

# ChemMedChem

Supporting Information

## **Multitarget Derivatives of D2AAK1 as Potential Antipsychotics: The Effect of Substitution in the Indole Moiety**

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**Content:**

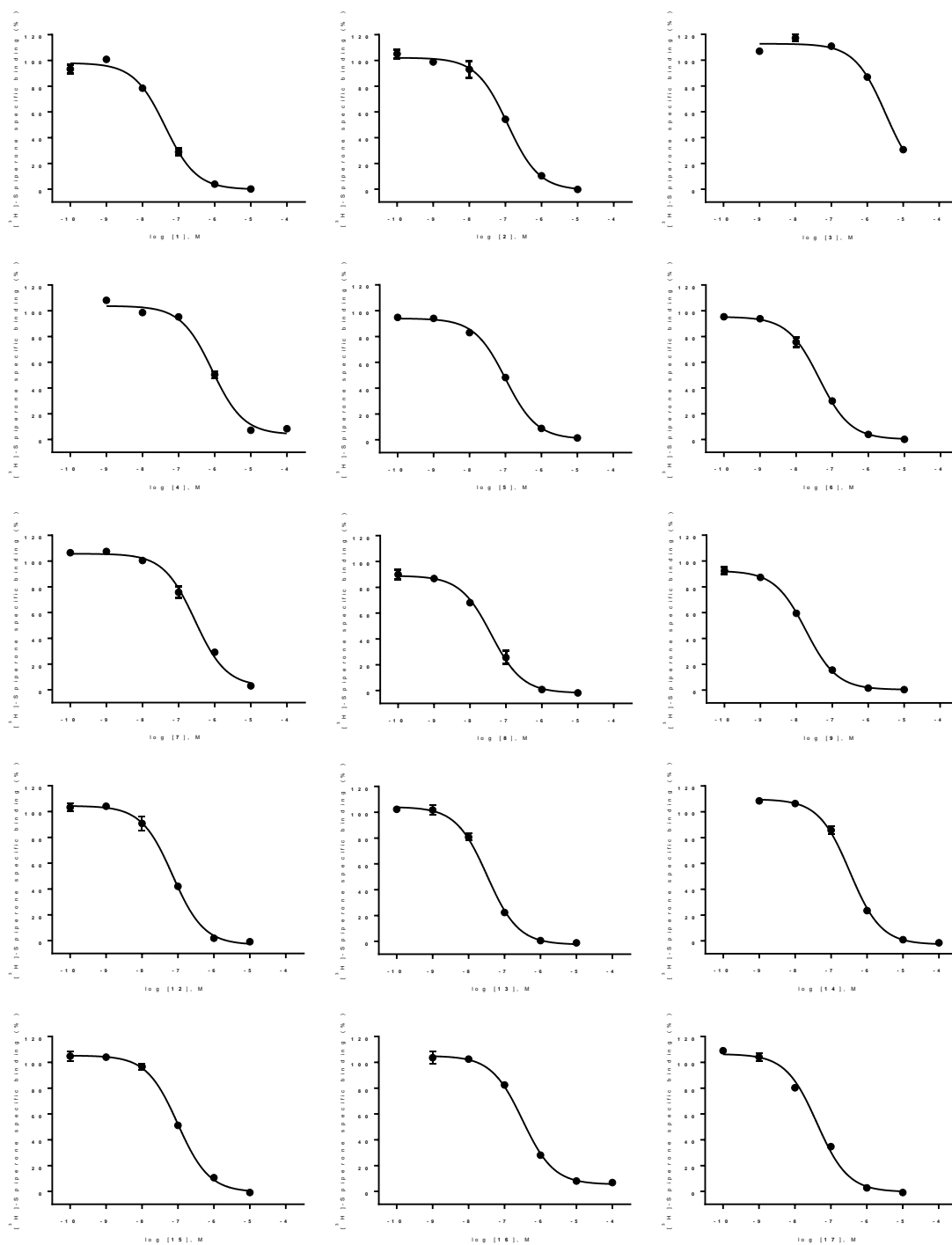
**Figure S1.** Competition radioligand binding curves for compounds **1-9** and **12-17** at human dopamine D<sub>2</sub> receptors.

**Figure S2.** Competition radioligand binding curves for compounds **1-3**, **6-9**, **12**, **13**, **15**, and **17** at human serotonin 5-HT<sub>1A</sub> receptors.

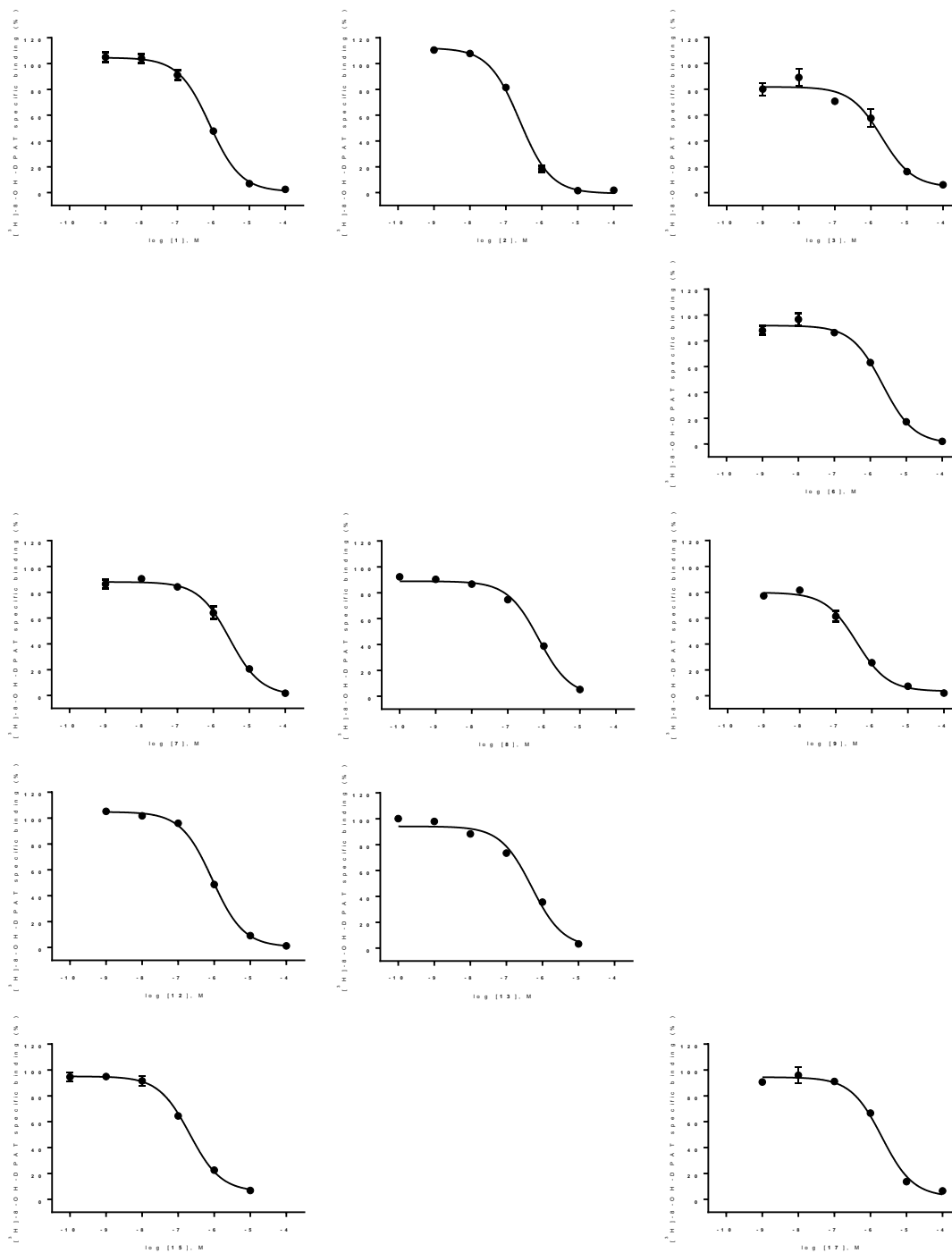
**Figure S3.** Competition radioligand binding curves for compounds **1**, **3**, **5**, **6**, **8**, **9**, and **12-17** at human serotonin 5-HT<sub>2A</sub> receptors.

**Table S1.** Detailed experimental conditions employed in radioligand binding assays.

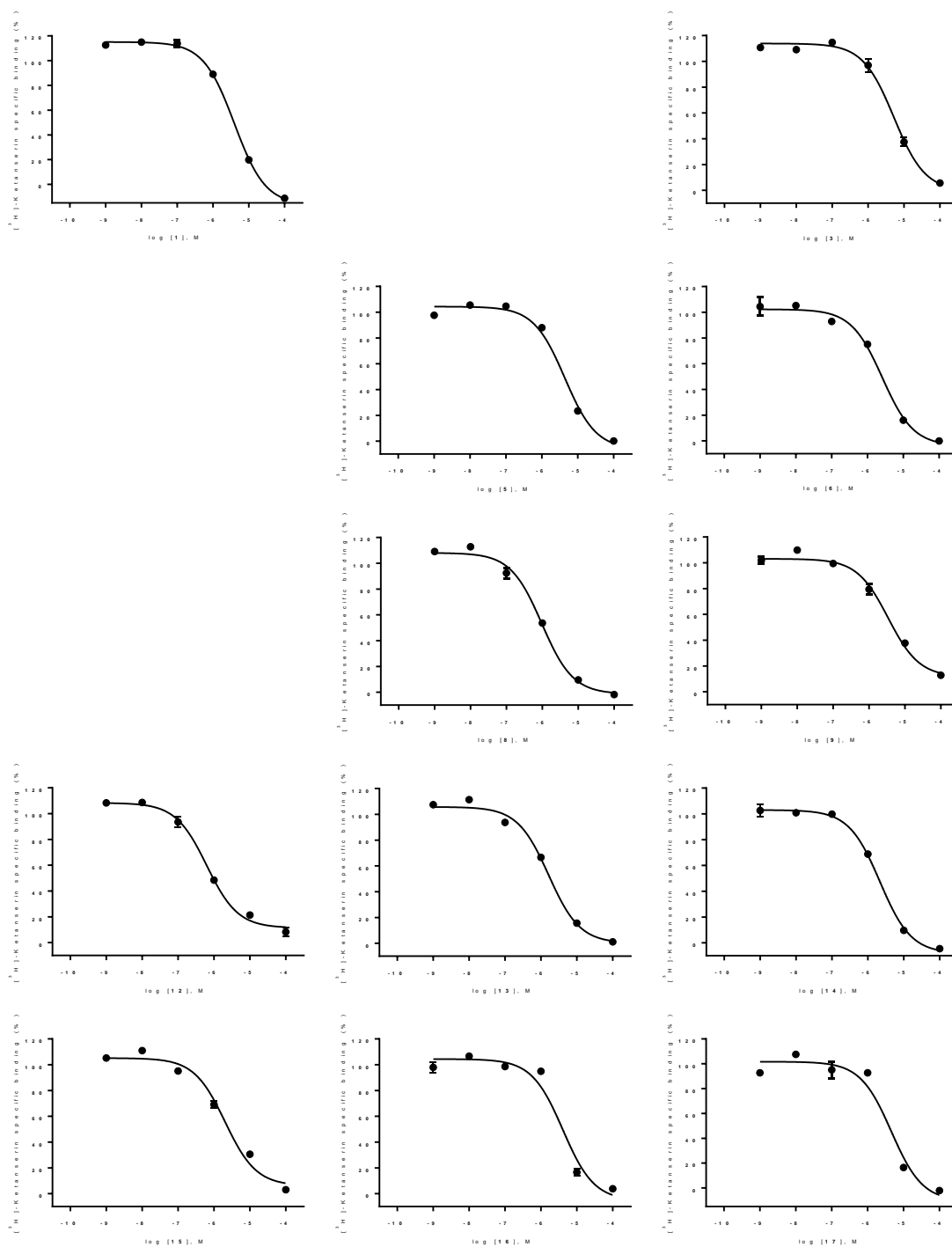
<sup>1</sup>H NMR, <sup>13</sup>C NMR spectra of reported compounds.



**Figure S1. Competition radioligand binding curves for compounds 1-9 and 12-17 in radioligand binding assays at D<sub>2</sub> receptors.** Concentration-dependent displacement of the specific binding of the D<sub>2</sub> radioligand [<sup>3</sup>H]-Spiperone by the compounds in membranes from CHO-K1 cells stably expressing the cloned human dopamine D<sub>2</sub> receptor (isoform D<sub>2short</sub>). The graphs show the data (mean ± SEM) of a representative experiment out of 2-3 independent experiments performed in duplicate.



**Figure S2. Competition radioligand binding curves for compounds 1-3, 6-9, 12, 13, 15, and 17 in radioligand binding assays at 5-HT<sub>1A</sub> receptors.** Concentration-dependent displacement of the specific binding of the 5- radioligand [<sup>3</sup>H]-8-OH-DPAT by the compounds in membranes from HEK293 cells stably expressing the cloned human serotonin 5-HT<sub>1A</sub> receptor. The graphs show the data (mean ± SEM) of a representative experiment out of 2 independent experiments performed in duplicate.



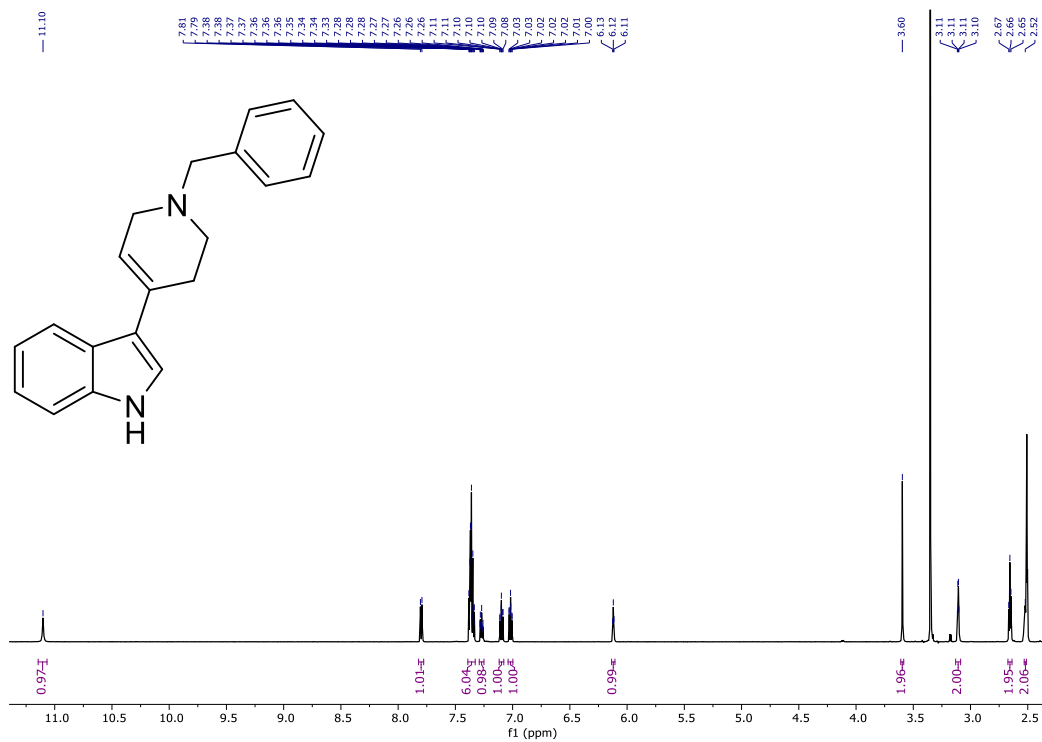
**Figure S3. Competition radioligand binding curves for compounds 1, 3, 5, 6, 8, 9, and 12-17 in radioligand binding assays at 5-HT<sub>2A</sub> receptors.** Concentration-dependent displacement of the specific binding of the radioligand [<sup>3</sup>H]-Ketanserin by the compounds in membranes from CHO-K1 cells stably expressing the cloned human serotonin 5-HT<sub>2A</sub> receptor. The graphs show the data (mean ± SEM) of a representative experiment out of 2-4 independent experiments performed in duplicate.

**Table S1.** Detailed experimental conditions employed in radioligand binding assays.

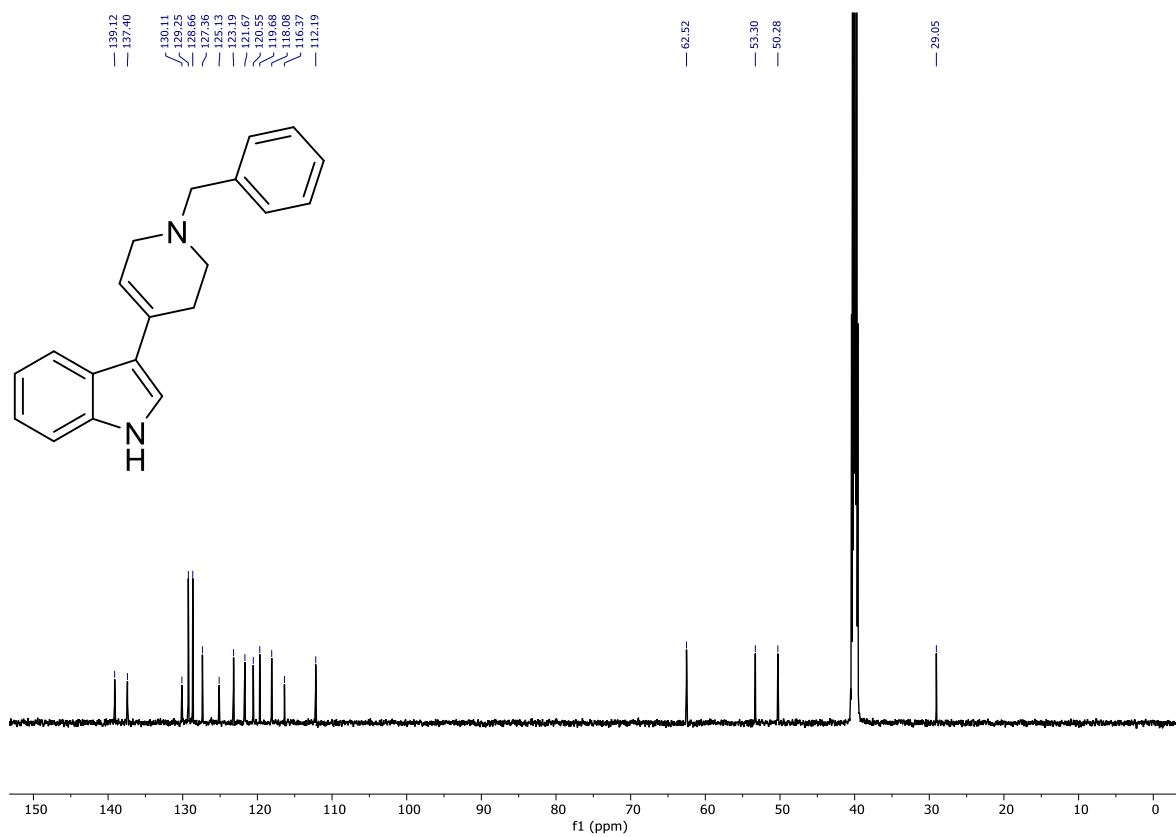
	<i>hD<sub>2</sub></i>	<i>h5-HT<sub>1A</sub></i>	<i>h5-HT<sub>2A</sub></i>	<i>hH<sub>1</sub></i>	<i>hM<sub>1</sub></i>
Protein per well	35 µg	10 µg	130 µg	50 µg	2.5 µg
Assay buffer	50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 5 mM MgCl <sub>2</sub> , 1 mM EDTA (pH = 7.4)	50 mM Tris-HCl, 5 mM MgSO <sub>4</sub> (pH = 7.4)	50 mM Tris-HCl (pH = 7.5)	41.4 mM Na <sub>2</sub> HPO <sub>4</sub> , 8.6 mM KH <sub>2</sub> PO <sub>4</sub> (pH = 7.5)	10 mM HEPES, 100 mM NaCl, 10 mM MgCl <sub>2</sub> , 0.2% BSA (pH = 7.4)
Radioligand	0.6 nM [ <sup>3</sup> H]-Spiperone <sup>a</sup>	1 nM [ <sup>3</sup> H]-8-OH-DPAT <sup>b</sup>	1.25 nM [ <sup>3</sup> H]-Ketanserin <sup>c</sup>	4 nM [ <sup>3</sup> H]-Pyrilamine <sup>d</sup>	7 nM [ <sup>3</sup> H]-Pirenzepine <sup>e</sup>
Nonspecific binding	100 µM Sulpiride	10 µM Serotonin	1 µM Methysergide	10 µM Triprolidine	200 µM Pirenzepine
Incubation	25°C/120 min	37°C/120 min	37°C/30 min	27°C/60 min	25°C/90 min
Filter plate	GF/C <sup>f</sup>	GF/C <sup>f</sup>	GF/B <sup>g</sup>	GF/B <sup>g</sup>	GF/C <sup>f</sup>
Filter plate pre-treatment	presoaked with 0.5% PEI <sup>h</sup> for 1 h and washed with 250 µl incubation buffer	presoaked with 0.5% PEI <sup>h</sup> for 1 h and washed with 250 µl incubation buffer	presoaked with 0.5% PEI <sup>h</sup> for 1 h and washed with 250 µl incubation buffer	presoaked with 0.1% Tween20 for 1 h and washed with 250 µl incubation buffer	presoaked with 0.1% Tween20 for 1 h and washed with 250 µl incubation buffer
Wash buffer	50 mM Tris-HCl, 0.9% NaCl (pH = 7.4)	50 mM Tris-HCl (pH = 7.4)	50 mM Tris-HCl (pH = 6.6)	41.4 mM Na <sub>2</sub> HPO <sub>4</sub> , 8.6 mM KH <sub>2</sub> PO <sub>4</sub> (pH = 7.5)	10 mM HEPES, 0.1% BSA (pH = 7.4)
Washing steps	4 x 250 µl wash buffer	4 x 250 µl wash buffer	6 x 250 µl wash buffer	6 x 250 µl wash buffer	4 x 250 µl wash buffer

<sup>a</sup> 54.3 Ci/mmol, 1 mCi/ml, PerkinElmer NET1187250UC. <sup>b</sup> 200 Ci/mmol, 1 mCi/ml, PerkinElmer NET929250UC. <sup>c</sup> 47.3 Ci/mmol, 1 mCi/ml, PerkinElmer NET791250UC. <sup>d</sup> 20.1 Ci/mmol, 1 mCi/ml, PerkinElmer NET594250UC; <sup>e</sup> 85.0 Ci/mmol, 1 mCi/ml, PerkinElmer NET780250UC. <sup>f</sup> MultiScreen<sub>HTS</sub> FB Filter Plate, PerkinElmer MSFBN6. <sup>g</sup> MultiScreen<sub>HTS</sub> FB Filter Plate, PerkinElmer MSFCN6. <sup>h</sup> PEI, Poly(ethyleneimine).

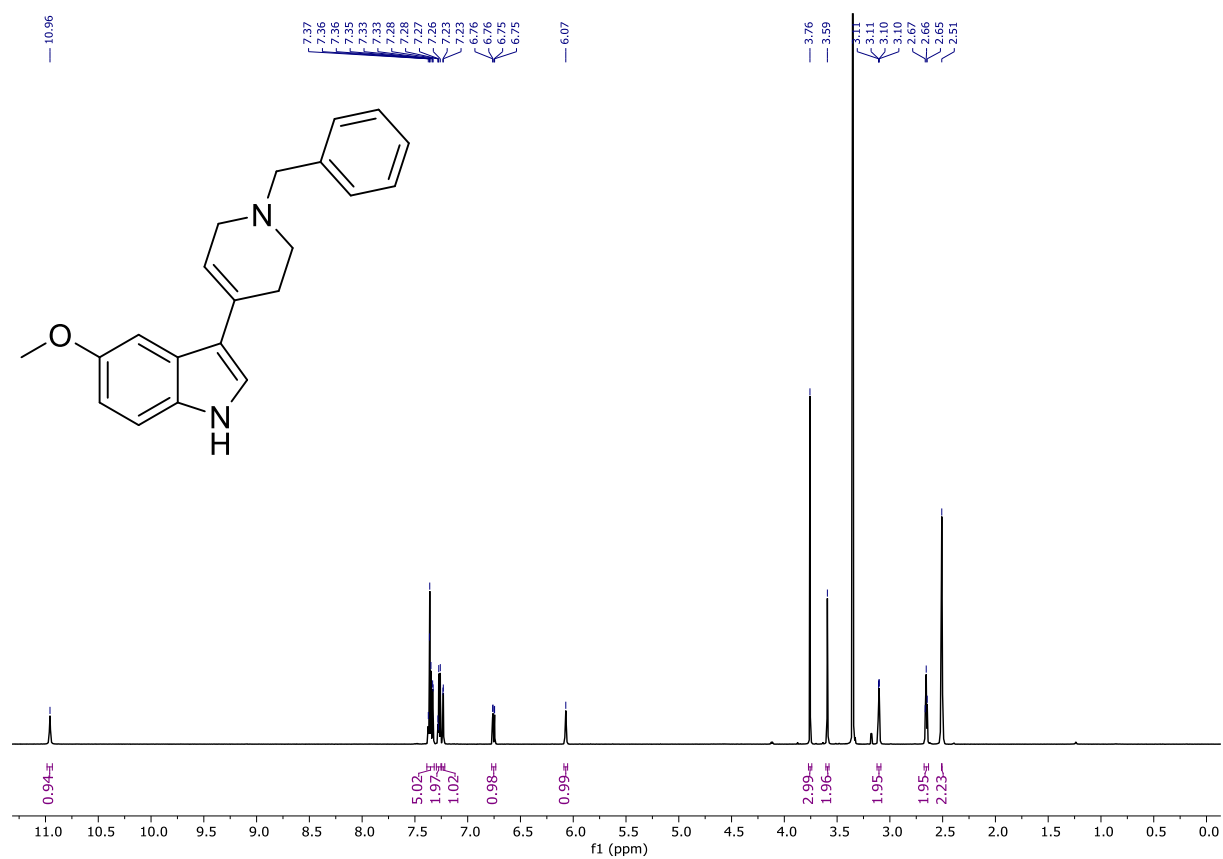
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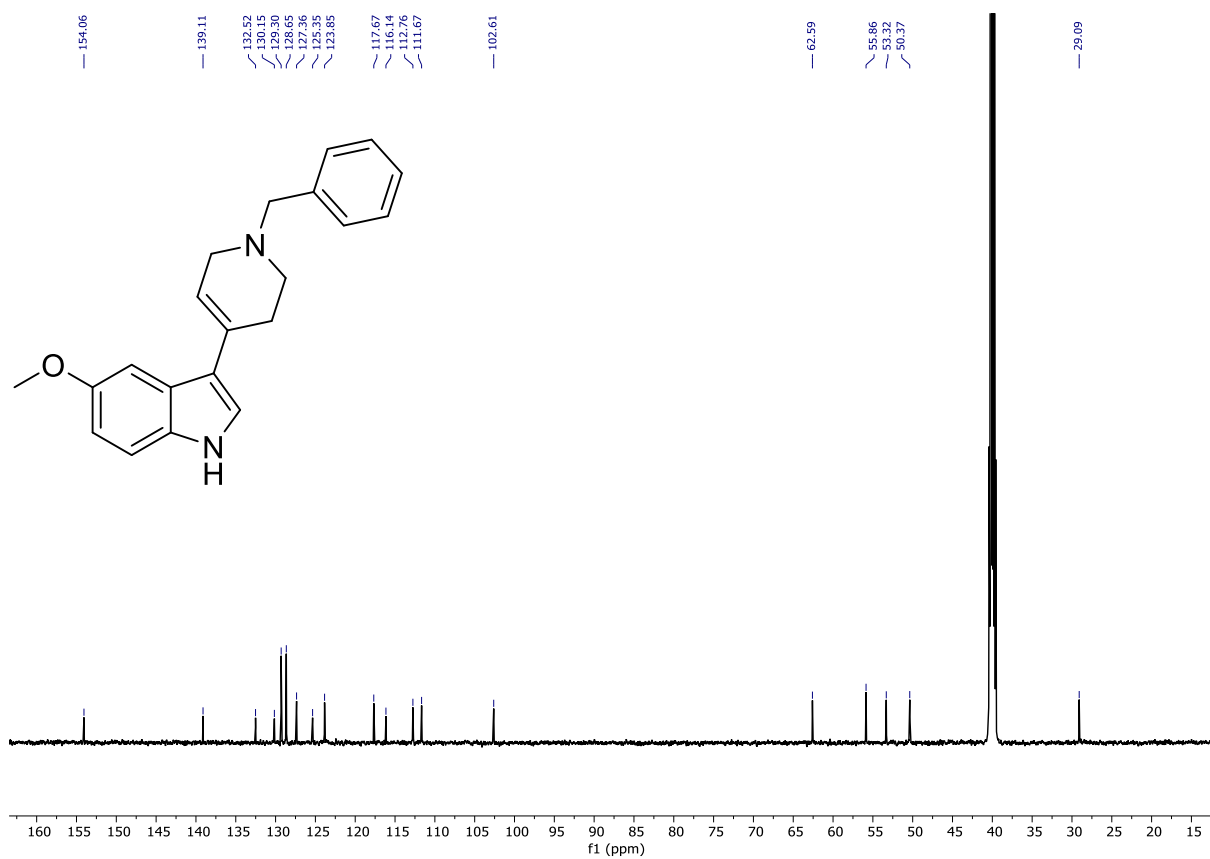
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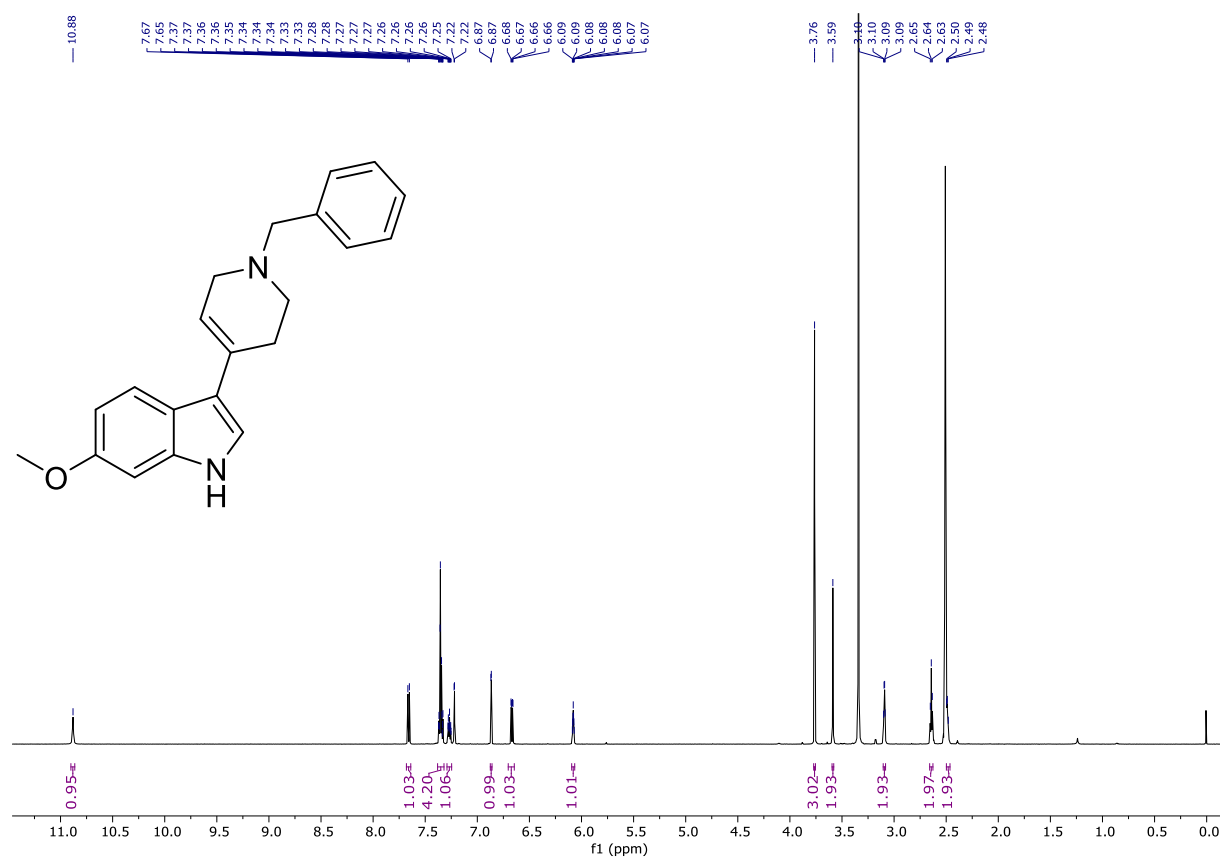
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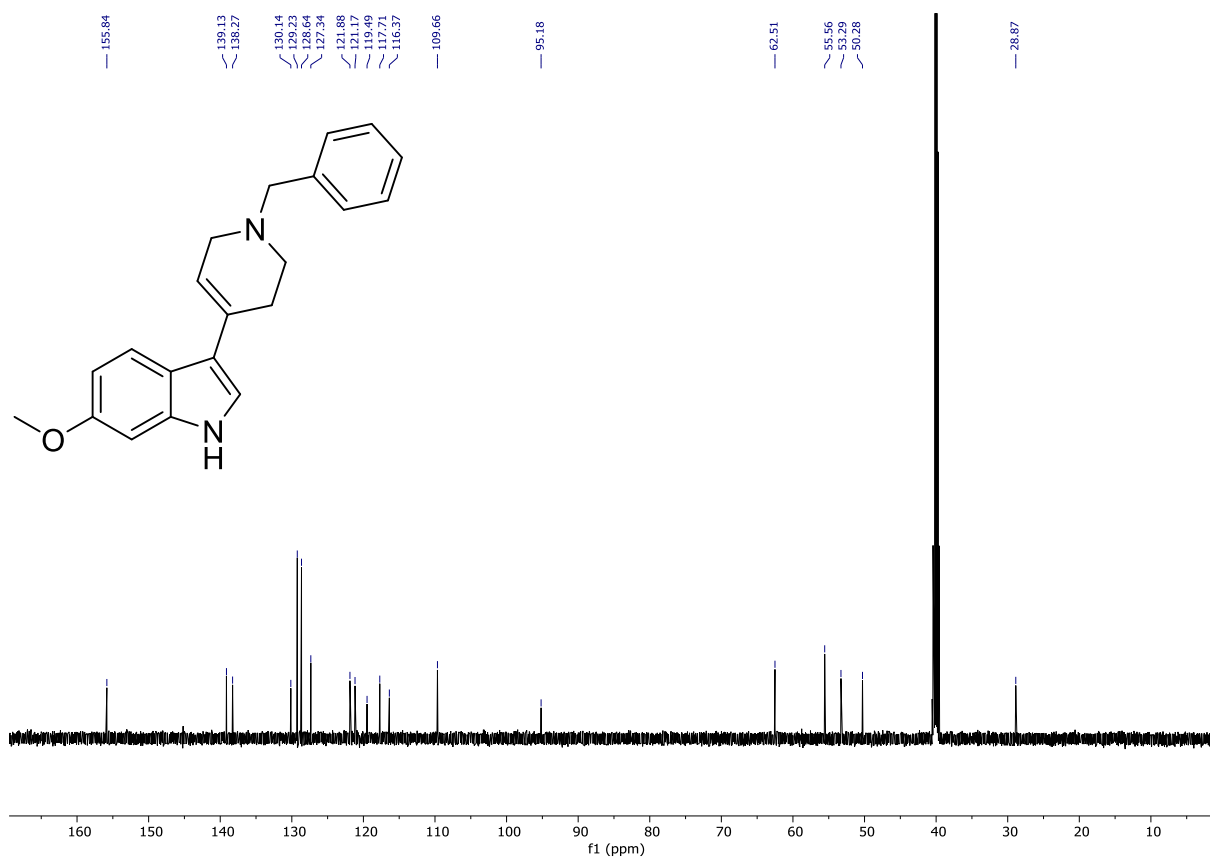
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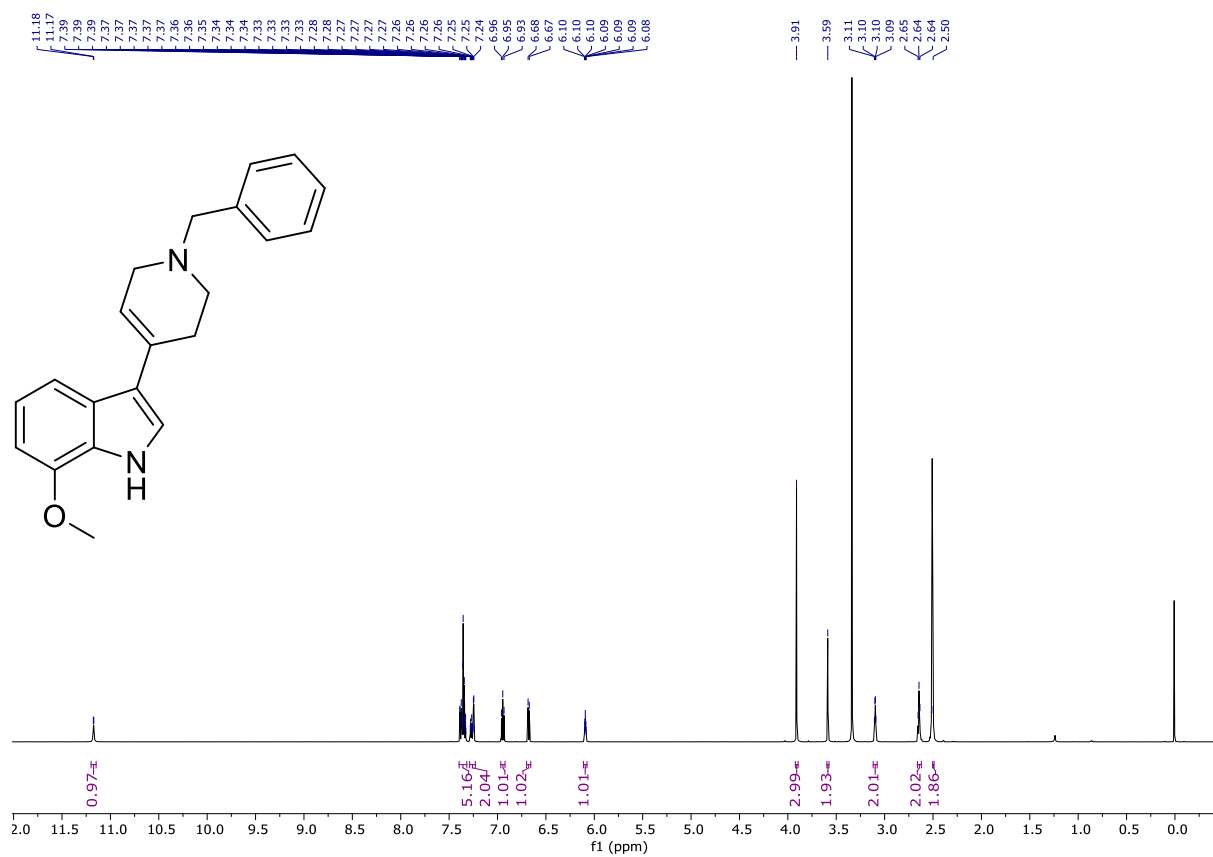
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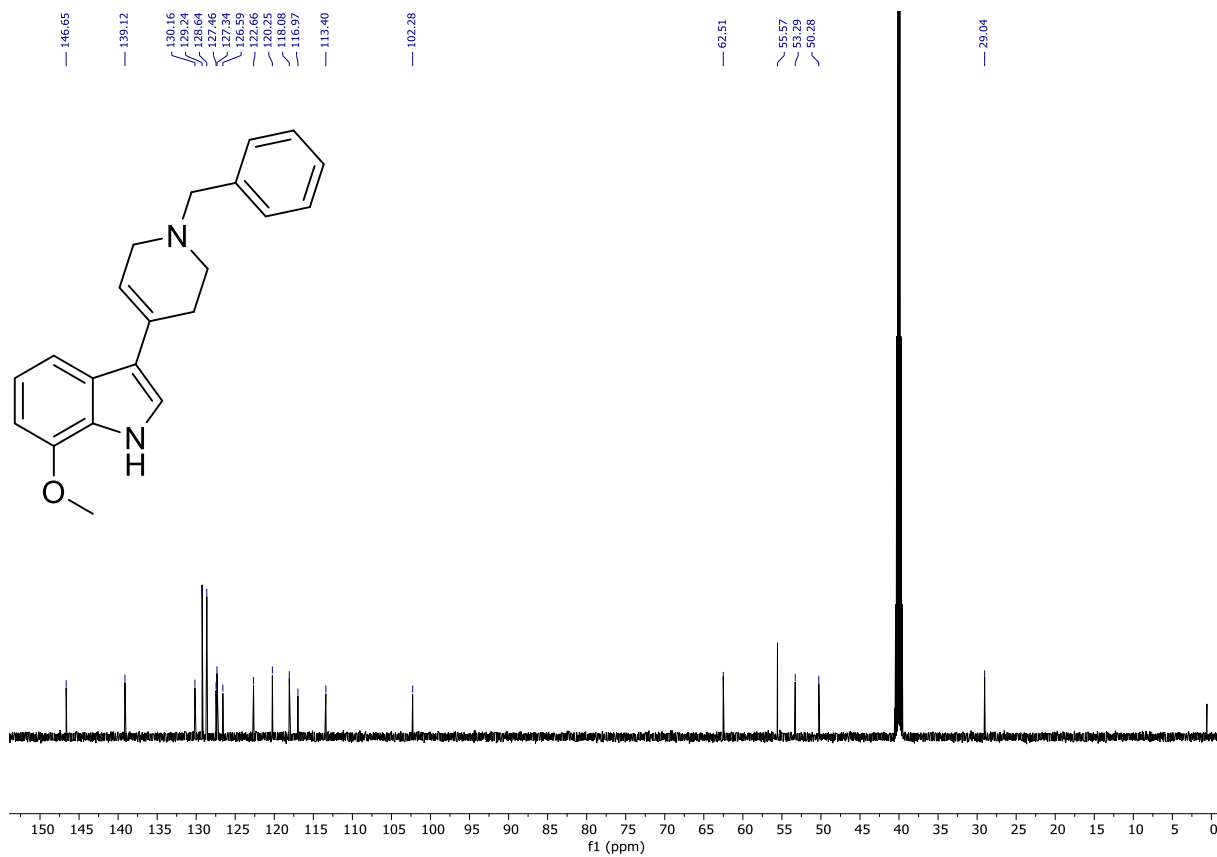
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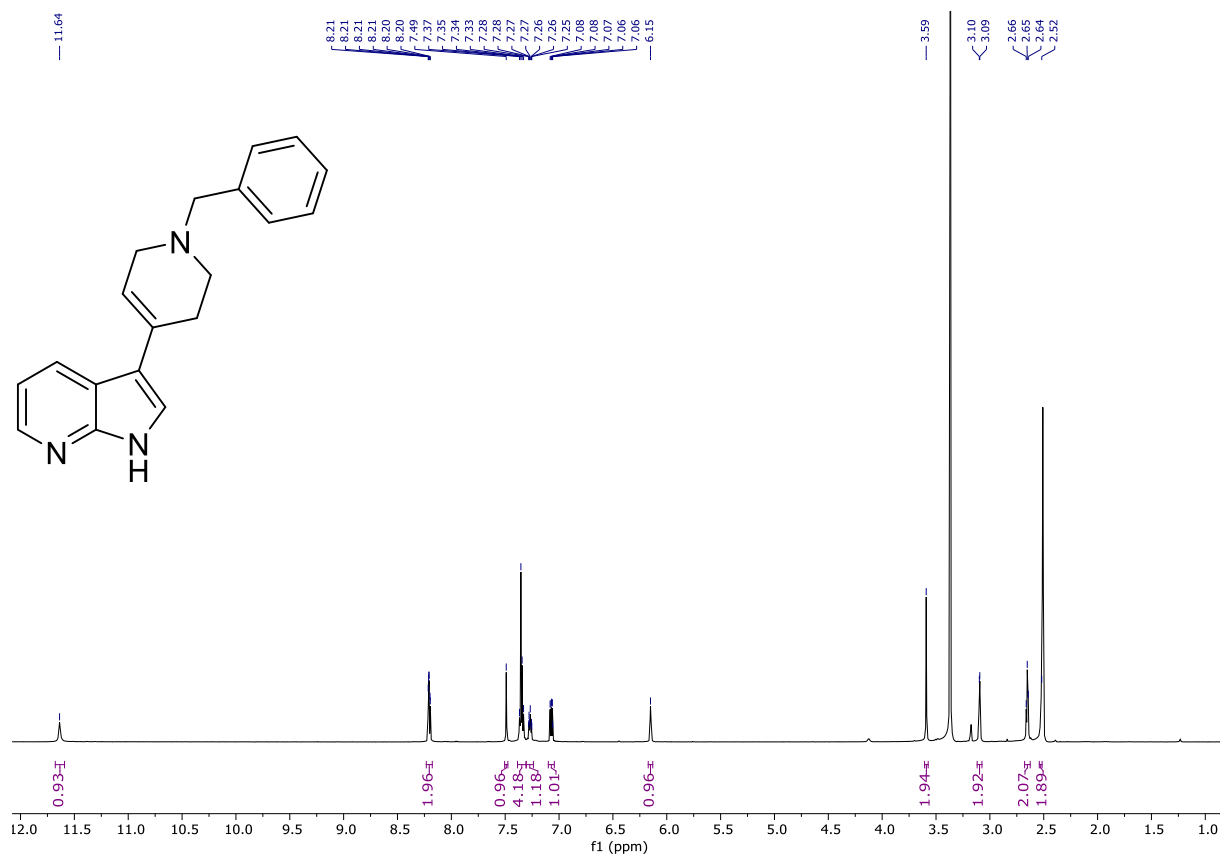
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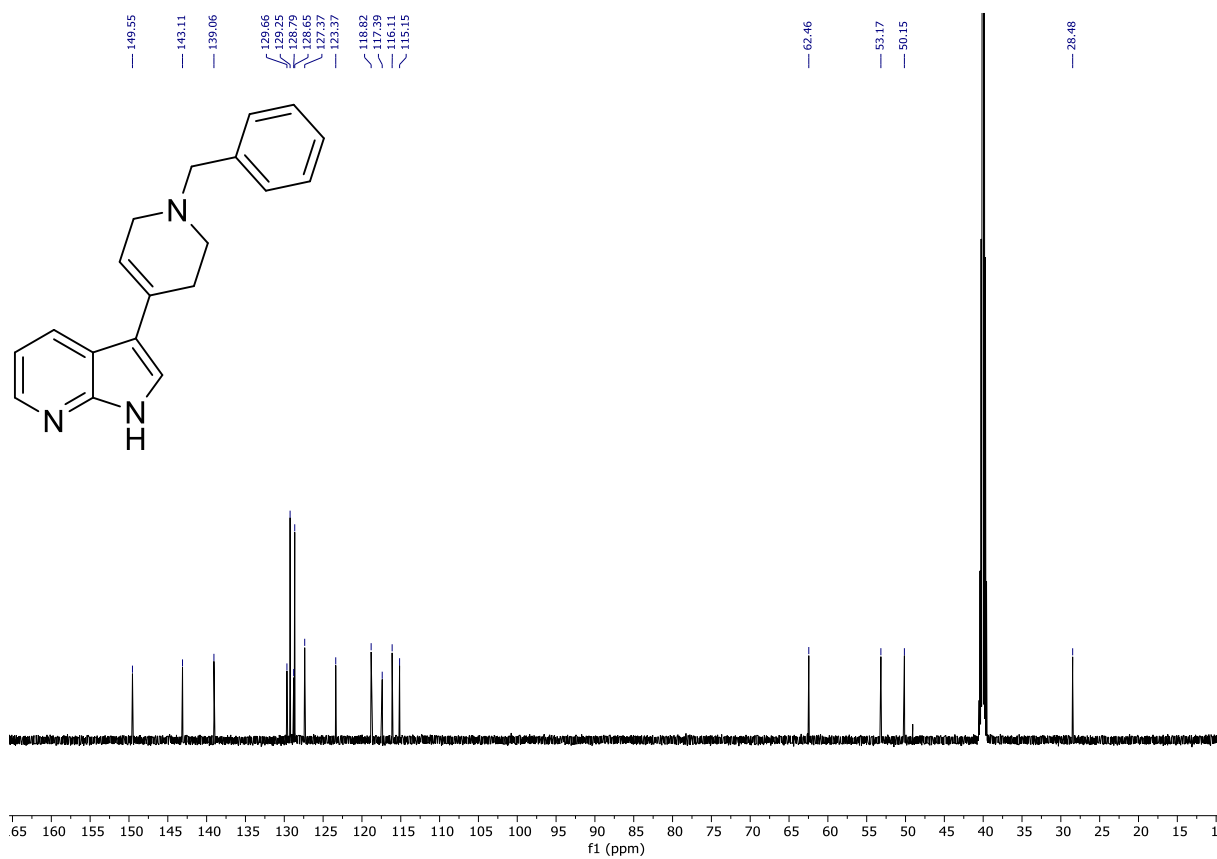
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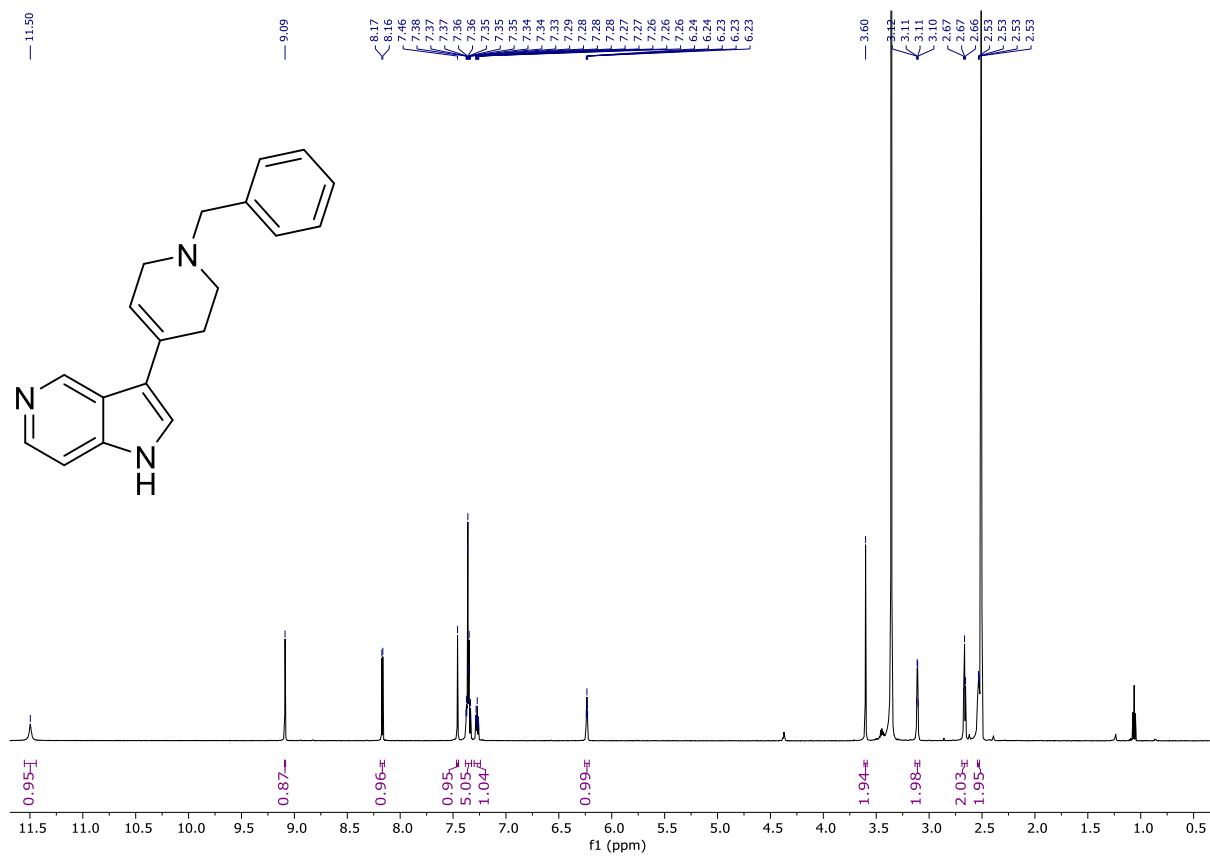
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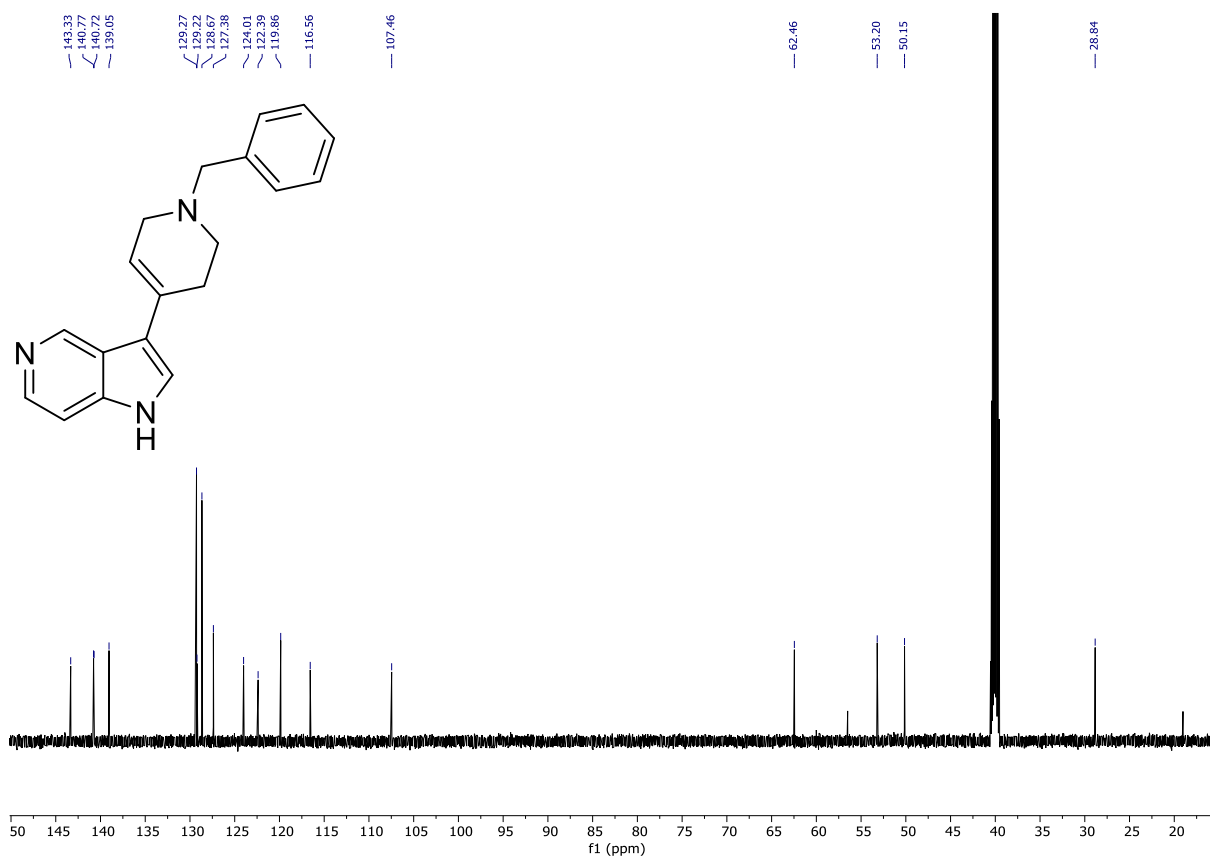
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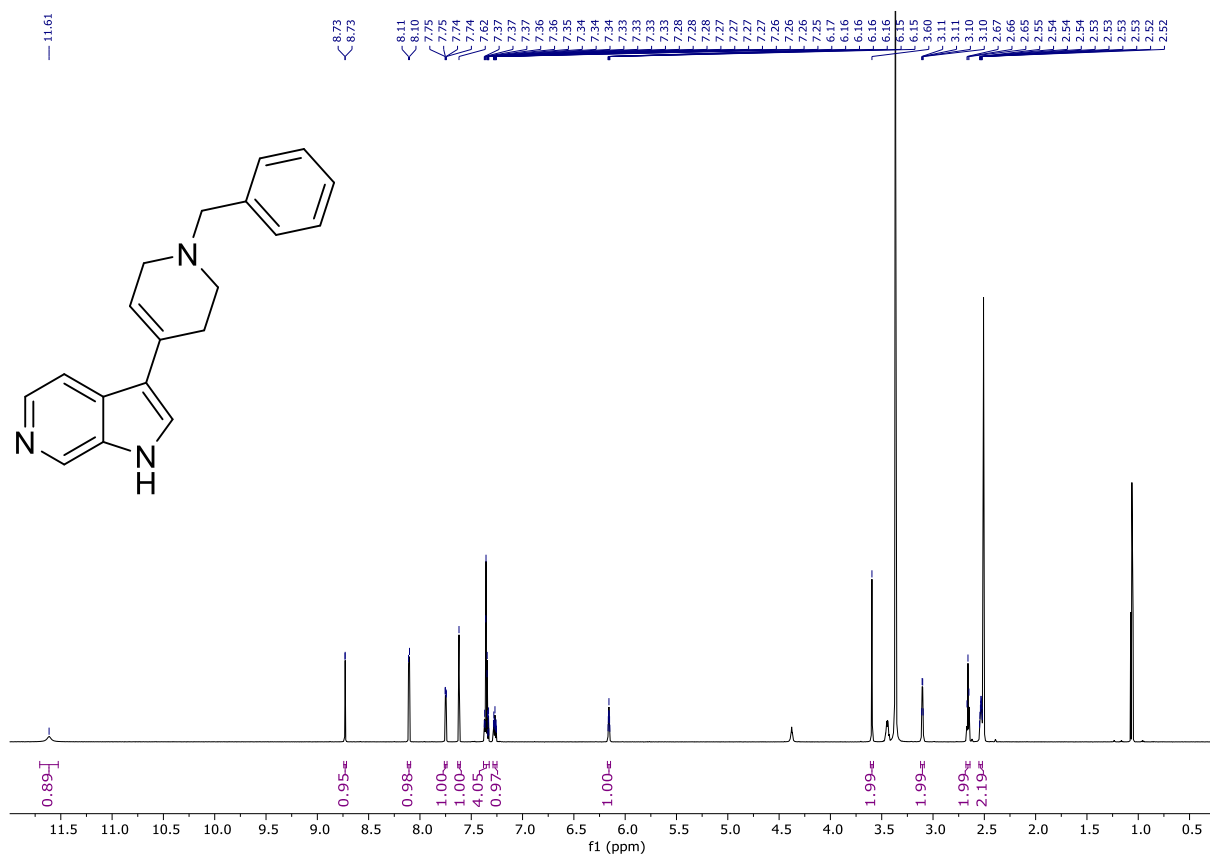
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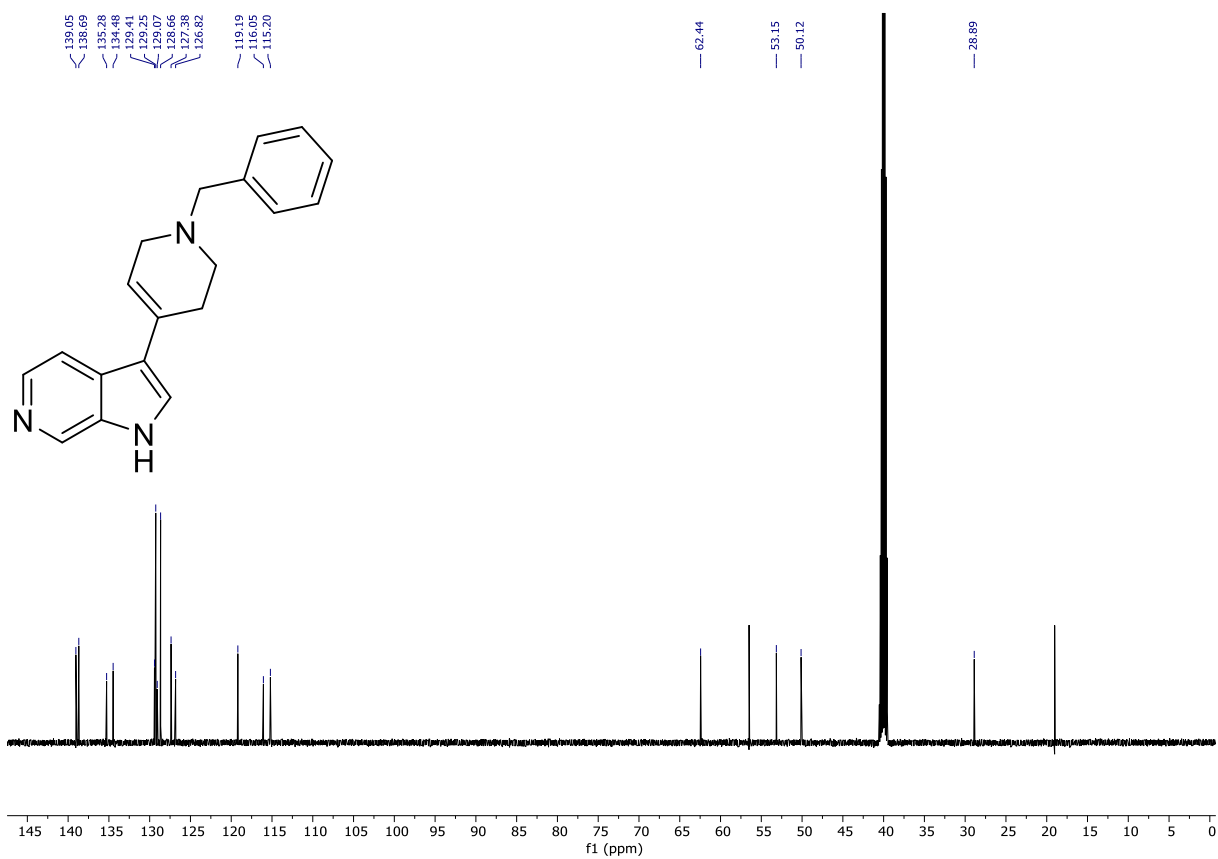
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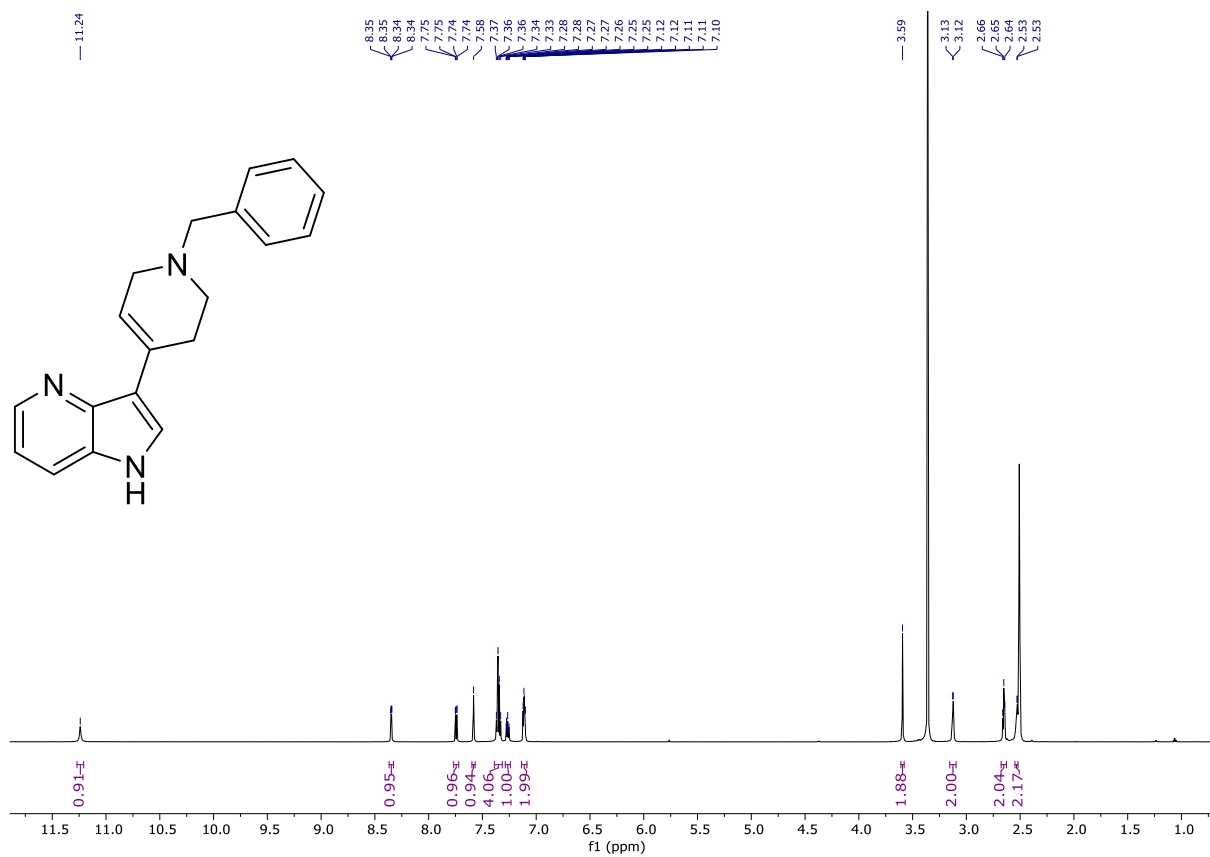
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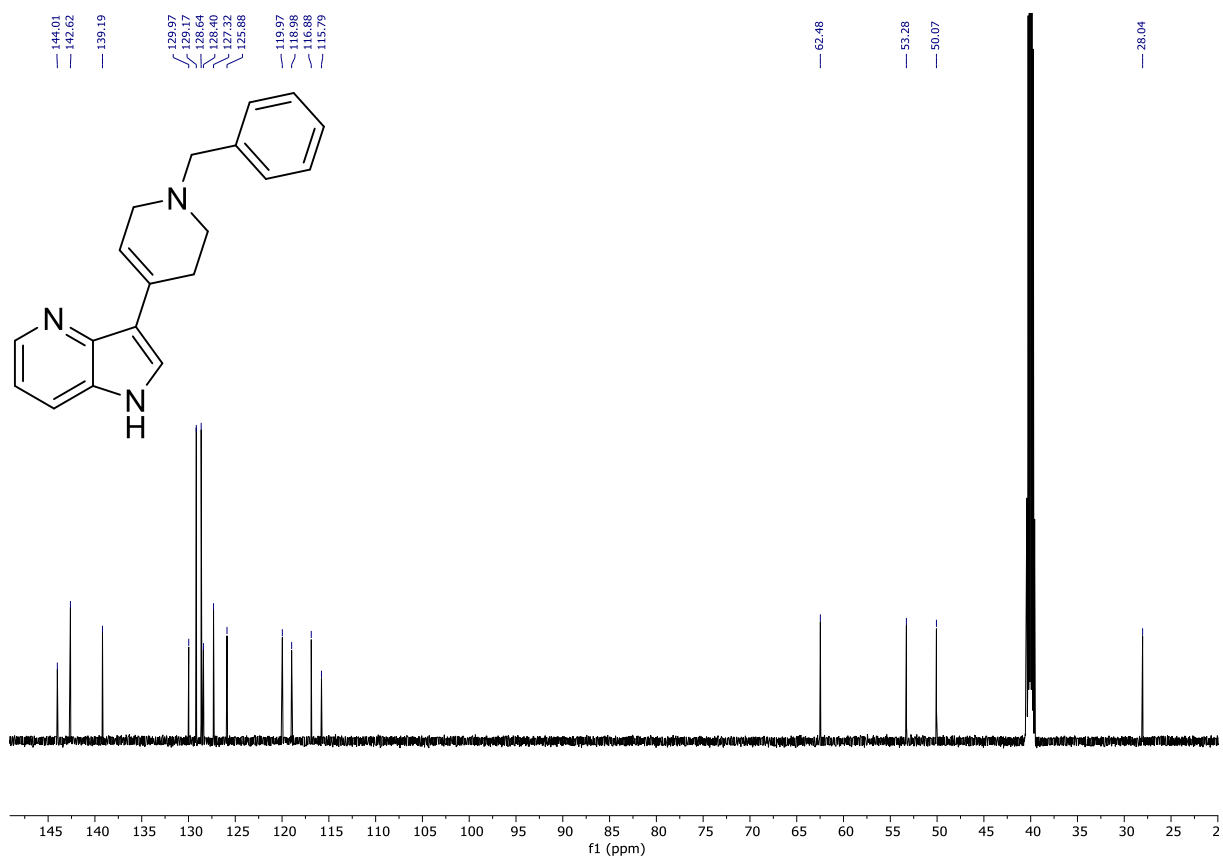
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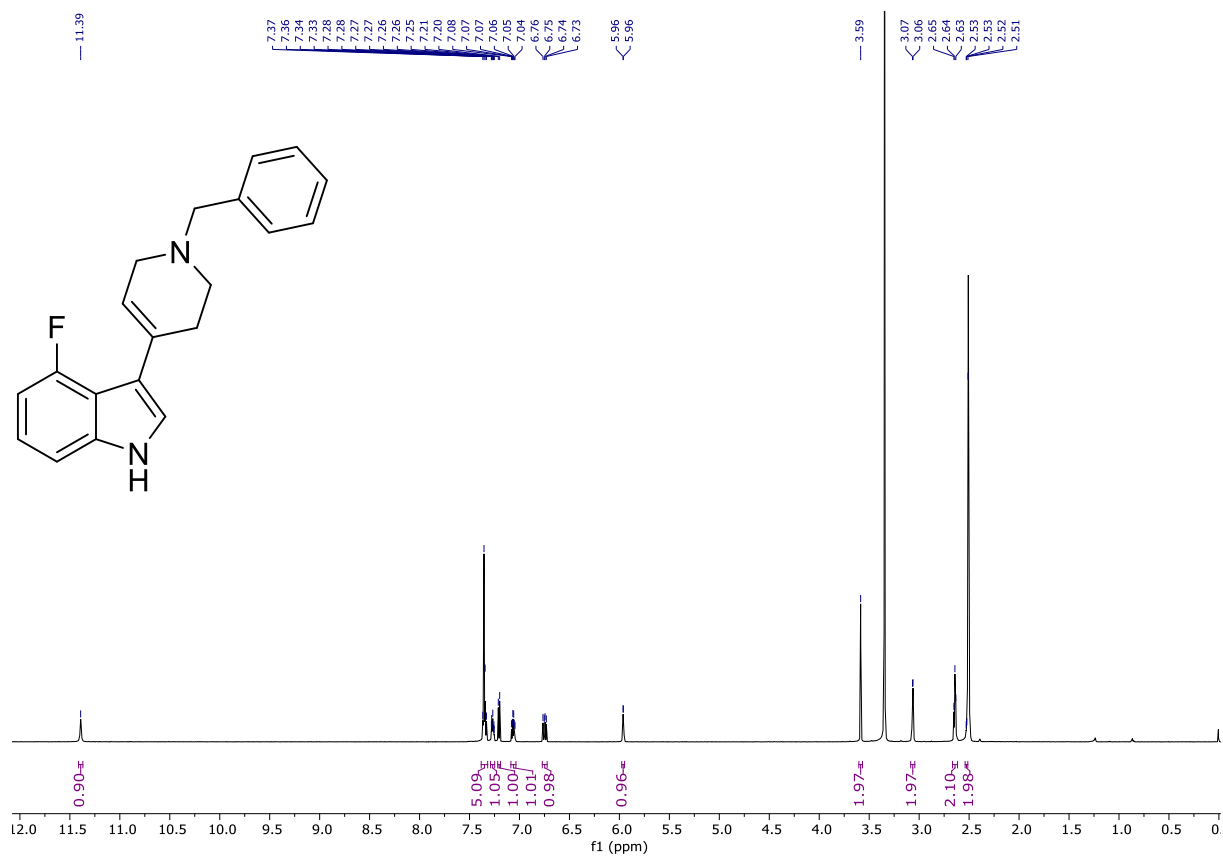
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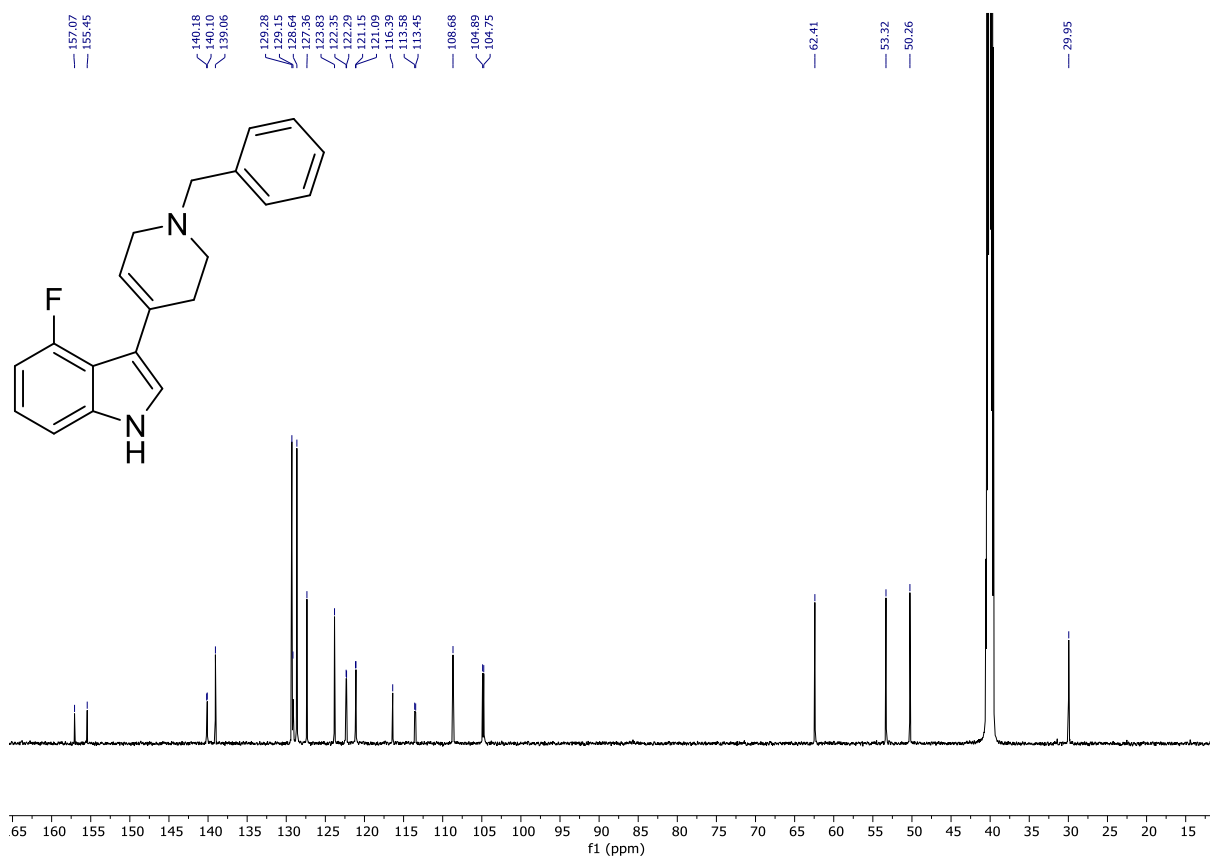
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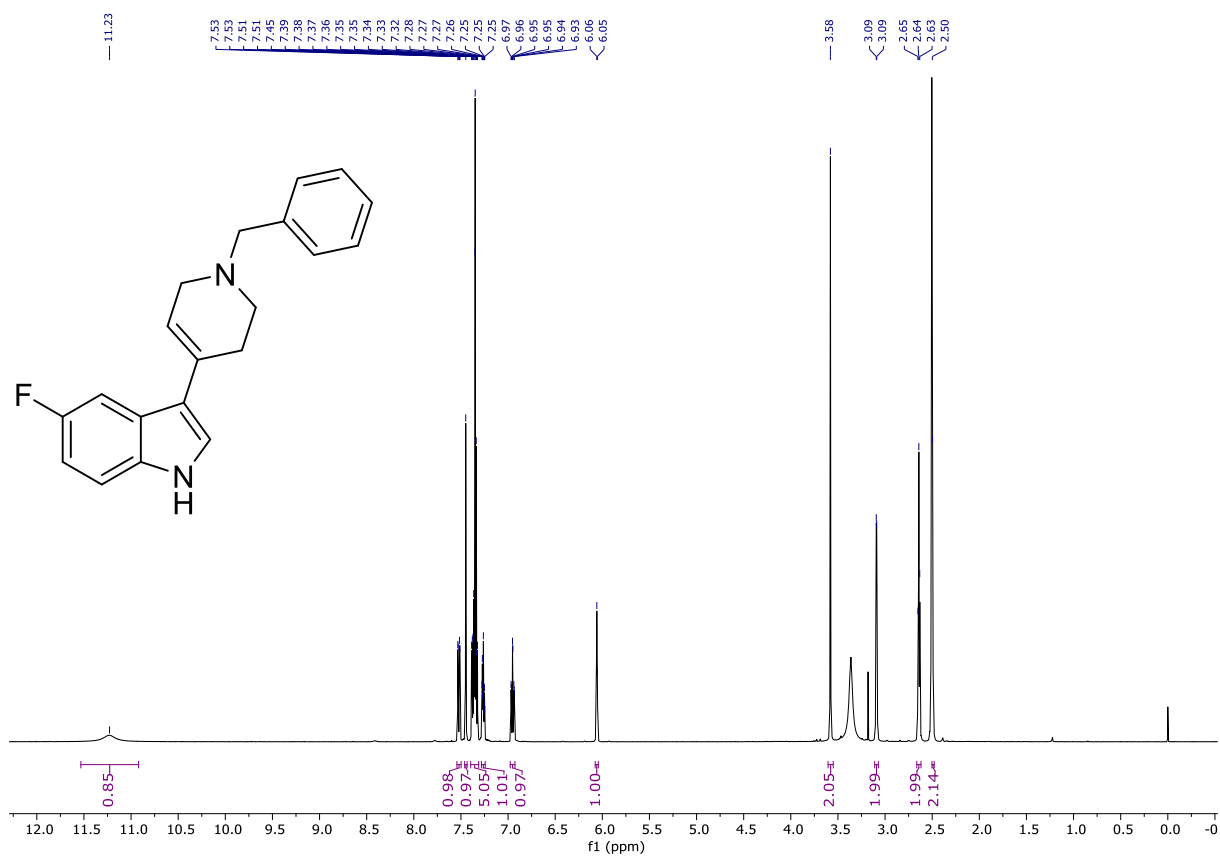
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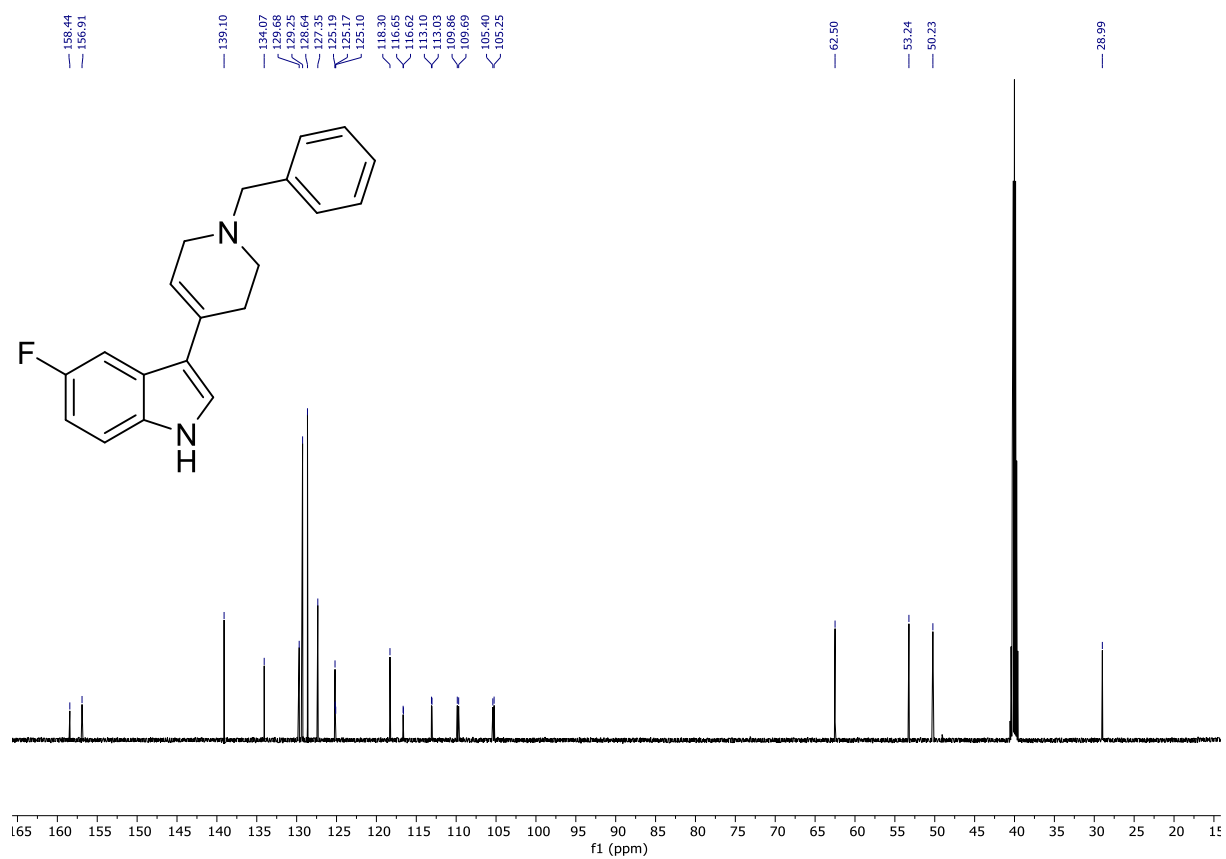
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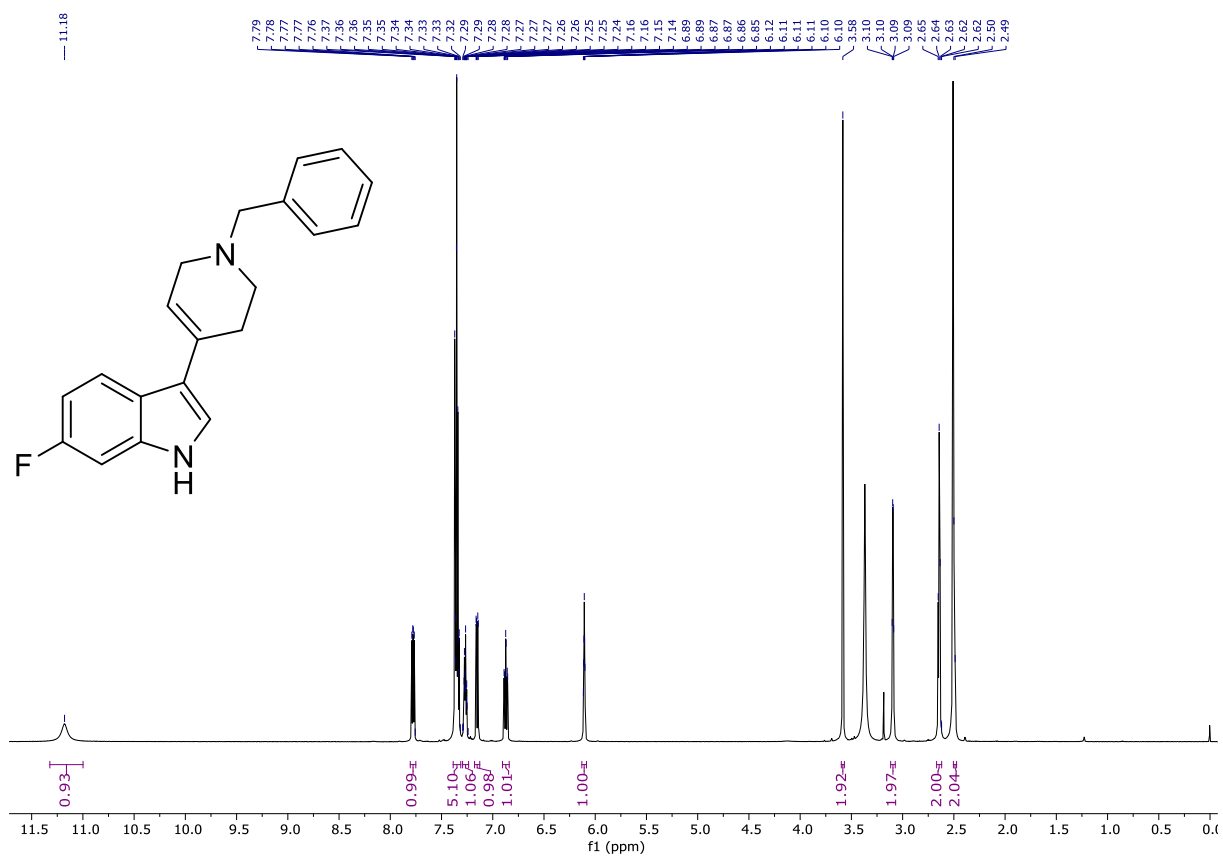
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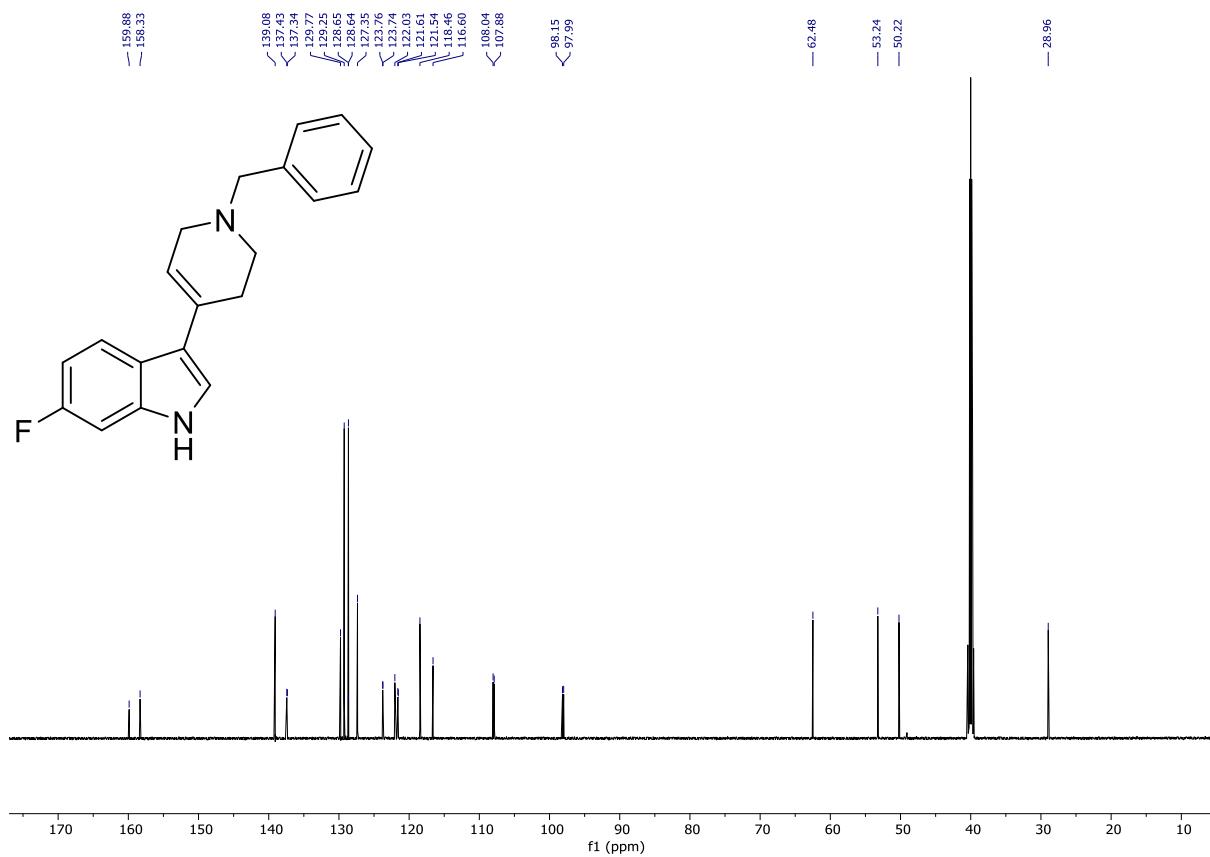
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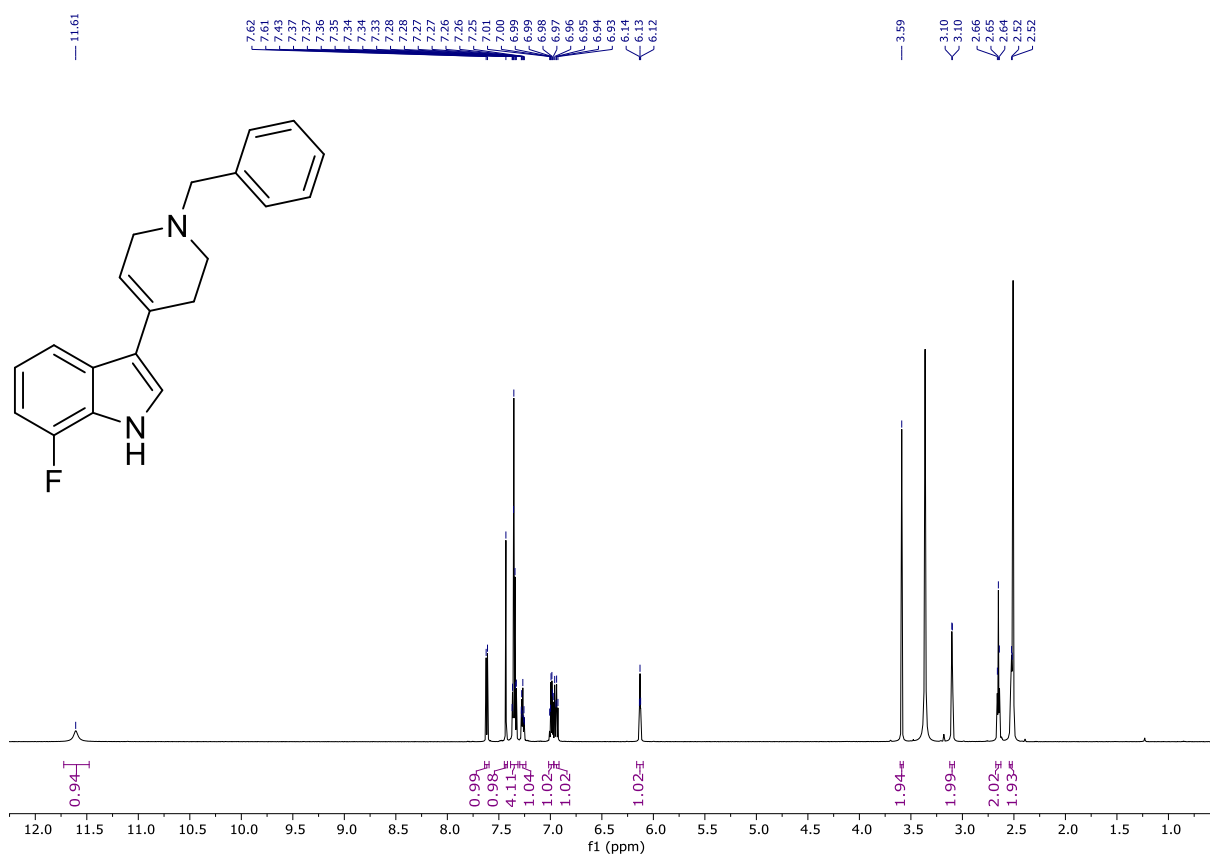
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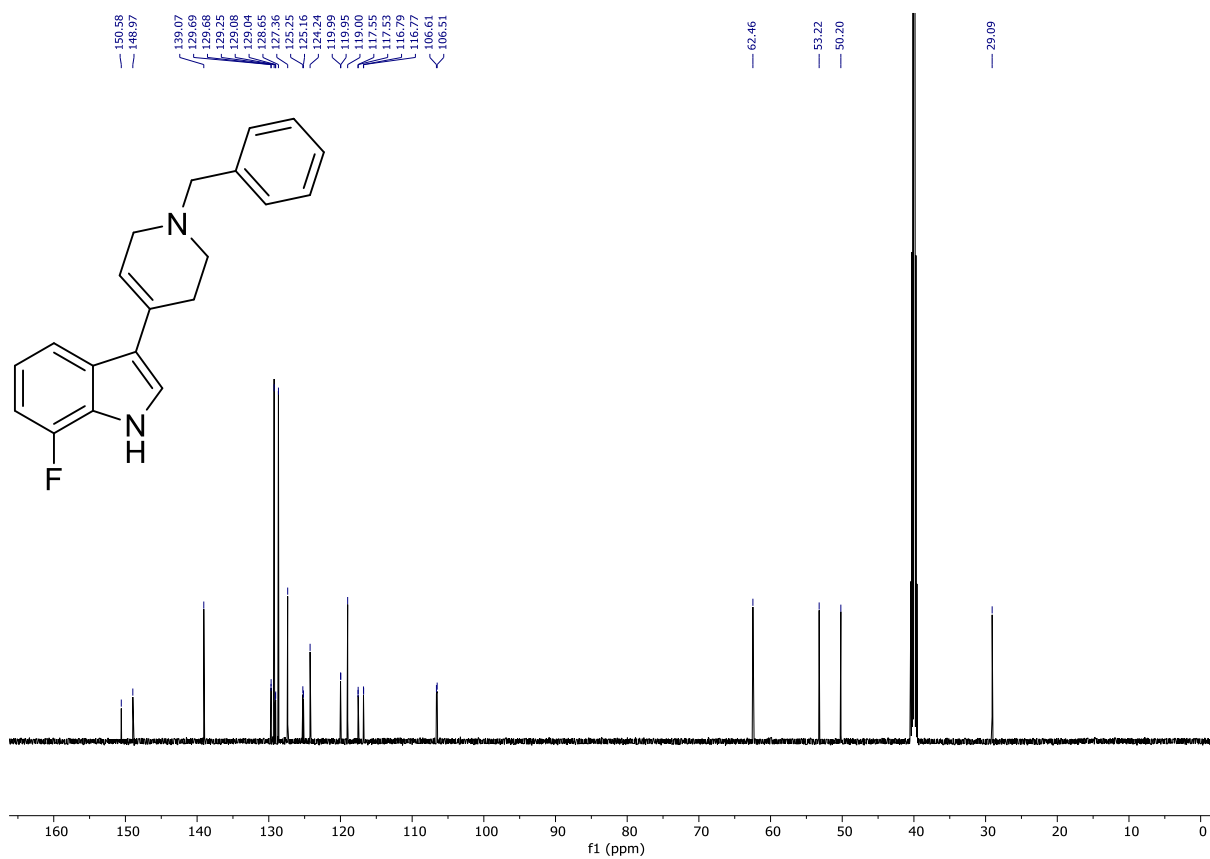
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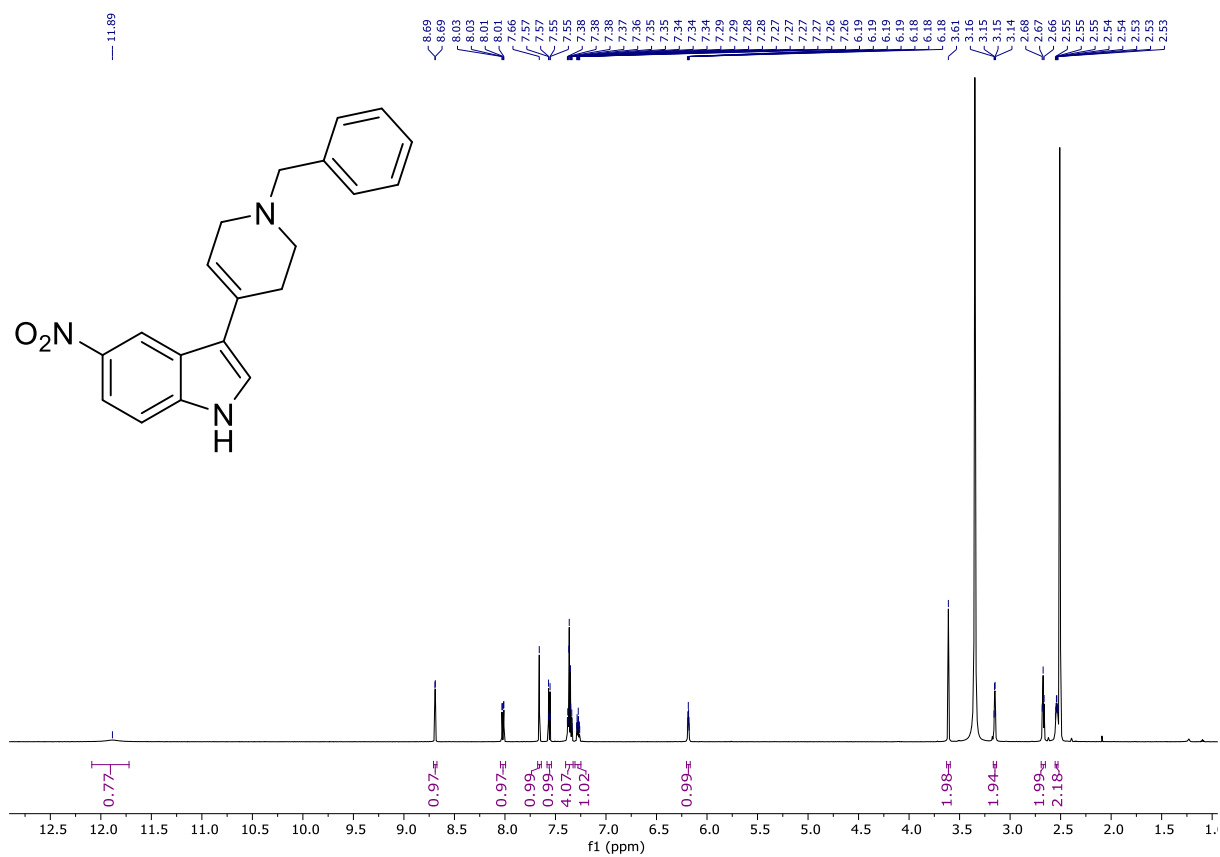
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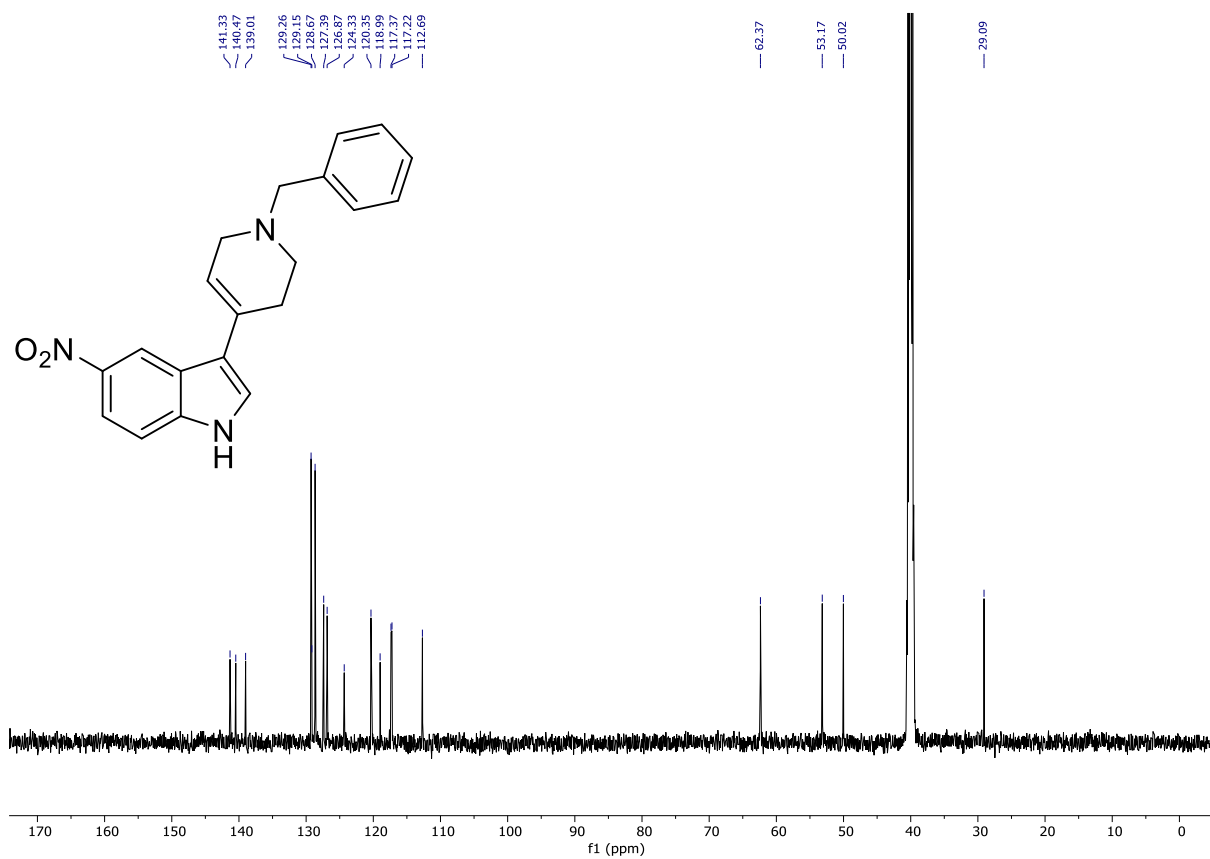
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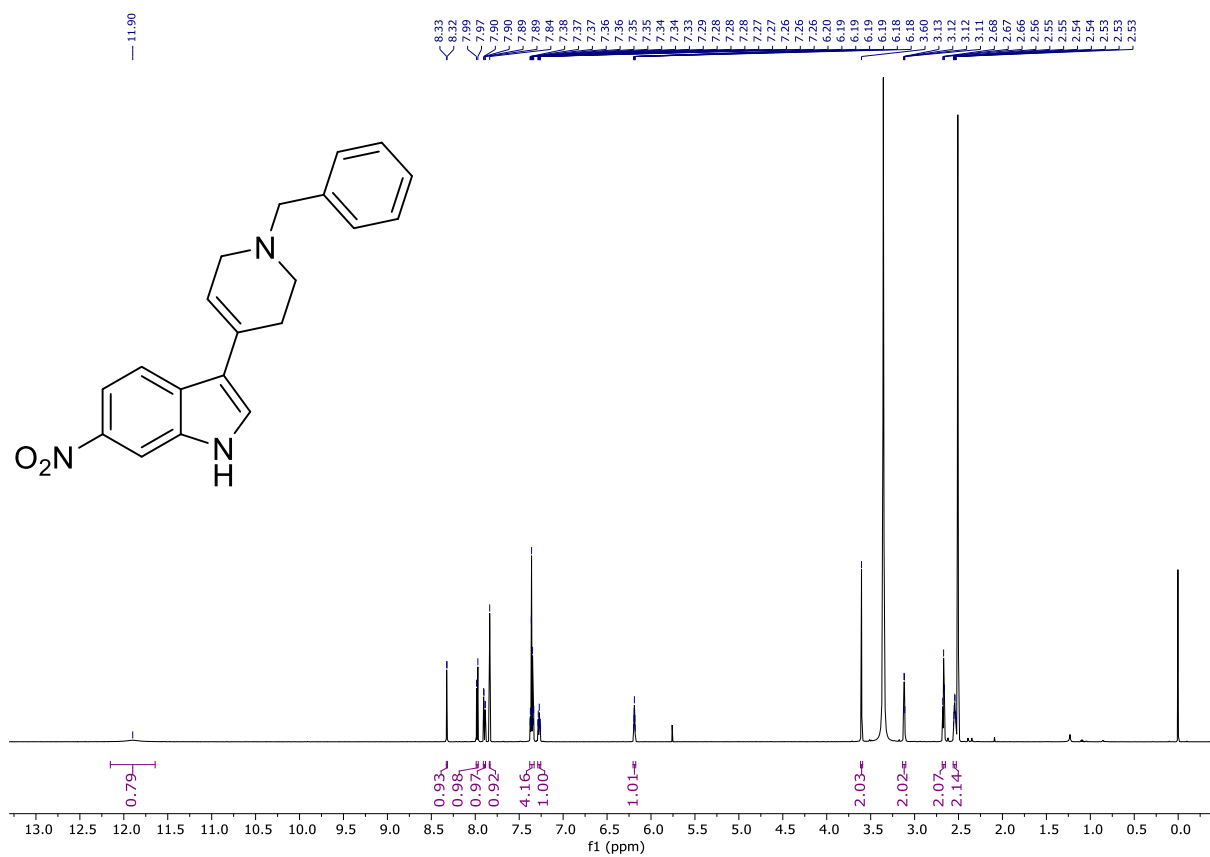
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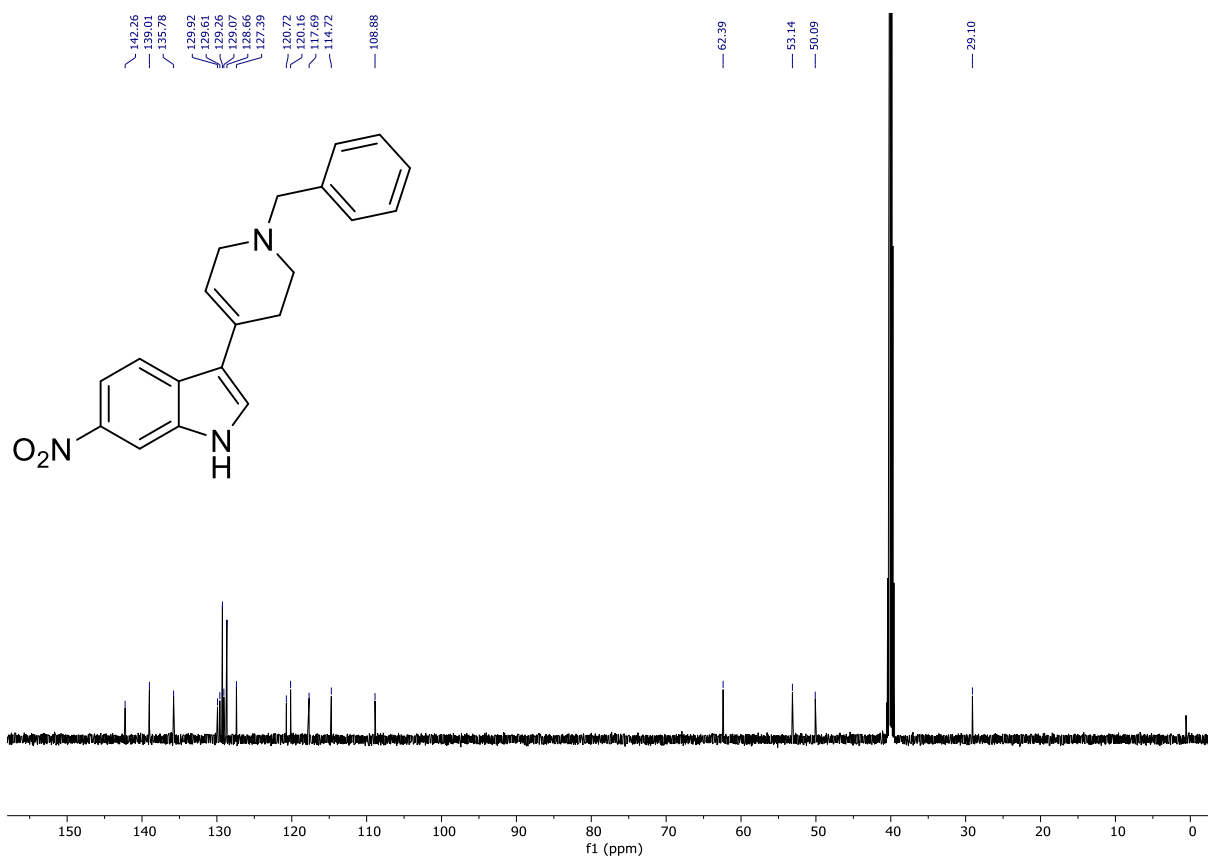
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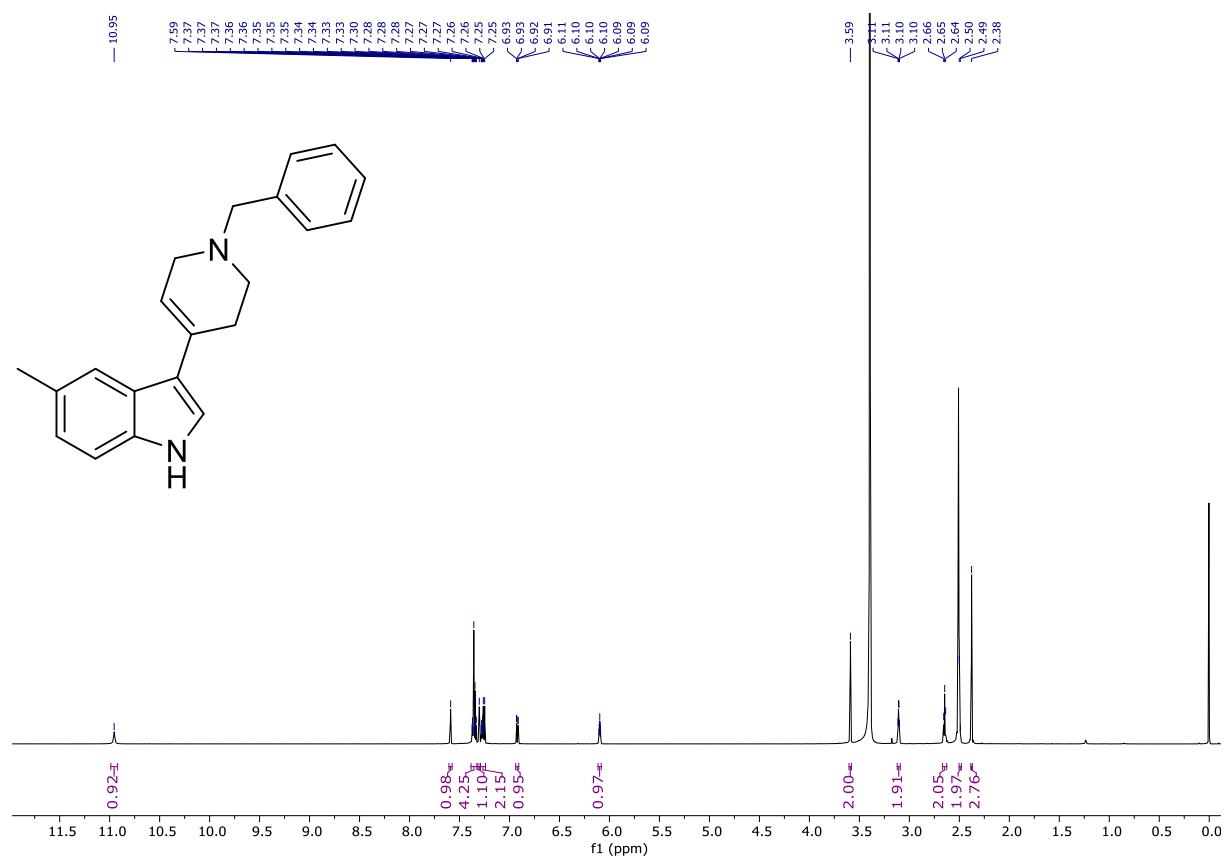
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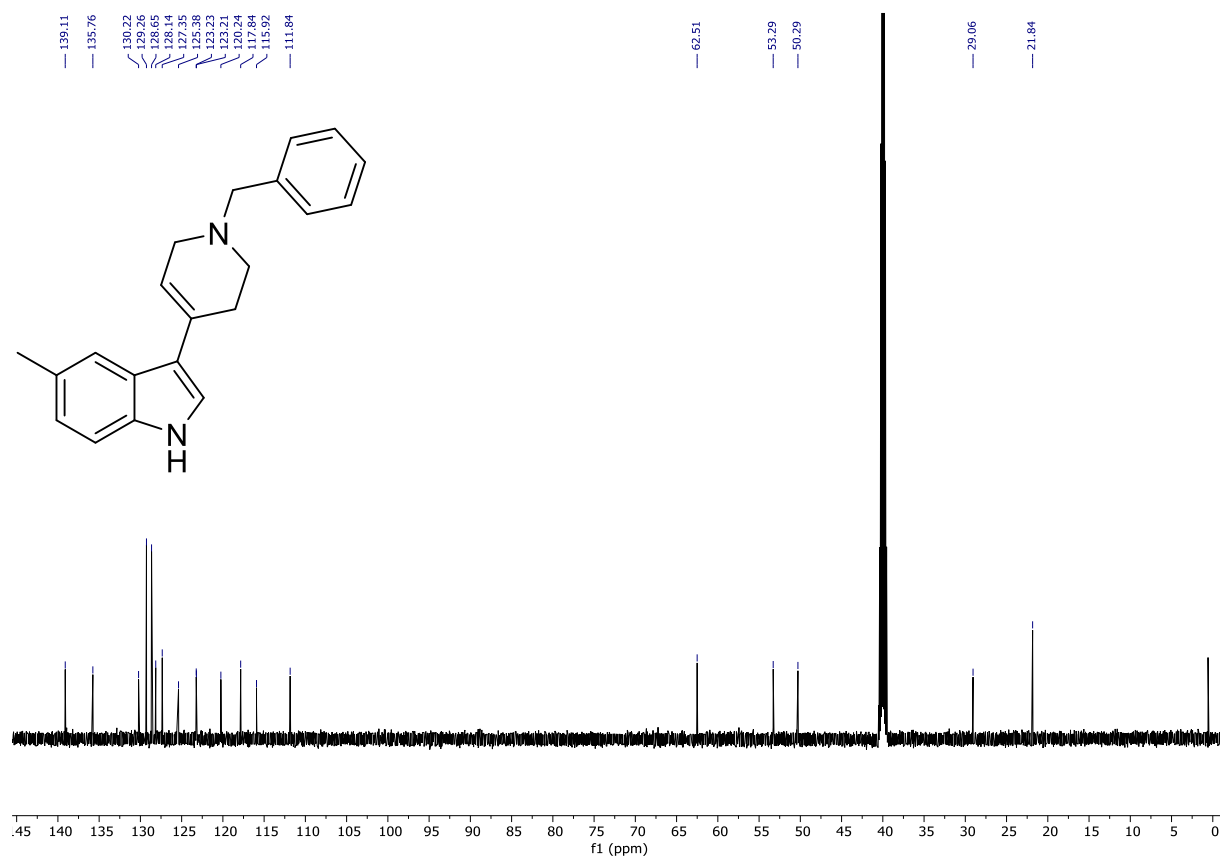
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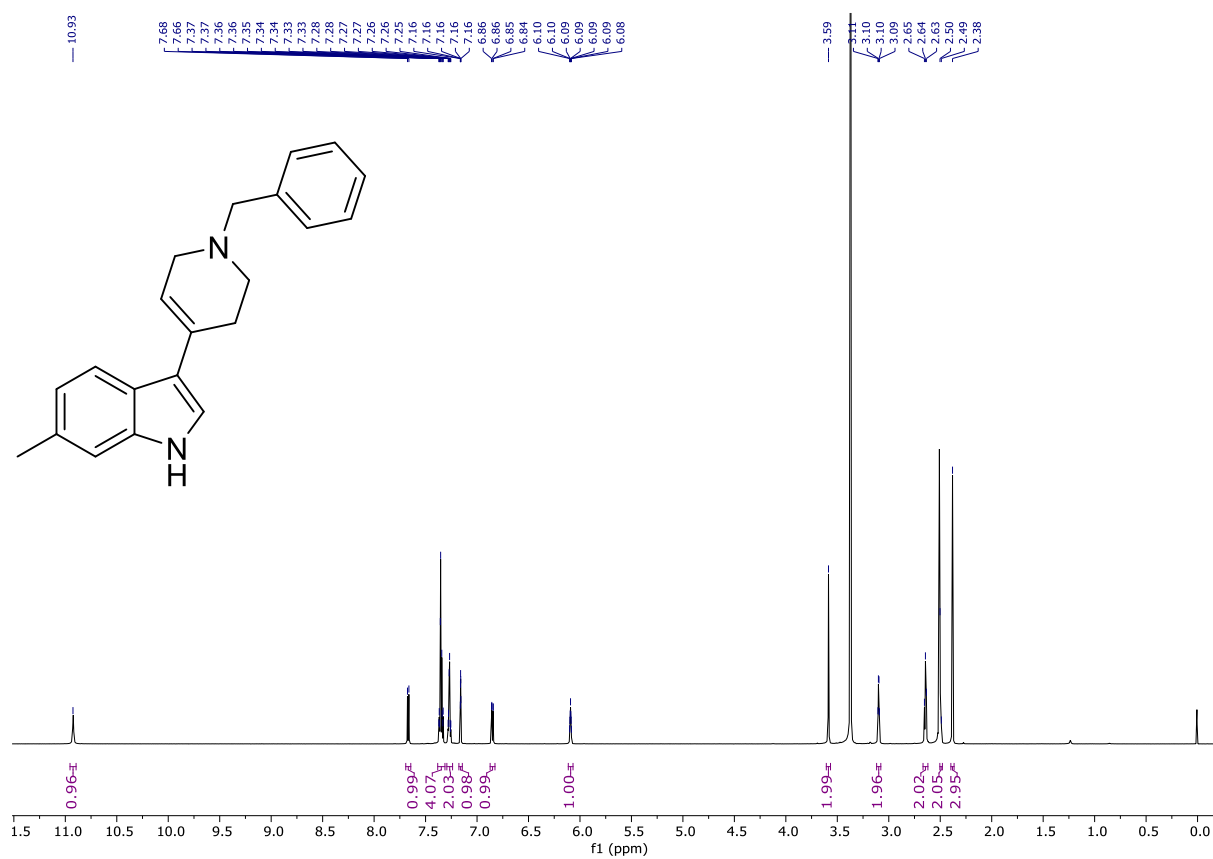
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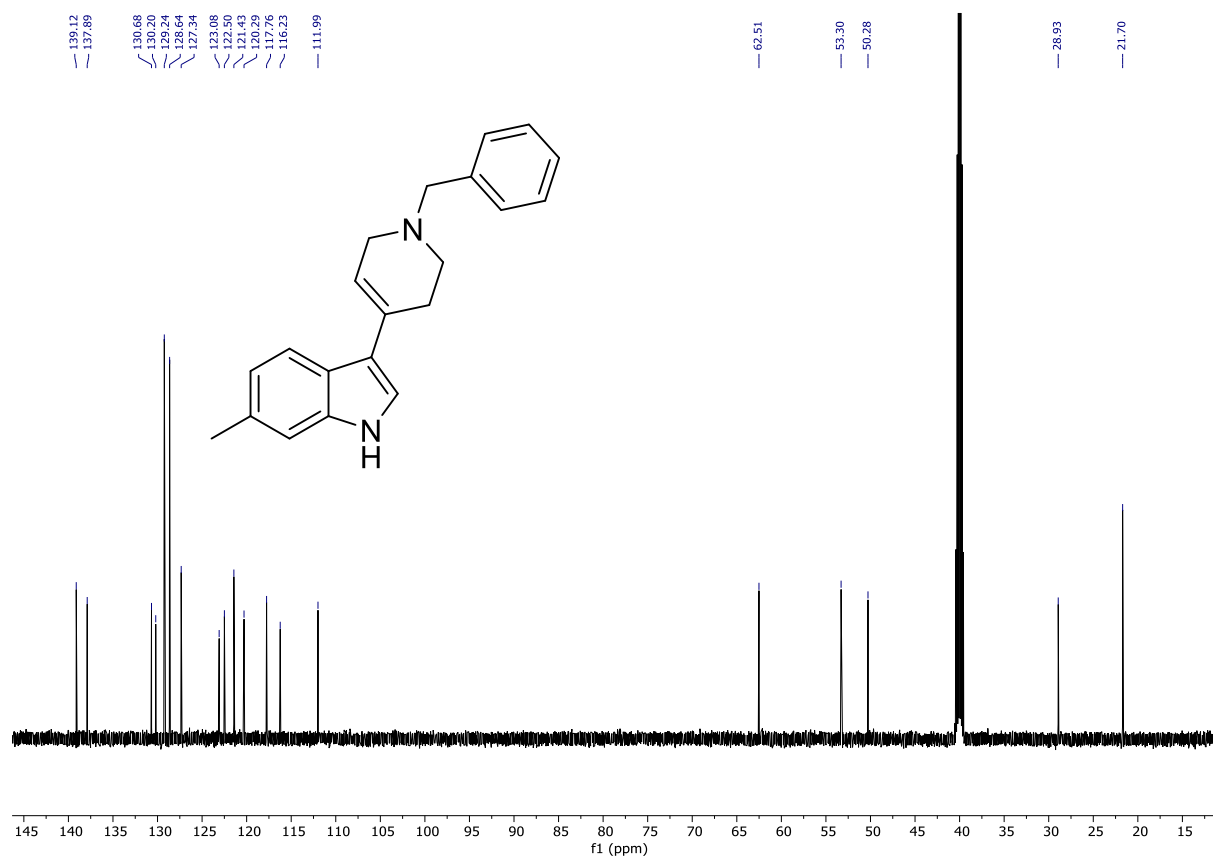
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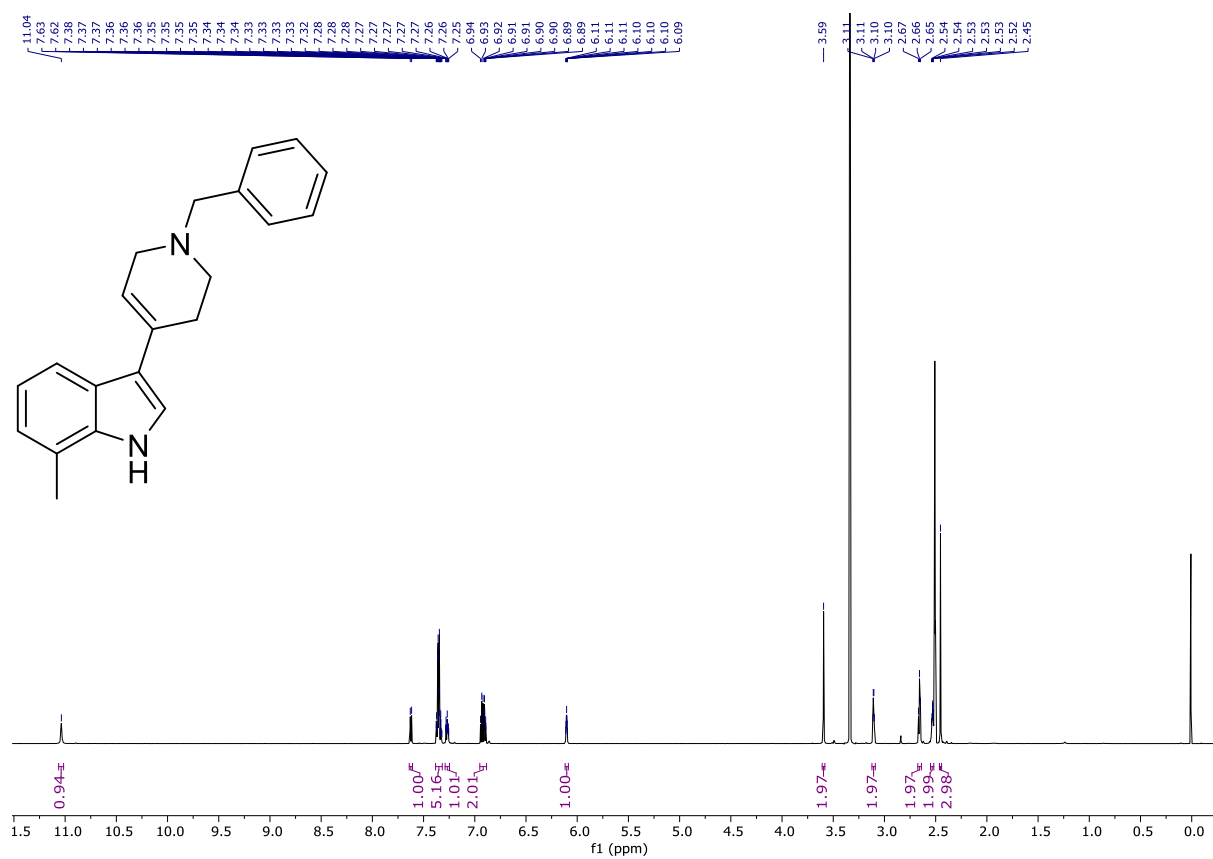
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### 3-(1-Benzyl-1,2,3,6-tetrahydropyridin-4-yl)-6-methyl-1H-indole (16)



### 3-(1-Benzyl-1,2,3,6-tetrahydropyridin-4-yl)-7-methyl-1H-indole (17)



### 3-(1-Benzyl-1,2,3,6-tetrahydropyridin-4-yl)-7-methyl-1H-indole (17)

