

## Supporting Information

# **Design, Synthesis and Preclinical Evaluation of a brain-permeable PET Tracer for P2Y<sub>12</sub> Receptor Imaging in the Brain**

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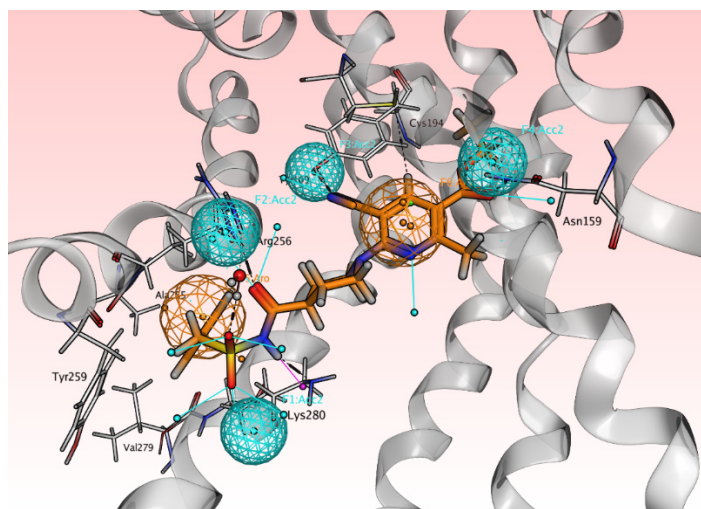
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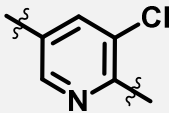

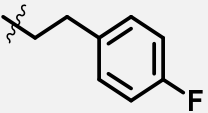
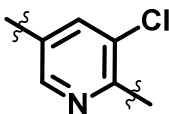

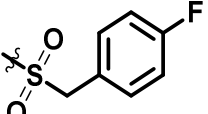
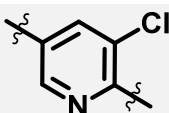
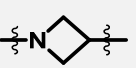
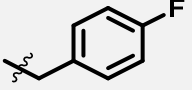
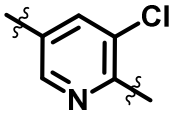
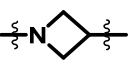
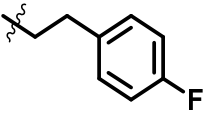
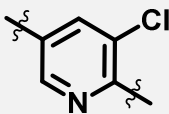
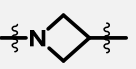
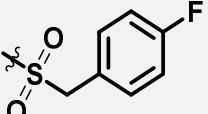
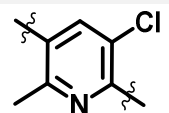

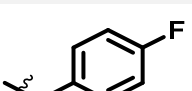
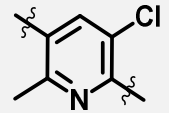
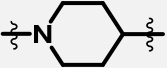
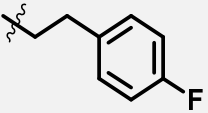
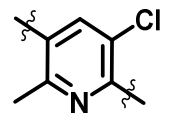

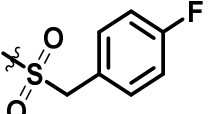
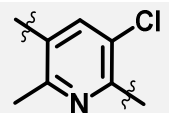
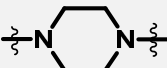
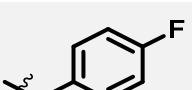
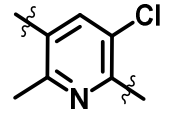

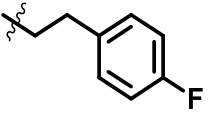
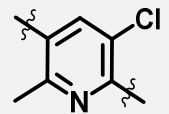

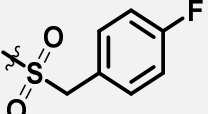
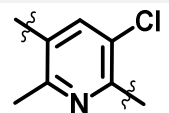
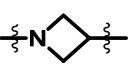
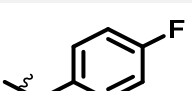
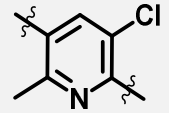
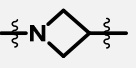
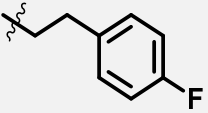
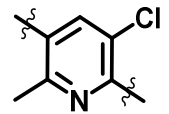
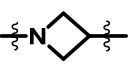
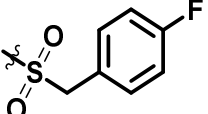
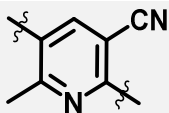
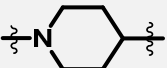
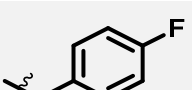
## Docking results and *in silico* brain permeability prediction



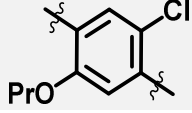

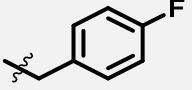
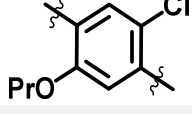

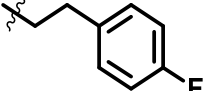
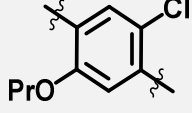
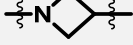
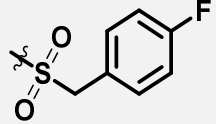
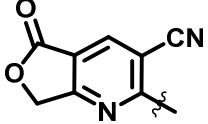
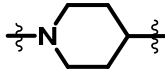
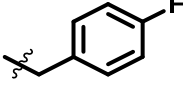
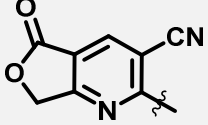
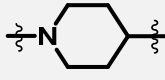
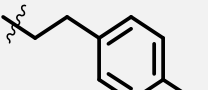
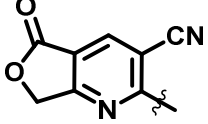
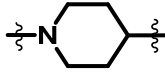
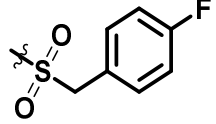
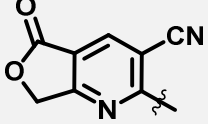
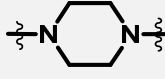
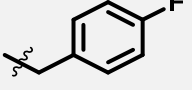
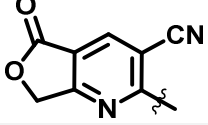
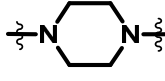
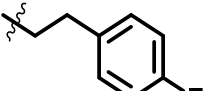
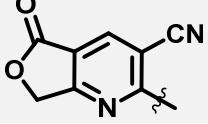
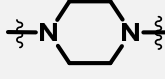
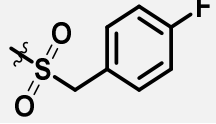
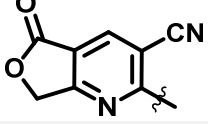

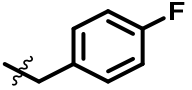
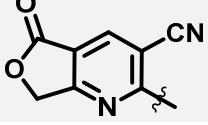

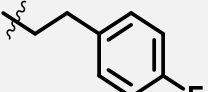
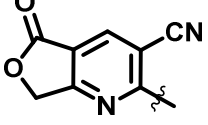

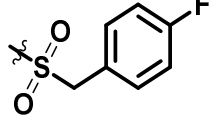
**Figure S1.** Pharmacophore for docking studies. 6 Features were defined based on central interactions: F1:Acc2 Lys280; F2:Acc2 Arg256; F3:Acc2 Tyr109; F4:Acc2 Asn159; F5:Aro Ar2; F6:Aro Ar1. (F = feature; Acc = hydrogen acceptor; Aro = aromatic interaction; Ar = aryl fragment).

**Table S1.** Docking Results. CNS MPO, BBB and S scores (RMSD = root mean square deviation).

Entry	Compound	Ar <sub>1</sub>	Amino Linker	Ar <sub>2</sub>	CNS MPO	BBB	S	RMSD
1	8				4.75	4.47	-9.23	1.22
2	14				4.36	4.44	-9.66	1.39
3					4.57	3.58	-10.23	1.78
4					5.15	4.42	-9.21	1.34
5					4.88	4.39	-9.54	1.09
6					4.46	3.54	-9.30	1.46
7	11				5.39	4.52	-9.14	1.04
8					5.13	4.49	-9.55	0.96
9					4.77	3.61	-9.83	1.24
10	9				4.29	4.74	-9.52	1.27
11	15				3.77	4.71	-9.92	1.34
12					4.20	3.86	-9.82	1.97
13	6				4.12	4.70	-9.19	0.80

14					4.26	4.66	-9.36	1.10
15					4.31	3.81	-9.81	1.42
16	12				4.91	4.79	-9.26	1.20
17					4.51	4.77	-9.55	0.94
18					4.63	3.90	-9.88	1.23
19	18				3.90	4.71	-9.79	1.24
20	17				3.61	4.67	-10.15	1.59
21					3.73	3.84	-10.56	1.70
22					4.41	4.66	-9.57	1.13
23					3.42	4.63	-9.80	1.47
24					4.21	3.79	-9.79	1.37
25					4.68	4.77	-9.69	0.88
26					4.65	4.74	-9.94	1.23
27					4.53	3.88	-9.91	1.47
28	10				4.54	4.17	-10.03	1.67

29	16				4.15	4.15	-10.44	1.02
30					3.92	3.01	-10.86	1.64
31	5				4.04	3.27	-10.63	1.12
32	7				4.76	4.13	-9.80	1.04
33					4.52	4.10	-9.64	0.89
34					3.91	3.01	-10.34	1.05
35	13				5.10	4.21	-9.91	1.58
36					4.85	4.19	-9.95	1.06
37					4.12	3.03	-10.59	1.03
38	19				3.00	4.65	-10.58	0.99
39	20				2.90	4.61	-11.03	1.22
40					2.86	3.81	-10.26	3.20
41					3.19	4.60	-10.58	1.07
42					2.95	4.56	-10.51	1.00
43					3.28	3.77	-10.78	1.85

44					4.03	4.73	-10.49	1.23
45					3.17	4.69	-10.80	1.26
46					3.44	3.86	-10.75	1.16
47	22				5.26	4.21	-8.91	1.35
48					5.01	4.19	-8.04	0.79
49					4.12	3.03	-7.84	1.22
50	21				5.30	4.16	-8.82	1.48
51					5.16	4.15	-9.18	1.07
52					4.12	3.03	-9.25	2.03
53					5.61	4.23	-8.76	1.71
54					5.50	4.22	-8.86	1.44
55					4.33	3.04	-8.91	1.22

## IC<sub>50</sub> Value

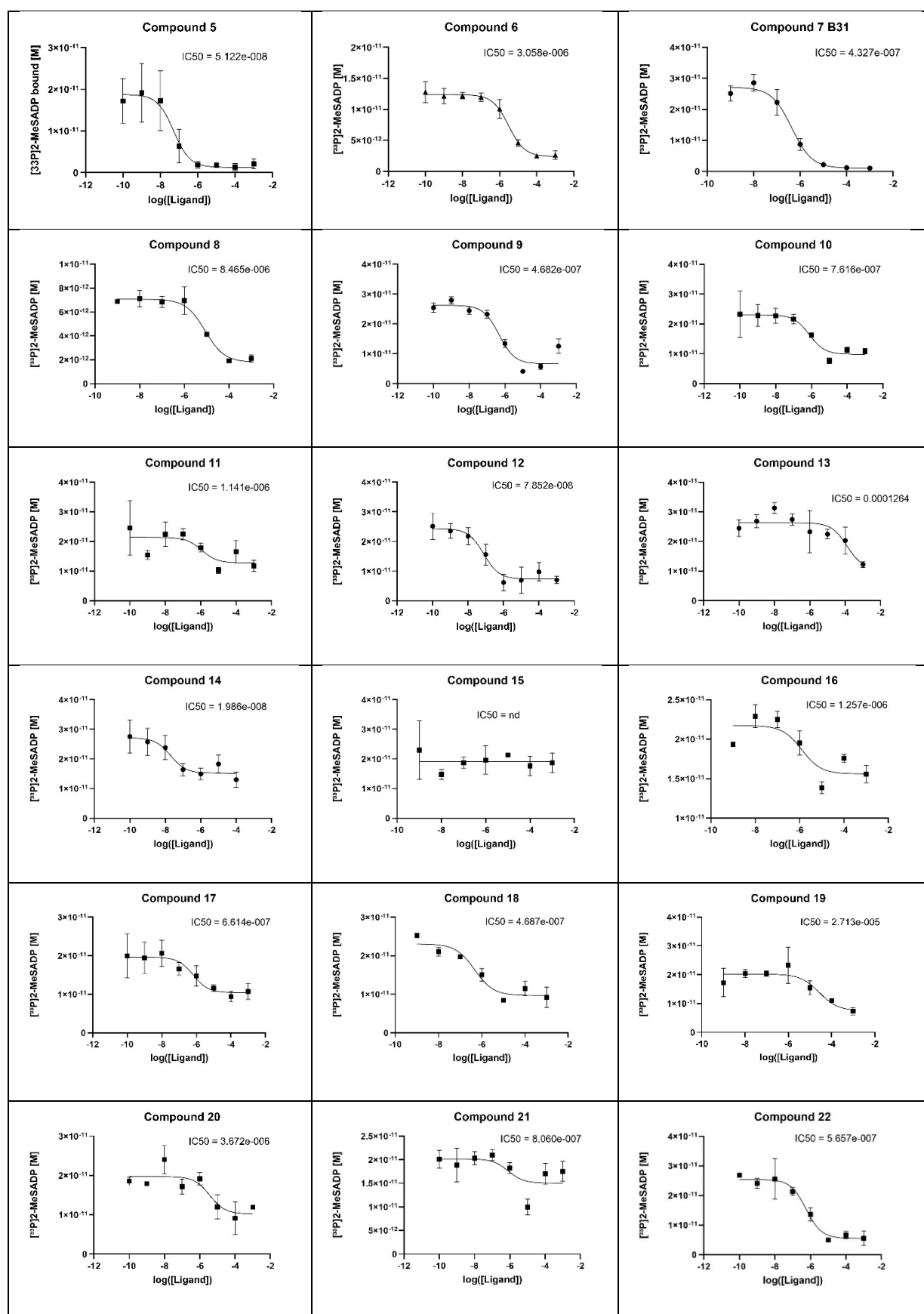


Figure S2. *In vitro* competitive binding curves for compounds 5-22.

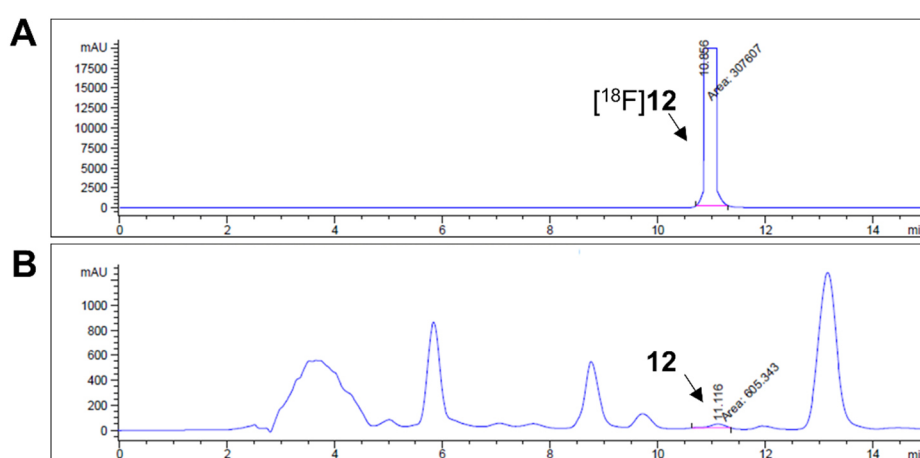


## HPLC Radiosynthesis

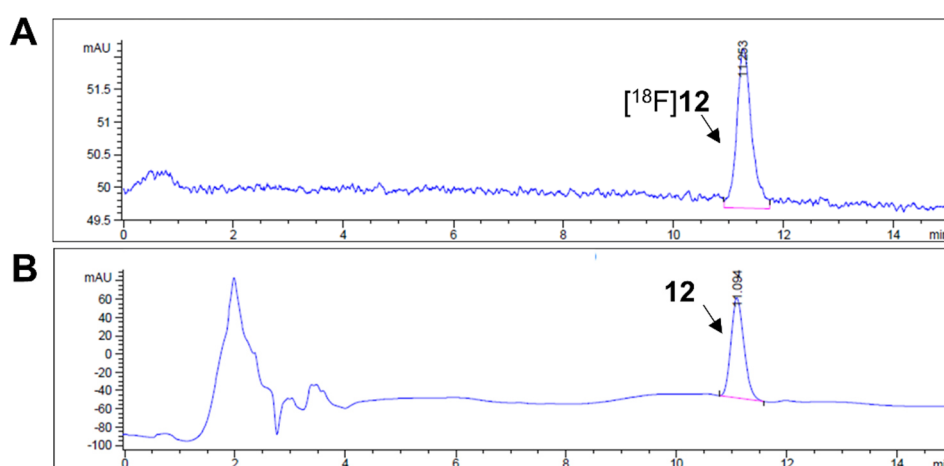
**HPLC Method A:** Luna<sup>®</sup> 5  $\mu$ m PFP(2) 100 Å, LC column 250 x 10 mm, 800  $\mu$ l injection, crude reaction solution in EtOH, 0 – 15 min 48% H<sub>2</sub>O (0.1% TFA), 52% MeCN (0.1% TFA), 5 mL/min.

**HPLC Method B:** Luna<sup>®</sup> 5  $\mu$ m PFP(2) 100 Å, LC column 250 x 4.6 mm, 100  $\mu$ l injection, product solution in PBS/EtOH (EtOH < 10%), 0 – 4 min 48% H<sub>2</sub>O (0.1% TFA), 52% MeCN (0.1% TFA); 4 – 11 min 40% H<sub>2</sub>O (0.1% TFA), 60% MeCN (0.1% TFA), 2 mL/min.

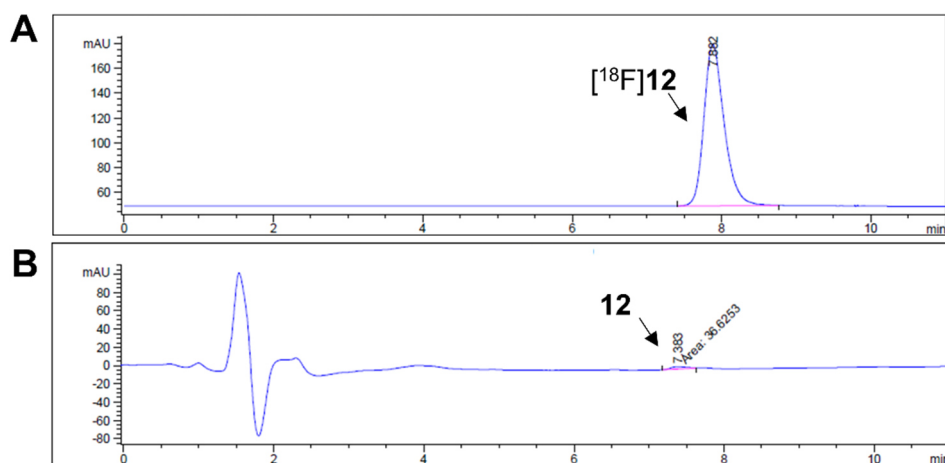
The purity of compound [<sup>18</sup>F]**12** for *in vitro* and *in vivo* testing was confirmed to be  $\geq 95\%$ .



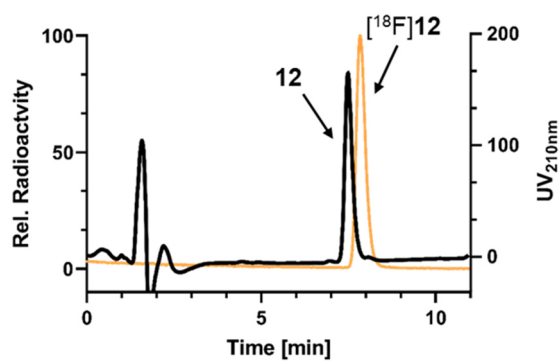
**Figure S3.** Preparative HPLC chromatogram of the crude reaction mixture of [<sup>18</sup>F]**12**; (A) radio signal, (B) UV at 210 nm; HPLC method A.



**Figure S4.** Preparative HPLC chromatogram of the product solution of [<sup>18</sup>F]**12** with cold reference **12** (1  $\mu$ g/ml); (A) radio signal, (B) UV at 210 nm; HPLC method A.

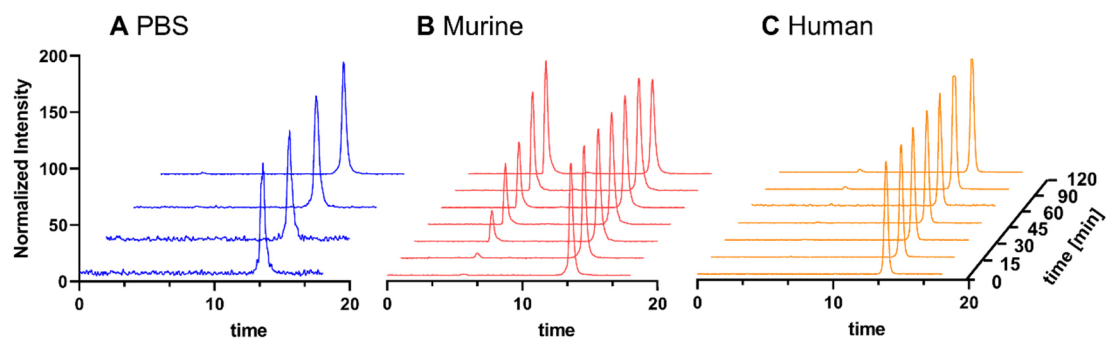


**Figure S5.** Analytical HPLC chromatogram of the product solution of  $[^{18}\text{F}]\mathbf{12}$ ; (A) radio signal, (B) UV at 210 nm; HPLC method B.



**Figure S6.** HPLC chromatogram of  $[^{18}\text{F}]\mathbf{12}$  (rel. radioactivity, orange) co-injected with reference compound  $\mathbf{12}$  (UV at 210 nm, black)

### Stability test



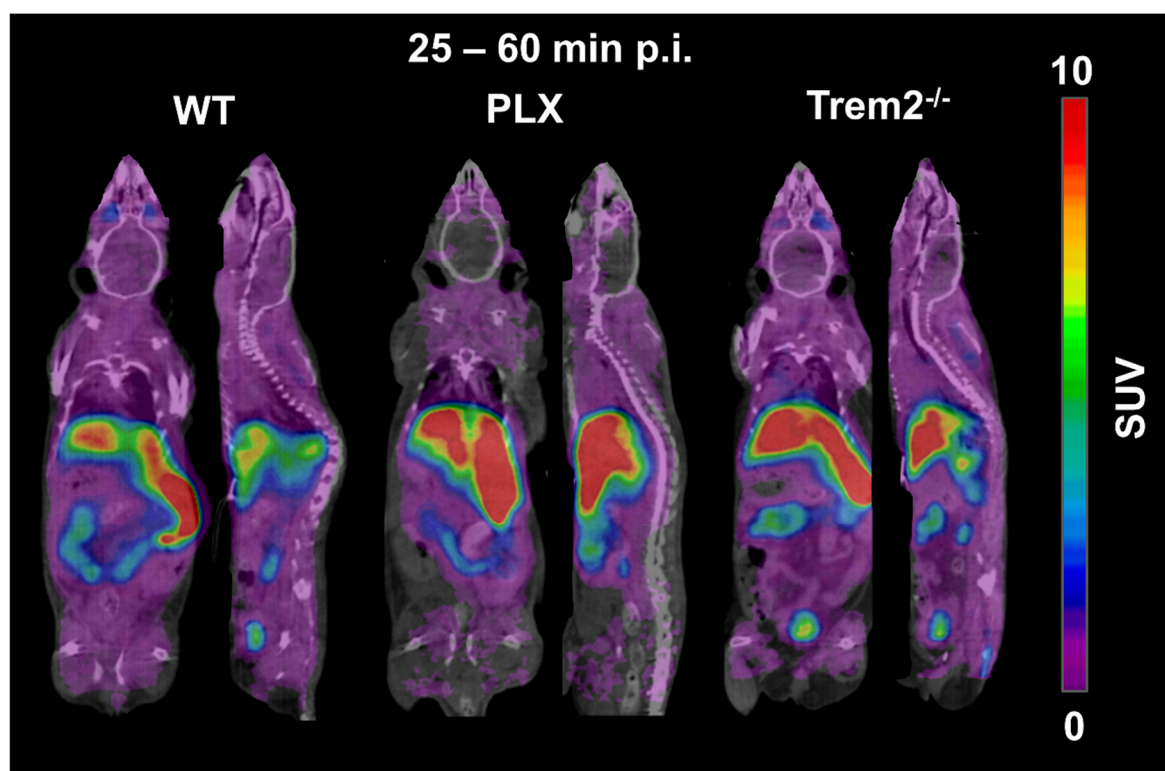
**Figure S7.** Stability of  $[^{18}\text{F}]\mathbf{12}$  in (A) PBS, (B) murine plasma and (C) human plasma measured by radio-HPLC.

## Biodistribution data

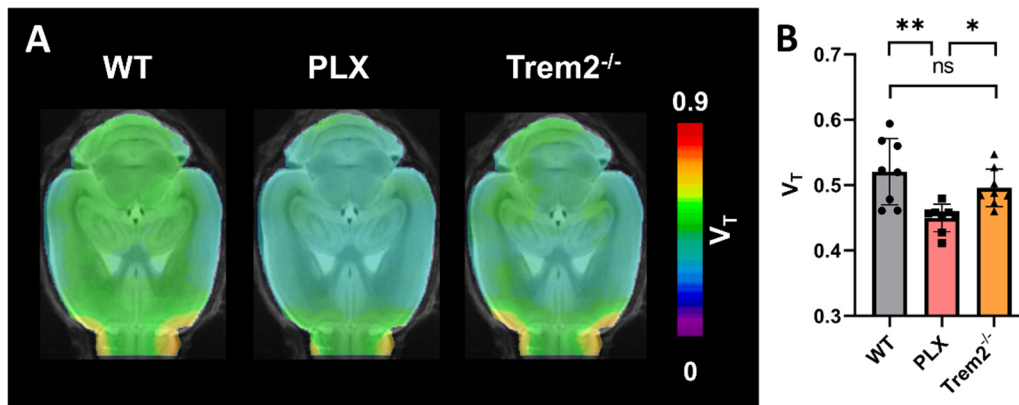
**Table S2.** Biodistribution of [ $^{18}\text{F}$ ]**12** in WT mice 60 min p.i., data expressed as decay-corrected mean %ID/g  $\pm$  SD.

Entry	Organ	n	%ID/g
1	Brain	12	$0.61 \pm 0.21$
2	Blood	12	$1.24 \pm 0.56$
3	Plasma	2	$1.58 \pm 0.78$
4	Heart	2	$1.30 \pm 0.51$
5	Lung	2	$0.68 \pm 0.15$
6	Spleen	2	$0.77 \pm 0.19$
7	Pancreas	2	$0.79 \pm 0.14$
8	Kidney	2	$5.51 \pm 1.08$
9	Liver	2	$19.62 \pm 4.75$

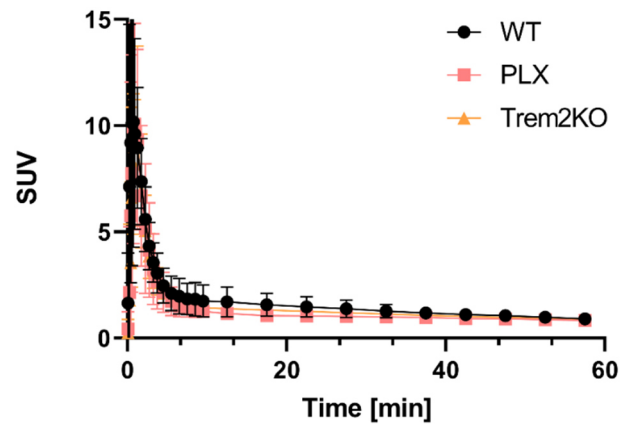
## PET Imaging



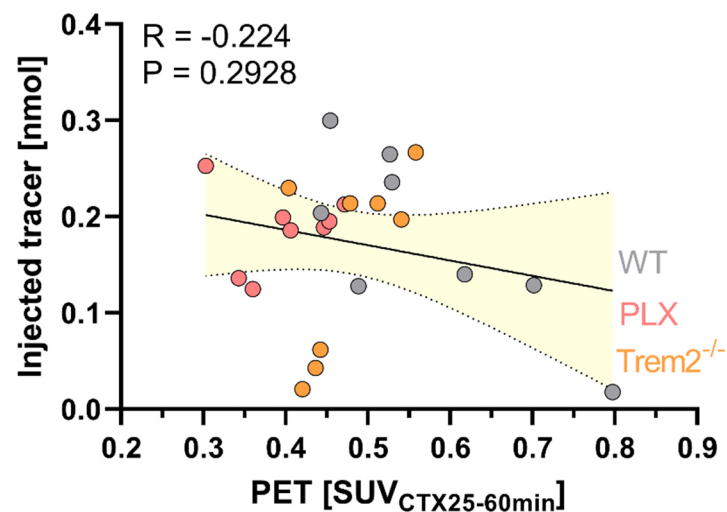
**Figure S8.** (A) Representative whole-body PET/CT image of WT, PLX and Trem2<sup>-/-</sup> mice, 25-60 min p.i.



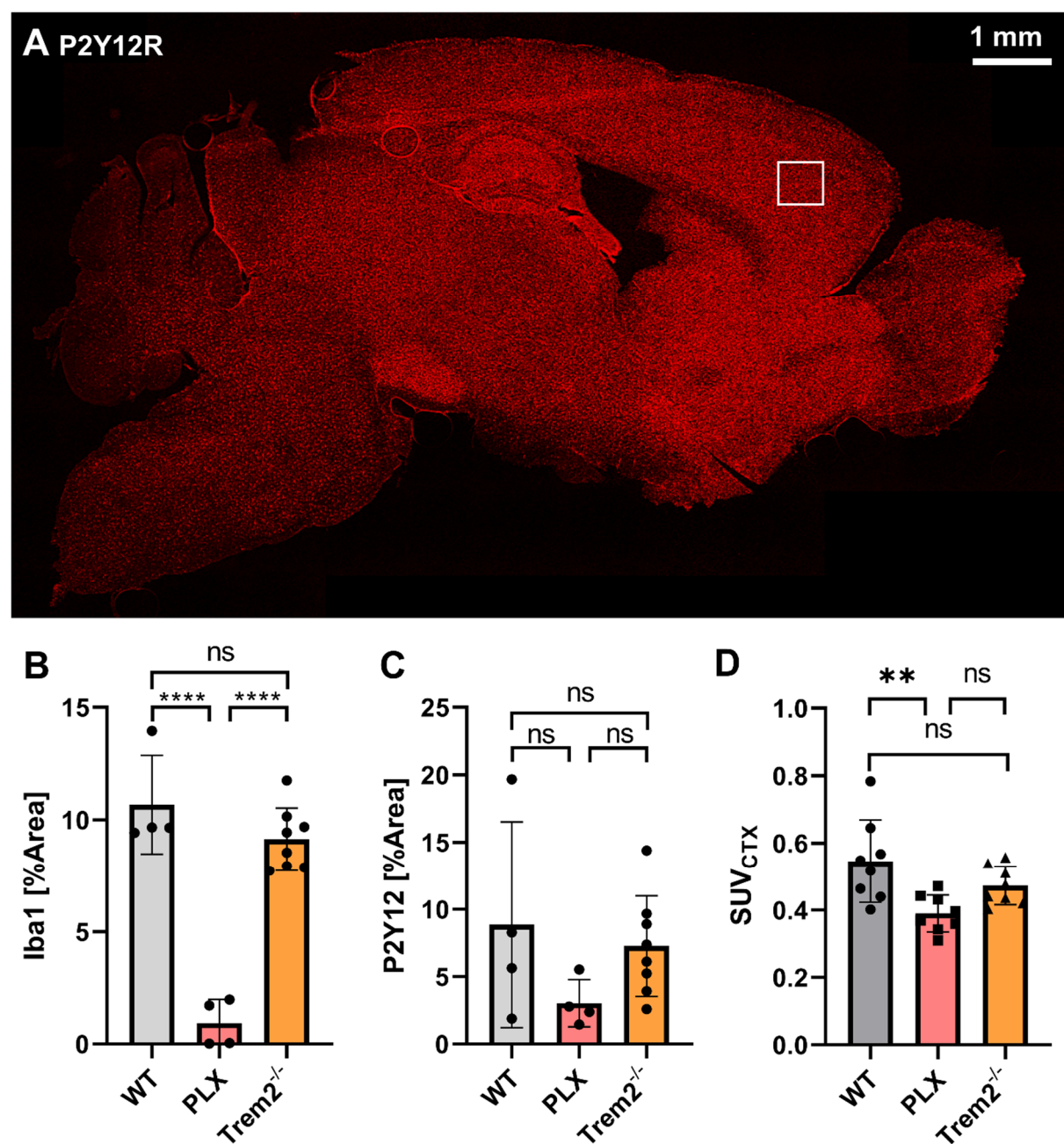
**Figure S9.** (A) Average  $V_T$  image of [ $^{18}\text{F}$ ]12 in WT, PLX and Trem2<sup>-/-</sup> mice (n = 8); (B) Tracer quantification of  $V_T$  whole brain, in WT, PLX treated and Trem2<sup>-/-</sup> mice. One-way ANOVA/Tukey's multiple comparisons test ( $F = 8.024$ ,  $p = 0.0026$ ),  $p > 0.05$  (ns),  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*), mean  $\pm$  SD.



**Figure S10.** Average ventricle TAC of WT, PLX and Trem2<sup>-/-</sup> mice.



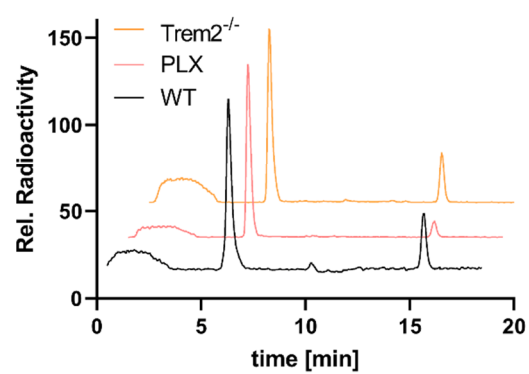
**Figure S11.** Correlation analysis of injected tracer amount and PET SUV; Linear regression,  $\alpha = 0.05$ , 95% CI.



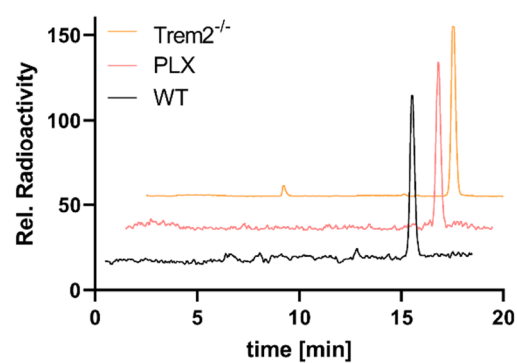
**Figure S12.** (A) IHC staining of the P2Y12R in a WT mouse; scale bar = 1 mm; the square represents the cortical area shown in the enlarged images in Figure 6A. (B) Iba1 immunofluorescence signal (%Area) in the CTX of WT, PLX and Trem2<sup>-/-</sup> mice. One-way ANOVA/Tukey's multiple comparisons test ( $F = 48.56$ ,  $p < 0.0001$ ); (C) P2Y12R immunofluorescence signal (%Area) in the CTX section of WT, PLX and Trem2<sup>-/-</sup> mice. One-way ANOVA/Tukey's multiple comparisons test ( $F = 1.724$ ,  $p = 0.2168$ ); (D) PET tracer quantification in the CTX of WT, PLX and Trem2<sup>-/-</sup> mice 25-60 min p.i. One-way ANOVA/Tukey's multiple comparisons test ( $F = 6.792$ ,  $p = 0.0053$ ),  $p > 0.05$  (ns),  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*),  $p \leq 0.001$  (\*\*\*),  $p \leq 0.0001$  (\*\*\*\*), mean  $\pm$  SD.

## Metabolite studies

### A Plasma



### B Brain



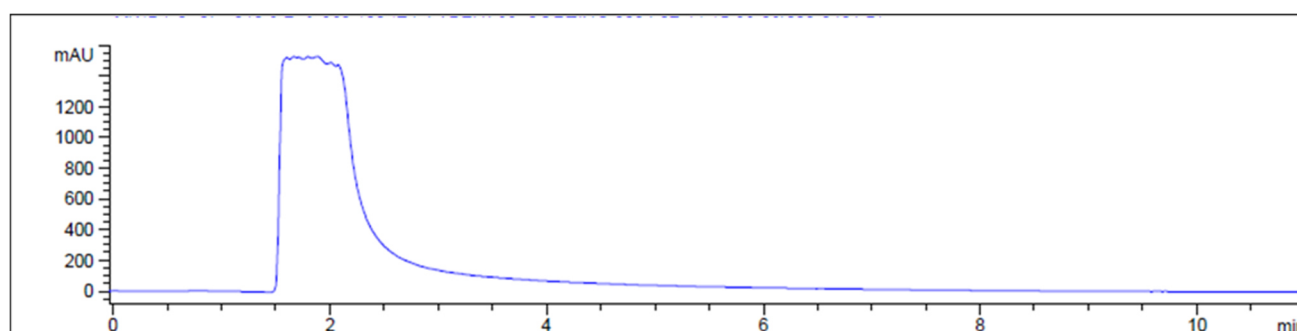
**Figure S13.** (A) Representative radio-HPLC chromatograms of plasma samples of WT, PLX and Trem2<sup>-/-</sup> mice 60 min p.i.; (B) Representative radio-HPLC chromatograms of brain samples of WT, PLX and Trem2<sup>-/-</sup> mice 60 min p.i.

## Representative HPLC Spectra for test compounds.

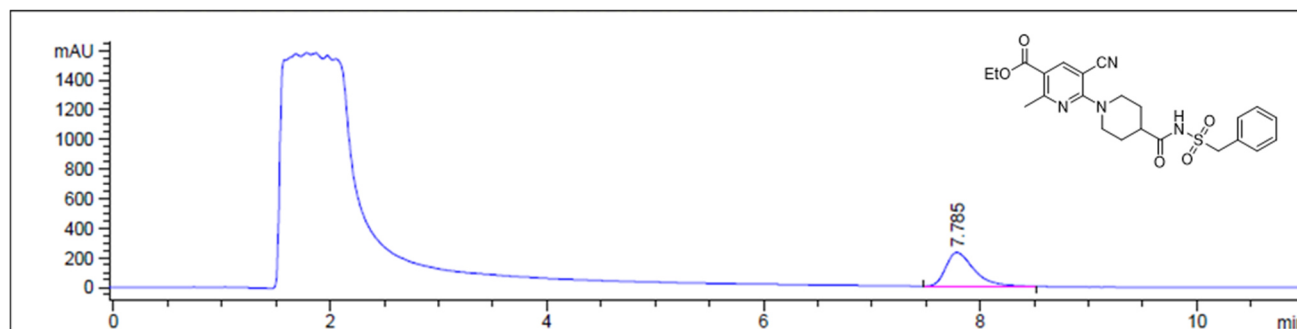
**HPLC Method C:** Luna 5  $\mu$ m PFP(2) 100 Å, LC Column 250 x 4.6 mm, 25  $\mu$ l injection, 1 mM test compound, solution in DMSO, 0 – 4 min 48% H<sub>2</sub>O (0.1% TFA), 52% MeCN (0.1% TFA), 2 ml/min; 4 – 11 min 40% H<sub>2</sub>O (0.1% TFA), 60% MeCN (0.1% TFA), 2 ml/min.

**HPLC Method D:** Luna 5  $\mu$ m PFP(2) 100 Å, LC Column 250 x 4.6 mm, 25  $\mu$ l injection, 1 mM test compound, solution in DMSO, 0 – 4 min 65% H<sub>2</sub>O (0.1% TFA), 35% MeCN (0.1% TFA), 2 ml/min; 4 – 11 min 50% H<sub>2</sub>O (0.1% TFA), 50% MeCN (0.1% TFA), 2 ml/min.

The purity of compounds for *in vitro* and *in vivo* testing was confirmed to be  $\geq 95\%$ .



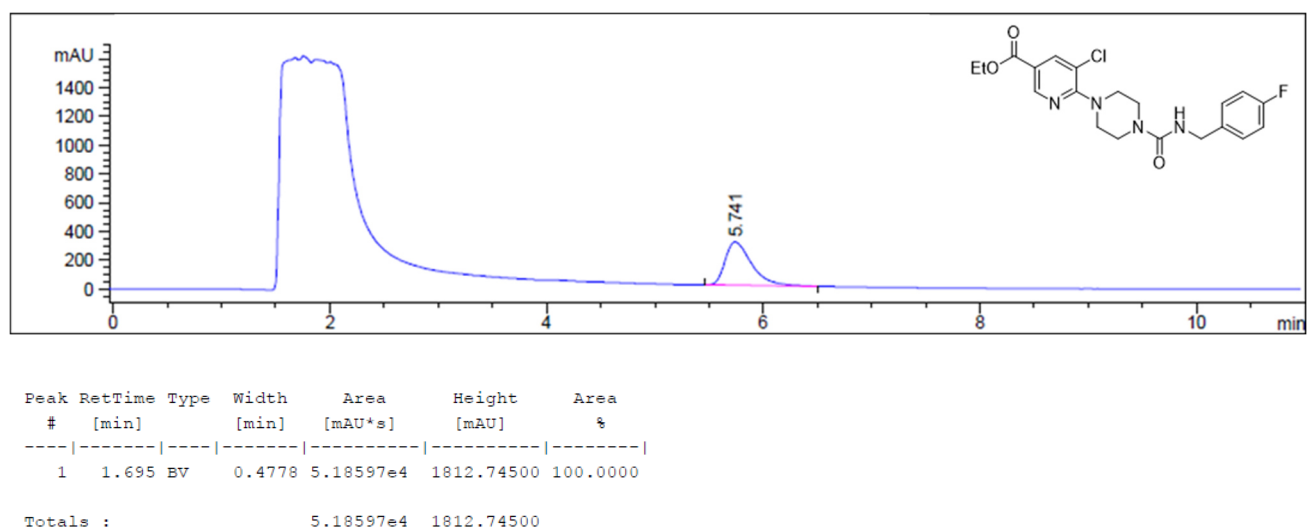
**Figure S14.** Analytical HPLC chromatogram of DMSO blank (UV 210 nm, HPLC method C).



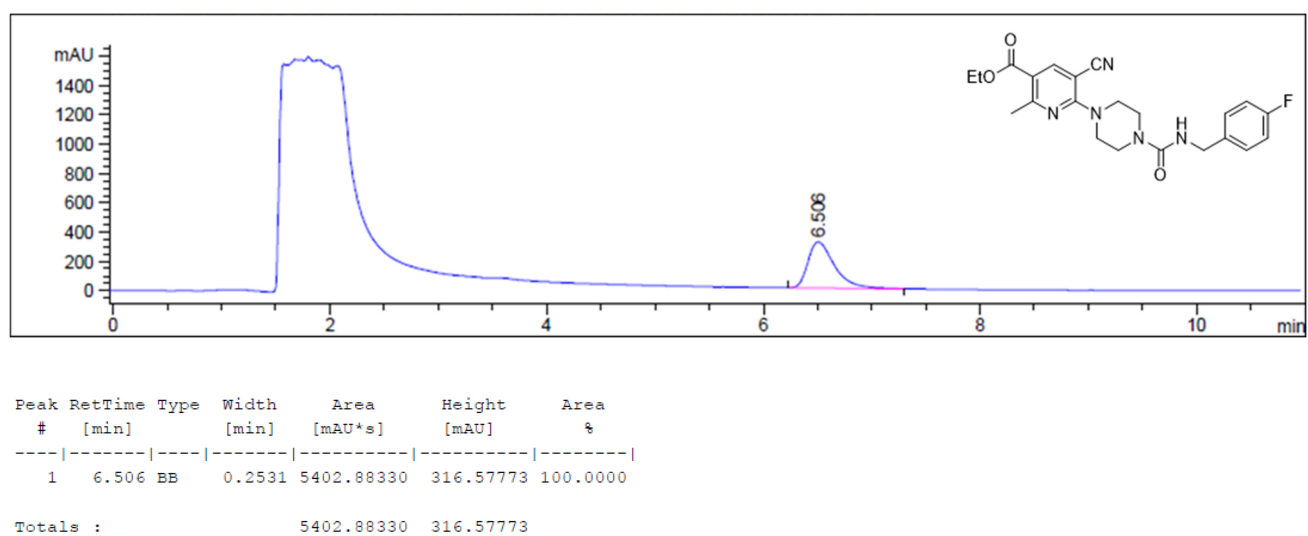
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.785	BB	0.2813	4176.67480	228.45567	100.0000

Totals : 4176.67480 228.45567

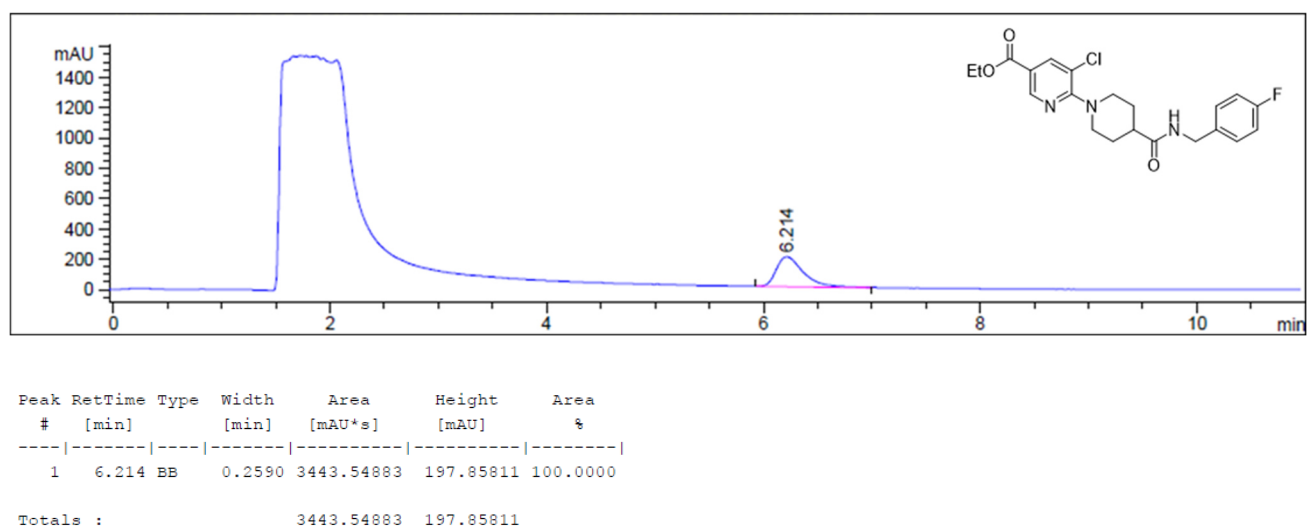
**Figure S15.** Analytical HPLC chromatogram of compound **5** AZD1283 (UV 210 nm, HPLC method C).



**Figure S16.** Analytical HPLC chromatogram of compound **6** (UV 210 nm, HPLC method C).

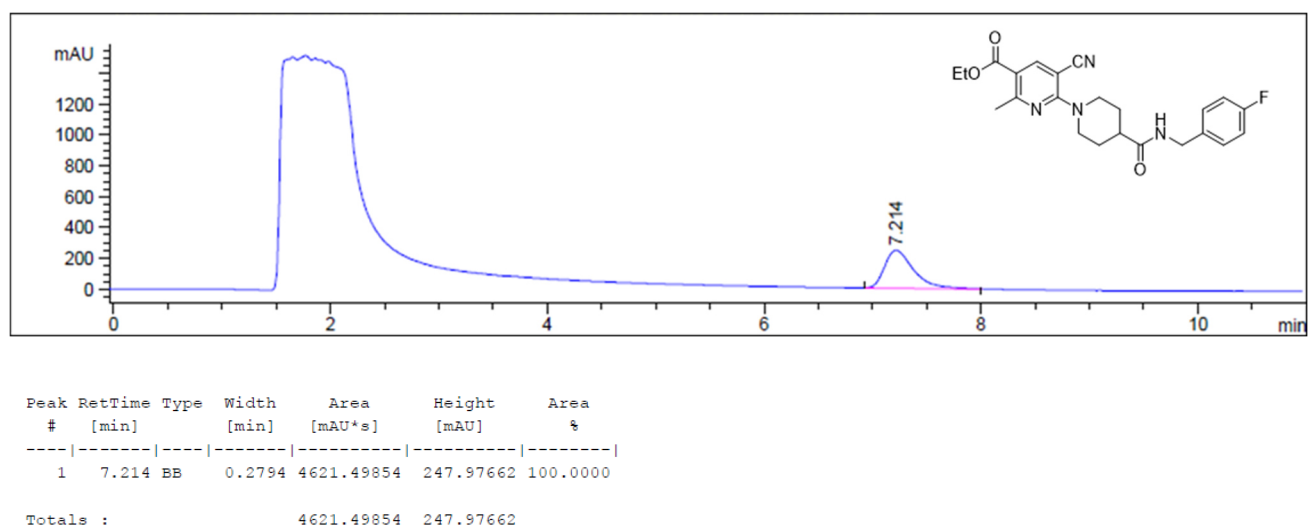


**Figure S17.** Analytical HPLC chromatogram of compound **7** (UV 210 nm, HPLC method C).

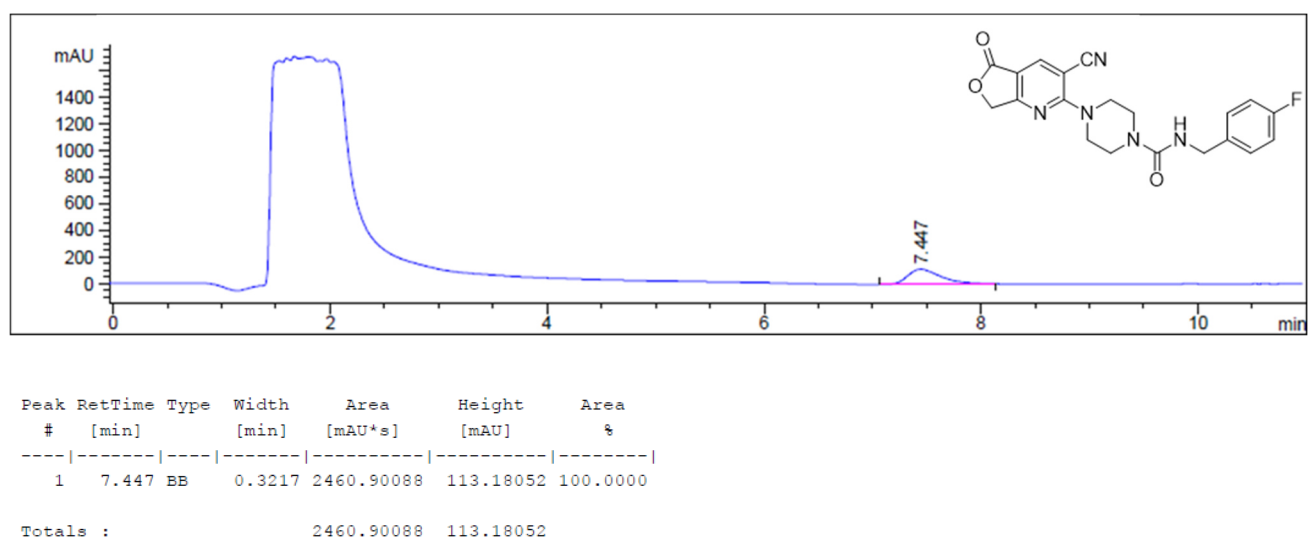


**Figure S18.** Analytical HPLC chromatogram of compound **10** (UV 210 nm, HPLC method C).

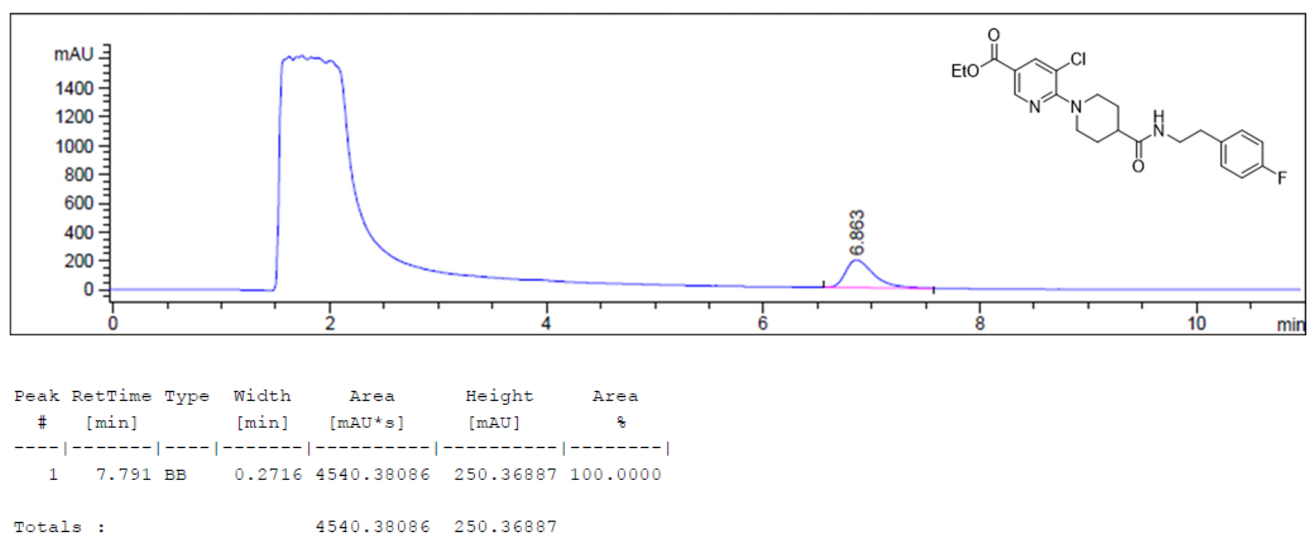




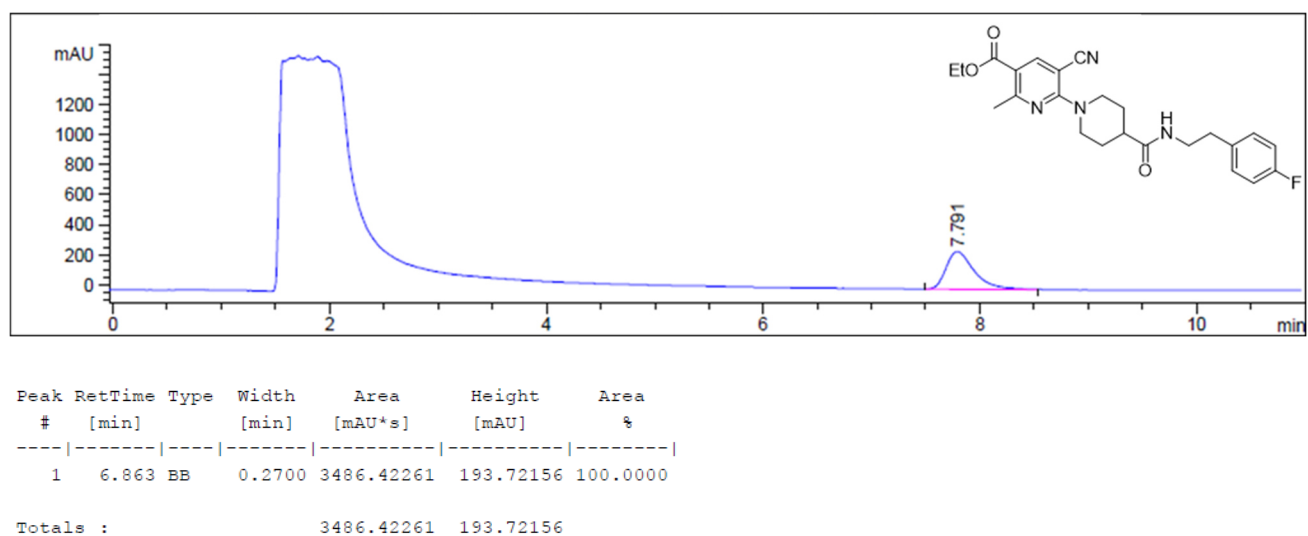
**Figure S19.** Analytical HPLC chromatogram of compound **12** (UV 210 nm, HPLC method C).



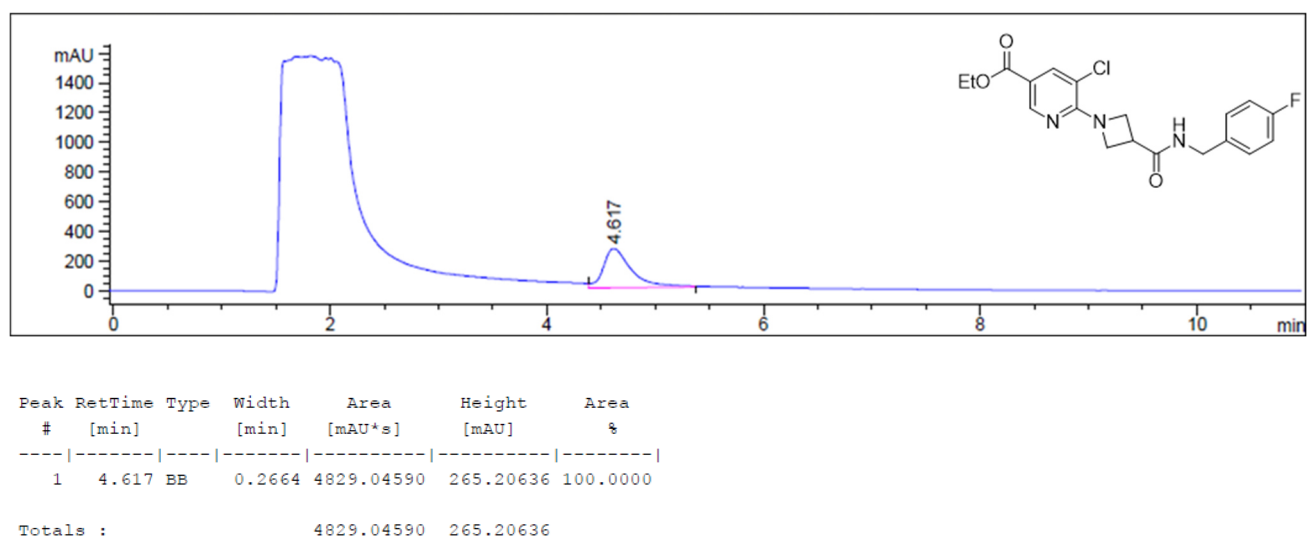
**Figure S20.** Analytical HPLC chromatogram of compound **14** (UV 210 nm, HPLC method D).



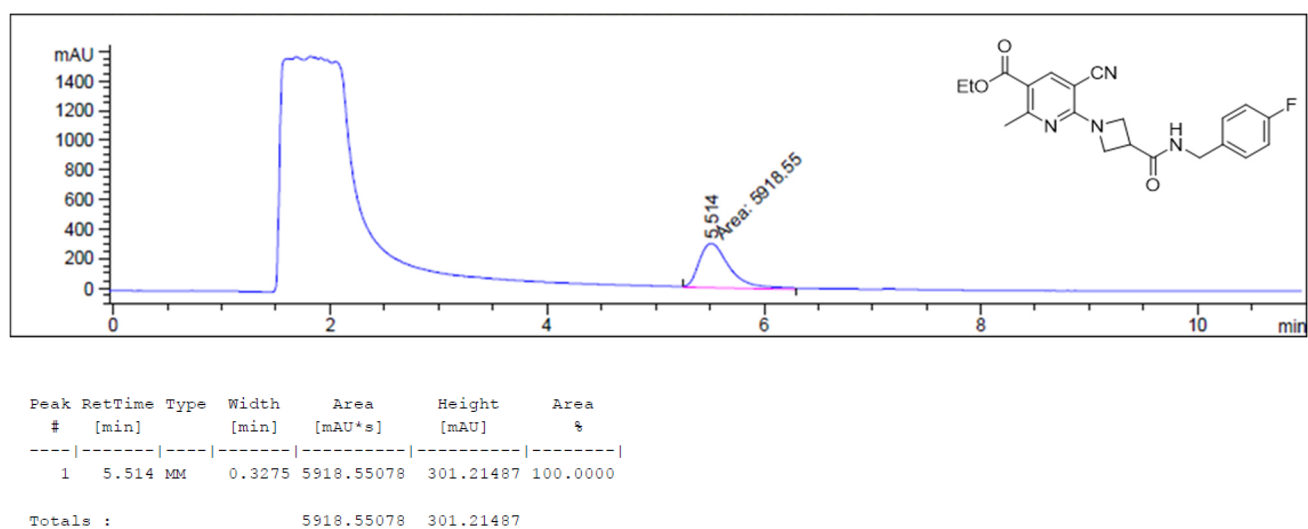
**Figure S21.** Analytical HPLC chromatogram of compound **16** (UV 210 nm, HPLC method C).



**Figure S22.** Analytical HPLC chromatogram of compound **18** (UV 210 nm, HPLC method C).



**Figure S23.** Analytical HPLC chromatogram of compound **21** (UV 210 nm, HPLC method C).

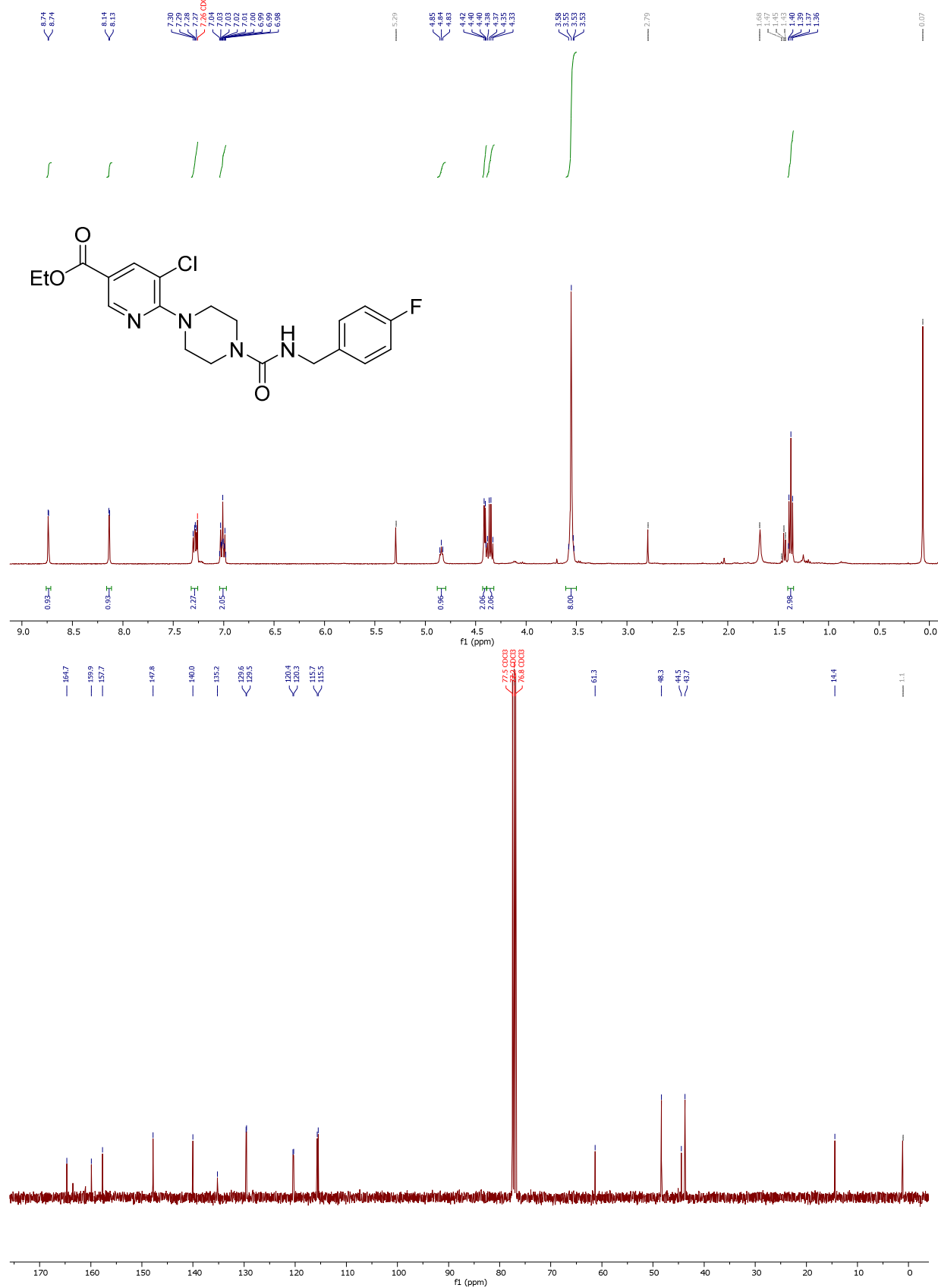


**Figure S24.** Analytical HPLC chromatogram of compound **22** (UV 210 nm, HPLC method C).

## NMR spectra

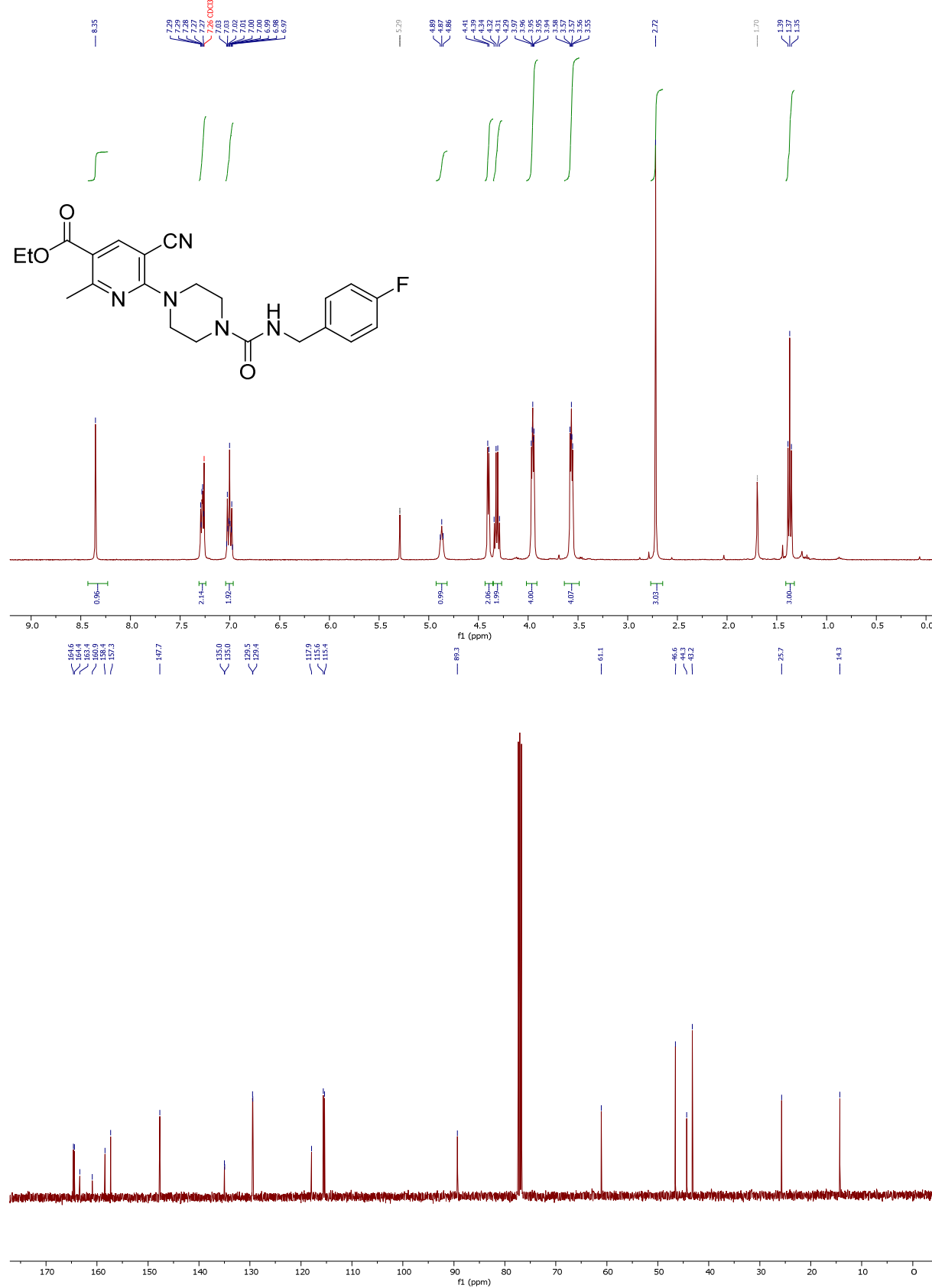
*Ethyl 5-chloro-6-(4-((4-fluorobenzyl)carbamoyl)piperazin-1-yl)nicotinate (6)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



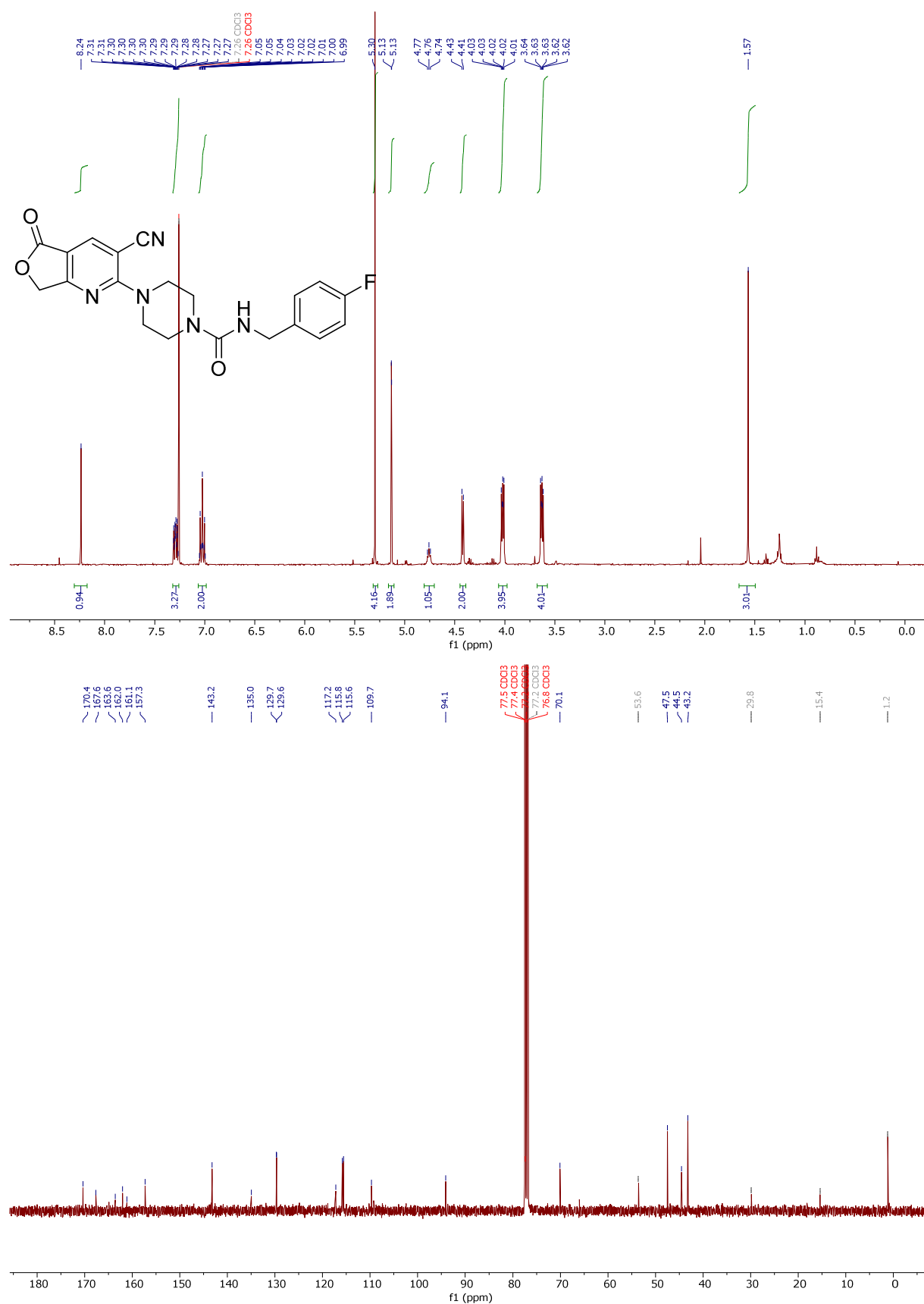
*Ethyl 5-cyano-6-((4-(4-fluorobenzyl)carbamoyl)piperazin-1-yl)-2-methylnicotinate (7)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



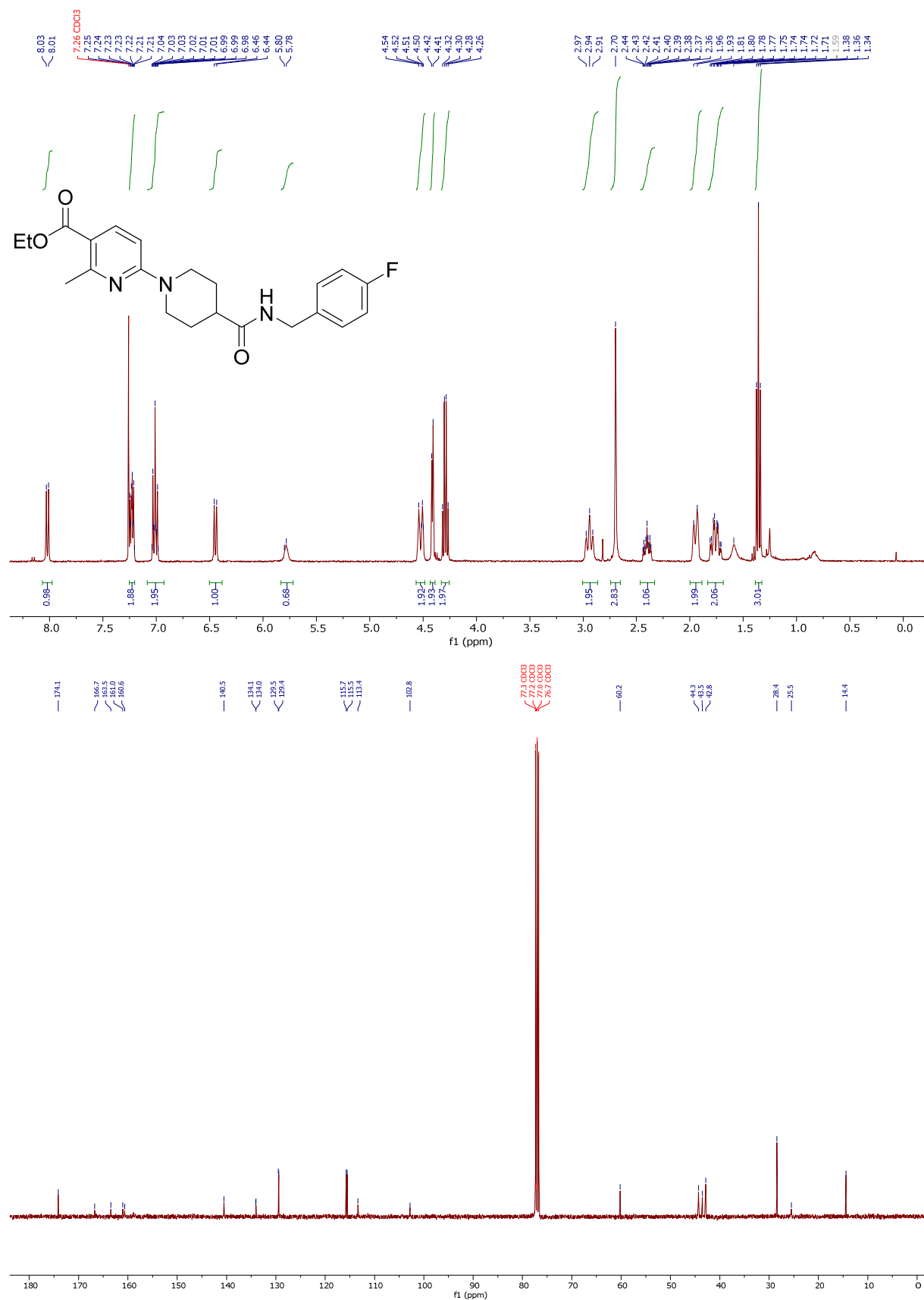
*1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-(4-fluorobenzyl)piperidine-4-carboxamide (8)*

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)



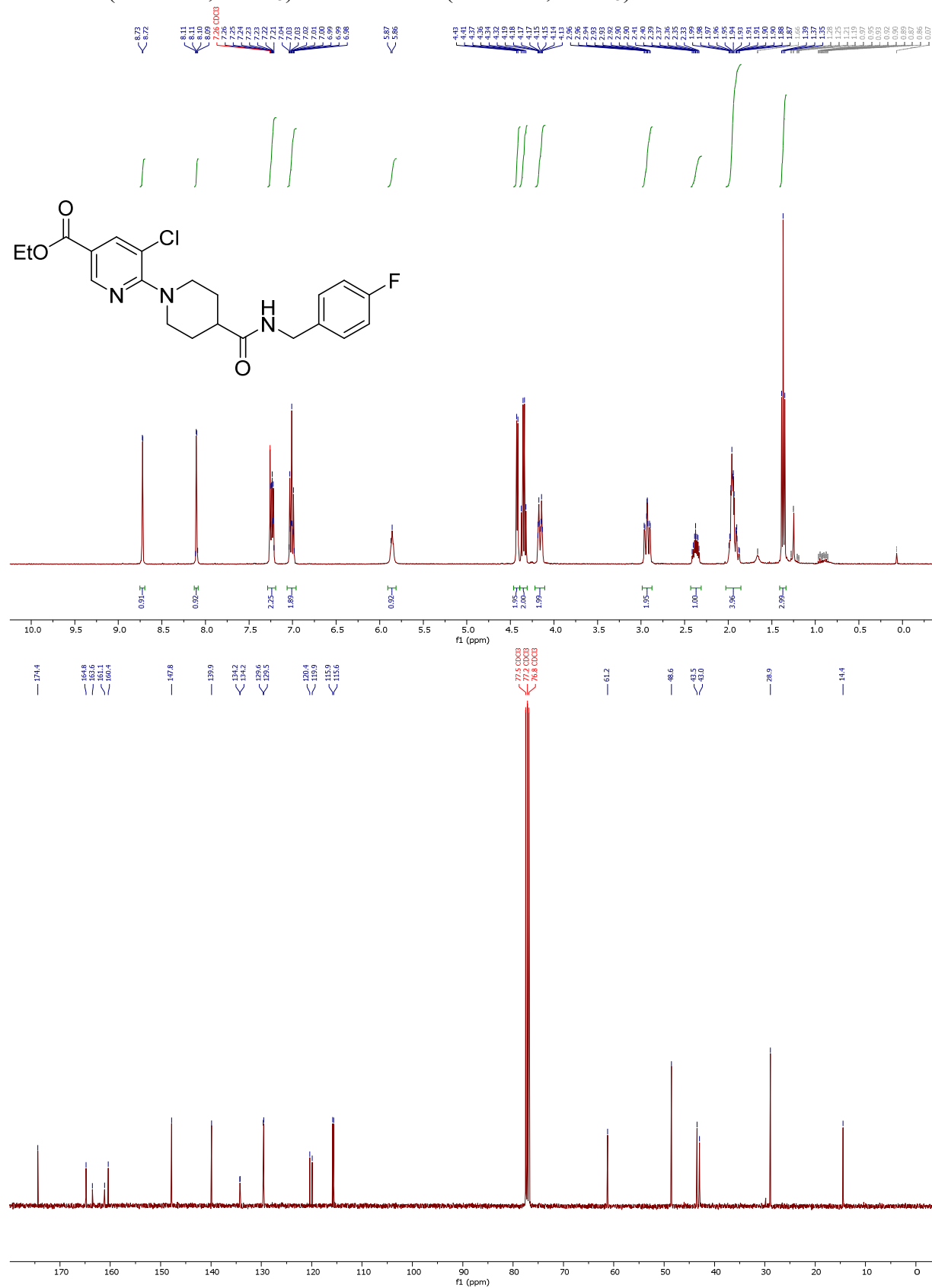
*Ethyl 6-((4-(4-fluorobenzyl)carbamoyl)piperidin-1-yl)-2-methylnicotinate (9)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



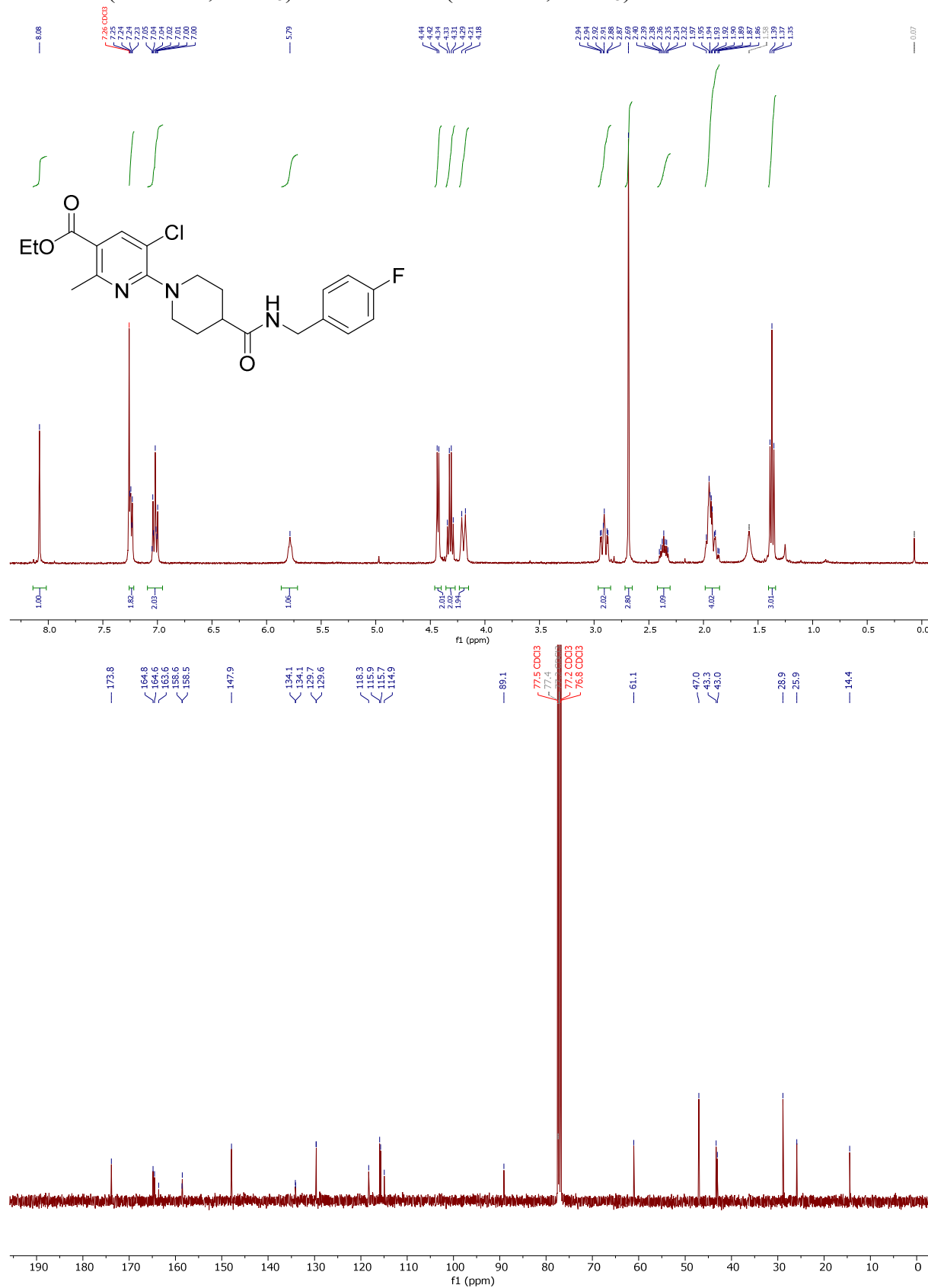
*Ethyl 5-chloro-6-(4-((4-fluorobenzyl)carbamoyl)piperidin-1-yl)nicotinate (10)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



*Ethyl 5-chloro-6-(4-((4-fluorobenzyl)carbamoyl)piperidin-1-yl)-2-methylnicotinate (11)*

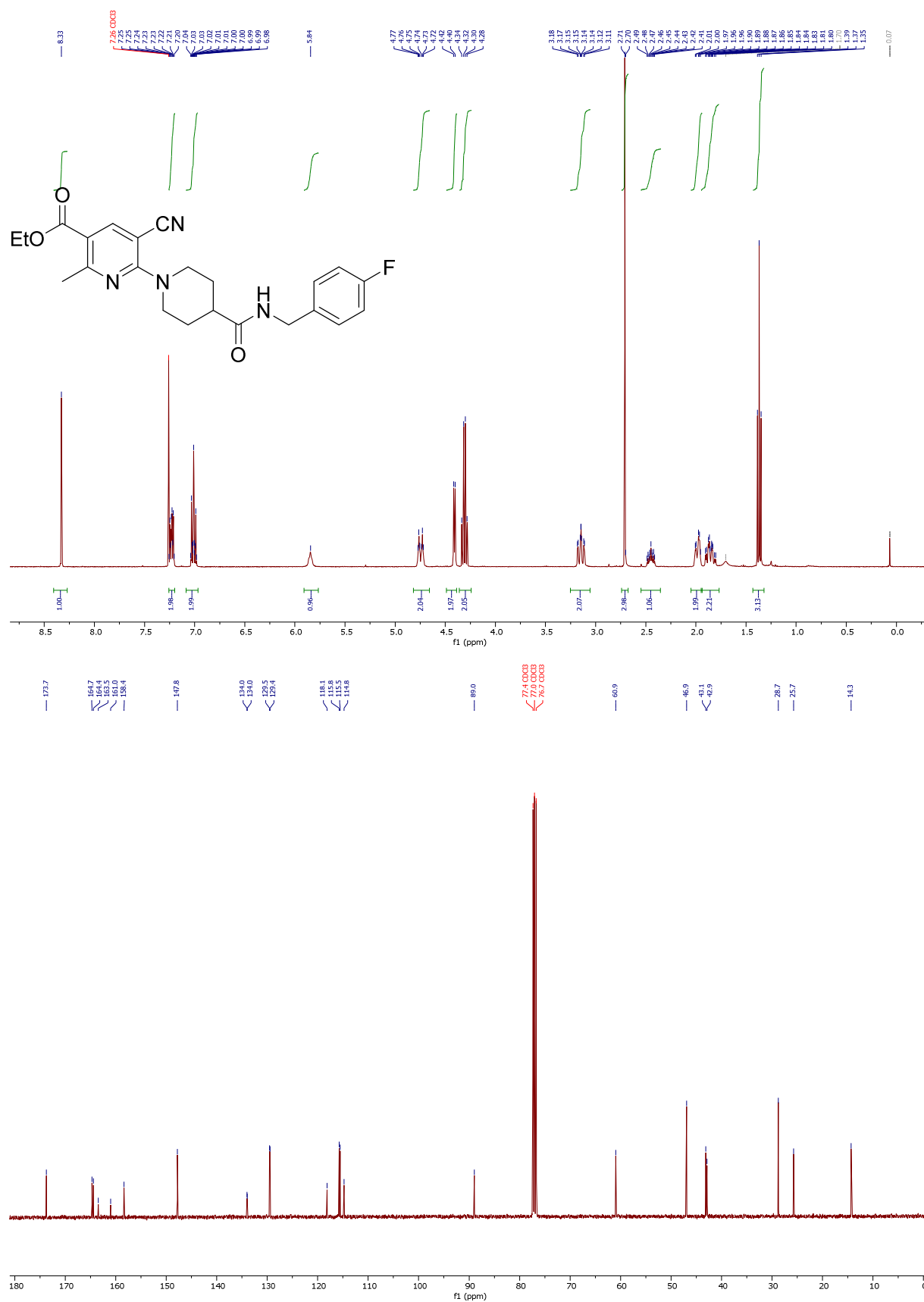
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )





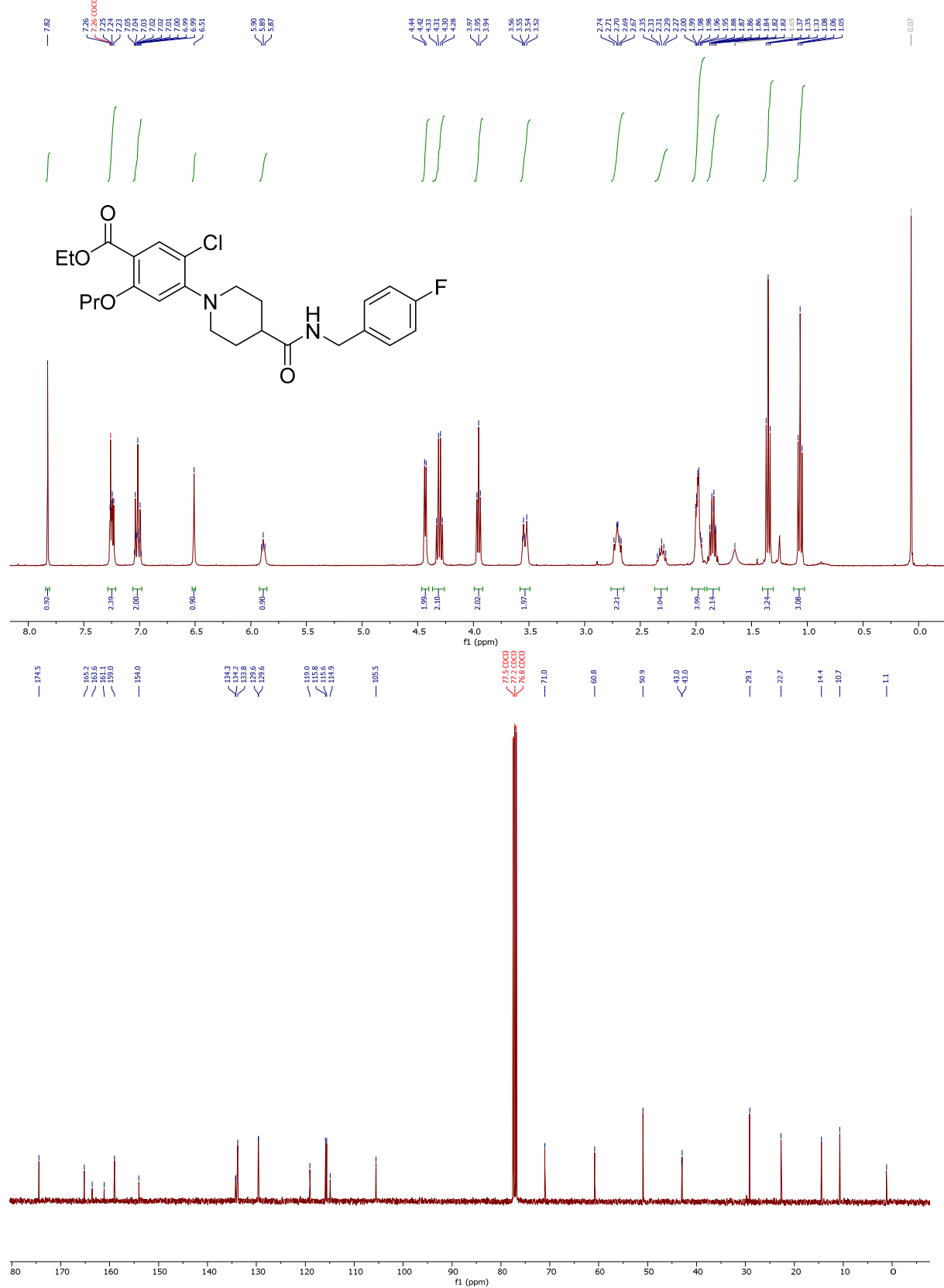
*Ethyl 5-cyano-6-((4-(4-fluorobenzyl)carbamoyl)piperidin-1-yl)-2-methylnicotinate (12)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



*Ethyl 5-chloro-4-(4-((4-fluorobenzyl)carbamoyl)piperidin-1-yl)-2-propoxybenzoate (13)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )

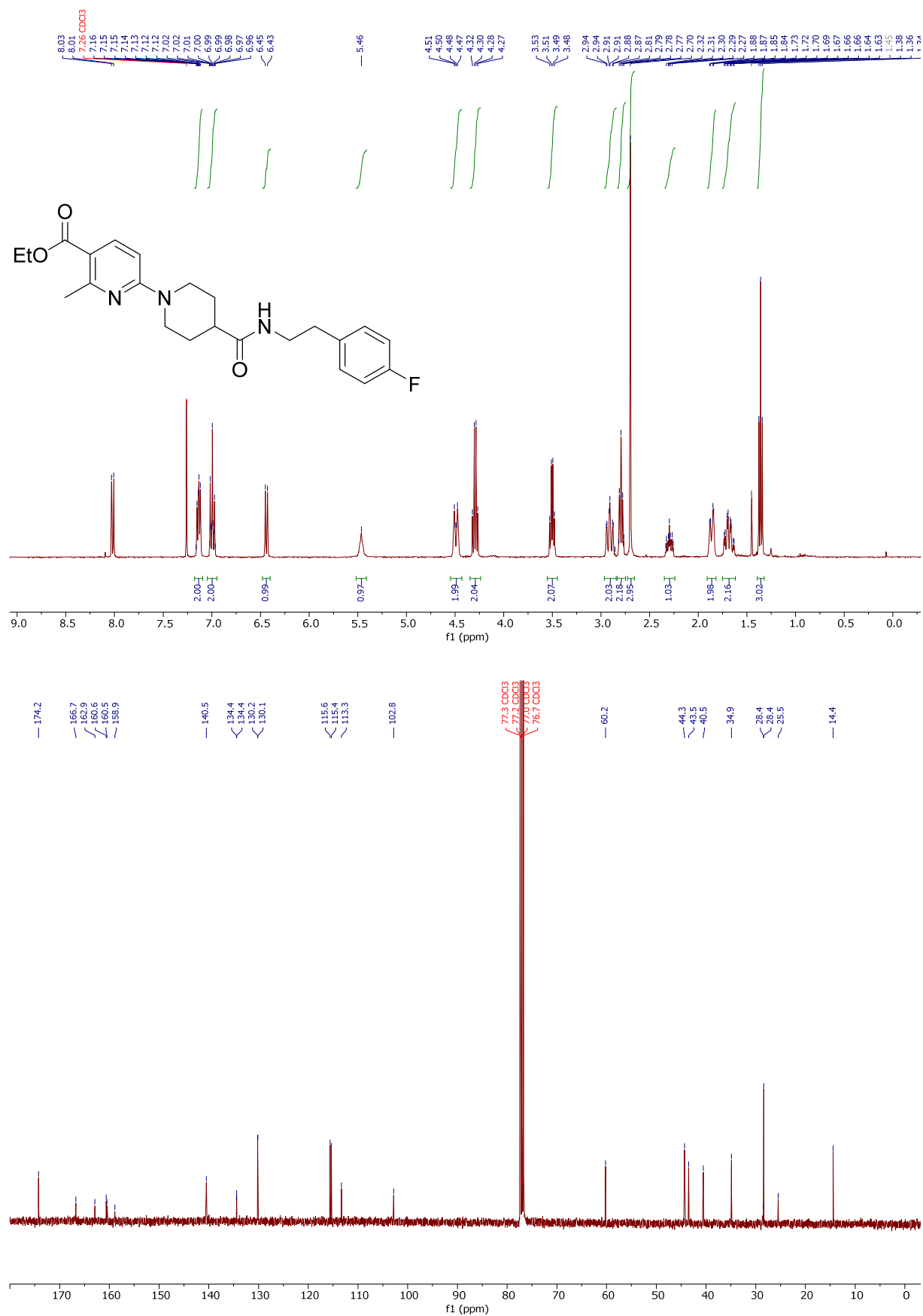


**<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>) and **<sup>13</sup>C NMR** (100 MHz, DMSO-*d*<sub>6</sub>)



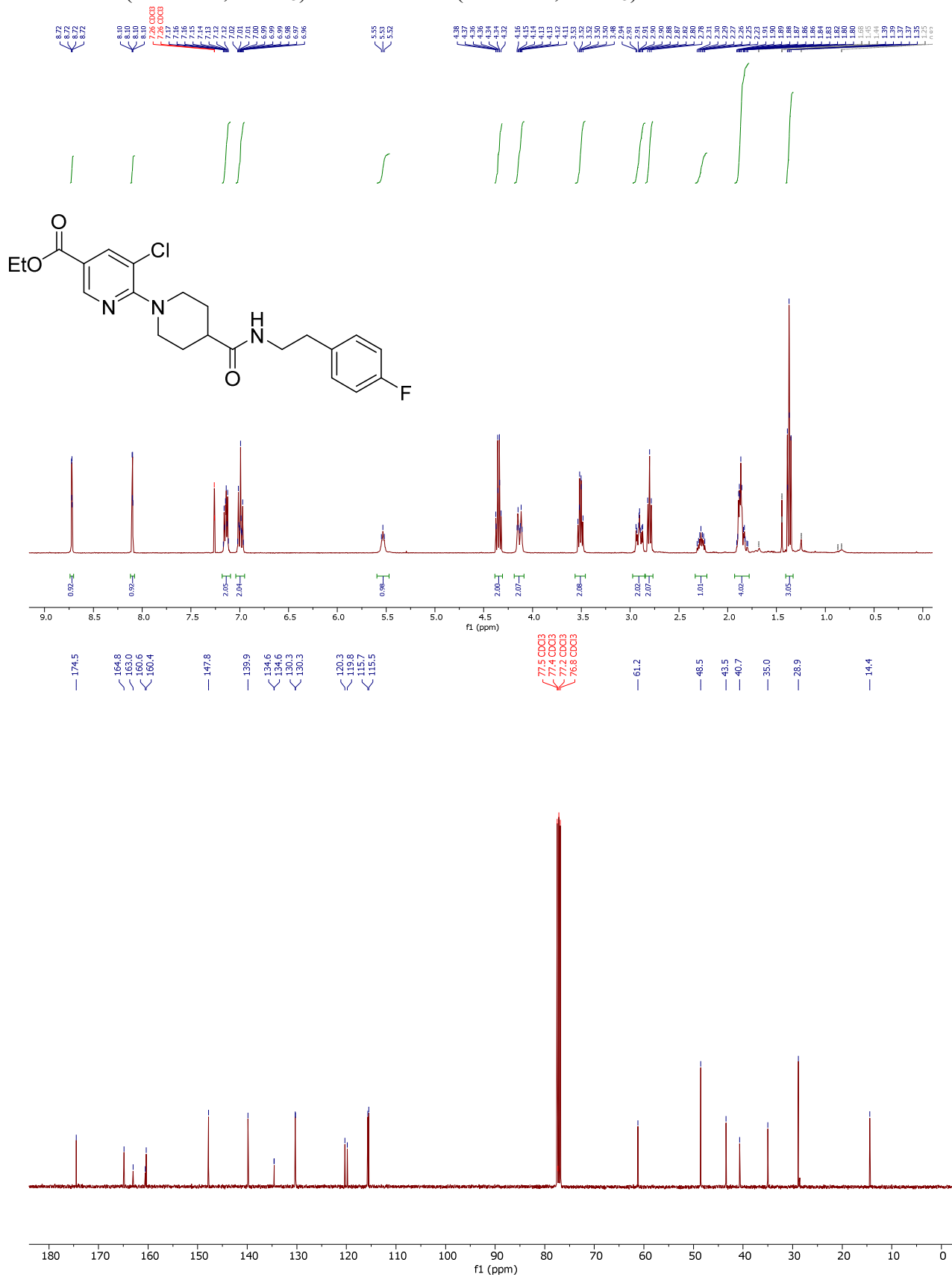
*Ethyl 6-((4-(4-fluorophenethyl)carbamoyl)piperidin-1-yl)-2-methylnicotinate (15)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



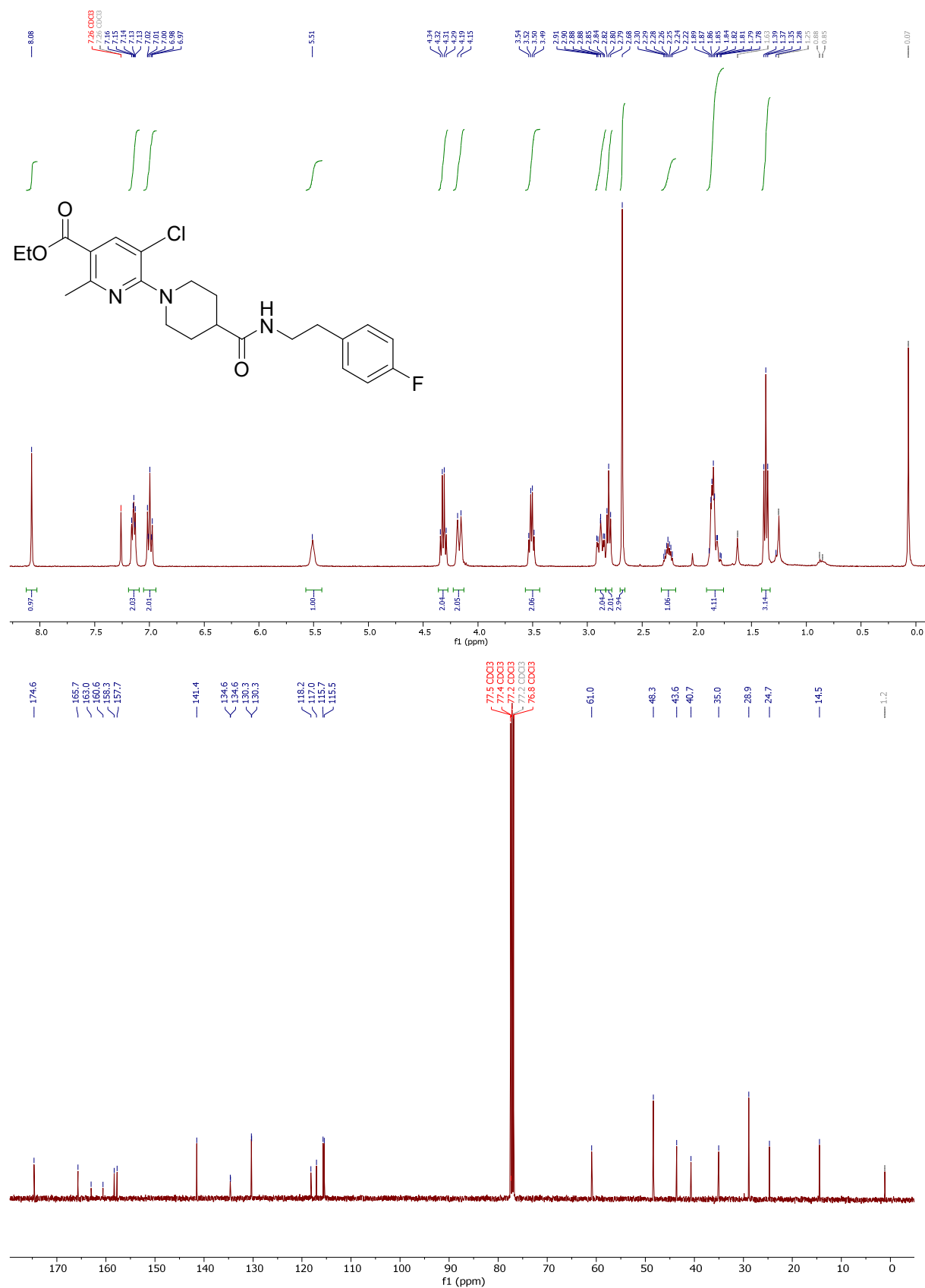
*Ethyl 5-chloro-6-(4-((4-fluorophenethyl)carbamoyl)piperidin-1-yl)nicotinate (16)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



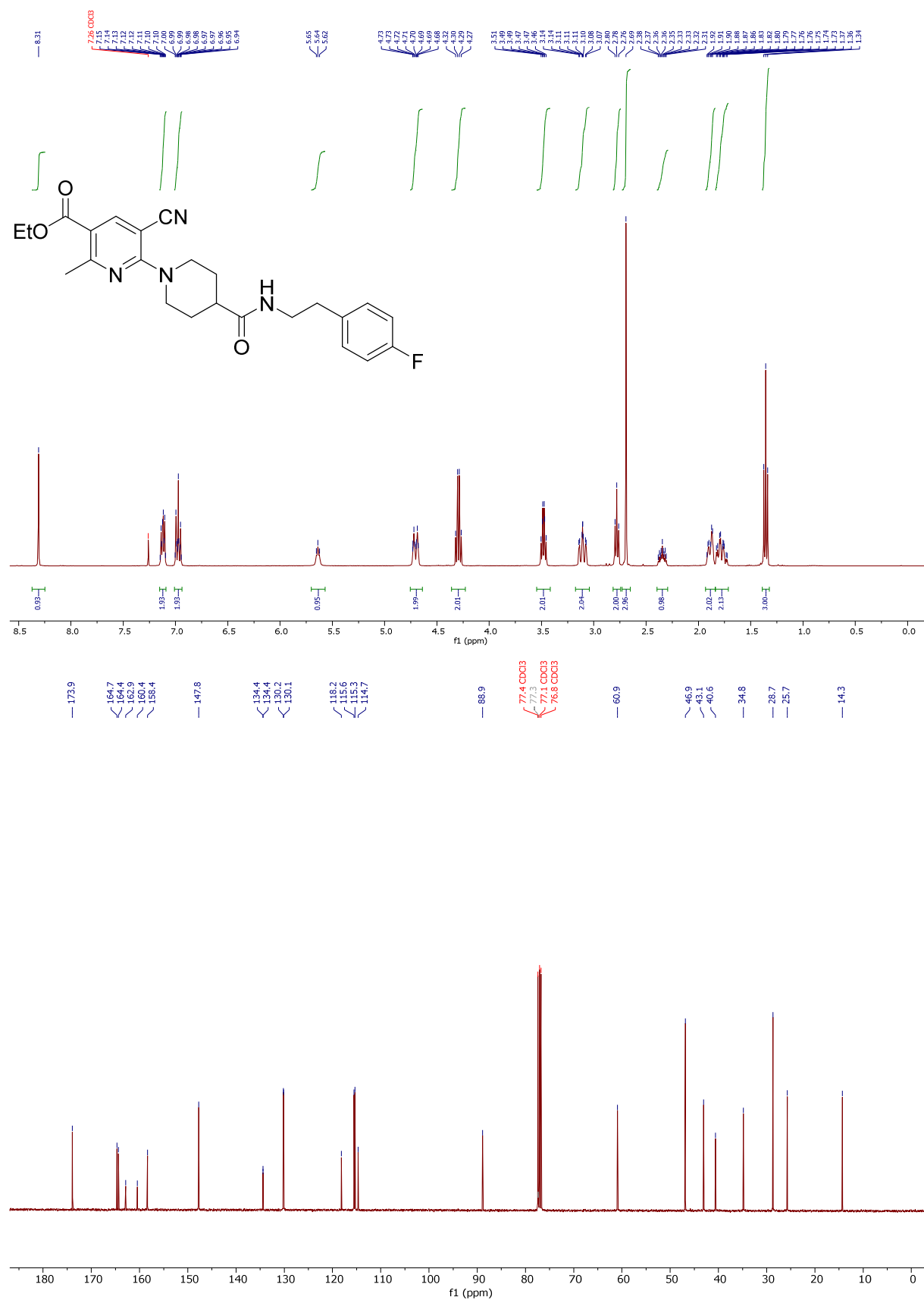
*Ethyl 5-chloro-6-(4-((4-fluorophenethyl)carbamoyl)piperidin-1-yl)-2-methylnicotinate (17)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



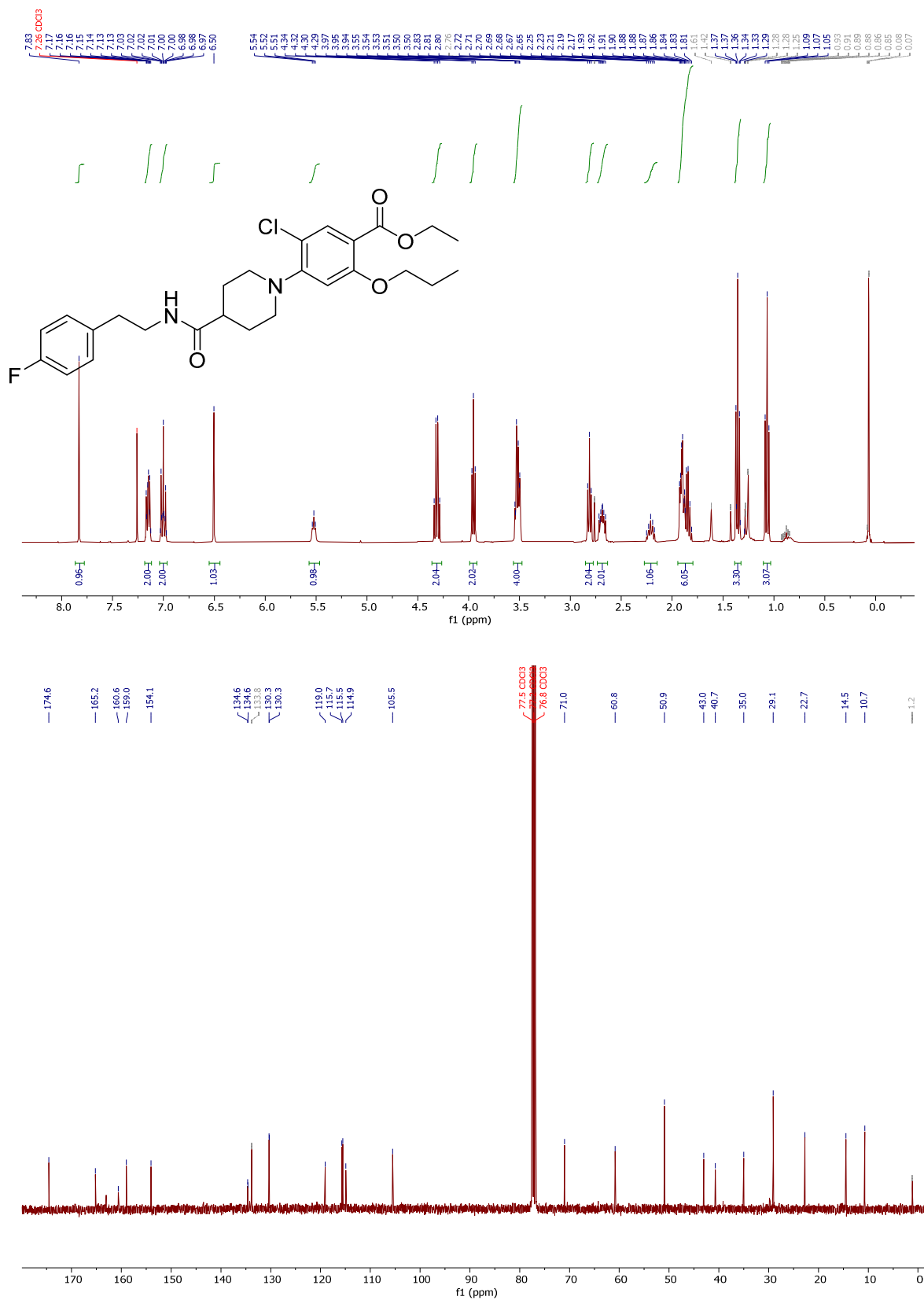
*Ethyl 5-cyano-6-((4-((4-fluorophenethyl)carbamoyl)piperidin-1-yl)-2-methylnicotinate (18)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



*Ethyl 5-chloro-4-((4-(4-fluorophenethyl)carbamoyl)piperidin-1-yl)-2-propoxybenzoate (19)*

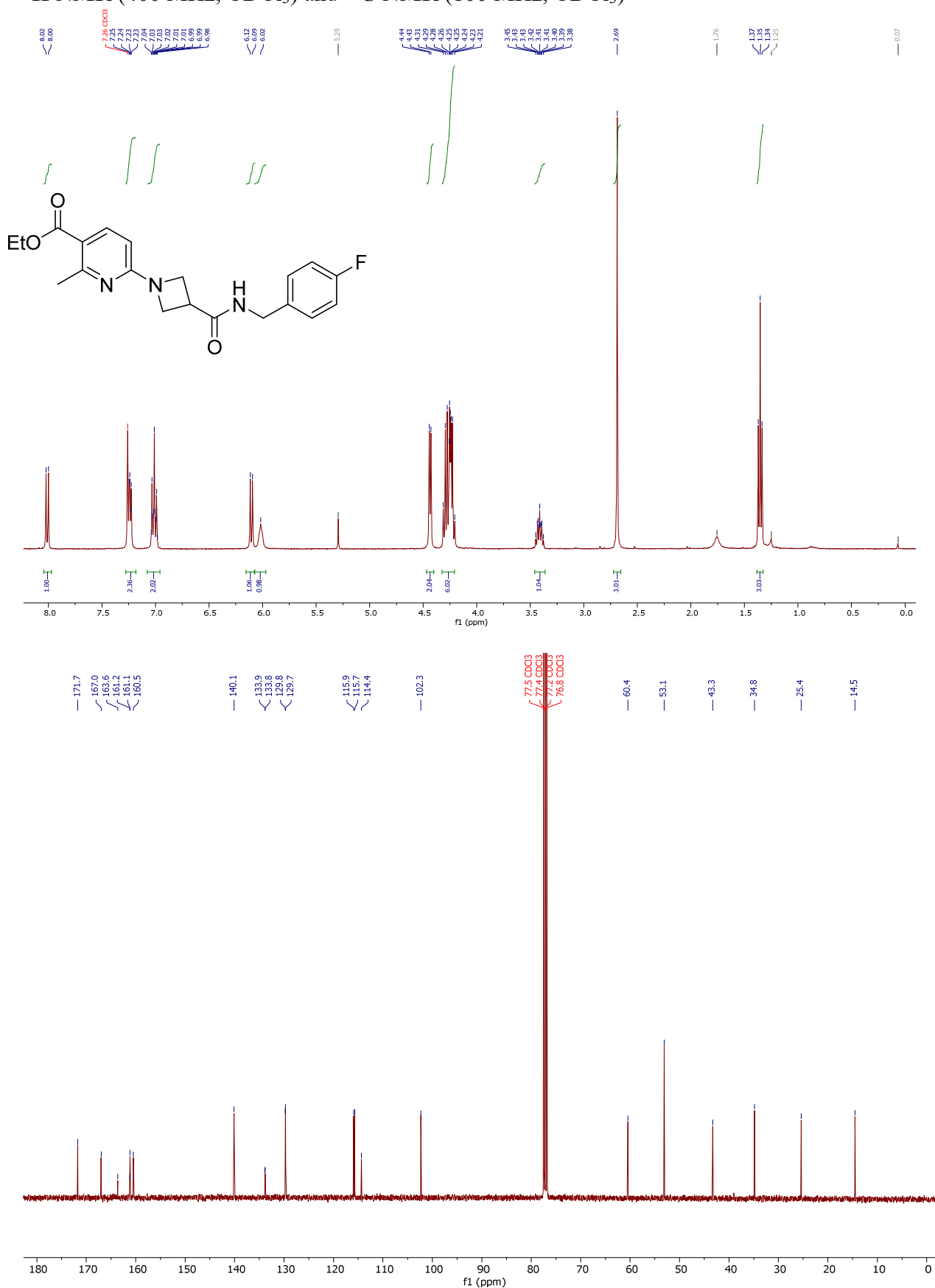
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )





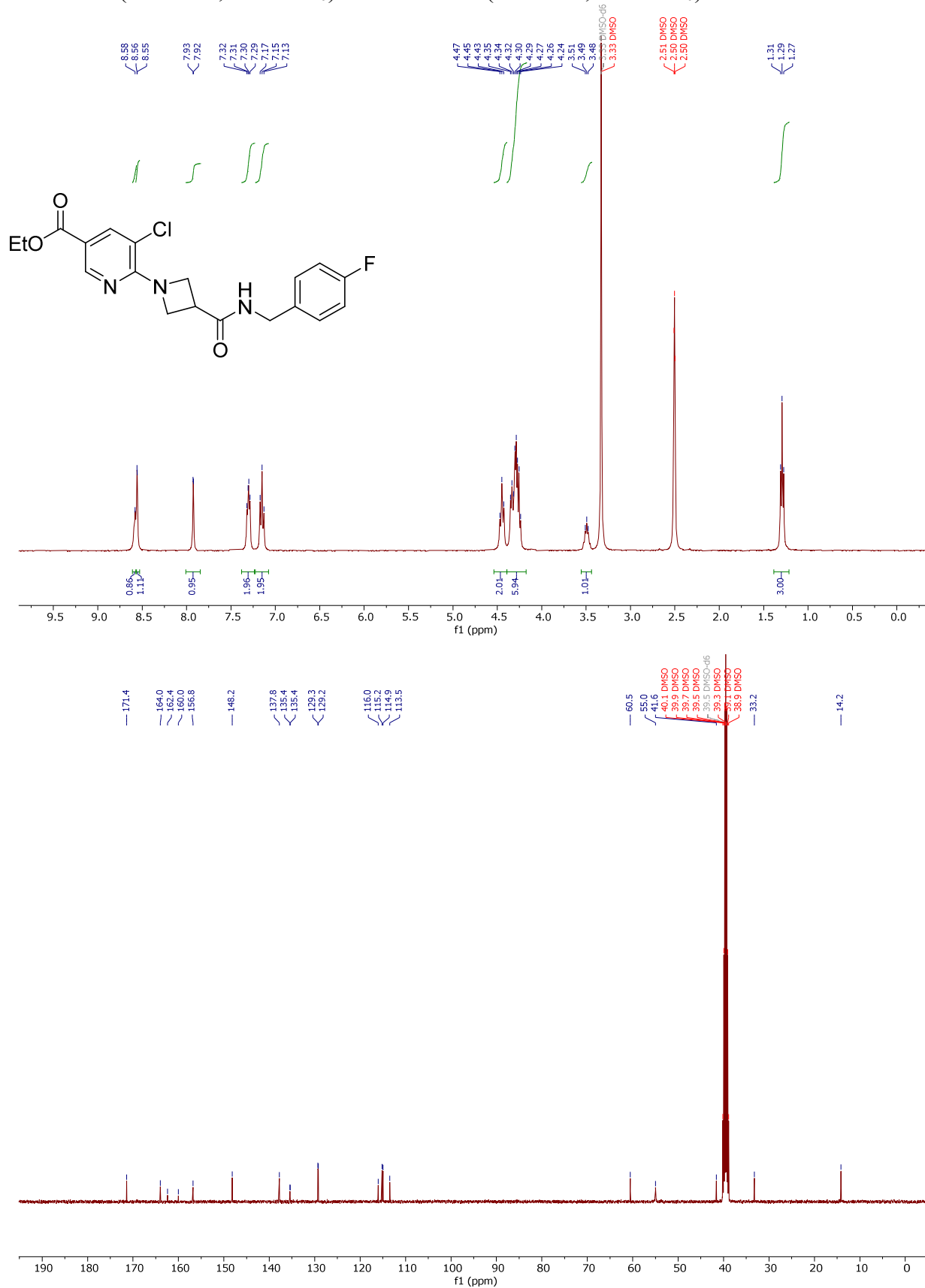
*tert*-Butyl 4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)carbamoyl)piperidine-1-carboxylate (20)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)

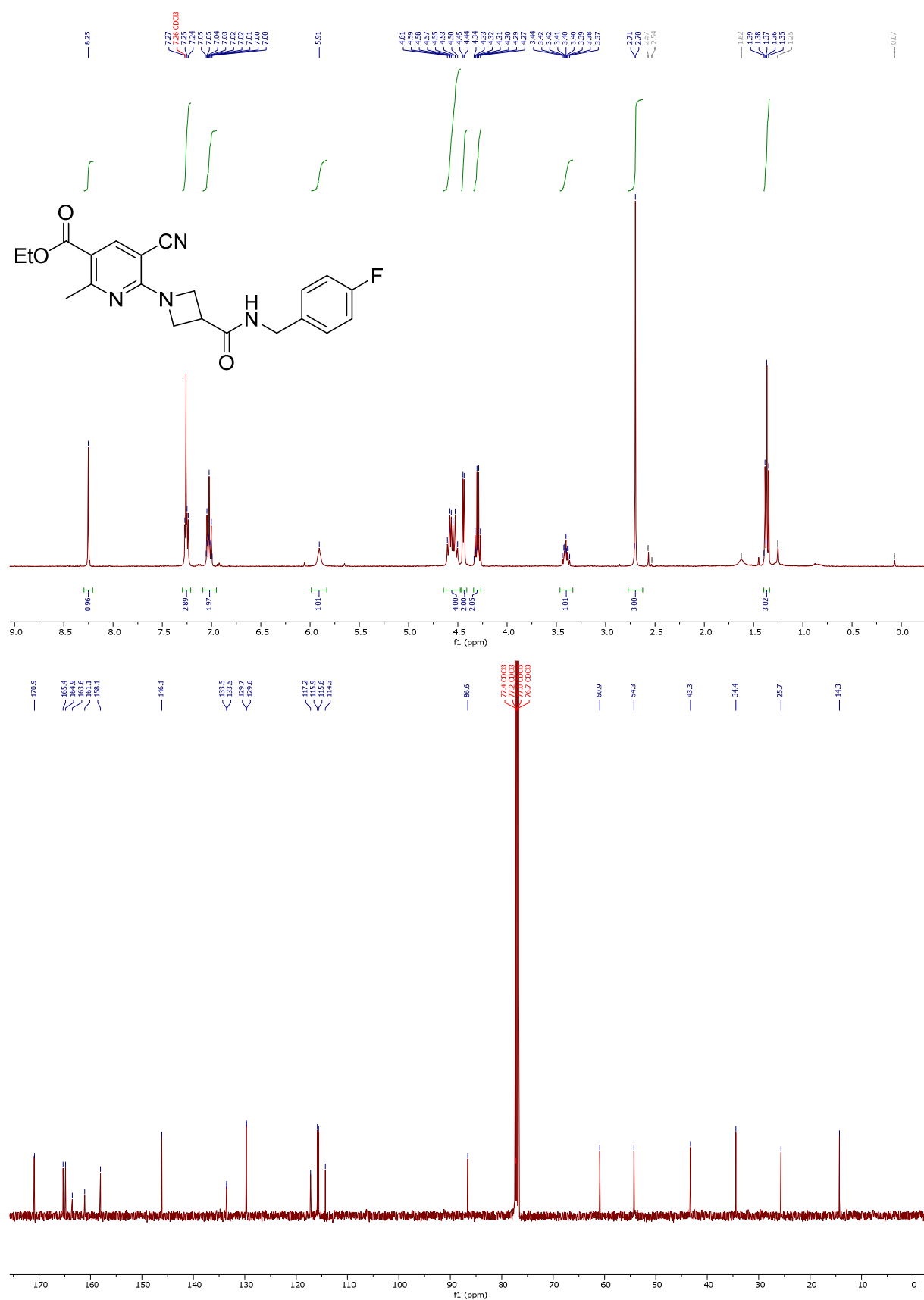


*Ethyl 5-chloro-6(3-((4-fluorobenzyl)carbamoyl)azetidine-1-yl)nicotinate (21)*

$^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )

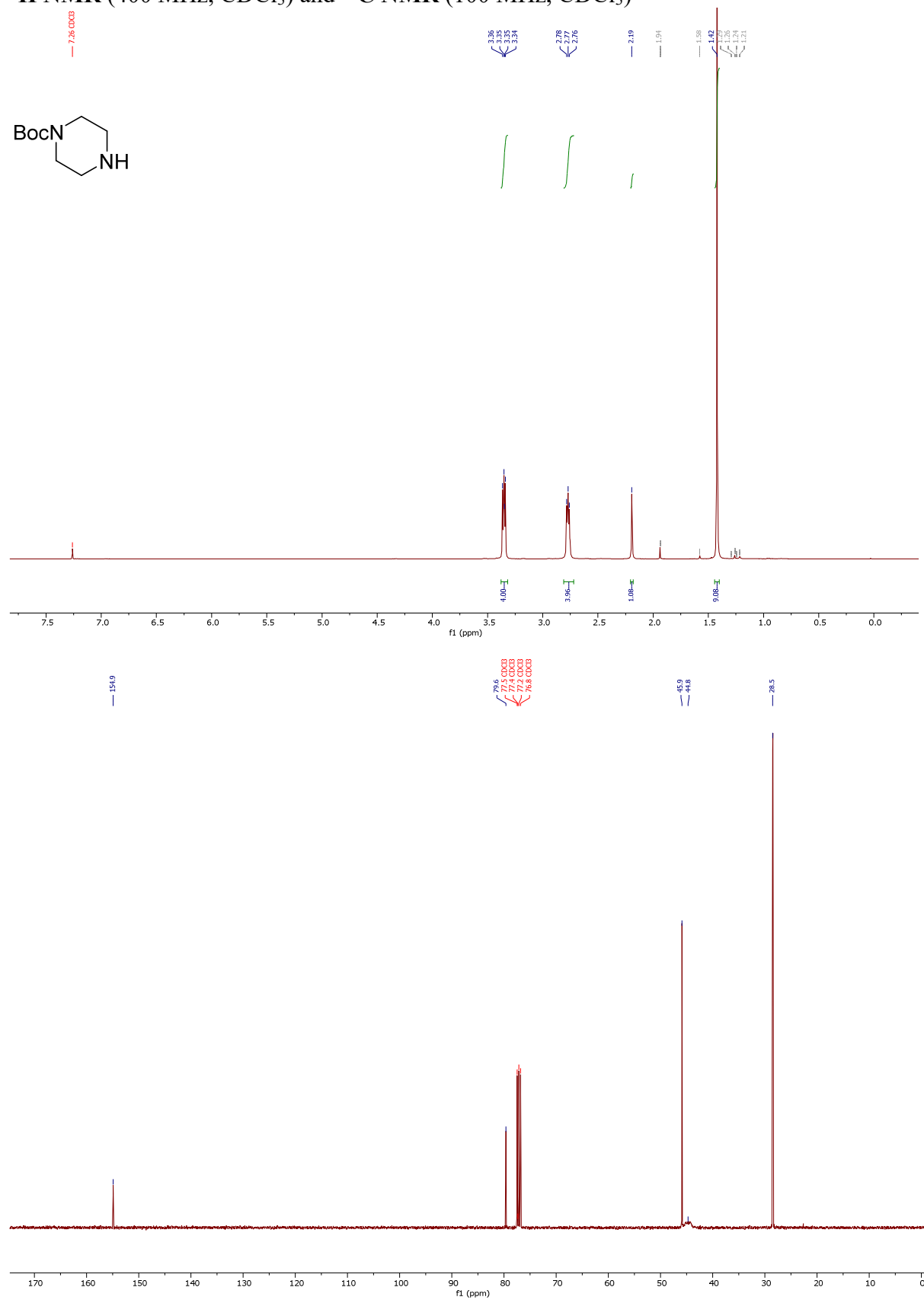


*Ethyl 5-cyano-6-(3-((4-fluorobenzyl)carbamoyl)azetidin-1-yl)-2-methylnicotinate (22)*



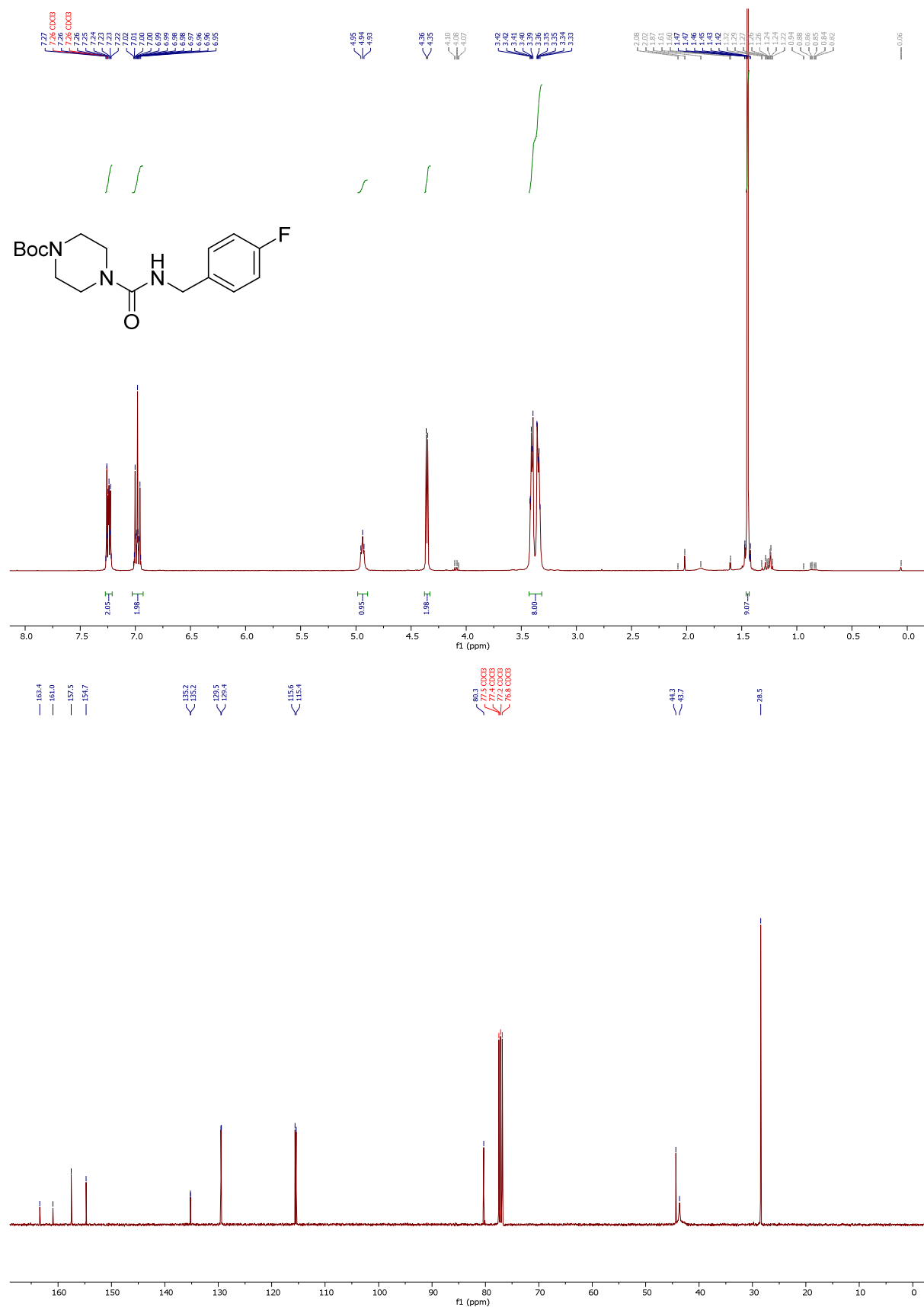
*tert*-Butyl piperazine-1-carboxylate (**23**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



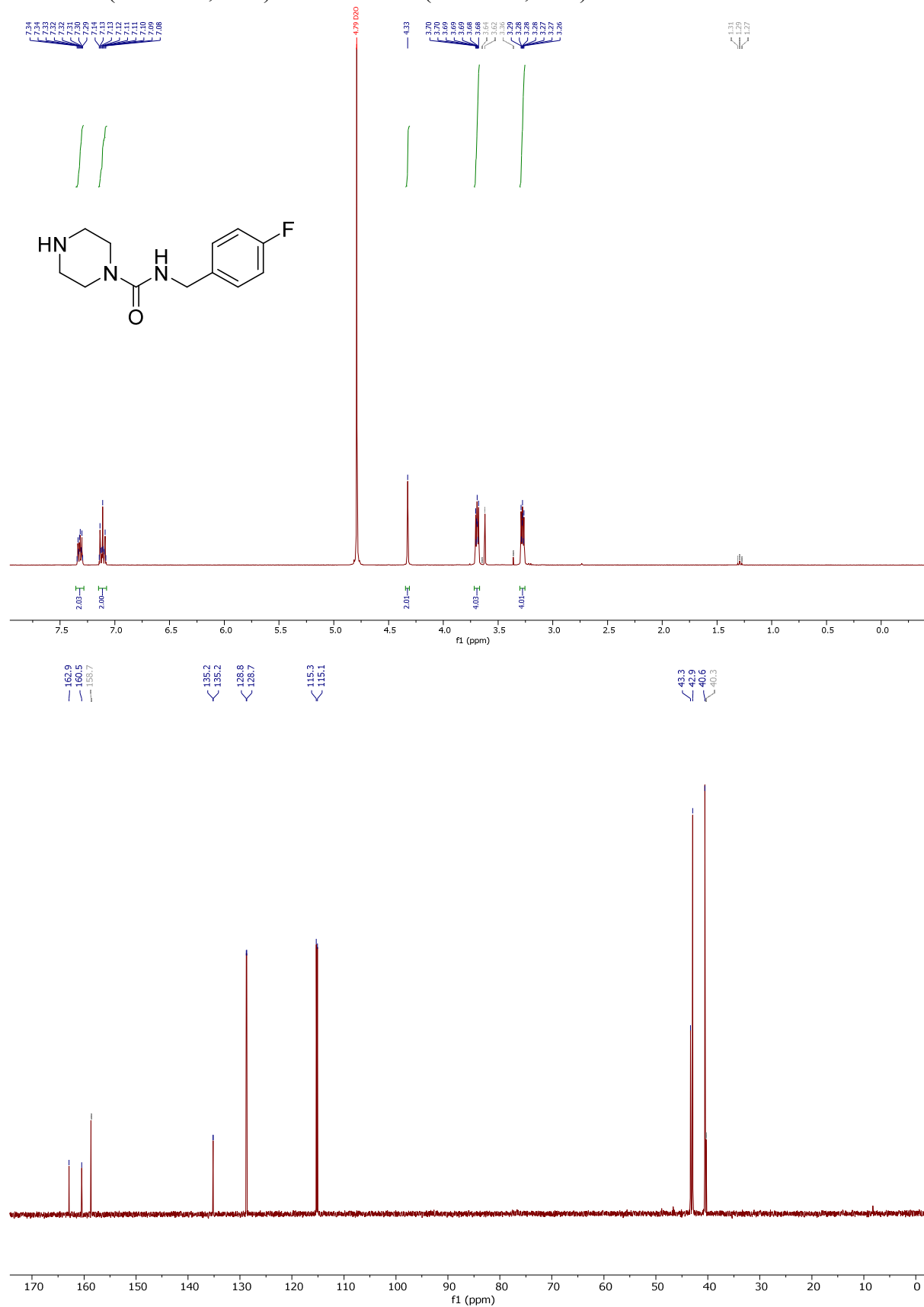
*tert*-Butyl 4-((4-fluorobenzyl)carbamoyl)piperazine-1-carboxylate (**24**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)



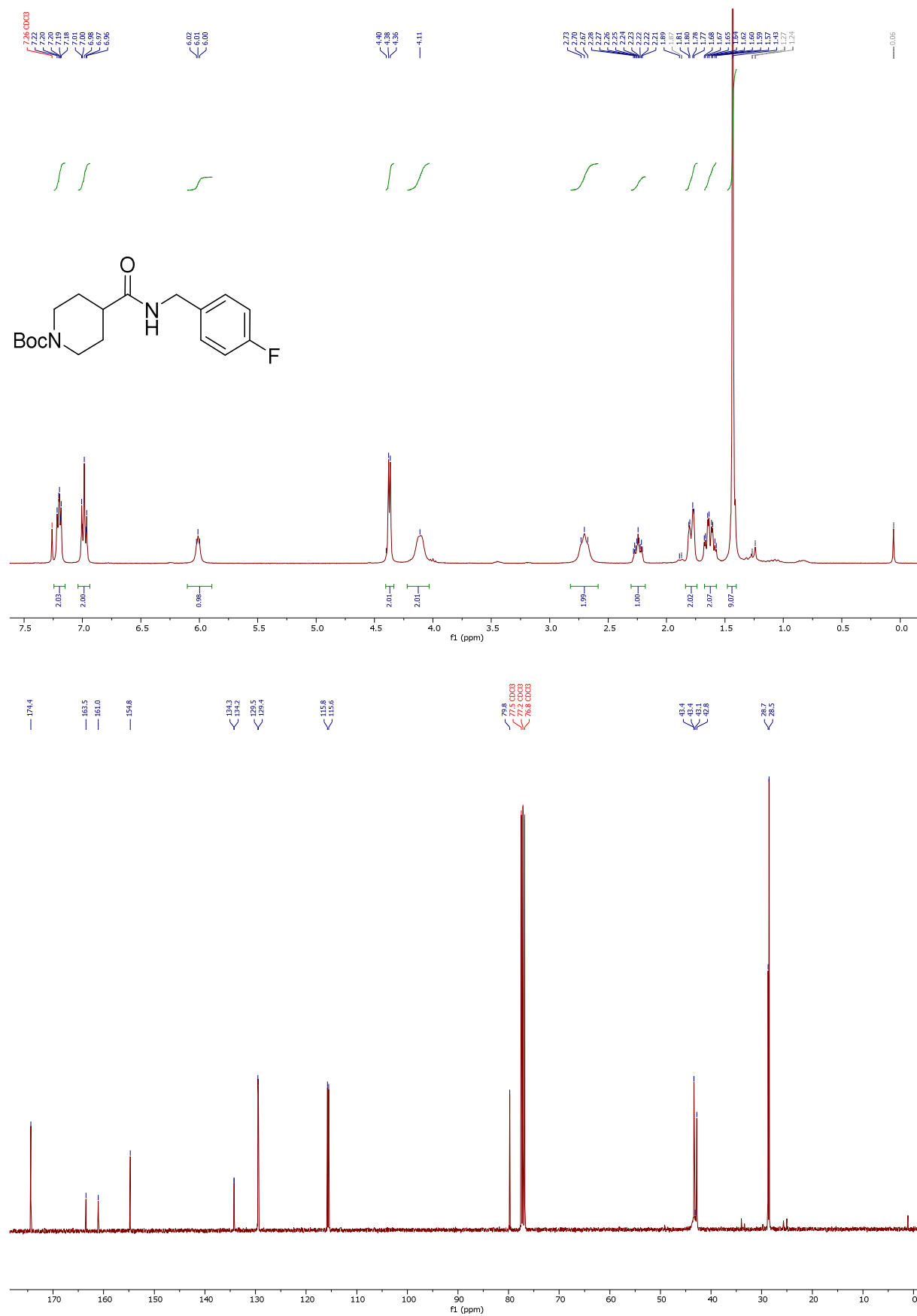
*N*-(4-Fluorobenzyl)piperazine-1-carboxamide (**25**)

$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )



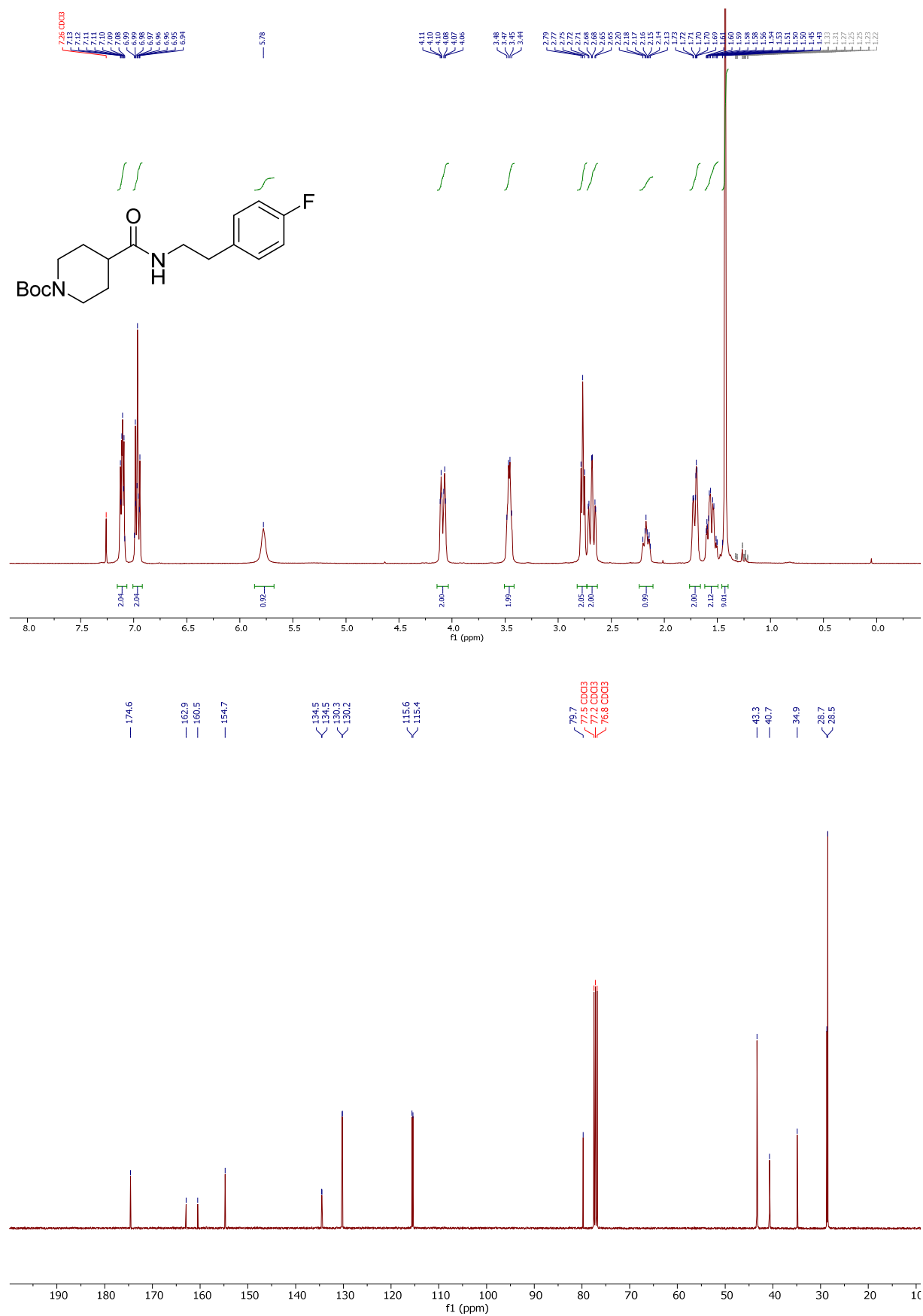
*tert*-Butyl 4-((4-fluorobenzyl)carbamoyl)piperidine-1-carboxylate (**28**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)



*tert*-Butyl 4-((4-fluorophenethyl)carbamoyl)piperidine-1-carboxylate (**29**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)



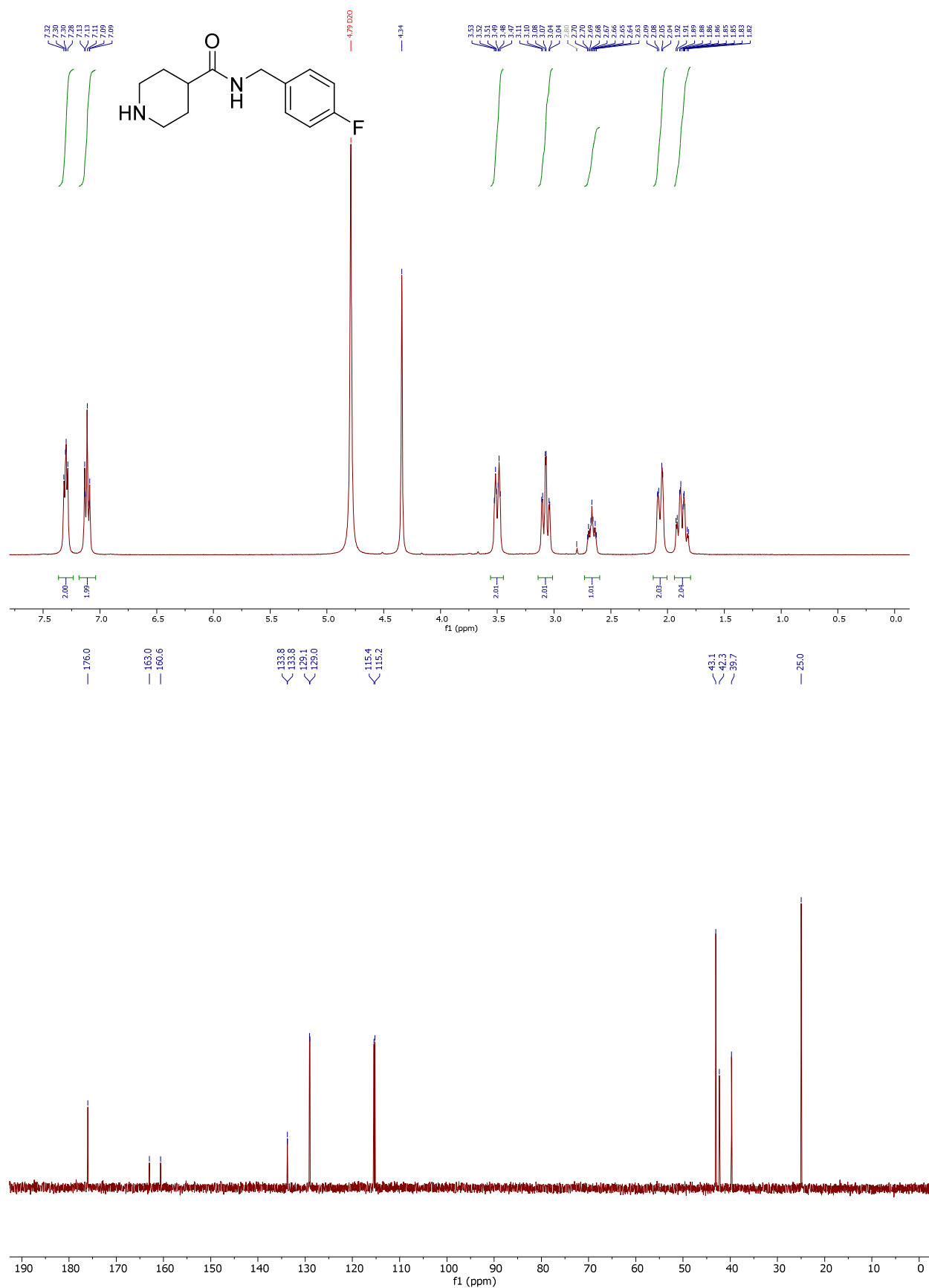


**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) and **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>)



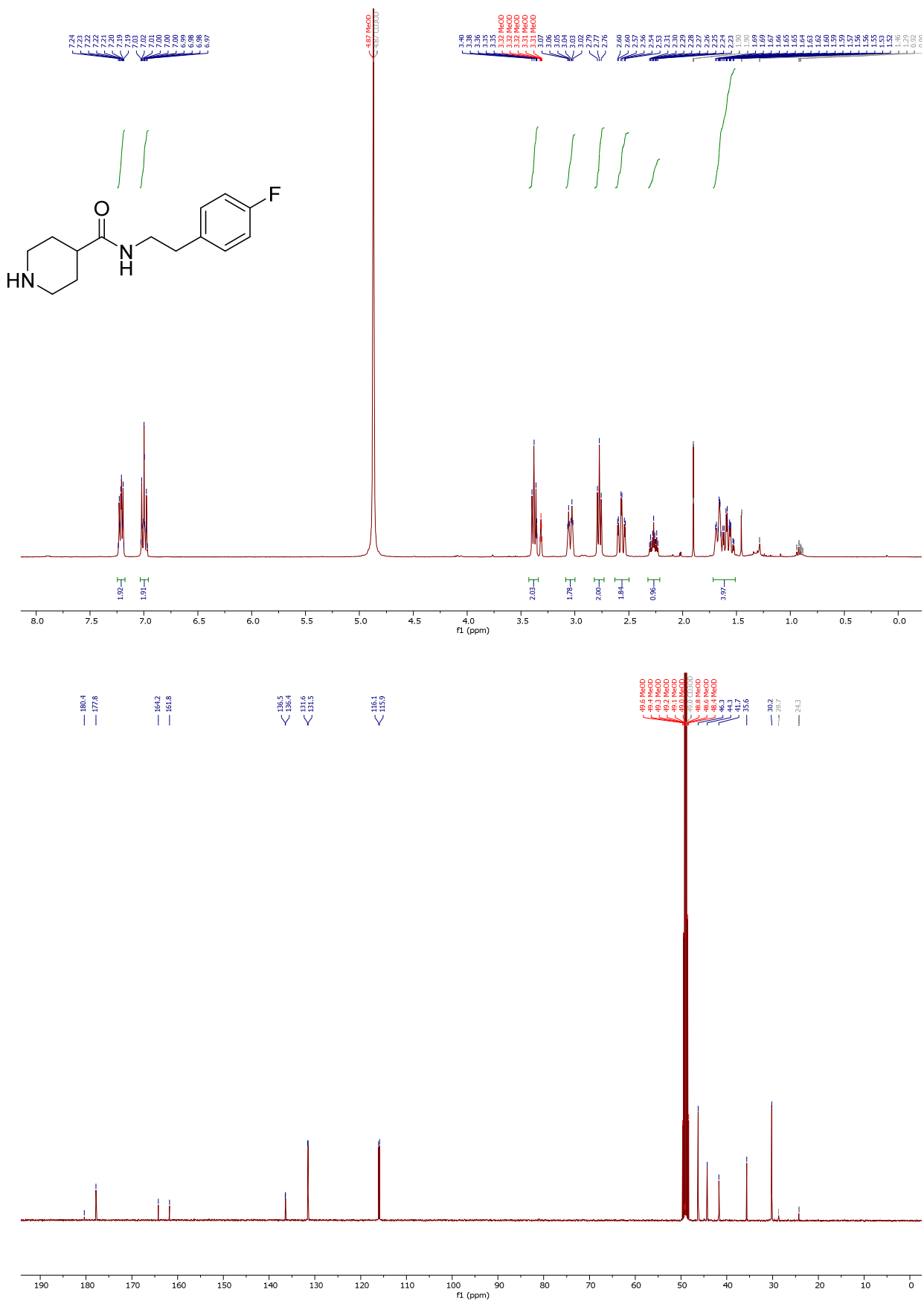
*N*-(4-Fluorobenzyl)piperidine-4-carboxamide (**31**)

$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )



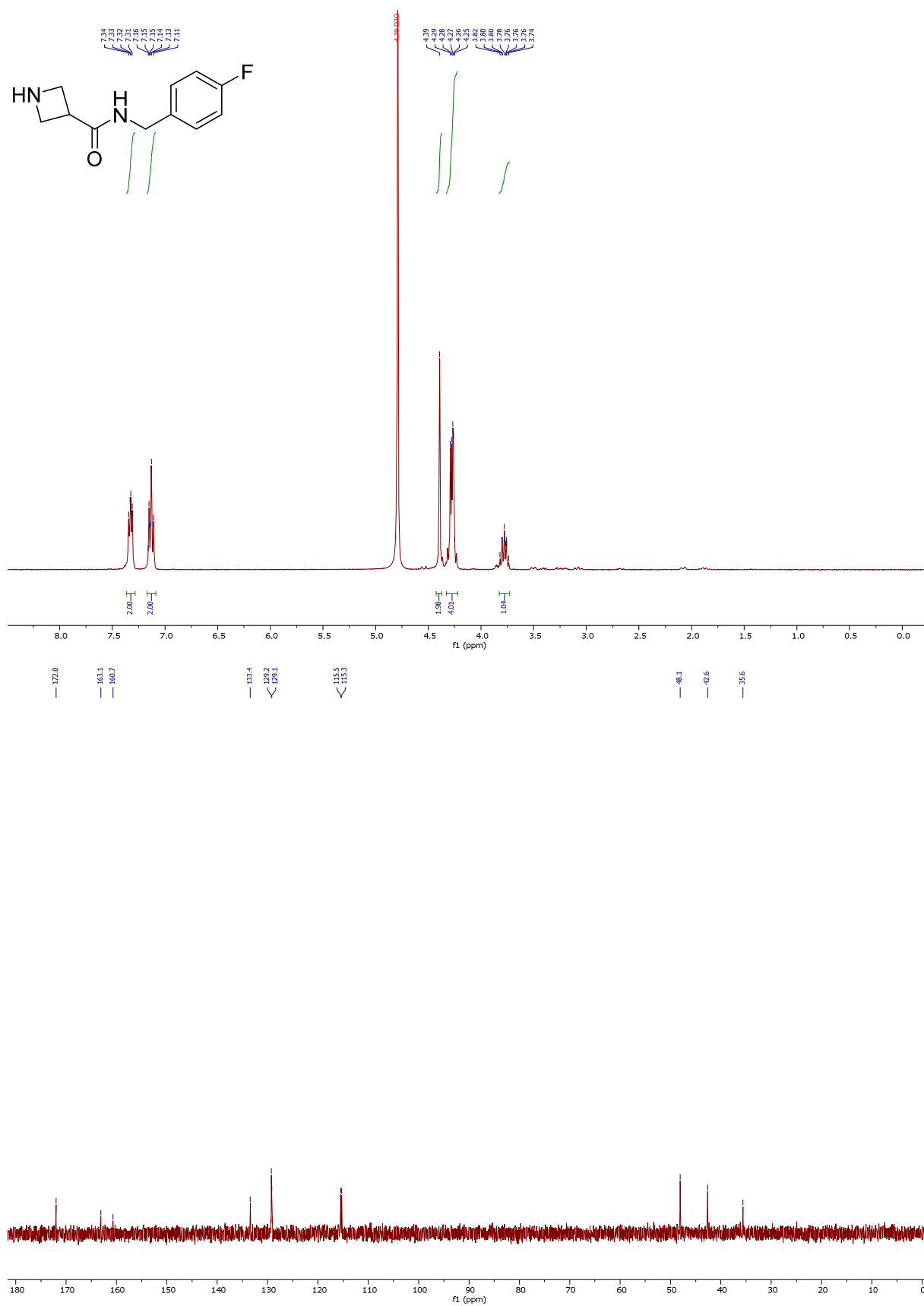
*N*-(4-Fluorophenethyl)piperidine-4-carboxamide (**32**)

$^1\text{H}$  NMR (400 MHz,  $\text{MeOD}_4$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{MeOD}_4$ )



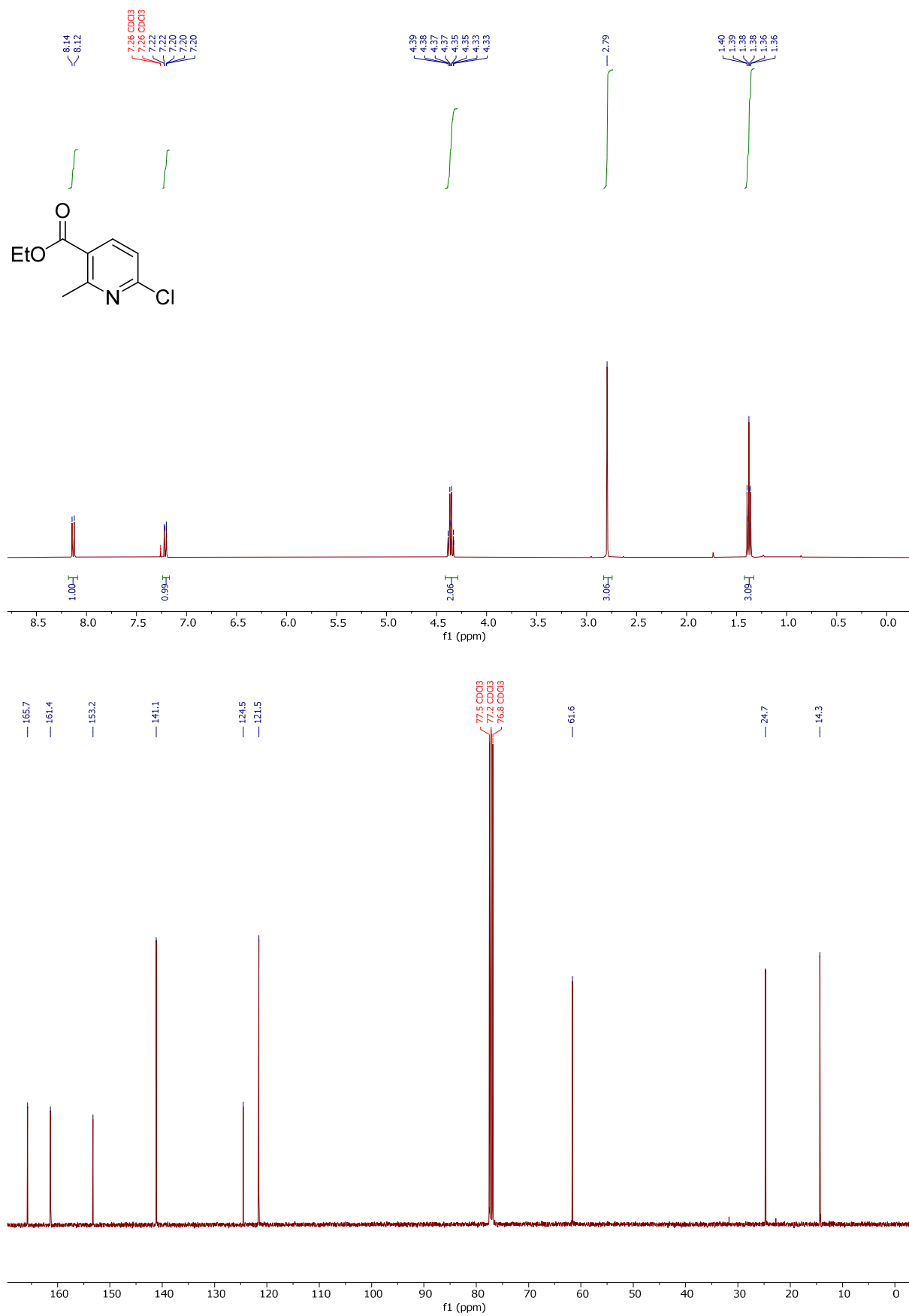
*N*-(4-Fluorobenzyl)azetidine-3-carboxamide (**33**)

$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )



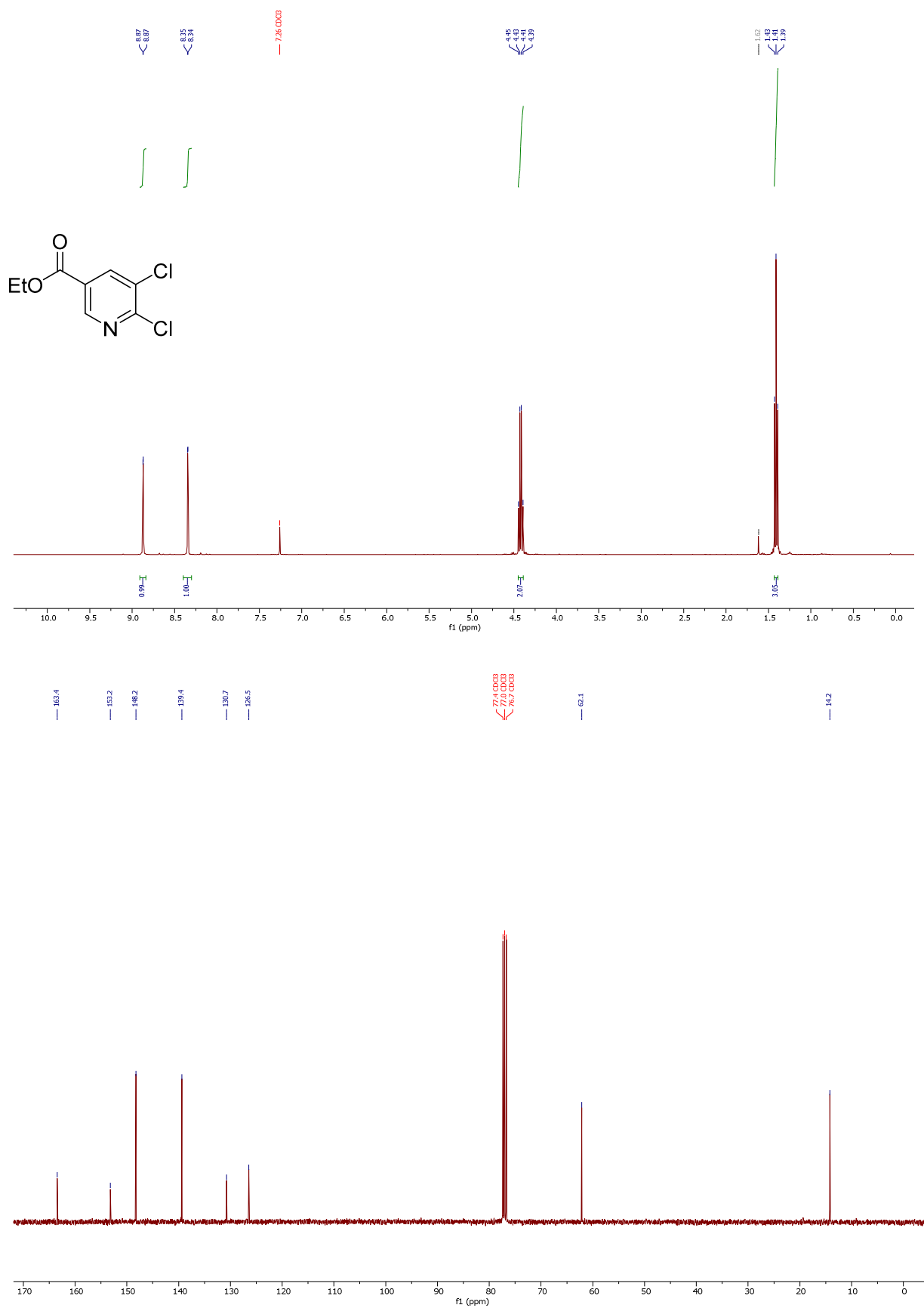
*Ethyl 6-chloro-2-methylnicotinate (34)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



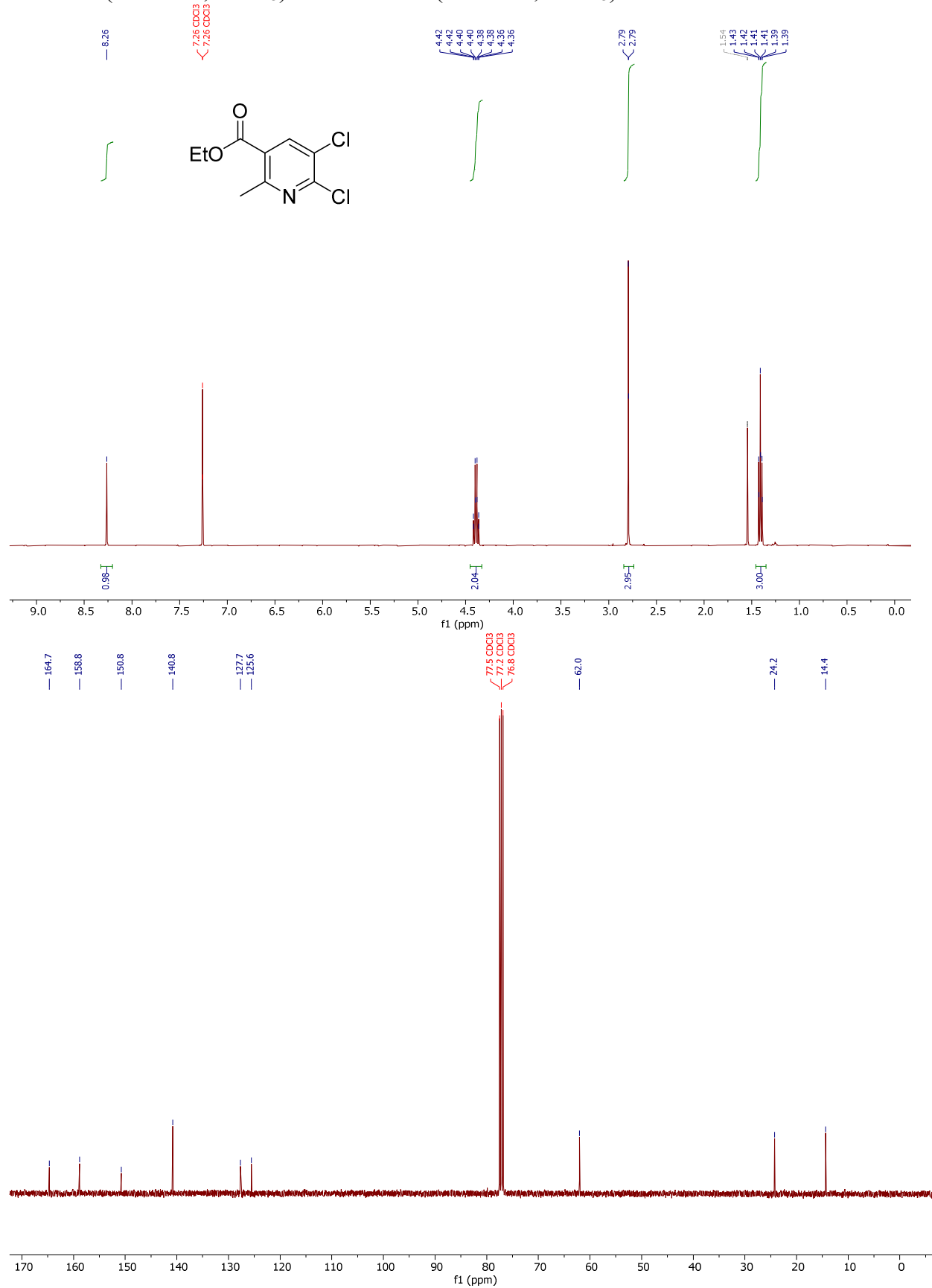
*Ethyl 5,6-dichloronicotinate (35)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )



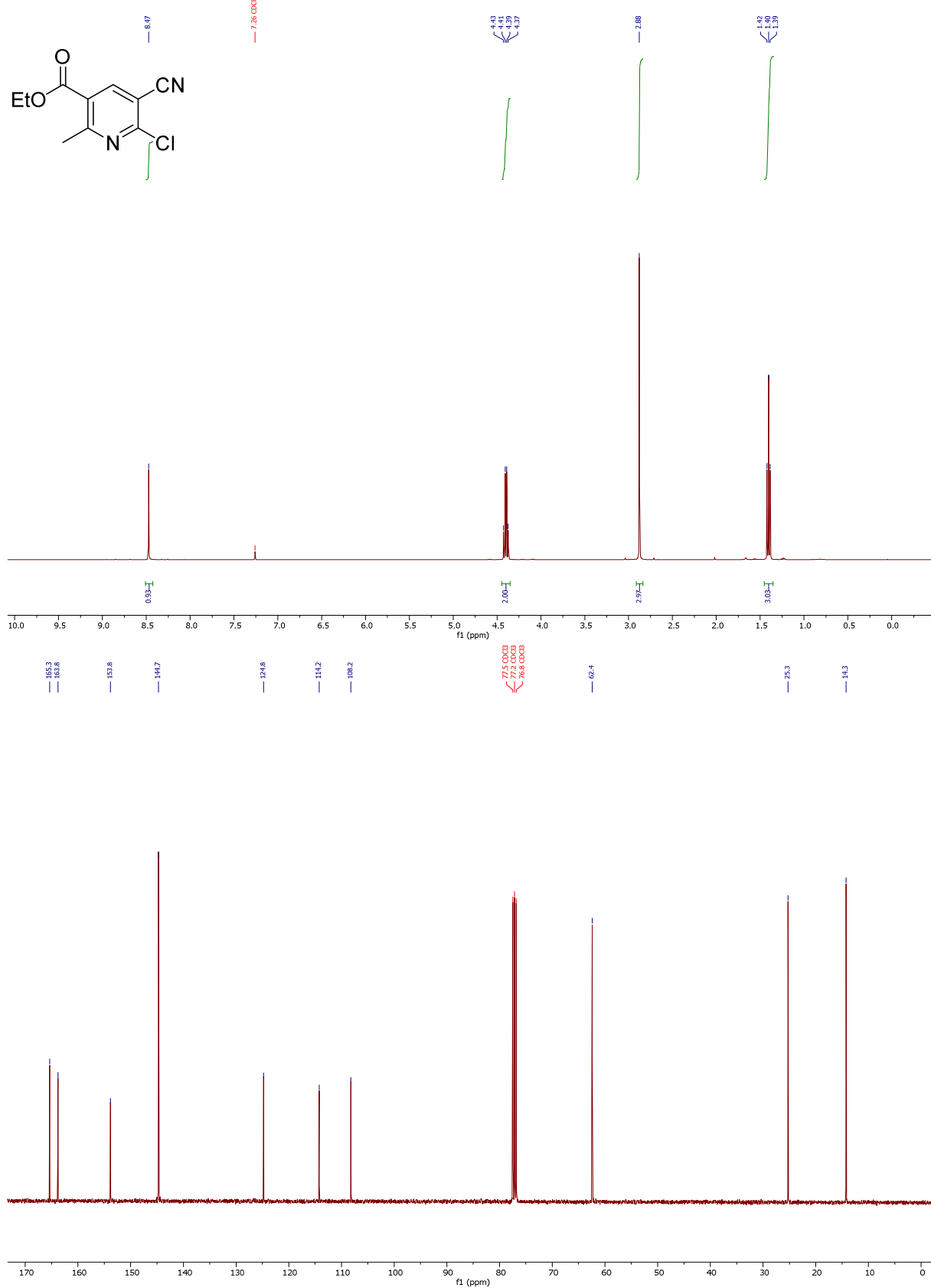
*Ethyl 5,6-dichloro-2-methylnicotinate (36)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



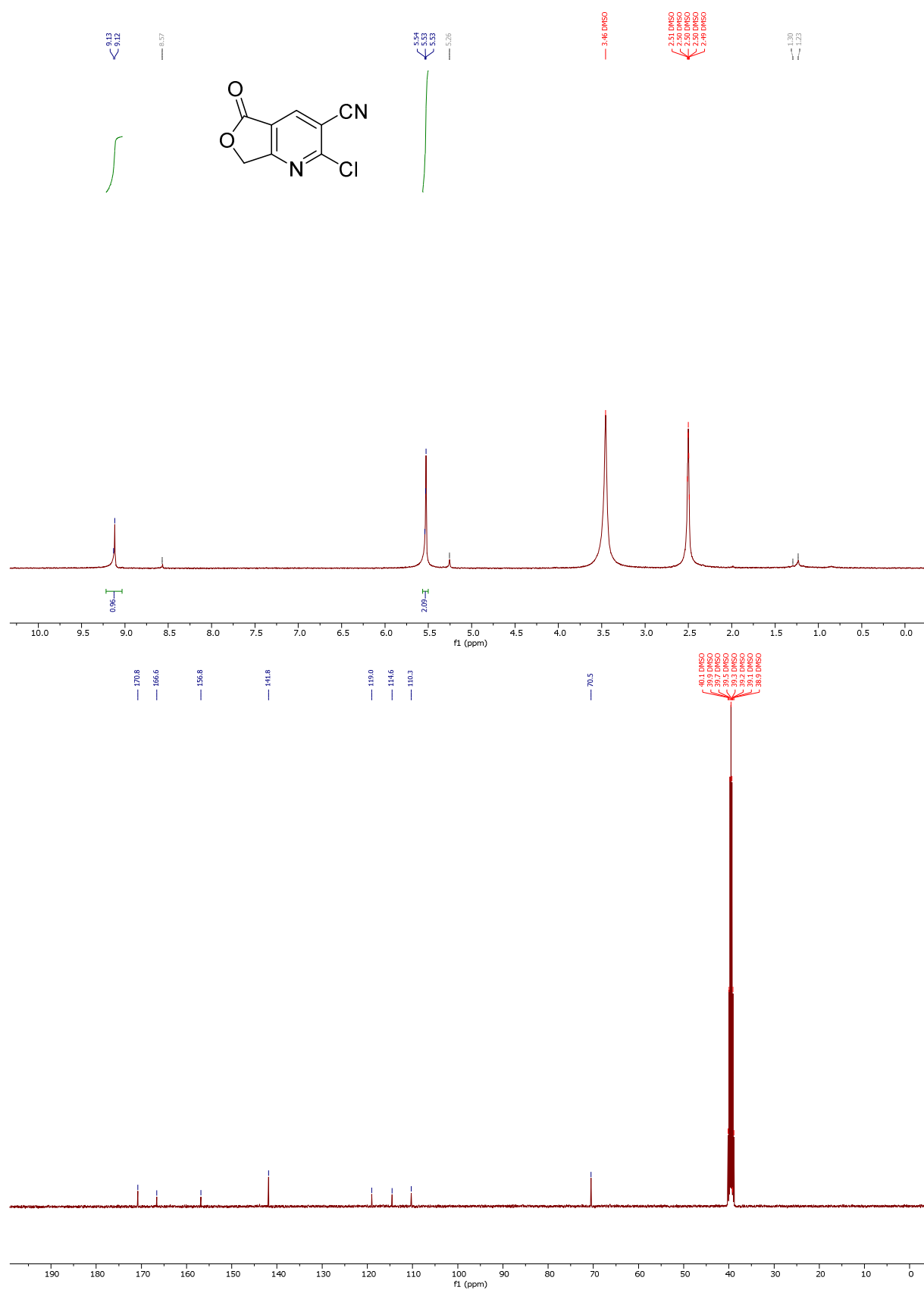
*Ethyl 6-chloro-5-cyano-2-methylnicotinate (37)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



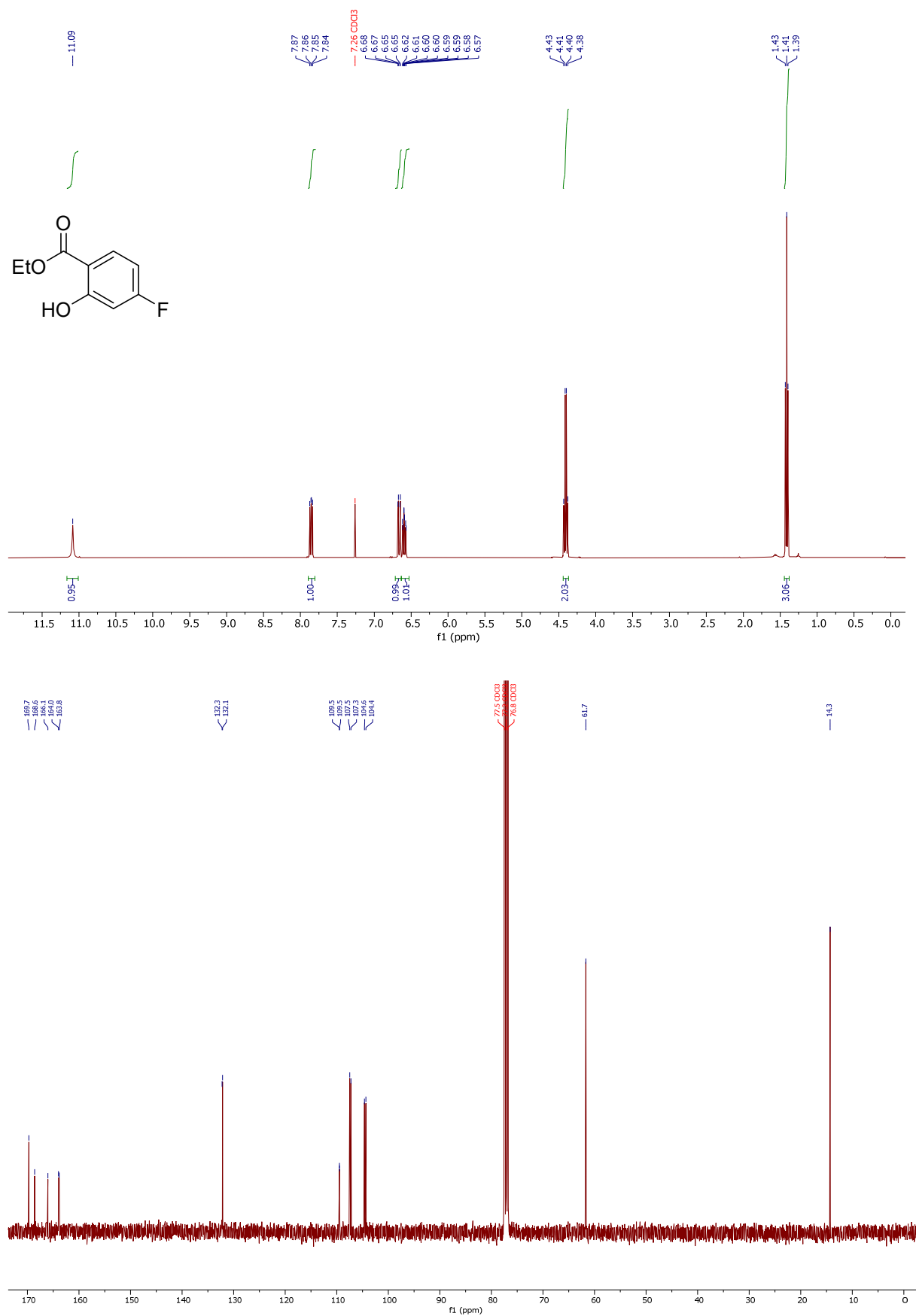


*2-Chloro-5-oxo-5,7-dihydrofuro[3,4-b]pyridine-3-carbonitrile (38)*



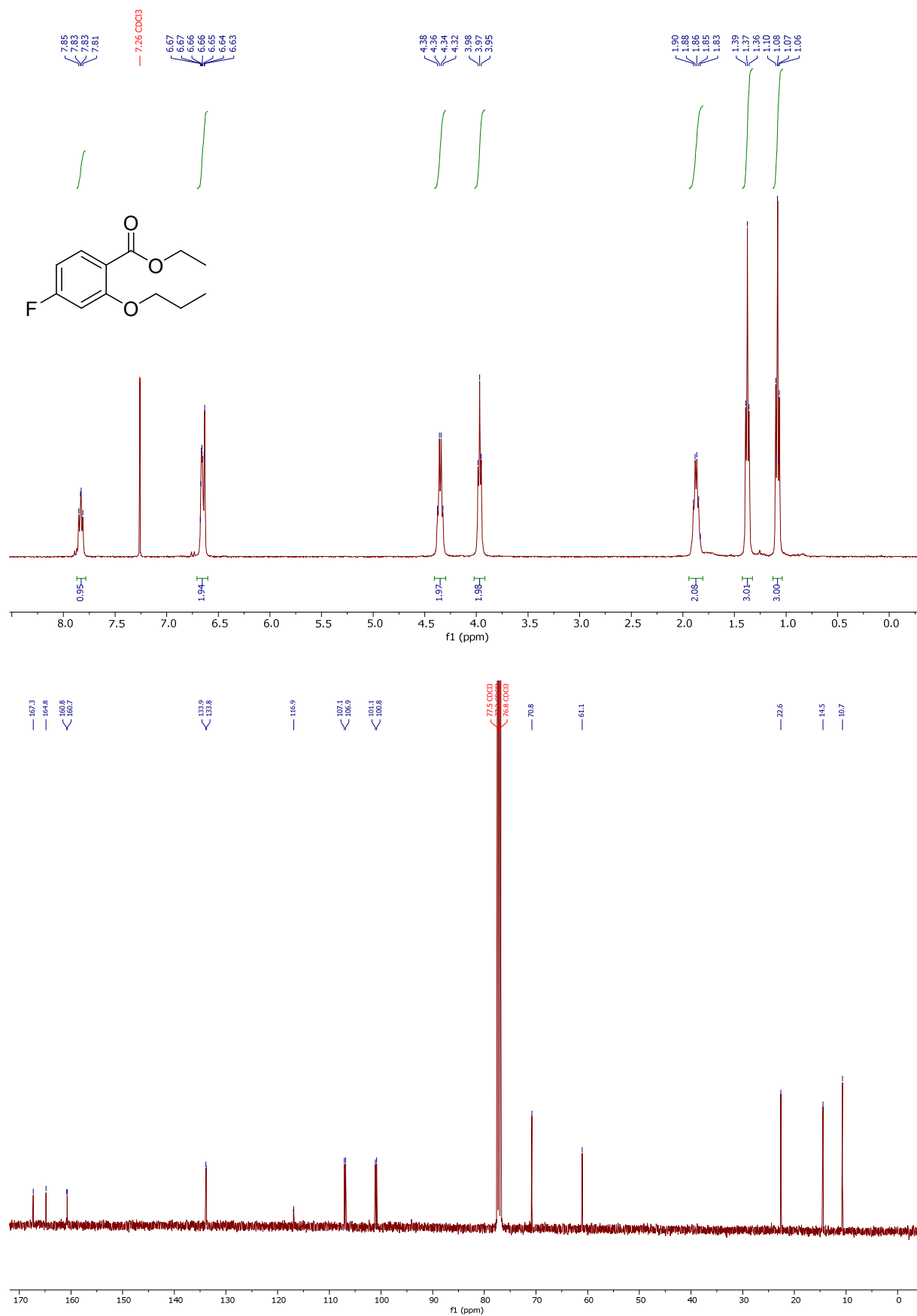
*Ethyl 4-fluoro-2-hydroxybenzoate (40)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



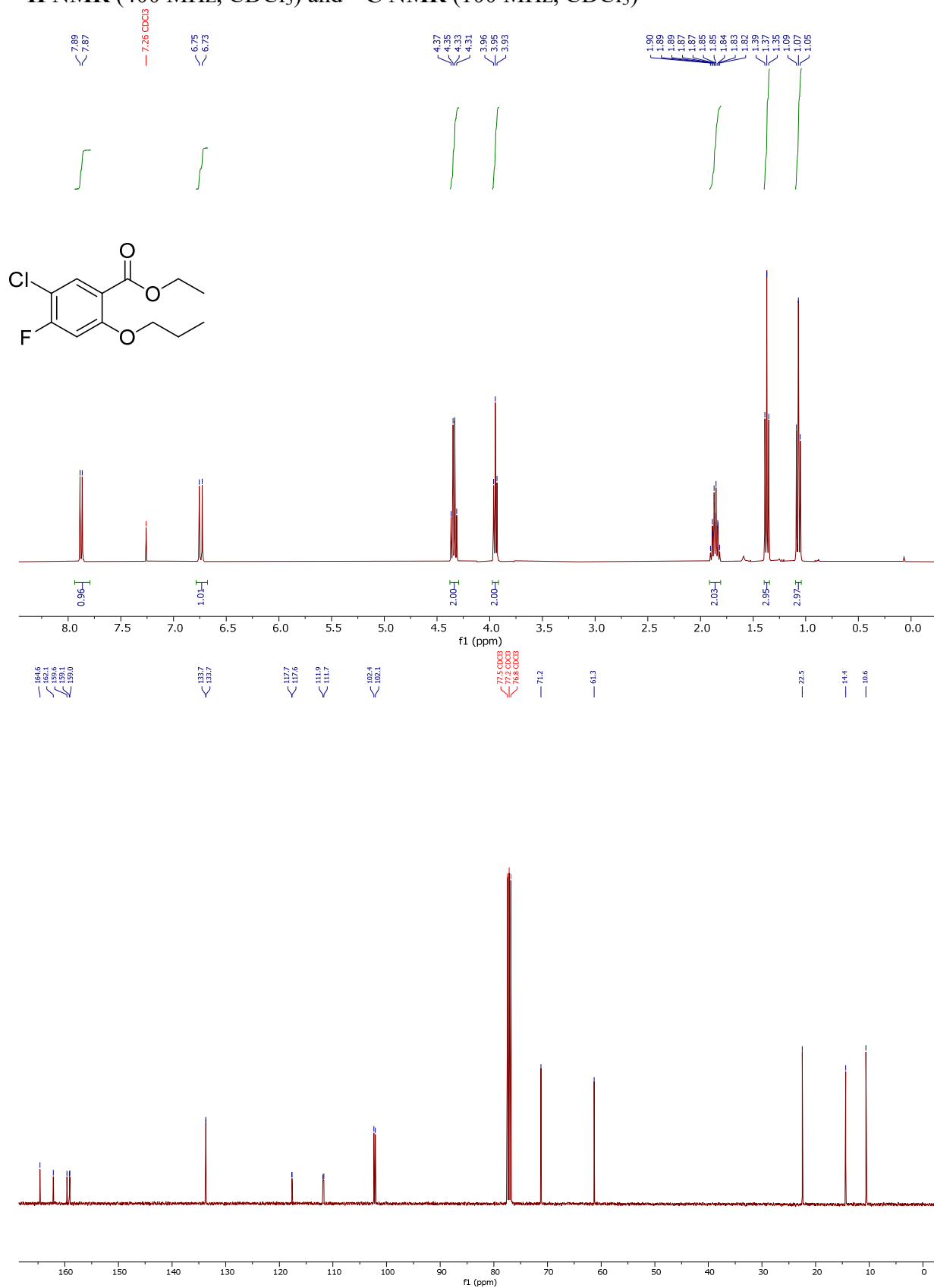
*Ethyl 4-fluoro-2-propoxybenzoate (41)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



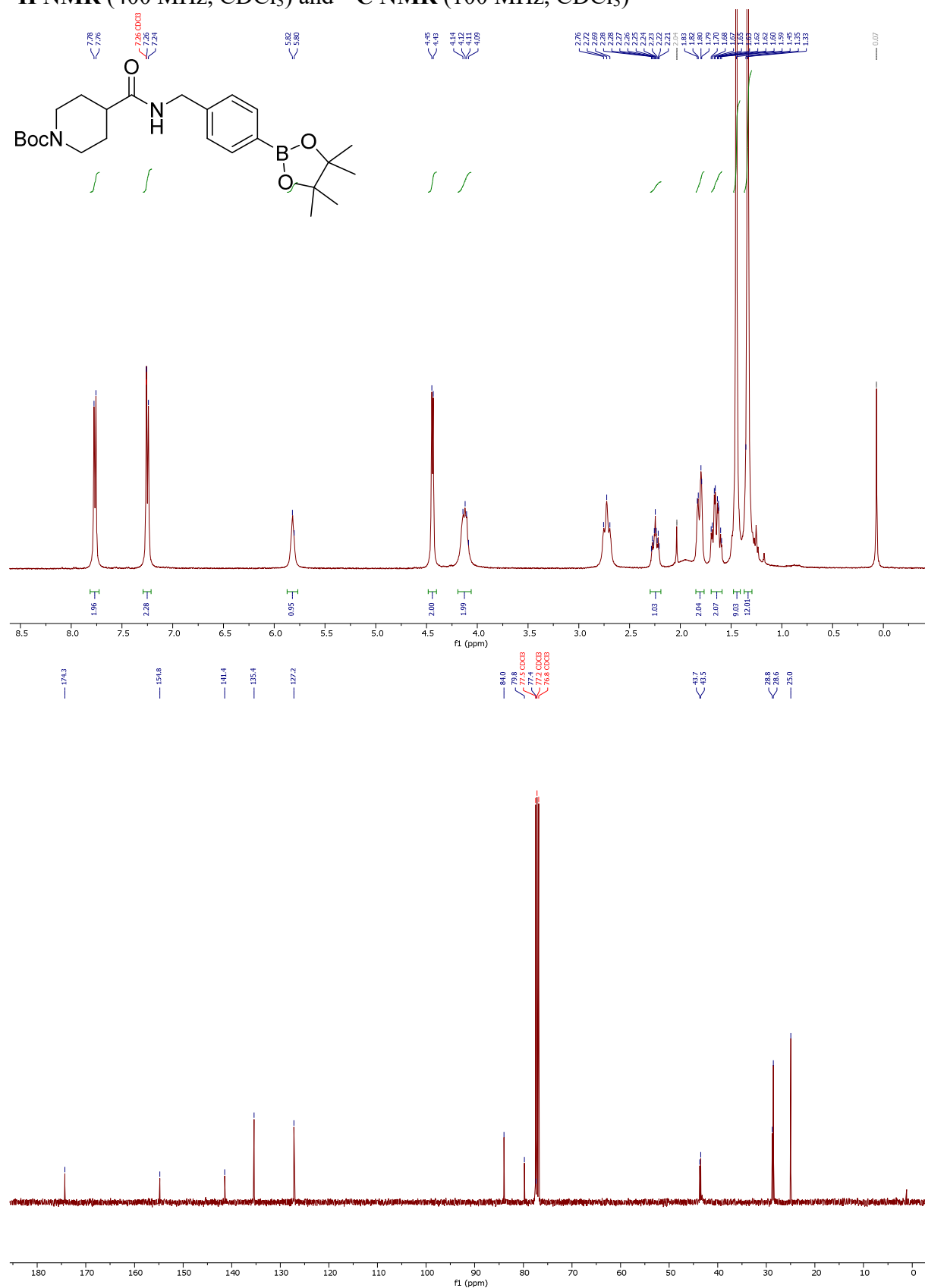
*Ethyl 4-fluoro-2-propoxybenzoate (42)*

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )



*tert*-Butyl 4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)carbamoyl)piperidine-1-carboxylate (**43**)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)



<sup>1</sup>H NMR (400 MHz, MeOD<sub>4</sub>) and <sup>13</sup>C NMR (100 MHz, MeOD<sub>4</sub>)

*Ethyl 5-cyano-2-methyl-6-((4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)carbamoyl)piperidin-1-yl)nicotinate (45)*

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)

