GSK4112, a Small Molecule Chemical Probe for the Cell Biology of the Nuclear Heme Receptor Rev-erbα

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SUPPORTING INFORMATION

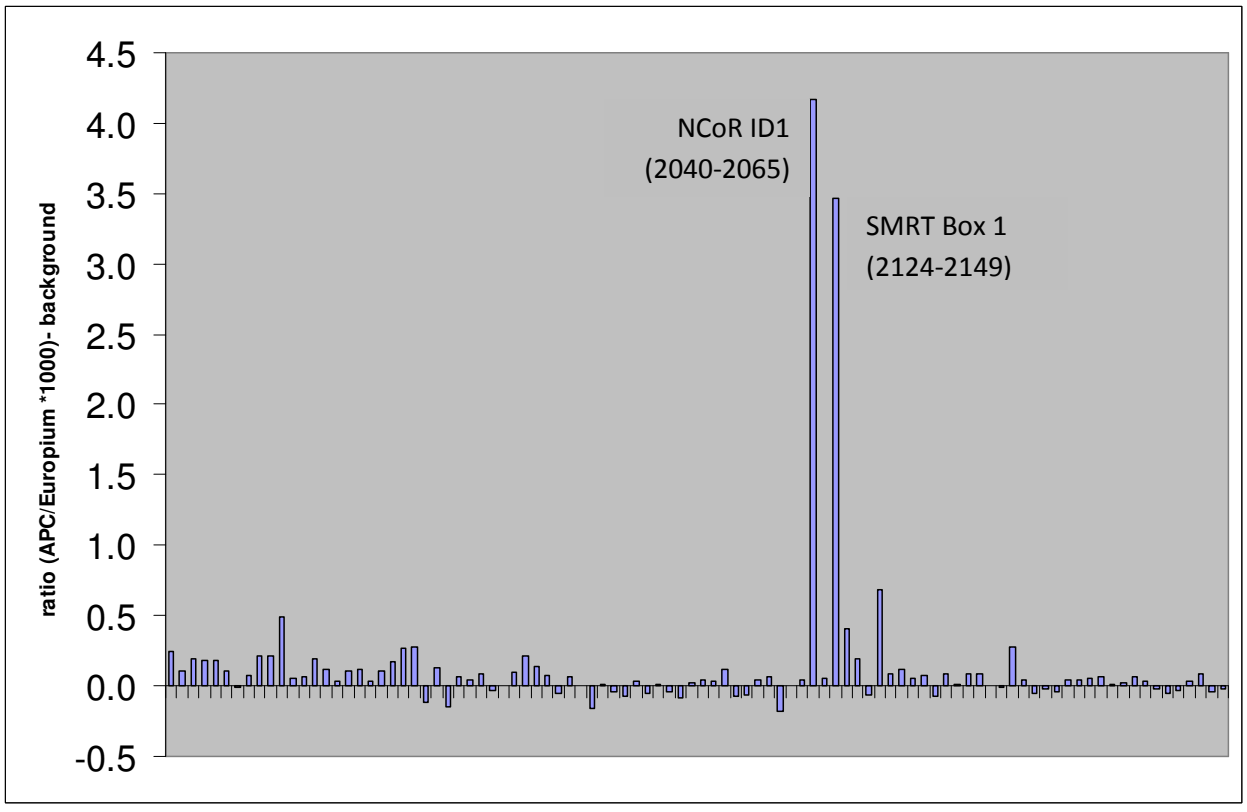


Figure S1. Results of FRET peptide scan using biotinylated fragments from known NR cofactors conjugated with europium chelate.

Compound Preparation. Solvents and reagents were reagent grade and used without purification unless otherwise noted. All ^{1}H NMR spectra were recorded on a Varian 400MHz spectrometer. Chemical shifts (δ) are reported downfield from tetramethylsilane (Me_4Si) in parts per million (ppm) of the applied field. Peak multiplicities are abbreviated: singlet, s; broad singlet, bs; doublet, d; triplet, t; quartet, q; multiplet, m. Coupling constants (*J*) are reported in hertz. LCMS analyses were conducted using a Waters Acquity UPLC system with UV detection performed from 210 to 350 nm with the MS detection performed on a Waters Acquity SQD spectrometer.

***tert*-Butyl *N*-[(4-chlorophenyl)methyl]-N-[(5-nitro-2-thienyl)carbonyl]glycinate (2).** 5-Nitrothiophenecarboxylic acid (100 mg, 0.578 mmol) was dissolved in thionyl chloride (2mL, 27.4 mmol) and heated to reflux for 1 hour. The thionyl chloride was then removed under reduced pressure leaving the acid chloride as a yellow crystalline solid. A solution of N,N-diisopropylethylamine (0.068 mL, 0.391 mmol) and *tert*-butyl N-[(4-chlorophenyl)methyl]glycinate (100 mg, 0.391 mmol) in dichloromethane (4 mL) was added to the flask containing the acid chloride. The reaction mixture was stirred at room temperature for 10 minutes. The reaction mixture was then evaporated to near dryness and partitioned between aqueous NaOH (1N, 50 mL) and Et_2O (50 mL) the organic fraction was then washed with aqueous HCl (1N, 50 mL), saturated aqueous sodium bicarbonate (50 mL) and dried over Na_2SO_4. The crude product was purified by silica gel chromatography (EtOAc in Hexanes, 0-20%) to yield the title product as a pale yellow oil (151 mg, 0.349 mmol, 89 % yield). ^{1}H NMR (400 MHz, $SO(CD_3)_2$, 85 °C) δ 1.38 (s, 9H), 4.17 (s, 2H), 4.74 (s, 2H), 7.31-7.45 (m, 5H), 8.02 (d, *J* = 4.0 Hz, 1H); ^{13}C (100 MHz, $SO(CD_3)_2$, 85 °C) δ 28.1, 82.3, 128.7, 129.0, 129.6, 130.2, 132.8, 135.8, 143.8, 153.0, 163.0, 167.9 (despite heating the sample to 85 °C peaks in the region of 48-56 ppm failed to resolve): MS (ESI): *m/z* 411 $(M+H)^+$.

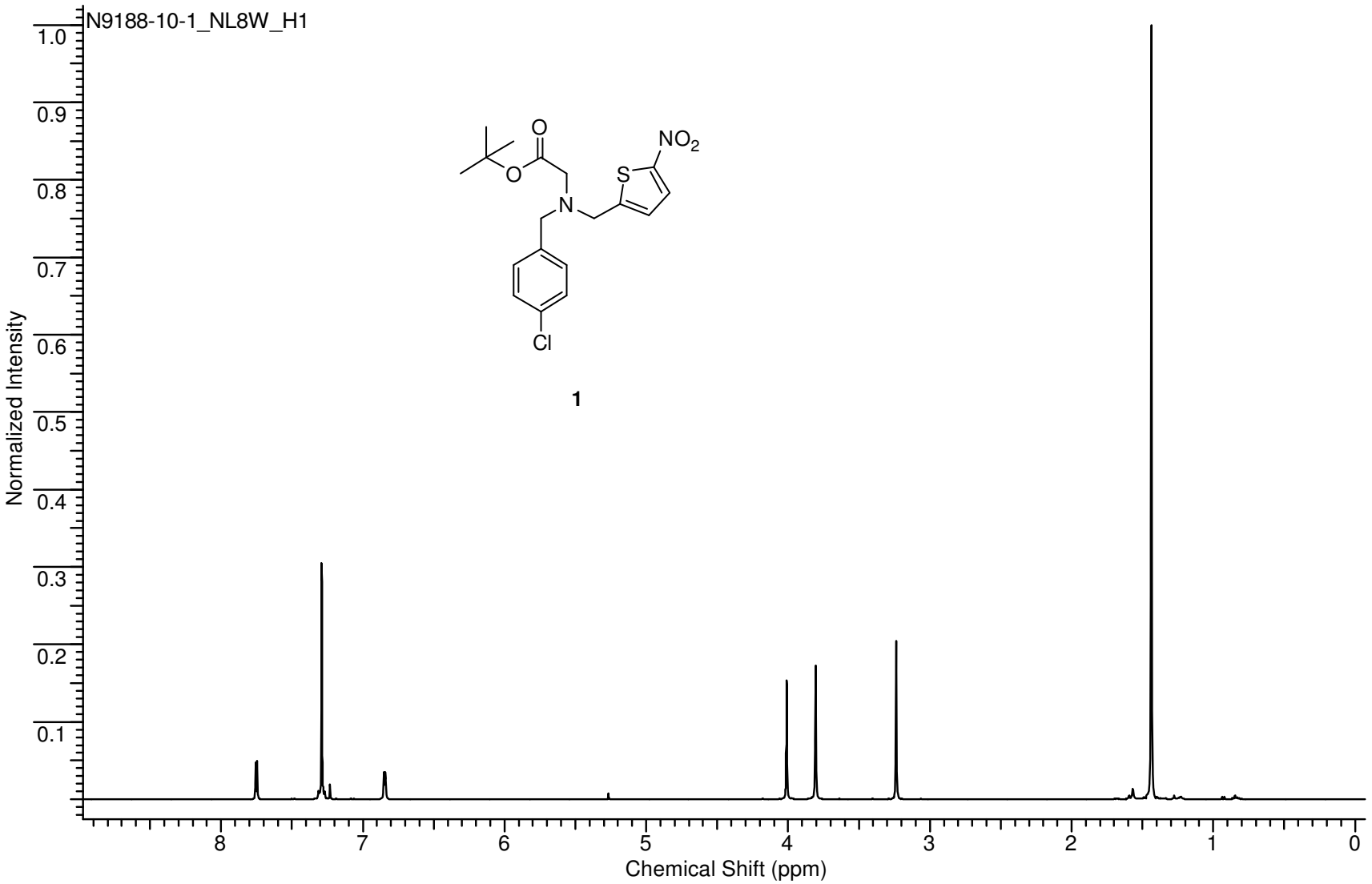
***N*-Benzyl-*N*-(4-chlorobenzyl)-1-(5-nitrothiophen-2-yl)methanamine (3).** To a stirred solution of the appropriately substituted 4-chlorobenzylamine (432 μL, 3.5 mmol) in *N,N*-dimethylformamide (5 mL) was added successively benzaldehyde (356 μL, 3.5 mmol) and sodium triacetoxyborohydride (1.1 g, 5.5 mmol). The reaction mixture was allowed to stir for 5 hours at room temperature. To this reaction mixture was added successively 5-nitro-2-thiophenecarboxaldehyde (555 mg, 3.5 mmol) and sodium triacetoxyborohydride (1.1 g, 5.5 mmol) the reaction mixture was stirred overnight at room temperature. The reaction mixture was then partitioned between saturated aqueous sodium bicarbonate solution (40 mL) and EtOAc (3 x 50 mL), the organic fractions were combined, washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The crude products were purified by silica gel column chromatography (EtOAc in Hexanes, 0-10%). 610 mg, 164 mmol, 46.3 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59 (s, 2H), 3.62 (s, 2H), 3.72 (s, 2H), 6.84 (d, *J* = 3.9 Hz, 1 H), 7.24 - 7.41 (m, 9H), 7.76 (d, *J*=3.9 Hz, 1H); ^{13}C (100 MHz, $CDCl_3$) δ 52.9, 57.6, 58.3, 124.3, 127.8, 128.8, 128.9, 128.9, 130.2, 133.4, 137.0, 138.1, 151.0, 154.3. MS (ESI): *m/z* 373 $(M+H)^+$.

***N*-Benzyl-*N*-(3,4-dichlorobenzyl)-1-(5-nitrothiophen-2-yl)methanamine (4).** Prepared in a manner similar to **3**. The crude products were purified by silica gel column chromatography (EtOAc in Hexanes,

0-10%). 578 mg, 1.405 mmol, 49.5 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.58 (s, 2H), 3.63 (s, 2H), 3.73 (s, 2H), 6.86 (d, *J* = 4.3 Hz, 1H), 7.26 - 7.31 (m, 2H), 7.33 – 7.41 (m, 4H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.48 (d, *J* = 1.95 Hz, 1H), 7.78 (d, *J* = 4.1 Hz, 1H); ^{13}C (100 MHz, $CDCl_3$) δ 53.0, 57.2, 58.4, 124.6, 127.9, 128.1, 128.9, 130.7, 130.8, 131.6, 132.8, 137.8, 138.9, 151.1, 153.8. MS (ESI): *m/z* 407 $(M+H)^+$.

***N*-[(4-Chlorophenyl)methyl]-*N*-[(5-nitro-2-thienyl)methyl]glycinate (5).** To a solution of 1,1-dimethylethyl N-[(4-chlorophenyl)methyl]-N-[(5-nitro-2-thienyl)methyl]glycinate (80 mg, 0.20 mmol) in methanol (15 mL) was added HCl in dioxane (4.0 M, 0.5 mL, 2.000 mmol). The reaction mixture was heated to reflux for 6 hours before being cooled, evaporated to dryness, taken up in EtOAc (40 mL) and washed with saturated aqueous sodium bicarbonate solution (40 mL) and brine (40 mL). The organic fraction was then concentrated under reduced pressure, and the crude product was purified by silica gel column chromatography (EtOAc in Hexanes, 0-15%) to yield the title product as a colorless oil (40 mg, 0.107 mmol, 53.1 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39 (s, 2H), 3.70 (s, 3H), 3.84 (s, 2H), 4.03 (s, 2H), 6.87 (d, *J* = 4.11 Hz, 1 H), 7.31 (s, 4h), 7.77 (d, *J* = 4.11 Hz, 1 H); ^{13}C (100 MHz, $CDCl_3$) δ 51.8, 52.9, 53.0, 57.2, 124.7, 128.9, 129.0, 130.3, 133.7, 136.3, 151.3, 171.1; MS (ESI): *m/z* 355 $(M+H)^+$.

2-((4-chlorobenzyl)((5-nitrothiophen-2-yl)methyl)amino)-N,N-dimethylacetamide (6). To a solution of 2-((4-chlorobenzyl)amino)-N,N-dimethylacetamide (524 mg, 2.3 mmol) in DMF (5 mL) was added successively acetic acid (0.5 mL), 5-nitro-2-thiophenecarboxaldehyde (363 mg, 2.3 mmol), and sodium triacetoxyborohydride (735 mg, 3.5 mmol). The reaction was allowed to stir overnight. The reaction mixture was then diluted with EtOAc (100 mL) and washed with saturated aqueous sodium bicarbonate solution (50 mL) and brine (50 mL). After drying over $MgSO_4$ and concentration under reduced pressure, the resulting yellow oil was purified by silica gel chromatography (EtOAc in Hexanes, 0-15%) to yield the title product as a pale yellow oil (182 mg, 0.466 mmol, 20.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 (s, 3H) 2.93 (s, 3H) 3.40 (s, 2H) 3.87 (s, 2H) 4.12 (s, 2H) 6.89 (d, *J* = 4.0 Hz, 1H) 7.34 (s, 4 H) 7.80 (d, *J* = 4.0 Hz, 1H); ^{13}C (100 MHz, $CDCl_3$) δ 35.4, 36.7, 53.2, 53.5, 57.4, 124.3, 128.8, 128.8, 130.3, 133.4, 136.6, 154.4, 169.7. MS (ESI): *m/z* 368 $(M+H)^+$.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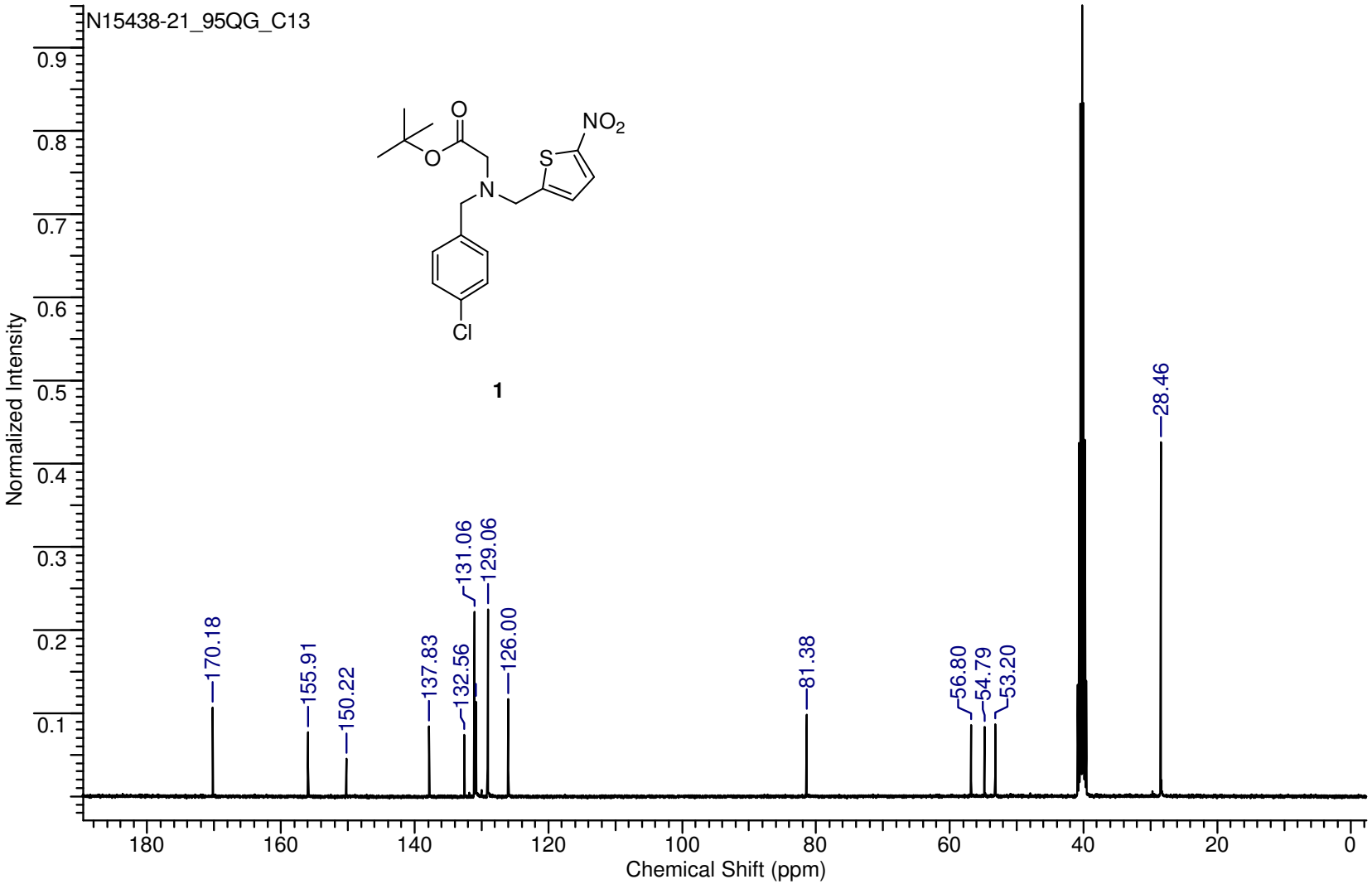
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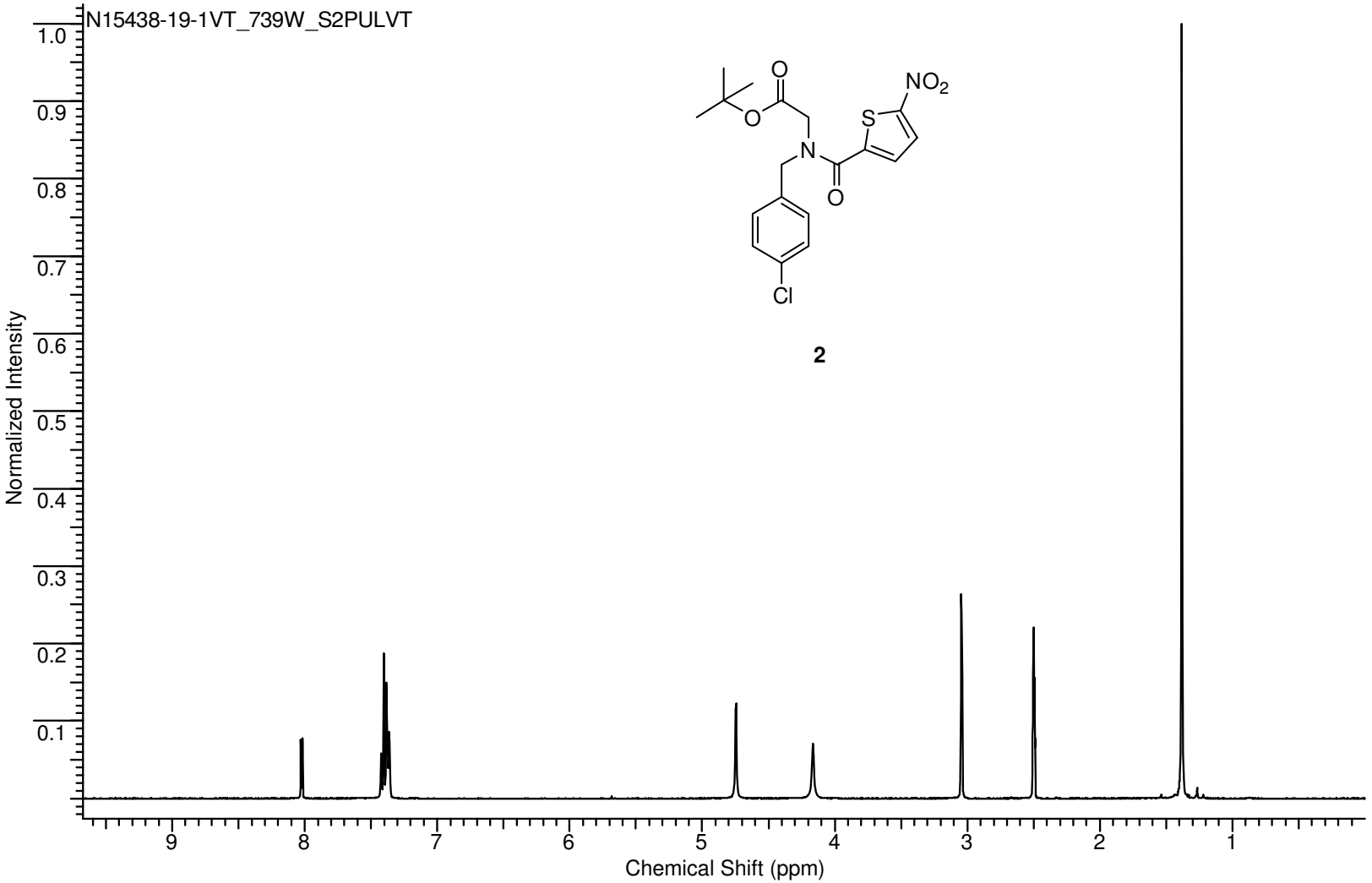
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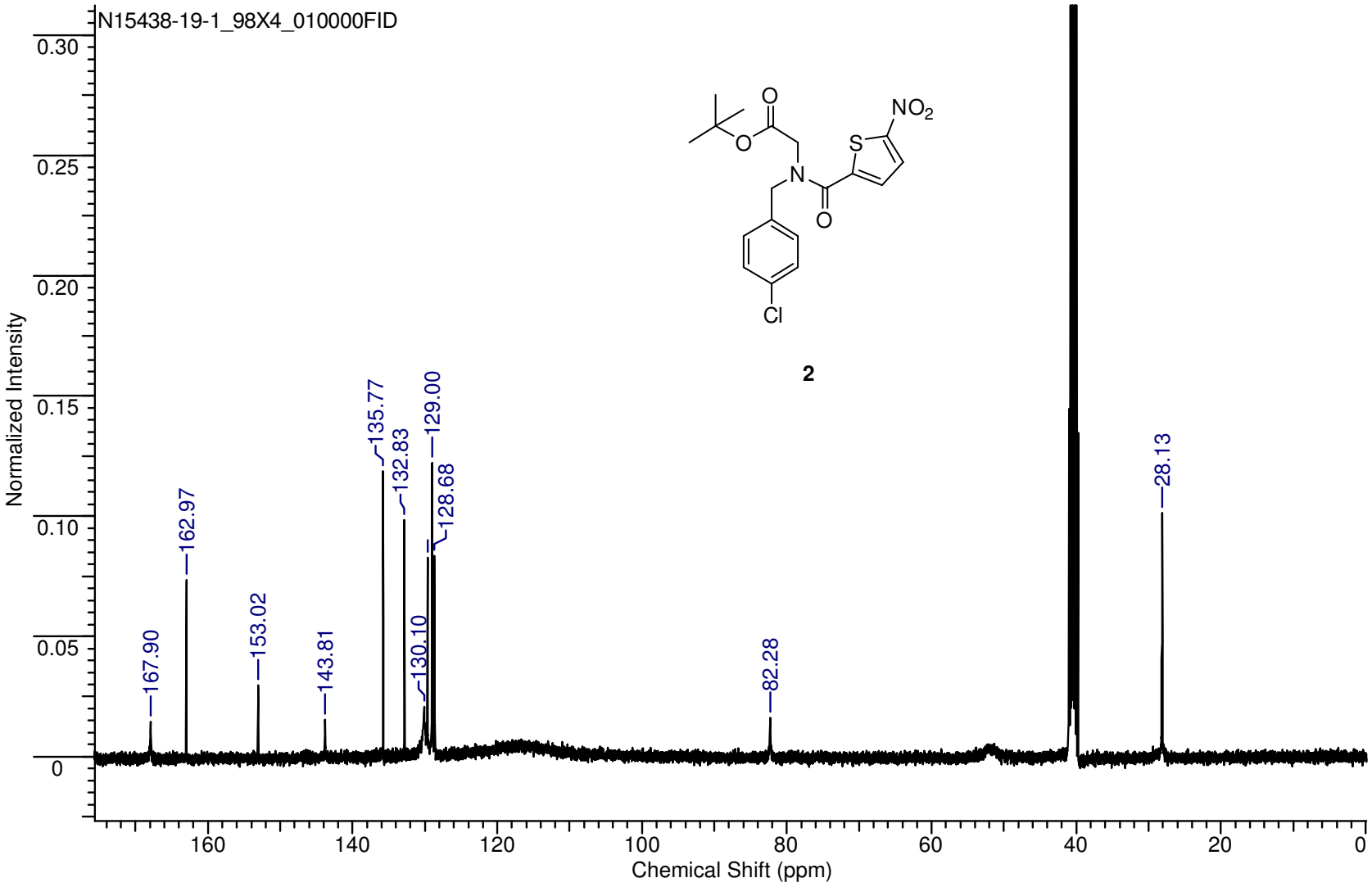
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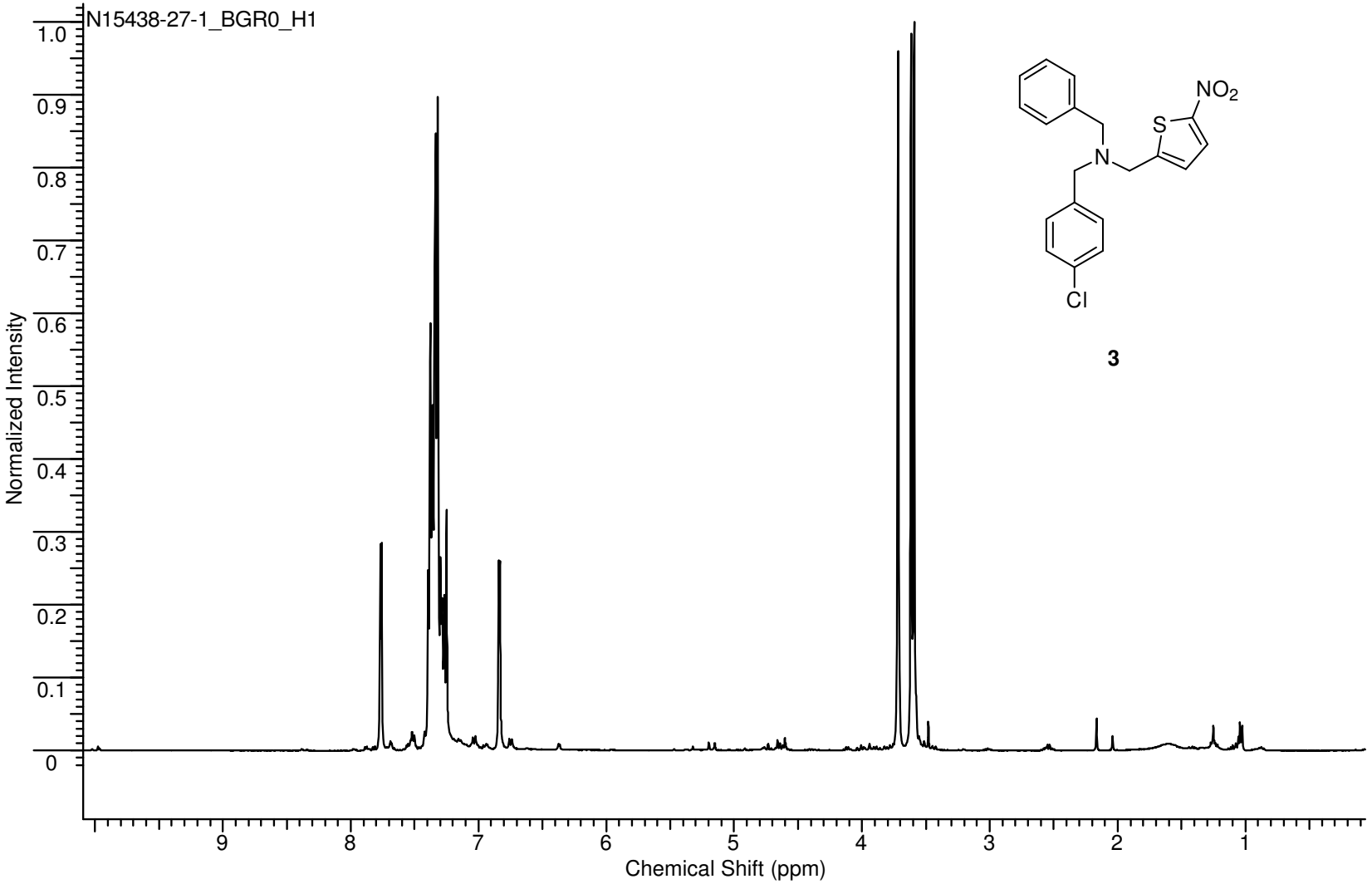
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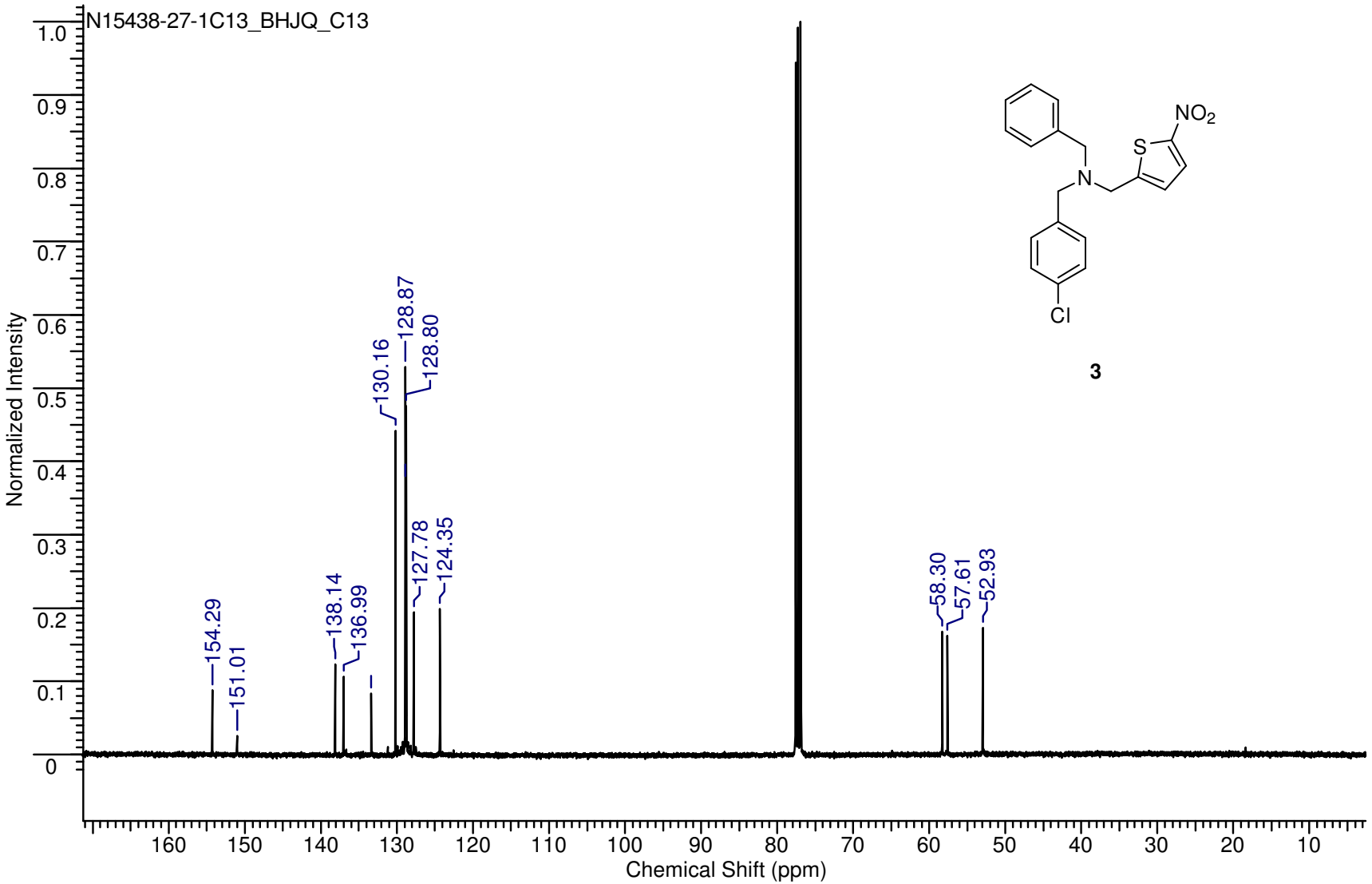
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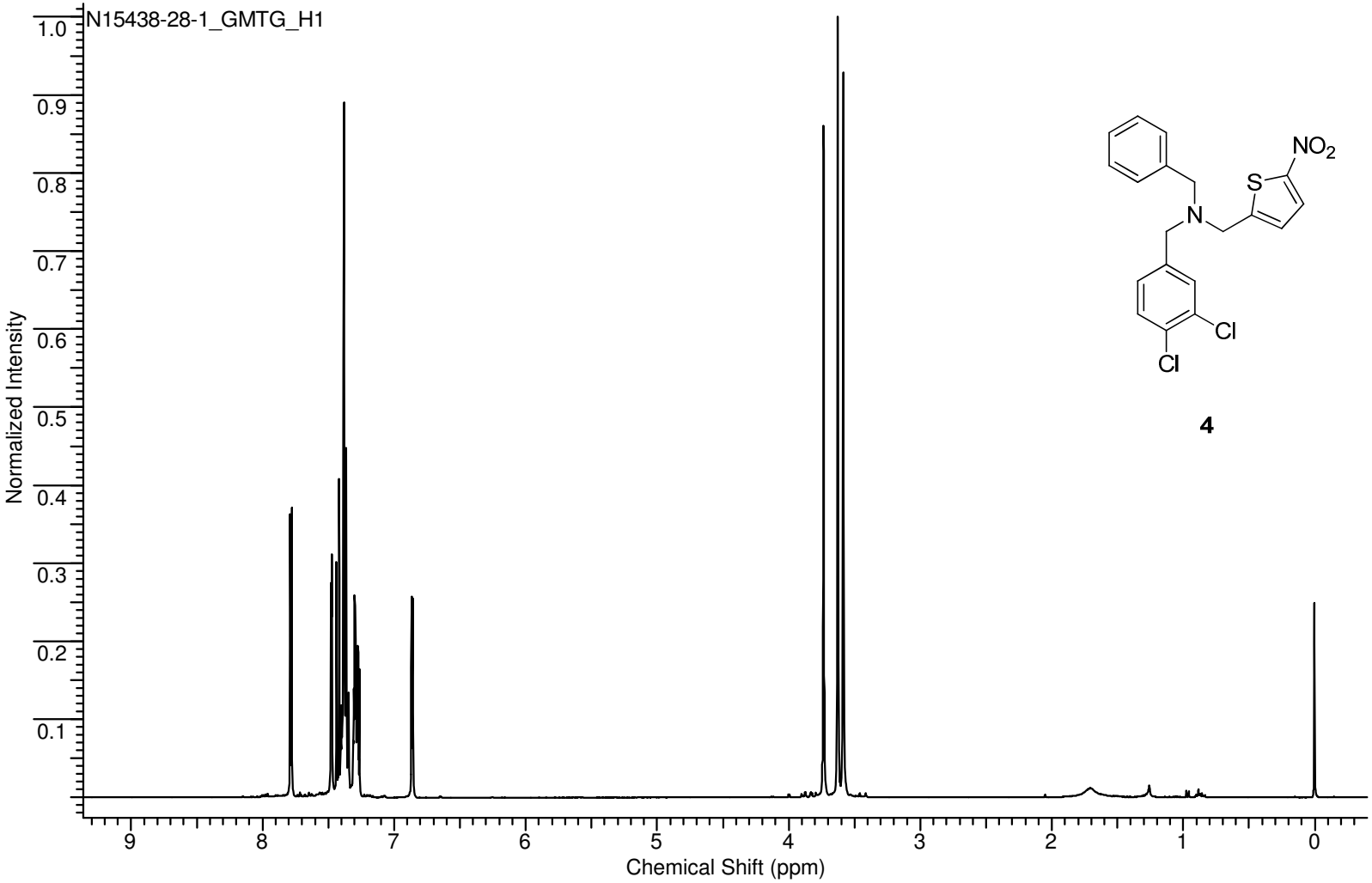
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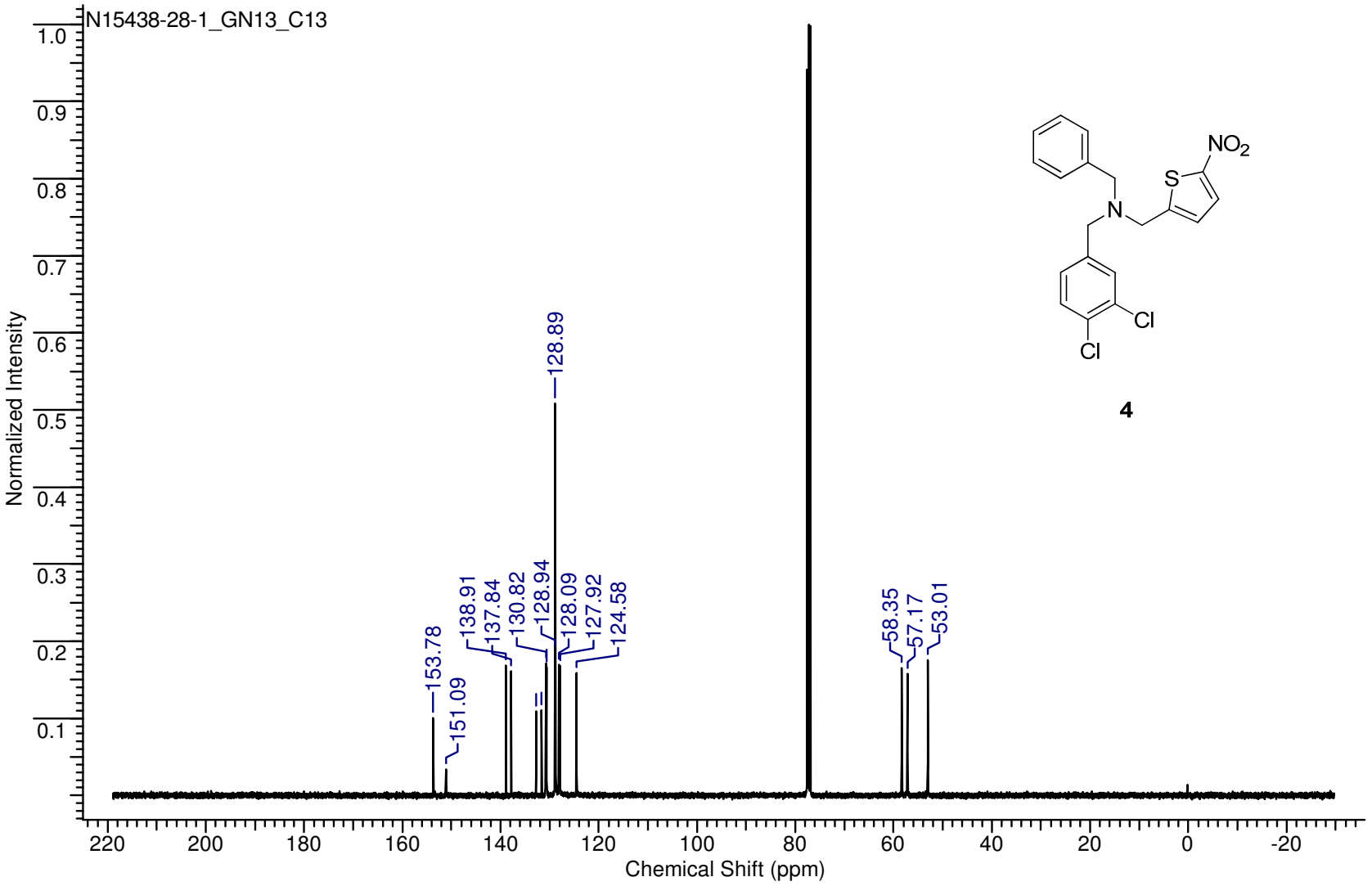
4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128.09
127.92
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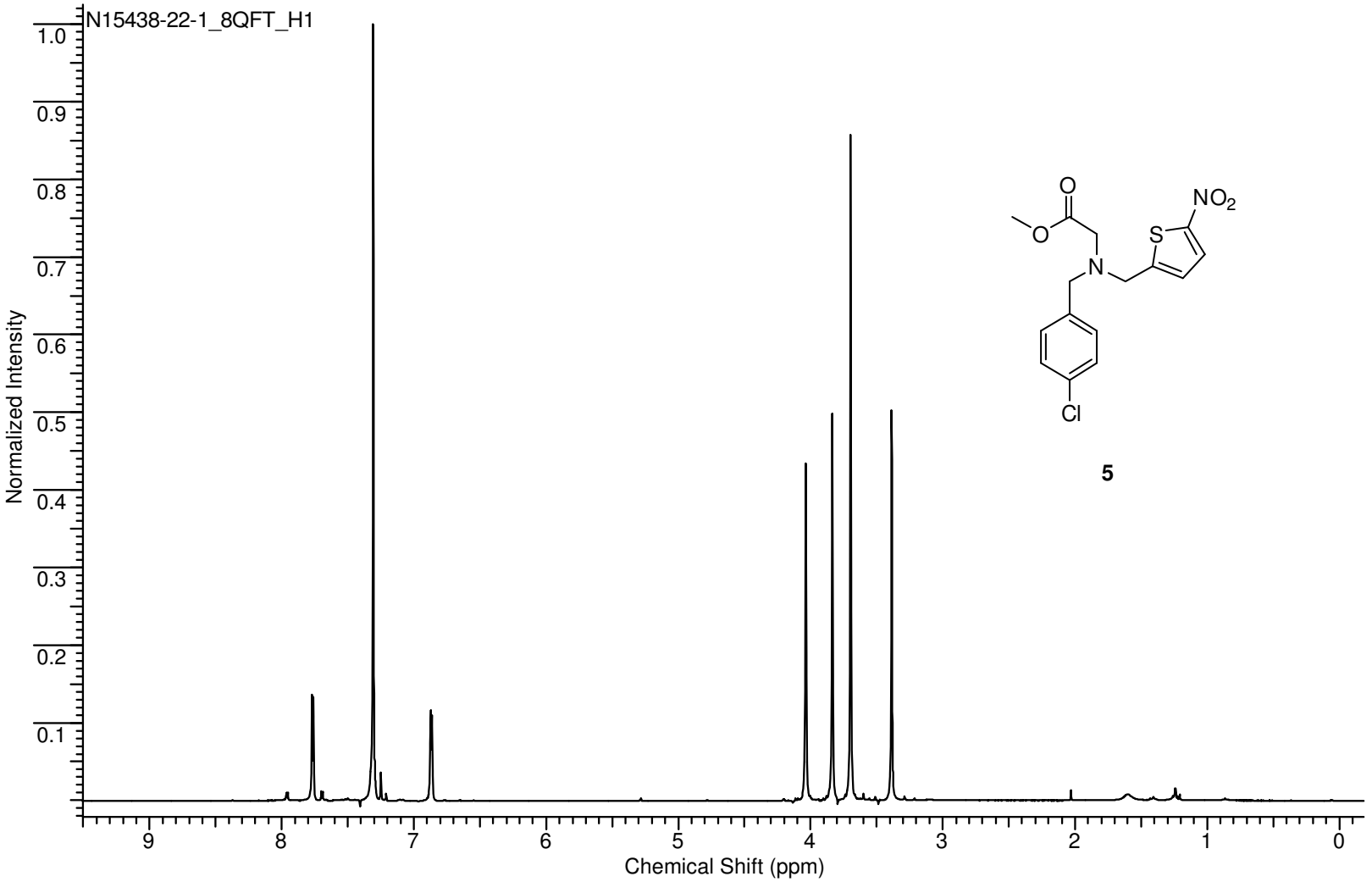
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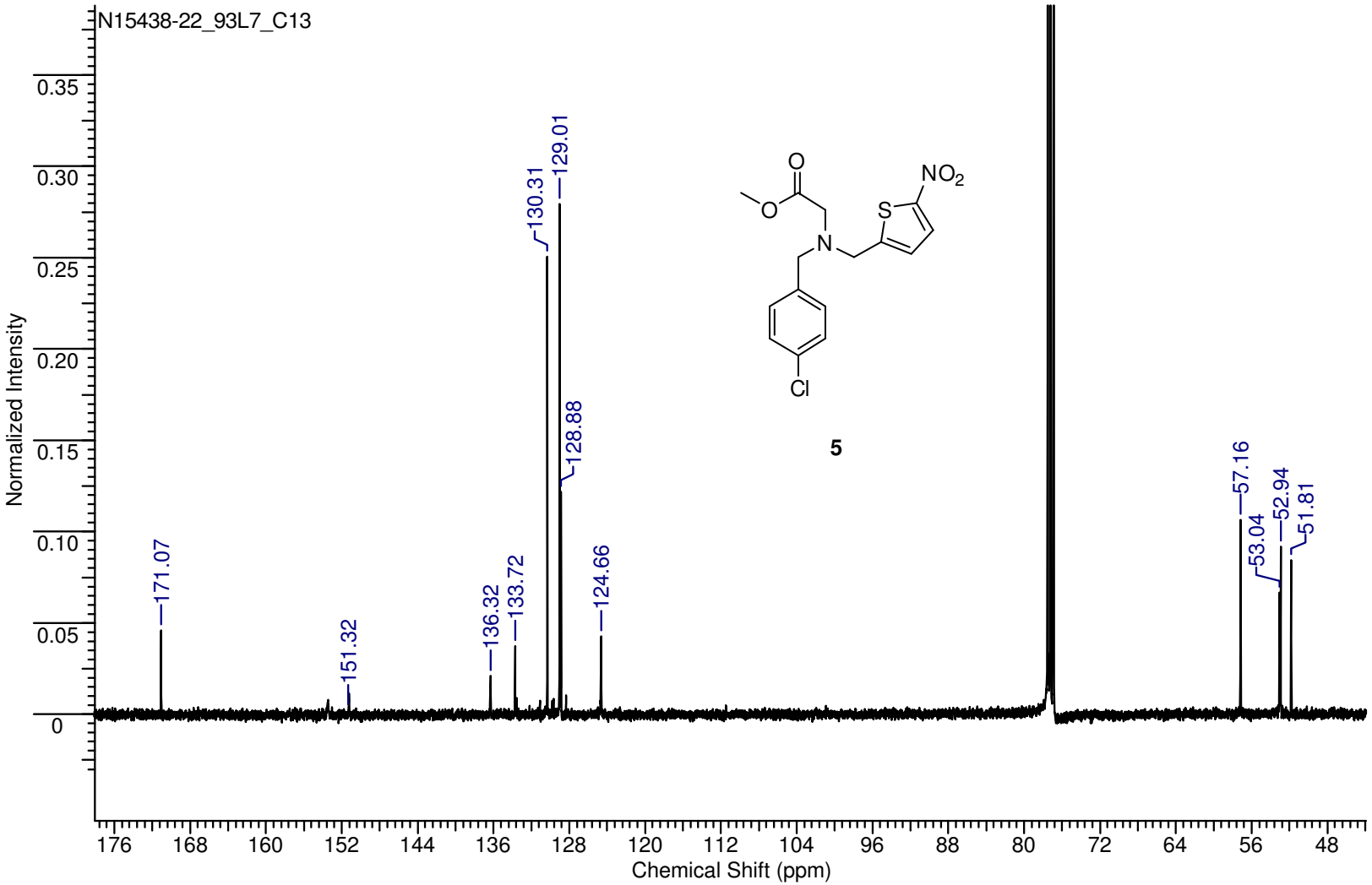
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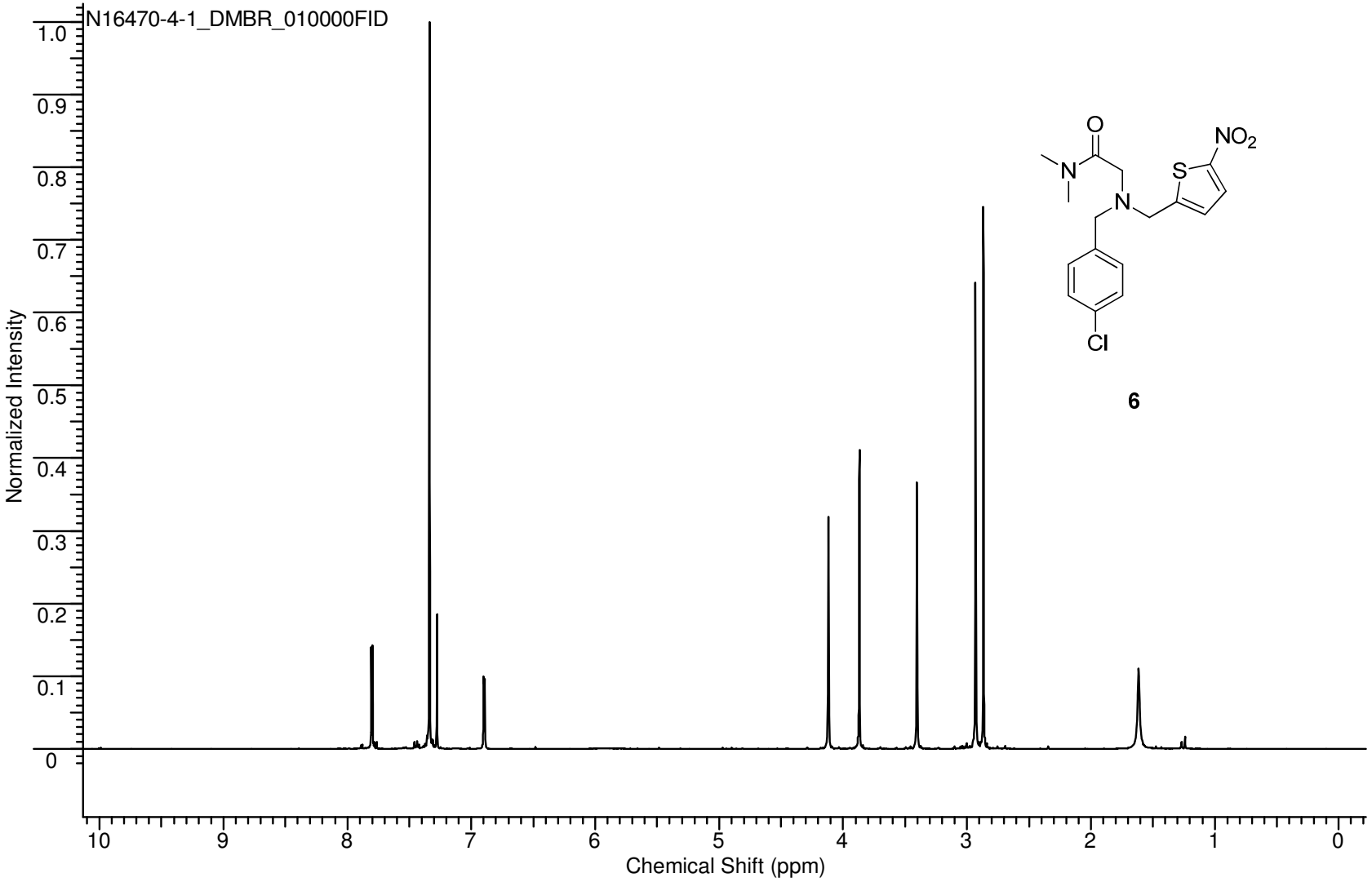
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NO2
Cl

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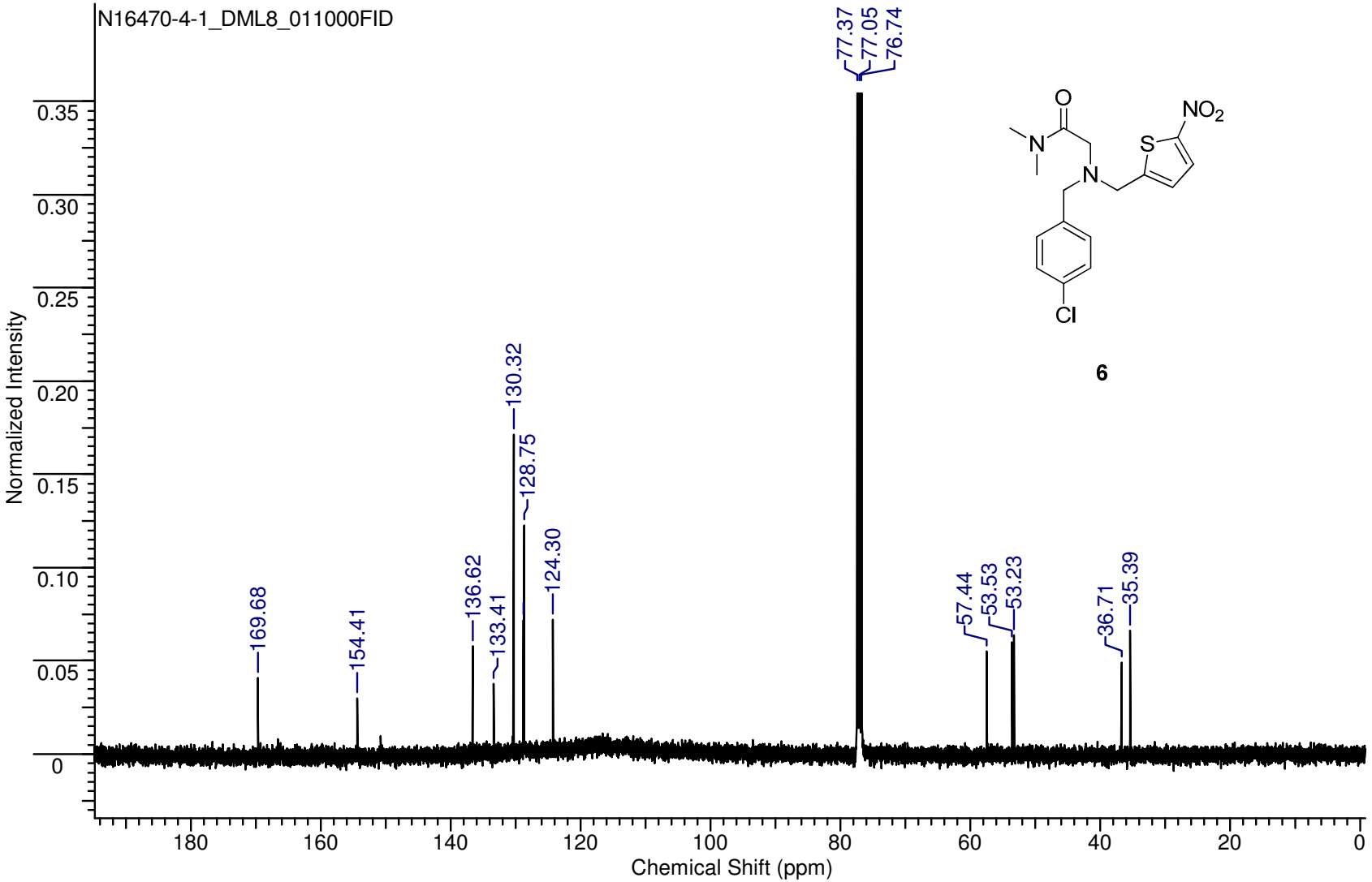
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