ABSTRACT

Targeting Tumor Hypoxia with Potent Vascular Disrupting Agents

Bunnarack Kuch

Director: Kevin G. Pinney

Current therapeutic methods highlight the use of vascular disrupting agents (VDAs) in anti-cancer therapy. Through a binding interaction with the colchicine binding site on beta-tubulin, VDAs function as inhibitors of tubulin polymerization, resulting in morphological changes to the endothelial cells lining tumor-feeding vessels, which selectively disrupts blood flow to tumors and results in tumor necrosis. An active area of research inquiry centers on methods to further enhance selectivity of therapeutic agents and strategies towards tumors and targets within the tumor microenvironment, including vasculature. Bioreductively activatable prodrug conjugates (BAPCs) represent one such strategy. BAPCs utilize potent anti-cancer agents as prodrugs to selectively target tumor hypoxia. In order to reduce toxicity and enhance targeting, VDAs are coupled with bioreductive triggers to mask the ability of the resultant BAPCs to interact with the tubulin-microtubule protein system. Under hypoxic conditions which are inherent to many solid tumor cancers, the trigger compounds are cleaved, thus releasing the cytotoxic VDAs site-specific to the tumor. Synthetic pathways for these BAPCs have been developed to effectively couple highly active VDAs with bioreductive triggers.

APPROVED BY DIRECTOR OF HONORS THESIS:

_____

Dr. Kevin G. Pinney, Department of Chemistry and Biochemistry

APPROVED BY THE HONORS PROGRAM:

_____

Dr. Elizabeth Corey, Director

DATE: _____

TARGETING TUMOR HYPOXIA WITH POTENT VASCULAR DISRUPTING AGENTS

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Bunnarack Kuch

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CHAPTER 1

Introduction

The tumor vasculature is a promising target for anti-cancer therapy due to its importance in tumor cell survival.[1,2] Solid tumors require functional blood supply as well as a steady oxygen flow in order to maintain their state of continuous growth.[2] Therefore, the ability to deprive tumors of their nutrient source opens up numerous means of combating cancer.[3] Therapeutic methods have so far concentrated on two different methods of targeting tumor vasculature; an anti-angiogenic approach that utilizes angiogenesis inhibiting agents (AIAs) and an antivascular approach that highlights the use of vascular disrupting agents (VDAs) to rapidly and selectively shut down existing tumor vasculature.[3–5] VDAs can disrupt tumor vasculature due to their primary function as inhibitors of tubulin polymerization.[4,6–8] Since tubulin assembly is so crucial to the dynamic function of the cell cytoskeleton, the inhibition mechanism will lead to morphological changes that results in vascular collapse, reduced blood flow, and eventually secondary tumor necrosis.[7,9]

Shown in Figure 1, combretastatin A-1 (CA1) and combretastatin A-4 (CA4), isolated from the African bush willow tree *Combretum caffrum* Kuntze (Combretacae), are natural products, isolated and characterized by Pettit and his coworkers, that function as VDAs.[10–16]

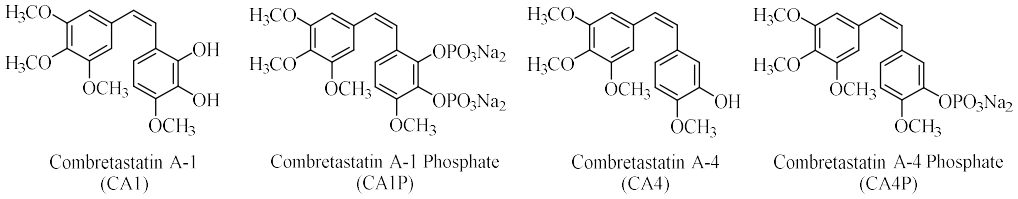


Figure 1. Combretastatin A-1, Combretastatin A-4, and their Phosphate Prodrugs.[11,12,17]

In an attempt to improve the solubility of the combretastatin natural products, phosphate prodrugs of CA1 and CA4, referred to as combretastatin A-1 phosphate and combretastatin A-4 phosphate (CA1P and CA4P, respectively), were synthesized and have thus demonstrated remarkable efficacy at disrupting tumor vasculature in both preclinical and clinical studies.[14,17–21] Known to selectively bind to the colchicine binding site, these agents (like many VDAs that bind to the colchicine binding site) initiate a cascade of cell signaling events that causes morphological changes to the endothelial cells of tumor blood vessels.[7,14,22,23] This leads to a complete collapse of the tumor vasculature, depriving the tumor its essential nutrients, shown in Figure 2.[7,24–26]

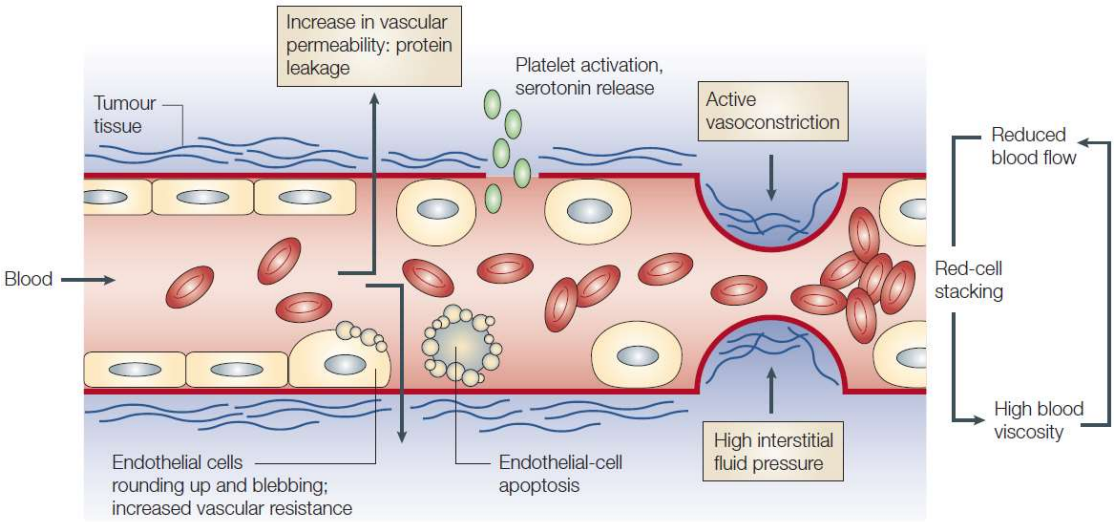


Figure 2. Proposed mechanism for rapid shutdown of tumor vasculature after treatment with CA4-P or DMXAA.[27] (Reproduced directly from reference 27)

However, the tumor microenvironment poses a significant threat to the effective application of these drugs.[28,29] The vasculature in tumors is known to be highly disorganized and abnormal compared to that of healthy tissues.[28,30] It is well-known that tumors are very proliferative in nature, therefore, to satisfy the oxygen and nutrient demands, tumors create their own vascular network.[27,31,32] However, endothelial cell division cannot keep up with the demand for new vasculature and, as a result, produce poorly developed, immature, discontinuous blood vessels.[27] Irregularly shaped endothelial cells and the uneven luminal layer of the blood vessels can lead to increased interstitial fluid pressure as a result of an increase in fluid permeability.[22,27] Furthermore, vascular networks in tumors are chaotic, exhibiting complex branching patterns and irregular vessel lengths and diameters which contributes to a high resistance of blood flow in tumor masses.[27,33] Oxygen diffusion limitation and the inability to rectify the oxygen deficit leads to varying concentrations of oxygen within tumor, resulting in regional areas of hypoxia. Tumor hypoxia is problematic because it confers resistance to conventional methods of radiotherapy and chemotherapy.[27,34]

Although the presence of tumor hypoxia can be detrimental to the efficacy of various VDAs as well as other anticancer agents, it can also serve as a high-priority target for combating solid tumors.[27,35,36] Low oxygen concentration is a characteristic unique to only solid tumors, and not normally present in healthy tissues.[28,34,36,37] As a result, a new strategy seeks to exploit this characteristic by using tumor hypoxia as a target in selective anticancer therapy.[35,38,39] Research in the field of hypoxia-activated prodrugs, also known as bioreductively activatable prodrug conjugates (BAPCs), offers promising potentials in anticancer therapy.[33,36] In well-oxygenated conditions, these prodrug

conjugates are inactive and their effects are masked.[36] However, in hypoxic conditions they are activated by enzymatic reduction through one- or two- electron reductases such as NAD(P)H-dependent flavoproteins (one electron reductase) and cytochrome P450s (two electron reductase).[33]

Tirapazamine (TPZ), shown in Figure 3, a benzotriazine di-*N*-oxide and hypoxia-selective cytotoxin, is one type of BAPC selectively activated by one- and two- electron reductases.[33,36,40] In Phase II and III clinical trials, TPZ demonstrated significant activity in non-small lung, head, and neck cancers when used in combination with radiotherapy and cisplatin-based chemotherapy.[36] Under hypoxia conditions, the drug is reduced by intracellular reductase(s) to form cytotoxic radicals that function by acting as alkylating agents; leading to double-stranded DNA breaks, single-stranded DNA breaks, damage to DNA bases, and poisoning of topoisomerase II.[33] Despite the promising results, further clinical studies using TPZ were halted due to implications of survival benefits.[41,42] However, it has become the standard for comparison of many newly synthesized BAPCs.[41]

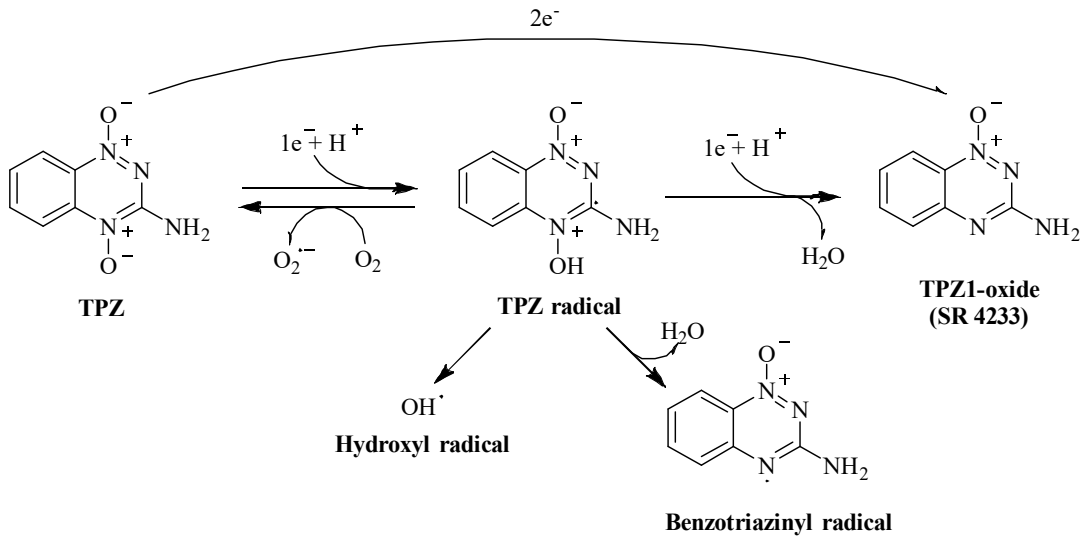


Figure 3. Reduction of TPZ to generate a DNA-reactive free radical.[33,36,41]

Current emphasis on another type of BAPCs focuses on the use of 5-nitrothiophene analogues as potential bioreductive triggers.[43] Depicted in Figure 4, these bioreductive triggers are attached to the pharmacophore, masking its effect. Upon reduction in hypoxic conditions, the trigger is cleaved and the pharmacophore is released into the tumor microenvironment.[9,33]

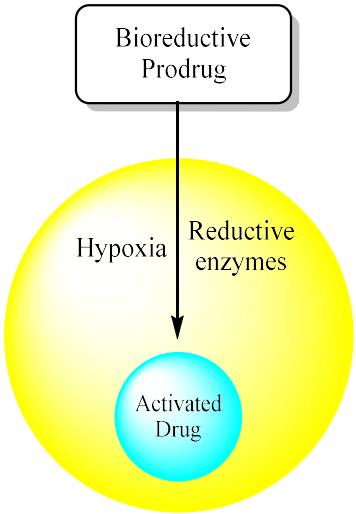


Figure 4. Generalized scheme for activation of BAPC in hypoxic conditions.[33]

Nitroaromatic compounds, such as the 5-nitrothiophenes, have long been known to possess redox properties similar to that of BAPCs, yet no such compounds have reached clinical trials.[9] Using the powerful anti-proliferative activity of CA4 as a lead, Peter Davis and coworkers synthesized 5-nitrothiophene analogues of CA4, consisting of *nor*-, *mono*-, and *gem*-dimethyl triggers, displayed in Figure 5.[43]

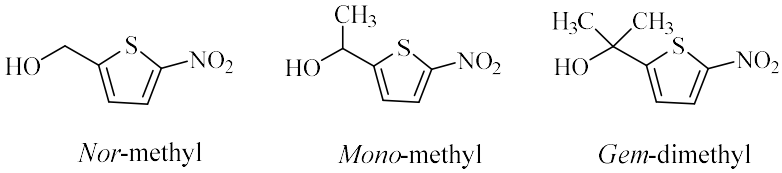


Figure 5. Nitrothiophene-based triggers used in the synthesis of CA1 and CA4 BAPCs.[43]

The *nor*-, *mono*-, and *gem*-dimethyl nitrothienyl BAPCs were evaluated by Peter Davis and coworkers based on multiple criteria.[43,44] Results demonstrated that the *gem*-dimethyl CA4 BAPC not only possessed the greatest fragmentation efficiency when releasing CA4 under hypoxic conditions, but was also successfully able to mask the effects of CA4 until the trigger was cleaved by NADPH:cytochrome P450R or NADPH:cytochrome P450R 3A4 and maintain structural integrity in oxygen-rich environments due to its resistance to aerobic metabolism.[43,45]

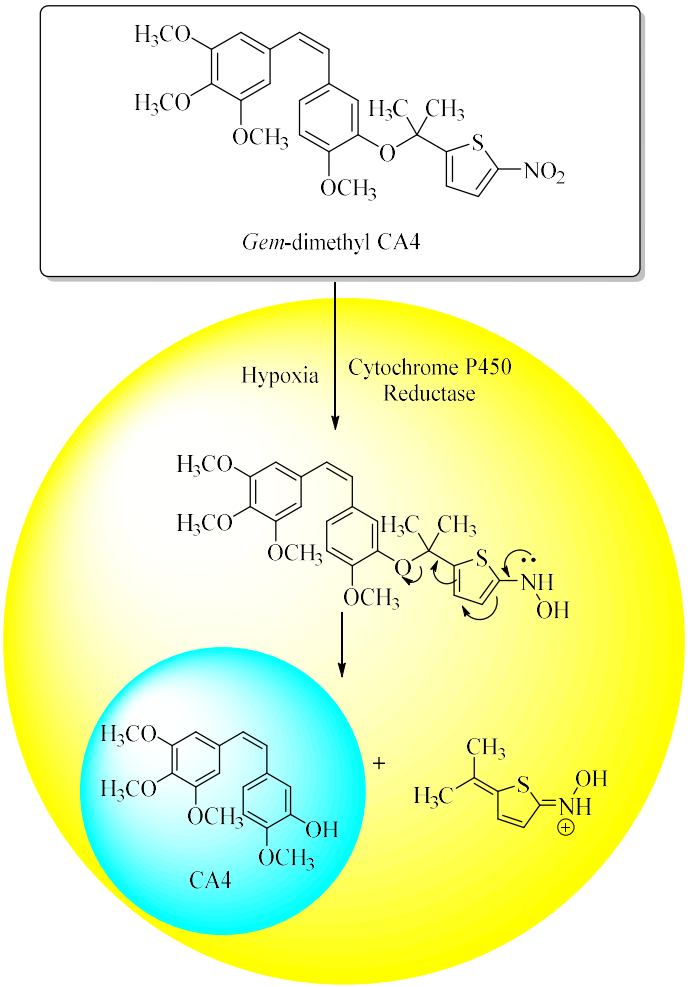


Figure 6. Reduction and Cleavage of *gem*-dimethyl CA4 by *NADPH*-Cytochrome P450 Reductase in Hypoxic Conditions.[33]

For the research described herein, a selective protection strategy devised by the Pinney Group will use *tert*-butyldimethylsilyl (TBS) to protect CA1, allowing for selective attachment of the *nor*- and *gem*-dimethyl nitrothiophene trigger to the C-2 and C-3 positions.[46–49] The nitrothiophene triggers used by Peter Davis and coworkers, *nor*-, *mono*-, and *gem*-dimethyl substituted, were synthesized using a new synthetic strategy with potentials to afford greater yields. The newly synthesized BAPCs of CA1 will be evaluated under hypoxic and normoxic conditions to demonstrate the effectiveness of these CA1-trigger compounds as possible hypoxia-activated prodrugs.

CHAPTER 2

Results and Discussion

The synthesis of CA1-BAPCs is highlighted by four reactions which serve as crucial points in achieving formation of the final product: 1) a Wittig olefination reaction results in the synthesis diTBS-Protected CA1 with preference for the cis product, 2) a deprotection of the TBS group at either the C-2 or C-3 position, 3) a Mitsunobu reaction to couple monoTBS-protected-CA1 with nitrothiophene triggers, and 4) a deprotection of monoTBS-CA1-BAPCs to achieve the final product.

Outlined in **Figure 7**, the synthesis of monoTBS-protected-CA1 (**6** and **7**) can be accomplished by selective demethylation of aldehyde **1** at the C-2 and C-3 position using boron trichloride to yield the catechol compound **2**.[11] The hydroxyl groups at the C-2 and C-3 position of catechol **2** were protected using *tert*-butyldimethylsilyl chloride (TBSCl), in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine (Et_3N), to generate compound **3**. Through a Wittig olefination reaction between compound **3** and phosphonium salt **4**, diTBS-protected CA1 **5** was generated as a mixture of E- and Z-isomers with a 9:1 Z:E ratio. The desired Z-isomers were isolated using flash column chromatography. Compound **5** underwent partial deprotection in the presence of tetrabutylammonium fluoride (TBAF) to afford a mixture of monoTBS-Protected-CA1 **6** and **7** in equal proportions, in addition to compound **8**, CA1. Compounds **6**, **7**, were then separated from **8** using flash column chromatography, with **6** and **7** carried forward to the Mitsunobu reaction.

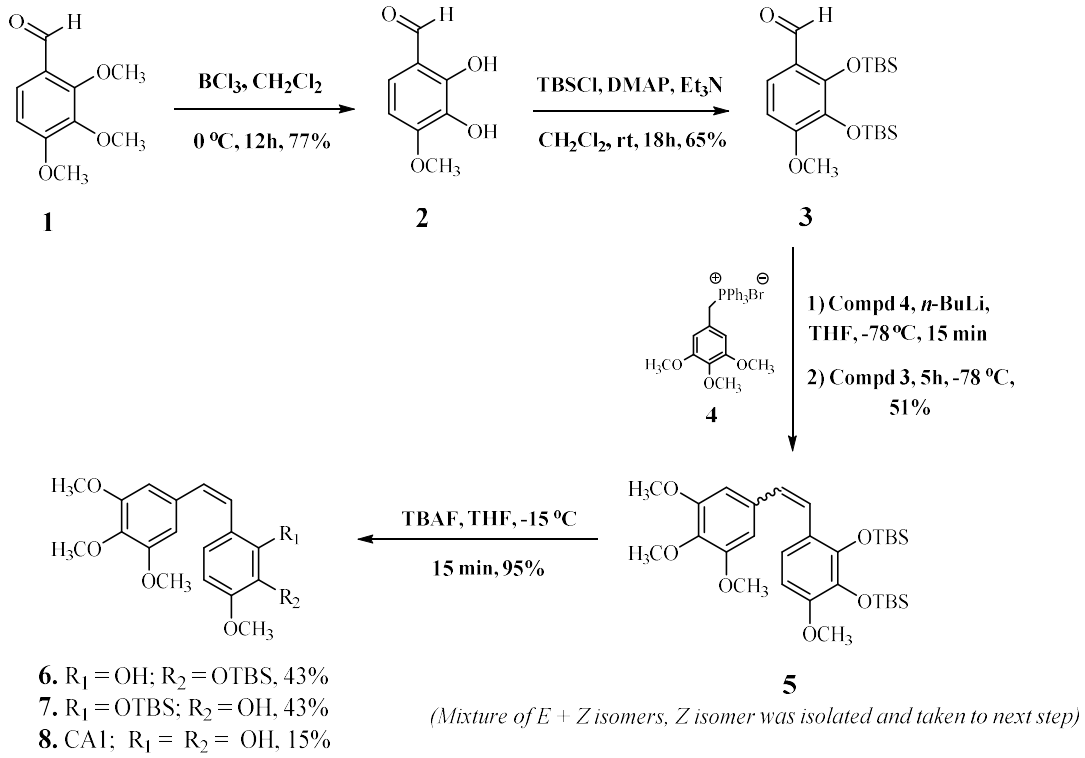


Figure 7. Synthesis of mono TBS-Protected CA1

Figure 8 details the synthesis of the *nor*-methyl, *mono*-methyl, and *gem*-dimethyl nitrothiophene triggers proposed by Peter Davis and coworkers.[43] Using his route, the primary 5-nitrothienyl alcohol, **10**, was prepared by reduction of commercially available 5-nitrothiophene-2-carboxaldehyde **9** using sodium borohydride ($NaBH_4$). Similarly, the secondary 5- nitrothienyl alcohol, **12**, was prepared by sodium borohydride reduction of 2-acetyl-5-nitrothiophene **11**. The g*em*-dimethyl alcohol, **14**, required methyllithium as a strong nucleophilic methylating agent to methylate 2-acetylthiophene followed by careful nitration of the resulting compound **13** using fuming nitric acid and acetic anhydride to yield g*em*-dimethyl alcohol **14**.

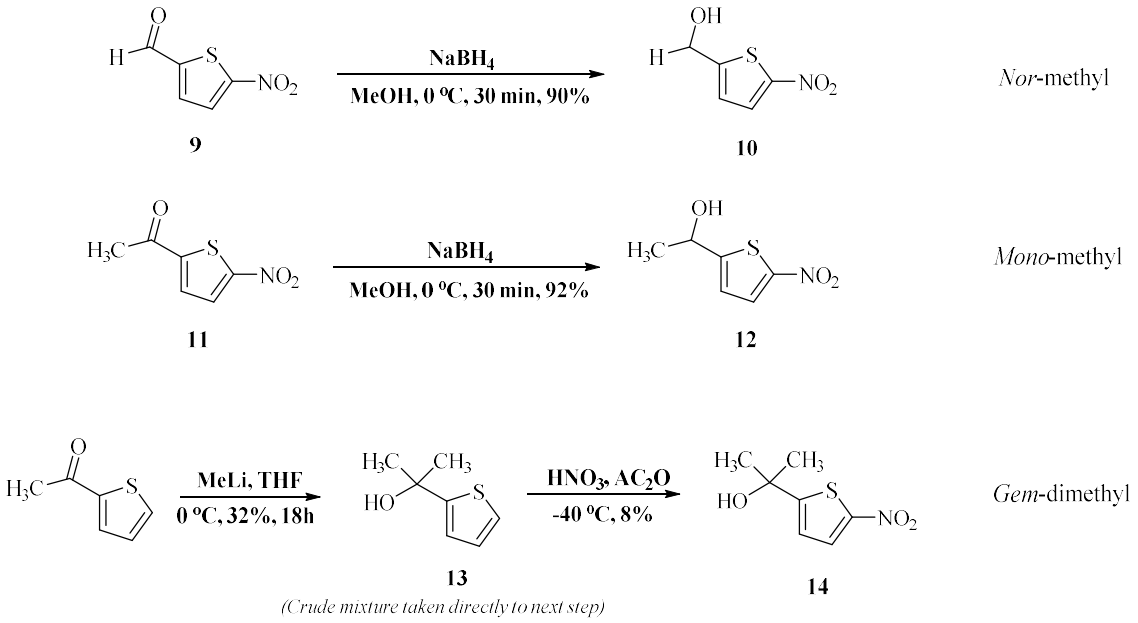


Figure 8. Synthesis of *Nor*-methyl, *Mono*-methyl, and *Gem*-dimethyl Nitrothiophene Triggers, Published by Peter Davis.[43]

Despite the successes of the route proposed by Peter Davis in the synthesis of compounds **10** and **12**, the formation of compound **14** proved to be challenging as the process required two consecutive low-yielding steps, most notably the nitration step that afforded merely an 8% yield. This, in addition, to the discontinuation of compound **11** from commercial sources propelled us to design a new and improved approach to the synthesis of *gem*-dimethyl alcohol **14**, outlined in **Figure 9**.

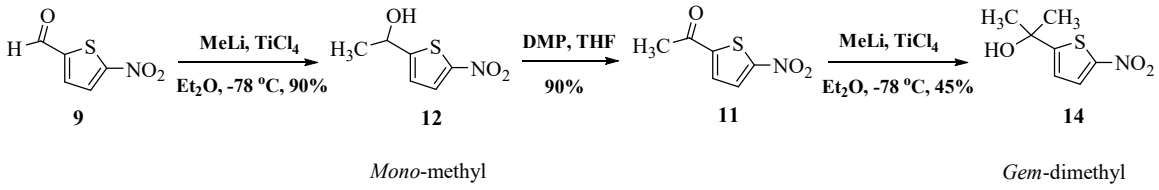


Figure 9. New Route for the Synthesis of *Nor*-methyl, *Mono*-methyl, and *Gem*-dimethyl Nitrothiophene Triggers.[50]

The new synthetic approach allowed for the synthesis of all three nitrothiophene triggers from a single starting material, compound **9**. The *nor*-methyl trigger remained unchanged and followed the same sodium borohydride reduction procedures described by Peter Davis. Methylation of 5-nitrothiophene-2-carboxaldehyde **9** with methyllithium and titanium tetrachloride ($TiCl_4$) in diethyl ether (Et_2O) yielded the *mono*-methyl nitrothiophene trigger **12**. Subsequent oxidation of compound **12** with Dess-Martin periodinane (DMP) yielded compound **11** as an intermediate. Finally, methylation of compound **11** using methyllithium and titanium tetrachloride in diethyl ether granted us the *gem*-dimethyl nitrothiophene trigger **14**.

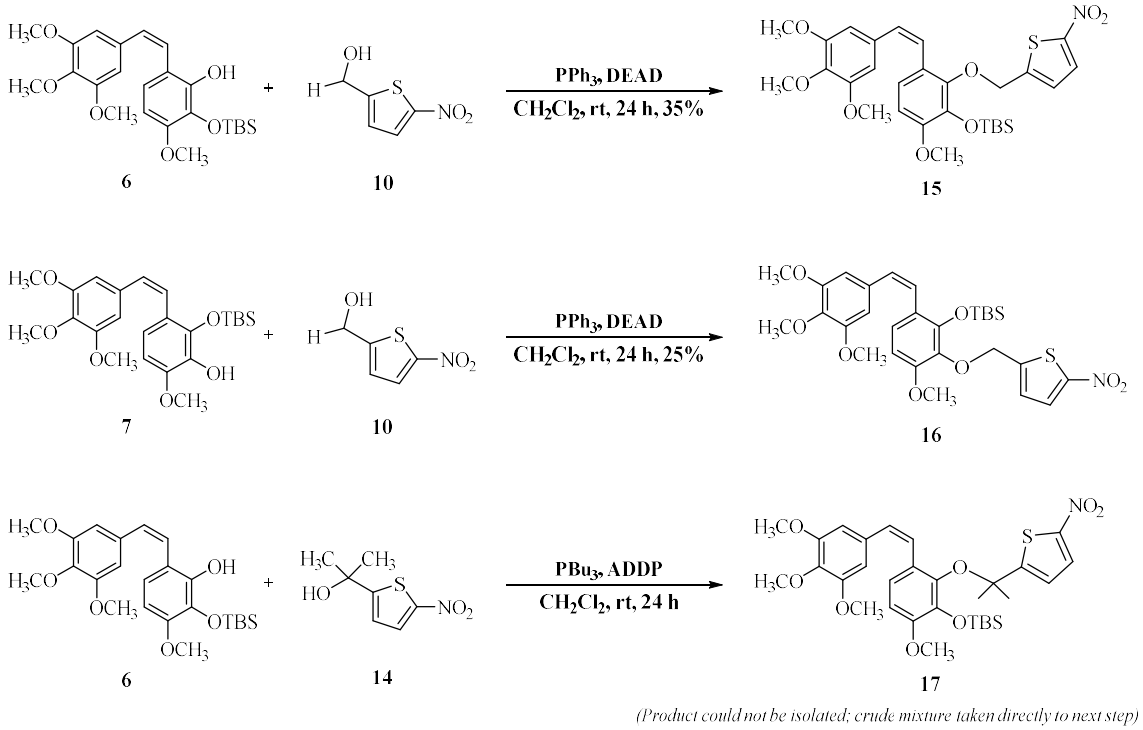


Figure 10. Synthesis of TBS-Protected CA1-Nitrothiophene BAPCs Using *Nor*-methyl and *Gem*-dimethyl Nitrothiophene Triggers

Two different sets of Mitsunobu conditions were used to couple stilbene compounds **6** and **7** to their nitrothiophene triggers **10** and **14**, as shown in **Figure 10**. The reagent mixture of diethyl azodicarboxylate (DEAD) and triphenylphosphine (PPh_3) was used to generate the primary alkyl-aryl ethers **15** and **16** by Mitsunobu coupling of compounds **6** and **7**, respectively, with the *nor*-methyl nitrothiophene trigger **10**.[47,48] In order to successfully synthesize the hindered tertiary alkyl-aryl ether **17**, a reagent mixture of 1-1'-(azodicarbonyl)-dipeperidine (ADDP) and tributylphosphine (PBu_3) was used to allow coupling of compound **6** with *gem*-dimethyl trigger **14**.[47,49] Compounds **15** and **16** were isolated using flash column chromatography. Compound **17**, however, could not be isolated and was taken directly to the next step as a crude mixture.

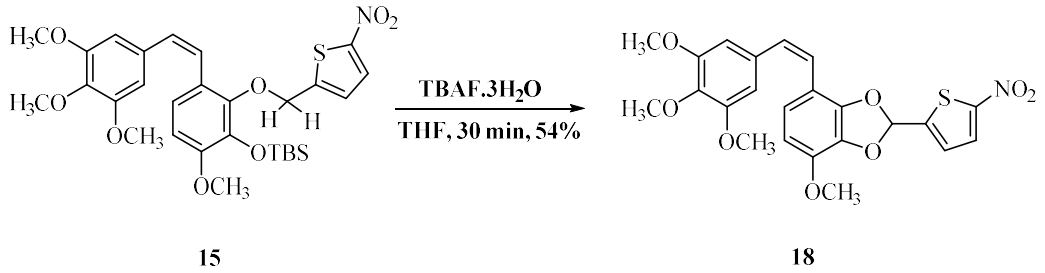


Figure 11. Formation of Cyclized Product **18** after TBS-deprotection Using TBAF

Interestingly, using TBAF for TBS-deprotection of compounds **15** and **16** did not yield the desired *nor*-methyl trigger CA1 BAPC. Despite the conventionality of the process outlined in **Figure 11**, we observed the formation of a new dioxole ring **18**, rather than the expected TBS-deprotection. Detailed in **Figure 12**, it was only after switching to HCl (2M) and acetic acid (AcOH) were we able to successfully remove the TBS groups, granting the formation of *nor*-methyl trigger CA1 BAPCs **19** and **20**.

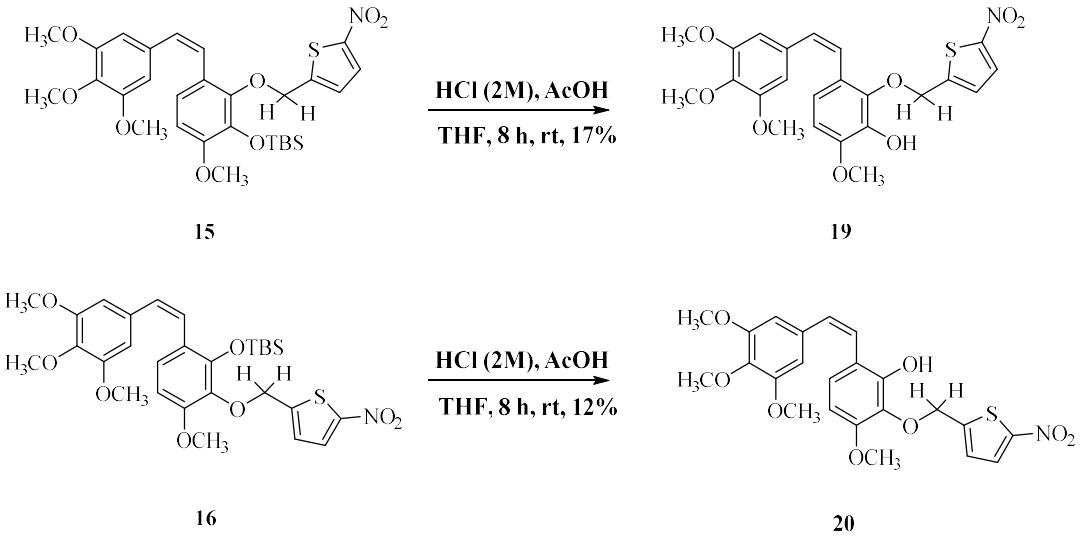


Figure 12. TBS-deprotection Yielding Selected Regioisomers of *Nor*-methyl CA1 BAPC

Contrary to the interactions observed in **Figures 11** and **12**, the formation of the *gem*-dimethyl CA1 BAPC does not require the use of HCl and acetic acid, as shown in **Figure 13**. The hindered tertiary alkyl-aryl ether **17** appears to lack the ability to cyclize due to the presence of the two geminal methyl groups. Following conventional routes, TBAF was used in the TBS-deprotection of compound **17** to afford the *gem*-dimethyl CA1 BAPC **21**.

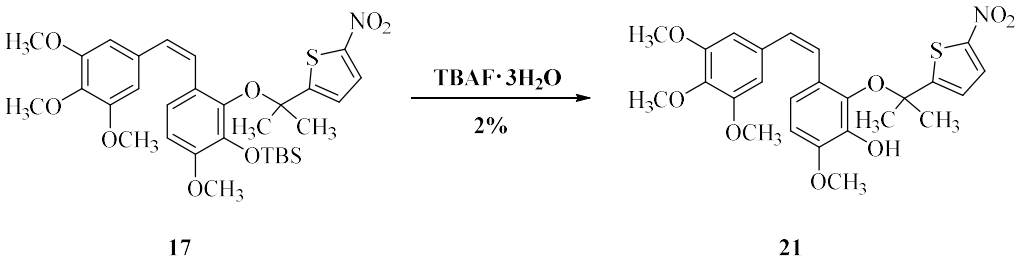


Figure 13. TBS-deprotection Yielding *Gem*-dimethyl CA1 BAPC

Biological Evaluation[44]

Table 1. Inhibition of Tubulin Polymerization and Percent Inhibition of Colchicine Binding. Courtesy of Dr. Ernest Hamel (National Cancer Institute)

Compound	Inhibition of Tubulin Polymerization IC_{50}	Inhibition of Colchicine Binding % Inhibition	
	(μM)±SD	1 μM	5 μM±SD
8 (CA1)	1.9	ND	99.6±0.7
CA4	0.64	84±2	97±0.7
18	1.7±0.2	ND	25±3
19	1.7±0.01	53±3	92±0.5
20	4.3±0.4	ND	58±4
21	1.3±0.08	ND	43±4

ND= Data not available

The newly synthesized BAPCs were evaluated for their ability to inhibit tubulin polymerization and in a competitive binding assay (with colchicine) for the colchicine site on the tubulin heterodimer. Shown in **Table 1**, the results were assessed using their parent drugs, CA1 and CA4, as standards for the determination of effectiveness. Compounds **18**, **19**, and **21** displayed excellent inhibition of tubulin polymerization, comparable to that of CA1. Of the BAPCs that were synthesized, compound **19** showed colchicine binding inhibition similar to that of CA1 and CA4. However, since these evaluations were performed under normoxic conditions, compounds **18** and **21** display the most remarkable characteristics. Their low percent inhibition values are indicative of the effectiveness of the triggers. Under normoxic conditions, these nitrothiophene BAPCs should remain inactive. However, indicated by their low IC_{50} values, once compounds such as **19** and **21** bind to the colchicine binding site they are more effective at inhibiting tubulin polymerization than CA1.

CHAPTER 3

Materials and Methods

Chemistry

Acetic Acid (AcOH), Dichloromethane (CH_2Cl_2), methanol, nitric acid, and tetrahydrofuran (THF) were used in their anhydrous forms or as obtained from the chemical suppliers. Unless specified, reactions were performed under nitrogen gas. Purification of intermediates and products was carried out with a Biotage Isolera flash purification system using silica gel (200−400 mesh, 60 Å) or RP-18 pre-packed columns. Intermediates and products synthesized were characterized on the basis of their ^{1}H NMR (600 MHz), ^{13}C NMR (125 MHz) and ^{31}P NMR (240 MHz) spectroscopic data using a Bruker DRX 600 MHz instrument. Spectra were recorded in $CDCl_3$, $(CD_3)_2CO$. All chemical shifts are expressed in ppm (δ), and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), sextet (sext), septet (sept), double doublet (dd), double double doublet (ddd), and multiplet (m). Mass spectrometry was carried out using a Thermo Scientific LTQ Orbitrap Discovery instrument under positive (+ve) and negative (-ve) ESI (Electrospray Ionization). Purity of the final compounds was further analyzed at 25 °C using an Agilent 1200 HPLC system with a diode-array detector (λ = 190−400 nm), a Zorbax XDB-C18 HPLC column (4.6 mm Å~ 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; solvent A acetonitrile, solvent B H_2O; Method A: H_2O; gradient, 10% A/90% B to 100% A/0% B over 0 to 40 min; post-time 10 min, Method B: H_2O; gradient, 50% A/50% B to 90% A/10% B over 0 to 30 min; post-time 10 min; flow rate 1.0 mL/min; injection volume 20 μL; monitored at

wavelengths of 210, 230, 254, 280, and 320 nm.

Synthesis

2,3-Dihydroxy-4-methoxybenzaldehyde (2)[31]

2,3,4-Trimethoxybenzaldehyde **1** (4.00 g, 20.4 mmol) was added to dry CH_2Cl_2 (80 mL) in an ice bath (0 °C). Boron trichloride (45 mL, 45 mmol, 1.0 M) was added dropwise to the reaction mixture, followed by stirring for 12 h. The reaction was quenched with $NaHCO_3$ and acidified to pH 2 with HCl (12 M). The product was then extracted with EtOAc, dried with sodium sulfate, and evaporated under reduced pressure. The crude mixture was filtered through silica gel in a frit funnel using CH_2Cl_2 and evaporated under reduced pressure. Flash chromatography of the crude product was performed using a prepacked 100 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 → 69% A/31% B over 13.12 min (10 CV), 69% A/31% B over 2.38 min (2 CV); flow rate 50.0 mL/min; monitored at λ 254 and 280 nm] yielded compound **2** (2.64 g, 15.7 mmol, 77%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.12 (1H, s), 9.76 (1H, s), 7.15 (1H, d, J = 8.5 Hz), 6.63 (1H, d, J = 8.5 Hz), 5.46 (1H, s), 3.99 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.2, 153.0, 149.0, 133.0, 126.1, 116.1, 103.6, 56.4.

2,3-bis((tert-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (3)[44]

2,3-Dihydroxy-4-methoxybenzaldehyde **2** (1.00 g, 5.95 mmol) was dissolved in dry CH_2Cl_2 (30 mL). Et_3N (2.00 mL, 14.3 mmol) and DMAP (0.025 g, 0.200 mmol) were added to the solution. Next, TBSCl (2.10 g, 13.9 mmol) was dissolved in DMF and added drop-wise into reaction mixture, followed by stirring for 18 h at room temperature.

H_2O was used to quench the reaction and the crude product was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 40%A / 60%B (10 CV), 40%A / 60%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] yielding compound **3** (0.650 g, 1.64 mmol, 65%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (1H, s), 7.48 (1H, d, *J*=8.8 Hz), 6.62 (1H, d, *J*=8.8 Hz), 3.84 (3H, s), 1.04 (9H, s), 0.99 (9H, s), 0.13 (12H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 189.64, 157.88, 151.32, 137.10, 123.64, 121.69, 105.73, 77.57, 77.36, 77.15, 55.53, 26.51, 26.36, 19.07, 18.89, -3.51.

3,4,5-Trimethoxybenzyltriphenylphosphonium bromide (4)[25]

3,4,5-Trimethoxybenzylbromide, obtained from manufacturer, (11.00 g, 42.1 mmol) and PPh_3 (12.1 g, 46.3 mmol) was added to a solution of acetone (100 mL, anhydrous). The reaction was allowed to stir for 5 h under nitrogen gas. After 5 h, the resulting mixture was filtered using a Buchner funnel. The solid was washed with acetone (100 mL) and hexanes (50 mL) and dried under vacuum to afford compound **4** (20.3 g, 38.2 mmol, 92% yield) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74 – 7.64 (9H, m, Ar*H*), 7.58 – 7.50 (6H, m, Ar*H*), 6.43 (2H, d, *J*=2.6 Hz), 5.29 (2H, d, *J*=14.1 Hz, benzylic CH_2), 3.70 (3H, d, *J*=3.4 Hz), 3.43 (6H, d, *J*=3.7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 153.0 (C, C-3, C-5), 137.6 (C, C-4), 134.8 [CH, Ph(C-4)], 134.6 [CH, Ph(C-3, C-5)], 130.0 [CH, Ph(C-2, C-6)], 122.4 (C, C-1), 117.8 [C, Ph(C-1)], 108.8 (CH, C-2, C-6), 60.8 (CH_3, O*C*H_3-4), 56.2 (CH_3, O*C*H_3-3, -5), 30.8 (CH_2, -*C*H_2P). ^{31}P NMR (243 MHz, $CDCl_3$) δ 23.2.

(Z)-((3-methoxy-6-(3,4,5-trimethoxystyryl)-1,2-phenylene)bis(oxy))bis(tert-butyldimethylsilane) (5)[44]

n-Butyllithium (11.4 mL, 2.5M) was added to a solution of compound **4** (11.2 g, 21.4 mmol) in THF (350 mL). The solution was allowed to stir for 15 min at -78 °C. Compound (**3**) (5.66 g, 14.3 mmol) was dissolved in THF and added drop-wise using a dropping funnel, followed by stirring for 5 h. After 5 h, H_2O was used to quench the reaction and the residue was extracted with Et_2O. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a pre-packed 340 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 30%A / 70%B (10 CV), 30%A / 70%B (2 CV); flow rate: 85 mL/min; monitored at 254 and 280 nm] affording compound **5** (2.89 g, 5.15 mmol, 51%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (1H, d, *J*=8.6 Hz), 6.62 (2H, s), 6.58 (1H, d, *J*=12.2 Hz), 6.37 (1H, d, *J*=9.2 Hz), 6.37 (1H, d, *J*=12 Hz), 3.83 (3H, s), 3.74 (3H, s), 3.67 (6H, s), 1.04 (9H, s), 1.00 (9H, s), 0.19 (6H, s), 0.10 (6H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.0, 152.0, 146.5, 137.1, 137.1, 133.1, 128.0, 127.7, 123.5, 122.5, 106.2, 104.5, 77.6, 77.4, 77.1, 61.2, 56.1, 55.3, 26.7, 26.4, 19.1, 18.9, -2.9, -3.6.

(Z)-2-((tert-butyldimethylsilyl)oxy)-3-methoxy-6-(3,4,5-trimethoxystyryl)phenol (6)[44]

(Z)-2-((tert-butyldimethylsilyl)oxy)-6-methoxy-3-(3,4,5-trimethoxystyryl)phenol (7)[44]

Compound **5** (2.00 g, 3.57 mmol) was dissolved in THF (150 mL) and allowed to cool to -15 °C in a salt and ice bath. TBAF· $3H_2O$ (1.01 g, 3.20 mmol) was also dissolved in

THF (10 mL) and added drop-wise to the reaction. After 30 min, the reaction was quenched with H_2O, THF was evaporated off completely, and the crude residue was extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 70%A / 30%B (13 CV), 70%A / 30%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] yielding an inseparable mixture of compounds **6** and **7** (0.860 g, 2.59 mmol, 43%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (1H, d, *J*=8.7 Hz), 6.71 (1H, d, *J*=8.7 Hz), 6.58 (2H, d, *J*=12.0 Hz), 6.52 (4H, s), 6.47 (1H, d, *J*=12.1 Hz), 6.41 (1H, d, *J*=12.2 Hz), 6.36 (1H, d, *J*=8.5 Hz), 6.30 (1H, d, *J*=8.6 Hz), 5.66 (1H, s), 5.45 (1H, s), 3.81 (6H, s), 3.78 (3H, s), 3.74 (3H, s), 3.64 (12H, d, *J*=2.2 Hz), 1.01 (9H, d, *J*=5.2 Hz), 1.00 (9H, s), 0.22 (6H, s), 0.19 (6H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.7, 152.7, 149.3, 146.9, 145.9, 141.2, 137.0, 137.0, 136.8, 132.9, 132.8, 131.6, 129.6, 129.0, 126.8, 124.5, 123.2, 122.0, 120.1, 117.1, 106.1, 106.0, 103.8, 103.0, 60.9, 60.8, 56.1, 55.8, 55.7, 55.2, 26.0, 26.0, 18.6, 18.6, -3.9, -4.4.

(Z)-3-methoxy-6-(3,4,5-trimethoxystyryl)benzene-1,2-diol (8)[11]

Deprotection of both TBS groups on compound **5** using TBAF, purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 70%A / 30%B (13 CV), 70%A / 30%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm], yielded compound **8** (CA1) (0.179 mg, 0.538 mmol, 15%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (1H, d, *J*=8.6 Hz), 6.59 (1H, d, *J*=12.1 Hz), 6.54 (1H, d, *J*=11.9 Hz), 6.52

(2H, s), 6.39 (1H, d, *J*=8.6 Hz), 5.39 (2H, s), 3.86 (3H, s), 3.83 (3H, s), 3.67 (6H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.9, 146.5, 141.7, 137.4, 132.7, 132.6, 130.5, 124.2, 120.5, 118.0, 106.1, 103.1, 77.4, 77.2, 77.2, 76.9, 61.0, 56.3, 56.0. HRMS: *m*/*z*: obsd 355.154 $[M+Na]^+$, calcd for $C_{18}H_{20}O_6^+$, 332.1260. HPLC (Method A): 11.3 min.

(Z)-tert-butyl(6-methoxy-2-((5-nitrothiophen-2-yl)methoxy)-3-(3,4,5-trimethoxystyryl)-phenoxy)dimethylsilane (15)[44]

The inseparable mixture of compounds **6** and **7** (1.00 g, 2.24 mmol) was dissolved in CH_2Cl_2 (50 mL). The *nor*-methyl trigger **10** (0.428 g, 2.69 mmol) and DIAD (0.867 mL) was added to the reaction mixture. Lastly, PPh_3 (1.47 g, 5.60 mmol) was added drop-wise to the mixture, followed by stirring for 24 h at room temperature. After 24 h, H_2O (40 mL) was used to quench the reaction and the resulting residue was extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 40%A / 60%B (10 CV), 40%A / 60%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] yielding isomer **15** (0.350 g, 0.739 mmol, 35%) as a brownish-yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76 (1H, d, *J*=4.1 Hz), 6.91 (1H, d, *J*=4.1 Hz), 6.87 (1H, dd, *J*=8.6, 0.8 Hz), 6.57 (1H, d, *J*=8.6 Hz), 6.50 (1H, d, *J*=12.0 Hz), 6.45 (1H, d, *J*=12.2 Hz), 6.44 (1H, s), 5.12 (2H, s), 3.82 (3H, s), 3.79 (3H, s), 3.65 (6H, s), 0.99 (9H, s), 0.13 (6H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.7, 151.6, 151.4, 148.6, 147.6, 138.4, 137.1, 132.4, 130.4, 128.2, 125.1, 124.9, 124.4, 122.3, 107.5, 105.9, 68.5, 60.9, 55.8, 55.4, 25.8, 18.6, -4.6.

(Z)-tert-butyl(3-methoxy-2-((5-nitrothiophen-2-yl)methoxy)-6-(3,4,5-trimethoxystyryl)phenoxy)dimethylsilane (16)[44]

The inseparable mixture of compounds **6** and **7** (1.00 g, 2.24 mmol) was dissolved in CH_2Cl_2 (50 mL). The *nor*-methyl trigger **10** (0.428 g, 2.69 mmol) and DIAD (0.867 mL) was added to the reaction mixture. Lastly, PPh_3 (1.47 g, 5.60 mmol) was added drop-wise to the mixture. The reaction was allowed to stir for 24 h at room temperature. After 24 h, H_2O (40 mL) was used to quench the reaction and the resulting residue was extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 40%A / 60%B (10 CV), 40%A / 60%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] yielding isomer **16** (0.250 g, 0.425 mmol, 25%) as a brownish-yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (1H, d, *J*=4.1 Hz), 7.00 (1H, d, *J*=8.7 Hz), 6.96 (1H, d, *J*=4.1 Hz), 6.56 (1H, d, *J*=12.2 Hz), 6.52 (2H, s), 6.44 (1H, d, *J*=11.6 Hz), 6.42 (1H, d, *J*=8.5 Hz), 5.30 (2H, s), 3.83 (3H, s), 3.80 (3H, s), 3.67 (6H, s), 1.01 (9H, s), 0.18 (6H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.4, 152.8, 152.7, 148.8, 147.8, 138.4, 137.0, 132.6, 129.1, 128.2, 126.3, 125.9, 125.4, 123.3, 105.9, 104.9, 68.8, 60.9, 55.9, 55.8, 26.1, 18.6, -3.9.

(Z)-4-methoxy-2-(5-nitrothiophen-2-yl)-7-(3,4,5-trimethoxystyryl)-benzo[d][1,3]dioxole (18)[44]

Compound **15** (0.095 g, 0.162 mmol) was dissolved in THF (10 mL) and allowed to cooled to 0 °C in an ice bath. TBAF· $3H_2O$ (0.0672 g, 0.213 mmol) was also dissolved in

THF (10 mL) and added drop-wise to the reaction mixture. The reaction was stirred 30 min and quenched with H_2O (5 mL). THF was evaporated off completely under reduced pressure. The residue was extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude organic product was purified by flash column chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (13 CV), 60%A / 40%B (2 CV); flow rate: 20 mL/min; monitored at 254 and 280 nm] affording compound **18** (0.0510 g, 0.108 mmol, 54%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (1H, d, *J*=4.2 Hz), 7.15 (1H, d, *J*=4.2 Hz), 7.07 (1H, s), 6.86 (1H, d, *J*=8.8 Hz), 6.56 (1H, d, *J*=12.0 Hz), 6.50 (2H, s), 6.48 (1H, d, *J*=8.8 Hz), 6.44 (1H, d, *J*=12.0 Hz), 3.90 (3H, s), 3.83 (3H, s), 3.69 (6H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.9, 146.0, 145.2, 143.2, 137.3, 133.8, 132.6, 131.2, 128.1, 126.0, 123.4, 121.7, 113.5, 107.8, 105.7, 105.6, 105.2, 60.9, 56.6, 55.9. ^{13}C NMR DEPT ($CDCl_3$, 151 MHz) δ 131.2, 128.1, 126.0, 123.4, 121.7, 107.8, 105.6, 105.2, 60.9, 56.6, 55.9. HRMS: *m*/*z*: obsd 494.0881 $[M+23]^+$, calcd for $C_{23}H_{21}NO_8S^+$, 471.0988. HPLC (Method A): 17.2 min.

(Z)-6-methoxy-2-((5-nitrothiophen-2-yl)methoxy)-3-(3,4,5-trimethoxystyryl)phenol (19)[44]

To a solution of compound **15** (0.115 g, 0.196 mmol) dissolved in THF (30 mL), AcOH (7 mL) and HCl (5 mL, 2M) was added drop-wise at room temperature. After 8 h, the reaction was quenched with H_2O (40 mL), THF was evaporated off completely, and the residue was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The

crude product was purified by flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10%A / 90%B (1 CV), 10%A / 90%B → 80%A / 20%B (10 CV), 80%A / 20%B (2 CV); flow rate: 20 mL/min; monitored at 254 and 280 nm] yielding compound **19** (0.020 g, 0.0422 mmol, 17%) as a brown oil. ^{1}H NMR (600 MHz, Acetone) δ 8.15 (1H, s), 7.93 (1H, d, *J*=4.2 Hz), 7.21 (1H, d, *J*=4.1 Hz), 6.95 (1H, d, *J*=8.7 Hz), 6.58 (2H, s), 6.54 (1H, d, *J*=12.2 Hz), 6.49 (1H, d, *J*=8.7 Hz), 6.44 (1H, d, *J*=12.2 Hz), 5.29 (2H, s), 3.86 (3H, s), 3.68 (3H, s), 3.62 (6H, s). ^{13}C NMR (151 MHz, Acetone) δ 153.1, 152.3, 149.2, 148.6, 137.4, 134.1, 132.7, 129.0, 128.6, 126.5, 125.1, 124.5, 124.5, 117.9, 106.2, 103.0, 68.5, 59.6, 55.4, 55.2. ^{13}C NMR DEPT (151 MHz, Acetone) δ 129.0, 128.6, 126.5, 125.1, 124.5, 106.2, 103.0, 68.5, 59.6, 55.4, 55.2. HRMS: *m*/*z*: obsd 496.1034 $[M+Na]^+$, calcd for $C_{23}H_{23}NO_8S^+$, 473.1144. HPLC (Method B): 10.0 min.

(Z)-3-methoxy-2-((5-nitrothiophen-2-yl)methoxy)-6-(3,4,5-trimethoxystyryl)phenol (20)[44]

To a solution of compound **16** (0.250 g, 0.425 mmol) dissolved in THF (25 mL), AcOH (10 mL) and HCl (10 mL, 2M) was added drop-wise at room temperature. After 8 h, the reaction was quenched with H_2O (40 mL), THF was evaporated off completely, and the residue was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10%A / 90%B (1 CV), 10%A / 90%B → 80%A / 20%B (10 CV), 80%A / 20%B (2 CV); flow rate: 20 mL/min; monitored at 254 and 280 nm] yielding compound **20** (0.030 g, 0.0634 mmol, 12%) as a

brown oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.77 (1H, d, *J*=4.1 Hz), 6.97 (1H, d, *J*=4.1 Hz), 6.79 (1H, d, *J*= 8.4 Hz), 6.56 (1H, d, *J*= 8.4 Hz), 6.55 (1H, d, *J*=12.2 Hz), 6.50 (1H, d, *J*=12.2 Hz), 6.45 (2H, s), 5.59 (1H, s), 5.24 (2H, s), 3.88 (3H, s), 3.82 (3H, s), 3.65 (6H, s). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 152.8, 151.8, 148.5, 147.0, 142.6, 138.4, 137.2, 132.4, 130.7, 128.2, 125.5, 124.6, 124.3, 120.5, 106.6, 106.0, 68.7, 60.9, 56.4, 55.8. HRMS: *m*/*z*: obsd 496.1033 $[M+Na]^+$, calcd for $C_{23}H_{23}NO_8S^+$, 473.1144. HPLC (Method B): 12.5 min.

(Z)-6-methoxy-2-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)-3-(3,4,5-trimethoxystyryl)phenol (21)[44]

Compound **17** (2.35 g, 3.82 mmol) was dissolved in THF (250 mL) and cooled to -15 °C using a salt and ice bath. TBAF· $3H_2O$ (1.32 g, 4.19 mmol) was also dissolved in THF (10 mL) and added drop-wise to the reaction mixture. After 1 h, H_2O (40 mL) was used to quench the reaction, THF was evaporated off completely, and the residue was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (13 CV), 60%A / 40%B (2 CV); flow rate: 20 mL/min; monitored at 254 and 280 nm] yielding compound **21** (0.050 g, 0.980 mmol, 2%) as a brownish-yellow solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.77 (1H, d, *J*=4.2 Hz), 6.93 (1H, d, *J*=4.2 Hz), 6.84 (1H, d, *J*=8.6 Hz), 6.56 (1H, d, *J*=8.6 Hz), 6.52 (2H, s), 6.48 (1H, d, *J*=12.2 Hz), 6.30 (1H, d, *J*=12.2 Hz), 5.47 (1H, s), 3.86 (3H, s), 3.84 (3H, s), 3.67 (6H, s), 1.79 (6H, s). ^{13}C NMR ($CDCl_3$, 151 MHz) δ 161.5, 152.9, 150.6, 147.1, 140.6, 140.2, 137.3, 132.5, 129.3, 128.4,

127.2, 126.6, 122.2, 120.6, 106.9, 106.0, 81.9, 77.4, 77.2, 76.9, 61.1, 56.4, 56.0, 29.5. HRMS: *m/z*: obsd 524.1352 $[M+Na]^+$, calcd for $C_{25}H_{27}NO_8S^+$, 501.1457. HPLC (Method B): 12.3 min

Preparation of Compounds 10, 12 and 14 Using Route Published by Peter Davis.[43]

(5-nitrothiophen-2-yl)methanol (10)[43]

5-Nitrothiophene-2-carboxaldehyde (1.00 g, 6.38 mmol) was dissolved in anhydrous methanol (20 mL) and cooled to 0 °C in an ice bath. Sodium borohydride (0.270 g, 7.14 mmol) was added to the reaction, followed by stirring for 2 h. Ice was added to the solution, following completion, and the solution was acidified to pH 7 with HCl (3M). The reaction was extracted with EtOAc, dried with sodium sulfate, 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 → 65% A/35% B over 13.12 min (10 CV), 65% A/35% B over 2.38 min (2 CV); flow rate 50.0 mL/min; monitored at λ 254 and 280 nm] yielded compound **10** (0.914 g, 5.74 mmol, 90%) as a brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (1H, d, J = 4 Hz), 6.96 (1H, d, J = 4 Hz), 4.91 (2H, d, J = 5.5), 2.20 (1H,s). ^{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 (12)[43]

2-Acetyl-5-nitrothiophene (1.00 g, 5.85 mmol) was dissolved in anhydrous methanol (20 mL) and cooled to 0 °C in an ice bath. Sodium borohydride (0.259 g, 6.71 mmol) was added to the reaction, followed by stirring for 2 h. Ice was added to the solution, following completion, and the solution was acidified to pH 7 with HCl (3M). The

solution was then extracted with EtOAc, dried with sodium sulfate, 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 λ 254 and 280 nm] yielded compound **12** (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), 6.90 (1H, d, J = 4 Hz), 5.15 (1H, dq, J = 6 Hz, J = 5 Hz), 2.23 (1H, d, J = 5 Hz), 1.63 (3H, d, J = 6 Hz). ^{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 (13)[43]

2-Acetylthiophene (10.0 g, 79.2 mmol) was dissolved in dry THF (100 mL) and cooled to 0 °C in an ice bath. Methyllithium (64 mL, 103 mmol, 1.6 M) was added dropwise to the reaction, followed by stirring for 18 h. H_2O (40 mL) was used to quench the reaction, THF was evaporated off completely, and the residue was extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated 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 λ 254 and 280 nm] yielded compound **13** (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), 6.97 (2H, m), 2.04 (1H, s), 1.68 (6H, s).

2-(5-nitrothiophen-2-yl)propan-2-ol (14)[43]

Compound **13** (6.22 g, 4.37 mmol) was dissolved in Ac_2O (67 mL) and allowed to cool to -78 °C in a dry-ice bath. Fuming nitric acid (25 mL) was added drop wise to the reaction mixture. After 2 h, the reaction mixture was allowed to warm to -15 °C. Ice (200 g) was added to the solution and stirred for 40 minutes. The reaction mixture was extracted with EtOAc (3×75 mL), washed repeatedly with brine and water. The combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10%A / 90%B (1 CV), 10%A / 90%B → 70%A / 30%B (13 CV), 70%A / 30%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] yielding compound **14** (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), 6.87 (1H, d, *J*=4.2 Hz), 2.13 (1H, s), 1.67 (3H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.6, 133.9, 128.9, 121.4, 77.4, 77.2, 76.9, 72.0, 32.2.

Synthesis of Compounds 10, 12 and 14 Using New Route[50]

1-(5-nitrothiophen-2-yl)ethan-1-ol (12)[43,44]

Titanium tetrachloride (7.84 g, 41.3 mmol) was added slowly and dropwise into a flask of Et_2O (80 mL) at -78 °C. Following the addition, methyllithium (1.6 M, 25.8 mL, 41.3 mmol) was added slowly and dropwise into the mixture. This mixture was stirred for 1.5 h. 5-nitro-2-thiophenecarboxaldehyde **9**, obtained commercially, (5.00g, 31.8 mmol) was dissolved in Et_2O (120 mL) and added dropwise to the reaction using a syringe. The reaction was allowed to stir for 12 h. The reaction was quenched with H_2O (50 mL) and the residue was extracted with EtOAc (6 × 40 mL). The combined extracts were washed

with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10%A / 90%B (1 CV), 10%A / 90%B → 73%A / 27%B (13 CV), 73%A / 27%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] affording compound **12** (4.95 g, 28.6 mmol, 90%) as a dark brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (1H, d, *J* = 4 Hz), 6.90 (1H, d, *J* = 4 Hz), 5.15 (1H, dq, *J* = 6 Hz, *J* = 5 Hz), 2.23 (1H, d, *J* = 5 Hz), 1.63 (3H, d, *J* = 6 Hz). ^{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 (11)[43, 44]

Compound **12** (1.04 g, 6.00 mmol) was dissolved in CH_2Cl_2 (70 mL). Solid Dess-Martin periodinane (DMP) (3.82 g, 9.00mmol) was added to the solution and the reaction was stirred for 1 h at room temperature. The reaction was quenched with saturated sodium thiosulfate solution (50 mL) and saturated sodium bicarbonate solution (50 mL). The residue was extracted with EtOAc (4 x 30 mL) and the combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10%A / 90%B (1 CV), 10%A / 90%B → 80%A / 20%B (13 CV), 80%A / 20%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] yielding compound **11** (0.873 g, 5.10 mmol, 90%) as yellow-orange crystals. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 (1H, d, *J*=4.3 Hz), 7.58 (1H, d, *J*=4.3 Hz), 2.60 (3H, s). ^{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 (14)[43, 44]

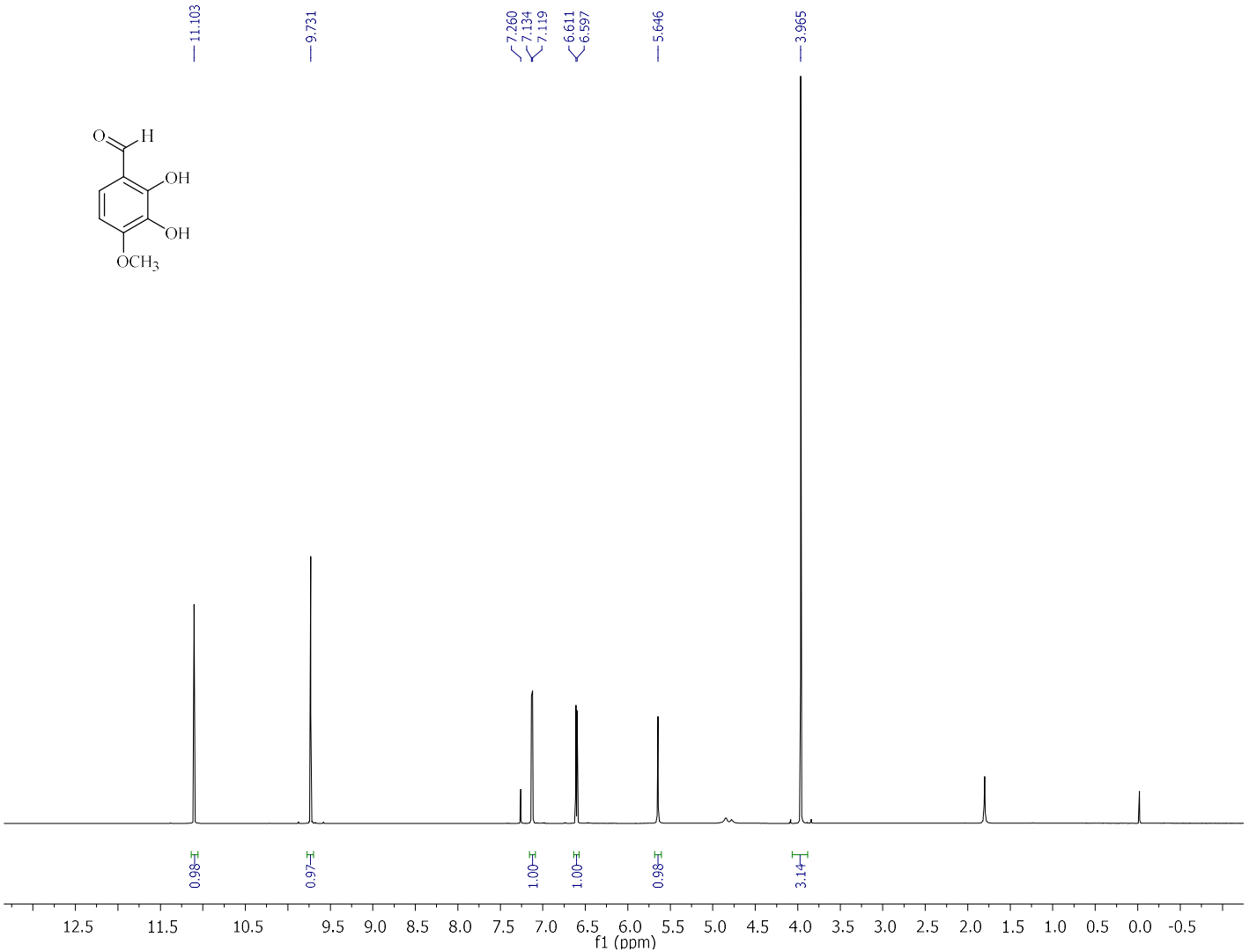
Titanium tetrachloride (3.62 g, 19.1 mmol) was added slowly and dropwise into a flask of Et_2O (80 mL) at -78 °C. Following the addition, methyllithium (1.6 M, 11.9 mL, 19.1 mmol) was added slowly and dropwise into the mixture, stirred for 1.5 h. 2-acetyl-5-nitrothiophene **11** (2.50 g, 14.7 mmol) was dissolved in Et_2O (140 mL) and added dropwise to the reaction using a syringe. The reaction was allowed to stir for 12 h. The reaction was quenched with H_2O (50 mL) and the residue was extracted with EtOAc (6 × 40 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10%A / 90%B (1 CV), 10%A / 90%B → 70%A / 30%B (13 CV), 70%A / 30%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] affording compound **14** (1.61 g, 8.60 mmol, 45%) as a dark orange oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.79 (1H, d, *J*=4.2 Hz), 6.87 (1H, d, *J*=4.2 Hz), 2.13 (1H, s), 1.67 (3H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.6, 133.9, 128.9, 121.4, 77.4, 77.2, 76.9, 72.0, 32.2.

Tubulin Studies[7,51,52]

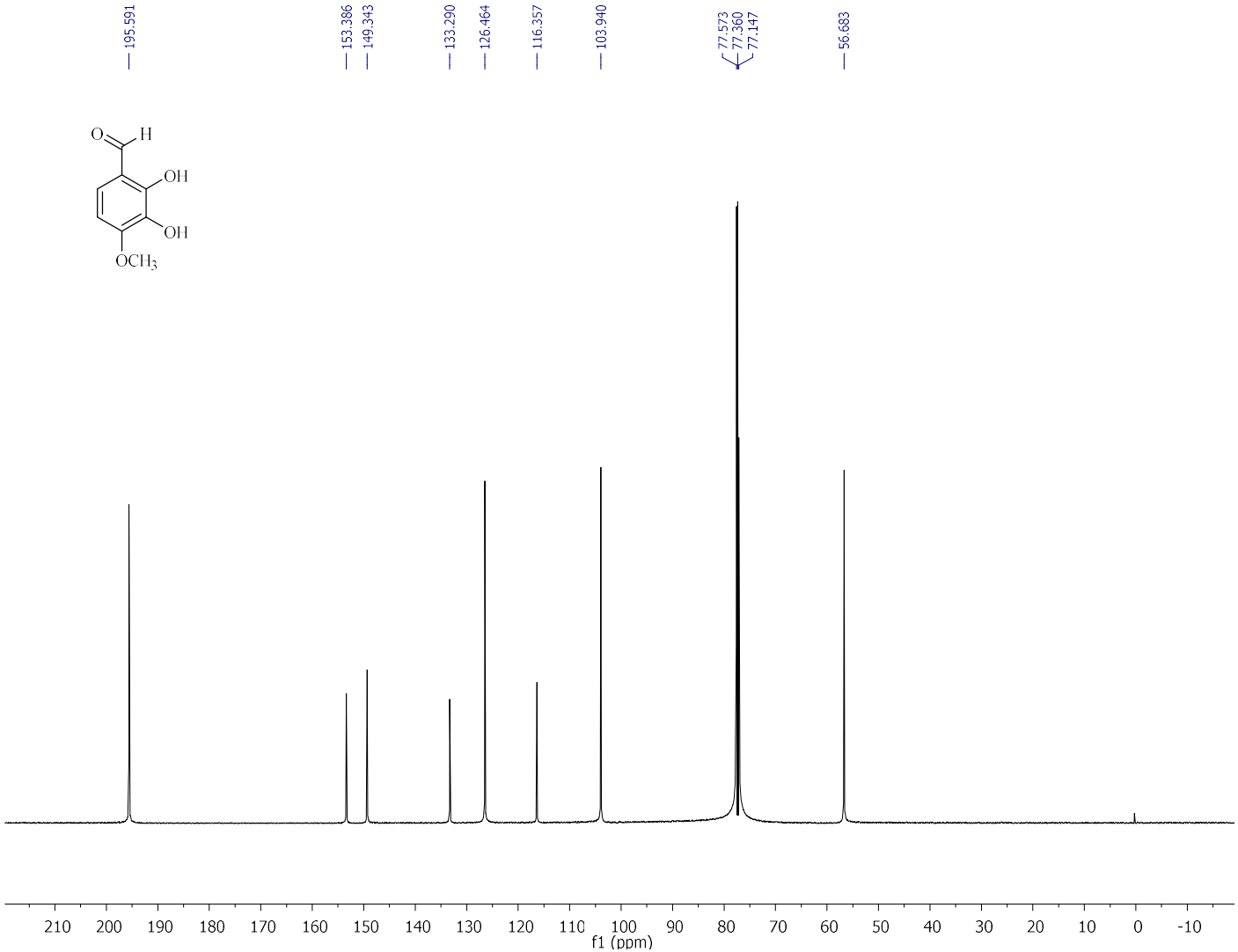
All biological evaluations were performed by Dr. Ernest Hamel (National Cancer Institute). Procedures for the evaluations can be found in the references listed above.

APPENDIX

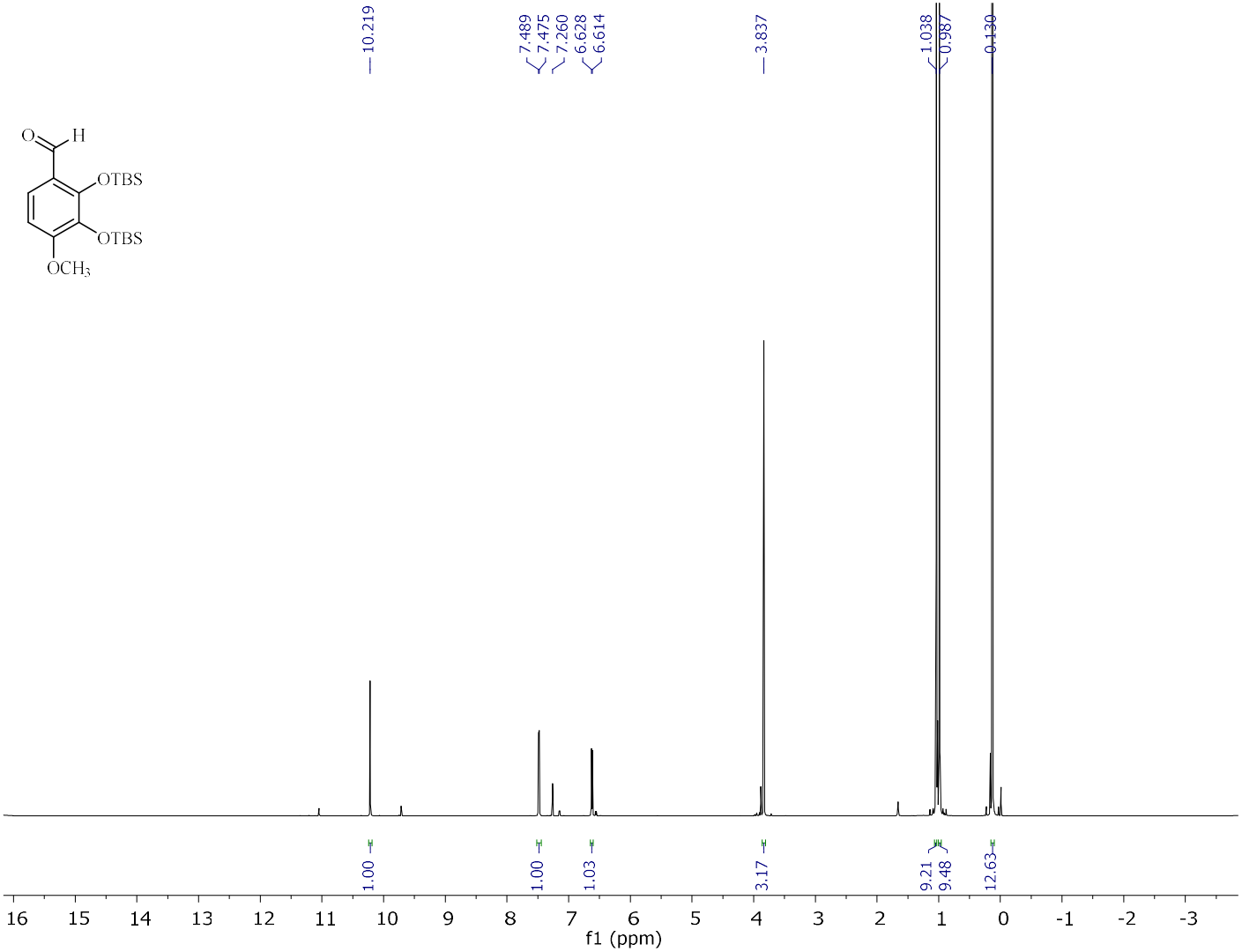
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **2**



^{13}C NMR (151 MHz, $CDCl_3$) for Compound **2**



^{1}H NMR (600 MHz, $CDCl_3$) for Compound **3**



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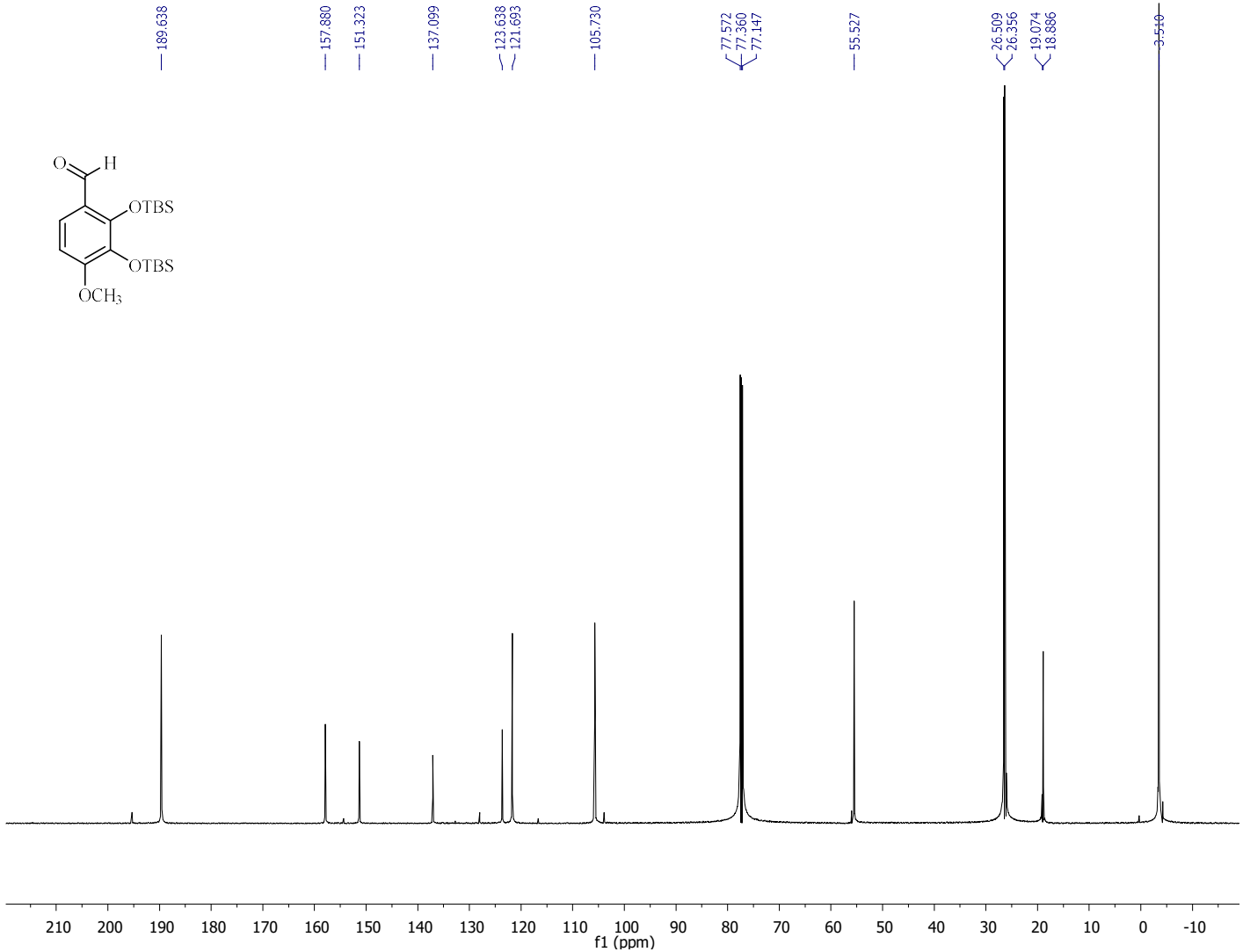
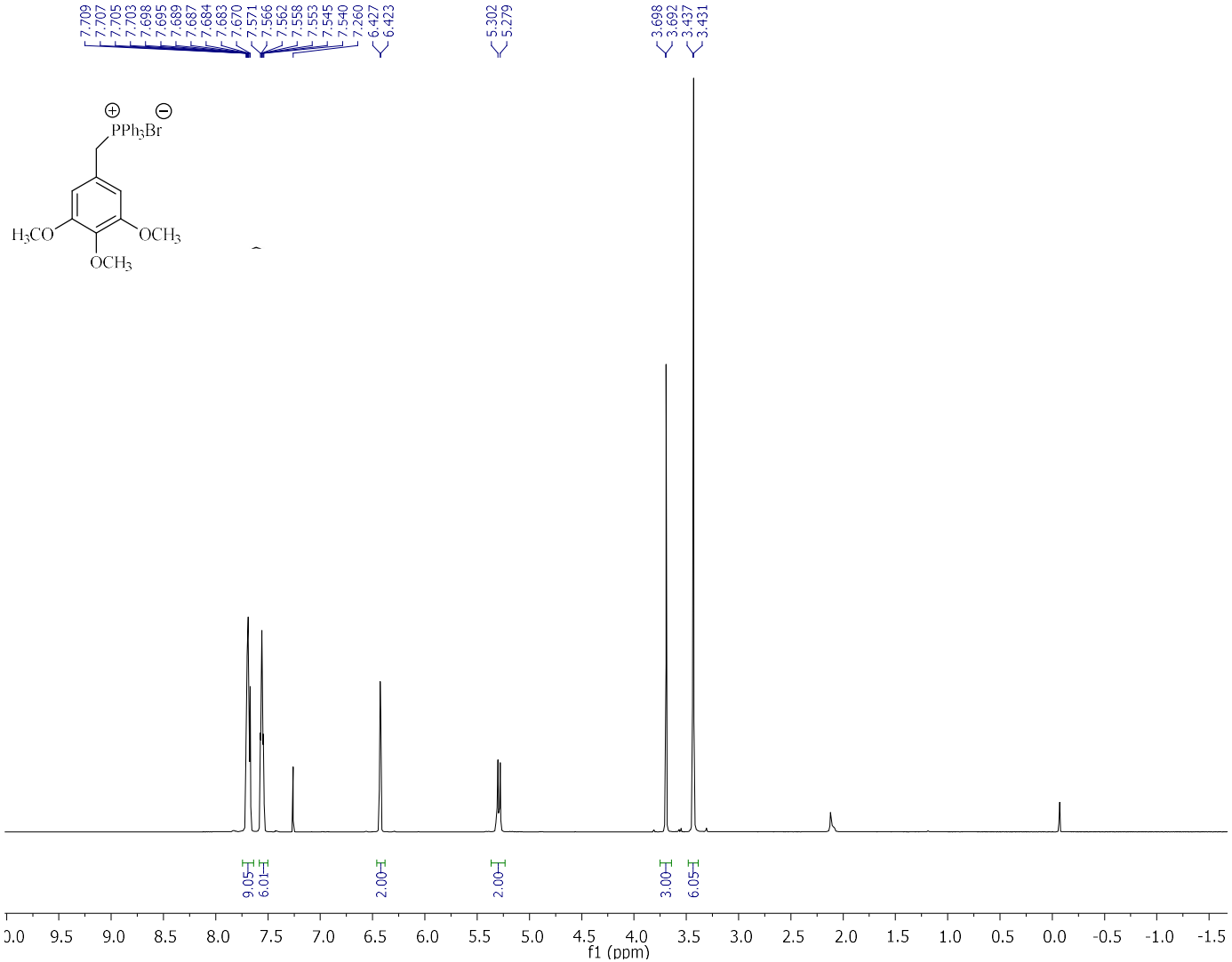
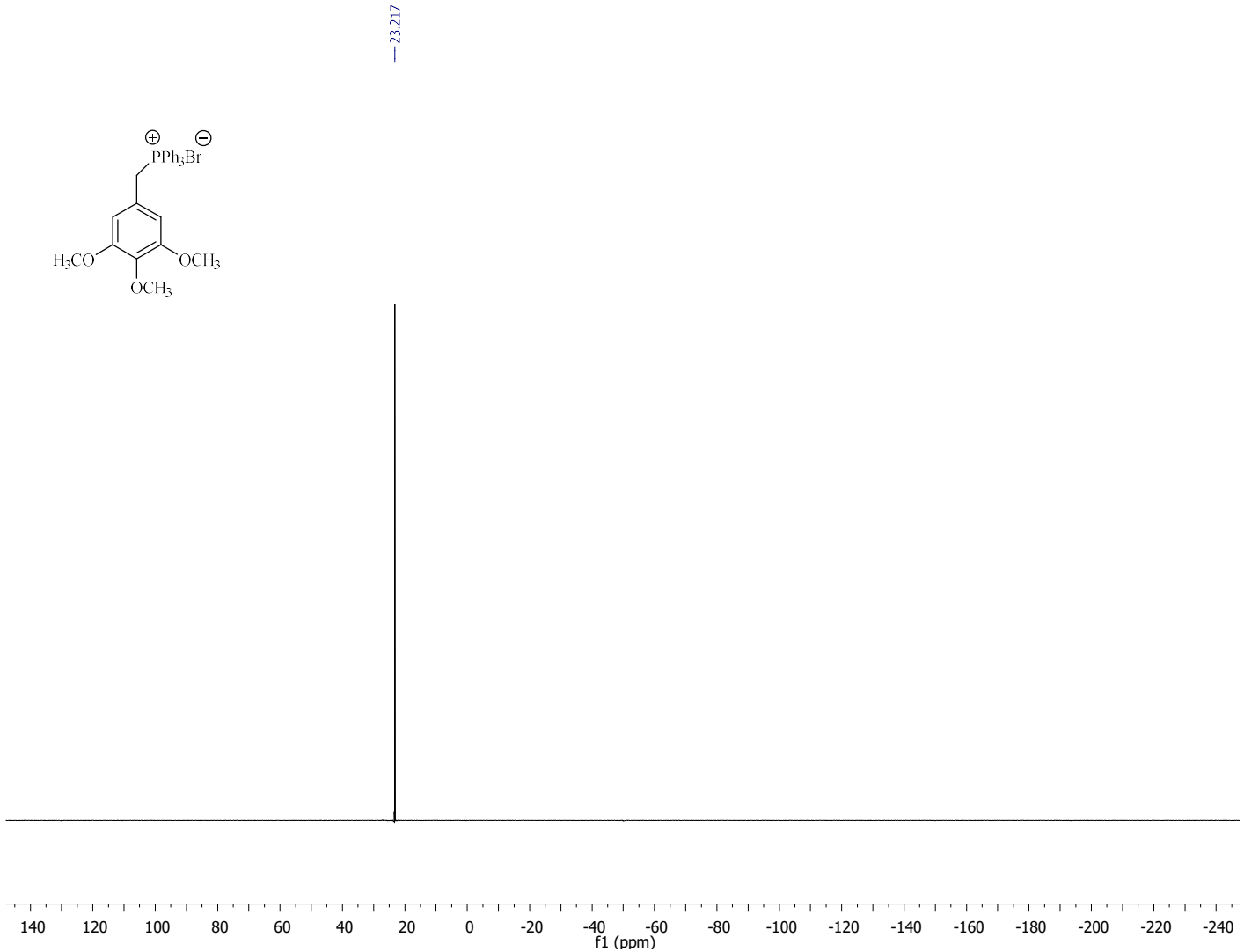


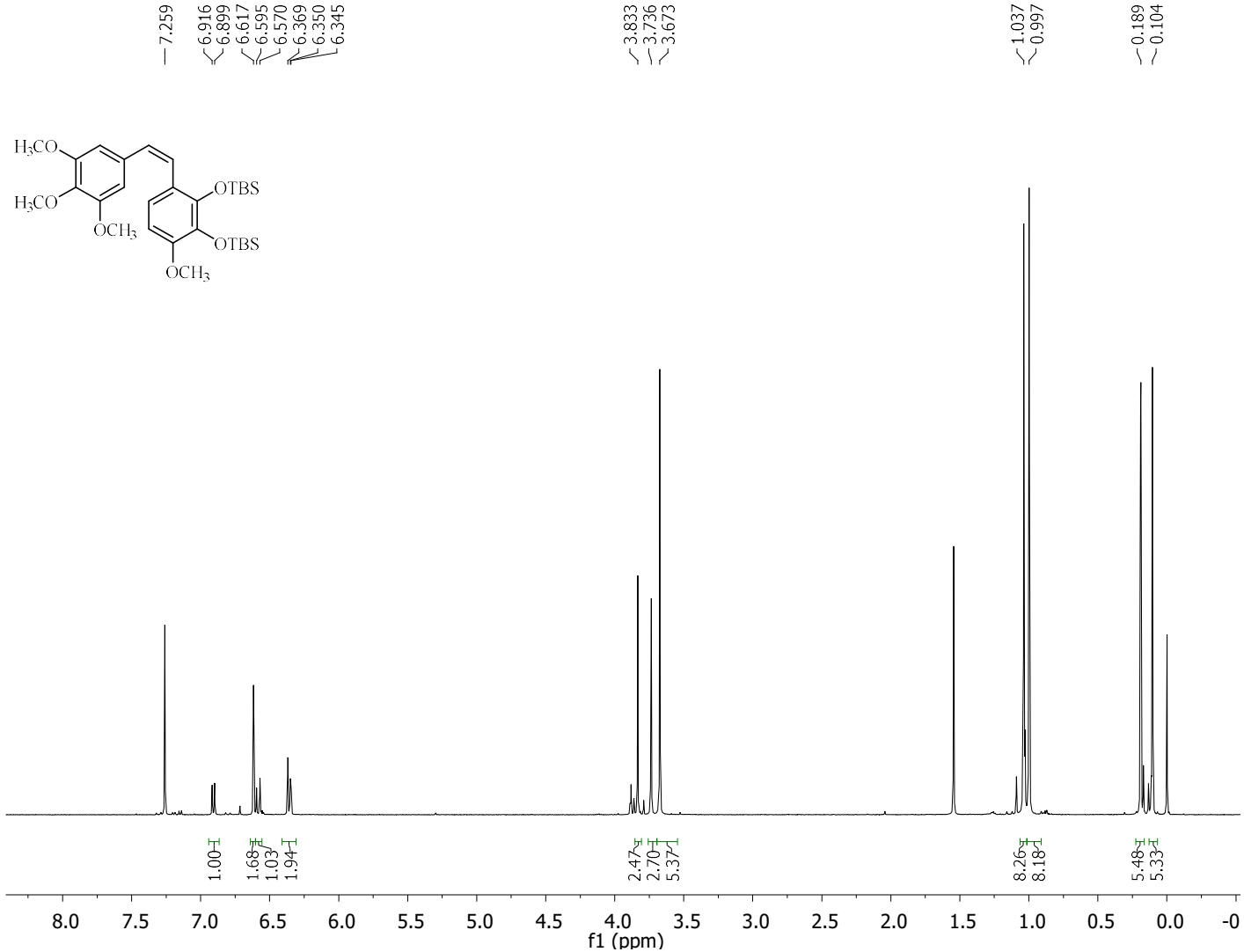
^{1}H NMR (600 MHz, $CDCl_3$) Compound **4**



^{31}P NMR (240 MHz, $CDCl_3$) Compound **4**



^{1}H NMR (500 MHz, $CDCl_3$) for Compound **5**



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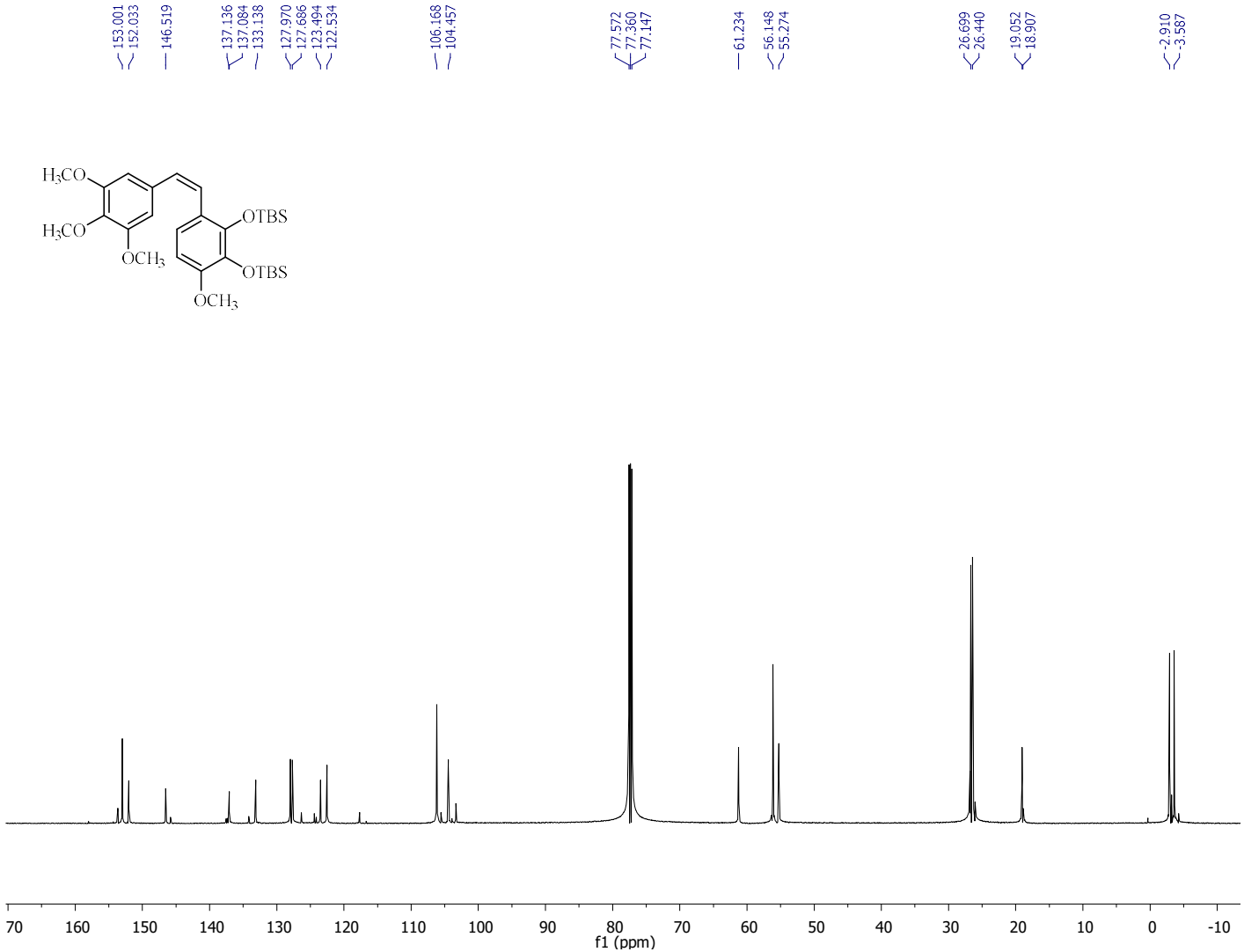
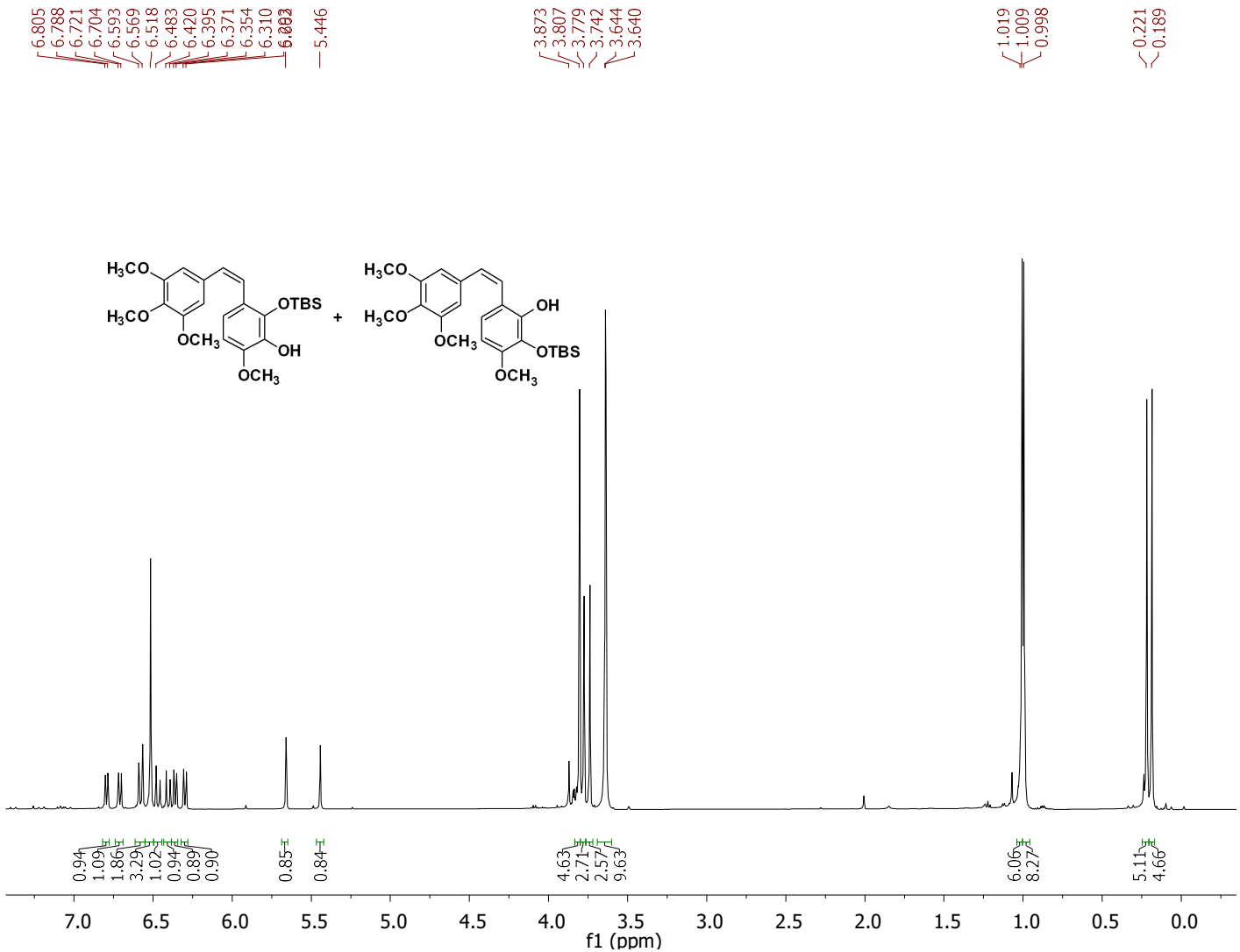
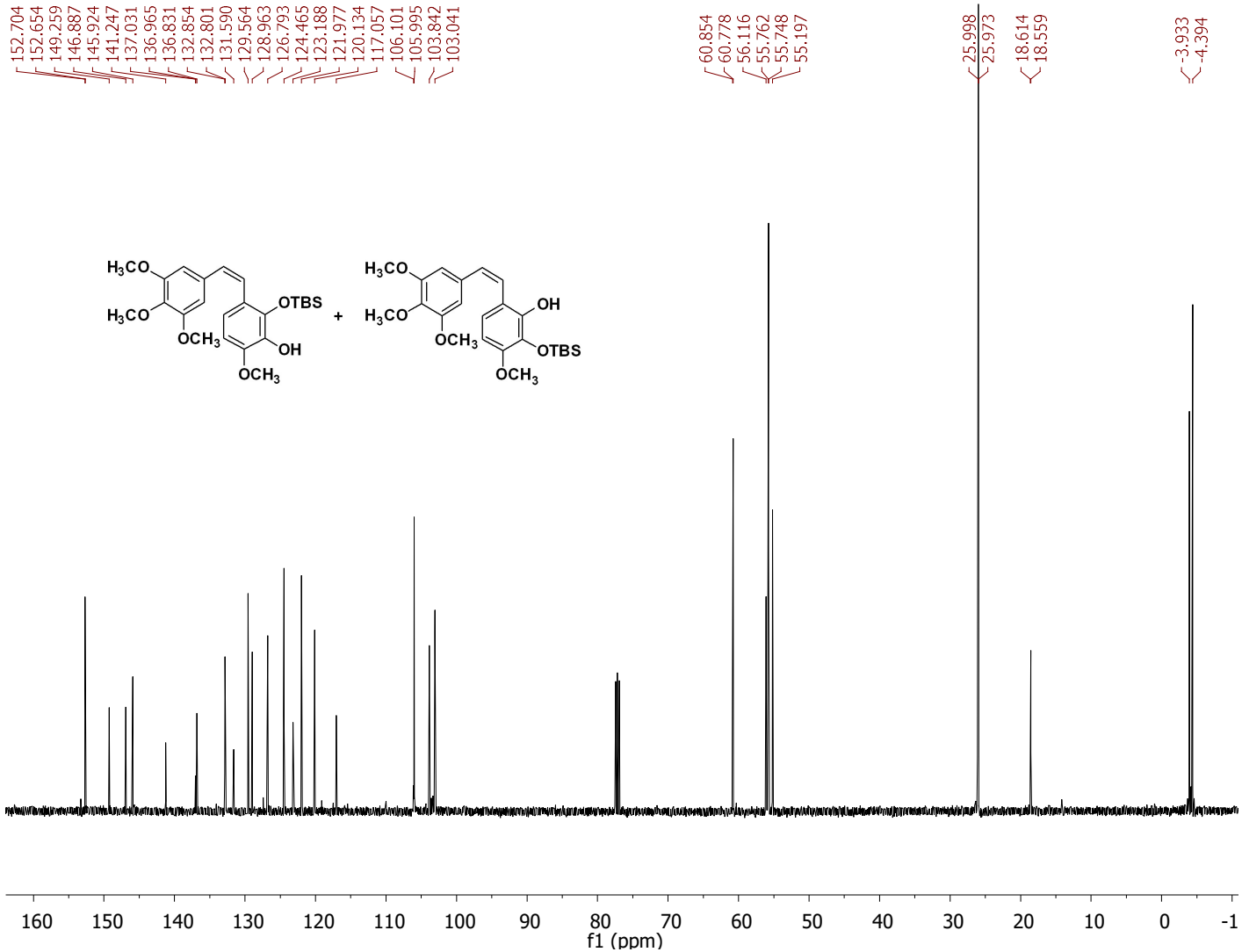


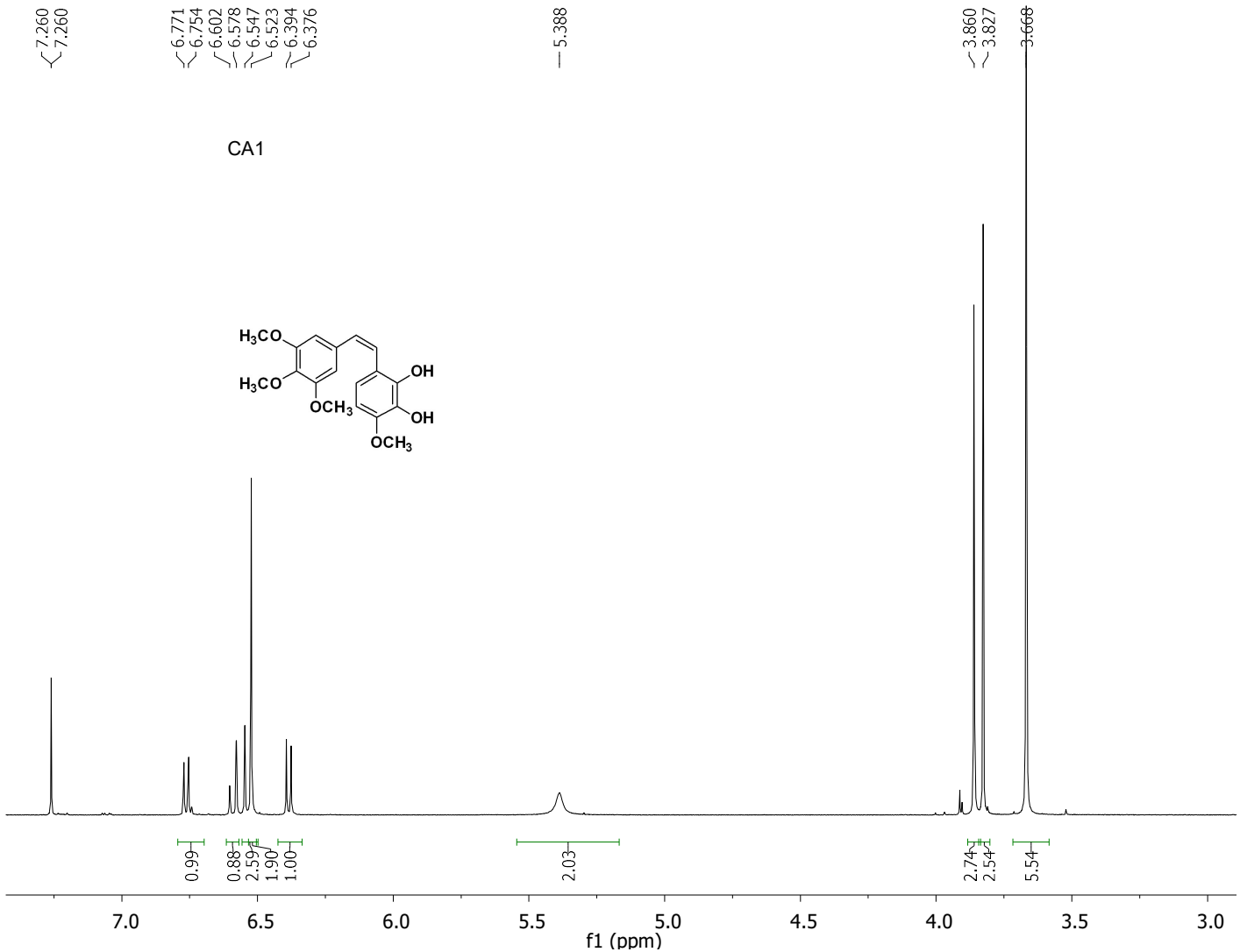
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **6** and **7**



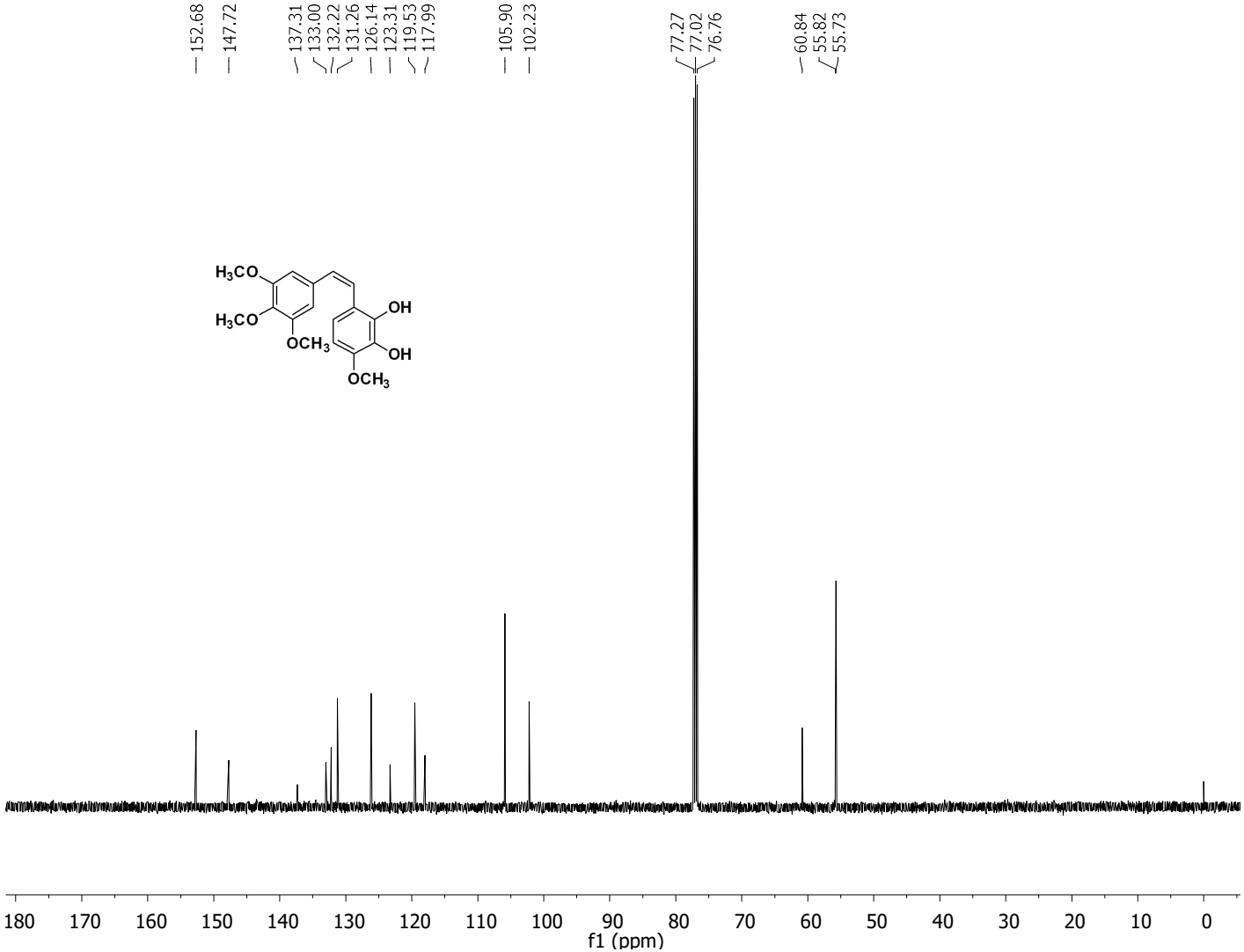
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **6** and **7**



^{1}H NMR (500 MHz, $CDCl_3$) for Compound **8**



^{13}C NMR (125 MHz, $CDCl_3$) for Compound **8**



HPLC Traces of Compound **8**

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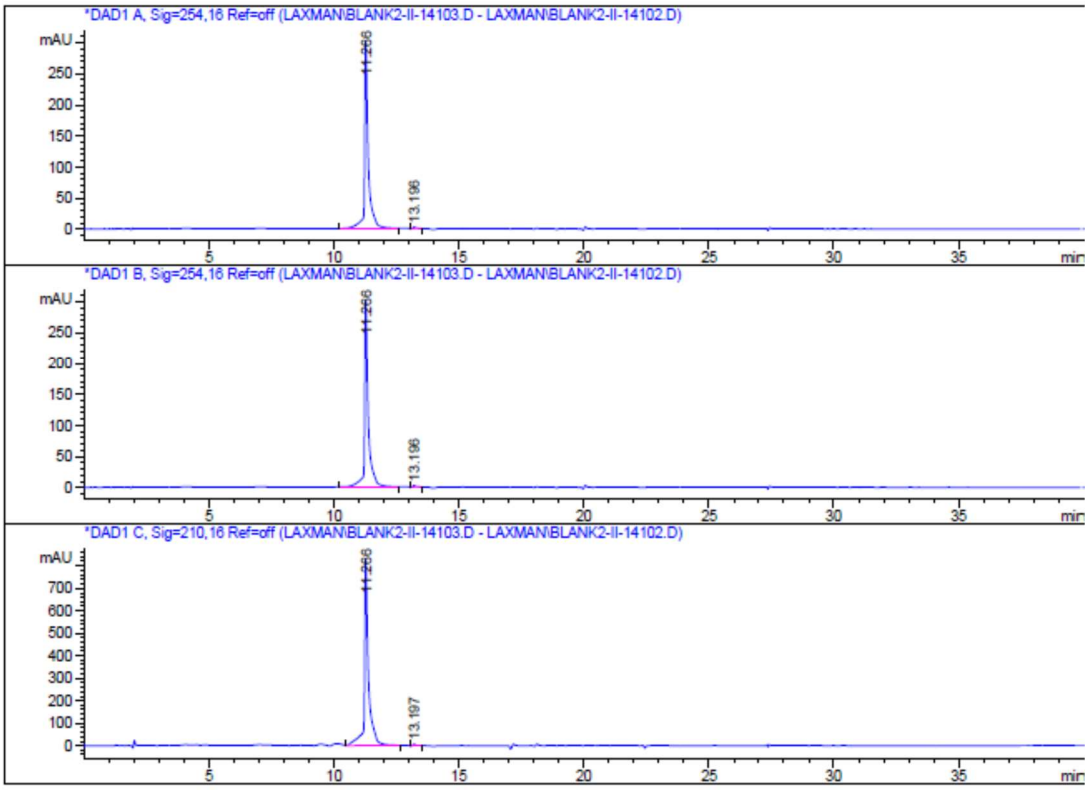


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Instrument 1 10/31/2012 2:15:33 PM Laxman                    Page   1 of 5
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HPLC Traces of Compound **8**

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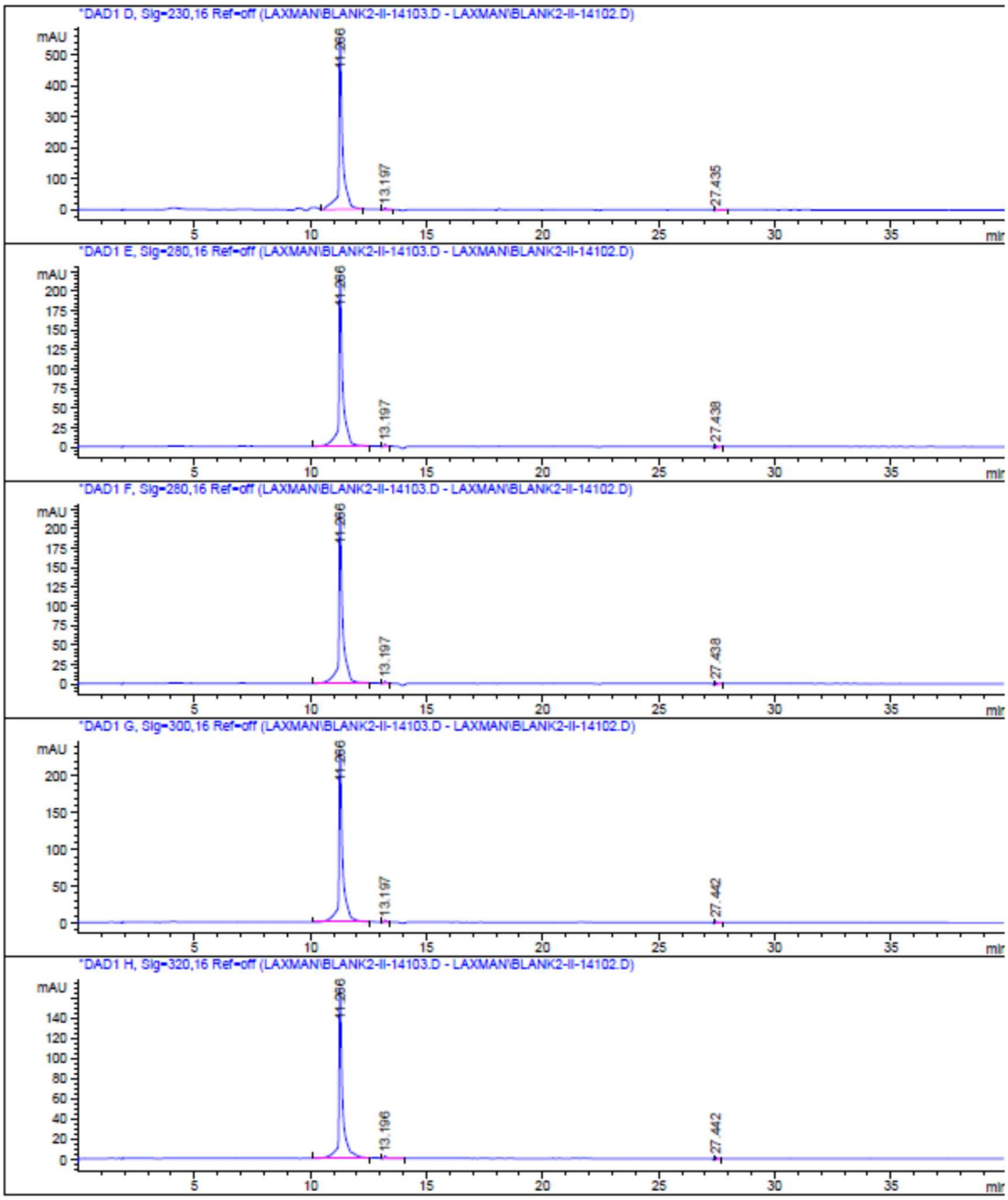

Instrument 1 10/31/2012 2:15:33 PM Laxman Page 2 of 5

HPLC Traces of Compound **8**

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Sample Name: LD-II-141-1blank2

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

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   Dilution              :      1.0000
   Use Multiplier & Dilution Factor with ISTDs


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      2  13.196 BB    0.1167   23.85802    2.93930   0.7785

   Totals :                  3064.69640  305.53552



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      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
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   Totals :                  9469.33689  842.84096



Instrument 1 10/31/2012 2:15:33 PM Laxman                          Page   3 of 5
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HPLC Traces of Compound **8**

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
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Signal 5: DAD1 E, Sig=280,16 Ref=off
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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
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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
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Instrument 1 10/31/2012 2:15:33 PM Laxman Page 4 of 5

HPLC Traces of Compound **8**

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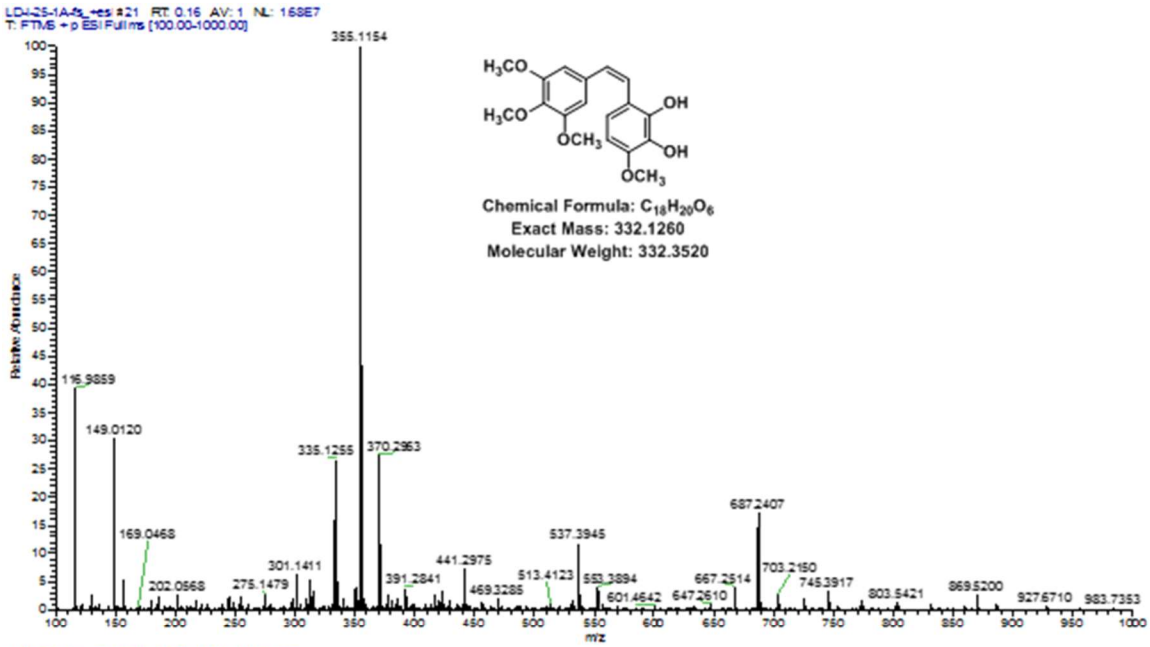
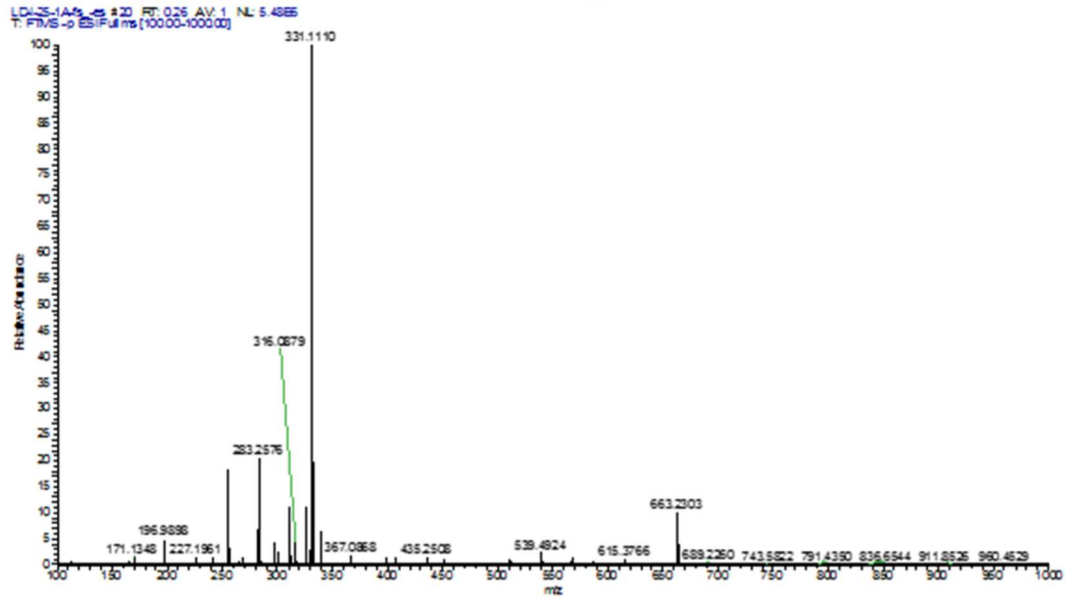
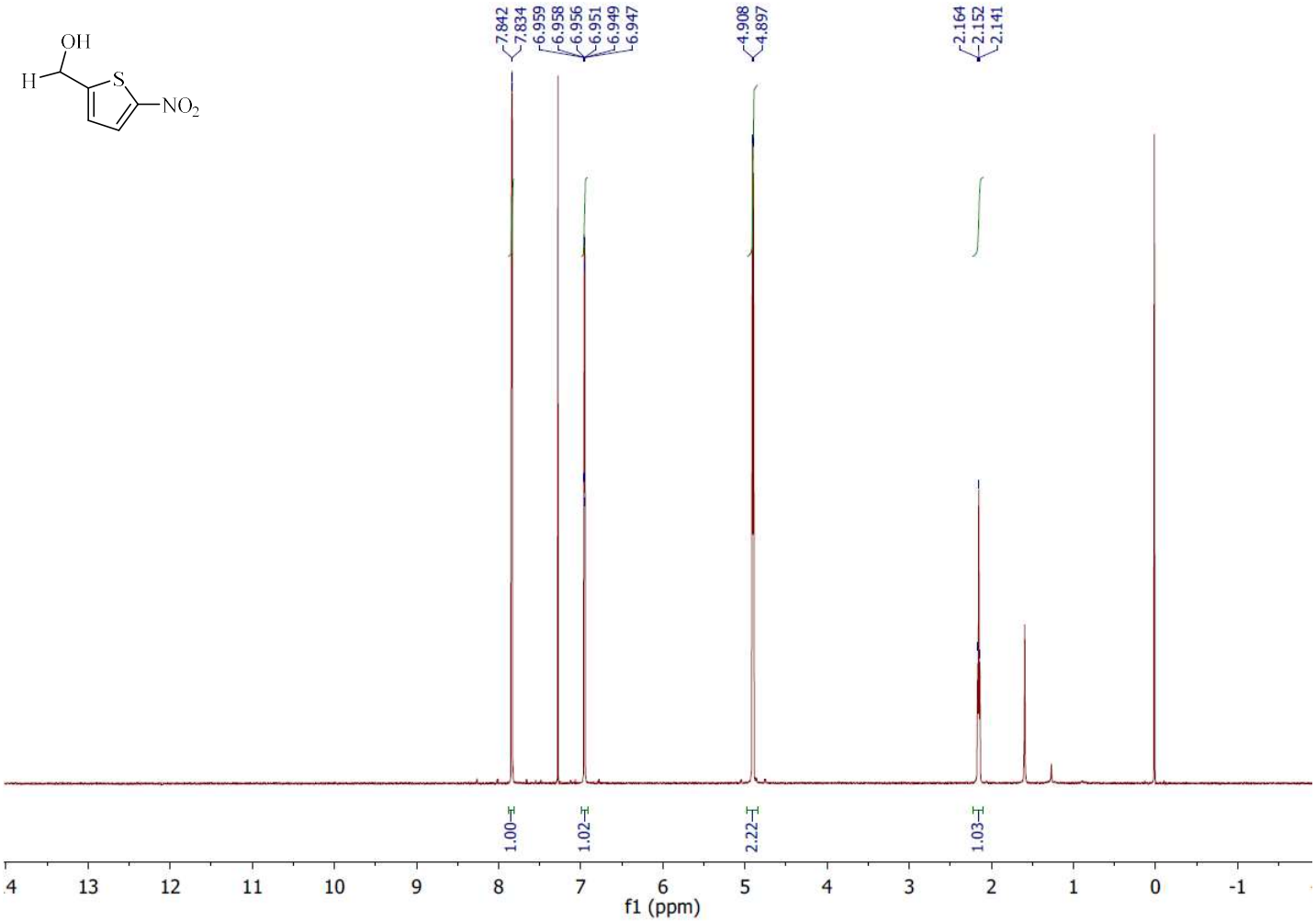




^{1}H NMR (600 MHz, $CDCl_3$) for Compound **10**



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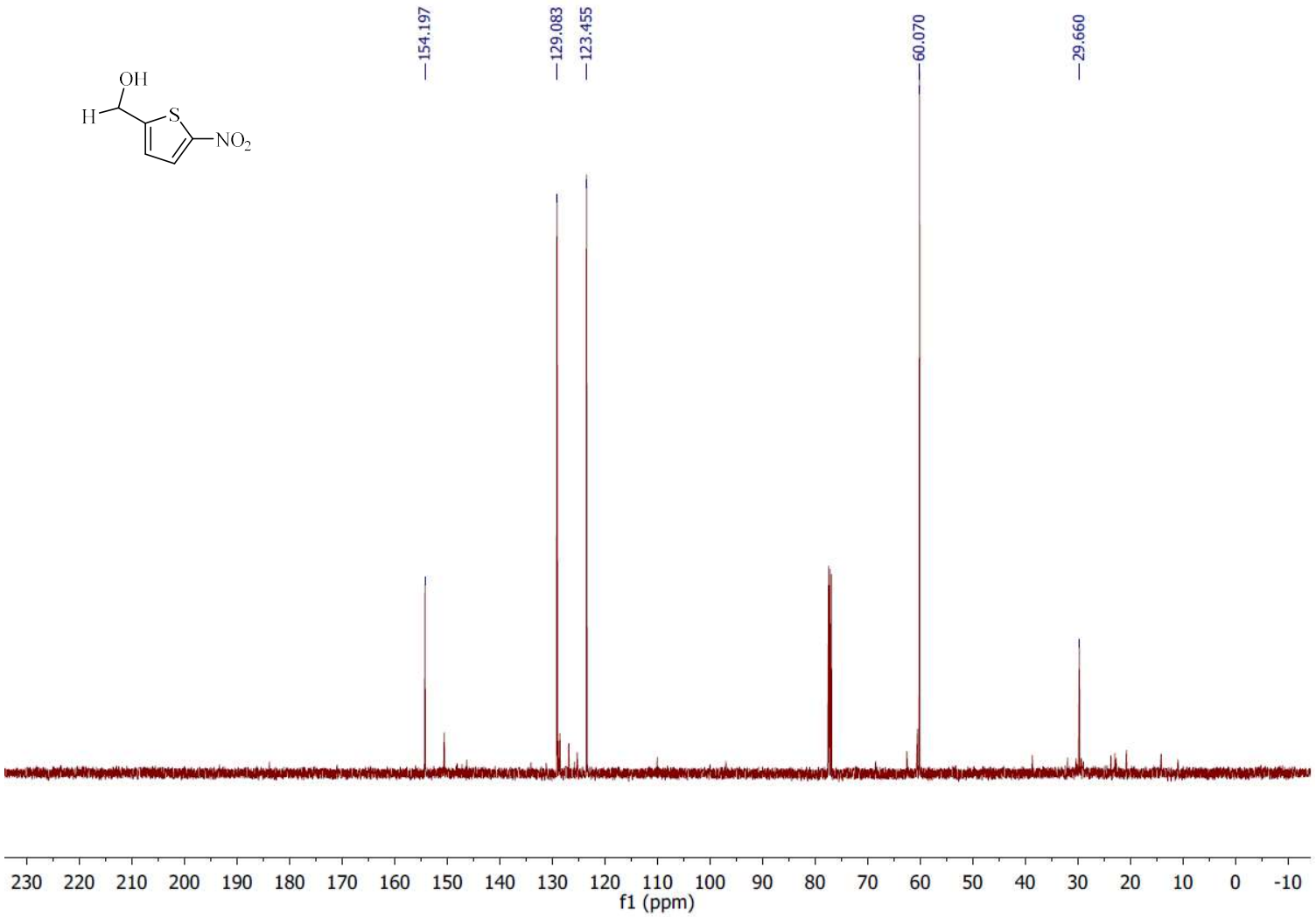

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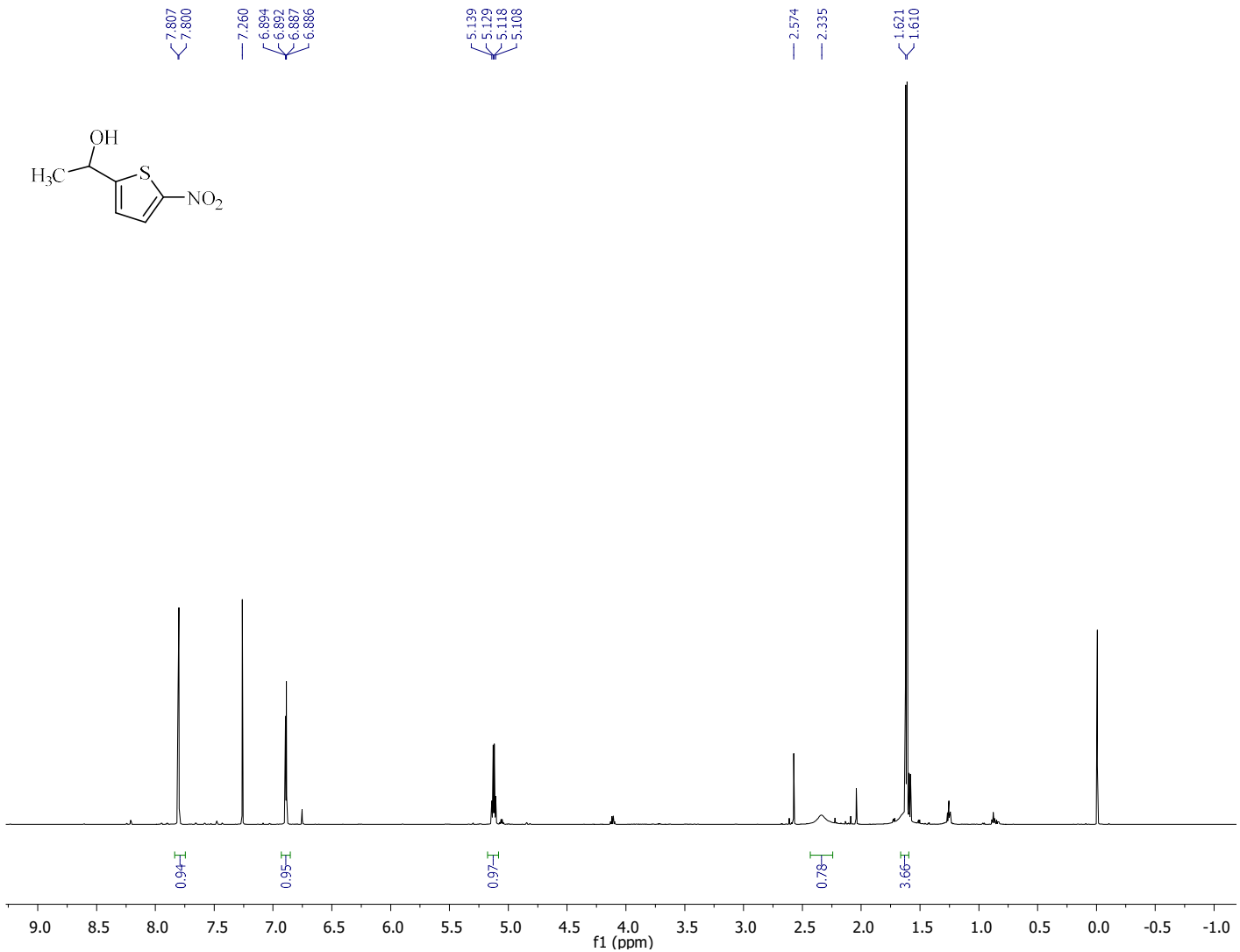

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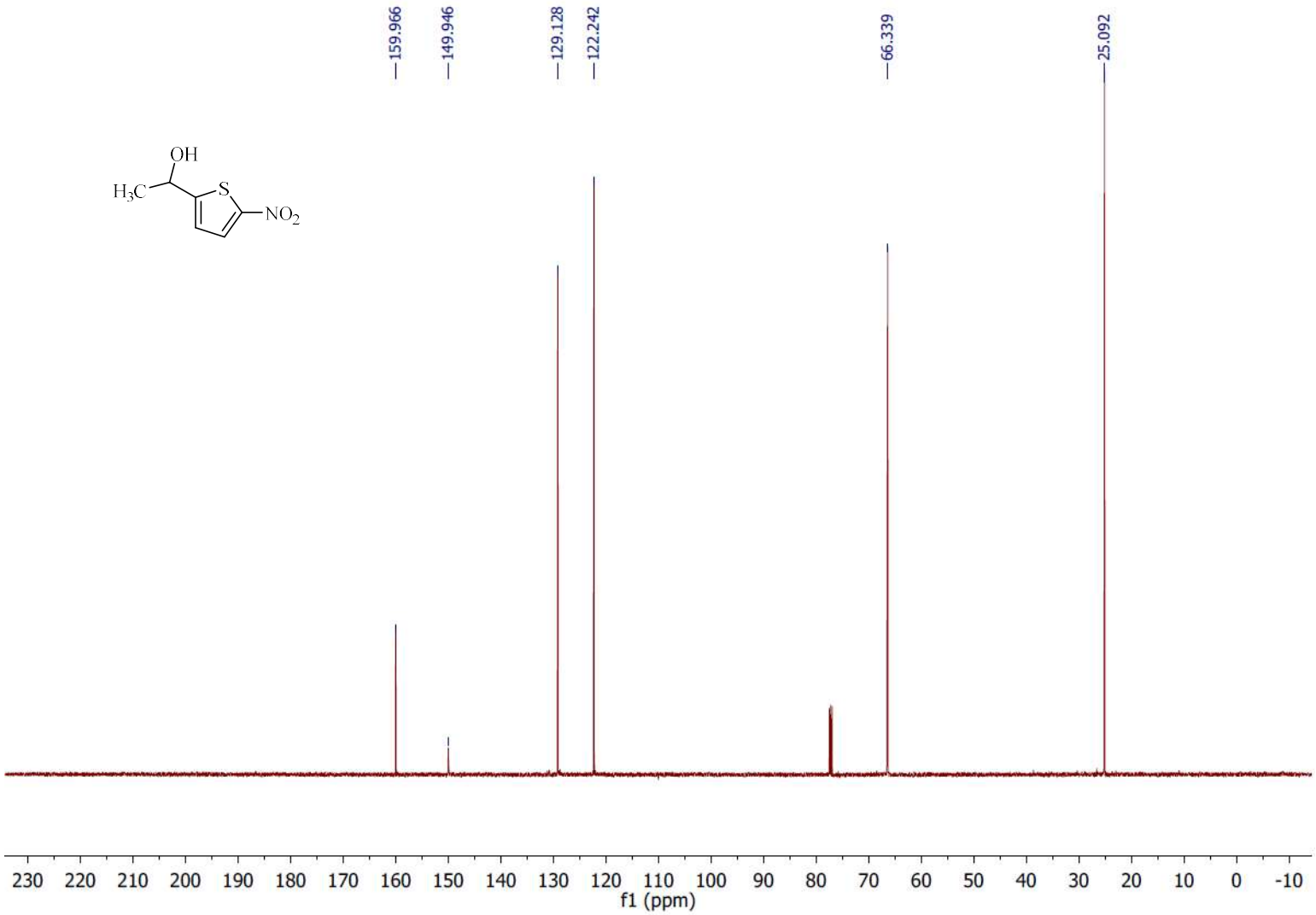

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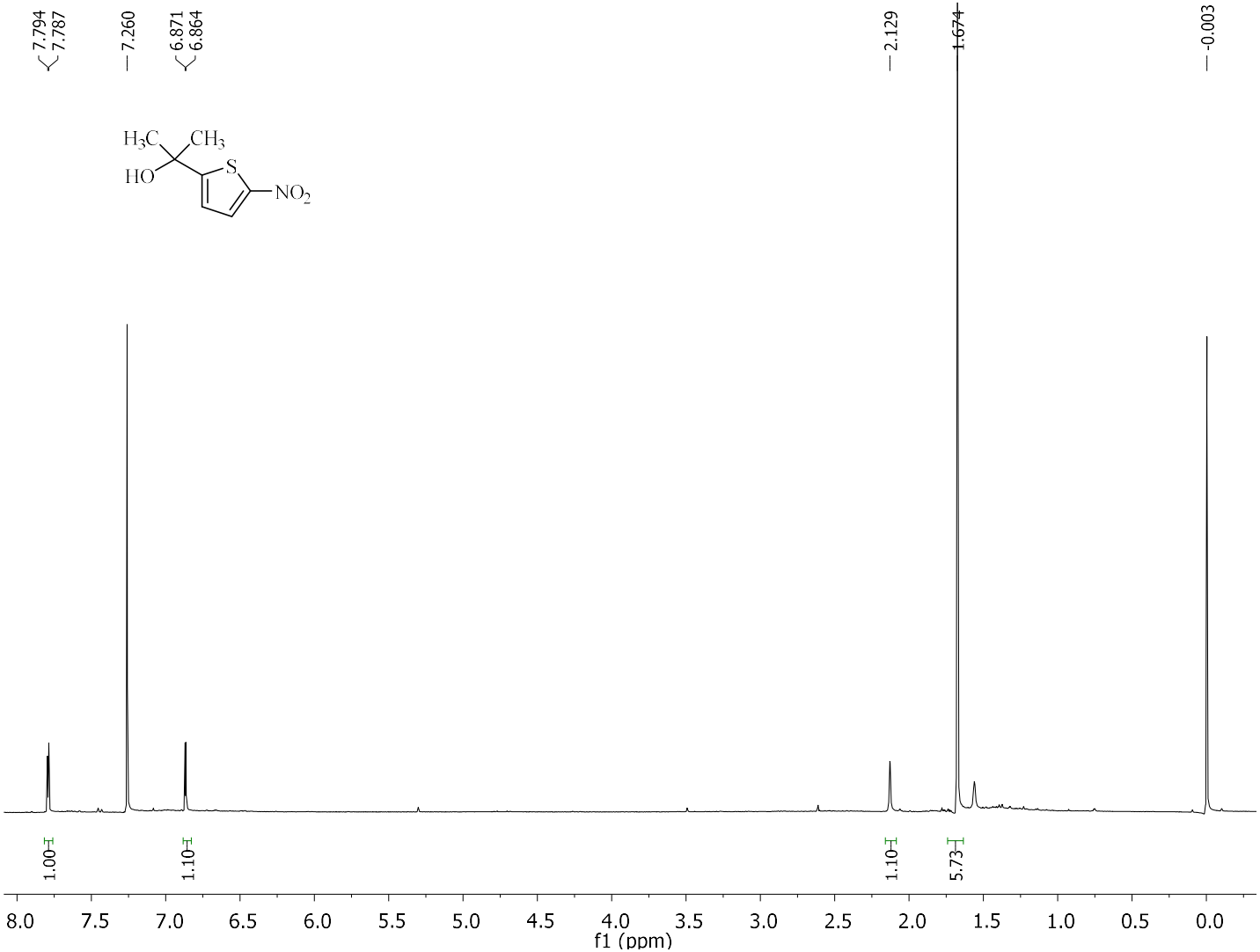

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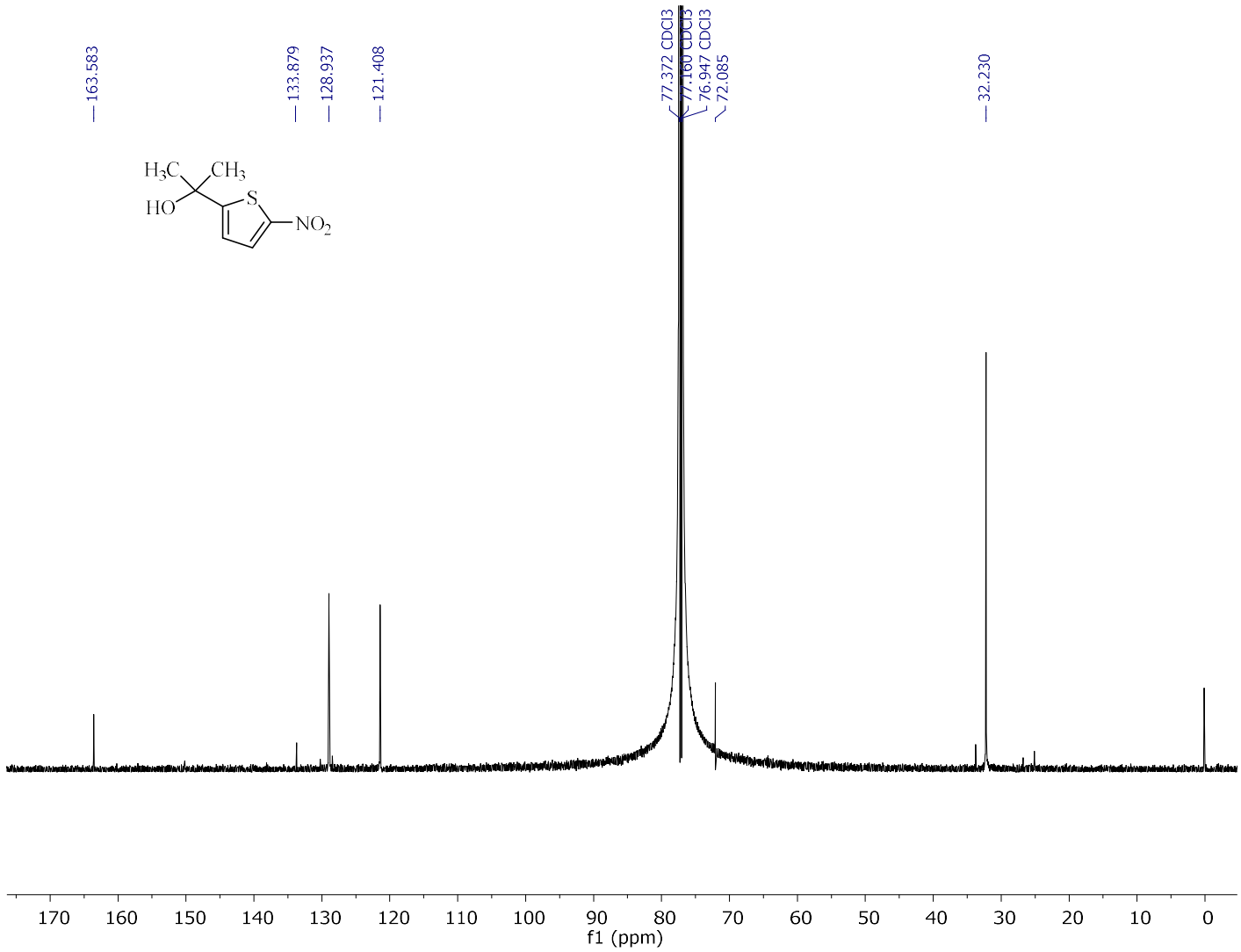

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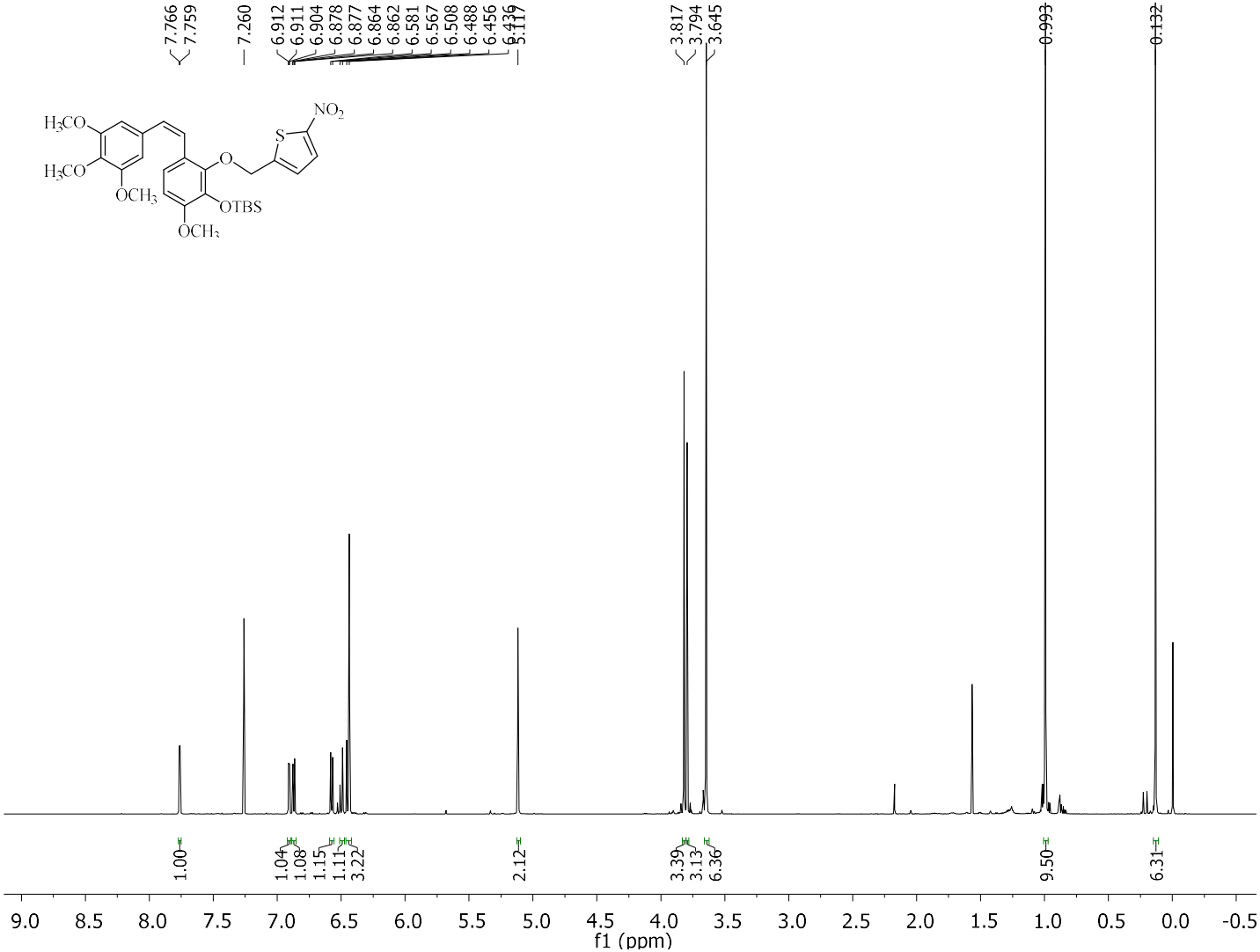

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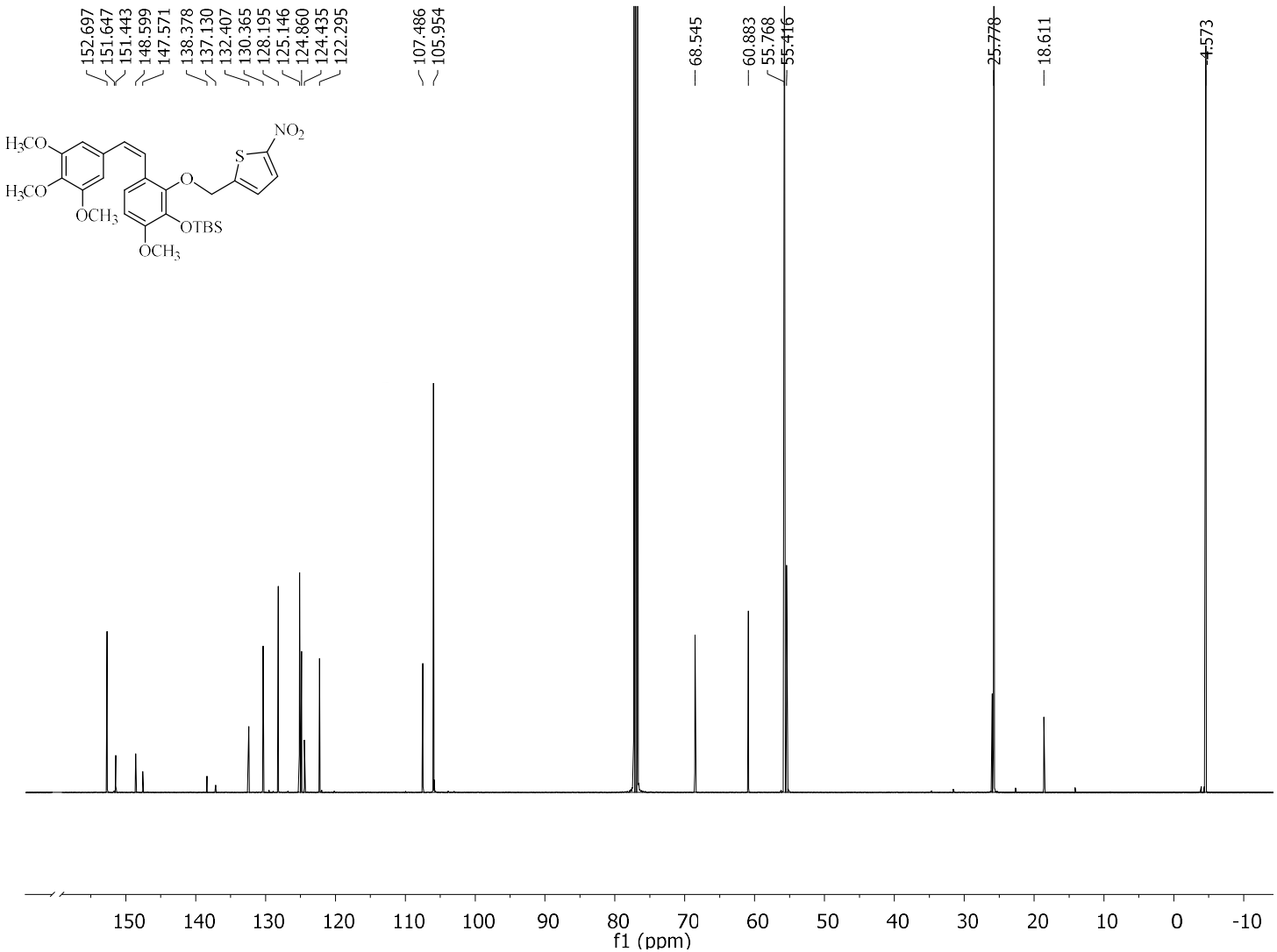

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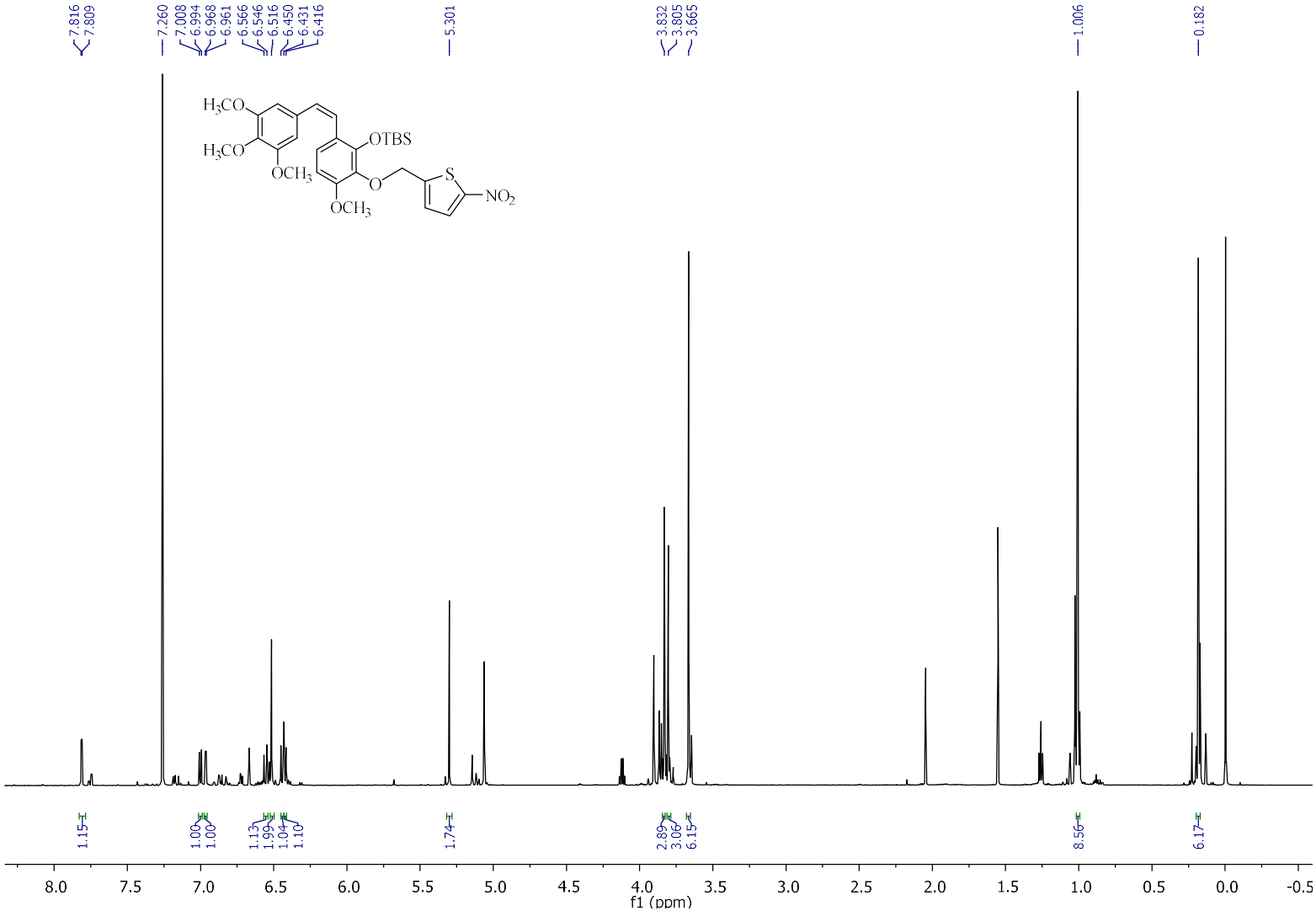

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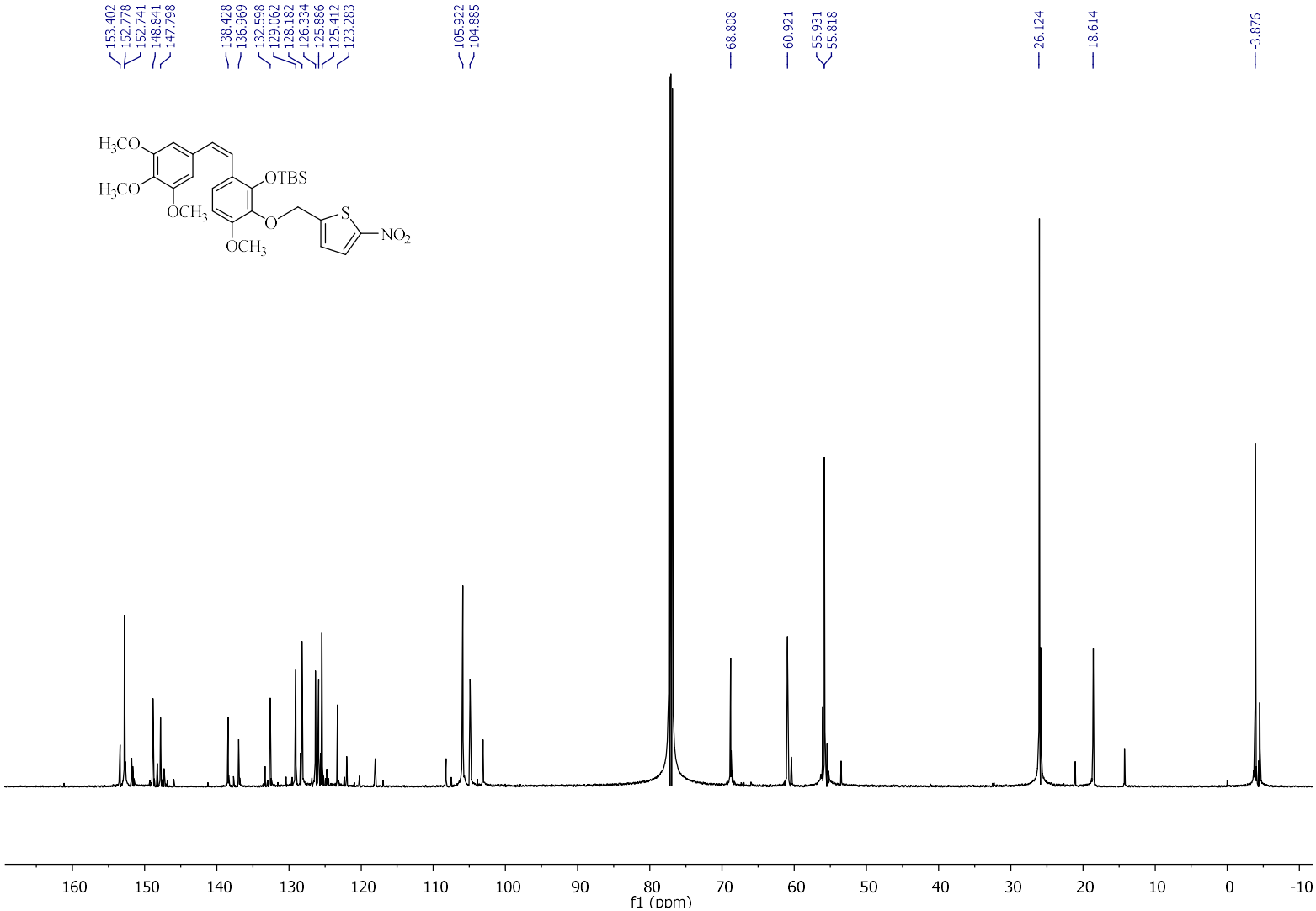

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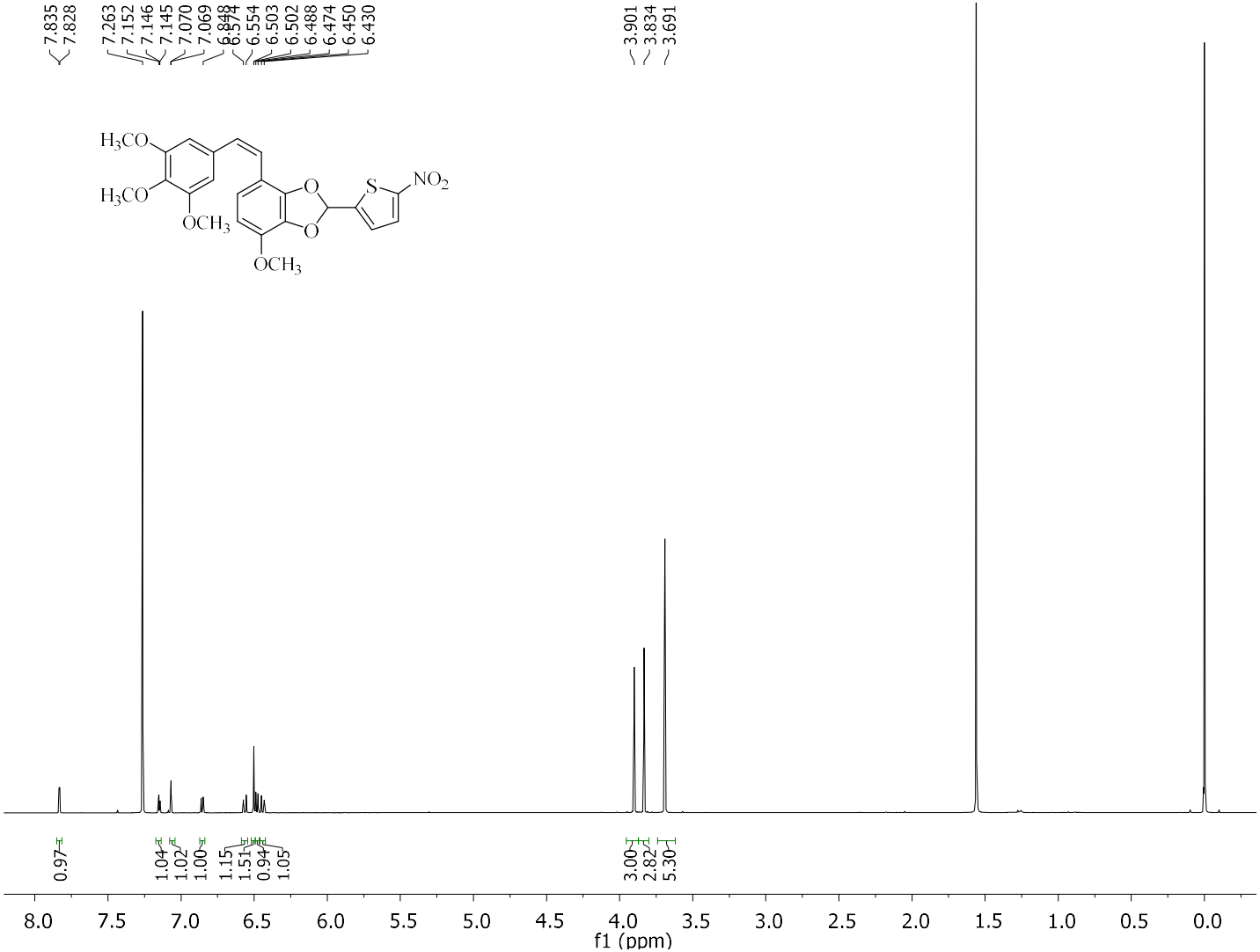

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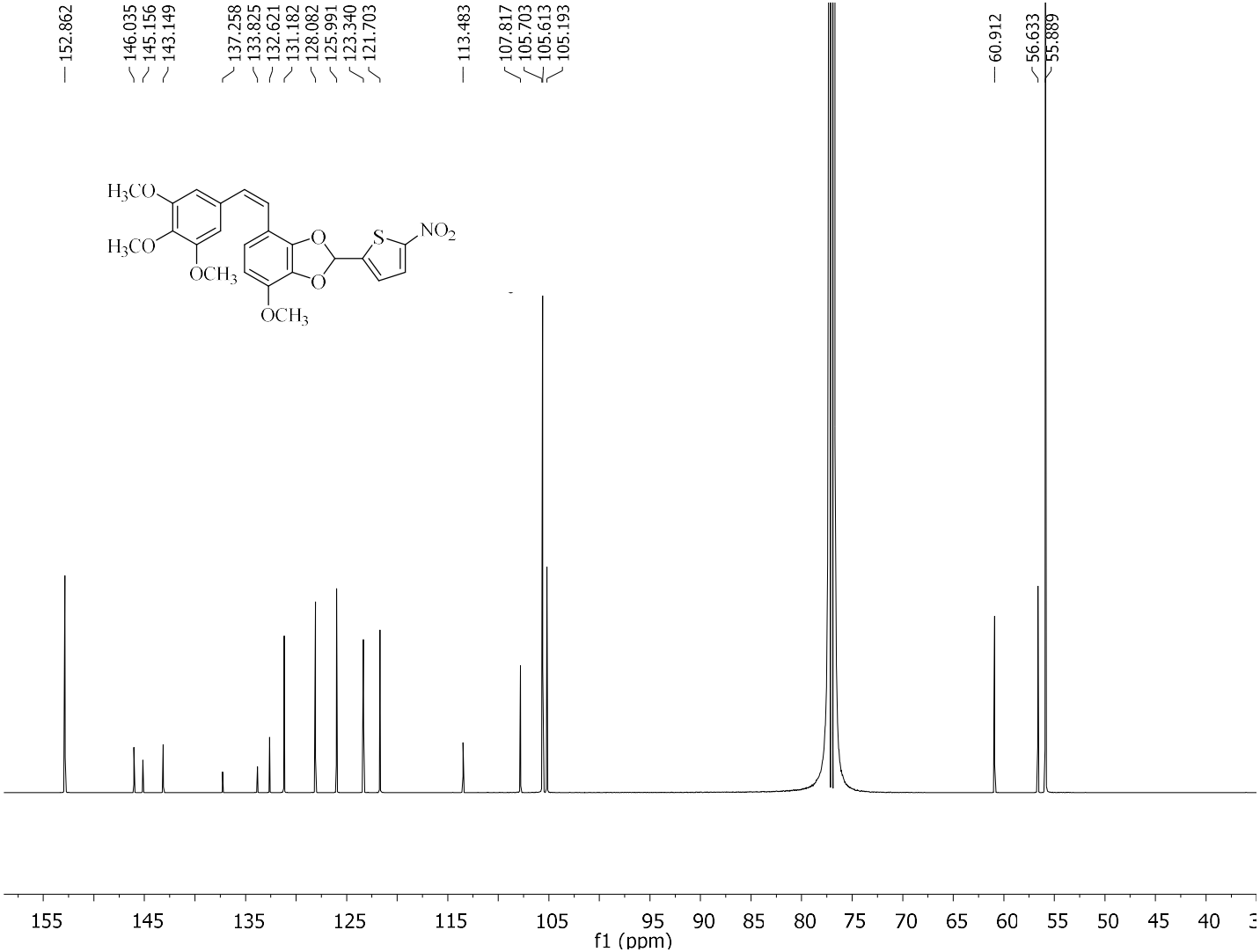
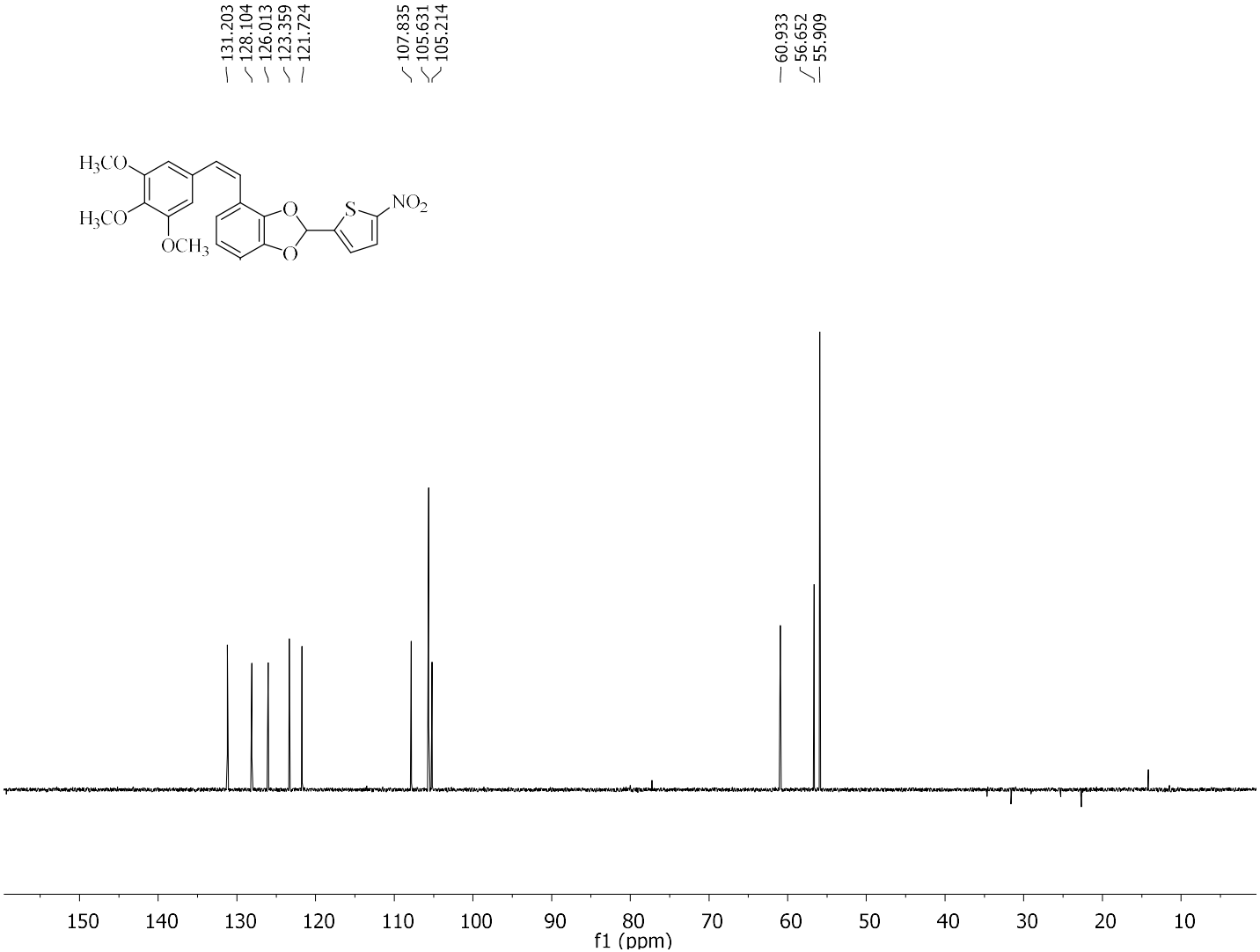


^{13}C DEPT NMR (125 MHz, $CDCl_3$) for Compound **18**



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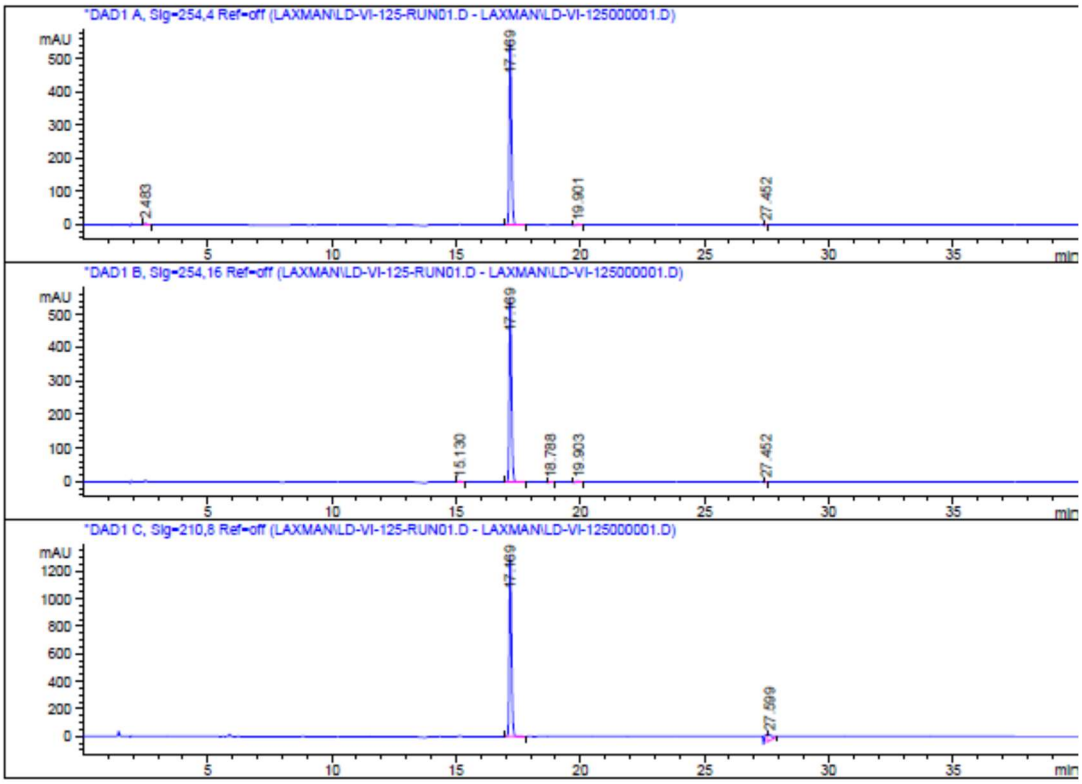

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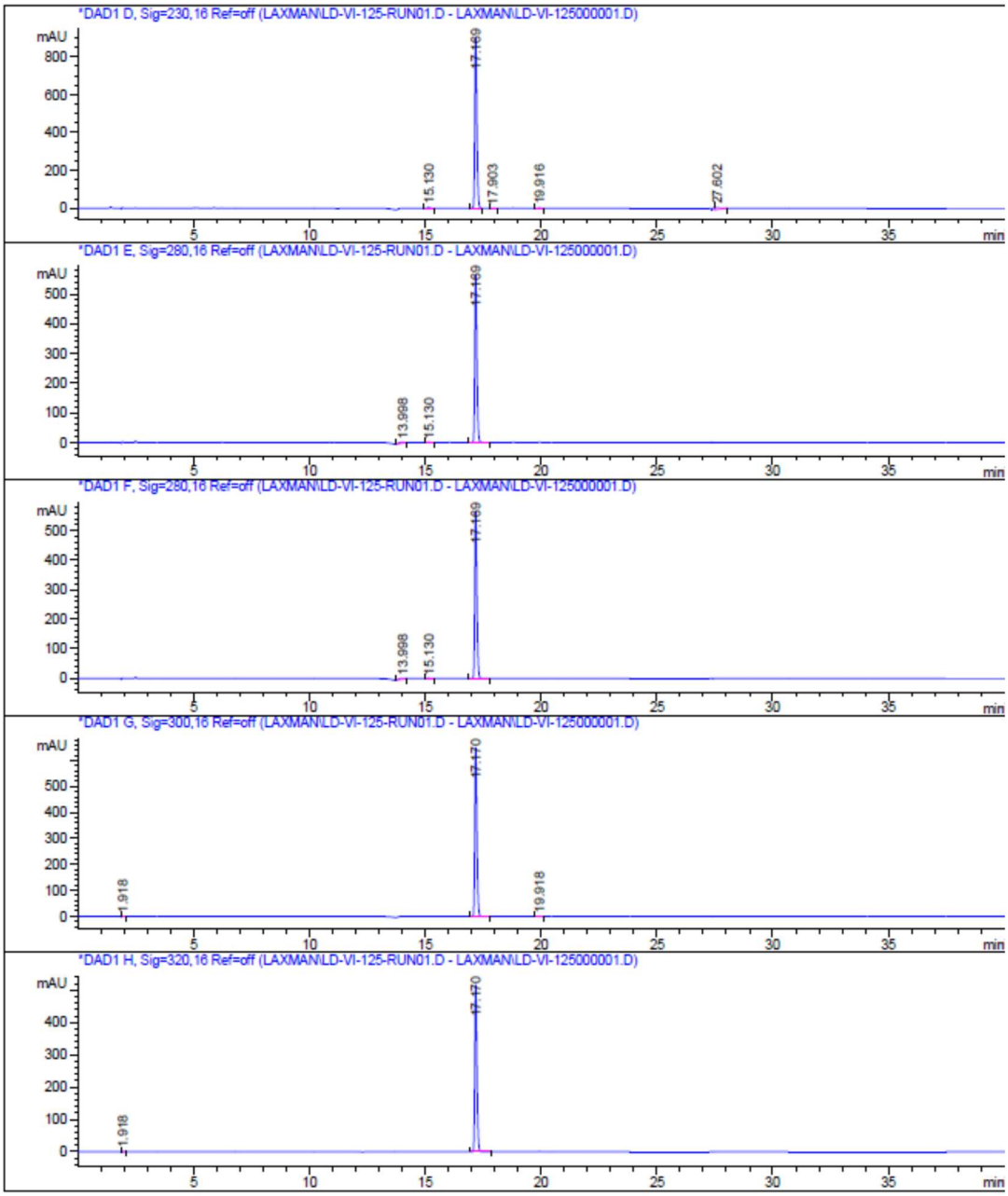

Instrument 1 12/9/2014 12:40:25 PM FDTC1 D Page 2 of 5

HPLC Traces for Compound **18**

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

HPLC Traces for Compound **18**

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

HPLC Traces for Compound **18**

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

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [mAU*s]     [mAU]       %
   ----|-------|----|-------|----------|----------|--------|
      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 Type  Width     Area      Height     Area
     #   [min]        [min]   [mAU*s]     [mAU]       %
   ----|-------|----|-------|----------|----------|--------|
      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 ***
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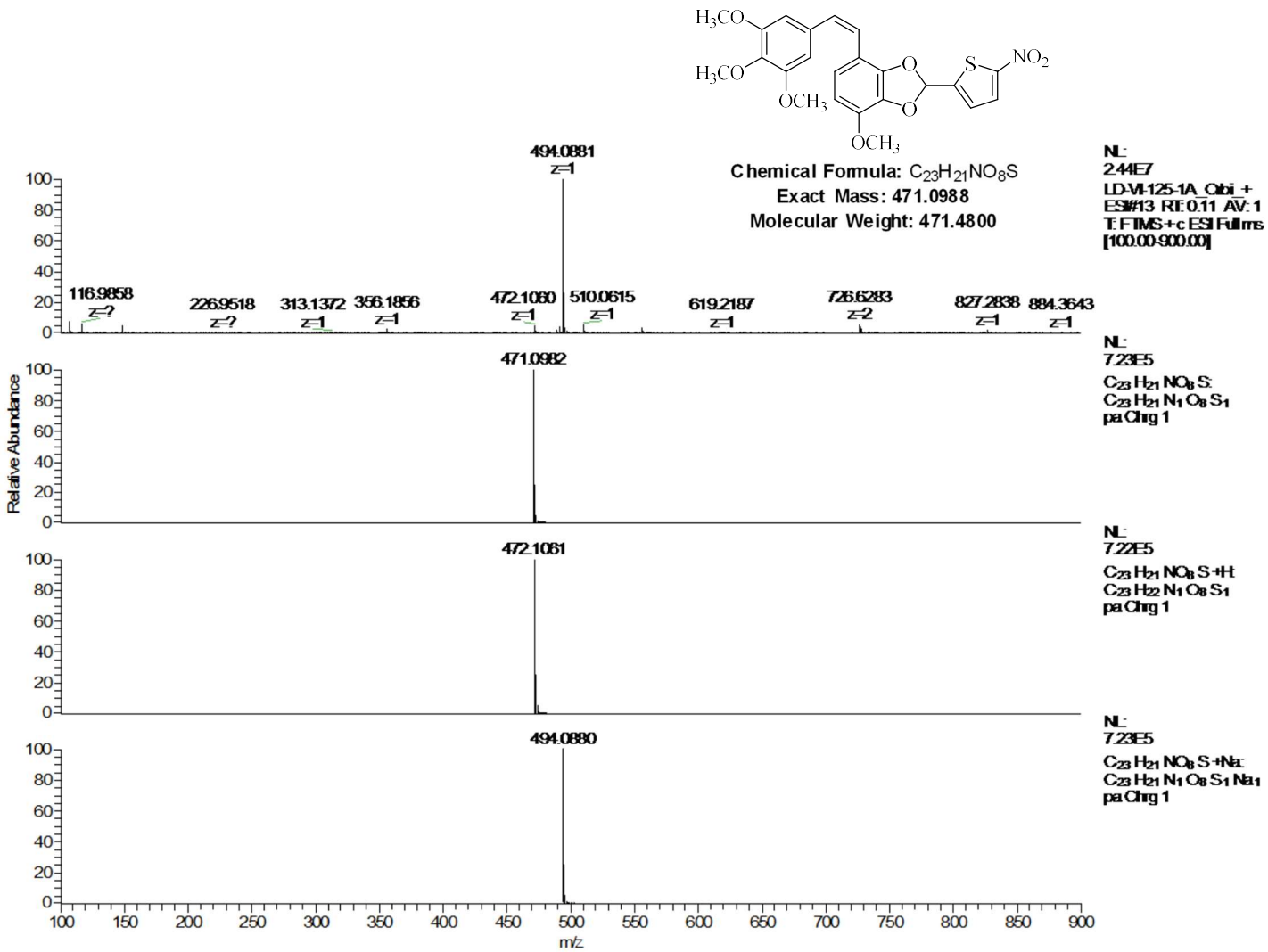
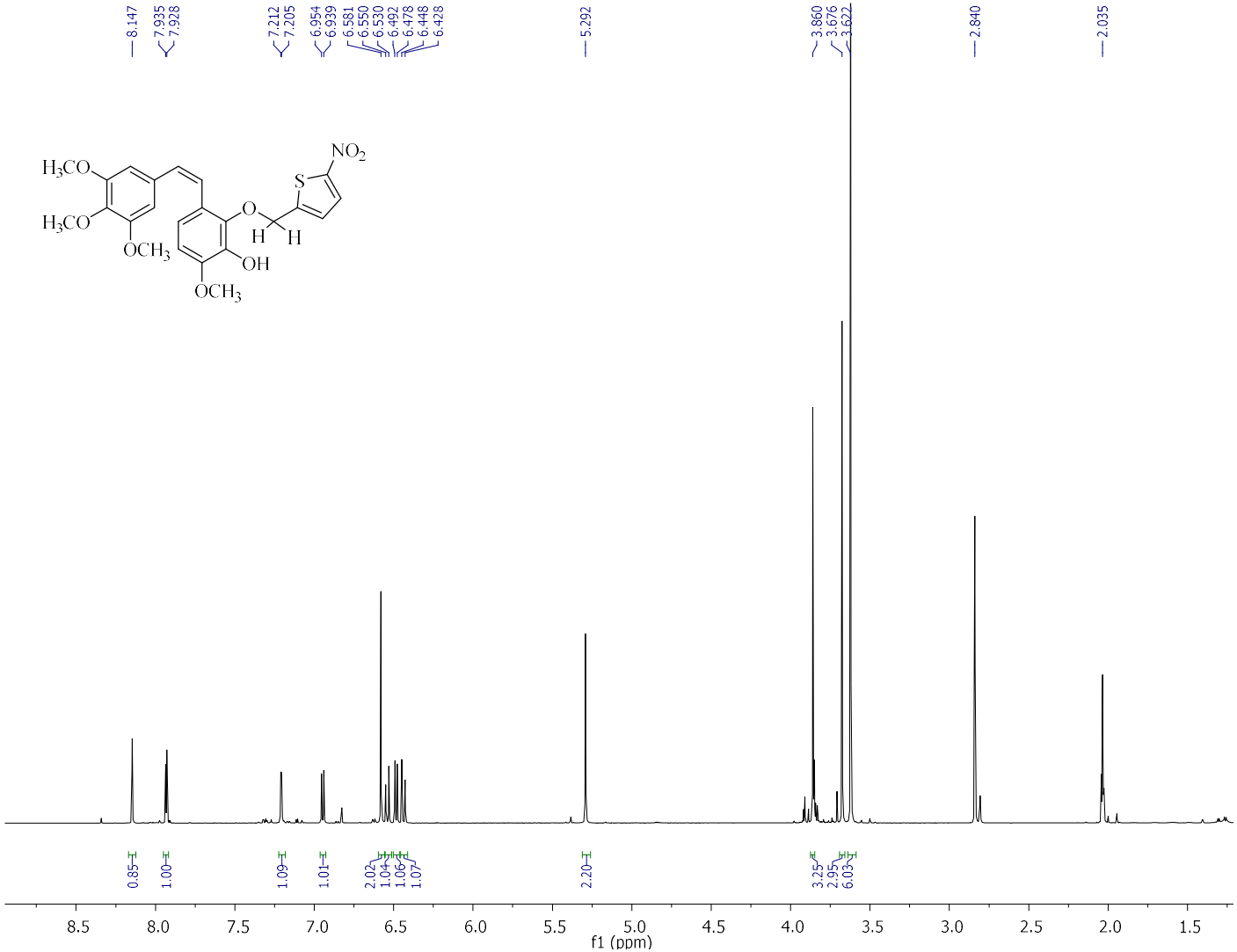
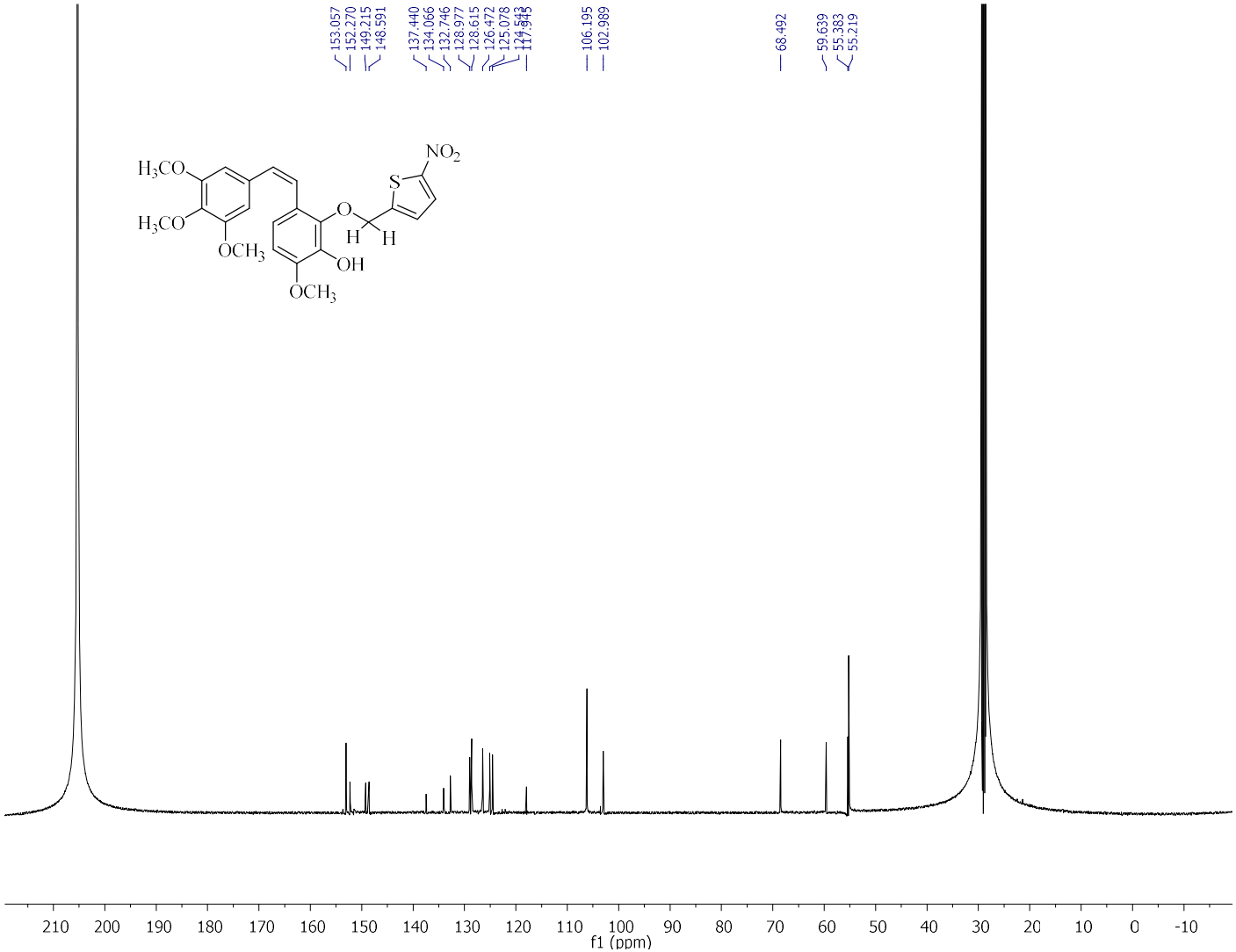
HRMS Traces for Compound **18**



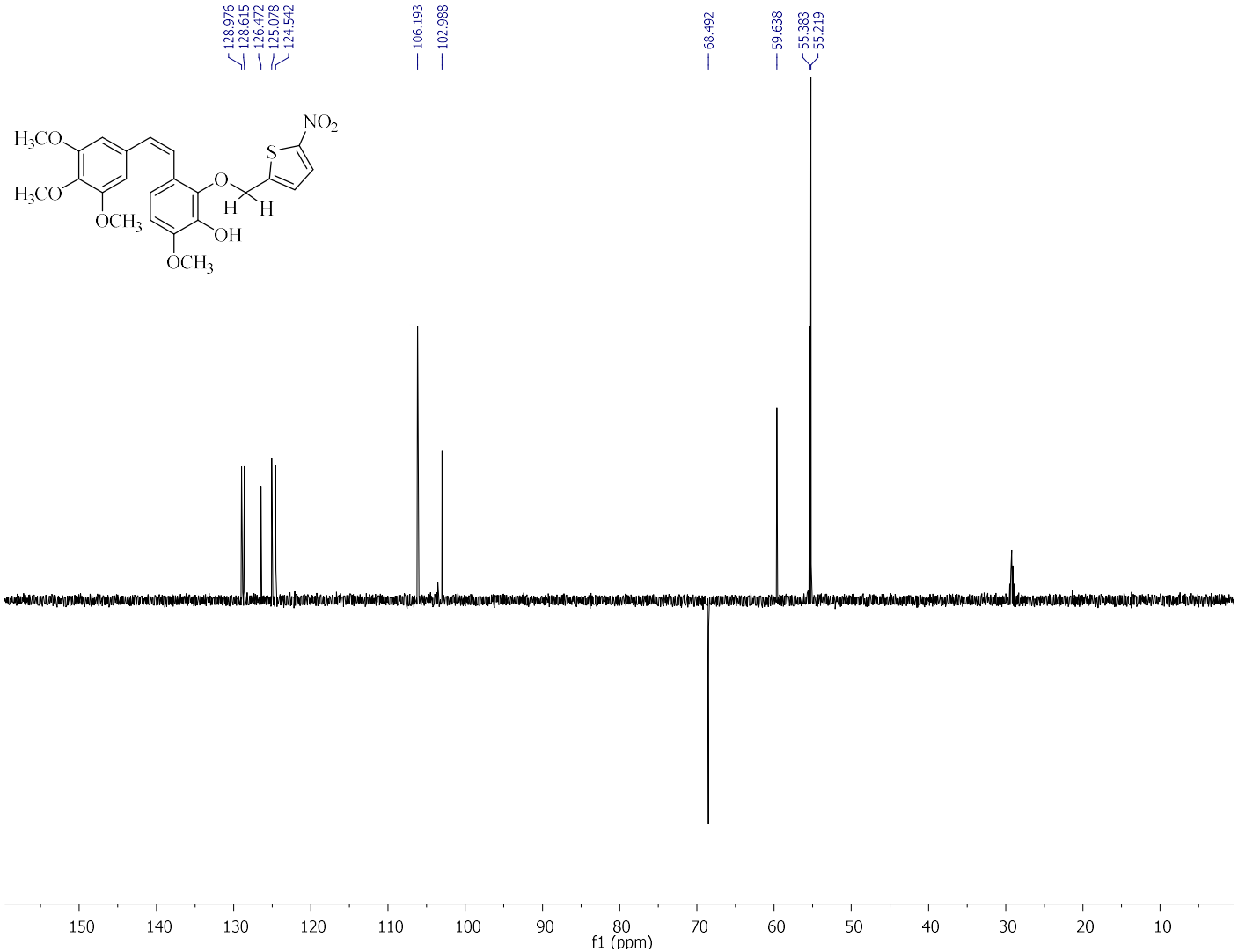
^{1}H NMR (600 MHz, Acetone) for Compound **19**



^{13}C NMR (151 MHz, Acetone) for Compound **19**



^{13}C DEPT NMR (125 MHz, Acetone) for Compound **19**



HPLC Traces for Compound **19**

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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
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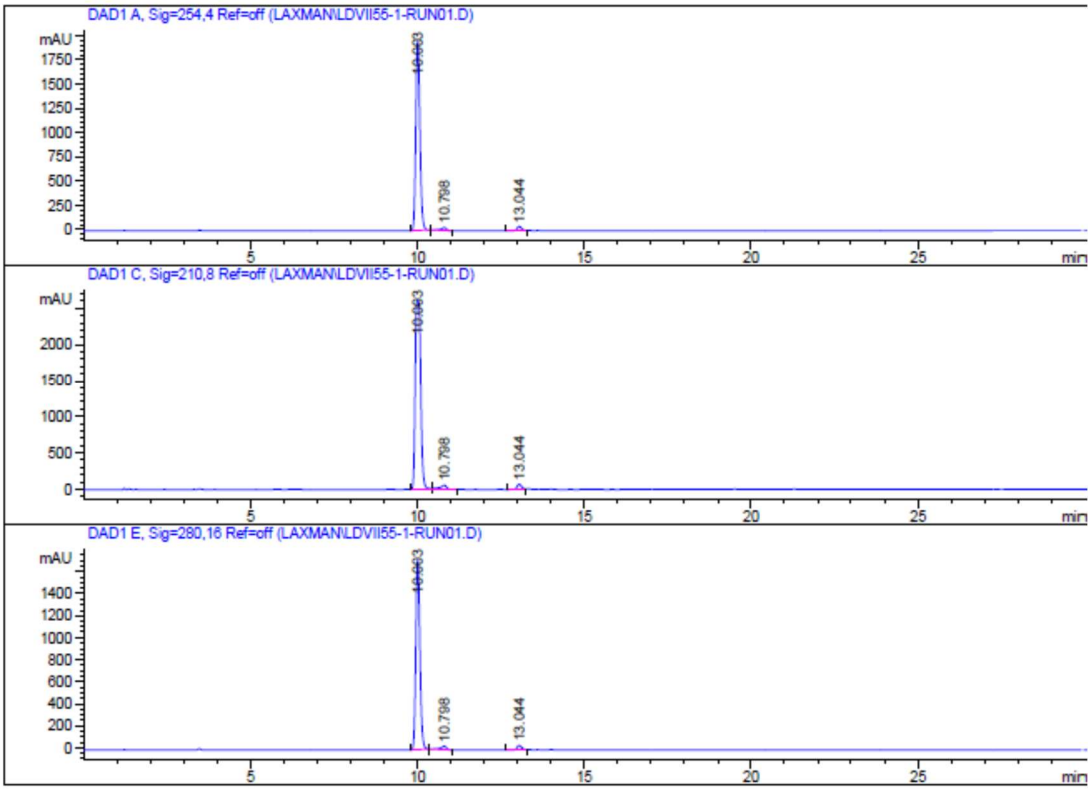


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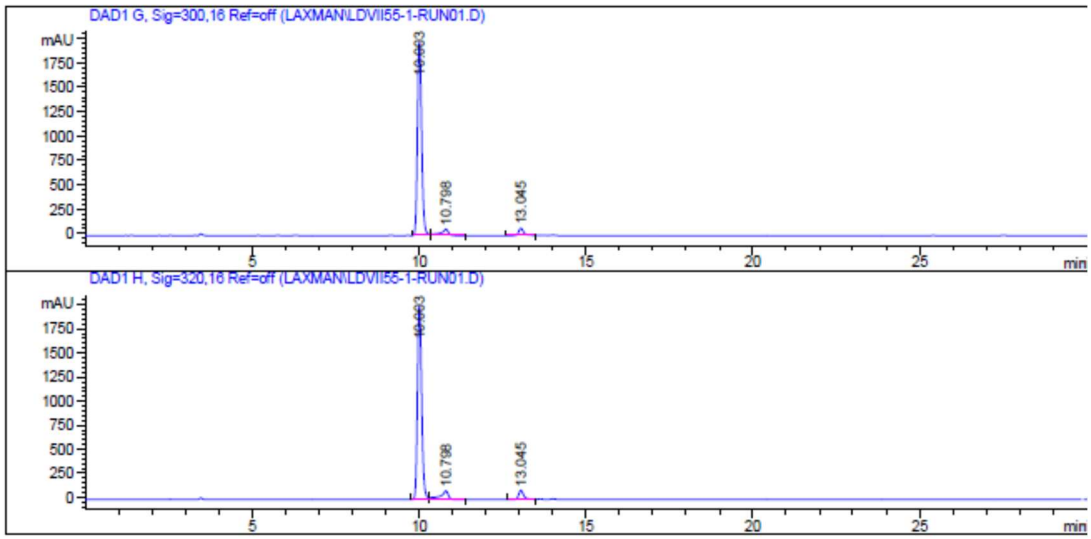
Instrument 1 4/9/2015 11:52:45 AM Graham                    Page   1 of 3
```

HPLC Traces for Compound **19**

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



==========
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

HPLC Traces for Compound **19**

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

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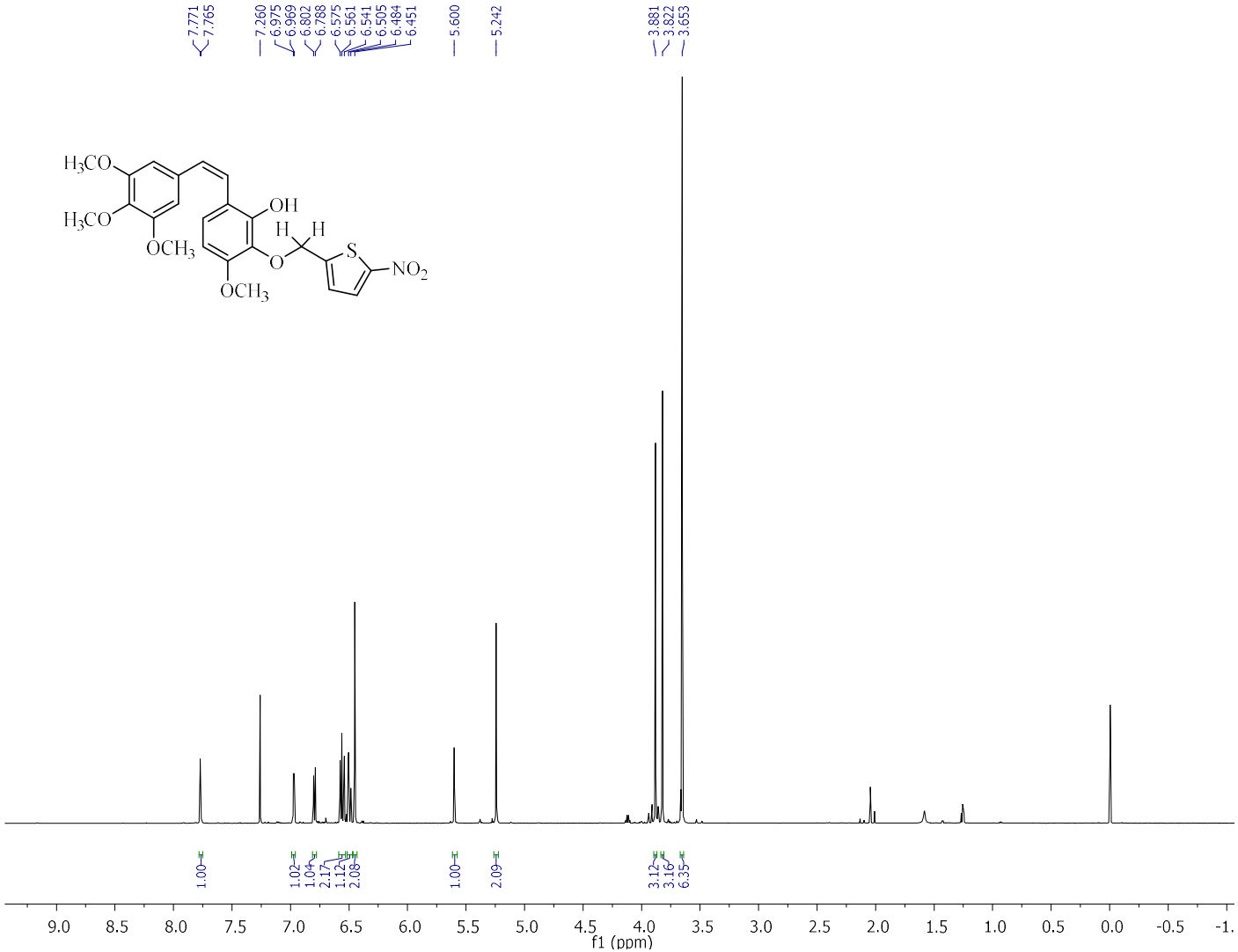
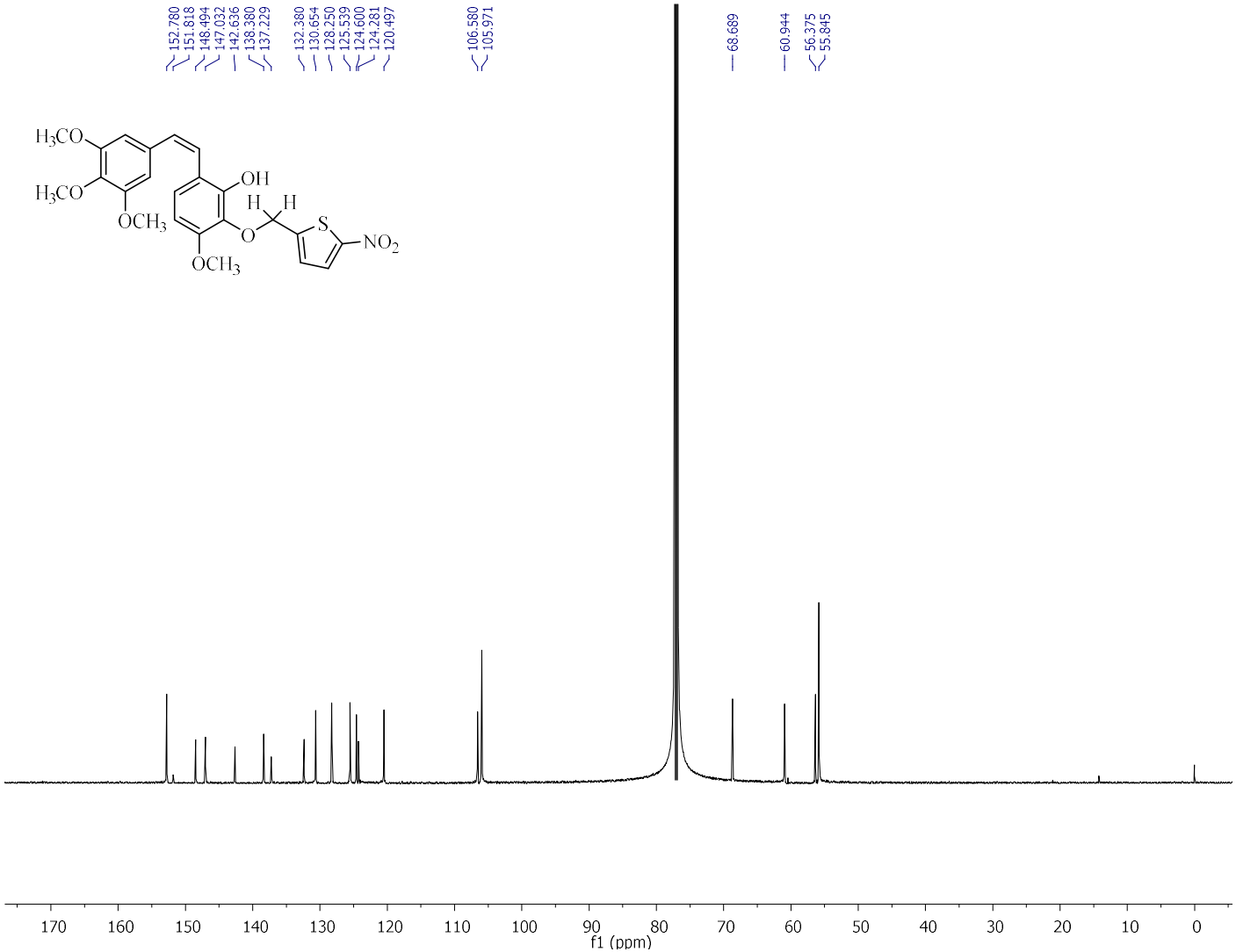
*** End of Report ***

Instrument 1 4/9/2015 11:52:45 AM Graham Page 3 of 3

^{1}H NMR (600 MHz, $CDCl_3$) for Compound **20**



^{13}C NMR (151 MHz, $CDCl_3$) for Compound **20**



HPLC Traces for Compound **20**

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

    ==================================================================
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    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
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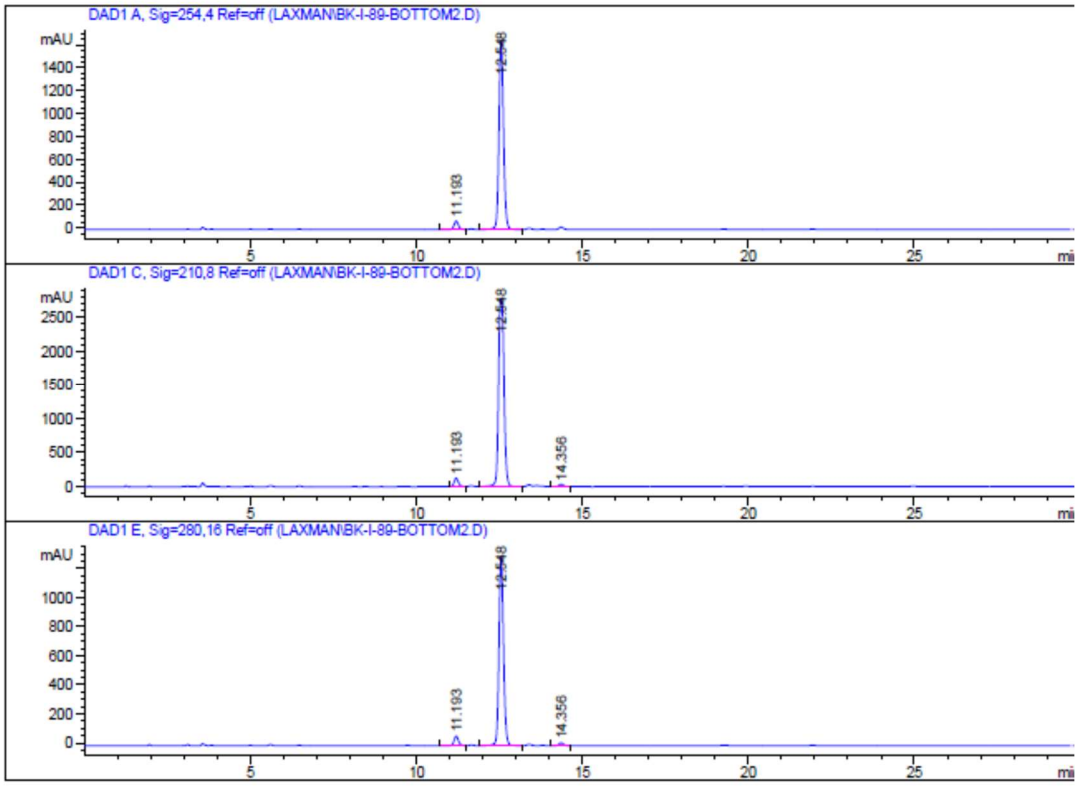


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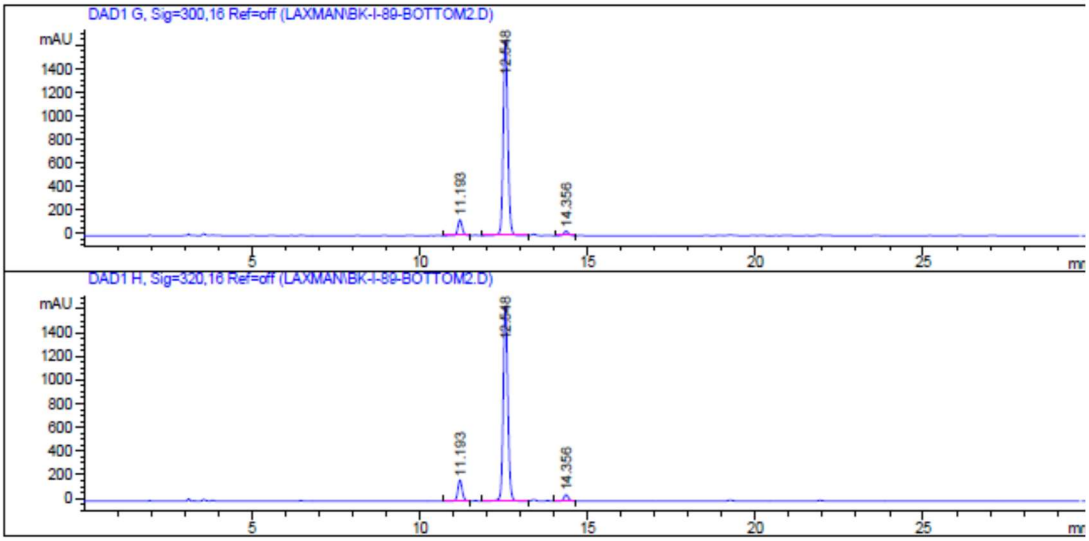
Instrument 1 7/8/2015 3:31:02 PM Laxman                    Page   1 of 3
```

HPLC Traces for Compound **20**

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



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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	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

Instrument 1 7/8/2015 3:31:02 PM Laxman Page 2 of 3

HPLC Traces for Compound **20**

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	

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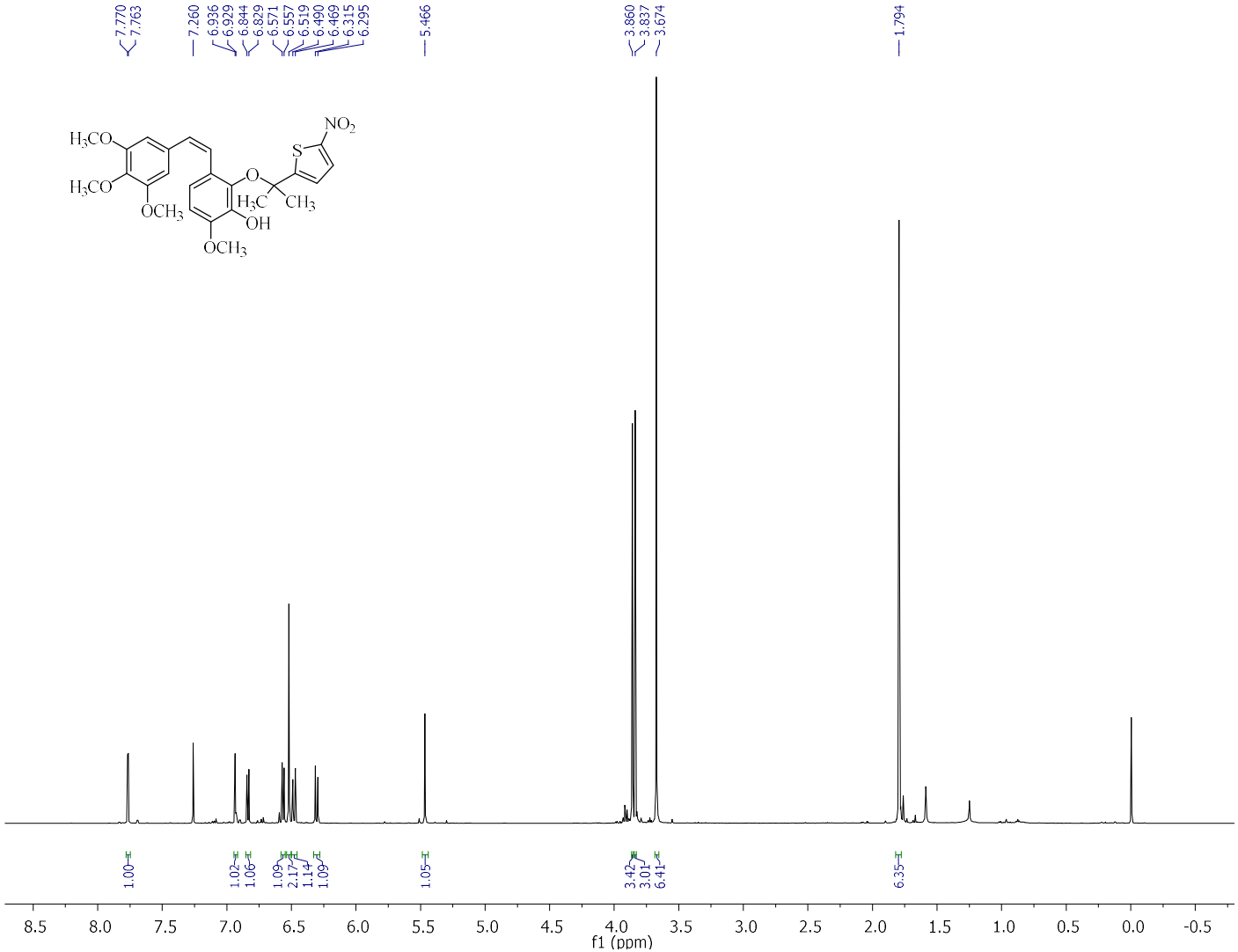
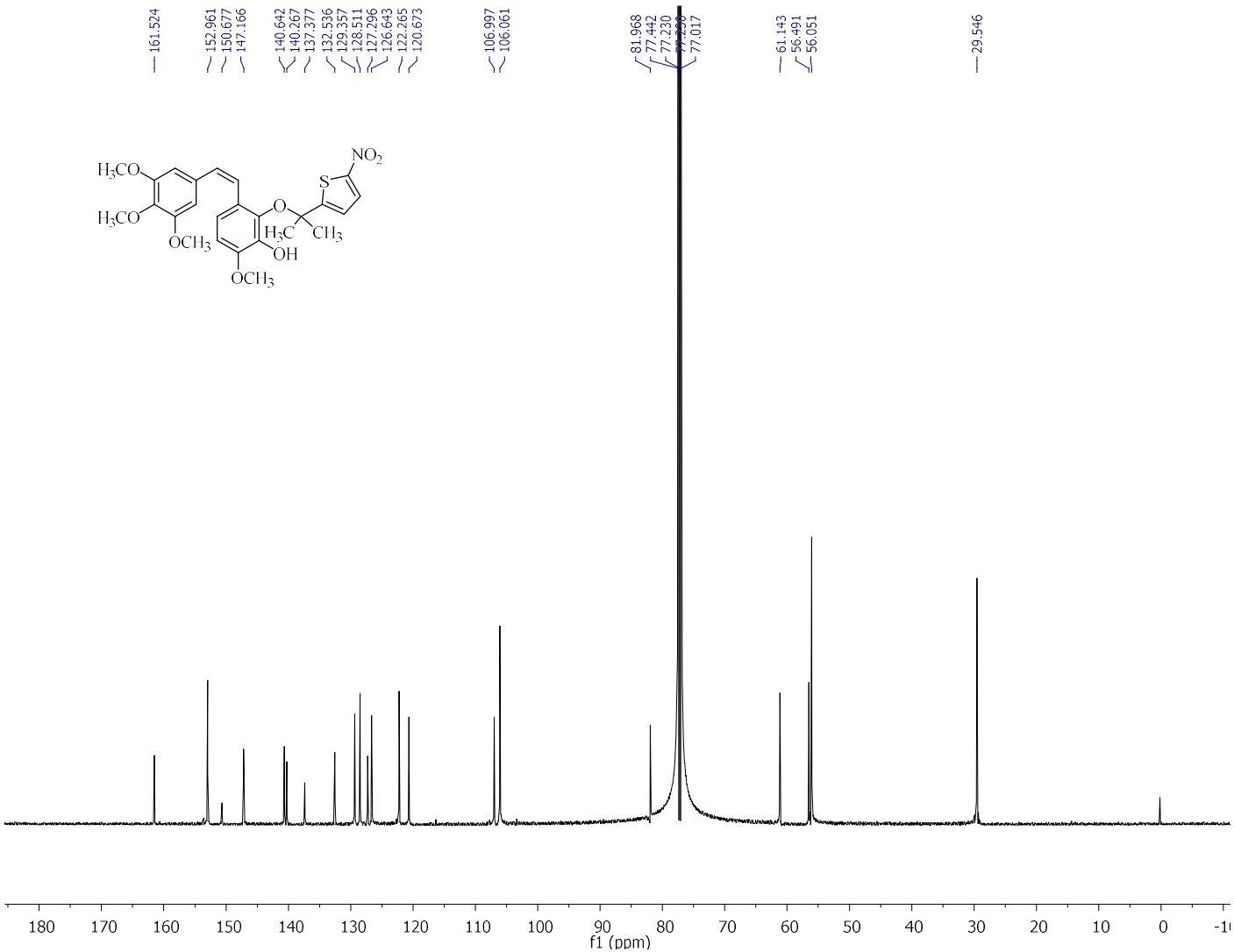
*** End of Report ***

^{1}H NMR (600 MHz, $CDCl_3$) for Compound **21**



^{13}C NMR (151 MHz, $CDCl_3$) for Compound **21**



HPLC Traces for Compound **21**

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

    ==================================================================
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    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
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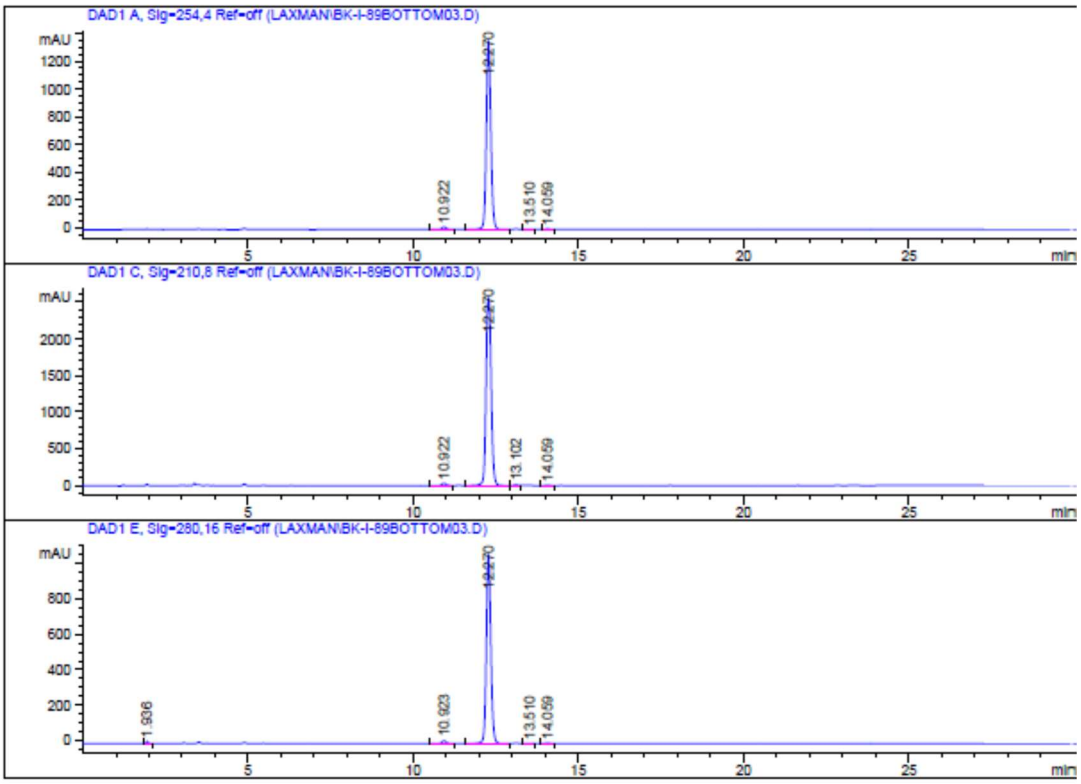


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Instrument 1 7/10/2015 12:43:01 PM Laxman                    Page   1 of 4
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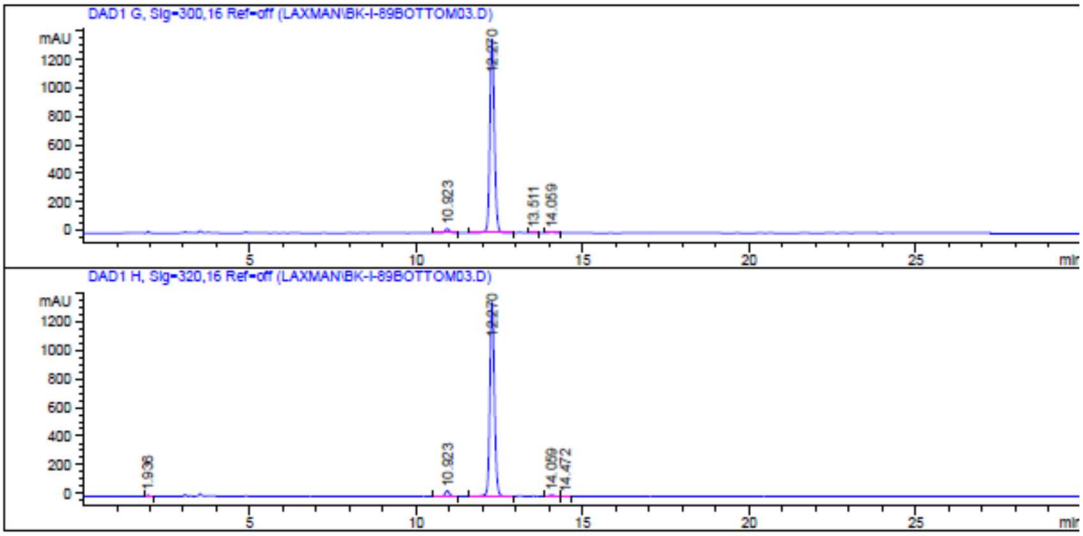
HPLC Traces for Compound **21**

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



==========
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	10.922	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

HPLC Traces for Compound **21**

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

Instrument 1 7/10/2015 12:43:01 PM Laxman Page 3 of 4

HPLC Traces for Compound **21**

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

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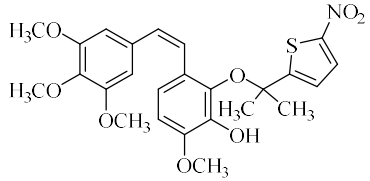
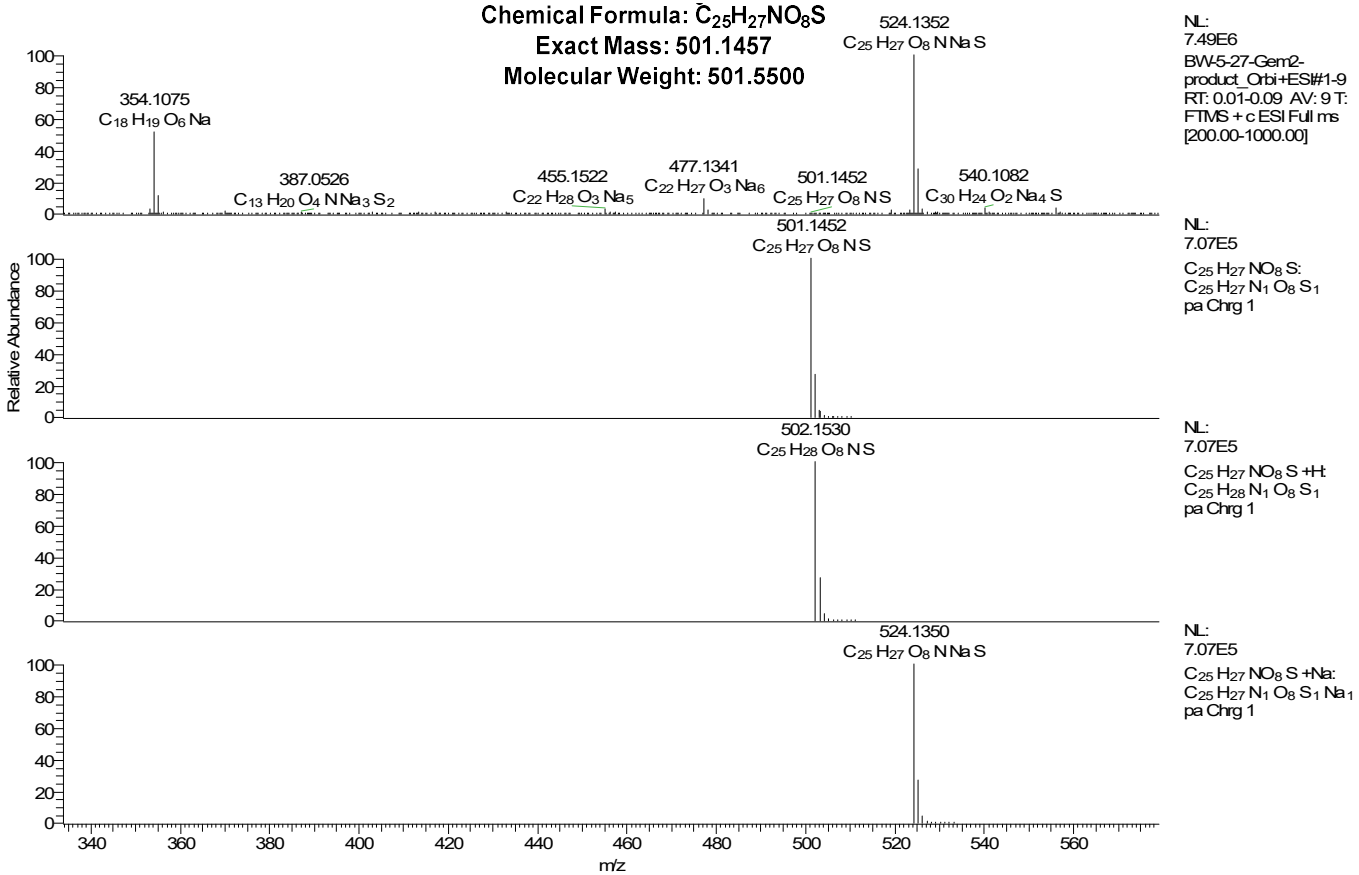
*** End of Report ***

HRMS Traces for Compound **21**



Chemical Formula: $C_{25}H_{27}NO_8S$
Exact Mass: 501.1457
Molecular Weight: 501.5500



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