Supporting Information

Bioreductively Activatable Prodrug Conjugates of Combretastatin A-1 and Combretastatin A-4 as Anticancer Agents Targeted Towards Tumor Hypoxia

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Figures and Schemes

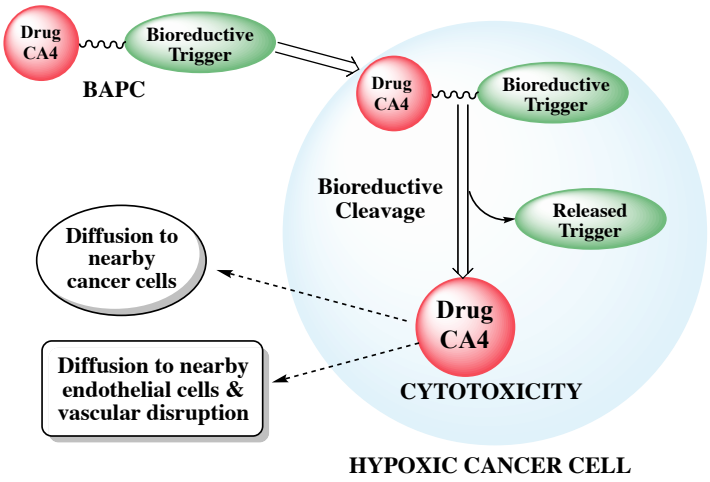


Figure S1. Selective release of cytotoxic agent (CA4) from non-toxic BAPC under tumor hypoxia. BAPCs are designed to activate selectively in the hypoxic tumor microenvironment, thereby releasing their cytotoxic anticancer agent (payload).[S1]

The disorganized, leaky tumor-associated capillaries with shunts and blind ends lead to diminished blood flow in the central mass of the tumor and an increased average diffusion distance for oxygen and nutrients to reach tumor cells.[S2] Furthermore, there is a distinct oxygen concentration gradient present in a significant percentage of solid tumors, varying from normoxic to hypoxic to anoxic.[S3] Tumor-associated hypoxia is believed to be one of the significant contributing factors to treatment failure and relapse of solid tumors in cancer patients, as the tumor cells in the hypoxic region have been implicated in resistance.[S3–S6]

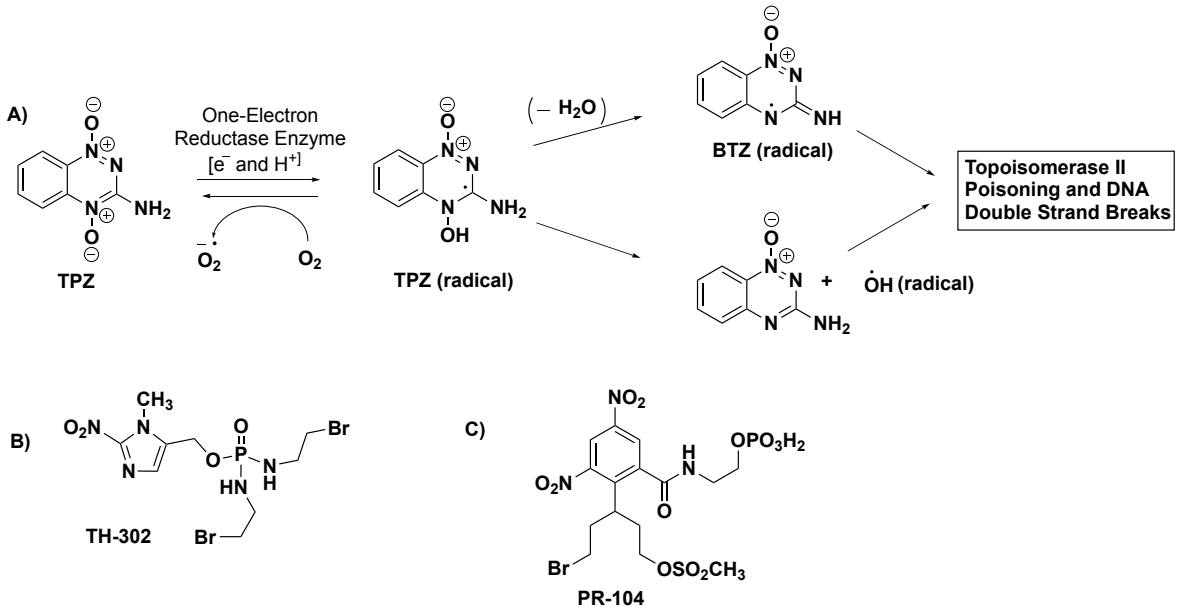


Figure S2. A) Mechanism of action for tirapazamine with hypoxic cells; B) Structure of TH-302; C**)** Structure of PR-104.[S7–S10]

Tirapazamine represents one type of hypoxia-selective therapeutic agent. Reduction of its triazine moiety to a free radical leads to DNA damage and poisoning of topoisomerase II (Figure S2, A).[S7,S8] While Phase I and Phase II clinical trials for tirapazamine had positive results, a Phase III clinical trial utilizing the combination of tirapazamine with the conventional anticancer agent cisplatin to treat advanced non-small cell lung cancer was unsuccessful,[S3] due largely to dose-limiting toxicity.[S11,S12] The high degree of hypoxia-selective activation coupled with its performance in early clinical trials resulted in tirapazamine being viewed as a promising positive control against which new hypoxia-selective therapeutic agents are often compared.[S2] TH-302 (evofosfamide),[S13,S14] a 2-nitroimidazole-based nitrogen mustard prodrug (Figure S2, B) that releases its parent drug bromoisophosphoramide mustard under hypoxic conditions, advanced to Phase III human clinical trials.[S9,S15] Unlike the Phase I and II studies, the results of the Phase III clinical trials showed no statistical significance for TH-302 against pancreatic adenocarcinoma and soft tissue sarcoma. [S16–S19] PR-104 (Figure S2, C) is a phosphate ester pre-prodrug which

contains a nitrogen mustard moiety that becomes active and induces DNA cross-linking under hypoxic conditions.[S2,S10,S20] Nitroreduction can act as an electronic switch to activate a reactive center, as in the case of the reduction of the PR-104 alcohol to form the cytotoxic hydroxylamine (or amine), or initiate fragmentation from the radical anion or hydroxylamine to release the trigger and generate the (non-radical) parent cytotoxin, the nitrogen mustard in the case of TH-302.[S9,S21,S22]

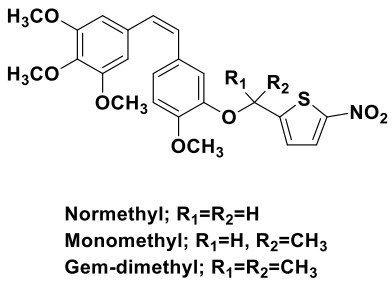
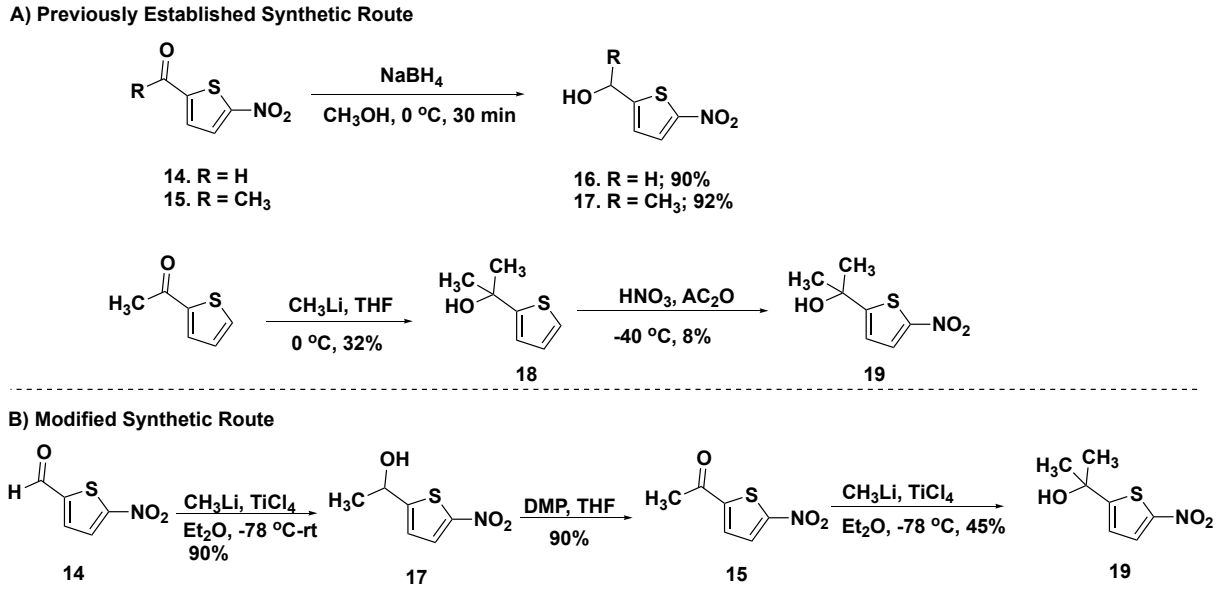
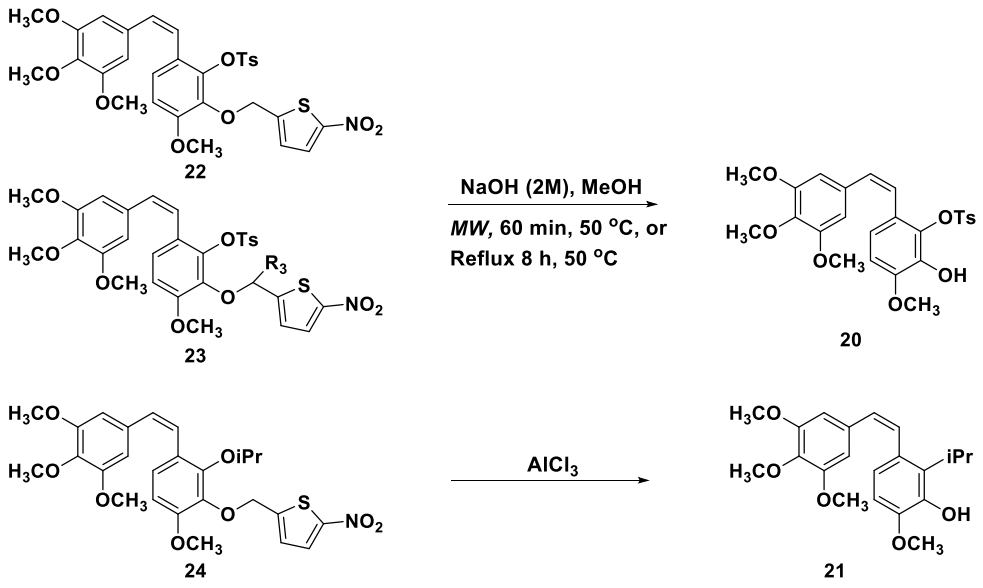


Figure S3. Combretastatin A-4 (CA4) incorporating nitrothiophene-based bioreductive triggers.[S23]



Scheme S1. Synthesis of nitrothiophene triggers: A) Previously established synthetic route (by Davis and co-workers); [S23] B) Modified synthetic route.[S24, S25]

The synthetic route reported by Davis and co-workers was utilized in the synthesis of the *nor*- and *mono*-methyl nitrothiophene triggers **16** and **17**, which involved reduction of aldehyde **14** and ketone **15**, respectively (Scheme **S1**).[S23] However, in our hands, the synthesis of the *gem*-dimethyl nitrothiophene trigger **19** (Scheme **S1**) suffered from two consecutive low-yielding steps, which included methylation of the carbonyl group followed by nitration at the C5 position. In order to obtain a sufficient quantity of compound **19**, it proved efficacious to develop a modified synthetic route that provided all three triggers (*nor*-, *mono*- and *gem*-) from a single starting material (aldehyde **14**).[S1,S25] Methylation of aldehyde **14** furnished *mono*-methyl trigger **17**, which, upon subsequent oxidation and methylation, yielded *gem*-dimethyl trigger **19** in good yield (Scheme **S1**).



Scheme **S2**. Attempted Deprotection of Compounds **22**, **23** and **24**

Solubilization Vehicles for in vivo Studies

Since BAPC **45** was insoluble in buffered saline or water, it was necessary to develop a suitable vehicle to solubilize this agent for in vivo use. While BAPC **45** proved soluble in DMSO, there are limits (in terms of volume tolerability) associated with using DMSO alone in mice. A solubilization study identified several potential vehicles [including: 90% DMSO / 10% PBS; 33.3% DMSO / 66.7% sesame oil; 50% DMSO / 50% Tween 80; 12% DMSO / 12% Tween 80 / 71% PEG 400 / 5% PBS; 11% DMSO / 11% Tween 80 / 67% PEG 400 / 11% ethanol; and 10% DMSO / 55% sesame oil / 35% PEG 400 (referred to as DSP)] from which the latter was chosen for in vivo studies based on its improved ability to solubilize BAPC **45**.

Synthesis of Bioreductive Triggers

Synthesis of Compounds **16**, **17** and **19** Using the Previously Established Synthetic Route (Davis' Route)[S23]

(5-Nitrothiophen-2-yl)methanol (16) [S23]**.** This bioreductive trigger was prepared following the synthetic protocol reported by Pinney and Co-workers.[S25] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (1H, d, *J* = 4 Hz, ArH), 6.96 (1H, d, *J* = 4 Hz, ArH), 4.91 (2H, d, *J* = 5.5, CH_2), 2.20 (1H, s, OH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.4, 150.9, 128.9, 123.6, 60.4.

1-(5-Nitrothiophen-2-yl)ethan-1-ol (17)[S23]**.** 2-Acetyl-5-nitrothiophene (1.00 g, 5.85 mmol) was dissolved in dry methanol (20 mL) in an ice bath (0 °C). $NaBH_4$ (0.259 g, 6.71 mmol) was added, and the reaction mixture was stirred for 2 h. Ice was added to the reaction mixture, and it was acidified to neutral pH with 3 M HCl. The solution was then extracted with EtOAc, and the organic phase was dried with Na_2SO_4 and evaporated under reduced pressure. Flash chromatography of the crude product using a prepacked 50 g silica column [eluents: solvent A, EtOAc; solvent B, hexanes; gradient, 10% A/90% B over 1.19 min (1 CV), 10% A/90% B → 64% A/36% B over 13.12 min (10 CV), 64% A/36% B over 2.38 min (2 CV); flow rate 50.0 mL/min; monitored at 254 and 280 nm] yielded mono methyl trigger **17** (0.932 g, 5.38 mmol, 92%) as a brown oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (1H, d, *J* = 4 Hz, , ArH), 6.90 (1H, d, *J* = 4 Hz, , ArH), 5.15 (1H, dq, *J* = 6 Hz, *J* = 5 Hz, CH), 2.23 (1H, d, *J* = 5 Hz, OH), 1.63 (3H, d, *J* = 6 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.0, 149.9, 129.1, 122.2, 66.3, 25.1.

2-(Thiophen-2-yl)propan-2-ol (18)[S23]**.** 2-Acetylthiophene (10.0 g, 79.2 mmol) was dissolved in dry THF (100 mL) in an ice bath (0 °C). CH_3Li (64 mL, 103 mmol, 1.6 M) was added dropwise, and the reaction mixture was stirred for 18 h. The reaction was quenched with water, and volatile

components were evaporated under reduced pressure. The reaction mixture was then extracted with EtOAc, and the organic phase was dried with Na_2SO_4 and evaporated under reduced pressure. Flash chromatography of the crude product using a prepacked 100 g silica column [eluents: solvent A, EtOAc; solvent B, hexanes; gradient, 12% A/88% B over 1.19 min (1 CV), 12% A/88% B → 100% A/0% B over 13.12 min (10 CV), 100% A/0% B over 2.38 min (2 CV); flow rate 50.0 mL/min; monitored at 254 and 280 nm] yielded 2-(thiophen-2-yl)propan-2-ol (**18**) (3.60 g, 25.3 mmol 32%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (1H, dd, *J* = 5 Hz, *J* = 1.5 Hz, ArH), 6.97 (2H, m, ArH), 2.04 (1H, s, OH), 1.68 (6H, s, CH_3).

2-(5-Nitrothiophen-2-yl)propan-2-ol (19)[S23]**.** The tertiary alcohol **18** (6.22 g, 4.37 mmol) was dissolved in Ac_2O (67 mL) and cooled to -78 °C. Fuming HNO_3 (25 mL) was added dropwise, and the reaction mixture was stirred for 2 h while allowing the reaction mixture to warm to -15° C. Ice (200 g) was added to the solution, which was stirred for 40 min. The reaction mixture was extracted with EtOAc (3×75 mL), and the organic phase was washed repeatedly with brine, water and saturated sodium bicarbonate, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified using flash column chromatography affording the alcohol product **19** (0.655 g, 0.35 mmol, 8%) as an orange wax: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.79 (1H, d, *J* = 4.2 Hz, ArH), 6.87 (1H, d, *J* = 4.2 Hz, ArH), 2.13 (1H, s, OH), 1.67 (6H, s, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.6, 133.9, 128.9, 121.4, 72.0, 32.2.

Synthesis of Compounds **15**, **17** and **19** Using the Modified Synthetic Route (Titanium Tetrachloride Route) [S24, S25]

1-(5-Nitrothiophen-2-yl)ethan-1-ol (17). [S24, S25] This bioreductive trigger was prepared following the synthetic protocol reported by Pinney and Co-workers.[S25] ^{1}H NMR (500 MHz,

$CDCl_3$) δ 7.81 (1H, d, *J* = 4 Hz, ArH), 6.90 (1H, d, *J* = 4 Hz, ArH), 5.15 (1H, dq, *J* = 6 Hz, *J* = 5 Hz, CH), 2.23 (1H, d, *J* = 5 Hz, OH), 1.63 (3H, d, *J* = 6 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.0, 149.9, 129.1, 122.2, 66.3, 25.1.

1-(5-Nitrothiophen-2-yl)ethan-1-one (15). This bioreductive trigger was prepared following the synthetic protocol reported by Pinney and Co-workers.[S25] ^1H NMR (600 MHz, $CDCl_3$) δ 7.89 (1H, d, *J* = 4.3 Hz, ArH), 7.58 (1H, d, *J* = 4.3 Hz, ArH), 2.60 (3H, s, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.5, 156.5, 148.2, 130.2, 128.4, 26.6.

2-(5-Nitrothiophen-2-yl)propan-2-ol (19). [S24, S25] This bioreductive trigger was prepared following the synthetic protocol reported by Pinney and Co-workers.[S25] ^1H NMR ($CDCl_3$, 600 MHz) δ 7.79 (1H, d, *J* = 4.2 Hz, ArH), 6.87 (1H, d, *J* = 4.2 Hz, ArH), 2.13 (1H, s, OH), 1.67 (3H, s, CH_3), ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.6, 133.9, 128.9, 121.4, 72.0, 32.2.

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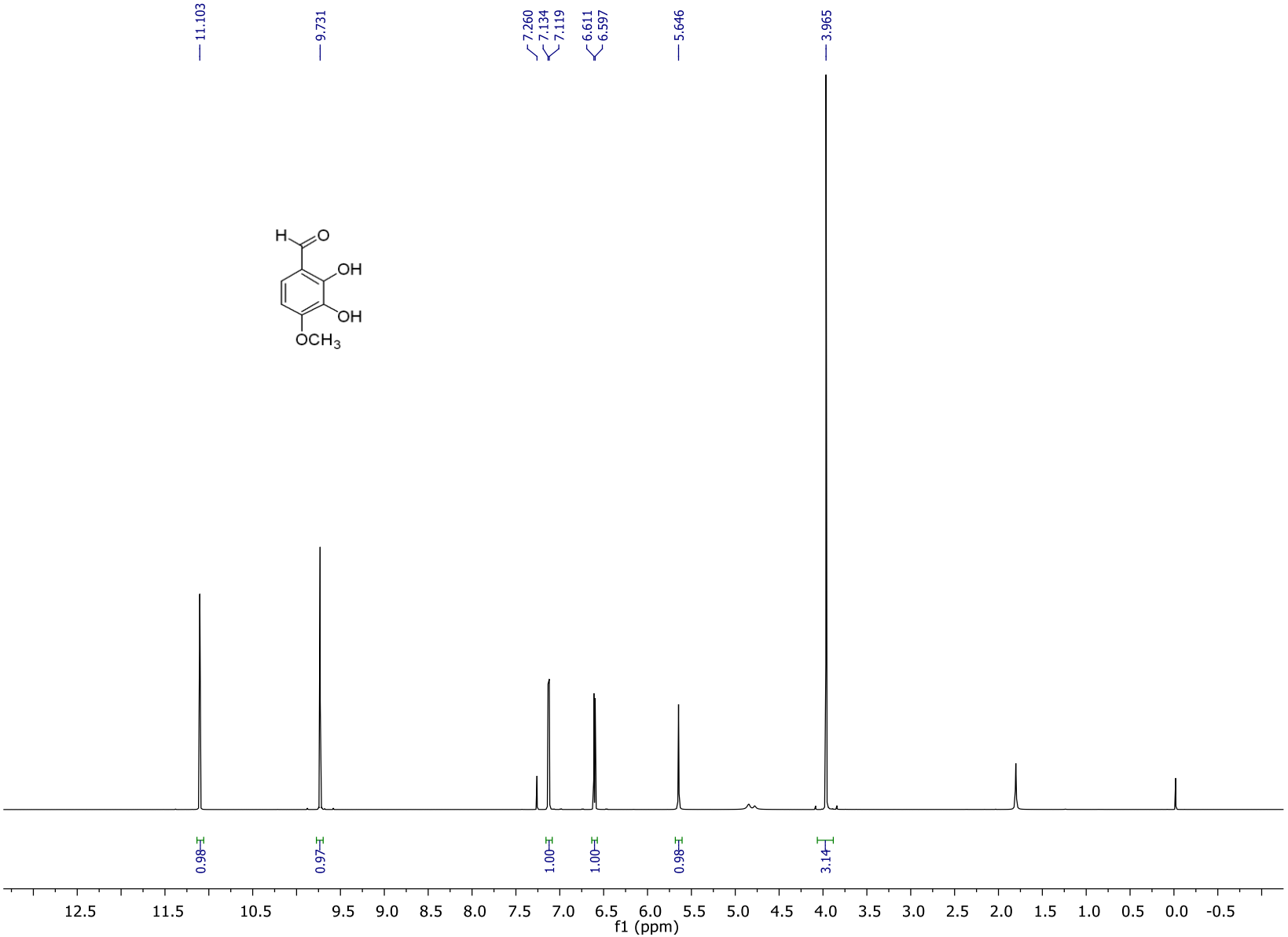
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **2**



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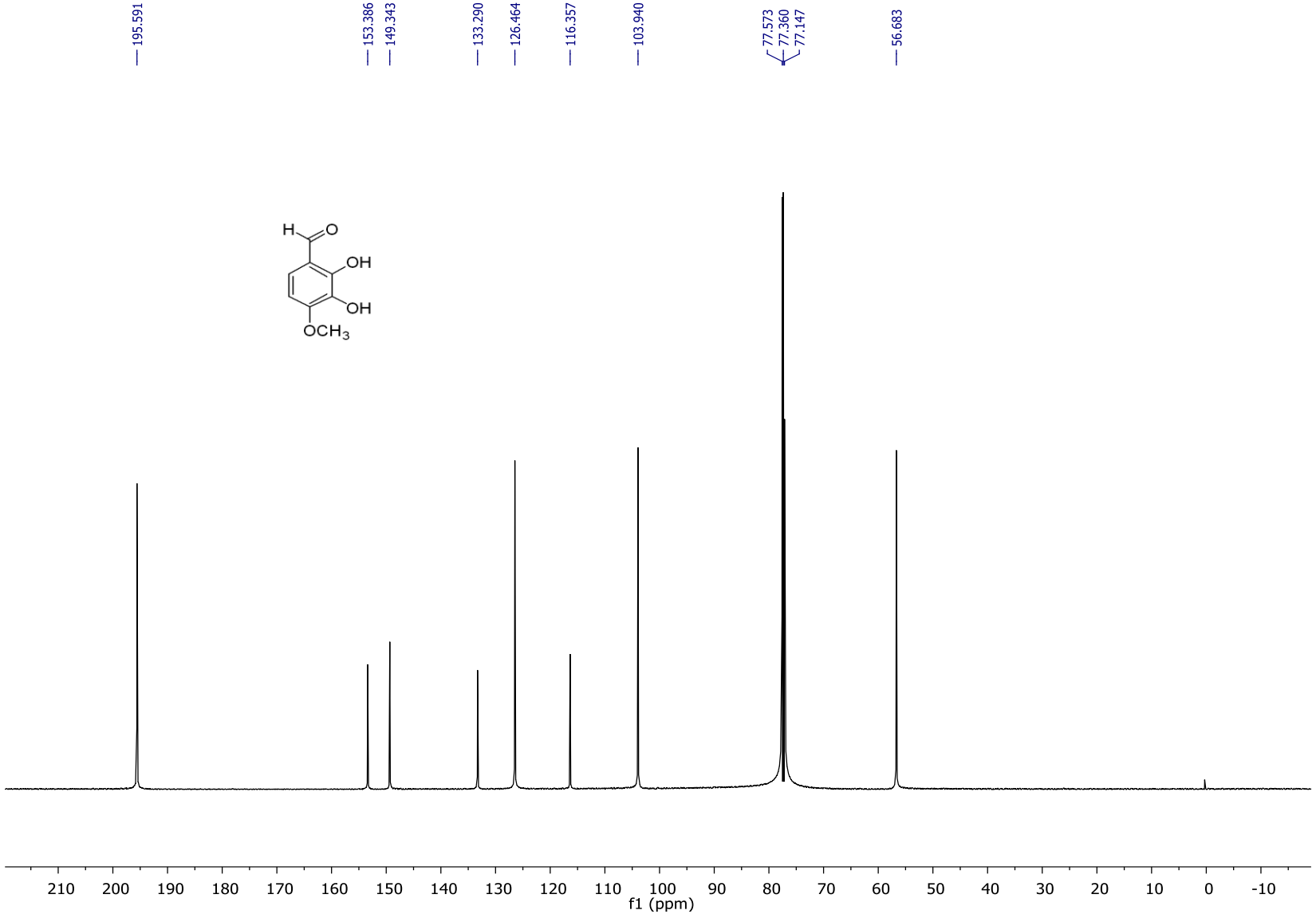

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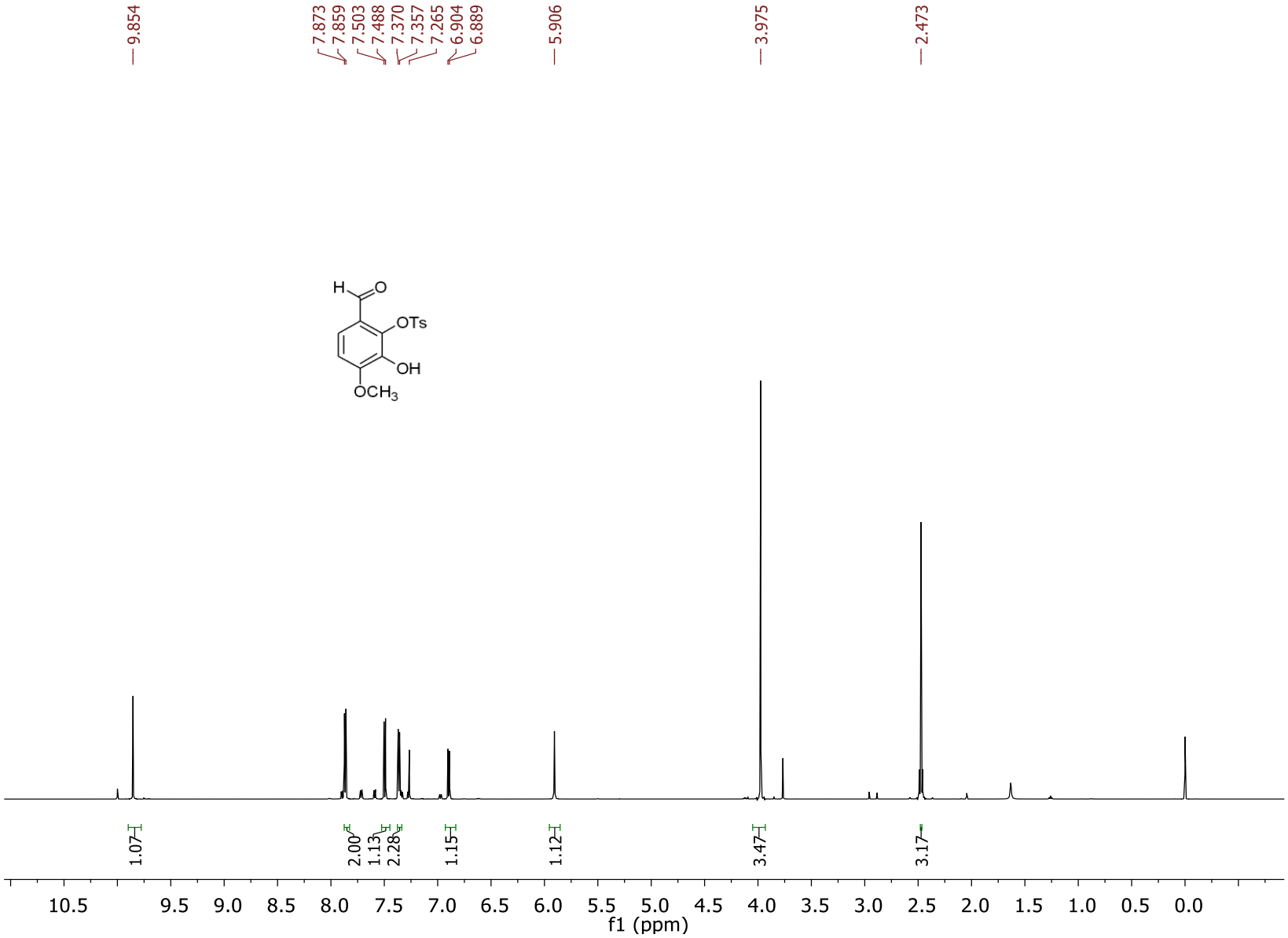

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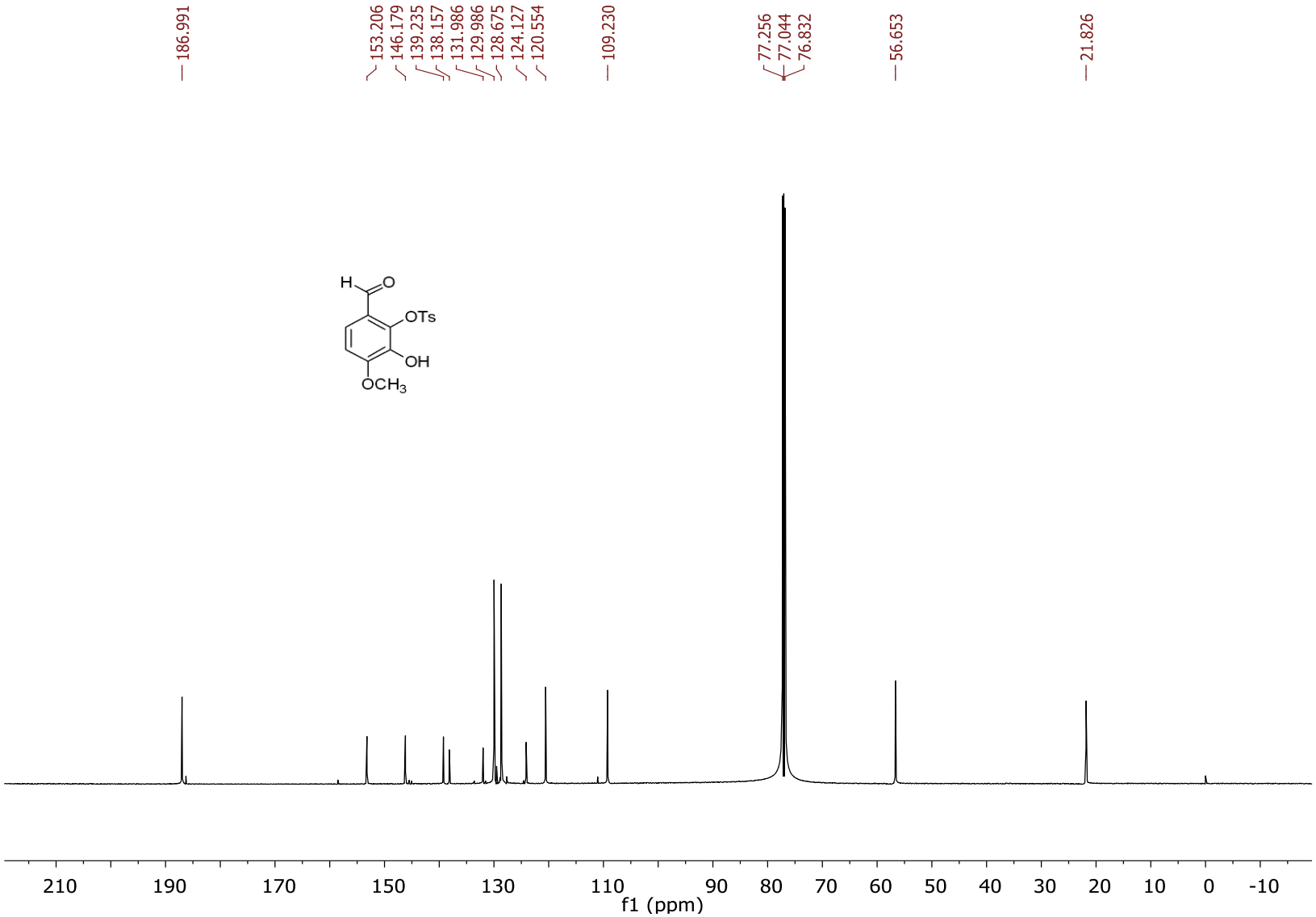

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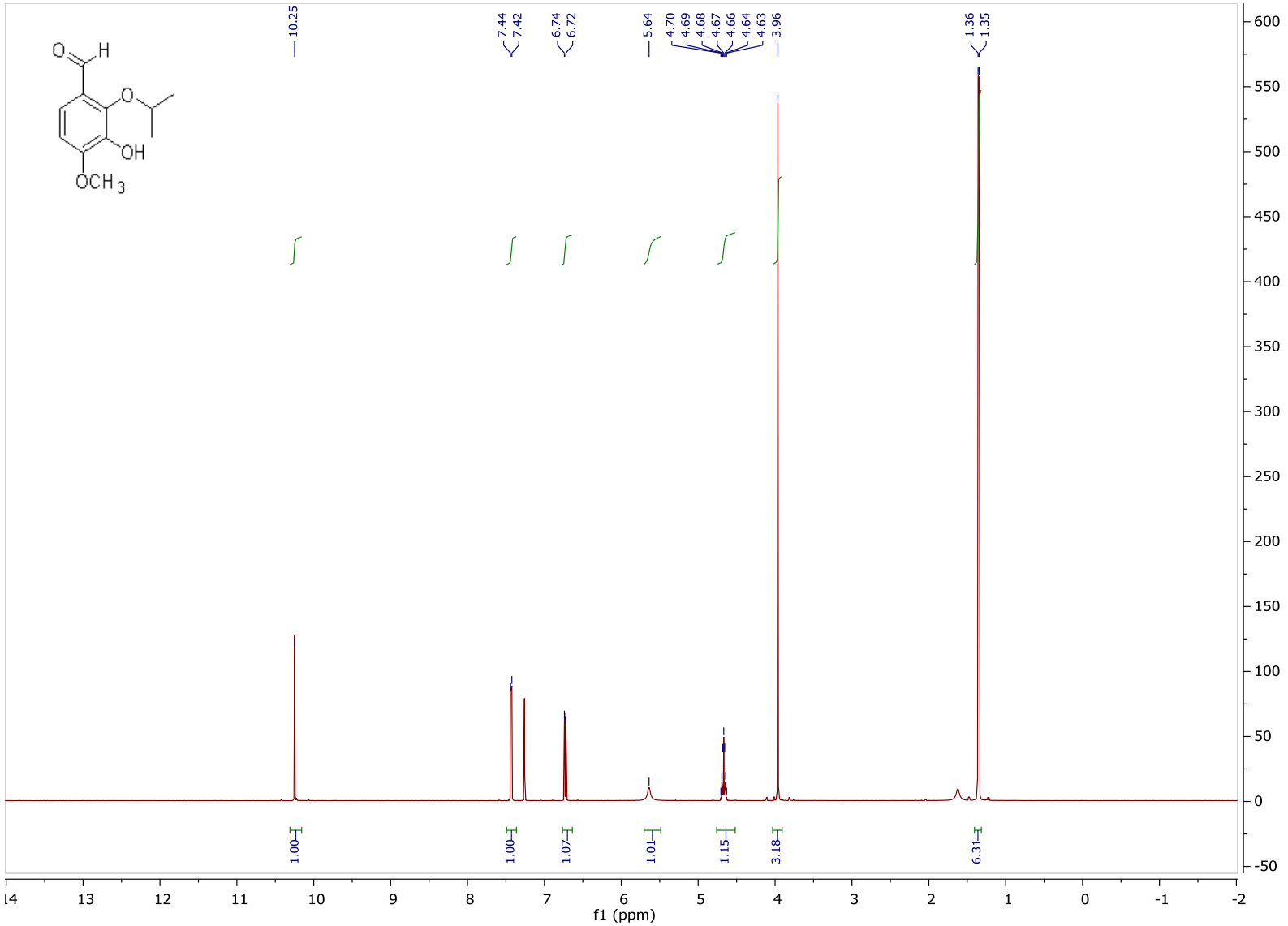

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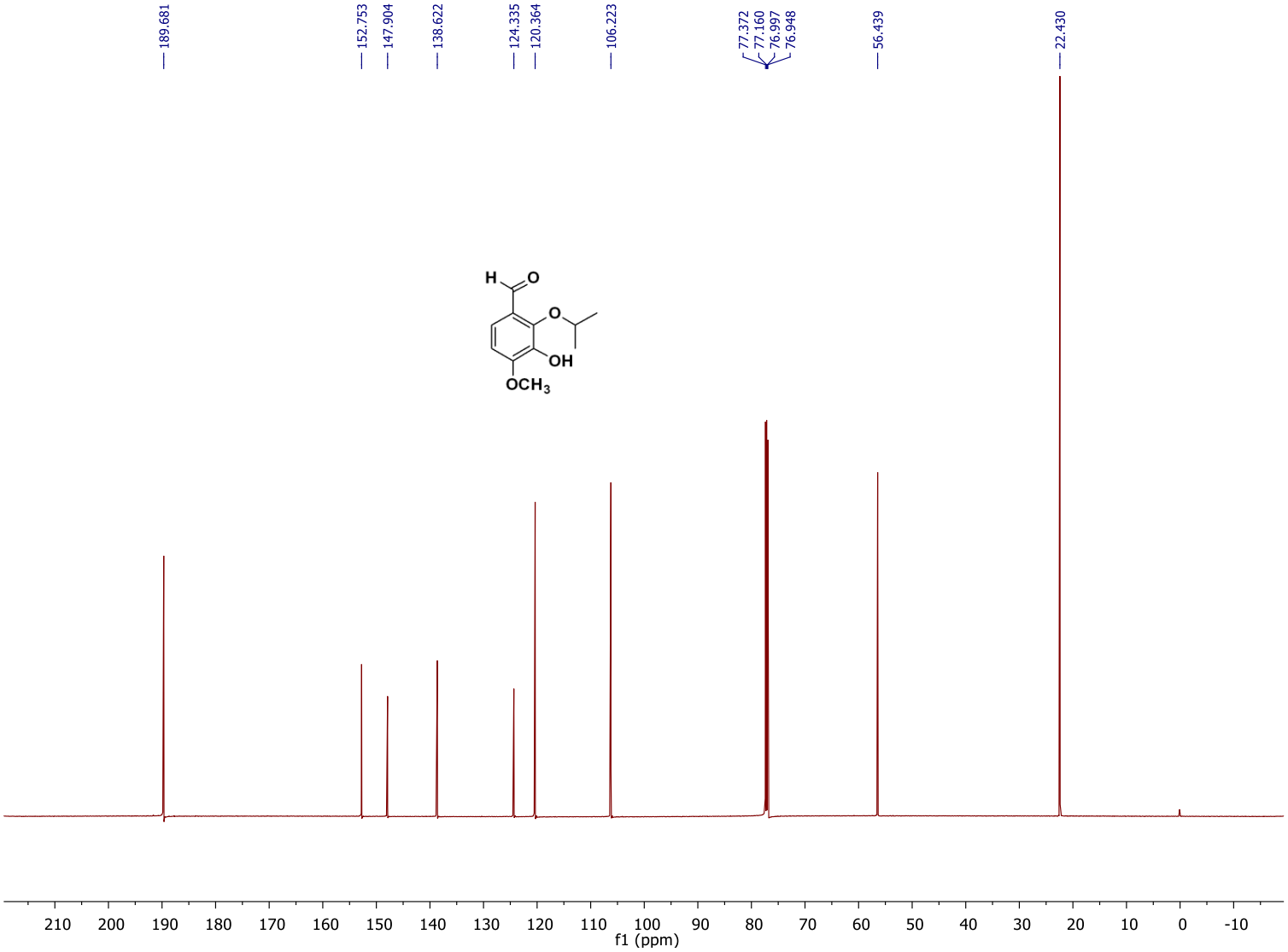

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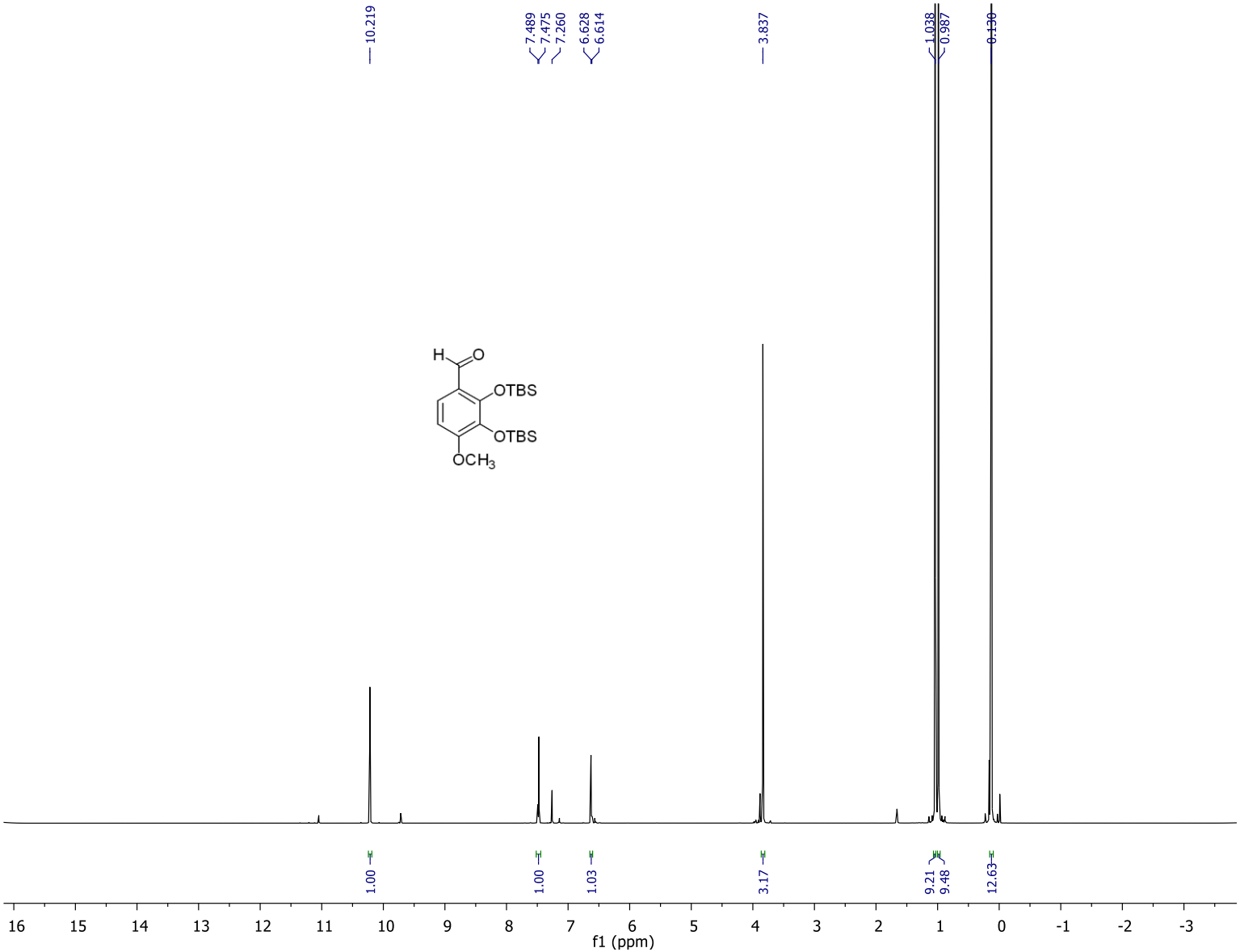

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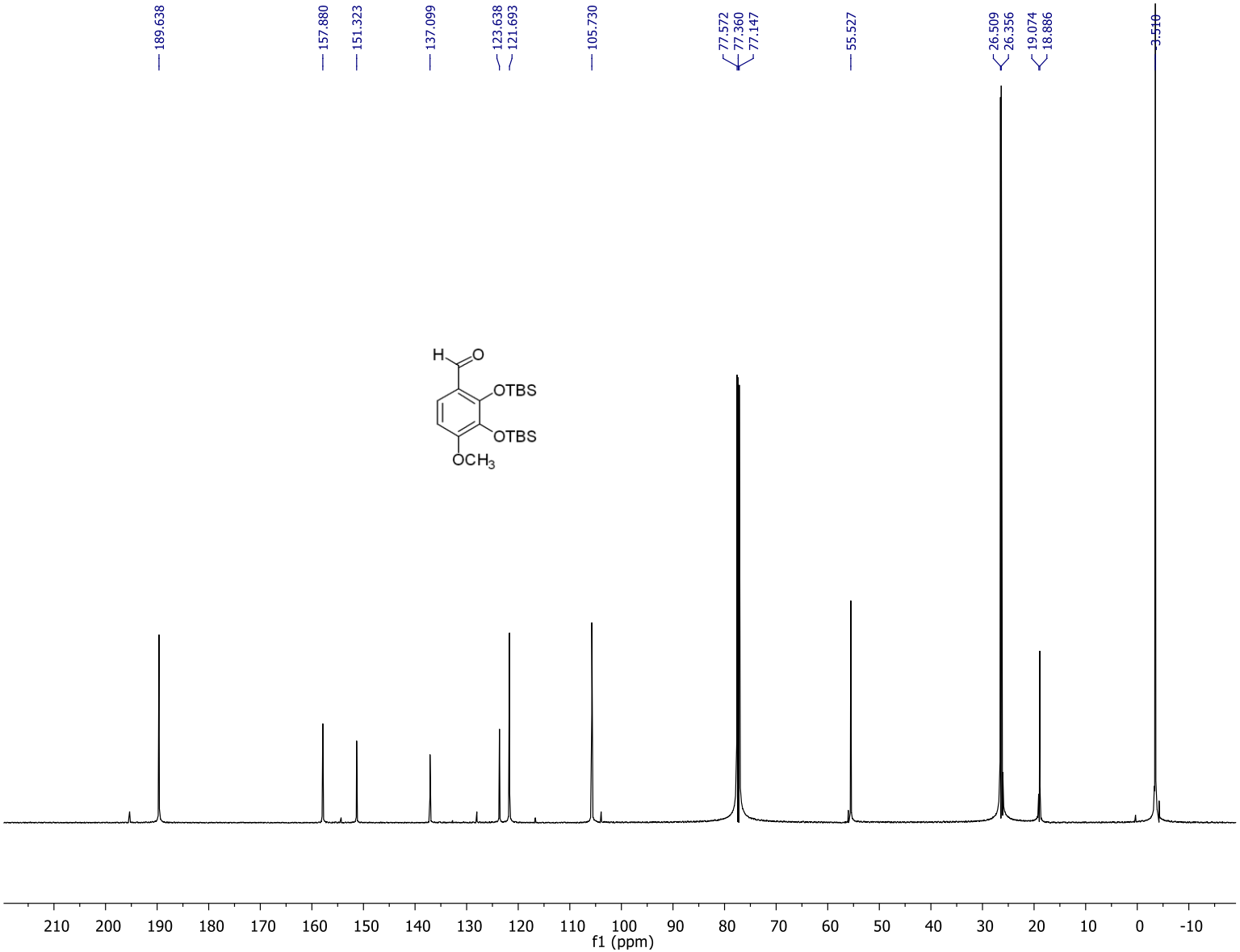

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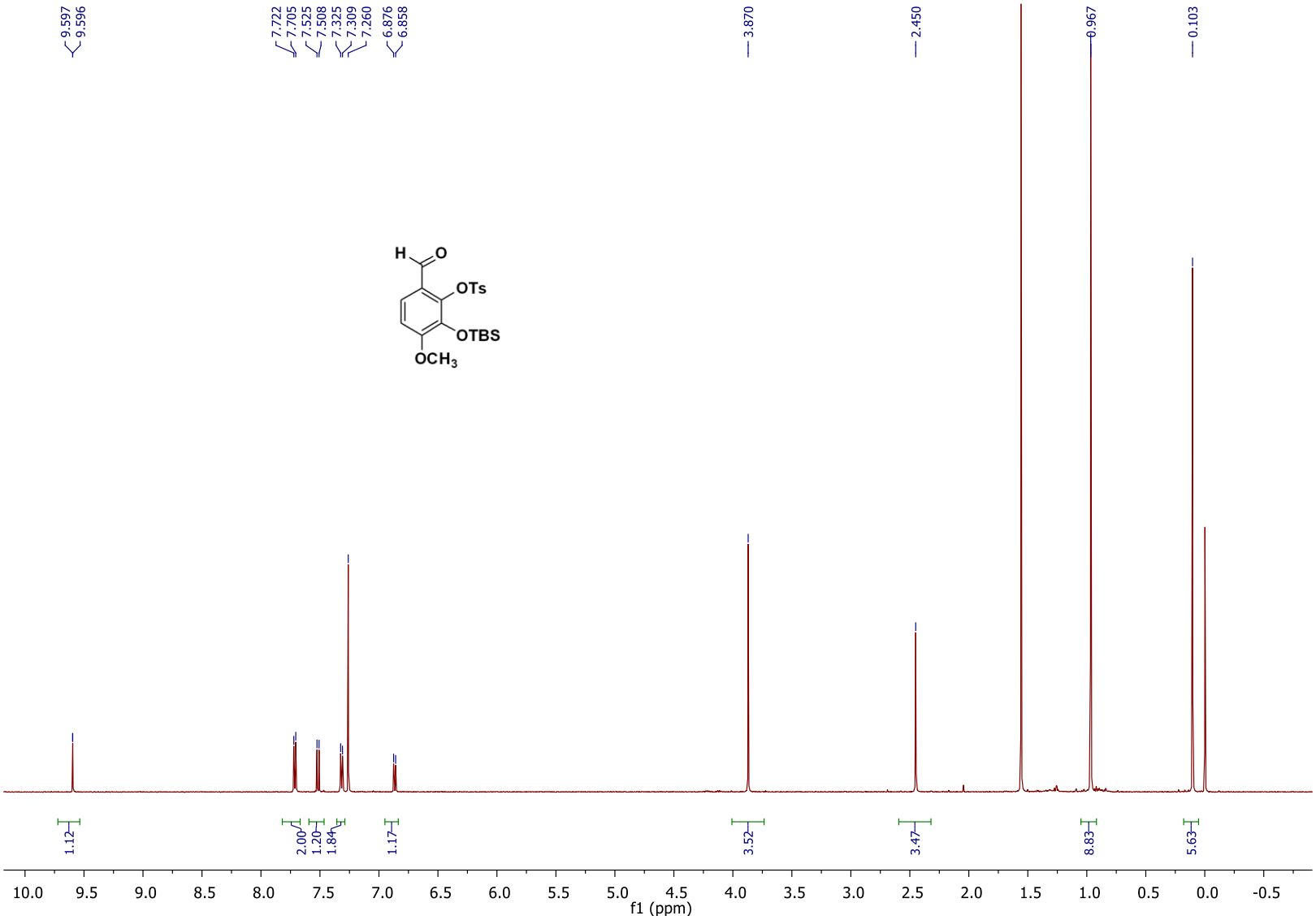

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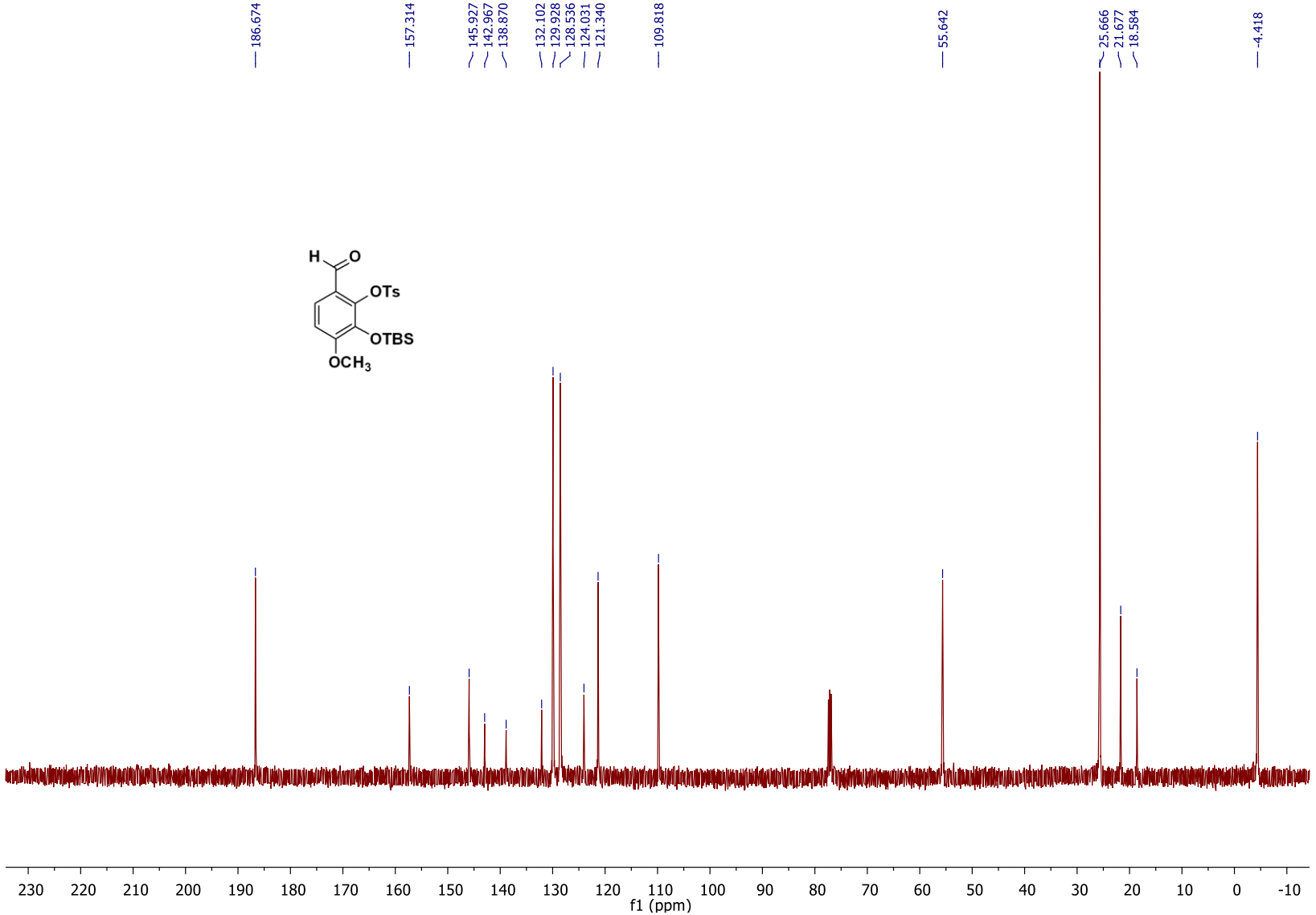

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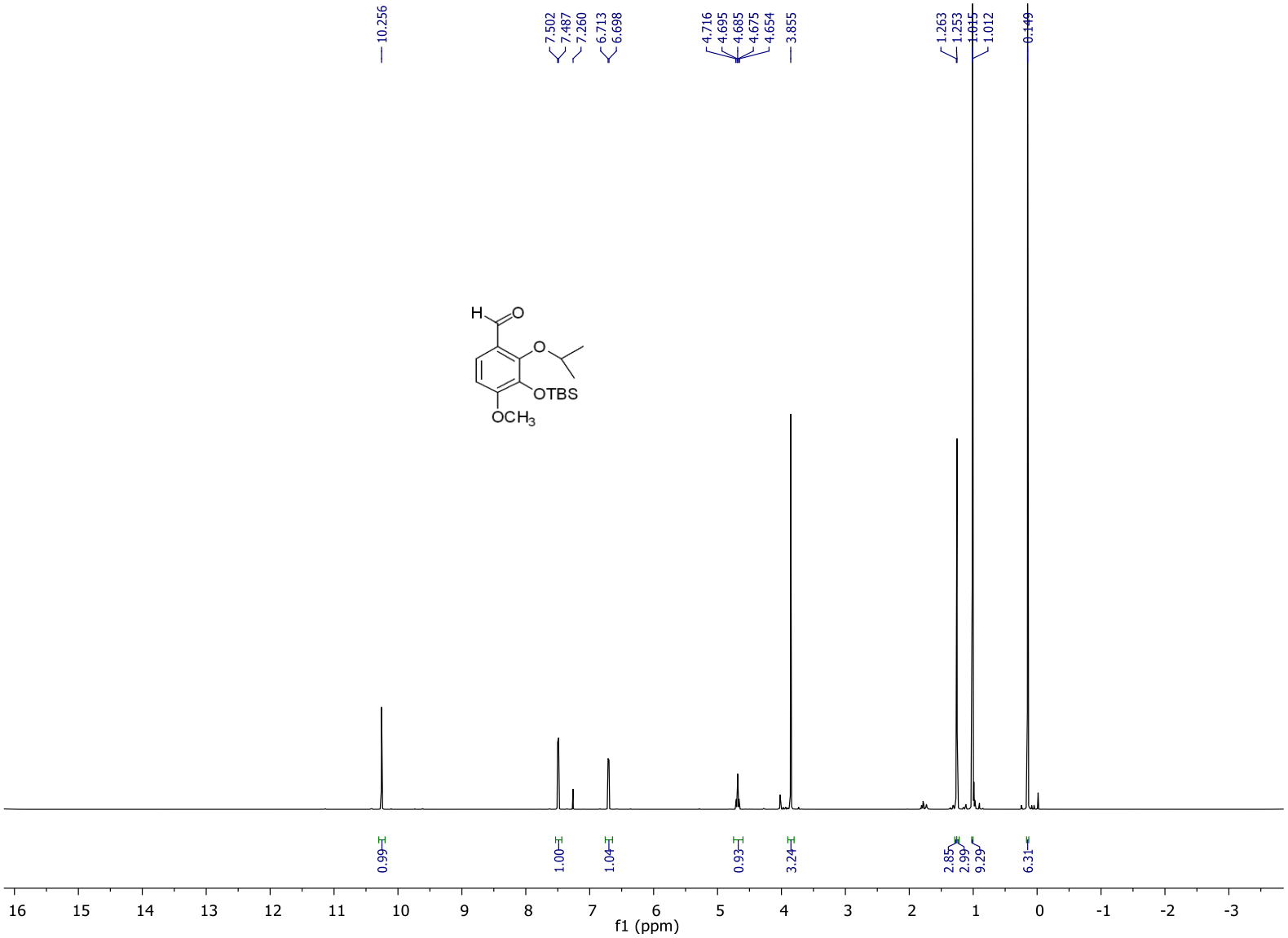

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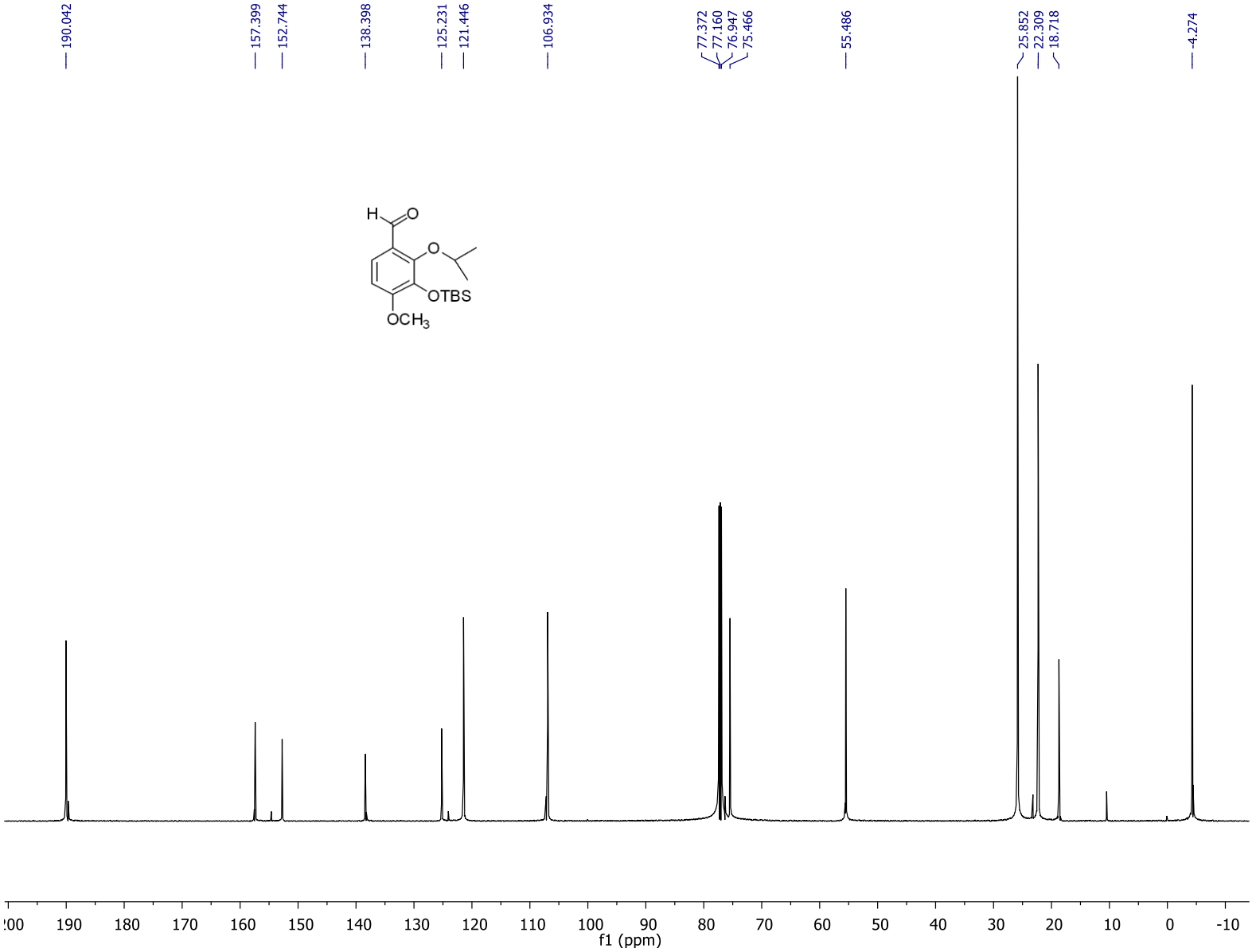
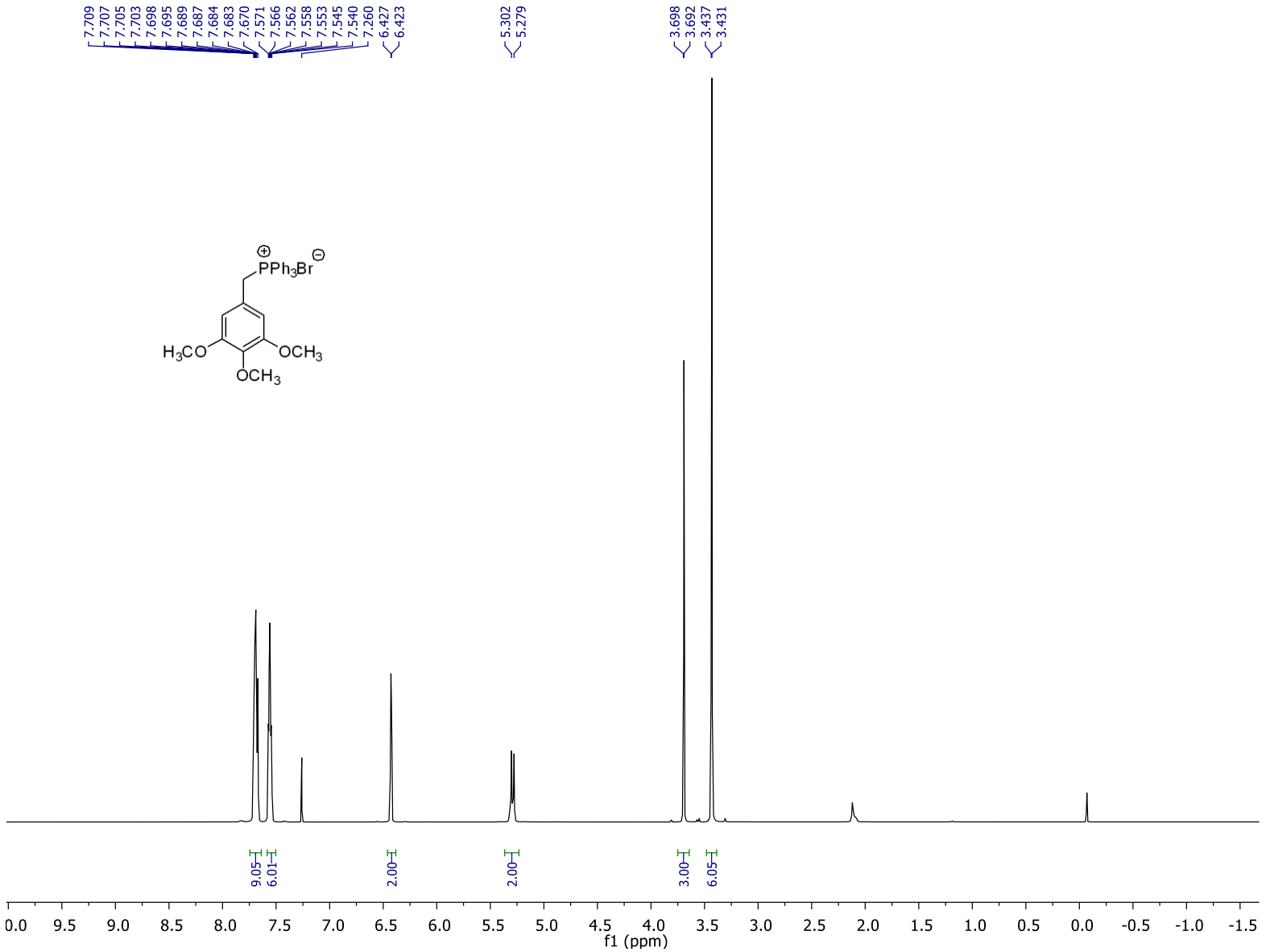
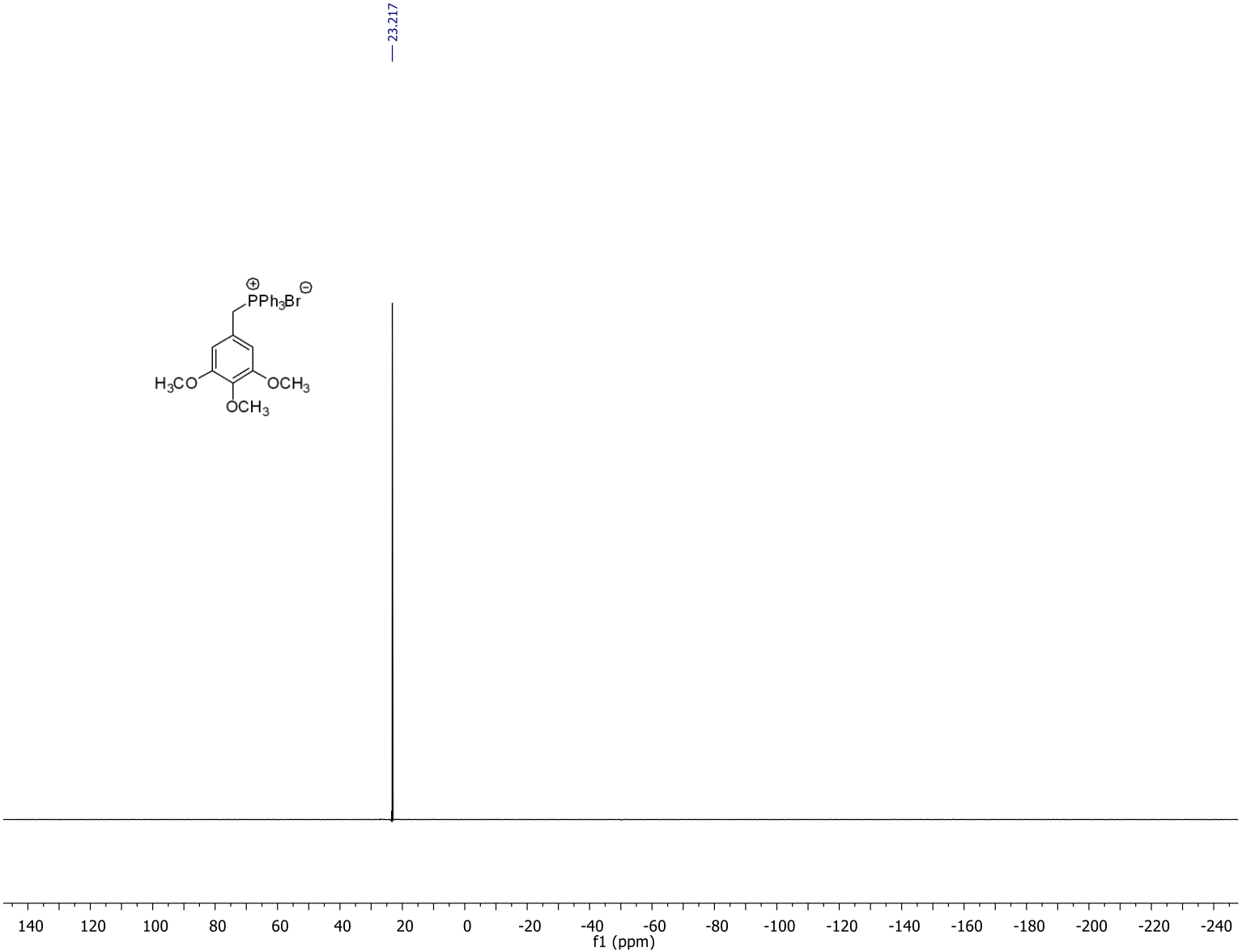


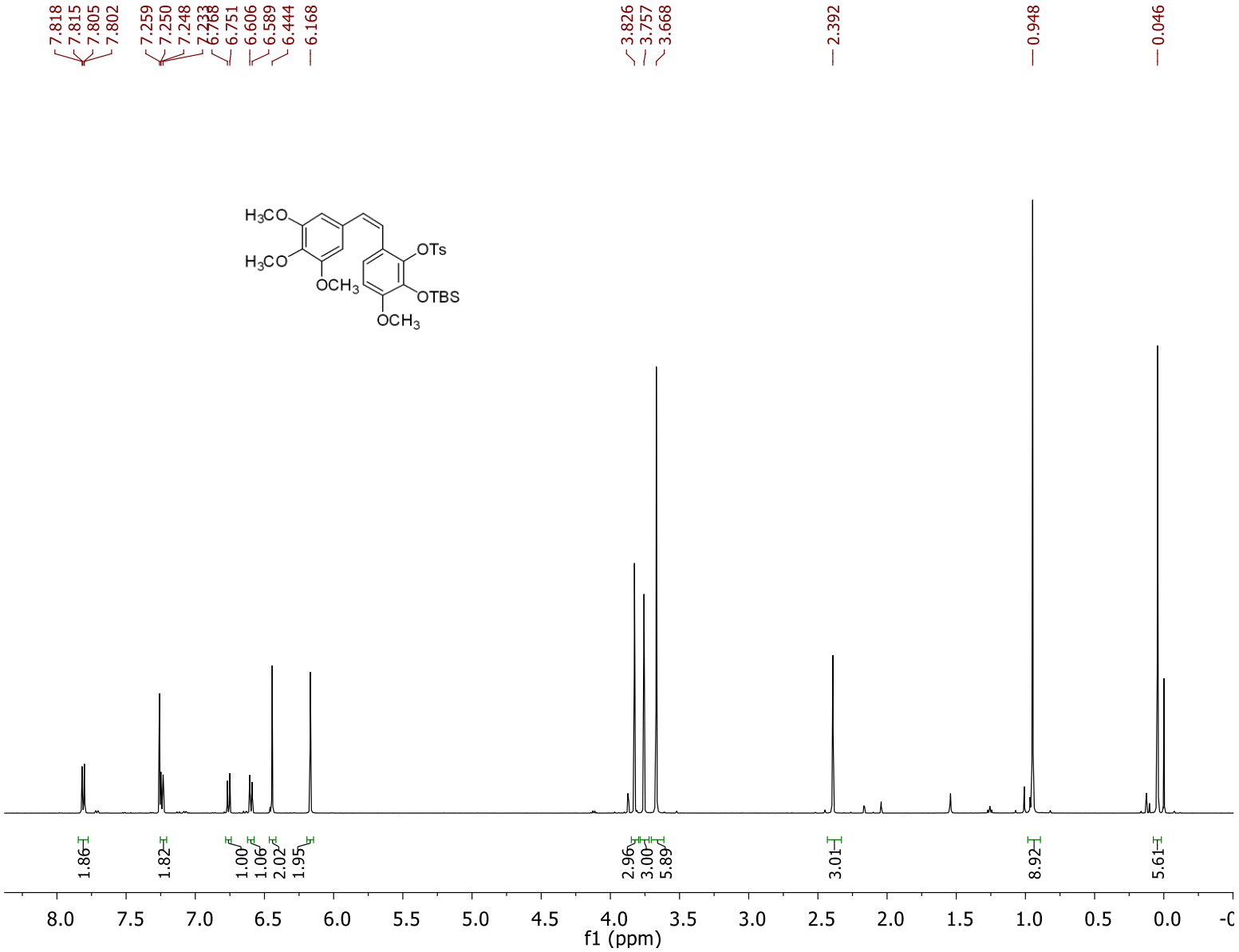
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **10**



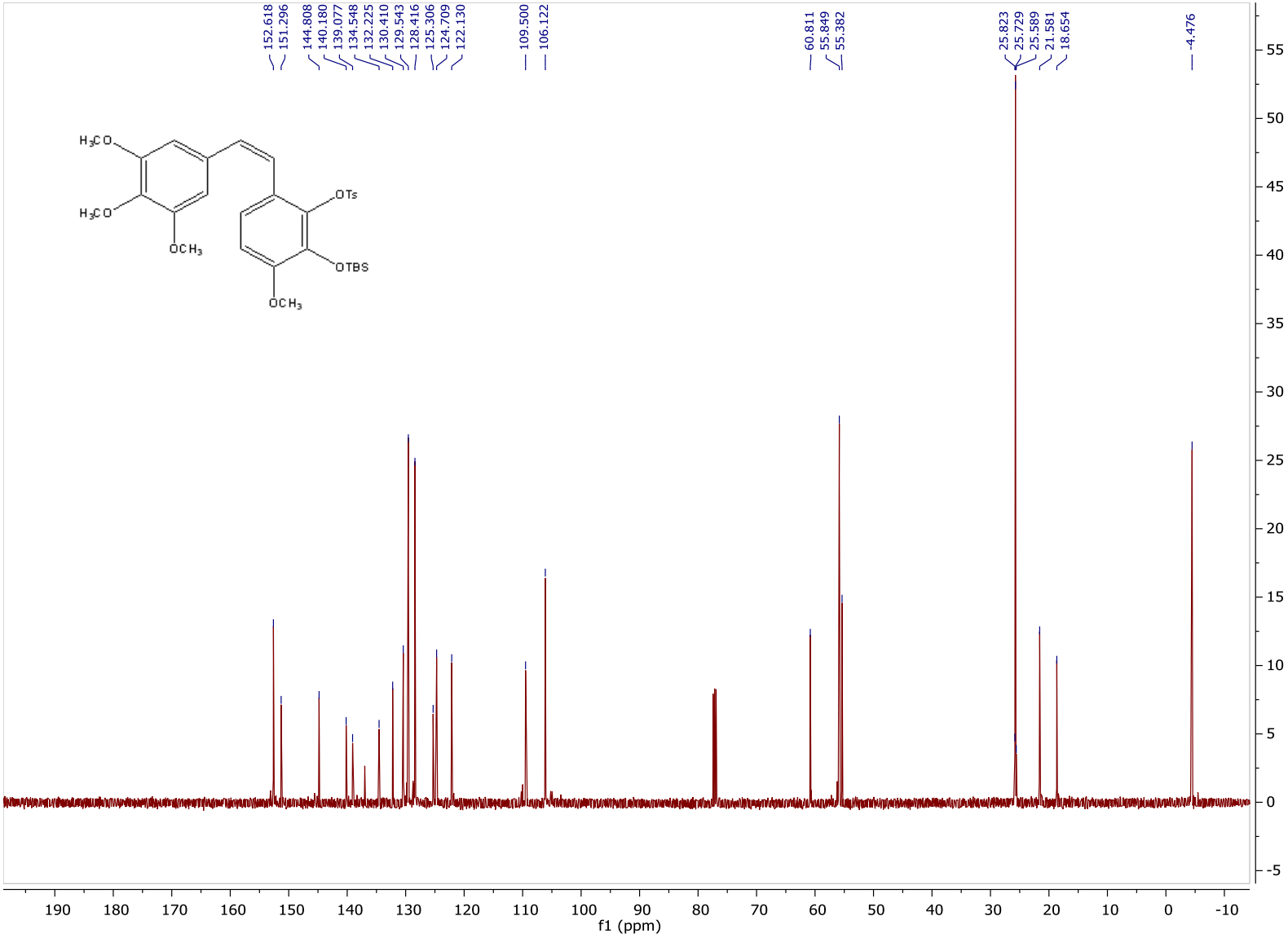
^{31}P NMR (240 MHz, $CDCl_3$) for Compound **10**



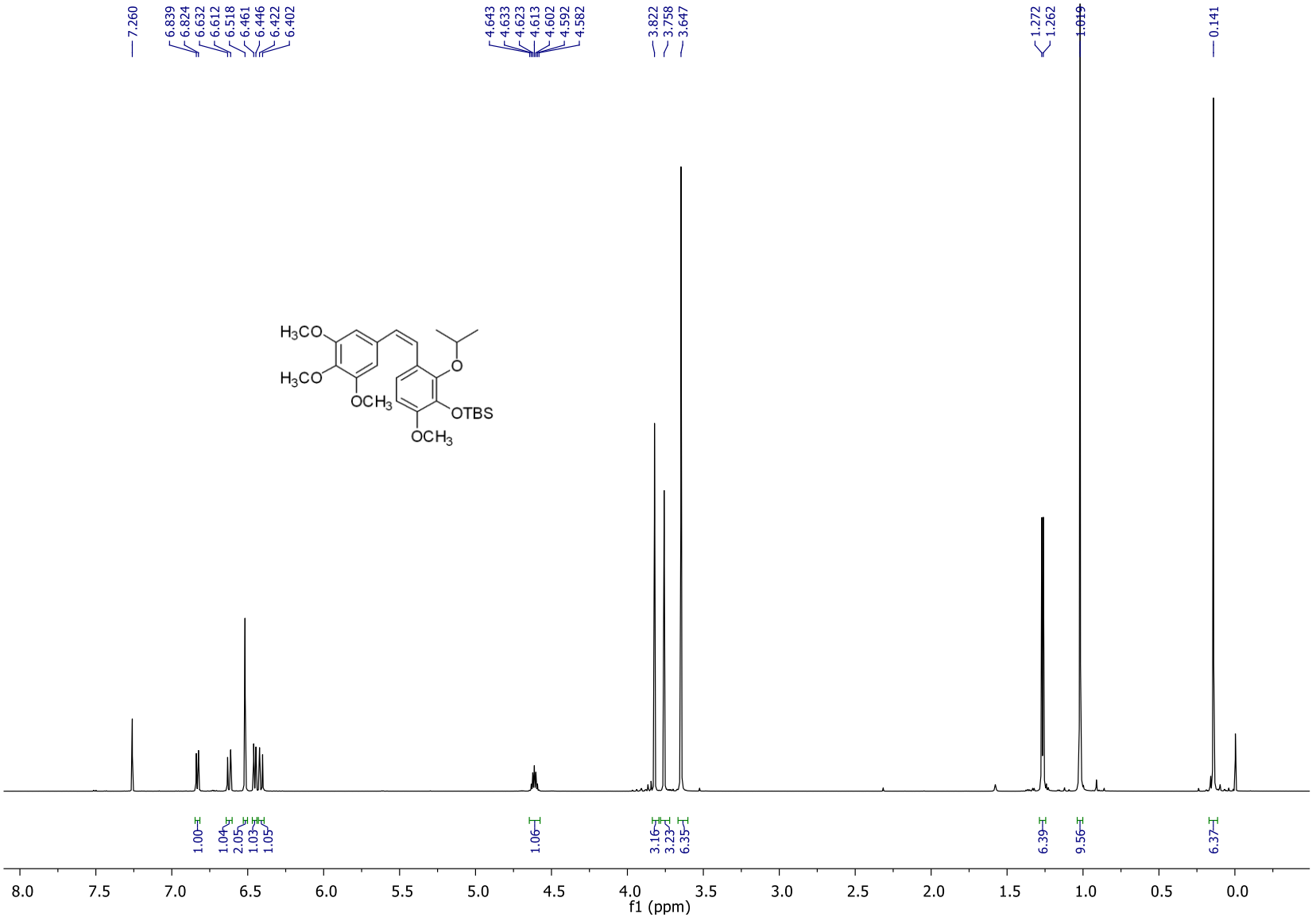
^{1}H NMR (500 MHz, $CDCl_3$) Compound **11**



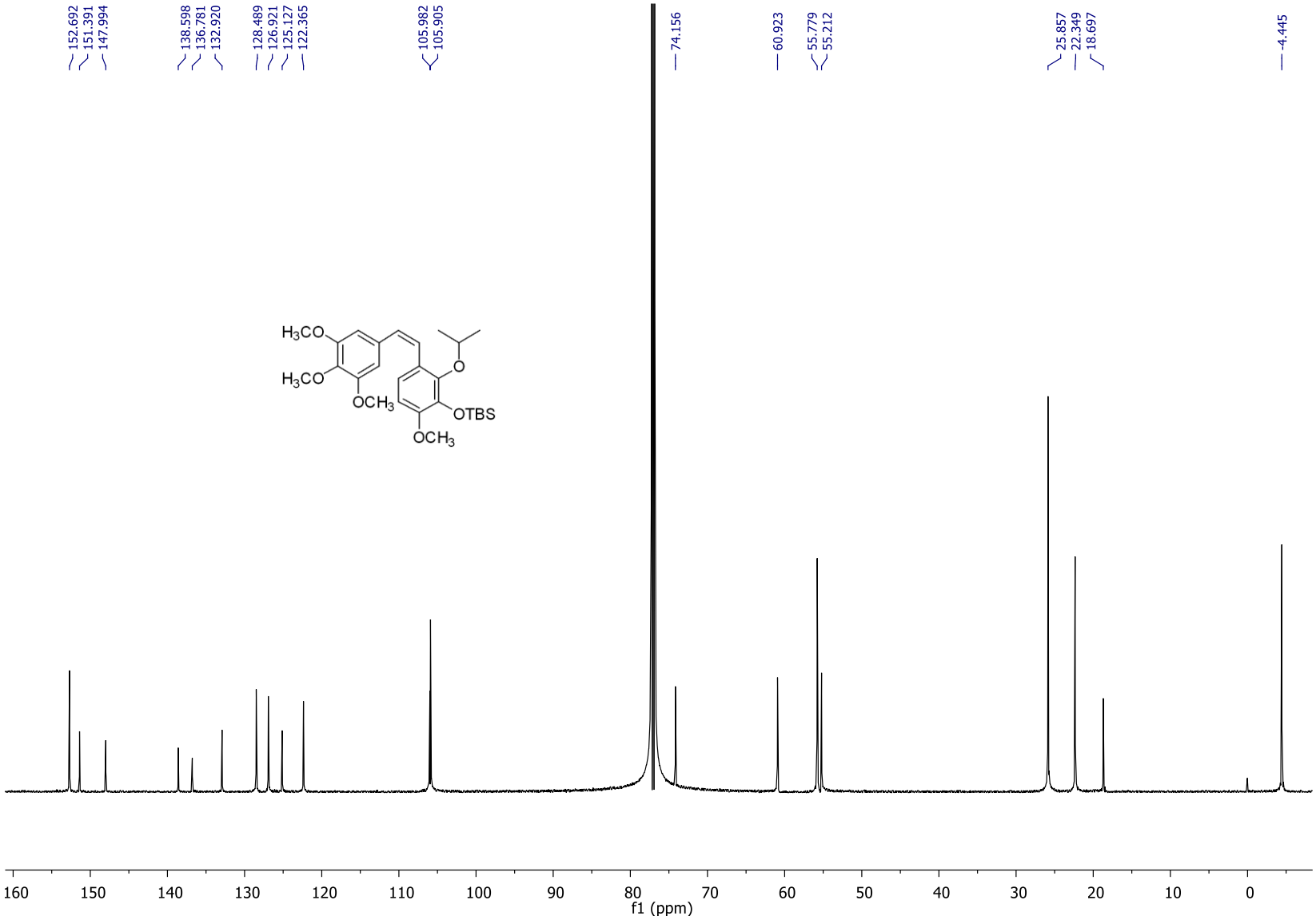
^{13}C NMR (126 MHz, $CDCl_3$) for Compound **11**



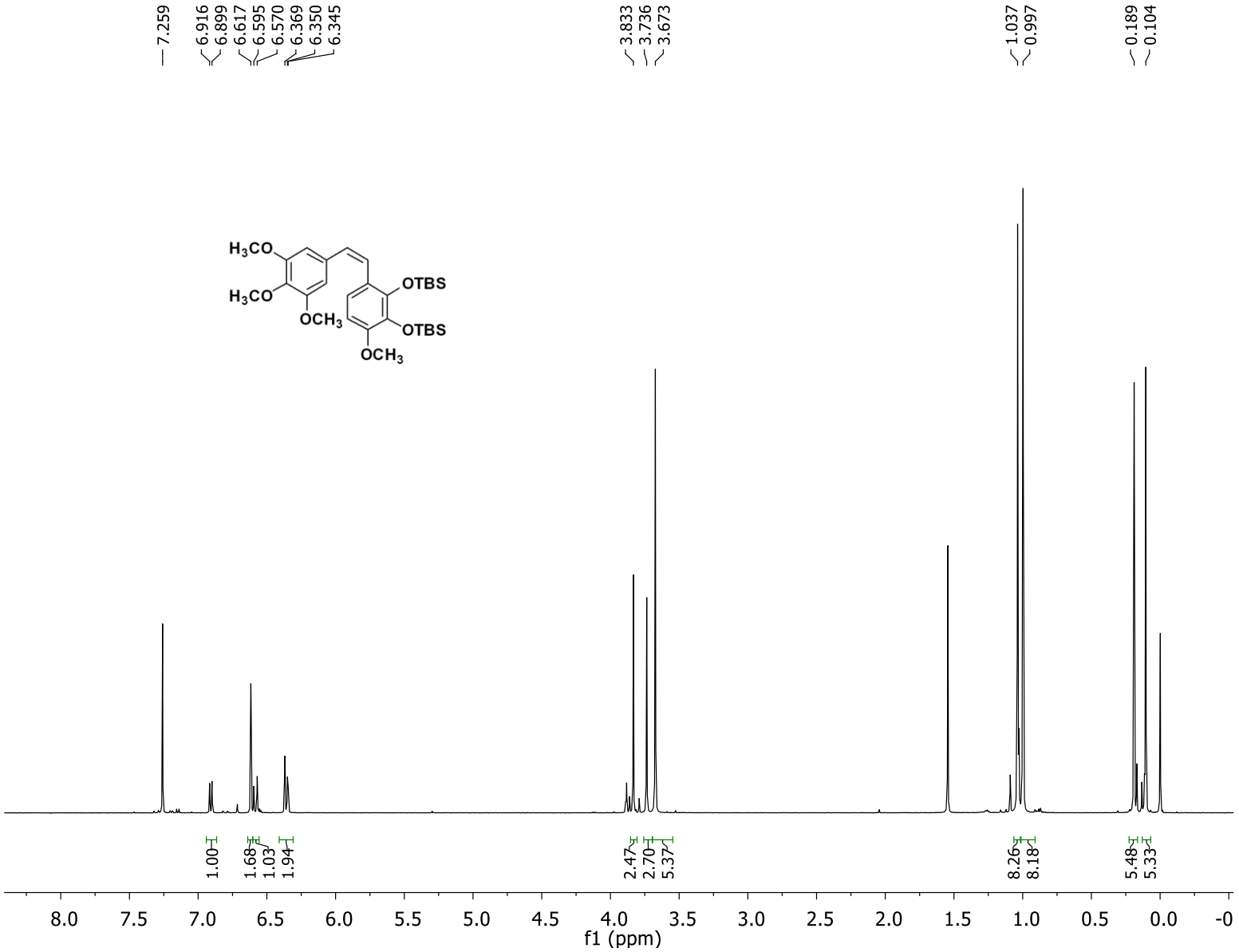
^{1}H NMR (600 MHz, $CDCl_3$) Compound **12**



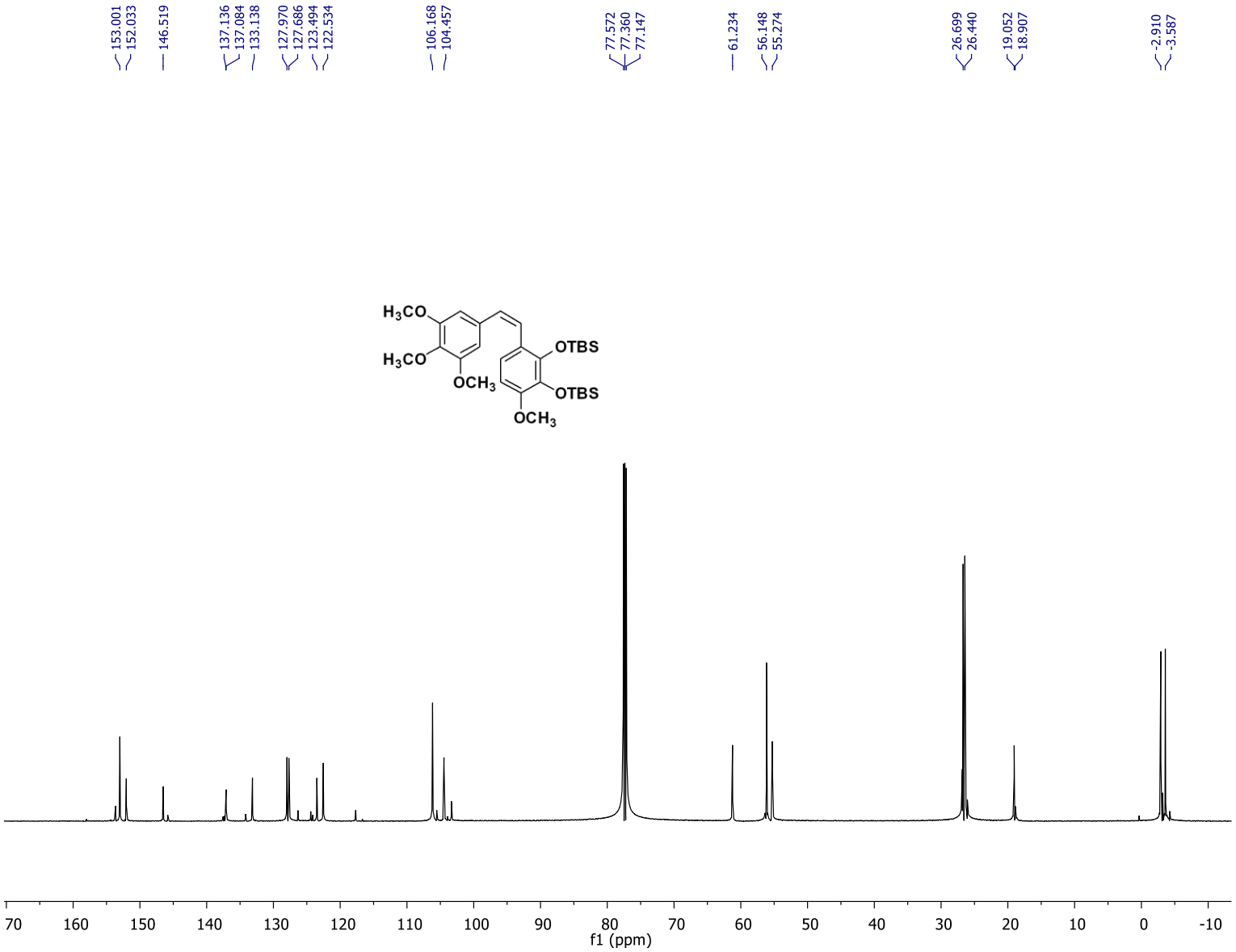
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **12**



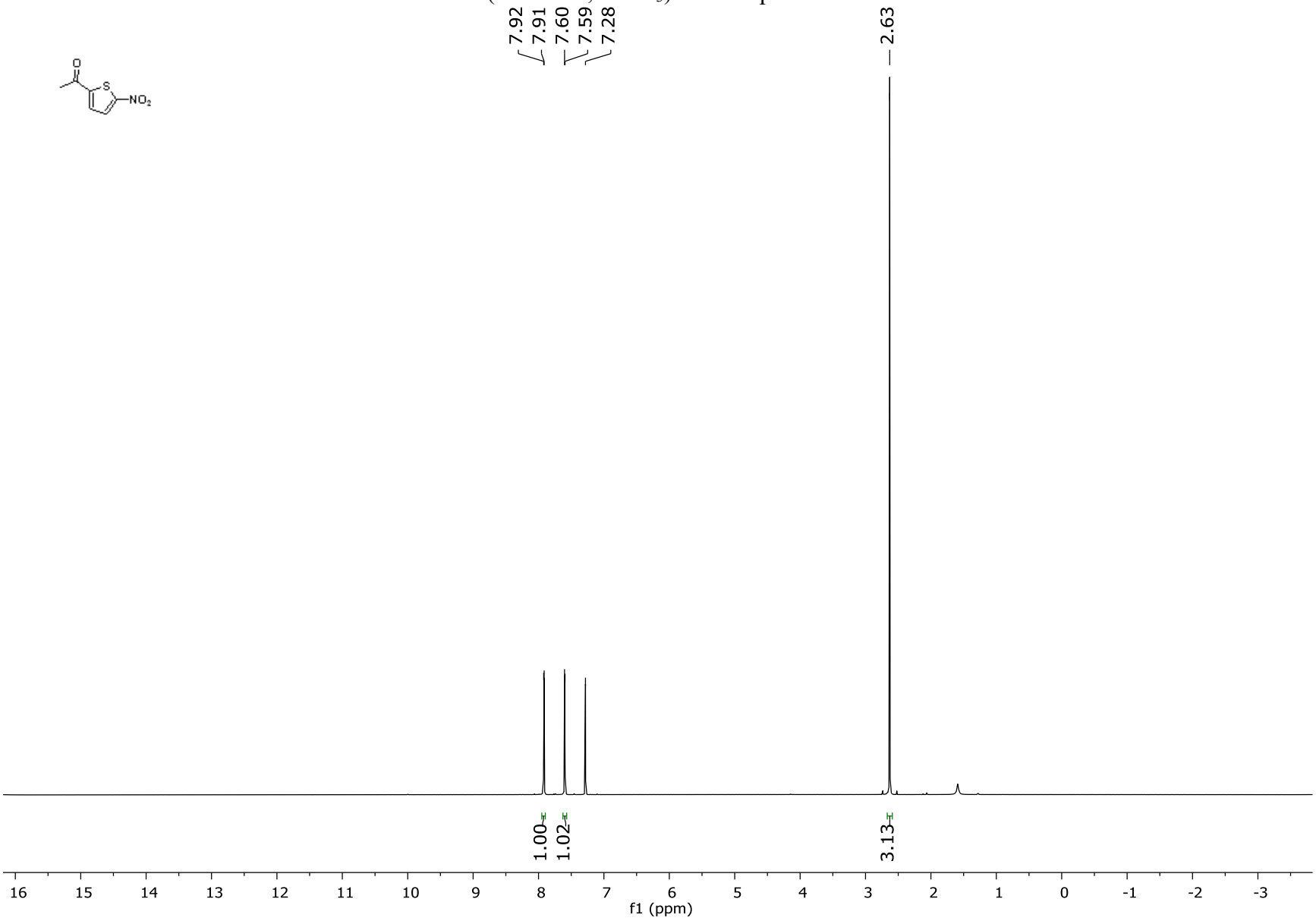
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **13**



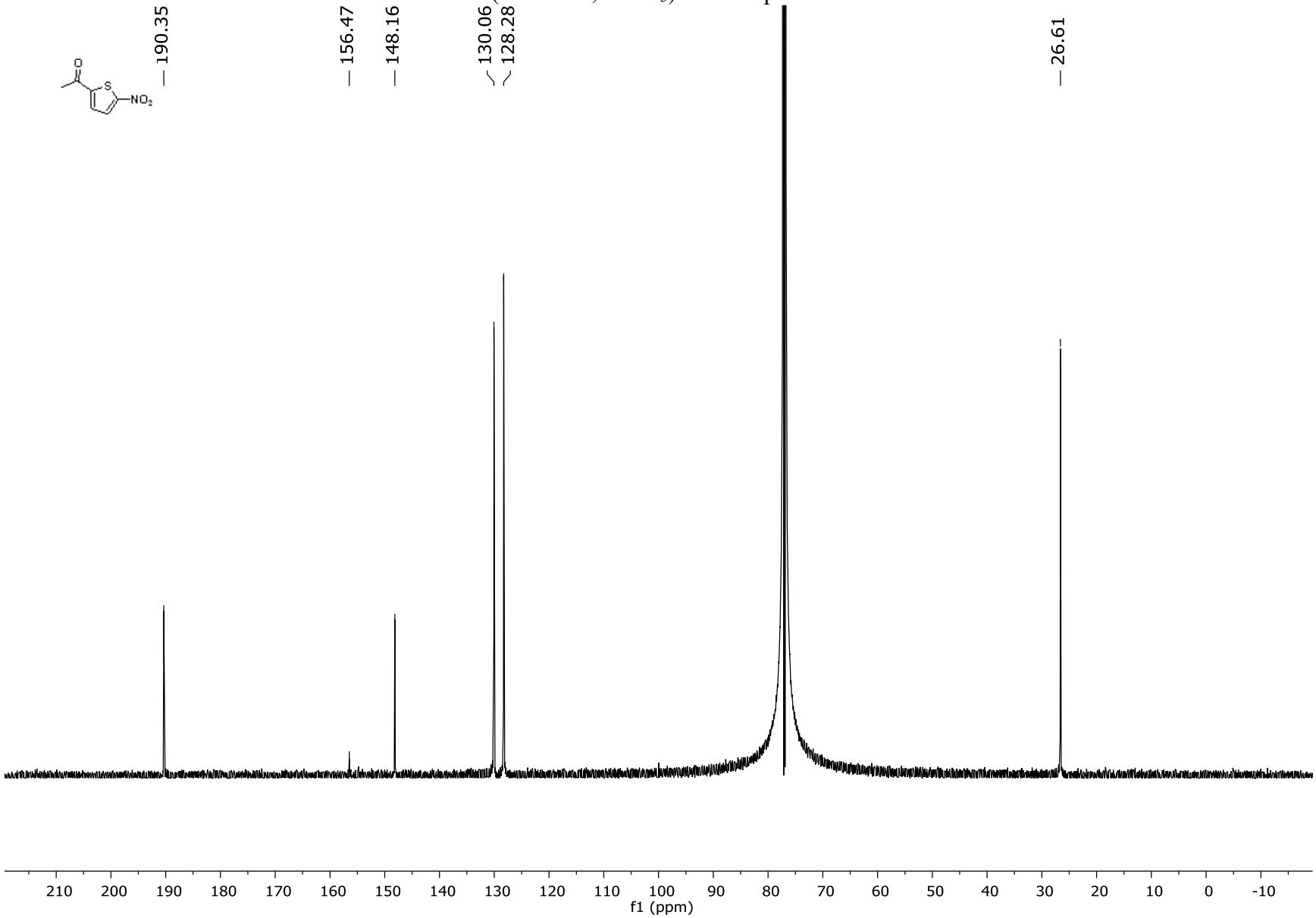
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **13**



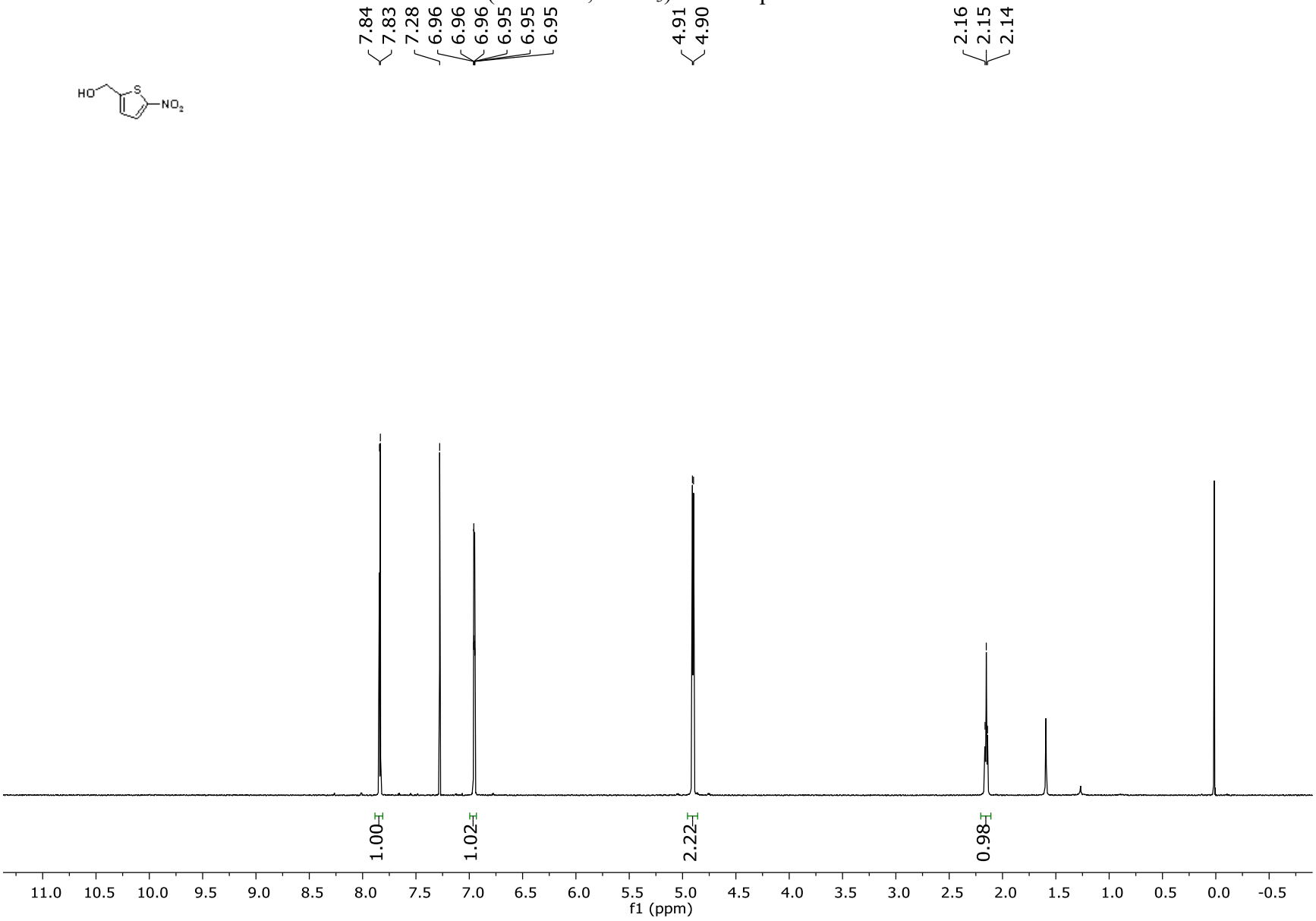
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **15**



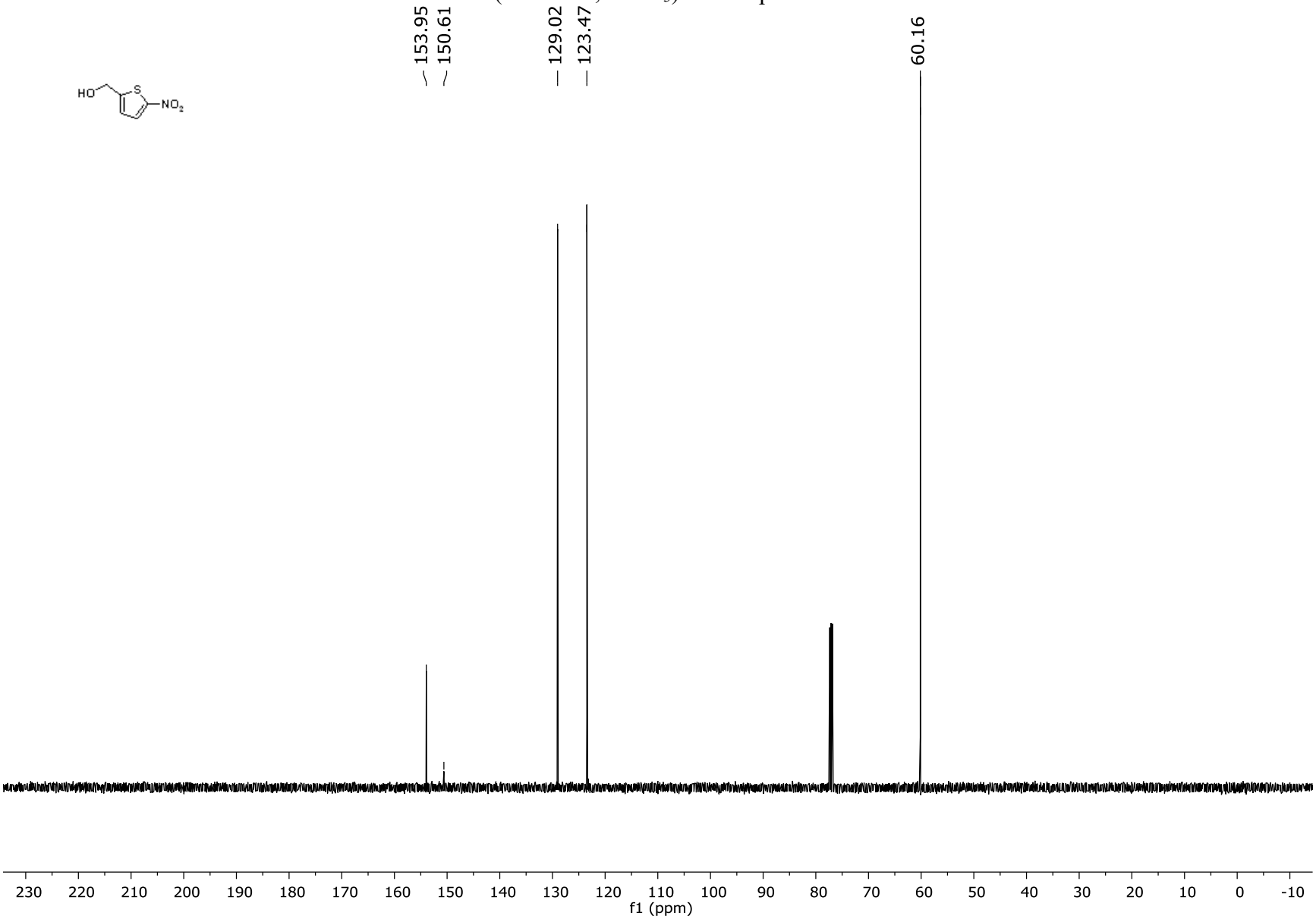
^{13}C NMR (500 MHz, $CDCl_3$) for Compound **15**



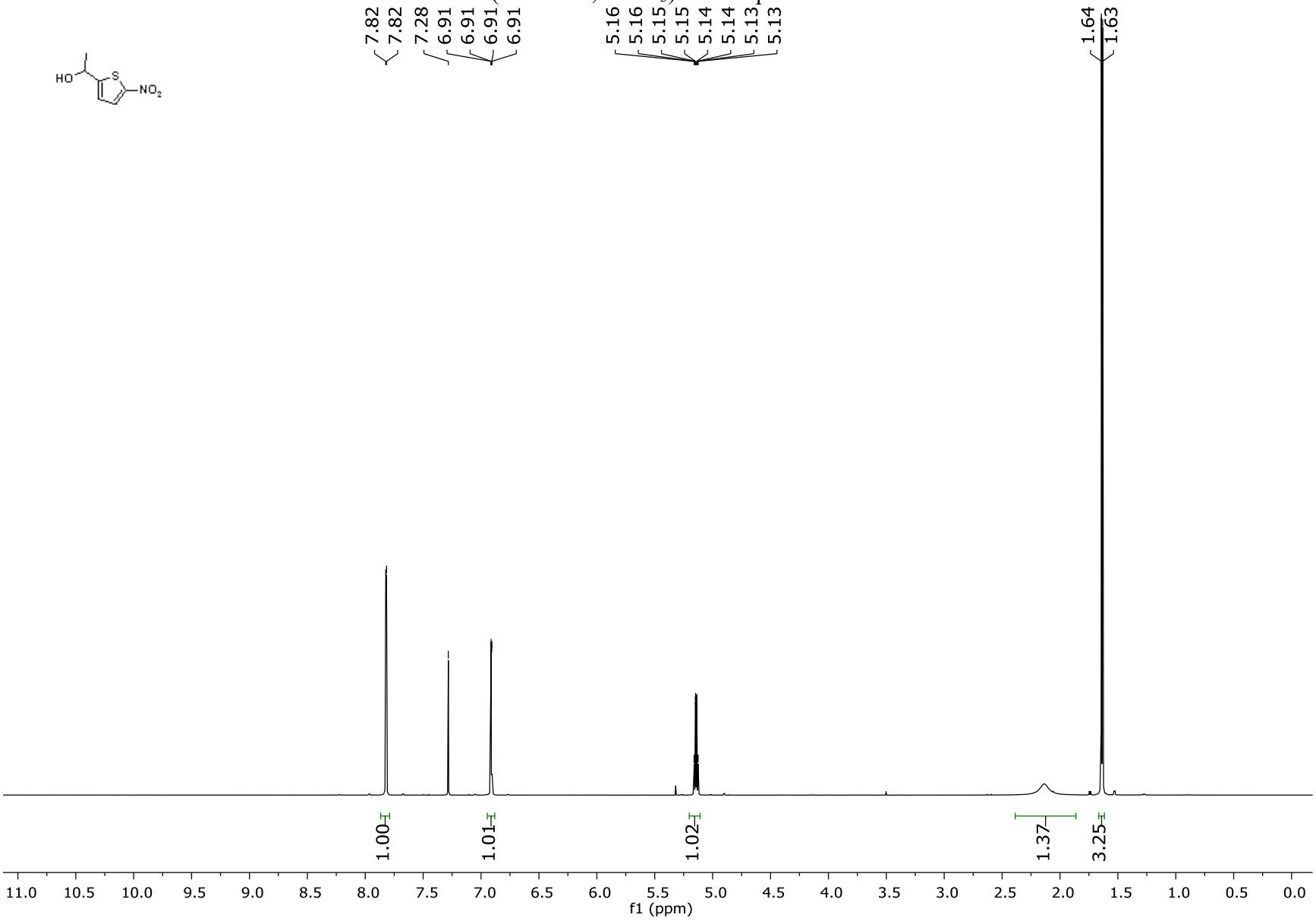
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **16**



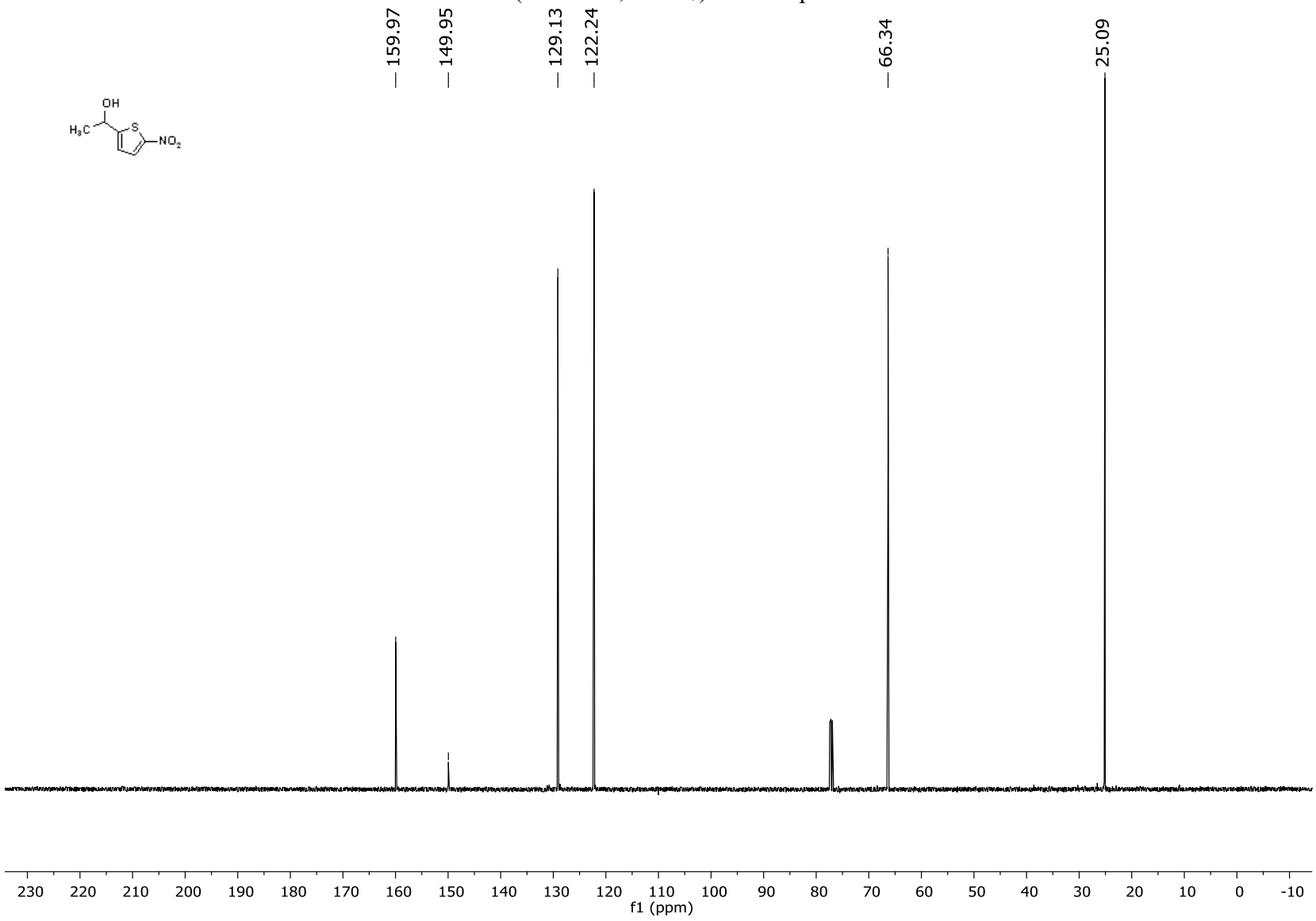
^{13}C NMR (500 MHz, $CDCl_3$) of Compound **16**



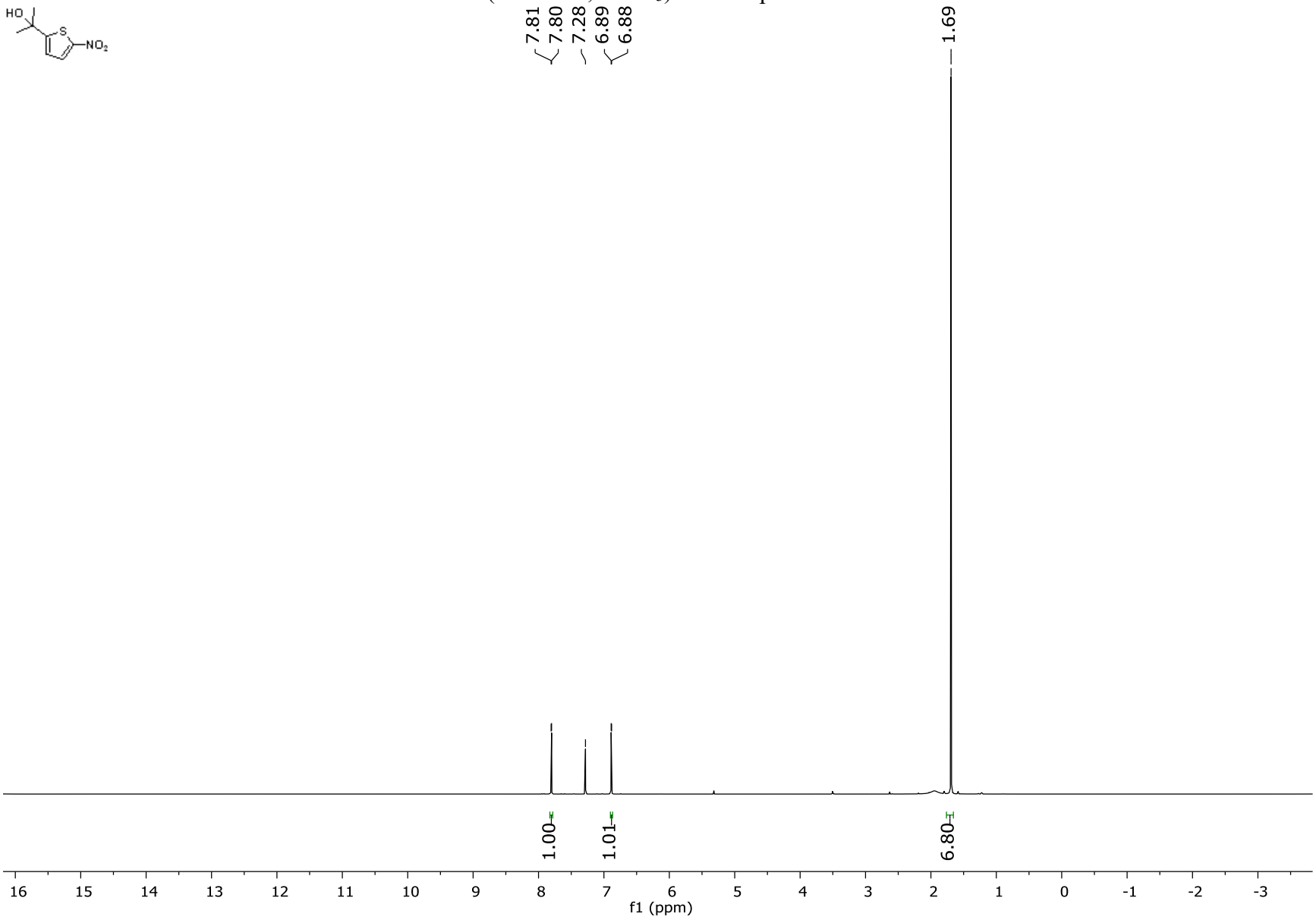
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **17**



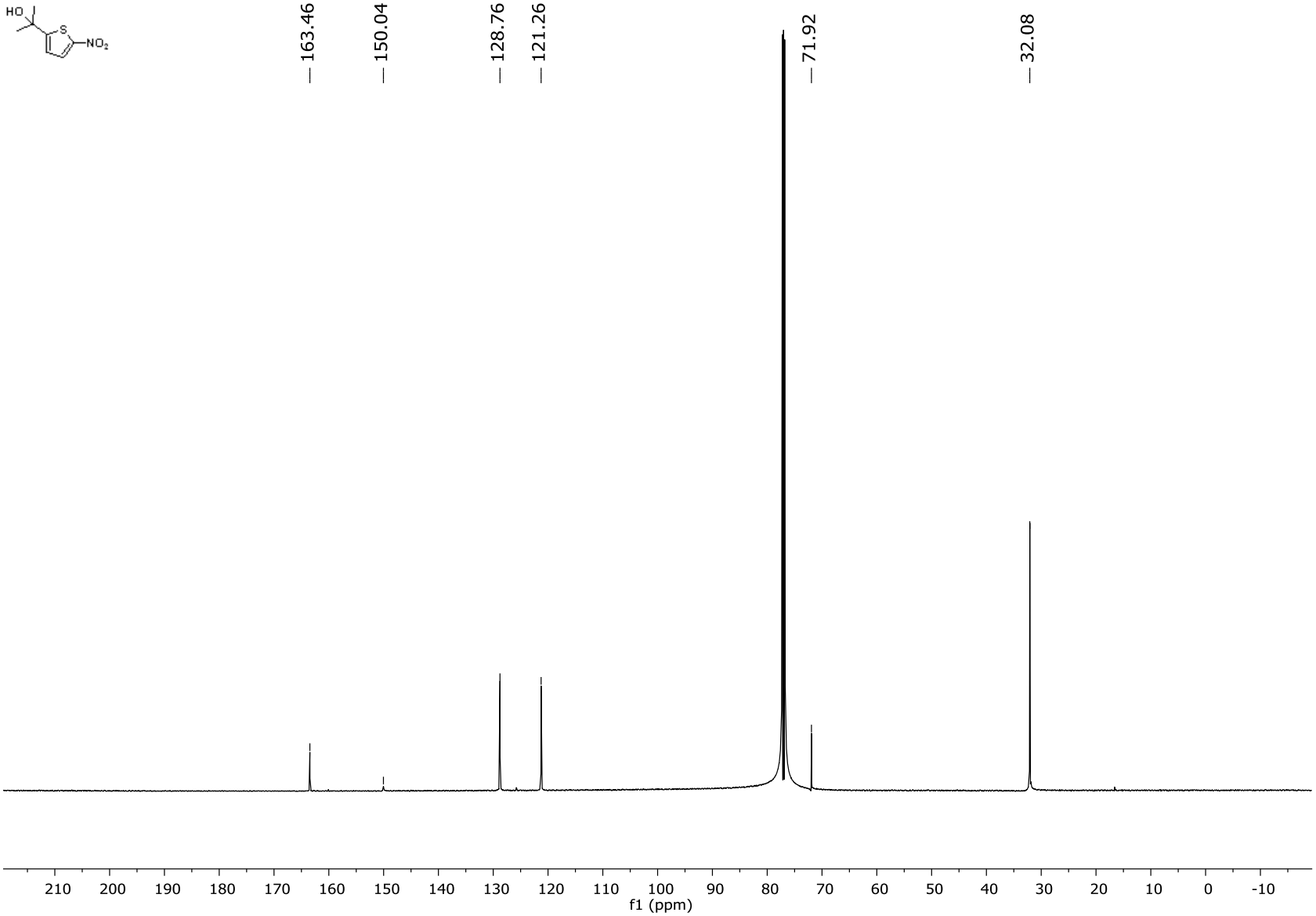
^{13}C NMR (500 MHz, $CDCl_3$) for Compound **17**



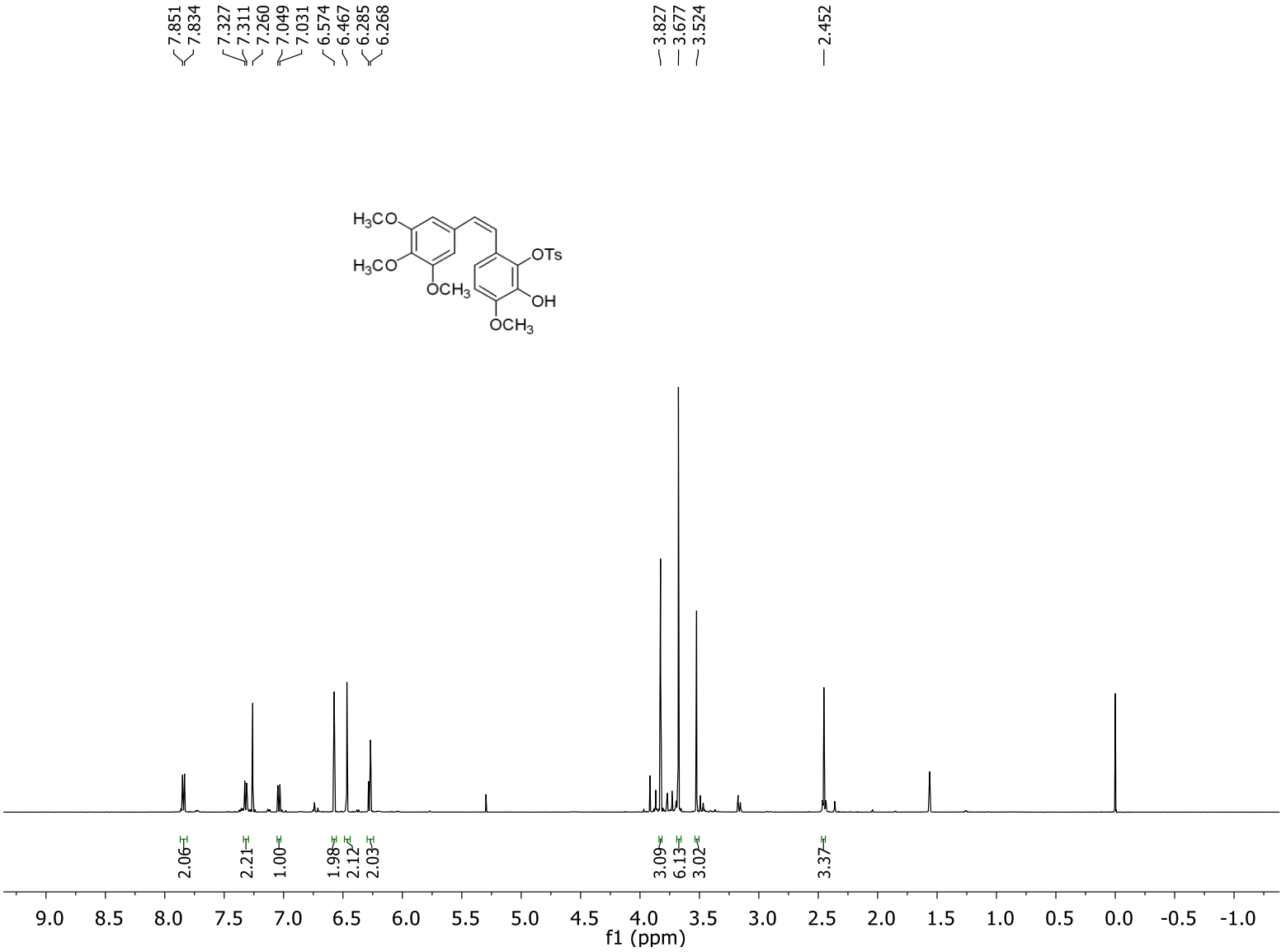
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **19**



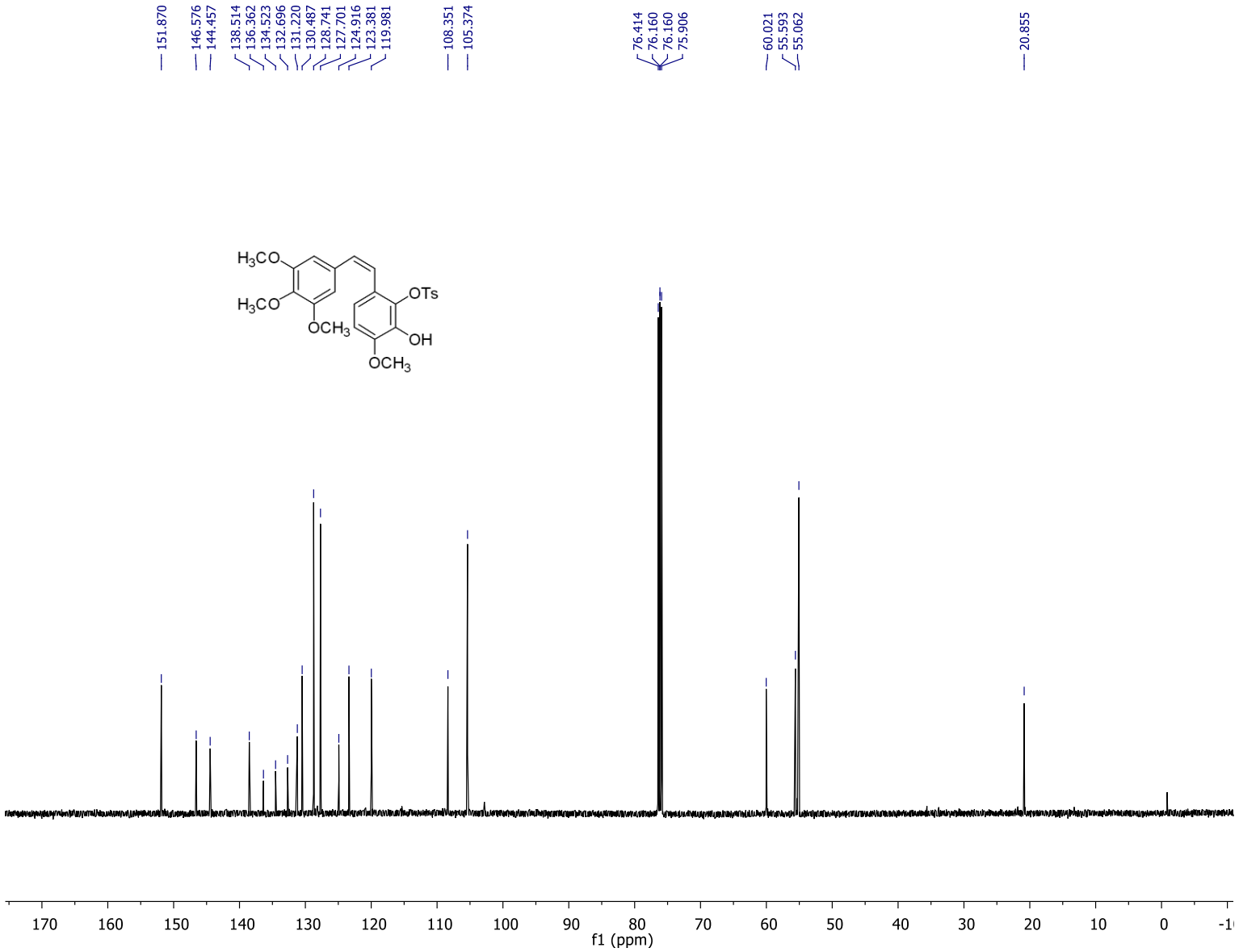
^{13}C NMR (500 MHz, $CDCl_3$) for Compound **19**



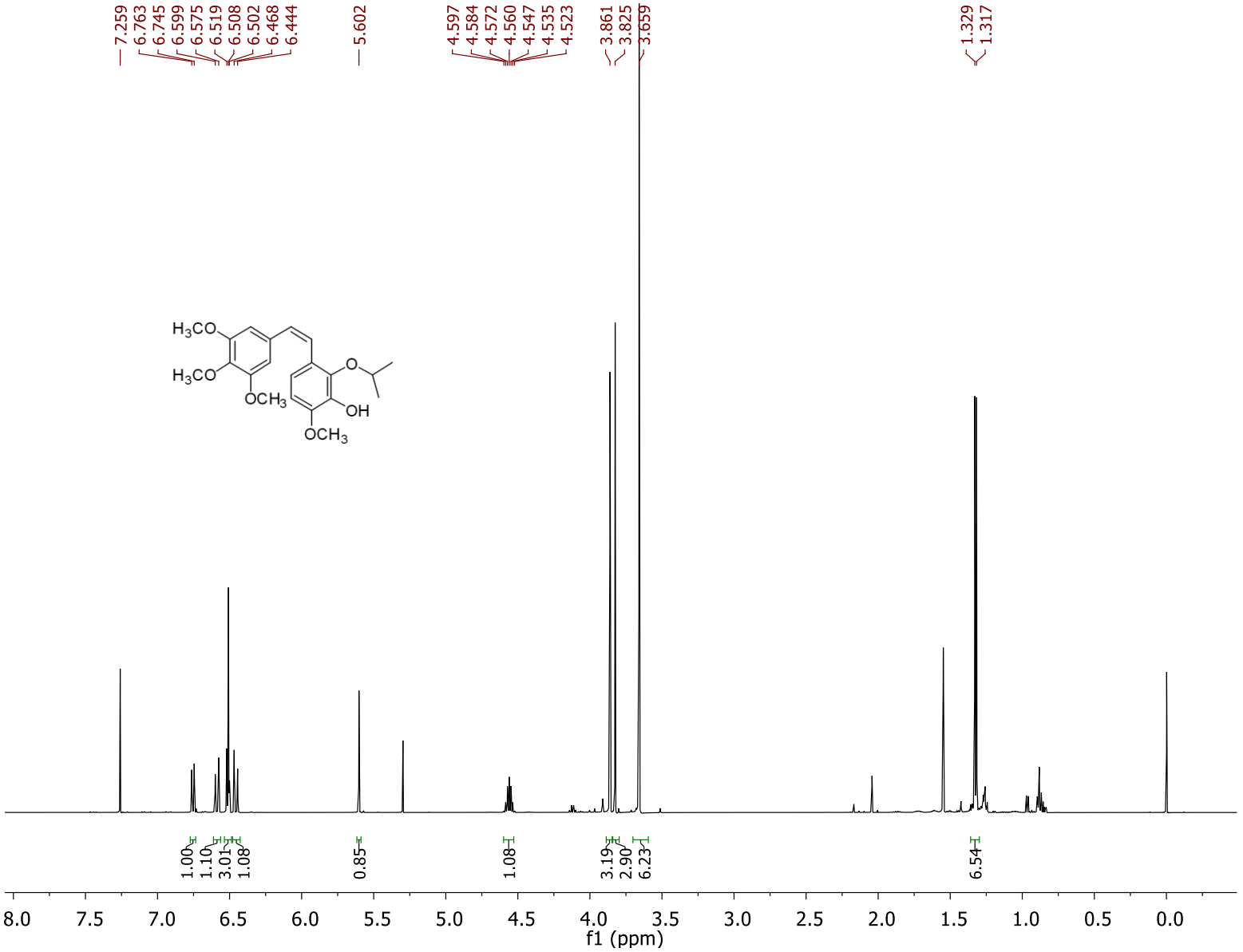
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **20**



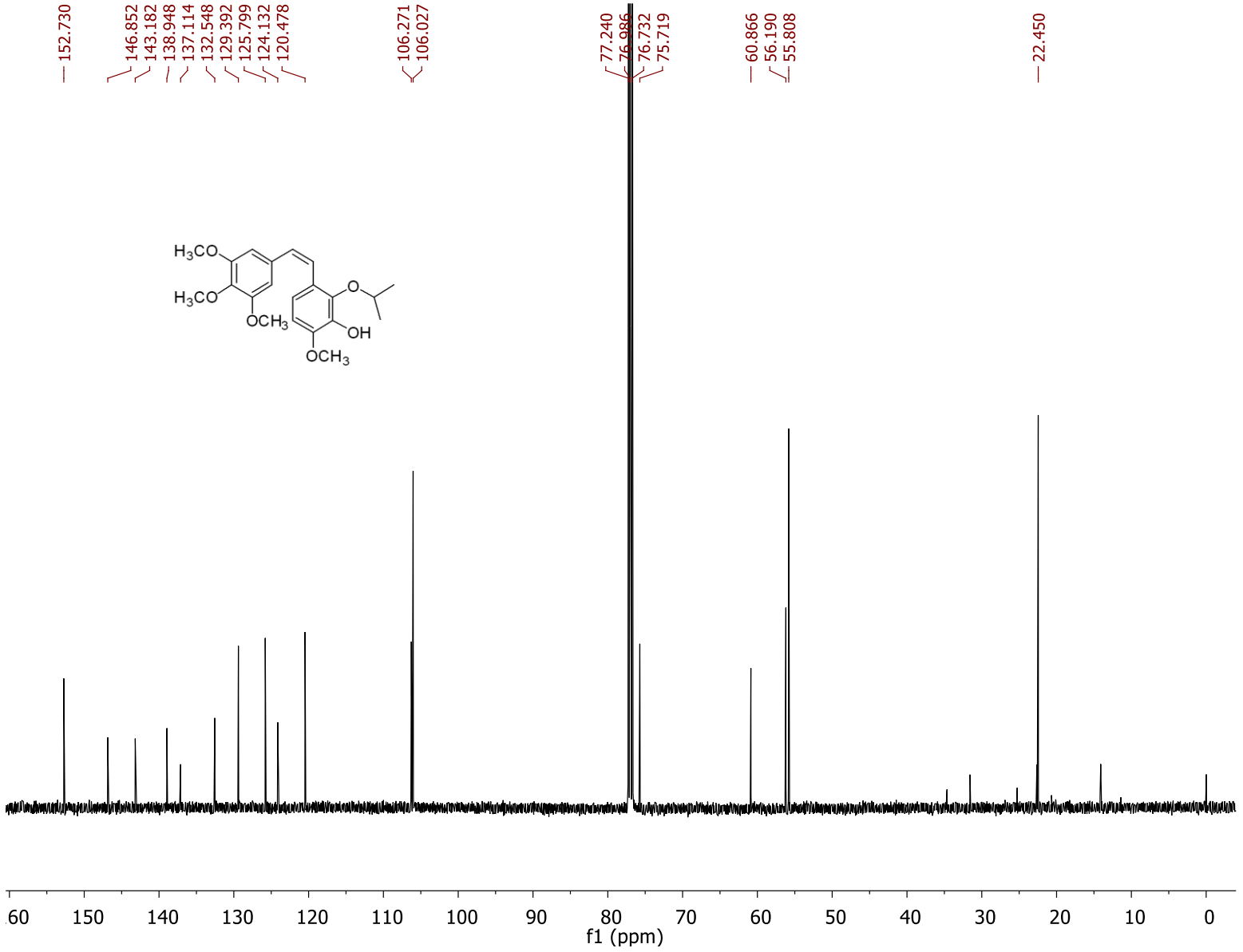
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **20**



^{1}H NMR (500 MHz, $CDCl_3$) for Compound **21**



^{13}C NMR (125 MHz, $CDCl_3$) for Compound **21**



HPLC trace of Compound **21**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-11-1A\LDV-11-1ARUN001.D
Sample Name: LD-V-11-1A-run1

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    Acq. Instrument : Instrument 1                    Location :    -
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    Acq. Method      : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed     : 2/4/2014 12:46:52 PM by Laxman
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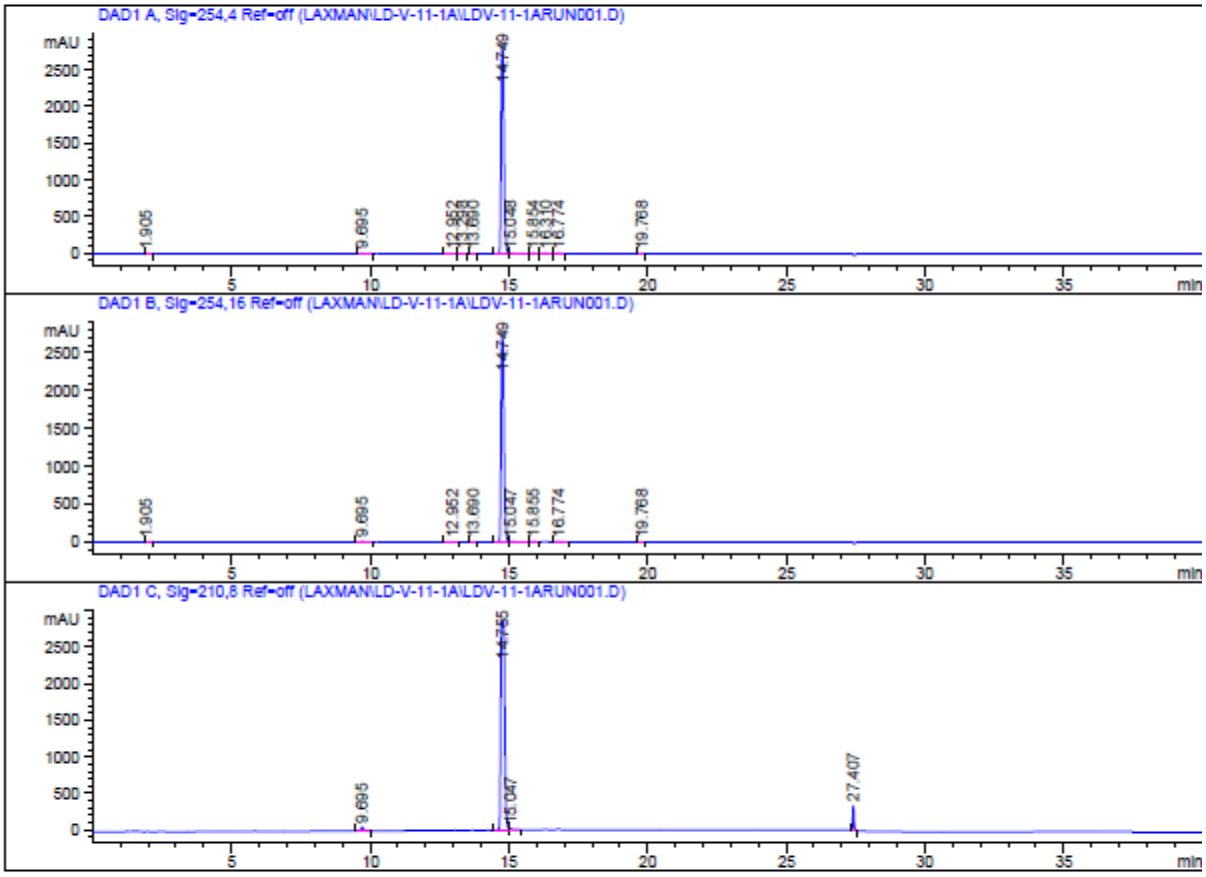

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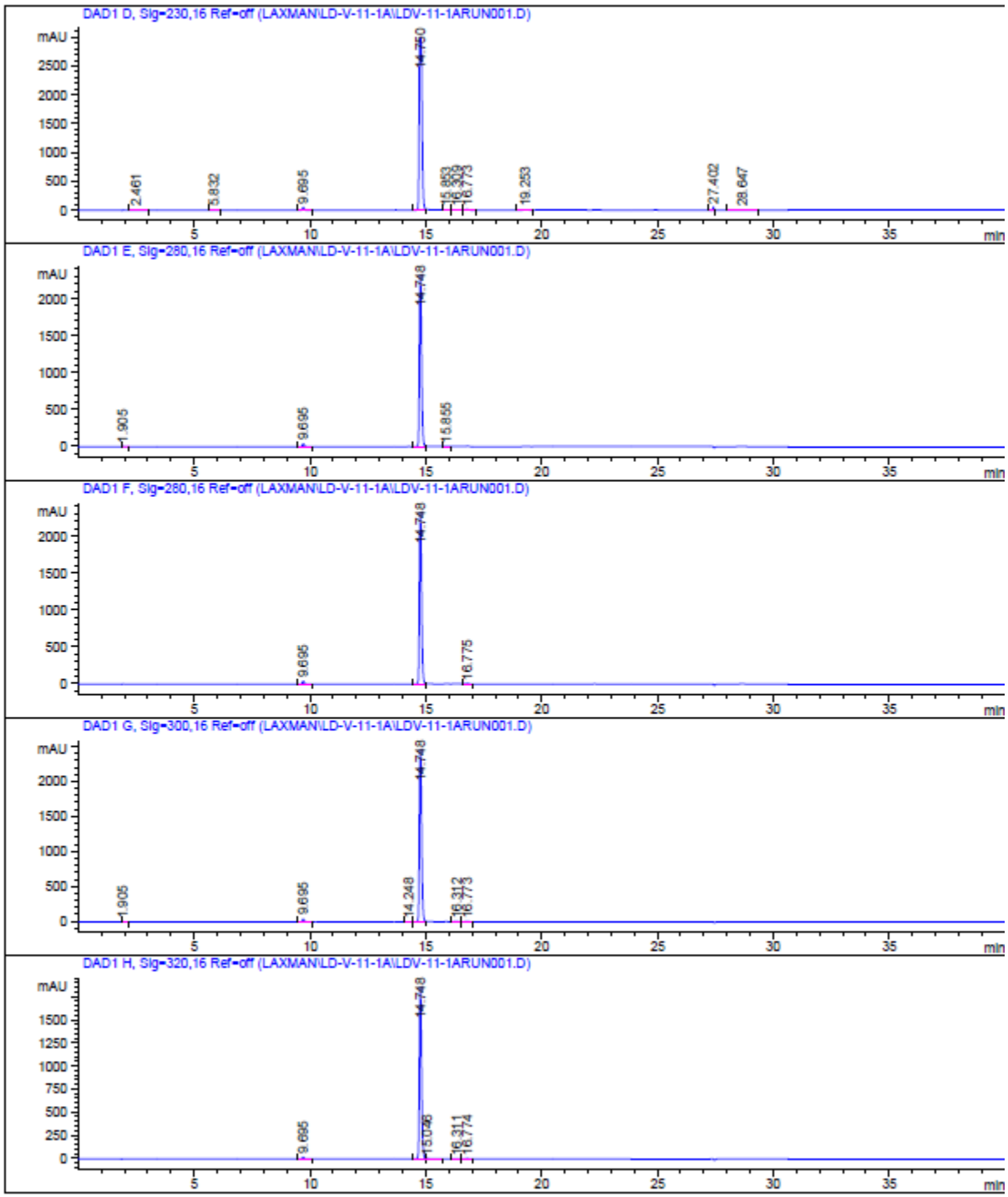
Sample Name: LD-V-11-1A-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-11-1A\LDV-11-1ARUN001.D
Sample Name: LD-V-11-1A-run1

==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.905	BB	0.1050	63.49230	8.32688	0.3077
2	9.695	BB	0.0838	39.29815	7.18572	0.1905
3	12.952	BV	0.1532	21.31435	1.92459	0.1033
4	13.298	VB	0.1364	20.15860	2.21022	0.0977
5	13.690	BB	0.0898	6.70567	1.15370	0.0325
6	14.749	BV	0.1128	2.02511e4	2852.08301	98.1487
7	15.048	VB	0.1430	86.96108	8.25082	0.4215
8	15.854	BV	0.1257	47.48036	5.67062	0.2301
9	16.310	VB	0.1784	36.28421	2.81407	0.1759
10	16.774	BB	0.1202	53.41854	6.91010	0.2589
11	19.768	BB	0.0976	6.86424	1.08825	0.0333
Totals :				2.06331e4	2897.61796	

Signal 2: DAD1 B, Sig=254,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.905	BB	0.1050	62.44164	8.18640	0.3122
2	9.695	BB	0.0836	61.18841	11.22495	0.3060
3	12.952	BV	0.1563	20.12209	1.77477	0.1006
4	13.690	BB	0.0892	6.66208	1.15644	0.0333
5	14.749	BV	0.1127	1.96340e4	2770.85913	98.1825
6	15.047	VB	0.1425	84.68669	8.06554	0.4235
7	15.855	BV	0.1314	54.17786	6.11229	0.2709
8	16.774	VB	0.1354	67.86295	7.50964	0.3394
9	19.768	BB	0.0975	6.31473	1.00224	0.0316
Totals :				1.99975e4	2815.89140	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-11-1A\LDV-11-1ARUN001.D
Sample Name: LD-V-11-1A-run1

Signal 3: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.695	BB	0.0833	279.00644	51.44276	0.9452
2	14.755	BV	0.1586	2.80721e4	2858.40137	95.1058
3	15.047	VB	0.1125	153.97672	19.41314	0.5217
4	27.407	BB	0.0490	1011.62750	337.22437	3.4273
Totals :				2.95167e4	3266.48163	

Signal 4: DAD1 D, Sig=230,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.461	BB	0.1822	14.68391	1.06963	0.0556
2	5.832	BB	0.0800	11.26862	2.11878	0.0427
3	9.695	BB	0.0838	260.12378	47.56544	0.9848
4	14.750	BV	0.1376	2.56213e4	3001.83618	96.9993
5	15.853	BB	0.1186	20.32653	2.61849	0.0770
6	16.309	BV	0.2044	92.46318	6.11626	0.3501
7	16.773	VB	0.1353	111.28555	12.33039	0.4213
8	19.253	BB	0.1732	14.00256	1.08039	0.0530
9	27.402	BB	0.0492	191.40921	60.00833	0.7247
10	28.647	BB	0.4277	77.03762	2.63050	0.2917
Totals :				2.64139e4	3137.37440	

Signal 5: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.905	BB	0.1052	49.02067	6.41397	0.3222
2	9.695	BB	0.0835	215.97261	39.69125	1.4193
3	14.748	VV	0.0984	1.49142e4	2339.12183	98.0145
4	15.855	BV	0.1255	37.12501	4.44043	0.2440
Totals :				1.52163e4	2389.66748	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-11-1A\LDV-11-1ARUN001.D
Sample Name: LD-V-11-1A-run1

Signal 6: DAD1 F, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.695	BB	0.0835	215.97261	39.69125	1.4225
2	14.748	VV	0.0984	1.49142e4	2339.12183	98.2322
3	16.775	BB	0.1217	52.42577	6.66968	0.3453
Totals :				1.51826e4	2385.48276	

Signal 7: DAD1 G, Sig=300,16 Ref=off

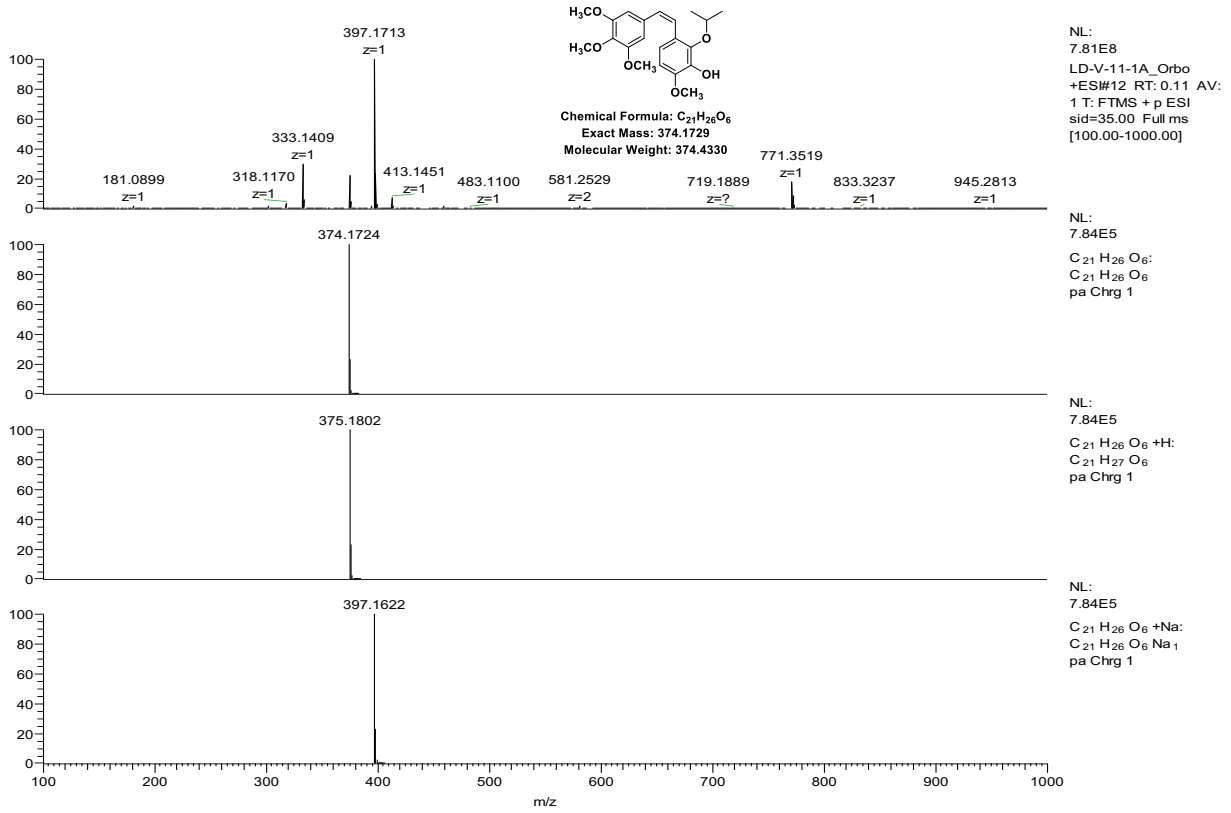
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.905	BB	0.1016	36.36117	4.95634	0.2178
2	9.695	BB	0.0835	209.04446	38.38157	1.2521
3	14.248	BB	0.1195	9.78438	1.27535	0.0586
4	14.748	BV	0.1033	1.63664e4	2471.26636	98.0315
5	16.312	BB	0.1665	21.17754	1.75824	0.1268
6	16.773	BB	0.1221	52.27137	6.61828	0.3131
Totals :				1.66951e4	2524.25614	

Signal 8: DAD1 H, Sig=320,16 Ref=off

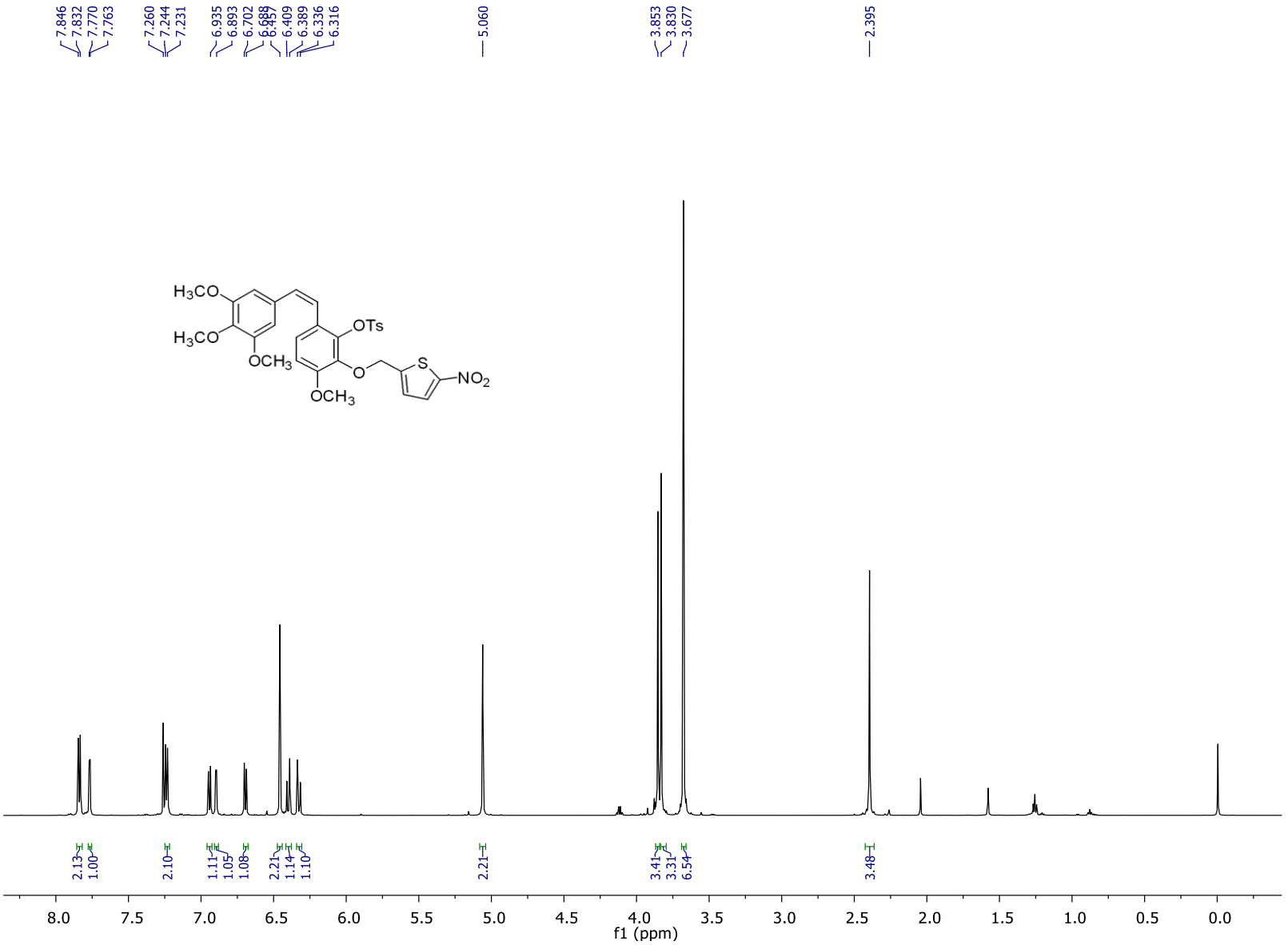
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.695	BB	0.0836	103.53847	18.99932	0.8508
2	14.748	BV	0.0987	1.19672e4	1867.92029	98.3422
3	15.046	VV	0.1531	53.64690	4.63198	0.4409
4	16.311	BB	0.1646	11.93031	1.00390	0.0980
5	16.774	BB	0.1222	32.61986	4.12577	0.2681
Totals :				1.21689e4	1896.68126	

*** End of Report ***

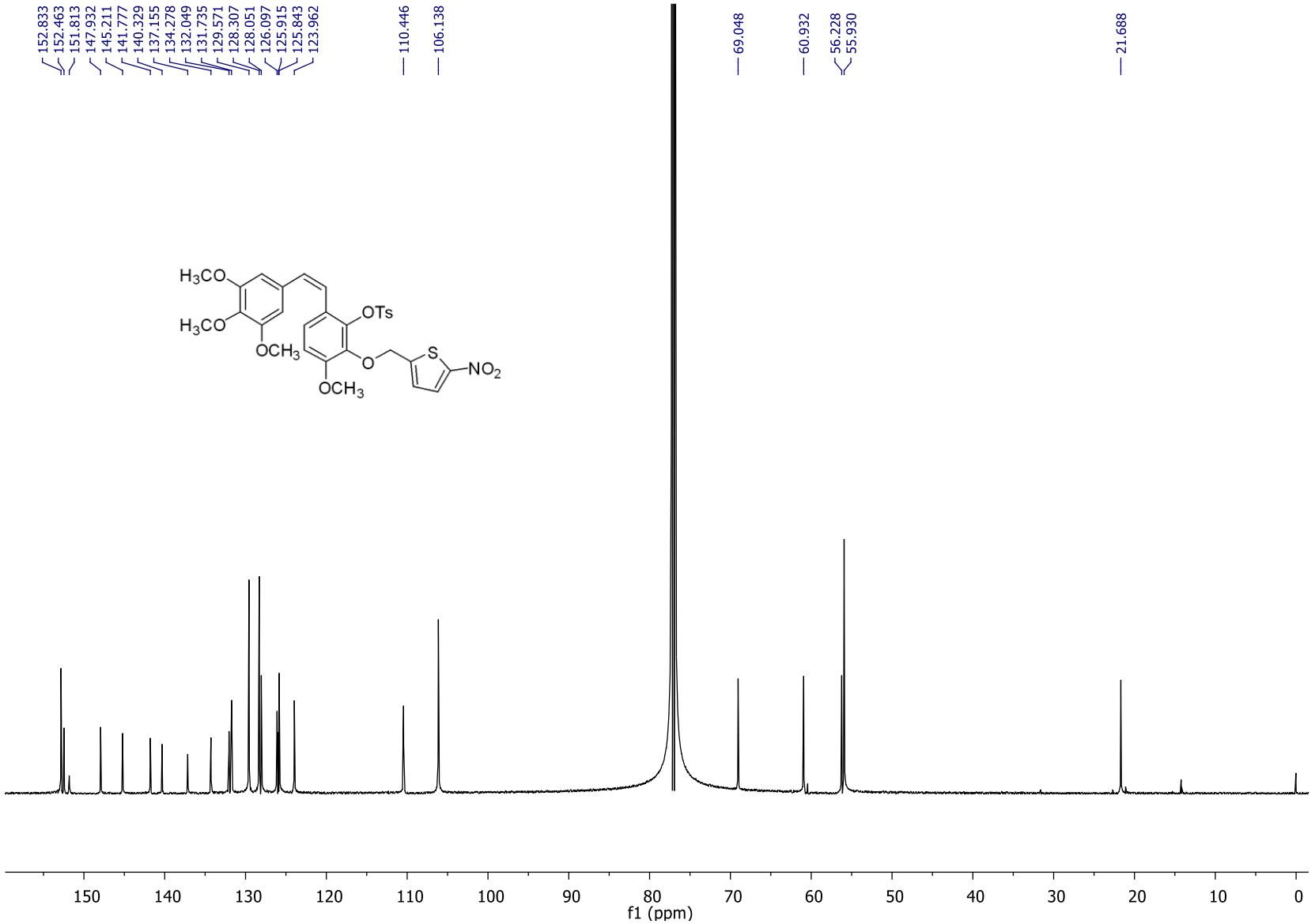
HRMS Traces of Compound **21**



^{1}H NMR (600 MHz, $CDCl_3$) for Compound **22**



^{13}C NMR (151 MHz, $CDCl_3$) for Compound **22**



HPLC Traces of Compound **22**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D
Sample Name: LD-III-43-1A-rerun-run1

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    Acq. Instrument : Instrument 1                    Location :    -
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    Acq. Method      : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed     : 2/20/2013 10:20:33 AM by Laxman
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    Last changed     : 2/20/2013 1:53:17 PM by Laxman
                      (modified after loading)
    Sample Info      : run1
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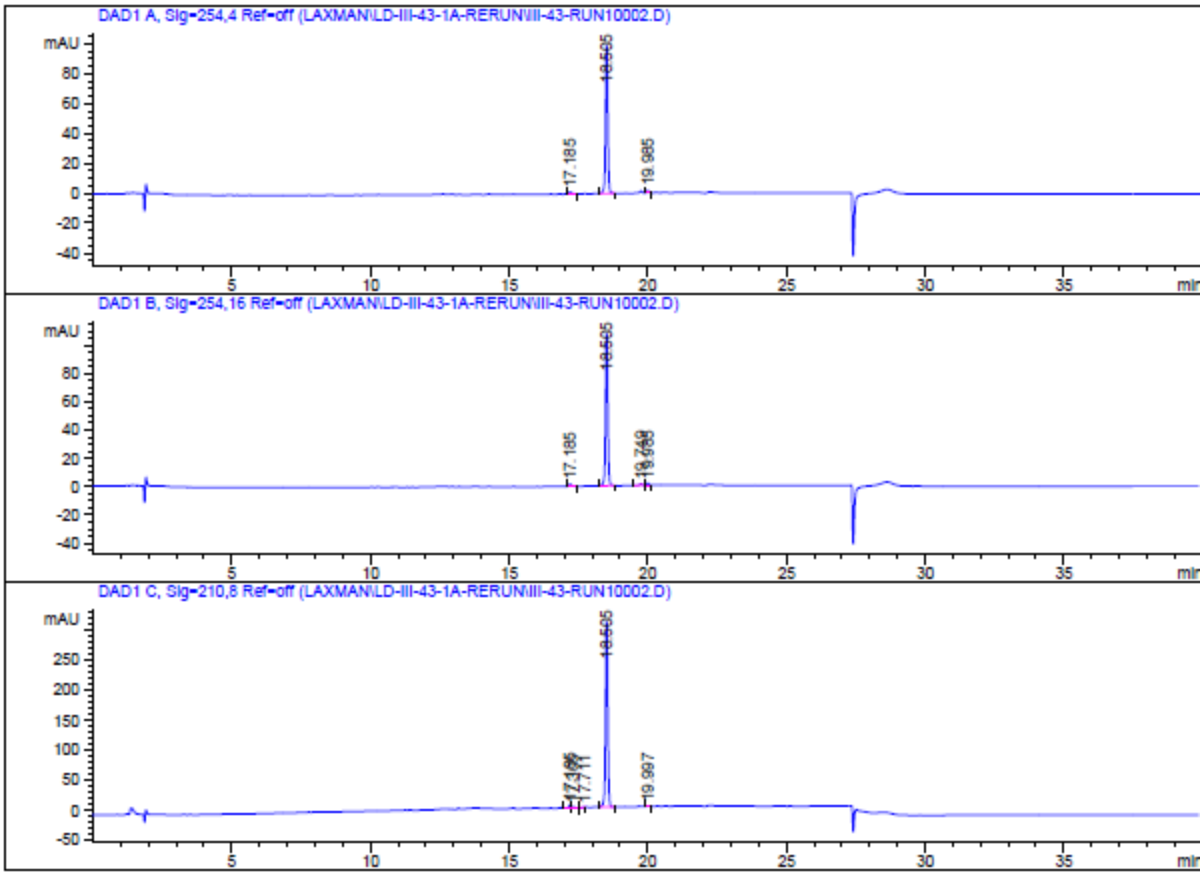
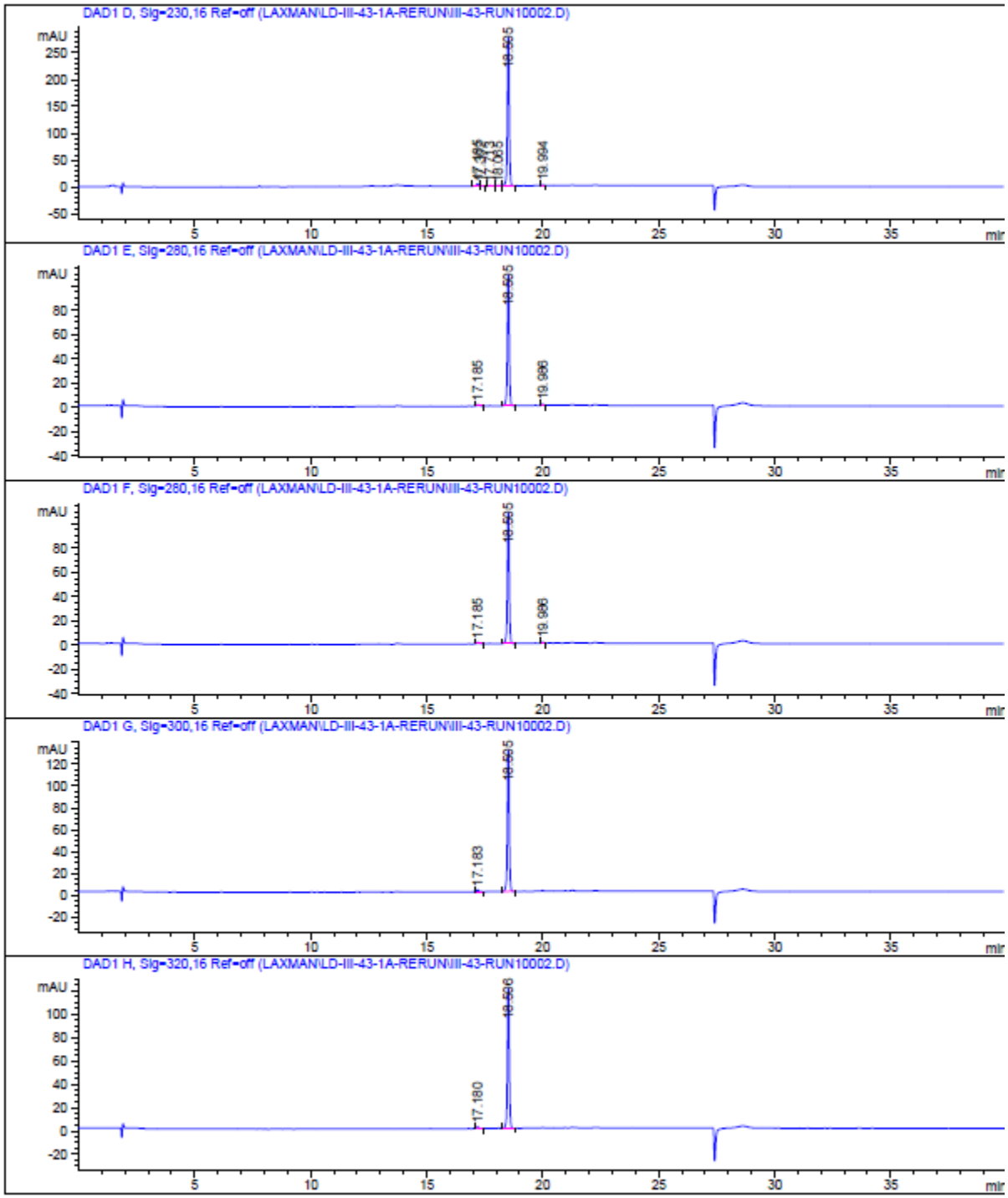


Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D
Sample Name: LD-III-43-1A-rerun-run1



Instrument 1 2/20/2013 1:55:35 PM Laxman Page 2 of 5

Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D
Sample Name: LD-III-43-1A-rerun-run1

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.185	BB	0.0973	9.40742	1.41932	1.6521
2	18.505	BB	0.0847	552.32959	99.64810	96.9969
3	19.985	BB	0.0787	7.69327	1.52861	1.3510
Totals :				569.43028	102.59603	

Signal 2: DAD1 B, Sig=254,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.185	BB	0.0975	10.34489	1.55665	1.6471
2	18.505	BB	0.0846	601.68658	108.63660	95.8011
3	19.749	BB	0.0994	8.24213	1.21077	1.3123
4	19.985	BB	0.0792	7.78437	1.53429	1.2394
Totals :				628.05797	112.93830	

Signal 3: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.185	VV	0.0913	30.09891	4.92847	1.6977
2	17.305	VB	0.0784	7.90903	1.47888	0.4461
3	17.711	BV	0.0716	5.09792	1.07028	0.2875
4	18.505	VB	0.0846	1723.29053	311.30359	97.2001
5	19.997	BB	0.0837	6.53474	1.23493	0.3686
Totals :				1772.93112	320.01615	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D
Sample Name: LD-III-43-1A-rerun-run1

Signal 4: DAD1 D, Sig=230,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.185	BV	0.0911	28.28330	4.63913	1.7630
2	17.302	VB	0.0714	6.18744	1.25790	0.3857
3	17.713	BV	0.1039	7.61578	1.03443	0.4747
4	18.065	VV	0.1511	10.39987	1.01869	0.6482
5	18.505	VB	0.0846	1544.25427	278.70560	96.2568
6	19.994	BB	0.0834	7.56541	1.43652	0.4716
Totals :				1604.30607	288.09227	

Signal 5: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.185	BB	0.0998	9.16147	1.33870	1.4730
2	18.505	BB	0.0851	607.04138	108.86829	97.6000
3	19.986	BB	0.0793	5.76582	1.13497	0.9270
Totals :				621.96867	111.34196	

Signal 6: DAD1 F, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.185	BB	0.0998	9.16147	1.33870	1.4730
2	18.505	BB	0.0851	607.04138	108.86829	97.6000
3	19.986	BB	0.0793	5.76582	1.13497	0.9270
Totals :				621.96867	111.34196	

Signal 7: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.183	BB	0.0982	10.97565	1.63835	1.4851
2	18.505	BB	0.0854	728.06720	129.90228	98.5149
Totals :				739.04285	131.54064	

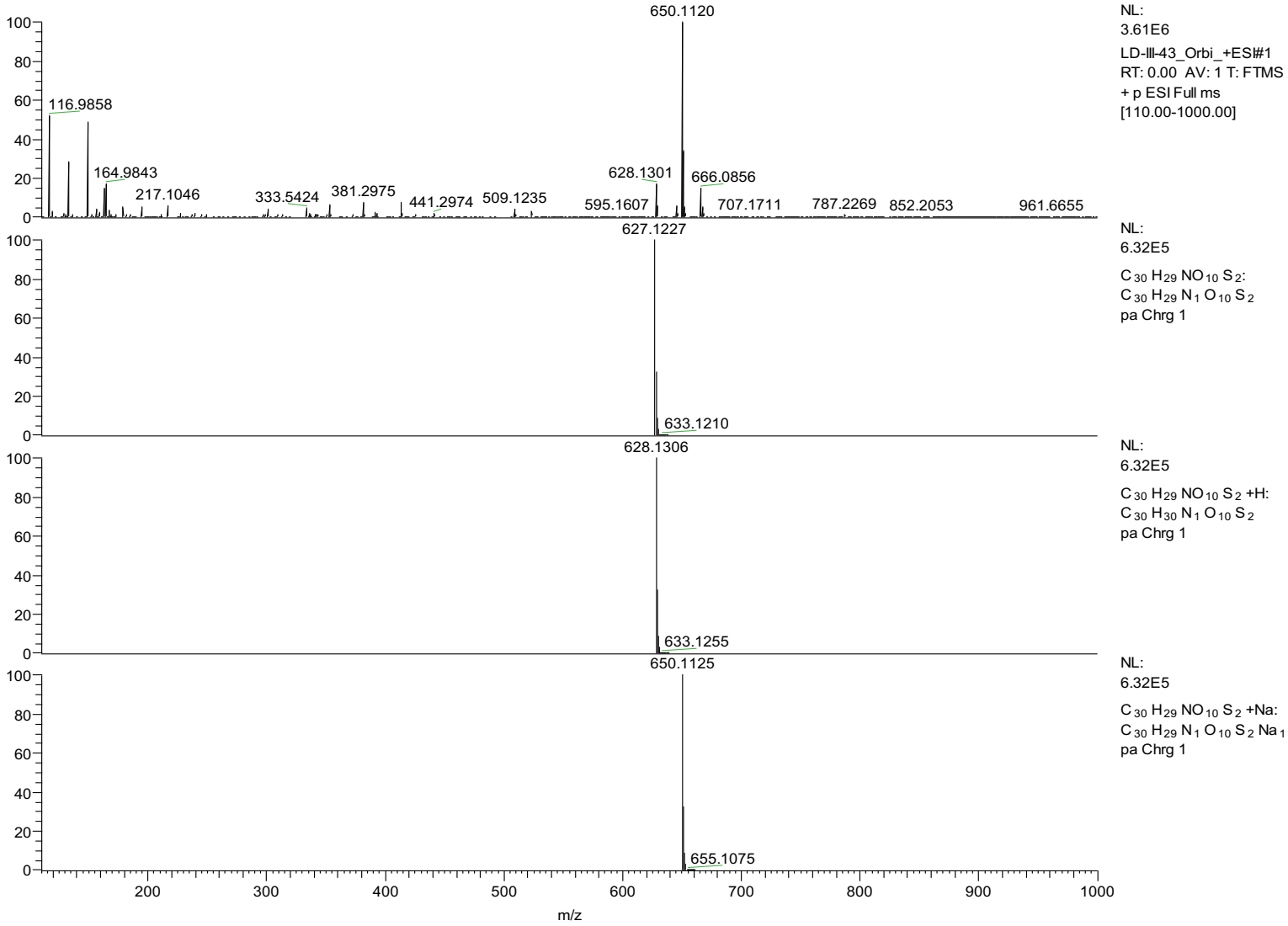
Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D
Sample Name: LD-III-43-1A-rerun-run1

Signal 8: DAD1 H, Sig=320,16 Ref=off

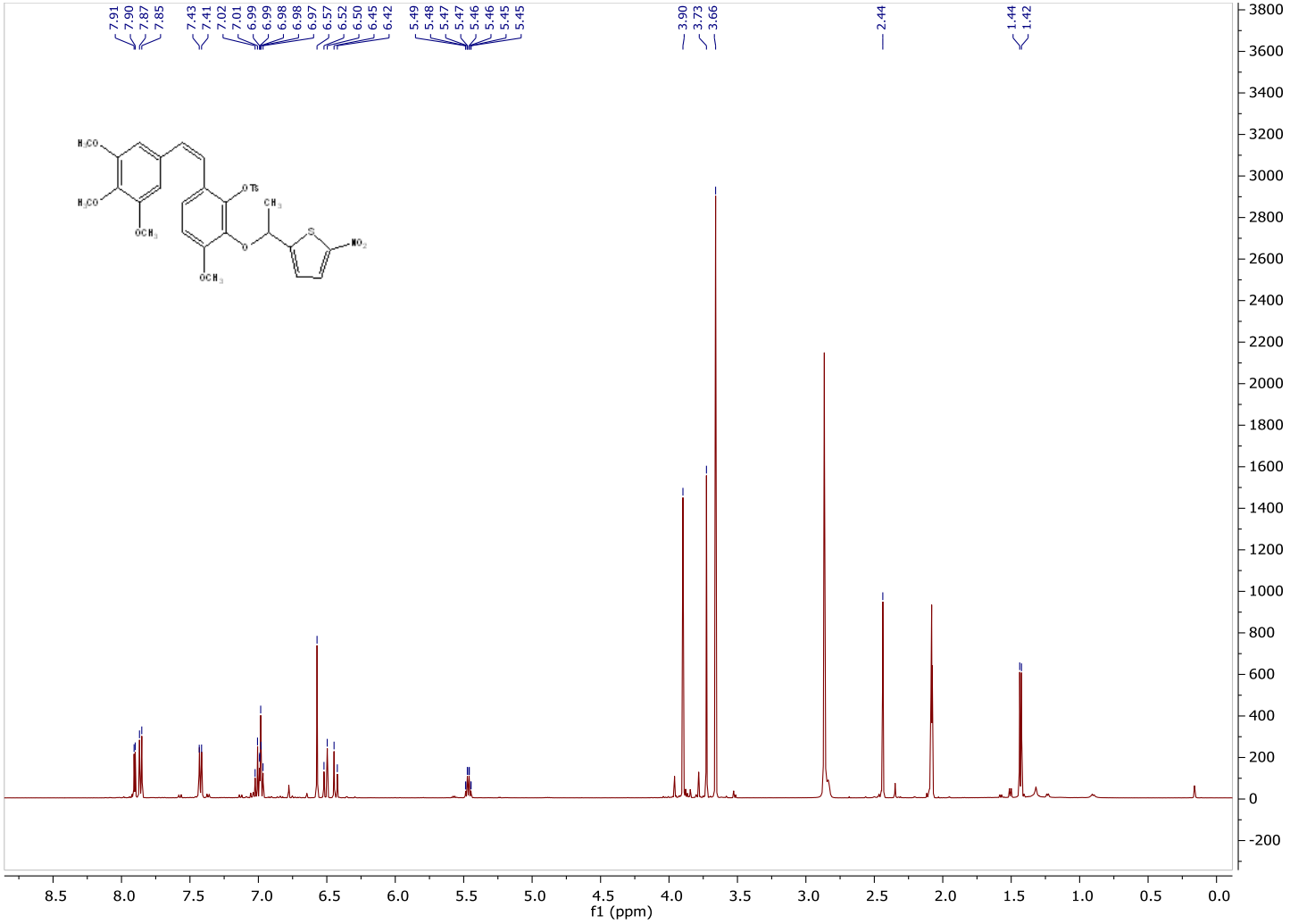
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.180	BB	0.0952	8.94699	1.42591	1.3034
2	18.506	BB	0.0853	677.49988	121.10182	98.6966
Totals :				686.44687	122.52773	

*** End of Report ***

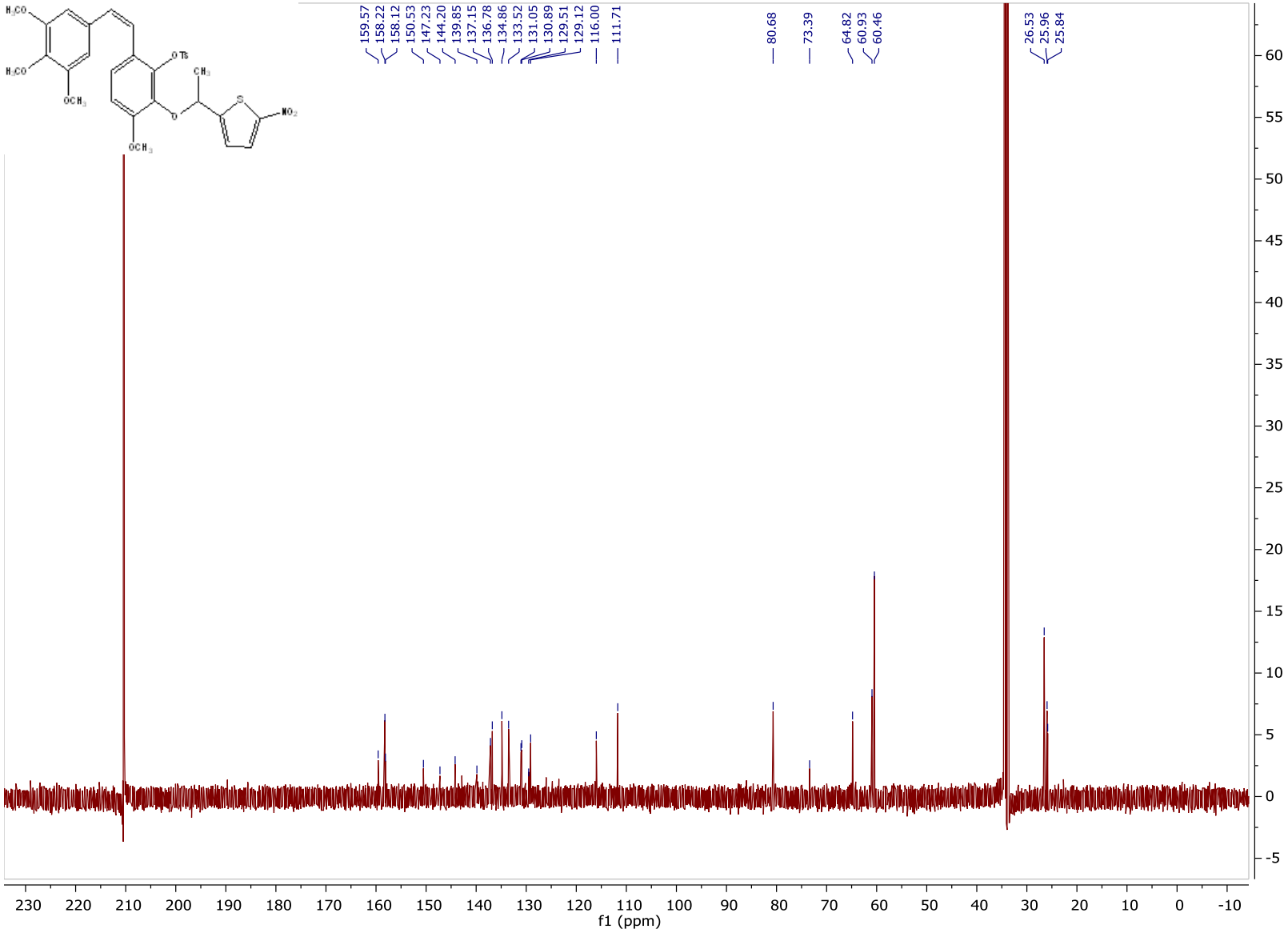
Mass Spectrum of Compound **22**



^{1}H NMR (500 MHz, $CDCl_3$) of (Z)-3-Methoxy-2-(2-(5-nitrothiophen-2-yl)propoxy)-6-(3,4,5-trimethoxystyryl)-phenyl-4-methylbenzenesulfonate (**23**)

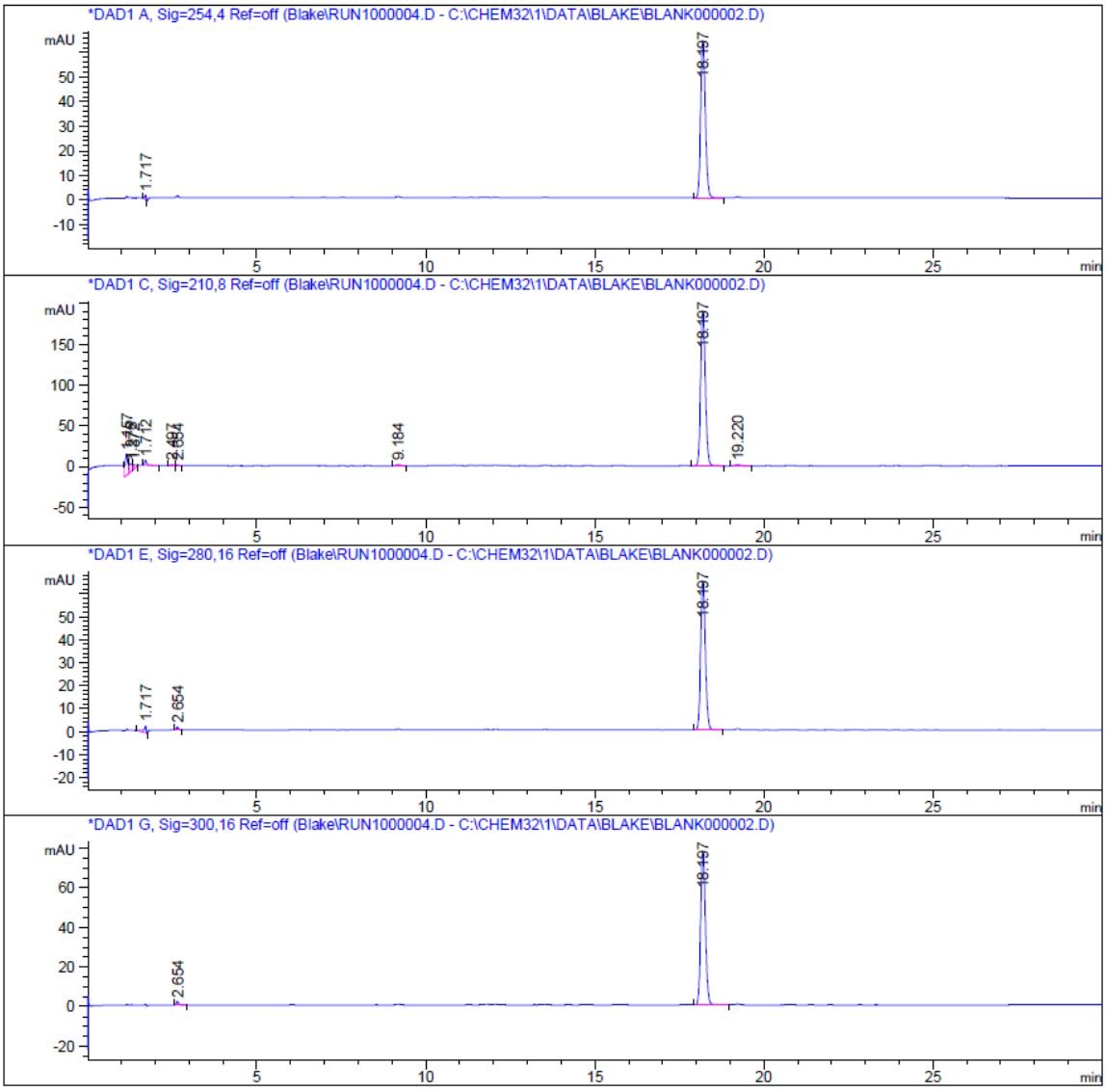


^{13}C NMR (500 MHz, $CDCl_3$) of (Z)-3-Methoxy-2-(2-(5-nitrothiophen-2-yl)propoxy)-6-(3,4,5-trimethoxystyryl)-phenyl-4-methylbenzenesulfonate (**23**)



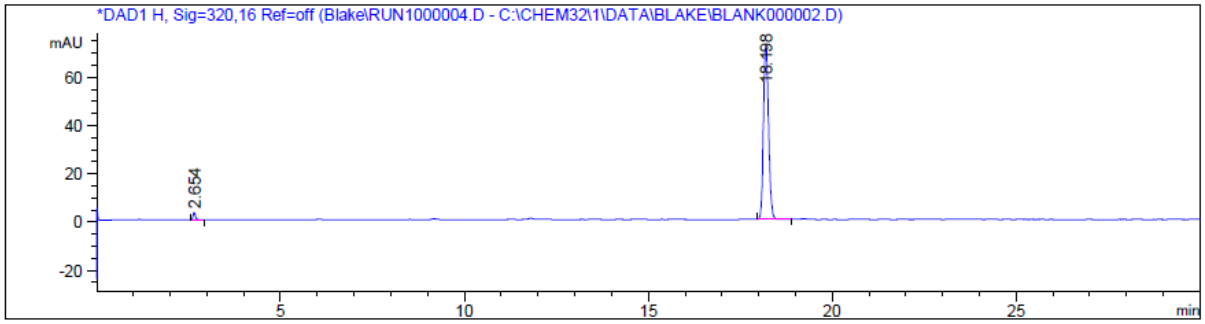
HPLC trace of compound **23**

```
Data File C:\Chem32\1\Data\Blake\RUN1000004.D
Sample Name: TosylMono CA1 Run 1

    ======================================================================
    Acq. Operator    : Blake
    Acq. Instrument  : Instrument 1                    Location :     -
    Injection Date   : 3/5/2015 8:21:32 PM
    Acq. Method      : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
    Last changed     : 3/5/2015 7:55:58 PM by Blake
    Analysis Method  : C:\CHEM32\1\METHODS\RT-ACNWASH 2.M
    Last changed     : 7/9/2015 2:27:22 PM by Blake
    Method Info      : General Column Wash Method

    Sample Info      : Tosyl Mono Trigger CA1 Run 1

    Additional Info  : Peak(s) manually integrated
```




```
1200 HPLC 9/1/2016 1:28:01 PM SYSTEM                    Page   1 of 3
```

Data File C:\Chem32\1\Data\Blake\RUN1000004.D
Sample Name: TosylMono CA1 Run 1




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

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=off
 Signal has been modified after loading from rawdata file!

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]    [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.717 BB    0.0497    6.60572    2.04210   1.0763
   2  18.197 BB    0.1470  607.16217   63.78918  98.9237

Totals :                   613.76789   65.83128



Signal 2: DAD1 C, Sig=210,8 Ref=off
 Signal has been modified after loading from rawdata file!

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]    [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.157 VV    0.0747  140.96169   26.23448   6.7080
   2   1.278 VV    0.0986   64.48904    9.56787   3.0689
   3   1.372 VB    0.0760   27.96374    5.09453   1.3307
   4   1.712 BB    0.0664   27.76718    6.42144   1.3214
   5   2.497 BV    0.0845    7.12432    1.28859   0.3390
   6   2.654 VB    0.0630    5.25895    1.30582   0.2503
   7   9.184 BB    0.1264   13.37233    1.61839   0.6364
   8  18.197 BB    0.1469 1798.89990  189.18680  85.6047
   9  19.220 BB    0.1466   15.56711    1.64256   0.7408

Totals :                  2101.40426  242.36048
```

Data File C:\Chem32\1\Data\Blake\RUN1000004.D
Sample Name: TosylMono CA1 Run 1

Signal 3: DAD1 E, Sig=280,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.717	BB	0.0774	16.70851	2.98108	2.6269
2	2.654	BB	0.0649	5.25675	1.25308	0.8265
3	18.197	BB	0.1470	614.09607	64.56446	96.5467
Totals :				636.06133	68.79862	

Signal 4: DAD1 G, Sig=300,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.654	BB	0.0678	8.59714	1.93753	1.1541
2	18.197	BB	0.1472	736.32220	77.24043	98.8459
Totals :				744.91934	79.17796	

Signal 5: DAD1 H, Sig=320,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.654	BB	0.0673	12.99429	2.95627	1.8475
2	18.198	BB	0.1476	690.34473	72.19302	98.1525
Totals :				703.33902	75.14929	

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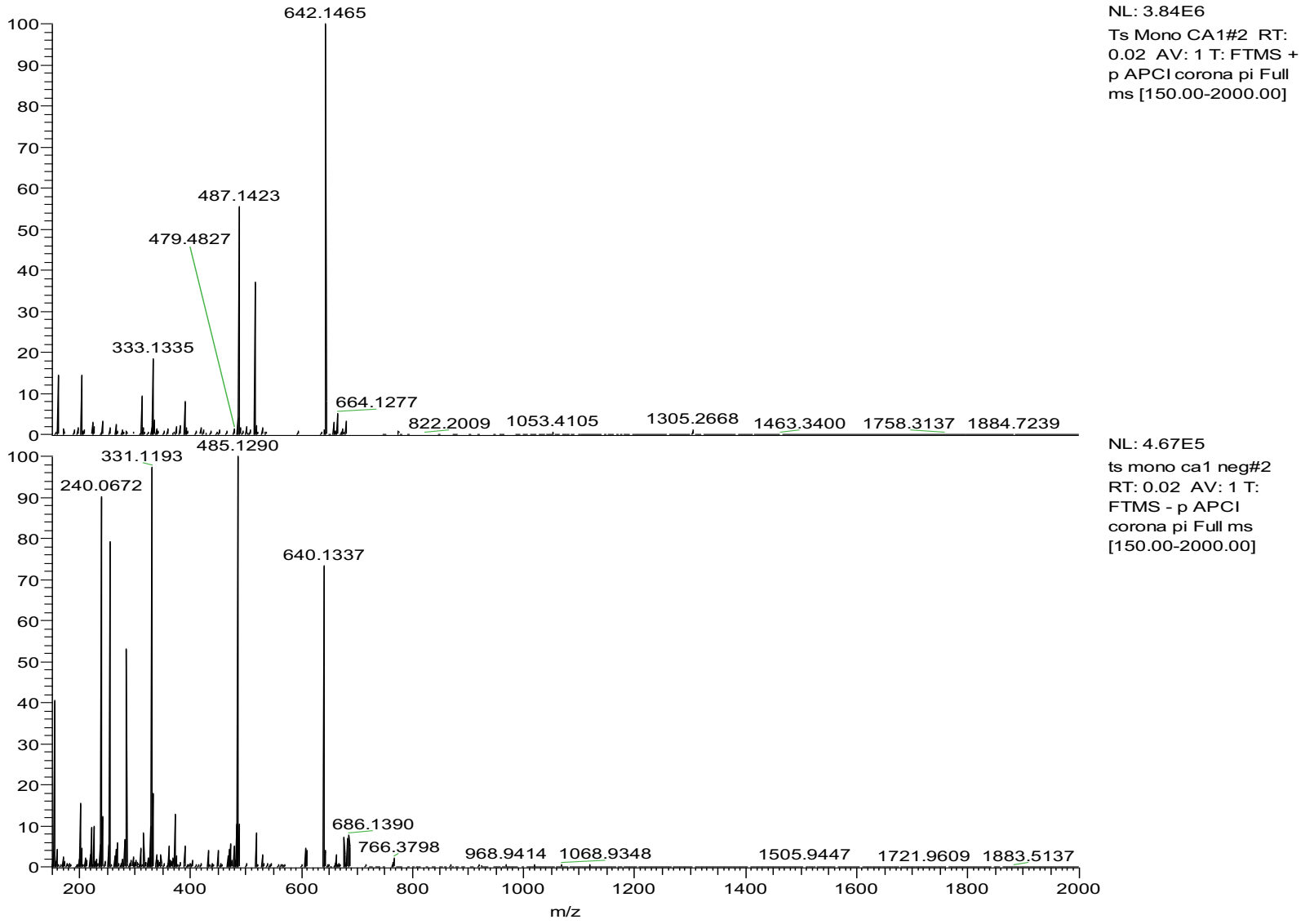
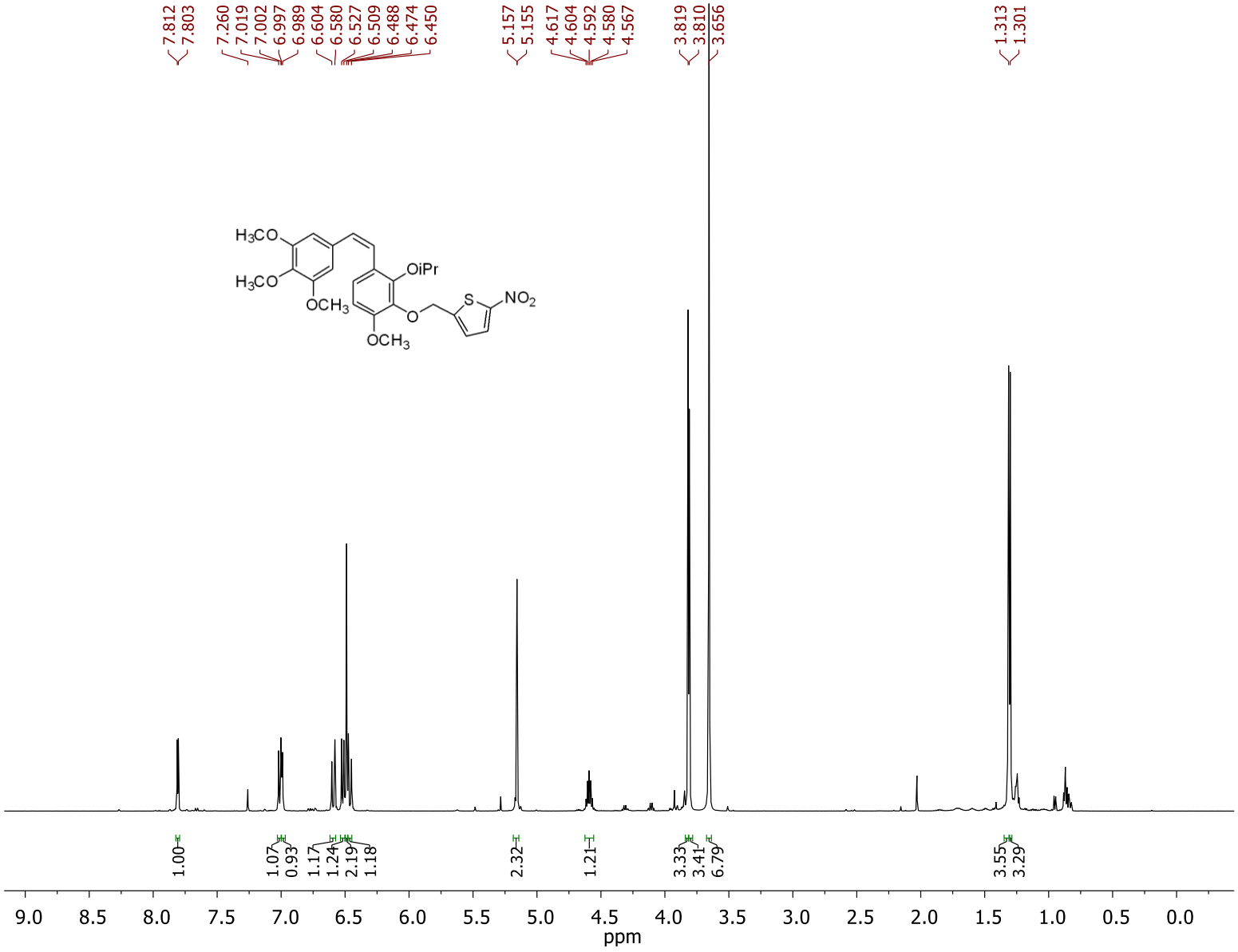
*** End of Report ***

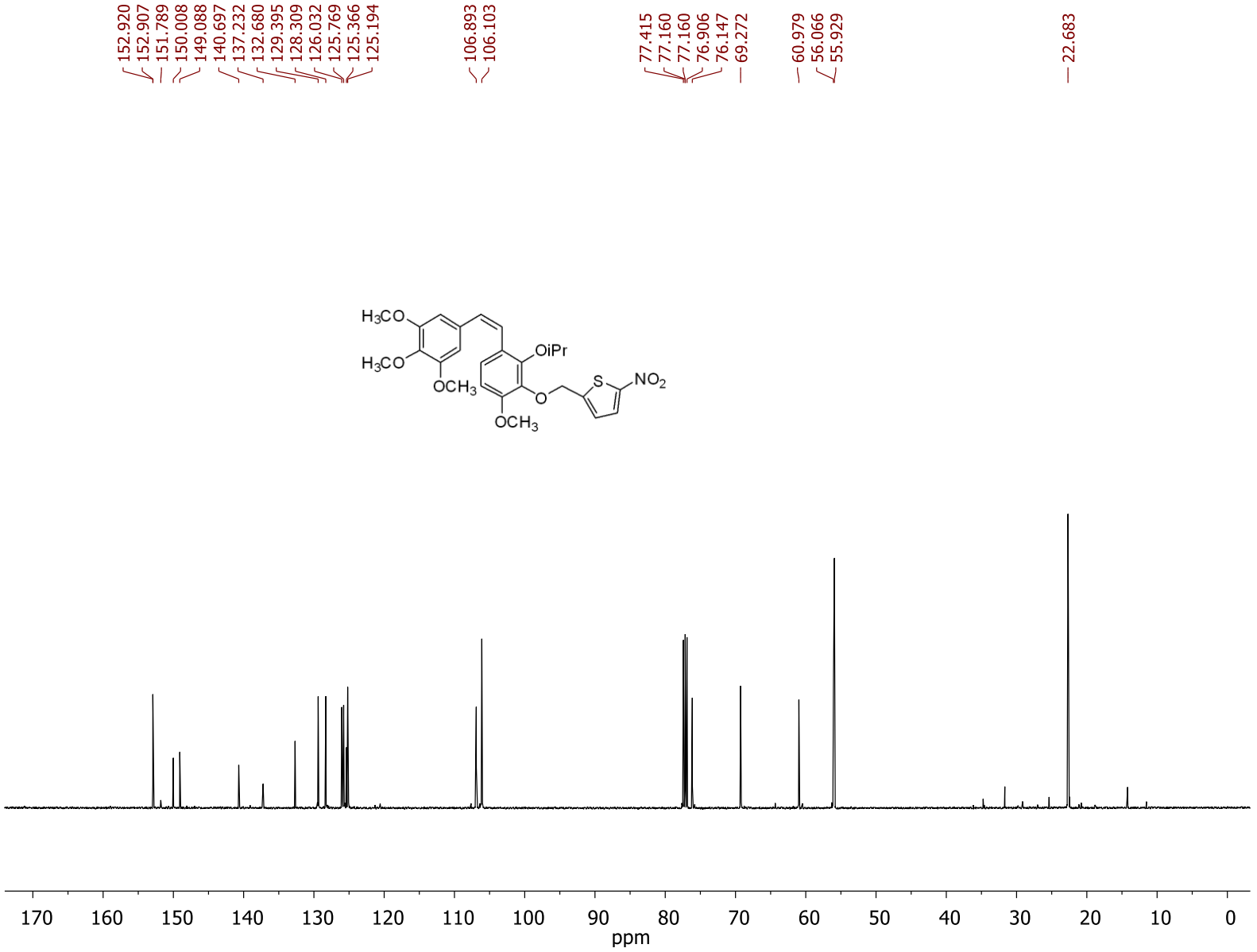
Mass Spectrum of Compound **23**



^{1}H NMR (500 MHz, $CDCl_3$) for Compound **24**



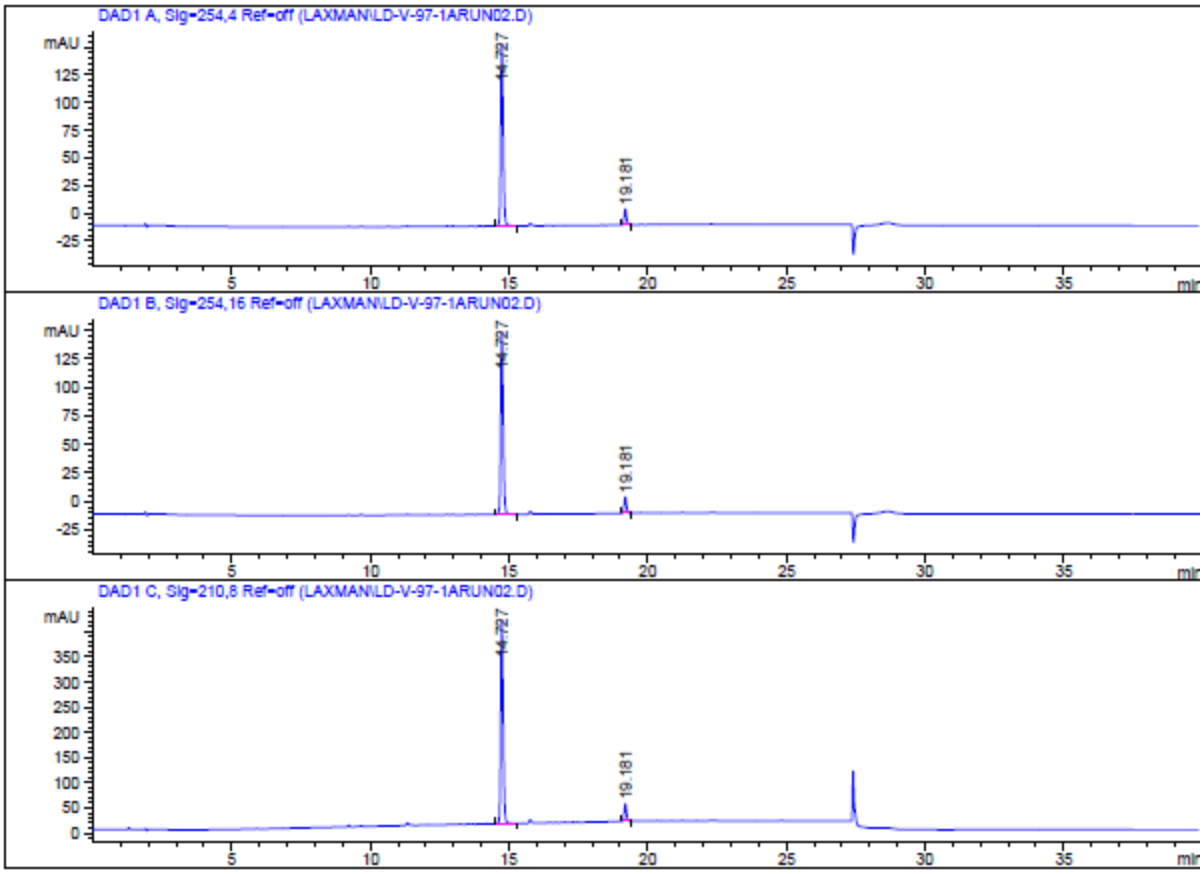
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **24**



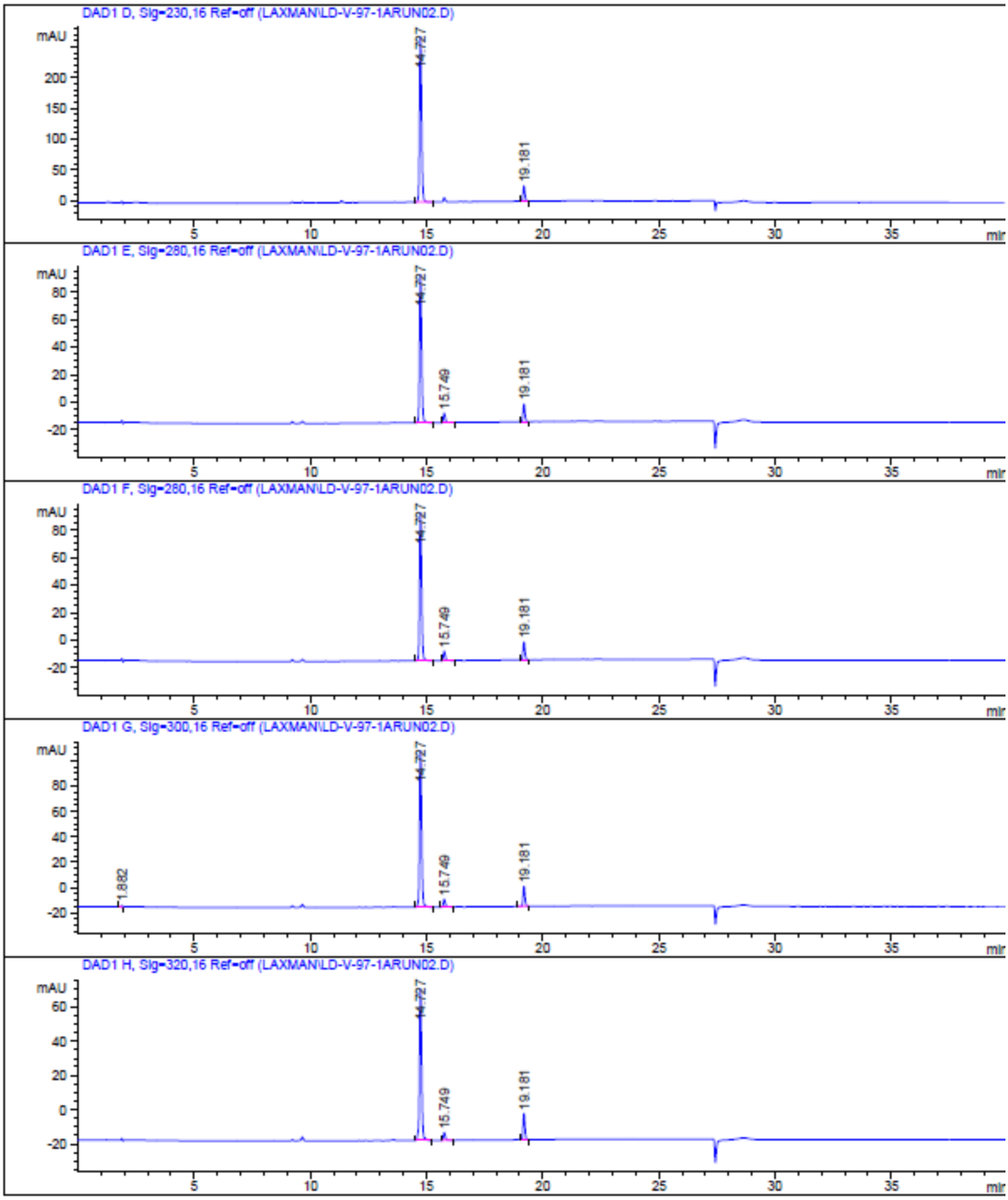
HPLC Traces of Compound **24**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D
Sample Name: LD-V-97-1A-run2

    ======================================================================
    Acq. Operator    : Casey
    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date   : 6/11/2014 12:23:01 PM
    Acq. Method      : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed     : 6/11/2014 12:20:19 PM by Casey
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D\DA.M (MASTERMETHOD.M)
    Last changed     : 3/6/2015 11:26:20 AM by Blake
                       (modified after loading)
    Sample Info      : LD-V-97-1A-run2
                       Mastermethod
```



Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D
Sample Name: LD-V-97-1A-run2



Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D
Sample Name: LD-V-97-1A-run2

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

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.727	BB	0.0937	1013.76843	165.00717	92.8924
2	19.181	BB	0.0863	77.56749	14.08282	7.1076
Totals :				1091.33592	179.08999	

Signal 2: DAD1 B, Sig=254,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.727	BB	0.0937	987.21277	160.64627	92.7104
2	19.181	BB	0.0863	77.62172	14.09153	7.2896
Totals :				1064.83449	174.73780	

Signal 3: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.727	BB	0.0932	2476.75439	405.73618	92.9730
2	19.181	VB	0.0864	187.19514	33.90105	7.0270
Totals :				2663.94954	439.63723	

Signal 4: DAD1 D, Sig=230,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.727	BB	0.0935	1653.68958	269.86240	92.4120
2	19.181	VB	0.0865	135.78531	24.57749	7.5880

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D
Sample Name: LD-V-97-1A-run2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				1789.47488	294.43989	

Signal 5: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.727	BB	0.0939	663.65198	107.74279	85.4036
2	15.749	BB	0.0929	42.01422	6.91261	5.4067
3	19.181	BB	0.0862	71.41132	12.97093	9.1897
Totals :				777.07752	127.62633	

Signal 6: DAD1 F, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.727	BB	0.0939	663.65198	107.74279	85.4036
2	15.749	BB	0.0929	42.01422	6.91261	5.4067
3	19.181	BB	0.0862	71.41132	12.97093	9.1897
Totals :				777.07752	127.62633	

Signal 7: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.882	BB	0.0563	5.62916	1.48090	0.6242
2	14.727	BB	0.0948	770.54956	123.48886	85.4397
3	15.749	BB	0.0922	36.64074	6.09094	4.0628
4	19.181	BB	0.0877	89.04414	15.81382	9.8733
Totals :				901.86360	146.87453	

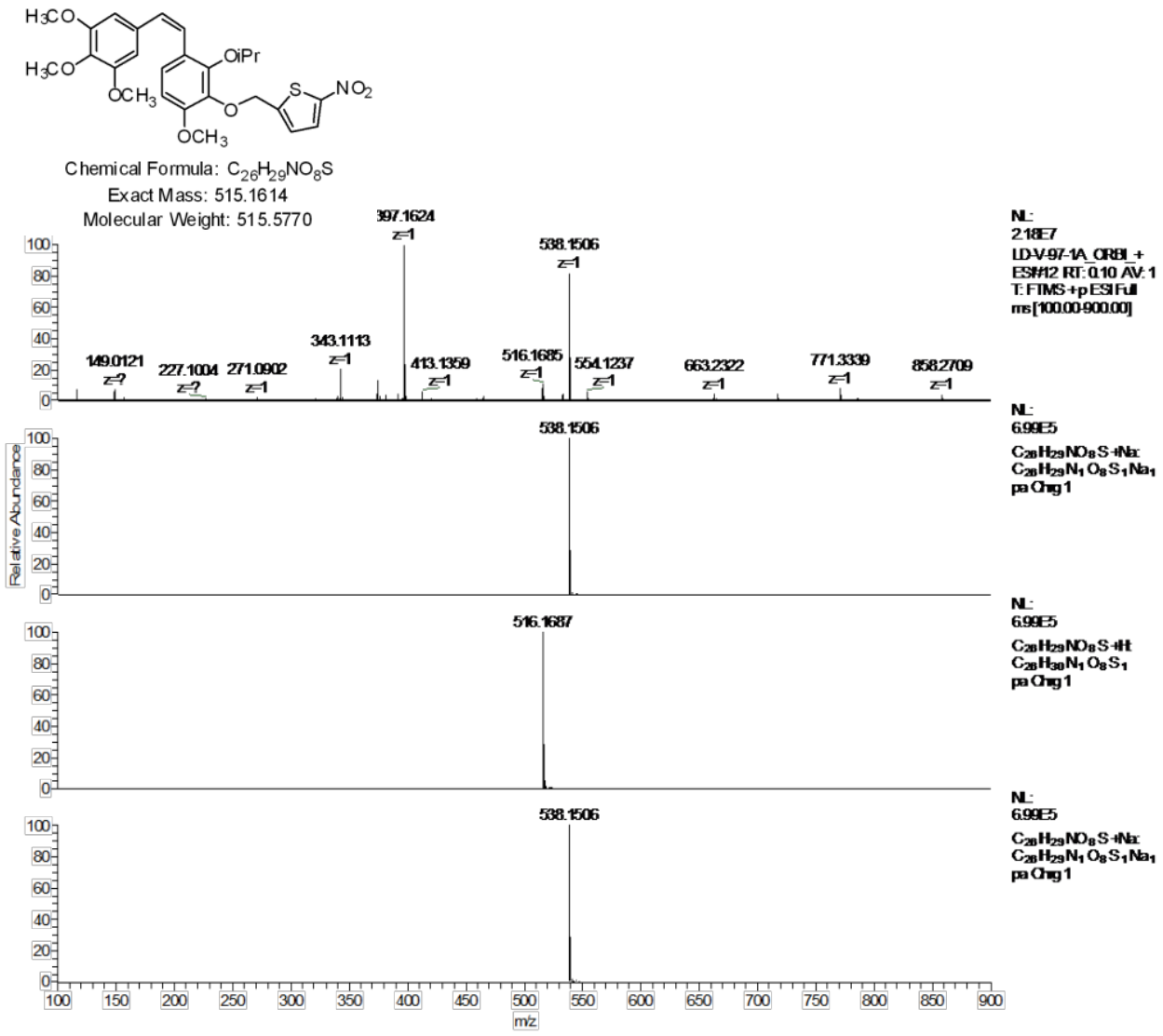
Signal 8: DAD1 H, Sig=320,16 Ref=off

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D
Sample Name: LD-V-97-1A-run2

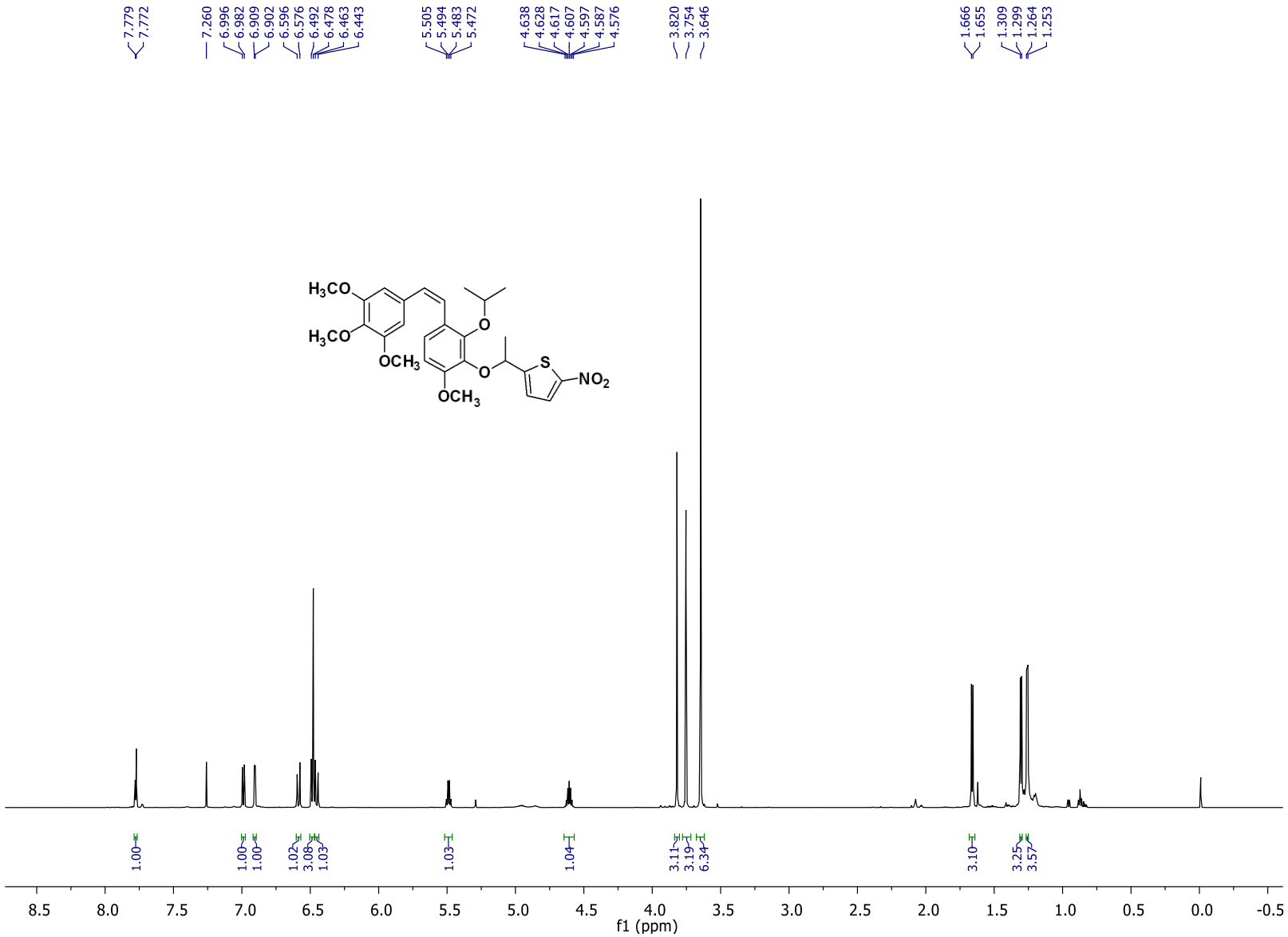
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.727	BB	0.0968	568.63849	88.65764	83.4444
2	15.749	BB	0.0922	27.48027	4.56638	4.0326
3	19.181	VB	0.0867	85.33914	15.39945	12.5230
Totals :				681.45790	108.62348	

*** End of Report ***

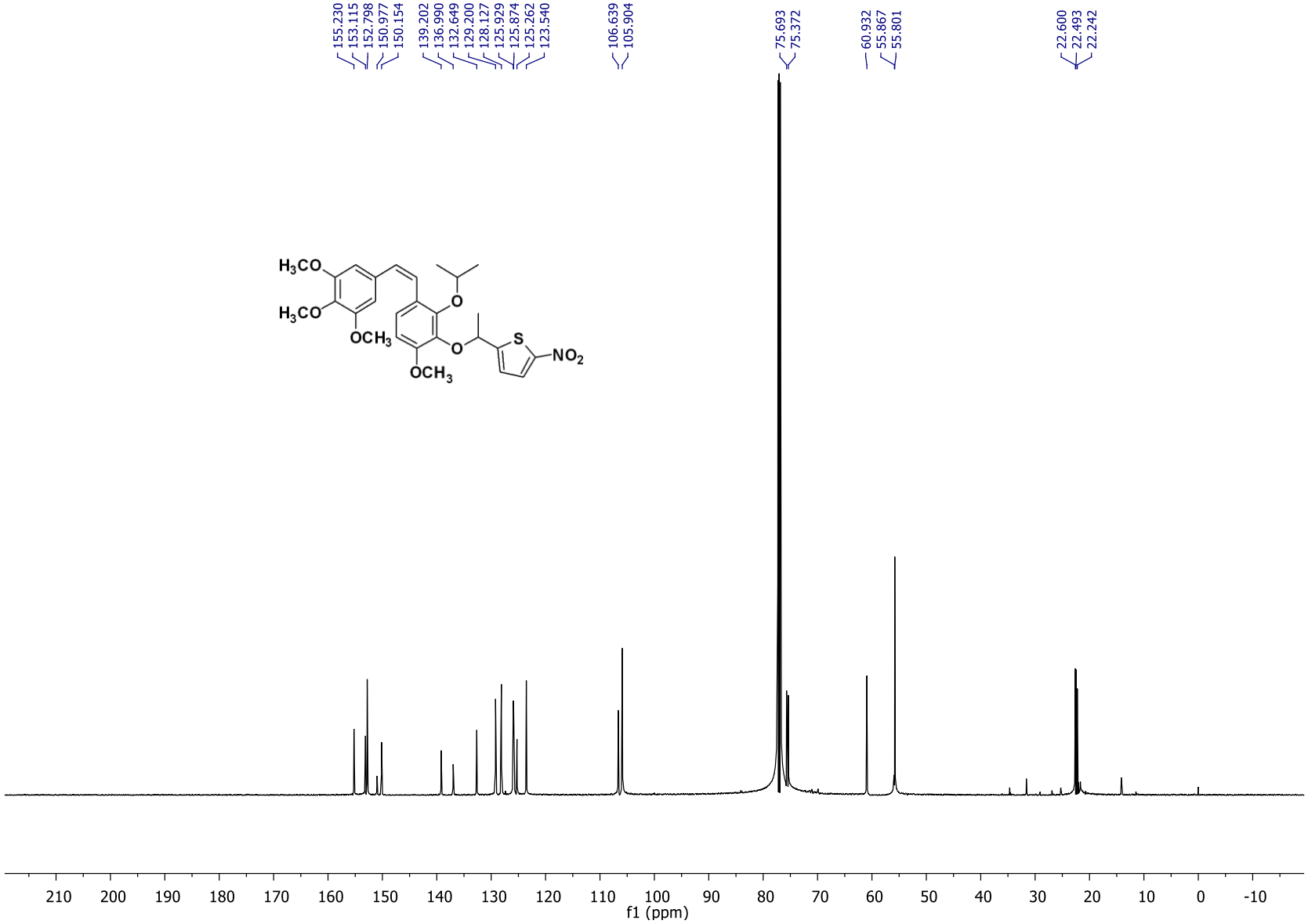
Mass Spectrum of Compound **24**



^{1}H NMR (600 MHz, $CDCl_3$) for Compound **25**



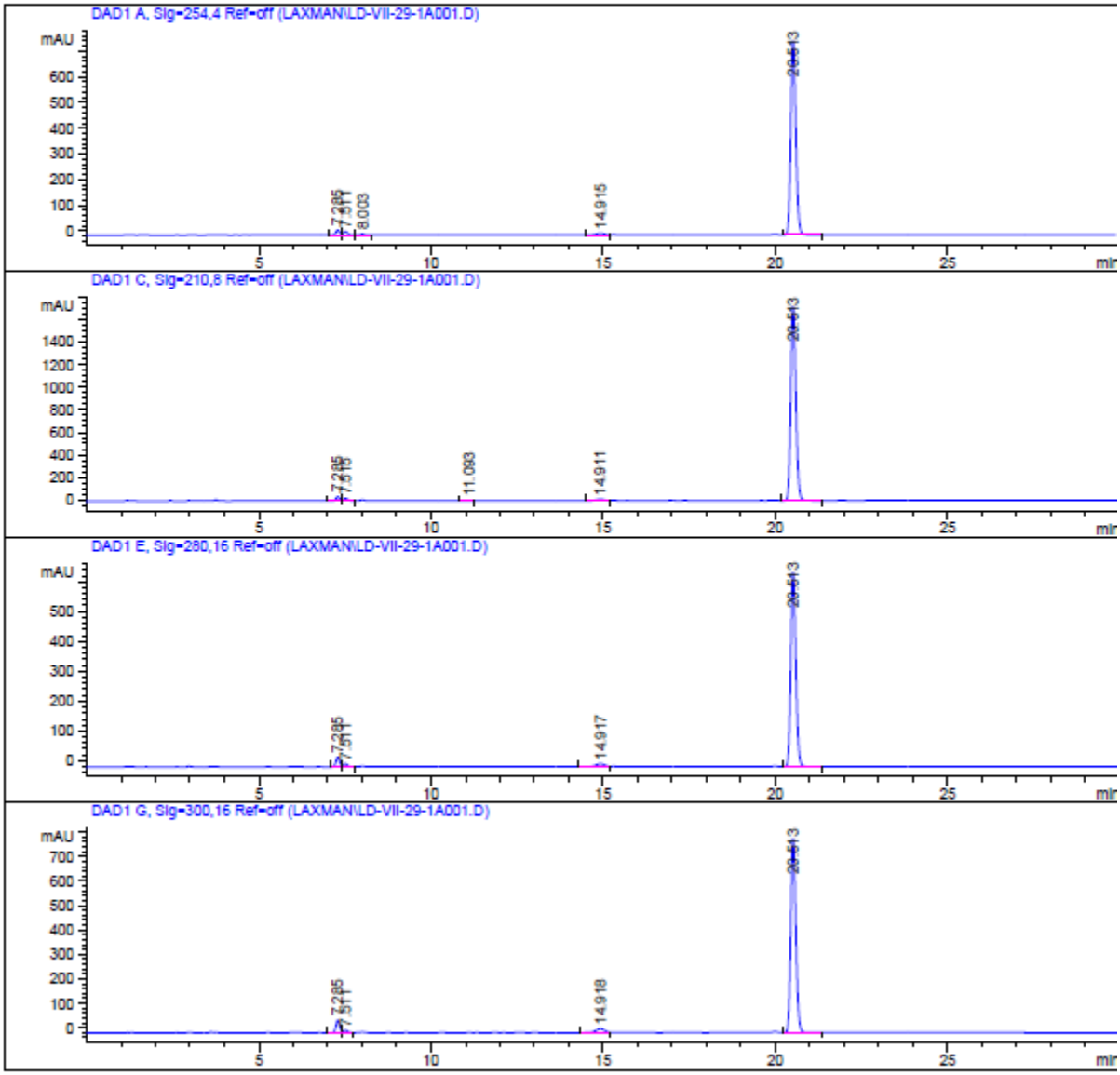
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **25**



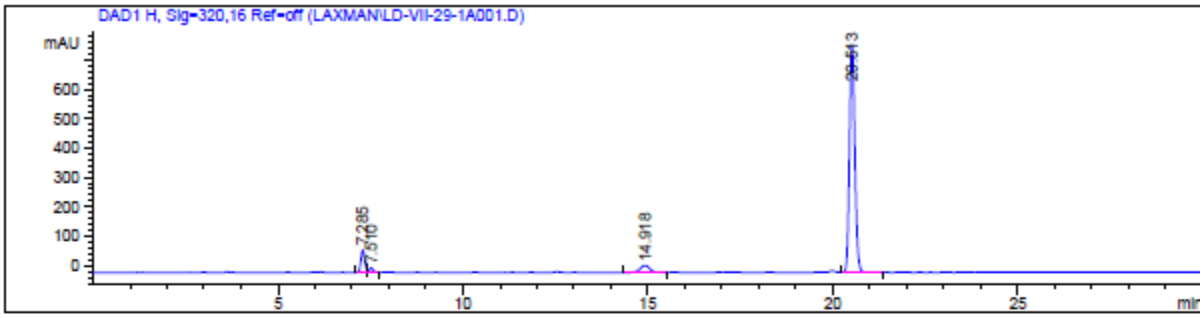
HPLC Traces for Compound **25**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-29-1A001.D
Sample Name: LD-VII-29-1A

======================================================================
Acq. Operator    : Laxman
Acq. Instrument : Instrument 1                    Location :   -
Injection Date  : 3/1/2015 3:44:20 PM
Acq. Method      : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
Last changed     : 3/1/2015 3:36:56 PM by Laxman
Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-VII-29-1A001.D\DA.M (GRAD 2 50-90 ACN.M)
Last changed     : 3/1/2015 4:24:33 PM by Laxman
```



Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-29-1A001.D
Sample Name: LD-VII-29-1A



==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1134	137.70985	18.81188	1.7190
2	7.511	VB	0.1180	108.58200	14.07325	1.3554
3	8.003	BB	0.1245	30.20725	3.72818	0.3771
4	14.915	BV	0.2773	103.74889	5.89437	1.2951
5	20.513	VB	0.1577	7630.70166	756.65521	95.2534

Totals : 8010.94966 799.16290

Signal 2: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1181	282.01685	36.51880	1.5456
2	7.515	VB	0.1310	187.78674	21.27100	1.0291
3	11.093	BV	0.1711	14.92364	1.34912	0.0818
4	14.911	BV	0.2798	225.43584	12.77740	1.2355
5	20.513	VB	0.1588	1.75367e4	1721.62097	96.1080

Totals : 1.82469e4 1793.53730

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-29-1A001.D
Sample Name: LD-VII-29-1A

Signal 3: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1138	238.84053	32.47009	3.3890
2	7.511	VB	0.1171	77.61353	9.94490	1.1013
3	14.917	BV	0.2830	175.57196	9.80124	2.4912
4	20.513	VB	0.1575	6555.55762	650.98096	93.0185
Totals :				7047.58364	703.19719	

Signal 4: DAD1 G, Sig=300,16 Ref=off

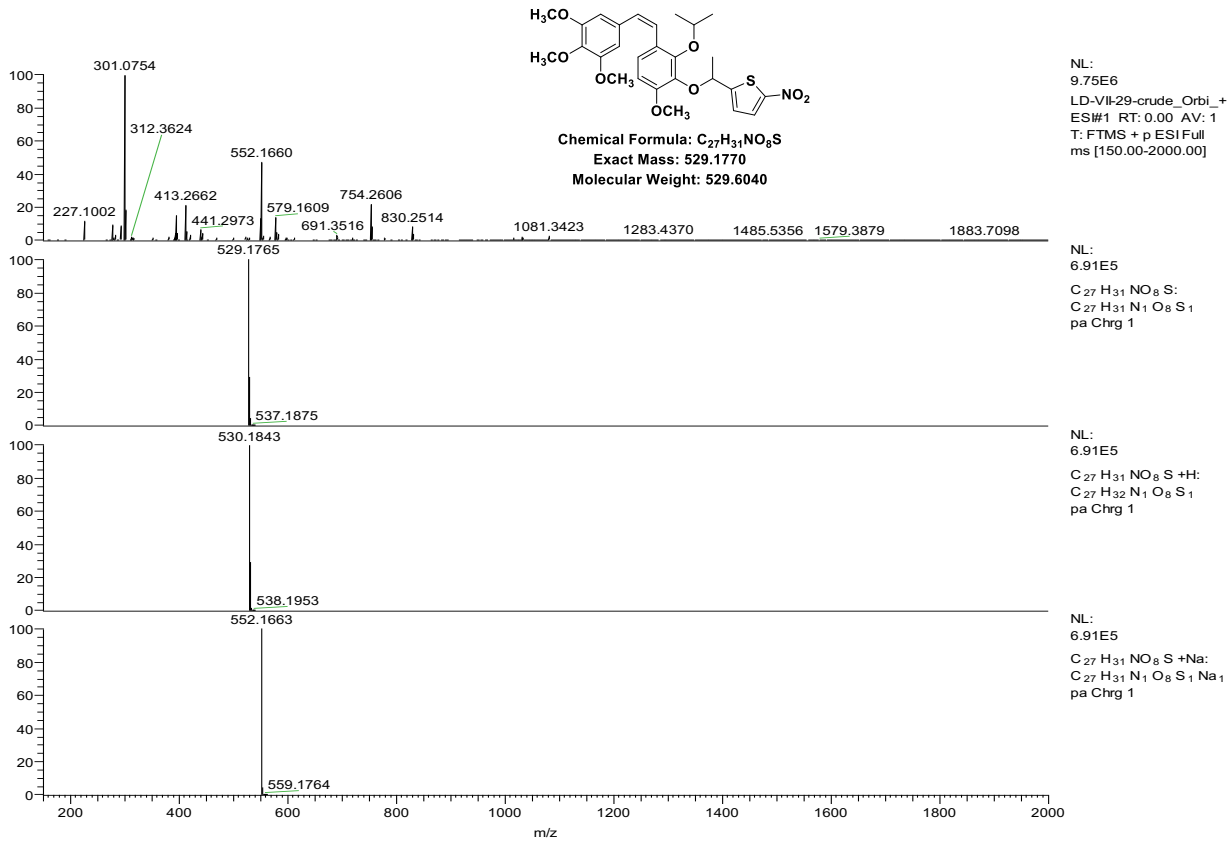
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1139	376.73462	51.17566	4.2972
2	7.511	VB	0.1162	81.67451	10.56421	0.9316
3	14.918	BV	0.2823	290.15118	16.09673	3.3096
4	20.513	VB	0.1556	8018.51367	795.31226	91.4617
Totals :				8767.07399	873.14884	

Signal 5: DAD1 H, Sig=320,16 Ref=off

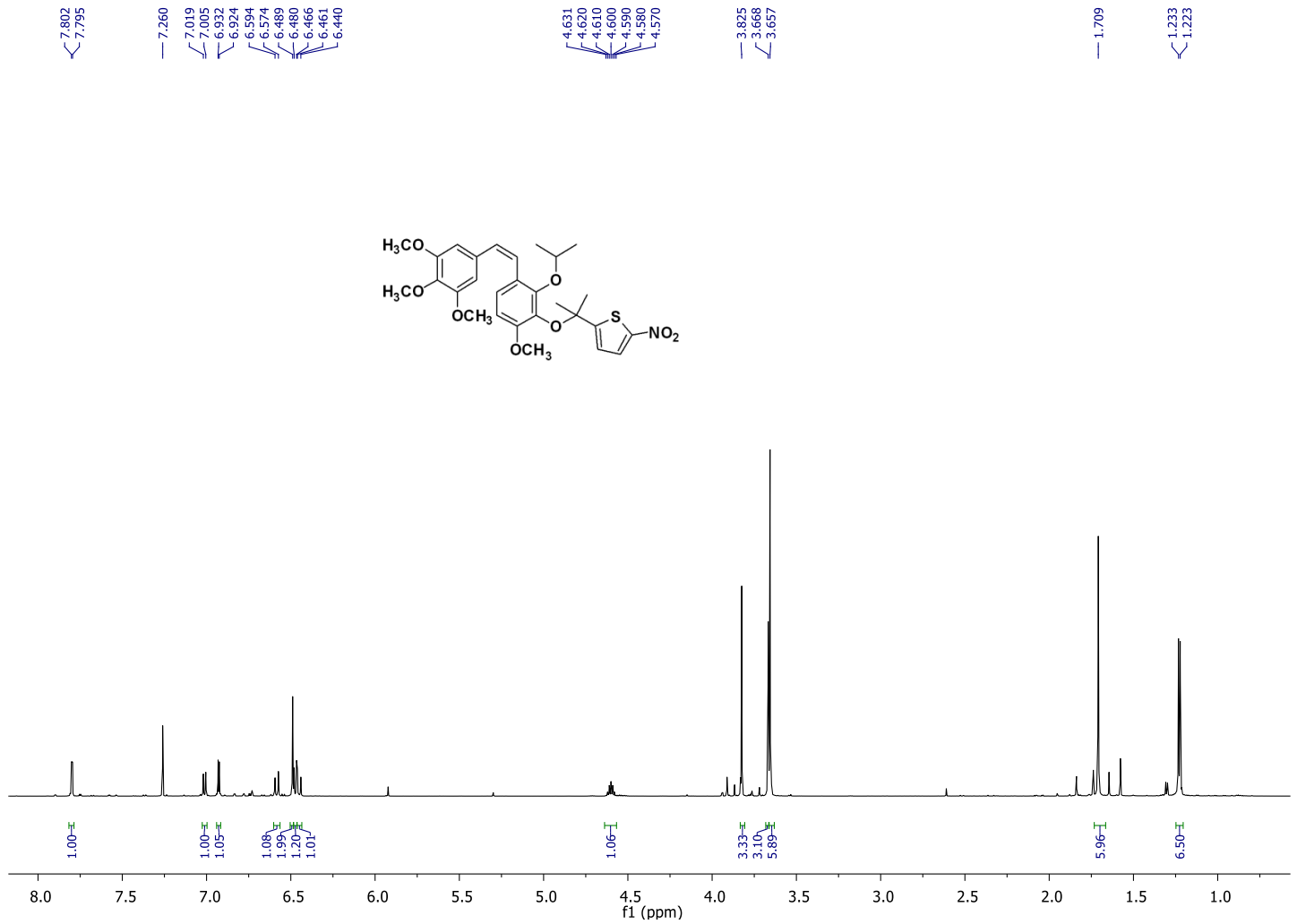
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1139	556.89746	75.66253	6.2256
2	7.510	VB	0.1160	116.93579	15.15809	1.3072
3	14.918	BB	0.2899	437.53549	23.64482	4.8912
4	20.513	VB	0.1578	7833.95703	775.83319	87.5760
Totals :				8945.32578	890.29863	

*** End of Report ***

Mass Spectrum of Compound **25**



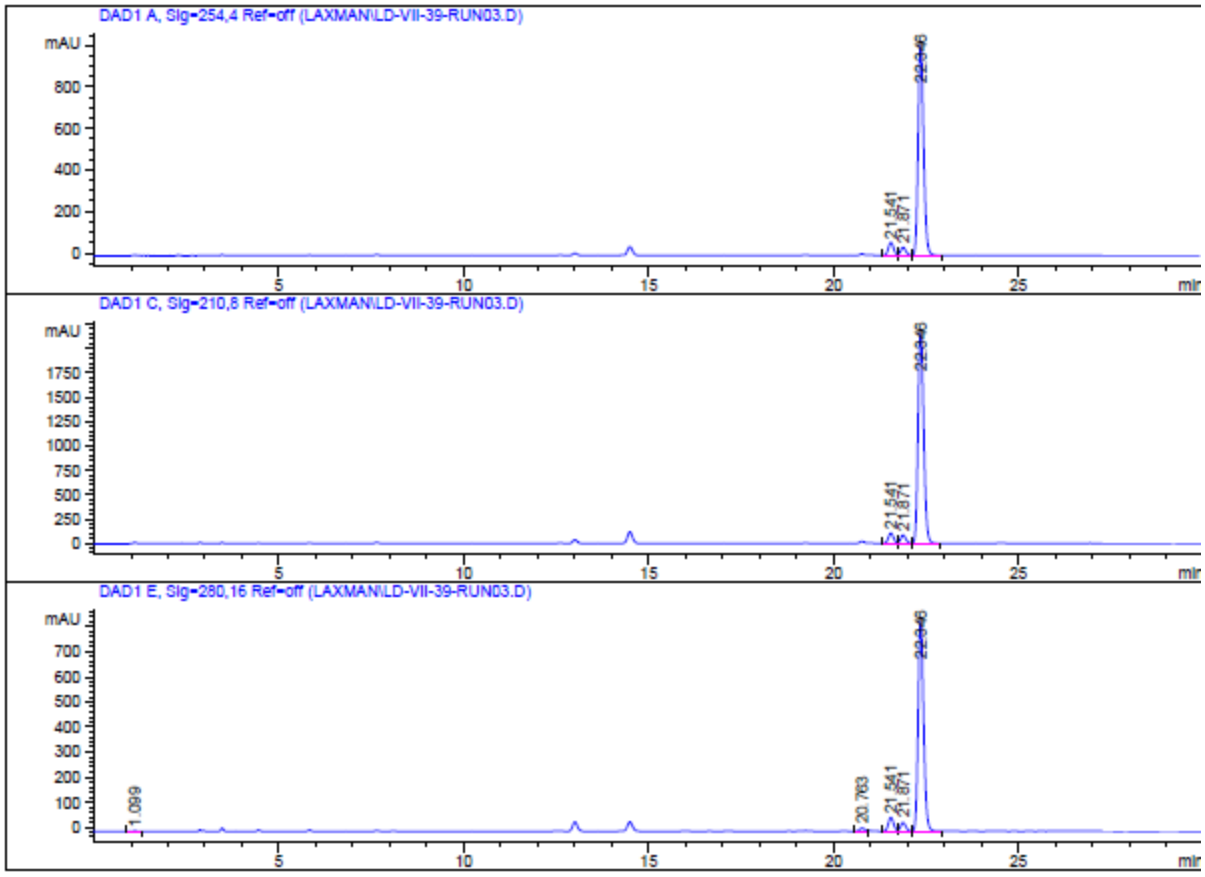
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **26**



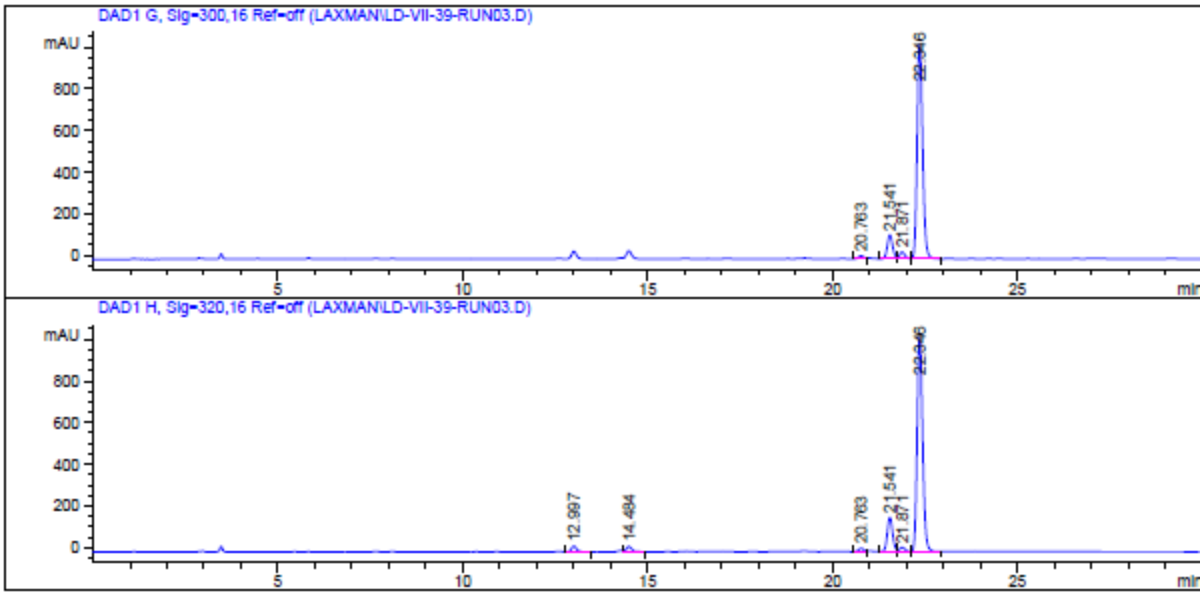
HPLC Traces of Compound **26**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-39-RUN03.D
Sample Name: LD-VII-39-run3

    ======================================================================
    Acq. Operator    : Laxman
    Acq. Instrument  : Instrument 1                    Location :    -
    Injection Date   : 3/15/2015 10:57:34 AM
    Acq. Method      : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
    Last changed     : 3/15/2015 10:37:39 AM by Laxman
    Analysis Method  : C:\CHEM32\1\DATA\LAXMAN\LD-VII-39-RUN03.D\DA.M (GRAD 2 50-90 ACN.M)
    Last changed     : 3/16/2015 6:22:00 PM by Laxman
    Sample Info      : Method-Grad2 50-90% ACN
```



Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-39-RUN03.D
Sample Name: LD-VII-39-run3



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Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	21.541	BV	0.1566	625.99896	62.65627	5.4443
2	21.871	VV	0.1579	398.06259	38.72581	3.4619
3	22.346	VB	0.1602	1.04742e4	1016.64459	91.0938
Totals :				1.14983e4	1118.02668	

Signal 2: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	21.541	BV	0.1564	1048.61108	105.09589	4.1849
2	21.871	VV	0.1576	866.73926	84.56084	3.4591

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-39-RUN03.D
Sample Name: LD-VII-39-run3

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
3	22.346	VB	0.1687	2.31415e4	2164.44214	92.3560
Totals :				2.50569e4	2354.09888	

Signal 3: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.099	BV	0.0821	22.67783	3.88244	0.2335
2	20.763	BV	0.1483	119.33536	12.40154	1.2289
3	21.541	BV	0.1567	553.77106	55.33903	5.7025
4	21.871	VV	0.1578	351.39407	34.20695	3.6185
5	22.346	VB	0.1601	8663.92285	841.87061	89.2167
Totals :				9711.10117	947.70056	

Signal 4: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.763	BV	0.1474	140.45000	14.70714	1.1420
2	21.541	BV	0.1571	1151.50391	114.67712	9.3632
3	21.871	VV	0.1580	327.26285	31.81455	2.6611
4	22.346	VB	0.1604	1.06790e4	1034.58325	86.8338
Totals :				1.22982e4	1195.78207	

Signal 5: DAD1 H, Sig=320,16 Ref=off

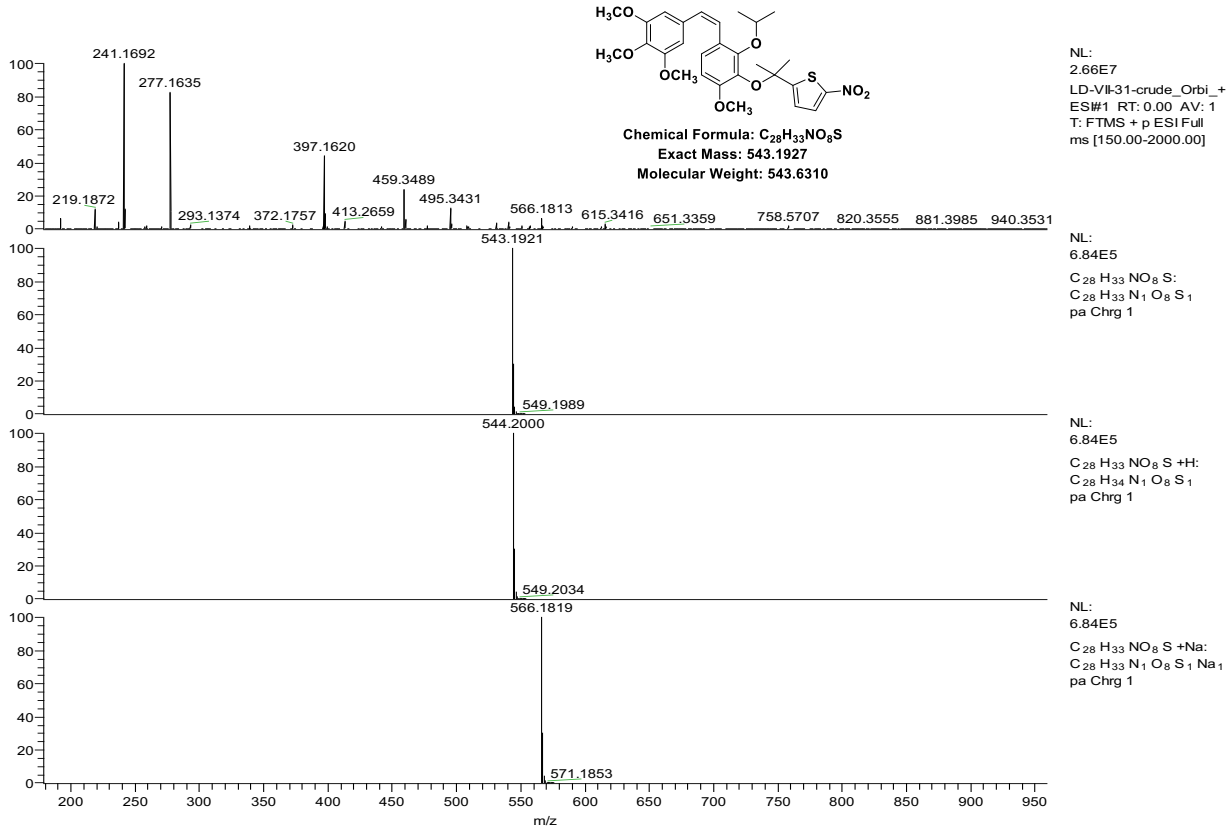
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.997	VB	0.1451	252.68199	27.01931	1.9125
2	14.484	VB	0.1580	257.79825	25.06436	1.9512
3	20.763	BV	0.1472	158.41324	16.62563	1.1990
4	21.541	BV	0.1574	1657.25757	164.71658	12.5434
5	21.871	VV	0.1556	215.00967	20.96922	1.6274
6	22.346	VB	0.1605	1.06710e4	1033.17529	80.7665
Totals :				1.32122e4	1287.57039	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-39-RUN03.D
Sample Name: LD-VII-39-run3

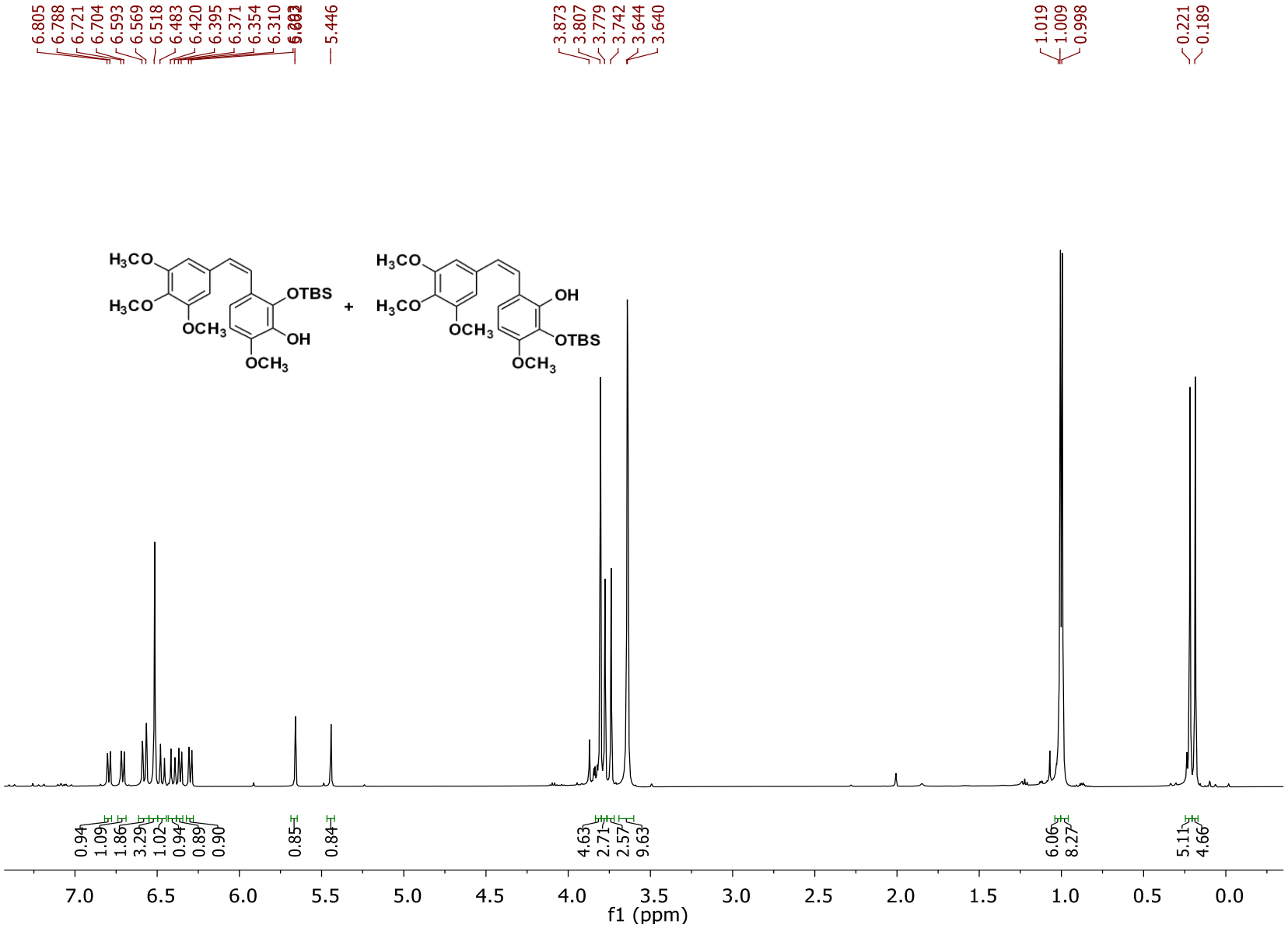
==========

*** End of Report ***

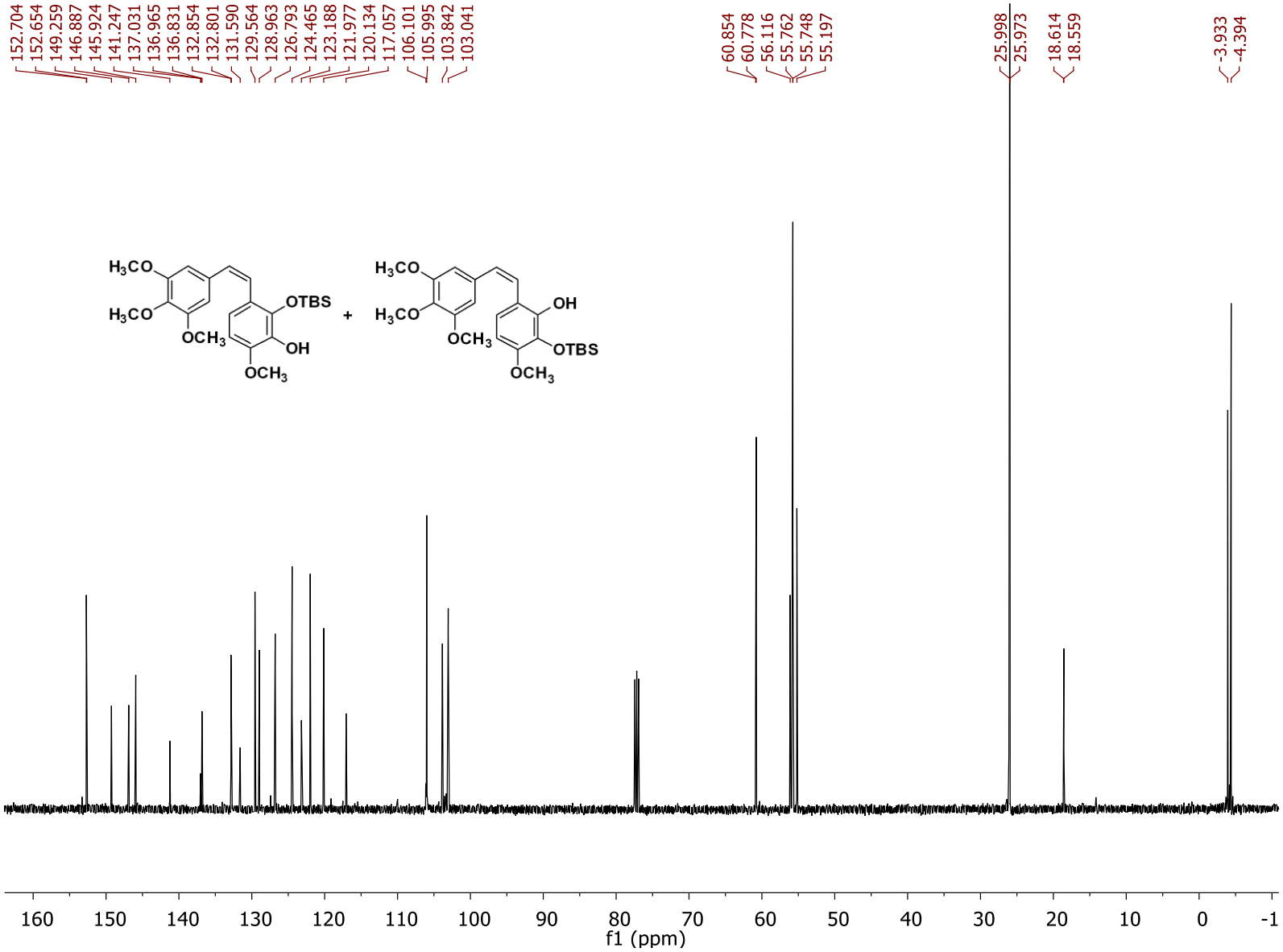
Mass Spectrum of Compound **26**



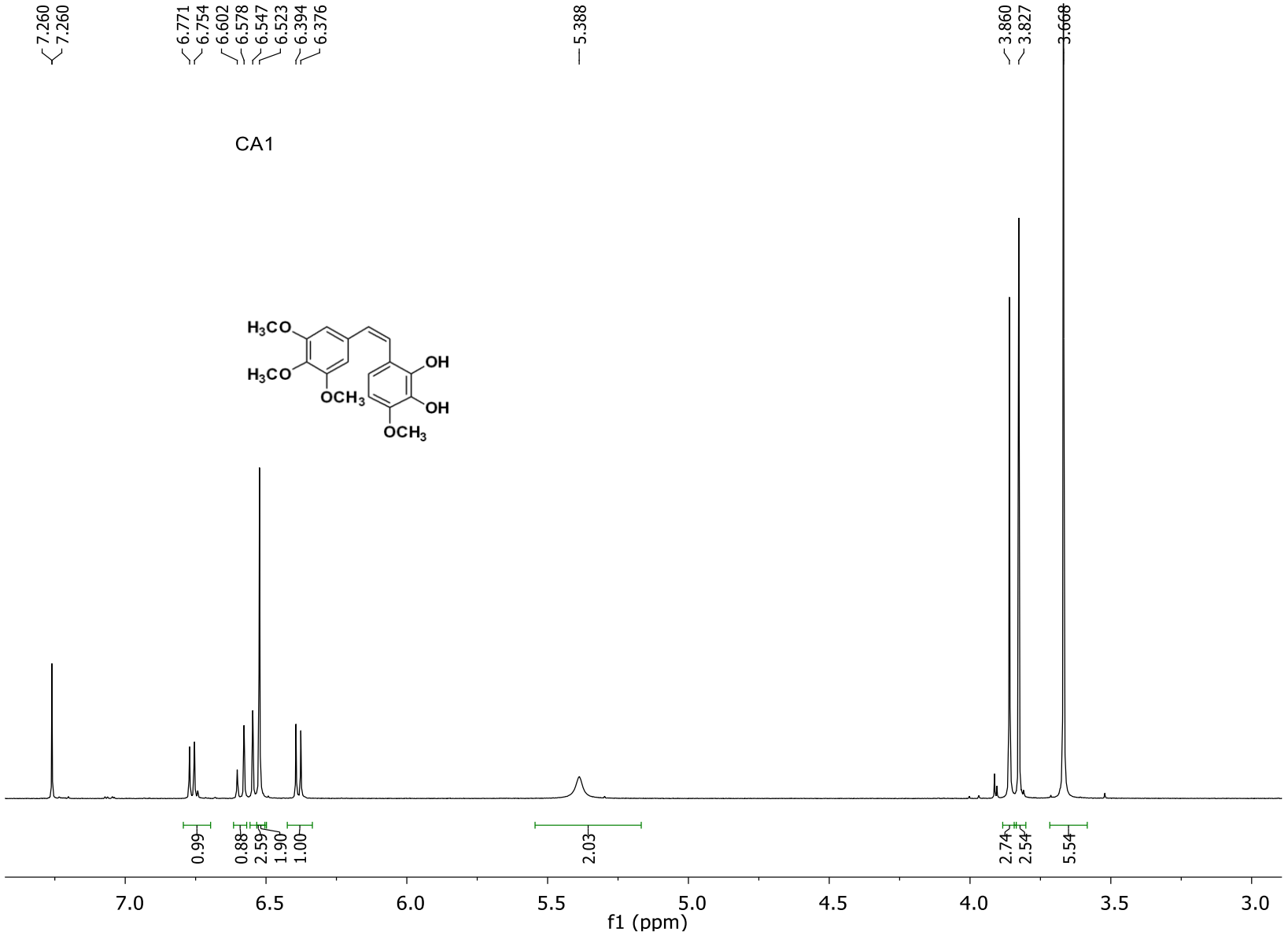
^{1}H NMR (500 MHz, $CDCl_3$) for Compounds **27** and **28**



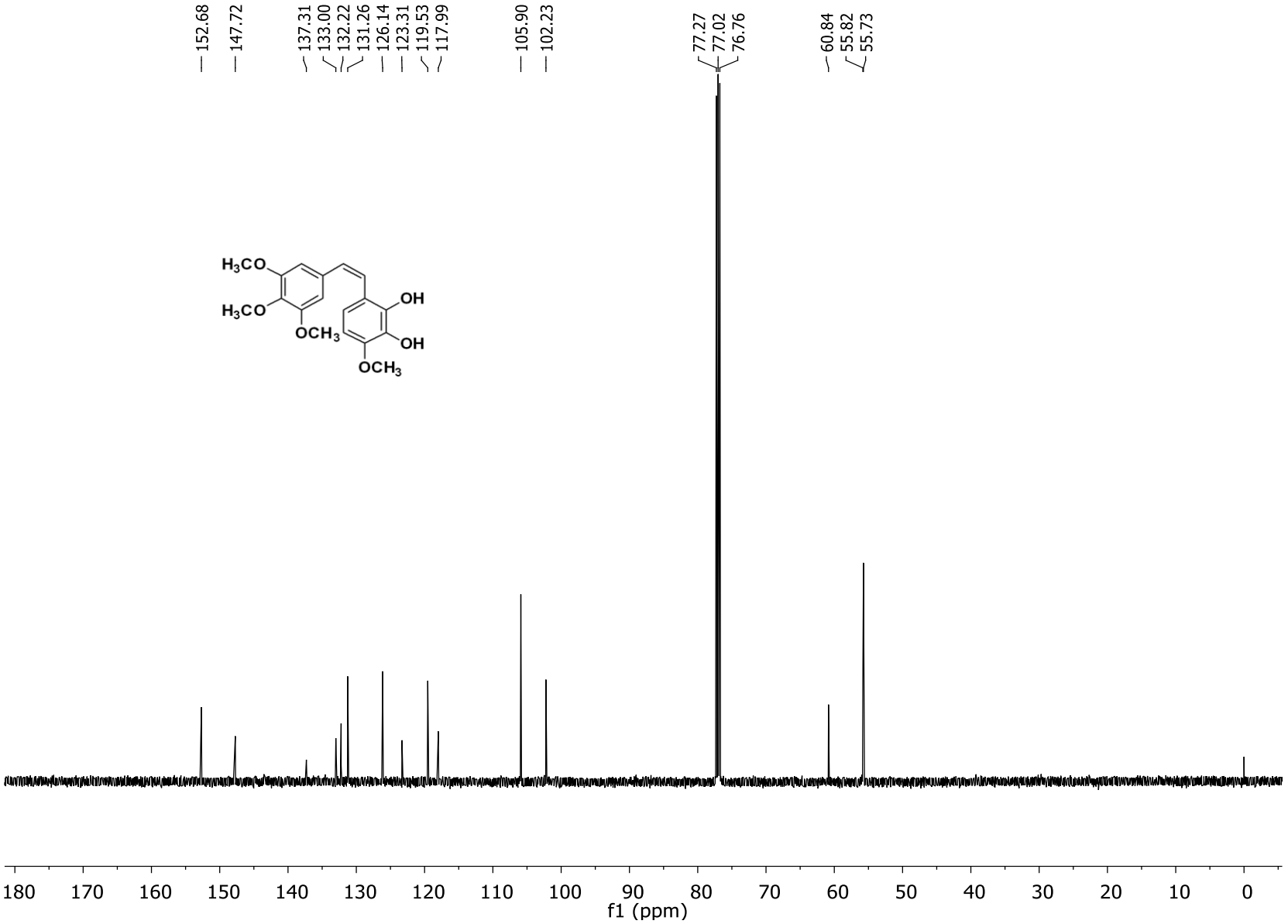
^{13}C NMR (125 MHz, $CDCl_3$) for Compounds **27** and **28**



^{1}H NMR (500 MHz, $CDCl_3$) for Compound **29**



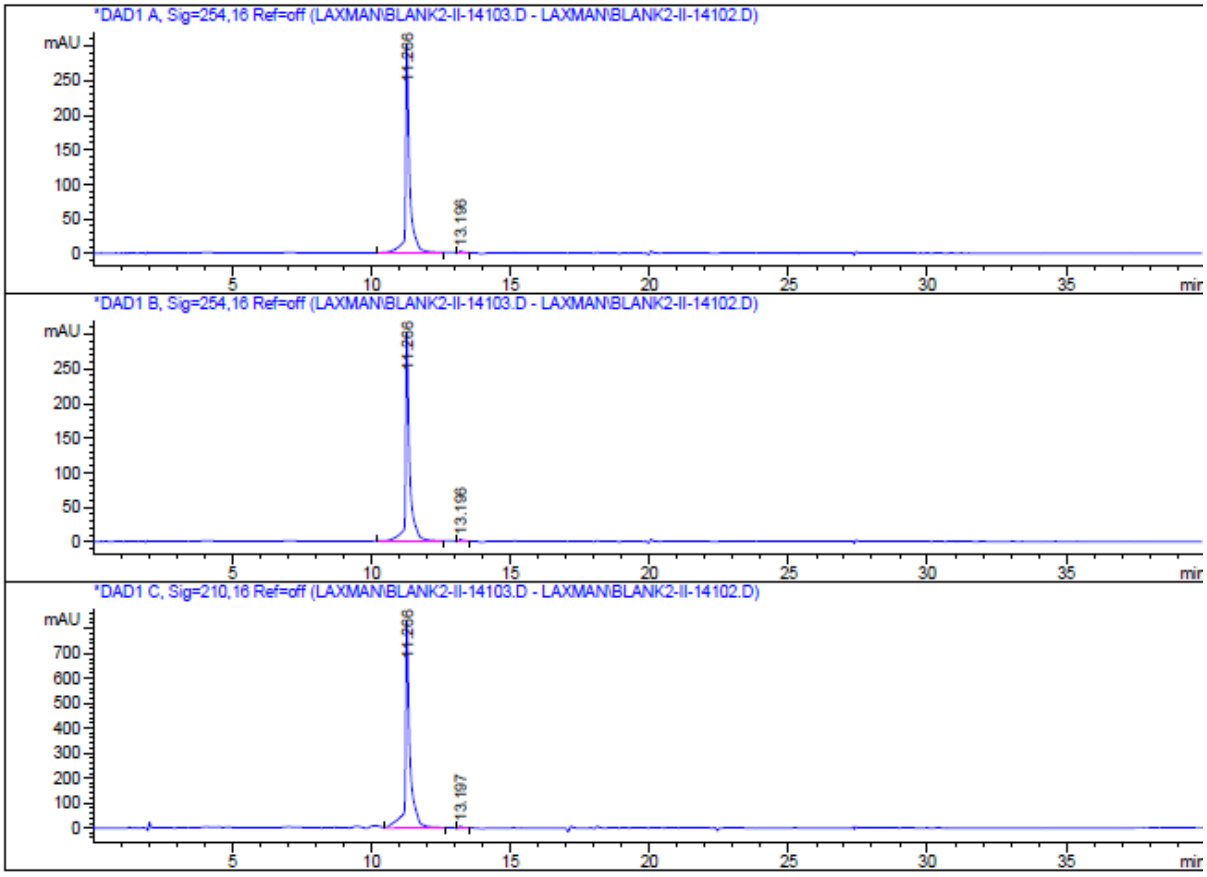
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **29**



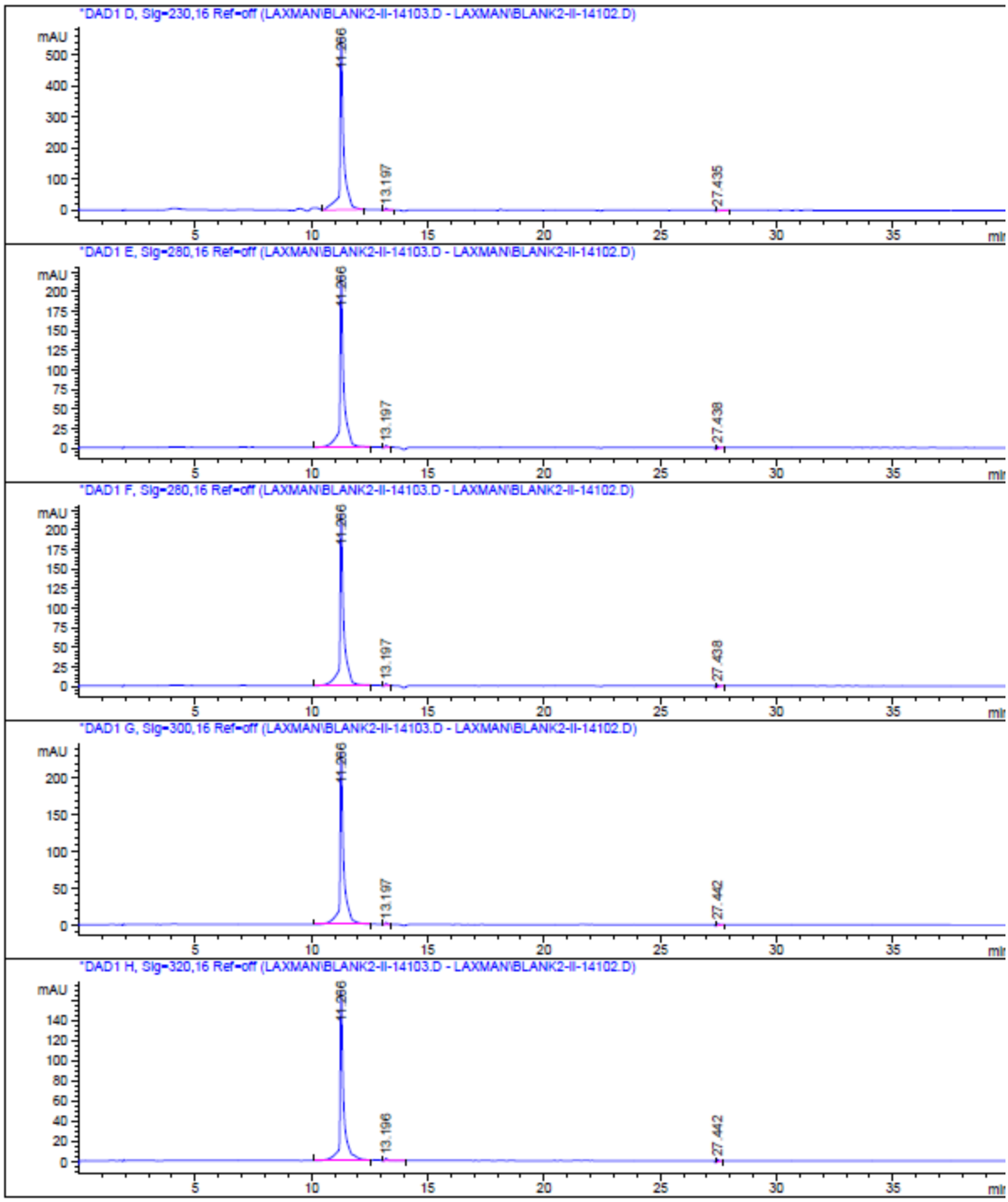
HPLC Traces of Compound **29**

```
Data File C:\CHEM32\1\DATA\LAXMAN\BLANK2-II-14103.D
Sample Name: LD-II-141-1blank2

    ======================================================================
    Acq. Operator   : Laxman
    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 10/31/2012 1:13:11 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 10/31/2012 10:10:23 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\BLANK2-II-14103.D\DA.M (MASTERMETHOD.M)
    Last changed    : 10/31/2012 2:13:03 PM by Laxman
    Sample Info     : 10% ACN in water
```



Data File C:\CHEM32\1\DATA\LAXMAN\BLANK2-II-14103.D
Sample Name: LD-II-141-1blank2



Data File C:\CHEM32\1\DATA\LAXMAN\BLANK2-II-14103.D
Sample Name: LD-II-141-1blank2

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	BB	0.1372	3040.63838	302.59622	99.2215
2	13.196	BB	0.1167	23.85802	2.93930	0.7785
Totals :				3064.69640	305.53552	

Signal 2: DAD1 B, Sig=254,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	BB	0.1372	3040.63838	302.59622	99.2215
2	13.196	BB	0.1167	23.85802	2.93930	0.7785
Totals :				3064.69640	305.53552	

Signal 3: DAD1 C, Sig=210,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	VB	0.1514	9399.36523	834.36102	99.2611
2	13.197	BB	0.1183	69.97166	8.47993	0.7389
Totals :				9469.33689	842.84096	

Data File C:\CHEM32\1\DATA\LAXMAN\BLANK2-II-14103.D
Sample Name: LD-II-141-1blank2

Signal 4: DAD1 D, Sig=230,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	VB	0.1531	6351.90918	556.84320	98.0392
2	13.197	BB	0.1182	53.54675	6.49505	0.8265
3	27.435	BB	0.1898	73.49426	5.11005	1.1344
Totals :				6478.95018	568.44830	

Signal 5: DAD1 E, Sig=280,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	BB	0.1545	2534.90454	219.80244	98.0531
2	13.197	BB	0.1035	18.30730	2.62107	0.7081
3	27.438	BB	0.1365	32.02457	3.32076	1.2387
Totals :				2585.23642	225.74427	

Signal 6: DAD1 F, Sig=280,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	BB	0.1545	2534.90454	219.80244	98.0531
2	13.197	BB	0.1035	18.30730	2.62107	0.7081
3	27.438	BB	0.1365	32.02457	3.32076	1.2387
Totals :				2585.23642	225.74427	

Signal 7: DAD1 G, Sig=300,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	BB	0.1517	2648.87939	234.58089	98.3005
2	13.197	BB	0.1041	19.77978	2.81020	0.7340
3	27.442	BB	0.1246	26.01523	2.74336	0.9654

Data File C:\CHEM32\1\DATA\LAXMAN\BLANK2-II-14103.D
Sample Name: LD-II-141-1blank2

Totals : 2694.67441 240.13445

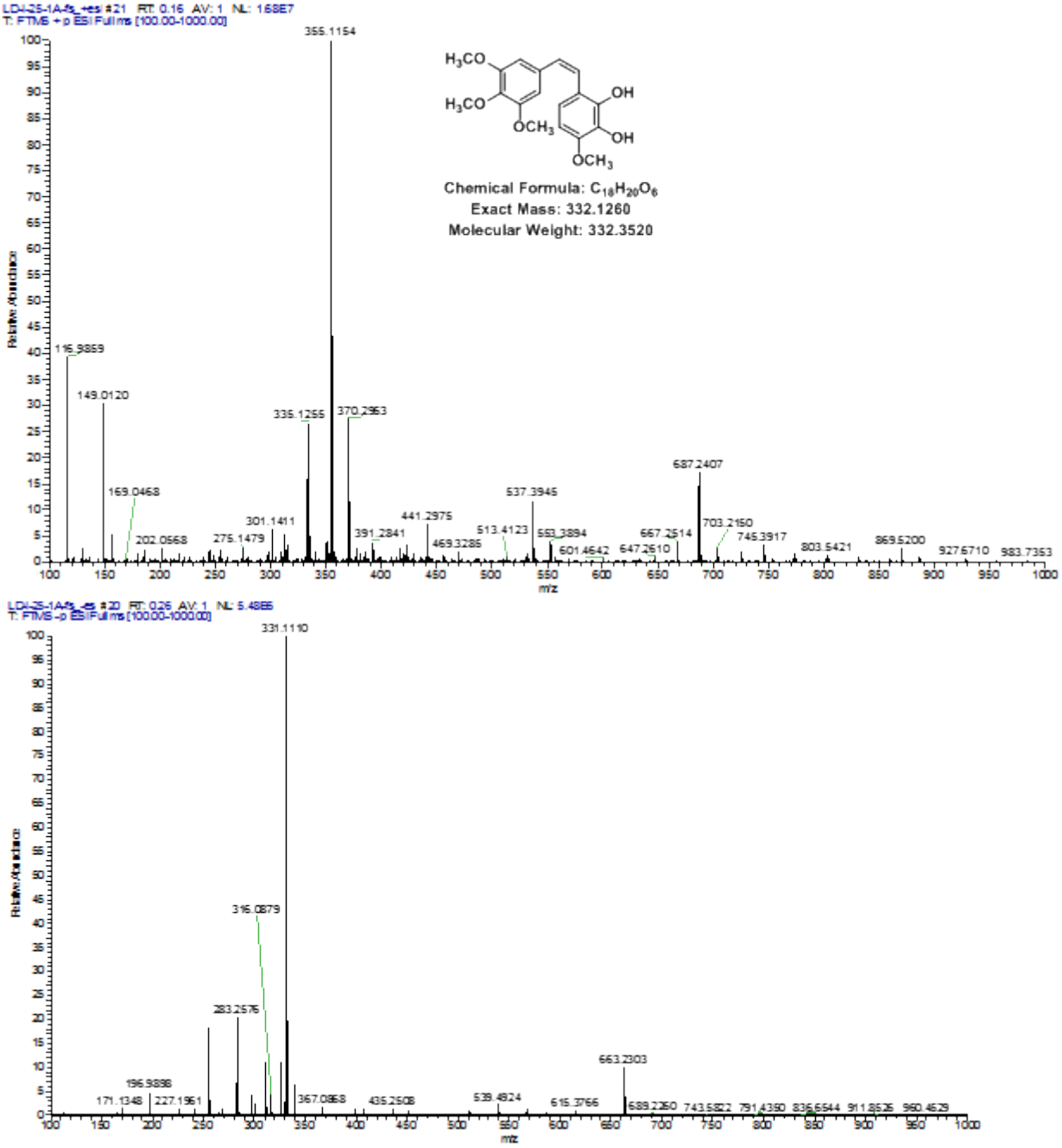
Signal 8: DAD1 H, Sig=320,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	BB	0.1474	1847.67163	169.15536	97.3511
2	13.196	BB	0.1628	29.10647	2.41100	1.5336
3	27.442	BB	0.1115	21.16774	2.53313	1.1153

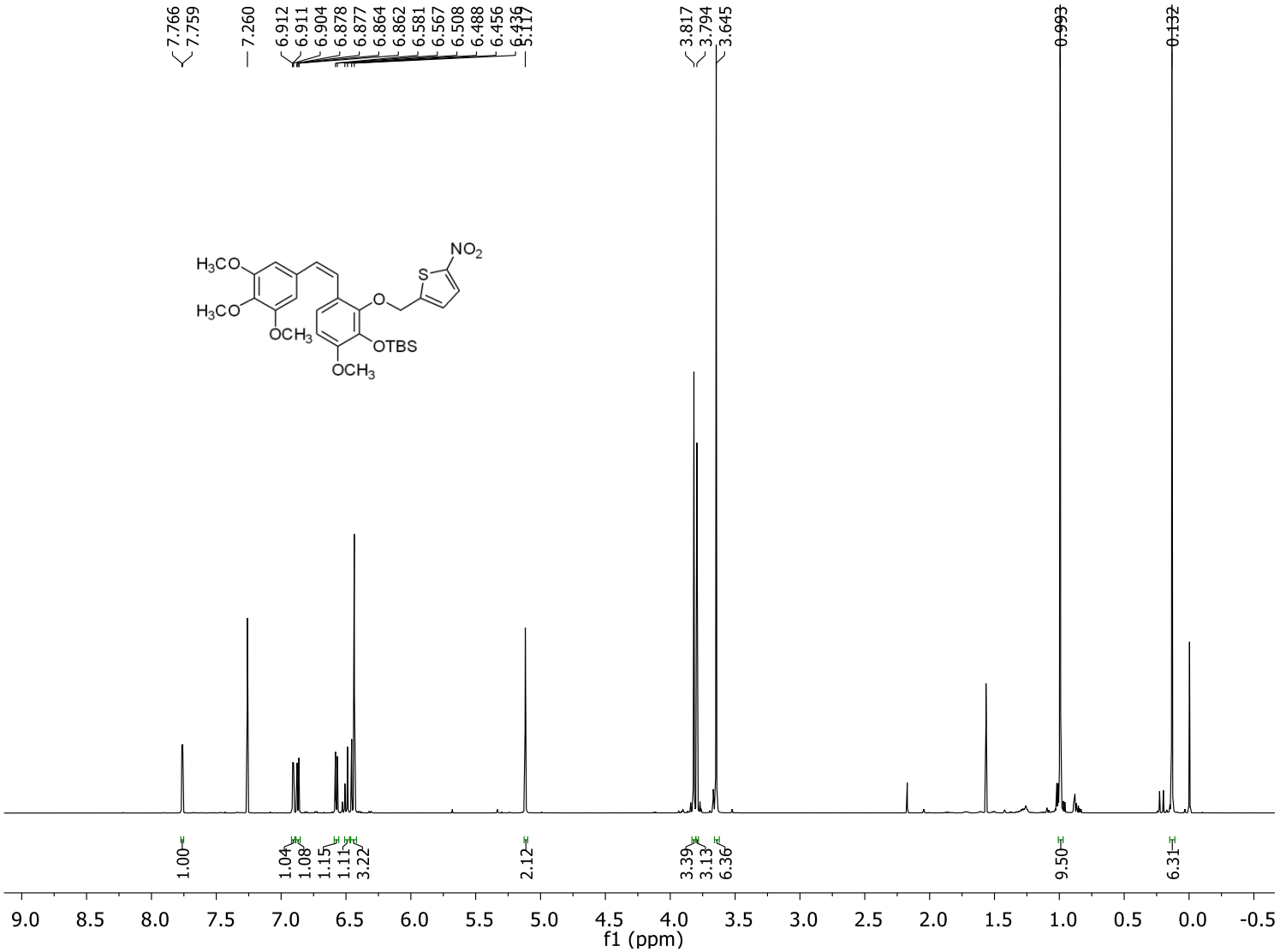
Totals : 1897.94584 174.09949

*** End of Report ***

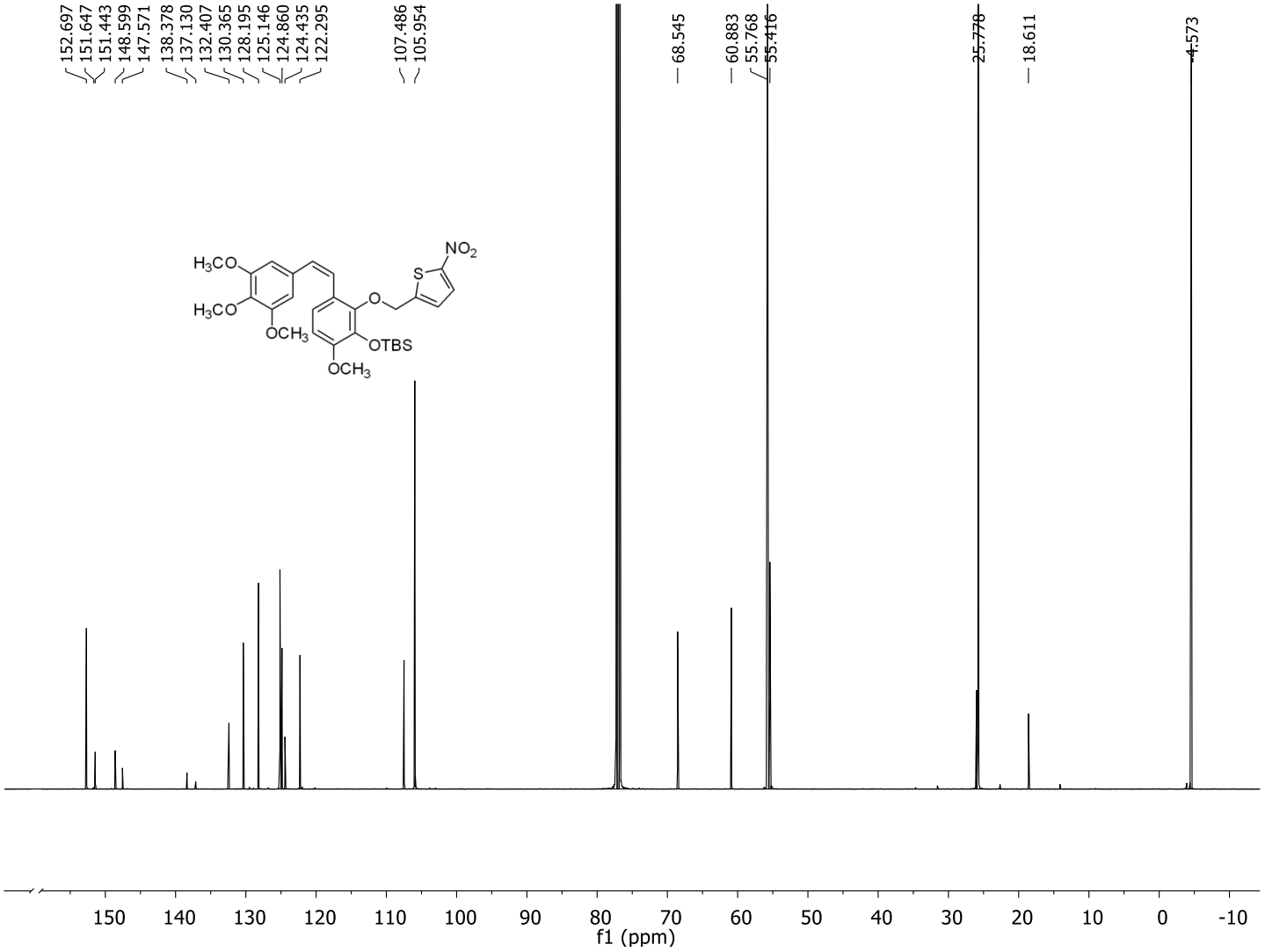
Mass Spectrum of Compound **29**



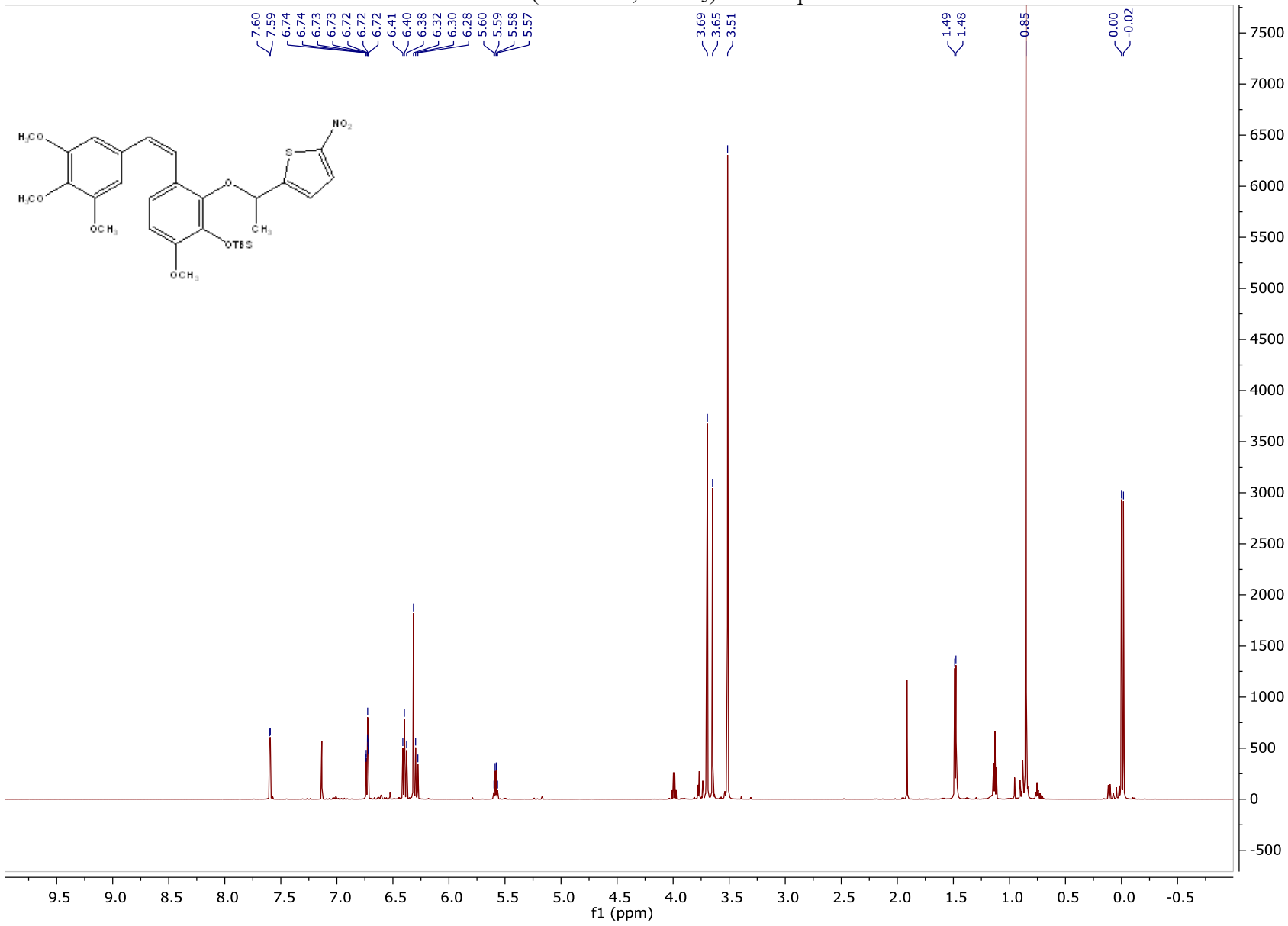
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **30**



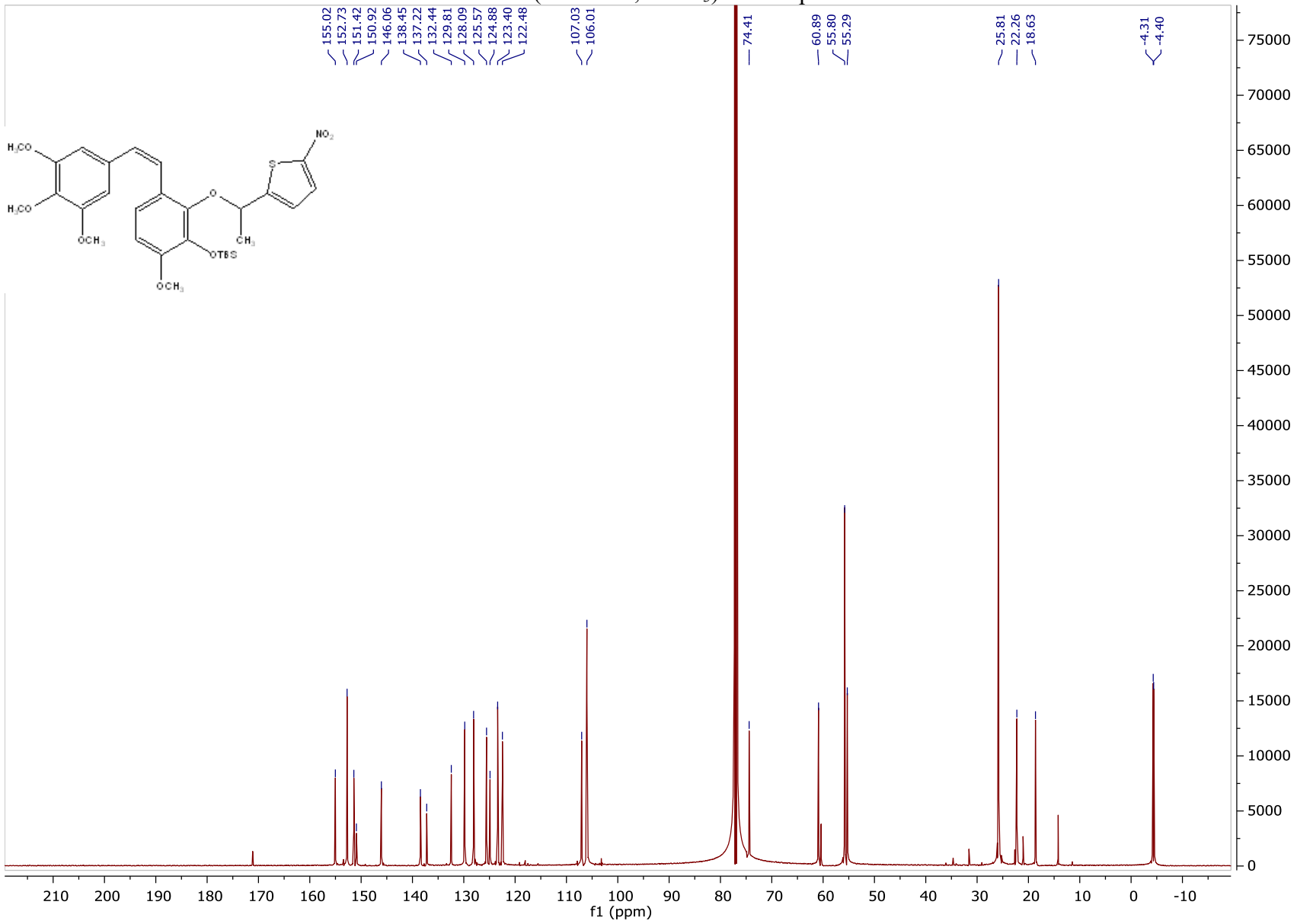
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **30**



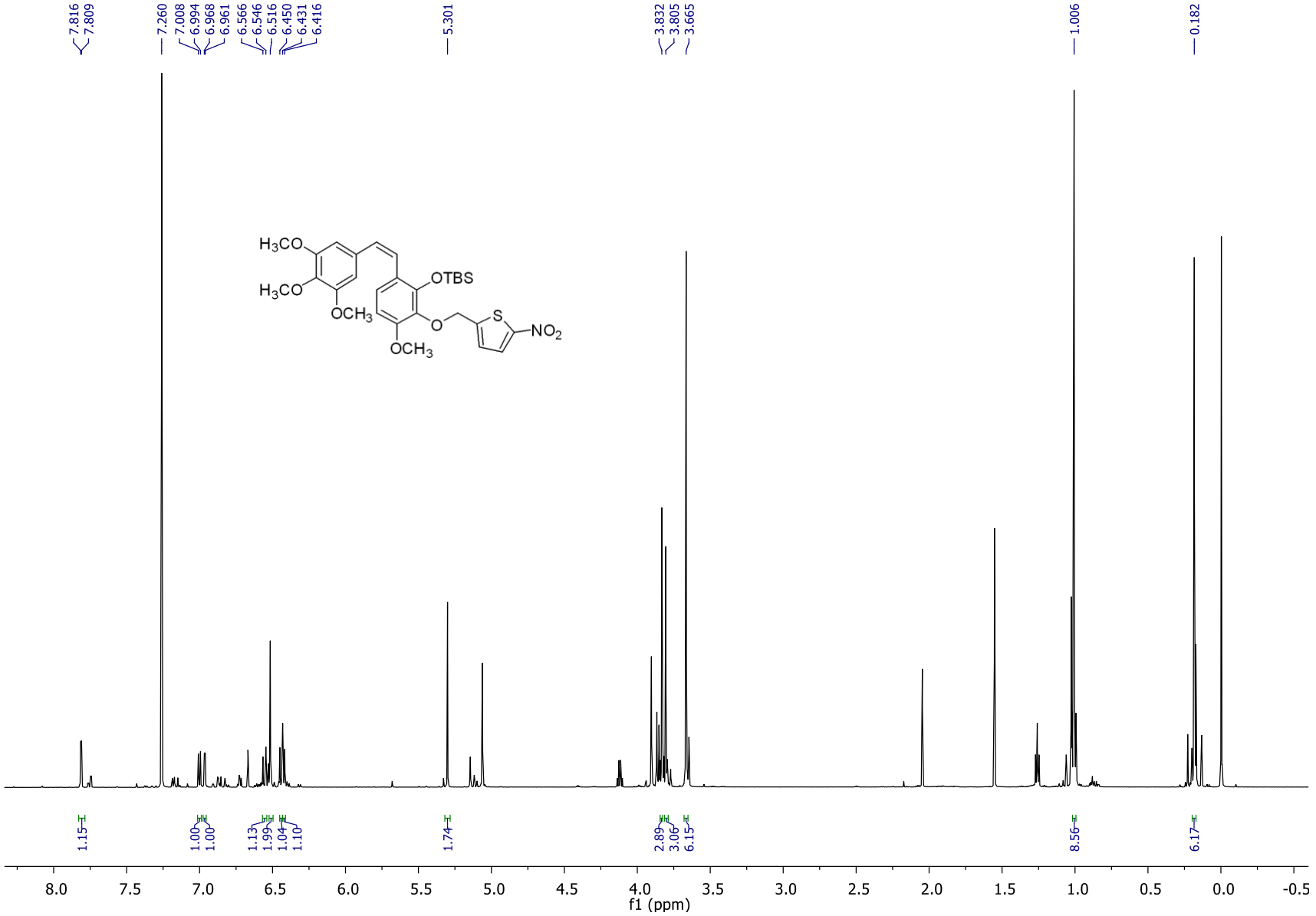
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **31**



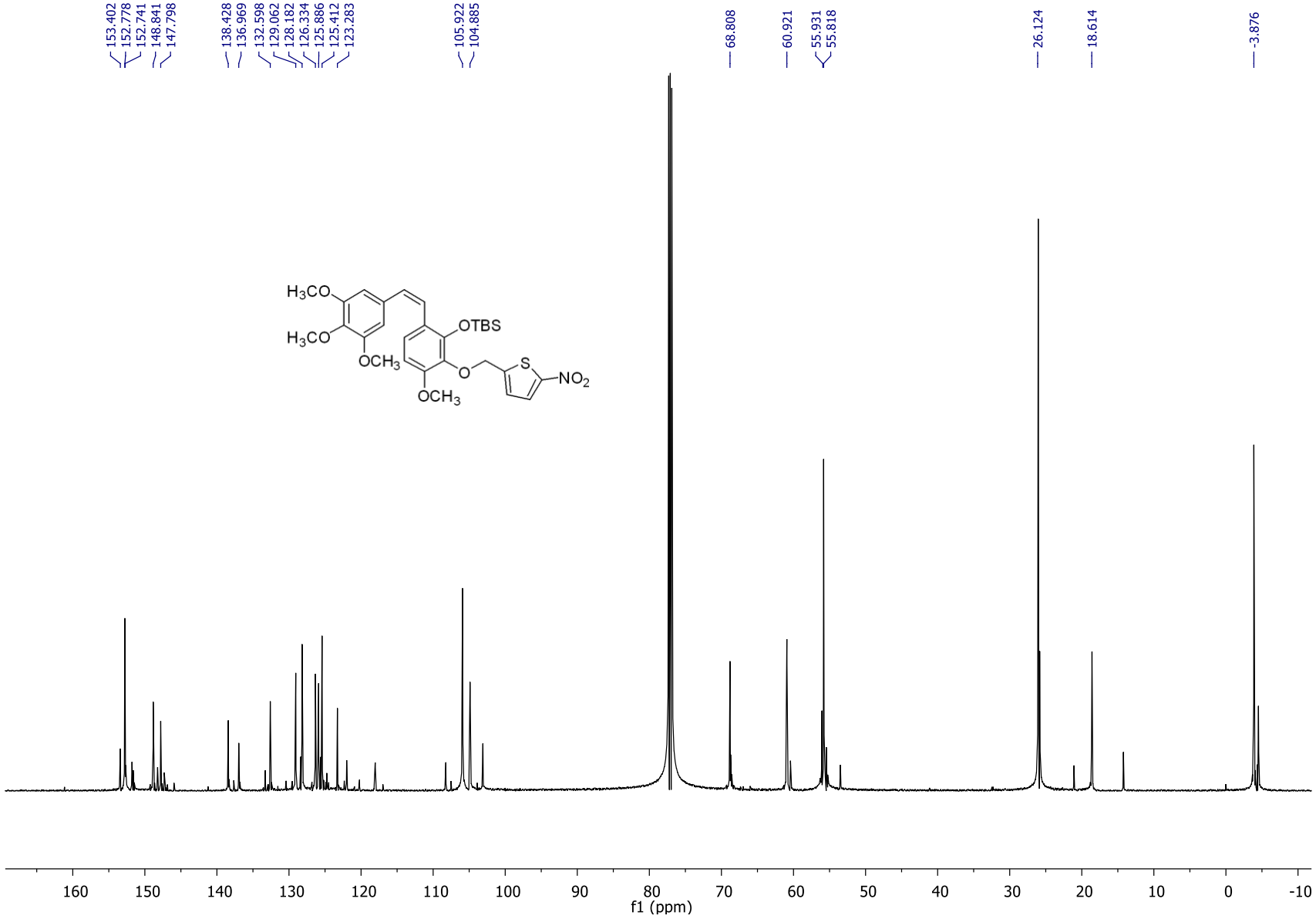
^{13}C NMR (500 MHz, $CDCl_3$) of Compound **31**



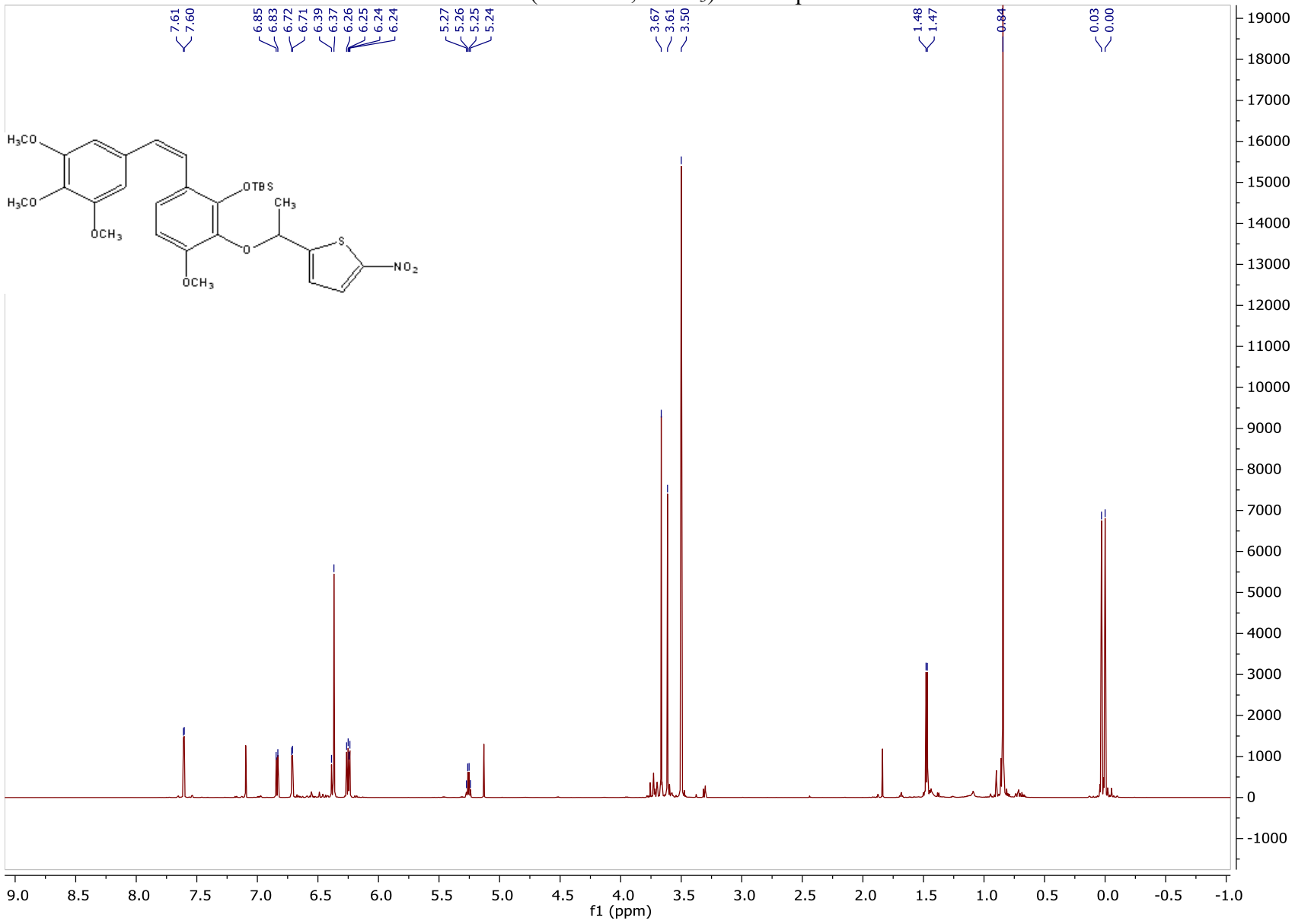
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **33**



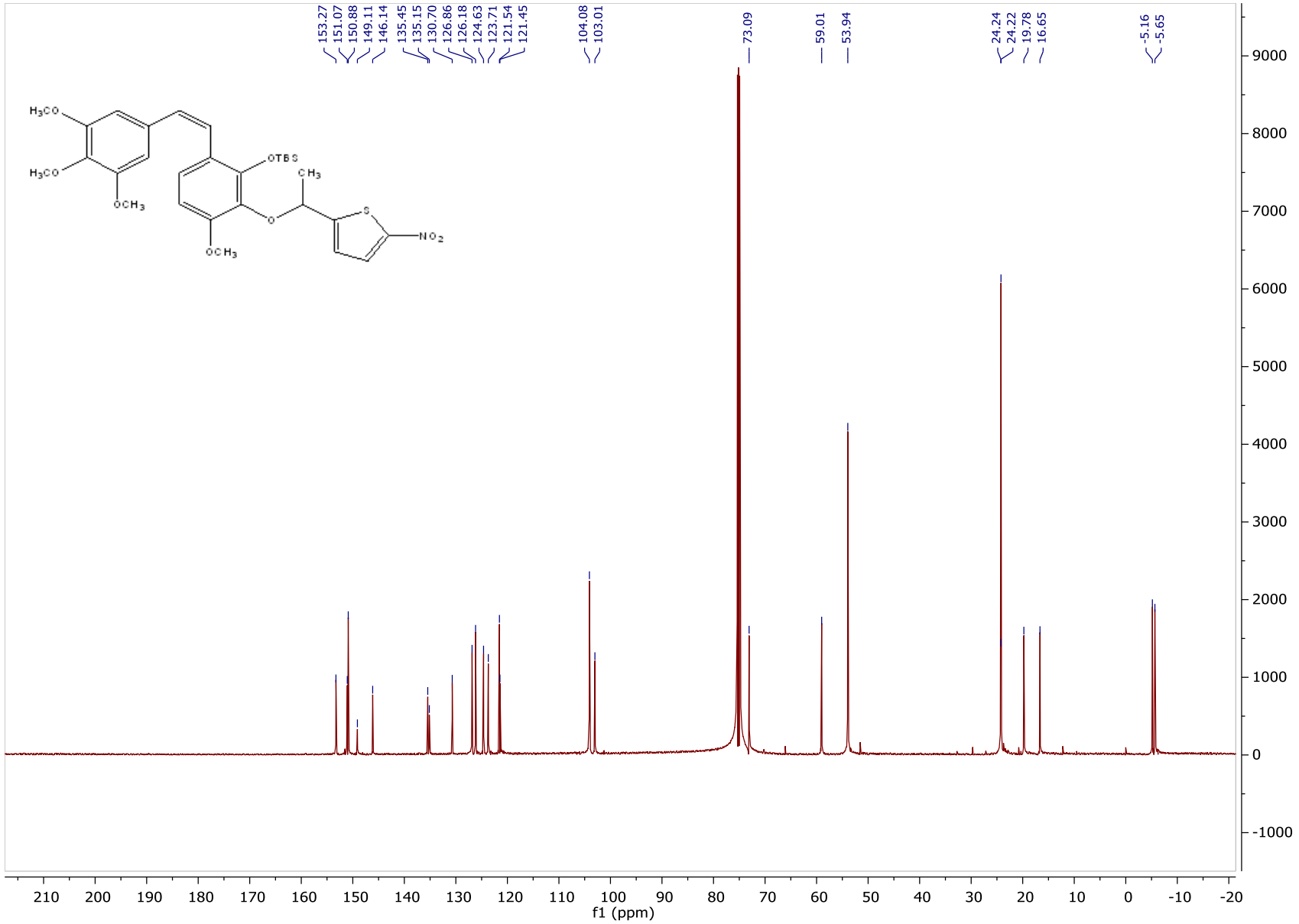
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **33**



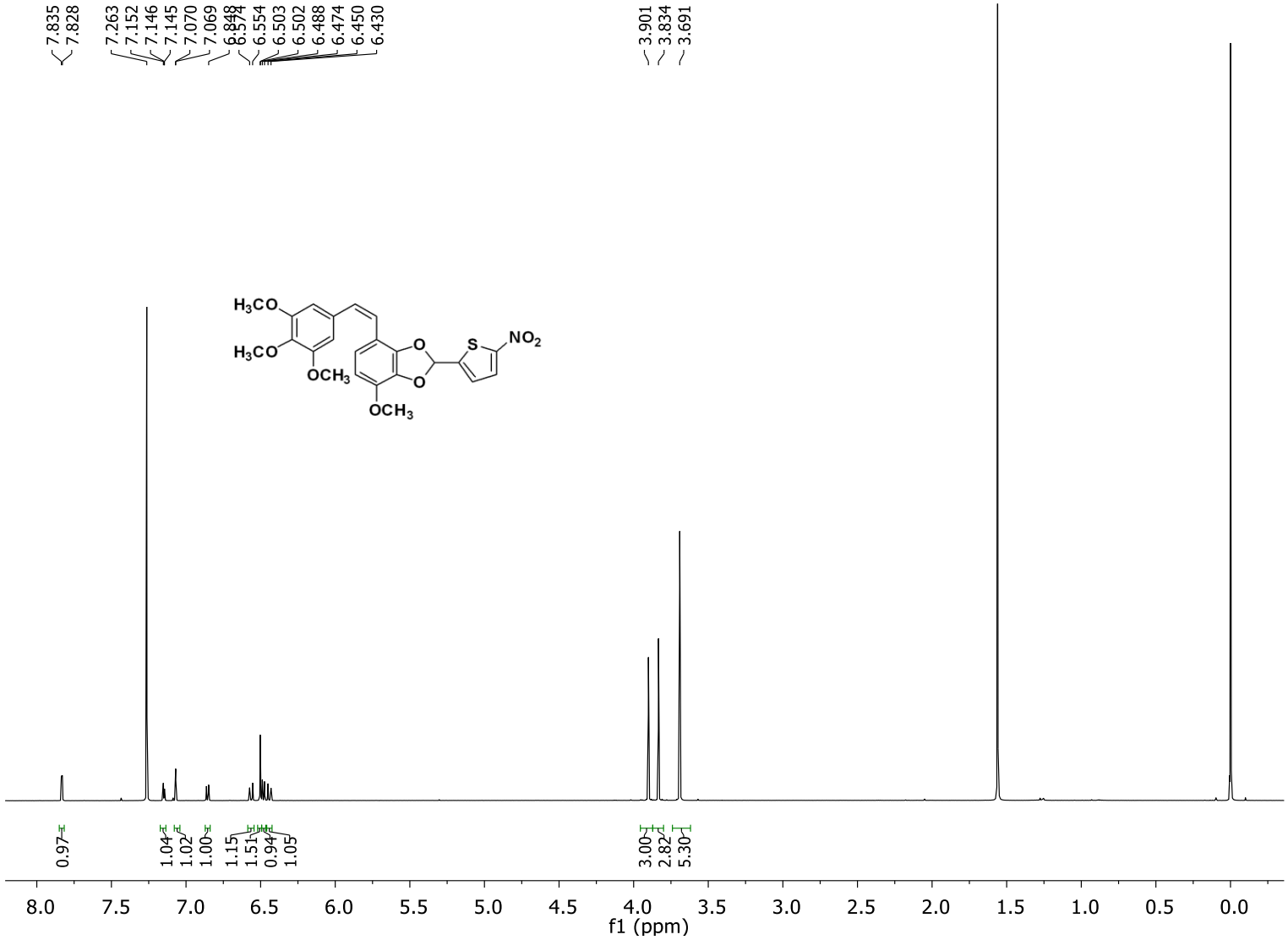
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **34**



^{13}C NMR (500 MHz, $CDCl_3$) of Compound **34**

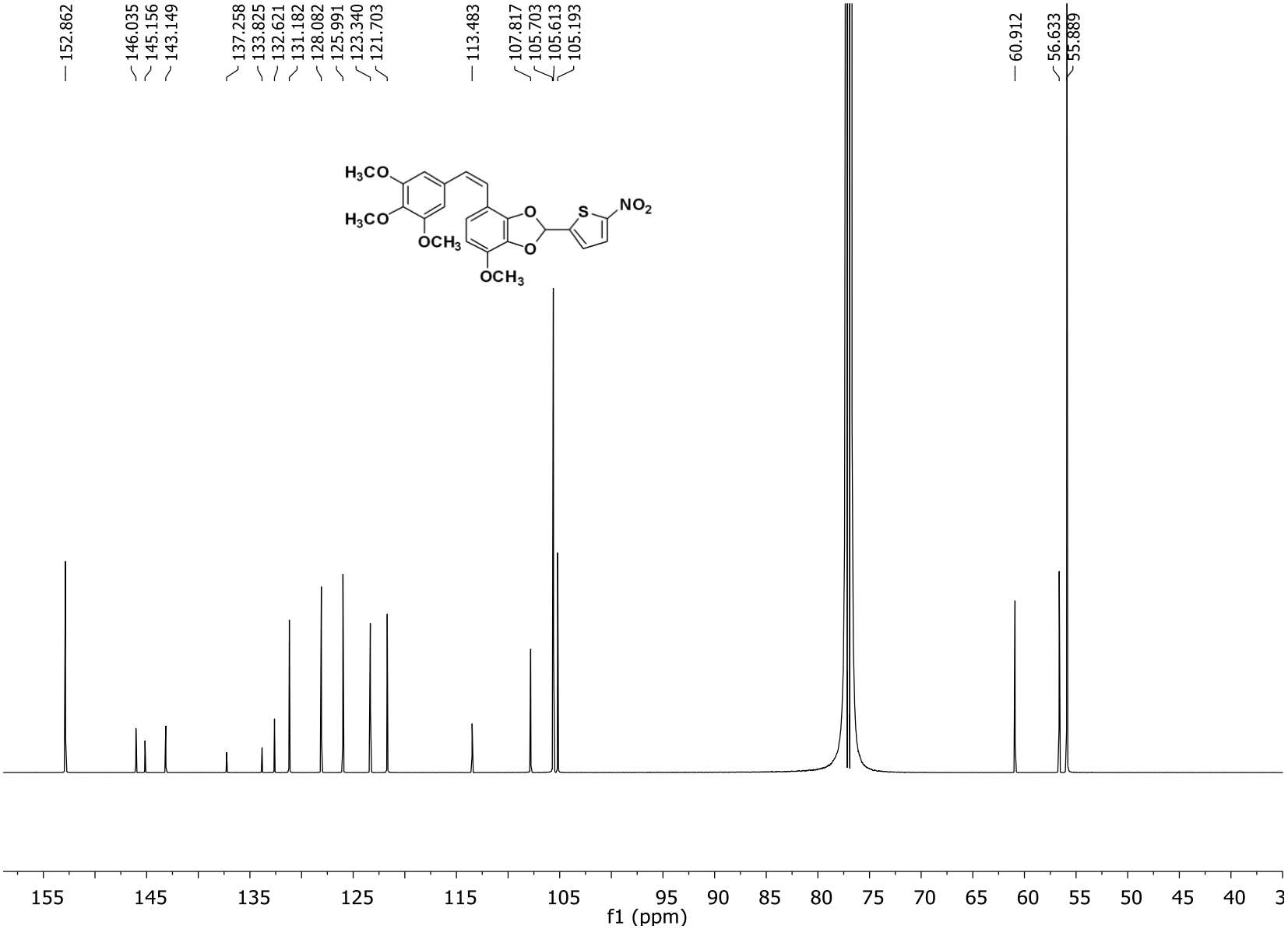


^{1}H NMR (500 MHz, $CDCl_3$) for Compound **35**

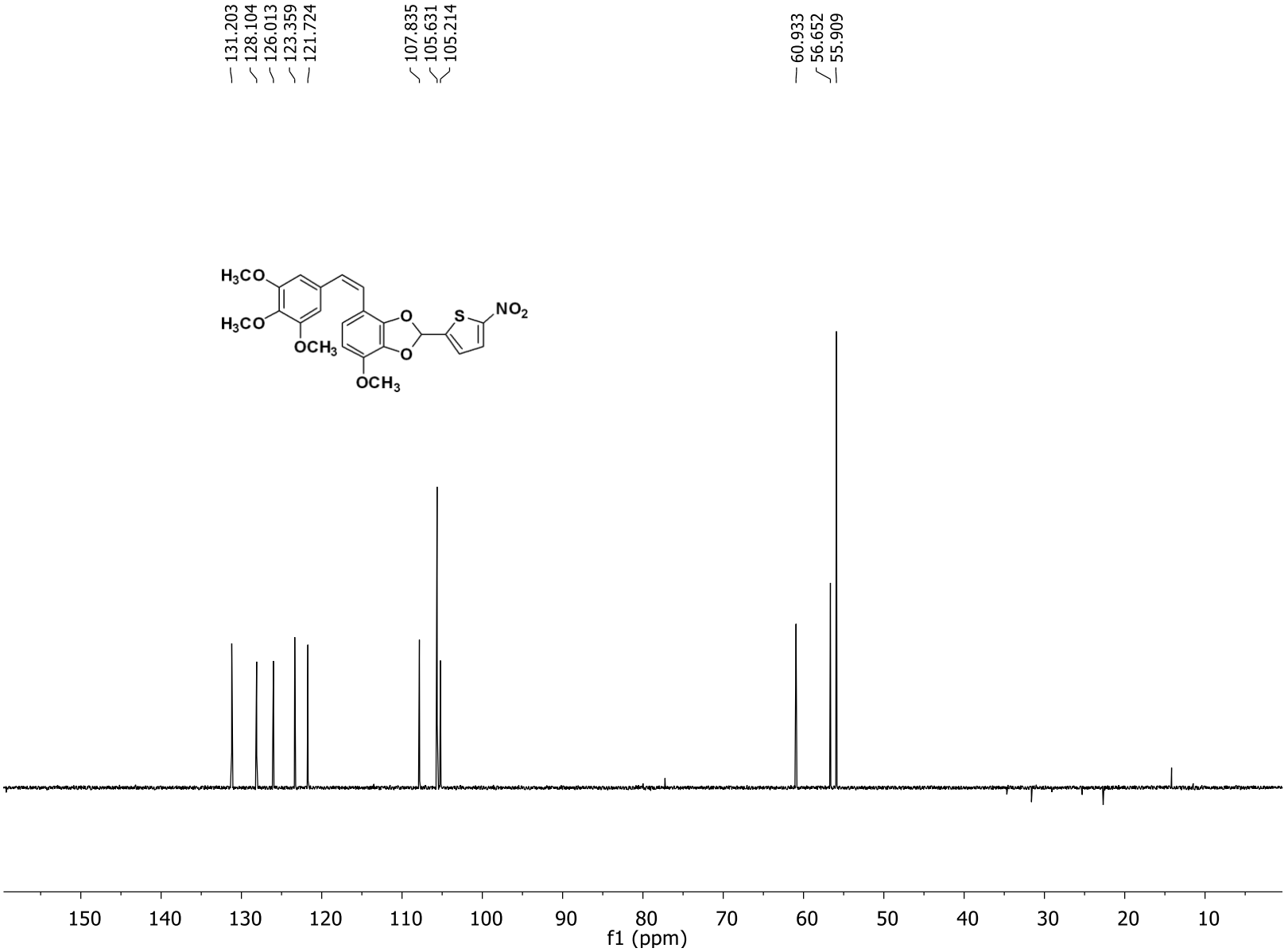


Note: The proton count drops at the non-aromatic nitrothiophene carbon for the nor-methyl cyclic structure **35**.

^{13}C NMR (125 MHz, $CDCl_3$) for Compound **35**



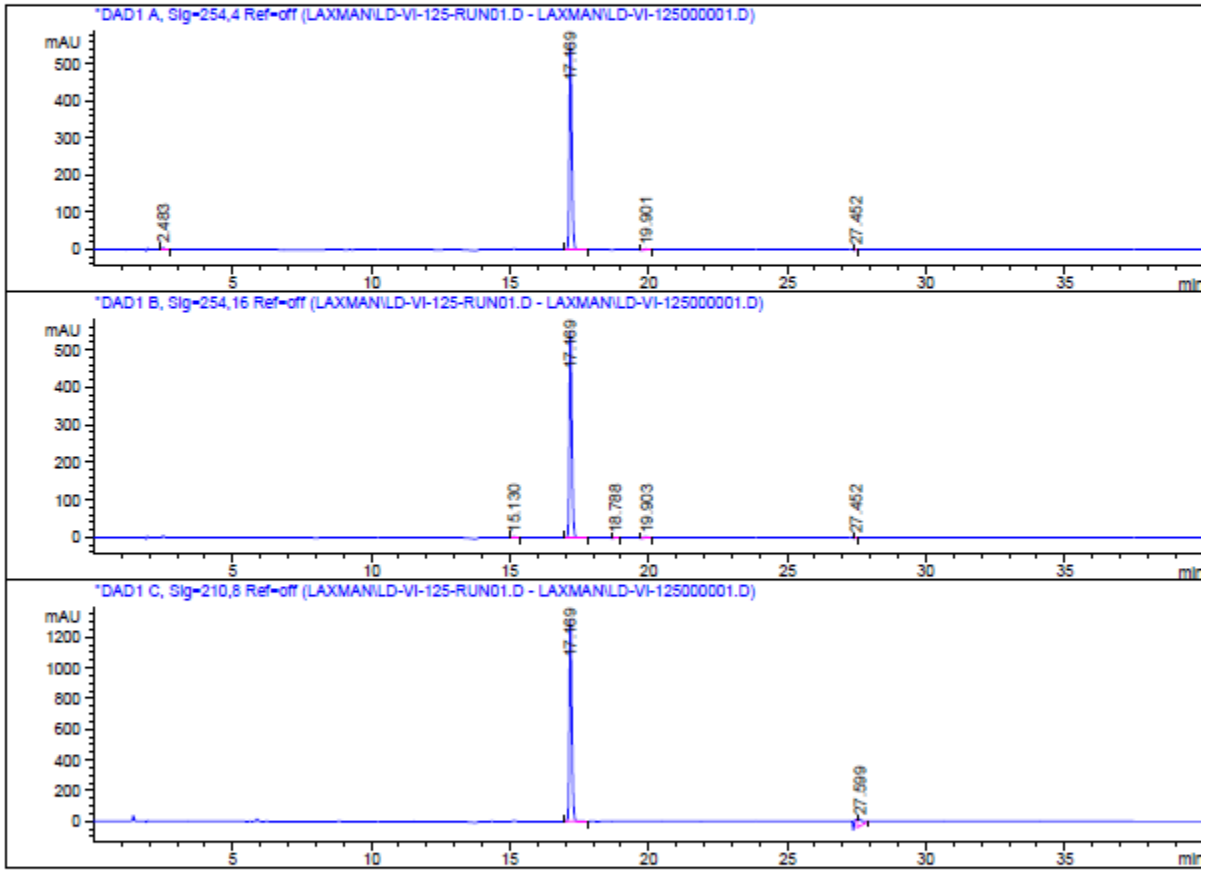
^{13}C DEPT NMR (125 MHz, $CDCl_3$) for Compound **35**



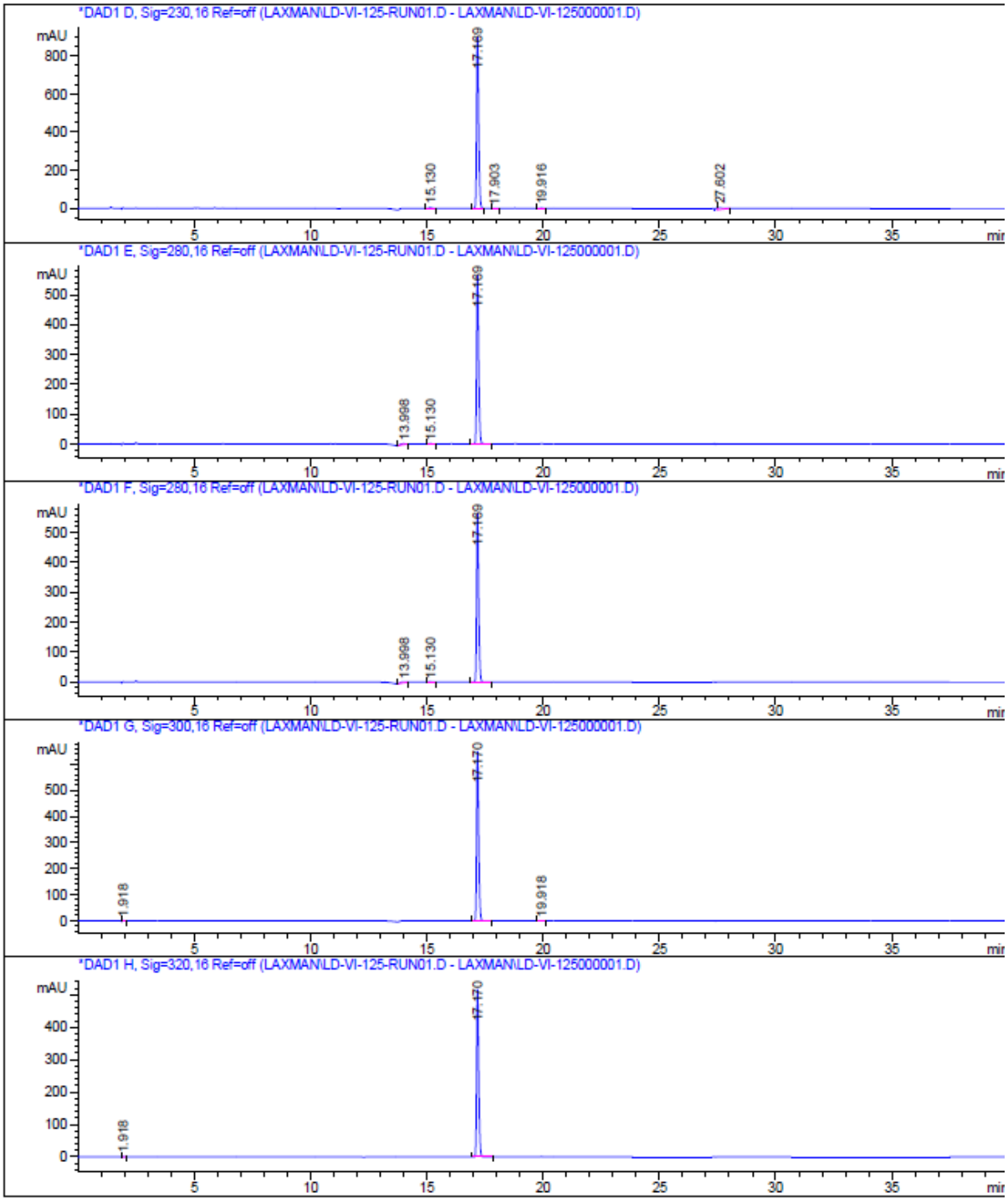
HPLC Traces for Compound **35**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-125-RUN01.D
Sample Name: LD-VI-125-1A-run1

    ======================================================================
    Acq. Operator    : Laxman
    Acq. Instrument : Instrument 1                    Location :    -
    Injection Date   : 12/9/2014 11:32:27 AM
    Acq. Method      : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed     : 12/9/2014 11:26:37 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-VI-125-RUN01.D\DA.M (MASTERMETHOD.M)
    Last changed     : 12/9/2014 12:37:21 PM by ERICA P
    Sample Info      : Method:Mastermethod
```



Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-125-RUN01.D
Sample Name: LD-VI-125-1A-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-125-RUN01.D
Sample Name: LD-VI-125-1A-run1

==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.483	BB	0.0712	21.61808	4.74181	0.6184
2	17.169	BB	0.0939	3441.88940	558.35913	98.4640
3	19.901	BB	0.1673	24.80698	1.93890	0.7097
4	27.452	BB	0.0756	7.26803	1.52501	0.2079
Totals :				3495.58249	566.56485	

Signal 2: DAD1 B, Sig=254,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.130	BB	0.1068	15.60068	2.14649	0.4513
2	17.169	BB	0.0939	3399.50537	551.31744	98.3460
3	18.788	BB	0.1392	12.07905	1.36572	0.3494
4	19.903	BB	0.1665	22.60753	1.80040	0.6540
5	27.452	BB	0.0773	6.88721	1.45335	0.1992
Totals :				3456.67985	558.08340	

Signal 3: DAD1 C, Sig=210,8 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.169	BB	0.0946	8231.09180	1323.41785	92.6451
2	27.599	VB	0.1942	653.44891	46.99881	7.3549
Totals :				8884.54071	1370.41666	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-125-RUN01.D
Sample Name: LD-VI-125-1A-run1

Signal 4: DAD1 D, Sig=230,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.130	BB	0.1026	29.53756	4.27385	0.5128
2	17.169	BB	0.0936	5548.04736	903.54651	96.3151
3	17.903	BB	0.1581	17.66217	1.58403	0.3066
4	19.916	BB	0.1198	11.05690	1.31928	0.1919
5	27.602	VB	0.2266	154.00311	9.04747	2.6735
Totals :				5760.30710	919.77115	

Signal 5: DAD1 E, Sig=280,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.998	BB	0.2883	63.81478	3.37971	1.7886
2	15.130	BB	0.1106	10.40754	1.40286	0.2917
3	17.169	BB	0.0939	3493.63037	567.18445	97.9197
Totals :				3567.85270	571.96701	

Signal 6: DAD1 F, Sig=280,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.998	BB	0.2883	63.81478	3.37971	1.7886
2	15.130	BB	0.1106	10.40754	1.40286	0.2917
3	17.169	BB	0.0939	3493.63037	567.18445	97.9197
Totals :				3567.85270	571.96701	

Signal 7: DAD1 G, Sig=300,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.918	BB	0.0755	18.05997	3.31663	0.4456
2	17.170	BB	0.0942	4028.34277	651.30194	99.3971

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-125-RUN01.D
Sample Name: LD-VI-125-1A-run1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
3	19.918	BB	0.0962	6.37247	1.00239	0.1572
Totals :				4052.77521	655.62096	

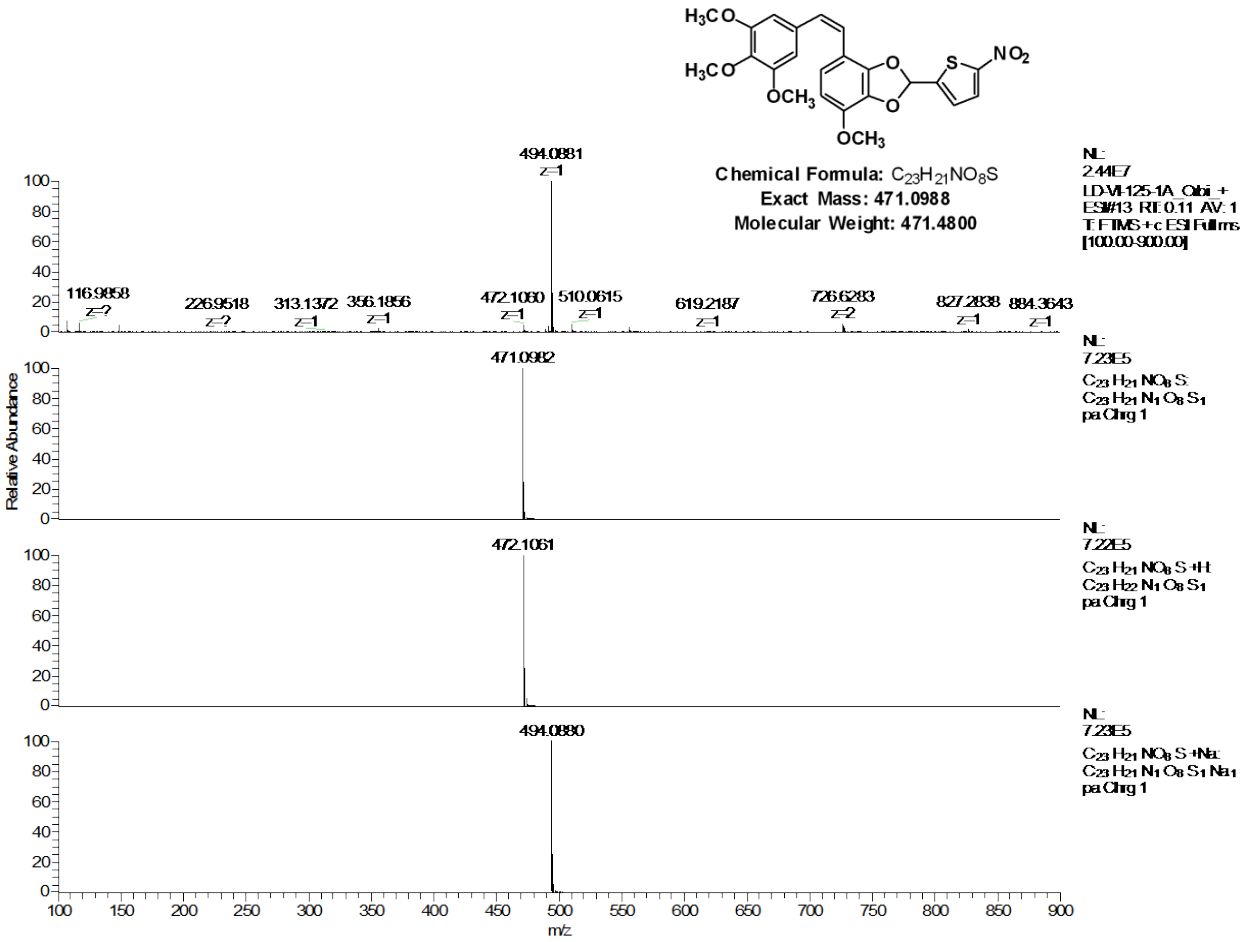
Signal 8: DAD1 H, Sig=320,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.918	BB	0.0723	15.04224	2.90981	0.4641
2	17.170	BB	0.0947	3226.43042	517.99158	99.5359
Totals :				3241.47266	520.90138	

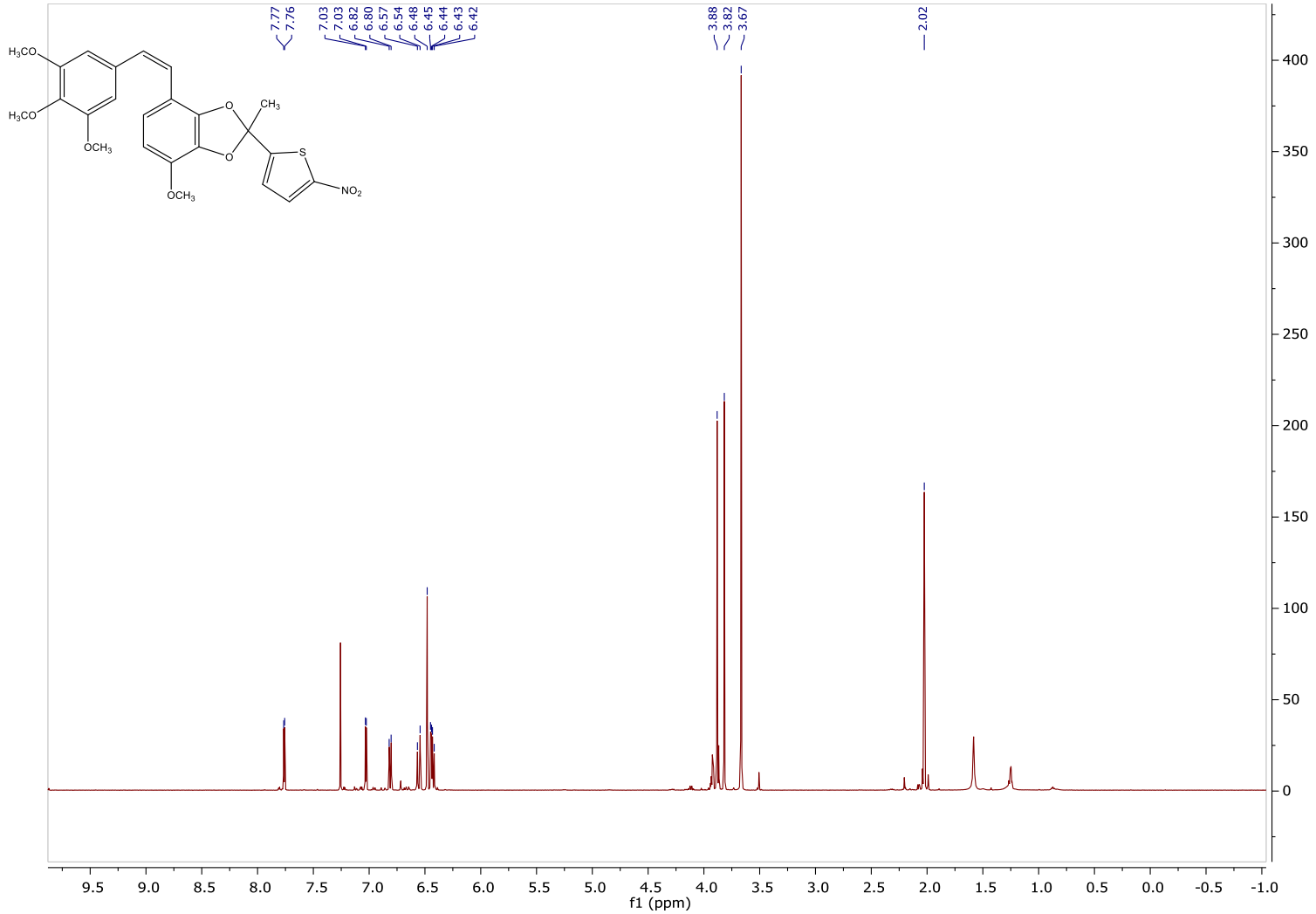
==========

*** End of Report ***

Mass Spectrum for Compound **35**

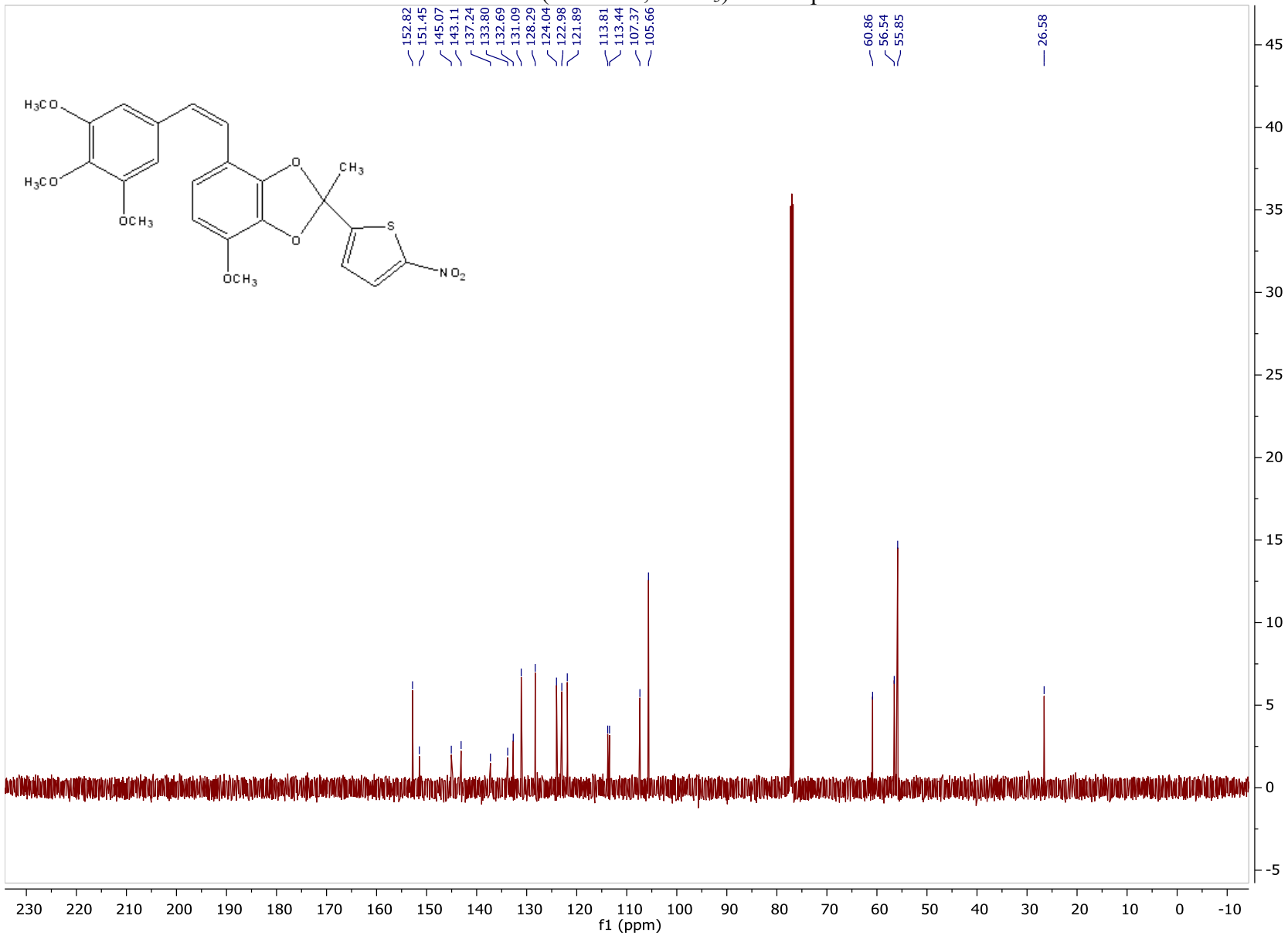


^{1}H NMR (500 MHz, $CDCl_3$) of Compound **36**



Note: The proton signal at the non-aromatic nitrothiophene carbon disappears for the *mono*-methyl cyclic structure **36**.

^{13}C NMR (500 MHz, $CDCl_3$) of Compound **36**



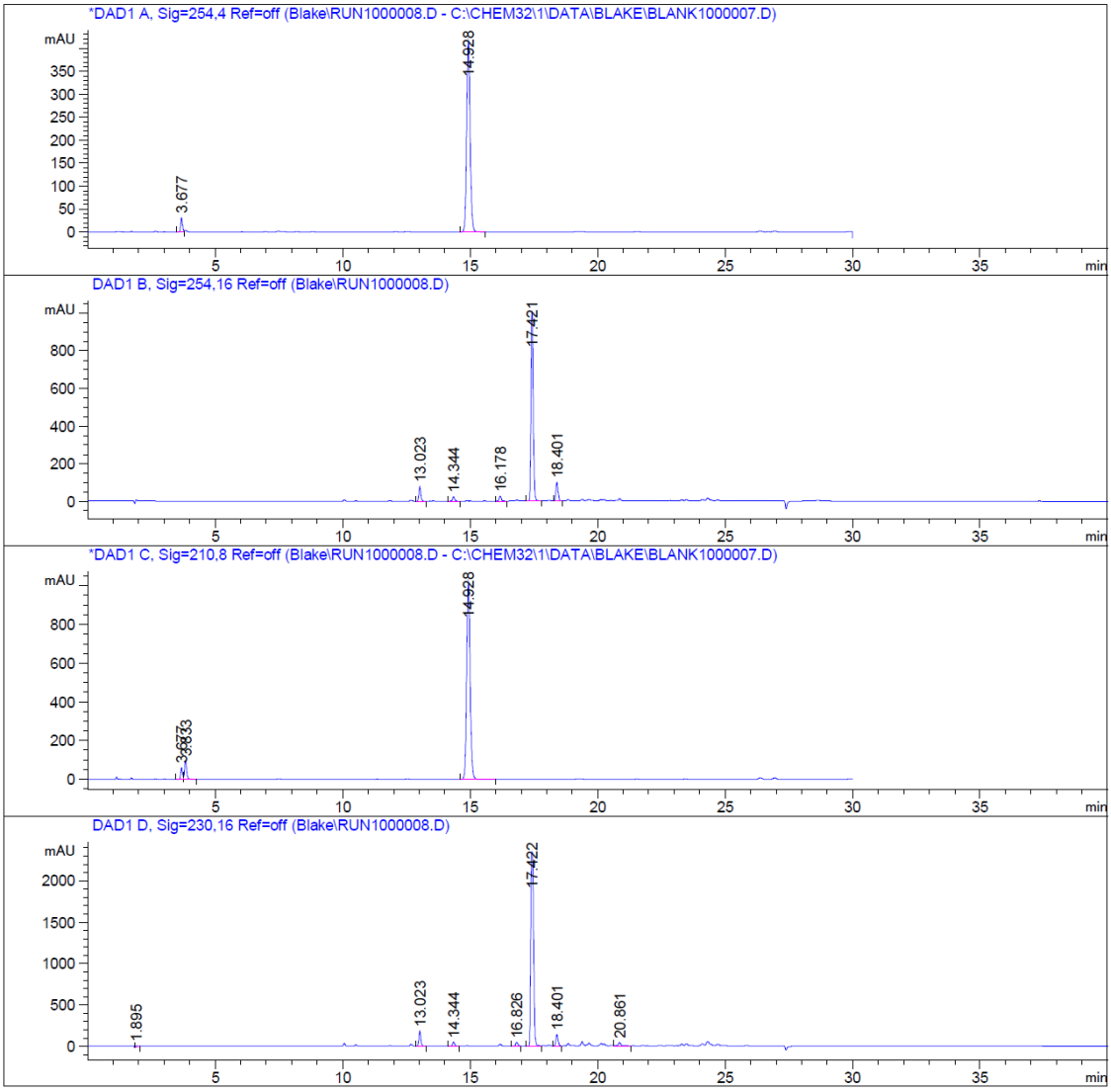
HPLC Trace of Compound **36**

Data File C:\Chem32\1\Data\Blake\RUN1000008.D
Sample Name: DiboundMonoCA1 Run1

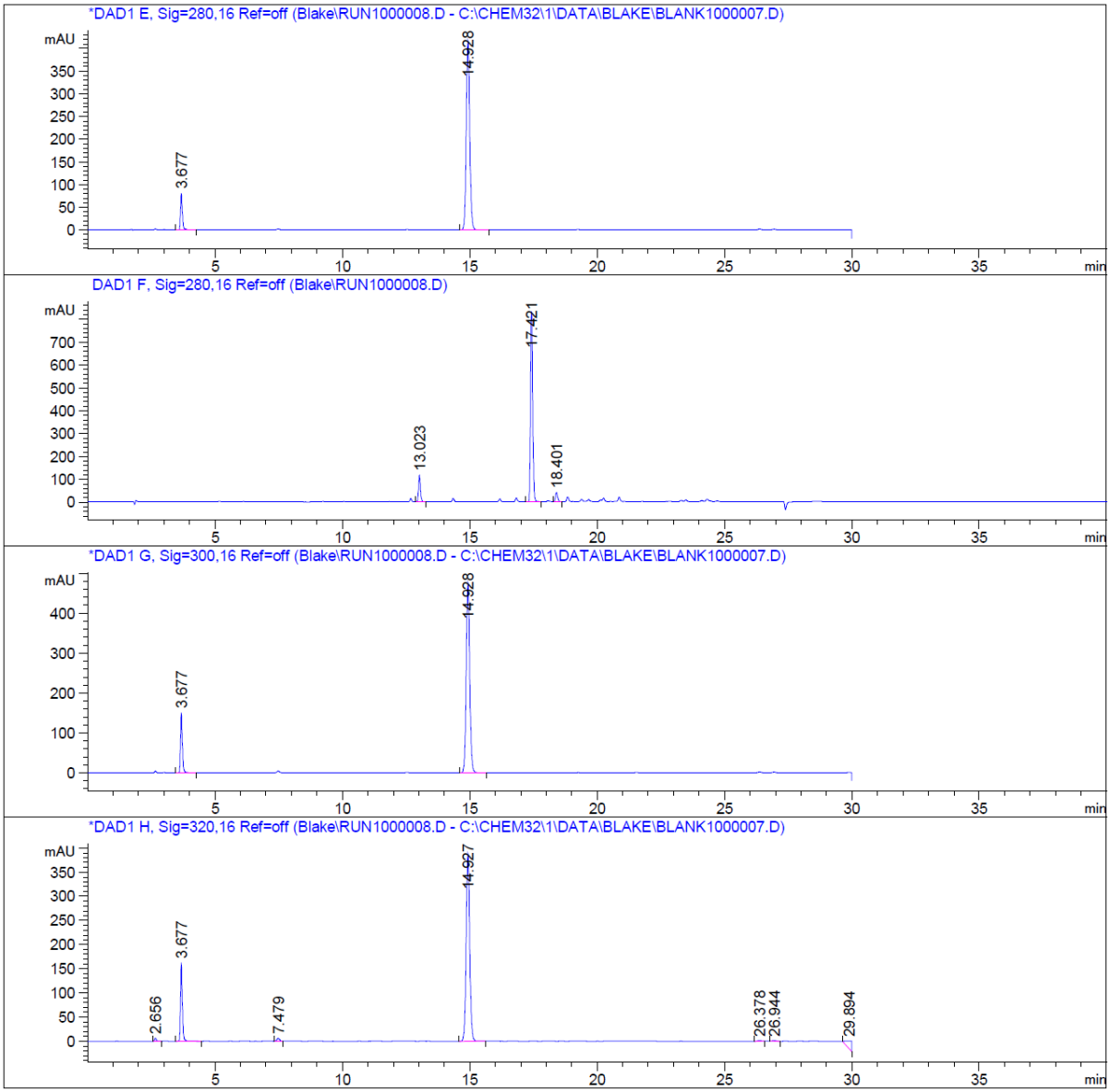
```
==========================================================================
Acq. Operator   : Blake
Acq. Instrument : Instrument 1                    Location :     -
Injection Date  : 3/5/2015 10:53:54 PM
Acq. Method     : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
Last changed    : 3/5/2015 10:44:03 PM by Blake
Analysis Method : C:\CHEM32\1\METHODS\RT-ACNWASH 2.M
Last changed    : 7/9/2015 2:27:22 PM by Blake
Method Info     : General Column Wash Method

Sample Info     : Dibound Mono Trigger CA1 Run 1

Additional Info : Peak(s) manually integrated
```



Data File C:\Chem32\1\Data\Blake\RUN1000008.D
Sample Name: DiboundMonoCA1 Run1



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Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Data File C:\Chem32\1\Data\Blake\RUN1000008.D
Sample Name: DiboundMonoCA1 Run1

Signal 1: DAD1 A, Sig=254,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.677	BV	0.0751	154.96552	31.65848	3.7729
2	14.928	BB	0.1464	3952.39966	417.61395	96.2271
Totals :				4107.36517	449.27243	

Signal 2: DAD1 B, Sig=254,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.023	BB	0.0792	412.37903	78.59989	5.5884
2	14.344	BB	0.0864	140.51746	24.69783	1.9042
3	16.178	BB	0.0870	150.26970	26.17360	2.0364
4	17.421	BV	0.0921	6080.55078	1011.67804	82.4012
5	18.401	VB	0.0914	595.48279	100.03498	8.0697
Totals :				7379.19975	1241.18435	

Signal 3: DAD1 C, Sig=210,8 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.677	BV	0.0737	292.19226	61.24960	2.7847
2	3.833	VB	0.0865	548.90851	96.23808	5.2313
3	14.928	BB	0.1461	9651.77344	1023.13129	91.9841
Totals :				1.04929e4	1180.61897	

Signal 4: DAD1 D, Sig=230,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.895	BB	0.0813	104.26080	18.61610	0.5003
2	13.023	BB	0.0792	970.77802	184.92006	4.6584
3	14.344	BB	0.0886	307.83749	52.38946	1.4772
4	16.826	VV	0.0895	281.78674	47.30568	1.3522
5	17.422	BV	0.1256	1.80297e4	2346.77515	86.5168
6	18.401	VB	0.0910	824.25653	139.37587	3.9553
7	20.861	VV	0.1067	320.90729	43.16537	1.5399
Totals :				2.08395e4	2832.54768	

Data File C:\Chem32\1\Data\Blake\RUN1000008.D
Sample Name: DiboundMonoCA1 Run1

Signal 5: DAD1 E, Sig=280,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.677	BB	0.0774	414.77179	81.48527	9.4891
2	14.928	BB	0.1464	3956.28125	417.95911	90.5109
Totals :				4371.05304	499.44437	

Signal 6: DAD1 F, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.023	BB	0.0793	614.59393	117.06161	10.6630
2	17.421	BV	0.0908	4903.08594	831.98090	85.0673
3	18.401	VV	0.0920	246.09302	41.01019	4.2697
Totals :				5763.77289	990.05270	

Signal 7: DAD1 G, Sig=300,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.677	BB	0.0764	758.47052	151.60106	14.2607
2	14.928	BB	0.1475	4560.13281	476.96796	85.7393
Totals :				5318.60333	628.56902	

Signal 8: DAD1 H, Sig=320,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.656	BB	0.0669	29.37908	6.73783	0.5950
2	3.677	BB	0.0764	815.72125	162.95384	16.5208
3	7.479	BB	0.1111	41.89455	5.88280	0.8485
4	14.927	BB	0.1491	3772.36133	389.08377	76.4016
5	26.378	BB	0.1392	15.31042	1.69743	0.3101
6	26.944	BB	0.1383	14.27374	1.59684	0.2891
7	29.894	BBA	0.2220	248.60039	16.66612	5.0349
Totals :				4937.54077	584.61863	

Data File C:\Chem32\1\Data\Blake\RUN1000008.D
Sample Name: DiboundMonoCA1 Run1

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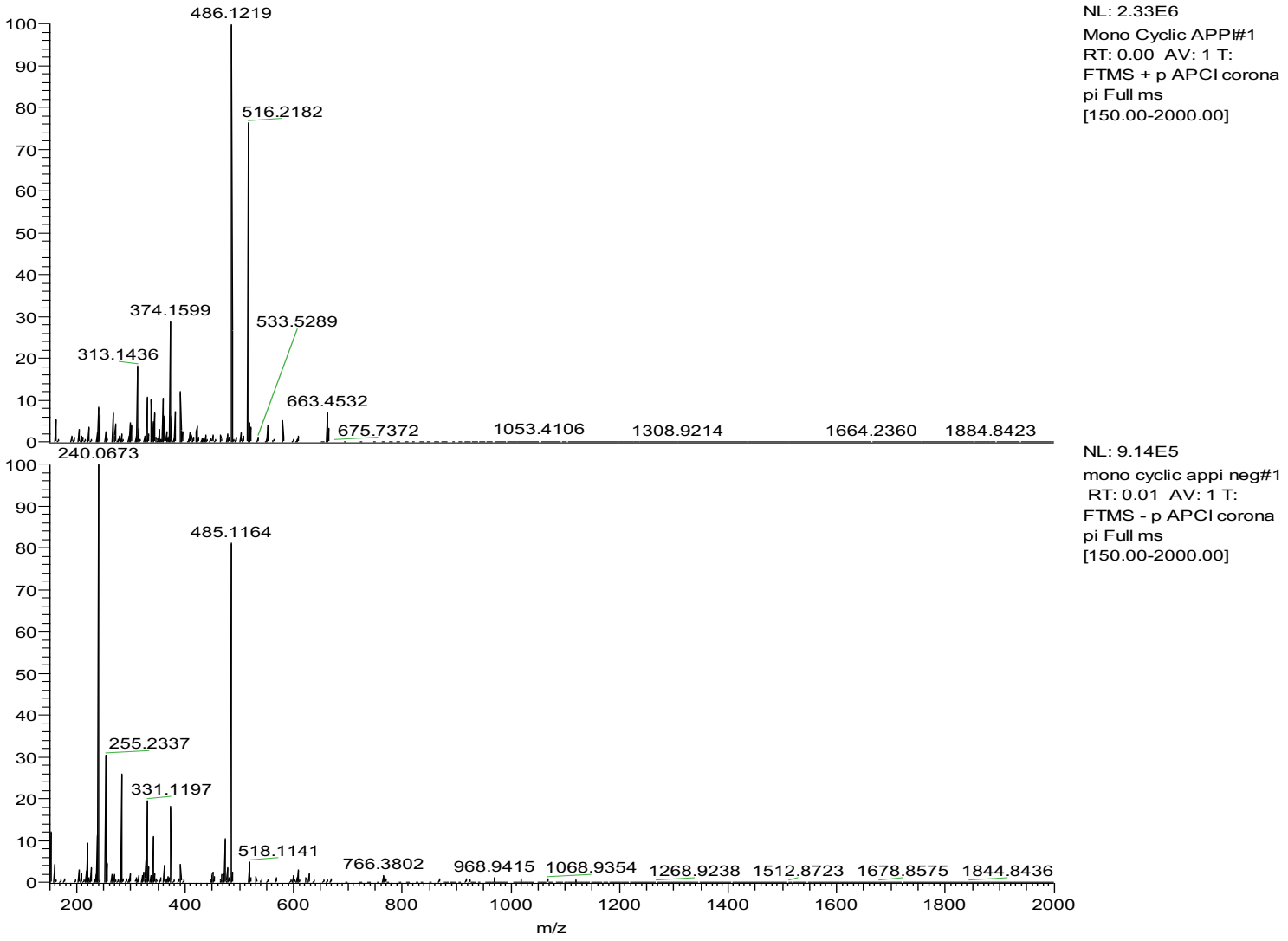
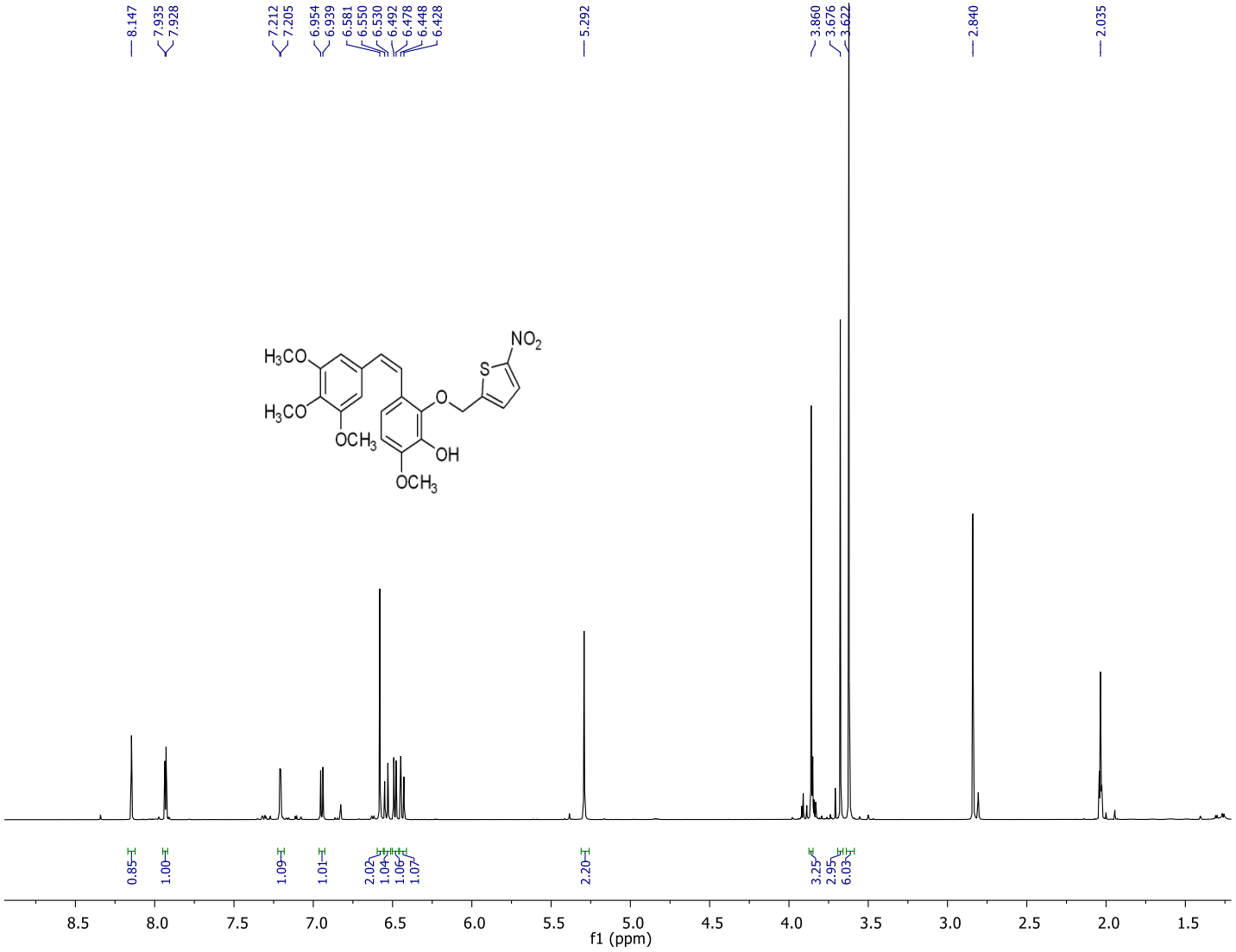
*** End of Report ***

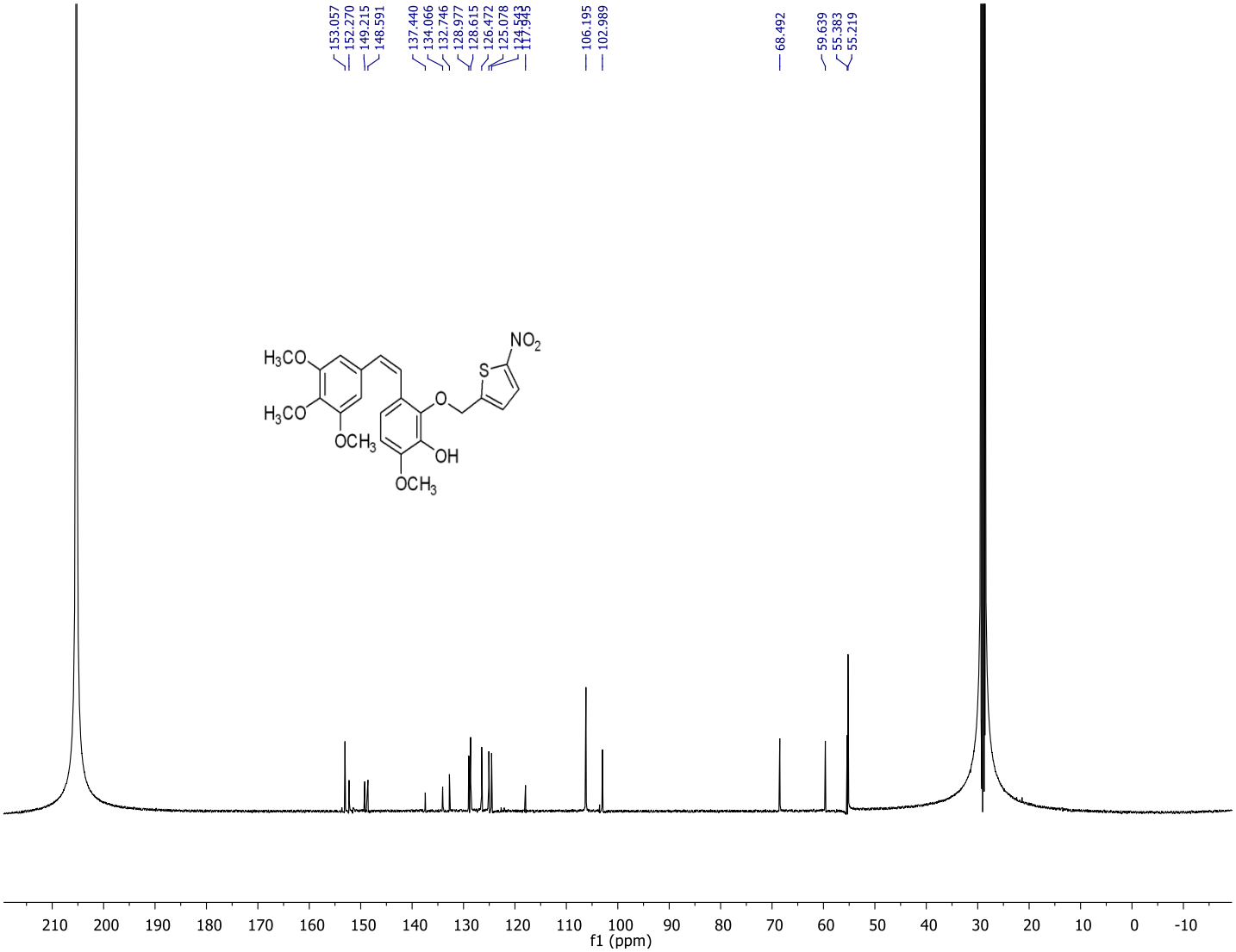
Mass Spectrum of Compound **36**



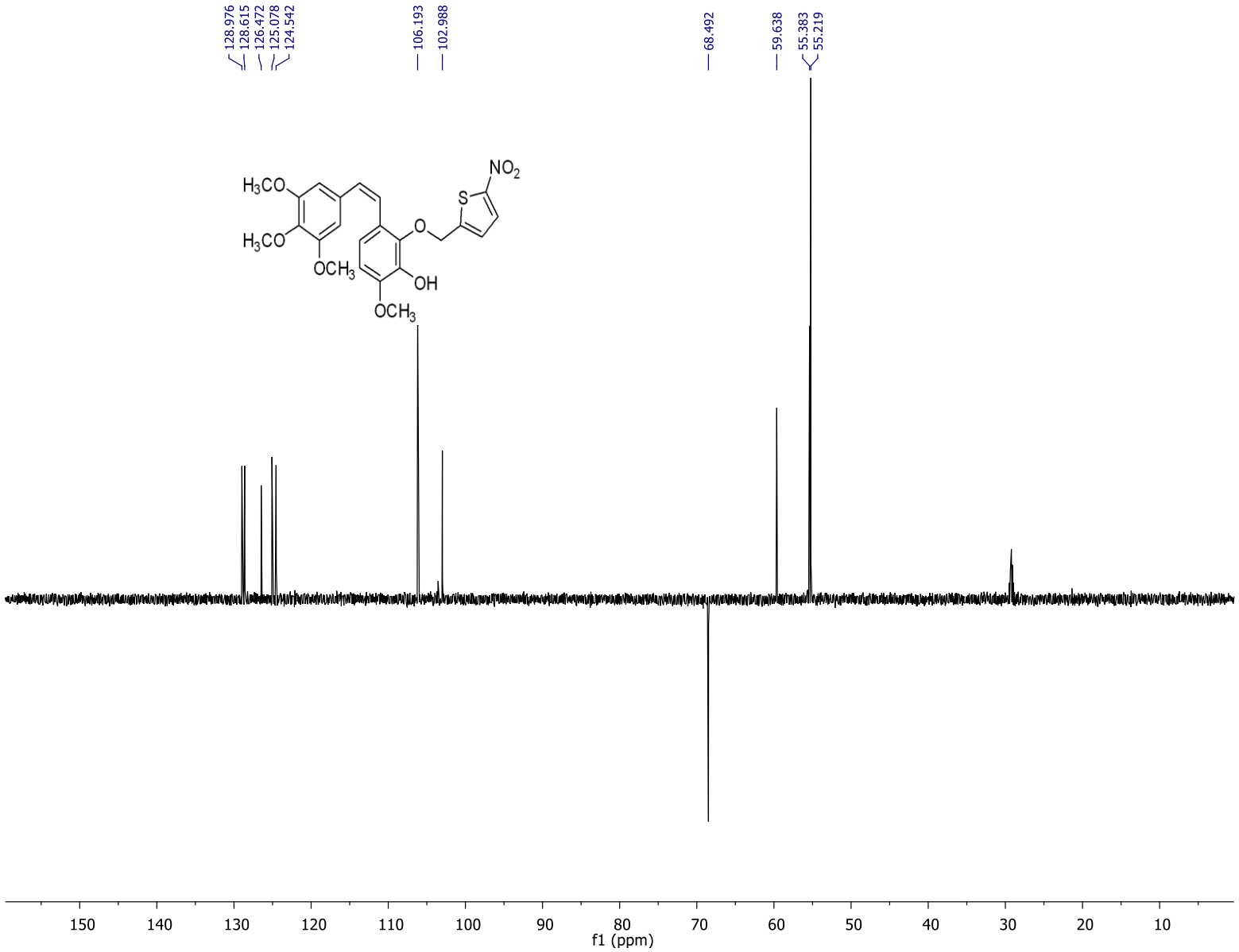
^{1}H NMR (600 MHz, Acetone) for Compound **37**



^{13}C NMR (151 MHz, Acetone) for Compound **37**



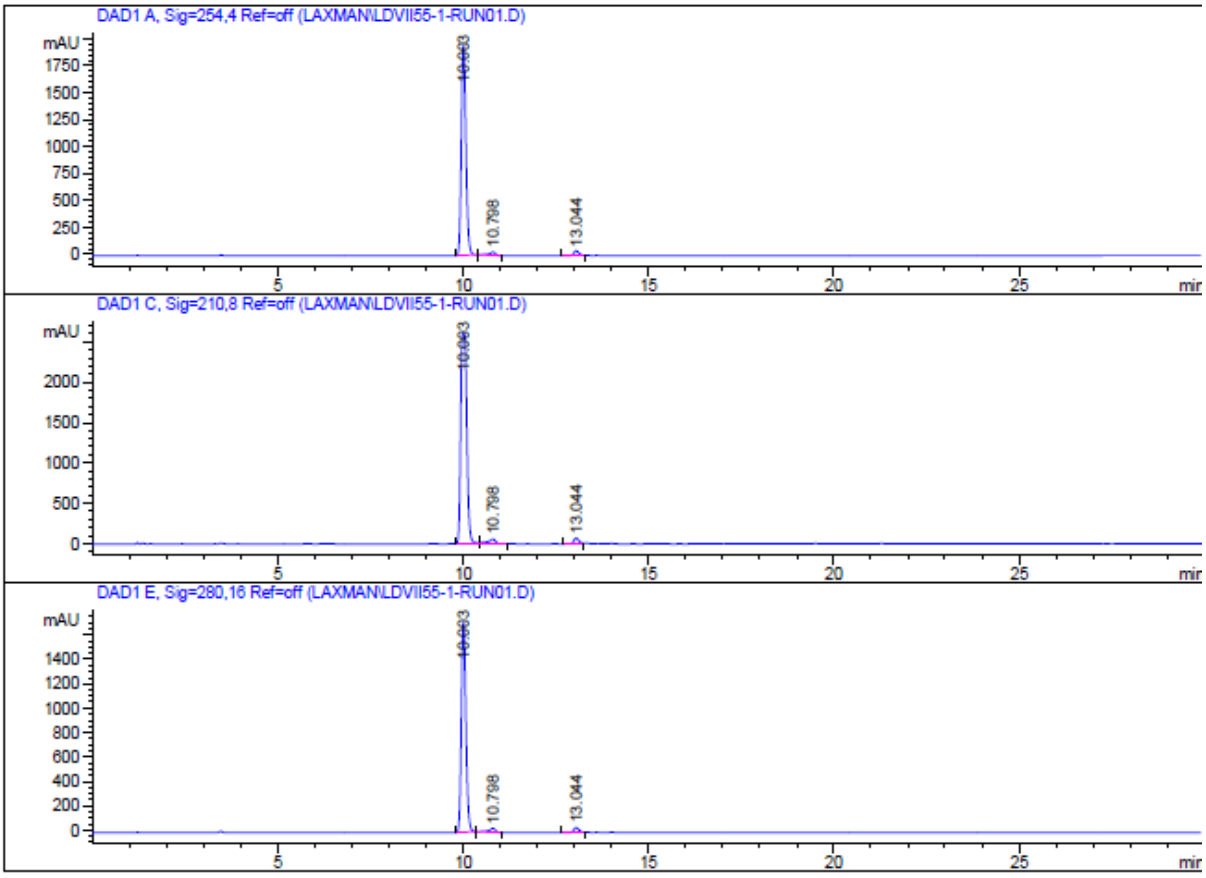
^{13}C DEPT NMR (125 MHz, Acetone) for Compound **37**



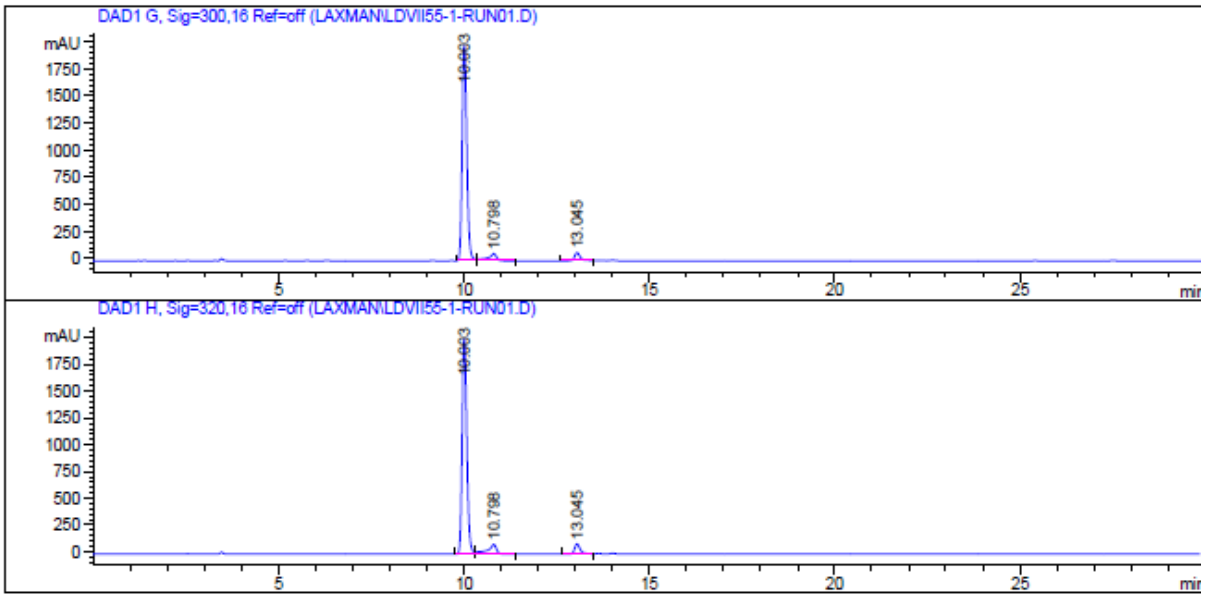
HPLC Traces for Compound **37**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LDVII55-1-RUN01.D
Sample Name: LD-VII-55-1A-run1

    ======================================================================
    Acq. Operator   : Laxman
    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 4/9/2015 11:04:03 AM
    Acq. Method     : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
    Last changed    : 4/9/2015 10:42:01 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LDVII55-1-RUN01.D\DA.M (GRAD 2 50-90 ACN.M)
    Last changed    : 4/9/2015 11:51:08 AM by Graham
    Sample Info     : Method- GRAD 2 50-90% ACN
```



Data File C:\CHEM32\1\DATA\LAXMAN\LDVII55-1-RUN01.D
Sample Name: LD-VII-55-1A-run1



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.003	BB	0.1338	1.67595e4	1959.17419	95.2846
2	10.798	BB	0.1757	362.35123	29.84251	2.0601
3	13.044	BV	0.1528	467.03598	45.85099	2.6553
Totals :				1.75889e4	2034.86770	

Signal 2: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.003	BV	0.1807	2.97224e4	2613.60645	94.6144
2	10.798	VV	0.2239	966.52844	59.38451	3.0767

Data File C:\CHEM32\1\DATA\LAXMAN\LDVII55-1-RUN01.D
Sample Name: LD-VII-55-1A-run1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
3	13.044	BV	0.1521	725.30652	71.63013	2.3088
Totals :				3.14143e4	2744.62109	

Signal 3: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.003	BB	0.1331	1.46707e4	1727.81067	94.7674
2	10.798	BB	0.1781	385.23578	31.18816	2.4885
3	13.044	BV	0.1532	424.80612	41.59309	2.7441
Totals :				1.54808e4	1800.59192	

Signal 4: DAD1 G, Sig=300,16 Ref=off

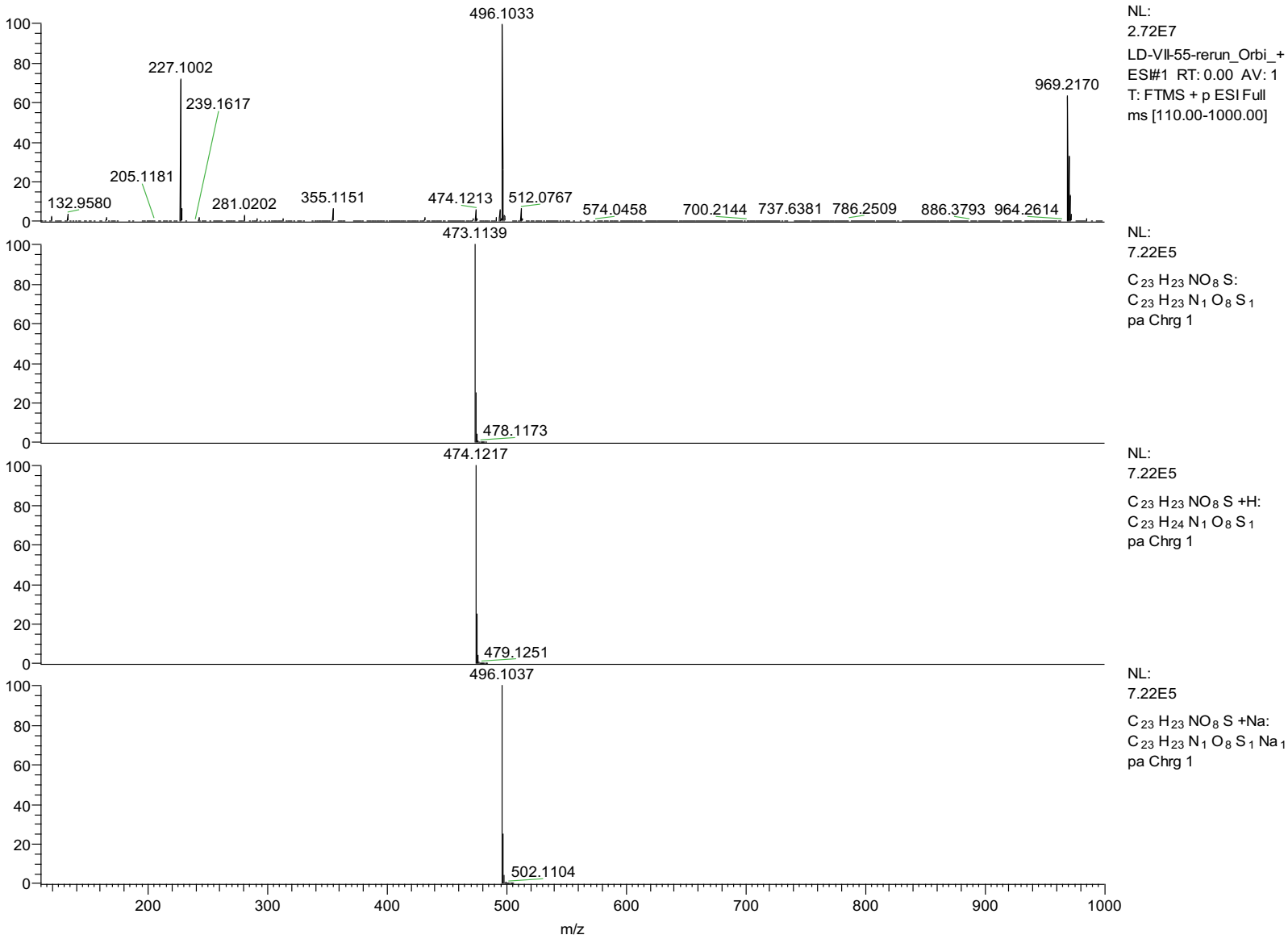
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.003	VV	0.1358	1.73904e4	1992.41077	89.9899
2	10.798	VB	0.2316	1108.33960	65.42634	5.7353
3	13.045	BV	0.1613	826.08325	75.69793	4.2747
Totals :				1.93248e4	2133.53503	

Signal 5: DAD1 H, Sig=320,16 Ref=off

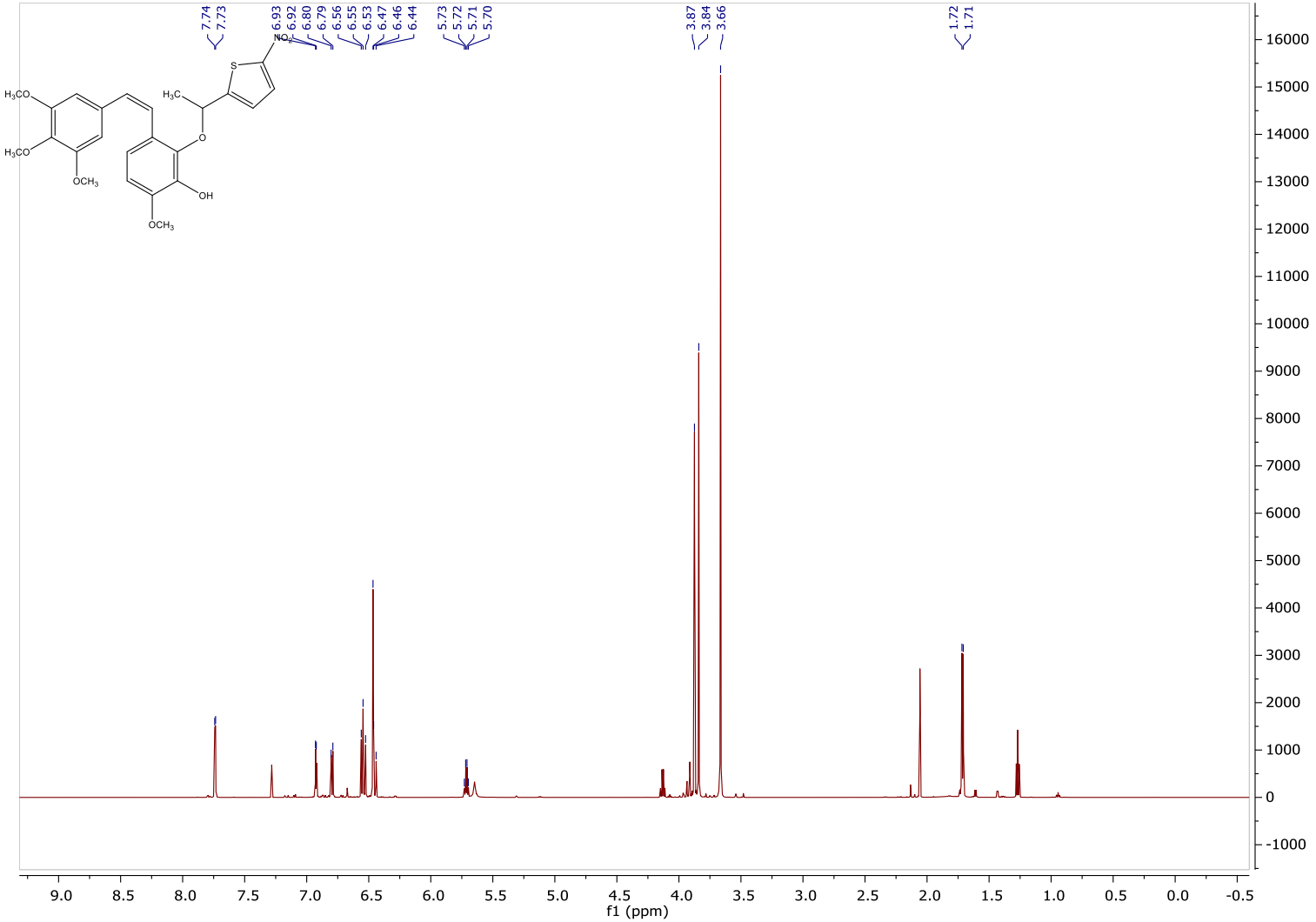
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.003	VV	0.1365	1.77261e4	2018.29065	87.3120
2	10.798	VB	0.2320	1536.88574	90.55610	7.5701
3	13.045	BV	0.1596	1039.03064	96.51353	5.1179
Totals :				2.03020e4	2205.36028	

*** End of Report ***

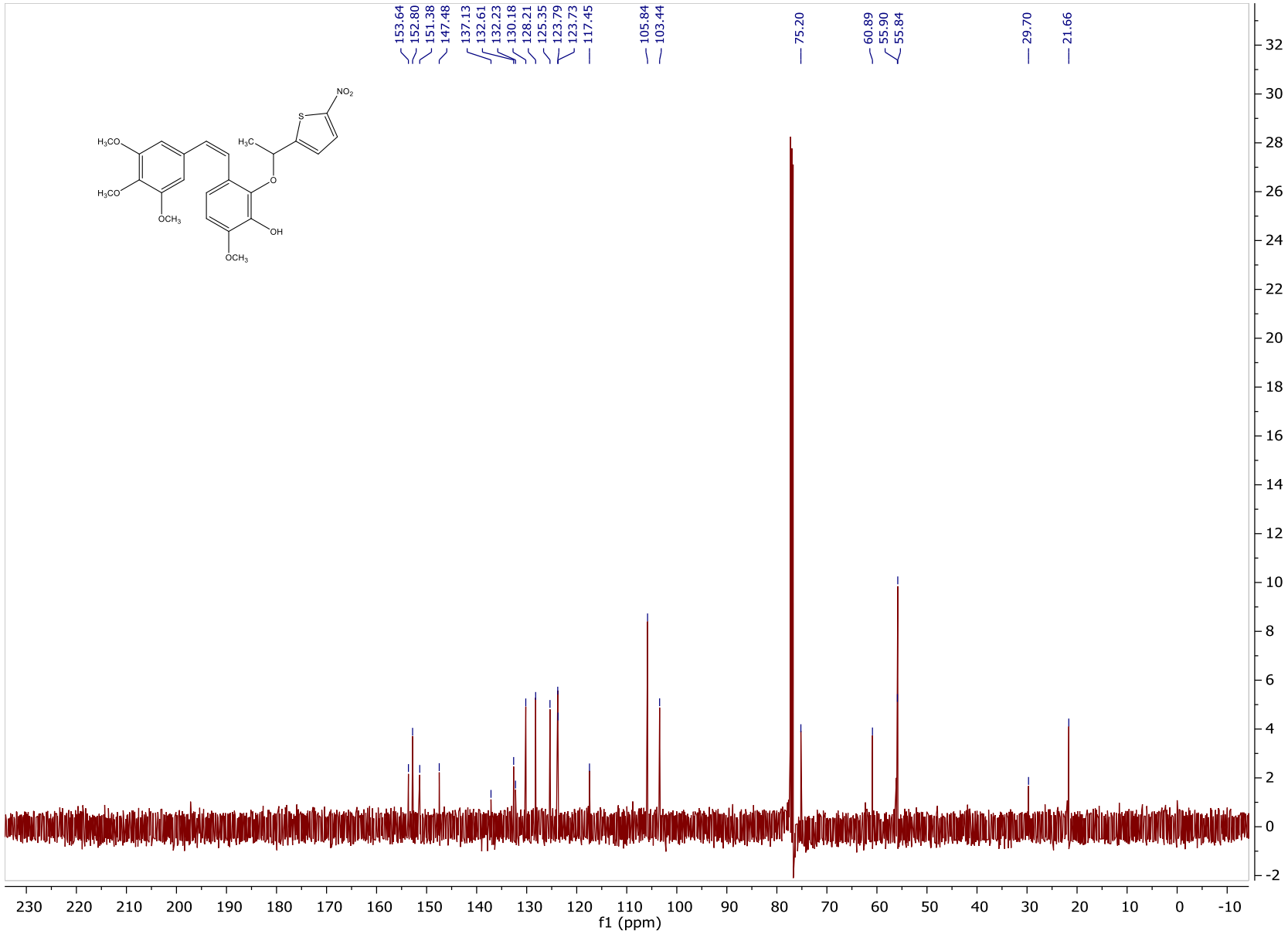
Mass Spectrum of Compound **37**



^{1}H NMR (500 MHz, $CDCl_3$) of Compound **38**



^{13}C NMR (500 MHz, $CDCl_3$) of Compound **38**

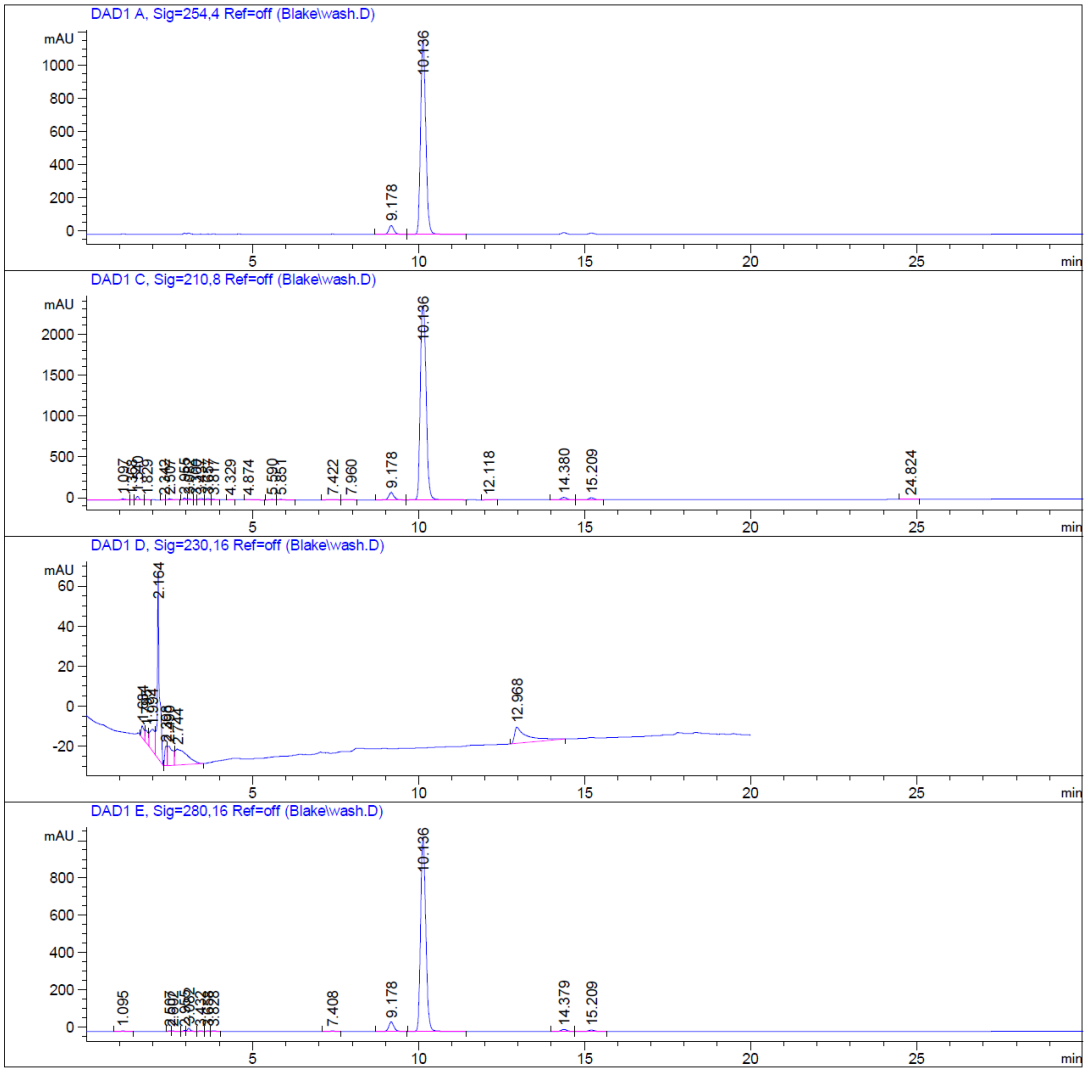


HPLC Trace of Compound **38**

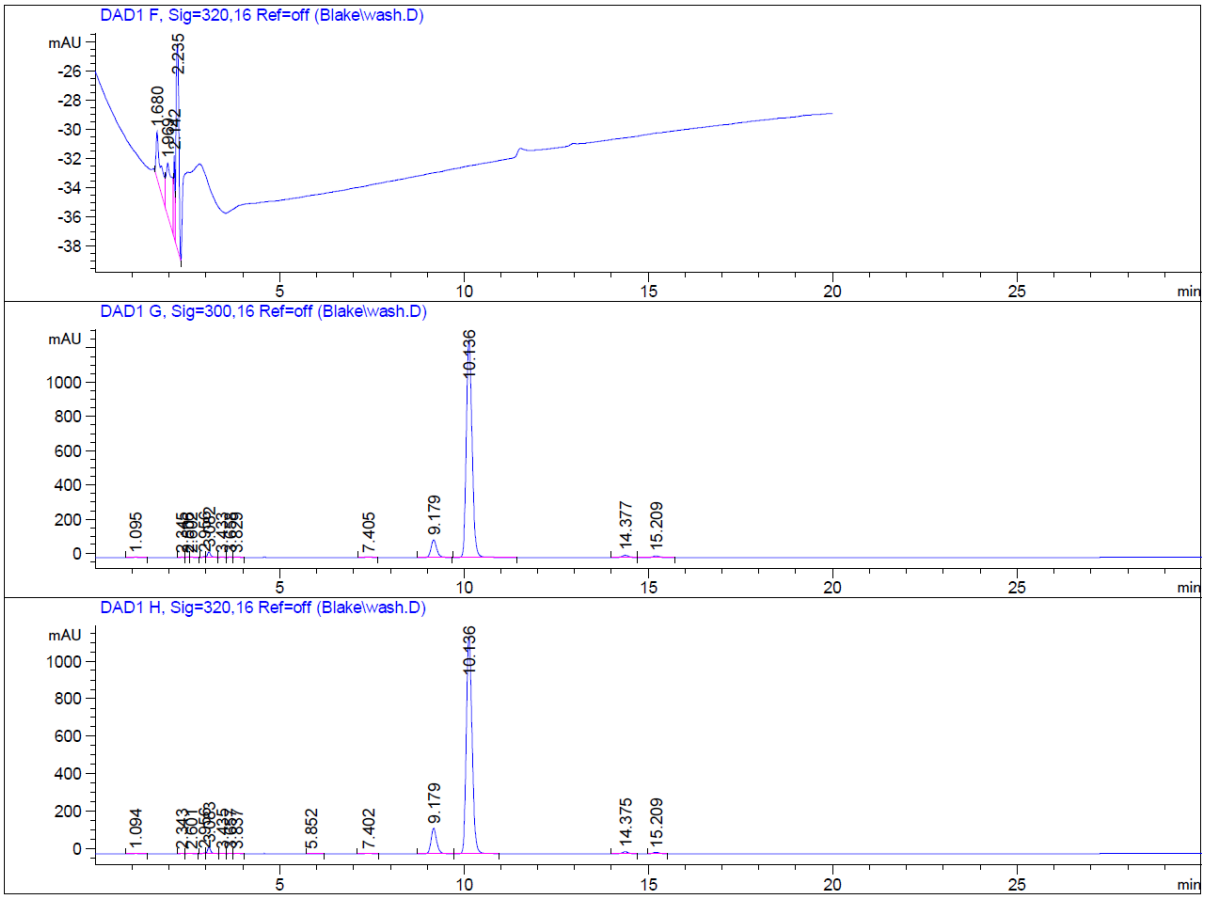
Data File C:\Chem32\1\Data\Blake\wash.D
Sample Name: wash

```
    ======================================================================
    Acq. Operator   : SYSTEM
    Sample Operator : SYSTEM
    Acq. Instrument : 1200 HPLC                    Location :     -
    Injection Date  : 9/27/2016 8:32:29 PM
                                                   Inj Volume : No inj
    Acq. Method     : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
    Last changed    : 4/30/2014 1:53:57 AM by ERICAP
    Analysis Method : C:\CHEM32\1\METHODS\RT-ACNWASH 2.M
    Last changed    : 7/9/2015 2:27:22 PM by Blake
    Method Info     : General Column Wash Method

    Additional Info : Peak(s) manually integrated
```



Data File C:\Chem32\1\Data\Blake\wash.D
Sample Name: wash



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Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.178	BB	0.1647	575.78760	53.86548	4.3780
2	10.136	BB	0.1669	1.25761e4	1174.37427	95.6220

Totals : 1.31518e4 1228.23975

Data File C:\Chem32\1\Data\Blake\wash.D
Sample Name: wash

Signal 2: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.097	BV	0.1320	136.03398	13.68161	0.4357
2	1.368	VV	0.0703	12.33870	2.55526	0.0395
3	1.540	VV	0.0834	244.72534	45.03909	0.7839
4	1.829	VB	0.1006	9.03021	1.34108	0.0289
5	2.342	BV	0.0697	10.77329	2.34061	0.0345
6	2.507	VB	0.1222	104.57416	11.93351	0.3350
7	2.955	BV	0.0832	102.11187	18.84634	0.3271
8	3.082	VB	0.0869	83.05157	14.47536	0.2660
9	3.300	BV	0.0611	8.05128	2.08455	0.0258
10	3.437	VV	0.1378	106.61871	12.46211	0.3415
11	3.657	VV	0.1006	80.88427	12.31509	0.2591
12	3.817	VB	0.1048	42.89491	6.04386	0.1374
13	4.329	BB	0.1209	23.56181	3.16331	0.0755
14	4.874	BB	0.2170	19.74468	1.20575	0.0632
15	5.590	BV	0.1370	55.60751	6.29734	0.1781
16	5.851	VB	0.1489	69.15494	7.02185	0.2215
17	7.422	BV	0.2089	74.75564	5.22441	0.2394
18	7.960	VB	0.2152	45.88793	3.02121	0.1470
19	9.178	BV	0.1670	978.68933	89.93841	3.1348
20	10.136	VB	0.1901	2.84074e4	2364.92432	90.9899
21	12.118	BB	0.1855	14.03423	1.19036	0.0450
22	14.380	BV	0.1724	301.84442	26.99912	0.9668
23	15.209	VB	0.1833	263.51685	21.76087	0.8441
24	24.824	BB	0.1987	25.10380	1.94365	0.0804
Totals :				3.12204e4	2675.80906	

Signal 3: DAD1 D, Sig=230,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.684	BV	0.0840	35.62299	5.93305	2.9175
2	1.795	VV	0.0921	42.89725	6.25040	3.5133
3	1.994	VV	0.1386	112.01411	11.02189	9.1740
4	2.164	VB	0.0678	449.19000	93.95271	36.7888
5	2.398	BV	0.0788	50.80936	9.74313	4.1613
6	2.490	VV	0.1384	102.15647	9.73474	8.3666
7	2.744	VB	0.3176	206.77625	7.99775	16.9350
8	12.968	BB	0.3564	221.53159	8.16957	18.1435
Totals :				1220.99802	152.80325	

Data File C:\Chem32\1\Data\Blake\wash.D
Sample Name: wash

Signal 4: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.095	BB	0.1203	23.23680	2.59926	0.1911
2	2.507	VV	0.0825	13.25547	2.39738	0.1090
3	2.602	VB	0.0799	11.86713	2.16566	0.0976
4	2.955	BV	0.0778	23.27455	4.69450	0.1914
5	3.082	VB	0.0850	99.69138	17.36660	0.8198
6	3.432	BB	0.1141	8.99181	1.30945	0.0739
7	3.658	BV	0.0895	8.01711	1.42729	0.0659
8	3.828	VB	0.1285	17.14674	2.11773	0.1410
9	7.408	BB	0.1763	18.24699	1.56190	0.1501
10	9.178	BB	0.1644	553.62714	51.93246	4.5528
11	10.136	BB	0.1669	1.11720e4	1043.97192	91.8739
12	14.379	BB	0.1690	122.91336	11.11768	1.0108
13	15.209	BB	0.1837	87.87356	7.23654	0.7226
Totals :				1.21602e4	1149.89837	

Signal 5: DAD1 F, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.680	BV	0.1282	31.92470	3.26162	19.7916
2	1.969	VV	0.1516	42.04145	3.61721	26.0634
3	2.142	VV	0.0422	14.54720	5.74288	9.0185
4	2.235	VB	0.0862	72.79123	14.09685	45.1266
Totals :				161.30458	26.71855	

Signal 6: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.095	BB	0.1158	20.06848	2.34592	0.1311
2	2.345	BV	0.0891	8.57328	1.44754	0.0560
3	2.506	VV	0.0789	9.90580	1.83700	0.0647
4	2.602	VB	0.0802	12.84264	2.33244	0.0839
5	2.956	BV	0.0738	24.54047	5.12679	0.1603
6	3.082	VB	0.0868	182.88321	31.91949	1.1949
7	3.433	BB	0.1152	8.80266	1.29695	0.0575
8	3.658	BV	0.0890	7.88855	1.41603	0.0515
9	3.829	VB	0.1365	16.91241	2.00245	0.1105
10	7.405	BB	0.1704	26.67171	2.35090	0.1743
11	9.179	BB	0.1639	1102.77478	103.80939	7.2051
12	10.136	BB	0.1673	1.36339e4	1269.69946	89.0786
13	14.377	BB	0.1741	145.54846	12.85734	0.9510
14	15.209	BB	0.1804	104.16794	8.77950	0.6806

Data File C:\Chem32\1\Data\Blake\wash.D
Sample Name: wash

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				1.53055e4	1447.22120	

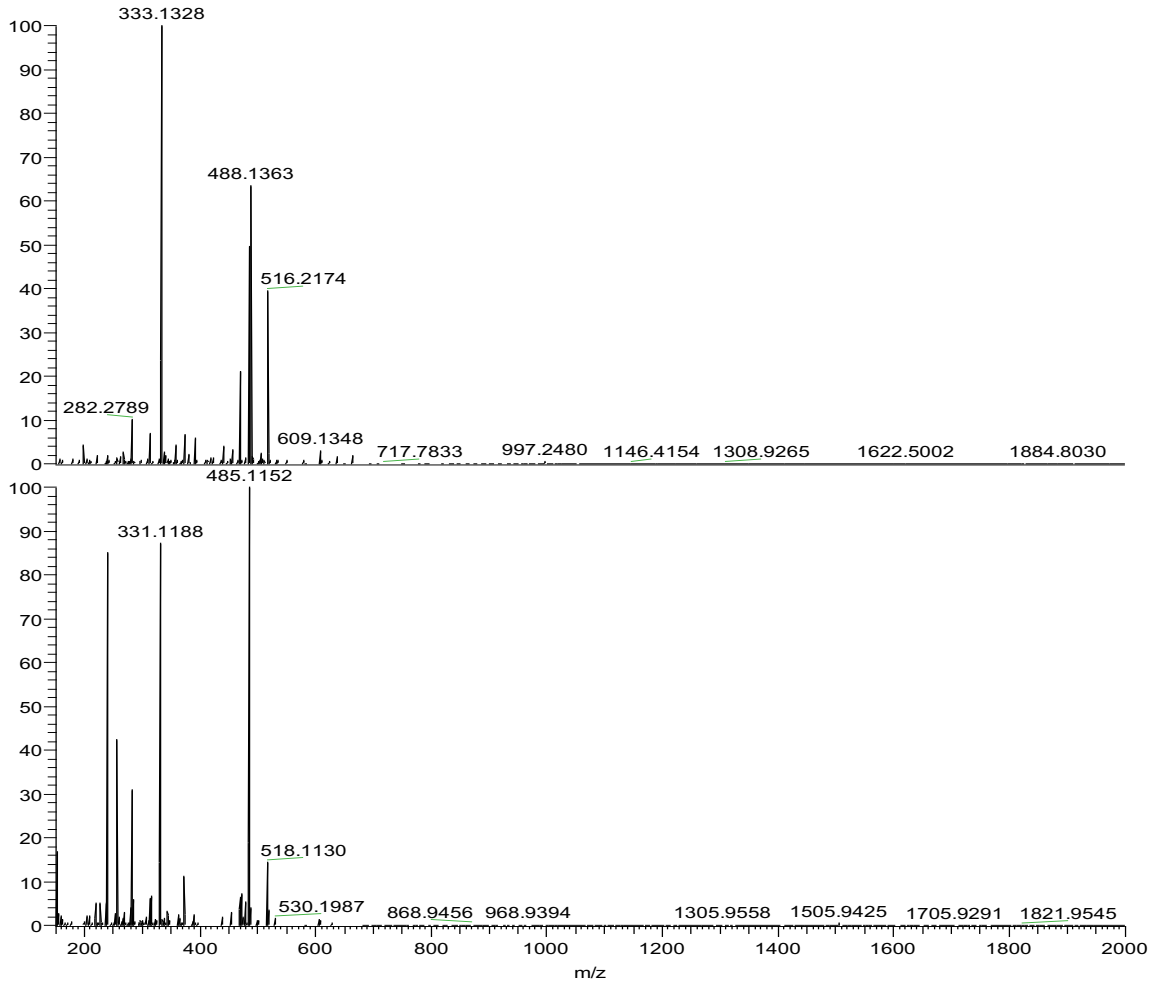
Signal 7: DAD1 H, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.094	BB	0.1136	17.27962	2.06573	0.1198
2	2.343	BV	0.0916	11.41241	1.86050	0.0791
3	2.601	VB	0.1189	17.75151	2.05259	0.1231
4	2.956	BV	0.0724	18.20128	3.90547	0.1262
5	3.083	VV	0.0884	202.91699	34.59048	1.4070
6	3.435	VB	0.1226	12.80356	1.72437	0.0888
7	3.657	BV	0.0893	8.89841	1.58915	0.0617
8	3.837	VB	0.1539	11.76254	1.24904	0.0816
9	5.852	VB	0.1474	10.19680	1.04921	0.0707
10	7.402	BB	0.1734	30.37993	2.61731	0.2106
11	9.179	BB	0.1638	1439.05432	135.63530	9.9782
12	10.136	BB	0.1671	1.24273e4	1159.30432	86.1692
13	14.375	BB	0.1776	123.96412	10.66818	0.8595
14	15.209	BB	0.1679	90.05003	8.34193	0.6244
Totals :				1.44220e4	1366.65357	

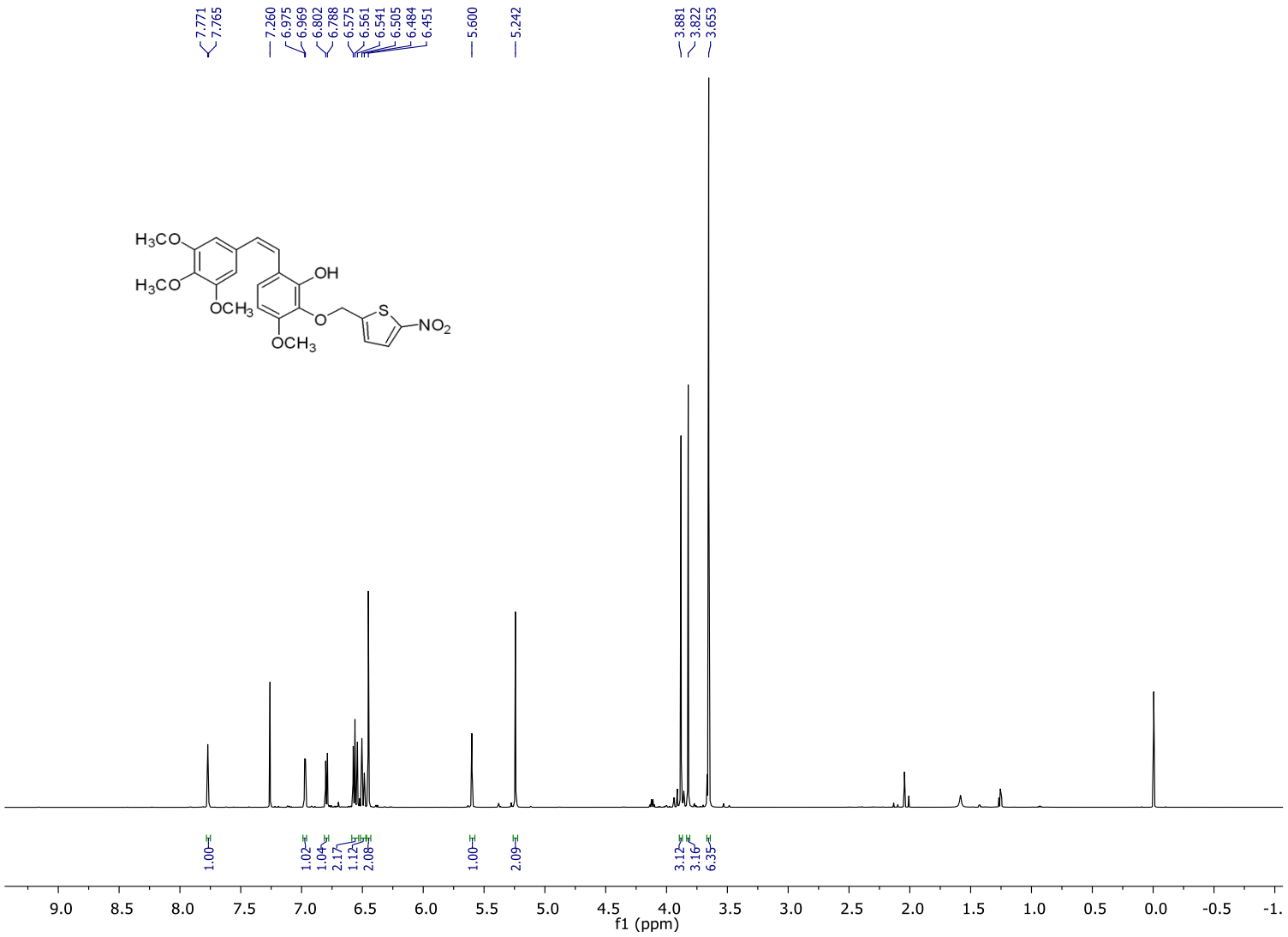
==========

*** End of Report ***

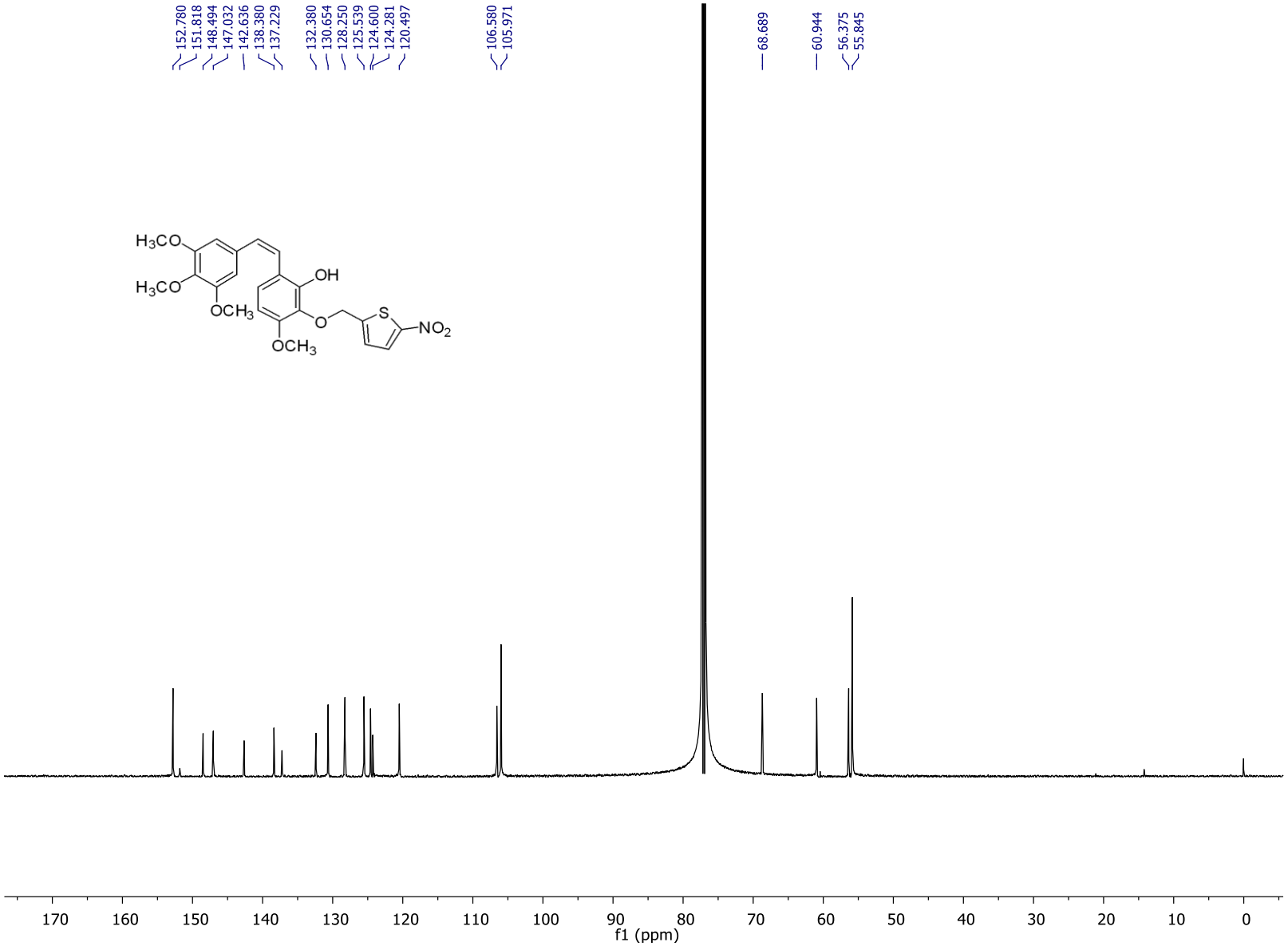
Mass Spectrum of Compound **38**



^{1}H NMR (600 MHz, $CDCl_3$) for Compound **39**



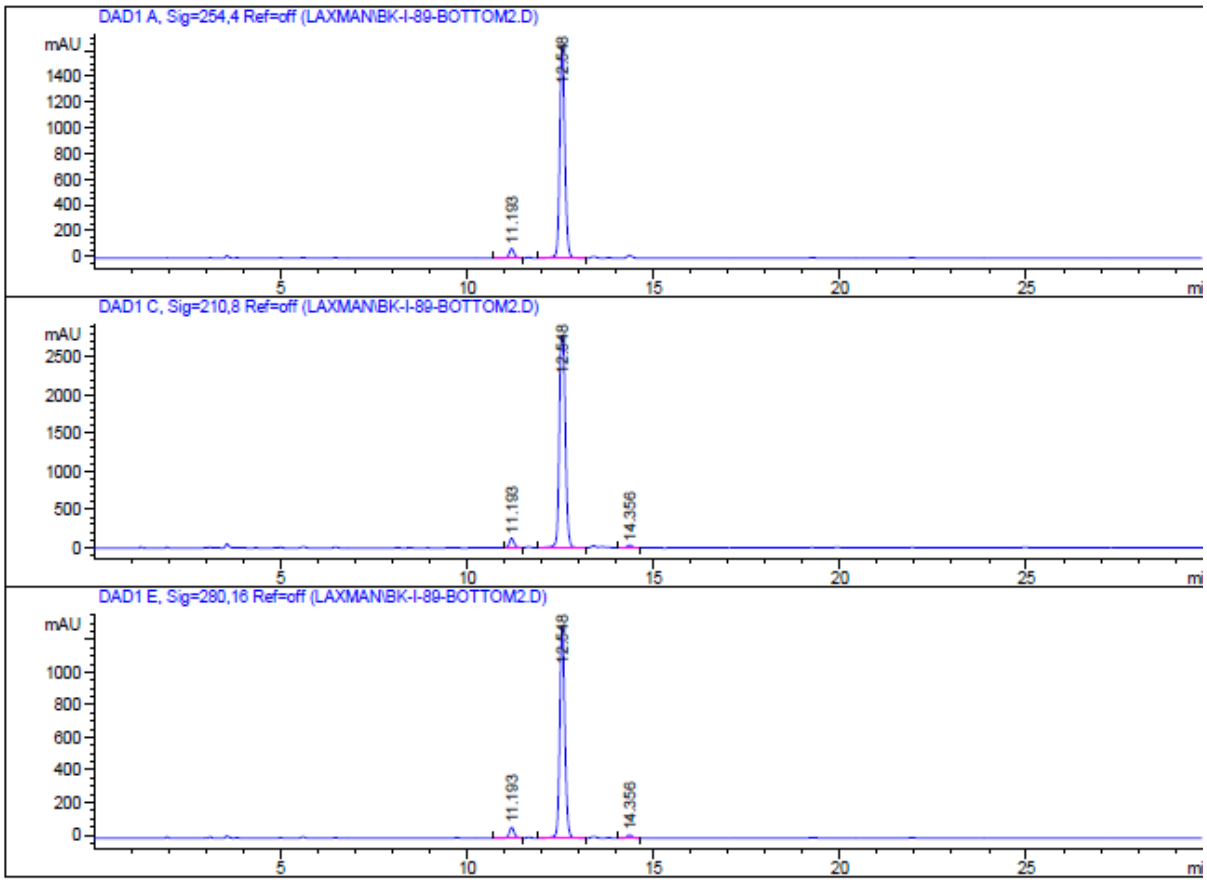
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **39**



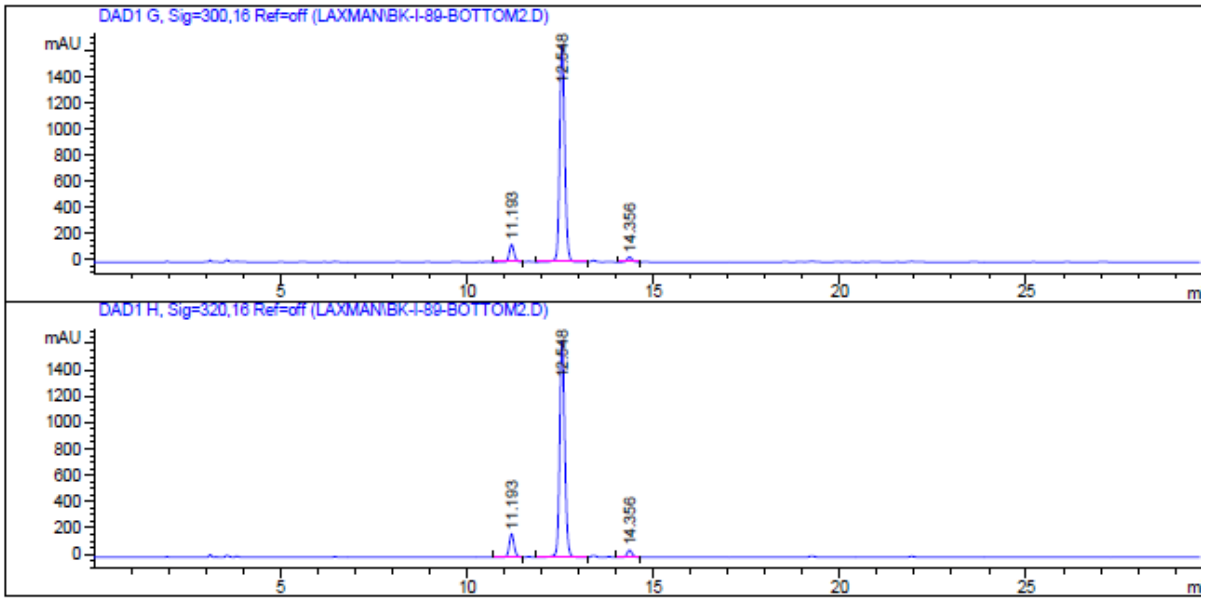
HPLC Traces for Compound **39**

```
Data File C:\CHEM32\1\DATA\LAXMAN\BK-I-89-BOTTOM2.D
Sample Name: BK-I-89-bottom-isomer-rerun

    ======================================================================
    Acq. Operator    : Laxman
    Acq. Instrument : Instrument 1                    Location :    -
    Injection Date   : 7/8/2015 2:42:58 PM
    Acq. Method      : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
    Last changed     : 7/8/2015 2:37:39 PM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\BK-I-89-BOTTOM2.D\DA.M (GRAD 2 50-90 ACN.M)
    Last changed     : 7/8/2015 3:28:55 PM by Laxman
    Sample Info      : Method-Grad2 50-90% ACN
```



Data File C:\CHEM32\1\DATA\LAXMAN\BK-I-89-BOTTOM2.D
Sample Name: BK-I-89-bottom-isomer-rerun



==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.193	BV	0.1415	686.84894	74.56828	4.1781
2	12.548	BV	0.1472	1.57524e4	1652.59790	95.8219
Totals :				1.64393e4	1727.16618	

Signal 2: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.193	VB	0.1392	1117.05908	123.83302	3.3745
2	12.548	BB	0.1808	3.16479e4	2780.26611	95.6037
3	14.356	BV	0.1445	338.26584	36.38631	1.0219

Data File C:\CHEM32\1\DATA\LAXMAN\BK-I-89-BOTTOM2.D
Sample Name: BK-I-89-bottom-isomer-rerun

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				3.31032e4	2940.48544	

Signal 3: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.193	BV	0.1410	610.41125	66.56650	4.6274
2	12.548	BV	0.1468	1.23966e4	1305.82520	93.9761
3	14.356	BB	0.1443	184.21126	19.83845	1.3965
Totals :				1.31912e4	1392.23014	

Signal 4: DAD1 G, Sig=300,16 Ref=off

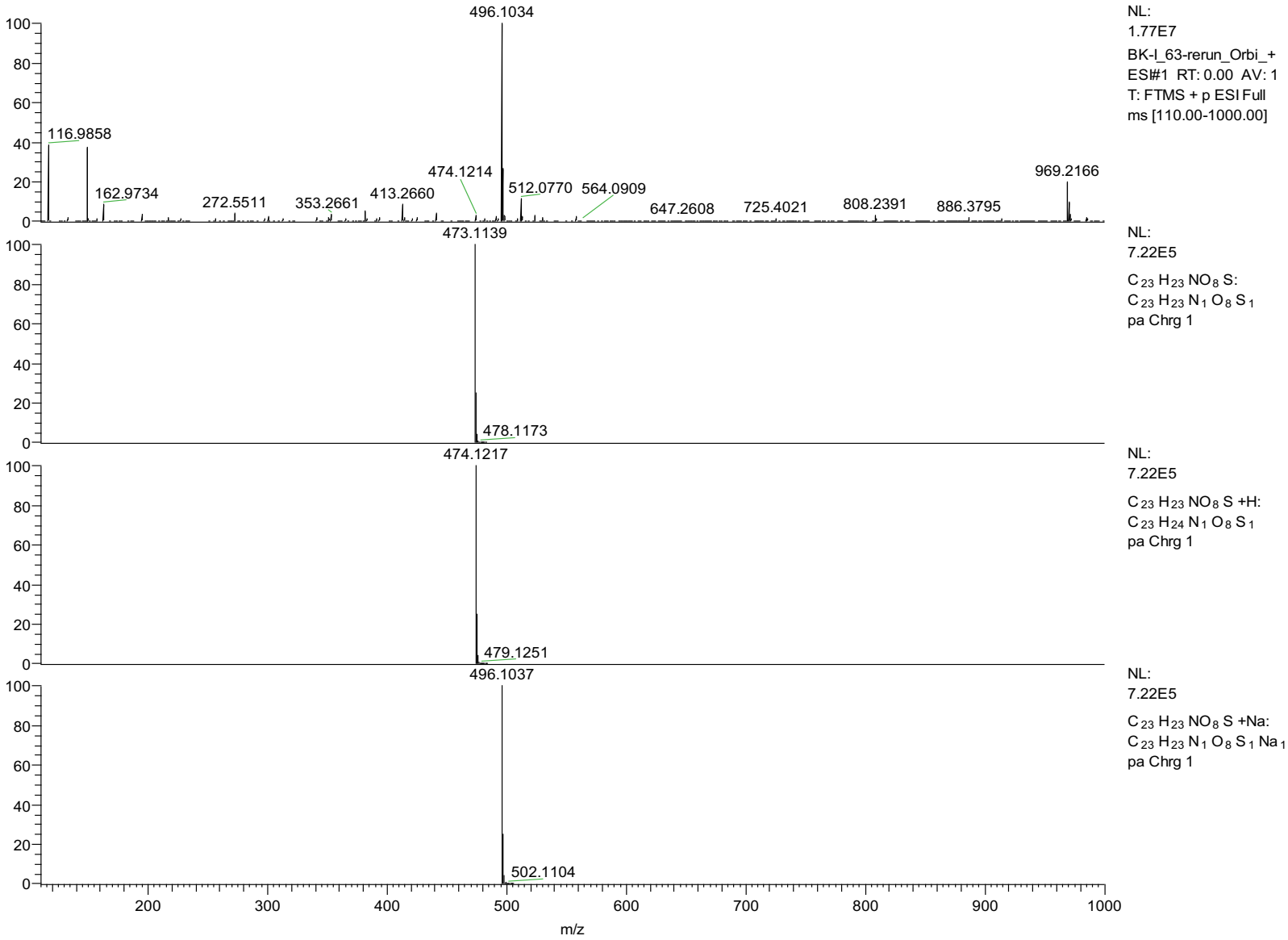
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.193	BV	0.1404	1228.46973	134.74460	7.0437
2	12.548	BV	0.1473	1.58569e4	1661.58582	90.9187
3	14.356	BV	0.1442	355.36490	38.30421	2.0376
Totals :				1.74407e4	1834.63463	

Signal 5: DAD1 H, Sig=320,16 Ref=off

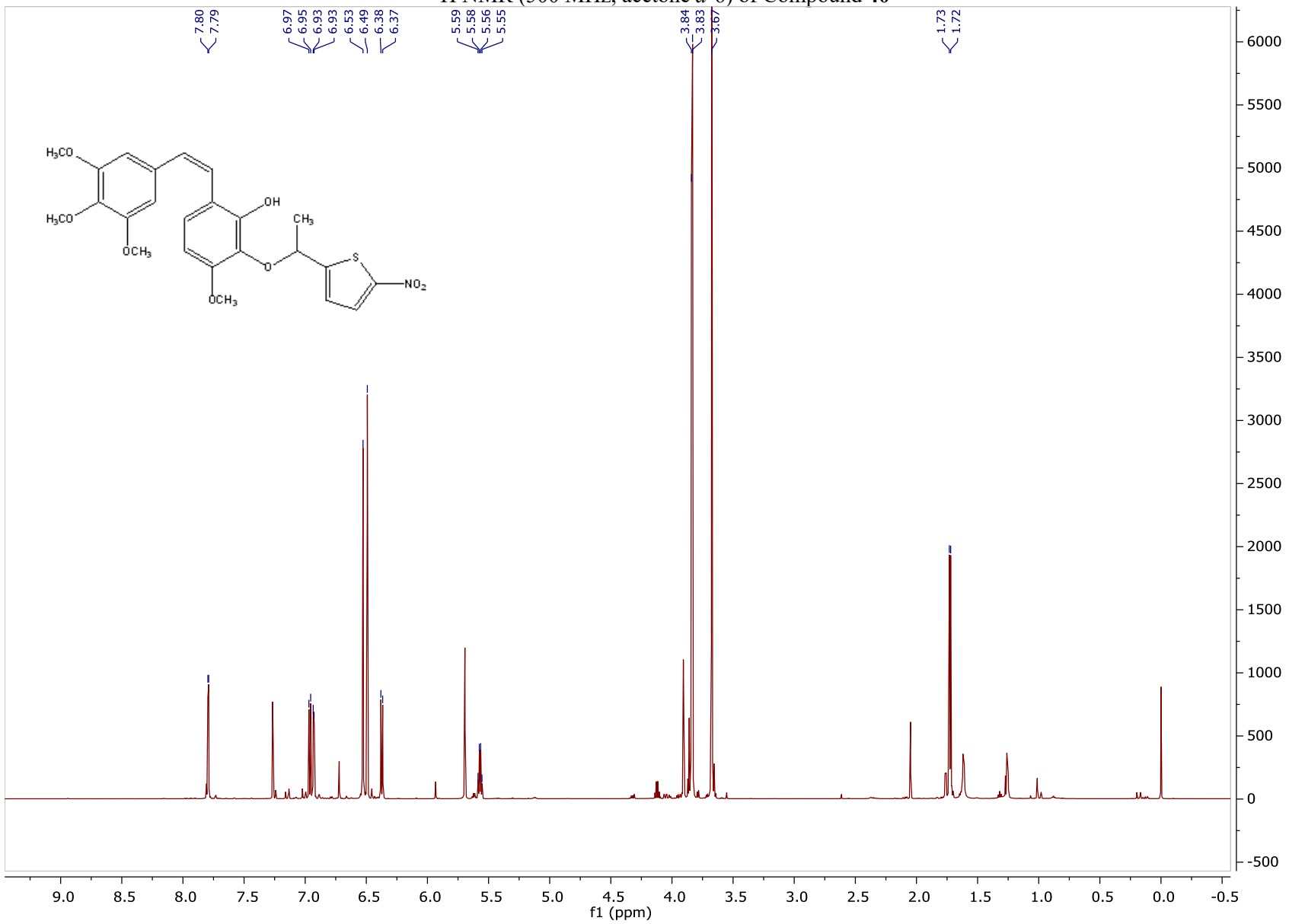
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.193	BV	0.1401	1616.99170	177.81357	9.0370
2	12.548	BV	0.1474	1.57714e4	1651.39465	88.1433
3	14.356	BV	0.1443	504.52426	54.32704	2.8197
Totals :				1.78929e4	1883.53526	

*** End of Report ***

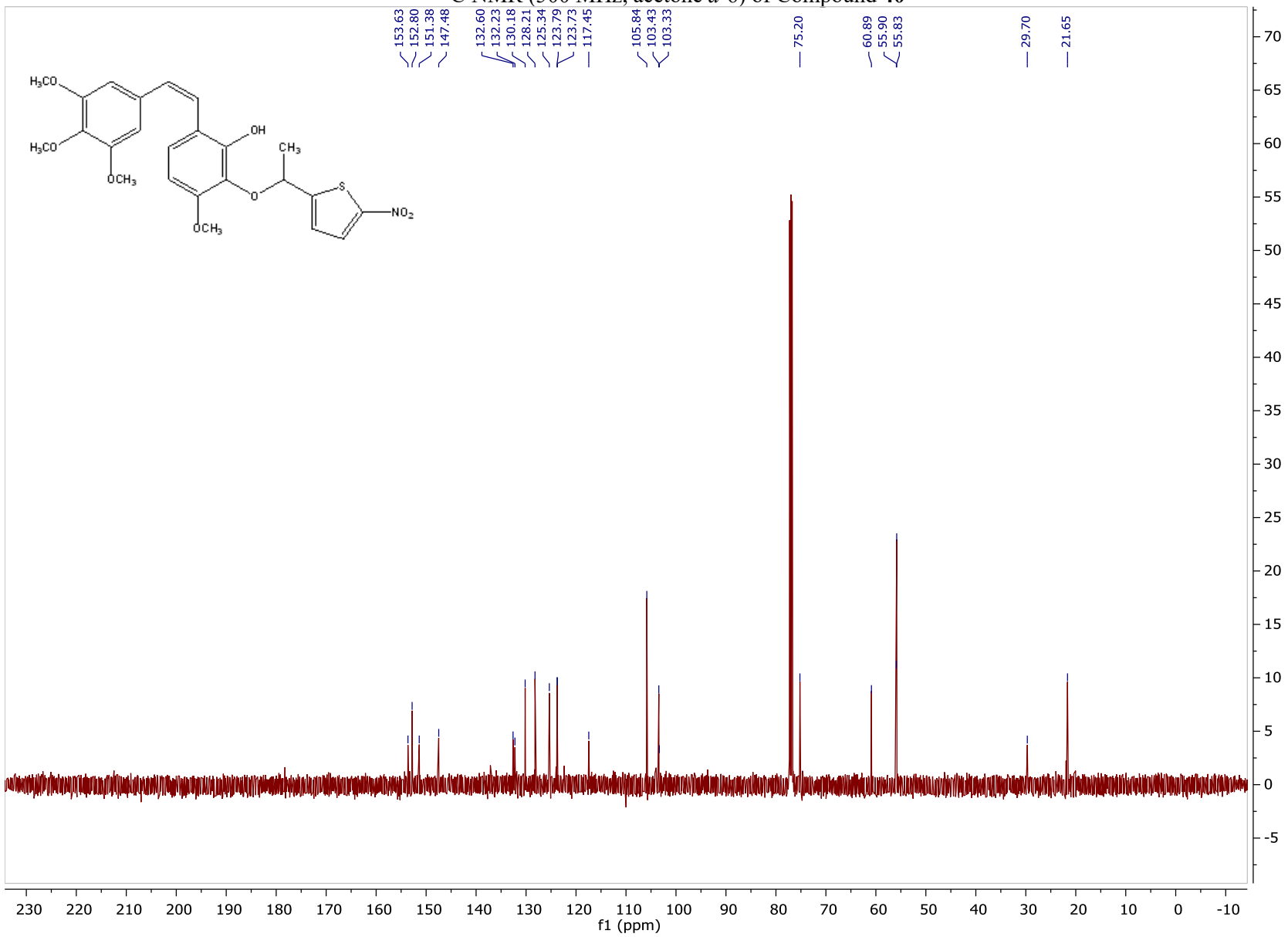
Mass Spectrum of Compound **39**



^{1}H NMR (500 MHz, acetone *d*-6) of Compound **40**



^{13}C NMR (500 MHz, acetone *d*-6) of Compound **40**



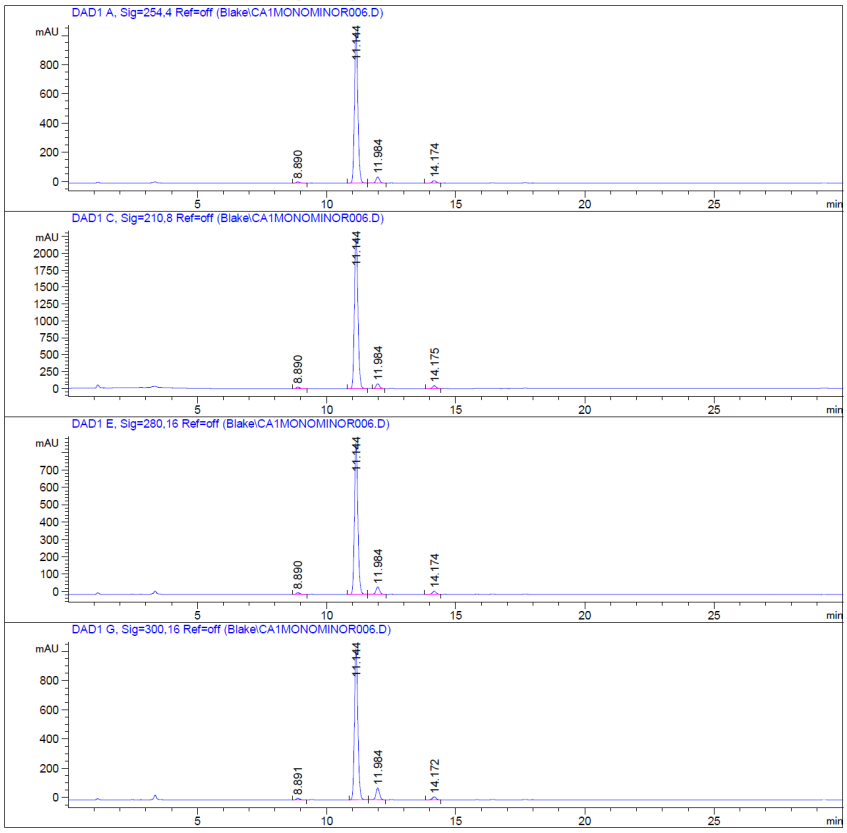
HPLC Trace of Compound **40**

Data File C:\Chem32\1\Data\Blake\CA1MONOMINOR006.D
Sample Name: CA1MonoMinorIsomer

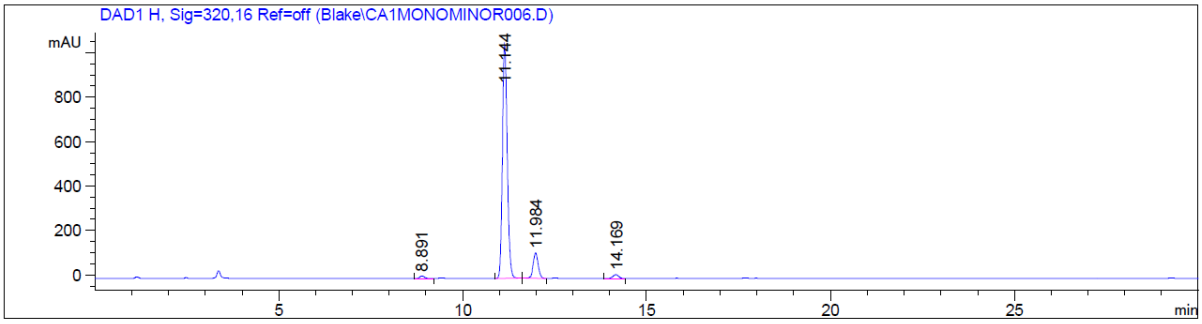
```
    ================================================================
    Acq. Operator   : BLAKE
    Acq. Instrument : Instrument 1                    Location :     -
    Injection Date  : 5/11/2016 3:35:08 PM
    Acq. Method     : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
    Last changed    : 5/11/2016 3:29:22 PM by BLAKE
    Analysis Method : C:\CHEM32\1\METHODS\RT-ACNWASH 2.M
    Last changed    : 7/9/2015 2:27:22 PM by Blake
    Method Info     : General Column Wash Method

    Sample Info     : Purest Frac

    Additional Info : Peak(s) manually integrated
```



Data File C:\Chem32\1\Data\Blake\CA1MONOMINOR006.D
Sample Name: CA1MonoMinorIsomer



==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.890	BB	0.1415	98.29693	10.66364	0.9651
2	11.144	BV	0.1412	9471.36328	1031.05286	92.9948
3	11.984	VB	0.1531	435.87039	43.43245	4.2796
4	14.174	BV	0.1531	179.30080	17.85905	1.7605
Totals :				1.01848e4	1103.00799	

Signal 2: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.890	BB	0.1418	248.90137	26.94793	1.0891
2	11.144	BV	0.1507	2.14740e4	2223.11255	93.9608
3	11.984	VB	0.1474	705.34277	73.89548	3.0863
4	14.175	BV	0.1507	425.95596	43.32686	1.8638
Totals :				2.28542e4	2367.28281	

Signal 3: DAD1 E, Sig=280,16 Ref=off

Data File C:\Chem32\1\Data\Blake\CA1MONOMINOR006.D
Sample Name: CA1MonoMinorIsomer

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.890	BB	0.1418	87.92610	9.51514	1.0175
2	11.144	BV	0.1410	7950.11084	866.50201	91.9969
3	11.984	VB	0.1518	424.61340	42.77497	4.9135
4	14.174	BV	0.1530	179.06296	17.85389	2.0721
Totals :				8641.71330	936.64601	

Signal 4: DAD1 G, Sig=300,16 Ref=off

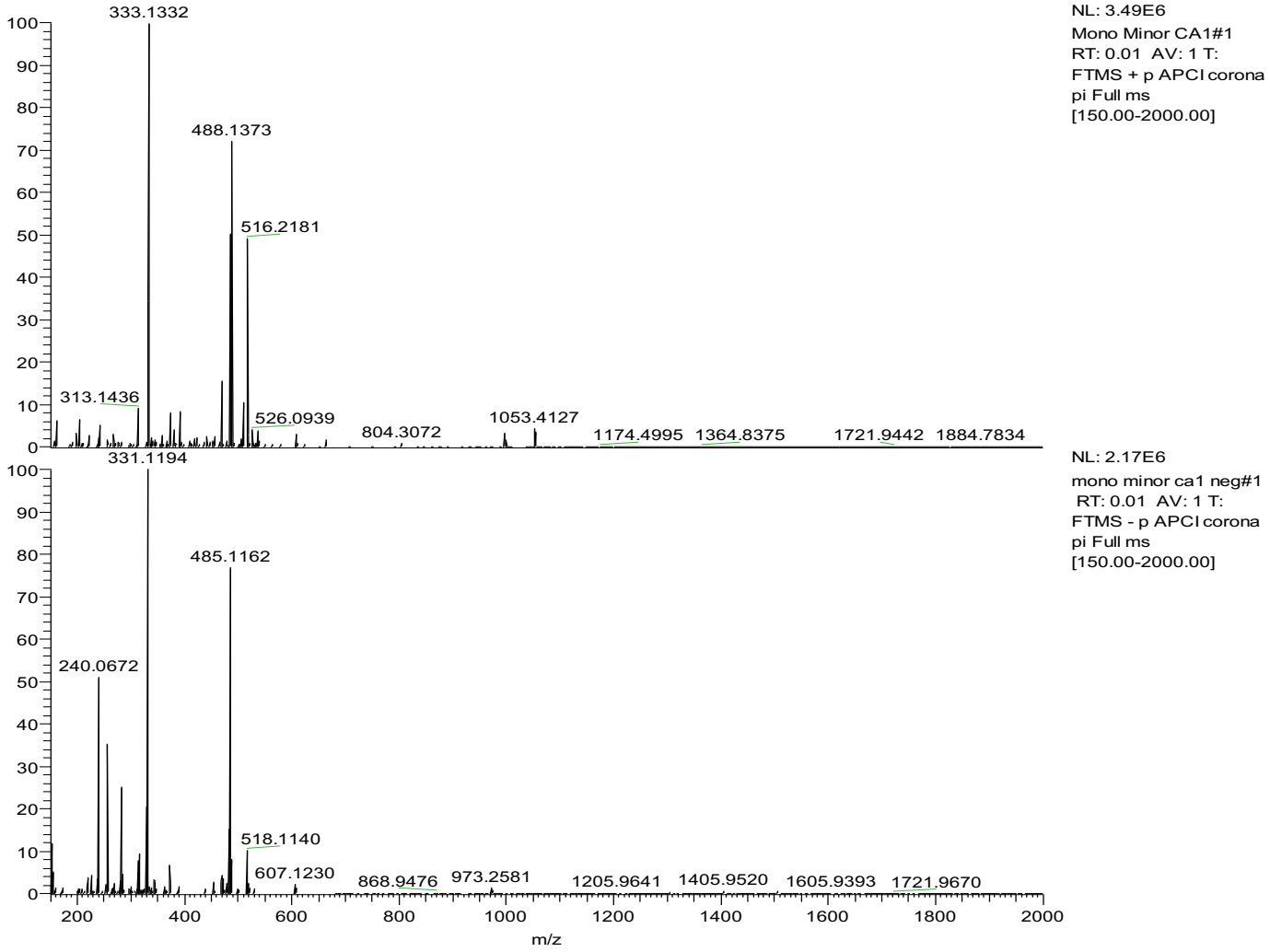
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.891	BB	0.1416	101.99403	11.06105	0.9697
2	11.144	BB	0.1410	9427.53125	1028.07629	89.6329
3	11.984	BB	0.1464	772.94672	81.67284	7.3488
4	14.172	BV	0.1590	215.45932	20.77102	2.0485
Totals :				1.05179e4	1141.58120	

Signal 5: DAD1 H, Sig=320,16 Ref=off

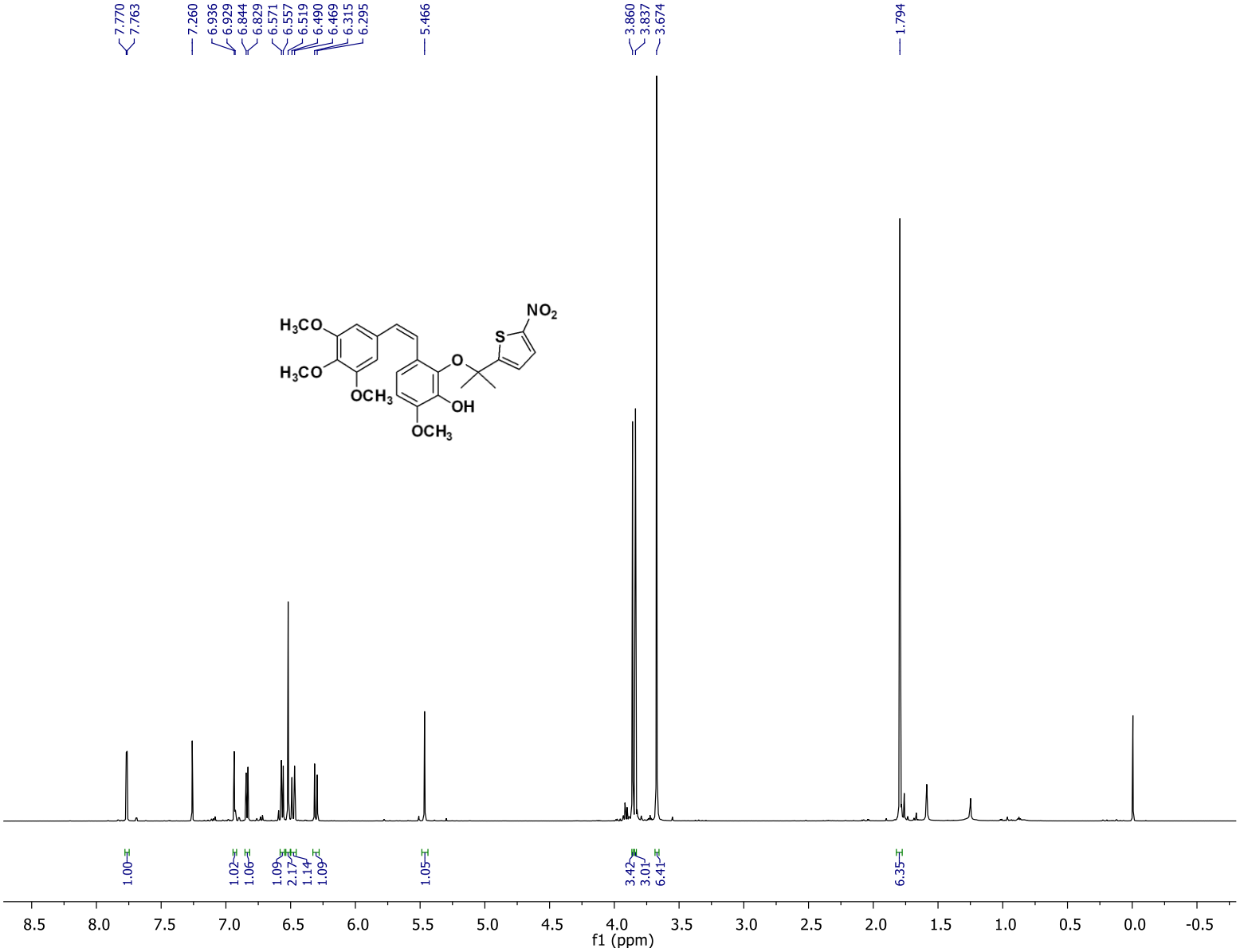
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.891	BB	0.1415	108.28686	11.75676	0.9815
2	11.144	BB	0.1411	9654.57617	1051.61963	87.5111
3	11.984	BB	0.1464	1080.27380	114.20609	9.7918
4	14.169	BV	0.1644	189.26395	17.46828	1.7155
Totals :				1.10324e4	1195.05076	

*** End of Report ***

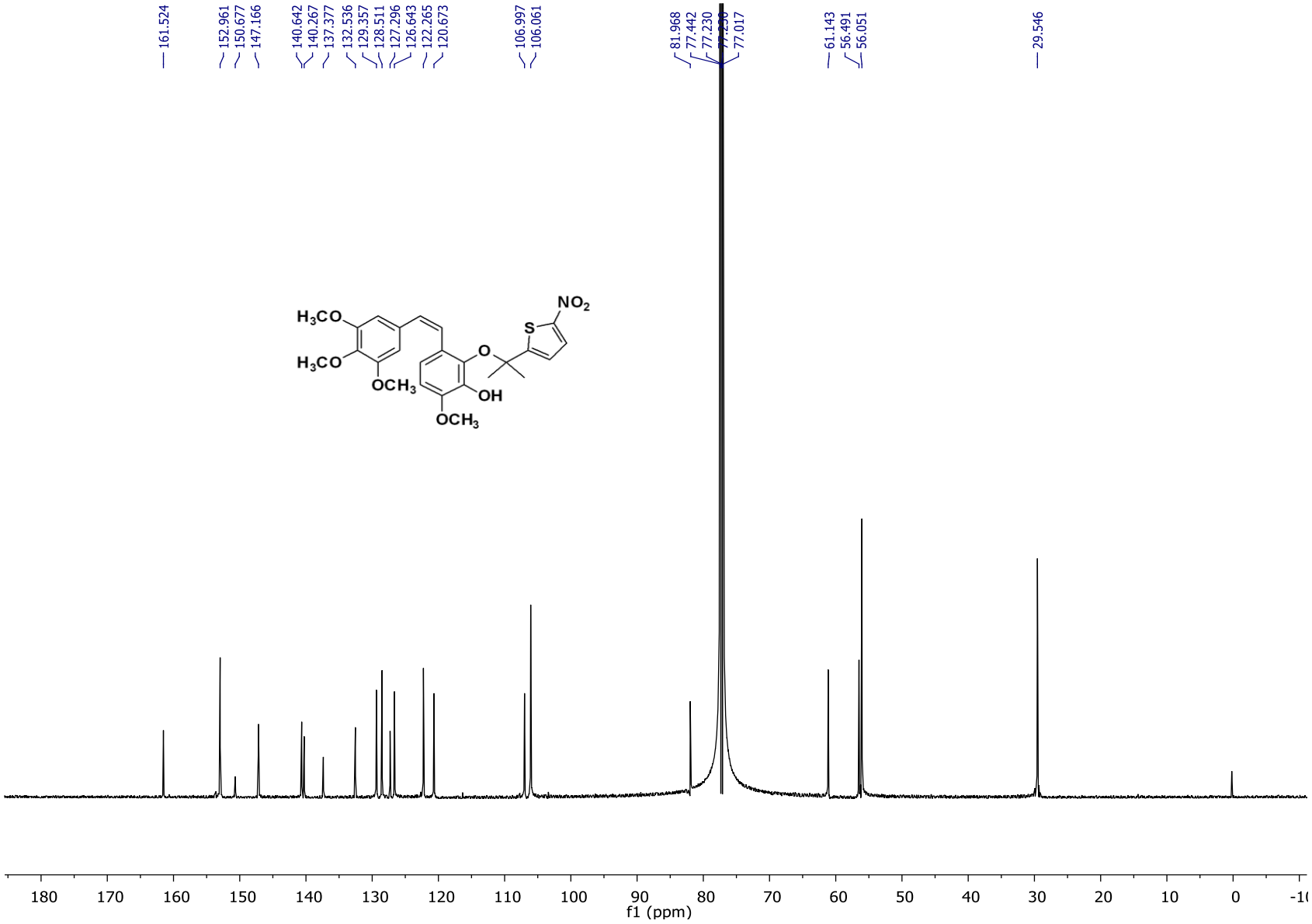
Mass Spectrum of Compound **40**



^{1}H NMR (600 MHz, $CDCl_3$) for Compound **41**



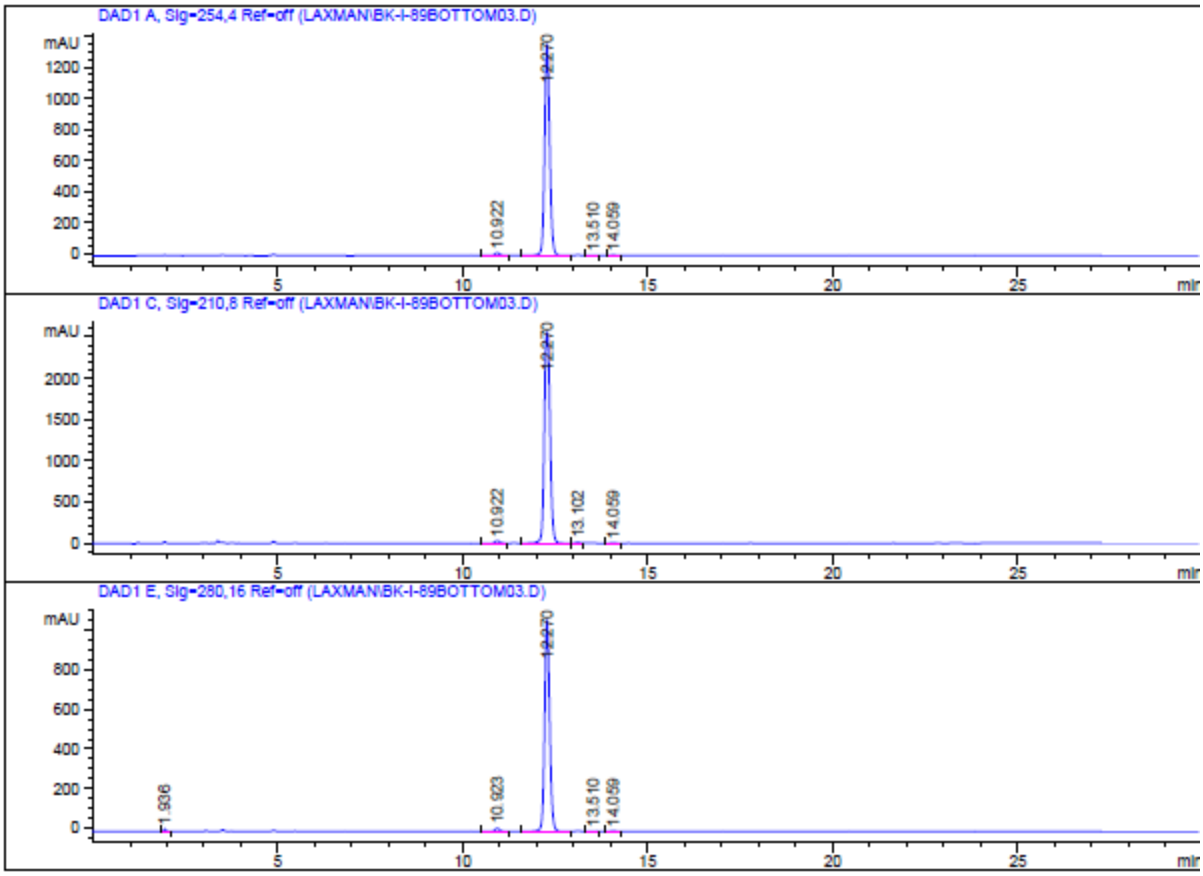
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **41**



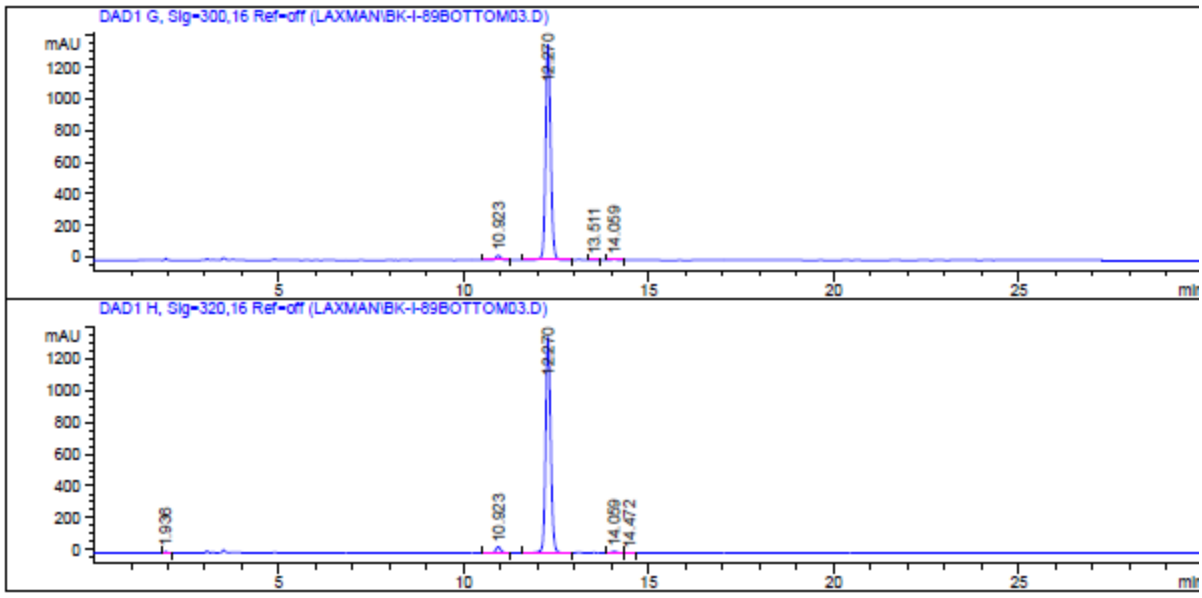
HPLC Traces for Compound **41**

```
Data File C:\CHEM32\1\DATA\LAXMAN\BK-I-89BOTTOM03.D
Sample Name: BK-I-89bottom-rerun3

    ======================================================================
    Acq. Operator    : Laxman
    Acq. Instrument : Instrument 1                    Location :    -
    Injection Date  : 7/10/2015 11:55:32 AM
    Acq. Method      : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
    Last changed     : 7/10/2015 10:49:26 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\BK-I-89BOTTOM03.D\DA.M (GRAD 2 50-90 ACN.M)
    Last changed     : 7/10/2015 12:41:23 PM by Laxman
    Sample Info      : Method-Grad2 50-90% ACN
```



Data File C:\CHEM32\1\DATA\LAXMAN\BK-I-89BOTTOM03.D
Sample Name: BK-I-89bottom-rerun3



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.922	BV	0.1421	168.96503	18.23570	1.2853
2	12.270	BB	0.1469	1.29245e4	1359.97986	98.3160
3	13.510	BB	0.1399	17.83705	1.96581	0.1357
4	14.059	BB	0.1396	34.57308	3.82064	0.2630

Totals : 1.31458e4 1384.00200

Signal 2: DAD1 C, Sig=210,8 Ref=off

Data File C:\CHEM32\1\DATA\LAXMAN\BK-I-89BOTTOM03.D
Sample Name: BK-I-89bottom-rerun3

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.922	BV	0.1429	300.59656	32.20519	1.0795
2	12.270	BB	0.1691	2.73845e4	2553.27441	98.3416
3	13.102	BV	0.1368	101.43078	11.51260	0.3643
4	14.059	BB	0.1415	59.76510	6.60854	0.2146
Totals :				2.78463e4	2603.60075	

Signal 3: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.936	BB	0.0606	53.48746	13.38945	0.5150
2	10.923	BV	0.1396	146.41867	15.88057	1.4099
3	12.270	BB	0.1466	1.01396e4	1069.87317	97.6365
4	13.510	BB	0.1376	13.06543	1.47166	0.1258
5	14.059	BB	0.1398	32.48272	3.58338	0.3128
Totals :				1.03851e4	1104.19823	

Signal 4: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.923	BV	0.1389	280.53387	30.61063	2.0978
2	12.270	BV	0.1470	1.30084e4	1367.02710	97.2770
3	13.511	BB	0.1406	20.27245	2.26112	0.1516
4	14.059	BB	0.1405	63.33008	6.93787	0.4736
Totals :				1.33726e4	1406.83672	

Signal 5: DAD1 H, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.936	VB	0.0602	37.33607	9.43805	0.2777
2	10.923	BV	0.1388	361.49524	39.48792	2.6884
3	12.270	BV	0.1471	1.29486e4	1359.96545	96.2983
4	14.059	BB	0.1407	90.02934	9.84660	0.6695
5	14.472	BB	0.1373	8.87988	1.02271	0.0660
Totals :				1.34464e4	1419.76074	

Data File C:\CHEM32\1\DATA\LAXMAN\BK-I-89BOTTOM03.D
Sample Name: BK-I-89bottom-rerun3

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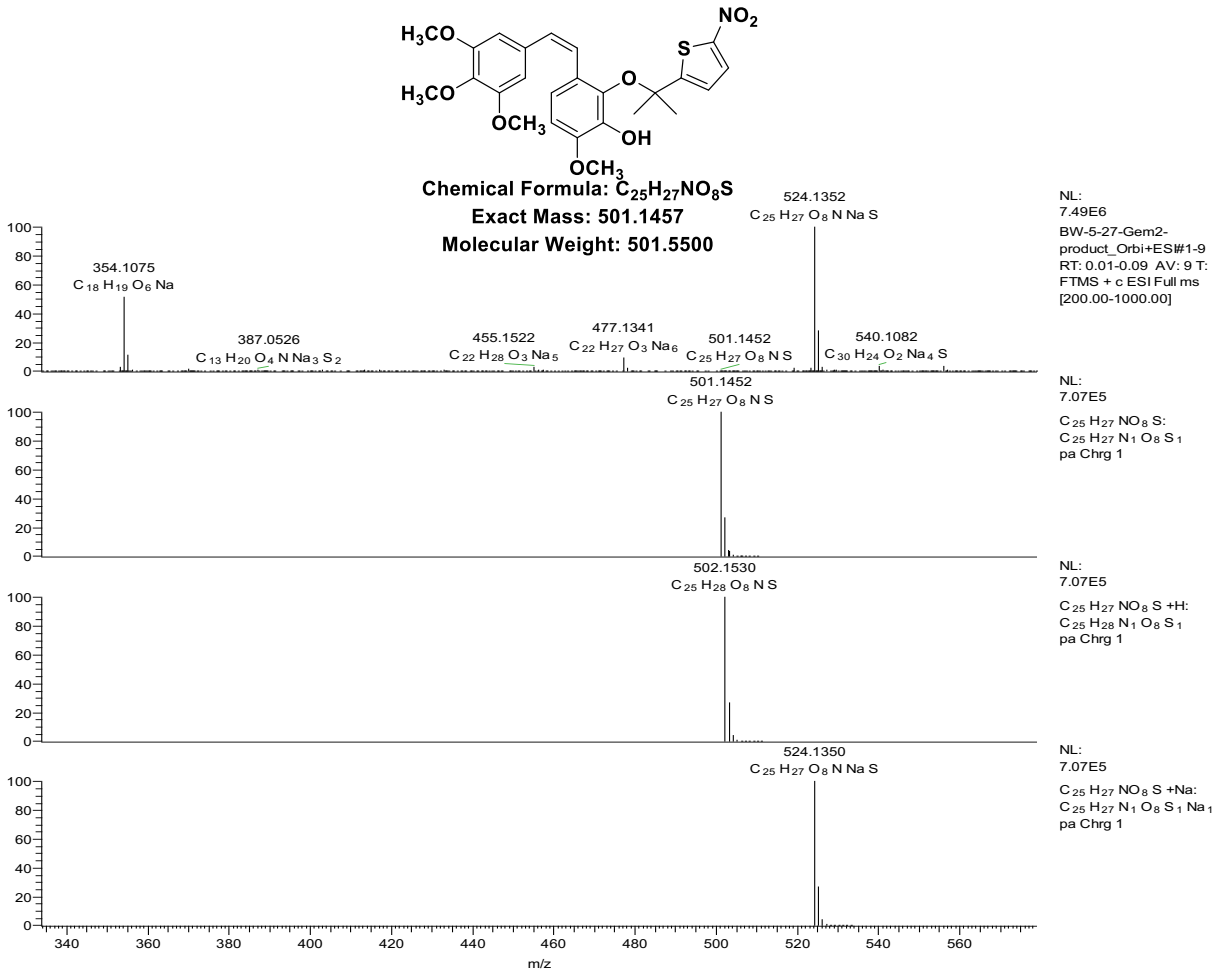
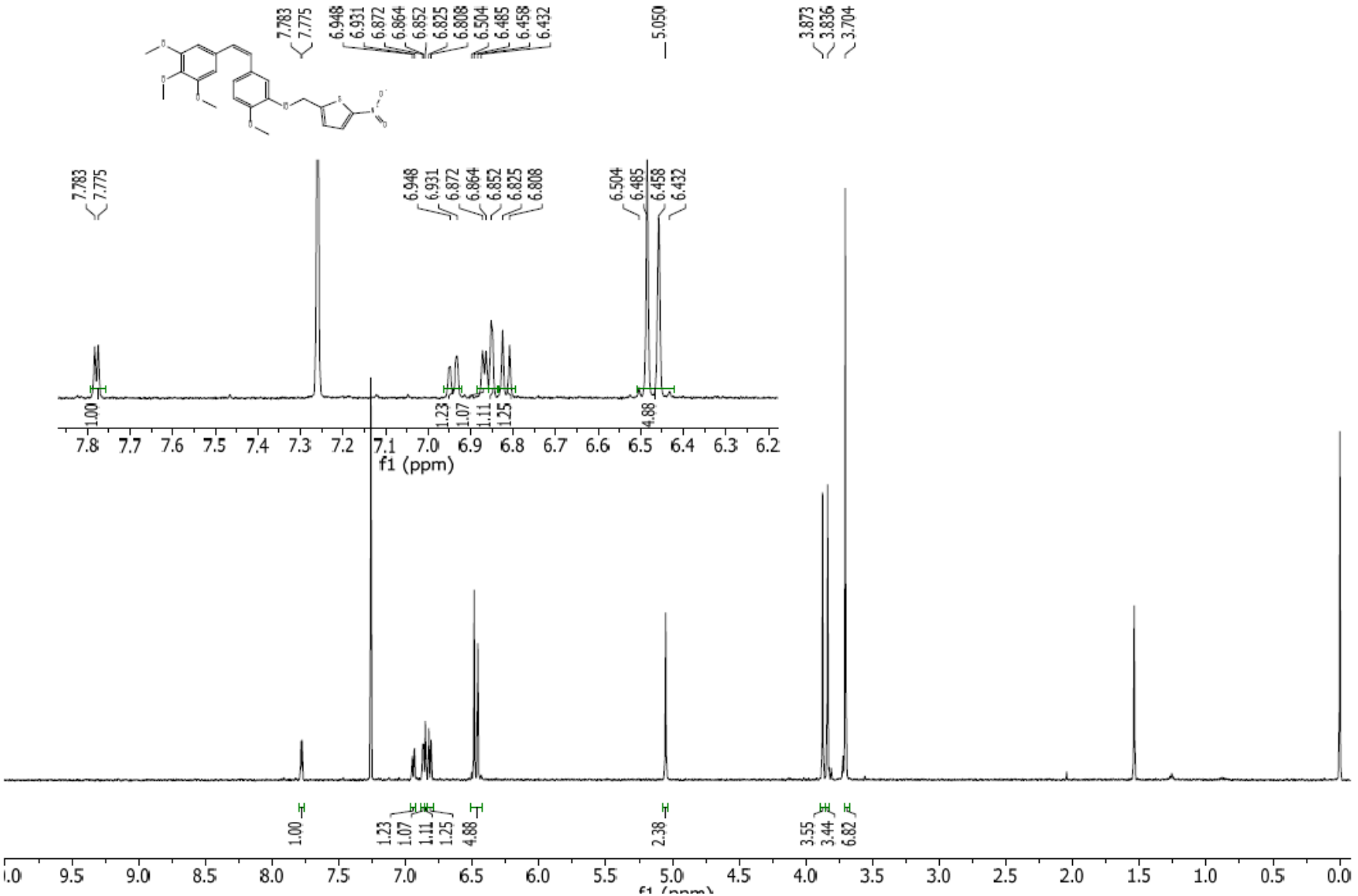
*** End of Report ***

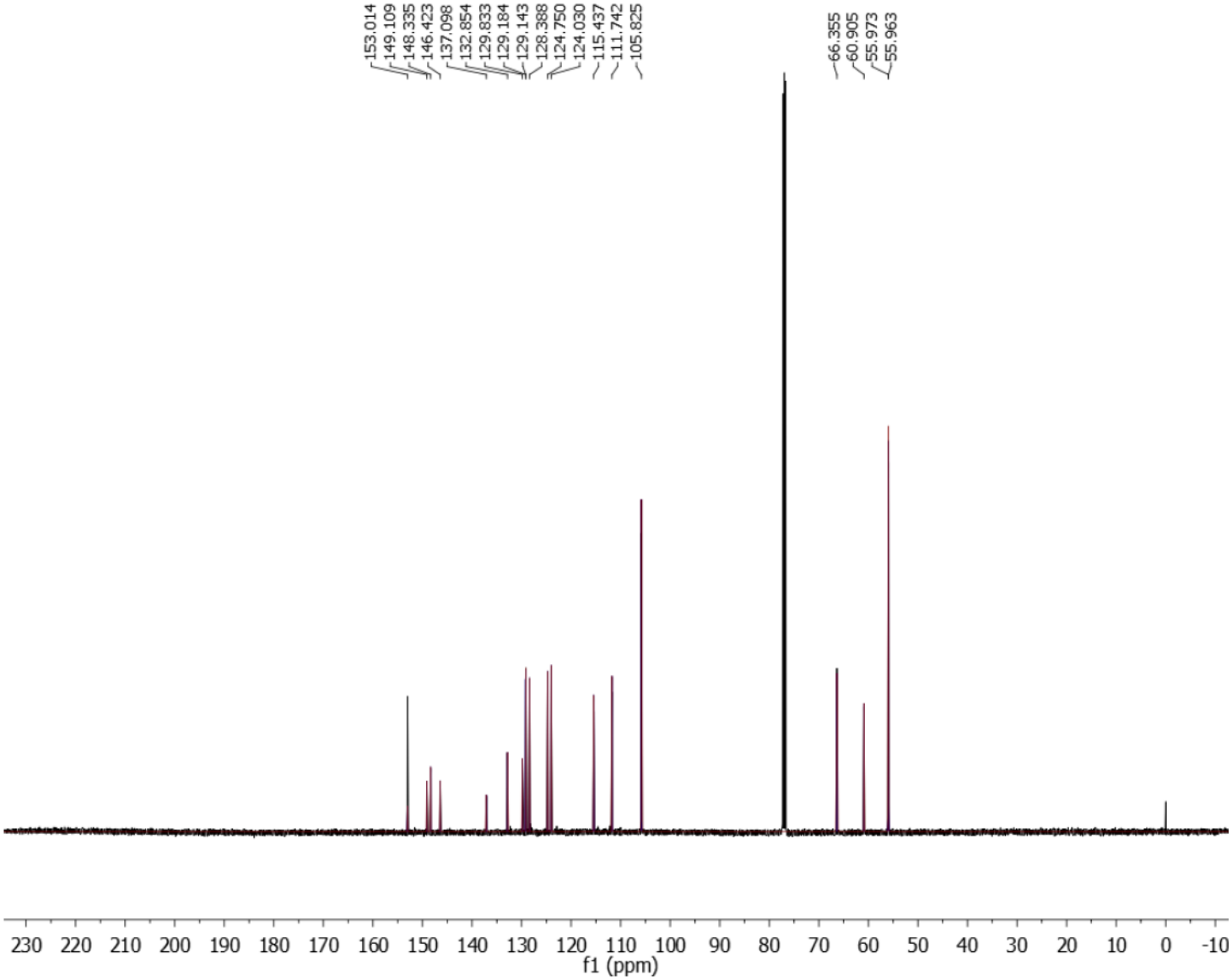
Mass Spectrum for Compound **41**



^{1}H NMR (500 MHz, $CDCl_3$) of Compound **43**



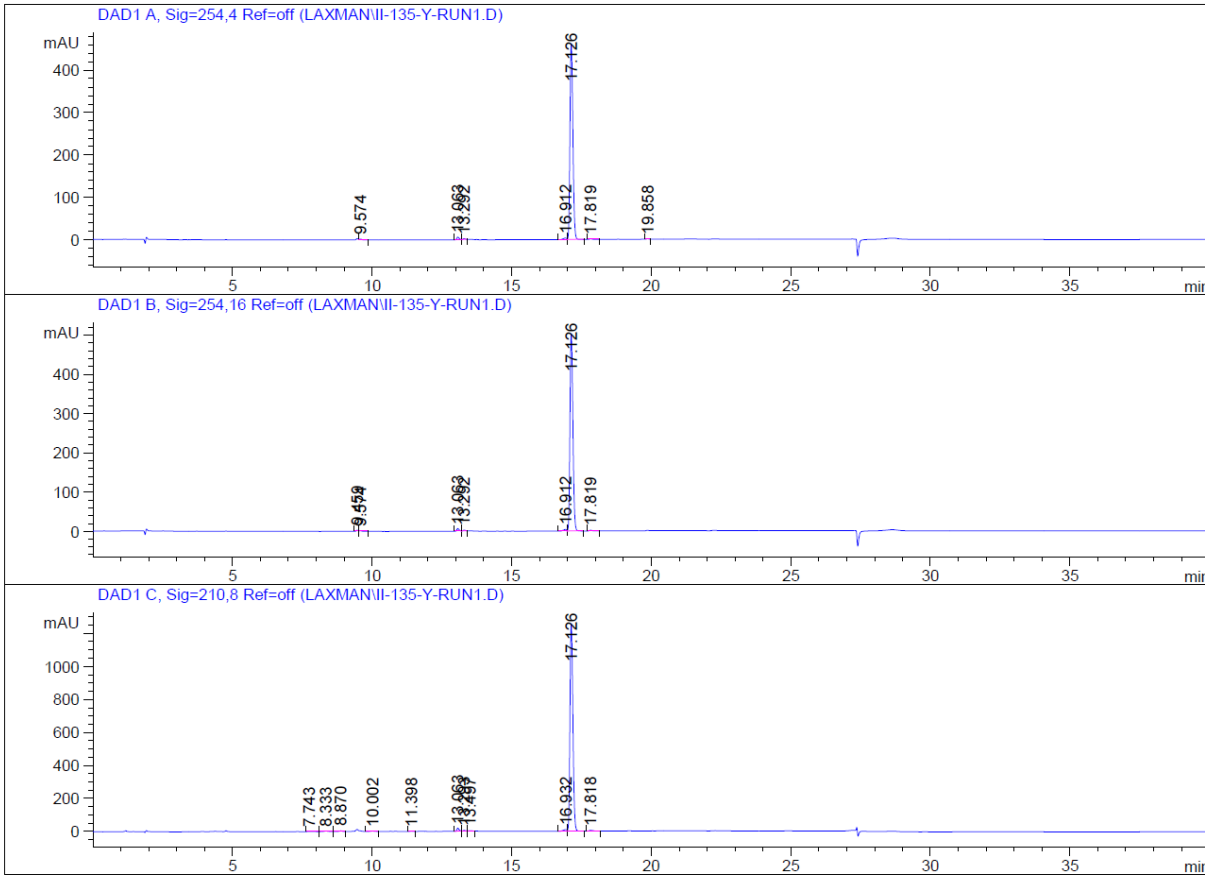
^{13}C NMR (500 MHz, $CDCl_3$) of Compound **43**



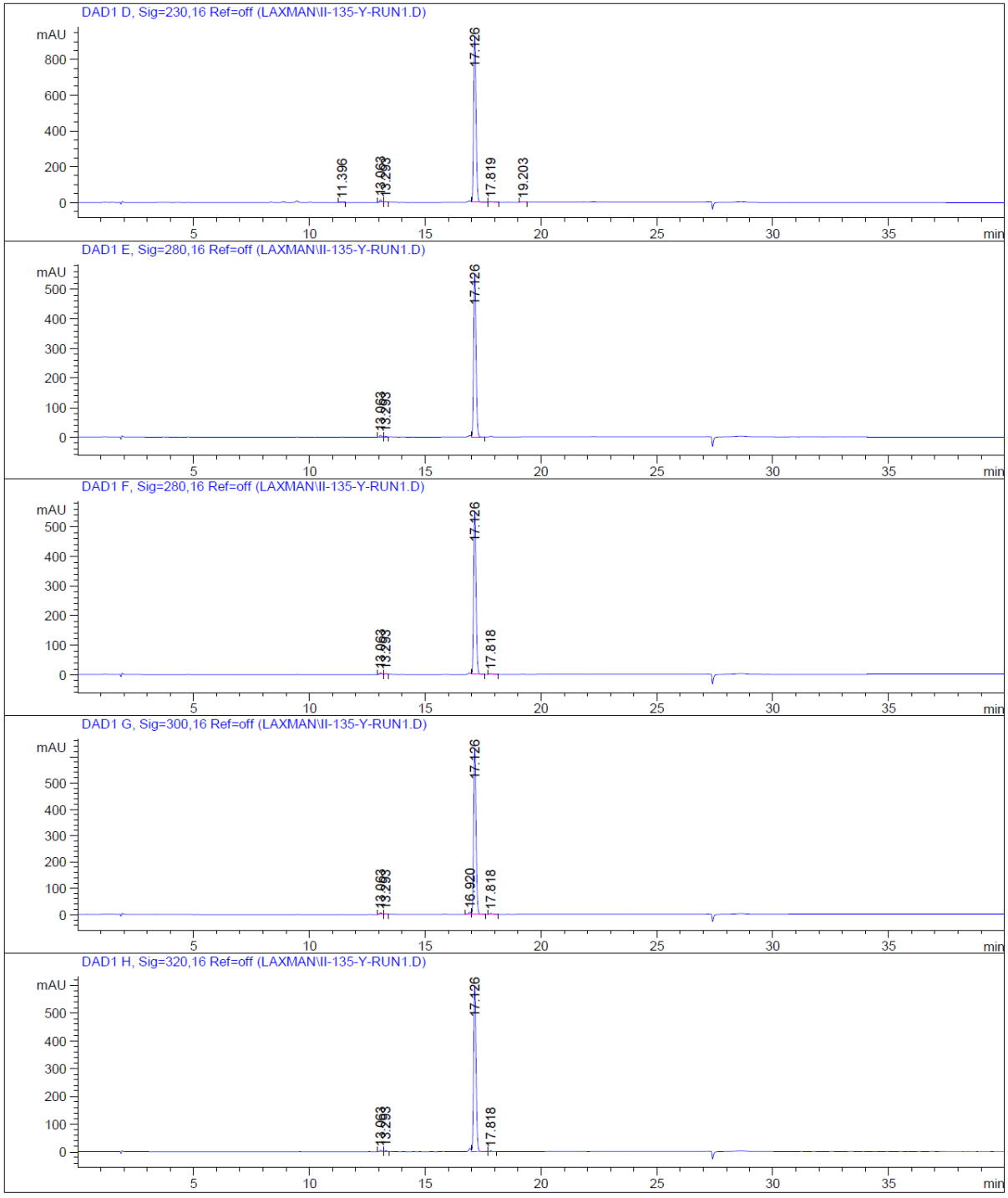
HPLC Trace of Compound **43**

```
Data File C:\CHEM32\1\DATA\LAXMAN\II-135-Y-RUN1.D
Sample Name: LD-II-135-Y- Blank1

    ======================================================================
    Acq. Operator   : Laxman
    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 11/14/2012 12:51:23 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 11/14/2012 12:27:15 PM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\II-135-Y-RUN1.D\DA.M (MASTERMETHOD.M)
    Last changed    : 11/14/2012 1:41:39 PM by Laxman
    Sample Info     :
                      10% ACN in Water
```



Data File C:\CHEM32\1\DATA\LAXMAN\II-135-Y-RUN1.D
Sample Name: LD-II-135-Y- Blank1



Data File C:\CHEM32\1\DATA\LAXMAN\II-135-Y-RUN1.D
Sample Name: LD-II-135-Y- Blank1

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.574	VB	0.0884	12.42534	2.05785	0.3582
2	13.063	BV	0.0886	36.53085	6.21524	1.0531
3	13.292	VB	0.0823	13.20063	2.39415	0.3805
4	16.912	BV	0.1250	32.23994	3.79975	0.9294
5	17.126	VB	0.1146	3354.70923	462.85483	96.7093
6	17.819	BB	0.1066	13.03892	1.79827	0.3759
7	19.858	BB	0.0861	6.71396	1.22175	0.1935
Totals :				3468.85887	480.34183	

Signal 2: DAD1 B, Sig=254,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.459	BV	0.0768	14.51984	2.87982	0.3841
2	9.574	VB	0.0887	10.97569	1.81098	0.2904
3	13.063	BV	0.0884	39.76277	6.77752	1.0519
4	13.292	VB	0.0819	13.63666	2.48918	0.3608
5	16.912	BV	0.1249	35.15076	4.14536	0.9299
6	17.126	VB	0.1145	3651.35840	503.87845	96.5989
7	17.819	BB	0.1055	14.51247	2.02653	0.3839
Totals :				3779.91658	524.00784	

Signal 3: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.743	BB	0.1630	14.03030	1.23105	0.1463
2	8.333	BB	0.1290	35.60196	3.81120	0.3712
3	8.870	BB	0.1127	31.62539	4.06788	0.3298
4	10.002	BB	0.1266	20.58630	2.29664	0.2147

Data File C:\CHEM32\1\DATA\LAXMAN\II-135-Y-RUN1.D
Sample Name: LD-II-135-Y- Blank1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
5	11.398	BB	0.0819	8.22434	1.54954	0.0858
6	13.063	BV	0.0929	118.07655	18.90557	1.2313
7	13.293	VV	0.1031	44.22927	6.06295	0.4612
8	13.497	VV	0.2122	41.45767	3.09574	0.4323
9	16.932	BV	0.1304	92.42262	10.32446	0.9638
10	17.126	VB	0.1167	9135.98730	1257.78174	95.2681
11	17.818	BB	0.1249	47.52321	5.38835	0.4956
Totals :				9589.76493	1314.51514	

Signal 4: DAD1 D, Sig=230,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.396	BB	0.0826	7.50027	1.39710	0.1082
2	13.063	BV	0.0929	92.58957	14.82736	1.3352
3	13.293	VV	0.1024	34.48166	4.76518	0.4973
4	17.126	VB	0.1145	6759.72949	932.89081	97.4820
5	17.819	BB	0.1088	31.06098	4.07915	0.4479
6	19.203	BB	0.1156	8.97497	1.16906	0.1294
Totals :				6934.33694	959.12866	

Signal 5: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.063	BV	0.0883	35.15522	6.00424	0.8637
2	13.293	VB	0.0801	15.65592	2.94128	0.3846
3	17.126	VB	0.1145	4019.61060	554.99536	98.7517
Totals :				4070.42174	563.94088	

Signal 6: DAD1 F, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.063	BV	0.0883	35.15522	6.00424	0.8596
2	13.293	VB	0.0801	15.65592	2.94128	0.3828
3	17.126	VB	0.1145	4019.61060	554.99536	98.2804
4	17.818	BB	0.1008	19.51952	2.96627	0.4773

Data File C:\CHEM32\1\DATA\LAXMAN\II-135-Y-RUN1.D
Sample Name: LD-II-135-Y- Blank1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				4089.94126	566.90715	

Signal 7: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.063	BV	0.0884	38.27963	6.52826	0.8106
2	13.293	VB	0.0803	11.99059	2.24650	0.2539
3	16.920	BV	0.1100	61.49498	8.53992	1.3022
4	17.126	VB	0.1146	4588.46826	633.00854	97.1655
5	17.818	BB	0.0978	22.08729	3.49350	0.4677
Totals :				4722.32075	653.81673	

Signal 8: DAD1 H, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.063	BV	0.0881	30.74878	5.26413	0.6958
2	13.293	VB	0.0793	20.63757	3.92874	0.4670
3	17.126	VB	0.1147	4350.68311	599.46283	98.4526
4	17.818	BB	0.0972	16.99543	2.70743	0.3846
Totals :				4419.06489	611.36313	

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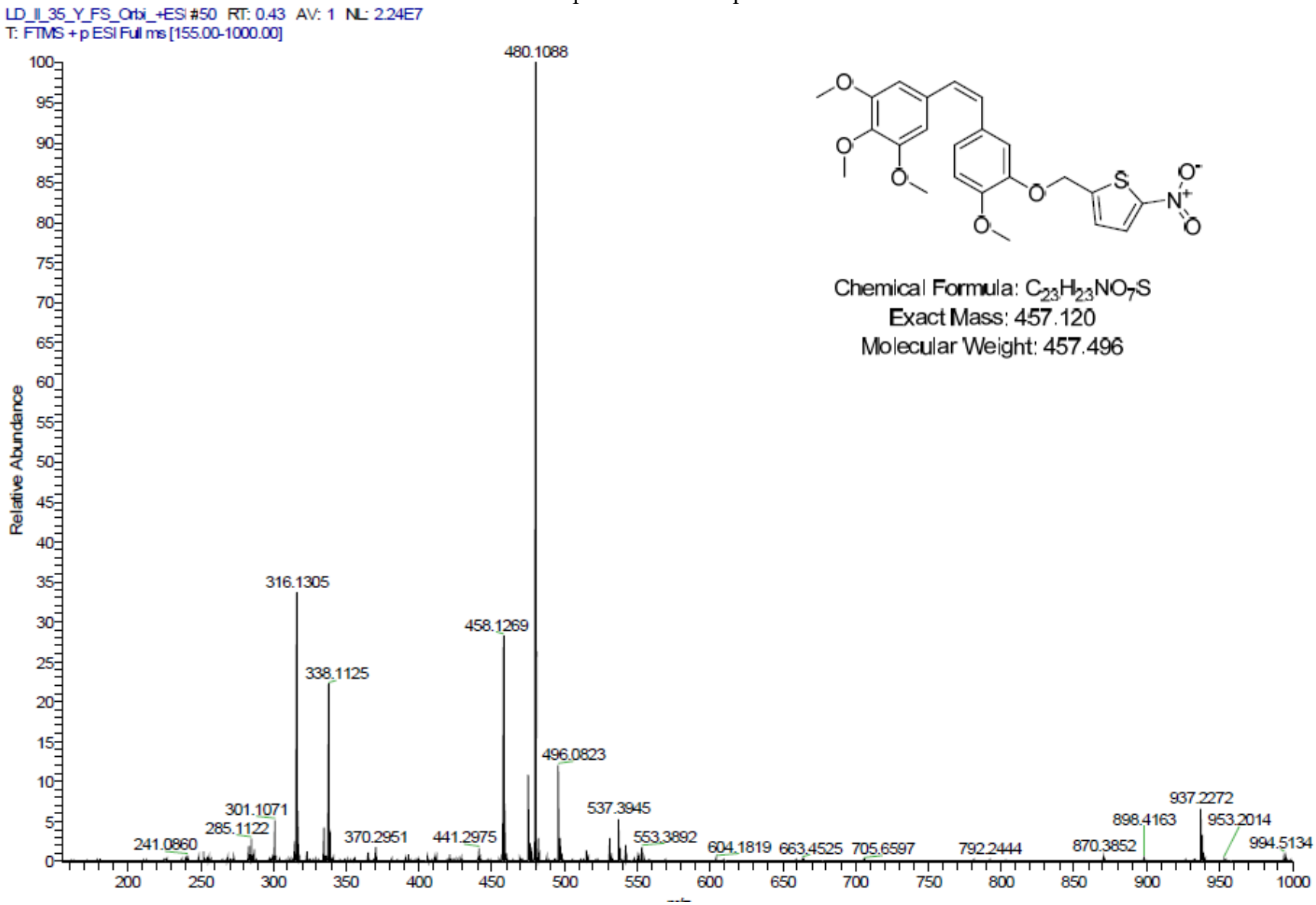
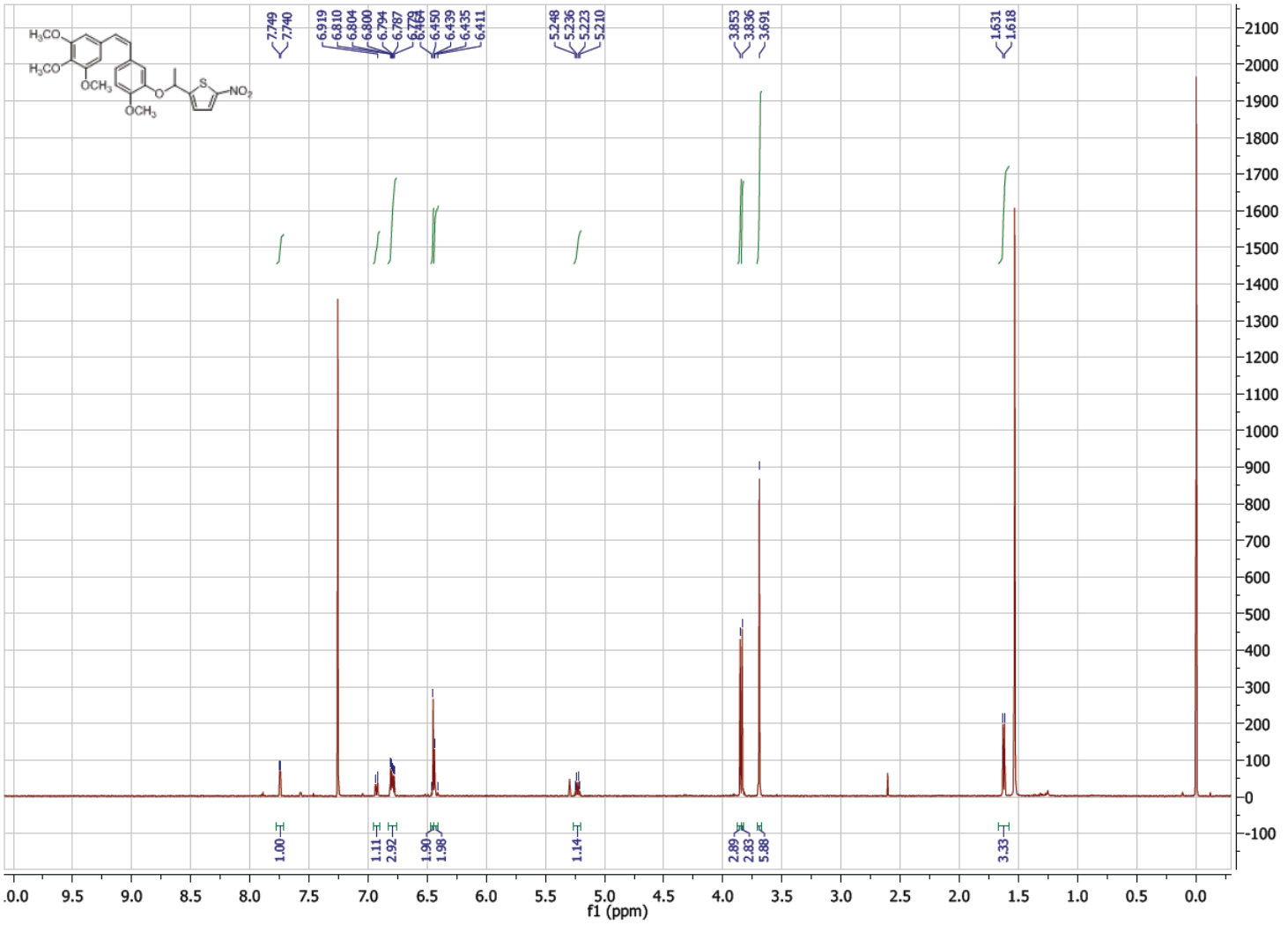
*** End of Report ***

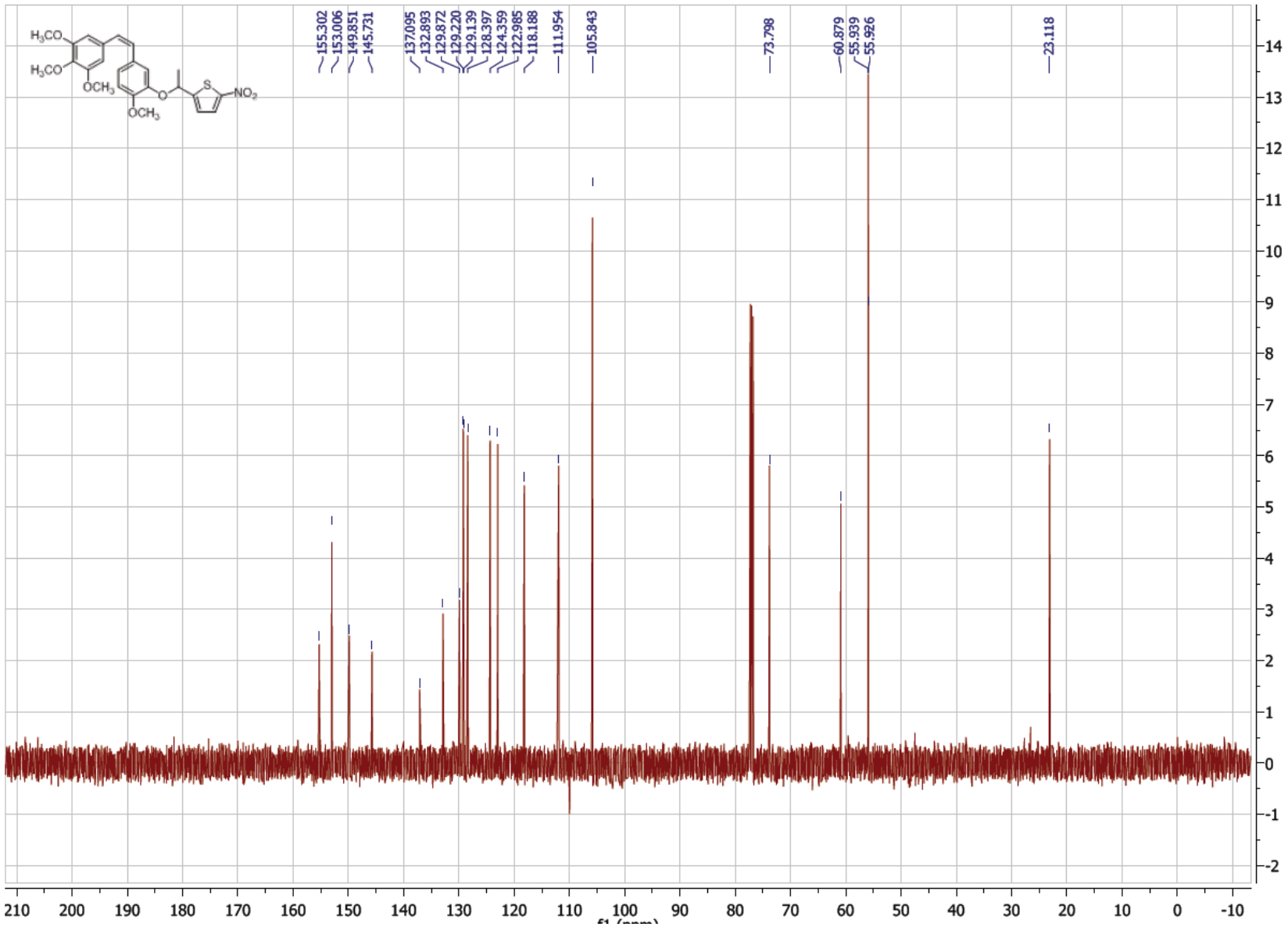
Mass Spectrum of Compound **43**



^{1}H NMR (500 MHz, $CDCl_3$) of Compound **44**



^{13}C NMR (500 MHz, $CDCl_3$) of Compound **44**



HPLC Trace of compound **44**

Data File C:\Chem32\1\Data\Blake\CA4 Monothio pTLC5.D
Sample Name: CA4 Monothio pTLC5

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======================================================================
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Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC                    Location :   1
Injection Date  : 7/8/2016 4:47:42 PM
                                               Inj Volume : No inj
Acq. Method     : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
Last changed    : 4/30/2014 1:53:57 AM by ERICAP
Analysis Method : C:\CHEM32\1\METHODS\RT-ACNWASH 2.M
Last changed    : 7/9/2015 2:27:22 PM by Blake
Method Info     : General Column Wash Method

Additional Info : Peak(s) manually integrated
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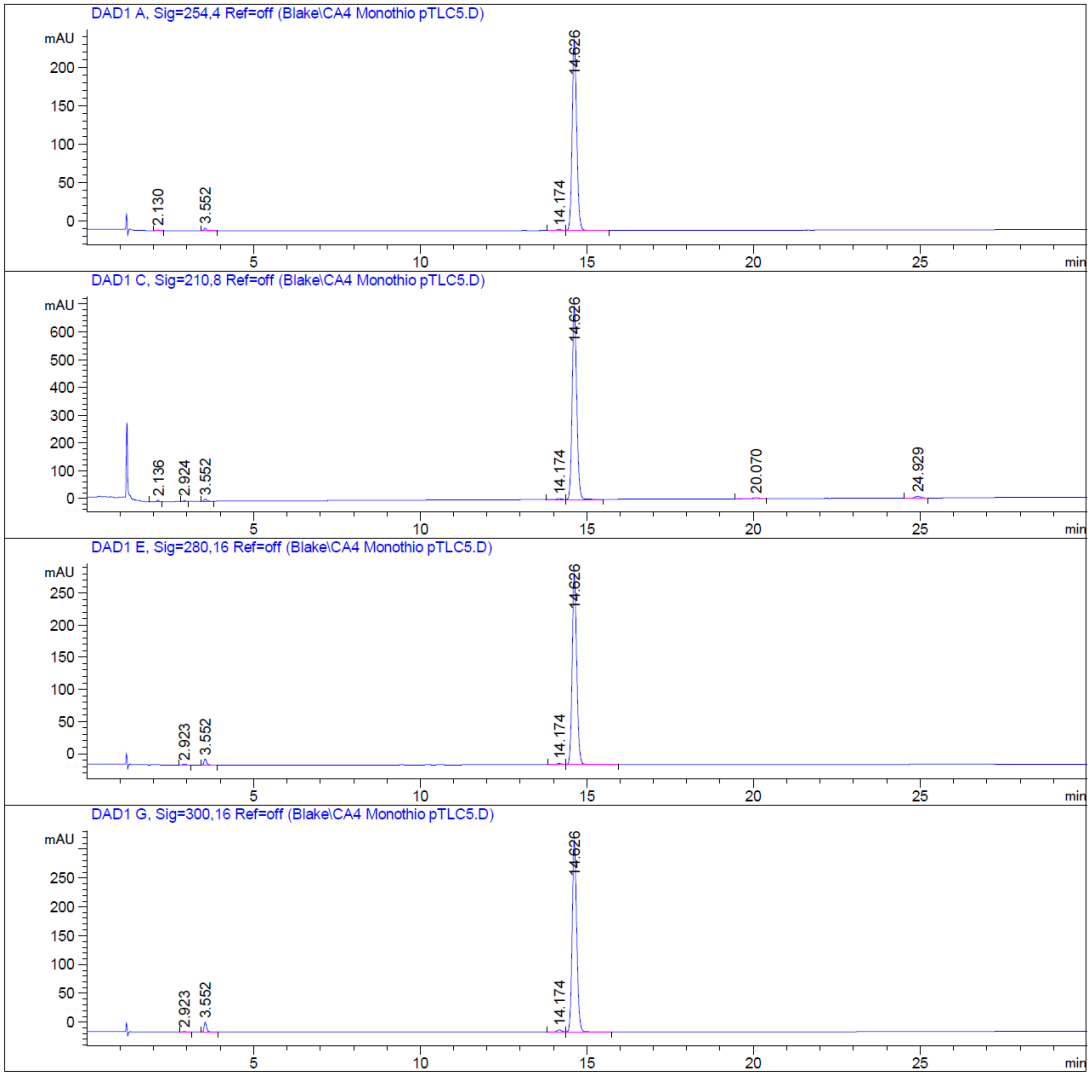
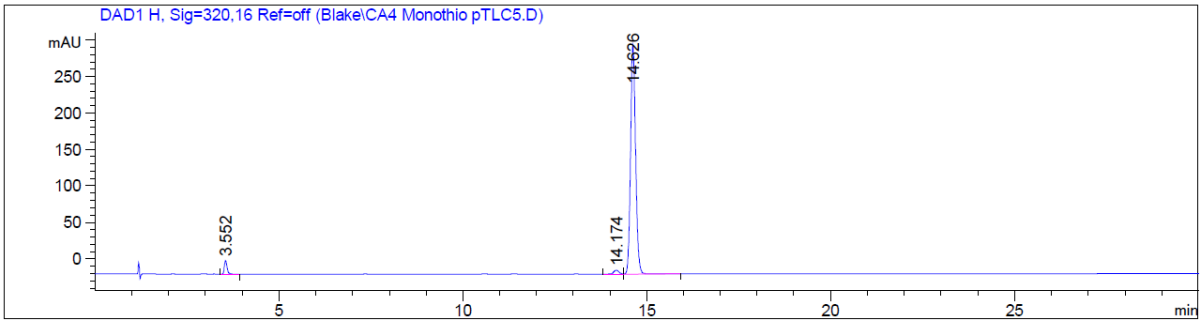


Data File C:\Chem32\1\Data\Blake\CA4 Monothio pTLC5.D
Sample Name: CA4 Monothio pTLC5



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.130	BB	0.0777	5.62220	1.06346	0.2320
2	3.552	BB	0.0820	18.60733	3.50377	0.7677
3	14.174	BV	0.1472	13.58555	1.40050	0.5605
4	14.626	VB	0.1481	2385.85034	248.38174	98.4398

Totals : 2423.66542 254.34947

Signal 2: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.136	BB	0.0801	28.96852	5.26916	0.4191
2	2.924	BB	0.0764	14.33322	2.96729	0.2074
3	3.552	BB	0.0816	36.36889	6.88614	0.5262
4	14.174	BV	0.1490	31.27967	3.17406	0.4525
5	14.626	VB	0.1481	6678.81738	694.79736	96.6230
6	20.070	BB	0.1771	53.05204	4.58050	0.7675
7	24.929	BB	0.1923	69.42081	5.53725	1.0043

Totals : 6912.24055 723.21177

Data File C:\Chem32\1\Data\Blake\CA4 Monothio pTLC5.D
Sample Name: CA4 Monothio pTLC5

Signal 3: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.923	BB	0.0811	6.66962	1.23359	0.2272
2	3.552	BB	0.0819	49.89929	9.39798	1.7000
3	14.174	BV	0.1489	19.37604	2.00204	0.6601
4	14.626	VB	0.1481	2859.24146	297.65536	97.4126
Totals :				2935.18641	310.28898	

Signal 4: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.923	BB	0.0843	7.20654	1.26827	0.2164
2	3.552	BB	0.0820	91.39571	17.19951	2.7441
3	14.174	BV	0.1448	36.30665	3.82295	1.0901
4	14.626	VB	0.1481	3195.77393	332.57687	95.9495
Totals :				3330.68283	354.86761	

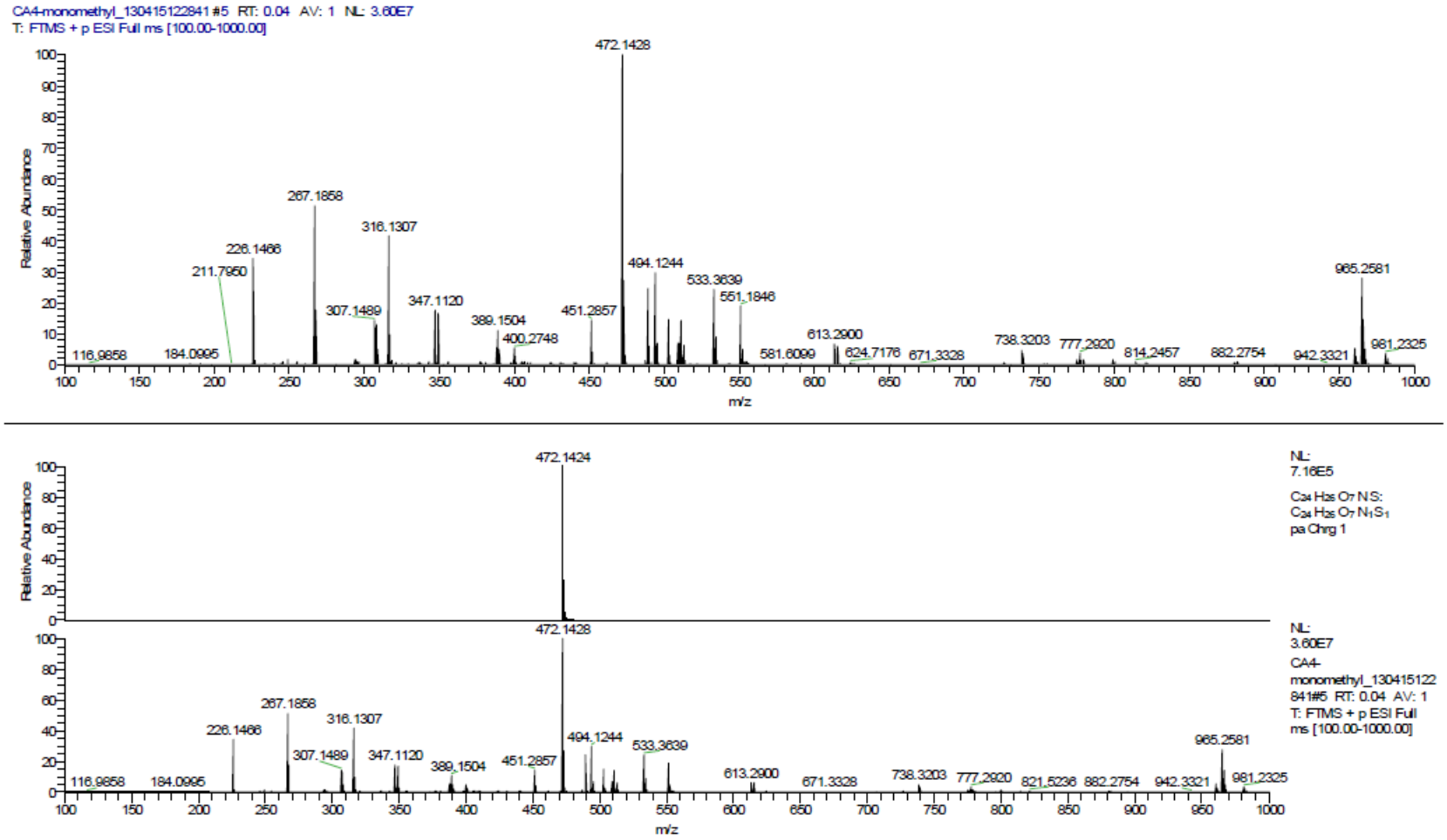
Signal 5: DAD1 H, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.552	BB	0.0820	96.86775	18.21322	3.0557
2	14.174	BV	0.1463	49.83819	5.27211	1.5721
3	14.626	VB	0.1482	3023.37158	314.28720	95.3722
Totals :				3170.07752	337.77254	

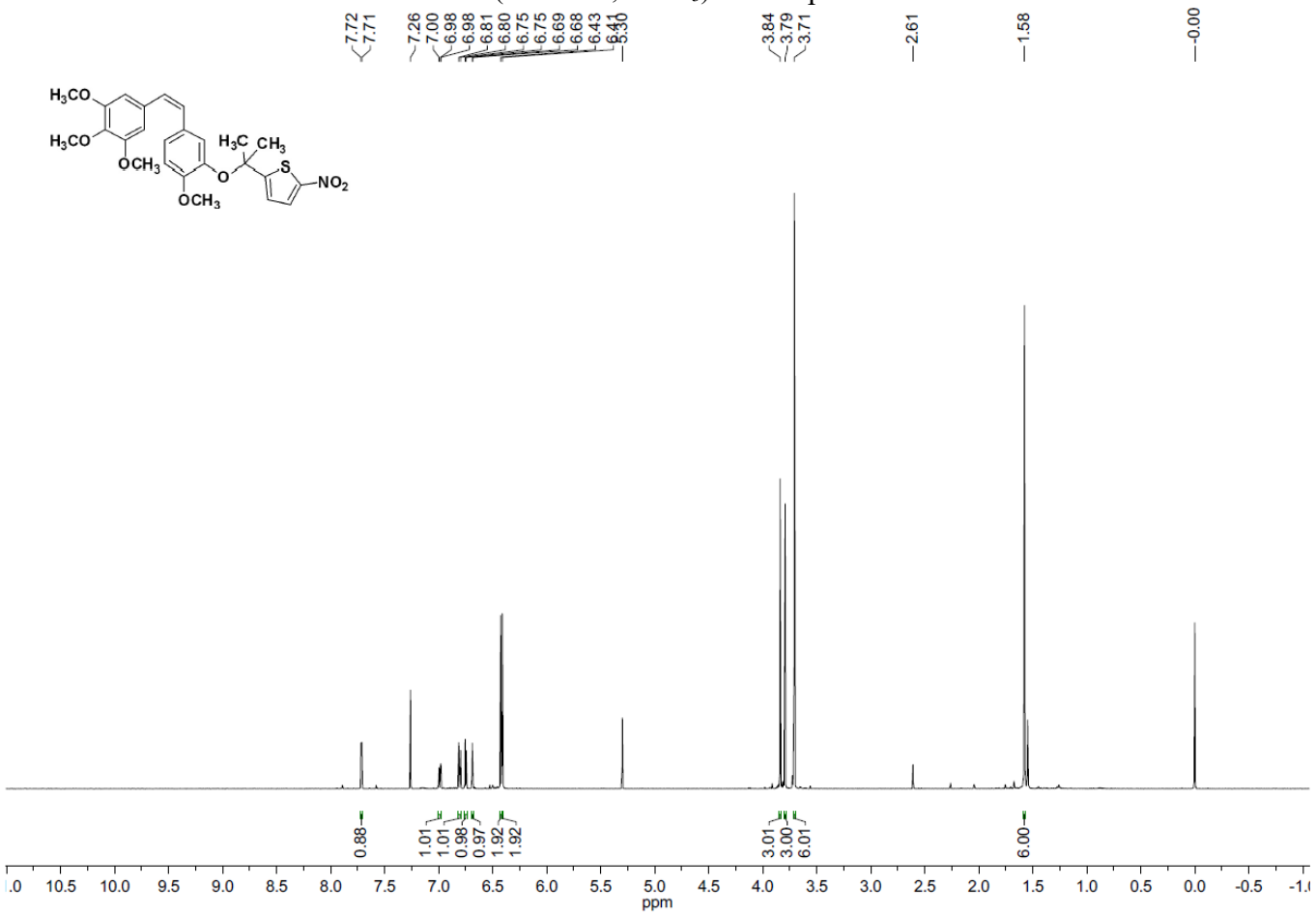
*** End of Report ***

Mass Spectrum of Compound **44**

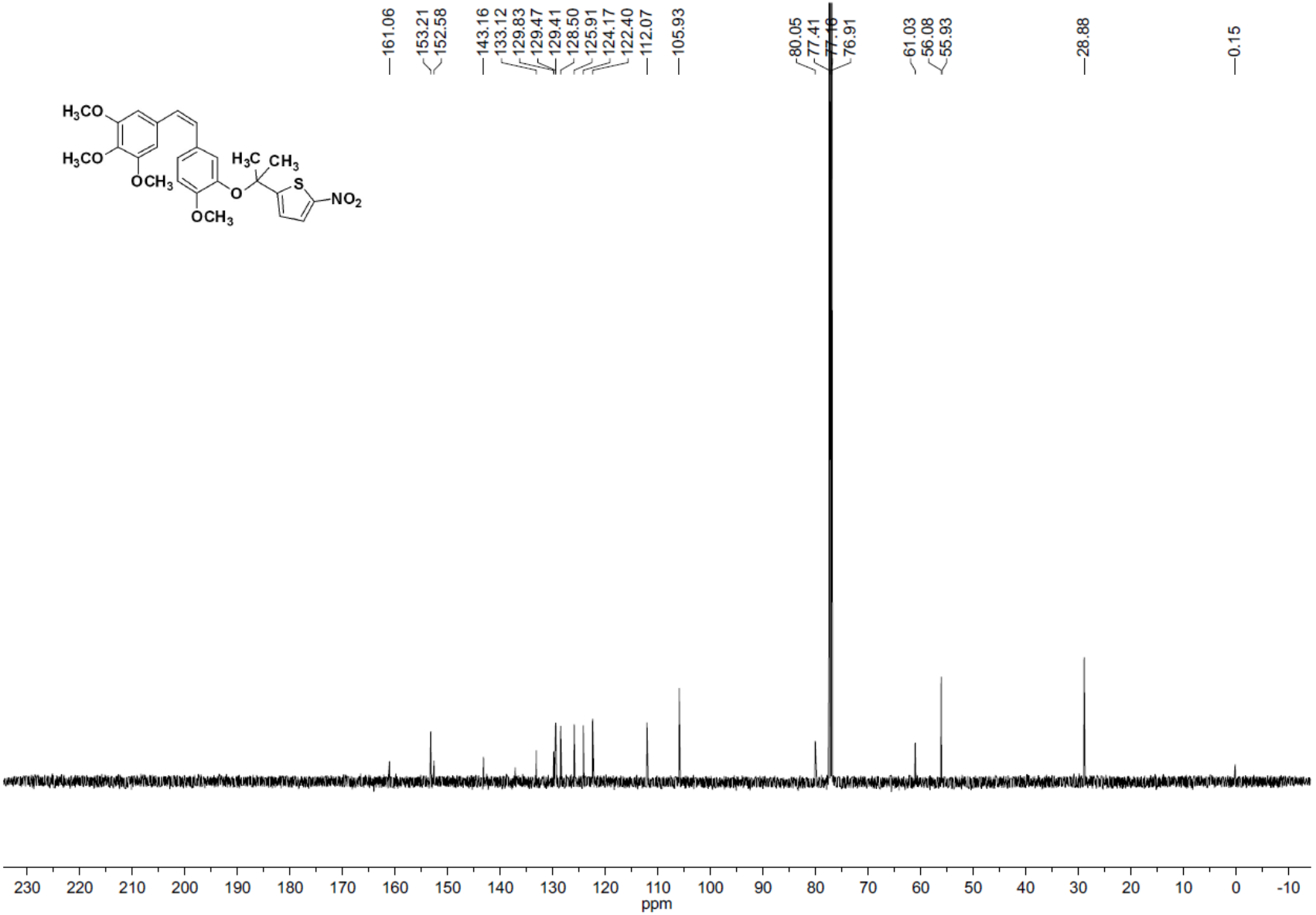
CA4-monomethyl_130415122841 4/15/2013 12:28:42 PM BWH-101-42



^{1}H NMR (500 MHz, $CDCl_3$) of Compound **45**



^{13}C NMR (500 MHz, $CDCl_3$) of Compound **45**



HPLC Trace of Compound **45**

Data File C:\Chem32\1\Data\Debo\MT_IV_30_July232016.D
Sample Name: MT_IV_30_July232016

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    ======================================================================
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    Sample Operator : SYSTEM
    Acq. Instrument : 1200 HPLC                    Location :   1
    Injection Date  : 7/23/2016 9:42:36 AM
                                                   Inj Volume : No inj
    Method          : C:\Chem32\1\Methods\GRAD 2 50-90 ACN.M
    Last changed    : 4/30/2014 1:53:57 AM by ERICAP
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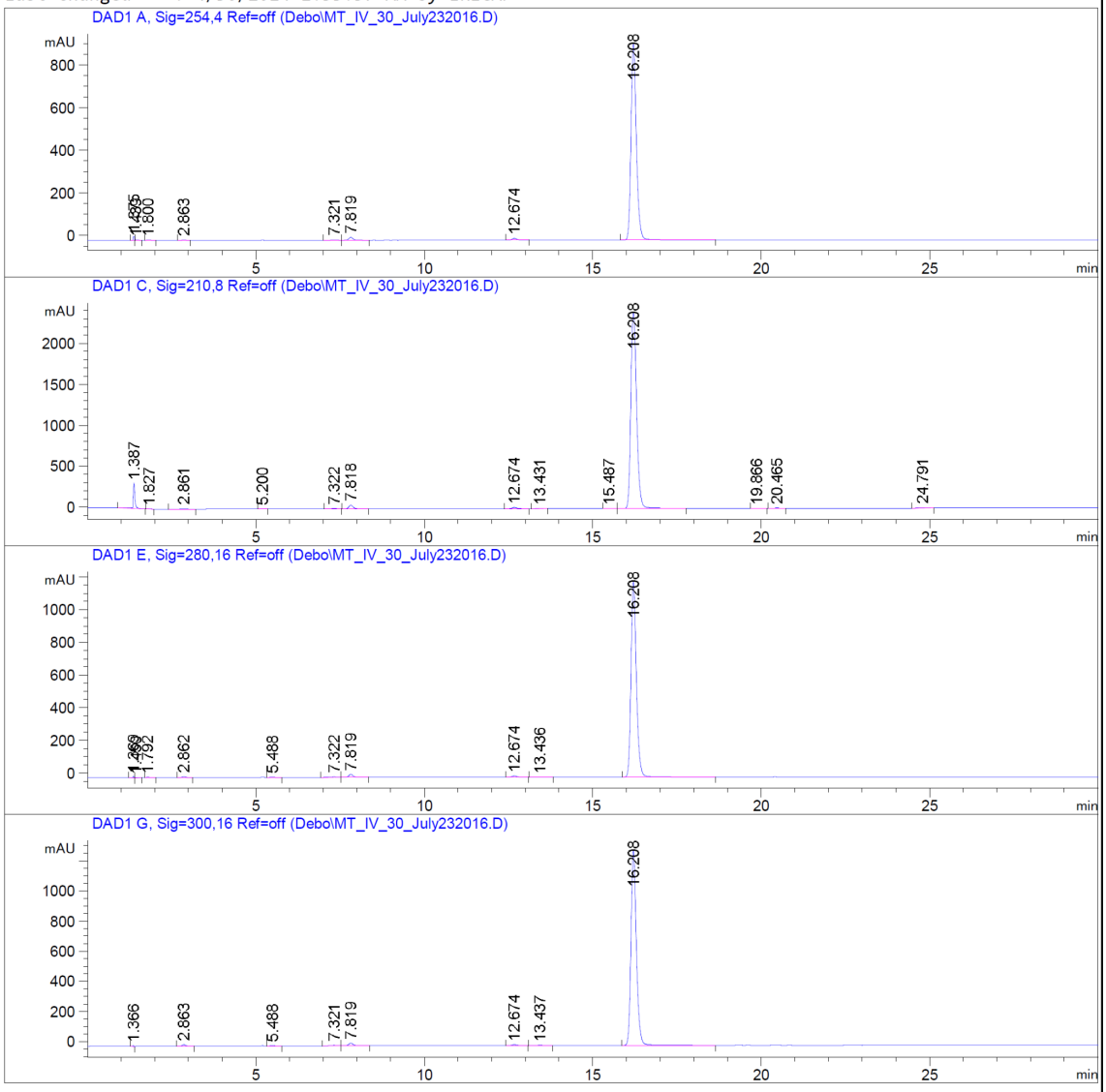
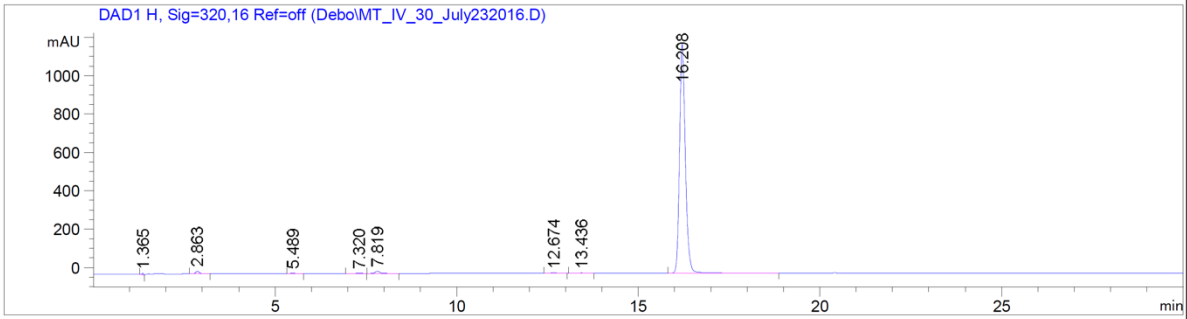


Data File C:\Chem32\1\Data\Debo\MT_IV_30_July232016.D
Sample Name: MT_IV_30_July232016



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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.375	BB	0.0395	47.84431	20.32474	0.4520
2	1.439	BB	0.0639	8.20352	1.77653	0.0775
3	1.800	BB	0.1336	10.87313	1.30011	0.1027
4	2.863	BB	0.1040	22.44121	3.27424	0.2120
5	7.321	BB	0.1386	22.19114	2.47454	0.2096
6	7.819	BB	0.1408	126.82910	13.59680	1.1981
7	12.674	BB	0.1491	83.87212	8.50225	0.7923
8	16.208	BB	0.1699	1.02636e4	921.47638	96.9558
Totals :				1.05859e4	972.72559	

Signal 2: DAD1 C, Sig=210,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.387	BB	0.0530	1065.00549	303.36530	3.4833
2	1.827	BB	0.1043	35.50399	5.15995	0.1161
3	2.861	BB	0.1512	77.23377	7.08497	0.2526
4	5.200	BB	0.1118	18.82471	2.62088	0.0616
5	7.322	BB	0.1355	70.29004	7.92301	0.2299
6	7.818	BB	0.1396	405.49631	43.97836	1.3263
7	12.674	BB	0.1489	133.60876	13.56780	0.4370
8	13.431	BB	0.1597	12.15954	1.14720	0.0398
9	15.487	BB	0.1574	21.32000	2.11807	0.0697
10	16.208	BB	0.1878	2.86536e4	2391.41113	93.7182
11	19.866	BB	0.1788	18.68639	1.64127	0.0611

Data File C:\Chem32\1\Data\Debo\MT_IV_30_July232016.D
Sample Name: MT_IV_30_July232016

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
12	20.465	BB	0.2256	28.00288	1.99733	0.0916
13	24.791	BB	0.2067	34.46542	2.53411	0.1127
Totals :				3.05742e4	2784.54936	

Signal 3: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.369	BB	0.0424	31.02741	9.94166	0.2251
2	1.450	BB	0.0881	22.86131	3.60076	0.1659
3	1.792	BB	0.1071	9.47275	1.24050	0.0687
4	2.862	BB	0.1074	41.54430	5.81458	0.3014
5	5.488	BB	0.1153	8.60729	1.12513	0.0625
6	7.322	BV	0.1411	31.28039	3.34435	0.2270
7	7.819	VB	0.1429	178.90128	18.81951	1.2981
8	12.674	BB	0.1480	80.48096	8.23416	0.5840
9	13.436	BB	0.1775	15.14640	1.28551	0.1099
10	16.208	BB	0.1701	1.33622e4	1198.67053	96.9574
Totals :				1.37815e4	1252.07668	

Signal 4: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.366	BB	0.0420	26.20239	8.49298	0.1760
2	2.863	BB	0.1074	62.68039	8.76715	0.4210
3	5.488	BB	0.1181	13.51595	1.75055	0.0908
4	7.321	BB	0.1379	28.18667	3.10416	0.1893
5	7.819	BB	0.1413	166.55807	17.77341	1.1187
6	12.674	BB	0.1481	56.69556	5.79743	0.3808
7	13.437	BB	0.1756	19.28821	1.65920	0.1295
8	16.208	BB	0.1707	1.45158e4	1295.72217	97.4939
Totals :				1.48890e4	1343.06704	

Signal 5: DAD1 H, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.365	BB	0.0423	24.93155	8.01595	0.1815
2	2.863	BB	0.1072	98.64788	13.83761	0.7182
3	5.489	BB	0.1194	20.06679	2.56164	0.1461
4	7.320	BB	0.1469	19.44419	2.00913	0.1416

Data File C:\Chem32\1\Data\Debo\MT_IV_30_July232016.D
Sample Name: MT_IV_30_July232016

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
5	7.819	BB	0.1450	110.30787	11.59118	0.8031
6	12.674	BB	0.1484	33.36906	3.40343	0.2430
7	13.436	BB	0.1734	18.38263	1.58354	0.1338
8	16.208	BB	0.1712	1.34095e4	1192.82092	97.6326
Totals :				1.37347e4	1235.82340	

*** End of Report ***

X-Ray Crystallography Data for Compound **45**

Crystallographic data for compound **45** (CCDC-1502328) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax:+44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk). Compound **45** is referred to as KP61 in the tables on pages S176-S183.

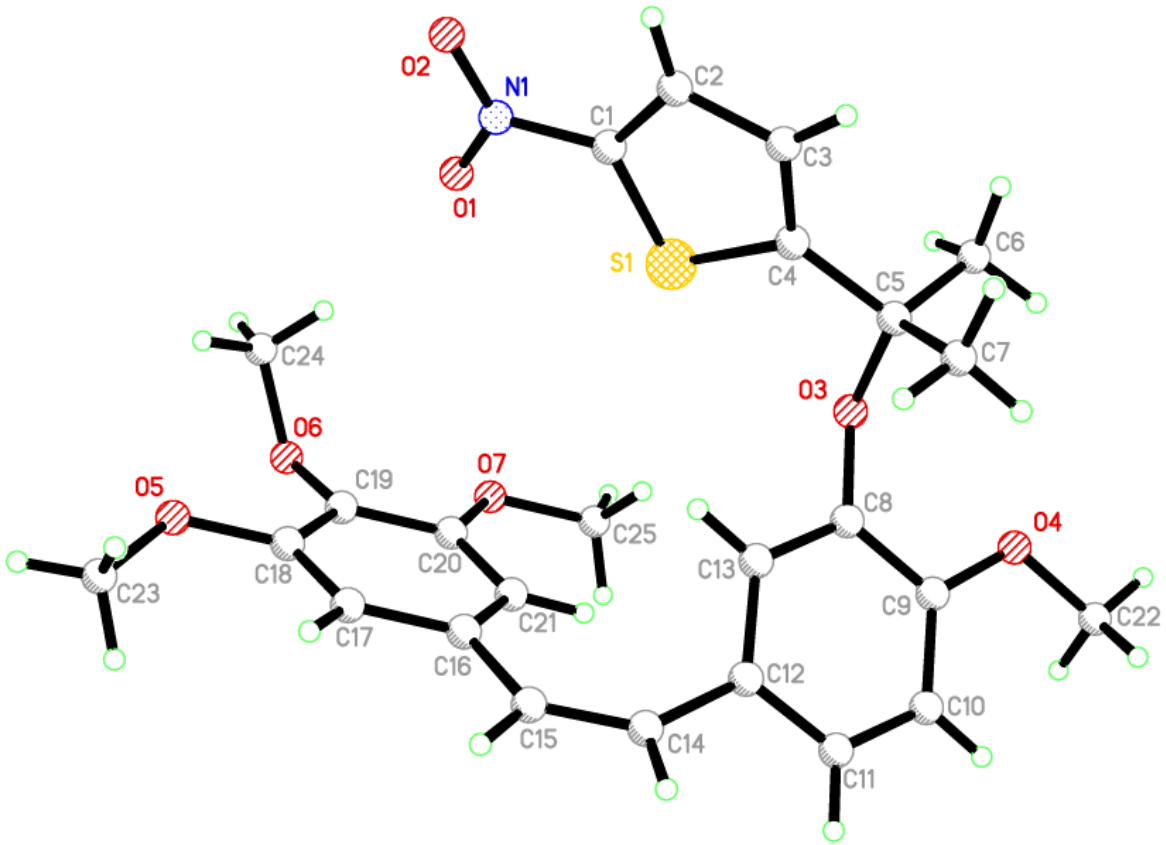


Table S1. Crystal data and structure refinement for kp61.

Identification code	kp61	
Empirical formula	C25 H27 N O7 S	
Formula weight	485.54	
Temperature	110(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 8.0895(7) Å	α= 100.514(5)°.
	b = 12.0696(10) Å	β= 104.240(5)°.
	c = 14.0112(12) Å	γ = 108.180(5)°.
Volume	1208.84(18) $Å^3$	
Z	2	
Density (calculated)	1.334 Mg/m^3	
Absorption coefficient	0.179 mm^{-1}	
F(000)	512	
Crystal size	0.31 x 0.28 x 0.11 mm^3	
Theta range for data collection	2.06 to 26.46°.	
Index ranges	-9<=h<=10, -15<=k<=15, -16<=l<=17	
Reflections collected	12273	
Independent reflections	4851 [R(int) = 0.0427]	
Completeness to theta = 26.46°	97.5 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9802 and 0.9465	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4851 / 0 / 313	
Goodness-of-fit on F^2	1.028	
Final R indices [I>2sigma(I)]	R1 = 0.0457, wR2 = 0.1101	
R indices (all data)	R1 = 0.0772, wR2 = 0.1277	
Largest diff. peak and hole	0.158 and -0.218 $e.Å^{-3}$	

Table S2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for kp61. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
S(1)	3104(1)	5110(1)	1330(1)	61(1)
O(1)	975(3)	2648(2)	59(2)	112(1)
O(2)	88(3)	3044(2)	-1374(1)	94(1)
O(3)	5098(2)	7059(1)	3092(1)	52(1)
O(4)	7261(2)	8749(1)	4853(1)	66(1)
O(5)	-4646(2)	1366(2)	1585(1)	79(1)
O(6)	-1425(2)	1040(1)	2114(1)	71(1)
O(7)	1682(2)	2868(1)	3284(1)	65(1)
N(1)	959(3)	3351(2)	-460(2)	73(1)
C(1)	2038(3)	4618(2)	40(2)	60(1)
C(2)	2242(4)	5513(3)	-422(2)	80(1)
C(3)	3320(4)	6639(2)	290(2)	80(1)
C(4)	3899(3)	6577(2)	1275(2)	53(1)
C(5)	5164(3)	7583(2)	2234(2)	56(1)
C(6)	7146(3)	7905(2)	2232(2)	84(1)
C(7)	4646(4)	8688(2)	2330(2)	69(1)
C(8)	4284(3)	7434(2)	3785(1)	45(1)
C(9)	5429(3)	8284(2)	4725(2)	49(1)
C(10)	4634(3)	8594(2)	5448(2)	56(1)
C(11)	2758(3)	8086(2)	5236(2)	56(1)
C(12)	1602(3)	7231(2)	4313(2)	49(1)
C(13)	2427(3)	6927(2)	3591(2)	47(1)
C(14)	-406(3)	6740(2)	4103(2)	61(1)
C(15)	-1683(3)	5628(2)	3628(2)	60(1)
C(16)	-1551(3)	4457(2)	3224(2)	50(1)
C(17)	-3142(3)	3499(2)	2579(2)	57(1)
C(18)	-3120(3)	2368(2)	2196(2)	56(1)
C(19)	-1484(3)	2175(2)	2432(2)	54(1)
C(20)	127(3)	3133(2)	3083(2)	51(1)
C(21)	85(3)	4252(2)	3489(2)	52(1)
C(22)	8469(3)	9566(2)	5828(2)	74(1)

C(23)	-6392(4)	1422(3)	1509(2)	99(1)
C(24)	-1556(5)	656(3)	1080(2)	98(1)
C(25)	3340(3)	3811(2)	3954(2)	66(1)

Table S3. Bond lengths [Å] and angles [°] for kp61.

S(1)-C(1)	1.699(2)
S(1)-C(4)	1.707(2)
O(1)-N(1)	1.215(3)
O(2)-N(1)	1.222(3)
O(3)-C(8)	1.387(2)
O(3)-C(5)	1.462(2)
O(4)-C(9)	1.362(2)
O(4)-C(22)	1.430(3)
O(5)-C(18)	1.374(3)
O(5)-C(23)	1.413(3)
O(6)-C(19)	1.380(2)
O(6)-C(24)	1.405(3)
O(7)-C(20)	1.369(2)
O(7)-C(25)	1.419(3)
N(1)-C(1)	1.438(3)
C(1)-C(2)	1.344(3)
C(2)-C(3)	1.393(3)
C(3)-C(4)	1.368(3)
C(4)-C(5)	1.510(3)
C(5)-C(7)	1.511(3)
C(5)-C(6)	1.528(3)
C(8)-C(13)	1.368(3)
C(8)-C(9)	1.399(3)
C(9)-C(10)	1.383(3)
C(10)-C(11)	1.378(3)
C(11)-C(12)	1.389(3)
C(12)-C(13)	1.397(3)
C(12)-C(14)	1.476(3)
C(14)-C(15)	1.335(3)
C(15)-C(16)	1.470(3)
C(16)-C(17)	1.389(3)
C(16)-C(21)	1.395(3)
C(17)-C(18)	1.380(3)
C(18)-C(19)	1.386(3)

C(19)-C(20)	1.399(3)
C(20)-C(21)	1.380(3)

C(1)-S(1)-C(4)	90.52(11)
C(8)-O(3)-C(5)	120.04(14)
C(9)-O(4)-C(22)	117.76(18)
C(18)-O(5)-C(23)	117.7(2)
C(19)-O(6)-C(24)	115.77(18)
C(20)-O(7)-C(25)	117.19(16)
O(1)-N(1)-O(2)	124.0(2)
O(1)-N(1)-C(1)	117.4(2)
O(2)-N(1)-C(1)	118.6(2)
C(2)-C(1)-N(1)	125.5(2)
C(2)-C(1)-S(1)	114.02(19)
N(1)-C(1)-S(1)	120.41(17)
C(1)-C(2)-C(3)	110.8(2)
C(4)-C(3)-C(2)	113.7(2)
C(3)-C(4)-C(5)	129.0(2)
C(3)-C(4)-S(1)	110.99(18)
C(5)-C(4)-S(1)	119.94(15)
O(3)-C(5)-C(4)	106.26(15)
O(3)-C(5)-C(7)	113.24(16)
C(4)-C(5)-C(7)	111.97(19)
O(3)-C(5)-C(6)	104.83(18)
C(4)-C(5)-C(6)	109.06(18)
C(7)-C(5)-C(6)	111.10(19)
C(13)-C(8)-O(3)	121.15(17)
C(13)-C(8)-C(9)	120.55(18)
O(3)-C(8)-C(9)	118.14(18)
O(4)-C(9)-C(10)	124.90(19)
O(4)-C(9)-C(8)	116.78(18)
C(10)-C(9)-C(8)	118.3(2)
C(11)-C(10)-C(9)	120.4(2)
C(10)-C(11)-C(12)	122.11(19)
C(11)-C(12)-C(13)	116.73(19)
C(11)-C(12)-C(14)	120.37(19)

C(13)-C(12)-C(14)	122.79(19)
C(8)-C(13)-C(12)	121.85(19)
C(15)-C(14)-C(12)	131.8(2)
C(14)-C(15)-C(16)	132.1(2)
C(17)-C(16)-C(21)	118.51(19)
C(17)-C(16)-C(15)	118.41(19)
C(21)-C(16)-C(15)	123.0(2)
C(18)-C(17)-C(16)	121.3(2)
O(5)-C(18)-C(17)	124.6(2)
O(5)-C(18)-C(19)	115.2(2)
C(17)-C(18)-C(19)	120.2(2)
O(6)-C(19)-C(18)	121.8(2)
O(6)-C(19)-C(20)	118.94(19)
C(18)-C(19)-C(20)	119.0(2)
O(7)-C(20)-C(21)	124.03(19)
O(7)-C(20)-C(19)	115.48(18)
C(21)-C(20)-C(19)	120.48(19)
C(20)-C(21)-C(16)	120.5(2)

Symmetry transformations used to generate equivalent atoms:

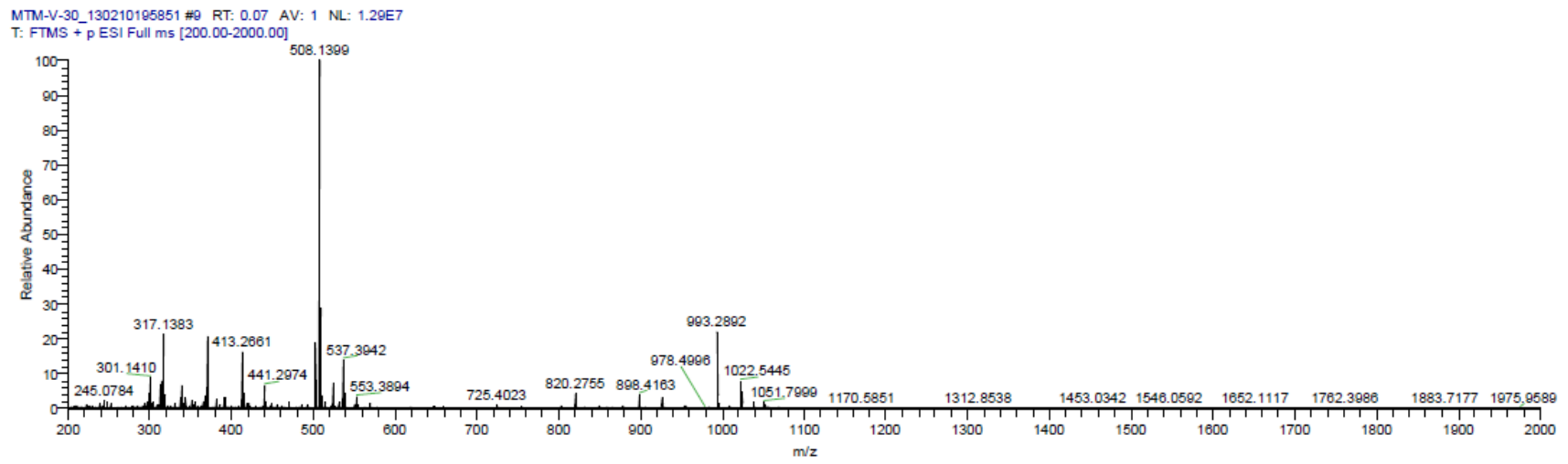
Table S4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for kp61. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

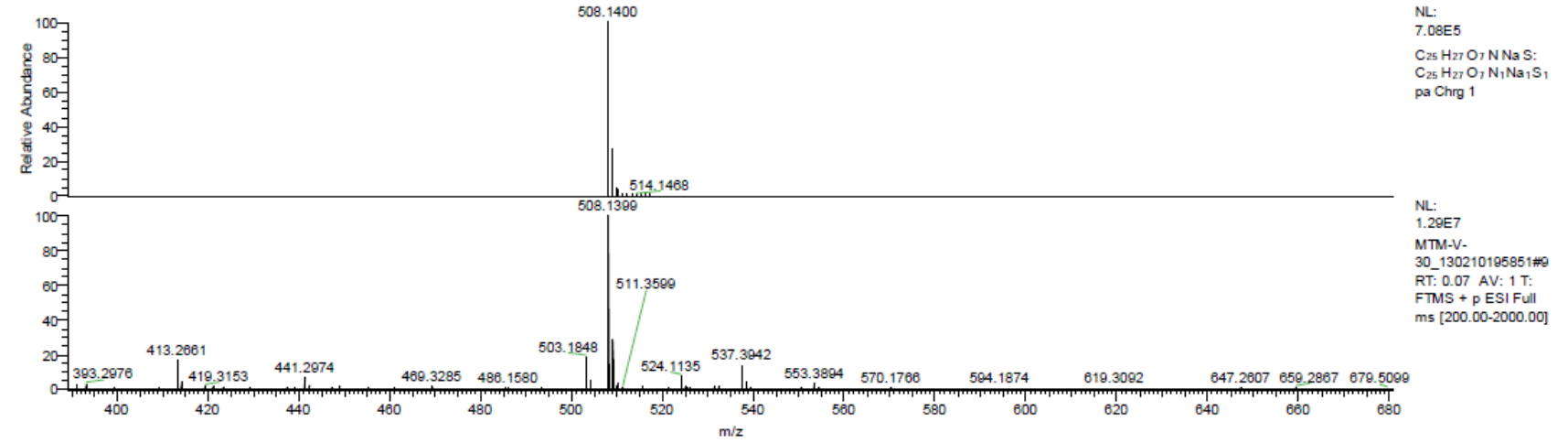
	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S(1)	80(1)	59(1)	49(1)	21(1)	21(1)	30(1)
O(1)	149(2)	71(1)	90(2)	22(1)	16(1)	25(1)
O(2)	85(1)	116(2)	59(1)	-3(1)	11(1)	37(1)
O(3)	63(1)	57(1)	49(1)	18(1)	25(1)	33(1)
O(4)	54(1)	66(1)	65(1)	8(1)	12(1)	18(1)
O(5)	56(1)	72(1)	86(1)	8(1)	11(1)	13(1)
O(6)	84(1)	48(1)	77(1)	17(1)	20(1)	24(1)
O(7)	55(1)	51(1)	89(1)	20(1)	14(1)	26(1)
N(1)	75(2)	84(2)	60(1)	12(1)	22(1)	36(1)
C(1)	71(2)	69(2)	47(1)	16(1)	25(1)	32(1)
C(2)	102(2)	98(2)	48(1)	29(1)	30(1)	41(2)
C(3)	111(2)	76(2)	62(2)	36(1)	34(2)	36(2)
C(4)	64(1)	61(1)	53(1)	24(1)	32(1)	32(1)
C(5)	64(1)	54(1)	61(1)	22(1)	33(1)	25(1)
C(6)	67(2)	96(2)	98(2)	34(2)	46(2)	25(2)
C(7)	92(2)	56(1)	78(2)	30(1)	41(1)	34(1)
C(8)	57(1)	42(1)	46(1)	15(1)	21(1)	25(1)
C(9)	55(1)	44(1)	54(1)	17(1)	16(1)	22(1)
C(10)	71(2)	50(1)	46(1)	9(1)	16(1)	27(1)
C(11)	81(2)	53(1)	52(1)	19(1)	34(1)	37(1)
C(12)	60(1)	46(1)	58(1)	22(1)	28(1)	29(1)
C(13)	54(1)	42(1)	47(1)	13(1)	16(1)	21(1)
C(14)	68(2)	60(1)	78(2)	25(1)	39(1)	39(1)
C(15)	55(1)	64(1)	76(2)	28(1)	32(1)	32(1)
C(16)	52(1)	55(1)	56(1)	27(1)	24(1)	25(1)
C(17)	52(1)	68(1)	60(1)	30(1)	22(1)	26(1)
C(18)	52(1)	59(1)	53(1)	20(1)	15(1)	15(1)
C(19)	62(2)	48(1)	55(1)	22(1)	20(1)	20(1)
C(20)	53(1)	50(1)	59(1)	27(1)	22(1)	23(1)
C(21)	49(1)	48(1)	60(1)	22(1)	18(1)	17(1)
C(22)	68(2)	59(1)	73(2)	14(1)	1(1)	12(1)

C(23)	56(2)	108(2)	102(2)	4(2)	7(2)	19(2)
C(24)	130(3)	84(2)	97(2)	23(2)	60(2)	46(2)
C(25)	50(1)	66(1)	87(2)	25(1)	20(1)	26(1)

Mass Spectrum of Compound **45**

C:\Xcalibur\...\MTM-V-30_130210195851 2/10/2013 7:58:51 PM MTM-V-30

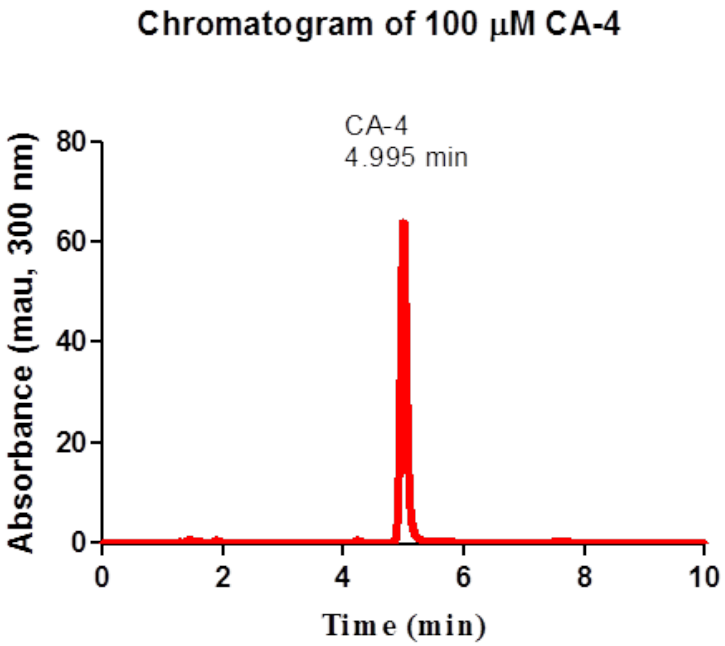


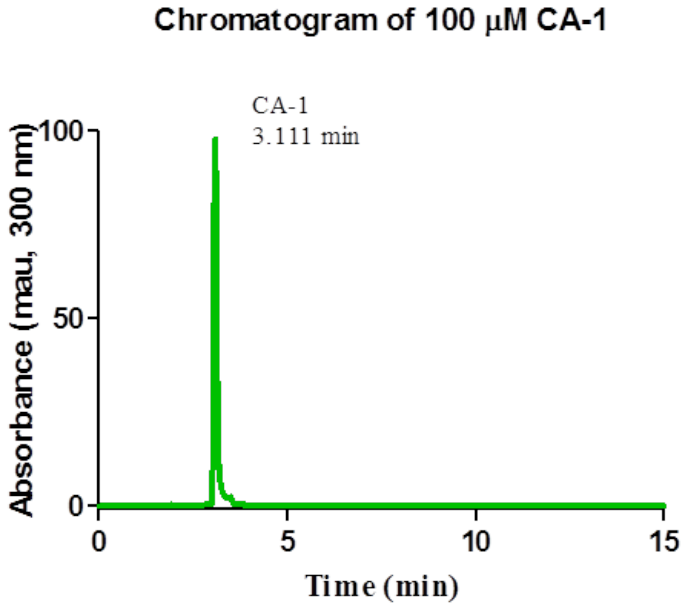


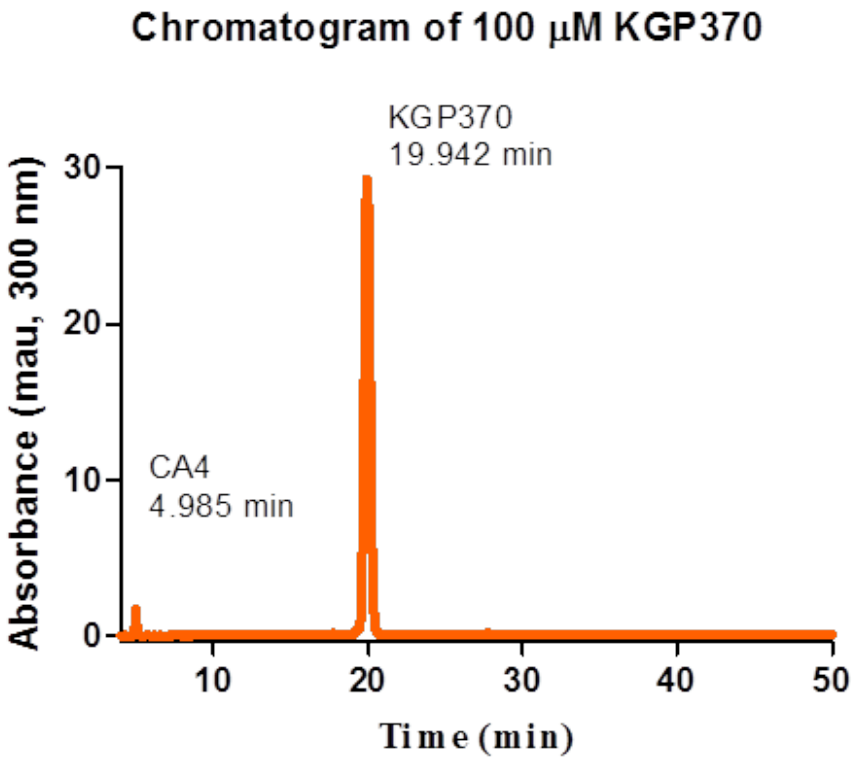
NADPH Cytochrome P450 Oxidoreductase Cleavage Assay

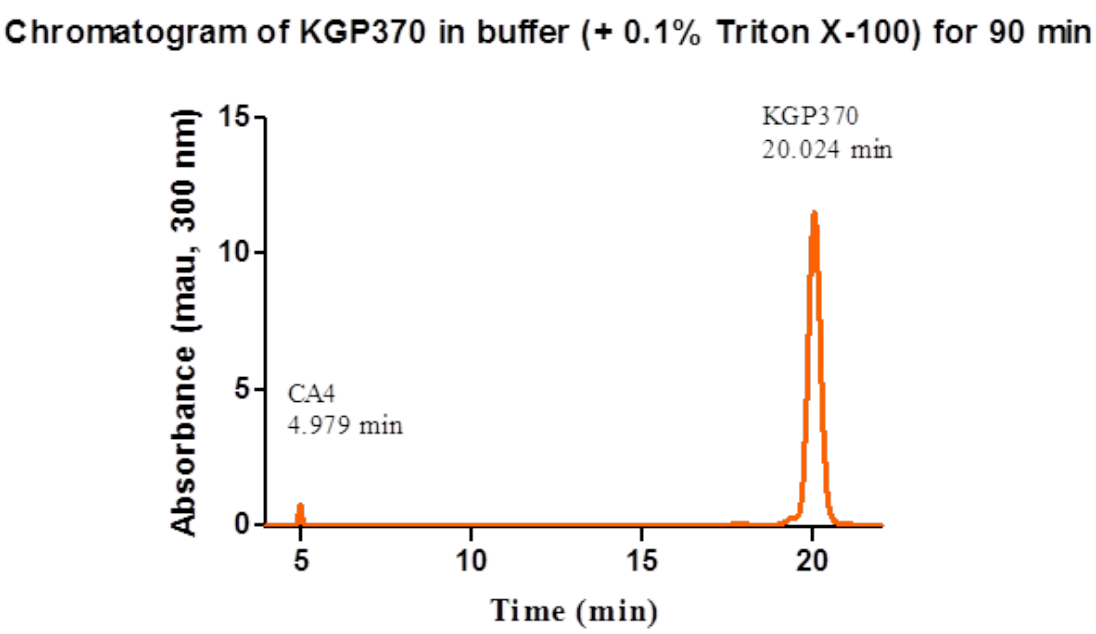
HPLC Conditions:

Solvent: 55% Acetonitrile/water isocratic; detection wavelength: 300 nm; flow rate: 1 mL/min.

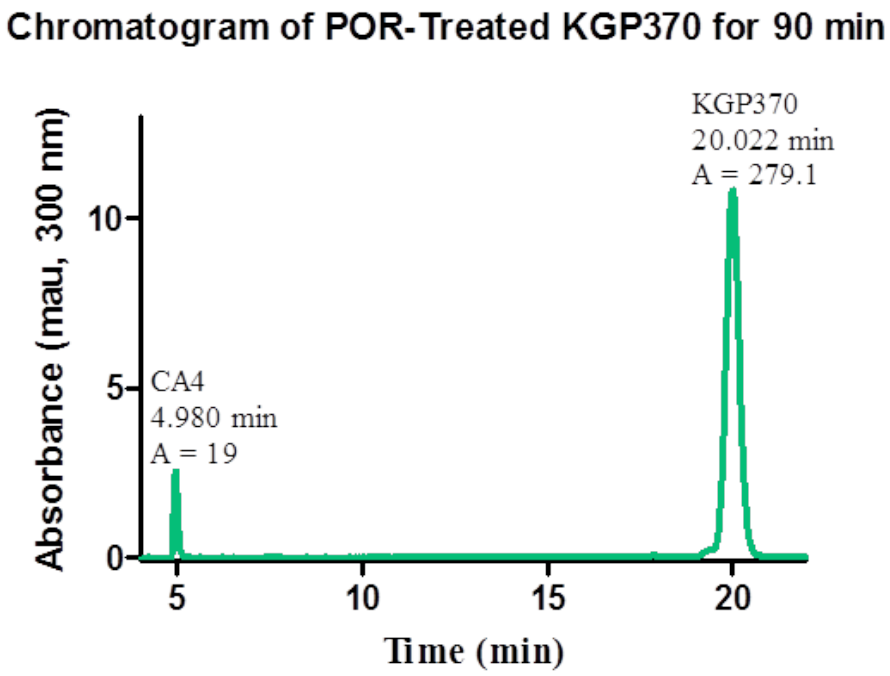


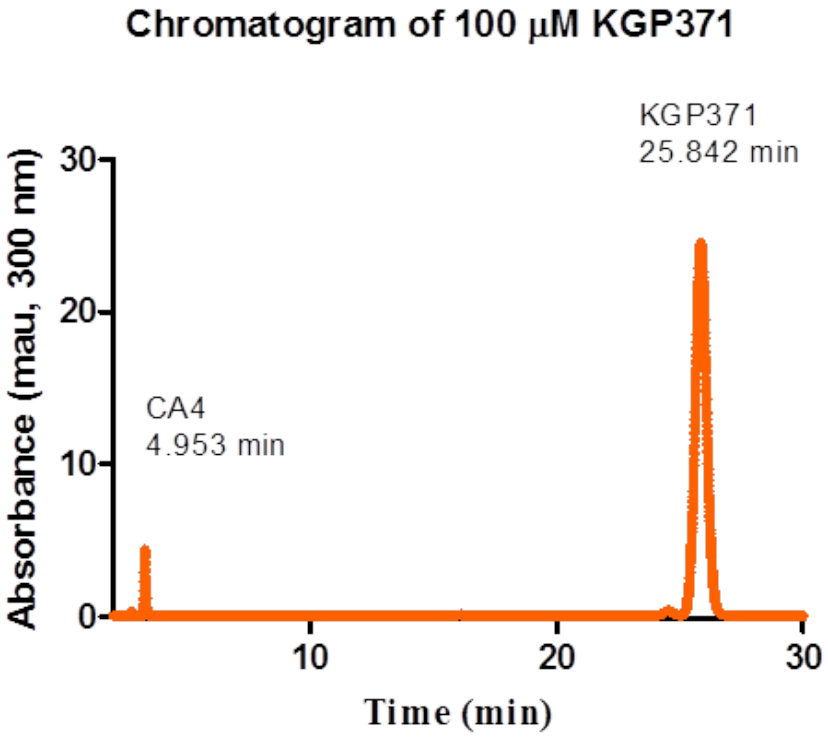




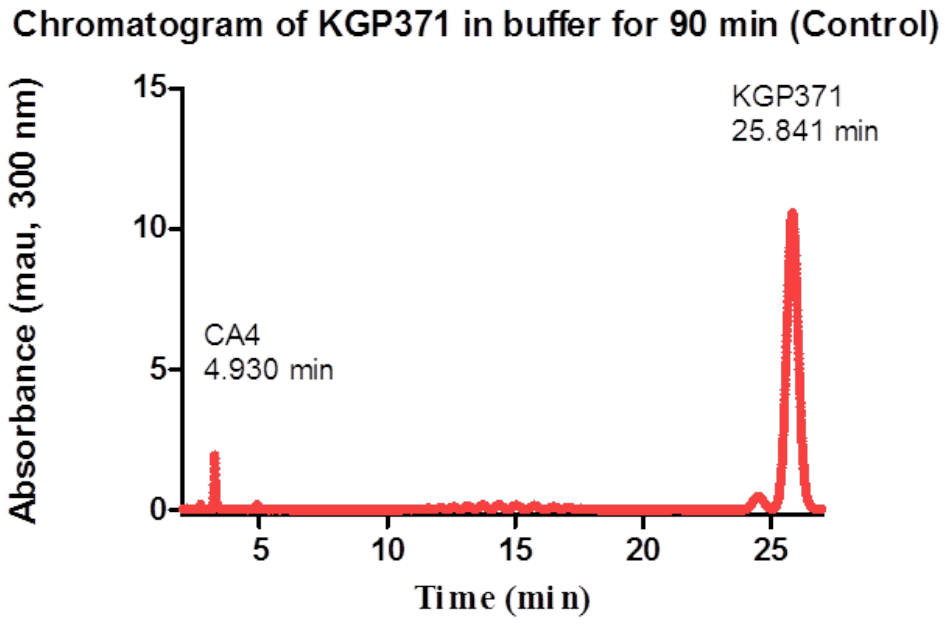


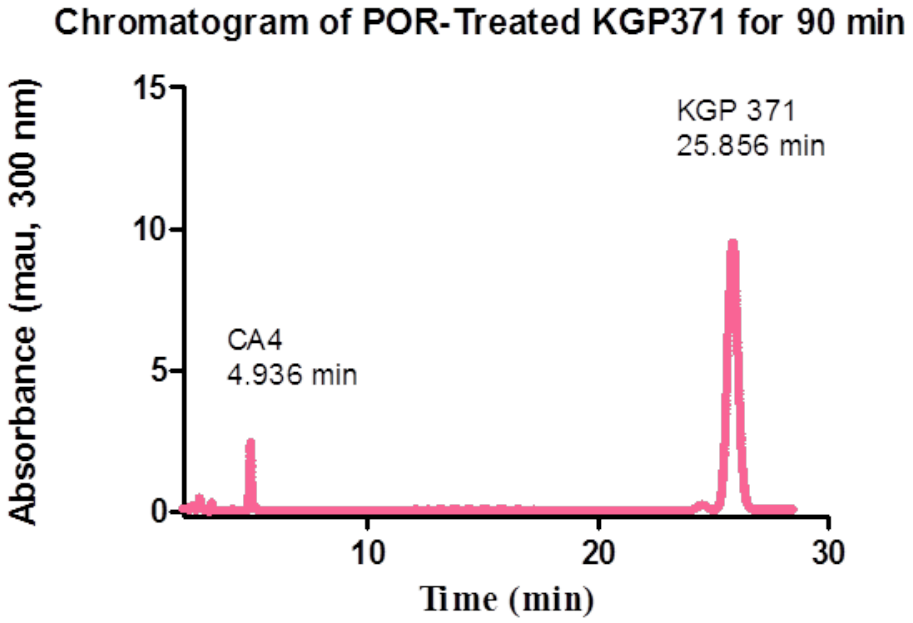
NOTE: KGP370 in the HPLC traces refers to compound **43** in the manuscript.





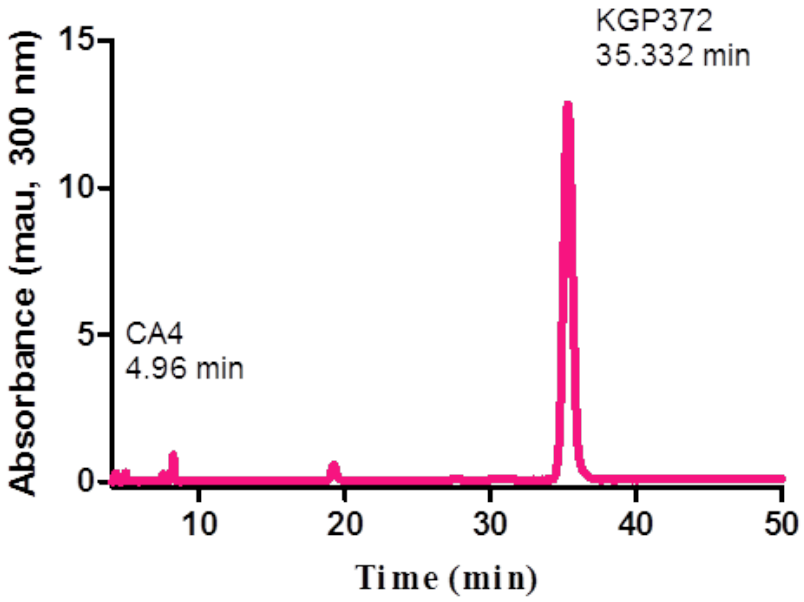
NOTE: KGP370 in the HPLC traces refers to compound **43** in the manuscript. KGP371 in the HPLC traces refers to compound **44** in the manuscript.



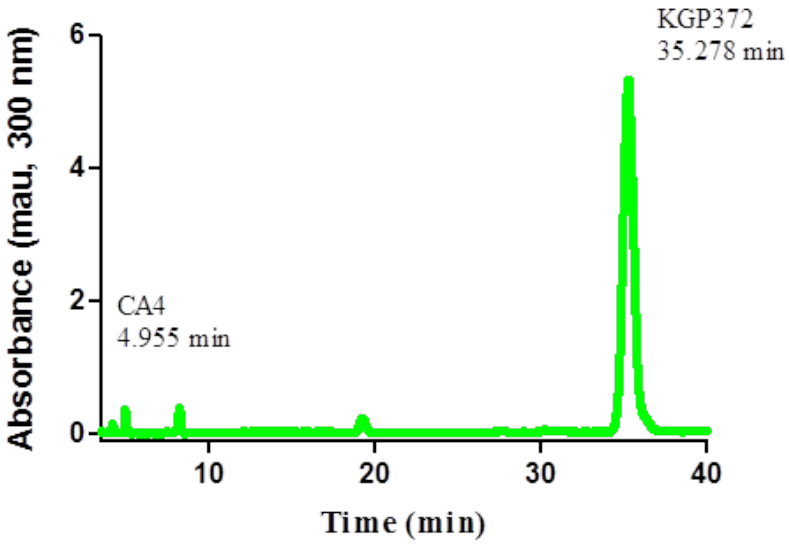


NOTE: KGP371 in the HPLC traces refers to compound **44** in the manuscript.

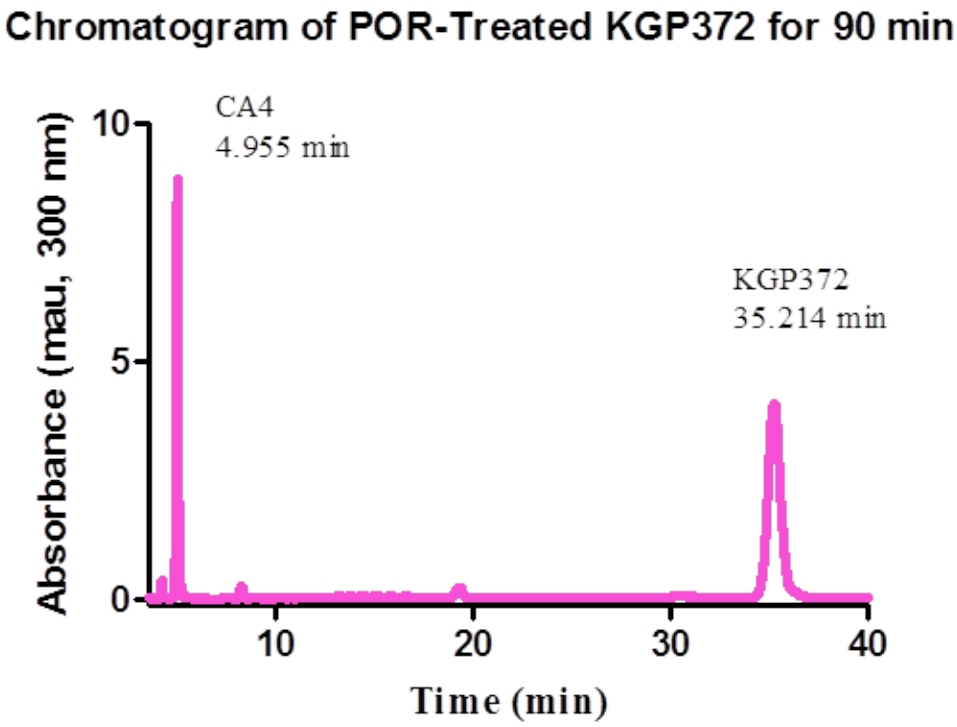
Chromatogram of 100 μM KGP372

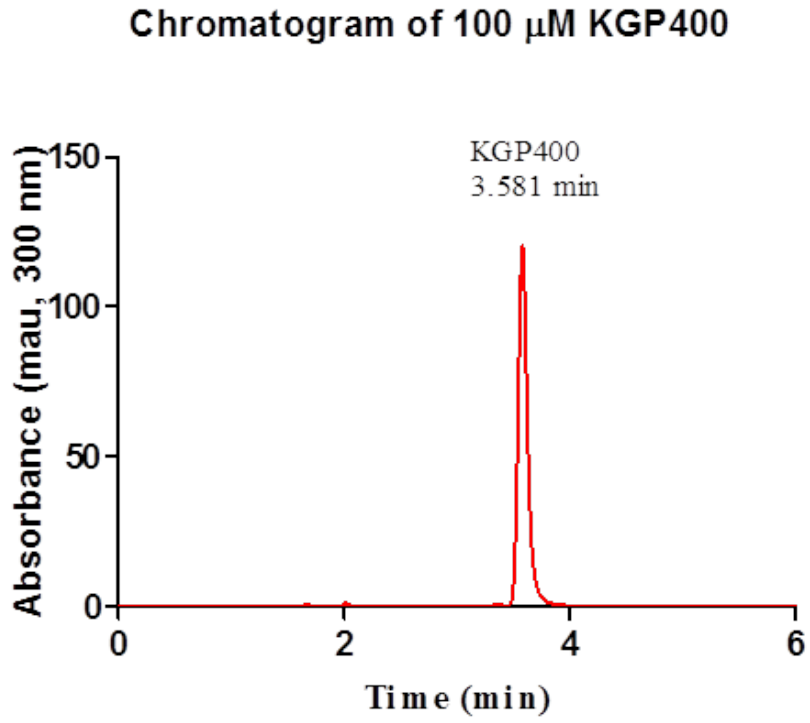


Chromatogram of KGP372 in buffer (+ 0.1% Triton X-100) for 90 min

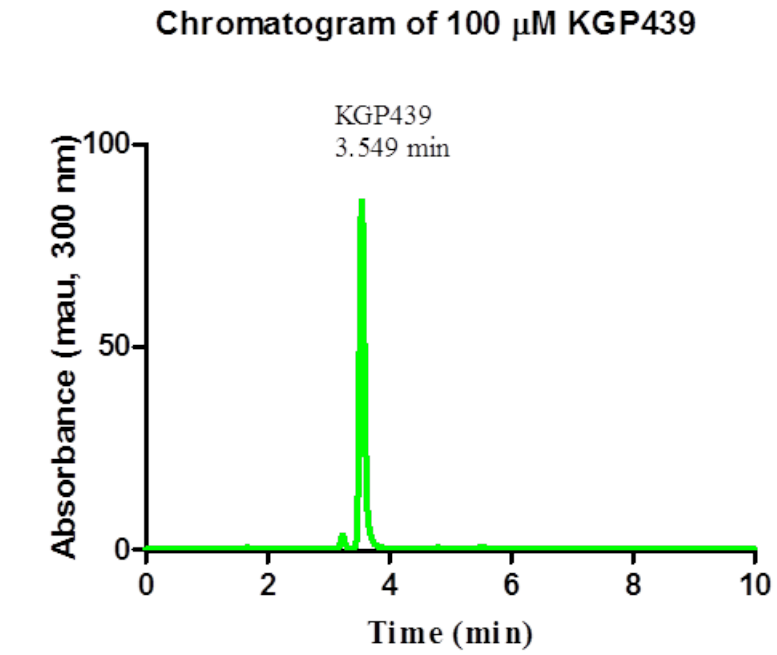


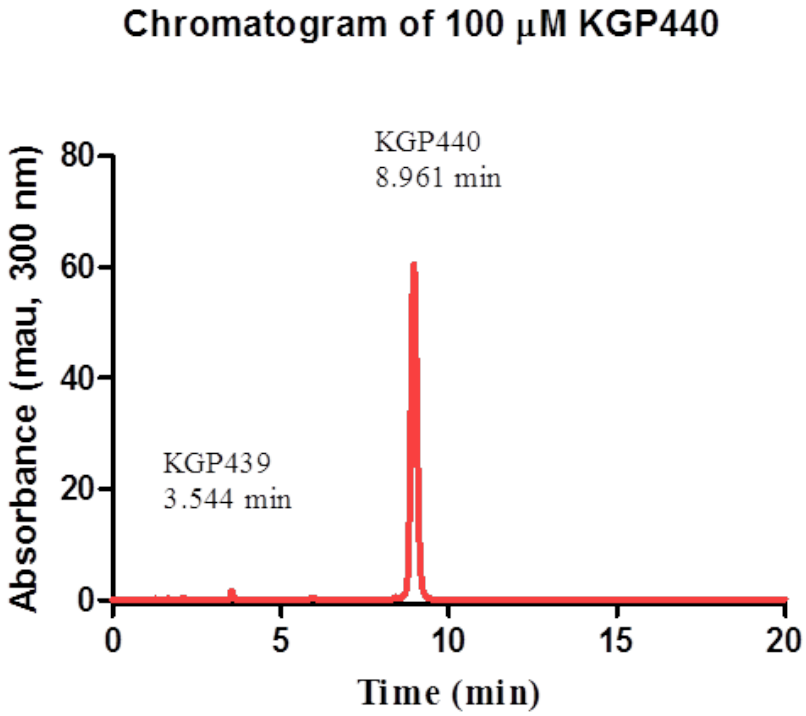
NOTE: KGP372 in the HPLC traces refers to compound **45** in the manuscript.





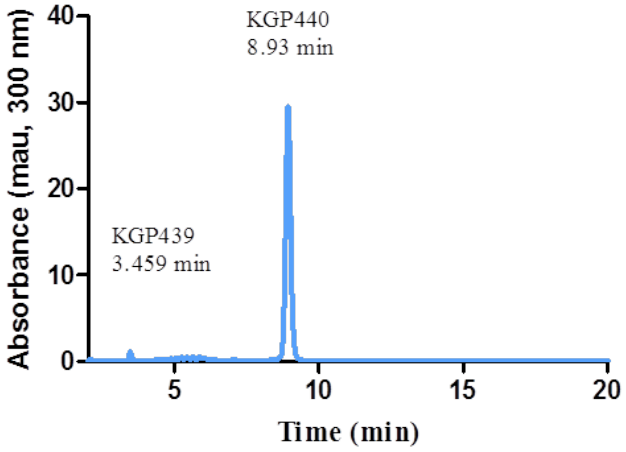
NOTE: KGP372 in the HPLC traces refers to compound **45** in the manuscript. KGP400 in the HPLC traces refers to compound **21** in the manuscript.



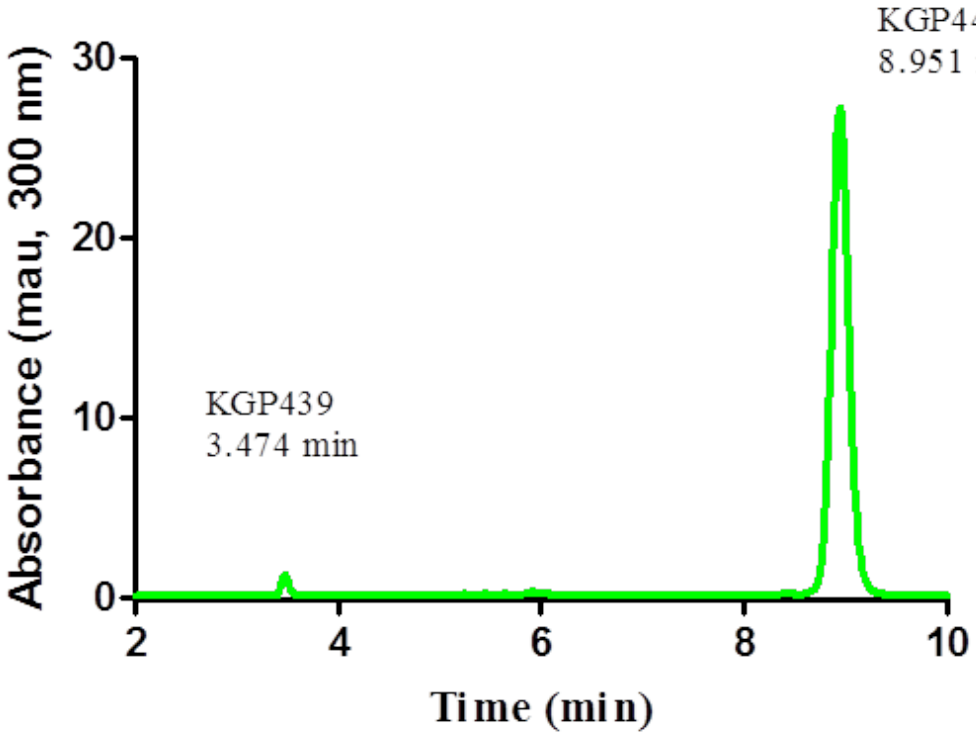


NOTE: KGP439 in the HPLC traces refers to compound **20** in the manuscript. KGP440 in the HPLC traces refers to compound **22** in the manuscript.

Chromatogram of KGP440 in buffer (approx 50 μM, + 0.1% Triton X-100) for 90 min

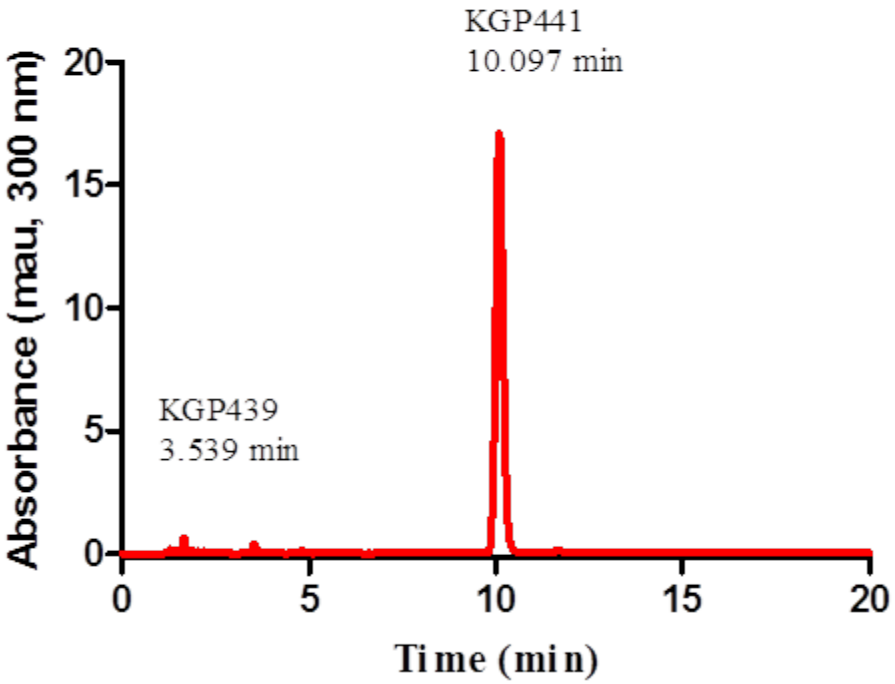


Chromatogram of POR-Treated KGP440 for 90 min

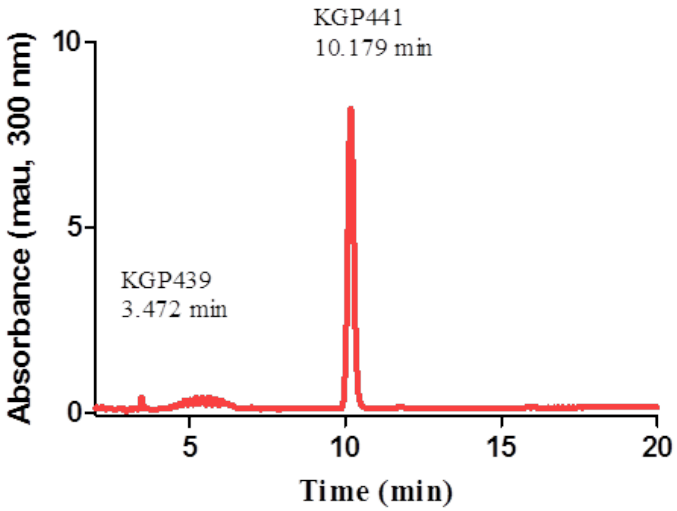


Note: KGP440 in the HPLC traces refers to compound **22** in the manuscript.

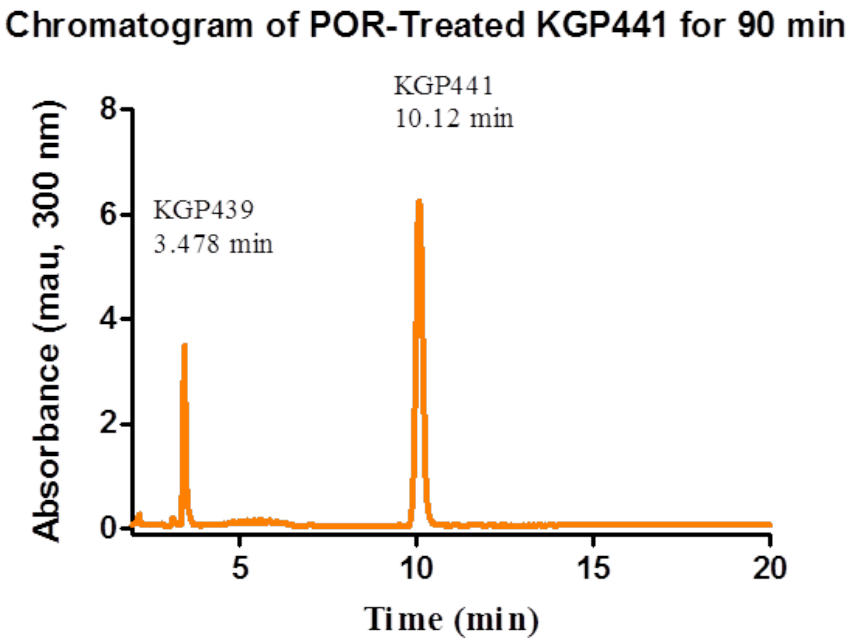
Chromatogram of 100 μM KGP441

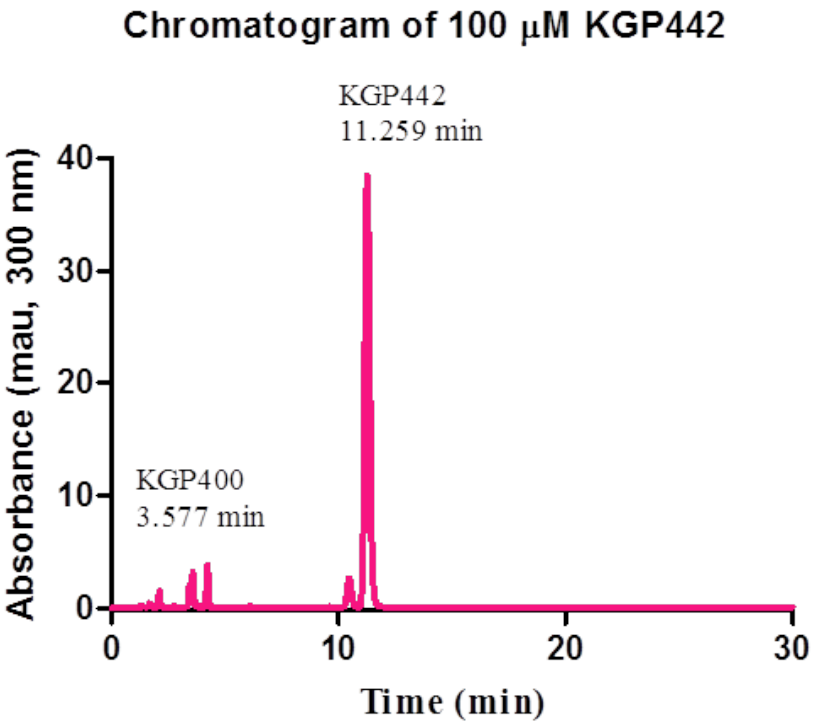


Chromatogram of KGP441 in buffer (approx 50 μM, + 0.1% Triton X-100) for 90 min



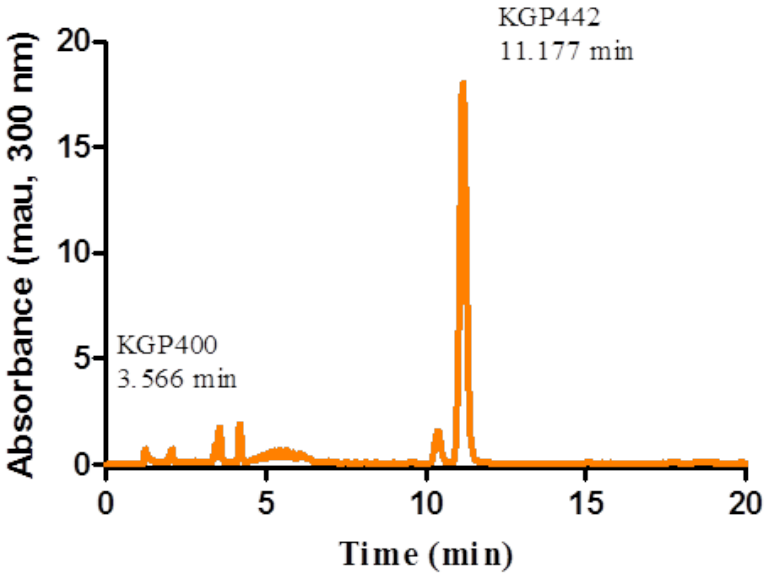
NOTE: KGP441 in the HPLC traces refers to compound **23** in the manuscript.



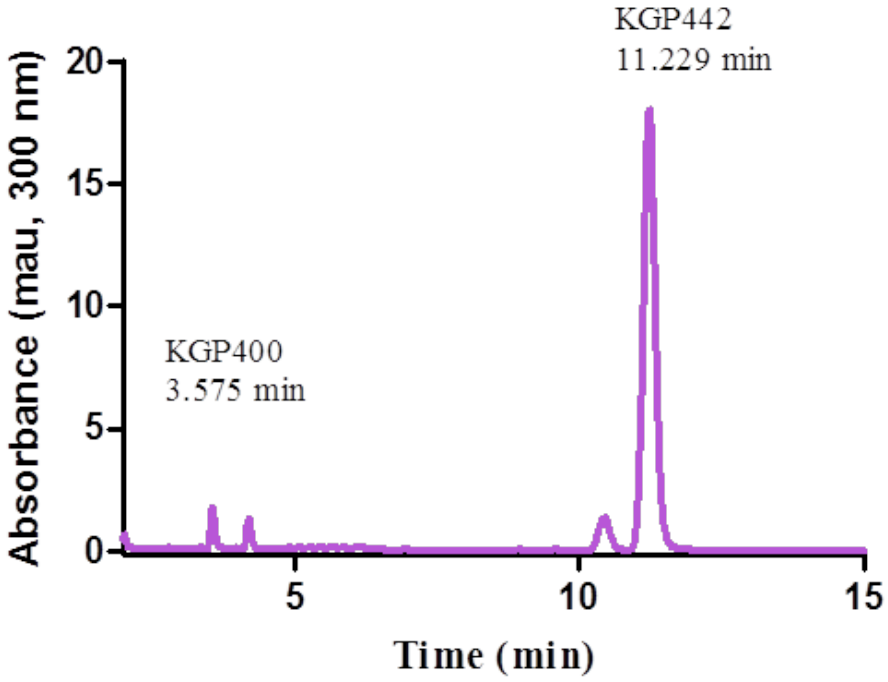


NOTE: KGP441 in the HPLC traces refers to compound **23** in the manuscript. KGP442 in the HPLC traces refers to compound **24** in the manuscript.

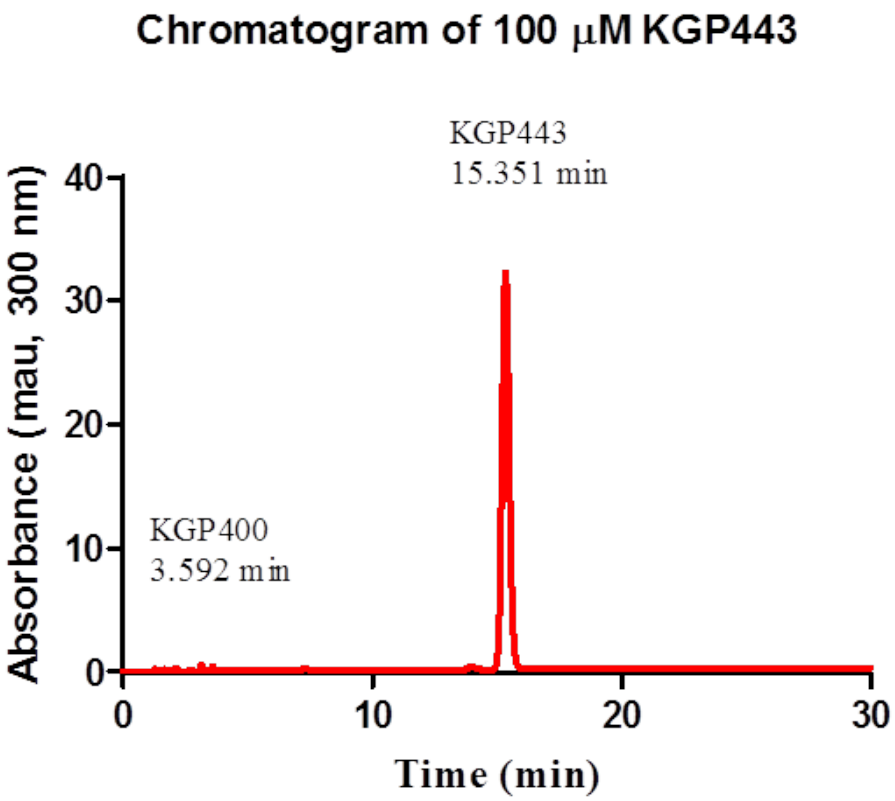
Chromatogram of KGP442 in buffer (+ 0.1% Triton X-100) for 90 min

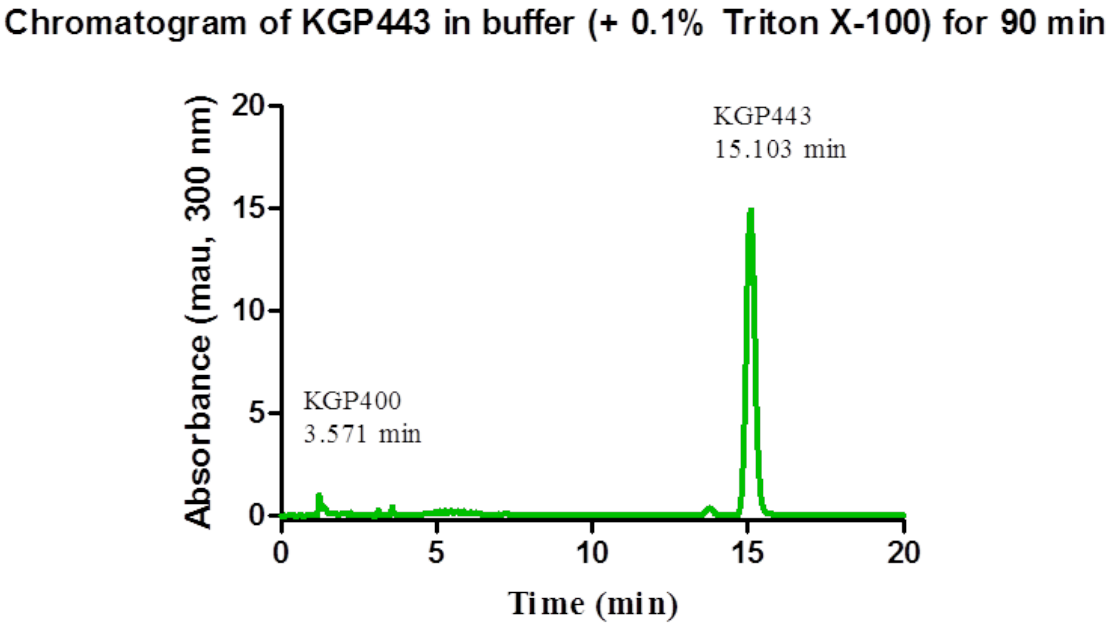


Chromatogram of POR-Treated KGP442 for 90 min

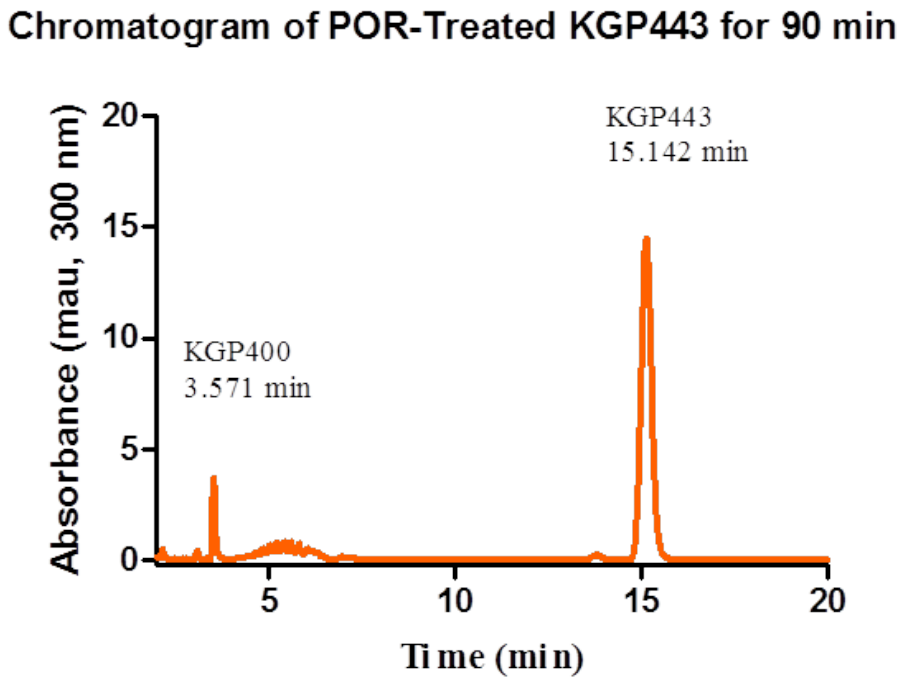


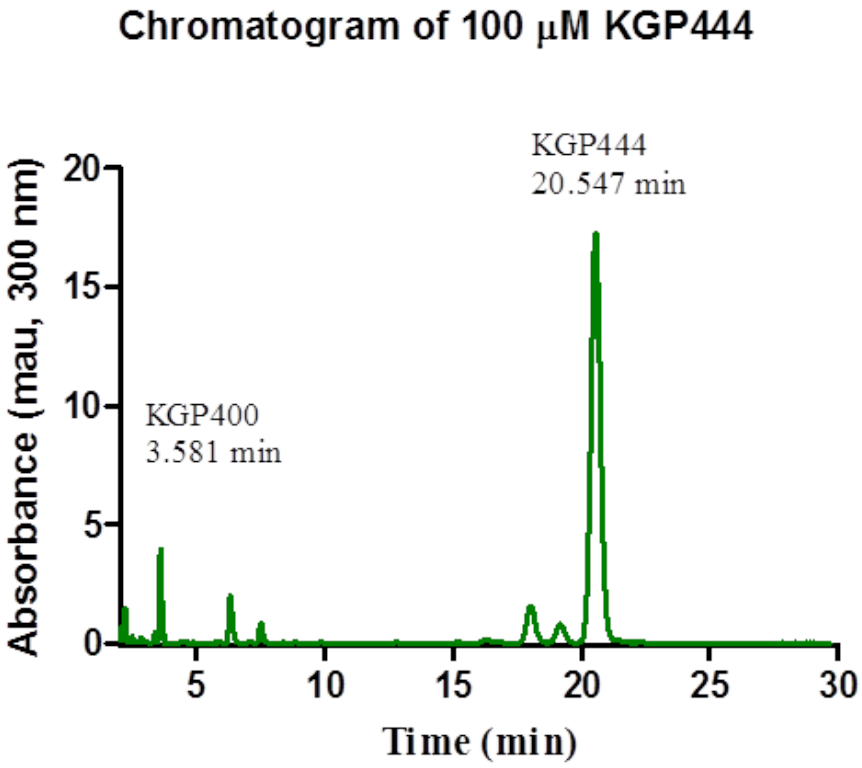
NOTE: KGP442 in the HPLC traces refers to compound **24** in the manuscript.





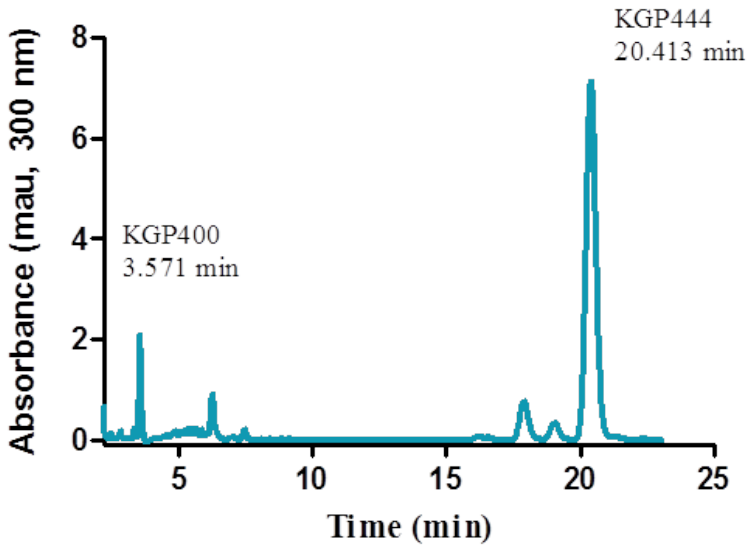
NOTE: KGP443 in the HPLC traces refers to compound **25** in the manuscript.



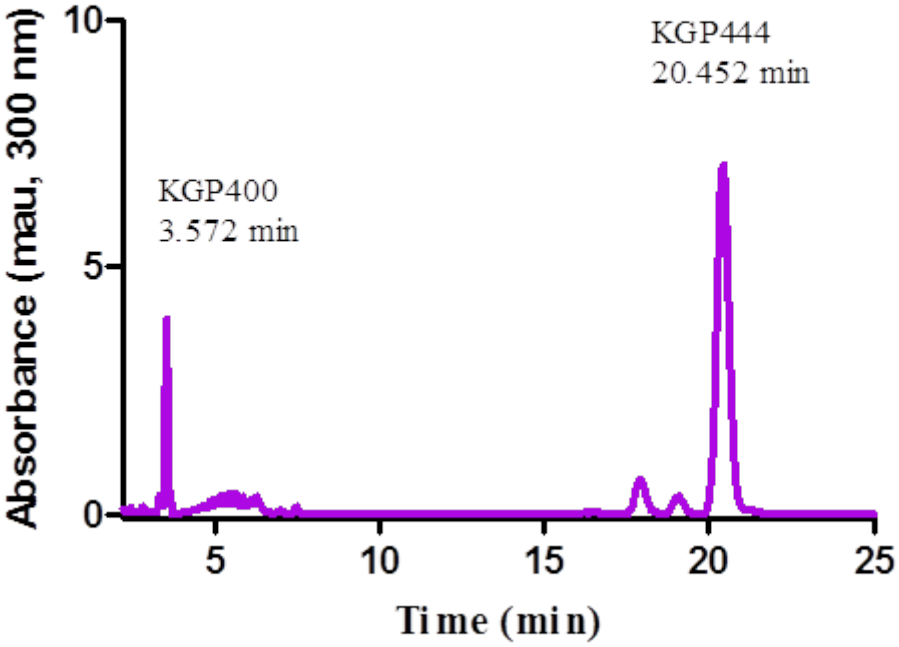


NOTE: KGP443 in the HPLC traces refers to compound **25** in the manuscript. KGP444 in the HPLC traces refers to compound **26** in the manuscript.

Chromatogram of KGP444 in buffer (+ 0.1% Triton X-100) for 90 min

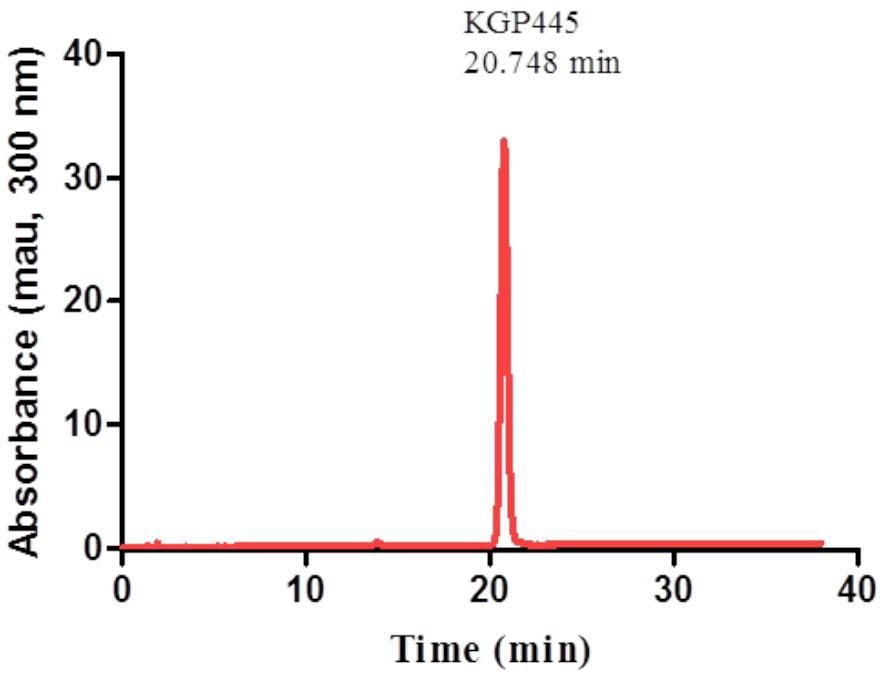


Chromatogram of POR-Treated KGP444 for 90 min

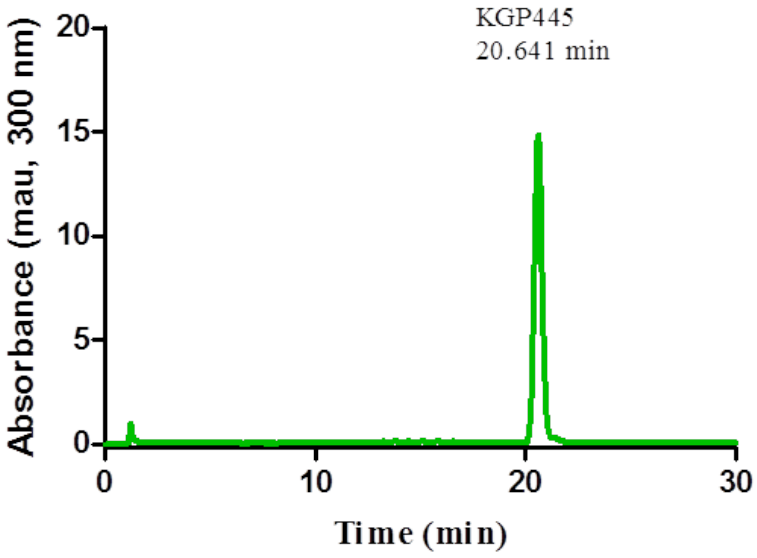


NOTE: KGP444 in the HPLC traces refers to compound **26** in the manuscript.

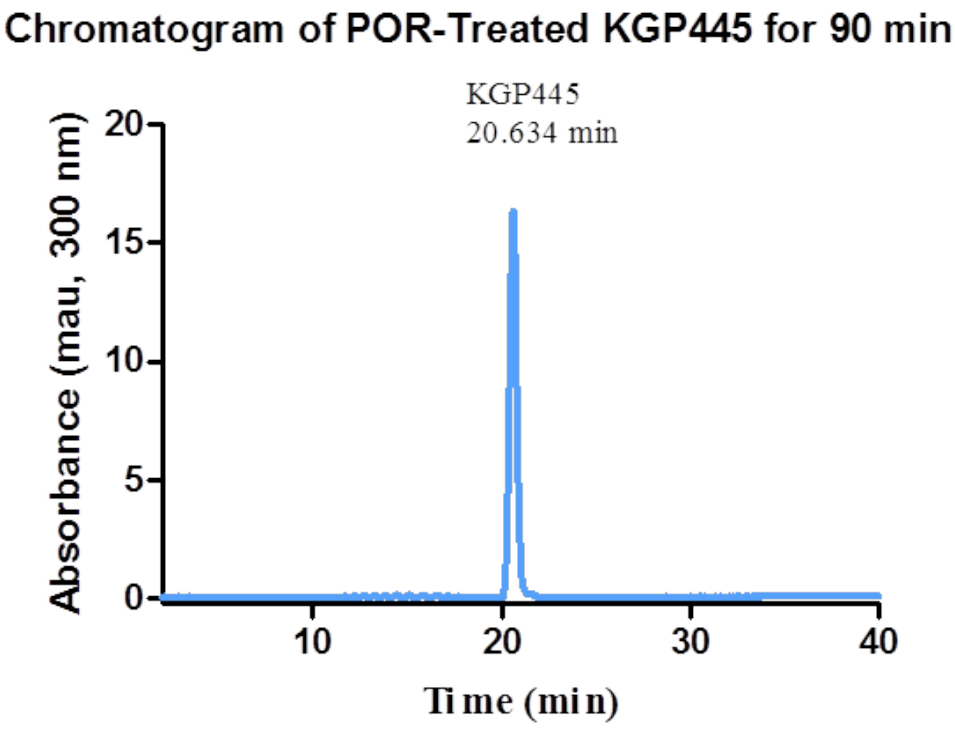
Chromatogram of 100 μM KGP445

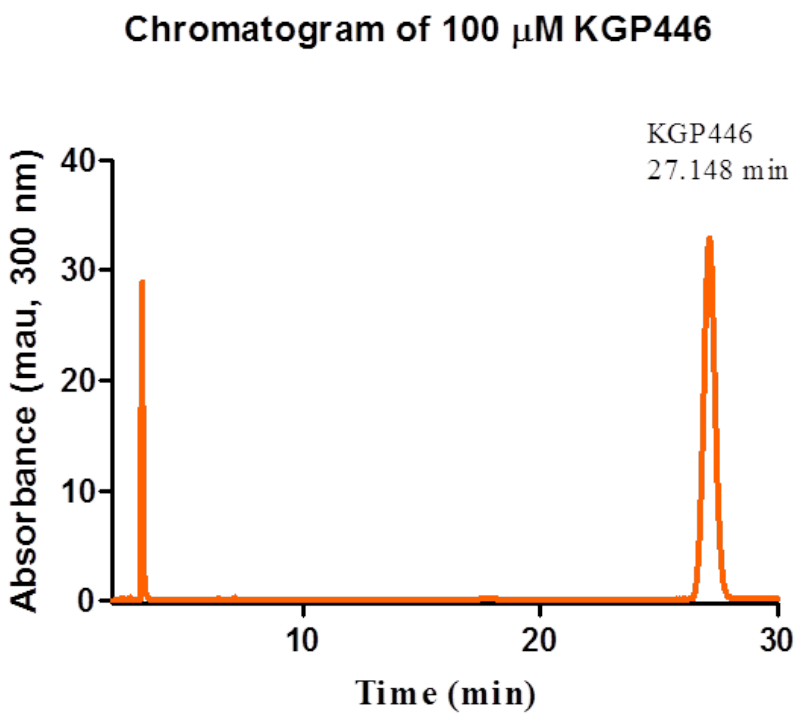


Chromatogram of KGP445 in buffer (+ 0.1% Triton X-100) for 90 min



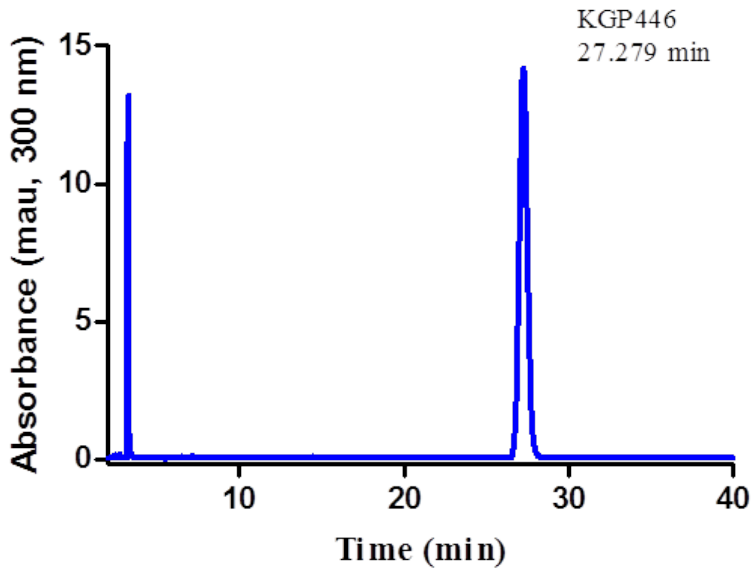
NOTE: KGP445 in the HPLC traces refers to compound **35** in the manuscript.



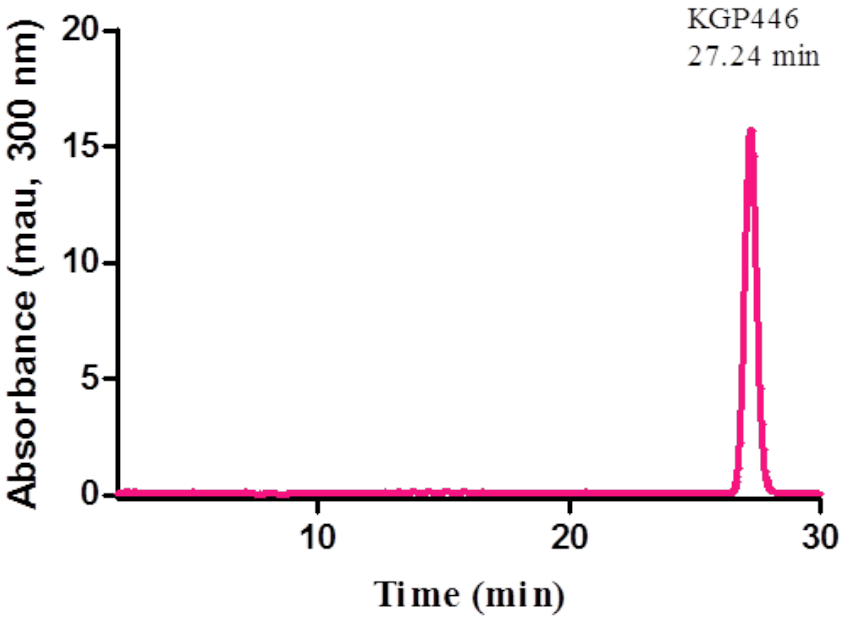


NOTE: KGP445 in the HPLC traces refers to compound **35** in the manuscript. KGP446 in the HPLC traces refers to compound **36** in the manuscript.

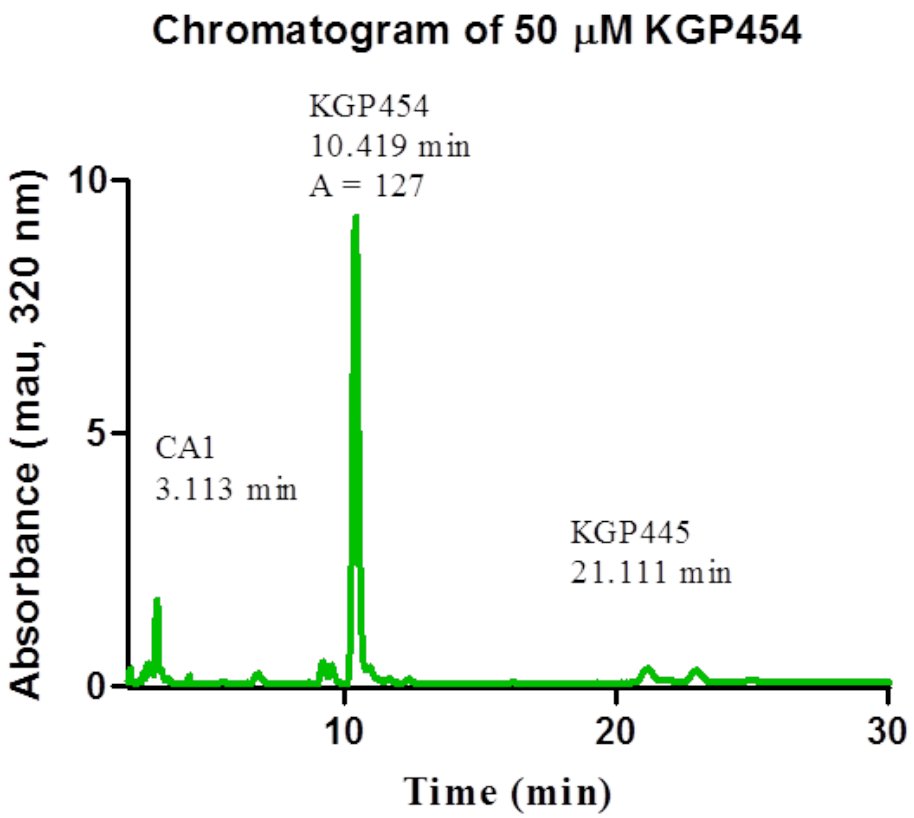
Chromatogram of KGP446 in buffer (+ 0.1% Triton X-100) for 90 min



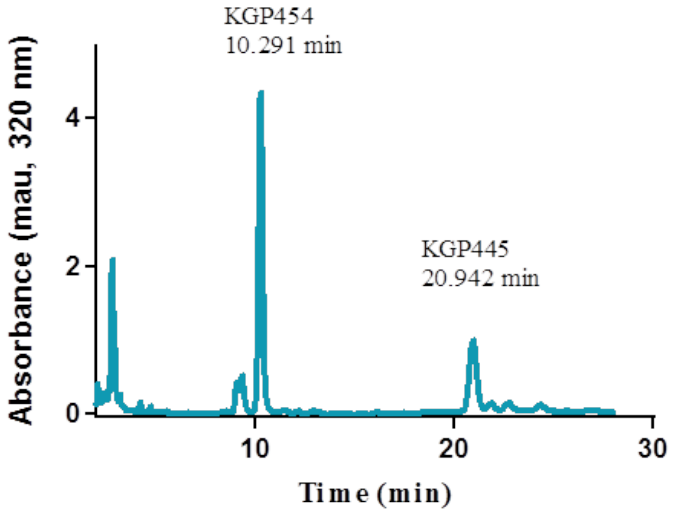
Chromatogram of POR-Treated KGP446 for 90 min



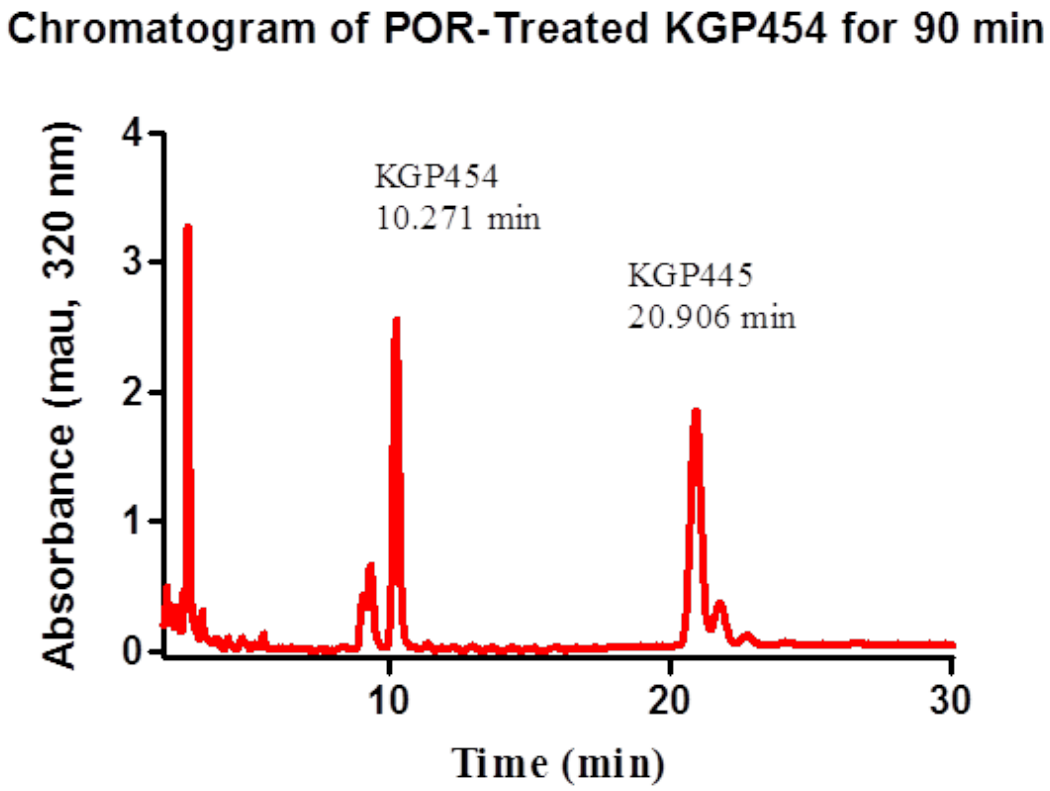
NOTE: KGP446 in the HPLC traces refers to compound **36** in the manuscript.



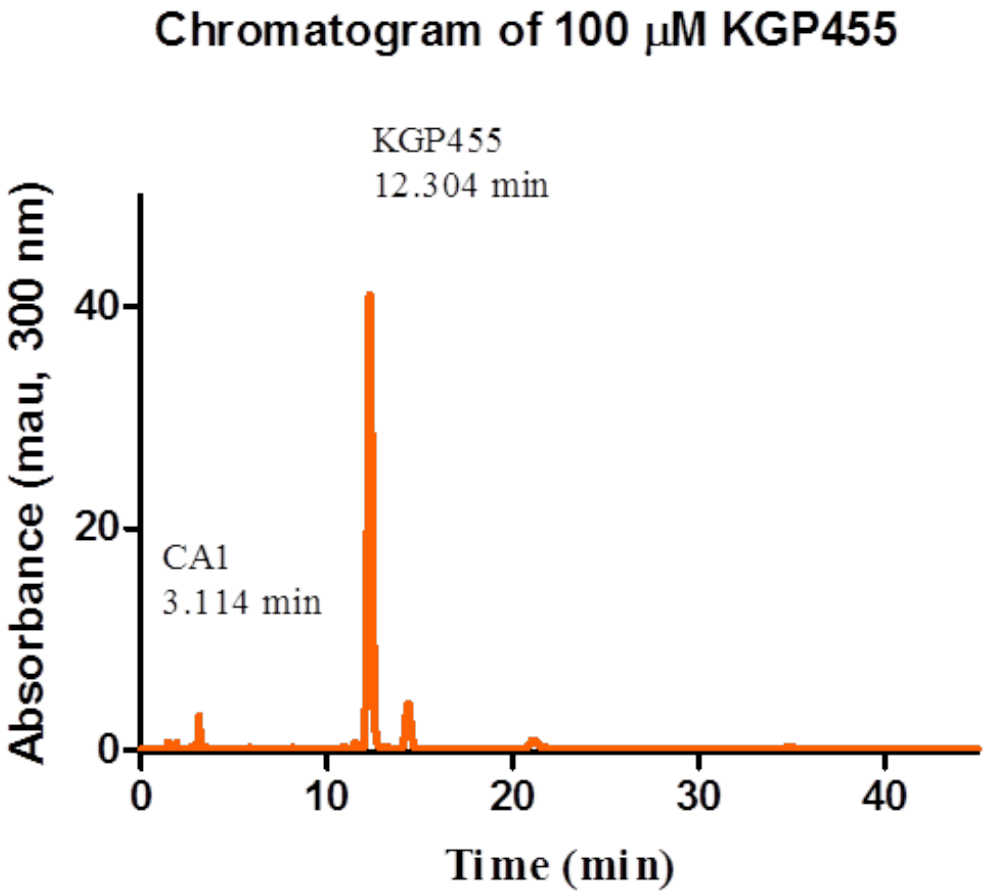
Chromatogram of KGP454 in buffer (approx 50 μM, + 0.1% Triton X-100) for 90 min

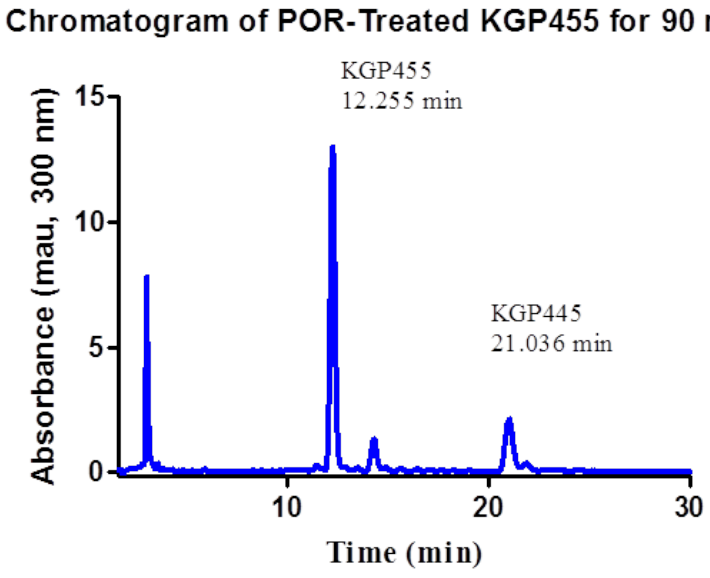


NOTE: KGP454 in the HPLC traces refers to compound **39** in the manuscript. KGP445 in the HPLC traces refers to compound **35** in the manuscript.

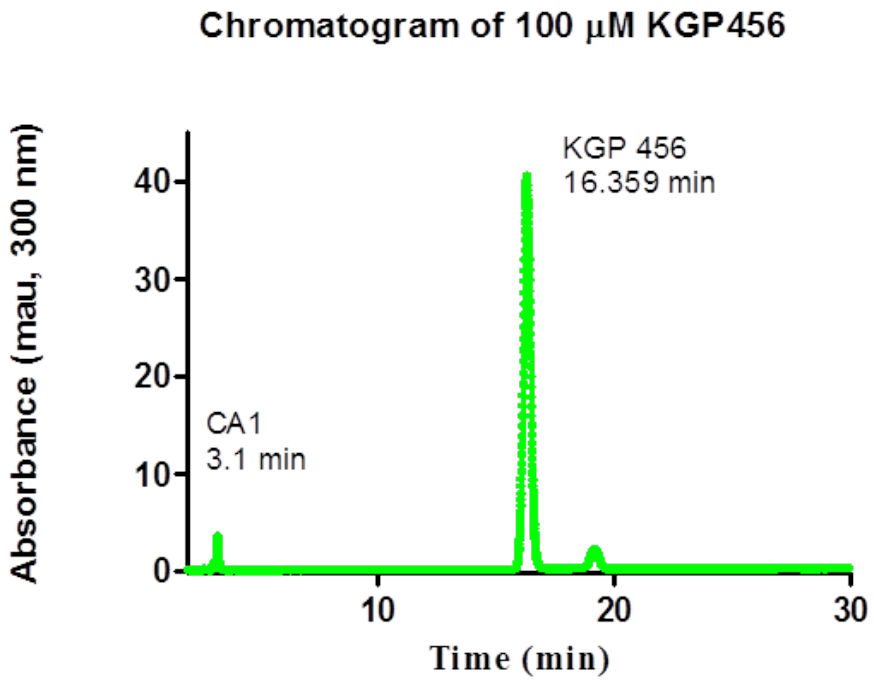


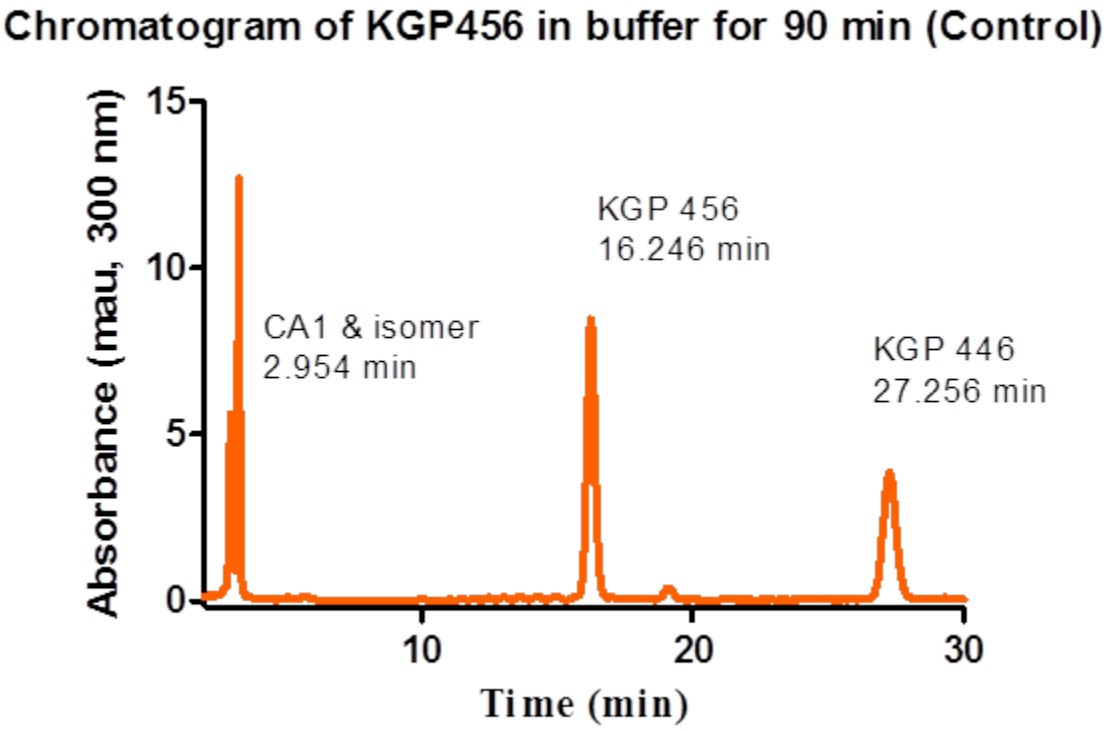
NOTE: KGP454 in the HPLC traces refers to compound **39** in the manuscript. KGP445 in the HPLC traces refers to compound **35** in the manuscript.



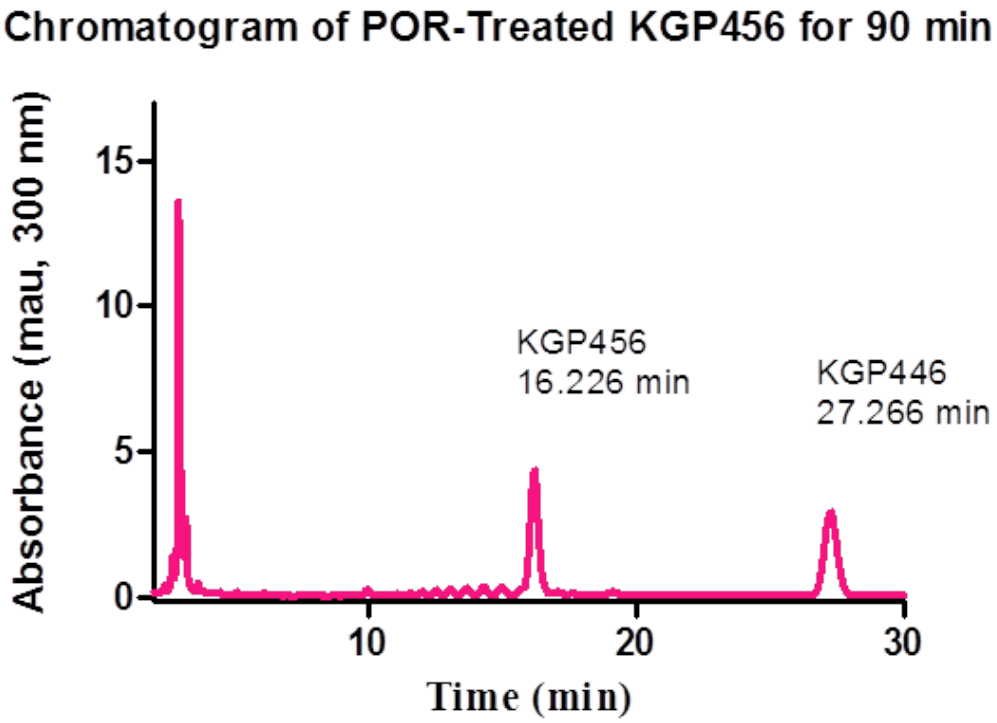


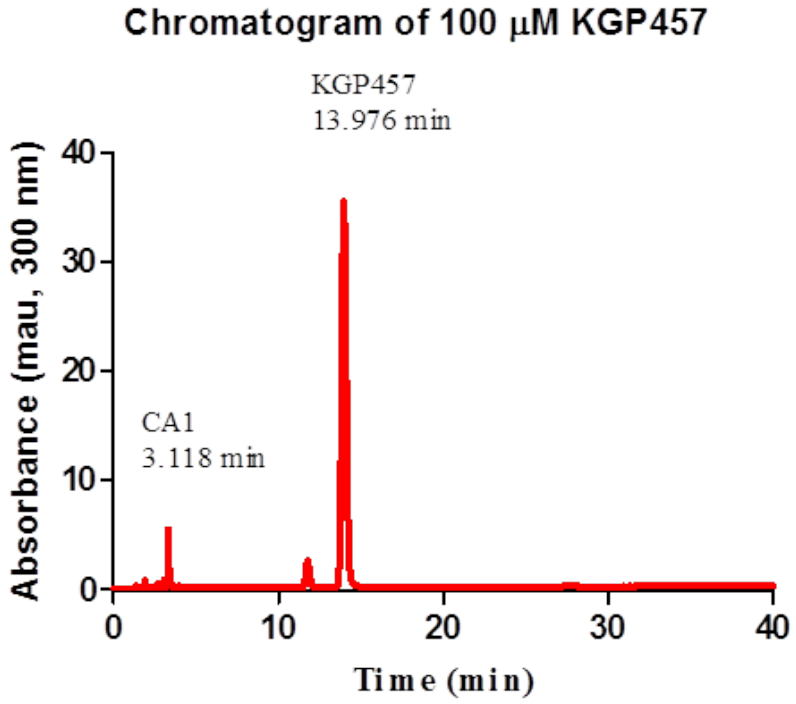
NOTE: KGP455 in the HPLC traces refers to compound **37** in the manuscript. KGP445 in the HPLC traces refers to compound **35** in the manuscript.



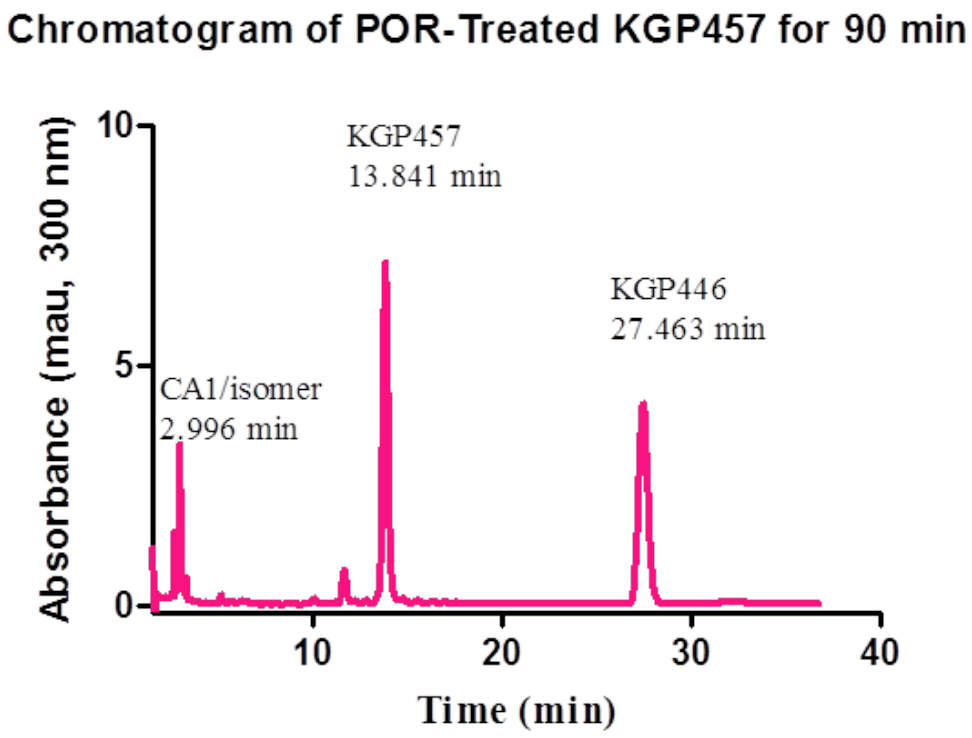


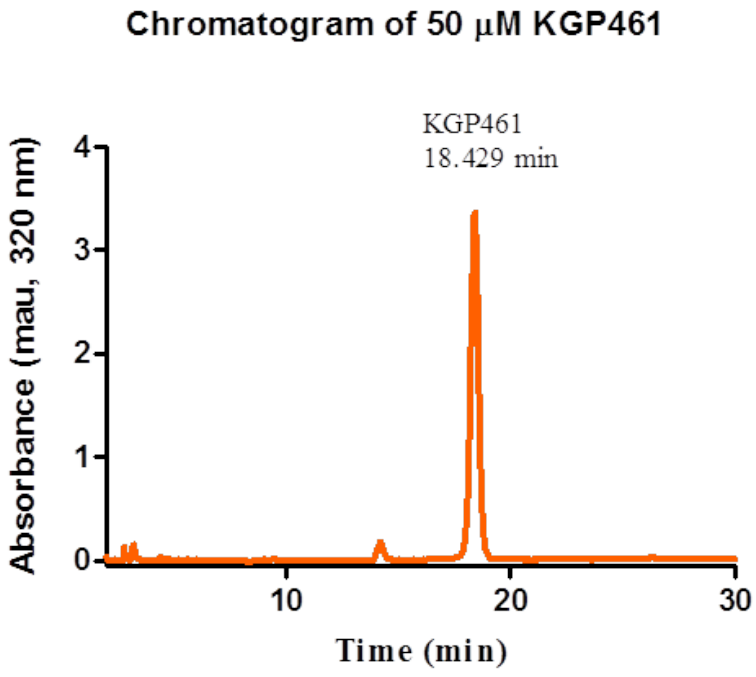
NOTE: KGP456 in the HPLC traces refers to compound **40** in the manuscript. KGP446 in the HPLC traces refers to compound **36** in the manuscript.



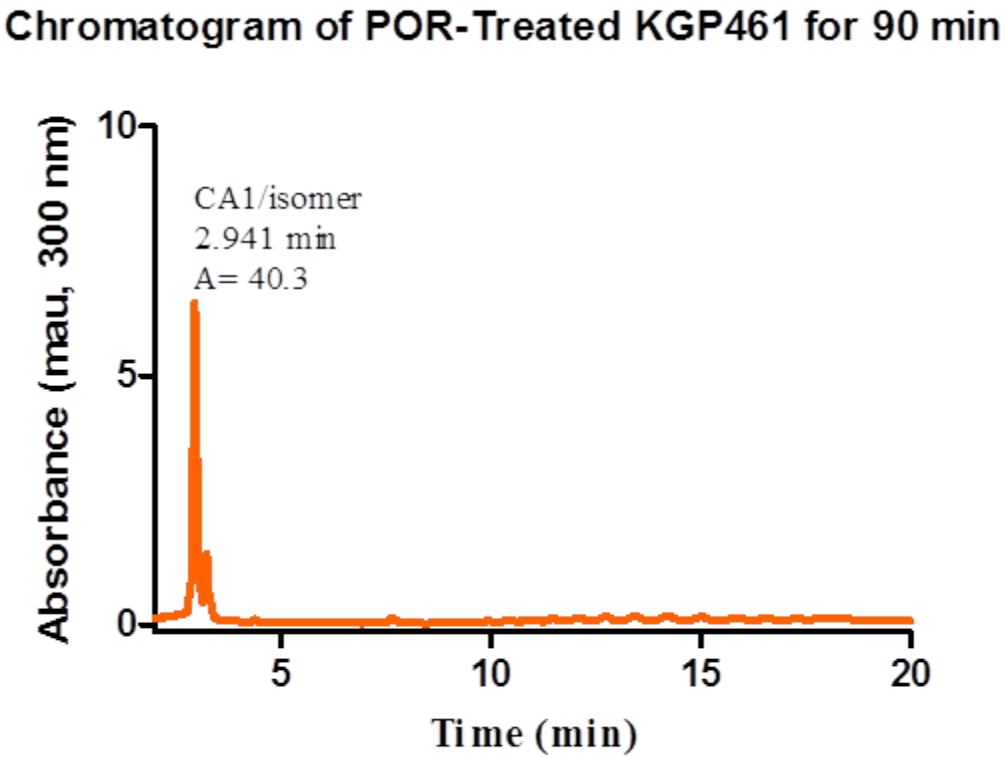


NOTE: KGP456 in the HPLC traces refers to compound **40** in the manuscript. KGP446 in the HPLC traces refers to compound **36** in the manuscript. KGP457 in the HPLC traces refers to compound **38** in the manuscript.





NOTE: KGP446 in the HPLC traces refers to compound **36** in the manuscript. KGP457 in the HPLC traces refers to compound **38** in the manuscript. KGP461 in the HPLC traces refers to compound **41** in the manuscript.



NOTE: KGP461 in the HPLC traces refers to compound **41** in the manuscript.

NOE Spectra Analysis for Compounds **37**, **38** and **41**

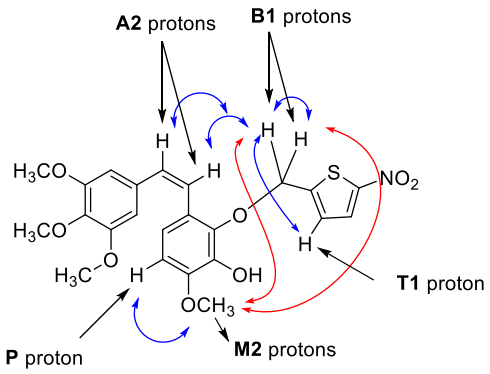
To determine the regioselectivity of the TBS deprotection step, Mitsunabu reactions to install trigger (nitrothiophene group) in the TBS deprotected compounds (Scheme 4), we did NOE study of three final compounds **37**, **38** and **41** (Scheme 6) to determine the connectivity of the trigger to the molecules and regioselctivity of the reactions.

NOE spectra of compound **37**: Structure of compound **37** (established after NOE study) with important NOE interaction has been shown in page 202. We were looking for positive NOE bridge alkene protons A2 and benzylic protons B1 (protons are assigned arbitrarily with A, B, T etc) and between benzylic protons B1 and methoxy M2 protons. Positive NOE and negative or no NOE have been shown in blue and red colors duble headed arrows respectively. Theoratically if the nitrothiphene trigger is connected to hydroxyl group next to methoxy group (M2), in NOE spectra there should be positive NOE between B1 protons and M2 proteons. Similaryly positive NOE should be observed between A2 and B1 protons if the trigger is connected to hydroxyl group close to bridge alkene protons A2. In the actual NOE study when M2 protons was irrradiated, only one positive NOE was observed for P proton (red color spectra in page 202). No positive NOEs were observed for B1 or T1 protons. But when B1 protons were irradiated, positive NOEs were observed only for A2 protons and T1 proton (blue spectra in page 202). Therefore, this NOE study confirm the structure of **37**.

NOE spectra of compound **38**: NOE spectra of compound **38** has been shown in page 203. When compound **38**, G1 protons were irradiated, positive NOE were observed for A1 protons, B and T protons (red spectra). On irradiation of B proton positive NOE were observed for A1, G1 and T protons (green spectra). This NOE spectra thus clearly established the structure of compound **38**.

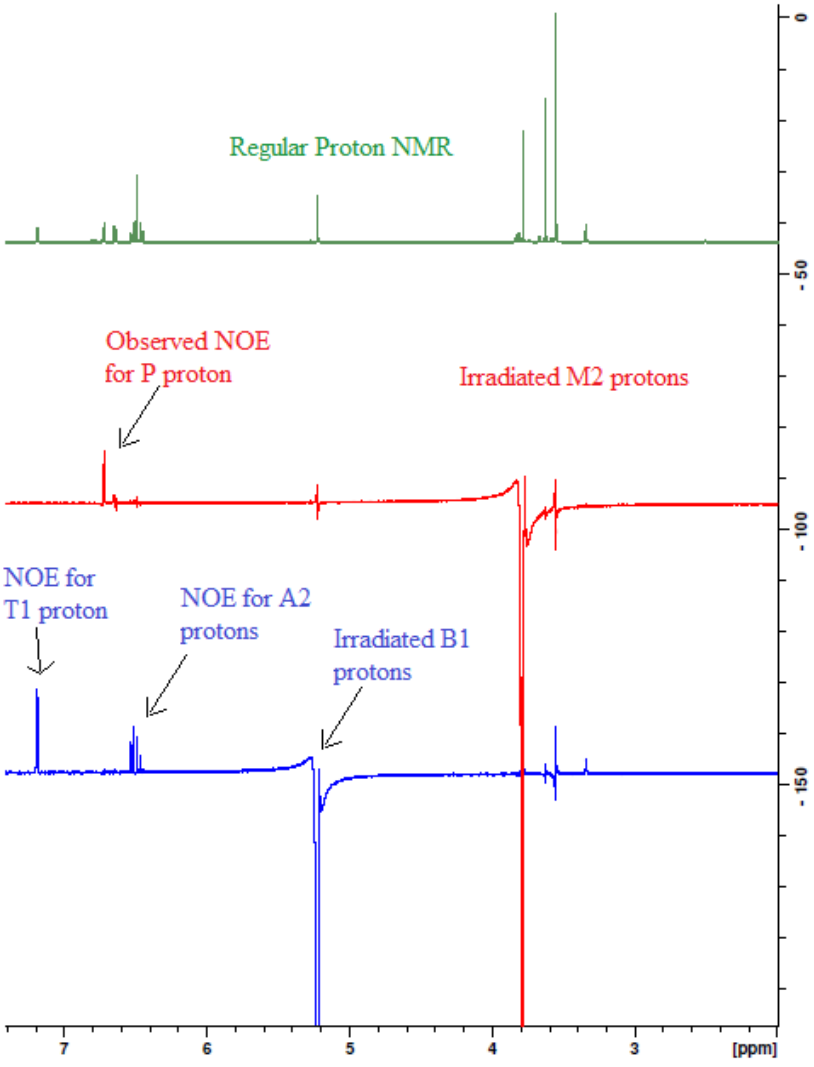
NOE Spectra of Compound **41**: NOE spectra of compound **41** has been shown in page 204. Irradiation of both A protons produced positive NOE for G protons (purple and green spectra). But when M protons was irradiated no positive NOE was observed for G protons. This clearly indicated that nitrophenyl trigger is connected to the hydroxyl group closer to the bridge alkene protons (or second from the methoxy group).

NOE Spectra for Compound **37**

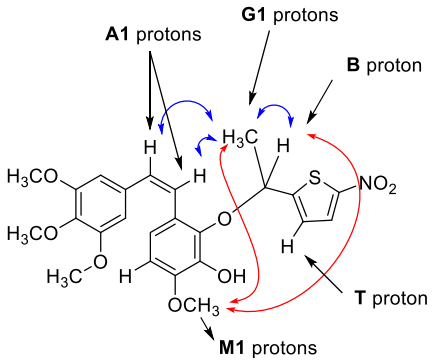


Blue arrow: NOE observed
Red arrow: No NOE observed

37

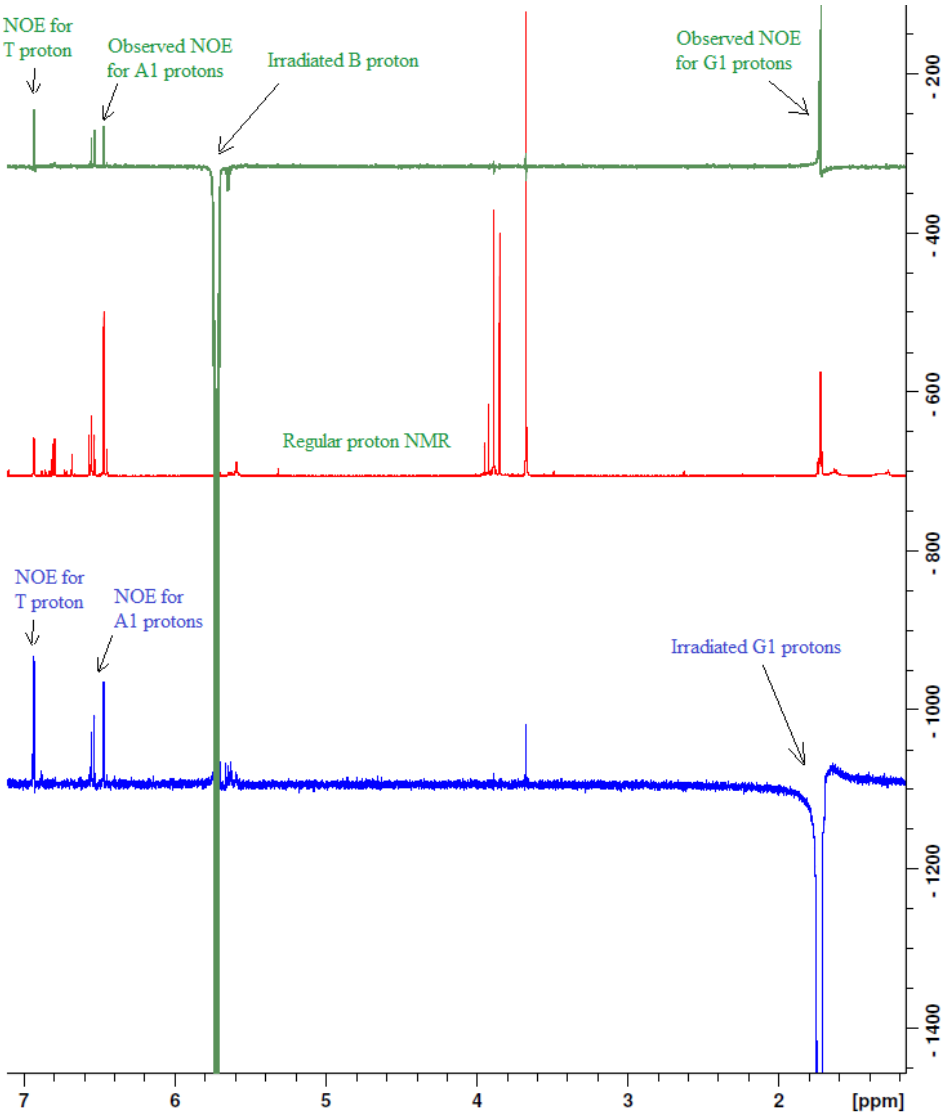


NOE for Compound **38**

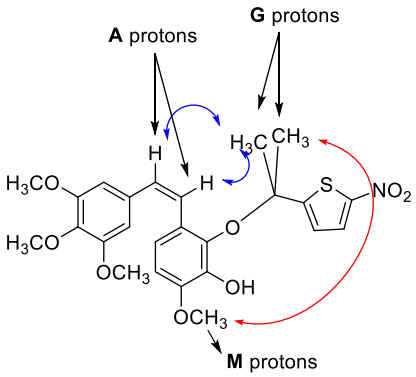


Blue arrow: NOE observed
Red arrow: No NOE observed

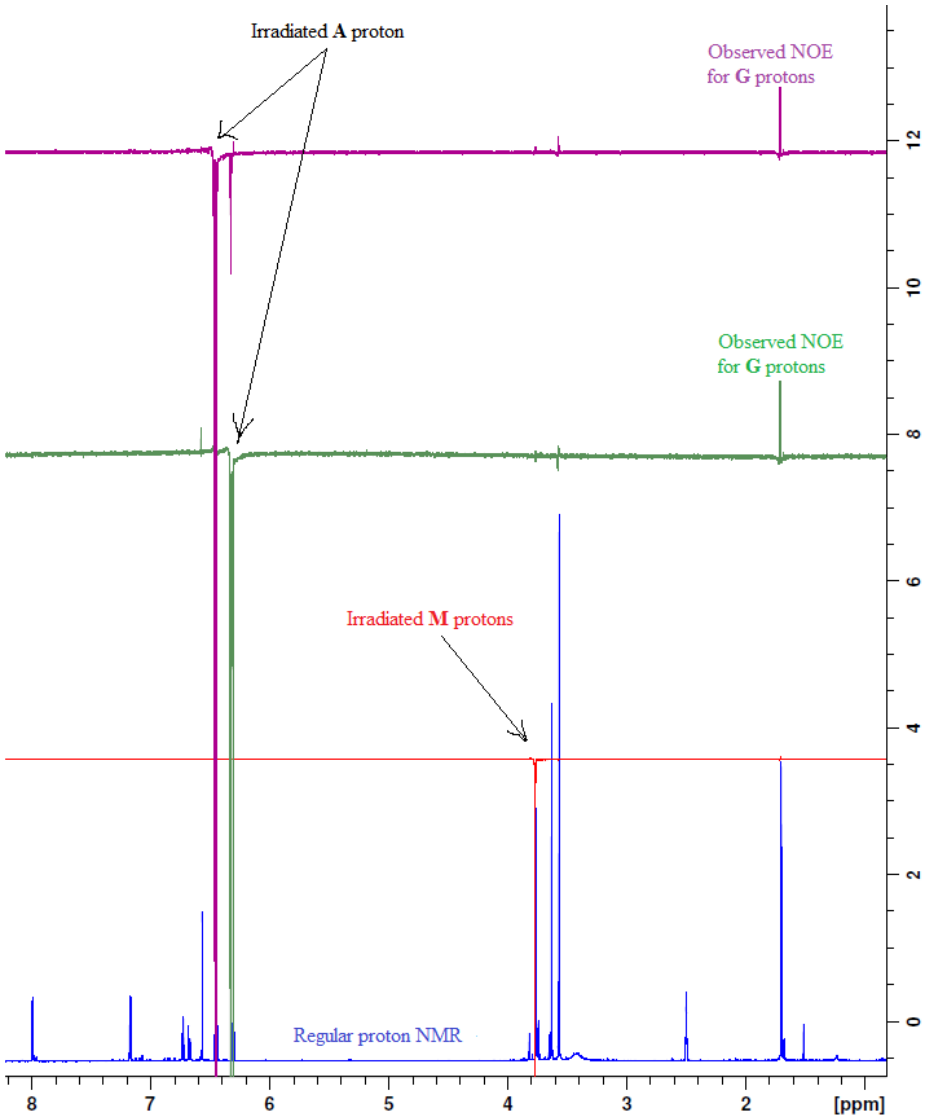
38



NOE spectra of Compound **41**



Blue arrow: NOE observed
Red arrow: No NOE observed



Preliminary Pharmacokinetic (PK) Study

LC-ESI MS Analysis: Samples were analyzed on an Accela liquid chromatograph coupled to an LTQ Orbitrap Discovery mass spectrometer (Thermo Electron, Bremen, Germany) using positive electrospray ionization (+ESI). Samples were diluted tenfold into mobile phase and were injected (10 µL) onto the LC system consisting of a 15 cm x 2.1 mm (5 µm, 80 Å) Extended-C18 column (Agilent Technologies). A binary mobile phase gradient containing 0.1 % (v/v) formic acid (Fisher) in water (A) and acetonitrile (B) was applied as follows: 45 % A / 55 % B for 0.5 min, ramp to 2 % A / 98 % B over 3.50 min, held for 1.50 min at 2% A/ 98% B, return to 45 % A/ 55 % B in 0.1 min, and equilibrated for 1.40 min at 45 % A/ 55 % B. Additional chromatographic parameters were as follows: column temperature, 30 °C; flow rate, 350 µL/min. Full-scan accurate mass spectra (*m/z* range: 50-700) of eluting compounds were obtained at high resolution (30,000 FWHM) on the Orbitrap mass analyzer using internal calibration (accuracy of measurements < 2 ppm) and processed using Xcalibur v.2.0.7 software. Electrospray source conditions were: sheath and auxiliary gas flow 50 and 5 arbitrary units (a.u.), respectively; heated capillary temperature 300 °C; electrospray voltage 4.5 kV; capillary voltage 43 V; tube lens voltage 205 V. The MS spectra were analyzed at exact masses for KGP18 [the internal standard (IS)] at *m/z* 357.1684-357.1720 [M+H]; BAPC **45** at *m/z* 508.1375-508.1425 [M+Na], and CA4 at *m/z* 317.1368-317.1400 [M+H] for peaks at the respective elution times.

Standard Curves: Multiple replicates (n >3) of standard curves for CA4 and **45** were obtained from 8-point serial dilutions (spiked with IS KGP18) of stock solutions using the following concentrations (Table S5).

Table S5.	BAPC **45** (KGP372) Conc.	CA4	KGP18
Standard	(ppb)	Conc. (ppb)	Conc. (ppb)
8	1.14	1.17	1.27
7	2.28	2.34	2.54
6	5.68	5.86	6.36
5	11.36	11.73	12.73
4	14.20	14.66	15.91
3	28.41	29.32	31.82
2	56.82	58.64	63.64
1	113.64	117.27	127.27

After analysis by LC/MS, these standard curves were determined to be linear. The limit of quantification (LOQ) is represented by the lowest point [1.14 ppb KGP372 (BAPC **45**); 1.17 ppb CA4; 1.27 ppb KGP18 (IS)] on each standard curve (Figure S4A, B).

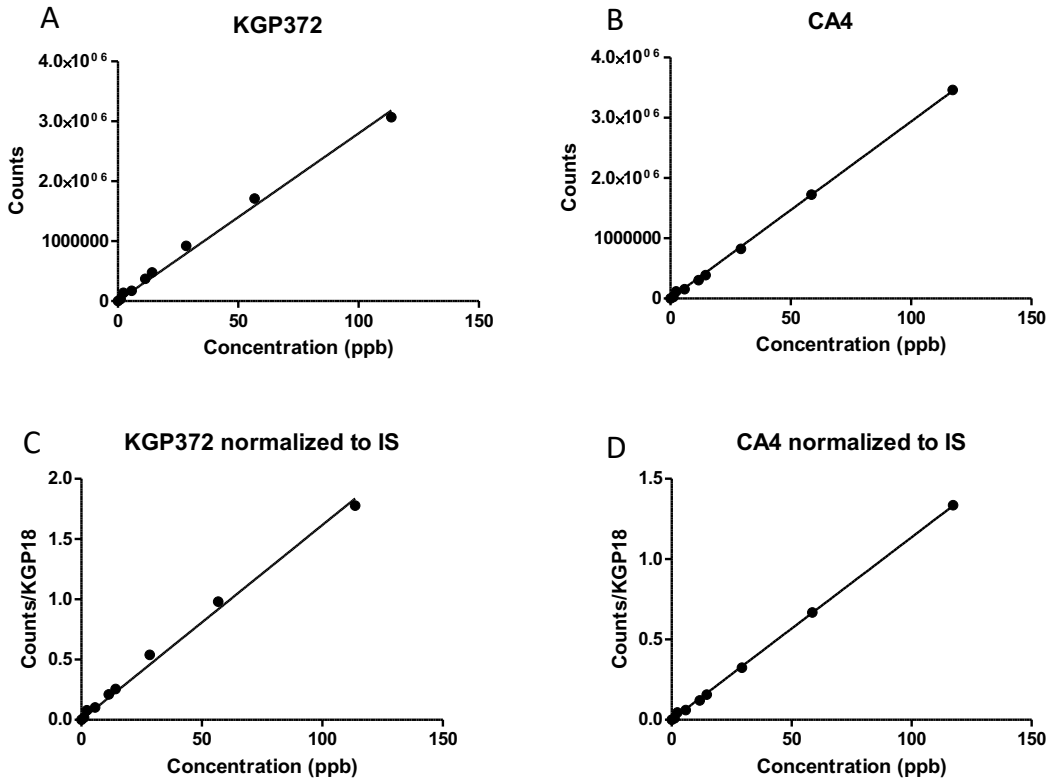


Figure S4. Representative standard curves for LC-MS. (A) BAPC **45** (KGP372); (B) CA4; (C) BAPC **45** (KGP372) standard curve normalized to internal standard KGP18 (IS); (D) CA4 standard curve normalized to internal standard KGP18 (IS).

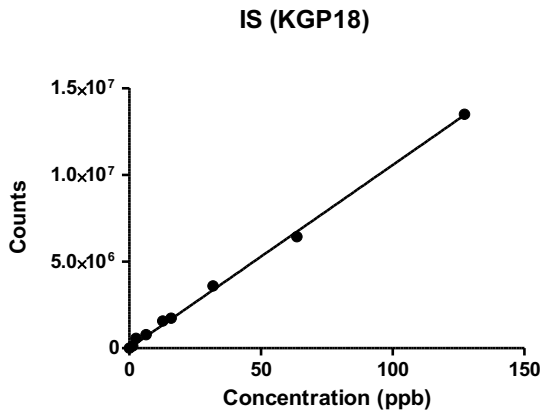


Figure S5. Standard curve for internal standard (IS) KGP18.

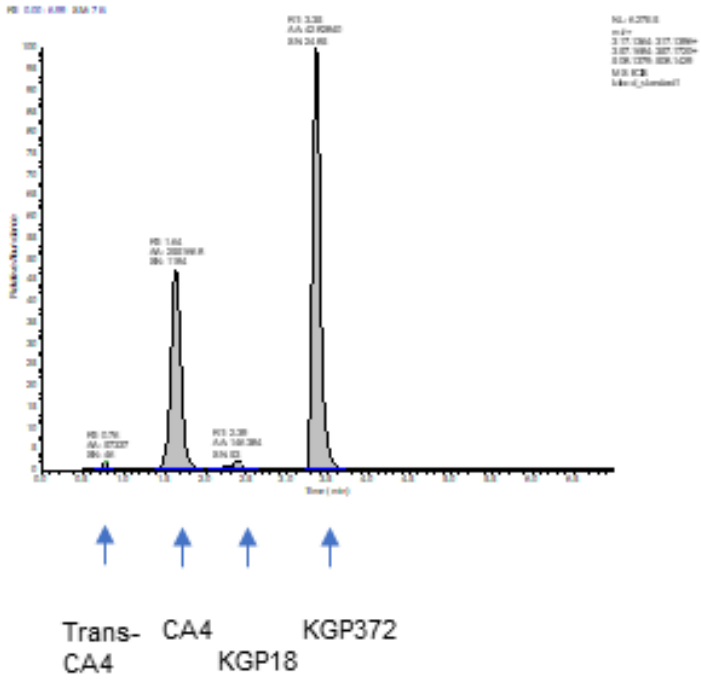


Figure S6. Elution of standards from LC, detection by exact molecular ion mass by MS. Retention times: *trans*-CA4 (0.76 min), CA4 (1.63 min), internal standard KGP18 (2.39 min), and KGP372 (BAPC **45**, 3.35 min).

In Vivo Mouse Dosing: A dose of 150 mg/kg BAPC **45** in DPS vehicle (120-135 μL depending on mouse weight) was administered IP using a 29 G insulin syringe to three adult female BALB/C mice (24-27.2 g, UTSW breeding colony) and samples (50 μL) were drawn at various times (30 min, 1 h, 2 h, 4 h, 8 h and 24 h) by retro-orbital bleed with heparin (~5 μL) and kept chilled at 4° C until frozen at -80° C.

Sample Preparation: Blood samples (50 μL) were diluted with 50 μL of water (Fisher) and proteins were precipitated by the addition of acetonitrile (Fisher). Samples were eluted from 0.3 mL supported liquid extraction Chem-Elut columns (Agilent) with acetonitrile, concentrated to dryness under nitrogen and reconstituted with 500 μL acetonitrile. Samples were filtered through a 0.20 μm syringe filter (Millex), and spiked with an internal standard (IS, KGP18).[S26] Extraction recoveries from spiked samples of blood from untreated mice were 94% for CA4, and 53% for BAPC **45**.

Results: All components separated upon analysis by LC/MS as described above for standards. None of these compounds (CA4, BAPC **45**, (KGP372), or KGP18 (IS) were detected in extracted, untreated mouse blood (data not shown). Quality control standards consisted of solutions spiked with known amounts of KGP18 internal standard and were run every 12 samples throughout the assay. BAPC **45** was measurable in all post injection samples, with increases at the 4 and 8 h time points. By the 24 h time point, blood concentrations of BAPC **45** had decreased (see Table S6 and Figures S7-S8). The release of CA4 was observed in blood levels at 4 h, which increased at 8 and 24 h (refer to Figure S7-S8 and Table S6). The mechanism for CA4 release was undetermined. In vitro (control) experiments with BAPC **45** in pH 7.4 phosphate buffer demonstrated minimal cleavage (0.69%) during a 48 h timeframe.

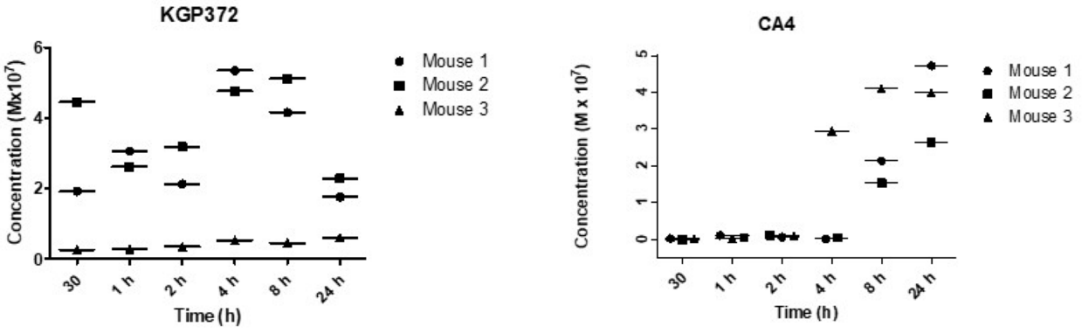


Figure S7. BAPC **45** (KGP372) detected in three individual mice dosed at 150 mg/kg of BAPC **45** (KGP372) in a vehicle of 10%DMSO / 55% sesame oil / 35% PEG400 at designated time points (30 min, 1 h, 2 h, 4 h, 8 h, and 24 h) post-treatment. (left) KGP372 (BAPC **45**) concentrations; (right) CA4 concentrations.

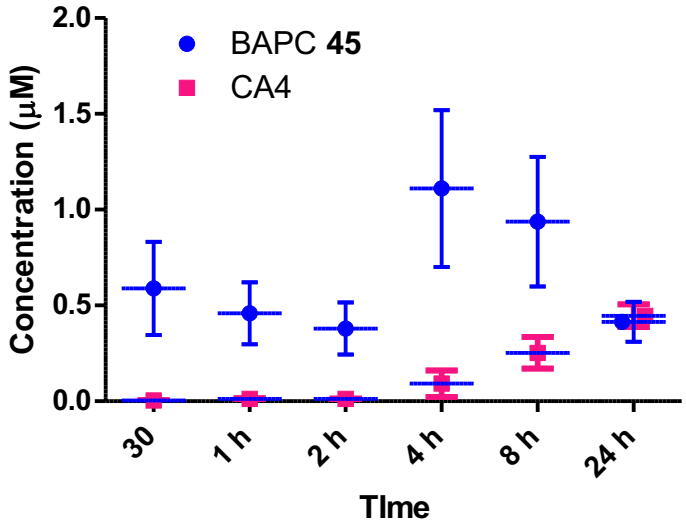


Figure S8. Combined data from Figure S7 and Table S6. BAPC **45** (KGP372) detected in three individual female mice dosed at 150 mg/kg of BAPC **45** (KGP372) in a vehicle of 10%DMSO / 55% sesame oil / 35% PEG400 at designated time points (30 min, 1 h, 2 h, 4 h, 8 h, and 24 h) post-treatment.

Table S6. Mean values (μM ± SEM) for BAPC **45** and CA4 concentrations in blood samples from mice treated with BAPC **45** (See Figures S7 and S8).

Time	BAPC **45** Avg ± SEM (μM)	CA4 Avg ± SEM (μM)
30 min	0.59 ± 0.24	< LOD[a]
1 h	0.46 ± 0.16	< LOD[a]
2 h	0.38 ± 0.14	detectable < LOQ[b]
4 h	1.1 ± 0.41	0.092 ± 0.069
8 h	0.94 ± 0.34	0.25 ± 0.083
24 h	0.42 ± 0.10	0.45 ± 0.059

[a] LOD = limit of detection

[b] LOQ = limit of quantification

Hypoxia Cytotoxicity Ratio (HCR) Determined in A549 Lung Cancer Cell Line

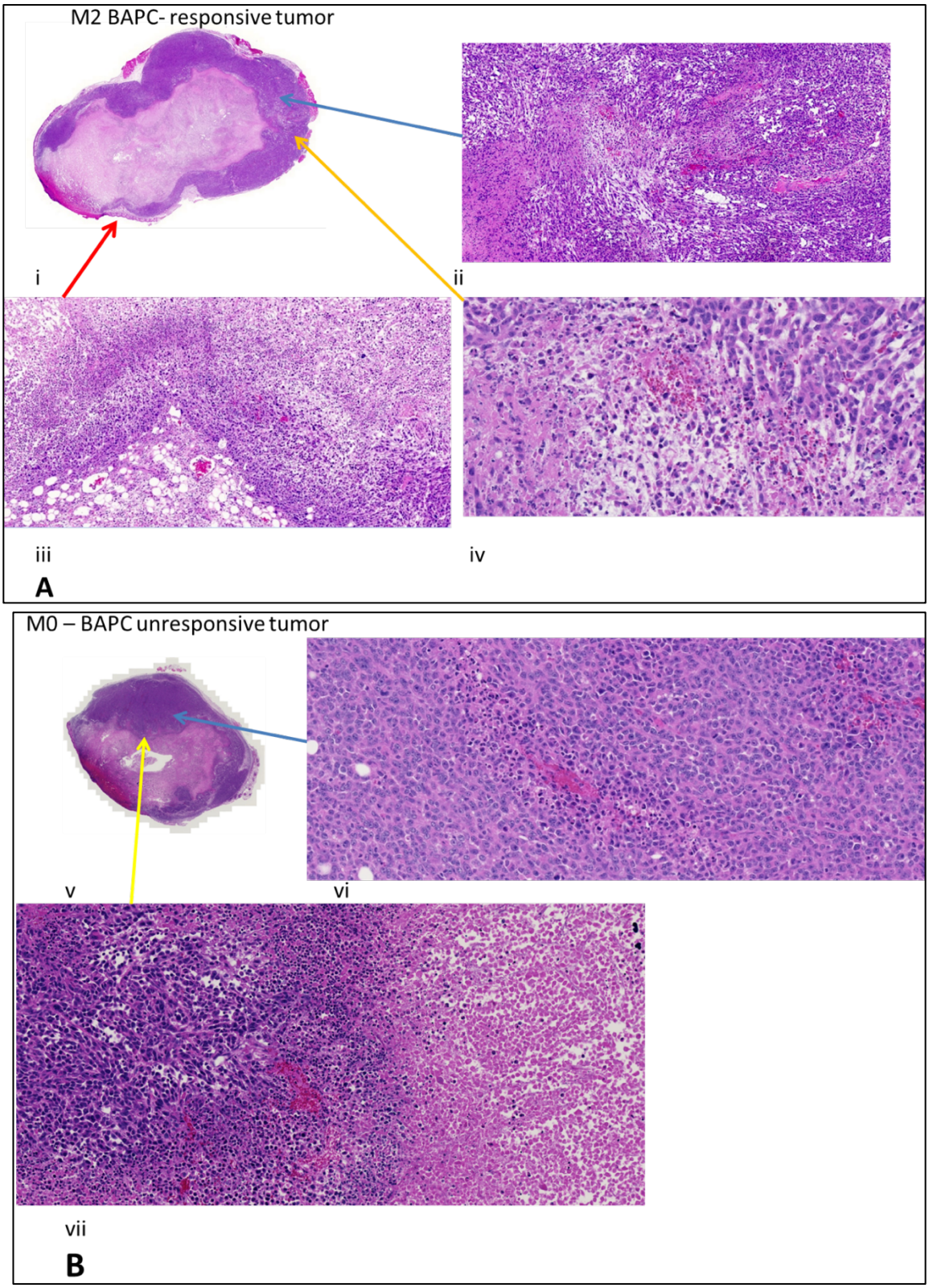
Since the parent agents, CA4 and CA1, released from their corresponding BAPCs in hypoxic conditions are diffusable antimitotic agents, the hypoxia cytotoxicity assay was modified to eliminate drug removal after the 4 h hypoxic (or normoxic) treatment. With these conditions, the HCR for tirapazamine was much lower than reported in the literature (Table 3). For experiments in which the drug was washed out after the 4 h hypoxic (or normoxic) treatment, the HCR value was much higher and comparable to literature values (Table S7).

Table S7. In Vitro Potency and Hypoxia Cytotoxicity Ratio (HCR) of Tirapazamine and RB6145 in the A549 Human Cancer Cell Line

Compound	GI_{50} [oxic][a] (μM)±SEM	GI_{50} [anoxic][a] (μM)±SEM	HCR
RB6145	>130	36±6.4	>3.6
Tirapazamine	>203	3.3±1.3	>62

[a] Average of n ≥ 3 independent determinations

Additional Histology Related to BAPC **45**



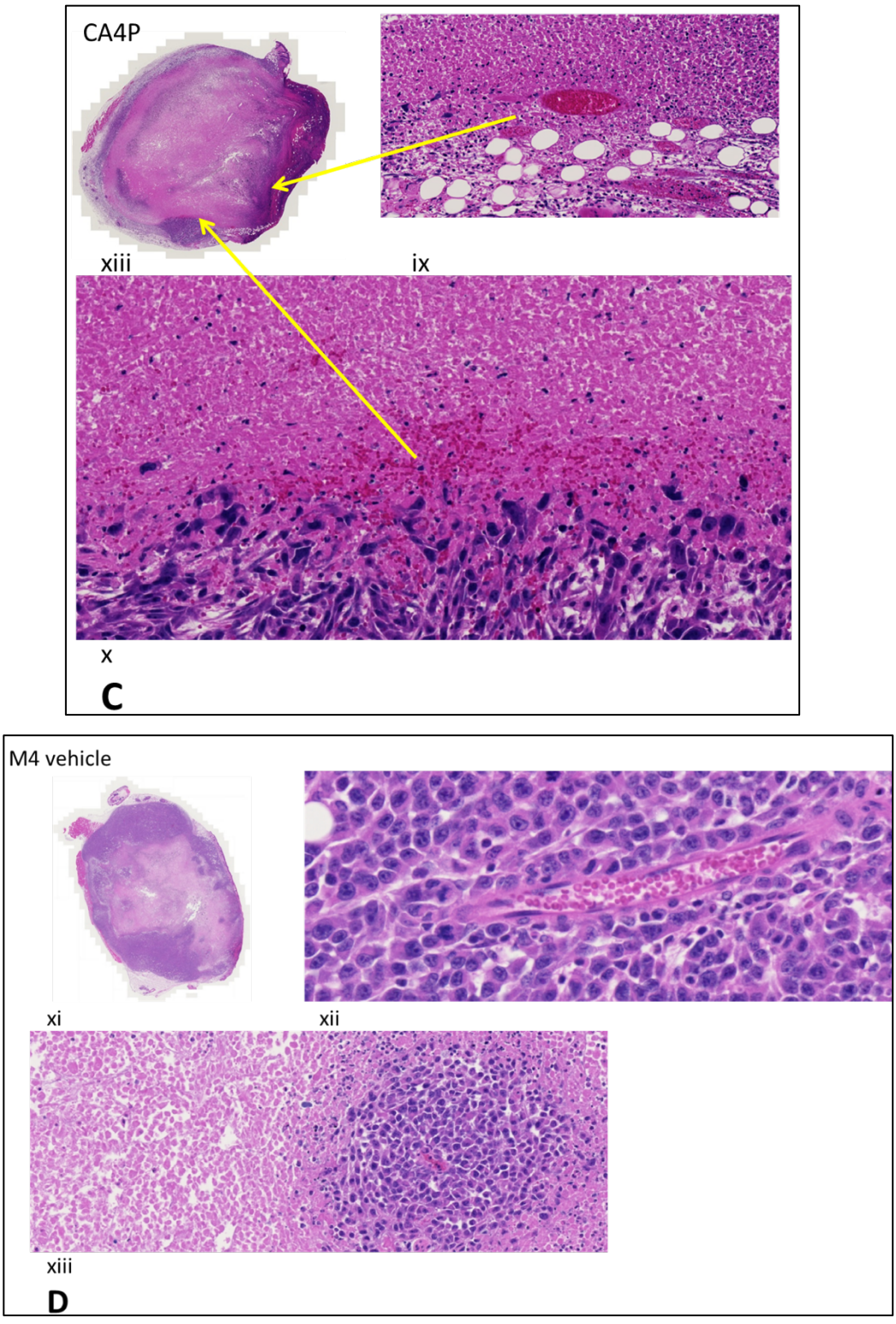


Figure S9. Histology revealing hemorrhage in 4T1 tumors following vascular damage. H&E stained sections of the four 4T1 tumors shown in Figure 5 (main manuscript) with magnified inserts to reveal vascular damage. All tumors showed extensive necrosis. A) M2 tumor responsive to BAPC 45. I) whole mount section; ii- iv) magnified insets

showing congested and hemorrhagic blood vessels. B) M0 tumor unresponsive to BAPC showing substantial necrosis but largely intact blood vessels. C) Tumor M1 following CA4P showing extensive hemorrhage particularly at the interface of viable and necrotic tissue. D) M4 receiving vehicle only showing intact blood vessels and classic viable tumor tissue chords, but no obvious hemorrhage despite extensive inherent necrosis.

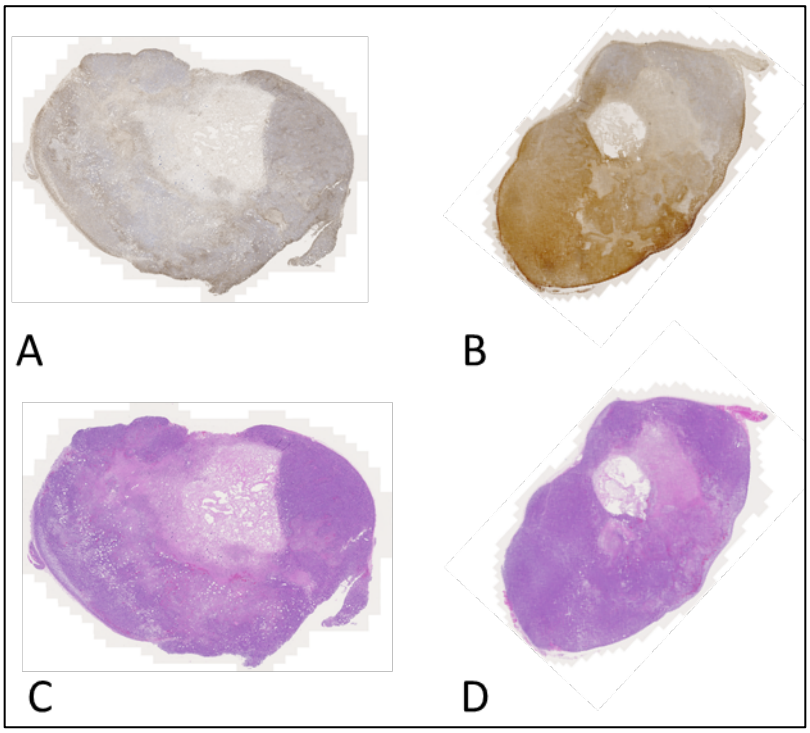


Figure S10. Hypoxia in control untreated orthotopic 4T1 tumors. Based on standard procedures,[S27] two 4T1 tumor-bearing mice received intravenous infusion of pimonidazole (60 mg/kg; Hypoxyprobe™-1 Plus Kit; Hypoxyprobe Inc., Burlington, MA, USA). Sixty minutes later mice were anesthetized and the tumors excised and rapidly immersed in 4% paraformaldehyde with overnight fixation followed by a series of hydrations within 24 hr., before they were submitted for routine paraffin embedding, sectioning, and H&E staining (Histo Pathology Core, UT Southwestern). Pimonidazole was stained in 5 µm paraffin sections using a Hypoxyprobe™-1 Plus Kit according to the manufacturer's protocol for paraffin-embedded tissue. Whole mount images were obtained using a Zeiss Axio Scan.Z1 (Zeiss, Peabody, MA, USA). Both tumors were similar volume about 0.44 cm^3. **A** and **B** show sections from respective tumors exhibiting very different levels of hypoxia. **B** and **D** show the corresponding H&E slides indicating some necrosis in each tumor.