Supporting Information

Indole Chloropyridinyl Ester-Derived SARS-CoV-2 3CLpro Inhibitors: Enzyme Inhibition, Antiviral Efficacy, Structure-Activity and X-ray Structural Studies

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General Methods.

All reactions were carried out under an argon atmosphere in either flame or oven-dried (120 °C) glassware. All reagents and chemicals were purchased from commercial suppliers and used without further purification unless otherwise noted. Anhydrous solvents were obtained as follows: Dichloromethane from calcium hydride, diethyl ether and tetrahydrofuran from Na/Benzophenone, methanol and ethanol from activated magnesium under argon. All purification procedures were carried out with reagent grade solvents (purchased form VWR) in air. TLC analysis was conducted using glass-backed Thin-Layer Silica Gel Chromatography Plates (60 Å, 250 μm thickness, F-254 indicator). Column chromatography was performed using 230-400 mesh, 60 Å pore diameter silica gel. ^{1}H, ^{13}C NMR spectra were recorded at room temperature on a Bruker AV-III-400 and AV-III-800. Chemical shifts (δ values) are reported in parts per million, and are referenced to the deuterated residual solvent peak. NMR data is reported as: δ value (chemical shift, J-value (Hz), integration, where s = singlet, d = doublet, t = triplet, q = quartet, brs = broad singlet). LRMS and HRMS spectra were recorded at the Purdue University Department of Chemistry Mass Spectrometry Center. HPLC analysis was done an on Agilent 1260 series instrument using a YMC Pack ODS-A column of 4.6 mm ID for analysis. The purity of all test compounds was determined by HPLC analysis to be ≥90% pure.

Determination of X-ray structure of 3CL protease-inhibitor complex

Table S1: Crystallographic Data Collection and Refinement Statistics

3CLpro-Inhibitor Complex	Compound **2** (GRL-01720) bound to SARS-CoV-2	Compound **9d** (GRL-09120) bound to SARS-CoV-2	Compound 7**b** (GRL-0686) bound to SARS-CoV
Resolution range (Å)	27.08 - 1.649 (1.708 - 1.649)	29.83 - 1.646 (1.705 - 1.646)	30.58 - 1.63 (1.688 - 1.63)
Space group	C 1 2 1	C 1 2 1	C 1 2 1
Unit cell (Å)			
a	96.50	97.20	106.73
b	82.14	82.43	84.47
c	54.38	54.28	53.50
β (degrees)	116.9	117.2°	105.1°
Total reflections	218414 (22139)	220813 (21442)	402081 (24801)
Unique reflections	45449 (4502)	45866 (4440)	56039 (4949)
Multiplicity	4.8 (4.9)	4.8 (4.8)	7.2 (5.0)
Completeness (%)	99.79 (99.40)	99.53 (96.86)	97.67 (85.93)
Mean I/sigma(I)	16.18 (2.42)	17.86 (2.15)	23.62 (2.00)
Wilson B-factor (Å^2)	20.98	22.09	27.55
R-merge	0.07787 (0.5886)	0.07269 (0.7296)	0.05835 (0.5505)
R-meas	0.08759 (0.6578)	0.08167 (0.8192)	0.0633 (0.6047)
R-pim	0.03933 (0.2891)	0.03674 (0.3683)	0.02408 (0.2451)

CC1/2	0.997 (0.86)	0.998 (0.777)	0.998 (0.938)
CC*	0.999 (0.962)	1 (0.935)	0.999 (0.984)
Reflections used in refinement	45440 (4498)	45864 (4440)	55826 (4910)
Reflections used for R-free	2000 (198)	2000 (194)	1990 (174)
R-work	0.1712 (0.2307)	0.1572 (0.2197)	0.1905 (0.3228)
R-free	0.1940 (0.2776)	0.1841 (0.2597)	0.2219 (0.3472)
CC(work)	0.961 (0.918)	0.972 (0.905)	0.940 (0.917)
CC(free)	0.958 (0.782)	0.969 (0.827)	0.914 (0.925)
Number of non-hydrogen atoms	2580	2668	2806
macromolecules	2353	2386	2436
ligands	11	11	43
solvent	216	271	327
Protein residues	301	302	306
RMS(bonds)	0.011	0.011	0.006
RMS(angles)	1.43	1.39	0.77
Ramachandran favored (%)	98.33	98.67	98.36
Ramachandran allowed (%)	1.34	1.33	1.64
Ramachandran outliers (%)	0.33	0.00	0.00
Rotamer outliers (%)	1.53	2.26	0.73
Clashscore	2.78	3.59	7.13
Average B-factor (Å^2)	35.79	34.85	45.47
macromolecules	35.45	33.92	43.97
ligands	40.97	58.67	68.45
solvent	39.18	42.04	53.60
Number of TLS groups	1	1	15

Cells, viruses, and antiviral activity.

VeroE6 cells and TMPRSS2-overexpressing VeroE6 (VeroE6TMPRSS2) cells were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan). VeroE6 cells were maintained in Dulbecco's modified Eagle's medium (d-MEM) supplemented with 10% fetal bovine serum (FCS), 100 μg/ml of penicillin, and 100 μg/ml of streptomycin. VeroE6TMPRSS2 cells were maintained in d-MEM as reported (ref.1) in the presence of 1 mg/ml of G418. SARS-CoV-2 strain JPN/TY/WK-521 (SARS-CoV-2WK-521) was obtained from the National Institute of Infectious Diseases (Tokyo, Japan).

Antiviral assay was carried out as described recently (ref 1): Cells were seeded in a 96-well plate ($2x10^4$ cells/well) and incubated. After 24 h, virus was inoculated into cells at multiplicity of infection (MOI) of 0.05. After an additional 72 h, cell culture supernatants were harvested and viral RNA was extracted using a QIAamp viral RNA minikit (Qiagen, Hilden, Germany), and

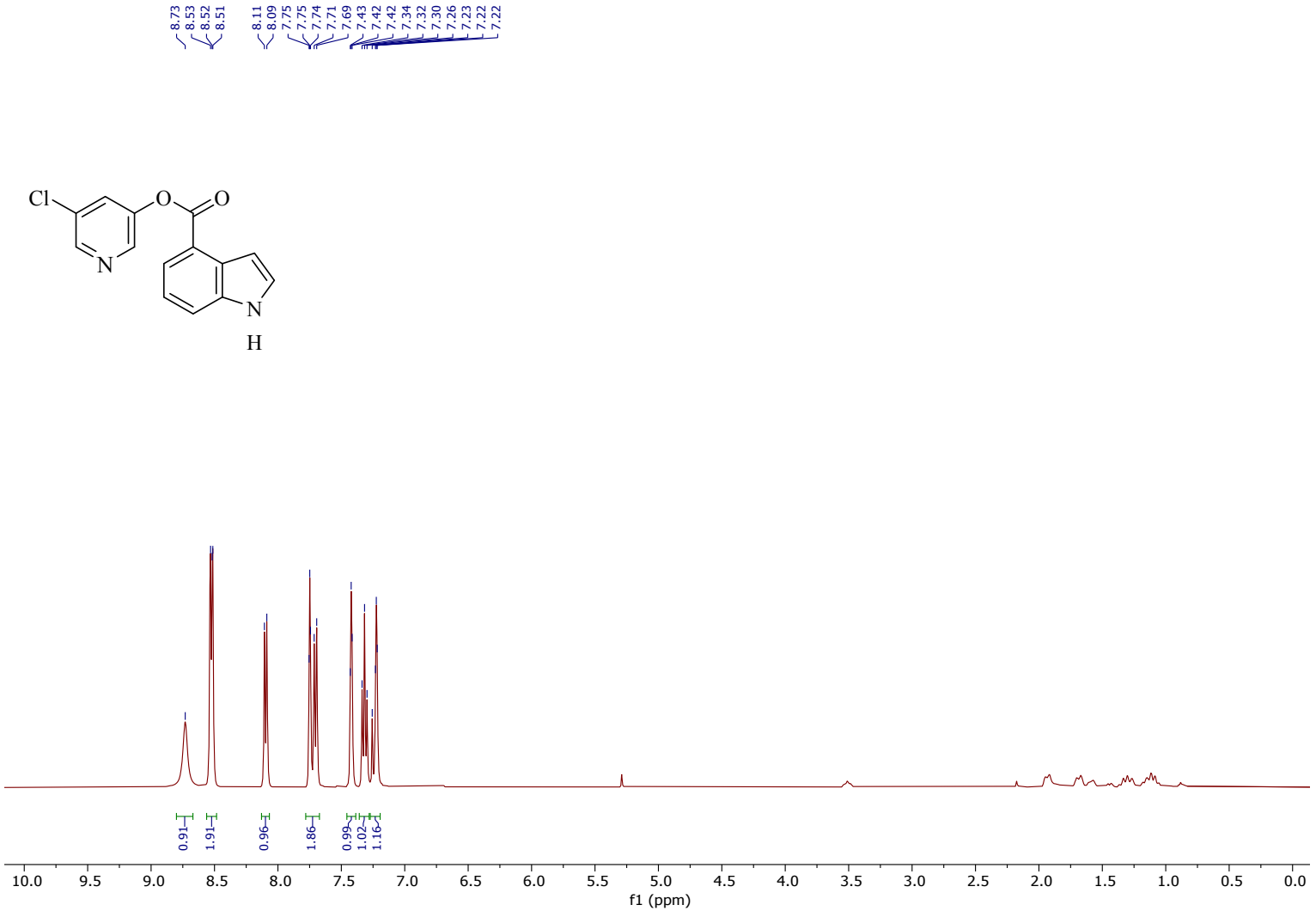
quantitative RT-PCR (RT-qPCR) was then performed using One Step PrimeScript III RT-qPCR mix (TaKaRa Bio, Shiga, Japan) following the instructions of the manufacturers. The primers and probe used for detecting SARS-CoV-2 envelope (6) were 5=-ACT TCT TTT TCT TGC TTT CGT GGT-3= (forward), 5=-GCA GCA GTA CGC ACA CAA TC-3= (reverse), and 5=-FAM-CTA GTT ACA CTA GCC ATC CTT ACT GC-black hole quencher 1 (BHQ1)-3= (probe). To determine the cytotoxicity of each compound, cells were seeded in a 96-well plate (2_104 cells/well). One day later, various concentrations of each compound were added, and cells were incubated for additional 3 days. The 50% cytotoxic concentrations (CC50) values were determined using the WST-8 assay and Cell Counting Kit-8 (Dojindo, Kumamoto, Japan).

Immunochemistry.

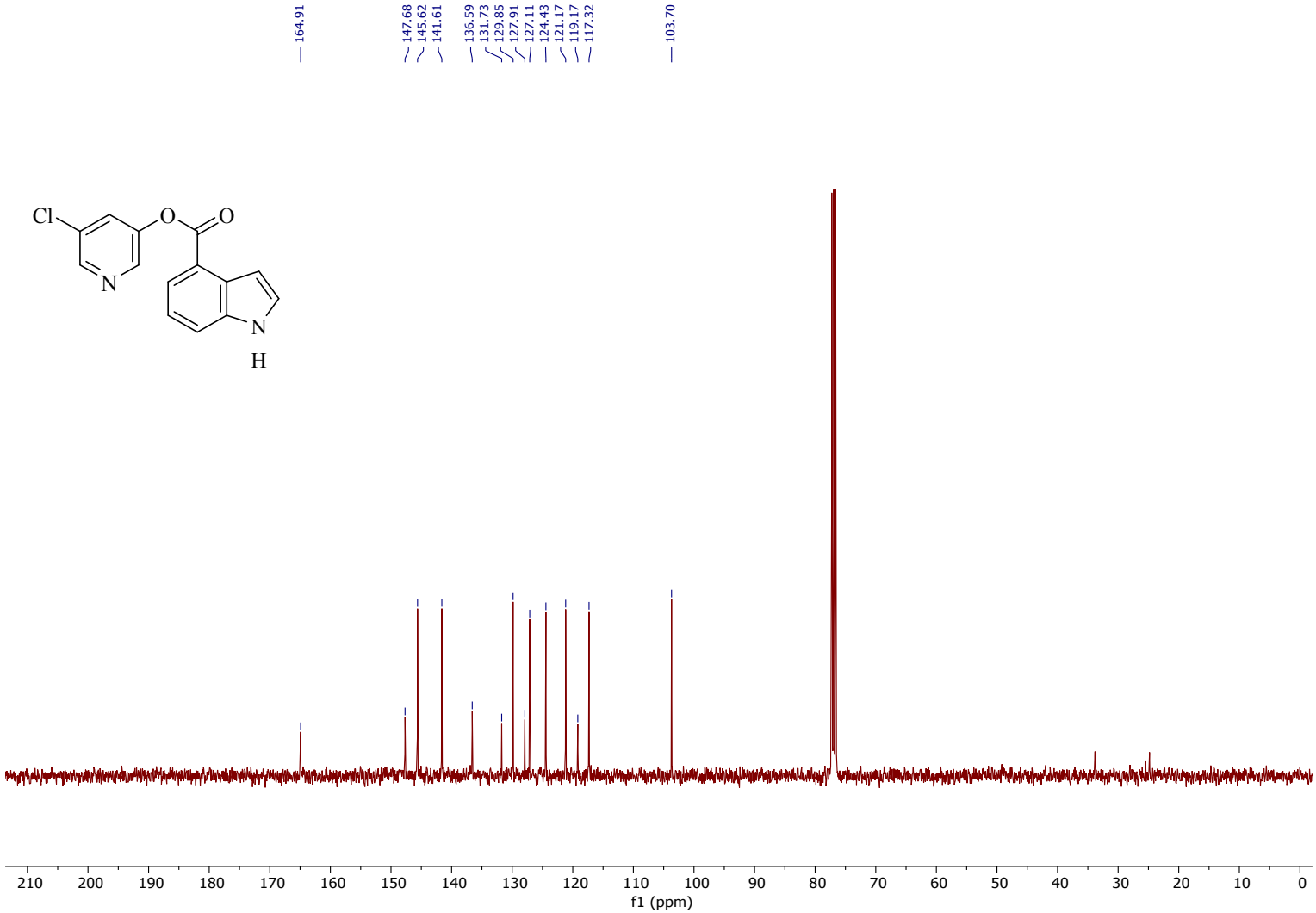
Details are reported in a recent paper (ref.1). As described, cells in a 96-well microtiter culture plate were fixed with 4% paraformaldehyde–phosphate-buffered saline (PBS) for 15 min, washed with PBS (300 µl/well) three times for 5 min each time, and then blocked with a blocking buffer (10% goat serum, 1% bovine serum albumin [BSA], 0.3% Triton X-100, PBS 1x) for 1 h. The blocking buffer was removed and the cells were immediately stained with the primary antibody mouse monoclonal anti-SARS-CoV/SARS-CoV-2 (COVID-19) spike antibody (1A9) (GeneTex, Alton Pkwy Irvine, CA, USA) or a convalescent IgG fraction, which was isolated from serum of a convalescent COVID-19 individual using a spin column-based antibody purification kit (Cosmo Bio, Tokyo, Japan) overnight at 4°C. The stained cells were washed with PBS (300 µl/well) three times for 5 min each time, and the cells were incubated with secondary antibody goat polyclonal anti-mouse IgG-Alexa Fluor 488 antibody (Thermo Fisher Scientific, Waltham, MA, USA), or goat polyclonal anti-human IgG-Alexa Fluor 488 Fab fragment antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA), together with Texas Red-X dye-conjugated phalloidin (Thermo Fisher Scientific) for F-actin visualization for 2 h. The cells were washed with PBS (300 µl/well) three times for 5 min each time, DAPI (4=,6-diamidino-2-phenylindole) solution (Thermo Fisher Scientific)–PBS (50 µl/well) was added to stain nuclei. Signals were acquired with a Cytation 5 cell imaging multimode reader (BioTek,Winooski, VT, USA).

References:

[1] Reference: Hattori, S.-i.; Higshi-Kuwata, N.; Raghavaiah, J.; Das, D.; Bulut, H.; Davis, D. A.; Takamatsu, Y.; Matsuda, K.; Takamune, N.; Kishimoto, N.; Okamura, T.; Misumi, S.; Yarchoan, R.; Maeda, K.; Ghosh, A. K.; Mitsuya, H. GRL-0920, an Indole Chloropyridinyl Ester, Completely Blocks SARS-CoV-2 Infection. *mBio* **2020**, *11*, e01833-20.



^{1}H NMR (400 MHz, $CDCl_3$) of Compound 1



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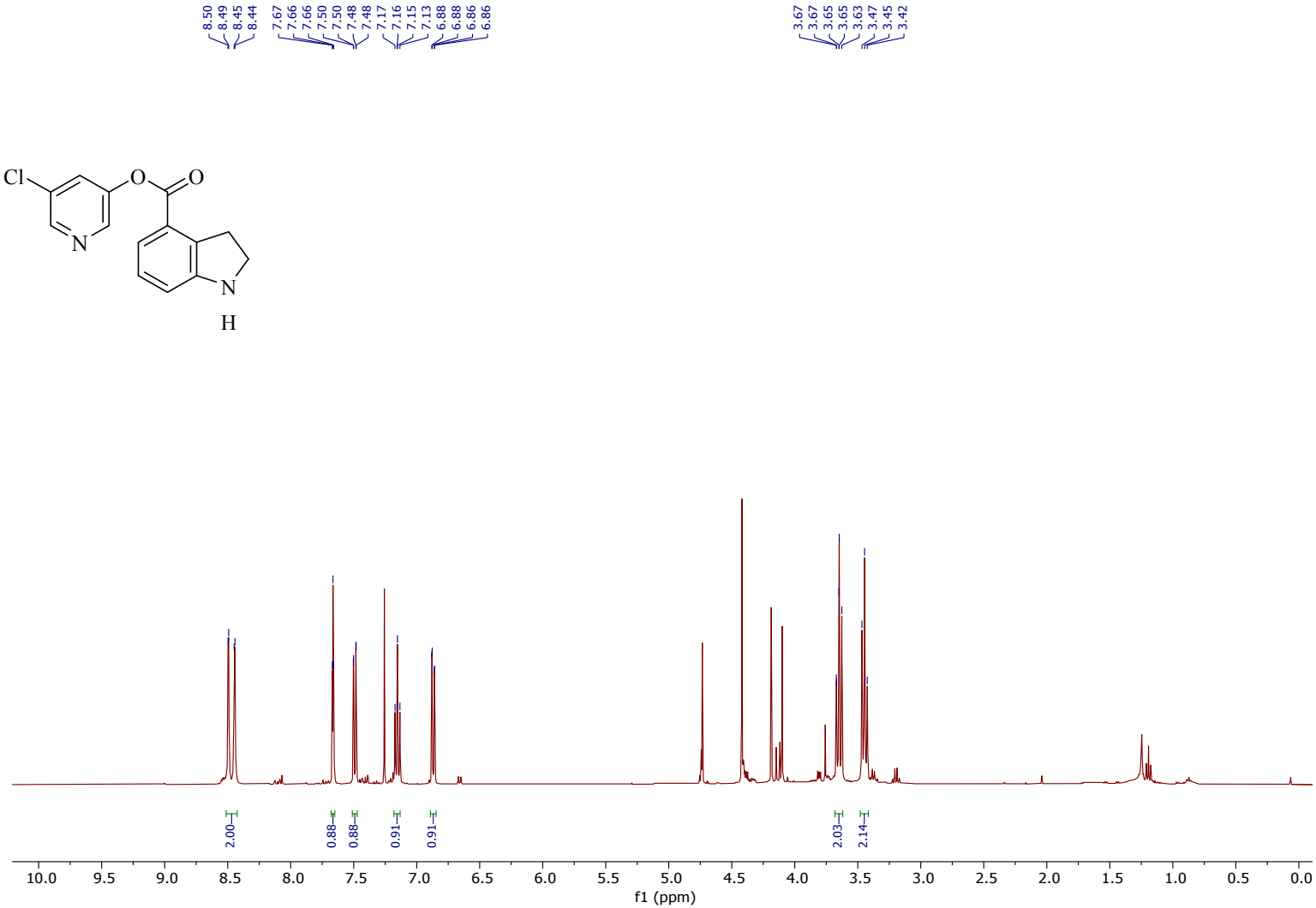

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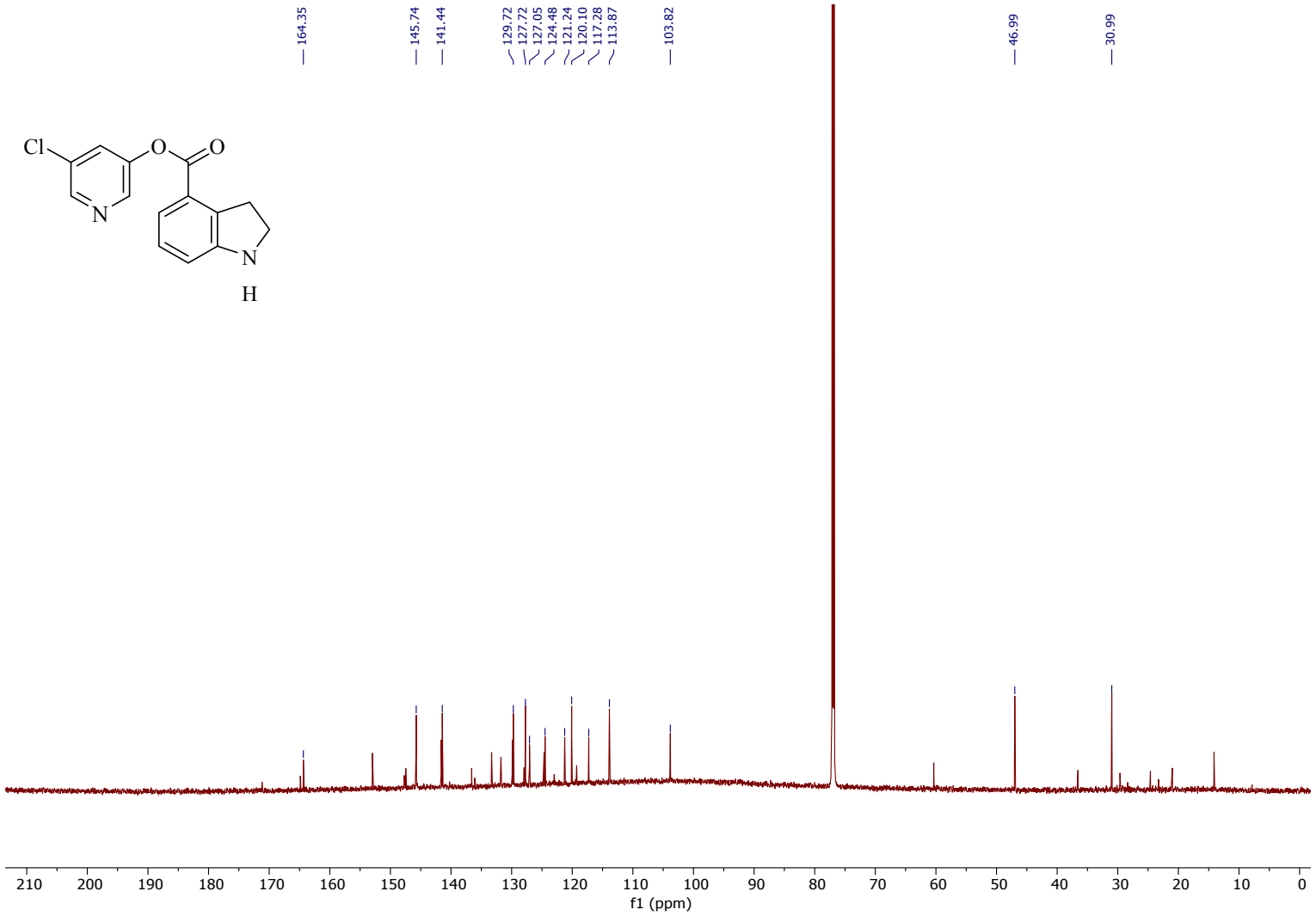

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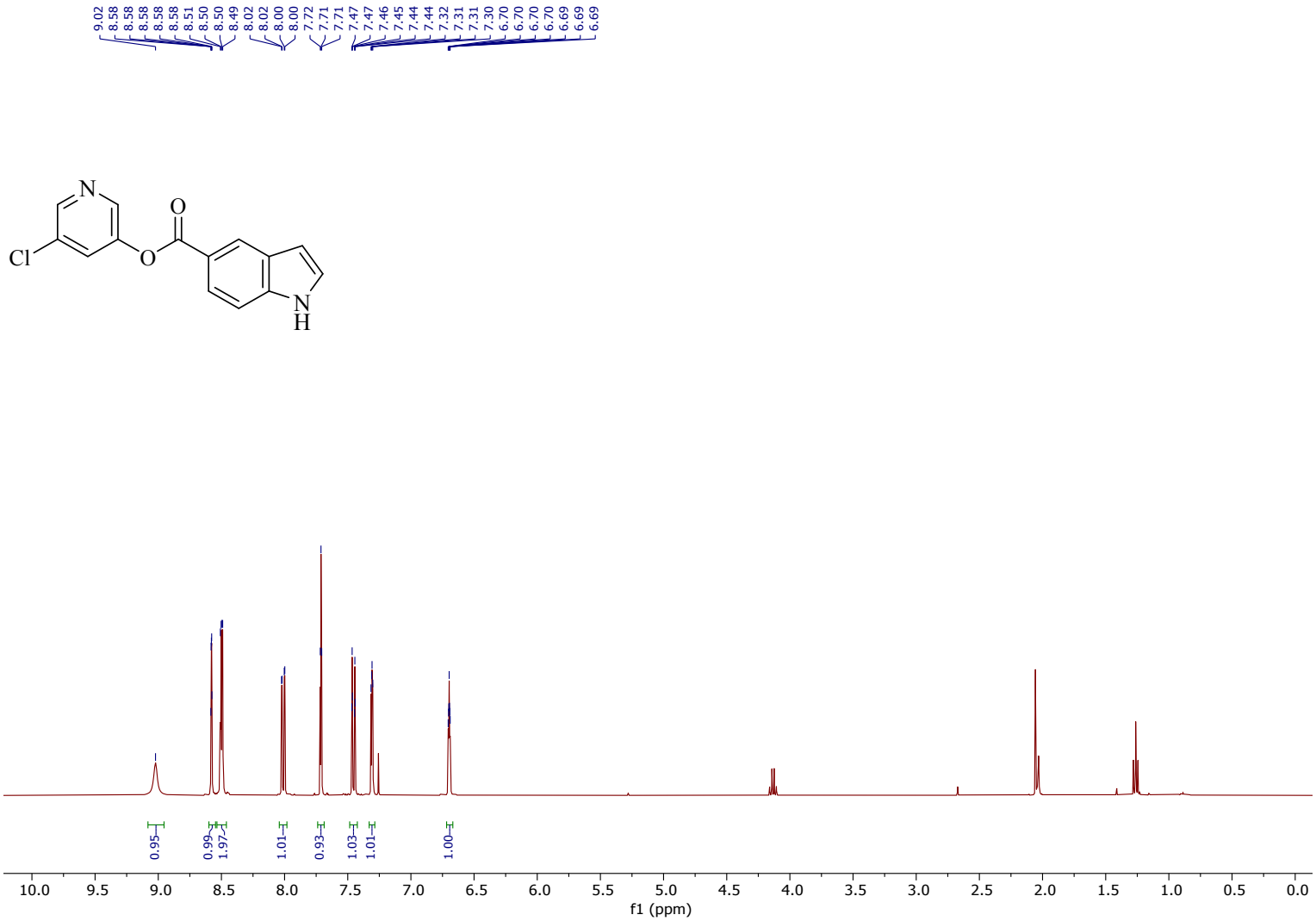
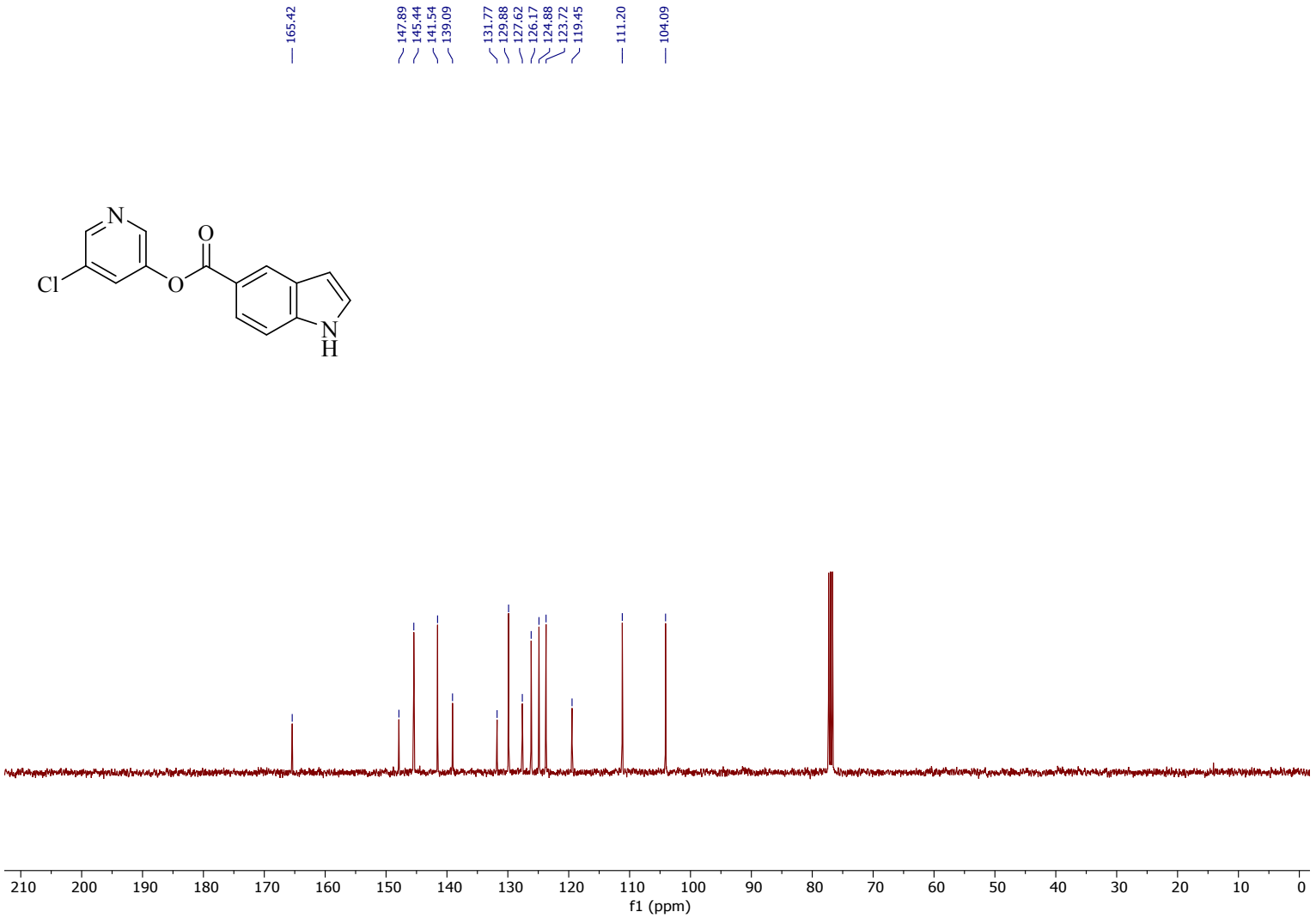


^{1}H NMR (400 MHz, $CDCl_3$) of Compound 7a



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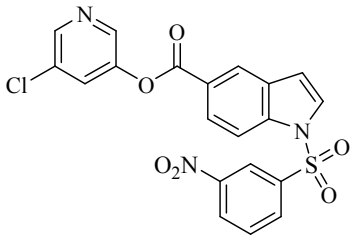

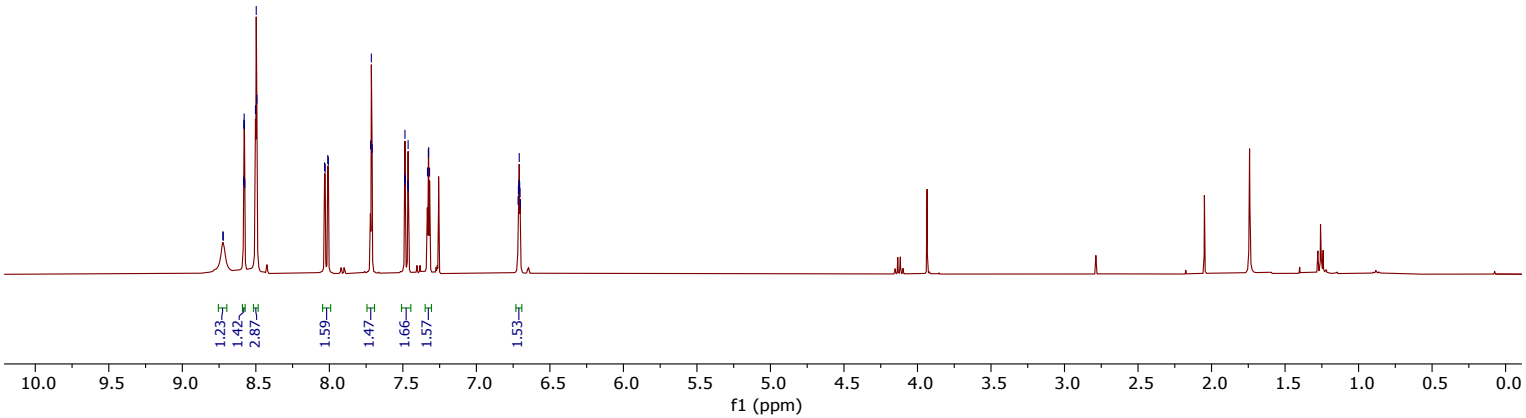

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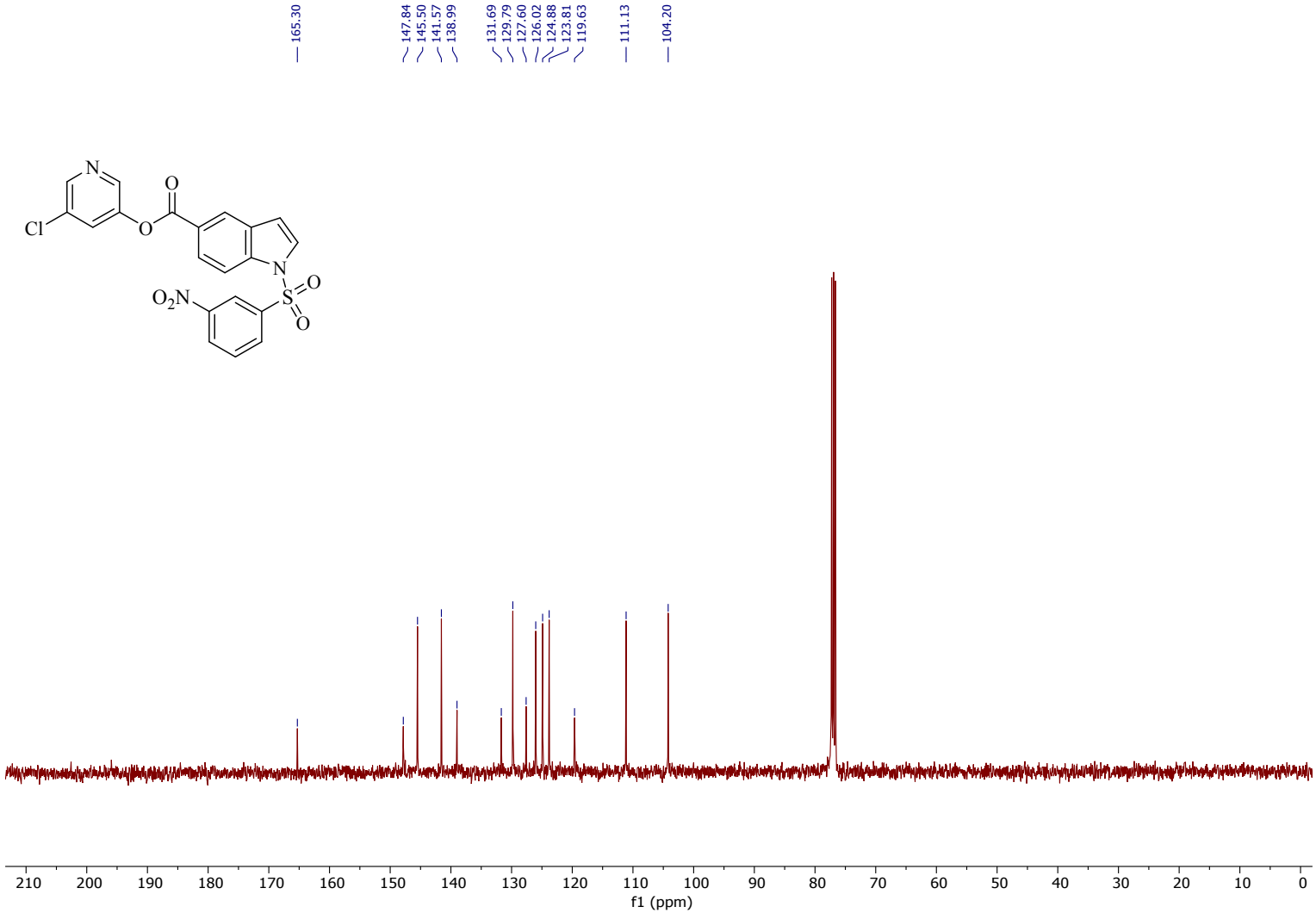

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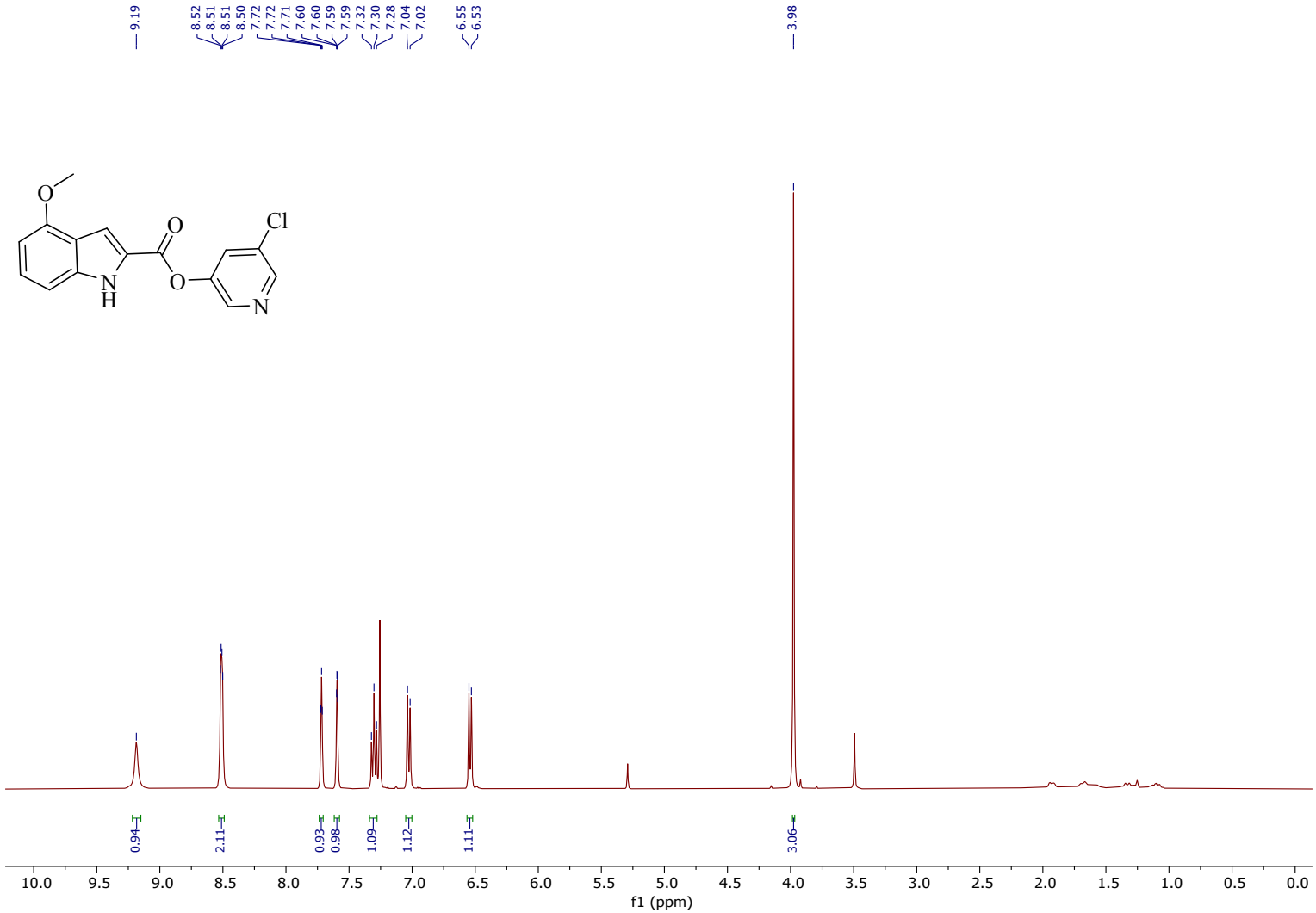

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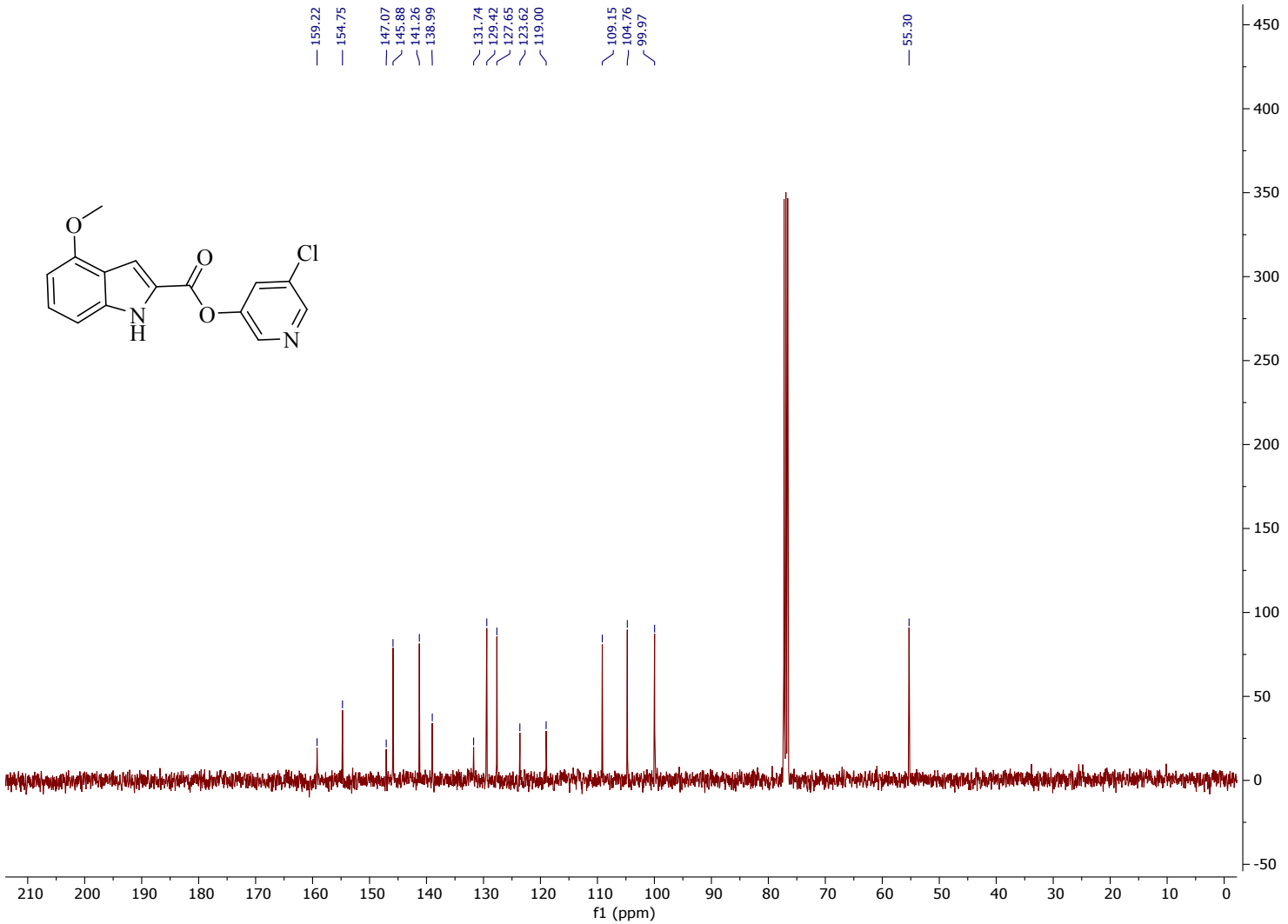

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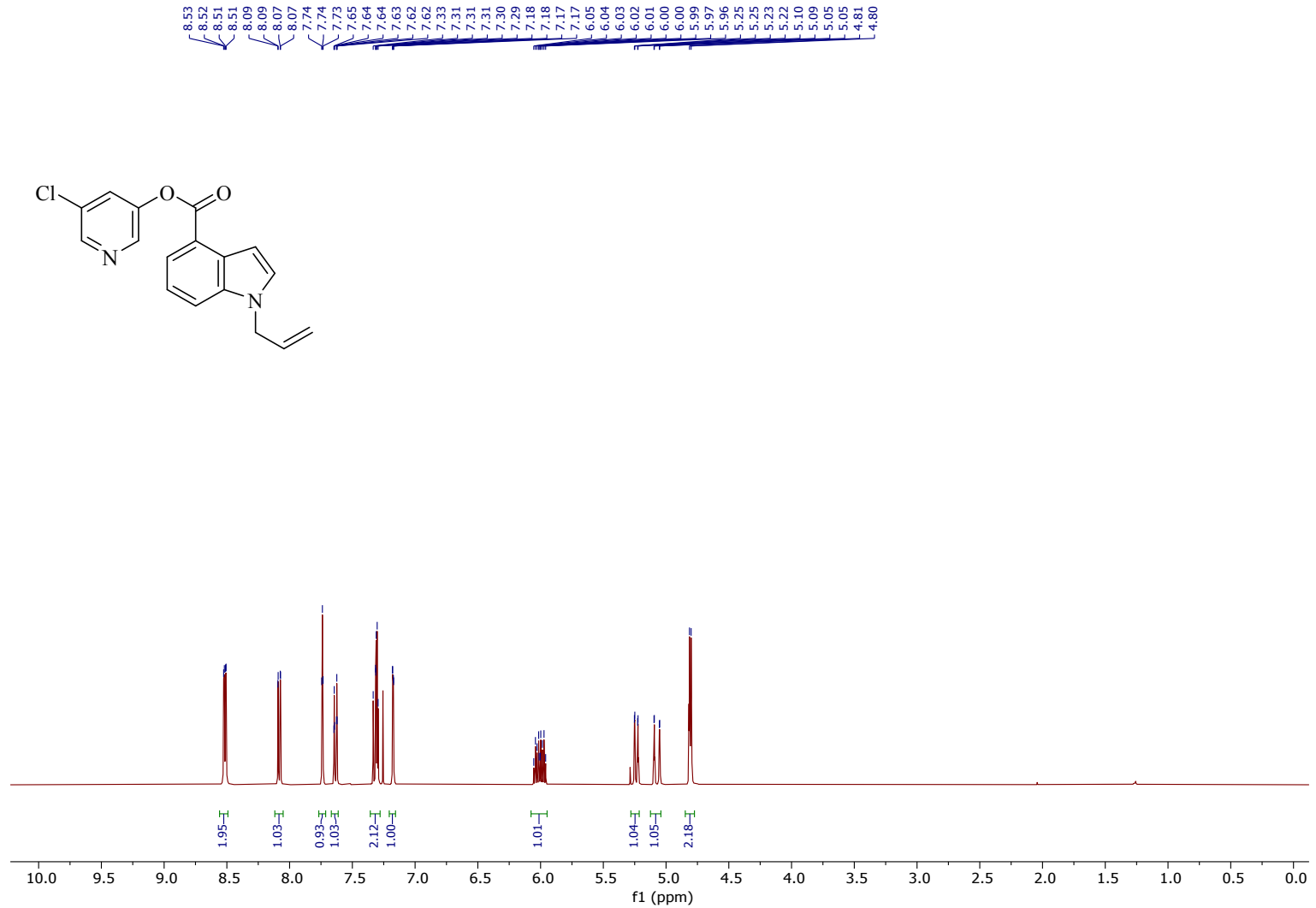

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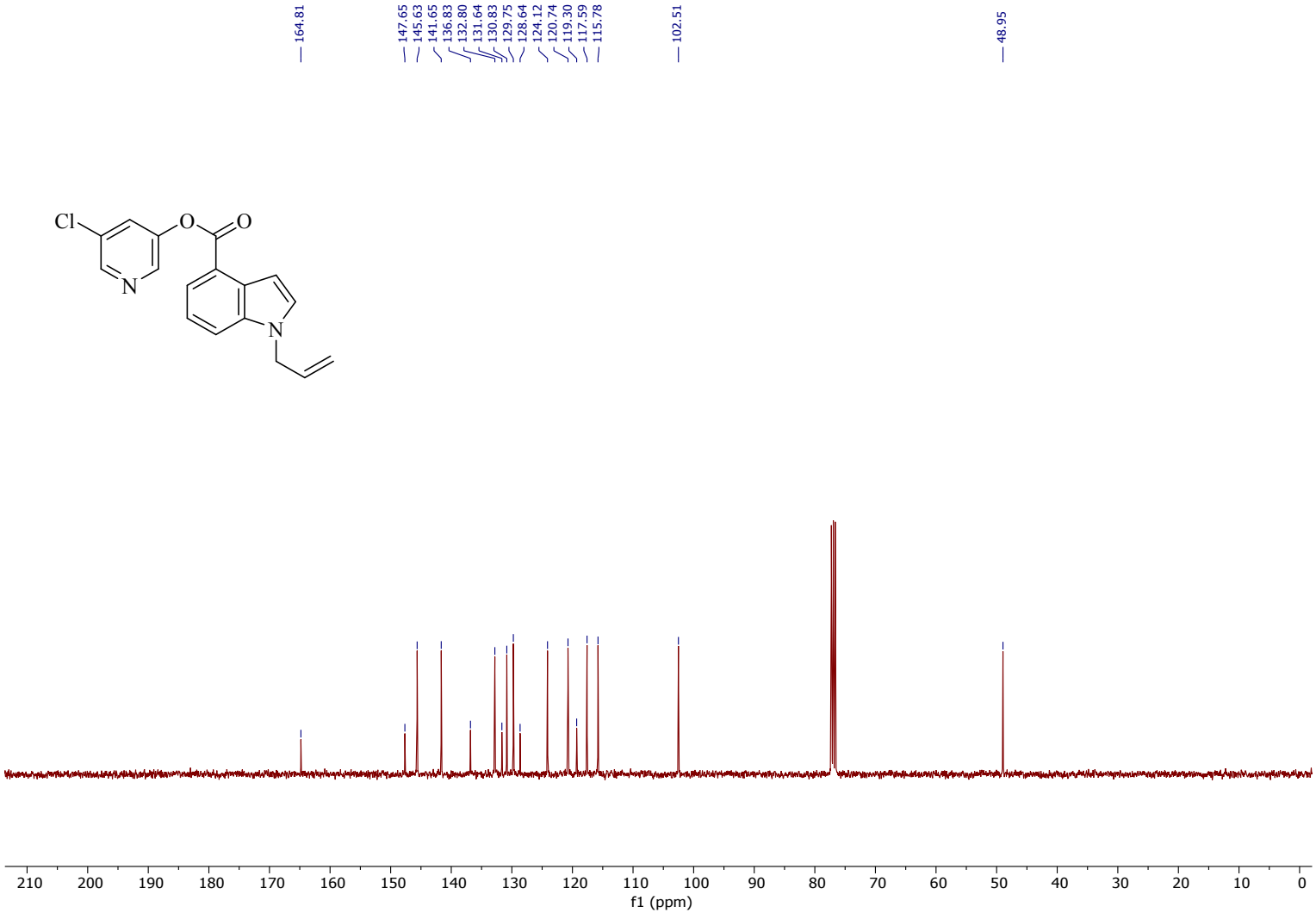

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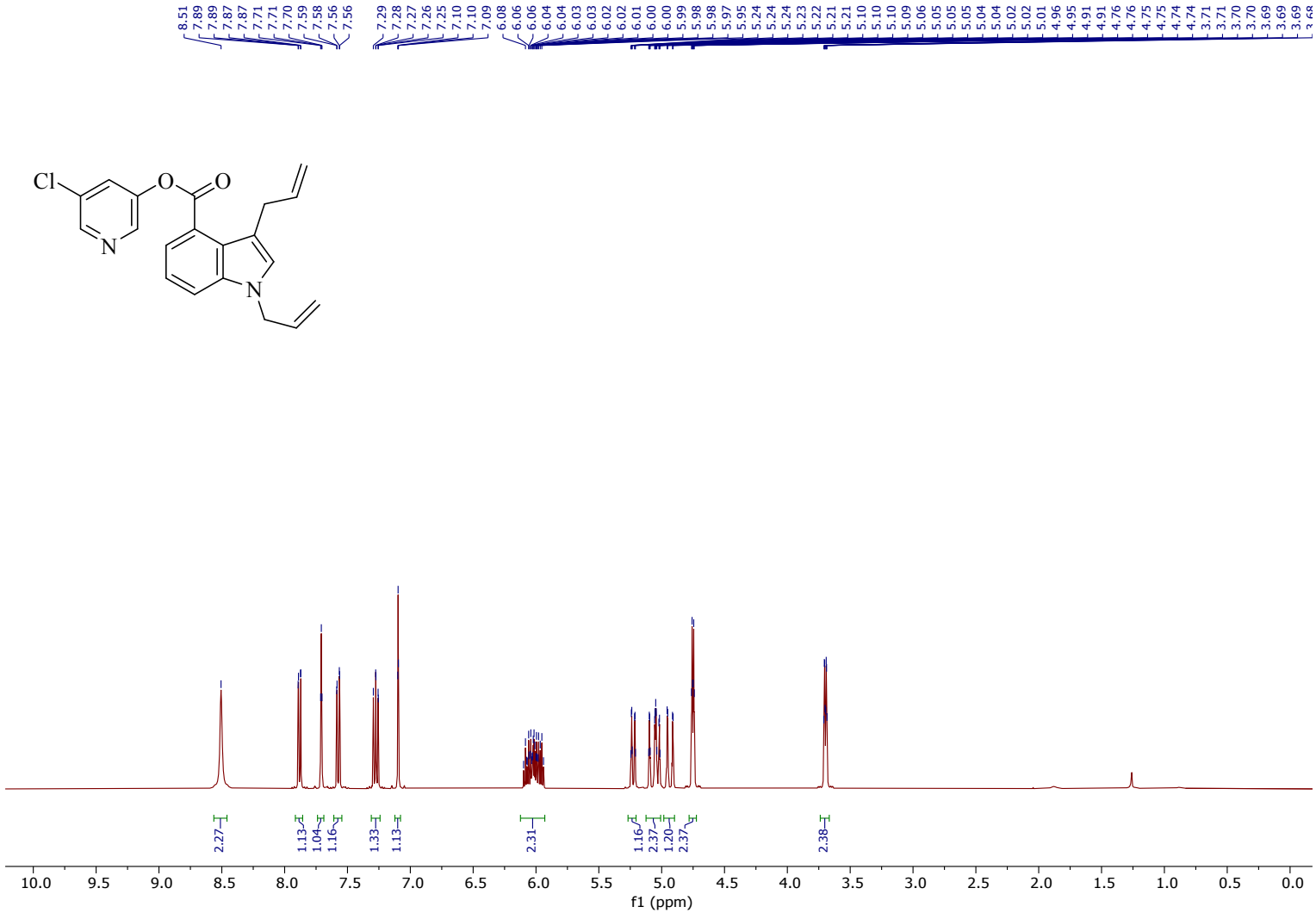

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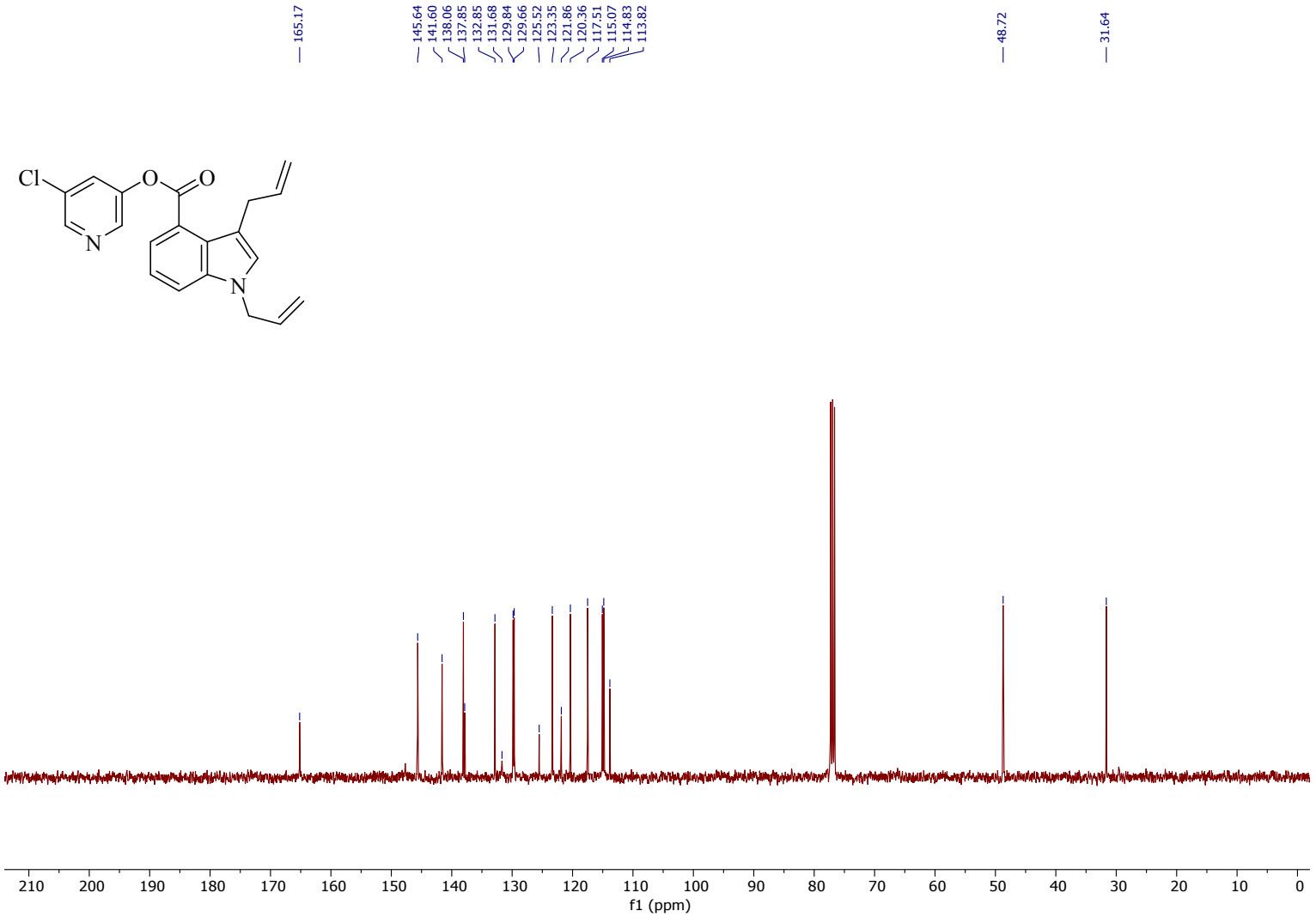

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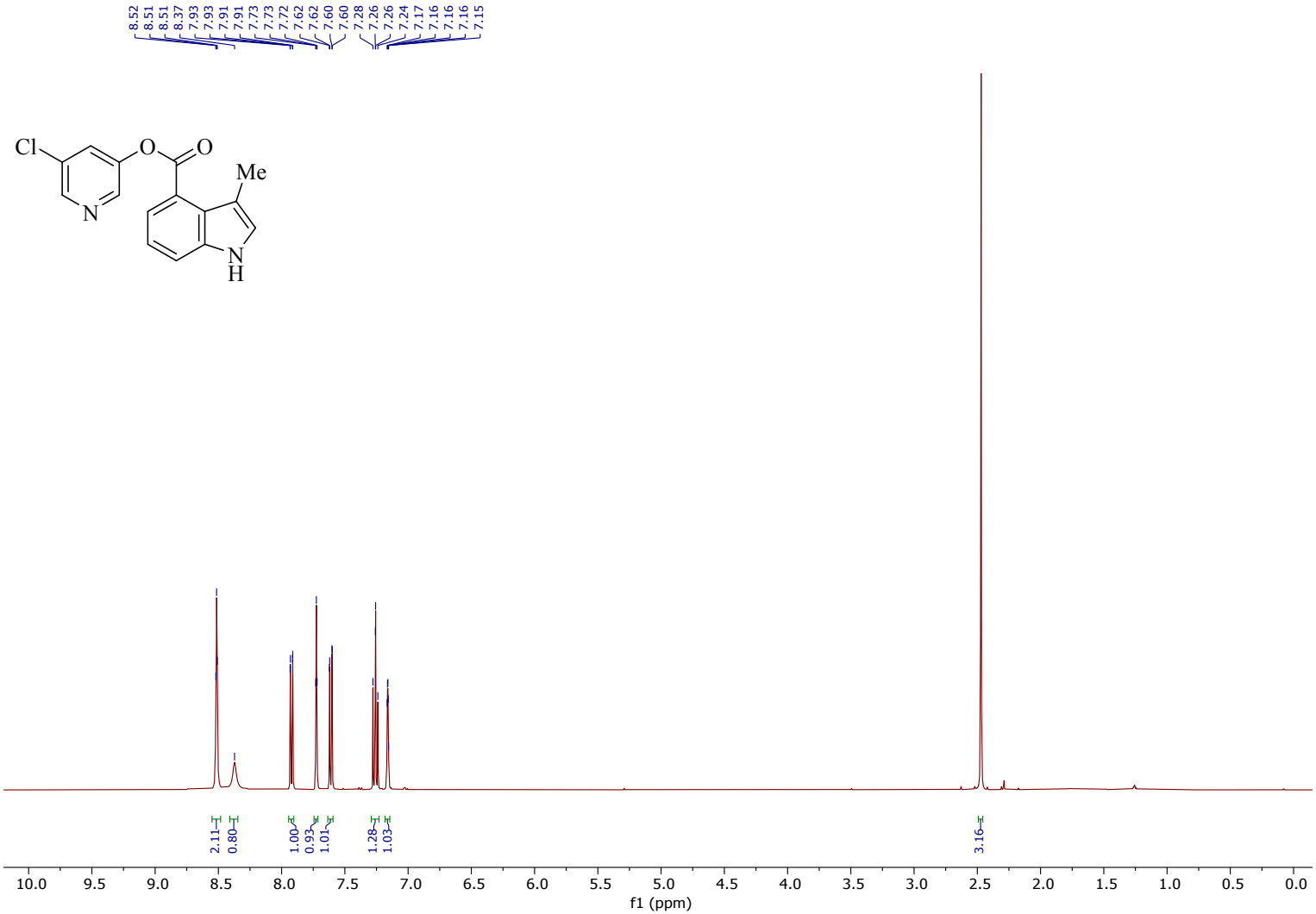

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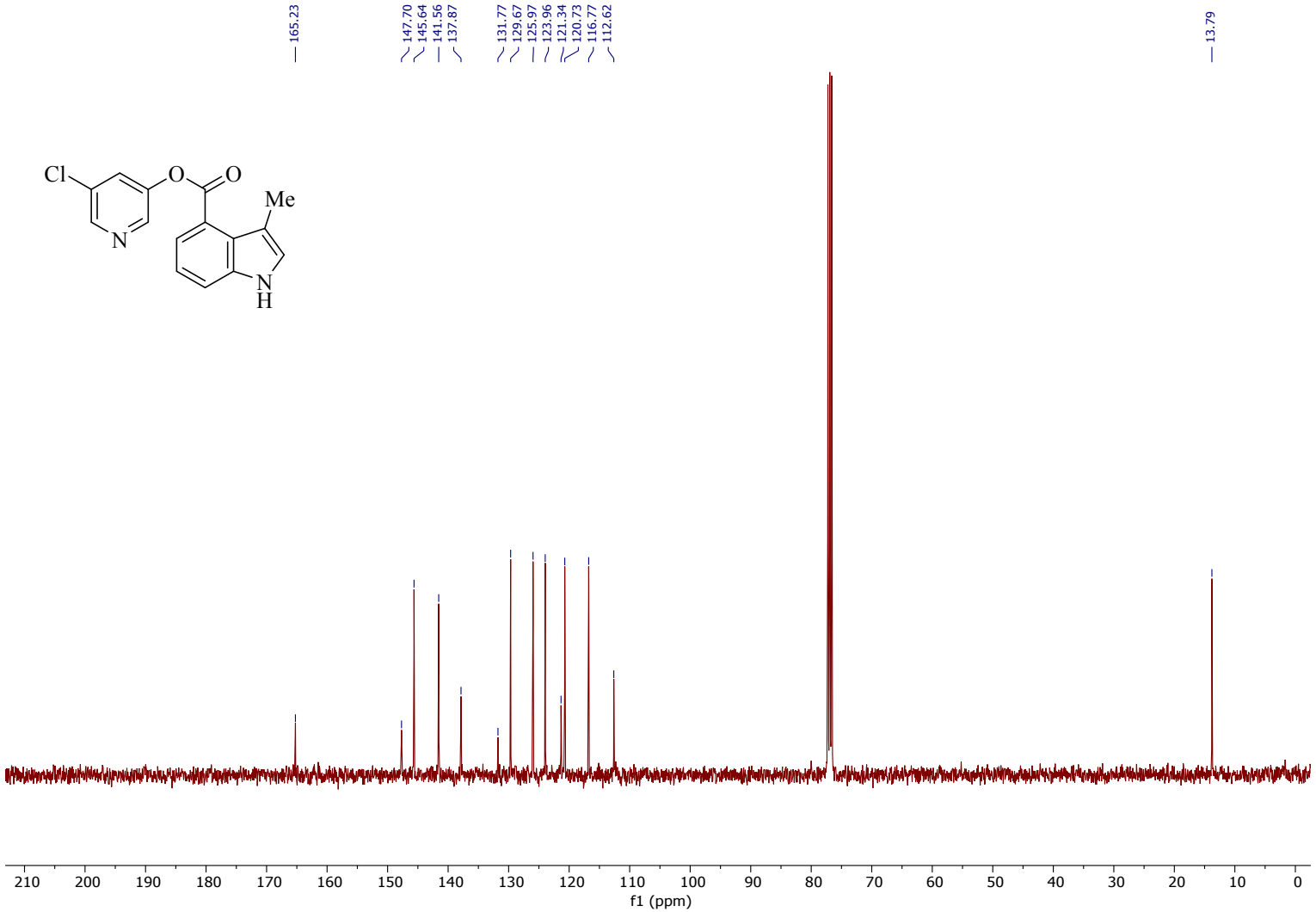

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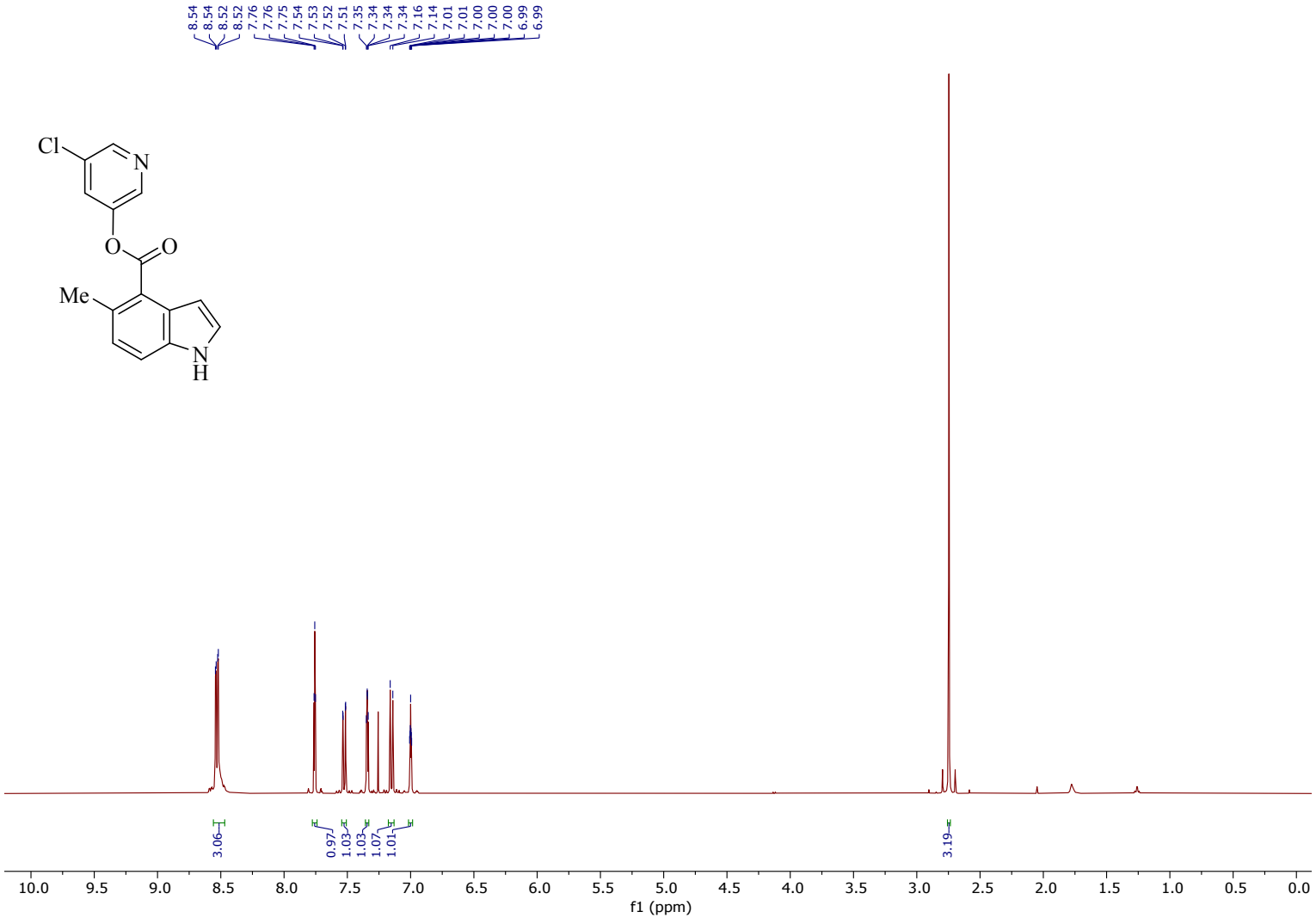

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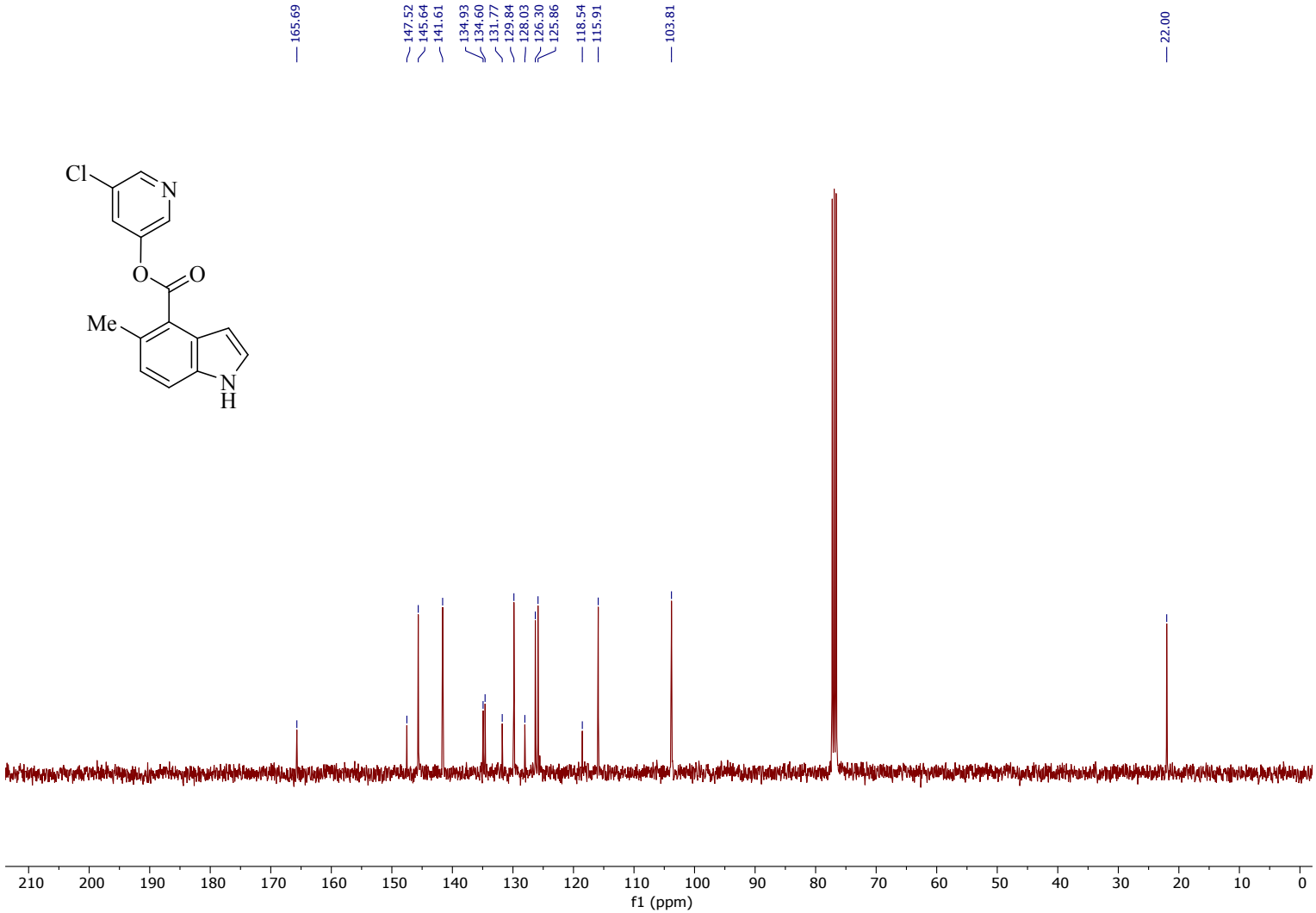

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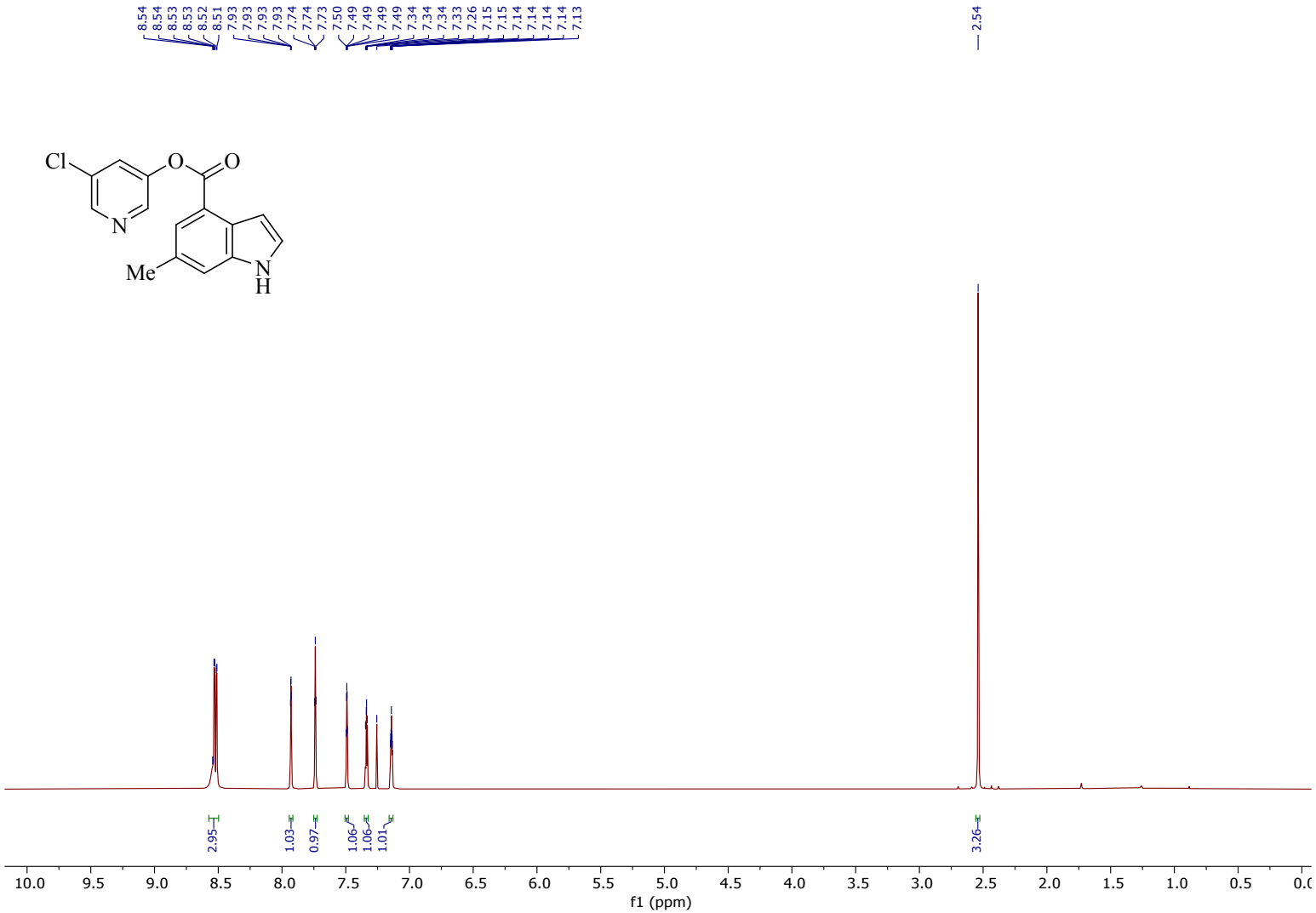

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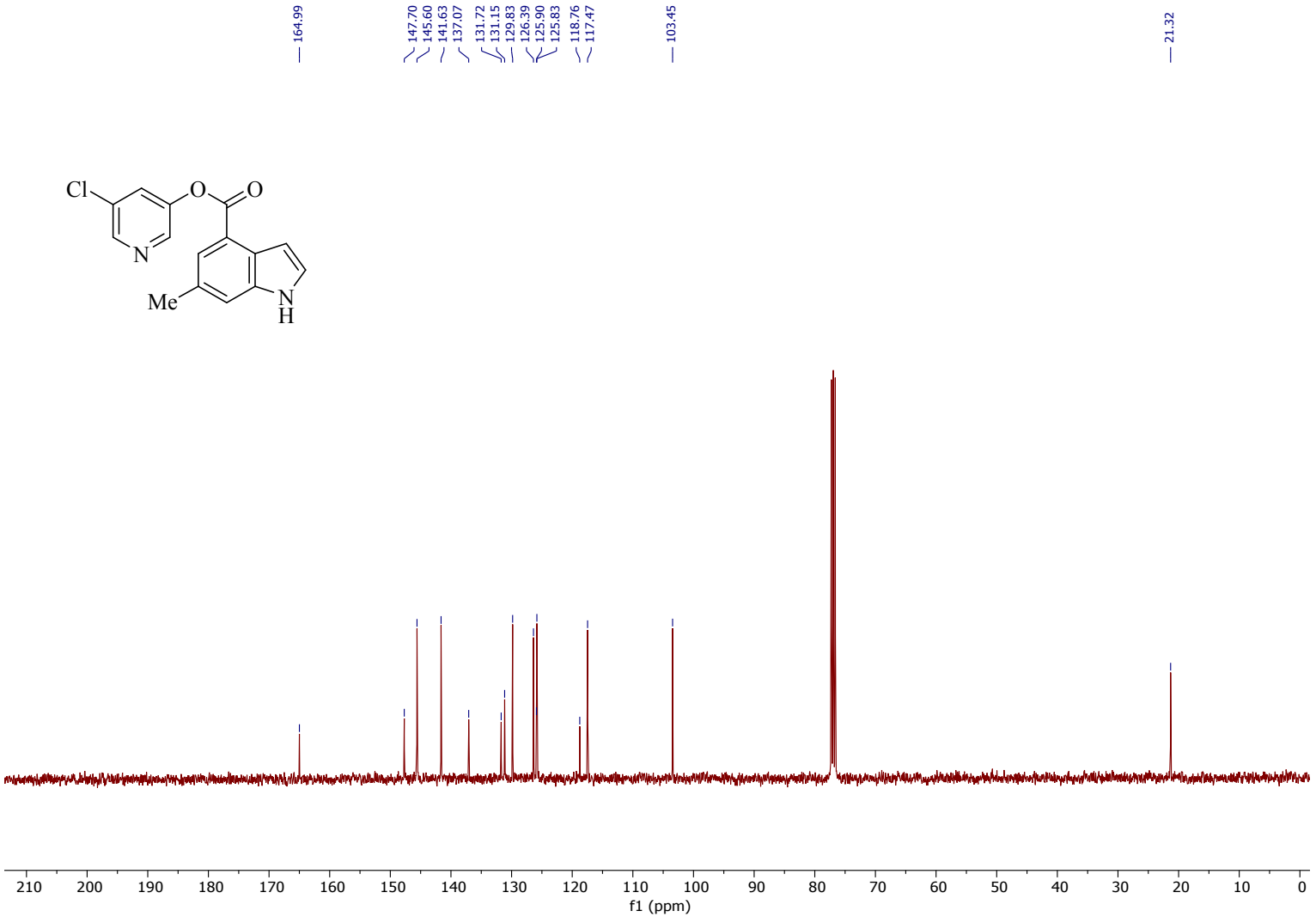

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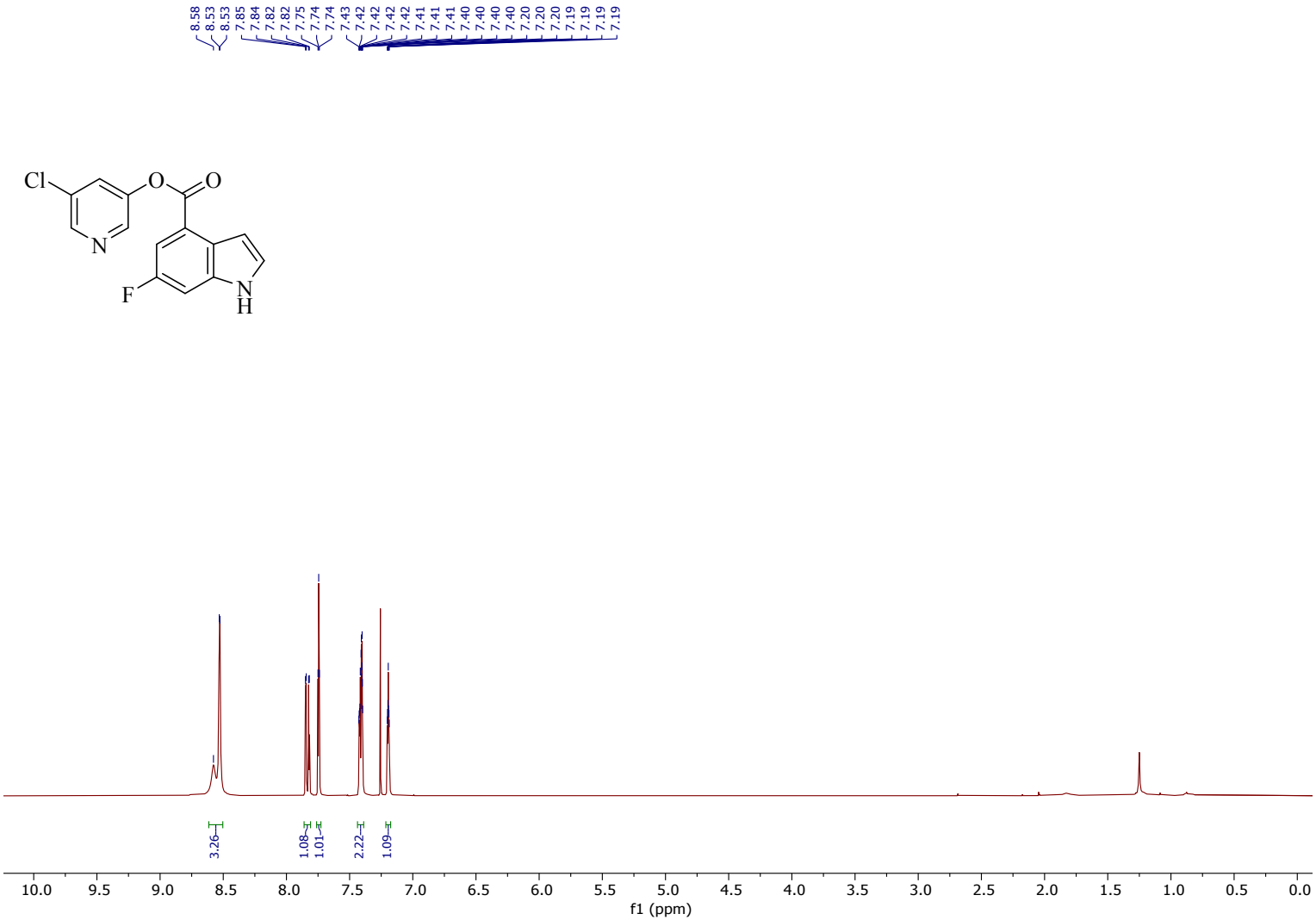

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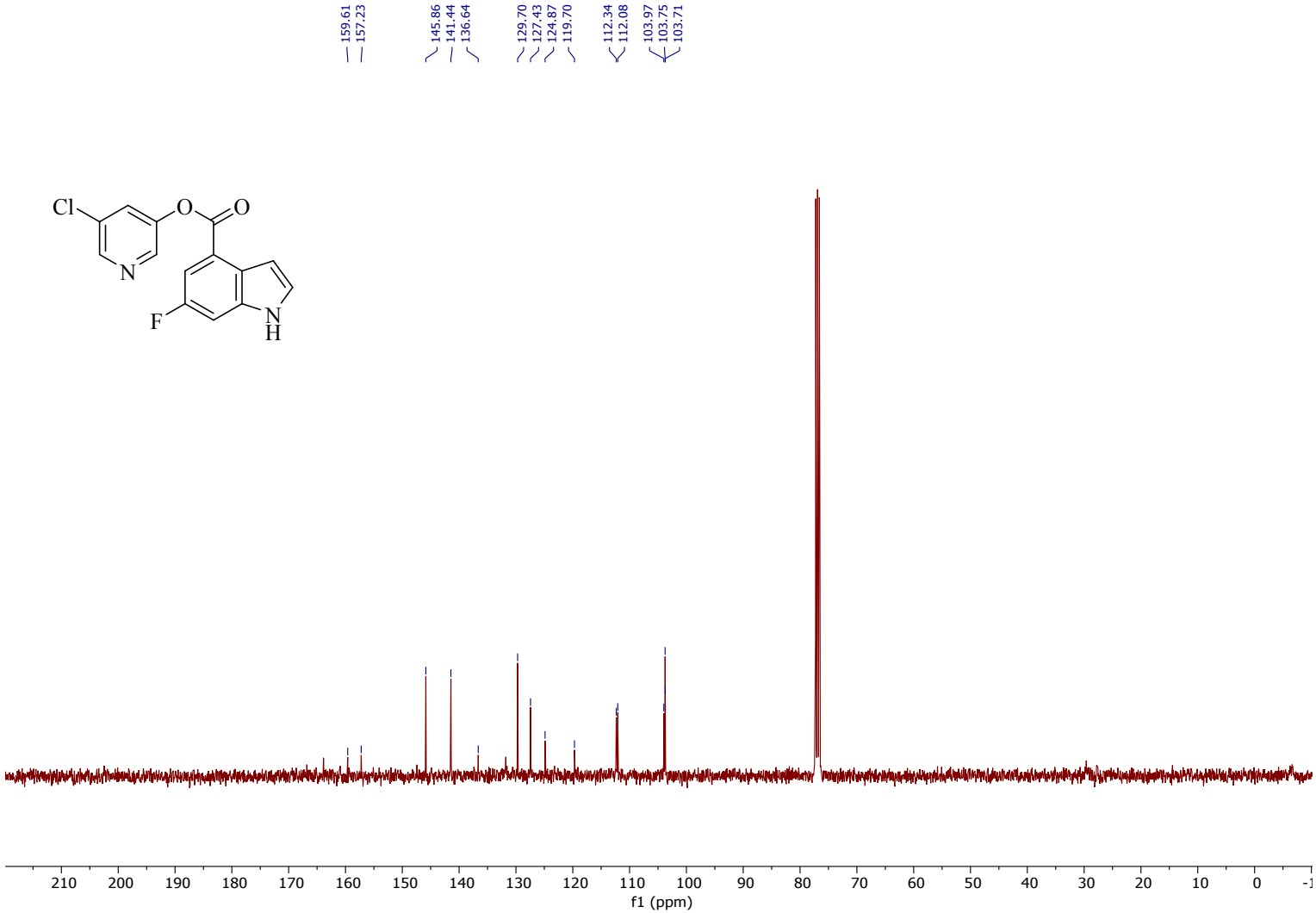

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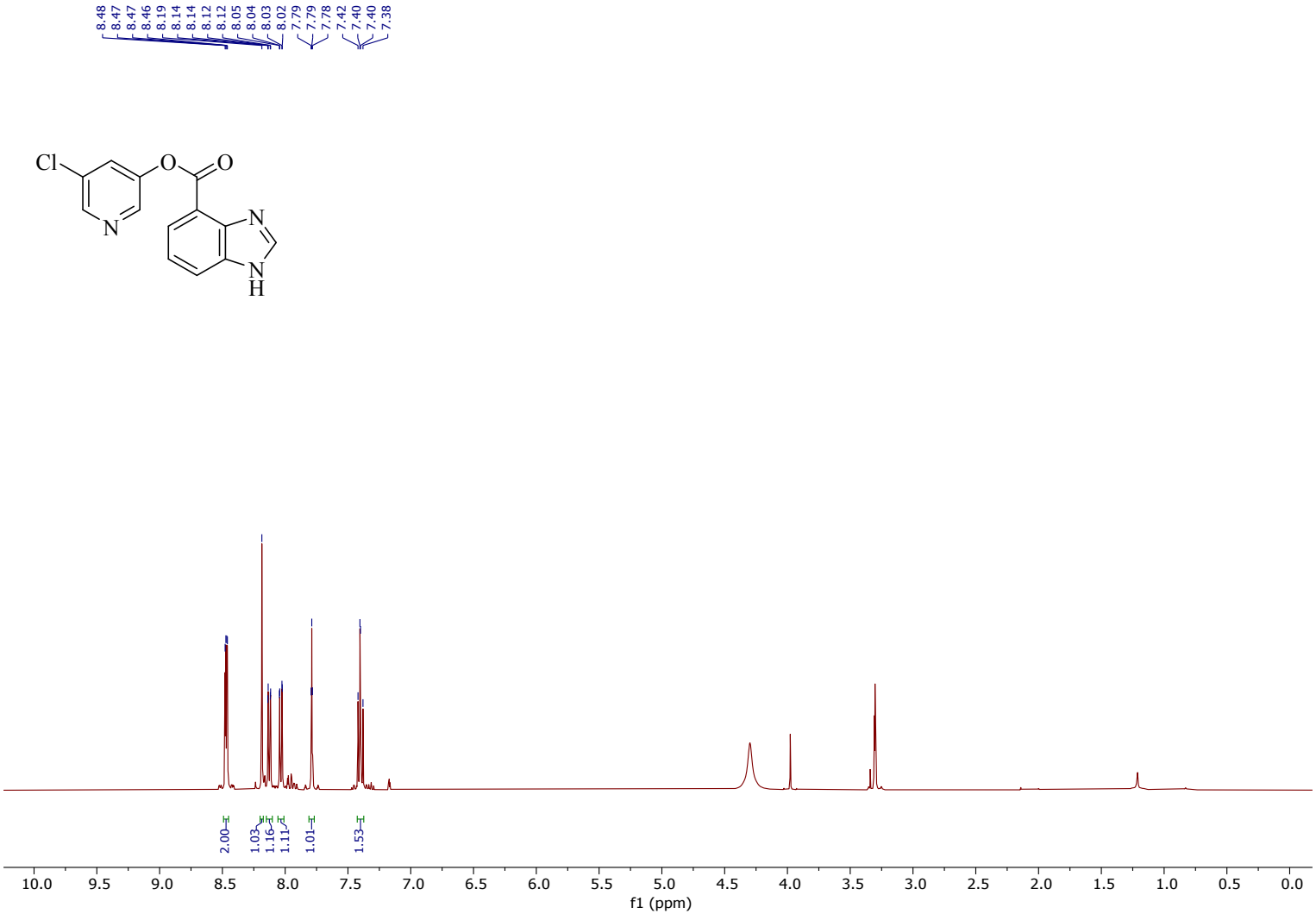

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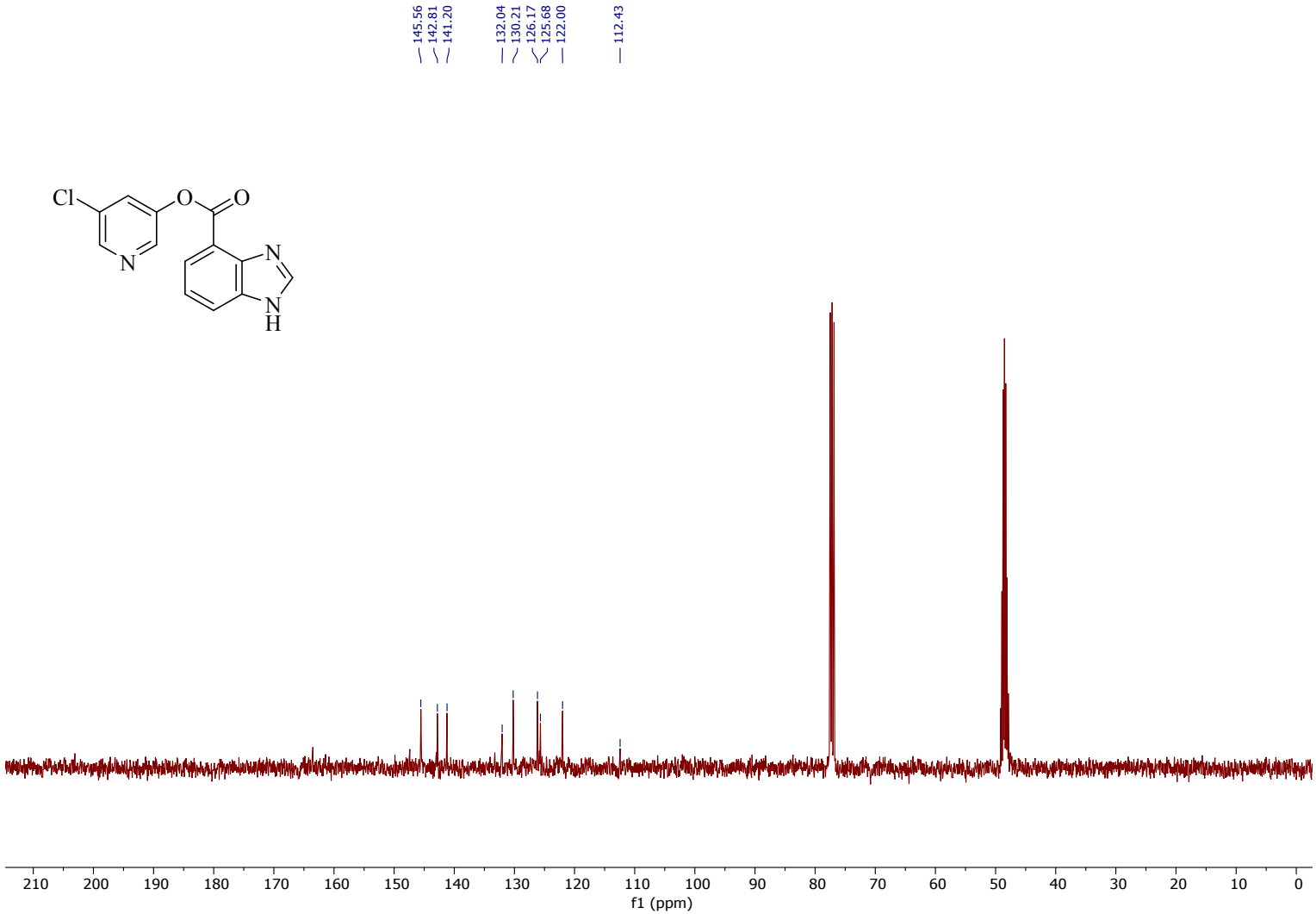

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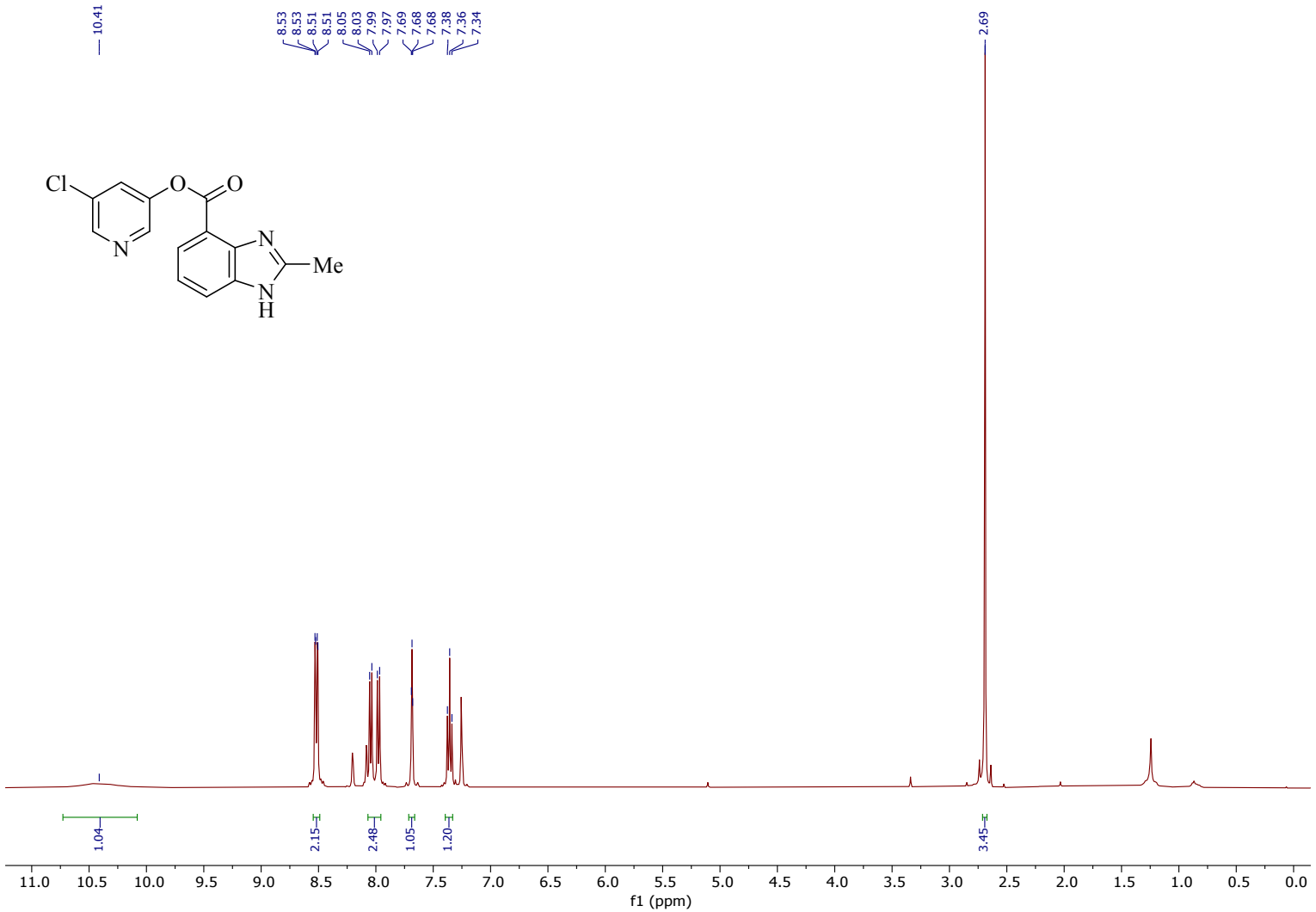

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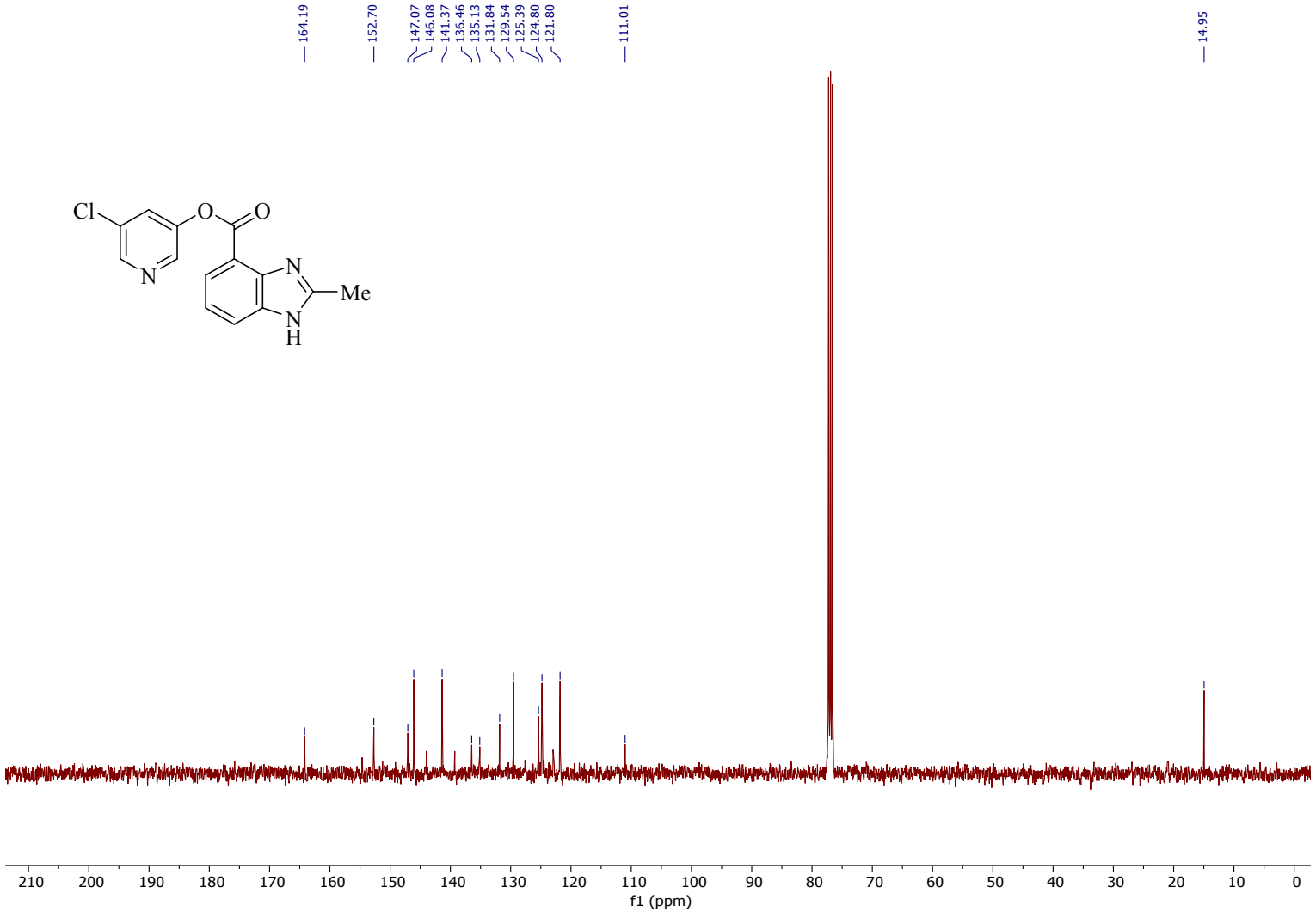

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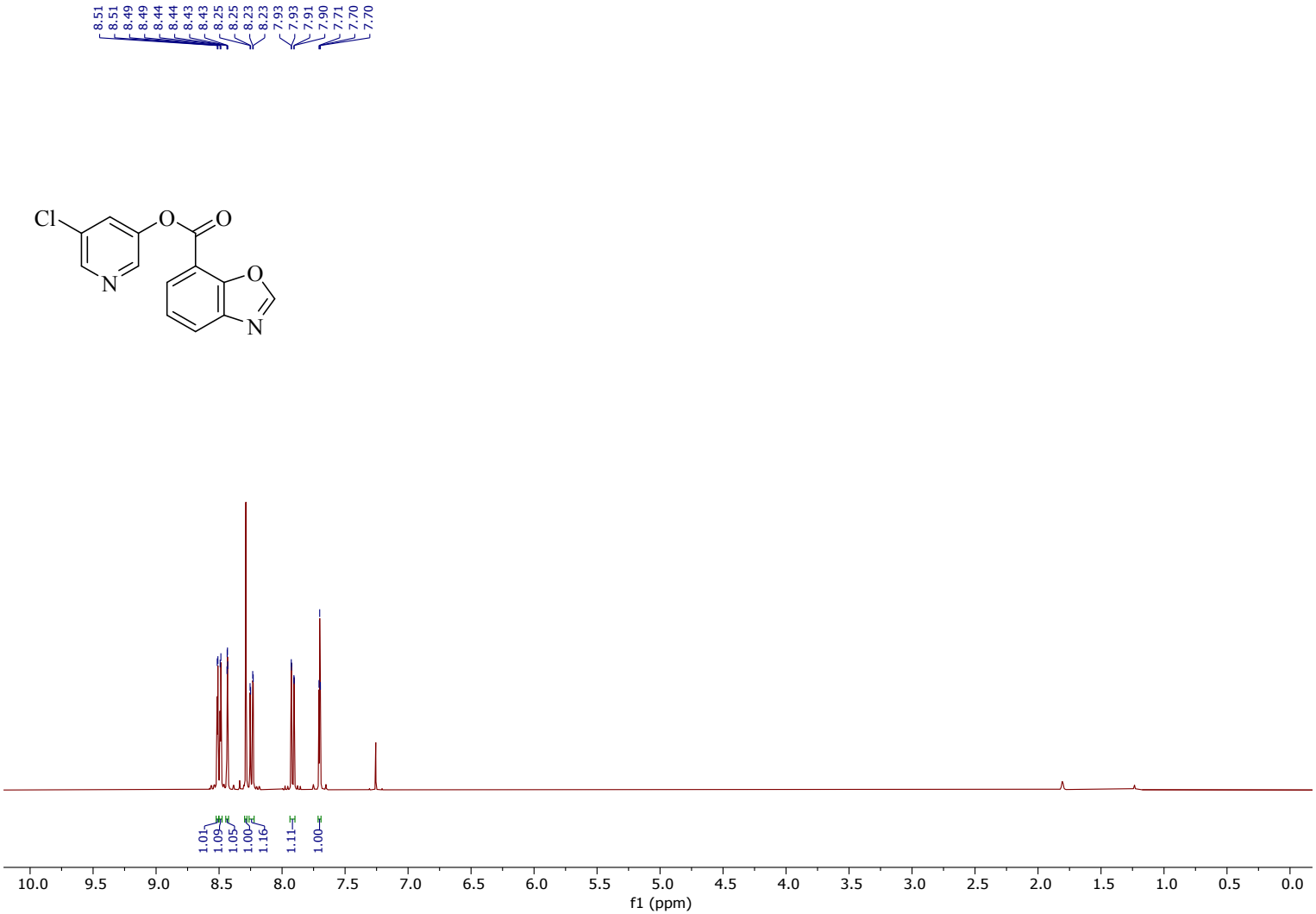

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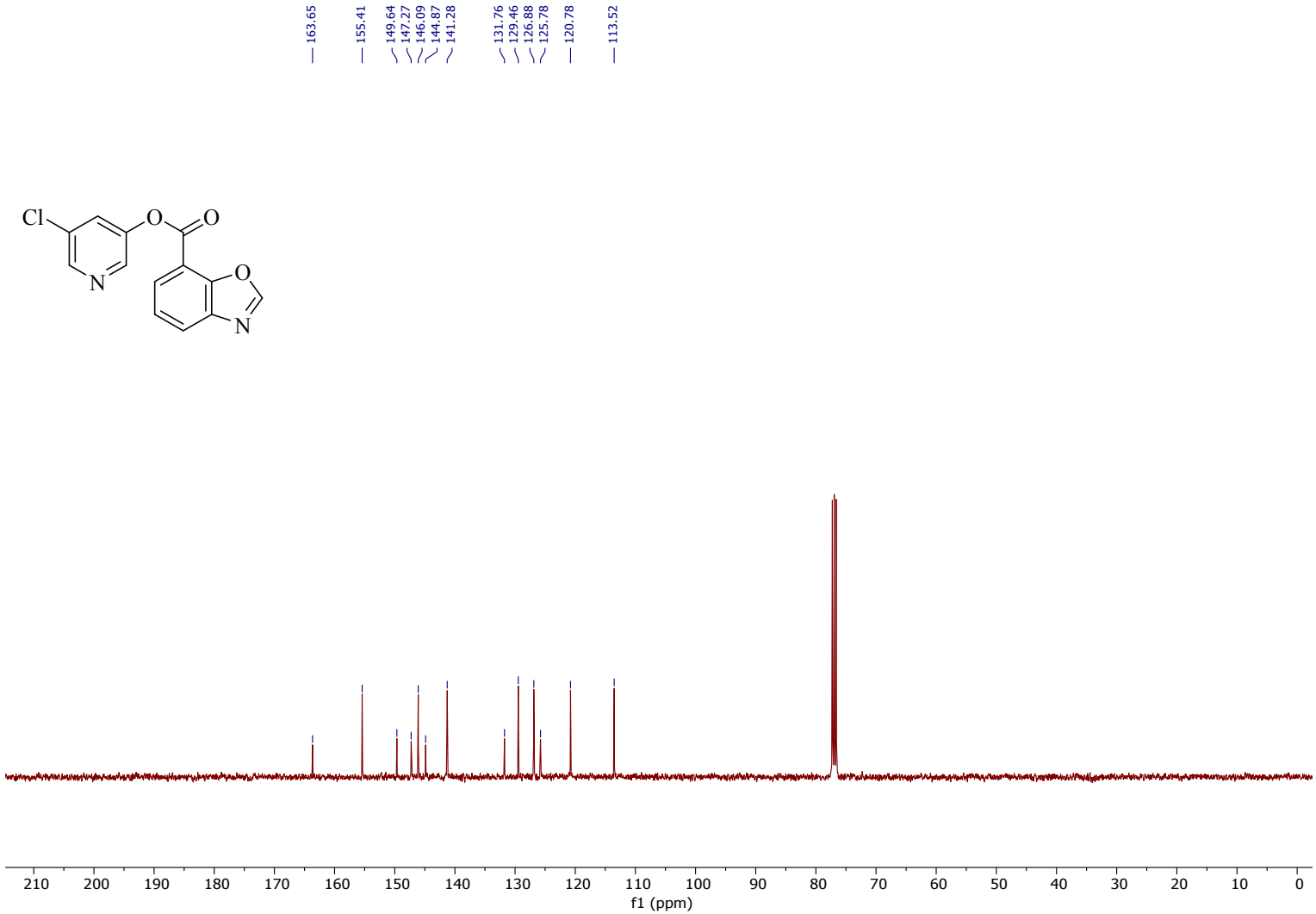

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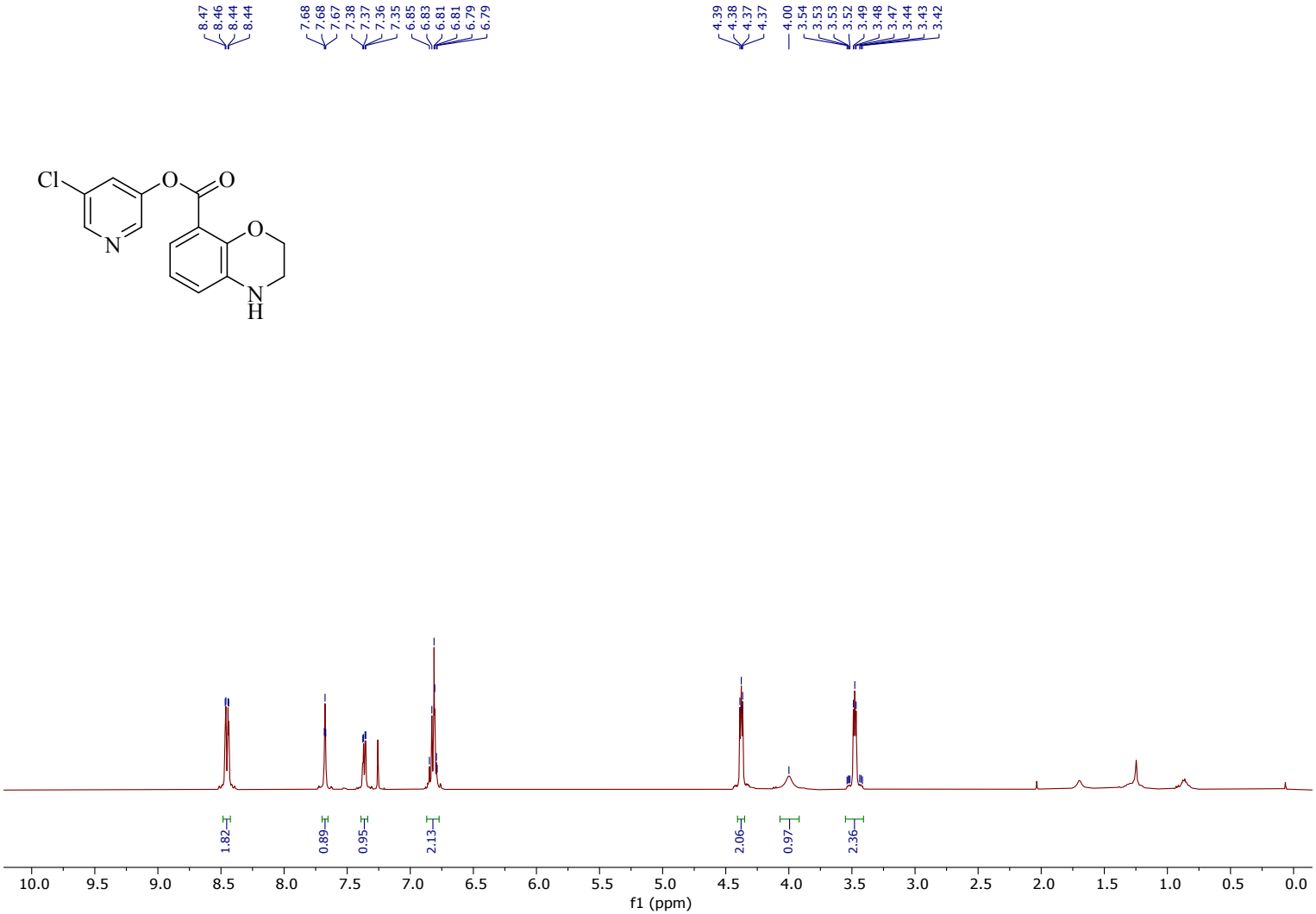

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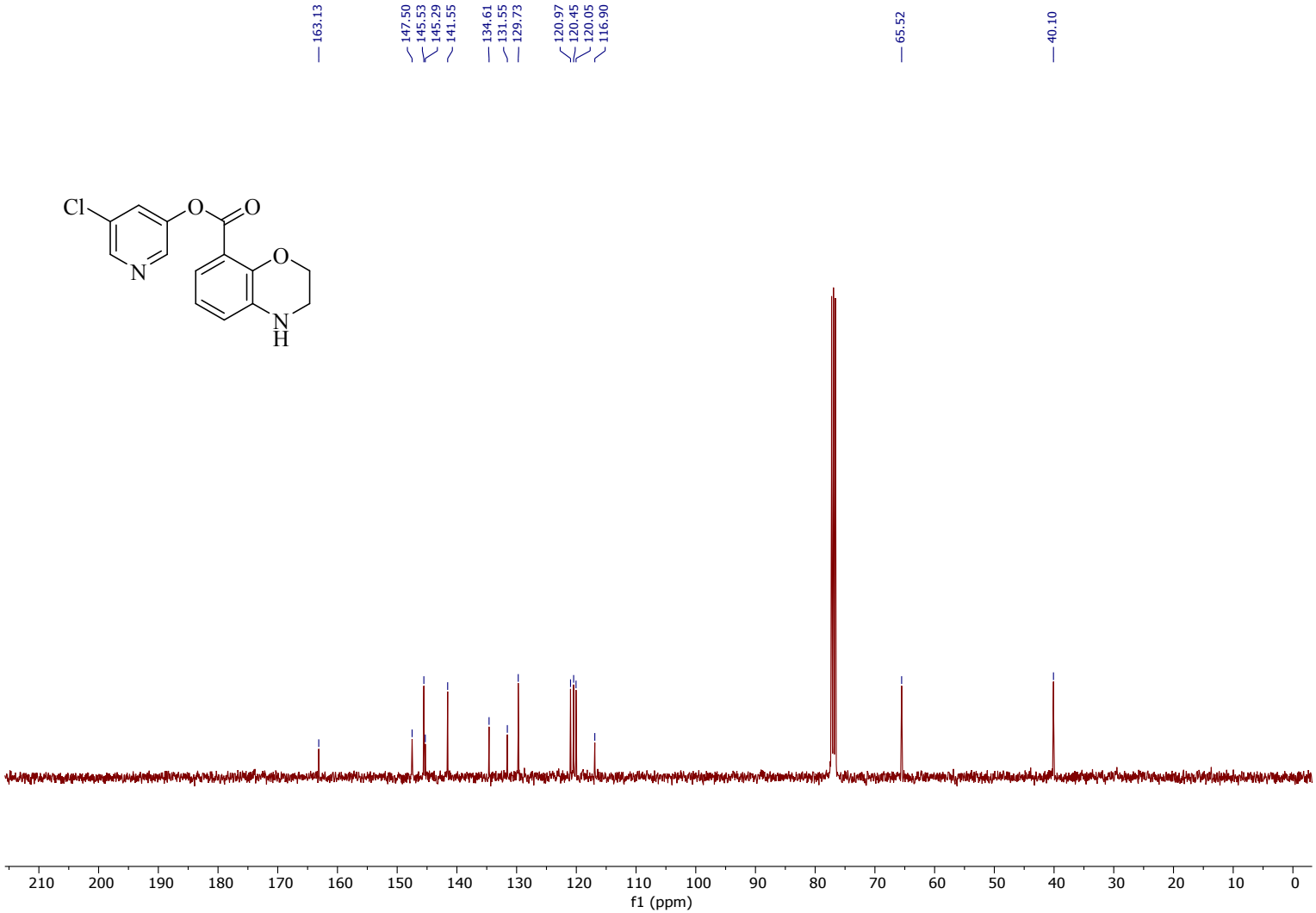

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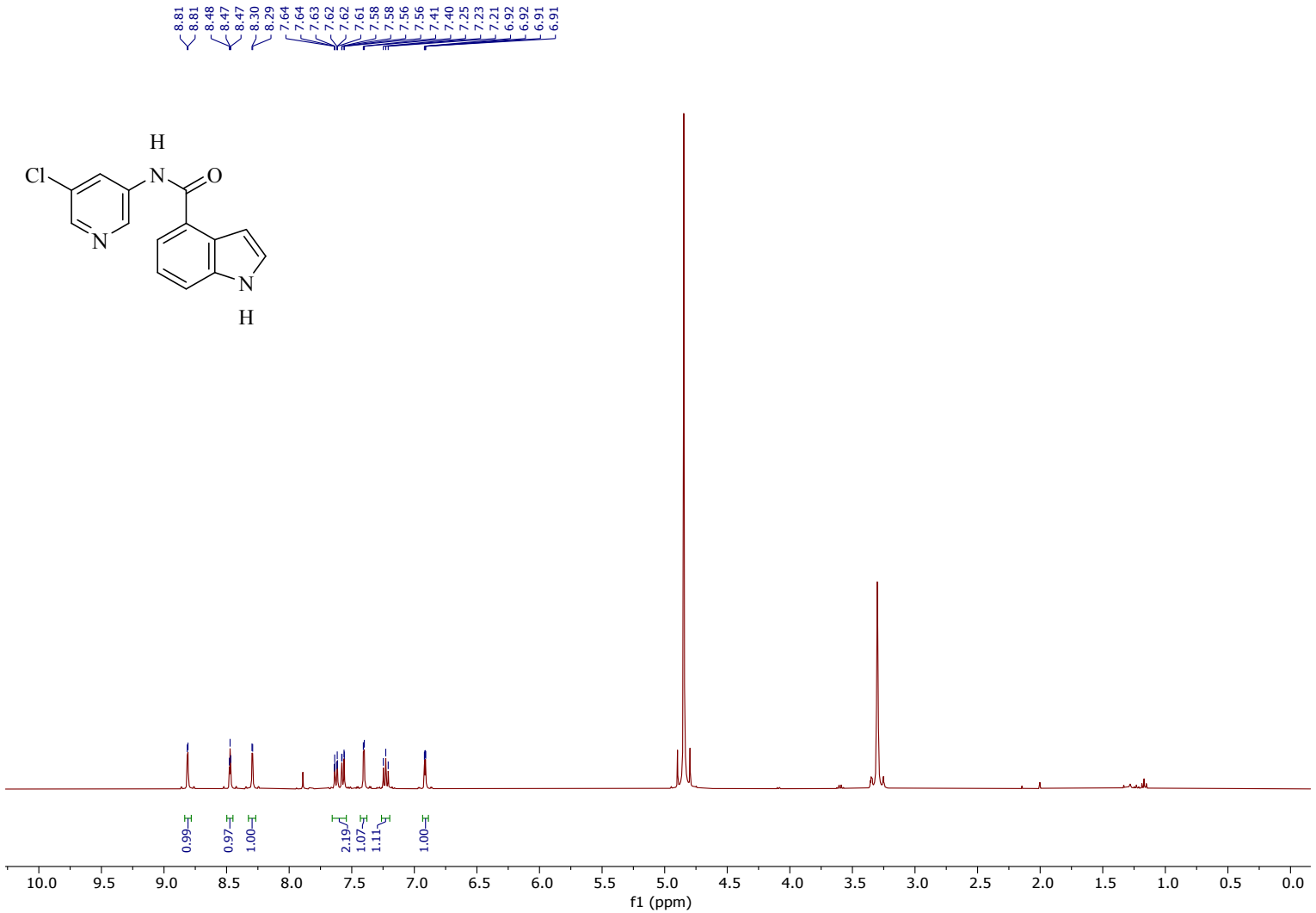
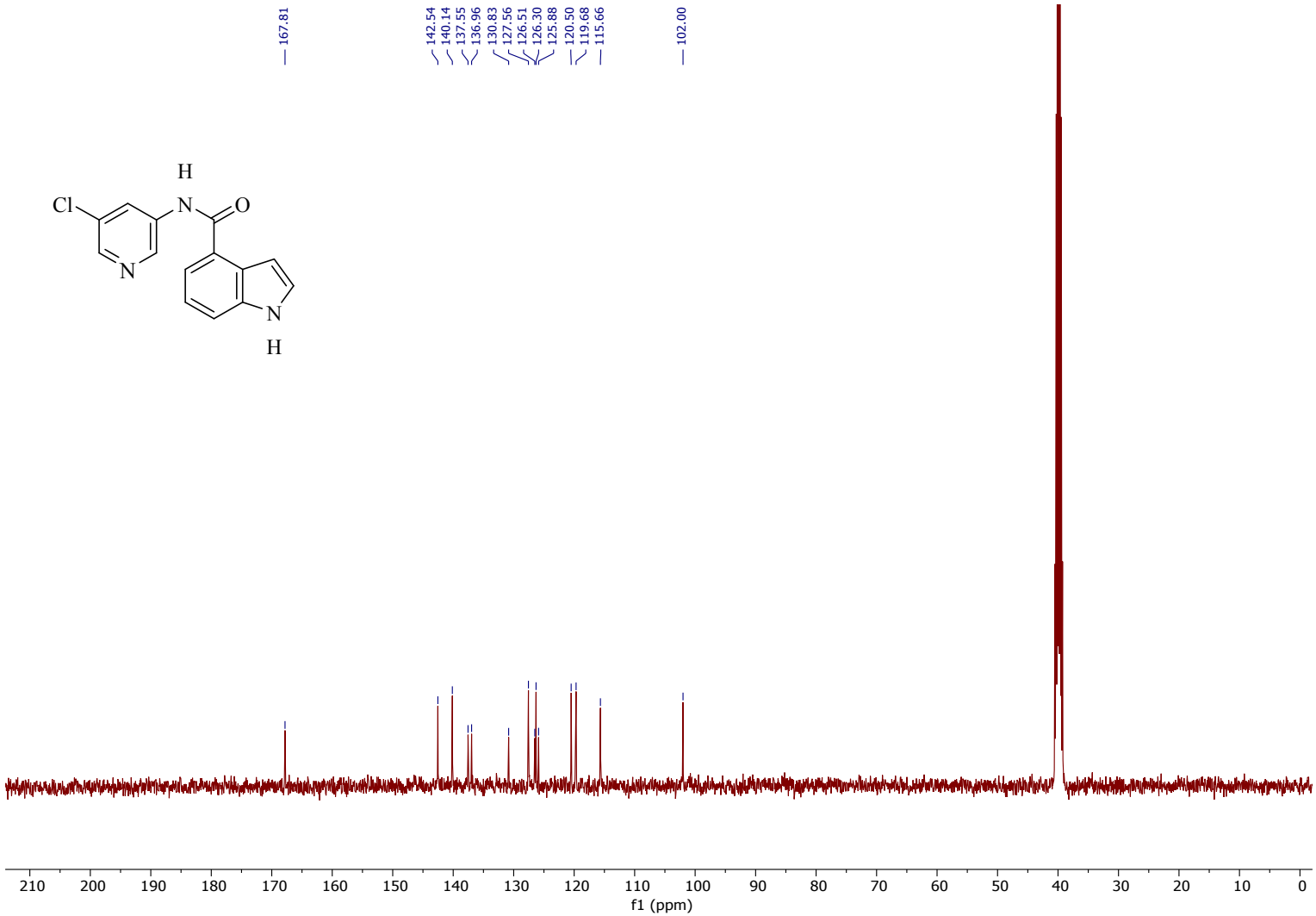
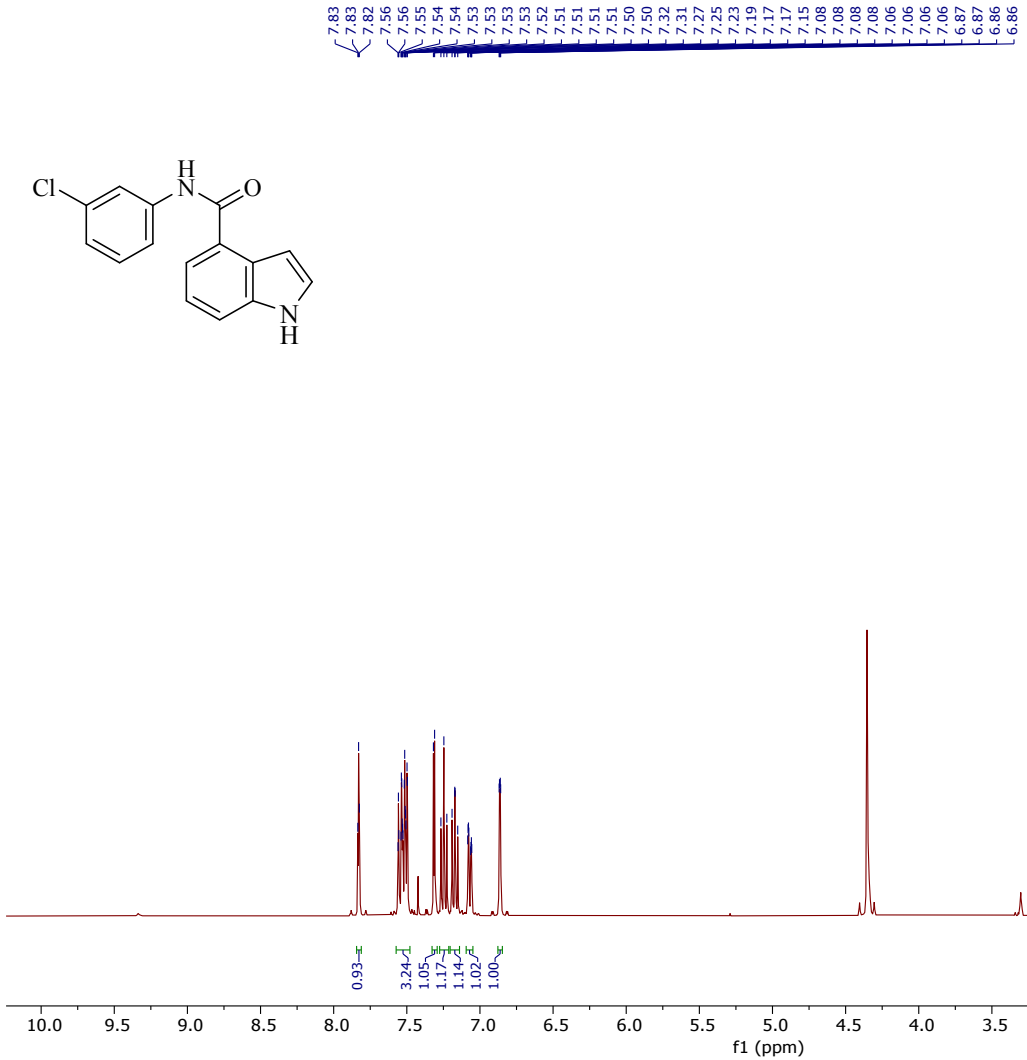


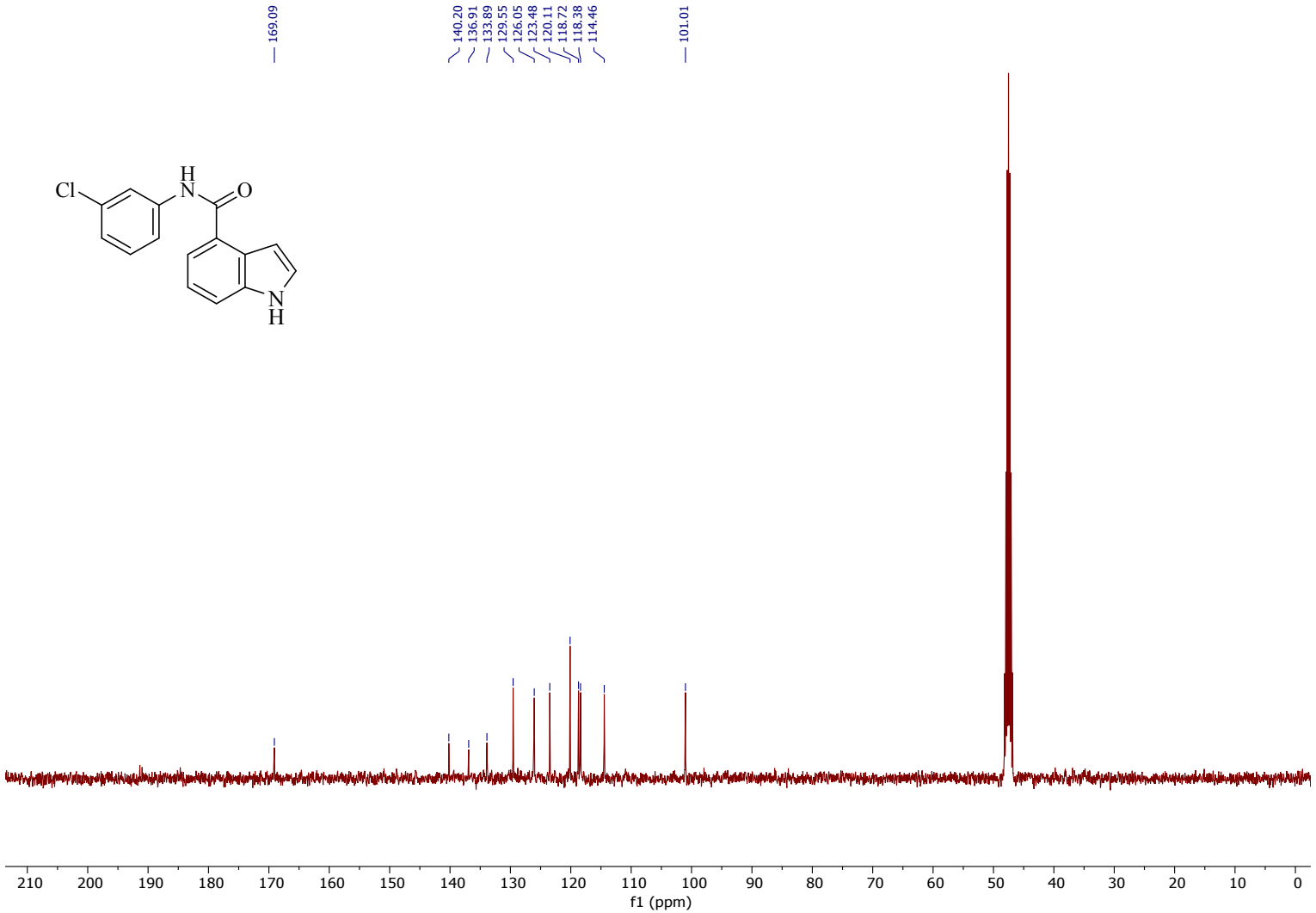
^{1}H NMR (400 MHz, MeOD) of Compound 9a



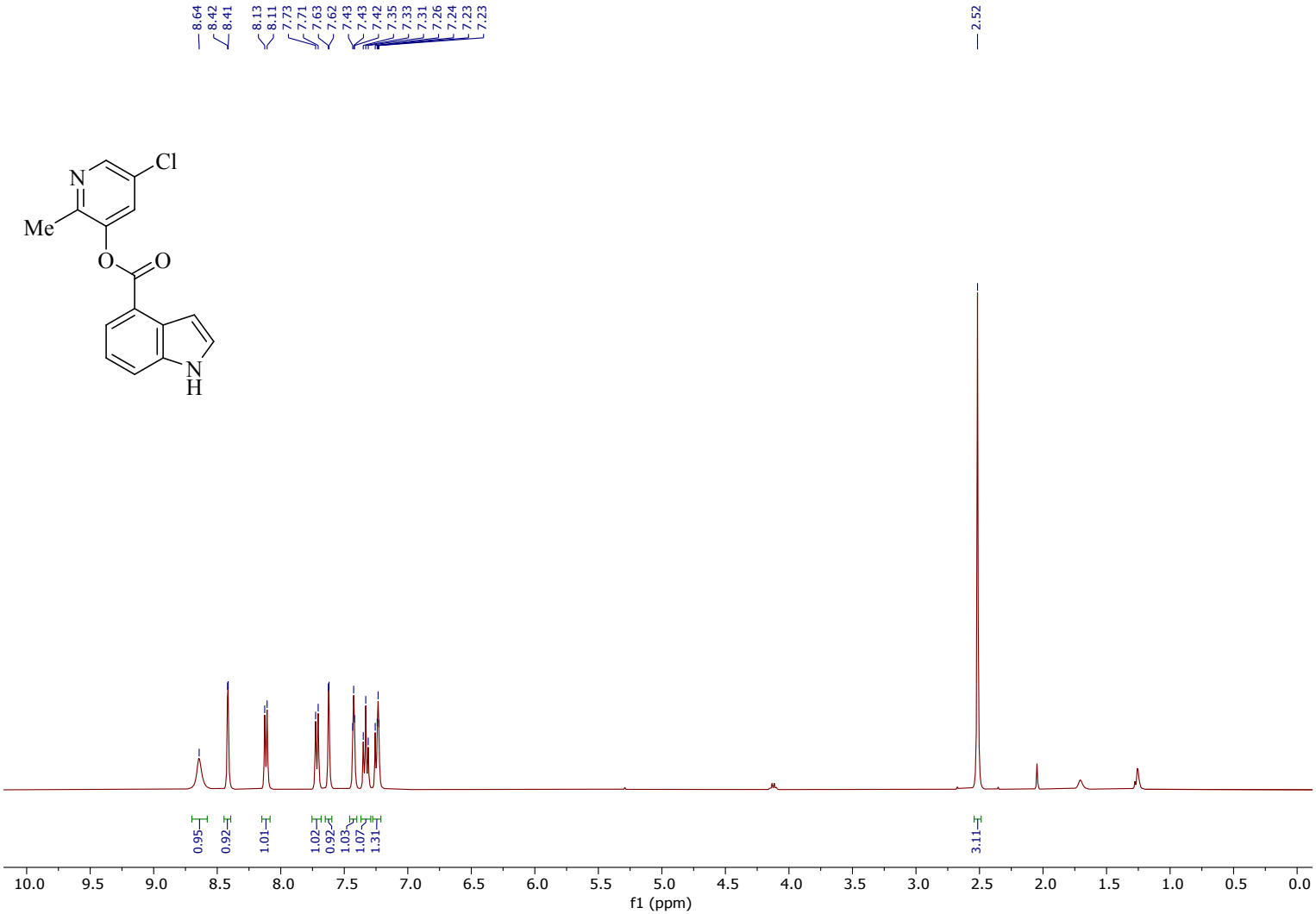
^{13}C NMR (100 MHz, DMSO) of Compound 9a



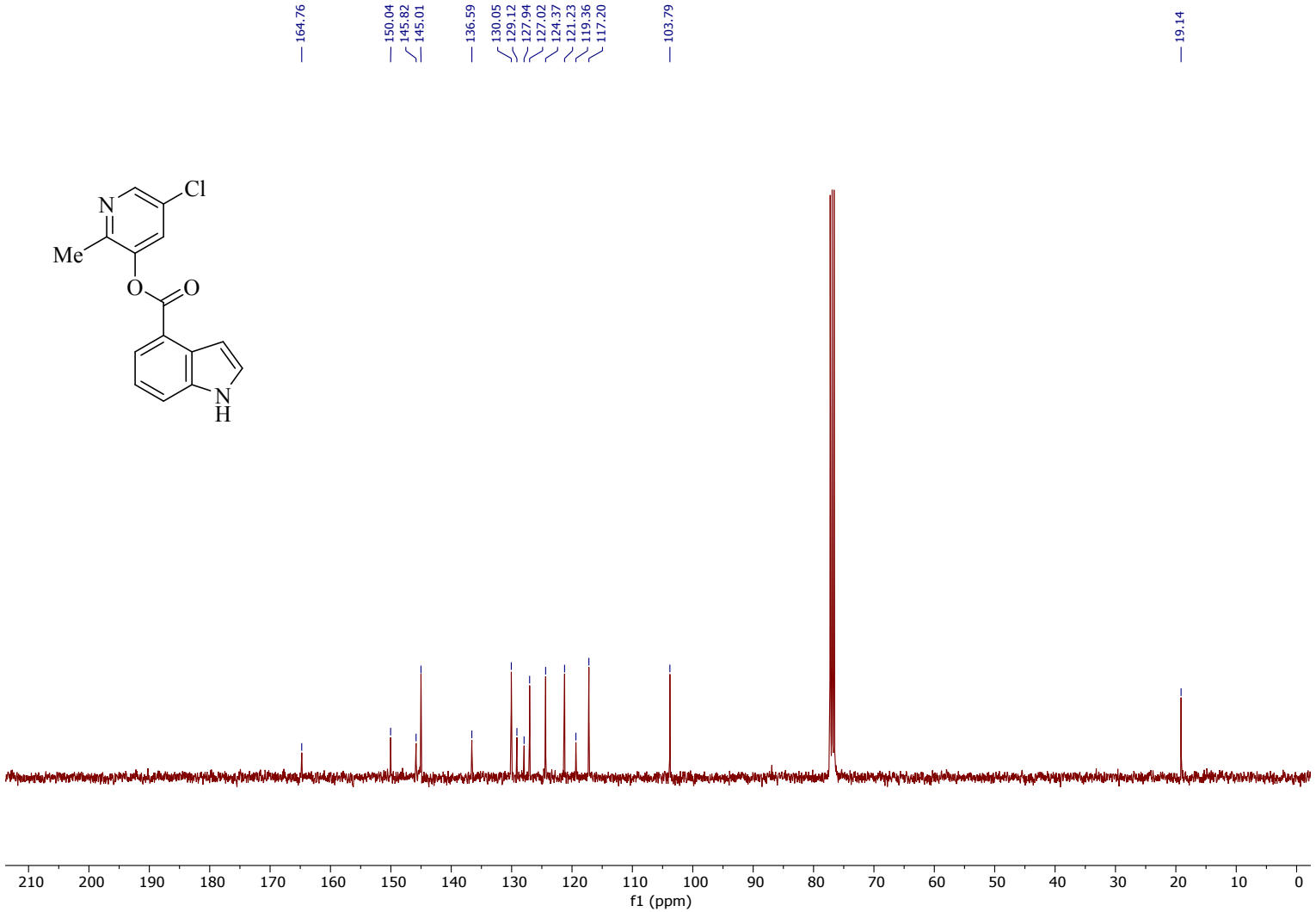
^{1}H NMR (400 MHz, MeOD) of Compound 9b



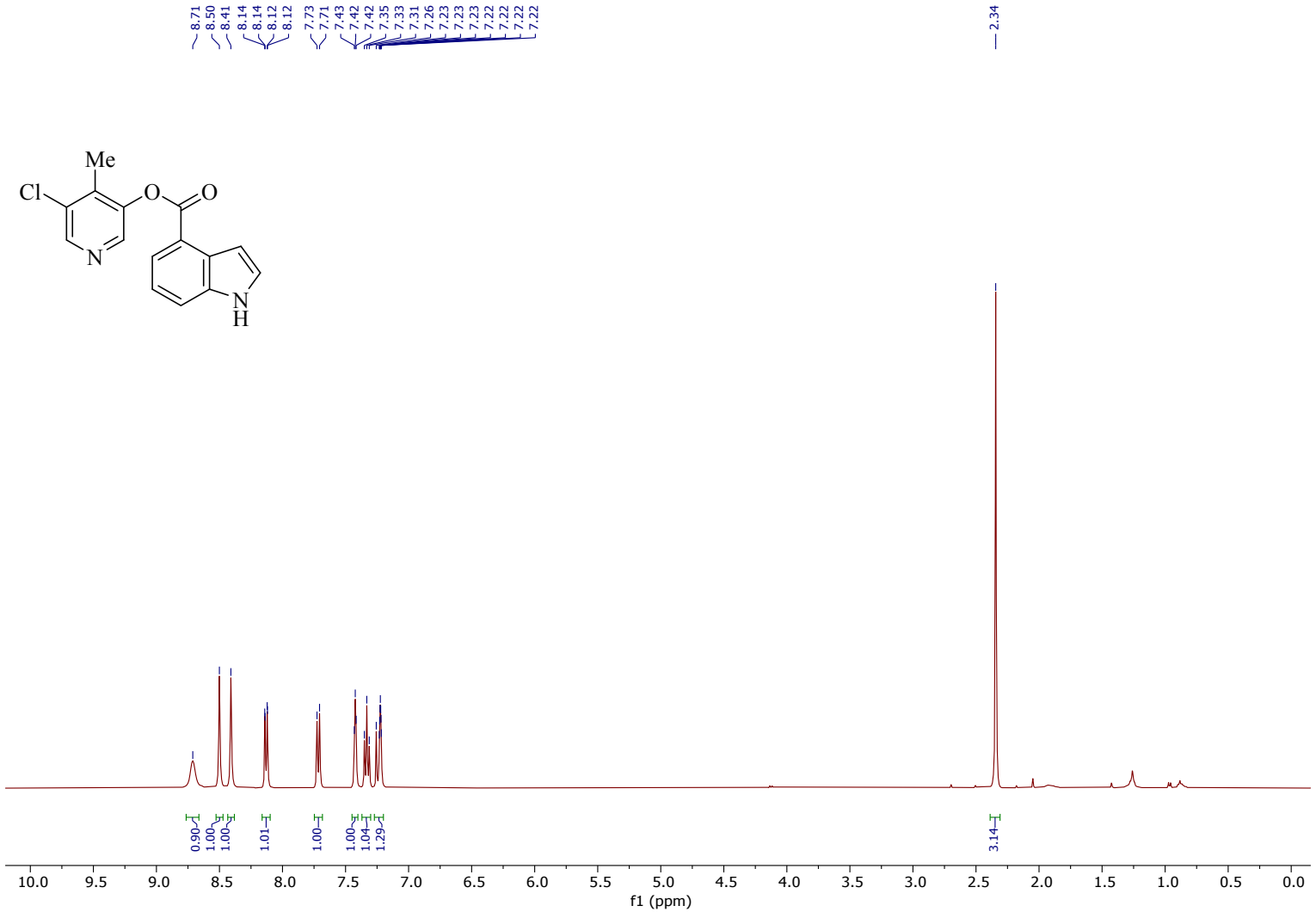
^{13}C NMR (100 MHz, MeOD) of Compound 9b



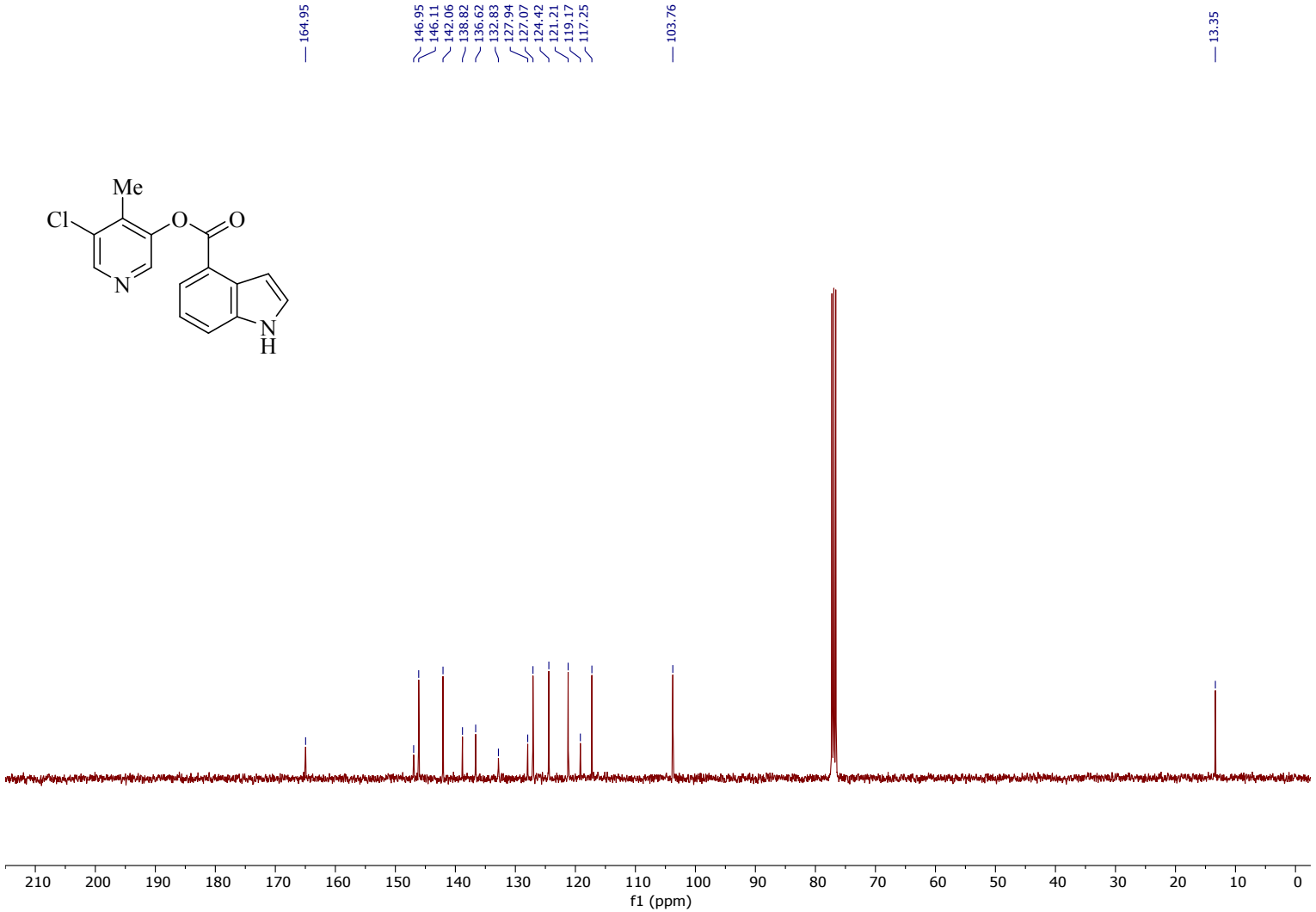
^{1}H NMR (400 MHz, $CDCl_3$) of Compound 9c



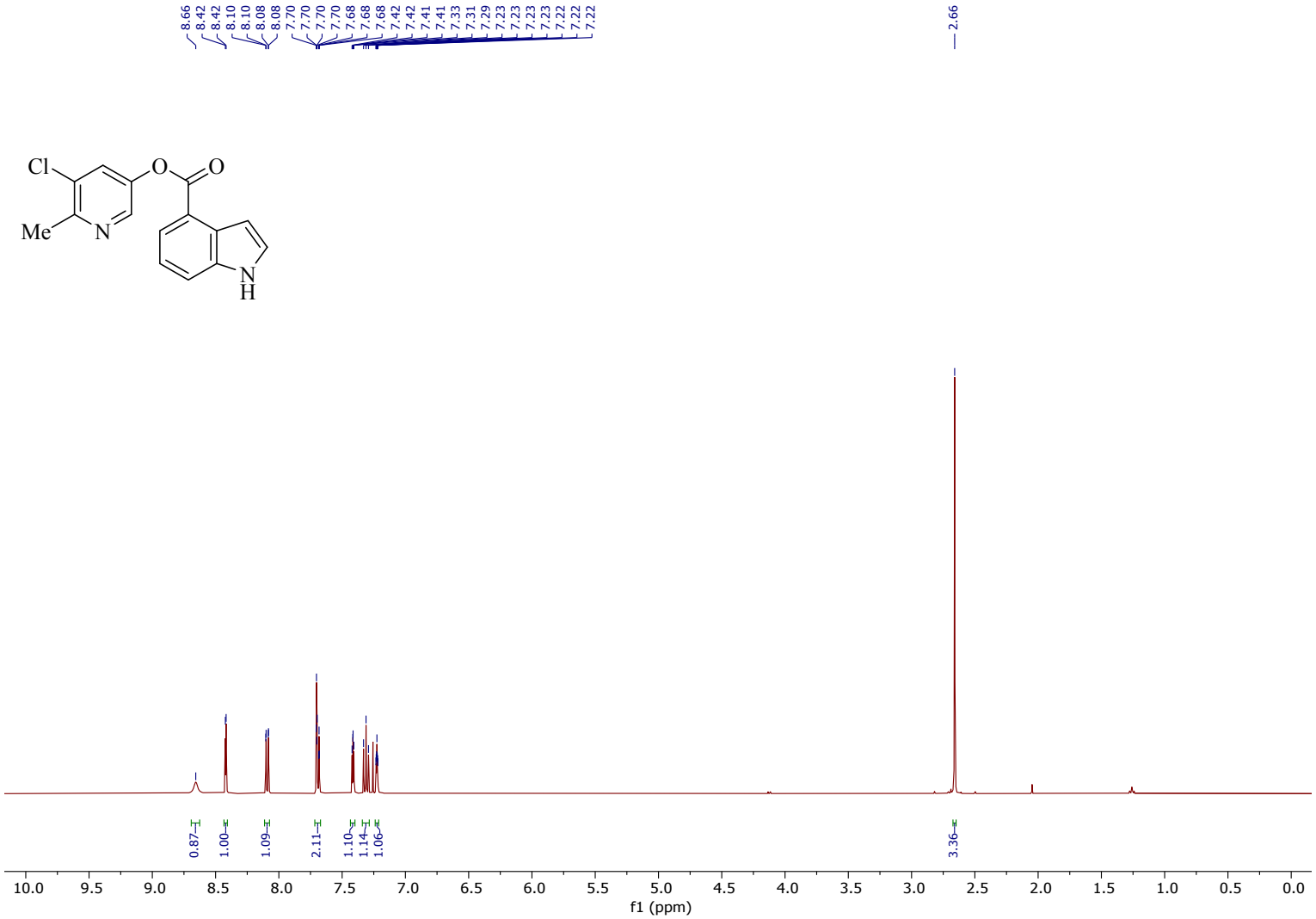
^{13}C NMR (100 MHz, $CDCl_3$) of Compound 9c



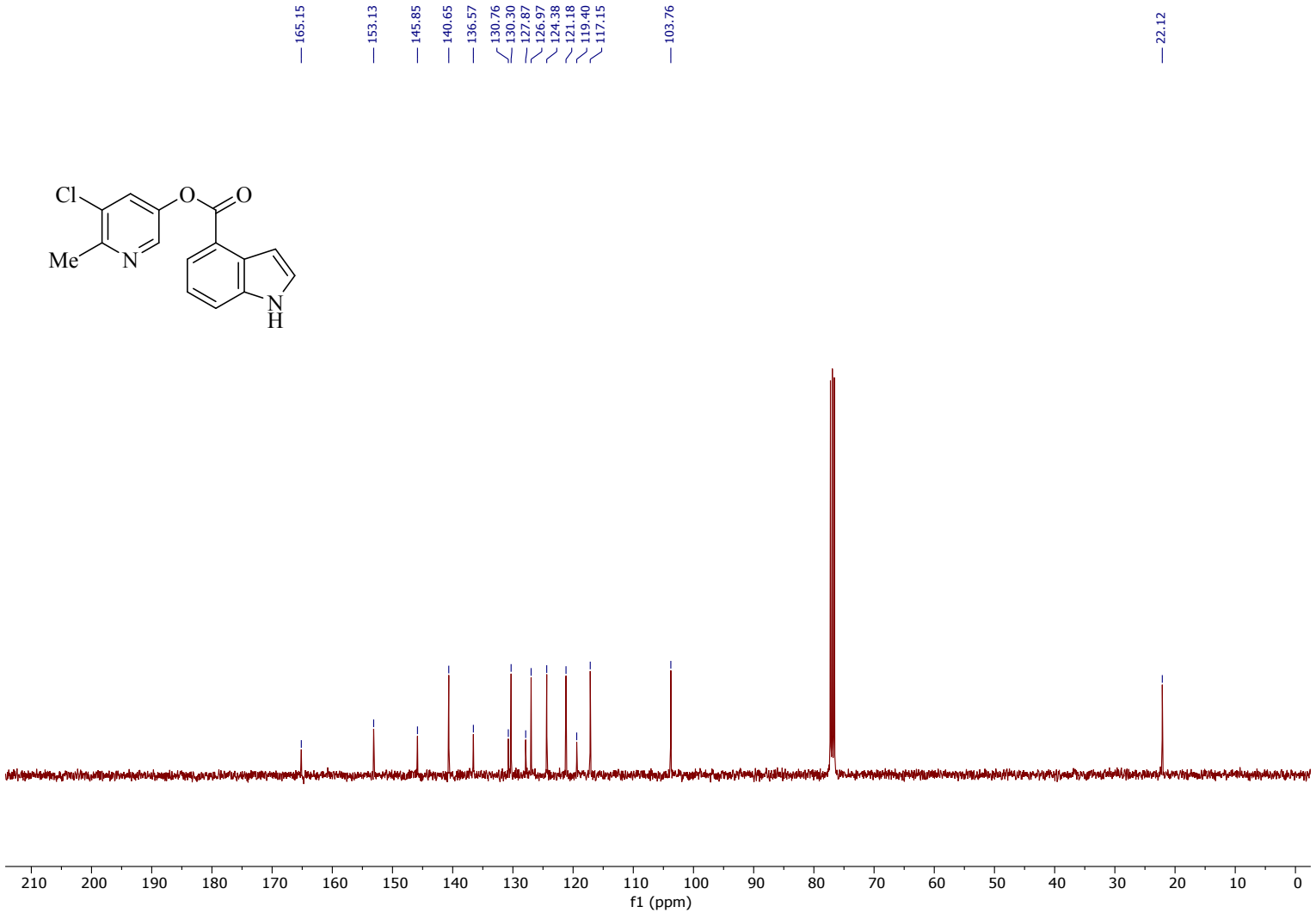
^{1}H NMR (400 MHz, $CDCl_3$) of Compound 9d



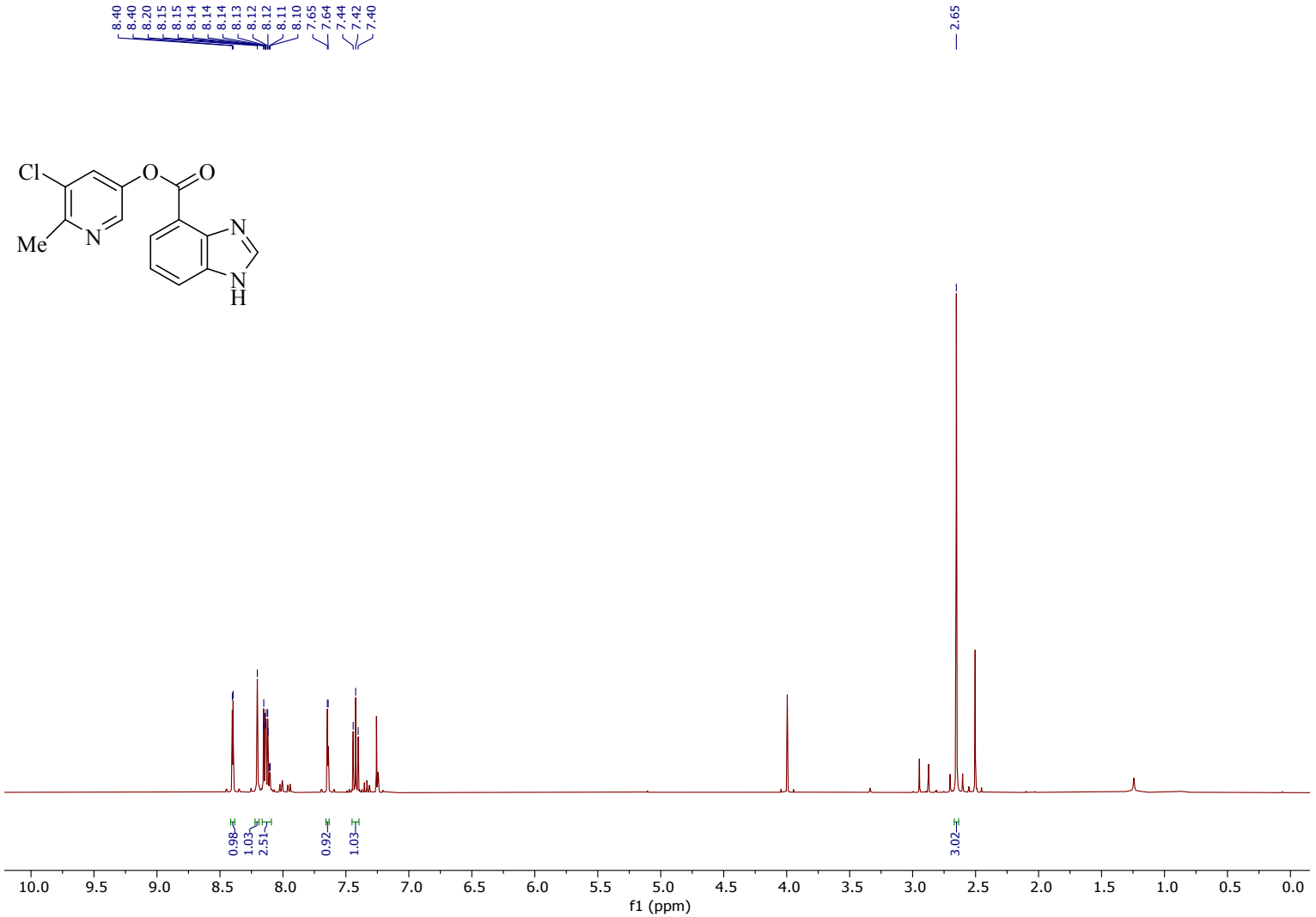
^{13}C NMR (100 MHz, $CDCl_3$) of Compound 9d



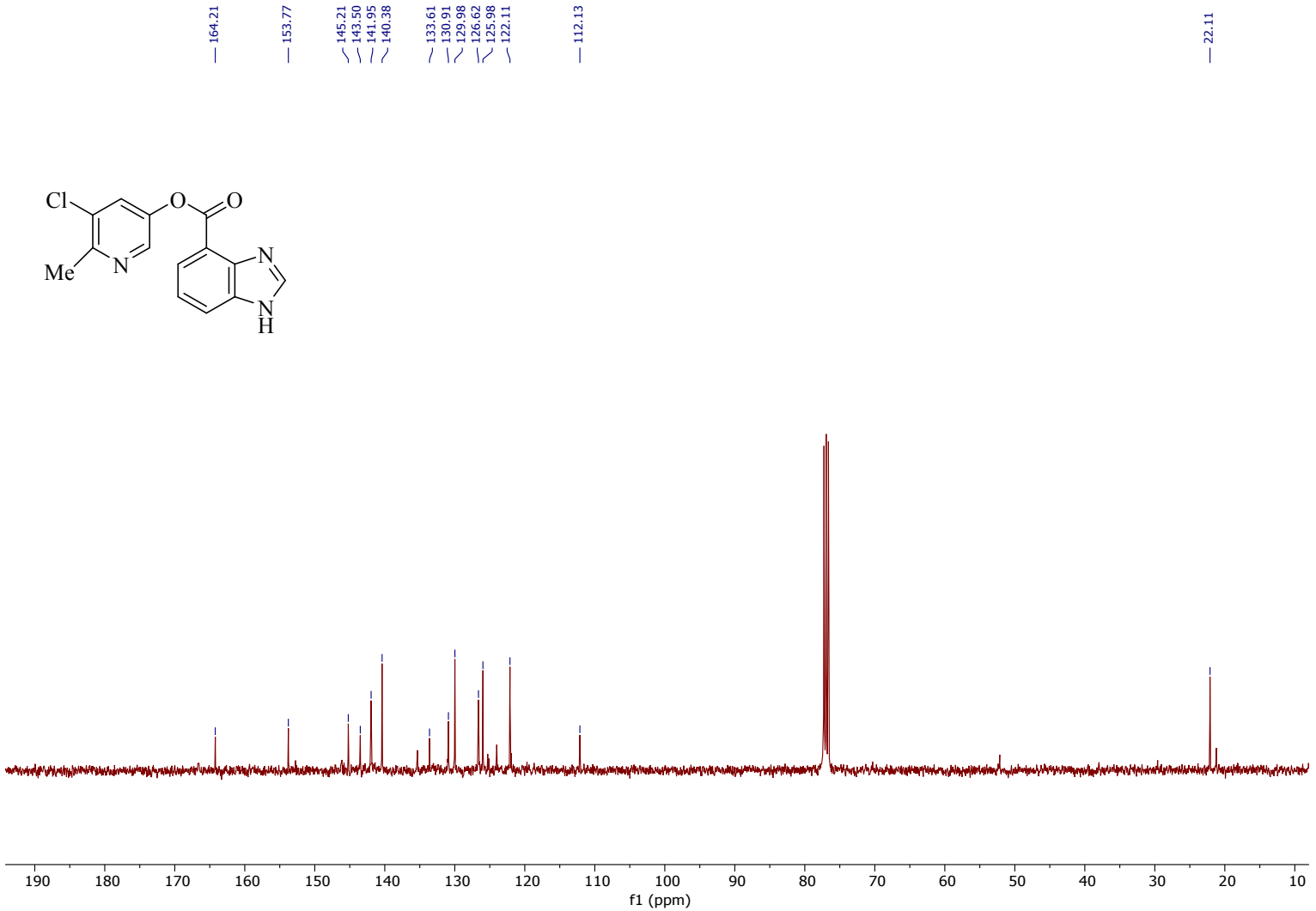
^{1}H NMR (400 MHz, $CDCl_3$) of Compound 9e



^{13}C NMR (100 MHz, $CDCl_3$) of Compound 9e



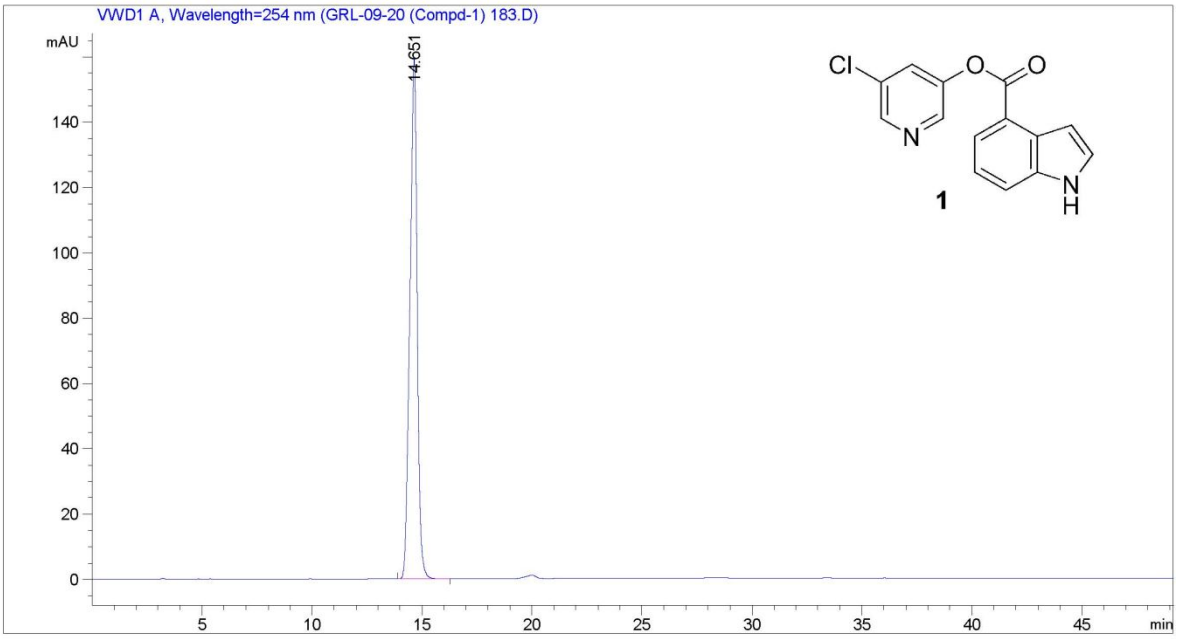
^{1}H NMR (400 MHz, $CDCl_3$) of Compound 9f



^{13}C NMR (100 MHz, $CDCl_3$) of Compound 9f

Data File C:\Chem32\1\Data\GRL-09-20 (Compd-1) 183.D
Sample Name: GRL-09-20 (Compd-1)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 7/30/2021 1:23:20 PM
                                             Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 7/30/2021 1:22:33 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```




```
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No Fractions found.
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                         Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-09-20 (Compd-1) 183.D
Sample Name: GRL-09-20 (Compd-1)

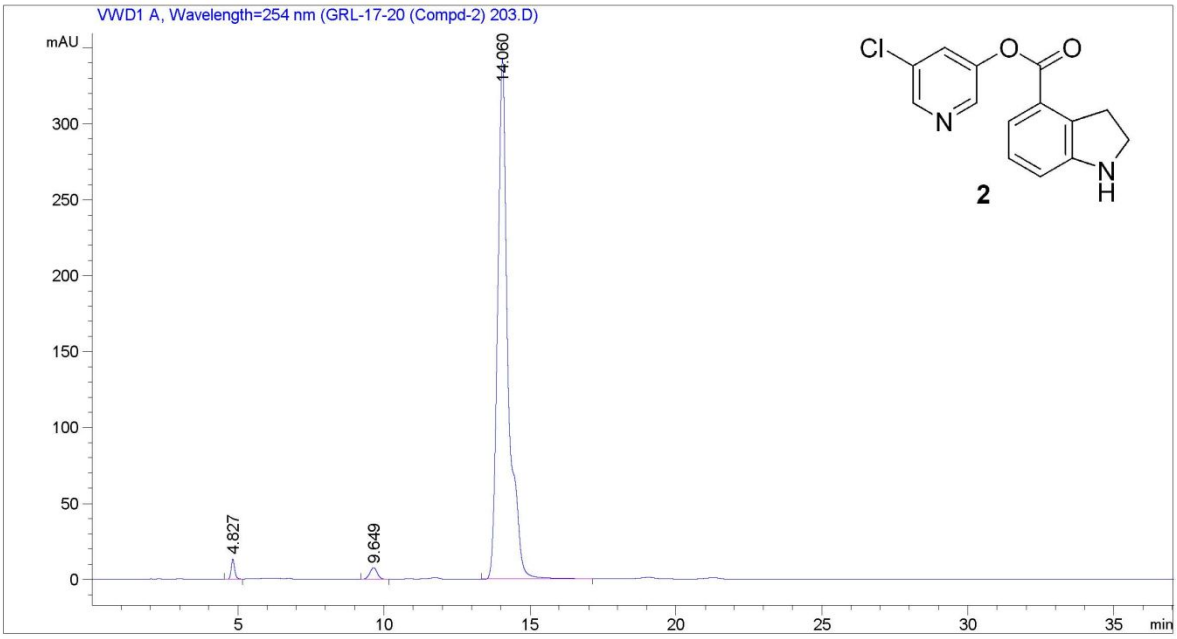
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.651	BB	0.3326	3662.51416	159.10939	100.0000
Totals :				3662.51416	159.10939	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-17-20 (Compd-2) 203.D
Sample Name: GRL-17-20 (Compd-2)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/4/2021 5:35:25 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/4/2021 4:51:51 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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                       Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-17-20 (Compd-2) 203.D
Sample Name: GRL-17-20 (Compd-2)

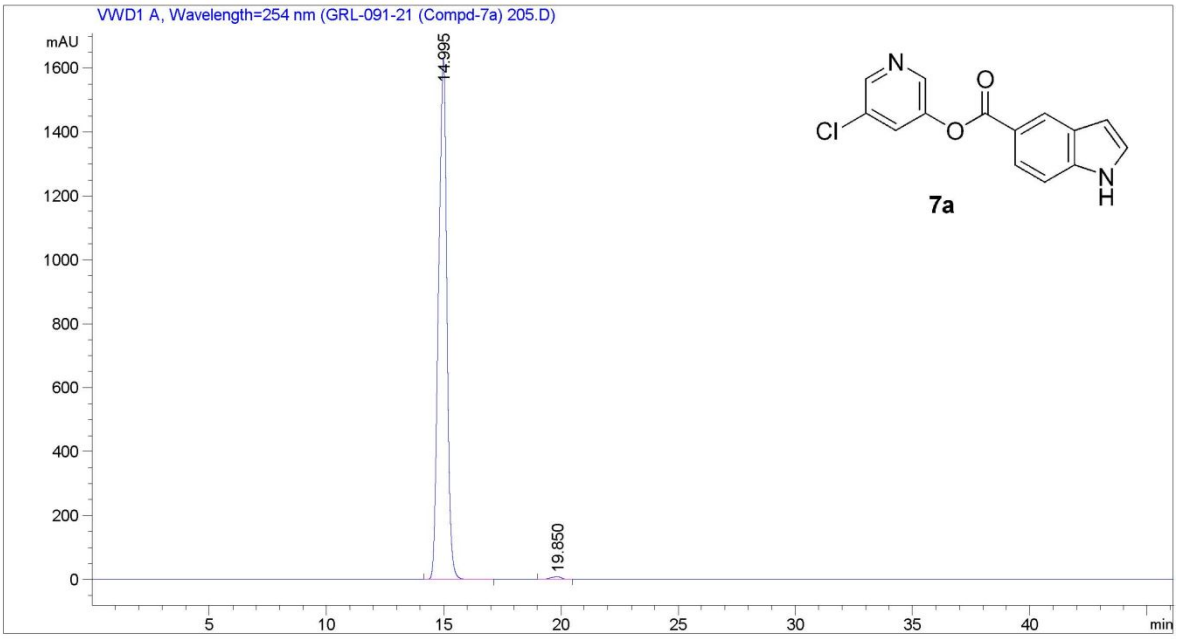
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.827	BB	0.1242	107.90797	13.22163	1.2268
2	9.649	BB	0.2729	132.48586	7.54188	1.5062
3	14.060	BB	0.3555	8555.67383	342.41159	97.2670
Totals :				8796.06766	363.17510	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-091-21 (Compd-7a) 205.D
Sample Name: GRL-091-21 (Compd-7a)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/4/2021 8:52:43 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/4/2021 8:33:40 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-091-21 (Compd-7a) 205.D
Sample Name: GRL-091-21 (Compd-7a)

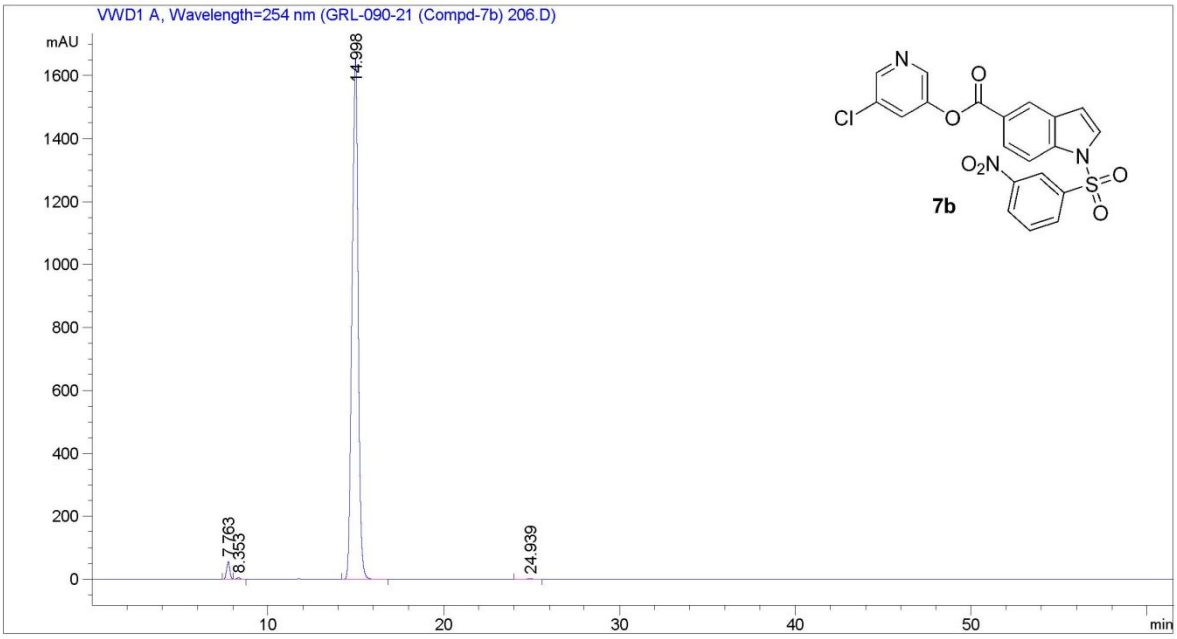
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.995	BB	0.4056	4.01335e4	1628.01428	99.2866
2	19.850	BB	0.4433	288.36905	9.31197	0.7134
Totals :				4.04219e4	1637.32625	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-090-21 (Compd-7b) 206.D
Sample Name: GRL-090-21 (Compd-7b)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/5/2021 10:33:22 AM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/4/2021 8:33:40 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-090-21 (Compd-7b) 206.D
Sample Name: GRL-090-21 (Compd-7b)

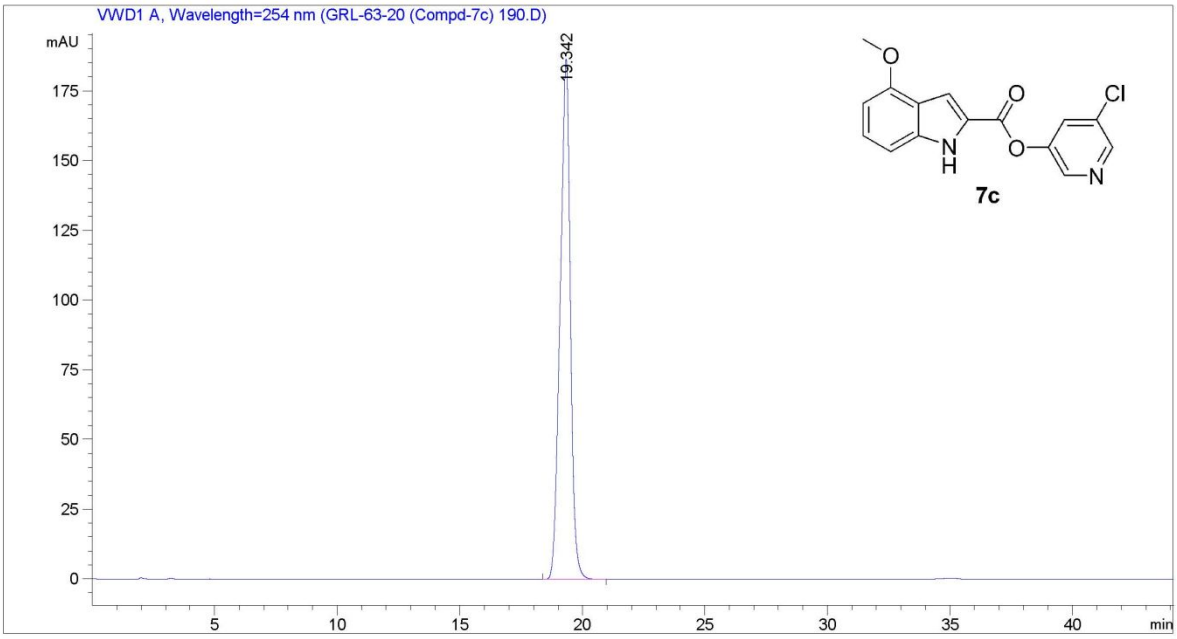
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.763	BV R	0.1940	704.71271	57.08348	1.7267
2	8.353	VB E	0.1960	74.28173	5.62638	0.1820
3	14.998	BB	0.3950	3.99600e4	1653.33862	97.9129
4	24.939	BB	0.5246	72.77259	1.92280	0.1783
Totals :				4.08118e4	1717.97128	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-63-20 (Compd-7c) 190.D
Sample Name: GRL-63-20 (Compd-7c)

```
======================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/2/2021 11:16:37 AM
                                             Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/2/2021 10:32:47 AM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-63-20 (Compd-7c) 190.D
Sample Name: GRL-63-20 (Compd-7c)

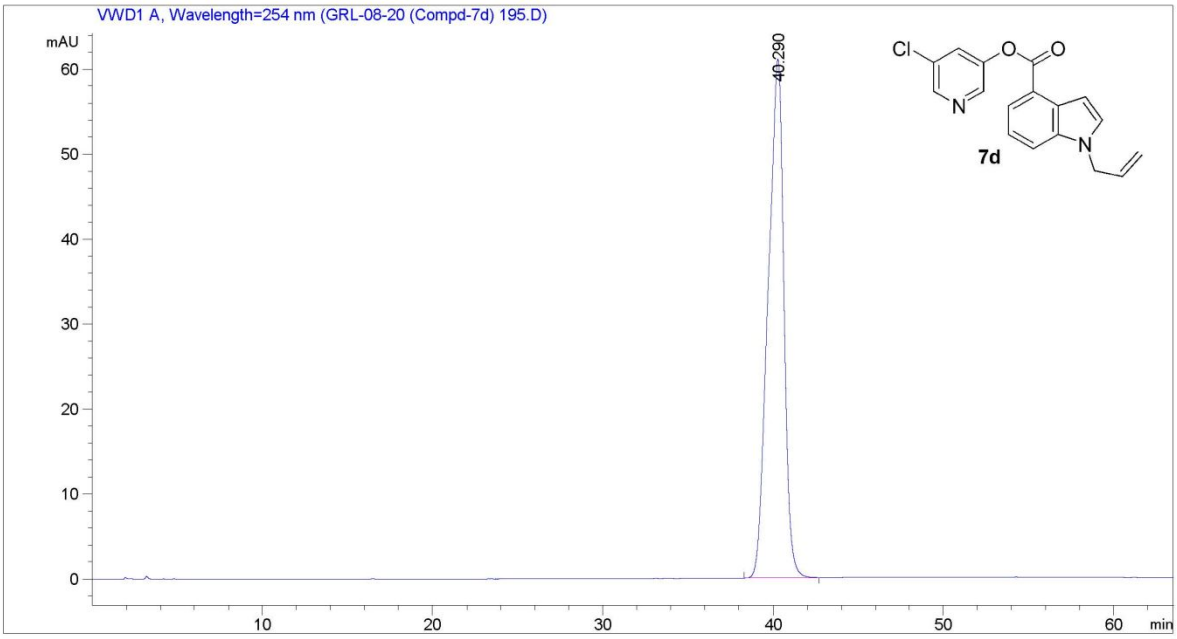
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.342	BB	0.4222	5408.72852	186.57550	100.0000
Totals :				5408.72852	186.57550	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-08-20 (Compd-7d) 195.D
Sample Name: GRL-08-20 (Compd-7d)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/3/2021 11:52:24 AM
                                             Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/3/2021 11:11:10 AM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-08-20 (Compd-7d) 195.D
Sample Name: GRL-08-20 (Compd-7d)

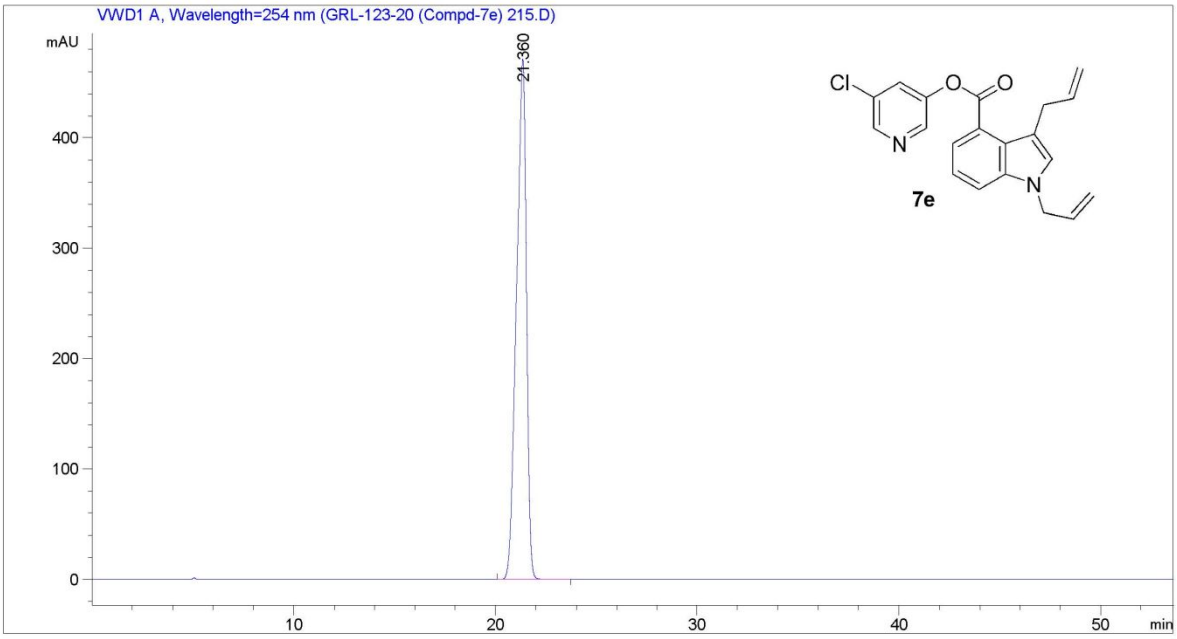
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	40.290	BB	0.9119	3906.91724	61.02806	100.0000
Totals :				3906.91724	61.02806	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-123-20 (Compd-7e) 215.D
Sample Name: GRL-123-20 (Compd-7e)

```
=====================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/7/2021 5:56:38 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/7/2021 4:59:13 PM by SYSTEM
                  (modified after loading)
Sample Info     : 65% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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No Fractions found.
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                      Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-123-20 (Compd-7e) 215.D
Sample Name: GRL-123-20 (Compd-7e)

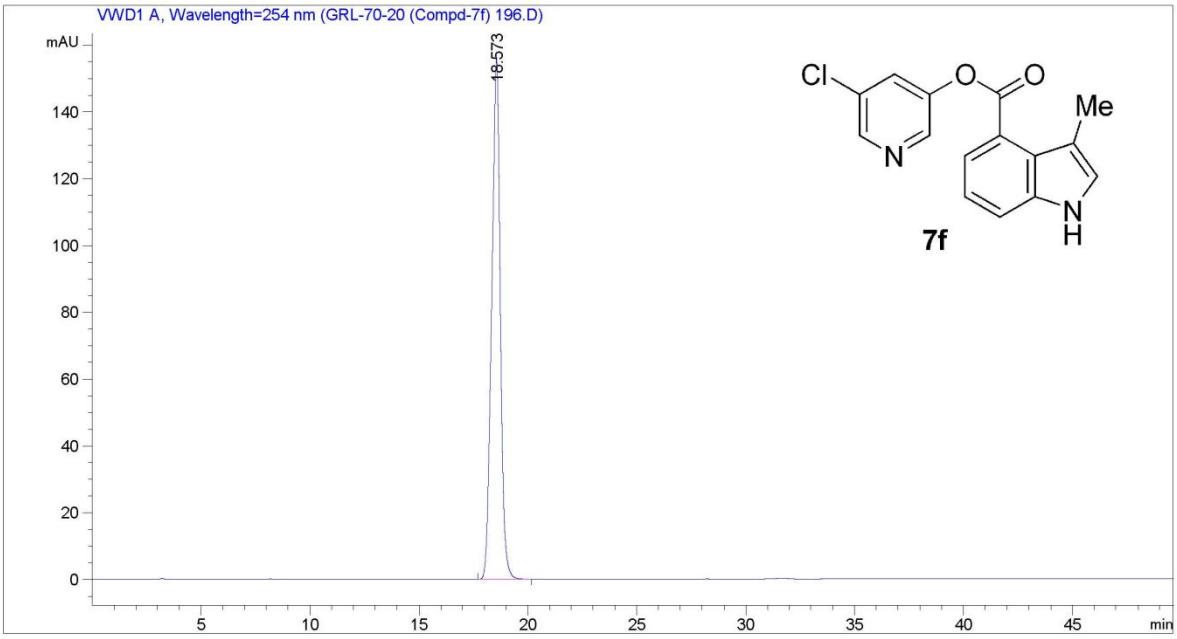
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	21.360	BB	0.4864	1.60585e4	471.40701	100.0000
Totals :				1.60585e4	471.40701	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-70-20 (Compd-7f) 196.D
Sample Name: GRL-70-20 (Compd-7f)

```
=====================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/3/2021 2:40:16 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/3/2021 1:48:59 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-70-20 (Compd-7f) 196.D
Sample Name: GRL-70-20 (Compd-7f)

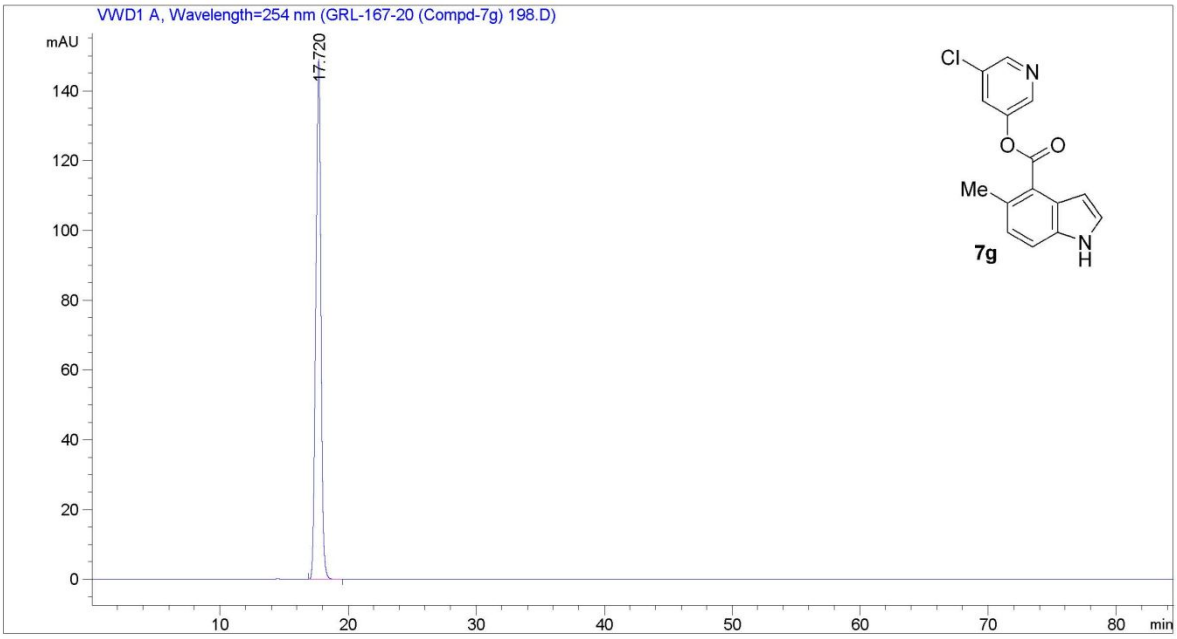
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.573	BB	0.4012	4274.72607	155.73651	100.0000
Totals :				4274.72607	155.73651	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-167-20 (Compd-7g) 198.D
Sample Name: GRL-167-20 (Compd-7g)

```
=====================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/3/2021 6:22:38 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/3/2021 5:32:08 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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=============================================================================
                        Fraction Information
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No Fractions found.
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                     Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-167-20 (Compd-7g) 198.D
Sample Name: GRL-167-20 (Compd-7g)

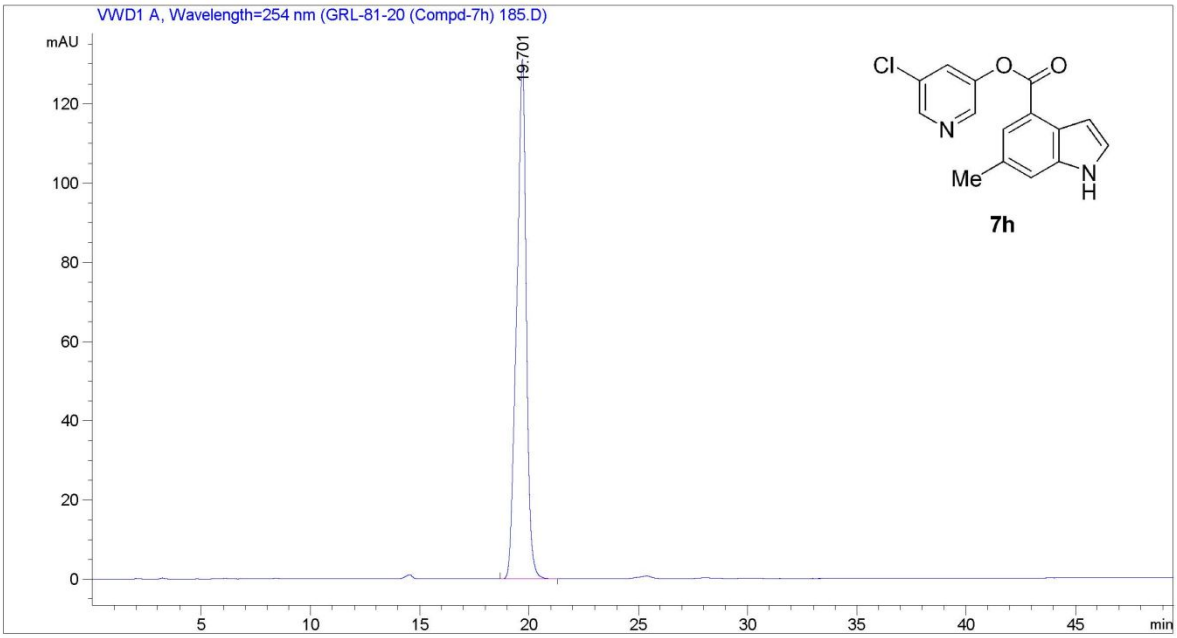
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.720	BB	0.4045	4120.62256	149.04968	100.0000
Totals :				4120.62256	149.04968	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-81-20 (Compd-7h) 185.D
Sample Name: GRL-81-20 (Compd-7h)

```
======================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 7/30/2021 4:14:42 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 7/30/2021 3:57:32 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```




```
================================================================================
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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-81-20 (Compd-7h) 185.D
Sample Name: GRL-81-20 (Compd-7h)

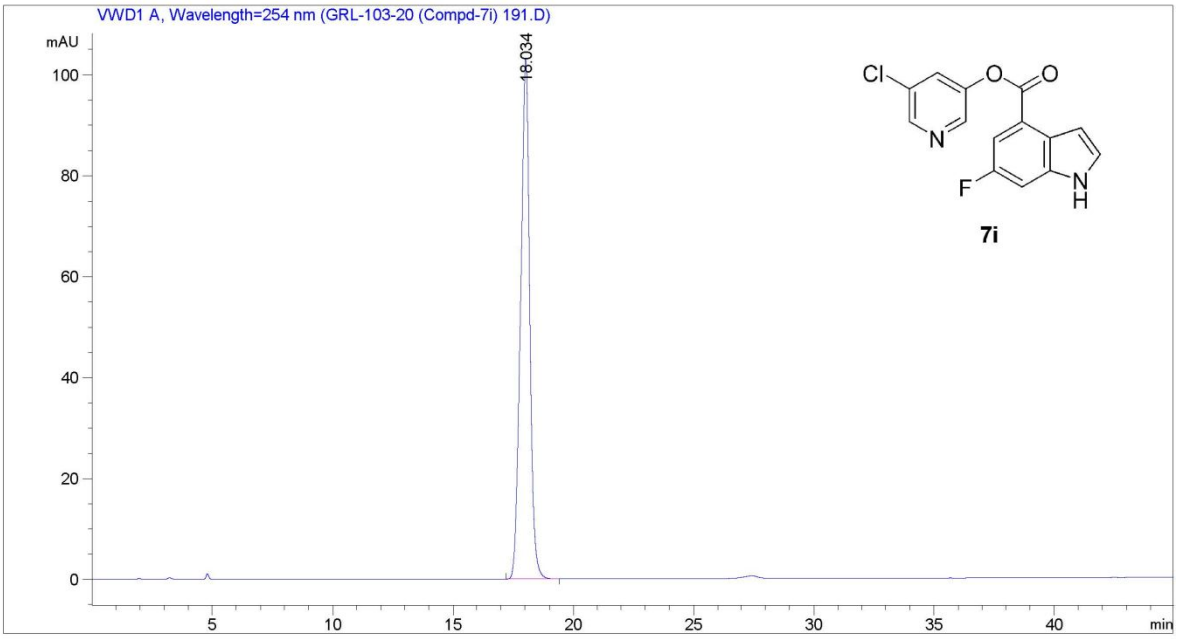
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.701	BB	0.4360	3989.20605	131.11749	100.0000
Totals :				3989.20605	131.11749	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-103-20 (Compd-7i) 191.D
Sample Name: GRL-103-20 (Compd-7i)

```
======================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/2/2021 1:48:11 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/2/2021 1:05:23 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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================================================================================
                          Fraction Information
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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-103-20 (Compd-7i) 191.D
Sample Name: GRL-103-20 (Compd-7i)

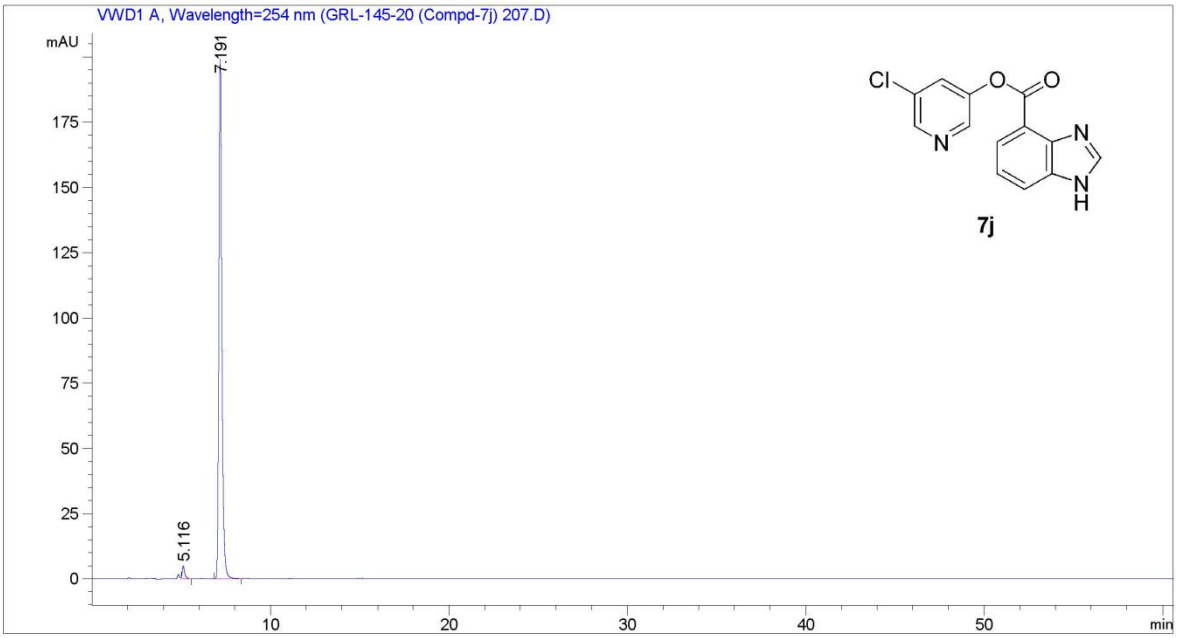
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.034	BB	0.3860	2720.54126	103.04881	100.0000
Totals :				2720.54126	103.04881	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-145-20 (Compd-7j) 207.D
Sample Name: GRL-145-20 (Compd-7j)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/5/2021 12:19:28 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/4/2021 8:33:40 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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No Fractions found.
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                         Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-145-20 (Compd-7j) 207.D
Sample Name: GRL-145-20 (Compd-7j)

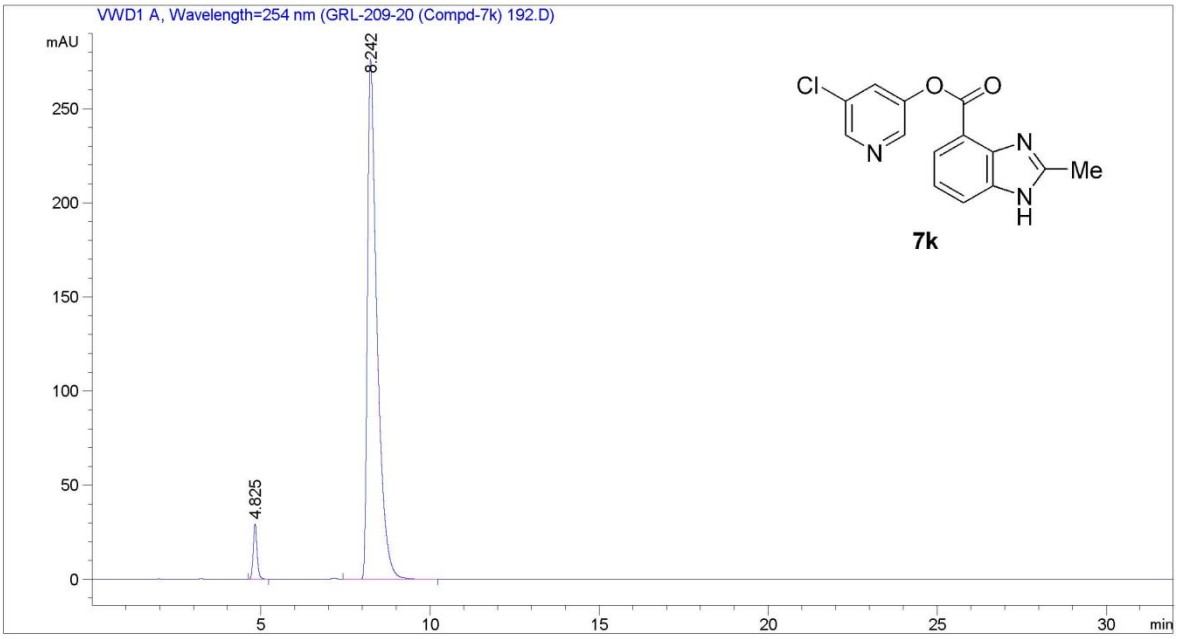
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.116	VB	0.1410	46.86685	4.92609	1.9715
2	7.191	BB	0.1746	2330.38794	199.09828	98.0285
Totals :				2377.25479	204.02438	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-209-20 (Compd-7k) 192.D
Sample Name: GRL-209-20 (Compd-7k)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/2/2021 4:10:31 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/2/2021 3:00:57 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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                            Fraction Information
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No Fractions found.
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                         Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-209-20 (Compd-7k) 192.D
Sample Name: GRL-209-20 (Compd-7k)

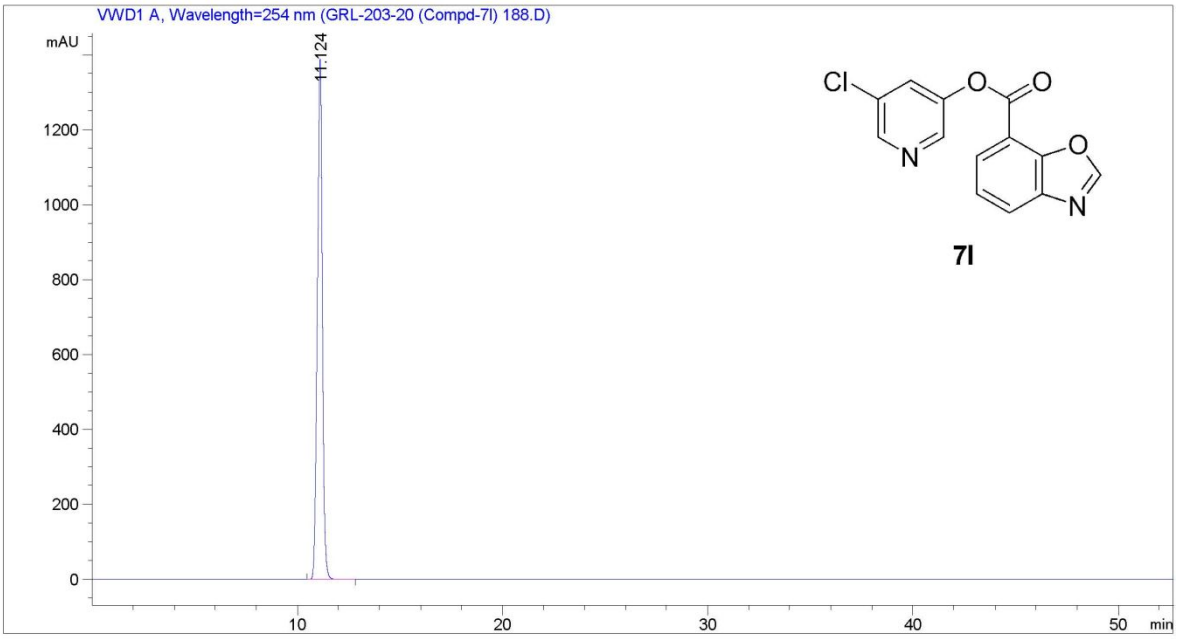
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.825	BB	0.1185	229.21916	29.23641	4.0551
2	8.242	BB	0.2842	5423.39258	276.08188	95.9449
Totals :				5652.61174	305.31829	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-203-20 (Compd-7l) 188.D
Sample Name: GRL-203-20 (Compd-7l)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 7/31/2021 4:00:10 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 7/31/2021 3:20:42 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```



7l

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================================================================================
                          Fraction Information
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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-203-20 (Compd-7l) 188.D
Sample Name: GRL-203-20 (Compd-7l)

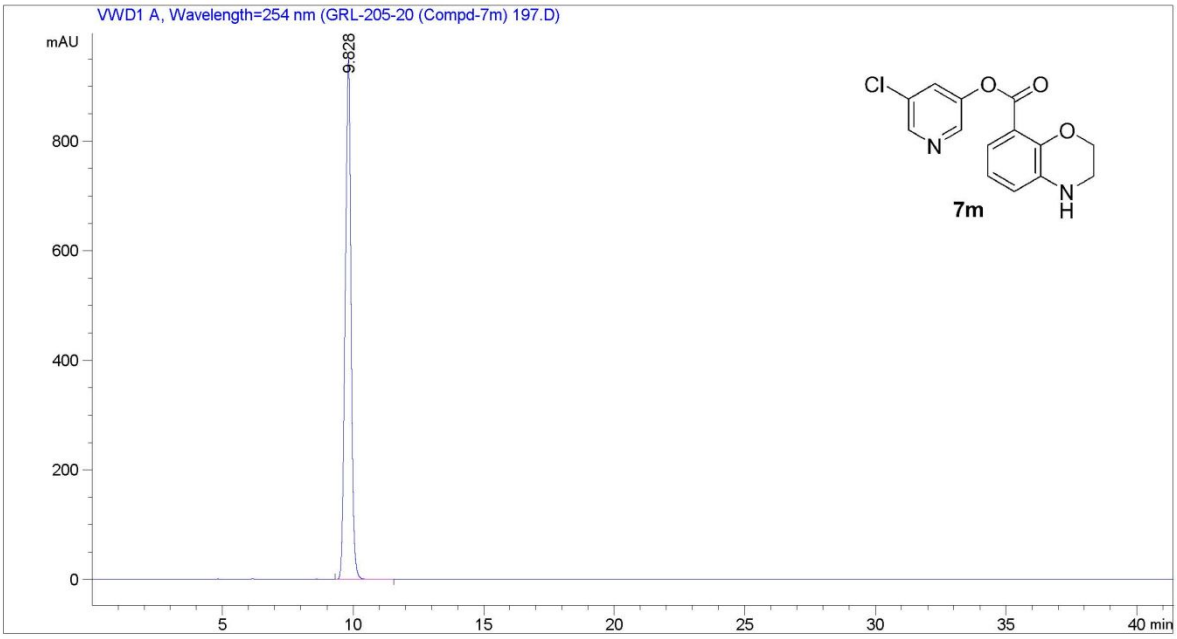
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.124	BB	0.2478	2.34398e4	1389.63794	100.0000
Totals :				2.34398e4	1389.63794	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-205-20 (Compd-7m) 197.D
Sample Name: GRL-205-20 (Compd-7m)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/3/2021 4:26:51 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/3/2021 3:43:52 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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================================================================================
                          Fraction Information
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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-205-20 (Compd-7m) 197.D
Sample Name: GRL-205-20 (Compd-7m)

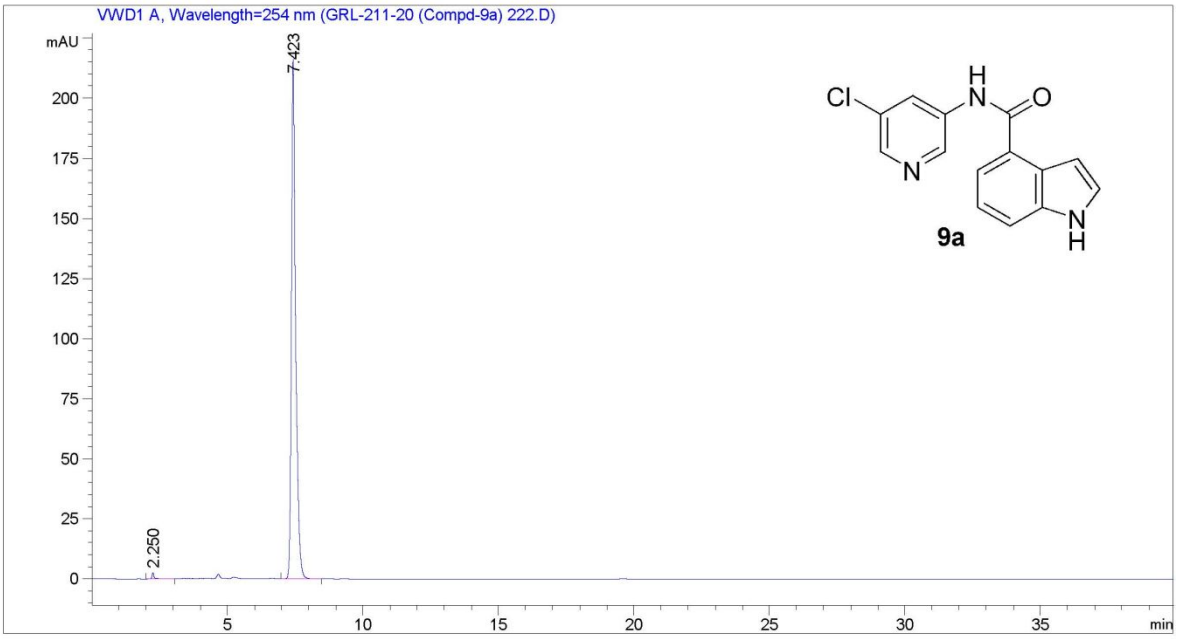
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.828	BB	0.2288	1.47161e4	949.55652	100.0000
Totals :				1.47161e4	949.55652	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-211-20 (Compd-9a) 222.D
Sample Name: GRL-211-20 (Compd-9a)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/11/2021 11:54:11 AM
                                             Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/10/2021 7:56:13 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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==============================================================================
                          Fraction Information
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No Fractions found.
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=====================================================================
                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-211-20 (Compd-9a) 222.D
Sample Name: GRL-211-20 (Compd-9a)

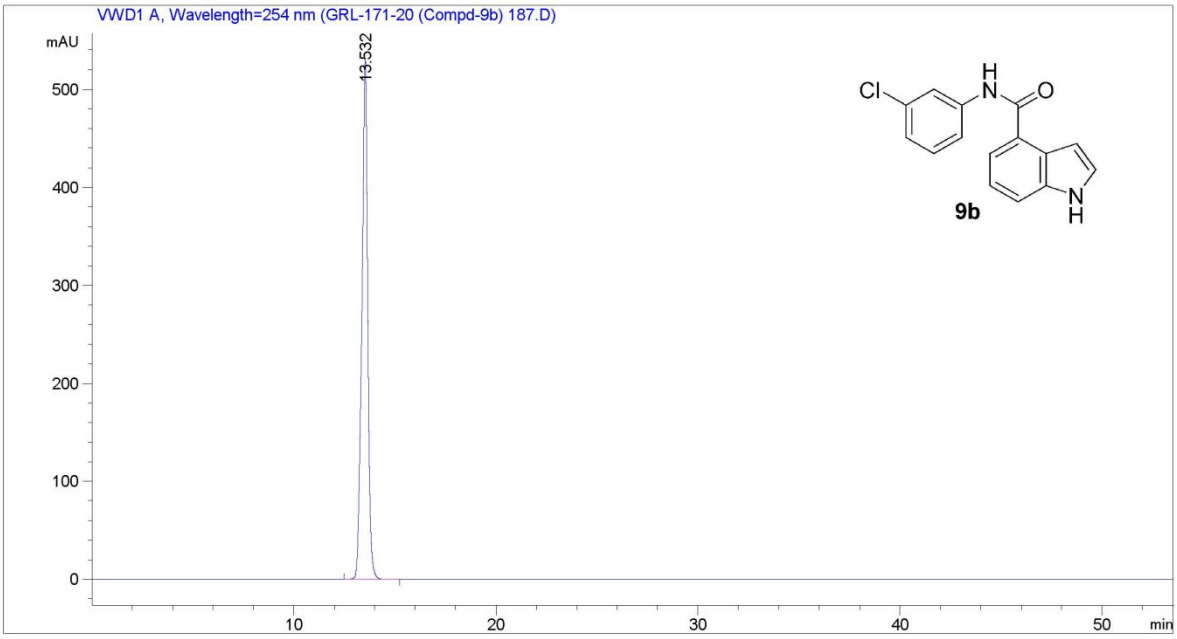
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.250	BV R	0.0846	15.72284	2.59787	0.6703
2	7.423	BB	0.1537	2329.96753	216.25853	99.3297
Totals :				2345.69037	218.85640	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-171-20 (Compd-9b) 187.D
Sample Name: GRL-171-20 (Compd-9b)

```
=====================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 7/30/2021 8:21:00 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 7/30/2021 7:52:04 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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=============================================================================
                        Fraction Information
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No Fractions found.
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                      Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-171-20 (Compd-9b) 187.D
Sample Name: GRL-171-20 (Compd-9b)

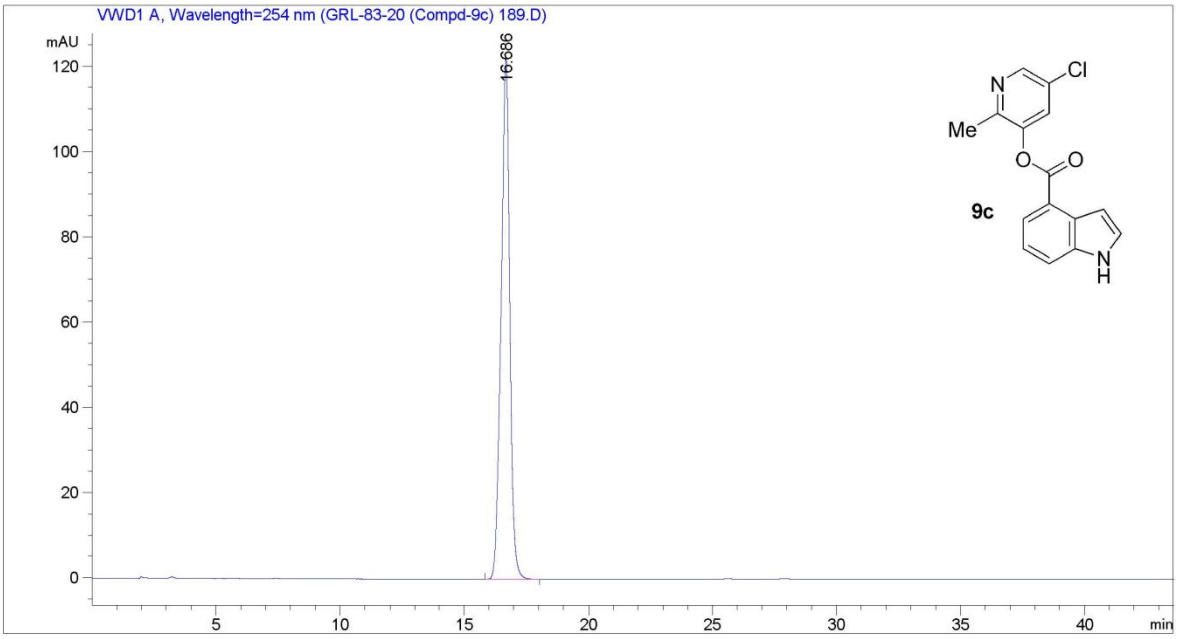
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.532	BB	0.2934	1.07554e4	530.89288	100.0000
Totals :				1.07554e4	530.89288	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-83-20 (Compd-9c) 189.D
Sample Name: GRL-83-20 (Compd-9c)

```
=====================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 7/31/2021 6:10:08 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 7/31/2021 5:42:56 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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=============================================================================
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No Fractions found.
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                      Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-83-20 (Compd-9c) 189.D
Sample Name: GRL-83-20 (Compd-9c)

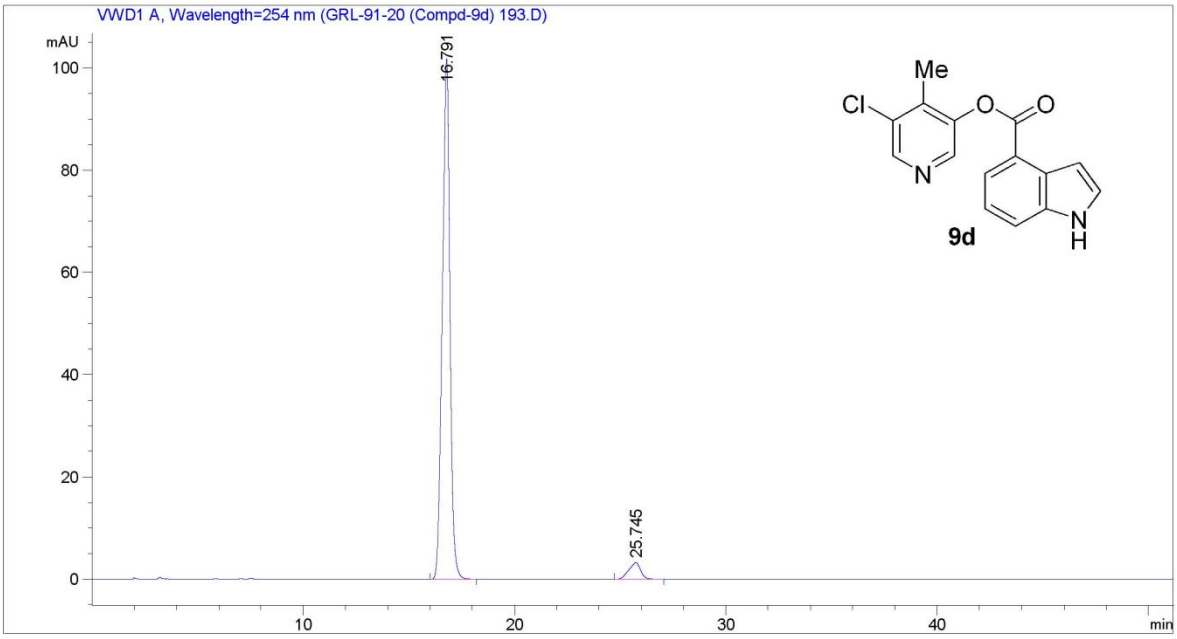
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.686	BB	0.3546	2963.87207	121.94595	100.0000
Totals :				2963.87207	121.94595	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-91-20 (Compd-9d) 193.D
Sample Name: GRL-91-20 (Compd-9d)

```
=====================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/2/2021 6:47:14 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/2/2021 3:00:57 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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=============================================================================
                        Fraction Information
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No Fractions found.
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                      Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-91-20 (Compd-9d) 193.D
Sample Name: GRL-91-20 (Compd-9d)

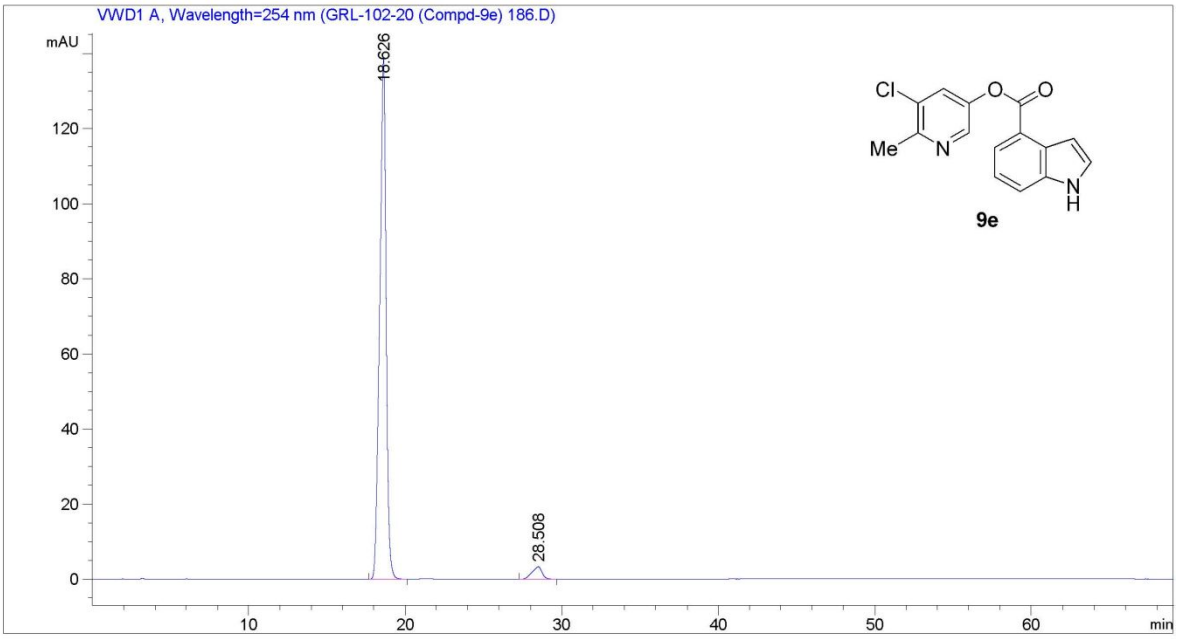
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	16.791	BB	0.3658	2543.07446	101.68309	95.2569
2	25.745	BB	0.5571	126.62586	3.24617	4.7431
Totals :				2669.70033	104.92926	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-102-20 (Compd-9e) 186.D
Sample Name: GRL-102-20 (Compd-9e)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 7/30/2021 6:08:56 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 7/30/2021 5:36:01 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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                          Fraction Information
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No Fractions found.
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                       Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-102-20 (Compd-9e) 186.D
Sample Name: GRL-102-20 (Compd-9e)

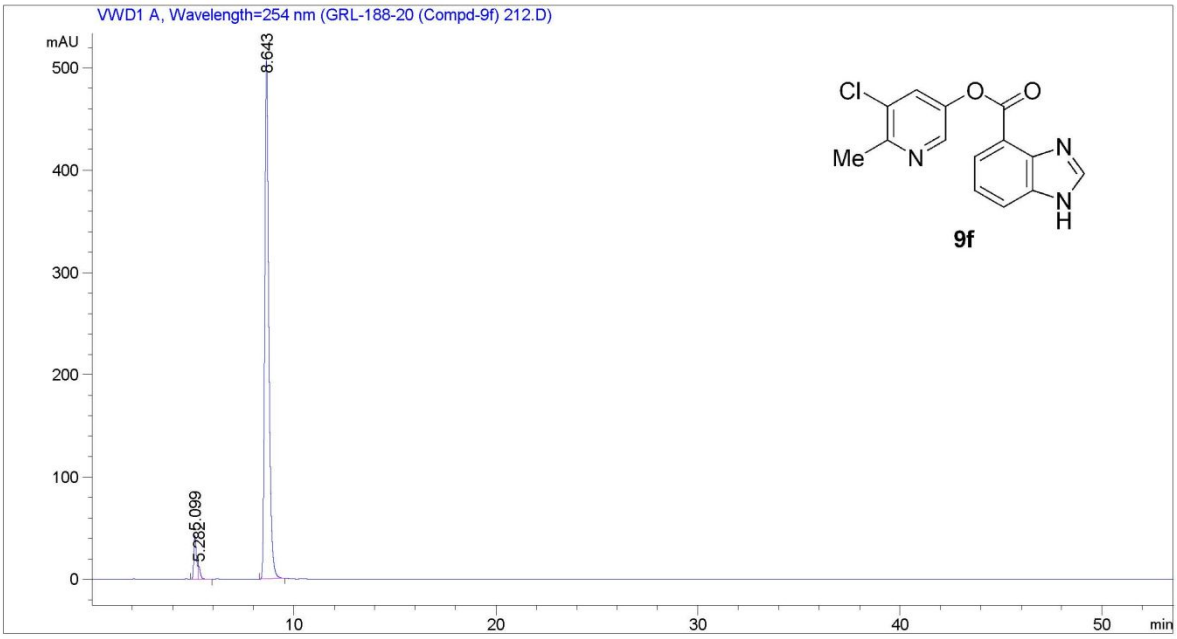
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.626	BB	0.4097	3947.97852	138.42316	96.3732
2	28.508	BB	0.6239	148.57428	3.35968	3.6268
Totals :				4096.55280	141.78283	

*** End of Report ***

Data File C:\Chem32\1\Data\GRL-188-20 (Compd-9f) 212.D
Sample Name: GRL-188-20 (Compd-9f)

```
==========================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 8/6/2021 3:28:56 PM
                                              Inj Volume : 5.000 µl
Method          : C:\CHEM32\1\METHODS\HMS_ZORBAXNH2.M
Last changed    : 8/6/2021 2:41:32 PM by SYSTEM
                  (modified after loading)
Sample Info     : 50% MeCN/H2O
                  0.8 mL/min
                  254 nm
                  5 uL inj.
                  1 mg/mL
                  YMCPAK ODS-A
```

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                       Area Percent Report
=======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:                :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

Data File C:\Chem32\1\Data\GRL-188-20 (Compd-9f) 212.D
Sample Name: GRL-188-20 (Compd-9f)

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.099	BV	0.1345	385.71869	42.66505	5.0942
2	5.282	VB	0.1137	97.85328	12.73054	1.2923
3	8.643	BB	0.2087	7088.20361	508.35724	93.6135
Totals :				7571.77558	563.75283	

*** End of Report ***