

SUPPORTING INFORMATION

Synthesis and pharmacological characterization of conformationally restricted retigabine analogues as novel neuronal Kv7 channels activators

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1. Supplementary schemes and figures:

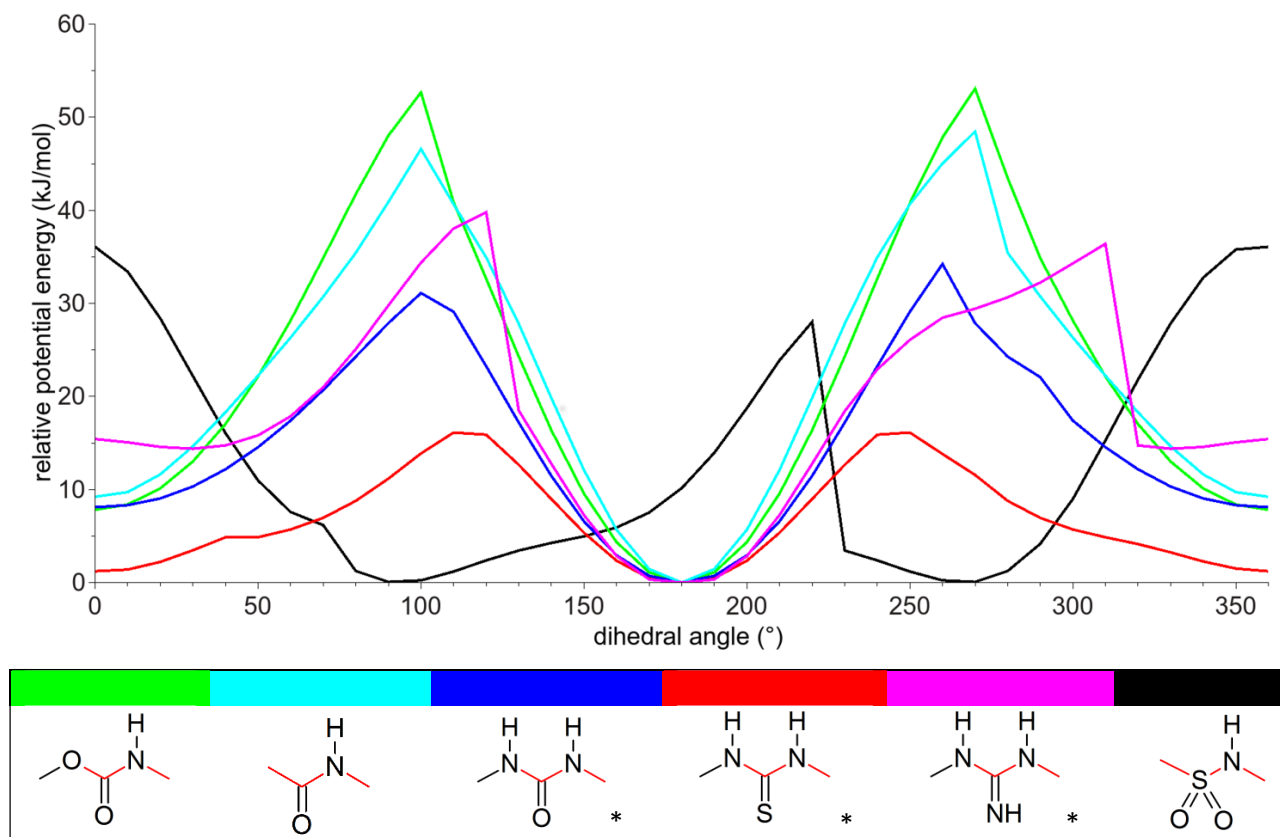


Figure S1. Relative potential energies for the rotation of the bond connecting the hydrogen bond donor group to the hydrogen bond acceptor one, according to the OPLS3 forcefield. Scanned dihedral angles are red-highlighted in the legend. *For these molecules, due to the presence of a second NH group, a two-coordinate scan was employed. (see materials and methods)

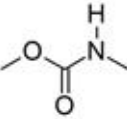
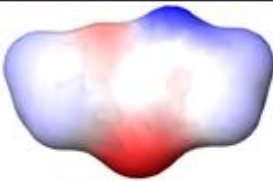
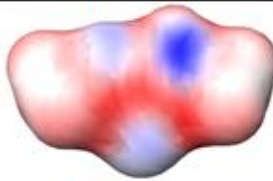
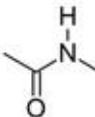
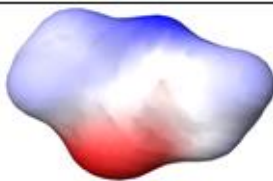
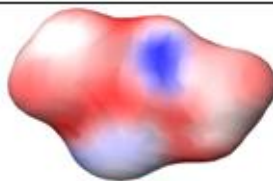
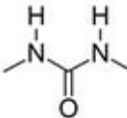
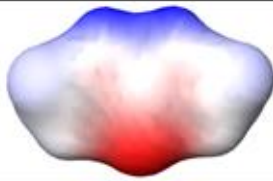
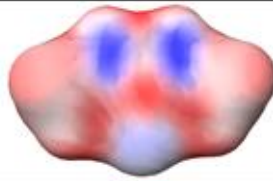
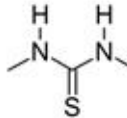
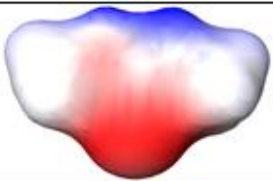
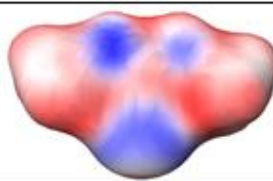
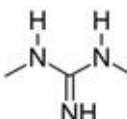
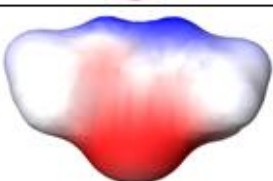
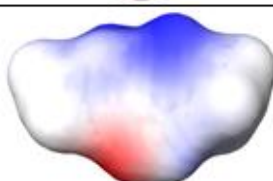
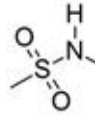
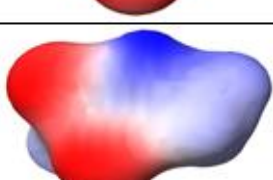
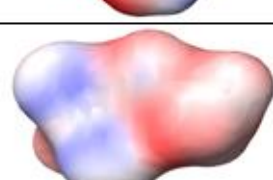
Linker moiety	Electrostatic potentials ^a	Electron densities ^b
		
		
		
		
		
		

Table S1. Linker moieties connecting the 5-amino group to R1 group. ^aRelative electrostatic potential surfaces. Relative potentials are color coded by a red-white-blue color ramp, from the most negative to the most positive value. ^bRelative electron densities surfaces. Relative densities are color coded by a red-white-blue color ramp, from to lowest to the highest electron density.

Table S2: Effect of tested compounds on ramp-evoked currents of Kv 7.2

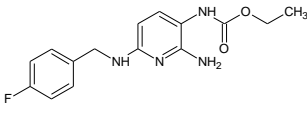
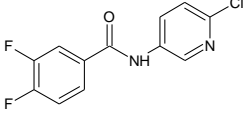
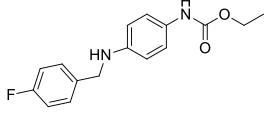
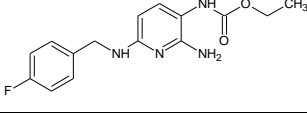
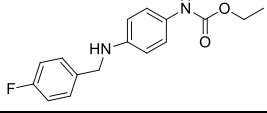
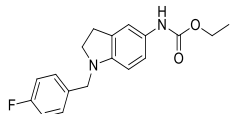
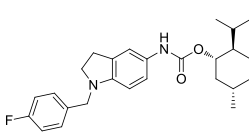
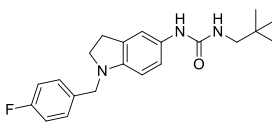
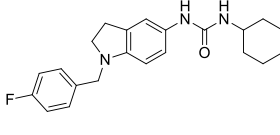
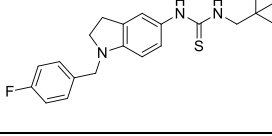
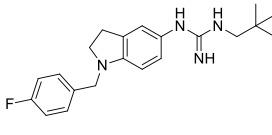
NAME	STRUCTURE	I_{drug}/I_{ctl} at -40 mV	I_{drug}/I_{ctl} at 0 mV	N
Flupirtine		2.47±0.23	1.24±0.08	5
ICA27243		8.97±0.89	2.84±0.34	5
Retigabine (1 µM)		3.96±0.51	1.82±0.16	11
Flupirtine		2.47±0.23	1.24±0.08	5
Retigabine		6.20±1.03	1.95±0.12	38
4a		2.01±0.58	1.26±0.13	6
4b		0.80±0.14	0.48±0.07	5
5a		3.31±0.61	1.40±0.08	6
5b		0.91±0.21	0.81±0.11	5
6		1.77±0.77	1.04±0.24	3
7		0.98±0.09	0.84±0.09	3

Table S2: continue

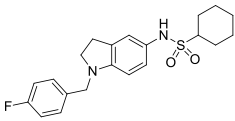
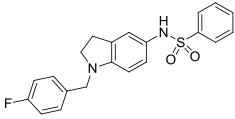
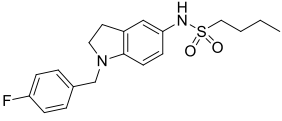
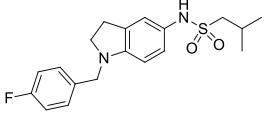
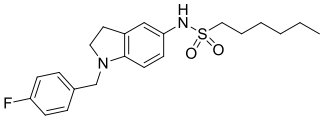
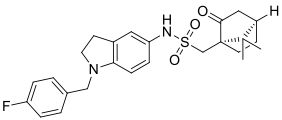
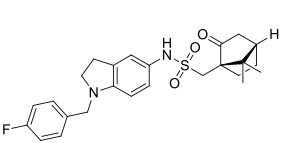
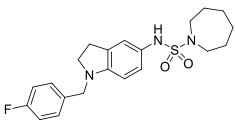
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8b		1.15±0.28	0.64±0.14	3
8c		2.03±0.19	1.55±0.12	5
8d		2.55±0.79	1.93±0.32	5
8e		2.48±0.20	2.18±0.09	3
8f		1.12±0.05	0.99±0.06	3
8g		1.49±0.28	1.16±0.07	5
8h		0.89±0.12	0.98±0.11	5

Table S2: continue

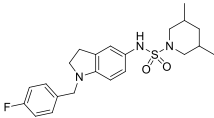
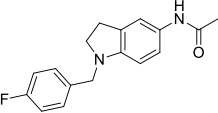
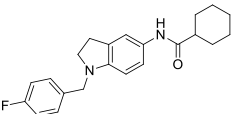
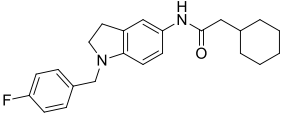
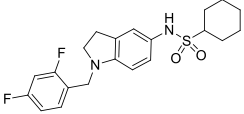
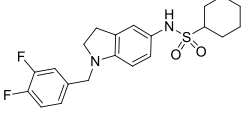
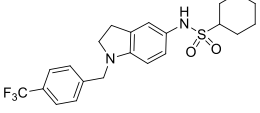
8i		1.91±0.44	1.87±0.22	5
9a		1.88±0.14	1.64±0.20	4
9b		1.32±0.21	0.66±0.07	5
9c		2.37±0.40	1.71±0.12	4
13a		5.82±1.38	3.79±1.09	3
13b		4.56±1.11	2.11±0.18	3
13c		3.62±0.20	2.37±0.58	3

Table S2: continue

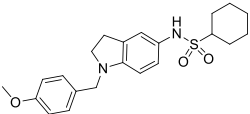
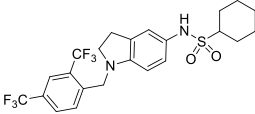
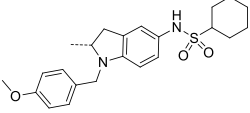
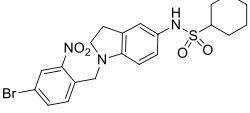
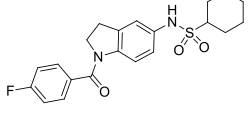
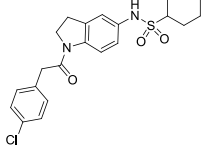
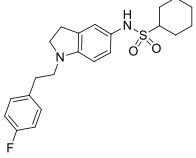
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13e		1.53±0.39	1.22±0.11	5
13f		1.13±0.15	0.92±0.13	3
13g		7.27±1.32	3.09±0.52	4
16a		1.85±0.30	1.22±0.07	4
16b		1.45±0.08	1.18±0.08	3
19		3.24±1.52	1.37±0.17	4

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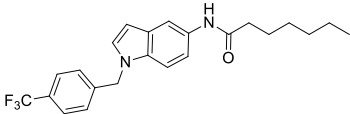
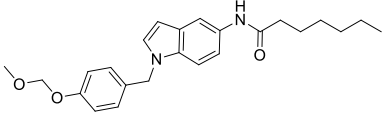
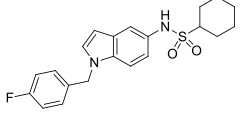
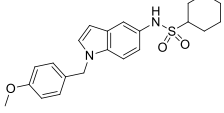
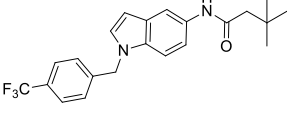
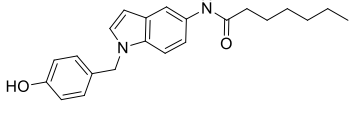
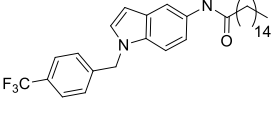
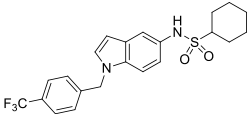
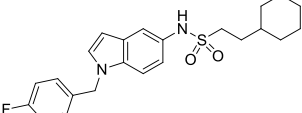
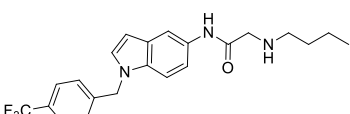
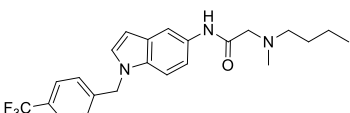
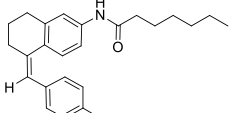
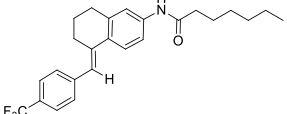
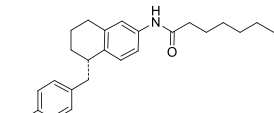
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23b		6.31±1.89	2.20±0.12	4
23c		4.71±1.31	2.76±0.19	7
23d		2.00±0.36	1.63±0.06	4
24a		8.23±1.36	3.54±0.88	3
24b		4.50±1.20	2.72±0.92	4
25a		1.29±0.18	1.02±0.05	3

Table S2: continue

26a		2.27±0.49	1.93±0.19	3
27a		0.94±0.16	0.8±0.13	3
28a		1.31±0.22	1.10±0.04	4
29a		1.60±0.30	1.30±0.11	4
33a		1.90±0.40	1.43±0.16	3
33b		1.34±0.47	0.95±0.15	3
34		1.67±0.18	1.04±0.05	3

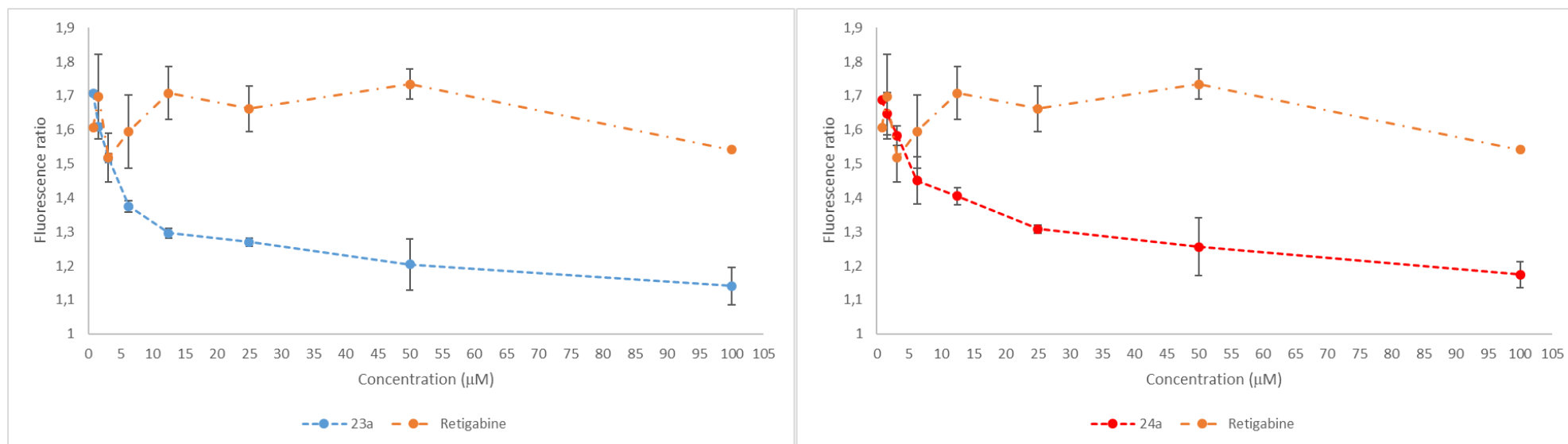


Figure S2. Fluorescence intensity ratio at two different wavelengths (I=372 nm and III=383 nm) for **23a**, **24a** and retigabine using pyrene as fluorescent probe.

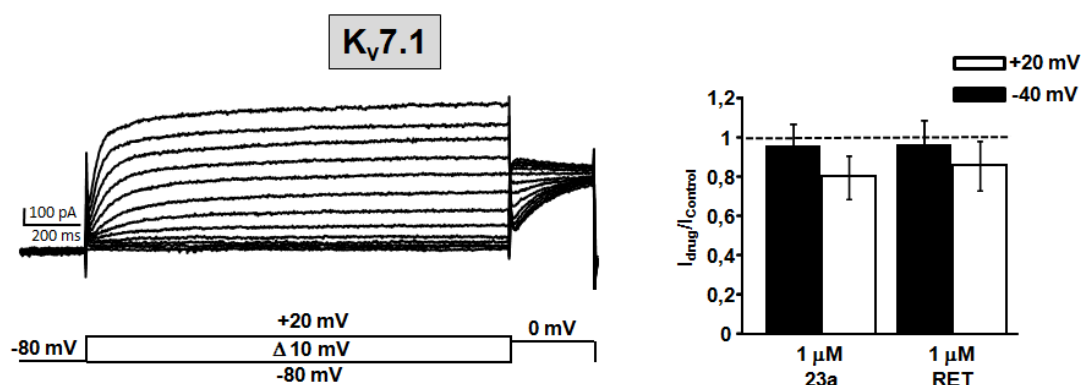


Figure S3. Effect of retigabine and **23a** on homomeric Kv7.1 channels

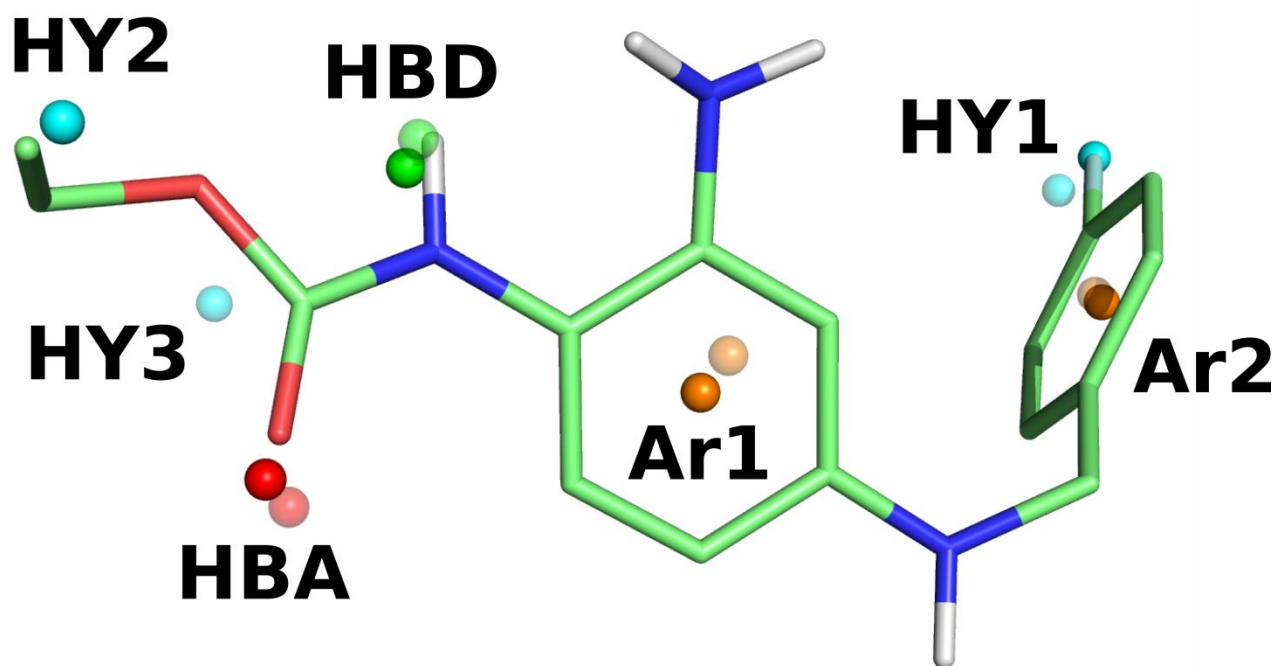


Figure S4. Superimposition of models B (solid spheres) and C (transparent spheres). Pharmacophore features are color-coded as follows: Hydrophobic (HY – cyan), Aromatic (Ar – orange), Hydrogen Bond Donor (HBD – green), Hydrogen Bond Donor (HBD – green). Retigabine is depicted in green sticks as reference.

<i>Molecule</i>	<i>Model A</i>	<i>Model B</i>	<i>Model C</i>
8a	X		X
13a	X		X
13b	X		X
13d	X		X
13g	X		X
23a	X	X	
23c	X		X
24a	X	X	
Retigabine	X	X	
23b	X	X	X
24b	X	X	X
25a	X	X	X
27a	X	X	X
28a	X	X	X
29a	X	X	X
33a	X	X	X
33b	X	X	X
34	X	X	X

Table S3. Training sets of models A, B and C. Active compounds are typed in green, while inactive ones are in red. Crosses indicate whether the compound was used for a model or not.

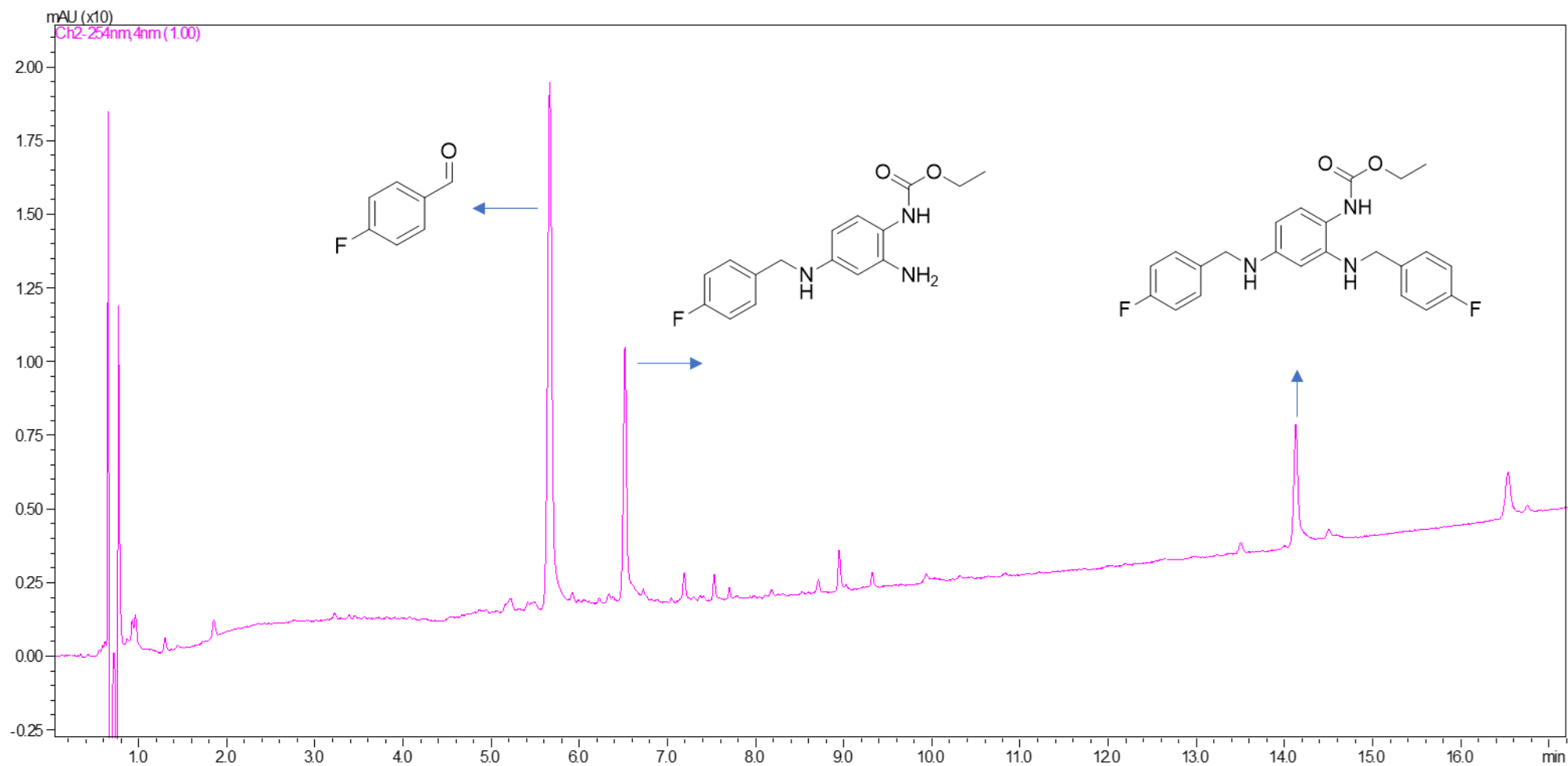


Figure S5. UHPLC profile of a retigabine batch after 6h visible light irradiation

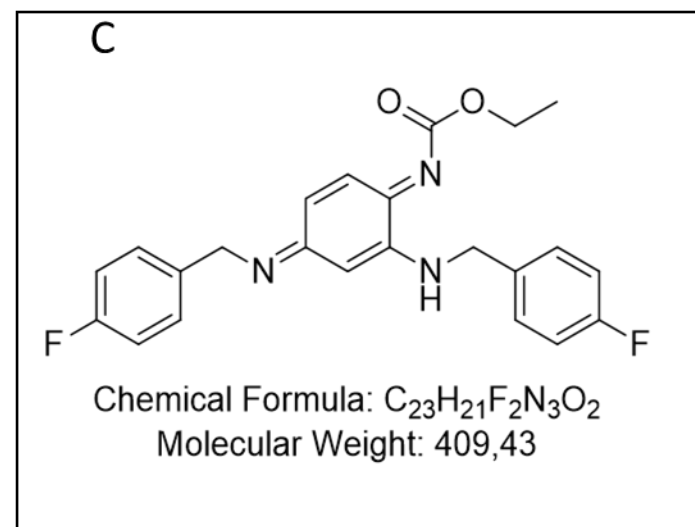
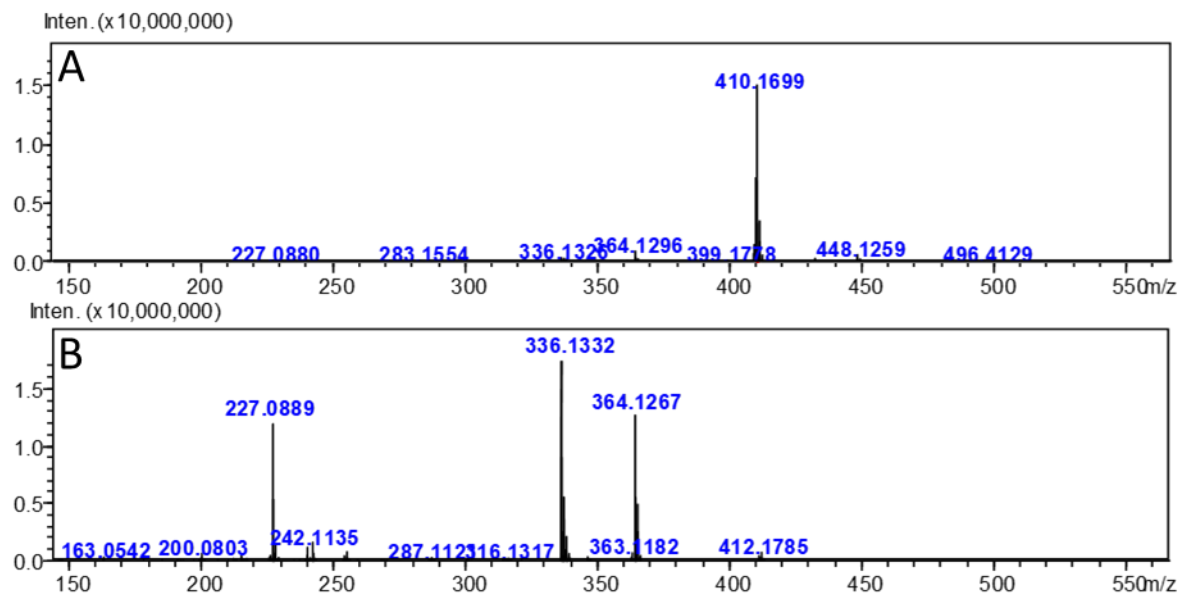


Figure S6. Full scan profile (A), MS/MS profile (B) and predicted structure of retigabine photoxidative byproduct eluting at 14.23 minutes (C).

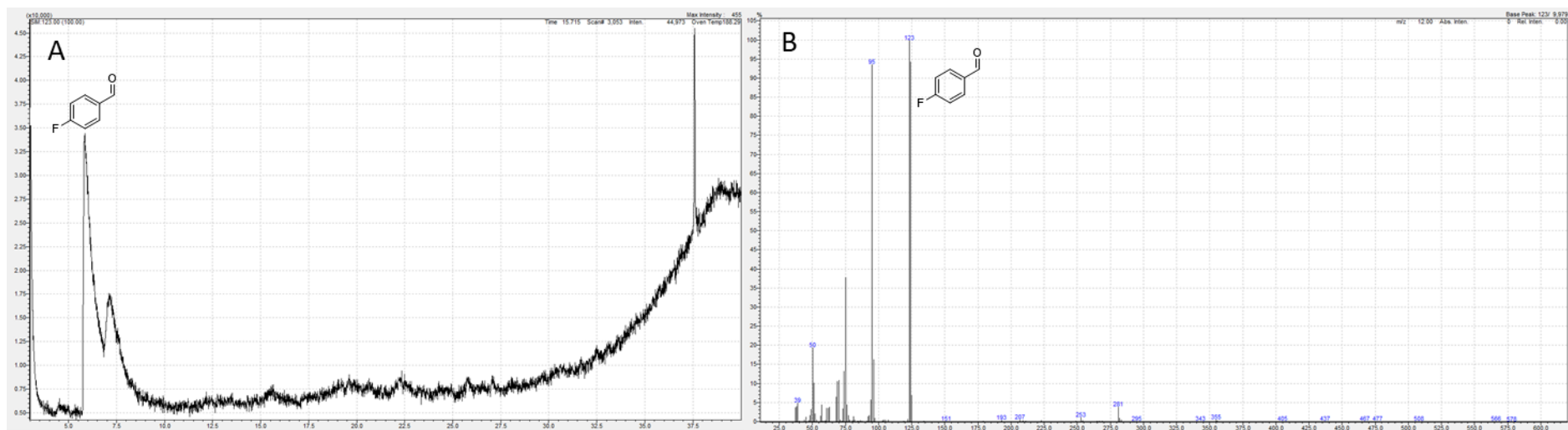


Figure S7. (A) Retigabine GC profile and (B) full scan mass spectra of the main peak (4-fluorobenzaldehyde)

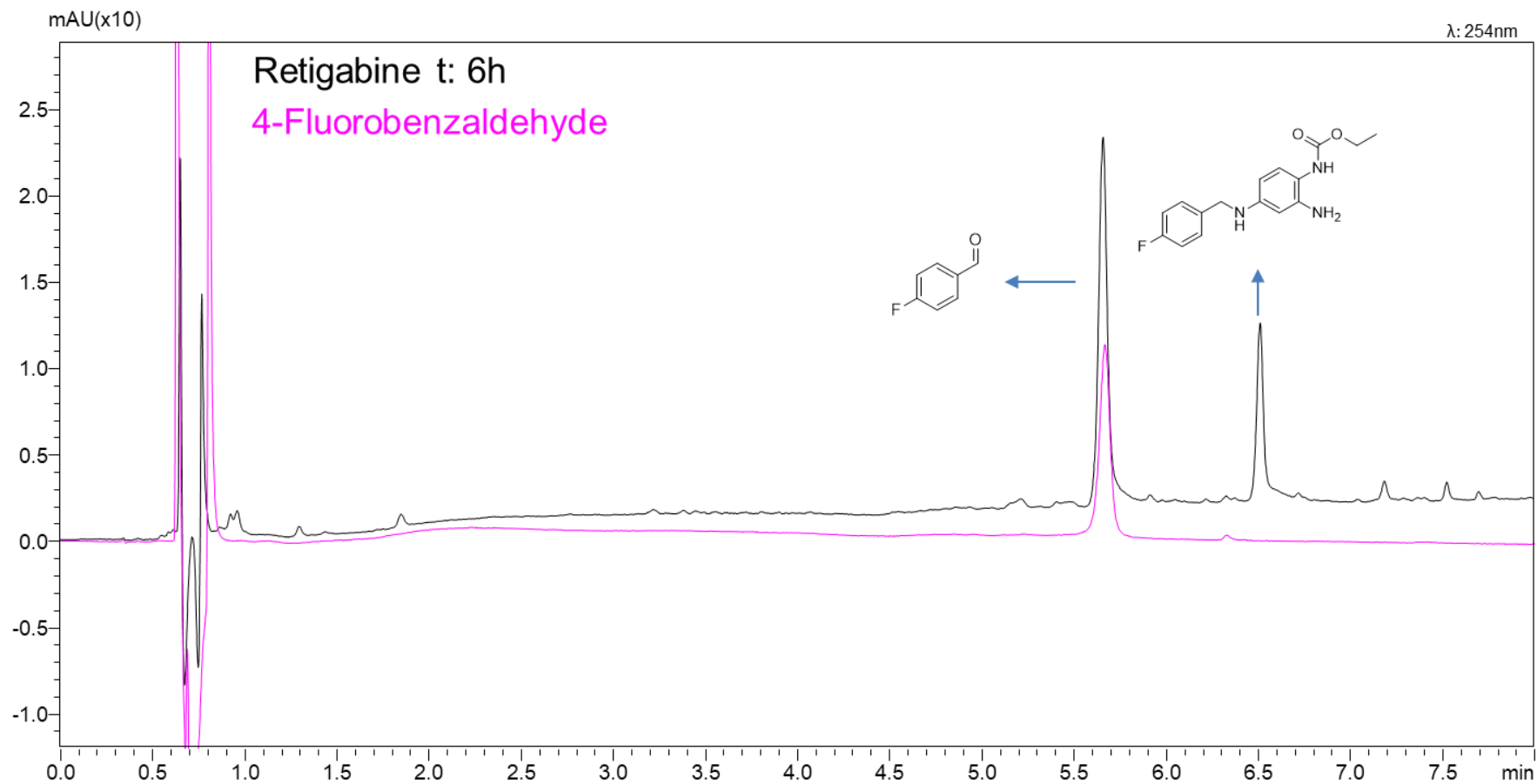


Figure S8. Overlapping HPLC traces of a 4-fluorobenzaldehyde standard solution and a photodegraded retigabine batch

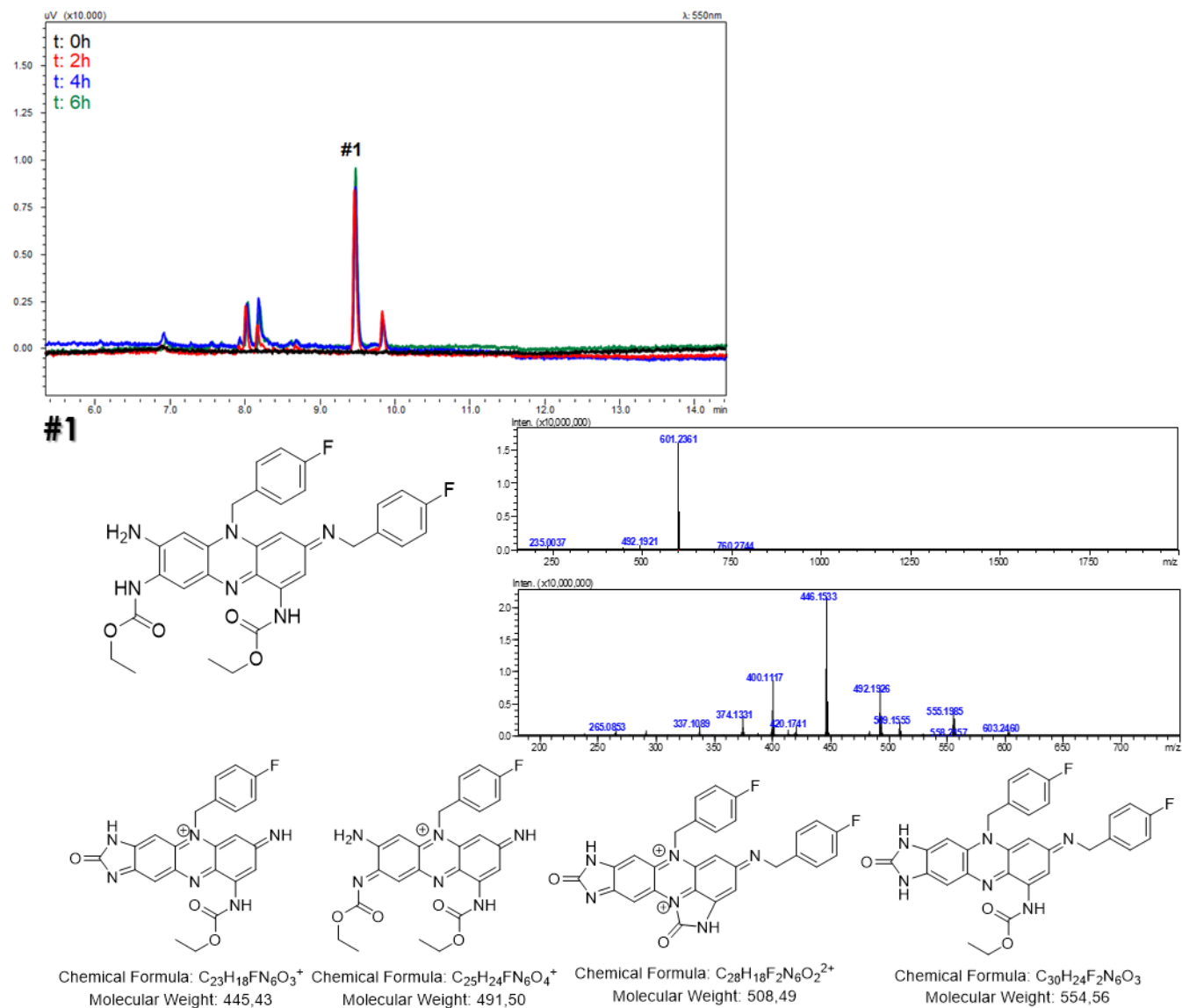
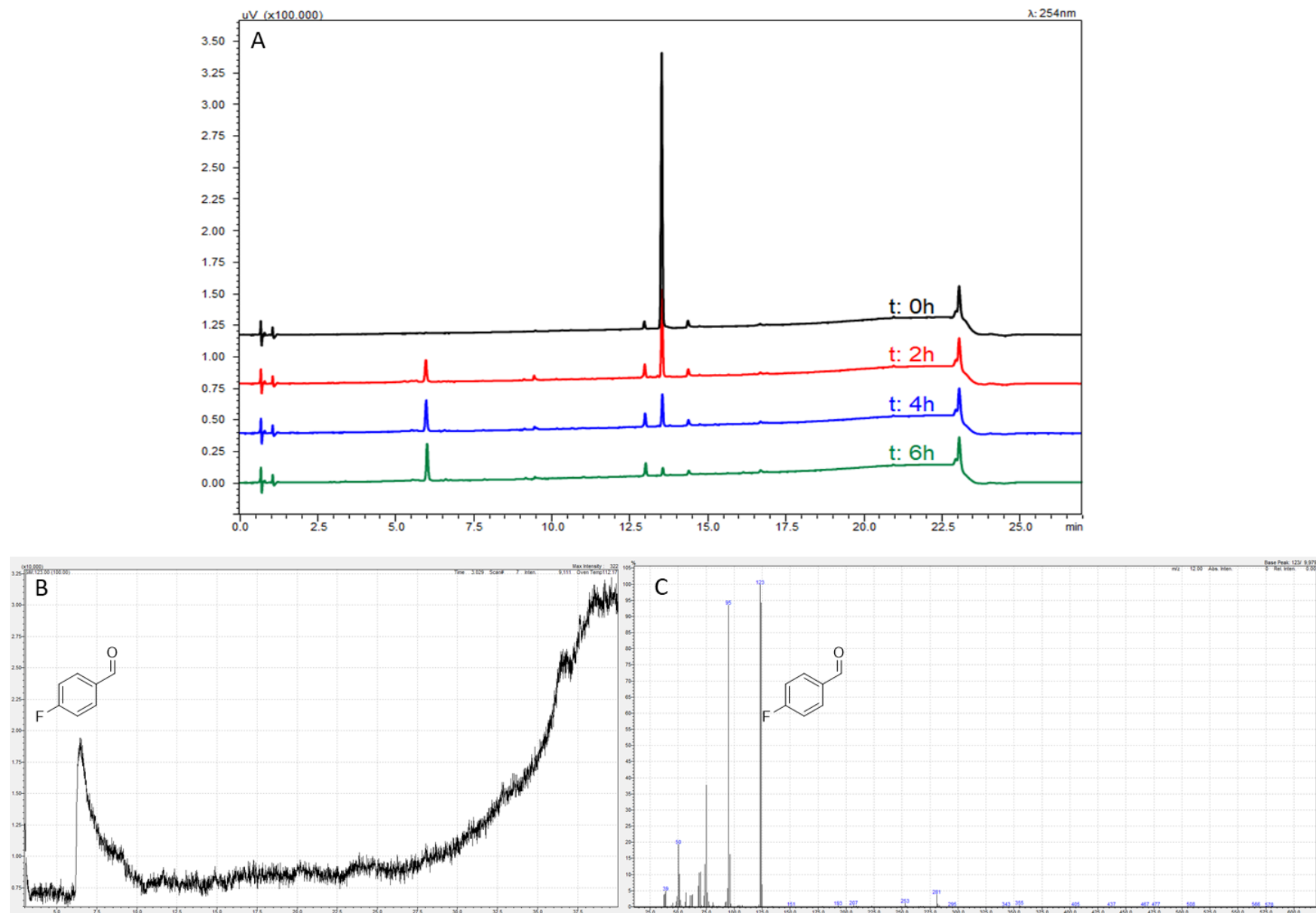


Figure S9. MS/MS profile for unknown compound **#1** and the corresponding putative molecular structures



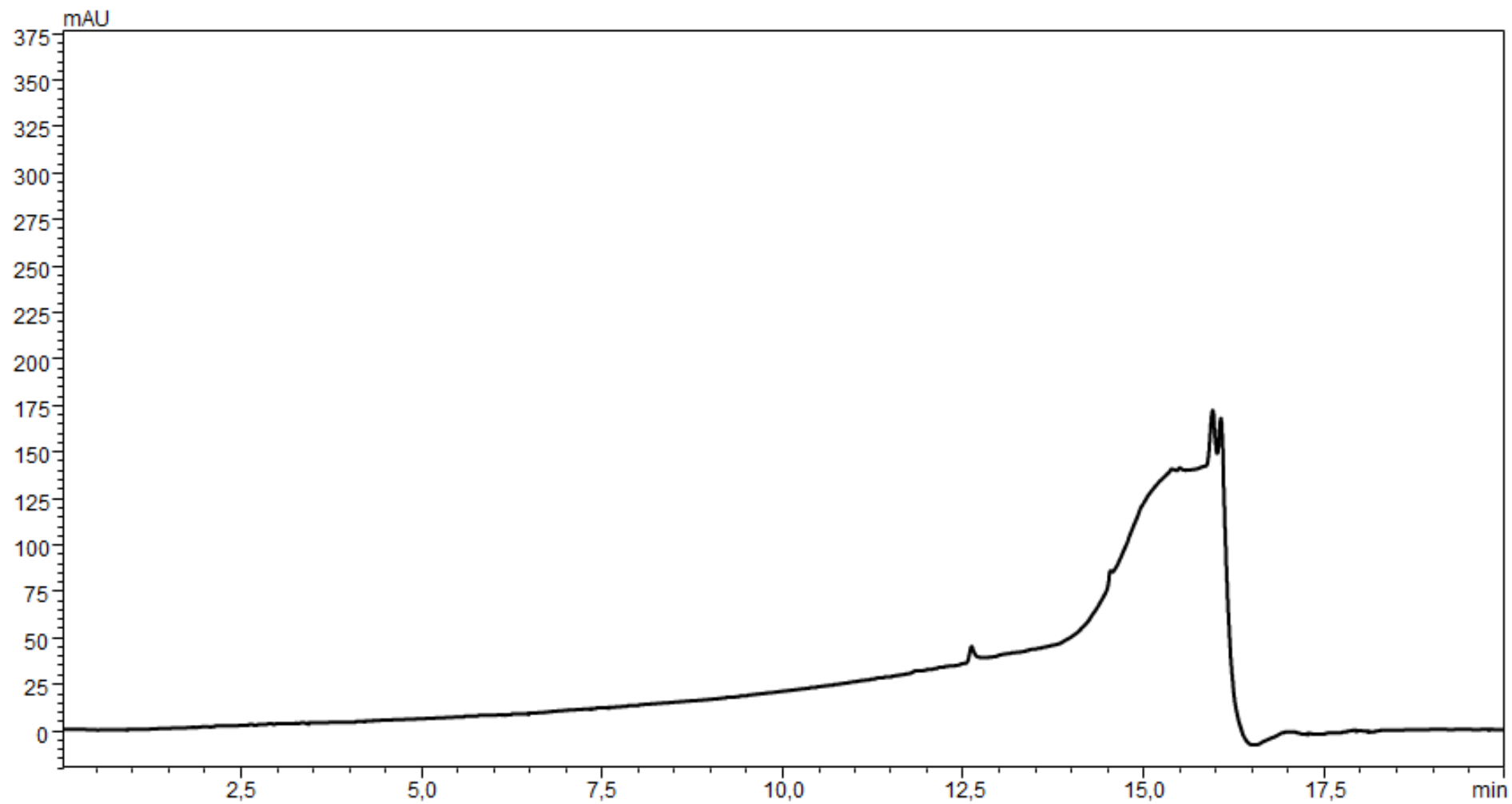


Figure S11. Blank RP-HPLC trace of the analytical method used for purity determination of compounds **4a-b**, **5a-b**, **6-7**, **8a-i**, **9a-c**, **13a-g**, **16a-b**, **19**, **23a-d**, **24a-b**, **25a-29a** and **34**

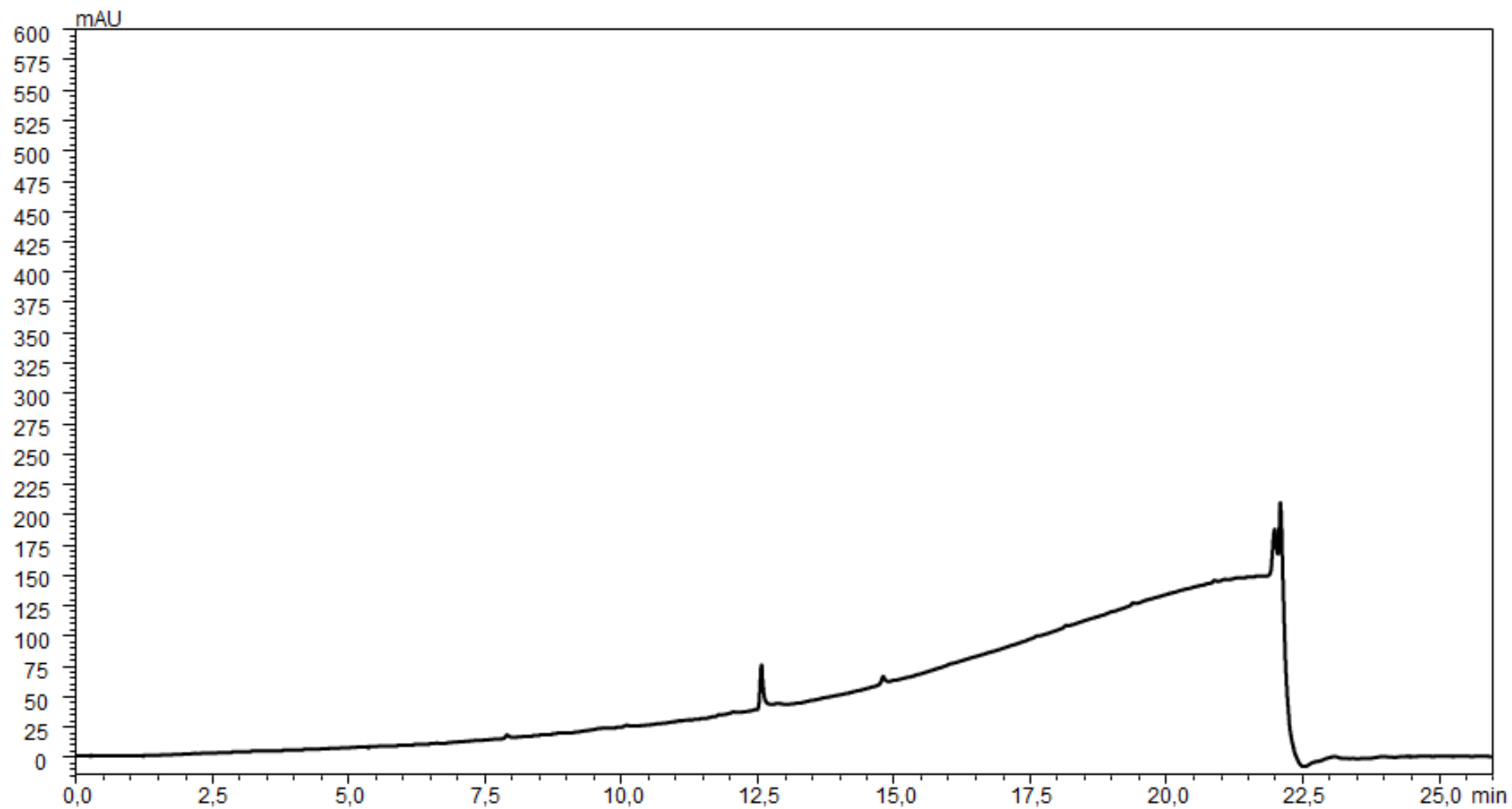
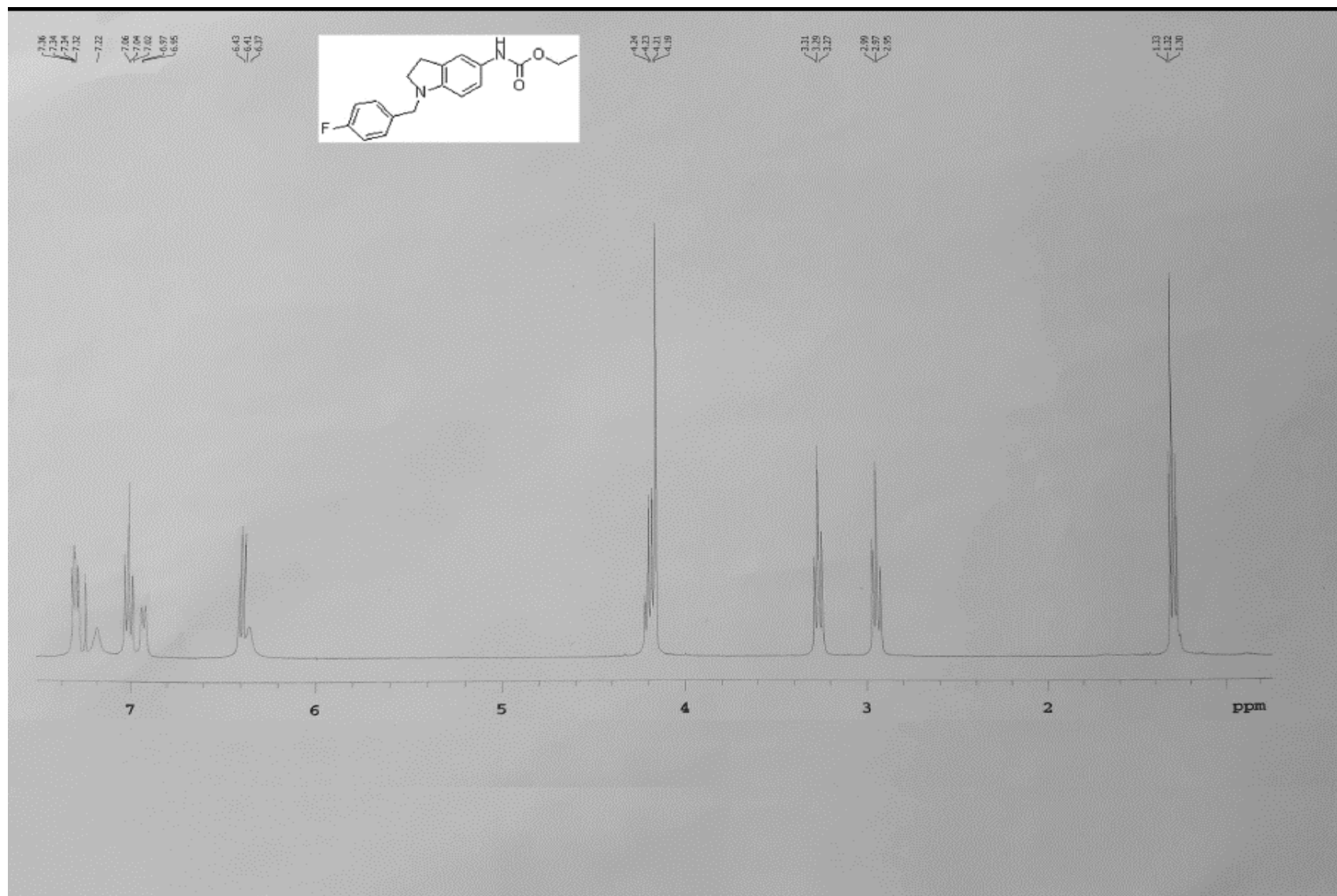
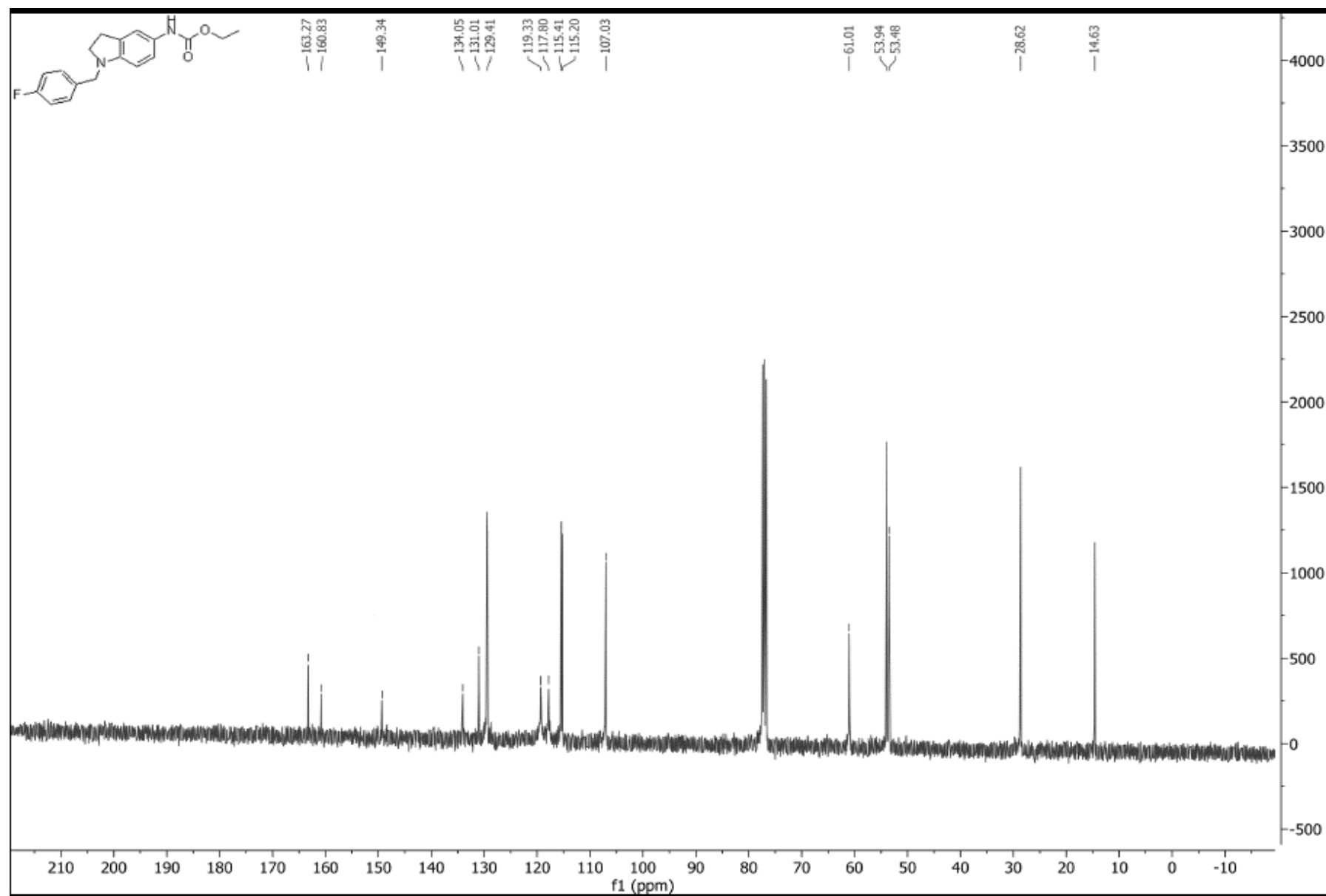


Figure S12. Blank RP-HPLC trace for the analytical method used for purity determination of compounds **33a** and **33b**.

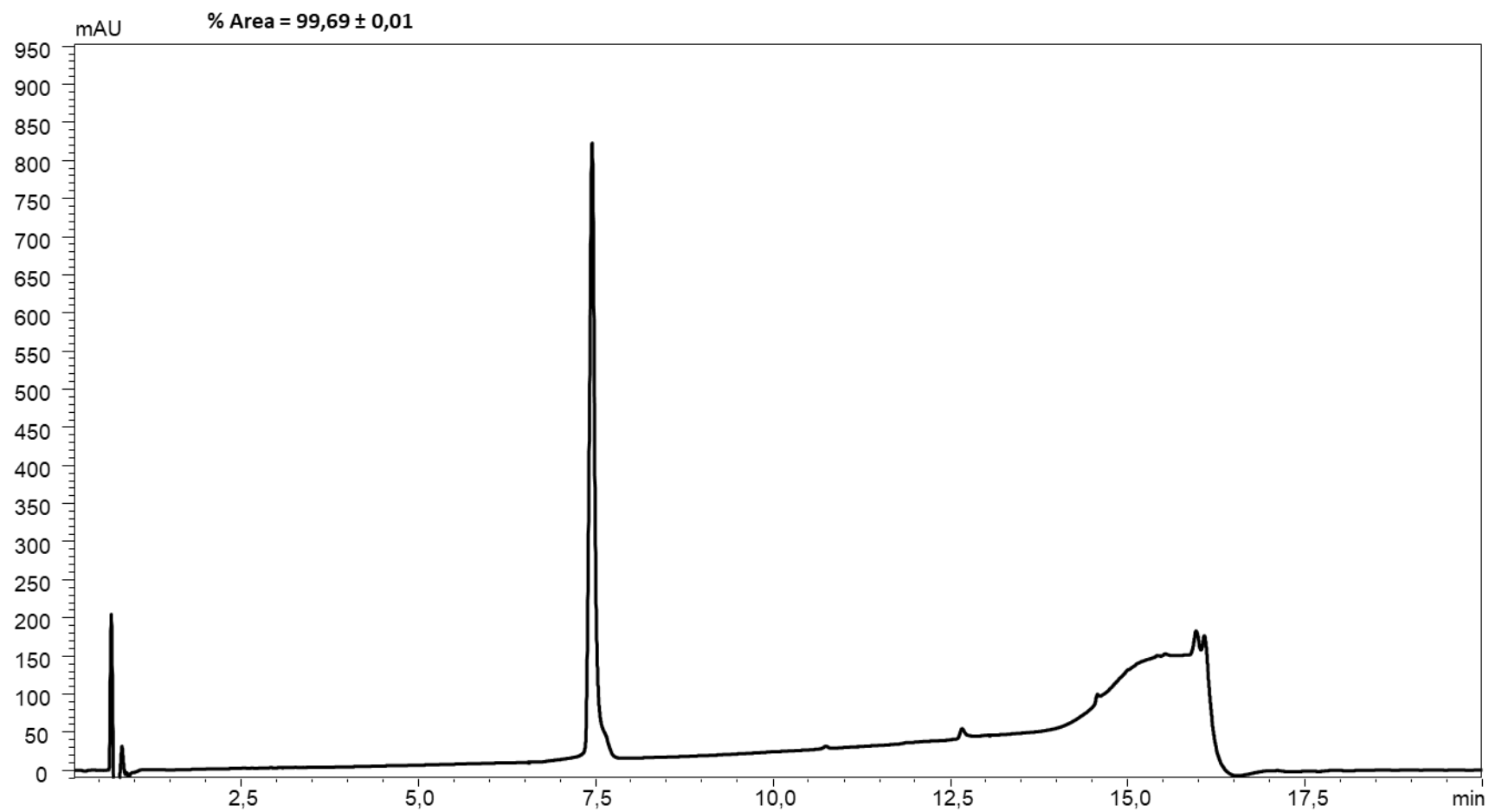
2. NMR spectra and HPLC traces of synthesized compounds



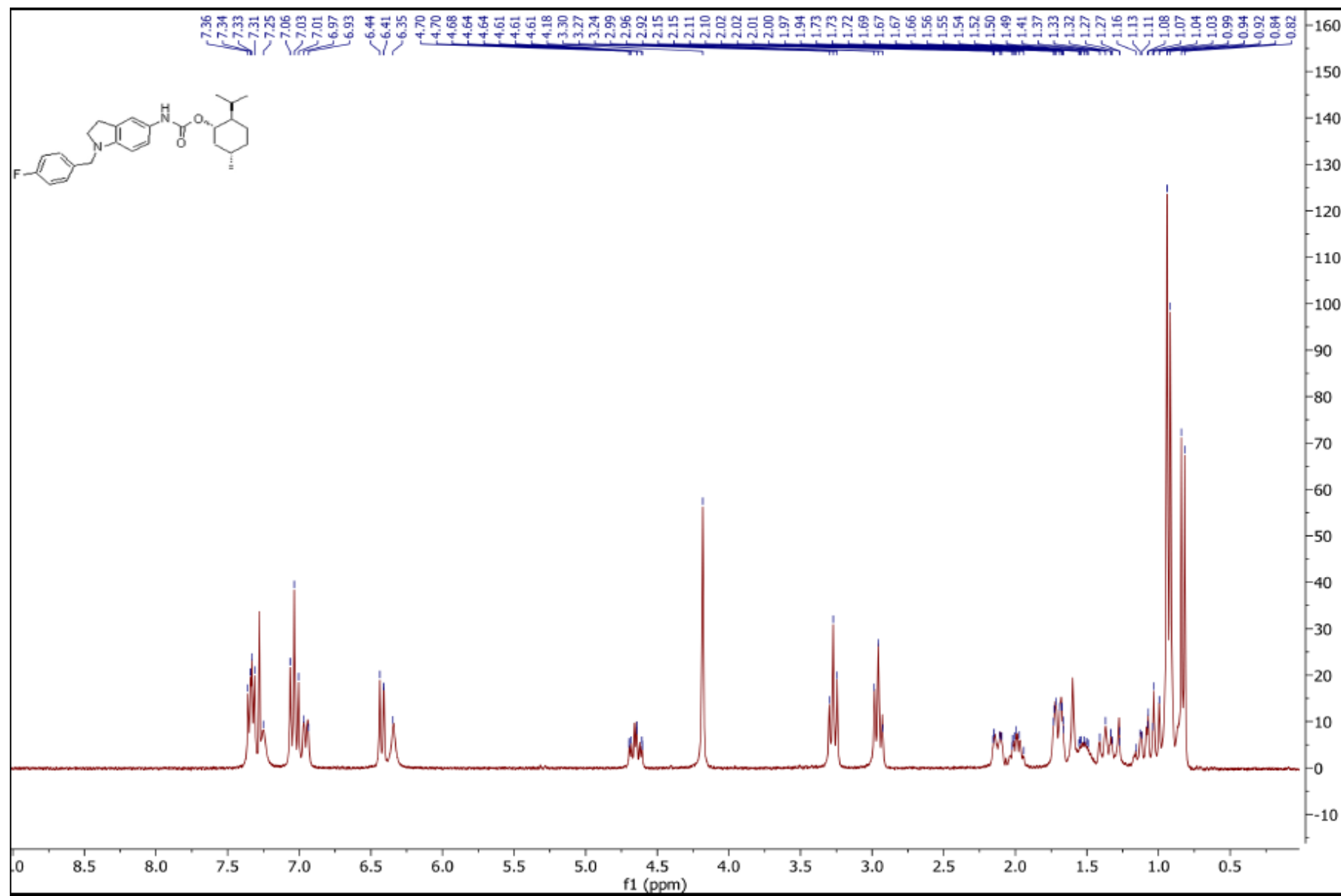
¹H NMR of compound **4a**



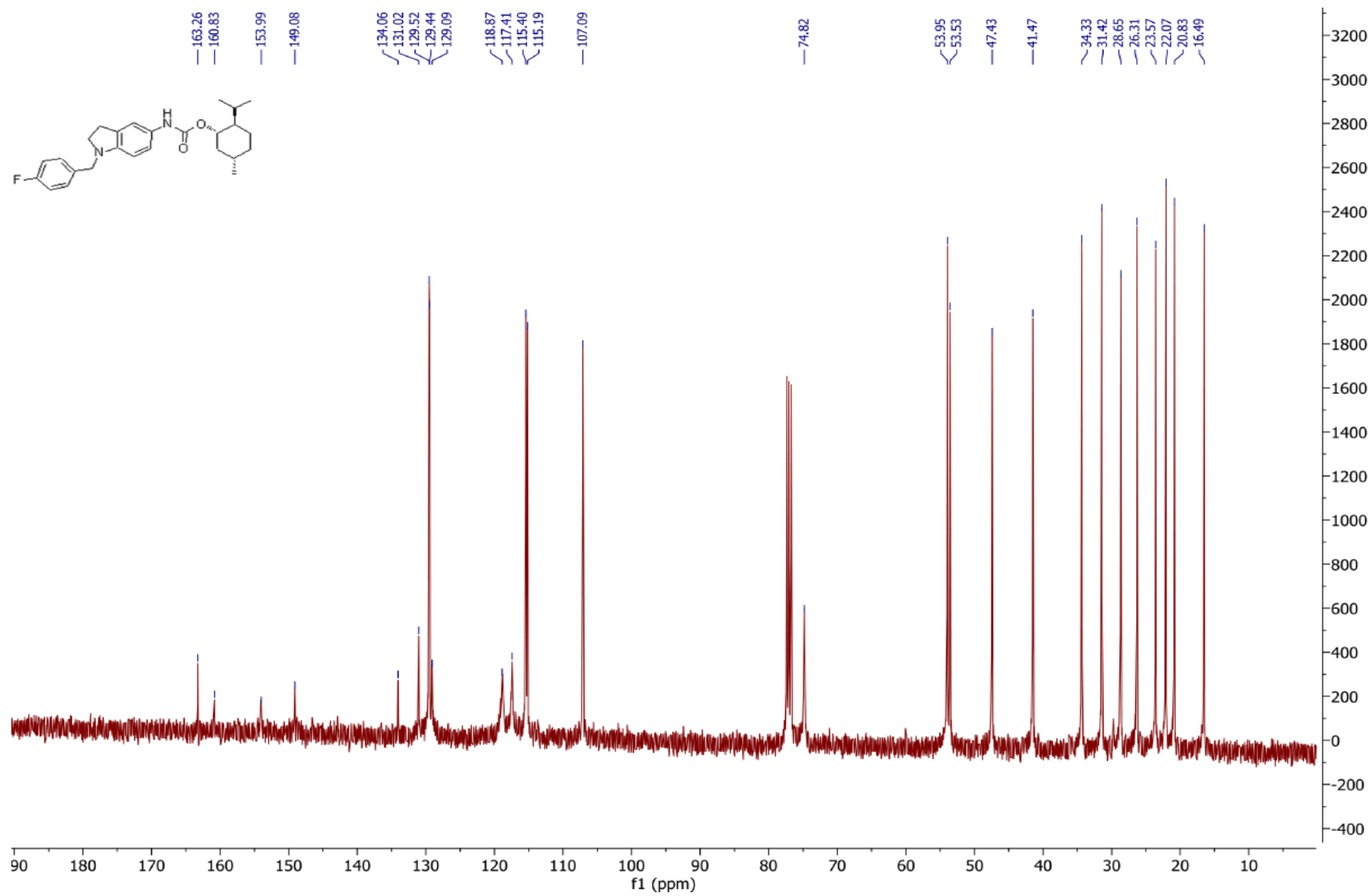
¹³C NMR of compound **4a**



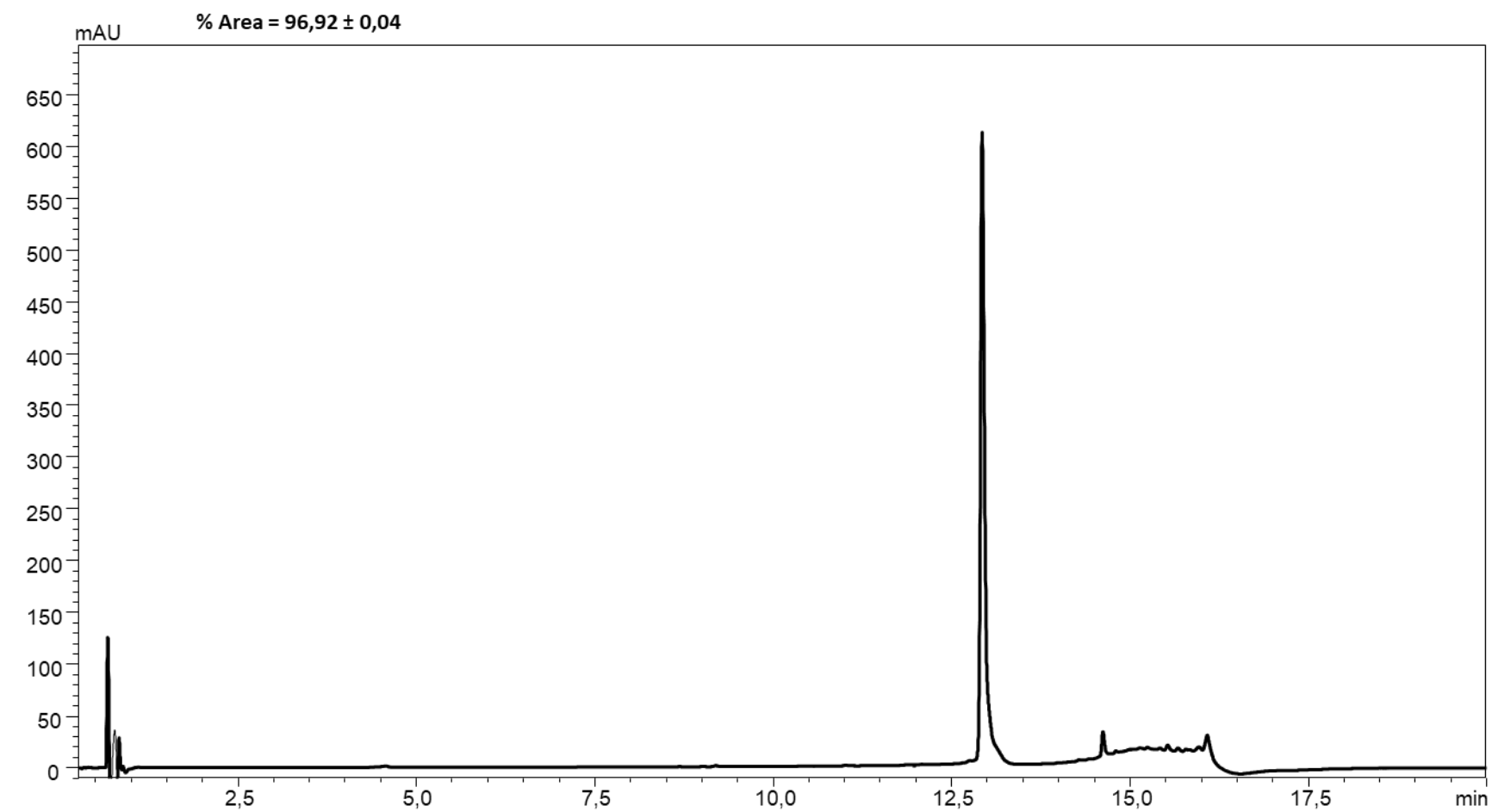
RP-HPLC trace of compound **4a**



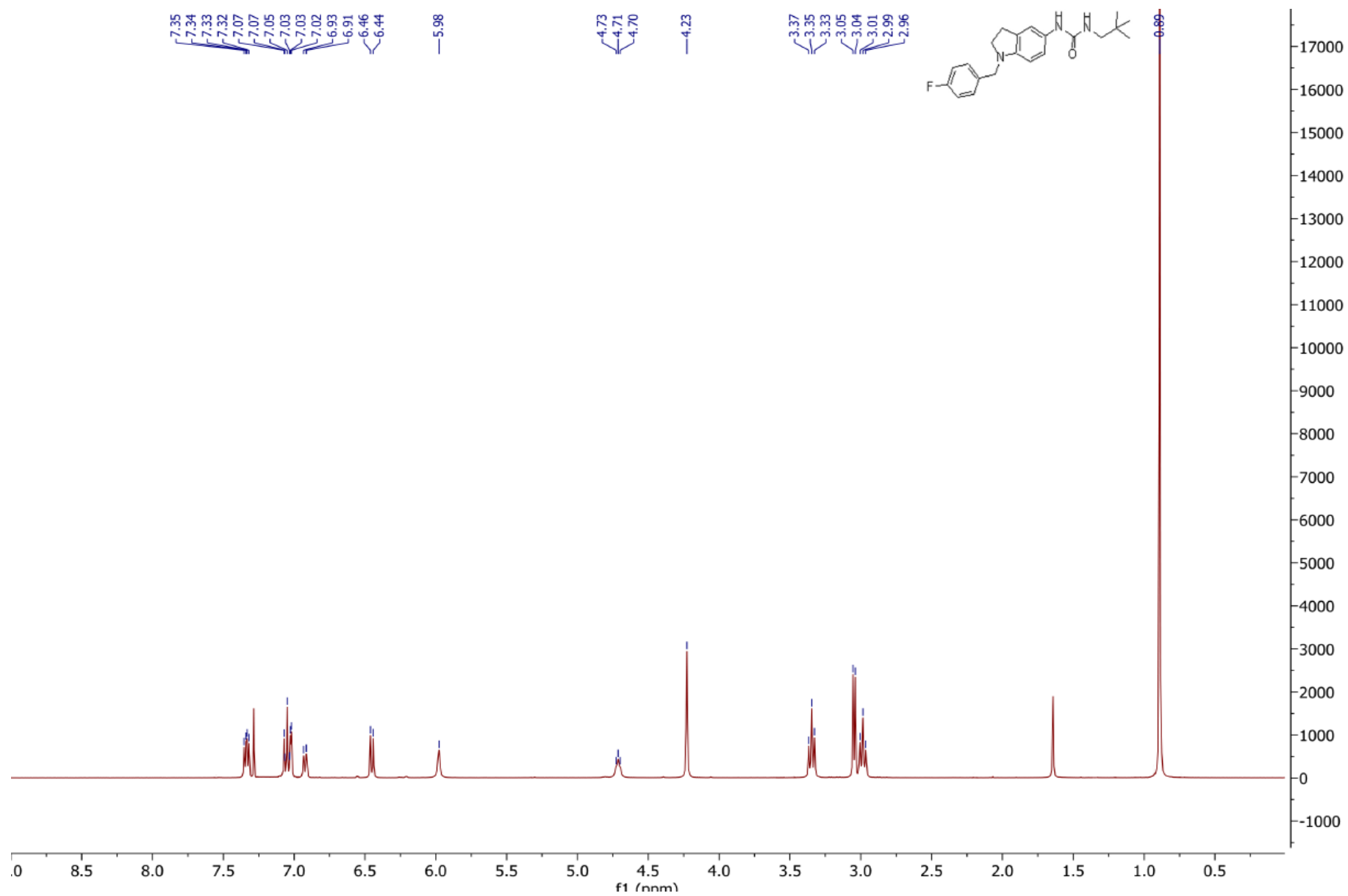
¹H NMR of compound **4b**



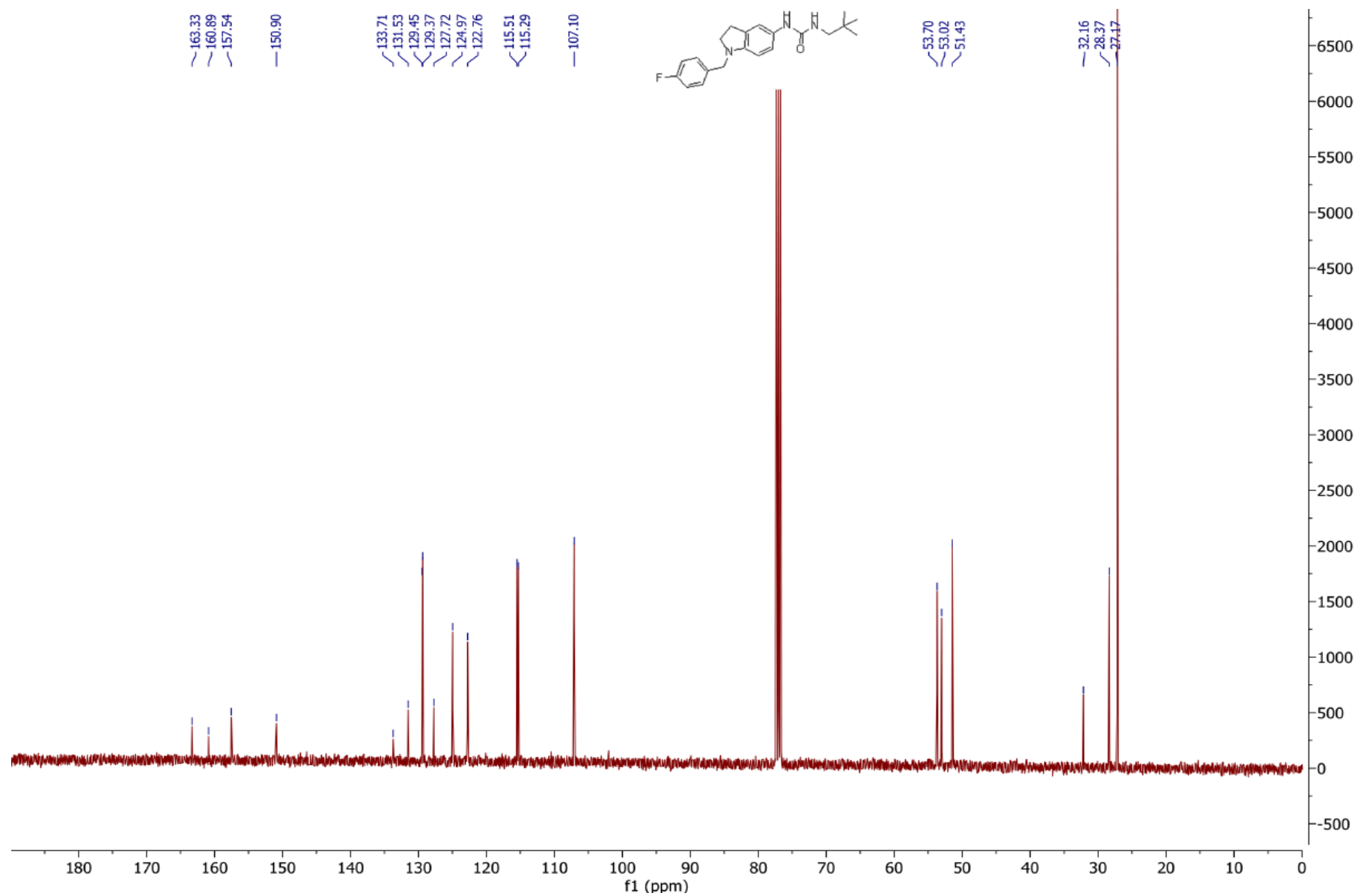
¹³C NMR of compound **4b**



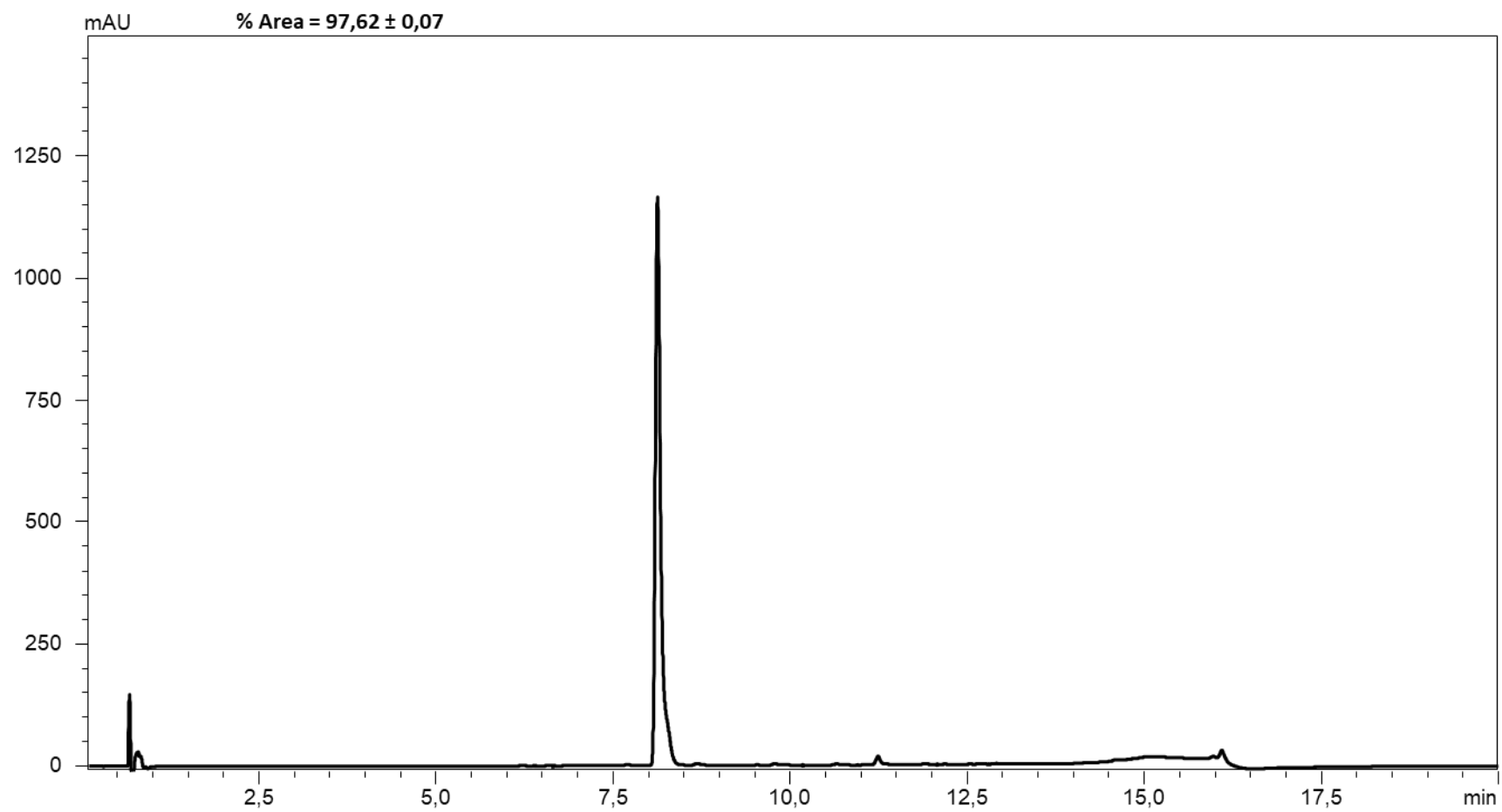
RP-HPLC trace of compound **4b**



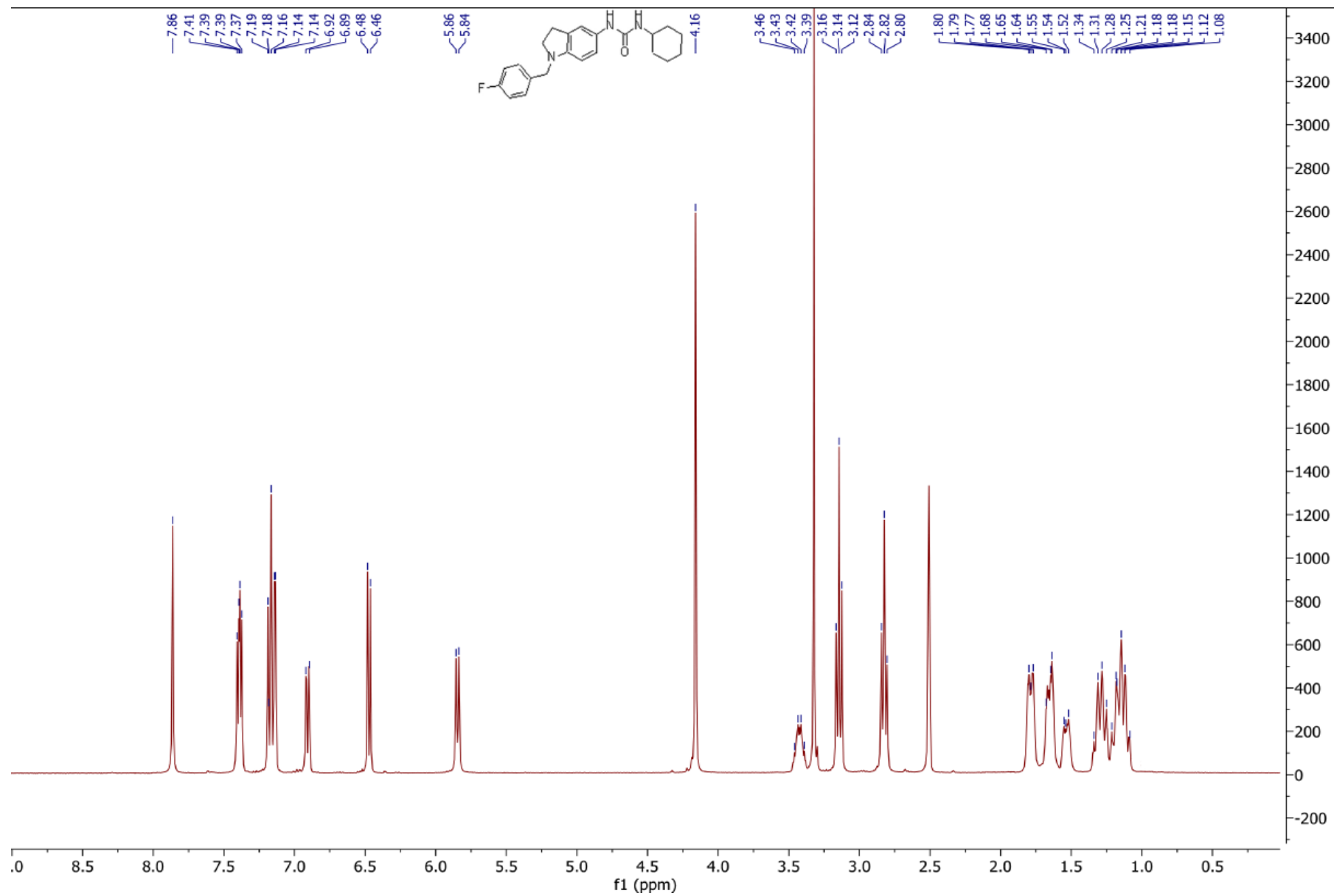
¹H NMR of compound **5a**



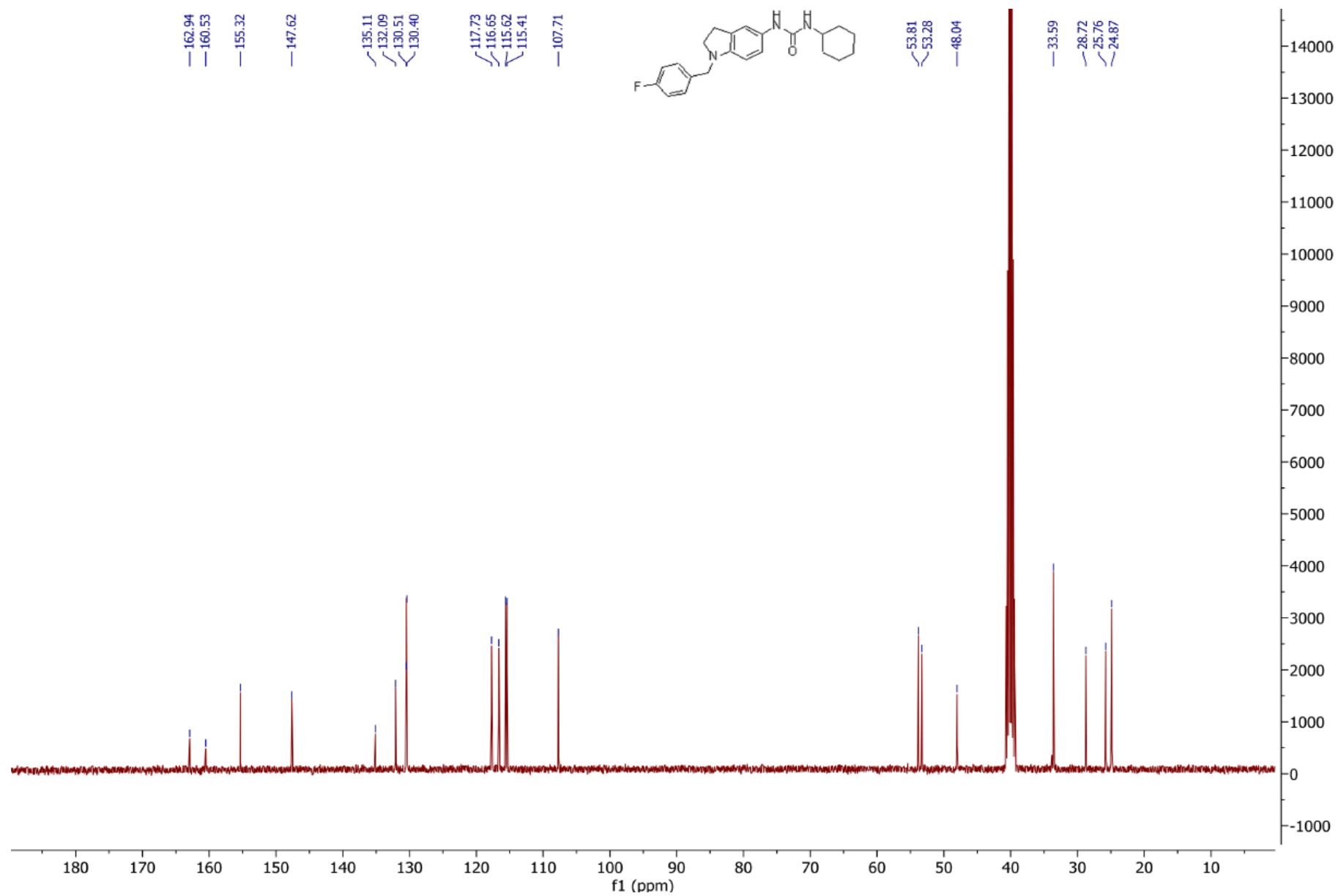
¹³C NMR of compound **5a**



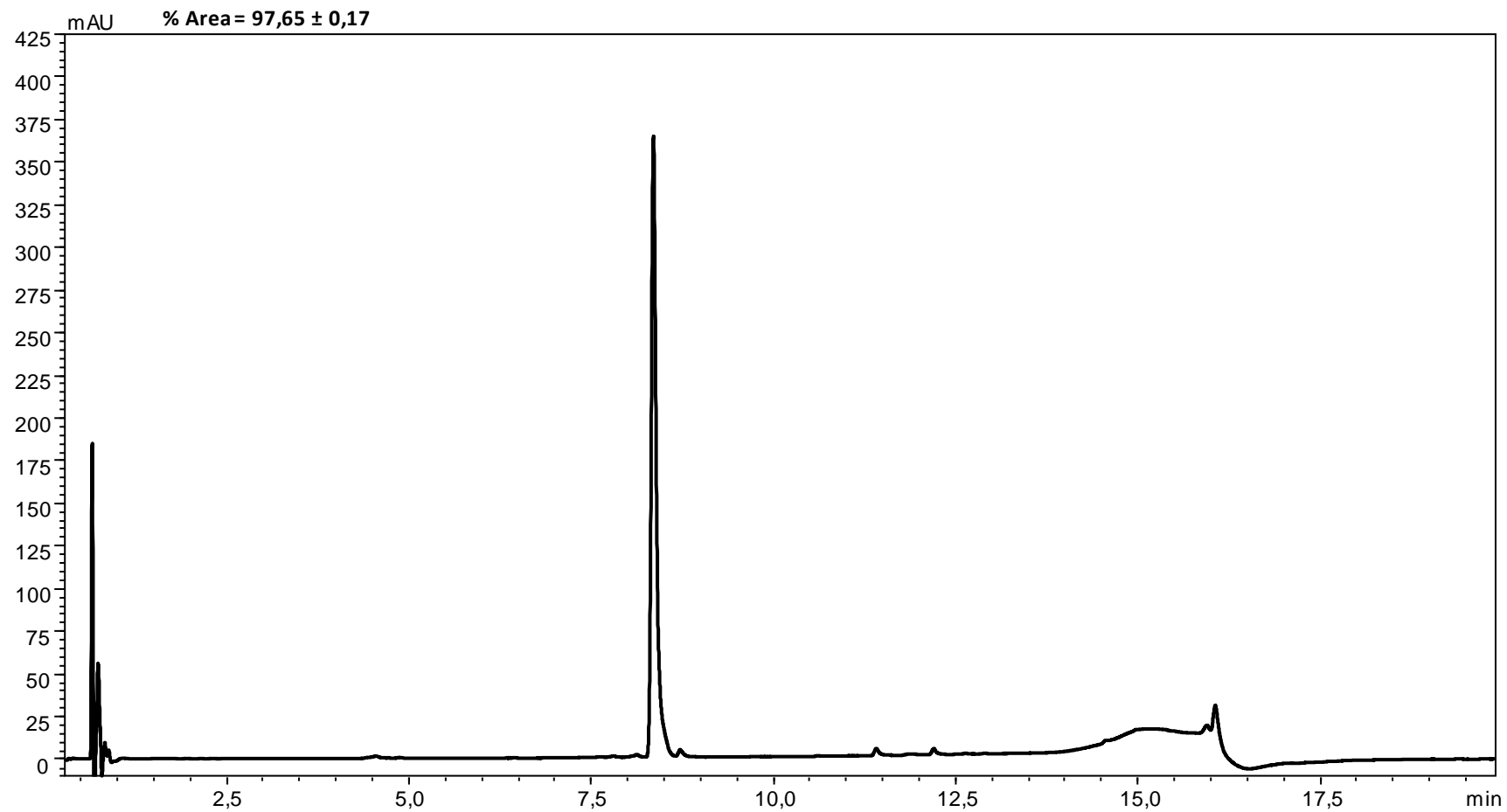
RP-HPLC trace of compound **5a**



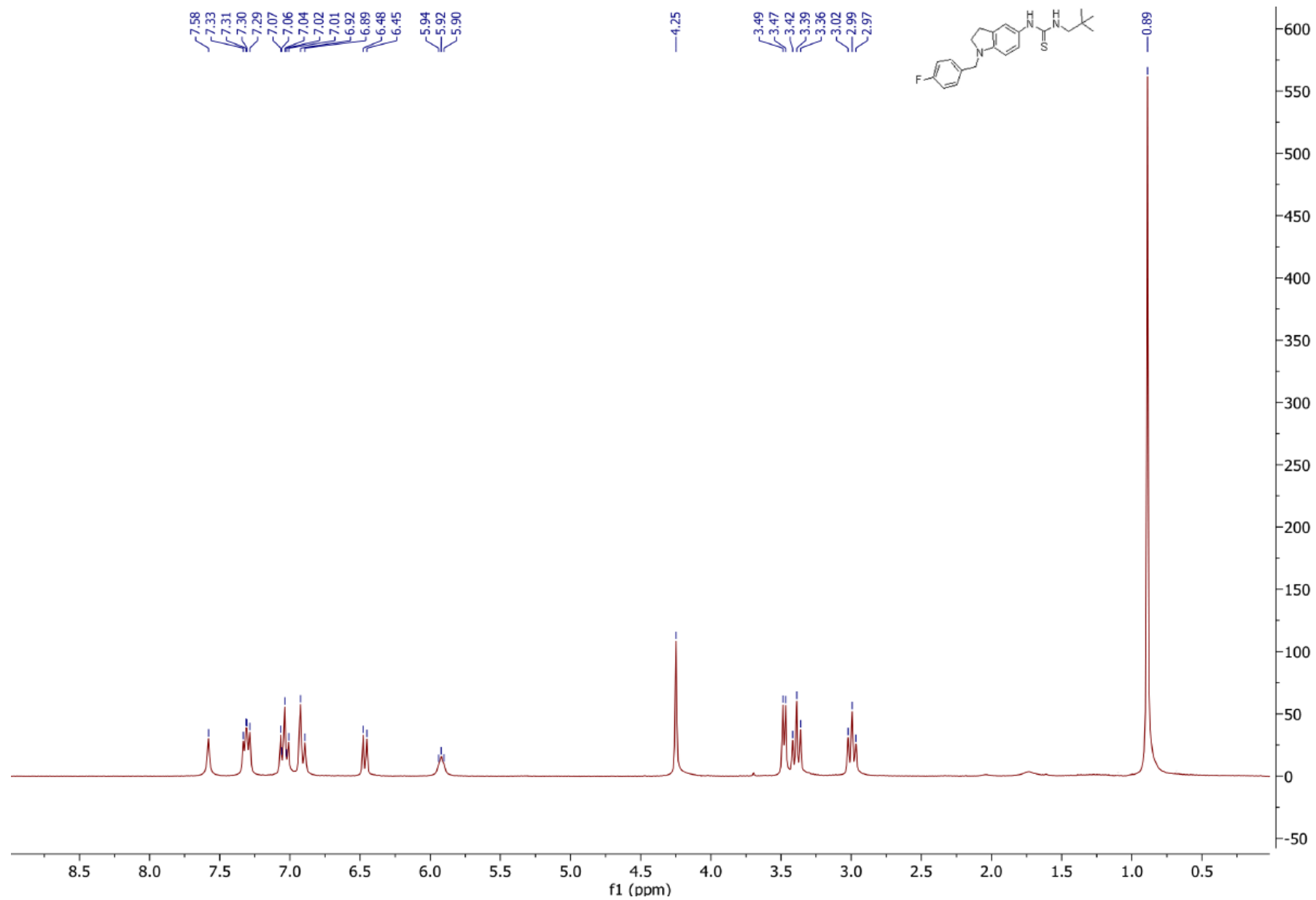
¹H NMR of compound **5b**



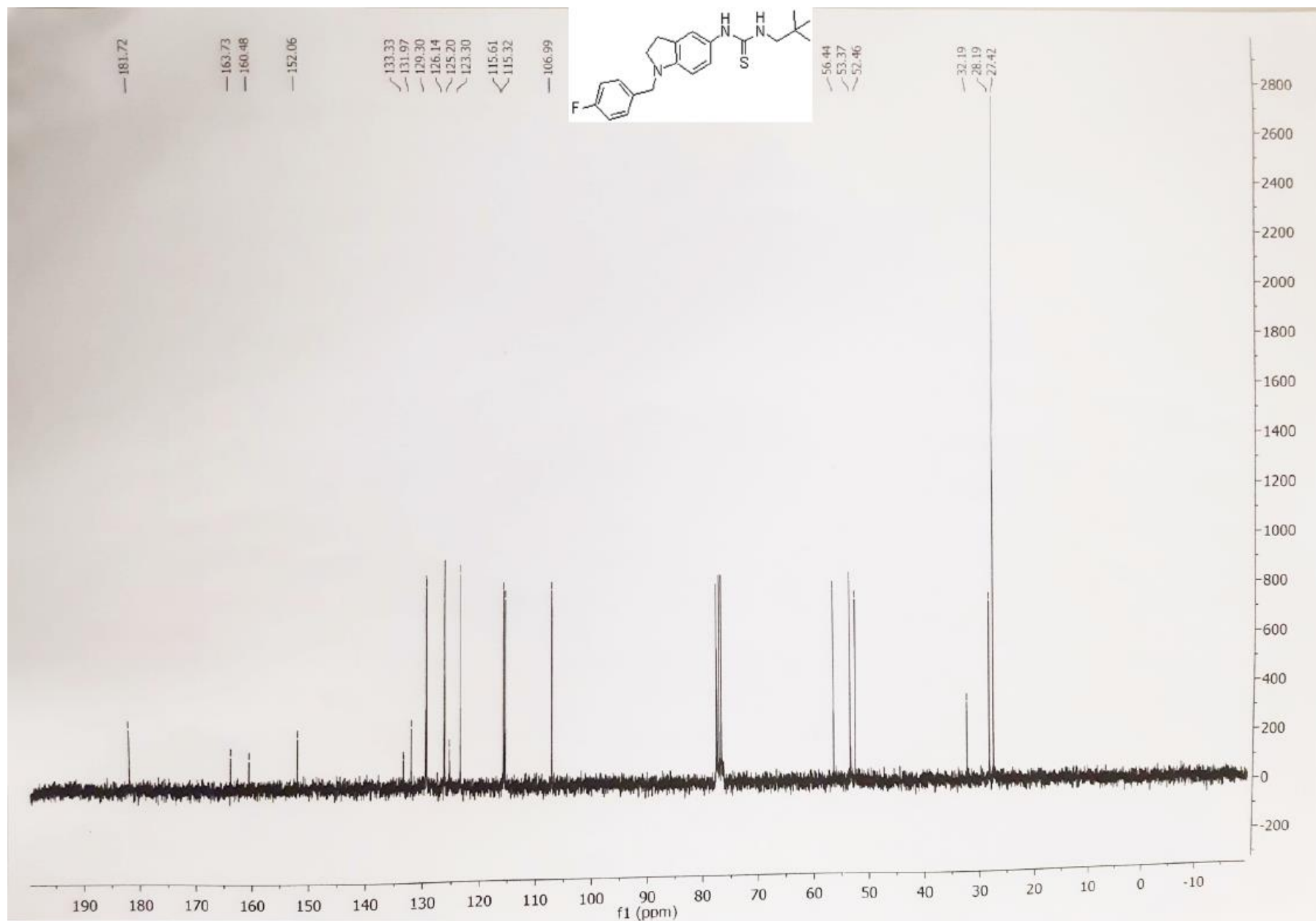
¹³C NMR of compound **5b**



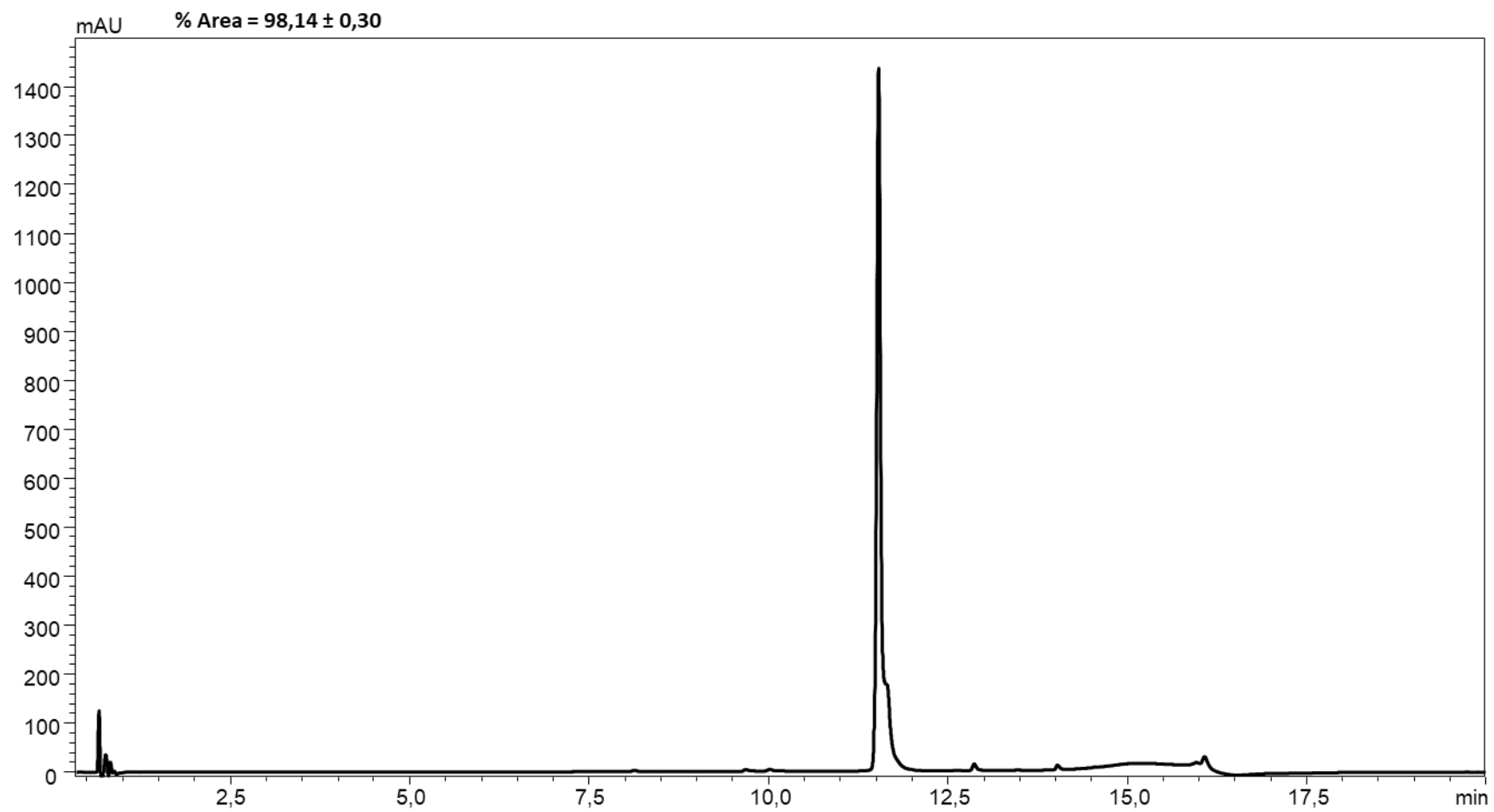
RP-HPLC trace of compound **5b**



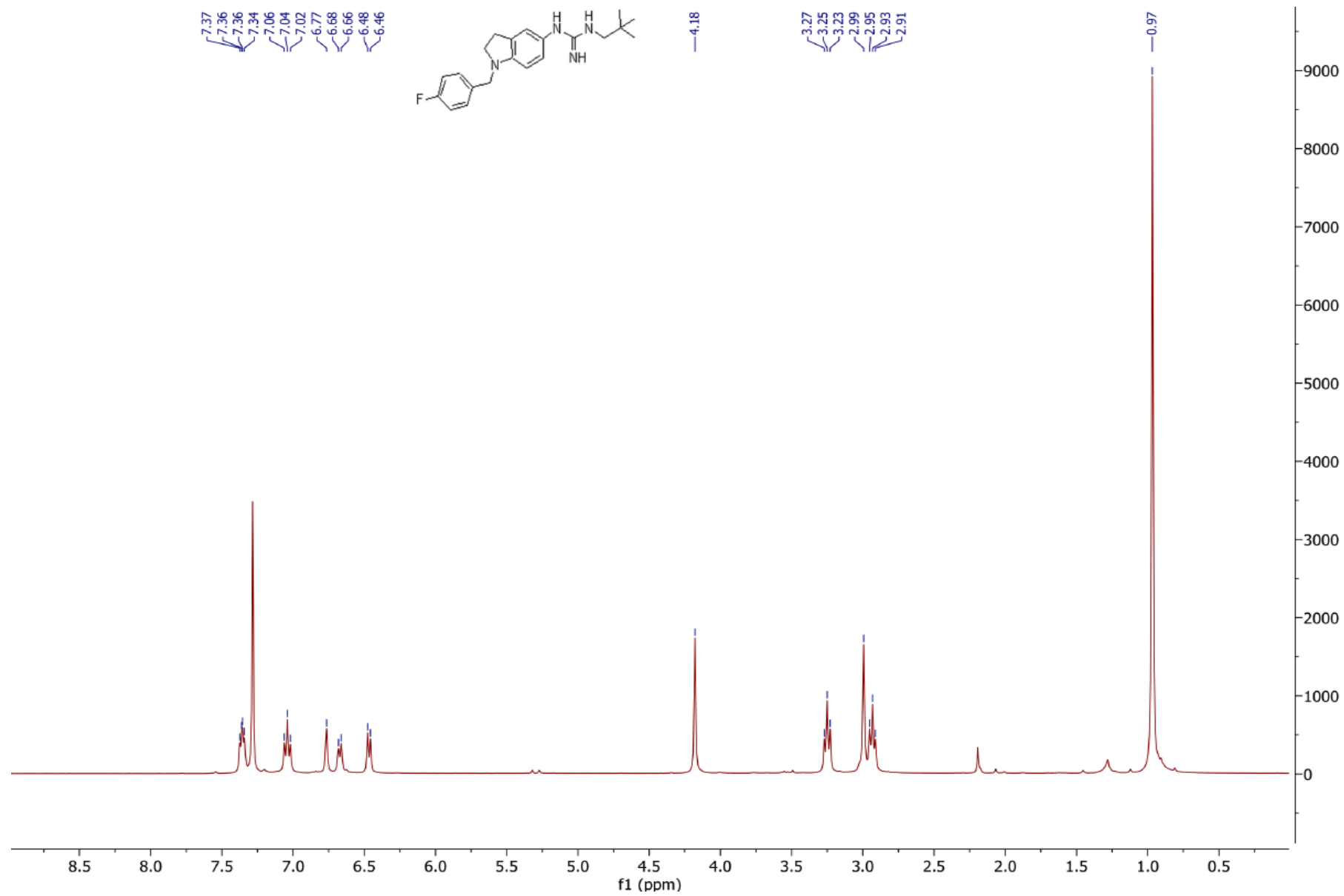
¹H NMR of compound **6**



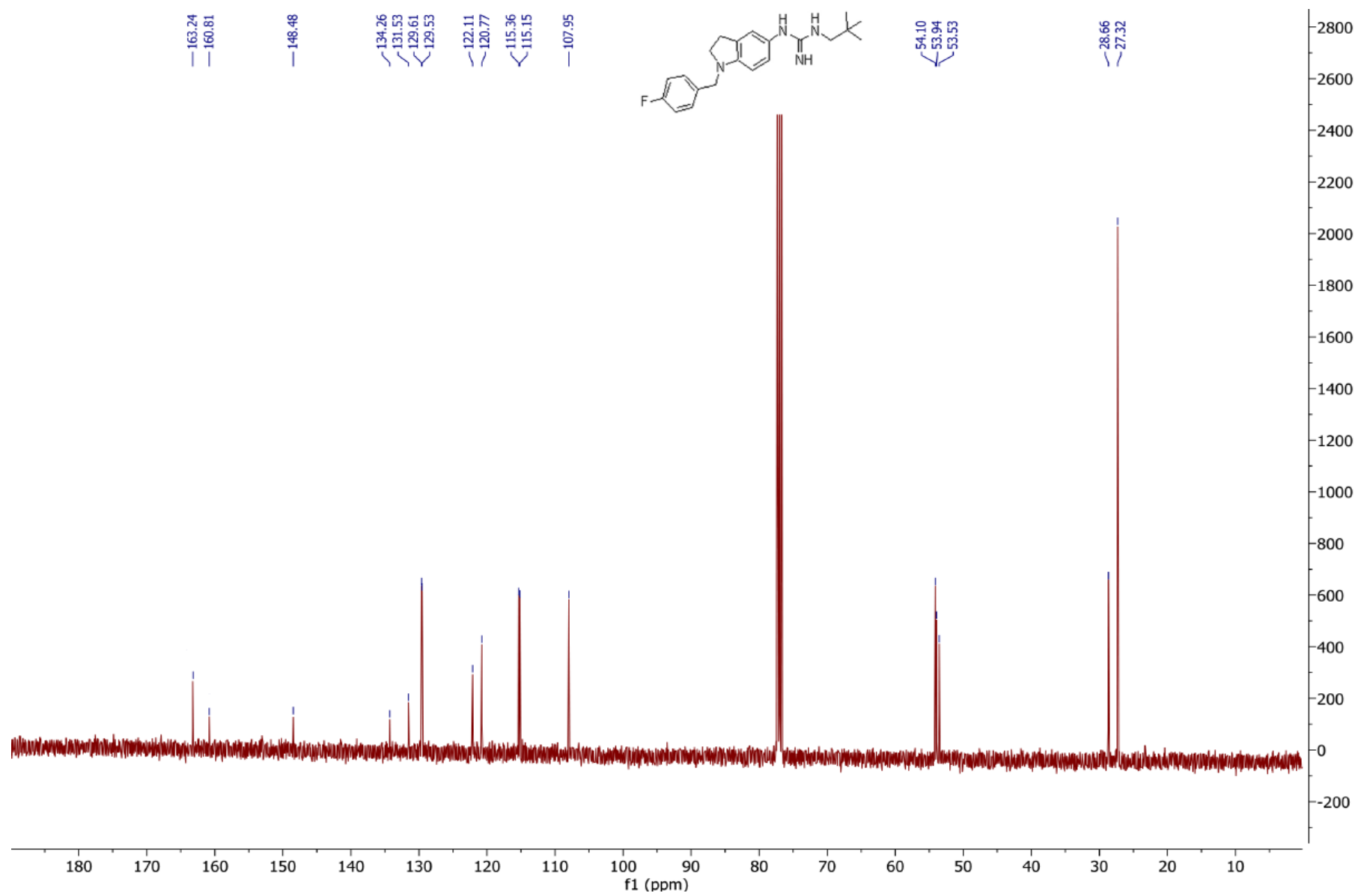
¹³C NMR of compound 6



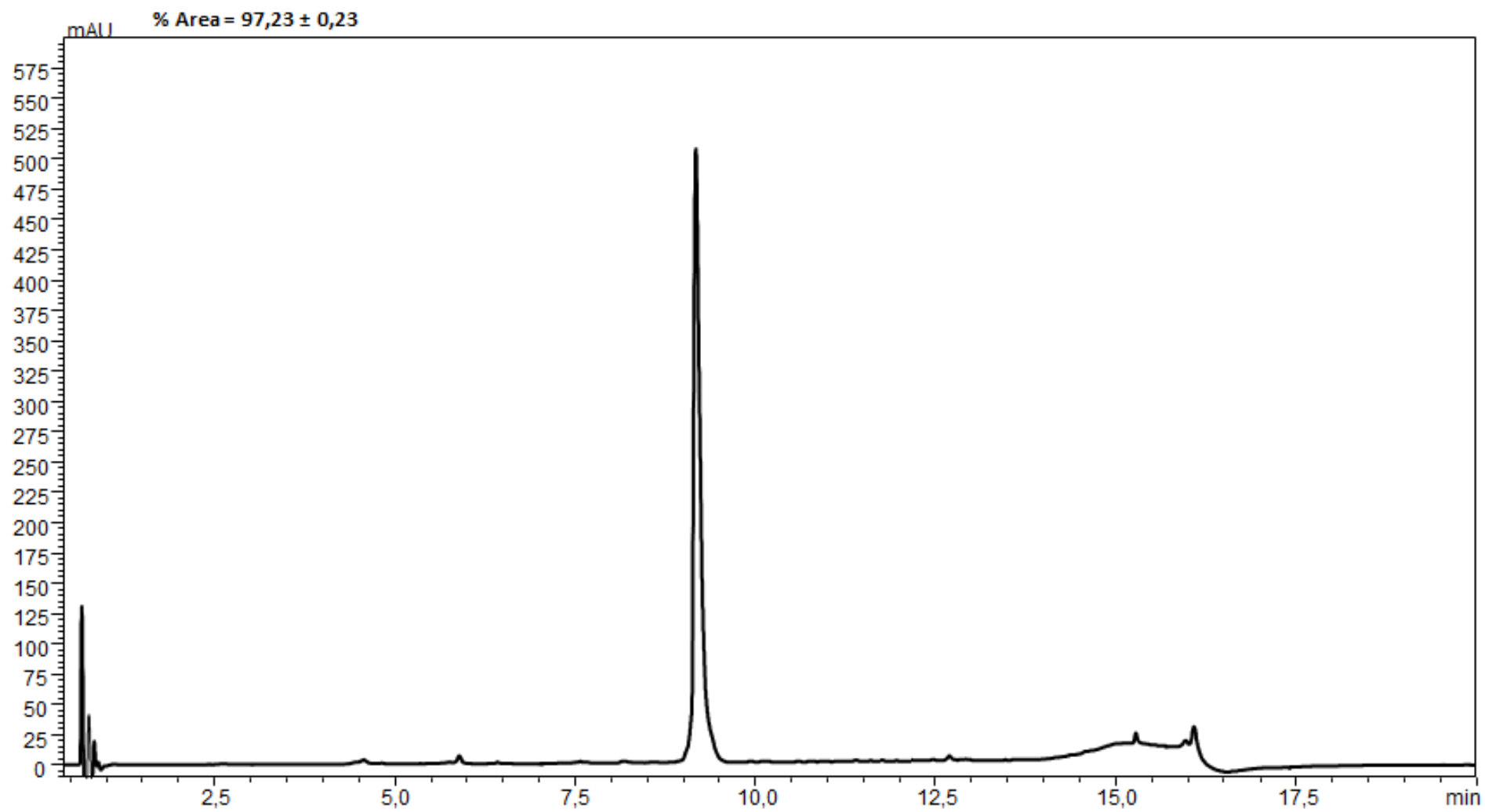
RP-HPLC trace of compound **6**



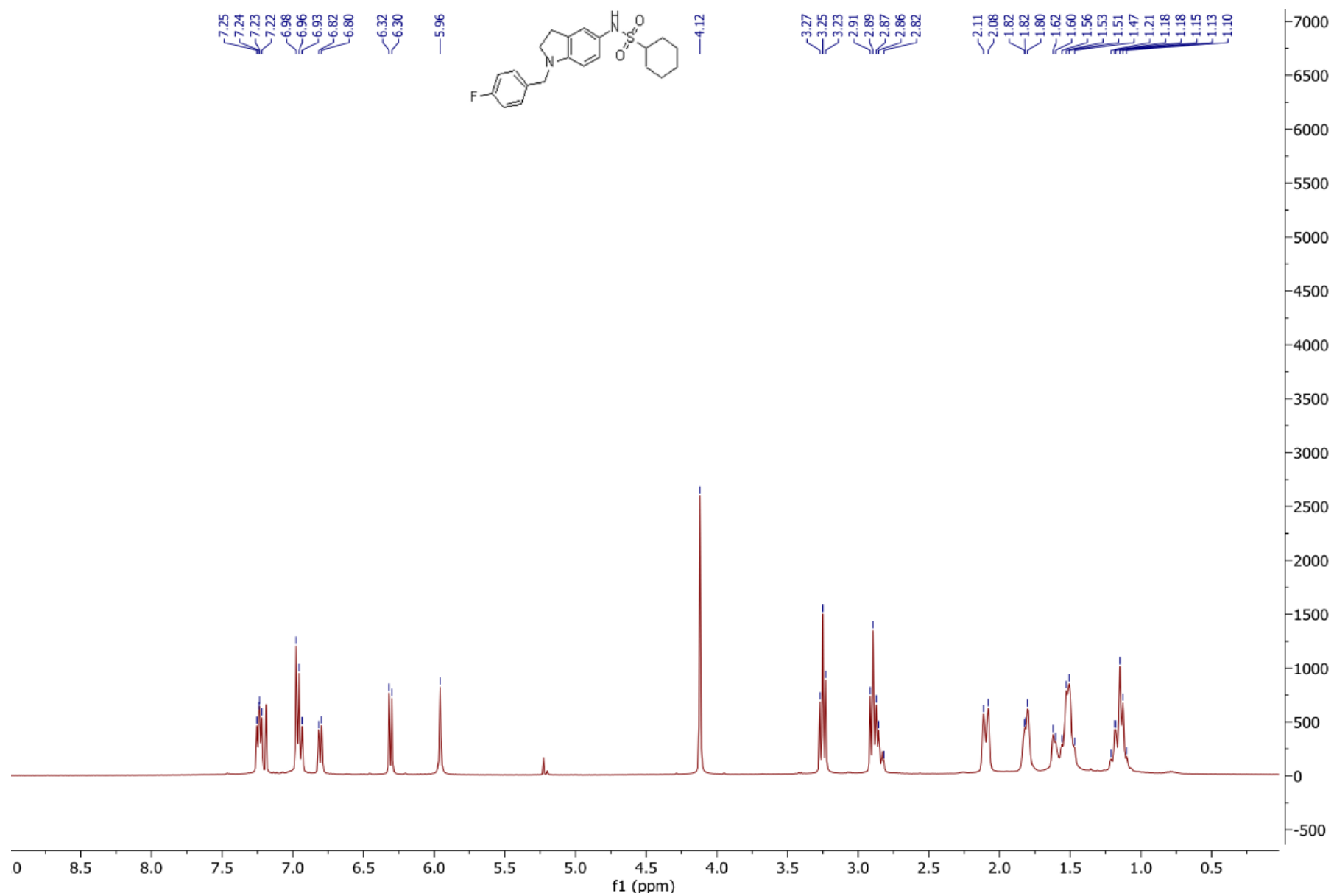
¹H NMR of compound 7



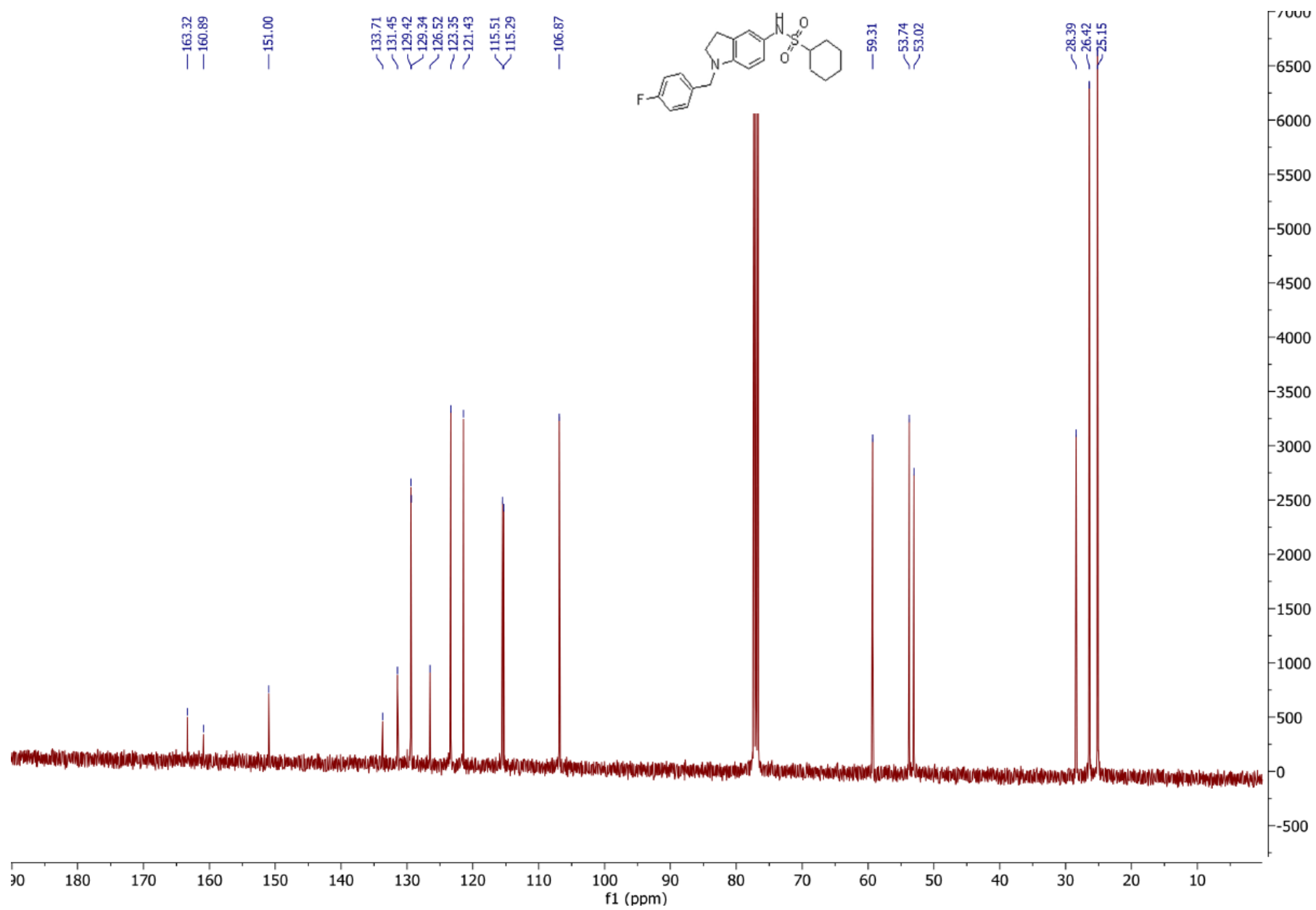
¹³C NMR of compound 7



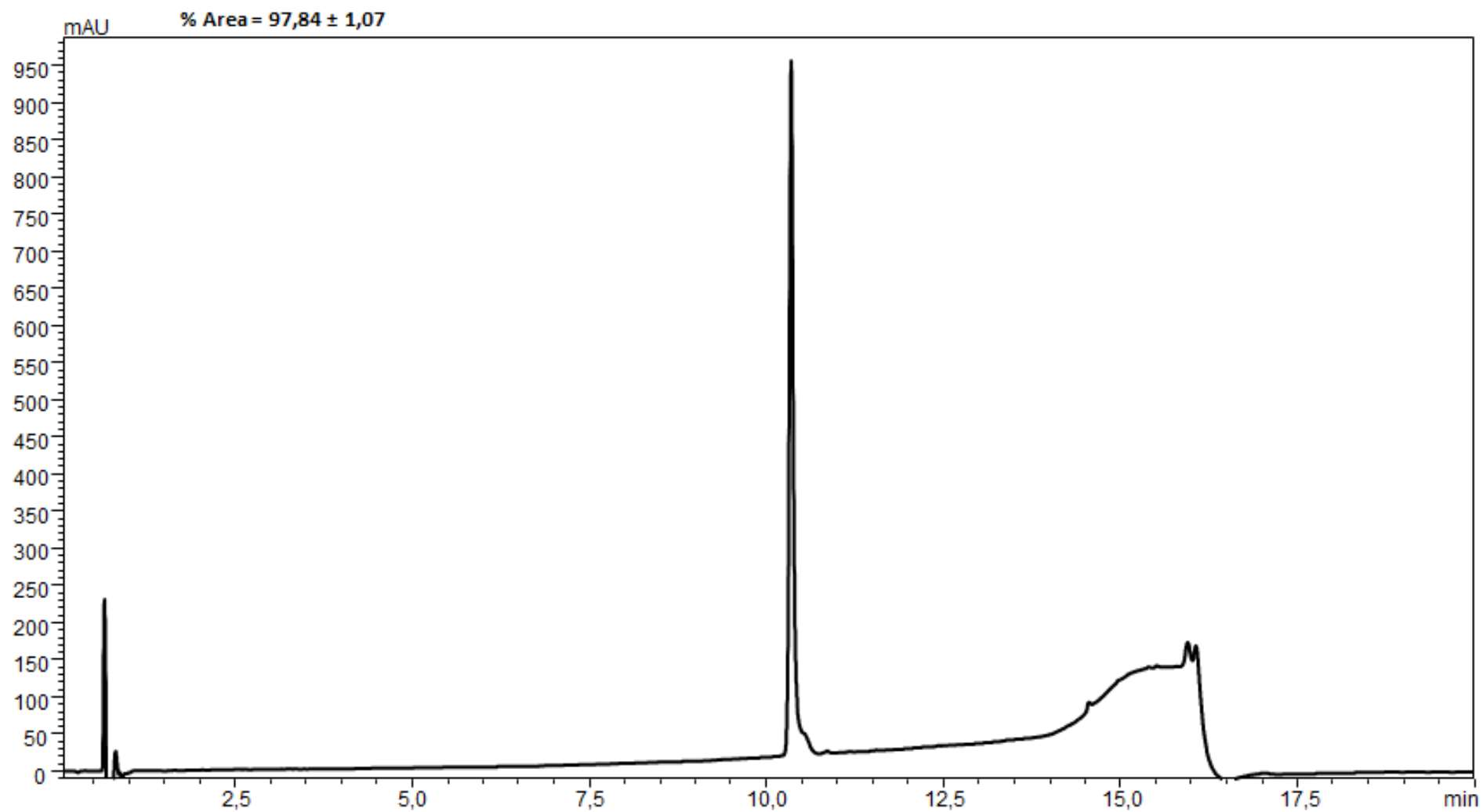
RP-HPLC trace of compound **7**



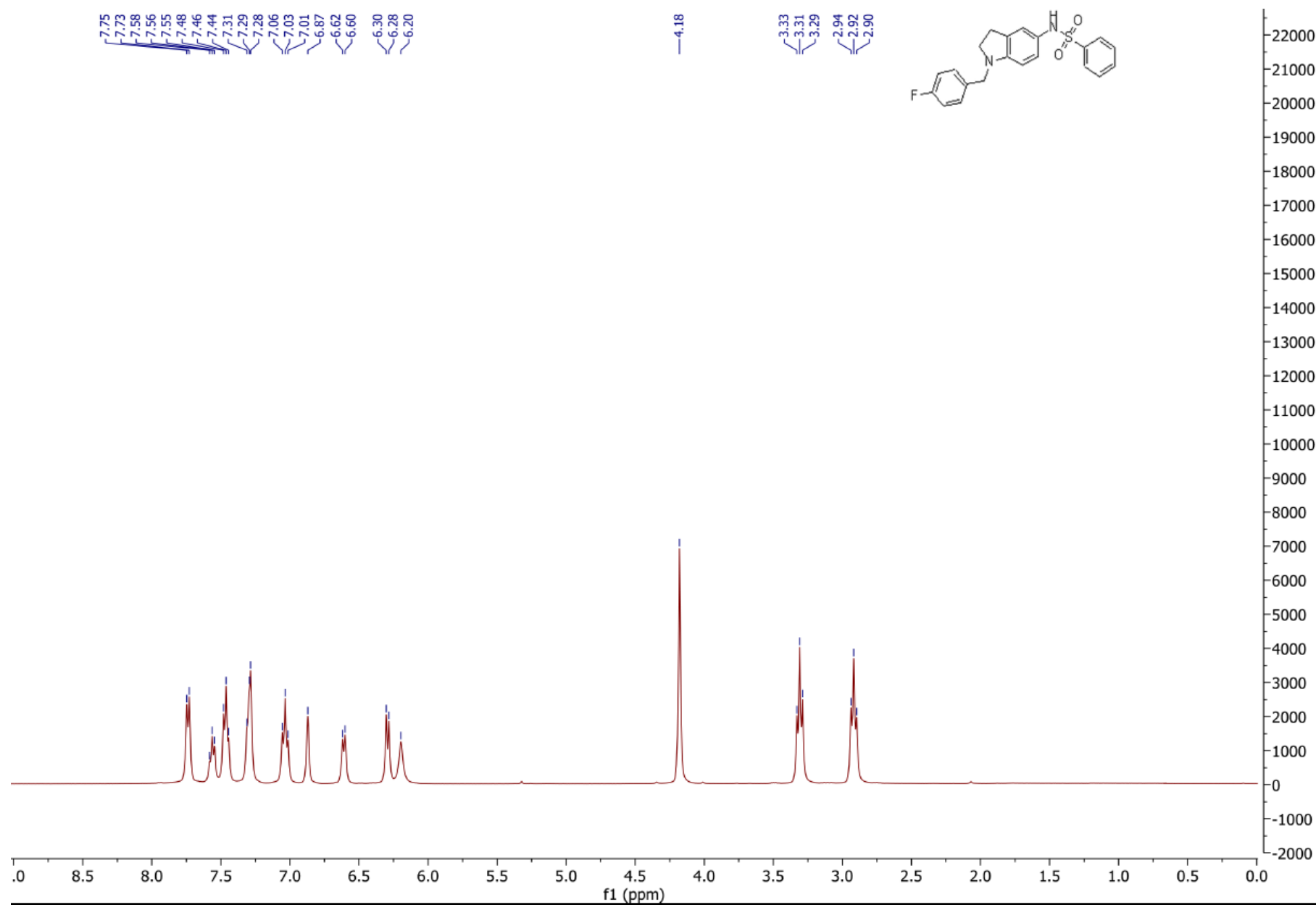
¹H NMR of compound **8a**



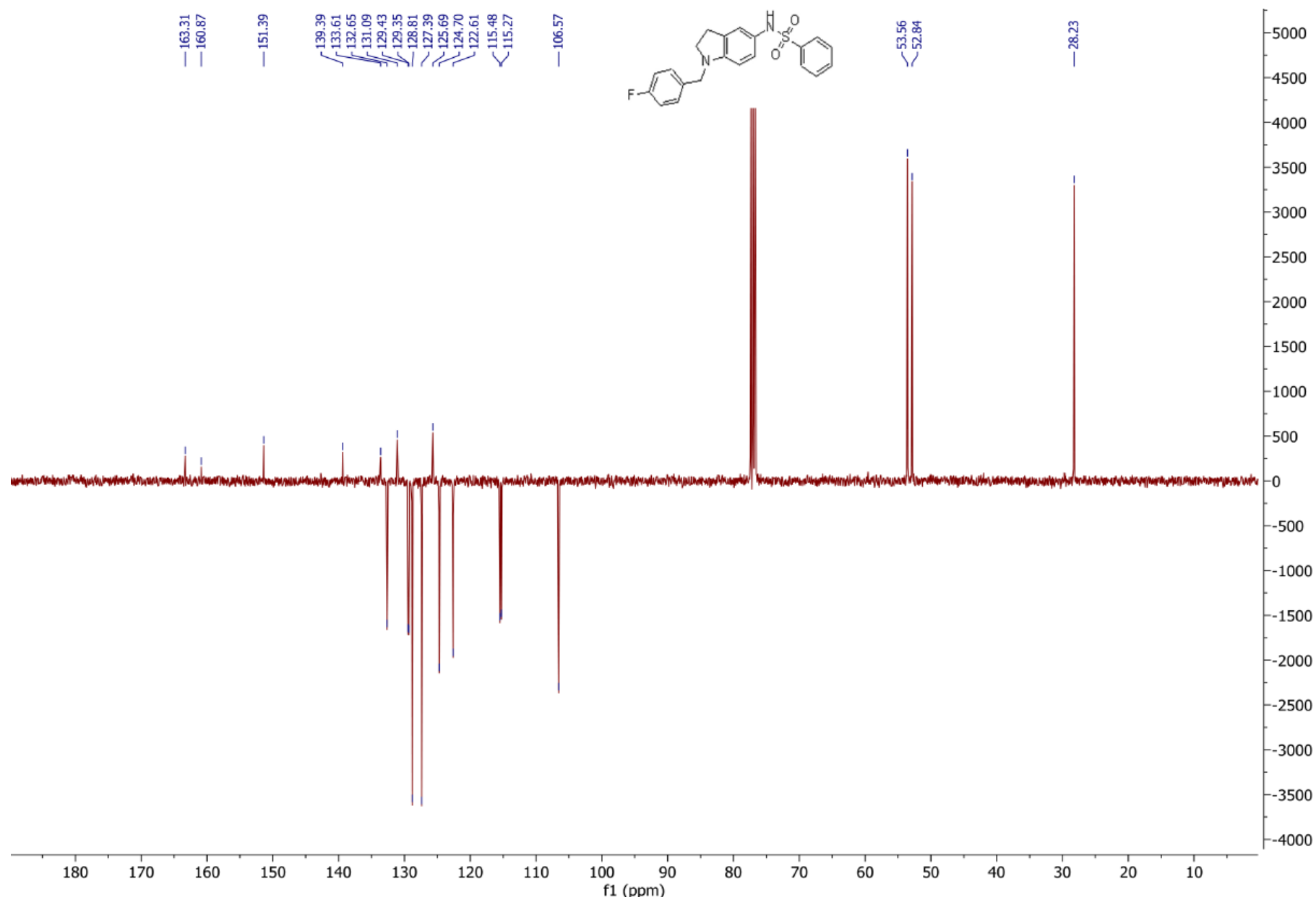
¹³C NMR of compound **8a**

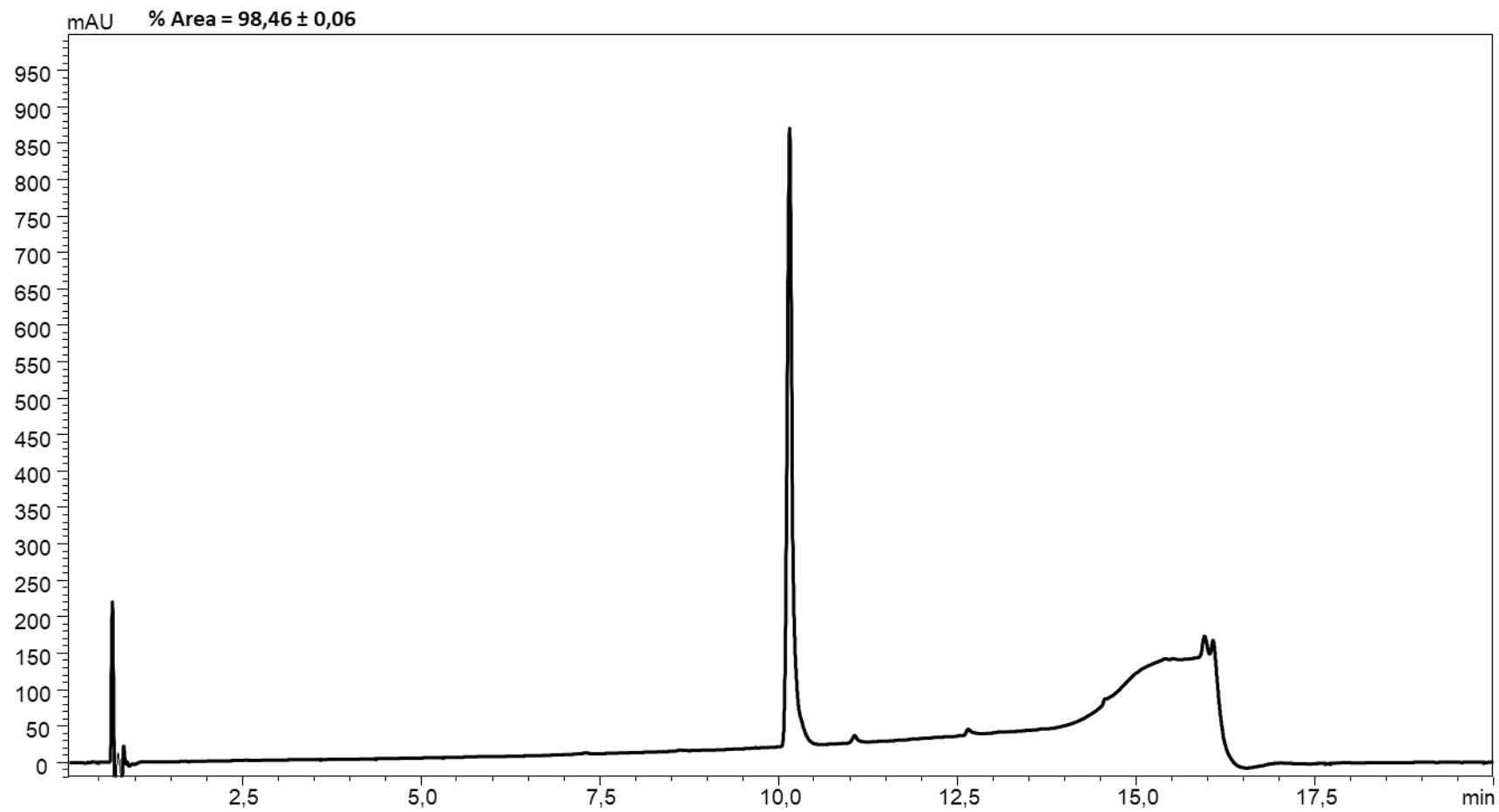


RP-HPLC trace of compound **8a**

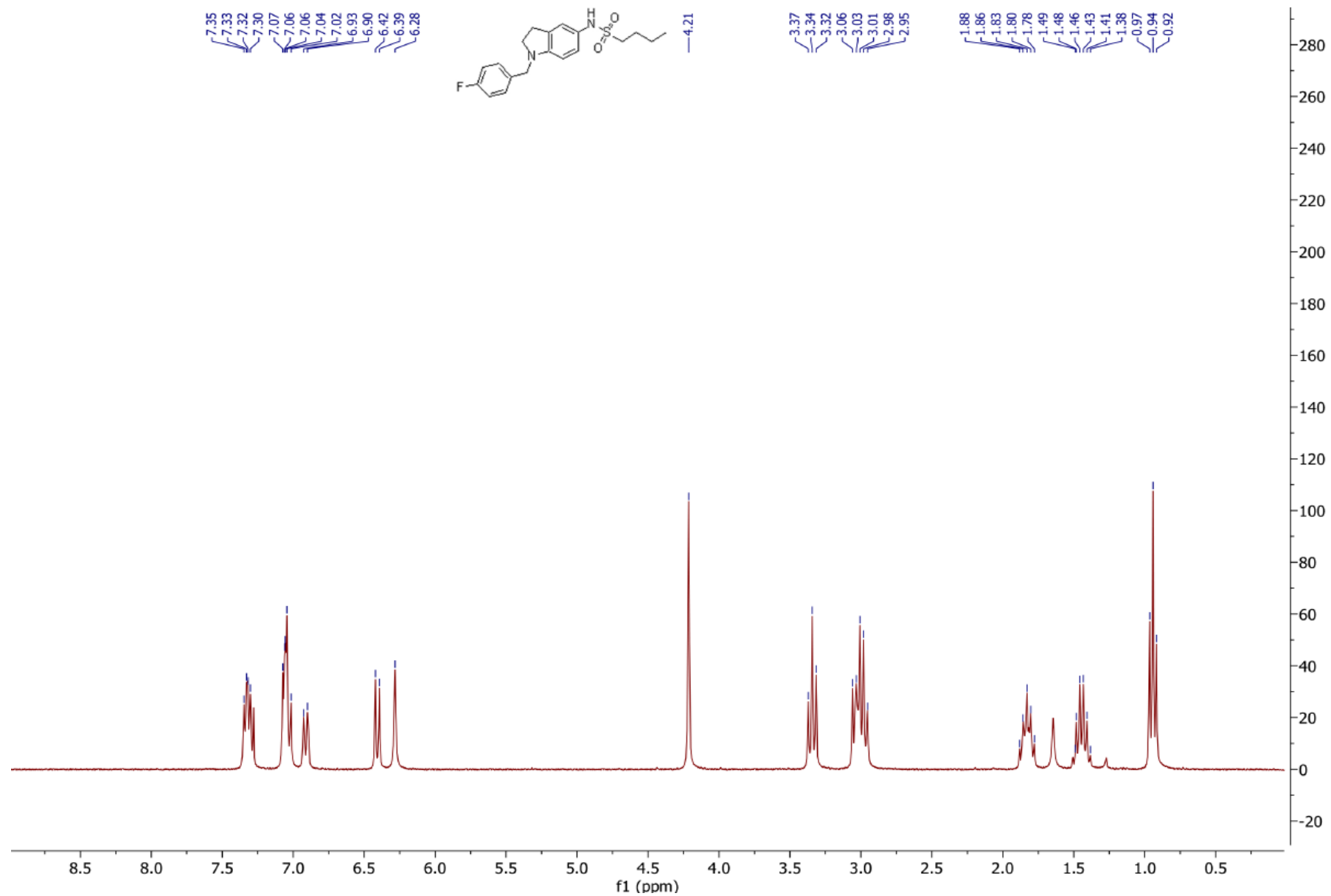


¹H NMR of compound **8b**

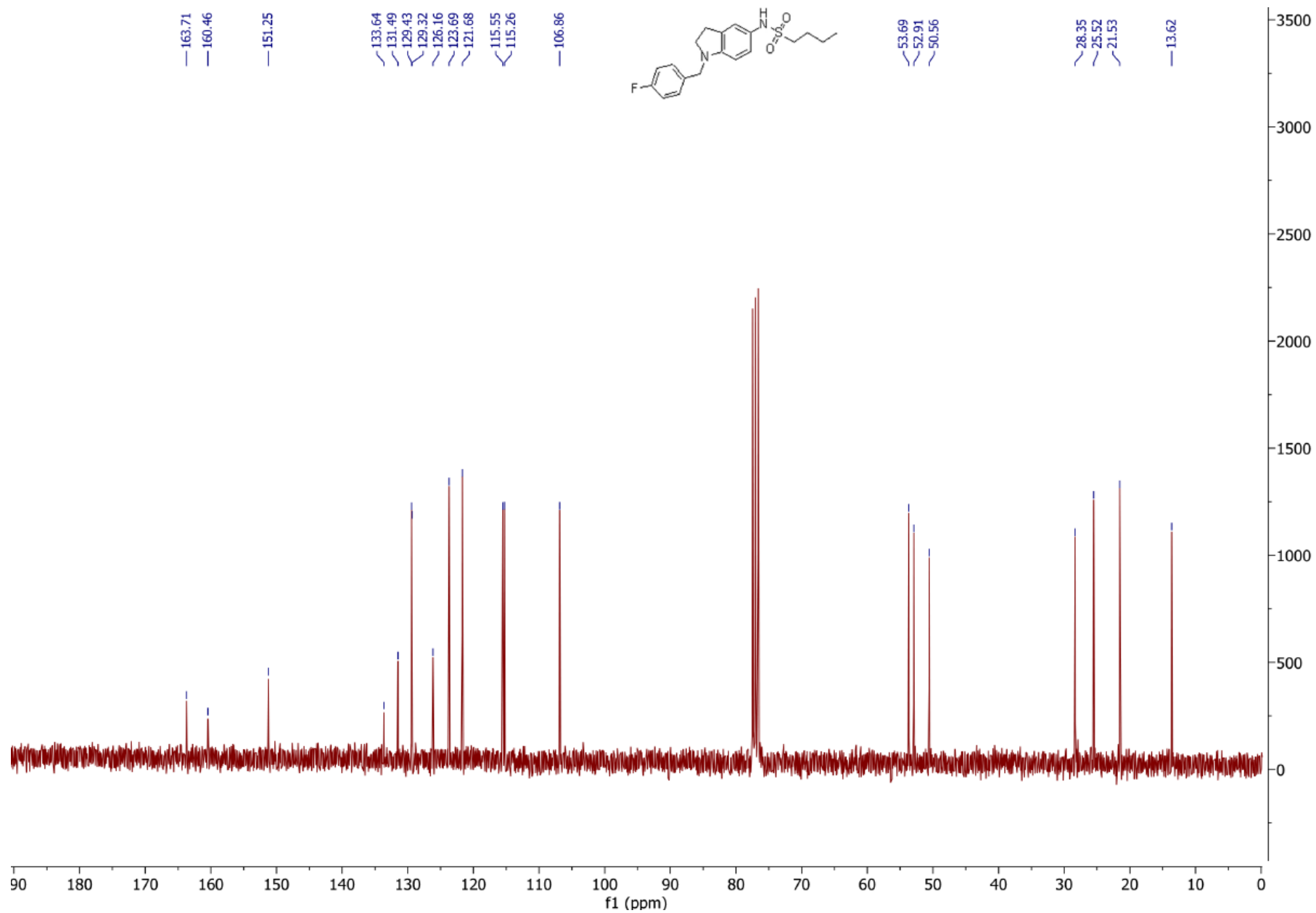




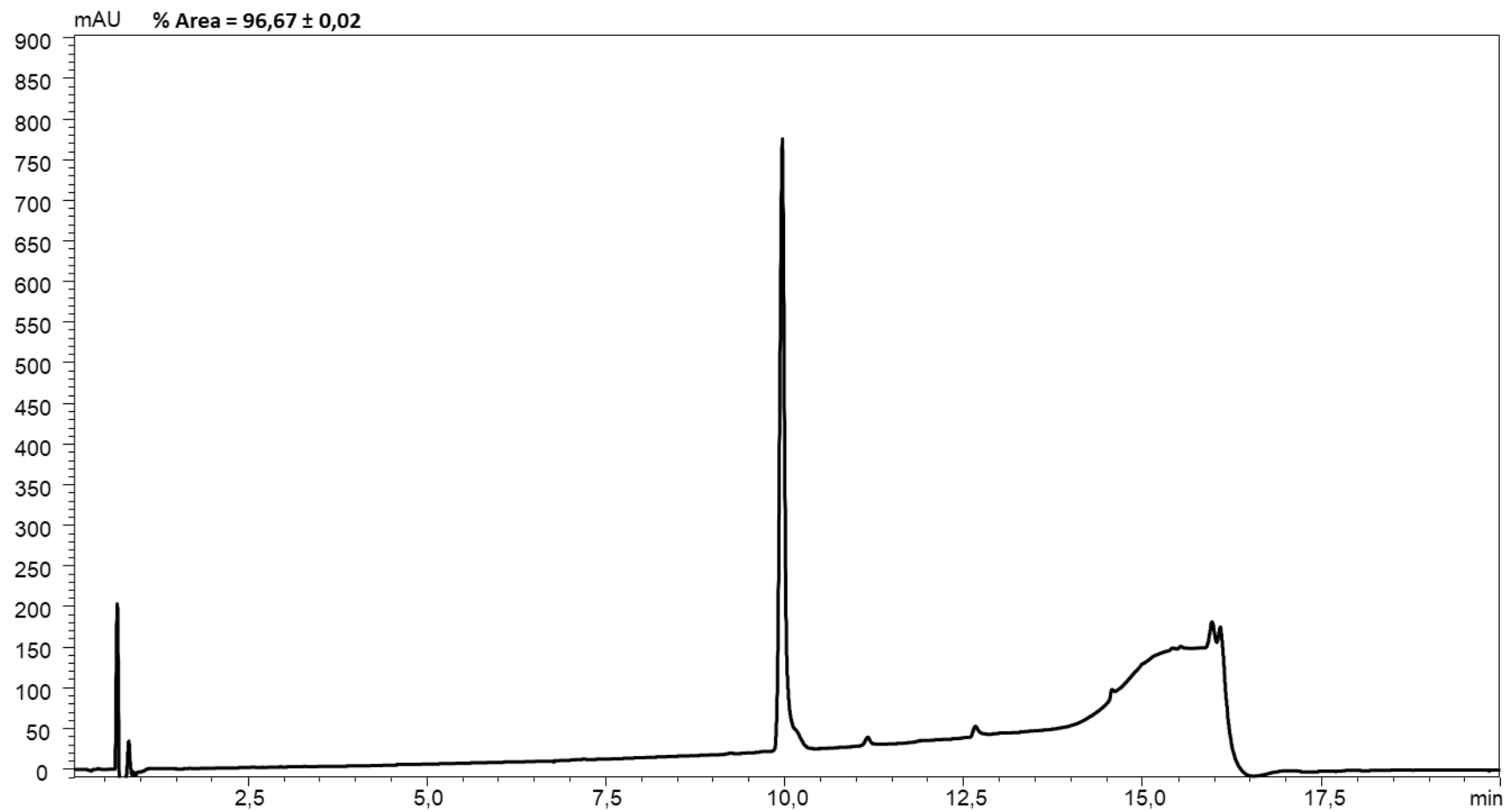
RP-HPLC trace of compound **8b**



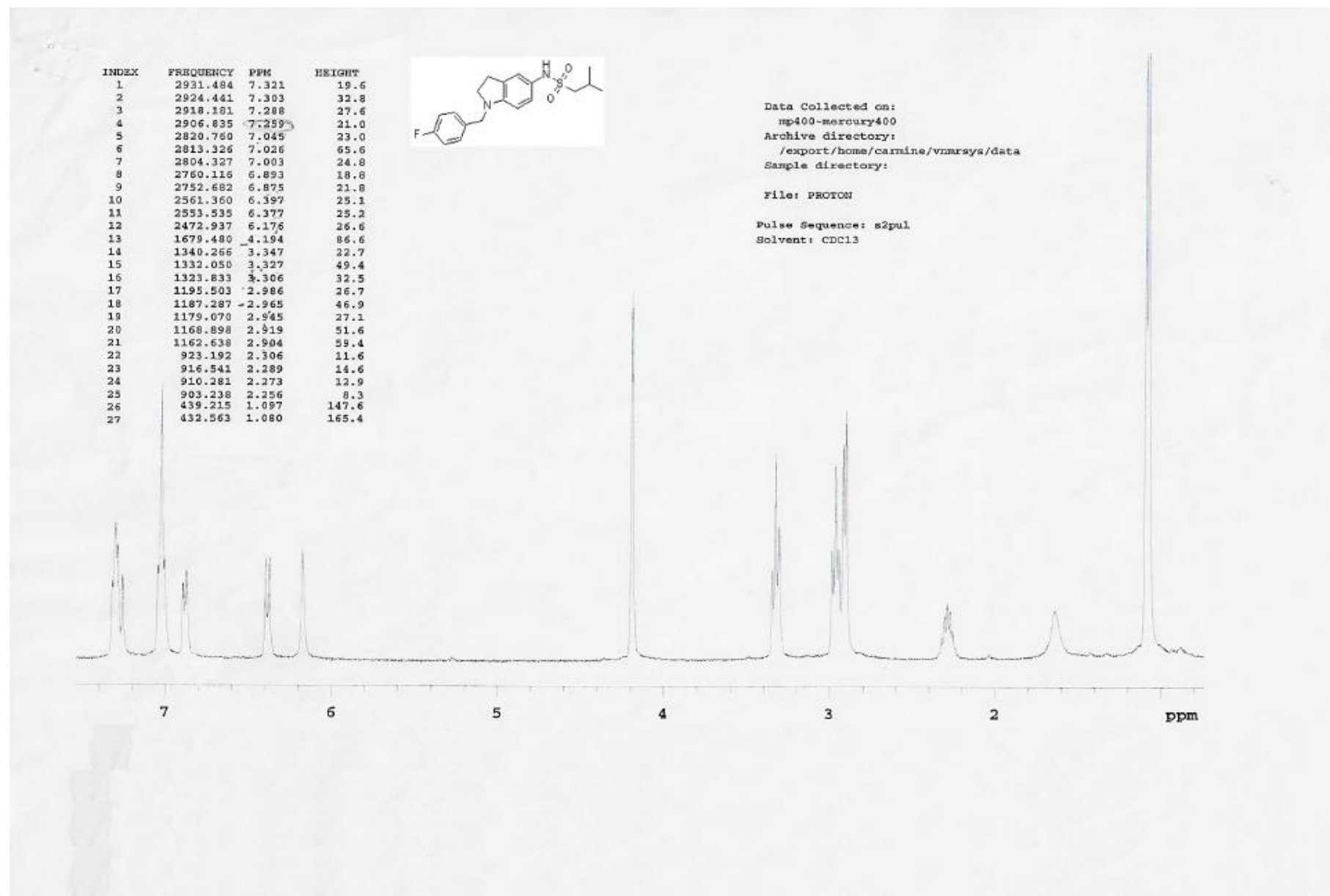
¹H NMR of compound **8c**



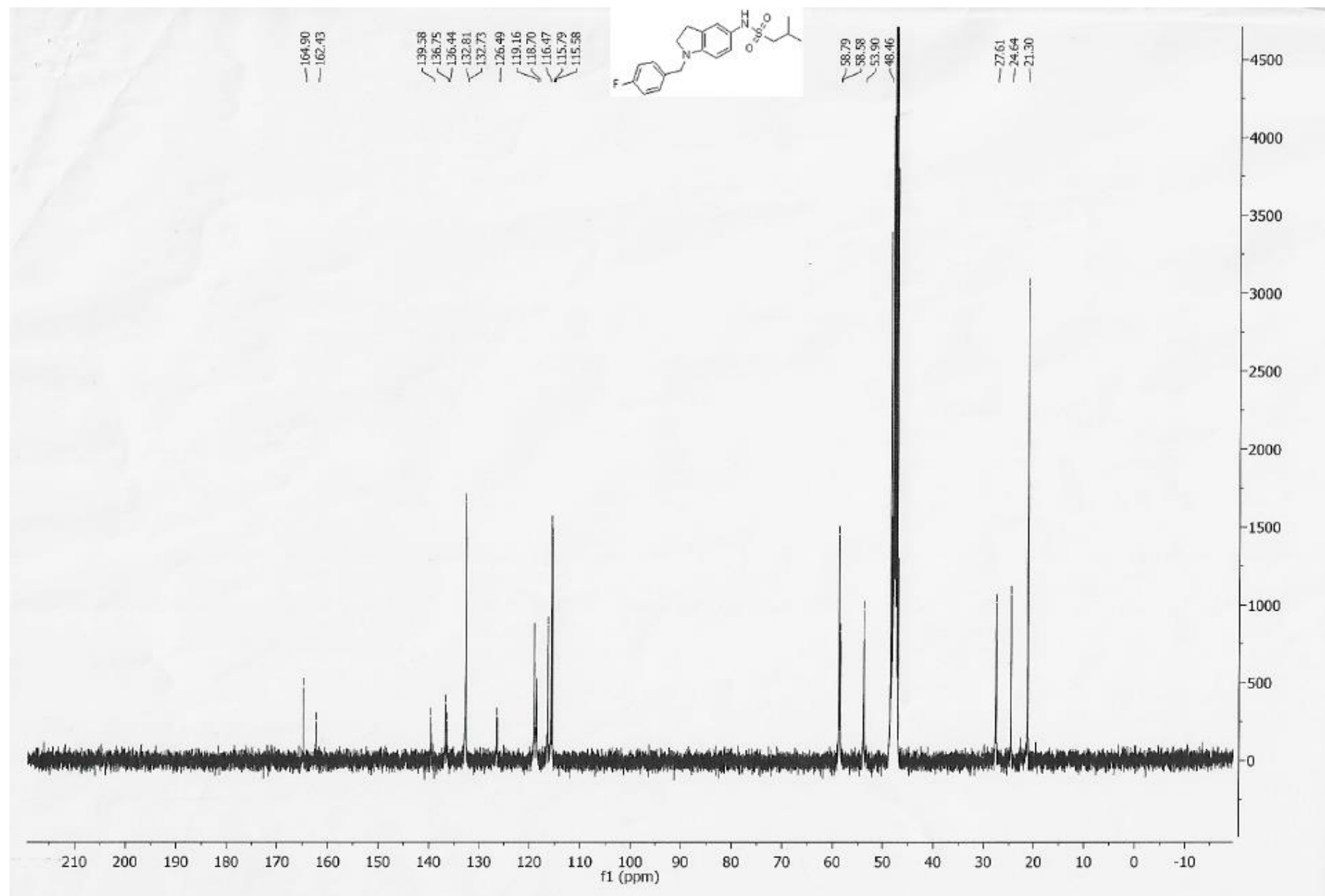
13C NMR of compound 8c



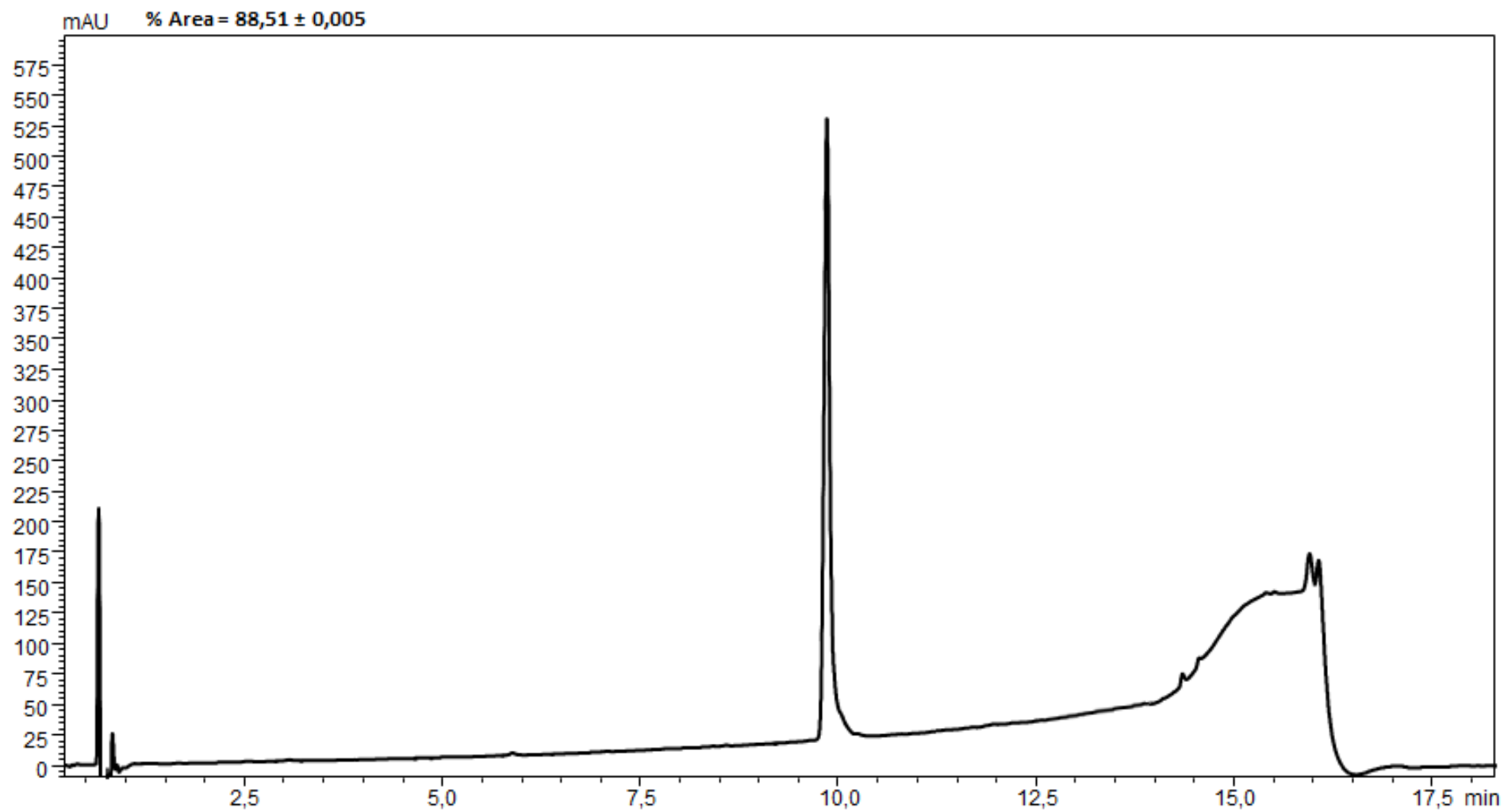
RP-HPLC trace of compound **8c**



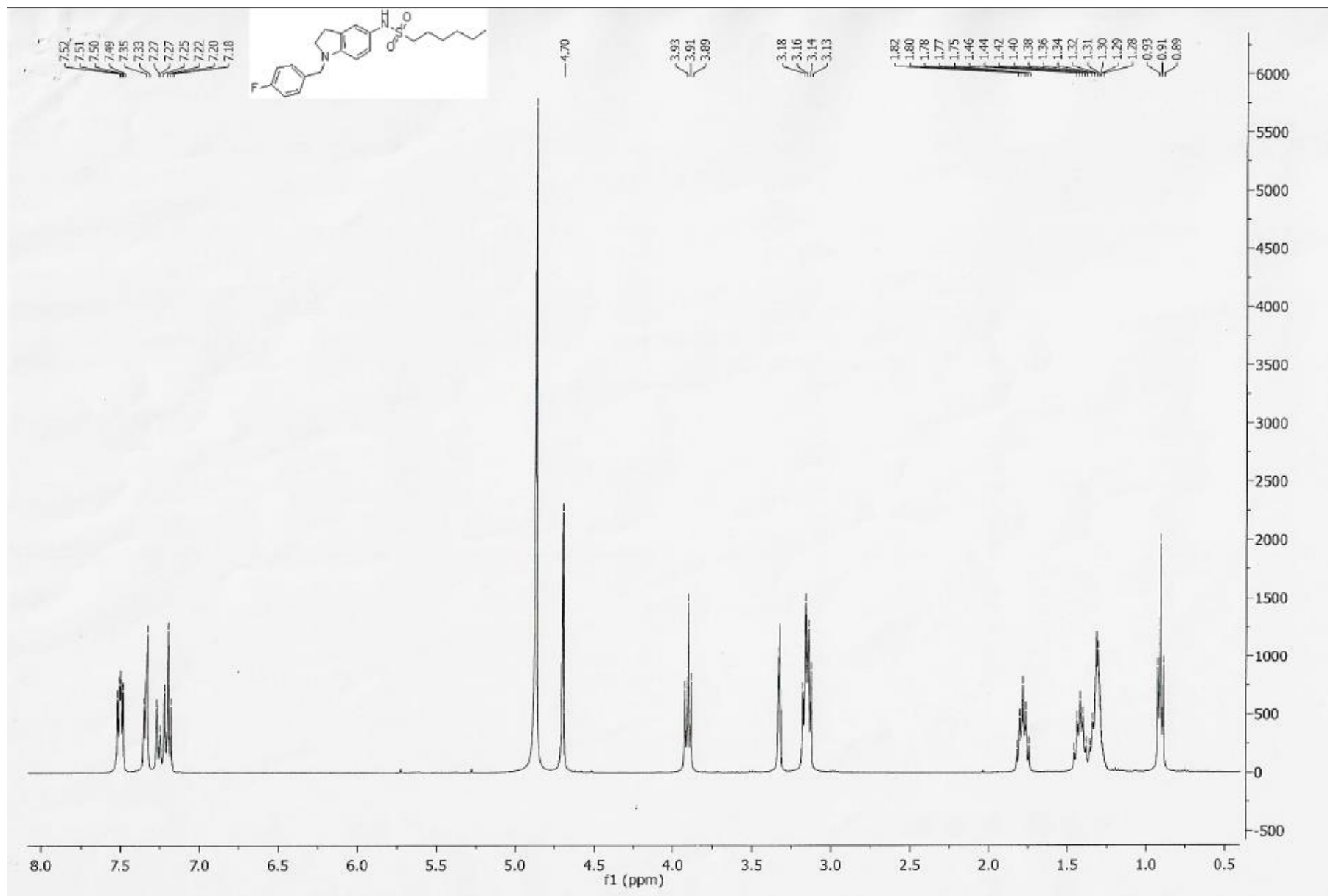
¹H NMR of compound **8d**



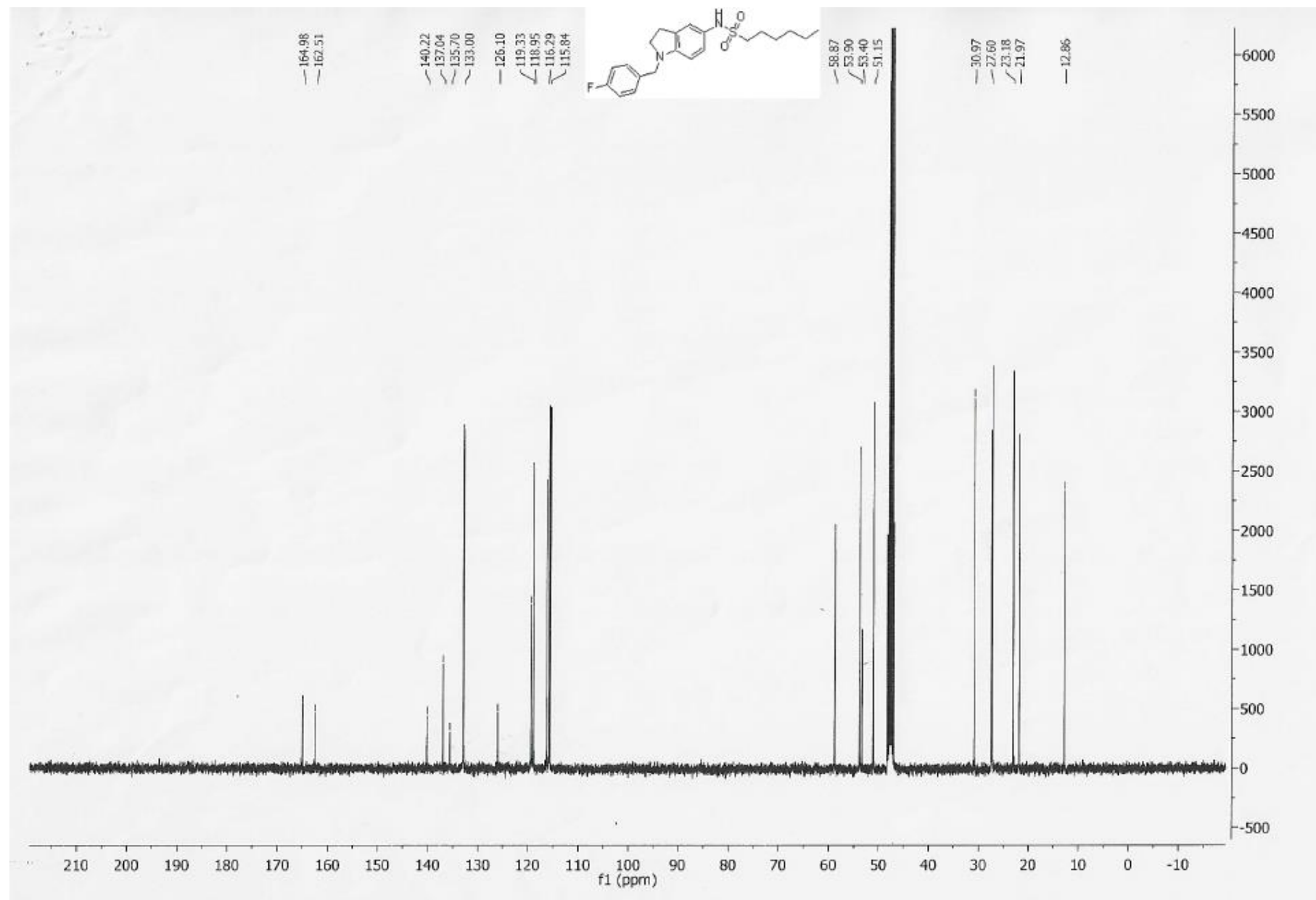
¹³C NMR of compound 8d



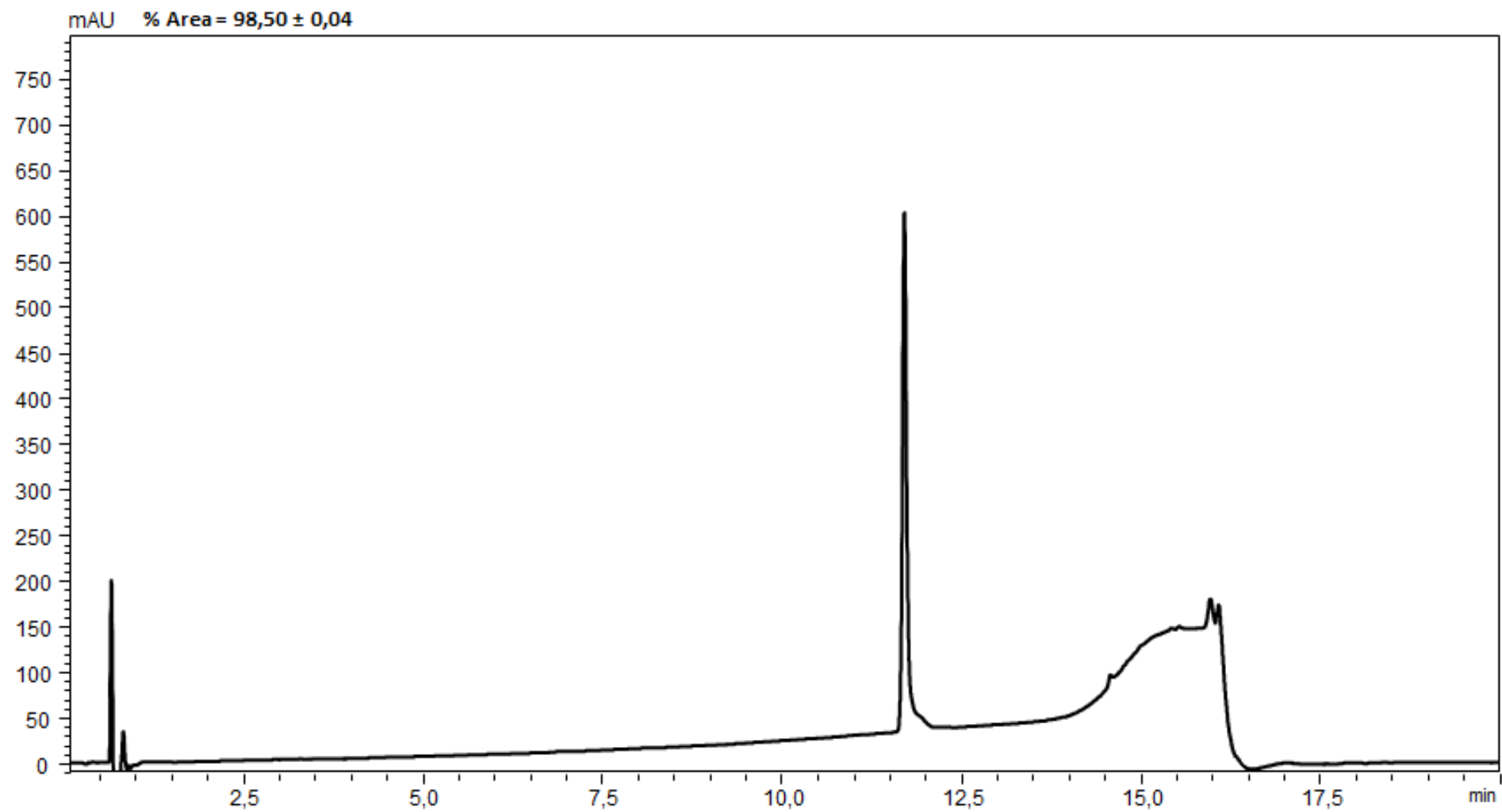
RP-HPLC trace of compound **8d**



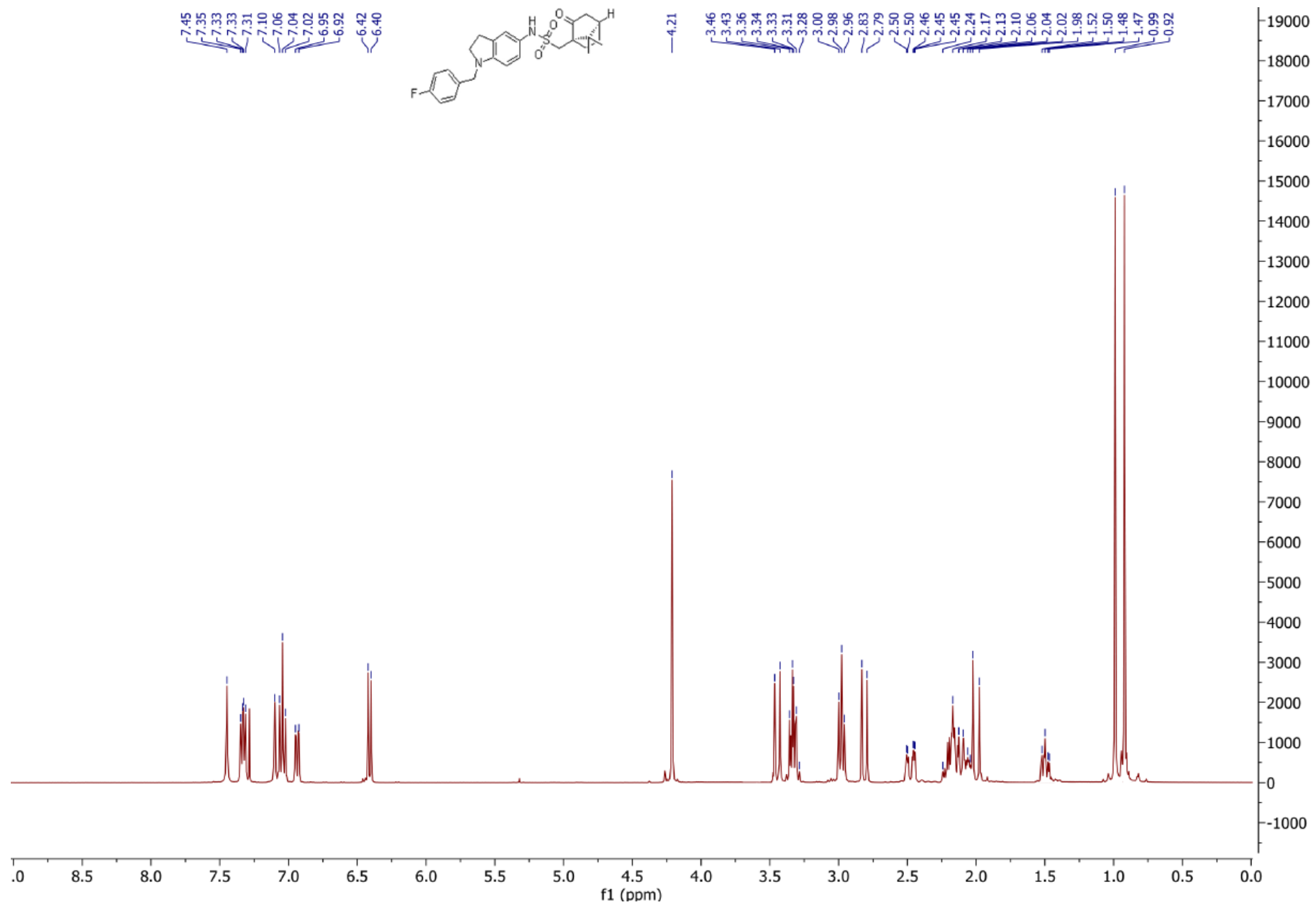
^1H NMR of compound **8e**



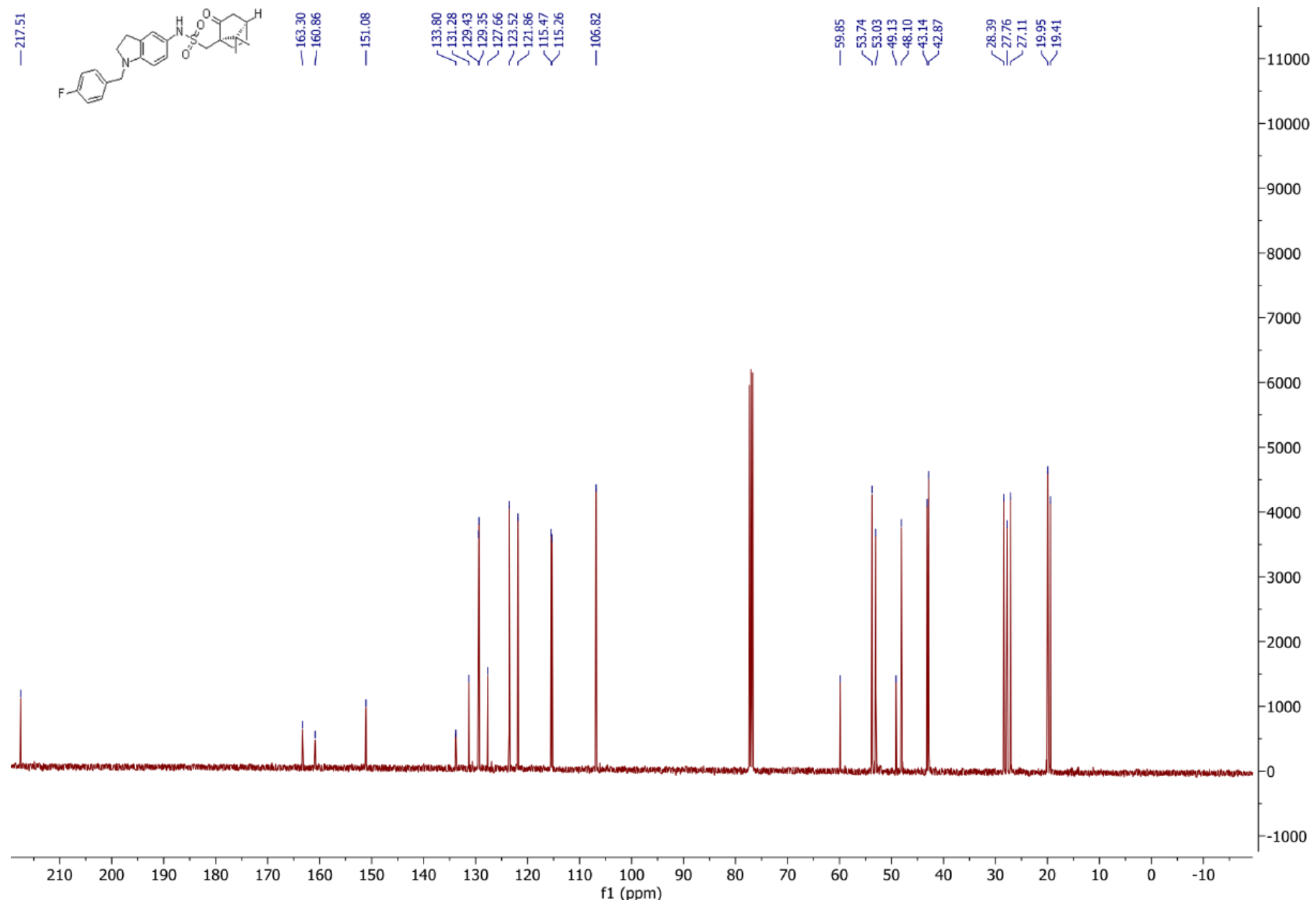
¹³C NMR of compound **8e**



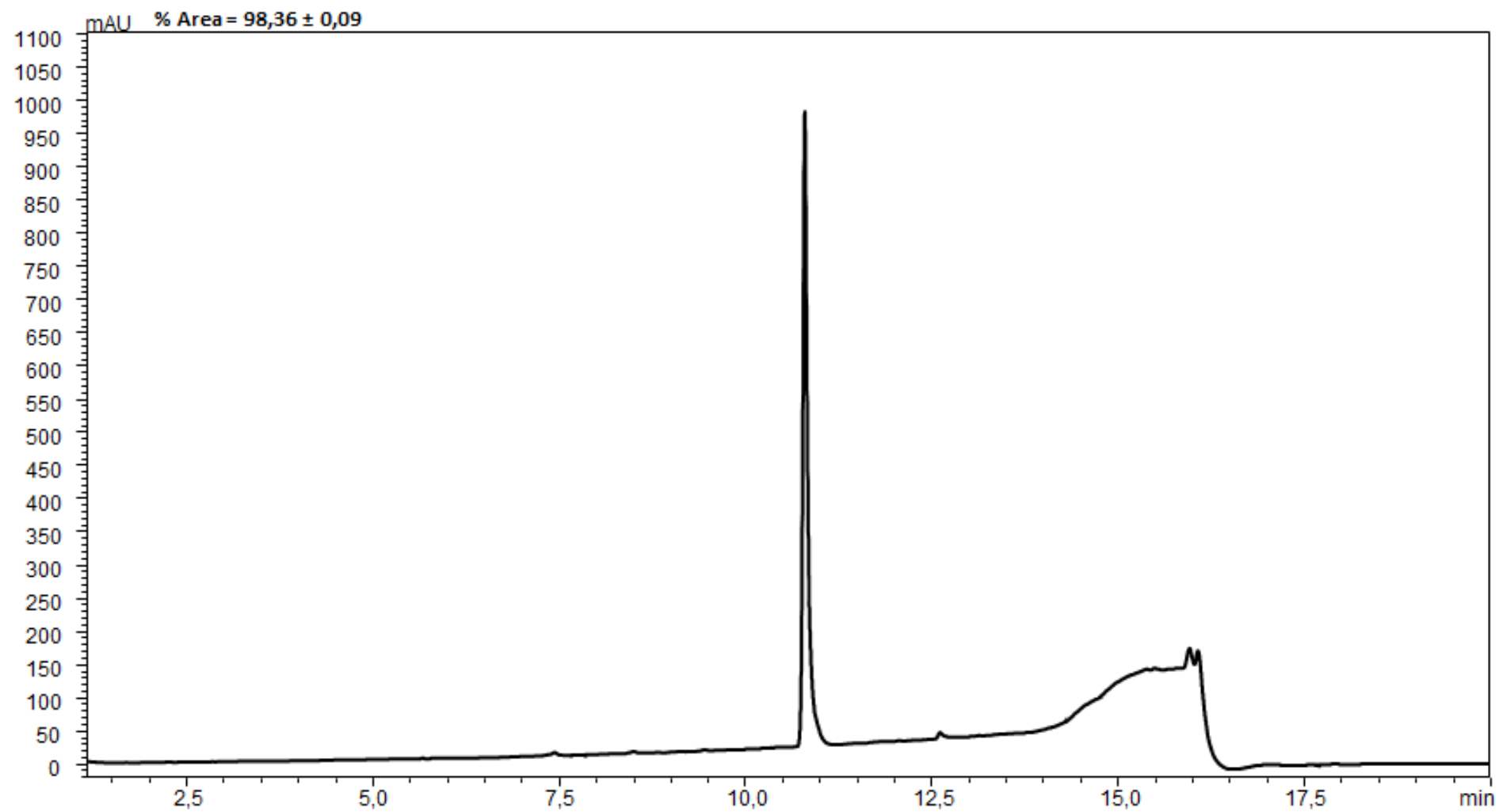
RP-HPLC trace of compound **8e**



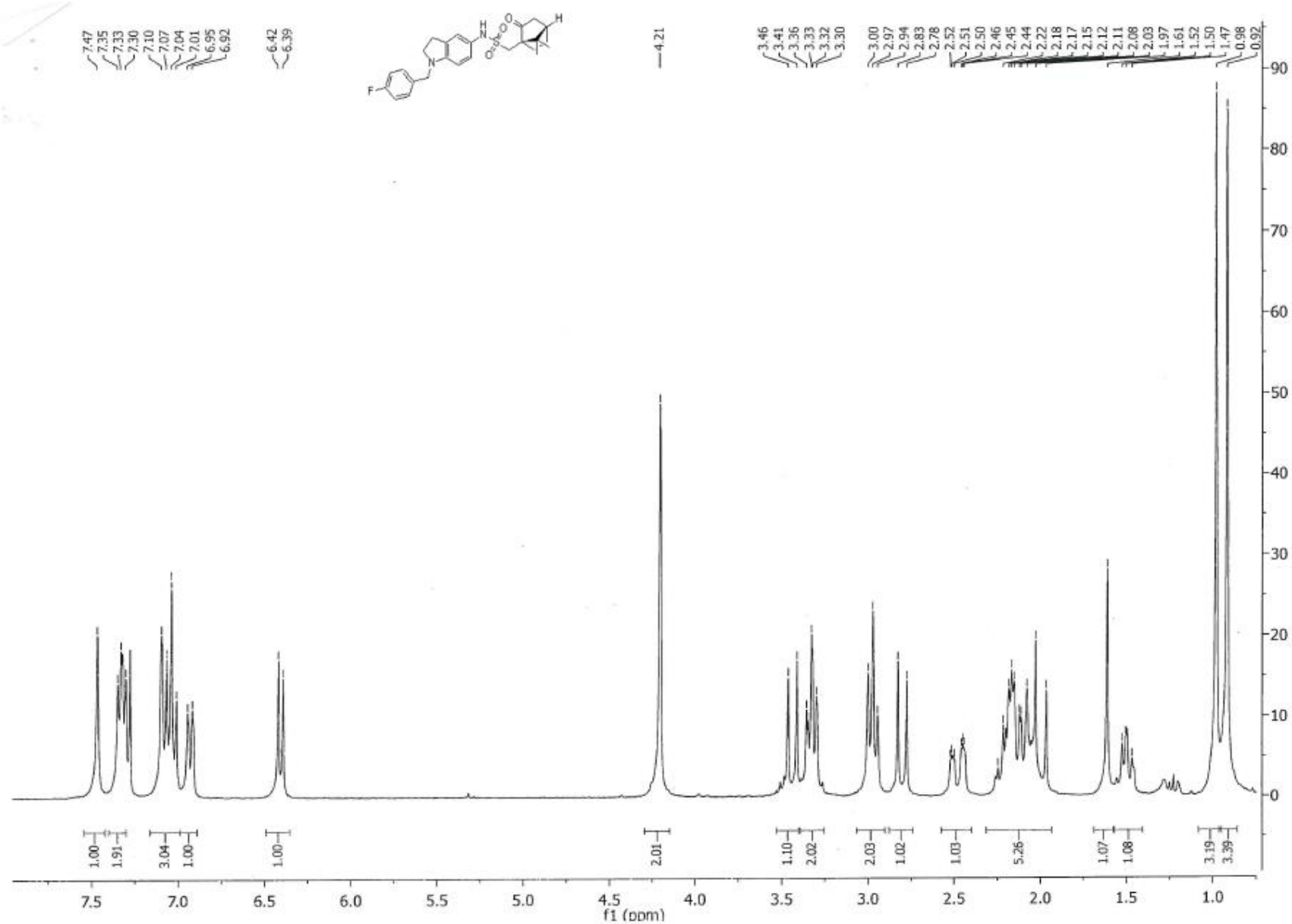
¹H NMR of compound **8f**



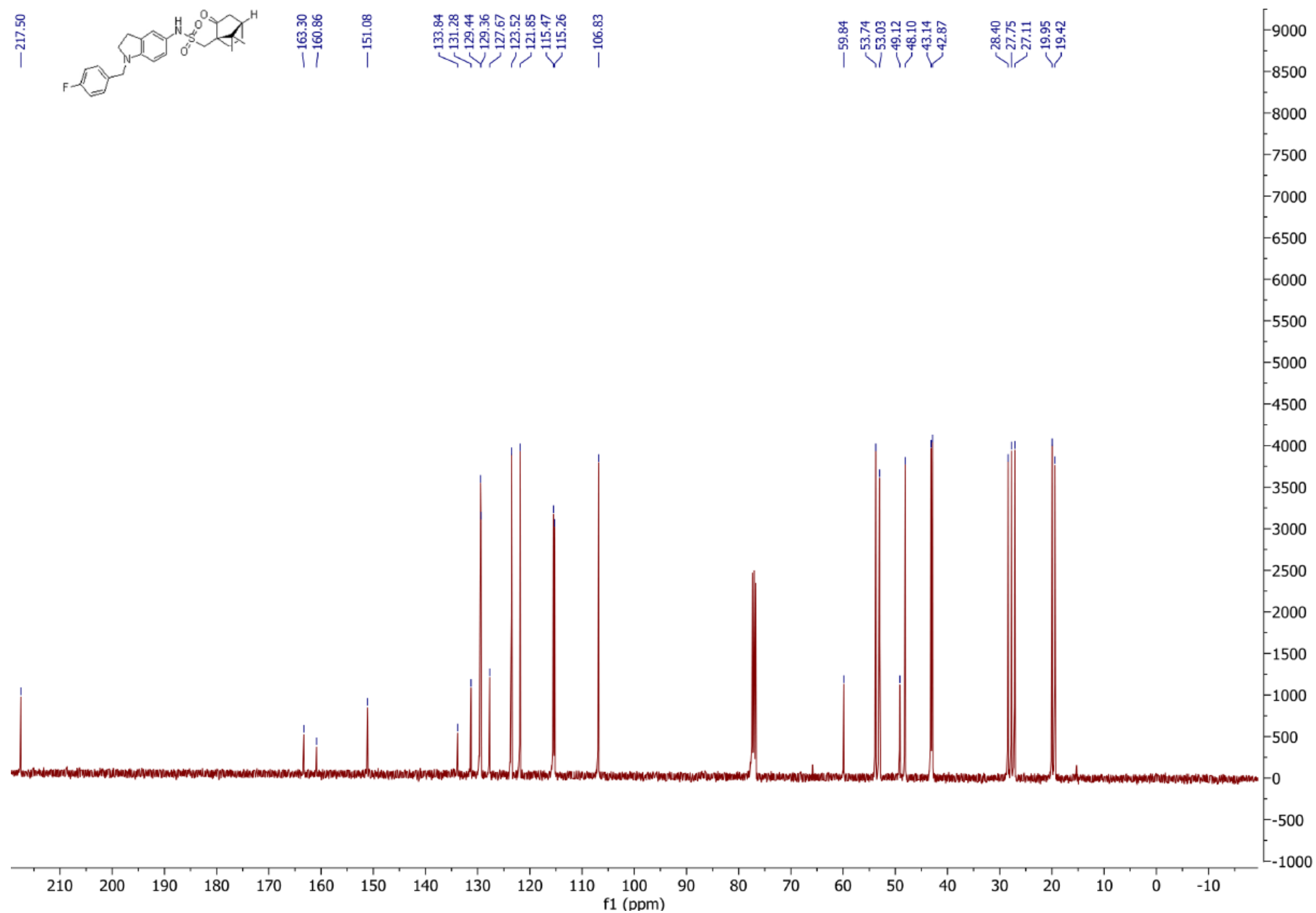
^{13}C NMR of compound **8f**



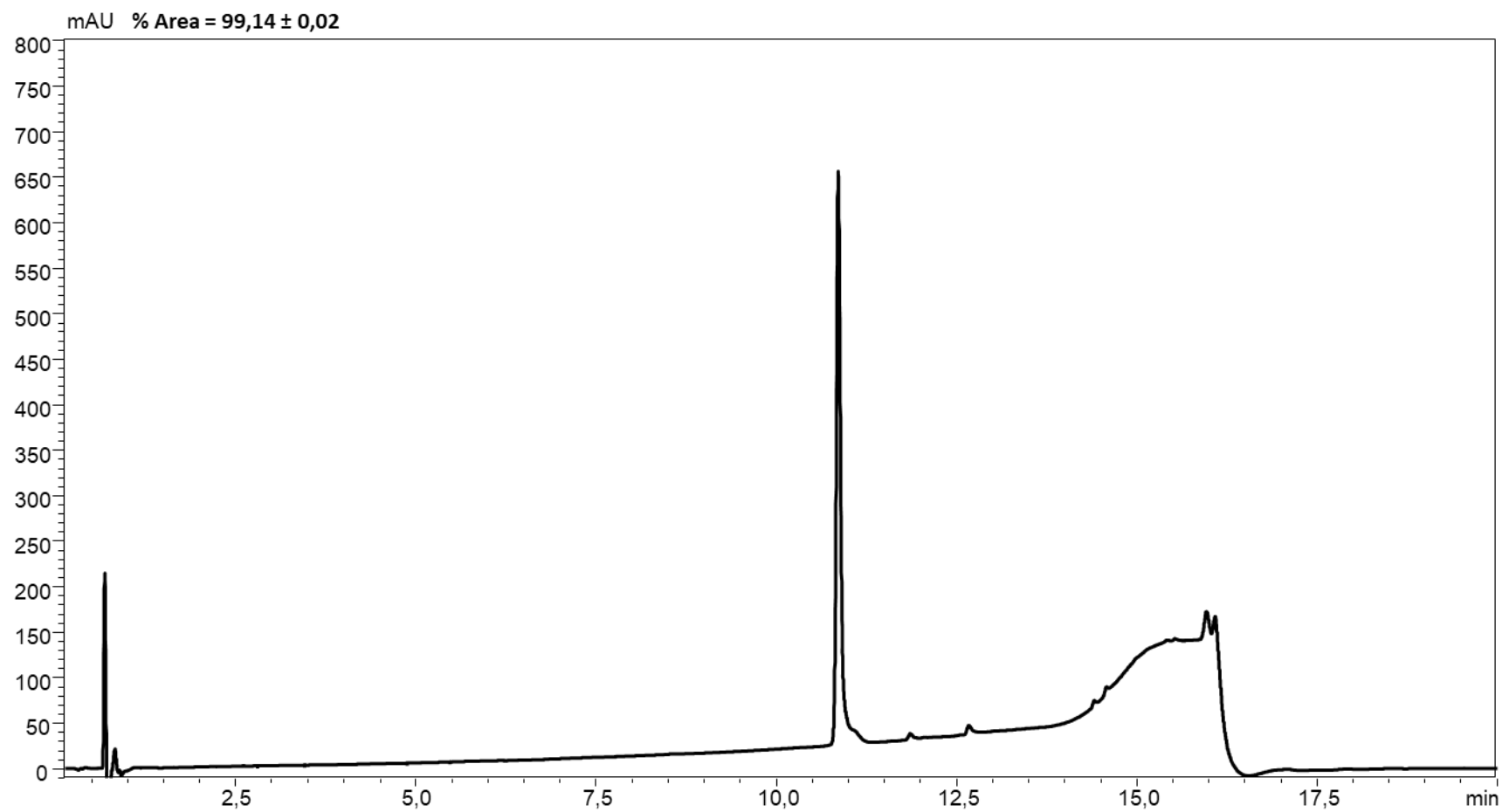
RP-HPLC of compound **8f**



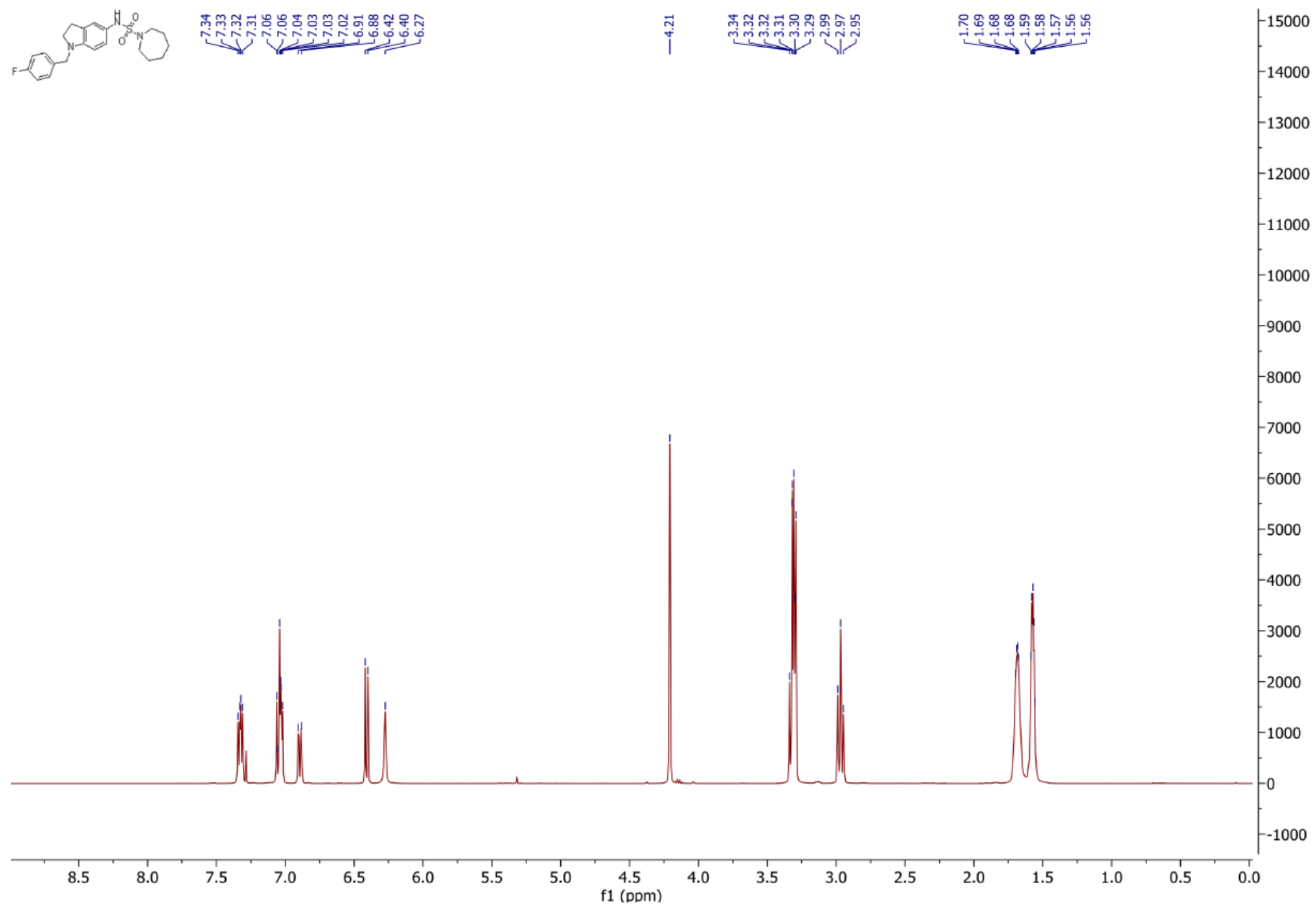
^1H NMR of compound **8g**

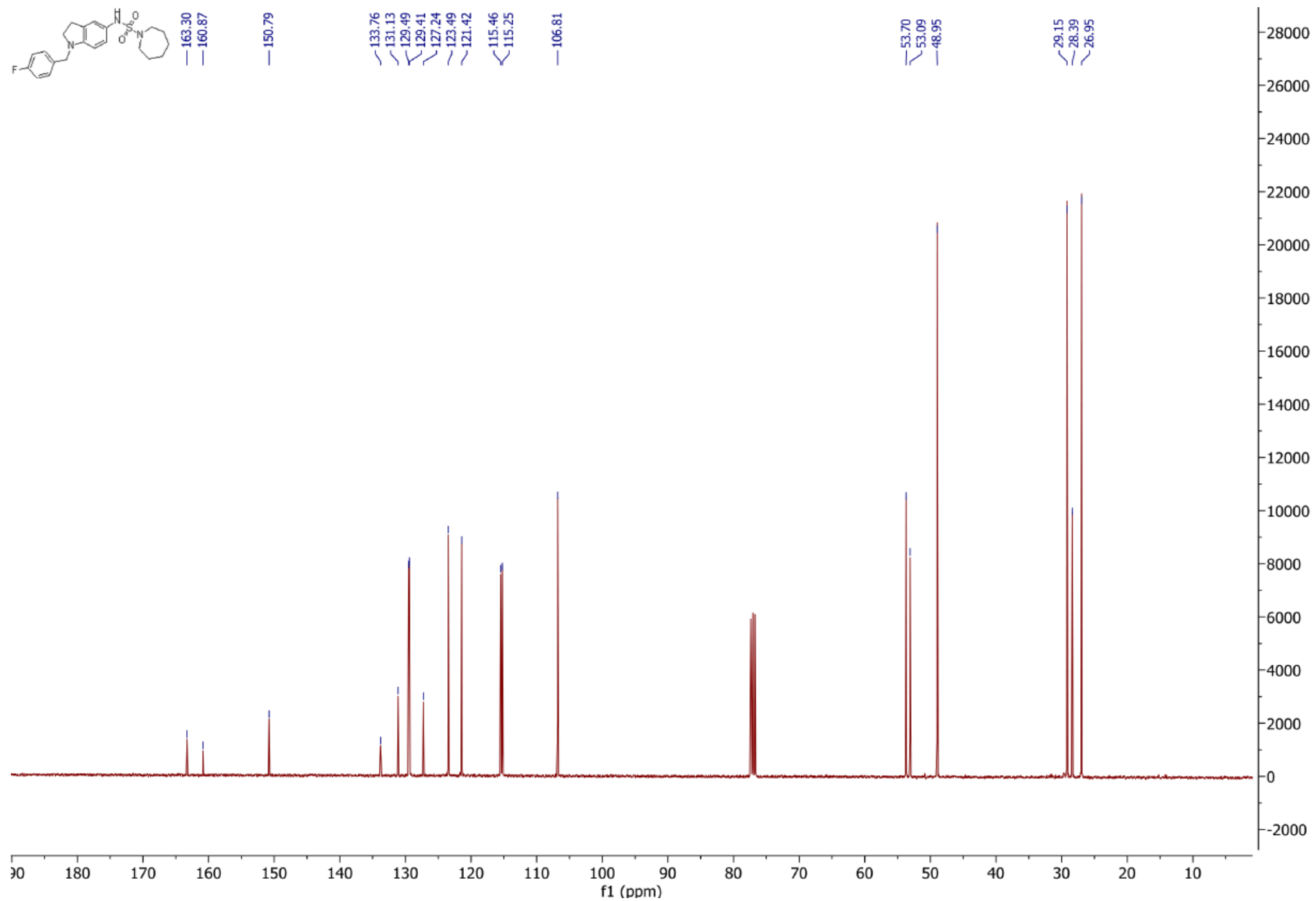


¹³C NMR of compound **8g**

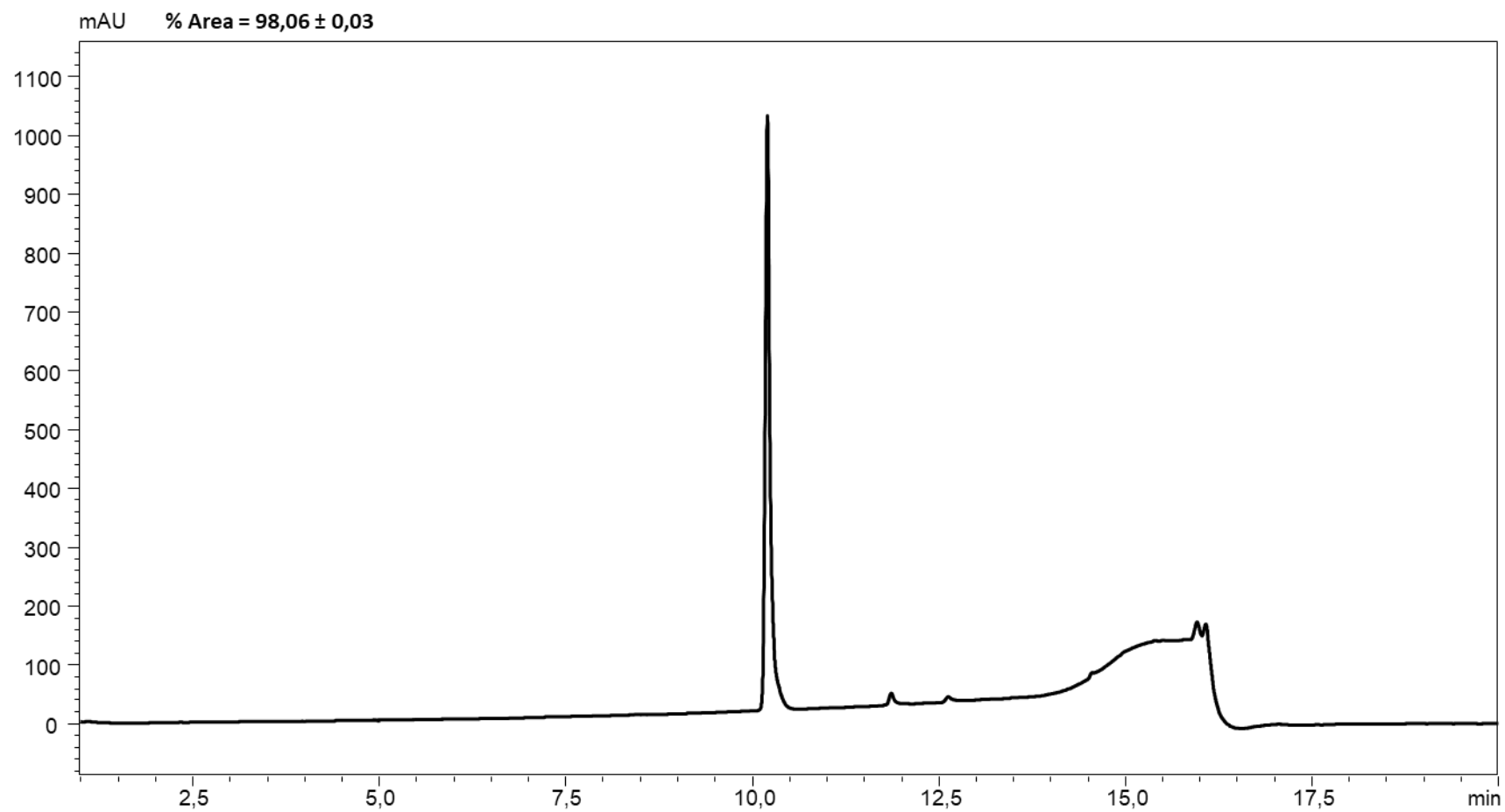


RP-HPLC trace of compound **8g**

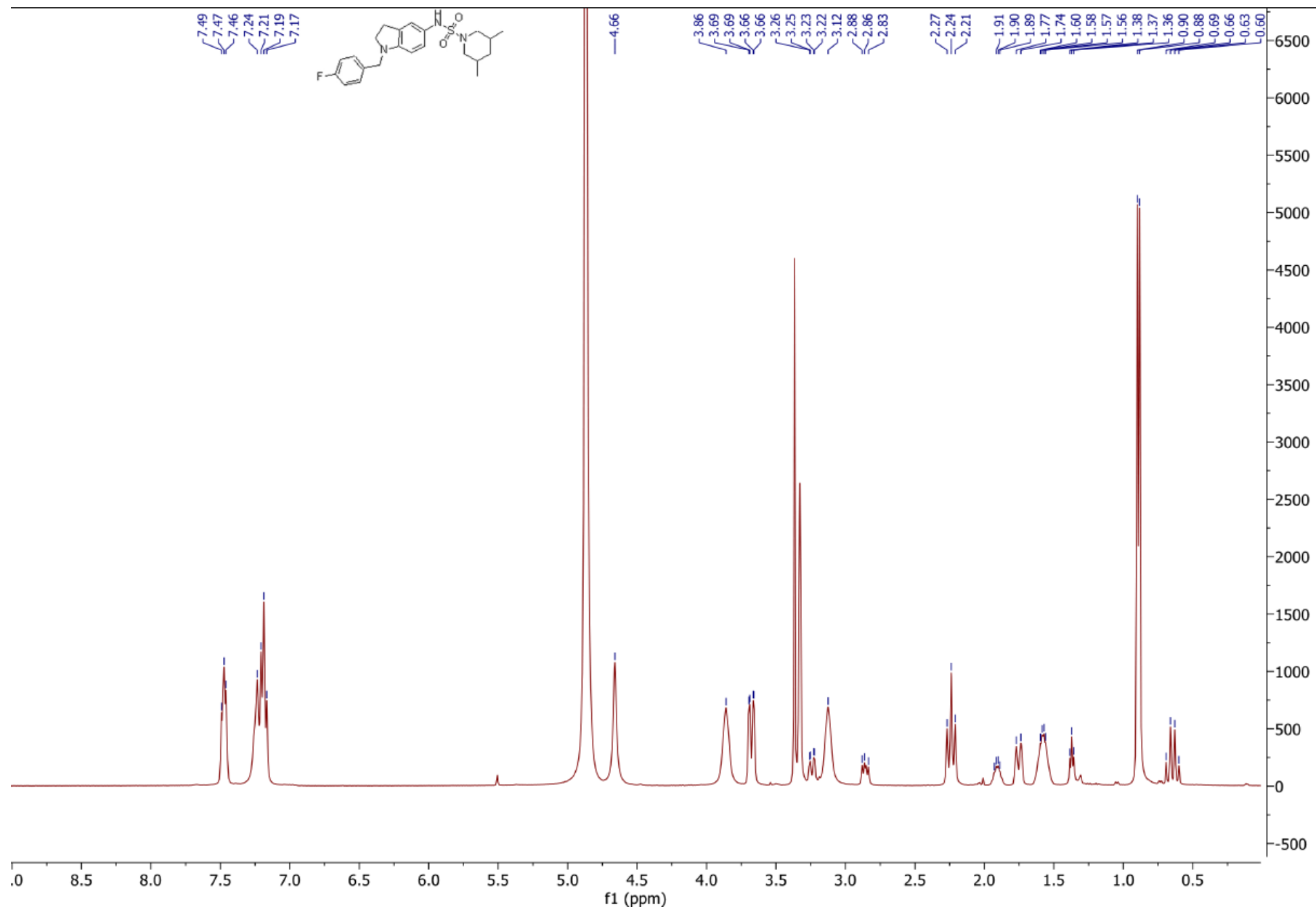




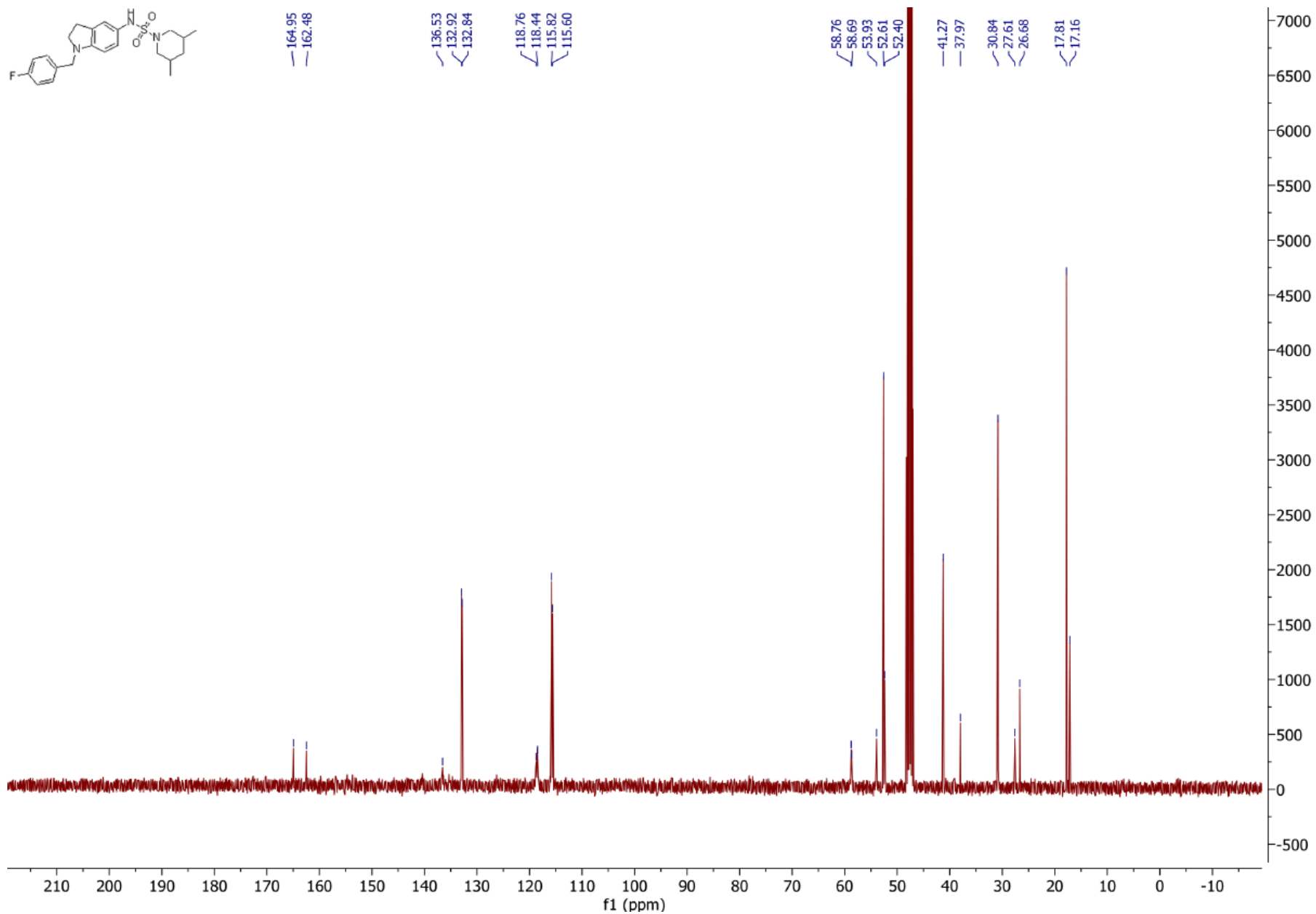
¹³C NMR of compound **8h**

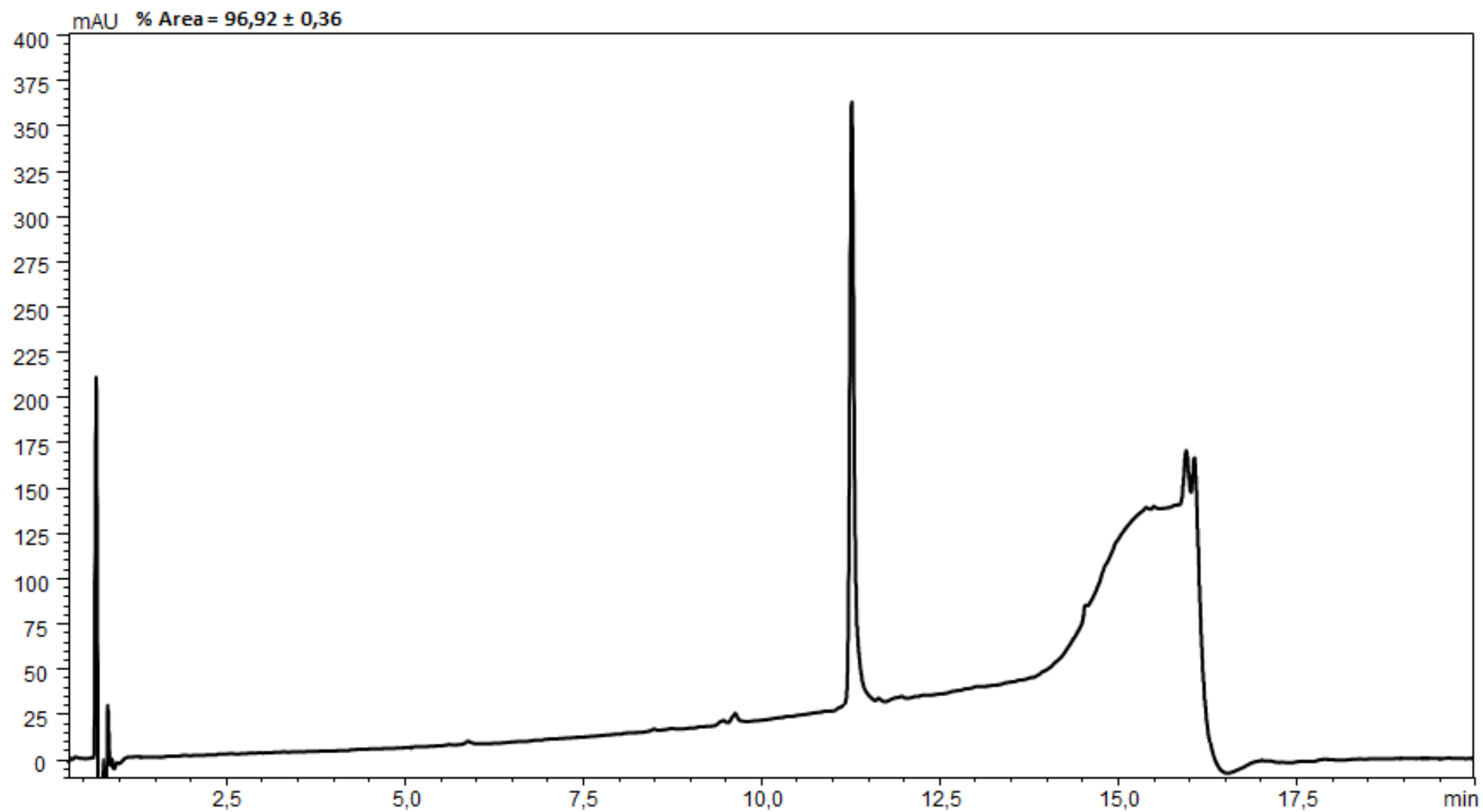


RP-HPLC trace of compound **8h**

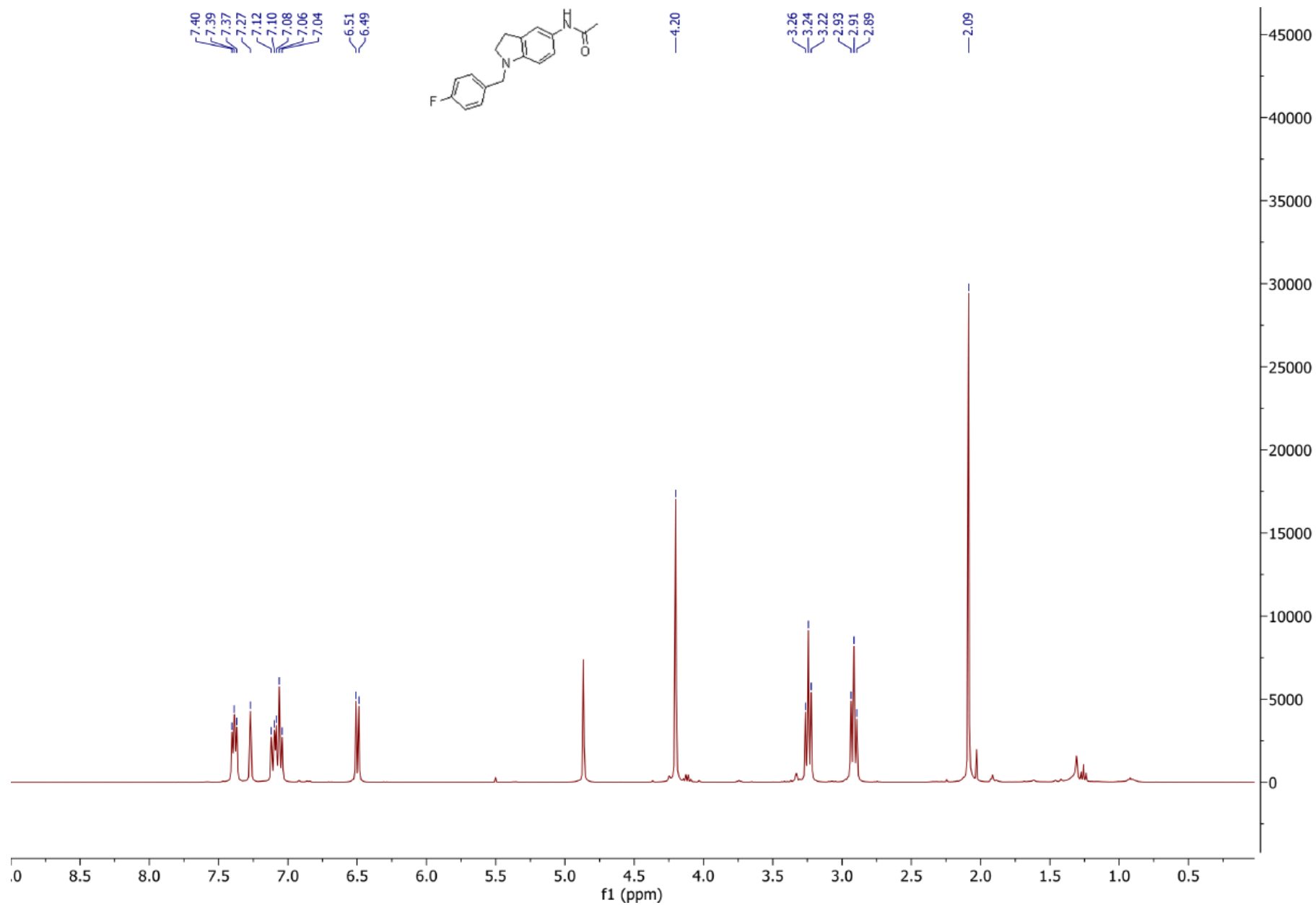


¹H NMR of compound **8i**

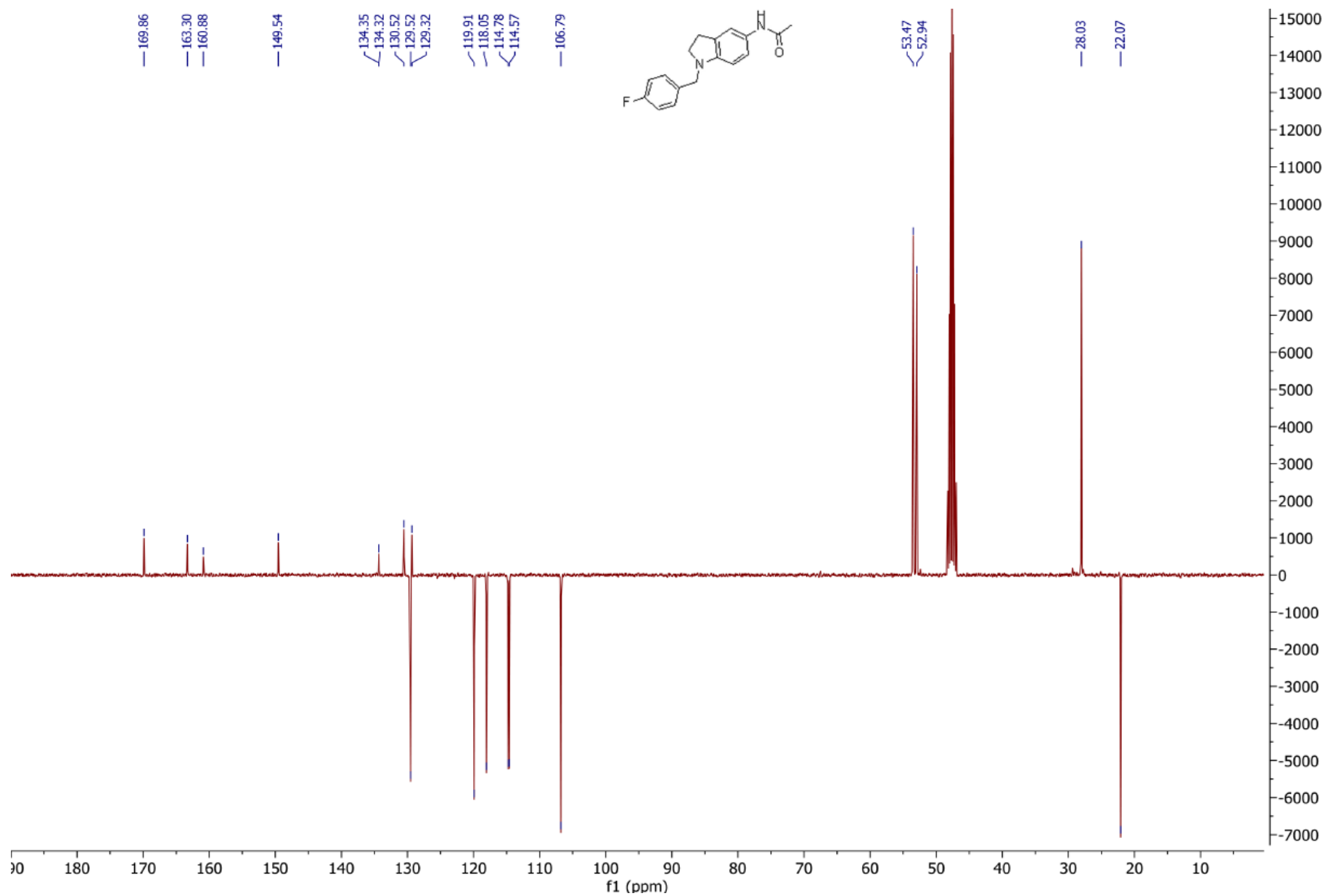




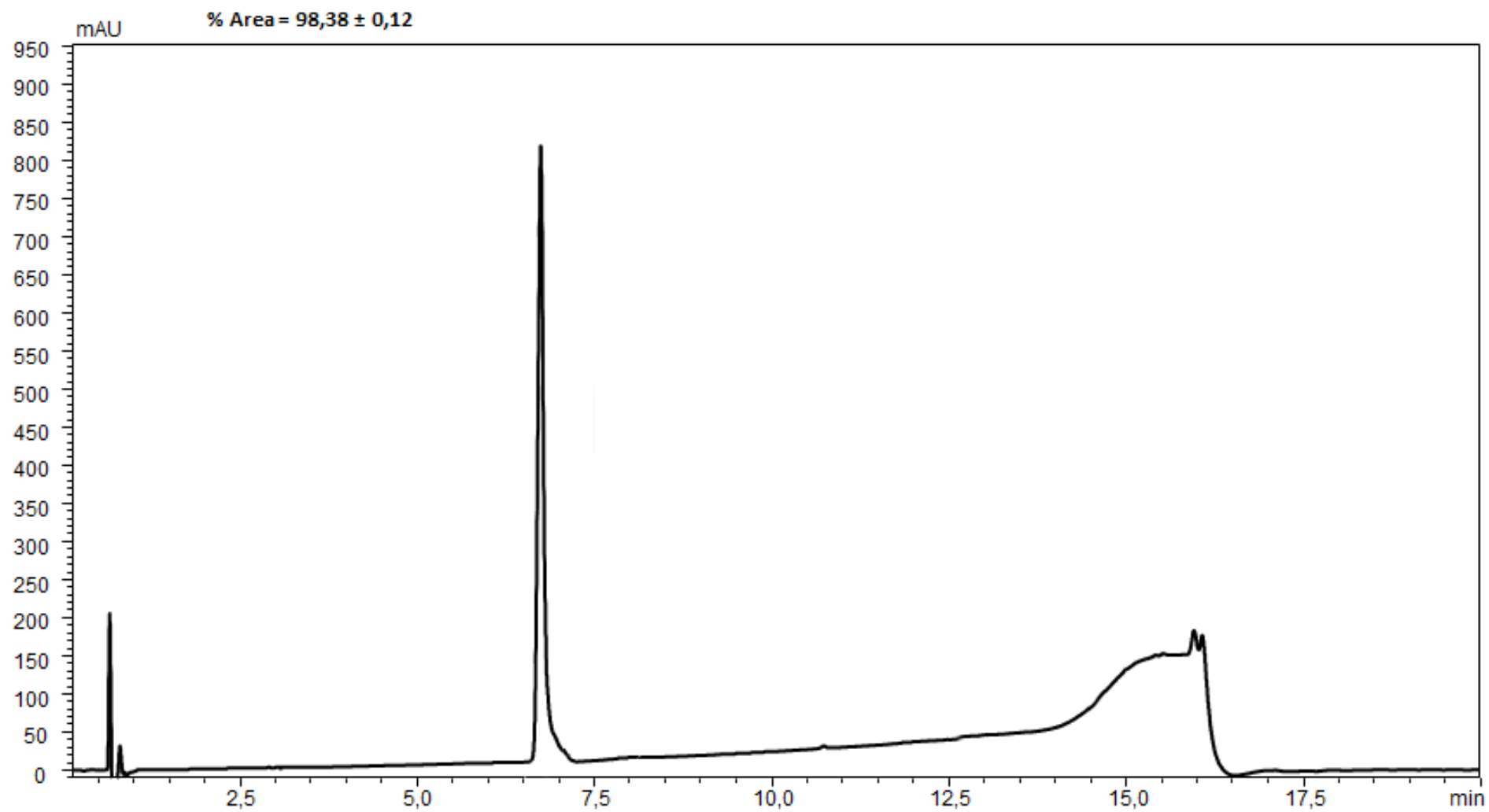
RP-HPLC of compound **8i**



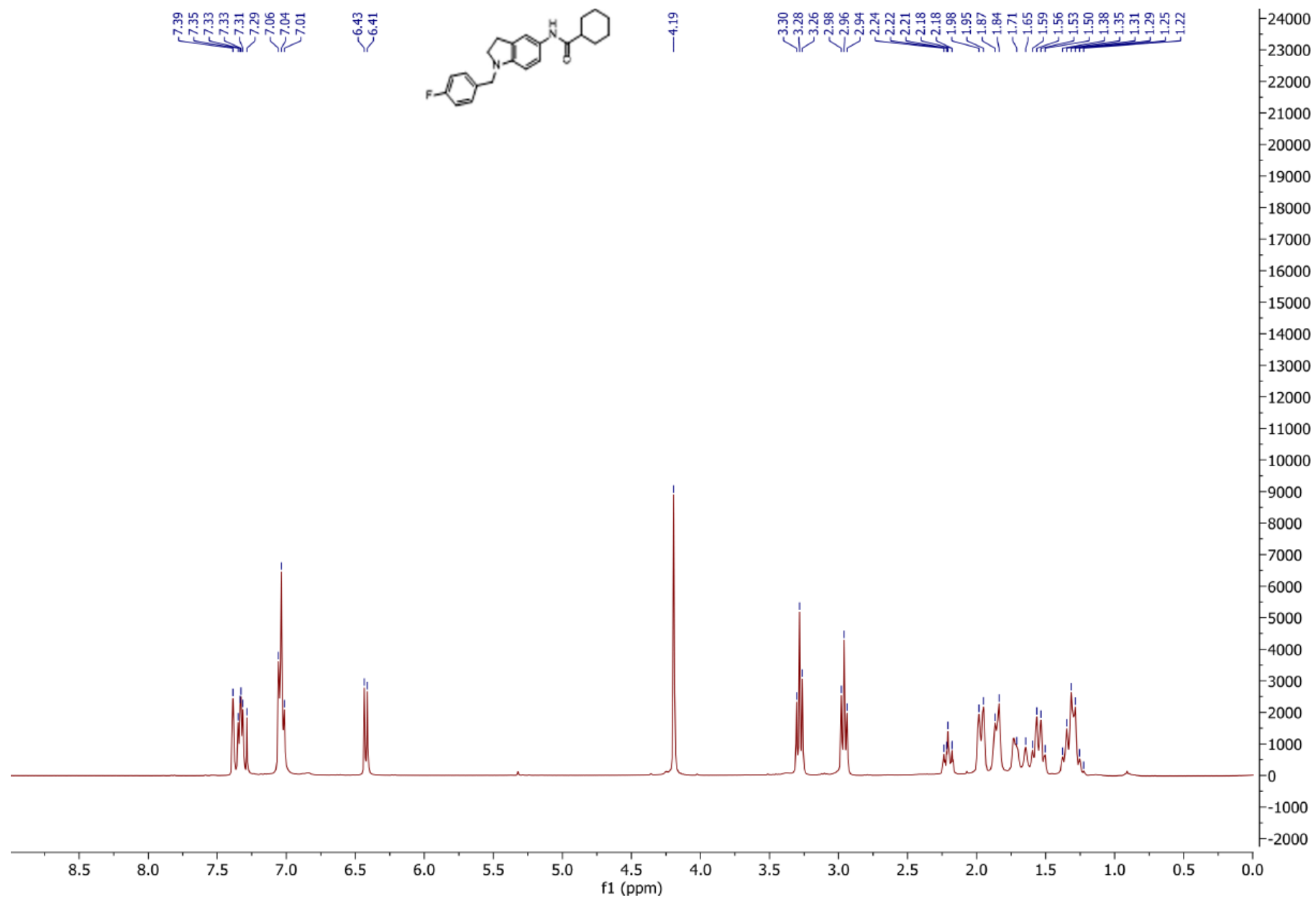
¹H NMR of compound **9a**



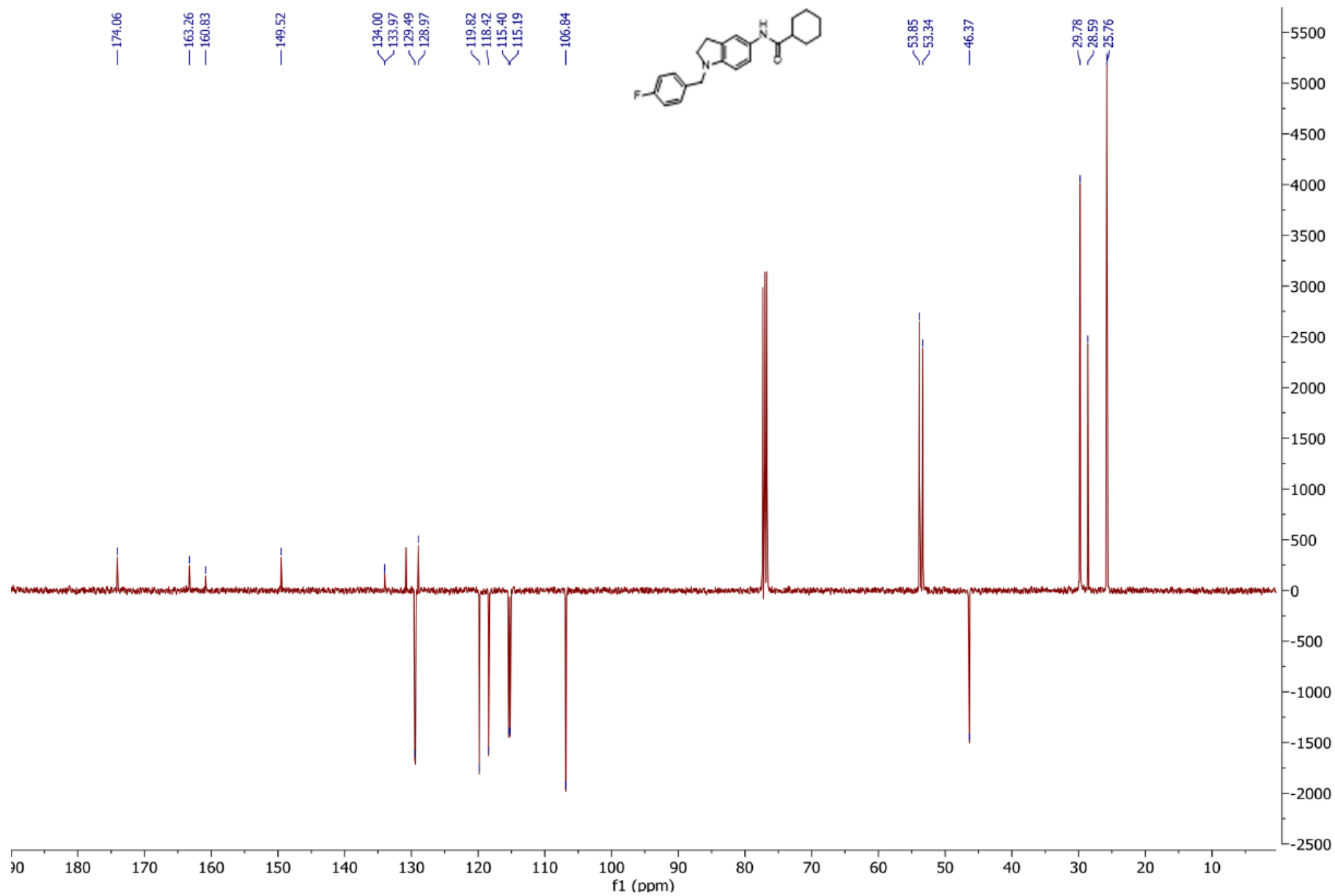
qDEPT NMR of compound **9a**



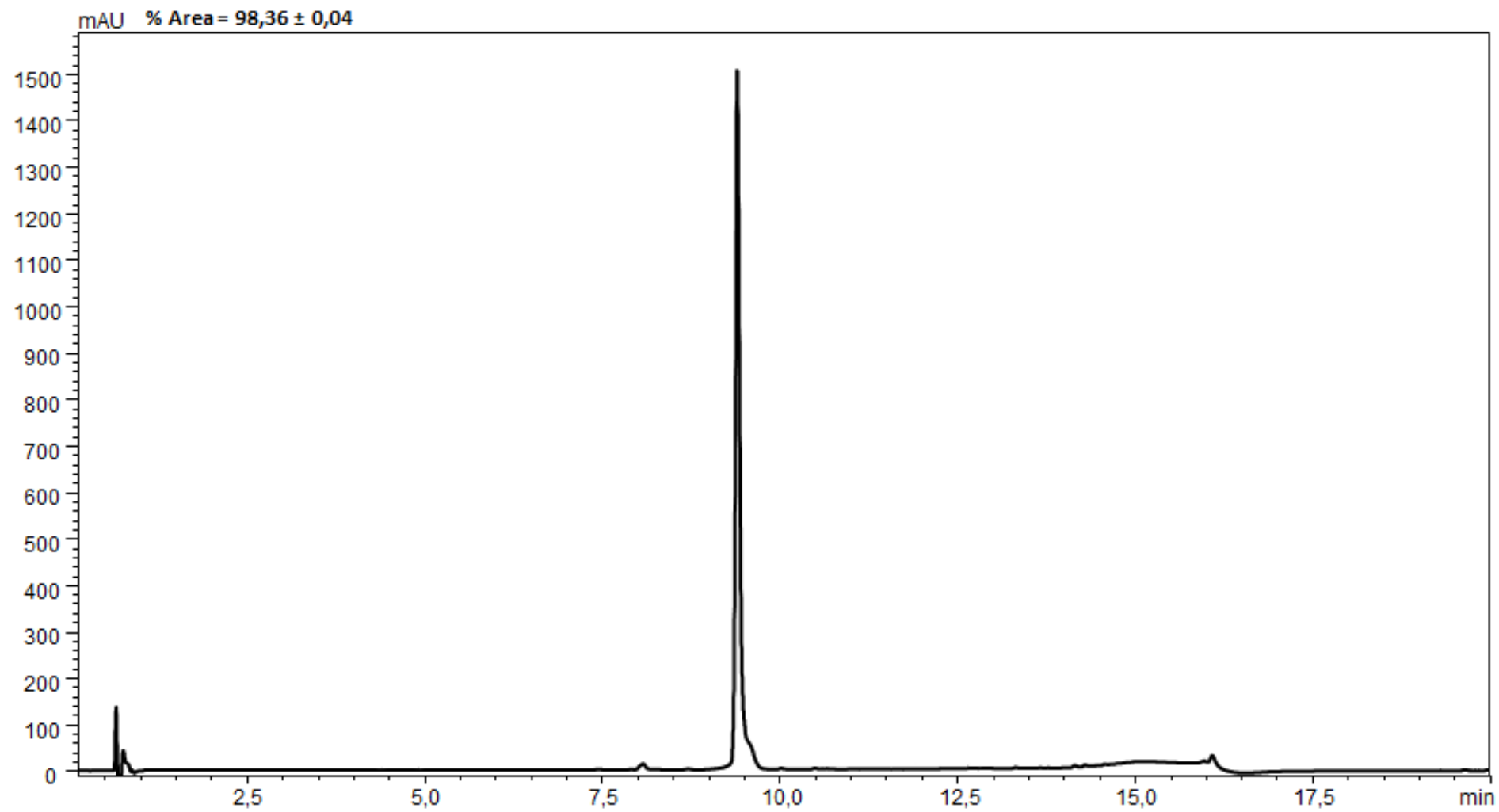
RP-HPLC of compound **9a**



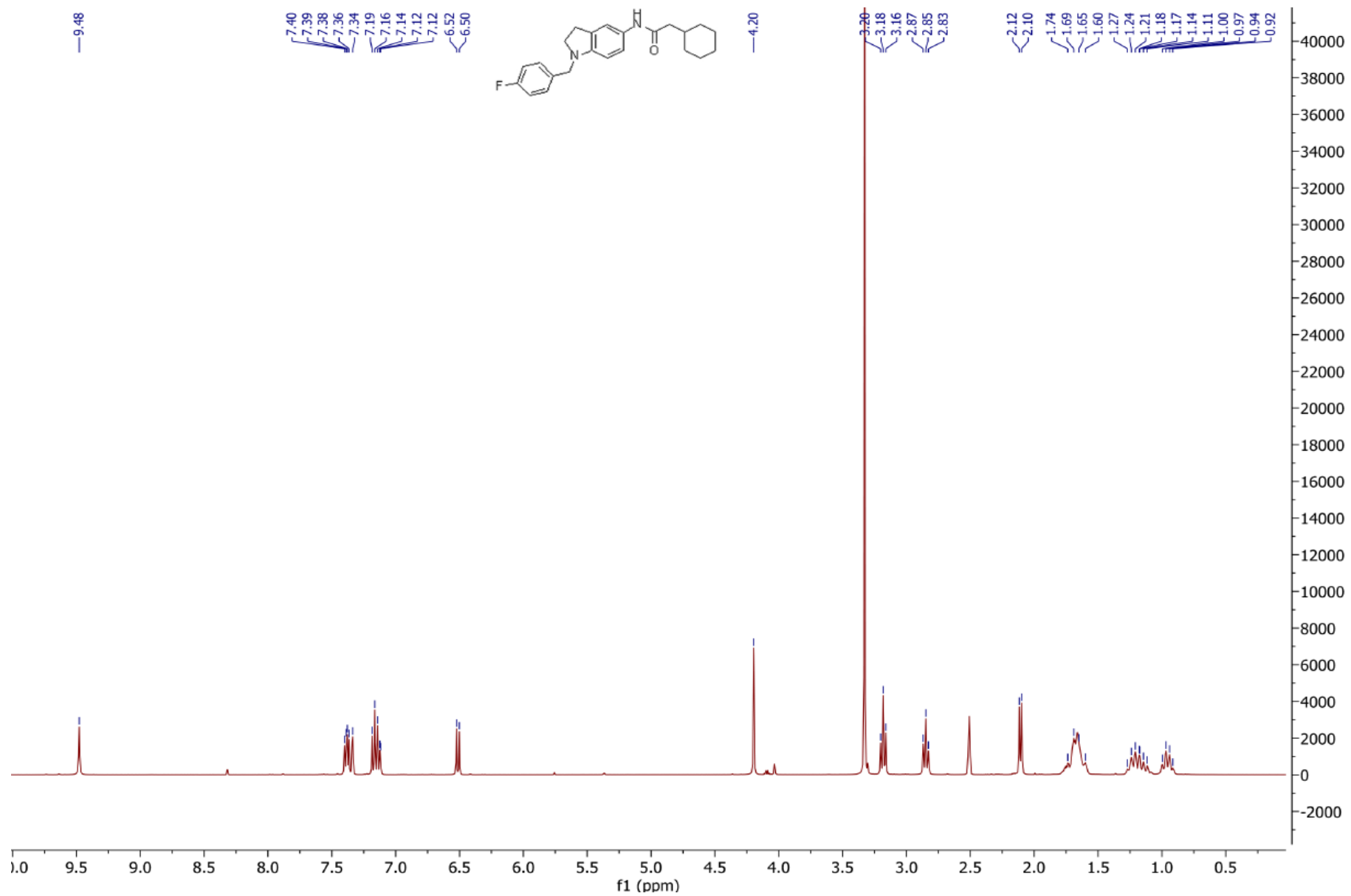
¹H NMR of compound **9b**



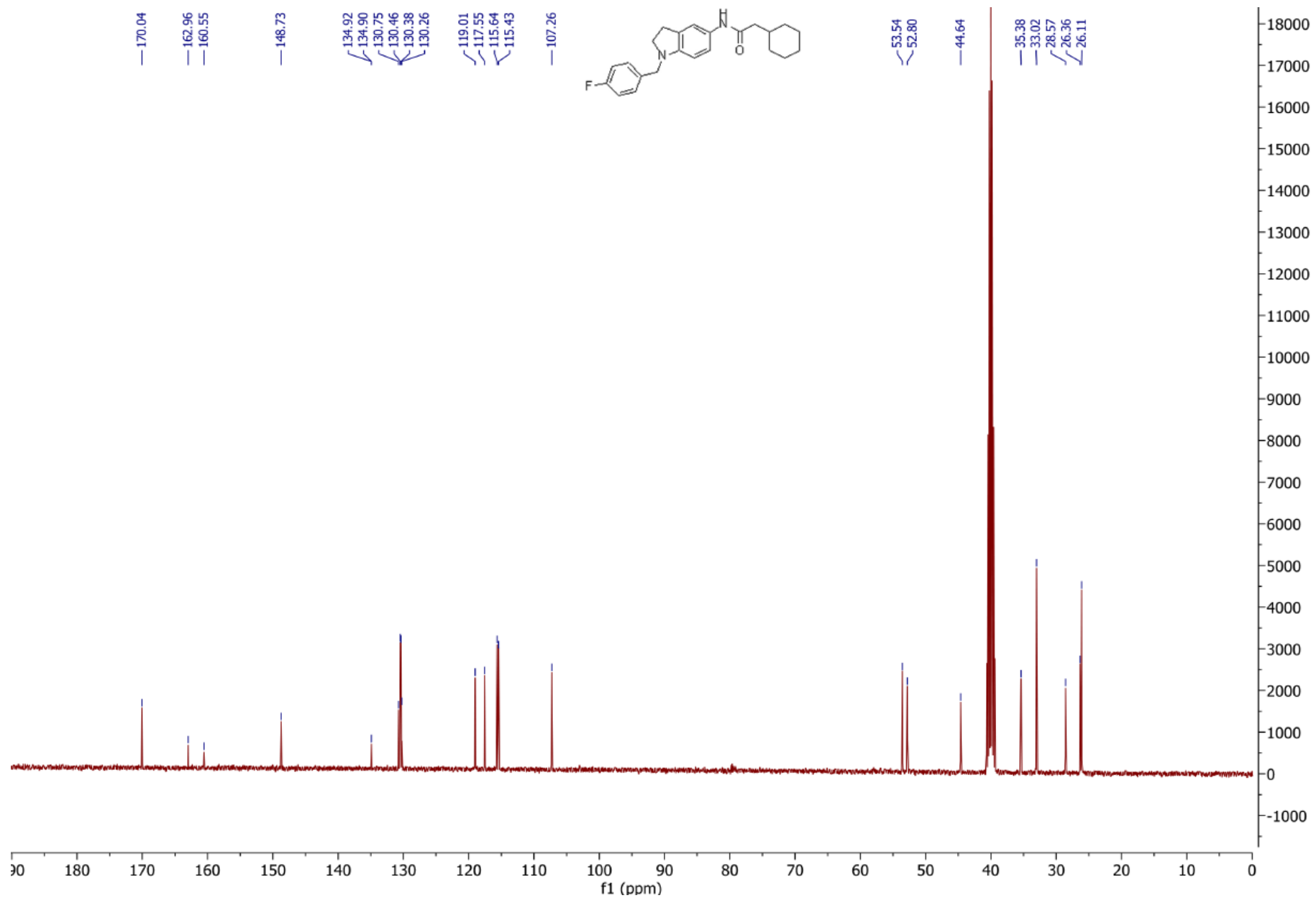
qDEPT NMR of compound **9b**



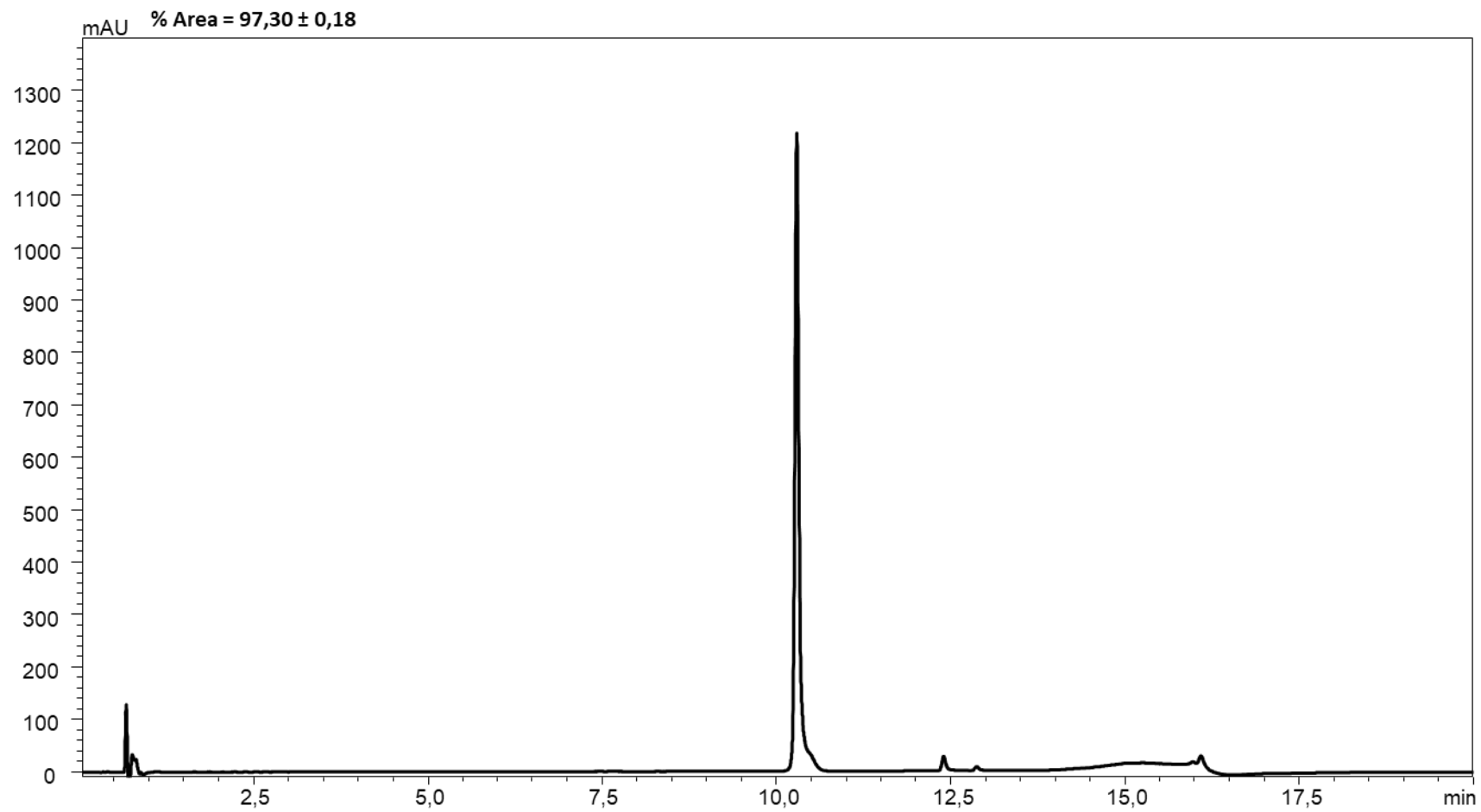
RP-HPLC trace of compound **9b**



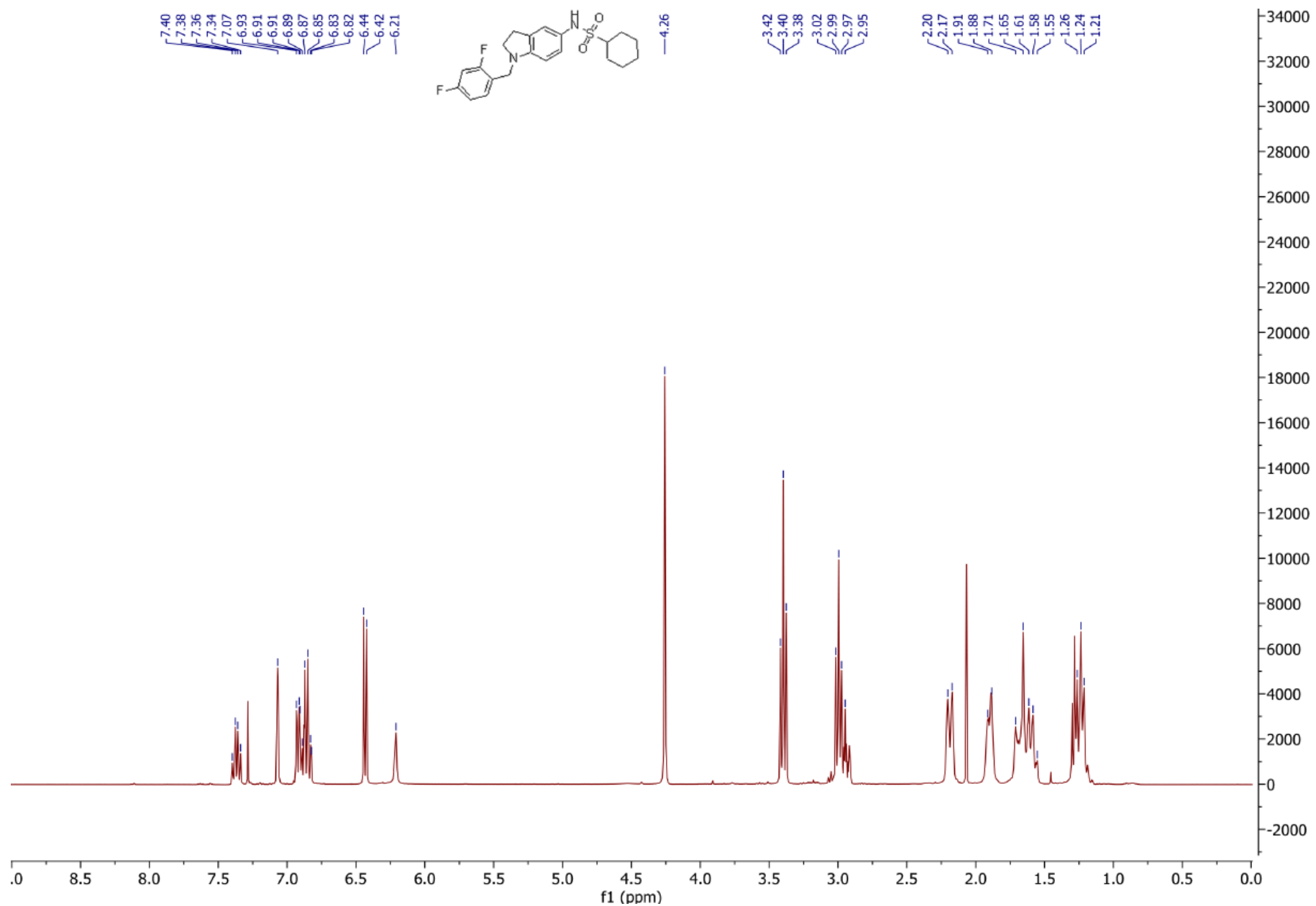
¹H NMR of compound **9c**



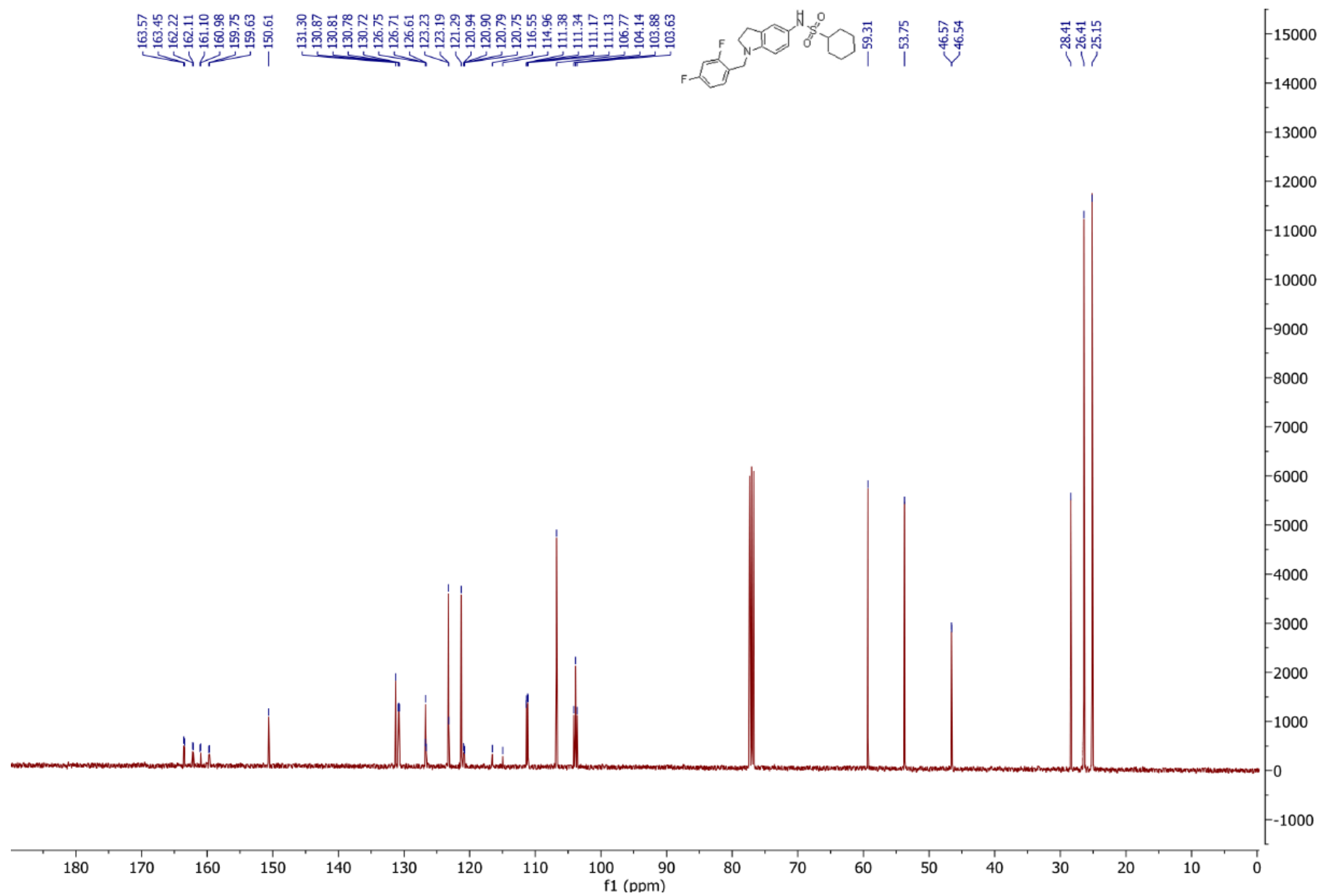
¹³C NMR of compound **9c**



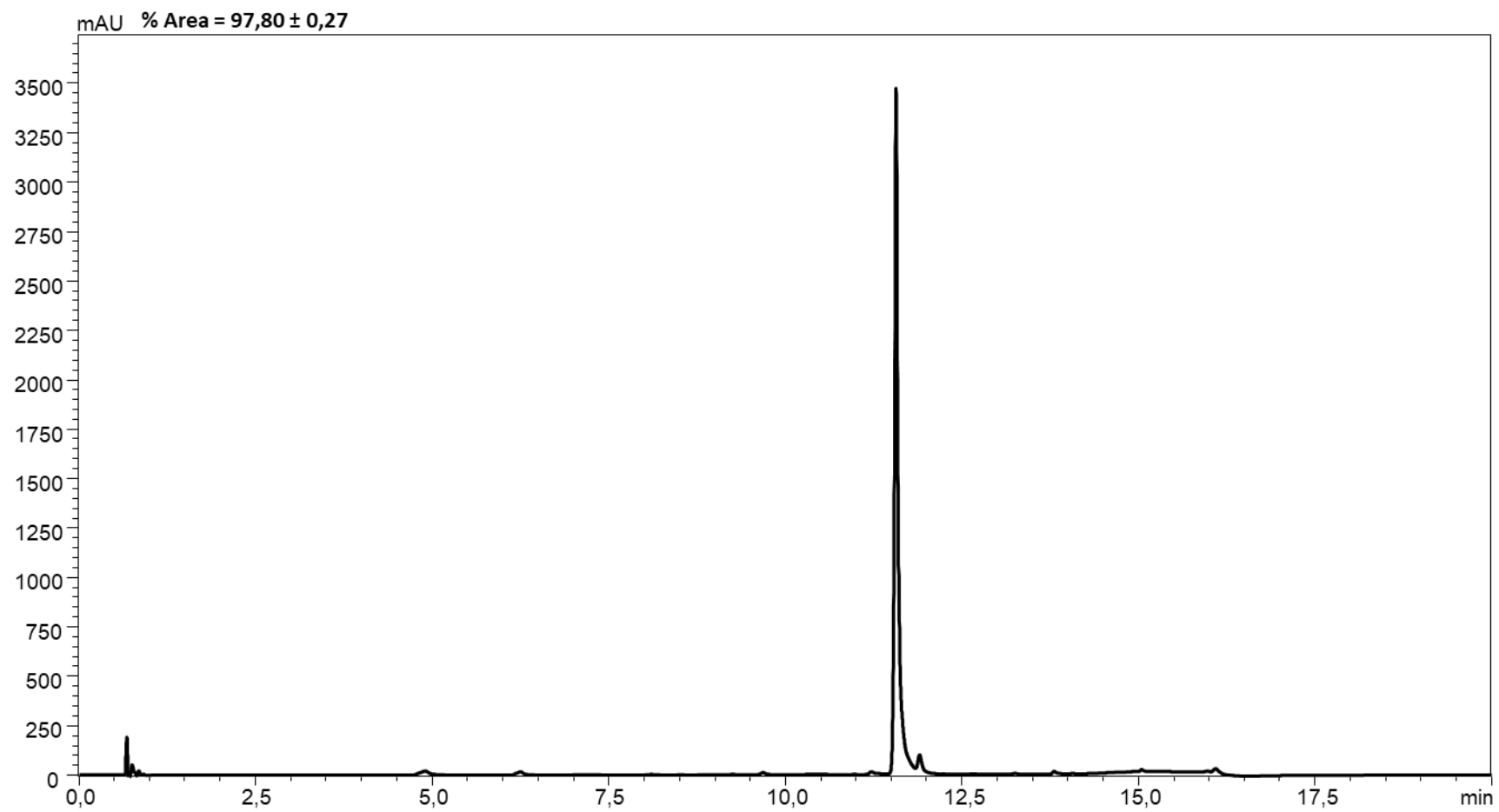
RP-HPLC trace of compound **9c**



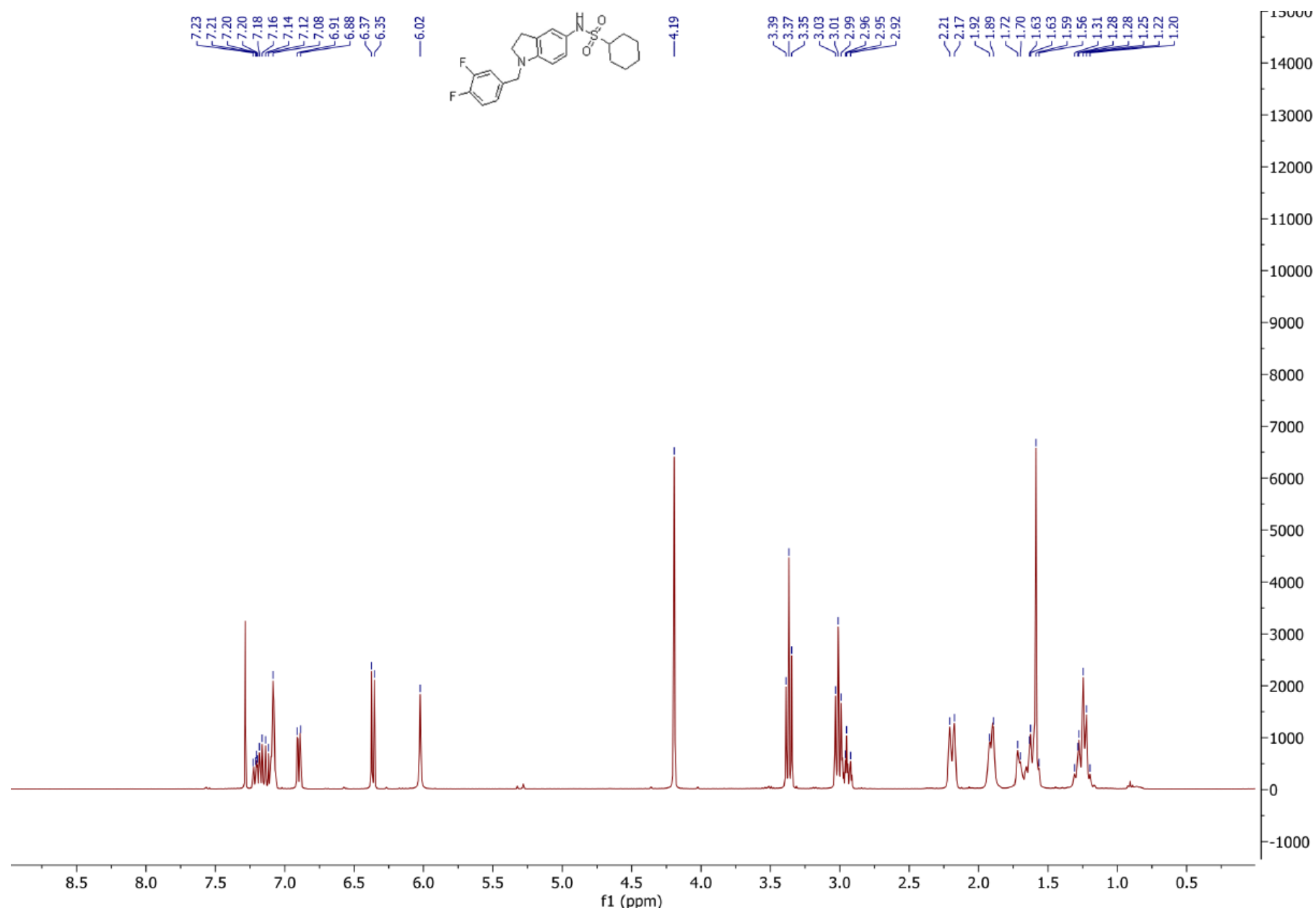
¹H NMR of compound **13a**



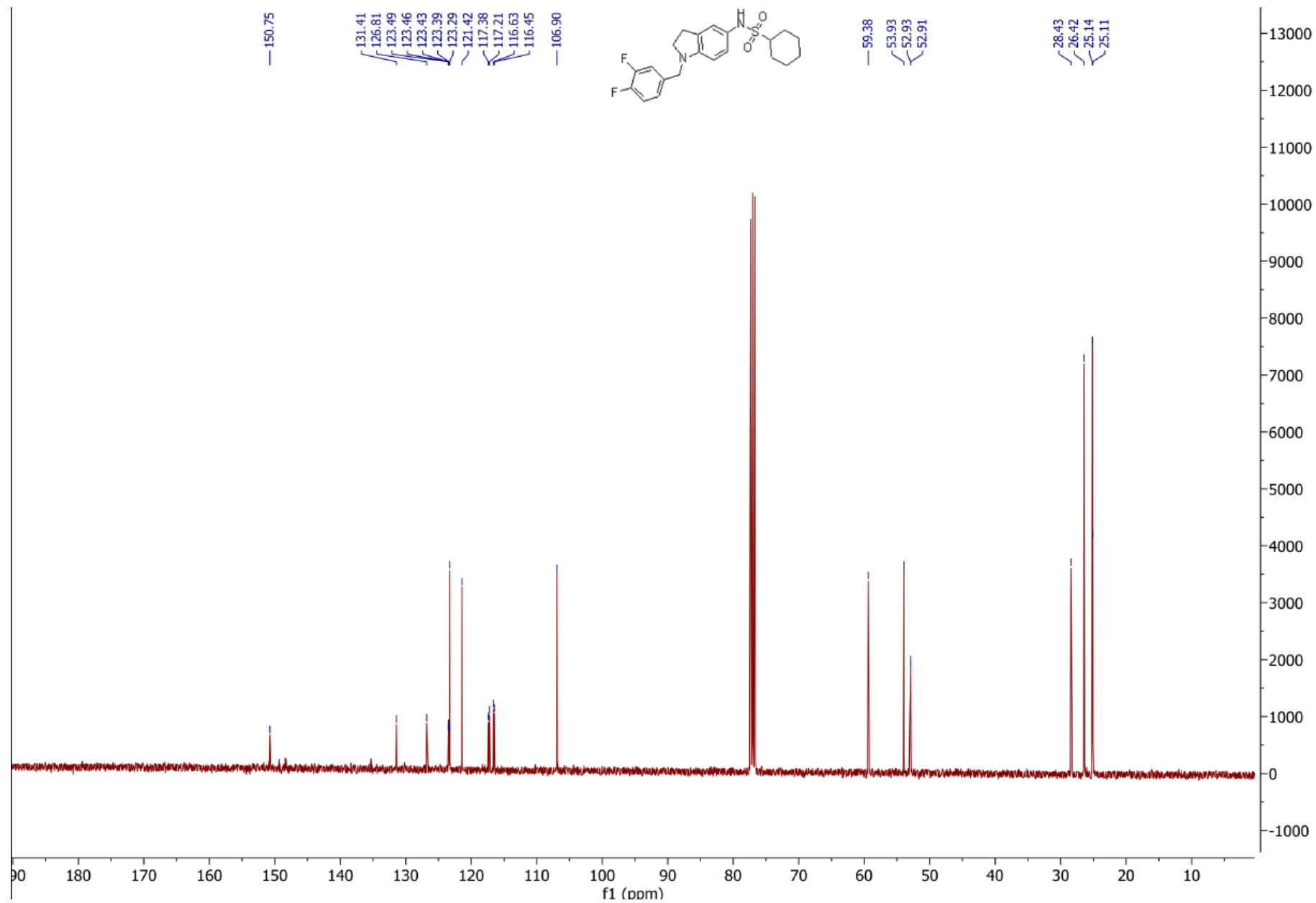
^{13}C NMR of compound **13a**



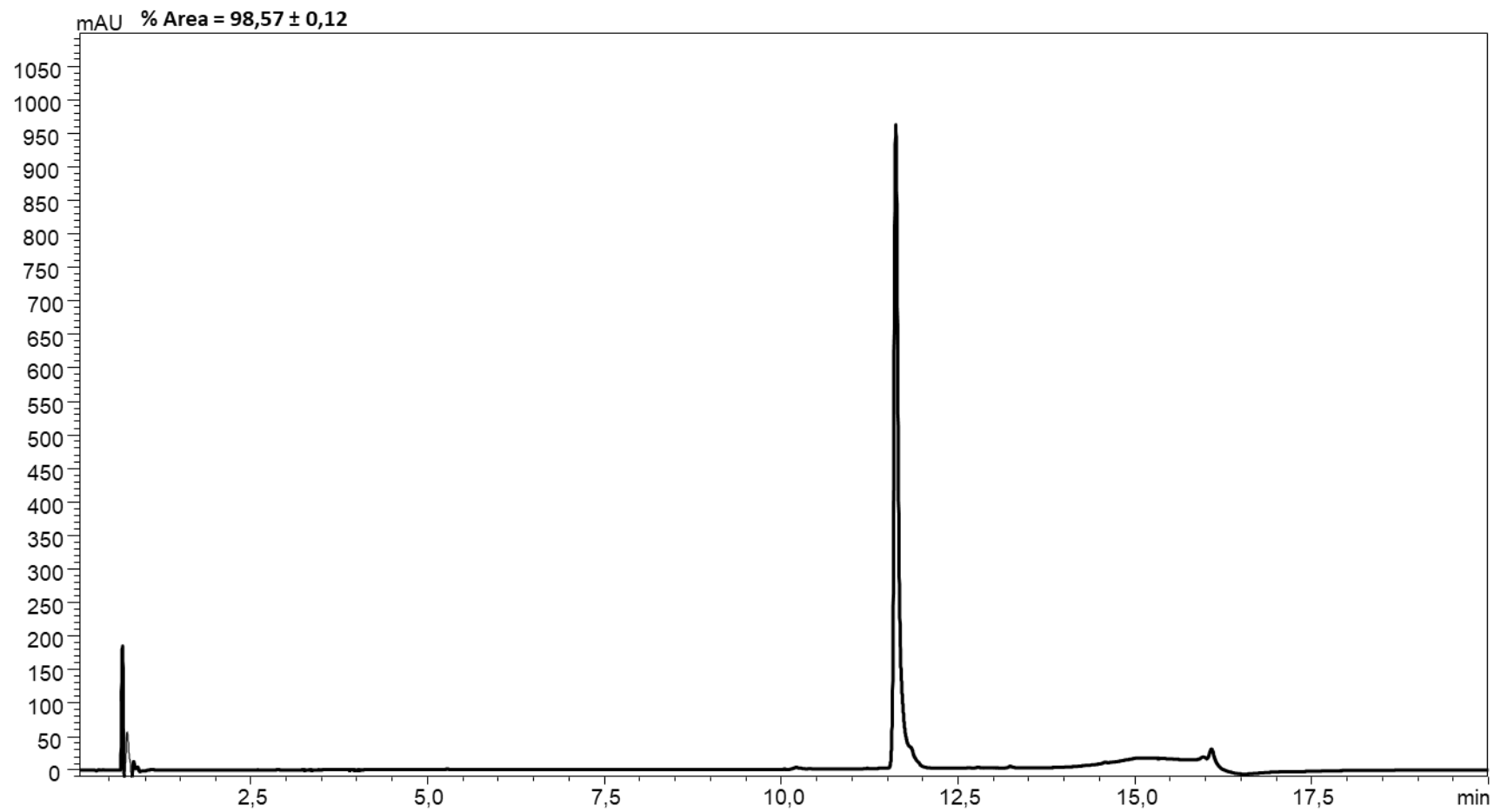
RP-HPLC trace of compound **13a**



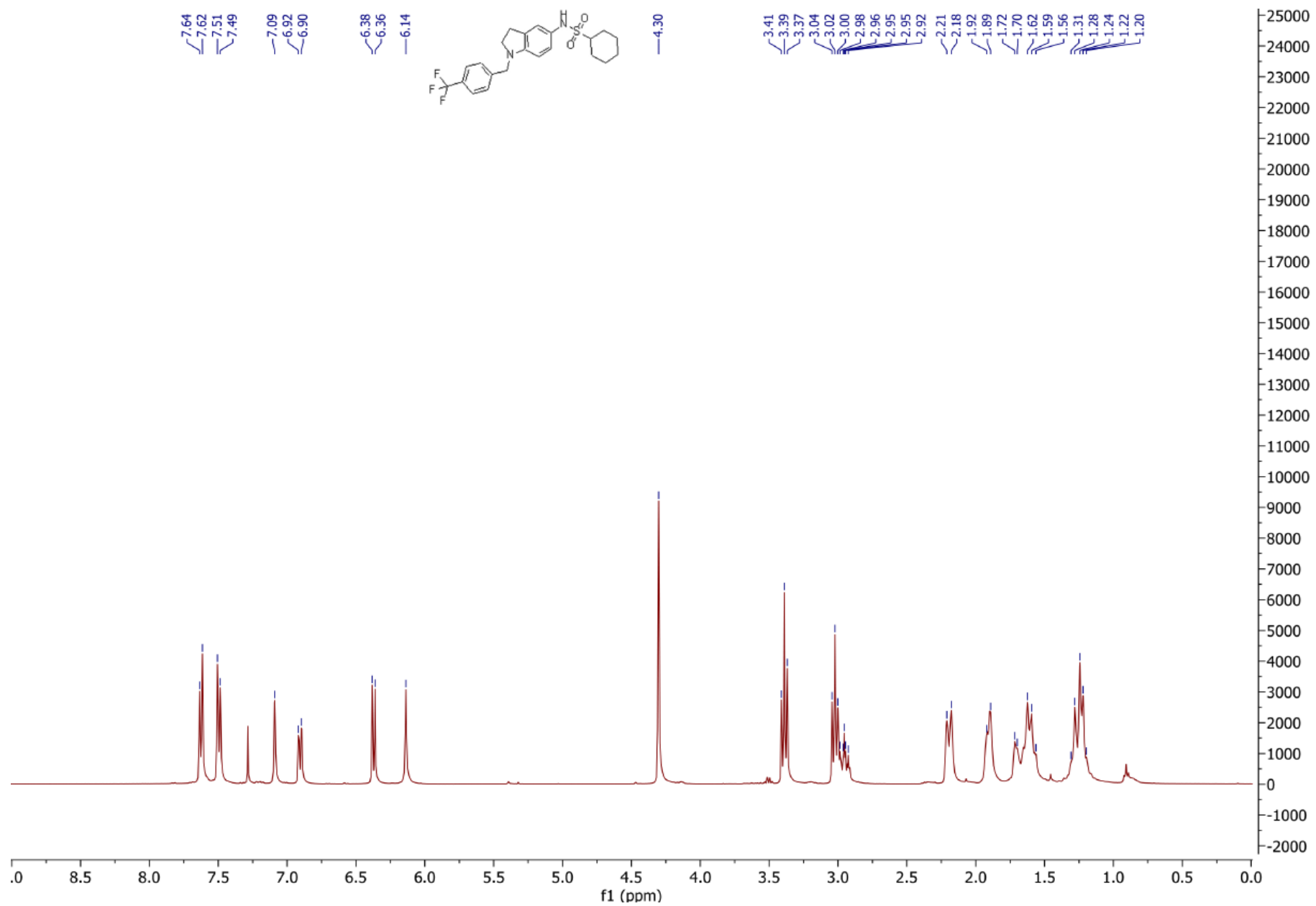
¹H NMR of compound **13b**



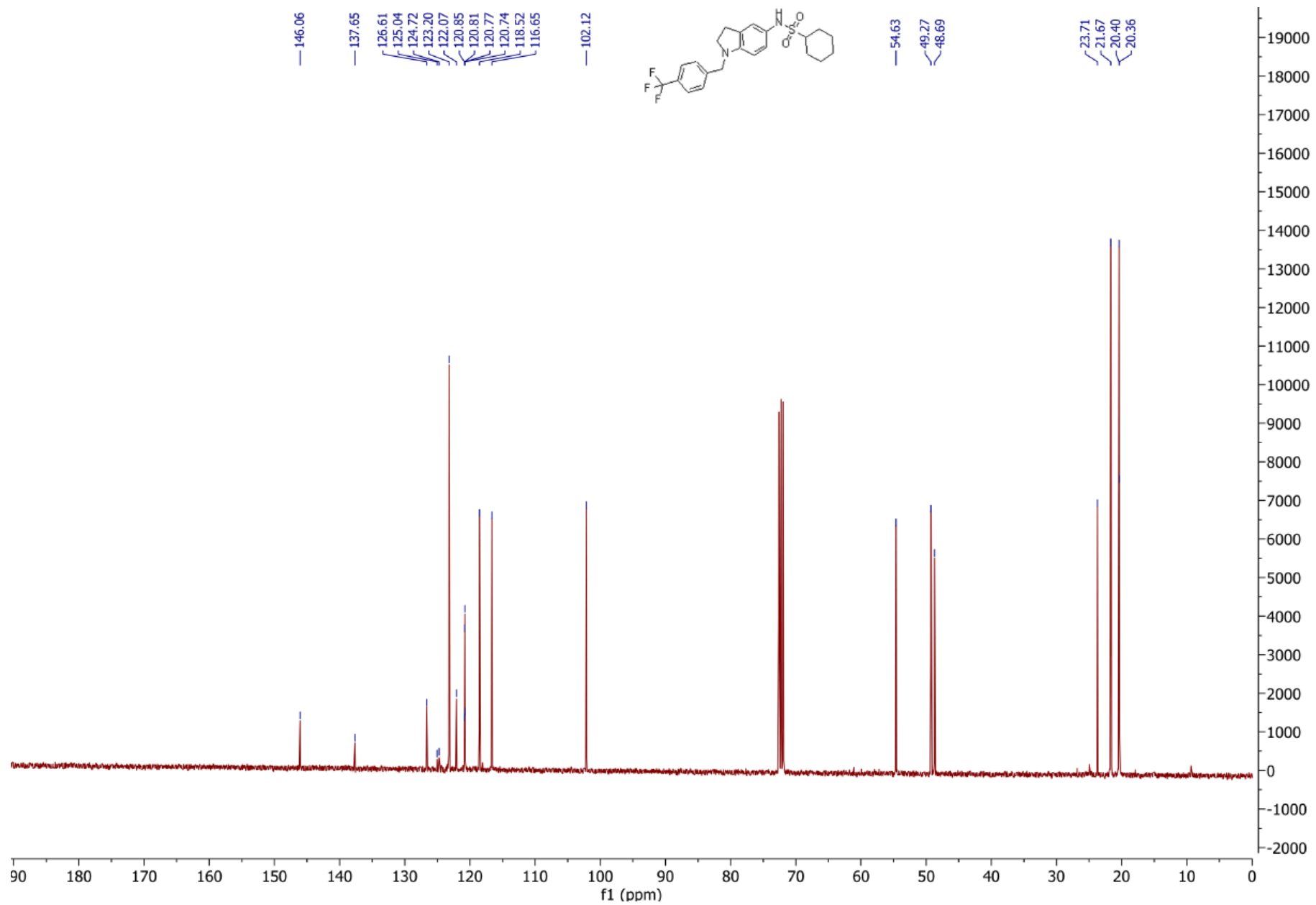
¹³C NMR of compound **13b**



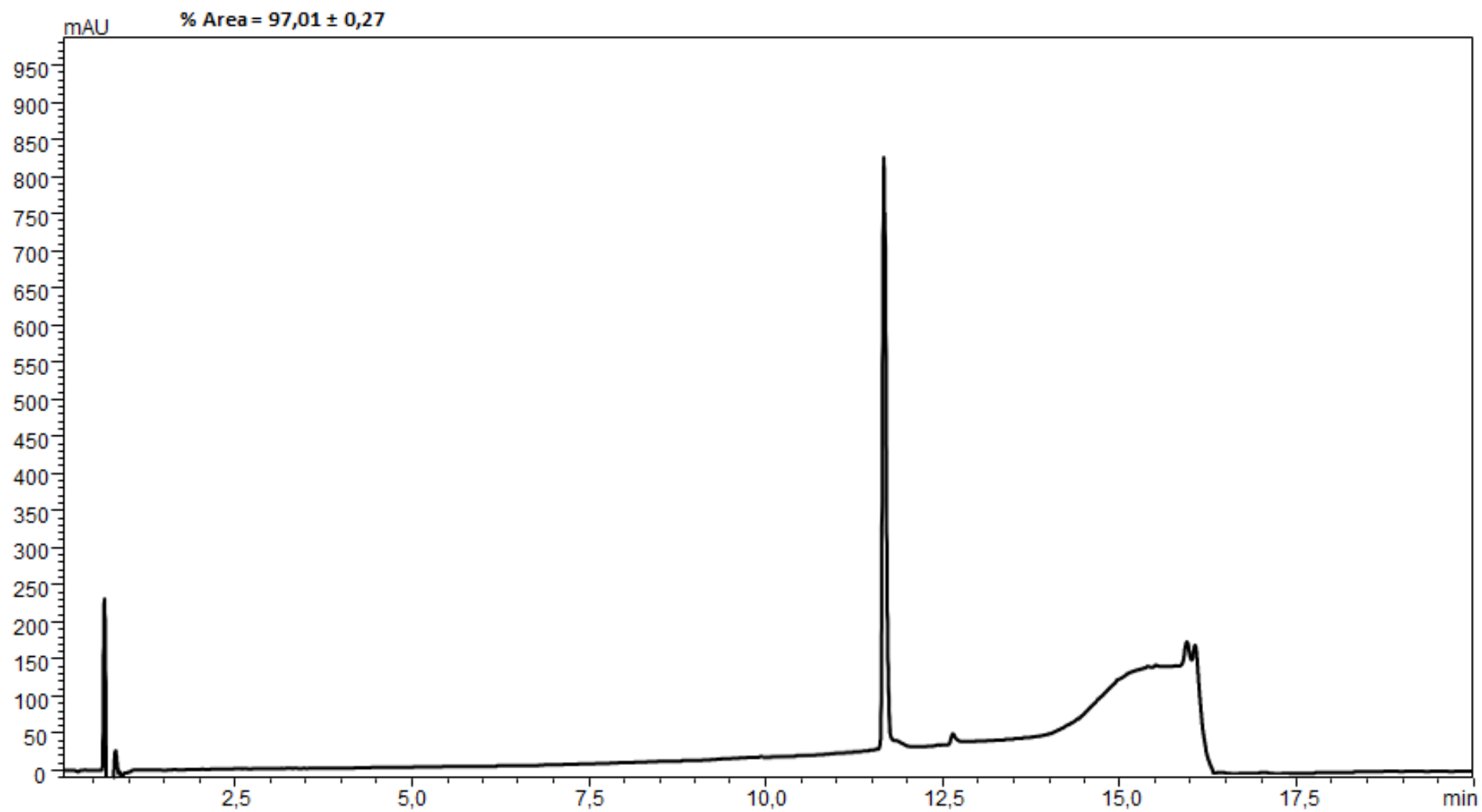
RP-HPLC trace of compound **13b**



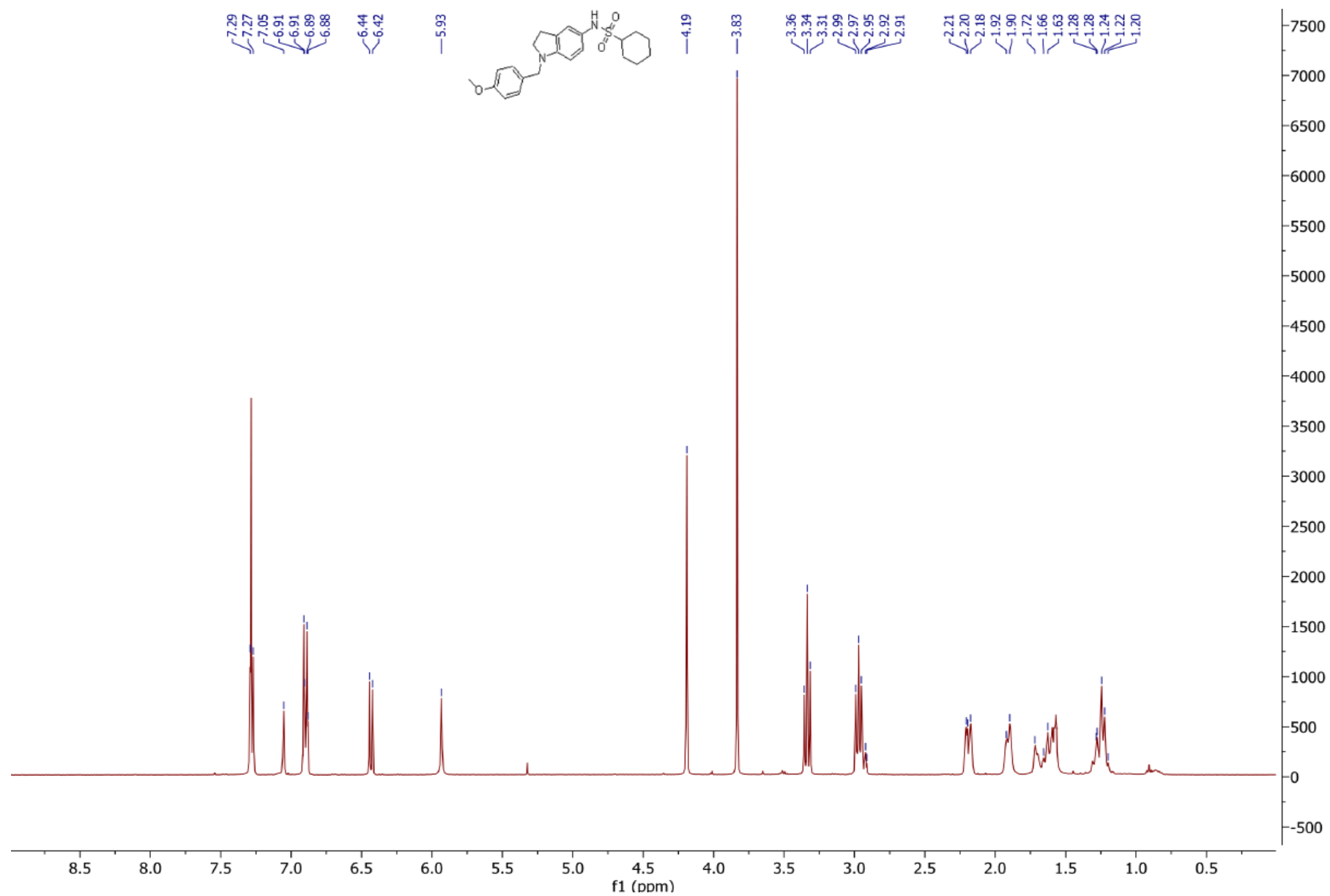
¹H NMR of compound **13c**



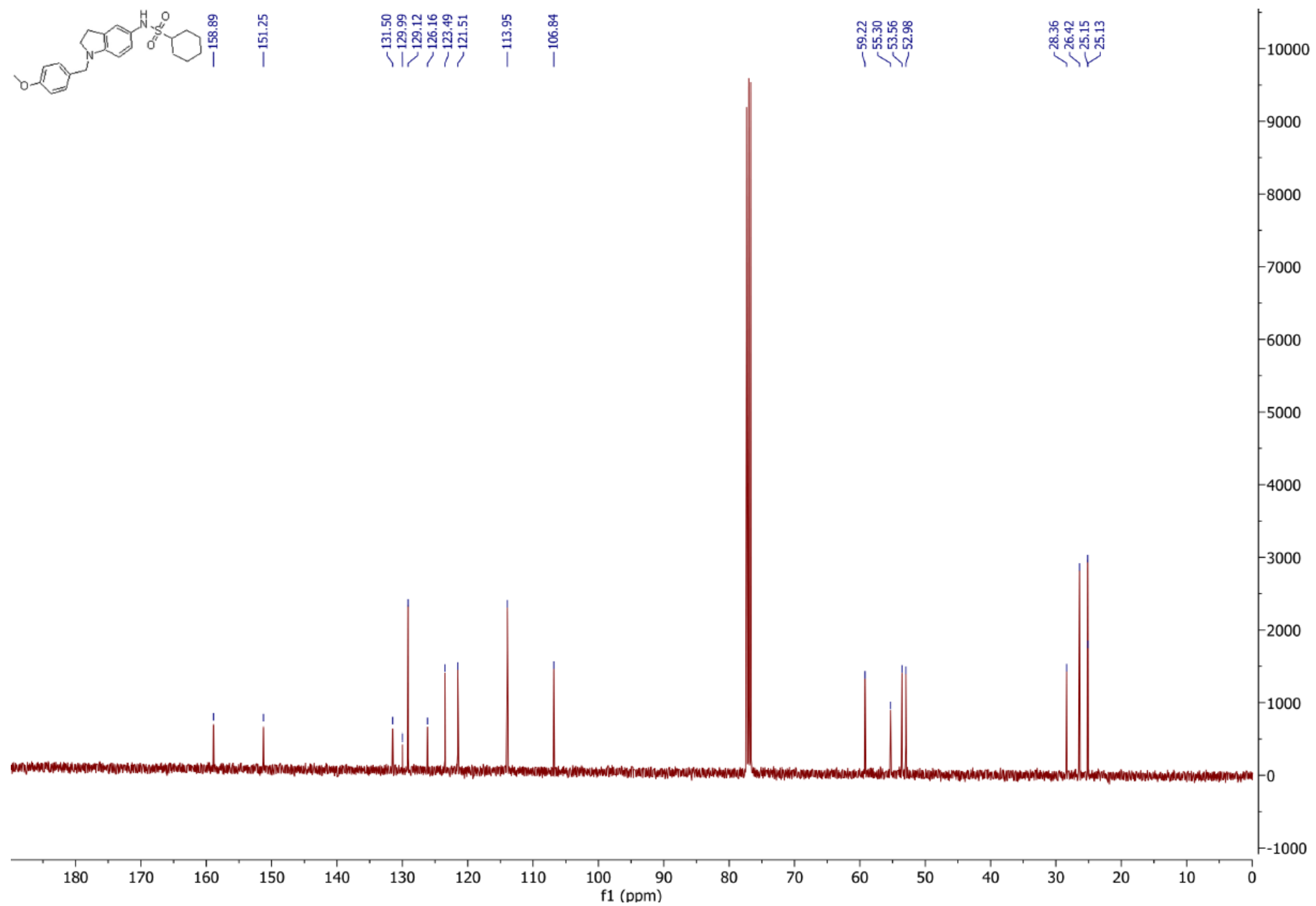
¹H NMR of compound **13c**



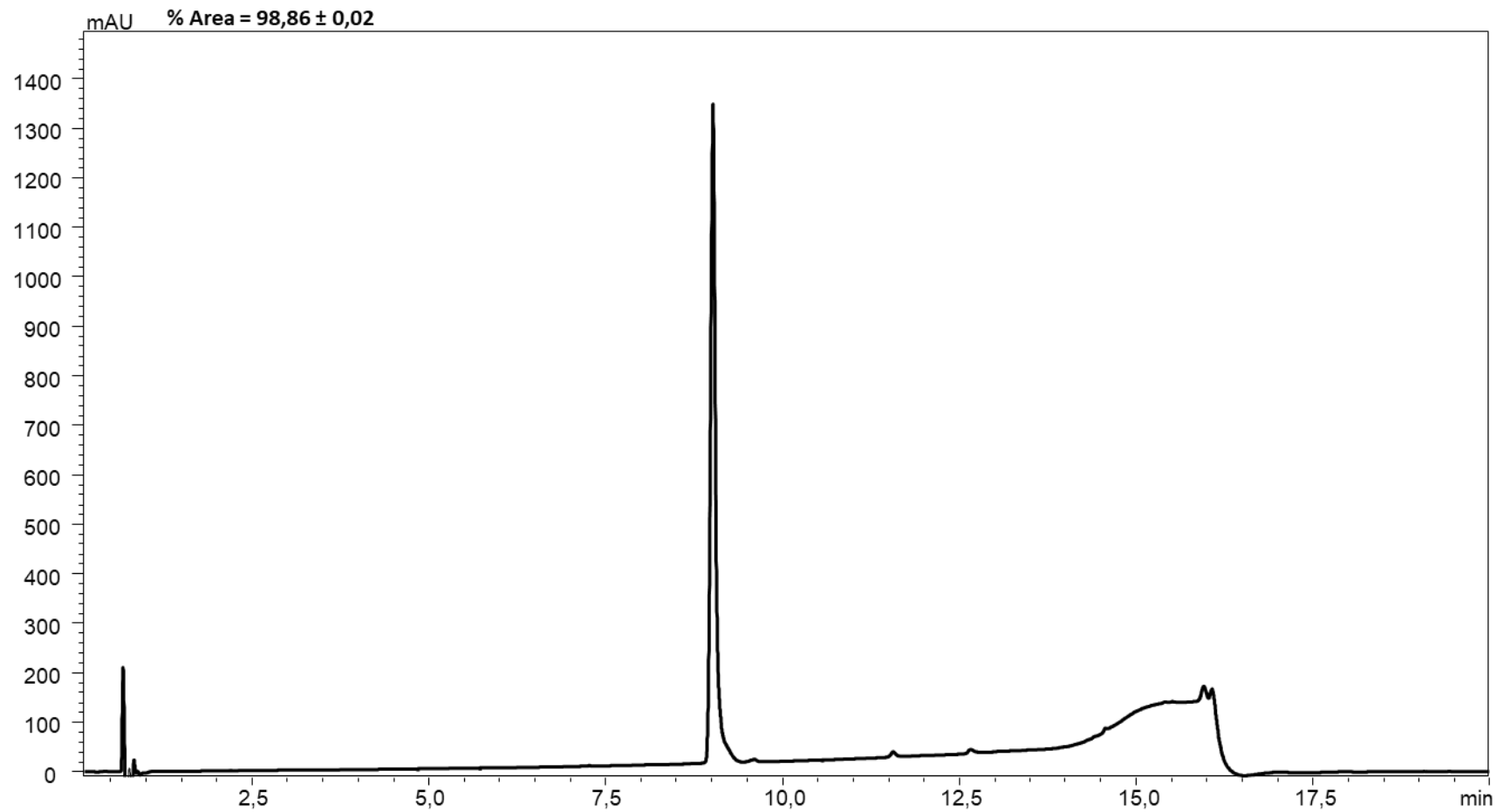
^1H NMR of compound **13c**



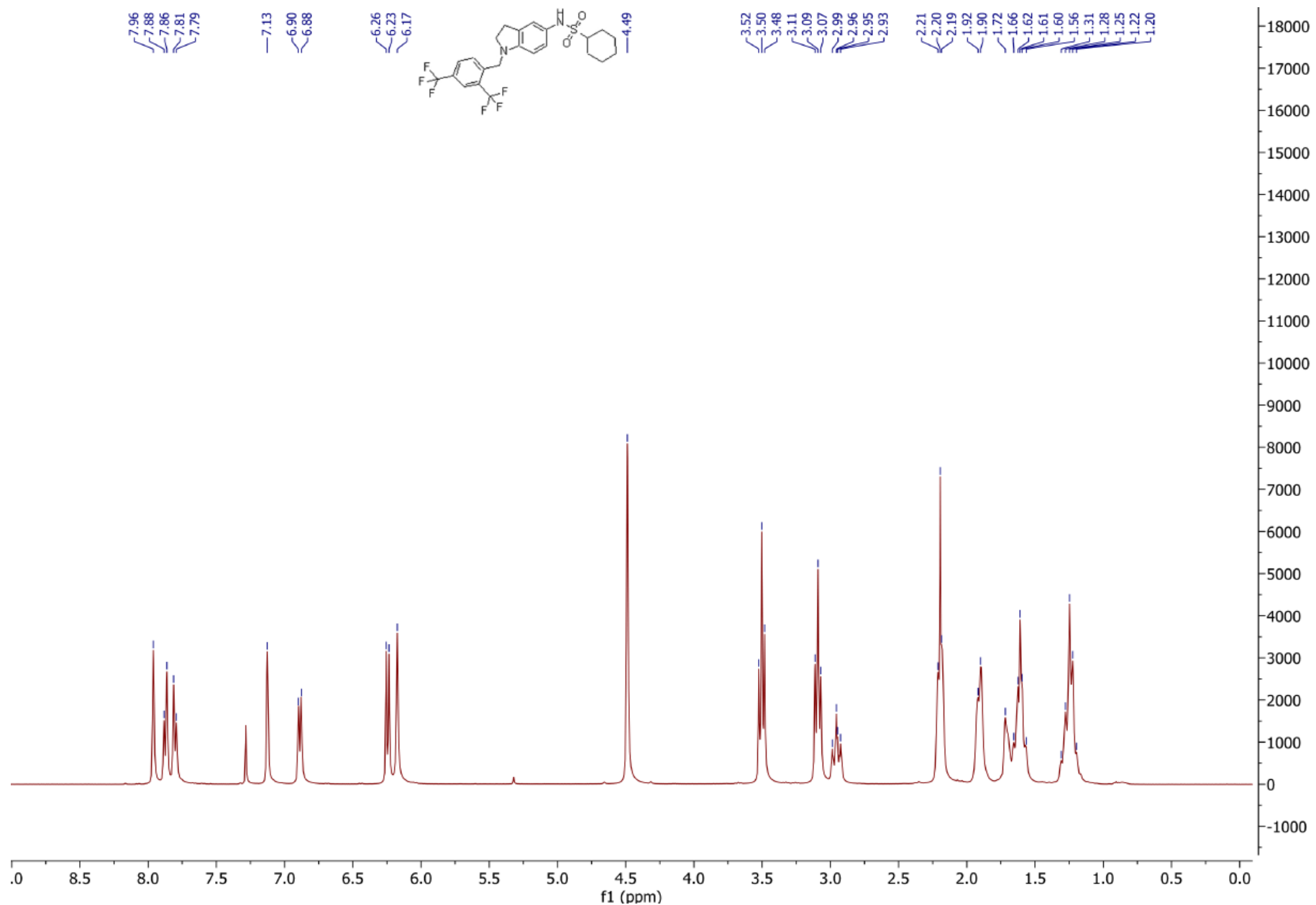
¹H NMR of compound **13d**



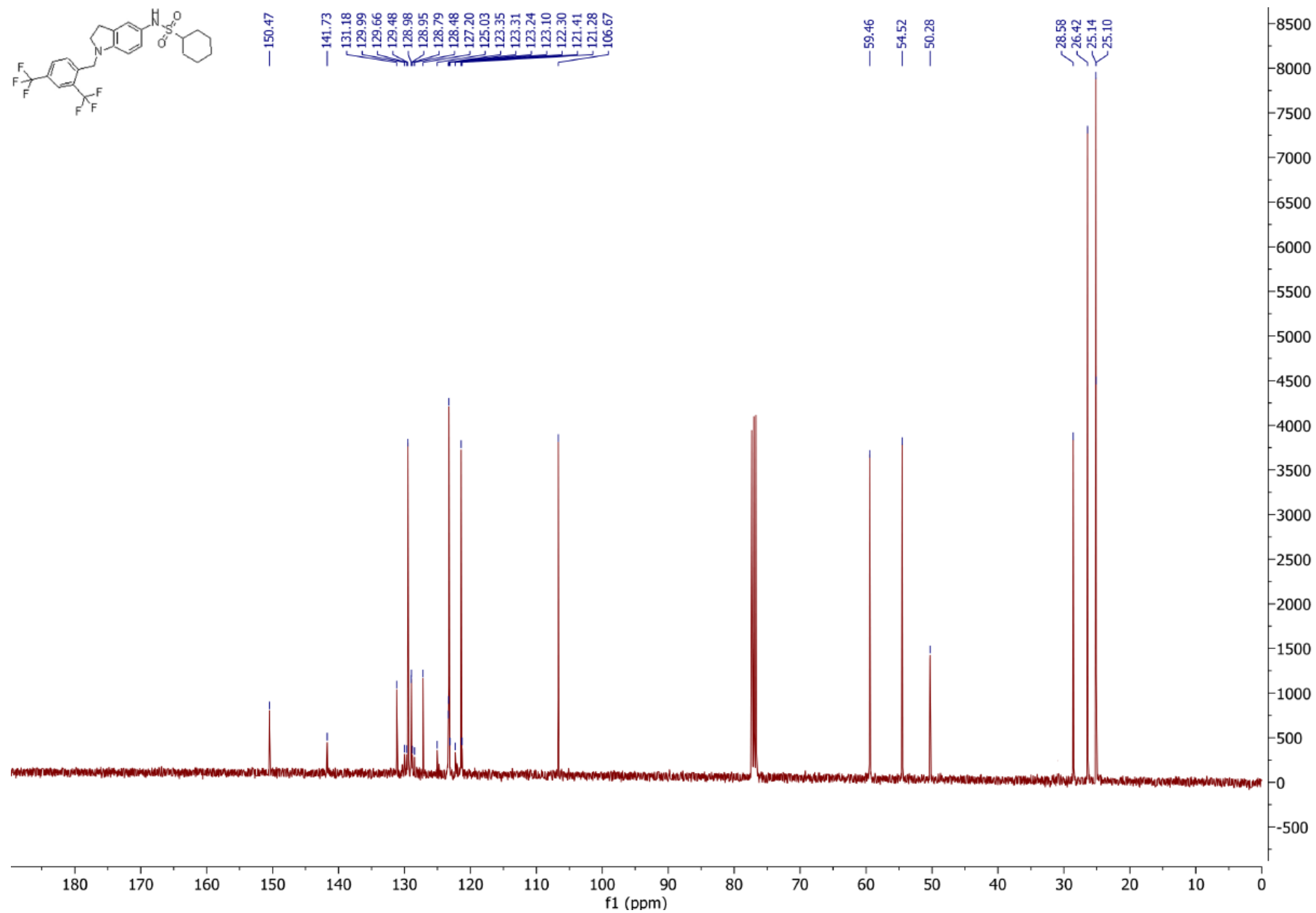
¹³C NMR of compound **13d**



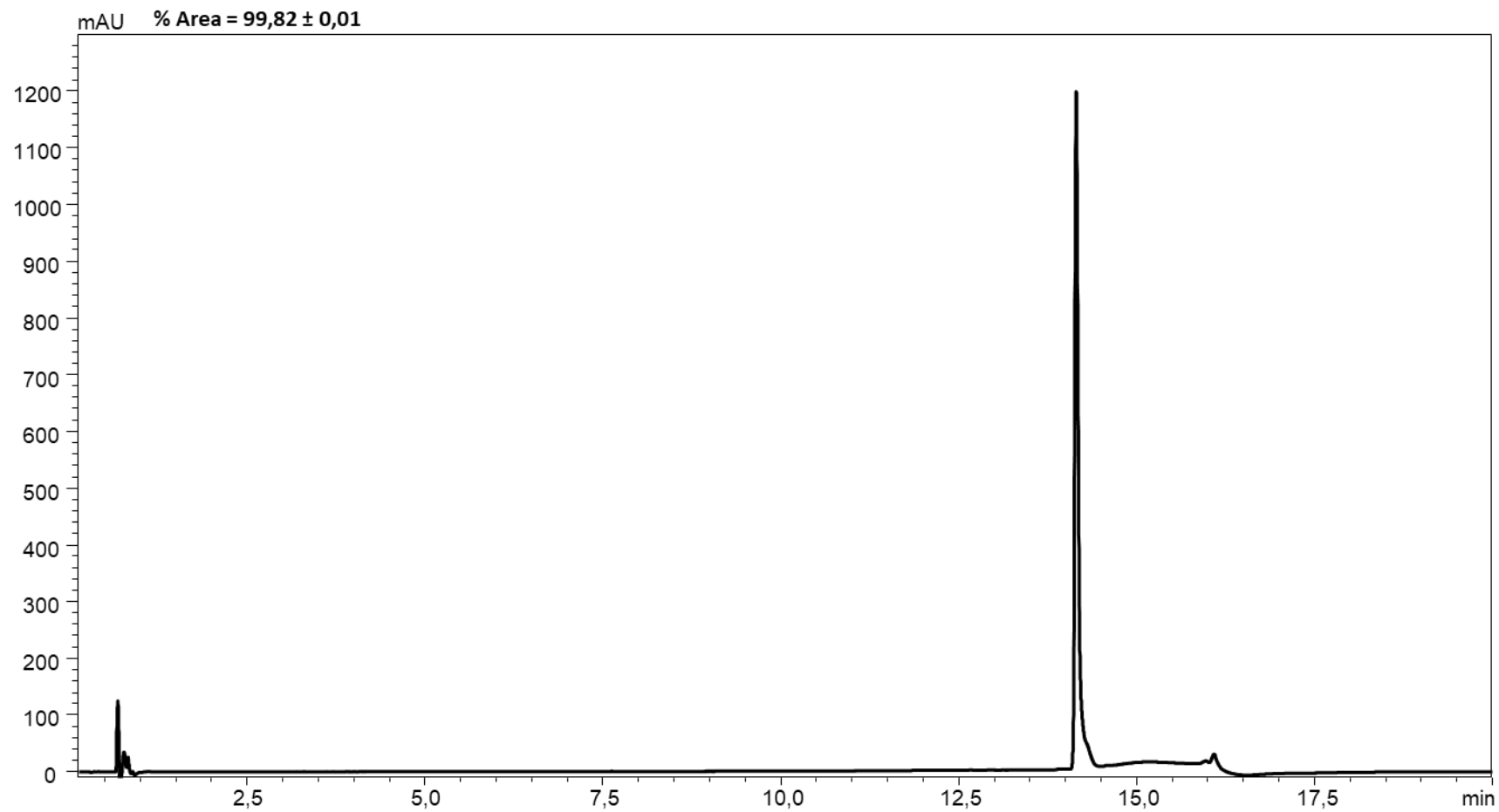
RP-HPLC trace of compound **13d**



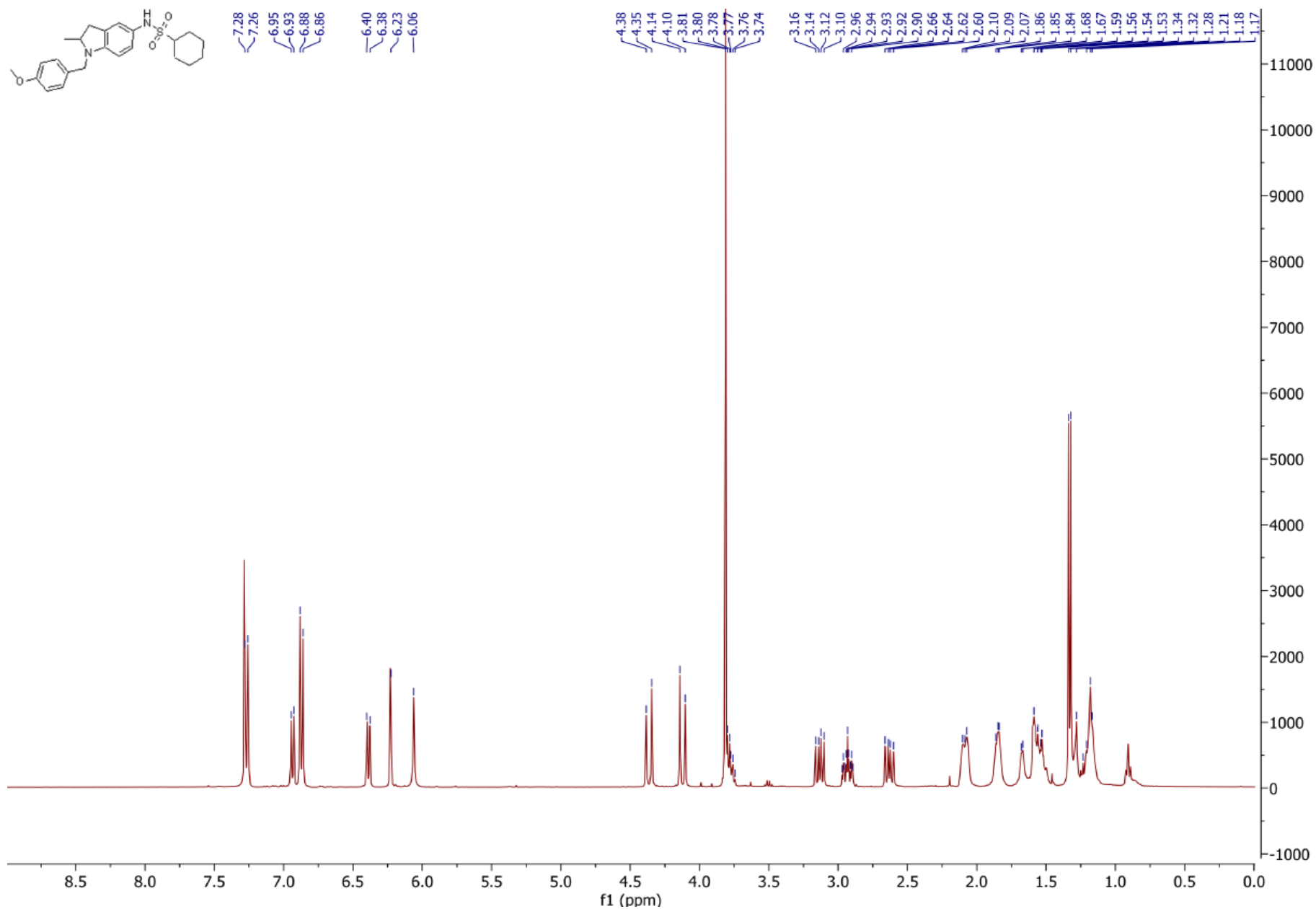
1H NMR of compound 13e



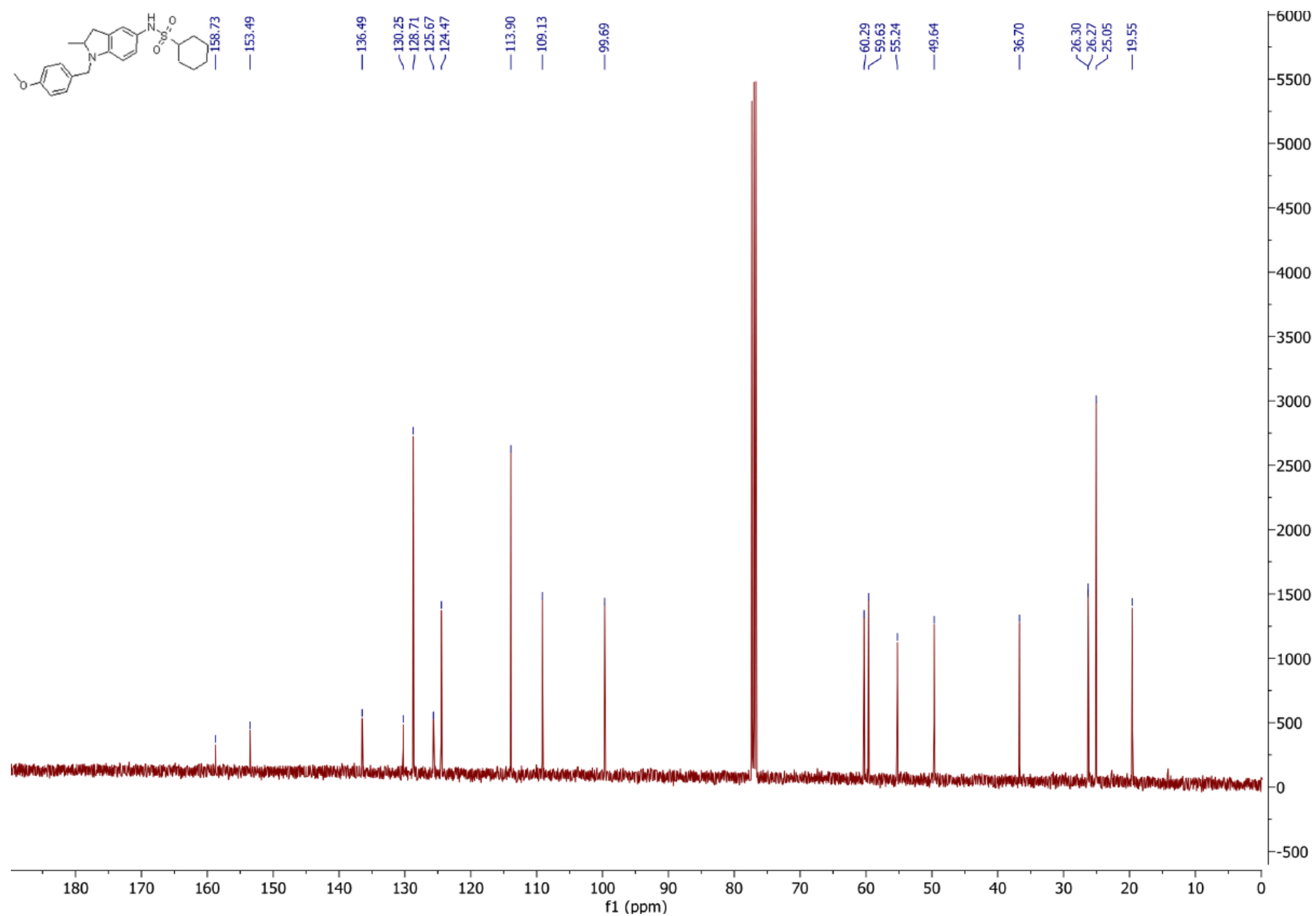
¹³C NMR of compound **13e**



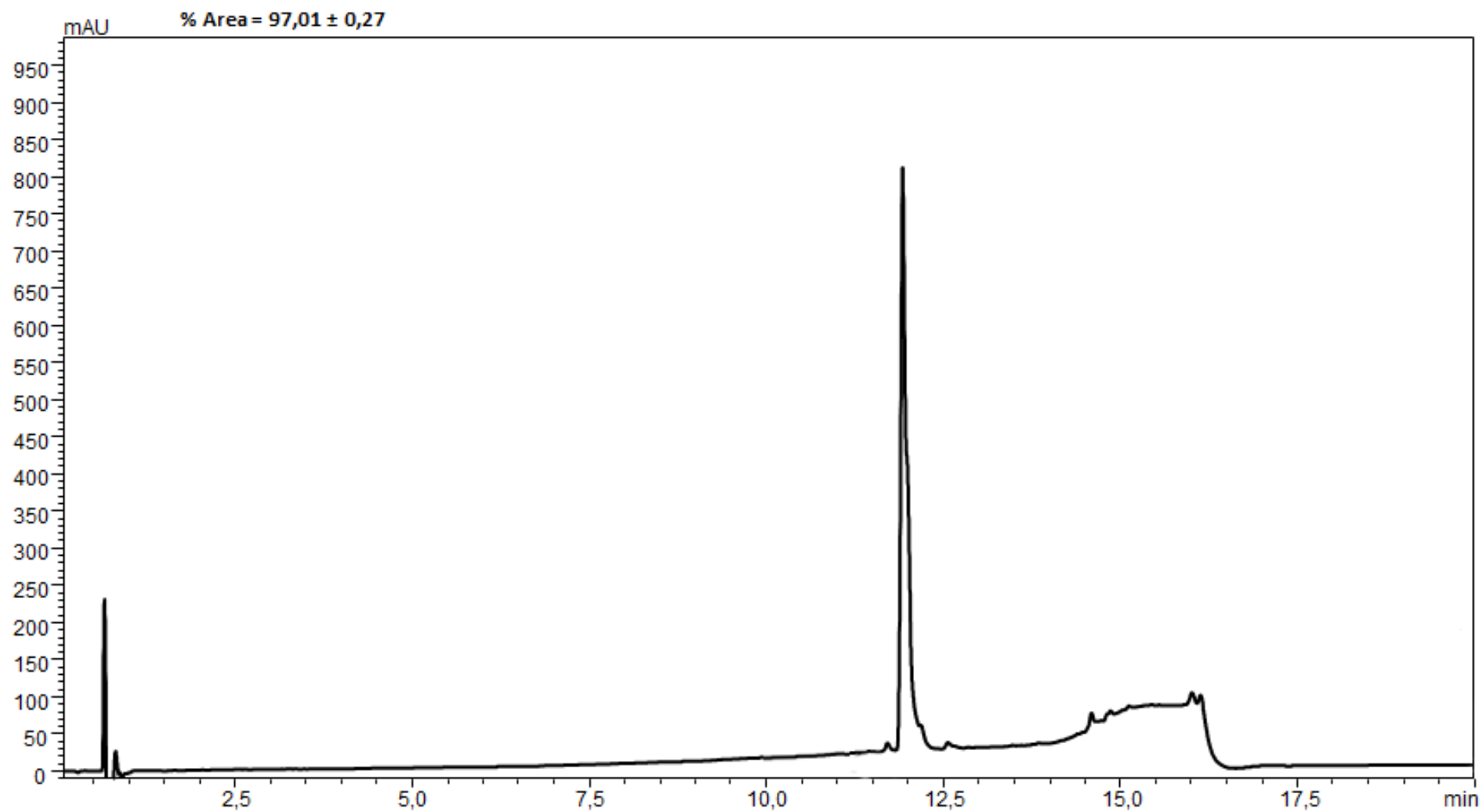
RP-HPLC of compound **13e**



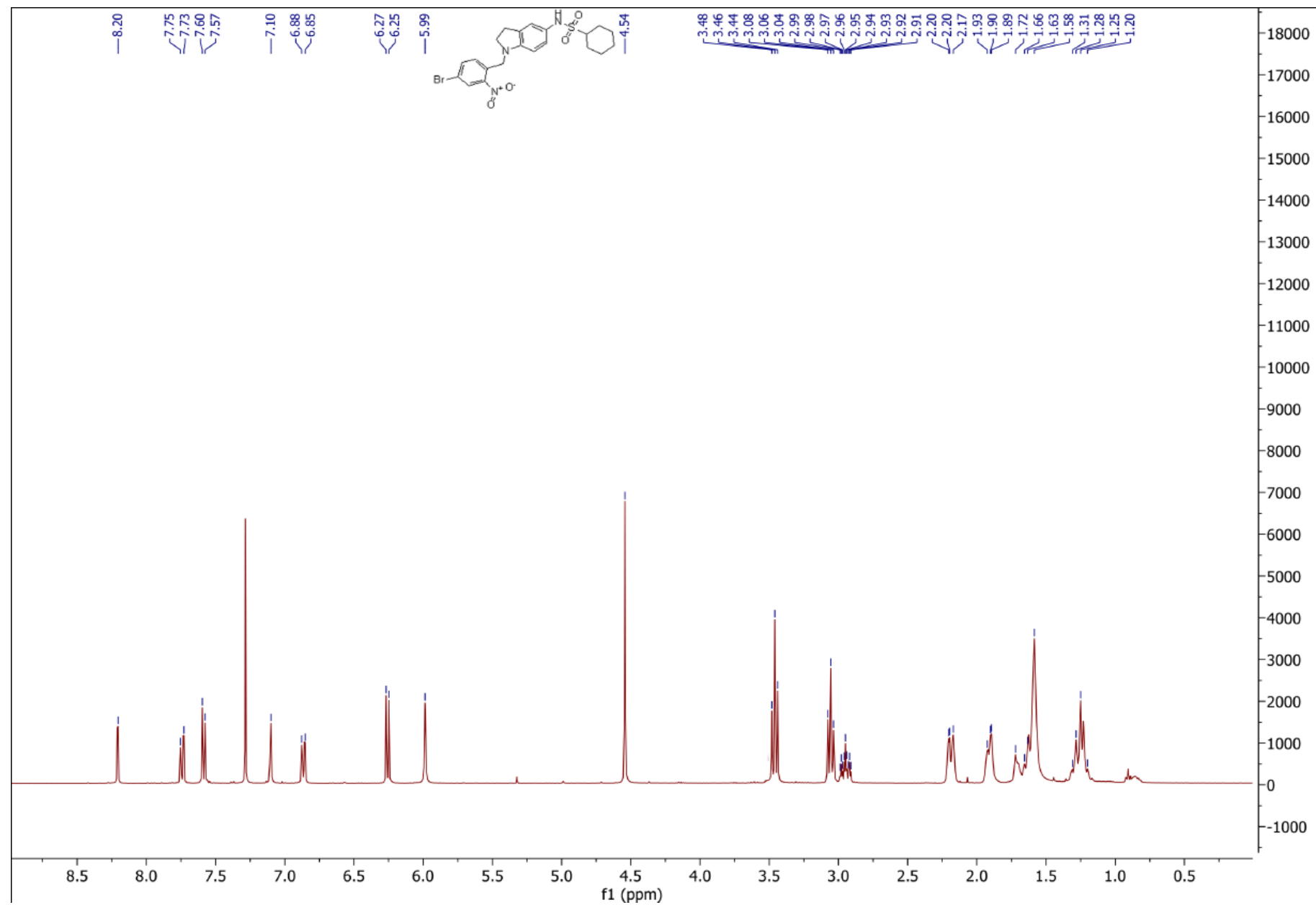
¹H NMR of compound **13f**



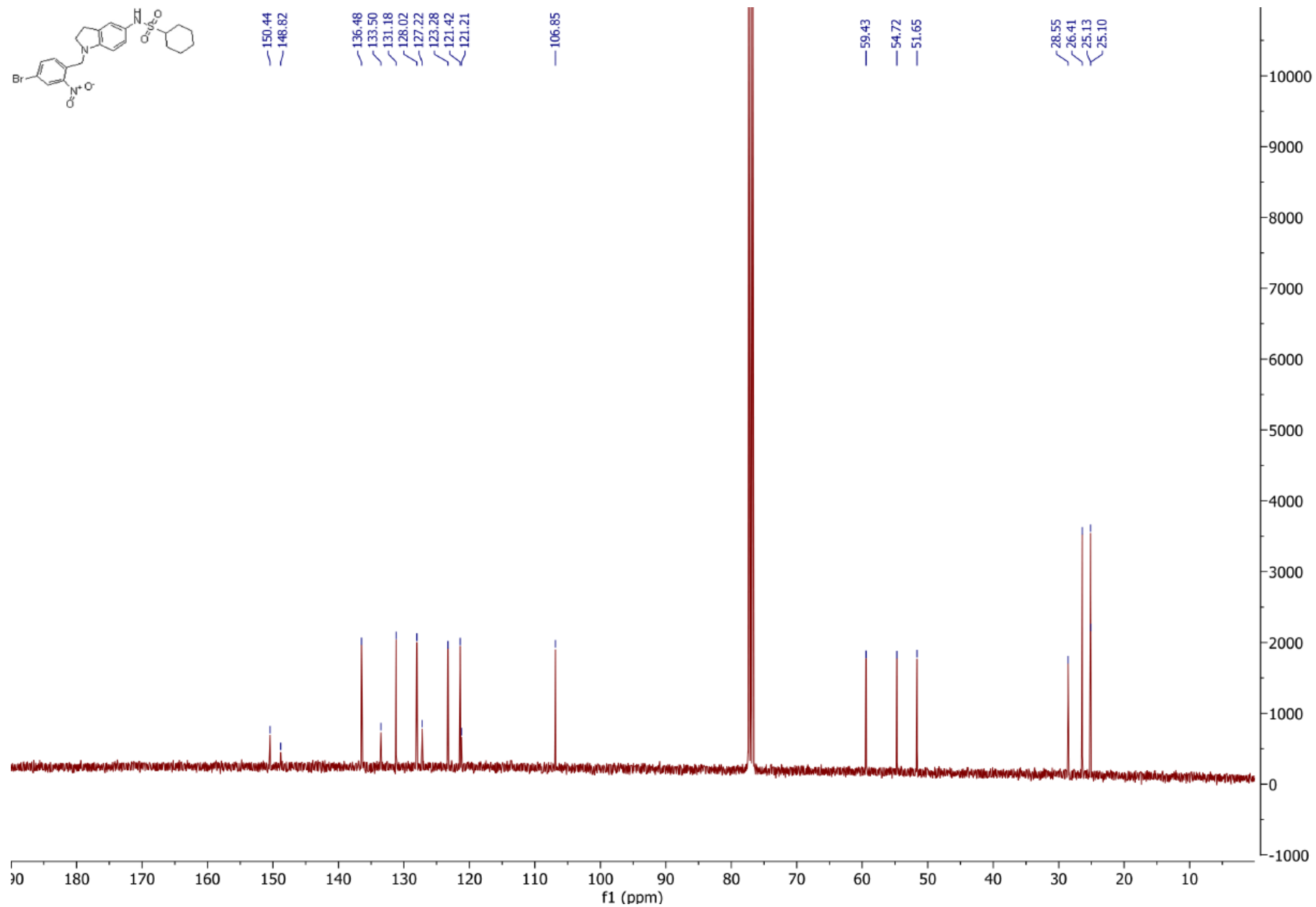
¹³C NMR of compound **13f**



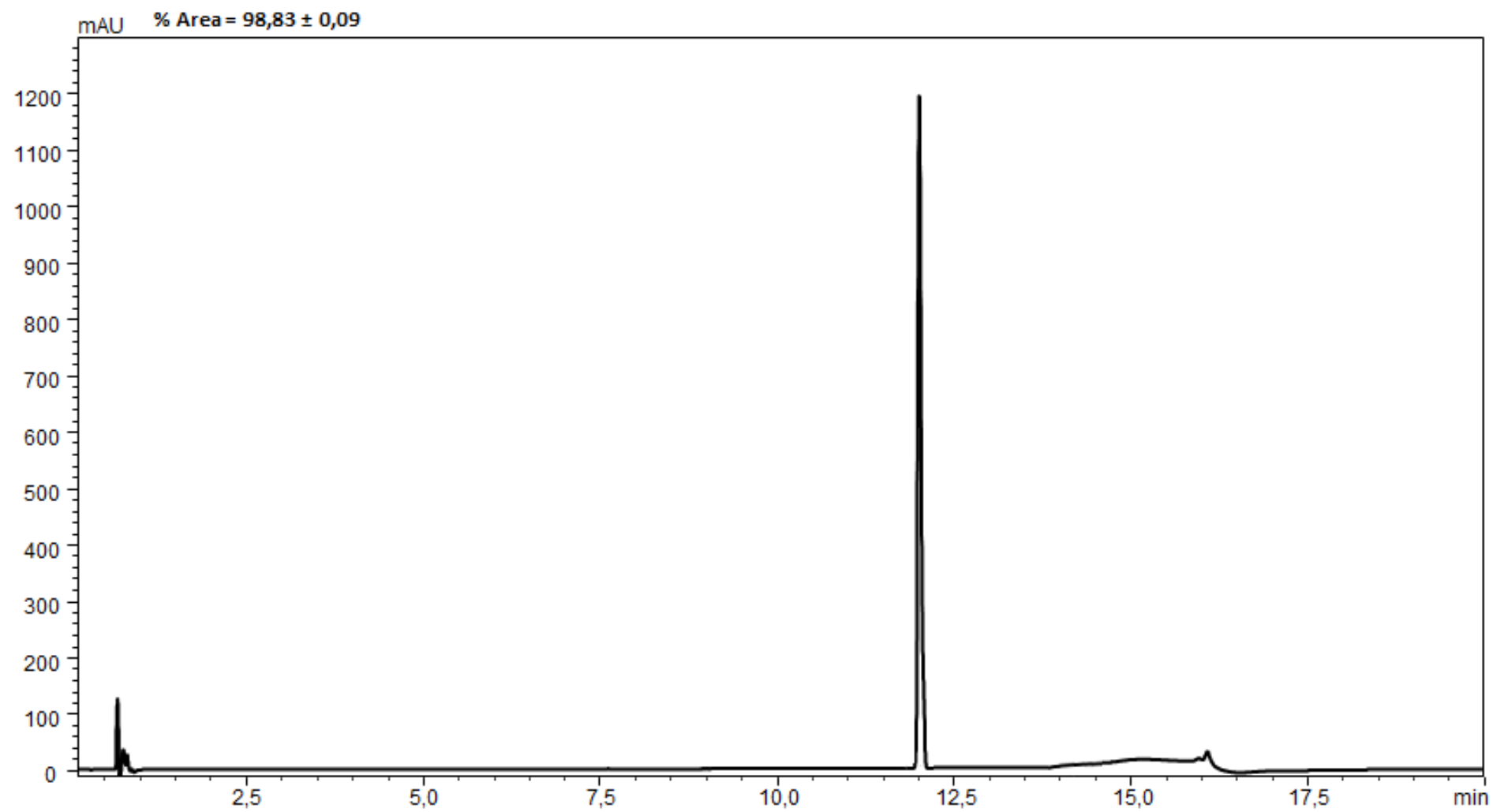
RP-HPLC of compound **13f**



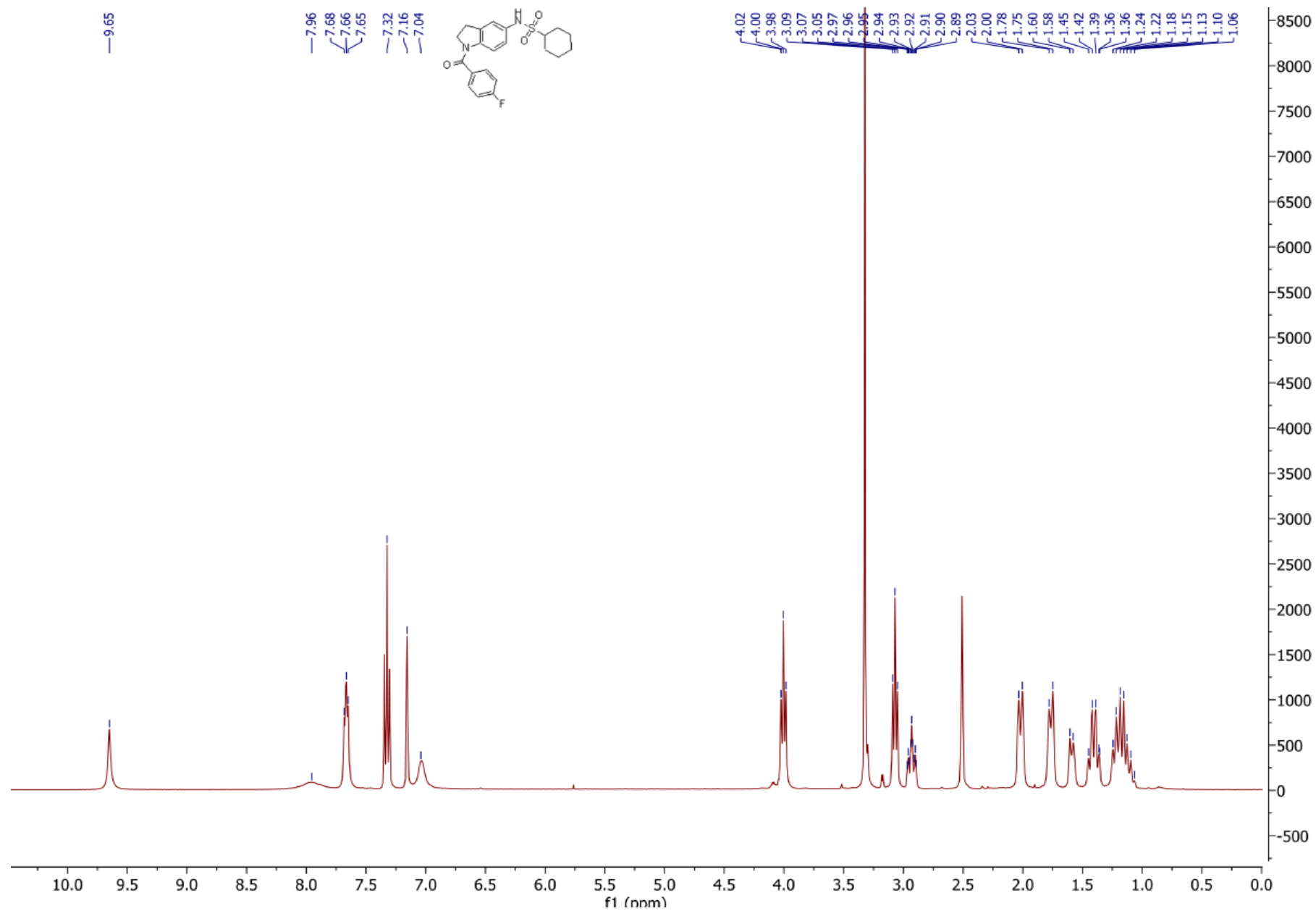
¹H NMR of compound 13g



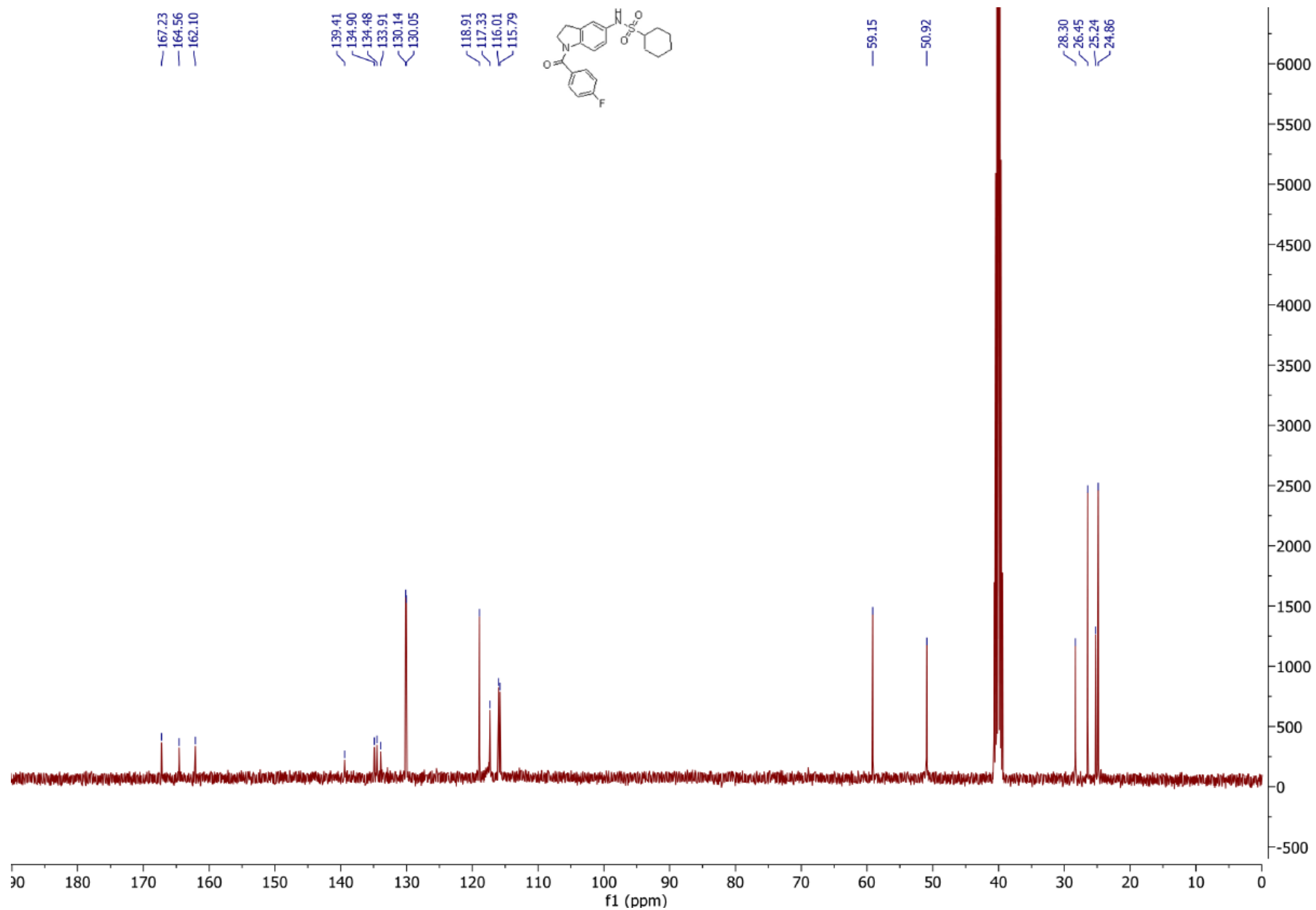
¹³C NMR of compound **13g**



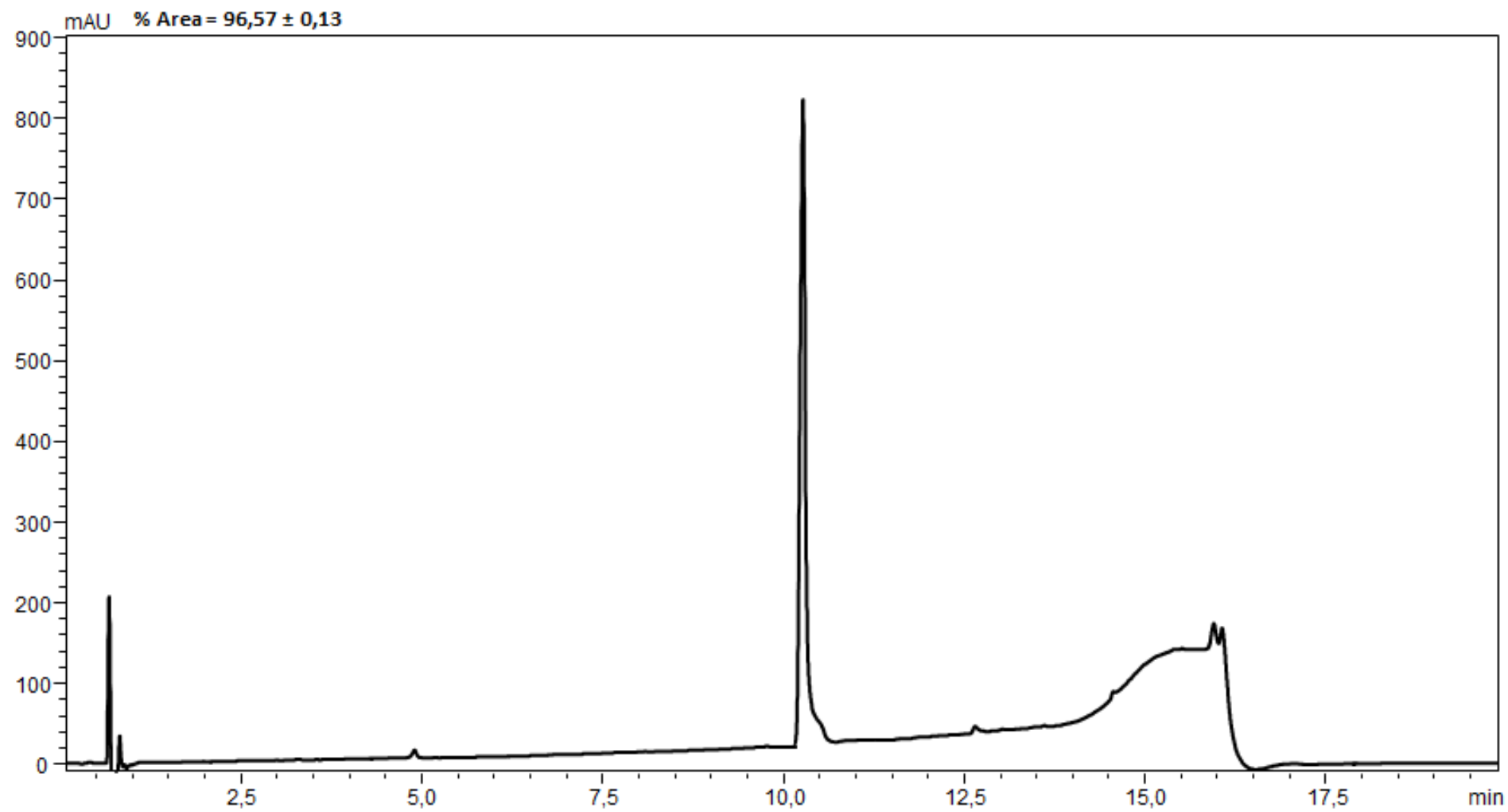
RP-HPLC trace of compound **13g**

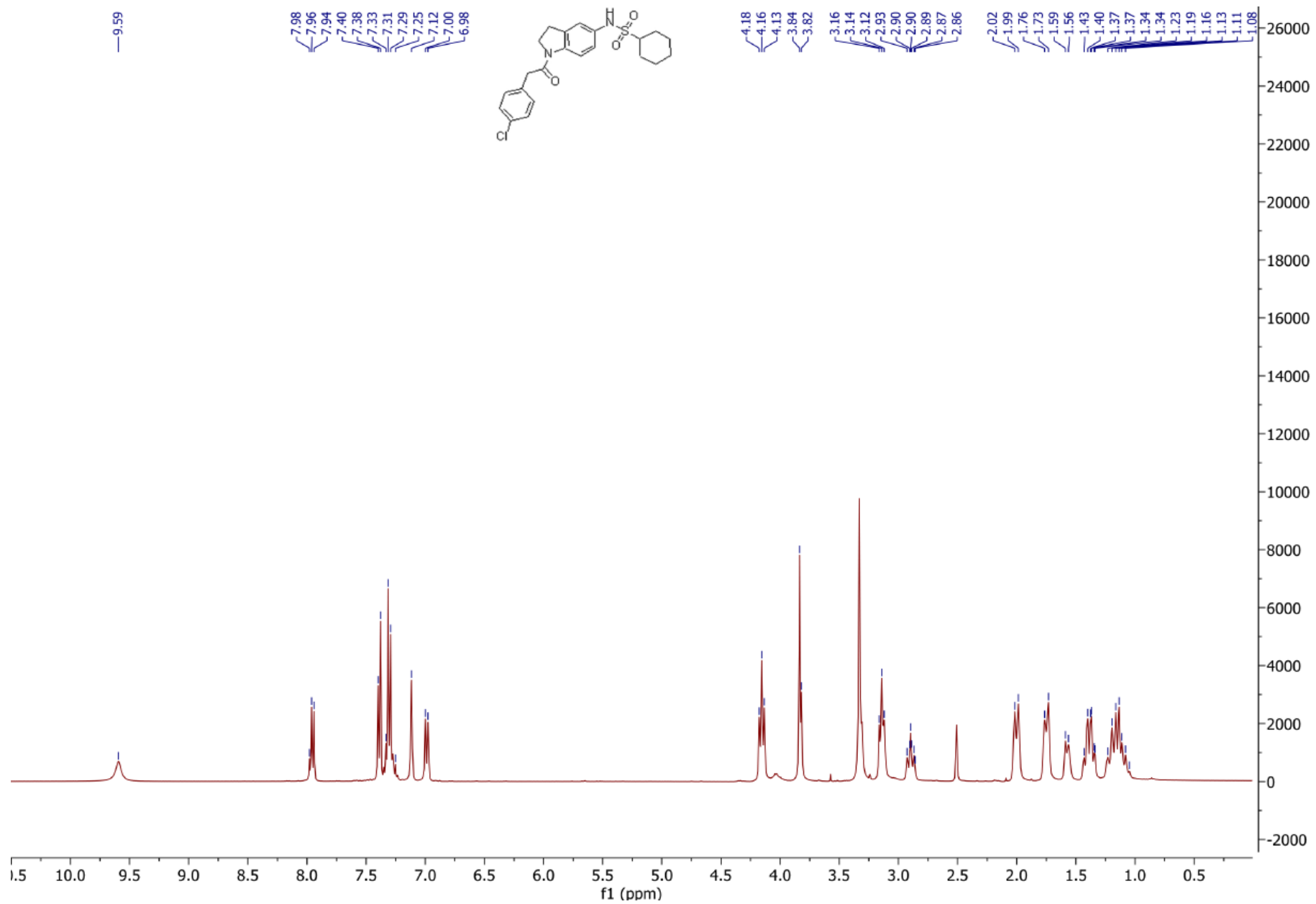


¹H NMR of compound **16a**

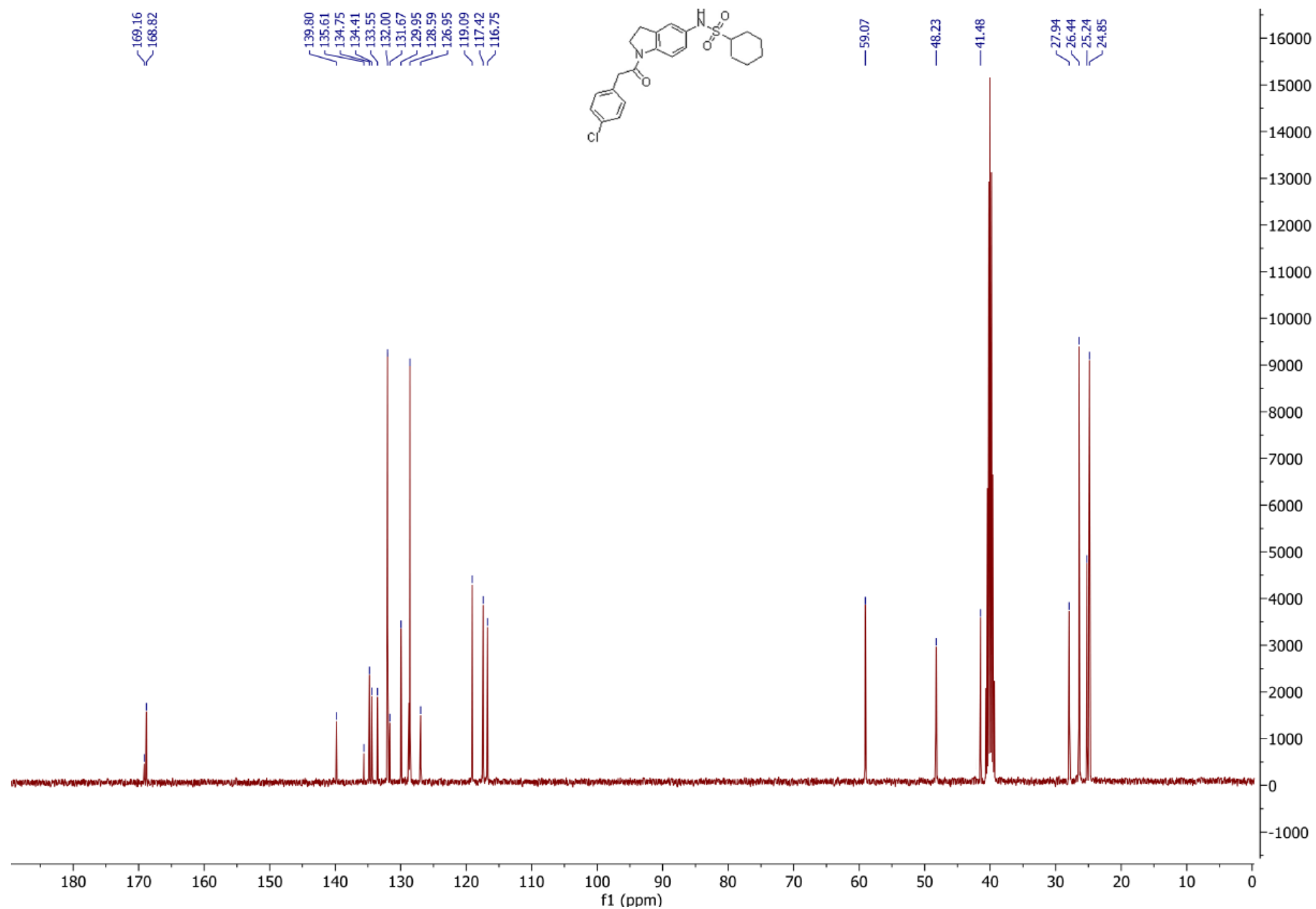


¹³C NMR of compound **16a**

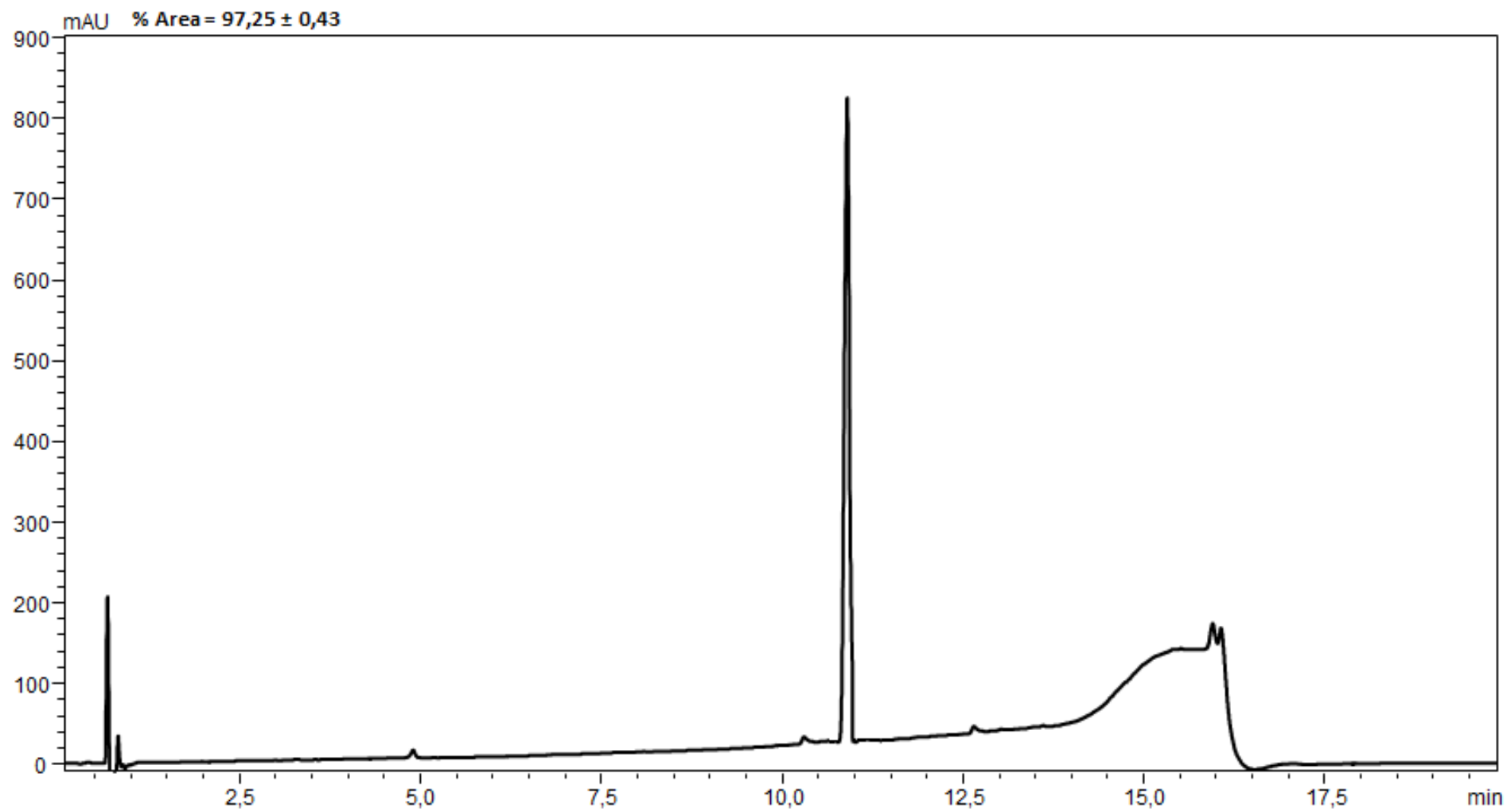




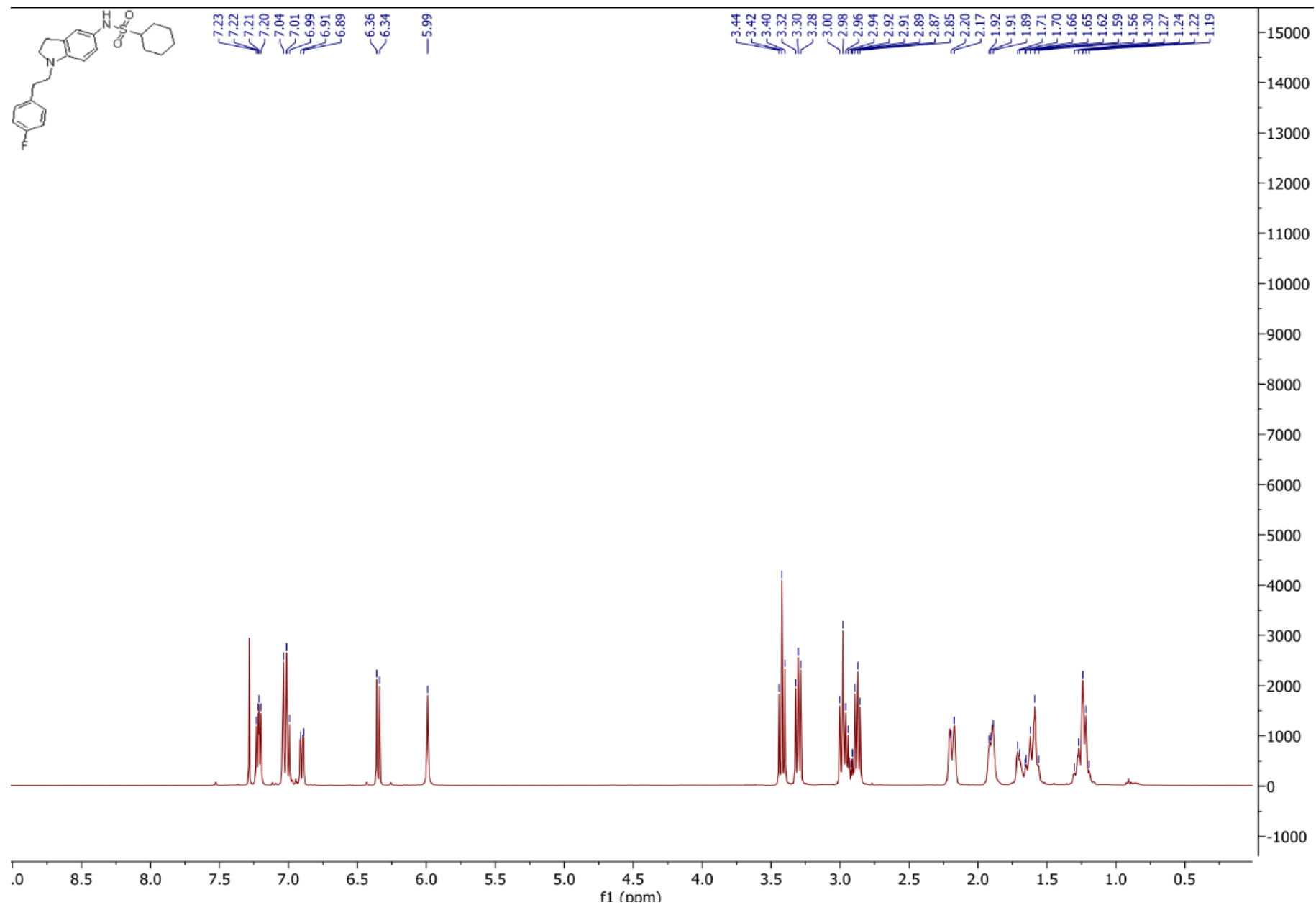
¹H NMR of compound **16b**



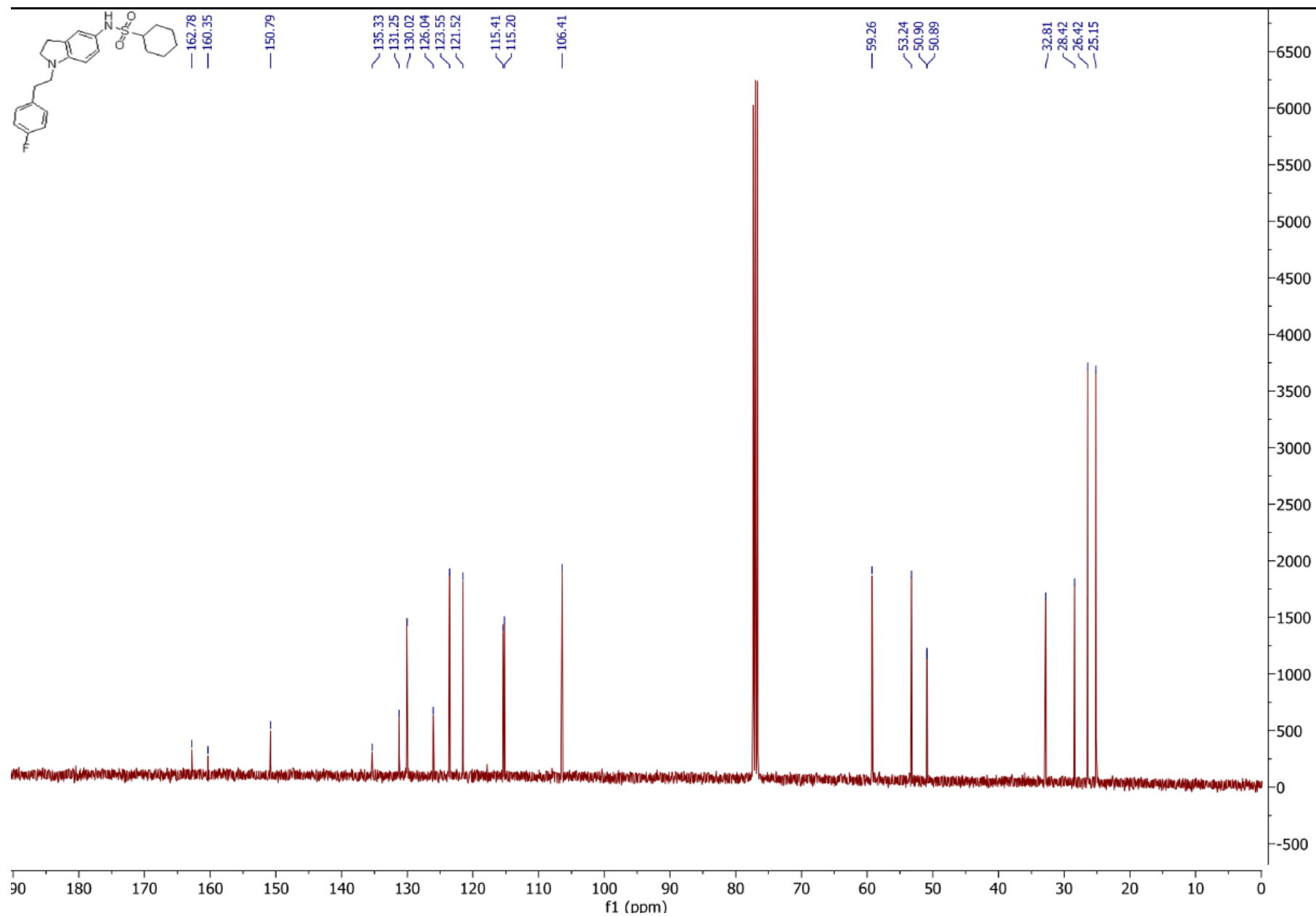
¹³C NMR of compound **16b**



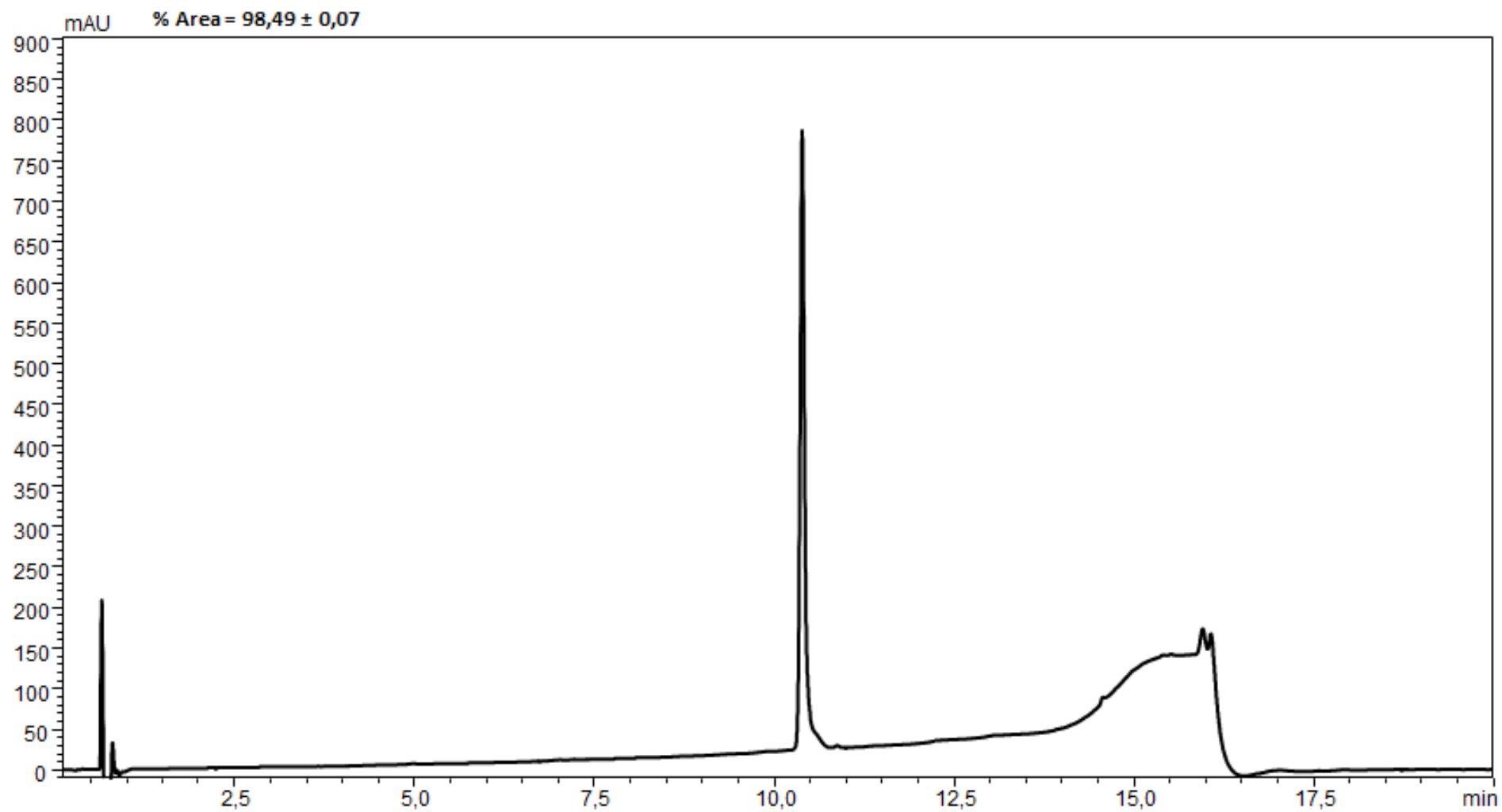
RP-HPLC trace of compound **16b**



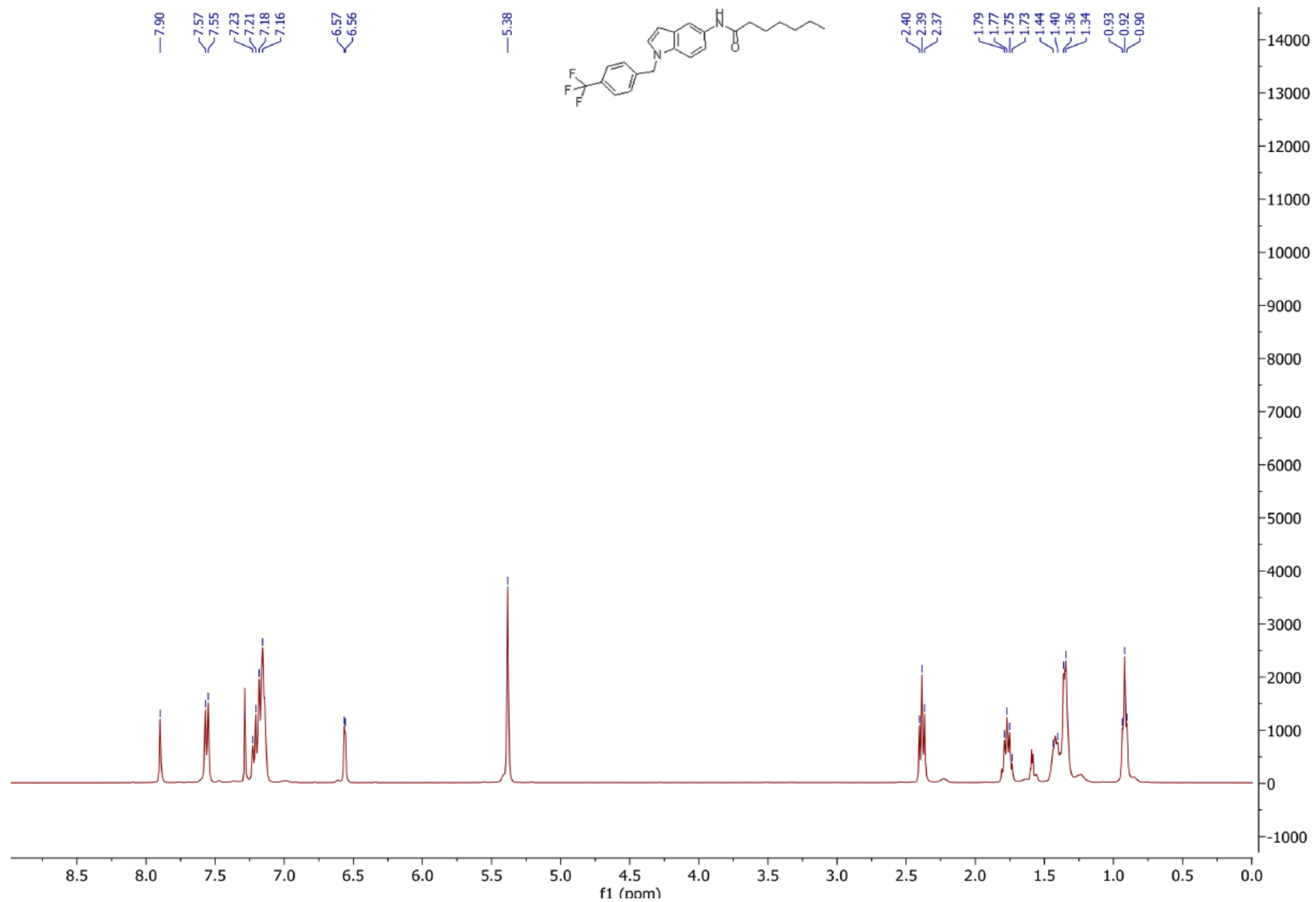
¹H NMR of compound **19**



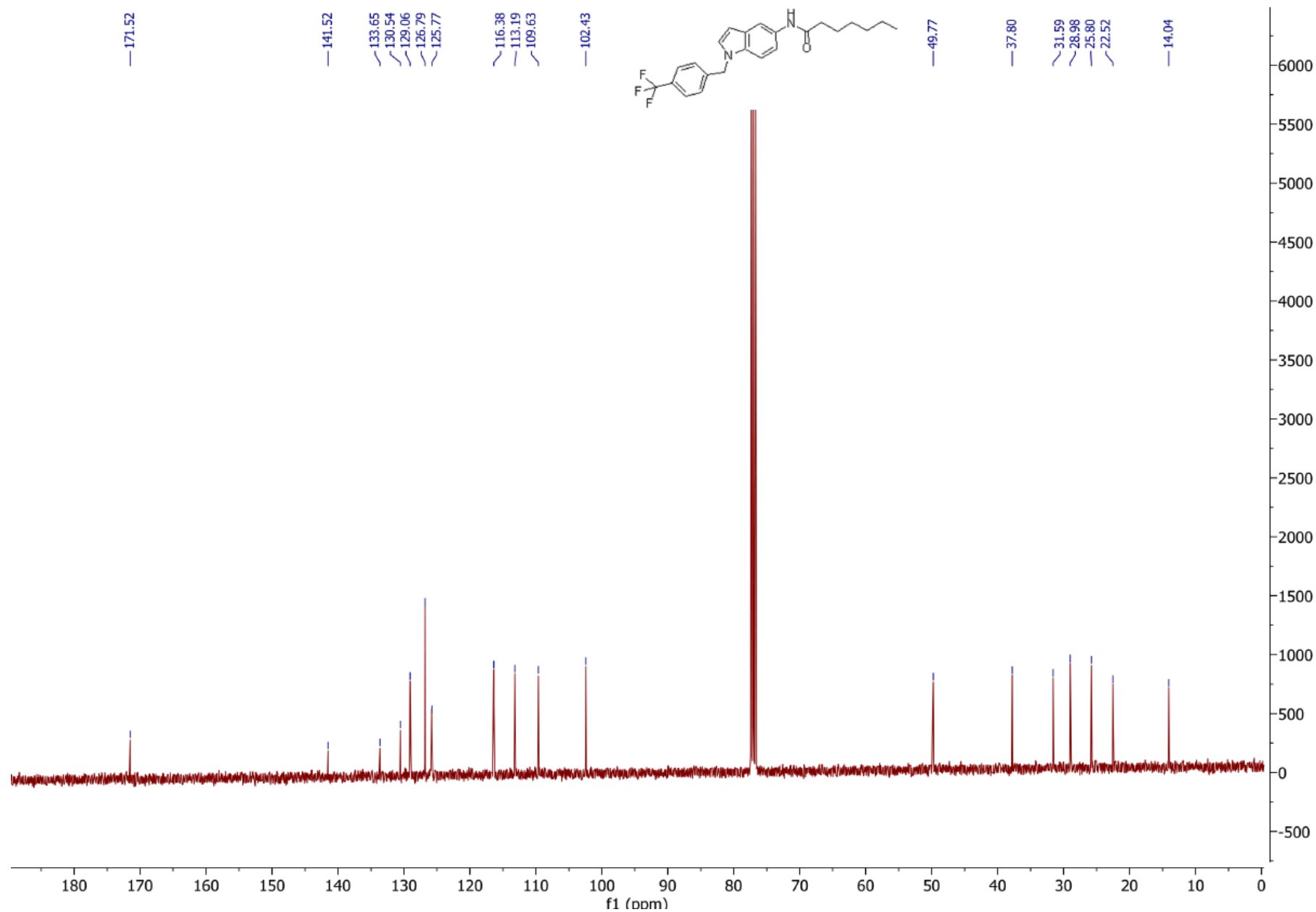
¹³C NMR of compound **19**



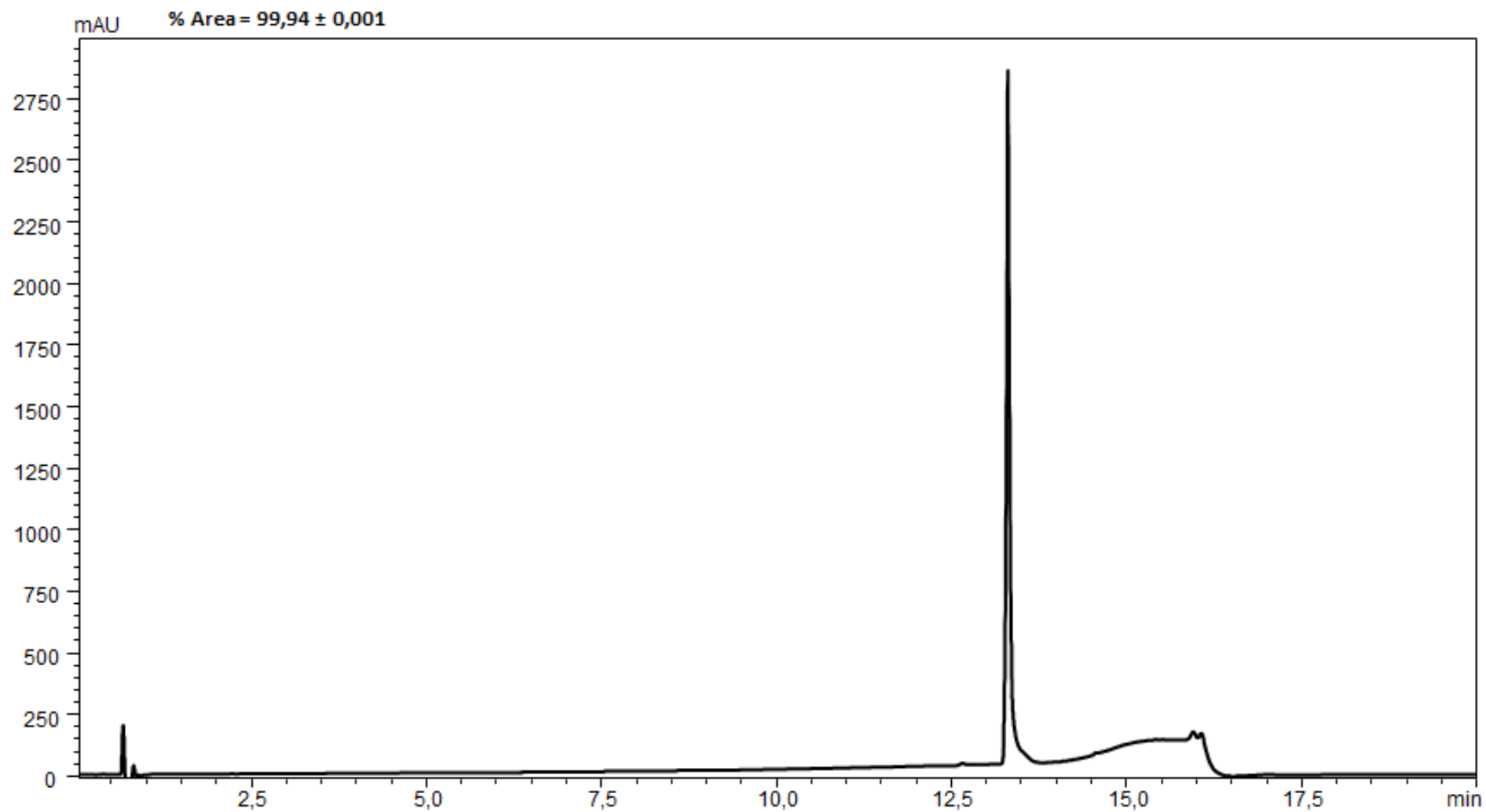
RP-HPLC trace of compound **19**



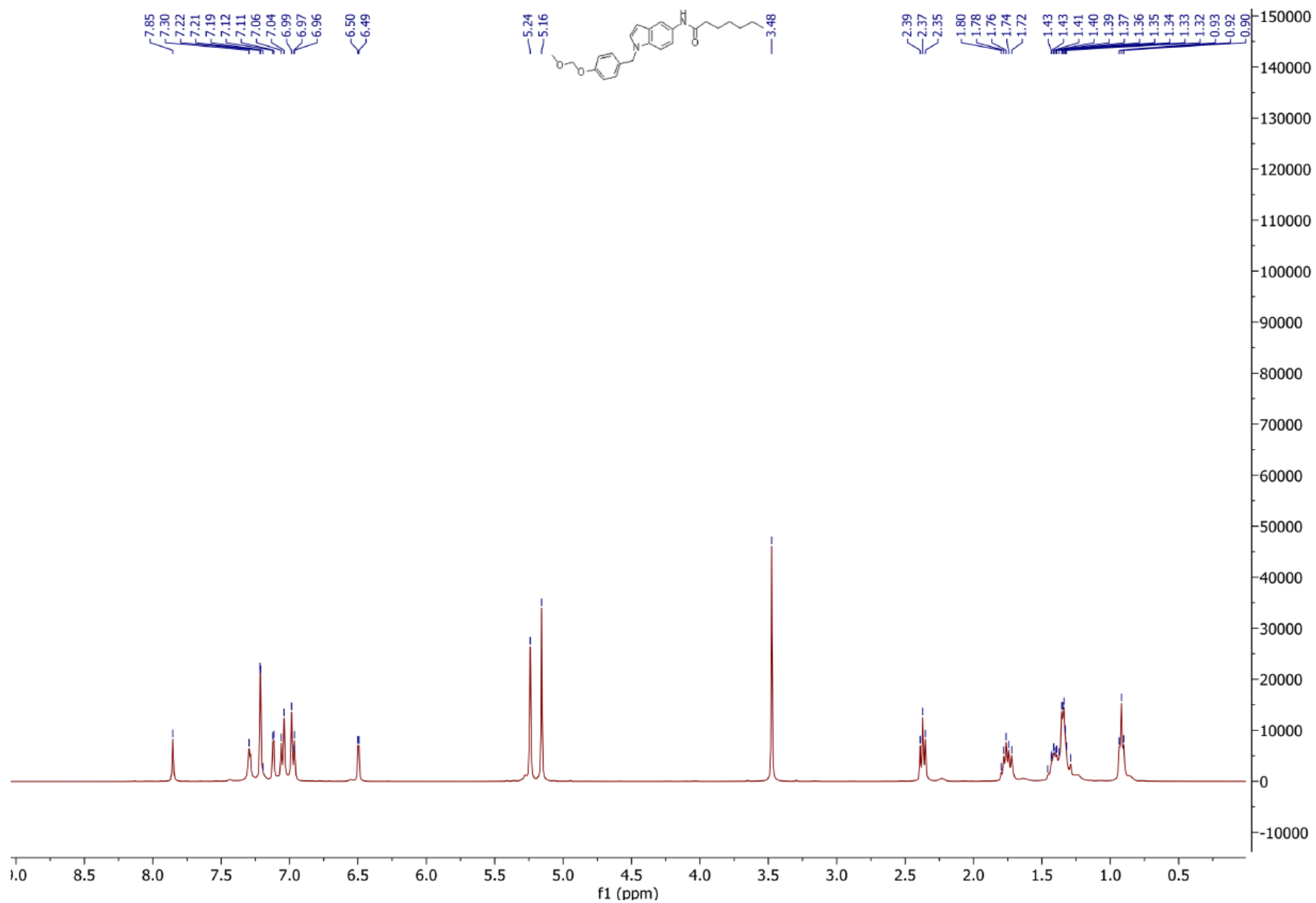
¹H NMR of compound **23a**



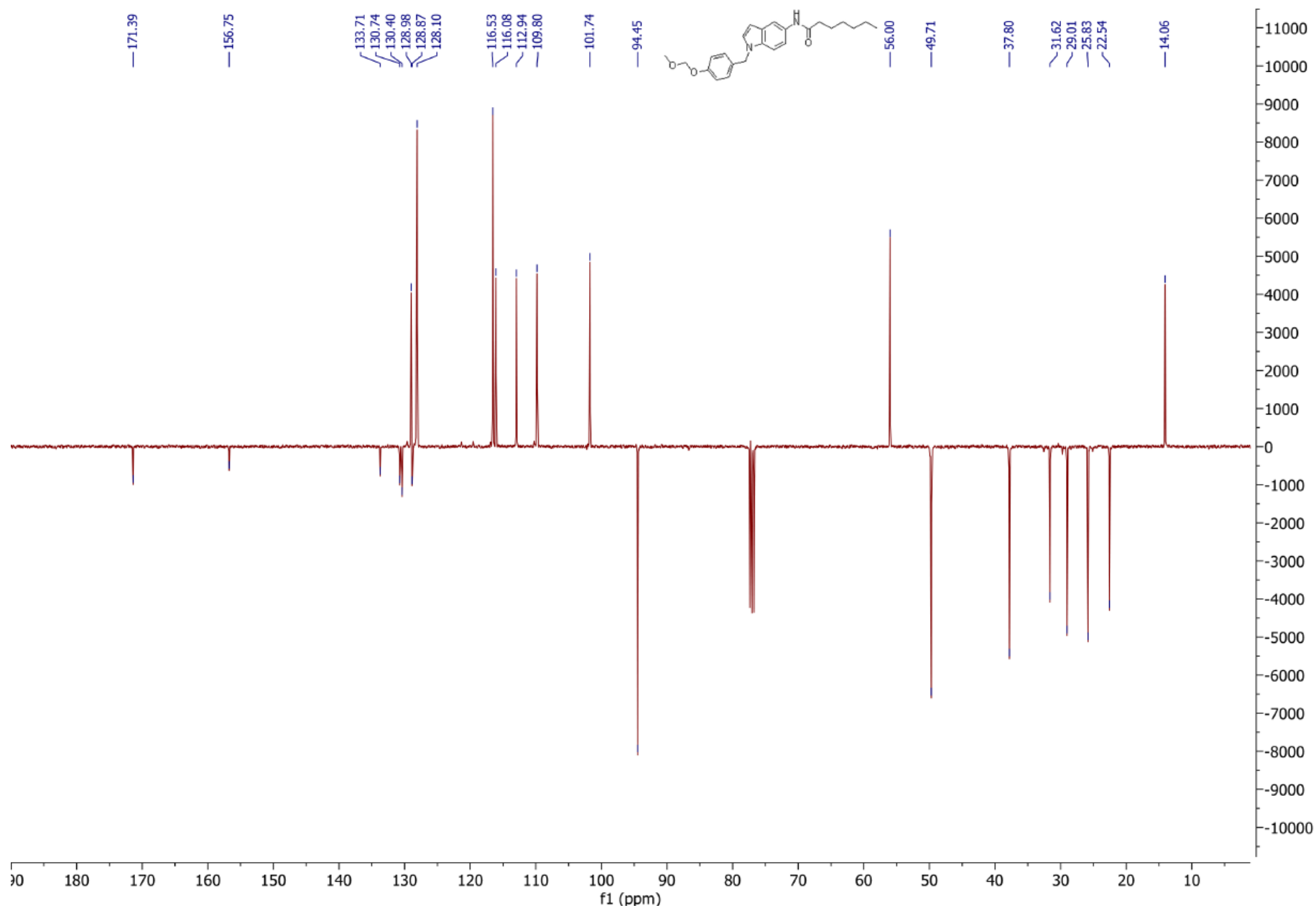
¹³C NMR of compound **23a**



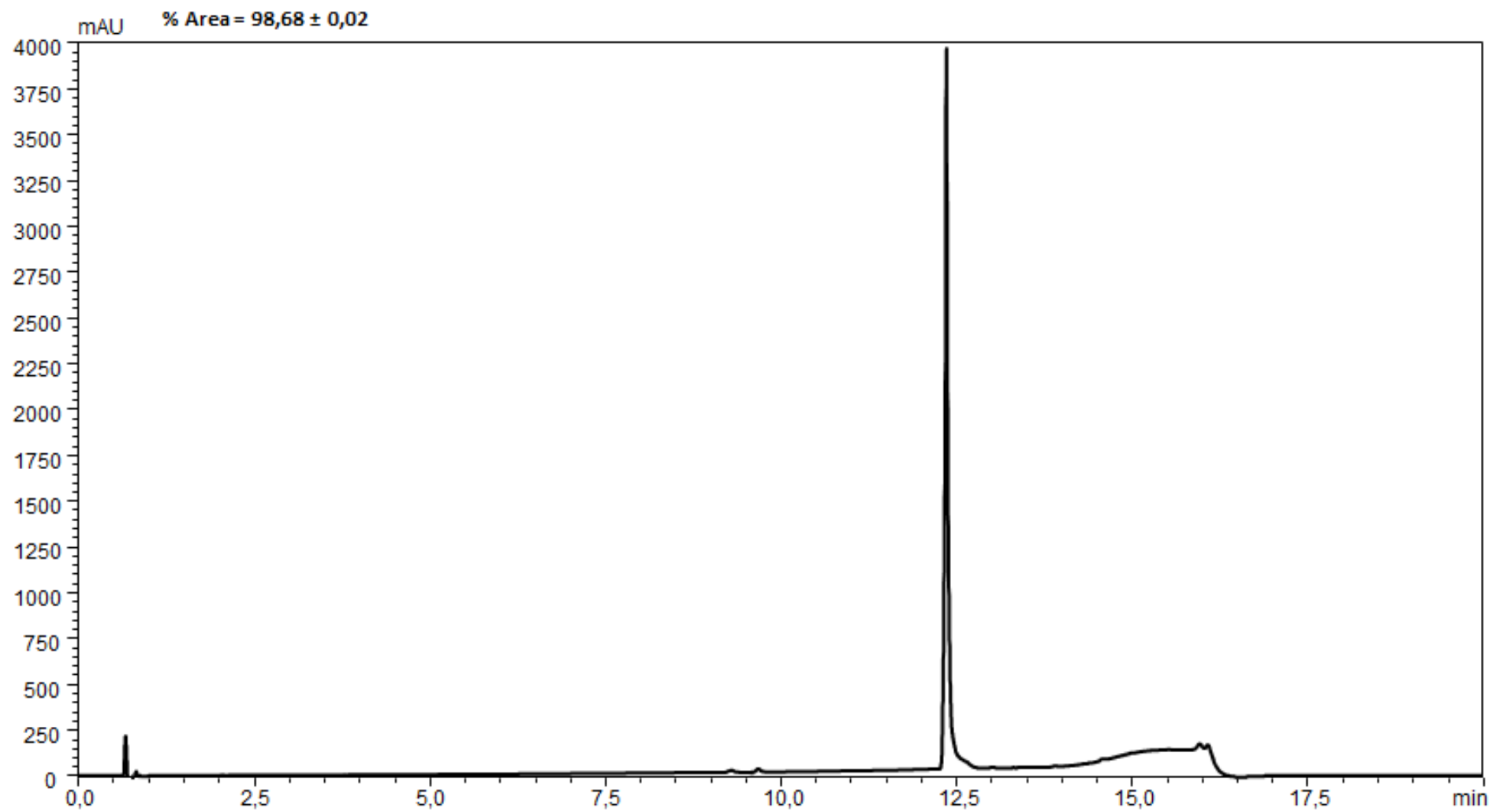
RP-HPLC of compound **23a**



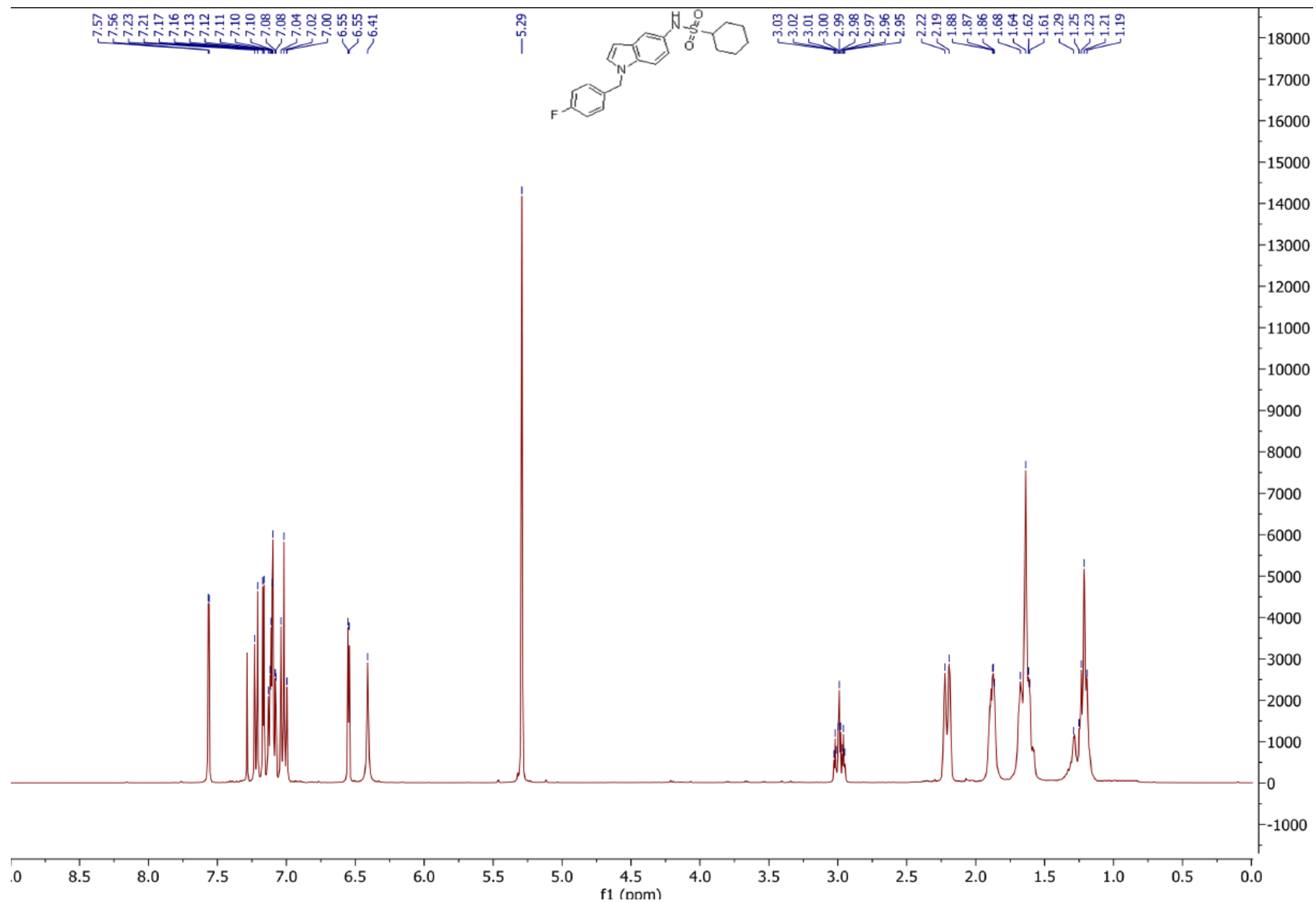
¹H NMR of compound 23b



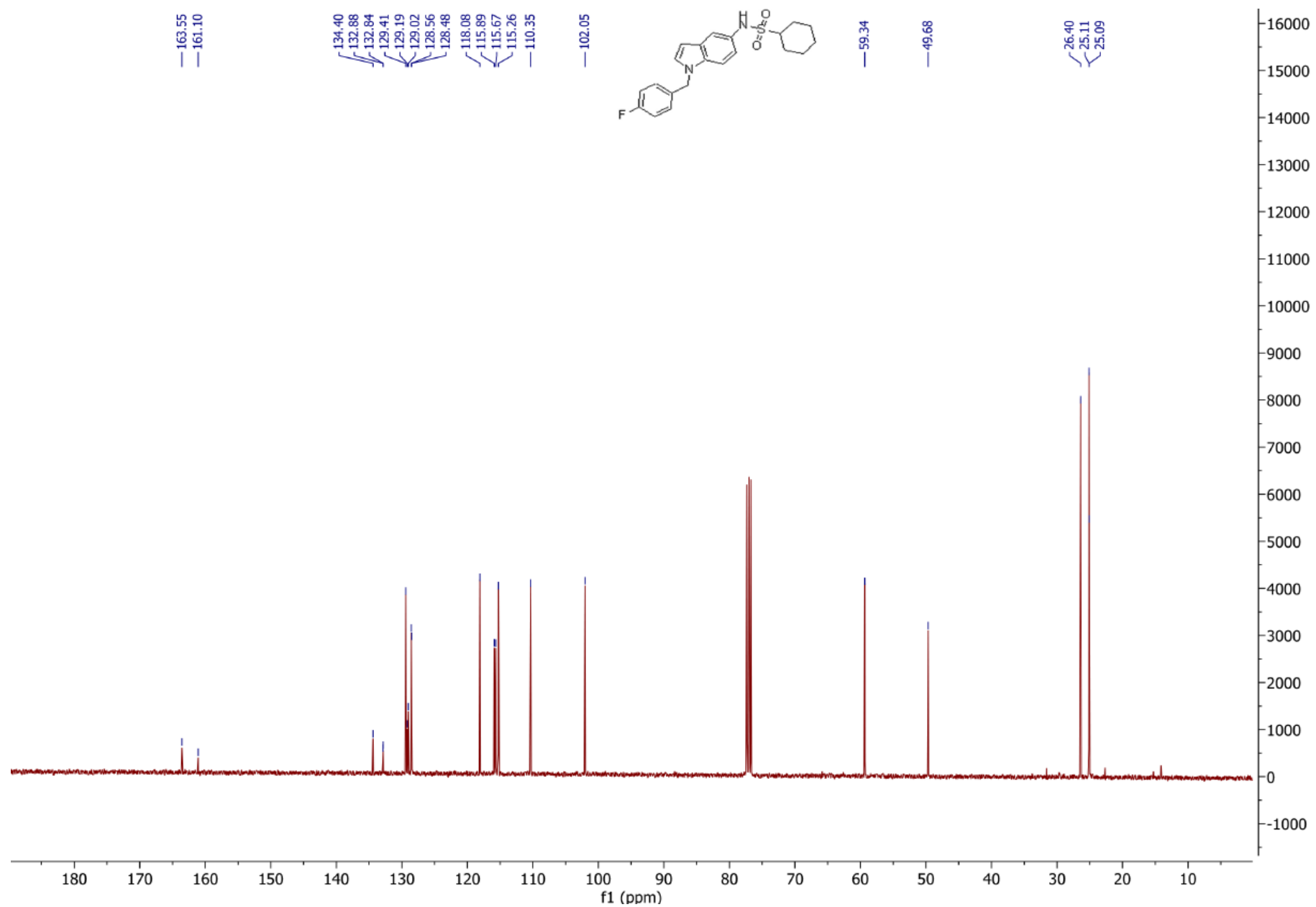
qDEPT NMR of compound **23b**



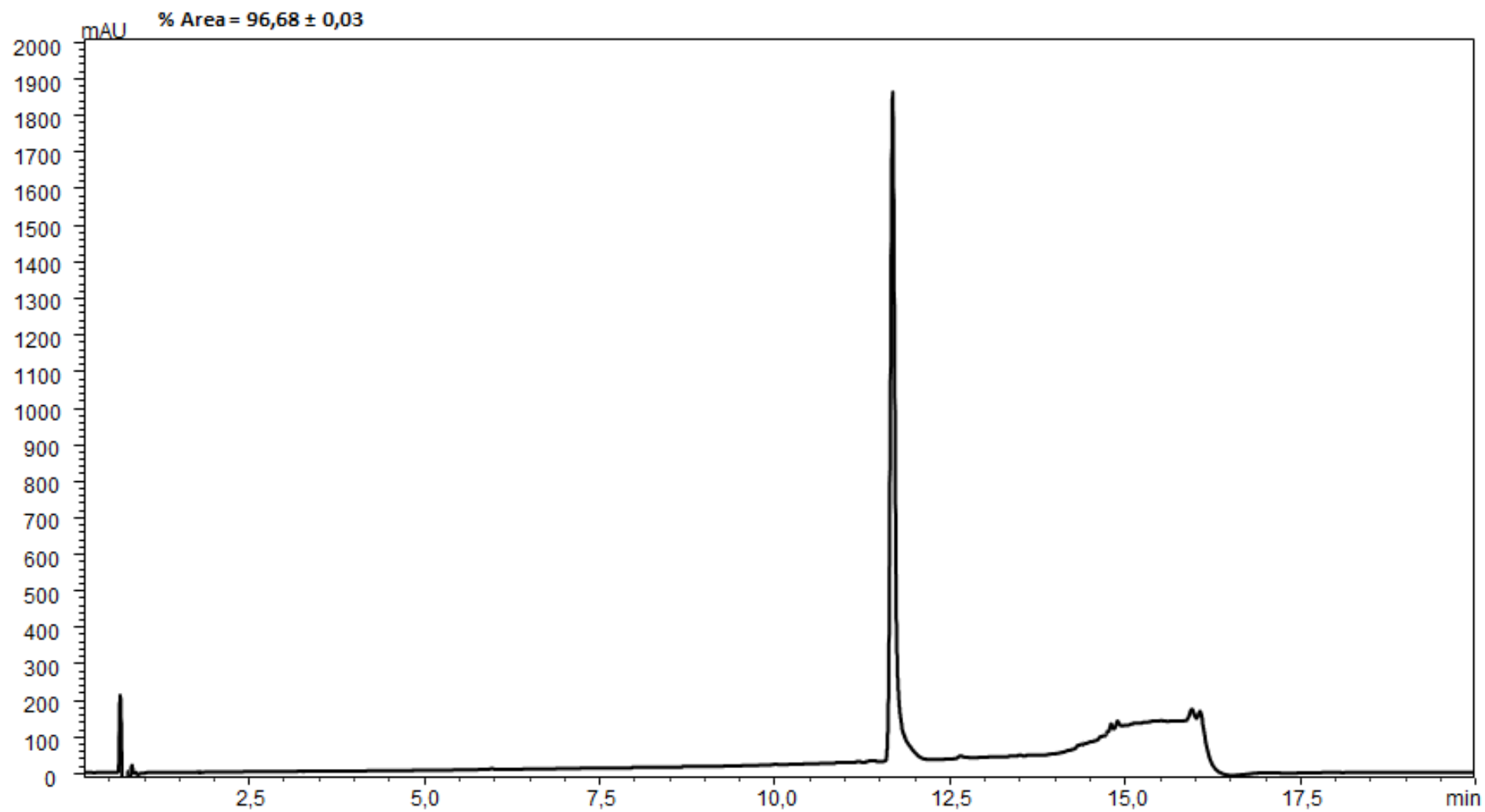
RP-HPLC trace of compound **23b**

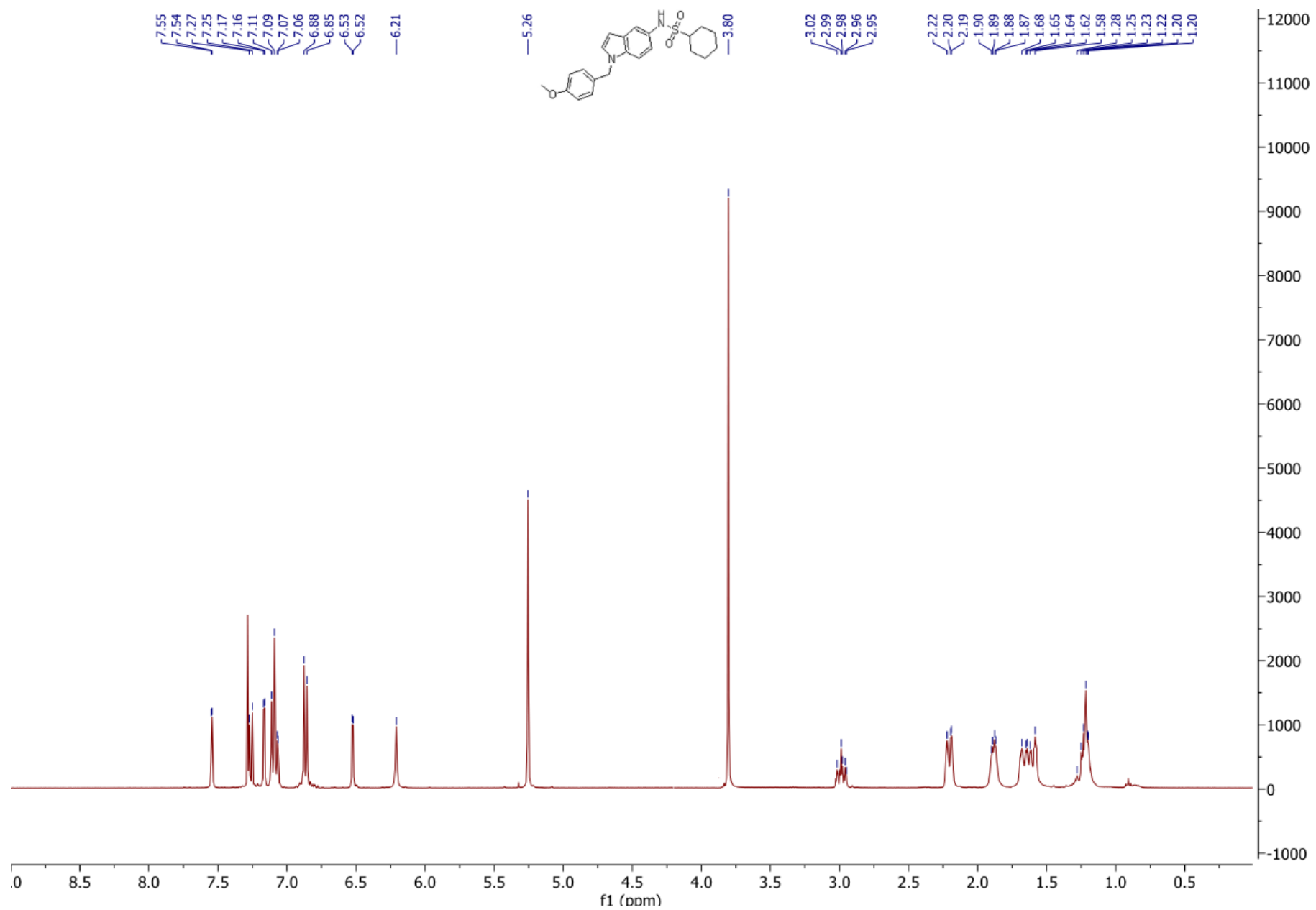


¹H NMR of compound **23c**

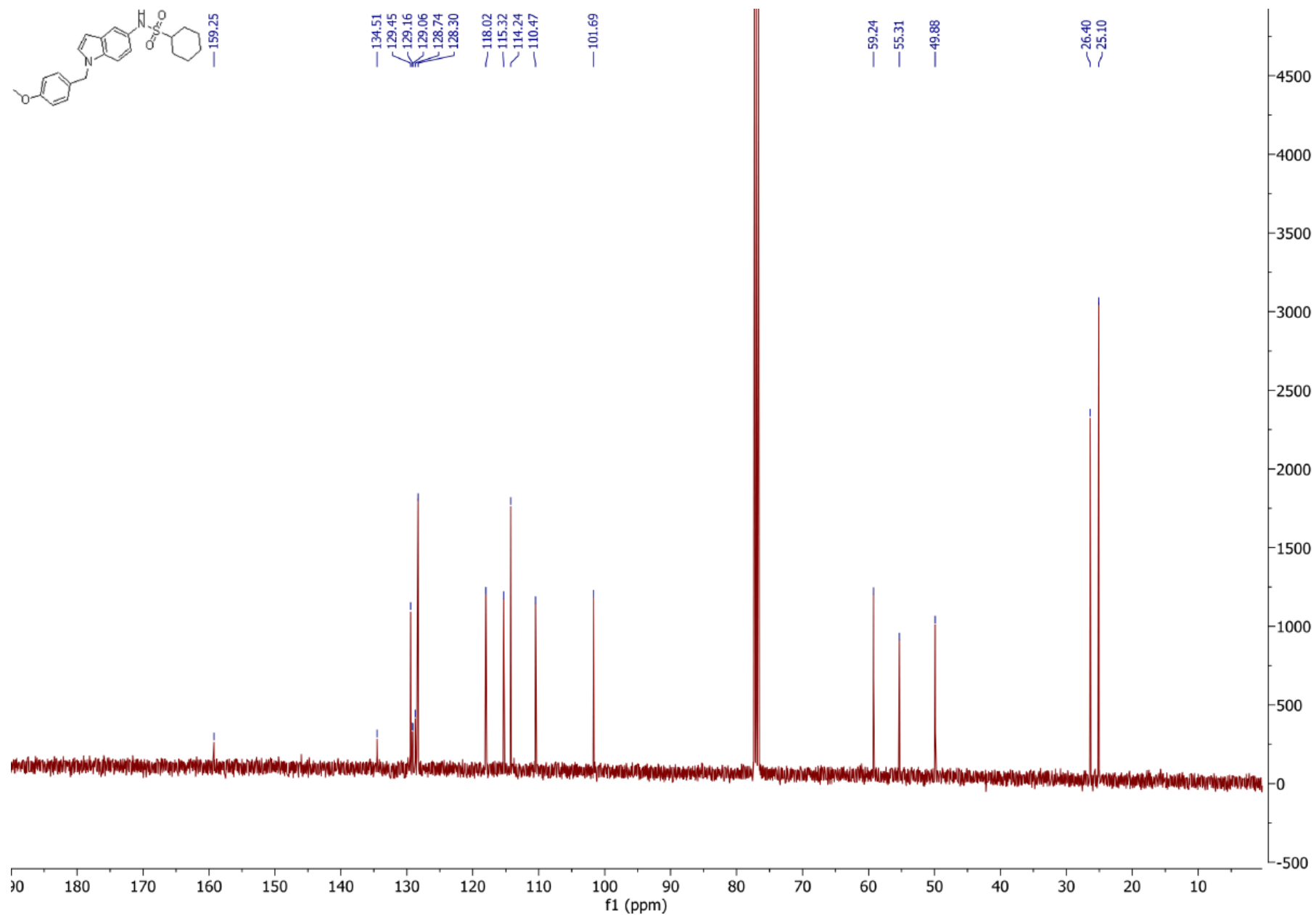


¹³C NMR of compound **23c**

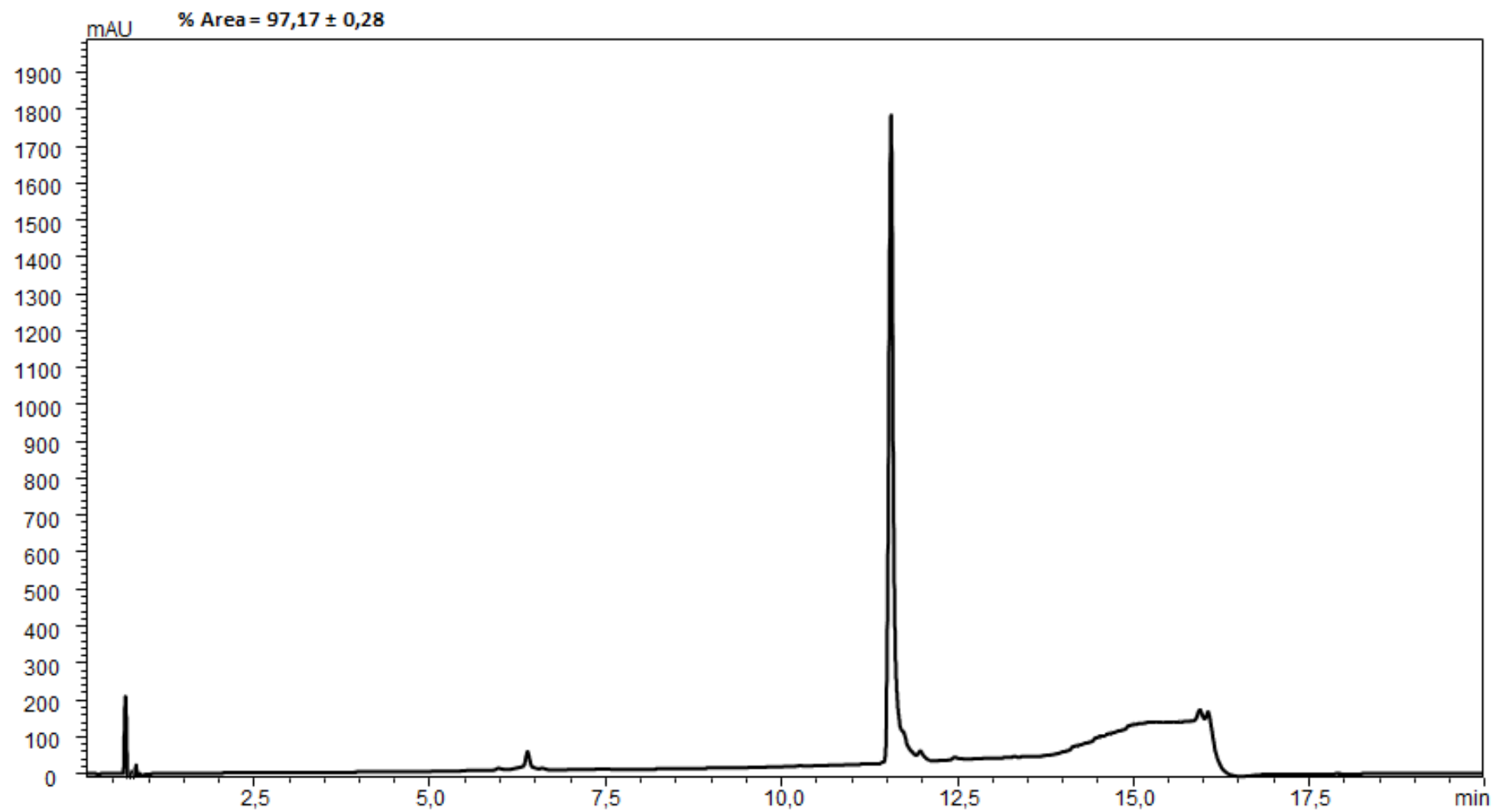




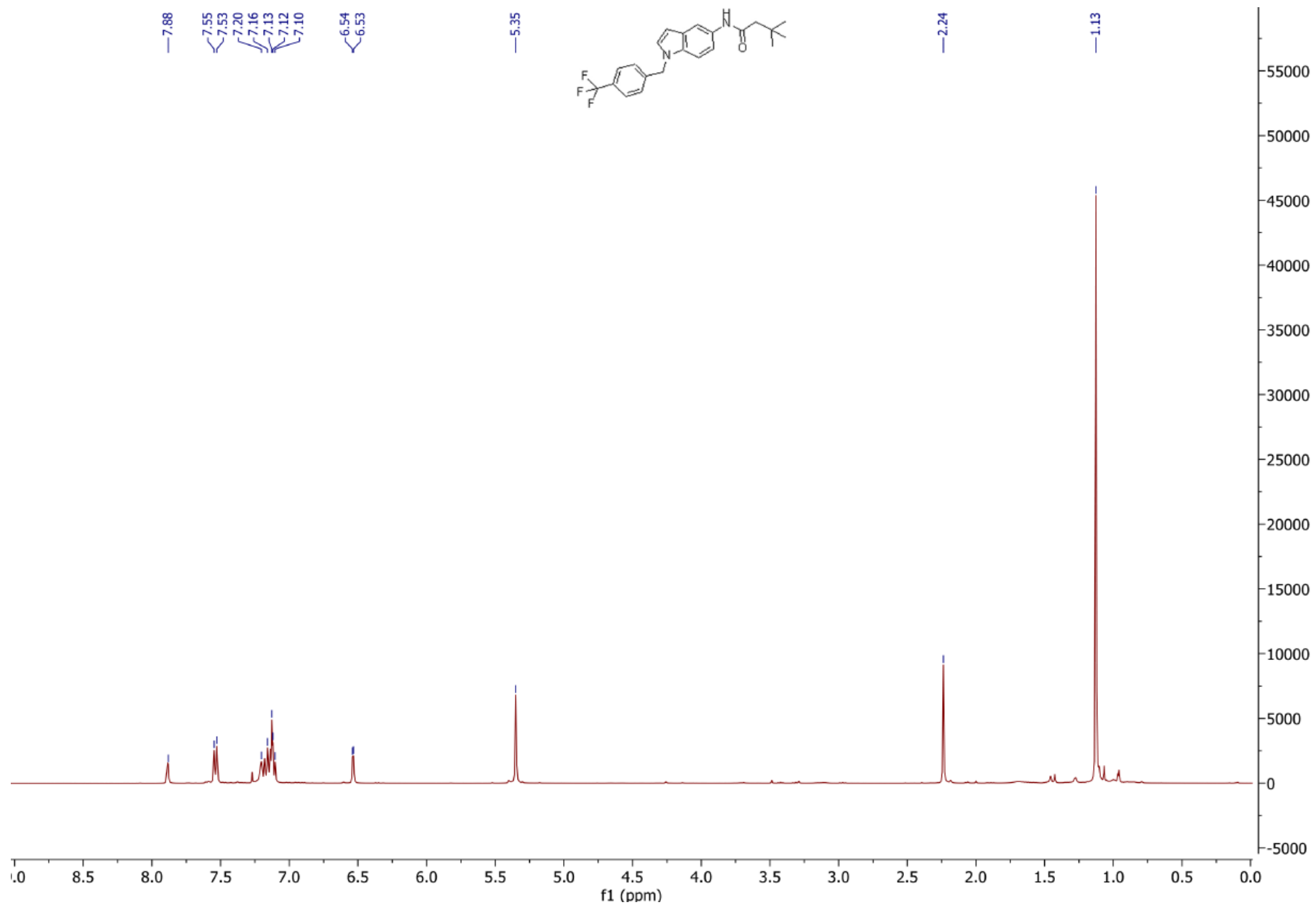
¹H NMR of compound **23d**



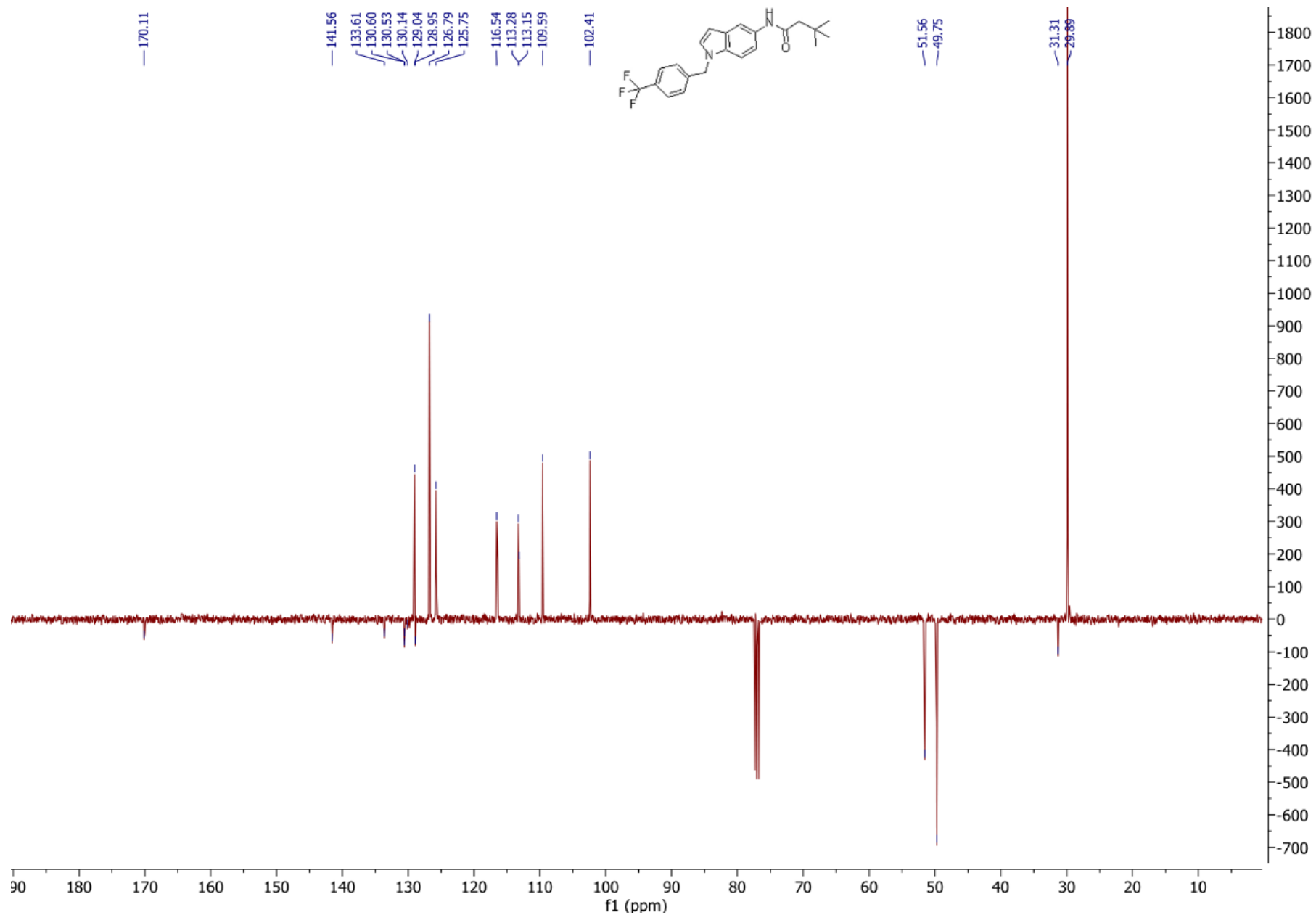
^{13}C NMR of compound **23d**



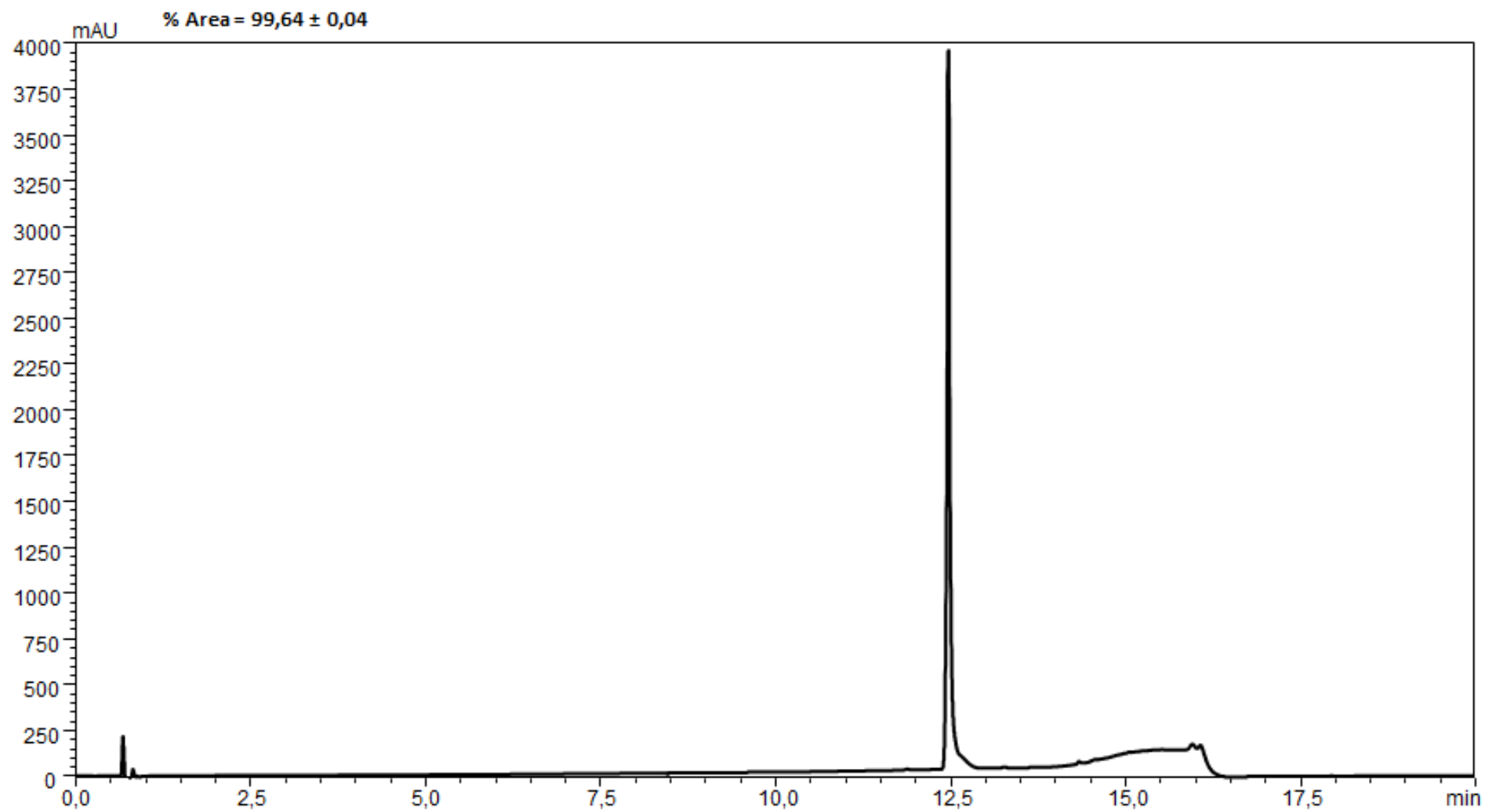
RP-HPLC trace of compound **23d**



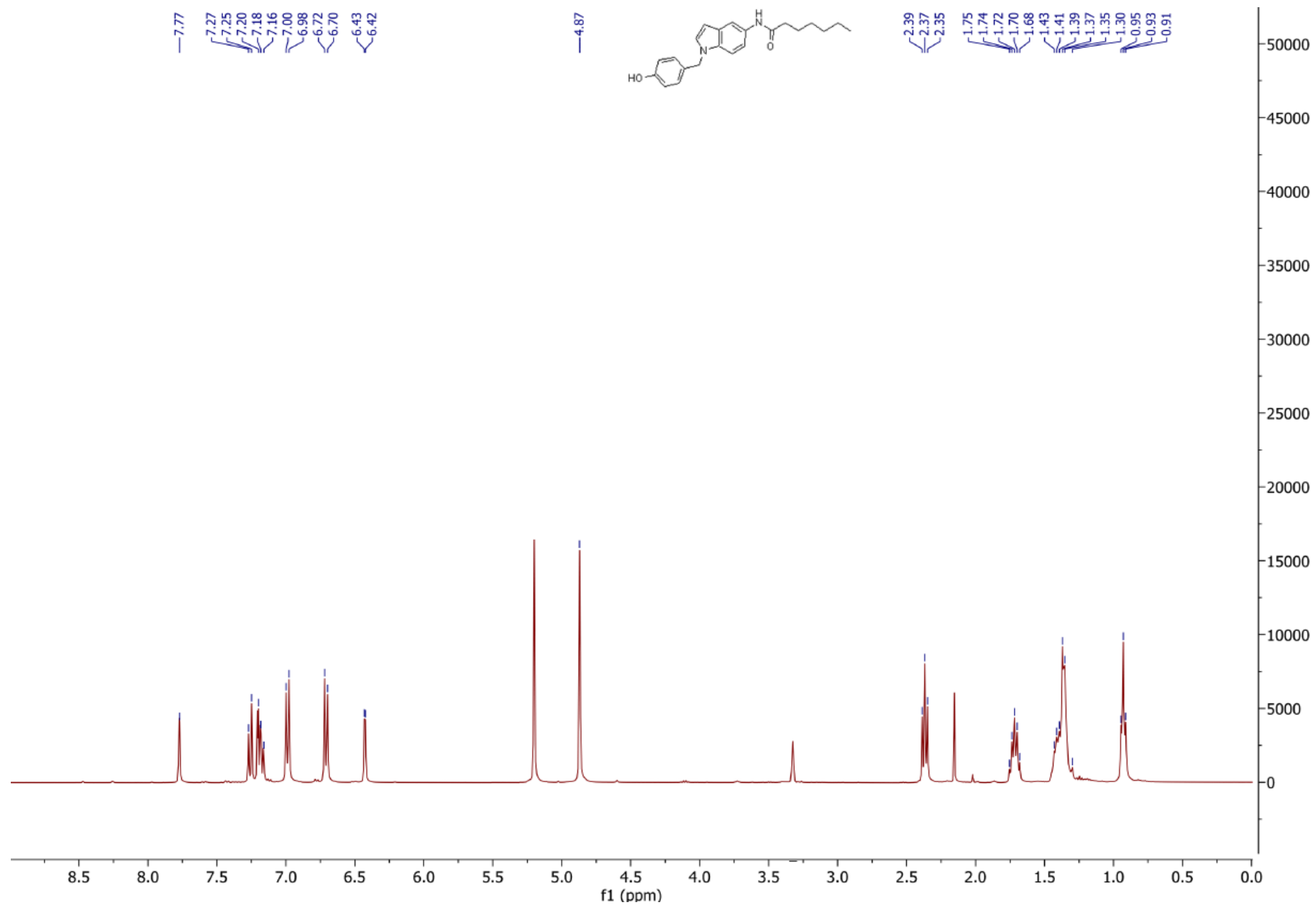
¹H NMR of compound **24a**



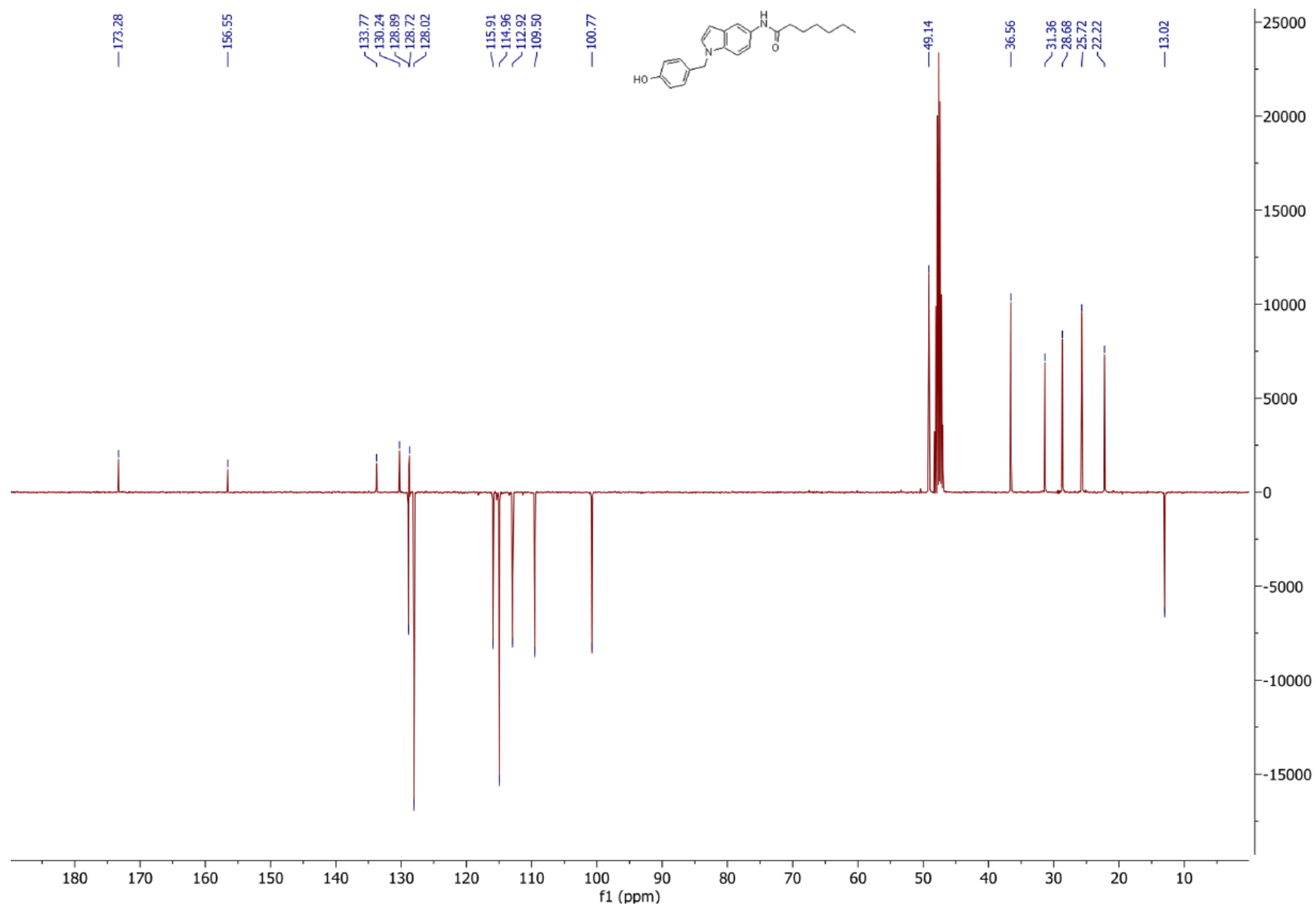
qDEPT NMR of compound **24a**



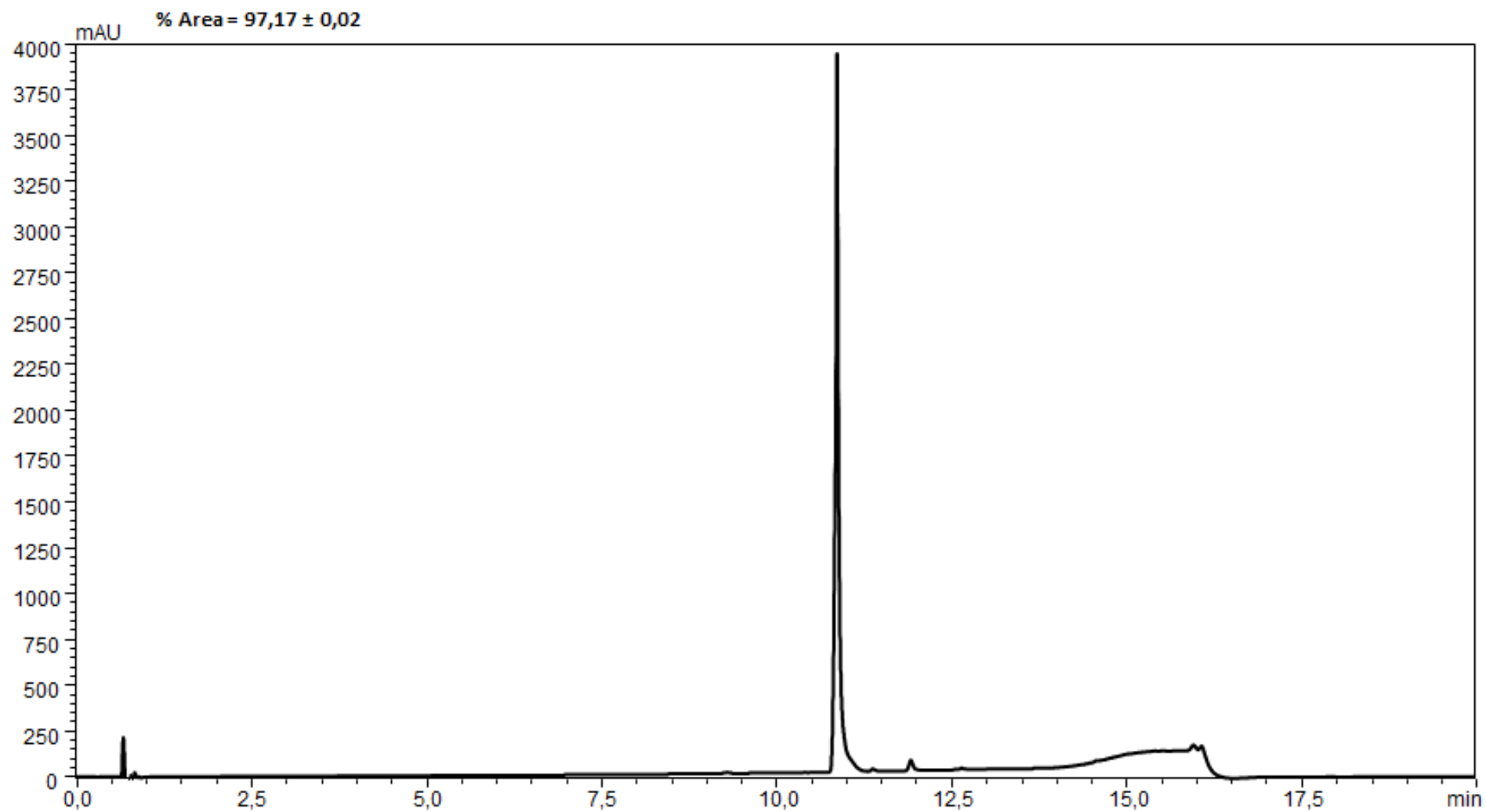
RP-HPLC trace of compound **24a**



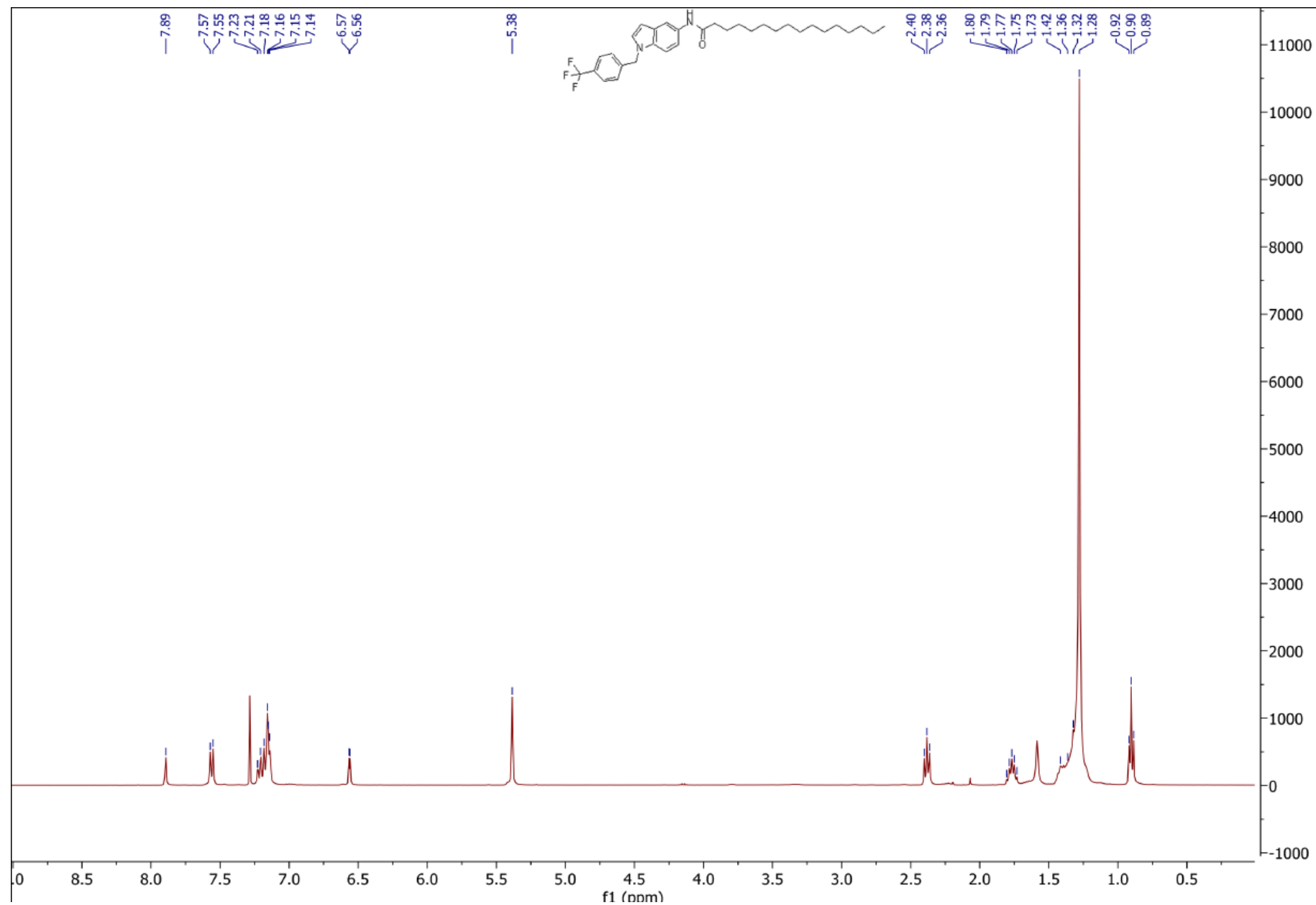
¹H NMR of compound **24b**



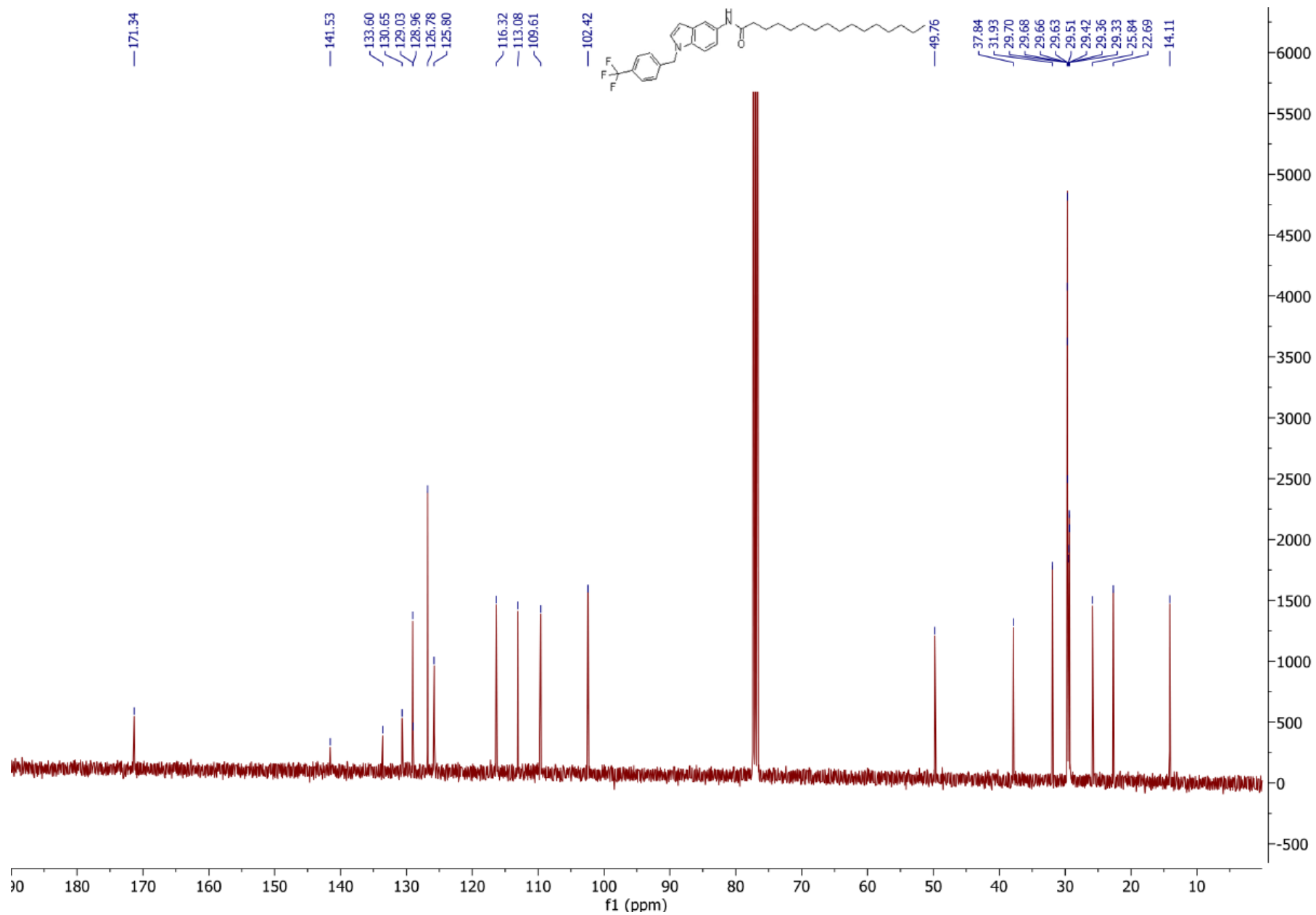
qDEPT NMR of compound **24b**



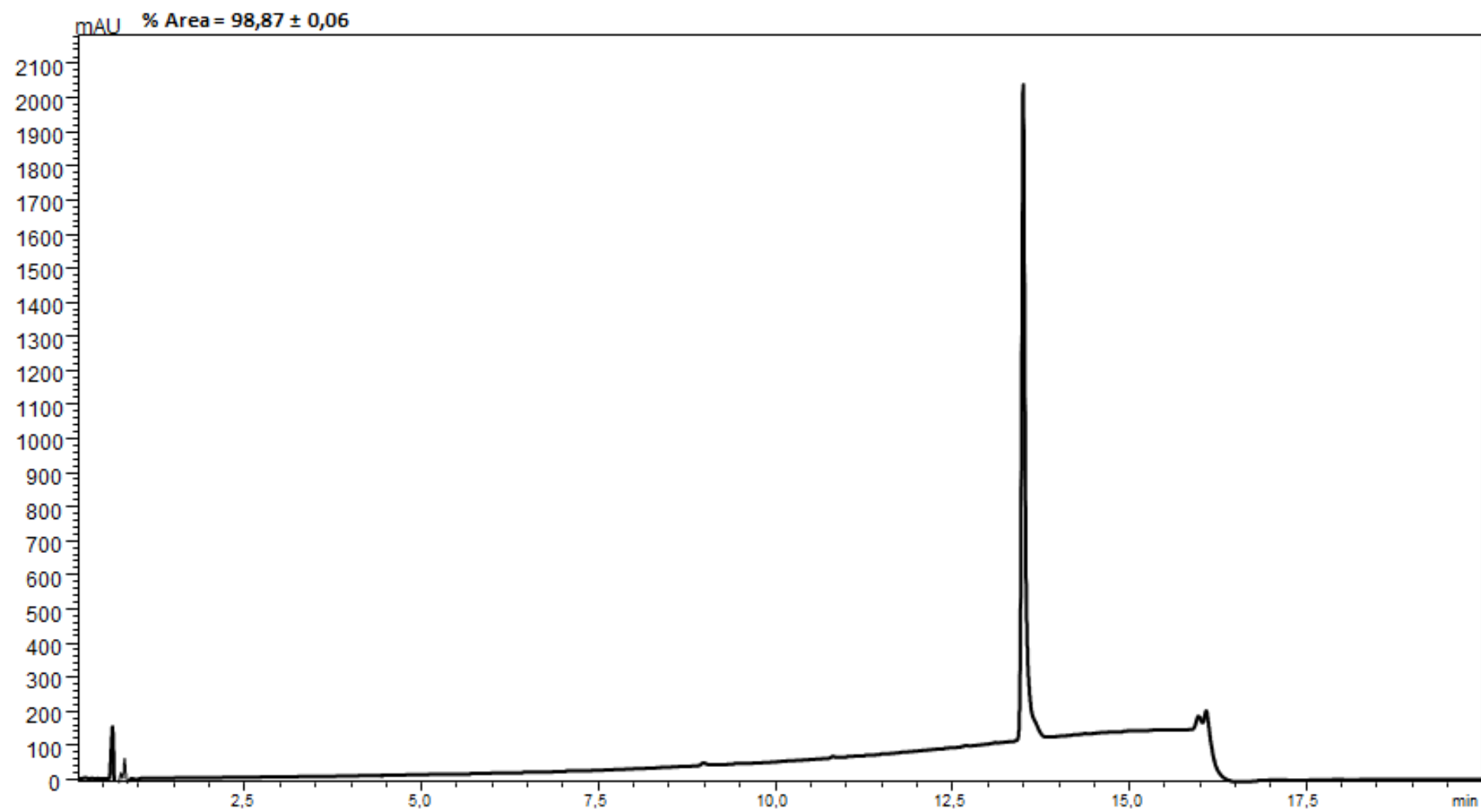
RP-HPLC of compound **24b**

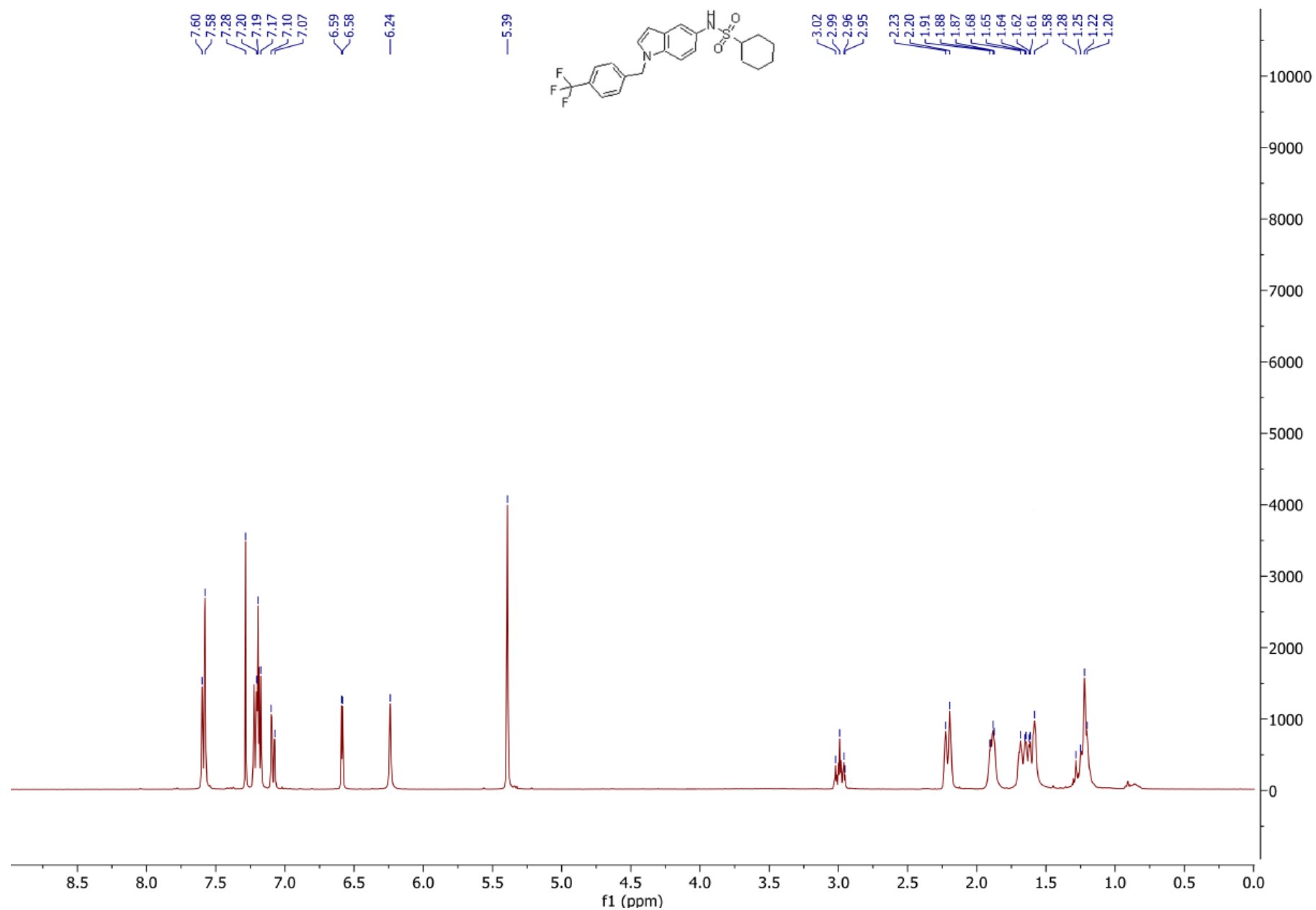


¹H NMR of compound **25a**

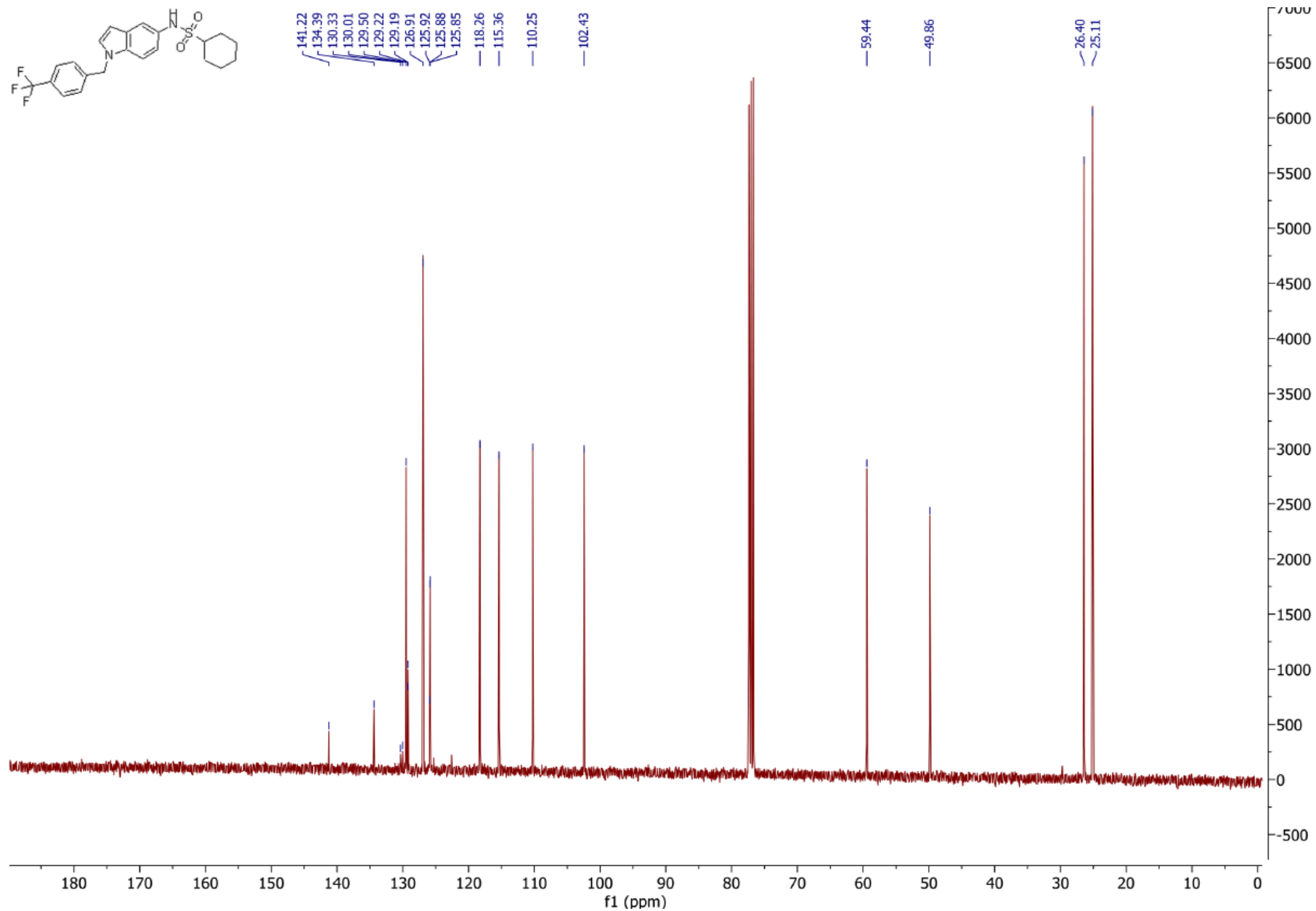


¹³C NMR of compound **25a**

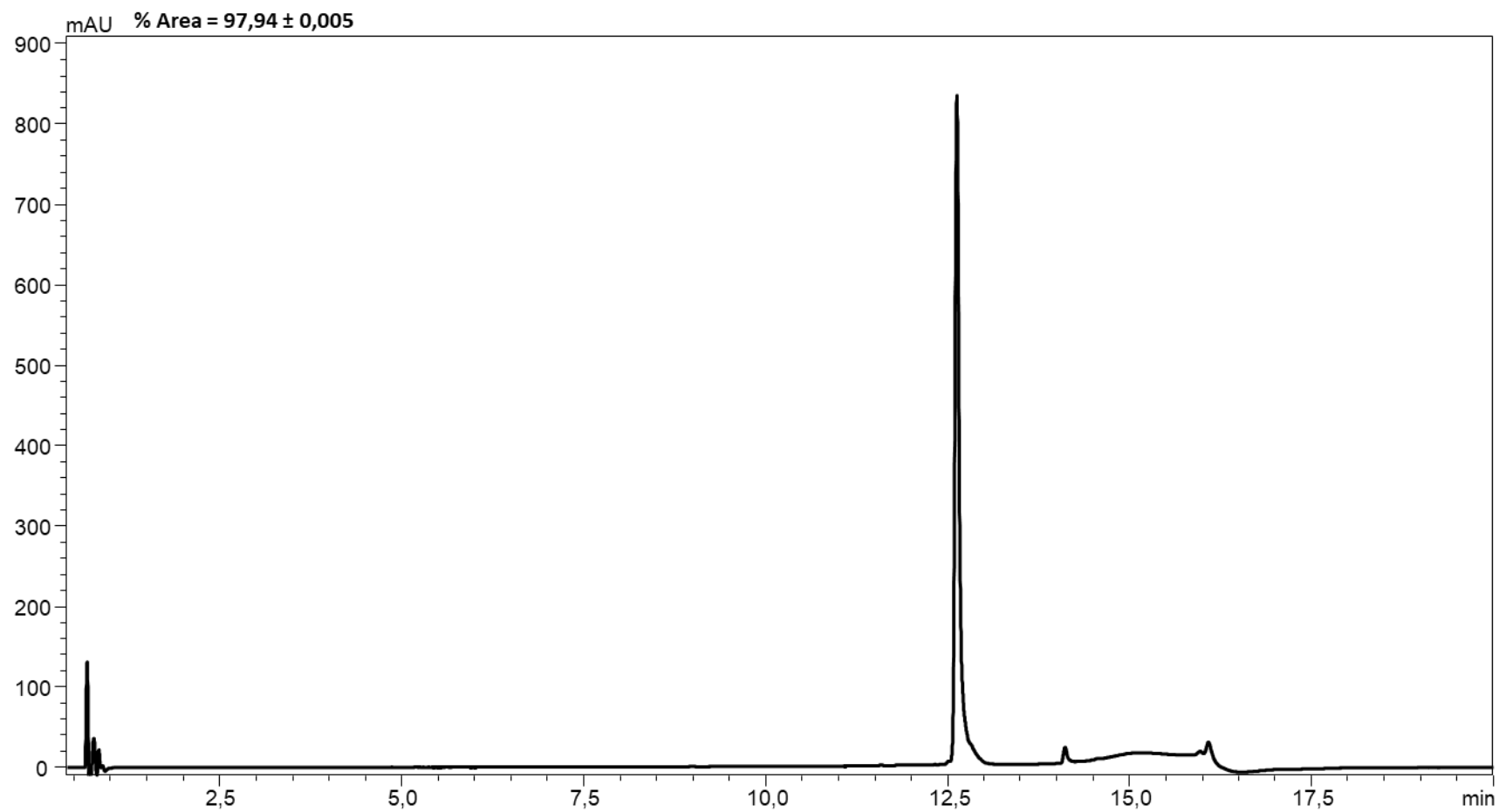




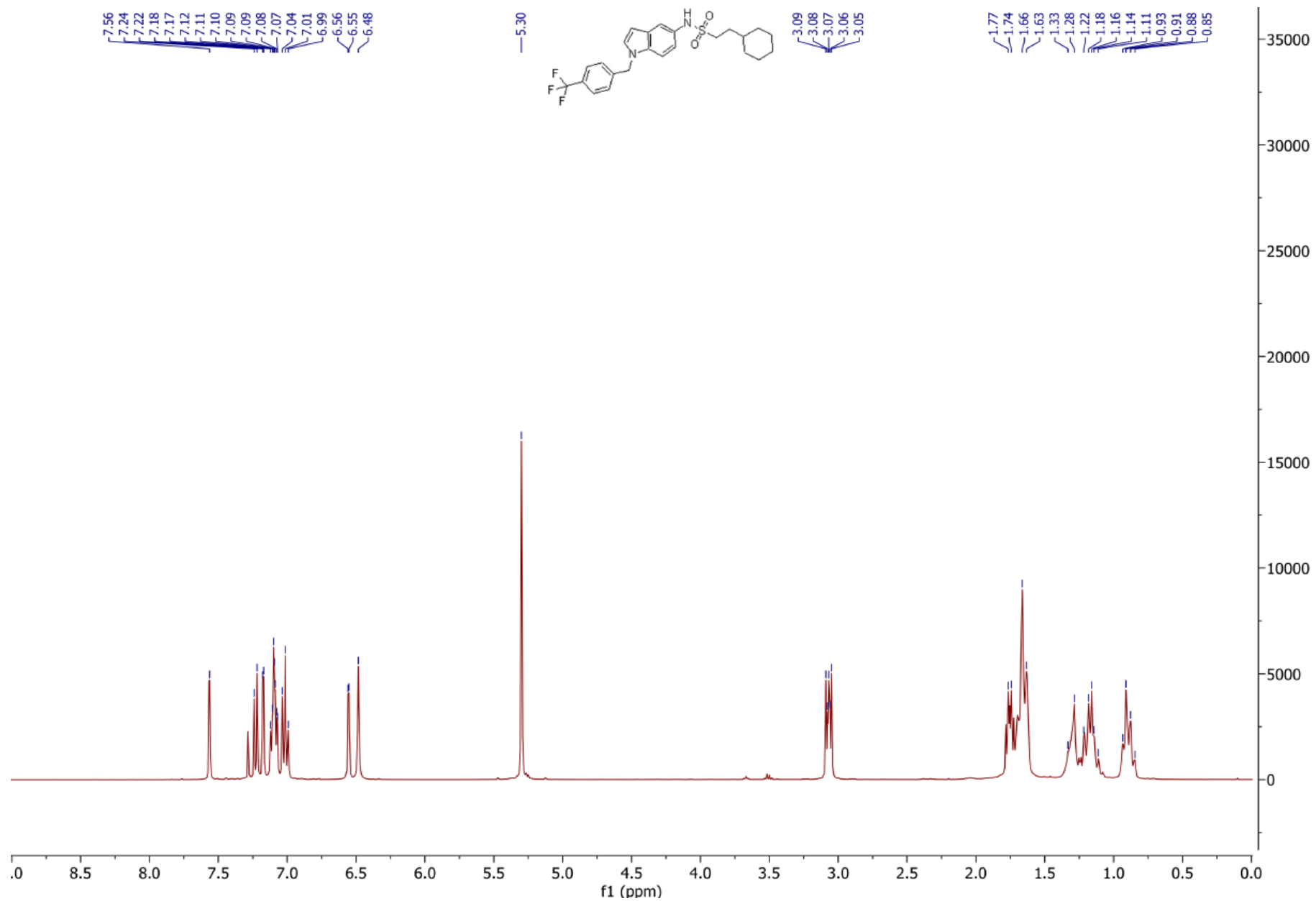
¹H NMR of compound **26a**



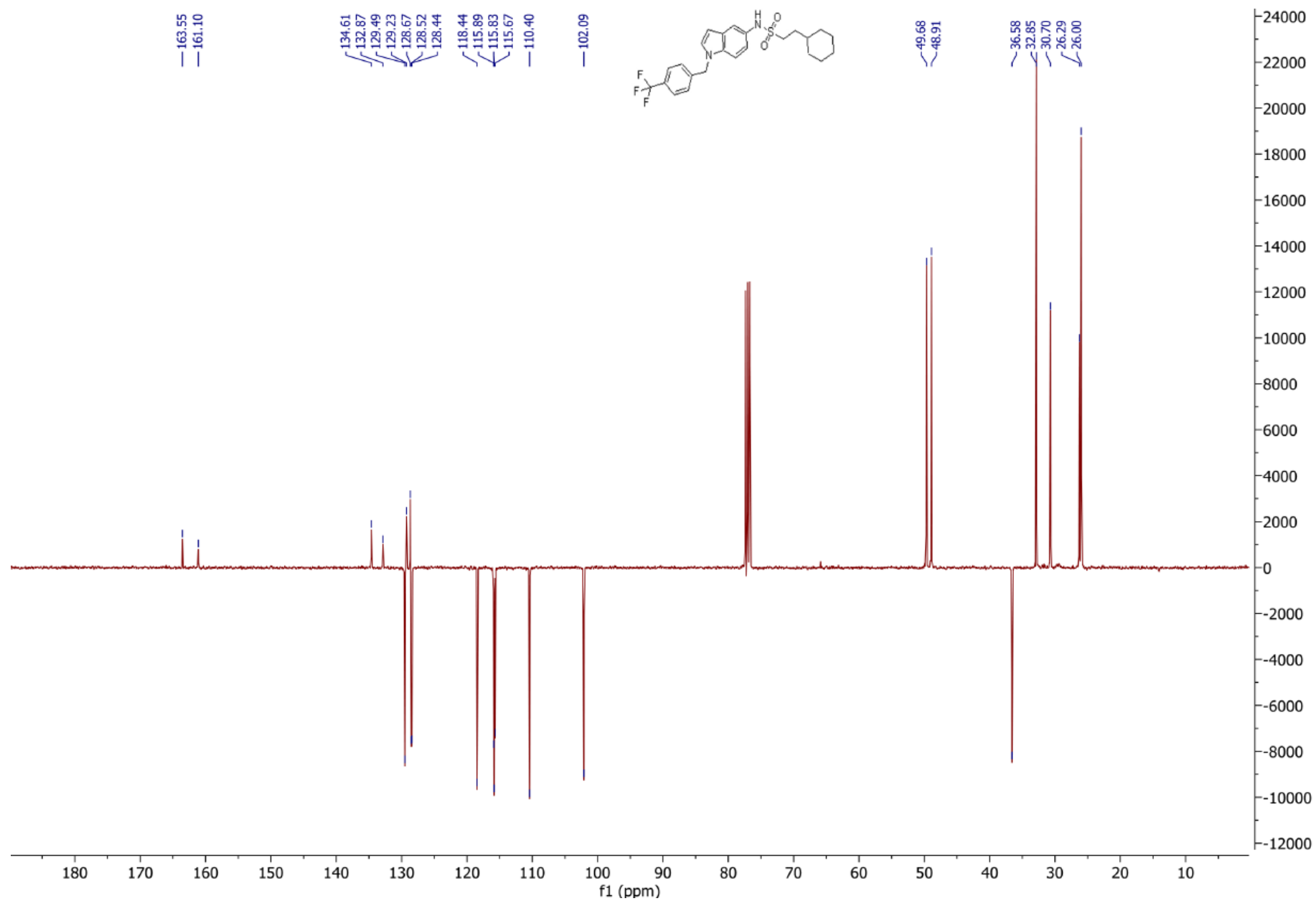
^{13}C NMR of compound **26a**



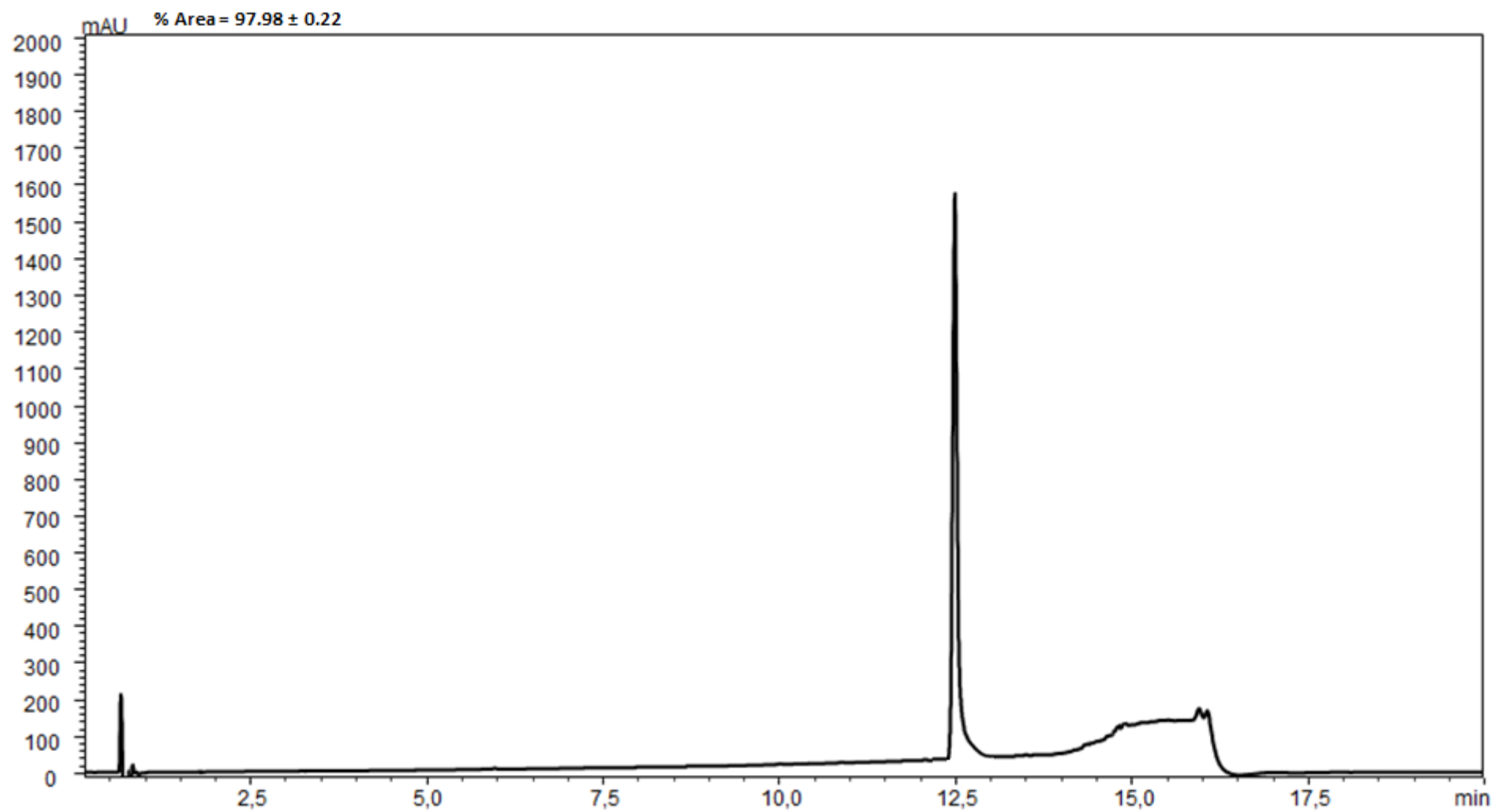
RP-HPLC trace of compound **26a**

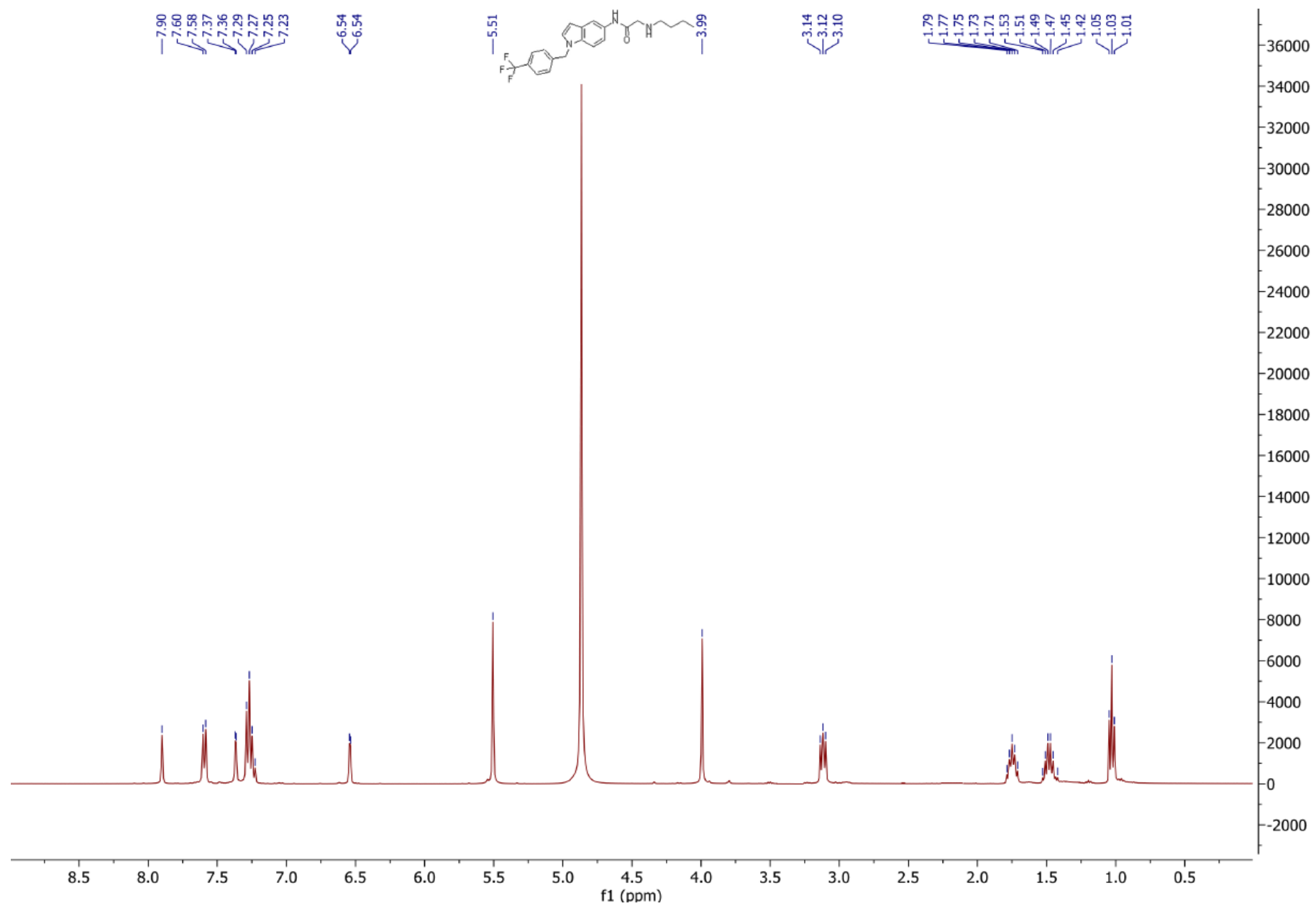


¹H NMR of compound **27a**

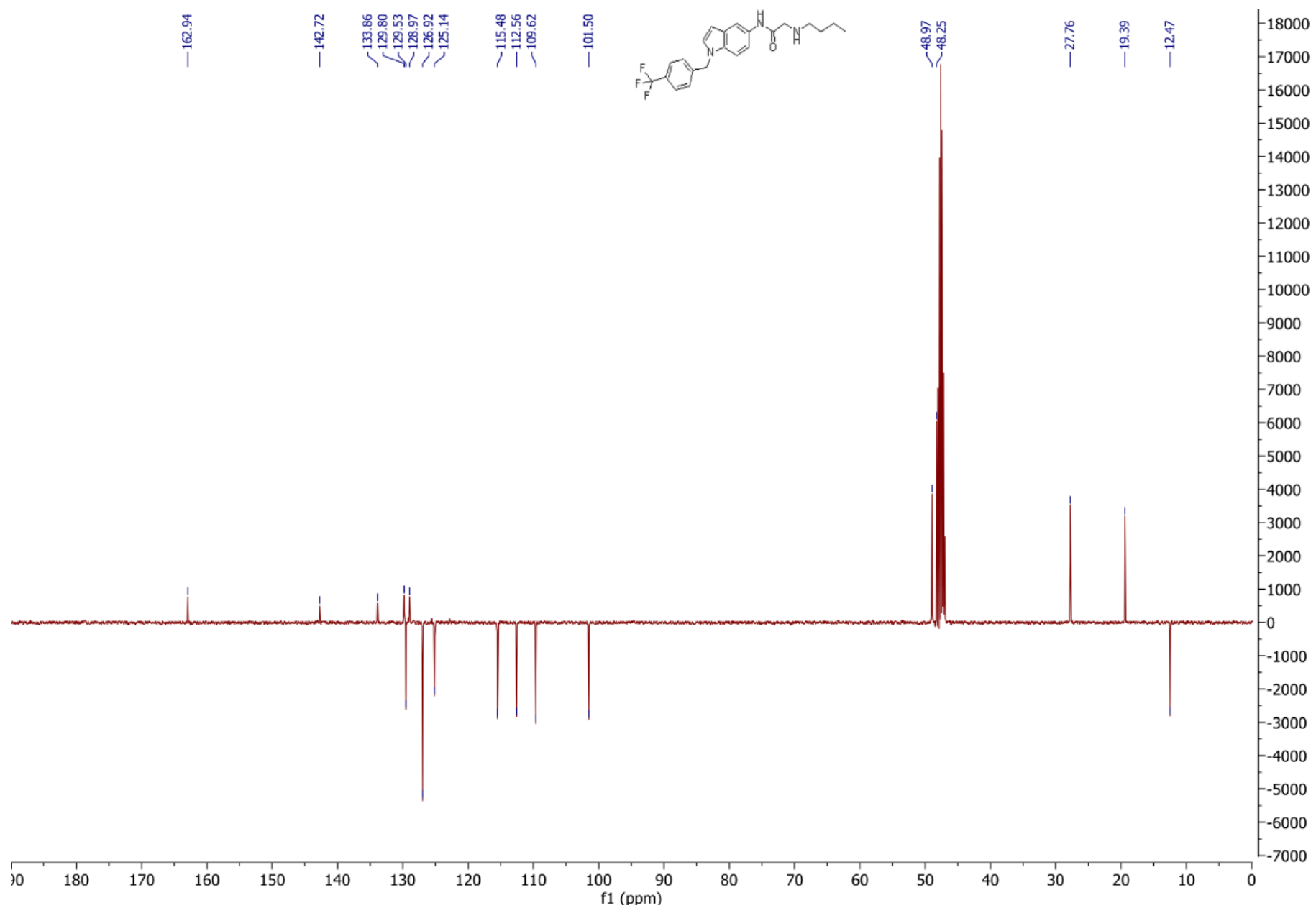


qDEPT NMR of compound **27a**

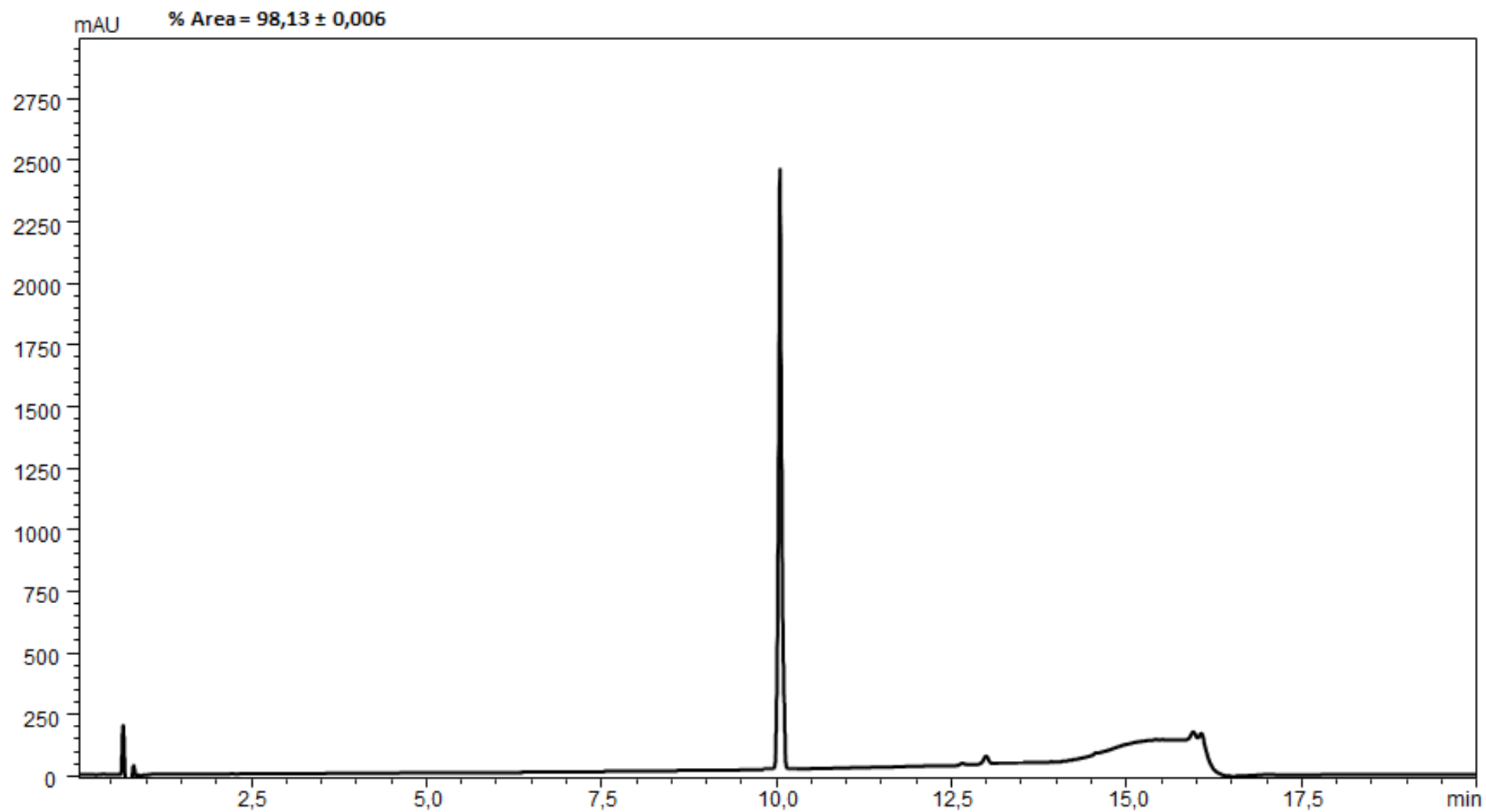




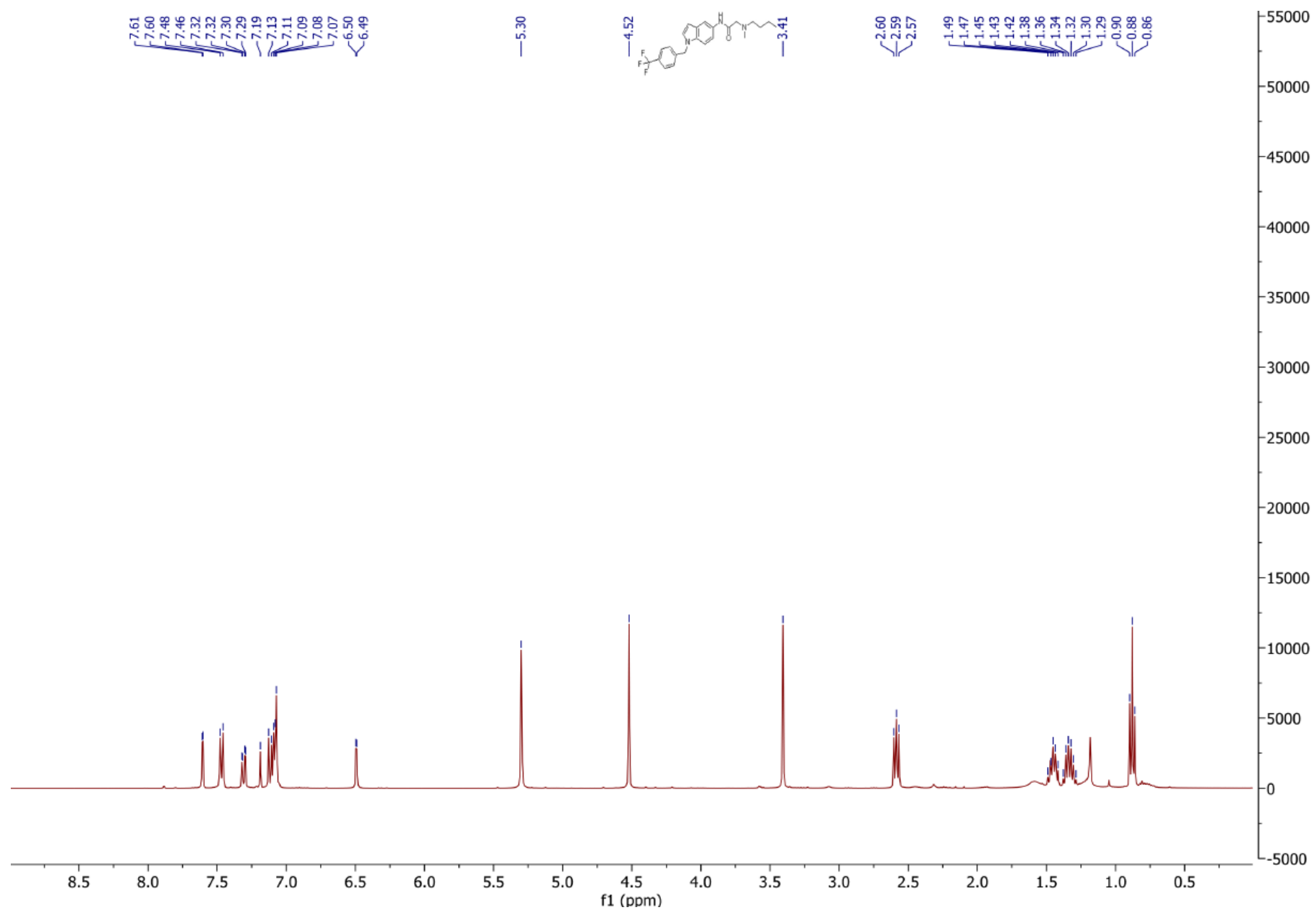
¹H NMR of compound **28a**



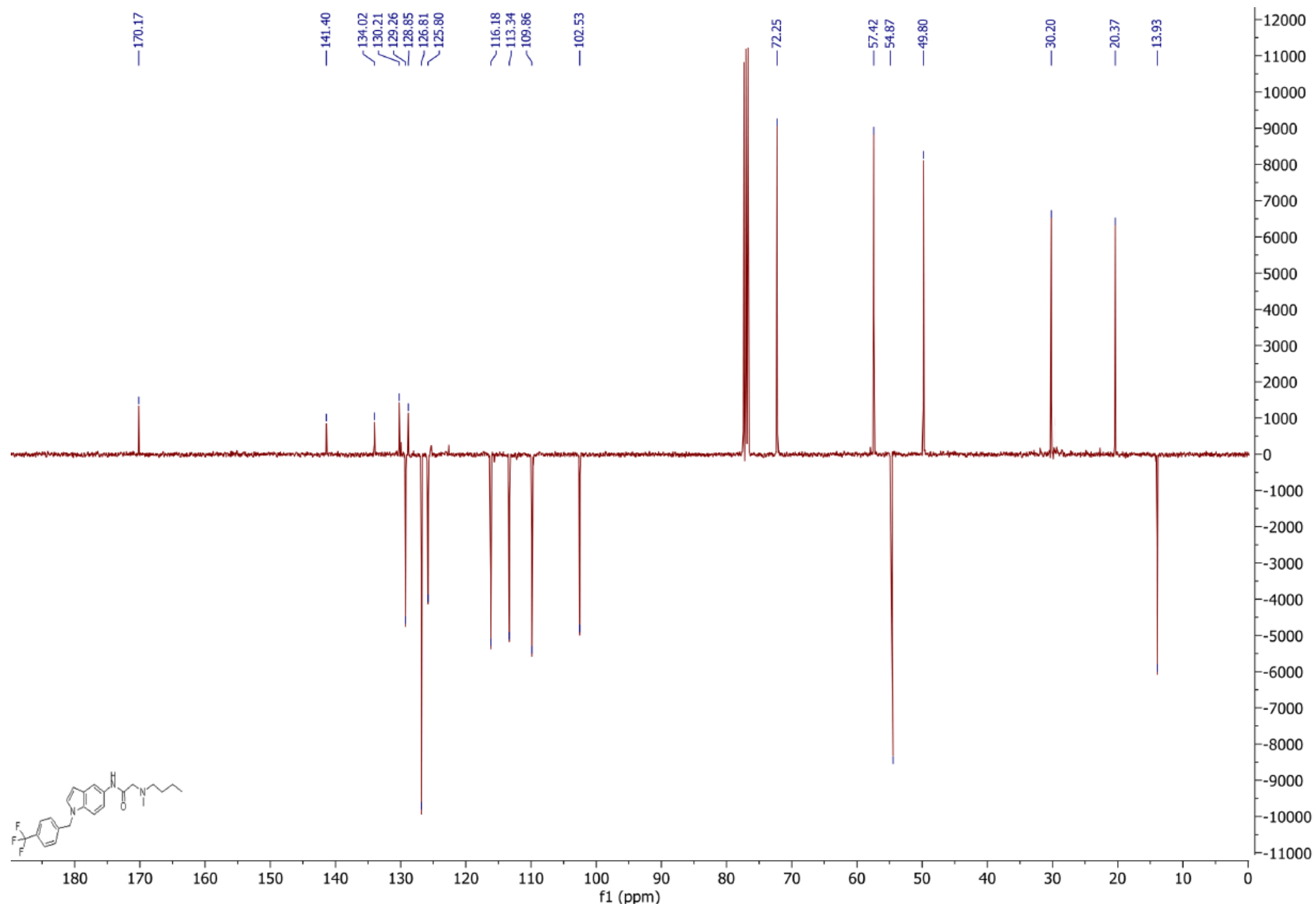
qDEPT NMR of compound **28a**



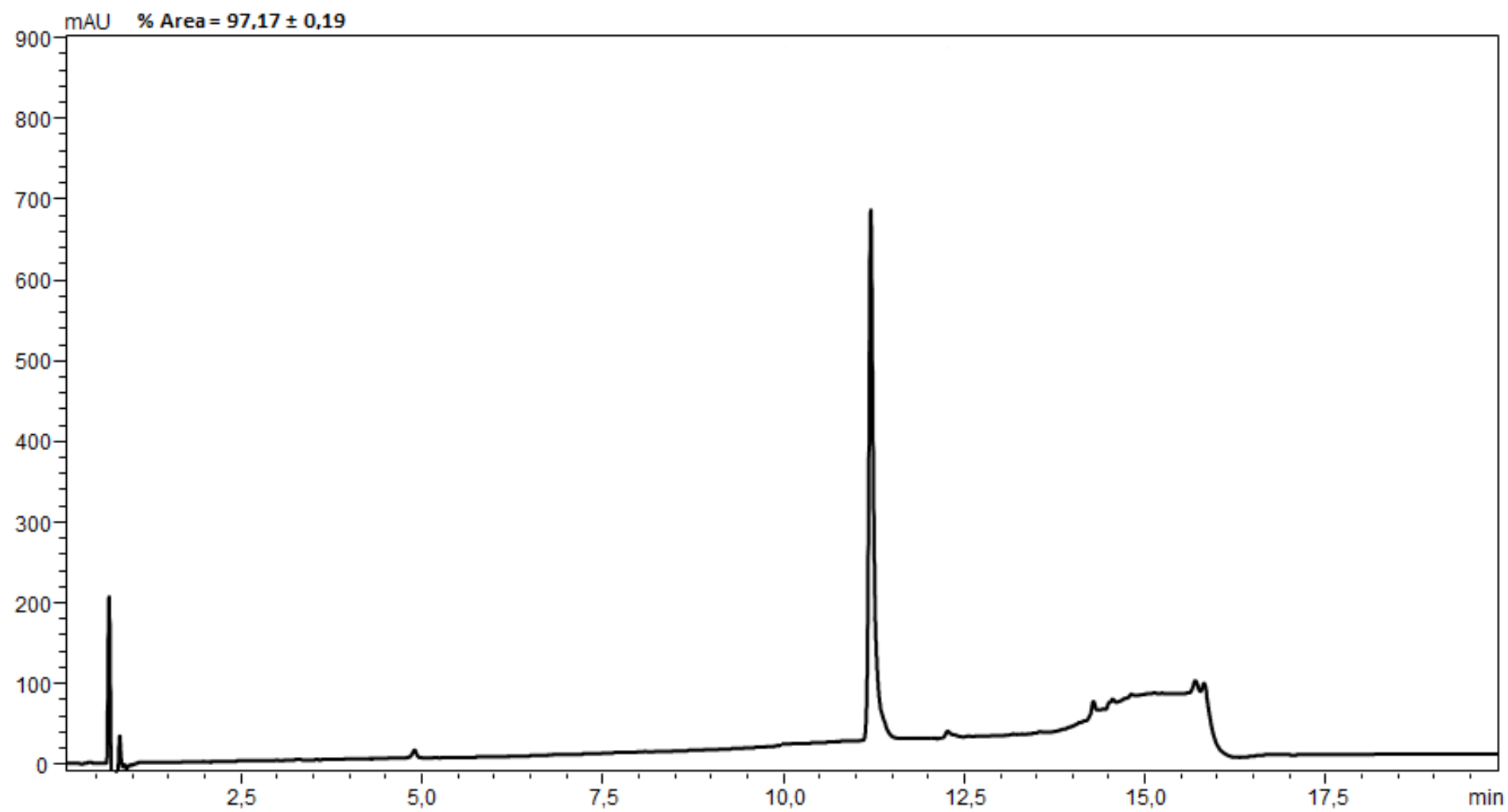
RP-HPLC trace of compound **28a**

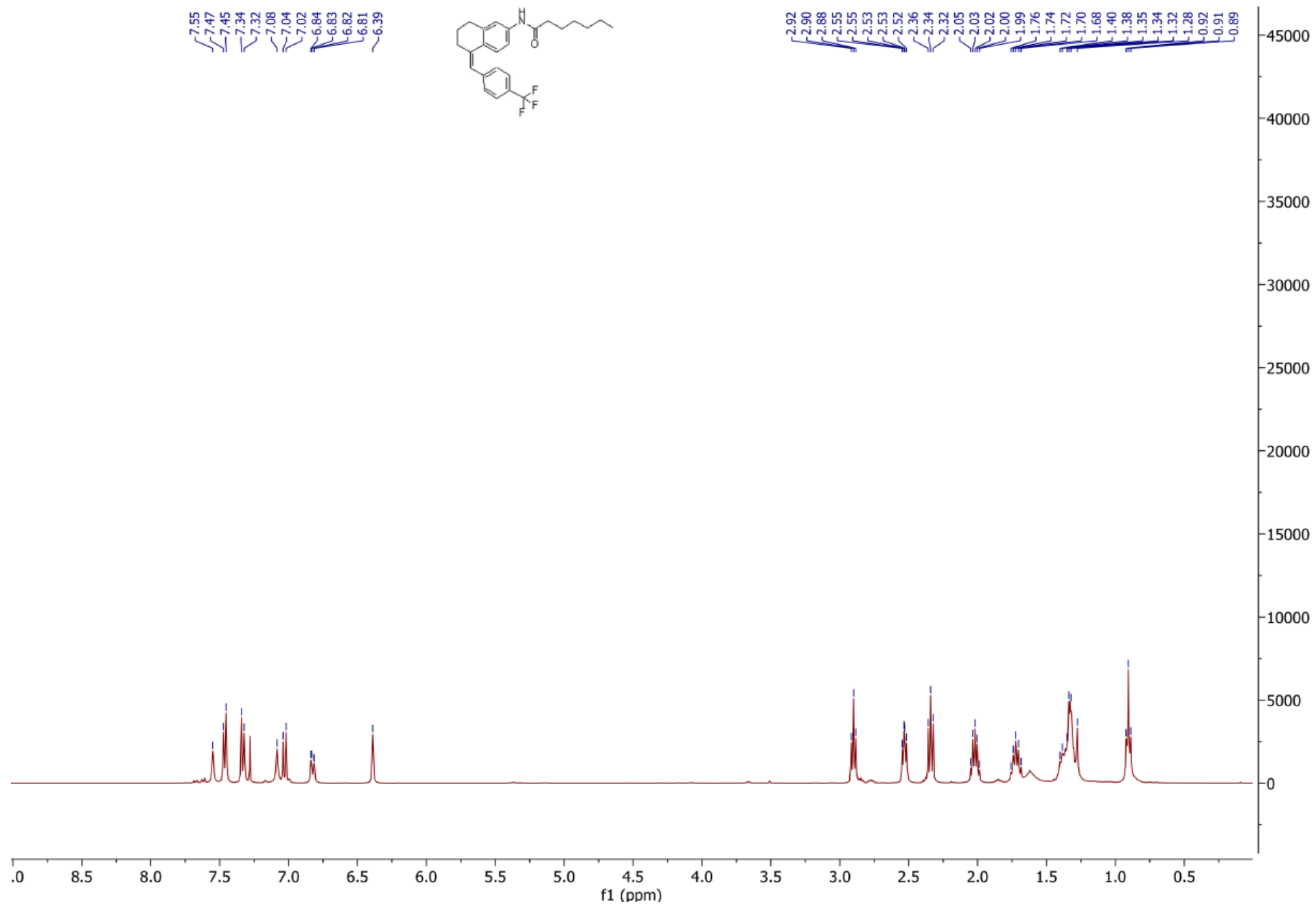


¹H NMR of compound 29a

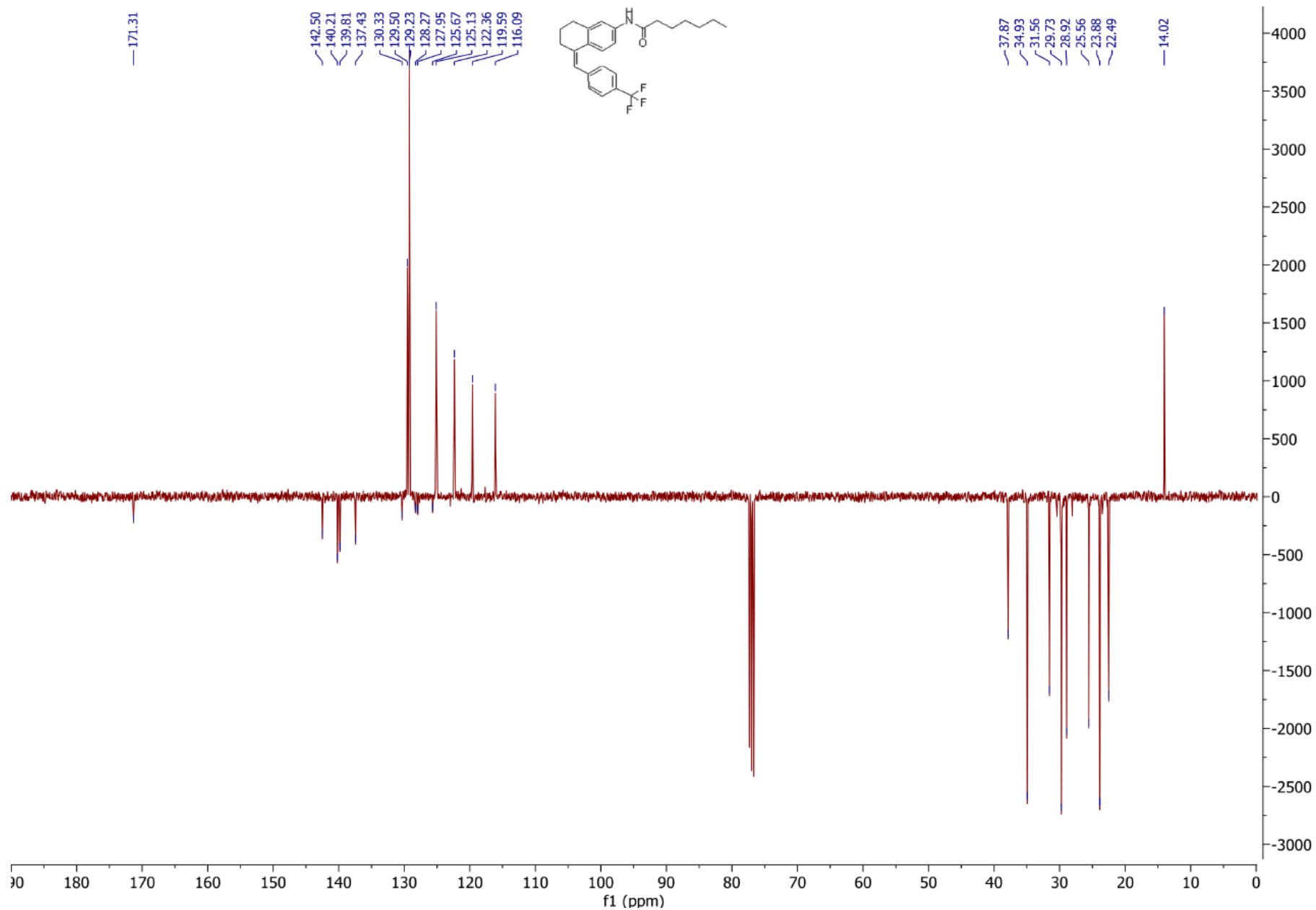


¹³C NMR of compound **29a**

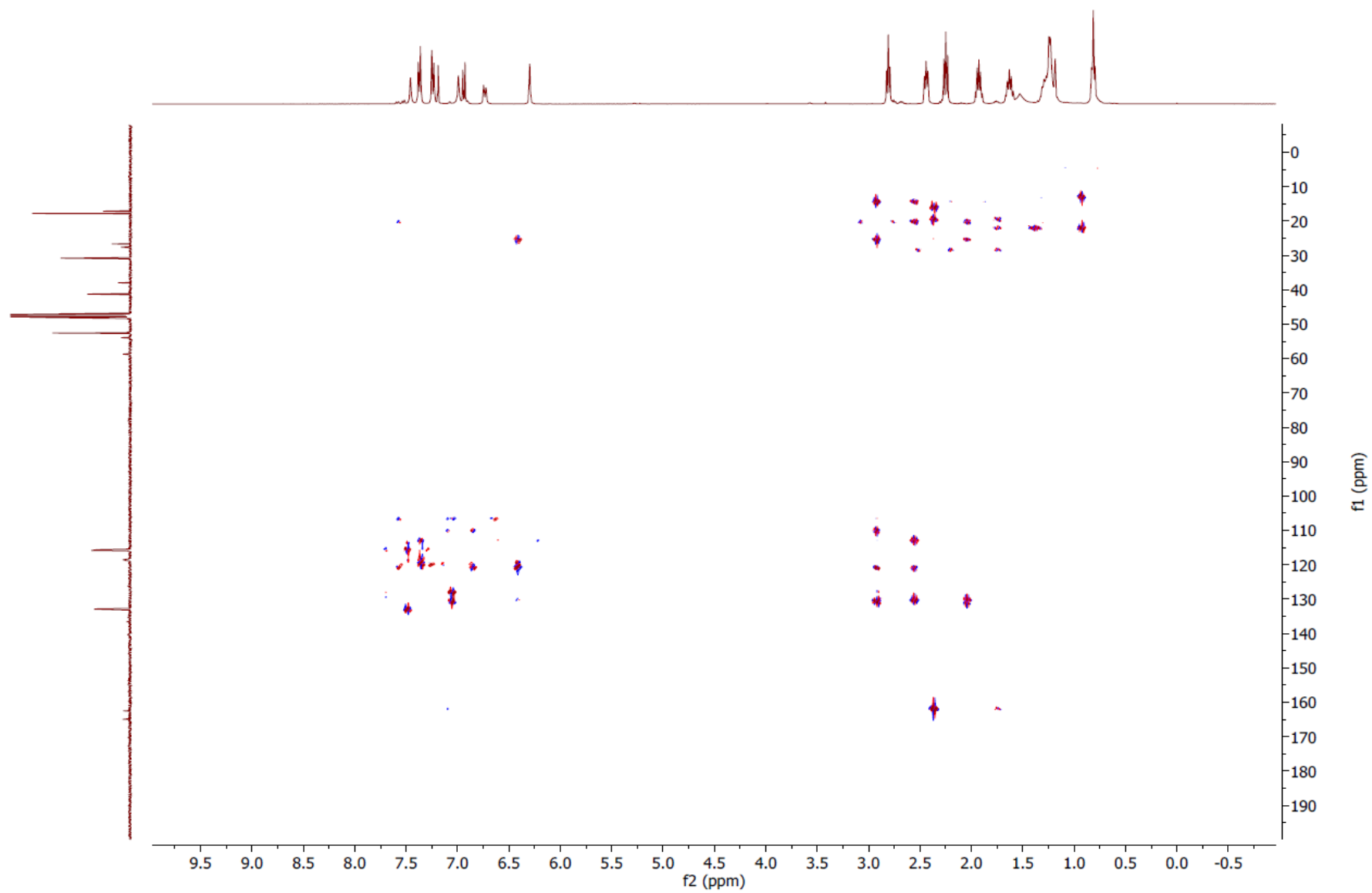




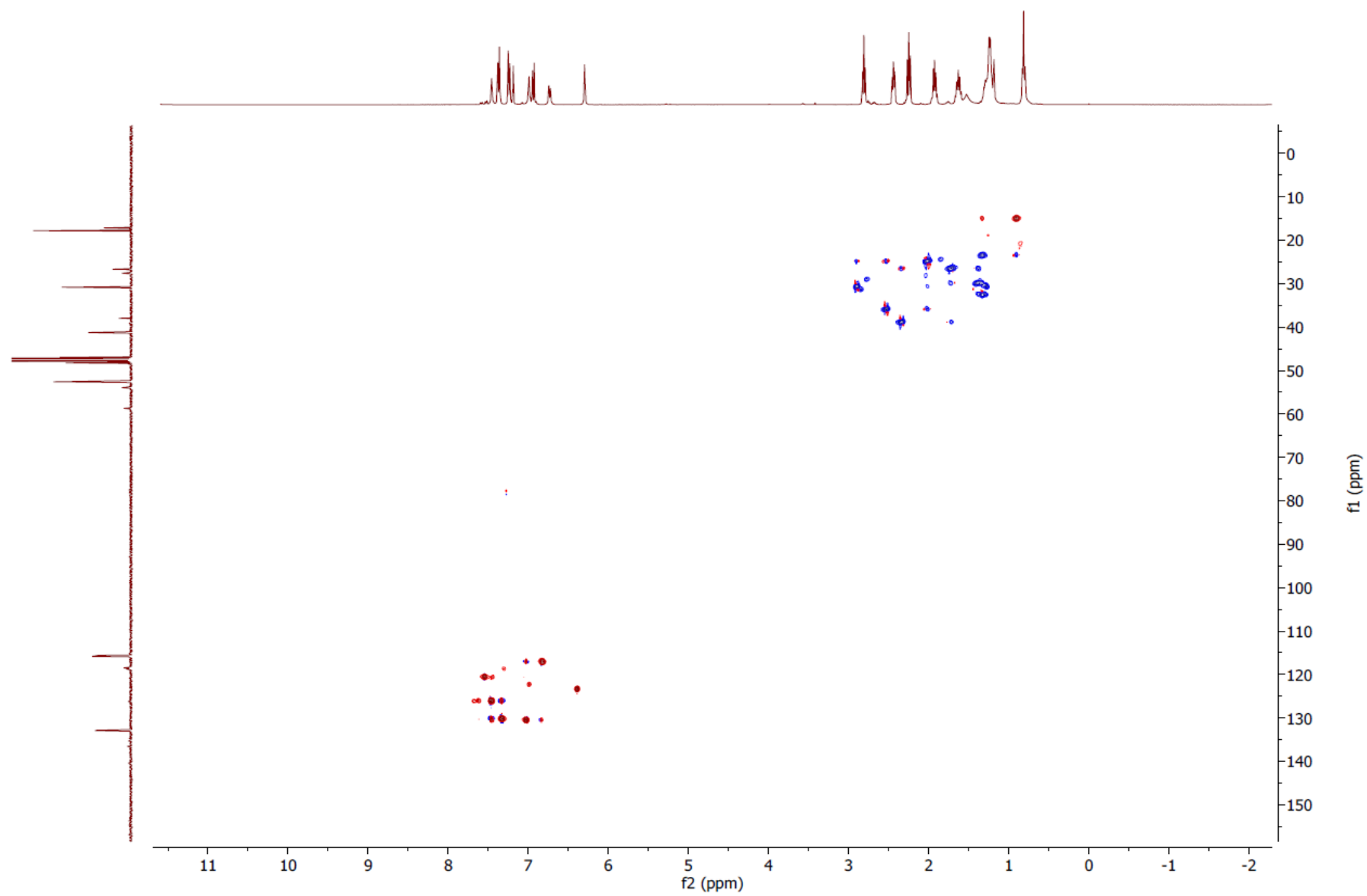
¹H NMR of compound **33a**



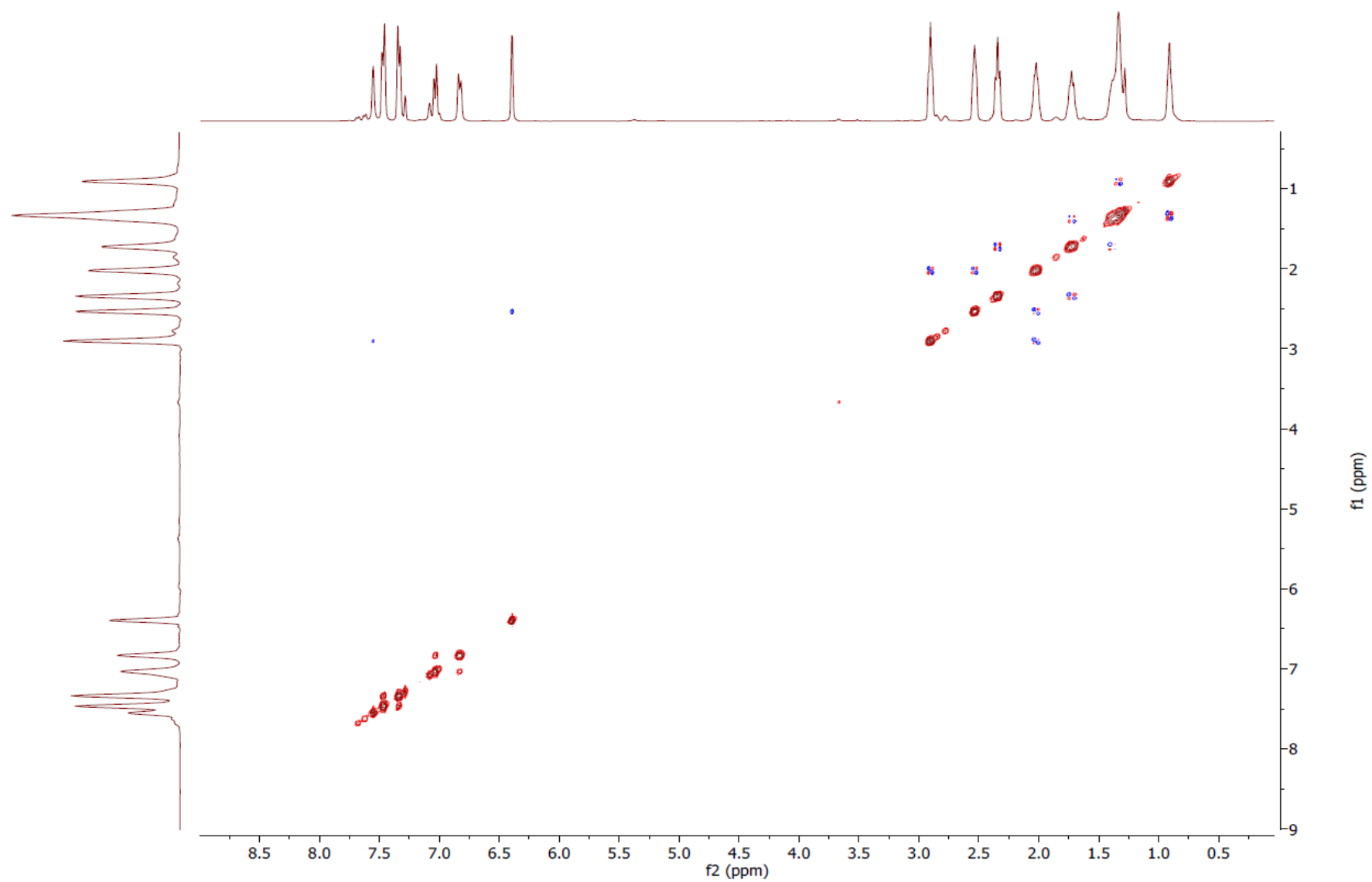
qDEPT NMR of compound **33a**



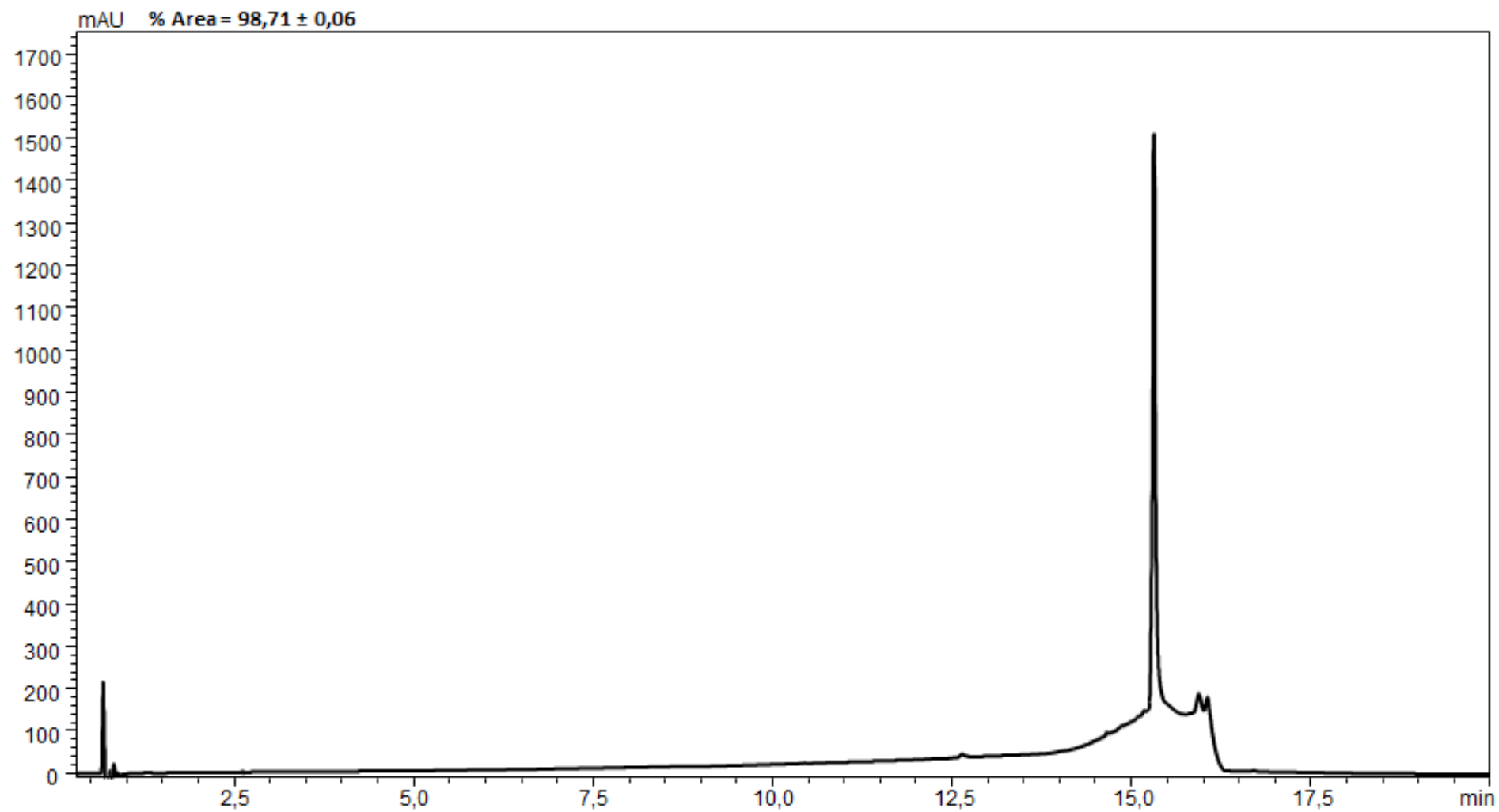
HMBC NMR spectra of compound **33a**



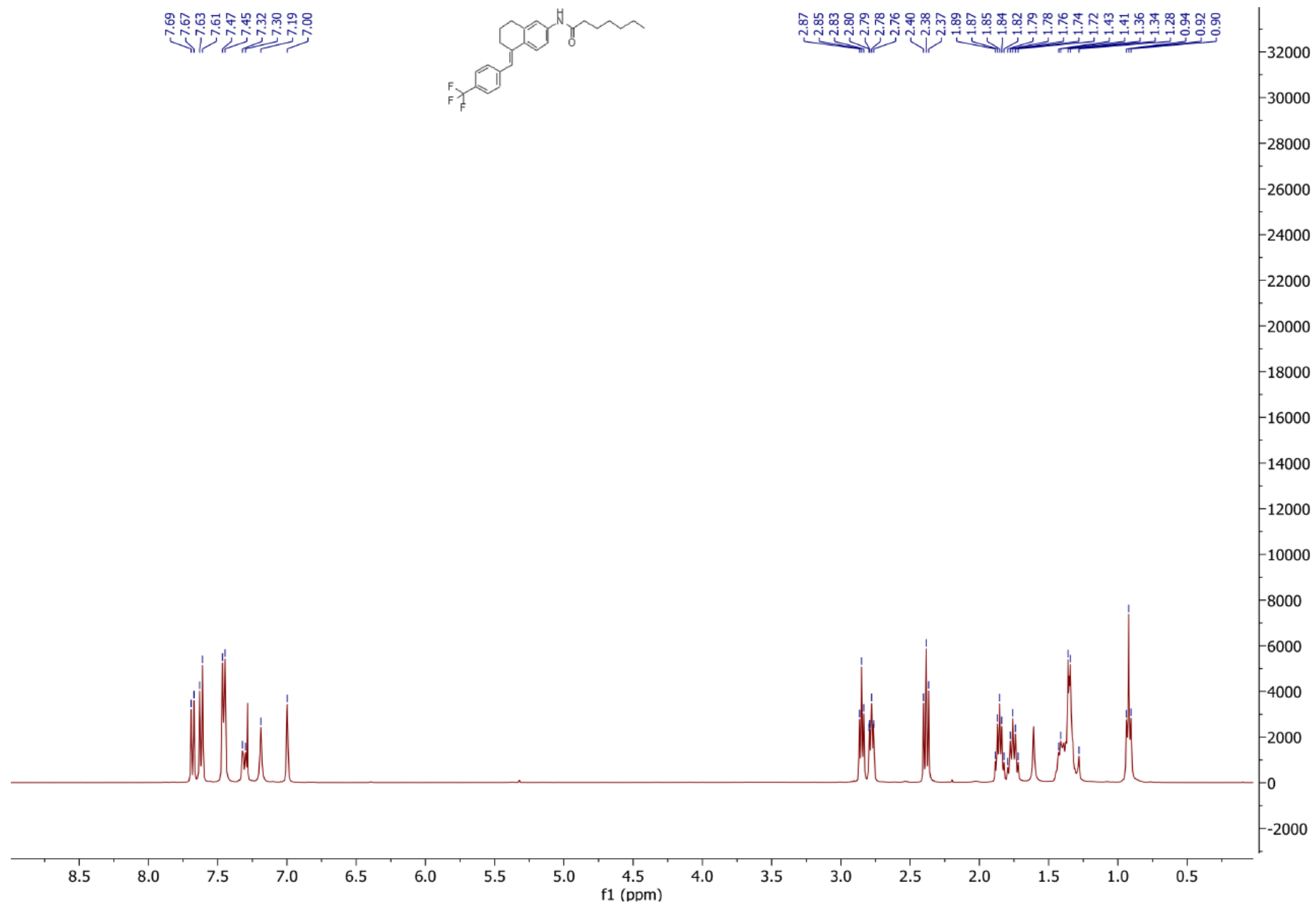
HMQC NMR spectra of compound **33a**



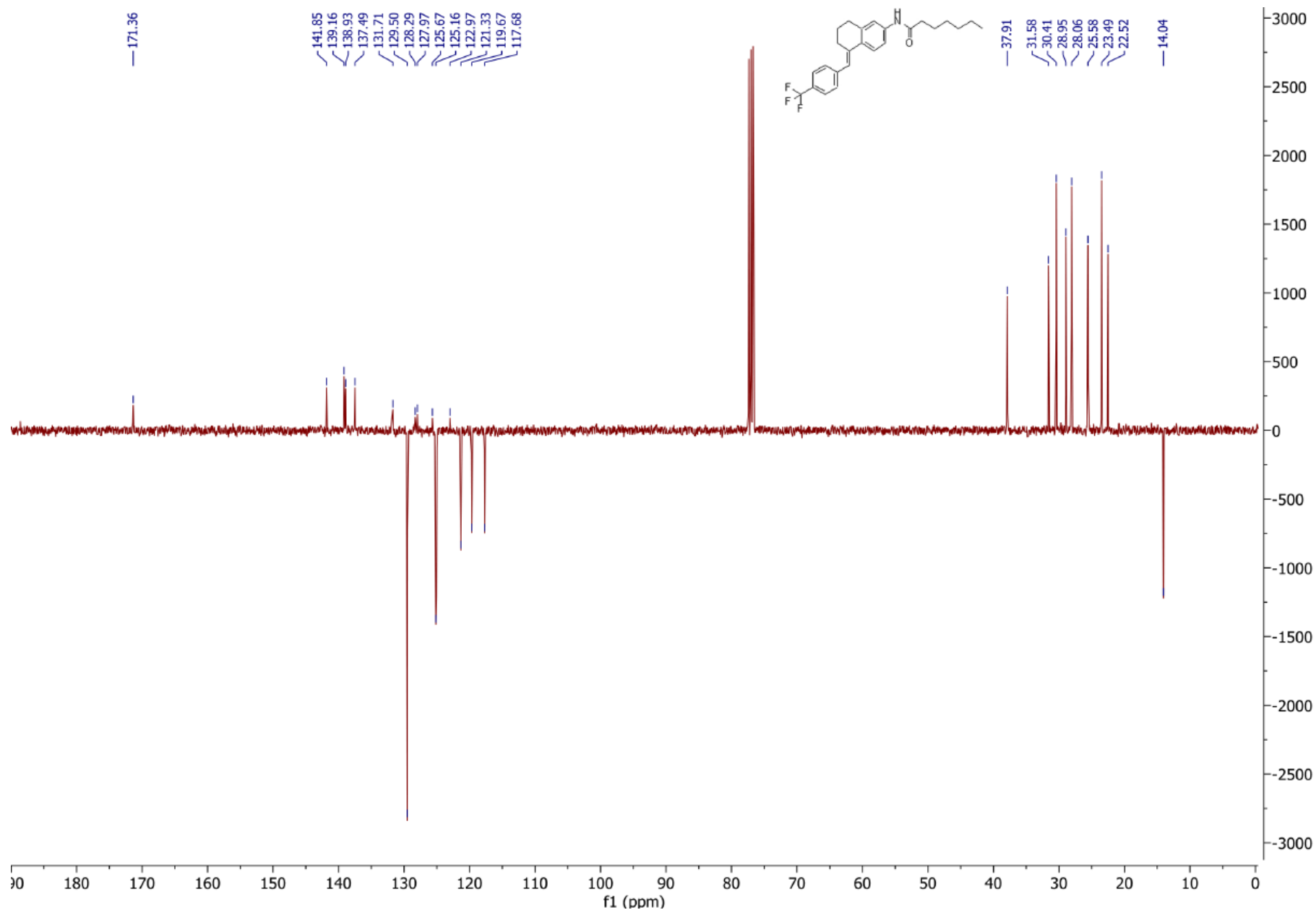
ROESY NMR spectra of compound **33a**



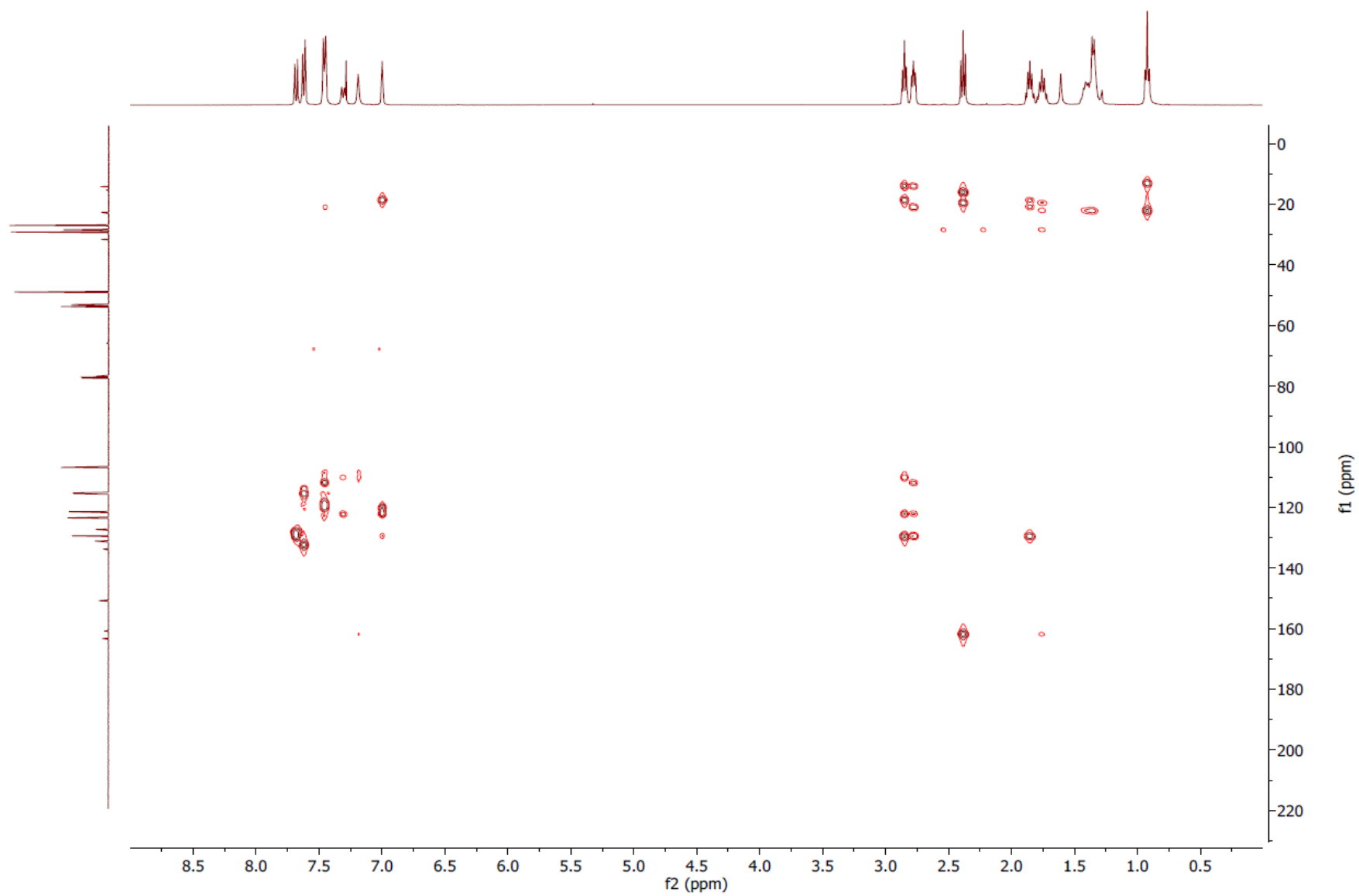
RP-HPLC trace of compound **33a**



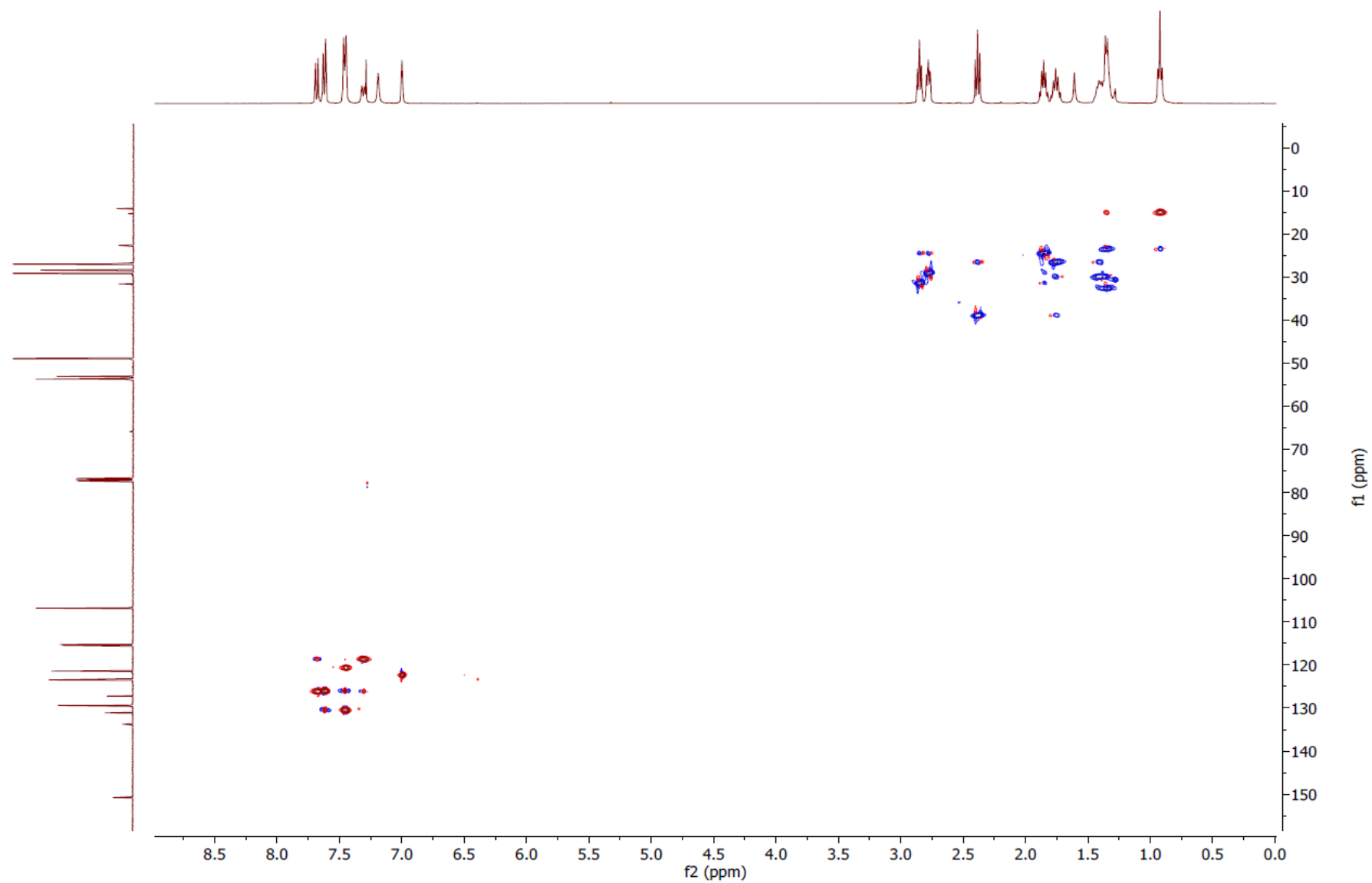
¹H NMR of compound **33b**



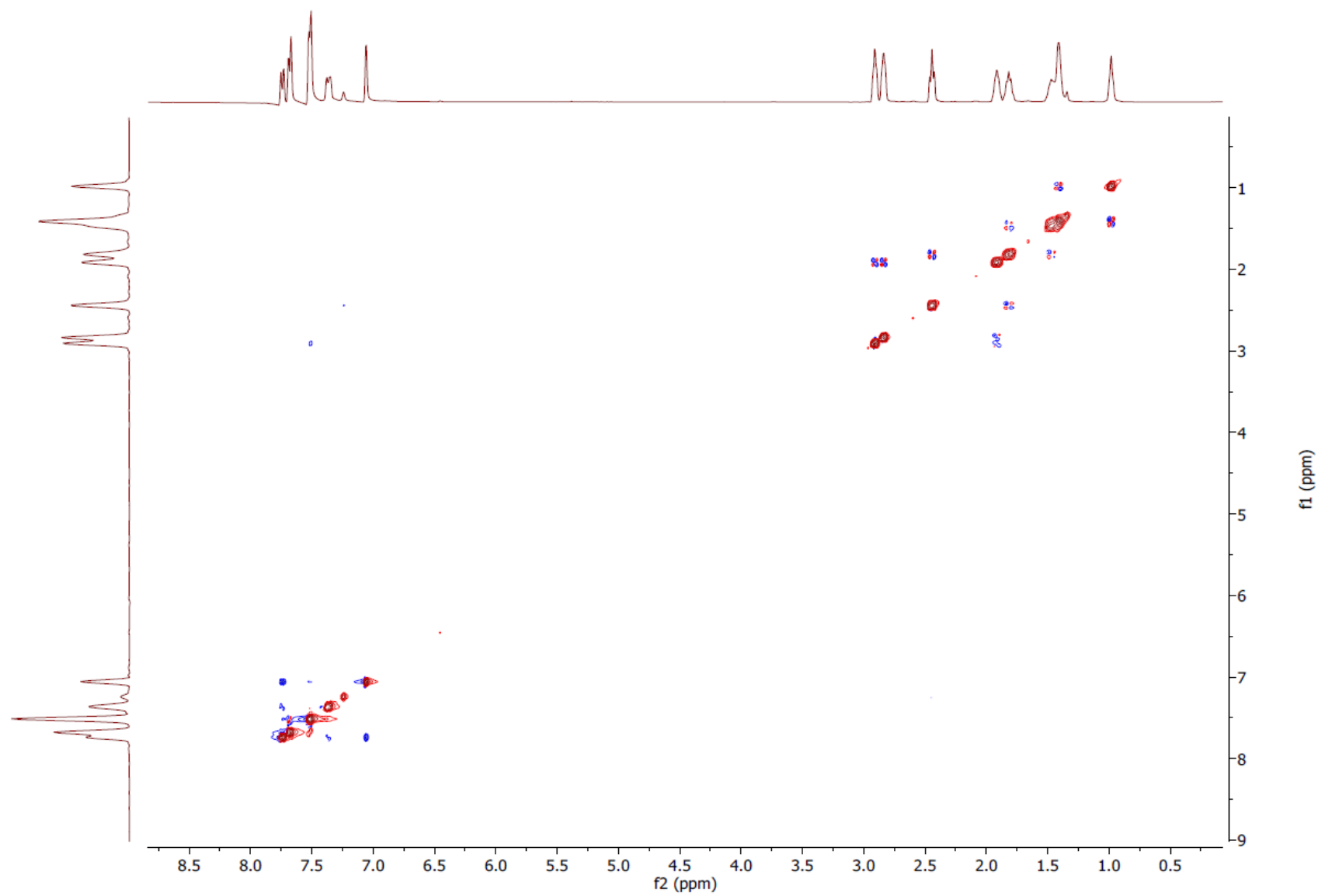
qDEPT NMR of compound **33b**



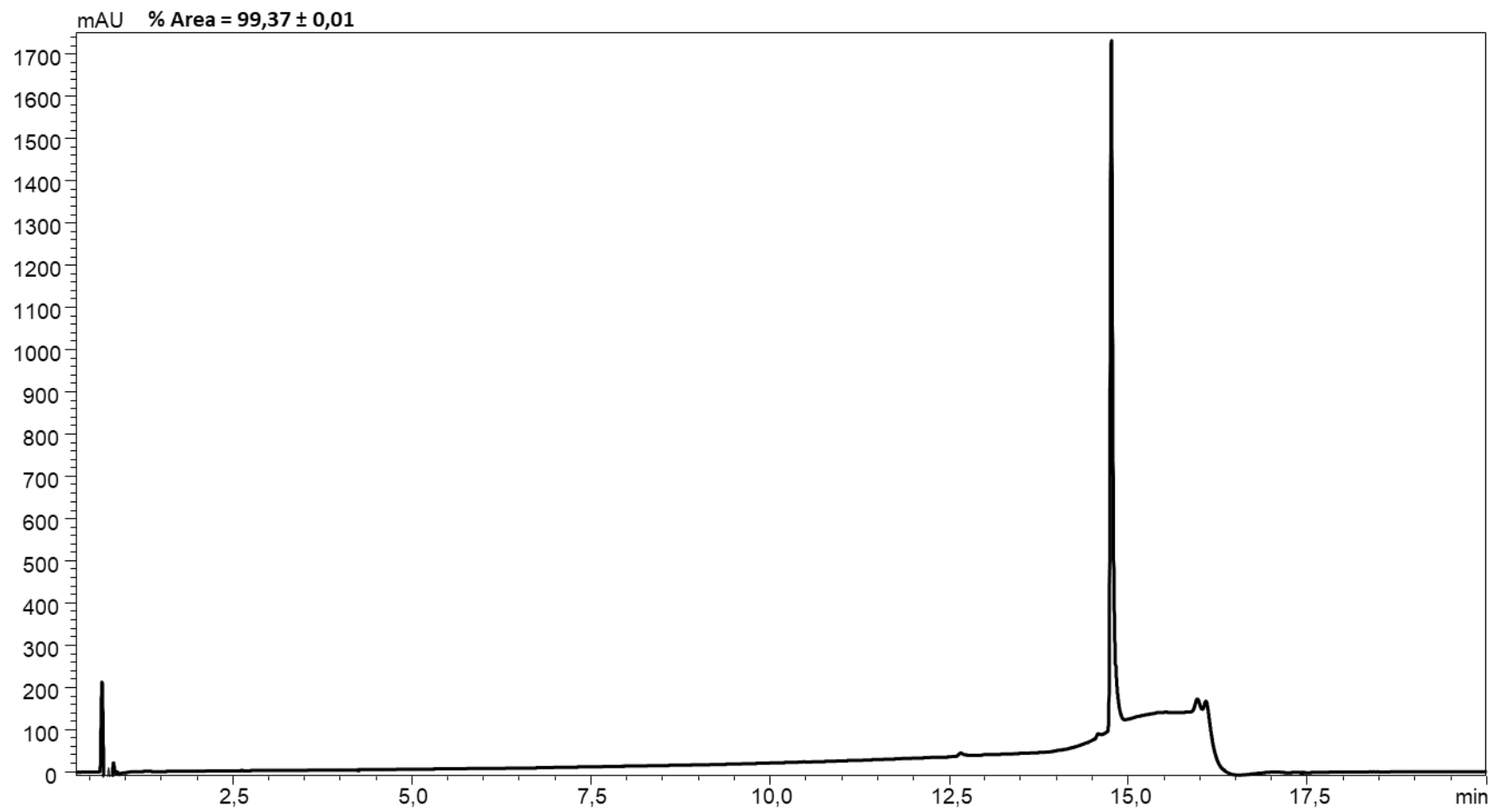
HMBC NMR spectra of compound **33b**

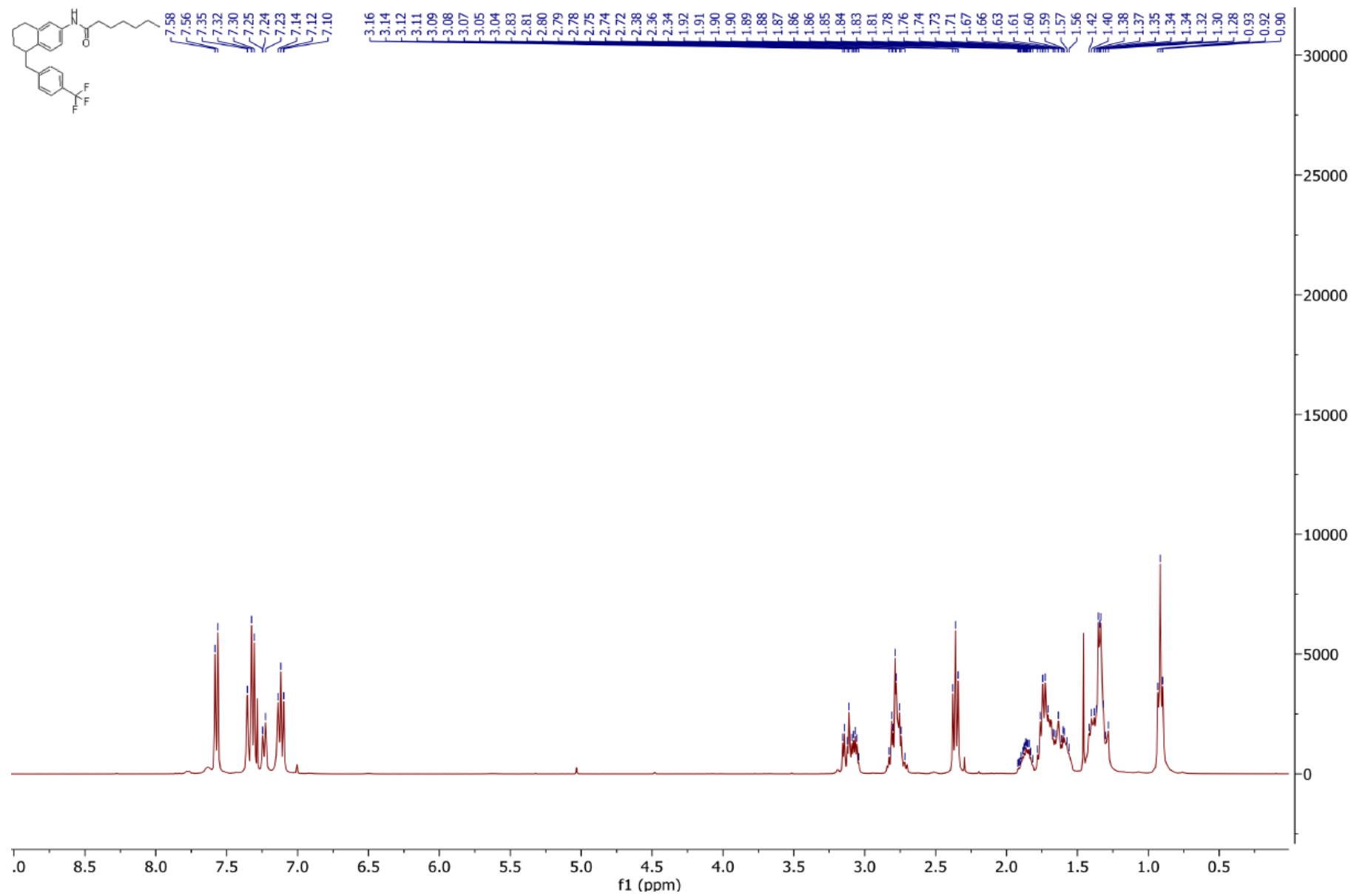


HMQC NMR spectra of compound **33b**

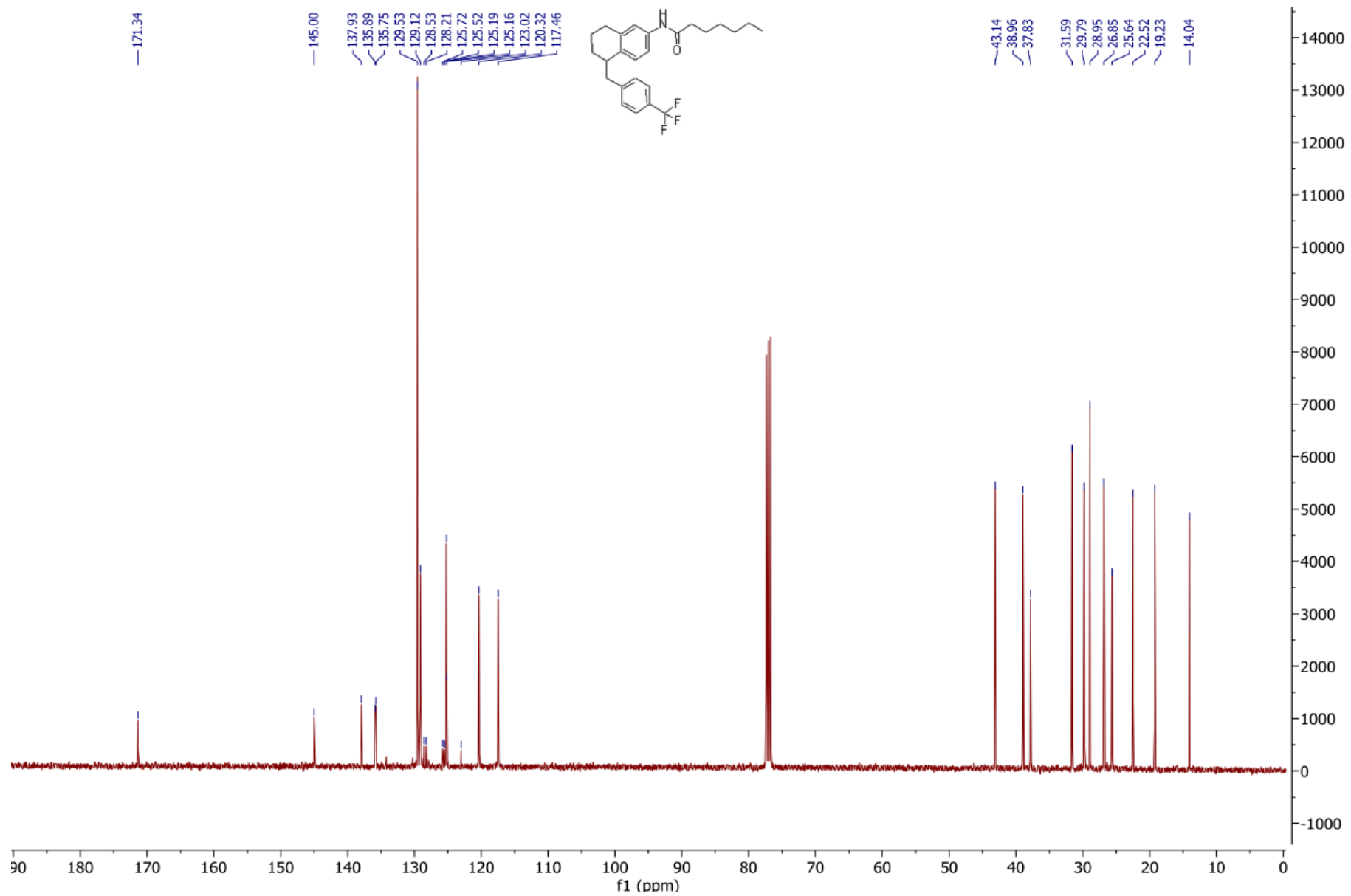


ROESY NMR spectra of compound **33b**

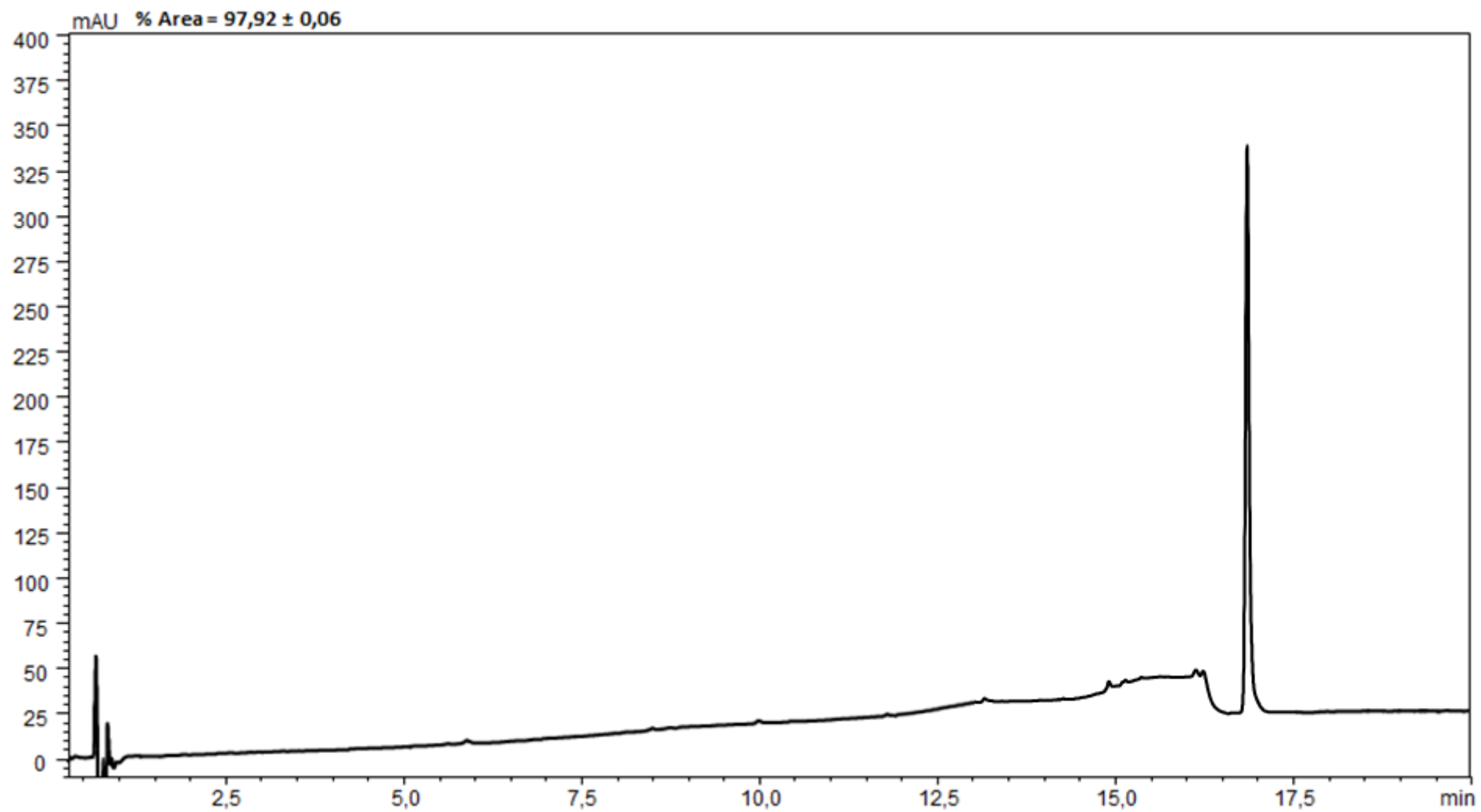




¹H NMR of compound 34



¹³C NMR of compound **34**



RP-HPLC trace of compound **34**