

Supporting Information

for

Neuroprotective Effects of σ_2 R/TMEM97 Receptor Modulators in Neuronal Model of Huntington's Disease

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Table S1. σ_2 R/TMEM97 and σ_1 R binding affinities. ^a								
Compound number	K_i (nM) σ_2 R/TMEM97(r) ^b	$pK_i \pm SD$ σ_2 R/TMEM97(r) ^b	K_i (nM) σ_1 R(gp) ^b	$pK_i \pm SD$ σ_1 R(gp) ^b	K_i (nM) σ_2 R/TMEM97(h) ^c	$pK_i \pm SD$ σ_2 R/TMEM97(h) ^c	K_i (nM) σ_1 R(h) ^c	$pK_i \pm SD$ σ_1 R(h) ^c
DKR-1051	61	7.23 ± 0.09	556	6.25 ± 0.07				
UKH-1114	46	7.3±0.2	1279	5.9±0.15	110.6	6.96±0.59		
AMA-1127	11	8.0±0.1	207	6.68±0.08				
DKR-1677	5.1	8.3±0.1	230	6.64±0.06	11	7.99±0.09	31	7.52±0.08
JJS-1678					3.3	8.58±0.09	106	7.08±0.12
BJM-1679					5.5	8.26±0.22	624	6.21±0.07
EES-1686					6.0	8.34±0.18	97	7.03±0.08
BEA-1687					26	7.6±0.1	185	6.7±0.1
MPC-1154	166	6.8±0.1	5.9	8.23±0.06				
HLJ-1560	116	6.94±0.12	12	7.9±0.07				

^a K_i values determined from average pK_i obtained from non-linear regression of radioligand competition binding isotherms run at least in triplicate by PDSP. ^b σ_2 R/TMEM97 was sourced from rat PC12 cells and σ_1 R was sourced from guinea pig brain. ^c σ_2 R/TMEM97 and σ_1 R were sourced from HEK293T transfected with human σ_1 R and σ_2 R/TMEM97.

Table S2. DKR-1051 binding profile at non-sigma receptor sites.^a

Target	<i>K_i</i> (nM)	Target	<i>K_i</i> (nM)
5HT _{1A}	*	Beta2	*
5HT _{1B}	*	Beta3	*
5HT _{1D}	*	BZP Rat Brain	*
5HT _{1e}	*	Calcium Channel	*
5HT _{2A}	*	D ₁	*
5HT _{2B}	2902*	D ₂	*
5HT _{2C}	*	D ₃	*
5HT ₃	*	D ₄	*
5HT _{5a}	*	D ₅	*
5HT ₆	*	DAT	1264
5HT ₇	*	DOR	*
A2B2	*	GabaA	*
A2B4	*	H ₁	1676
A3B2	*	H ₃	*
A3B4	5202	KOR	331
A4B2	*	M ₁	*
A4B2**	*	M ₂	*
A4B4	>10,000	M ₃	*
A7	>10,000	M ₄	*
A7**	*	M ₅	*
Alpha _{1a}	*	MOR	881
Alpha _{1b}	*	NET	301
Alpha _{1d}	*	NMDA	*
Alpha _{2a}	*	PBR	*
Alpha _{2b}	2307	SERT	*
Alpha _{2c}	*	V1A	*
AMPA	*	V1B	*
Beta1	*	V2	*

* < 50% inhibition of radioligand binding at 10 μ M.

** sourced from rodent brain

^a Receptor binding assays were performed by the Psychoactive Drug Screening Program (PDSP) at Chapel Hill, North Carolina.

Table S3. UKH-1114 binding profile at non-sigma receptor sites.^a

Target	<i>K_i</i> (nM)	Target	<i>K_i</i> (nM)
5HT _{1A}	*	Beta3	*
5HT _{1B}	*	BZP Rat Brain	*
5HT _{1D}	*	Calcium Channel	>10,000
5HT _{1e}	*	D ₁	*
5HT _{2A}	*	D ₂	*
5HT _{2B}	*	D ₃	*
5HT _{2C}	*	D ₄	*
5HT ₃	*	D ₅	*
5HT _{5a}	*	DAT	*
5HT ₆	*	DOR	*
5HT ₇	*	GabaA	*
A2B2	*	H ₁	*
A2B4	*	H ₃	*
A3B2	*	hERG	549
A3B4	*	KOR	1383
A4B2	*	M ₁	*
A4B2**	*	M ₂	*
A4B4	*	M ₃	*
A7	*	M ₄	*
A7**	*	M ₅	*
Alpha _{1a}	*	MOR	*
Alpha _{1b}	*	NET	1,046
Alpha _{1d}	*	NMDA	6,724
Alpha _{2a}	*	PBR	*
Alpha _{2b}	*	SERT	*
Alpha _{2c}	*	V1A	>10,000
AMPA	>10,000	V1B	>10,000
Beta1	*	V2	>10,000
Beta2	*		

* < 50% inhibition of radioligand binding at 10 μM.

** sourced from rodent brain

^a Receptor binding assays were performed by the Psychoactive Drug Screening Program (PDSP) at Chapel Hill, North Carolina.

Table S4. AMA-1127 binding profile at non-sigma receptor sites.^a

Target	<i>K_i</i> (nM)	Target	<i>K_i</i> (nM)
5HT _{1A}	38	BZP Rat Brain	*
5HT _{1B}	*	D ₂	*
5HT _{1D}	1492	D ₃	670
5HT _{1e}	1204	D ₄	568
5HT _{2A}	719	DAT	906
5HT _{2B}	819	DOR	*
5HT _{2C}	460	GabaA	*
5HT ₃	741	H ₂	456
5HT _{5a}	*	H ₄	*
5HT ₆	*	KOR	*
5HT ₇	441	M ₄	*
Alpha _{1a}	*	M ₅	*
Alpha _{1b}	*	MOR	*
Alpha _{1d}	3241	NET	165
Alpha _{2a}	194		
Alpha _{2b}	1818	PBR	*
Alpha _{2c}	5756	SERT	*
Beta1	*		
Beta2	*		
Beta3	*		

* <50% inhibition of radioligand binding at 10 μM.

** sourced from rodent brain

^a Receptor binding assays were performed by the Psychoactive Drug Screening Program (PDSP) at Chapel Hill, North Carolina.

Table S5. BJM-1679 binding profile at non-sigma receptor sites.^a

Target	<i>K_i</i> (nM)	Target	<i>K_i</i> (nM)
5HT _{1A}	*	D ₁	*
5HT _{1B}	*	D ₂	*
5HT _{1D}	*	D ₃	740
5HT _{1e}	*	D ₄	*
5HT _{2A}	2042	D ₅	*
5HT _{2B}	1703	DAT	488
5HT _{2C}	232	DOR	*
5HT ₃	*	GabaA	*
5HT _{5a}	*	H ₁	*
5HT ₆	2286	H ₂	*
5HT _{7A}	*	H ₃	2217
Alpha _{1a}	*	H ₄	
Alpha _{1b}	*	KOR	*
Alpha _{1d}	*	M ₁	1438
Alpha _{2a}	2123	M ₂	*
Alpha _{2b}	*	M ₃	*
Alpha _{2c}	*	M ₄	*
Beta ₁	*	M ₅	787
Beta ₂	*	MOR	*
Beta ₃	*	NET	263
BZP Rat Brain	*	PBR	*
		SERT	1823

* < 50% inhibition of radioligand binding at 10 μ M.

** sourced from rodent brain

^a Receptor binding assays were performed by the Psychoactive Drug Screening Program (PDSP) at Chapel Hill, North Carolina.

Table S6. EES-1686 binding profile at non-sigma receptor sites.^a

Target	<i>K_i</i> (nM)	Target	<i>K_i</i> (nM)
5HT _{1A}	*	D ₁	*
5HT _{1B}	*	D ₂	*
5HT _{1D}	*	D ₃	991
5HT _{1E}	*	D ₄	*
5HT _{2A}	2166	D ₅	944
5HT _{2B}	*	DAT	*
5HT _{2C}	201	DOR	*
5HT ₃	*	GabaA	*
5HT _{5a}	*	H ₁	*
5HT ₆	3918	H ₂	2248
5HT _{7A}	*	H ₃	2484
Alpha _{1a}	*	H ₄	
Alpha _{1b}	3837	KOR	*
Alpha _{1d}	*	M ₁	*
Alpha _{2a}	8220	M ₂	*
Alpha _{2b}	*	M ₃	*
Alpha _{2c}	*	M ₄	*
Beta1	*	M ₅	2254
Beta2	*	MOR	*
Beta3	*	NET	421
BZP Rat Brain	*	PBR	*
		SERT	*

* < 50% inhibition of radioligand binding at 10 μ M.

** sourced from rodent brain

^a Receptor binding assays were performed by the Psychoactive Drug Screening Program (PDSP) at Chapel Hill, North Carolina.

Table S7. BEA-1687 binding profile at non-sigma receptor sites.^a

Target	<i>K_i</i> (nM)	Target	<i>K_i</i> (nM)
5HT _{1A}	*	D ₁	*
5HT _{1B}	*	D ₂	*
5HT _{1D}	1522	D ₃	*
5HT _{1E}	*	D ₄	525
5HT _{2A}	*	D ₅	*
5HT _{2B}	*	DAT	*
5HT _{2C}	2165	DOR	*
5HT ₃	*	GabaA	*
5HT _{5A}	*	H ₁	*
5HT ₆	*	H ₂	*
5HT _{7A}	*	H ₃	*
Alpha _{1a}	*	H ₄	*
Alpha _{1b}	*		
Alpha _{1d}	3044	KOR	*
Alpha _{2a}	*	M ₁	*
Alpha _{2b}	*	M ₂	*
Alpha _{2c}	*	M ₃	*
AMPA	*	M ₄	*
Beta ₁	*	M ₅	*
Beta ₂	*	MOR	*
Beta ₃	*	NET	*
BZP Rat Brain	*	NMDA	*
		PBR	*
		SERT	*

* < 50% inhibition of radioligand binding at 10 μM.

** sourced from rodent brain

^a Receptor binding assays were performed by the Psychoactive Drug Screening Program (PDSP) at Chapel Hill, North Carolina.

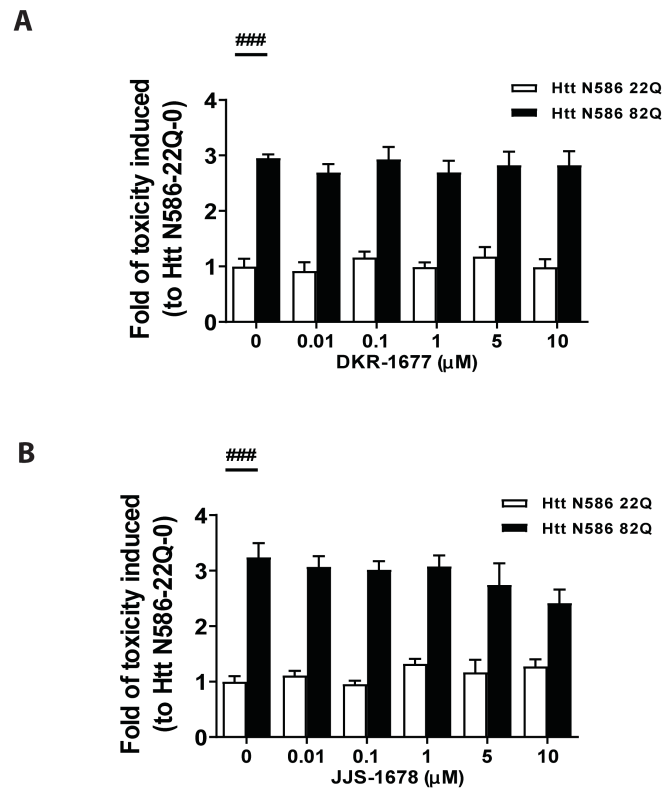


Figure S1. $\sigma_2\text{R}/\text{TMEM97}$ -selective modulators did not show protective effect on mHTT induced toxicity. $\sigma_2\text{R}/\text{TMEM97}$ -selective modulators, **DKR-1677 (A)** and **JJS-1678 (B)** had no effect on mHTT induced cell toxicity. ^{###} $p < 0.001$ vs Htt N586-22Q.

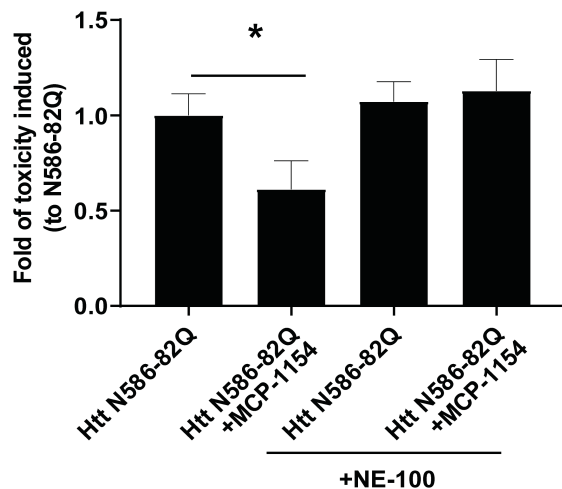
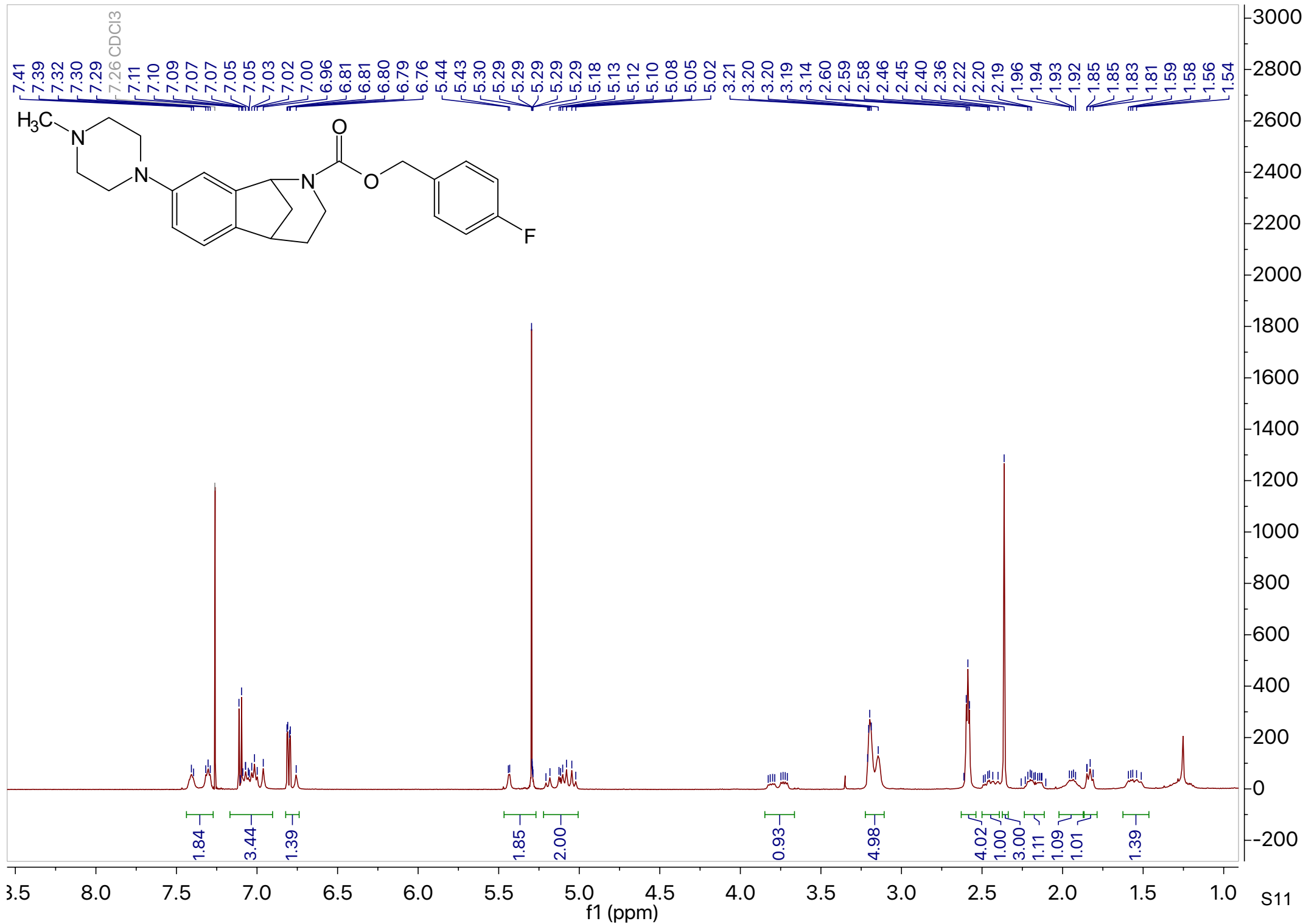
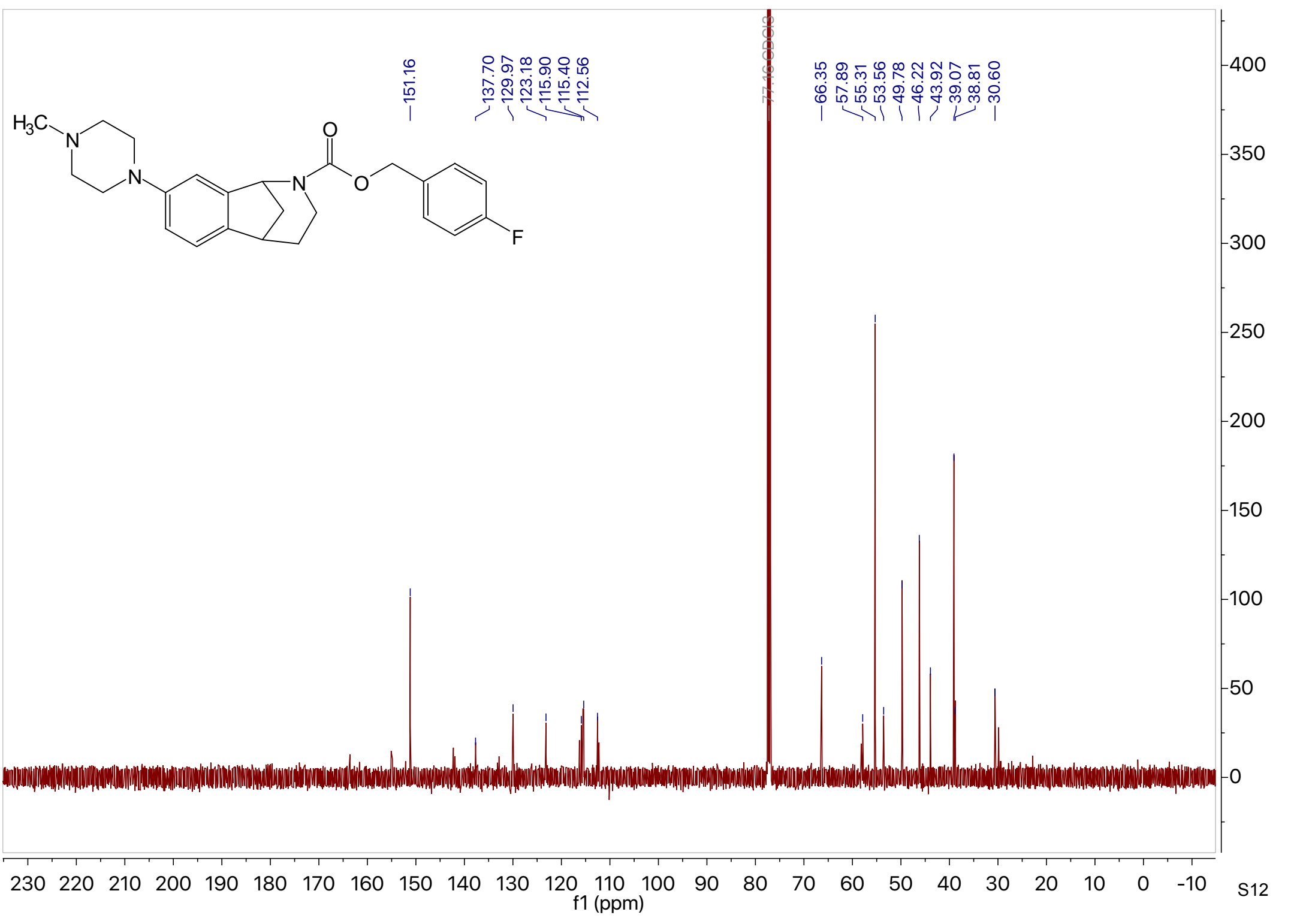
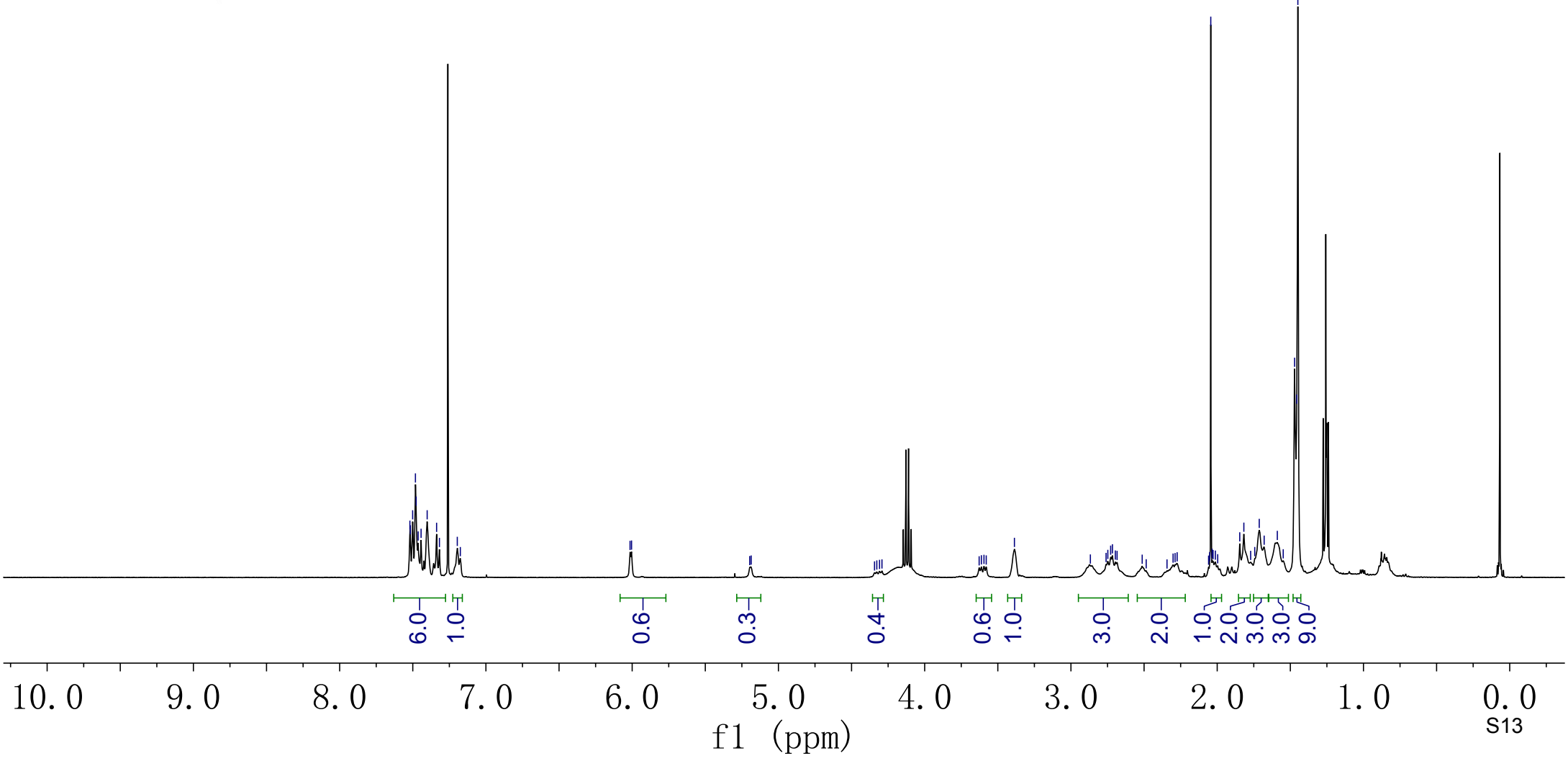
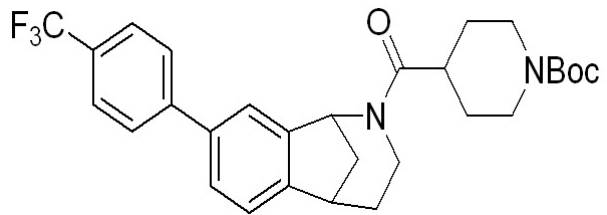


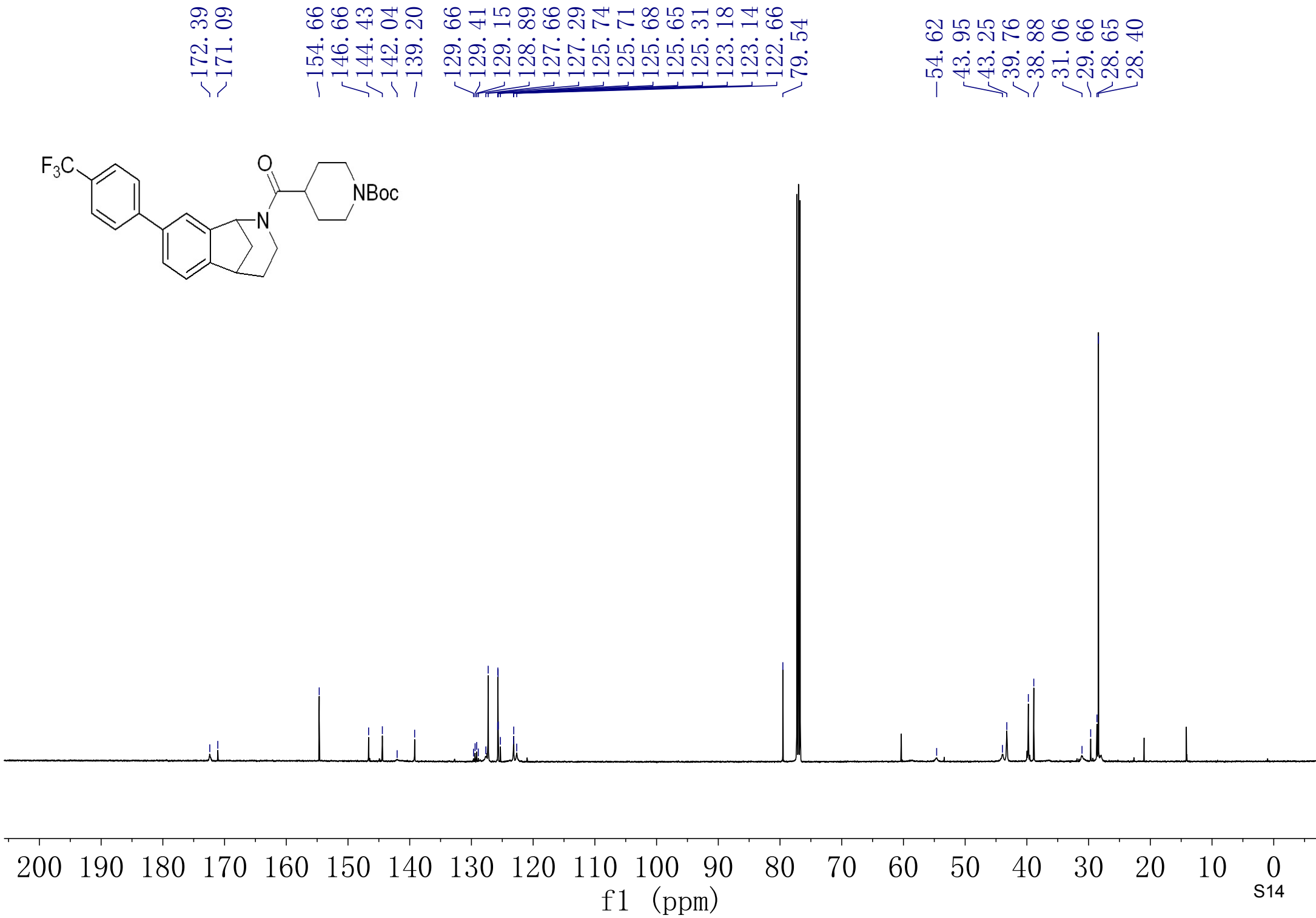
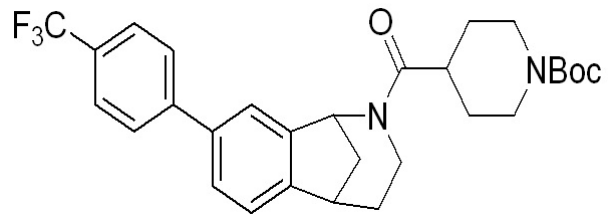
Figure S2. Specificity of MCP-1154. NE-100 was used in the primary cortical neurons treated $\sigma_1\text{R}$ modulator, **MCP-1154**. Primary cortical neurons were co-transfected with Htt N586-82Q and GFP. Four hours after transfection, neurons were treated with modulators with or without a pretreatment with $1 \mu\text{M}$ of NE-100. Forty-eight hours later, neurons were fixed and nuclei were stained. The protective effect of **MCP-1154** was blocked by NE-100. * $p < 0,05$ vs Htt N586 82Q. $n = 3$.

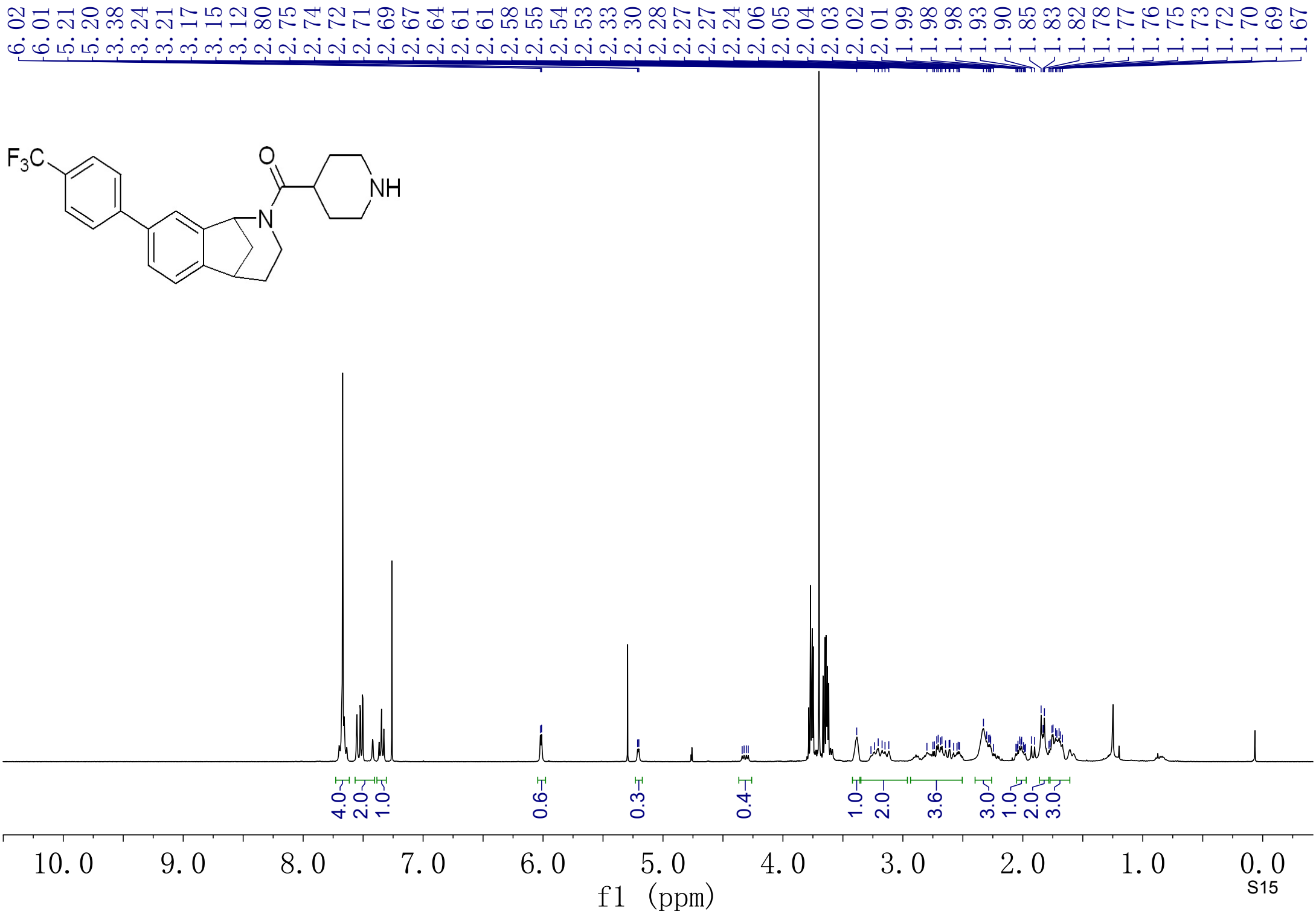
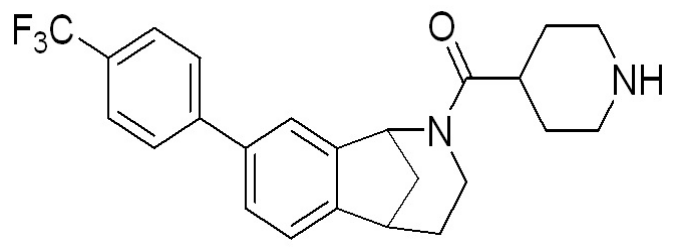


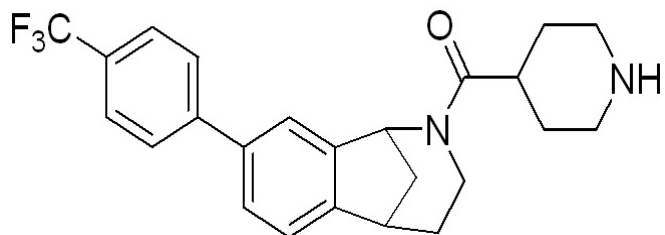


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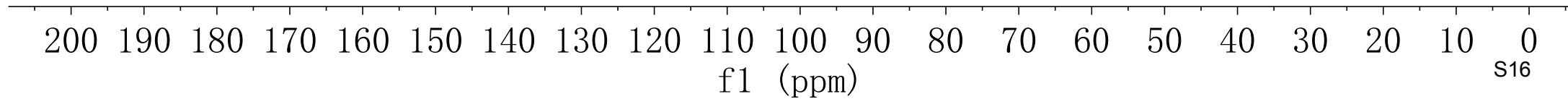


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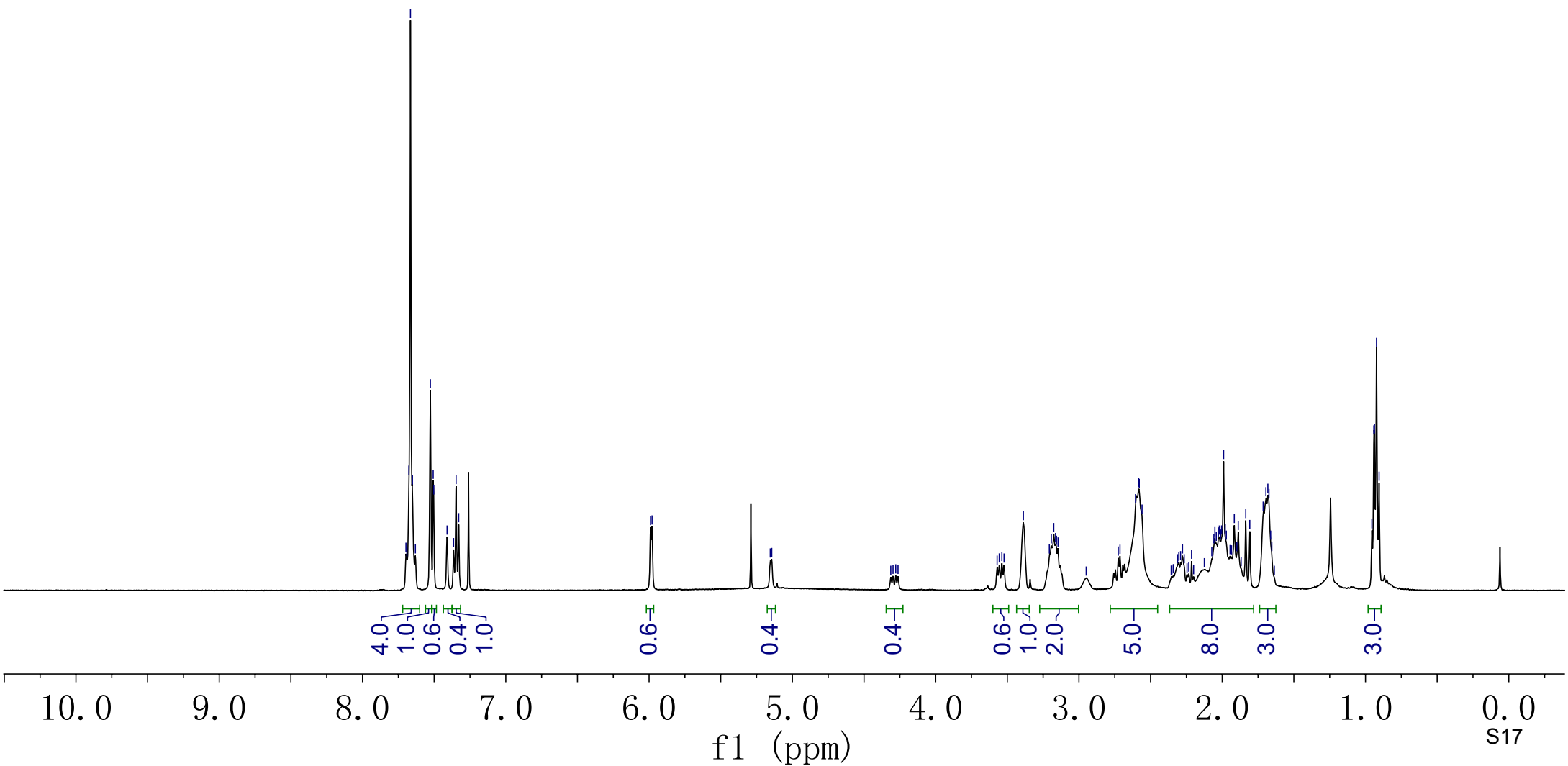
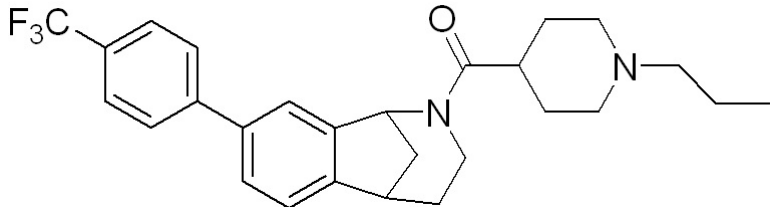
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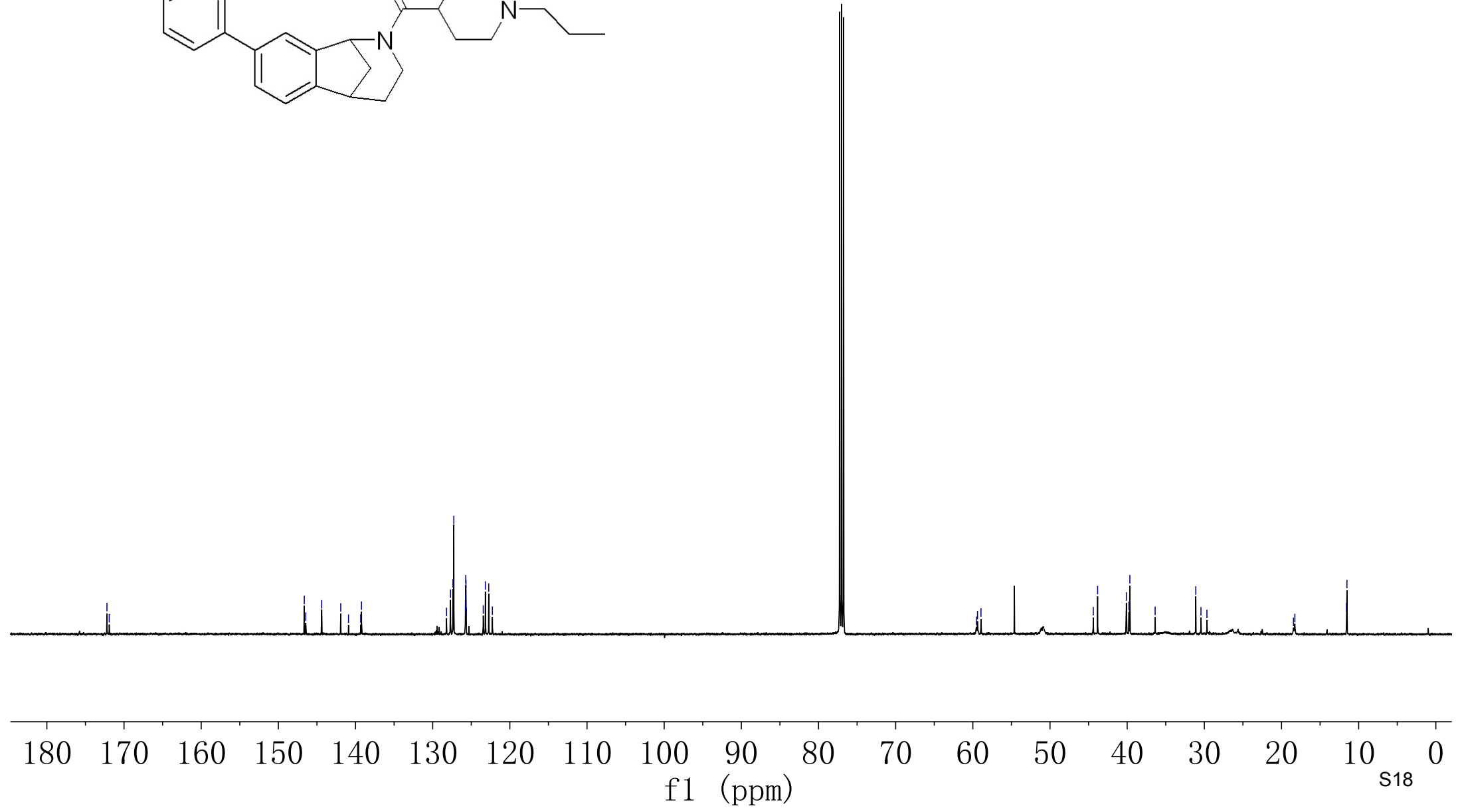
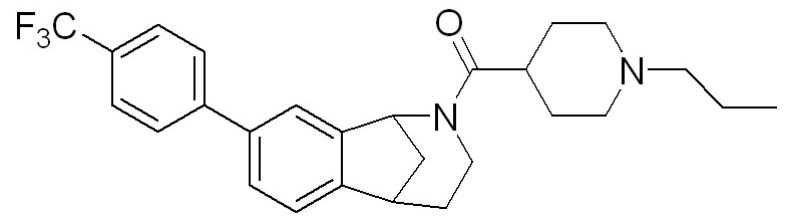
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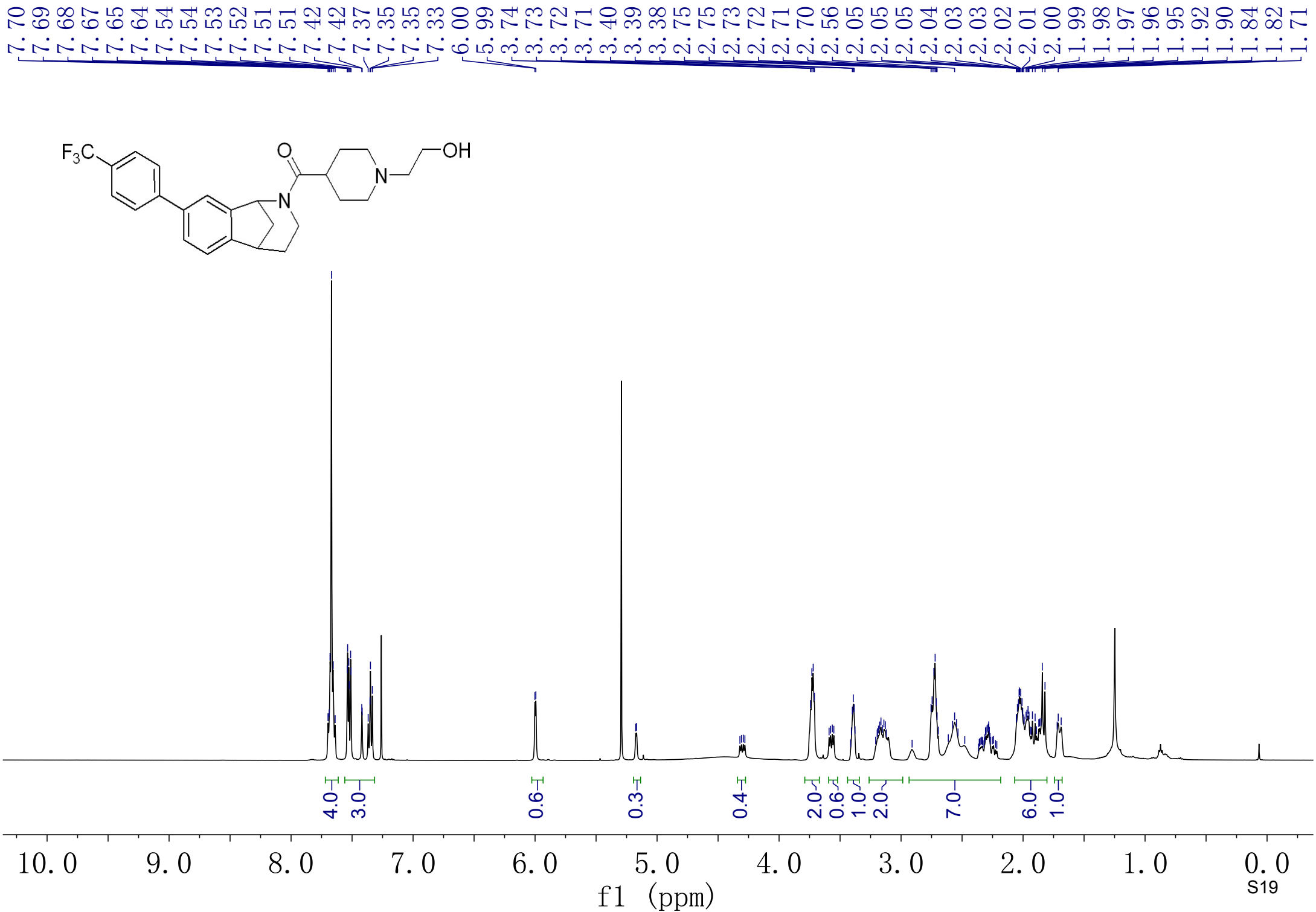
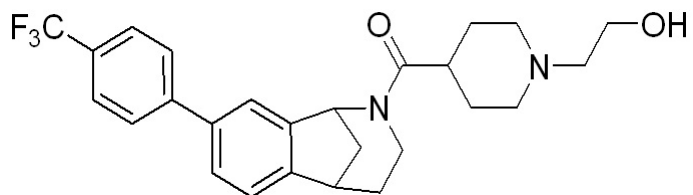


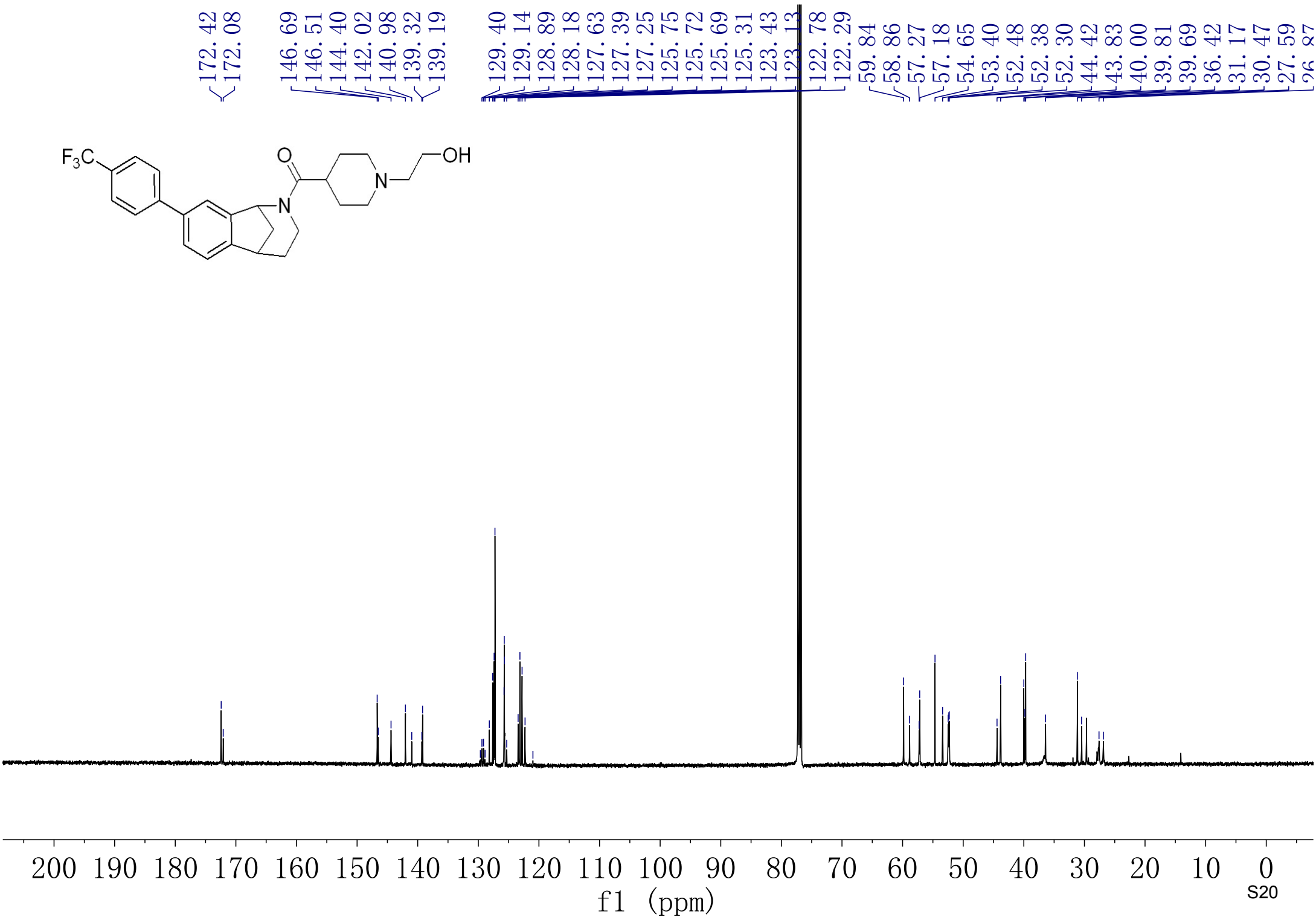
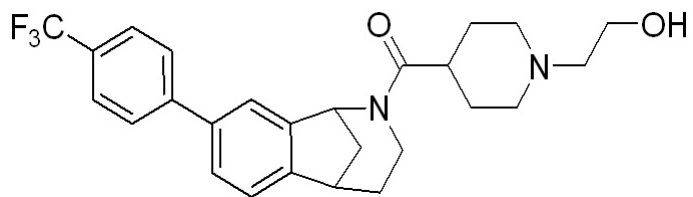
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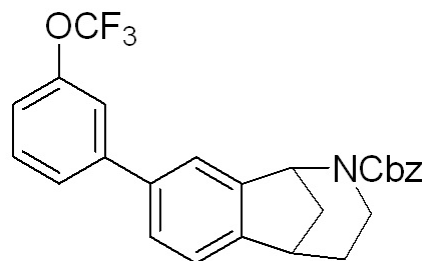
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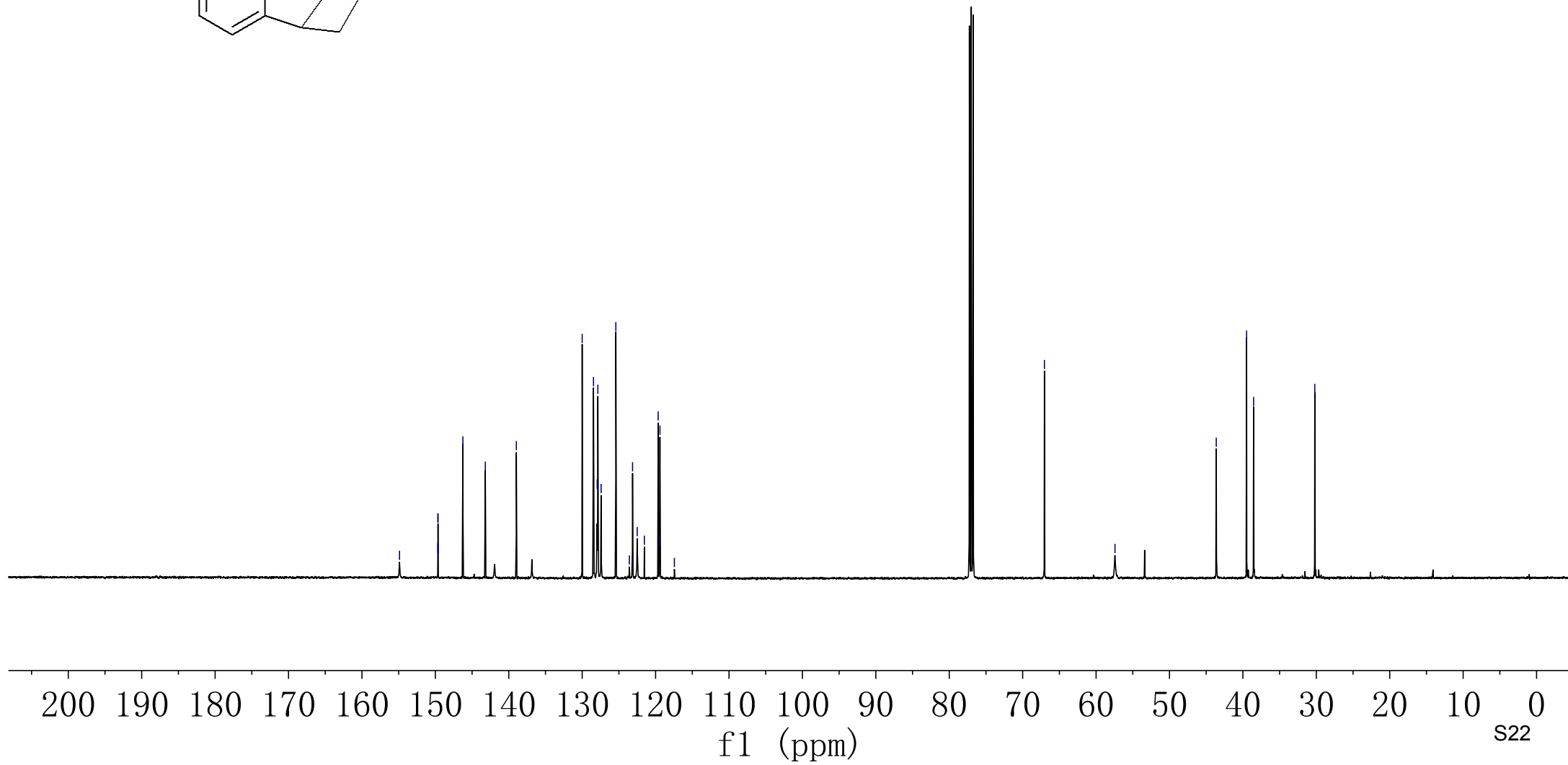
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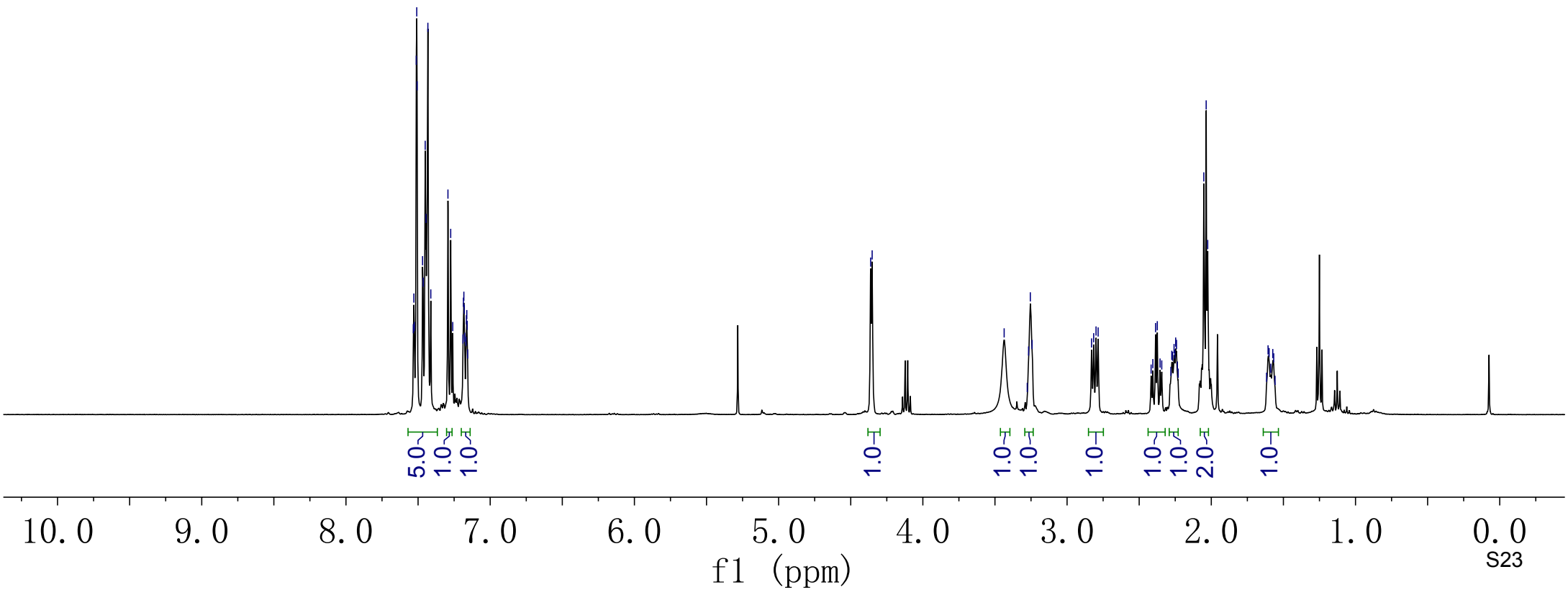
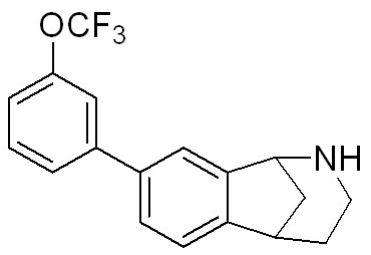
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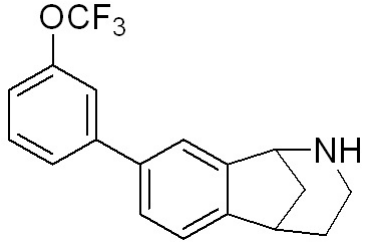
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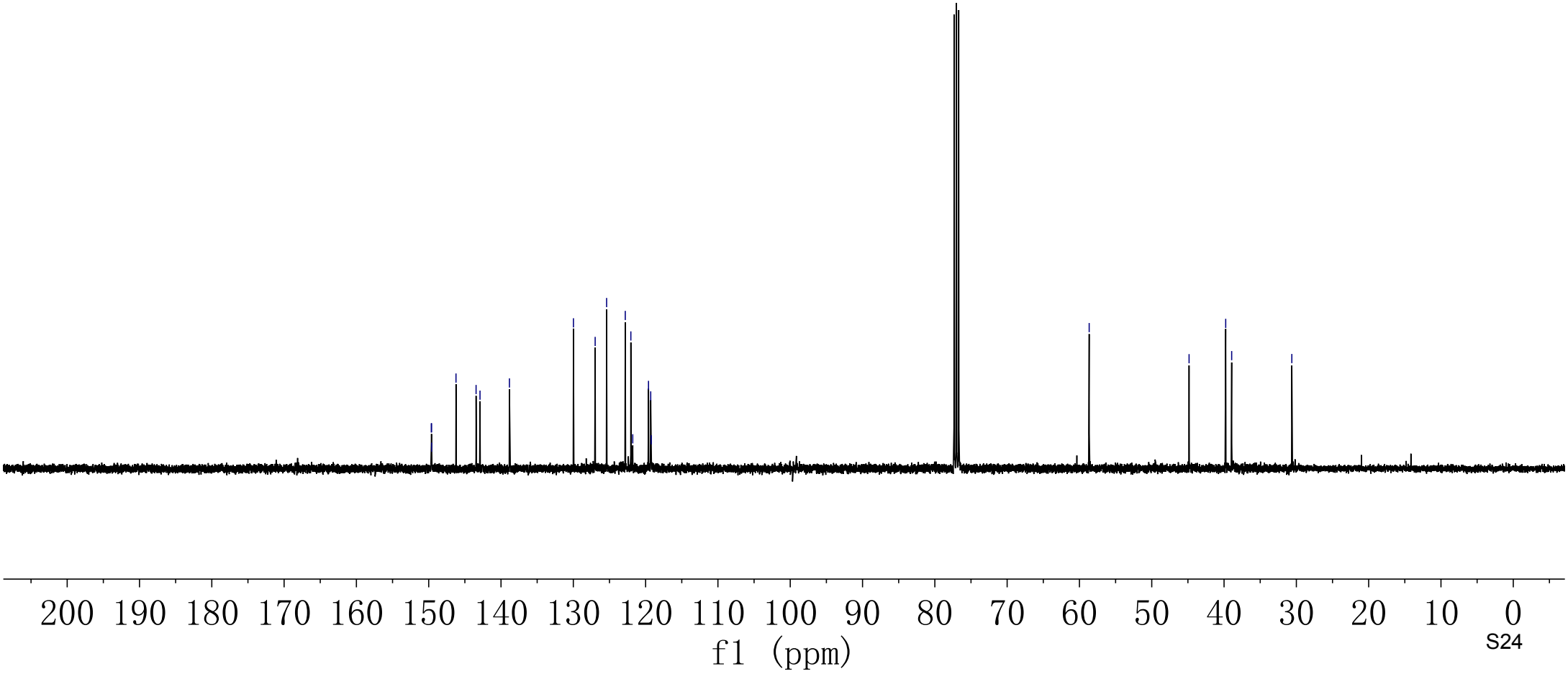
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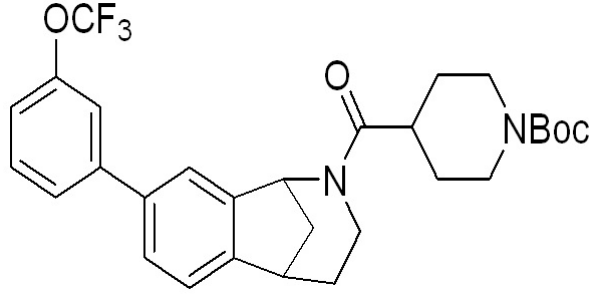
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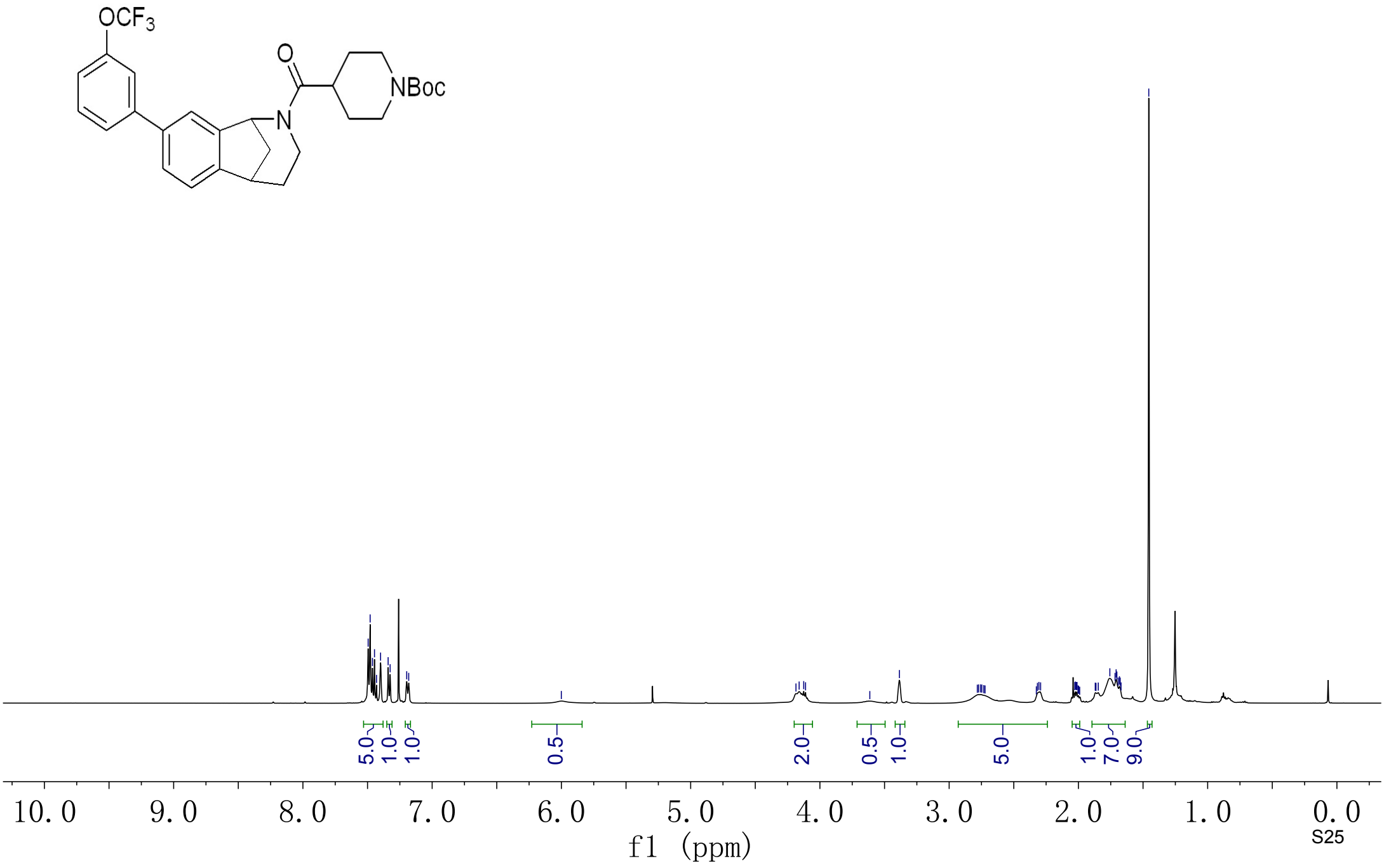
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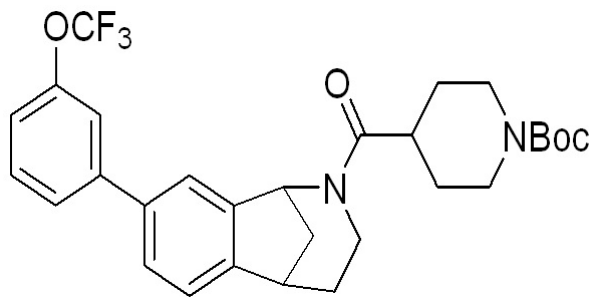
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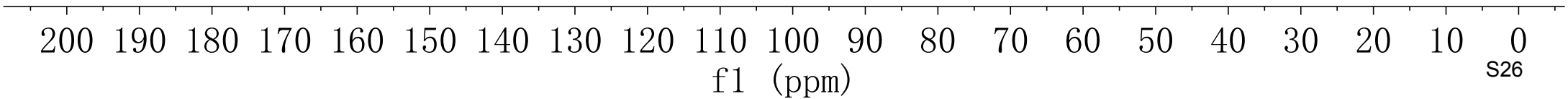




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149.67
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123.15
122.61
121.52
119.63
119.53
119.48
117.43

-79.56

-54.76
44.03
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39.77
38.91
31.04
29.69
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28.43



7.511
7.506
7.495
7.486
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7.475
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7.462
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