Supplementary Materials

Pursuing the Complexity of Alzheimer's Disease: Discovery of Fluoren-9-Amines as Selective Butyrylcholinesterase Inhibitors and *N*-Methyl-D-Aspartate Receptor Antagonists

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Table of contents

1. Physiochemical properties and drug-likeness of final compounds

For the prediction of physiochemical properties (Table S1) was used MarvinSketch 20.4.0, ChemAxon Ltd. Drug-likeness of designed derivatives was evaluated by SwissADME online tool.[1]

Table S1. Physiochemical parameters of compounds **3a-o**, THA and Memantine.

Compound	MW	pKa	logP	HBA	HBD	TPSA
3a	259.78	9.06	3.76	1	1	12.03
3b	231.72	8.89	2.99	1	1	12.03
3c	299.84	9.34	4.79	1	1	12.03
3d	257.76	8.65	3.46	1	1	12.03
3e	271.79	8.96	3.90	1	1	12.03
3f	285.82	9.66	4.22	1	0	3.24
3g	275.78	8.33	2.94	2	1	21.26
3h	245.70	8.97	3.35	1	1	12.03
3i	273.80	9.60	4.09	1	0	3.24
3j	337.29	8.32	3.22	2	0	6.48
3k	314.85	8.39	3.58	2	0	6.48
3l	287.79	7.45	3.15	2	0	12.47
3m	273.80	9.15	4.31	1	1	12.03
3n	273.80	9.22	4.29	1	1	12.03
3o	271.78	9.80	3.78	1	0	3.24
THA	198.26	8.95	2.63	2	1	38.91
Memantine	179.30	10.70	2.07	1	1	26.02

MW = molecular weight, pKa = acid dissociation constant, logP = partition coefficient, HBA = hydrogen bond acceptor, HBB = hydrogen bond donor, TPSA = topological polar surface area

Table S2. Drug-likeness of derivatives **3a-o**, THA and memantine.

	Drug-likeness				
Compound	**Lipinski** [a]	**Ghose** [b]	**Veber** [c]	**Egan** [d]	**Muegge** [e]
3a	Yes	Yes	Yes	Yes	No [f]
3b	Yes	Yes	Yes	Yes	No [g]
3c	Yes	Yes	Yes	Yes	No [f]
3d	Yes	Yes	Yes	Yes	No [f]
3e	Yes	Yes	Yes	Yes	No [f]
3f	Yes	Yes	Yes	Yes	No [f]
3g	Yes	Yes	Yes	Yes	Yes
3h	Yes	Yes	Yes	Yes	No [f]
3i	Yes	Yes	Yes	Yes	No [f]
3j	Yes	Yes	Yes	Yes	Yes
3k	Yes	Yes	Yes	Yes	Yes
3l	Yes	Yes	Yes	Yes	Yes
3m	Yes	Yes	Yes	Yes	No [f]
3n	Yes	Yes	Yes	Yes	No [f]
3o	Yes	Yes	Yes	Yes	No [f]
THA	Yes	Yes	Yes	Yes	No [f]
Memantine	Yes	Yes	Yes	Yes	No [g]

[a] Lipinski represent,[2] MW < 500 Da, logP <5, HBD <5, HBA <10; [b] Ghose,[3] MW = 180 – 480, MR (molar refractivity) = 40 – 130, logP = -0.4 – +5.6, number of atoms = 20 – 70; [c] Veber,[4] rotatable bonds <10, TPSA <140; [d] Egan,[5] TPSA <132, logP <5.88; [e] Muegge,[6] MW = 200 – 500, log P = -2 - +5, HBD <5, HBA <10, MR = 40 – 130, rotatable bonds <8, heavy atoms = 20 – 70, TPSA <120, net charge -2 - +2; [f] 1 violation, [g] 2 violations.

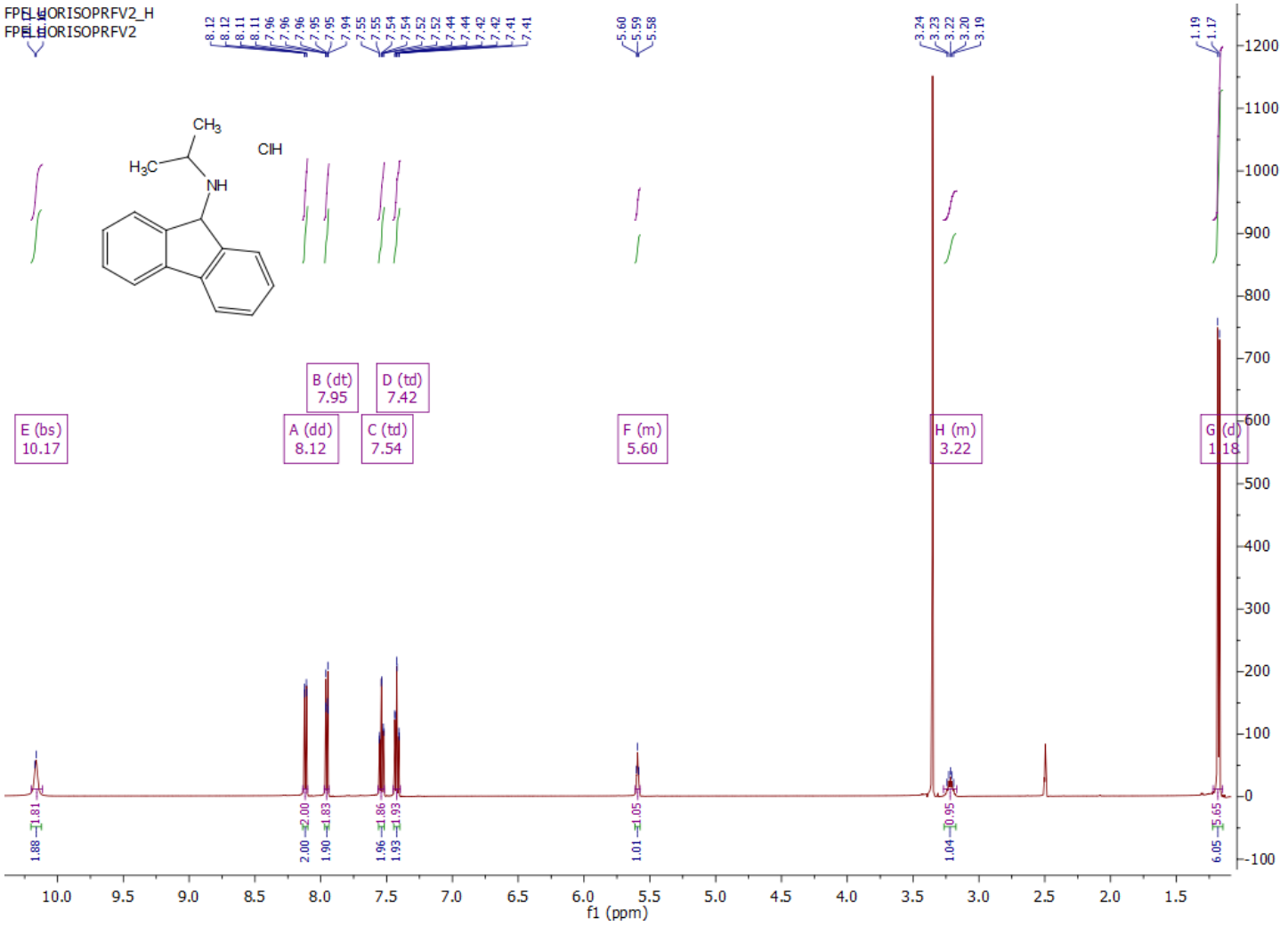
2. MTT cell viability test prior the BBB evaluation using hCMEC/D3 cells

Table S3. Effect of tested compounds on the cell viability of the hCMEC/D3 cells

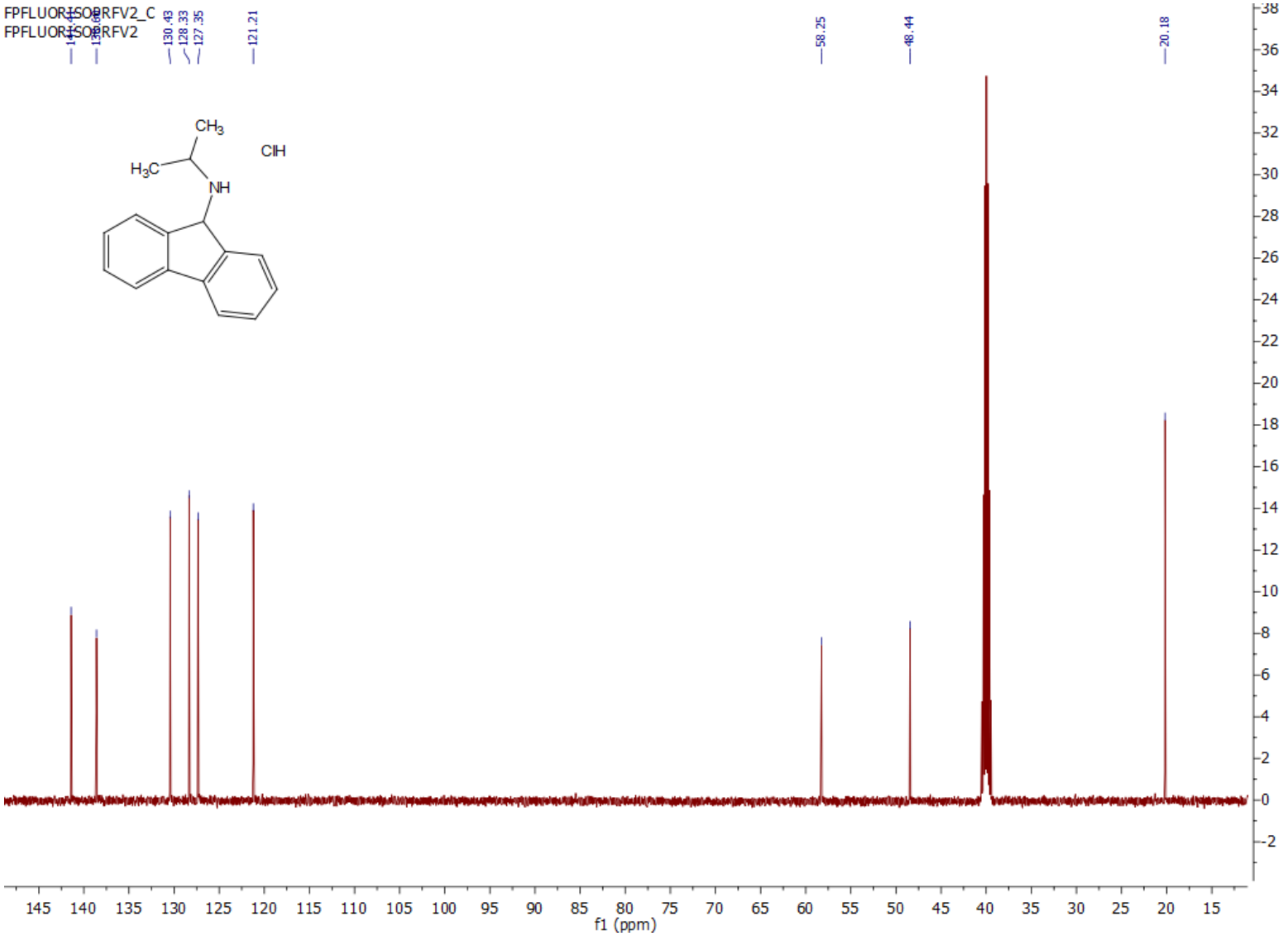
	Conc.	% viable cells	SD	n
3c	**100 μM**	82.87	11.19	3
	50 μM	93.44	1.66	4
3e	**100 μM**	92.34	9.43	3
	50 μM	92.79	4.11	3
3m	**100 μM**	80.10	6.22	3
	50 μM	82.08	4.34	4
3n	**100 μM**	92.67	11.81	3
	50 μM	98.20	20.59	3
Tacrine	**100 μM**	94.47	22,25	4
	50 μM	91.11	17.02	3

3. ^{1}H and ^{13}C NMR spectra

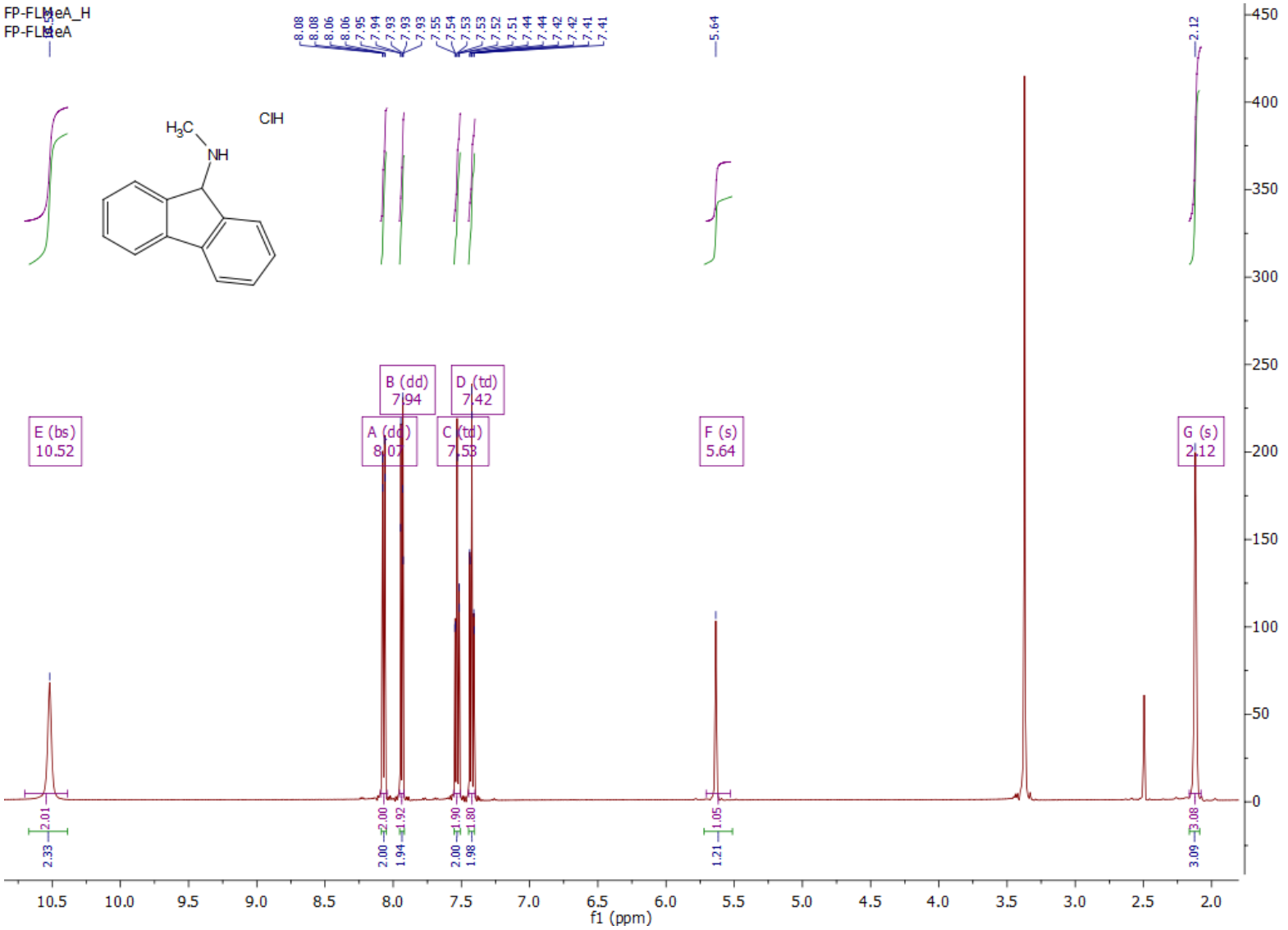
^{1}H NMR of *N*-propan-2-yl-9*H*-fluoren-9-amine hydrochloride (3a):



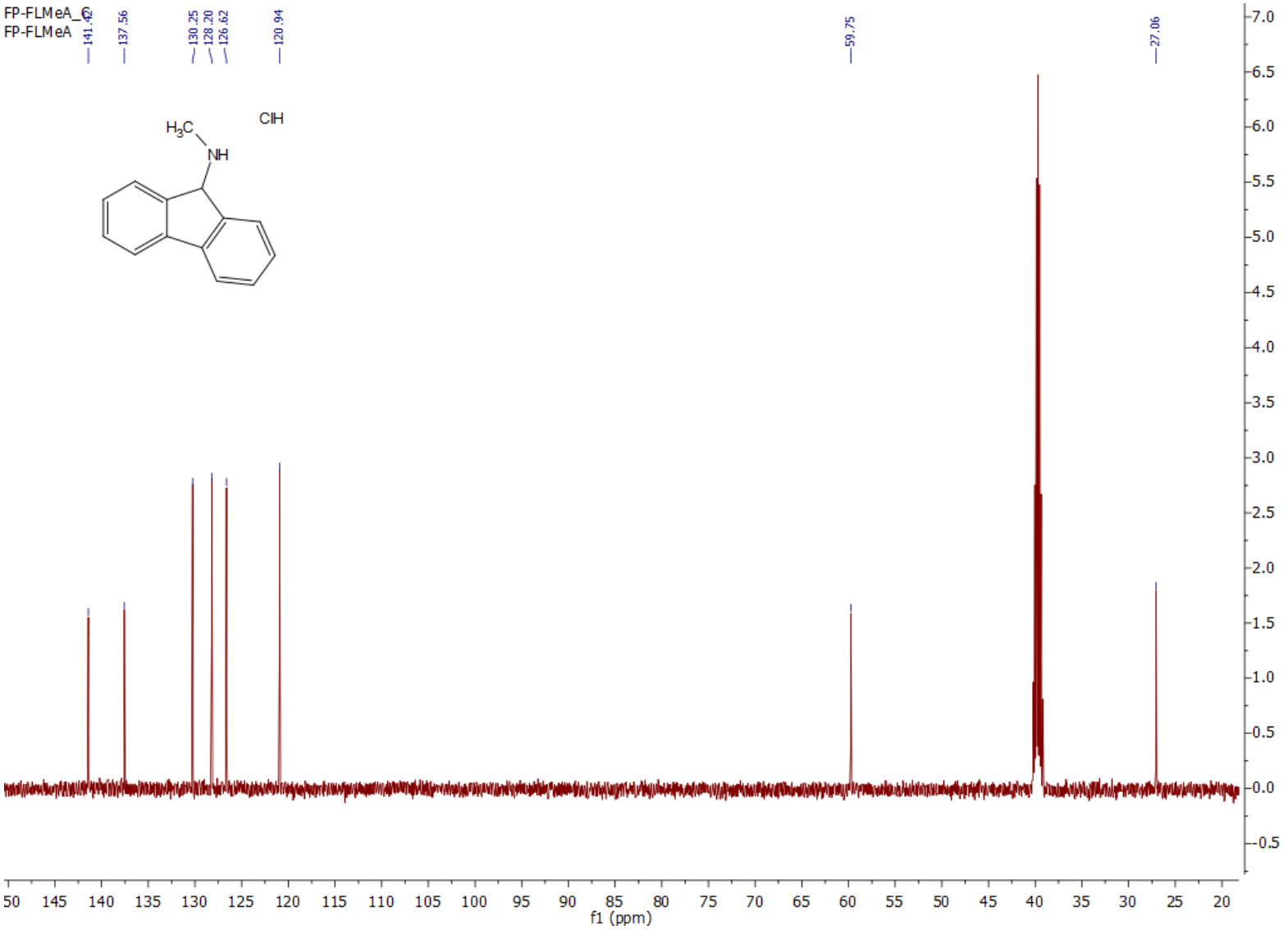
^{13}C NMR of *N*-propan-2-yl-9*H*-fluoren-9-amine hydrochloride (3a):



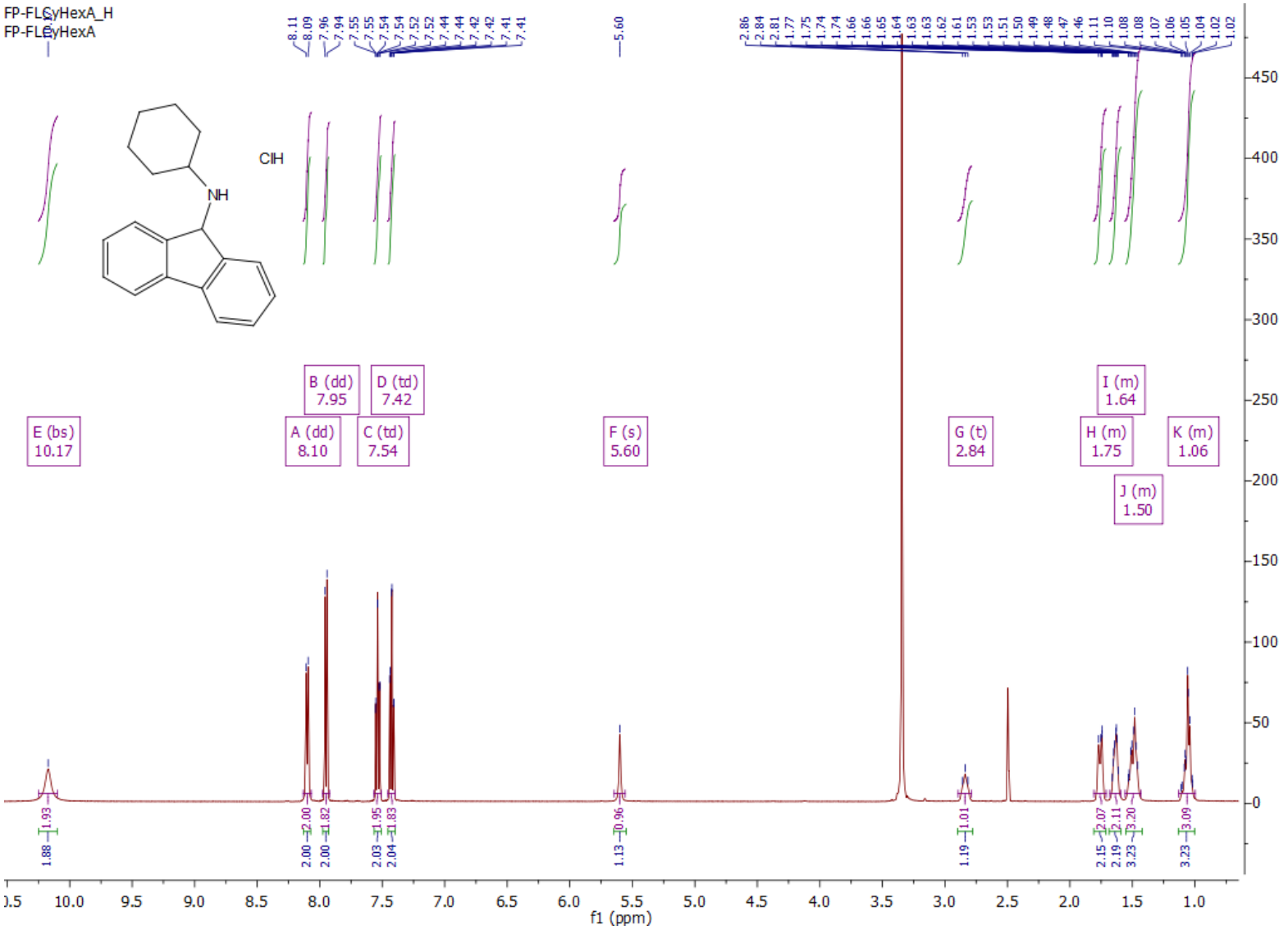
^{1}H NMR of *N*-methyl-9*H*-fluoren-9-amine hydrochloride (3b):



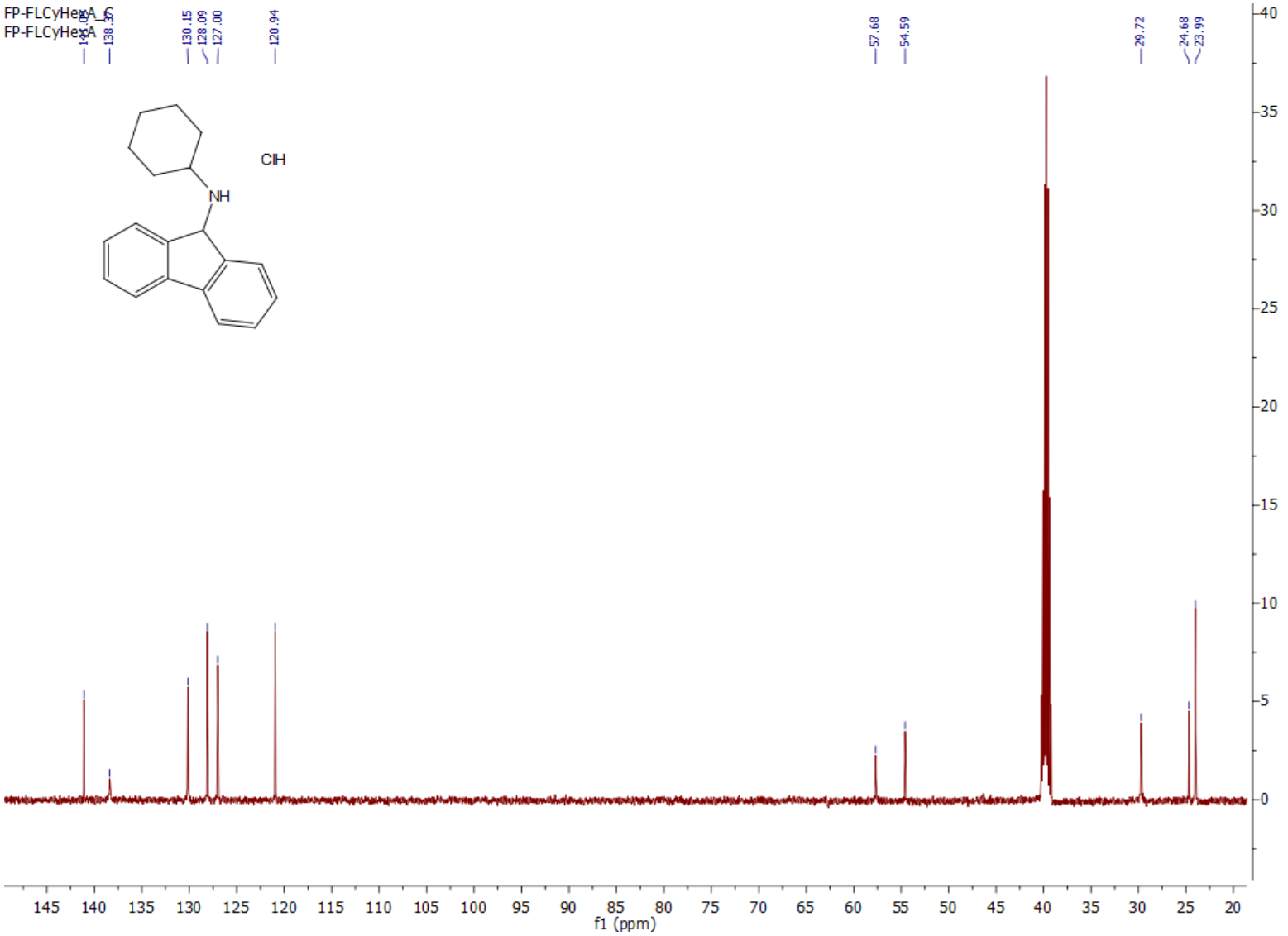
^{13}C NMR of *N*-methyl-9*H*-fluoren-9-amine hydrochloride (3b):



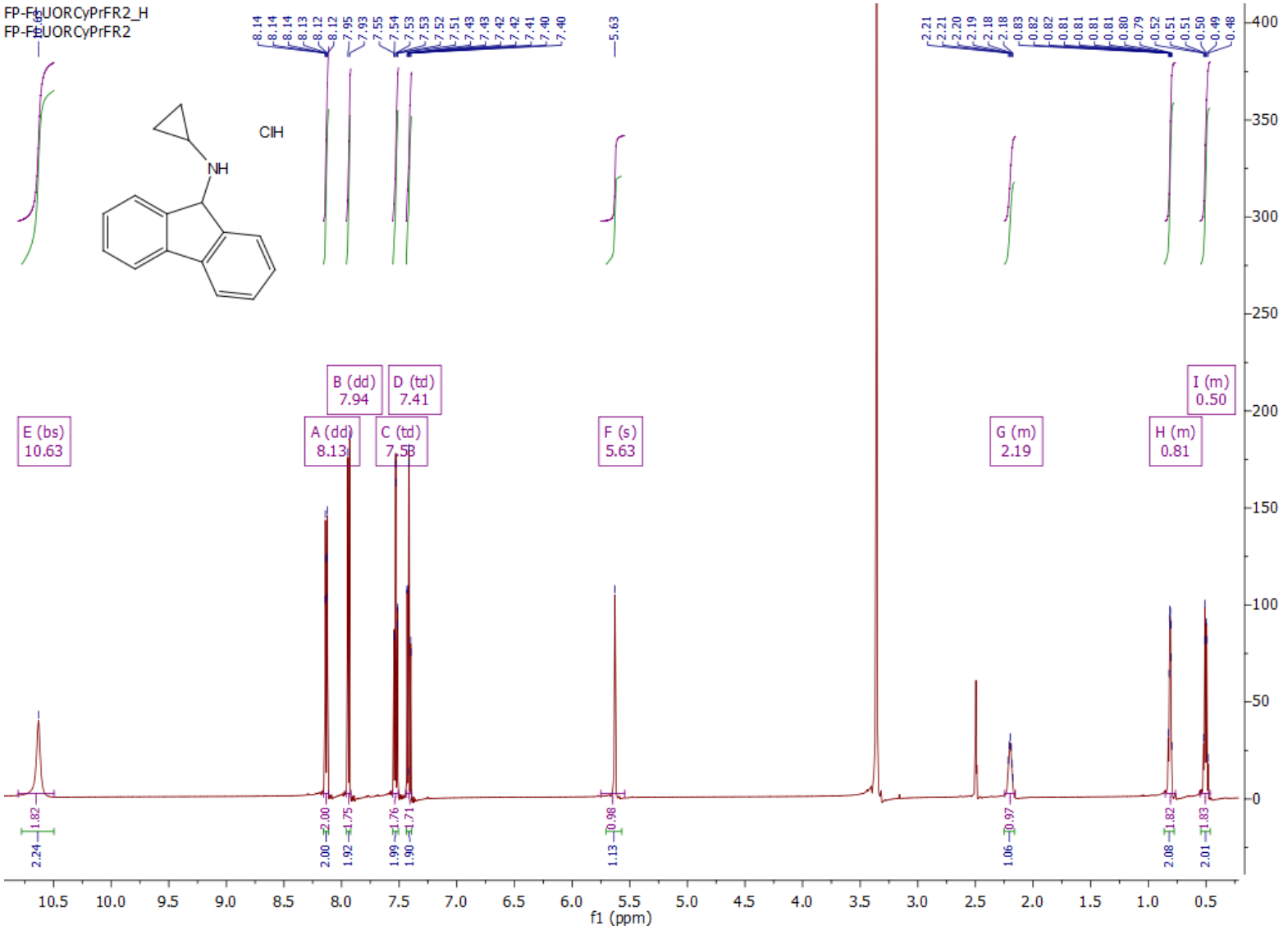
[1]H NMR of *N*-cyclohexyl-9*H*-fluoren-9-amine hydrochloride (3c):



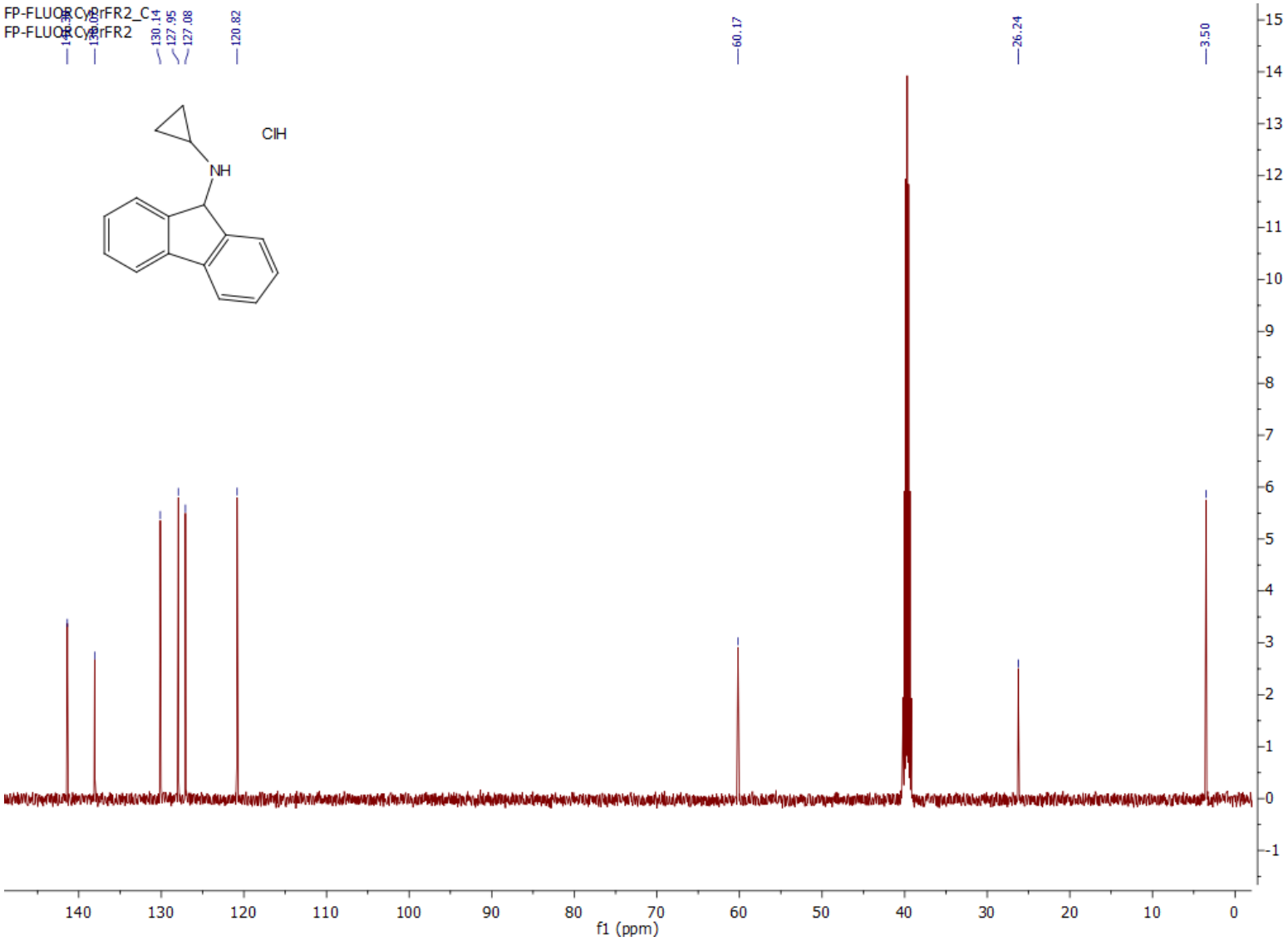
^{13}C NMR of *N*-cyclohexyl-9*H*-fluoren-9-amine hydrochloride (3c):



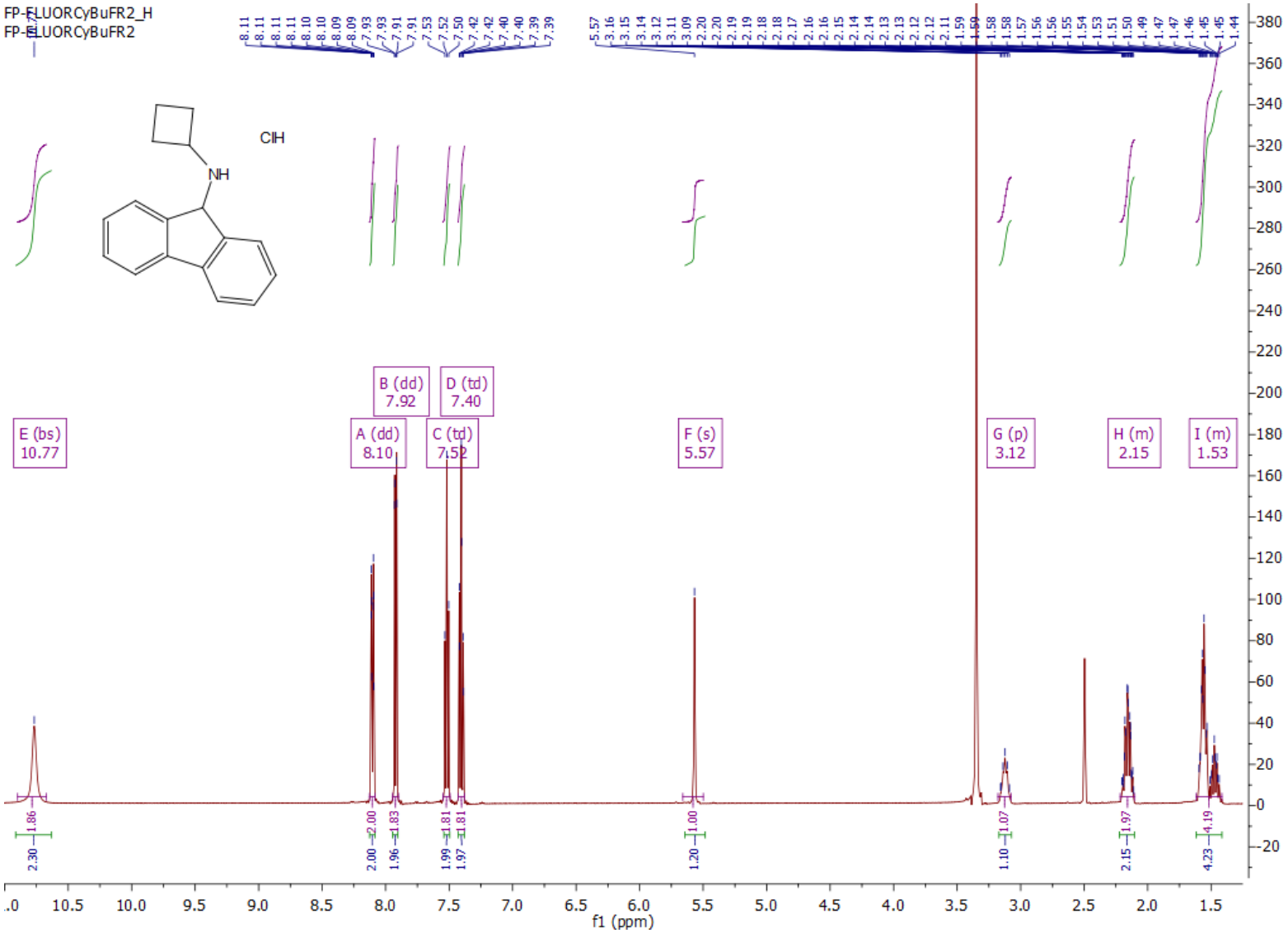
[1]H NMR of *N*-cyclopropyl-9*H*-fluoren-9-amine hydrochloride (3d):



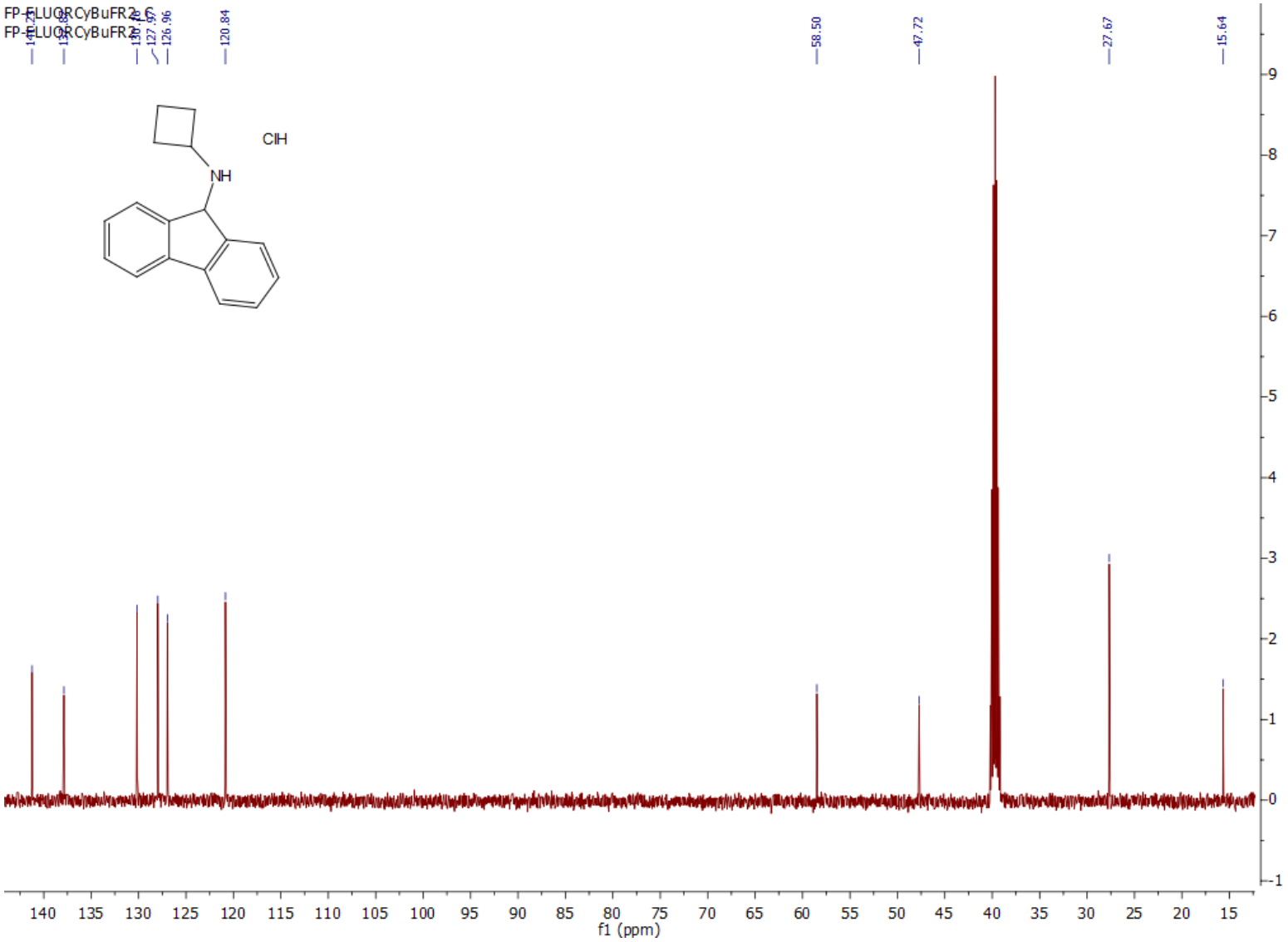
^{13}C NMR of *N*-cyclopropyl-9*H*-fluoren-9-amine hydrochloride (3d):



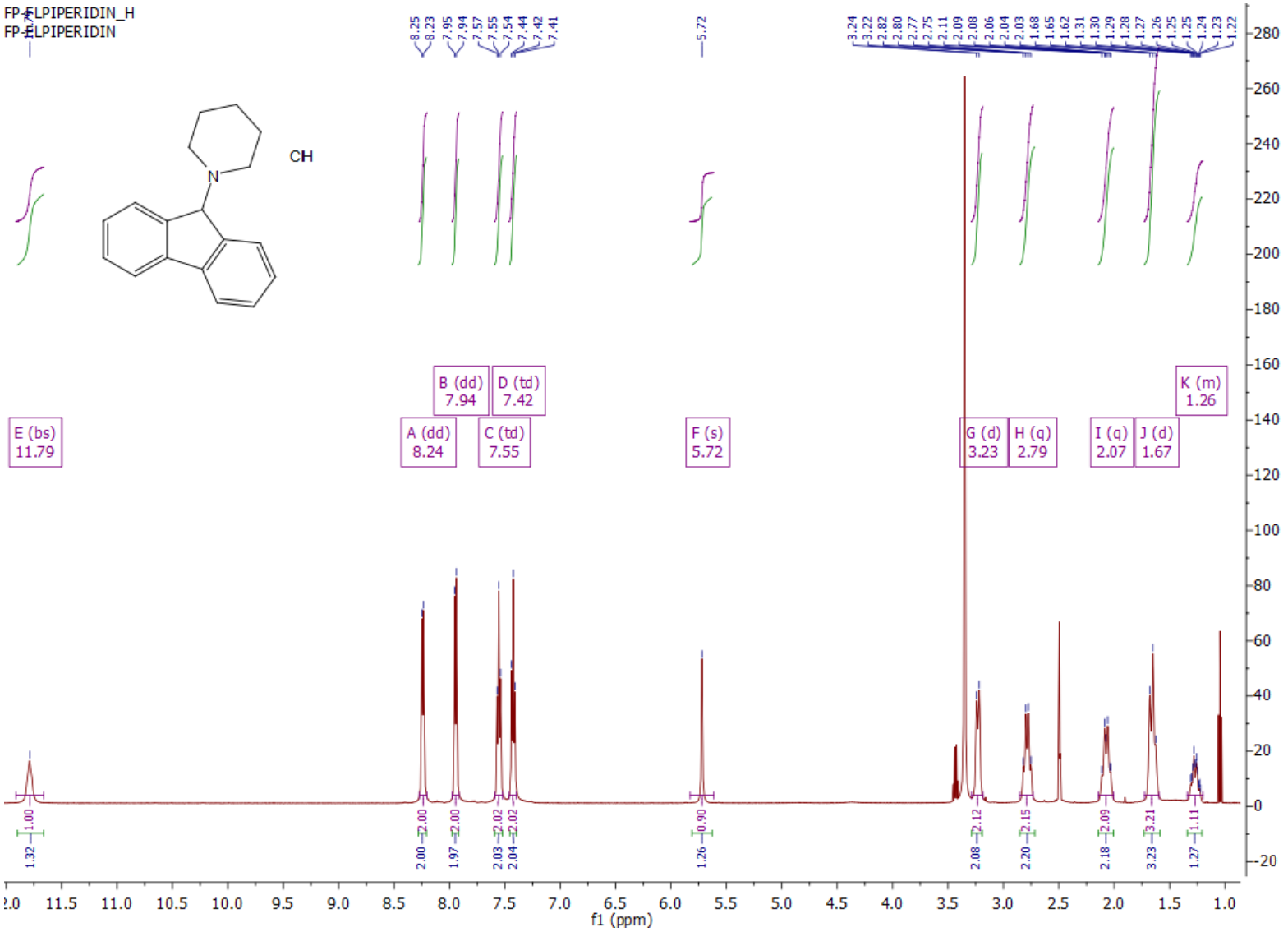
^{1}H NMR of *N*-cyclobutyl-9*H*-fluoren-9-amine hydrochloride (3e):



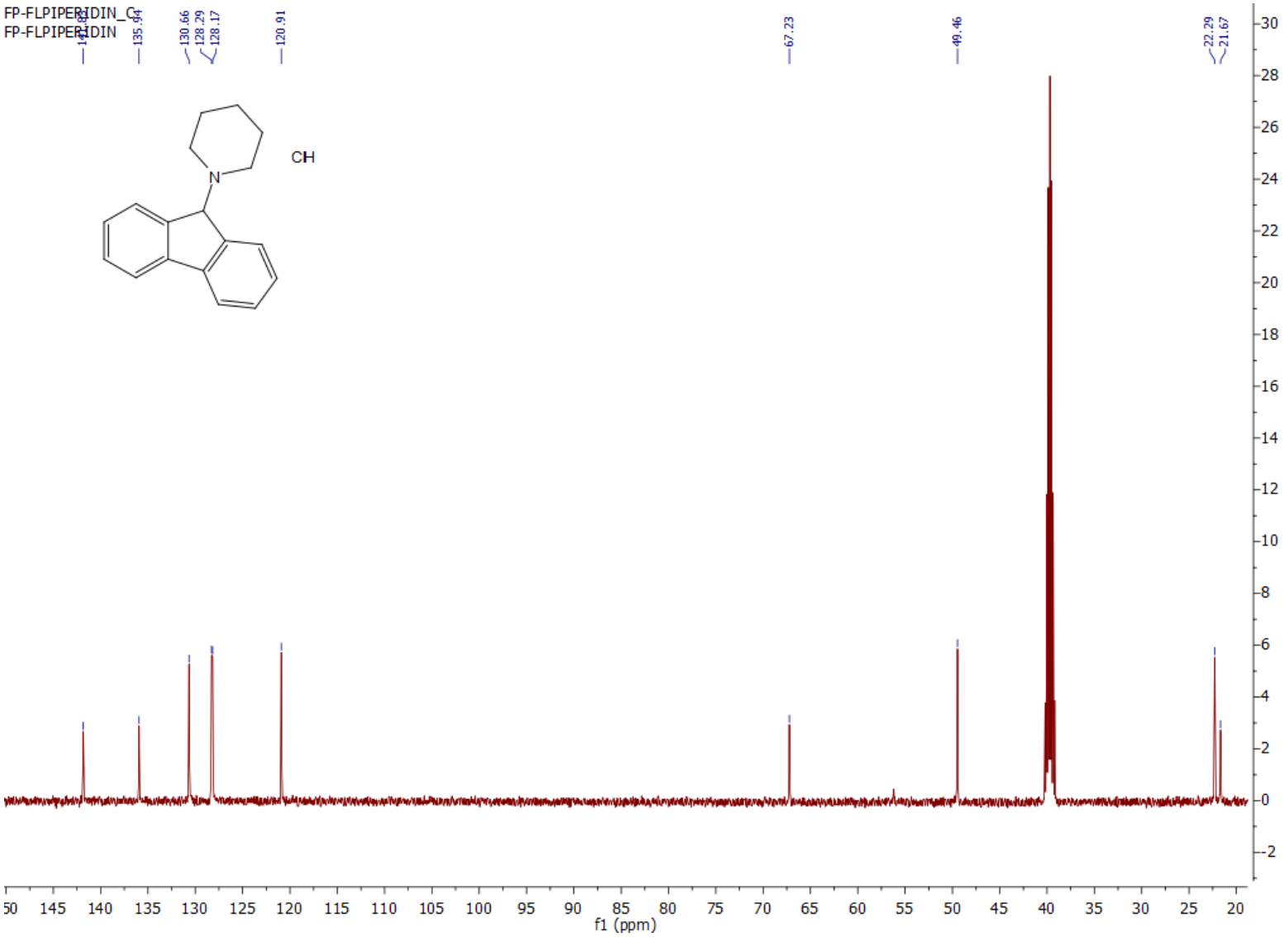
^{13}C NMR of *N*-cyclobutyl-9*H*-fluoren-9-amine hydrochloride (3e):



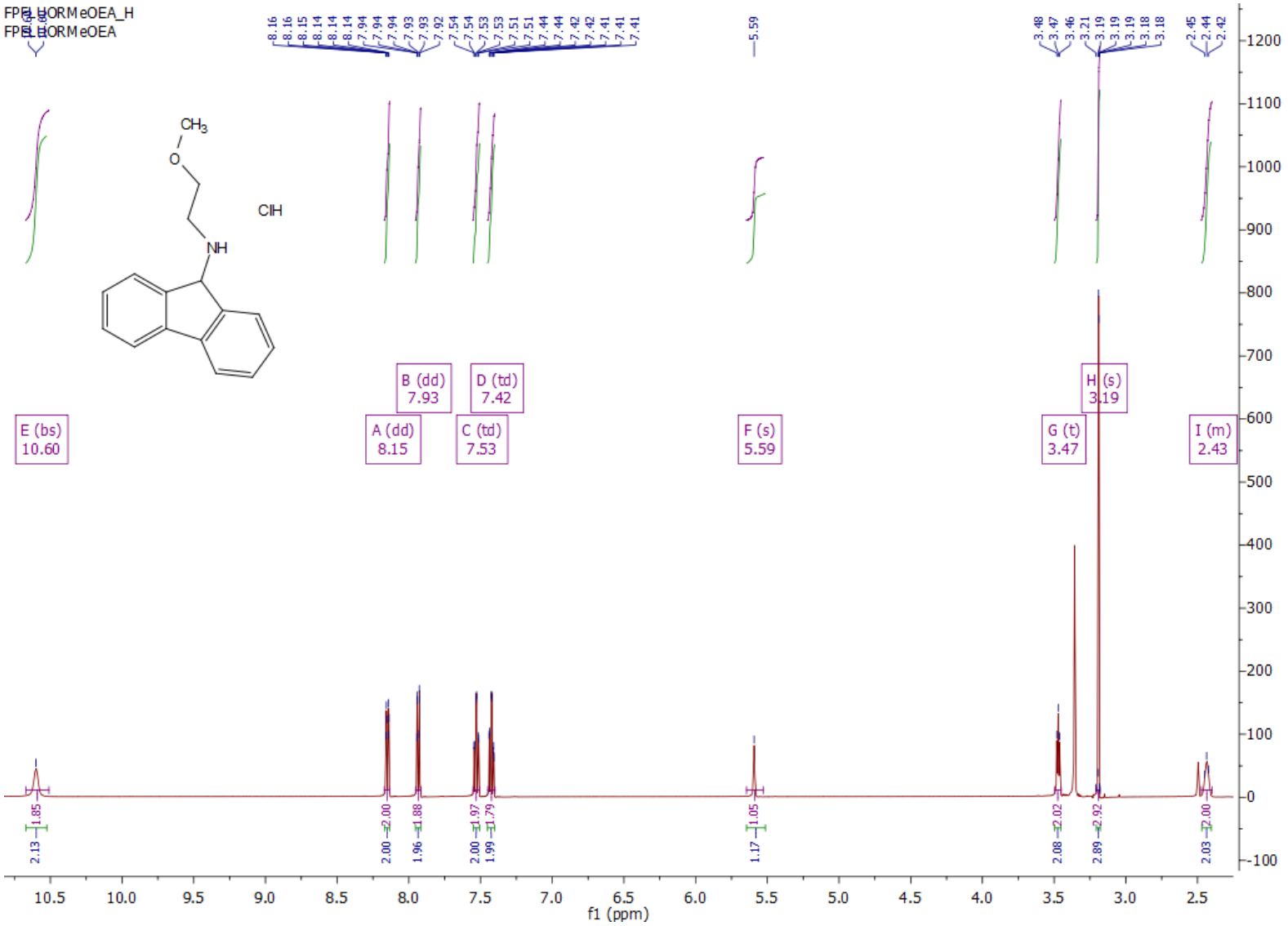
^{1}H NMR of 1-(9*H*-fluoren-9-yl)piperidine hydrochloride (3f):



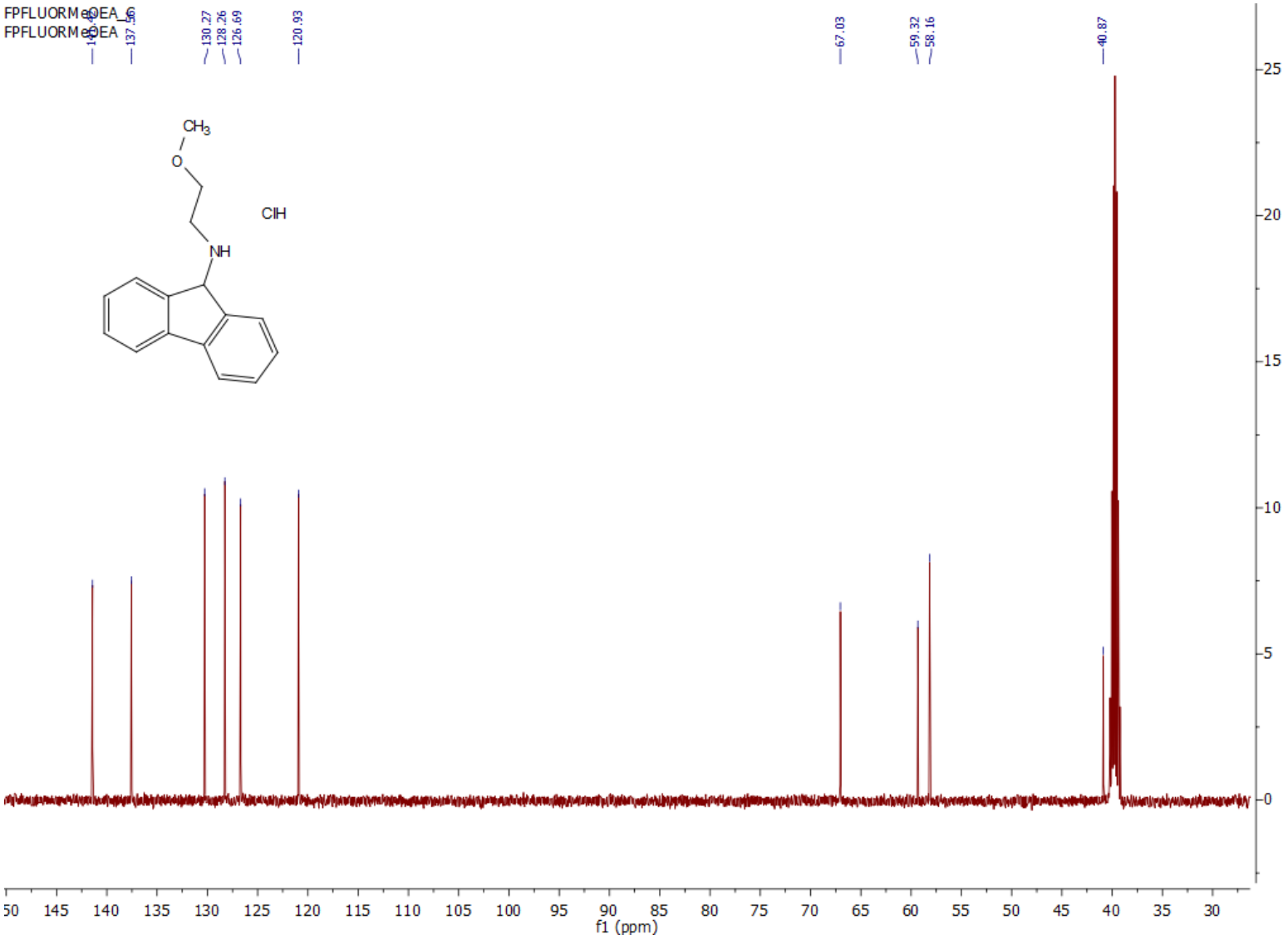
^{13}C NMR of 1-(9*H*-fluoren-9-yl)piperidine hydrochloride (3f):



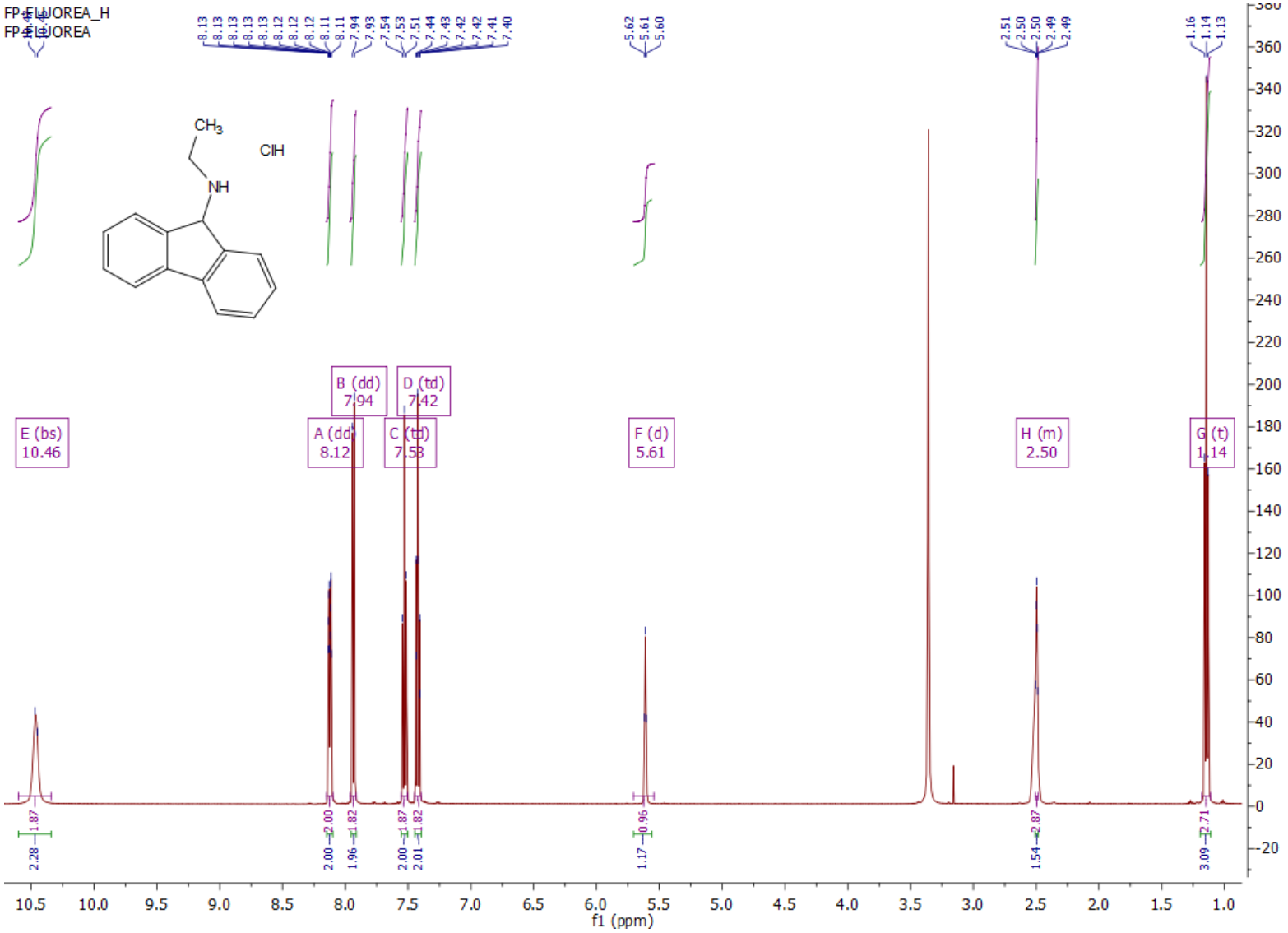
^{1}H NMR of *N*-(2-methoxyethyl)-9*H*-fluoren-9-amine hydrochloride (3g):



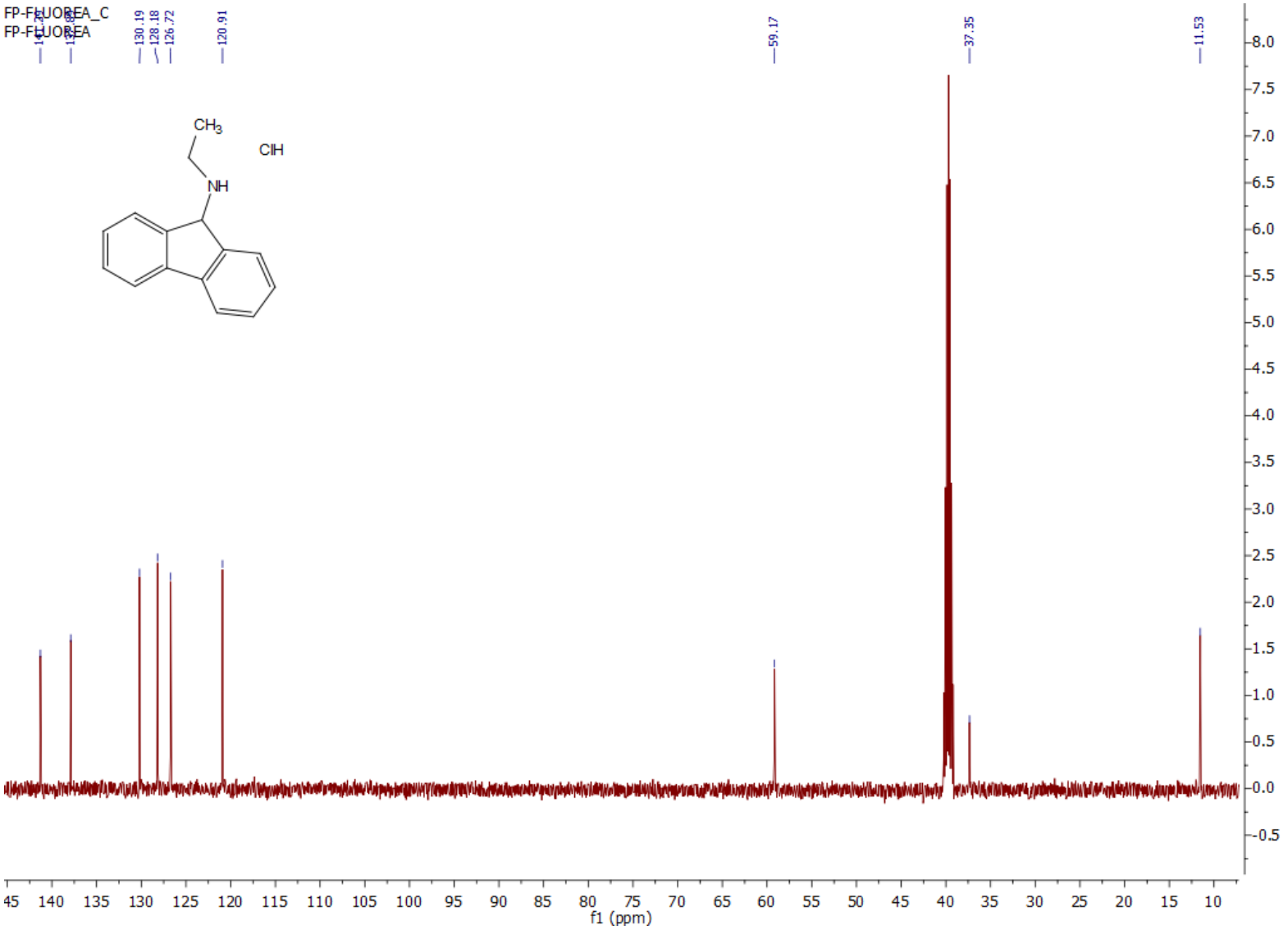
^{13}C NMR of *N*-(2-methoxyethyl)-9*H*-fluoren-9-amine hydrochloride (3g):



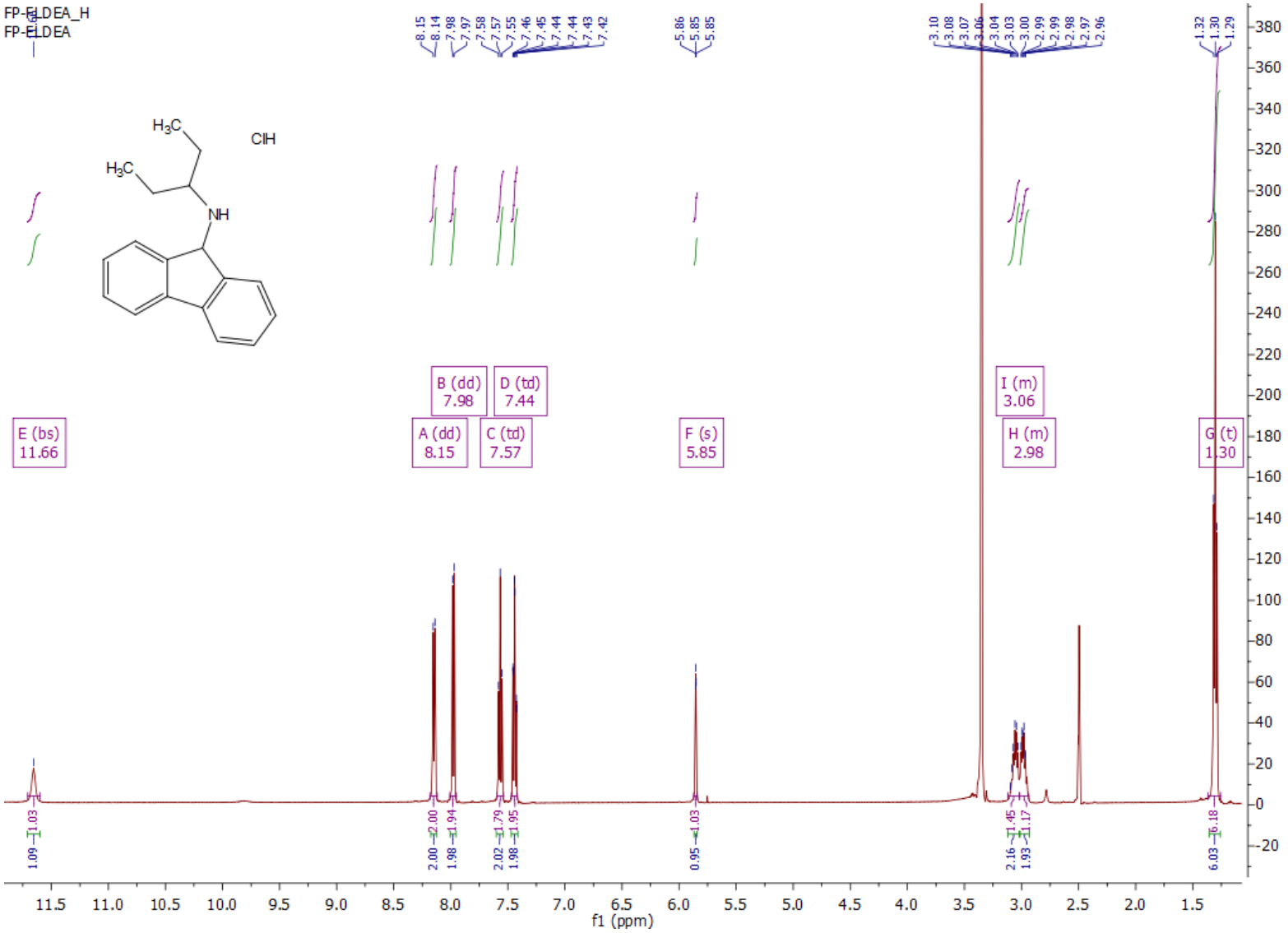
^{1}H NMR of *N*-ethyl-9*H*-fluoren-9-amine hydrochloride (3h):



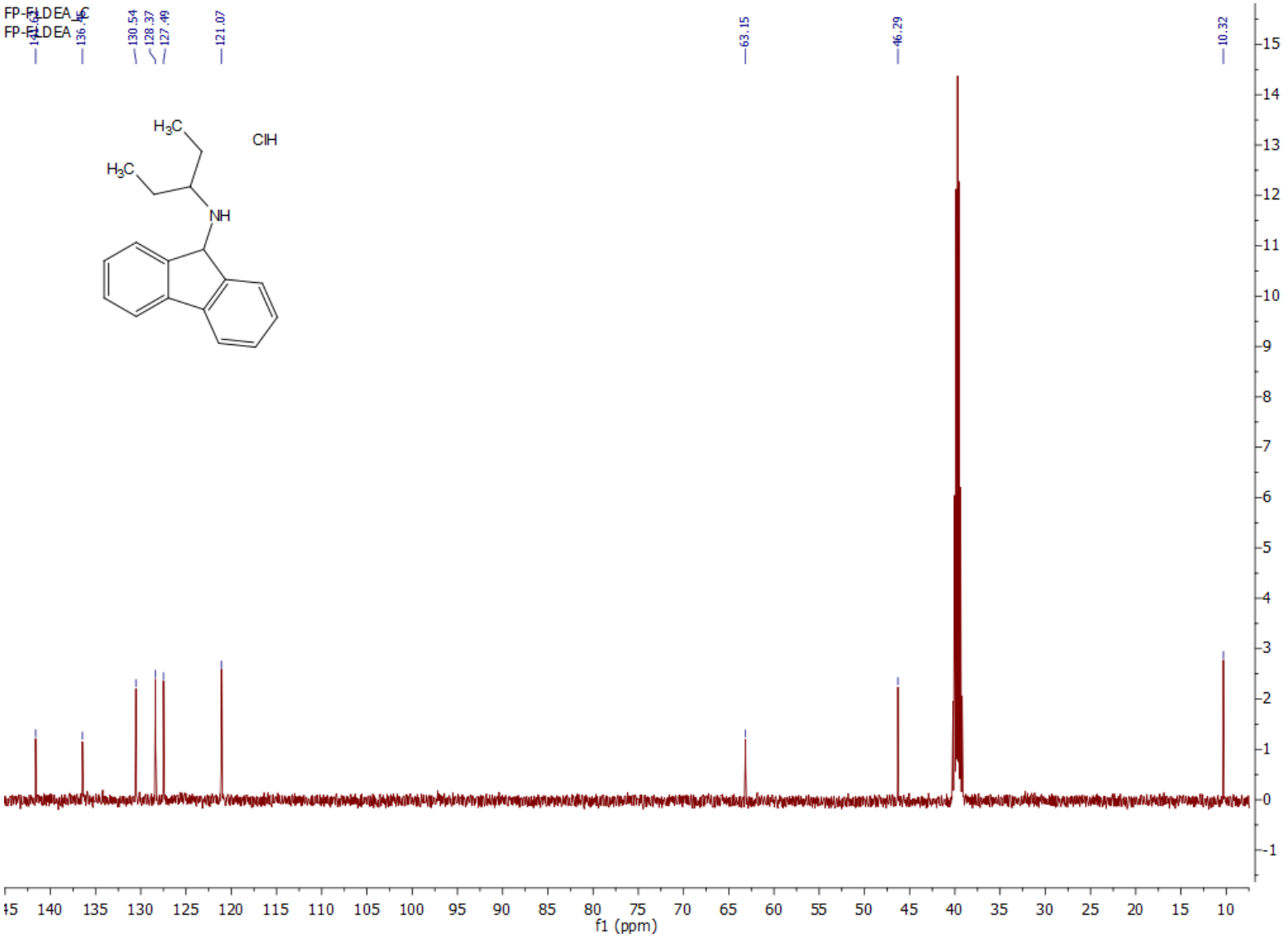
^{13}C NMR of *N*-ethyl-9*H*-fluoren-9-amine hydrochloride (3h):



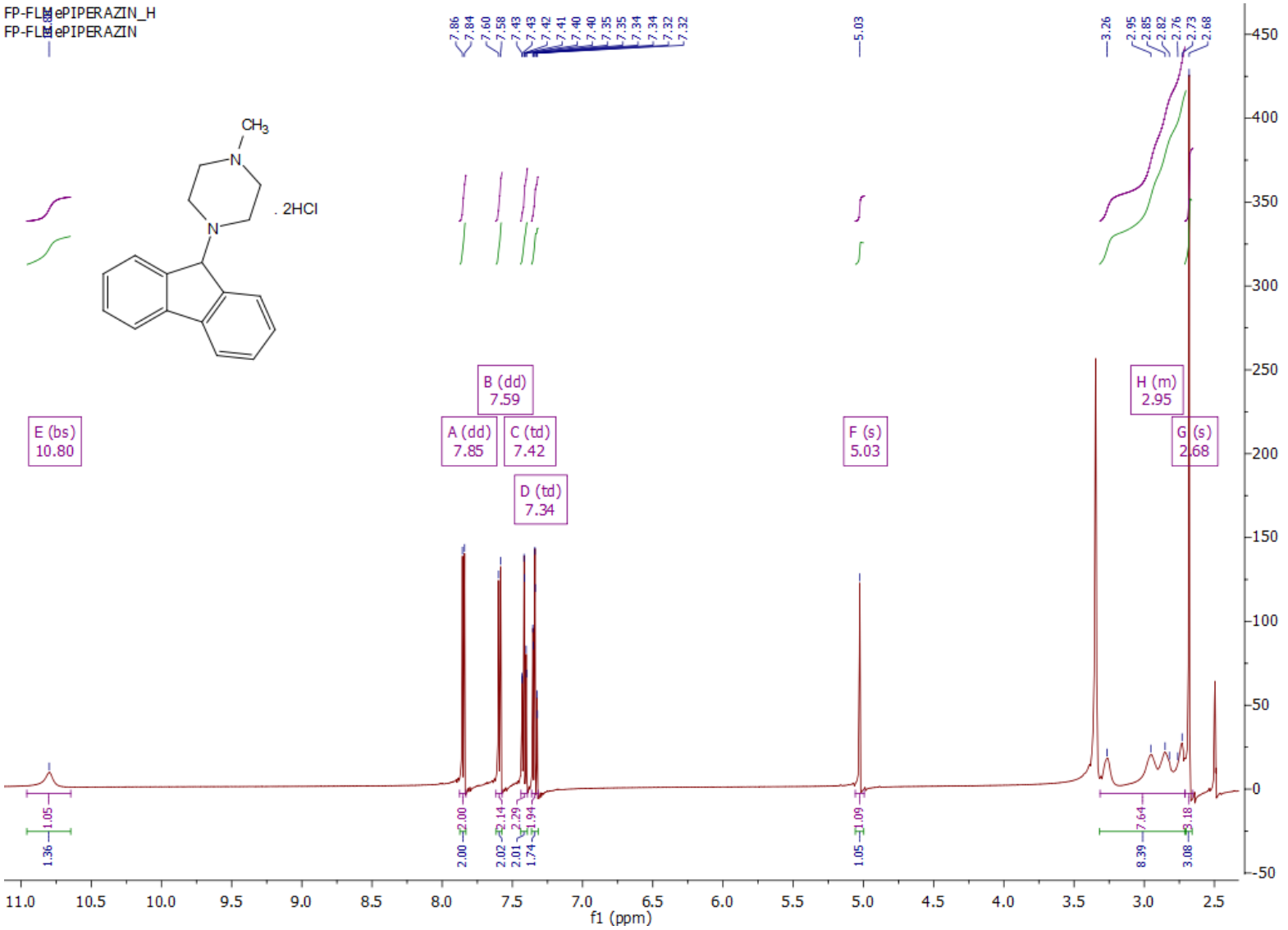
^{1}H NMR of *N,N*-diethyl-9*H*-fluoren-9-amine hydrochloride (3i):



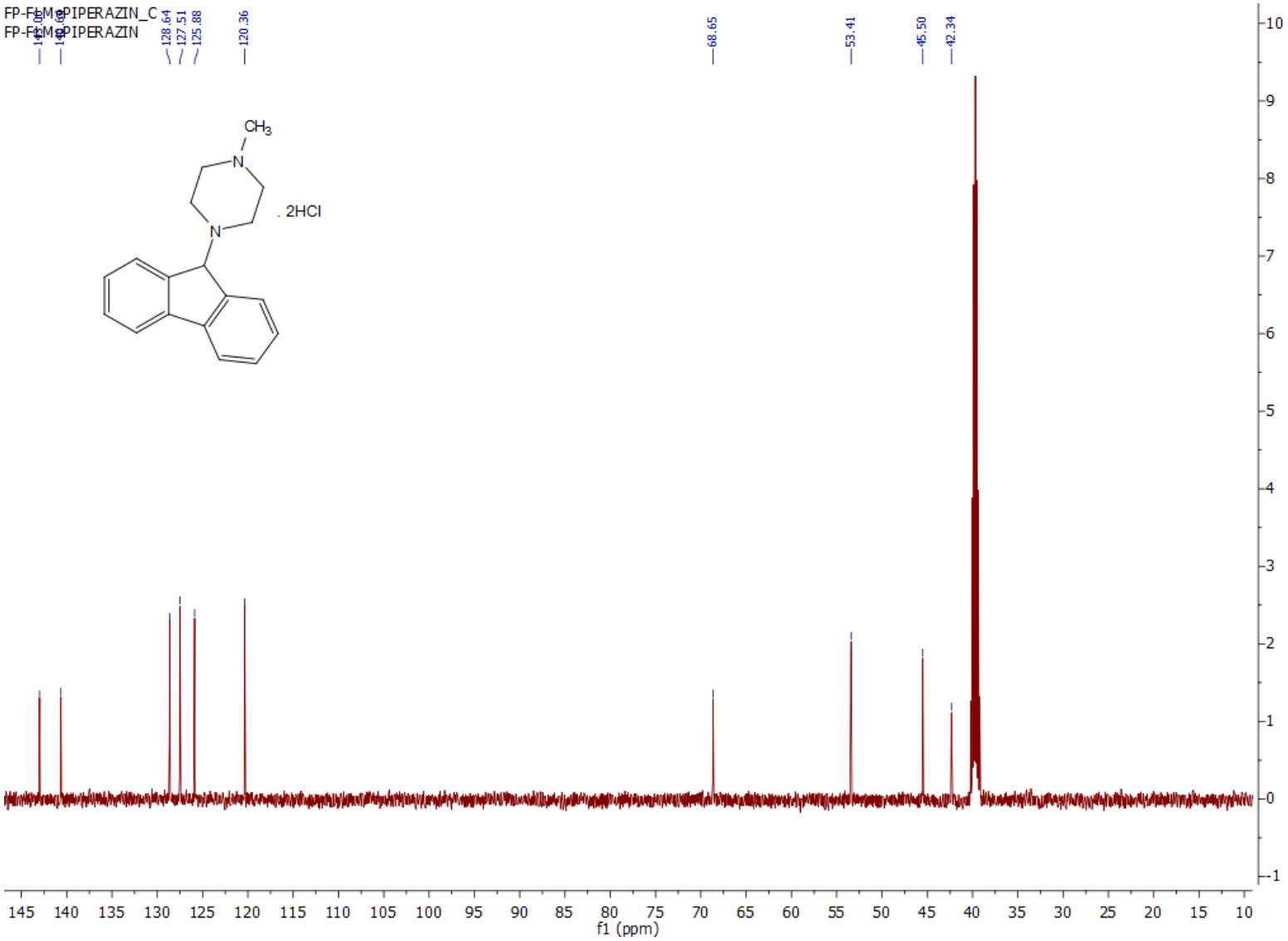
^{13}C NMR of *N,N*-diethyl-9*H*-fluoren-9-amine hydrochloride (3i):



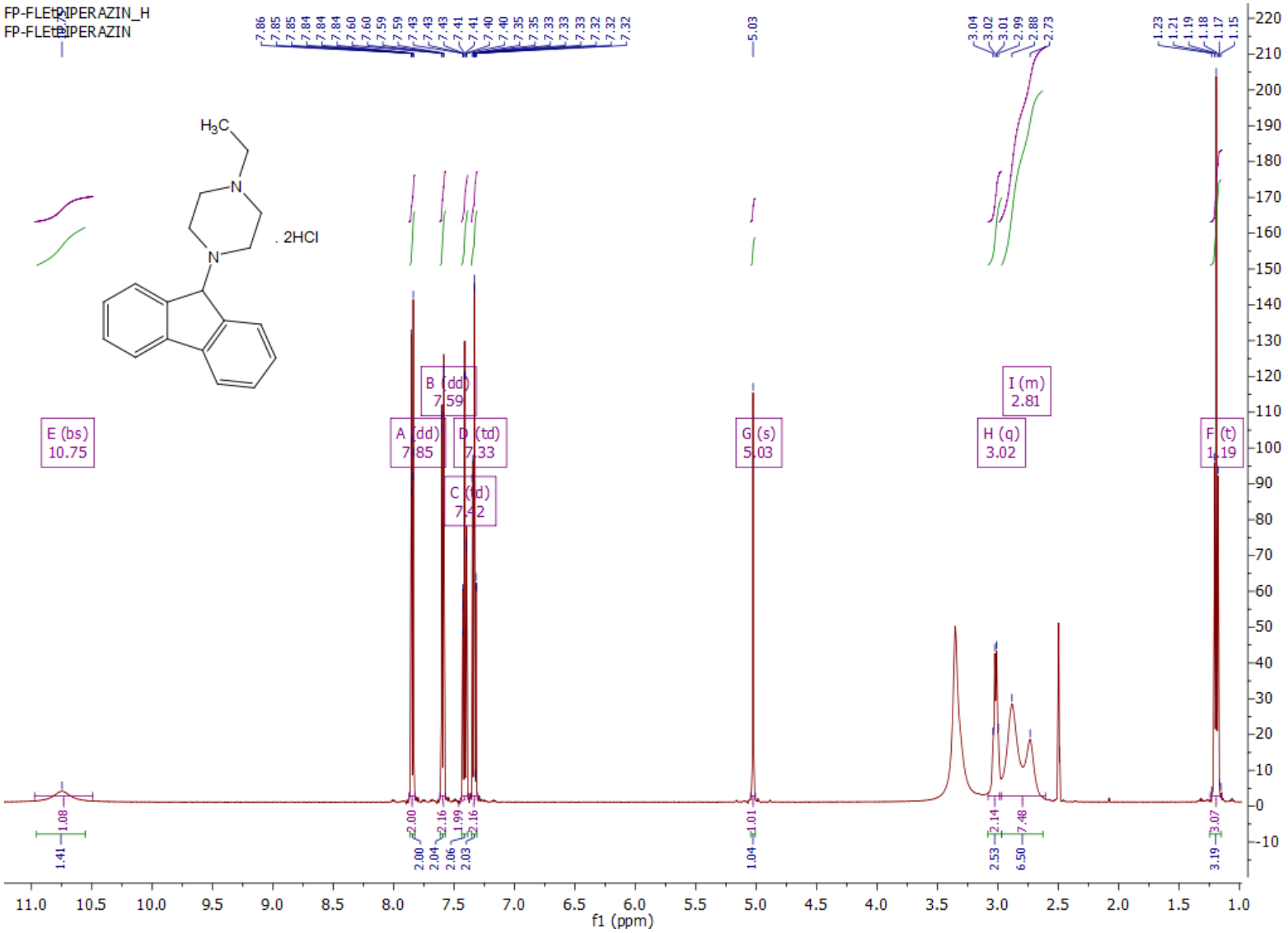
[1]H NMR of 1-(9*H*-fluorene-9-yl)-4-methylpiperazine hydrochloride (3j):



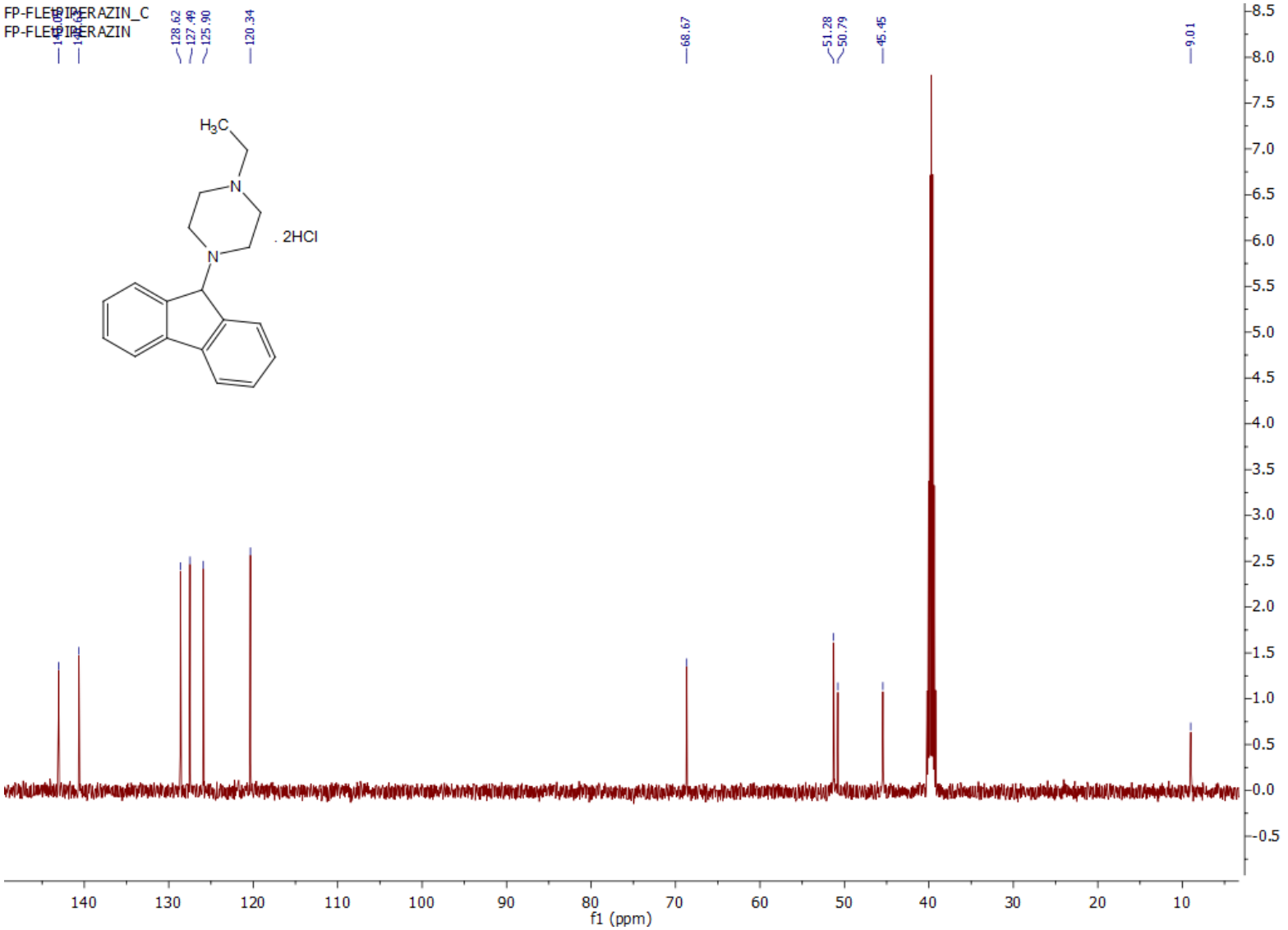
^{13}C NMR of 1-(9*H*-fluorene-9-yl)-4-methylpiperazine hydrochloride (3j):



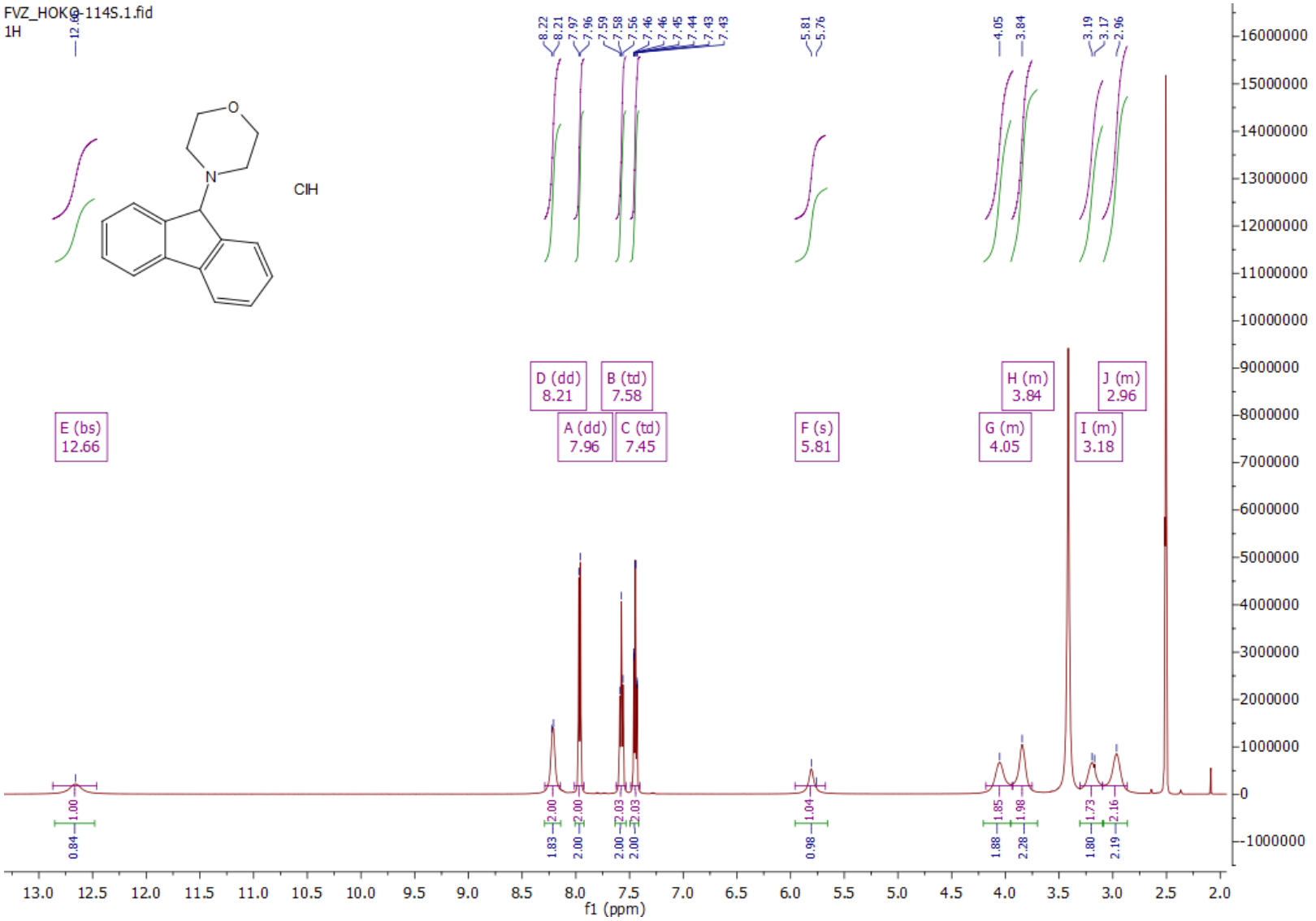
^{1}H NMR of 1-(9*H*-fluorene-9-yl)-4-ethylpiperazine hydrochloride (3k):



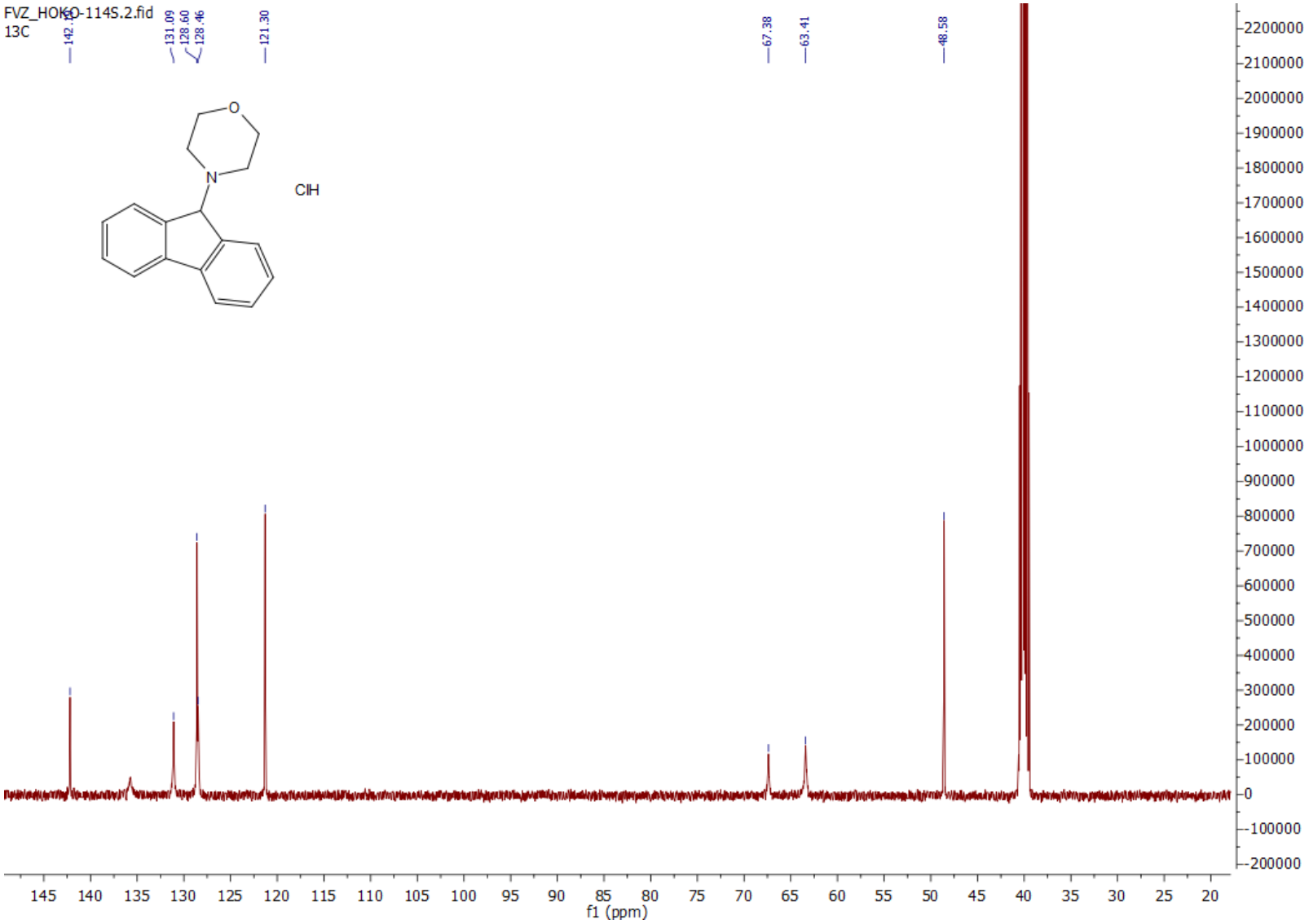
^{13}C NMR of 1-(9*H*-fluorene-9-yl)-4-ethylpiperazine hydrochloride (3k):



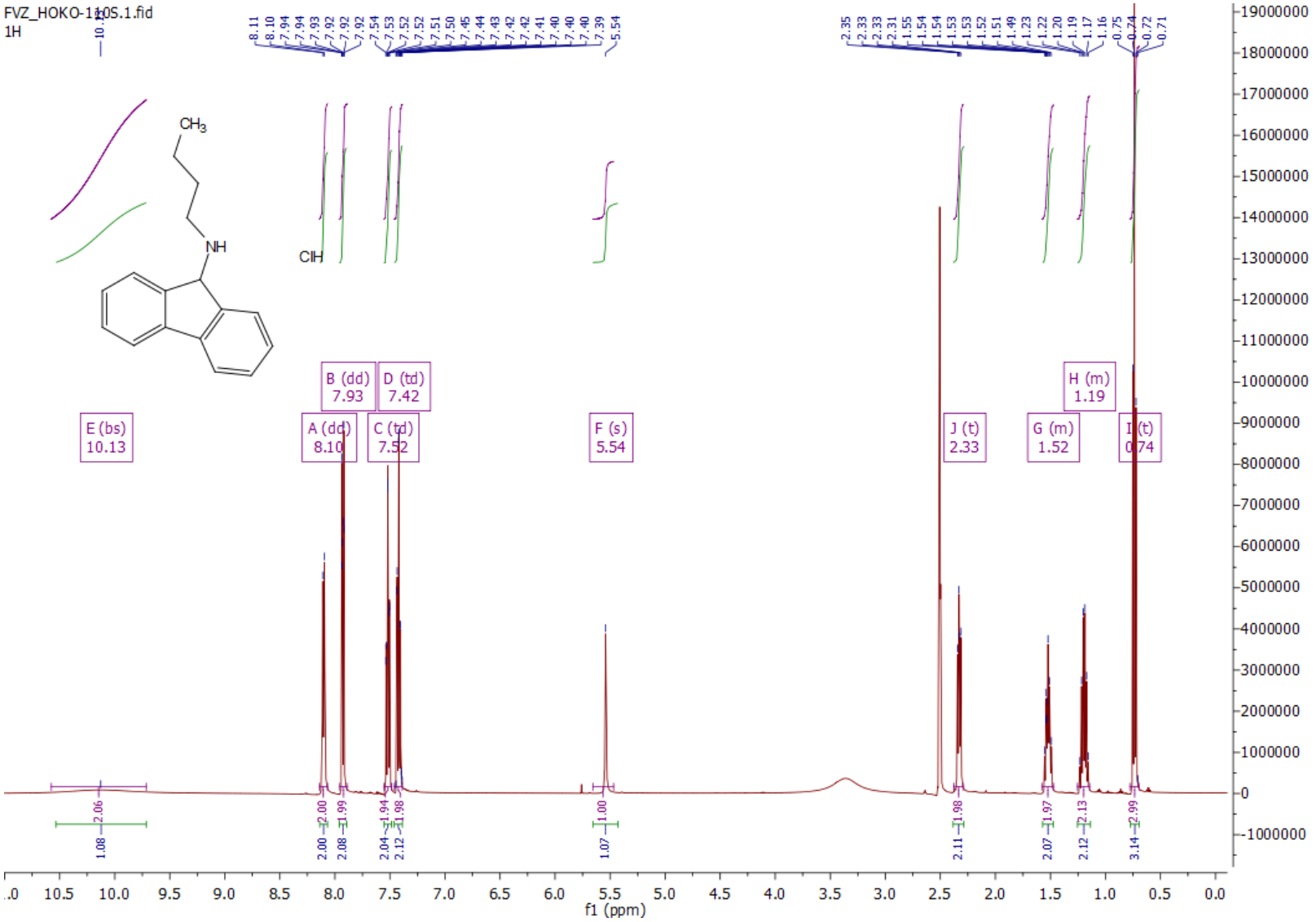
^{1}H NMR of 4-(9*H*-fluorene-9-yl)morpholine hydrochloride (3l):



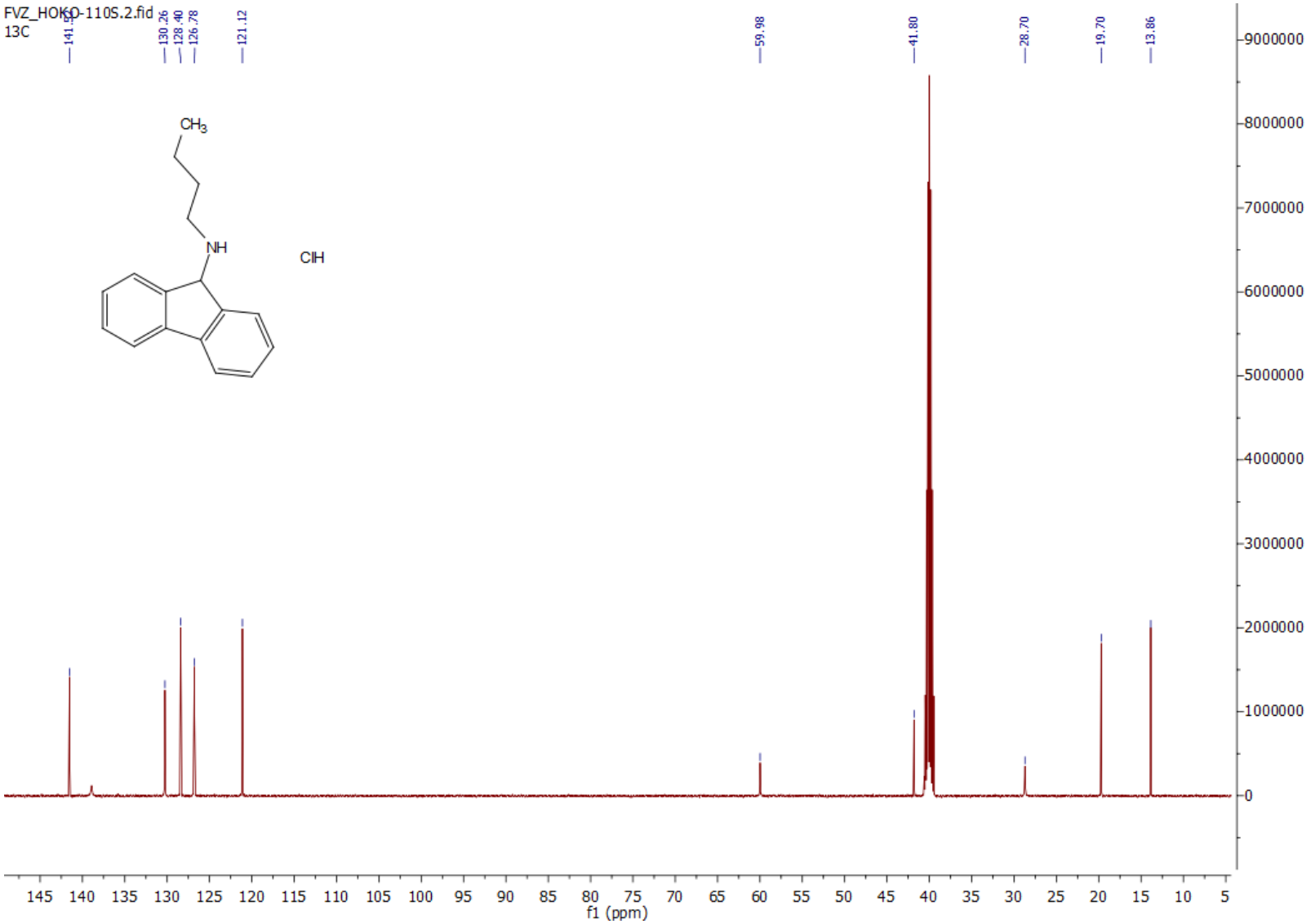
^{13}C NMR of 4-(9*H*-fluorene-9-yl)morpholine hydrochloride (3l):



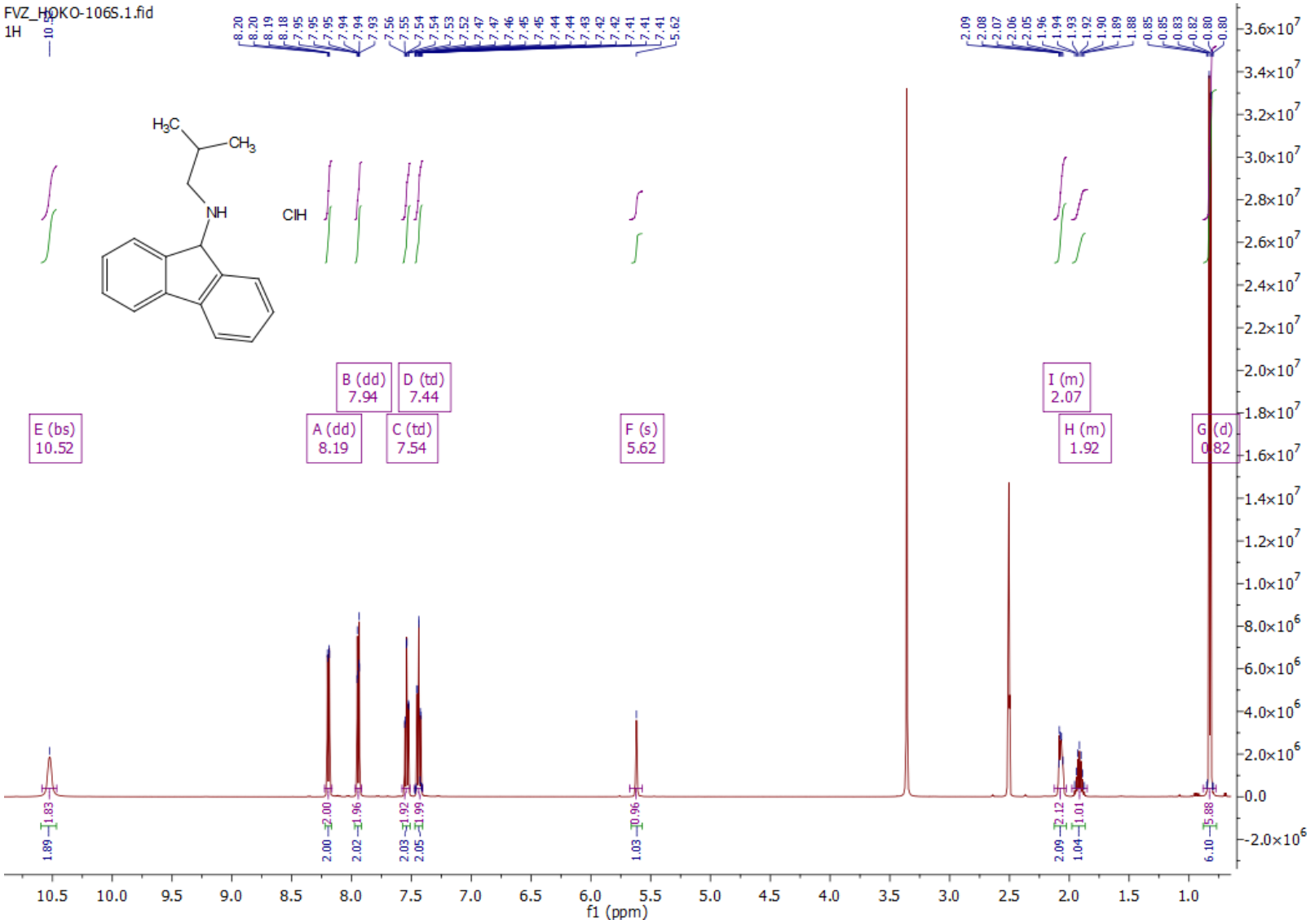
^{1}H NMR of *N*-butyl-9*H*-fluoren-9-amine hydrochloride (3m):



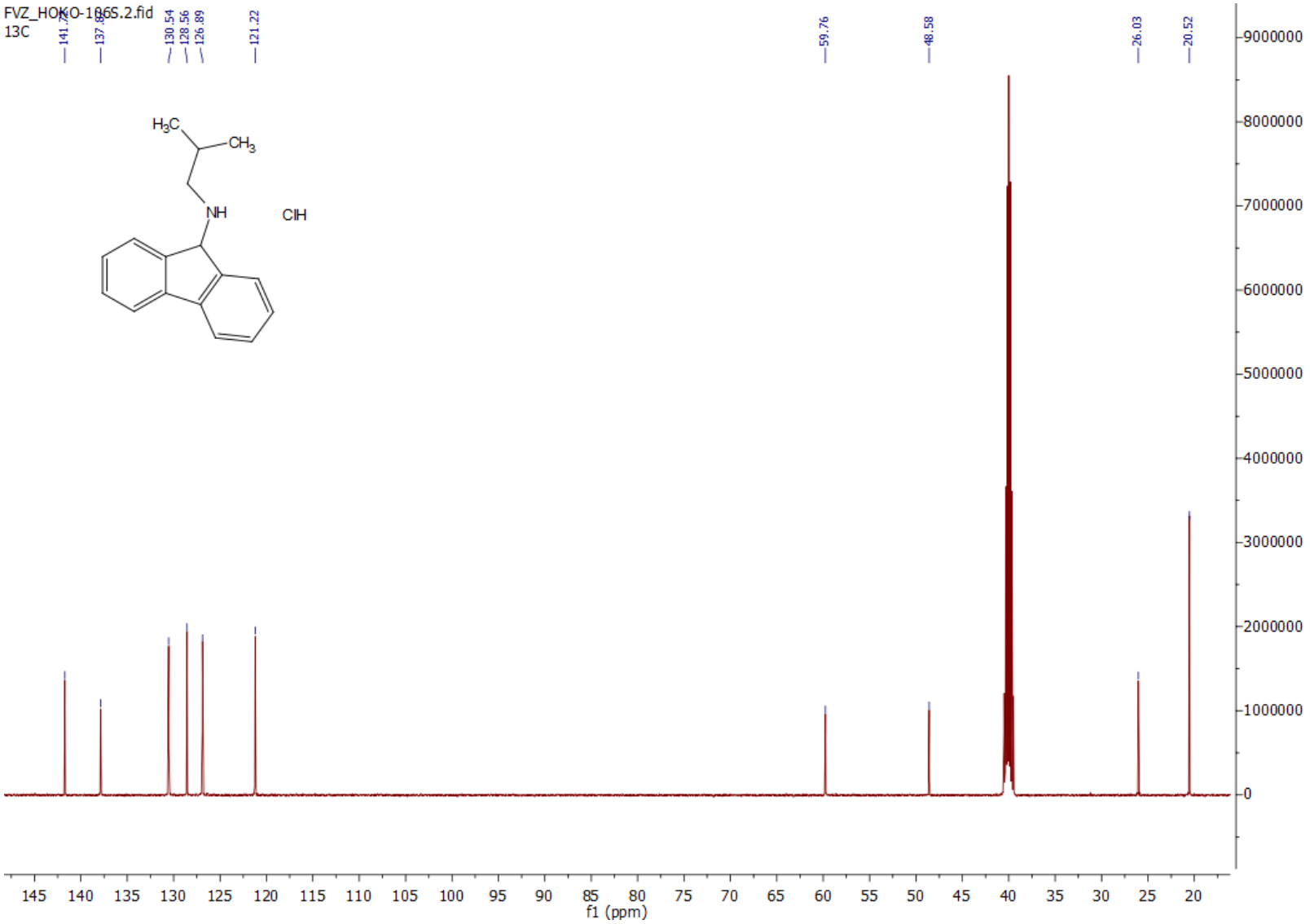
^{13}C NMR of *N*-butyl-9*H*-fluoren-9-amine hydrochloride (3m):



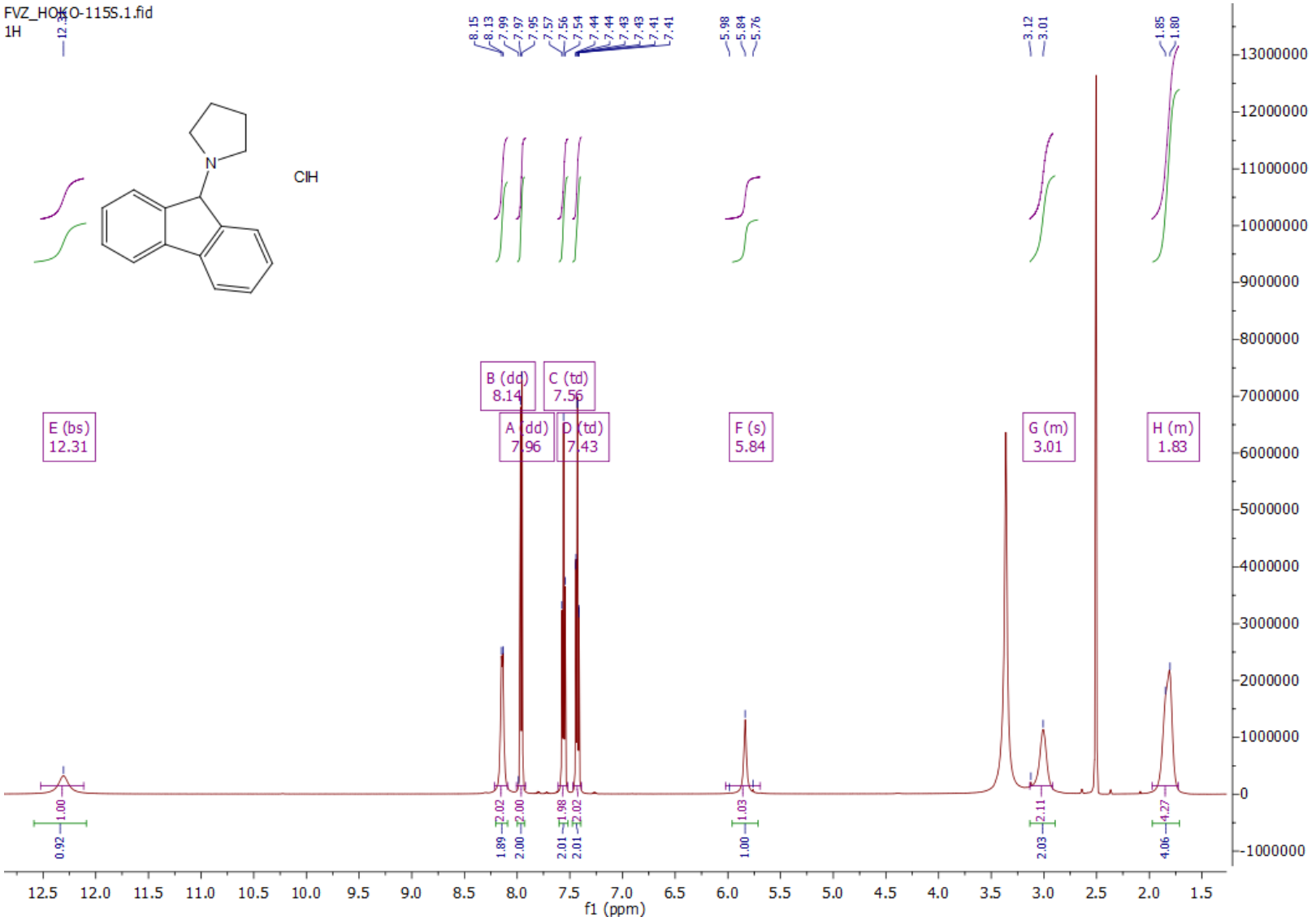
^{1}H NMR of *N*-(2-methylpropyl)-9*H*-fluoren-9-amine hydrochloride (3n):



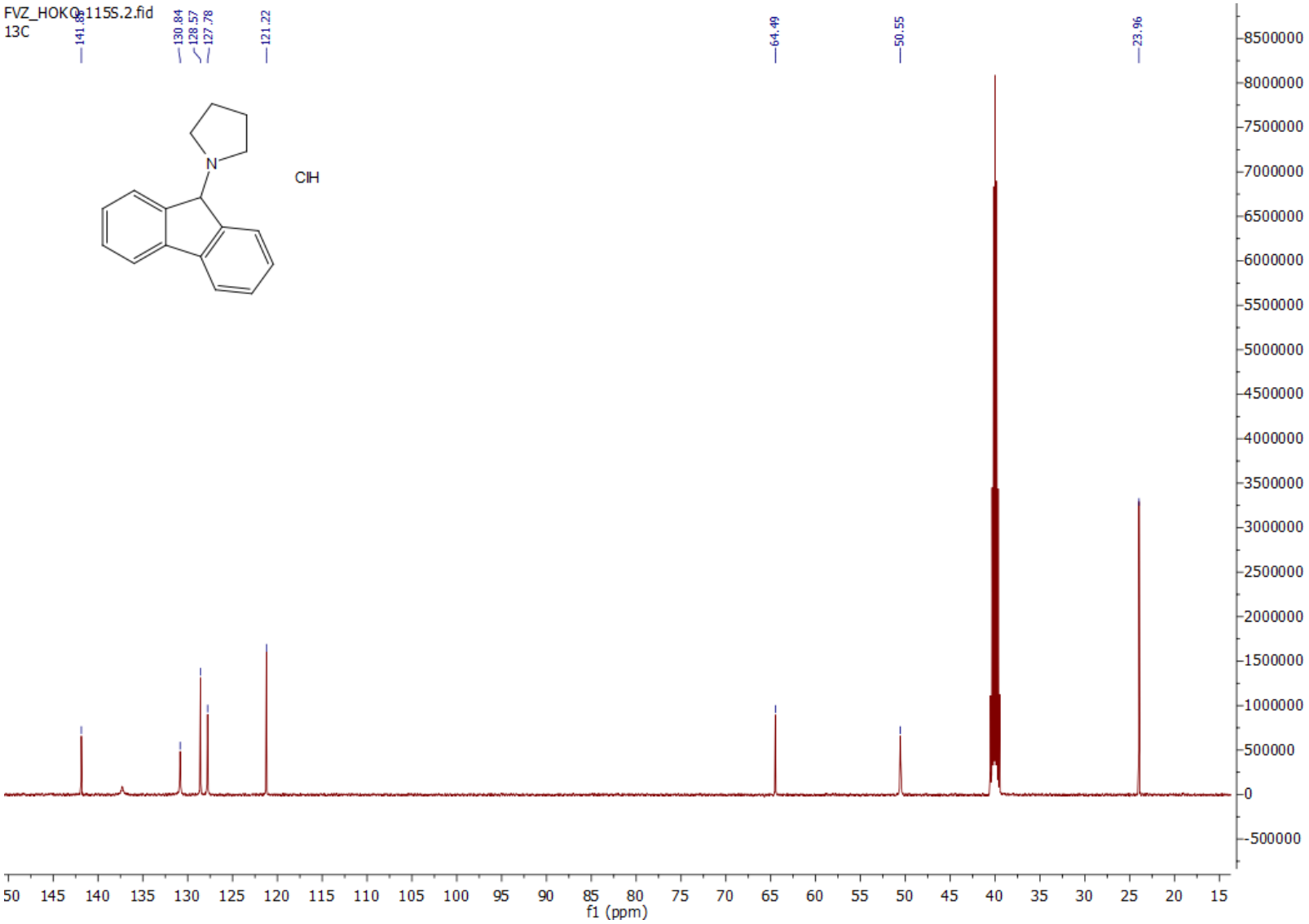
^{13}C NMR of *N*-(2-methylpropyl)-9*H*-fluoren-9-amine hydrochloride (3n):



[1]H NMR of 1-(9*H*-fluoren-9-yl)pyrrolidine hydrochloride (3o):

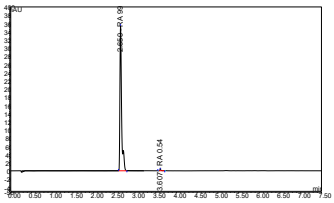


^{13}C NMR of 1-(9*H*-fluoren-9-yl)pyrrolidine hydrochloride (3o):

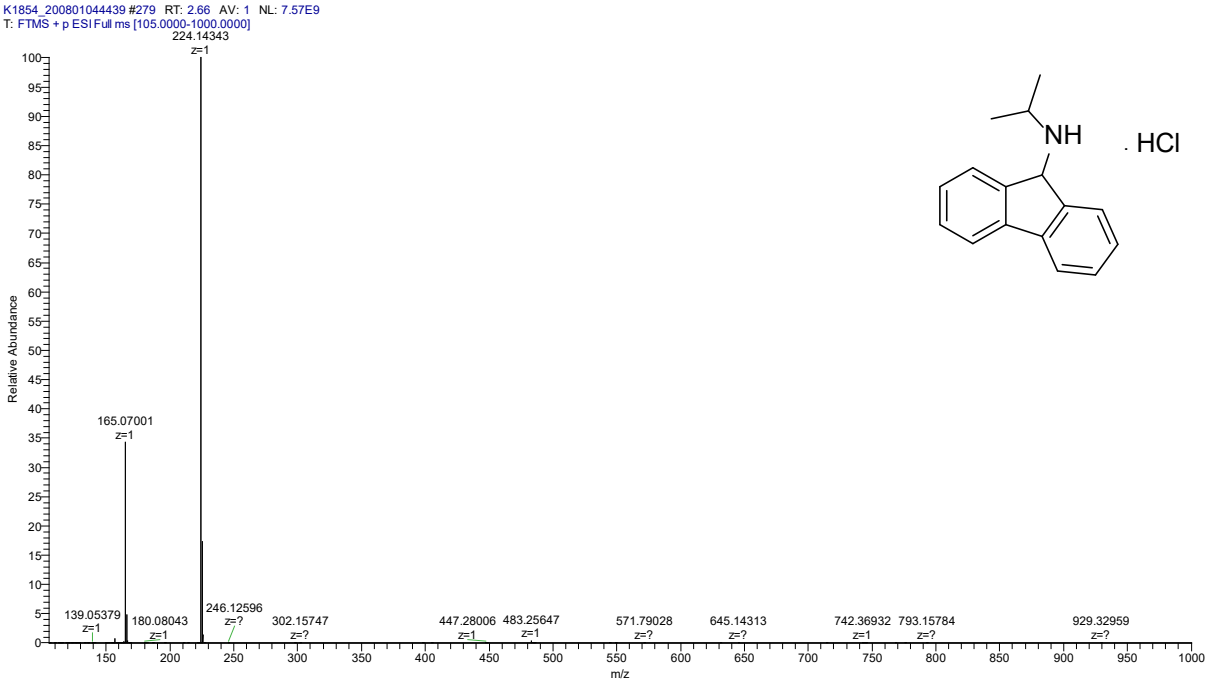


4. HPLC chromatograms and MS spectra of final compounds

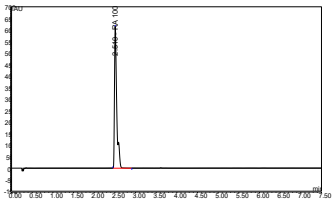
HPLC chromatogram (UV 254 nm) of *N*-propan-2-yl-9*H*-fluoren-9-amine hydrochloride (3a)



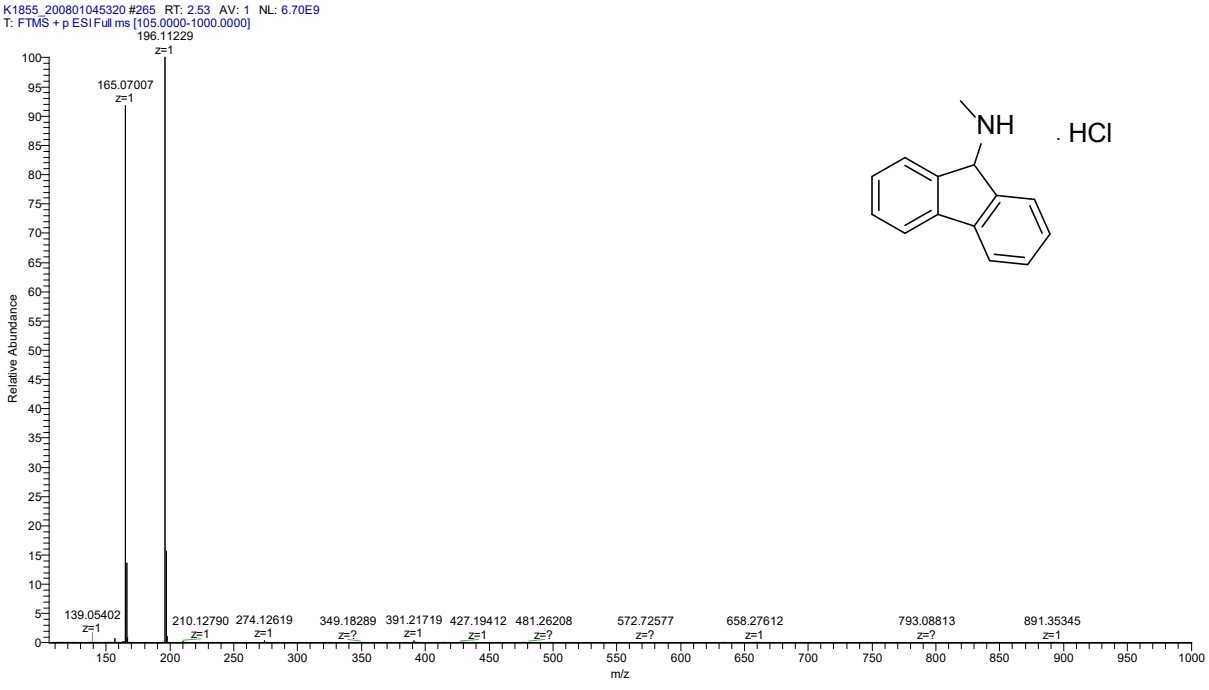
MS spectrum of *N*-propan-2-yl-9*H*-fluoren-9-amine hydrochloride (3a) at Rt: 2.66 min:



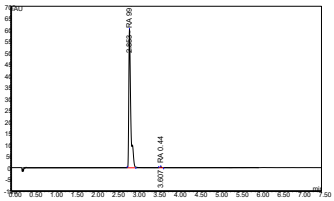
HPLC chromatogram (UV 254 nm) of *N*-methyl-9*H*-fluoren-9-amine hydrochloride (3b):



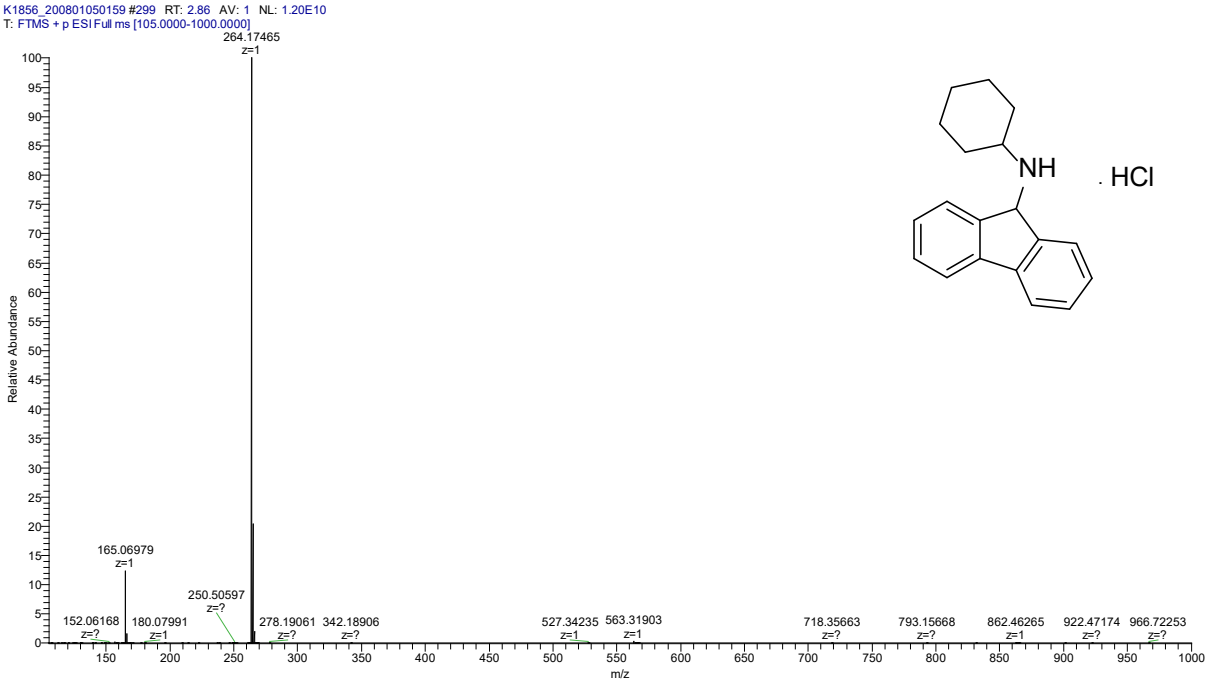
MS spectrum of *N*-methyl-9*H*-fluoren-9-amine hydrochloride (3b) at R_t: 2.53 min:



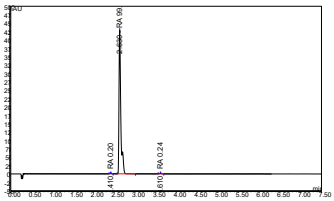
HPLC chromatogram (UV 254 nm) of *N*-cyclohexyl-9*H*-fluoren-9-amine hydrochloride (3c):



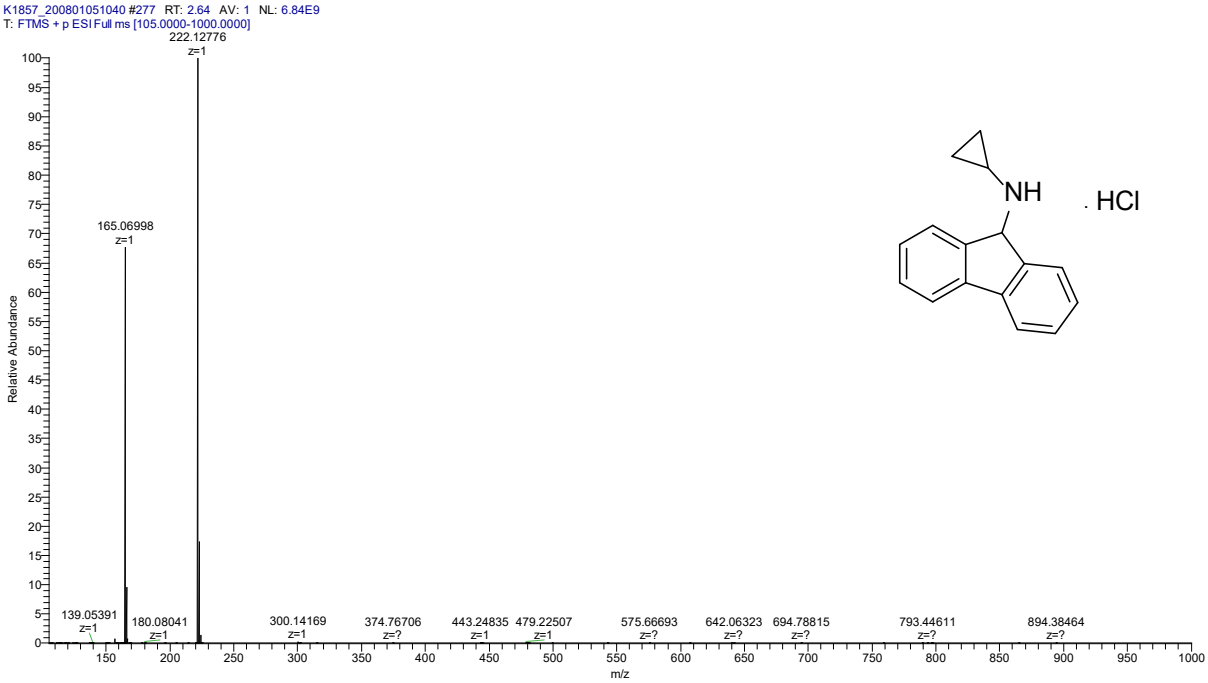
MS spectrum of *N*-cyclohexyl-9*H*-fluoren-9-amine hydrochloride (3c) at R_t: 2.86 min:



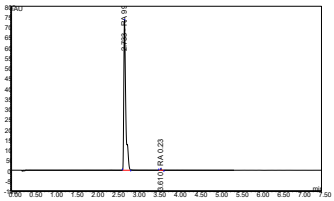
HPLC chromatogram (UV 254 nm) of *N*-cyclopropyl-9*H*-fluoren-9-amine hydrochloride (3d):



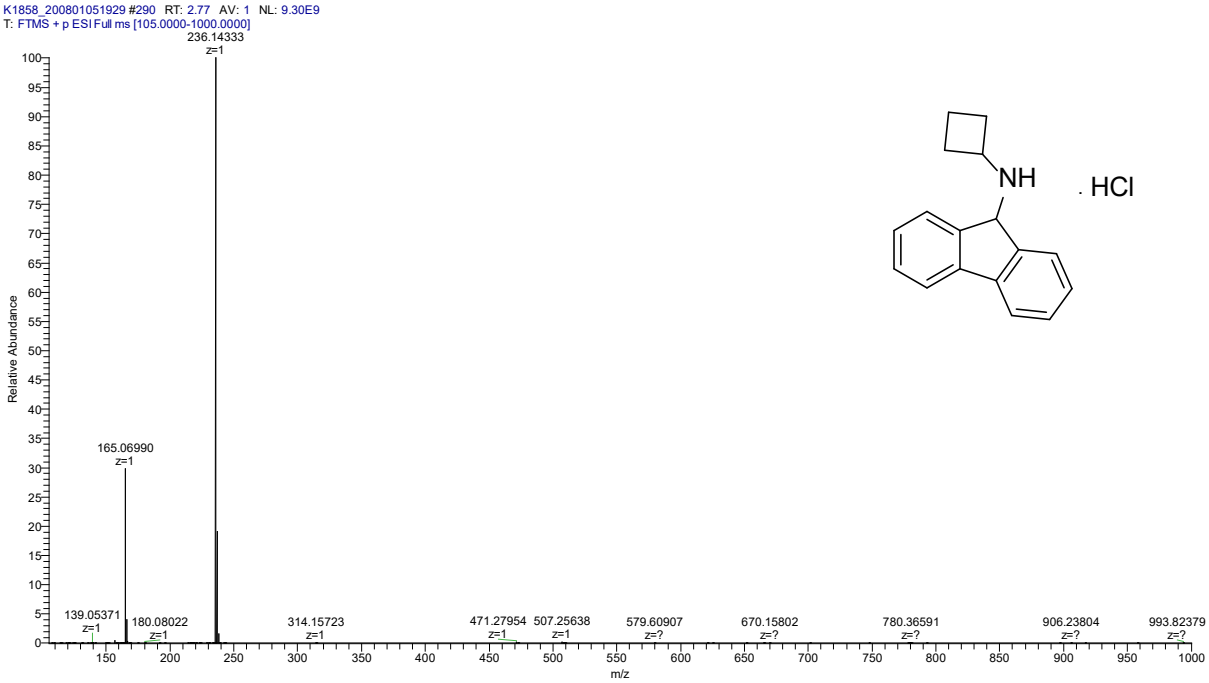
MS spectrum of *N*-cyclopropyl-9*H*-fluoren-9-amine hydrochloride (3d) at R_t: 2.64 min:



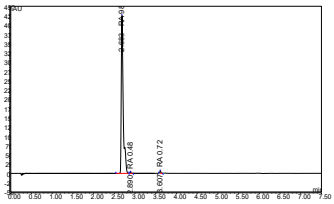
HPLC chromatogram (UV 254 nm) of *N*-cyclobutyl-9*H*-fluoren-9-amine hydrochloride (3e):



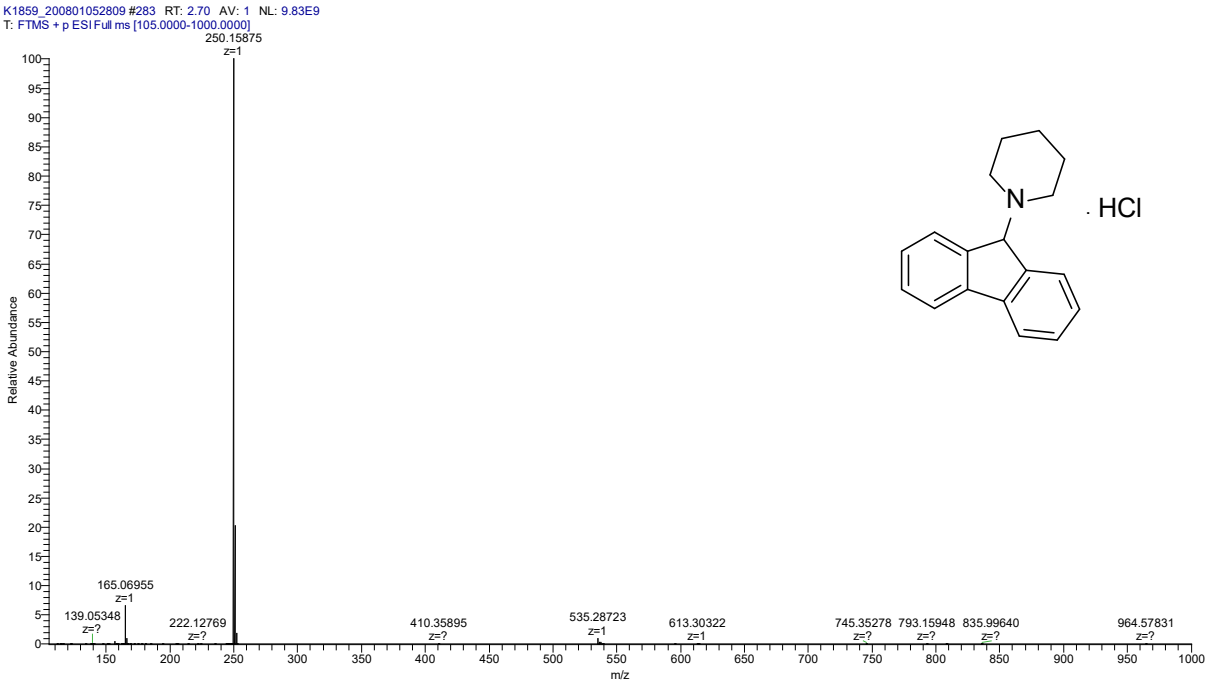
MS spectrum of *N*-cyclobutyl-9*H*-fluoren-9-amine hydrochloride (3e) at R_t: 2.77 min:



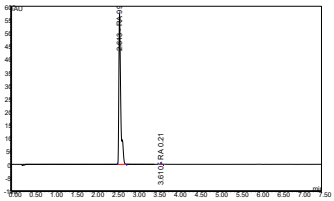
HPLC chromatogram (UV 254 nm) of 1-(9*H*-fluoren-9-yl)piperidine hydrochloride (3f):



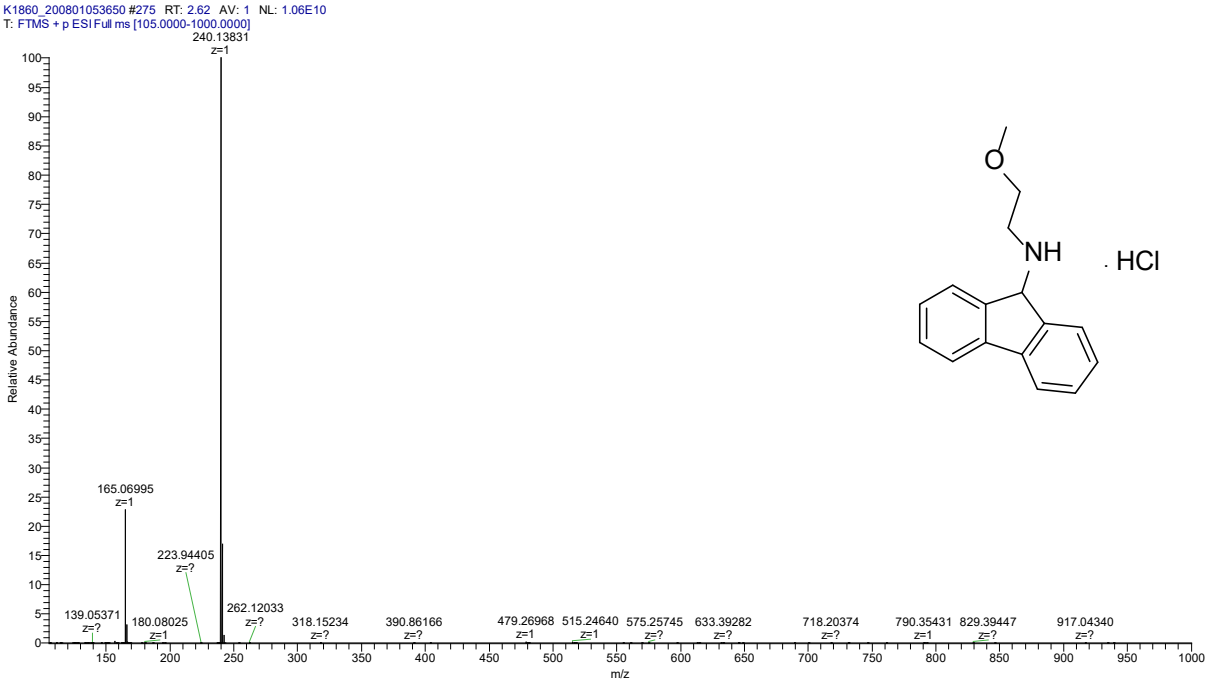
MS spectrum of 1-(9*H*-fluoren-9-yl)piperidine hydrochloride (3f) at R_t: 2.70 min:



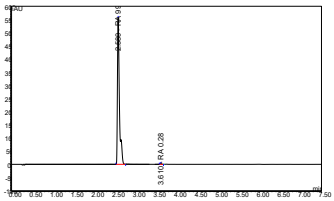
HPLC chromatogram (UV 254 nm) of *N*-(2-methoxyethyl)-9*H*-fluoren-9-amine hydrochloride (3g):



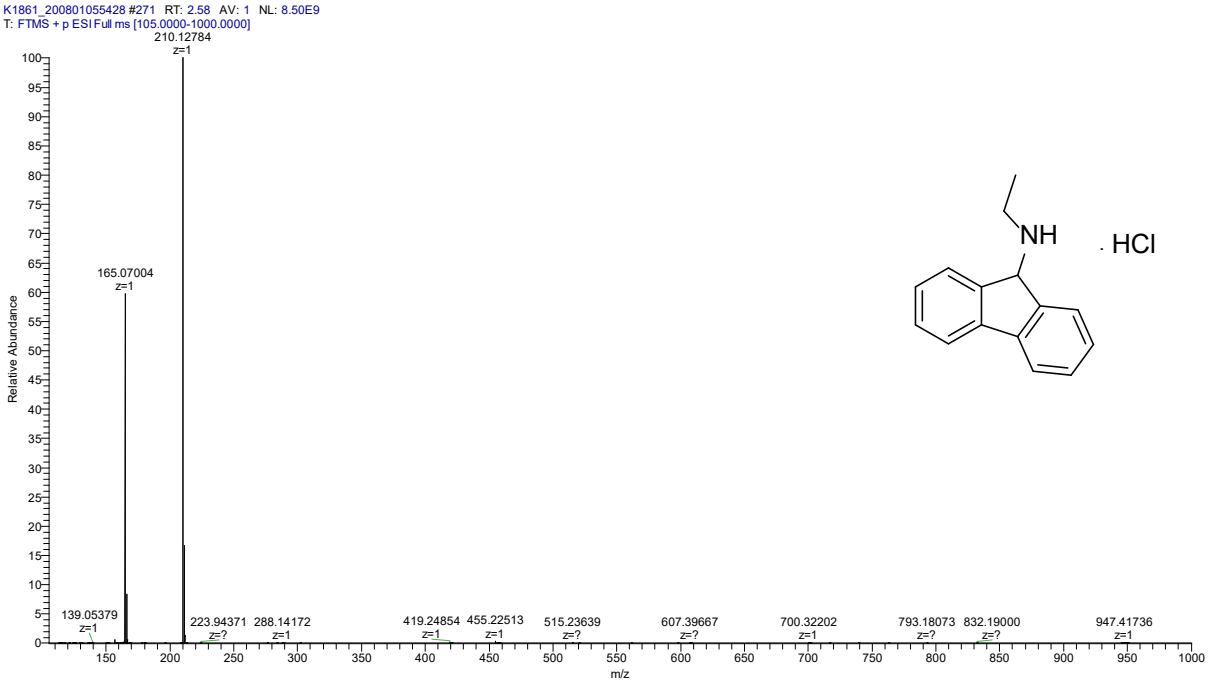
MS spectrum of *N*-(2-methoxyethyl)-9*H*-fluoren-9-amine hydrochloride (3g) at R_t: 2.62 min:



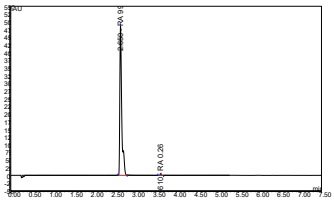
HPLC chromatogram (UV 254 nm) of *N*-ethyl-9*H*-fluoren-9-amine hydrochloride (3h):



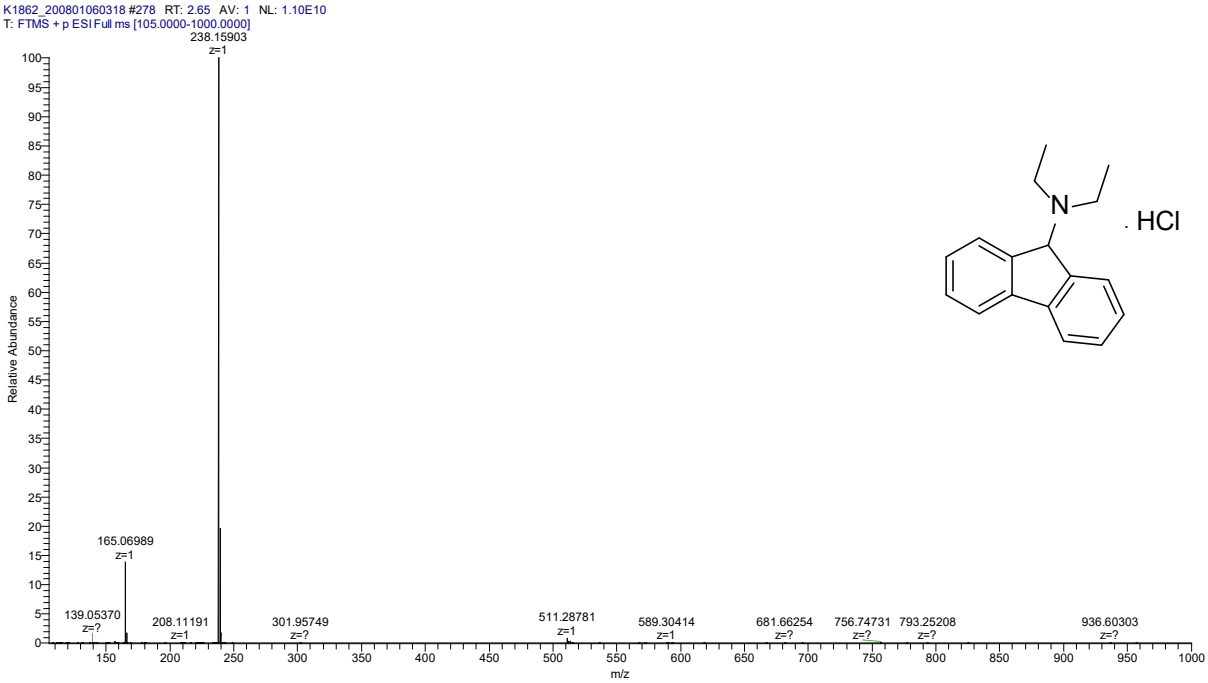
MS spectrum of *N*-ethyl-9*H*-fluoren-9-amine hydrochloride (3h) at Rt: 2.58 min:



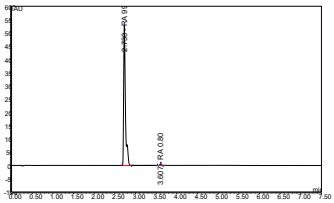
HPLC chromatogram (UV 254 nm) of *N,N*-diethyl-9*H*-fluoren-9-amine hydrochloride (3i):



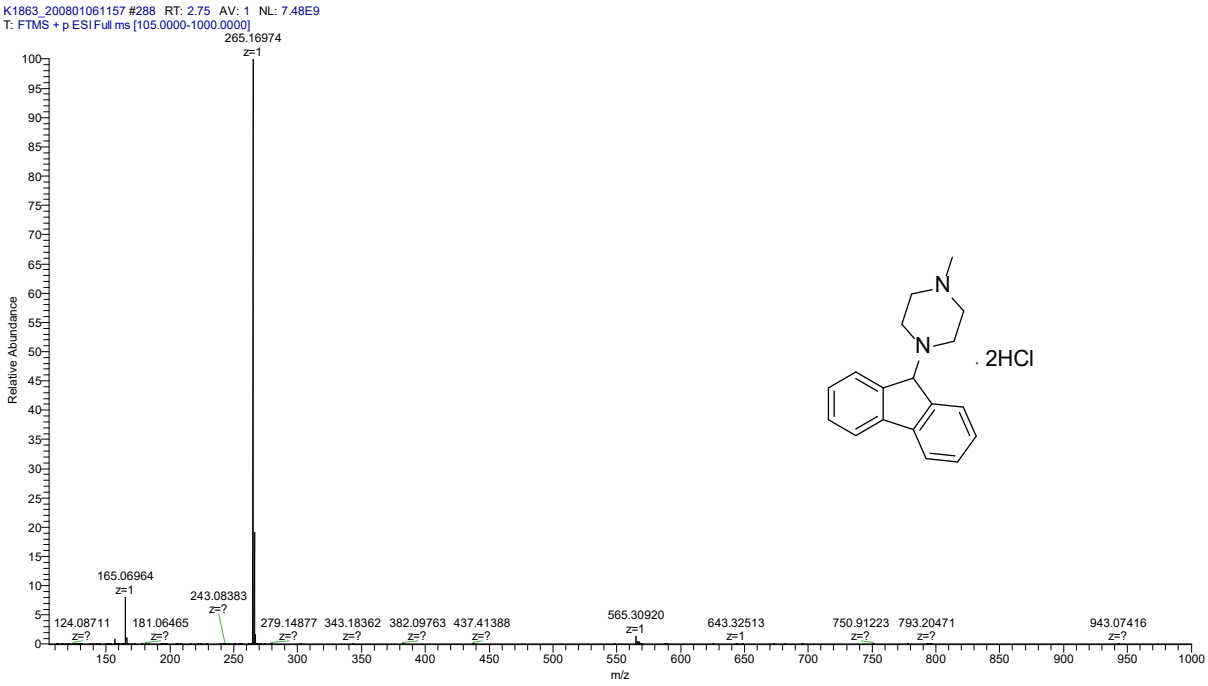
MS spectrum of *N,N*-diethyl-9*H*-fluoren-9-amine hydrochloride (3i) at R_t: 2.65 min:



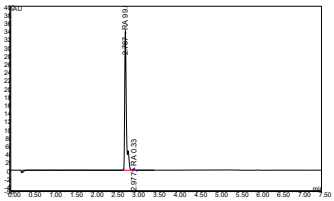
HPLC chromatogram (UV 254 nm) of 1-(9*H*-fluorene-9-yl)-4-methylpiperazine hydrochloride (3j):



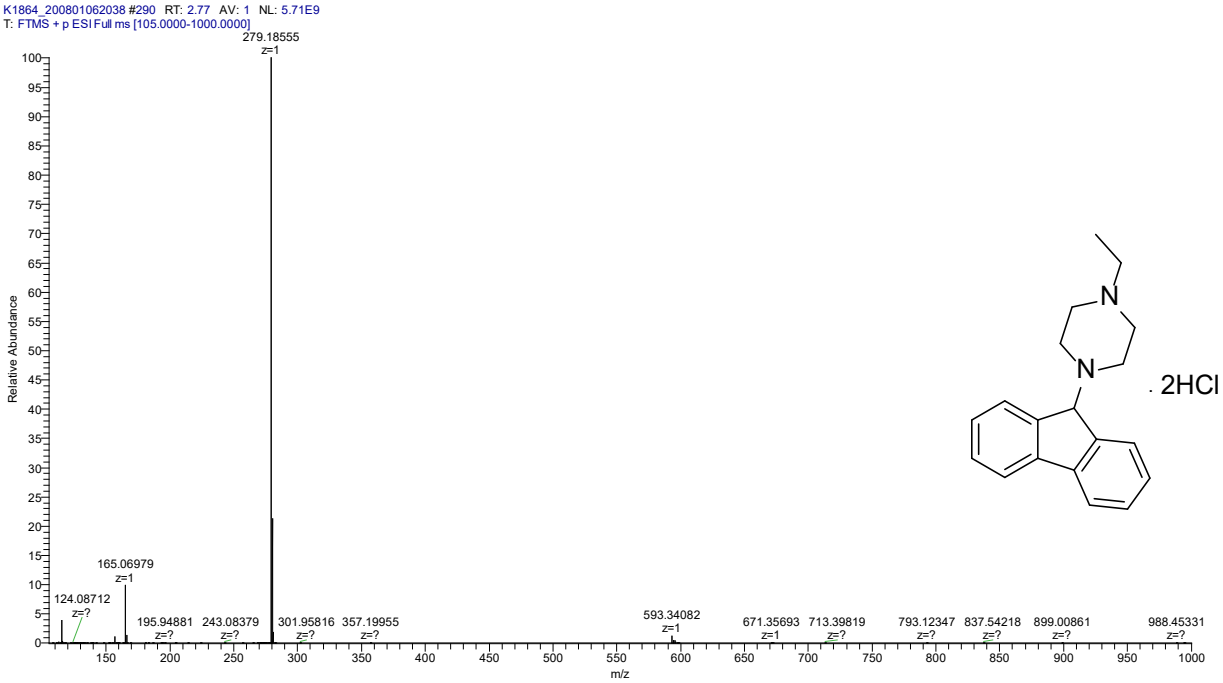
MS spectrum of 1-(9*H*-fluorene-9-yl)-4-methylpiperazine hydrochloride (3j) at Rₜ: 2.75 min:



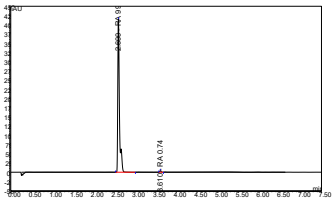
HPLC chromatogram (UV 254 nm) of 1-(9*H*-fluorene-9-yl)-4-ethylpiperazine hydrochloride (3k):



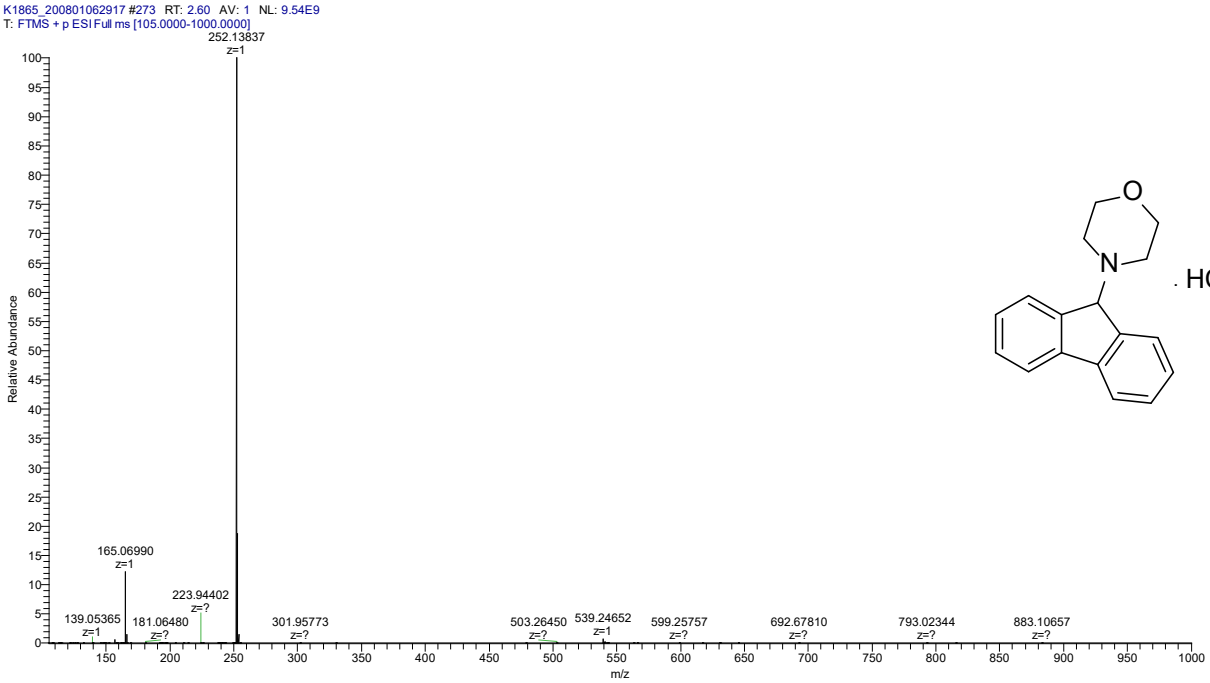
MS spectrum of 1-(9*H*-fluorene-9-yl)-4-ethylpiperazine hydrochloride (3k) at R_t: 2.77 min:



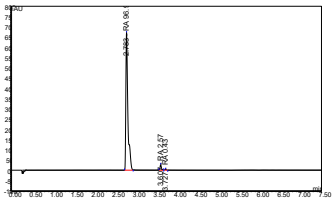
HPLC chromatogram (UV 254 nm) of 4-(9*H*-fluorene-9-yl)morpholine hydrochloride (3l):



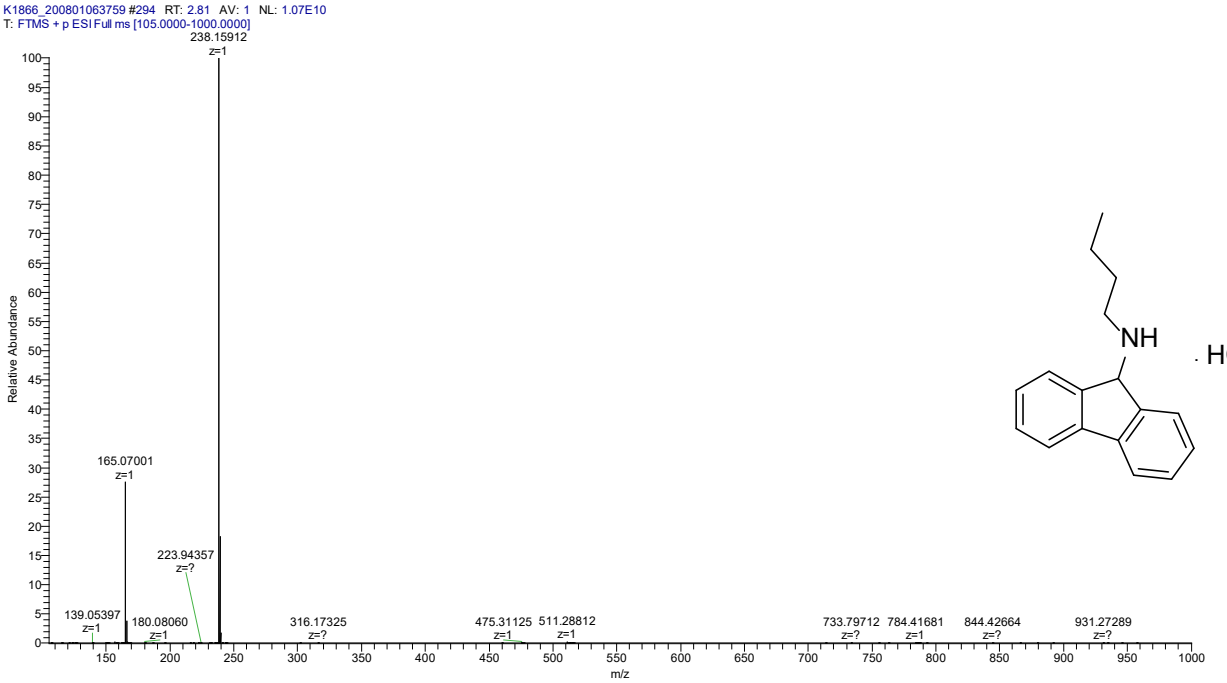
MS spectrum of 4-(9*H*-fluorene-9-yl)morpholine hydrochloride (3l) at R_t: 2.60 min:



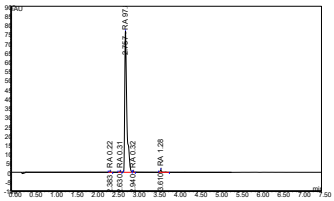
HPLC chromatogram (UV 254 nm) of *N*-butyl-9*H*-fluoren-9-amine hydrochloride (3m):



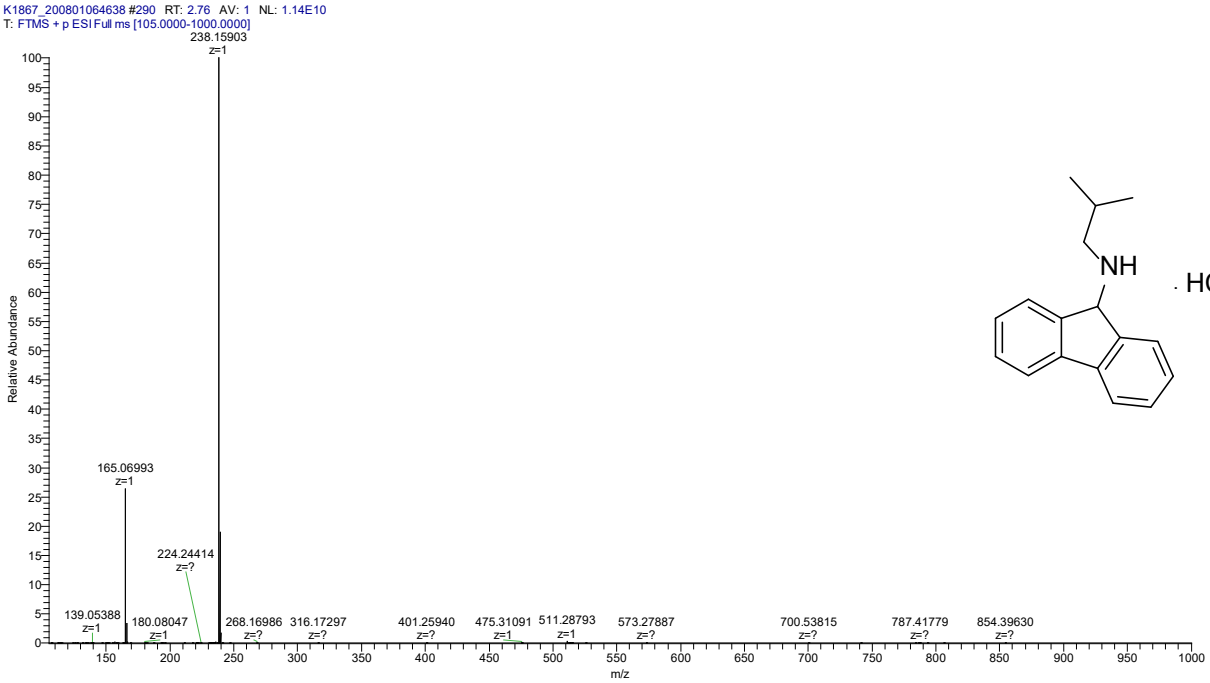
MS spectrum of *N*-butyl-9*H*-fluoren-9-amine hydrochloride (3m) at R_t: 2.81 min:



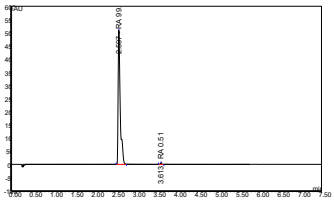
HPLC chromatogram (UV 254 nm) of *N*-(2-methylpropyl)-9*H*-fluoren-9-amine hydrochloride (3n):



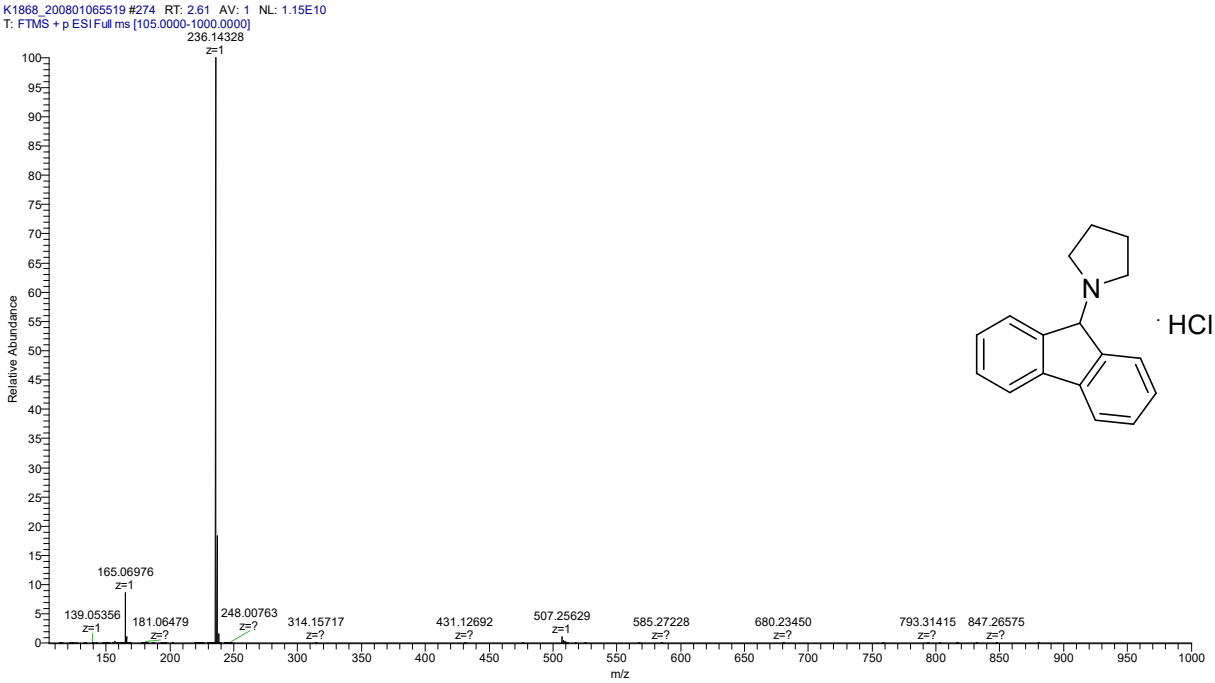
MS spectrum of *N*-(2-methylpropyl)-9*H*-fluoren-9-amine hydrochloride (3n) at R_t: 2.76 min:



HPLC chromatogram (UV 254 nm) of 1-(9*H*-fluoren-9-yl)pyrrolidine hydrochloride (3o):



MS spectrum of 1-(9*H*-fluoren-9-yl)pyrrolidine hydrochloride (3o) at R_t: 2.61 min:



5. References

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