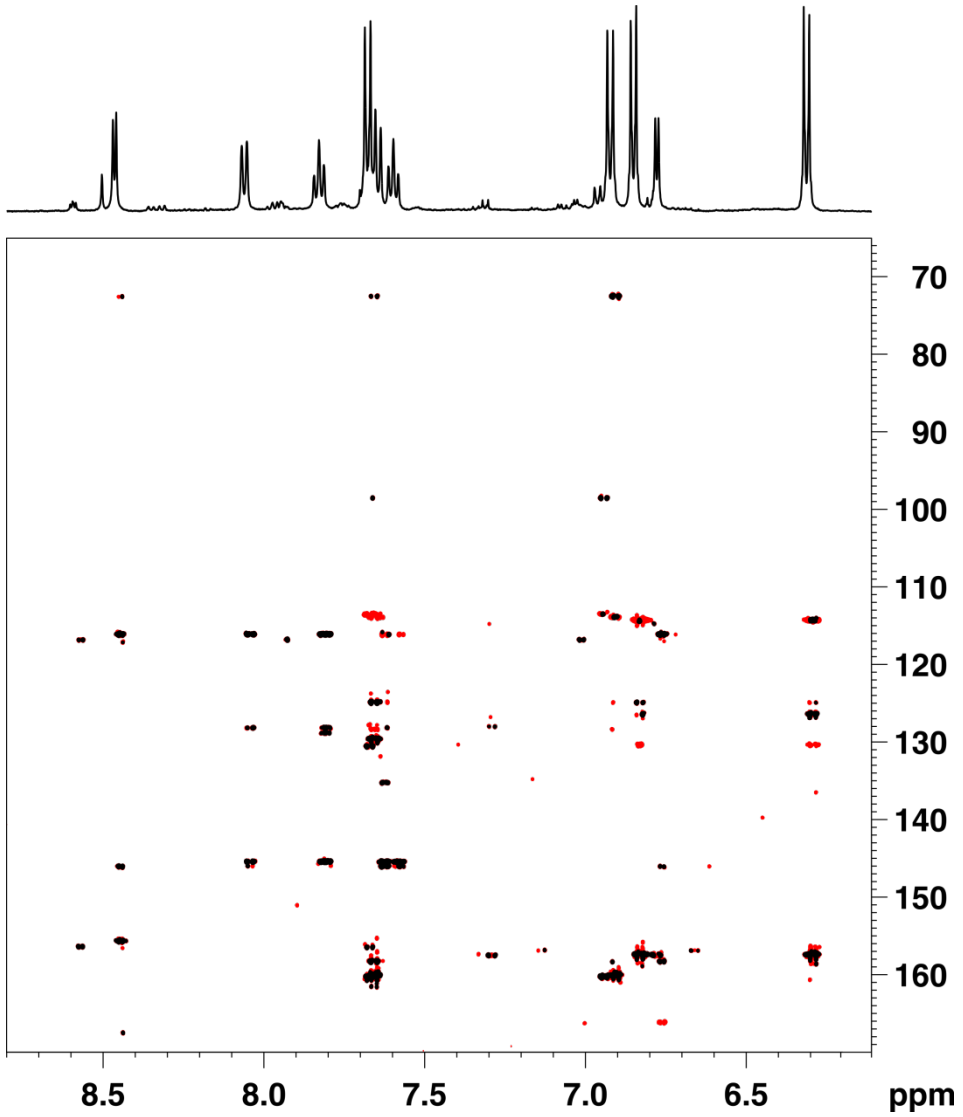
*Data also includes: molecular formula + NMR (^1H, ^{13}C, HSQC, COSY, ^1H-^{13}C HMBC, ^1H-^{15}N HMBC)

Long-Range LR-HSQMBC Correlations Observed for Eudistidine C (1)



Four-bond (green arrows) and five-bond (red arrows) carbon-proton correlations observed in a 2 Hz optimized LR-HSQMBC experiment digitized with 768 F1 increments.

LR-HSQMBC Spectrum of Eudistidine C (1)



2 Hz LR-HSQMBC on eudistidine C (**1**) natural product (CD_3OD). Data were acquired using 3072 × 768 points with 26 transients accumulated per each t_1 increment giving an overall acquisition time of 15 h 30 min. Data were processed by linear prediction to 1536 points in F1. Prior to Fourier transformation, zero-filling to 4096 points in F2 and 2048 points in F1 were applied and a shifted squared sine bell apodization function was used.

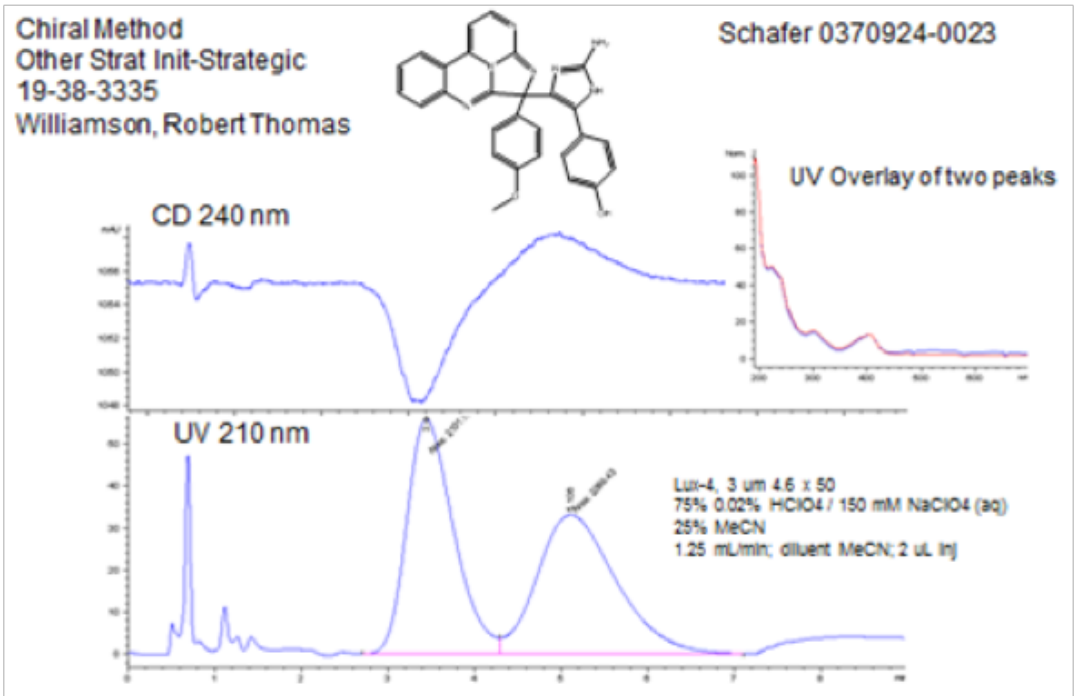
^{13}C NMR Chemical Shift Comparison of Eudistidine C Natural Product vs Synthetic Eudistidine C

position	Natural Product δ_C (ppm)	Synthetic Free Base	Synthetic TFA Salt
2	167.5	167.5	167.4
3	94.3	94.4	101.8
3a	147.4	147.5	147.8
3b	117.6	117.8	117.6
4	126.1	126.2	127.2
5	130.2	130.2	132.0
6	136.5	136.5	138.4
7	129.7	129.8	130.7
7a	147.0	147.1	146.7
8a	159.5	159.5	153.6
8c	157.3	157.4	155.0
10	73.7	73.7	69.6 (hmbc)
11	130.9	130.8	127.7
12/16	129.8	129.8	129.7
13/15	115.2	115.2	115.6
14	161.7	161.7	162.4
14-OMe	55.8	55.8	55.6
17	126.1	125.8	122.7
19	148.0	148.1	148.4
21	127.5	127.5	129.0
22	120.0	119.9	118.4
23/27	131.9	131.9	132.1
24/26	115.7	115.7	115.6
25	159.3	159.3	159.9

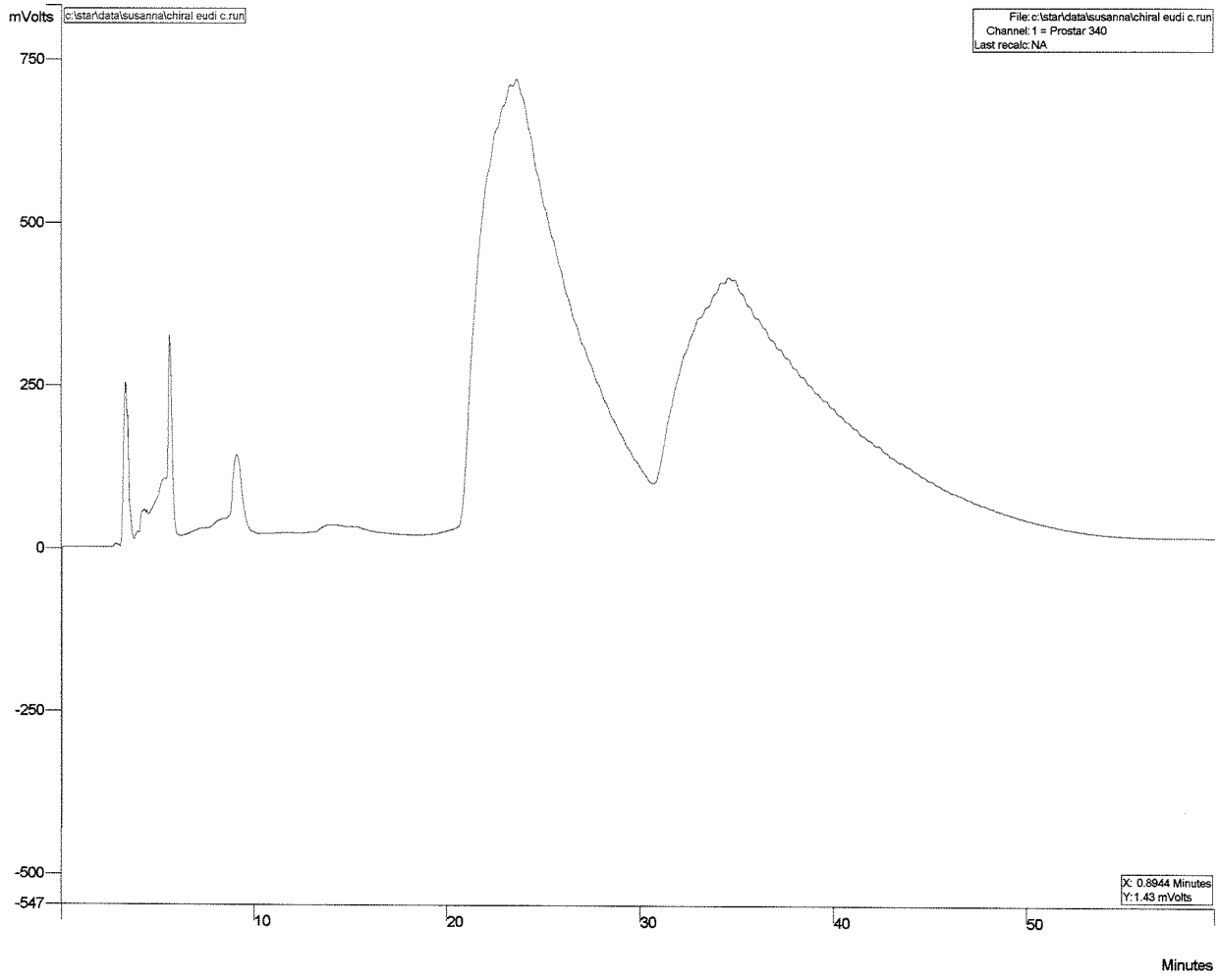
^{1}H NMR Chemical Shift Comparison of Eudistidine C Natural Product vs Synthetic Eudistidine C

position	Natural Product δ_H (ppm)	Synthetic Free Base	Synthetic TFA Salt
2	8.45	8.48	8.90
3	6.77	6.83	7.78
4	8.06	8.13	8.55
5	7.58	7.63	7.92
6	7.81	7.85	8.13
7	7.65	7.69	8.03
12/16	7.68	7.69	7.67
13/15	6.91	6.90	6.93
14-OMe	3.76	3.76	3.77
23/27	6.85	6.87	6.86
24/26	6.30	6.32	6.35

Chiral Resolution of Eudistidine C (1) Natural Product

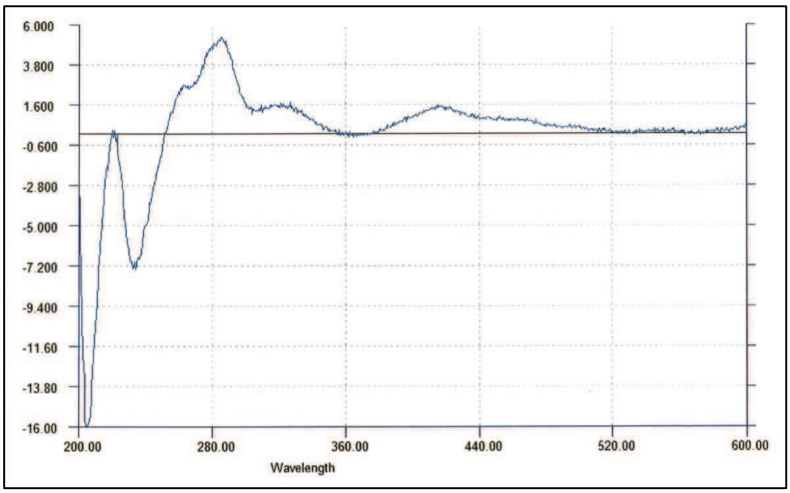


Preparative Chiral Separation of Synthetic Eudistidine C (1)

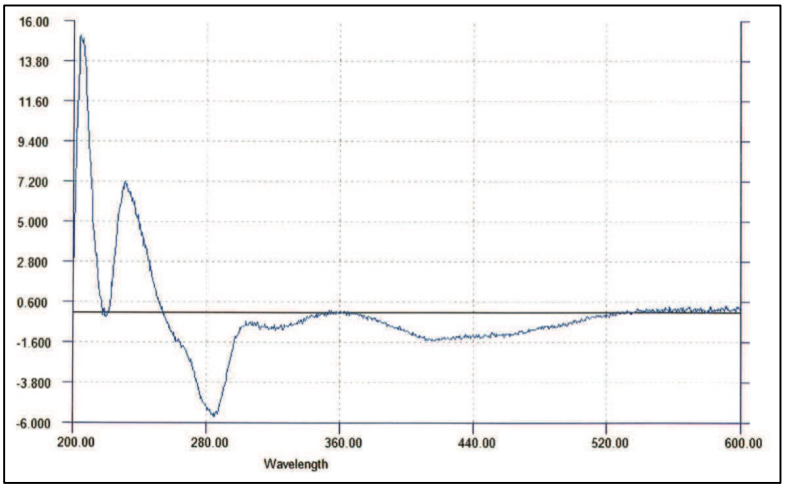


The two enantiomers were separated using a Phenomenex Lux 4 column (5 μm, 250 x 10 mm) employing an isocratic gradient of 25% MeCN / 75% 150 mM $NaClO_4$ (0.02% $HClO_4$) over 53 mins affording (+)-*R*-eudistidine C (**1a**) [7.8 mg, $[\alpha]_D$ +132.6 (c 0.1, MeOH)], and (-)-*S*-eudistidine C (**1b**) [7.7 mg, $[\alpha]_D$ -148.5 (c 0.1, MeOH)].

Experimental ECD Spectra for (+)-*R*-Eudistidine C (1a) and (-)-*S*-Eudistidine C (1b)



(+)-*R*-Eudistidine C (**1a**)

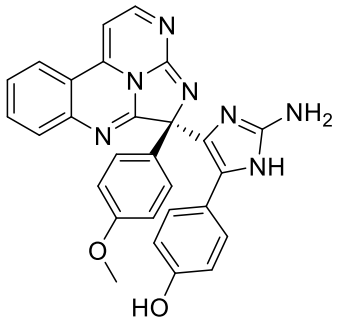


(-)-*S*-Eudistidine C (**1b**)

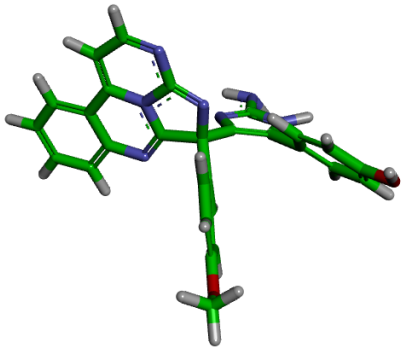
Conformational Analysis and ECD Calculation for (+)-*R*-Eudistidine C (1b)

Conformational Analysis and ECD Calculation

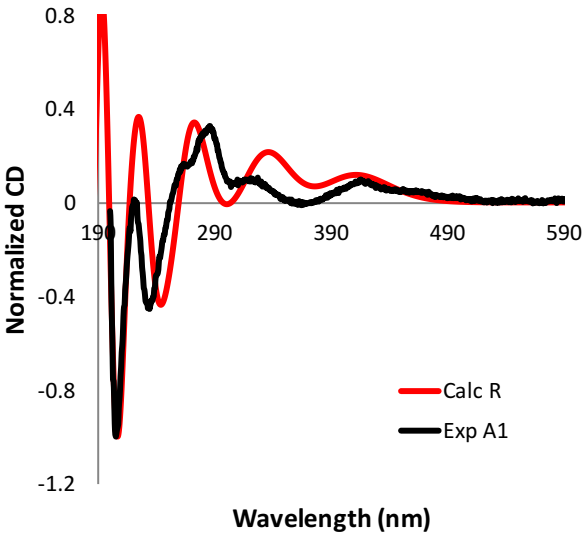
Chemical Structure of (R)-Eudistidine C:



Global Minimum (R)-Eudistidine C:



ECD Comparison:



Correlation between experimental peak A1 and the calculated (R)-Eudistidine C as a free-base

p300/HIF-1α Assay

Inhibition of HIF-1α binding to p300 was measured by displacement of GST-p300-CH1 (aa 323–423) from synthetic biotinylated HIF-1α C-TAD (aa 786–826; Peptide Protein Research Ltd., Fareham, UK) immobilized on 384-well streptavidin-coated plates. Bound GST-CH1 was detected using a Europium-labeled antibody to GST (PerkinElmer Life Sciences). 48.5 nM HIF-1α C-TAD was used to coat plates for 5 h at room temperature. Plates were washed four times with TBST (50 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 8.0) buffer. 7.35 nM GST-CH1 was added with along with the test compounds or control (1% DMSO) in TBST with 5% BSA, 0.5 mM DTT, and 10 uM $ZnCl_2$ and incubated overnight at 24 °C. Plates were washed four times with TBST, and Europium-labeled anti-GST (450 ng/mL) was added to plates in the same buffer used for GST-CH1 addition. After 2 h, plates were washed four times in TBST. DELFIA enhancement solution (PerkinElmer Life Sciences) was added and plates were placed on a rocker for 30 min, before reading with a Victor3 plate reader (PerkinElmer Life Sciences) or a Pherastar Plate Reader (BMG Labtech), using the Europium setting under time-resolved fluorescence. Values were corrected for background and expressed as a percentage of controls (DMSO) to provide the percentage of CH1 binding.

p300/ HIF-1α assay results

Compound	IC_{50} (μM)
1a	n.a.
1b	276
5	314
6	399
7	356
8	n.a.
9	n.a.
10	141

n.a. = not active (IC_{50} > 500 μM)

Antimalarial Screening Assay

The antimalarial activity was determined against chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum* by measuring plasmodial lactate dehydrogenase (LDH) activity according to the procedure of Makler and Hinrichs.[5] A suspension of red blood cells infected with the D6 or W2 strain of *P. falciparum* (200 µL, with 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 μg/mL Amikacin) was added to the wells of a 96-well plate containing 10 μL of serially diluted test samples. The plate was incubated at 37 ^{O}C, for 72 h in in an environment of 90% N_2, 5% O_2, and 5% CO_2. Plasmodial LDH activity was determined by mixing 20 μL of the incubation mixture with 100 μL of the Malstat reagent and incubating at room temperature for 30 min. Twenty microliters of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO) was added and the plate was further incubated in the dark for 1 h. The reaction was then stopped by adding 100 μL of a 5% acetic acid solution and the absorbance was read at 650 nm. Artemisinin and chloroquine were included as the drug controls. The *in vitro* cytotoxicity of samples to mammalian cells was also tested in order to determine the selectivity index of the antimalarial activity. Vero cells (monkey kidney fibroblasts) were seeded into a 96-well plate at a density of 25,000 cells/well and grown for 24 h. Test samples at different concentrations were added and cells were further incubated for 48 h. Cell viability was determined by the Neutral Red method.[6] Doxorubicin was included as the drug control. IC_{50} values were obtained from the dose response curves.

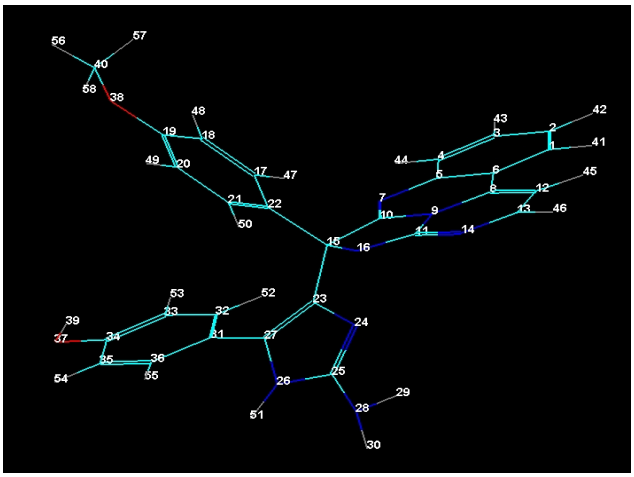
(5) Makler, M. T.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 205-210.
(6) Borenfreund, E.; Babich, H.; Martin-Alguacil, N. *In vitro Cell. Dev. Biol.* **1990**, *26*, 1030-1034.

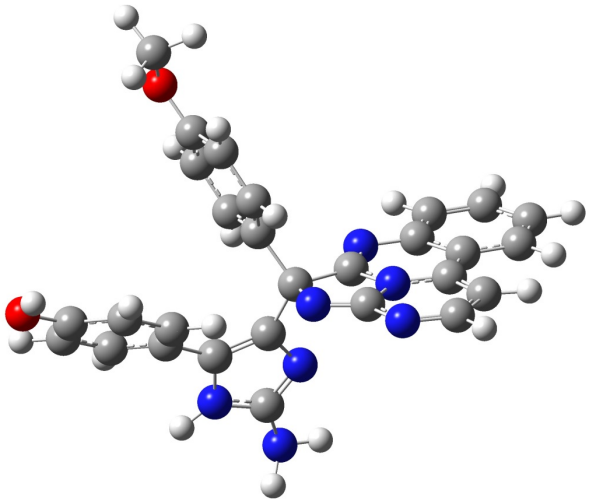
Antimalarial activity against P. falciparum **and cytotoxicity towards Vero cells**

Compound	D6 strain IC_{50} (μM)	W2 strain IC_{50} (μM)	Cytotoxicity
1a	2.8	1.5	n.c.
1b	4.2	2.5	n.c.
2	1.4	1.1	n.c.
6	n.a.	7.5	n.c.
7	2.4	1.0	n.c.
8	5.8	3.6	n.c.
9	6.6	5.6	n.c.
10	1.1	0.6	n.c.

n.a. = not active; n.c. = no cytotoxicity

Molecular Model of Eudistidine C (1)



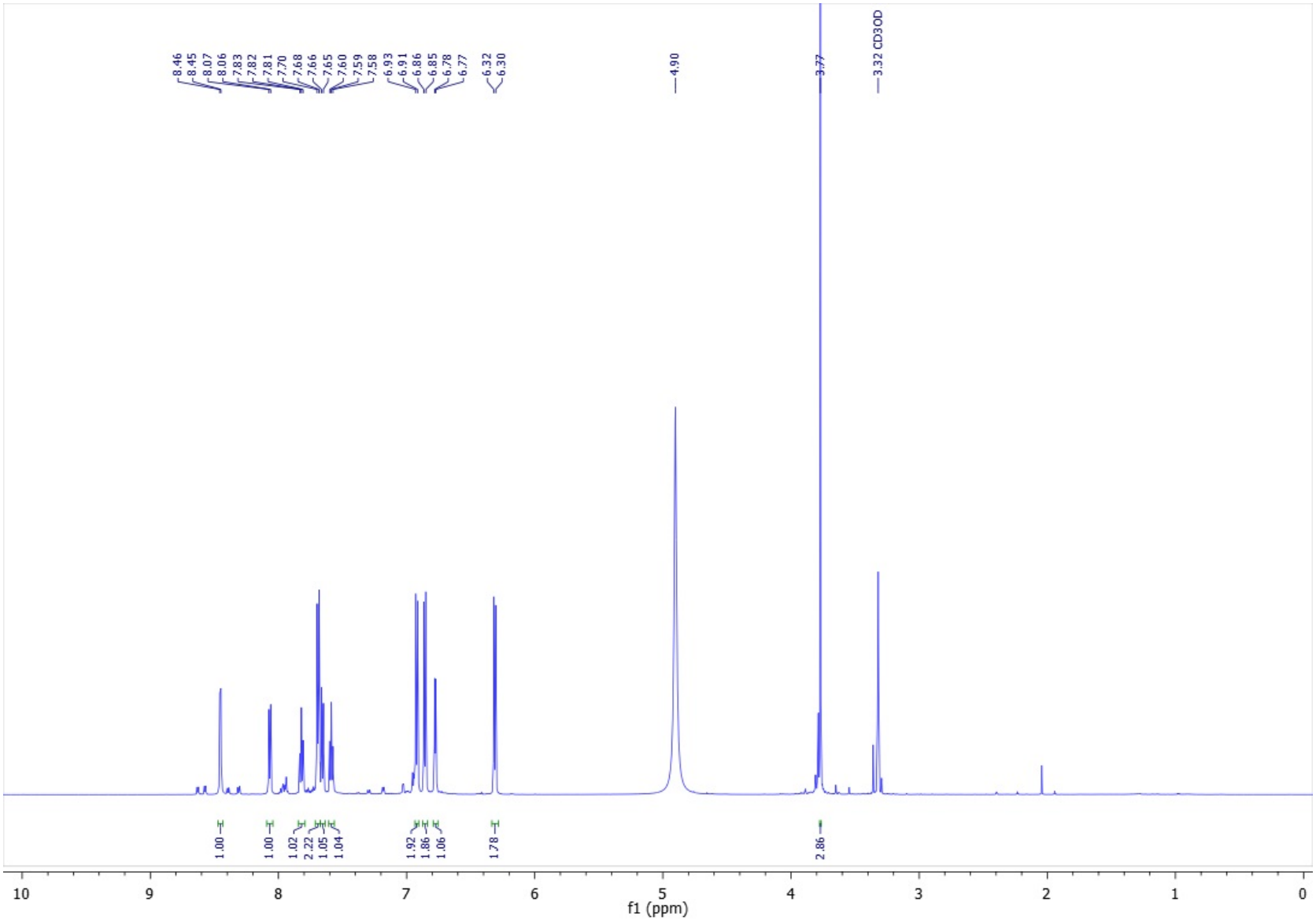


Atomic coordinates of molecular model of eudistidine C (1)

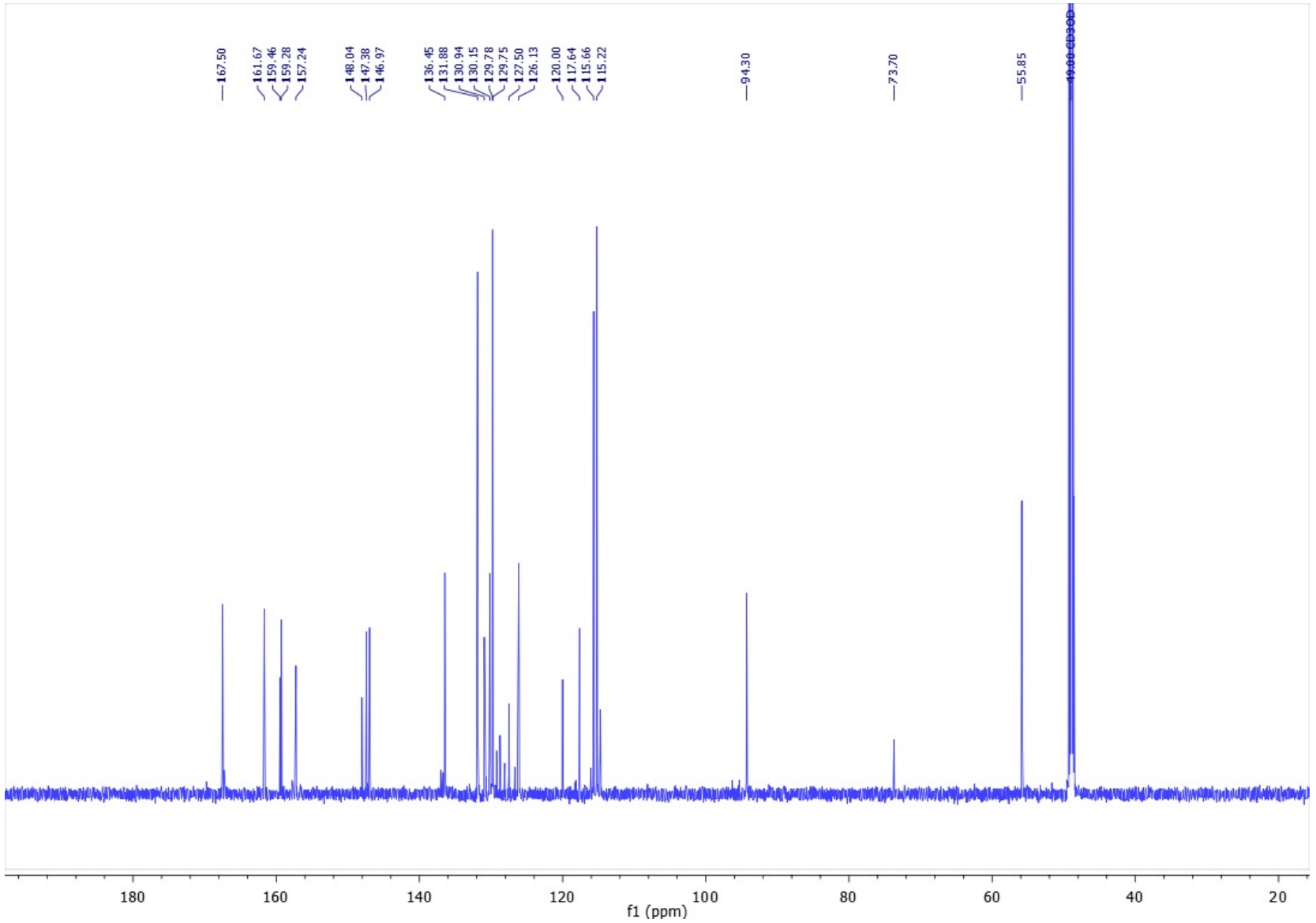
C	5.78711400	0.53043200	0.08790200
C	6.41643500	0.91459600	-1.08542500
C	5.65741200	1.13112500	-2.25189800
C	4.28240700	0.96097400	-2.23781200
C	3.61860600	0.56721300	-1.05735500
C	4.38974800	0.35102700	0.12541300
N	2.23706200	0.40977600	-1.11346600
C	3.68586300	-0.03484800	1.33411200
N	2.33524100	-0.17057000	1.15805100
C	1.66580000	0.03424900	-0.02226600
C	1.39466600	-0.50177600	2.18408000
C	4.14067800	-0.27542700	2.62031300
C	3.17712000	-0.62072500	3.60893800
N	1.87300800	-0.73590000	3.44373900
C	0.18278000	-0.24674900	0.30002600
N	0.17607600	-0.52219700	1.76231600
C	-1.08420900	1.87582000	0.94066100
C	-1.80182500	3.03517200	0.61922600
C	-2.08898400	3.32107100	-0.71922800
C	-1.65202900	2.44336800	-1.72390500
C	-0.94319000	1.29703600	-1.38928700
C	-0.65393100	0.99236400	-0.04868700
C	-0.24848500	-1.51187100	-0.42718900
N	0.71560800	-2.36313100	-0.94575400
C	0.05901600	-3.38155300	-1.45437900
N	-1.28672500	-3.23795900	-1.28883700
C	-1.51279900	-2.04179100	-0.59902200
N	0.61585700	-4.44845100	-2.15963200

H	1.62536300	-4.36048400	-2.18659400
H	0.35123500	-5.36425700	-1.81139400
C	-2.86810300	-1.65014000	-0.19509400
C	-3.11729600	-1.11435900	1.08034300
C	-4.41113500	-0.76912800	1.46818900
C	-5.48516100	-0.96730200	0.59453700
C	-5.25970600	-1.50579800	-0.67615100
C	-3.96310000	-1.83625200	-1.06151500
O	-6.77874000	-0.65339700	0.92785300
O	-2.78406300	4.41964900	-1.14900600
H	-6.80521800	-0.28166400	1.81934600
C	-3.25590400	5.34366400	-0.17825100
H	6.37291300	0.36522900	0.98657600
H	7.49341500	1.04902500	-1.10422200
H	6.15407100	1.43338200	-3.16922400
H	3.67963700	1.12115700	-3.12545200
H	5.18793100	-0.20133300	2.87863900
H	3.53463400	-0.81134300	4.62067900
H	-0.87399500	1.64879700	1.98024100
H	-2.12875600	3.69151300	1.41715400
H	-1.88126900	2.67903000	-2.75823900
H	-0.60825400	0.62955600	-2.17641400
H	-2.00270400	-3.87859000	-1.59763000
H	-2.29001400	-0.97917200	1.76966600
H	-4.58429300	-0.35601200	2.46001800
H	-6.09907600	-1.64301200	-1.35003500
H	-3.79766000	-2.21798800	-2.06606400
H	-3.77342300	6.12500500	-0.73661200
H	-2.42744200	5.78895000	0.38746800
H	-3.95829600	4.86884800	0.51875200

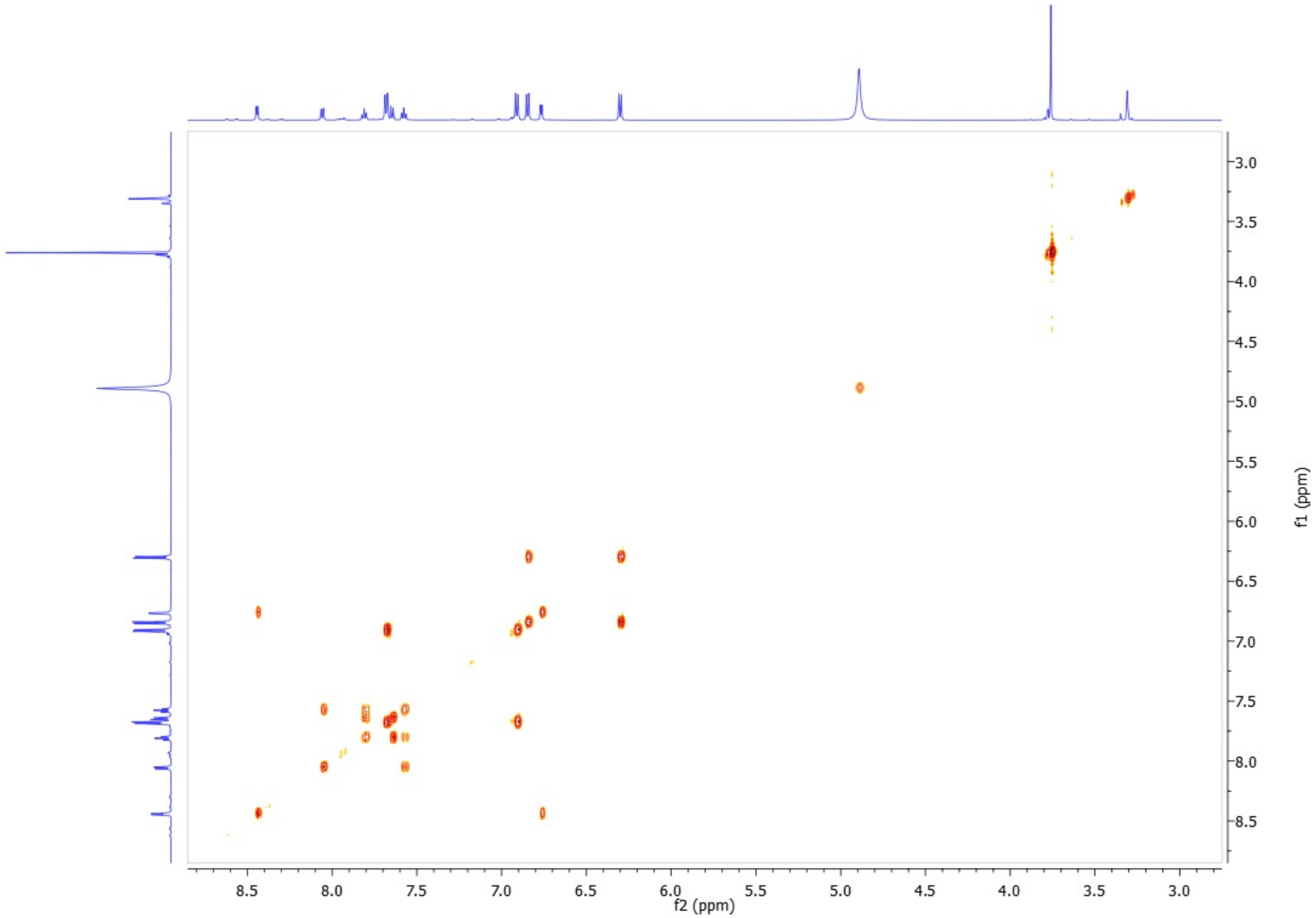
Eudistidine C (1) ^{1}H NMR Spectrum (600 MHz, CD_3OD)



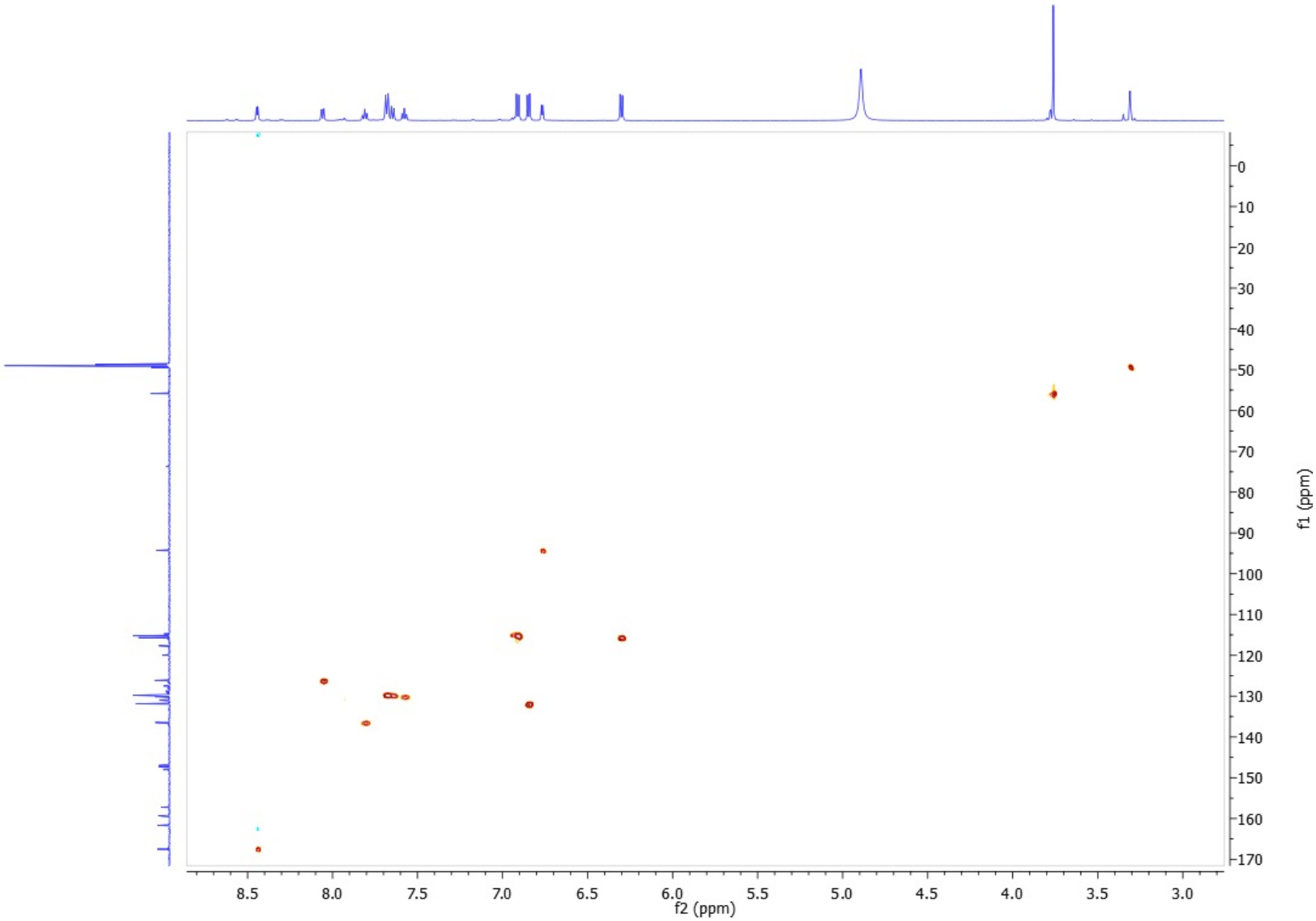
Eudistidine C (1) ^{13}C NMR Spectrum (150 MHz, CD_3OD)



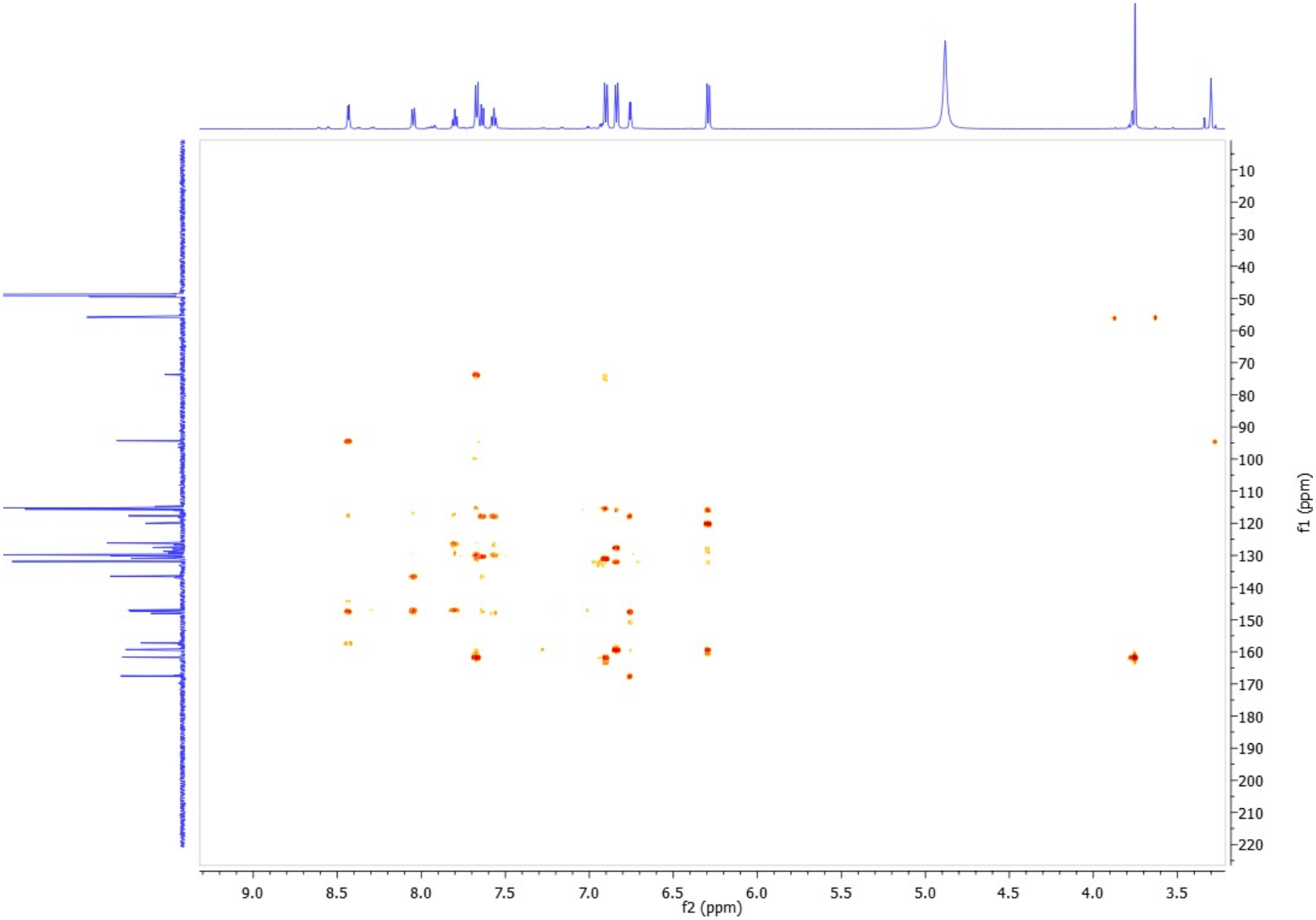
Eudistidine C (1) COSY Spectrum (CD_3OD)



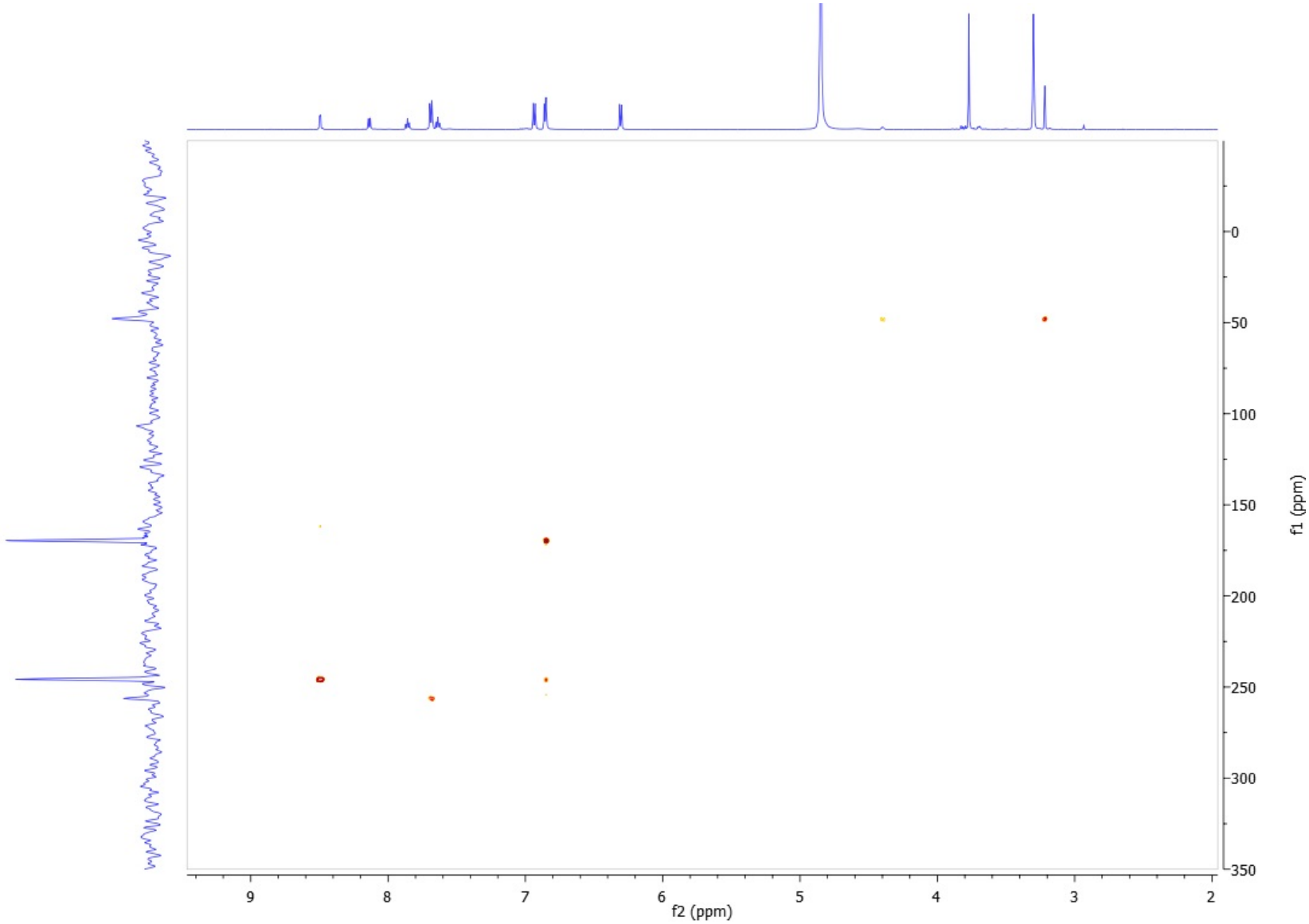
Eudistidine C (1) HSQC Spectrum (CD_3OD)



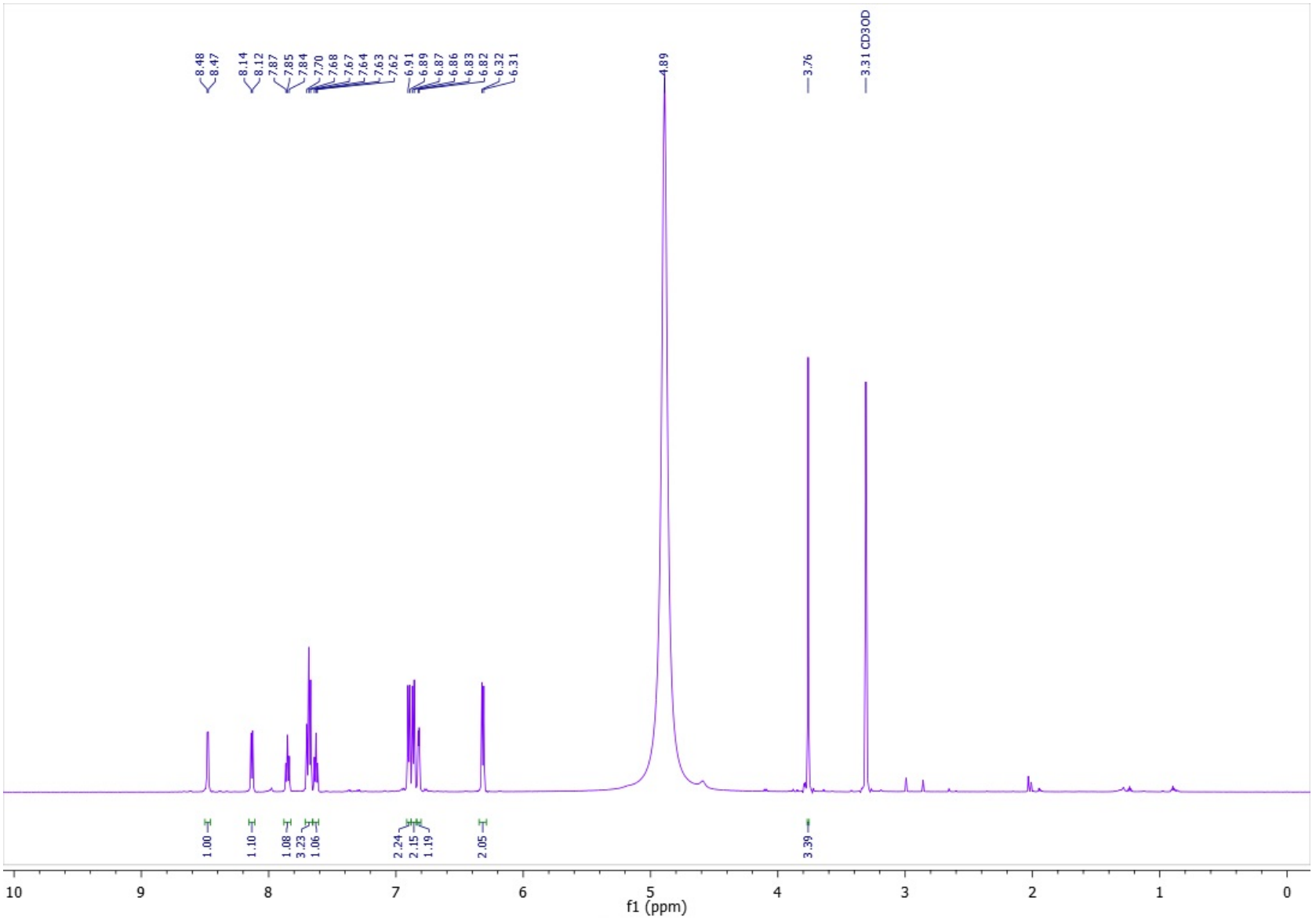
Eudistidine C (1) HMBC Spectrum (CD_3OD)



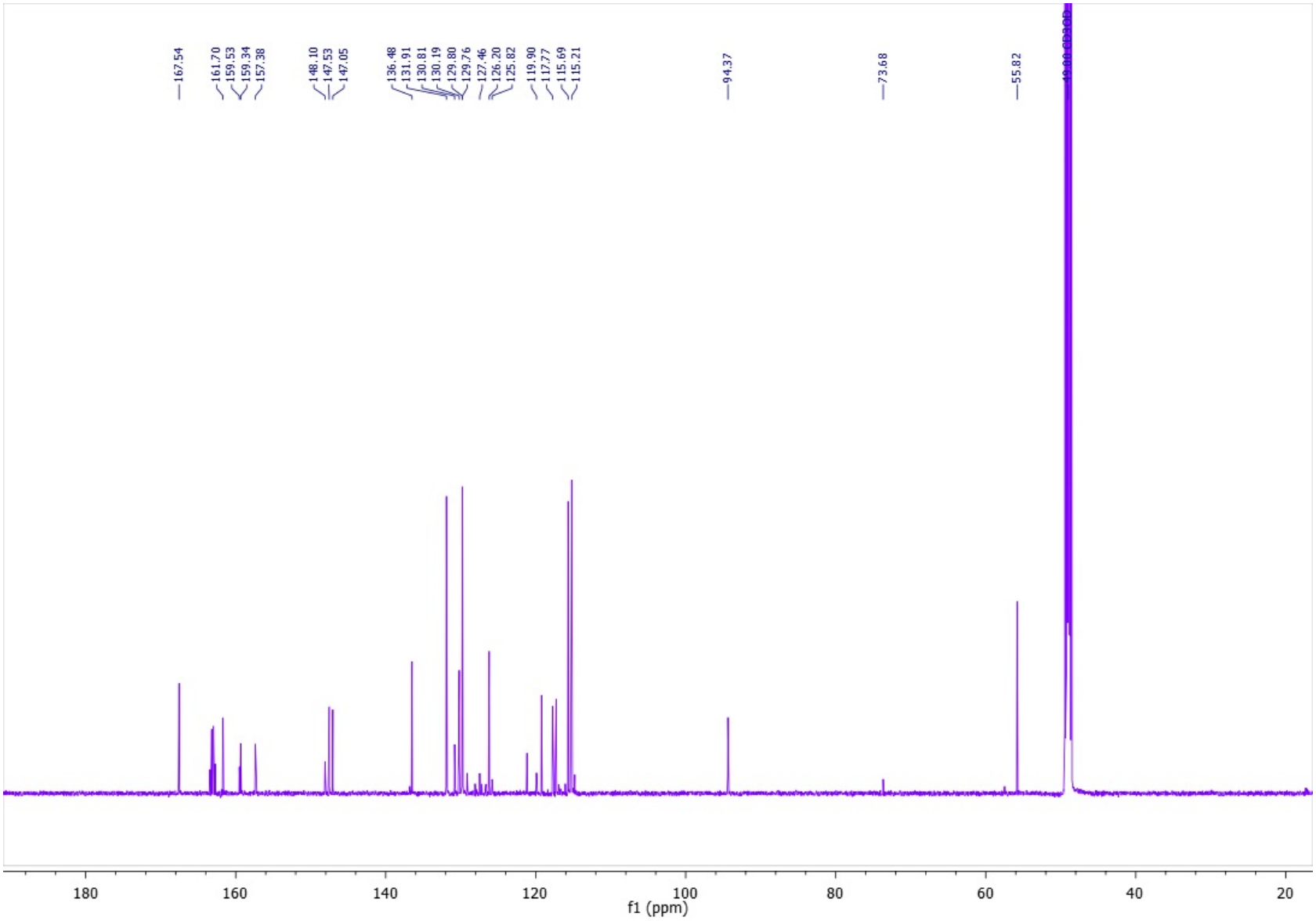
Eudistidine C (1) ^{15}N-^{1}H HMBC Spectrum (CD_3OD)



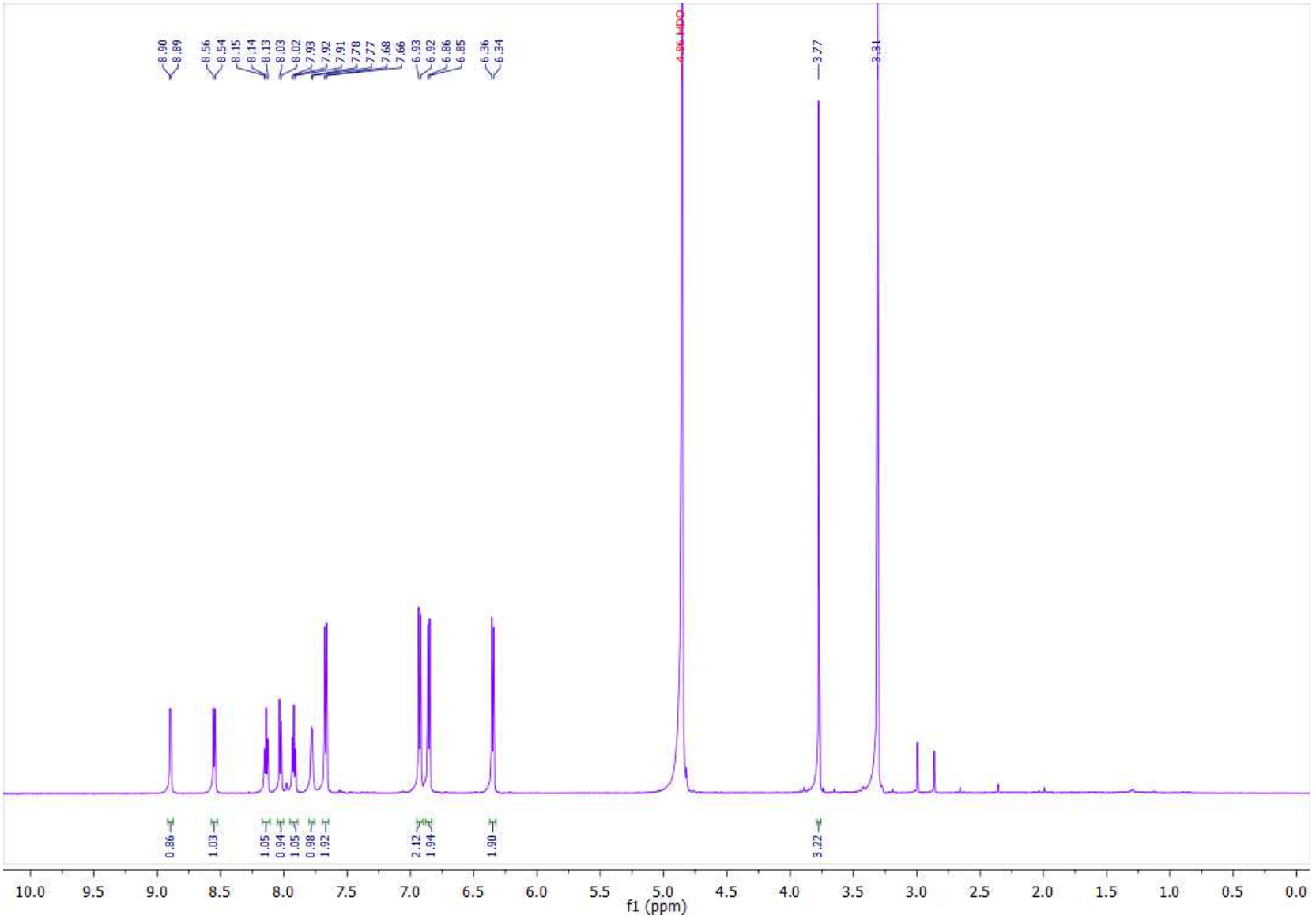
Synthetic Eudistidine C (1) Free Base ^{1}H NMR Spectrum (600 MHz, CD_3OD)



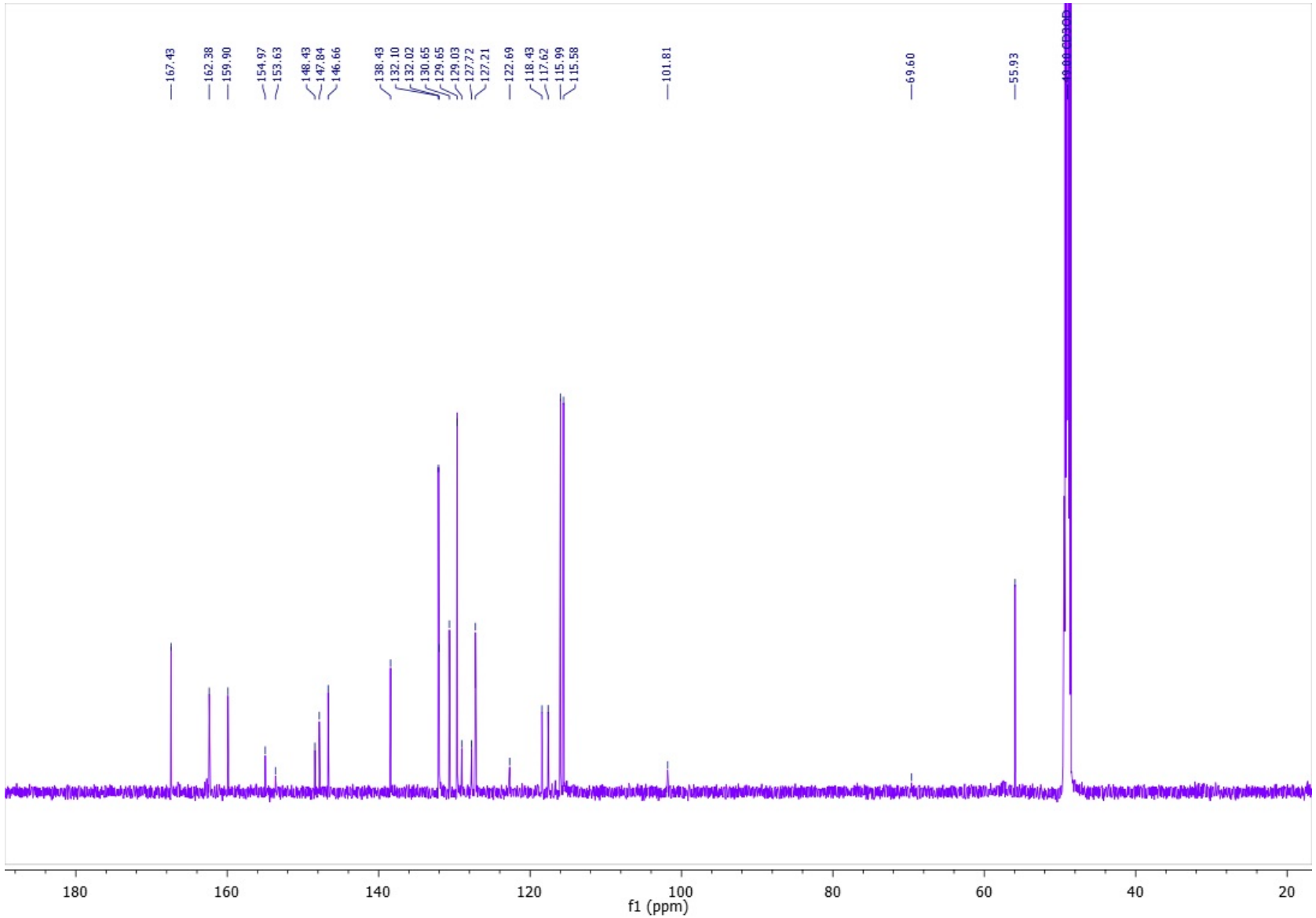
Synthetic Eudistidine C (1) Free Base ^{13}C NMR Spectrum (150 MHz, CD_3OD)



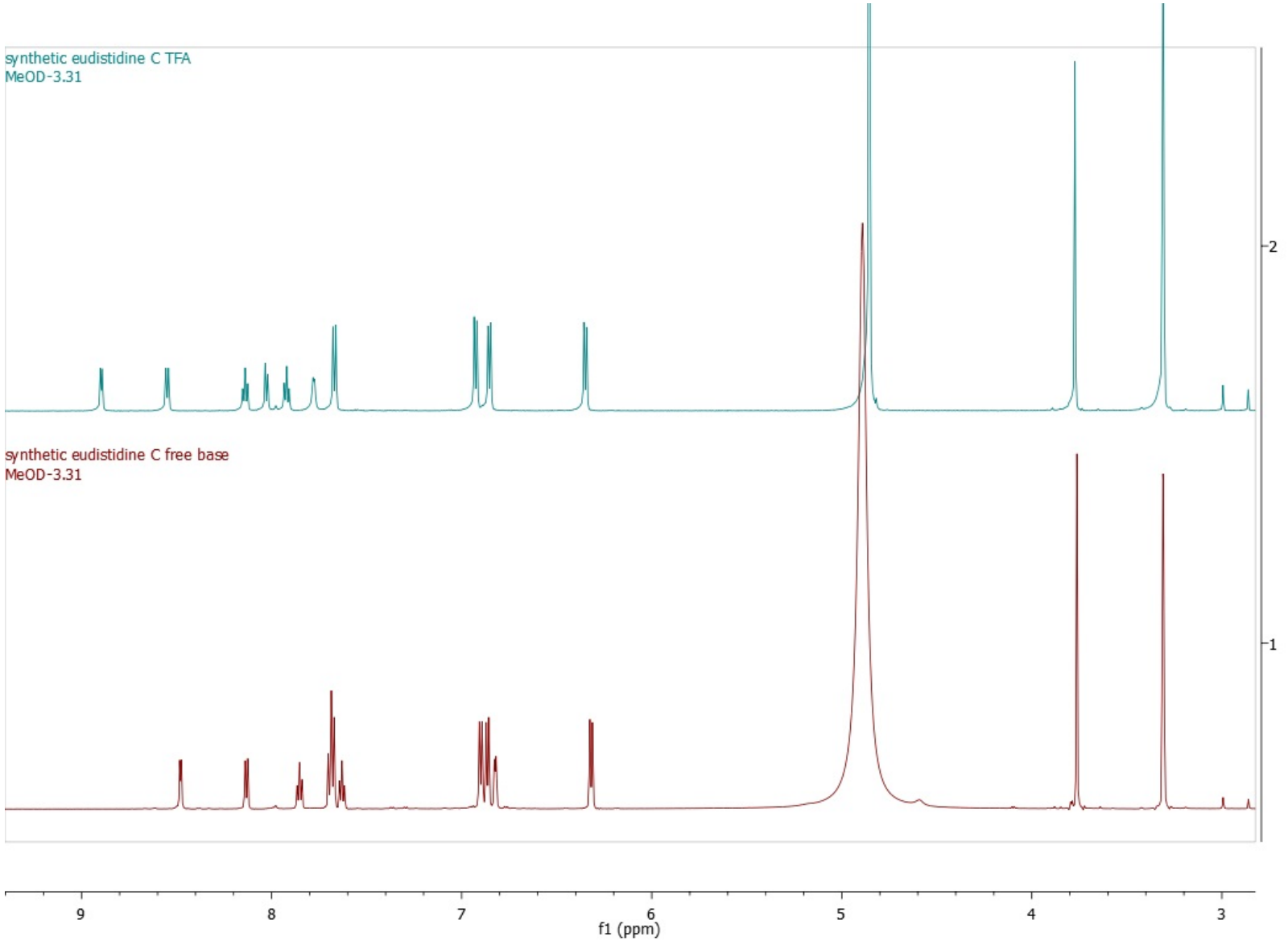
Synthetic Eudistidine C (1) TFA Salt ^{1}H NMR Spectrum (600 MHz, CD_3OD)



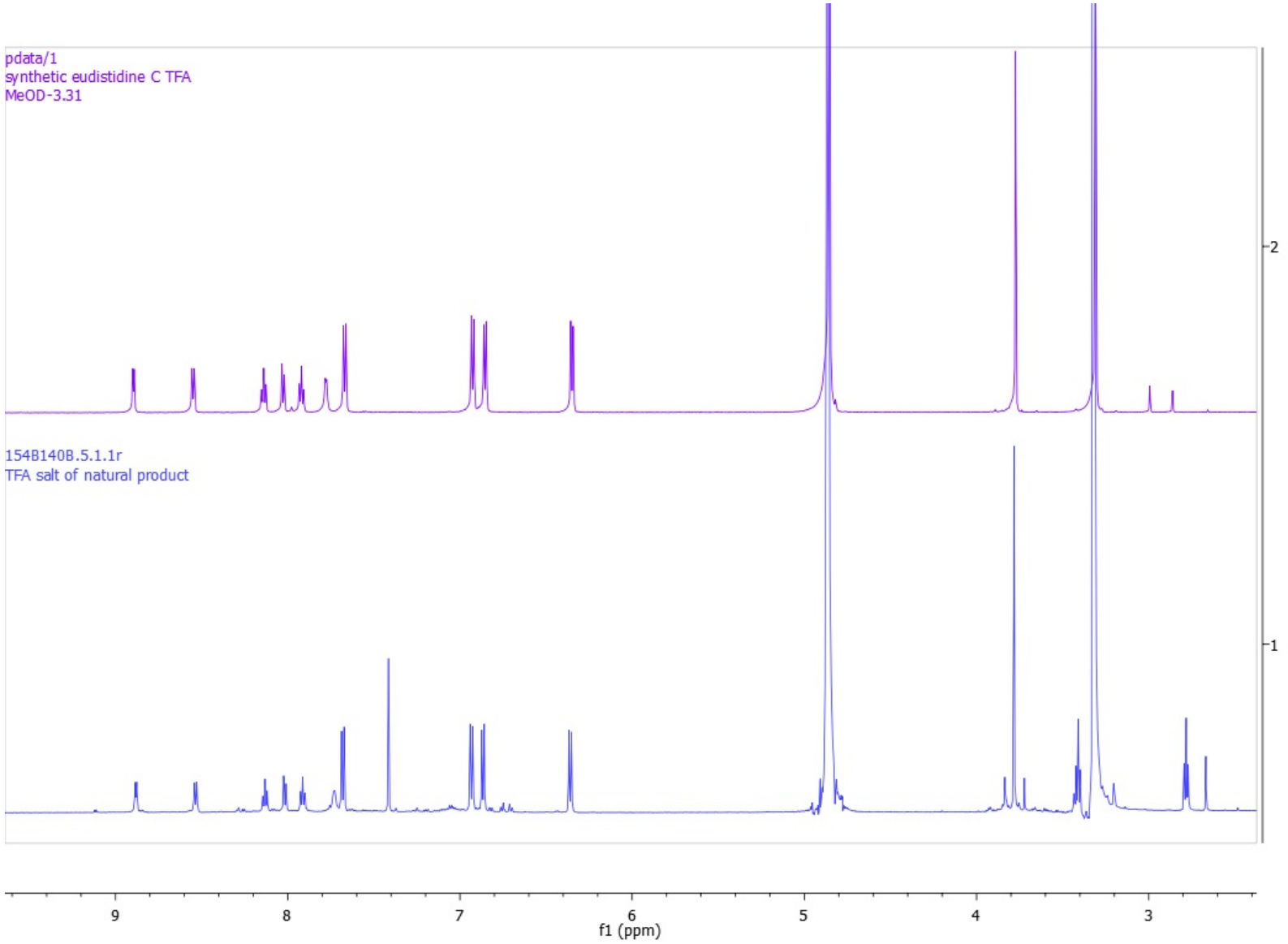
Synthetic Eudistidine C (1) TFA Salt ^{13}C NMR Spectrum (150 MHz, CD_3OD)



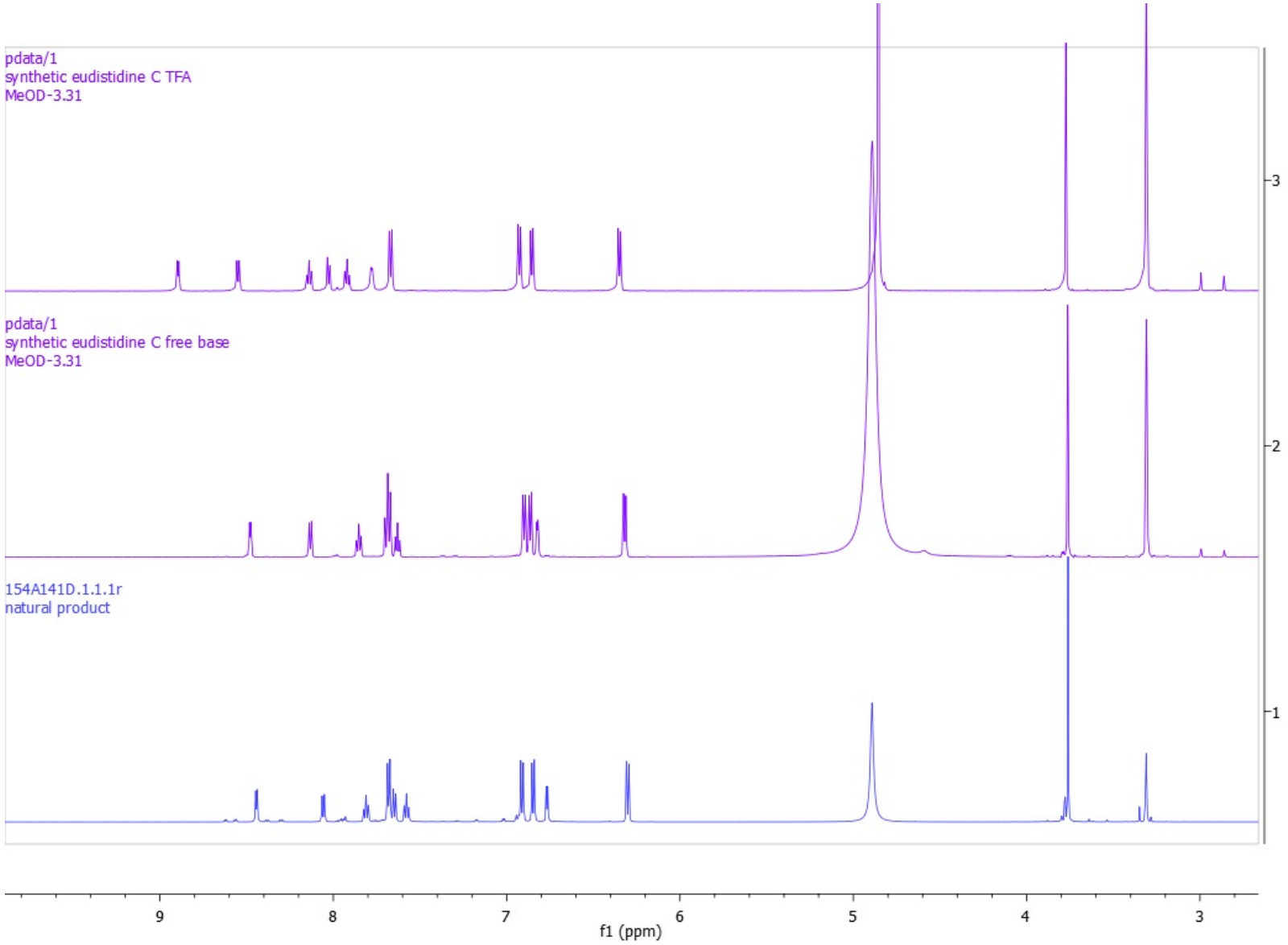
Synthetic Eudistidine C (1) Free Base and TFA Salt ^{1}H NMR Spectral Comparison (600 MHz, CD_3OD)



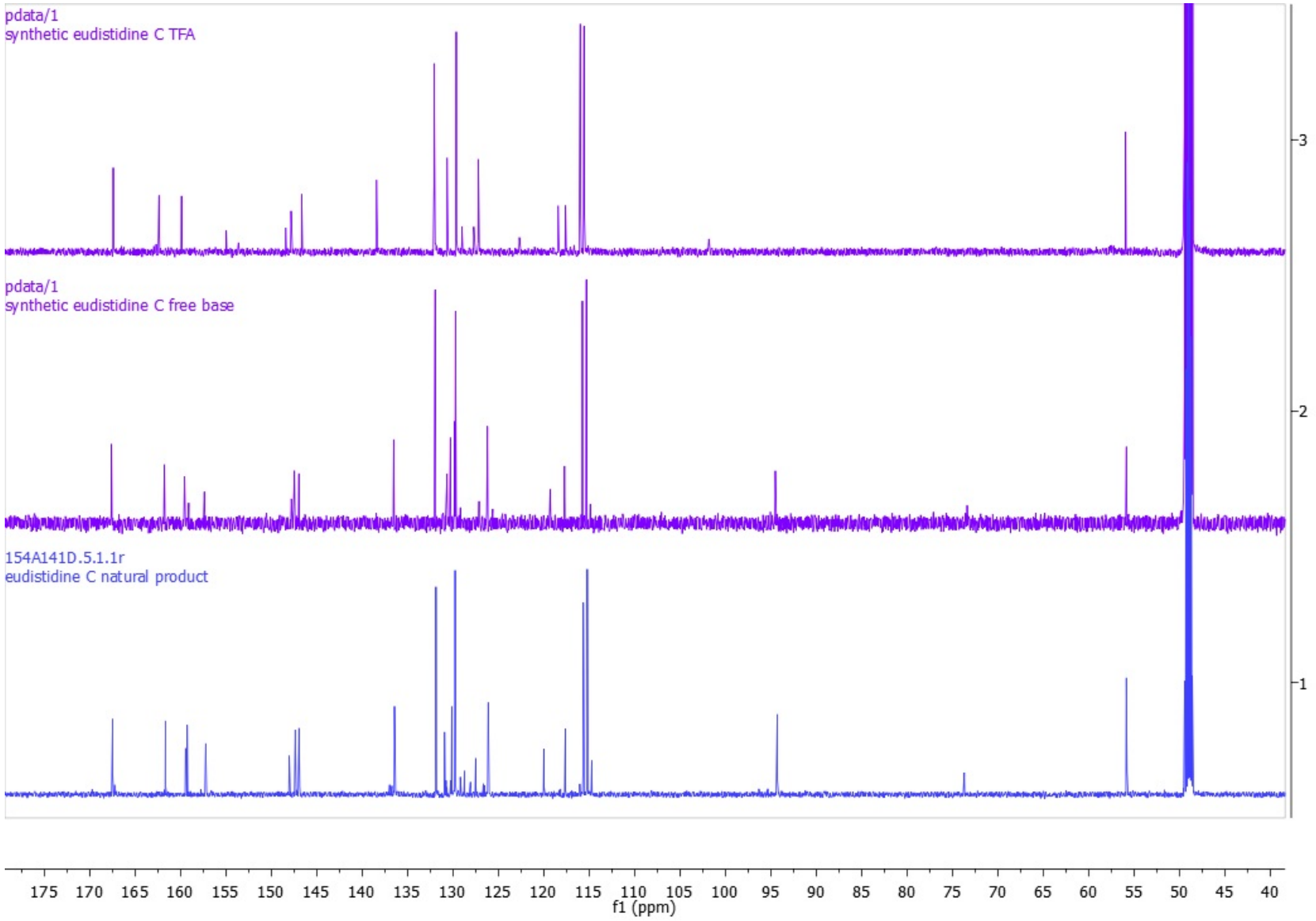
Eudistidine C (1) Natural Product and Synthetic TFA Salts ^{1}H NMR Spectral Comparison (600 MHz, CD_3OD)



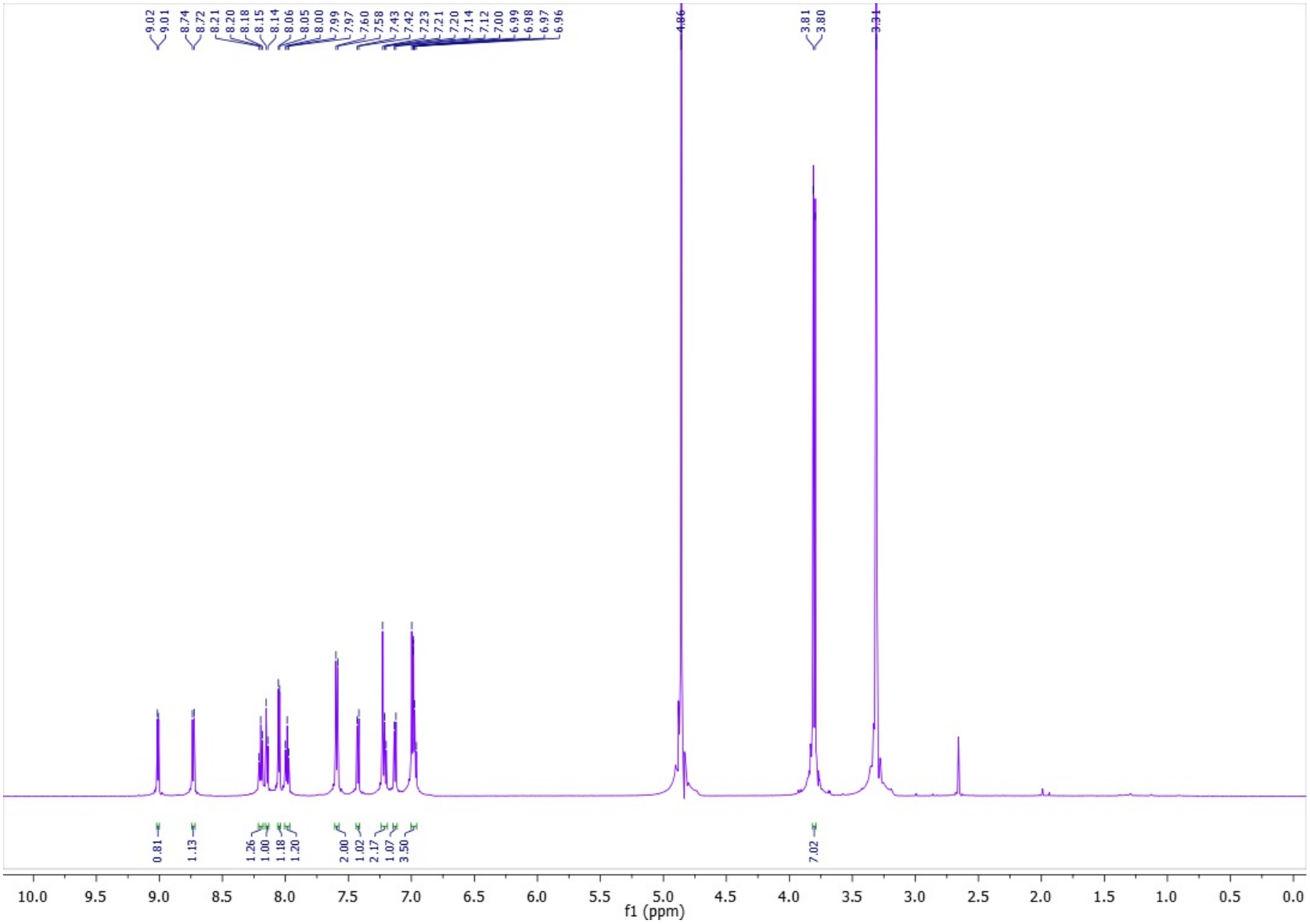
Eudistidine C (1) Natural Product and Synthetic Free Base & TFA Salt ^{1}H NMR Spectral Comparison (600 MHz, CD_3OD)



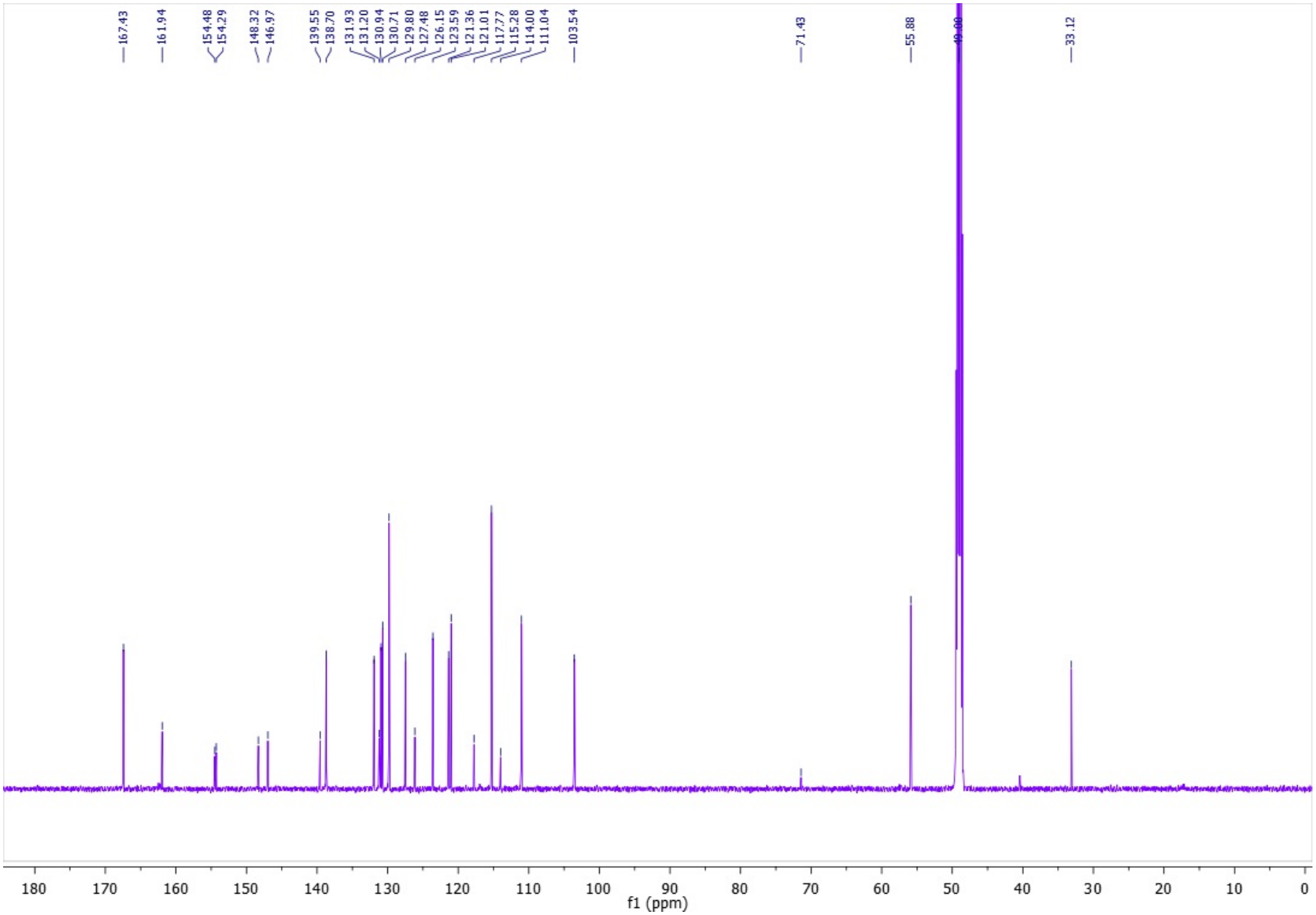
Eudistidine C (1) Natural Product and Synthetic Free Base and TFA Salt ^{13}C NMR Spectral Comparison (150 MHz, CD_3OD)



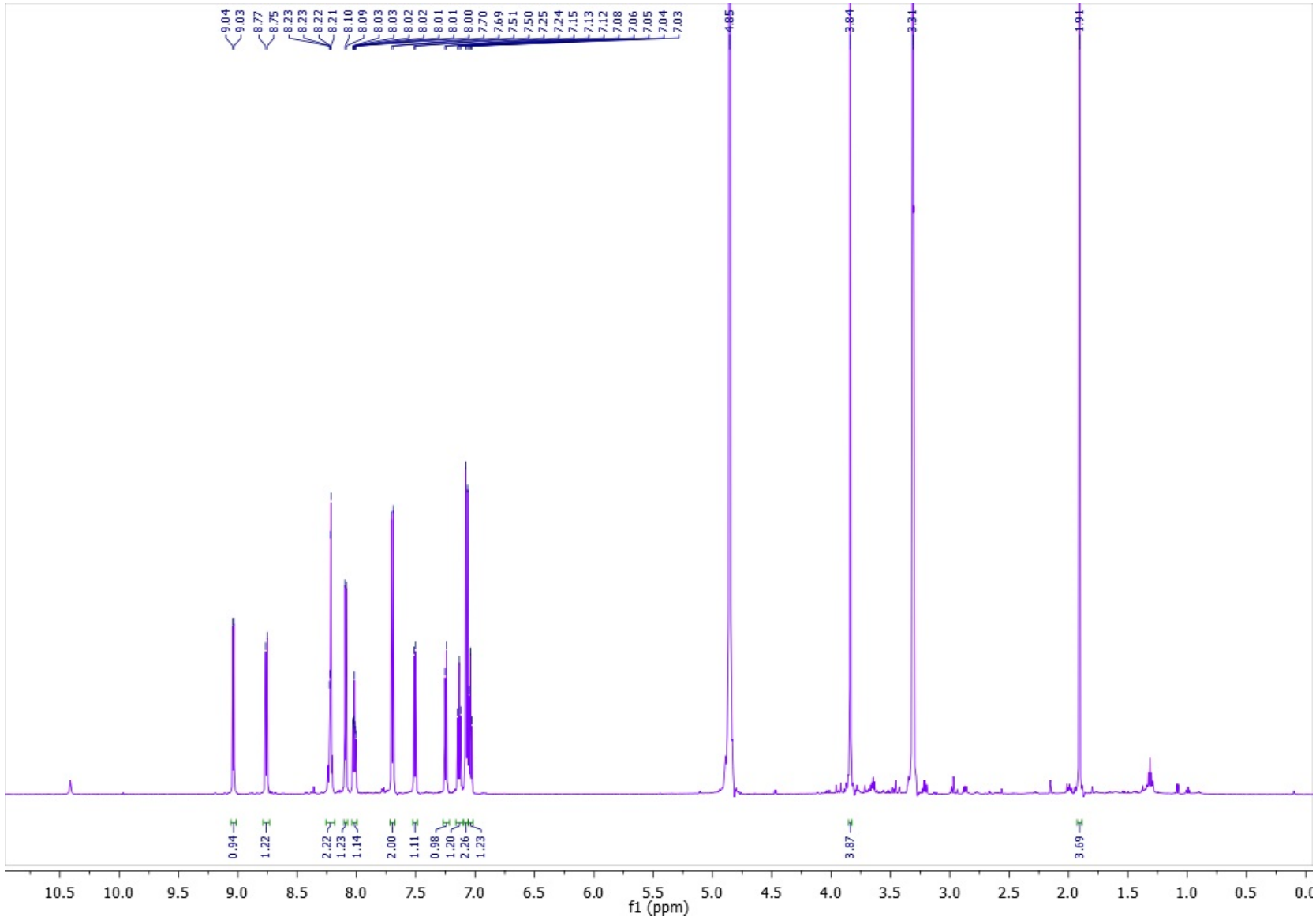
Synthetic Eudistidine C *N*-Methyl Indole Analogue (5) ^{1}H NMR Spectrum (600 MHz, CD_3OD)



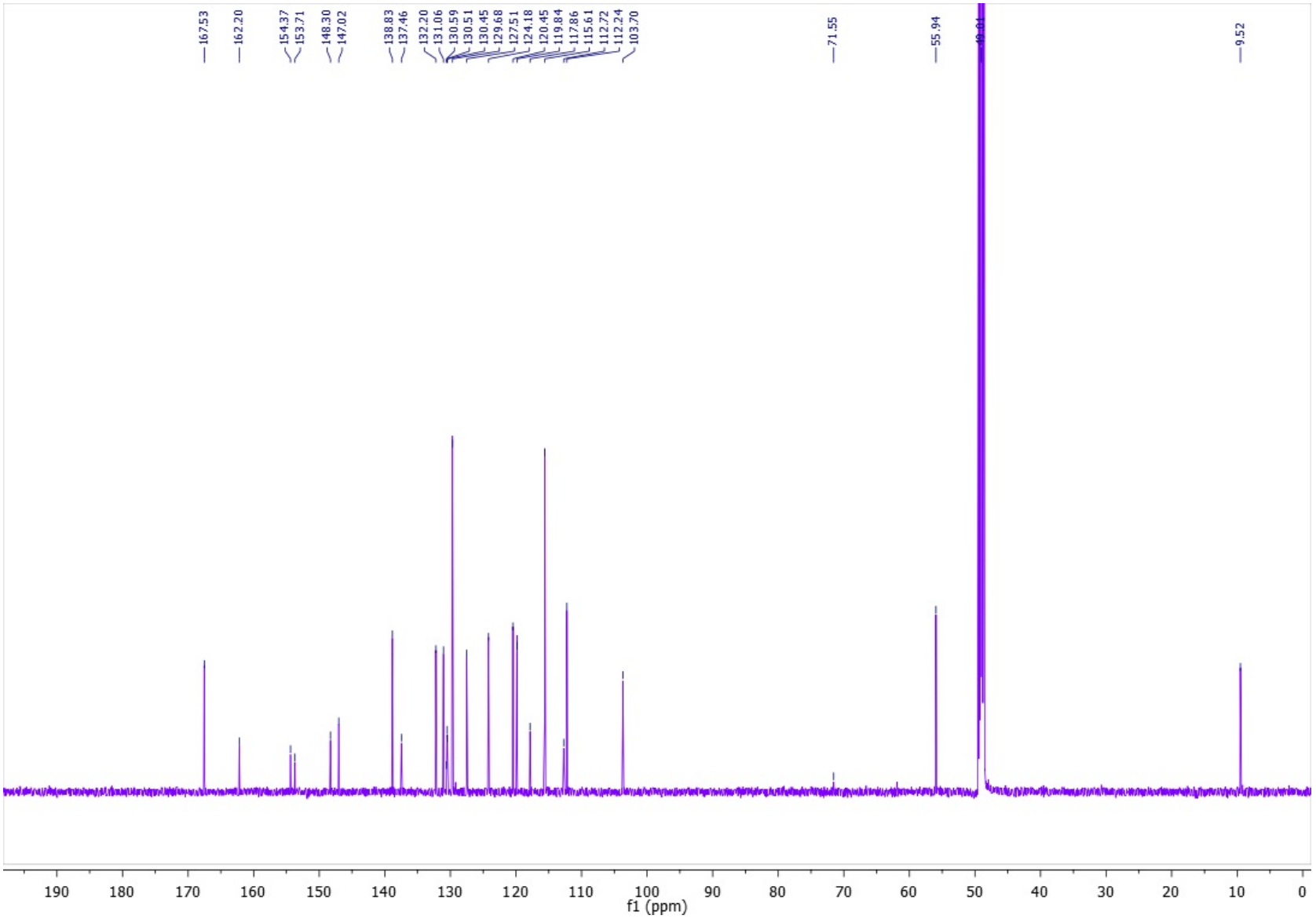
Synthetic Eudistidine C *N*-Methyl Indole Analogue (5) ^{13}C NMR Spectrum (150 MHz, CD_3OD)



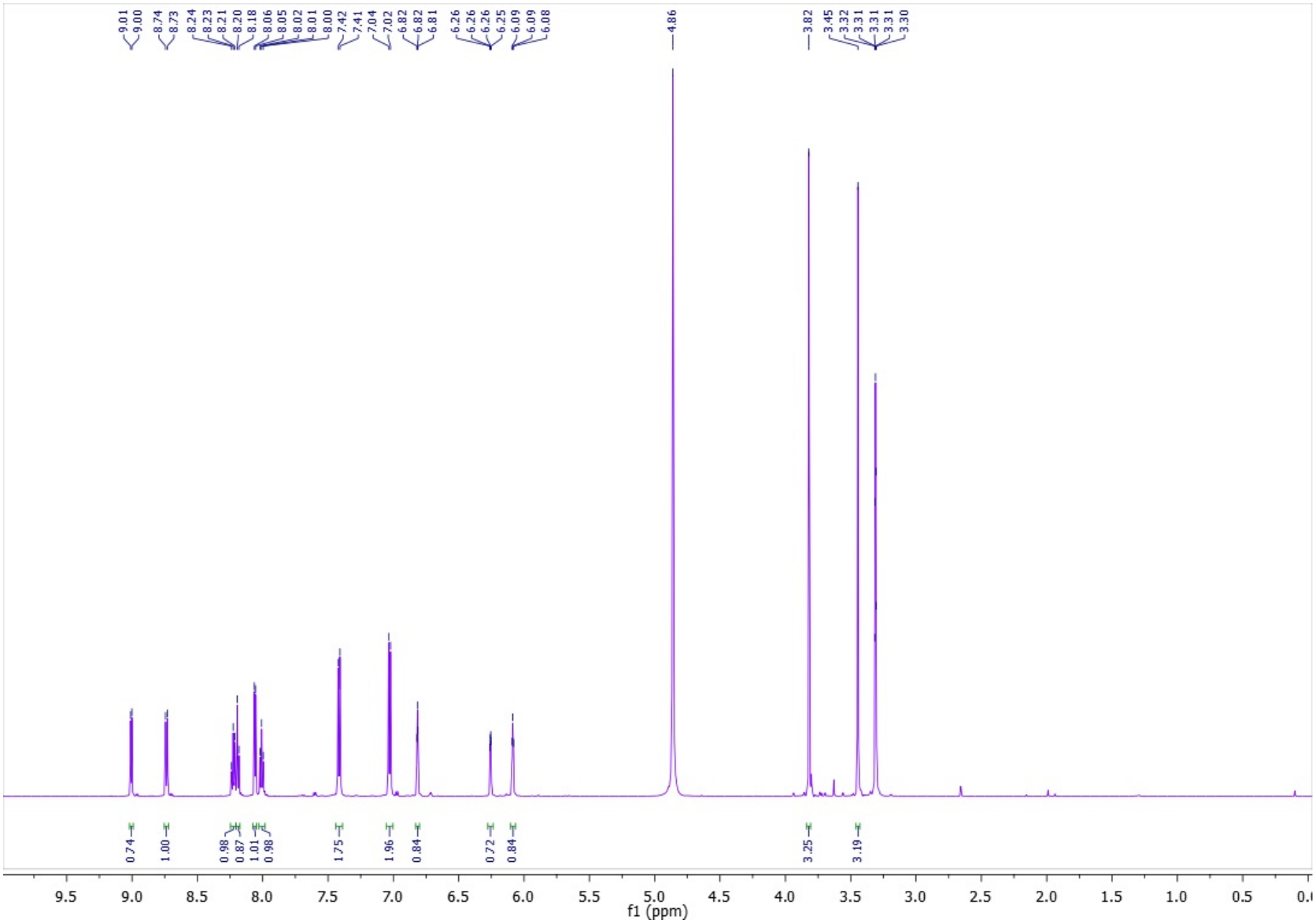
Synthetic Eudistidine C Skatole Analogue (6) ^{1}H NMR Spectrum (600 MHz, CD_3OD)



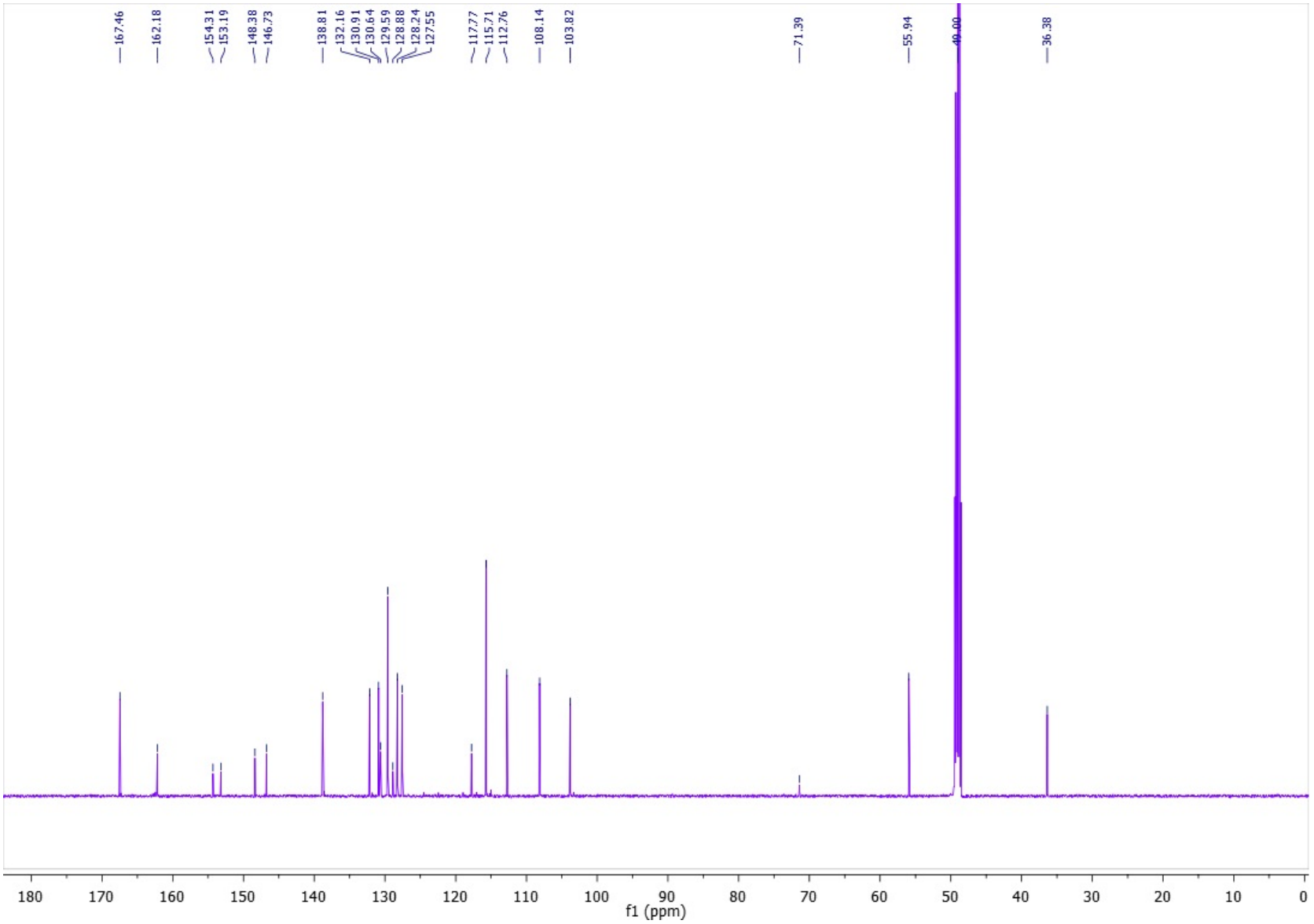
Synthetic Eudistidine C Skatole Analogue (6) ^{13}C NMR Spectrum (150 MHz, CD_3OD)



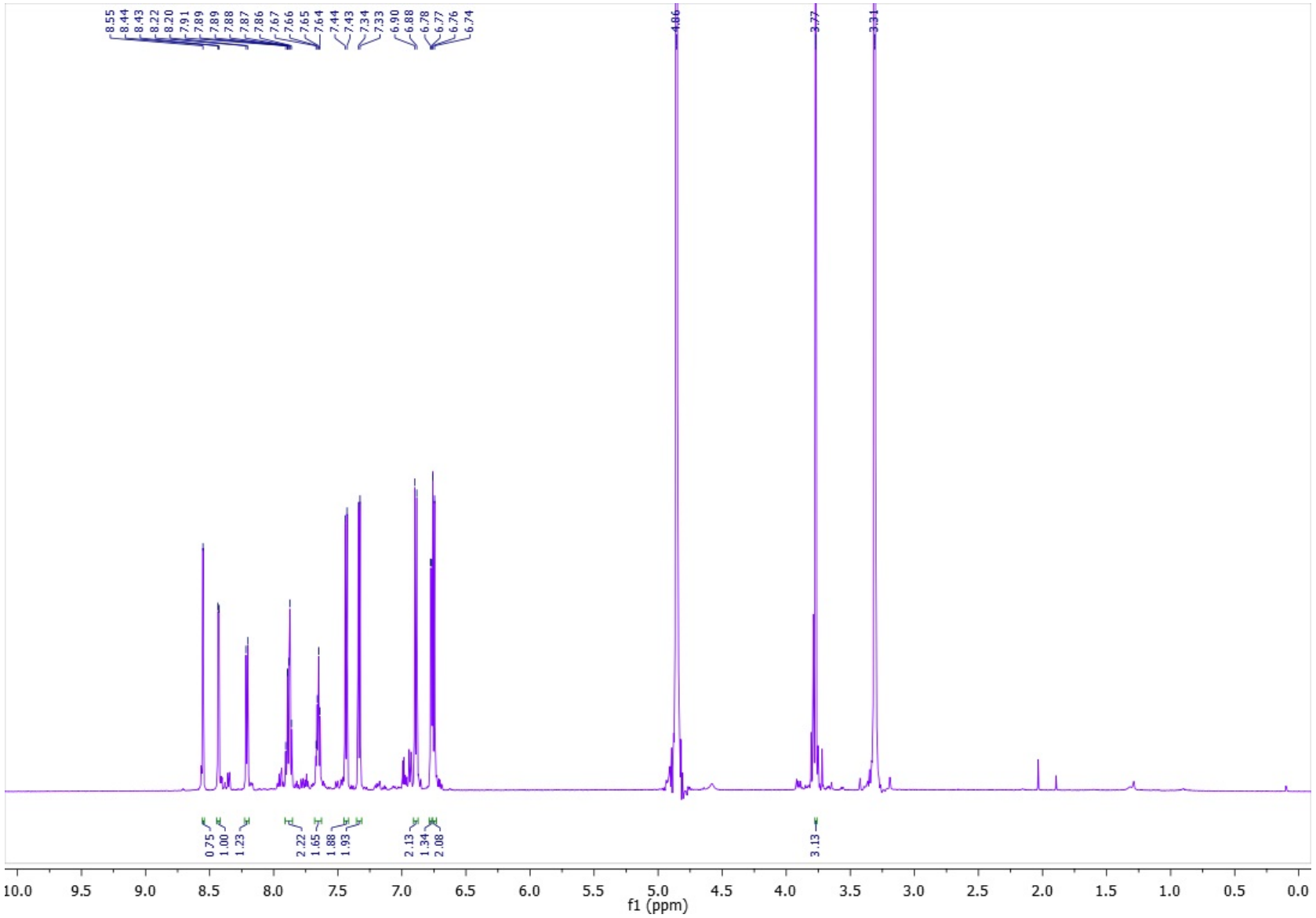
Synthetic Eudistidine C *N*-Methyl Pyrrole Analogue (7) ^{1}H NMR Spectrum (600 MHz, CD_3OD)



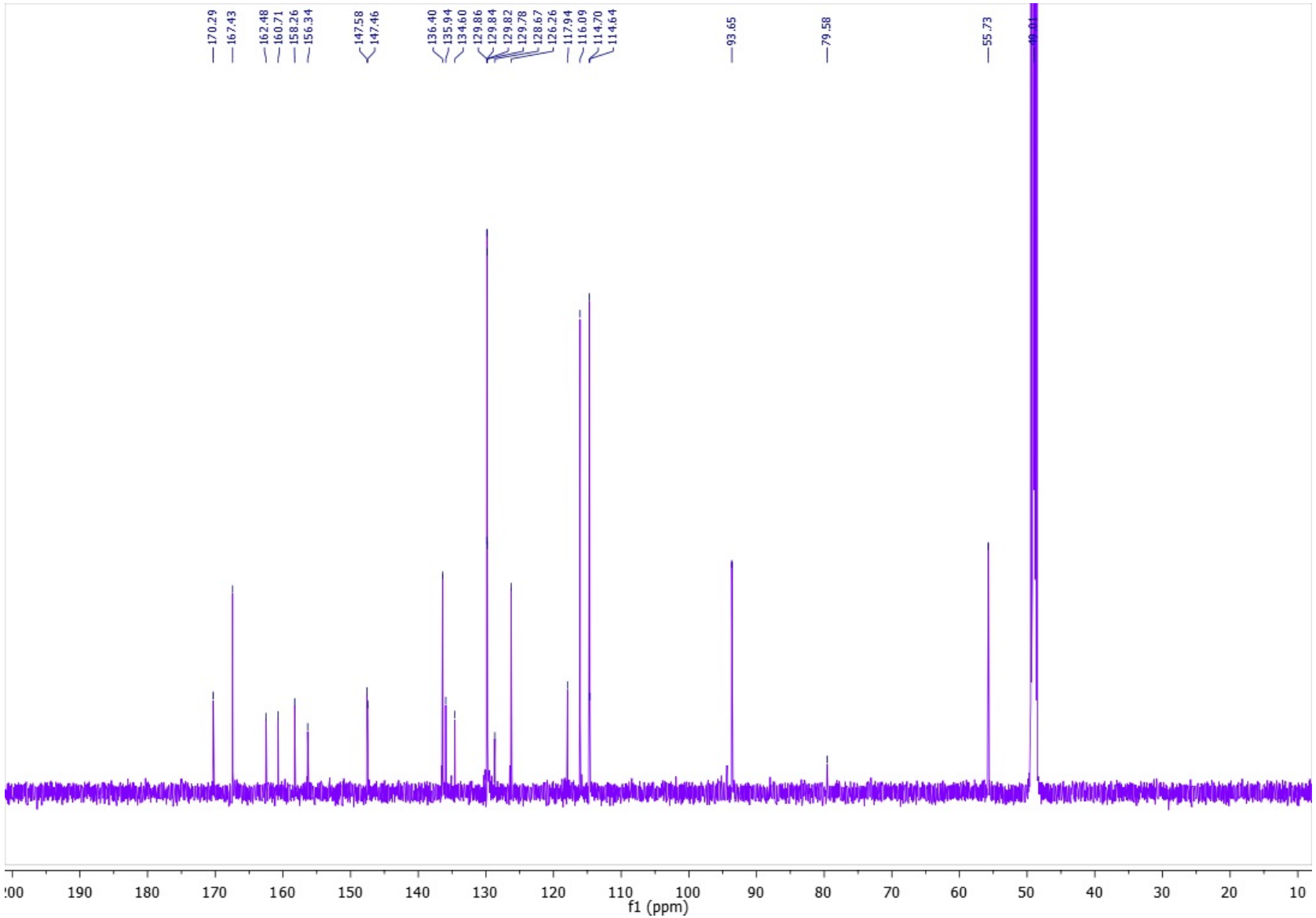
Synthetic Eudistidine C *N*-Methyl Pyrrole Analogue (7) ^{13}C NMR Spectrum (150 MHz, CD_3OD)



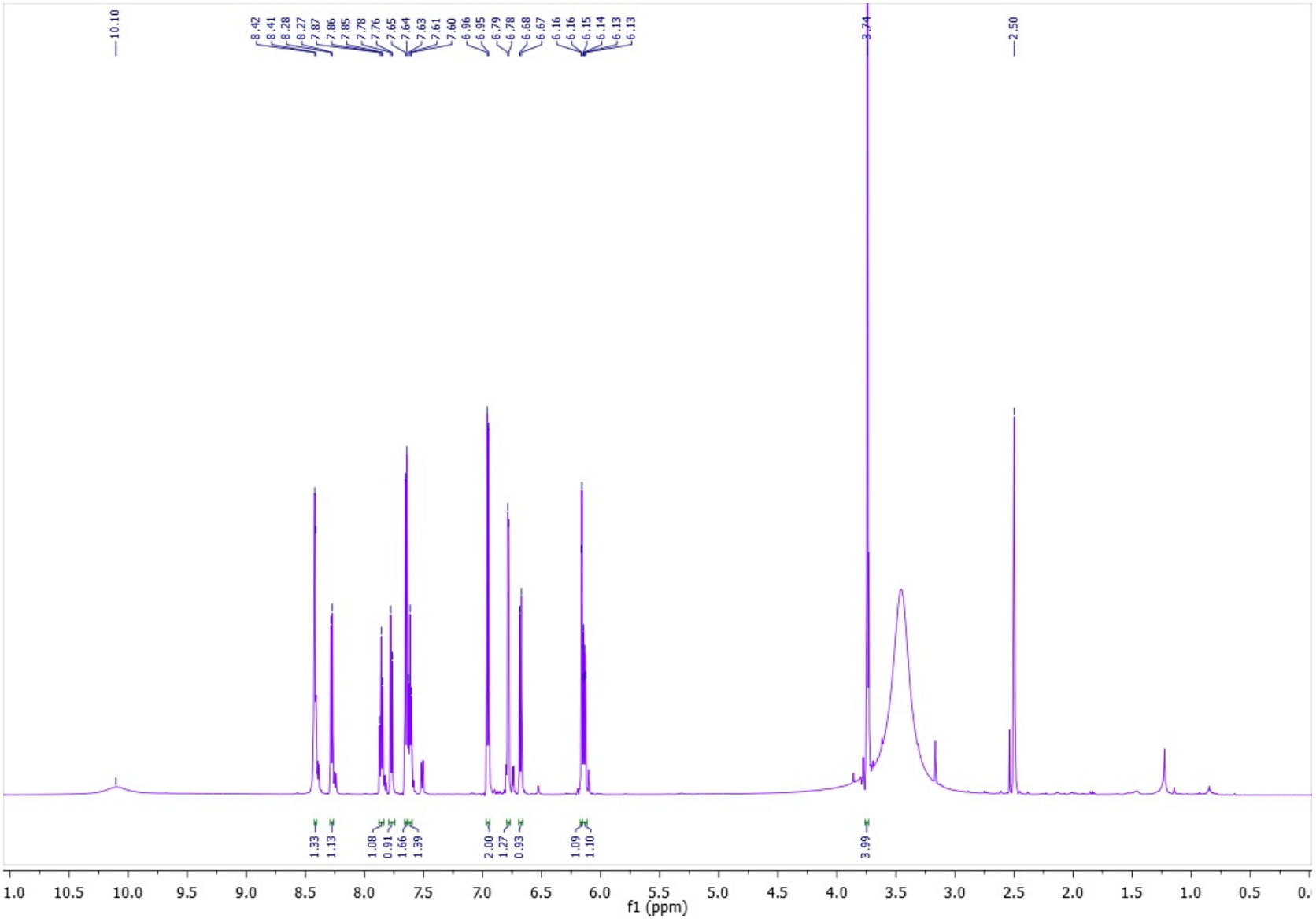
Synthetic Eudistidine C *p*-Phenol Analogue (8) ^{1}H NMR Spectrum (600 MHz, CD_3OD)



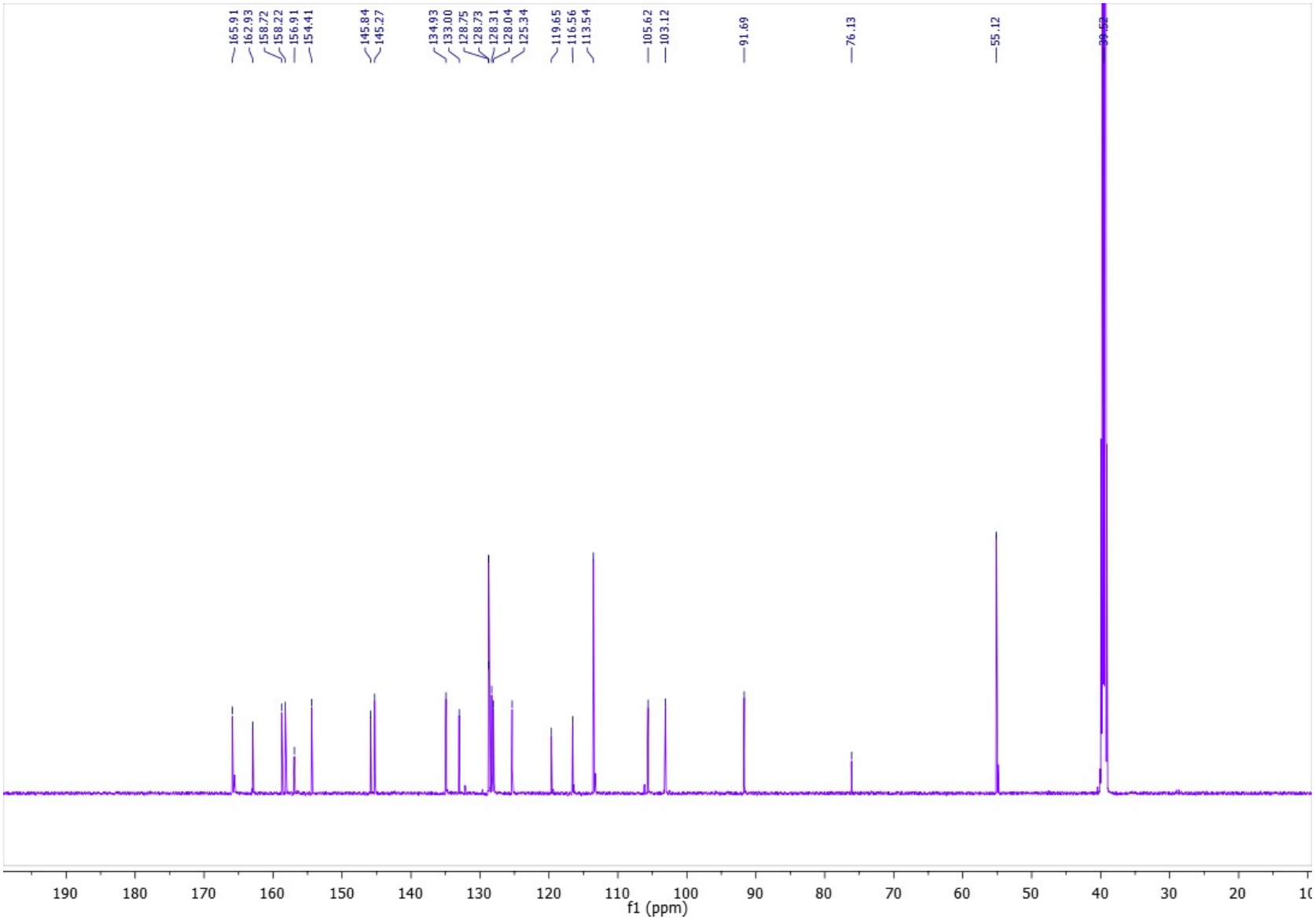
Synthetic Eudistidine C *p*-Phenol Analogue (8) ^{13}C NMR Spectrum (150 MHz, CD_3OD)



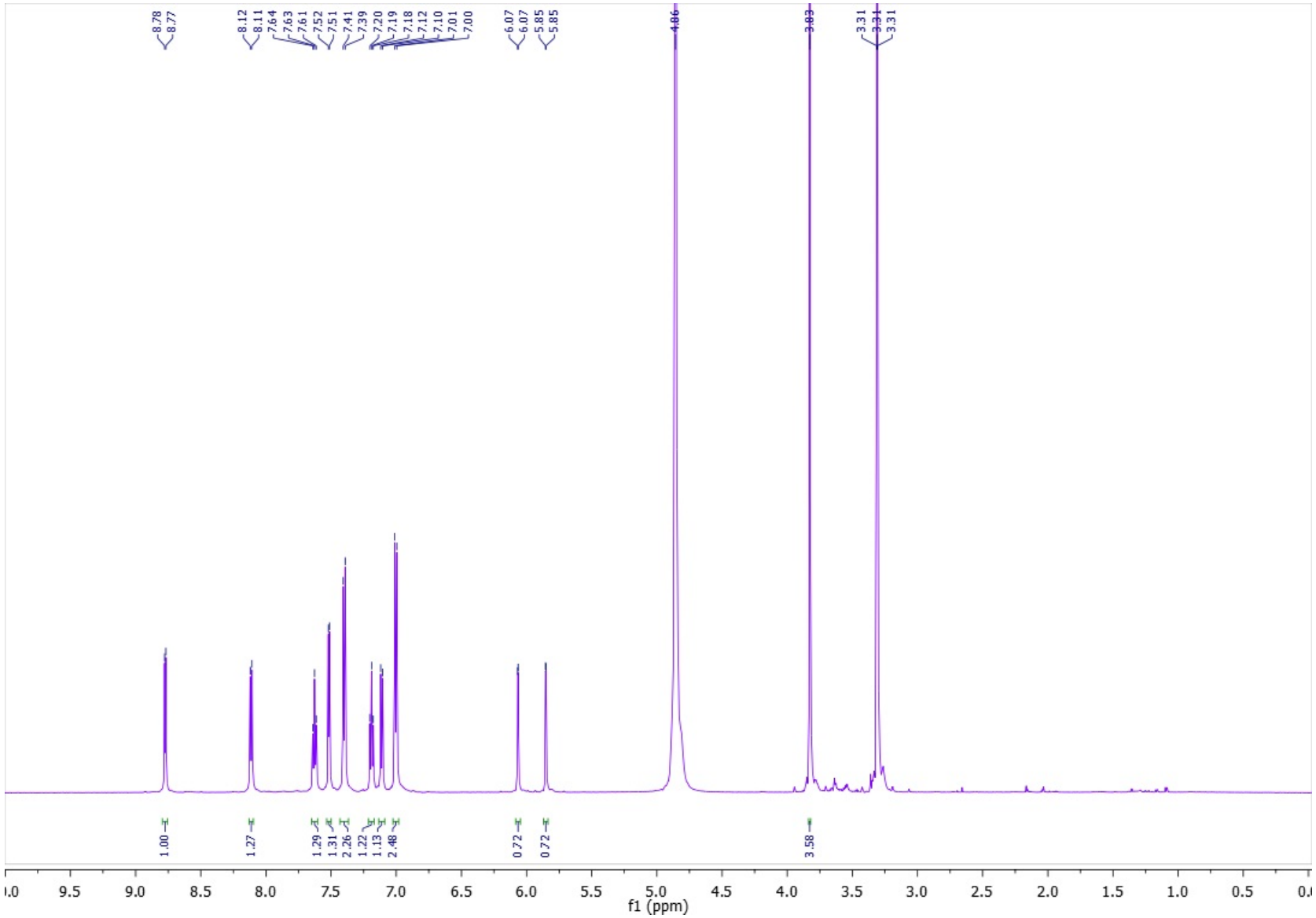
Synthetic Eudistidine C Resorcinol Analogue (9) ^{1}H NMR Spectrum (600 MHz, DMSO)



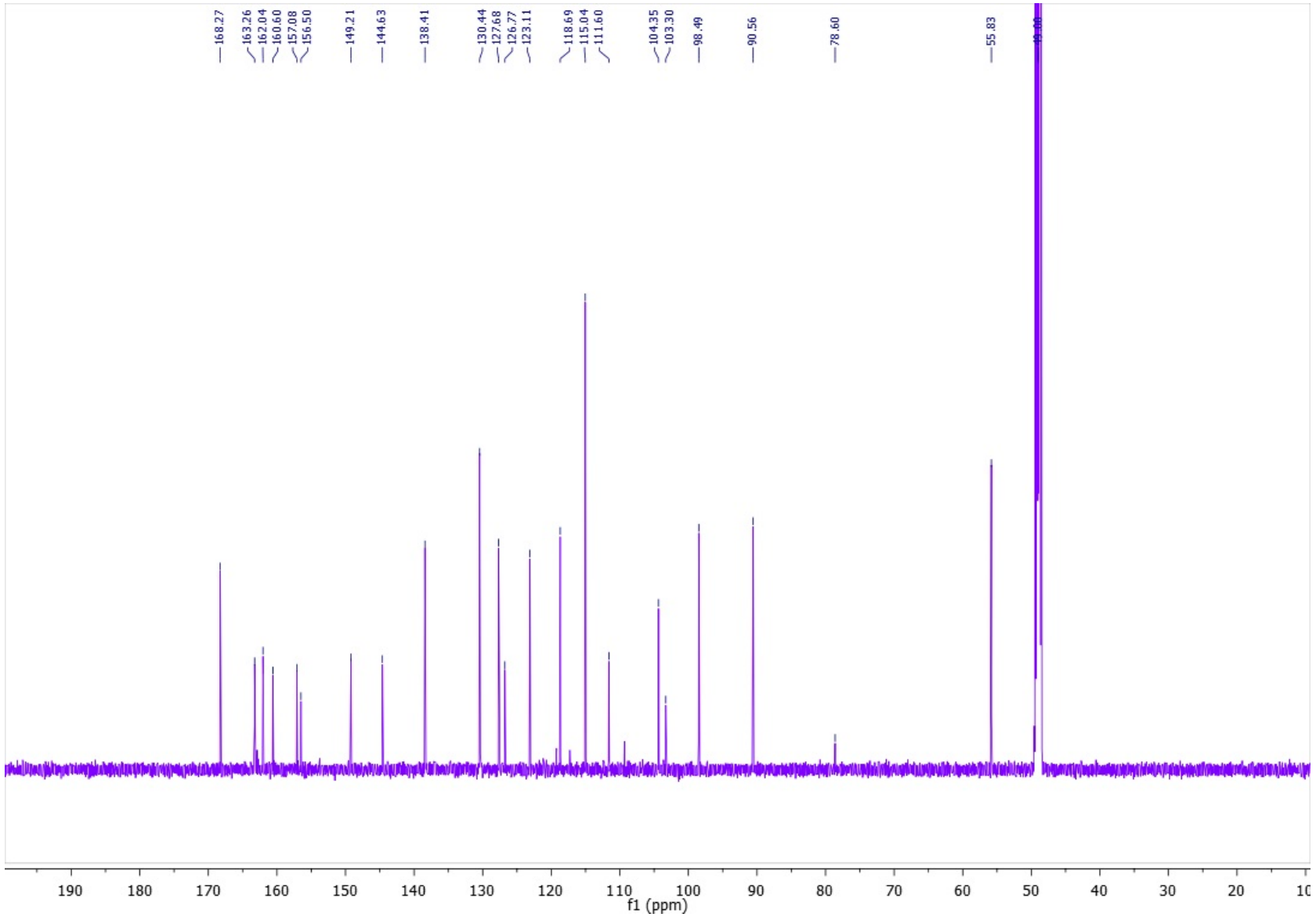
Synthetic Eudistidine C Resorcinol Analogue (9) ^{13}C NMR Spectrum (150 MHz, DMSO)



Synthetic Eudistidine C Phloroglucinol Analogue (10) ^{1}H NMR Spectrum (600 MHz, CD_3OD)



Synthetic Eudistidine C Phloroglucinol Analogue (10) ^{13}C NMR Spectrum (150 MHz, CD_3OD)



Imidazole 4 1H NMR spectrum (600 MHz, DMSO-d_6)