Synthesis and Preclinical Evaluation of Sulfonamido-based [^{11}C-*Carbonyl*]-Carbamates and Ureas for Imaging Monoacylglycerol Lipase

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Supplementary information

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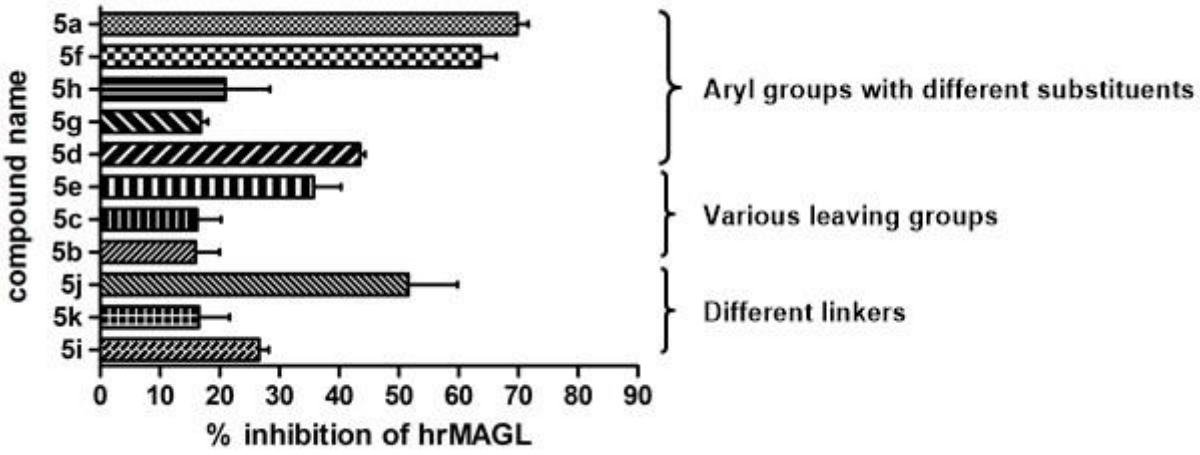


Figure S1: Single point inhibition assay of hrMAGL

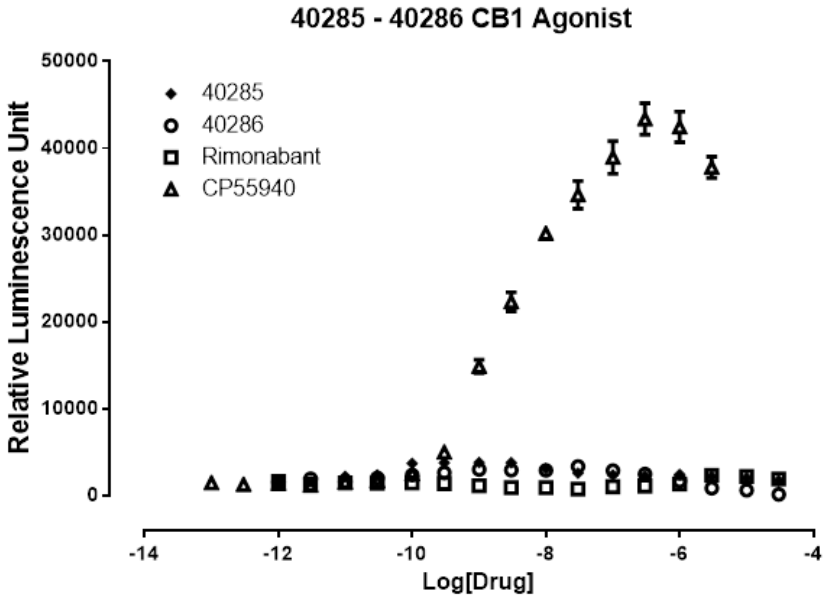


Figure S2: CB1/ CB2 assay data from NIH PDSP program.
(A) Test of compounds as CB1 agonist. 40285 = compound TZPU (5f) ; 40286 = compound SAR127303 (5a)

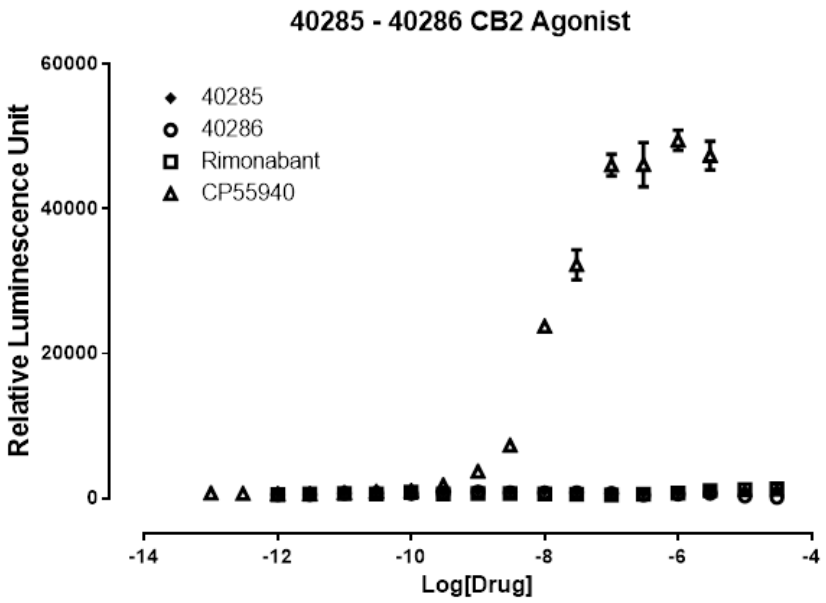


Figure S2: CB1/ CB2 assay data from NIH PDSP program.
(B) Test of compounds as CB2 agonist. 40285 = compound TZPU (5f) ; 40286 = compound SAR127303 (5a)

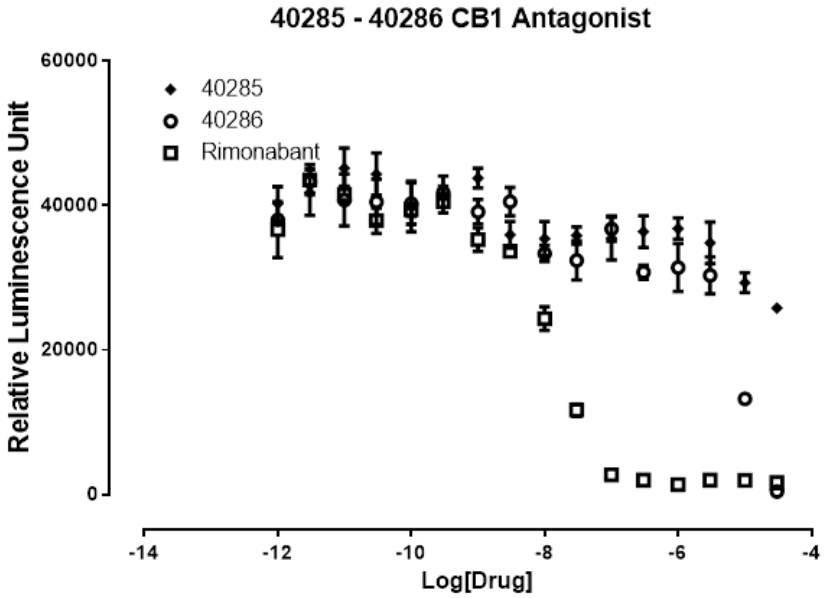


Figure S2: CB1/ CB2 assay data from NIH PDSP program.
(C) Test of compounds as CB1 antagonist. 40285 = compound TZPU (5f) ; 40286 = compound SAR127303 (5a)

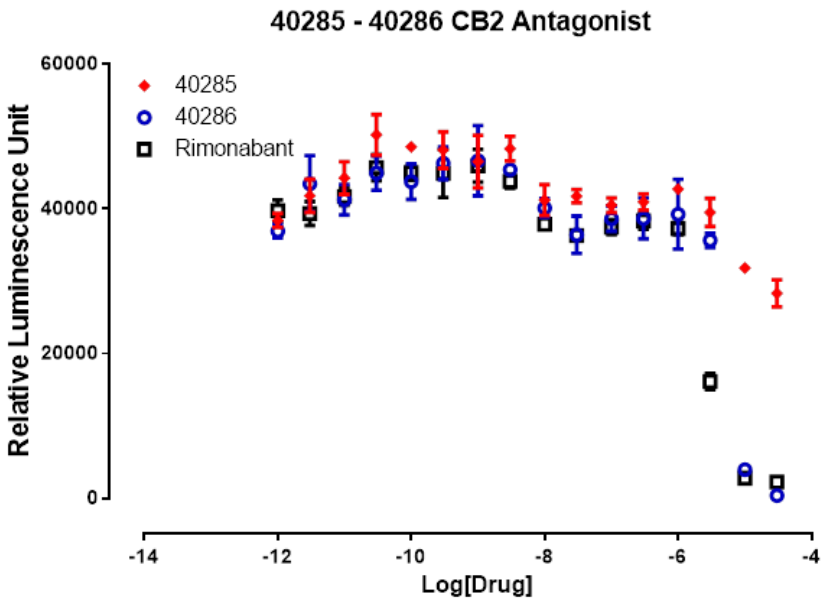


Figure S2: CB1/ CB2 assay data from NIH PDSP program.
(D) Test of compounds as CB2 antagonist. 40285 = compound TZPU (5f) ; 40286 = compound SAR127303 (5a)

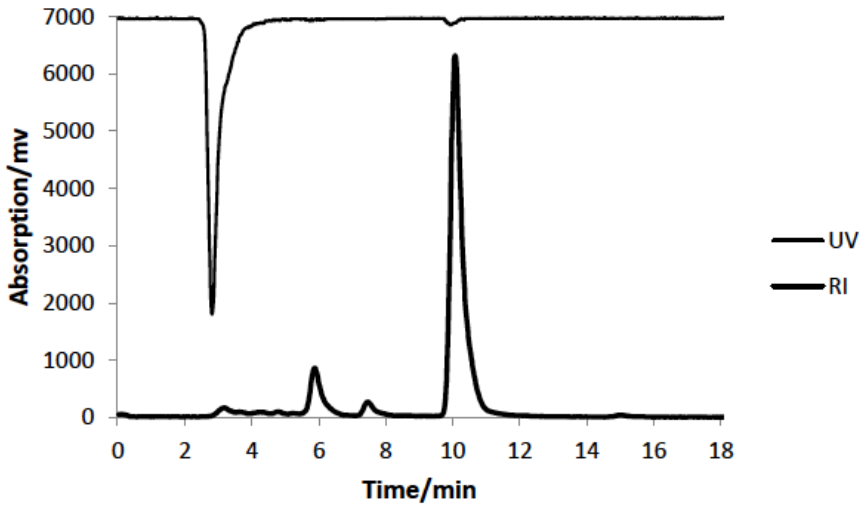


Figure S3: Analytical and semi-prep radioHPLC separations.
(A) Semi-prep HPLC trace for [^{11}C]SAR127303 ([^{11}C]5a)

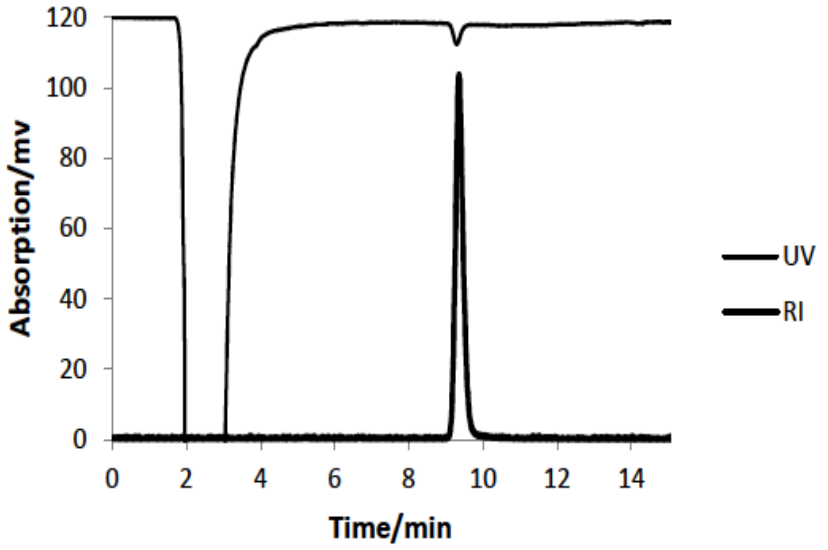


Figure S3: Analytical and Semi-prep radioHPLC separations
(B) Analytic HPLC trace for [^{11}C]SAR127303 ([^{11}C]5a) after separation

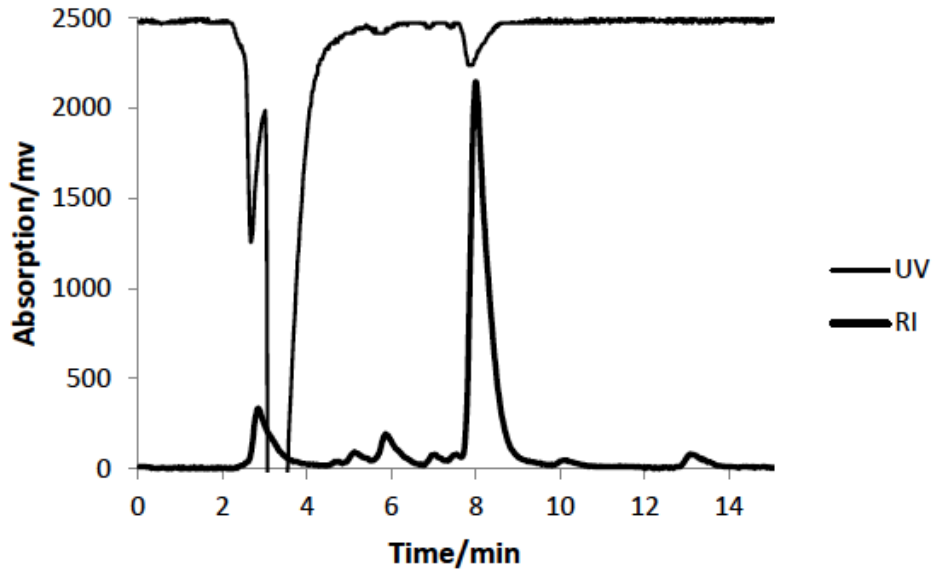


Figure S3: Analytical and Semi-prep radioHPLC separations
(C) Semi-prep HPLC trace for [^{11}C]TZPU ([^{11}C]5f)

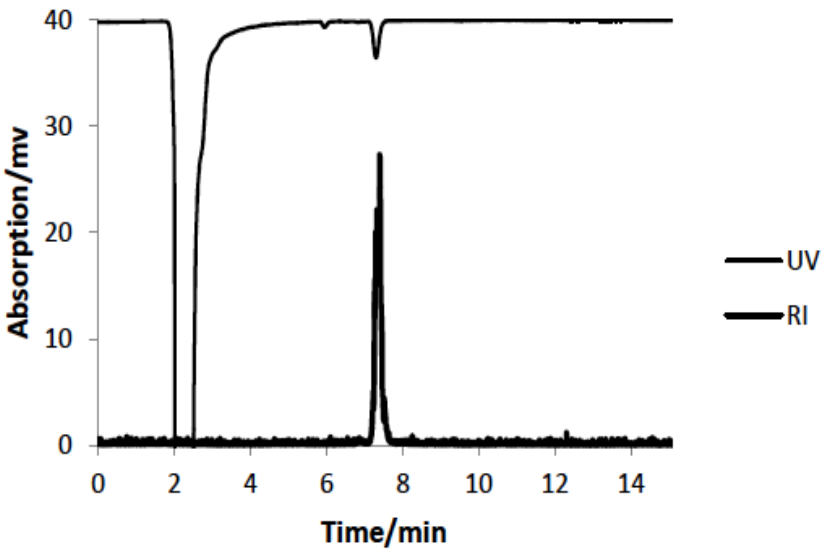


Figure S3: Analytical and Semi-prep radioHPLC separations
(D) Analytical HPLC trace for [^{11}C]TZPU ([^{11}C]5f) after separation

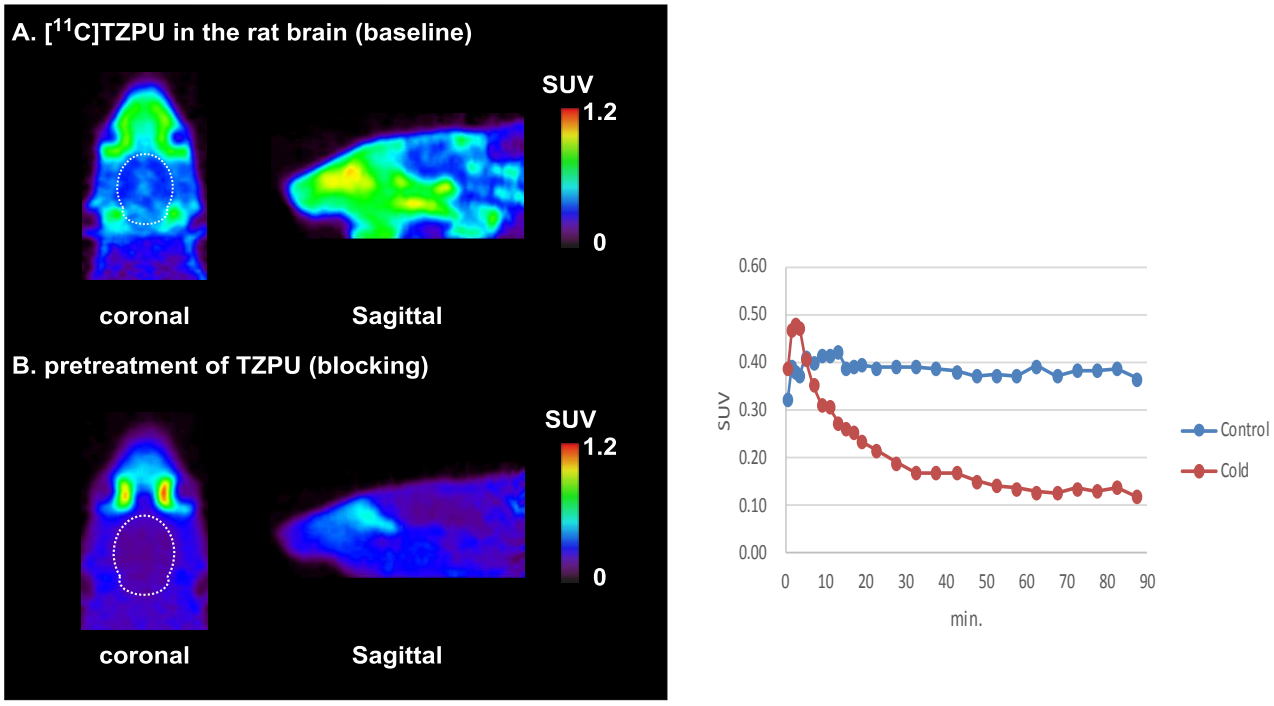


Figure S4: Whole brain PET images and time-activity curves of [^{11}C]TZPU ([^{11}C]5f).

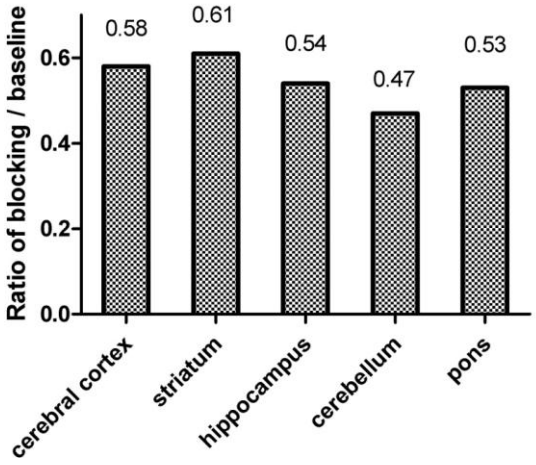


Figure S5: Ratio of [^{11}C]TZPU ([^{11}C]5f) uptake between blocking and baseline in different brain regions

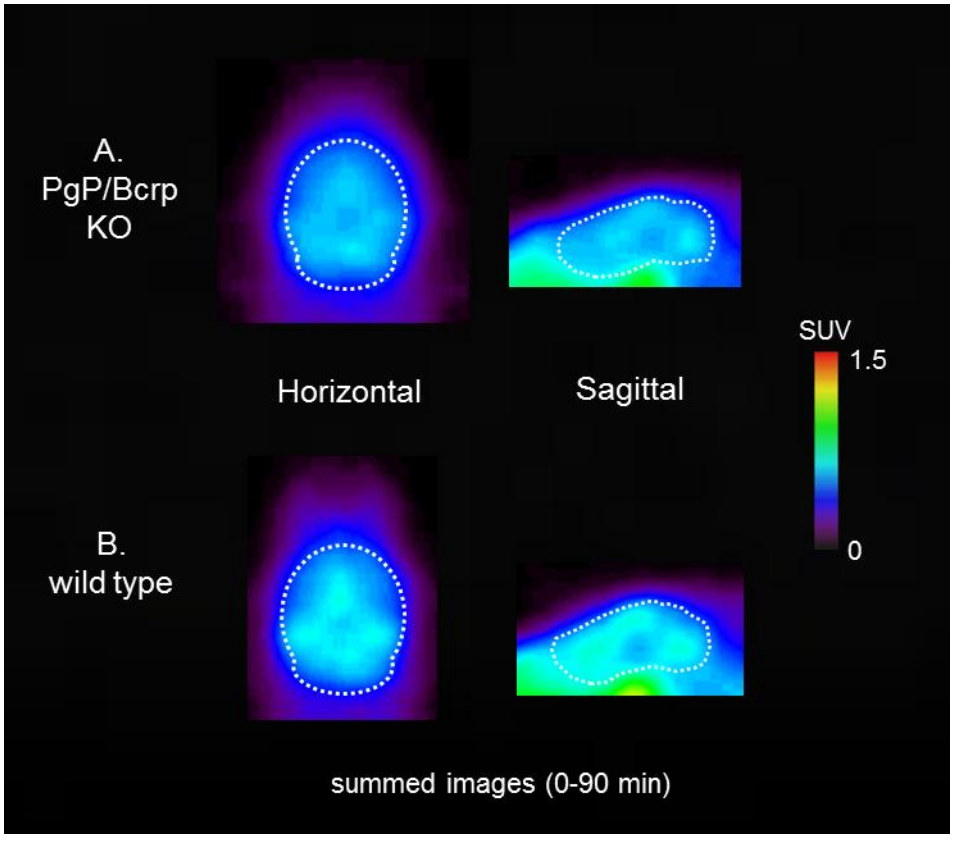


Figure S6: Whole brain PET images and time-activity curves of [^{11}C]TZPU ([^{11}C]5f) in PgP/Bcrp KO mice and wild-type control.

(A) PET images of [^{11}C]5f between PgP/Bcrp KO mice and wild type control

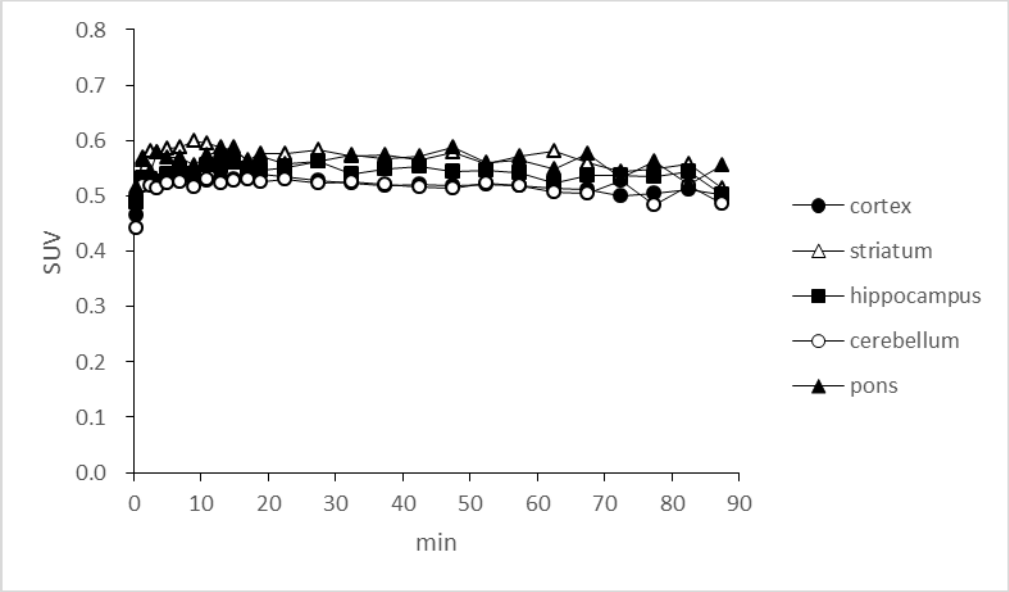


Figure S6: Whole brain PET images and time-activity curves of [^{11}C]TZPU ([^{11}C]5f) in different brain regions.
(B) Time activity curves of [^{11}C]5f in PgP/Bcrp KO mice

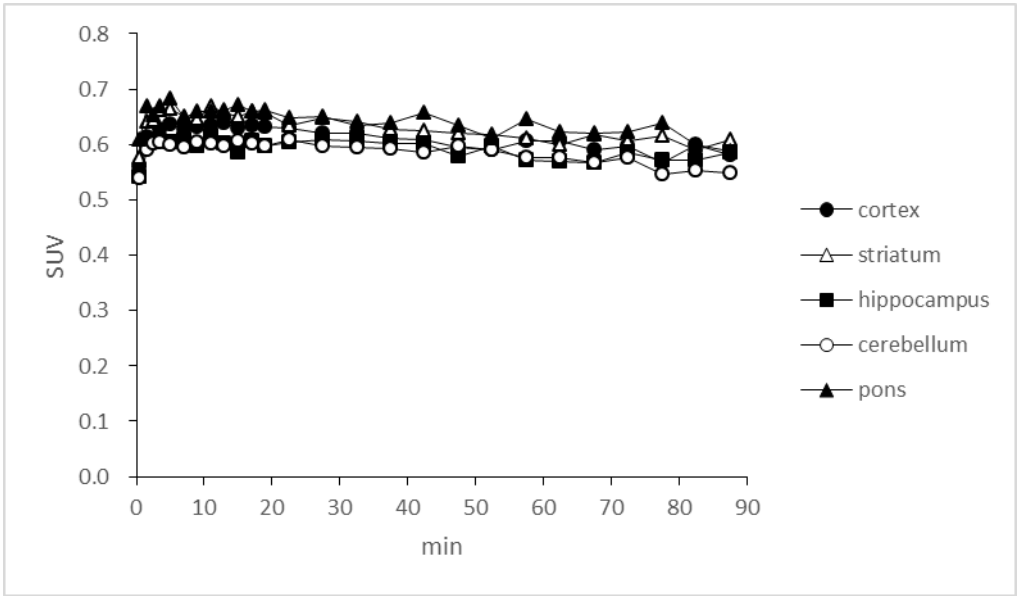


Figure S6: Whole brain PET images and time-activity curves of [^{11}C]TZPU ([^{11}C]5f) in different brain regions.
(C) Time activity curves of [^{11}C]5f in wild type control mice

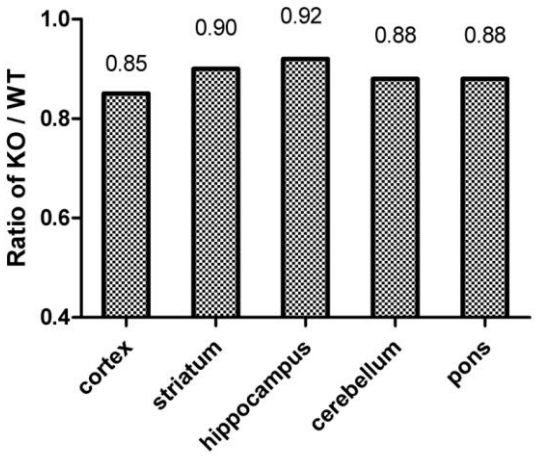


Figure S7: Ratio of [^{11}C]TZPU ([^{11}C]5f) uptake between PgP Bcrp KO and WT mice in different brain regions

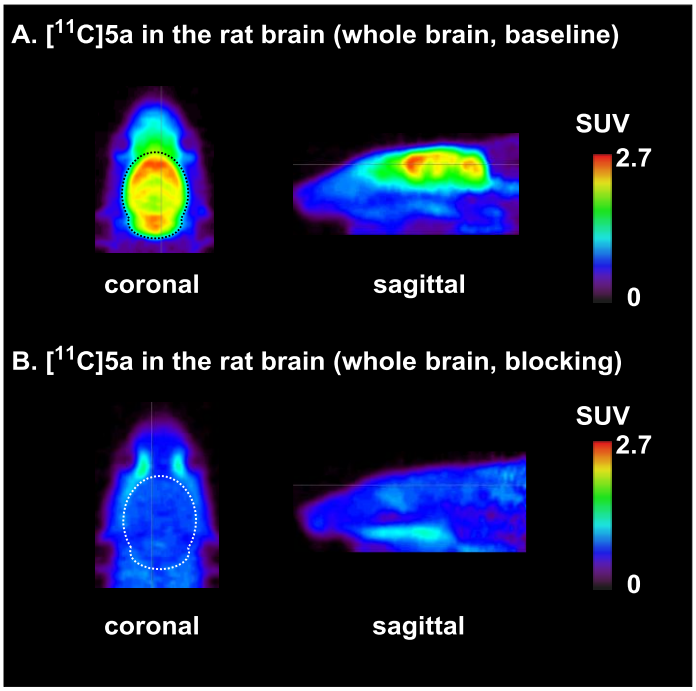


Figure S8: Whole brain PET images of [^{11}C]SAR127303 ([^{11}C]5a)

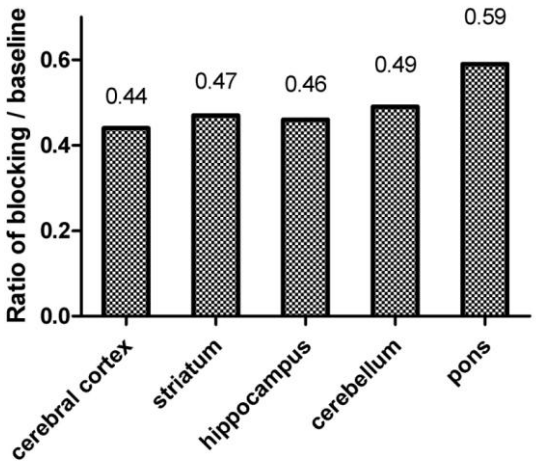


Figure S9: Ratio of [^{11}C]SAR127303 uptake between blocking and baseline in different rat brain regions

Metabolite assay for brain homogenate. Following the intravenous injection of [^{11}C]SAR127303, Sprague-Dawley rats (n = 3) were sacrificed at 30 min post injection. The rat brain were quickly removed and homogenized in an ice-cooled CH_3CN/H_2O (v/v 1/1, 1 mL) solution. The homogenate was centrifuged at 150,000 rpm for 2 min at 4 ℃ and the supernatant was collected. An aliquot of the supernatant (100 μL) obtained from the brain homogenate was injected into the radio-HPLC system, and analyzed under the similar analytical conditions described for [^{11}C]SAR127303 with the flow rate of 1.5 mL/min. The percentage of [^{11}C]SAR127303 to total radioactivity (corrected for decay) on the HPLC charts was calculated as (peak area for [^{11}C]MPPO/total peak area) × 100. The same procedure was used for metabolite analysis in plasma at 30 min post-injection and the percentage of parent tracer was 7%. A representative HPLC chromatogram of the brain metabolite analysis is shown below.

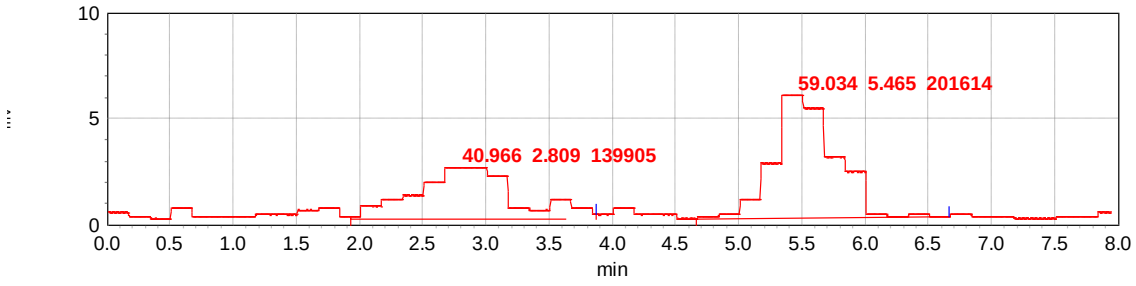


Figure S10: Radiometabolite analysis of [^{11}C]SAR127303

^{11}C-labeled MAGL tracer in the lit.[1]	cLogD by ADMET Predictor™	LogD measured by Wilson et al.[1]
Compound 14	3.27	2.76
KML29	4.59	4.6
JJKK0048	4.23	3.32
JW642	5.08	5.00
ML30	3.20	3.6
standard deviation of errors ($S_{y.x}$)	0.51	

Reference: [1] Hicks JW, Parkes J, Tong J, Houle S, Vasdev N, Wilson AA. Radiosynthesis and ex vivo evaluation of [^{11}C-carbonyl]carbamate- and urea-based monoacylglycerol lipase inhibitors. Nucl Med Biol. 2014; 41: 688-94.

Table S1: Comparison of cLogD estimated by commercial software and LogD values in the literature

Ex vivo biodistribution studies on mice
[^{11}C]SAR127303 ([^{11}C]5a): 2.27 Ci/μmol, 0.023 nmol/mouse
[^{11}C]TZPU ([^{11}C]5f): 0.89 Ci/μmol, 0.061 nmol/mouse
PET imaging studies in rat brain
baseline
[^{11}C]SAR127303 ([^{11}C]5a): 2.27-4.6 Ci/μmol, 0.26-0.54 nmol/rat
[^{11}C]TZPU ([^{11}C]5f): 0.53-2.73 Ci/μmol, 0.45-2.30 nmol/rat
blocking
[^{11}C]SAR127303 ([^{11}C]5a): 0.32-1.96 Ci/μmol, 0.62-3.75 nmol/rat
[^{11}C]TZPU ([^{11}C]5f): 0.44-1.02 Ci/μmol, 1.06-2.42 nmol/rat
PET imaging studies in NHP brain
[^{11}C]SAR127303 ([^{11}C]5a): 2.02 Ci/μmol, 1.85 nmol/monkey.

Table S2: Specific activity of [^{11}C]SAR127303 and [^{11}C]TZPU in PET imaging studies

tissue	1 min	5 min	15 min	30 min	60 min
blood	12.61 ± 0.95	11.23 ± 0.44	10.97 ± 0.67	10.79 ± 0.50	8.64 ± 0.32
heart	13.94 ± 1.90	12.50 ± 0.60	12.63 ± 0.97	10.94 ± 1.15	10.88 ± 0.95
lungs	33.98 ± 2.06	29.04 ± 5.53	32.15 ± 3.08	24.53 ± 2.09	26.33 ± 2.27
liver	2.49 ± 0.61	2.73 ± 0.09	2.51 ± 0.12	2.10 ± 0.07	1.96 ± 0.13
pancreas	2.97 ± 0.45	4.23 ± 0.73	4.26 ± 2.33	2.70 ± 0.70	3.61 ± 0.12
spleen	1.25 ± 0.33	2.12 ± 0.36	2.46 ± 0.85	1.90 ± 0.22	1.92 ± 0.21
adrenal glands	7.24 ± 3.47	5.31 ± 1.96	6.00 ± 2.46	4.54 ± 0.30	5.03 ± 0.98
kidneys	8.90 ± 0.83	8.59 ± 0.73	8.78 ± 4.21	5.56 ± 0.41	6.64 ± 1.30
small intestine	2.97 ± 0.18	3.92 ± 0.18	3.68 ± 1.40	2.68 ± 0.20	3.05 ± 0.18
testes	0.33 ± 0.04	0.46 ± 0.06	0.53 ± 0.07	0.90 ± 0.01	1.00 ± 0.02
muscle	2.42 ± 0.41	2.19 ± 0.60	1.77 ± 0.25	1.81 ± 0.11	1.89 ± 0.44
brain	0.98 ± 0.08	0.98 ± 0.14	1.08 ± 0.12	0.89 ± 0.07	0.82 ± 0.15
white adipose	0.18 ± 0.04	0.33 ± 0.06	0.29 ± 0.12	0.24 ± 0.05	0.21 ± 0.03
brown adipose	1.67 ± 0.28	6.23 ± 3.40	2.91 ± 1.62	5.30 ± 3.76	2.71 ± 0.82

Table S3: Ex vivo biodistribution of [^{11}C]TZPU ([^{11}C]5f) in mice. Date are expressed as %ID/g (mean ± SD, n = 3)

tissue	1 min	5 min	15 min	30 min	60 min
blood	4.18 ± 0.19	3.75 ± 0.14	3.66 ± 0.30	3.60 ± 0.19	2.86 ± 0.11
heart	4.63 ± 0.76	4.18 ± 0.24	4.20 ± 0.25	3.65 ± 0.41	3.60 ± 0.31
lungs	11.28 ± 0.94	9.71 ± 1.92	10.70 ± 0.94	8.19 ± 0.62	8.72 ± 0.86
liver	0.82 ± 0.18	0.91 ± 0.04	0.83 ± 0.03	0.70 ± 0.02	0.65 ± 0.06
pancreas	0.98 ± 0.13	1.41 ± 0.25	1.41 ± 0.76	0.90 ± 0.23	1.19 ± 0.02
spleen	0.41 ± 0.10	0.71 ± 0.11	0.82 ± 0.27	0.64 ± 0.07	0.63 ± 0.06
adrenal glands	2.42 ± 1.23	1.77 ± 0.66	1.99 ± 0.78	1.51 ± 0.10	1.66 ± 0.29
kidneys	2.95 ± 0.31	2.87 ± 0.26	2.91 ± 1.36	1.85 ± 0.11	2.20 ± 0.41
small intestine	0.99 ± 0.03	1.31 ± 0.07	1.22 ± 0.45	0.89 ± 0.07	1.01 ± 0.05
testes	0.11 ± 0.02	0.16 ± 0.02	0.18 ± 0.02	0.30 ± 0.00	0.33 ± 0.00
muscle	0.80 ± 0.16	0.73 ± 0.20	0.59 ± 0.08	0.60 ± 0.05	0.63 ± 0.16
brain	0.32 ± 0.03	0.33 ± 0.05	0.36 ± 0.04	0.30 ± 0.02	0.27 ± 0.05
white adipose	0.06 ± 0.01	0.11 ± 0.02	0.09 ± 0.04	0.08 ± 0.02	0.07 ± 0.01
brown adipose	0.55 ± 0.10	2.07 ± 1.11	0.96 ± 0.52	1.76 ± 1.23	0.90 ± 0.27

Table S4: Ex vivo biodistribution of [^{11}C]TZPU ([^{11}C]5f) in mice. Date are expressed as SUV (mean ± SD, n = 3)

tissue	1 min	5 min	15 min	30 min	60 min
blood	1.92 ± 0.11	1.52 ± 0.09	1.74 ± 0.14	1.69 ± 0.09	1.69 ± 0.02
heart	14.03 ± 0.29	5.80 ± 0.25	5.38 ± 0.12	5.12 ± 0.69	4.49 ± 0.35
lungs	13.17 ± 1.21	6.28 ± 1.58	5.32 ± 0.56	4.32 ± 0.14	3.48 ± 0.37
liver	3.59 ± 0.16	6.75 ± 0.66	8.86 ± 1.35	8.28 ± 1.18	6.77 ± 0.57
pancreas	5.42 ± 0.55	6.35 ± 0.31	4.21 ± 0.36	3.08 ± 0.18	2.52 ± 0.08
spleen	2.30 ± 0.54	2.52 ± 0.05	2.34 ± 0.09	2.25 ± 0.13	1.98 ± 0.23
adrenal glands	7.31 ± 1.10	9.71 ± 2.95	7.22 ± 0.39	7.14 ± 0.89	5.92 ± 0.68
kidneys	8.11 ± 0.45	4.98 ± 0.58	5.71 ± 0.88	5.26 ± 1.22	4.27 ± 0.93
small intestine	4.12 ± 0.11	4.33 ± 0.32	4.55 ± 0.40	3.86 ± 0.20	3.56 ± 0.58
testes	1.06 ± 0.15	1.38 ± 0.18	1.51 ± 0.11	1.50 ± 0.04	1.36 ± 0.14
muscle	4.00 ± 1.13	2.45 ± 0.28	1.68 ± 0.17	1.26 ± 0.11	1.14 ± 0.12
brain	3.89 ± 0.10	4.41 ± 0.29	4.52 ± 0.11	4.66 ± 0.25	4.49 ± 0.17
white adipose	0.34 ± 0.13	0.79 ± 0.05	1.23 ± 0.10	1.43 ± 0.24	1.39 ± 0.07
brown adipose	3.49 ± 1.47	17.10 ± 3.84	13.16 ± 5.66	13.87 ± 2.74	12.68 ± 3.04

Table S5: Ex vivo biodistribution of [^{11}C]SAR127303 ([^{11}C]5a) in mice. Date are expressed as %ID/g (mean ± SD, n = 3)

tissue	1 min	5 min	15 min	30 min	60 min
blood	0.63 ± 0.04	0.51 ± 0.02	0.59 ± 0.04	0.58 ± 0.03	0.56 ± 0.01
heart	4.63 ± 0.02	1.93 ± 0.07	1.82 ± 0.03	1.74 ± 0.25	1.50 ± 0.12
lungs	4.34 ± 0.32	2.09 ± 0.51	1.80 ± 0.18	1.47 ± 0.03	1.16 ± 0.13
liver	1.18 ± 0.05	2.25 ± 0.25	3.00 ± 0.43	2.81 ± 0.39	2.25 ± 0.16
pancreas	1.79 ± 0.17	2.12 ± 0.13	1.42 ± 0.12	1.05 ± 0.09	0.84 ± 0.04
spleen	0.76 ± 0.17	0.84 ± 0.03	0.79 ± 0.03	0.76 ± 0.06	0.66 ± 0.09
adrenal glands	2.41 ± 0.36	3.24 ± 1.03	2.44 ± 0.12	2.46 ± 0.38	1.97 ± 0.21
kidneys	2.67 ± 0.14	1.66 ± 0.21	1.93 ± 0.30	1.79 ± 0.45	1.42 ± 0.29
small intestine	1.36 ± 0.06	1.44 ± 0.13	1.54 ± 0.14	1.31 ± 0.07	1.18 ± 0.18
testes	0.35 ± 0.05	0.46 ± 0.06	0.51 ± 0.03	0.51 ± 0.02	0.45 ± 0.04
muscle	1.32 ± 0.39	0.81 ± 0.08	0.57 ± 0.05	0.43 ± 0.03	0.38 ± 0.04
brain	1.28 ± 0.03	1.47 ± 0.08	1.53 ± 0.05	1.58 ± 0.10	1.49 ± 0.05
white adipose	0.11 ± 0.04	0.26 ± 0.02	0.42 ± 0.03	0.49 ± 0.09	0.46 ± 0.02
brown adipose	1.14 ± 0.47	5.71 ± 1.37	4.46 ± 1.93	4.73 ± 1.07	4.21 ± 0.96

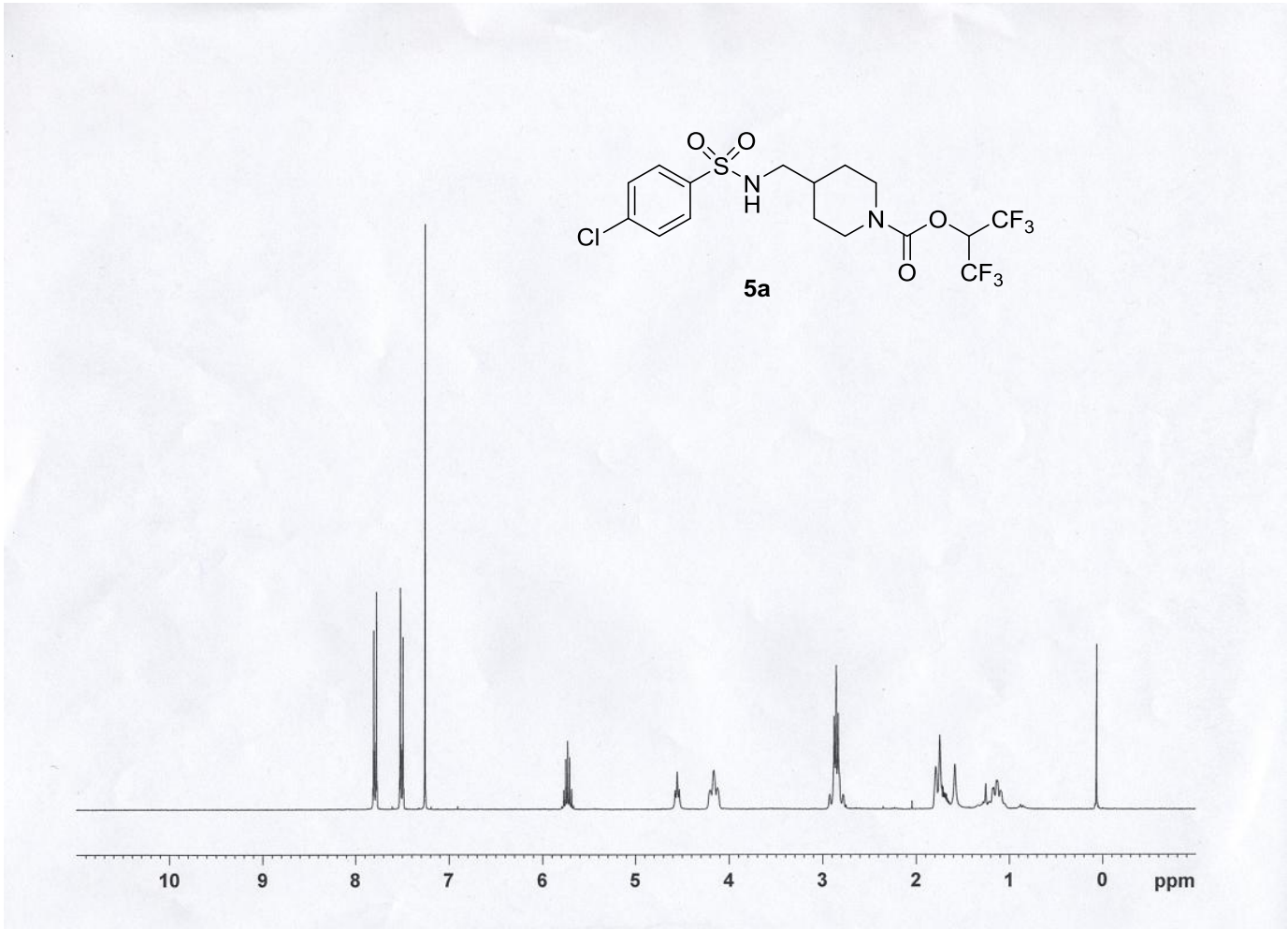
Table S6: Ex vivo biodistribution of [^{11}C]SAR127303 ([^{11}C]5a) in mice. Date are expressed as SUV (mean ± SD, n = 3)

Name	Melting point (°C)	Name	Melting point (°C)
Compound 5a (SAR127303)	**135-137**	Compound 5f (TZPU)	**109-110**
Compound 5b	**109-111**	Compound 5g	**152-153**
Compound 5c	**127-128**	Compound 5h	**168-170**
Compound 5d	**118-119**	Compound 5i	**150-152**
Compound 5e	**71-73**	Compound 5k	**90-92**

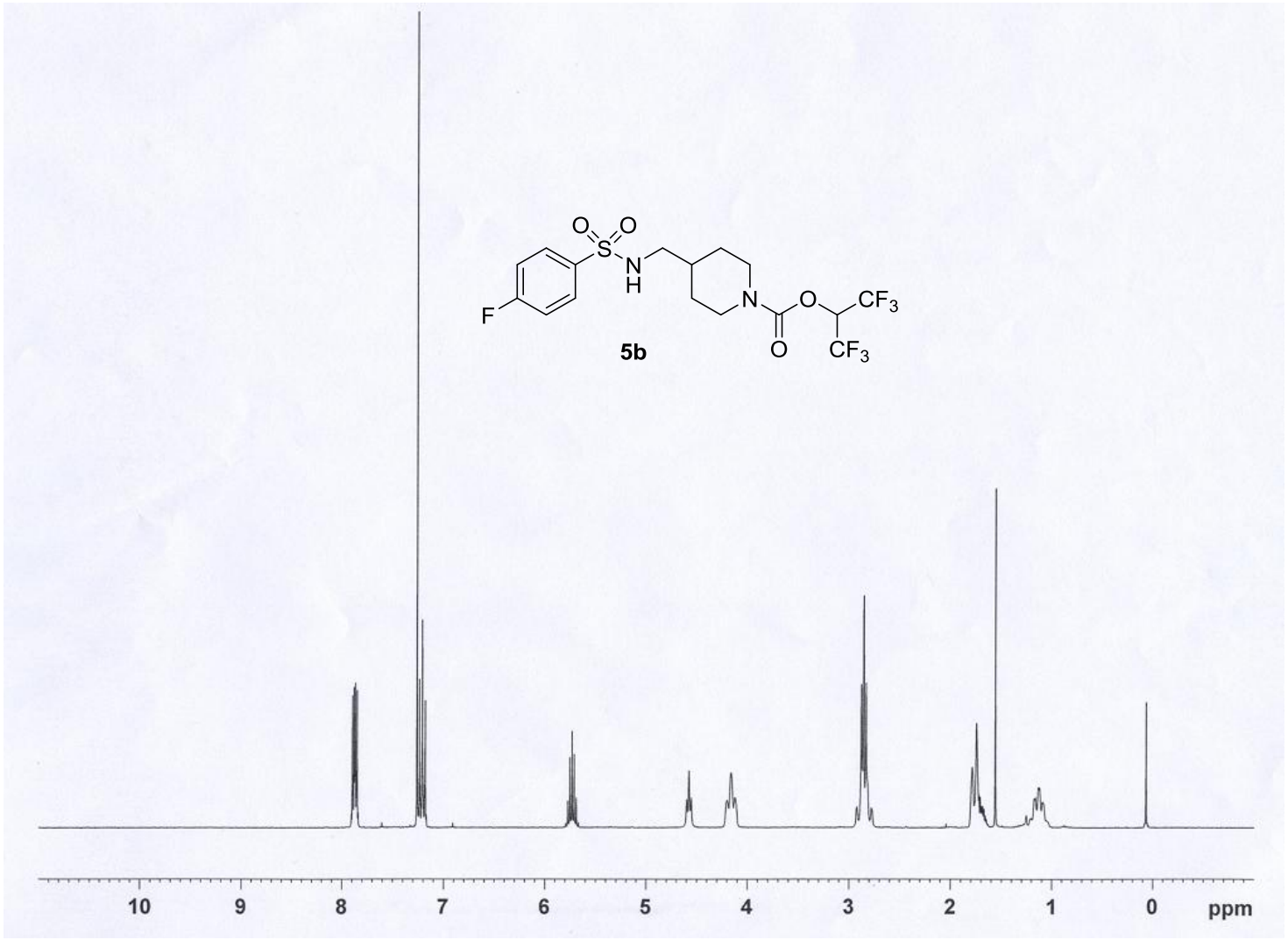
Table S7: Melting points of compounds 5a-5i and 5k

NMR spectra of compounds 5a-5k

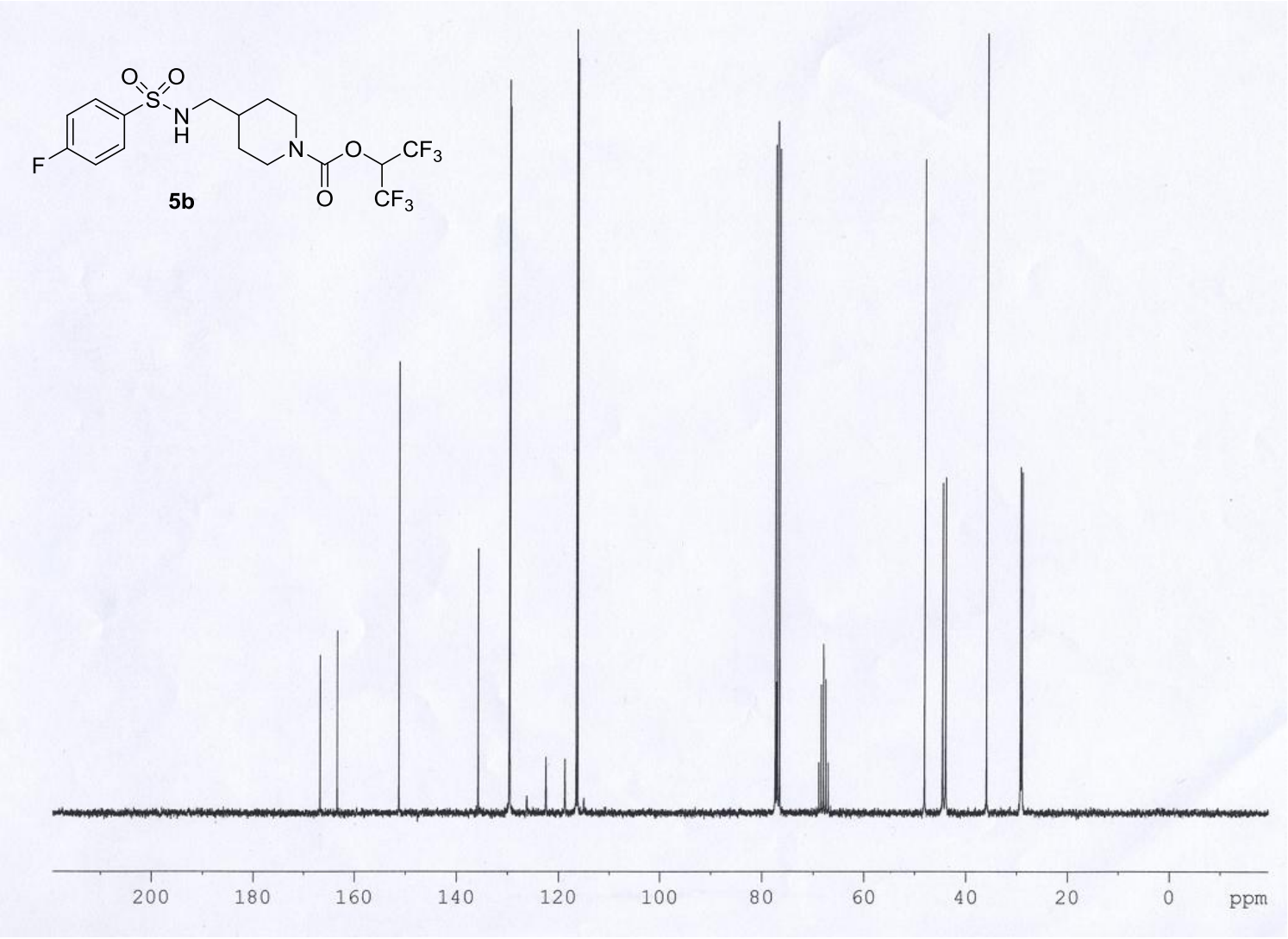
(A). ^{1}H spectrum of compound SAR127303 (5a)



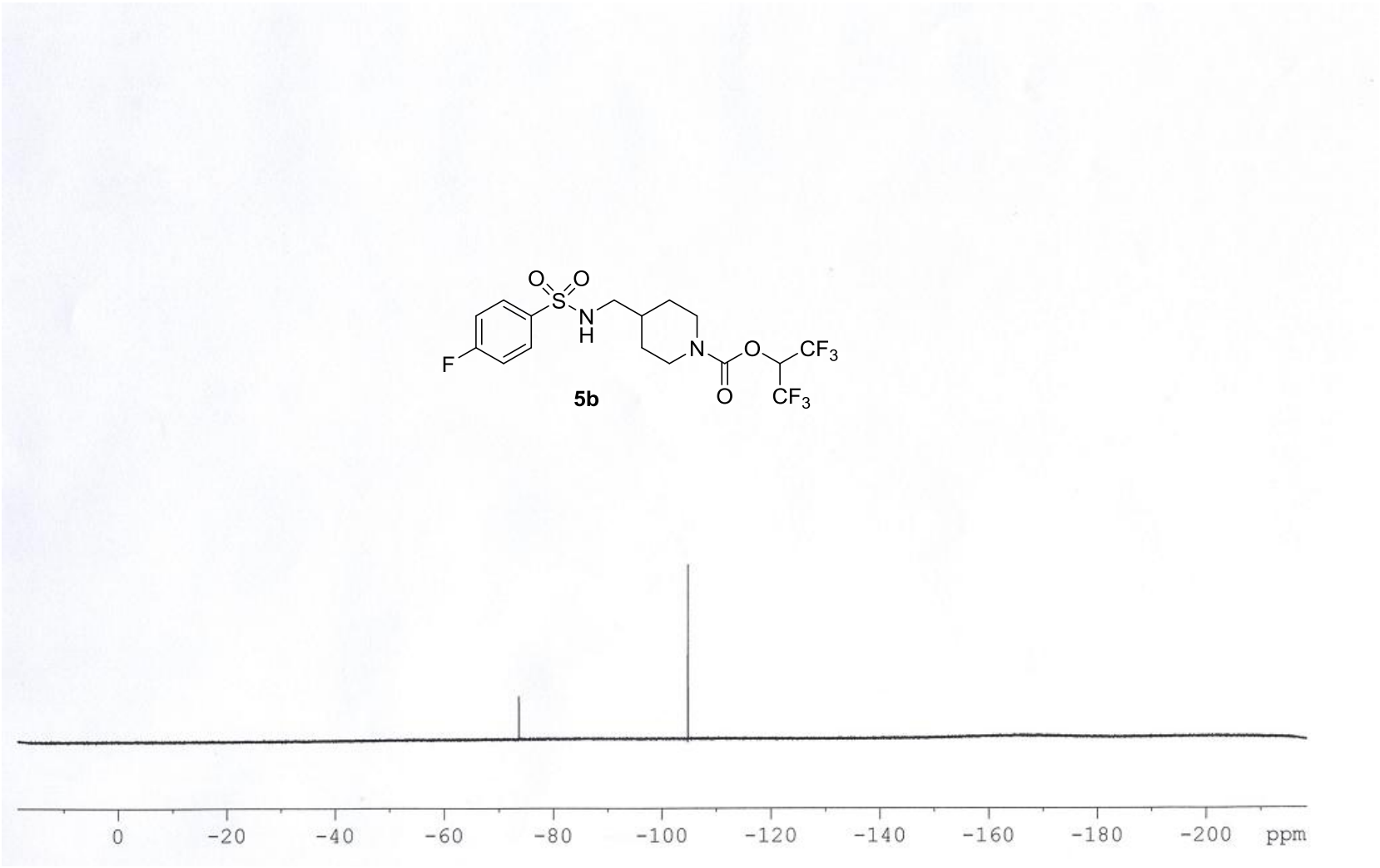
(B). ^{1}H spectrum of compound SAR127303 (5b)



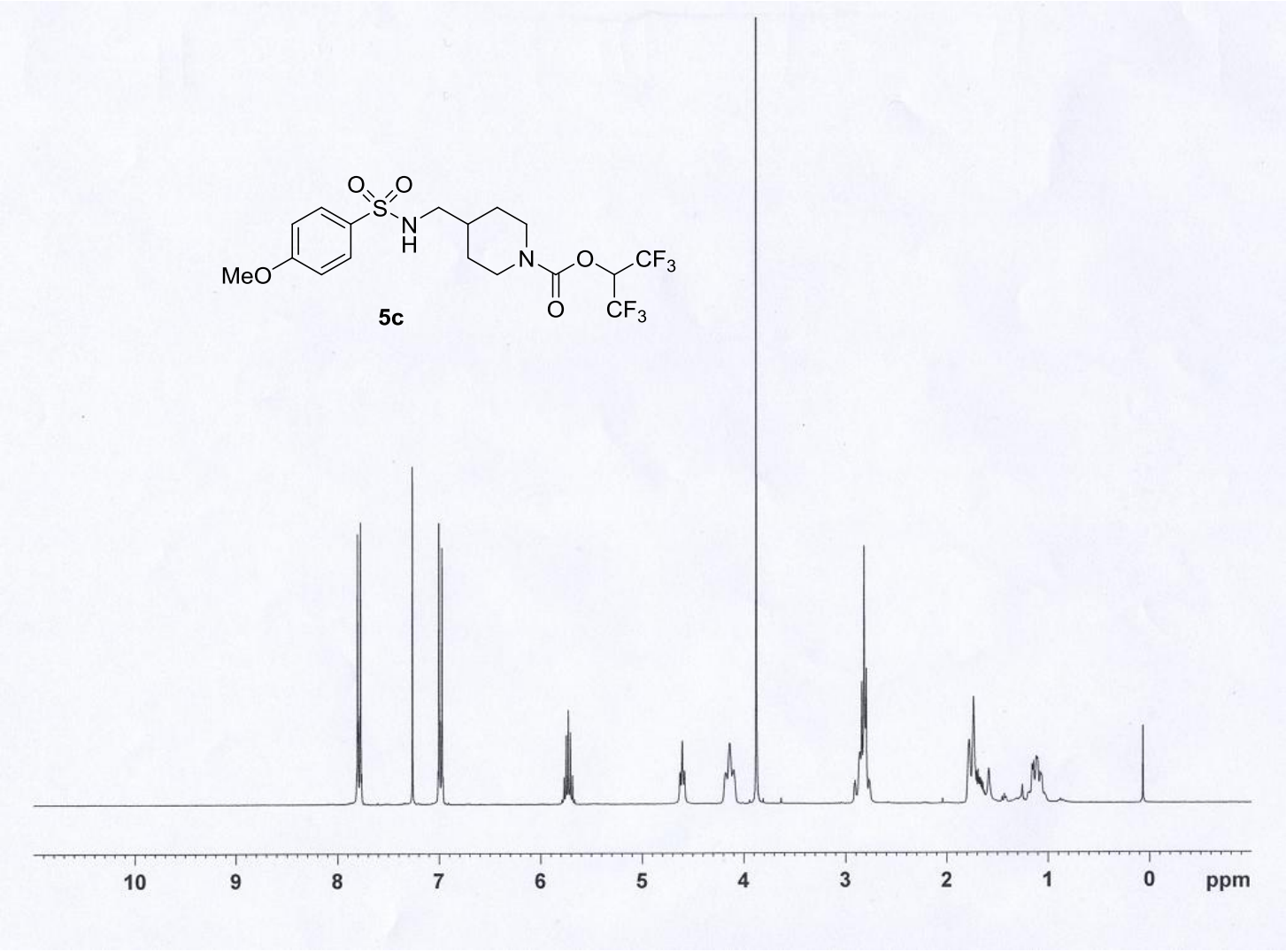
(C). ^{13}C NMR spectrum of compound 5b



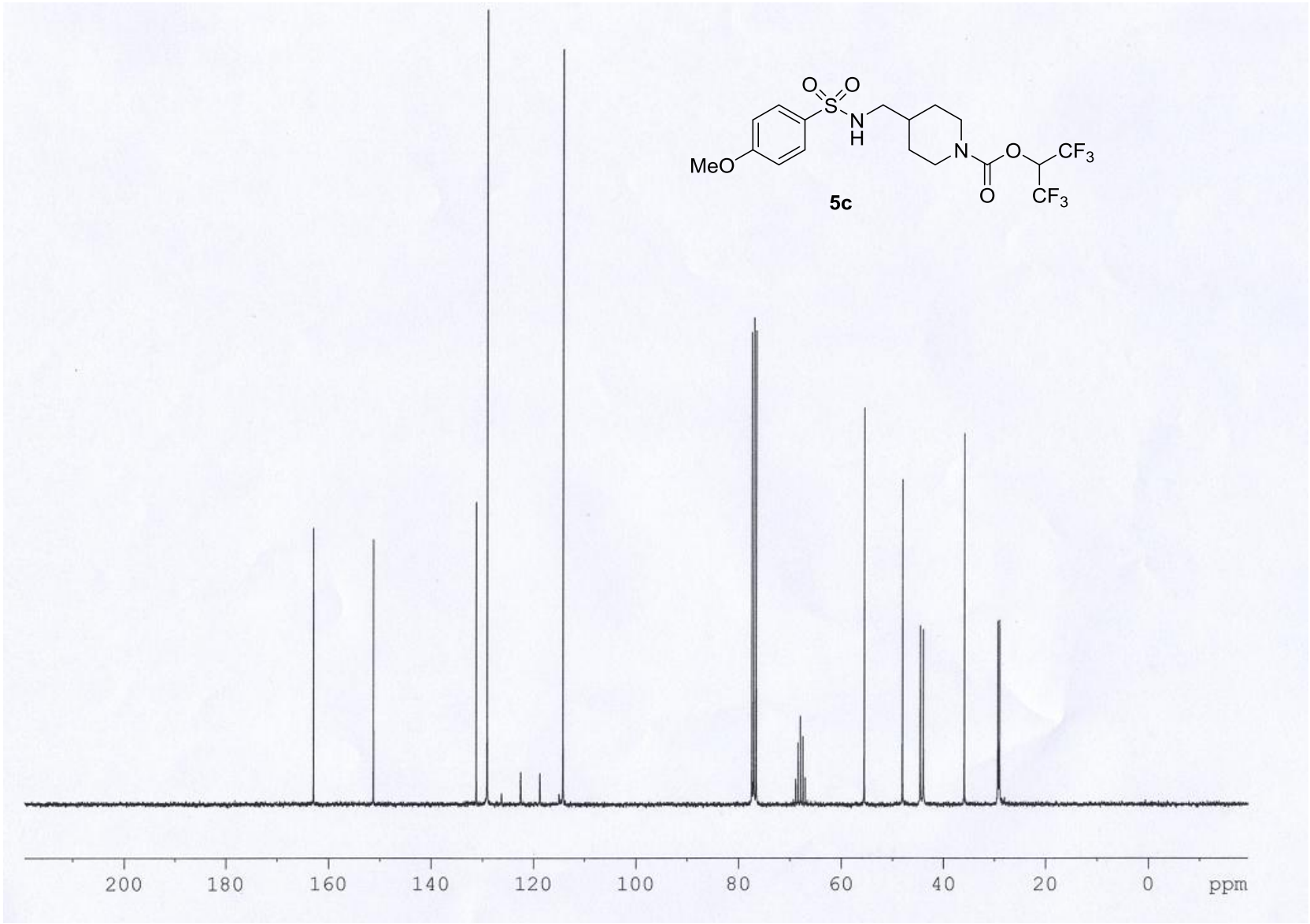
(D). ^{19}F NMR spectrum of compound 5b



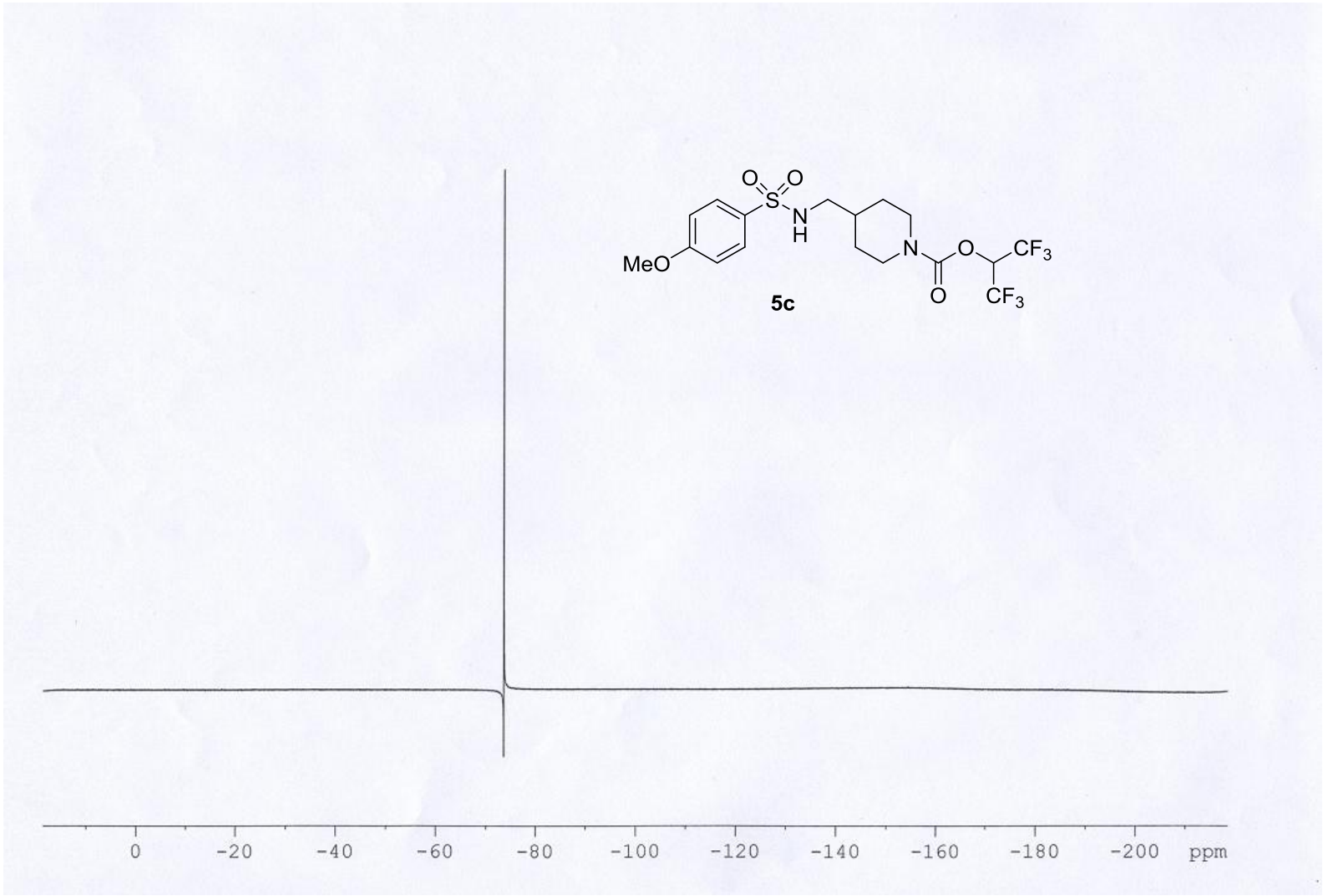
(E). ^{1}H spectrum of compound 5c



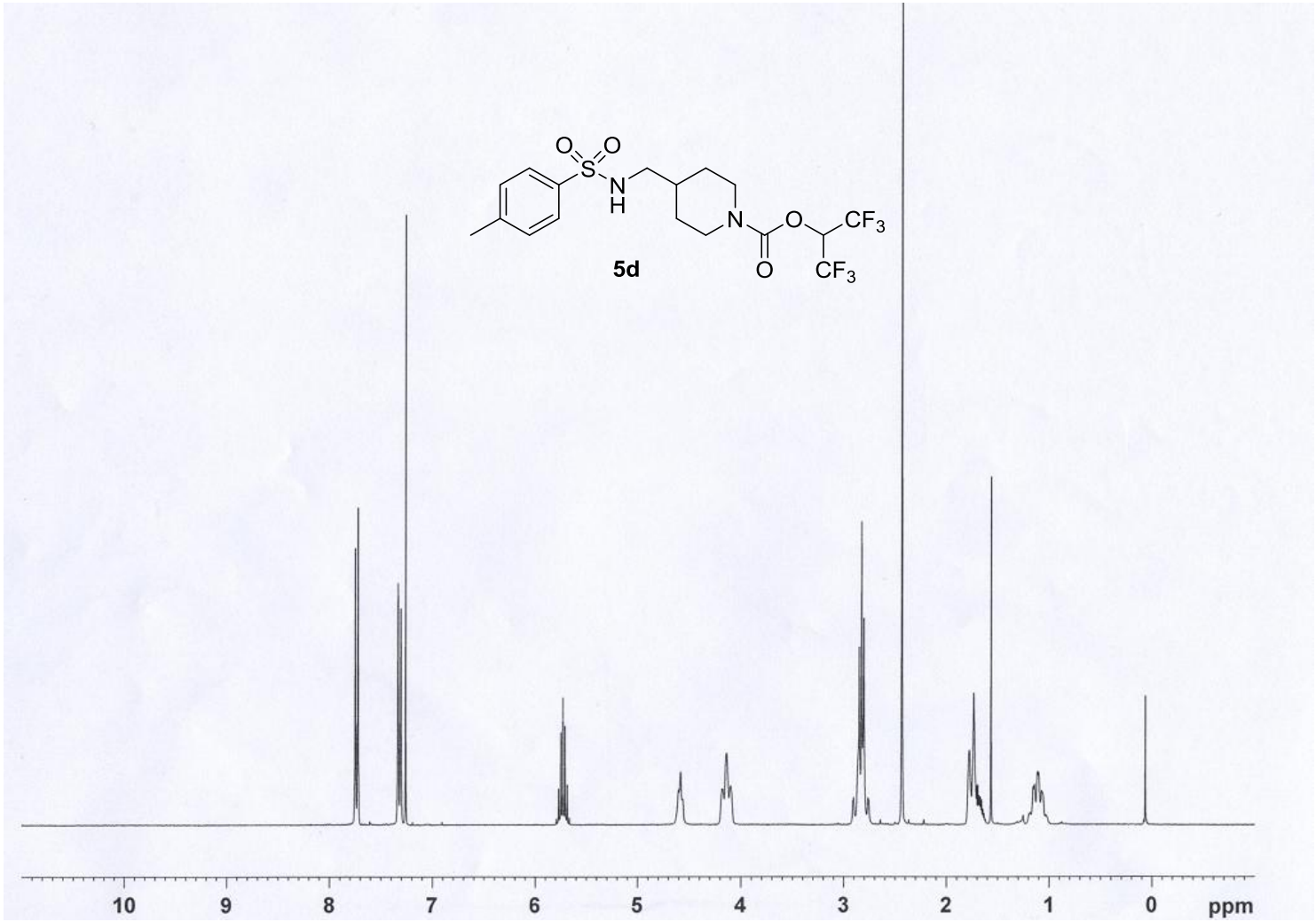
(F). ^{13}C NMR spectrum of compound 5c



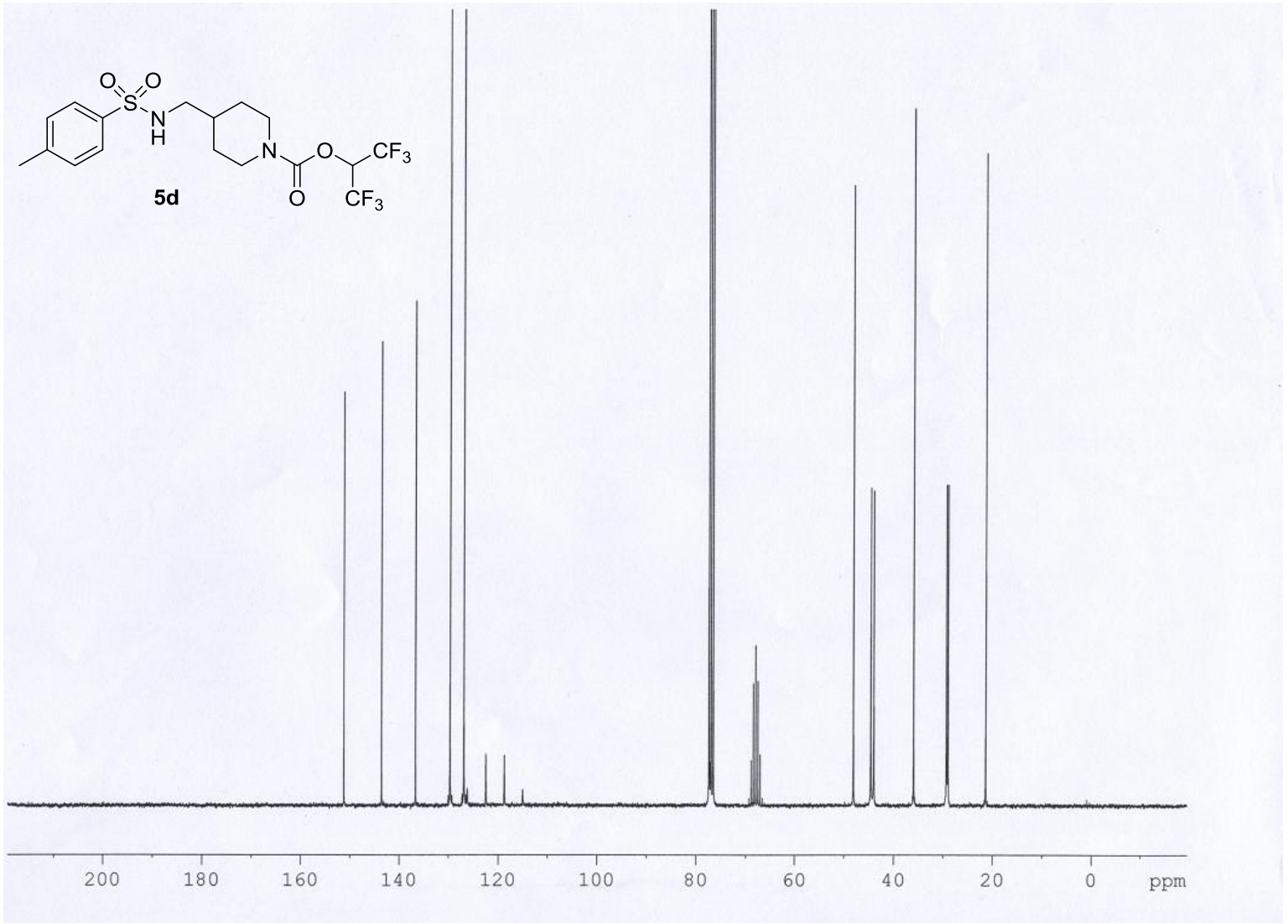
(G). ^{19}F NMR spectrum of compound 5c



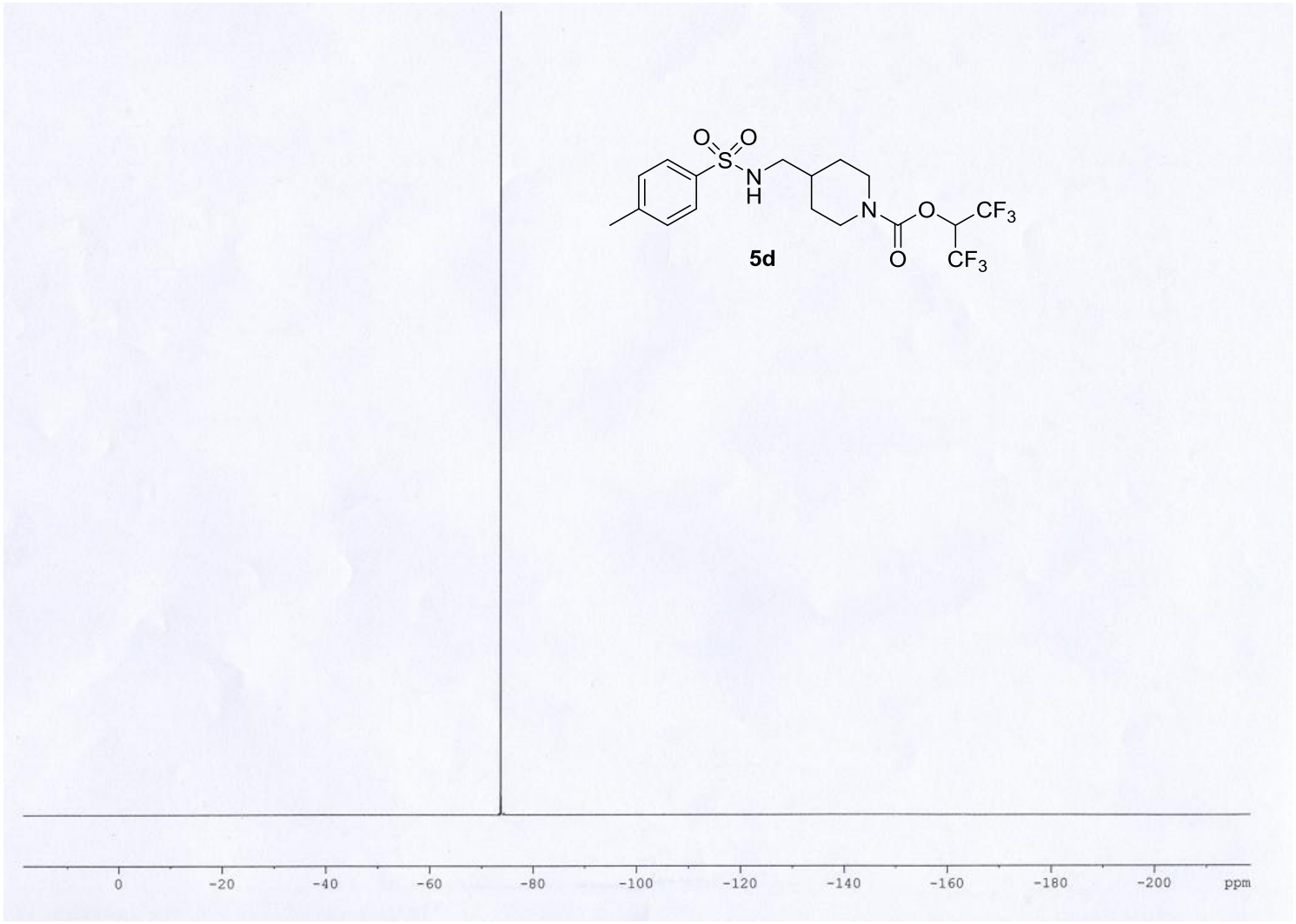
(H). ^{1}H spectrum of compound 5d



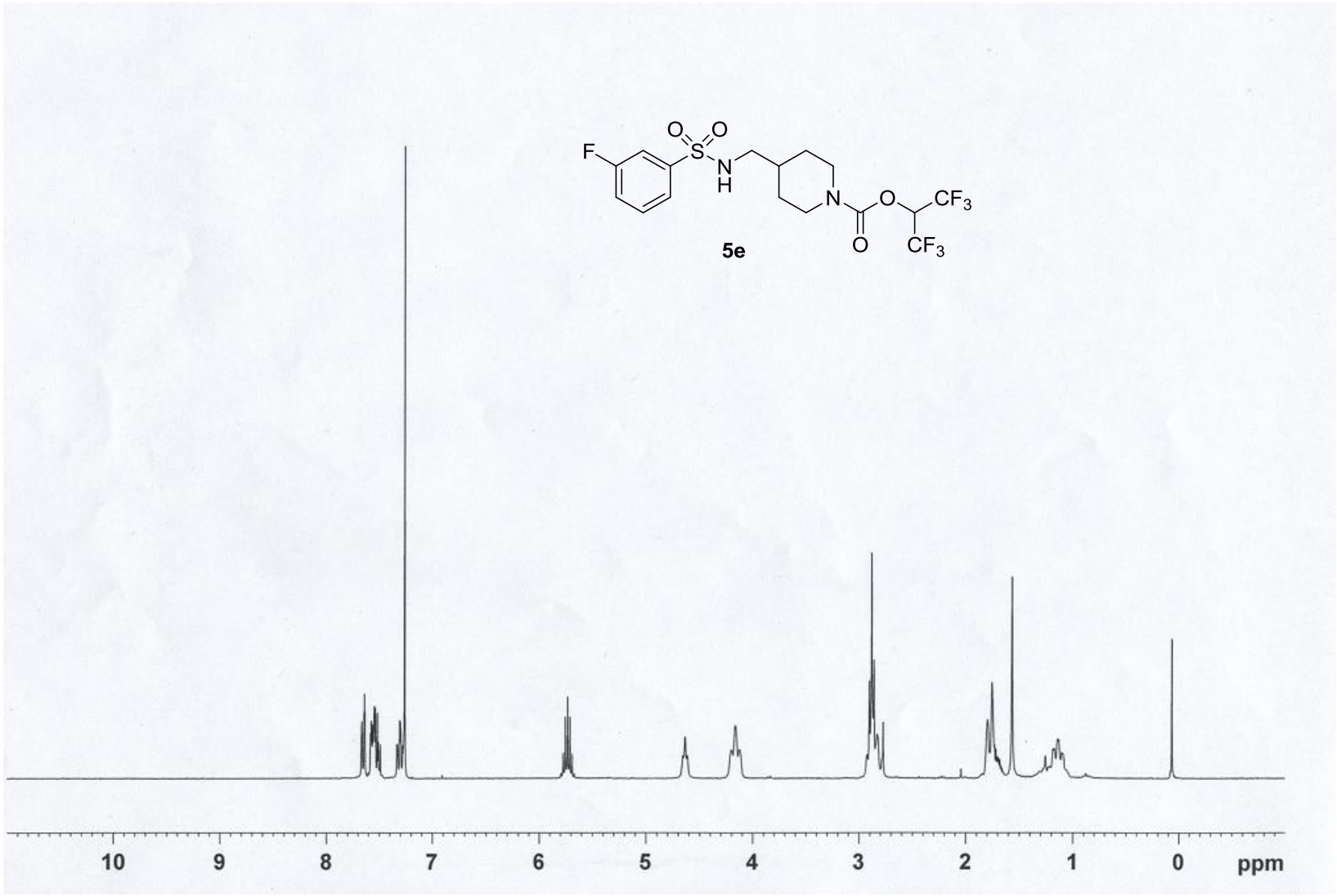
(I). ^{13}C NMR spectrum of compound 5d



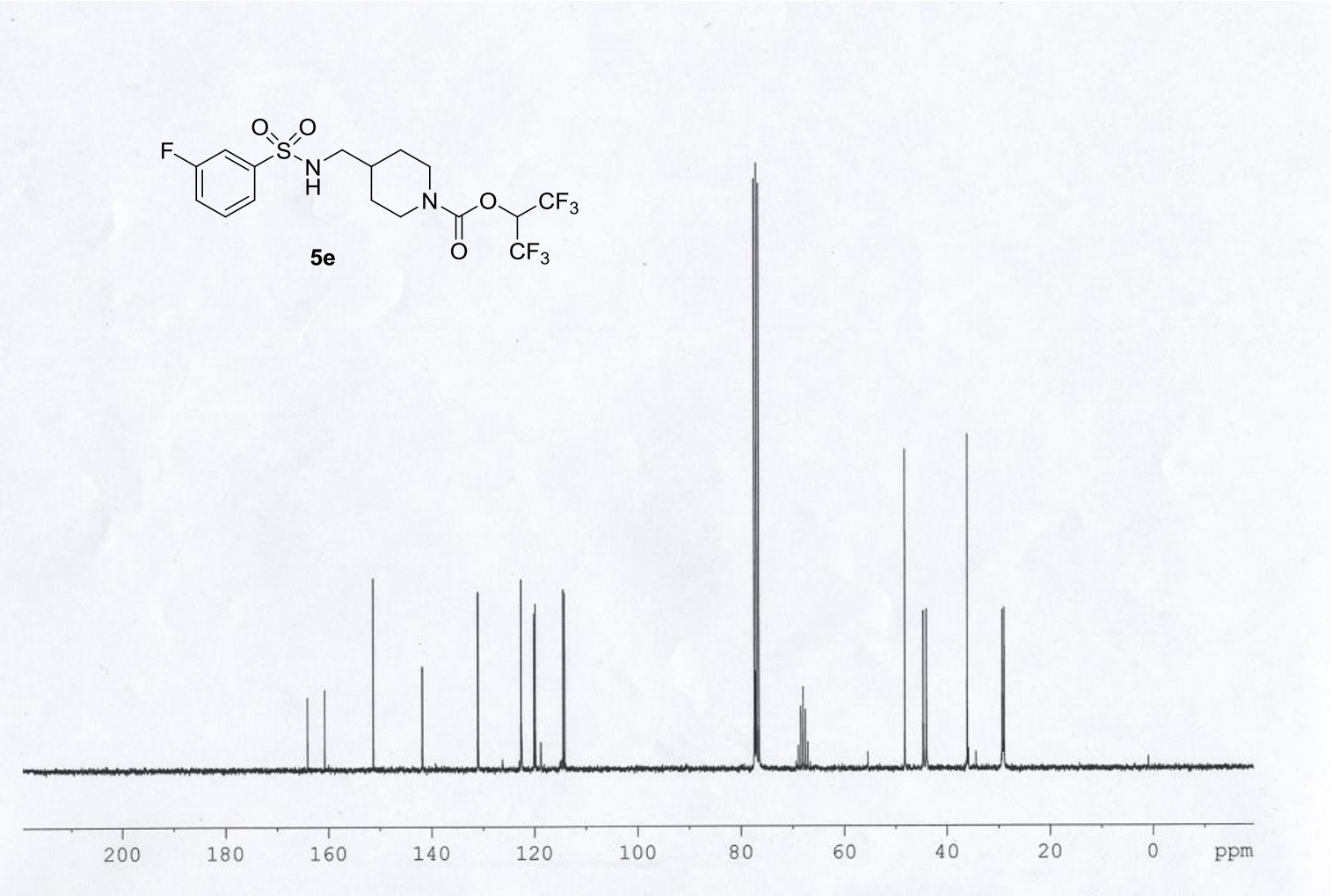
(J). ^{19}F NMR spectrum of compound 5d



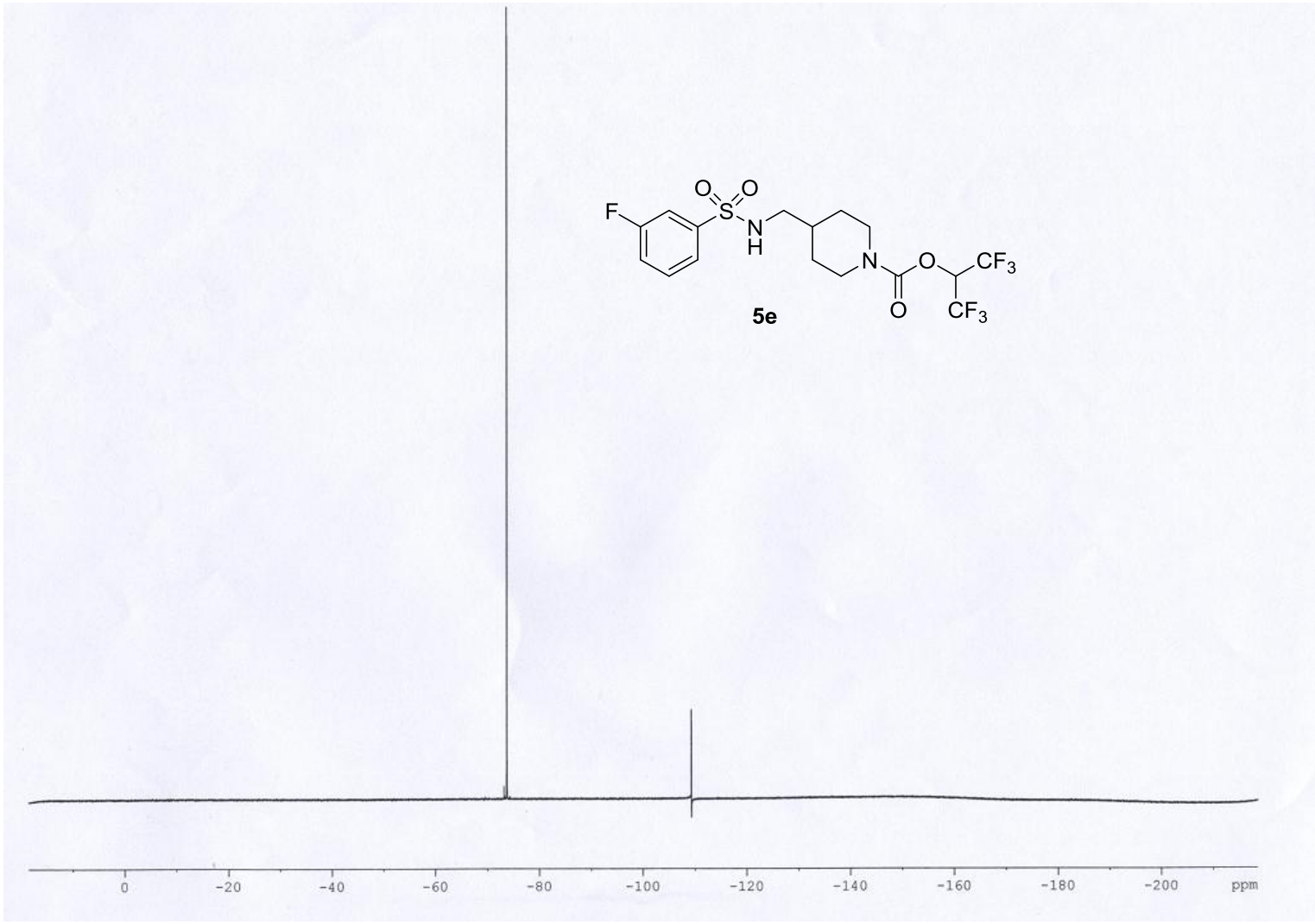
(K). ^{1}H spectrum of compound 5e



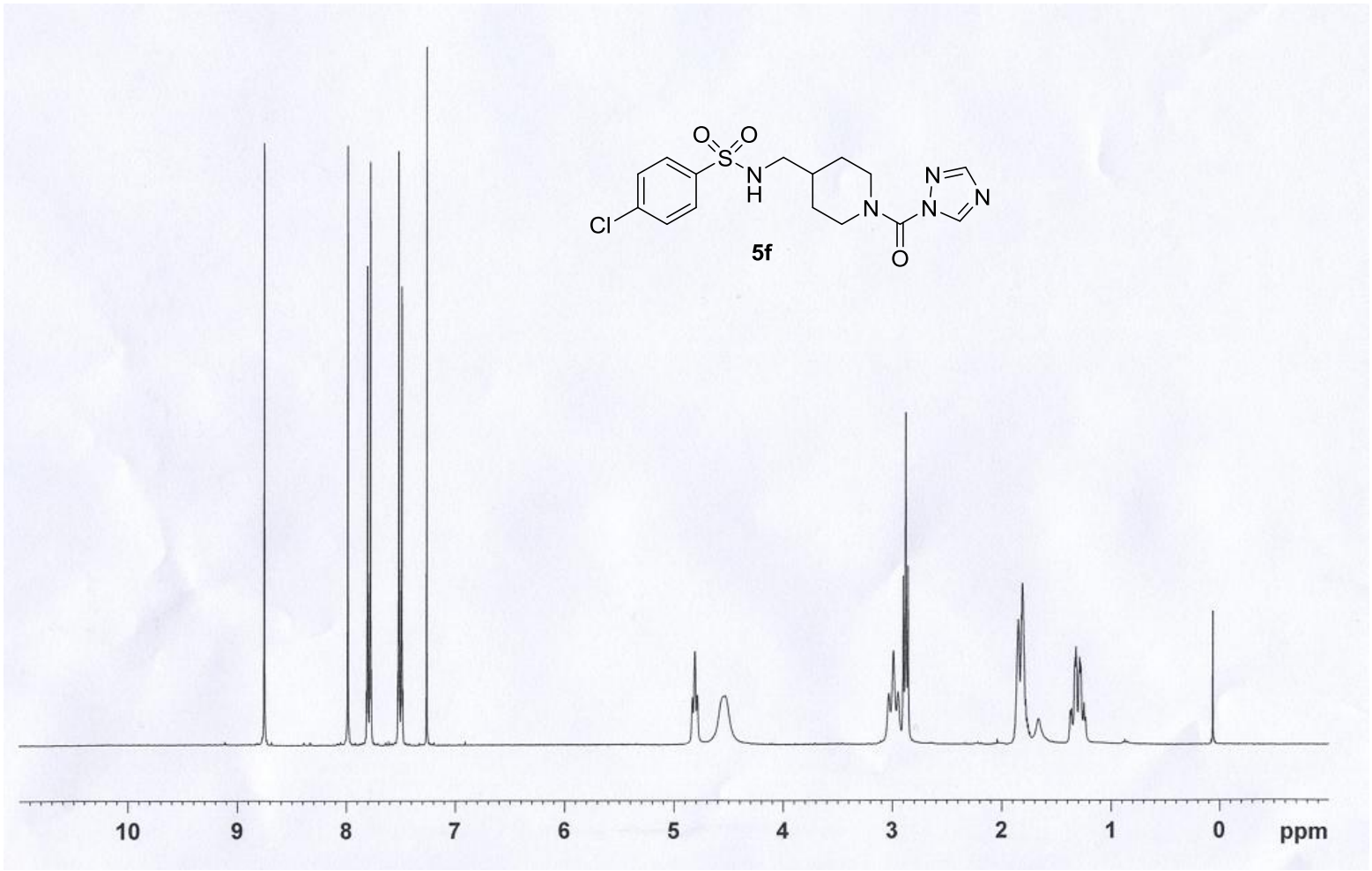
(L). ^{13}C NMR spectrum of compound 5e



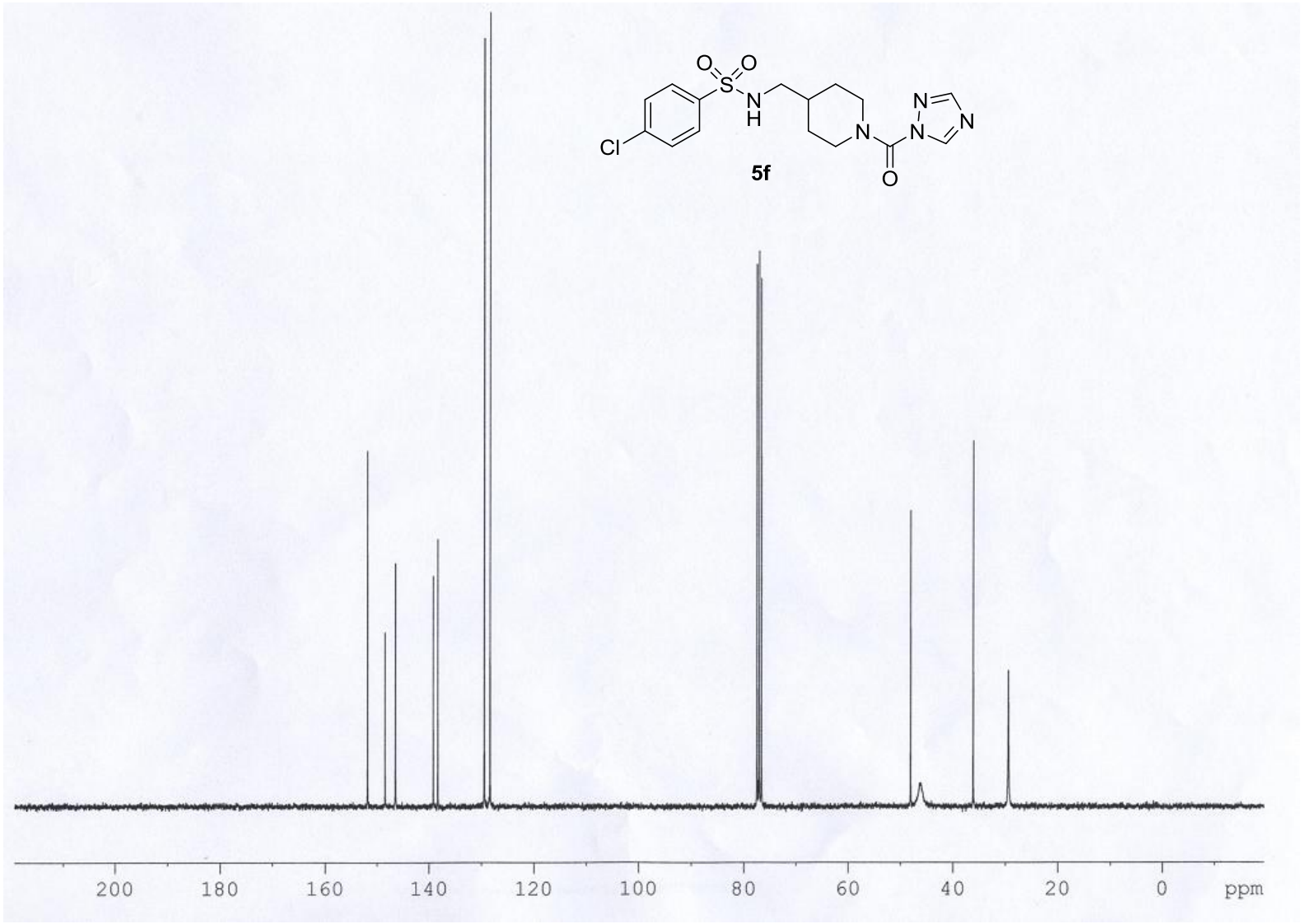
(M). ^{19}F NMR spectrum of compound 5e



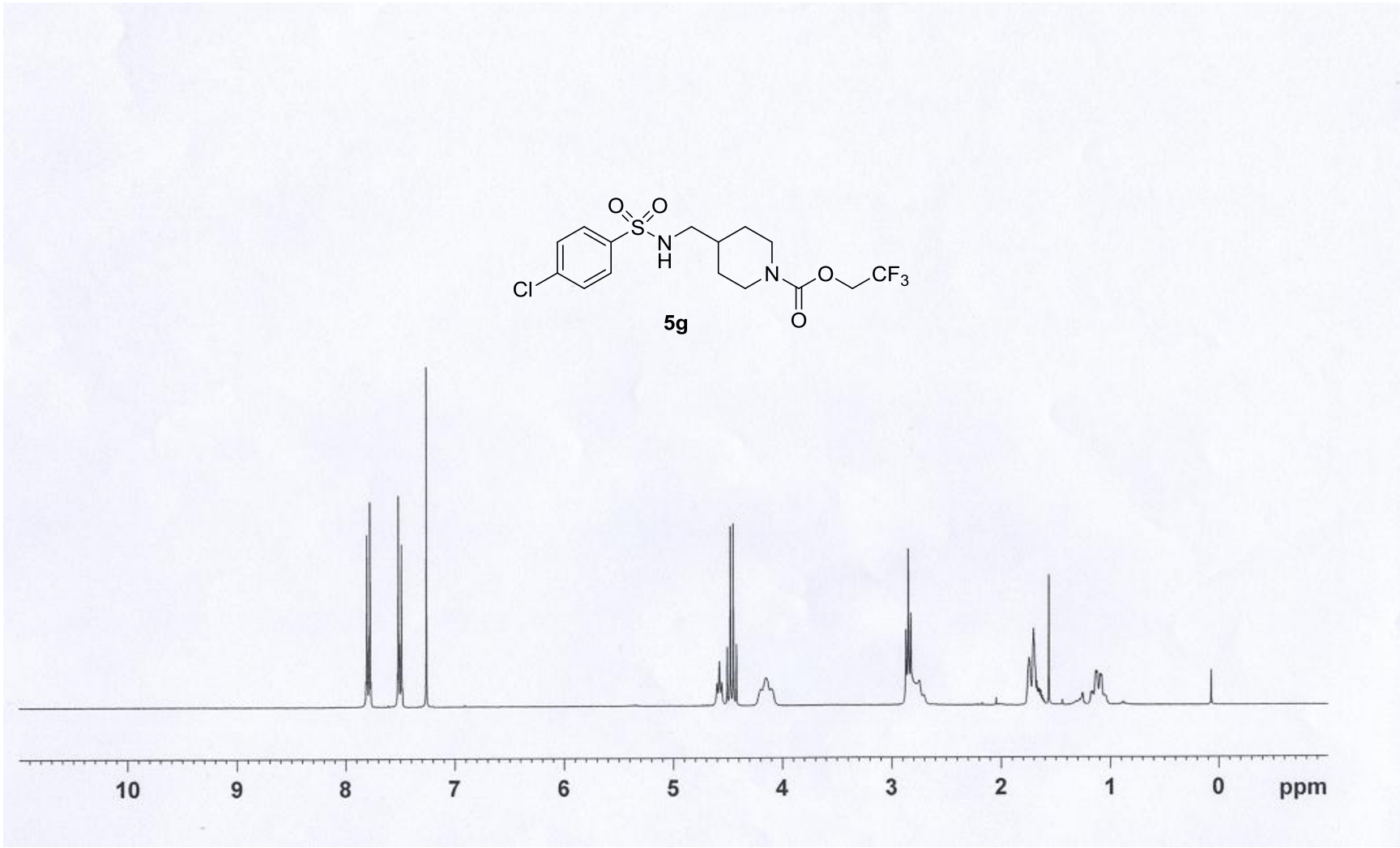
(N). ^{1}H spectrum of compound TZPU (5f)



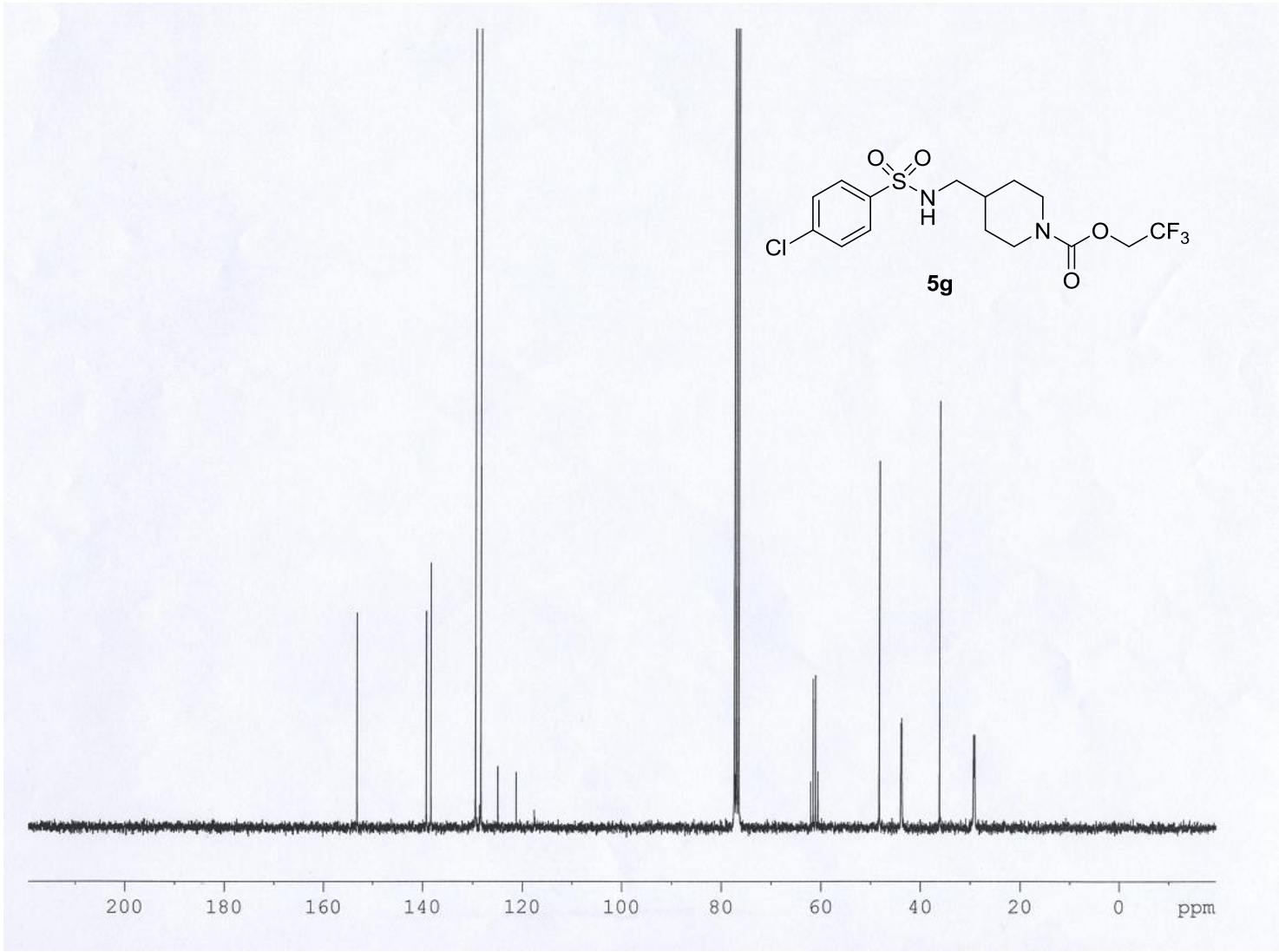
(O). ^{13}C NMR spectrum of compound TZPU (5f)



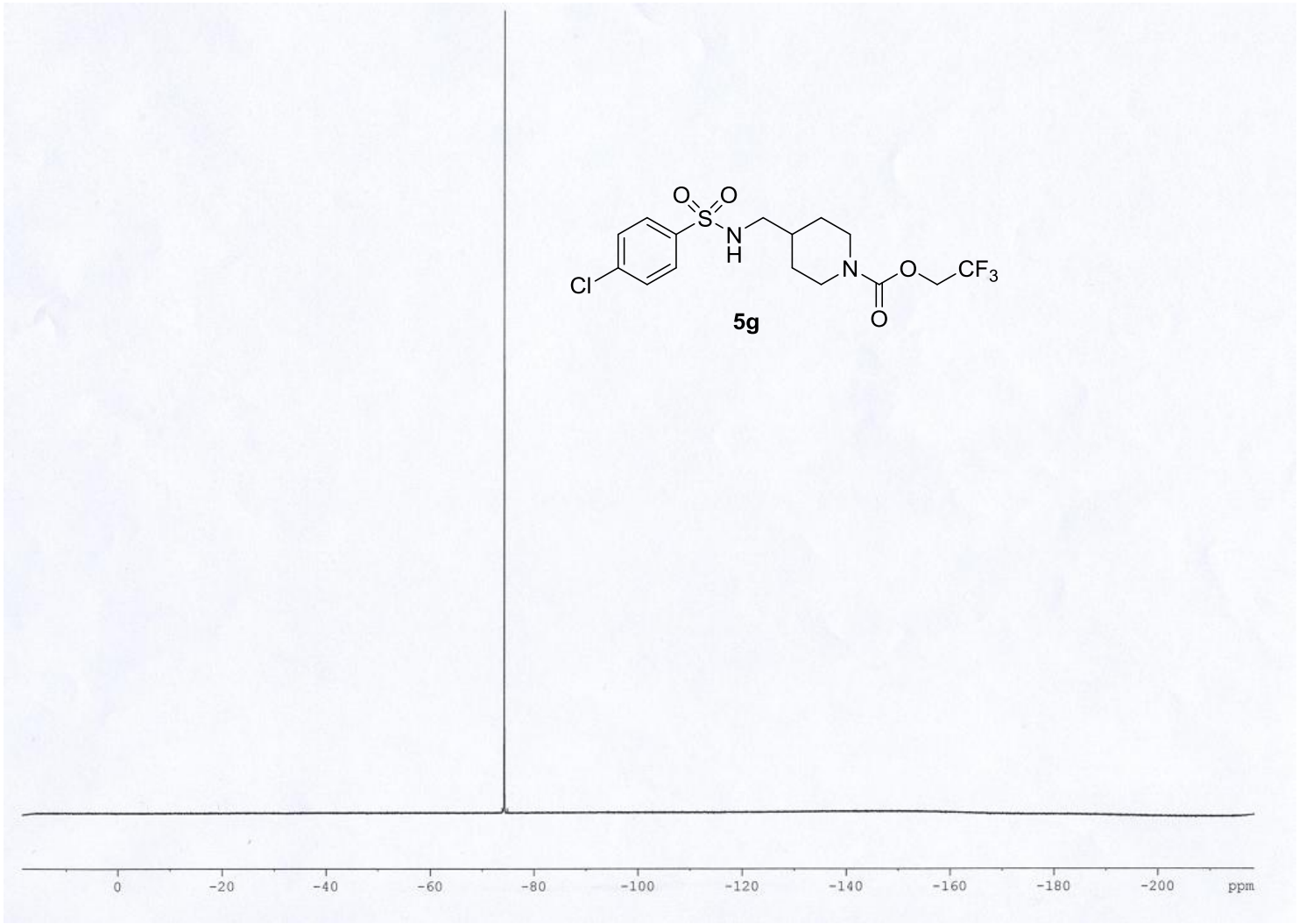
(P). ^{1}H spectrum of compound 5g



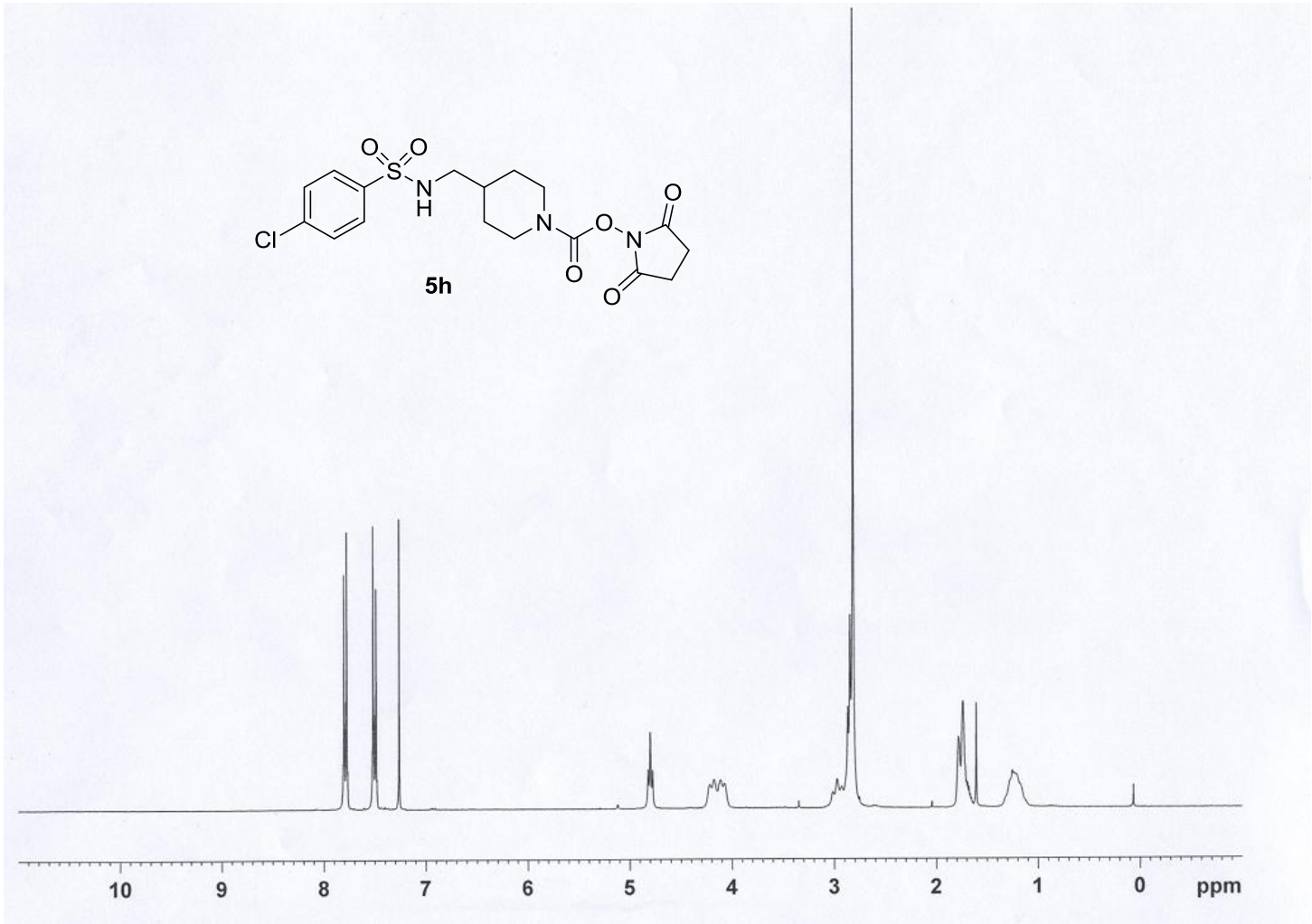
(Q). ^{13}C NMR spectrum of compound 5g



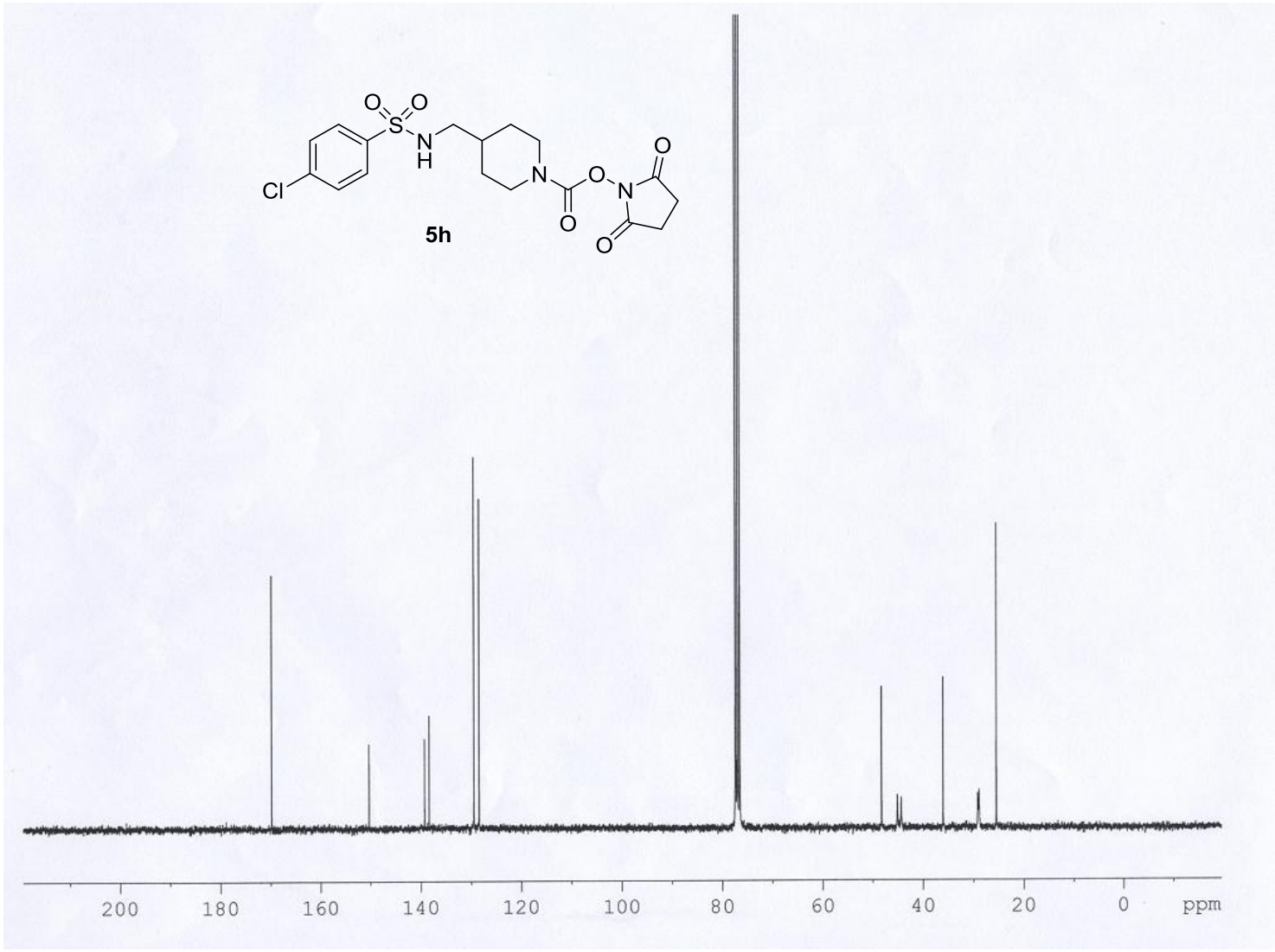
(R). ^{19}F NMR spectrum of compound 5g



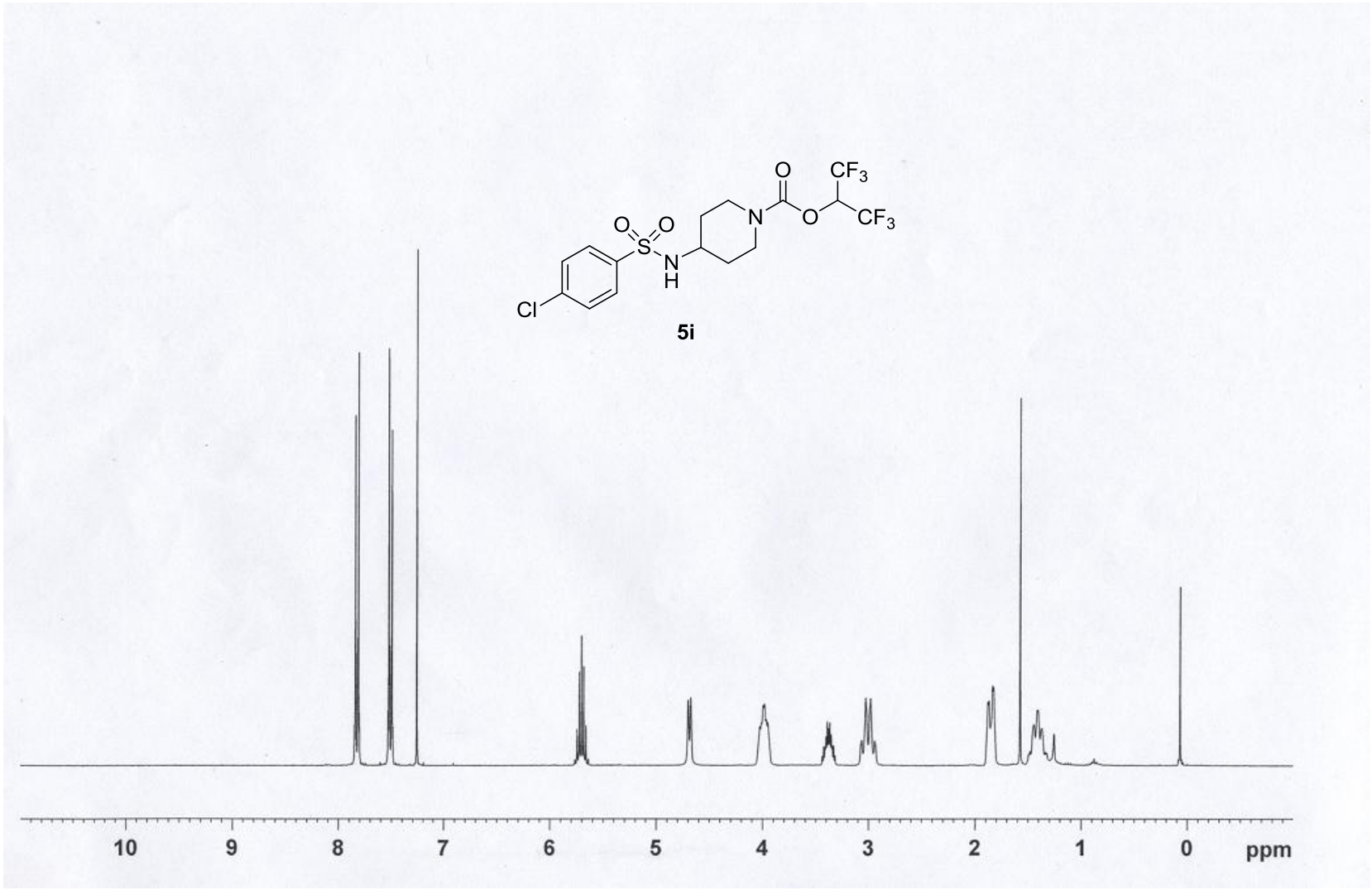
(S). ^{1}H spectrum of compound 5h



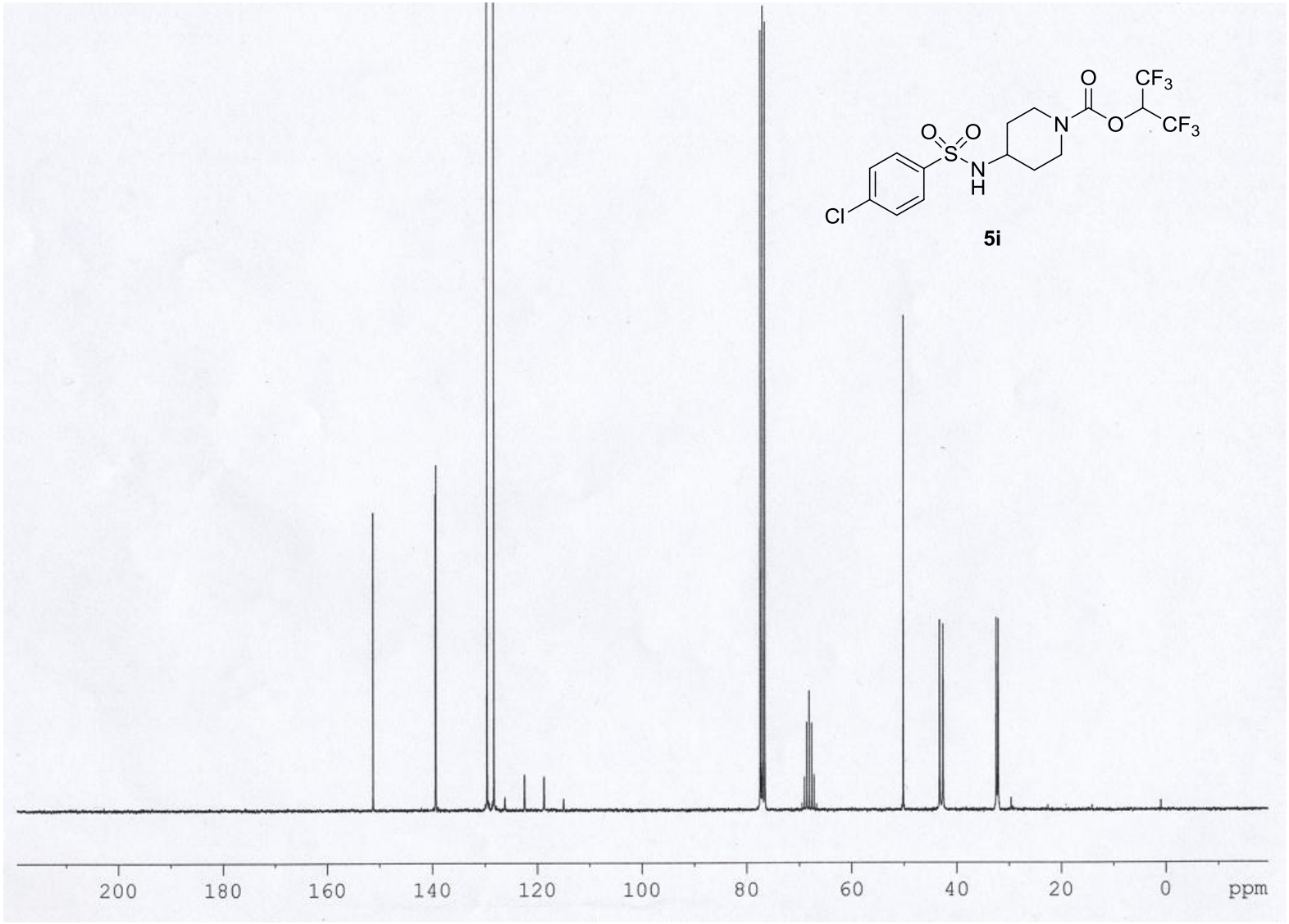
(T). ^{13}C NMR spectrum of compound 5h



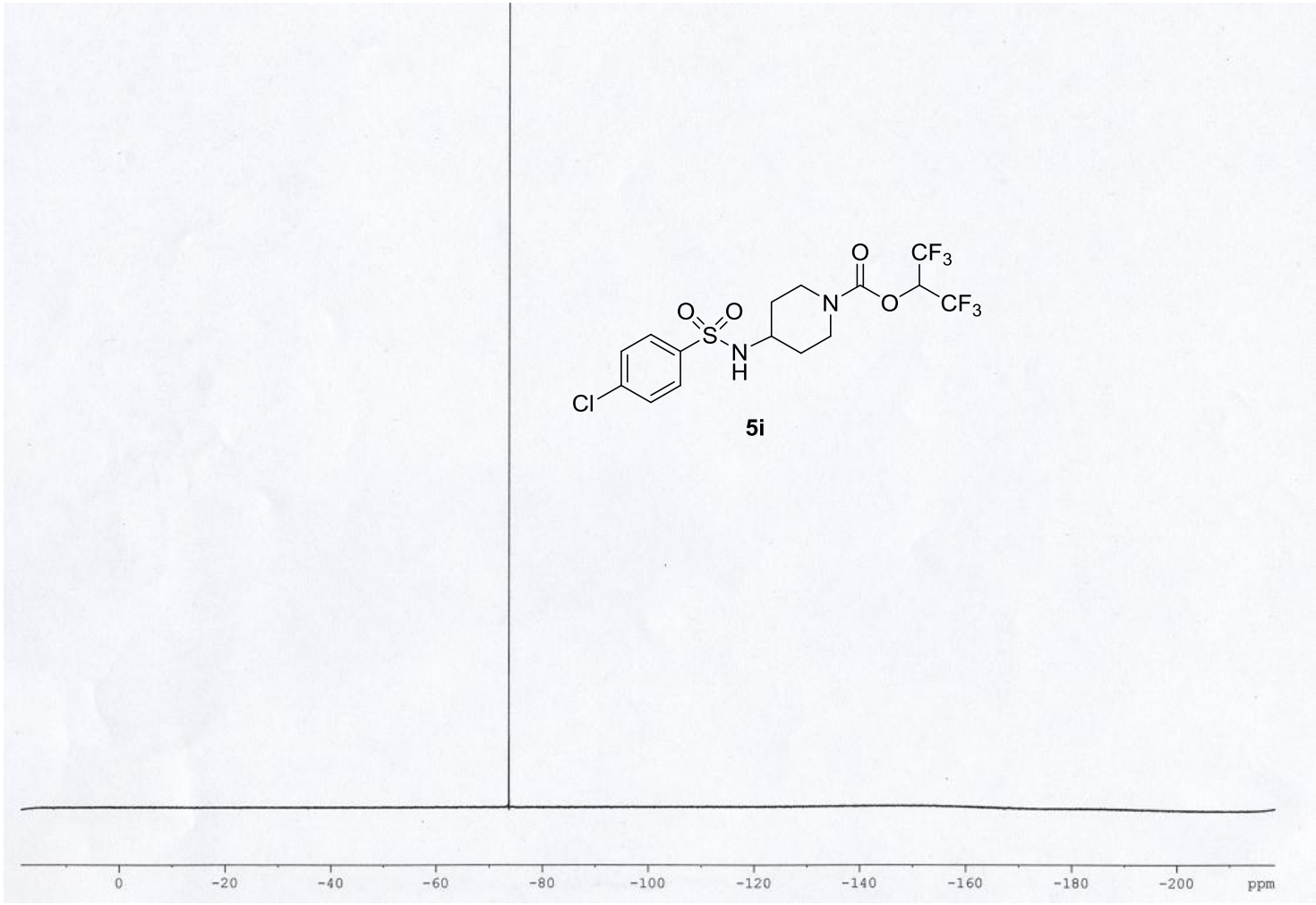
(U). ^{1}H spectrum of compound 5i



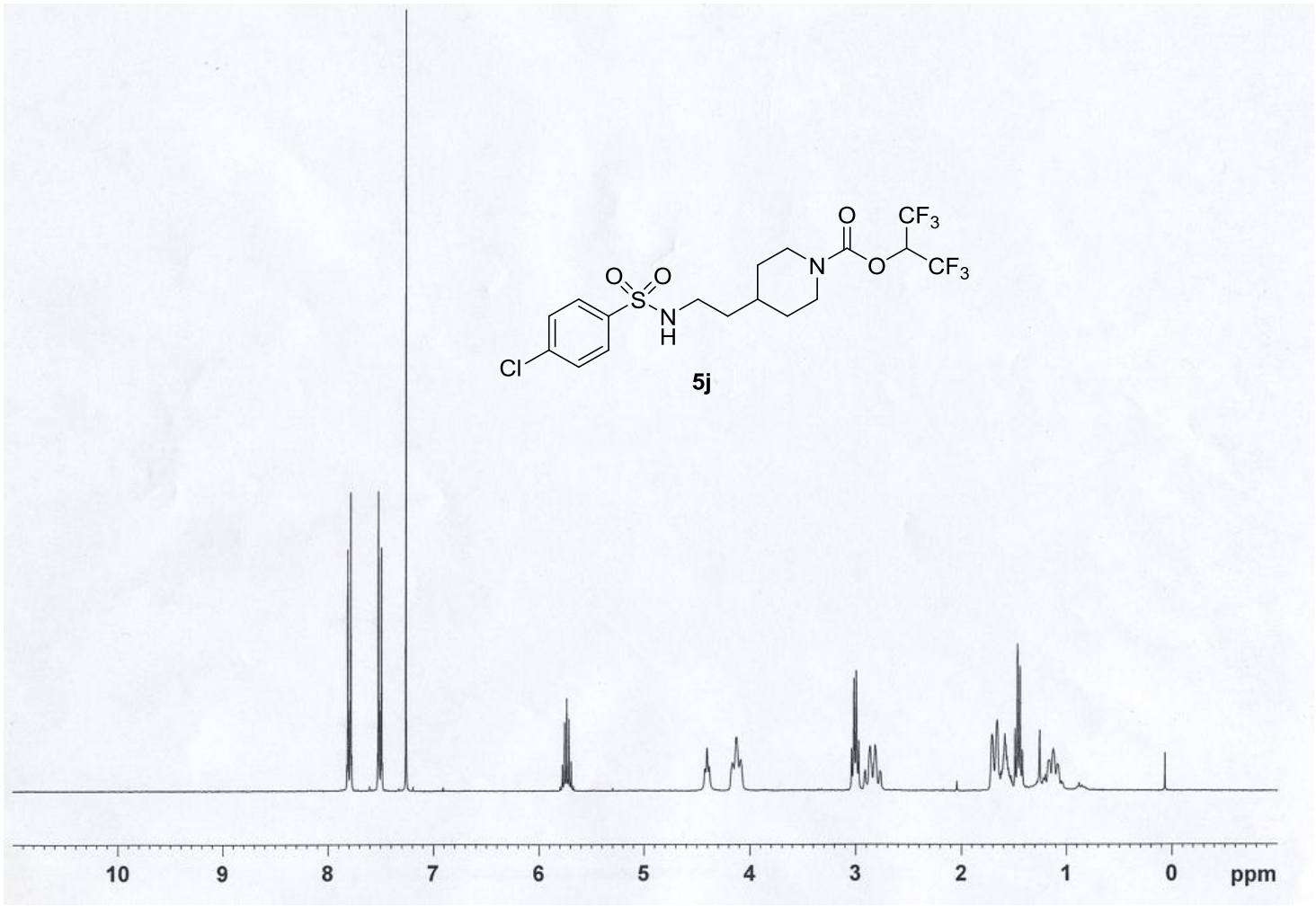
(V). ^{13}C NMR spectrum of compound 5i



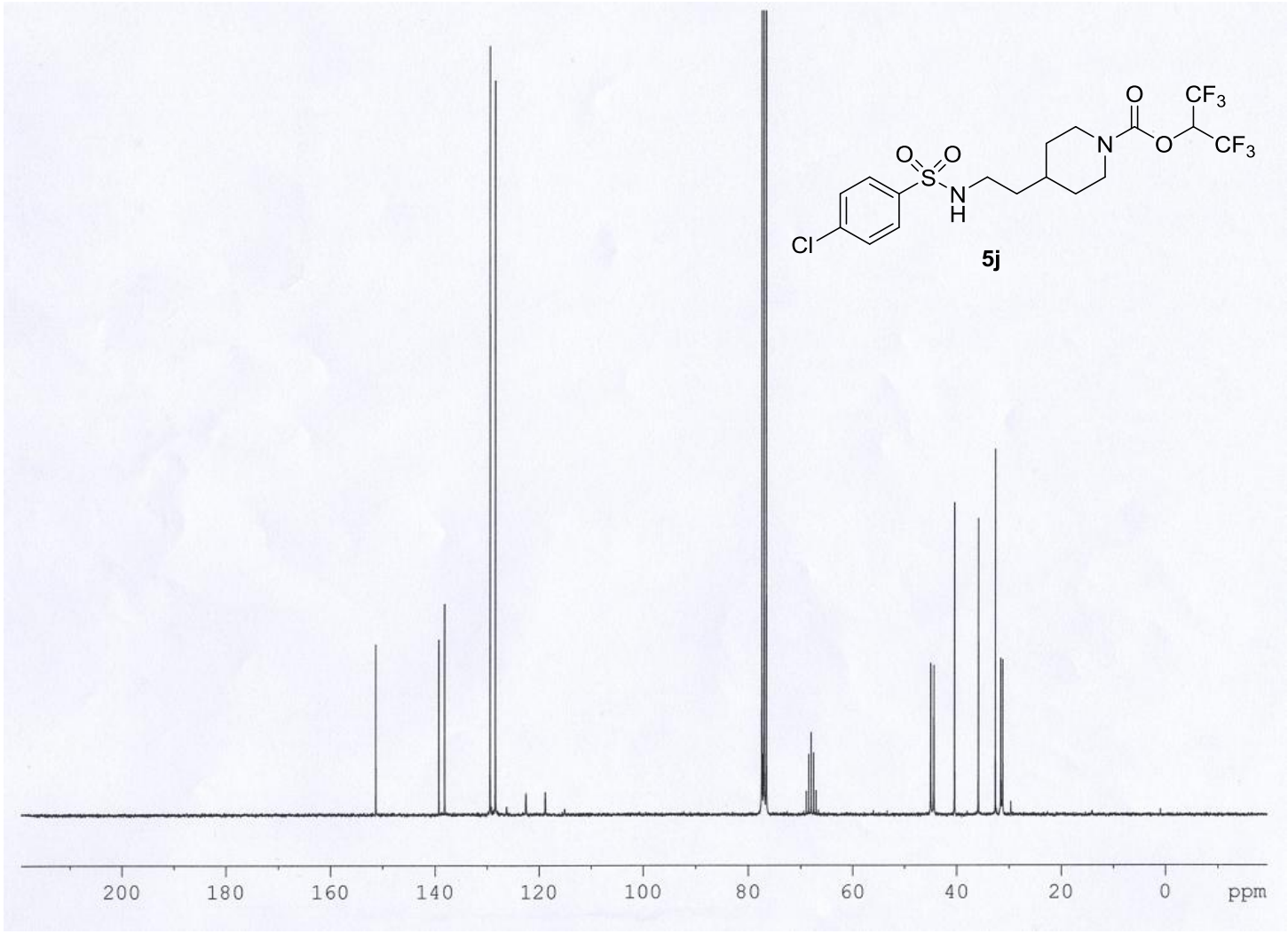
(W). ^{19}F NMR spectrum of compound 5i



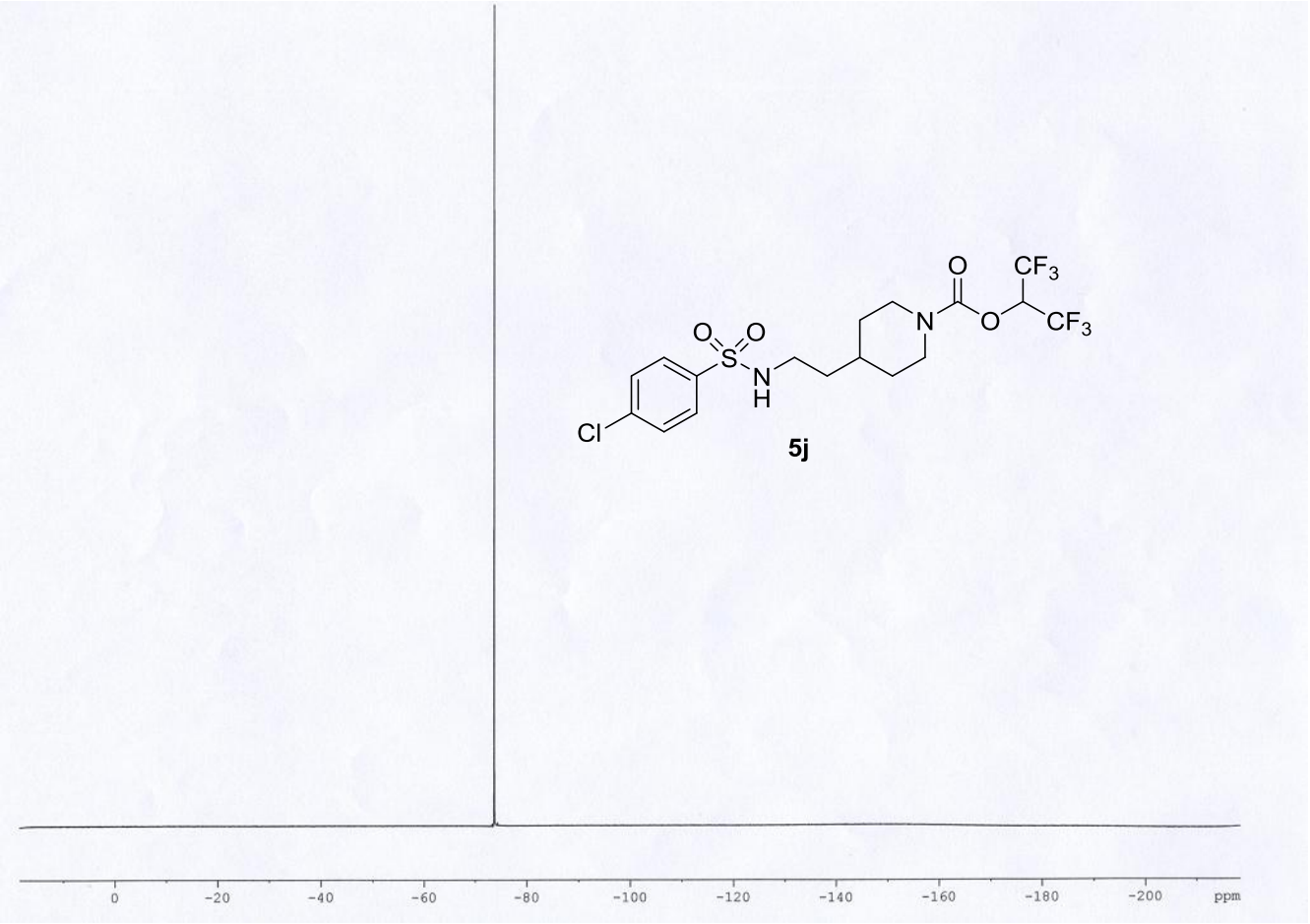
(X). ^{1}H spectrum of compound 5j



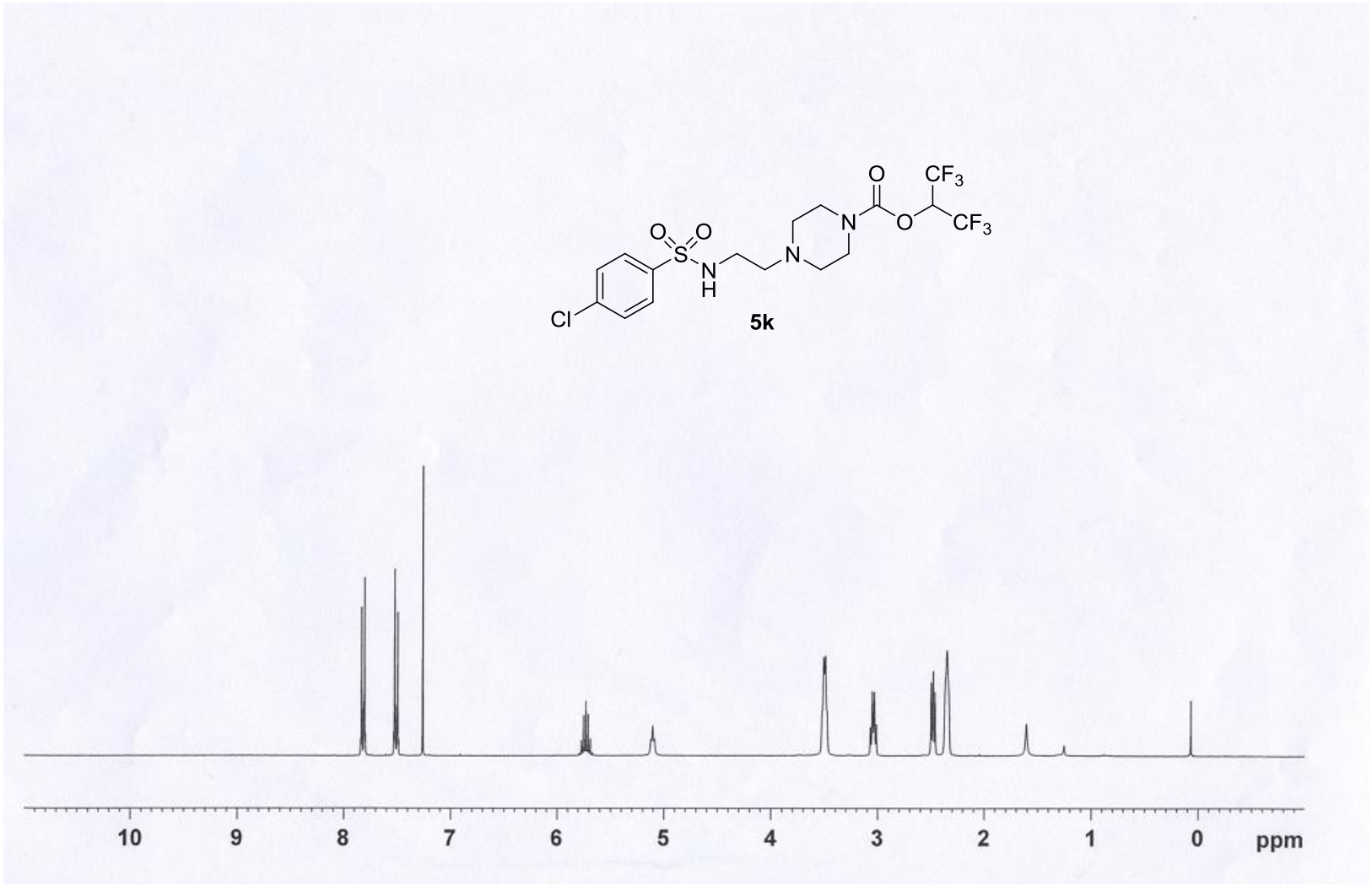
(Y). ^{13}C NMR spectrum of compound 5j



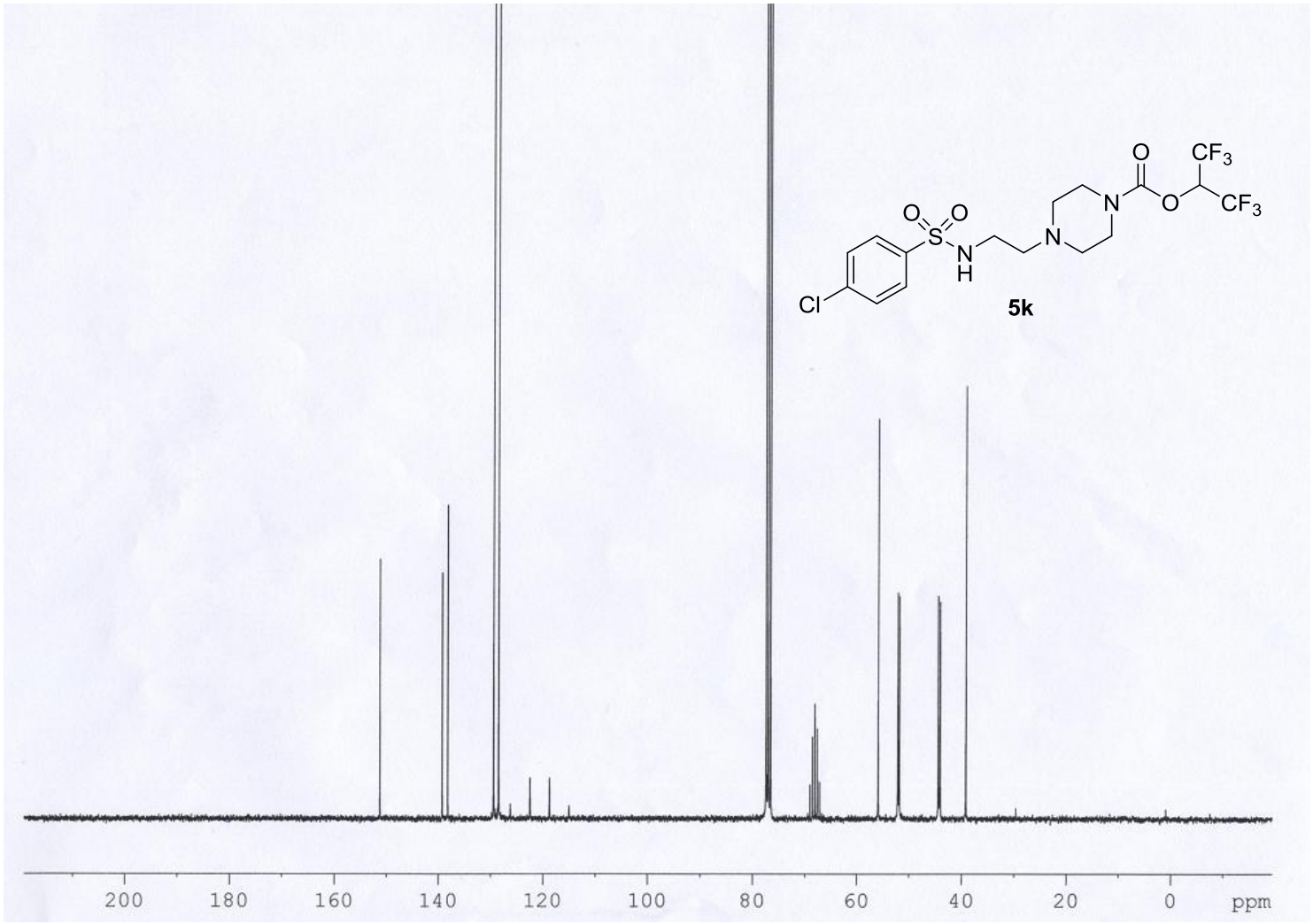
(Z). ^{19}F NMR spectrum of compound 5j



(AA). ^{1}H spectrum of compound 5k



(BB). ^{13}C NMR spectrum of compound 5k



(CC). ^{19}F NMR spectrum of compound 5k