ABSTRACT

Synthesis, Characterization, and Biological Evaluation of Bioreductively Activatable
Prodrug Conjugates (BAPCs) of Phenstatin, KGP18, OXi6196, Combretastatin A-1, and
Combretastatin A-4

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Mentor: Kevin G. Pinney, Ph.D.

Selective targeting of tumors with anticancer agents represents a universally
important strategy to improve efficacy and reduce patient side effects. Targeting tumor-
associated hypoxia (low oxygen tension) represents one type of promising therapeutic
regimen. Bioreductively activatable prodrug conjugates (BAPCs) are designed to be
biologically inert under normoxia, however in hypoxic environments they will selectively
release their parent anticancer agent. Inhibitors of tubulin polymerization (assembly) are
promising anticancer agents for functionalization as their corresponding BAPCs. Upon
hypoxia-selective release, these compounds function biologically as antimitotic agents
with a subset demonstrating dual mechanistic capability as potent vascular disrupting
agents (VDAs), which selectively damage tumor-associated vasculature leading to
enhanced tumor necrosis. Phenstatin, OXi6196, combretastatin A-1 (CA1),
combretastatin A-4 (CA4), and KGP18 are promising anticancer agents for development
as BAPCs. These compounds are effective inhibitors of tubulin assembly and
demonstrate potent activity in vitro against human cancer cell lines.

Synthetic pathways have been identified for the preparation of nitrothienyl prodrugs of CA1 and CA4 using the *nor*-methyl, *mono*-methyl, and *gem*-dimethyl nitrothiophene-based triggers. A regioselective protecting group strategy was utilized in order to synthesize the nitrothiophene triggers regioselectively to the C-2 and C-3 positions of CA1. Tosyl, isopropyl, and *tert*-butyldimethylsilyl protecting groups were important in establishing this CA1 regioselectivity. Several series of BAPCs were also developed based on phenstatin, KGP18, and Oxi6196 using *nor*-methyl, *mono*-methyl, and *gem*-dimethyl variants of the nitrothiophene, nitrobenzyl, nitroimidazole, and nitrofuran triggers. A selection of the CA1, CA4, and phenstatin BAPCs were evaluated biologically for their ability to inhibit tubulin assembly as well as their stability in aqueous conditions and their ability to undergo enzymatic cleavage in the presence of NADPH cytochrome P450 oxidoreductase.

Synthesis, Characterization, and Biological Evaluation of Bioreductively Activatable Prodrug
Conjugates (BAPCs) of Phenstatin, KGP18, OXi6196, Combretastatin A-1, and Combretastatin A-4

by

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None of this would have been possible without the loving support of my father, mother, and brother, all of whom encouraged me to follow my dreams. Thank you.

DEDICATION

Craig, Katharine, and Ryan Winn

In Memory of

Don and Patricia Brenner

CHAPTER ONE

Introduction

Healthy and structured vasculature is required to efficiently deliver nutrients to and eliminate waste products from cells throughout the body.[1-11] Vasculature in healthy tissue is a well-organized system of vessels ranging in size from capillaries to arteries and veins (Figure 1.1).[1-11] In contrast, tumor-associated vasculature is a disorganized system, spread in a more random fashion and is subject to weak and inconsistent blood flow, reducing the overall nutrient delivery to the tumor.[1-11] Poorly structured tumor-associated vasculature offers an effective target to neutralize the nutrient-starved tumor, reducing or possibly eliminating blood flow to the tumor through vascular disruption or destruction.[1-11]
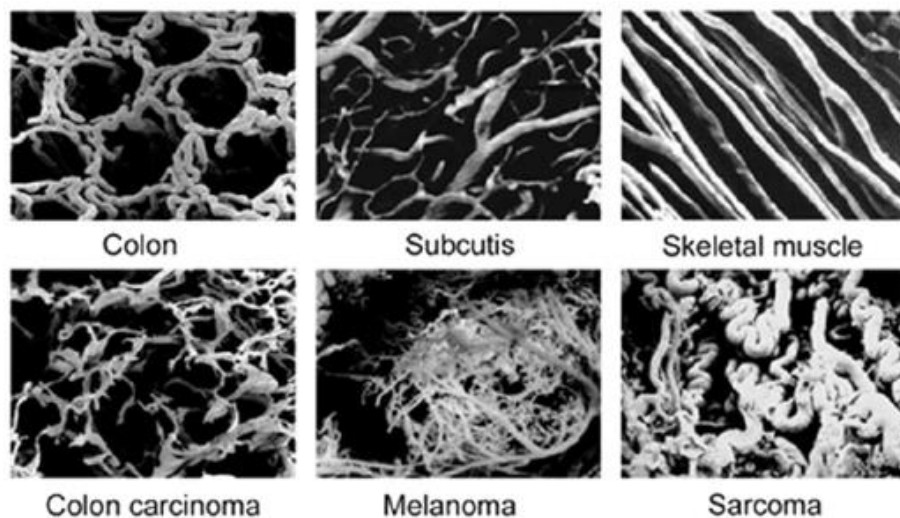
Figure 1.1. Comparative Images of Vasculature in Healthy Tissue Versus Tumor-Associated Vasculature.[3,9]

Vascular Targeting Agents

One therapeutic option to target tumor-associated vasculature is the employment of vascular targeting agents (VTAs).[1-11] Vascular targeting agents are divided into two distinct classes of compounds: angiogenesis-inhibiting agents (AIAs), which disrupt the formation of new vasculature in the tumor, and vascular disrupting agents (VDAs), which collapse and destroy existing tumor vasculature (Figure 1.2).[1-11]
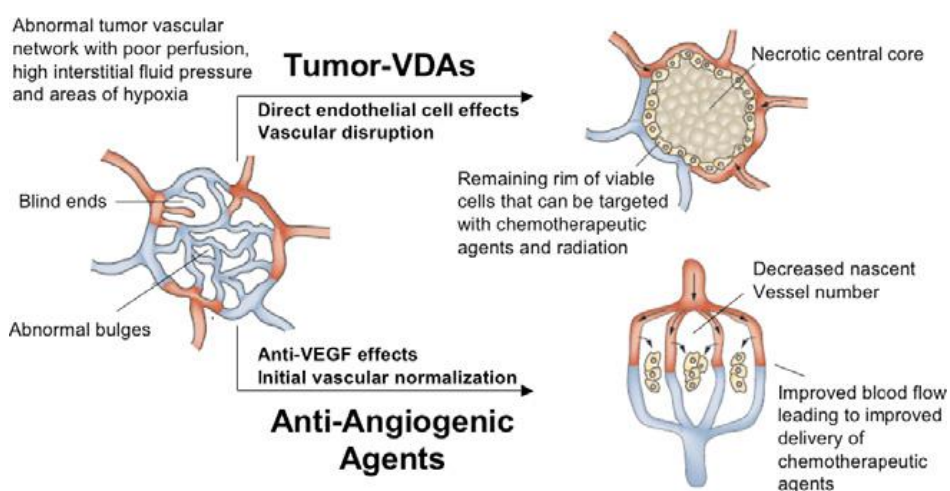
Figure 1.2. The Effects of Vascular Disrupting Agents and Angiogenesis-Inhibiting Agents on Tumor-Associated Vasculature.[2]

Tumor angiogenesis is the development of new vasculature within the tumor, which can be attributed to the presence of vascular endothelial growth factor-A (VGEF-A).[2,12-13] As most of the new vasculature in the tumor is generated at the periphery, AIAs are most active inhibiting the newly forming vessels in that region.[2,12-13] With fewer overall blood vessels forming in the tumor, the remaining vasculature has less competition for blood flow, enhancing overall blood flow and nutrient delivery in the tumor (Figure 1.2).[1-13] This enhanced blood flow allows for the improved delivery of

cytotoxic agents and radiotherapy treatments.[1-13] Thus, AIAs are most effective in combination regimens, enhancing the efficacy of established cytotoxic agents such as carboplatin and paclitaxel in solid tumors, a difficult therapeutic target.[2,12-13]

Vascular Disrupting Agents

Vascular disrupting agents represent another therapeutic option for targeting blood vessels in tumors, functioning by collapsing the existing vasculature.[1-11] VDAs typically target the endothelial cells lining the vessel walls, subduing or eliminating blood flow through the damaged vessel (Figure 1.3).[1-11] One subset of VDAs contains small molecules that act through microtubule disruption in the endothelial cells lining the vessel walls.[1-11] The disruption of microtubule assembly causes the cells to lose shape and round up, removing the structural integrity of the endothelial layer inside the vessel walls and allowing the body's interstitial pressure to collapse the vessel (Figure 1.4).[1-11] VDAs work well in concert with AIAs, as they only act on existing vasculature and cannot prevent vessel regrowth.[14-19] Combinatorial therapies have shown promising results, reducing overall tumor growth.[14-19]
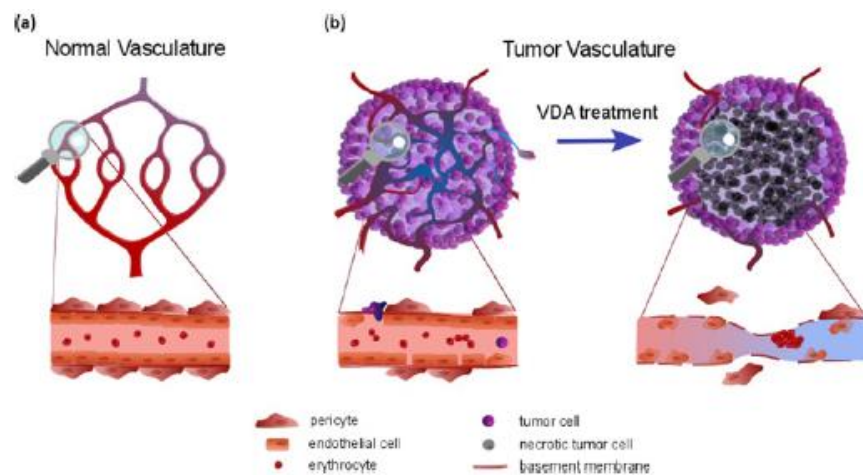
Figure 1.3. Comparison of Healthy Vasculature and Tumor-Associated Vasculature. Effects of Vascular Disrupting Agents on Tumor-Associated Vasculature.[7]

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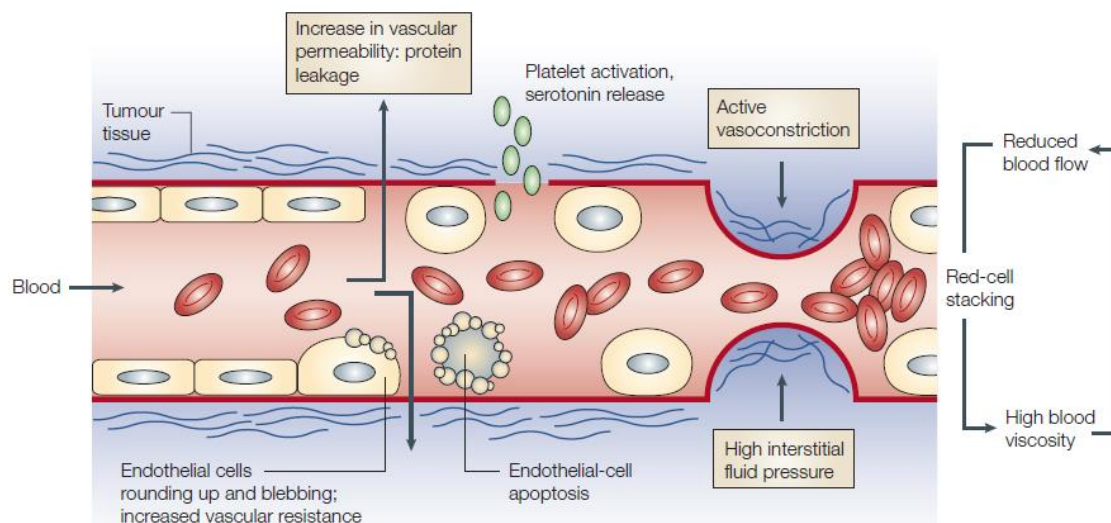
Figure 1.4. Results of Endothelial Cell Disruption by VDAs on Vasculature.[11]

Combretastatin A-1 and Combretastatin A-4

Combretastatin A-1 (CA1) and combretastatin A-4 (CA4), natural products discovered by Pettit and co-workers, are two potent vascular disrupting agents that inhibit microtubule assembly.[20-25] CA1 and CA4 (Figure 1.5) were first isolated and characterized by the Pettit group from the African bush willow tree *Combretum caffrum* Kuntze (Combretacae).[20-25] *Combretum caffrum* bark has been historically utilized by the Zulu tribe as a charm to ward off enemies.[26] The two natural products, CA1 and CA4, have the same biological mechanism of action, interacting with the colchicine binding site on tubulin to disrupt microtubule assembly, leading to a loss a defined cellular structure and collapsing the vasculature.[20-25] With the aid of enhanced solubility, the phosphate salt prodrugs of CA1 (OXi4503) and CA4 (Zybrestat™) have shown potency in clinical trials, and Zybrestat™ has reached advanced clinical trials.[18,19,27-32]

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CA4, R$_1$ = H, R$_2$ = OH
CA4P, R$_1$ = H, R$_2$ = OPO$_3$Na$_2$
CA1, R$_1$ = R$_2$ = OH
CA1P, R$_1$ = R$_2$ = OPO3Na2

Phenstatin, R = OH
Phenstatin Phosphate, R = OPO$_3$Na$_2$

KGP18, R = OH
KGP265, R = OPO$_3$Na$_2$

Oxi6196, R = OH
Oxi6197, R = OPO$_3$Na$_2$
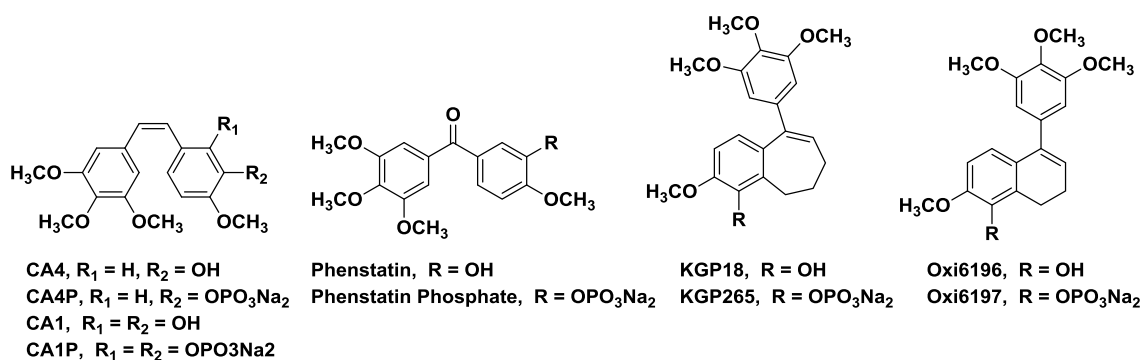
Figure 1.5. Structures of CA1, CA4, Phenstatin, KGP18, and OXi6196 and Their Phosphate Salts

Phenstatin

Phenstatin, first synthesized by Pettit and co-workers, is a potent cytotoxic agent and an inhibitor of tubulin polymerization, collapsing tumor vasculature (Figure **1.5**).[28,33] Discovered serendipitously by the Pettit group during an attempt to synthesize an epoxide at the *Z*-stilbenoid bridge of CA4, phenstatin was first obtained as the surprising result of a Jacobsen epoxidation reaction (Figure 1.6).[33] A diarylacetaldehyde was formed after a phenyl shift during the epoxidation, and subsequent oxidative cleavage yielded the silylated phenstatin.[33] Phenstatin has the same biological mechanism of action as CA4, disrupting microtubule assembly through interactions with the colchicine binding site on tubulin.[33] In preliminary biological evaluations to determine the ability of phenstatin to inhibit tubulin polymerization activities of phenstatin and its corresponding phosphate prodrug in cell assays are very similar to those of CA4 and combretastatin A-4 phosphate (CA4P).[28,33]
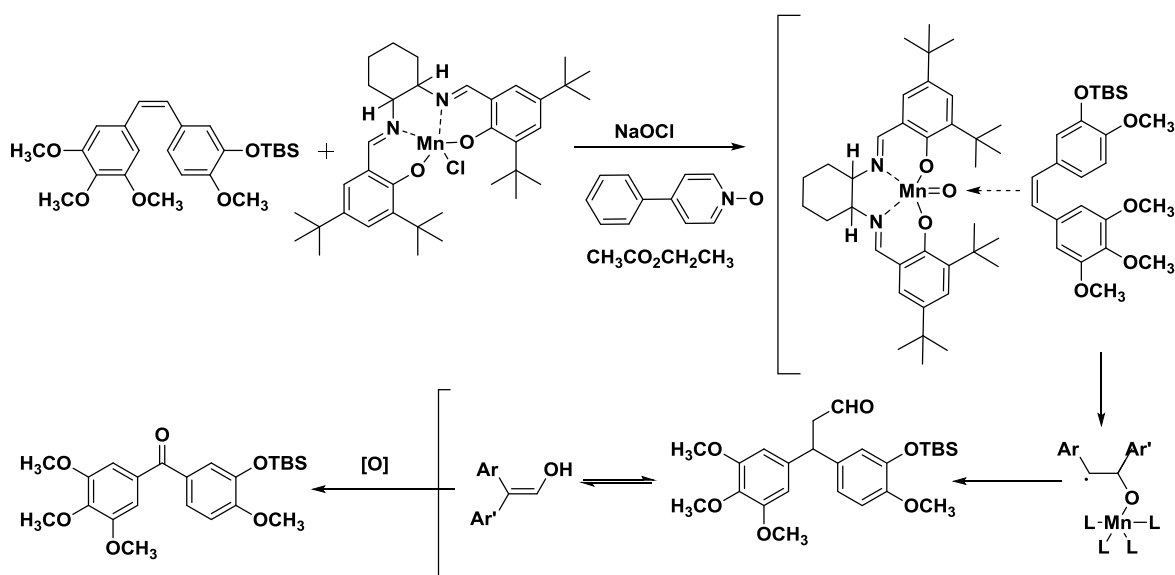
Figure 1.6. Proposed Mechanism for the Formation of Phenstatin from CA4 during a Jacobsen Epoxidation Reaction[33]

Structurally Modified Analogues of CA4

The relative simplicity of the *Z*-stilbenoid molecular architecture inherent to CA4 had led to many structural modifications. The Pinney Research Group (Baylor University) has a well-established program that centers on the design and synthesis of new small-molecule inhibitors of tubulin polymerization that are inspired by natural products, including CA4, CA1, and colchicine. KGP18, for example, is a benzosuberene-based CA4 analogue that functions biologically with a similar mechanism of action to that inherent to CA1 and CA4, collapsing tumor-associated vasculature (Figure 1.5).[34-36] In preliminary biological testing, KGP18 and its corresponding phosphate salt have been shown to be effective inhibitors of tubulin assembly and VDAs, displaying potent cytotoxicity across a number of human cancer cell lines such as SK-OV-3, NCI-H460, and DU-145.[34-36]

OXi6196, the 6-membered ring analog of KGP18, was first synthesized by the Pinney group in 2004 (Figure 1.5).[34,37] Owing in part to a nearly identical structure to KGP18, OXi6196 is also a highly active inhibitor of tubulin assembly.[34,37] The preliminary biological data for OXi6196 is promising, showing excellent inhibition of tubulin assembly and potent cytotoxicity against human cancer cell lines.[37]

Tumor Vasculature

Tumor-associated vasculature is an attractive therapeutic target due to its distinct differences from blood vessels feeding healthy tissue.[3,8,38-49] Due to the rapid growth of the tumor tissue in comparison to the cellular division rate of the endothelial cells of blood vessels, tumor-associated vasculature tends to be poorly formed.[3,8,38-49] The tumor-associated vasculature not only lacks the overall structure and organization of vasculature feeding healthy tissue, but also can be irregularly shaped with inconsistent vessel diameter and wall thickness. The vessels in tumors can feature blind ends, occlusions, and bulges (Figure 1.7).[3,8,38-49] Inconsistent vessel wall thickness, leading to thin spots in the walls, can cause increased interstitial pressure due to fluid permeability.[3,8,38-49] Leaky vessel walls are common due to the disjointed nature of the endothelial cells and luminal layer.[3,8,38-49]

All of these conditions in addition to irregular branching among the vessels causes the blood flow in tumor-associated vasculature to be inconsistent, sometimes even flowing in opposing directions within a blood vessel.[3,8,38-49] Poor blood flow in addition to varying distances between tumor-associated vasculature leads to regions of pronounced hypoxia, as the vessel distance becomes greater than the diffusion distance of oxygen (Figure 1.8).[3,8,38-49] Solid tumor microenvironments can also experience pH and

catabolite gradients as well as reduced cell proliferation. Hypoxic regions can become more acidic in nature than standard tissue.[3,8,38-49]
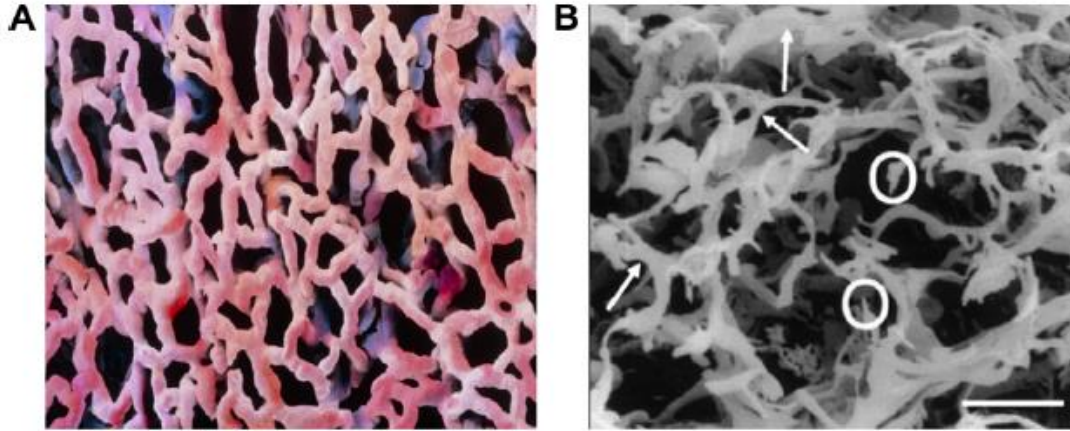
Figure 1.7. Microvascular Cast of Healthy Lung Tissue Versus Human Sigmoidal Adenocarcinoma [blind ends circled, abnormal bulges noted with arrows] as Seen Through Scanning Electron Microscopy[2,50]
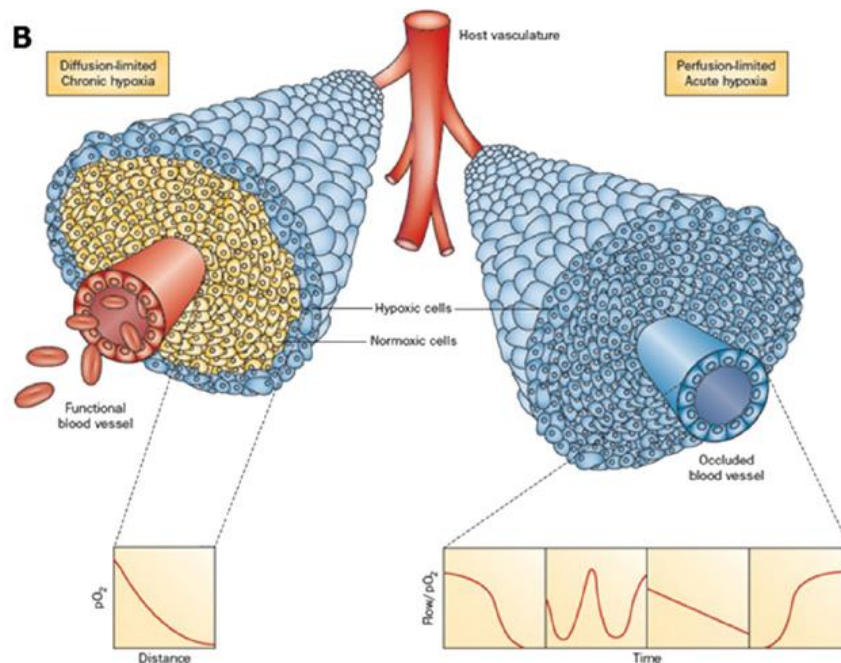
Figure 1.8. Characterization Displaying the Difference Between Tumor Tissue Growing Around Standard Tumor Vasculature Versus Damaged Vasculature.[3]

Tumor Hypoxia

Tumor heterogeneity, particularly due to hypoxia, poses a unique obstacle in the clinical management of neoplastic disease based on traditional interventions, provided divergent biological characteristics on which these are rationalized (Figure 1.9).[8,38-49] Hypoxia in tumors incites a number of biological responses, ranging from increased metastasis and partial arrest of DNA repair mechanisms to aberrant genomic regulation of pro-apoptotic signaling, leading to suppression of apoptosis and an initiation of autophagy.[8,38-49] By convention, the underlying mechanisms traditional therapies are targeted to cannot be relied on in normal solid tumor biology for comprehensive curative effects.[8,38-49] Radiotherapy as well as a number of cytotoxic agents such as taxanes and platinum-based agents have been shown to be less effective against hypoxic tissues and solid tumors.[8,38-49] Since hypoxia diminishes traditional therapeutic efficacy, it has become a tempting target for new anticancer agents and treatments.[8,38-49]
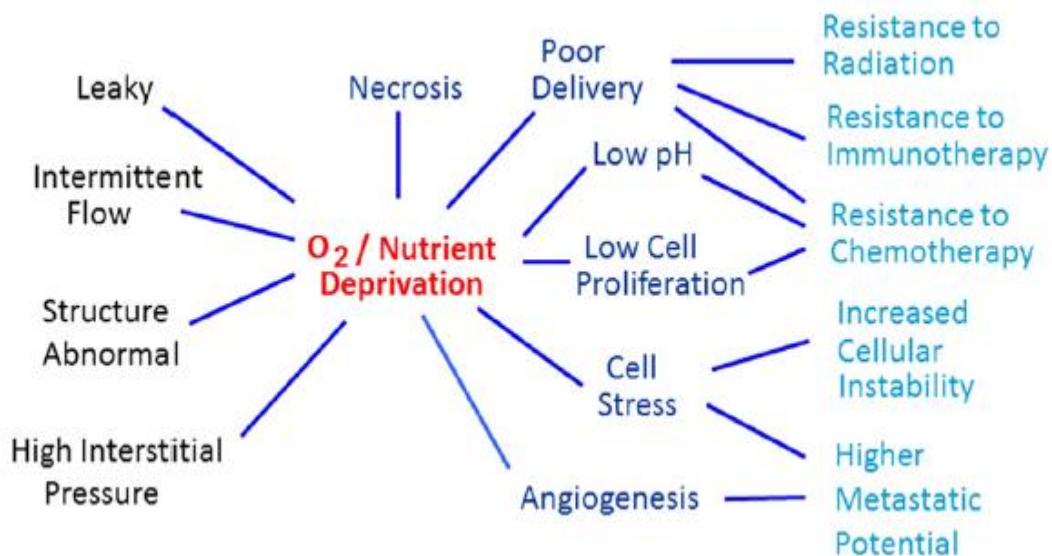
Figure 1.9. Intrinsic Features of Tumor-Associated Vasculature and the Biological Consequences of Those Conditions.[3]

Hypoxia activated prodrugs (HAPs) / bioreductively activatable prodrug conjugates (BAPCs) are a promising therapeutic option for targeting tumor hypoxia.[8,38] HAPs and BAPCs utilize bioreductive initiation mechanisms through enzymatic activity, typically NADPH cytochrome P450 oxidoreductase (POR) and NAD(P)H dependent flavoproteins, activating selectively in low oxygen environments.[8,38]

Ideally, HAPs and BAPCs have a number of requirements to ensure maximum effectiveness, from favored activation in low oxygen environments over normoxic conditions to cytotoxicity against non-proliferative cells in hypoxic regions and the capacity to diffuse to the low oxygen areas of the tumor while active.[8,38] A number of HAPs and BAPCs have reached the point of clinical trials, including tirapazamine, TH-302, PR-104, and AQ4N.[8,38] Although several of the BAPCs have displayed promising results in Phase I and Phase II trials, no BAPC has yet made it past Phase III trials.[8,38]
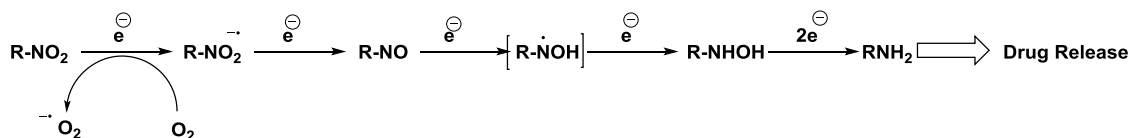
Figure 1.10. Reduction Pathway of Nitro to Amine for Bioreductively Activatable Prodrugs Via NADPH Cytochrome P450 Oxidoreductase[49]

Tirapazamine

Tirapazamine, an aromatic N-oxide synthesized by Zeman et al., was one of the trailblazers for the BAPC field.[8,51-57] Activated by one electron reductases such as cytochrome P450 oxidoreductase, tirapazamine is first radicalized by POR, which splits into a hydroxyl radical and a benzotriazinyl radical (Figure 1.11).[8,54-57] Both the hydroxyl radical and benzotriazinyl radical can oxidize DNA and damage the strand.[8,54-57]

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If tirapazamine reacts with a two electron reductase, it will be reduced into the mono N-oxide, rendering it effectively non-toxic.[8] Since this reduction pathway eliminates the tirapazamine's toxicity, it can be viewed as a bioprotective step in the body.[8] Due to promising results in cell line testing, tirapazamine has been taken into clinical trials.[8] While Phase I and Phase II trials yielded positive results, multiple Phase III trials showed little to no benefit or increased survival duration in several cell lines.[8]
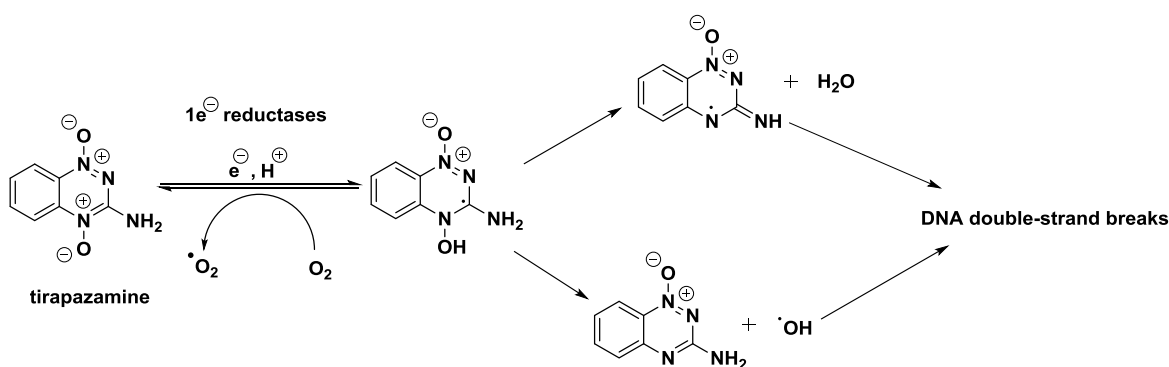
Figure 1.11. Bioreductive Activation of Tirapazamine[8]

TH-302, AQ4N, and PR-104

TH-302, synthesized and tested by Threshold Pharmaceuticals, is a 2-nitroimidazole based BAPC attached to DNA alkylating agent bromo-isophosphoramide.[8,58,59] The prodrug is activated by one electron reductases such as cytochrome P450 reductase, reducing the nitro group on the nitroimidazole trigger to an amine (Figure 1.12).[8,58,59]

The free electron pair on the amine can then push into the ring, causing an electron cascade that cleaves the trigger, releasing the active drug in the hypoxic regions of the tumor.[8,58,59] Highly effective in *in vitro* studies and early *in vivo* studies in mice

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with HCR ratios as high as 600, TH-302 has been taken into Phase III clinical trials.[8,60-64] After showing promising results in Phase I and Phase II clinical trials, TH-302 was unsuccessful in a Phase III clinical trial, displaying no statistically significant anticancer activity against soft tissue sarcoma and pancreatic adenocarcinoma.[8,60-64]
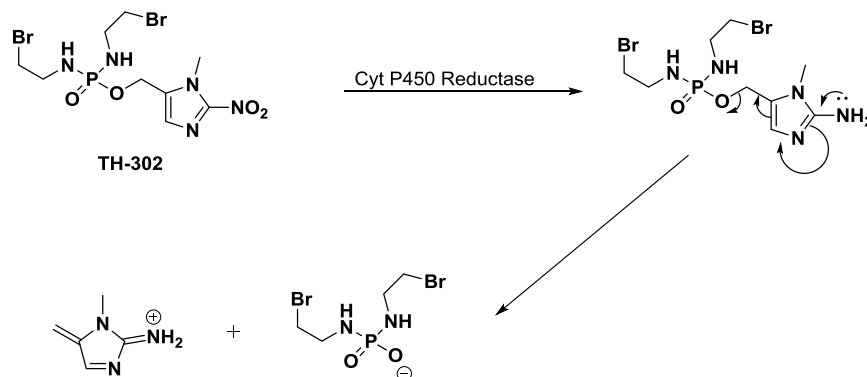
Figure 1.12. Bioreductive Trigger Release from TH-302[8]

AQ4N, synthesized by McKeown *et. al.*, is an aliphatic N-oxide prodrug that upon enzymatic activation is converted to the topoisomerase II inhibitor AQ4.[8,65-75] AQ4N is first converted to the mono-N-oxide AQ4M via a two electron reduction.[8,65-75] A subsequent two electron reduction of AQ4M generates the active form AQ4 (Figure 1.13).[8,65-75] The activated drug AQ4 acts in the hypoxic regions by non-covalent binding to DNA, inhibiting topoisomerase activity as the cells attempt to replicate.[8,65-75] In aerobic conditions, AQ4N also displays some anti-angiogenic properties, targeting endothelial cells.[8,65-75]

In terms of its anti-angiogenic properties, the mechanism of action for the drug is currently unknown, although extensive microtubule network disruption was detected.[8,65-75] The drug is only active in hypoxic regions due to its inability to compete with oxygen

at the active site of cytochrome P450 reductase, preventing reduction in the normoxic regions of the body.[8,65-75] AQ4N has progressed to Phase I and Phase II clinical trials, but has not as of yet progressed to Phase III trials.[76-77]
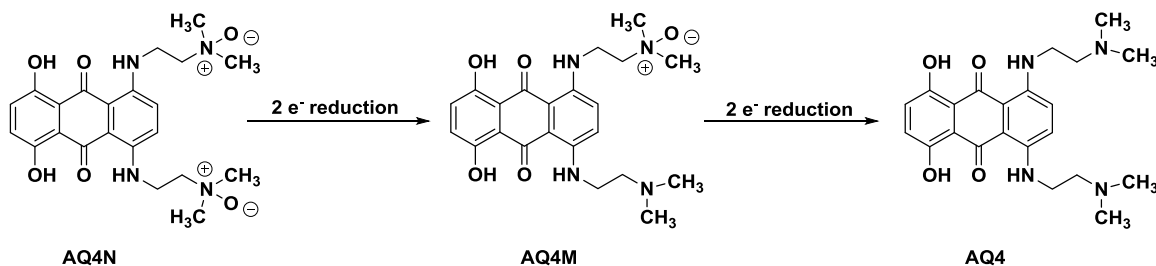
Figure 1.13. Bioreductive Activation of AQ4 from AQ4N[8]

PR-104, synthesized and tested by Wilson *et. al.* at the University of Auckland is a nitroaromatic preprodrug of PR-104A, eventually reducing to its active form PR-104M.[8,78-85] PR-104 is first hydrolyzed by phosphatases to reveal prodrug PR-104A (Figure 1.14).[8,78-85] One and two electron reductases reduce PR-104A into the active form PR-104M, which can then interact with DNA.[8,78-85]

The cytotoxicity associated with PR-104M derives from its ability to form interstrand DNA crosslinks.[8,78-85] PR-104 has been taken into Phase I and II clinical trials, yielding promising results in a Phase I/II trial against leukemia.[8,86-87] The prodrug has some dose-limiting toxicity issues when paired with gemcitabine and docetaxel, possibly due to the glucuronidation of PR-104A that diminishes the clearance of the drug from the patient's system.[8,86-87]

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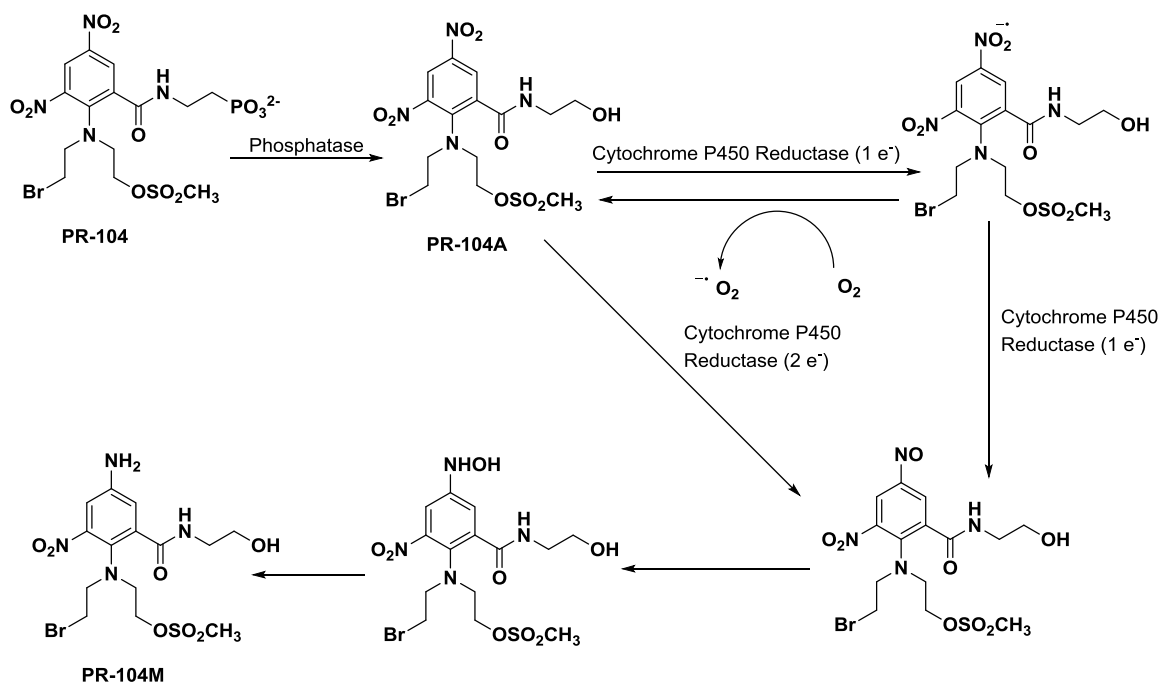
Figure 1.14. Bioreductive Activation of PR-104 to PR-104M[8]

CA4-BAPCs

In an attempt to generate a cytotoxic hypoxia-selective VDA, CA4 was linked to nitrothiophene triggers by Davis and co-workers.[88,89] Davis and coworkers synthesized *nor*-methyl, *mono*-methyl, and *gem*-dimethyl nitrothiophene triggers (Figure 1.15) and utilized the Mitsunobu reaction to covalently attach them to CA4.[88,89] The *gem*-dimethyl CA4 prodrug proved to be the most active of the trio, maintaining the highest HCR values.[88]

The *gem*-dimethyl trigger provided the greatest resistance to cleavage under normoxic conditions, keeping the CA4-BAPC prodrug intact while the *nor*-methyl and *mono*-methyl trigger CA4-BAPCs underwent partial cleavage under normoxic conditions.[88] Also of note was the overall lack of activity of the CA4-*gem*-dimethyl

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trigger prodrug in normoxic conditions, as the compound only became active under

hypoxic conditions, displaying high selectivity for low-oxygen environments.[88]
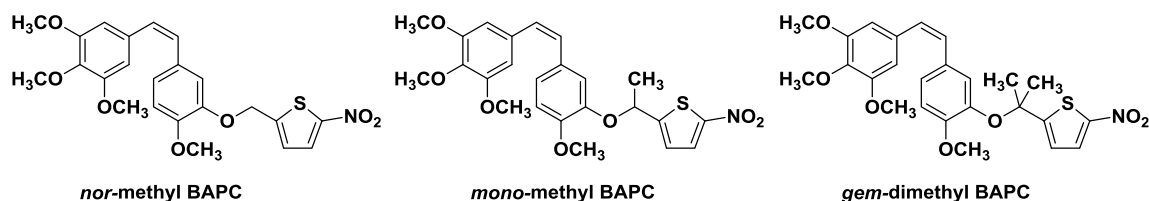
Figure 1.15. *Nor*-methyl, *Mono*-methyl, and *Gem*-dimethyl Nitrothienyl CA4-BAPCs[88]

Nitrothiophene Triggers

The mechanism of cleavage for the nitrothiophene triggers is similar in nature to

the cleavage of nitroimidazole triggers with a one electron reductase such as cytochrome

P450 reductase reducing the nitro group on the trigger.[88,90-100] Once the nitro has been

reduced, an electron cascade through the thiophene ring will lead to the trigger cleaving

from CA4, releasing the active drug (Figure 1.16).[88] Once released from the

nitrothiophene trigger, CA4 acts in hypoxic regions as a VDA, interacting with the

colchicine binding site on tubulin, disrupting the microtubules in endothelial cells which

leads to the collapse of the tumor vasculature.[88]
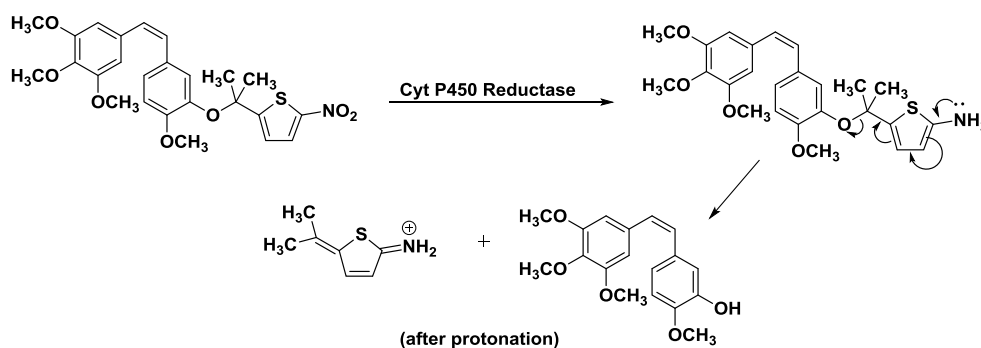
Figure 1.16. *Gem*-dimethyl Nitrothienyl CA4-BAPC Trigger Cleavage[88]

For the research described herein, inspired by the BAPC tumor targeting strategy, we developed BAPCs for KGP18, Oxi6196, CA4, CA1, and phenstatin. The bioreductive triggers utilized in the BAPC synthesis varied from the nitrothiophene, nitroimidazole, nitrobenzyl, and nitrofuran. These four trigger sets were covalently linked to the anticancer agents through the use of the Mitsunobu reaction. The newly prepared CA4, CA1, KGP18, Oxi6196, and phenstatin BAPCs were evaluated under hypoxic and normoxic conditions in order to determine their differential from their respective base anticancer agent (collaboration with the Trawick Research Group, Baylor University).

CHAPTER TWO

Bioreductively Activatable Prodrug Conjugates of Phenstatin
Designed to Target Tumor Hypoxia

The author Blake A. Winn contributed to this manuscript through the synthesis of all eleven final compounds including characterization, which included proton and carbon NMRs, HPLC, and HRMS. In addition, Blake A. Winn contributed a significant amount to the writing and editing of the manuscript, as well as the preparation of the supporting data.

Abstract

A variety of solid tumor cancers contain significant regions of hypoxia, which provide unique challenges for targeting by potent anticancer agents. Bioreductively activatable prodrug conjugates (BAPCs) represent a promising strategy for therapeutic intervention. BAPCs are designed to be biologically inert until they come into contact with low oxygen tension, at which point reductase enzyme mediated cleavage releases the parent anticancer agent in a tumor-specific manner. Phenstatin is a potent inhibitor of tubulin polymerization, mimicking the chemical structure and biological activity of the natural product combretastatin A-4. Synthetic approaches have been established for nitrobenzyl, nitroimidazole, nitrofuranyl, and nitrothienyl prodrugs of phenstatin

incorporating *nor*-methyl, *mono*-methyl, and *gem*-dimethyl variants of the attached nitro compounds. A series of BAPCs based on phenstatin have been prepared by chemical synthesis and evaluated against the tubulin-microtubule protein system. In a preliminary study using anaerobic conditions, the *gem*-dimethyl nitrothiophene and *gem*-dimethyl nitrofuran analogues were shown to undergo efficient enzymatic cleavage in the presence of NADPH cytochrome P450 oxidoreductase. Each of the eleven BAPCs evaluated in this study demonstrated significantly reduced inhibitory activity against tubulin in comparison to the parent anticancer agent phenstatin (IC_{50} = 1.0 µM). In fact, the majority of the BAPCs (seven of the eleven analogs) were not inhibitors of tubulin polymerization (IC_{50} > 20 µM), which represents an anticipated (and desirable) attribute for these prodrugs, since they are intended to be biologically inactive prior to enzyme-mediated cleavage to release phenstatin.

Introduction

Tumor-associated vasculature has emerged as a promising target for anticancer therapies due to its marked differences from vasculature feeding healthy tissue.[1-8,10,11] Vasculature associated with healthy tissue forms a well-organized delivery network for oxygen and nutrients to cells.[1-8,10,11] In contrast, tumor-associated vasculature is forced to develop rapidly to meet the enhanced demand for significant amounts of nutrients and oxygen required by tumors.[1-8,10,11]

The rapid growth of tumor-associated vasculature results in compromised structural integrity, which is characterized by weakened vessel walls, increased interstitial pressure, blind ends, and bulges.[1-8,10,11] Tumor-associated vasculature is generated rapidly, leading to groups of vessels spaced far apart from each other, and this results in

regions of hypoxia that develop when this distance is greater than the diffusion distance of oxygen.[1-8,10,11]

One promising therapeutic option for targeting tumor-associated vasculature involves treatment with vascular targeting agents (VTAs), which include angiogenesis-inhibiting agents (AIAs) and vascular disrupting agents (VDAs).[1-8,10,11] AIAs, which represent a fairly well investigated therapeutic strategy, act by inhibiting angiogenesis, the formation of new tumor-associated vasculature, while leaving existing vessels intact.[4,12-13]

Inhibition of angiogenesis limits tumor growth and also leads to increased blood flow in the remaining vasculature, allowing for increased delivery of chemotherapy and potentially enhanced tumor damage from radiotherapy to an otherwise difficult therapeutic target due to hypoxia.[4,12-13] VDAs impact tumor vasculature from a mechanistic approach that is distinct from AIAs. One subset of VDAs is comprised of small-molecule inhibitors of tubulin polymerization that target existing tumor-associated vasculature by causing rapid morphology changes (flat to round) of the endothelial cells lining these vessels.[1-8,10,11] This leads to irreversible vessel damage and tumor necrosis.[1-8,10,11]

A representative clinically relevant small-molecule VDA that disrupts microtubule formation is the natural product combretastatin A-4 (CA4) (Figure 2.1). First isolated from the African bush willow tree *Combretum caffrum Kuntze* by the Pettit group, CA4 is a potent inhibitor of tubulin polymerization, functioning through a binding interaction at the colchicine site on tubulin.[20-23,30] Its corresponding water-soluble phosphate prodrug salt [combretastatin A-4P (CA4P)] has reached advanced clinical

trials as a promising VDA.[14,15,24,27,31] However, no small-molecule VDA has yet been

approved by the FDA.

Combretastatin A-4 and its Prodrug
Combretastatin A-4 (CA4) : R = OH
Combretastatin A-4P (CA4P) : R = OPO_3Na_2

Phenstatins and their Prodrugs
Hydroxyphenstatin: $R^1 = R^2 = OH$
Hydroxyphenstatin Diphosphate : $R^1 = R^2 = OPO_3Na_2$
Phenstatin: $R^1 = H$; $R^2 = OH$
Phenstatin Phosphate: $R^1 = H$; $R^2 = OPO_3Na_2$
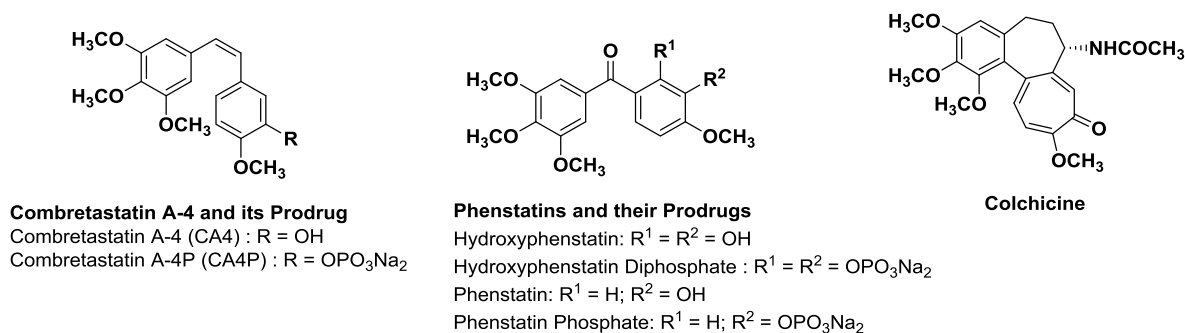
Colchicine

Figure 2.1. Colchicine, Phenstatin, and Combretastatin Natural Products and their
Corresponding Phosphate Salts

Phenstatin, originally synthesized by the Pettit group in 1998, is another potent

inhibitor of tubulin polymerization with pronounced anticancer activity against human

cancer cell lines (Figure **1**).[28,33] Phenstatin was discovered serendipitously by the Pettit

group during an attempt to prepare a CA4 analogue bearing an epoxide moiety as a

replacement for the ethylene bridge.[33] The ketone functionality of phenstatin was the

surprising result of a Jacobsen epoxidation reaction intended to form an epoxide from the

corresponding olefin.[33] Phenstatin mirrors the biological mechanism of action of CA4,

disrupting microtubule assembly through a binding interaction with the colchicine site on

tubulin.[28] Phenstatin and its corresponding water-soluble phosphate salt prodrug

counterpart demonstrate pronounced cytotoxicity against human cancer cell lines.[28]

While hydroxyphenstatin, the diol analog of phenstatin, displays potent inhibition of

tubulin polymerization, its diphosphate salt analog is less active against in vitro cancer

cell lines and as an inhibitor of tubulin polymerization than its phenstatin phosphate counterpart.[28]

Solid tumors represent inherently challenging therapeutic targets due, in part, to the significant differences between vasculature feeding healthy tissue versus tumor-associated vasculature, which is a contributing factor to the profound regions of hypoxia that often characterize tumors.[3,8,38-49] A combination of blind ends, leaky vessel walls, occlusions, and kinked vessels, along with the increased average distance between capillaries, leads to hypoxic regions where many common radiotherapies and chemotherapeutic options are less effective.[8,38-49] The challenges created by hypoxia and large diffusion distances offer a unique opportunity for targeted therapeutic intervention.[8,38] Bioreductively activatable prodrug conjugates (BAPCs) represent a possible hypoxia-activated treatment method.[8,38] BAPCs are designed to be activated by reductase enzymes, such as NADPH cytochrome P450 oxidoreductase (POR) in regions of hypoxia in the tumor microenvironment, releasing a potent anticancer agent in a tumor-specific manner.[8,38]

A series of BAPCs utilizing CA4 as the parent anticancer agent and incorporating *nor*-, *mono*-, and *gem*-dimethyl nitrothiophene triggers was reported in 2006 by Davis and co-workers.[88-89] The CA4-BAPC bearing the *gem*-dimethyl nitrothiophene trigger proved to be the most active of the trio, demonstrating the greatest resistance to cleavage under normoxic conditions *in vitro*, in effect displaying a high selectivity for low-oxygen tumor environments.[88] While the *nor*-methyl nitrothiophene BAPC was only activated at very low oxygen concentrations (<0.01% O_2), the bioreductive triggers for *mono*-methyl and *gem*-dimethyl BAPCs were cleaved over a greater range of oxygen concentrations.[88]

The *gem*-dimethyl CA4-BAPC was significantly more effective in hypoxic environments in vitro compared to the *nor*- and *mono*-methyl CA4-BAPCs, releasing approximately 50% CA4 at 0.5% O_2 with the aid of POR.[88]

The mechanism of cleavage (under hypoxia) for the nitrothiophene trigger begins with a one electron reductase such as POR reducing the nitro group on the trigger (Figure 2.2).[88] Once the nitro group on the trigger has been reduced, an electron cascade through the thiophene ring leads to trigger detachment, releasing the active VDA (CA4 in this example).[88] In comparison, under normal oxygen tension, the species obtained after the initial one-electron reduction is simply re-oxidized (by molecular oxygen) and thus does not lead to cleavage.[88]
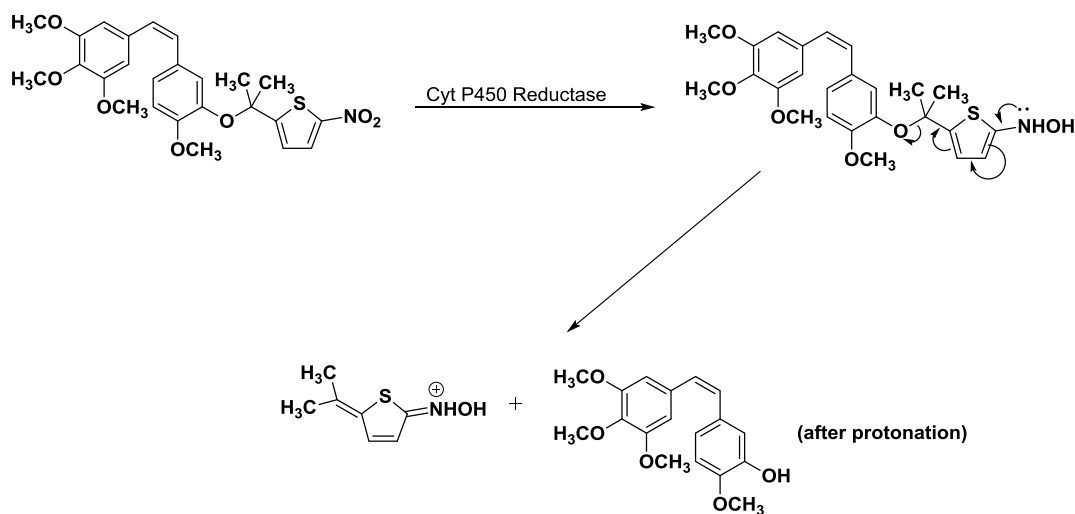
Figure 2.2. *Gem*-dimethyl Nitrothienyl Trigger Release from CA4[88]

Although no BAPC has yet been approved by the FDA, two BAPCs that have reached advanced clinical trials are TH-302 and PR-104. TH-302 (from Threshold Pharmaceuticals) is a 2-nitroimidazole based BAPC attached to the DNA alkylating agent

bromo-isophosphoramide (Figure 2.3).[59,101] The prodrug is activated by one electron

reductases such as POR, reducing the nitro group on the nitroimidazole trigger in a

similar mechanistic pathway to the nitrothienyl trigger cleavage.[8] Highly effective in *in*

vitro studies and early *in vivo* studies in mice with hypoxia cytotoxicity ratios (HCR) as

high as 600, TH-302 has advanced to Phase III clinical trials after successful Phase I and

II studies, although the results of the first Phase III trial were not statistically

significant.[60-62] PR-104 (Figure 2.3), synthesized and biologically evaluated by Wilson *et*

al. at the University of Auckland, is a nitroaromatic preprodrug of PR-104A, eventually

being reduced to the active forms PR104H and PR-104M.[8,78-80] The cytotoxicity of PR-

104M derives from its ability to form interstrand DNA crosslinks.[8] PR-104 was taken into

Phase I and II clinical trials, yielding promising results in Phase I trials but stalled at

Phase II due to dose-limiting toxicity and overall efficacy issues.[8,86,87]
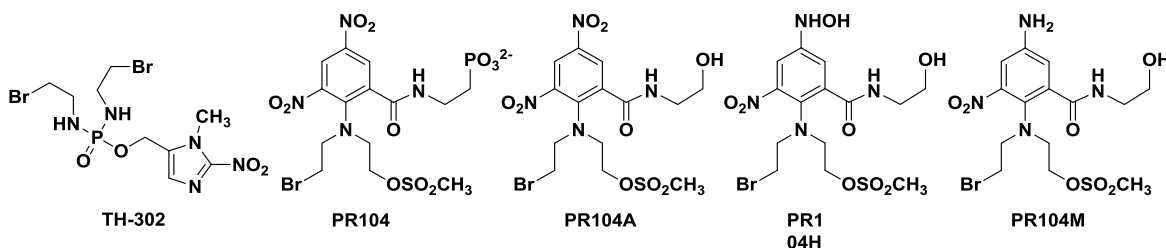
Figure 2.3. TH-302, PR104, PR104A, PR104H, and PR104M[8]

Intrigued by the concept of targeting tumor hypoxia with BAPCs, a series of such

prodrugs were prepared by chemical synthesis based on the unique tubulin-active

anticancer agent, phenstatin. Utilizing a combination of synthetic pathways previously

described in the literature along with our modifications designed to improve yield and

reaction efficiency, a selected subset of *nor-*, *mono-*, and *gem*-dimethyl nitrothiophene,

nitrobenzyl, nitroimidazole, and nitrofuran triggers were synthesized and linked to phenstatin.[88,89] Preliminary biological assessment of these phenstatin BAPCs evaluated their ability to inhibit tubulin polymerization, and a subset were evaluated for their suitability as substrates for enzymatic-mediated cleavage to release the parent anticancer agent, phenstatin.

Results and Discussion

While each of the phenstatin-based BAPCs represents a new chemical entity, phenstatin and the prodrug triggers were synthesized utilizing methodology previously described (Scheme 2.1).[33,102] Isovanillin was protected as its corresponding *tert*-butyldimethylsilyl ether, aldehyde **2**.[33,102] Halogen-metal exchange of aryl bromide **3**, followed by the introduction of aldehyde **2**, afforded the secondary alcohol **4**, which, upon oxidation, generated phenstatin precursor **5**.[33,102] Removal of the TBS protecting group yielded phenstatin **6**.[33,102]
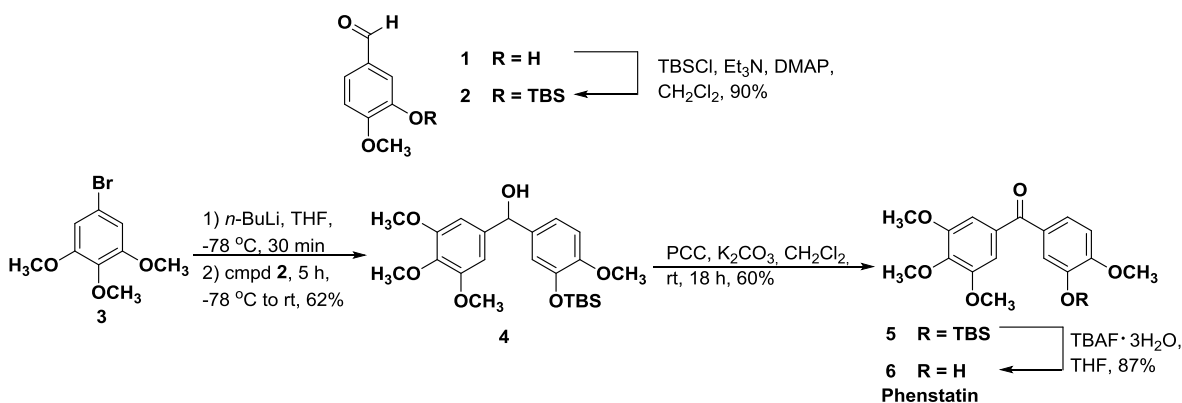
Scheme 2.1. Synthesis of Phenstatin **6**[33,102]

The *nor*-methyl nitrothiophene trigger **8** was generated in excellent yield through reduction of aldehyde **7**, as reported by Davis *et al*. (Scheme 2.2).[88] The Davis route to the *gem*-dimethyl nitrothiophene trigger **11** proved less effective in our hands, generating the product but only in low yield. This motivated us to consider a modified synthetic methodology toward the *gem*-dimethyl trigger **11**. Methylation conditions described by Reetz *et al.* provided an improved synthetic route that generated both *mono-* and *gem-* dimethyl nitrothiophene triggers from aldehyde **7** in good yield.[103] *Mono*-methyl trigger **9** was synthesized through methylation of aldehyde **7** with methyllithium and titanium tetrachloride.[103] Oxidation with Dess-Martin periodinane (DMP) generated ketone **10** in high yield, and further methylation of ketone **10** furnished the *gem*-dimethyl trigger **11** (Scheme 2.2).[103,104] Further investigation determined that trimethyl aluminum was a more effective methylating agent for the conversion of aldehyde **7** and ketone **10** to their corresponding *mono-* and *gem-* triggers **9** and **11**, respectively, in comparison to the methyllithium / titanium tetrachloride method (see Supplementary data).
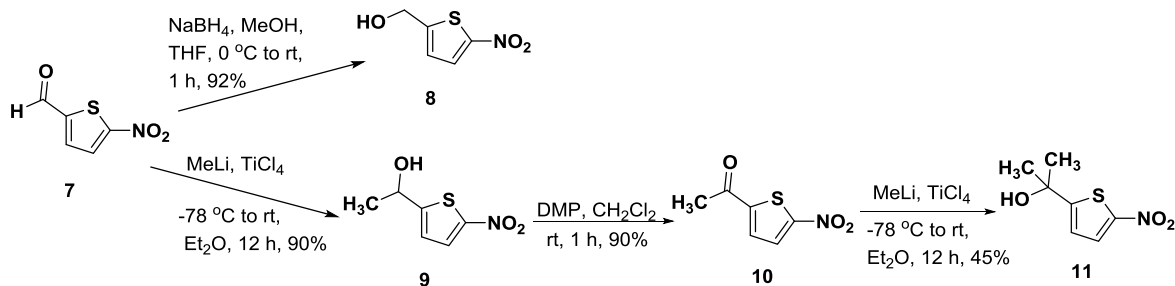
Scheme 2.2. Synthesis of Nitrothiophene Triggers[88,103,104]

A Mitsunobu reaction was utilized to conjugate the bioreductive triggers to phenstatin to generate the requisite BAPCs (Scheme 2.3).[88,102,105,106] Depending on the

reactivity of the bioreductive triggers involved in each reaction, a combination of either diethyl azodicarboxylate (DEAD), diisopropyl azodicarboxylate (DIAD), or 1,1'-(azodicarbonyl)dipiperidine (ADDP) and triphenylphosphine or tributylphosphine were employed to generate the ether linkage.[88,105,106] Synthesis of the phenstatin *nor*-methyl nitrothiophene BAPC **12** and its corresponding *mono*-methyl BAPC **13** involved the reaction of phenstatin with nitrothiophene **8** (or **9**), DIAD, and triphenylphosphine.[88,102] The phenstatin gem-dimethyl BAPC **14** was synthesized in a Mitsunobu reaction utilizing ADDP, tributylphosphine, phenstatin, and nitrothiophene trigger **11**.[88,102]

(**A**) cmpd **12**: DIAD, P(Ph)$_3$, CH$_2$Cl$_2$, cmpd **8**, rt, 2 d, 34% (R$_1$ = R$_2$ = H); cmpd **13**: DIAD, P(Ph)$_3$, CH$_2$Cl$_2$, cmpd **9**, rt, 2 d, 30% (R$_1$ = H, R$_2$ = CH$_3$); cmpd **14**: ADDP, P(Bu)$_3$, CH$_2$Cl$_2$, cmpd **11**, rt, 2 d, 7% (R$_1$ = R$_2$ = CH$_3$);
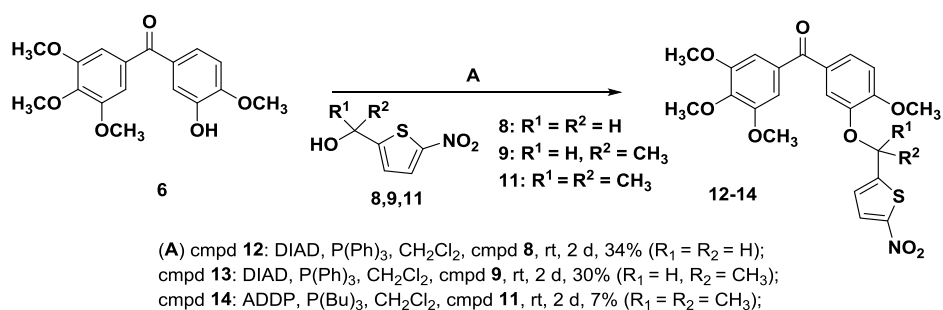
Scheme 2.3. Synthesis of the Phenstatin Nitrothiophene BAPCs[88,102,105,106]

The phenstatin nitrobenzyl BAPCs were synthesized in a similar fashion to the phenstatin nitrothiophene BAPCs, utilizing a Mitsunobu reaction to generate the critical ether linkage (Scheme A.1, Appendix A).[88,102,105,106] Synthesis of the *nor*-methyl nitrobenzyl BAPC **20** and the *mono*-methyl nitrobenzyl BAPC **21** was achieved through the reaction of phenstatin, DIAD, and triphenylphosphine, with the appropriate trigger (4-nitrobenzyl alcohol **19** and *mono*-methyl trigger **16**, respectively).[88,102] A reaction of tributylphosphine, ADDP, phenstatin, and the *gem*-dimethyl nitrobenzyl trigger **18** furnished the *gem*-dimethyl nitrobenzyl BAPC **22**.[88,102] The synthetic route reported by

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Reetz and co-workers was utilized for the synthesis of the nitrobenzyl triggers **16** and **18** (Scheme A.2, Appendix A).[103]

The nitroimidazole phenstatin BAPCs (Scheme A.3, Appendix A) were generated through a Mitsunobu reaction analogous to the chemistry described previously for the nitrobenzyl and nitrothiophene BAPCs.[88,102,105,106] The *nor*-methyl nitroimidazole BAPC **31** was synthesized utilizing a Mitsunobu reaction with phenstatin, DIAD, triphenylphosphine, and nitroimidazole **28**.[88,102] Triphenylphosphine, DIAD, phenstatin, and nitroimidazole **30** reacted to yield the *mono*-methyl nitroimidazole BAPC **32**.[88,102] The synthesis of the nitroimidazole triggers followed a route developed by Conway *et al.* (Scheme A.4, Appendix A).[104,107,108] Despite several attempts directed towards methylation of the nitroimidazole ketone, the *gem*-dimethyl nitroimidazole trigger was not successfully synthesized in our hands (Scheme A.5**,** Appendix A).[103,104,55-63]

The synthetic route for the nitrofuran bioreductive triggers (Scheme 2.4) was based on the new route to the nitrothiophene triggers shown in Scheme 2.2.[88,103,104,108] The *nor*-methyl nitrofuran trigger **34** was generated through the reduction of aldehyde **33**.[88] Aldehyde **33** was methylated to yield *mono*-methyl nitrofuran trigger **35**.[103,104] The synthesis of the *gem*-dimethyl nitrofuran trigger **37** was achieved in high yield by oxidation of *mono*-methyl trigger **35** to its corresponding ketone **36**, followed by methylation to generate the *gem*-dimethyl nitrofuran trigger **37**.[103,104]
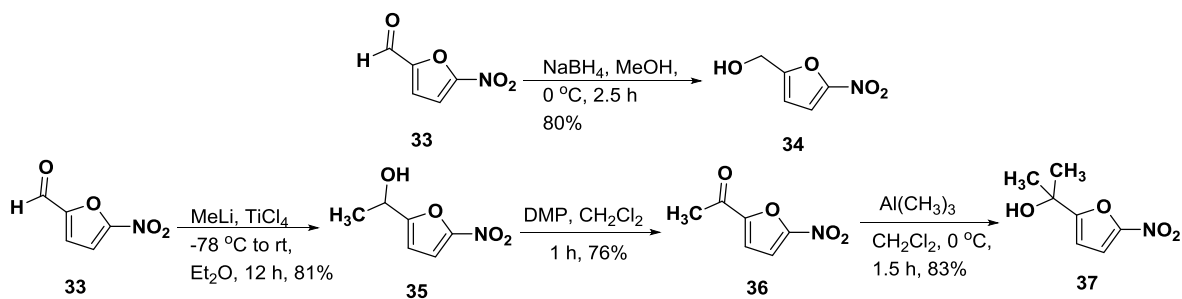
Scheme 2.4. Synthesis of the Nitrofuran Triggers[88,103,104,108]

Mitsunobu chemistry was once again employed to form the requisite ether linkage between the nitrofuran triggers and phenstatin, generating the nitrofuran BAPCs (Scheme 2.5).[88,102,107,108] The *nor*-methyl nitrofuran BAPC **38** and the *mono*-methyl nitrofuran BAPC **39** were generated through the reaction of phenstatin, DIAD, and triphenylphosphine with the appropriate trigger (*nor*-methyl nitrofuran **34** and *mono*-methyl nitrofuran **35**, respectively).[88,102] The *gem*-dimethyl nitrofuran BAPC **40** was likewise synthesized via a Mitsunobu reaction with nitrofuran **37**.[88,102]

(a) cmpd **38**: DIAD, P(Ph)$_3$, CH$_2$Cl$_2$, cmpd **34**, rt, 2 d, 41% (R^1 = R^2 = H);
cmpd **39**: DIAD, P(Ph)$_3$, CH$_2$Cl$_2$, cmpd **35**, rt, 2 d, 47% (R^1 = H, R^2 = CH$_3$);
cmpd **40**: ADDP, P(Bu)$_3$, CH$_2$Cl$_2$, cmpd **37**, rt, 2 d, 8% (R^1 = R^2 = CH$_3$);
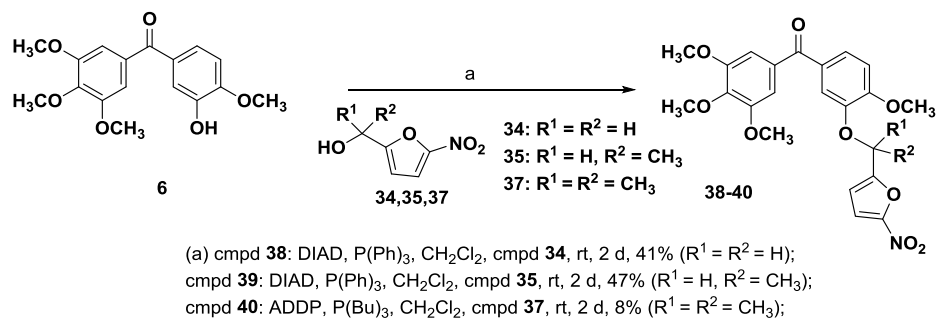
Scheme 2.5. Synthesis of the Phenstatin Nitrofuran BAPCs[88,102,107,108]

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cmpd **12,20,31,38**: R^1 = R^2 = H
cmpd **13,21,32,39**: R^1 = H, R^2 = CH$_3$
cmpd **14,22,40**: R^1 = R^2 = CH$_3$
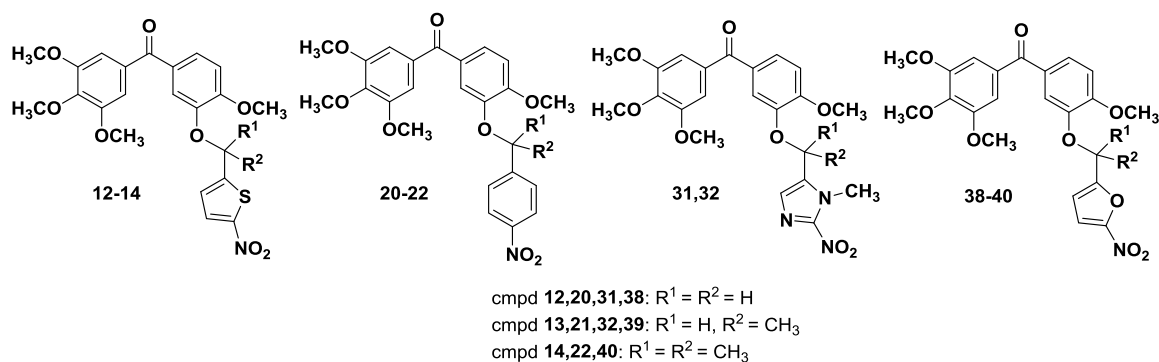
Figure 2.4. Phenstatin BAPCs Prepared by Chemical Synthesis

Biological Evaluation

The phenstatin BAPCs (Figure 2.4), as well as phenstatin, were evaluated for their ability to inhibit tubulin polymerization and compete for the colchicine binding site (Table 2.1). In addition to the *mono*-methyl nitrothiophene BAPC **13** and the *gem*-dimethyl nitrofuran BAPC **40**, the entire nitrobenzyl series (**20,21,22**) and the nitroimidazole series (**31,32**) all proved to be inactive (IC$_{50}$ >20 µM) as inhibitors of tubulin polymerization. This is an important and desirable attribute for these phenstatin-based BAPCs, since they are designed to be biologically inactive until enzyme-mediated prodrug cleavage releases the active anticancer agent (phenstatin), which itself is a potent inhibitor of tubulin polymerization (IC$_{50}$ = 1.0 µM).

The other four BAPCs (**12**, **14**, **38**, **39**) evaluated in this study demonstrated significantly reduced (IC$_{50}$ range of 9-16 µM) inhibition of tubulin polymerization, in comparison to phenstatin. The limited activity of these four BAPCs might be attributed to partial trigger cleavage under the assay conditions, leading to generation of the tubulin-active parent anticancer agent phenstatin. This hypothesis of partial cleavage has not been confirmed or further investigated to date.

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Based on the pioneering work by Davis and co-workers with CA4-BAPCs, which demonstrated the potency of the *gem*-dimethyl variant (in comparison to the corresponding *nor*- and *mono*-methyl analogues), the three phenstatin-based *gem*-dimethyl BAPCs prepared in this study were subjected to further initial evaluation. A stability study carried out in pH 7.4 potassium phosphate buffer solution on the three *gem*-dimethyl trigger BAPCs (**14, 22, 40**) demonstrated promising results, with each BAPC remaining structurally intact over a 24 h period with no observable (by HPLC analysis) cleavage or degradation. These same three BAPCs (**14, 22, 40**) were treated (in separate experiments) with NADPH cytochrome P450 oxidoreductase (POR) to evaluate them as substrates for this enzyme under anoxic conditions.

The *gem*-dimethyl furan and thiophene compounds (**14** and **40**, respectively) were fully cleaved over the course of 24 h, while, interestingly, the *gem*-dimethyl benzyl compound **22** did not undergo cleavage in the 24 h assay (Table 2.2). The reduction potential for the nitrobenzyl trigger is less than the reduction potential (less electron-philic) than that of the nitrofuran, nitroimidazole, and nitrothiophene triggers, possibly explaining its resistance to cleavage by POR under these assay conditions.[108]

Table 2.1.
Inhibition of Tubulin Polymerization
and Percent Inhibition of Colchicine Binding

Compd	Inhibition of Tubulin Assembly IC_{50}	Inhibition of Colchicine Binding % Inhibition μM ±SD
	(μM)±SD	5 μM
42 CA4	0.73 ± 0.04	98 ± 0.1
6 Phenstatin	1.0 ± 0.2^a	85 ± 2
12	16 ± 0.6	19 ± 2
13	>20	15 ± 0.09
14	9.0 ± 1	16 ± 3
20	>20	11 ± 4
21	>20	5.8 ± 5
22	>20	8.8 ± 5
31	>20	7.1 ± 0.01
32	>20	11 ± 5
38	15 ± 1	17 ± 0.01
39	12 ± 0.5	16 ± 3
40	>20	13 ± 5

[a]data from reference **23**

Table 2.2
Bioreductive Trigger Hydrolysis (Untreated)
and Cleavage of POR-Treated BAPCs

Compd	Hydrolysis Percentage in pH 7.4 Phosphate Buffer for 24 h	Cleavage Percentage of POR-treated for 24 h[a]
14	0	100
22	0	0
40	0	100

[a]anoxic conditions

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In conclusion, a series of eleven promising phenstatin-based BAPCs were prepared by chemical synthesis and had little to on activity as inhibitors of tubulin assembly or binding of colchicine to tubulin, in comparison to the parent anticancer agent phenstatin, a potent tubulin inhibitor. In preliminary studies, the three phenstatin-based *gem*-dimethyl BAPCs (**14, 22, 40**) demonstrated aqueous solution stability (over 24 h), and two of the BAPCs (**14, 40**) were suitable substrates for POR. These BAPCs have the potential to be therapeutic agents that target hypoxic tumor cells.

Experimental Section

Chemistry

General Materials and Methods.[109,110] CH_2Cl_2 and tetrahydrofuran (THF) were used in their anhydrous forms, as obtained from the chemical suppliers. Reactions were performed under an inert atmosphere using N_2. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F254, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a flash purification system using silica gel (200-400 mesh, 60 Å) or RP-18 prepacked columns. Intermediates and products synthesized were characterized on the basis of their ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data. TMS was used as an internal standard for spectra recorded in $CDCl_3$. All the chemical shifts are expressed in ppm (δ), coupling constants (J) are presented in Hz, and peak patterns are reported as broad (br), singlet (s), doublet (d), doublet of doublets (dd) triplet (t), quartet (q), septet (sept), and multiplet (m). HRESIMS were obtained using positive or negative electrospray ionization (ESI) techniques using a Thermo Scientific LTQ OrbitrapDiscovery instrument. 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 x 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column. Flow rate 1.0 mL/min; injection volume 20 μL; monitored at 254 nm, 300 nm, 320 nm. Two different HPLC gradients were used for purity analysis; Method A: water/acetonitrile, gradient 10:90 to 90:10 from 0 to 25 min and isocratic 90:10 from 25 to 30 min; Method B: water/acetonitrile, gradient 50:50 to 90:10 from 0 to 25 min and isocratic 90:10 from 25 to 30 min (note: 4-dimethylaminopyridine is abbreviated DMAP, ethyl acetate is abbreviated EtOAc, *N,N*-dimethylformamide is abbreviated DMF, chloroform-d is abbreviated CDCl$_3$]

3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (**2**):[102] Isovanillin (2.01 g, 13.2 mmol), triethylamine (4.00 mL, 28.5 mmol), and DMAP (0.045 g, 0.37 mmol) were dissolved in dry CH$_2$Cl$_2$ (60 mL). *tert*-Butyldimethylsilyl chloride (2.214 g, 14.7 mmol) was added to the reaction mixture, which was stirred for 12 h. The reaction was quenched with water, extracted with diethyl ether (Et$_2$O), which was washed with water and brine, dried with Na$_2$SO$_4$, 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, 10% A/90% B (1 CV), 10% A/90% B → 27% A/73% B (10 CV), 27% A/73% B over (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm] yielded 3-((*tert*-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (**2**) (3.17 g, 11.9 mmol, 90%) as a yellow oil.

¹H NMR (500 MHz, CDCl₃) δ 9.82 (1H, s), 7.49 (1H, dd, *J* = 8.5 Hz, *J* = 2 Hz), 7.37 (1H, d, *J* = 2 Hz), 6.96 (1H, d, *J* = 8.5 Hz), 3.90 (3H, s), 1.00 (9H, s), 0.17 (6H, s).

¹³C NMR (125 MHz, CDCl₃) δ 190.9, 156.6, 145.3, 130.2, 126.3, 120.0, 111.1, 55.6, 25.6, 18.4, -4.6.

(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (**4**):[102] 1-Bromo-3,4,5-trimethoxybenzene (1.81 g, 7.31 mmol) was dissolved in dry THF (60 mL) in a dry ice/acetone bath (-78 °C). *n*-Butyllithium (2.8 mL, 7.0 mmol, 2.5 M) was added dropwise to the reaction mixture, which was stirred for 30 min. 3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (2.00 g, 7.50 mmol) dissolved in dry THF (20 mL) was added dropwise, and the reaction mixture was stirred for 5 h. The reaction was quenched with water, acidified to pH 7 with 3 M HCl, extracted with Et₂O, washed with water and brine, dried with Na₂SO₄, 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B (10 CV), 80% A/20% B over (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm] yielded (3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (**4**) (2.02 g, 4.65 mmol, 62%) as a pale yellow oil.

¹H NMR (500 MHz, CDCl₃) δ 6.89 (2H, m), 6.80 (1H, d, *J* = 8.5 Hz), 6.57 (2H, d, *J* = 4.5 Hz), 5.24 (1H, d, *J* = 4.5 Hz), 3.81 (3H, s), 3.77 (9H, s), 0.94 (9H, d, *J* = 3.5 Hz), 0.11 (6H, d, *J* = 2.5 Hz).

^{13}**C NMR** (125 MHz, CDCl$_3$) δ 153.0, 150.3, 144.7, 140.0, 136.5, 119.9, 119.4, 111.8, 103.4, 75.5, 60.7, 55.9, 55.5, 25.7, 18.4, -4.6.

(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (**5**):[102]   (3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (3.00 g, 6.90 mmol), Celite (2.45 g), and potassium carbonate [K$_2$CO$_3$] (2.46 g, 17.8 mmol) were dissolved in dry CH$_2$Cl$_2$ (130 mL) in an ice bath (0 °C). Pyridinium chlorochromate [PCC] (1.52 g, 7.04 mmol) was added in small increments and the reaction mixture was stirred for 18 h. The reaction mixture was filtered with CH$_2$Cl$_2$ in a frit funnel containing a 50/50 mixture of Celite and silica gel and then 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, 10% A/90% B (1 CV), 10% A/90% B → 45% A/55% B (10 CV), 45% A/55% B (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm] yielded (3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (**5**) (1.79 g, 4.14 mmol, 60%) as a yellow oil.

^{1}**H NMR** (500 MHz, CDCl$_3$) δ 7.40 (1H, d, *J* = 8 Hz), 7.33 (1H, s), 6.99 (2H, s), 6.87 (1H, d, *J* = 8.5 Hz), 3.88 (3H, s), 3.84 (3H, s), 3,83 (6H, s), 0.96 (9H, s), 0.14 (6H, s).

^{13}**C NMR** (125 MHz, CDCl$_3$) δ 194.5, 154.9, 152.7, 144.6, 141.5, 133.3, 130.4, 125.3, 122.3, 110.7, 107.4, 60.9, 56.2, 55.5, 25.6, 18.4, -4.6.

Phenstatin (**6**):[102] (3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (3.59 g, 8.31 mmol) was dissolved in dry THF (100 mL). Tetrabutylammonium fluoride trihydrate (3.93 g, 12.5 mmol) was added, and the reaction

mixture was stirred for 18 h. The reaction was quenched with water, acidified to pH 7 with 3 M HCl, and extracted with EtOAc, which 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 → 99% A/1% B over 13.12 min (10 CV), 99% A/1% B over 2.38 min (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm] yielded phenstatin (**6**) (2.06 g, 6.47 mmol, 78%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (1H, s), 7.37 (1H, d, J = 8.5 Hz). 7.01 (2H, s), 6.90 (1H, d, J = 8 Hz), 3.94 (3H, s), 3.90 (3H, s), 3.85 (6H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.7, 152.8, 150.2, 145.3, 141.6, 133.1, 131.0, 123.7, 116.2, 109.7, 107.5, 61.0, 56.3, 56.1.

HRMS [M+Na]$^+$: 341.0997 (calcd for [$C_{17}H_{18}O_6Na$]$^+$, 341.1103).

HPLC retention time (Method A): 15.35 min.

(5-Nitrothiophen-2-yl)methanol (**8**):[88] 5-Nitrothiophene-2-carboxaldehyde (1.00 g, 6.38 mmol) was dissolved in dry methanol (20 mL) in an ice bath (0 °C). $NaBH_4$ (0.270 g, 7.14 mmol) was added, and the reaction mixture was stirred for 2 h. Ice was added and the solution was acidified to pH 7 with 3 M HCl. The reaction mixture was extracted with EtOAc, 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 (1 CV), 10% A/90% B → 65% A/35% B (10 CV), 65% A/35% B (2 CV); flow rate 50.0 mL/min; monitored at 254 and 280 nm] yielded alcohol **8** (0.914 g, 5.74 mmol, 90%) as a brown oil.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.84 (1H, d, J = 4.1 Hz), 6.95 (1H, dt, J = 4.1, 1.0 Hz), 4.90 (2H, d, J = 5.2 Hz), 2.15 (1H, t, J = 5.8 Hz).

^{13}C NMR (126 MHz, CDCl$_3$) δ 154.0, 150.6, 129.0, 123.5, 60.2.

1-(5-Nitrothiophen-2-yl)ethan-1-ol (9):[103] TiCl$_4$ (7.84 g, 41.3 mmol) was added slowly dropwise into Et$_2$O (80 mL) at -78 °C, after which methyllithium (1.6 M, 25.8 mL, 41 mmol) was added drop-wise, and the reaction mixture was stirred for 1.5 h. 5-Nitro-2-thiophenecarboxaldehyde (5.00g, 31.8 mmol) was dissolved in Et$_2$O (120 mL) and added dropwise to the reaction mixture, which was stirred (12 h). H$_2$O (50 mL) was used to quench the reaction. The layers were partitioned, and the residue was extracted with EtOAc (6 × 40 mL). The combined extracts were washed with brine, dried over Na$_2$SO$_4$, 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 alcohol **9** (4.95 g, 28.6 mmol, 90%) as a dark brown oil.

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.82 (1H, d, J = 4.2 Hz), 6.91 (1H, dd, J = 4.2, 1.0 Hz), 5.14 (1H, qd, J = 6.4, 1.0 Hz), 2.14 (1H, s), 1.64 (3H, d, J = 6.5 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-ol (9) [Alternate Route]: 5-Nitro-2-thiophenecarboxaldehyde (1.00 g, 6.36 mmol) was dissolved in CH$_2$Cl$_2$ (50 mL) at 0 °C. Trimethylaluminum (2 M, 5.30 mL, 10.6 mmol) was added dropwise, and the reaction mixture was stirred for 2 h. The reaction was quenched with HCl (1 M, 40 mL) and the

layers were partitioned. The residue was extracted with CH_2Cl_2 (3 x 30 mL), and the combined organic phase was washed with brine (40 mL), dried over Na_2SO_4, and evaporated 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 alcohol **11** (1.01 g, 5.85 mmol, 92%) as yellow-orange crystals.

1-(5-Nitrothiophen-2-yl)ethan-1-one (10): 2-(1-Hydroxyethyl)-5-nitrothiophene (1.04 g, 6.00 mmol) was dissolved in 70 mL CH_2Cl_2 at rt. Dess-Martin periodinane (3.82 g, 9.00mmol) was added in portions to the solution, and the reaction mixture was stirred (1 h). Saturated solutions of sodium thiosulfate (50 mL) and $NaHCO_3$ (50 mL) were used to quench the reaction mixture. The layers were partitioned, and the residue was extracted with EtOAc (4 x 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 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] affording ketone **10** (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 190.35, 156.47, 148.16, 130.06, 128.28, 26.61.

2-(5-Nitrothiophen-2-yl)propan-2-ol (11):[103] TiCl$_4$ (3.62 g, 19.1 mmol) was slowly

added dropwise into Et$_2$O (80 mL) at -78 °C, after which methyllithium (1.6 M, 11.9 mL,

19 mmol) was added dropwise, and the reaction mixture was stirred for 1.5 h. 2-Acetyl-5-

nitrothiophene (2.50 g, 14.7 mmol) was dissolved in Et$_2$O (140 mL) and added dropwise

to the reaction mixture, which was stirred (12 h). H$_2$O (50 mL) was used to quench the

reaction mixture. The layers were partitioned, and the residue was extracted with

EtOAc (6 × 40 mL). The combined extracts were washed with brine, dried over Na$_2$SO$_4$,

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 alcohol **11** (1.61 g, 8.60 mmol, 45%) as a dark orange oil.

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.80 (1H, d, J = 4.2 Hz), 6.89 (1H, d, J = 4.2 Hz), 1.69

(6H, s).

^{13}C NMR (151 MHz, CDCl$_3$) δ 163.46, 150.04, 128.76, 121.26, 71.92, 32.08.

2-(5-Nitrothiophen-2-yl)propan-2-ol (11) [Alternate Route]: 2-Acetyl-5-

nitrothiophene (0.500 g, 2.92 mmol) was dissolved in CH$_2$Cl$_2$ (20 mL) at 0 °C.

Trimethylaluminum (2 M, 2.42 mL, 4.85 mmol) was added dropwise, and the reaction

mixture was stirred for 2 h. The reaction was quenched with HCl (1 M, 30 mL), and the

layers were partitioned. The residue was extracted with CH$_2$Cl$_2$ (3 x 20 mL), and the

combined organic extracts were washed with brine (20 mL), dried over Na$_2$SO$_4$, and

evaporated under reduced pressure. The crude product was purified by flash column

chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280 nm] affording alcohol **11** (0.365 g, 2.13 mmol, 73%) as bright orange crystals.

(4-Methoxy-3-((5-nitrothiophen-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (12):[88,102] Phenstatin (0.405 g, 1.27 mmol), DIAD (0.289 g, 1.43 mmol), and (5-nitrothiophen-2-yl)methanol (0.454 g, 2.85 mmol) were dissolved in dry CH_2Cl_2 (40 mL). Triphenylphosphine (0.574 g, 2.19 mmol) was added, and the reaction mixture was stirred for 2 d. The reaction was quenched with water (30 mL) and extracted with EtOAc (3 x 30 mL), which 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, 17% A/83% B (1 CV), 17% A/83% B → 100% A/0% B (10 CV), 100% A/0% B (2 CV); flow rate 35.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((5-nitrothiophen-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**12**) (0.198 g, 0.431 mmol, 34%) as a brown solid.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.83 (1H, d, J = 4.5 Hz), 7.51 (1H, d, J = 1.5 Hz), 7.49 (1H, dd, J = 8 Hz, J = 1.5 Hz), 7.06 (1H, d, J = 4.5), 6.99 (2H, s), 6.97 (1H, d, J = 8.5 Hz), 5.33 (2H, s), 3.98 (3H, s), 3.93 (3H, s), 3.87 (6H, s).

^{13}C NMR (125 MHz, CDCl$_3$) δ 194.3, 153.7, 152.8, 147.7, 146.9, 141.7, 133.0, 130.2, 128.4, 126.6, 125.2, 115.5, 110.6, 107.4, 66.3, 61.0, 56.3, 56.2.

HRMS [M+Na]$^+$: 482.0880 (calcd for [C$_{22}$H$_{21}$NNaO$_8$S]$^+$, 482.0880).

(4-Methoxy-3-(1-(5-nitrothiophen-2-yl)ethoxy)phenyl)(3,4,5-

trimethoxyphenyl)methanone (13):[88,102] Phenstatin (0.407 g, 1.28 mmol), DIAD (0.294

g, 1.45 mmol), and 1-(5-nitrothiophen-2-yl)ethanol (0.505 g, 2.92 mmol) were dissolved

in dry CH_2Cl_2 (40 mL). Triphenylphosphine (0.558 g, 2.13 mmol) was added, and the

reaction mixture was stirred for 2 d. The reaction was quenched with water and extracted

with EtOAc, which was dried with Na_2SO_4 and evaporated under reduced pressure. Flash

chromatography of the crude product using a prepacked 25 g silica column [eluents:

solvent A, EtOAc; solvent B, hexanes; gradient, 15% A/85% B (1 CV), 15% A/85% B →

100% A/0% B (10 CV), 100% A/0% B (2 CV); flow rate 20.0 mL/min; monitored at 254

and 280 nm] yielded (4-methoxy-3-(1-(5-nitrothiophen-2-yl)ethoxy)phenyl)(3,4,5-

trimethoxyphenyl)methanone (**13**) (0.179 g, 0.378 mmol, 30%) as a tan yellow solid.

¹H NMR (500 MHz, $CDCl_3$) δ 7.77 (1H, d, J = 4.2 Hz), 7.48 (1H, dd, J = 8.4, 2.0 Hz),

7.45 (1H, d, J = 2.0 Hz), 6.99 – 6.92 (4H, m), 5.60 (1H, q, J = 6.4 Hz), 3.96 (3H, s), 3.91

(3H, s), 3.84 (6H, s), 1.77 (3H, d, J = 6.4 Hz).

¹³C NMR (125 MHz, $CDCl_3$) δ 194.2, 154.8, 154.4, 152.8, 151.0, 145.9, 141.7, 133.0,

130.2, 128.4, 126.8, 123.4, 118.4, 110.9, 107.4, 73.6, 61.0, 56.3, 56.1, 23.2.

HRMS [M+Na]⁺: 496.1038 (calcd for $[C_{23}H_{23}NNaO_8S]^+$, 496.1037).

HPLC retention time (Method B): 10.33 min.

 (4-Methoxy-3-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-

trimethoxyphenyl)methanone (14):[88,102] Phenstatin (0.581 g, 1.83 mmol), ADDP (0.597

g, 2.38 mmol), and 2-(5-nitrothiophen-2-yl)propan-2-ol (0.410 g, 2.19 mmol) were dissolved in dry CH_2Cl_2 (80 mL). Tributylphosphine (0.752 mL, 3.06 mmol) was added dropwise, and the reaction mixture was stirred for 2 d. The reaction mixture was dried under reduced pressure. Flash chromatography of the crude product using a prepacked 25 g silica column [eluents: 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 50.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**14**) (0.062 g, 0.128 mmol, 7%) as an orange gum.

1H NMR (600 MHz, acetone-d_6) δ 7.93 (1H, d, J = 4.3 Hz), 7.67 (1H, dd, J = 8.5, 2.2 Hz), 7.41 (1H, d, J = 2.1 Hz), 7.22 (1H, d, J = 8.5 Hz), 7.19 (1H, d, J = 4.3 Hz), 7.02 (2H, s), 3.95 (3H, s), 3.87 (6H, s), 3.84 (3H, s), 1.79 (6H, s).

13C NMR (151 MHz, acetone-d_6) δ 192.94, 161.01, 157.41, 153.10, 150.43, 142.93, 141.89, 133.12, 129.98, 128.83, 127.80, 125.38, 123.07, 111.83, 107.31, 80.82, 59.80, 55.74, 55.44, 28.16.

HRMS [M+Na]$^+$: 510.1190 (calcd for [$C_{24}H_{25}NNaO_8S$]$^+$, 510.1193).

HPLC retention time (Method B): 11.49 min.

1-(4-Nitrophenyl)ethan-1-ol (16):[103] $TiCl_4$ (2.72 mL, 24.8 mmol) was added dropwise slowly to dry Et_2O (100 mL) in an acetone / dry ice bath (-78 °C). Methyllithium (15.5 mL, 25 mmol, 1.6 M) was then added dropwise slowly to the reaction mixture which was stirred for 1.5 h. 4-Nitrobenzaldehyde (2.88g, 19.1 mmol) dissolved in Et_2O (140 mL) was added dropwise to the reaction mixture, which was stirred for 18 h. The reaction was

quenched with water and extracted with CH_2Cl_2 (3 x 50 mL), which was washed with water and brine and dried over 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B over (10 CV), 80% A/20% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded 1-(4-nitrophenyl)ethan-1-ol (**16**) (2.49 g, 14.9 mmol, 78%) as a yellow-orange oil.

1H NMR (600 MHz, CDCl$_3$) δ 8.17 (2H, d, J = 8.7 Hz), 7.53 (2H, d, J = 8.6 Hz), 5.01 (1H, q, J = 6.5 Hz), 1.51 (3H, d, J = 6.6 Hz).

13C NMR (151 MHz, CDCl$_3$) δ 153.22, 147.09, 126.13, 123.71, 69.43, 25.44.

2-(4-Nitrophenyl)propan-2-ol (**18**):[103]   TiCl$_4$ (3.02 mL, 27.6 mmol) was added dropwise slowly to dry Et$_2$O (100 mL) in an acetone / dry ice bath (-78 °C). Methyllithium (17.2 mL, 28 mmol, 1.6 M) was then added dropwise slowly to the reaction mixture, which was stirred for 1.5 h. 4-Nitroacetophenone (3.50g, 21.2 mmol) dissolved in Et$_2$O (150 mL) was added dropwise to the reaction mixture, which was stirred for 18 h. The reaction was quenched with water, and the mixture was extracted with CH_2Cl_2 (3 x 50 mL), which was washed with water and brine, dried over 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, 10% A/90% B (1 CV), 10% A/90% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded 2-(4-nitrophenyl)propan-2-ol (**18**) (1.42 g, 7.84 mmol, 37%) as an orange oil.

^{1}H NMR (600 MHz, CDCl$_3$) δ 8.16 (2H, d, J = 8.9 Hz), 7.65 (2H, d, J = 8.9 Hz), 1.61 (7H, s).

^{13}C NMR (151 MHz, CDCl$_3$) δ 156.52, 146.64, 125.51, 123.45, 72.49, 31.69.

(4-methoxy-3-((4-nitrobenzyl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone

(20):[88,102,105,106] Phenstatin (0.500 g, 1.57 mmol), DIAD (0.35 mL, 1.9 mmol), and 4-nitrobenzyl alcohol (0.481 g, 3.14 mmol) were dissolved in dry CH$_2$Cl$_2$ (60 mL). Triphenylphosphine (0.700 g, 2.67 mmol) was added, and the reaction mixture was stirred for 2 d. The reaction was quenched with water, and the reaction mixture was extracted with EtOAc, which was dried with Na$_2$SO$_4$ 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B (10 CV), 80% A/20% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((4-nitrobenzyl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**20**) (0.462 g, 1.02 mmol, 65%) as a yellow solid.

^{1}H NMR (600 MHz, acetone-d_6) δ 8.31 (2H, d, J = 8.7 Hz), 7.84 (2H, d, J = 9.0 Hz), 7.54 (1H, d, J = 2.0 Hz), 7.52 (1H, dd, J = 8.3, 2.0 Hz), 7.18 (1H, d, J = 8.4 Hz), 7.04 (2H, s), 5.40 (2H, s), 4.00 (3H, s), 3.87 (6H, s), 3.84 (3H, s).

^{13}C NMR (151 MHz, acetone-d_6) δ 193.21, 153.71, 153.10, 147.67, 145.12, 141.81, 133.24, 130.25, 128.06, 125.41, 123.49, 114.93, 110.99, 107.37, 99.99, 69.45, 59.80, 55.70, 55.54. **HRMS** [M+Na]$^+$: 476.1315 (calcd for [C$_{24}$H$_{23}$NNaO$_8$]$^+$, 476.1316).

HPLC retention time (Method B): 9.55 min.

(4-Methoxy-3-(1-(4-nitrophenyl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (21):[88,102,105,106] Phenstatin (0.500 g, 1.57 mmol), DIAD (0.348 mL, 1.88 mmol), and 1-(4-nitrophenyl)ethan-1-ol (0.525 g, 3.14 mmol) were dissolved in dry CH_2Cl_2 (60 mL). Triphenylphosphine (0.700 g, 2.67 mmol) was added to the reaction mixture, which was stirred for 2 d. The reaction was quenched with water, and the reaction mixture was extracted with CH_2Cl_2 (3 x 40 mL), which 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B over (10 CV), 80% A/20% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-(1-(4-nitrophenyl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**21**) (0.315 g, 0.675 mmol, 43%) as white solid.

^{1}H NMR (600 MHz, acetone-d_6) δ 8.26 (2H, d, J = 8.8 Hz), 7.78 (2H, d, J = 8.4 Hz), 7.46 (1H, dd, J = 8.4, 2.0 Hz), 7.34 (1H, d, J = 2.0 Hz), 7.15 (1H, d, J = 8.4 Hz), 6.93 (2H, s), 5.72 (1H, q, J = 6.4 Hz), 4.00 (3H, s), 3.83 (3H, s), 3.81 (6H, s), 1.69 (3H, d, J = 6.5 Hz).

^{13}C NMR (151 MHz, CDCl$_3$) δ 194.27, 153.96, 152.80, 150.05, 147.48, 146.57, 141.76, 133.04, 130.14, 126.53, 125.83, 124.01, 116.93, 110.61, 107.50, 60.98, 56.36, 56.14, 23.86, 21.95. **HRMS** [M+Na]$^+$: 490.1471 (calcd for [C$_{25}$H$_{25}$NNaO$_8$]$^+$, 490.1472).

HPLC retention time (Method B): 10.05 min.

(4-Methoxy-3-((2-(4-nitrophenyl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (22):[88,102,105,106] Phenstatin (0.500 g, 1.57 mmol), ADDP

(0.475 g, 1.88 mmol), and 2-(4-nitrophenyl)propan-2-ol (0.569 g, 3.14 mmol) were dissolved in dry CH_2Cl_2 (70 mL). Tributylphosphine (0.66 mL, 2.67 mmol) was added dropwise to the reaction mixture, which was stirred for 2 d. The reaction was quenched with water, and the mixture was extracted with EtOAc, which was washed with water and brine, 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B (10 CV), 80% A/20% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((2-(4-nitrophenyl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**22**) (0.174 g, 0.361 mmol, 23%) as tan solid,

^{1}H NMR (600 MHz, acetone-d_6) δ 8.25 (2H, d, J = 8.9 Hz), 7.91 (2H, d, J = 8.9 Hz), 7.57 (1H, dd, J = 8.5, 2.1 Hz), 7.18 (1H, d, J = 8.5 Hz), 7.12 (1H, d, J = 2.1 Hz), 6.92 (2H, s), 3.96 (3H, s), 3.84 (6H, s), 3.83 (3H, s), 1.76 (6H, s).

^{13}C NMR (151 MHz, acetone-d_6) δ 193.05, 156.74, 154.53, 153.03, 147.02, 143.92, 141.76, 133.21, 129.72, 126.62, 126.34, 123.48, 123.30, 111.62, 107.16, 81.28, 59.77, 55.73, 55.43, 27.99.

HRMS [M+Na]$^+$: 504.1629 (calcd for [$C_{26}H_{27}NNaO_8$]$^+$, 504.1629).

HPLC retention time (Method B): 10.82 min.

Ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate (26):[104] To a suspension of sarcosine ethyl ester (4.00 g, 0.026 mol) in THF (90 mL) and ethyl formate (90 mL) was added NaH (60 % dispersion in mineral oil, 10.0 g, 0.25 mol) in several portions at room temperature. The reaction mixture was stirred for 3 h, and, during this time, a yellow

suspension formed. The reaction mixture was concentrated and triturated with hexane (2 x 150 mL). The hexane was decanted, and the resulting light tan solid was dried in *vacuo*. Ethanol (80 mL) and concentrated aqueous HCl (16 mL) were added to the solid, and the suspension was heated to reflux for 2 h. The reaction mixture was then filtered while hot, and the filter was rinsed with boiling ethanol (2 x 50 mL). The combined filtrate was concentrated to yield a brown oil. The oil was diluted with ethanol (140 mL) and water (60 mL), and the pH of the solution was adjusted to 3 by using NaOH solution (2 M). Cyanamide (2.18 g, 0.052 mol) was added, and the resulting solution was heated to reflux for 1.5 h. After being cooled to room temperature, the reaction mixture was concentrated to approximately 1/8 of its original volume. Solid K_2CO_3 was added to adjust the pH of the concentrated reaction mixture to 8-9, resulting in the formation of a yellow precipitate. The solid was removed by filtration, washed with a K_2CO_3 solution (1 M, 1 x 20 mL) and water (2 x 20 mL) and dried to afford a pale yellow solid (1.97 g, 12.0 mmol, 45%).

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.45 (1H, s), 4.27 (2H, q, *J* = 7.1 Hz), 4.25 (2H, s), 3.68 (3H, s), 1.34 (3H, t, *J* = 7.1 Hz).

^{13}C NMR (151 MHz, CDCl$_3$) δ 160.67, 151.89, 135.50, 119.05, 59.82, 30.55, 14.41.

Ethyl 1-methyl-2-nitro-1*H*-imidazole-5-carboxylate (27):[104,107] Aminoimidazole (0.700 g, 4.14 mmol) in acetic acid (7.3 mL) was added dropwise to an aqueous solution of sodium nitrite (3.6 mL, 11 M). The solution was stirred at room temperature for 4 h until no more N_2 was formed. The reaction mixture was extracted with CH$_2$Cl$_2$ (1 x 20 mL), washed with brine (1 x 20 mL) and a saturated aqueous solution of Na$_2$SO$_3$ (1 x 20

mL). The organic layer was then dried over Na_2SO_4, filtered and concentrated to afford a crude yellow solid. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 70 mL/min; monitored at 254 and 280 nm] afforded the nitroimidazole analogue **27** (0.510 g, 2.60 mmol, 63%) as a yellow solid.

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.74 (1H, s), 4.40 (2H, q, J = 7.1 Hz), 4.35 (3H, s), 1.41 (3H, t, J = 7.1 Hz).

^{13}C NMR (151 MHz, CDCl$_3$) δ 159.08, 147.46, 134.67, 126.29, 61.84, 35.39, 14.18.

(1-Methyl-2-nitro-1H-imidazol-5-yl)methanol (28):[104,107] A suspension of the nitroimidazole ethyl ester (0.796 g, 4.00 mmol) in 0.75 M NaOH solution (16 mL) was stirred at room temperature overnight to give a clear light yellow solution. The pH of the reaction mixture was adjusted to 1 by adding concentrated HCl. The resulting solution was extracted with EtOAc (5 x 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to afford a light yellow solid. The solid was dissolved in THF (8 mL) with triethylamine (0.880 mL, 6.30 mmol). Isobutylchloroformate (0.820 mL, 6.30 mmol) was added dropwise at -40 ^{o}C, and the reaction mixture was stirred at room temperature for 1 h. NaBH$_4$ (0.794 g, 21.0 mmol) was added to the solution, followed by dropwise addition of water (7 mL) over 1 h while maintaining the temperature around 0 ^{o}C. The reaction mixture was extracted with Et_2O (3 x 20 mL), which was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: methanol; solvent B: CH$_2$Cl$_2$; gradient:

1%A / 99%B (4 CV), 1%A / 99%B → 15%A / 85%B (10 CV), 15%A / 85%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] afforded the normethyl nitroimidazole trigger (**28**) (0.449 g, 2.86 mmol, 71%) as a pale yellow solid.

¹H NMR (600 MHz, Methanol-d_4) δ 7.11 (1H, s), 4.68 (2H, s), 4.06 (3H, s).

¹³C NMR (151 MHz, MeOD) δ 145.82, 137.93, 126.02, 53.16, 33.40.

1-Methyl-2-nitro-1*H*-imidazole-5-carbaldehyde (29): Normethyl nitroimidazole trigger **28** (359 mg, 2.28 mmol) was dissolved in CH_2Cl_2 (10 mL). Dess–Martin periodinane (1.16 g, 2.74 mmol) was added and the reaction mixture was stirred for 1 h at room temperature. Saturated solutions of $NaHCO_3$ (20 mL) and sodium thiosulfate (20 mL) were added to the reaction mixture, which was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (1 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] afforded imidazole analogue (346 mg, 2.23 mmol, 98%) as a yellow solid**.**

¹H NMR (600 MHz, CDCl₃) δ 9.94 (1H, s), 7.82 (1H, s), 4.36 (3H, s).

¹³C NMR (151 MHz, CDCl₃) δ 180.39, 148.35, 139.38, 132.38, 35.57.

1-(1-Methyl-2-nitro-1*H*-imidazol-5-yl)ethan-1-ol (30):[103] $TiCl_4$ (1.3 mL, 11 mmol) in Et_2O (60 mL) was treated with methyllithium (7.1 mL, 1.6M, 11 mmol) at -78 °C, and the resulting solution was stirred for 1 h. A THF (15 mL) solution of imidazole aldehyde

analogue (0.884 g, 5.70 mmol) was added dropwise and the reaction mixture was stirred for 24 h. Water (50 mL) was added and the resulting solution was extracted with EtOAc (3 x 50 mL), which was dried over Na_2SO_4 and concentrated to afford a crude brown oil. Purification by flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 17%A / 83%B (1 CV), 17%A / 83%B → 100%A / 0%B (7 CV), 100%A / 0%B (5 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] afforded the monomethyl nitroimidazole trigger (**30**) (400 mg, 2.34 mmol, 41%) as a yellow solid.

¹H NMR (600 MHz, acetone-d_6) δ 7.07 (1H, s), 5.01 (1H, p, *J* = 6.2 Hz), 4.64 (1H, d, *J* = 6.0 Hz), 4.09 (3H, s), 1.63 (3H, d, *J* = 6.6 Hz).

¹³C NMR (151 MHz, acetone-d_6) δ 146.4, 141.6, 124.7, 60.4, 33.9, 21.1

(4-Methoxy-3-((1-methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**31**):[88,102,105,106] Phenstatin (0.500 g, 1.57 mmol), (1-methyl-2-nitro-1*H*-imidazol-5-yl)methanol (0.296 g, 1.89 mmol), and DIAD (0.40 mL, 2.04 mmol) were dissolved in CH_2Cl_2. Triphenylphosphine (0.825 g, 3.14 mmol) was added to the mixture, and the reaction mixture was stirred for 24 h. The reaction mixture was then 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, 17%A/83%B over 1.19 min (1 CV), 17%A/83%B → 100%A/0%B over 8.33 min (7 CV), 100%A / 0%B over 5.95 min (5 CV); flow rate 100 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((1-methyl-2-nitro-1*H*-imidazol-5-

yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**31**) (0.346 g, 0.757 mmol, 48%) as a pale yellow-white solid.

¹H NMR (600 MHz, CDCl₃) δ 7.62 (1H, d, *J* = 1.7 Hz), 7.52 (1H, dd, *J* = 8.3, 1.7 Hz), 7.24 (1H, s), 7.04 (2H, s), 6.97 (1H, d, *J* = 8.4 Hz), 5.18 (2H, s), 4.16 (3H, s), 3.97 (3H, s), 3.96 (3H, s), 3.91 (6H, s).

¹³C NMR (151 MHz, CDCl₃) δ 194.16, 153.97, 152.91, 146.74, 141.88, 132.92, 132.30, 130.43, 129.31, 127.00, 116.43, 110.61, 107.52, 99.98, 61.24, 61.01, 56.39, 56.05, 34.54.

HRMS [M+Na]⁺: 480.1376 (calcd for [C₂₂H₂₃N₃NaO₈]⁺, 480.1377).

HPLC retention time (Method B): 4.66 min.

 (4-methoxy-3-(1-(1-methyl-2-nitro-1*H*-imidazol-5-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**32**):[88,102,105,106] Phenstatin (0.250 g, 0.786 mmol), DIAD (0.19 mL, 1.02 mmol), and 1-(1-methyl-2-nitro-1*H*-imidazol-5-yl)ethan-1-ol (0.161 g, 0.943 mmol) were added to dry CH₂Cl₂ (50 mL). Triphenylphosphine (0.412 g, 1.57 mmol) was added, and the reaction mixture was stirred for 2 d. The reaction solvent was 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, 15%A/85%B over 1.19 min (1 CV), 15%A/85%B → 100%A/0%B over 8.33 min (7 CV), 100%A / 0%B over 14.28 min (12 CV); flow rate 100 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-(1-(1-methyl-2-nitro-1*H*-imidazol-5-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**32**) (0.119 g, 0.252 mmol, 32%) as a pale yellow-white solid.

¹H NMR (600 MHz, CDCl₃) δ 7.57 (1H, d, *J* = 2.0 Hz), 7.55 (1H, dd, *J* = 8.3, 2.0 Hz), 7.21 (1H, s), 7.02 (2H, s), 6.99 (1H, d, *J* = 8.4 Hz), 5.59 (1H, q, *J* = 6.5 Hz), 4.13 (3H, s), 3.97 (3H, s), 3.96 (3H, s), 3.91 (6H, s), 1.81 (3H, d, *J* = 6.5 Hz).

¹³C NMR (151 MHz, CDCl₃) δ 194.09, 154.63, 152.90, 145.23, 141.89, 137.09, 132.88, 130.47, 127.15, 118.90, 110.95, 107.51, 99.98, 68.42, 61.01, 56.39, 56.04, 34.70, 18.55.

HRMS [M+Na]⁺: 494.1533 (calcd for [C₂₃H₂₅N₃NaO₈]⁺ 494.1534).

HPLC retention time (Method B): 5.17 min.

(5-Nitrofuran-2-yl)methanol (34):[88] 5-Nitrofuran-2-carbaldehyde (4.00 g, 28 mmol) was dissolved in anhydrous methanol (80 mL) and cooled to 0 °C. NaBH₄ (1.17 g, 31 mmol) was added to the reaction mixture, which was stirred for 2.5 h. The reaction was quenched with an HCl solution (1 M, 40 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to afford a crude yellow oil. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] afforded (5-nitrofuran-2-yl)methanol (**34**) (3.23 g, 22.6 mmol, 80%) as a pale yellow oil.

¹H NMR (600 MHz, CDCl₃) δ 7.31 (1H, d, *J* = 3.6 Hz), 6.58 (1H, d, *J* = 3.6 Hz), 4.74 (2H, s), 2.09 (1H, s).

¹³C NMR (151 MHz, CDCl₃) δ 157.37, 151.92, 112.40, 110.61, 57.45.

1-(5-Nitrofuran-2-yl)ethan-1-ol (**35**):[103] TiCl$_4$ (0.78 mL, 7.1 mmol) in Et$_2$O (35 mL) was treated with methyllithium (4.4 mL, 1.6 M, 7.1 mmol) at -78 ^{o}C. The resulting solution was stirred for 1 h. A THF (10 mL) solution of 5-nitrofuran-2-carbaldehyde (0.500 g, 3.5 mmol) was added dropwise, and the reaction mixture was stirred for 24 h. Water (30 mL) was added and the resulting solution was extracted with EtOAc (3 x 30 mL), which was dried over Na$_2$SO$_4$ and concentrated to afford a crude brown oil. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] afforded 1-(5-nitrofuran-2-yl)ethan-1-ol (**35**) (449 mg, 2.86 mmol, 81%) as a brown oil.

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.29 (1H, d, J = 4.1 Hz), 6.52 (1H, d, J = 4.6 Hz), 4.96 (1H, q, J = 7.1 Hz), 2.57 (1H, s), 1.61 (3H, d, J = 6.8 Hz).

^{13}C NMR (151 MHz, CDCl$_3$) δ 161.27, 151.59, 112.51, 108.57, 63.66, 21.38.

1-(5-Nitrofuran-2-yl)ethan-1-one (**36**): Dess-Martin periodinane (8.62 g, 20.4 mmol) was added to 1-(5-nitrofuran-2-yl)ethan-1-ol (3.20 g, 20.4 mmol) dissolved in CH$_2$Cl$_2$ (250 mL), and the reaction mixture was stirred for 1 h. The reaction was quenched with saturated solutions of sodium thiosulfate and NaHCO$_3$, then extracted with CH$_2$Cl$_2$ (3 x 50 mL), which was washed with water and brine, dried with Na$_2$SO$_4$, 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, 7% A/93% B over 1.19 min (1 CV), 7% A/93% B → 50% A/50% B over 13.12 min (10 CV), 50%

A/50% B over 2.38 min (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm]

yielded 1-(5-nitrofuran-2-yl)ethan-1-one (**36**) (2.98 g, 19.2 mmol, 94%) as yellow solid.

¹H NMR (600 MHz, CDCl$_3$) δ 7.38 (1H, d, J = 3.8 Hz), 7.28 (1H, d, J = 3.7 Hz), 2.61 (3H, s).

¹³C NMR (151 MHz, CDCl$_3$) δ 186.73, 151.91, 151.48, 116.79, 111.94, 26.27.

2-(5-Nitrofuran-2-yl)propan-2-ol (37): 1-(5-Nitrofuran-2-yl)ethan-1-one (3.00 g, 19.3 mmol) in CH$_2$CI$_2$ (120 mL) was treated dropwise at 0 °C with trimethylaluminium (16.0 mL, 2.0 M, 32 mmol), and the resulting yellow solution was stirred for 90 min at 0 °C. Sat. aq. NH$_4$Cl was added to the reaction mixture, which was extracted with CH$_2$Cl$_2$ (3 x 50 mL). The combined organic layers were dried over Na$_2$SO$_4$ and filtered, and the solvent was removed under reduced pressure to give a yellow oil. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 1000mL/min; monitored at 254 and 280 nm] afforded 2-(5-nitrofuran-2-yl)propan-2-ol (**37**) (2.75 g, 16.1 mmol, 83%) as a yellow oil.

¹H NMR (600 MHz, CDCl$_3$) δ 7.27 (1H, d, J = 3.7 Hz), 6.49 (1H, d, J = 3.7 Hz), 2.36 (1H, s), 1.65 (7H, s).

¹³C NMR (151 MHz, CDCl$_3$) δ 164.05, 151.36, 112.55, 107.37, 69.30, 28.67.

(4-Methoxy-3-((5-nitrofuran-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (38):[88,102,105,106] Phenstatin (0.250 g, 0.786 mmol), DEAD (0.16 mL, 1.02 mmol), and (5-nitrofuran-2-yl)methanol (0.135 g, 0.943 mmol) were

dissolved in dry CH_2Cl_2 (60 mL). Triphenylphosphine (0.412 g, 1.57 mmol) was added, and the reaction mixture was stirred for 2 d. The solvent was 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 → 80% A/20% B over 13.12 min (10 CV), 80% A/20% B over 2.38 min (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((5-nitrofuran-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**38**) (0.143 g, 0.322 mmol, 41%) as a white solid.

¹H NMR (600 MHz, acetone-d_6) δ 7.59 (1H, d, J = 2.0 Hz), 7.56 – 7.52 (2H, m), 7.17 (1H, d, J = 8.4 Hz), 7.06 (2H, s), 6.95 (1H, d, J = 3.7 Hz), 5.32 (2H, s), 3.96 (3H, s), 3.88 (6H, s), 3.85 (3H, s).

¹³C NMR (151 MHz, acetone-d_6) δ 193.12, 154.11, 153.89, 153.12, 147.13, 141.85, 133.19, 130.26, 126.00, 124.87, 116.00, 113.41, 112.45, 111.18, 107.39, 63.08, 59.80, 55.72, 55.47. **HRMS** [M+Na]⁺: 466.1107 (calcd for [$C_{22}H_{21}NNaO_9$]⁺, 466.1109).

HPLC retention time (Method B): 6.81 min.

(4-Methoxy-3-(1-(5-nitrofuran-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (39):[88,102,105,106] Phenstatin (0.250 g, 0.786 mmol), DIAD (0.20 mL, 1.02 mmol), and 1-(5-nitrofuran-2-yl)ethan-1-ol (0.148 g, 0.943 mmol) were dissolved in dry CH_2Cl_2 (60 mL). Triphenylphosphine (0.412 g, 1.57 mmol) was added, and the reaction mixture was stirred for 2 d. The solvent was 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 → 80% A/20% B over 13.12 min (10 CV), 80% A/20% B over 2.38 min (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-(1-(5-nitrofuran-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**39**) (0.169 g, 0.369 mmol, 47%) as a white solid.

^{1}H NMR (600 MHz, acetone-d_6) δ 7.55 (1H, dd, J = 8.4, 2.1 Hz), 7.51 (1H, d, J = 2.1 Hz), 7.49 (1H, d, J = 3.7 Hz), 7.17 (1H, d, J = 8.4 Hz), 7.02 (2H, s), 6.83 (1H, d, J = 3.5 Hz), 5.65 (1H, q, J = 6.5 Hz), 3.97 (3H, s), 3.87 (6H, s), 3.84 (3H, s), 1.77 (3H, d, J = 6.6 Hz).

^{13}C NMR (151 MHz, acetone-d_6) δ 193.05, 158.26, 154.84, 153.11, 151.79, 146.11, 141.89, 133.16, 130.29, 126.49, 119.30, 112.44, 111.54, 111.02, 107.39, 70.96, 59.80, 55.73, 55.52, 18.82.

HRMS [M+Na]$^+$: 480.1263 (calcd for [$C_{23}H_{23}NNaO_9$]$^+$, 480.1265).

HPLC retention time (Method B): 7.86 min.

(4-Methoxy-3-((2-(5-nitrofuran-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (40):[88,102,105,106] Phenstatin (1.00 g, 3.14 mmol), ADDP (1.03 g, 4.08 mmol), and 2-(5-nitrofuran-2-yl)propan-2-ol (0.646 g, 3.77 mmol) were dissolved in dry THF (80 mL). Tributylphosphine (1.55 mL, 6.28 mmol) was added dropwise, and the reaction mixture was stirred for 2 d. The solvent was 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B (10 CV), 80% A/20% B (0.2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((2-(5-nitrofuran-2-

yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**40**) (0.118 g, 0.251 mmol, 8%) as a colorless solid.

¹H NMR (600 MHz, acetone-d6) δ 7.58 (1H, dd, J = 8.5, 2.1 Hz), 7.40 (1H, d, J = 3.7 Hz), 7.14 – 7.06 (2H, m), 6.94 (2H, s), 6.69 (1H, d, J = 3.7 Hz), 3.83 (6H, s), 3.81 (3H, s), 3.80 (3H, s), 1.75 (6H, s).

¹³C NMR (151 MHz, acetone-d_6) δ 192.97, 160.47, 157.53, 153.09, 151.48, 143.09, 141.85, 133.15, 129.94, 127.80, 125.96, 112.31, 111.61, 110.81, 107.25, 77.40, 59.80, 55.74, 55.38, 25.03.

HRMS [M+Na]$^+$: 494.1422 (calcd for [C$_{24}$H$_{25}$NNaO$_9$]$^+$ 494.1422).

HPLC retention time (Method B): 8.26 min

Biological Evaluations

Colchicine Binding Assay. Inhibition of [^{3}H]colchicine binding to tubulin was determined using 0.1 mL reaction mixtures. Each reaction mixture contained 1.0 μM tubulin, 5.0 μM [^{3}H]colchicine (from Perkin–Elmer), 5% (v/v) dimethyl sulfoxide, potential inhibitors at 5.0 μM and components that were previously demonstrated to stabilize the colchicine binding activity of tubulin[111] (1.0 M monosodium glutamate [adjusted to pH 6.6 with HCl in a 2.0 M stock solution], 0.5 mg/mL bovine serum albumin, 0.1 M glucose-1-phosphate, 1.0 mM MgCl$_2$, and 1.0 mM GTP). Incubation was for 10 min at 37°C, a time point at which the binding reaction in the control is 40–60% complete. Reactions were stopped by adding 2.0 mL of ice-cold water and placing the samples on ice. Each sample was poured onto a stack of two DEAE-cellulose filters, followed immediately by 6 mL of ice-cold water, and the water was aspirated under reduced vacuum. The filters were

washed with 2 mL water X 3 and, following removal of excess water under a strong vacuum, placed into vials containing 5 mL of Biosafe II scintillation cocktail. Samples were counted the next day in a Beckman scintillation counter. Samples with potential inhibitors were compared to controls with no inhibitor to determine percent inhibition. All samples were corrected for the amount of colchicine that bound to the filters in the absence of tubulin.

Inhibition of Tubulin Polymerization[113] Tubulin assembly experiments were performed using 0.25 mL reaction mixtures (final volume).[112] The mixtures contained 1 mg/mL (10 µM) purified bovine brain tubulin, 0.8 M monosodium glutamate (pH 6.6, as above), 4% (v/v) dimethyl sulfoxide, 0.4 mM GTP, and varying compound concentrations. Initially, all components except GTP were preincubated for 15 min at 30 °C in 0.24 mL. After chilling the mixtures on ice, 10 µL of 10 mM GTP was added. The reaction mixtures were then transferred to cuvettes held at 0 °C in Beckman DU-7400 and DU-7500 spectrophotometers equipped with electronic temperature controllers. The temperature was jumped to 30 °C over about 30 s, and polymerization was followed turbidimetrically at 350 nm for 20 min. Each reaction set included a reaction mixture without compound, and the IC50 was defined as the compound concentration that inhibited extent of assembly by 50% after 20 min at 30 °C.

NADPH Cytochrome P450 Oxidoreductase Cleavage Assay[114,115] Rat NADPH cytochrome P450 oxidoreductase (POR) and protocatechuate 3,4-dioxygenase (PCD) were purchased from Corning® and Sigma-Aldrich, respectively, and their enzymatic

activities were determined in terms of enzyme units (U). All bioreductive prodrugs were dissolved in DMSO as 10 mM stock solutions.

An aliquot (5 µL) of the 10 mM compound DMSO stock solution along with 0.5 µL 0.1% Triton X-100 were added to 395.5 µl 200 mM pH 7.4 potassium phosphate buffer containing 400 µM freshly made protocatechuic acid (PCA). The components were fully mixed in a microvessel capped with a rubber septum stopper and subjected to three cycles of evacuation and flushing with N_2 using a manifold, followed by sparging with N_2 for an additional 20 min. PCD (0.08 units) was added by Hamilton syringe, and the solution was scrubbed for 10 min to allow for sufficient O_2 digestion by PCA/PCD. POR stock (0.006 units) was introduced followed by NADPH (0.8 mM final concentration) into the vial followed by an additional round of N_2 sparging. The reaction mixture was incubated for 24 h at 37 °C, cooled on ice and treated with an equal volume of acetonitrile. After centrifugation and filtration, the samples were analyzed by HPLC. Solutions without POR were used as controls.

CHAPTER THREE

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

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Mason,[2] Mary L. Trawick[1*], Kevin G. Pinney[1*] Bioreductively Activatable Prodrug
Conjugates of Combretastatin A-1 and Combretastatin A-4 as Anticancer Agents Targeted
towards Tumor Hypoxia

The author Blake A. Winn contributed to this manuscript through the synthesis of six of

the final compounds including full characterization, which included proton and carbon

NMRs, HPLC, and HRMS. In addition, Blake A. Winn significantly contributed to the

writing and editing of the manuscript, as well as the preparation of the supporting data.

Abstract

Targeting tumor-associated hypoxia with small-molecule anticancer agents represents a

promising strategy to potentially improve treatment efficacy and reduce patient side-

effect profiles. Bioreductively activatable prodrug conjugates (BAPCs), which

incorporate a potent anticancer agent with a bioreductive trigger, are designed to be

substrates for reductase enzymes operating in regions of tumor hypoxia. While inert

under normoxic conditions, these BAPCs are intended to be cleaved in low oxygen

environments (hypoxia) to release their parent anticancer agent. Synthetic pathways have

been identified for the preparation of nitrothienyl prodrugs of the natural product

combretastatin A-1 (CA1) that incorporate *nor*-methyl, *mono*-methyl, and *gem*-dimethyl

nitrothiophene triggers. A regioselective protecting group strategy (tosyl, isopropyl, and *tert*-butyldimethylsilyl) was utilized to establish regioselective control around the catechol functionality inherent to CA1, thus facilitating incorporation of these nitrothiophene triggers at the C-2 and C-3 positions of CA1. A related series of BAPCs based on the natural product combretastatin A-4 (CA4), which was originally reported by Davis and co-workers, were synthesized as comparison standards. CA4 and CA1 function biologically as potent inhibitors of tubulin polymerization and effective vascular disrupting agents (VDAs). These series of CA1 and CA4 BAPCs (15 compounds in total) were evaluated biologically for their ability to inhibit tubulin polymerization and for their differential cytotoxicity (normoxia versus hypoxia) against the A549 human lung cancer cell line. In addition, they were evaluated as substrates for the reductase enzyme NADPH cytochrome P450 oxidoreductase (POR). The CA4-*gem*-dimethylnitrothiophene BAPC (**45**) proved exemplary in comparison to its *nor*-methyl and *mono*-methyl CA4-BAPC cogeners (**43** and **44**, respectively). It was stable to hydrolysis conditions (24 h), was cleaved by POR (25% at 90 min), was inactive (desirable prodrug attribute) as an inhibitor of tubulin polymerization ($IC_{50} > 20$ μM), and demonstrated hypoxia-selective activation in the A549 cell line [hypoxia cytotoxicity ratio (HCR) = 40]. The related CA1-*gem*-dimethylnitrothiophene BAPC (**41**) was also promising with HCR = 30 and complete cleavage observed upon treatment with POR. However, BAPC **41** was also labile under hydrolysis conditions, suggesting that pharmacokinetic (PK) considerations may prove crucial for the successful future development of these (and related) BAPCs as therapeutic agents.

Introduction

The tumor microenvironment exemplifies a number of unique attributes that distinguish it from the microenvironment associated with healthy tissue. One of these key differences centers on vascular architecture and associated blood flow dynamics.[1-3] Solid tumors, once they reach approximately 2-5 mm^3 in size, must establish their own vascular network in order to meet their rapidly accelerating demand for oxygen and nutrients.[116-123] This rapid angiogenic development of tumor-associated vasculature results in disorganized, fragile, and leaky vessels, thus providing a target for therapeutic intervention.[1-3,124-126] Vascular disrupting agents (VDAs) are compounds that selectively damage established tumor-associated vasculature, thus denying necessary oxygen and nutrients, and ultimately resulting in necrosis.[1,4-6] The natural products combretastatin A-1 (CA1, Figure 3.1) and combretastatin A-4 (CA4, Figure 3.1), isolated from the bark of the African bush willow tree *Combretum caffrum* Kuntze (Combretacae) by Pettit and co-workers, are potent inhibitors of tubulin polymerization (binding at the colchicine site) and function biologically as VDAs.[22,23,25,32] As tubulin binding inhibitors, they cause rapid morphological changes to the endothelial cells lining tumor-associated blood vessels, causing vascular collapse, which leads to starvation of tumor cells from oxygen and nutrient depravation.[2,11,136] The corresponding water-soluble phosphate prodrugs of CA1 and CA4 [referred to as CA1P (also known as OXi4503) and CA4P (also known as Zybrestat[TM]) respectively, Fig. 1] have advanced through preclinical and clinical trials.[4,18,19,27-32]
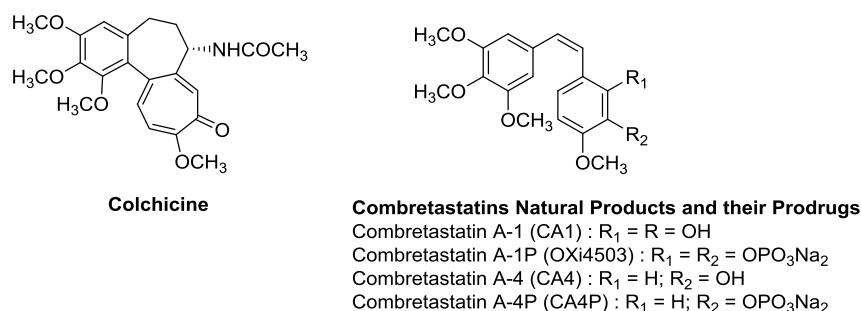
Figure 3.1. Colchicine and Combretastatin Natural Products and their Corresponding Phosphate Prodrug Salts

In addition to an aberrant vascular network and elevated interstitial pressure due to immature and leaky vasculature, the tumor microenvironment is further characterized by a pH gradient, with cells distant from blood vessels being acidic in nature.[3,9] The high probability for existing tumor-associated capillaries to be kinked and distant leads to an increased average diffusion distance for oxygen and nutrients to reach tumor cells as well as poor blood flow in the central mass of the tumor.[38] Furthermore, there is a distinct oxygen concentration gradient inherent to a significant percentage of solid tumors, varying from normoxic to hypoxic to anoxic.[3] Tumor 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 are considered to be resistant to many conventional anticancer therapies.[3,41-43]

Importantly, the presence of low oxygen concentrations and cell necrosis are unique features of solid tumors, not naturally occurring in normal tissue.[3] The presence of pronounced regions of hypoxia in tumors offers an opportunity for targeting through the selective delivery of potent anticancer agents utilizing appropriate prodrug strategies.[3,38,43] These conjugates activate under hypoxic conditions, similar to those in the tumor microenvironment, releasing their accompanying potent anticancer agent in a

64

selective fashion. Such compounds include hypoxia-activated agents as well as prodrugs,[3,38,43,44] which are also referred as bioreductively activated prodrug conjugates (BAPCs).[137] These hypoxia-selective agents undergo activation through either one- or two-electron enzymes, principally NAD(P)H-dependent flavoproteins for one-electron processes and cytochrome P450s for two-electron processes.[3,38]

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 3.2).[51,52] 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,[3] displaying dose-limiting toxicity.[128,138] 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 compared.[38] TH-302,[101] a 2-nitroimidazole-based nitrogen mustard prodrug (Figure 3.2. B) that releases its parent drug bromoisophosphoramide mustard under hypoxic conditions, advanced to Phase III human clinical trials.[59,138] Unlike the Phase I and II studies, the results of the Phase III clinical trial showed no statistical significance for TH-302 against pancreatic adenocarcinoma and soft tissue sarcoma.[60-62]
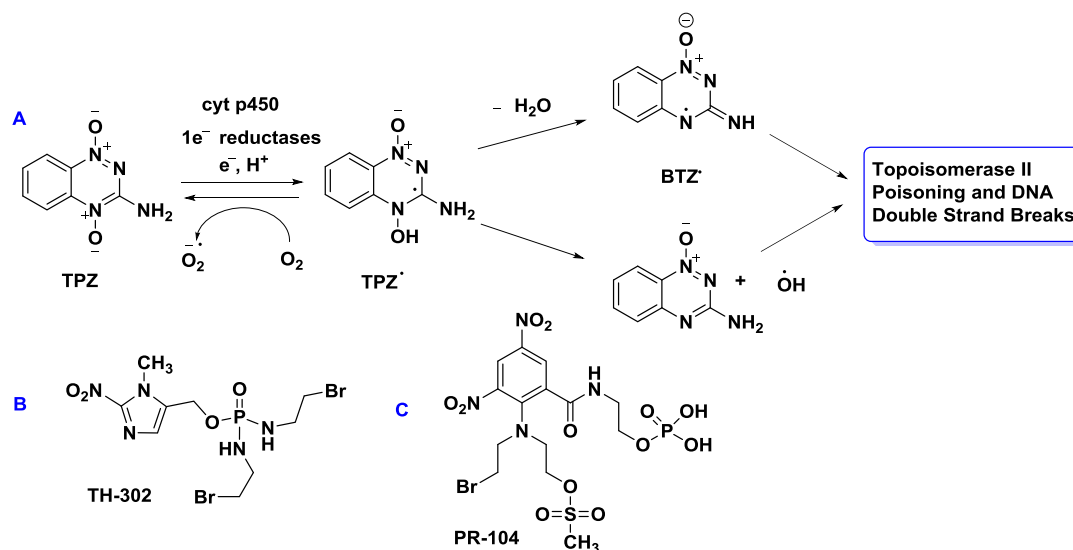
Figure 3.2. A. The Mechanism by which Tirapazamine Selectively Kills Hypoxic Cells.
B. Structure of TH-302. **C.** Structure of PR-104.[51,52]

Another class of BAPCs incorporates a bioreductive trigger, which can be cleaved
to selectively release the active anticancer agent in hypoxic conditions. Once cleaved, the
therapeutic agents are released and diffuse into the tumor microenvironment (Fig. 3.3).
Since hypoxia is a condition that is commonly associated with solid tumors, it provides
an excellent opportunity for selective targeting.[3,38]
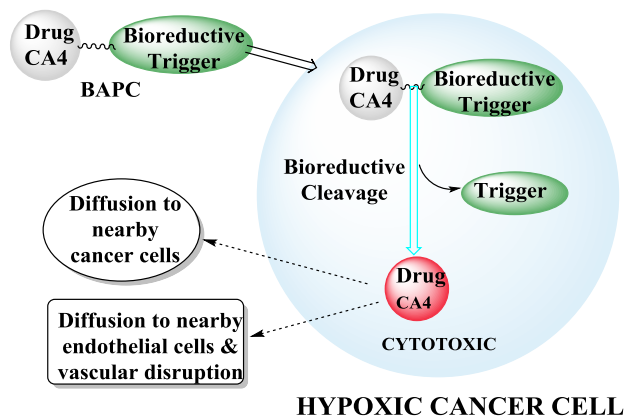
Figure 3.3. Selective Release of Cytotoxic Agent (CA4) from Non-Toxic BAPC under
Tumor Hypoxia. BAPCs are designed to activate selectively in the hypoxic tumor
microenvironment, thereby releasing their cytotoxic anticancer agent (payload).[139]

Davis and co-workers prepared a series of *nor-*, *mono-*, and *gem*-dimethyl-nitrothienyl BAPCs that incorporate CA4, and demonstrated their ability to release CA4 from the bioreductive triggers in A549 cells under hypoxic conditions.[88]
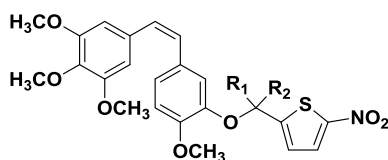
Normethyl; $R_1=R_2=H$
Monomethyl; $R_1=H$, $R_2=CH_3$
Gem-dimethyl; $R_1=R_2=CH_3$

Figure 3.4. Combretastatin A-4 (CA4) Incorporating Nitrothiophene-Based Bioreductive Triggers[88]

The efficacy of these BAPCs was evaluated by determining their cytotoxicity in normoxia and CA4 release under normoxia versus hypoxia, in the A549 human cancer cell line.[88]
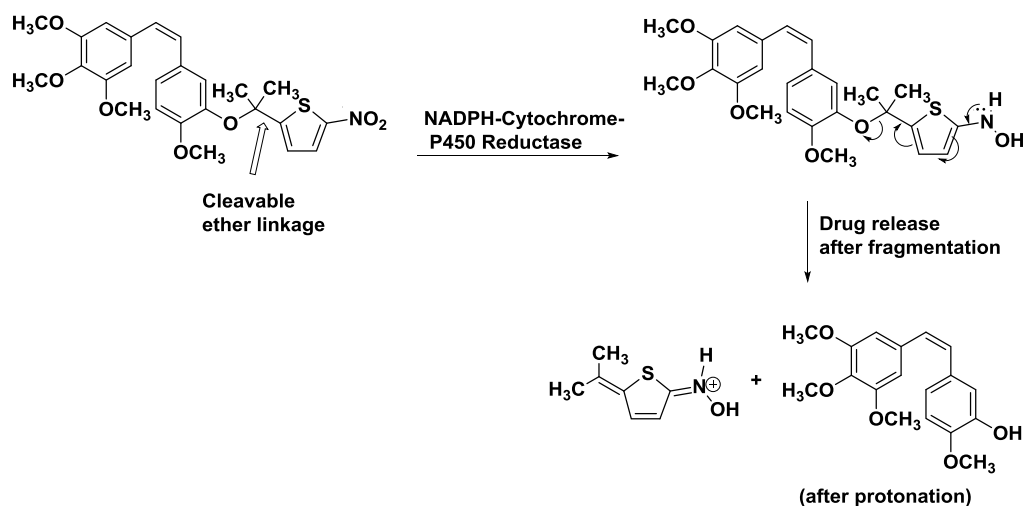
Scheme. 3.1. Biological Reduction and Cleavage of CA4 *gem*-Dimethyl Nitrothiophene Trigger Releasing CA4[88]

It was determined that the *gem*-dimethyl-nitrothiophene trigger CA4 prodrug (Scheme **1**) was the most resistant to aerobic metabolism (in comparison to the *nor-* and *mono*-methyl-nitrothiophene trigger CA4 prodrugs) and the *gem*-dimethyl CA4-BAPC remained intact in high oxygen environments.[88] While the *gem-* and *mono*-substituted CA4-BAPCs were effective across a range of oxygen concentrations, the unsubstituted (*nor*-methyl) was specifically effective under extreme hypoxia (<0.01% O_2).[88]

Inspired by the promise of targeting tumor hypoxia for the selective delivery of tubulin-active VDAs, and building on the encouraging results reported for the CA4-BAPCs, we have designed and synthesized a series of BAPCs that incorporate the natural product CA1, and evaluated them in preliminary studies to access their efficacy as a new anticancer therapeutic regimen. A regioselective protecting group strategy (incorporating *tert*-butyldimethylsilyl, isopropyl, and tosyl groups) that we previously developed for another application was utilized to differentiate the catechol functionality (C-2 and C-3 positions) inherent to CA1.[33,128,129,140] The nitrothiophene triggers previously described by Davis and co-workers were synthesized using a new synthetic strategy.[88] The resultant CA1 BAPCs were evaluated for their ability to inhibit tubulin polymerization and to function as substrates for the reductase enzyme cytochrome P450 oxidoreductase (POR). In addition, differential cytotoxicity studies (normoxia versus hypoxia) suggested which BAPCs held the most promise for the targeting of tumor hypoxia.

Synthesis

The CA1-BAPCs were synthesized by utilizing two key reactions- a Wittig olefination to generate regioselectively protected CA1, and a Mitsunobu reaction between the tosyl, isopropyl, and *tert*-butyldimethylsilyl protected CA1 analogues (**20**, **21**, **27**, and **28** respectively, Schemes 3.4 and 3.6) and the nitrothienyl triggers (**16**, **17** and **19**, Scheme 3.3).[1,2] Synthesis of the regioselectively protected *Z*-CA1 analogues (**11-13**, Scheme 3.2) was successfully executed utilizing the Wittig olefination reaction between aldehydes **5-7** (Scheme 3.2) and the requisite triphenyl phosphonium salt **10**.[1] The Wittig reaction produced both the *Z*- and *E*- isomers of the stilbene, but favored the *Z*- isomer (Scheme 3.2).[1-4,135]

Selective demethoxylation of aldehyde **1** using boron trichloride yielded catechol **2**, which generated selectively protected aldehydes **3-7** (Scheme 3.2) using a previously reported synthetic strategy.[1,3,135] Phosphonium salt **10** was generated after bromination of benzyl alcohol **8** using phosphorous tribromide followed by a reaction with triphenyl phosphine. A Wittig reaction between the suitably protected aldehydes (**5-7**) with Wittig salt **10** yielded both *Z*- and *E*- stilbene isomers (**11**-**13**, favoring the *Z*- isomer), which were separated using flash column chromatography. Synthesis of the three nitrothiophene triggers utilized in the Mitsunobu reactions is detailed in Scheme 3.3.
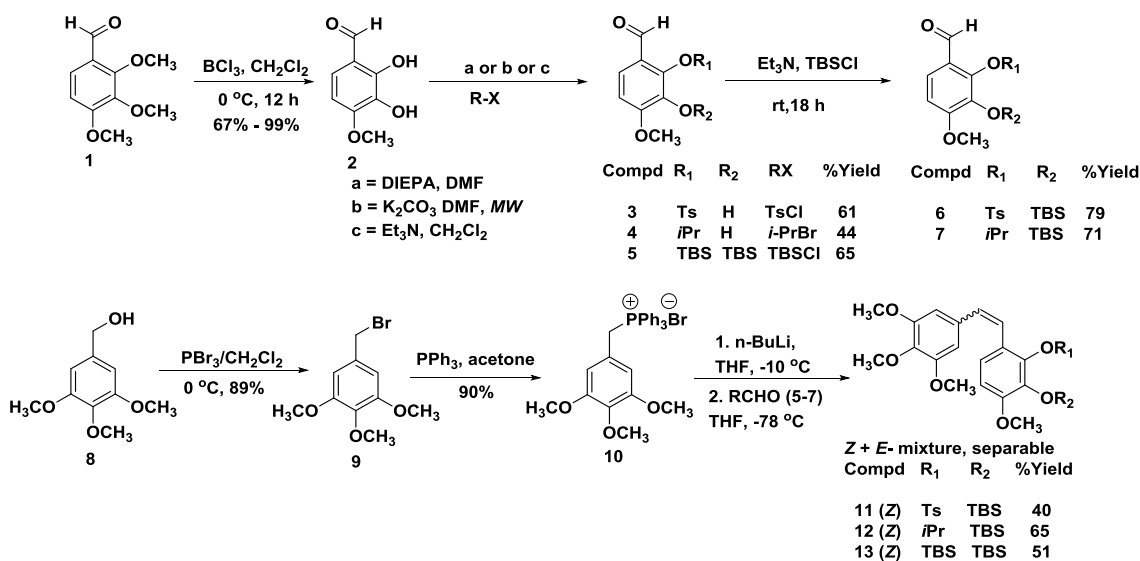
Scheme 3.2. Synthesis of Regioselectively Protected CA1 Analogues **11-13**[128,129]

The synthetic route reported by Davis and co-workers highlights the synthesis of the *nor-* and *mono*-methyl nitrothiophene triggers **16** and **17** in good yield through the reduction of aldehyde **14** and ketone **15** respectively (Scheme 3.3).[5,88] However, in our hands (Scheme 3.3), the synthesis of the *gem*-dimethyl nitrothiophene trigger **19** suffered with two consecutive low yielding steps, which included methylation of the carbonyl group followed by nitration at the C5 position. In order to scale up the production of compound **19**, it was imperative for us to develop an improved synthetic route. The new synthetic route provided all three triggers (*nor-*, *mono-* and *gem-*) from a single starting material **14**. Methylation of aldehyde **14** furnished *mono*-methyl trigger **17**, which on subsequent oxidation and methylation yielded *gem*-dimethyl trigger **19** in good yield (Scheme 3.3).

Old Route

14. R = H
15. R = CH3

16. R = H; 90%
17. R = CH$_3$; 92%

18

19

New Synthetic Route

14

17

15

19
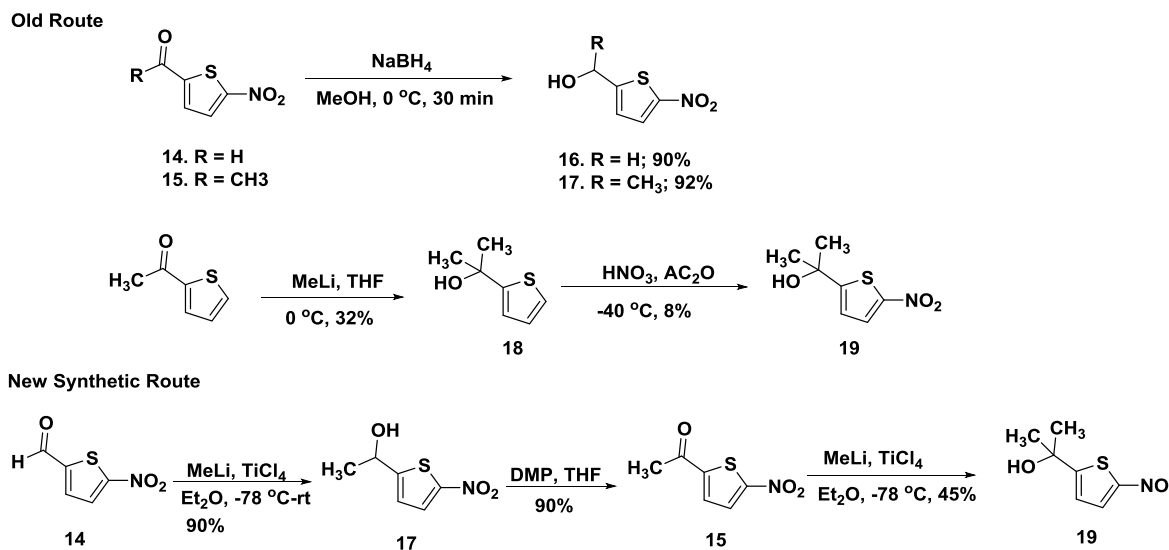
Scheme 3.3. Synthesis of Nitrothiophene Triggers Using Old Route (Proposed by Peter Davis and Co-Workers) and New Routes[88]

Deprotection of regioselective CA1 analogues **11** and **12** using TBAF yielded their corresponding phenols **20** and **21** respectively, which were subjected to Mitsunobu conditions that further incorporated nitrothiophene triggers (**16, 17** and **19**), phosphine reagents (PPh$_3$ or PBu$_3$) and azo compounds (diethylazodicarboxylate [DEAD], diisopropylazodicarboxylate [DIAD] or 1,1'-(azodicarbonyl)-dipiperidine [ADDP]) to generate BAPCs **22**-**26** (Scheme 3.4).

11. R$_1$ = Ts
12. R$_1$ = *i*Pr

20. R$_1$ = Ts, 70%
21. R$_1$ = *i*Pr, 90%

trigger	phosphine	azo
16	PPh$_3$	DEAD
17	PPh$_3$	DIAD

Compd	R$_1$	R$_2$	R$_3$	%Yield
22.	Ts	H	H	47%
23.	Ts	H	CH$_3$	61%
24.	*i*Pr	H	H	17%
25.	*i*Pr	H	CH$_3$	47%
26.	*i*Pr	CH$_3$	CH$_3$	13%
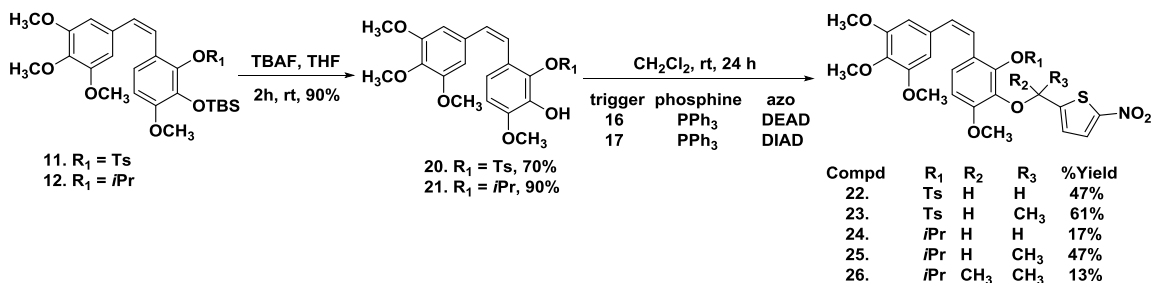
Scheme 3.4. Synthesis of Regioselectively Protected CA1-BAPCs **22**-**26**[88,143]

The attempted deprotection of compounds **22** and **23** with NaOH (2M) under either microwave or reflux conditions did not yield the desired product, but instead cleaved the nitrothiophene trigger from the starting material, regenerating compound **20** (see Appendix B**).** Similarly, compound **24** regenerated compound **21** upon attempted deprotection using $AlCl_3$ (see Appendix B). In an effort to solve this problem, we attempted to partially cleave the *bis*-TBS protected CA1 **13** using a deficiency of TBAF, which resulted in a mixture of regioisomers **27** and **28** (Scheme 3.5), which proved inseparable, in our hands, by flash column chromatography. While the mixture of regioisomers **27** and **28** produced their respective Mitsunobu products **30**-**34**, CA1 analogue **29** was unreactive under these conditions. The protected CA1-BAPC **32** proved difficult to purify, in our hands, through column chromatography, so the crude product was taken to the next step. Interestingly, the conventional TBS-deprotection of compounds **30** and **31** using TBAF yielded ring-cyclized products **35** and **36** (proposed structures based on analysis of NMR and HRMS data) without producing any other discernable side products (Scheme 3.6).
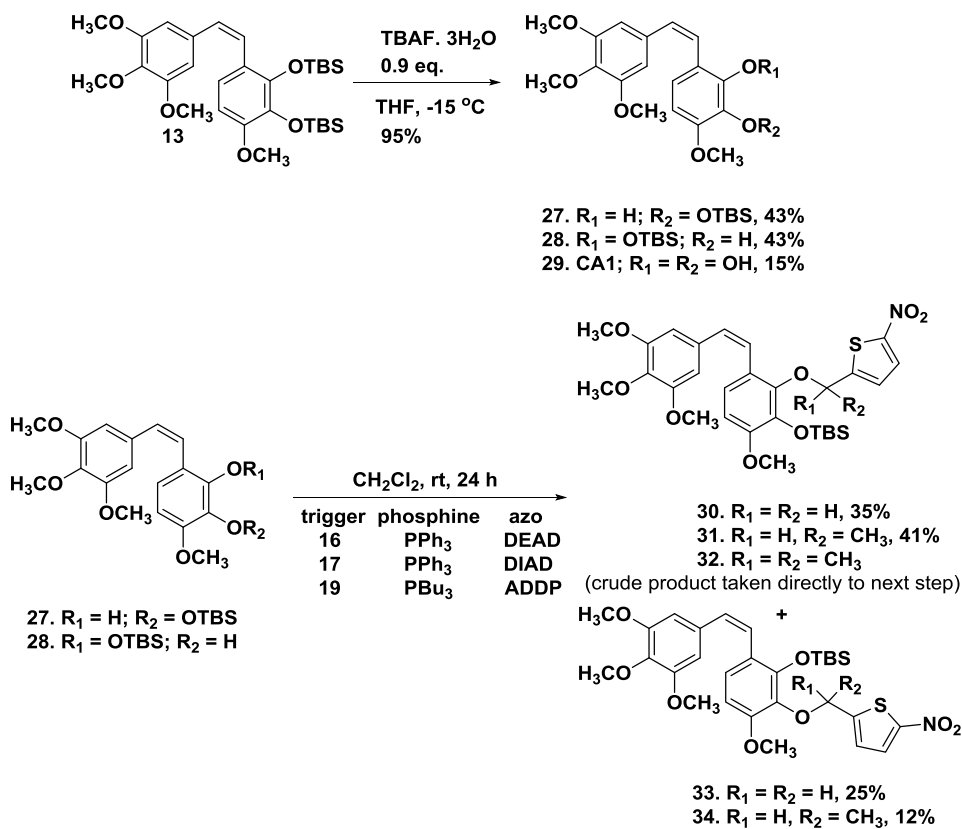
Scheme 3.5. Synthesis of TBS-Protected CA1-BAPCs **30-34**
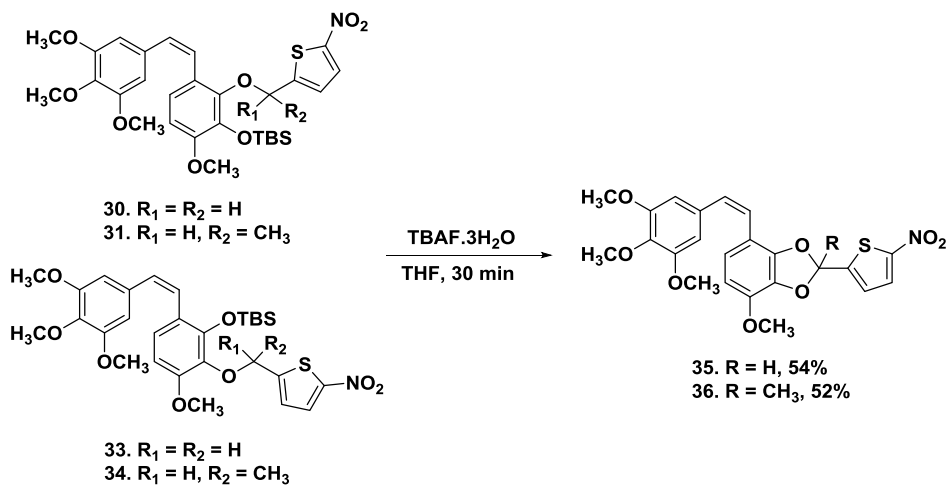
Scheme 3.6. TBS-Deprotection to Generate Ring-Cyclized Products **35** and **36**

However, upon modification of the deprotection conditions [HCl (2M)/AcOH, instead of TBAF], the desired CA1-BAPCs **37**-**40** were obtained (regiochemistry was determined through 1D NOE NMR) (Scheme 3.7). Intrigued by this unusual cyclization (that produced **35** and **36**), we investigated whether exposure of phenolic compounds **30** and **31** to strong base would facilitate a similar cyclization reaction and this indeed proved to be the case (Scheme 3.8).
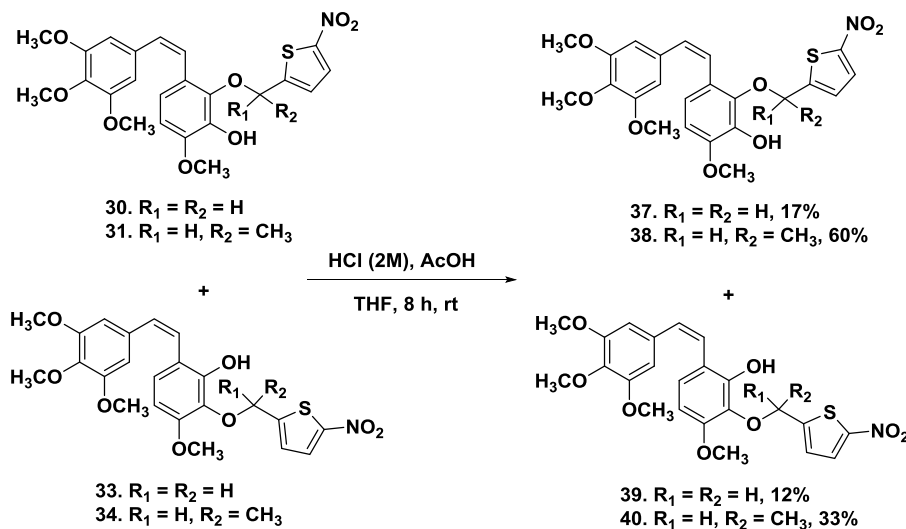
Scheme 3.7. Synthesis of *Nor-* and *Mono-* Methyl CA1-BAPCs **37**-**40**
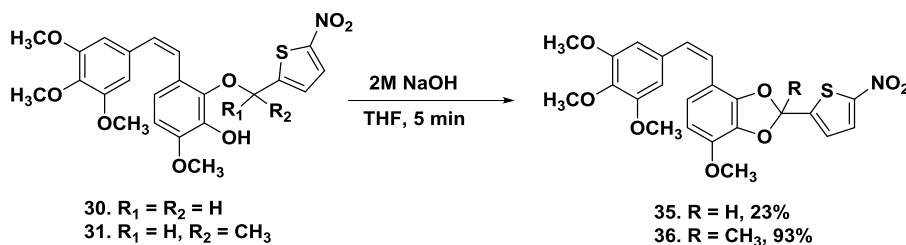
Scheme 3.8. Base Generating Ring-Cyclized Products **35** and **36**

Since purification of the TBS-protected *gem*-dimethyl CA1 BAPC **32** by column chromatography proved to be unsuccessful, it was taken directly to the deprotection step

(Scheme 3.9) to generate *gem*-dimethyl CA1 BAPC **41**. As opposed to the previous use

of HCl (2M)/AcOH in the deprotection of the silyl group (Scheme 3.7), TBAF was used

as the reagent for the deprotection (regiochemistry was determined through 1D NOE

NMR). While the overall yield for this deprotection was quite low, the remaining

material balance included only starting material, and no cyclized byproducts or *E*- isomer

were detected.
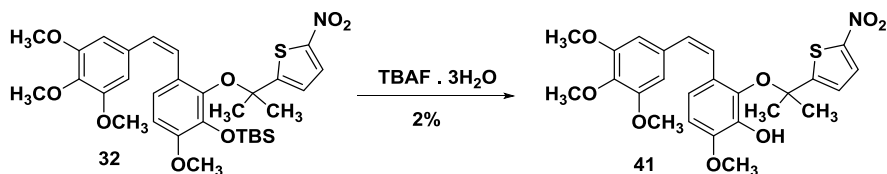
Scheme 3.9. Synthesis of Gem-Dimethyl CA1-BAPC **41**

A series of CA4-BAPCs were synthesized under conditions similar to those

previously reported[88] by Davis *et al* but with some modifications (Scheme 3.10).[88] CA4-

BAPC **43** was synthesized through a Mitsunobu reaction heated to 50 °C with

nitrothiophene **16**.[88] CA4 and nitrothiophene **17** were reacted with DIAD and

triphenylphosphine to generate BAPC **44**.[88] The *gem*-dimethyl CA4-BAPC **45** was

synthesized from CA4, ADDP, nitrothiophene **19**, and tributylphosphine.[88] In order to

improve the yield for the *gem*-dimethyl CA4-BAPC, subsequent Mitsunobu reactions

were performed in toluene.[88] While the overall yield was improved, the new method

required a more intensive purification procedure to remove the remaining CA4 and *gem*-

dimethyl thiophene trigger which had nearly identical chromatographic retention times to

the desired CA4-BAPC. The reaction mixture was subjected to chemical modification to

facilitate chromatographic separation during purification. The phenolic moiety of CA4

was converted to its corresponding silyl ether (TBS) and the unreacted *gem*-dimethyl

trigger was subsequently acetylated, allowing both of these compounds to be successfully

separated chromatographically from the desired CA4 *gem*-dimethyl-nitrothiophene

BAPC.

Reagents and conditions:
16, DEAD, PPh$_3$, THF, 50 °C, 13 h
17, DIAD, PPh$_3$, THF, r.t., 2d
19, ADDP, PBu$_3$, benzene or toluene, r.t., 1d

43. R$_1$ = R$_2$ = H; 50%
44. R$_1$ = H, R$_2$ = CH$_3$; 26%
45. R$_1$ = R$_2$ = CH$_3$; 23% (benzene), 31% (toluene)
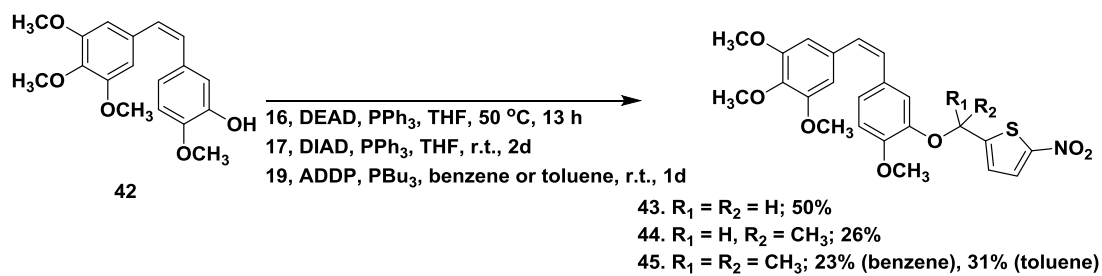
Scheme 3.10. Synthesis of CA4-BAPCs[88]

A.

Compd	R_1	R_2	R_3
22.	Ts	H	H
23.	Ts	H	CH_3
24.	iPr	H	H
25.	iPr	H	CH_3
26.	iPr	CH_3	CH_3

35. R = H
36. R = CH_3

37. $R_1 = R_2 = H$,
38. $R_1 = H$, $R_2 = CH_3$
41. $R_1 = R_2 = CH_3$

39. $R_1 = R_2 = H$
40. $R_1 = H$, $R_2 = CH_3$

43. $R_1 = R_2 = H$; 50%
44. $R_1 = H$, $R_2 = CH_3$; 26%
45. $R_1 = R_2 = CH_3$; 23%

B.

Compd 20

Compd 21

Compd 29
CA1

Compd 42
CA4
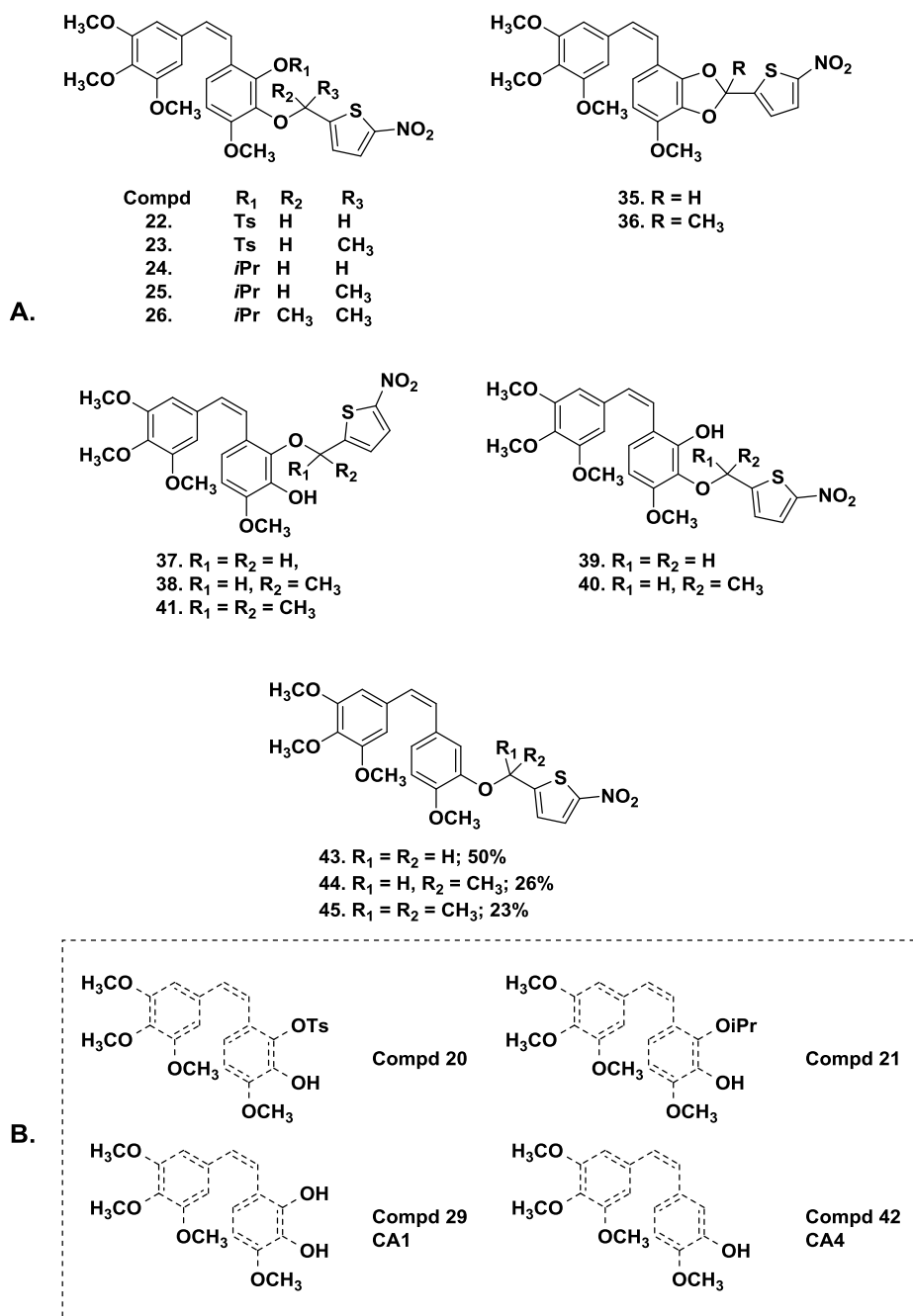
Figure 3.5. A. Compilation of Parent Anticancer Agents and their Corresponding BAPCs Utilized in this Study B. Parent CA1 and CA4 Anticancer Agents

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Biological Evaluation

Table 3.1.
Inhibition of Tubulin Polymerization
and Percent Inhibition of Colchicine Binding

Compound	Inhibition of Tubulin Polymerization IC$_{50}$	Inhibition of Colchicine Binding % Inhibition μM ±SD	
	(μM)±SD	1 μM	5 μM
29 (CA1)[b]	1.9[c]	ND	99.6±0.7
CA4[d]	0.64	84±2	97±0.7
20 KGP439	0.84±0.1	50±5	84±1
21 KGP400	0.82±0.04	72±4	94±0.7
22 KGP440	>20	ND	ND
23 KGP441	>20	ND	ND
25 KGP442	12±1	ND	ND
26 KGP443	>20	ND	ND
27 KGP444	9.5±0.9	ND	ND
35 KGP445	1.7±0.2	ND	25±3
36 KGP446	>20	ND	ND
37 KGP455	1.7±0.01	53±3	92±0.5
38 KGP457	0.84±0.1	34±3	90±0.7

39 KGP454	4.3±0.4	ND	58±4
40 KGP456	6.2±0.3	ND	72±3
41 KGP461	1.3±0.08	ND	43±4
43 KGP 370	>20	ND	26±1
44 KGP 371	>20	ND	15±5
45 KGP 372	>20	ND	33±3

[a] Average of n ≥ 3 independent determinations
[b] Data from ref. 110, see ref. 27 for additional data
[c] Data from ref. 32, see ref. 27 for additional data
[d] For additionl data, see ref. 27
ND= Data not available

The BAPCs and their parent anticancer agents (CA4 and CA1) were evaluated for

their ability to inhibit tubulin polymerization and colchicine binding (Table 3.1). The

parent anticancer agents [CA4, CA1, tosyl protected CA1 (**20**), and isopropyl protected

CA1 (**21**)] utilized in this study were potent inhibitors of tubulin polymerization (IC_{50} =

1.9, 0.64, 0.84 and 0.82 μM respectively) and strongly inhibited colchicine binding. The

TBS-protected CA1 analogue **27** was only moderate as an inhibitor of tubulin

polymerization (IC_{50} = 9.5 μM). Ideally, the BAPCs prepared from these parent

anticancer agents would be protected from binding to tubulin until cleaved (*in vivo*) to

generate their corresponding anticancer agents. Considering the collective group of

fifteen BAPCs synthesized for this study, seven BAPCs (**22**, **23**, **26**, **36**, **43**, **44**, **45**) were

inactive (IC_{50} > 20 μM) as inhibitors of tubulin polymerization while three BAPCs (**25**,

39, **40**) were moderate inhibitors (IC$_{50}$ > 3 μM but < 20 μM) and four BAPCs (**35**, **37**, **38**, **41**) proved to be potent inhibitors (IC$_{50}$ < 3 μM). It is important to note that the BAPCs that proved active as inhibitors of tubulin polymerization might have undergone partial cleavage to the parent anticancer agent in the buffered (cell-free) assay conditions, although a hydrolysis experiment (Table 3.2) demonstrated that only BAPC **41** underwent significant cleavage (100% after 48 h).

Table 3.2**.**
Bioreductive Trigger Hydrolysis (Untreated)
and Cleavage of Cytochrome P450 Reductase Treated BAPCs

Compound	Hydrolysis Percentage in pH 7.4 phosphate buffer for 48 hrs	Cleavage Percentage of POR-treated for 90 min
20 KGP439	ND	ND
21 KGP400	ND	ND
22 KGP440	0.25	NC
23 KGP441	0.84	13.5
25 KGP442	1.59	1.1
26 KGP443	0.69	3.8
27 KGP444	4.03	7.6
35 KGP445	0	NC
36 KGP446	0	NC
37 KGP455	0	14.2 (23.8% cyclization of 455 to 445)
38 KGP457	ND	5.6 (47.8% cyclization of 457 to 446)
39 KGP454	0	17.9 (45.9% cyclization of 454 to 445)

40	0	25.5
KGP456		(34.8% cyclization of 456 to 446)
41	100	100
KGP461		
43	0.35	2.7
KGP 370		
44	ND	4.1
KGP 371		
45	0.69	25.4
KGP 372		

ND= Data not available

NC= No cleavage

In preliminary studies, the previously described CA4-BAPCs were treated with POR (Table 3.2). After incubation (90 min) in the presence of a PCA/PCD oxygen scrubbing system, **43** and **44** underwent minimal cleavage (2.7% and 4.1% respectively) while **45** was more efficiently cleaved (25.4%). These results are in accordance with the previously reported results from Davis and co-workers that utilized supersomal POR with compounds **43**, **44**, and **45**, and demonstrated that **45** cleaved more readily (to release CA4) than **43** and **44**.[49] This trend of increased cleavage (from *gem*-dimethyl to *mono*-methyl to *nor*-methyl) to generate the corresponding parent anticancer agent (CA4 in this case) was also observed in the POR treated CA1-BAPCs. The *gem*-dimethyl CA1-BAPC **41** and the isopropyl-protected *gem*-dimethyl BAPC (**27**) were cleaved more extensively in comparison to their corresponding *mono*-methyl and *nor*-methyl BAPCs. The *mono*-methyl and *nor*-methyl CA1-BAPCs (**37**, **38**, **39**, **40**) were cleaved by POR to differential extents, depending on the position of the nitrothiophene side chain (bioreductive trigger) and the hydroxyl group. It should be noted that under these assay conditions, these four BAPCs (**37**, **38**, **39**, **40**) underwent cyclization to generate their corresponding cyclized analogues **35** or **36**. While the mechanism of this cyclization is unknown, it appears to

depend, at least to some extent, on the pH of the buffer solution, and under these assay

conditions cyclization that incorporates the bioreductive trigger was more favorable than

the desired cleavage of the prodrug trigger. BAPC **41** was the only *gem*-dimethyl BAPC

that was fully cleaved (100%) by POR (90 min) in this study, thus its hydrolytic stability

was further evaluated in the pH 7.4 buffer. The BAPC **41** showed no apparent

spontaneous hydrolysis at different time intervals for the first 150 min, but it was mostly

decomposed if incubated in the buffer for 24-48 h. Therefore, the cleavage (100%) of

BAPC **41** by POR (90 min) was not due to spontaneous hydrolysis in buffer.

Table 3.3.
In Vitro Potency and Hypoxia Cytotoxicity Ratio (HCR) of the CA4 and CA1-BAPCs in
the A549 Human Cancer Cell Line

Compound	IC_{50} [oxic][a] (μM)$\pm$SD	IC_{50} [anoxic][a] (μM)$\pm$SD	HCR
RB6145	>89.1	9.5$\pm$8.2	>9.4
Tirapazamine	66.5$\pm$41.3	7.7$\pm$2.2	8.6
29 **CA1**	1.2$\pm$1.9	0.8$\pm$0.5	1.5
CA4	0.005$\pm$0.0004	0.006$\pm$0.0008	0.8
22 KGP440	0.4$\pm$0.05	0.5$\pm$0.1	0.8
23 KGP441	4.6$\pm$0.3	3.3$\pm$0.9	1.4
25 KGP442	3.7$\pm$4.7	0.6$\pm$0.1	6.2
26 KGP443	2.9$\pm$0.7	1.2$\pm$0.6	2.4

27 KGP444	1.6±1.5	0.5±0.1	3.2
35 KGP445	ND	ND	ND
36 KGP446	0.6±0.06	0.7±0.4	0.9
37 KGP455	0.2±0.2	0.3±0.3	0.7
38 KGP457	0.2±0.2	0.3±0.3	0.7
39 KGP454	0.3±0.03	0.7±0.08	0.4
40 KGP456	ND	ND	ND
41 KGP461	6.0±11.1	0.2±0.1	30
43 KGP 370	0.1±0.1	0.03±0.02	3.3
44 KGP 371	0.2±0.1	0.03±0.01	6.7
45 KGP 372	2.1±3.0	0.05±0.04	42

[a] Average of n ≥ 3 independent determinations

The initial cytotoxicity data for the CA1 and CA4 BAPCs showed promise for differential activity between oxic and hypoxic environments, with several BAPCs demonstrating a positive hypoxia cytotoxicity ratio (HCR). Utilizing an HCR of 6.0 as a benchmark to rank effective versus less effective BAPCs, a number of prodrugs stood out, notably compounds **25**, **41**, **44**, and **45**. The most active prodrugs in the series were the *gem*-dimethyl BAPCs of CA1 (**41**) and CA4 (**45**) with HCRs of 30 and 42 respectively, which was consistent with previous studies by Davis and co-workers that

demonstrated the *gem*-dimethyl CA4-BAPC had greater resistance to cleavage in oxic environments, releasing the parent anticancer agent (CA4) selectively under hypoxic conditions.[88]

Materials and Methods

General Materials and Methods

Acetic acid (AcOH), acetic anhydride, acetonitrile, dichloromethane, dimethylformamide (DMF), ethanol, methanol, nitric acid, sulfuric acid, and tetrahydrofuran (THF) were used in their anhydrous forms or as obtained from the chemical suppliers. Reactions were performed under nitrogen gas. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F254, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a Biotage Isolera or Teledyne Combiflash flash purification system using silica gel (200−400 mesh, 60 Å) or RP-18 pre-packed columns or manually in glass columns.

Intermediates and products synthesized were characterized on the basis of their ^{1}H NMR (600 or 500 MHz), ^{13}C NMR (150, 125 or 90 MHz) and ^{31}P NMR (240 MHz) spectroscopic data using a Varian VNMRS 500 MHz or a Bruker DRX 600 MHz or a Bruker DPX 360 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).

Purity of the final compounds was further analyzed at 25 °C using an Agilent 1200 HPLC system with a diode-array detector ($\lambda = 190-400$ 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. Mass spectrometry was carried out under either positive or negative ESI (electrospray ionization) or positive or negative atmospheric pressure photoionization using a Thermo Scientific LTQ OrbitrapDiscovery instrument.

Experimental Section

2,3-Dihydroxy-4-methoxybenzaldehyde (2)[128,129]

2,3,4-Trimethoxybenzaldehyde (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 and it was stirred for 18 hours. The reaction was then quenched with $NaHCO_3$ and acidified to pH 2 with conc. HCl, The product was then extracted with ethyl acetate, dried with sodium sulfate, and evaporated under reduced pressure. The crude mixture was then filtered through silica gel in a frit funnel with CH_2Cl_2 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, 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

2,3-dihydroxy-4-methoxybenzaldehyde (**2**) (2.64 g, 15.7 mmol, 77%) as a yellow solid.

¹H NMR (500 MHz, CDCl₃) 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).

¹³C NMR (125 MHz, CDCl₃) δ 195.2, 153.0, 149.0, 133.0, 126.1, 116.1, 103.6, 56.4.

6-formyl-2-hydroxy-3-methoxyphenyl 4-methylbenzenesulfonate (3)[128,129]

To a solution of aldehyde **2** (1.15 g, 6.76 mmol), and DIPEA (2.50 mL, 14.3 mmol) in

anhydrous DMF (10 mL) at, p-TSCl (1.29g , 6.73 mmol) was added in portions while

stirring at room temperature. After stirring for 5 h, the reaction mixture was quenched

with H2O (20 mL), and extracted with EtOAc (3 × 25 mL). The combined organic phase

was washed with brine, dried over MgSO4, filtered, 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, 40% A/60% B over

1.19 min (1 CV), 40% A/60% B →100% A/0% B over 16.3 min (10 CV), 100% A/0% B

over 3.18 min (2 CV); flow rate 40.0 mL/min; monitored at λ 254 and 280 nm] afforded

aldehyde **3** (1.33 g, 4.3 mmol, 61% yield) as a white solid.

¹H NMR (600 MHz, CDCl₃) δ 9.85 (1H, s), 7.87 (2H, d, *J* = 8.3 Hz), 7.50 (1H, d, *J* = 8.6

Hz), 7.36 (2H, d, *J* = 8.0 Hz), 6.90 (1H, d, *J* = 8.6 Hz), 5.91 (1H, s), 3.97 (3H, s), 2.47

(3H, s).

¹³C NMR (151 MHz, CDCl₃) δ 187.0, 153.2, 146.2, 139.2, 138.2, 132.0, 130.0, 128.7,

124.1, 120.6, 109.2, 56.7, 21.8.

3-Hydroxy-2-isopropoxy-4-methoxybenzaldehyde (4)[128,129]

2,3-Dihydroxy-4-methoxybenzaldehyde (0.400 g, 2.34 mmol), potassium carbonate [K_2CO_3] (0.330 g, 2.38 mmol), and 2-bromopropane (0.21 mL, 2.3 mmol) were dissolved in dry DMF (5mL) in a 5 mL Biotage microwave vial. The reaction was run in a Biotage microwave reactor (2h, 90 °C, normal absorbance). The reaction was then quenched with water, then extracted with ethyl acetate, washed with water and brine, dried with sodium sulfate, and evaporated under reduced pressure. Flash column 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 → 54% A/46% B over 13.12 min (10 CV), 54% A/46% B over 2.38 min (2 CV); flow rate 40.0 mL/min; monitored at λ 254 and 280 nm] yielded 3-hydroxy-2-isopropoxy-4-methoxybenzaldehyde (4) (0.220 g, 1.05 mmol, 44%) as a tan solid.

^{1}H NMR (CDCl$_3$, 500 MHz) δ 10.25 (1H, s), 7.44 (1H, d, $J = 8.7$ Hz), 6.74 (1H, d, $J = 8.7$ Hz), 5.65 (1H, s), 4.70 (1H, hept, $J = 6.1$ Hz), 3.96 (3H, s), 1.36 (7H, d, $J = 6.1$ Hz)

^{13}C NMR (151 MHz, CDCl$_3$) δ 189.7, 152.8, 147.9, 138.6, 124.3, 120.4, 106.2, 77.0, 56.4, 22.4.

2,3-bis((tert-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (5)[128,129]

To a solution of 2,3-dihydroxy-4-methoxybenzaldehyde (1.00 g, 5.95 mmol), Et$_3$N (2.00 mL, 14.3 mmol), and DMAP (0.025 g, 0.200 mmol) in CH$_2$Cl$_2$ (30 mL), TBSCl (2.10 g, 13.9 mmol) was dissolved in DMF and added drop-wise. The reaction was allowed to stir for 12 h at room temperature. H$_2$O was used to quench the reaction and the residue was extracted with CH$_2$Cl$_2$ (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 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] affording 2,3-bis((tert-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (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 189.6, 157.9, 151.3, 137.1, 123.6, 121.7, 105.7, 55.5, 26.5, 26.4, 19.1, 18.9, -3.5.

2-((tert-butyldimethylsilyl)oxy)-6-formyl-3-methoxyphenyl 4-methylbenzenesulfonate (6)[128,129]

Aldehyde **3** (0.501 g, 1.77 mmol), Et_3N (2.00 mL, 14.3 mmol), and DMAP (0.035 g, 0.28 mmol) were dissolved in dry CH_2Cl_2 (45 mL). TBSCl (0.327 g, 2.17 mmol) was added and the reaction was stirred for 18 hours. The reaction was quenched with water, extracted with diethyl ether, washed with water and brine, dried with sodium sulfate, and evaporated under reduced pressure flash column chromatography of the crude product using a prepacked 50 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 → 54% A/46% B over 13.12 min (10 CV), 54% A/46% B over 2.38 min (2 CV); flow rate 35.0 mL/min; monitored at λ 254 and 280 nm] yielded aldehyde **6** (0.610 g, 1.40 mmol, 79%) as a white solid.

^{1}H NMR (500 MHz, CDCl$_3$) δ 9.60 (1H, d, J = 0.47 Hz), 7.71 (2H, d, J = 8.34 Hz), 7.52 (1H, d, J = 8.70 Hz), 7.32 (2H, d, J = 8.05 Hz), 6.87 (1H, d, J = 8.63 Hz), 3.87 (3H, s), 2.45 (3H, s), 0.97 (9H, s), 0.10 (6H, s).

^{13}C NMR (126 MHz, CDCl$_3$) δ 186.7, 157.3, 145.9, 143.0, 138.9, 132.1, 129.9, 128.5, 124.0, 121.3, 109.8, 55.6, 25.7, 21.7, 18.6, -4.4.

3-((tert-butyldimethylsilyl)oxy)-2-isopropoxy-4-methoxybenzaldehyde (7)[128,129]

Aldehyde **4** (1.39 g, 6.61 mmol), Et$_3$N (1.40 mL, 9.91 mmol), and DMAP (0.050 g, 0.40 mmol) were dissolved in dry CH$_2$Cl$_2$ (50 mL). TBSCl (1.50 g, 9.95 mmol) was added and the reaction was stirred for 18 hours. The reaction was quenched with water, extracted with diethyl ether, washed with water and brine, dried with sodium sulfate, and evaporated under reduced pressure. Flash column chromatography of the crude product using a prepacked 50 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 → 54% A/46% B over 13.12 min (10 CV), 54% A/46% B over 2.38 min (2 CV); flow rate 35.0 mL/min; monitored at λ 254 and 280 nm] yielded aldehyde **7** (1.53 g, 4.71 mmol, 71%) as a white solid.

^{1}H NMR (600 MHz, CDCl$_3$) δ 10.11 (1H, s), 7.35 (1H, d, J = 8.7 Hz), 6.56 (1H, d, J = 8.7 Hz), 4.60 – 4.45 (1H, m), 3.71 (3H, s), 1.11 (3H, s), 1.10 (3H, s), 0.86 (9H, d, J = 2.0 Hz), 0.00 (6H, s).

^{13}C NMR (151 MHz, CDCl$_3$) δ 190.0, 157.4, 152.7, 138.4, 125.2, 121.4, 106.9, 75.5, 55.5, 25.9, 22.3, 18.7, -4.3.

3,4,5-Trimethoxybenzylbromide (9)[128,129]

The mixture of 3,4,5-trimethoxybenzylalcolol (20.1g, 101.4 mmol) and PBr_3 (4.8 mL, 50.7 mmol) in anhydrous CH_2Cl_2 was stirred for 1 h at 0 °C under nitrogen. Water (10 mL) was added, and the organic layer was separated and extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. After the recrystallization of the crude solid with 10% (EtOAc/hexane), the off-white solid of bromide **9** (23.6 g, 90.3 mmol, 89% yield) was obtained, which needed no further purification.

^{1}H NMR (500 MHz, CDCl$_3$) δ 6.62 (2H, s, H-2, H-6), 4.47 (2H, s, benzylic CH_2), 3.87 (6H, s, C-3, C-5 OCH_3), 3.85 (3H, s, C-4 OCH_3).

^{13}C NMR (125 MHz, CDCl$_3$) δ 153.3 (C, C-3, C-5), 138.2 (C, C-4), 133.2 (C, C-1), 106.1 (CH, C-2, C-6), 60.9 (CH$_3$, OCH_3-4), 56.1 (CH$_3$, OCH_3-3, -5), 34.3 (CH$_2$, -CH_2Br).

3,4,5-Trimethoxybenzyltriphenylphosphonium Bromide (10)[128,129]

A mixture of bromide **9** (11.00 g, 42.1 mmol), and PPh$_3$ (12.1 g, 46.3 mmol) in acetone (100 mL, anhydrous) was stirred in a flask under N$_2$. After 5 h, the resulting suspension was filtered through a Buchner funnel, and the solid was washed with acetone (100 mL) and hexanes (50 mL) to afford an off-white solid. The solid was dried in vacuo to obtain the phosphonium salt **10** (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, ArH), 7.58 – 7.50 (6H, m, ArH), 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$_3$, OCH$_3$-4), 56.2 (CH$_3$, OCH$_3$-3, -5), 30.8 (CH$_2$, -CH$_2$P). ^{31}P NMR (243 MHz, CDCl$_3$) δ 23.2.

(Z)-2-((tert-butyldimethylsilyl)oxy)-3-methoxy-6-(3,4,5-trimethoxystyryl)phenyl 4-methylbenzenesulfonate (11)[128,129]

Triphenyl(3,4,5-trimethoxybenzyl)phosphonium bromide (3.25 g, 6.20 mmol) was dissolved in dry THF (90 mL) in an ice/salt bath (-10 °C). *n*-Butyllithium (2.4 mL, 6.0 mmol, 2.5 M) was added dropwise and the reaction was stirred for 30 minutes. The aldehyde **6** (2.01 g, 4.60 mmol) was dissolved in dry THF (30 mL), added dropwise to the reaction mixture, and stirred for 5 hours. The reaction was quenched with water, and the THF was evaporated under reduced pressure. The mixture was extracted with ethyl acetate, washed with water and brine, dried with sodium sulfate, 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, 10% A/90% B over 1.19 min (1 CV), 10% A/90% B → 80% A/20% B over 13.12 min (10 CV), 80% A/20% B over 2.38 min (2 CV); flow rate 35.0 mL/min; monitored at λ 254 and 280 nm] yielded *Z*-isomer **11** (1.11 g, 1.84 mmol, 40%) as a white solid.

^{1}**H NMR** (500 MHz, CDCl$_3$) δ 7.82 (2H, d, J = 8.5 Hz), 7.25 (2H, d, J = 8 Hz), 6.77 (1H, d, J = 8.5 Hz), 6.61 (1H, d, 8.5 Hz), 6.44 (2H, s), 6.19 (1H, d, J = 12 Hz), 6.16 (1H, d, J = 12 Hz), 3.82 (3H, s), 3.76 (3H, s), 3.67 (6H, s), 0.95 (9H, s), 0.04 (6H, s).

¹³C NMR (125 MHz, CDCl$_3$) δ 152.6, 151.3, 144.8, 140.2, 139.1, 134.5, 132.2, 130.4, 129.5, 128.4, 125.3, 124.7, 122.1, 109.5, 106.1, 60.8, 55.8, 55.4, 25.8, 25.7, 25.6, 21.6, 18.7, -4.5.

(Z)-tert-butyl(2-isopropoxy-6-methoxy-3-(3,4,5-trimethoxystyryl)phenoxy)-dimethylsilane (12)[128,129]

Triphenyl(3,4,5-trimethoxybenzyl)phosphonium bromide (1.94 g, 3.70 mmol) was dissolved in dry THF (50 mL) and cooled to -15 ^{o}C. *n*-Butyllithium (2.5 M in hexane, 1.78 mL, 4.44 mmol, 2.5 M) was added dropwise and the reaction was stirred for 25 minutes. The reaction mixture was cooled to -78 ^{o}C, and a solution of aldehyde **7** in THF (30 mL) was added drop wise and the reaction was stirred for 5 hours. The reaction was quenched with water, and the THF was evaporated under reduced pressure. The mixture was extracted with ethyl acetate, washed with water and brine, dried with sodium sulfate, and evaporated under reduced pressure. The crude product was purified using flash column chromatography to yield *Z*-isomer (after separating it from *E*-isomer) (0.982 g, 2.01 mmol, 65%) as a reddish/white solid.

¹H NMR (600 MHz, CDCl$_3$) δ 6.83 (1H, d, *J*=8.6 Hz), 6.62 (1H, d, *J*=12.1 Hz), 6.52 (2H, s), 6.45 (1H, d, *J*=8.6 Hz), 6.41 (1H, d, *J*=12.1 Hz), 4.61 (1H, hept, *J*=6.1 Hz), 3.82 (3H, s), 3.76 (3H, s), 3.65 (6H, s), 1.27 (6H, d, *J*=6.1 Hz), 1.02 (10H, s), 0.14 (6H, s).

¹³C NMR (151 MHz, CDCl$_3$) δ 152.7, 151.4, 148.0, 138.6, 136.8, 132.9, 128.5, 126.9, 125.1, 122.4, 106.0, 105.9, 74.2, 60.9, 55.8, 55.2, 25.9, 22.3, 18.7, -4.4.

(Z)-((3-methoxy-6-(3,4,5-trimethoxystyryl)-1,2-phenylene)bis(oxy))bis(tert-butyldimethylsilane) (13)[128,129]

n-Butyllithium (11.4 mL, 2.5M) was added to a solution of phosphonium salt (11.2 g, 21.4 mmol) in THF (350 mL). The resulting solution was allowed to stir for 15 min at -78 °C. Aldehyde **5** (5.66 g, 14.3 mmol) was dissolved in THF and added drop-wise using a dropping funnel. The reaction was allowed to stir for 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 **13** (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, 133.1, 128.0, 127.7, 123.5, 122.5, 106.2, 104.5, 61.2, 56.1, 55.3, 26.7, 26.4, 19.1, -2.9, -3.6.

Synthesis of Compounds **16**, **17** and **19** using Old Route[144]

(5-nitrothiophen-2-yl)methanol (16)[144]

5-Nitrothiophene-2-carboxaldehyde (1.00 g, 6.38 mmol) was dissolved in dry methanol (20 mL) in an ice bath (0 °C). Sodium borohydride (0.270 g, 7.14 mmol) was added and the reaction was stirred for two hours. Ice was added and the solution was acidified to pH 7 with 3 M HCl. The reaction was extracted with ethyl acetate, 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 alcohol **16** (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 (17)[144]

2-Acetyl-5-nitrothiophene (1.00 g, 5.85 mmol) was dissolved in dry methanol [MeOH] (20 mL) in an ice bath (0 °C). Sodium borohydride [NaBH$_4$] (0.259 g, 6.71 mmol) was added and the reaction was stirred for two hours. Ice was added to the reaction and it was acidified to neutral pH with 3 M HCl. The solution was then extracted with ethyl acetate, 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 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), 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 (18)[88]

2-Acetylthiophene (10.0 g, 79.2 mmol) was dissolved in dry THF (100 mL) in an ice bath (0 °C). Methyllithium (64 mL, 103 mmol, 1.6 M) was added dropwise and the reaction was stirred for 18 hours. The reaction was quenched with water and evaporated under reduced pressure. The reaction was then extracted with ethyl acetate, dried with sodium sulfate, 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 λ 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), 6.97 (2H, m), 2.04 (1H, s), 1.68 (6H, s).

2-(5-nitrothiophen-2-yl)propan-2-ol (19)[144]

The tertiary alcohol **18** (6.22 g, 4.37 mmol) was dissolved in Ac$_2$O (67 mL) and cooled to -78 °C. Fuming nitric acid (25 mL) was added drop wise and the reaction mixture was

stirred for 2 h allowing the reaction mixture to warm to -15 °C. Ice (200 g) was added to the solution and stirred for 40 min. The reaction mixture was extracted with EtOAc (3×75 mL) and washed repeadadly 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), 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, 72.0, 32.2.

Synthesis of Compounds **15**, **17** and **19** using New Route

1-(5-nitrothiophen-2-yl)ethan-1-ol (17)[144]

Titanium tetrachloride (7.84 g, 41.3 mmol) was added slowly dropwise into Et_2O (80 mL) at -78 °C, after which methyllithium (1.6 M, 25.8 mL, 41.3 mmol) was added dropwise and the reaction was stirred for 1.5 hours. 5-nitro-2-thiophenecarboxaldehyde (5.00g, 31.8 mmol) was dissolved in Et_2O (120 mL) and added dropwise to the reaction. The reaction was stirred (12 hr) and H_2O (50 mL) was used to quench the reaction. The layers were partitioned, 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 **17** (4.95 g, 28.6 mmol, 90%) as a dark brown oil.

¹H NMR (500 MHz, CDCl₃) 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).

¹³C NMR (125 MHz, CDCl₃) δ 160.0, 149.9, 129.1, 122.2, 66.3, 25.1.

1-(5-nitrothiophen-2-yl)ethan-1-one (15)

2-(1-hydroxyethyl)-5-nitrothiophene (1.04 g, 6.00 mmol) was dissolved in 70 mL CH_2Cl_2 at rt. Dess-Martin periodinane (3.82 g, 9.00mmol) was added in portions to the solution and the reaction was stirred (1 hr). Saturated sodium thiosulfate solution (50 mL) and saturated sodium bicarbonate solution (50 mL) were used to quench the reaction. The layers were partitioned, and the residue was extracted with EtOAc (4 x 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 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] affording compound **15** (0.873 g, 5.10 mmol, 90%) as a yellow-orange crystals.

¹H NMR (600 MHz, CDCl₃) δ 7.89 (1H, d, J=4.3 Hz), 7.58 (1H, d, J=4.3 Hz), 2.60 (3H, s)

¹³C NMR (151 MHz, CDCl₃) δ 190.5, 156.5, 148.2, 130.2, 128.4, 26.6.

2-(5-nitrothiophen-2-yl)propan-2-ol (19)[103]

Titanium tetrachloride (3.62 g, 19.1 mmol) was added slowly dropwise into Et_2O (80 mL) at -78 °C, after which methyllithium (1.6 M, 11.9 mL, 19.1 mmol) was added dropwise and the reaction was stirred for 1.5 hours. 2-acetyl-5-nitrothiophene (2.50 g, 14.7 mmol) was dissolved in Et_2O (140 mL) and added dropwise to the reaction. The reaction was stirred (12 hr) and H_2O (50 mL) was used to quench the reaction. The layers were partitioned, 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 **19** (1.61 g, 8.60 mmol, 45%) as a dark orange oil.

¹H 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).

¹³C NMR (151 MHz, CDCl$_3$) δ 163.6, 133.9, 128.9, 121.4, 72.0, 32.2.

(Z)-2-hydroxy-3-methoxy-6-(3,4,5-trimethoxystyryl)phenyl 4-methylbenzene-sulfonate (20)[128,129]

To a solution of *Z*-stilbene **11** (0.754 g, 1.26 mmol) in dry THF (40 mL) at -15 °C, TBAF· $3H_2O$ (3.8 mL, 3.8 mmol) was dissolved in THF (10 mL) and added drop-wise. The reaction was allowed to stir for 12 h. H_2O (40 mL) was used to quench the reaction, THF was evaporated off completely, and the residue was extracted with EtOAc (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 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (1 CV), 12%A / 88%B → 82%A / 18%B (10 CV), 82%A / 18%B (2 CV); flow rate: 35 mL/min; monitored at 254 and 280 nm] afforded compound **20** (0.429 g, 0.882 mmol, 70%) as a dark green solid.

¹H NMR (500 MHz, CDCl₃) δ 7.91 (1H, d, *J*=8.1 Hz), 7.29 (1H, d, *J*=8.0 Hz), 6.71 (1H, d, *J*=8.6 Hz), 6.62 (1H, d, *J*=8.6 Hz), 6.42 (1H, s), 6.36 (1H, d, *J*=12.0 Hz), 6.32 (1H, d, *J*=12.0 Hz), 5.89 (1H, s), 3.86 (3H, s), 3.82 (3H, s), 3.66 (6H, s), 2.42 (2H, s).

¹³C NMR (126 MHz, CDCl₃) δ 151.9, 146.6, 144.5, 138.5, 136.4, 134.5, 132.7, 131.2, 130.5, 128.7, 127.7, 124.9, 123.4, 119.9, 108.3, 105.4, 75.9, 60.0, 55.6, 55.0, 20.9.

(Z)-2-isopropoxy-6-methoxy-3-(3,4,5-trimethoxystyryl)phenol (21)[128,129]

To a solution of compound **12** (0.150 g, 0.251 mmol) in THF (5 mL) at room temperature, TBAF· $3H_2O$ (0.0952 g, 0.302 mmol) was dissolved in THF and added drop-wise. The reaction was allowed to stir for 0.5 h. H_2O (5 mL) was used to quench the reaction, THF was evaporated off completely, and the residue was extracted with EtOAc (3 × 10 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: 8 mL/min; monitored at 254 and 280 nm] affording compound **21** (0.135 g, 0.361 mmol, 90%) as a white solid.

¹H NMR (500 MHz, CDCl₃) δ 6.75 (1H, d, *J*=8.6 Hz), 6.59 (1H, d, *J*=12.1 Hz), 6.51

(1H, d, *J*=8.7 Hz), 6.51 (2H, s), 6.46 (1H, d, *J*=12.1 Hz), 5.60 (1H, s), 4.56 (1H, hept,

J=6.1 Hz), 3.86 (3H, s), 3.82 (3H, s), 3.66 (6H, s), 1.32 (6H, d, *J*=6.2 Hz).

¹³C NMR (126 MHz, CDCl₃) δ 152.7, 146.9, 143.2, 138.9, 137.1, 132.5, 129.4, 125.8,

124.1, 120.5, 106.3, 106.0, 75.7, 60.9, 56.2, 55.8, 22.5.

HRMS: *m/z*: obsd 397.1713 [M+Na]⁺, calcd for $C_{21}H_{26}O_6^+$, 397.1713.

HPLC (Method A): 14.7 min.

(Z)-3-methoxy-2-((5-nitrothiophen-2-yl)methoxy)-6-(3,4,5-trimethoxystyryl)phenyl

4-methylbenzenesulfonate (22)

To a solution of compound **20** (0.700 g, 1.44 mmol), *nor*-methyl trigger **16** (0.191 g, 1.20

mmol), and DIAD (0.32 mL) in CH₂Cl₂ (10 mL), PPh₃ (0.610 g, 2.33 mmol) was

dissolved in CH₂Cl₂ and added drop-wise. The reaction was allowed to stir for 12 h at

room temperature. H₂O was used to quench the reaction and the residue was extracted

with CH₂Cl₂ (3 × 30 mL). The combined extracts were washed with brine, dried over

Na₂SO₄, filtered, and concentrated 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: 10%A / 90%B (1 CV), 10%A / 90%B → 80%A /

20%B (10 CV), 80%A / 20%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280

nm] affording tosyl-protected CA1 nor-methyl BAPC **22** (0.125 g, 0.236 mmol, 47%) as

a tan-white solid.

¹H NMR (600 MHz, CDCl₃) δ 7.84 (2H, d, *J*=8.2 Hz), 7.77 (1H, d, *J*=4.1 Hz), 7.24 (2H,

d, *J*=8.1 Hz), 6.94 (1H, d, *J*=8.7 Hz), 6.90 (1H, d, *J*=4.1 Hz), 6.69 (1H, d, *J*=8.8 Hz), 6.46

(2H, s), 6.40 (1H, d, *J*=11.9 Hz), 6.33 (1H, d, *J*=11.9 Hz), 5.06 (2H, s), 3.85 (3H, s), 3.83 (3H, s), 3.68 (6H, s), 2.40 (3H, s).

^{13}C NMR (151 MHz, CDCl$_3$) δ 152.8, 152.5, 151.8, 147.9, 145.2, 141.8, 140.3, 137.2, 134.3, 132.0, 131.7, 129.6, 128.3, 128.1, 126.1, 125.9, 125.8, 124.0, 110.4, 106.1, 69.0, 60.9, 56.2, 55.9, 21.7.

HRMS: *m/z*: obsd 650.1120 [M+Na]$^+$, calcd for C$_{30}$H$_{29}$NO$_{10}$S$_2$$^+$, 627.1233.

HPLC (Method A): 18.5 min.

(Z)-3-Methoxy-2-(2-(5-nitrothiophen-2-yl)propoxy)-6-(3,4,5-trimethoxystyryl)-phenyl-4-methylbenzenesulfonate (23)

To a solution of compound **20** (0.200 g, 0.411 mmol), DIAD (0.100 g, 0.495 mmol), and 1-(5-nitrothiophen-2-yl) ethanol (0.059 g, 0.34 mmol) in CH$_2$Cl$_2$ (25 mL), triphenylphosphine (0.216 g, 0.822 mmol) was added and the reaction was stirred for 24 h. The reaction was quenched with water, extracted with ethyl acetate, dried with sodium sulfate, and evaporated under reduced pressure. Flash chromatography of the crude product using a prepacked 25 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 25.0 mL/min; monitored at λ 254 and 280 nm] yielded (*Z*)-3-methoxy-2-(2-(5-nitrothiophen-2-yl)propoxy)-6-(3,4,5-trimethoxystyryl)phenyl-4-methylbenzenesulfonate (**23**) (0.160 g, 0.249 mmol, 61%) as a yellow solid.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.90 (1H, d, *J* = 4.3 Hz), 7.86 (2H, d, *J* = 8.3 Hz), 7.44 (2H, d, *J* = 8.3 Hz), 7.01 (1H, d, *J* = 8.8 Hz), 6.99 (2H, m), 6.57 (2H, s), 6.51 (1H, d, *J* =

12.0 Hz), 6.44 (1H, d, $J = 11.9$ Hz), 5.47 (1H, q, $J = 6.5$ Hz), 3.90 (3H, s), 3.73 (3H, s),

3.66 (6H, s), 2.44 (3H, s), 1.43 (3H, d, $J = 6.5$ Hz).

¹³C NMR (125 MHz, CDCl₃) δ 159.6, 158.2, 158.1, 150.5, 147.2, 144.2, 139.9, 137.2,

136.8, 134.9, 133.5, 131.1, 130.9, 129.5, 129.1, 116.0, 111.7, 80.7, 73.4, 64.8, 60.9, 60.5,

26.5, 26.0, 25.8.

HRMS: *m/z*: obsd 642.1465 [M+H]⁺, calcd for $C_{31}H_{31}NO_{10}S_2^+$, 641.1389.

HPLC (Method A): 18.2 min.

(Z)-2-((2-isopropoxy-6-methoxy-3-(3,4,5-trimethoxystyryl)phenoxy)methyl)-5-

nitrothiophene (24)

To a solution of isopropyl protected CA1 **21** (0.350 g, 0.843 mmol), *nor*-methyl trigger

16 (0.162 g, 1.02 mmol), and DEAD (0.220 mL) in CH₂Cl₂ (10 mL), PPh₃ (0.430 g, 1.64

mmol) was dissolved in CH₂Cl₂ and added drop-wise. The reaction was allowed to stir for

24 h at room temperature. H₂O (40 mL) was used to quench the reaction and the residue

was extracted with CH₂Cl₂ (3 × 20 mL). The combined extracts were washed with brine,

dried over Na₂SO₄, filtered, and concentrated 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: 10%A / 90%B (1 CV), 10%A / 90%B → 80%A /

20%B (10 CV), 80%A / 20%B (2 CV); flow rate: 17 mL/min; monitored at 254 and 280

nm] affording CA1-BAPC **24** (0.0600 g, 0.116 mmol, 17%) as a yellow oil.

¹H NMR (500 MHz, CDCl3) δ 7.82 (1H, d, *J*=4.1 Hz), 7.02 (1H, d, *J*=8.8 Hz), 7.00 (1H,

d, *J*=4.2 Hz), 6.60 (1H, d, *J*=12.1 Hz), 6.53 (1H, d, *J*=8.7 Hz), 6.50 (2H, s), 6.47 (1H, d,

J=12.1 Hz), 5.17 (1H, s), 4.60 (1H, p, *J*=6.2 Hz), 3.83 (3H, s), 3.82 (3H, s), 3.67 (6H, s), 1.32 (3H, s), 1.31 (3H, s).

^{13}C NMR (126 MHz, CDCl$_3$) δ 152.8, 152.8, 151.7, 149.9, 149.0, 140.6, 137.1, 132.6, 129.3, 128.2, 125.9, 125.7, 125.3, 125.1, 106.8, 106.0, 76.0, 69.2, 60.9, 55.9, 55.8, 22.6.

HRMS: *m/z*: obsd 538.1506 [M+23]$^+$, calcd for C$_{26}$H$_{29}$NO$_8$S$^+$ 515.1614.

HPLC (Method A): 14.7 min.

(Z)-2-(1-(2-isopropoxy-6-methoxy-3-(3,4,5-trimethoxystyryl)phenoxy)ethyl)-5-nitrothiophene (25)

To a solution of isopropyl protected CA1 **21** (0.267 g, 0.715 mmol), *mono*-methyl trigger **17** (0.136 g, 0.785 mmol), and DIAD (0.190 mL) in CH$_2$Cl$_2$ (10 mL), PPh$_3$ (0.364 g, 1.39 mmol) was dissolved in CH$_2$Cl$_2$ and added drop-wise. The reaction was allowed to stir for 12 h at room temperature. H$_2$O was used to quench the reaction and the residue was extracted with CH$_2$Cl$_2$ (3 × 20 mL). The combined extracts were washed with brine, dried over Na$_2$SO$_4$, 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: 10%A / 90%B (1 CV), 10%A / 90%B → 80%A / 20%B (10 CV), 80%A / 20%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] yielding CA1-BAPC **25** (0.125 g, 0.236 mmol, 47%) as a yellow oil.

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.78 (1H, d, *J*=4.2 Hz), 6.99 (1H, d, *J*=8.7 Hz), 6.91 (1H, d, *J*=4.1 Hz), 6.59 (1H, d, *J*=12.1 Hz), 6.49 (1H, d, *J*=8.6 Hz), 6.47 (2H, s), 6.45 (1H, d, *J*=12.2 Hz), 5.49 (1H, q, *J*=6.4 Hz), 4.61 (1H, hept, *J*=6.1 Hz), 3.82 (3H, s), 3.75 (3H, s), 3.65 (6H, s), 1.66 (3H, d, *J*=6.5 Hz), 1.30 (3H, d, *J*=6.1 Hz), 1.26 (3H, d, *J*=6.1 Hz).

¹³C NMR (151 MHz, CDCl₃) δ 155.2, 153.1, 152.8, 151.0, 150.2, 139.2, 137.0, 132.6, 129.2, 128.1, 125.9, 125.9, 125.3, 123.5, 106.6, 105.9, 75.7, 75.4, 60.9, 55.9, 55.8, 22.6, 22.5, 22.2. **HRMS**: *m/z*: obsd 552.1660 [M+23]⁺, calcd for C₂₇H₃₁NO₈S⁺, 529.1766. **HPLC** (Method B): 20.5 min.

(Z)-2-(2-(2-isopropoxy-6-methoxy-3-(3,4,5-trimethoxystyryl)phenoxy)propan-2-yl)-5-nitrothiophene (26)

To a solution of isopropyl protected CA1 **21** (0.150 g, 0.402 mmol), g*em*-dimethyl trigger **19** (0.091 g, 0.486 mmol), and ADDP (0.137 g, 0.543 mmol) in CH₂Cl₂ (10 mL), PBu₃ (0.199 mL) was added drop-wise. The reaction was allowed to stir for 24 h at room temperature. H₂O was used to quench the reaction and the residue was extracted with CH₂Cl₂ (3 × 20 mL). The combined extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated 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: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] yielding CA1-BAPC **26** (0.020 g, 0.037 mmol, 13%) as an orange oil.

¹H NMR (600 MHz, CDCl₃) δ 7.80 (1H, d, *J*=4.2 Hz), 7.01 (1H, d, *J*=8.7 Hz), 6.93 (1H, d, *J*=4.3 Hz), 6.58 (1H, d, *J*=12.1 Hz), 6.49 (2H, s), 6.47 (1H, d, *J*=8.6 Hz), 6.45 (1H, d, *J*=12.1 Hz), 4.60 (1H, hept, *J*=6.0 Hz), 3.82 (3H, s), 3.67 (3H, s), 3.66 (6H, s), 1.71 (6H, s), 1.23 (6H, d, *J*=6.1 Hz).

13C NMR (151 MHz, CDCl$_3$) δ 161.4, 154.6, 152.8, 151.6, 150.4, 137.1, 137.0, 132.7, 129.0, 128.1, 126.4, 126.2, 125.3, 122.1, 106.4, 105.9, 81.7, 75.1, 60.9, 55.8, 55.5, 28.8, 22.4.

HRMS: *m/z*: obsd 566.1819 [M+23]$^+$, calcd for C$_{28}$H$_{33}$NO$_8$S$^+$, 543.1927.

HPLC (Method B): 22.3 min.

Synthesis of Compounds **27**, **28** and **29**

Deprotection of TBS group of compound 13 using TBAF (0.9 eq.) yielded an inseparable mixture of compound **27** and **28**. At the same time, about 15% CA1 (compound **29**) is also isolated.

(Z)-2-((tert-butyldimethylsilyl)oxy)-3-methoxy-6-(3,4,5-trimethoxystyryl)phenol (27) and (Z)-2-((tert-butyldimethylsilyl)oxy)-6-methoxy-3-(3,4,5-trimethoxystyryl)phenol (28)[128,129]

To a solution of di-TBS CA1 **13** (2.00 g, 3.57 mmol) in THF (150 mL) at -15 °C, TBAF· 3H$_2$O (1.01 g, 3.20 mmol) was dissolved in THF (10 mL) and added drop-wise. The reaction was allowed to stir for 0.5 h. H$_2$O was used to quench the reaction, THF was evaporated off completely, and the residue was extracted with EtOAc (3 × 30 mL). The combined extracts were washed with brine, dried over Na$_2$SO$_4$, 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] affording a mixture of compounds **27** and **28** (0.860 g, 2.59 mmol, 43%) as a white solid.

¹H NMR (500 MHz, CDCl₃) 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).

¹³C NMR (126 MHz, CDCl₃) 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 (29)

The combretastatin A-1 (CA1) **29** (0.179 mg, 0.538 mmol, 15%) was isolated as a white solid.

¹H NMR (500 MHz, CDCl₃) 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).

¹³C NMR (126 MHz, CDCl₃) 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, 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.

Synthesis of Compounds **30** and **33**

To a solution of mixture of compounds **27 and 28** (1.00 g, 2.24 mmol), *nor*-methyl trigger **16** (0.428 g, 2.69 mmol), and DIAD (0.867 mL) in CH₂Cl₂ (50 mL), PPh₃ (1.47 g, 5.60 mmol) was added drop-wise. The reaction mixture was stirred (24 h) at room

temperature. H_2O (40 mL) was added to quench the reaction 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 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].

(Z)-tert-butyl(6-methoxy-2-((5-nitrothiophen-2-yl)methoxy)-3-(3,4,5-trimethoxystyryl)-phenoxy)dimethylsilane (30)

This isomer **30** (0.350 g, 0.739 mmol, 35%) was isolated 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 (33)

This isomer **33** (0.250 g, 0.425 mmol, 25%) was isolated as a brownish-yellow oil.

¹H NMR (600 MHz, CDCl₃) 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).

¹³C NMR (151 MHz, CDCl₃) 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.

Synthesis of Compounds **31** and **34**

Mono TBS CA1 (0.680 g, 1.52 mmol), diisopropylazodicarboxylate (0.415 g, 2.05 mmol), and monomethyl trigger (0.317 g, 1.82 mmol) were dissolved in THF (50 mL). Triphenylphosphine (0.793 g, 3.04 mmol) was added and the reaction was stirred (3 d). The reaction was concentrated under reduced pressure and the crude product was purified by flash column chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 40%A / 60%B (13 CV), 40%A / 60%B (2 CV); flow rate: 80 mL/min; monitored at 254 and 280 nm].

(Z)-tert-butyl(6-methoxy-2-(1-(5-nitrothiophen-2-yl)ethoxy)-3-(3,4,5-trimethoxystyryl)phenoxy)dimethylsilane (31)

This isomer **31** (0.375 g, 0.623 mmol, 41%) was isolated as a yellow oil.

¹H NMR (600 MHz, CDCl₃) δ 7.60 (1H, d, *J* = 4.2 Hz), 6.76 – 6.69 (2H, m), 6.41 (1H, d, *J* = 8.2 Hz), 6.39 (1H, d, *J* = 11.9 Hz), 6.32 (2H, s), 6.29 (1H, d, *J* = 12.1 Hz), 5.58 (1H,

q, $J = 6.4$ Hz), 3.69 (3H, s), 3.65 (3H, s), 3.51 (6H, s), 1.48 (3H, d, $J = 6.4$ Hz), 0.85 (9H, s), 0.00 (3H, s), -0.02 (3H, s).

13C NMR (151 MHz, CDCl₃) δ 155.0, 152.7, 151.4, 150.9, 146.1, 138.5, 137.2, 132.4, 129.8, 128.1, 125.6, 124.9, 123.4, 122.5, 107.0, 106.0, 74.4, 60.9, 55.8, 55.3, 25.8, 22.3, 18.6, -4.3, -4.4.

(Z)-tert-butyl(3-methoxy-2-(1-(5-nitrothiophen-2-yl)ethoxy)-6-(3,4,5-trimethoxystyryl)phenoxy)dimethylsilane (34)

This isomer **34** (0.073 g, 0.122 mmol, 12%) was isolated as a yellow oil.

1H NMR (600 MHz, CDCl₃) δ 7.61 (1H, d, $J = 4.2$ Hz), 6.84 (1H, d, $J = 8.7$ Hz), 6.71 (1H, d, $J = 4.2$ Hz), 6.38 (1H, d, $J = 12.0$ Hz), 6.37 (2H, s), 6.26 (1H, d, $J = 12.3$ Hz), 6.24 (1H, d, $J = 8.6$ Hz), 5.26 (1H, q, $J = 6.5$ Hz), 3.67 (3H, s), 3.61 (3H, s), 3.50 (6H, s), 1.47 (3H, d, $J = 6.5$ Hz), 0.84 (9H, s), 0.03 (3H, s), 0.00 (3H, s).

13C NMR (151 MHz, CDCl₃) δ 153.3, 151.1, 150.9, 149.1, 146.1, 135.5, 135.2, 130.7, 126.9, 126.2, 124.6, 123.7, 121.5, 121.5, 104.1, 103.0, 73.1, 59.0, 53.9, 24.2, 24.2, 19.8, 16.7, -5.2, -5.7.

(Z)-tert-butyl(6-methoxy-2-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)-3-(3,4,5-trimethoxystyryl)phenoxy)dimethylsilane (32)

Mono TBS CA1 (1.07 g, 2.40 mmol), *gem*-dimethyl trigger (0.540 g, 2.88 mmol), and ADDP (0.832 g, 3.30 mmol) were dissolved in CH₂Cl₂ (100 mL). Tributylphosphine (1.26 mL, 5.04 mmol) was added drop-wise and the reaction was stirred (2d). The

reaction was then concentrated under reduced pressure. Flash chromatography yielded the crude product which was taken to the next step for deprotection.

(Z)-4-methoxy-2-(5-nitrothiophen-2-yl)-7-(3,4,5-trimethoxystyryl)-benzo[d][1,3]dioxole (35)

To a solution of **30** (0.095 g, 0.162 mmol) in THF (10 mL) at 0 °C, TBAF· 3H$_2$O (0.0672 g, 0.213 mmol) was dissolved in THF (10 mL) and added drop-wise. The reaction was stirred (30 min) and H$_2$O (5 mL) was added. THF was evaporated off completely, and the residue was extracted with CH$_2$Cl$_2$ (3 × 20 mL). The combined extracts were washed with brine, dried over Na$_2$SO$_4$, 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 **35** (0.0510 g, 0.108 mmol, 54%) as a yellow oil.

¹H 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).

¹³C 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.

¹³C 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)-4-methoxy-2-(5-nitrothiophen-2-yl)-7-(3,4,5-trimethoxystyryl)-benzo[d][1,3]dioxole (35) [Base cyclization method]

Compound **37** (0.0380 g, 0.0803 mmol) was dissolved in THF (5 mL) at room temperature. Sodium hydroxide (1 mL, 2M) was added drop-wise and the reaction was then stirred (5 m). THF was rotovapped off completely, and the residue was extracted with CH_2Cl_2 (3 x 10 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: 36 mL/min; monitored at 254 and 280 nm] affording compound **35** (0.0090 g, 0.019 mmol, 23%)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.

(Z)-4-methoxy-2-methyl-2-(5-nitrothiophen-2-yl)-7-(3,4,5-trimethoxystyryl)-benzo[d][1,3]dioxole (36)

Compound **31** (0.105 g, 0.174 mmol) was dissolved in CH_2Cl_2 (20 mL) at -10 ^{o}C. Tert-butylammonium fluoride trihydrate (0.0620 g, 0.191 mmol) was dissolved in CH_2Cl_2 (2 mL) and added slowly drop wise to the reaction which was then stirred (18 min). H_2O (5 mL) was used to quench the reaction and the layers were partitioned. The residue was extracted with CH_2Cl_2 (3 x 10 mL), washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (1 CV), 12%A / 88%B → 100%A / 0%B (13 CV), 100%A / 0%B (2 CV); flow rate: 10 mL/min; monitored at 254 and 280 nm] affording compound **36** (0.044 g, 0.0906 mmol, 52%) as a yellow oil.

^{1}H NMR (500 MHz, acetone) δ 7.76 (1H, d, J = 4.3 Hz), 7.03 (1H, d, J = 4.2 Hz), 6.81 (1H, d, J = 8.8 Hz), 6.56 (1H, d, J = 12.0 Hz), 6.48 (2H, s), 6.45 (1H, d, J = 8.8 Hz), 6.42 (1H, d, J = 11.9 Hz), 3.88 (3H, s), 3.82 (3H, s), 3.67 (6H, s), 2.02 (3H, s).

^{13}C NMR (126 MHz, acetone) δ 152.8, 151.5, 145.1, 143.1, 137.2, 133.8, 132.7, 131.1, 128.3, 124.0, 123.0, 121.9, 113.8, 113.4, 107.4, 105.7, 60.9, 56.5, 55.9, 26.6.

HRMS: m/z: obsd 486.1219 $[M+H]^+$, calcd for $C_{24}H_{23}NO_8S^+$, 485.1144.

HPLC (Method A): 14.9 min.

(Z)-4-methoxy-2-methyl-2-(5-nitrothiophen-2-yl)-7-(3,4,5-trimethoxystyryl)-benzo[d][1,3]dioxole (36)   [Base cyclization method]

Compound **38** (0.0500 g, 0.103 mmol) was dissolved in THF (5 mL) at room temperature. Sodium hydroxide (1 mL, 2M) was added drop-wise and the reaction was then stirred (5 m). THF was rotovapped off completely, and the residue was extracted

with CH_2Cl_2 (3 x 10 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: 36 mL/min; monitored at 254 and 280 nm] affording compound **36** (0.0470 g, 0.0964 mmol, 93%) as a yellow oil.

^{1}H NMR (500 MHz, acetone) δ 7.76 (1H, d, $J = 4.3$ Hz), 7.03 (1H, d, $J = 4.2$ Hz), 6.81 (1H, d, $J = 8.8$ Hz), 6.56 (1H, d, $J = 12.0$ Hz), 6.48 (2H, s), 6.45 (1H, d, $J = 8.8$ Hz), 6.42 (1H, d, $J = 11.9$ Hz), 3.88 (3H, s), 3.82 (3H, s), 3.67 (6H, s), 2.02 (3H, s).

^{13}C NMR (126 MHz, acetone) δ 152.8, 151.5, 145.1, 143.1, 137.2, 133.8, 132.7, 131.1, 128.3, 124.0, 123.0, 121.9, 113.8, 113.4, 107.4, 105.7, 60.9, 56.5, 55.9, 26.6.

(Z)-6-methoxy-2-((5-nitrothiophen-2-yl)methoxy)-3-(3,4,5-trimethoxystyryl)phenol (37)

AcOH (7 mL) and HCl (5 mL, 2M) was added drop-wise to a solution of compound **30** (0.115 g, 0.196 mmol) in THF (30 mL). The reaction as allowed to stir for 8 h at room temperature. 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: 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] affording compound **37** (0.020 g, 0.0422

mmol, 17%) as a brown oil.

¹H NMR (600 MHz, CDCl₃) δ 7.82 (1H, d, *J*=4.1 Hz), 7.00 (1H, d, *J*=4.1 Hz), 6.98 (1H,

d, *J*=8.7 Hz), 6.53 (2H, s), 6.50 (2H, s), 6.39 (1H, d, *J*=8.8 Hz), 5.70 (1H, s), 5.24 (2H, s),

3.87 (3H, s), 3.83 (3H, s), 3.68 (6H, s).

¹H 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).

¹³C 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.

¹³C 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)-6-methoxy-2-(1-(5-nitrothiophen-2-yl)ethoxy)-3-(3,4,5-trimethoxystyryl)phenol

(38)

Compound **31** (0.200 g, 0.333 mmol) was dissolved in THF (5 mL). Glacial acetic acid (7

mL) and hydrochloric acid (2 M, 4 mL) were added dropwise and the reaction was stirred

(30 min). Glacial acetic acid (4 mL) and hydrochloric acid (2 M, 2.5 mL) were added

dropwise and the reaction was stirred (8 hr). H_2O (30 mL) was used to quench the

reaction and it was concentrated under reduced pressure. The residue was extracted with

CH_2Cl_2 (3 x 30 mL), washed multiple times 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 50 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] affording compound **38** (0.094 g, 0.199 mmol, 60%) as a yellow oil.

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.74 (1H, d, J = 4.2 Hz), 6.93 (1H, d, J = 4.2 Hz), 6.80 (1H, d, J = 8.5 Hz), 6.55 (1H, d, J = 8.6 Hz), 6.53 (1H, d, J = 12.5 Hz), 6.47 (2H s), 6.45 (1H, d, J = 12.2 Hz), 5.71 (1H, q, J = 6.4 Hz), 3.87 (3H, s), 3.84 (3H, s), 3.66 (6H, s), 1.71 (3H, d, J = 6.4 Hz).

^{13}C NMR (126 MHz, CDCl$_3$) δ 153.6, 152.8, 151.4, 147.5, 137.1, 132.6, 132.2, 130.2, 128.2, 125.4, 123.8, 123.8, 117.5, 105.8, 103.4, 75.2, 60.9, 55.9, 55.8, 29.7, 21.7.

HRMS: *m*/*z*: obsd 488.1363 [M+H]$^+$, calcd for C$_{24}$H$_{25}$NO$_8$S$^+$, 487.1301.

HPLC (Method B): 10.1 min.

(Z)-3-methoxy-2-((5-nitrothiophen-2-yl)methoxy)-6-(3,4,5-trimethoxystyryl)phenol (39)

AcOH (10 mL) and HCl (10 mL, 2M) was added drop-wise to a solution of compound **33** (0.250 g, 0.425 mmol) in THF (25 mL). The reaction as allowed to stir for 8 h at room temperature. H$_2$O (40 mL) was used to quench the reaction, THF was evaporated off completely, and the residue was extracted with CH$_2$Cl$_2$ (3 × 20 mL). The combined extracts were washed with brine, dried over Na$_2$SO$_4$, 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] affording compound **33** (0.030 g, 0.0634 mmol, 12%) as a brown oil.

¹H NMR (CDCl₃, 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).

¹³C NMR (CDCl₃, 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 C₂₃H₂₃NO₈S⁺, 473.1144.

HPLC (Method B): 12.5 min.

(Z)-3-methoxy-2-(1-(5-nitrothiophen-2-yl)ethoxy)-6-(3,4,5-trimethoxystyryl)phenol (40)

Compound **34** (0.100 g, 0.167 mmol) was dissolved in THF (3 mL). Glacial acetic acid (5.6 mL) and hydrochloric acid (2 M, 3.3 mL) were added dropwise and the reaction was stirred (8 hr). H₂O (20 mL) was used to quench the reaction and it was concentrated under reduced pressure. The residue was extracted with CH₂Cl₂ (3 x 20 mL), washed multiple times with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by flash column 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 (13 CV), 80%A / 20%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] affording compound **40** (0.026 g, 0.055 mmol, 33%) as a yellow oil.

¹H NMR (600 MHz, CDCl₃) δ 7.79 (1H, d, *J* = 4.2 Hz), 6.96 (1H, d, *J* = 8.7 Hz), 6.93 (1H, d, *J* = 4.2 Hz), 6.53 (2H, d, *J* = 12.4 Hz), 6.49 (2H, s), 6.37 (1H, d, *J* = 8.8 Hz), 5.56 (1H, q, *J* = 6.5 Hz), 3.84 (3H, s), 3.83 (3H, s), 3.67 (6H, s), 1.73 (3H, d, *J* = 6.5 Hz).

¹³C NMR (126 MHz, CDCl₃) δ 153.6, 152.8, 151.4, 147.5, 132.6, 132.2, 130.2, 128.2, 125.3, 123.8, 123.7, 117.5, 105.8, 103.4, 103.3, 75.2, 60.9, 55.9, 55.8, 29.7, 21.7.

HRMS: *m*/*z*: obsd 488.1373 [M+H]⁺, calcd for C₂₃H₂₃NO₈S⁺, 487.1301.

HPLC (Method B): 11.1 min.

(Z)-6-methoxy-2-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)-3-(3,4,5-trimethoxystyryl)phenol (41)

To a solution of compound **32** (2.35 g, 3.82 mmol) in THF (250 mL) at -15 °C, TBAF· 3H₂O (1.32 g, 4.19 mmol) was dissolved in THF (10 mL) and added drop-wise. The reaction was allowed to stir for 1 h. H₂O (40 mL) was used to quench the reaction, THF was evaporated off completely, and the residue was extracted with CH₂Cl₂ (3 × 20 mL). The combined extracts were washed with brine, dried over Na₂SO₄, 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] affording compound **41** (0.050 g, 0.980 mmol, 2%) as a brownish-yellow solid.

¹H NMR (CDCl₃, 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).

¹³C NMR (CDCl₃, 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, 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

[(Z)-2-((2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)methyl)-5-nitrothiophene (43)[88]

(5-Nitrothiophen-2-yl)methanol (0.100 g, 0.628 mmol), triphenylphosphine (0.336 g, 1.28 mmol), and combretastatin A-4 (0.396 g, 1.25 mmol) were dissolved in tetrahydrofuran (2 mL). DEAD (0.218 g, 1.25 mmol) was added and the reaction was stirred for 4 hours at 50 °C. The reaction was then evaporated under reduced pressure. The product was then extracted with ethyl acetate, washed with water and brine, dried with sodium sulfate, 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, 10% A/90% B over 1.19 min (1 CV), 10% A/90% B → 67% A/33% B over 13.12 min (10 CV), 67% A/33% B over 2.38 min (2 CV); flow rate 50.0 mL/min; monitored at λ 254 and 280 nm] and recrystallization in ethyl acetate and hexanes yielded [(Z)-2-((2-methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)methyl)-5-nitrothiophene (43) (0.286 g, 0.625 mmol, 50%),

¹H NMR (500 MHz, CDCl₃) δ 7.80 (1H, d, *J* = 4 Hz), 6.97 (1H, dd, *J* = 8 Hz, *J* = 1.5 Hz), 6.89 (1H, d, *J* = 4 Hz), 6.87 (1H, d, *J* = 2 Hz), 6.84 (1H, d, *J* = 8.5 Hz), 6.50 (2H, s), 6.48 (2H, d, *J* = 12 Hz), 5.07 (2H, s), 3.89 (3H, s), 3.86 (3H, s), 3.72 (6H, s).

^{13}C NMR (125 MHz, CDCl₃) δ 153.0, 149.1, 148.3, 146.4, 137.1, 132.9, 129.8, 129.2, 128.4, 124.8, 124.0, 115.4, 111.7, 105.8, 66.4, 60.9, 56.0, 56.0.

HRMS: *m/z*: obsd 480.1088 [M+Na]⁺, calcd for C₂₃H₂₃NO₇S⁺, 457.1195. **HPLC** (Method A): 17.1 min.

(Z)-2-(1-(2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)ethyl)-5-nitrothiophene (44)[88]

Combretastatin A-4 (0.251 g, 0.79 mmol), triphenylphosphine (0.105 g, 0.400 mmol), and 1-(5-nitrothiophen-2-yl)ethanol (0.197 g, 1.14 mmol) were dissolved in dry THF (10 mL). Diethyl azodicarboxylate [DEAD] (0.155 g, 0.890 mmol) was added dropwise and the reaction was stirred for 24 hours. The reaction was quenched with water, partitioned, extracted with ethyl acetate, dried with sodium sulfate, and evaporated under reduced pressure. Flash chromatography of the crude product using a prepacked 25 g silica column [eluents: solvent A, EtOAc; solvent B, hexanes; gradient, 15% A/85% B over 1.19 min (1 CV), 15% A/85% B → 100% A/0% B over 13.12 min (10 CV), 100% A/0% B over 2.38 min (2 CV); flow rate 25.0 mL/min; monitored at λ 254 and 280 nm] to yield (Z)-2-(1-(2-methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)ethyl)-5-nitrothiophene **44** (0.090 g, 0.19 mmol, 24%),

^{1}H NMR (500 MHz, CDCl₃) δ 7.75 (1H, d, *J* = 4.5 Hz), 6.94 (1H, dd, *J* = 8 Hz, *J* = 2 Hz), 6.81 (3H, m), 6.46 (1H, d, *J* = 12.5 Hz), 6.45 (2H, s), 6.44 (1H, d, *J* = 12 Hz), 5.25 (1H, q, *J* = 6 Hz), 3.86 (3H, s), 3.84 (3H, s), 3.69 (6H, s), 1.63 (3H, d, *J* = 6.5)

^{13}C NMR (125 MHz, CDCl₃) δ 155.3, 153.0, 149.9, 145.7, 137.1, 132.9, 129.9, 129.2, 129.1, 128.4, 124.4, 123.0, 118.2, 112.0, 105.8, 73.8, 60.9, 55.9, 55.9, 23.1.

HRMS: *m/z*: obsd 472.1428 [M+H]$^+$, calcd for C$_{24}$H$_{25}$NO$_8$S$^+$, 471.1532. **HPLC** (Method A): 17.6 min.

[(*Z*)-2-(2-(2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)propan-2-yl)-5-nitrothiophene (45)[88]

Combretastatin A-4 (1.87 g, 5.91 mmol), 2-(5-Nitrothiophen-2-yl)propan-2-ol (1.17 g, 6.25 mmol), and 1,1'-(azodicarbonyl)-dipiperdine [ADDP] (1.46 g, 5.79 mmol) were dissolved in benzene (15 mL). Tributylphosphine (1.43 mL, 5.91 mmol) was added dropwise and the reaction was stirred for 24 hours. The reaction was quenched with water, extracted with ethyl acetate, dried with sodium sulfate, and evaporated under reduced pressure. Flash chromatography of the crude product using a prepacked 25 g silica column [eluents: solvent A, EtOAc; solvent B, hexanes; gradient, 15% A/85% B over 1.19 min (1 CV), 15% A/85% B → 100% A/0% B over 13.12 min (10 CV), 100% A/0% B over 2.38 min (2 CV); flow rate 25.0 mL/min; monitored at λ 254 and 280 nm] yielded (*Z*)-2-(2-(2-methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)propan-2-yl)-5-nitrothiophene (**45**) (0.670 g, 1.38 mmol, 23%) as a dark red oil.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.72 (1H, d, *J* = 4.2 Hz), 7.01 (1H, dd, *J* = 2.0 Hz, 8.4 Hz), 6.83 (1H, d, *J* = 8.4 Hz), 6.77 (1H, d, *J* = 4.2 Hz), 6.72 (1H, d, *J* = 2.0 Hz), 6.43 (4H, d, *J* = 2.2 Hz), 3.85 (3H, s), 3.80 (3H, s), 3.71 (6H, s), 1.59 (6H, s).

^{13}C NMR (125 MHz, CDCl$_3$): δ 161.0, 152.9, 152.4, 150.3, 142.8, 136.8, 132.9, 129.5, 129.2, 129.1, 128.3, 125.8, 124.0, 122.2, 111.8, 105.7, 79.9, 60.7, 55.8, 55.6, 28.6.

HRMS: *m/z*: obsd 508.1399 [M+Na]$^+$, calcd for C$_{25}$H$_{27}$NO$_8$S$^+$, 485.1508. **HPLC** (Method A): 18.7 min.

[(Z)-2-(2-(2-Methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)propan-2-yl)-5-nitrothiophene (**45**)[88] (Alternate Purification Route)

Combretastatin A-4 (1.61 g, 5.09 mmol), 2-(5-Nitrothiophen-2-yl)propan-2-ol (1.00 g, 5.34 mmol), and 1,1'-(azodicarbonyl)-dipiperdine [ADDP] (1.35 g, 5.34 mmol) were dissolved in toluene (101 mL). Tributylphosphine (1.3 mL, 5.34 mmol) was added dropwisely and the reaction was stirred for 20 hours. Reaction mixture was diluted with EtOAc and quenched with water. The resulting mixture was extracted with ethyl acetate (30 mL X 3), dried with sodium sulfate, and evaporated under reduced pressure. Product was purified by flash using 5-20% EtOAc-hexane. Column purified product contains free alcohol, CA4 and BAPC (product) in the ratio of 1.0:2.4:3.0. Amount of BAPC is 921mg, 1.90 mmol (calculated from NMR) (TY: 2.469 g). Amount of free alcohol is 118 mg, 0.63 mmol. Amount of CA-4 is 480 mg, 1.52 mmol. To a solution of mixture of three compounds in DCM (30.0 mL), imidazole (1.03 g, 15.2 mmol) was added followed by TBSCl (2.52 g, 16.7 mmol) and stirred at room temperature for 4 h. Reaction was quenched with water, extracted with ethyl acetate (30 mL X 3), dried with sodium sulfate, and evaporated under reduced pressure. Product was purified by flash using 5-20%. All CA-4 got removed. Now the isolated compound is mixture of BAPC and free alcohol in the ratio (3.0:1) by NMR. Amount of mixture is 910 mg. Amount of BAPC is 807 mg, 1.66 mmol and free alcohol 103 mg, 0.55 mmol. A solution of mixture of BAPC, free alcohol, DMAP (67 mg, 0.55 mmol) and TEA (0.85 mL, 6.05 mmol) in DCM (11 mL) was treated with acetic anhydride (0.52 mL, 5.5 mmol) and stirred at room temperature for 4.0 h. Reaction was quenched with water, extracted with ethyl acetate (30 mL X 3), dried with sodium sulfate, and evaporated under reduced pressure. Product was

purified by flash using 5-15% EtOAc-hexane. (*Z*)-2-(2-(2-methoxy-5-(3,4,5-trimethoxystyryl)phenoxy)propan-2-yl)-5-nitrothiophene (**45**) (0.760 g, 1.56 mmol, 31%) was isolated as a dark red oil. By NMR no more free alcohol was observed in the purified product.

¹H NMR (500 MHz, CDCl₃) δ 7.72 (1H, d, *J* = 4.2 Hz), 7.01 (1H, dd, *J* = 2.0 Hz, 8.4 Hz), 6.83 (1H, d, *J* = 8.4 Hz), 6.77 (1H, d, *J* = 4.2 Hz), 6.72 (1H, d, *J* = 2.0 Hz), 6.43 (4H, d, *J* = 2.2 Hz), 3.85 (3H, s), 3.80 (3H, s), 3.71 (6H, s), 1.59 (6H, s).

¹³C NMR (125 MHz, CDCl₃): δ 161.0, 152.9, 152.4, 150.3, 142.8, 136.8, 132.9, 129.5, 129.2, 129.1, 128.3, 125.8, 124.0, 122.2, 111.8, 105.7, 79.9, 60.7, 55.8, 55.6, 28.6.

Biological Evaluation

Cell Culture and SRB Cytotoxicity Assay[145-148]

  Three cancer cell lines (DU-145, prostate; SK-OV-3, ovarian; and NCI-H460, lung cancer) were grown and passaged using DMEM media supplemented with 10% FBS (Gibco One Shot®) and 1% gentamycin sulfate (Teknova, Hollister, CA). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO₂, up to passage 15 for use in these experiments. The sulforhodamine B (SRB) assay was used to assess inhibition of human cell line growth as previously described.[145-148] Briefly, cancer cells were plated at 7500 cells/well using DMEM supplemented with 5% FBS and 1% gentamycin sulfate in 96-well plates and incubated for 24 h. Subsequently, 10-fold serial dilutions of the compounds to be tested were then added to the wells. After 48 h, the cells were fixed with 10% trichloroacetic acid (final concentration), stained with sulforhodamine B (Acid

Red 52) (TKI, Tokyo), read at 540 nm, and normalized at 630 nm with an automated

Biotek Elx800 plate reader (Biotek, Winooski, VT). A growth inhibition of 50% (GI_{50} or

the drug concentration causing a 50% reduction in the net protein staining relative to

controls) was calculated from optical density data with Excel software.

Inhibition of Tubulin Polymerization[131]

Tubulin assembly experiments were performed using 0.25 mL reaction mixtures

(final volume),[131] which contained 1.0 mg/mL (10 μM) purified bovine brain tubulin, 0.8

M monosodium glutamate (pH 6.6 in a 2 M stock solution) 4% (v/v) dimethyl sulfoxide,

0.4 mM GTP, and varying compound concentrations. Initially, all components except

GTP were preincuabted for 15 min at 30 °C in 0.24 mL. After chilling the mixtures on

ice, 10 μL of 10 mM GTP was added. The reaction mixtures were placed in cuvettes held

at 0 °C in Beckman DU-7400 and DU-7500 spectrophotometers equipped with electronic

temperature controllers. The temperature was jumped to 30 °C over about 30 s, and

polymerization was followed turbidimetrically at 350 nM for 20 min. Each reaction set

included a reaction mixture without compound, and the IC_{50} was defined as the

compound concentration that inhibited extent of assembly by 50% after 20 min at 30 °C.

Colchicine Binding Assay[130]

Inhibition of [³H]colchicine binding to tubulin was determined using 100 μL

reaction mixtures, each containing 1.0 μM tubulin, 5.0 μM [³H]colchicine (from Perkin-

Elmer), 5% (v/v) dimethyl sulfoxide, potential inhibitors at 1.0 or 5.0 μM and

components demonstrated to stabilize the colchicine binding activity of tubulin[130] (1.0 M

monosodium glutamate [adjusted to pH 6.6 with HCl in a 2.0 M stock solution], 0.5

mg/mL bovine serum albumin, 0.1 M glucose-1-phosphate, 1.0 mM $MgCl_2$, and 1.0 mM

GTP). Incubation was for 10 min at 37 °C, a time point at which the binding reaction in

the control is 40-60% complete. Reactions were stopped by adding 2.0 mL of ice-cold

water and placing the samples on ice. Each sample was poured onto a stack of two

DEAE-cellulose filters, followed immediately by 6 mL of ice-cold water, and the water

was aspirated under reduced vacuum. The filters were washed three times with 2 mL of

water and, following removal of excess water under a strong vacuum, placed into vials

containing 5 mL of Biosafe II scintillation cocktail. Samples were counted the next day in

a Beckman scintillation counter. Samples with potential inhibitors were compared to

controls with no inhibitor to determine percent inhibition. All samples were corrected for

the amount of colchicine that bound to the filters in the absence of tubulin.

NADPH Cytochrome P450 Oxidoreductase Cleavage Assay[133,134]

Rat NADPH cytochrome P450 oxidoreductase (POR) and protocatechuate 3,4-

dioxygenase (PCD) were purchased from Corning® and Sigma-Aldrich, respectively, and

their enzymatic activities were determined in terms of enzyme units (U). All bioreductive

prodrugs were dissolved in DMSO as 10 mM stock solutions.

An aliquot (5 µL) of the 10 mM compound DMSO stock solution along with 0.5 µL

0.1% Triton X-100 were added to 395.5 µl 200 mM pH 7.4 potassium phosphate buffer

containing 400 µM freshly made protocatechuic acid (PCA). The components were fully

mixed in a microvessel capped with a rubber septum stopper and subjected to three cycles

of evacuation and flushing with N_2 using a manifold, followed by sparging with N_2 for an

additional 20 min. PCD (0.08 units) was added by Hamilton syringe, and the solution was scrubbed for 10 min to allow for sufficient O_2 digestion by PCA/PCD. POR stock (0.006 units) was introduced followed by NADPH (0.8 mM final concentration) into the vial followed by an additional round of N_2 sparging. The reaction mixture was incubated for 24 h at 37 °C, cooled on ice and treated with an equal volume of acetonitrile. After centrifugation and filtration, the samples were analyzed by HPLC. Solutions without POR were used as controls.

CHAPTER FOUR

Synthesis of KGP18, KGP18 Bioreductively Activatable Prodrug Conjugates, OXi6196 Bioreductively Activatable Prodrug Conjugates, and the Nitroimidazole Trigger

With the successful generation of BAPCs that incorporate the anticancer agents phenstatin, CA1[136], and CA4, the development of further BAPCs with other potent VDAs and inhibitors of tubulin assembly from the Pinney group was pursued.[149-154] The dihydronaphthalene OXi6196 and the benzosuberene analogue VDA KGP18, both inhibitors of tubulin polymerization, are potent small-molecule cytotoxic agents inspired by the natural products colchicine, CA1 and CA4.[150-155] KGP18 and OXi6196 were synthesized and BAPCs of each were generated through Mitsunobu reactions with nitrothiophene, nitrofuran, and nitroimidazole triggers.
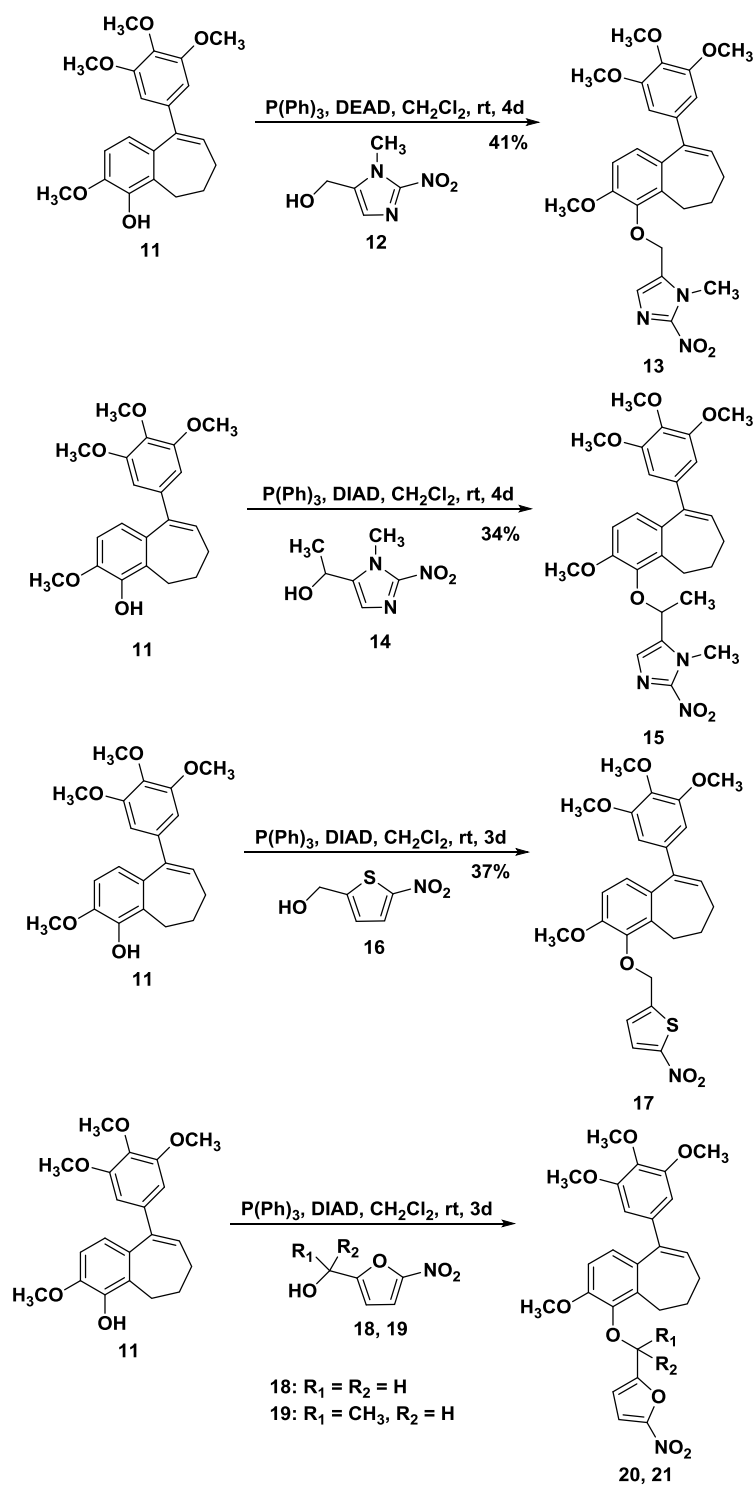
KGP18 and KGP18-BAPC Synthesis

The KGP18 synthesis developed by the Pinney Research Group utilizes two key reactions to generate the benzosuberene CA4 analogue- a cyclization employing Eaton's reagent to form the seven membered ring and a lithium-halogen exchange to install the trimethoxy aryl ring system.[36] The Wittig olefination reaction was used to generate alkene **3** from phosphonium salt **1** and aldehyde **2** (Scheme 4.1).[34-36] Reduction of alkene **3** yielded carboxylic acid **4**, which was then cyclized, upon treatment with Eaton's reagent, to produce benzosuberene **5**.[34-36] Benzosuberene **5** was demethylated to generate phenol **6**, which was silylated to form the protected benzosuberene **7**.[34-36]

A lithium-halogen exchange reaction was then performed with brominated coumpound **8**, which was then reacted with protected benzosuberene **7** to yield tertiary alcohol **9** (Scheme 4.1).[34-36] The tertiary alcohol **9** was then dehydrated to generate alkene **10**, which was then desilylated to yield KGP18.[34-36]

A Mitsunobu reaction was used to synthesize the KGP18-BAPCs (Scheme 4.2).[88,105,106] KGP18, DEAD, triphenylphosphine, and nitroimidazole trigger **12** were used to synthesize the normethyl nitroimidazole KGP18-BAPC **13**.[88,105,106] KGP18 BAPCs **15** and **17** were generated by reacting DIAD, triphenylphosphine, KGP18, and nitroimidazole trigger **14** and nitrothiophene trigger **16** respectively.[88,105,106] The *nor-*methyl and *mono*-methyl nitrofuran triggers **18** and **19** were reacted with DIAD, triphenylphosphine, and KGP18 to yield the BAPCs **20** and **21** respectively.[88,105,106] The *gem*-dimethyl nitrofuran KGP18-BAPC was also attempted, but the reaction yielded only starting material.[88,105,106]
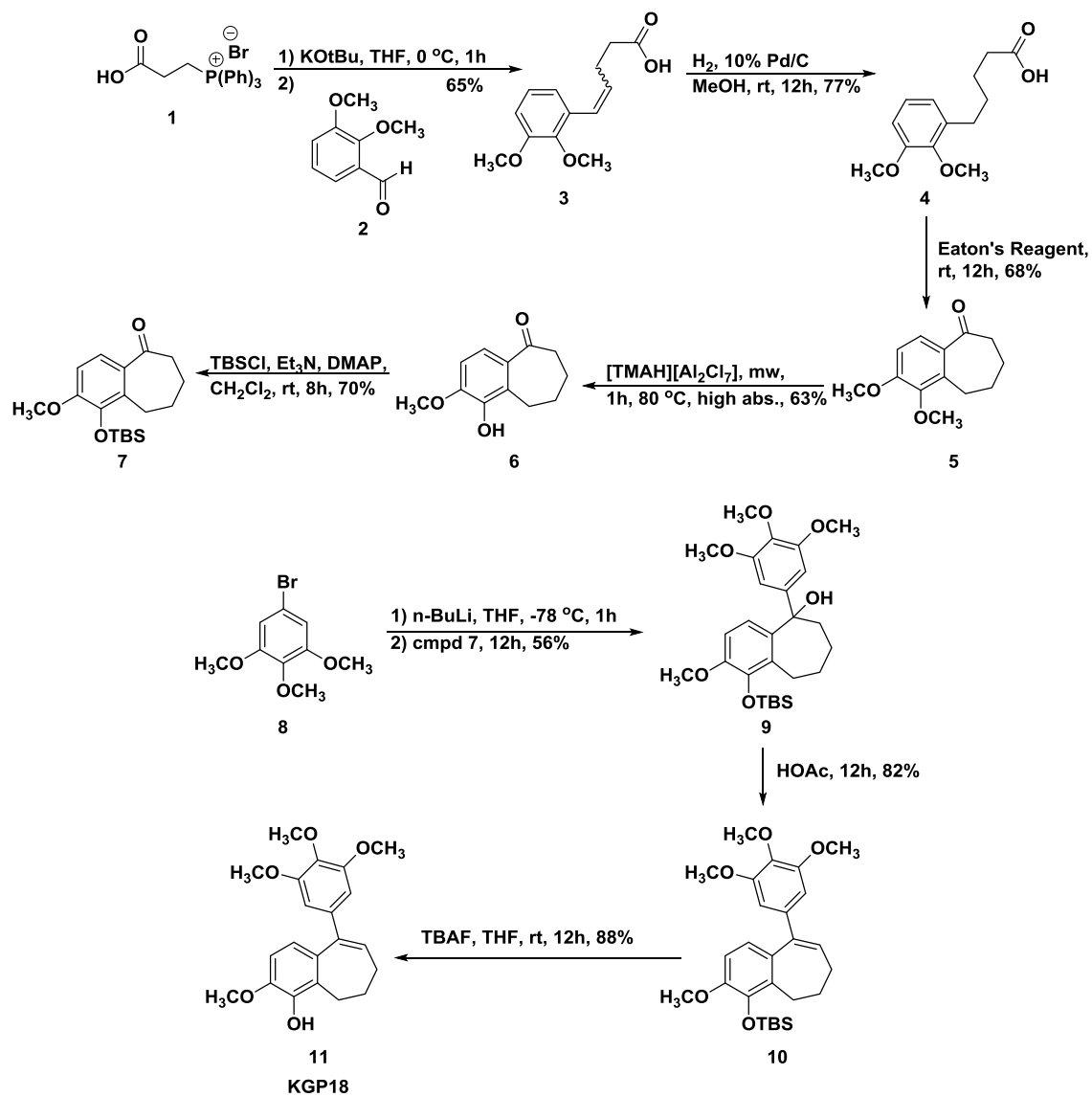
Scheme 4.1. KGP18 Synthesis[34-36]

Scheme 4.2. KGP18-BAPC Synthesis[88,105,106]

The OXi6196-BAPC syntheses were achieved through Mitsunobu reactions with OXi6196 generated by Casey Maguire, a graduate student in the Pinney Research Group who has developed efficient and novel routes to synthesize OXi6196.[34,37,156,157] DIAD, OXi6196, triphenylphosphine, and nitroimidazole triggers **12** and **14** were reacted to yield BAPCs **23** and **24** respectively (Scheme 4.3).[88,105,106] The *gem*-dimethyl nitrothiophene OXi6196 BAPC **26** was generated through the reaction of nitrothiophene trigger **25**, ADDP, tributylphosphine, and OXi6196.[88,105,106]
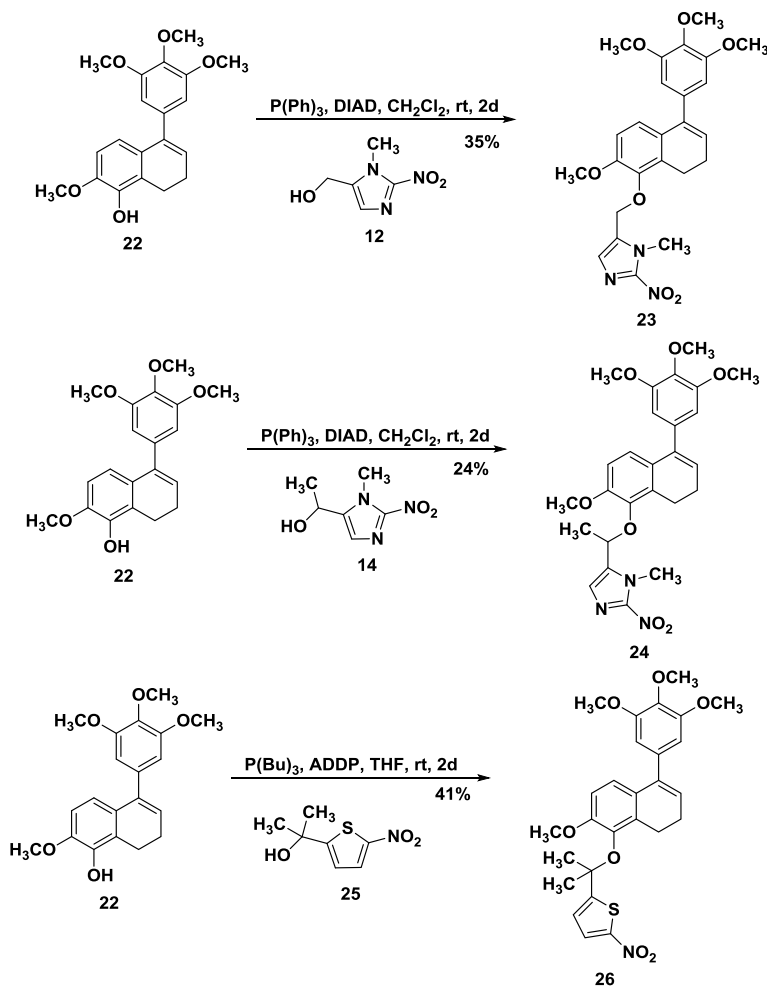
Scheme 4.3. OXi6196-BAPC Synthesis[88,105,106]

Nitroimidazole Bioreductive Trigger Synthesis

The patent route[107] to generate the nitroimidazole trigger was initially utilized to generate the cyclized aminoinidazole **30**, but after poor yields, the Conway *et. al* route was adapted to generate the nitroimidazole trigger.[104]

The patent route to the aminoimidazole intermediate **30** (Scheme 4.4) began with the formylation of sarcosine methyl ester **27** to generate aldehyde **28**.[107] An enol group was attached to **28** to yield the organic salt **29**, which was then deformylated and cyclized to generate aminoimidazole **30** in poor yield.[107]

Once the Conway *et. al* route was successfully utilized to generate both the *nor*-methyl nitroimidazole trigger and the *mono*-methyl nitroimidazole trigger **31**,[104] the next goal was to complete the series through the synthesis of the *gem*-dimethyl nitroimidazole trigger **33**. The initial attempts were to generate the *gem*-dimethyl nitroimidazole trigger through the same route as the nitrofurans and the nitrothiophenes: oxidize the *mono*-methyl trigger into the ketone, and then methylate the ketone to yield the *gem*-dimethyl trigger **33**.[103,104] The synthesis began with the successful oxidation of the *mono*-methyl nitroimidazole trigger **31** to ketone **32** (Scheme 4.5).[103,104] The methylation step proved difficult, however, as none of the methylation methods attempted generated product or any byproduct, only returning starting material **32**.[103,104]

After attempting the methylation with five different reagents in six different ways (Scheme 4.5) with no success, a different approach was required. As the nitrothiophene ketone was available and easier to generate, the Wittig reaction was run with the nitrothiophene **35** in a test system. The generation of the terminal alkene was attempted next to no avail, as the reaction of nitrothiophene ketone **35** with phosphonium salt **34** in
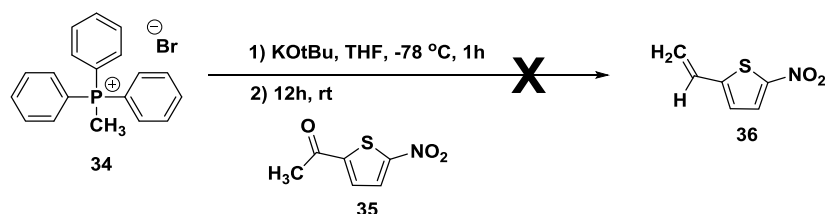
Wittig conditions did not yield the alkene **36**, returning only starting material (Scheme 4.6). With the failure of the Wittig chemistry in the test system, the Wittig reaction was not attempted on the nitroimidazole ketone.
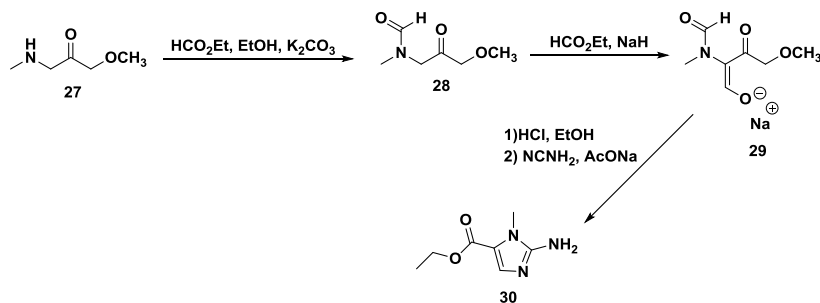
Scheme 4.4. Nitroimidazole Patent Route Synthesis[107]
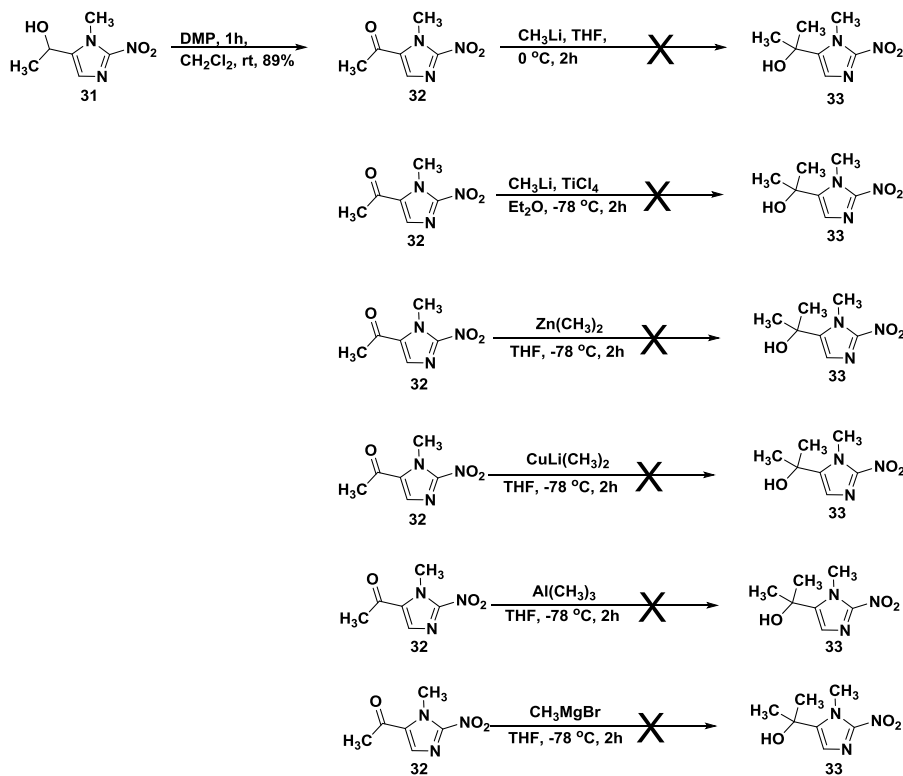
Scheme 4.5. Attempted Methylation of Nitroimidazole Ketone[103,104]

Scheme 4.6. Attempted Wittig with Nitrothiophene Ketone

CA1 Tosyl BAPC Synthesis

The Mitsunobu reaction was attempted with the *gem*-dimethyl nitrothiophene trigger on the C2 tosyl / C3 OH CA1 analog, since the *nor*-methyl and *mono*-methyl nitrothiophene tosyl CA1 (C2 tosyl / C3 trigger) had been successfully synthesized. The reaction was attempted to complete the tosyl / nitrothiophene trigger CA1 series, but the reaction to generate the *gem*-dimethyl nitrothiophene tosyl CA1 BAPC **38** was ultimately unsuccessful after multiple attempts, all of which only returned starting material (Scheme 4.7).[88,105,106]
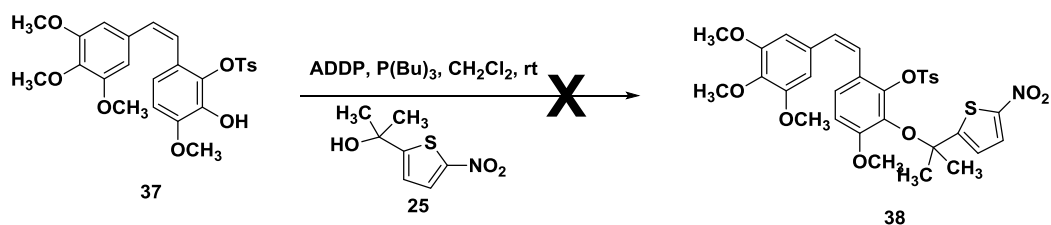
Scheme 4.7. Tosyl *Gem*-dimethyl-CA1-BAPC Attempted Synthesis[88,105,106]

Conclusions

In conclusion, several KGP18 and OXi6196-based BAPCs were synthesized utilizing the Mitsunobu reaction to covalently link KGP18 and OXi6196 to nitrothiophene, nitroimidazole, and nitrofuran triggers. This series of OXi6196 and

KGP18-BAPCs will undergo preliminary biological evaluation to determine their cytotoxicity in normoxic versus hypoxic conditions, hydrolysis in aqueous solution, bioreductive trigger cleavage upon treatment with POR, (in collaboration with Trawick Research Group, Baylor University) and their ability to inhibit tubulin polymerization and compete for the colchicine binding site (in collaboration with Ernest Hamel, NCI). The *gem*-dimethyl nitroimidazole trigger was not successfully synthesized, but a published route by Cavalleri and co-workers[127] to the *gem*-dimethyl nitroimidazole trigger will be attempted to generate the trigger.

Materials and Methods

5-(2,3-dimethoxyphenyl)pent-4-enoic acid (3)[34-36]

(2-carboxyethyl)triphenylphosphonium bromide (16.7 g, 39.0 mmol) was dissolved in THF (400 mL). Potassium *tert*-butoxide (11.7 g, 104 mmol) was added to the reaction and it was stirred for 1 h at room temperature. 2,3-Dimethoxybenzaldehyde (5.39 g, 32.0 mmol) was added to the reaction mixture and it was stirred for 8 h. The reaction was then quenched with HCl (2 M, 50 mL) and evaporated under reduced pressure. EtOAc (80 mL) was added to the residue and the layers were partitioned. The aqueous layer was extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and evaporated 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 → 50%A / 50%B (13 CV), 50%A / 50%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280

nm] to afford compound **3** (6.00 g, 25.4 mmol, 65%) as a light yellow solid. NMR characterization was conducted after the next step.

5-(2,3-dimethoxyphenyl)pentanoic acid (4)[34-36]

5-(2,3-dimethoxyphenyl)pent-4-enoic acid (5.39 g, 21.5 mmol) was added to an empty flask flushed under N_2, followed by 10 % palladium on carbon (0.430 g, 0.404 mmol). Methanol (100 mL) was added slowly to the reagents, and then the flask was purged under N_2. The flask was placed under vacuum, and then H_2 was added and the reaction was stirred for 12 h at room temperature. The reaction was then filtered through Celite in a frit funnel, rinsed with EtOAc (3 x 50 mL), and the combined organic phases were evaporated 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 7%A / 93%B (1 CV), 7%A / 93%B → 40%A / 60%B (13 CV), 40%A / 60%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford compound **4** (6.00 g, 25.4 mmol, 77%) as a clear oil.

^{1}H NMR (500 MHz, CDCl$_3$) δ 6.97 (1H, t, J = 7.9 Hz), 6.76 (2H, m), 3.85 (3H, s), 3.81 (3H, s), 2.64 (2H, t, J = 7.3 Hz), 2.38 (2H, t, J = 7.1 Hz), 1.74 – 1.60 (4H, m).

^{13}C NMR (125 MHz, CDCl$_3$) δ 179.5, 152.7, 147.0, 135.9, 123.8, 121.8, 110.1, 60.6, 55.6, 33.8, 30.1, 29.4, 24.5.

1,2-dimethoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (5)[34-36]

5-(2,3-dimethoxyphenyl)pentanoic acid (3.55 g, 14.9 mmol) was dissolved in Eaton's reagent (29 mL, 3 g/mmol of compound **4**) and stirred for 12 h at room temperature. The reaction was poured over ice and neutralized with saturated sodium bicarbonate. The

layers were partitioned, then the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, and evaporated 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 7%A / 93%B (1 CV), 7%A / 93%B → 40%A / 60%B (13 CV), 40%A / 60%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford compound **5** (2.22 g, 10.1 mmol, 68%) as a yellow solid.

¹H NMR (500 MHz, $CDCl_3$) δ 7.53 (1H, d, J = 8.6 Hz), 6.84 (1H, d, J = 8.6 Hz), 3.90 (3H, s), 3.79 (3H, s), 3.00 (2H, t, J = 6.1 Hz), 2.69 (2H, t, J = 6.1 Hz), 1.89 – 1.81 (2H, m), 1.81 – 1.72 (2H, m).

¹³C NMR (125 MHz, $CDCl_3$) δ 204.9, 156.1, 145.9, 135.7, 132.8, 125.5, 109.7, 61.1, 55.8, 40.6, 24.9, 23.2, 20.9.

1-hydroxy-2-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (6)[34-36]

[TMAH][Al_2Cl_7] (18.3 mL, 9.08 mmol) was added to 1,2-dimethoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (1.01 g, 4.54 mmol) in a 20 mL microwave vial. The reaction mixture was then exposed to microwave irradiation for 1 h on high absorbance at 80 °C. The reaction was then poured into water (50 mL) and EtOAc (40 mL) was added. The layers were partitioned and the aqueous layer was extracted (3 x 40 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280 nm] to afford compound **6** (0.590 g, 2.86

mmol, 63%) as a yellow solid.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.34 (1H, d, J = 8.5 Hz), 6.79 (1H, d, J = 8.5 Hz), 5.77

(1H, s), 3.94 (3H, s), 3.01 (2H, dd, J = 7.2, 5.0 Hz), 2.76 – 2.66 (2H, m), 1.83 (4H, m).

^{13}C NMR (125 MHz, CDCl$_3$) δ 205.1, 149.2, 142.4, 133.3, 127.7, 120.8, 107.9, 56.1,

40.8, 24.5, 23.0, 21.3.

1-((*tert*-butyldimethylsilyl)oxy)-2-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (7)[34-36]

1-hydroxy-2-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (2.00 g, 9.70 mmol)

was dissolved in CH$_2$Cl$_2$ (80 mL). Triethylamine (1.64 mL, 11.6 mmol), *tert*-

butyldimethylsilyl chloride (1.61 g, 10.7 mmol) and DMAP (0.0650 g, 0.532 mmol) were

added to the reaction mixture and it was stirred for 8 h. The reaction was quenched with

water (40 mL) and the layers were partitioned. The aqueous layer was extracted with

CH$_2$Cl$_2$ (3 x 40 mL). The combined organic layers were washed with brine (50 mL),

dried over Na$_2$SO$_4$, and evaporated under reduced pressure. The crude product was

purified by flash chromatography using a pre-packed 50 g silica column [solvent A:

EtOAc; solvent B: hexanes; gradient 2%A / 98%B (1 CV), 2%A / 98%B → 20%A /

80%B (13 CV), 20%A / 80%B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280

nm] to afford compound **7** (2.18 g, 6.79 mmol, 70%) as light tan crystals.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37 (1H, d, J = 8.5 Hz), 6.76 (1H, d, J = 8.6 Hz), 3.82

(3H, s), 3.00 (2H, dd, J = 7.0, 5.1 Hz), 2.69 (2H, dd, J = 7.3, 4.4 Hz), 1.84 – 1.73 (4H,

m), 1.01 (9H, s), 0.18 (6H, s).

¹³C NMR (125 MHz, CDCl₃) δ 205.3, 153.2, 141.7, 133.1, 133.1, 122.3, 108.7, 54.8, 40.7, 26.1, 25.6, 24.7, 23.9, 21.2, -3.9.

1-((*tert*-butyldimethylsilyl)oxy)-2-methoxy-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol (9)[34-36]

3,4,5-trimethoxybromobenzene (4.13 g, 16.7 mmol) was dissolved in THF (80 mL) at -78 °C. n-Butyllithium (1.6M, 7.06 mL, 16.8 mmol) was added dropwise to the reaction mixture and it was stirred for 1 h. 1-((*tert*-Butyldimethylsilyl)oxy)-2-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (3.99 g, 12.4 mmol) was added to the reaction mixture and it was stirred for 8 h while warming from -78 °C to room temperature. The reaction was quenched with water (50 mL) and the layers were partitioned. The aqueous layer was extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with brine (60 mL), dried over Na₂SO₄, and evaporated 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 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (13 CV), 60%A / 40%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford compound **9** (4.57 g, 9.35 mmol, 56%) as a clear oil. NMR characterization was performed after the next step.

tert-butyl((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)dimethylsilane (10)[34-36]

 1-((*tert*-Butyldimethylsilyl)oxy)-2-methoxy-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol (4.57 g, 9.35 mmol) was dissolved in glacial acetic acid (50 mL) and the reaction mixture was stirred for 12 h at room temperature. The

reaction was quenched with water (50 mL), the mixture was evaporated under reduced

pressure, and the residue was dissolved in EtOAc (60 mL) and water (40 mL). The layers

were partitioned, and then the aqueous layer was extracted with EtOAc (3 x 50 mL). The

combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and

evaporated 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 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (13 CV), 60%A /

40%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford

compound **10** (3.61 g, 7.67 mmol, 82%) as a clear oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.69 (1H d, J = 8.5 Hz), 6.61 (1H, d, J = 8.4 Hz), 6.48

(2H, s), 6.32 (1H, t, J = 7.3 Hz), 3.85 (3H, s), 3.80 (3H, s), 3.79 (6H, s), 2.76 (2H, t, J =

6.9 Hz), 2.10 (2H, p, J = 7.1 Hz), 1.95 (2H, q, J = 7.2 Hz), 1.04 (9H, s), 0.23 (6H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 152.8, 148.6, 143.0, 141.5, 138.6, 137.2, 133.8, 133.3,

126.9, 122.4, 108.3, 105.2, 60.9, 56.1, 54.6, 33.9, 26.2, 25.6, 24.2, 19.0, 3.8.

tert-butyl((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)dimethylsilane (11)[34-36]

tert-Butyl((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)dimethylsilane (3.61 g, 7.67 mmol) was dissolved in THF (80 mL). TBAF (1M,

9.00 mL, 9.00 mmol) was added dropwise to the reaction mixture and it was stirred for

12 h at room temperature. The reaction was quenched with water (50 mL), the mixture

was evaporated under reduced pressure, and the residue was dissolved in EtOAc (60 mL)

and water (40 mL). The layers were partitioned, and then the aqueous layer was extracted

with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL),

dried over Na_2SO_4, and evaporated 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] to afford compound **11** (2.40 g, 6.73 mmol, 88%) as a white solid.

^{1}H NMR (500 MHz, CDCl$_3$) δ 6.71 (1H, d, *J* = 8.4 Hz), 6.57 (1H, d, *J* = 8.4 Hz), 6.50 (2H, s), 6.33 (1H, t, *J* = 7.4 Hz), 3.91 (3H, s), 3.86 (3H, s), 3.80 (6H, s), 2.76 (2H, t, *J* = 7.0 Hz), 2.14 (2H, p, *J* = 7.1 Hz), 1.96 (2H, q, *J* = 7.2 Hz).

^{13}C NMR (125 MHz, CDCl$_3$) δ 152.8, 145.0, 142.8, 142.3, 138.5, 137.2, 134.2, 127.7, 127.2, 120.8, 107.6, 105.2, 60.9, 56.1, 55.9, 33.6, 25.7, 23.5.

5-((((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)methyl)-1-methyl-2-nitro-1H-imidazole (13)[88,105,106]

KGP18 (0.250 g, 0.702 mmol), (1-methyl-2-nitro-1H-imidazol-5-yl)methanol (0.123 g, 0.842 mmol), and DEAD (0.144 mL, 0.913 mmol) were dissolved in CH_2Cl_2 (60 mL) at room temperature. Triphenylphosphine (0.368 g, 1.40 mmol) was added to the mixture and the reaction was stirred for 2 d. The reaction was evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280 nm] to afford compound **13** (0.143 g, 0.288 mmol, 41%) as orange crystals.

^{1}H NMR (500 MHz, CDCl$_3$) δ 6.71 (1H, d, J = 8.4 Hz), 6.57 (1H, d, J = 8.4 Hz), 6.50 (2H, s), 6.33 (1H, t, J = 7.4 Hz), 3.91 (3H, s), 3.86 (3H, s), 3.80 (6H, s), 2.76 (2H, t, J = 7.0 Hz), 2.14 (2H, p, J = 7.1 Hz), 1.96 (2H, q, J = 7.2 Hz).

^{13}C NMR (125 MHz, CDCl$_3$) δ 152.8, 145.0, 142.8, 142.3, 138.5, 137.2, 134.2, 127.7, 127.2, 120.8, 107.6, 105.2, 60.9, 56.1, 55.9, 33.6, 25.7, 23.5.

HRMS: m/z: obsd 518.1899 [M+Na]$^+$, calcd for C$_{26}$H$_{29}$N$_3$O$_7$$^+$, 495.2006.

HPLC (Method A): 10.7 min.

5-(1-((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)ethyl)-1-methyl-2-nitro-1H-imidazole (15)[88,105,106]

KGP18 (0.250, 0.702 mmol), 1-(1-methyl-2-nitro-1H-imidazol-5-yl)ethan-1-ol (0.144 g, 0.842 mmol), and DIAD (0.179 g, 0.913 mmol) were dissolved in CH$_2$Cl$_2$ (60 mL) at room temperature. Triphenylphosphine (0.368 g, 1.40 mmol) was added to the mixture and the reaction was stirred for 2 d. The reaction was evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280 nm] to afford compound **15** (2.18 g, 0.239 mmol, 34%) as orange crystals.

HRMS: m/z: obsd 532.2054 [M+Na]$^+$, calcd for C$_{27}$H$_{31}$N$_3$O$_7$$^+$, 509.2162.

HPLC (Method A): 11.5 min.

2-(((3-Methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)methyl)-5-nitrothiophene (17)[88,105,106]

KGP18 (0.167 g, 0.469 mmol), 5-nitrothiophene-2-carboxaldehyde (0.0896 g, 0.563 mmol), and DIAD (0.124 mL, 0.633 mmol) were dissolved in CH_2Cl_2 (50 mL) at room temperature. Triphenylphosphine (0.246 g, 0.938 mmol) was added to the mixture and the reaction mixture was stirred for 2 d. The reaction was 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 10%A / 90%B (1 CV), 10%A / 90%B → 80%A / 20%B (13 CV), 80%A / 20%B (2 CV); flow rate: 30 mL/min; monitored at 254 and 280 nm] to afford compound **7** (0.0866 g, 0.174 mmol, 37%) as an orange oil. The product is still in purification.

(4-Methoxy-3-((5-nitrofuran-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (20)[88,105,106]

KGP18 (0.300 g, 0.842 mmol), (5-nitrofuran-2-yl)methanol (0.143 g, 1.01 mmol), and DIAD (0.213 mL, 1.09 mmol) were dissolved in THF (50 mL) at room temperature. Triphenylphosphine (0.442 g, 1.68 mmol) was added to the mixture and the reaction was stirred for 3 d. The reaction was evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280 nm] to afford compound **20** as a crude yellow oil.

(4-methoxy-3-(1-(5-nitrofuran-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (21)[88,105,106]

KGP18 (0.300 g, 0.842 mmol), 1-(5-nitrofuran-2-yl)ethan-1-ol (0.159 g, 1.01 mmol), and

DIAD (0.213 mL, 1.09 mmol) were dissolved in THF (50 mL) at room temperature.

Triphenylphosphine (0.442 g, 1.68 mmol) was added to the mixture and the reaction was

stirred for 3 d. The reaction was evaporated under reduced pressure. The crude product

was purified by flash chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280

nm] to afford compound **21** as crude yellow crystals.

2-(2-((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)propan-2-yl)-5-nitrofuran[88,105,106]

KGP18 (0.329 g, 0.925 mmol), 2-(5-nitrofuran-2-yl)propan-2-ol (0.190 g, 1.11 mmol),

and ADDP (0.303 mL, 1.20 mmol) were dissolved in THF (50 mL) at room temperature.

Tributylphosphine (0.228 mL, 1.85 mmol) was added to the mixture and the reaction was

stirred for 3 d. The reaction was evaporated under reduced pressure. The crude product

was purified by flash chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280

nm]. ^{1}H and ^{13}C NMR showed no product formation on the resulting fractions, only

starting material.

5-((((2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)oxy)methyl)-1-methyl-2-nitro-1H-imidazole (23)[88,105,106]

Oxi 6196 (0.363 g, 1.06 mmol), (1-methyl-2-nitro-1H-imidazol-5-yl)methanol (0.200 g, 1.27 mmol), and DIAD (0.280 mL, 1.43 mmol) were dissolved in THF (70 mL) at room temperature. Triphenylphosphine (0.557 g, 2.12 mmol) was added and the reaction mixture was stirred for 2 d. The reaction was evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280 nm] to afford compound **23** (1.33 g, 0.371 mmol, 35%) as a yellow solid.

¹H NMR (600 MHz, CDCl₃) δ 7.14 (1H, s), 6.85 (1H, d, *J* = 8.5 Hz), 6.69 (1H, d, *J* = 8.5 Hz), 6.54 (2H, s), 5.97 (1H, t, *J* = 4.6 Hz), 5.01 (2H, s), 4.24 (3H, s), 3.89 (3H, s), 3.85 (3H, s), 3.85 (6H, s), 2.76 (2H, t, *J* = 7.9 Hz), 2.31 (2H, td, *J* = 7.8, 4.6 Hz).

¹³C NMR (151 MHz, acetone *d*-6) δ 153.3, 152.1, 143.3, 139.5, 137.7, 136.3, 134.5, 130.7, 128.7, 128.5, 124.9, 122.2, 109.2, 106.1, 62.7, 59.7, 55.5, 55.1, 34.0, 22.6, 20.9.

HRMS: *m/z*: obsd 482.1921 [M+H]⁺, calcd for $C_{25}H_{27}N_3O_7^+$, 481.1849.

HPLC (Method A): 7.3 min.

5-(1-((2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)oxy)ethyl)-1-methyl-2-nitro-1H-imidazole (24)[88,105,106]

Oxi6196 (0.200 g, 0.585 mmol), 1-(1-methyl-2-nitro-1H-imidazol-5-yl)ethan-1-ol (0.120 g, 0.702 mmol), and DIAD (0.150 mL, 0.761 mmol) were dissolved in THF (60 mL) at room temperature. Triphenylphosphine (0.307 g, 1.17 mmol) was added to the reaction mixture and it was stirred for 2 d. The reaction was evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 g silica

column [solvent A: EtOAc; solvent B: hexanes; gradient 10%A / 90%B (1 CV), 10%A / 90%B → 79%A / 21%B (13 CV), 79%A / 21%B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford compound **24** (0.0694 g, 0.140 mmol, 24%) as a yellow solid.

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.14 (1H, s), 6.77 (1H, d, J = 8.5 Hz), 6.62 (1H, d, J = 8.6 Hz), 6.47 (2H, s), 5.91 (1H, t, J = 4.6 Hz), 5.53 (1H, q, J = 6.6 Hz), 4.11 (3H, s), 3.82 (3H, s), 3.79 (3H, s), 3.78 (6H, s), 2.69 (2H, td, J = 9.3, 8.9, 6.8 Hz), 2.27 – 2.14 (2H, m), 1.61 (2H, d, J = 6.6 Hz).

^{13}C NMR (126 MHz, acetone d-6) δ 153.3, 152.0, 141.6, 139.6, 138.8, 137.6, 136.3, 131.6, 128.7, 126.3, 125.0, 122.0, 109.2, 106.0, 68.9, 59.7, 55.5, 55.1, 34.1, 22.6, 21.7, 17.6.

HRMS: m/z: obsd 496.2077 [M+H]$^+$, calcd for C$_{26}$H$_{29}$N$_3$O$_7$$^+$, 495.2006.

HPLC (Method A): 10.0 min.

2-(2-((2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)oxy)propan-2-yl)-5-nitrothiophene (26)[88,105,106]

Oxi6196 (0.200 g, 0.584 mmol), 2-(5-nitrothiophen-2-yl)propan-2-ol (0.131 g, 0.701 mmol), and ADDP (0.192 mmol, 0.759 mmol) were dissolved in THF (50 mL) at room temperature. Tributylphosphine (0.241 mL, 0.975 mmol) was added drop-wise and the reaction mixture was stirred for 2 d. The reaction was then 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 10%A / 90%B (1 CV), 10%A / 90%B → 80%A / 20%B (13 CV), 80%A / 20%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] to yield a crude product. The crude product was then

dissolved in CH_2Cl_2 (40 mL). Triethylamine (0.240 mL, 1.75 mmol), DMAP (0.0710 g, 0.584 mmol), and *tert*-butyldimethylsilyl chloride (0.106 g, 0.701 mmol) were added to the reaction and it was stirred for 12 h. The reaction was quenched with water (40 mL) and then the layers were partitioned. The aqueous layer was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 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] to afford compound **26** (0.122 g, 0.239 mmol, 41%) as an orange solid.

¹H NMR (600 MHz, acetone*d*-6) 7.87 (1H, d, *J* = 4.3 Hz), 7.03 (1H, d, *J* = 4.3 Hz), 6.70 (1H, d, *J* = 8.4 Hz), 6.58 (2H, s), 6.54 (1H, d, *J* = 8.4 Hz), 5.95 (1H, t, *J* = 4.7 Hz), 3.82 (3H, s), 3.80 (6H, s), 3.75 (3H, s), 2.81 (2H, t, *J* = 7.9 Hz), 2.30 (2H, td, *J* = 7.9, 4.7 Hz), 1.63 (6H, s).

¹³C NMR (126 MHz, acetone *d*-6) δ 166.2, 153.1, 146.6, 142.5, 139.9, 137.5, 136.8, 129.3, 128.6, 124.5, 122.4, 121.4, 117.1, 107.8, 106.1, 70.9, 59.7, 55.5, 55.3, 31.4, 31.3, 22.7, 20.2.

HPLC (Method A): 7.0 min.

Methyl N-formyl-N-methylglycinate (28)[107]

Sarcosine methyl ester • HCl (16.0 g, 155 mmol) and potassium carbonate (16.4 g, 119 mmol) were dissolved in ethanol (80 mL) at room temperature. Ethyl formate (68 mL, 842 mmol) was added and the reaction was stirred for 18 h. The reaction was filtered through a frit funnel, the residue was rinsed with EtOAc (3 x 70 mL), and the combined

organic layers were evaporated under reduced pressure. Water (70 mL) and EtOAc (100 mL) were added to the residue and the layers were partitioned. The aqueous layer was extracted with EtOAc (3 x 60 mL). The combined organic fractions were washed with brine (80 mL), dried with Na_2SO_4, and evaporated under reduced pressure. After evaporation, methyl N-formyl-N-methylglycinate (**24**) (15.5 g, 118 mmol, 76%) was recovered as a pale tan oil. [product left in crude state, taken on to next reaction with no further purification]

Sodium (E)-3-methoxy-2-(N-methylformamido)-3-oxoprop-1-en-1-olate (29)[107]

Methyl N-formyl-N-methylglycinate (22.0 g, 183 mmol) was dissolved in ethyl formate (106 mL, 1.08 mol) at 0 °C. Sodium hydride (60% in mineral oil, 9.07 g, 219 mmol) was added to the reaction mixture in several small aliquots over 30 minutes, and the reaction was stirred for 12 h. The reaction mixture was triturated with hexanes (2 x 100 mL) in a frit funnel, and then the solid was dried by vacuum overnight to yield sodium €-3-methoxy-2-(N-methylformamido)-3-oxoprop-1-en-1-olate (**29**) (18.0 g, 117 mmol, 64%) as an off-white powder. [crude product taken to next step]

Ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate (30)[107]

Sodium (*E*)-3-methoxy-2-(N-methylformamido)-3-oxoprop-1-en-1-olate (0.507 g, 3.29 mmol) was dissolved in ethanol (8 mL) and concentrated HCl (3.5 mL). The reaction mixture was refluxed for 1 h. The reaction was then cooled to room temperature and the mixture was filtered through a frit funnel. The solid residue was rinsed with methanol (2 x 40 mL) and the combined organic layers were evaporated under reduced pressure. Water (30 mL) and CH_2Cl_2 (30 mL) were added to the residue and the layers were

partitioned. The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were then washed with saturated sodium bicarbonate (1 x 20 mL), dried over Na_2SO_4, and evaporated under reduced pressure to yield a thick brown oil. The oil was then dissolved in 10 % acetic acid in water (20 mL). Cyanamide (0.230 g, 5.47 mmol) and sodium acetate (0.596 g, 7.26 mmol) were dissolved in the reaction mixture and it was refluxed for 1 h. The reaction was then allowed to cool to room temperature and evaporated under reduced pressure to one half of its original volume. The reaction was then neutralized to pH 9 with sodium carbonate. The layers were partitioned and the aqueous layer was extracted with EtOAc (5 x 20 mL). The combined organic layers were washed with brine (1 x 30 mL), dried over Na_2SO_4, and evaporated under reduced pressure. Recrystallization was attempted on the residue, but no product was obtained.

1-(1-methyl-2-nitro-1H-imidazol-5-yl)ethan-1-one (32)[103,104]

1-(1-methyl-2-nitro-1H-imidazol-5-yl)ethan-1-ol (0.920 g, 5.38 mmol) was dissolved in CH_2Cl_2 (80 mL) at room temperature. DMP (2.74 g, 6.46 mmol) was added to the mixture and the reaction was stirred for 1 h. Saturated solutions of $NaHCO_3$ (30 mL) and sodium thiosulfate (30 mL) were added to the reaction mixture, which was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (1 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] afforded compound **32** (0.810 g, 4.79 mmol, 89%) as a yellow solid**.**

¹H NMR (500 MHz, CDCl₃) δ 7.76 (1H, s), 4.29 (3H, s), 2.57 (3H, s).

¹³C NMR (125 MHz, CDCl₃) δ 189.0, 135.5, 132.0, 68.5, 35.6, 28.4.

(Z)-3-methoxy-2-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)-6-(3,4,5-trimethoxystyryl)phenyl 4-methylbenzenesulfonate (38)[88,105,106]

(Z)-2-hydroxy-3-methoxy-6-(3,4,5-trimethoxystyryl)phenyl 4-methylbenzenesulfonate (0.480 g, 0.986 mmol), 2-(5-nitrothiophen-2-yl)propan-2-ol (0.222 g, 1.18 mmol), and ADDP (0.280 g, 1.11 mmol) were dissolved in THF (50 mL) at room temperature. Tributylphosphine (0.406 mL, 1.65 mmol) was added to the reaction mixture drop-wise and the reaction was stirred for 5 d. The reaction was evaporated under reduced pressure. TLC and crude NMR analysis confirmed no product was generated.

APPENDICES

The author Blake A. Winn contributed to this manuscript through the synthesis of all eleven final compounds including characterization, which included proton and carbon NMRs, HPLC, and HRMS. In addition, Blake A. Winn contributed a significant amount to the writing and editing of the manuscript, as well as the preparation of the supporting data. Zhe Shi contributed through the preparation of the supporting data as well as the synthesis of the nitroimidazole and nitrofuran triggers. Graham Carlson contributed through the synthesis of phenstatin and the phenstatin BAPCs **12**, **13**, **31**, and **32**. Evan Kelly contributed through the synthesis of phenstatin and the synthesis of phenstatin BAPCs **20-22**. David Ross contributed through the synthesis of phenstatin. Benson Nguyen contributed through developing the original Pinney Research Group route to phenstatin and the initial synthesis of phenstatin BAPC **14**. Ernest Hamel contributed through the preliminary biological evaluation of the phenstatin BAPCs ability to inhibit tubulin polymerization and compete for the colchicine binding site. Yifan Wang contributed through the preliminary biological evaluation of the enzymatic cleavage of the phenstatin BAPCs by POR and the hydrolysis of the phenstatin BAPCs.

Supplementary Data

Bioreductively Activatable Prodrug Conjugates of Phenstatin Designed to Target Tumor Hypoxia

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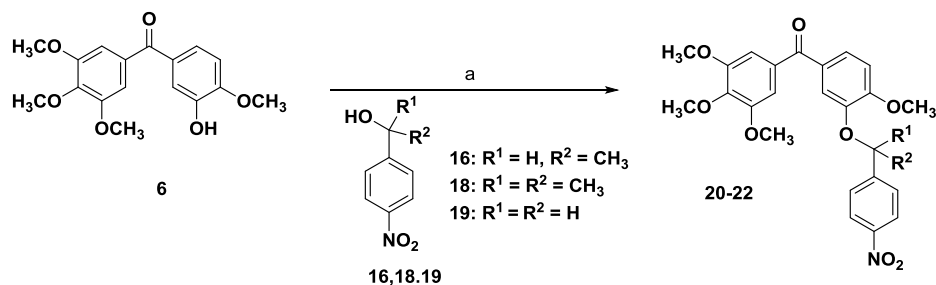
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Schemes

(a) cmpd **20**: DIAD, P(Ph)$_3$, CH$_2$Cl$_2$, cmpd **19**, rt, 2 d, 65% (R^1 = R^2 = H);
cmpd **21**: DIAD, P(Ph)$_3$, CH$_2$Cl$_2$, cmpd **16**, rt, 2 d, 43% (R^1 = H, R^2 = CH$_3$);
cmpd **22**: ADDP, P(Bu)$_3$, CH$_2$Cl$_2$, cmpd **18**, rt, 2 d, 23% (R^1 = R^2 = CH$_3$);

Scheme S1. Synthesis of the Phenstatin Nitrobenzyl BAPCs[S2-S5]
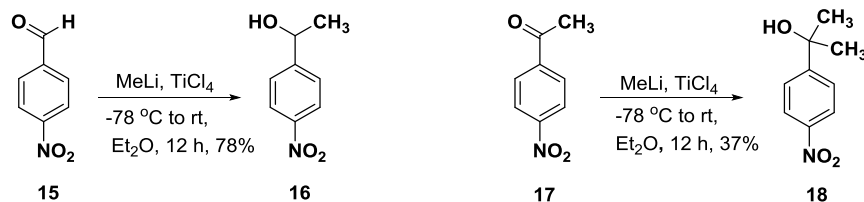
Scheme S2. Synthesis of the Nitrobenzyl Triggers[S1]

Mono-methyl nitrobenzyl trigger **16** and *gem*-dimethyl trigger **18** were synthesized

through methylation of aldehyde **15** and ketone **17**, respectively.[S1]
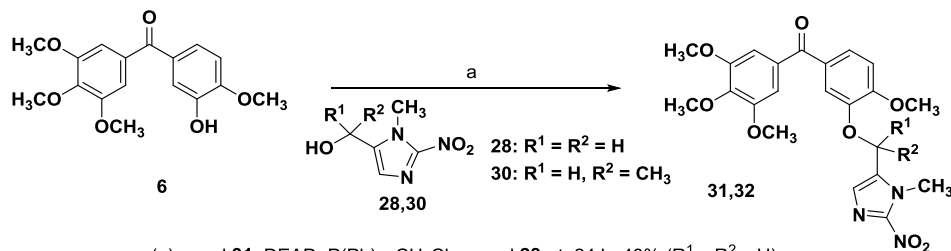
(a) cmpd **31**: DEAD, P(Ph)$_3$, CH$_2$Cl$_2$, cmpd **28**, rt, 24 h, 48% (R^1 = R^2 = H);
cmpd **32**: DIAD, P(Ph)$_3$, CH$_2$Cl$_2$, cmpd **30**, rt, 2 d, 32% (R^1 = H, R^2 = CH$_3$);

Scheme S3. Synthesis of the Phenstatin Nitroimidazole BAPCs[S2-S5]

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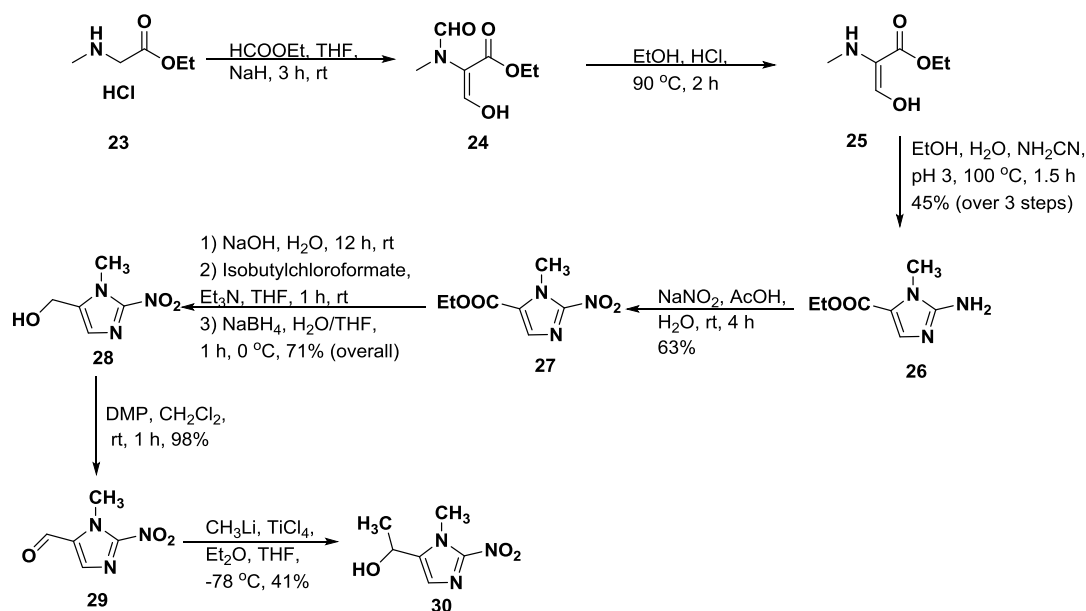
Scheme S4. Synthesis of the Nitroimidazole Triggers[S1,S6,S7]

In a three step process, sarcosine ethyl ester HCl was first formylated, then deformylated, and cyclized in order to generate ester **26** in reasonable yield over the three steps.[S6,S7] The amine **26** was oxidized to nitro ester **27**, which was hydrolyzed and then reacted with isobutylchloroformate to form a carbonate, which was subsequently reduced to the *nor*-methyl nitroimidazole trigger **28**.[S6,S7] Alcohol **28** was then oxidized to aldehyde **29** upon treatment with DMP.[S6,S7] Finally, aldehyde **29** was methylated to yield the *mono*-methyl nitroimidazole trigger **30**.[S1,S6,S7]
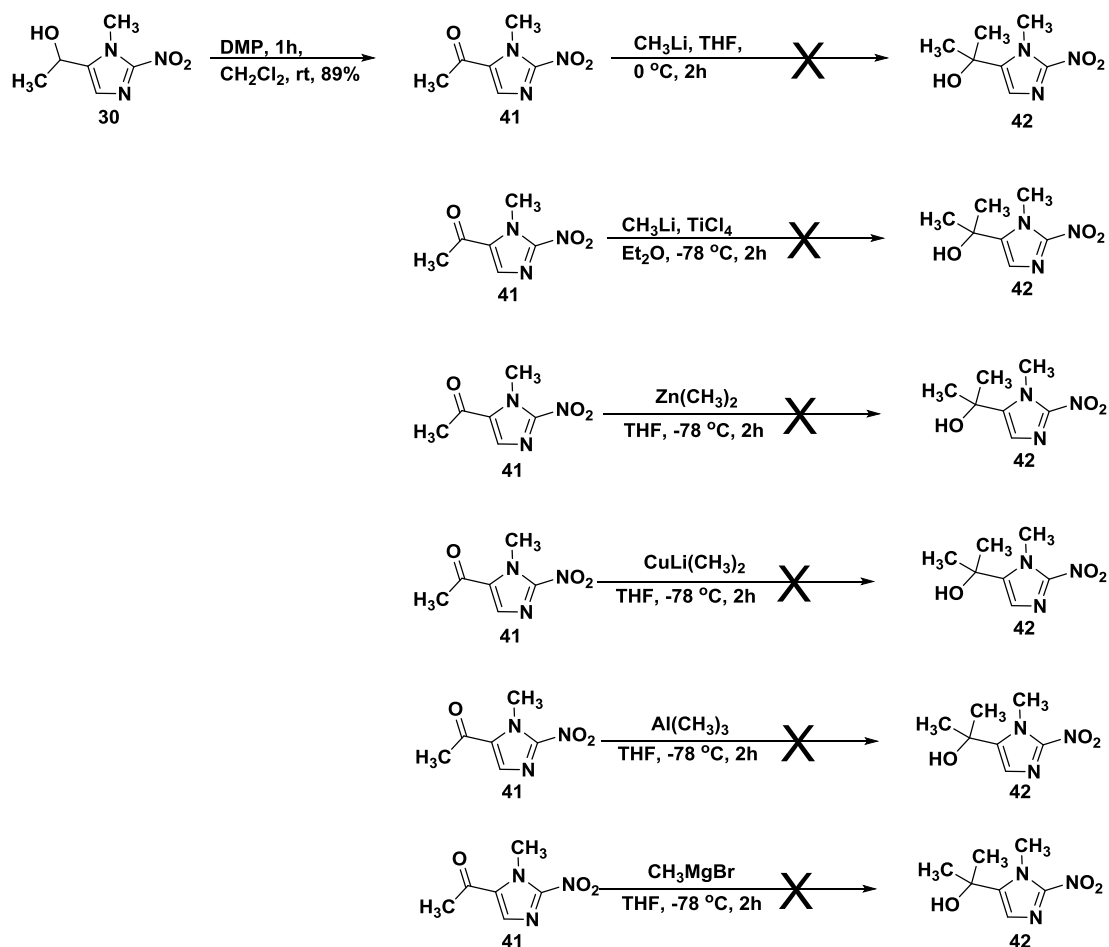
Scheme S5. Attempted Methylation of Nitroimidazole Ketone[S1,S6]

The oxidation of the *mono*-methyl nitroimidazole **30** yielded the ketone **41**. Several methylation methods were attempted on the nitroimidazole ketone **41** to generate the *gem*-dimethyl nitroimidazole **42**, but each method attempted only returned starting material. While the *gem*-dimethyl nitroimidazole trigger has been previously reported in the literature, only one (to the best of our knowledge) of the known reports (from among a very limited sub-set) provided a synthetic protocol[S9], and in an effort to avoid the large scale use of KCN and picric acid, we did not utilize this procedure.[S8-S17]

Experimental Procedure

General Experimental Procedures.[S18,S19] CH_2Cl_2 and tetrahydrofuran (THF) were used in their anhydrous forms, as obtained from the chemical suppliers. Reactions were performed under an inert atmosphere using N_2. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F254, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a flash purification system using silica gel (200-400 mesh, 60 Å) or RP-18 prepacked columns. Intermediates and products synthesized were characterized on the basis of their ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data. TMS was used as an internal standard for spectra recorded in $CDCl_3$. All the chemical shifts are expressed in ppm (δ), coupling constants (J) are presented in Hz, and peak patterns are reported as broad (br), singlet (s), doublet (d), doublet of doublets (dd) triplet (t), quartet (q), septet (sept), and multiplet (m). HRESIMS were obtained using positive or negative electrospray ionization (ESI) techniques using a Thermo Scientific LTQ OrbitrapDiscovery instrument. 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 x 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column. Flow rate 1.0 mL/min; injection volume 20 μL; monitored at 254 nm, 300 nm, 320 nm. Two different HPLC gradients were used for purity analysis; Method A: water/acetonitrile, gradient 10:90 to 90:10 from 0 to 25 min and isocratic 90:10 from 25 to 30 min; Method B: water/acetonitrile, gradient 50:50 to 90:10 from 0 to 25 min and isocratic 90:10 from 25 to 30 min (note: 4-dimethylaminopyridine is abbreviated DMAP, ethyl acetate is

abbreviated EtOAc, *N,N*-dimethylformamide is abbreviated DMF, and chloroform-d is abbreviated CDCl$_3$).

3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (2):[S3] Isovanillin (2.01 g, 13.2 mmol), triethylamine (4.00 mL, 28.5 mmol), and DMAP (0.045 g, 0.37 mmol) were dissolved in dry CH$_2$Cl$_2$ (60 mL). *tert*-Butyldimethylsilyl chloride (2.214 g, 14.7 mmol) was added to the reaction mixture, which was stirred for 12 h. The reaction was quenched with water, extracted with diethyl ether (Et$_2$O), which was washed with water and brine, dried with Na$_2$SO$_4$, 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, 10% A/90% B (1 CV), 10% A/90% B → 27% A/73% B (10 CV), 27% A/73% B over (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm] yielded 3-((*tert*-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (**2**) (3.17 g, 11.9 mmol, 90%) as a yellow oil.

^{1}H NMR (500 MHz, CDCl$_3$) δ 9.82 (1H, s), 7.49 (1H, dd, *J* = 8.5 Hz, *J* = 2 Hz), 7.37 (1H, d, *J* = 2 Hz), 6.96 (1H, d, *J* = 8.5 Hz), 3.90 (3H, s), 1.00 (9H, s), 0.17 (6H, s).

^{13}C NMR (125 MHz, CDCl$_3$) δ 190.9, 156.6, 145.3, 130.2, 126.3, 120.0, 111.1, 55.6, 25.6, 18.4, -4.6.

(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (4):[S3] 1-Bromo-3,4,5-trimethoxybenzene (1.81 g, 7.31 mmol) was dissolved in dry THF (60 mL) in a dry ice/acetone bath (-78 °C). *n*-

Butyllithium (2.8 mL, 7.0 mmol, 2.5 M) was added dropwise to the reaction mixture, which was stirred for 30 min. 3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (2.00 g, 7.50 mmol) dissolved in dry THF (20 mL) was added dropwise, and the reaction mixture was stirred for 5 h. The reaction was quenched with water, acidified to pH 7 with 3 M HCl, extracted with Et_2O, washed with water and brine, 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B (10 CV), 80% A/20% B over (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm] yielded (3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (**4**) as a pale yellow oil (2.02 g, 4.65 mmol, 62%) [1.58 g, 3.63 mmol, 48%, corrected for EtOAc].

¹H NMR (500 MHz, $CDCl_3$) δ 6.89 (2H, m), 6.80 (1H, d, *J* = 8.5 Hz), 6.57 (2H, d, *J* = 4.5 Hz), 5.24 (1H, d, *J* = 4.5 Hz), 3.81 (3H, s), 3.77 (9H, s), 0.94 (9H, d, *J* = 3.5 Hz), 0.11 (6H, d, *J* = 2.5 Hz).

¹³C NMR (125 MHz, $CDCl_3$) δ 153.0, 150.3, 144.7, 140.0, 136.5, 119.9, 119.4, 111.8, 103.4, 75.5, 60.7, 55.9, 55.5, 25.7, 18.4, -4.6.

(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (5):[S3] (3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (3.00 g, 6.90 mmol), Celite (2.45 g), and potassium carbonate [K_2CO_3] (2.46 g, 17.8 mmol) were dissolved in dry CH_2Cl_2 (130 mL) in an ice bath (0 °C). Pyridinium chlorochromate [PCC] (1.52 g, 7.04 mmol) was

added in small increments and the reaction mixture was stirred for 18 h. The reaction mixture was filtered with CH_2Cl_2 in a frit funnel containing a 50/50 mixture of Celite and silica gel and then 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, 10% A/90% B (1 CV), 10% A/90% B → 45% A/55% B (10 CV), 45% A/55% B (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm] yielded (3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (**5**) (1.79 g, 4.14 mmol, 60%) as a yellow oil.

1H NMR (500 MHz, $CDCl_3$) δ 7.40 (1H, d, J = 8 Hz), 7.33 (1H, s), 6.99 (2H, s), 6.87 (1H, d, J = 8.5 Hz), 3.88 (3H, s), 3.84 (3H, s), 3,83 (6H, s), 0.96 (9H, s), 0.14 (6H, s).

13C NMR (125 MHz, $CDCl_3$) δ 194.5, 154.9, 152.7, 144.6, 141.5, 133.3, 130.4, 125.3, 122.3, 110.7, 107.4, 60.9, 56.2, 55.5, 25.6, 18.4, -4.6.

Phenstatin (6):[S3] (3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (3.59 g, 8.31 mmol) was dissolved in dry THF (100 mL). Tetrabutylammonium fluoride trihydrate (3.93 g, 12.5 mmol) was added, and the reaction mixture was stirred for 18 h. The reaction was quenched with water, acidified to pH 7 with 3 M HCl, and extracted with EtOAc, which 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 → 99% A/1% B over 13.12 min (10 CV), 99% A/1% B over 2.38 min (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm] yielded phenstatin (**6**) (2.06 g, 6.47 mmol, 78%) as a white solid.

¹H NMR (500 MHz, CDCl₃) δ 7.42 (1H, s), 7.37 (1H, d, *J* = 8.5 Hz). 7.01 (2H, s), 6.90 (1H, d, *J* = 8 Hz), 3.94 (3H, s), 3.90 (3H, s), 3.85 (6H, s).

¹³C NMR (125 MHz, CDCl₃) δ 194.7, 152.8, 150.2, 145.3, 141.6, 133.1, 131.0, 123.7, 116.2, 109.7, 107.5, 61.0, 56.3, 56.1.

HRMS [M+Na]⁺: 341.0997 (calcd for [C₁₇H₁₈O₆Na]⁺, 341.1103).

HPLC retention time (Method A): 15.35 min [97.1% at 254 nm].

(5-Nitrothiophen-2-yl)methanol (8):[S2] 5-Nitrothiophene-2-carboxaldehyde (1.00 g, 6.38 mmol) was dissolved in dry methanol (20 mL) in an ice bath (0 °C). NaBH₄ (0.270 g, 7.14 mmol) was added, and the reaction mixture was stirred for 2 h. Ice was added and the solution was acidified to pH 7 with 3 M HCl. The reaction mixture was extracted with EtOAc, dried with Na₂SO₄, 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 (1 CV), 10% A/90% B → 65% A/35% B (10 CV), 65% A/35% B (2 CV); flow rate 50.0 mL/min; monitored at 254 and 280 nm] yielded alcohol **8** (0.914 g, 5.74 mmol, 90%) as a brown oil.

¹H NMR (500 MHz, CDCl₃) δ 7.84 (1H, d, *J* = 4.1 Hz), 6.95 (1H, dt, *J* = 4.1, 1.0 Hz), 4.90 (2H, d, *J* = 5.2 Hz), 2.15 (1H, t, *J* = 5.8 Hz).

¹³C NMR (126 MHz, CDCl₃) δ 154.0, 150.6, 129.0, 123.5, 60.2.

1-(5-Nitrothiophen-2-yl)ethan-1-ol (9):[S1] TiCl₄ (7.84 g, 41.3 mmol) was added slowly dropwise into Et₂O (80 mL) at -78 °C, after which methyllithium (1.6 M, 25.8 mL, 41

mmol) was added drop-wise, and the reaction mixture was stirred for 1.5 h. 5-Nitro-2-thiophenecarboxaldehyde (5.00g, 31.8 mmol) was dissolved in Et_2O (120 mL) and added dropwise to the reaction mixture, which was stirred (12 h). H_2O (50 mL) was used to quench the reaction. The layers were partitioned, 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 alcohol **9** (4.95 g, 28.6 mmol, 90%) as a dark brown oil.

¹H NMR (600 MHz, $CDCl_3$) δ 7.82 (1H, d, $J = 4.2$ Hz), 6.91 (1H, dd, $J = 4.2, 1.0$ Hz), 5.14 (1H, qd, $J = 6.4, 1.0$ Hz), 2.14 (1H, s), 1.64 (3H, d, $J = 6.5$ Hz).

¹³C 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-ol (9) [Alternate Route]: 5-Nitro-2-thiophenecarboxaldehyde (1.00 g, 6.36 mmol) was dissolved in CH_2Cl_2 (50 mL) at 0 °C. Trimethylaluminum (2 M, 5.30 mL, 10.6 mmol) was added dropwise, and the reaction mixture was stirred for 2 h. The reaction was quenched with HCl (1 M, 40 mL) and the layers were partitioned. The residue was extracted with CH_2Cl_2 (3 x 30 mL), and the combined organic phase was washed with brine (40 mL), dried over Na_2SO_4, and evaporated 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),

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70%A / 30%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] affording alcohol **11** (1.01 g, 5.85 mmol, 92%) as yellow-orange crystals.

1-(5-Nitrothiophen-2-yl)ethan-1-one (10): 2-(1-Hydroxyethyl)-5-nitrothiophene (1.04 g, 6.00 mmol) was dissolved in 70 mL CH_2Cl_2 at rt. Dess-Martin periodinane (3.82 g, 9.00mmol) was added in portions to the solution, and the reaction mixture was stirred (1 h). Saturated solutions of sodium thiosulfate (50 mL) and $NaHCO_3$ (50 mL) were used to quench the reaction mixture. The layers were partitioned, and the residue was extracted with EtOAc (4 x 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 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] affording ketone **10** (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 190.35, 156.47, 148.16, 130.06, 128.28, 26.61.

2-(5-Nitrothiophen-2-yl)propan-2-ol (11):[S1] $TiCl_4$ (3.62 g, 19.1 mmol) was slowly added dropwise into Et_2O (80 mL) at -78 °C, after which methyllithium (1.6 M, 11.9 mL, 19 mmol) was added dropwise, and the reaction mixture was stirred for 1.5 h. 2-Acetyl-5-nitrothiophene (2.50 g, 14.7 mmol) was dissolved in Et_2O (140 mL) and added dropwise to the reaction mixture, which was stirred (12 h). H_2O (50 mL) was used to quench the

reaction mixture. The layers were partitioned, 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 alcohol **11** (1.61 g, 8.60 mmol, 45%) as a dark orange oil.

^{1}H NMR (600 MHz, CDCl₃) δ 7.80 (1H, d, $J = 4.2$ Hz), 6.89 (1H, d, $J = 4.2$ Hz), 1.69 (6H, s).

^{13}C NMR (151 MHz, CDCl₃) δ 163.46, 150.04, 128.76, 121.26, 71.92, 32.08.

2-(5-Nitrothiophen-2-yl)propan-2-ol (11) [Alternate Route]: 2-Acetyl-5-nitrothiophene (0.500 g, 2.92 mmol) was dissolved in CH_2Cl_2 (20 mL) at 0 °C. Trimethylaluminum (2 M, 2.42 mL, 4.85 mmol) was added dropwise, and the reaction mixture was stirred for 2 h. The reaction was quenched with HCl (1 M, 30 mL), and the layers were partitioned. The residue was extracted with CH_2Cl_2 (3 x 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by flash column chromatography using a pre-packed 50 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: 50 mL/min; monitored at 254 and 280 nm] affording alcohol **11** (0.365 g, 2.13 mmol, 73%) as bright orange crystals.

(4-Methoxy-3-((5-nitrothiophen-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (12):[S2,S3] Phenstatin (0.405 g, 1.27 mmol), DIAD (0.289 g, 1.43 mmol), and (5-nitrothiophen-2-yl)methanol (0.454 g, 2.85 mmol) were dissolved in dry CH_2Cl_2 (40 mL). Triphenylphosphine (0.574 g, 2.19 mmol) was added, and the reaction mixture was stirred for 2 d. The reaction was quenched with water (30 mL) and extracted with EtOAc (3 x 30 mL), which 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, 17% A/83% B (1 CV), 17% A/83% B → 100% A/0% B (10 CV), 100% A/0% B (2 CV); flow rate 35.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((5-nitrothiophen-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**12**) (0.198 g, 0.431 mmol, 34%) as a brown solid.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.83 (1H, d, J = 4.5 Hz), 7.51 (1H, d, J = 1.5 Hz), 7.49 (1H, dd, J = 8 Hz, J = 1.5 Hz), 7.06 (1H, d, J = 4.5), 6.99 (2H, s), 6.97 (1H, d, J = 8.5 Hz), 5.33 (2H, s), 3.98 (3H, s), 3.93 (3H, s), 3.87 (6H, s).

^{13}C NMR (125 MHz, CDCl$_3$) δ 194.3, 153.7, 152.8, 147.7, 146.9, 141.7, 133.0, 130.2, 128.4, 126.6, 125.2, 115.5, 110.6, 107.4, 66.3, 61.0, 56.3, 56.2.

HRMS [M+Na]$^+$: 482.0880 (calcd for [C$_{22}$H$_{21}$NNaO$_8$S]$^+$, 482.0880).

HPLC retention time (Method B): 9.10 min [95.1% at 254 nm].

(4-Methoxy-3-(1-(5-nitrothiophen-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (13):[S2,S3] Phenstatin (0.407 g, 1.28 mmol), DIAD (0.294

g, 1.45 mmol), and 1-(5-nitrothiophen-2-yl)ethanol (0.505 g, 2.92 mmol) were dissolved in dry CH_2Cl_2 (40 mL). Triphenylphosphine (0.558 g, 2.13 mmol) was added, and the reaction mixture was stirred for 2 d. The reaction was quenched with water and extracted with EtOAc, which was dried with Na_2SO_4 and evaporated under reduced pressure. Flash chromatography of the crude product using a prepacked 25 g silica column [eluents: solvent A, EtOAc; solvent B, hexanes; gradient, 15% A/85% B (1 CV), 15% A/85% B → 100% A/0% B (10 CV), 100% A/0% B (2 CV); flow rate 20.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-(1-(5-nitrothiophen-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**13**) (0.179 g, 0.378 mmol, 30%) as a tan yellow solid.

¹H NMR (500 MHz, CDCl₃) δ 7.77 (1H, d, *J* = 4.2 Hz), 7.48 (1H, dd, *J* = 8.4, 2.0 Hz), 7.45 (1H, d, *J* = 2.0 Hz), 6.99 – 6.92 (4H, m), 5.60 (1H, q, *J* = 6.4 Hz), 3.96 (3H, s), 3.91 (3H, s), 3.84 (6H, s), 1.77 (3H, d, *J* = 6.4 Hz).

¹³C NMR (125 MHz, CDCl₃) δ 194.2, 154.8, 154.4, 152.8, 151.0, 145.9, 141.7, 133.0, 130.2, 128.4, 126.8, 123.4, 118.4, 110.9, 107.4, 73.6, 61.0, 56.3, 56.1, 23.2.

HRMS [M+Na]⁺: 496.1038 (calcd for $[C_{23}H_{23}NNaO_8S]^+$, 496.1037).

HPLC retention time (Method B): 10.33 min [98.1% at 254 nm].

 (4-Methoxy-3-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (14):[S2,S3] Phenstatin (0.581 g, 1.83 mmol), ADDP (0.597 g, 2.38 mmol), and 2-(5-nitrothiophen-2-yl)propan-2-ol (0.410 g, 2.19 mmol) were dissolved in dry CH_2Cl_2 (80 mL). Tributylphosphine (0.752 mL, 3.06 mmol) was added

dropwise, and the reaction mixture was stirred for 2 d. The reaction mixture was dried under reduced pressure. Flash chromatography of the crude product using a prepacked 25 g silica column [eluents: 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 50.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**14**) as an orange gum (0.062 g, 0.128 mmol, 7%) [0.050 g, 0.104 mmol, 6%, corrected for CH_2Cl_2].

¹H NMR (600 MHz, acetone-d_6) δ 7.93 (1H, d, J = 4.3 Hz), 7.67 (1H, dd, J = 8.5, 2.2 Hz), 7.41 (1H, d, J = 2.1 Hz), 7.22 (1H, d, J = 8.5 Hz), 7.19 (1H, d, J = 4.3 Hz), 7.02 (2H, s), 3.95 (3H, s), 3.87 (6H, s), 3.84 (3H, s), 1.79 (6H, s).

¹³C NMR (151 MHz, acetone-d_6) δ 192.94, 161.01, 157.41, 153.10, 150.43, 142.93, 141.89, 133.12, 129.98, 128.83, 127.80, 125.38, 123.07, 111.83, 107.31, 80.82, 59.80, 55.74, 55.44, 28.16.

HRMS [M+Na]$^+$: 510.1190 (calcd for [$C_{24}H_{25}NNaO_8S$]$^+$, 510.1193).

HPLC retention time (Method B): 11.49 min [96.3% at 254 nm].

1-(4-Nitrophenyl)ethan-1-ol (16):[S1] $TiCl_4$ (2.72 mL, 24.8 mmol) was added dropwise slowly to dry Et_2O (100 mL) in an acetone / dry ice bath (-78 °C). Methyllithium (15.5 mL, 25 mmol, 1.6 M) was then added dropwise slowly to the reaction mixture which was stirred for 1.5 h. 4-Nitrobenzaldehyde (2.88g, 19.1 mmol) dissolved in Et_2O (140 mL) was added dropwise to the reaction mixture, which was stirred for 18 h. The reaction was quenched with water and extracted with CH_2Cl_2 (3 x 50 mL), which was washed with

water and brine and dried over 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, 10% A/90% B (1 CV), 10% A/90% B →
80% A/20% B over (10 CV), 80% A/20% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded 1-(4-nitrophenyl)ethan-1-ol (**16**) (2.49 g, 14.9 mmol, 78%) as a yellow-orange oil.

^{1}H NMR (600 MHz, CDCl$_3$) δ 8.17 (2H, d, J = 8.7 Hz), 7.53 (2H, d, J = 8.6 Hz), 5.01 (1H, q, J = 6.5 Hz), 1.51 (3H, d, J = 6.6 Hz).

^{13}C NMR (151 MHz, CDCl$_3$) δ 153.22, 147.09, 126.13, 123.71, 69.43, 25.44.

2-(4-Nitrophenyl)propan-2-ol (18):[S1]   TiCl$_4$ (3.02 mL, 27.6 mmol) was added dropwise slowly to dry Et$_2$O (100 mL) in an acetone / dry ice bath (-78 °C). Methyllithium (17.2 mL, 28 mmol, 1.6 M) was then added dropwise slowly to the reaction mixture, which was stirred for 1.5 h. 4-Nitroacetophenone (3.50g, 21.2 mmol) dissolved in Et$_2$O (150 mL) was added dropwise to the reaction mixture, which was stirred for 18 h. The reaction was quenched with water, and the mixture was extracted with CH$_2$Cl$_2$ (3 x 50 mL), which was washed with water and brine, dried over 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, 10% A/90% B (1 CV), 10% A/90% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded 2-(4-nitrophenyl)propan-2-ol (**18**) (1.42 g, 7.84 mmol, 37%) as an orange oil.

¹H NMR (600 MHz, CDCl₃) δ 8.16 (2H, d, *J* = 8.9 Hz), 7.65 (2H, d, *J* = 8.9 Hz), 1.61 (7H, s).

¹³C NMR (151 MHz, CDCl₃) δ 156.52, 146.64, 125.51, 123.45, 72.49, 31.69.

(4-methoxy-3-((4-nitrobenzyl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (20):[S2-S5] Phenstatin (0.500 g, 1.57 mmol), DIAD (0.35 mL, 1.9 mmol), and 4-nitrobenzyl alcohol (0.481 g, 3.14 mmol) were dissolved in dry CH₂Cl₂ (60 mL). Triphenylphosphine (0.700 g, 2.67 mmol) was added, and the reaction mixture was stirred for 2 d. The reaction was quenched with water, and the reaction mixture was extracted with EtOAc, which was dried with Na₂SO₄ 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B (10 CV), 80% A/20% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((4-nitrobenzyl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**20**) (0.462 g, 1.02 mmol, 65%) as a yellow solid.

¹H NMR (600 MHz, acetone-*d₆*) δ 8.31 (2H, d, *J* = 8.7 Hz), 7.84 (2H, d, *J* = 9.0 Hz), 7.54 (1H, d, *J* = 2.0 Hz), 7.52 (1H, dd, *J* = 8.3, 2.0 Hz), 7.18 (1H, d, *J* = 8.4 Hz), 7.04 (2H, s), 5.40 (2H, s), 4.00 (3H, s), 3.87 (6H, s), 3.84 (3H, s).

¹³C NMR (151 MHz, acetone-*d₆*) δ 193.21, 153.71, 153.10, 147.67, 145.12, 141.81, 133.24, 130.25, 128.06, 125.41, 123.49, 114.93, 110.99, 107.37, 99.99, 69.45, 59.80, 55.70, 55.54. **HRMS** [M+Na]⁺: 476.1315 (calcd for [C₂₄H₂₃NNaO₈]⁺, 476.1316).

HPLC retention time (Method B): 9.55 min [100% at 254 nm].

(4-Methoxy-3-(1-(4-nitrophenyl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone

(21):[S2-S5] Phenstatin (0.500 g, 1.57 mmol), DIAD (0.348 mL, 1.88 mmol), and 1-(4-nitrophenyl)ethan-1-ol (0.525 g, 3.14 mmol) were dissolved in dry CH_2Cl_2 (60 mL). Triphenylphosphine (0.700 g, 2.67 mmol) was added to the reaction mixture, which was stirred for 2 d. The reaction was quenched with water, and the reaction mixture was extracted with CH_2Cl_2 (3 x 40 mL), which 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B over (10 CV), 80% A/20% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-(1-(4-nitrophenyl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**21**) (0.315 g, 0.675 mmol, 43%) as white solid.

^{1}H NMR (600 MHz, acetone-d_6) δ 8.26 (2H, d, J = 8.8 Hz), 7.78 (2H, d, J = 8.4 Hz), 7.46 (1H, dd, J = 8.4, 2.0 Hz), 7.34 (1H, d, J = 2.0 Hz), 7.15 (1H, d, J = 8.4 Hz), 6.93 (2H, s), 5.72 (1H, q, J = 6.4 Hz), 4.00 (3H, s), 3.83 (3H, s), 3.81 (6H, s), 1.69 (3H, d, J = 6.5 Hz).

^{13}C NMR (151 MHz, CDCl$_3$) δ 194.27, 153.96, 152.80, 150.05, 147.48, 146.57, 141.76, 133.04, 130.14, 126.53, 125.83, 124.01, 116.93, 110.61, 107.50, 60.98, 56.36, 56.14, 23.86, 21.95.

HRMS [M+Na]$^+$: 490.1471 (calcd for [C$_{25}$H$_{25}$NNaO$_8$]$^+$, 490.1472).

HPLC retention time (Method B): 10.05 min [96.7% at 254 nm].

(4-Methoxy-3-((2-(4-nitrophenyl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (22):[S2-S5] Phenstatin (0.500 g, 1.57 mmol), ADDP (0.475 g, 1.88 mmol), and 2-(4-nitrophenyl)propan-2-ol (0.569 g, 3.14 mmol) were dissolved in dry CH_2Cl_2 (70 mL). Tributylphosphine (0.66 mL, 2.67 mmol) was added dropwise to the reaction mixture, which was stirred for 2 d. The reaction was quenched with water, and the mixture was extracted with EtOAc, which was washed with water and brine, 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B (10 CV), 80% A/20% B (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((2-(4-nitrophenyl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**22**) (0.174 g, 0.361 mmol, 23%) as tan solid,

^{1}H NMR (600 MHz, acetone-d_6) δ 8.25 (2H, d, J = 8.9 Hz), 7.91 (2H, d, J = 8.9 Hz), 7.57 (1H, dd, J = 8.5, 2.1 Hz), 7.18 (1H, d, J = 8.5 Hz), 7.12 (1H, d, J = 2.1 Hz), 6.92 (2H, s), 3.96 (3H, s), 3.84 (6H, s), 3.83 (3H, s), 1.76 (6H, s).

^{13}C NMR (151 MHz, acetone-d_6) δ 193.05, 156.74, 154.53, 153.03, 147.02, 143.92, 141.76, 133.21, 129.72, 126.62, 126.34, 123.48, 123.30, 111.62, 107.16, 81.28, 59.77, 55.73, 55.43, 27.99.

HRMS [M+Na]$^+$: 504.1629 (calcd for [$C_{26}H_{27}NNaO_8$]$^+$, 504.1629).

HPLC retention time (Method B): 10.82 min [97.1% at 254 nm].

Ethyl 2-amino-1-methyl-1*H*-imidazole-5-carboxylate (26):[S6] To a suspension of

sarcosine ethyl ester (4.00 g, 0.026 mol) in THF (90 mL) and ethyl formate (90 mL) was

added NaH (60 % dispersion in mineral oil, 10.0 g, 0.25 mol) in several portions at room

temperature. The reaction mixture was stirred for 3 h, and, during this time, a yellow

suspension formed. The reaction mixture was concentrated and triturated with hexane (2

x 150 mL). The hexane was decanted, and the resulting light tan solid was dried in *vacuo*.

Ethanol (80 mL) and concentrated aqueous HCl (16 mL) were added to the solid, and the

suspension was heated to reflux for 2 h. The reaction mixture was then filtered while hot,

and the filter was rinsed with boiling ethanol (2 x 50 mL). The combined filtrate was

concentrated to yield a brown oil. The oil was diluted with ethanol (140 mL) and water

(60 mL), and the pH of the solution was adjusted to 3 by using NaOH solution (2 M).

Cyanamide (2.18 g, 0.052 mol) was added, and the resulting solution was heated to reflux

for 1.5 h. After being cooled to room temperature, the reaction mixture was concentrated

to approximately 1/8 of its original volume. Solid K_2CO_3 was added to adjust the pH of

the concentrated reaction mixture to 8-9, resulting in the formation of a yellow

precipitate. The solid was removed by filtration, washed with a K_2CO_3 solution (1 M, 1 x

20 mL) and water (2 x 20 mL) and dried to afford a pale yellow solid (1.97 g, 12.0 mmol,

45%).

¹H NMR (600 MHz, CDCl₃) δ 7.45 (1H, s), 4.27 (2H, q, *J* = 7.1 Hz), 4.25 (2H, s), 3.68

(3H, s), 1.34 (3H, t, *J* = 7.1 Hz).

¹³C NMR (151 MHz, CDCl₃) δ 160.67, 151.89, 135.50, 119.05, 59.82, 30.55, 14.41.

Ethyl 1-methyl-2-nitro-1*H*-imidazole-5-carboxylate (27):[S6,S7] Aminoimidazole (0.700 g, 4.14 mmol) in acetic acid (7.3 mL) was added dropwise to an aqueous solution of sodium nitrite (3.6 mL, 11 M). The solution was stirred at room temperature for 4 h until no more N_2 was formed. The reaction mixture was extracted with CH_2Cl_2 (1 x 20 mL), washed with brine (1 x 20 mL) and a saturated aqueous solution of Na_2SO_3 (1 x 20 mL). The organic layer was then dried over Na_2SO_4, filtered and concentrated to afford a crude yellow solid. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 70 mL/min; monitored at 254 and 280 nm] afforded the nitroimidazole analogue **27** (0.510 g, 2.60 mmol, 63%) as a yellow solid.

¹H NMR (600 MHz, CDCl₃) δ 7.74 (1H, s), 4.40 (2H, q, *J* = 7.1 Hz), 4.35 (3H, s), 1.41 (3H, t, *J* = 7.1 Hz).

¹³C NMR (151 MHz, CDCl₃) δ 159.08, 147.46, 134.67, 126.29, 61.84, 35.39, 14.18.

(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methanol (28):[S6,S7] A suspension of the nitroimidazole ethyl ester (0.796 g, 4.00 mmol) in 0.75 M NaOH solution (16 mL) was stirred at room temperature overnight to give a clear light yellow solution. The pH of the reaction mixture was adjusted to 1 by adding concentrated HCl. The resulting solution was extracted with EtOAc (5 x 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to afford a light yellow solid. The solid was dissolved in THF (8 mL) with triethylamine (0.880 mL, 6.30 mmol). Isobutylchloroformate (0.820 mL, 6.30 mmol) was added dropwise at -40 °C, and the reaction mixture was stirred at room

temperature for 1 h. NaBH$_4$ (0.794 g, 21.0 mmol) was added to the solution, followed by dropwise addition of water (7 mL) over 1 h while maintaining the temperature around 0 $^{\circ}$C. The reaction mixture was extracted with Et$_2$O (3 x 20 mL), which was dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: methanol; solvent B: CH$_2$Cl$_2$; gradient: 1%A / 99%B (4 CV), 1%A / 99%B → 15%A / 85%B (10 CV), 15%A / 85%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] afforded the normethyl nitroimidazole trigger (**28**) (0.449 g, 2.86 mmol, 71%) as a pale yellow solid.

^{1}H NMR (600 MHz, Methanol-d_4) δ 7.11 (1H, s), 4.68 (2H, s), 4.06 (3H, s).

^{13}C NMR (151 MHz, MeOD) δ 145.82, 137.93, 126.02, 53.16, 33.40.

1-Methyl-2-nitro-1H-imidazole-5-carbaldehyde (29): Normethyl nitroimidazole trigger **28** (359 mg, 2.28 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL). Dess–Martin periodinane (1.16 g, 2.74 mmol) was added and the reaction mixture was stirred for 1 h at room temperature. Saturated solutions of NaHCO$_3$ (20 mL) and sodium thiosulfate (20 mL) were added to the reaction mixture, which was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over Na$_2$SO$_4$ and filtered, and the solvent was removed under reduced pressure. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (1 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] afforded imidazole analogue (346 mg, 2.23 mmol, 98%) as a yellow solid**.**

^{1}H NMR (600 MHz, CDCl$_3$) δ 9.94 (1H, s), 7.82 (1H, s), 4.36 (3H, s).

¹³C NMR (151 MHz, CDCl₃) δ 180.39, 148.35, 139.38, 132.38, 35.57.

1-(1-Methyl-2-nitro-1*H*-imidazol-5-yl)ethan-1-ol (30):[S1] TiCl₄ (1.3 mL, 11 mmol) in Et₂O (60 mL) was treated with methyllithium (7.1 mL, 1.6M, 11 mmol) at -78 °C, and the resulting solution was stirred for 1 h. A THF (15 mL) solution of imidazole aldehyde analogue (0.884 g, 5.70 mmol) was added dropwise and the reaction mixture was stirred for 24 h. Water (50 mL) was added and the resulting solution was extracted with EtOAc (3 x 50 mL), which was dried over Na₂SO₄ and concentrated to afford a crude brown oil. Purification by flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 17%A / 83%B (1 CV), 17%A / 83%B → 100%A / 0%B (7 CV), 100%A / 0%B (5 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] afforded the monomethyl nitroimidazole trigger (**30**) (400 mg, 2.34 mmol, 41%) as a yellow solid.

¹H NMR (600 MHz, acetone-*d₆*) δ 7.07 (1H, s), 5.01 (1H, p, *J* = 6.2 Hz), 4.64 (1H, d, *J* = 6.0 Hz), 4.09 (3H, s), 1.63 (3H, d, *J* = 6.6 Hz).

¹³C NMR (151 MHz, acetone-*d₆*) δ 146.4, 141.6, 124.7, 60.4, 33.9, 21.1

(4-Methoxy-3-((1-methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (31):[S2-S5] Phenstatin (0.500 g, 1.57 mmol), (1-methyl-2-nitro-1*H*-imidazol-5-yl)methanol (0.296 g, 1.89 mmol), and DIAD (0.40 mL, 2.04 mmol) were dissolved in CH₂Cl₂. Triphenylphosphine (0.825 g, 3.14 mmol) was added to the mixture, and the reaction mixture was stirred for 24 h. The reaction mixture was then 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, 17%A/83%B over 1.19 min (1 CV), 17%A/83%B → 100%A/0%B over 8.33 min (7 CV), 100%A / 0%B over 5.95 min (5 CV); flow rate 100 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((1-methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**31**) as a pale yellow-white solid (0.346 g, 0.757 mmol, 48%) [0.284 g, 0.621 mmol, 39%, corrected for EtOAc].

1H NMR (600 MHz, CDCl$_3$) δ 7.62 (1H, d, *J* = 1.7 Hz), 7.52 (1H, dd, *J* = 8.3, 1.7 Hz), 7.24 (1H, s), 7.04 (2H, s), 6.97 (1H, d, *J* = 8.4 Hz), 5.18 (2H, s), 4.16 (3H, s), 3.97 (3H, s), 3.96 (3H, s), 3.91 (6H, s).

13C NMR (151 MHz, CDCl$_3$) δ 194.16, 153.97, 152.91, 146.74, 141.88, 132.92, 132.30, 130.43, 129.31, 127.00, 116.43, 110.61, 107.52, 99.98, 61.24, 61.01, 56.39, 56.05, 34.54.

HRMS [M+Na]$^+$: 480.1376 (calcd for [C$_{22}$H$_{23}$N$_3$NaO$_8$]$^+$, 480.1377).

HPLC retention time (Method B): 4.66 min [100% at 254 nm].

(4-methoxy-3-(1-(1-methyl-2-nitro-1*H*-imidazol-5-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (32):[S2-S5] Phenstatin (0.250 g, 0.786 mmol), DIAD (0.19 mL, 1.02 mmol), and 1-(1-methyl-2-nitro-1*H*-imidazol-5-yl)ethan-1-ol (0.161 g, 0.943 mmol) were added to dry CH$_2$Cl$_2$ (50 mL). Triphenylphosphine (0.412 g, 1.57 mmol) was added, and the reaction mixture was stirred for 2 d. The reaction solvent was 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, 15%A/85%B over 1.19 min (1 CV), 15%A/85%B → 100%A/0%B over 8.33 min (7

CV), 100%A / 0%B over 14.28 min (12 CV); flow rate 100 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-(1-(1-methyl-2-nitro-1*H*-imidazol-5-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**32**) as a pale yellow-white solid (0.119 g, 0.252 mmol, 32%) [0.101 g, 0.214 mmol, 27%, corrected for EtOAc].

^{1}H NMR (600 MHz, CDCl$_3$) δ 7.57 (1H, d, *J* = 2.0 Hz), 7.55 (1H, dd, *J* = 8.3, 2.0 Hz), 7.21 (1H, s), 7.02 (2H, s), 6.99 (1H, d, *J* = 8.4 Hz), 5.59 (1H, q, *J* = 6.5 Hz), 4.13 (3H, s), 3.97 (3H, s), 3.96 (3H, s), 3.91 (6H, s), 1.81 (3H, d, *J* = 6.5 Hz).

^{13}C NMR (151 MHz, CDCl$_3$) δ 194.09, 154.63, 152.90, 145.23, 141.89, 137.09, 132.88, 130.47, 127.15, 118.90, 110.95, 107.51, 99.98, 68.42, 61.01, 56.39, 56.04, 34.70, 18.55.

HRMS [M+Na]$^+$: 494.1533 (calcd for [C$_{23}$H$_{25}$N$_3$NaO$_8$]$^+$ 494.1534).

HPLC retention time (Method B): 5.17 min [98.6% at 254 nm].

(5-Nitrofuran-2-yl)methanol (**34**):^{S2} 5-Nitrofuran-2-carbaldehyde (4.00 g, 28 mmol) was dissolved in anhydrous methanol (80 mL) and cooled to 0 ^{o}C. NaBH$_4$ (1.17 g, 31 mmol) was added to the reaction mixture, which was stirred for 2.5 h. The reaction was quenched with an HCl solution (1 M, 40 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layer was dried over Na$_2$SO$_4$ and concentrated under reduced pressure to afford a crude yellow oil. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] afforded (5-nitrofuran-2-yl)methanol (**34**) (3.23 g, 22.6 mmol, 80%) as a pale yellow oil.

¹H NMR (600 MHz, CDCl₃) δ 7.31 (1H, d, *J* = 3.6 Hz), 6.58 (1H, d, *J* = 3.6 Hz), 4.74 (2H, s), 2.09 (1H, s).

¹³C NMR (151 MHz, CDCl₃) δ 157.37, 151.92, 112.40, 110.61, 57.45.

1-(5-Nitrofuran-2-yl)ethan-1-ol (**35**):[S1] TiCl₄ (0.78 mL, 7.1 mmol) in Et₂O (35 mL) was treated with methyllithium (4.4 mL, 1.6 M, 7.1 mmol) at -78 °C. The resulting solution was stirred for 1 h. A THF (10 mL) solution of 5-nitrofuran-2-carbaldehyde (0.500 g, 3.5 mmol) was added dropwise, and the reaction mixture was stirred for 24 h. Water (30 mL) was added and the resulting solution was extracted with EtOAc (3 x 30 mL), which was dried over Na₂SO₄ and concentrated to afford a crude brown oil. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] afforded 1-(5-nitrofuran-2-yl)ethan-1-ol (**35**) (449 mg, 2.86 mmol, 81%) as a brown oil.

¹H NMR (600 MHz, CDCl₃) δ 7.29 (1H, d, *J* = 4.1 Hz), 6.52 (1H, d, *J* = 4.6 Hz), 4.96 (1H, q, *J* = 7.1 Hz), 2.57 (1H, s), 1.61 (3H, d, *J* = 6.8 Hz).

¹³C NMR (151 MHz, CDCl₃) δ 161.27, 151.59, 112.51, 108.57, 63.66, 21.38.

1-(5-Nitrofuran-2-yl)ethan-1-one (**36**): Dess-Martin periodinane (8.62 g, 20.4 mmol) was added to 1-(5-nitrofuran-2-yl)ethan-1-ol (3.20 g, 20.4 mmol) dissolved in CH₂Cl₂ (250 mL), and the reaction mixture was stirred for 1 h. The reaction was quenched with saturated solutions of sodium thiosulfate and NaHCO₃, then extracted with CH₂Cl₂ (3 x

50 mL), which was washed with water and brine, 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, 7% A/93% B over 1.19 min (1 CV), 7% A/93% B → 50% A/50% B over 13.12 min (10 CV), 50% A/50% B over 2.38 min (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded 1-(5-nitrofuran-2-yl)ethan-1-one (**36**) (2.98 g, 19.2 mmol, 94%) as yellow solid.

¹H NMR (600 MHz, CDCl₃) δ 7.38 (1H, d, J = 3.8 Hz), 7.28 (1H, d, J = 3.7 Hz), 2.61 (3H, s).

¹³C NMR (151 MHz, CDCl₃) δ 186.73, 151.91, 151.48, 116.79, 111.94, 26.27.

2-(5-Nitrofuran-2-yl)propan-2-ol (37): 1-(5-Nitrofuran-2-yl)ethan-1-one (3.00 g, 19.3 mmol) in CH_2Cl_2 (120 mL) was treated dropwise at 0 °C with trimethylaluminium (16.0 mL, 2.0 M, 32 mmol), and the resulting yellow solution was stirred for 90 min at 0 °C. Sat. aq. NH₄Cl was added to the reaction mixture, which was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure to give a yellow oil. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 1000mL/min; monitored at 254 and 280 nm] afforded 2-(5-nitrofuran-2-yl)propan-2-ol (**37**) (2.75 g, 16.1 mmol, 83%) as a yellow oil.

¹H NMR (600 MHz, CDCl₃) δ 7.27 (1H, d, J = 3.7 Hz), 6.49 (1H, d, J = 3.7 Hz), 2.36 (1H, s), 1.65 (7H, s).

¹³C NMR (151 MHz, CDCl₃) δ 164.05, 151.36, 112.55, 107.37, 69.30, 28.67.

(4-Methoxy-3-((5-nitrofuran-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (38):[S2-S5] Phenstatin (0.250 g, 0.786 mmol), DEAD (0.16 mL, 1.02 mmol), and (5-nitrofuran-2-yl)methanol (0.135 g, 0.943 mmol) were dissolved in dry CH₂Cl₂ (60 mL). Triphenylphosphine (0.412 g, 1.57 mmol) was added, and the reaction mixture was stirred for 2 d. The solvent was 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 → 80% A/20% B over 13.12 min (10 CV), 80% A/20% B over 2.38 min (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((5-nitrofuran-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**38**) (0.143 g, 0.322 mmol, 41%) as a white solid.

¹H NMR (600 MHz, acetone-d_6) δ 7.59 (1H, d, J = 2.0 Hz), 7.56 − 7.52 (2H, m), 7.17 (1H, d, J = 8.4 Hz), 7.06 (2H, s), 6.95 (1H, d, J = 3.7 Hz), 5.32 (2H, s), 3.96 (3H, s), 3.88 (6H, s), 3.85 (3H, s).

¹³C NMR (151 MHz, acetone-d_6) δ 193.12, 154.11, 153.89, 153.12, 147.13, 141.85, 133.19, 130.26, 126.00, 124.87, 116.00, 113.41, 112.45, 111.18, 107.39, 63.08, 59.80, 55.72, 55.47. **HRMS** [M+Na]⁺: 466.1107 (calcd for [C₂₂H₂₁NNaO₉]⁺, 466.1109).

HPLC retention time (Method B): 6.81 min [99.1% at 254 nm].

(4-Methoxy-3-(1-(5-nitrofuran-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (39):[S2-S5] Phenstatin (0.250 g, 0.786 mmol), DIAD (0.20

mL, 1.02 mmol), and 1-(5-nitrofuran-2-yl)ethan-1-ol (0.148 g, 0.943 mmol) were dissolved in dry CH_2Cl_2 (60 mL). Triphenylphosphine (0.412 g, 1.57 mmol) was added, and the reaction mixture was stirred for 2 d. The solvent was 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 → 80% A/20% B over 13.12 min (10 CV), 80% A/20% B over 2.38 min (2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-(1-(5-nitrofuran-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**39**) (0.169 g, 0.369 mmol, 47%) as a white solid.

¹H NMR (600 MHz, acetone-d_6) δ 7.55 (1H, dd, J = 8.4, 2.1 Hz), 7.51 (1H, d, J = 2.1 Hz), 7.49 (1H, d, J = 3.7 Hz), 7.17 (1H, d, J = 8.4 Hz), 7.02 (2H, s), 6.83 (1H, d, J = 3.5 Hz), 5.65 (1H, q, J = 6.5 Hz), 3.97 (3H, s), 3.87 (6H, s), 3.84 (3H, s), 1.77 (3H, d, J = 6.6 Hz).

¹³C NMR (151 MHz, acetone-d_6) δ 193.05, 158.26, 154.84, 153.11, 151.79, 146.11, 141.89, 133.16, 130.29, 126.49, 119.30, 112.44, 111.54, 111.02, 107.39, 70.96, 59.80, 55.73, 55.52, 18.82.

HRMS [M+Na]⁺: 480.1263 (calcd for [$C_{23}H_{23}NNaO_9$]⁺, 480.1265).

HPLC retention time (Method B): 7.86 min [100% at 254 nm].

(4-Methoxy-3-((2-(5-nitrofuran-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (40):[S2-S5] Phenstatin (1.00 g, 3.14 mmol), ADDP (1.03 g, 4.08 mmol), and 2-(5-nitrofuran-2-yl)propan-2-ol (0.646 g, 3.77 mmol) were dissolved in

dry THF (80 mL). Tributylphosphine (1.55 mL, 6.28 mmol) was added dropwise, and the reaction mixture was stirred for 2 d. The solvent was 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, 10% A/90% B (1 CV), 10% A/90% B → 80% A/20% B (10 CV), 80% A/20% B (0.2 CV); flow rate 100.0 mL/min; monitored at 254 and 280 nm] yielded (4-methoxy-3-((2-(5-nitrofuran-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**40**) as a colorless solid (0.118 g, 0.251 mmol, 8%) [0.100 g, 0.213 mmol, 7%, corrected for CH_2Cl_2].

^{1}H NMR (600 MHz, acetone-d6) δ 7.58 (1H, dd, J = 8.5, 2.1 Hz), 7.40 (1H, d, J = 3.7 Hz), 7.14 − 7.06 (2H, m), 6.94 (2H, s), 6.69 (1H, d, J = 3.7 Hz), 3.83 (6H, s), 3.81 (3H, s), 3.80 (3H, s), 1.75 (6H, s).

^{13}C NMR (151 MHz, acetone-d_6) δ 192.97, 160.47, 157.53, 153.09, 151.48, 143.09, 141.85, 133.15, 129.94, 127.80, 125.96, 112.31, 111.61, 110.81, 107.25, 77.40, 59.80, 55.74, 55.38, 25.03.

HRMS [M+Na]$^+$: 494.1422 (calcd for [$C_{24}H_{25}NNaO_9$]$^+$ 494.1422).

HPLC retention time (Method B): 8.26 min [93.9% at 254 nm].

Biology

Colchicine Binding Assay[S22]

Inhibition of [^{3}H]colchicine binding to tubulin was determined using 0.1 mL reaction mixtures. Each reaction mixture contained 1.0 µM tubulin, 5.0 µM [^{3}H]colchicine (from Perkin–Elmer), 5% (v/v) dimethyl sulfoxide, potential inhibitors at 5.0 µM and components that were previously demonstrated to stabilize the colchicine binding activity of tubulin[S10] (1.0 M monosodium glutamate [adjusted to pH 6.6 with HCl in a 2.0 M stock solution], 0.5 mg/mL bovine serum albumin, 0.1 M glucose-1-phosphate, 1.0 mM MgCl$_2$, and 1.0 mM GTP). Incubation was for 10 min at 37°C, a time point at which the binding reaction in the control is 40–60% complete. Reactions were stopped by adding 2.0 mL of ice-cold water and placing the samples on ice. Each sample was poured onto a stack of two DEAE-cellulose filters, followed immediately by 6 mL of ice-cold water, and the water was aspirated under reduced vacuum. The filters were washed with 2 mL water X 3 and, following removal of excess water under a strong vacuum, placed into vials containing 5 mL of Biosafe II scintillation cocktail. Samples were counted the next day in a Beckman scintillation counter. Samples with potential inhibitors were compared to controls with no inhibitor to determine percent inhibition. All samples were corrected for the amount of colchicine that bound to the filters in the absence of tubulin.

Inhibition of Tubulin Polymerization[S22]

Tubulin assembly experiments were performed using 0.25 mL reaction mixtures (final volume).[S11] The mixtures contained 1 mg/mL (10 µM) purified bovine brain tubulin, 0.8

M monosodium glutamate (pH 6.6, as above), 4% (v/v) dimethyl sulfoxide, 0.4 mM GTP, and varying compound concentrations. Initially, all components except GTP were preincubated for 15 min at 30 °C in 0.24 mL. After chilling the mixtures on ice, 10 µL of 10 mM GTP was added. The reaction mixtures were then transferred to cuvettes held at 0 °C in Beckman DU-7400 and DU-7500 spectrophotometers equipped with electronic temperature controllers. The temperature was jumped to 30 °C over about 30 s, and polymerization was followed turbidimetrically at 350 nm for 20 min. Each reaction set included a reaction mixture without compound, and the IC50 was defined as the compound concentration that inhibited extent of assembly by 50% after 20 min at 30 °C.

NADPH Cytochrome P450 Oxidoreductase Cleavage Assay [S23,S24]

Rat NADPH cytochrome P450 oxidoreductase (POR) and protocatechuate 3,4-dioxygenase (PCD) were purchased from Corning® and Sigma-Aldrich, respectively, and their enzymatic activities were determined in terms of enzyme units (U). All bioreductive prodrugs were dissolved in DMSO as 10 mM stock solutions.

An aliquot (5 µL) of the 10 mM compound DMSO stock solution along with 0.5 µL 0.1% Triton X-100 were added to 395.5 µl 200 mM pH 7.4 potassium phosphate buffer containing 400 µM freshly made protocatechuic acid (PCA). The components were fully mixed in a microvessel capped with a rubber septum stopper and subjected to three cycles of evacuation and flushing with N_2 using a manifold, followed by sparging with N_2 for an additional 20 min. PCD (0.08 units) was added by Hamilton syringe, and the solution was scrubbed for 10 min to allow for sufficient O_2 digestion by PCA/PCD. POR stock (0.006 units) was introduced followed by NADPH (0.8 mM final concentration) into the vial

followed by an additional round of N_2 sparging. The reaction mixture was incubated for 24 h at 37 °C, cooled on ice and treated with an equal volume of acetonitrile. After centrifugation and filtration, the samples were analyzed by HPLC. Solutions without POR were used as controls.
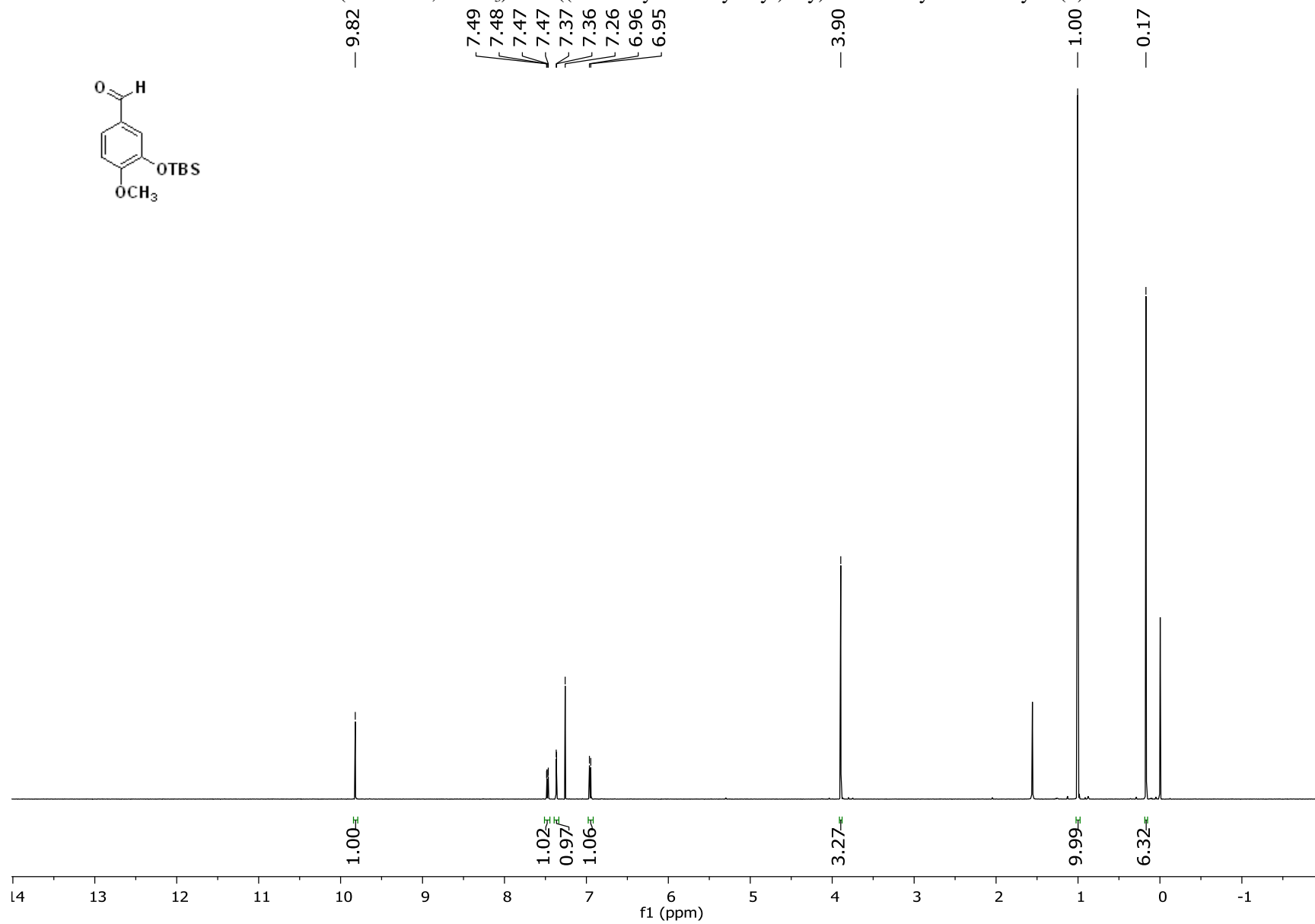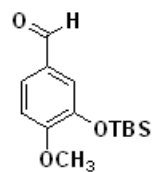
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^{1}H NMR (500 MHz, CDCl$_3$) of 3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (**2**)
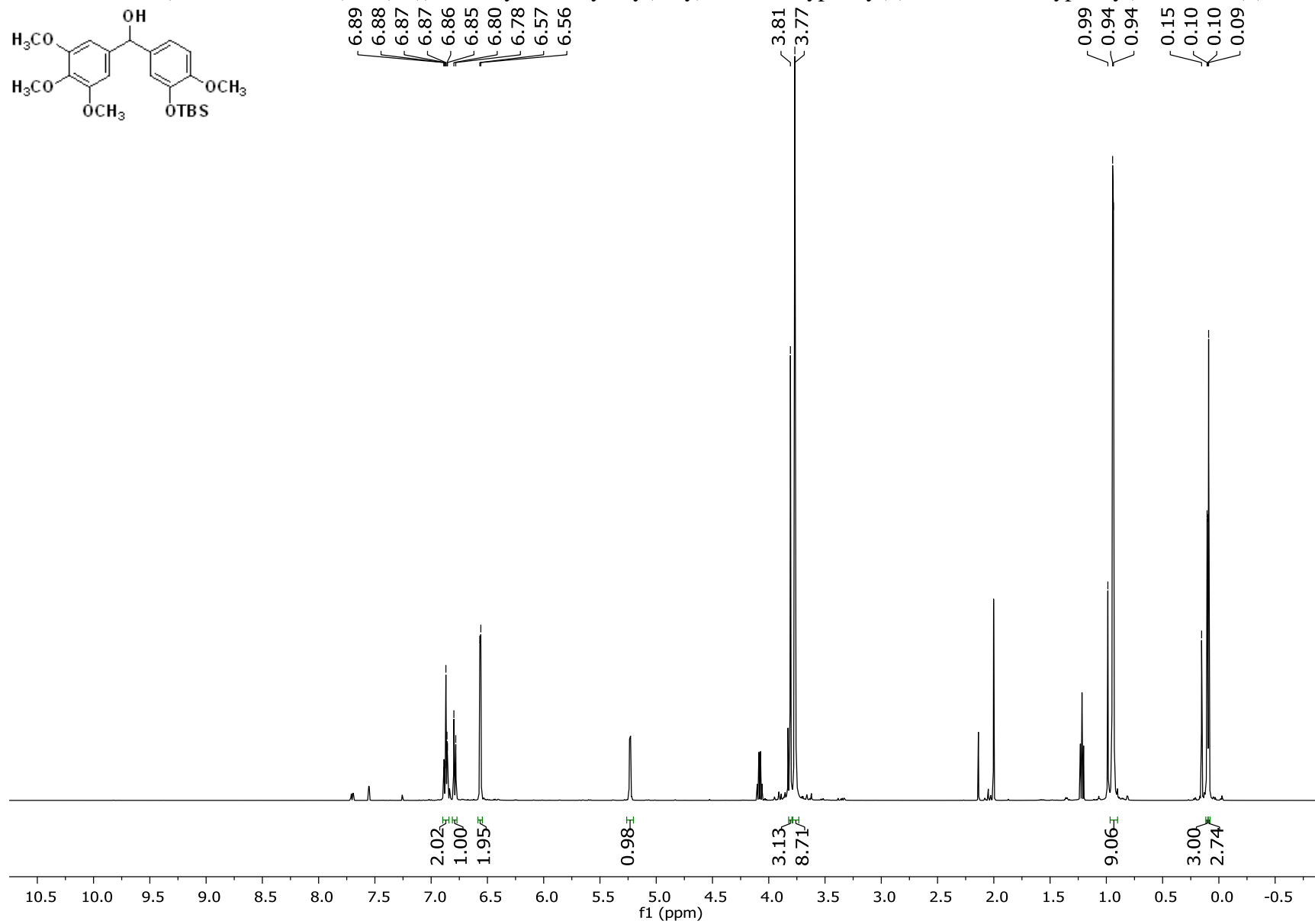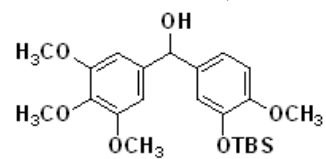
189

¹³C NMR (500 MHz, CDCl₃) of 3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (**2**)

190.9
156.6
145.5
130.2
126.3
120.0
111.1
77.3
77.0
76.8
55.6
25.6
18.4
-4.6

f1 (ppm)

190
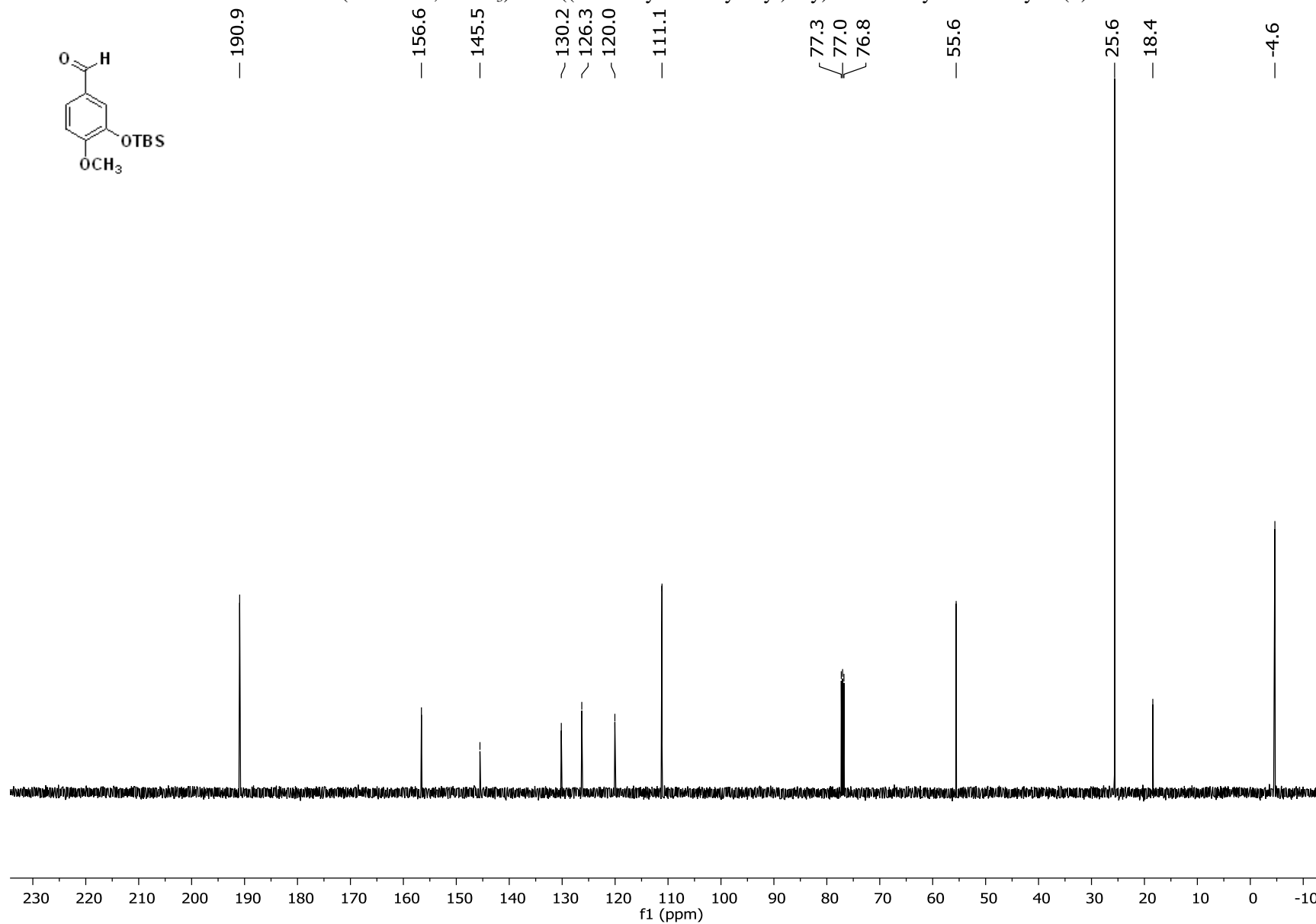
^{1}H NMR (500 MHz, CDCl$_3$) of (3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (**4**)

^{13}C NMR (500 MHz, CDCl$_3$) of (3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (**4**)

152.98
150.28
144.75
139.96
136.82
136.54
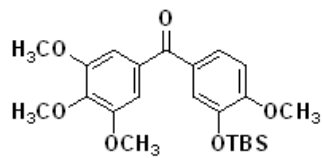119.87
119.40
111.82
103.35
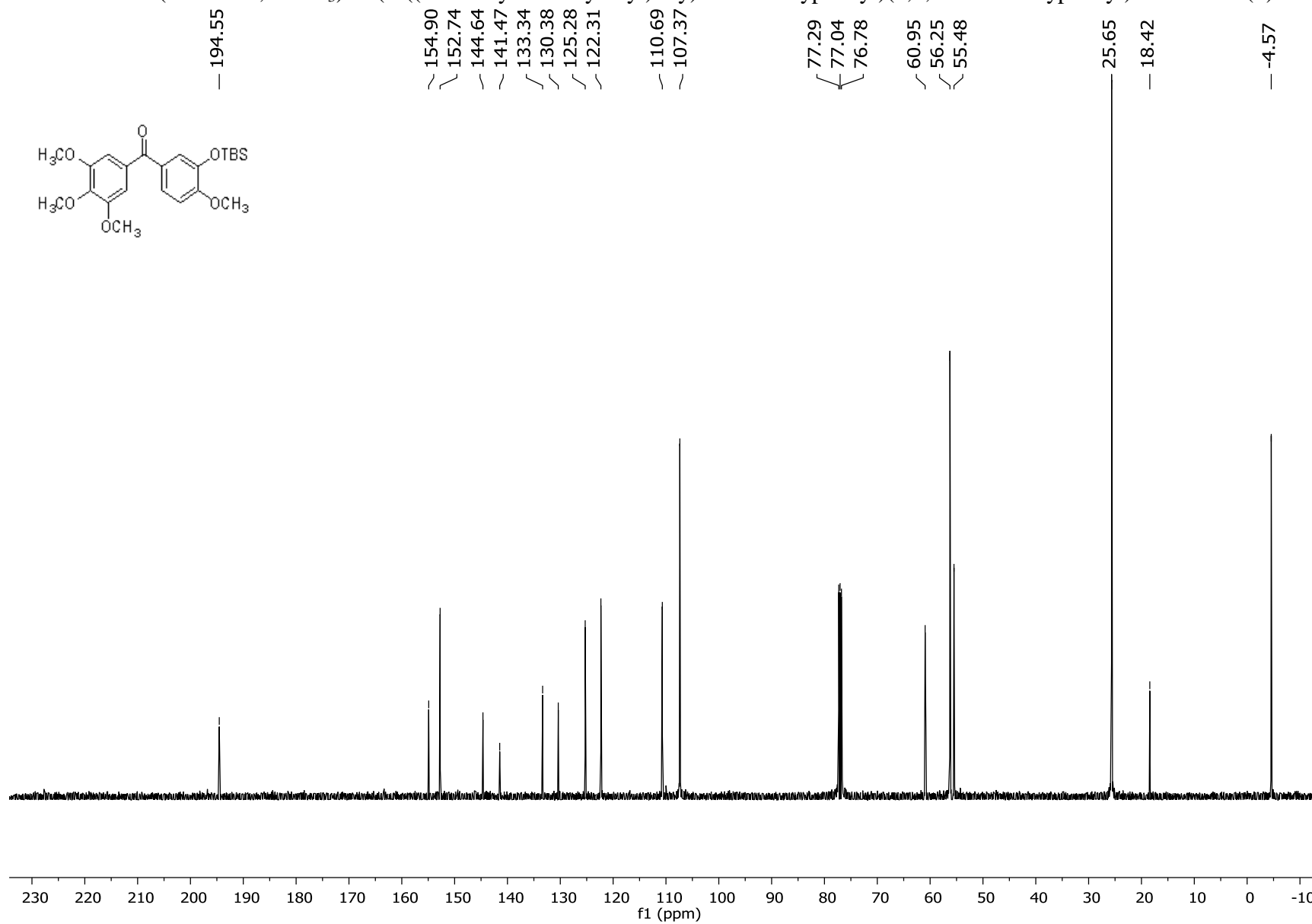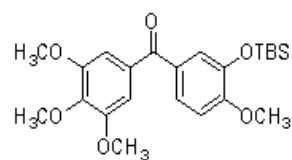75.52
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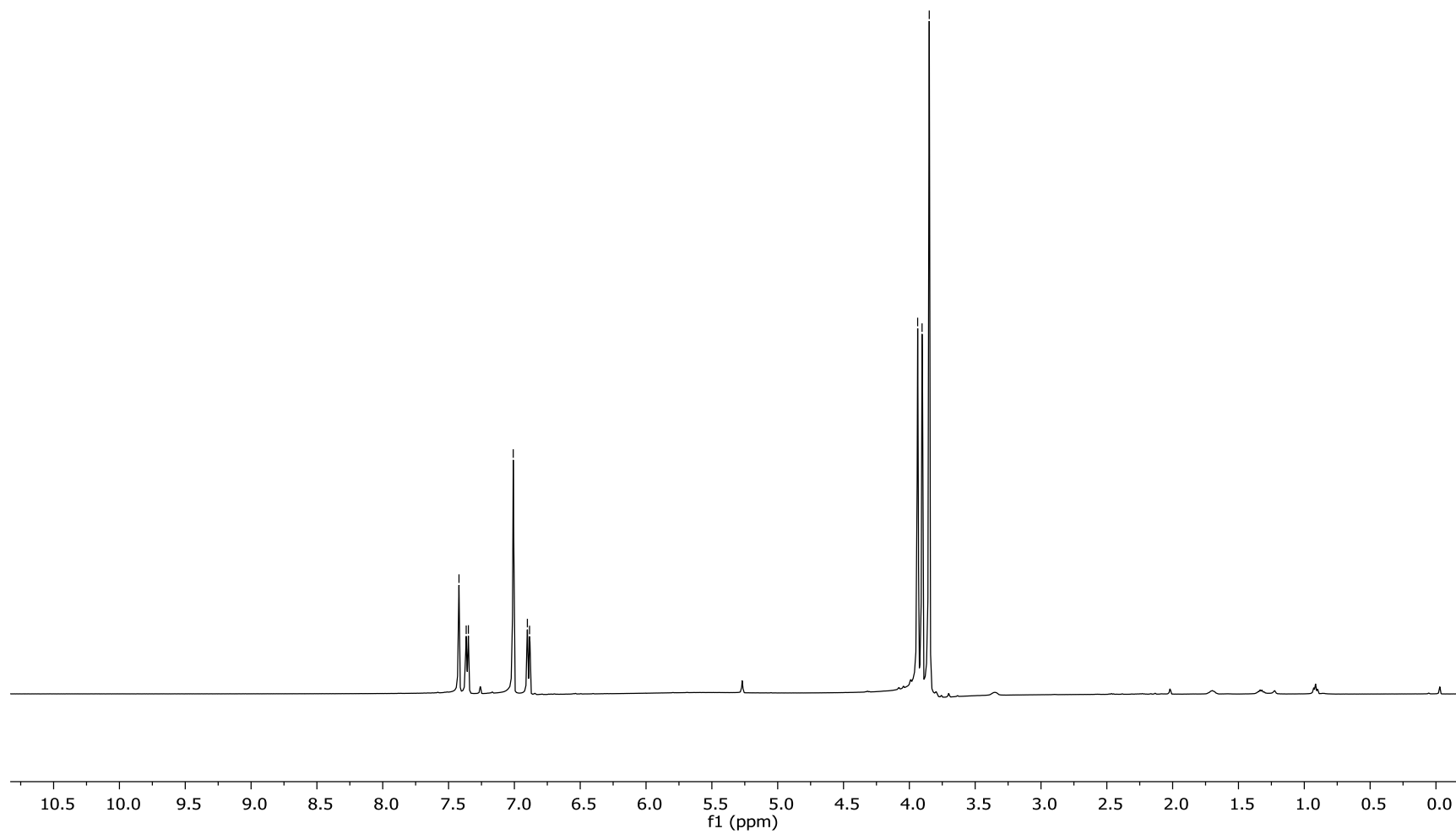55.93
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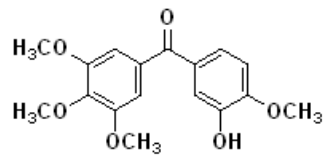18.42
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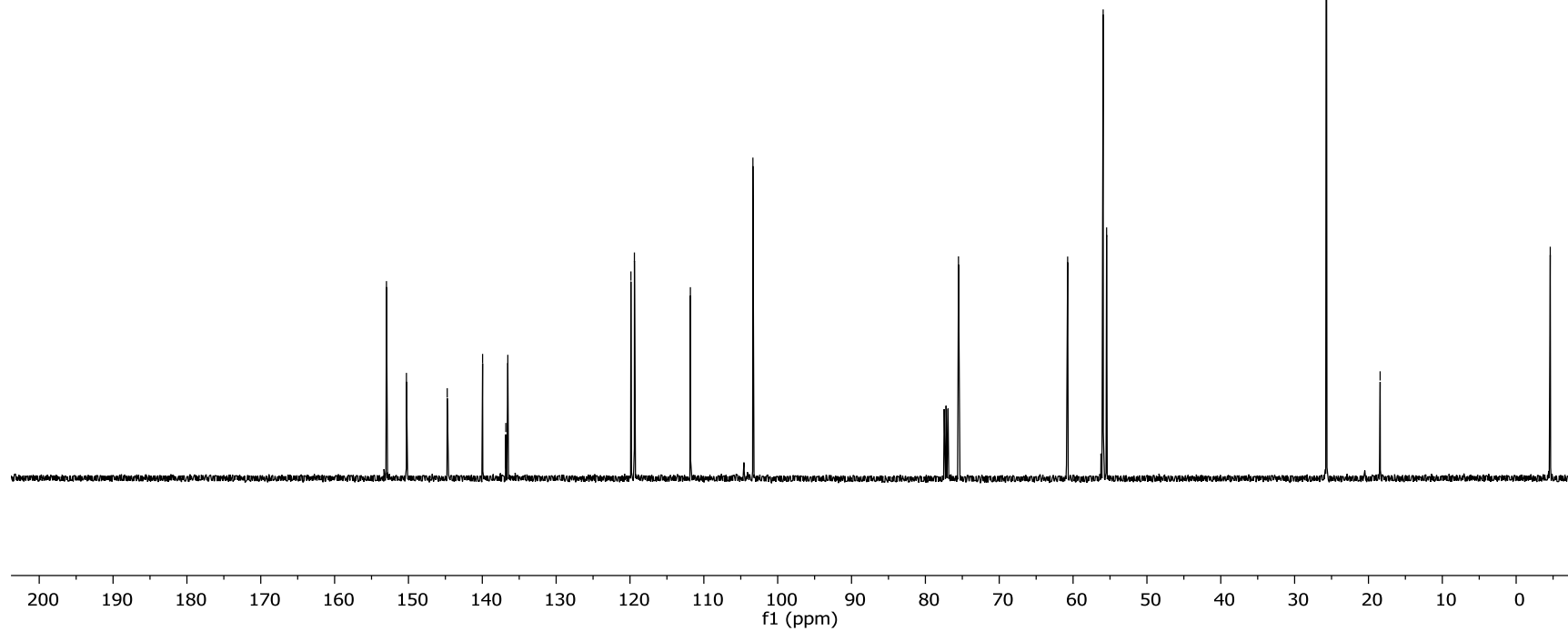
f1 (ppm)

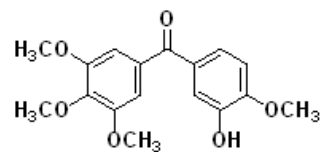¹H NMR (500 MHz, CDCl₃) of (3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (**5**)

^{13}C NMR (500 MHz, CDCl$_3$) of (3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (**5**)

194.55

154.90
152.74
144.64
141.47
133.34
130.38
125.28
122.31

110.69
107.37

77.29
77.04
76.78

60.95
56.25
55.48

25.65

18.42

-4.57

194

7.42
7.37
7.35
7.01
6.90
6.88
3.94
3.90
3.85

H$_3$CO

H$_3$CO

OCH$_3$

OCH$_3$

O

OCH$_3$

OH

f1 (ppm)
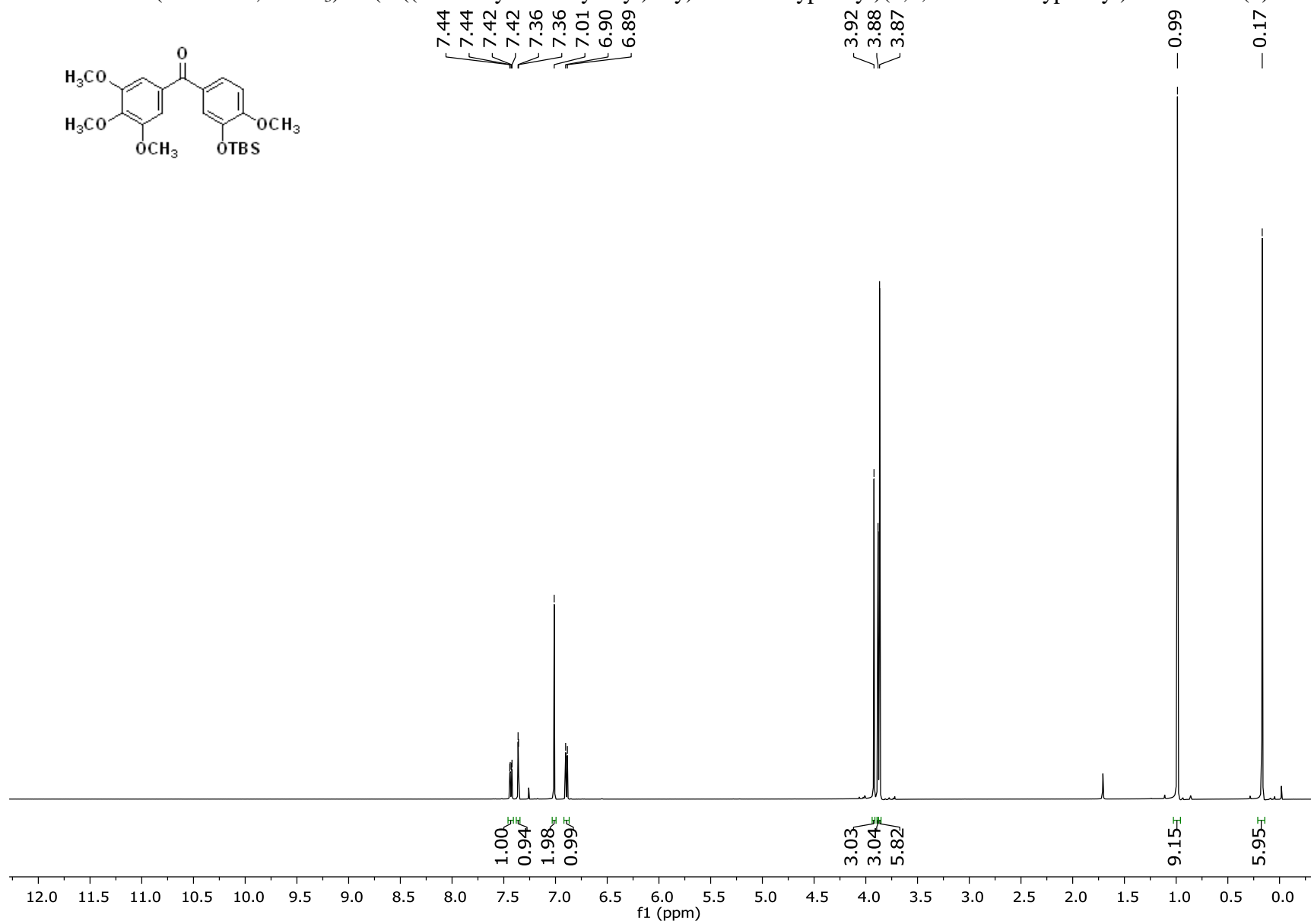
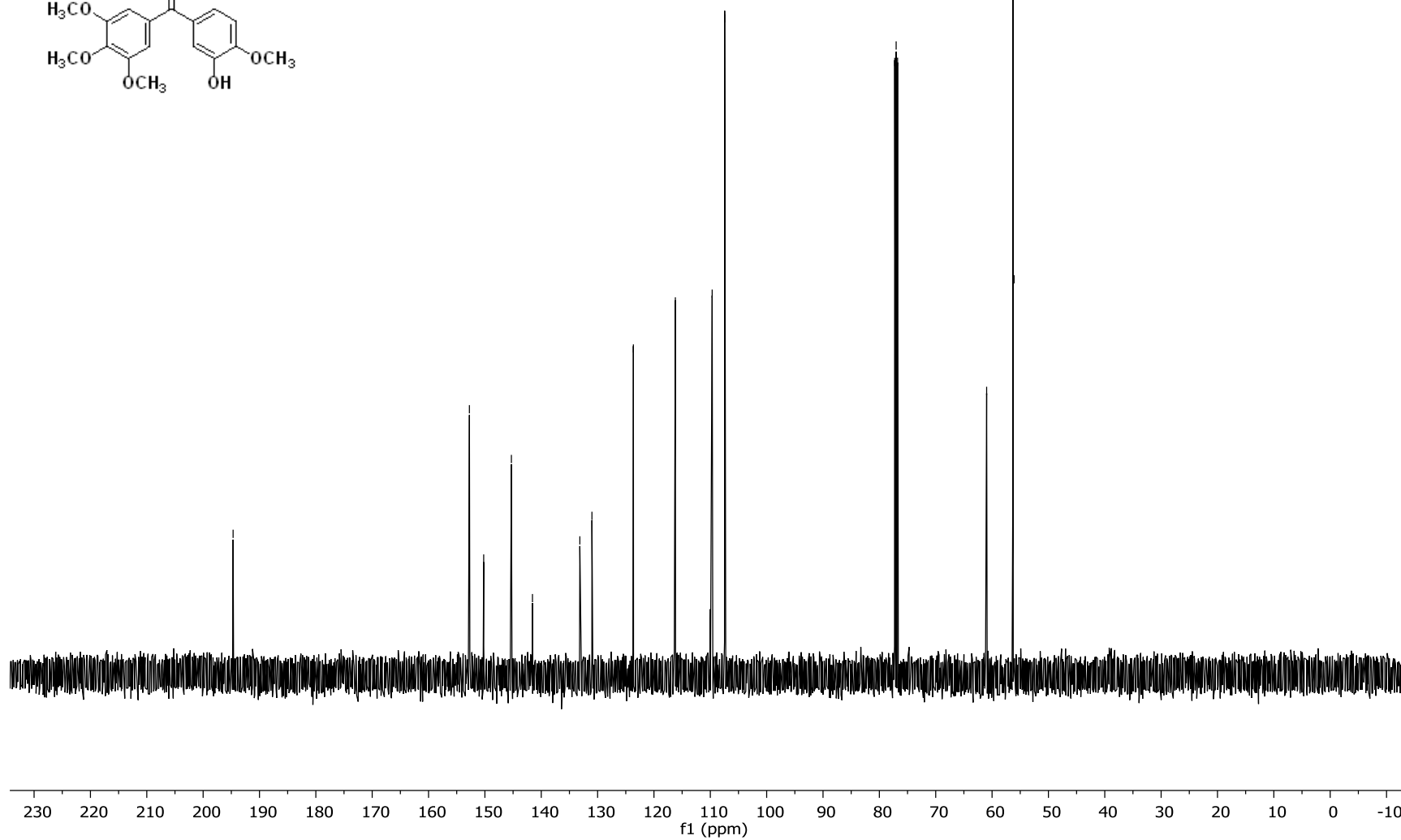^{13}C NMR (500 MHz, CDCl$_3$) of Phenstatin (**6**)

194.68

152.75
150.21
145.30
141.56
133.15
131.01
123.65
116.20
109.67
107.45

77.28
77.02
76.77

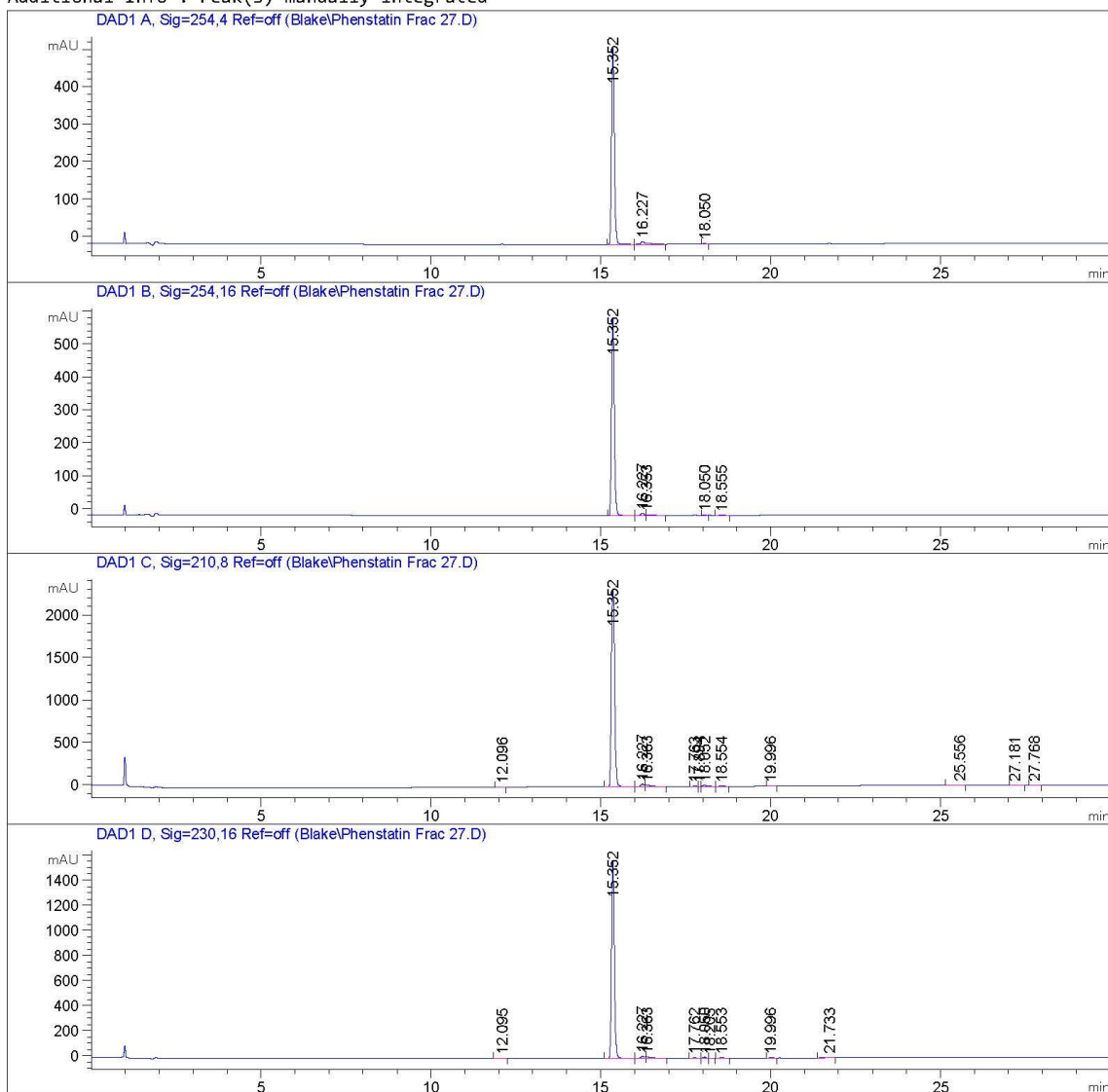60.96
56.28
56.09

f1 (ppm)

HPLC trace of Phenstatin (**6**)

197

DAD1 E, Sig=240,4 Ref=off (Blake\Phenstatin Frac 27.D)

DAD1 F, Sig=280,16 Ref=off (Blake\Phenstatin Frac 27.D)

DAD1 G, Sig=300,16 Ref=off (Blake\Phenstatin Frac 27.D)

DAD1 H, Sig=320,16 Ref=off (Blake\Phenstatin Frac 27.D)

```
==================================================================
                   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\Phenstatin Frac 27.D
Sample Name: Phenstatin Frac 27

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.352	BB	0.0883	2998.22803	527.79993	97.0908
2	16.227	BB	0.1495	80.98984	7.40825	2.6227
3	18.050	BB	0.0799	8.84660	1.72420	0.2865

Totals :   3088.06446   536.93238

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.352	BB	0.0882	3405.82935	599.99316	97.1155
2	16.227	BV	0.1020	51.16637	7.46052	1.4590
3	16.353	VB	0.1415	31.42821	2.96785	0.8962
4	18.050	BB	0.0799	9.80479	1.91018	0.2796
5	18.555	BB	0.1243	8.75974	1.10709	0.2498

Totals :   3506.98845   613.43880

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.096	BB	0.0949	9.64612	1.58789	0.0596
2	15.352	BB	0.1061	1.55548e4	2323.01147	96.1075
3	16.227	BV	0.0988	178.61842	27.14570	1.1036
4	16.363	VB	0.1623	172.26620	14.12024	1.0644
5	17.763	BV	0.0889	31.45477	5.32568	0.1943
6	17.894	VV	0.0683	6.48385	1.39280	0.0401
7	18.052	VB	0.1191	105.30116	12.65261	0.6506
8	18.554	BB	0.1161	46.81201	6.34585	0.2892
9	19.996	BV	0.1214	27.42125	3.21699	0.1694
10	25.556	BB	0.1151	21.76441	2.91681	0.1345
11	27.181	BB	0.2071	15.59067	1.17250	0.0963
12	27.768	BB	0.1430	14.63652	1.62679	0.0904

Totals :   1.61848e4   2400.51533

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

Data File C:\Chem32\1\Data\Blake\Phenstatin Frac 27.D
Sample Name: Phenstatin Frac 27

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.095	BB	0.0966	8.57315	1.37859	0.0918
2	15.352	BB	0.0890	9038.75781	1574.79211	96.7403
3	16.227	BV	0.0997	95.78050	14.37671	1.0251
4	16.363	VB	0.1708	92.87306	7.18653	0.9940
5	17.762	BV	0.0948	17.57367	2.73993	0.1881
6	18.050	VV	0.0871	33.97832	5.91144	0.3637
7	18.205	VB	0.0882	6.12776	1.04777	0.0656
8	18.553	BB	0.1149	24.83188	3.33253	0.2658
9	19.996	BV	0.1195	14.76467	1.76638	0.1580
10	21.733	BB	0.1416	10.06033	1.01494	0.1077

Totals :   9343.32115 1613.54694

Signal 5: DAD1 E, Sig=240,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.094	BB	0.1019	8.27689	1.23929	0.1207
2	15.352	BB	0.0883	6674.82422	1175.24585	97.3737
3	16.227	BV	0.0998	65.54812	9.82930	0.9562
4	16.359	VB	0.1528	50.34048	4.35634	0.7344
5	17.762	BB	0.0916	12.16266	1.98189	0.1774
6	18.050	BB	0.0800	18.94939	3.68454	0.2764
7	18.553	BB	0.1149	17.23790	2.31336	0.2515
8	19.996	BB	0.1039	7.51576	1.07042	0.1096

Totals :   6854.85542 1199.72099

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.094	BB	0.0992	6.63490	1.02998	0.1126
2	15.352	BB	0.0882	5749.51270	1013.11633	97.5483
3	16.227	BV	0.0990	46.42280	7.03225	0.7876
4	16.361	VB	0.1538	38.99524	3.39929	0.6616
5	17.762	BB	0.0927	9.66788	1.55119	0.1640
6	18.050	BB	0.0798	15.40553	3.00338	0.2614
7	18.553	BB	0.1146	14.79325	1.99385	0.2510
8	19.999	BV	0.1144	12.58604	1.58908	0.2135

Totals :   5894.01833 1032.71536

Data File C:\Chem32\1\Data\Blake\Phenstatin Frac 27.D
Sample Name: Phenstatin Frac 27

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.094	BB	0.0965	17.44289	2.80617	0.2470
2	15.352	BB	0.0883	6885.30078	1211.84802	97.4829
3	16.227	BV	0.0987	51.85132	7.88619	0.7341
4	16.362	VB	0.1552	46.17934	3.98537	0.6538
5	17.762	BB	0.0923	11.81466	1.90724	0.1673
6	18.050	BB	0.0818	19.25880	3.75469	0.2727
7	18.552	BB	0.1134	18.33415	2.50517	0.2596
8	19.996	BB	0.1034	12.90264	1.84833	0.1827

Totals :   7063.08459 1236.54119

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.094	BB	0.0961	29.14829	4.71398	0.4211
2	15.352	BB	0.0883	6756.80127	1189.29700	97.6206
3	16.227	BV	0.0983	42.30829	6.47003	0.6113
4	16.362	VB	0.1544	37.17588	3.22716	0.5371
5	17.762	BB	0.0935	11.34051	1.85076	0.1638
6	18.050	BB	0.0819	15.76419	3.07128	0.2278
7	18.552	BB	0.1103	15.57444	2.15592	0.2250
8	19.994	BB	0.1216	13.38015	1.56657	0.1933

Totals :   6921.49302 1212.35270

===
                    *** End of Report ***

Mass Spectrum of Phenstatin **6**

phestatin_160801120253 #1-20 RT: 0.01-0.22 A
T: FTMS + p ESI Full ms [150.00-700.00]

NL:
1.88E6
phestatin_160801120253
#1-20 RT: 0.01-0.22
AV: 20 T: FTMS + p ESI
Full ms [150.00-700.00]

NL:
8.19E5

$C_{17}H_{18}NaO_6$:
$C_{17}H_{18}Na_1O_6$
pa Chrg 1

^{1}H NMR (500 MHz, CDCl$_3$) of (5-nitrothiophen-2-yl)methanol (**8**)

203

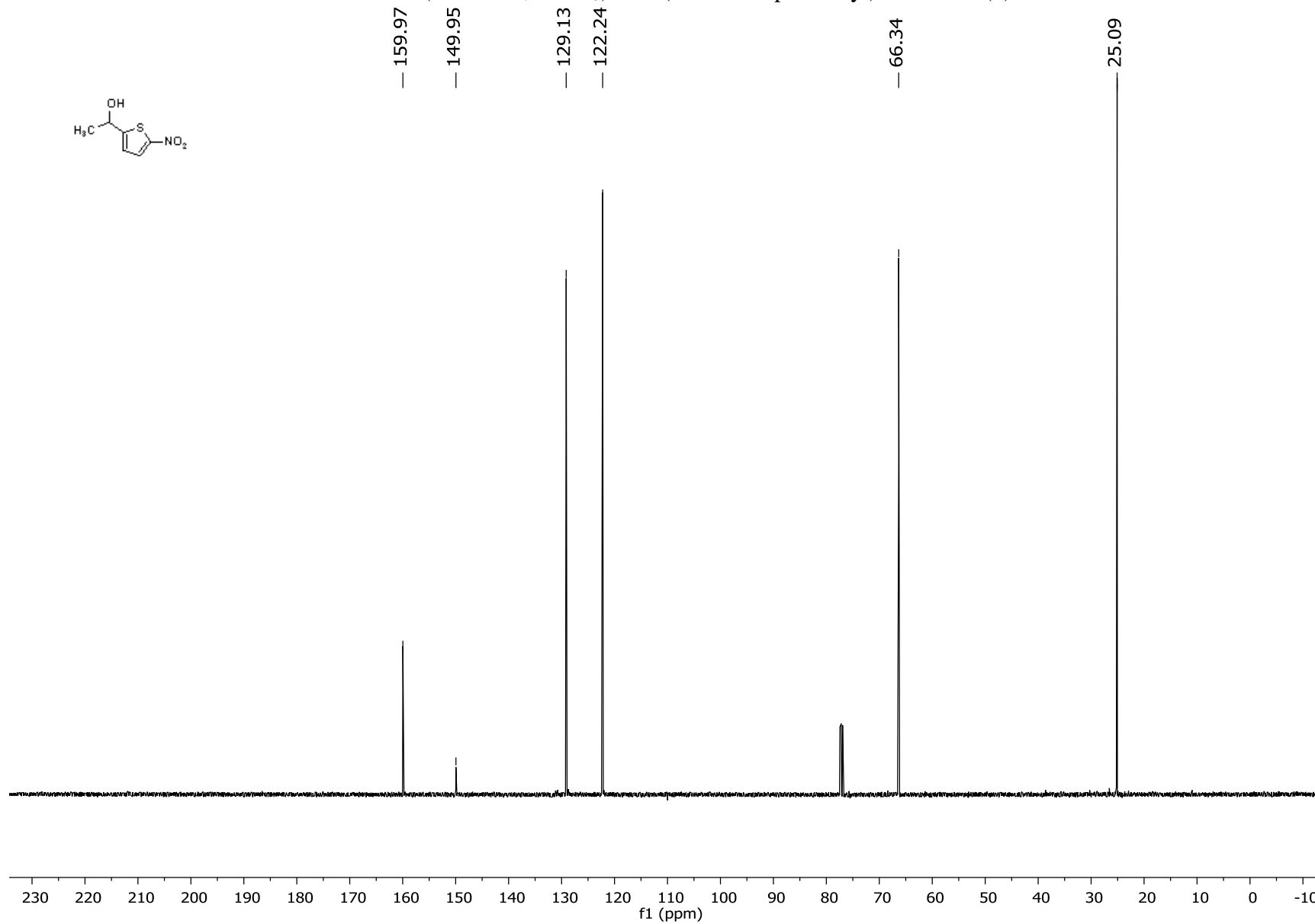^{13}C NMR (500 MHz, CDCl$_3$) of (5-nitrothiophen-2-yl)methanol (**8**)

¹H NMR (500 MHz, CDCl₃) of 1-(5-nitrothiophen-2-yl)ethan-1-ol (**9**)

7.82
7.82
7.28
6.91
6.91
6.91
6.91
5.16
5.16
5.15
5.15
5.14
5.14
5.13
5.13
1.64
1.63

1.00
1.01
1.02
1.37
3.25

11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
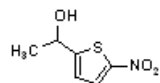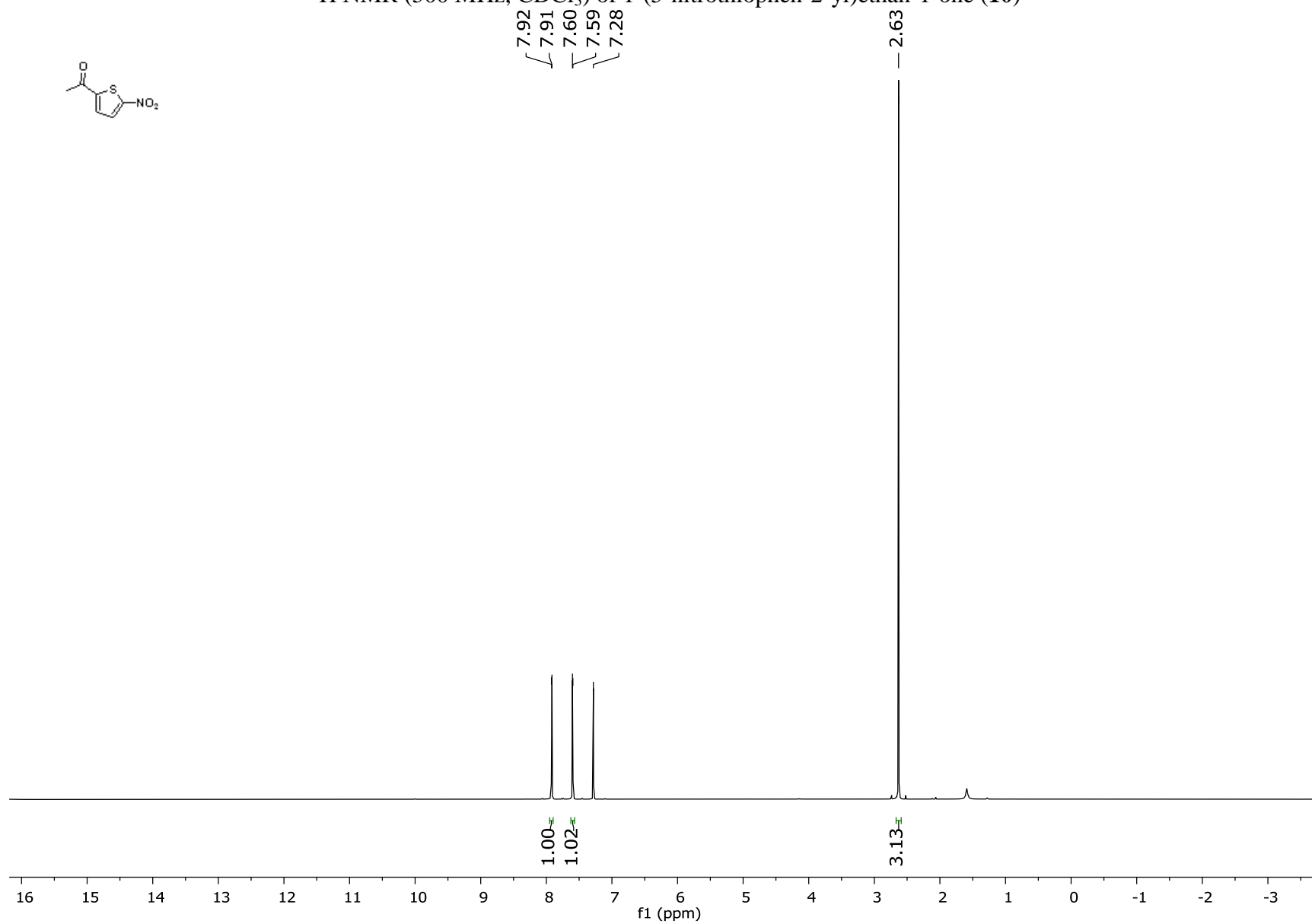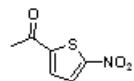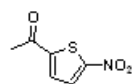f1 (ppm)

^{13}C NMR (500 MHz, CDCl$_3$) of 1-(5-nitrothiophen-2-yl)ethan-1-ol (**9**)

206

^{1}H NMR (500 MHz, CDCl$_3$) of 1-(5-nitrothiophen-2-yl)ethan-1-one (**10**)

7.92
7.91
7.60
7.59
7.28

2.63

1.00
1.02

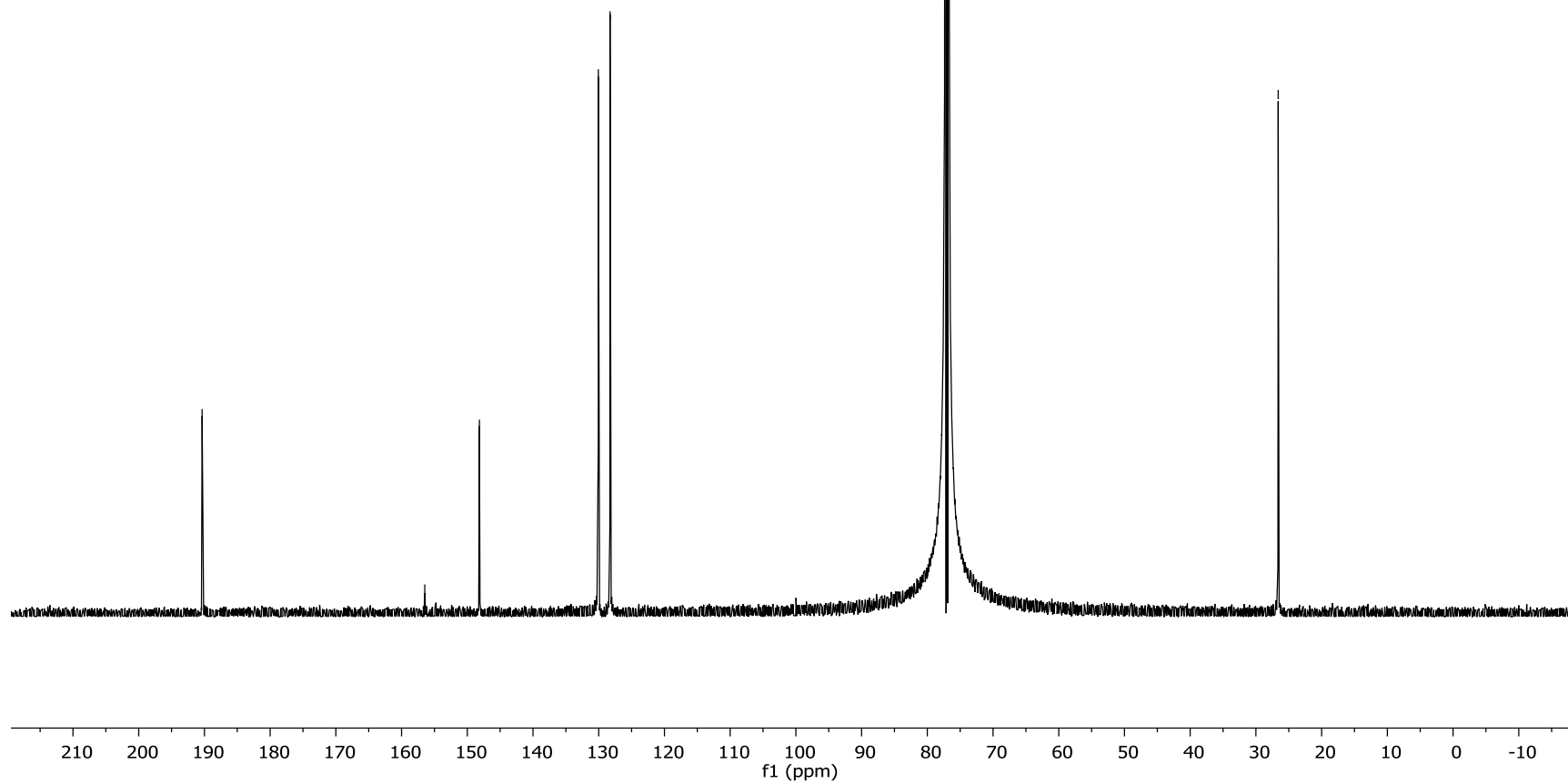3.13

f1 (ppm)
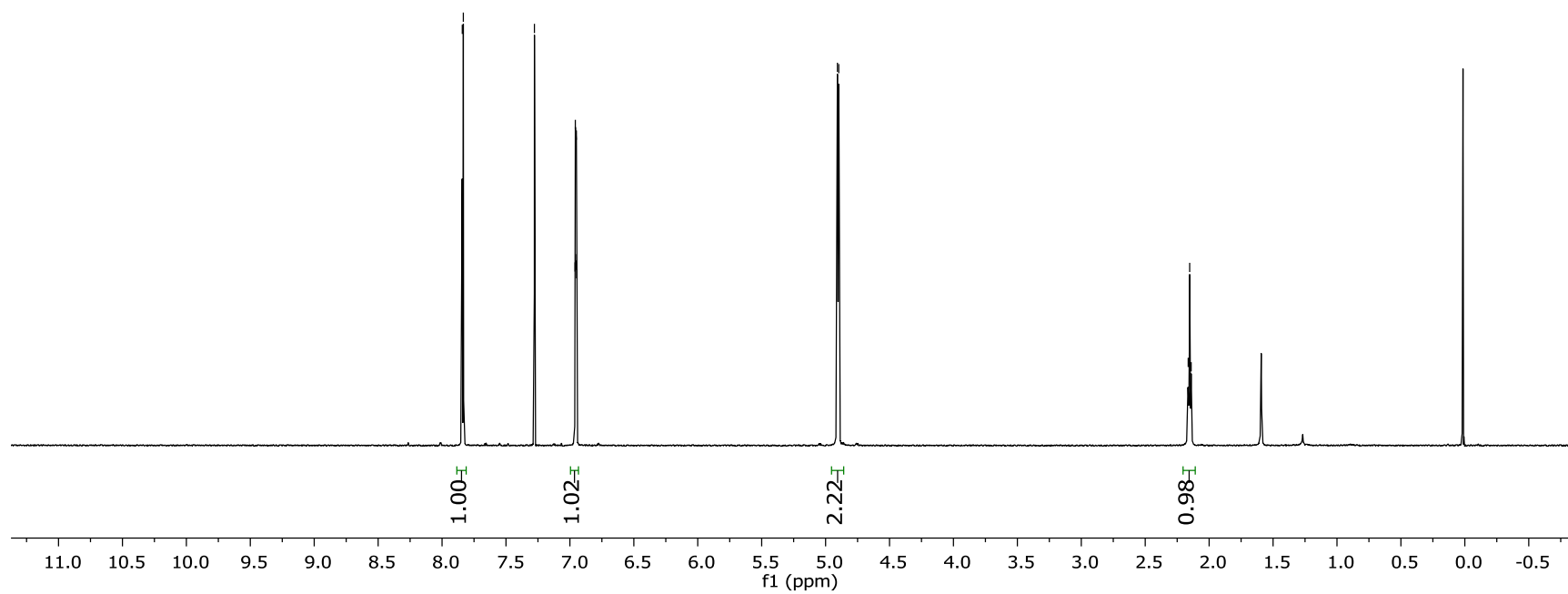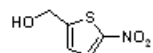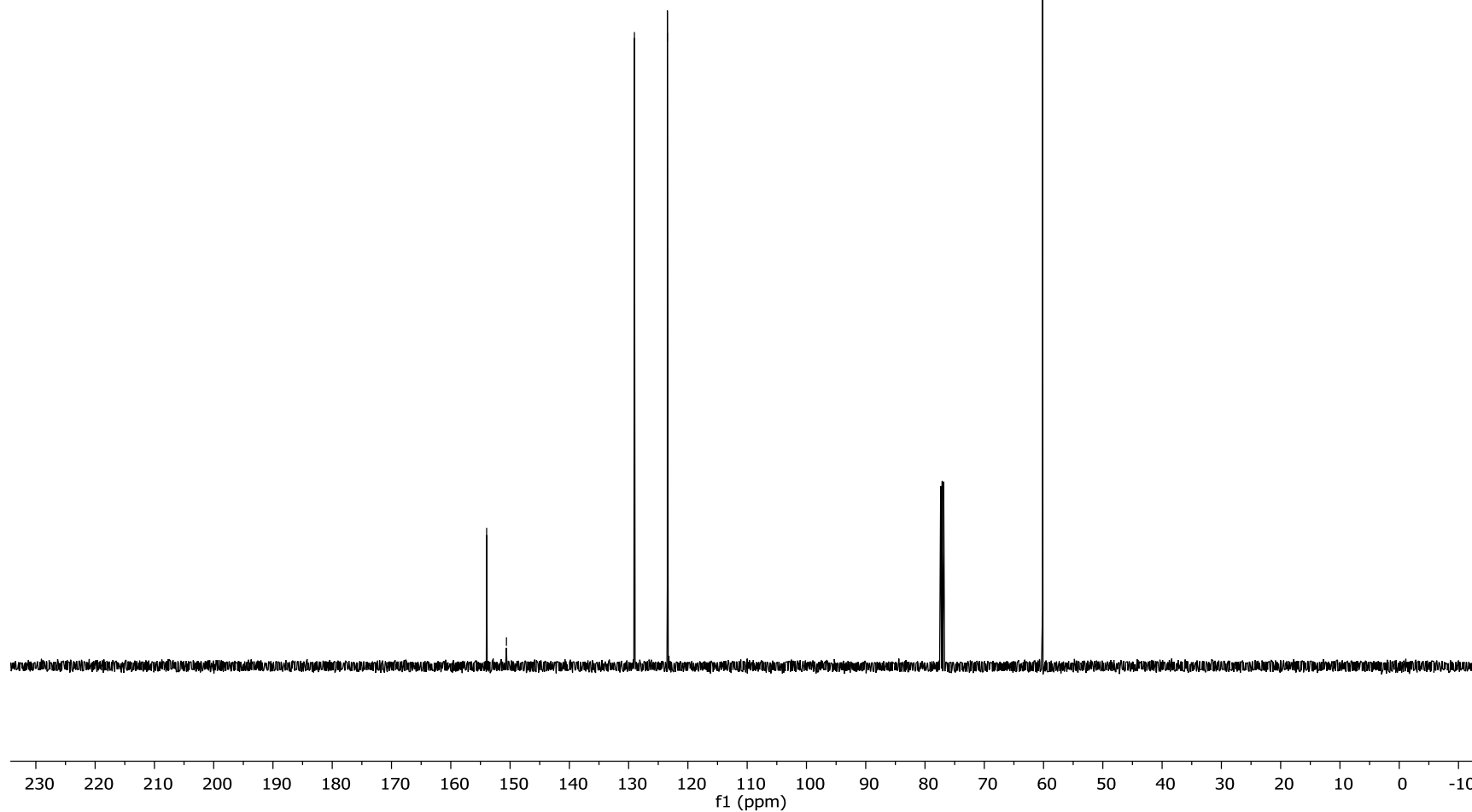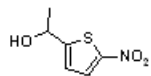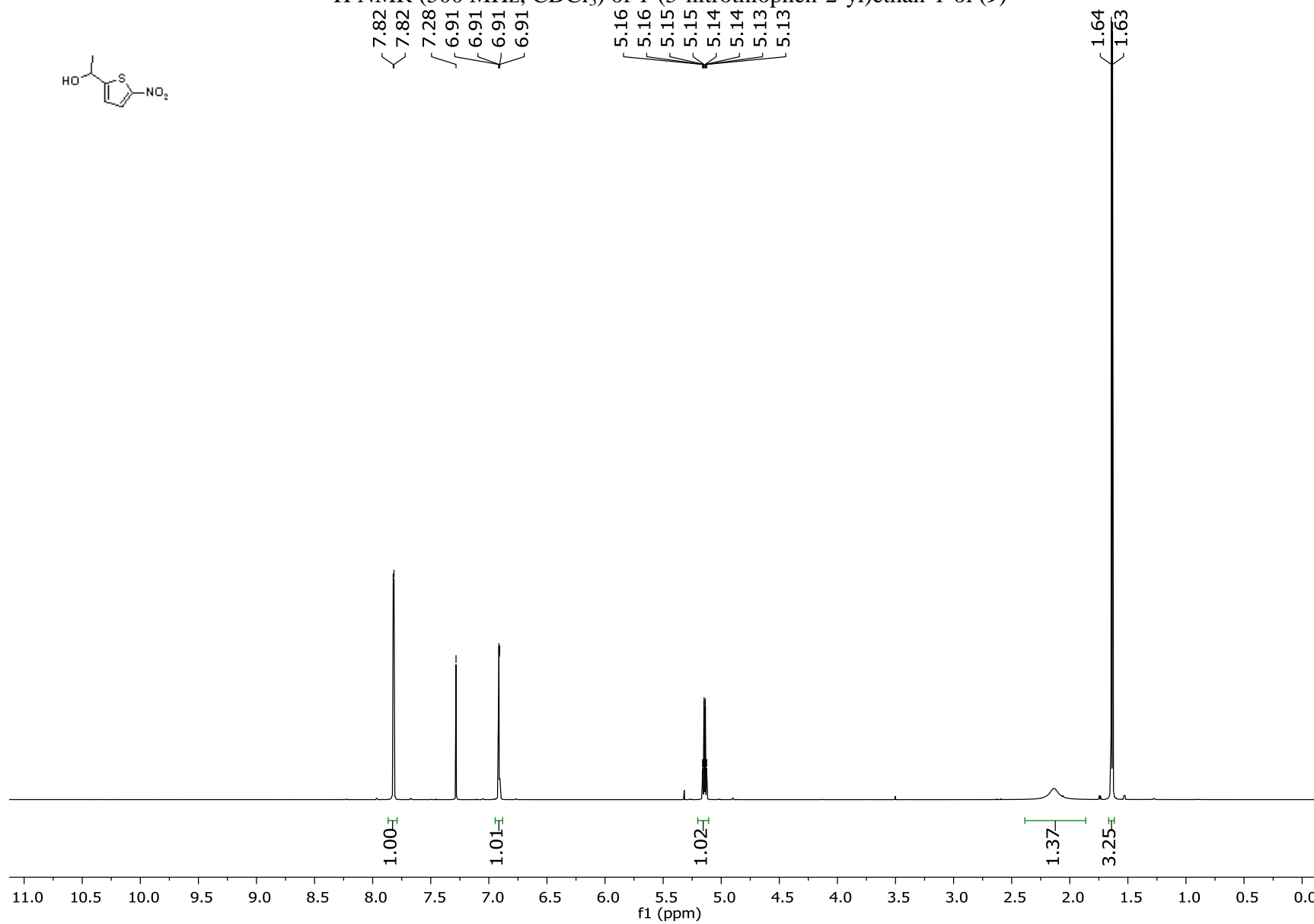
^{13}C NMR (500 MHz, CDCl$_3$) of 1-(5-nitrothiophen-2-yl)ethan-1-one (**10**)

190.35
156.47
148.16
130.06
128.28
26.61

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

208
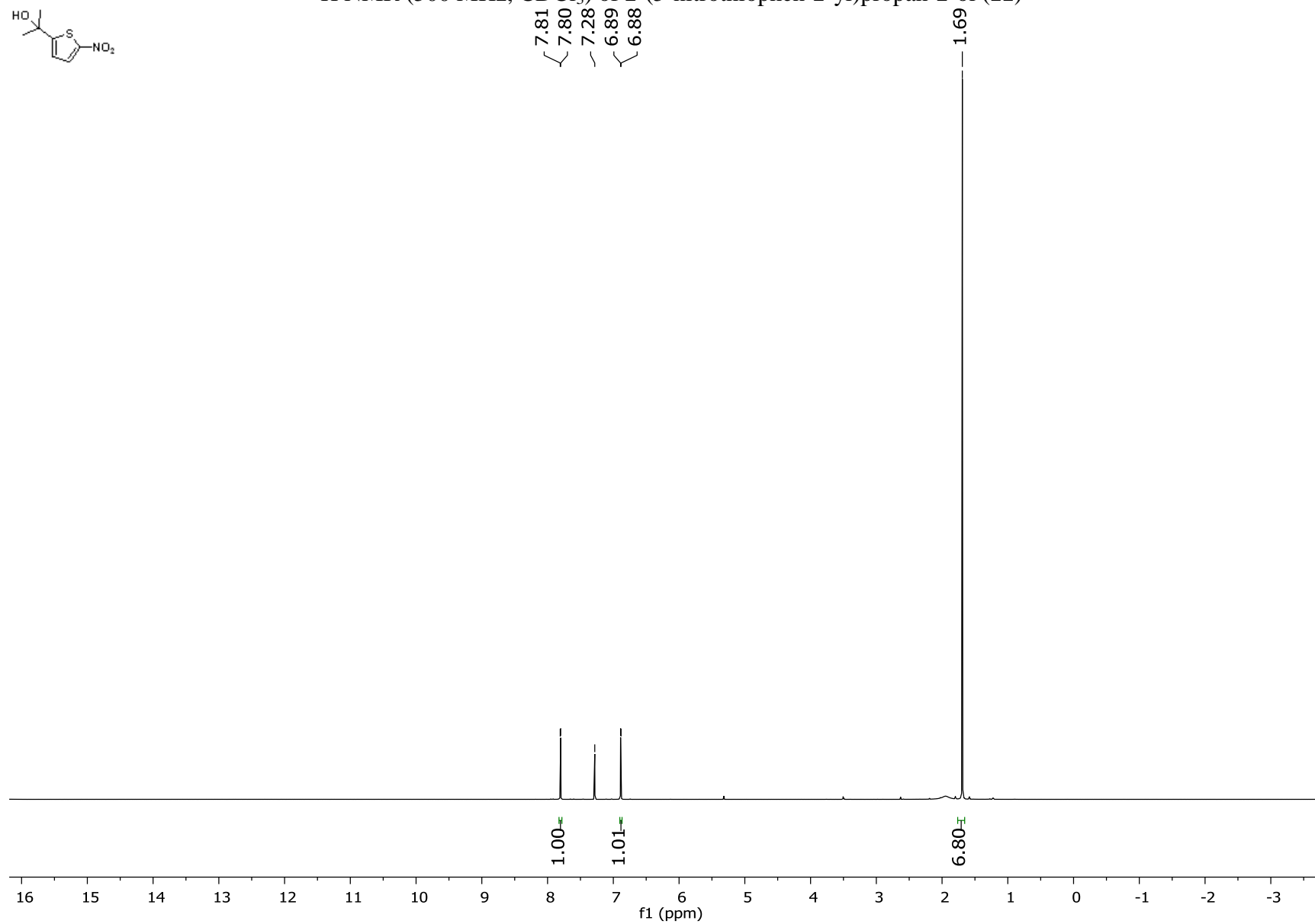
^{1}H NMR (500 MHz, CDCl$_3$) of 2-(5-nitrothiophen-2-yl)propan-2-ol (**11**)

7.81
7.80
7.28
6.89
6.88

1.69

1.00
1.01
6.80

f1 (ppm)

209
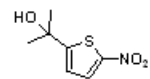
^{13}C NMR (500 MHz, CDCl$_3$) of 2-(5-nitrothiophen-2-yl)propan-2-ol (**11**)

— 163.46

— 150.04

— 128.76
— 121.26
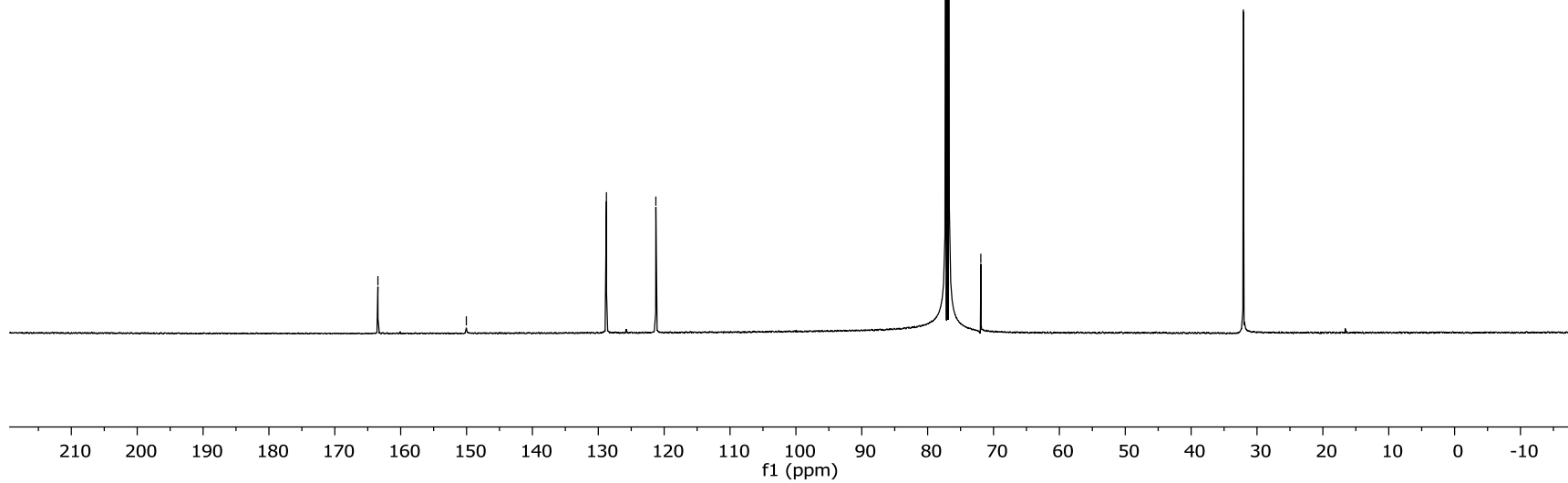
— 71.92

— 32.08

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

210

^{1}H NMR (500 MHz, CDCl$_3$) of (4-Methoxy-3-((5-nitrothiophen-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**12**)

7.83
7.82
7.51
7.51
7.49
7.49
7.48
7.47
7.06
7.06
6.99
6.97
6.95

5.33

3.98
3.93
3.87

1.00
1.10
1.11
1.03
2.20
1.14

2.22

3.42
3.34
6.84

f1 (ppm)

^{13}C NMR (500 MHz, CDCl$_3$) of (4-Methoxy-3-((5-nitrothiophen-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**12**)

194.25

153.68
152.83
147.70
146.90
141.72
132.98
130.21
128.38
126.60
125.22
115.55
110.61
107.42

66.31
60.99
56.34
56.18

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

f1 (ppm)
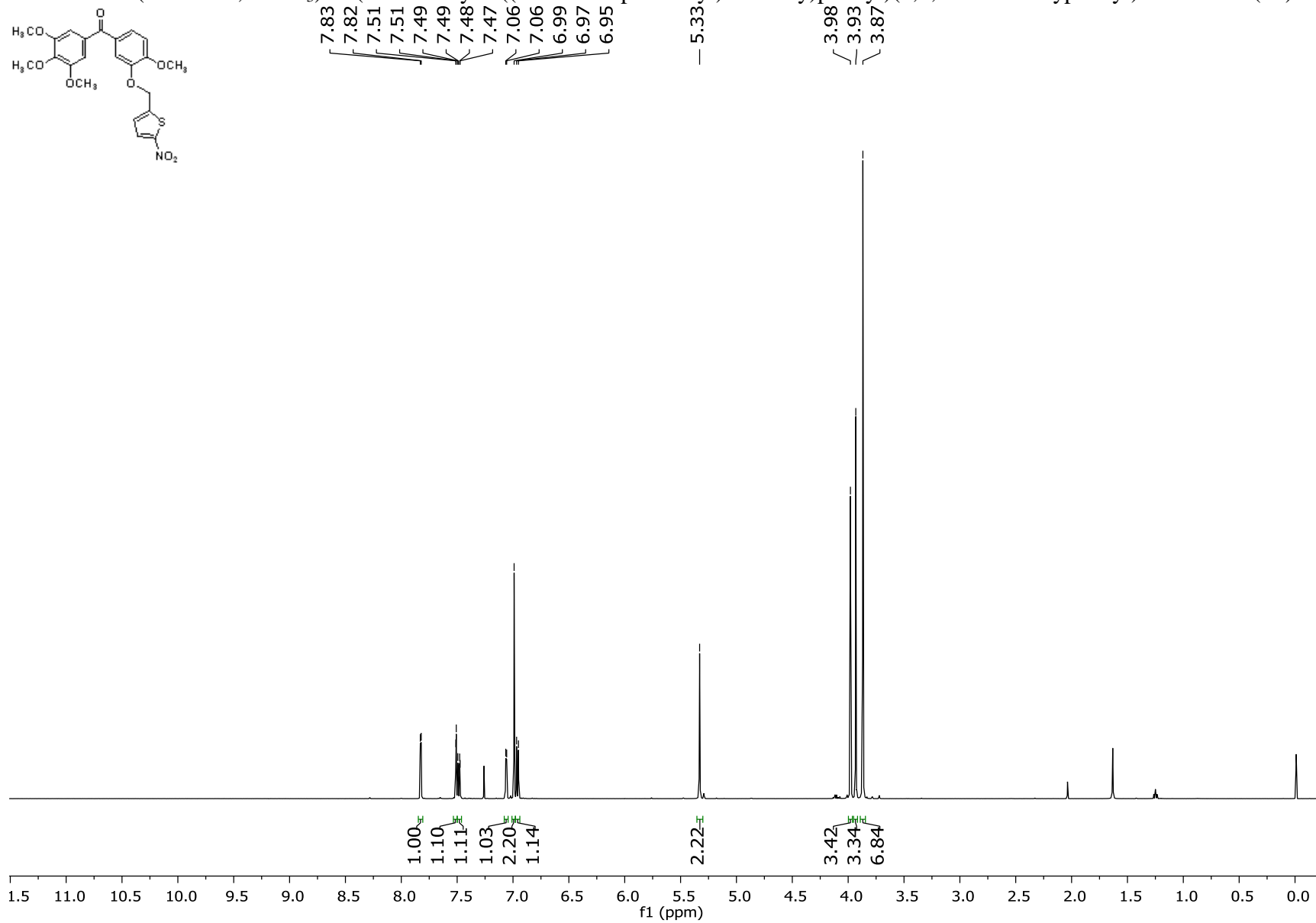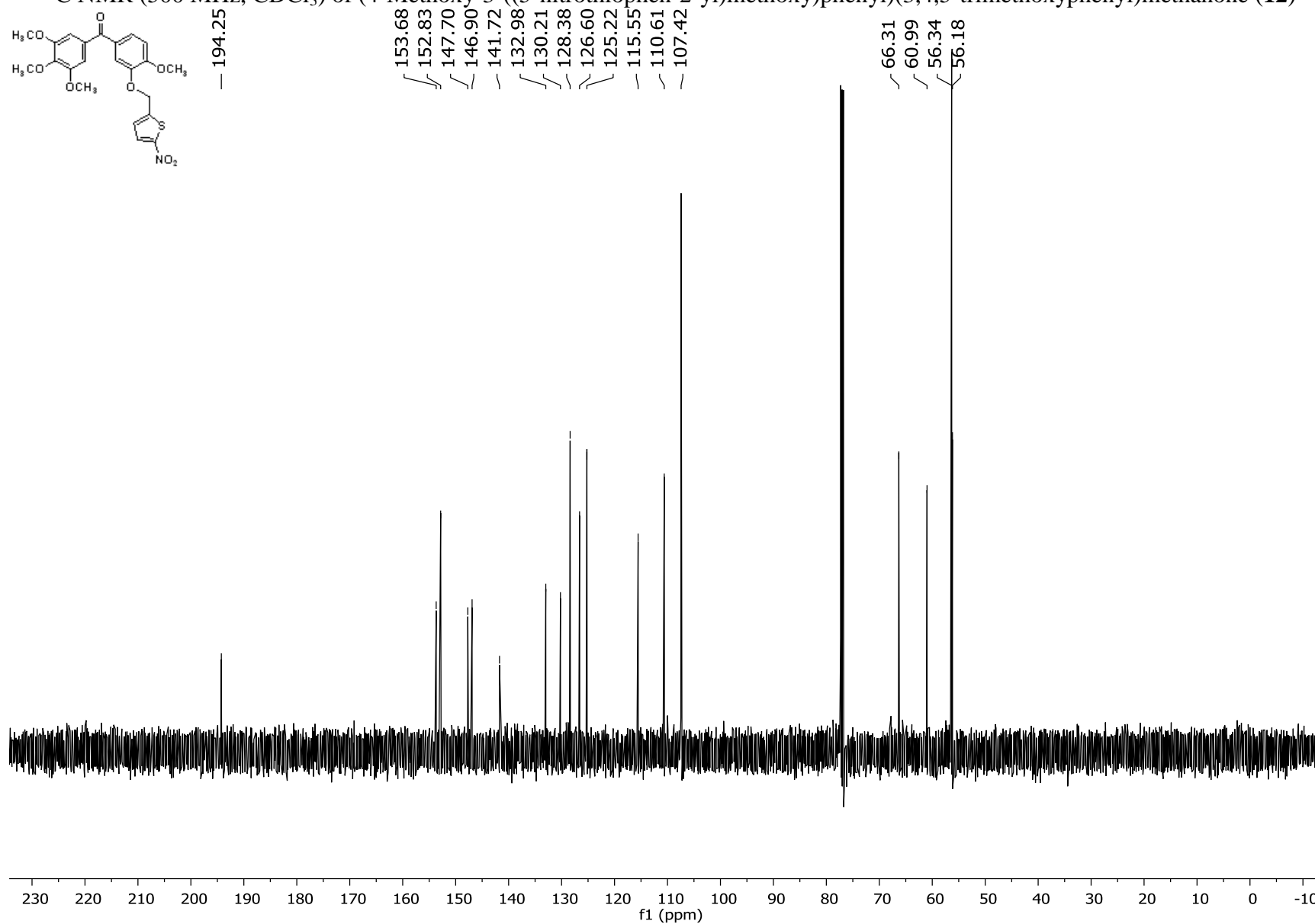
HPLC trace of compound **12**

Data File C:\Chem32\1\Data\Blake\Phenstatin TrueNormethyl Thio 2016-06-26 17-27-20.D
Sample Name: Phenstatin TrueNormethyl Thio

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Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC                 Location :   1
Injection Date  : 6/26/2016 5:27:20 PM
                                            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
Additional Info : Peak(s) manually integrated
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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 Type  Width     Area       Height     Area
 #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
  1   3.026 BB   0.0949   21.20730    3.39320   2.2973
  2   8.459 BB   0.1262   24.35077    2.95249   2.6378
  3   9.096 BB   0.1304  877.57184  104.09017  95.0648

Totals :                 923.12991  110.43585



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

Peak RetTime Type  Width     Area       Height     Area
 #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
  1   1.128 BV   0.0548   17.71029    4.83216   0.3212
  2   1.195 VV   0.0619   28.47927    6.65998   0.5165
  3   1.396 VB   0.1287   26.71319    2.67025   0.4845
  4   2.239 BB   0.0673    7.55711    1.71990   0.1371
  5   2.575 BB   0.0661    5.67737    1.32215   0.1030
  6   3.026 BV   0.0961  142.63956   22.44806   2.5870
  7   4.425 BB   0.0954   14.30667    2.33626   0.2595
  8   5.082 BB   0.1200   14.31204    1.73992   0.2596
  9   8.459 BB   0.1250  115.74354   14.21608   2.0992
 10   9.096 BB   0.1305 5048.74268  598.24670  91.5667
 11  10.801 BB   0.1971   18.62814    1.33238   0.3378
 12  11.307 BV   0.1543   14.42214    1.47258   0.2616
 13  11.599 VB   0.1482   12.39582    1.26680   0.2248
 14  20.083 BB   0.1788   11.77514    1.03427   0.2136
 15  24.964 BB   0.1962   34.62818    2.68930   0.6280
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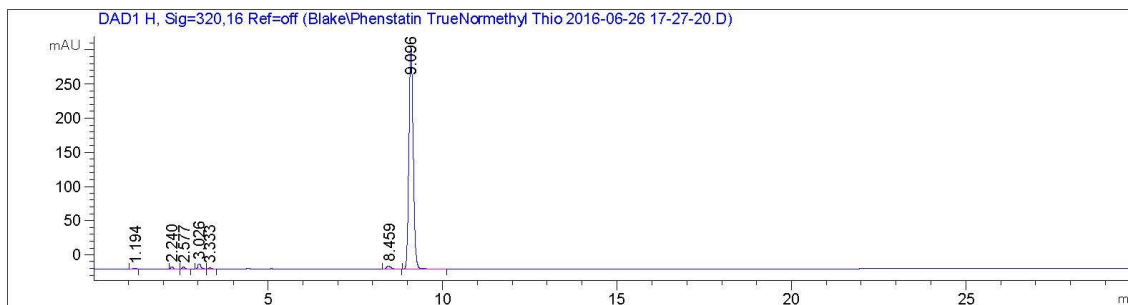
214

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     Totals :                5513.73112   663.98679
```

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.196	BB	0.0649	9.62400	2.12148	0.4471
2	2.239	BB	0.0674	7.57322	1.71800	0.3518
3	2.577	BB	0.0717	6.89039	1.44361	0.3201
4	3.026	BV	0.0956	41.57518	6.59008	1.9315
5	3.333	VB	0.0797	5.72017	1.08077	0.2658
6	8.459	BV	0.1276	46.38280	5.54183	2.1549
7	9.096	VB	0.1303	2034.68677	241.40295	94.5288

```
     Totals :                2152.45254   259.89872
```

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.195	BB	0.0675	8.49393	1.85242	0.3024
2	2.240	BB	0.0682	11.51104	2.57334	0.4098
3	2.577	BB	0.0716	10.29631	2.16045	0.3666
4	3.026	BV	0.0958	49.71453	7.86376	1.7699
5	3.334	VB	0.0793	10.78817	2.05226	0.3841
6	8.459	BV	0.1275	46.46970	5.55905	1.6544
7	9.096	VB	0.1305	2671.54370	316.35538	95.1128

```
     Totals :                2808.81739   338.41667
```

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.194	BB	0.0700	6.73554	1.40302	0.2340
2	2.240	BB	0.0686	17.09729	3.79214	0.5941
3	2.577	BB	0.0719	13.32974	2.78342	0.4632
4	3.026	BV	0.0960	48.87596	7.70904	1.6983
5	3.333	VB	0.0798	11.42673	2.15557	0.3970
6	8.459	BB	0.1272	37.23368	4.46768	1.2937
7	9.096	BB	0.1305	2743.29541	324.87579	95.3197

```
     Totals :                2877.99436   347.18666
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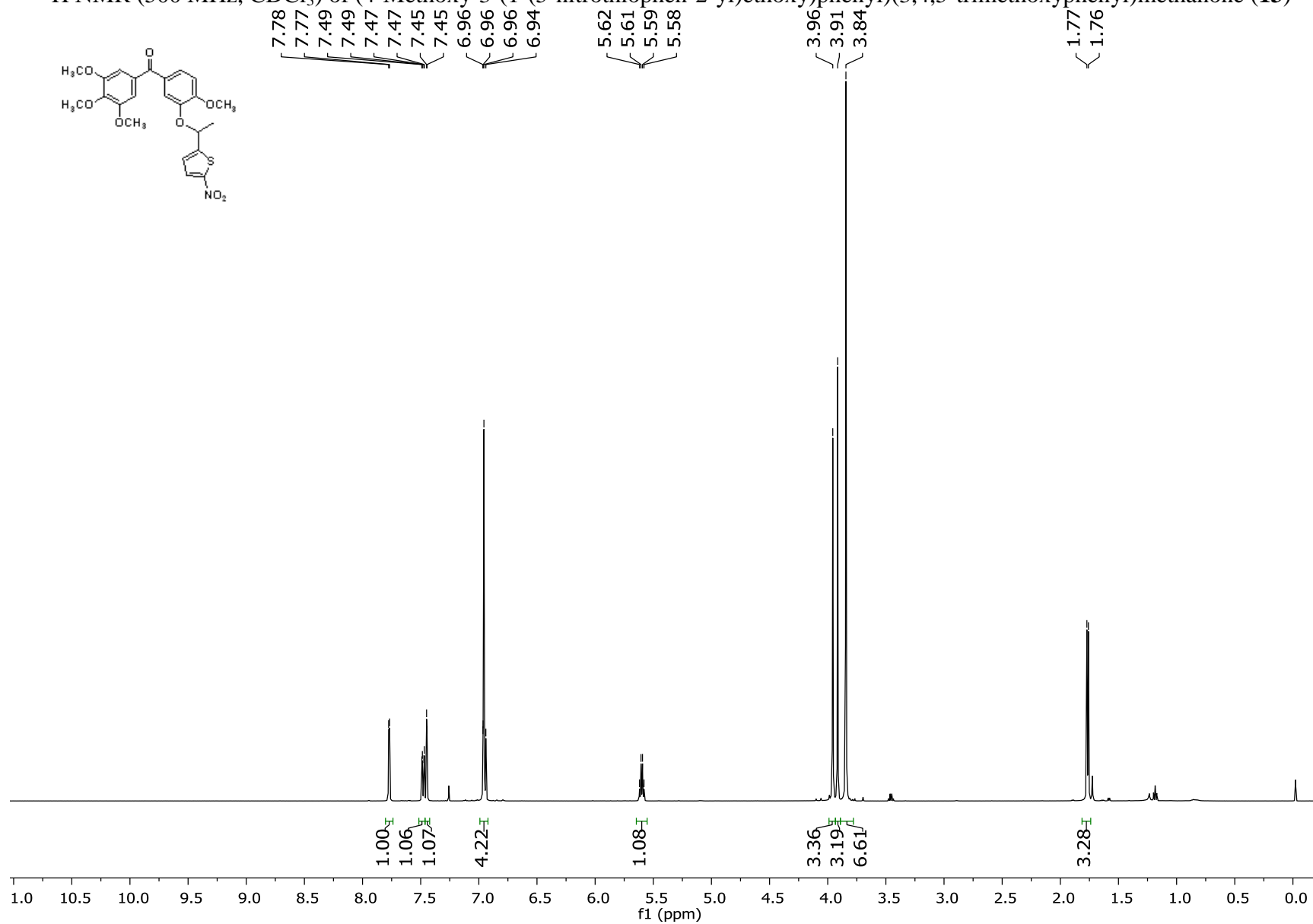
Mass Spectrum of Compound **12**

nor thio_Orbi_+ESI #2-8   RT: 0.02-0.09   AV: 7
T: FTMS + p ESI Full ms [120.00-1000.00]
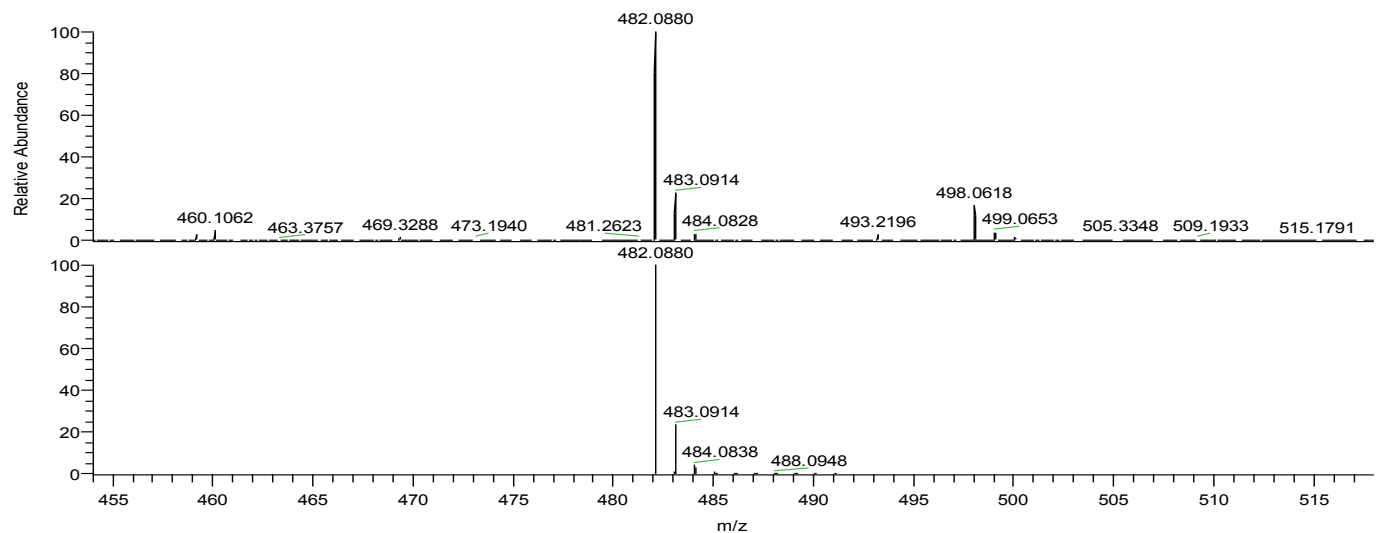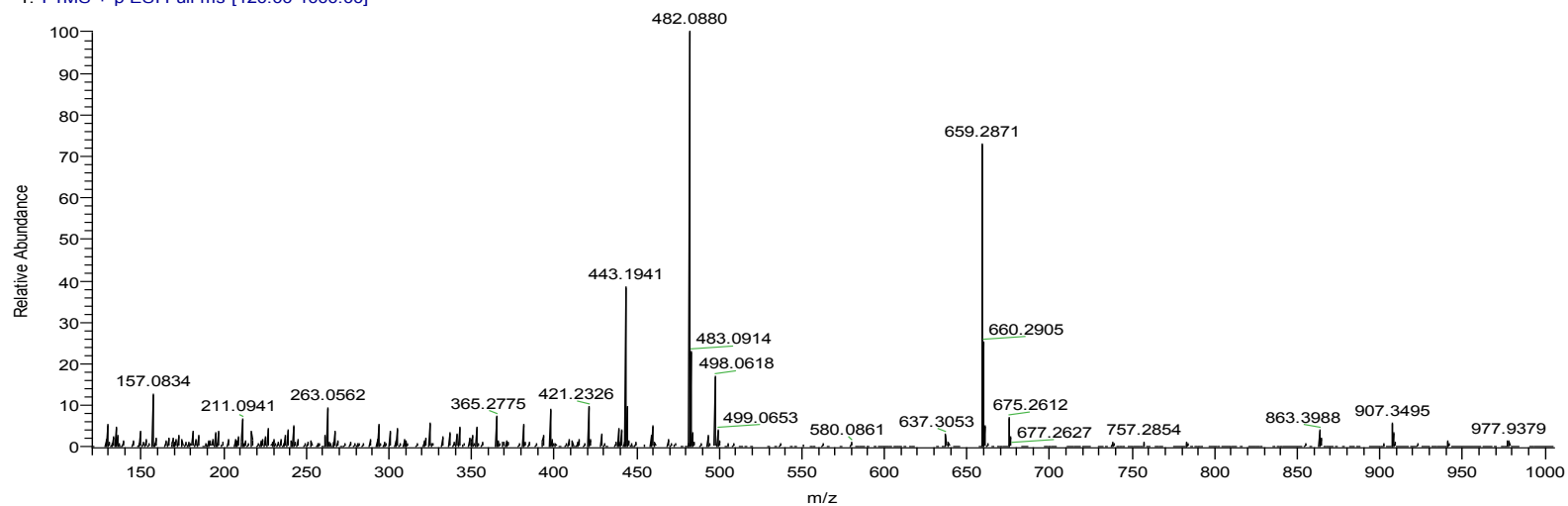
NL:
4.39E5
nor
thio_Orbi_+ESI#2-8
RT: 0.02-0.09 AV: 7 T:
FTMS + p ESI Full ms
[120.00-1000.00]

NL:
7.30E5
C$_{22}$H$_{21}$NNaO$_8$S:
C$_{22}$H$_{21}$N$_1$Na$_1$O$_8$S$_1$
pa Chrg 1

216

^{1}H NMR (500 MHz, CDCl$_3$) of (4-Methoxy-3-(1-(5-nitrothiophen-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**13**)

^{13}C NMR (500 MHz, CDCl$_3$) of (4-Methoxy-3-(1-(5-nitrothiophen-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**13**)
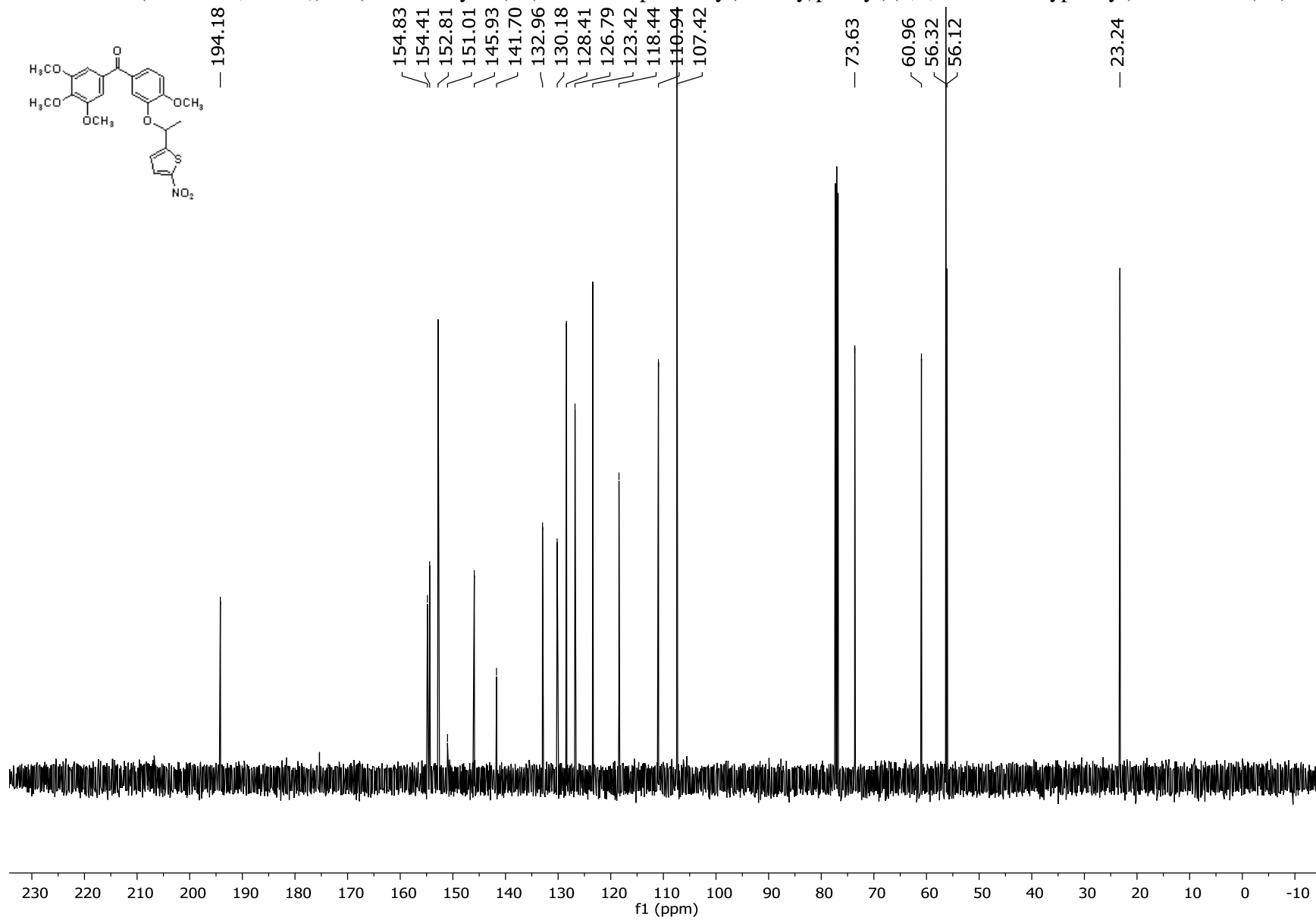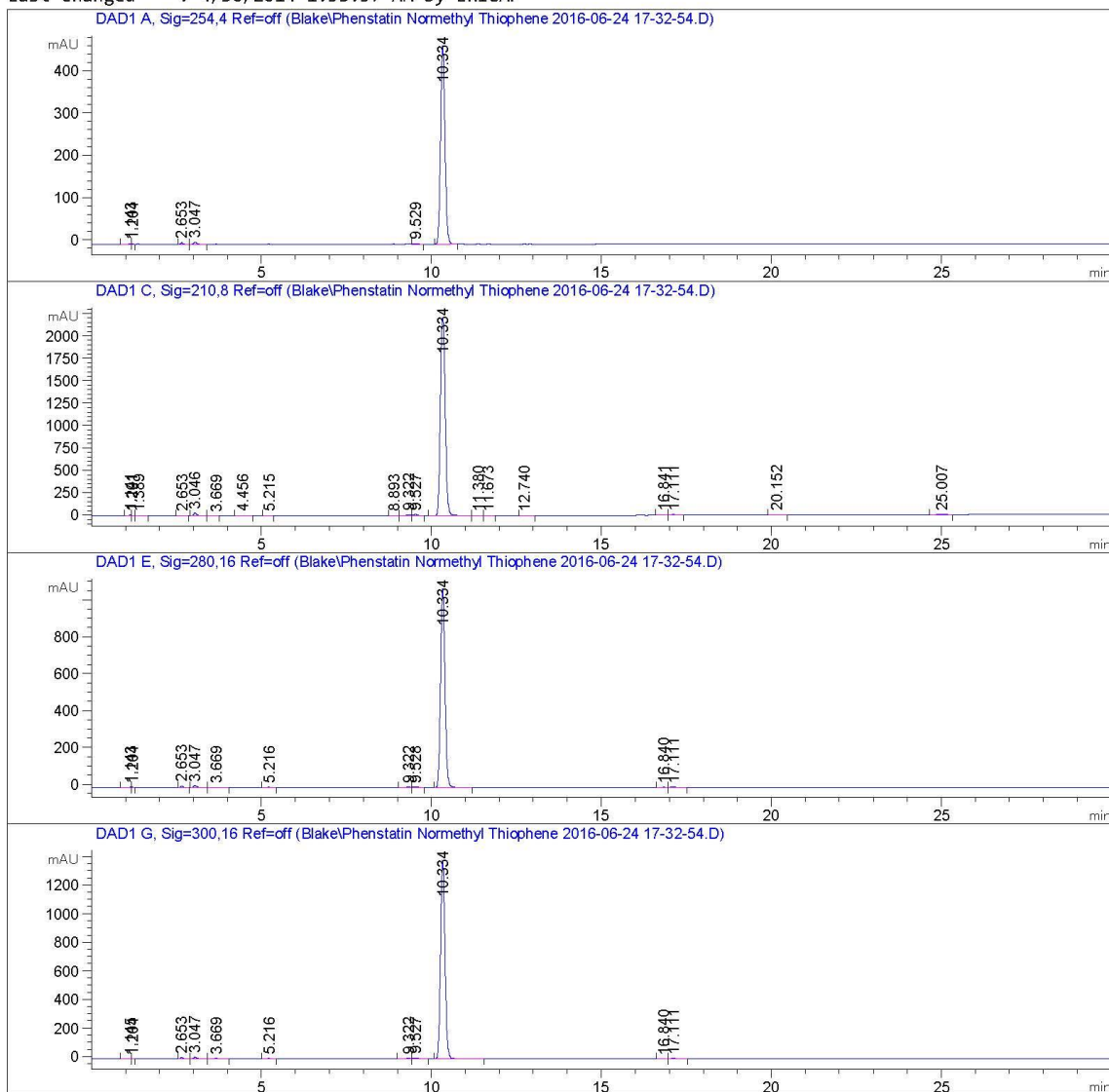
HPLC trace of compound **13**

Data File C:\Chem32\1\Data\Blake\Phenstatin Normethyl Thiophene 2016-06-24 17-32-54.D
Sample Name: Phenstatin Normethyl Thiophene

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Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC                    Location :   5
Injection Date  : 6/24/2016 5:32:54 PM
                                          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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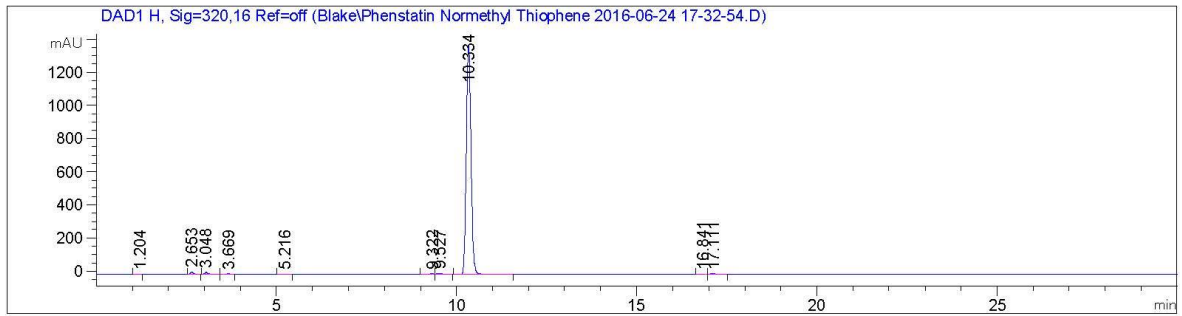
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                      Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   1.143 BV   0.0600    8.58213    2.08675   0.2039
  2   1.204 VB   0.0545    7.41947    2.13649   0.1763
  3   2.653 BB   0.0745   19.34395    3.99119   0.4595
  4   3.047 BB   0.0990   33.57935    5.08965   0.7977
  5   9.529 VB   0.1380   13.11411    1.44370   0.3115
  6  10.334 BB   0.1366 4127.41455  469.37863  98.0511

Totals :                4209.45357  484.12642



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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   1.141 BV   0.0633   43.73419    9.94480   0.1963
  2   1.201 VV   0.0535   26.91598    7.21480   0.1208
  3   1.389 VB   0.1294   32.97990    3.51567   0.1480
  4   2.653 BB   0.0740   35.44312    7.38718   0.1591
  5   3.046 BB   0.0988  215.30690   32.68957   0.9665
  6   3.669 BB   0.1041    8.01449    1.11129   0.0360
  7   4.456 BB   0.1097   19.01512    2.58725   0.0854
  8   5.215 BV   0.1157   37.60667    4.78377   0.1688
  9   8.893 BV   0.1182   16.32542    2.15833   0.0733
 10   9.322 VV   0.1420   63.16067    6.69659   0.2835
 11   9.527 VB   0.1380  102.39282   11.26775   0.4596
 12  10.334 BV   0.1534 2.14802e4  2210.79102  96.4232
 13  11.380 VV   0.1657   20.20461    1.84608   0.0907
```

Data File C:\Chem32\1\Data\Blake\Phenstatin Normethyl Thiophene 2016-06-24 17-32-54.D
Sample Name: Phenstatin Normethyl Thiophene

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
14	11.673	VB	0.1434	13.54844	1.47139	0.0608
15	12.740	BB	0.1591	13.56217	1.22604	0.0609
16	16.841	BV	0.1456	26.25587	2.79532	0.1179
17	17.111	VB	0.1507	55.89478	5.68751	0.2509
18	20.152	BB	0.1787	20.39025	1.76636	0.0915
19	25.007	BB	0.1970	46.04347	3.55750	0.2067

Totals :   2.22770e4 2318.49823

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.143	BV	0.0596	6.53655	1.60214	0.0674
2	1.204	VB	0.0552	6.66955	1.89072	0.0687
3	2.653	BB	0.0745	34.21731	7.06578	0.3526
4	3.047	BB	0.0983	64.12563	9.80002	0.6608
5	3.669	BB	0.0935	8.80327	1.39808	0.0907
6	5.216	BB	0.1159	13.75031	1.74597	0.1417
7	9.322	BV	0.1463	19.20857	1.92744	0.1979
8	9.528	VB	0.1379	32.43538	3.57494	0.3342
9	10.334	BB	0.1369	9488.83789	1075.78284	97.7831
10	16.840	BV	0.1428	9.43340	1.01169	0.0972
11	17.111	VB	0.1522	19.94340	2.00216	0.2055

Totals :   9703.96125 1107.80177

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.145	BV	0.0589	5.11084	1.27143	0.0406
2	1.204	VB	0.0561	6.30989	1.74947	0.0501
3	2.653	BB	0.0746	51.72796	10.66139	0.4106
4	3.047	BB	0.0982	76.94297	11.77816	0.6107
5	3.669	BB	0.0885	14.61334	2.48778	0.1160
6	5.216	BB	0.1149	17.59590	2.25759	0.1397
7	9.322	BV	0.1466	25.10475	2.46867	0.1993
8	9.527	VB	0.1390	48.79895	5.32161	0.3873
9	10.334	BB	0.1374	1.23120e4	1389.67432	97.7275
10	16.840	BV	0.1447	12.81279	1.37492	0.1017
11	17.111	VB	0.1516	27.28168	2.75248	0.2166

Totals :   1.25983e4 1431.79783

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.204	BB	0.0733	8.29830	1.57813	0.0660
2	2.653	BB	0.0747	78.90384	16.22612	0.6276
3	3.048	BB	0.0982	76.45702	11.70042	0.6082
4	3.669	BB	0.0846	14.34650	2.59015	0.1141
5	5.216	BB	0.1126	12.35557	1.62760	0.0983
6	9.322	BV	0.1481	24.54387	2.38473	0.1952
7	9.527	VB	0.1390	48.20722	5.25499	0.3835
8	10.334	BB	0.1375	1.22687e4	1383.28394	97.5908
9	16.841	BV	0.1445	12.23663	1.31579	0.0973
10	17.111	VB	0.1512	27.52718	2.78858	0.2190

Totals :   1.25716e4 1428.75044

===
                       *** End of Report ***

Mass Spectrum of Compound **13**
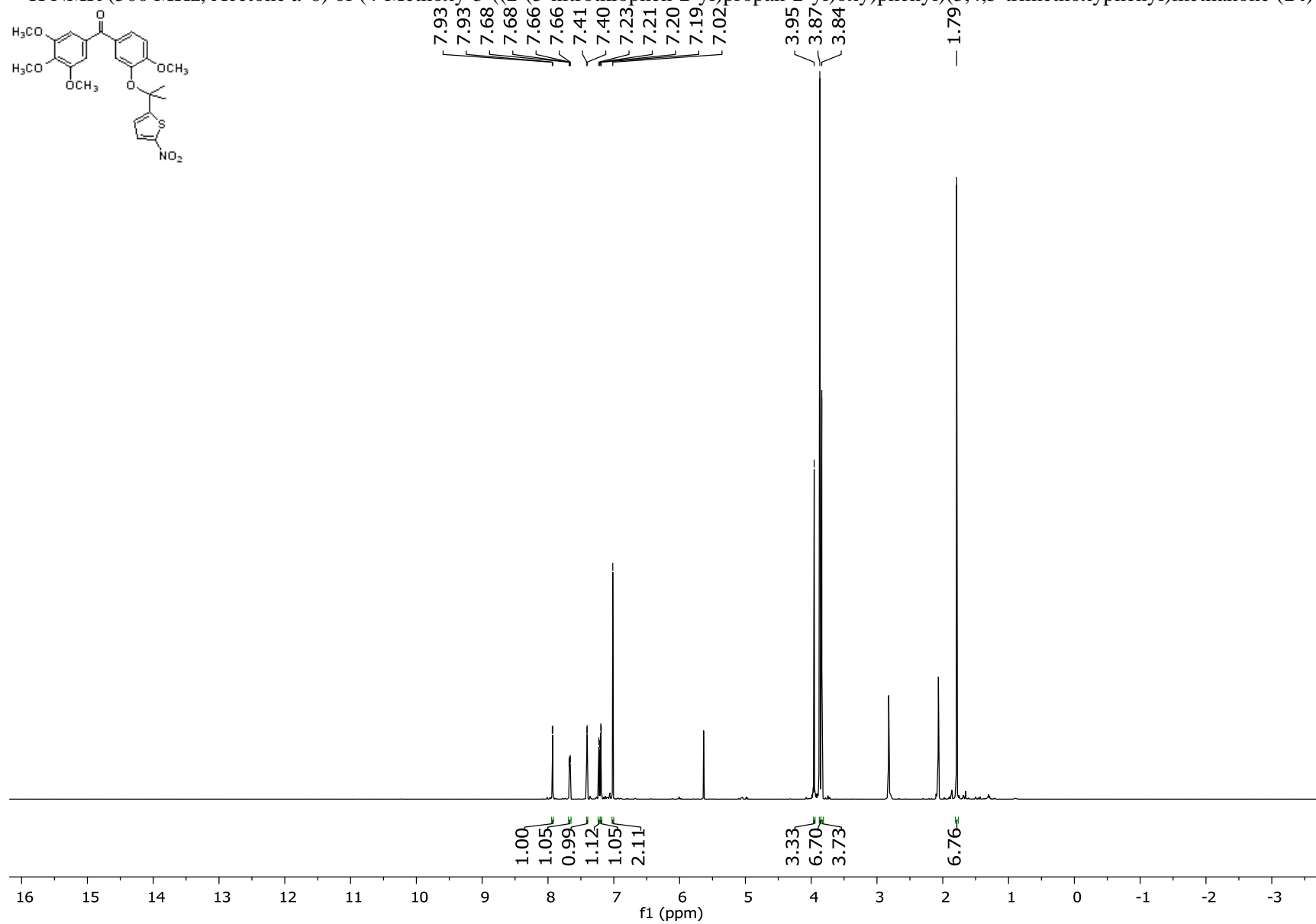
6/27/2016 3:22:29 PM

mono thio_Orbi_+ESI #2-20   RT: 0.01-0.16   AV:
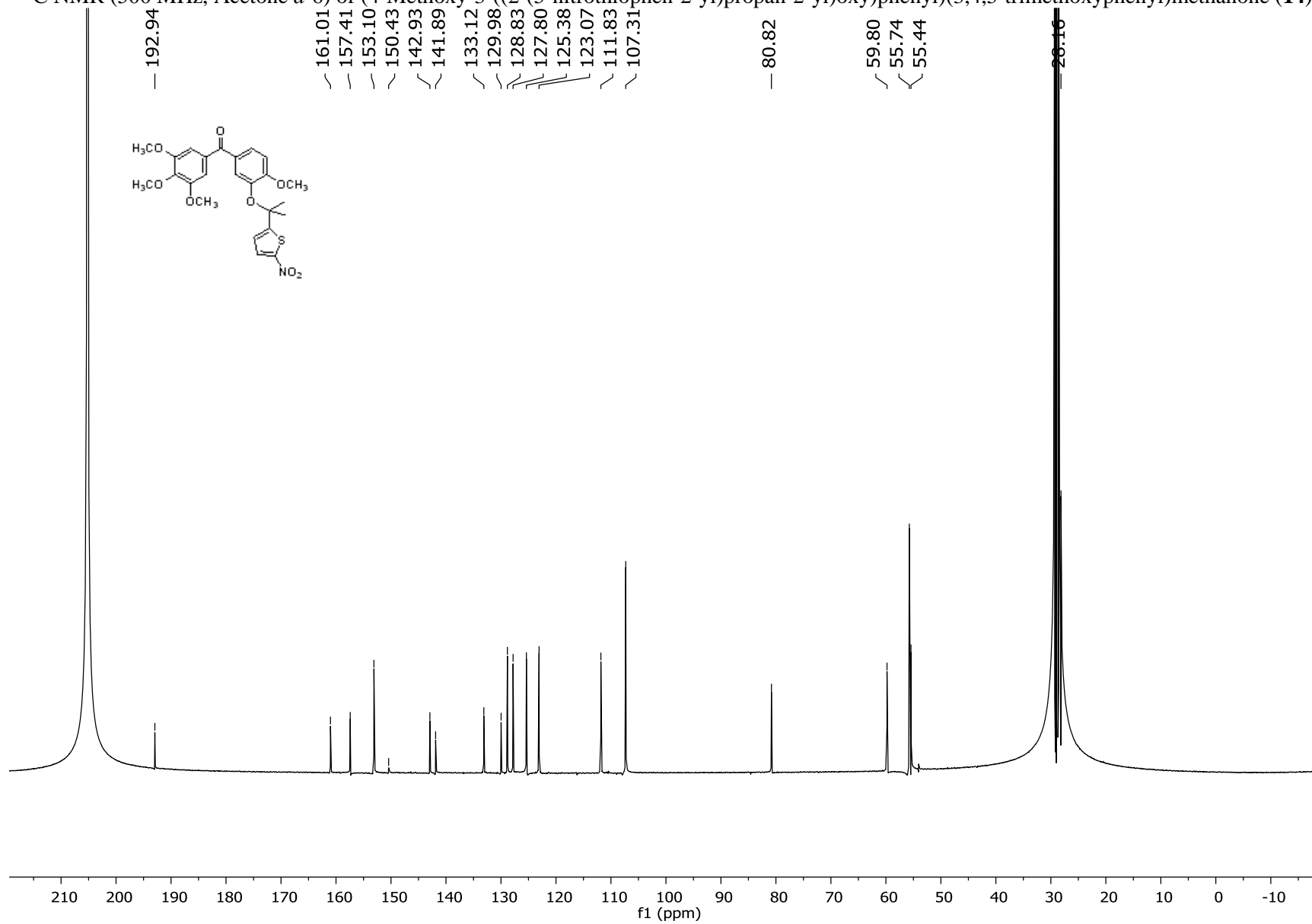T: FTMS + p ESI Full ms [120.00-1000.00]
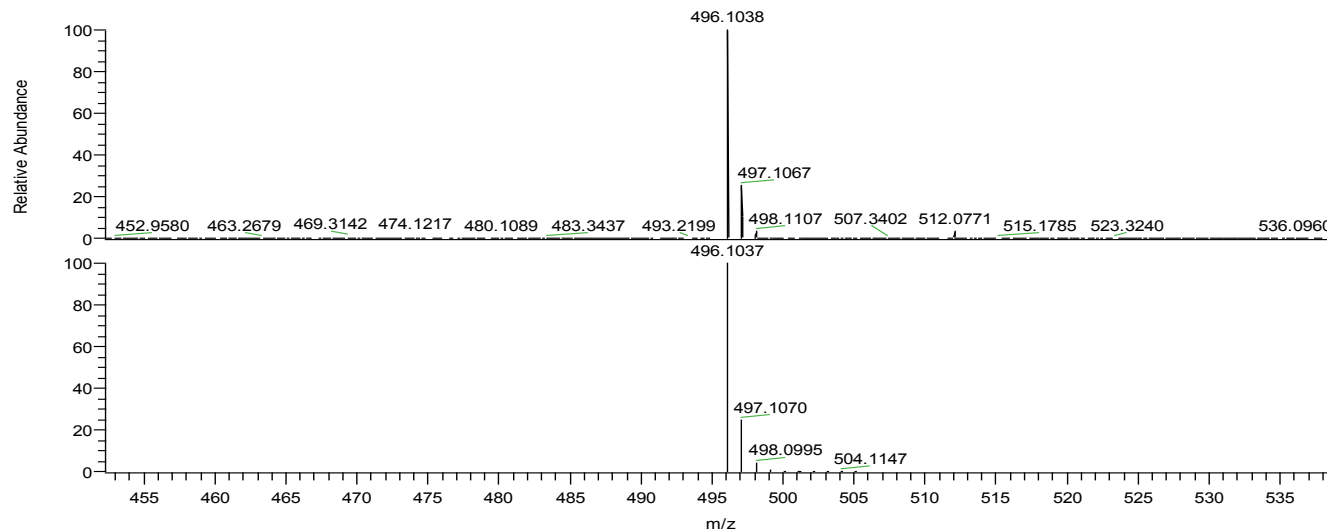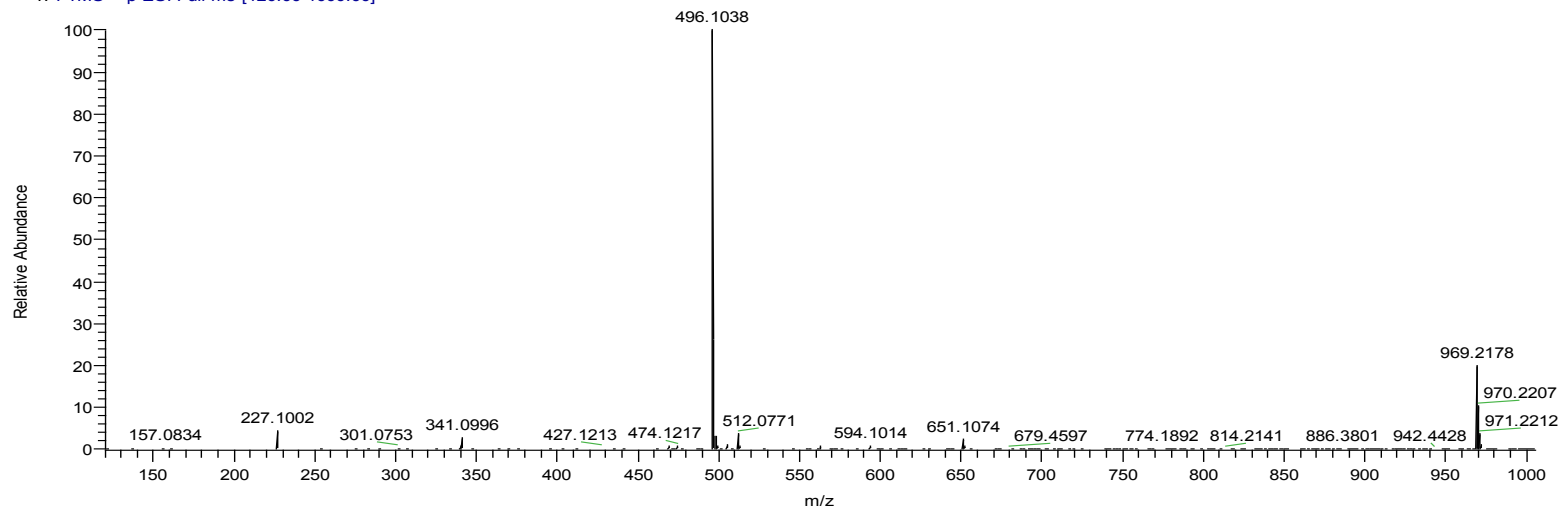
NL:
3.02E7
mono
thio_Orbi_+ESI#2-20
RT: 0.01-0.16 AV: 19
T: FTMS + p ESI Full
ms [120.00-1000.00]

NL:
7.22E5

$C_{23}H_{23}NNaO_8S$:
$C_{23}H_{23}N_1Na_1O_8S_1$
pa Chrg 1

223

¹H NMR (500 MHz, Acetone *d*-6) of (4-Methoxy-3-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**14**)
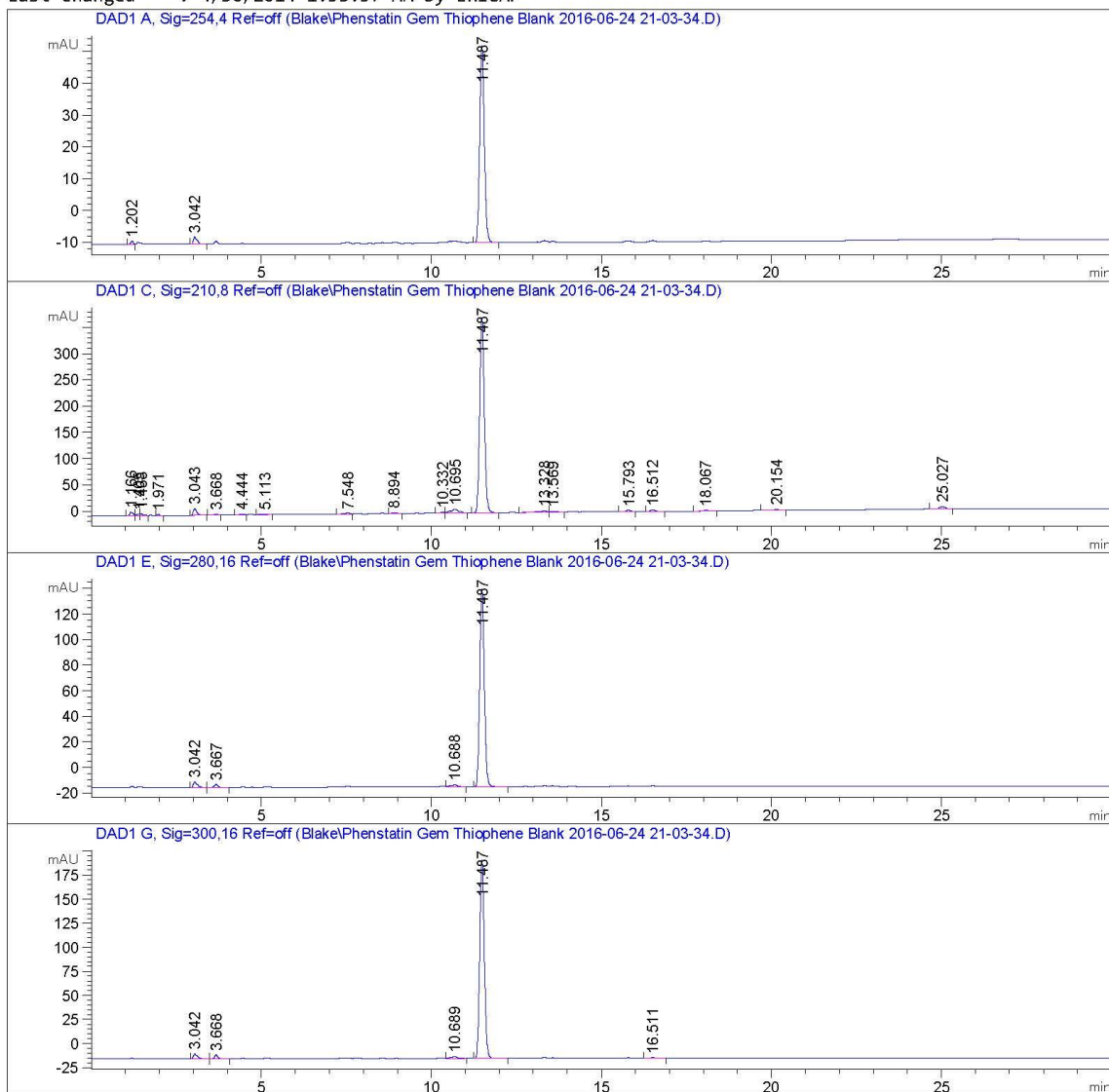
¹³C NMR (500 MHz, Acetone *d*-6) of (4-Methoxy-3-((2-(5-nitrothiophen-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**14**)

192.94

161.01
157.41
153.10
150.43
142.93
141.89
133.12
129.98
128.83
127.80
125.38
123.07
111.83
107.31

80.82

59.80
55.74
55.44

f1 (ppm)

HPLC trace of Compound **14**

```
==================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC                 Location :   1
Injection Date  : 6/24/2016 9:03:34 PM
                                        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
```
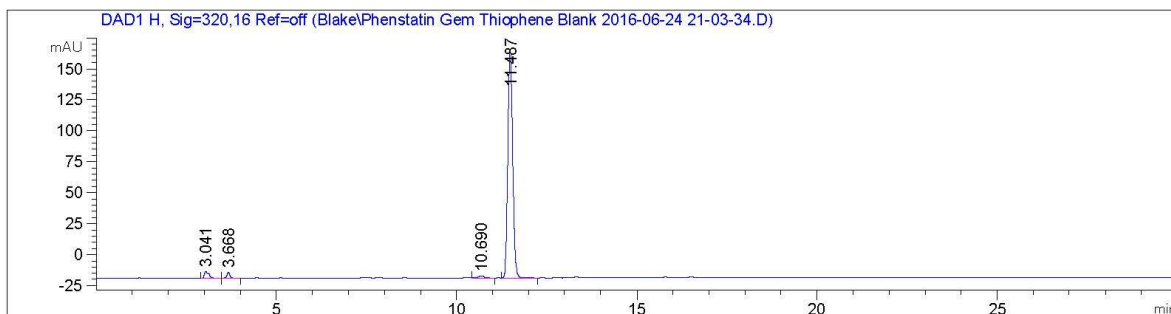
```
================================================================
                      Area Percent Report
================================================================

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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   1.202 BB   0.0857    5.33667    1.04275   0.8958
  2   3.042 BB   0.1101   16.95223    2.19617   2.8454
  3  11.487 BB   0.1420  573.47900   61.97279  96.2588

Totals :                 595.76790   65.21172



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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   1.166 BV   0.0850   44.55954    7.31982   1.0974
  2   1.405 VV   0.0847   20.22405    3.15720   0.4981
  3   1.468 VB   0.0751   18.11991    3.35221   0.4463
  4   1.971 BB   0.0663    9.28121    2.15234   0.2286
  5   3.043 BB   0.1037   92.63680   13.22735   2.2815
  6   3.668 BB   0.0852    9.84668    1.71010   0.2425
  7   4.444 BB   0.1046   10.00656    1.48508   0.2464
  8   5.113 BB   0.1511   16.65965    1.50521   0.4103
  9   7.548 BV   0.1487   20.27444    1.96016   0.4993
 10   8.894 VB   0.1489   15.76609    1.62993   0.3883
 11  10.332 BV   0.1426   17.45869    1.87600   0.4300
 12  10.695 VB   0.2301  120.72125    7.10826   2.9732
 13  11.487 BB   0.1441 3449.42578  372.38168  84.9533
 14  13.328 BV   0.2266   49.34008    2.98908   1.2152
 15  13.569 VB   0.1593   22.95136    2.13762   0.5653
 16  15.793 BB   0.1609   23.96850    2.31205   0.5903
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
17	16.512	BB	0.1592	27.70535	2.66782	0.6823
18	18.067	BB	0.1889	13.71129	1.07445	0.3377
19	20.154	BB	0.1812	20.82122	1.77091	0.5128
20	25.027	BB	0.1975	56.90002	4.44180	1.4013

Totals :   4060.37847   436.25907

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.042	BB	0.1081	31.74127	4.20423	2.1287
2	3.667	BB	0.0900	14.44893	2.40980	0.9690
3	10.688	VB	0.1976	23.67714	1.68805	1.5879
4	11.487	BB	0.1410	1421.26025	154.94855	95.3145

Totals :   1491.12759   163.25062

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.042	BB	0.1090	40.59677	5.20452	2.0351
2	3.668	BB	0.0877	25.26894	4.35773	1.2667
3	10.689	VB	0.1918	27.81913	2.00638	1.3946
4	11.487	BB	0.1410	1890.40027	206.06499	94.7647
5	16.511	BB	0.1622	10.75020	1.02629	0.5389

Totals :   1994.83530   218.65992

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.041	BB	0.1163	46.65141	5.53500	2.6037
2	3.668	BB	0.0883	27.14539	4.63966	1.5150
3	10.690	VB	0.1811	23.42678	1.80868	1.3075
4	11.487	BB	0.1410	1694.52991	184.68036	94.5738

Totals :   1791.75348   196.66370

===
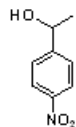*** End of Report ***

Mass Spectrum of Compound **14**

middle_con_Orbi_+ESI #2-20   RT: 0.01-0.19   AV
T: FTMS + p ESI Full ms [50.00-800.00]
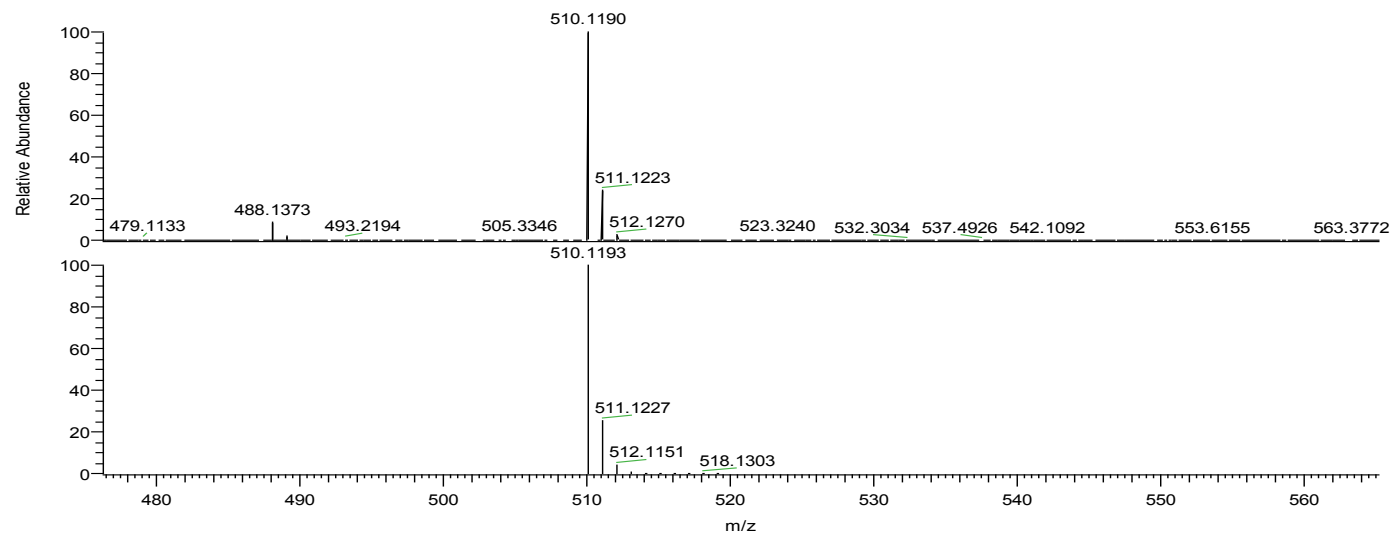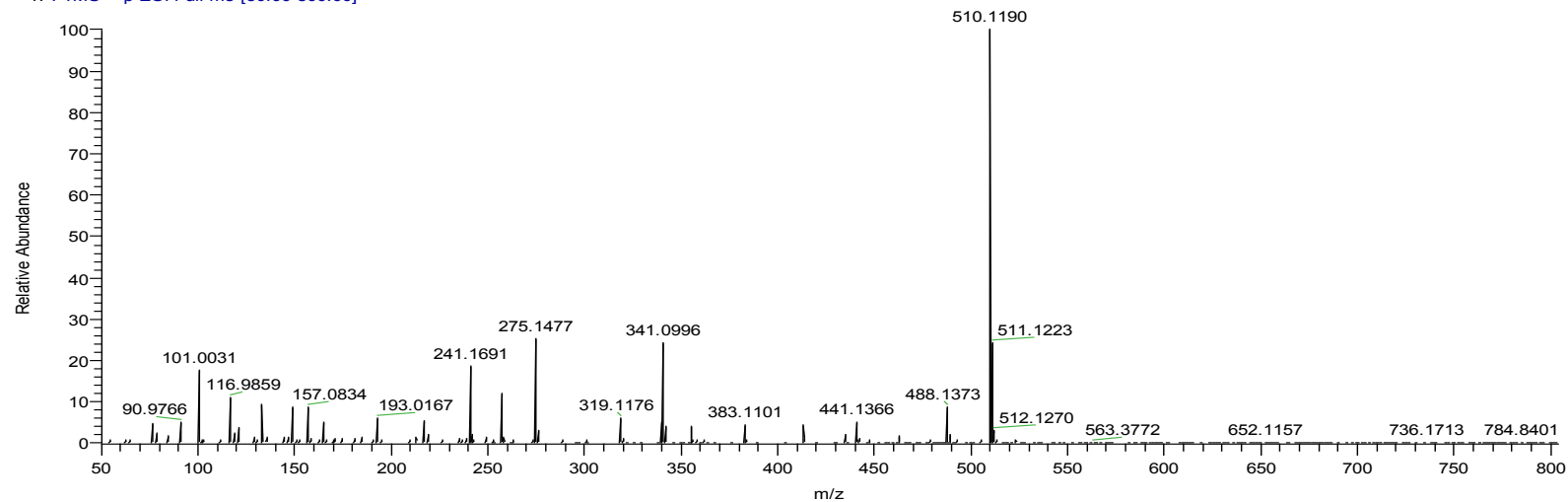
NL:
7.59E6
middle_con_Orbi_+
ESI#2-20 RT:
0.01-0.19 AV: 19 T:
FTMS + p ESI Full ms
[50.00-800.00]

NL:
7.14E5

$C_{24}H_{25}NNaO_8S$:
$C_{24}H_{25}N_1Na_1O_8S_1$
pa Chrg 1

229

^{1}H NMR (500 MHz, CDCl$_3$) of 1-(4-nitrophenyl)ethan-1-ol (**16**)
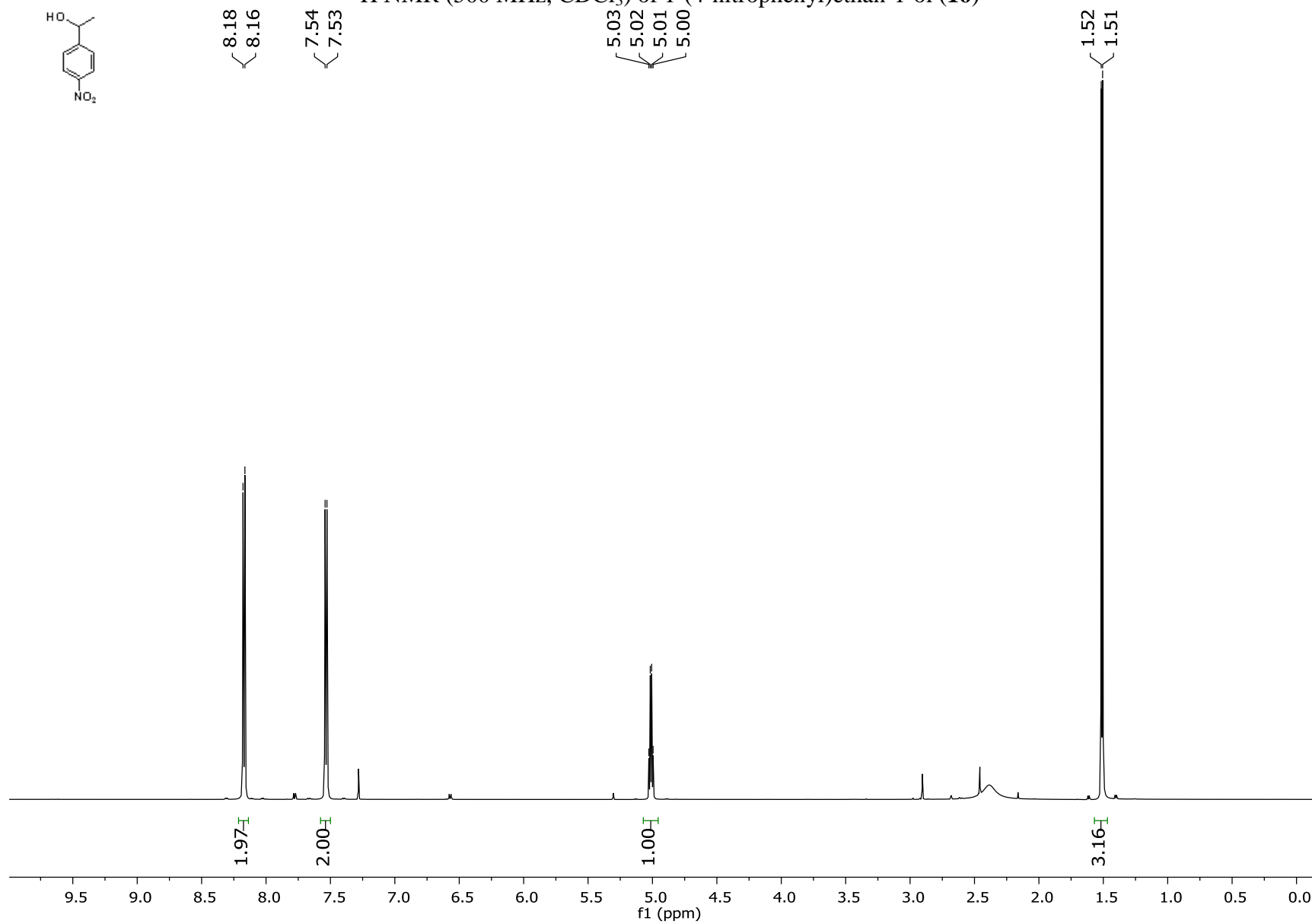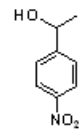
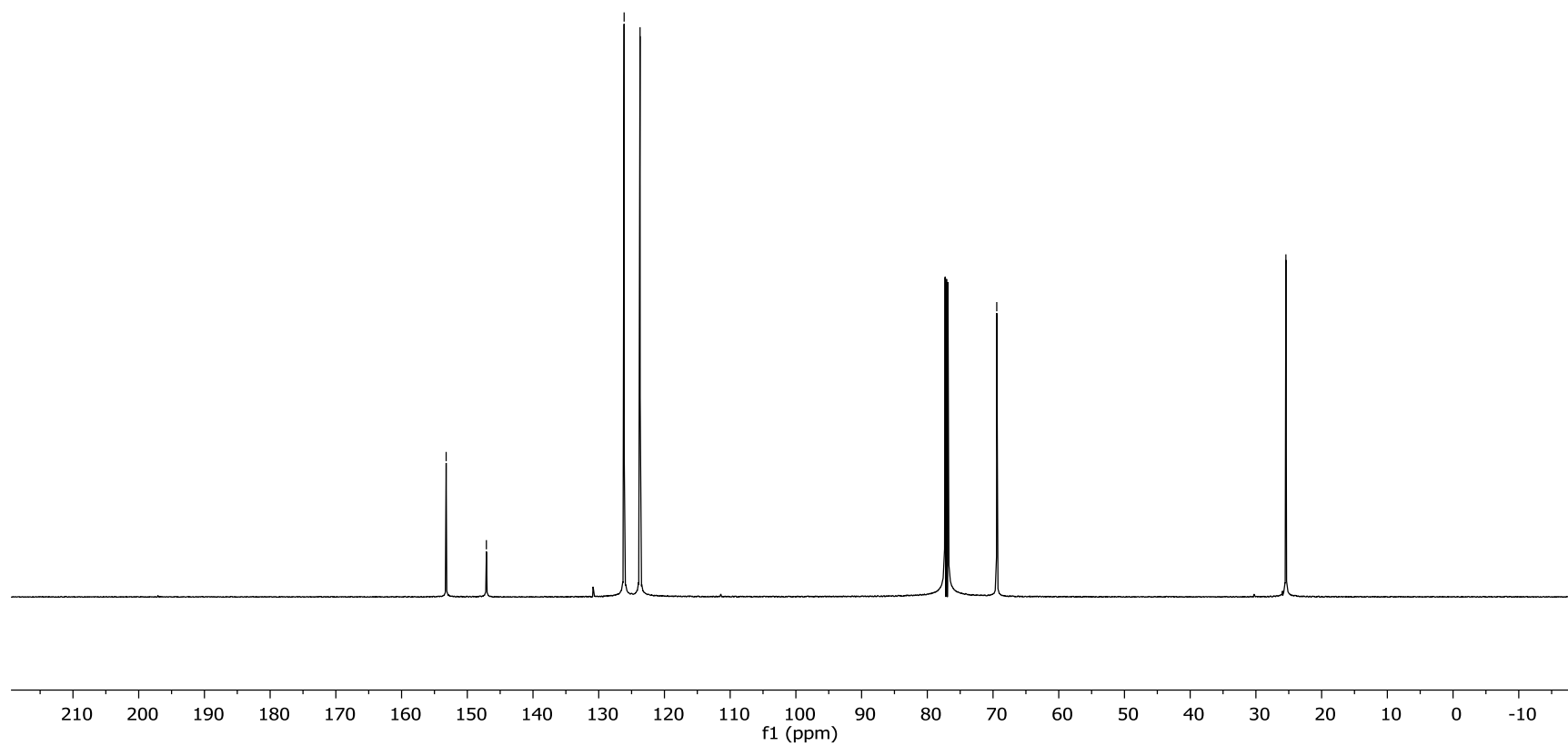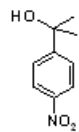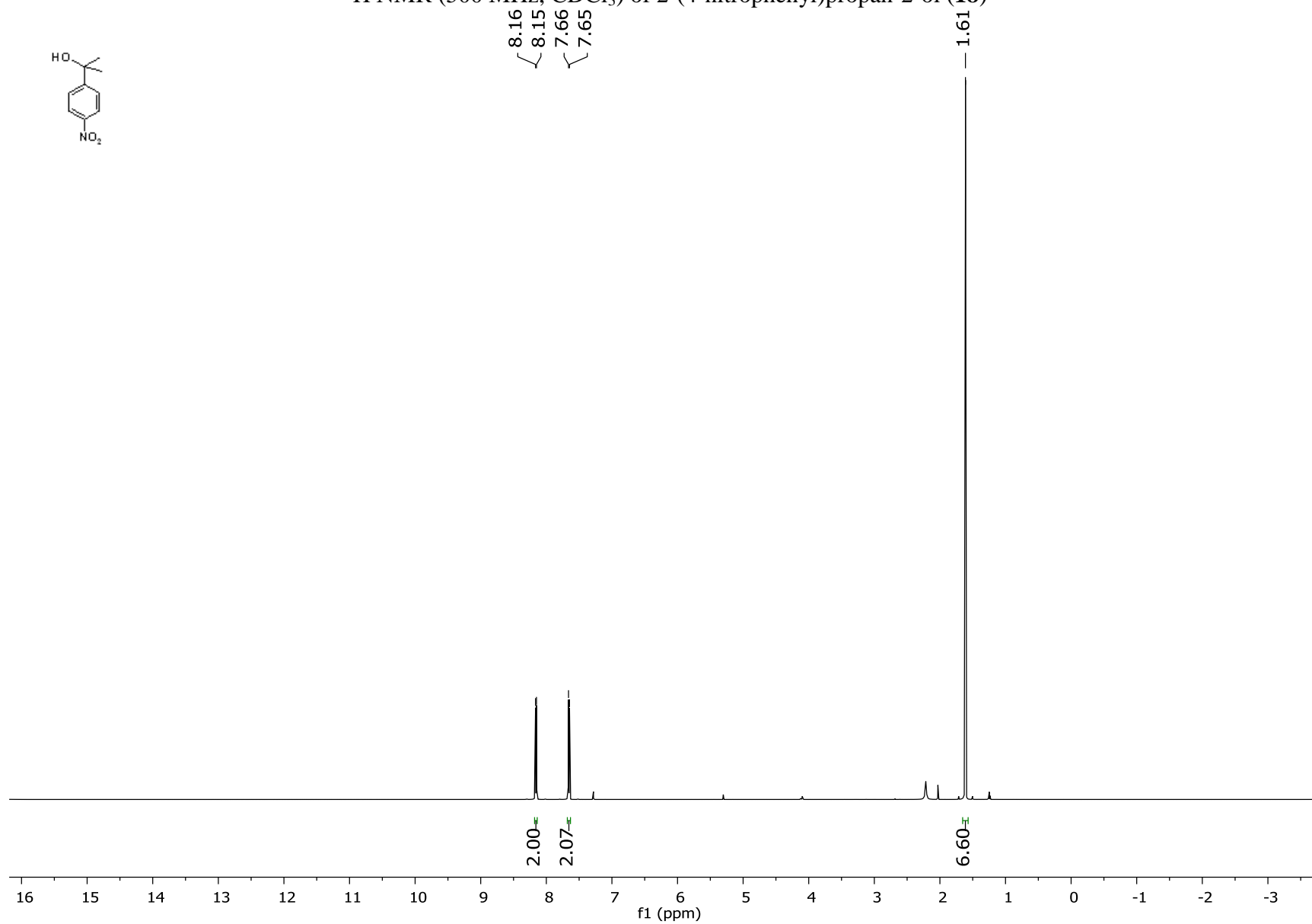^{13}C NMR (500 MHz, CDCl$_3$) of 1-(4-nitrophenyl)ethan-1-ol (**16**)

153.22
147.09
126.13
123.71
69.43
25.44
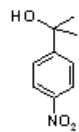
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

^{1}H NMR (500 MHz, CDCl$_3$) of 2-(4-nitrophenyl)propan-2-ol (**18**)

232

^{13}C NMR (500 MHz, CDCl$_3$) of 2-(4-nitrophenyl)propan-2-ol (**18**)

156.52
146.64
125.51
123.45
72.49
31.69

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
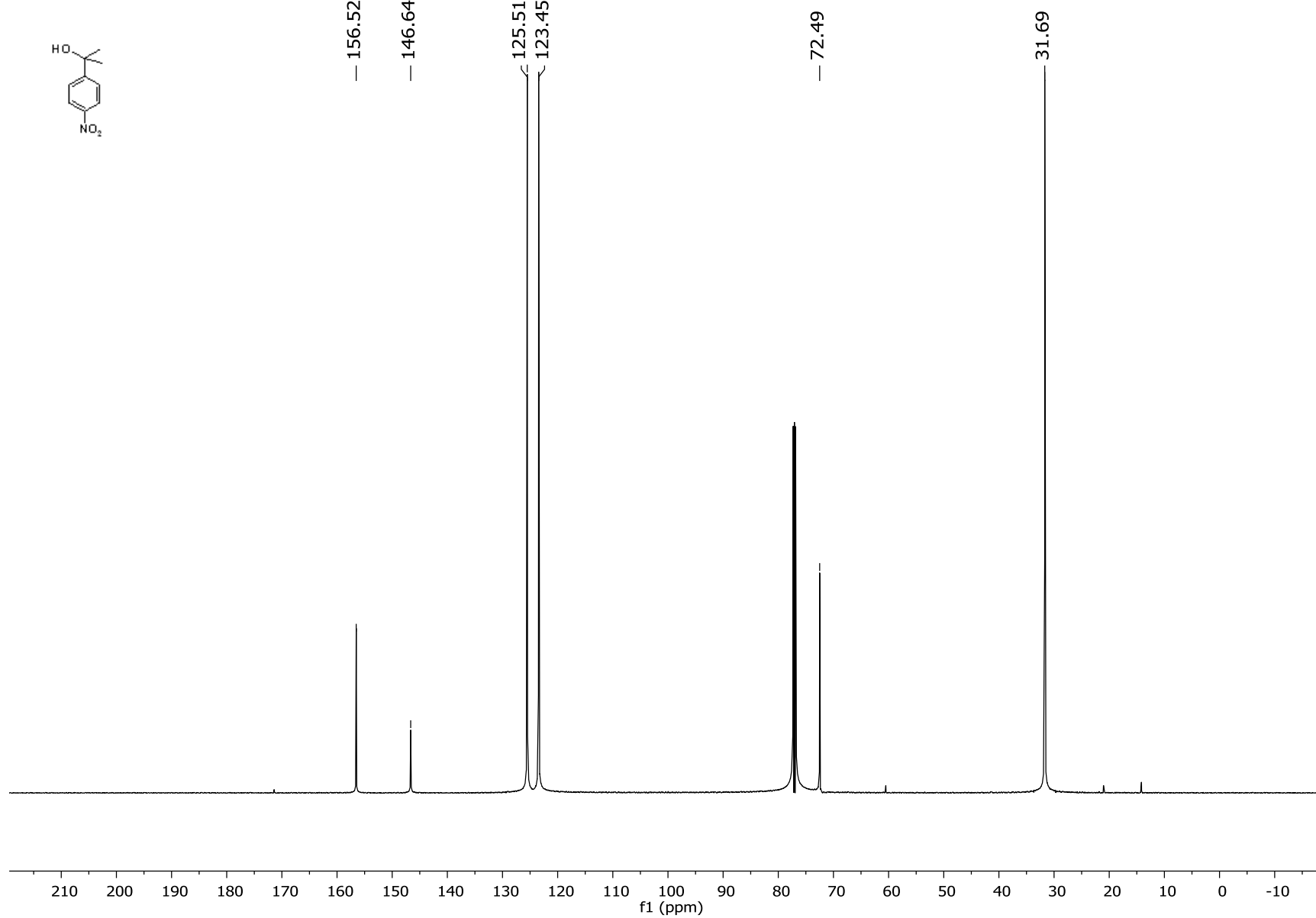
^{1}H NMR (500 MHz, CDCl$_3$) of (4-methoxy-3-((4-nitrobenzyl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**20**)

^{13}C NMR (500 MHz, CDCl$_3$) of (4-methoxy-3-((4-nitrobenzyl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**20**)
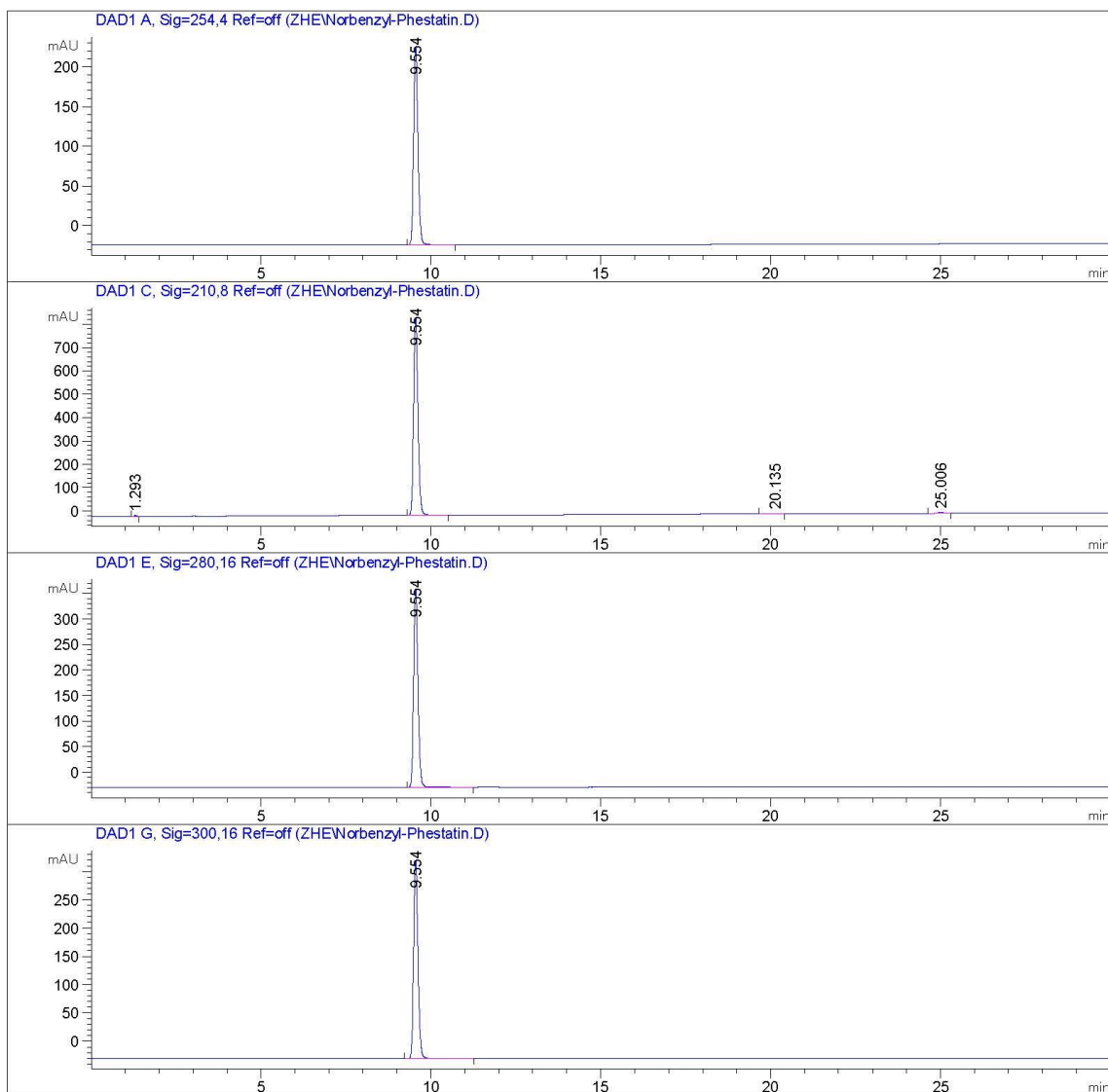

f1 (ppm)

235

HPLC trace of Compound 20

DAD1 H, Sig=320,16 Ref=off (ZHE\Norbenzyl-Phestatin.D)

```
===================================================================
                    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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   9.554 BB    0.1339 2177.75610  249.31061 100.0000

Totals :                 2177.75610  249.31061




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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   1.293 BV    0.0727   19.11470    3.67168   0.2548
  2   9.554 BB    0.1363 7414.29053  845.71783  98.8449
  3  20.135 BB    0.1791   27.84004    2.40504   0.3712
  4  25.006 BB    0.1964   39.68525    3.11932   0.5291

Totals :                 7500.93052  854.91388




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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   9.554 BB    0.1341 3399.72144  388.38205 100.0000

Totals :                 3399.72144  388.38205
```

Data File C:\Chem32\1\Data\ZHE\Norbenzyl-Phestatin.D
Sample Name: Norbenzyl-Phestatin

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

Peak RetTime Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
  1   9.554 BB   0.1341 3069.21484 350.57813 100.0000

Totals :   3069.21484 350.57813

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

Peak RetTime Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
  1   9.554 BB   0.1342 2399.73413 274.06729 100.0000

Totals :   2399.73413 274.06729

===
                        *** End of Report ***

Mass Spectrum of Compound **20**
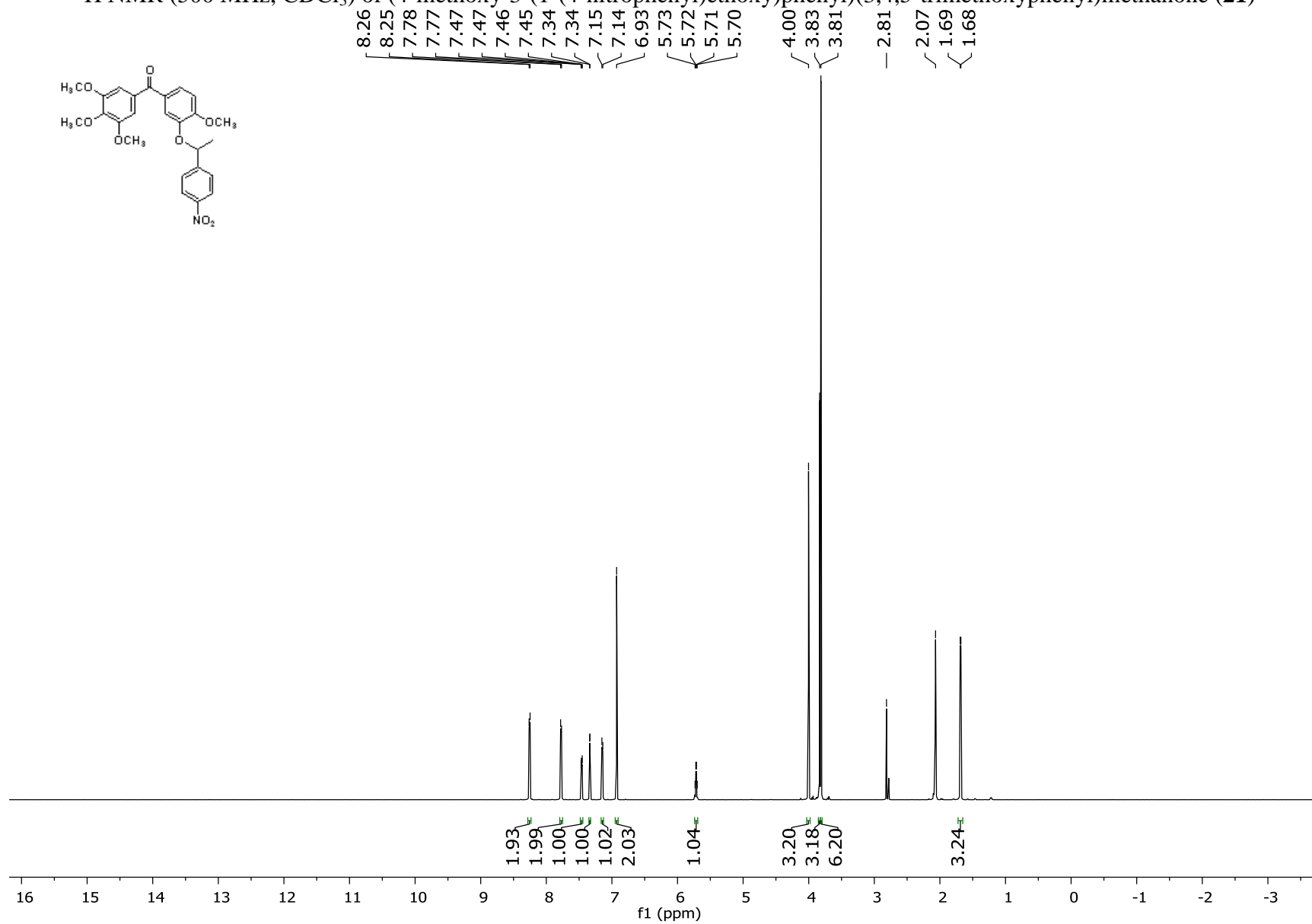
6/27/2016 3:35:19 PM

nor benzyl_Orbi_+ESI #1-20   RT: 0.01-0.20   AV:
T: FTMS + p ESI Full ms [120.00-1000.00]
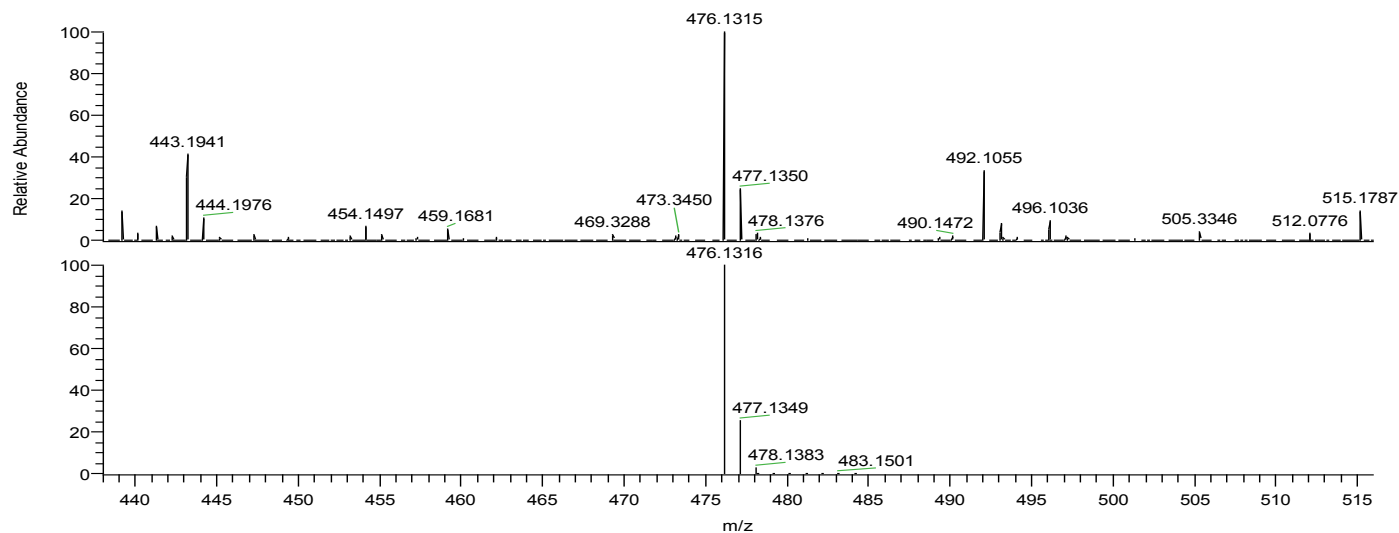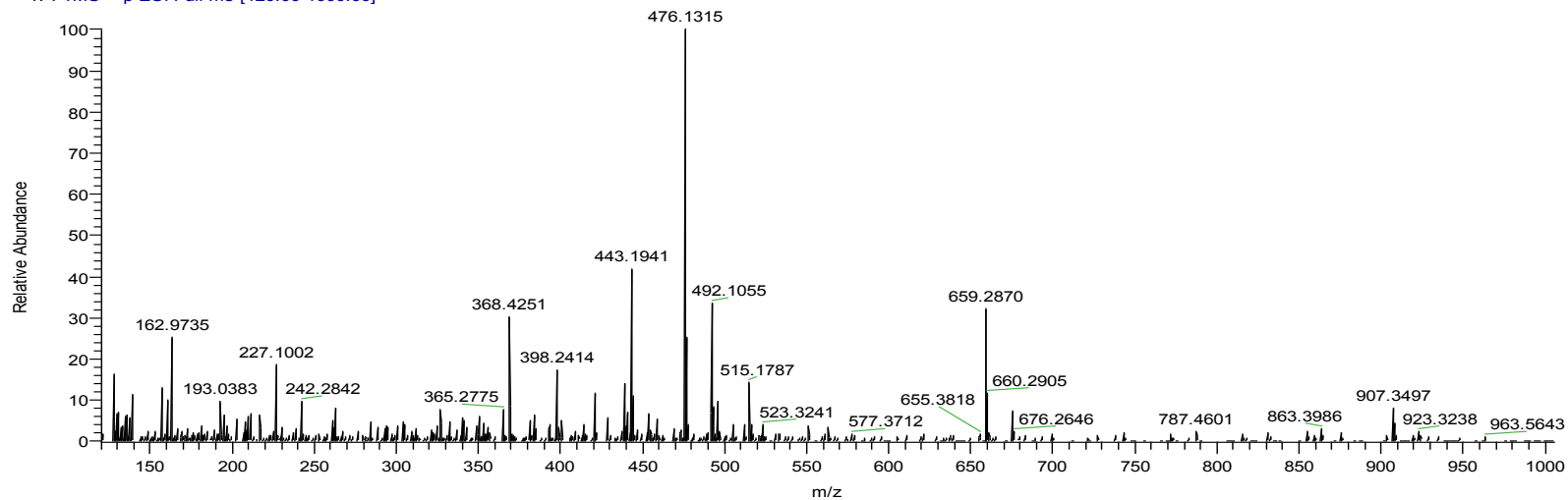
NL:
5.15E5
nor
benzyl_Orbi_+ESI#1-20
RT: 0.01-0.20 AV: 20 T:
FTMS + p ESI Full ms
[120.00-1000.00]

NL:
7.53E5

$C_{24}H_{23}NNaO_8$:
$C_{24}H_{23}N_1Na_1O_8$
pa Chrg 1

239

¹H NMR (500 MHz, CDCl₃) of (4-methoxy-3-(1-(4-nitrophenyl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**21**)

^{13}C NMR (500 MHz, CDCl$_3$) of (4-methoxy-3-(1-(4-nitrophenyl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**21**)

241
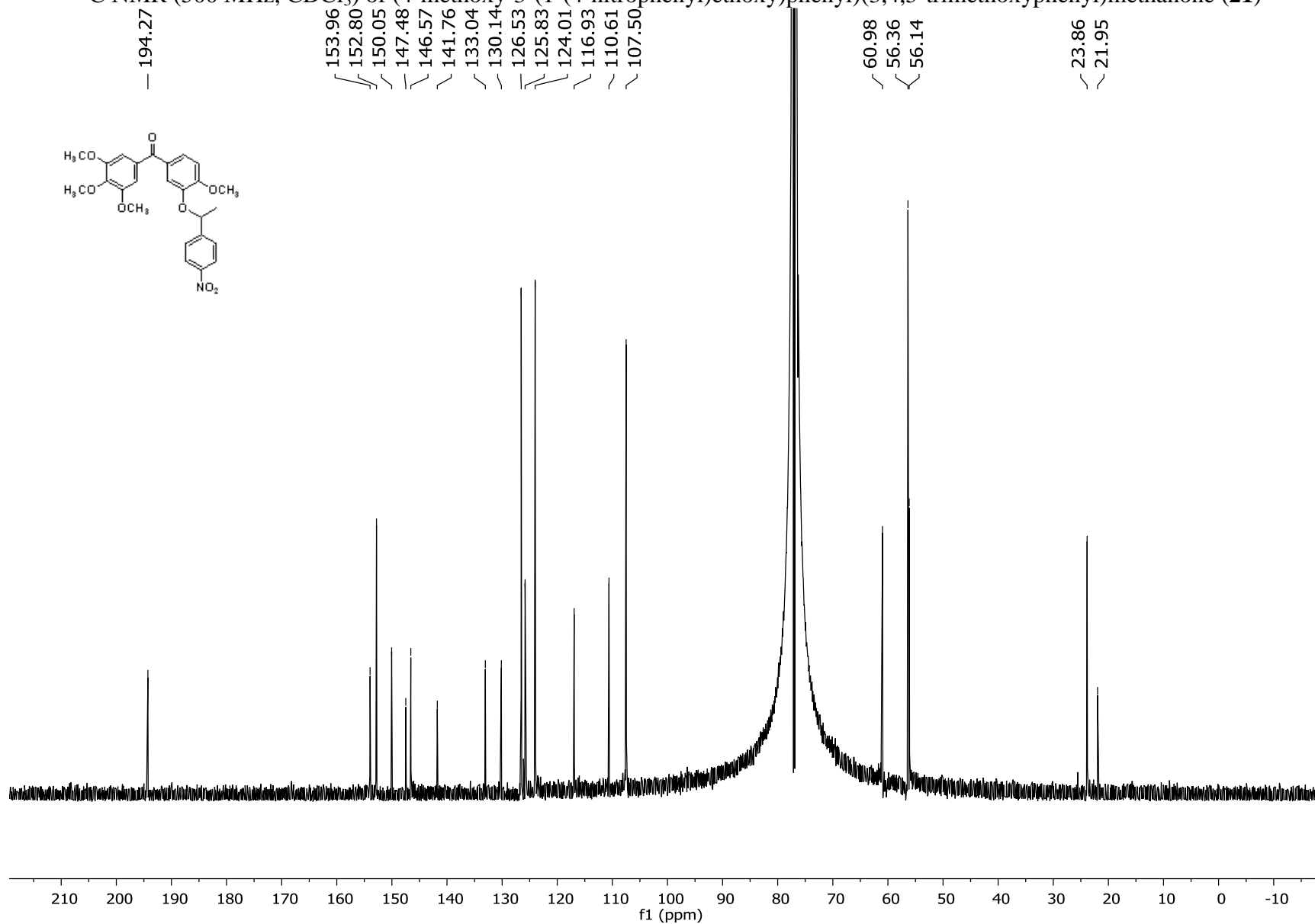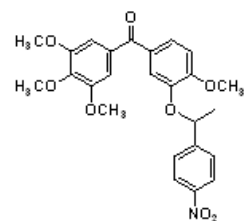
HPLC trace of compound 21

```
==================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC              Location :    4
Injection Date  : 6/24/2016 3:04:17 PM
                                         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
```

242

```
===================================================================
                      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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   2.623 VB   0.0705   97.26770   20.82153   2.3829
   2  10.050 BB   0.1355 3948.82544  445.16821  96.7381
   3  17.741 BV   0.1500   18.17998    1.89374   0.4454
   4  18.014 VB   0.1521   17.70173    1.77931   0.4337

Totals :                 4081.97484  469.66279




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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.164 BV   0.0694   33.68466    6.85008   0.2351
   2   1.400 VV   0.0721   12.71162    2.55051   0.0887
   3   2.485 BV   0.0637    7.93202    1.94075   0.0554
   4   2.623 VB   0.0704  125.16396   26.84341   0.8735
   5   8.888 BB   0.1209   15.13440    1.94245   0.1056
   6   9.570 BB   0.1334   17.91170    2.02143   0.1250
   7  10.050 BB   0.1415 1.38537e4  1531.66199  96.6844
   8  10.855 BB   0.1800   21.19083    1.69345   0.1479
   9  11.367 BV   0.1523   19.79466    2.02063   0.1381
  10  11.660 VB   0.1551   16.72773    1.66669   0.1167
  11  17.741 BV   0.1504   74.01029    7.68025   0.5165
  12  18.015 VB   0.1550   75.58664    7.41189   0.5275
  13  20.137 BB   0.1881   14.42555    1.21834   0.1007
  14  25.012 BB   0.2047   40.80371    3.07725   0.2848
```
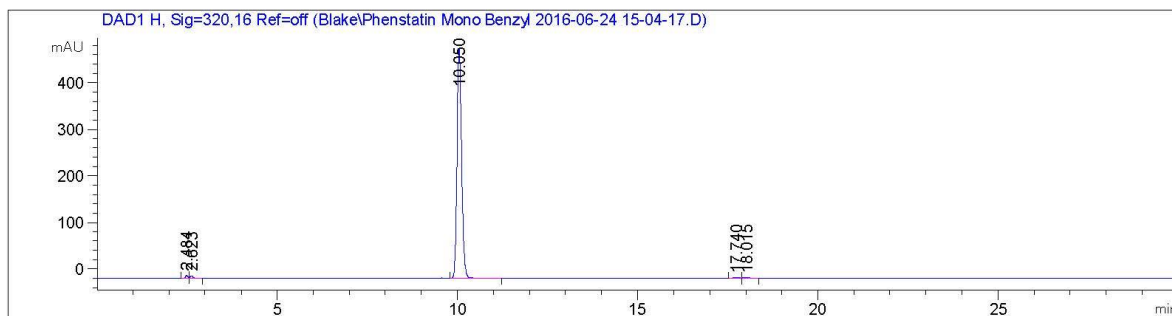
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Totals :                    1.43287e4  1598.57912
```

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.623	VB	0.0706	166.48155	35.57878	2.4453
2	10.050	VB	0.1357	6572.88330	739.32458	96.5442
3	17.741	BV	0.1502	33.93770	3.52851	0.4985
4	18.015	VB	0.1519	34.85859	3.50779	0.5120

```
Totals :                    6808.16114  781.93966
```

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.484	BV	0.0650	13.30461	3.16819	0.2212
2	2.623	VB	0.0709	97.07500	20.62395	1.6139
3	10.050	VB	0.1357	5846.73633	657.68555	97.2065
4	17.741	BV	0.1502	28.39500	2.95145	0.4721
5	18.014	VB	0.1519	29.24918	2.94428	0.4863

```
Totals :                    6014.76011  687.37341
```

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.484	BV	0.0668	27.32641	6.27228	0.6116
2	2.623	VB	0.0722	28.46769	5.90924	0.6372
3	10.050	VB	0.1357	4383.14844	493.09454	98.1016
4	17.740	BV	0.1504	14.43601	1.49873	0.3231
5	18.015	VB	0.1524	14.59014	1.46198	0.3265

```
Totals :                    4467.96868  508.23677
```

```
=====================================================================
                      *** End of Report ***
```

mono benzyl_Orbi_+ESI #1-20 RT: 0.01-0.16 A
T: FTMS + p ESI Full ms [120.00-1000.00]

227.1002

490.1471

205.1182
141.0633
249.0821
337.1046
431.2112
469.3288
492.1530
563.3764
639.1947
692.2424
764.3433
843.3063
915.6680
957.3048

Relative Abundance

m/z
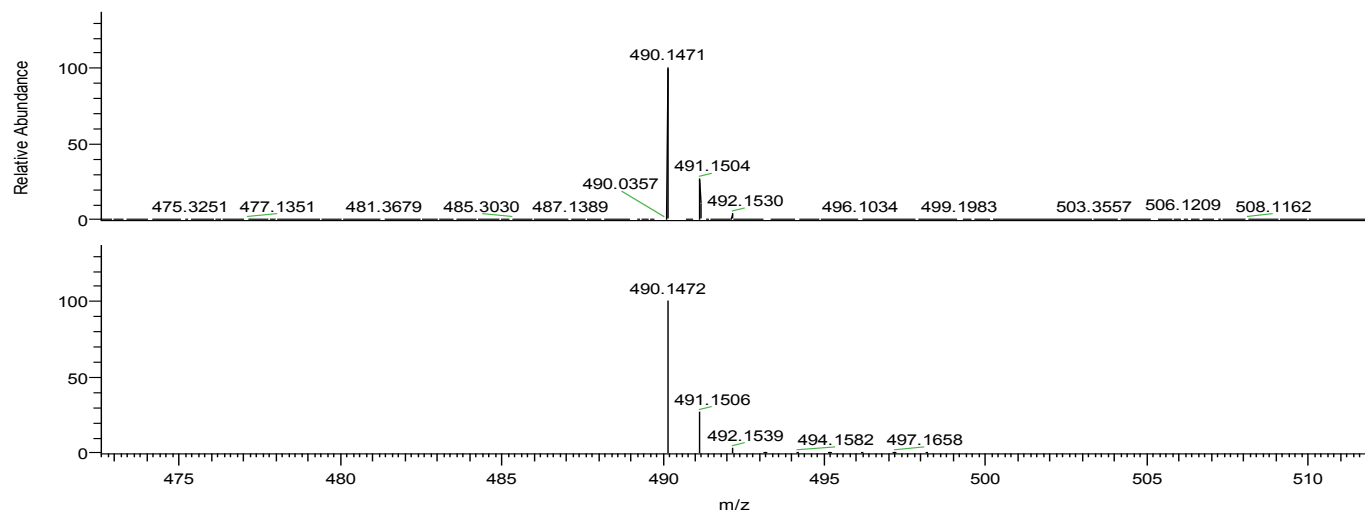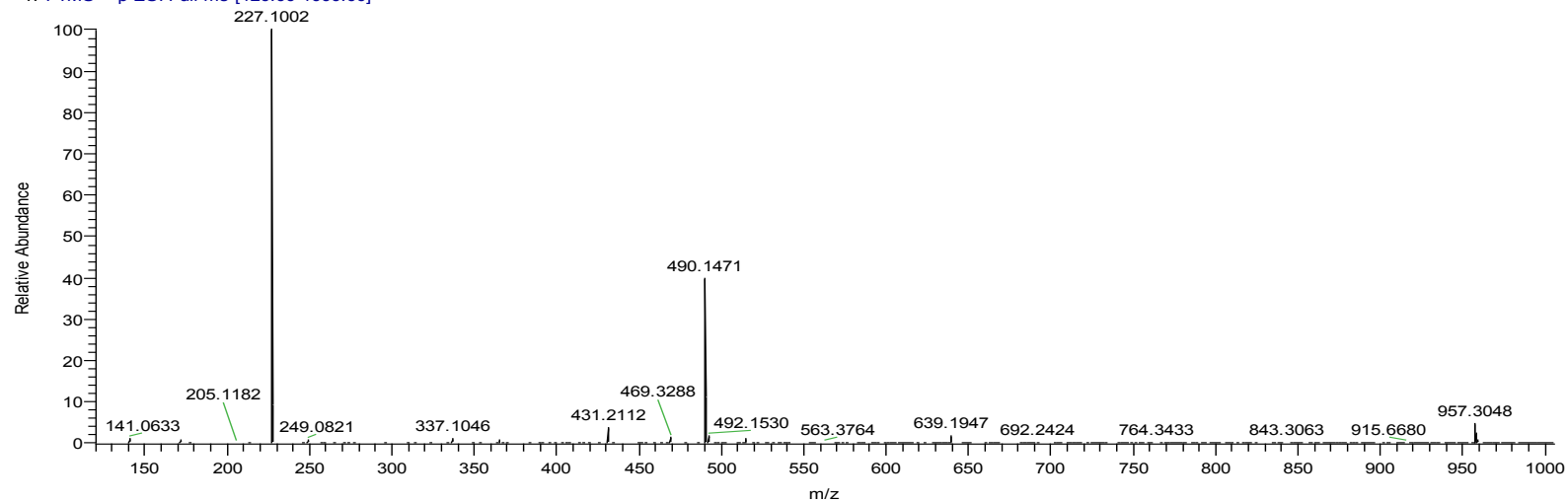
NL:
2.31E7
mono
benzyl_Orbi_+ESI#1-20
RT: 0.01-0.16 AV: 20 T:
FTMS + p ESI Full ms
[120.00-1000.00]

490.1471

475.3251 477.1351 481.3679 485.3030 487.1389
490.0357
491.1504
492.1530
496.1034 499.1983 503.3557 506.1209 508.1162

Relative Abundance

NL:
7.45E5

$C_{25} H_{25} NNaO_8$:
$C_{25} H_{25} N_1 Na_1 O_8$
pa Chrg 1

490.1472

491.1506
492.1539 494.1582 497.1658

Relative Abundance

m/z

245

¹H NMR (500 MHz, CDCl₃) of (4-methoxy-3-((2-(4-nitrophenyl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**22**)
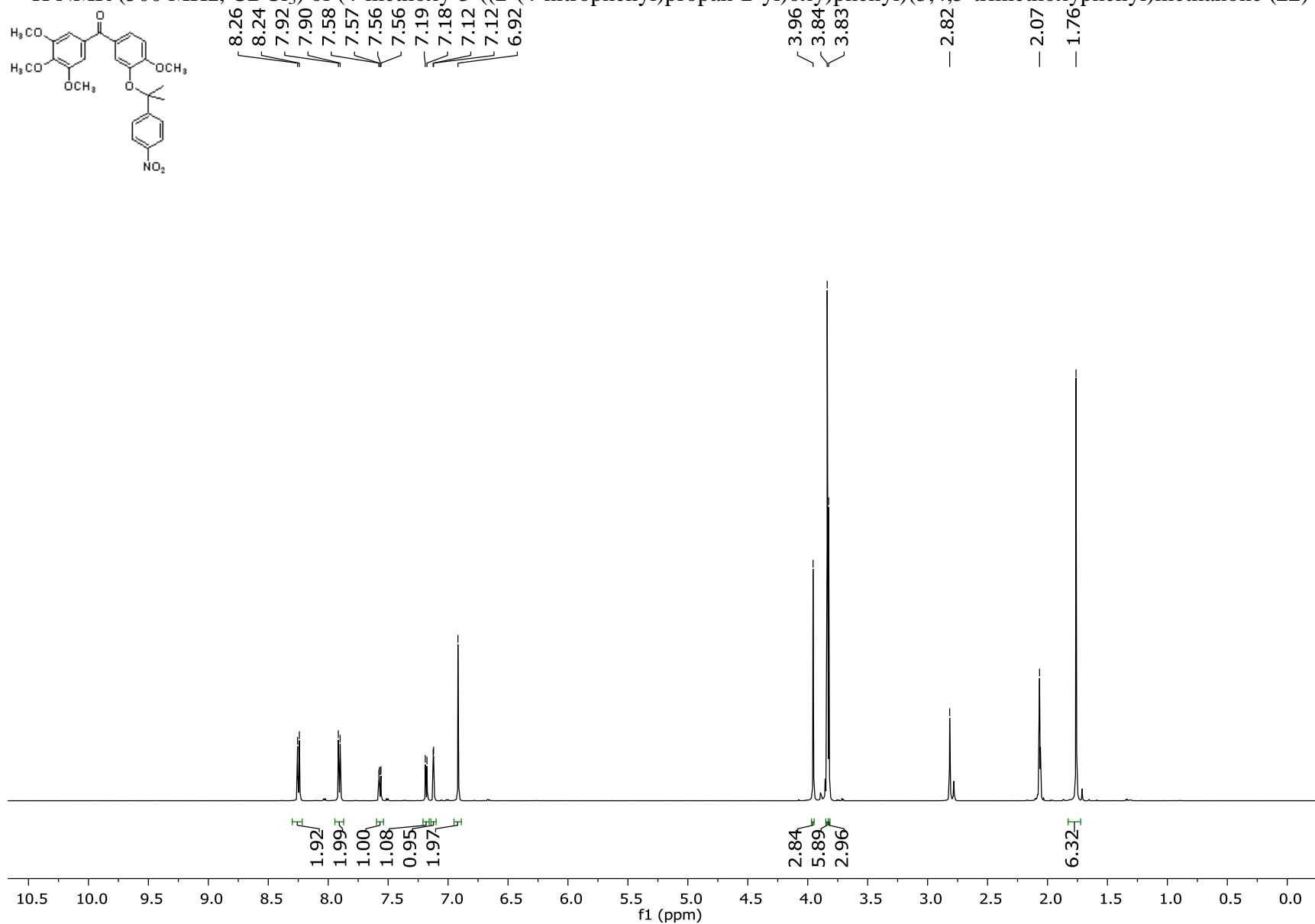
^{13}C NMR (500 MHz, CDCl$_3$) of (4-methoxy-3-((2-(4-nitrophenyl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**22**)
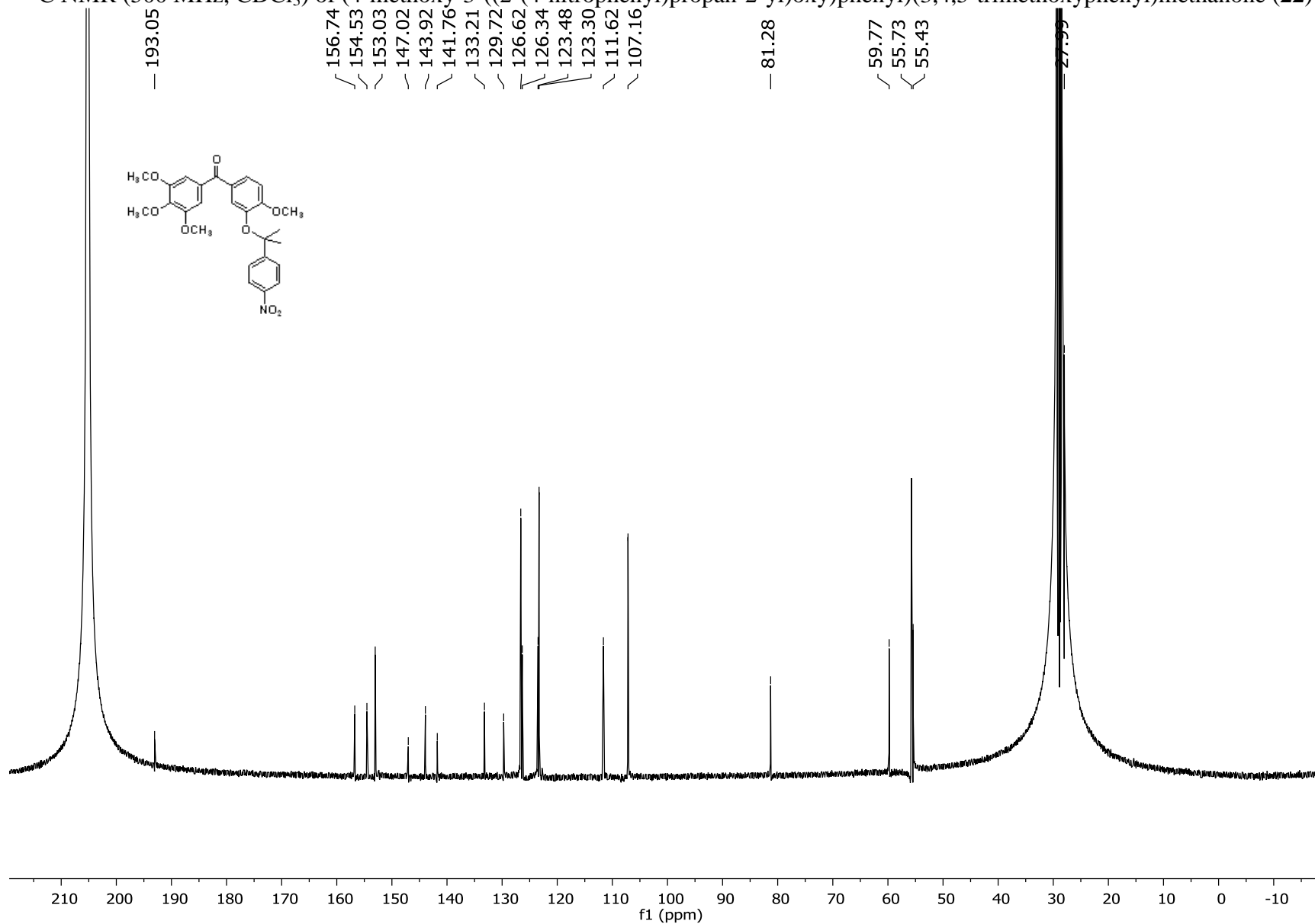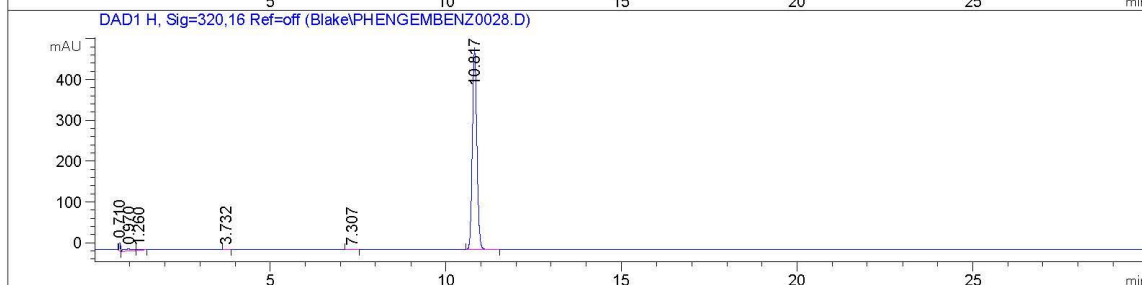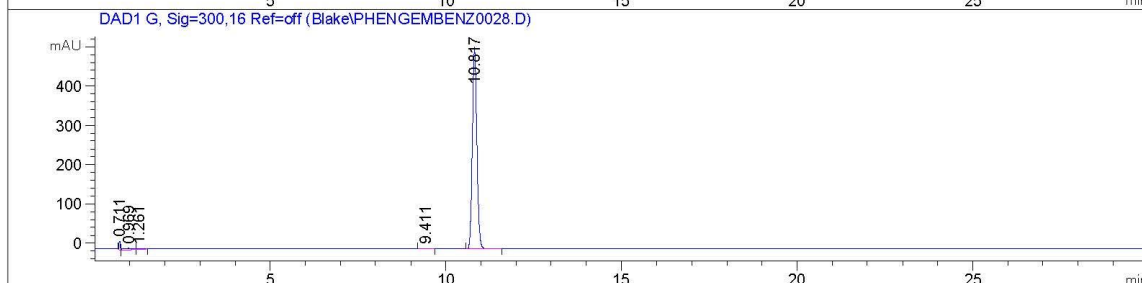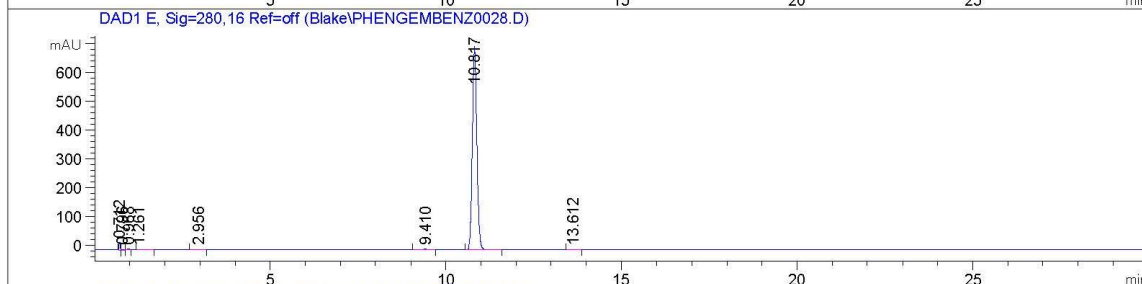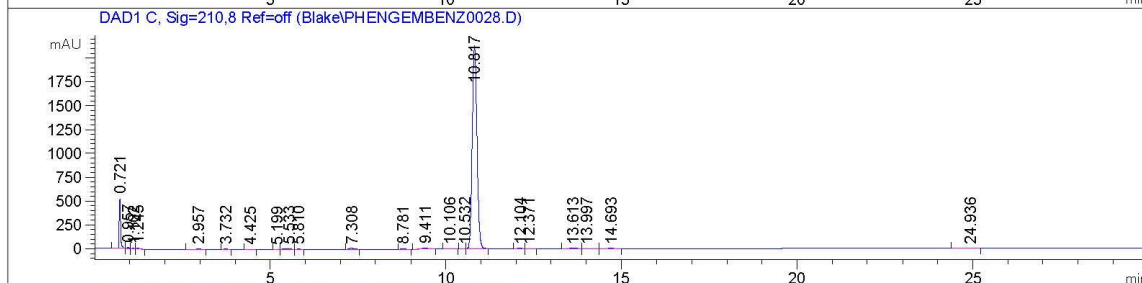
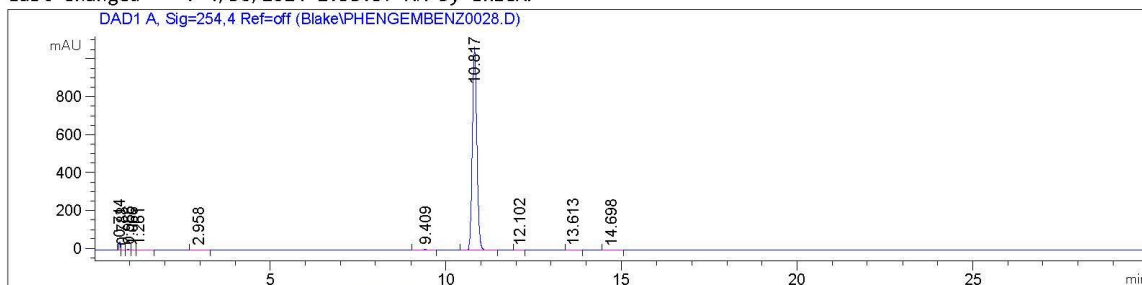# HPLC trace of Compound **22**

Data File C:\Chem32\1\Data\Blake\PHENGEMBENZ0028.D
Sample Name: PhenstatinGembenzyltrig

```
=====================================================================
                        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	0.714	BB	0.0395	102.19965	43.34666	1.0177
2	0.788	BV	0.0756	11.59024	2.19634	0.1154
3	0.966	VV	0.0785	23.34126	4.22338	0.2324
4	1.066	VB	0.0945	8.40134	1.47398	0.0837
5	1.261	BB	0.1233	14.02321	1.55368	0.1396
6	2.958	BB	0.1362	18.88556	2.11404	0.1881
7	9.409	BB	0.1514	48.99464	4.95399	0.4879
8	10.817	BB	0.1399	9753.58301	1074.87256	97.1235
9	12.102	BV	0.1431	19.73879	2.14927	0.1966
10	13.613	BB	0.1765	24.62234	2.10450	0.2452
11	14.698	BB	0.1917	17.07420	1.40602	0.1700

Totals :   1.00425e4   1140.39442

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.721	BV	0.0447	1461.52954	521.25201	6.5254
2	0.957	VB	0.0674	64.74452	14.14843	0.2891
3	1.102	BB	0.0571	15.24200	4.12479	0.0681
4	1.245	BB	0.0672	51.74103	11.34311	0.2310
5	2.957	BB	0.1593	54.75251	4.94014	0.2445
6	3.732	BB	0.1021	22.23397	3.40823	0.0993
7	4.425	BB	0.1374	10.97531	1.21530	0.0490
8	5.199	BV	0.1054	6.87328	1.01010	0.0307
9	5.533	VV	0.1920	37.97945	2.73436	0.1696
10	5.810	VB	0.1214	14.23922	1.81732	0.0636
11	7.308	BB	0.1311	62.97500	7.41682	0.2812
12	8.781	BB	0.1254	10.26077	1.28221	0.0458
13	9.411	BB	0.1549	112.26086	11.20404	0.5012
14	10.106	BB	0.1466	11.22099	1.18407	0.0501
15	10.532	BV	0.1069	18.19983	2.68996	0.0813
16	10.817	VB	0.1487	2.02327e4	2132.34351	90.3351
17	12.104	BV	0.1433	44.72208	4.86408	0.1997
18	12.371	VB	0.1659	11.08892	1.02774	0.0495
19	13.613	BV	0.1889	70.74862	5.54352	0.3159
20	13.997	VB	0.1912	17.23422	1.27911	0.0769
21	14.693	BB	0.1903	41.92006	3.43787	0.1872

Data File C:\Chem32\1\Data\Blake\PHENGEMBENZ0028.D
Sample Name: PhenstatinGembenzyltrig

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
22	24.936	BB	0.1983	23.75110	1.84351	0.1060

Totals :   2.23974e4 2740.11024

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.712	BB	0.0393	61.94859	26.47076	0.9453
2	0.796	BV	0.0805	16.87462	3.05010	0.2575
3	0.968	VB	0.0748	18.17283	3.48493	0.2773
4	1.261	BB	0.1265	10.94446	1.17680	0.1670
5	2.956	BB	0.1265	11.04522	1.30888	0.1685
6	9.410	BB	0.1523	31.17676	3.12741	0.4757
7	10.817	BB	0.1397	6391.30029	705.65088	97.5262
8	13.612	BB	0.1755	11.95675	1.02930	0.1825

Totals :   6553.41952 745.29904

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.711	BB	0.0393	51.22318	21.89296	1.0584
2	0.969	BV	0.2164	85.29426	4.91742	1.7624
3	1.261	VB	0.1406	22.31900	2.05697	0.4612
4	9.411	BB	0.1509	13.82055	1.40350	0.2856
5	10.817	BB	0.1396	4667.10986	515.53510	96.4325

Totals :   4839.76686 545.80594

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.710	BB	0.0391	45.33421	19.50666	0.9726
2	0.970	BV	0.2400	94.89272	4.89924	2.0358
3	1.260	VB	0.1407	23.02846	2.12033	0.4941
4	3.732	VB	0.1022	7.11667	1.06216	0.1527
5	7.307	BB	0.1299	8.64792	1.03083	0.1855
6	10.817	BB	0.1395	4482.10010	495.50699	96.1593

Totals :   4661.12007 524.12621

Data File C:\Chem32\1\Data\Blake\PHENGEMBENZ0028.D
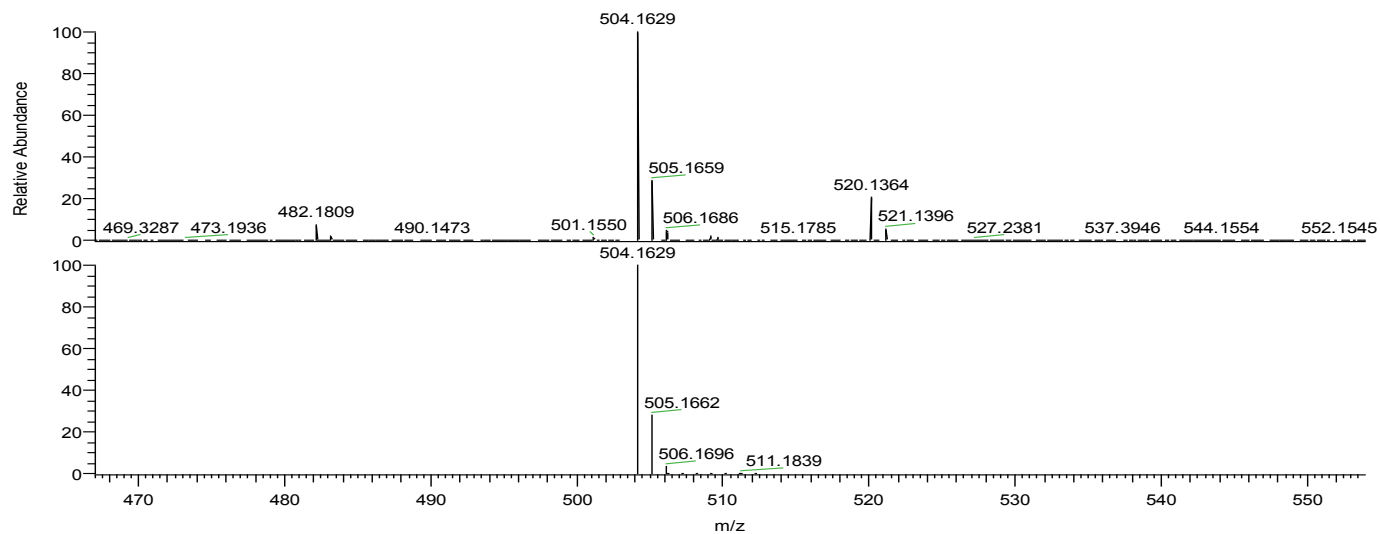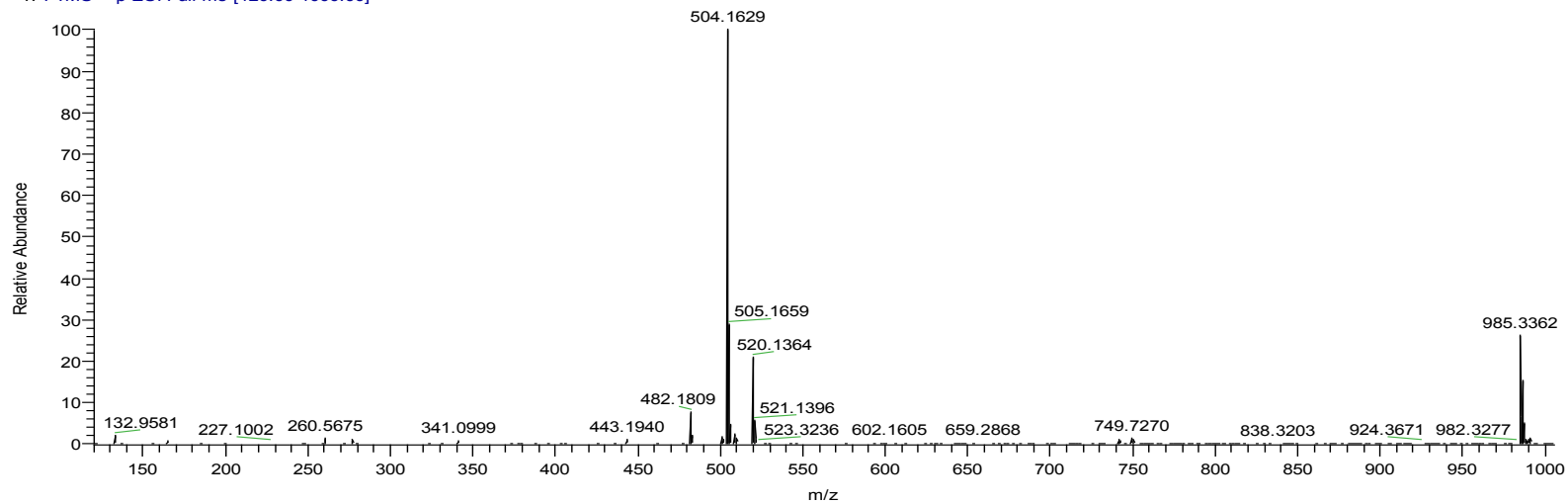Sample Name: PhenstatinGembenzyltrig

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*** End of Report ***

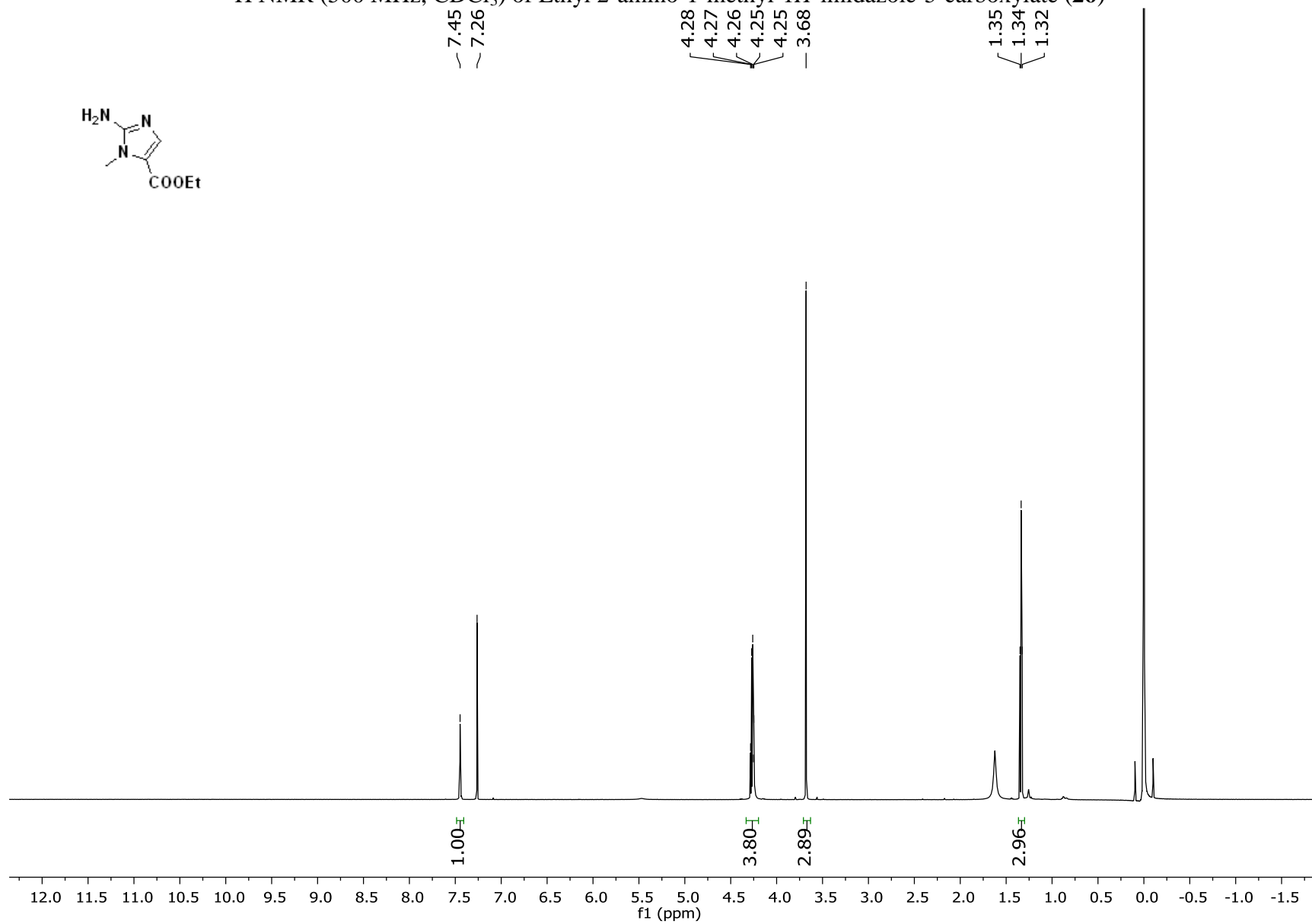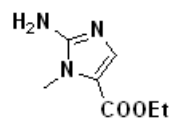# Mass Spectrum of Compound 22

6/27/2016 3:58:29 PM

gem benzyl_Orbi_+ESI #2-20   RT: 0.01-0.19   AV
T: FTMS + p ESI Full ms [120.00-1000.00]

NL:
7.47E6
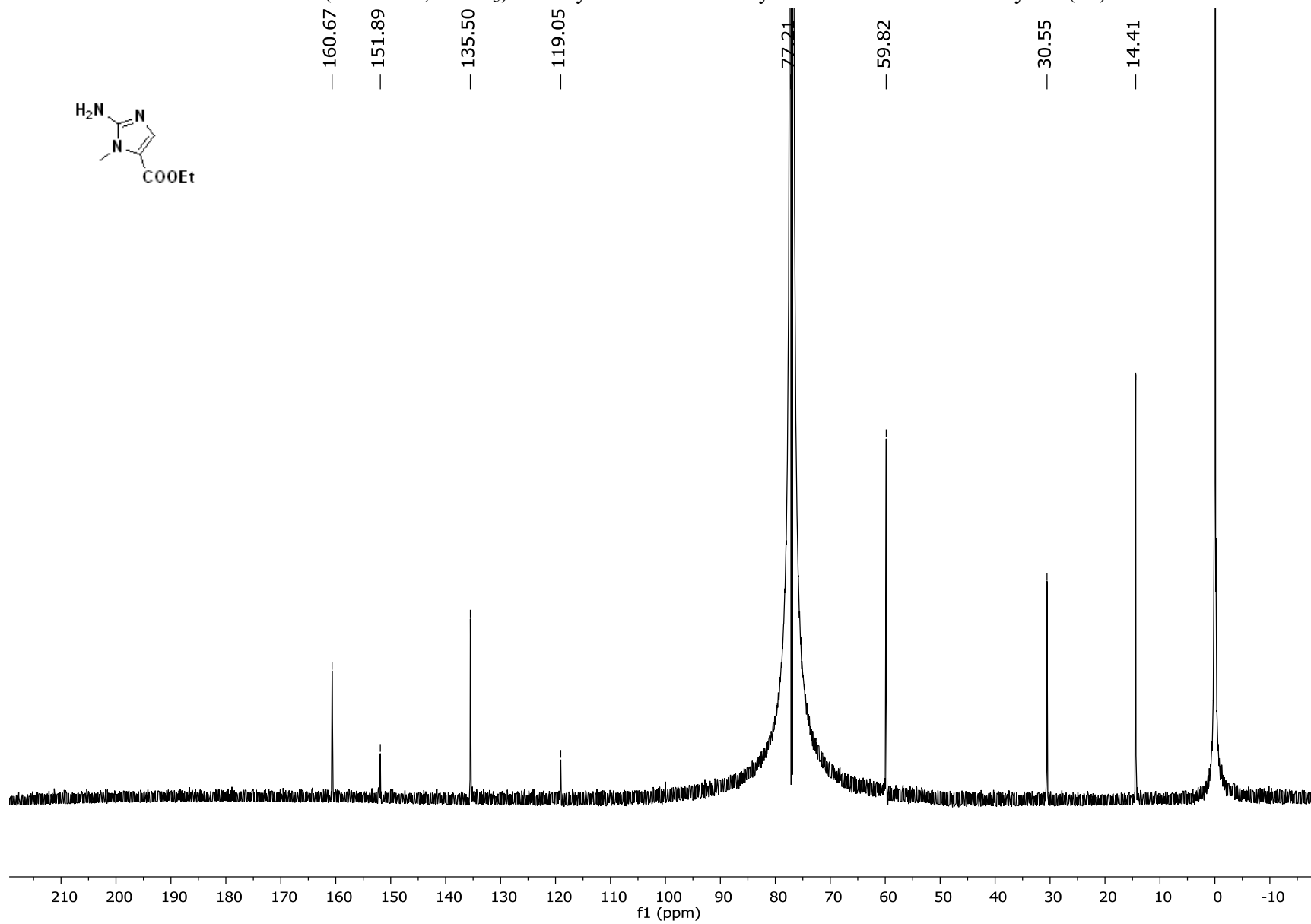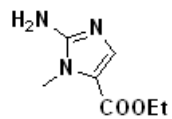gem benzyl_Orbi_+ESI#2-20 RT: 0.01-0.19 AV: 19 T: FTMS + p ESI Full ms [120.00-1000.00]

NL:
7.36E5

$C_{26}H_{27}NNaO_8$:
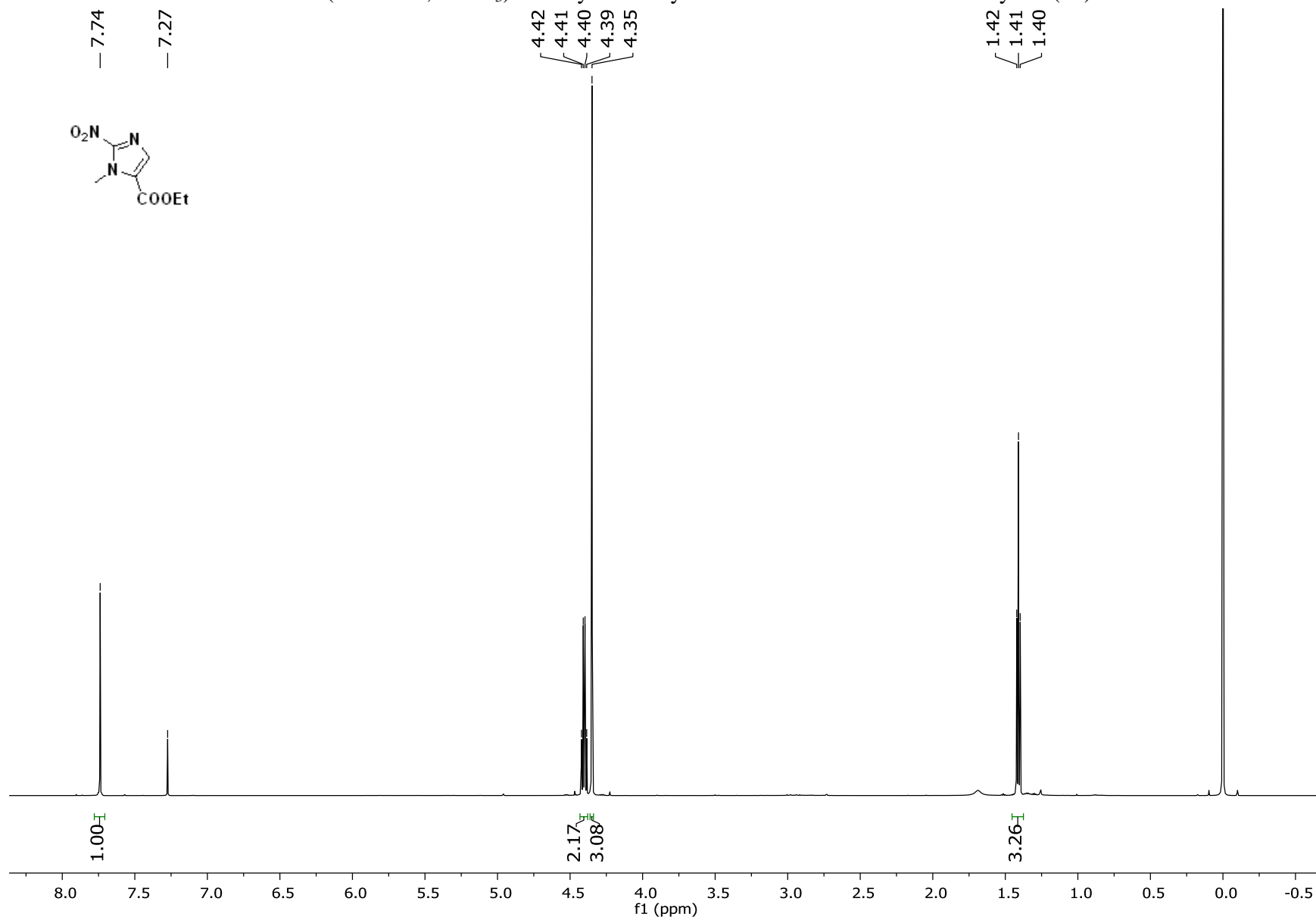$C_{26}H_{27}N_1Na_1O_8$
pa Chrg 1

252

^{1}H NMR (500 MHz, CDCl$_3$) of Ethyl 2-amino-1-methyl-1*H*-imidazole-5-carboxylate (**26**)
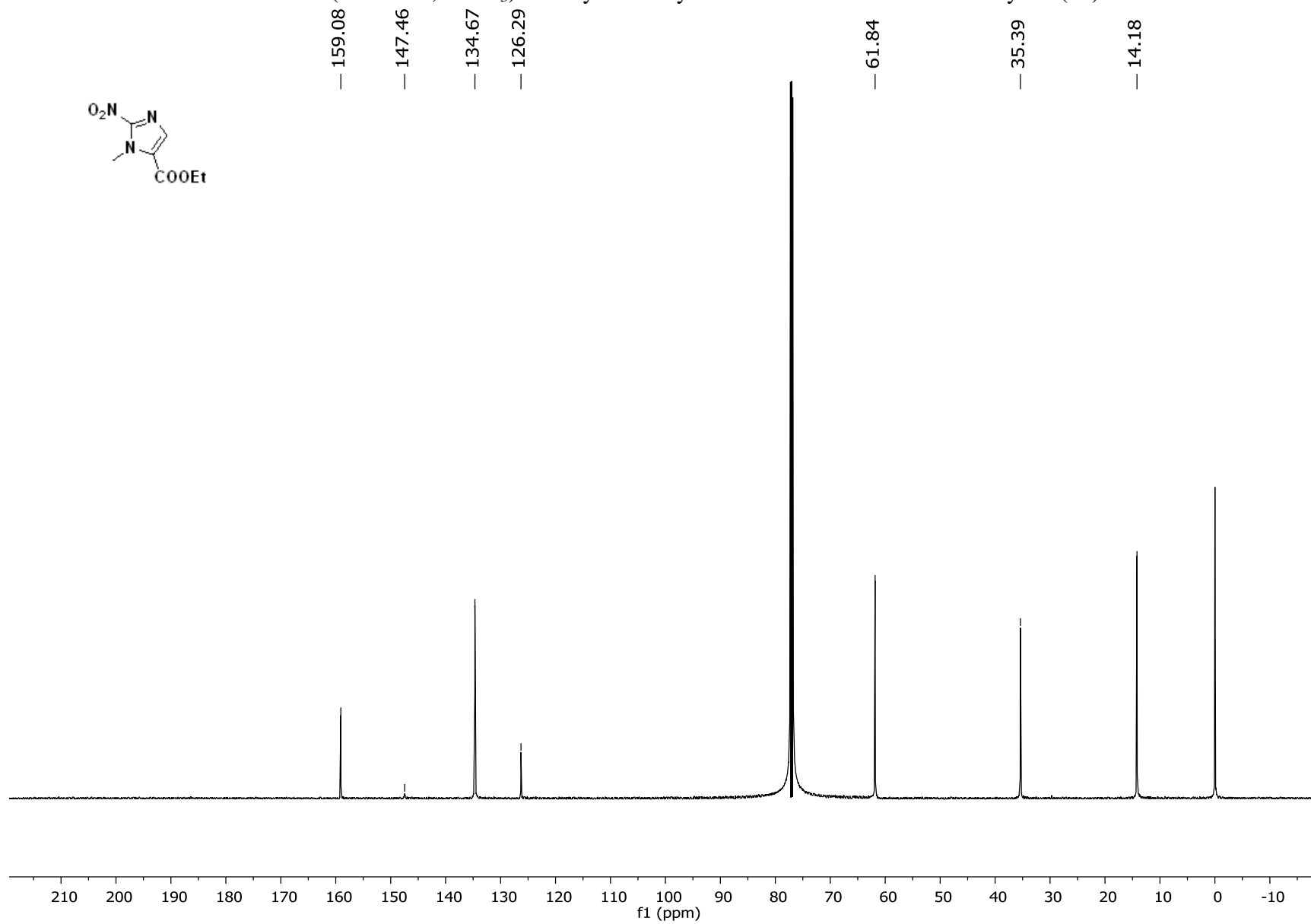
^{13}C NMR (500 MHz, CDCl$_3$) of Ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate (**26**)

^{1}H NMR (500 MHz, CDCl$_3$) of Ethyl 1-methyl-2-nitro-1H-imidazole-5-carboxylate (**27**)
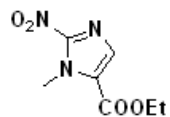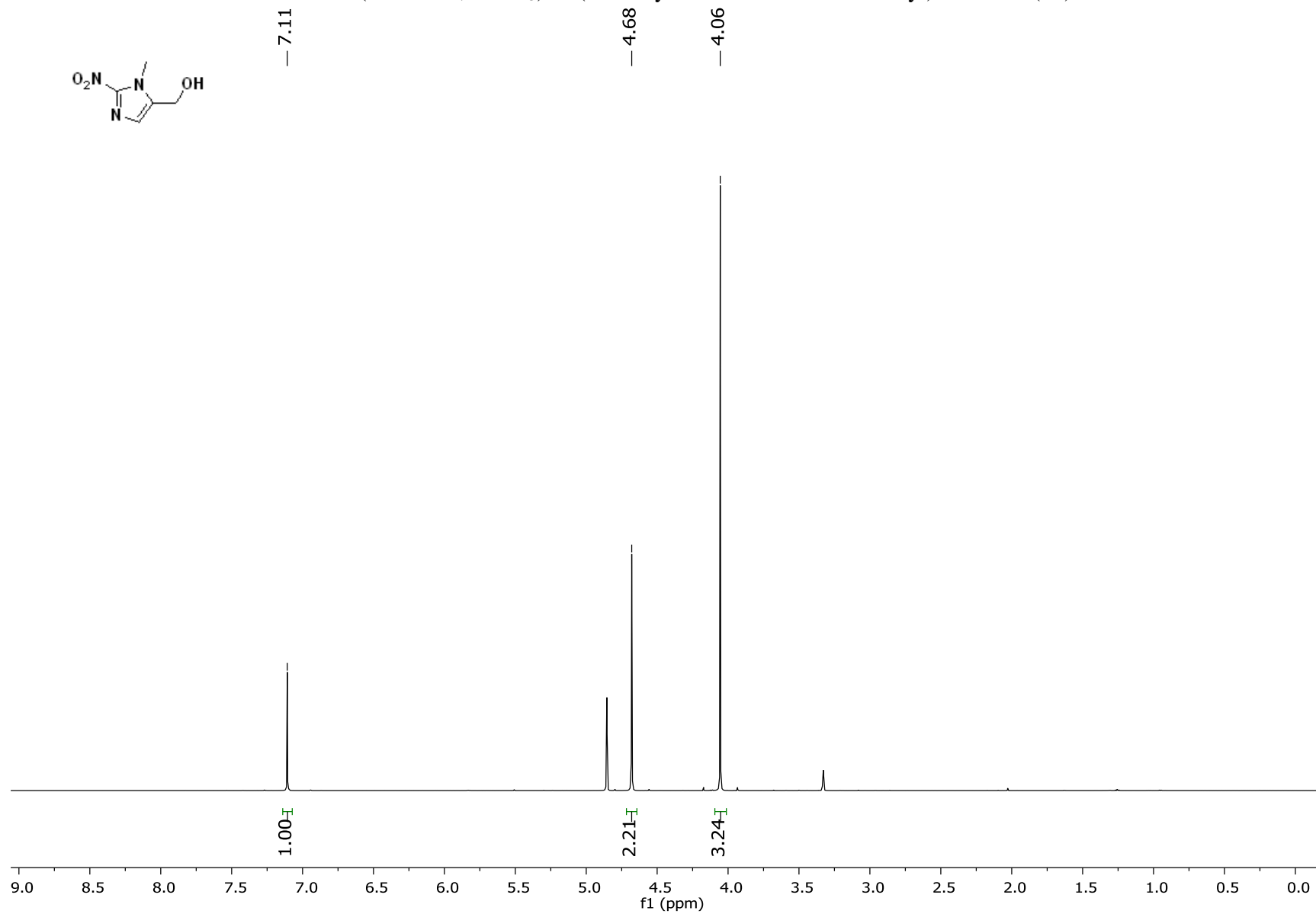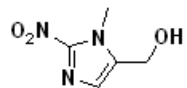
255

^{13}C NMR (500 MHz, CDCl$_3$) of Ethyl 1-methyl-2-nitro-1H-imidazole-5-carboxylate (**27**)

¹H NMR (500 MHz, CDCl₃) of (1-methyl-2-nitro-1*H*-imidazol-5-yl)methanol (**28**)

^{1}H NMR (500 MHz, CDCl$_3$) of (1-methyl-2-nitro-1H-imidazol-5-yl)methanol (**28**)

^{13}C NMR (500 MHz, CDCl$_3$) of (1-methyl-2-nitro-1H-imidazol-5-yl)methanol (**28**)

145.82
137.93
126.02
53.16
33.40

258

^{1}H NMR (500 MHz, CDCl$_3$) of 1-Methyl-2-nitro-1H-imidazole-5-carbaldehyde (**29**)

259
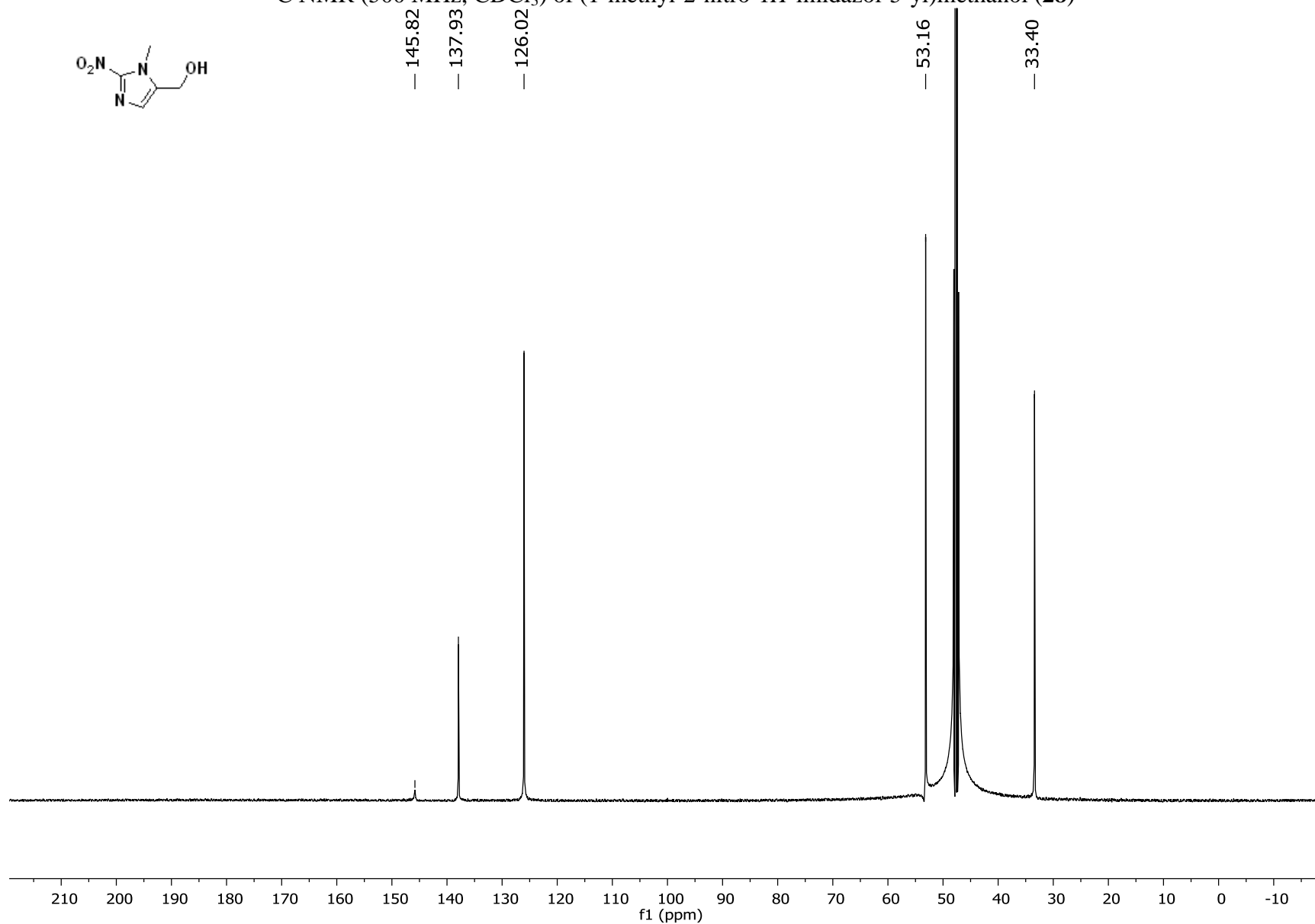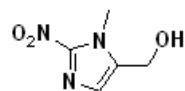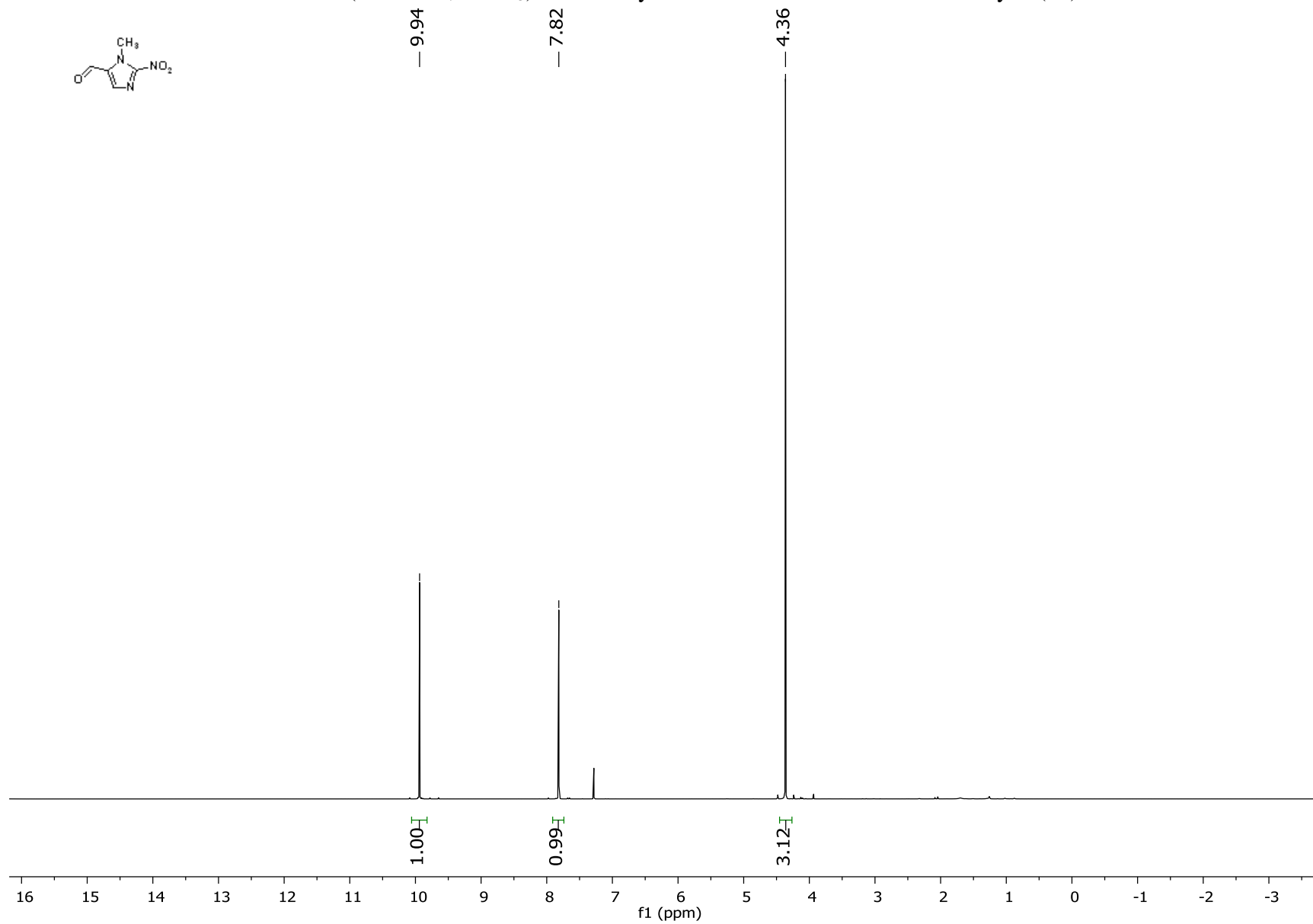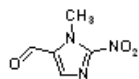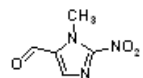
^{13}C NMR (500 MHz, CDCl$_3$) of 1-Methyl-2-nitro-1*H*-imidazole-5-carbaldehyde (**29**)

180.39

148.35

139.38

132.38

77.26
77.05
76.88
76.83

35.57

f1 (ppm)

260
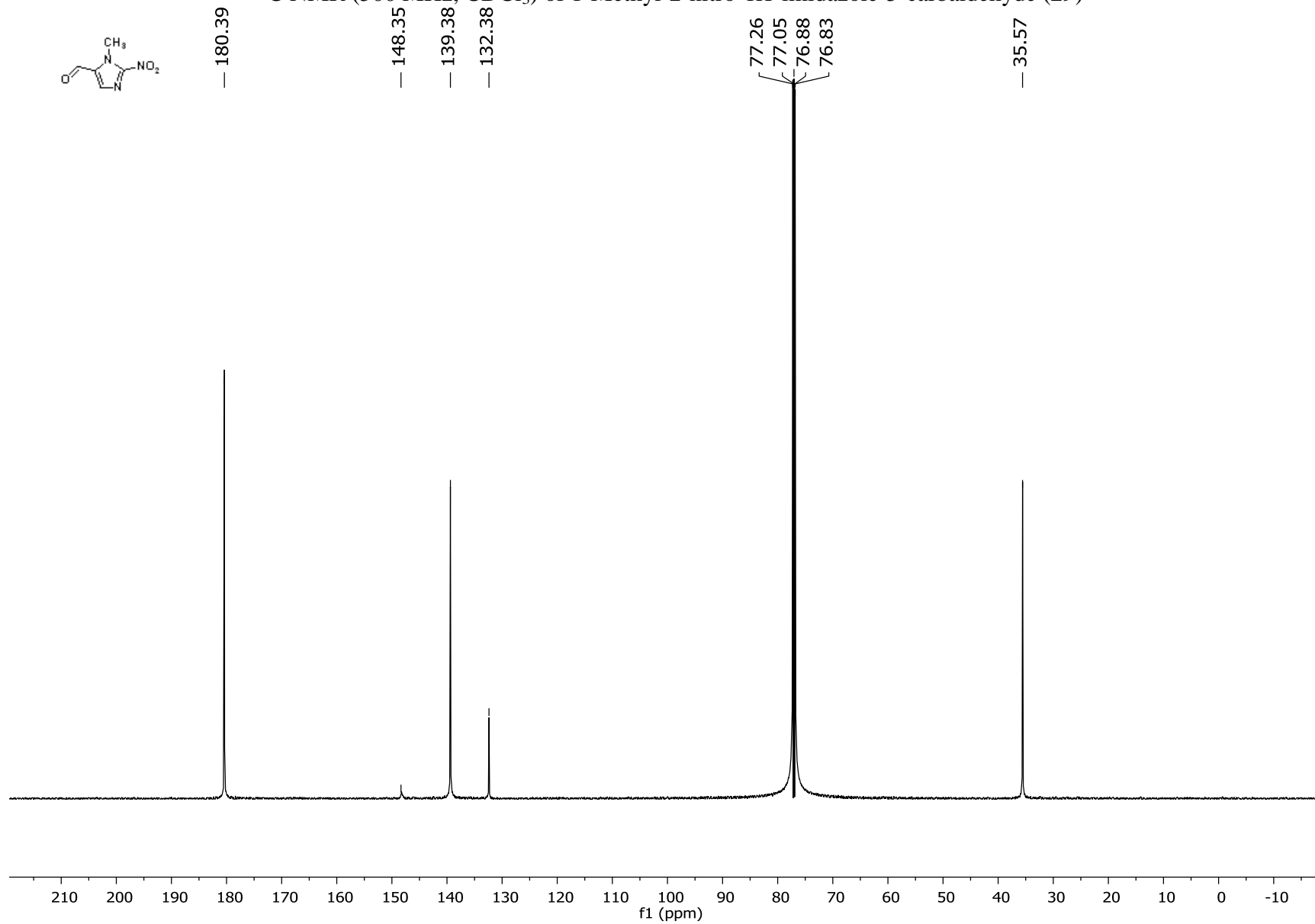
^{13}C NMR (500 MHz, Acetone d-6) of 1-(1-Methyl-2-nitro-1H-imidazol-5-yl)ethan-1-ol (**30**)
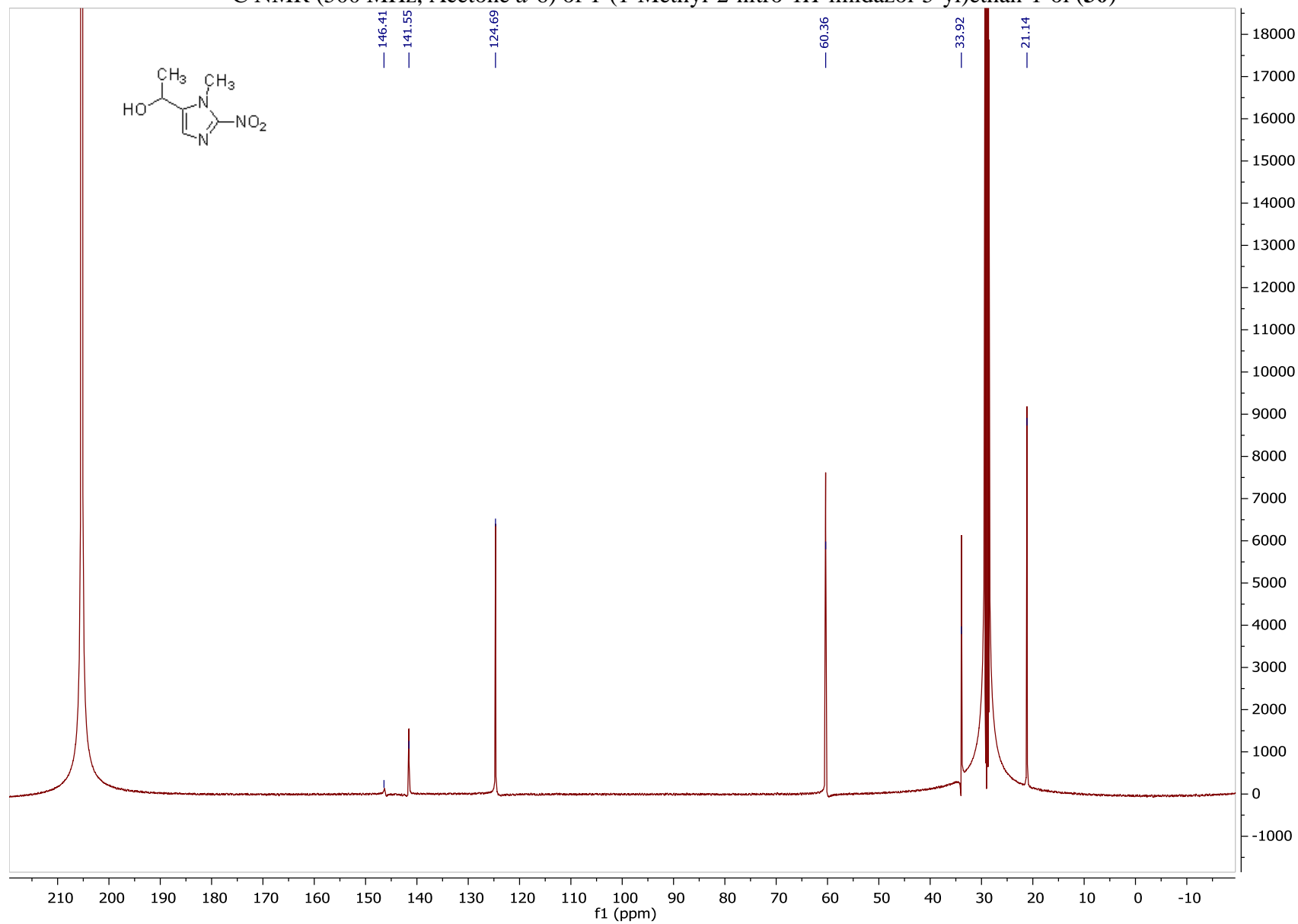
262

¹H NMR (500 MHz, CDCl₃) of (4-methoxy-3-((1-methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**31**)
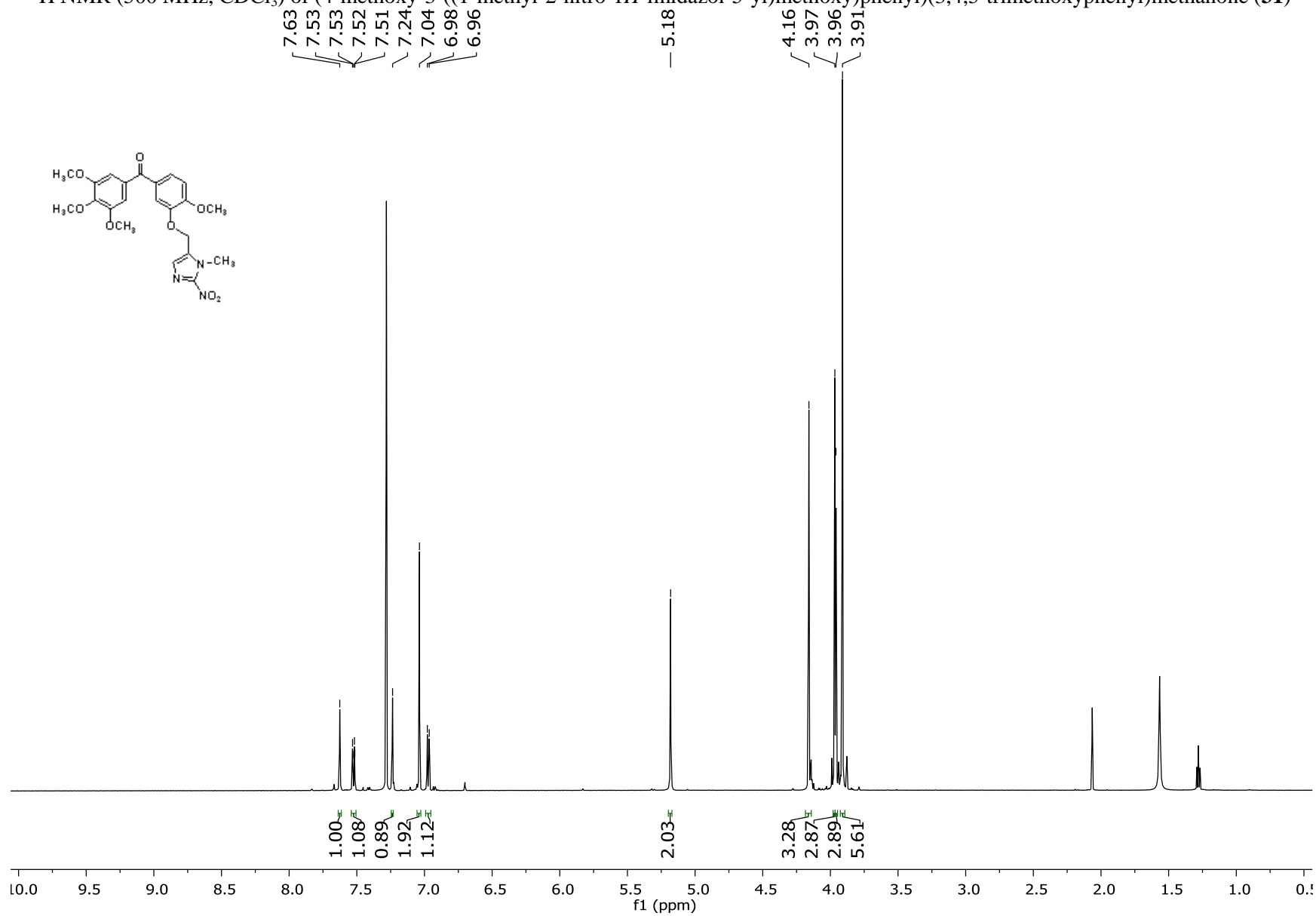
¹³C NMR (500 MHz, CDCl₃) of (4-methoxy-3-((1-methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**31**)
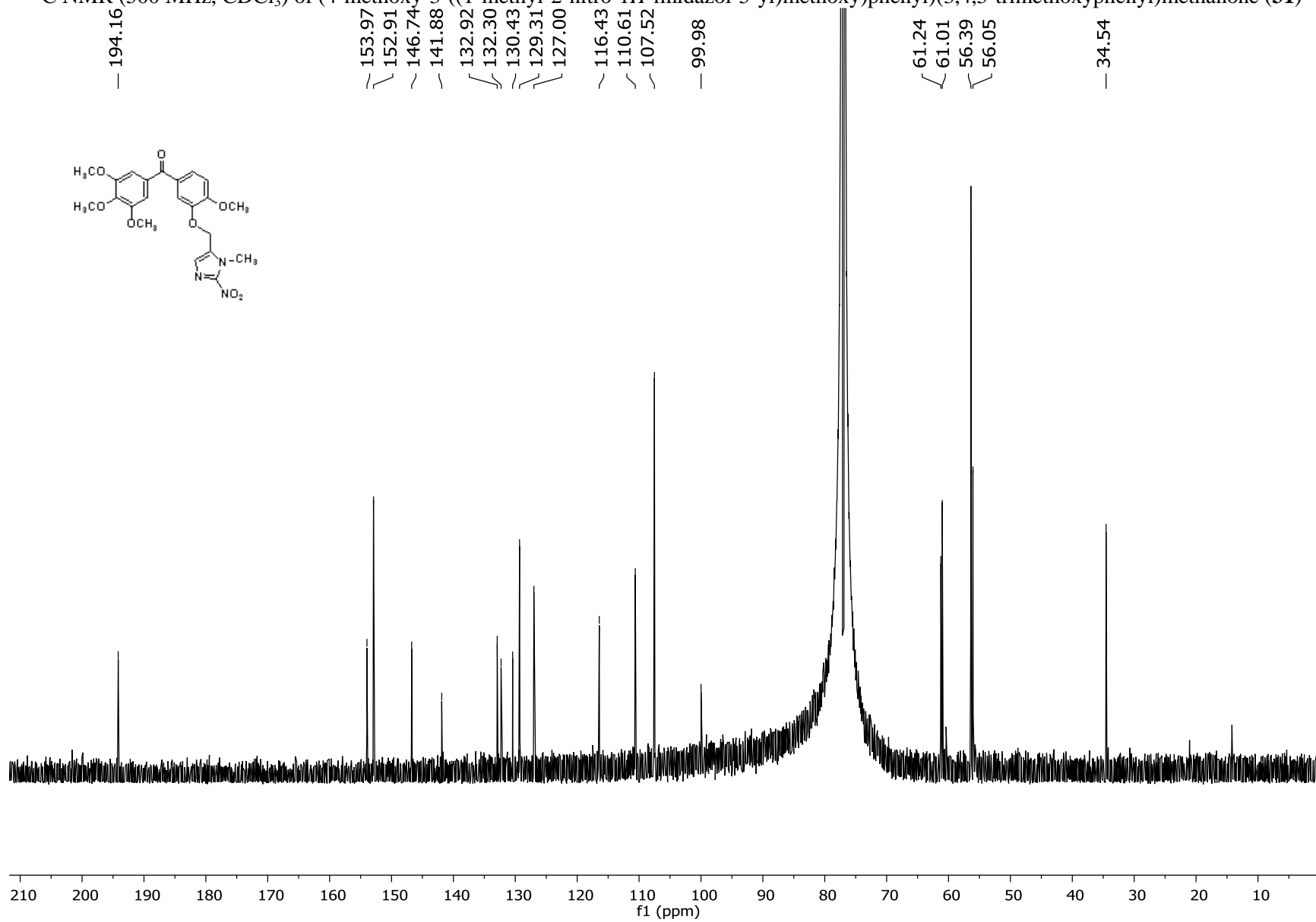
HPLC trace of Compound **31**

```
===================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC                 Location :   1
Injection Date  : 6/27/2016 10:16:41 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
```

DAD1 H, Sig=320,16 Ref=off (Graham\GJC-II-62 2016-06-27 10-16-41.D)
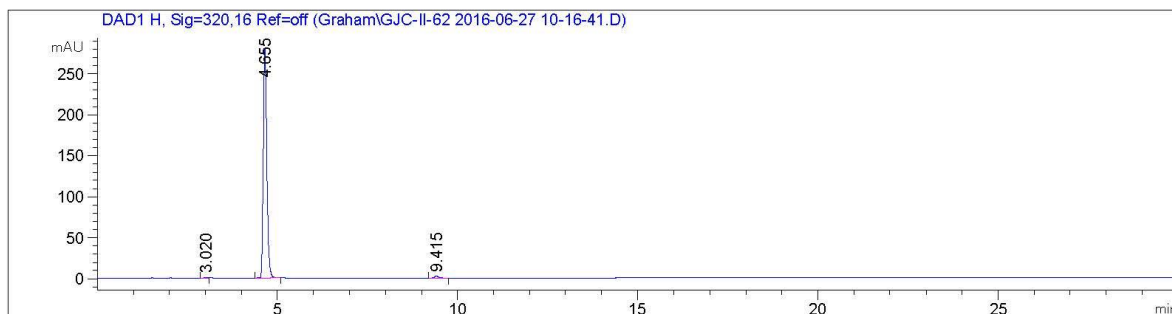
```
===================================================================
                      Area Percent Report
===================================================================

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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   4.655 BB   0.1037  492.90375   73.97382 100.0000

Totals :                  492.90375   73.97382



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

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   1.133 BB   0.0631   45.22847   10.32059   1.2369
  2   1.705 BB   0.0687   26.42975    5.85398   0.7228
  3   3.019 BB   0.1103   25.94071    3.35139   0.7094
  4   4.655 BB   0.1038 3395.83325  509.36142  92.8674
  5   9.415 BB   0.1376   60.64553    6.82763   1.6585
  6  20.074 BB   0.1800   41.57561    3.56745   1.1370
  7  24.961 BB   0.1989   60.99513    4.65493   1.6681

Totals :                 3656.64845  543.93739



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

```
Peak RetTime Type  Width     Area       Height     Area
  #   [min]       [min]   [mAU*s]    [mAU]      %
----|-------|----|-------|----------|----------|--------|
  1   4.655 BB   0.1017 1203.02307  180.74945  98.3370
  2   9.415 BB   0.1378   20.34402    2.28747   1.6630

Totals :                 1223.36709  183.03692
```

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

```
Peak RetTime Type  Width     Area       Height     Area
  #   [min]       [min]   [mAU*s]    [mAU]      %
----|-------|----|-------|----------|----------|--------|
  1   4.655 BB   0.1016 1759.79248  264.55835  98.5151
  2   9.415 BB   0.1385   26.52550    2.96211   1.4849

Totals :                 1786.31798  267.52046
```

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

```
Peak RetTime Type  Width     Area       Height     Area
  #   [min]       [min]   [mAU*s]    [mAU]      %
----|-------|----|-------|----------|----------|--------|
  1   3.020 BV   0.0863    6.43352    1.13186   0.3403
  2   4.655 BB   0.1017 1863.36987  279.61612  98.5610
  3   9.415 BB   0.1386   20.77160    2.31634   1.0987

Totals :                 1890.57500  283.06432
```

```
======================================================================
                      *** End of Report ***
```

267

Mass Spectrum of Compound **31**

nor imidazole_orbi+ESI #1-20 RT: 0.01-0.23 AV
T: FTMS + p ESI Full ms [150.00-700.00]
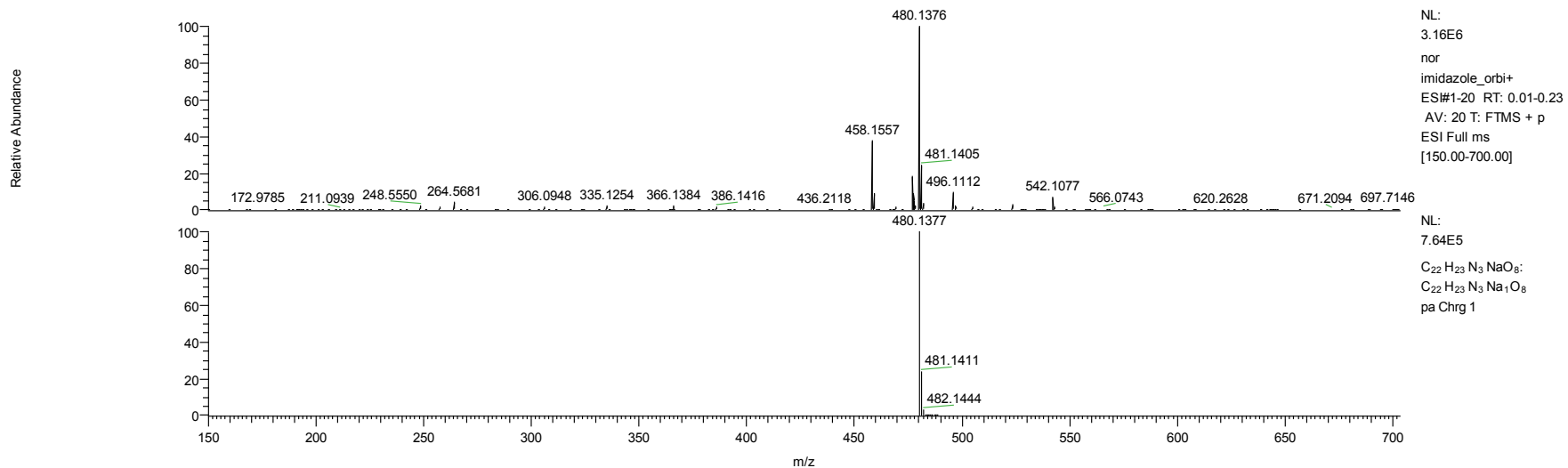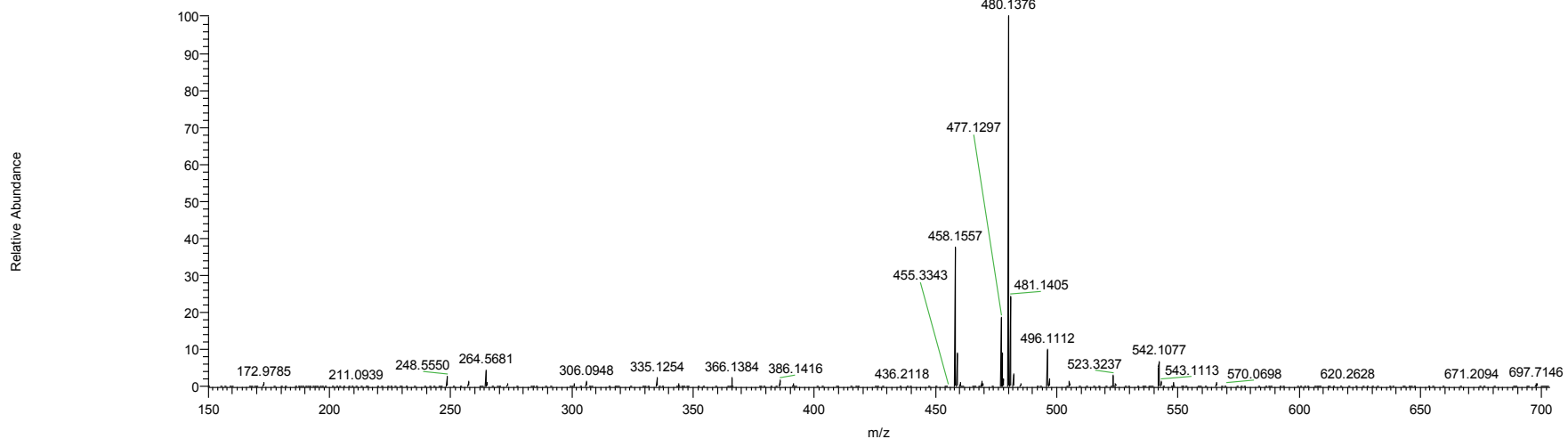
NL:
3.16E6
nor
imidazole_orbi+
ESI#1-20 RT: 0.01-0.23
AV: 20 T: FTMS + p
ESI Full ms
[150.00-700.00]

NL:
7.64E5

$C_{22}H_{23}N_3NaO_8$:
$C_{22}H_{23}N_3Na_1O_8$
pa Chrg 1

^{1}H NMR (500 MHz, CDCl$_3$) of (4-methoxy-3-(1-(1-methyl-2-nitro-1H-imidazol-5-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**32**)
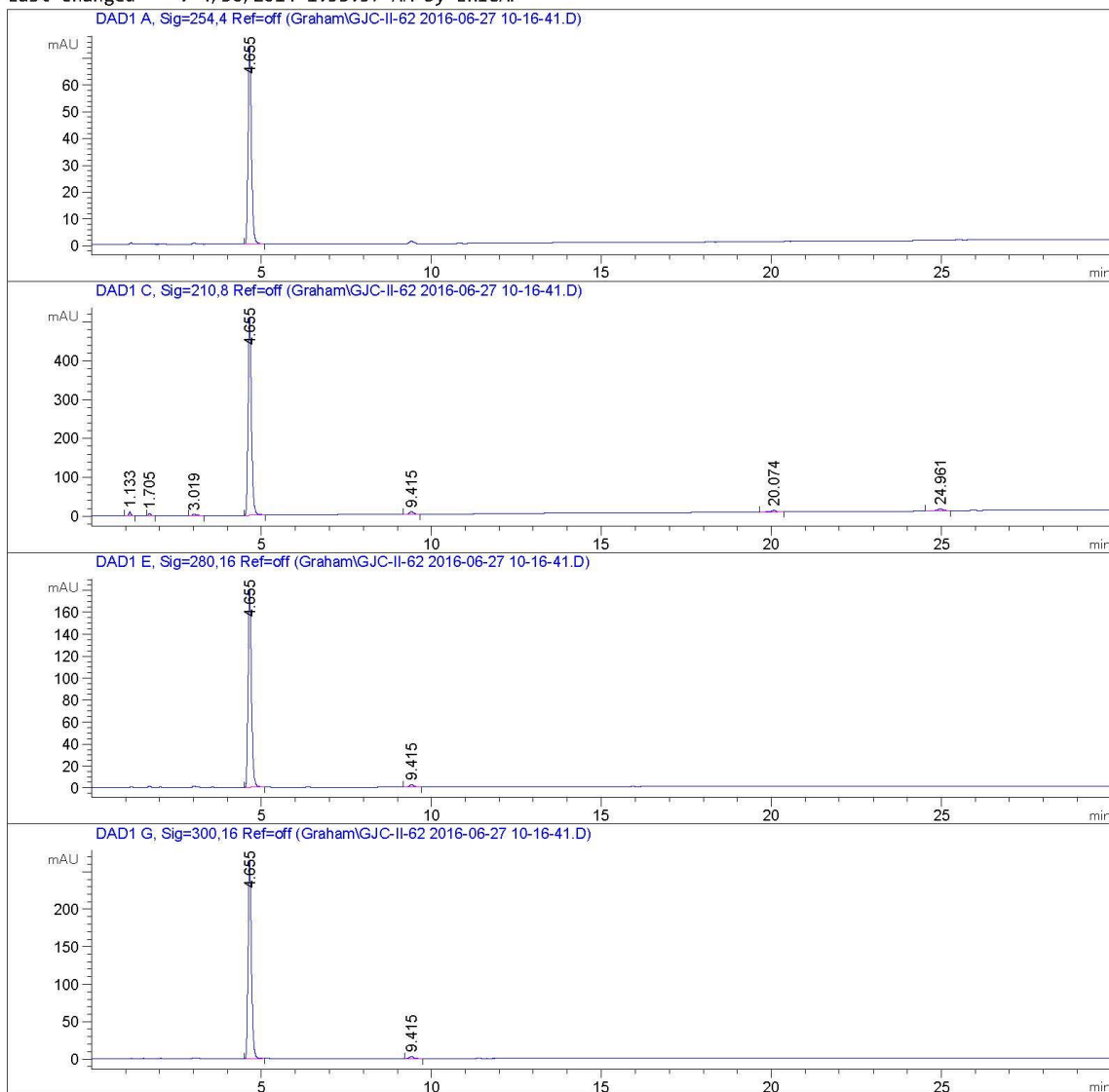
¹³C NMR (500 MHz, CDCl₃) of (4-methoxy-3-(1-(1-methyl-2-nitro-1*H*-imidazol-5-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**32**)
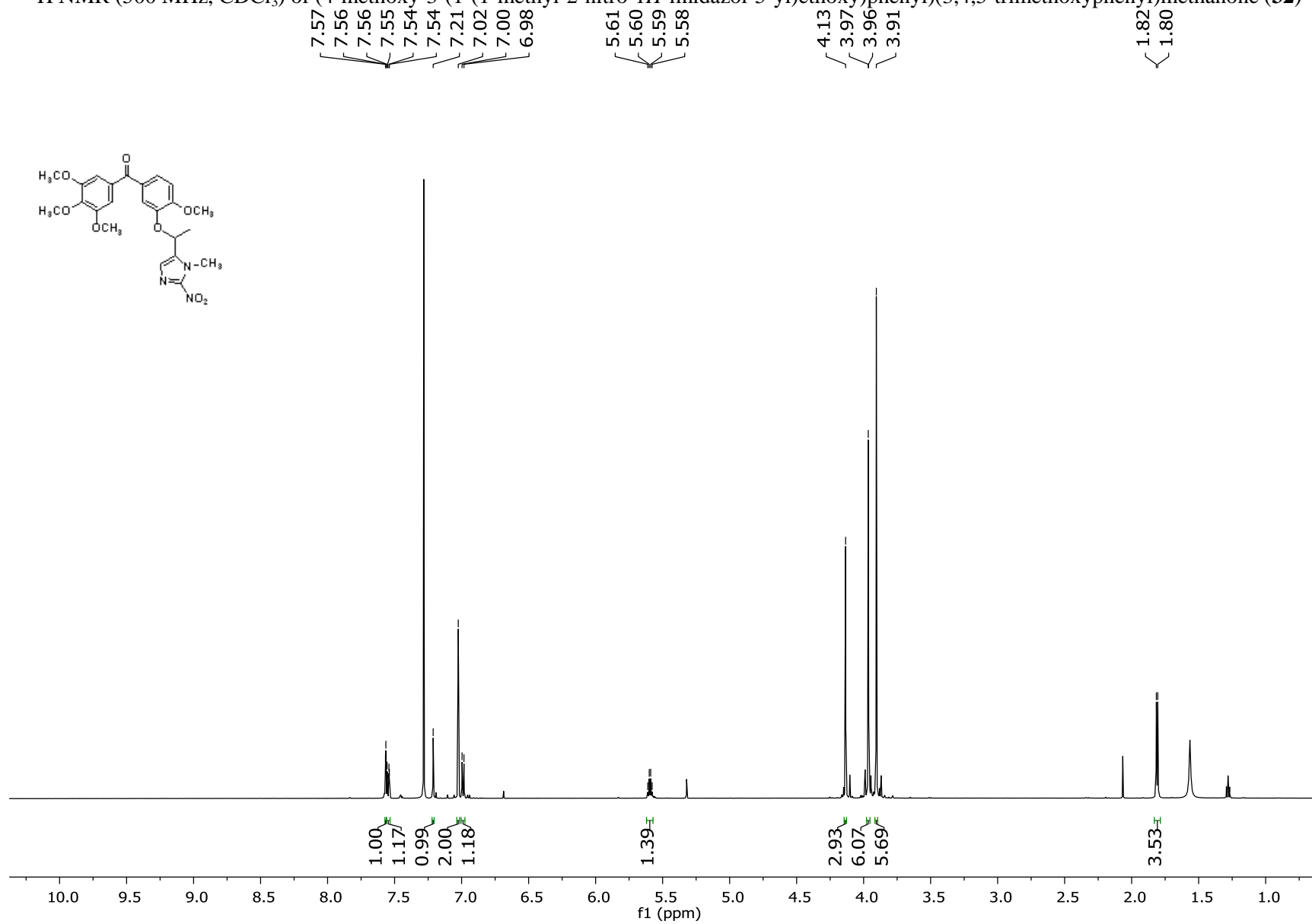
^{13}C NMR (500 MHz, CDCl$_3$) of (4-methoxy-3-(1-(1-methyl-2-nitro-1H-imidazol-5-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**32**)
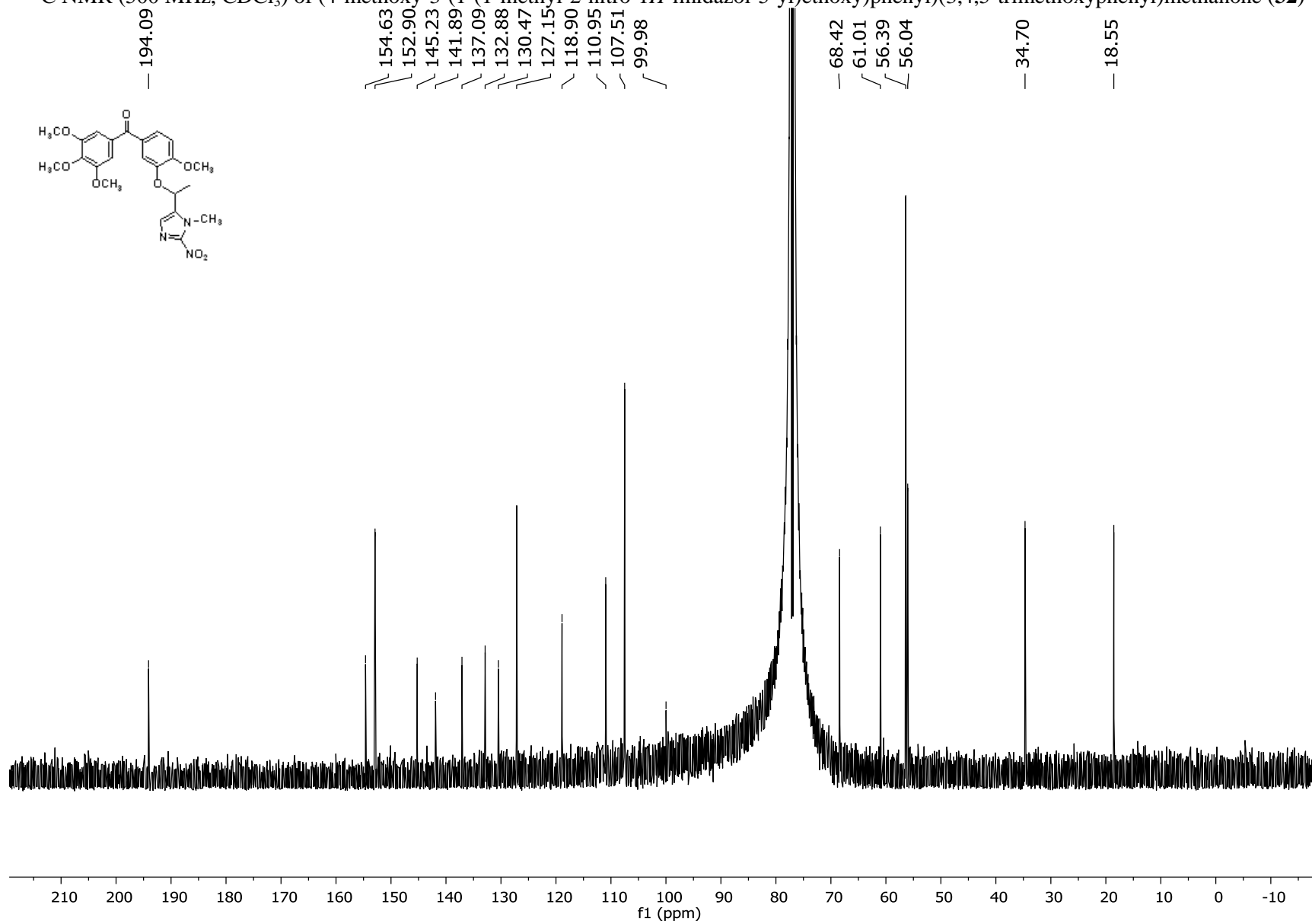
HPLC trace of compound **32**

```
==================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC              Location :      -
Injection Date  : 6/28/2016 9:33:37 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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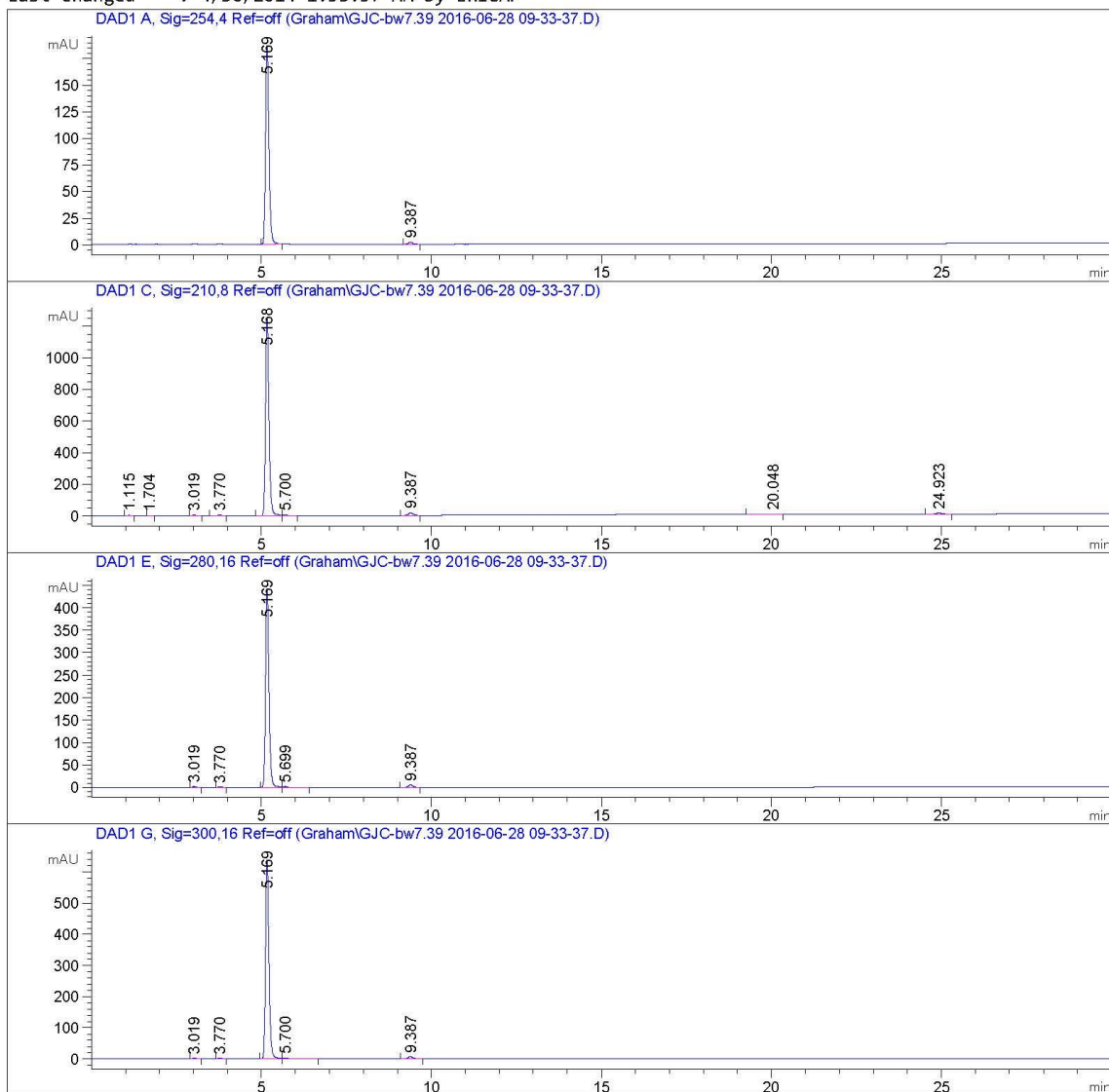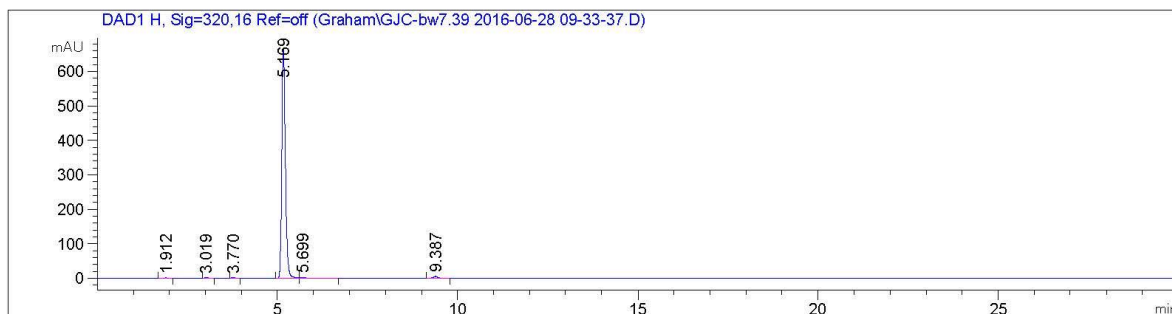
```
===================================================================
                        Area Percent Report
===================================================================

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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   5.169 BB    0.1079 1308.11023  186.41667  98.6448
  2   9.387 BB    0.1370   17.97061    1.99658   1.3552

Totals :                 1326.08084  188.41325




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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   1.115 BB    0.0636   20.10123    4.73168   0.2177
  2   1.704 BV    0.0693    8.56182    1.87530   0.0927
  3   3.019 BB    0.0982   41.66935    6.21576   0.4512
  4   3.770 BB    0.1098   35.03053    4.87756   0.3793
  5   5.168 BV    0.1083 8845.59863 1254.06128  95.7801
  6   5.700 VB    0.1295   45.40988    5.11972   0.4917
  7   9.387 BB    0.1394  138.76819   15.35983   1.5026
  8  20.048 BB    0.1804   40.14570    3.43492   0.4347
  9  24.923 BB    0.1998   60.03884    4.61281   0.6501

Totals :                 9235.32416 1300.28885
```

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.019	BB	0.0922	11.24072	1.81736	0.3528
2	3.770	BB	0.1068	8.59604	1.24050	0.2698
3	5.169	BV	0.1080	3104.03003	441.85226	97.4361
4	5.699	VB	0.1433	15.31075	1.52331	0.4806
5	9.387	BB	0.1395	46.52958	5.14450	1.4606

Totals :   3185.70712 451.57793

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.019	BB	0.0916	13.27996	2.16508	0.2894
2	3.770	BB	0.1061	11.34777	1.65200	0.2473
3	5.169	BV	0.1080	4477.59424	637.43597	97.5884
4	5.700	VB	0.1506	25.92229	2.42757	0.5650
5	9.387	BB	0.1397	60.09922	6.63421	1.3099

Totals :   4588.24348 650.31484

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.912	BB	0.0793	5.62801	1.03708	0.1181
2	3.019	BB	0.0924	13.15536	2.12034	0.2762
3	3.770	BB	0.1030	8.79059	1.29828	0.1845
4	5.169	BV	0.1080	4662.22168	663.61096	97.8683
5	5.699	VB	0.1514	26.86522	2.49865	0.5639
6	9.387	BB	0.1379	47.10875	5.18836	0.9889

Totals :   4763.76961 675.75368

===
*** End of Report ***

mono imidazole_orbi+ESI #2-20 RT: 0.01-0.20 .
T: FTMS + p ESI Full ms [150.00-700.00]
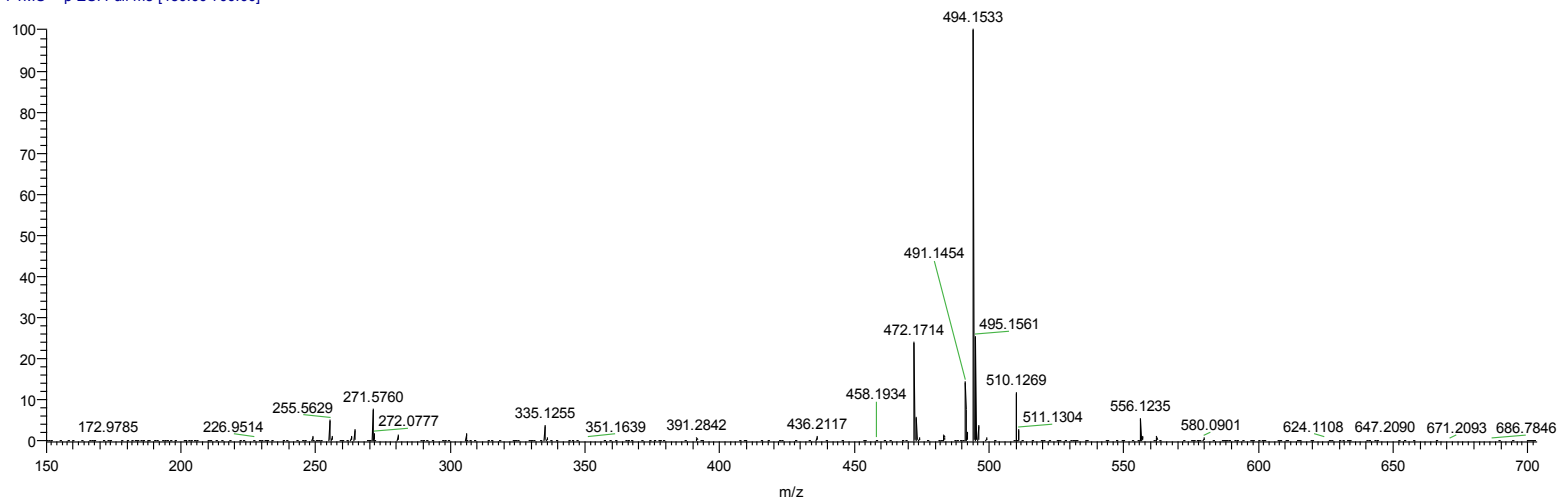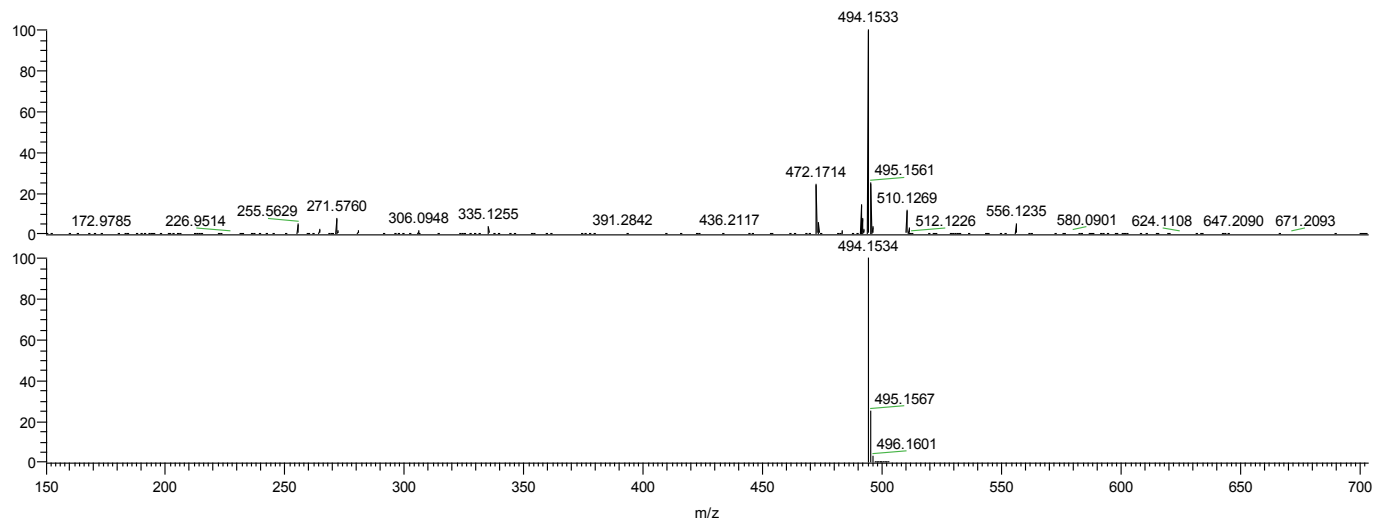
NL:
4.91E6
mono imidazole_orbi+ESI#2-20
RT: 0.01-0.20 AV: 19
T: FTMS + p ESI Full ms [150.00-700.00]

NL:
7.55E5
$C_{23}H_{25}N_3NaO_8$:
$C_{23}H_{25}N_3Na_1O_8$
pa Chrg 1

274

^{1}H NMR (500 MHz, CDCl$_3$) of (5-nitrofuran-2-yl)methanol (**34**)
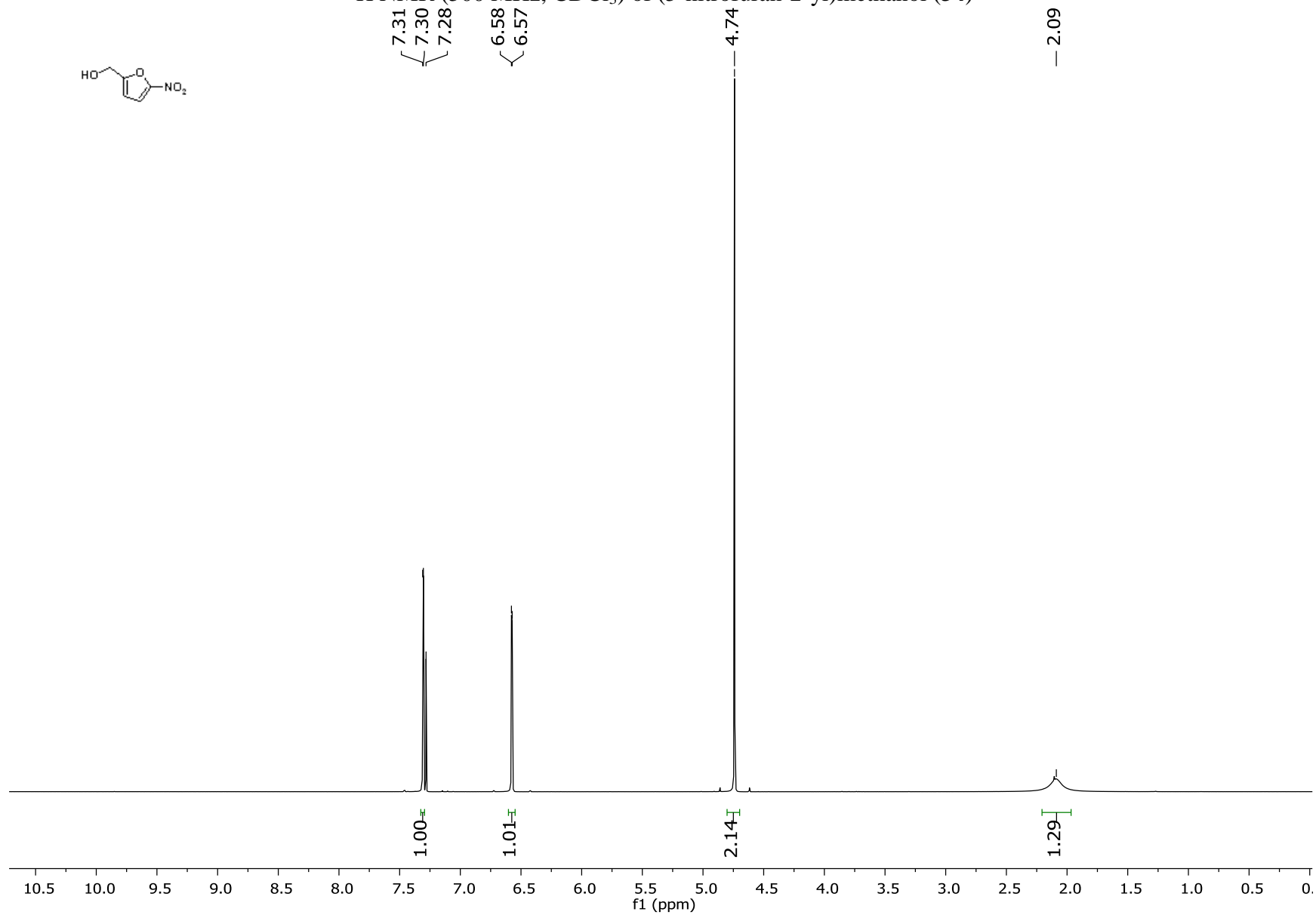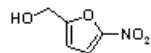
^{13}C NMR (500 MHz, CDCl$_3$) of (5-nitrofuran-2-yl)methanol (**34**)

157.4
151.9
112.4
110.6
77.2
77.0
76.8
57.4
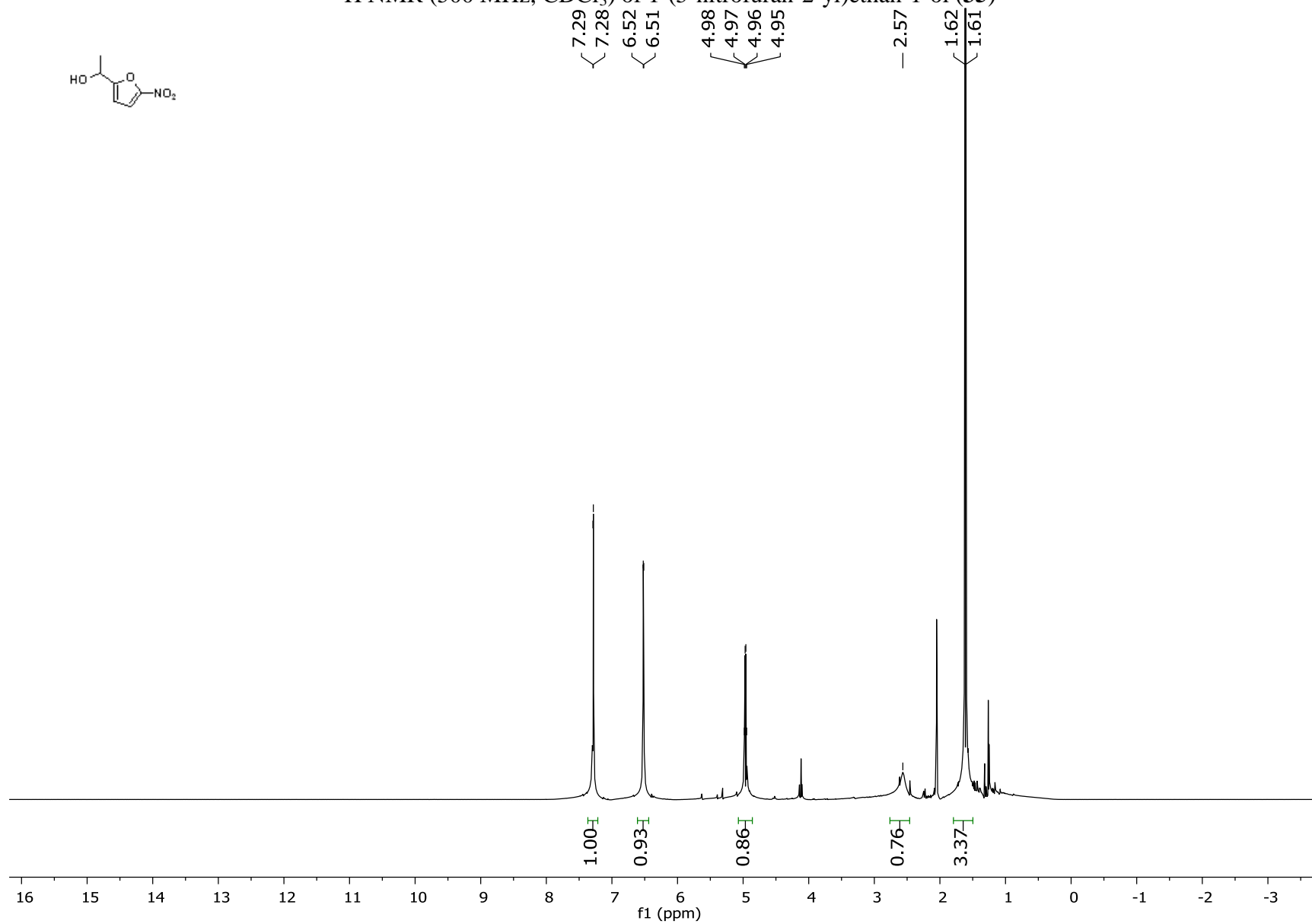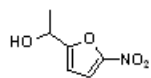
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

^{1}H NMR (500 MHz, CDCl$_3$) of 1-(5-nitrofuran-2-yl)ethan-1-ol (**35**)

^{13}C NMR (500 MHz, CDCl$_3$) of 1-(5-nitrofuran-2-yl)ethan-1-ol (**35**)
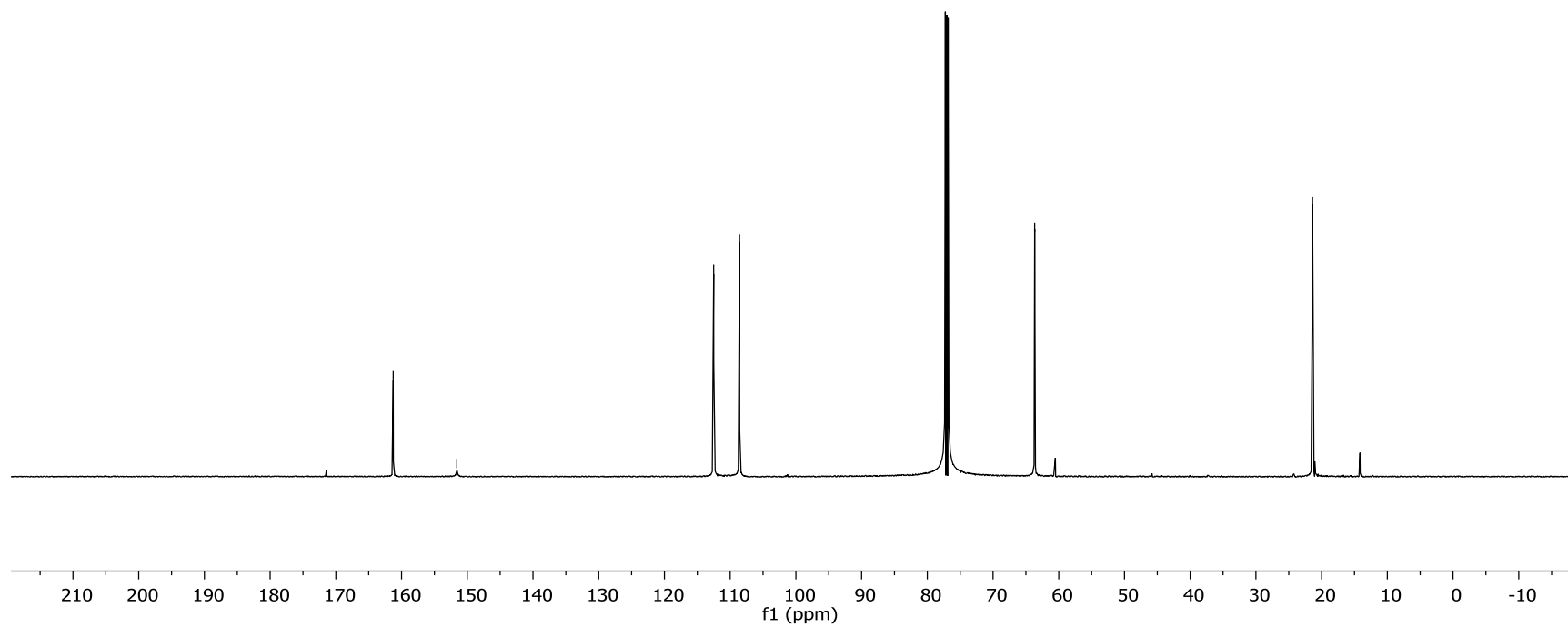
^{1}H NMR (500 MHz, CDCl$_3$) of 1-(5-nitrofuran-2-yl)ethan-1-one (**36**)

279
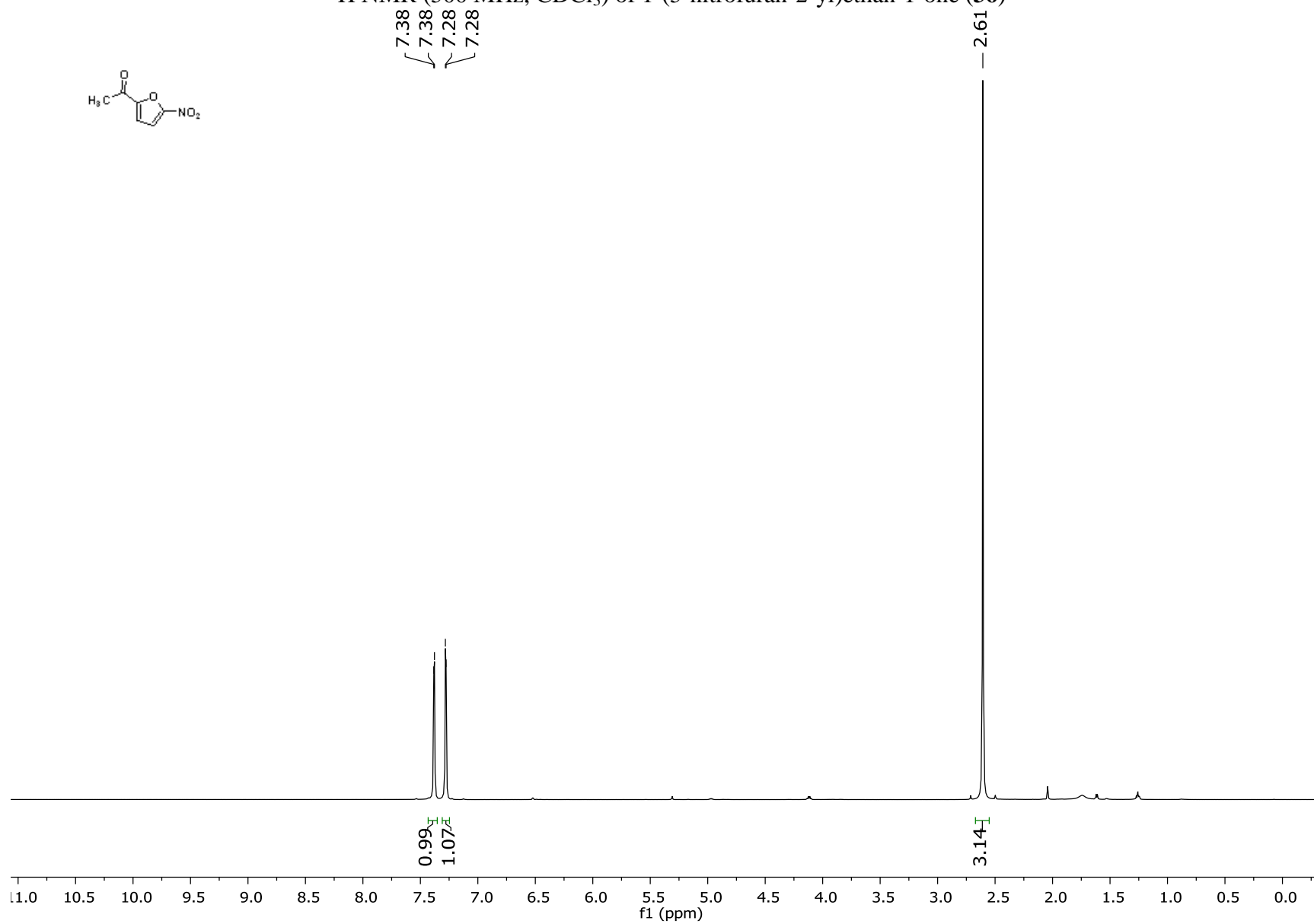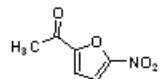
^{13}C NMR (500 MHz, CDCl$_3$) of 1-(5-nitrofuran-2-yl)ethan-1-one (**36**)

186.73

151.91
151.48
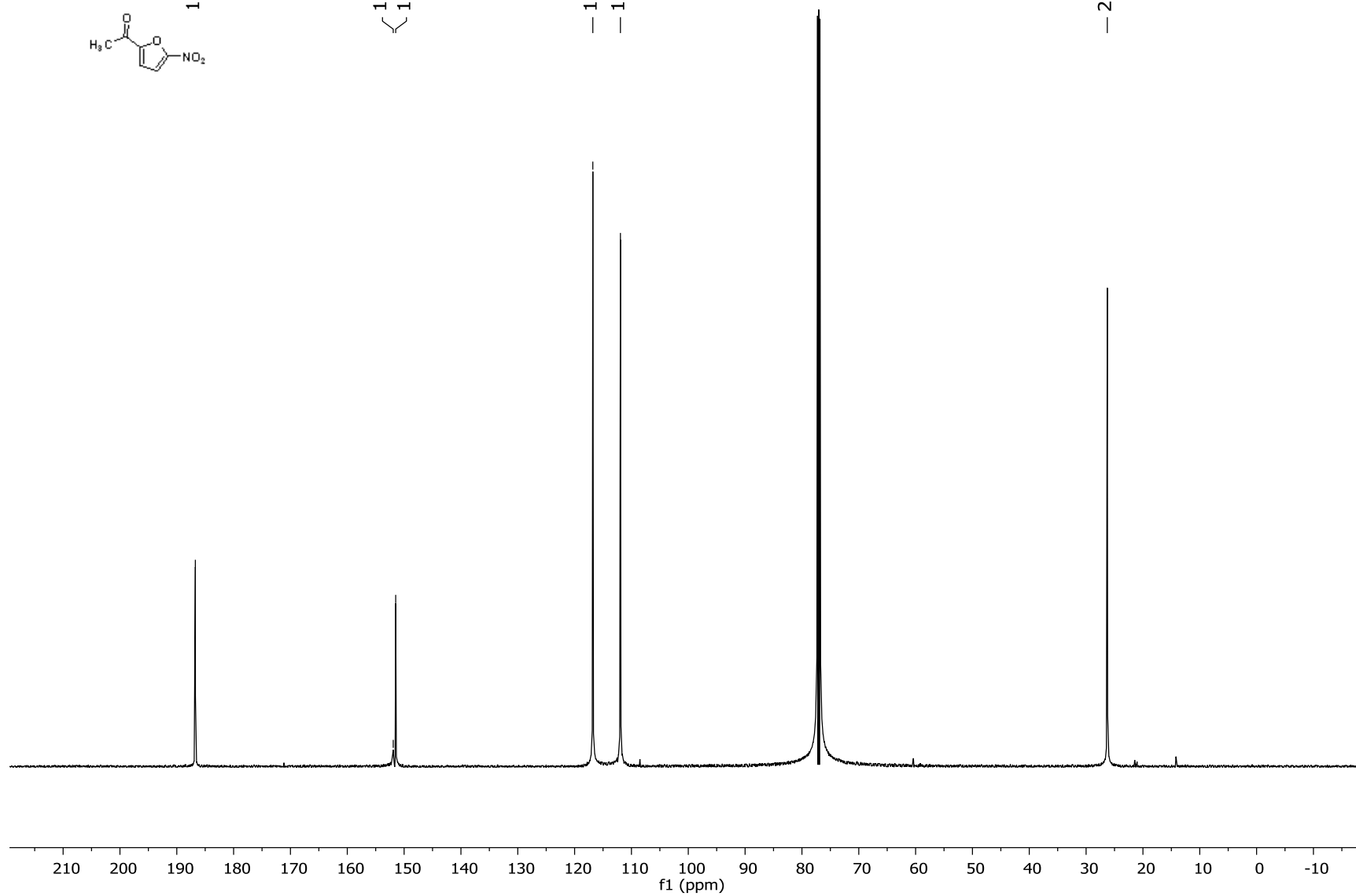
116.79
111.94

26.27

280

^{1}H NMR (500 MHz, CDCl$_3$) of 2-(5-nitrofuran-2-yl)propan-2-ol (**37**)

7.27
7.27
6.49
6.48
2.36
1.65

1.00
1.05
1.21
6.95

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
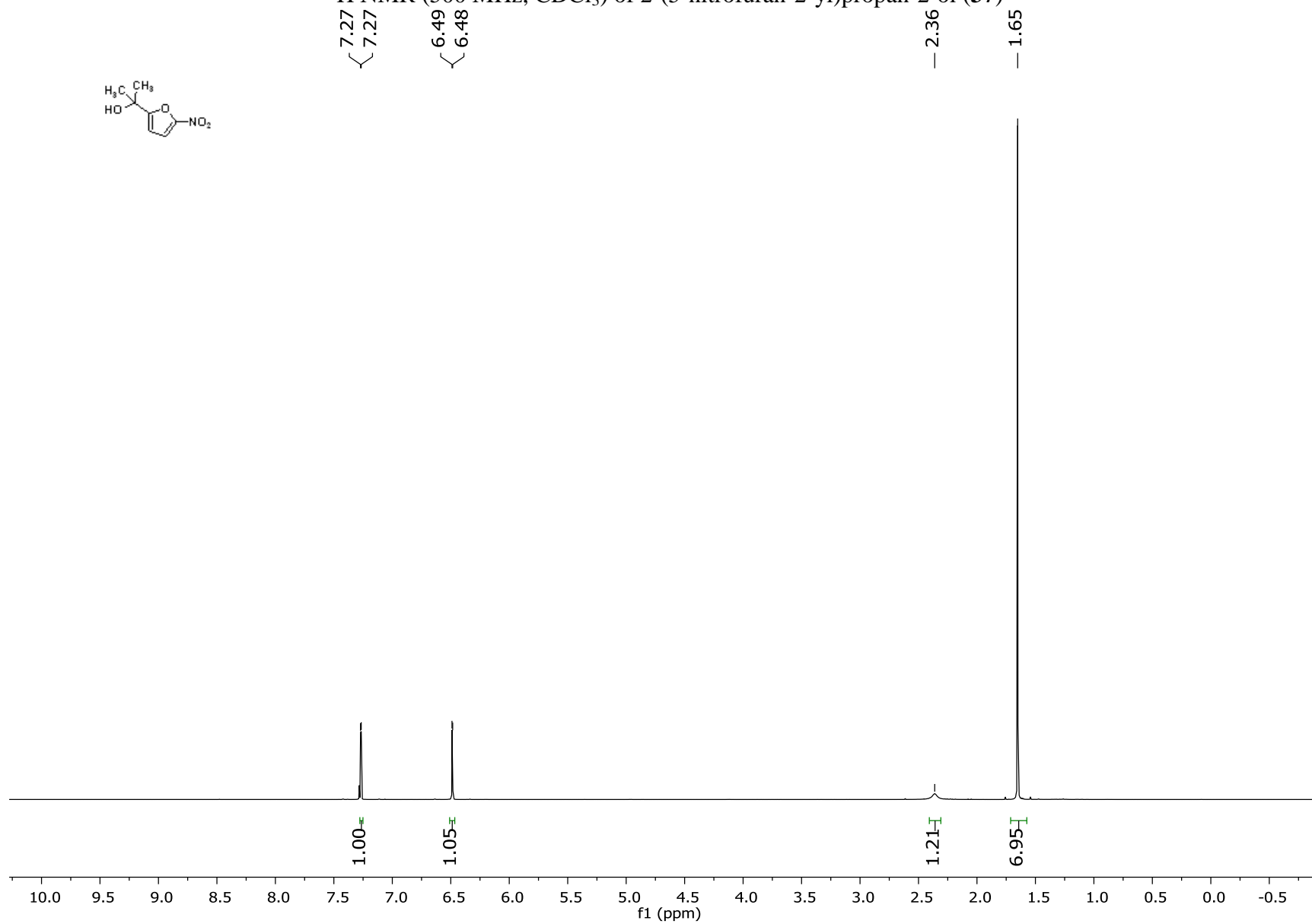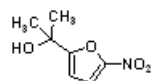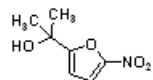
^{13}C NMR (500 MHz, CDCl$_3$) of 2-(5-nitrofuran-2-yl)propan-2-ol (**37**)

164.05
151.36
112.55
107.37
69.30
28.67
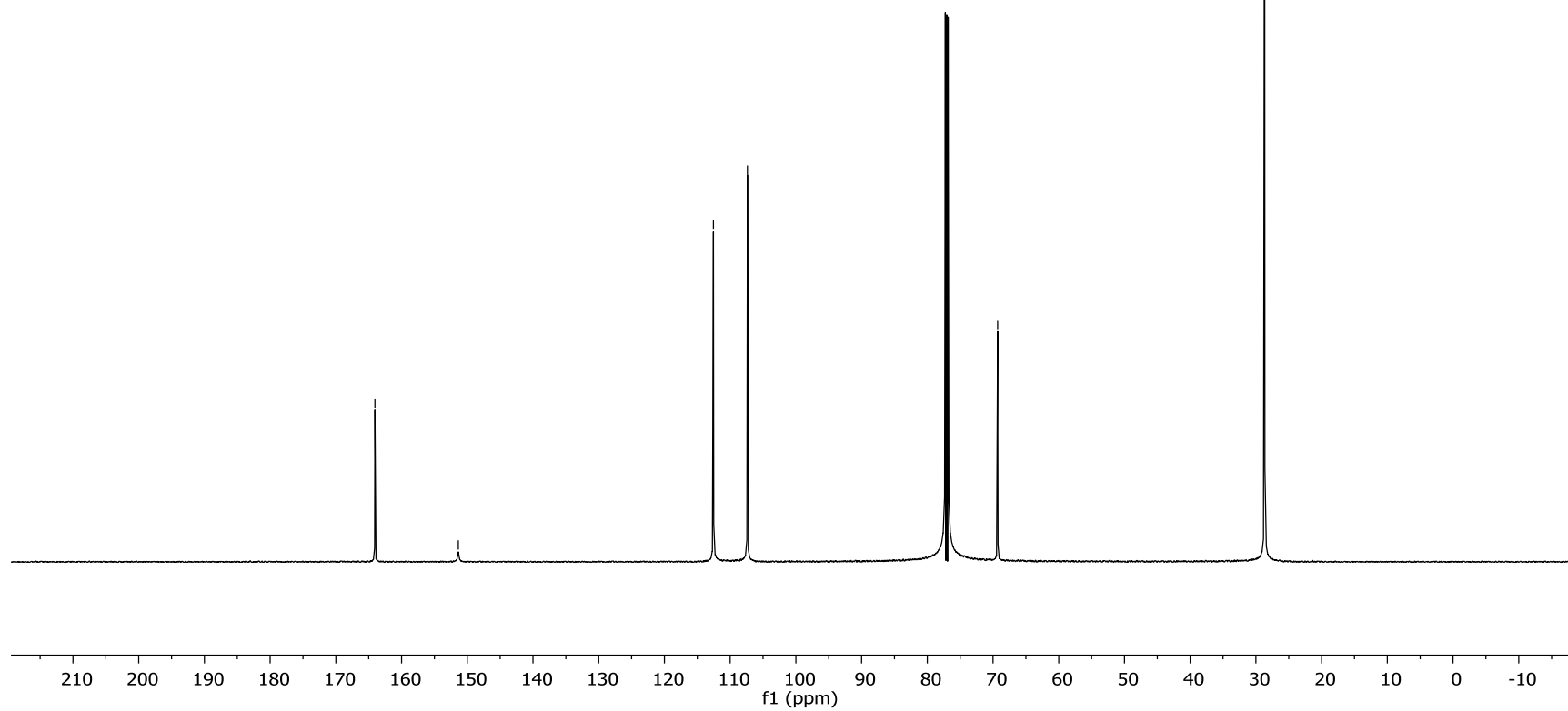
¹³C NMR (500 MHz, CDCl₃) of (4-methoxy-3-((5-nitrofuran-2-yl)methoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**38**)
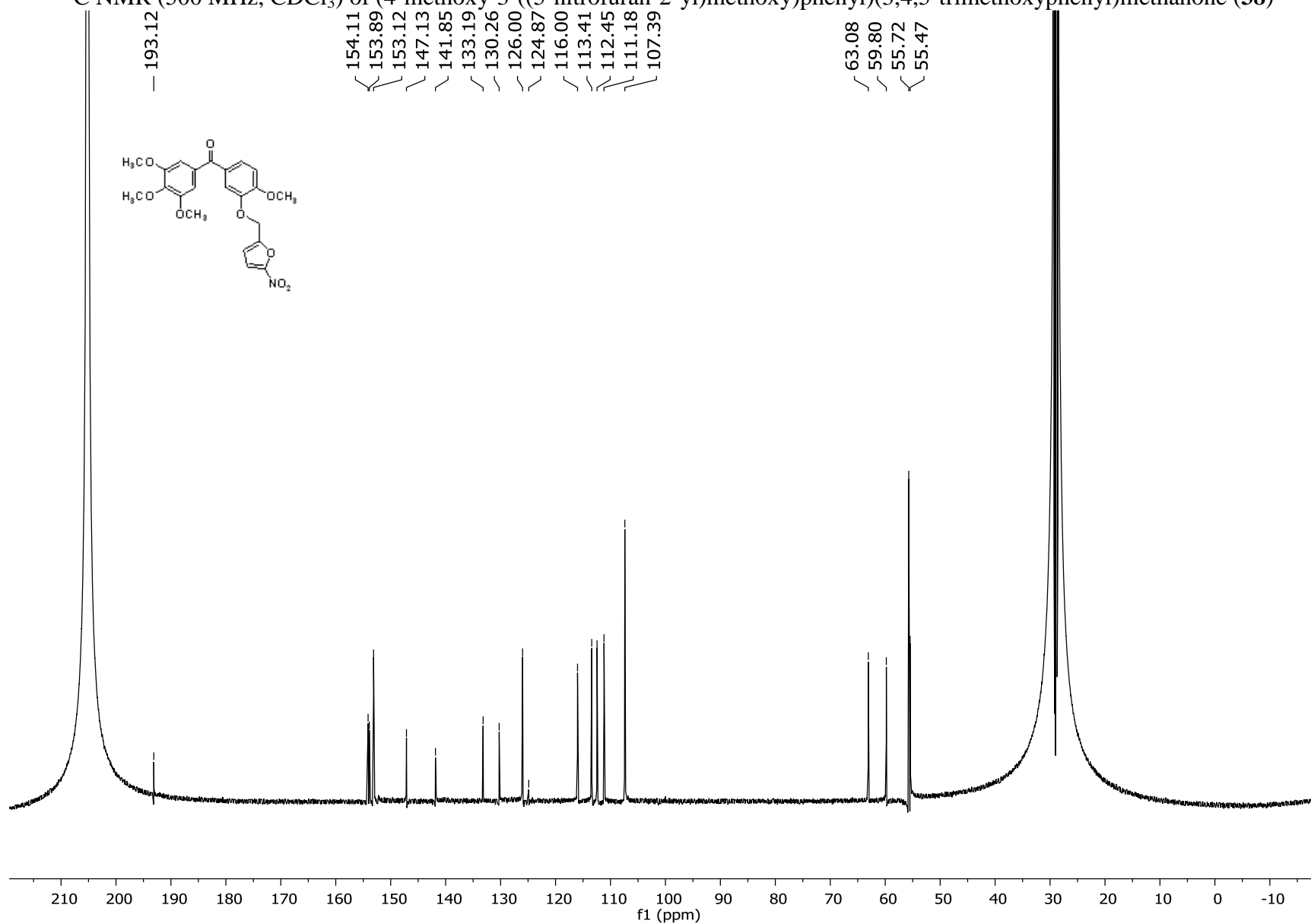
HPLC trace of compound **38**

Data File C:\Chem32\1\Data\Blake\Phenstatin Nor Furan RC Solid.D
Sample Name: Phenstatin Nor Furan RC Solid
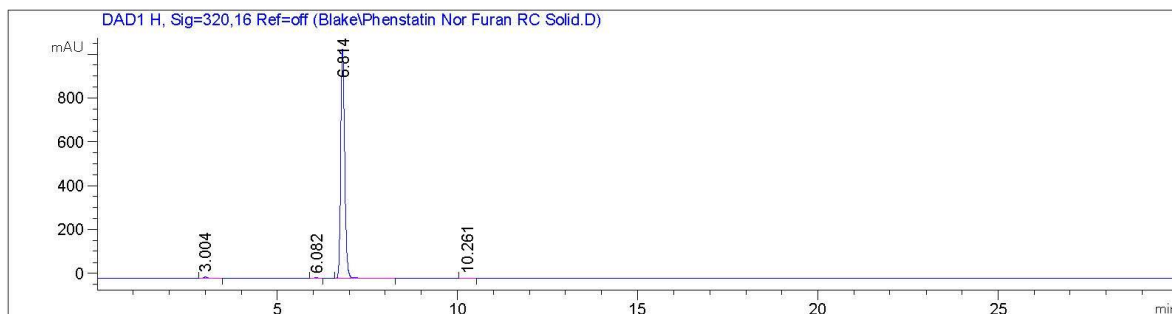
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===================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC                     Location :   1
Injection Date  : 7/11/2016 12:11:15 PM
                                                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
```

```
==================================================================
                    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	3.003	BB	0.0861	19.43468	3.42946	0.9099
2	6.814	BB	0.1169	2116.50854	277.77905	99.0901

Totals :   2135.94322   281.20851

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.312	BB	0.1514	38.18913	3.99850	0.2715
2	3.004	BB	0.0917	139.60963	22.71666	0.9926
3	5.017	BB	0.1257	19.12942	2.23739	0.1360
4	6.082	BB	0.1129	42.63132	5.85598	0.3031
5	6.814	BB	0.1213	1.36771e4	1748.27014	97.2408
6	10.263	BB	0.1351	19.30628	2.18430	0.1373
7	20.090	BB	0.1801	54.73910	4.69318	0.3892
8	24.956	BB	0.1972	74.47594	5.82237	0.5295

Totals :   1.40652e4   1795.77851

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.003	BB	0.0860	37.46567	6.62766	0.6635
2	6.082	BB	0.1126	13.18989	1.81989	0.2336
3	6.814	BB	0.1168	5595.62842	734.84283	99.1029

Totals :   5646.28397 743.29039

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.003	BB	0.0862	44.83185	7.90127	0.5356
2	6.082	BB	0.1122	16.83401	2.33198	0.2011
3	6.814	BB	0.1170	8296.80371	1087.12134	99.1219
4	10.262	BB	0.1325	11.83290	1.37401	0.1414

Totals :   8370.30247 1098.72860

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.004	BB	0.0873	44.69609	7.74583	0.5544
2	6.082	BB	0.1122	14.82649	2.05433	0.1839
3	6.814	BB	0.1170	7991.61816	1047.00903	99.1334
4	10.261	BB	0.1325	10.33726	1.20055	0.1282

Totals :   8061.47800 1058.00974

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*** End of Report ***

nor furan_Orbi_+ESI #2-20   RT: 0.01-0.18   AV: 1
T: FTMS + p ESI Full ms [120.00-1000.00]

NL:
3.45E6
nor
furan_Orbi_+ESI#2-20
RT: 0.01-0.18   AV: 19
T: FTMS + p ESI Full
ms [120.00-1000.00]

NL:
7.67E5

$C_{22}H_{21}NNaO_9$:
$C_{22}H_{21}N_1Na_1O_9$
pa Chrg 1
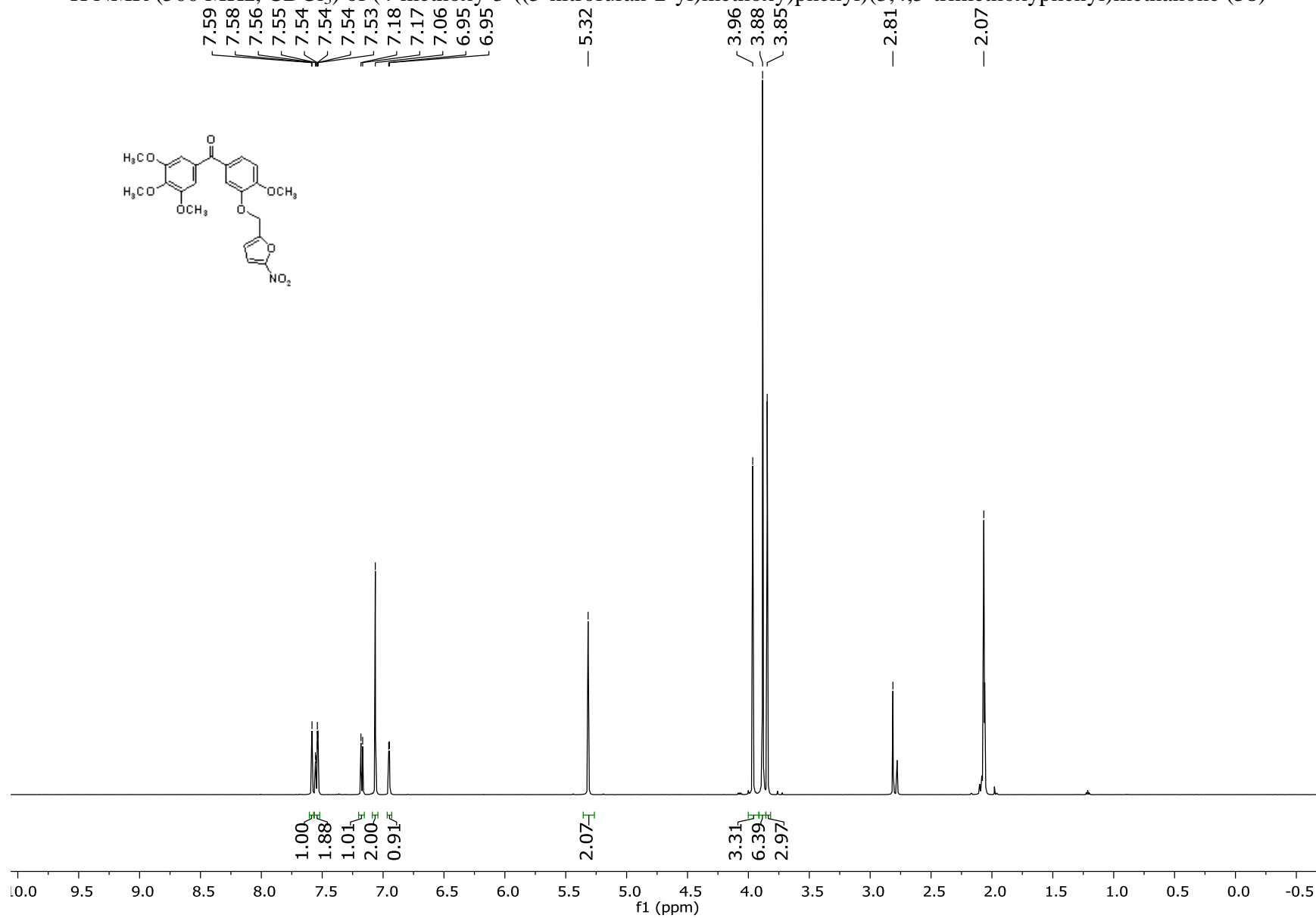
288
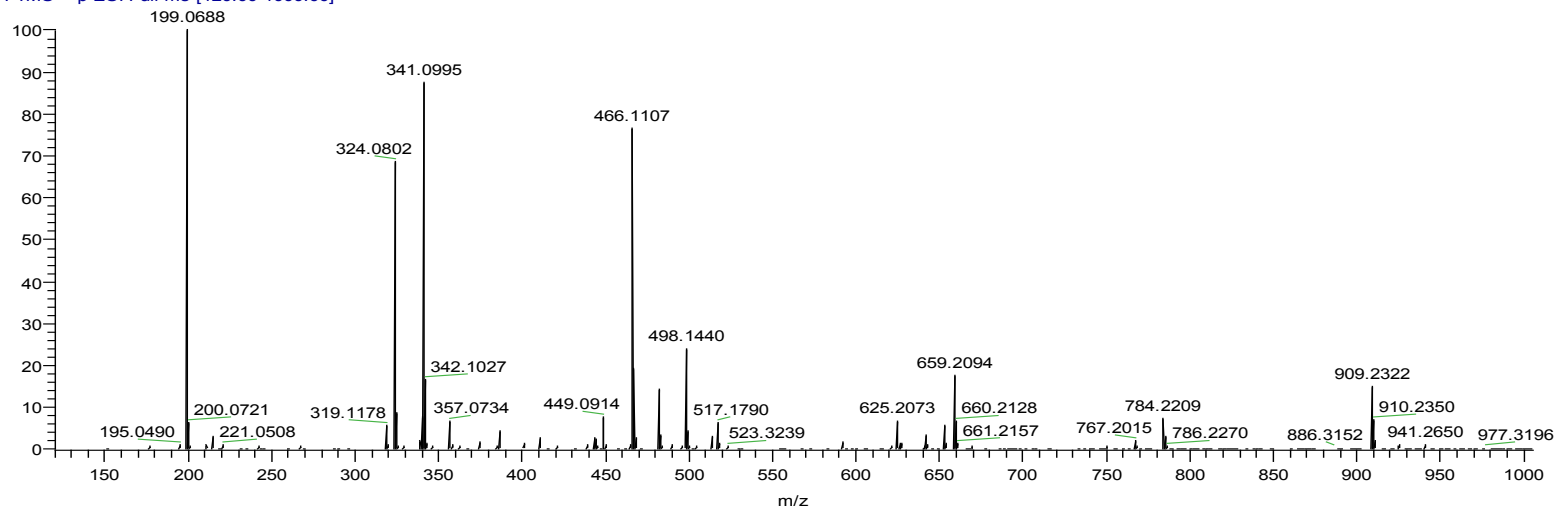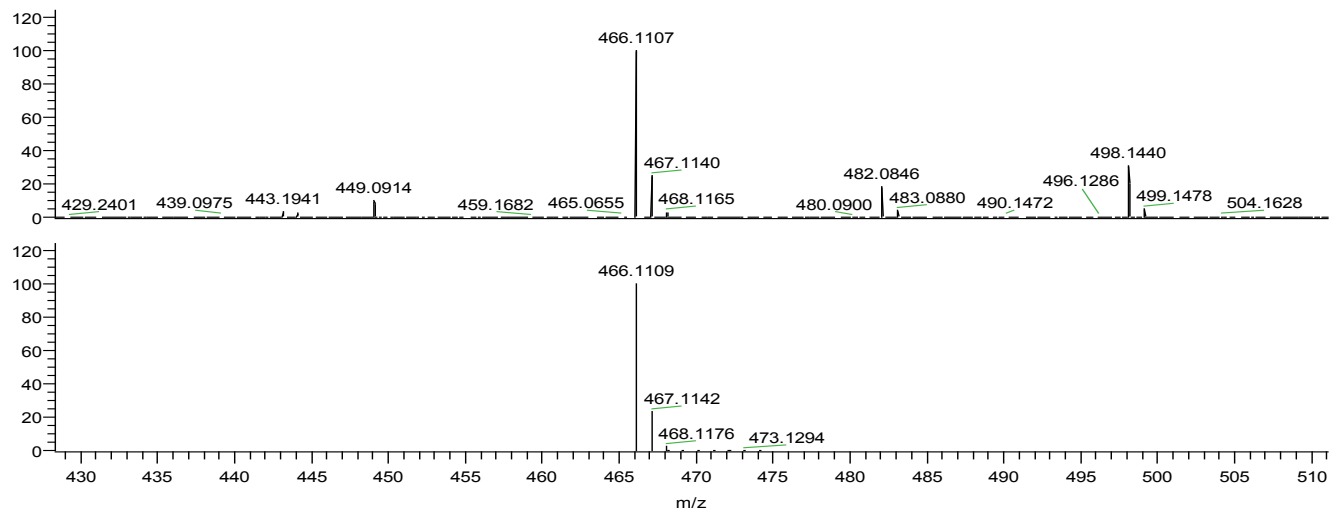
¹H NMR (500 MHz, CDCl₃) of (4-methoxy-3-(1-(5-nitrofuran-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**39**)

¹³C NMR (500 MHz, CDCl₃) of (4-methoxy-3-(1-(5-nitrofuran-2-yl)ethoxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**39**)
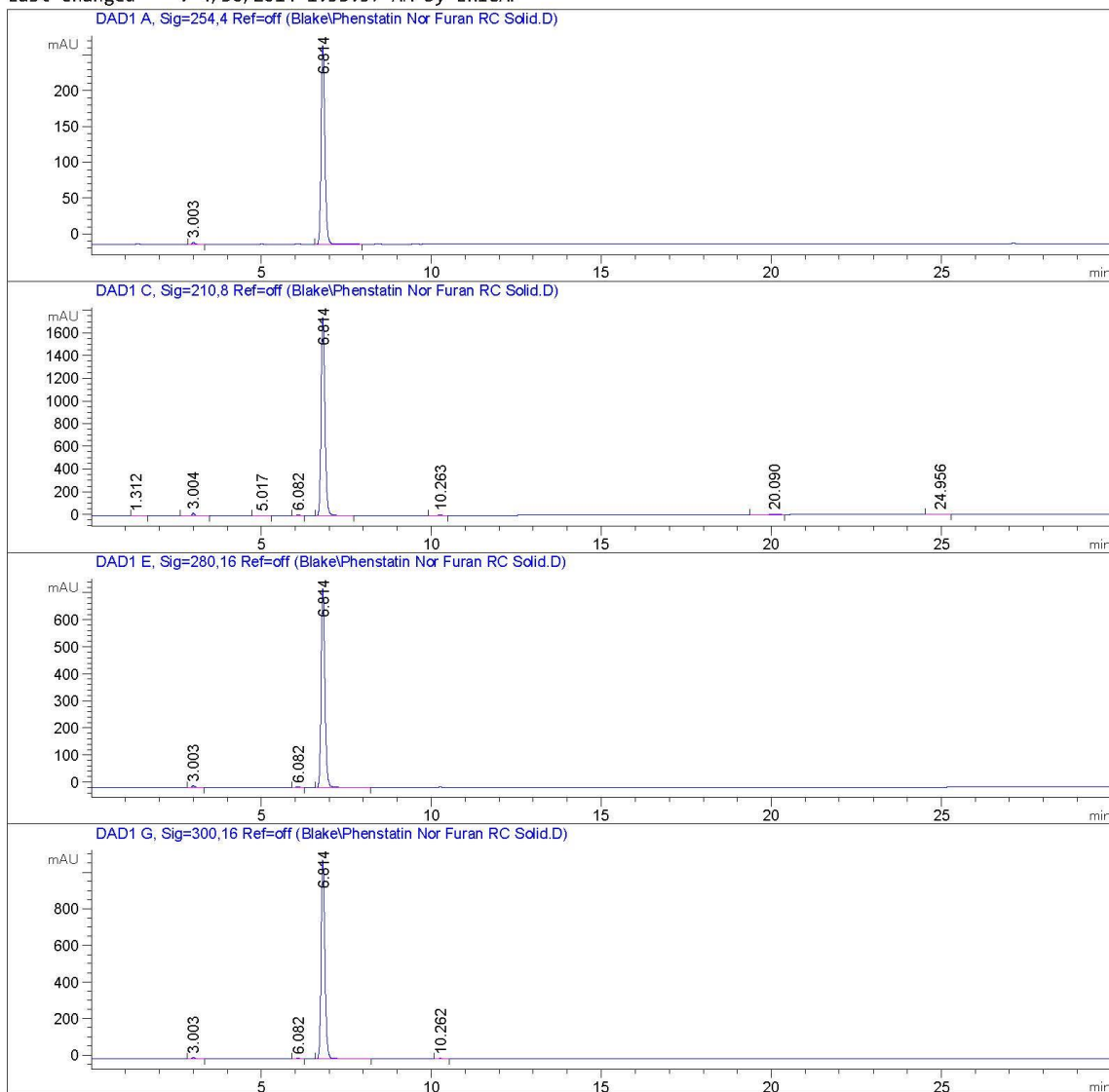
HPLC trace of compound **39**

DAD1 H, Sig=320,16 Ref=off (Blake\Phenstatin Mono Furan 2016-06-27 12-23-15.D)

```
===================================================================
                       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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
  1   7.859 BB   0.1215 2570.55176  320.66025 100.0000

Totals :                 2570.55176  320.66025



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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
  1   1.143 BV   0.0608   29.18751    7.28058   0.1716
  2   1.204 VV   0.0563   16.28708    4.28681   0.0957
  3   1.403 VV   0.1110   24.16215    2.84804   0.1420
  4   2.100 BB   0.0761   14.81390    2.87721   0.0871
  5   3.043 BB   0.1038   24.08342    3.43290   0.1416
  6   4.433 BB   0.0990    9.47919    1.51485   0.0557
  7   5.807 BB   0.1557   12.29721    1.10599   0.0723
  8   7.509 BV   0.1404    9.60507    1.03346   0.0565
  9   7.859 VV   0.1272 1.66029e4  2033.62903  97.5913
 10   9.112 VB   0.1640   41.80635    3.69494   0.2457
 11  10.842 BB   0.1713   23.59030    2.00501   0.1387
 12  11.339 BB   0.1653   46.23188    4.23744   0.2718
 13  11.639 BV   0.1197   11.18849    1.48851   0.0658
 14  11.864 VV   0.1333   56.25783    6.60982   0.3307
 15  12.099 VV   0.1291   36.92906    4.43911   0.2171
 16  20.131 BB   0.1803   16.98117    1.45313   0.0998
 17  25.014 BB   0.2001   36.88857    2.82995   0.2168
```

Data File C:\Chem32\1\Data\Blake\Phenstatin Mono Furan 2016-06-27 12-23-15.D
Sample Name: Phenstatin Mono Furan

Totals :   1.70126e4 2084.76680

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.100	BB	0.0656	8.17356	1.92110	0.1216
2	3.042	BB	0.1141	8.31909	1.03149	0.1238
3	7.859	BB	0.1213	6659.09961	832.68286	99.1019
4	9.111	BB	0.1340	11.07225	1.26691	0.1648
5	11.864	BV	0.1343	20.66961	2.40442	0.3076
6	12.099	VB	0.1294	12.11073	1.45069	0.1802

Totals :   6719.44486   840.75747

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.100	BB	0.0656	21.83418	5.13122	0.2203
2	3.042	BB	0.1077	9.10506	1.21092	0.0919
3	7.859	BB	0.1234	9787.00977	1222.91431	98.7672
4	9.110	BB	0.1339	16.66832	1.90752	0.1682
5	11.326	BB	0.1861	18.04028	1.36554	0.1821
6	11.864	BV	0.1363	32.98869	3.76236	0.3329
7	12.099	VB	0.1416	23.52586	2.50489	0.2374

Totals :   9909.17215 1238.79676

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.100	BV	0.0662	34.07635	7.91467	0.3584
2	2.481	VV	0.0843	6.13857	1.08086	0.0646
3	3.043	BB	0.1147	9.55276	1.17748	0.1005
4	7.859	BB	0.1234	9371.85645	1170.53589	98.5760
5	9.110	BB	0.1330	16.96758	1.95958	0.1785
6	11.326	BB	0.1880	18.39752	1.37558	0.1935
7	11.864	BV	0.1360	27.99903	3.20390	0.2945
8	12.099	VB	0.1417	22.25044	2.40969	0.2340

Totals :   9507.23871 1189.65765

===
                    *** End of Report ***

Mass Spectrum of Compound **39**

6/27/2016 4:06:54 PM
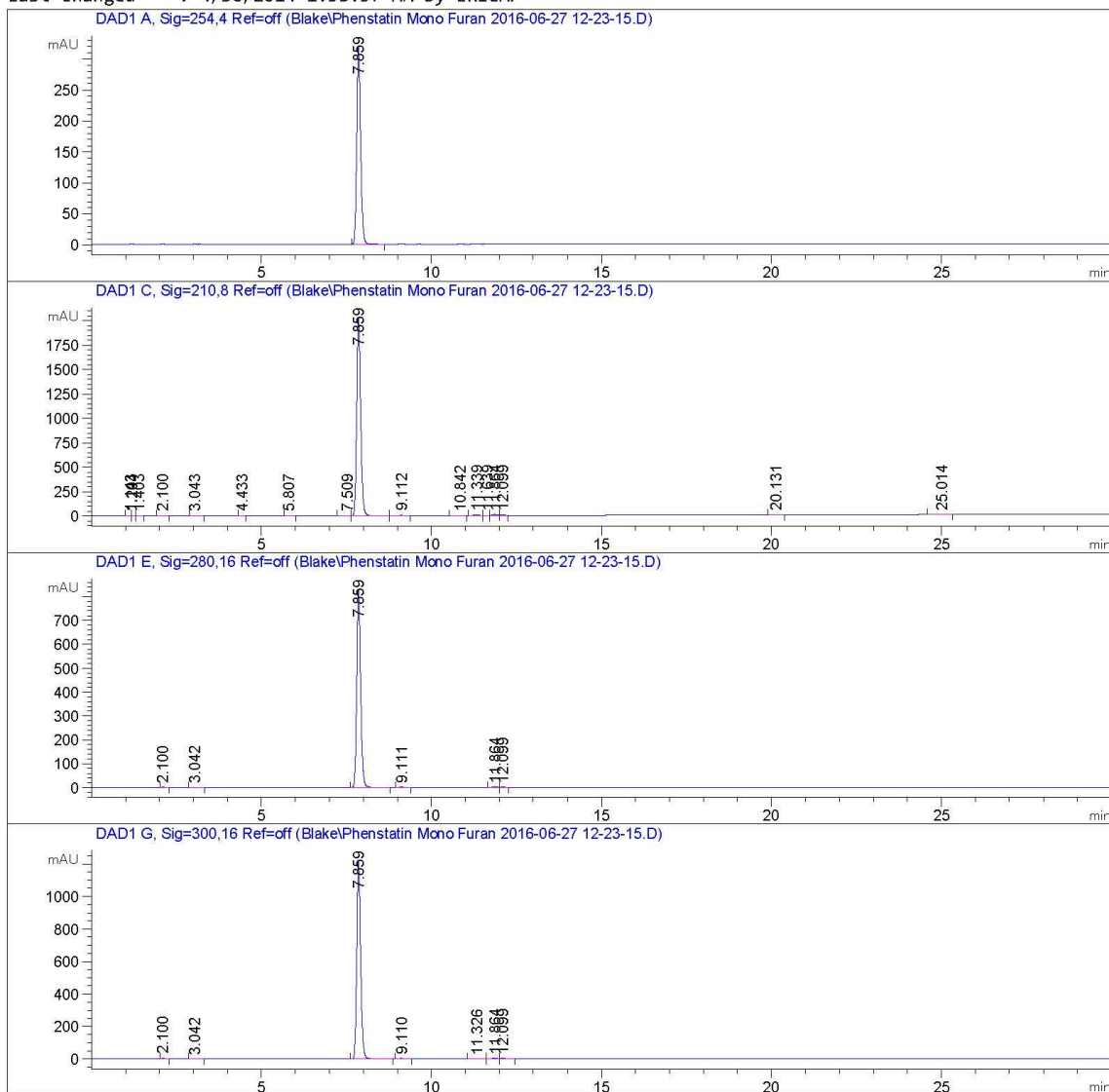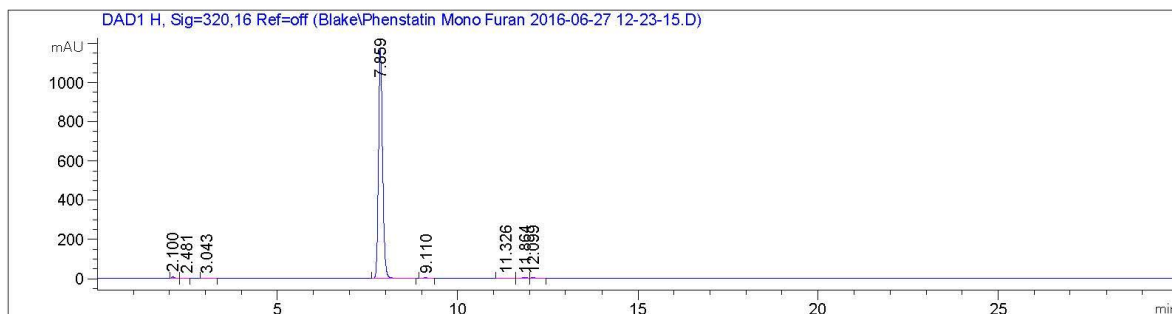
nor furan_Orbi_+ESI #2-20 RT: 0.01-0.18 AV: 1
T: FTMS + p ESI Full ms [120.00-1000.00]
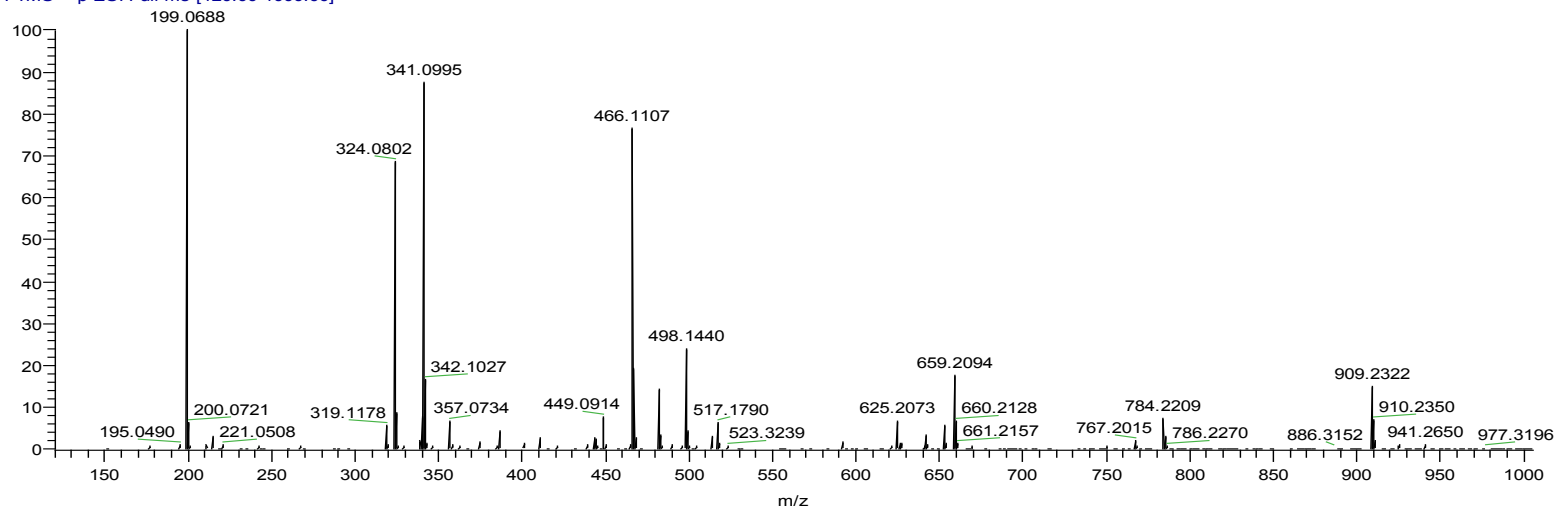
NL:
3.45E6
nor
furan_Orbi_+ESI#2-20
RT: 0.01-0.18 AV: 19
T: FTMS + p ESI Full
ms [120.00-1000.00]

NL:
7.67E5

$C_{22}H_{21}NNaO_9$:
$C_{22}H_{21}N_1Na_1O_9$
pa Chrg 1

294
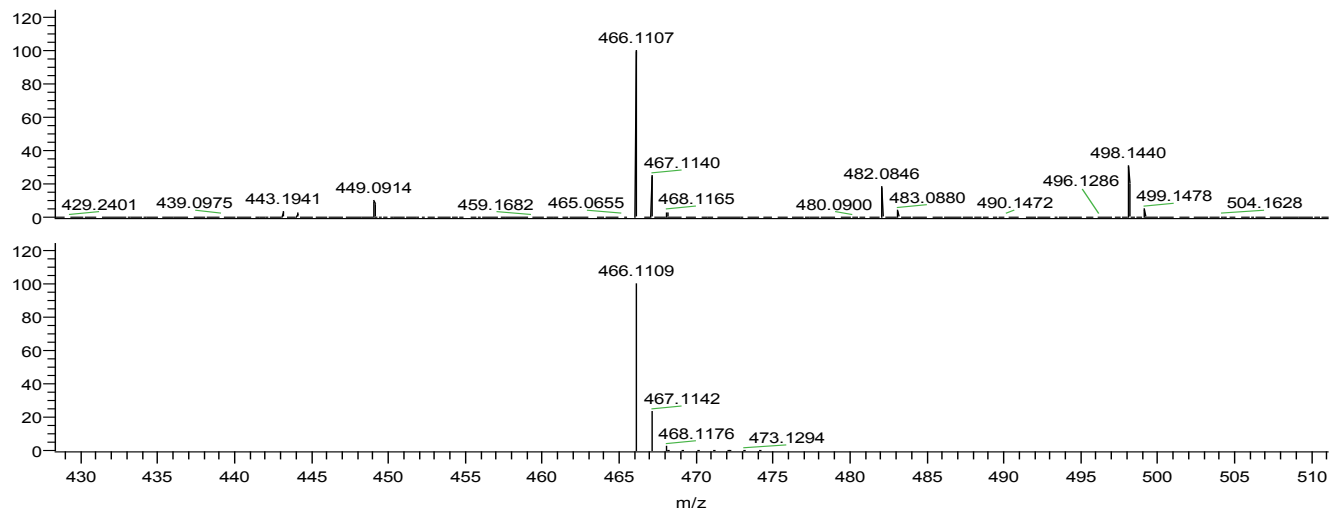
¹H NMR (500 MHz, Acetone *d*-6) of (4-methoxy-3-((2-(5-nitrofuran-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**40**)
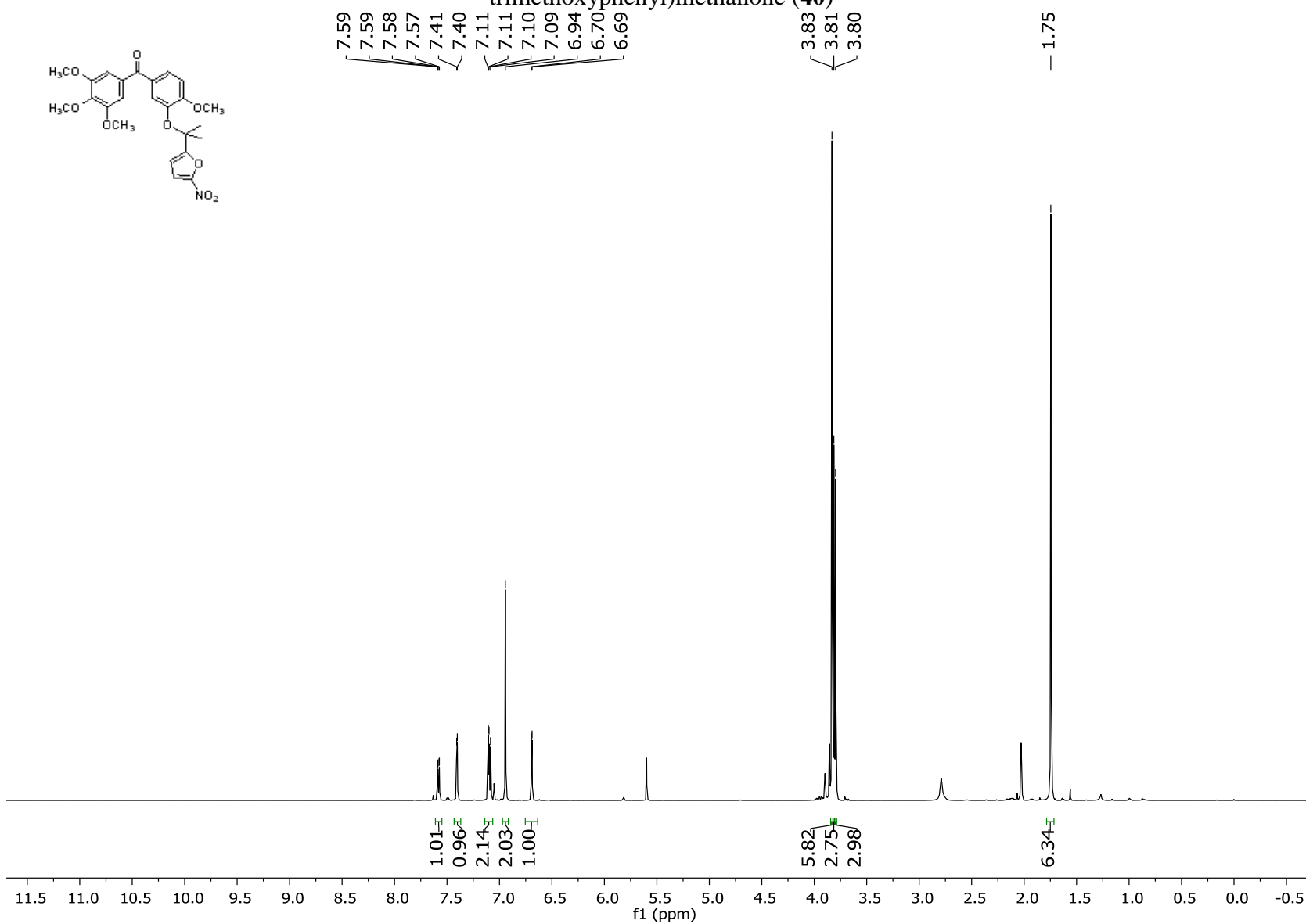
¹³C NMR (500 MHz, Acetone *d*-6) (4-methoxy-3-((2-(5-nitrofuran-2-yl)propan-2-yl)oxy)phenyl)(3,4,5-trimethoxyphenyl)methanone (**40**)

HPLC trace of compound **40**

```
==================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : 1200 HPLC                Location :   1
Injection Date  : 7/21/2016 10:17:15 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
Additional Info : Peak(s) manually integrated
```

DAD1 H, Sig=320,16 Ref=off (Blake\PhenGem Furan BW2 pTLC.D)

```
========================================================================
                          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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   5.985 BB   0.1196   60.40719    7.86837   6.0992
  2   8.262 BB   0.1267  930.00806  114.54267  93.9008

Totals :                 990.41525  122.41104



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

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   1.155 BV   0.0858   33.61111    5.45906   0.4613
  2   1.358 VB   0.0709   37.84983    7.76701   0.5194
  3   2.310 BV   0.0666    9.73679    2.24574   0.1336
  4   2.966 BB   0.0929   18.28416    2.92426   0.2509
  5   5.003 BB   0.1275   15.05728    1.66511   0.2066
  6   5.985 BB   0.1178  467.81430   60.77405   6.4201
  7   7.206 BV   0.1310   37.89943    4.37922   0.5201
  8   7.690 VB   0.1262   40.15192    4.86780   0.5510
  9   8.262 BB   0.1267 6484.05371  798.85181  88.9842
 10   9.351 BB   0.1343   16.43881    1.91213   0.2256
 11  12.061 BB   0.1437   14.32438    1.52408   0.1966
 12  19.927 BB   0.1889   45.66555    3.72825   0.6267
 13  24.823 BB   0.2168   65.85779    4.66276   0.9038

Totals :                7286.74504  900.76130
```

Data File C:\Chem32\1\Data\Blake\PhenGem Furan BW2 pTLC.D
Sample Name: PhenGem Furan BW2 pTLC

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.310	BB	0.0688	6.98924	1.54292	0.2483
2	5.985	BB	0.1175	144.75246	18.86642	5.1419
3	7.205	BB	0.1274	8.80737	1.05459	0.3129
4	7.690	BB	0.1237	15.07421	1.87737	0.5355
5	8.262	BB	0.1265	2639.51001	325.73743	93.7615

Totals :   2815.13329   349.07873

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.309	BB	0.0697	19.67340	4.27634	0.4790
2	2.967	BB	0.0890	5.96967	1.00962	0.1454
3	5.985	BB	0.1175	186.14351	24.25467	4.5325
4	7.205	BB	0.1267	10.81214	1.30512	0.2633
5	7.690	BB	0.1239	22.29101	2.77088	0.5428
6	8.262	BB	0.1265	3852.99243	475.58429	93.8181
7	9.352	BB	0.1334	8.99324	1.03437	0.2190

Totals :   4106.87539   510.23530

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.309	BB	0.0698	32.03522	6.94673	0.9125
2	5.985	BB	0.1175	165.23119	21.53300	4.7063
3	7.690	BB	0.1229	21.17863	2.66083	0.6032
4	8.262	BB	0.1265	3292.44238	406.37106	93.7781

Totals :   3510.88742   437.51163

==
*** End of Report ***

gem-furan_orbi+ESI #2-20 RT: 0.01-0.16 AV: 19
T: FTMS + p ESI Full ms [150.00-700.00]

Relative Abundance

494.1422

495.1452

491.1345

510.1157

411.1415

511.1190

472.1602

255.5573

271.5704

154.0497   214.1202 230.0701   306.0949   341.0997 383.1102 405.0632   449.6333   573.2805 592.1399 643.7093 685.2095

m/z

Relative Abundance

NL:
4.10E7
gem-furan_orbi+
ESI#2-20 RT:
0.01-0.16 AV: 19 T:
FTMS + p ESI Full ms
[150.00-700.00]

494.1422

495.1452
510.1157
491.1345
511.1190

411.1415

255.5573

271.5704

449.6333

154.0497 193.0383 230.0701   306.0949 341.0997   389.1595   573.2805 592.1399 643.7093 685.2095

NL:
7.51E5

C$_{24}$ H$_{25}$ NNaO$_9$:
C$_{24}$ H$_{25}$ N$_1$Na$_1$ O$_9$
pa Chrg 1

494.1422

495.1455

496.1489

m/z

300
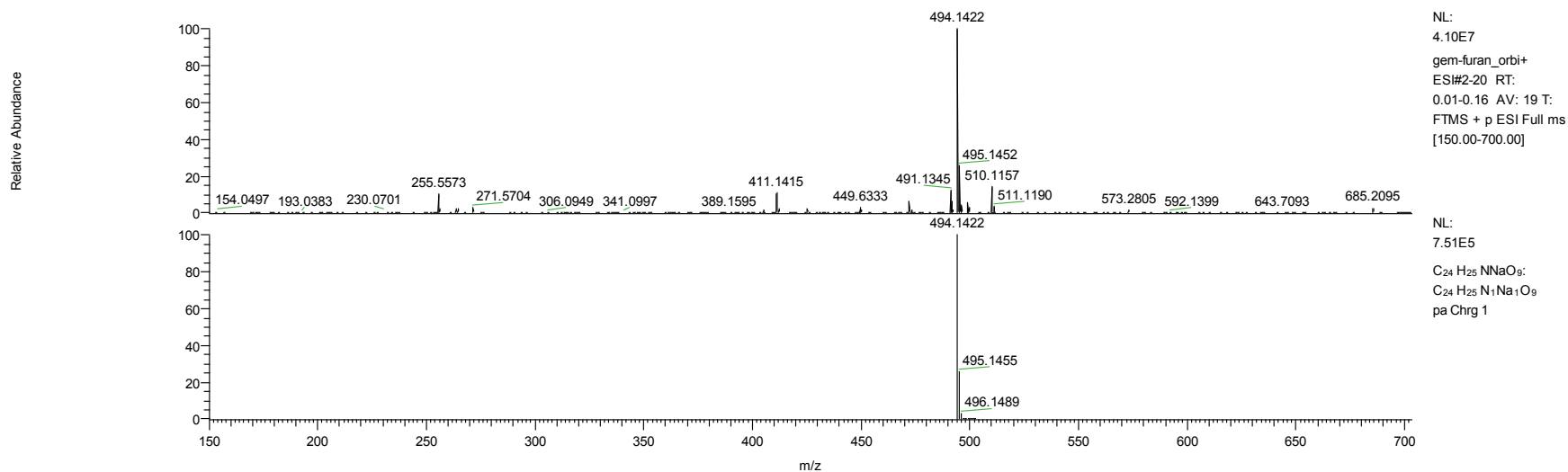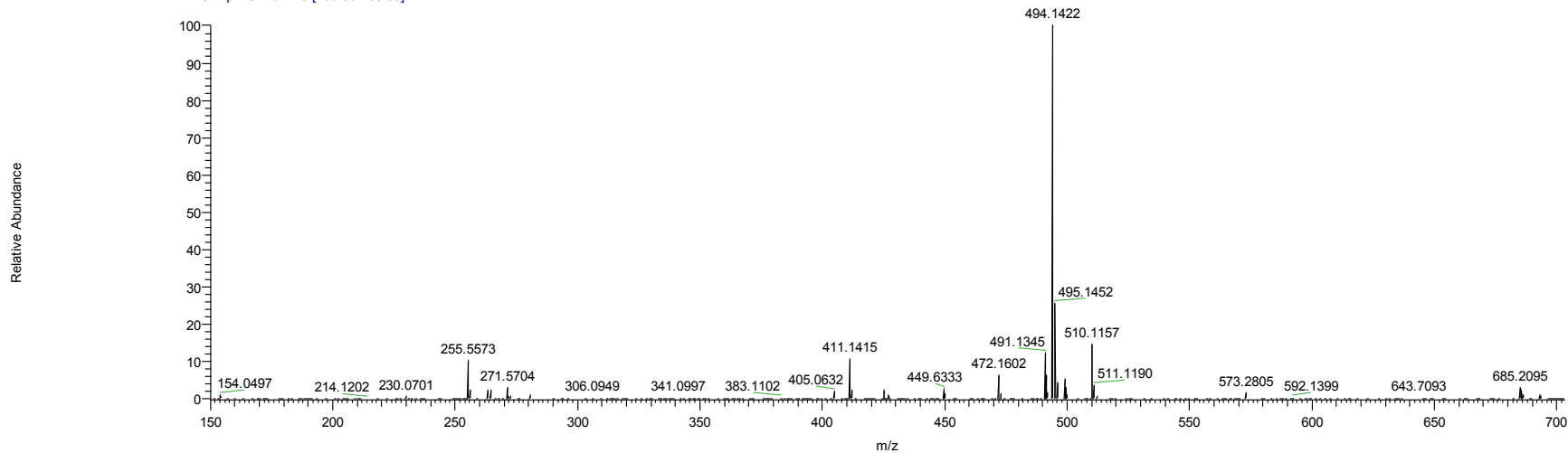
NADPH Cytochrome P450 Oxidoreductase Cleavage Assay

HPLC Conditions:

Solvent: 62% Acetonitrile/water isocratic; detection wavelength: 310 nm; flow rate: 1 mL/min.

[note: KGP493 is Compound **6**]

Chromatogram of 100uM KGP493
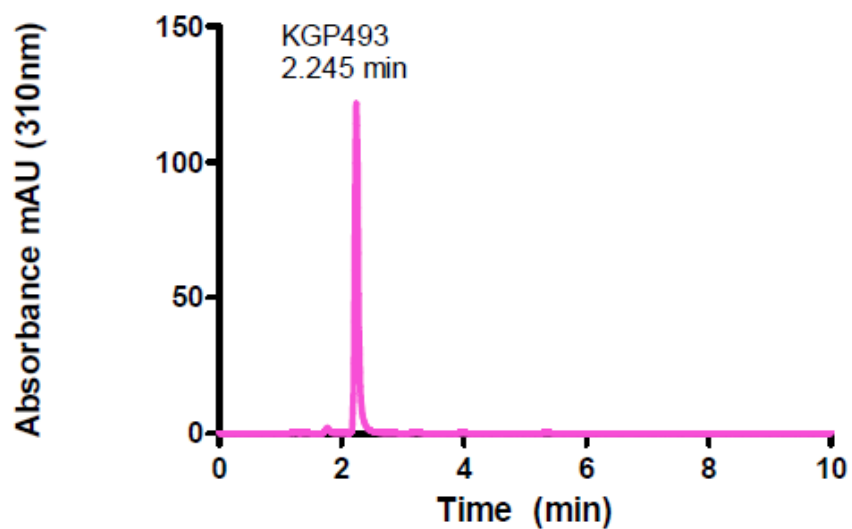
[note: KGP469 is Compound **14**]

Chromatogram of 100uM KGP469
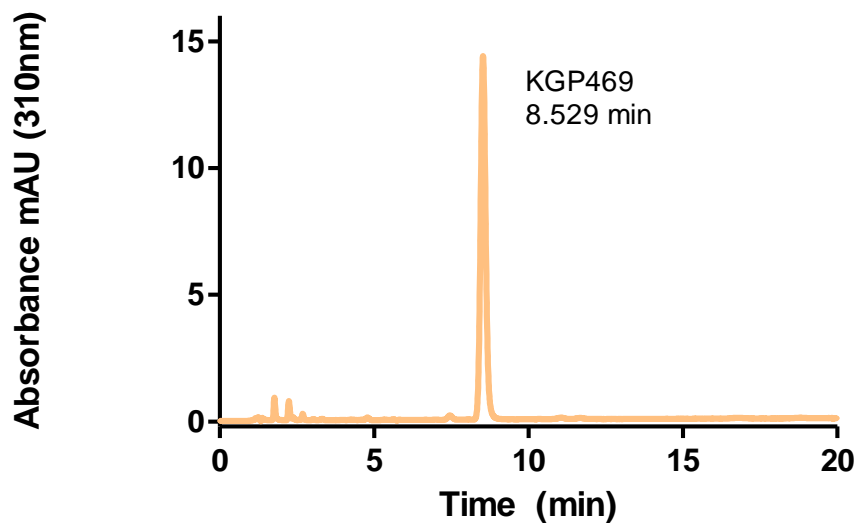
Chromatogram of KGP469 Control (24Hr)

KGP469
8.547 min
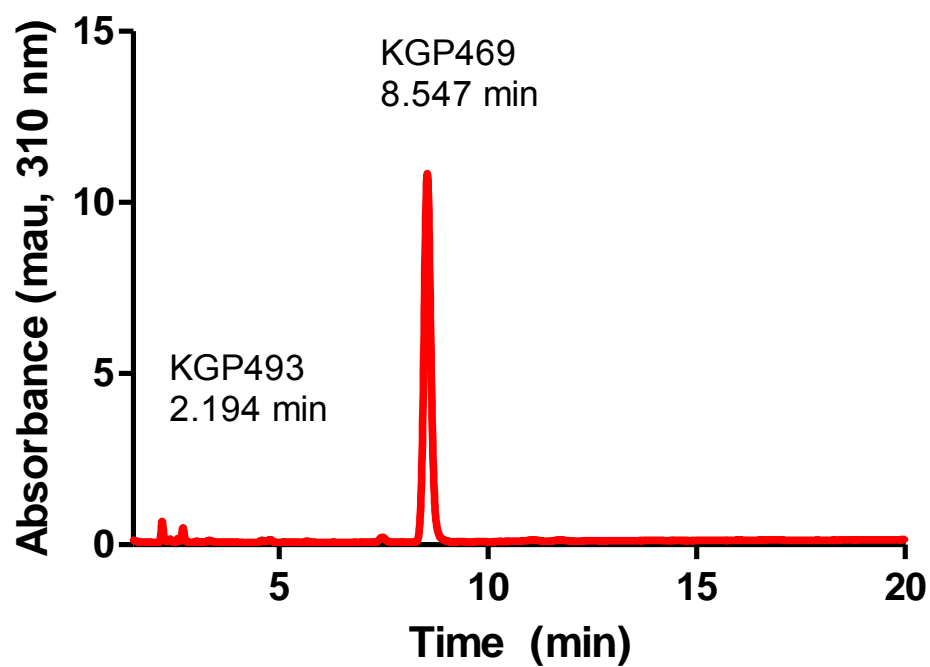
KGP493
2.194 min

Chromatogram of KGP469 Treated with POR (24Hr)

KGP493
2.198 min
A = 89.1

302
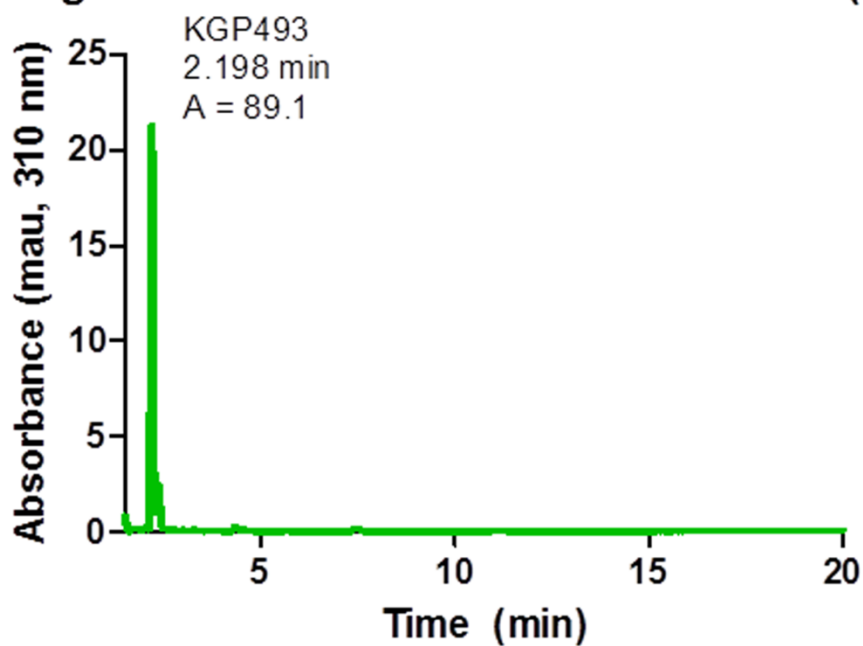
Chromatogram of 100uM KGP470

KGP470
8.069 min
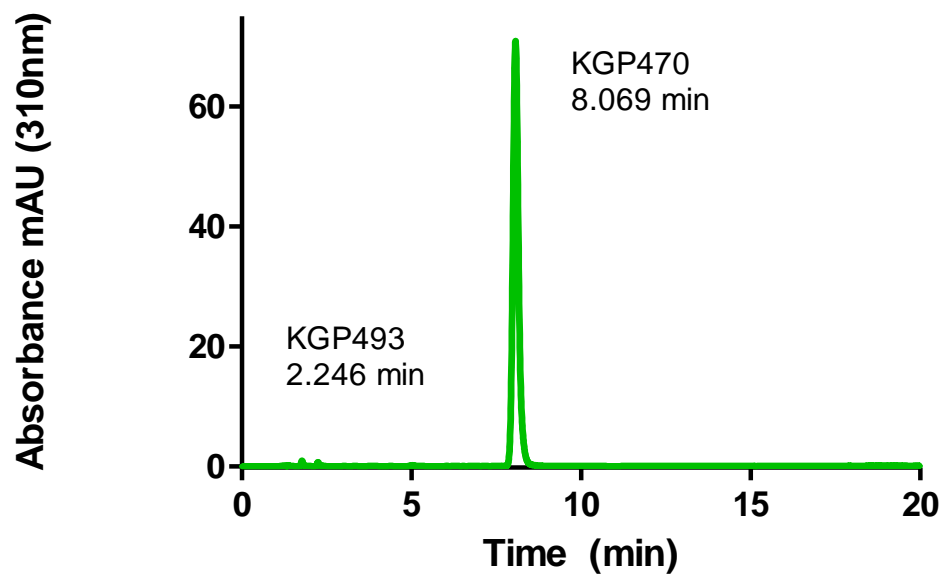
KGP493
2.246 min

Chromatogram of KGP470 Control (24Hr)

KGP470
8.080 min
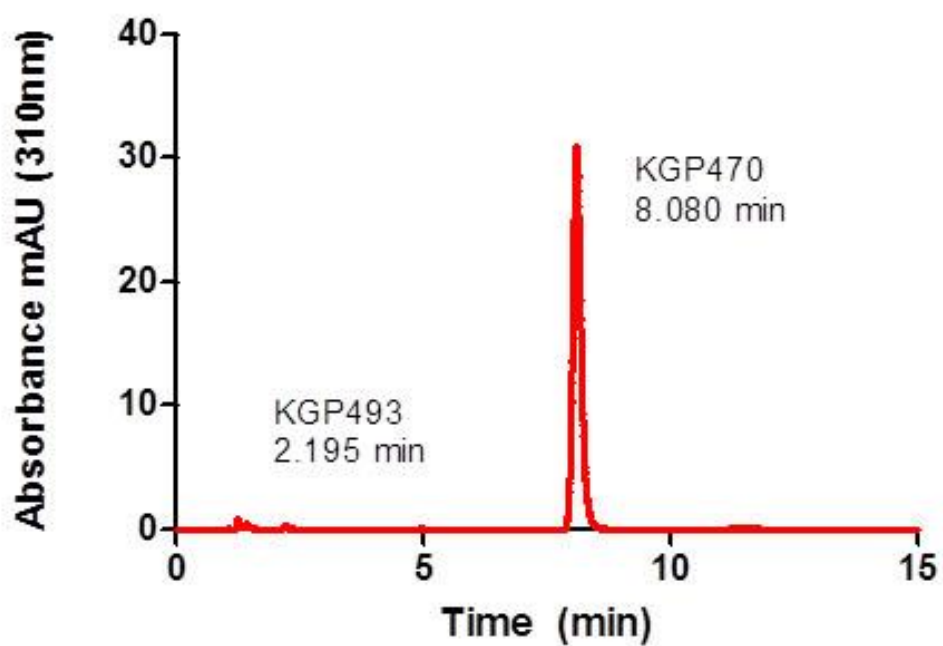
KGP493
2.195 min

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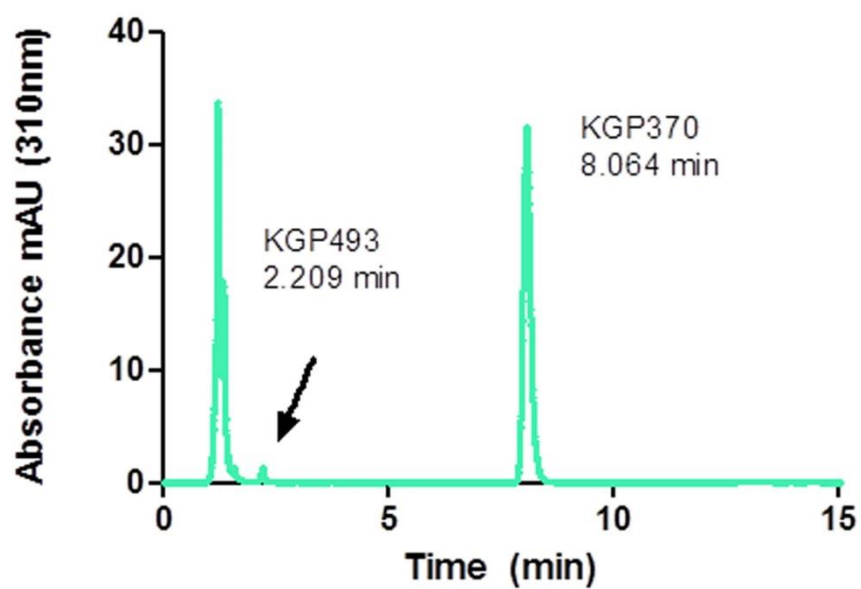
[note: KGP492 is Compound **40**]

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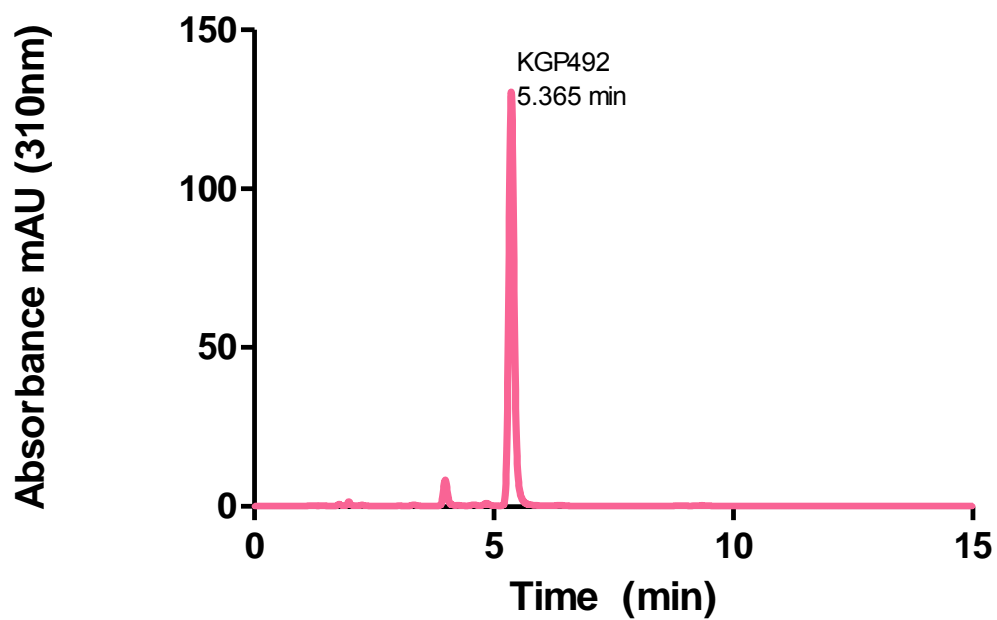
Chromatogram of KGP492 Control (24Hr)

KGP492
5.337 min
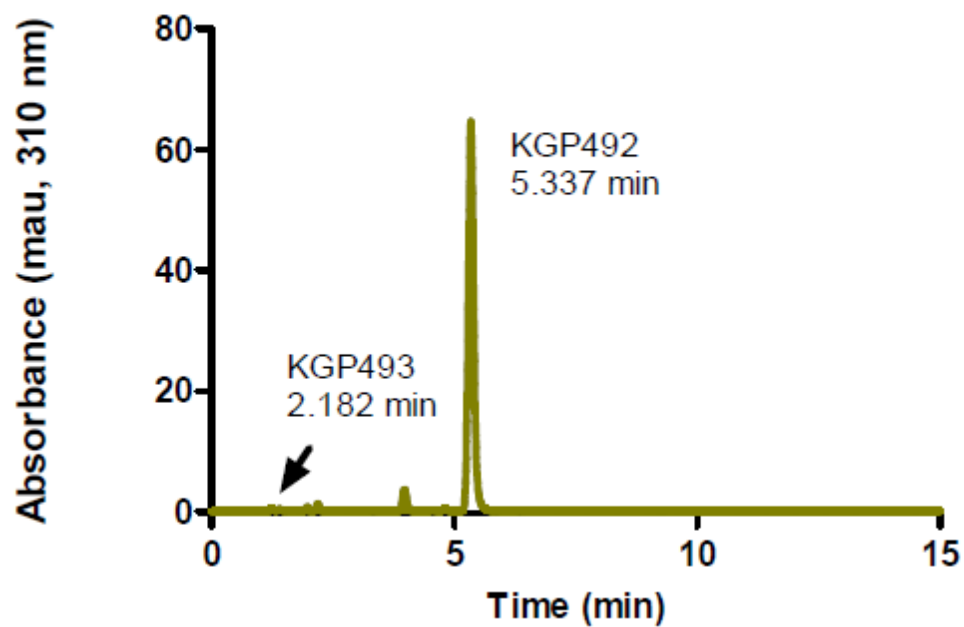
KGP493
2.182 min

Chromatogram of KGP492 Treated with POR (24Hr)
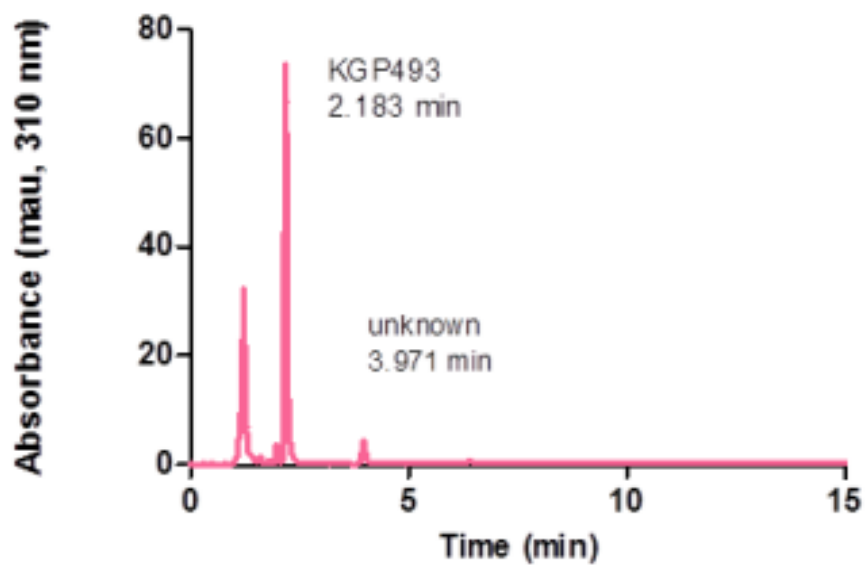
KGP493
2.183 min

unknown
3.971 min

Supporting Information: Bioreductively Activatable Prodrug Conjugates of Combretastatin A-1 and Combretastatin A-4 as Anticancer Agents Targeted towards Tumor Hypoxia

This appendix will be submitted to a peer reviewed journal with the following author list and title: Laxman Devkota[1†], Blake A. Winn[1†], Bunnarack Kuch[1], Matthew T. MacDonough[1], Tracy E. Strecker[1], Yifan Wang[1], Zhe Shi[1], Jeni Gerberich[2], Deboprosad Mondal[1], Rajeswari Mukherjee[1], Ernest Hamel[3], David J. Chaplin,[1,4] Peter Davis, Ralph P. Mason,[2] Mary L. Trawick[1*], Kevin G. Pinney[1*] Bioreductively Activatable Prodrug Conjugates of Combretastatin A-1 and Combretastatin A-4 as Anticancer Agents Targeted towards Tumor Hypoxia

The author Blake A. Winn contributed to this manuscript through the synthesis of six of the final compounds (**23**, **35**, **38**, **40**, **41**, **44**) including full characterization, which included proton and carbon NMRs, HPLC, and HRMS. In addition, Blake A. Winn significantly contributed to the writing and editing of the manuscript, as well as the preparation of the supporting data. Laxman Devkota contributed to this manuscript through the synthesis of nine of the final compounds (**22**, **24**, **25**, **26**, **35**, **37**, **39**, **41**, **43**) including full characterization, which included proton and carbon NMRs, HPLC, and HRMS. In addition, Laxman Devkota significantly contributed to the writing and editing of the manuscript, as well as the preparation of the supporting data. Bunnarack Kuch contributed through the synthesis of advanced intermediates and the CA1-BAPC **41**. Matthew MacDonough, Deboprosad Mondal, and Rajeswari Mukherjee contributed through the synthesis of the CA4 BAPC **45**. Zhe Shi and Deboprosad Mondal contributed to the spectral analysis of the CA1 BAPCs (1D NOE, HRMS). Ralph Mason and Jeni Gerberich contributed through the *in vivo* preliminary biological testing of the CA4-BAPC **45** in mice. Ernest Hamel contributed through the preliminary biological evaluation of the CA1 and CA4-BAPCs ability to inhibit tubulin polymerization and compete for the colchicine binding site. Tracy Strecker contributed

through the *in vitro* preliminary biological testing of the CA1 and CA4-BAPCs in normoxic and hypoxic environments in addition to SRB assays.

Supporting Information

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

Laxman Devkota[1†], Blake A. Winn[1†], Bunnarack Kuch[1], Matthew T. MacDonough[1], Tracy E. Strecker[1], Yifan Wang[1], Zhe Shi[1], Jeni Gerberich[2], Deboprosad Mondal[1], Rajeswari Mukherjee[1], Ernest Hamel[3], David J. Chaplin,[1,4] Peter Davis, Ralph P. Mason[2], Mary L. Trawick[1*], Kevin G. Pinney[1*]

[1]Department of Chemistry and Biochemistry, Baylor University, One Bear Place #97348, Waco,

Texas 76798-7348, United States

[2]Prognostic Imaging Research Laboratory, Department of Radiology, The University of Texas

Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9058, United

States

[3]Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer

Treatment and Diagnosis, National Cancer Institute, Frederick National Laboratory for Cancer

Research, National Institutes of Health, Frederick, MD 21702, United States

[4]OXiGENE Inc., 701 Gateway Boulevard, Suite 210, South San Francisco, California 94080,

United States

†Authors with equal contribution

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E-mail address: Kevin_Pinney@baylor.edu (K.G. Pinney).

Tel.: +1 254 710 6857.

E-mail address: Mary_Lynn_Trawick@baylor.edu (M.L. Trawick)

Table of Contents

Schemes

Scheme **S1**. Attempted Deprotection of Compounds **22**, **23** and **24**

^{1}H NMR (600 MHz, CDCl$_3$) for Compound **2**
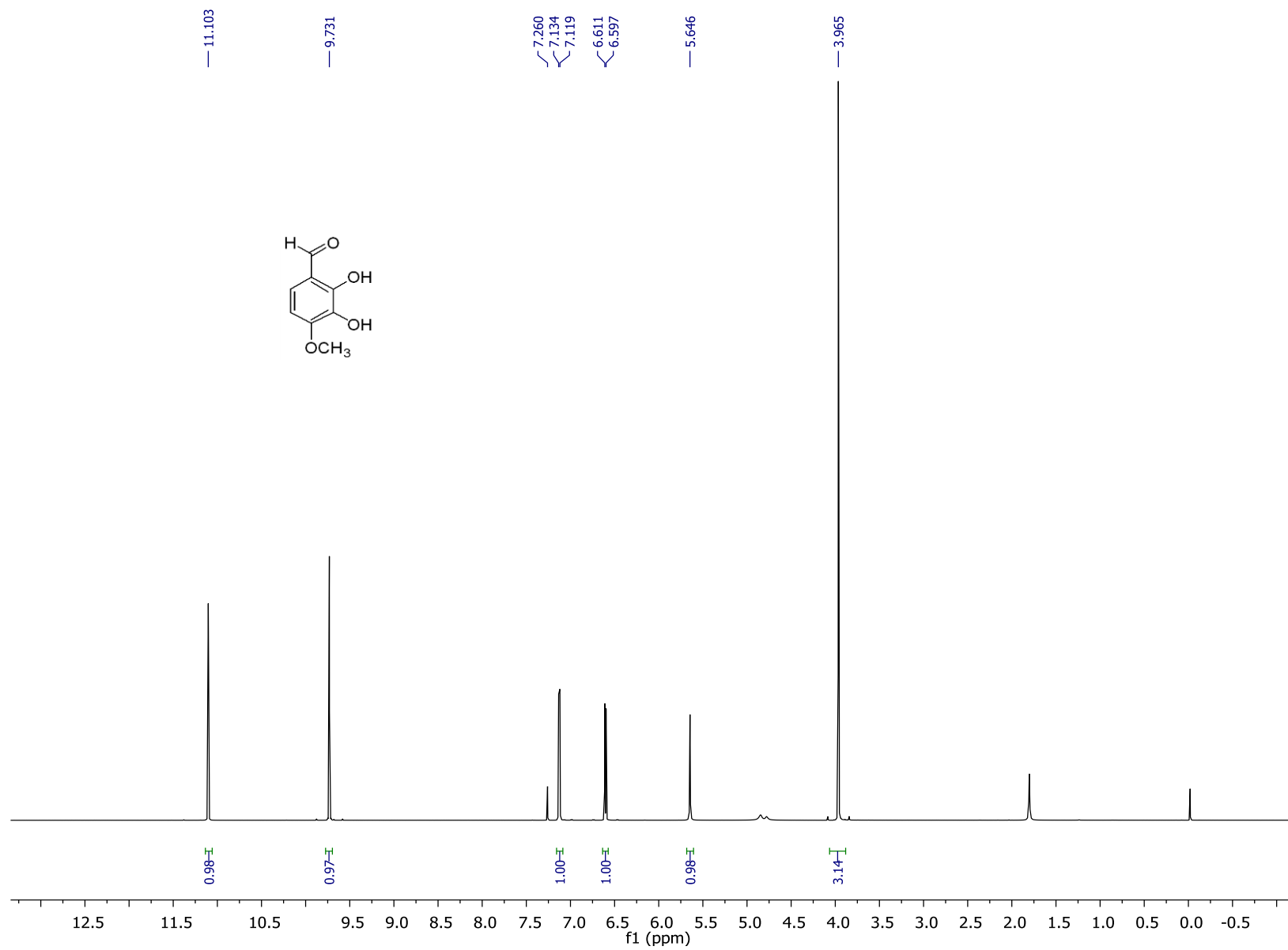
^{13}C NMR (151 MHz, CDCl$_3$) for Compound **2**

195.591

153.386
149.343

133.290
126.464

116.357

103.940

77.573
77.360
77.147

56.683

^{1}H NMR (600 MHz, CDCl$_3$) for Compound **3**

9.854

7.873
7.859
7.503
7.488
7.370
7.357
7.265
6.904
6.889

5.906

3.975

2.473

1.07

2.00
1.13
2.28

1.15

1.12

3.47

3.17

10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

^{13}C NMR (151 MHz, CDCl$_3$) for Compound **3**

186.991

153.206
146.179
139.235
138.157
131.986
129.986
128.675
124.127
120.554

109.230

77.256
77.044
76.832

56.653

21.826

f1 (ppm)

^{1}H NMR (500 MHz, CDCl$_3$) for Compound **4**

316
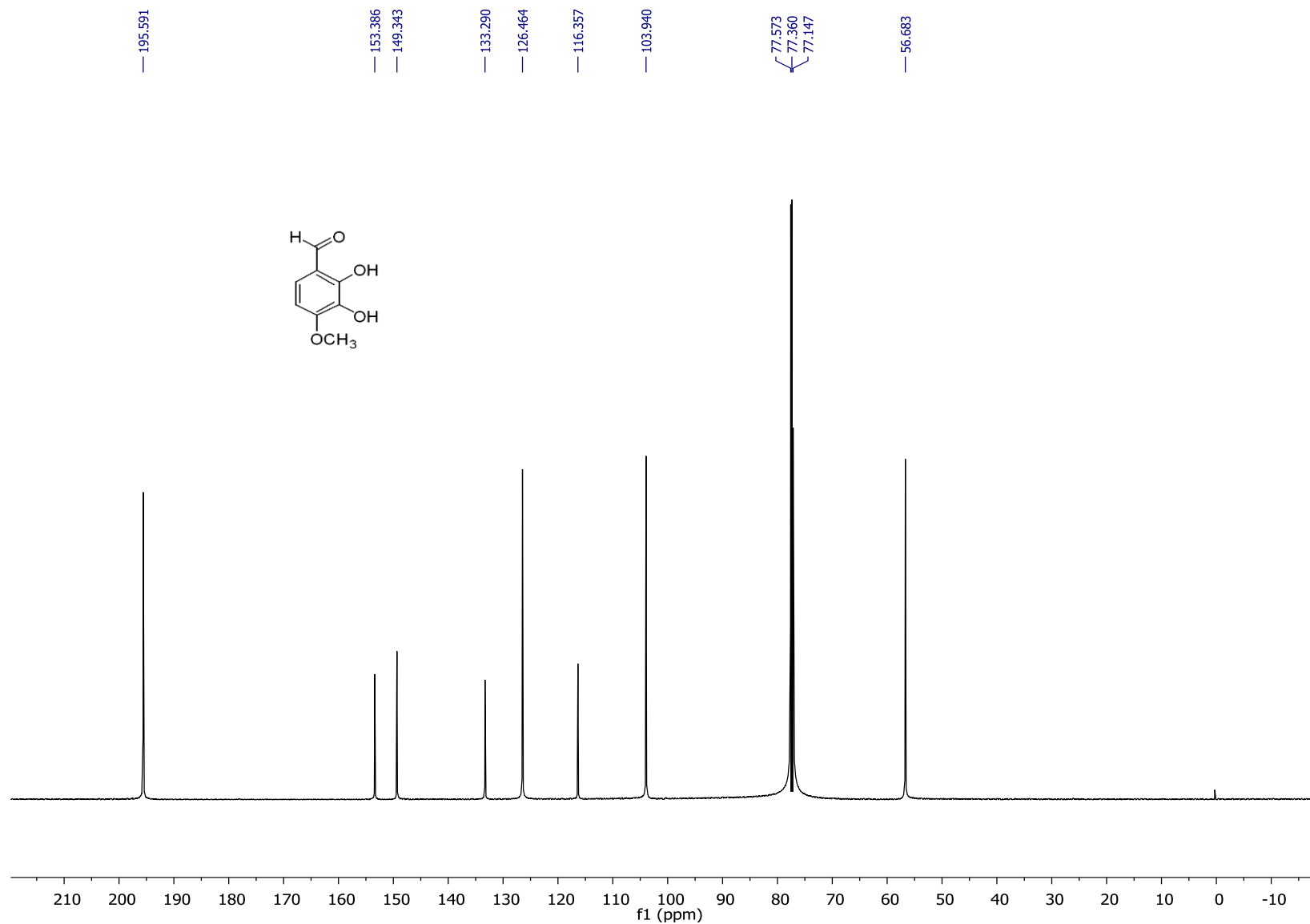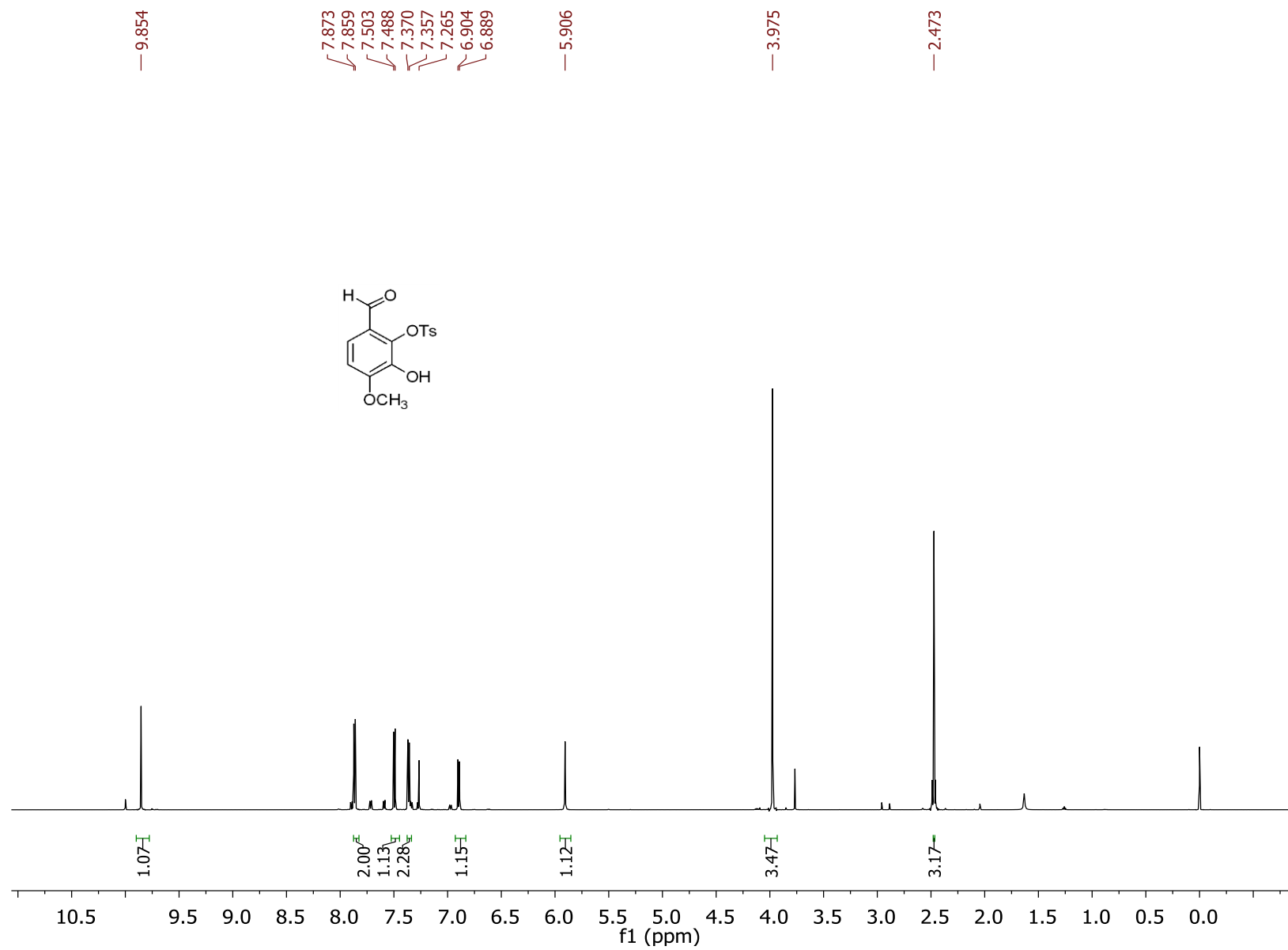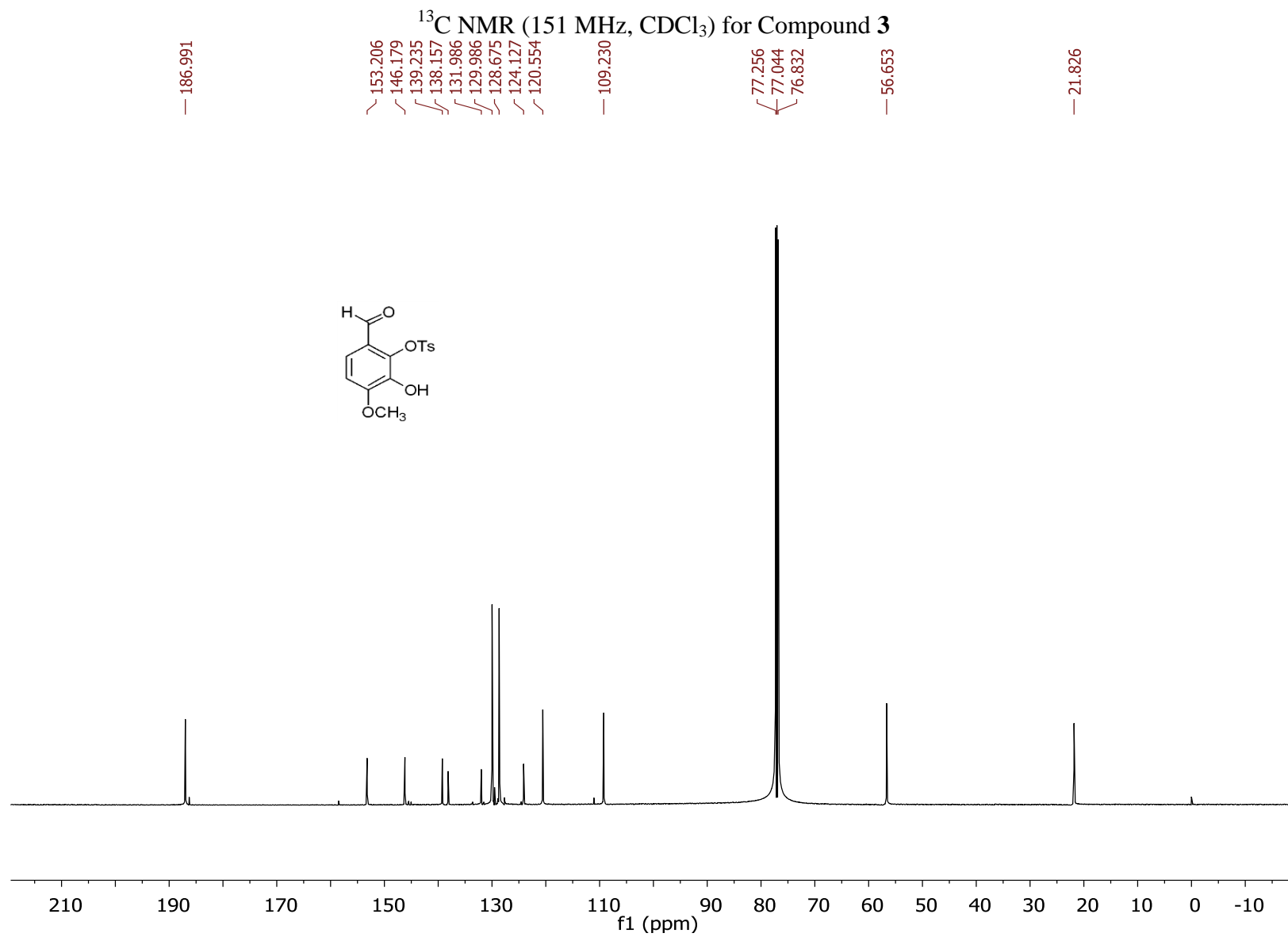
¹³C NMR (151 MHz, CDCl₃) for Compound **4**

^{13}C NMR (151 MHz, CDCl$_3$) for Compound **4**

189.681
152.753
147.904
138.622
124.335
120.364
106.223
77.372
77.160
76.997
76.948
56.439
22.430

f1 (ppm)
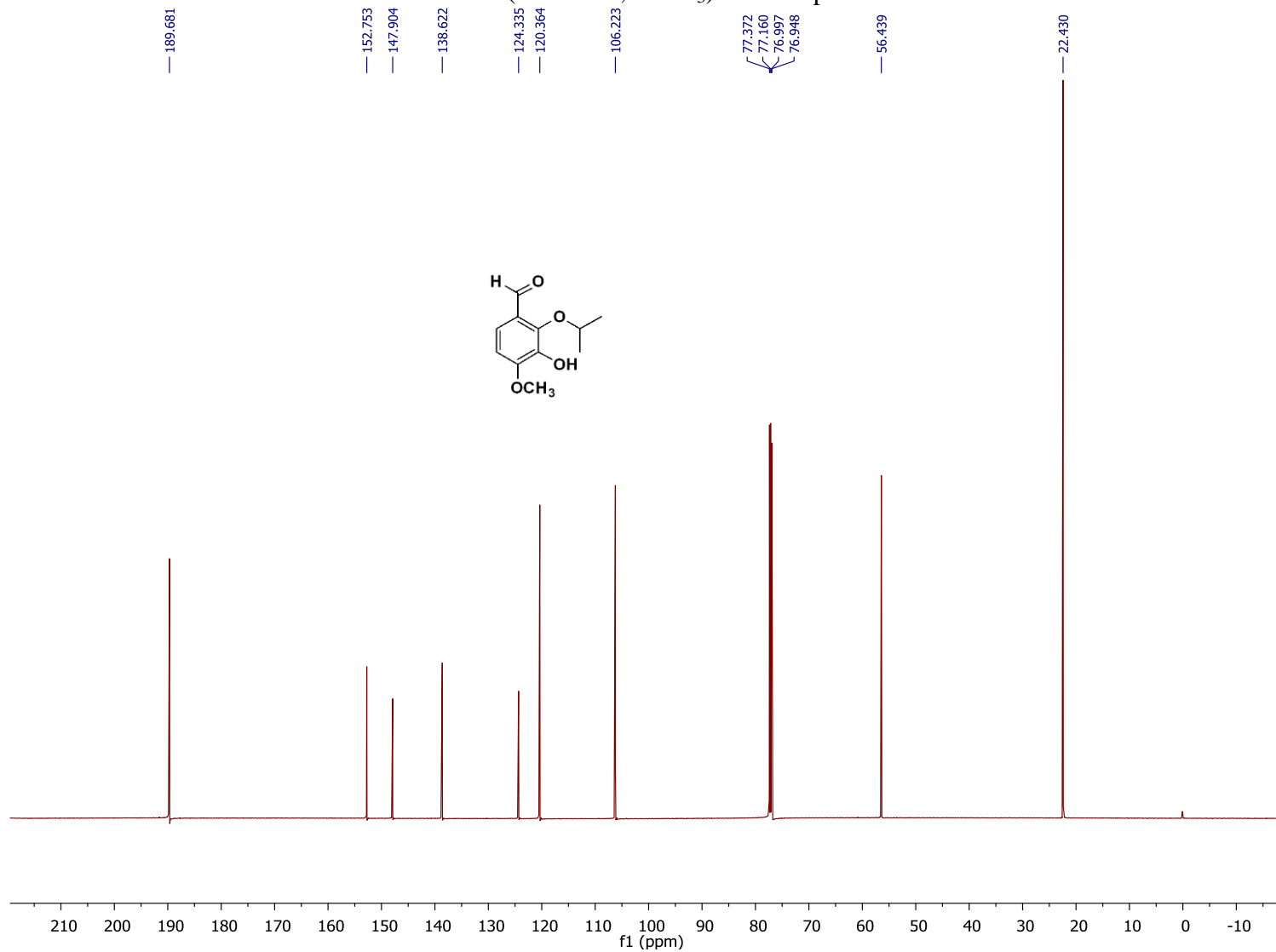
^{1}H NMR (600 MHz, CDCl$_3$) for Compound **5**

^{13}C NMR (151 MHz, CDCl$_3$) for Compound **5**

189.638

157.880
151.323

137.099

123.638
121.693

105.730

77.572
77.360
77.147

55.527

26.509
26.356

19.074
18.886

3.510

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
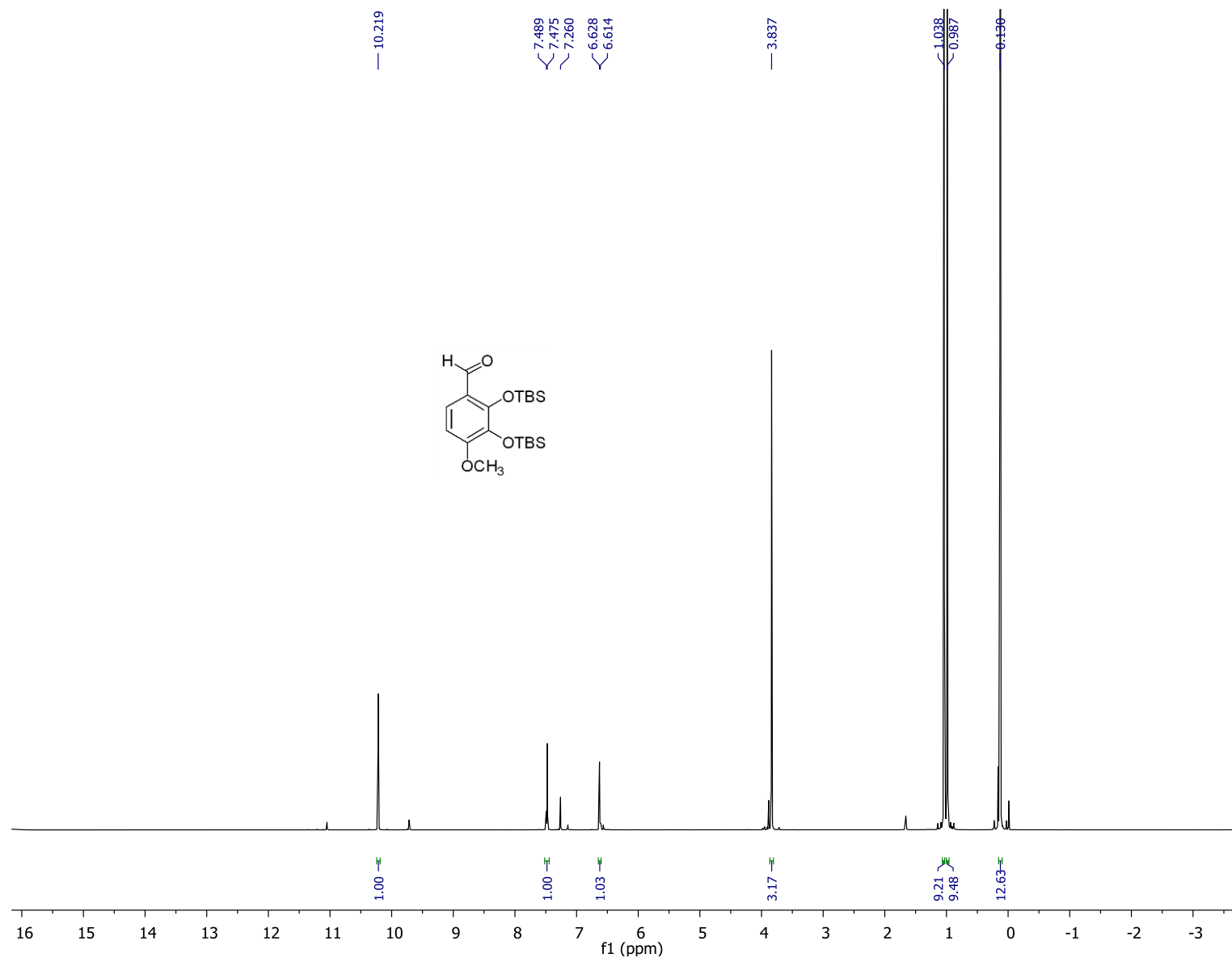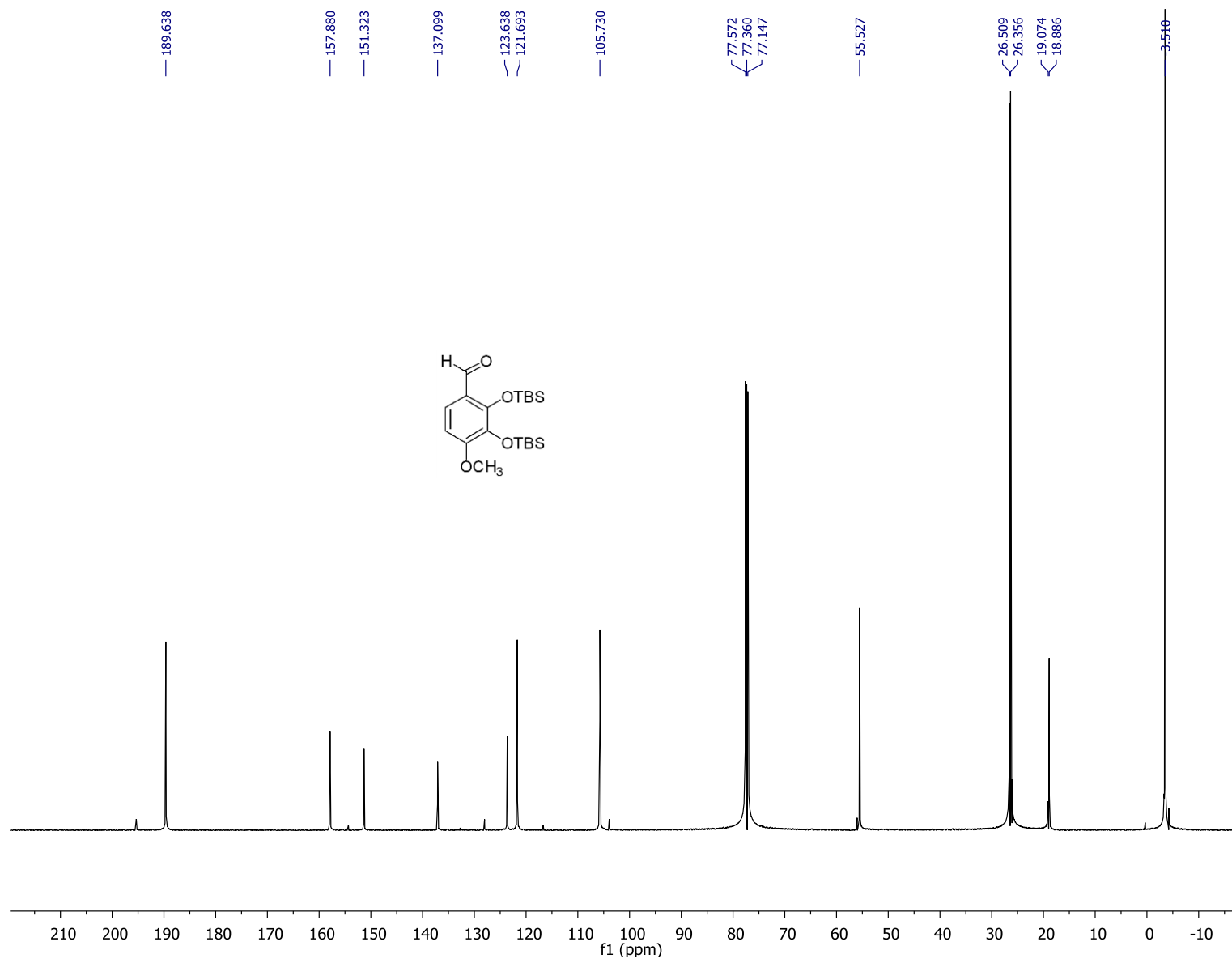
^{1}H NMR (500 MHz, CDCl$_3$) for Compound **6**
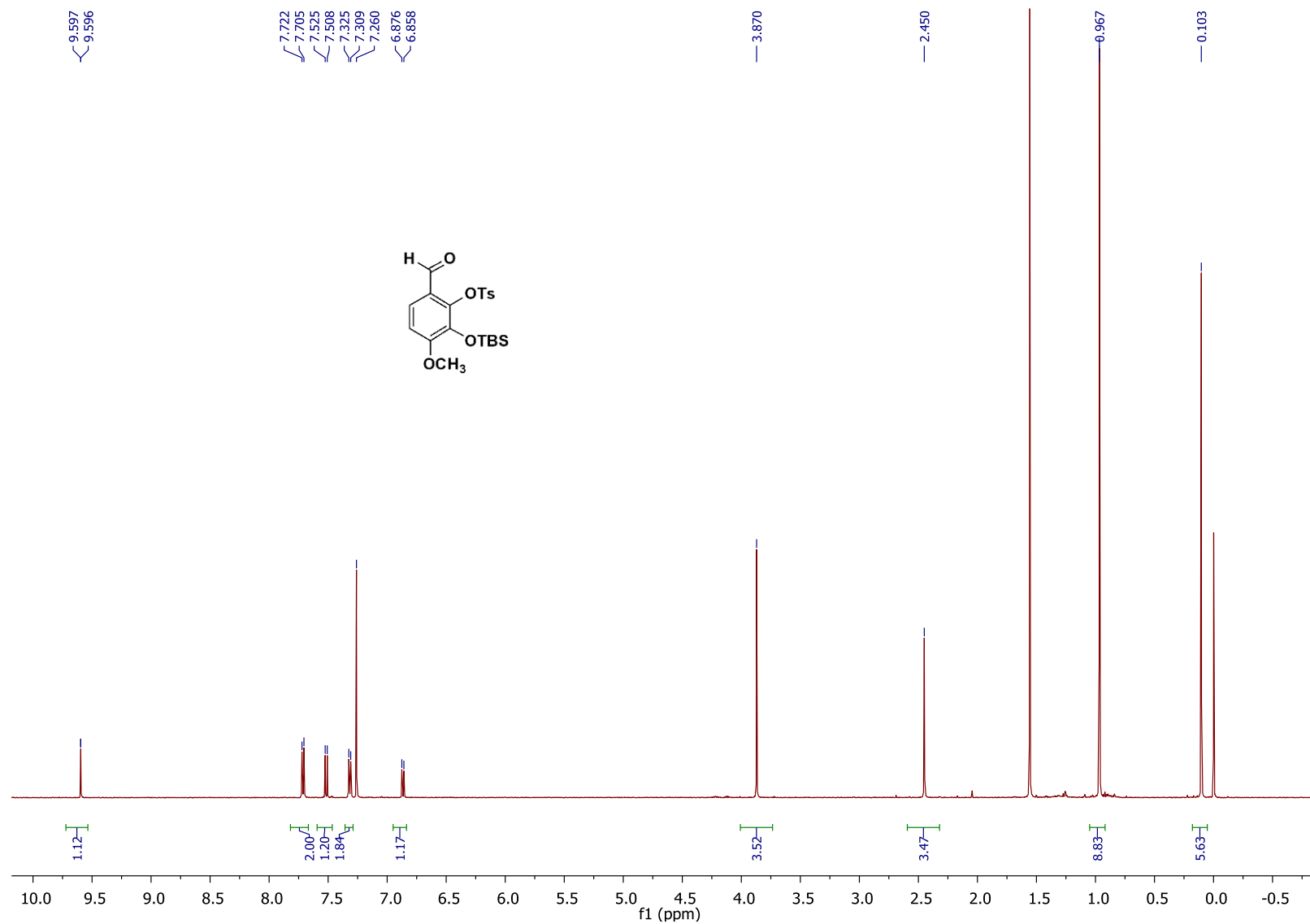
^{13}C NMR (126 MHz, CDCl$_3$) for Compound **6**

^{1}H NMR (600 MHz, CDCl$_3$) for Compound **7**

322

¹³C NMR (151 MHz, CDCl₃) for Compound **7**

^{1}H NMR (600 MHz, CDCl$_3$) for Compound **10**

— 23.217

| 140 | 120 | 100 | 80 | 60 | 40 | 20 | 0 | -20 | -40 | -60 | -80 | -100 | -120 | -140 | -160 | -180 | -200 | -220 | -240 |

f1 (ppm)
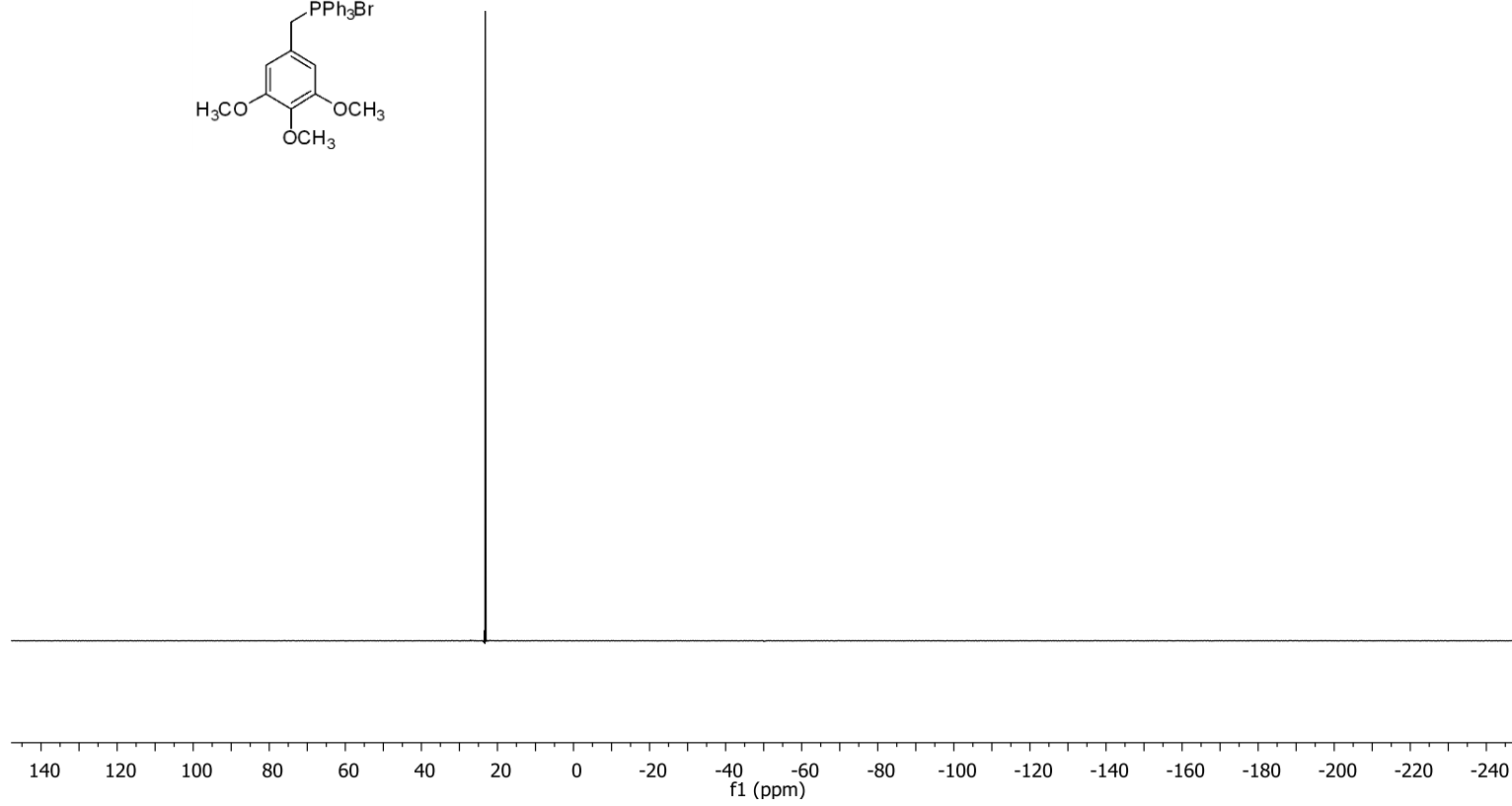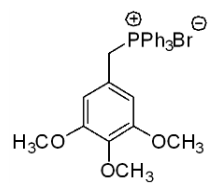
¹H NMR (500 MHz, CDCl₃) Compound **11**
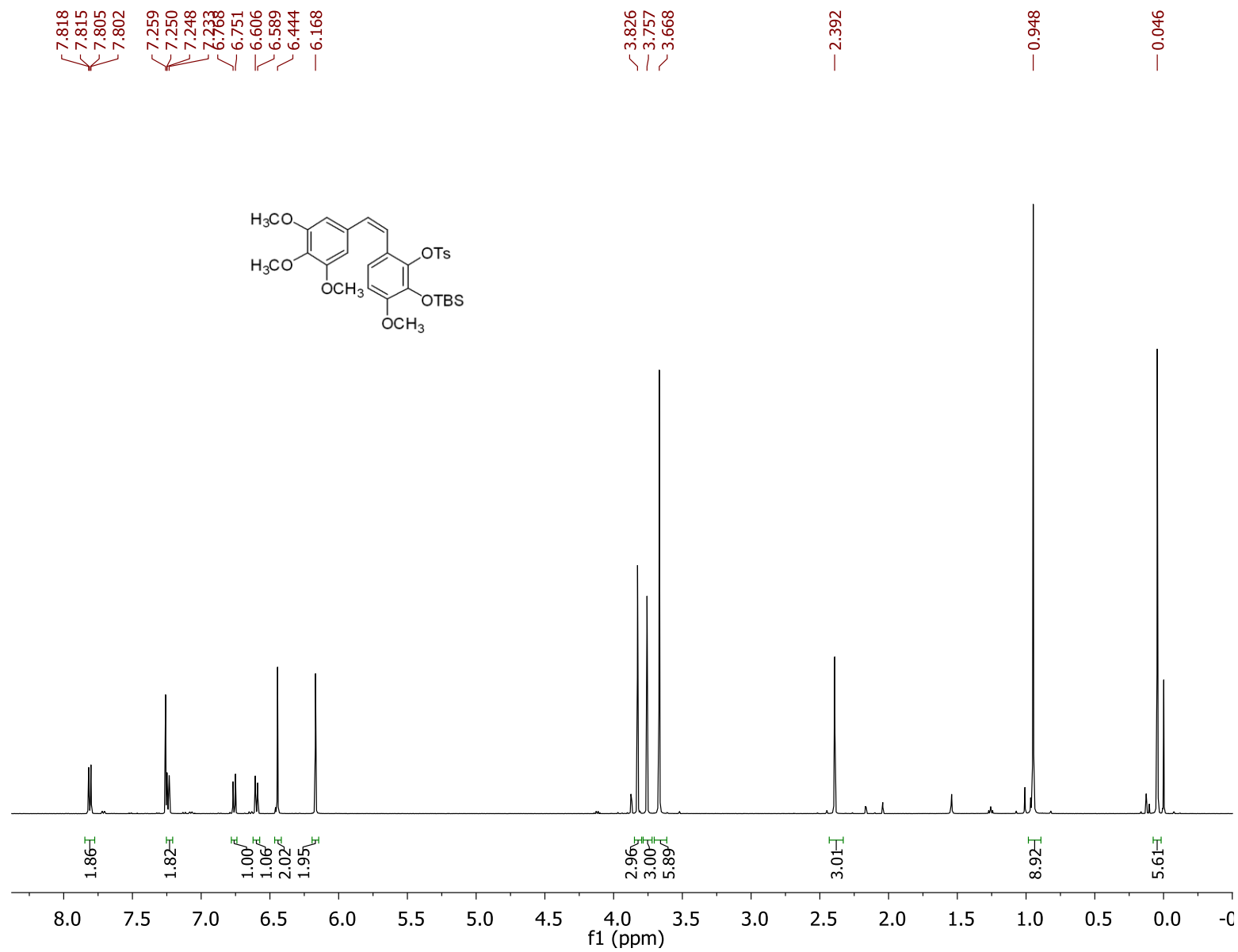
^{13}C NMR (126 MHz, CDCl$_3$) for Compound **11**
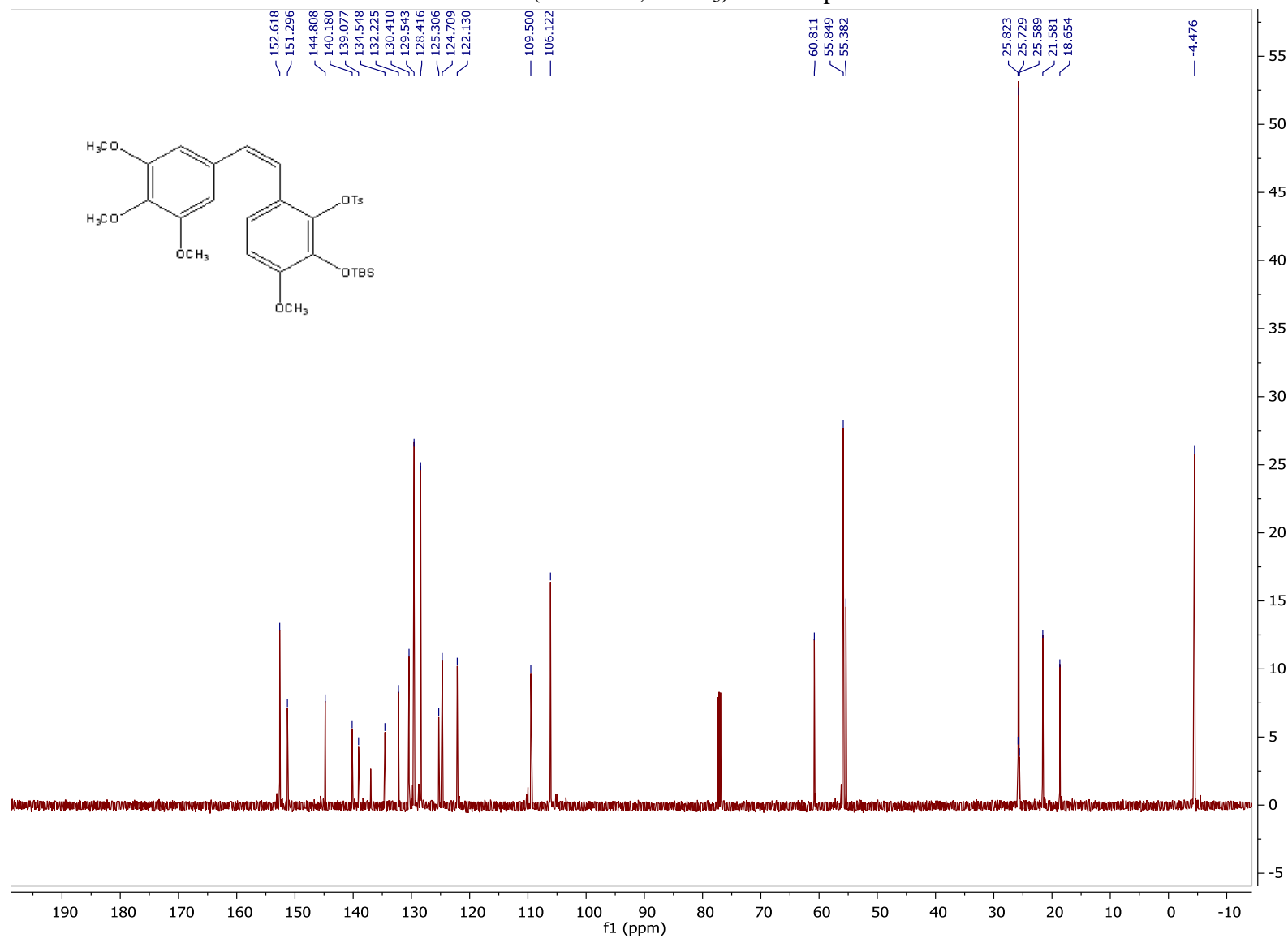
¹H NMR (600 MHz, CDCl₃) Compound **12**
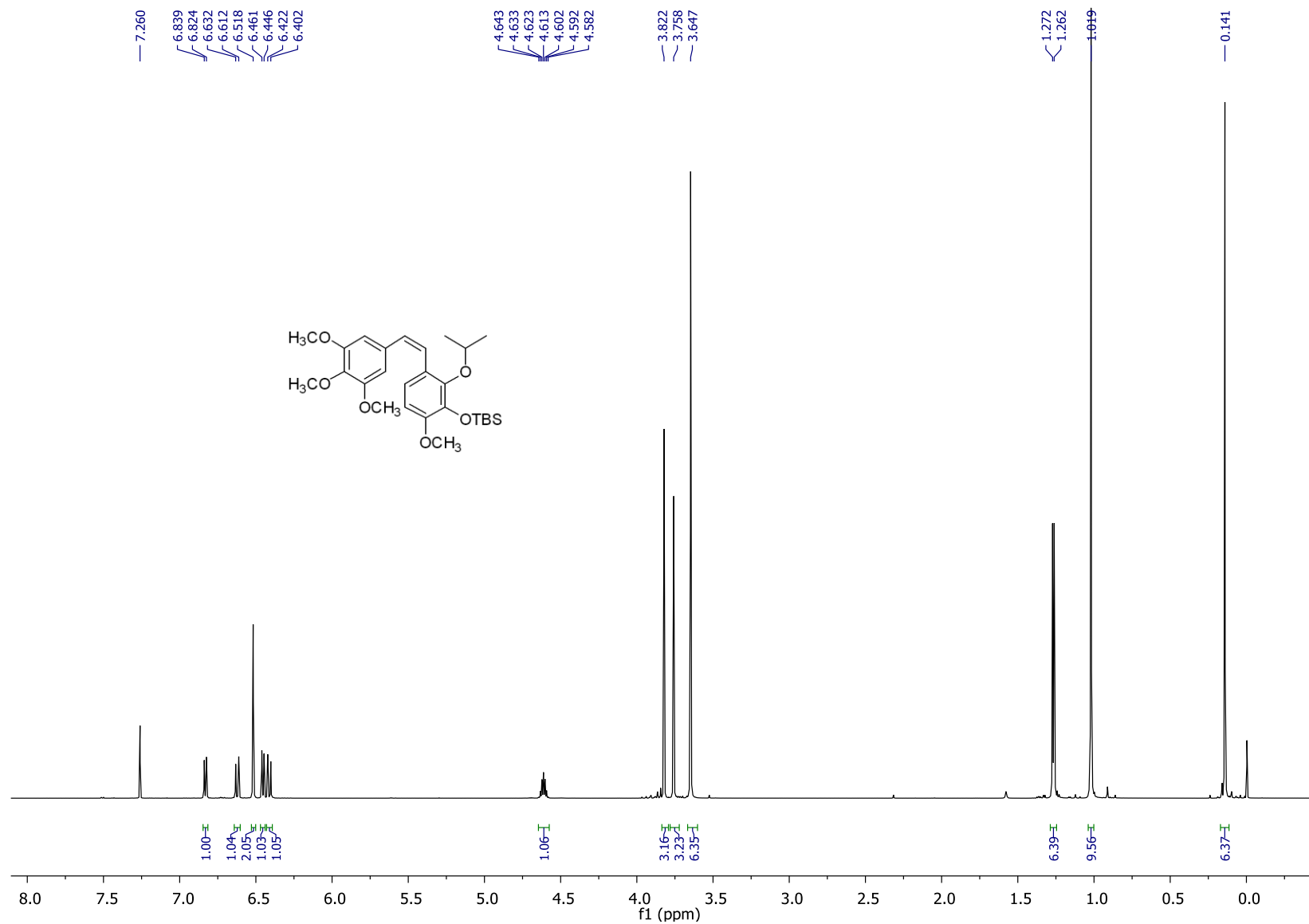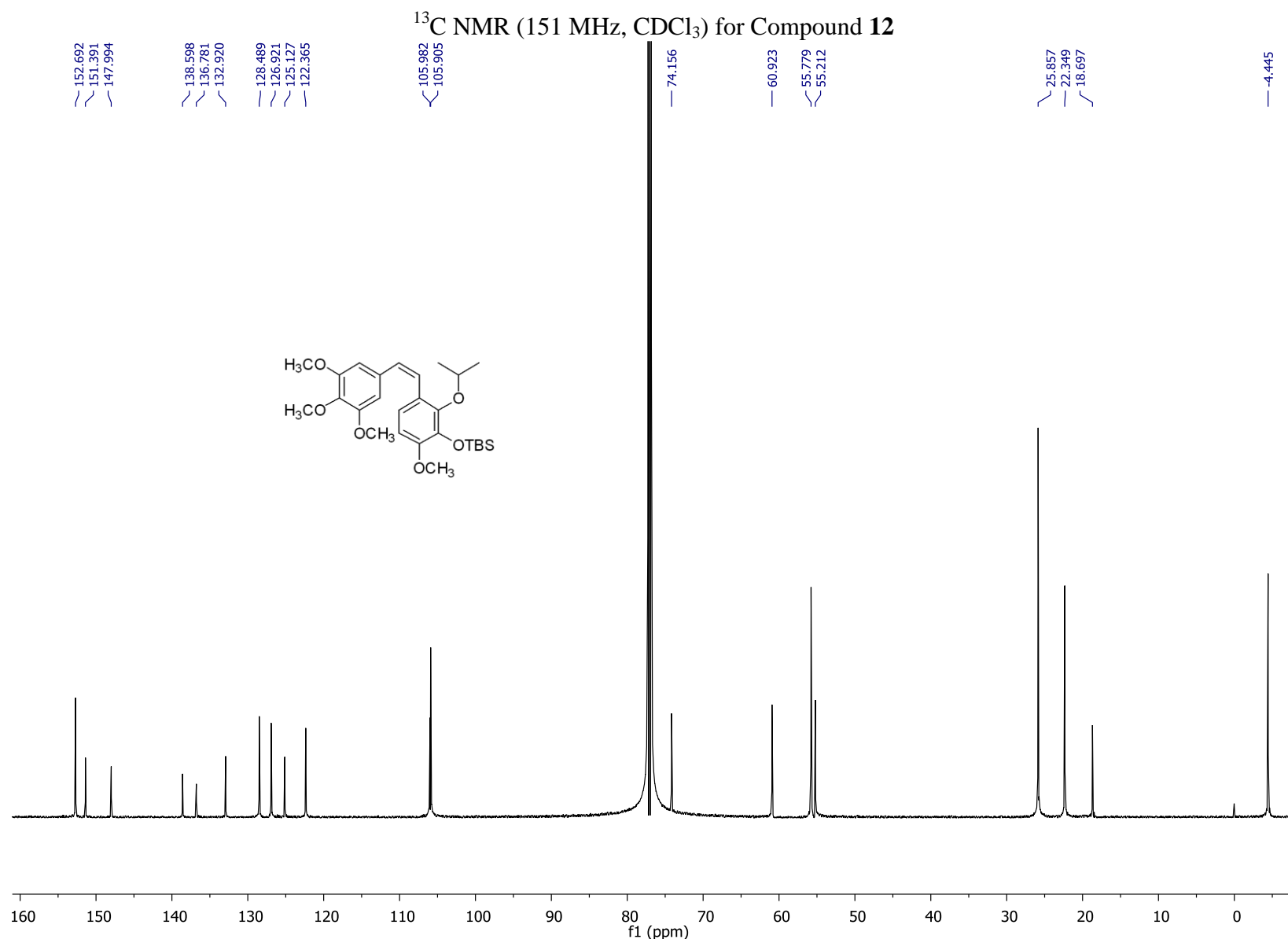
^{13}C NMR (151 MHz, CDCl$_3$) for Compound **12**

^{1}H NMR (500 MHz, CDCl$_3$) for Compound **13**
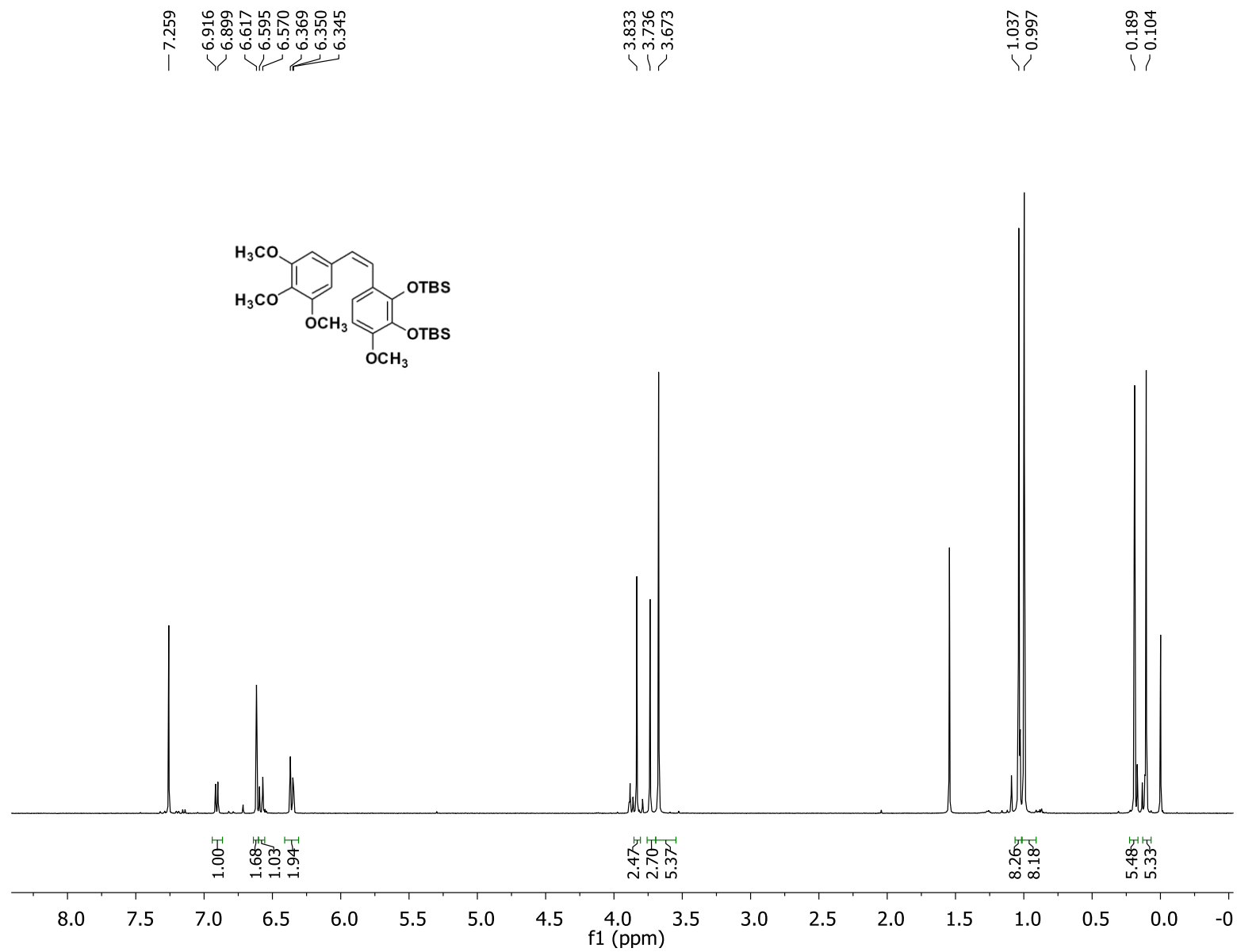
330

¹³C NMR (125 MHz, CDCl₃) for Compound **13**

153.001
152.033
146.519
137.136
137.084
133.138
127.970
127.686
123.494
122.534
106.168
104.457
77.572
77.360
77.147
61.234
56.148
55.274
26.699
26.440
19.052
18.907
-2.910
-3.587

70   160   150   140   130   120   110   100   90   80   70   60   50   40   30   20   10   0   -10
f1 (ppm)

^{1}H NMR (500 MHz, CDCl$_3$) for Compound **15**

7.92
7.91
7.60
7.59
7.28

2.63

1.00
1.02

3.13

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3

f1 (ppm)

^{13}C NMR (500 MHz, CDCl$_3$) for Compound **15**

190.35

156.47

148.16

130.06
128.28

26.61

333
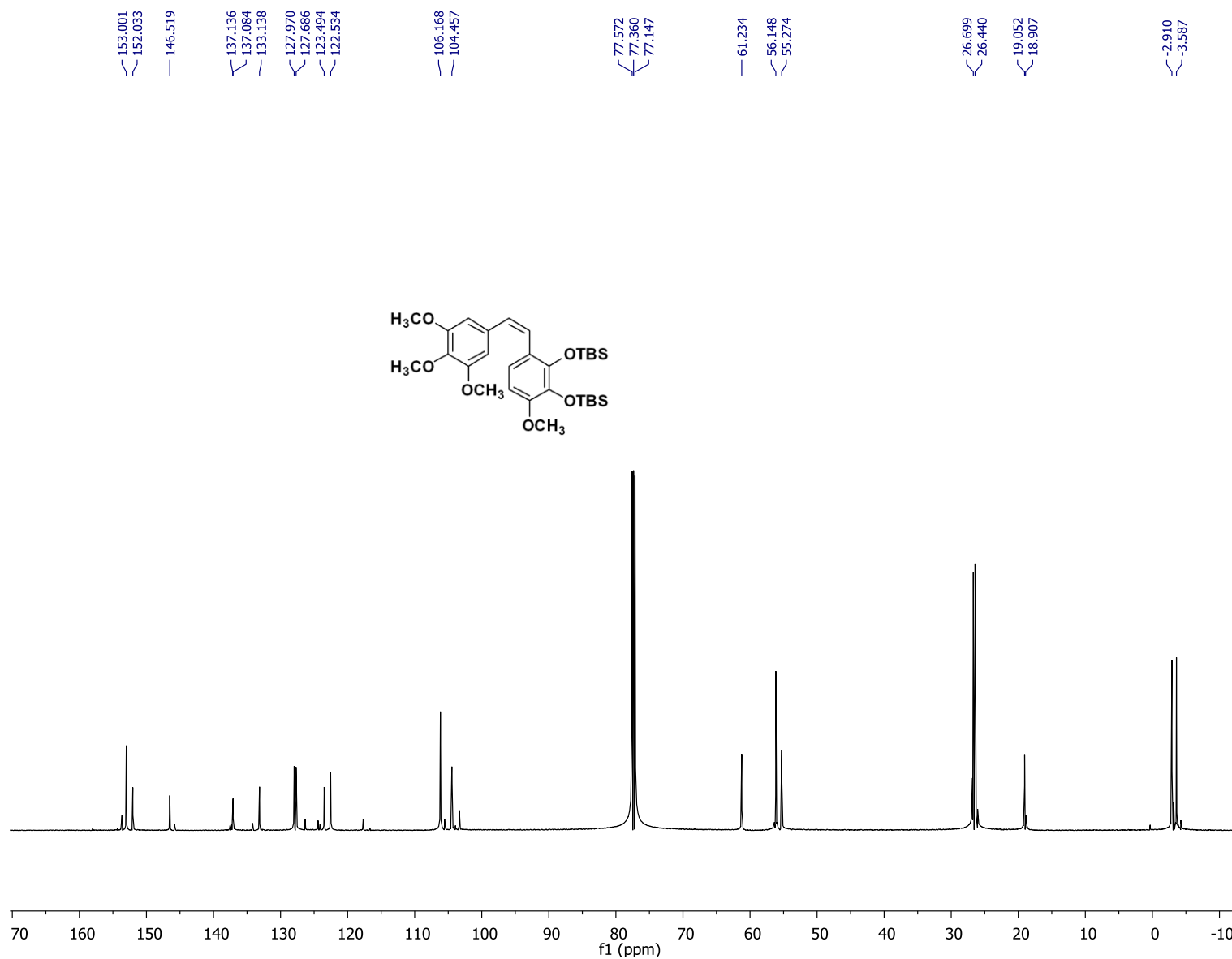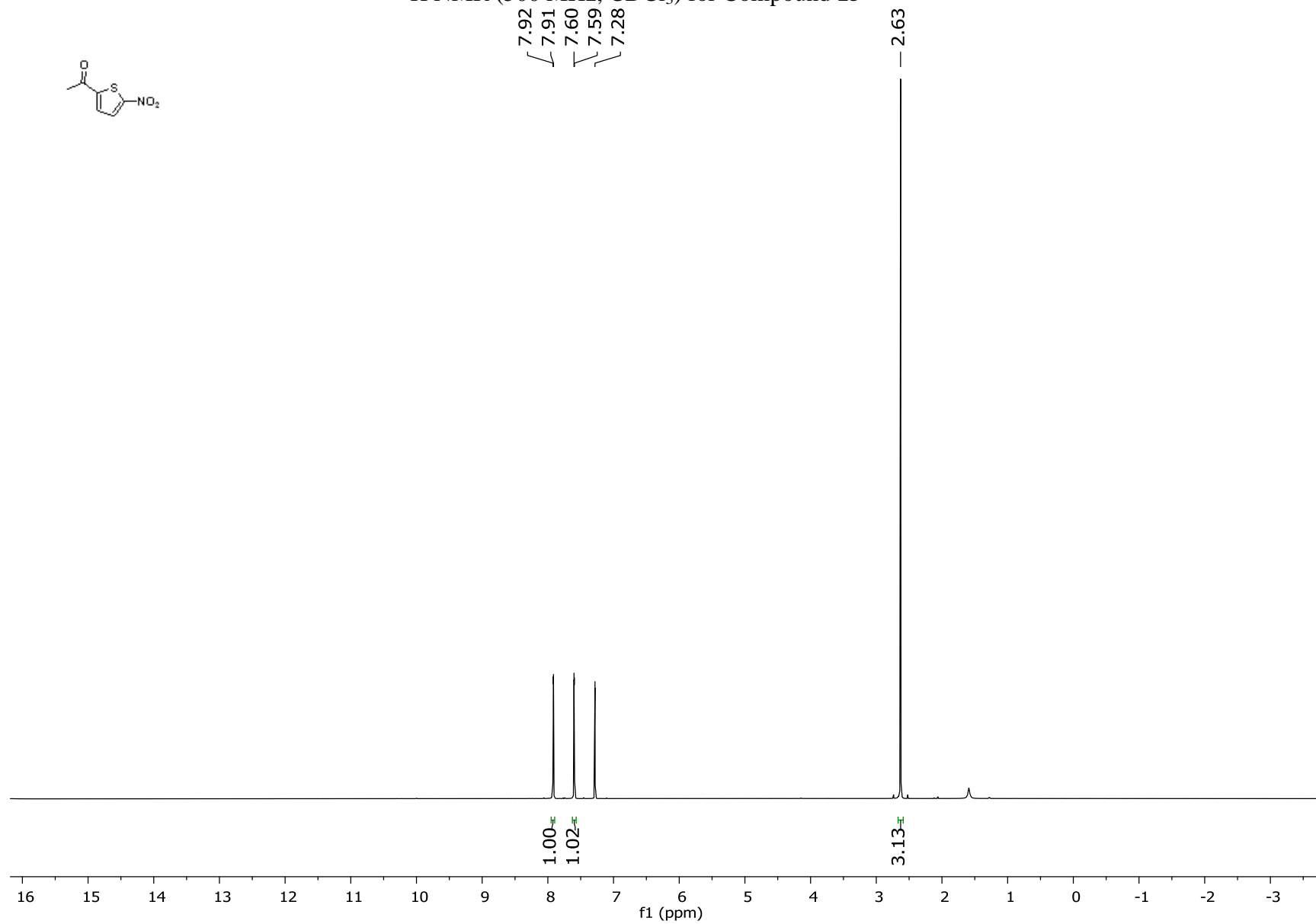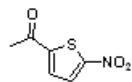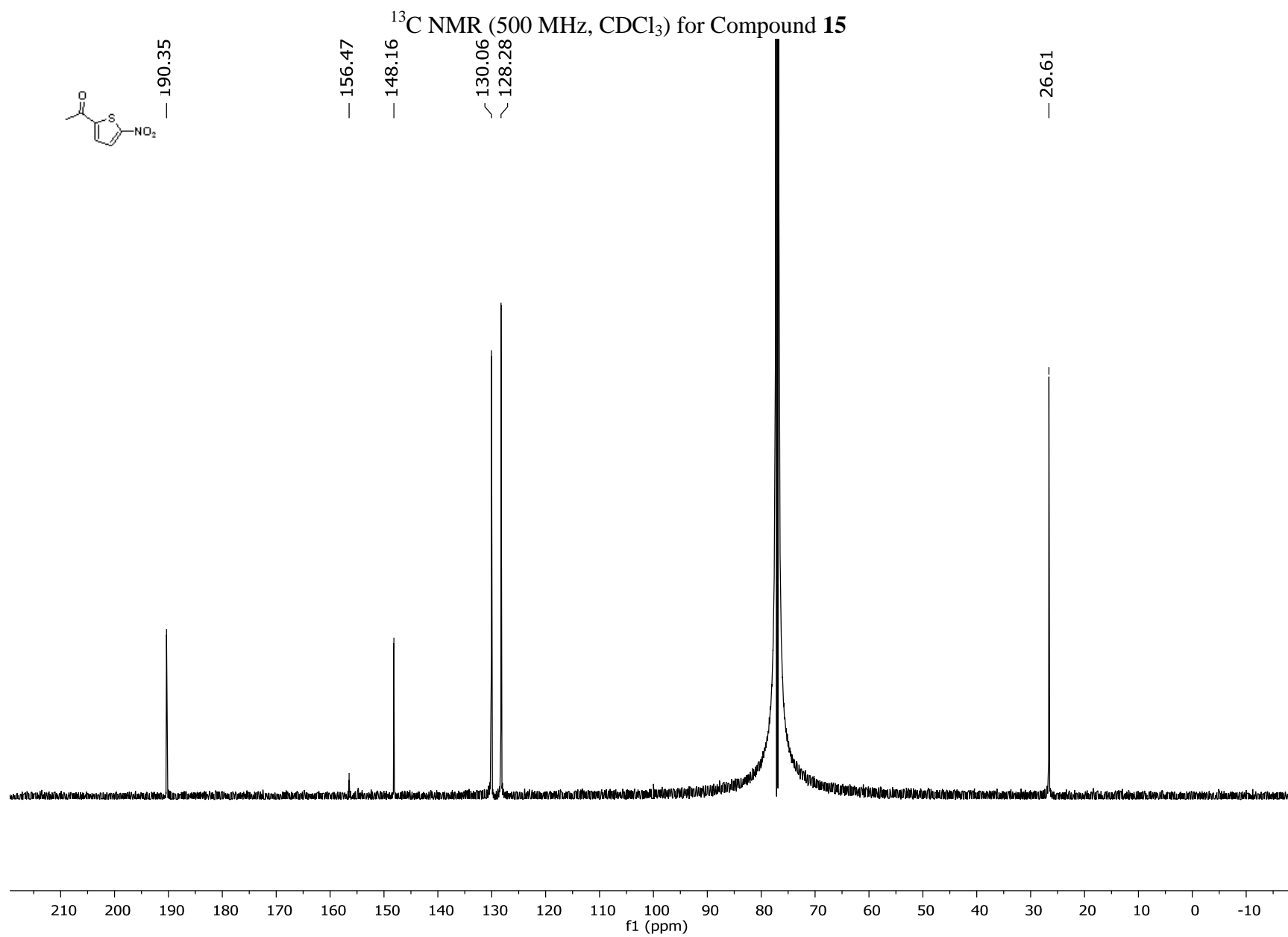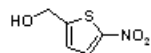
^{1}H NMR (500 MHz, CDCl$_3$) for Compound **16**

7.84
7.83
7.28
6.96
6.96
6.96
6.95
6.95
6.95
4.91
4.90
2.16
2.15
2.14
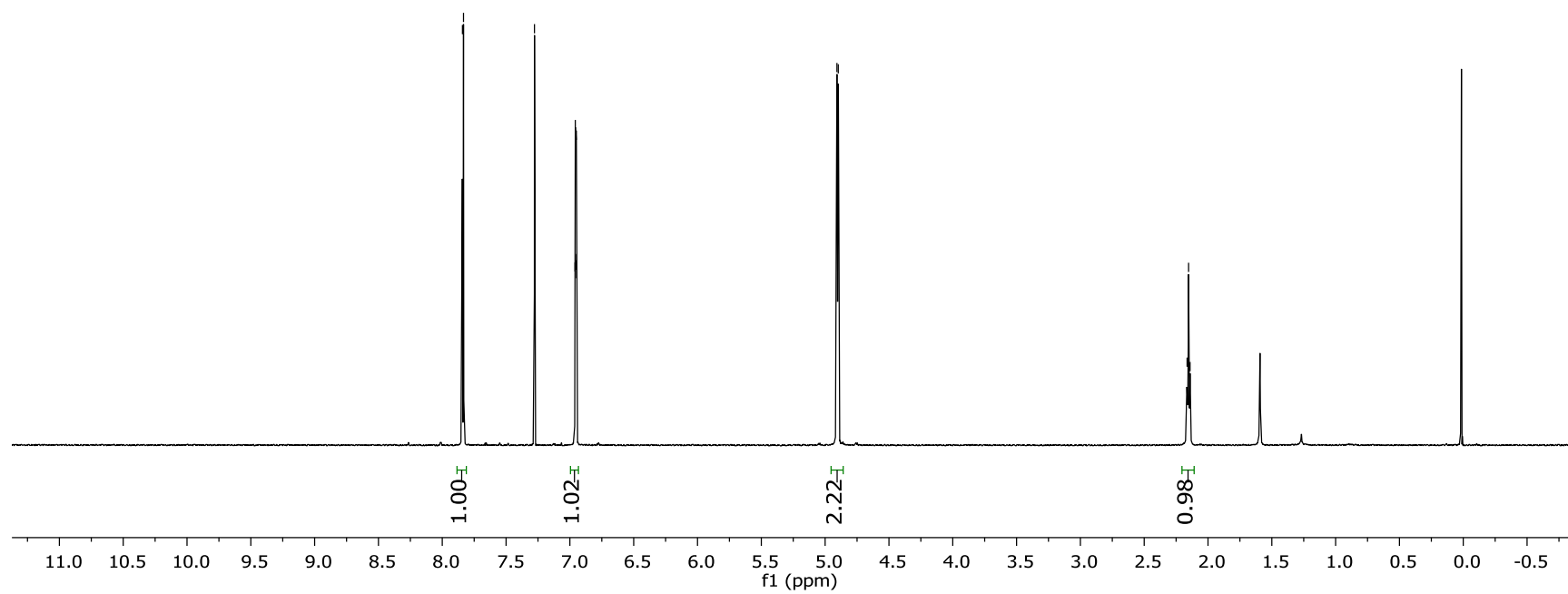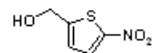
1.00
1.02
2.22
0.98
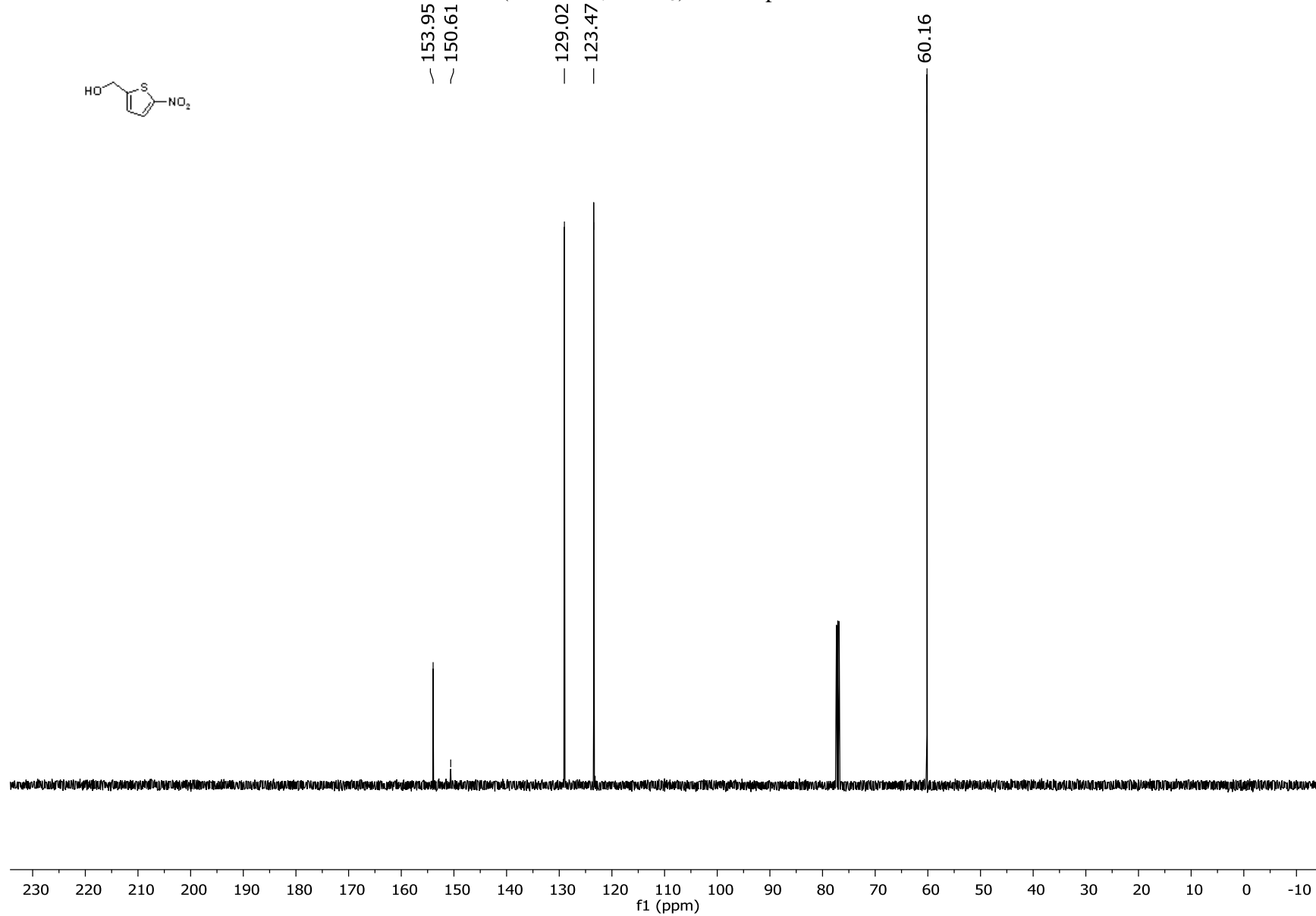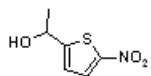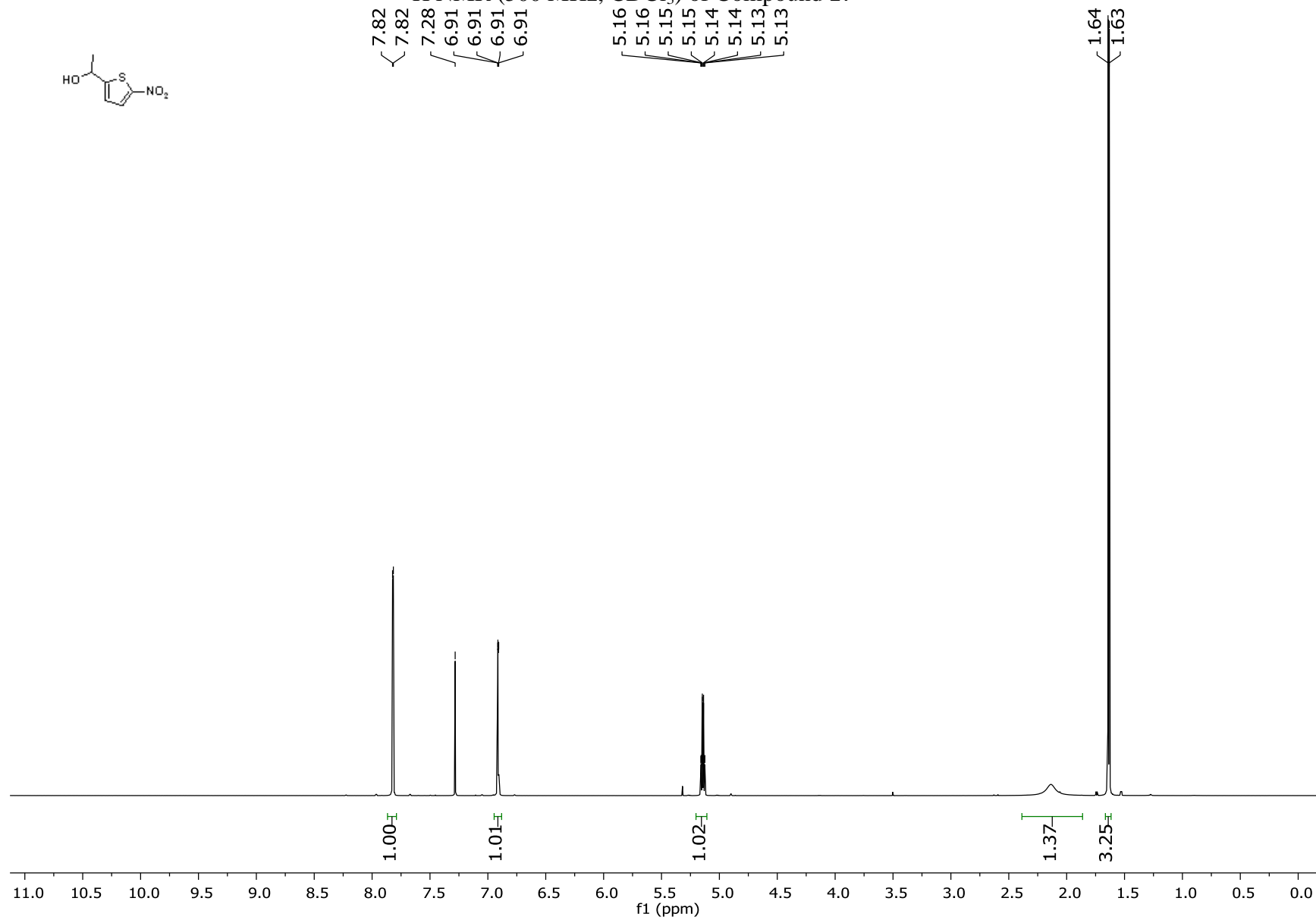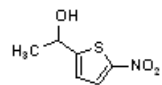
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **16**

153.95
150.61
129.02
123.47
60.16

f1 (ppm)

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **17**

7.82
7.82
7.28
6.91
6.91
6.91
6.91
5.16
5.16
5.15
5.15
5.14
5.14
5.13
5.13
1.64
1.63

1.00
1.01
1.02
1.37
3.25

11.0   10.5   10.0   9.5   9.0   8.5   8.0   7.5   7.0   6.5   6.0   5.5   5.0   4.5   4.0   3.5   3.0   2.5   2.0   1.5   1.0   0.5   0.0
f1 (ppm)

^{13}C NMR (500 MHz, CDCl$_3$) for Compound **17**

159.97
149.95
129.13
122.24
66.34
25.09

f1 (ppm)

337

^{1}H NMR (500 MHz, CDCl$_3$) for Compound **19**

7.81
7.80
7.28
6.89
6.88

1.69

1.00
1.01
6.80

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)

338

^{13}C NMR (500 MHz, CDCl$_3$) for Compound **19**

163.46
150.04
128.76
121.26
71.92
32.08
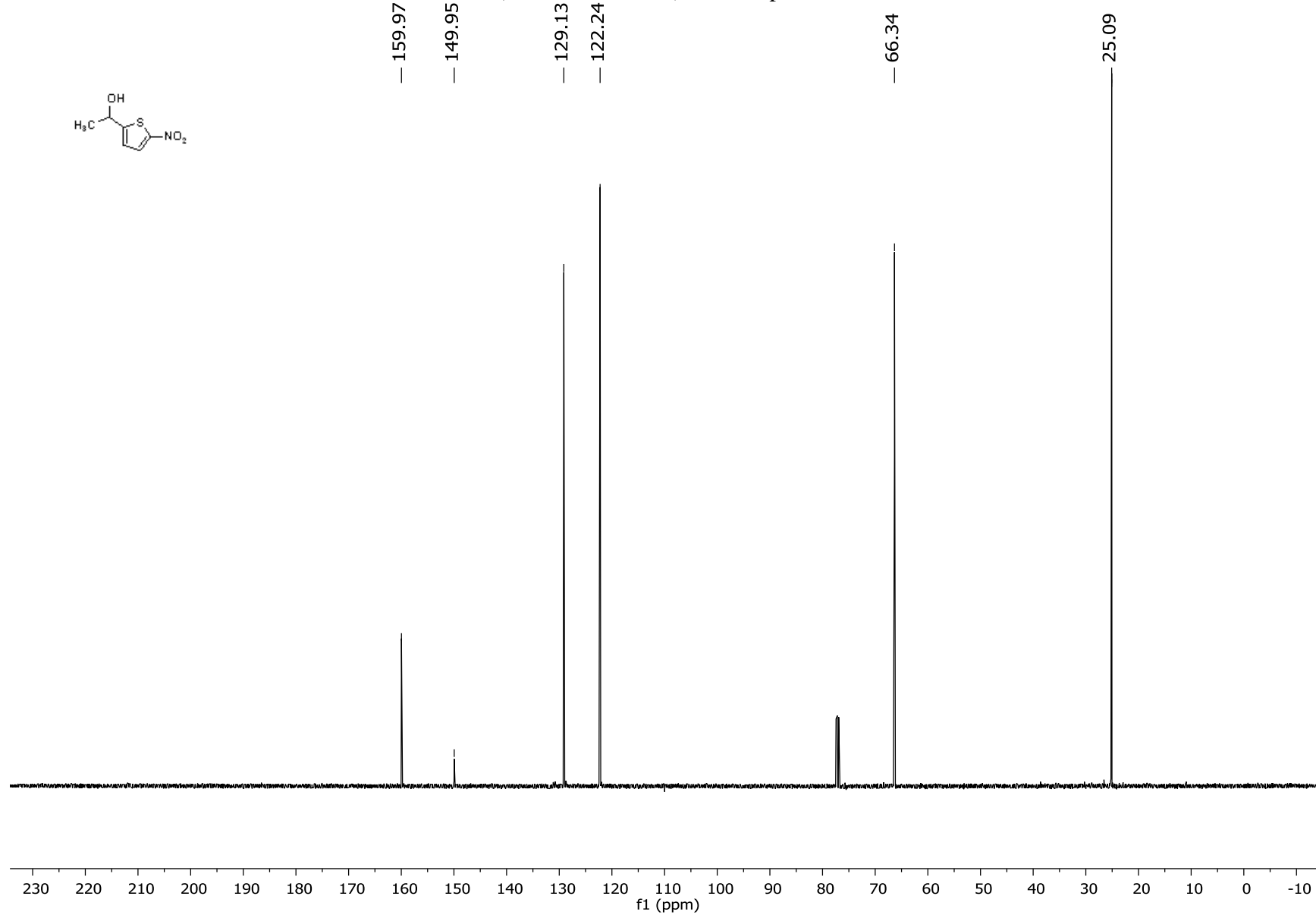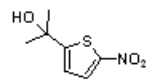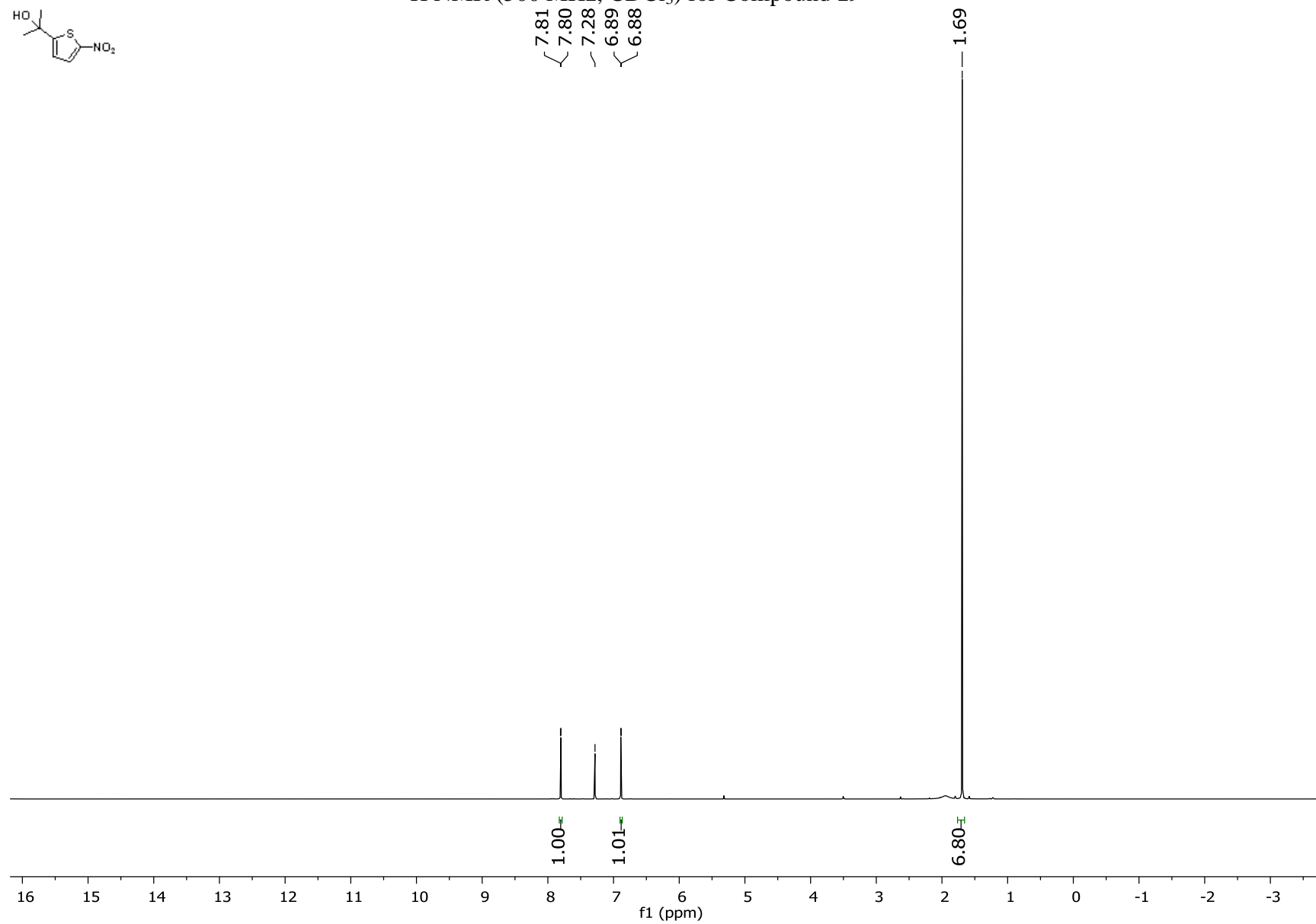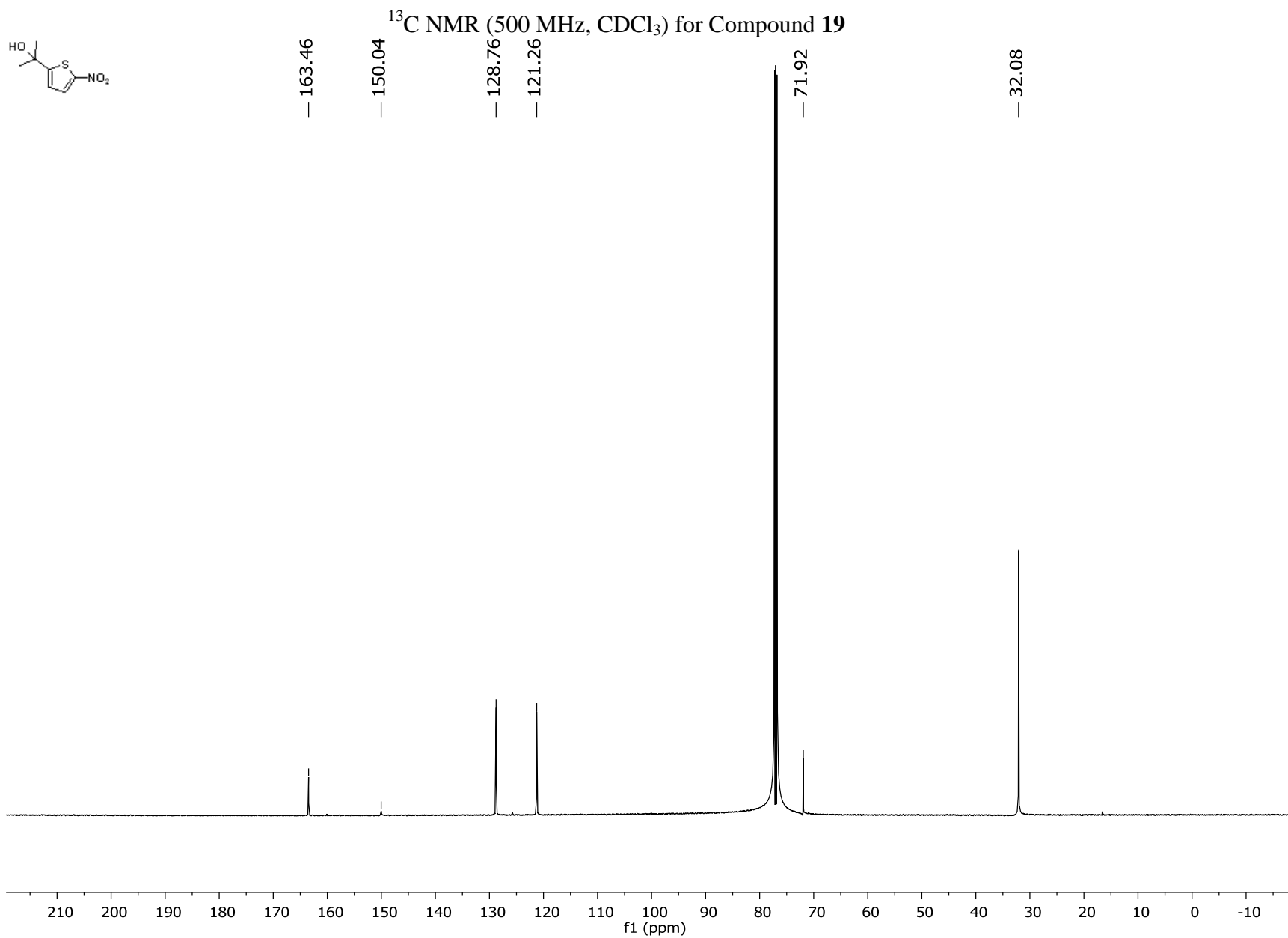
^{1}H NMR (500 MHz, CDCl$_3$) for Compound **20**
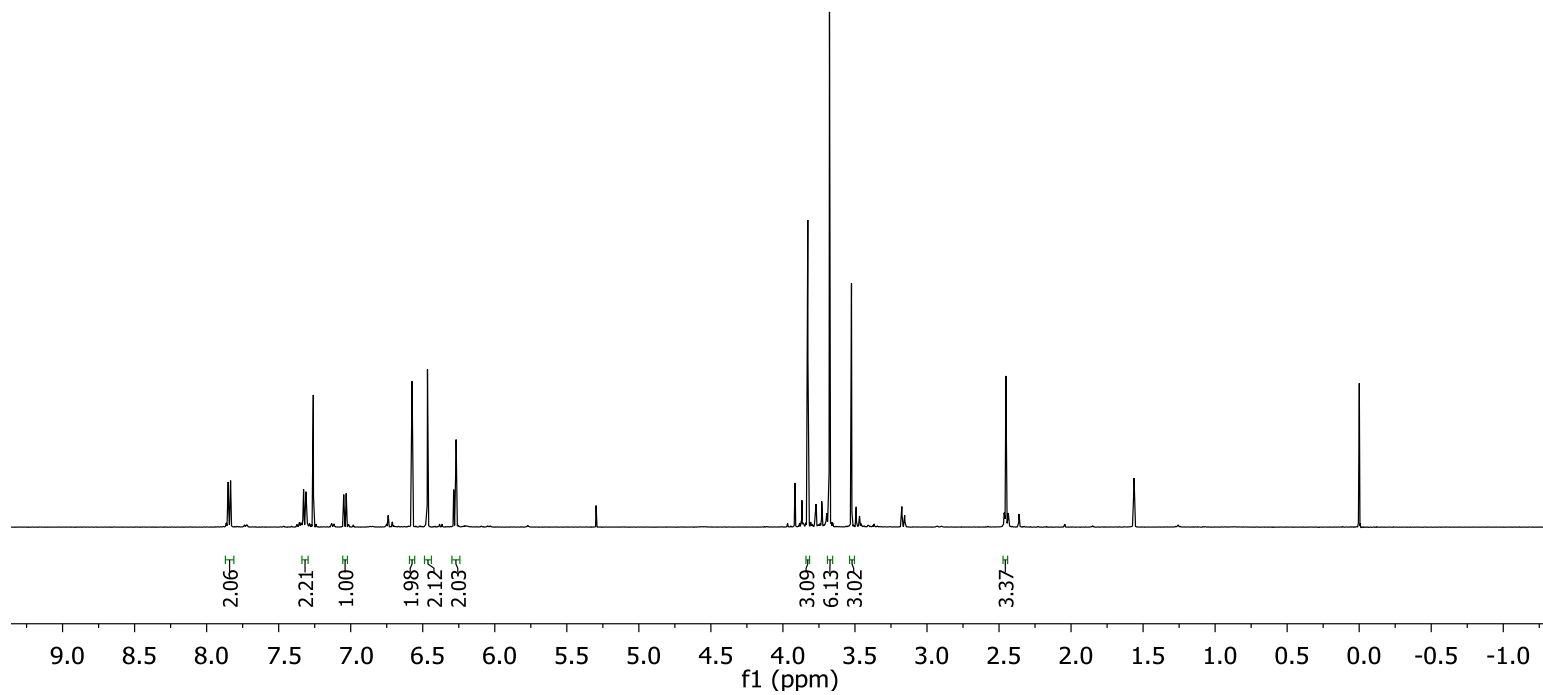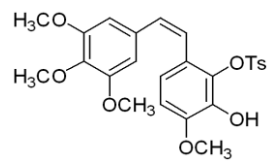
^{13}C NMR (125 MHz, CDCl$_3$) for Compound **20**
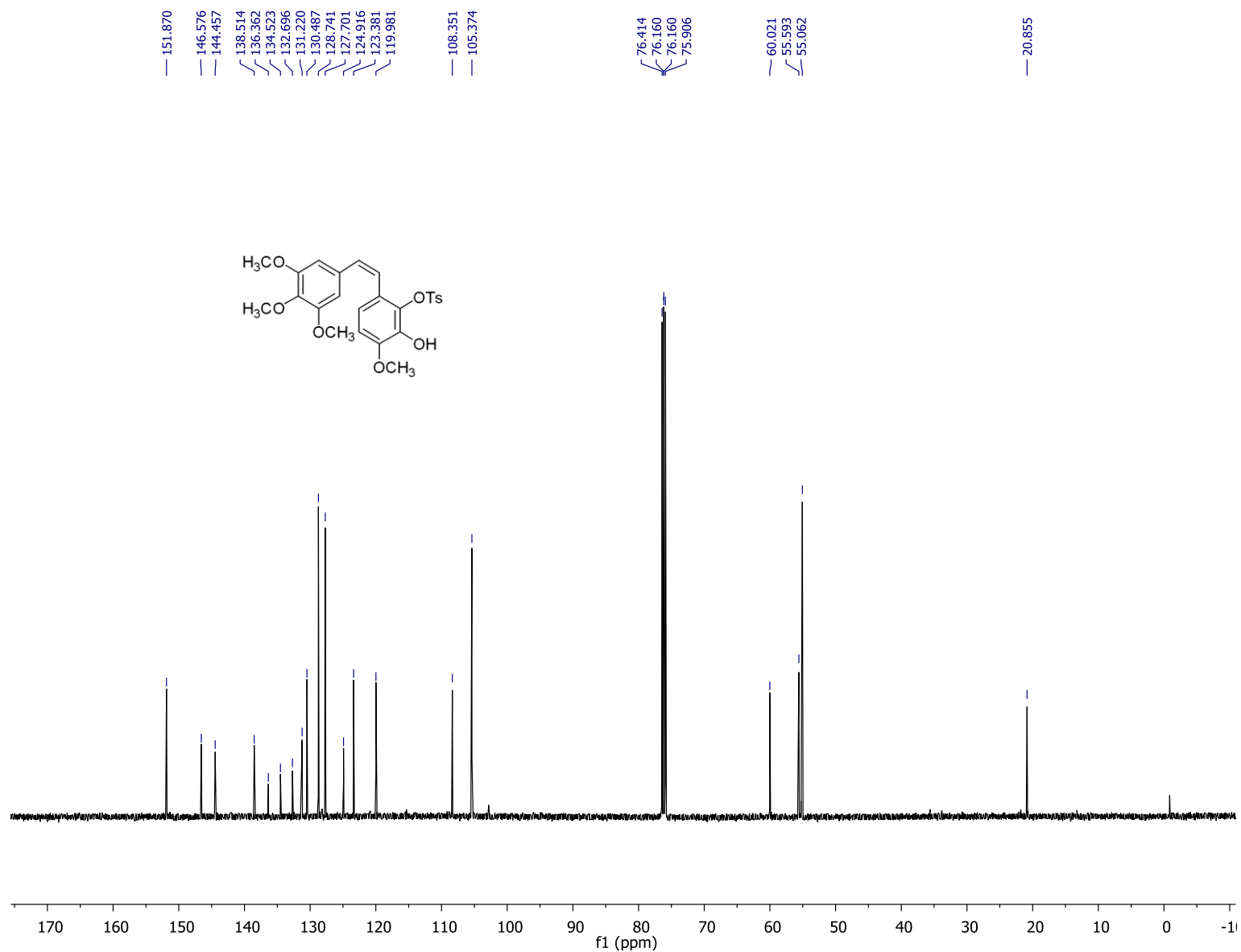
^{1}H NMR (500 MHz, CDCl$_3$) for Compound **21**

^{13}C NMR (125 MHz, CDCl$_3$) for Compound **21**

152.730

146.852
143.182
138.948
137.114
132.548
129.392
125.799
124.132
120.478

106.271
106.027

77.240
76.986
76.732
75.719

60.866
56.190
55.808

22.450

60   150   140   130   120   110   100   90   80   70   60   50   40   30   20   10   0
f1 (ppm)
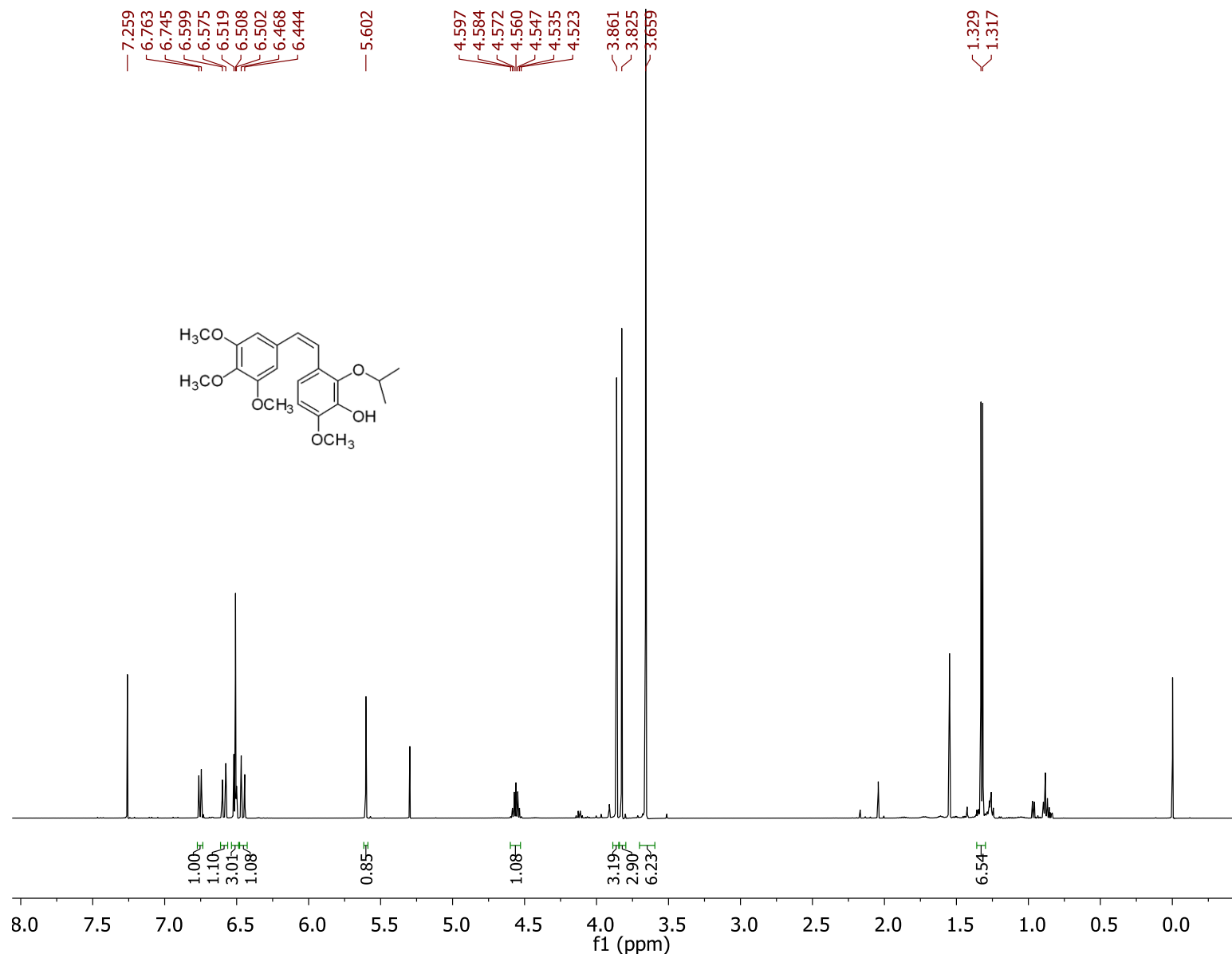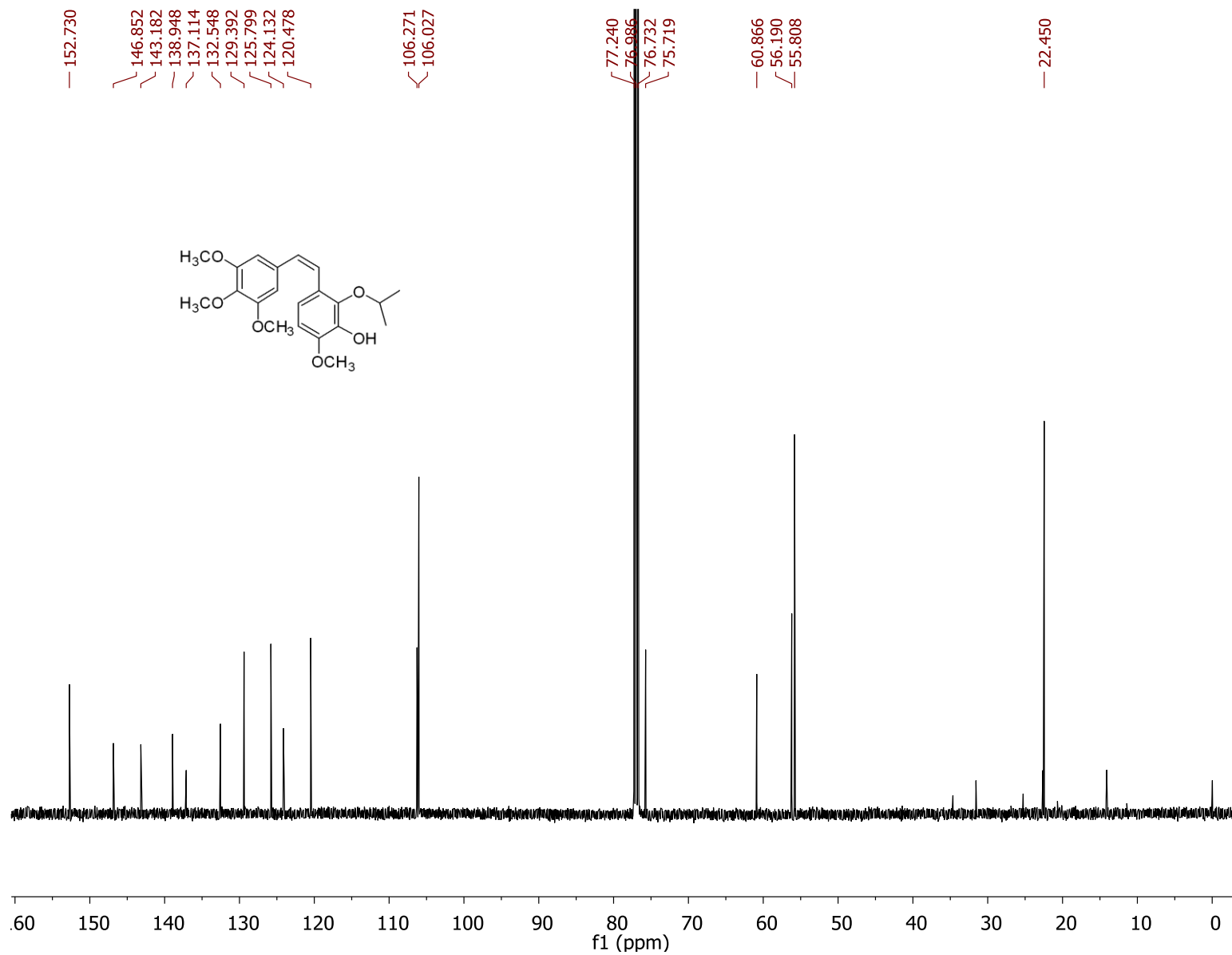
HPLC trace of Compound **21**

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-11-1A\LDV-11-1ARUN001.D
Sample Name: LD-V-11-1A-run1
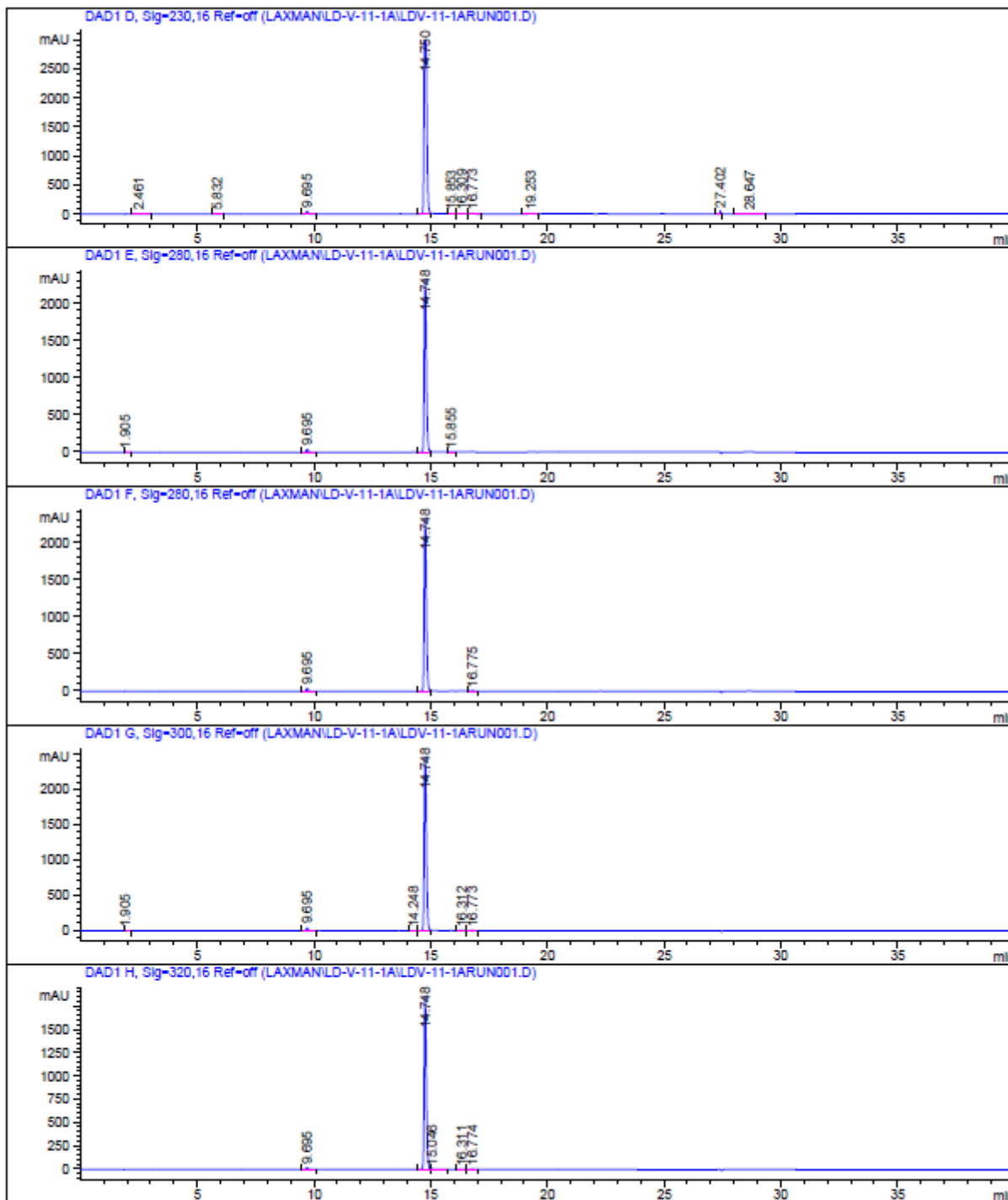
```
=================================================================
Acq. Operator   : Laxman
Acq. Instrument : Instrument 1              Location :   -
Injection Date  : 2/4/2014 12:51:42 PM
Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
Last changed    : 2/4/2014 12:46:52 PM by Laxman
Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-V-11-1A\LDV-11-1ARUN001.D\DA.M (MASTERMETHOD.M)
Last changed    : 2/4/2014 2:30:02 PM by Laxman
Sample Info     : 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
```

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

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

Chemical Formula: $C_{21}H_{26}O_6$
Exact Mass: 374.1729
Molecular Weight: 374.4330

NL:
7.81E8
LD-V-11-1A_Orbo
+ESI#12 RT: 0.11 AV:
1 T: FTMS + p ESI
sid=35.00 Full ms
[100.00-1000.00]

397.1713
z=1

333.1409
z=1

181.0899
z=1

318.1170
z=1

413.1451
z=1

483.1100
z=1

581.2529
z=2

719.1889
z=?

771.3519
z=1

833.3237
z=1

945.2813
z=1

NL:
7.84E5
$C_{21}H_{26}O_6$:
$C_{21}H_{26}O_6$
pa Chrg 1

374.1724

NL:
7.84E5
$C_{21}H_{26}O_6$ +H:
$C_{21}H_{27}O_6$
pa Chrg 1

375.1802

NL:
7.84E5
$C_{21}H_{26}O_6$ +Na:
$C_{21}H_{26}O_6Na_1$
pa Chrg 1

397.1622

Relative Abundance

m/z

^{1}H NMR (600 MHz, CDCl$_3$) for Compound **22**

350

^{13}C NMR (151 MHz, CDCl$_3$) for Compound **22**

152.833, 152.463, 151.813, 147.932, 145.211, 141.777, 140.329, 137.155, 134.278, 132.049, 131.735, 129.571, 128.307, 128.051, 126.097, 125.915, 125.843, 123.962, 110.446, 106.138, 69.048, 60.932, 56.228, 55.930, 21.688

351

HPLC Traces of Compound **22**

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. Operator    : Laxman
Acq. Instrument  : Instrument 1              Location :    -
Injection Date   : 2/20/2013 10:40:08 AM
Acq. Method      : C:\CHEM32\1\METHODS\MASTERMETHOD.M
Last changed     : 2/20/2013 10:20:33 AM by Laxman
Analysis Method  : C:\CHEM32\1\DATA\LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D\DA.M (
                   MASTERMETHOD.M)
Last changed     : 2/20/2013 1:53:17 PM by Laxman
                   (modified after loading)
Sample Info      : run1
                   10%ACN/H2O
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352

DAD1 D, Sig=230,16 Ref=off (LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D)

DAD1 E, Sig=280,16 Ref=off (LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D)

DAD1 F, Sig=280,16 Ref=off (LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D)

DAD1 G, Sig=300,16 Ref=off (LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D)

DAD1 H, Sig=320,16 Ref=off (LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D)

```
=====================================================================
                         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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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
```

354

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

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**

NL:
3.61E6

LD-III-43_Orbi_+ESI#1
RT: 0.00 AV: 1 T: FTMS
+ p ESI Full ms
[110.00-1000.00]

100 — 650.1120

80

60 — 116.9858

40

164.9843

20 — 628.1301 · 666.0856

217.1046 · 333.5424 · 381.2975 · 441.2974 · 509.1235 · 595.1607 · 707.1711 · 787.2269 · 852.2053 · 961.6655

NL:
6.32E5

$C_{30}H_{29}NO_{10}S_2$:
$C_{30}H_{29}N_1O_{10}S_2$
pa Chrg 1

100 — 627.1227

80

60

40

20

633.1210

NL:
6.32E5

$C_{30}H_{29}NO_{10}S_2$ +H:
$C_{30}H_{30}N_1O_{10}S_2$
pa Chrg 1

100 — 628.1306

80

60

40

20

633.1255

NL:
6.32E5

$C_{30}H_{29}NO_{10}S_2$ +Na:
$C_{30}H_{29}N_1O_{10}S_2Na_1$
pa Chrg 1

100 — 650.1125

80

60

40

20

655.1075

Relative Abundance

200 300 400 500 600 700 800 900 1000

m/z

^{13}C

NMR (500 MHz, CDCl₃) of (Z)-3-Methoxy-2-(2-(5-nitrothiophen-2-yl)propoxy)-6-(3,4,5-trimethoxystyryl)-phenyl-4-methylbenzenesulfonate (**23**)

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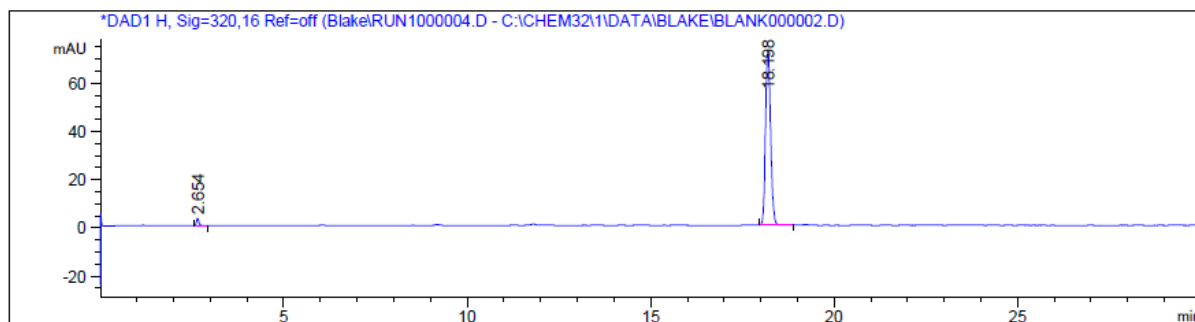
==

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

360

```
==================================================================
                     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
```

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

```
Peak RetTime Type  Width     Area      Height    Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.717 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 Type  Width     Area      Height    Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   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 Type  Width     Area      Height    Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   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
```

===
*** End of Report ***

Mass Spectrum of Compound **23**
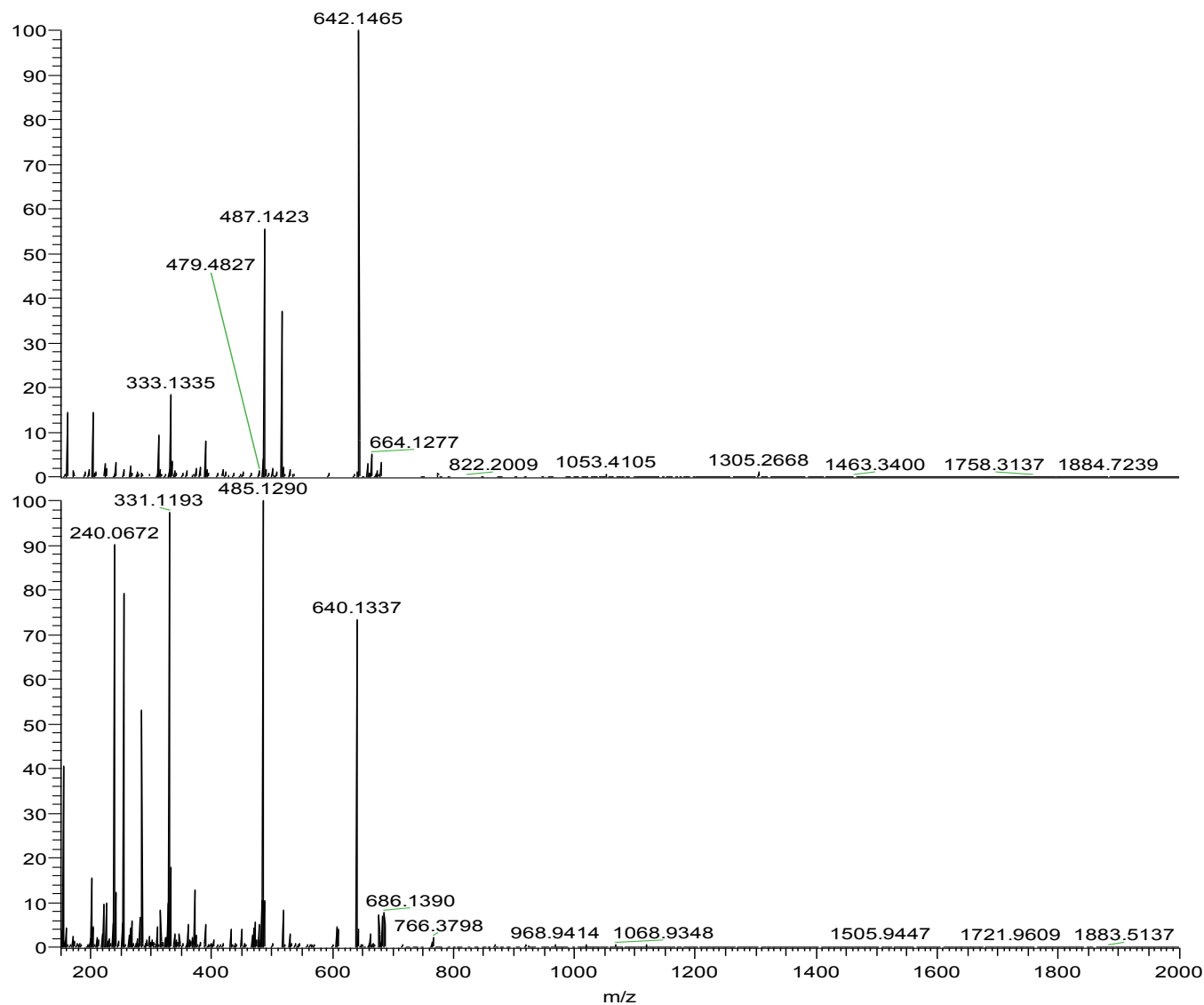
^{1}H NMR (500 MHz, CDCl$_3$) for Compound **24**

^{13}C NMR (125 MHz, CDCl$_3$) for Compound **24**

152.920
152.907
151.789
150.008
149.088
140.697
137.232
132.680
129.395
128.309
126.032
125.769
125.366
125.194

106.893
106.103

77.415
77.160
77.160
76.906
76.147
69.272

60.979
56.066
55.929

22.683
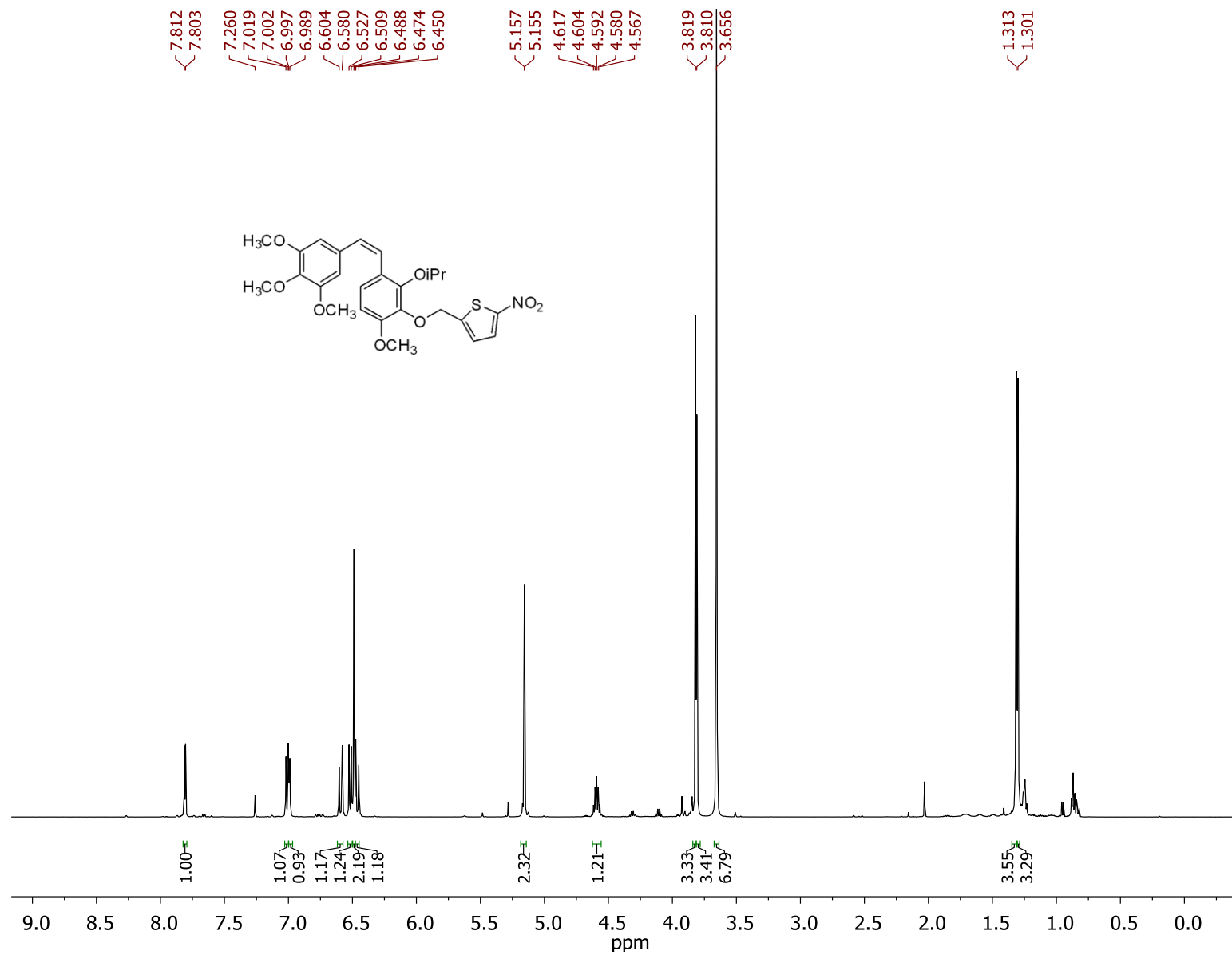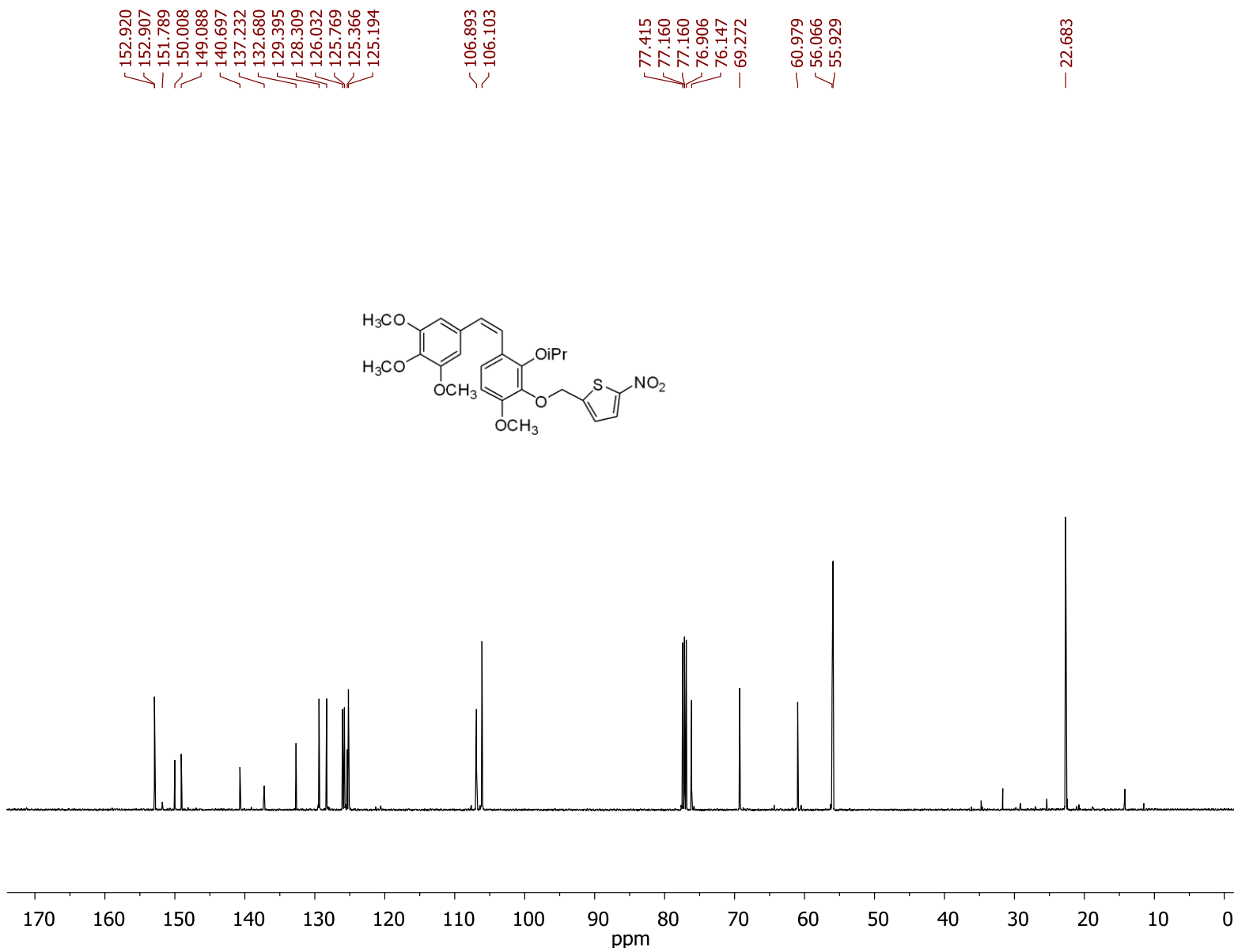
Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D
Sample Name: LD-V-97-1A-run2

===
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
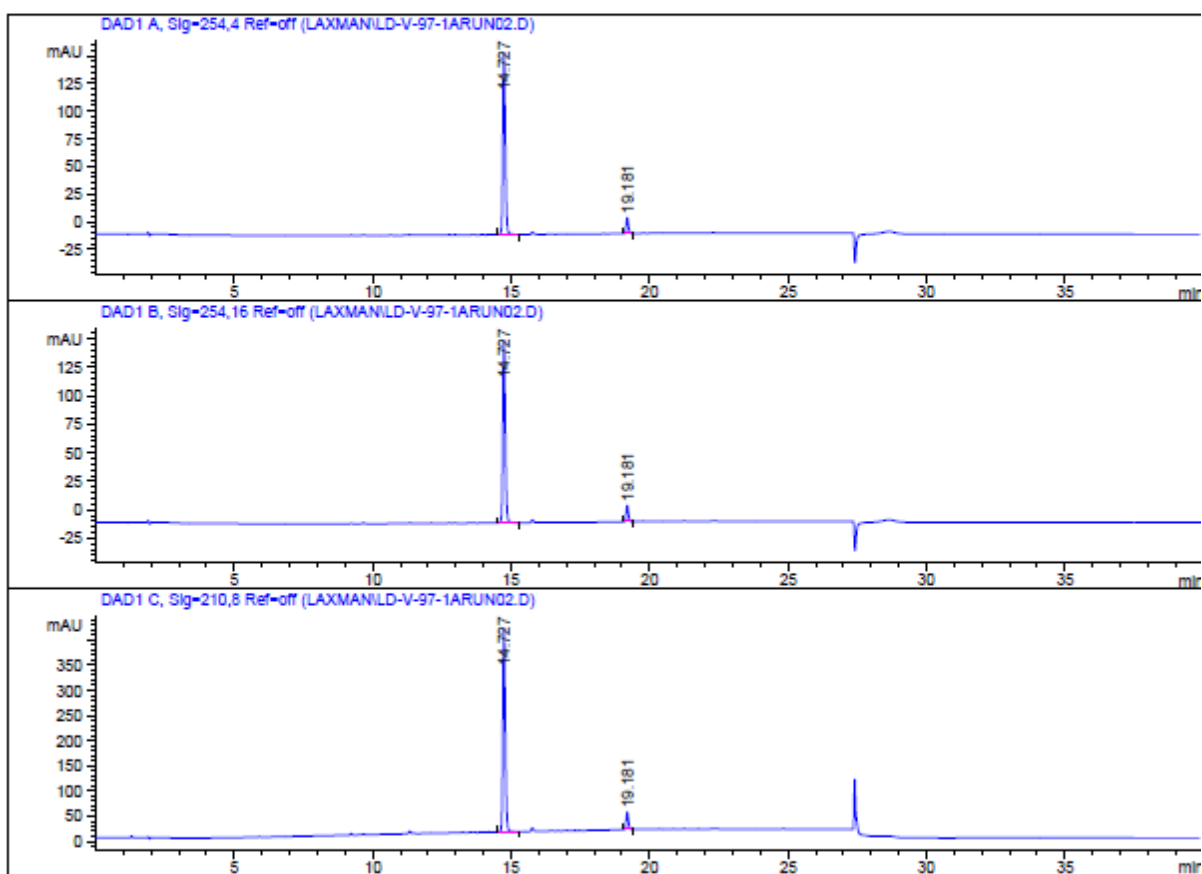
366

367
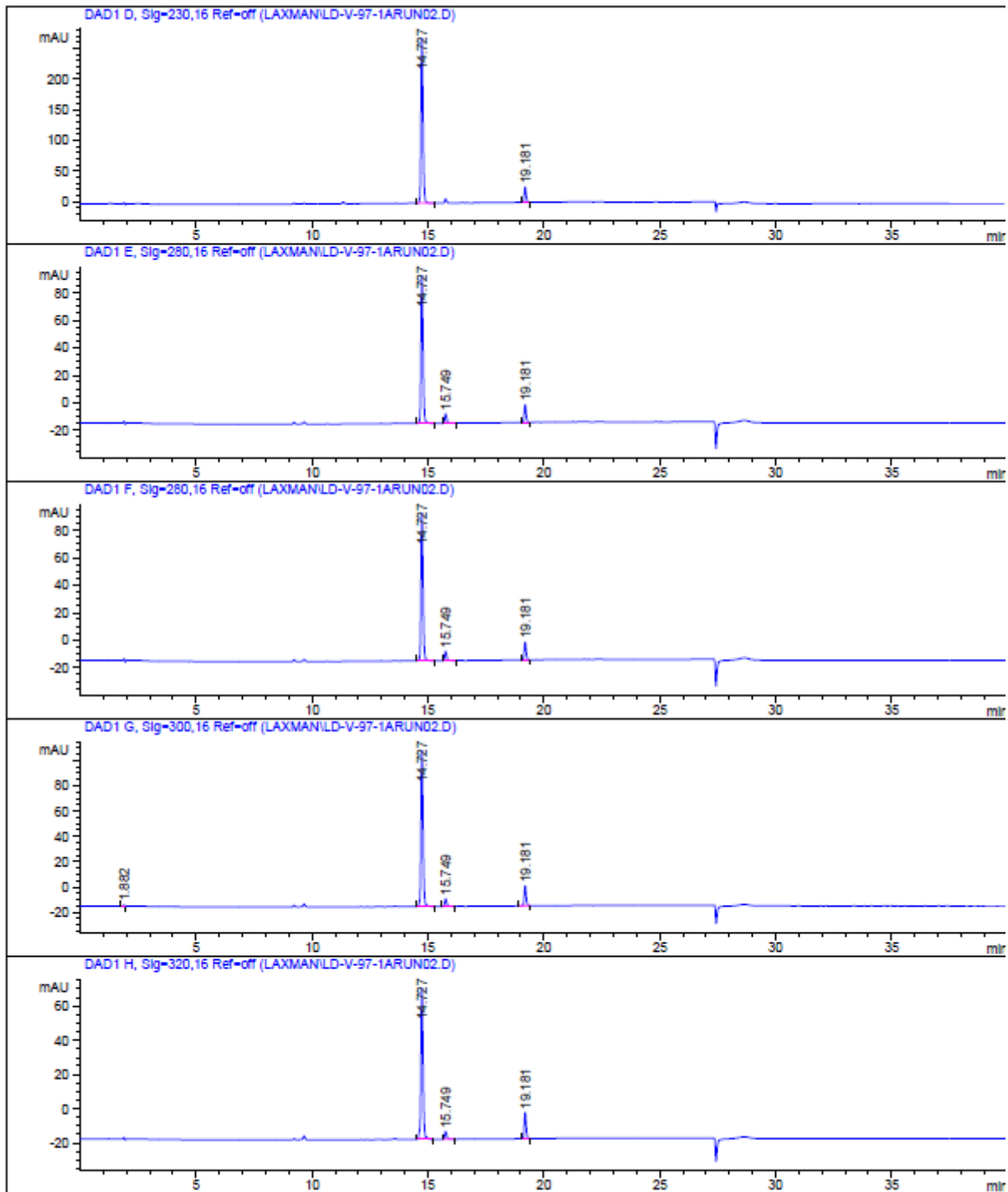
Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D
Sample Name: LD-V-97-1A-run2

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

Instrument 1 3/6/2015 11:26:26 AM Blake

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

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

```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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

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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 ***

370

Mass Spectrum of Compound **24**
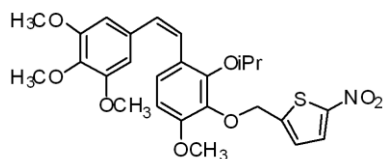
Chemical Formula: $C_{26}H_{29}NO_8S$
Exact Mass: 515.1614
Molecular Weight: 515.5770
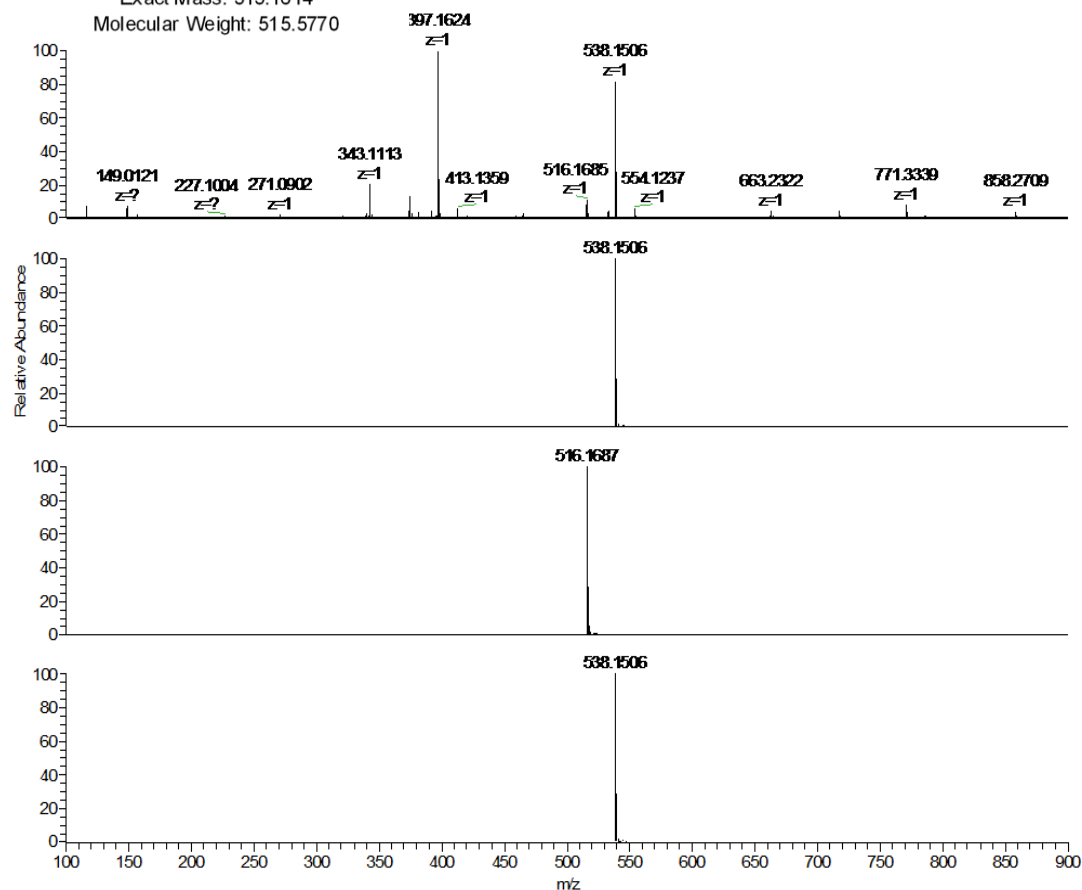
NL:
2.18E7
LD-V-97-1A_ORBI_+
ES#12 RT:0.10 AV:1
T: FTMS + p ESI Full
ms[100.00-900.00]

NL:
6.99E5
$C_{26}H_{29}NO_8S$ +Na:
$C_{26}H_{29}N_1O_8S_1Na_1$
pa Chrg 1

NL:
6.99E5
$C_{26}H_{29}NO_8S$ +H:
$C_{26}H_{30}N_1O_8S_1$
pa Chrg 1

NL:
6.99E5
$C_{26}H_{29}NO_8S$ +Na:
$C_{26}H_{29}N_1O_8S_1Na_1$
pa Chrg 1

^{1}H NMR (600 MHz, CDCl$_3$) for Compound **25**
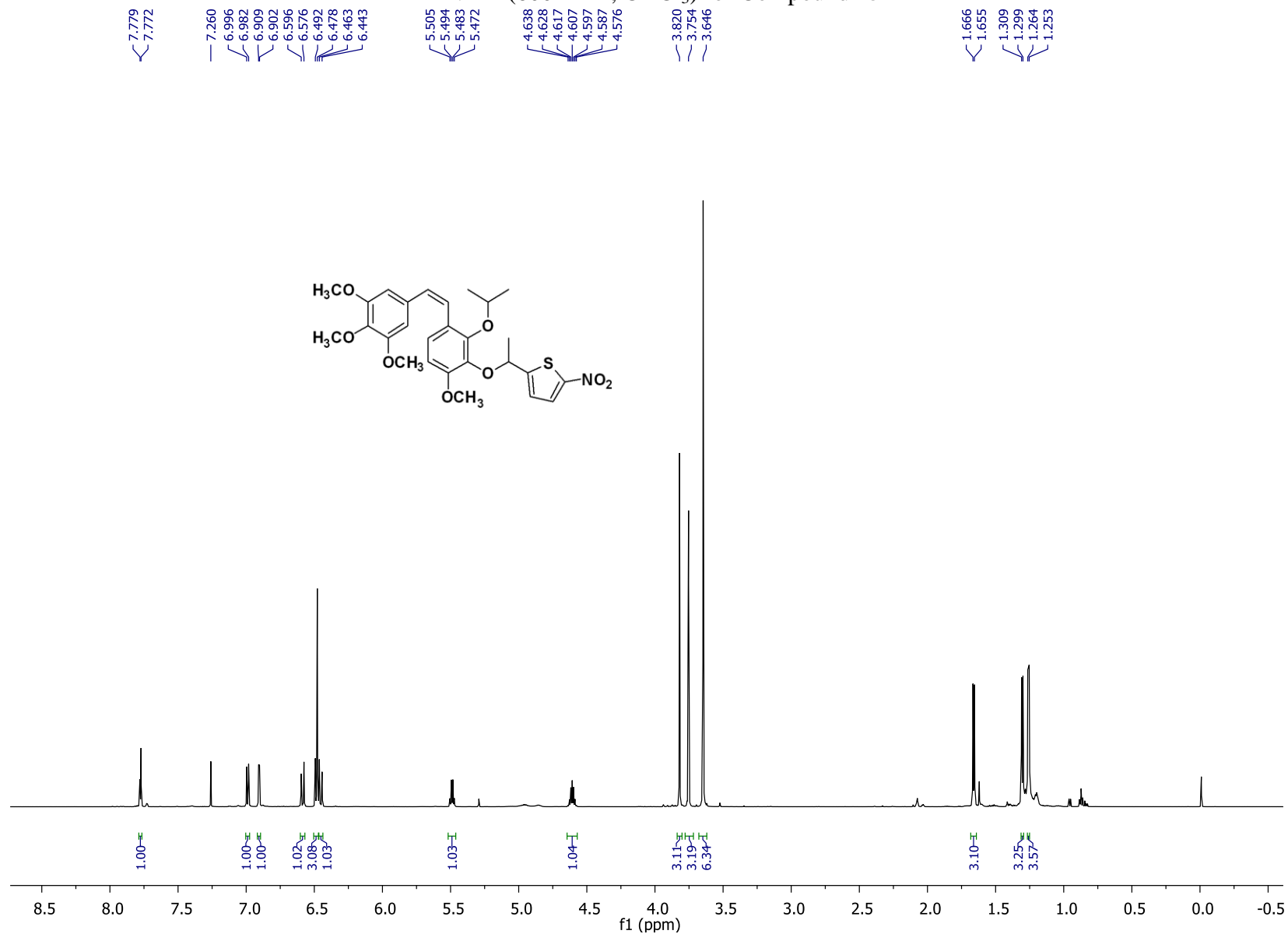
¹³C NMR (151 MHz, CDCl₃) for Compound 25
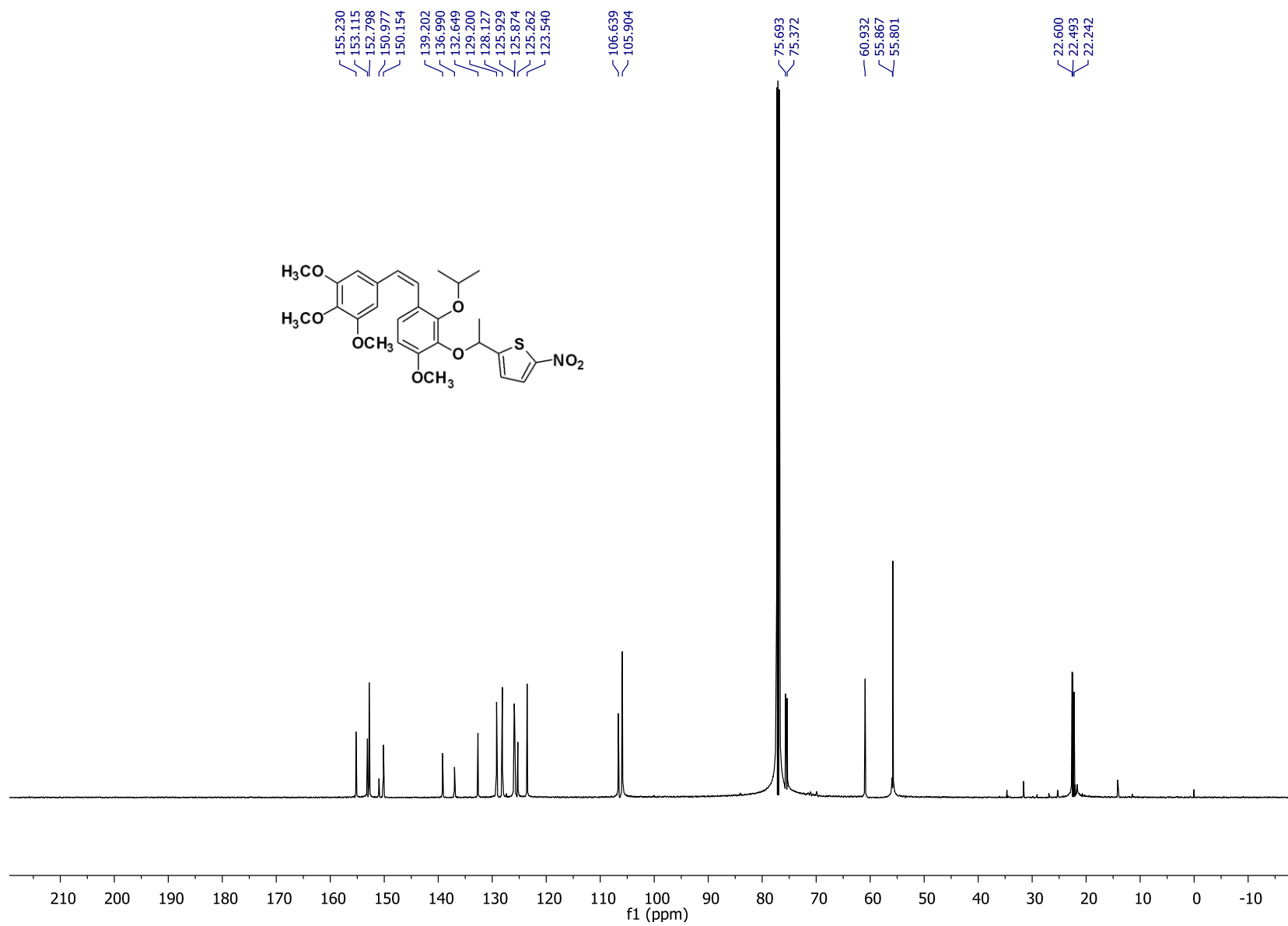
HPLC Traces for Compound **25**

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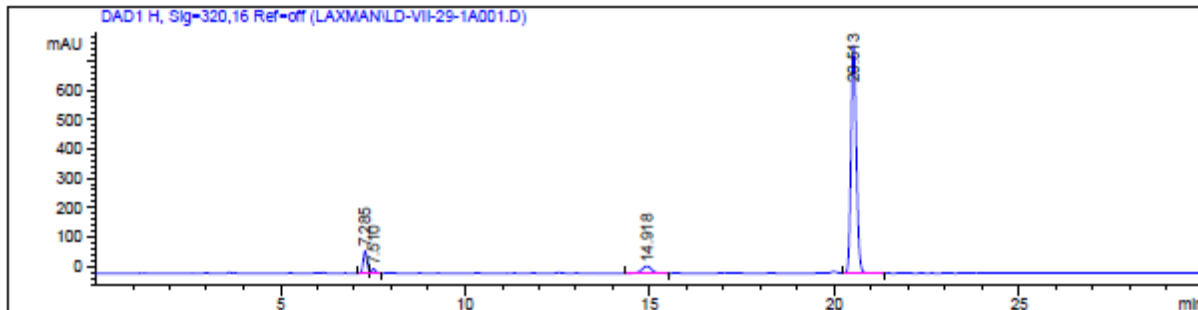
===
                    Area Percent Report
===

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 Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
   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 Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
   1   7.285 BV   0.1181 282.01685   36.51680   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

Instrument 1 3/1/2015 5:17:22 PM Graham   Page   2 of 3

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 :   8945.32576   890.29863

===
*** End of Report ***

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Mass Spectrum of Compound 25

Chemical Formula: $C_{27}H_{31}NO_8S$
Exact Mass: 529.1770
Molecular Weight: 529.6040

Spectrum 1 (NL: 9.75E6, LD-VII-29-crude_Orbi_+, ESI#1 RT: 0.00 AV: 1, T: FTMS + p ESI Full ms [150.00-2000.00]):
- 227.1002
- 301.0754
- 312.3624
- 413.2662
- 441.2973
- 552.1660
- 579.1609
- 691.3516
- 754.2606
- 830.2514
- 1081.3423
- 1283.4370
- 1485.5356
- 1579.3879
- 1883.7098

Spectrum 2 (NL: 6.91E5, $C_{27}H_{31}NO_8S$: $C_{27}H_{31}N_1O_8S_1$ pa Chrg 1):
- 529.1765
- 537.1875

Spectrum 3 (NL: 6.91E5, $C_{27}H_{31}NO_8S$ +H: $C_{27}H_{32}N_1O_8S_1$ pa Chrg 1):
- 530.1843
- 538.1953

Spectrum 4 (NL: 6.91E5, $C_{27}H_{31}NO_8S$ +Na: $C_{27}H_{31}N_1O_8S_1Na_1$ pa Chrg 1):
- 552.1663
- 559.1764

m/z

^{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
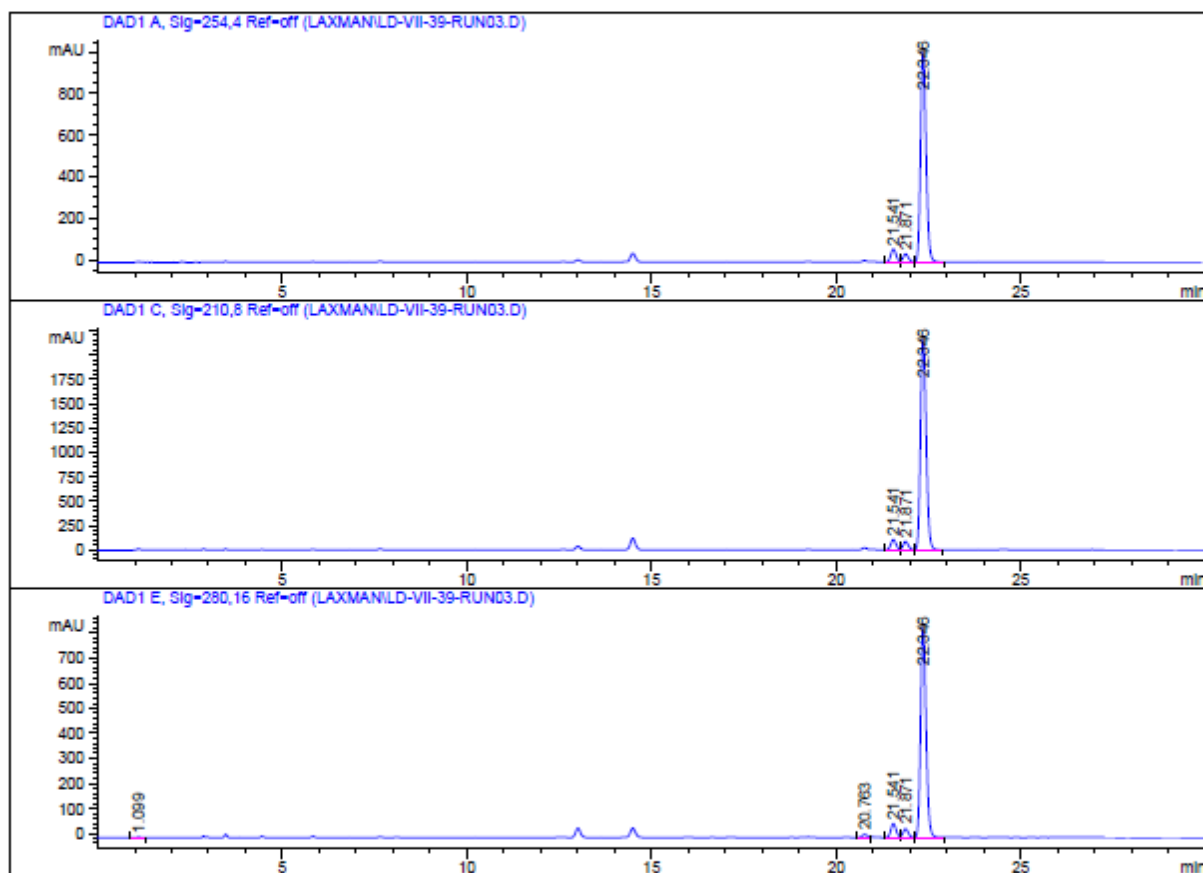
379
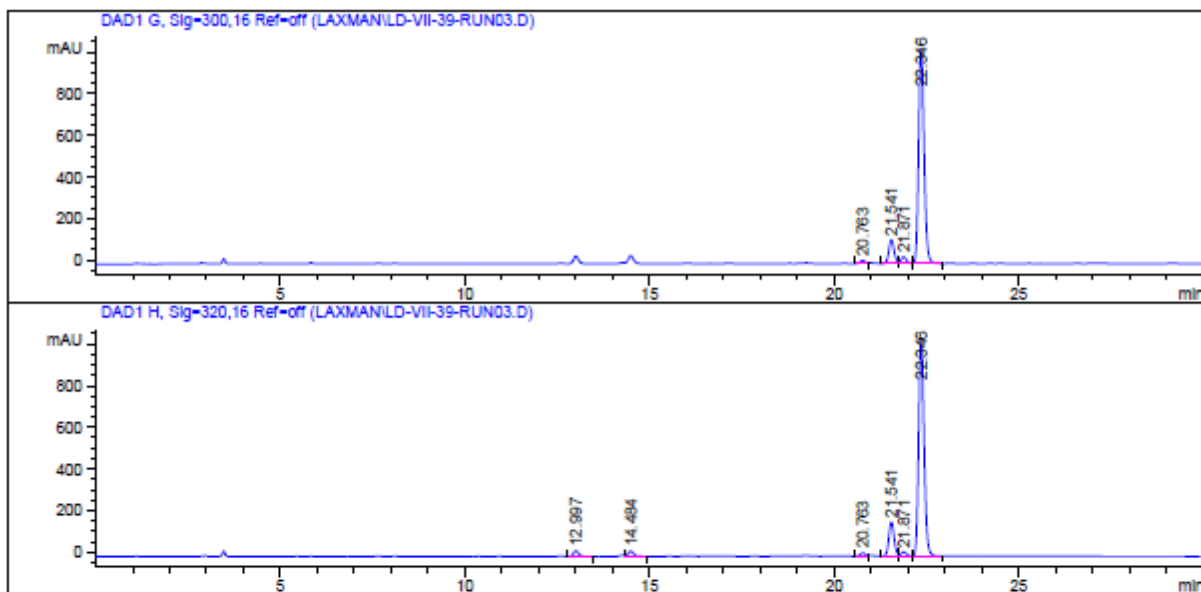
```
=================================================================
                        Area Percent Report
=================================================================

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

Instrument 1 3/16/2015 6:22:16 PM Laxman   Page   2 of 4

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

```
Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area        Height      Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area        Height      Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area        Height      Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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

381

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

Chemical Formula: $C_{28}H_{33}NO_8S$
Exact Mass: 543.1927
Molecular Weight: 543.6310

Relative Abundance

m/z

Panel 1 labels: 219.1872, 241.1692, 277.1635, 293.1374, 372.1757, 397.1620, 413.2659, 459.3489, 495.3431, 566.1813, 615.3416, 651.3359, 758.5707, 820.3555, 881.3985, 940.3531

Panel 2 labels: 543.1921, 549.1989

Panel 3 labels: 544.2000, 549.2034

Panel 4 labels: 566.1819, 571.1853

383

¹H NMR (500 MHz, CDCl₃) for Compound **27** and **28**

384
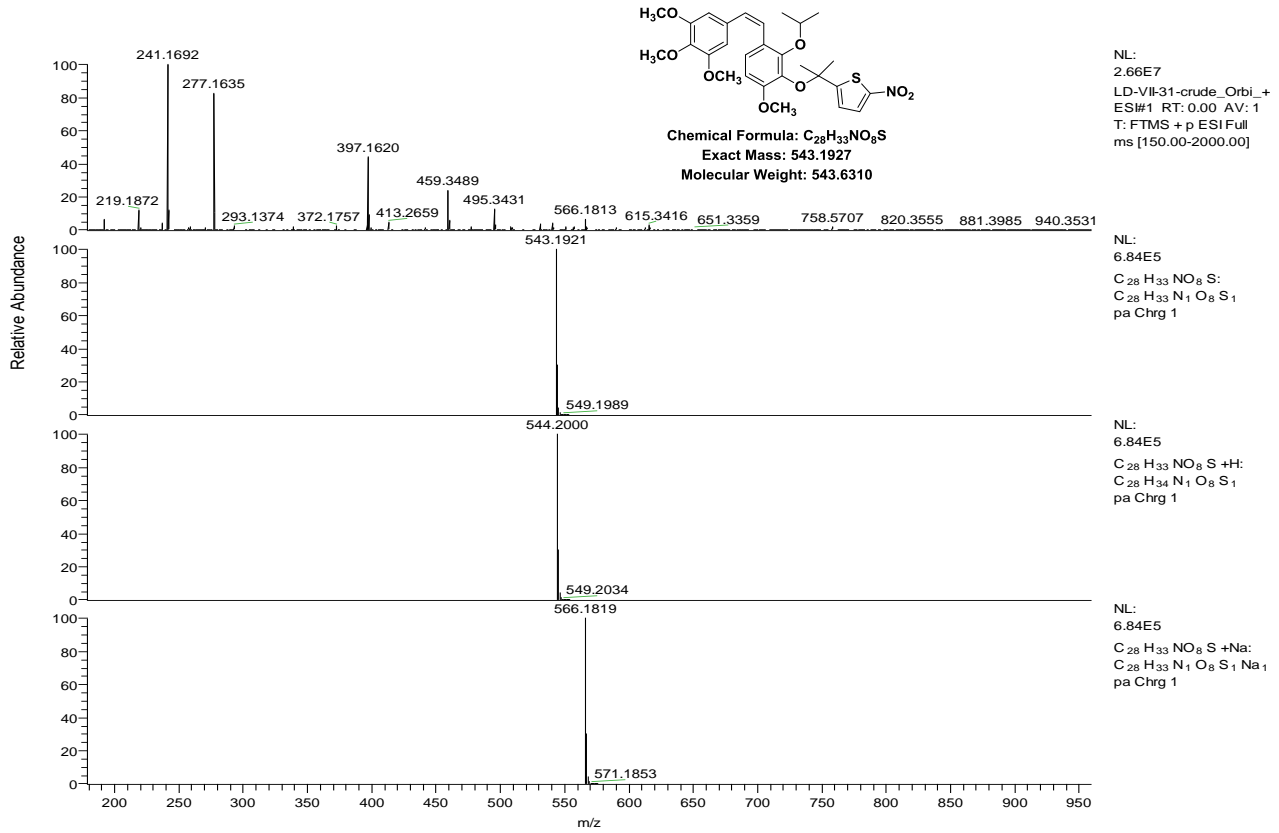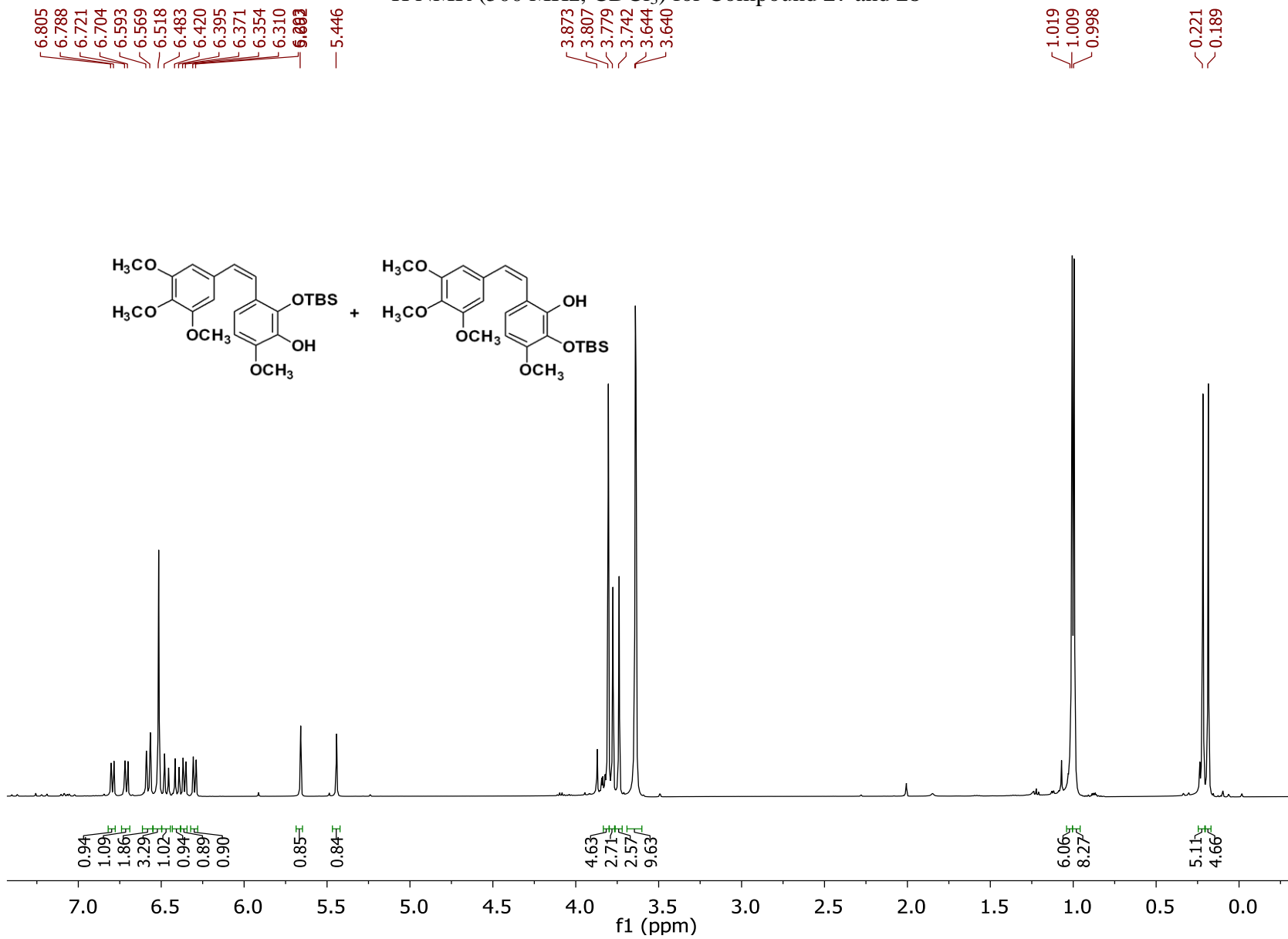
^{13}C NMR (125 MHz, CDCl$_3$) for Compound **27** and **28**

¹H NMR (500 MHz, CDCl₃) for Compound **29**

CA1

7.260
7.260
6.771
6.754
6.602
6.578
6.547
6.523
6.394
6.376
5.388
3.860
3.827
3.668

0.99
0.88
2.59
1.90
1.00
2.03
2.74
2.54
5.54

7.0   6.5   6.0   5.5   5.0   4.5   4.0   3.5   3.0
f1 (ppm)
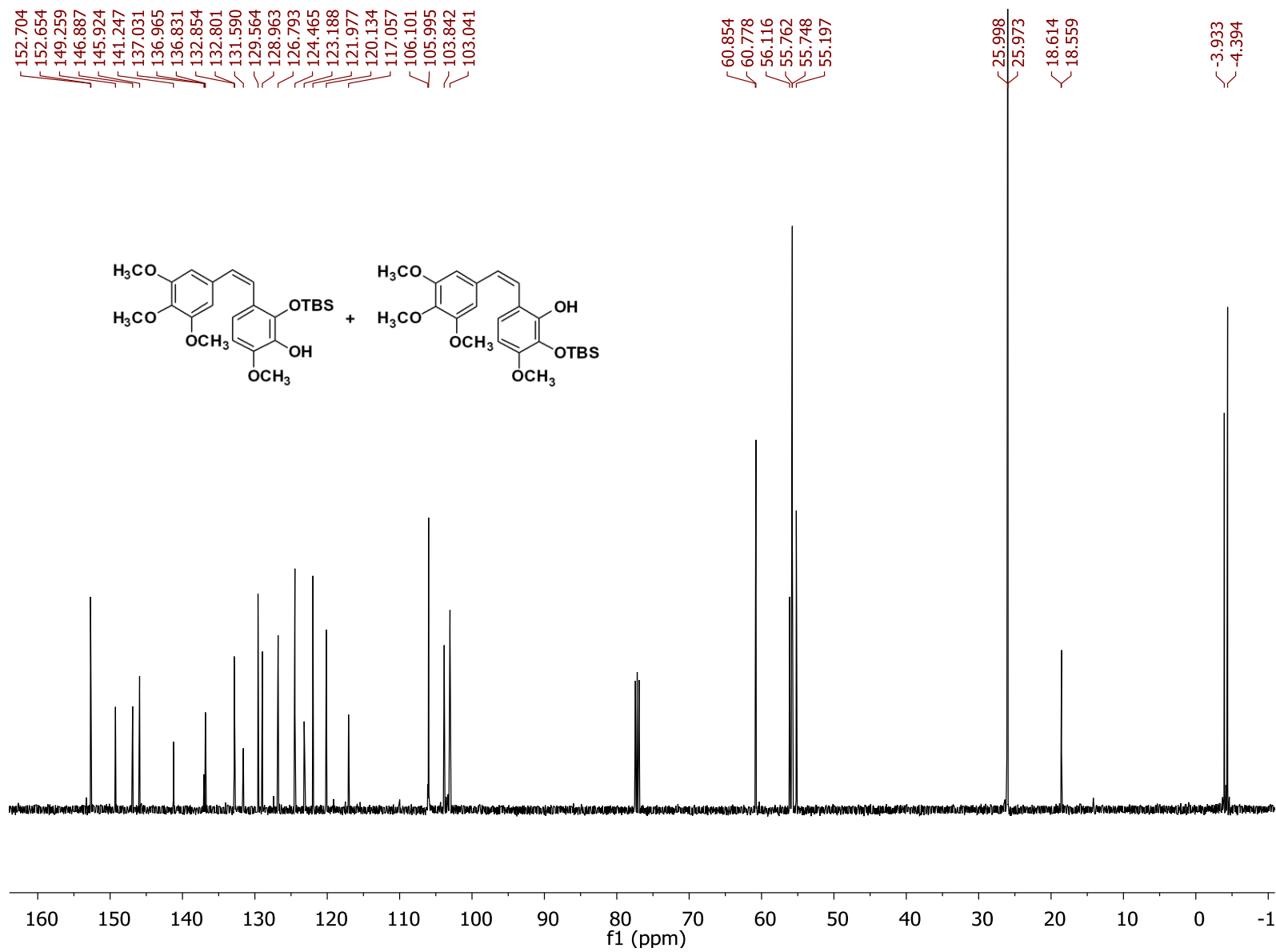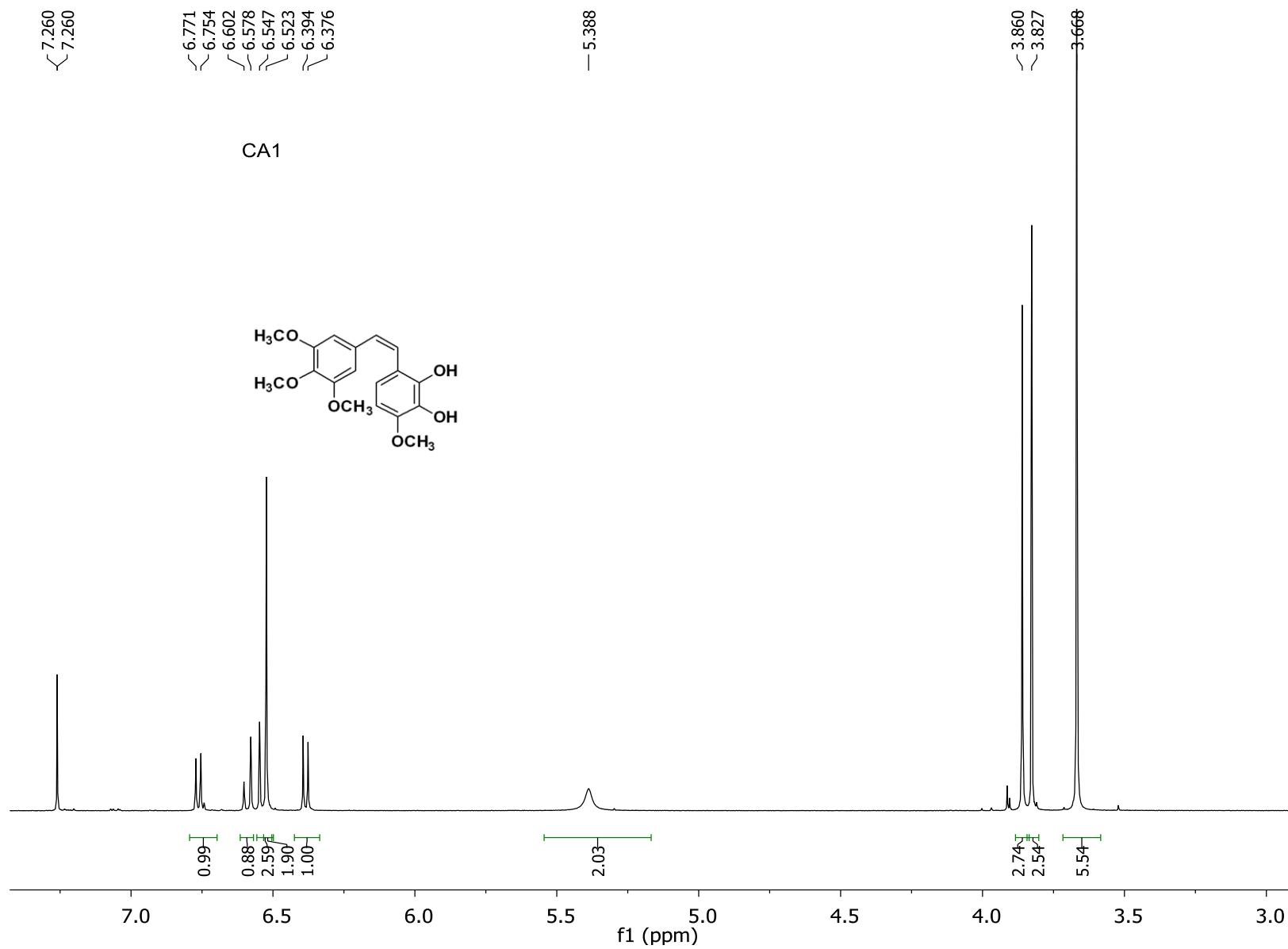
^{13}C NMR (125 MHz, CDCl$_3$) for Compound **29**
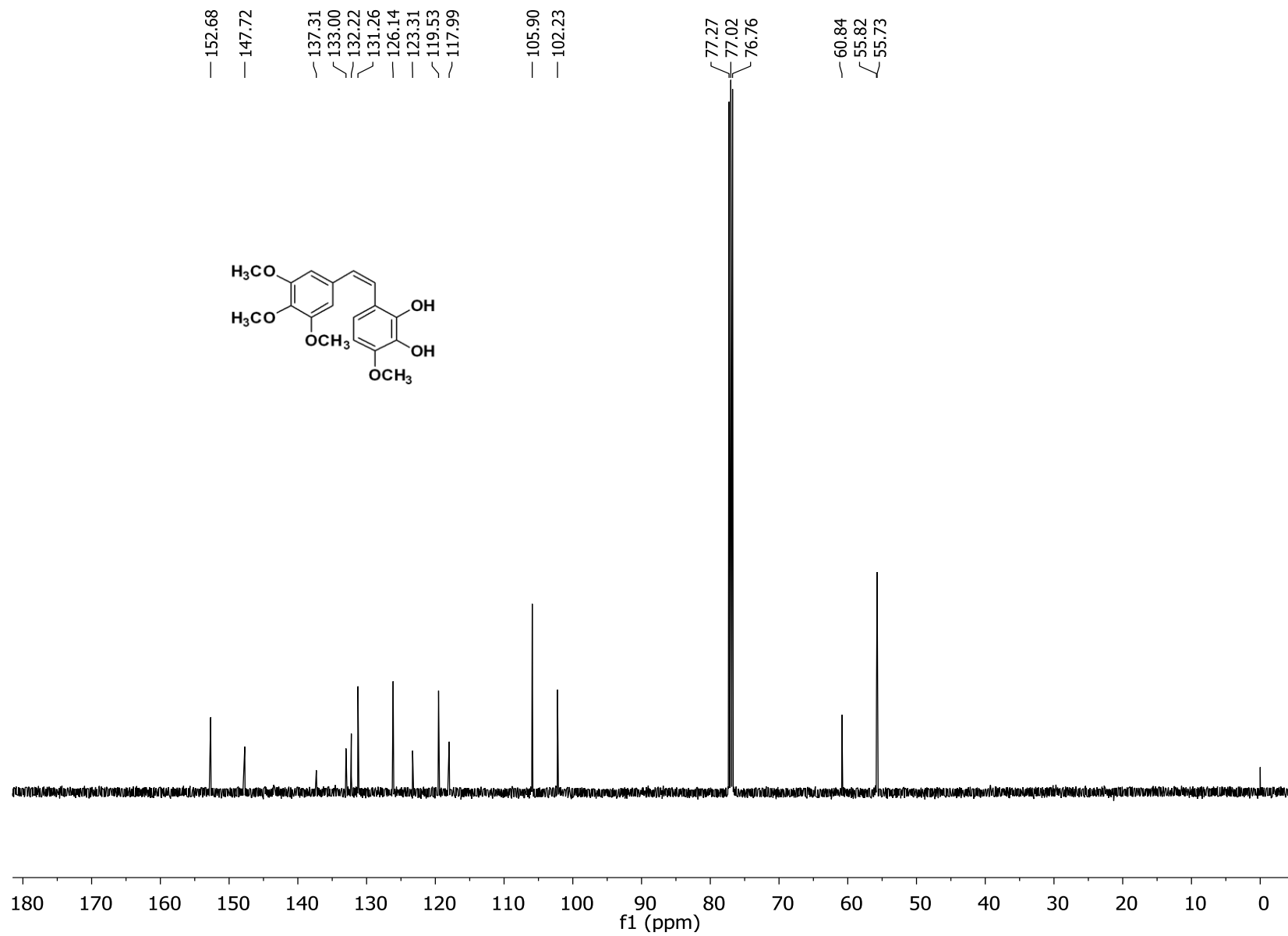
HPLC Traces of Compound **29**

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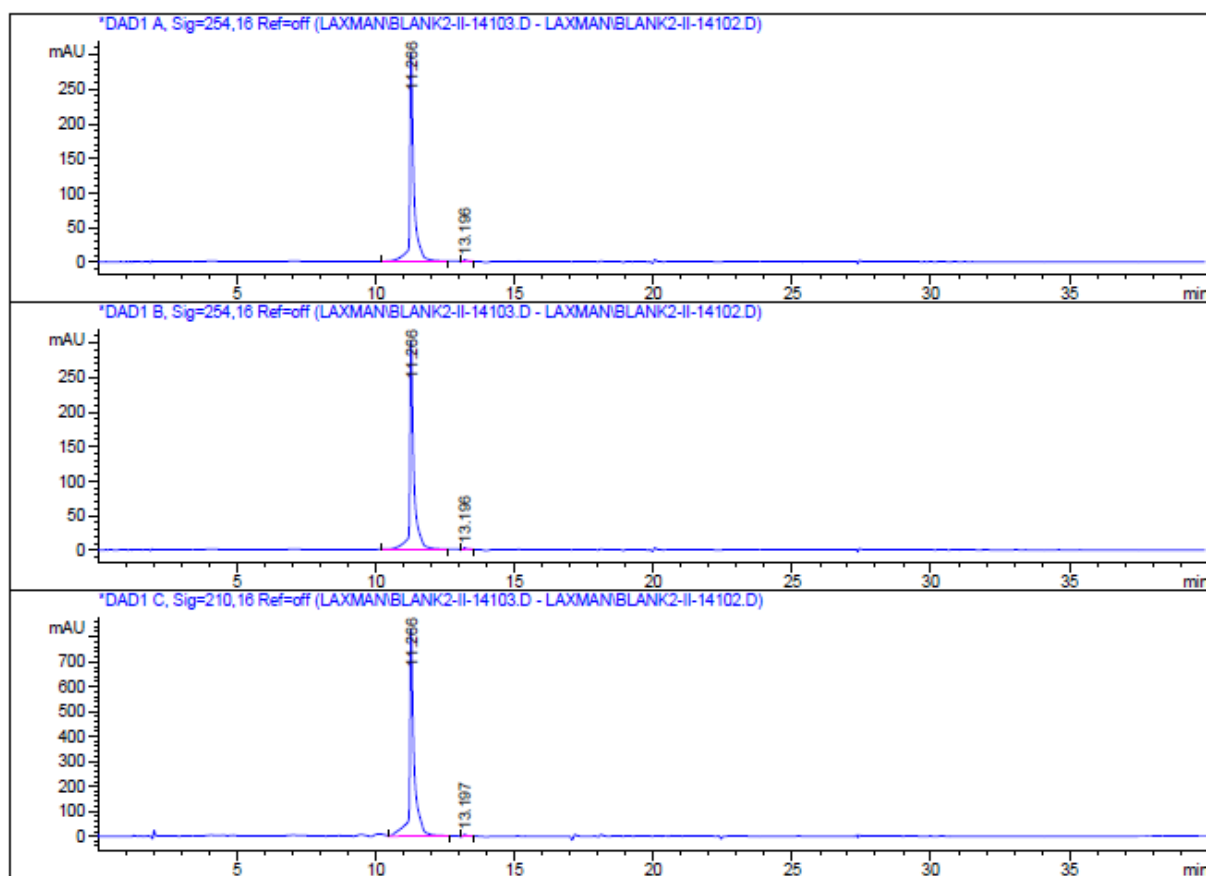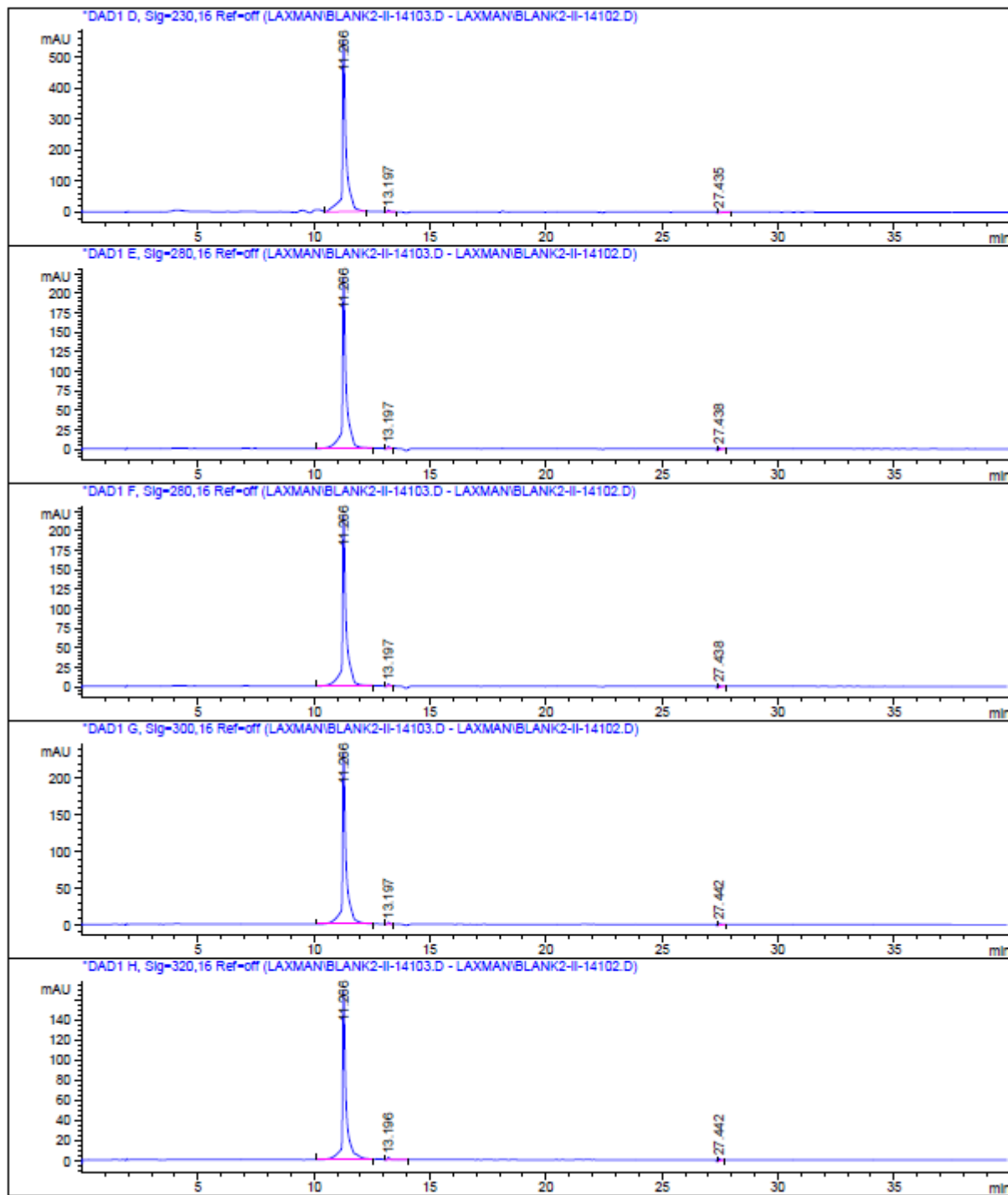
Data File C:\CHEM32\1\DATA\LAXMAN\BLANK2-II-14103.D
Sample Name: LD-II-141-1blank2

==
                          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 Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
   1 11.266 BB   0.1372 3040.83838 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 Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
   1 11.266 BB   0.1372 3040.83838 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 Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
   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 Type  Width      Area        Height     Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width      Area        Height     Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width      Area        Height     Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width      Area        Height     Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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
```

Totals :   2694.67441 240.13445

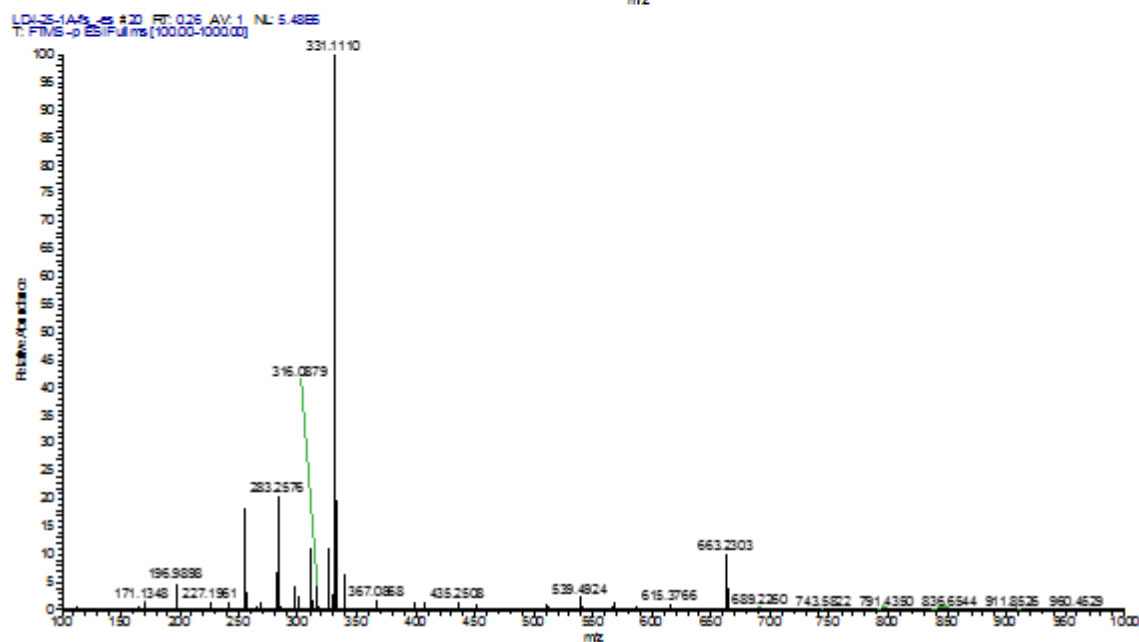Signal 8: DAD1 H, Sig=320,16 Ref=off
 Signal has been modified after loading from rawdata file!

```
Peak RetTime Type  Width     Area        Height     Area
 #   [min]        [min]   [mAU*s]       [mAU]       %
---|-------|----|-------|----------|----------|--------|
  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**

Chemical Formula: $C_{18}H_{20}O_6$
Exact Mass: 332.1260
Molecular Weight: 332.3520

^{1}H NMR (600 MHz, CDCl$_3$) for Compound **30**

¹³C NMR (125 MHz, CDCl₃) for Compound **30**

152.697
151.647
151.443
148.599
147.571
138.378
137.130
132.407
130.365
128.195
125.146
124.860
124.435
122.295
107.486
105.954
68.545
60.883
55.768
55.416
25.778
18.611
4.573

150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **31**

396
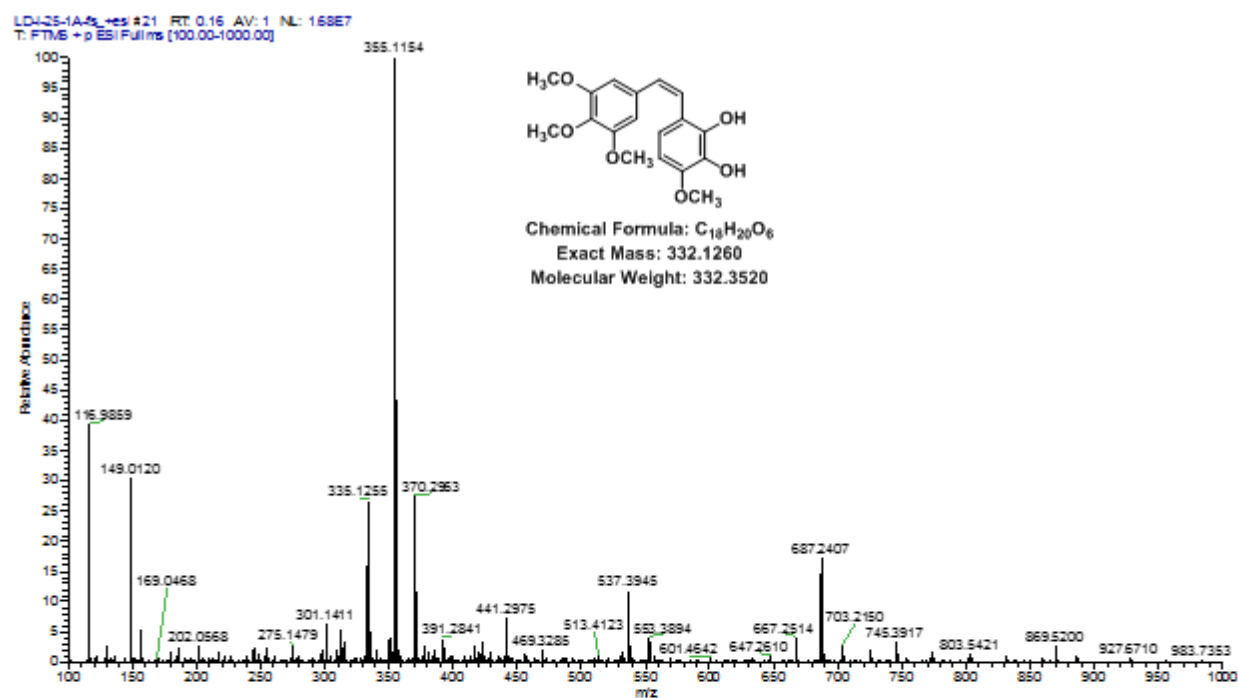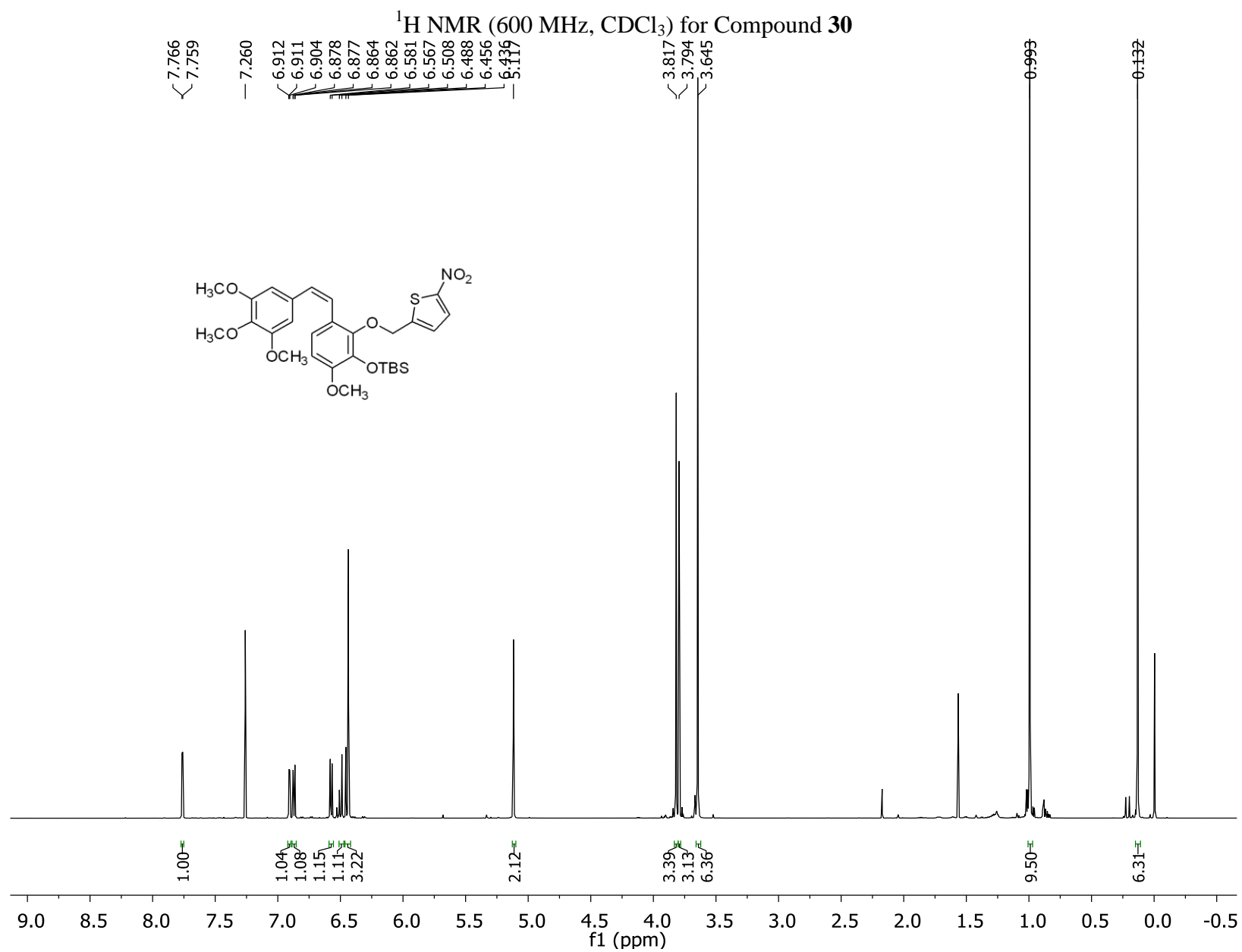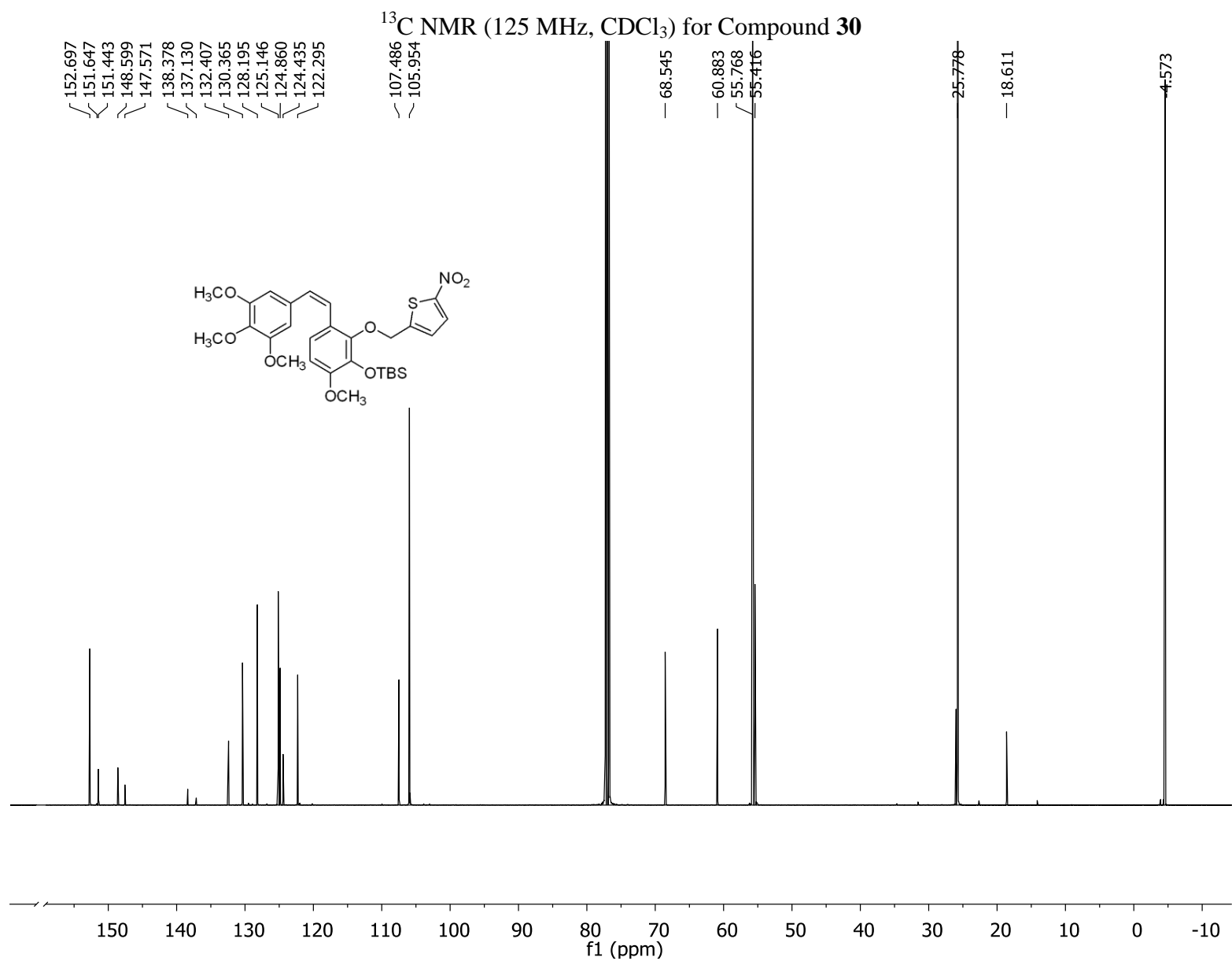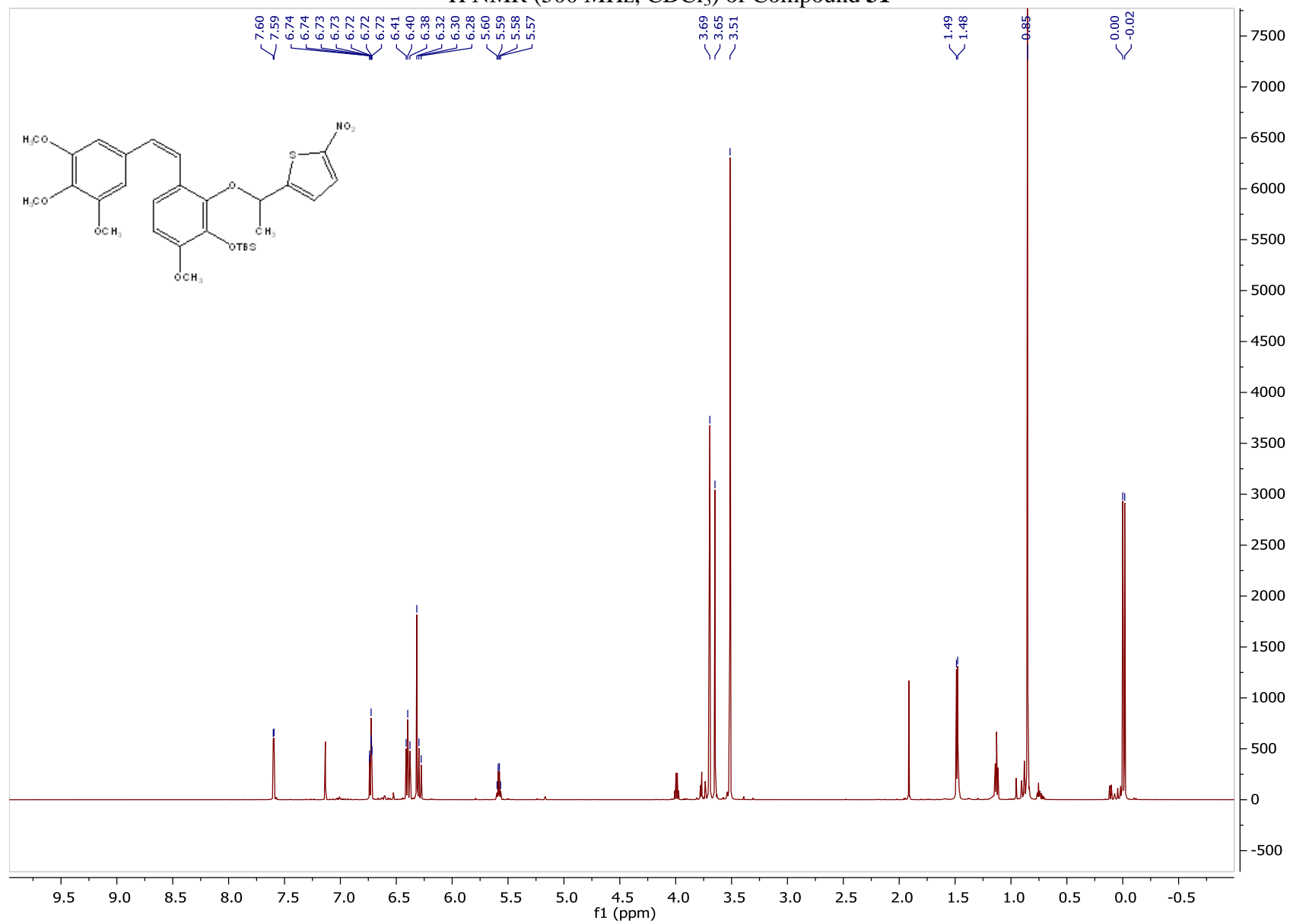
^{13}C NMR (500 MHz, CDCl$_3$) of Compound **31**

^{1}H NMR (600 MHz, CDCl$_3$) for Compound **33**
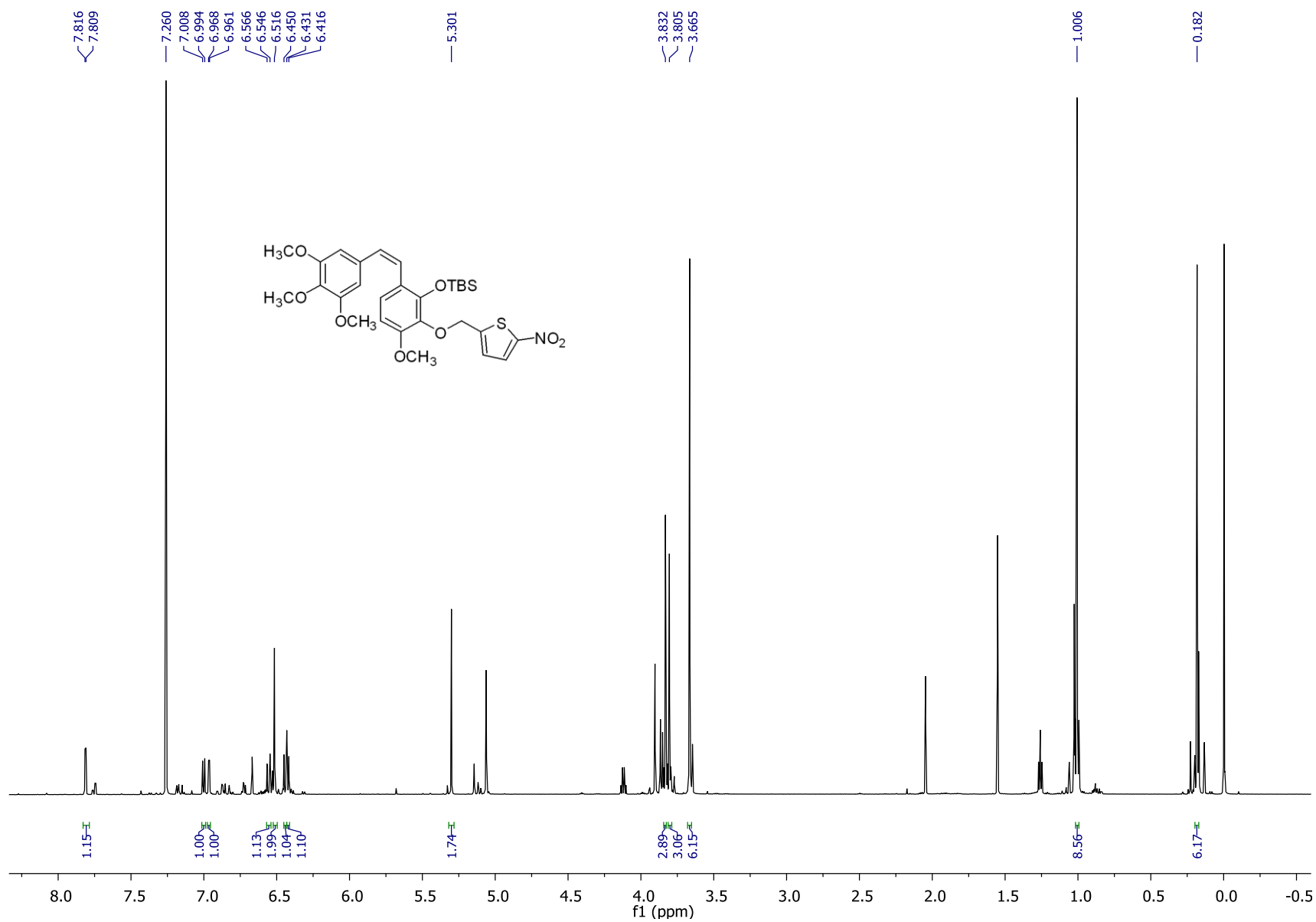
398

^{13}C NMR (151 MHz, CDCl$_3$) for Compound **33**

153.402
152.778
152.741
148.841
147.798
138.428
136.969
132.598
129.062
128.182
126.334
125.886
125.412
123.283
105.922
104.885
68.808
60.921
55.931
55.818
26.124
18.614
-3.876
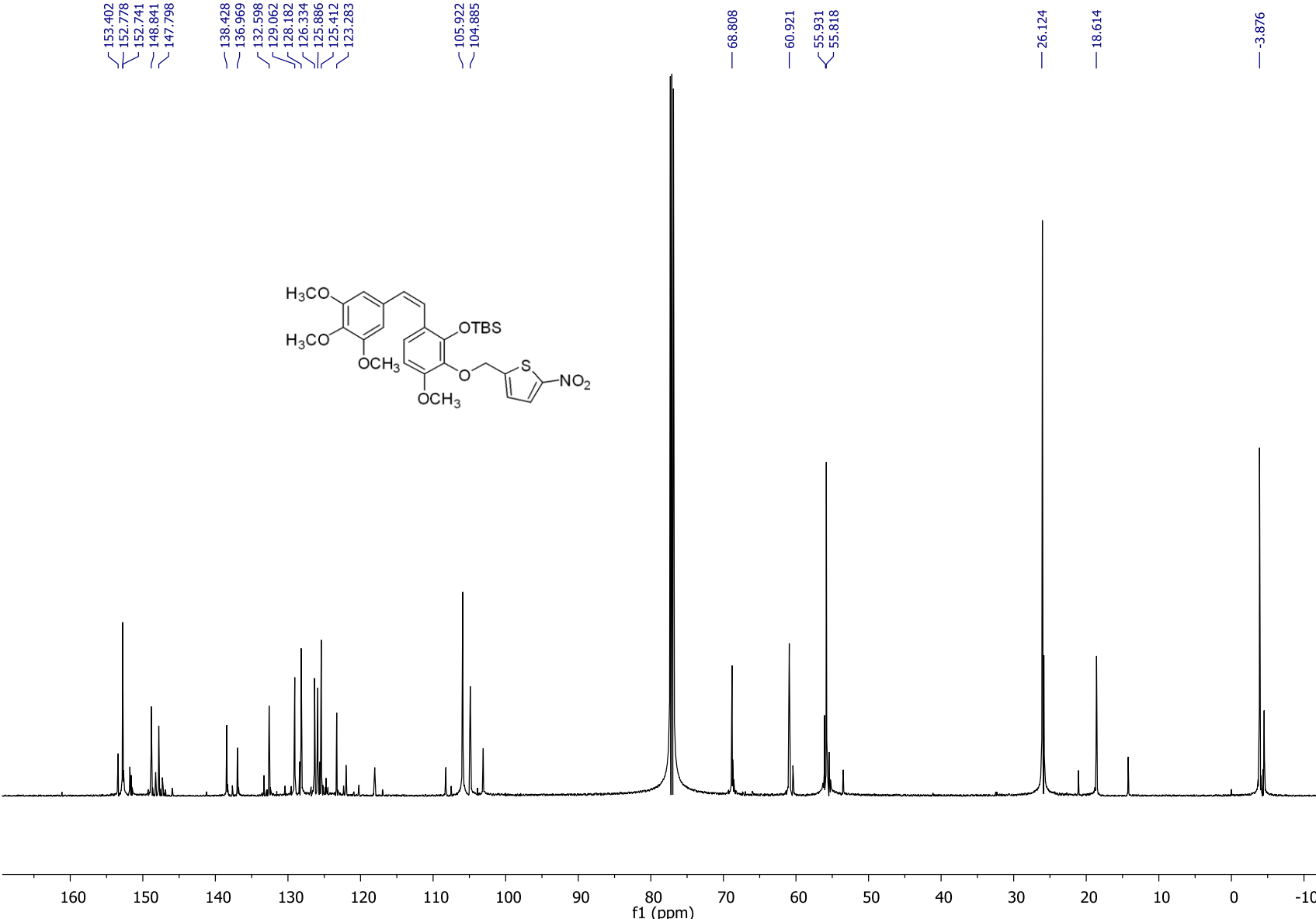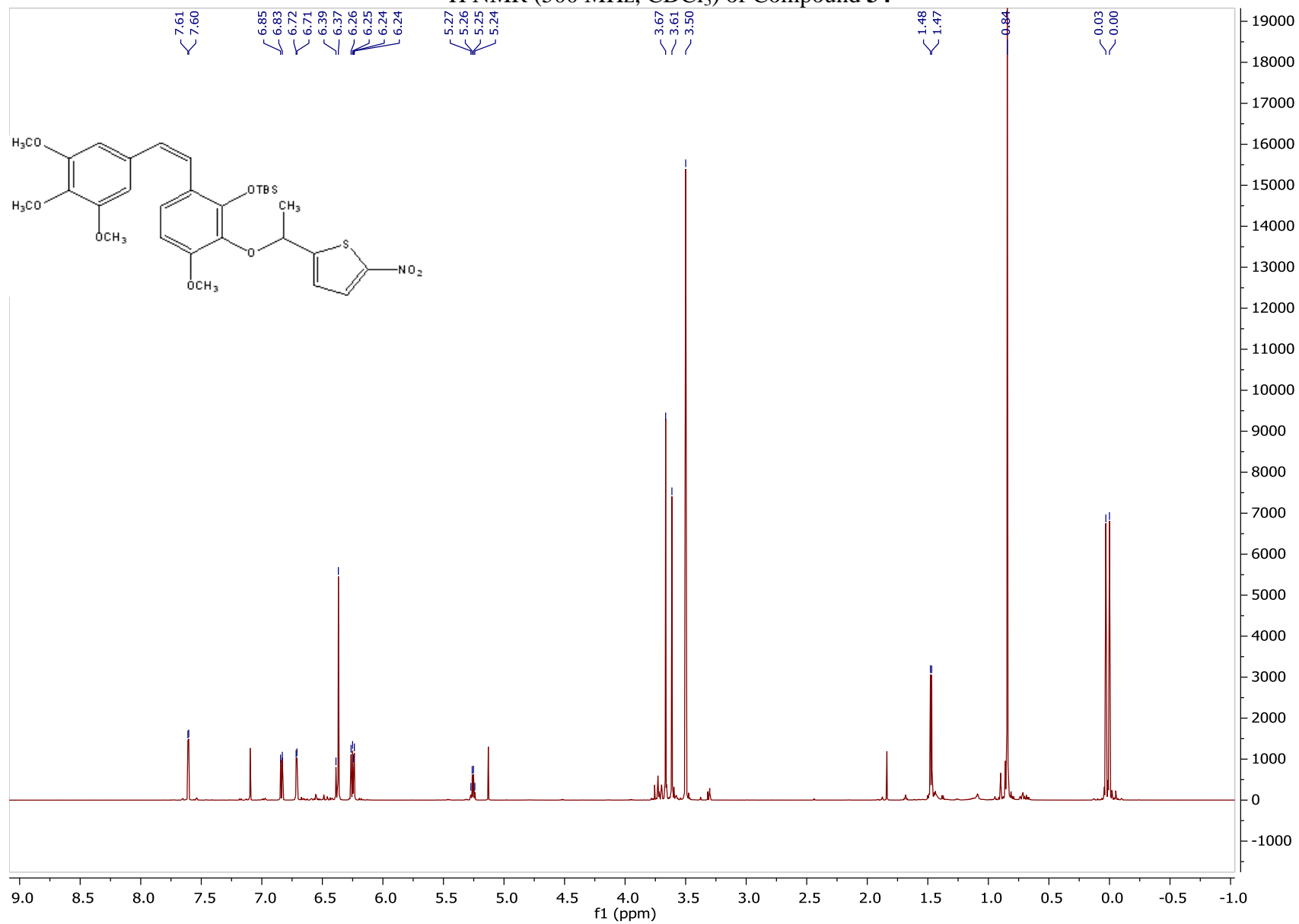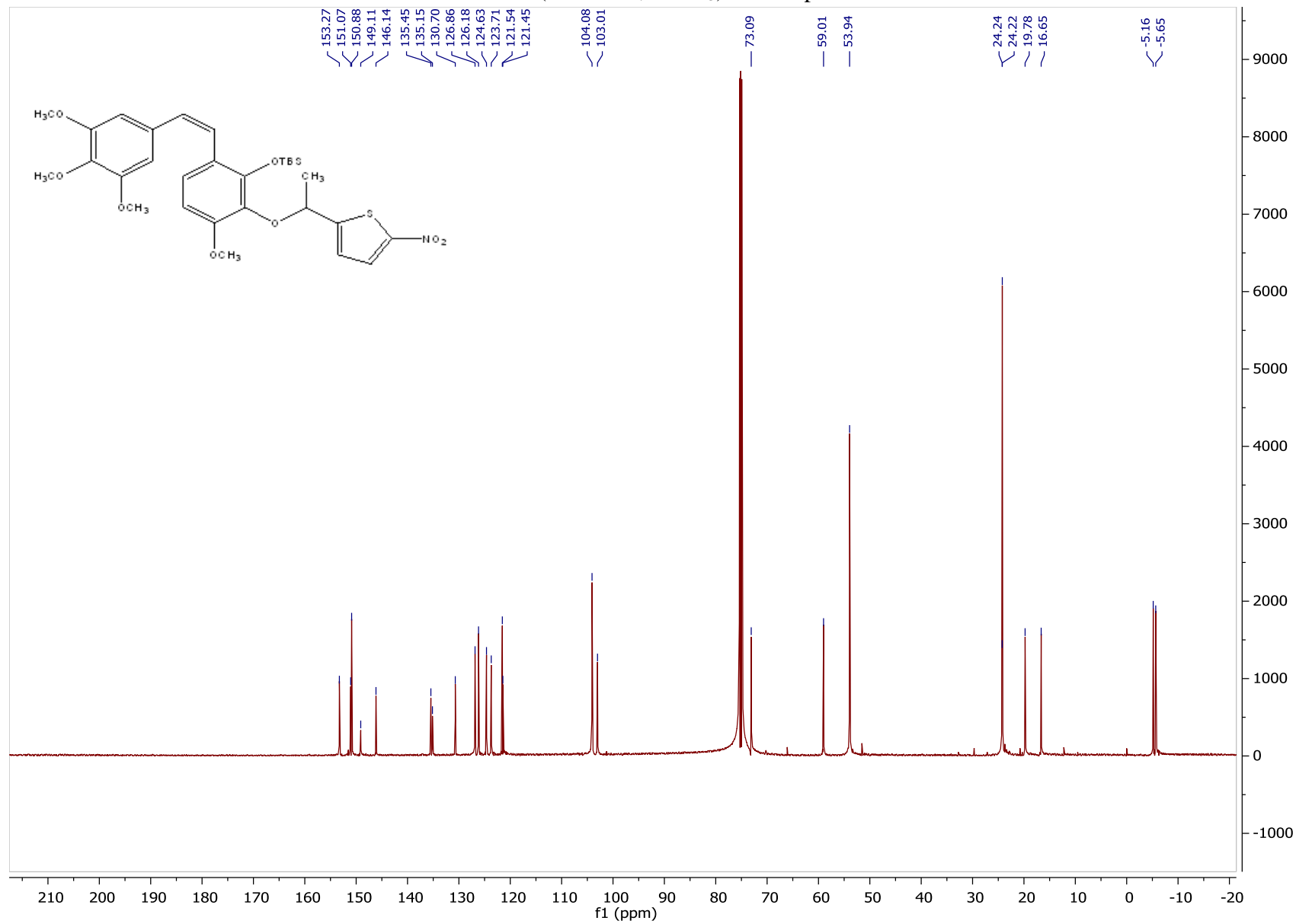
^{1}H NMR (500 MHz, CDCl$_3$) of Compound **34**

¹H NMR (500 MHz, CDCl₃) for Compound **35**

7.835
7.828
7.263
7.152
7.146
7.145
7.070
7.069
6.848
6.574
6.554
6.503
6.502
6.488
6.474
6.450
6.430
3.901
3.834
3.691

0.97

1.04
1.02
1.00
1.15
1.51
0.94
1.05

3.00
2.82
5.30

8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
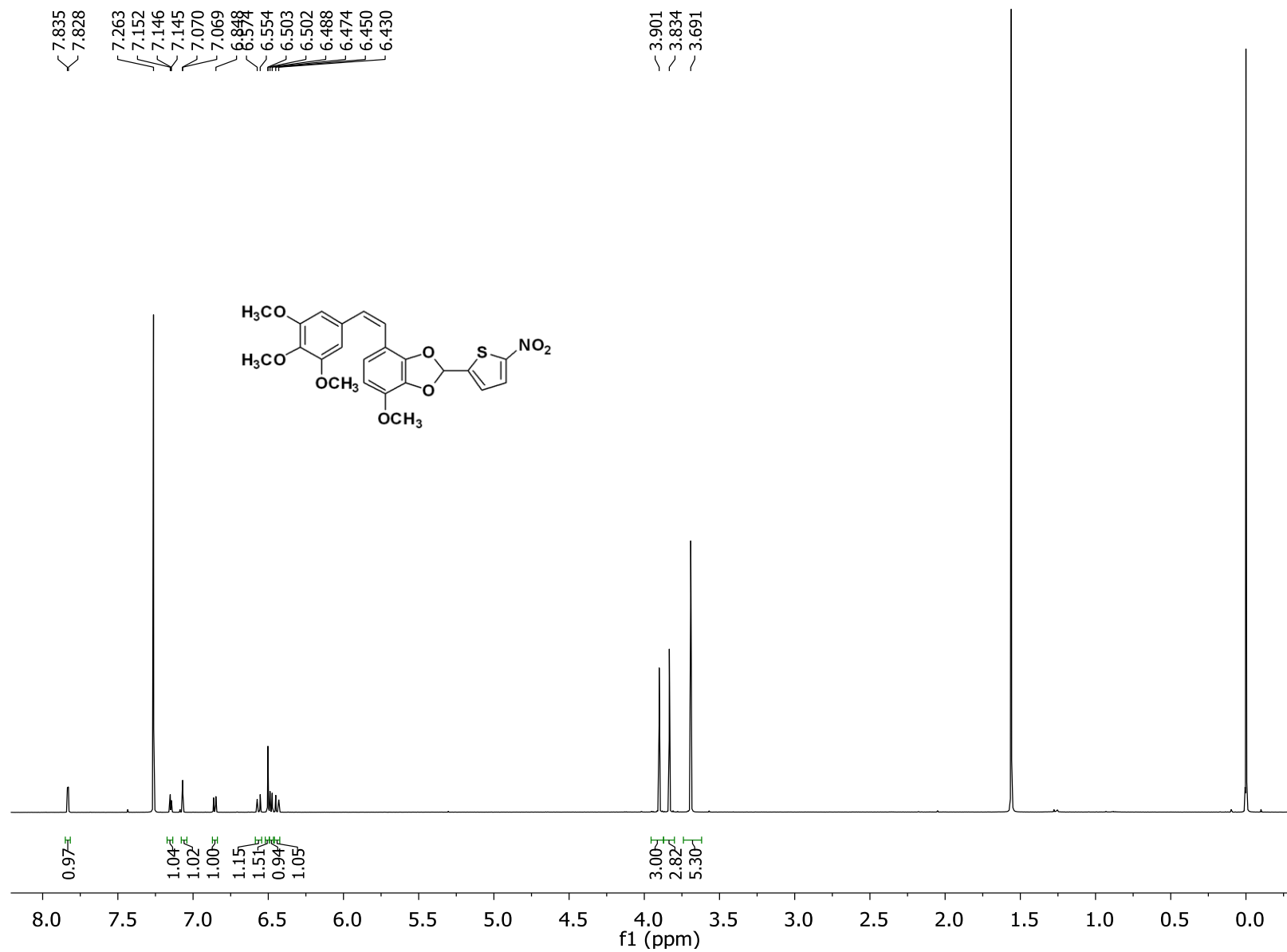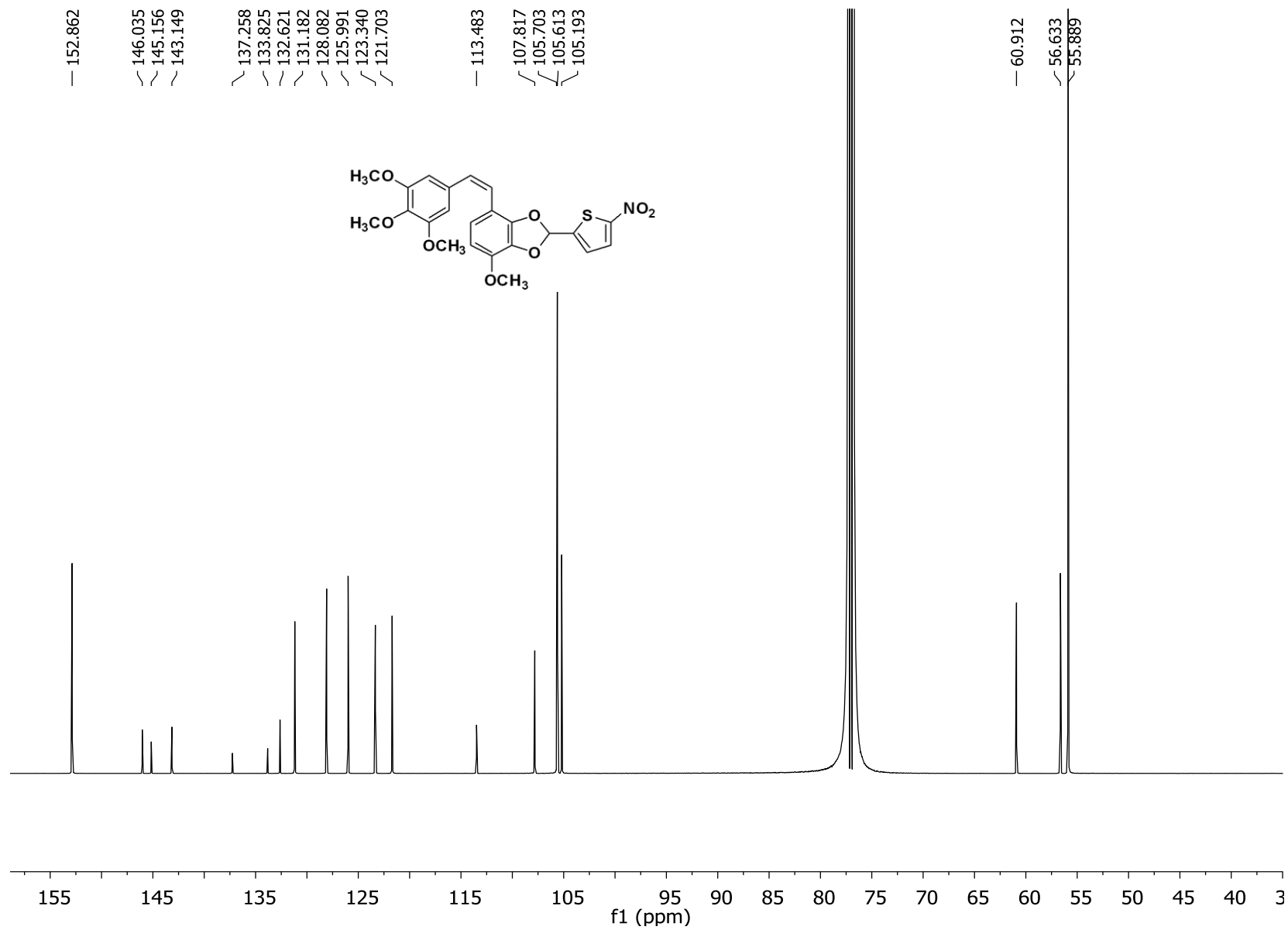
^{13}C NMR (125 MHz, CDCl$_3$) for Compound **35**

155   145   135   125   115   105   95   90   85   80   75   70   65   60   55   50   45   40   3
f1 (ppm)
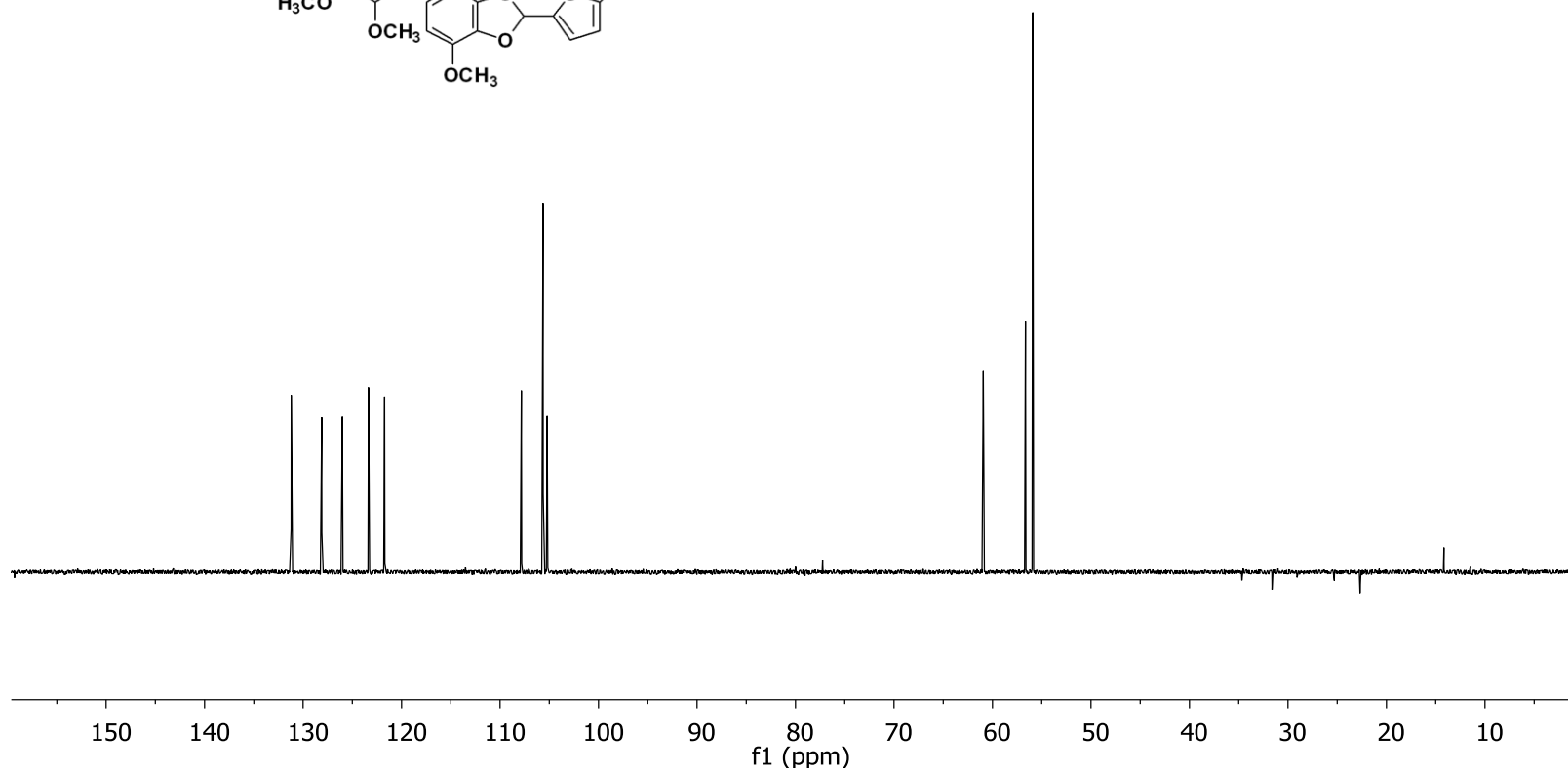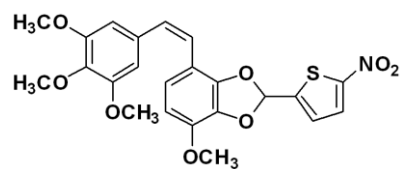
HPLC Traces for Compound **35**

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
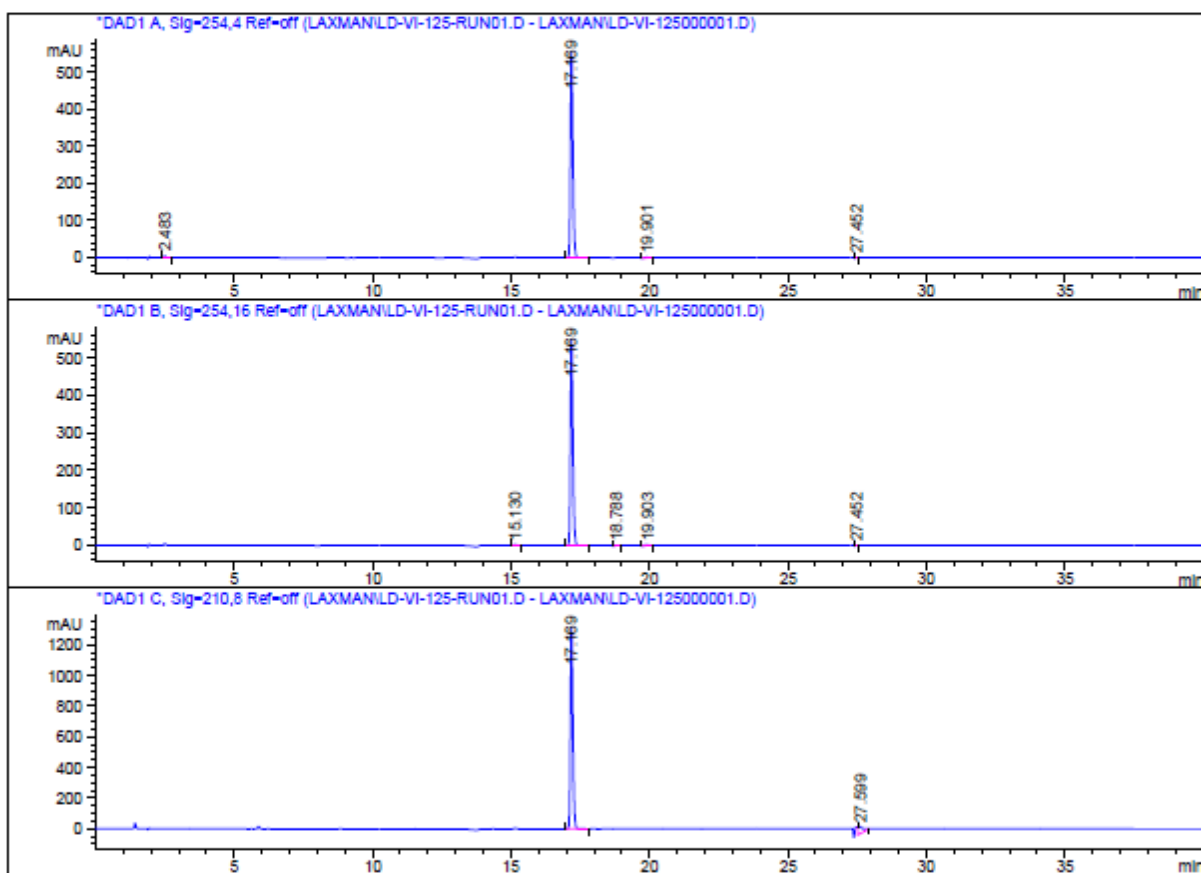
405

406
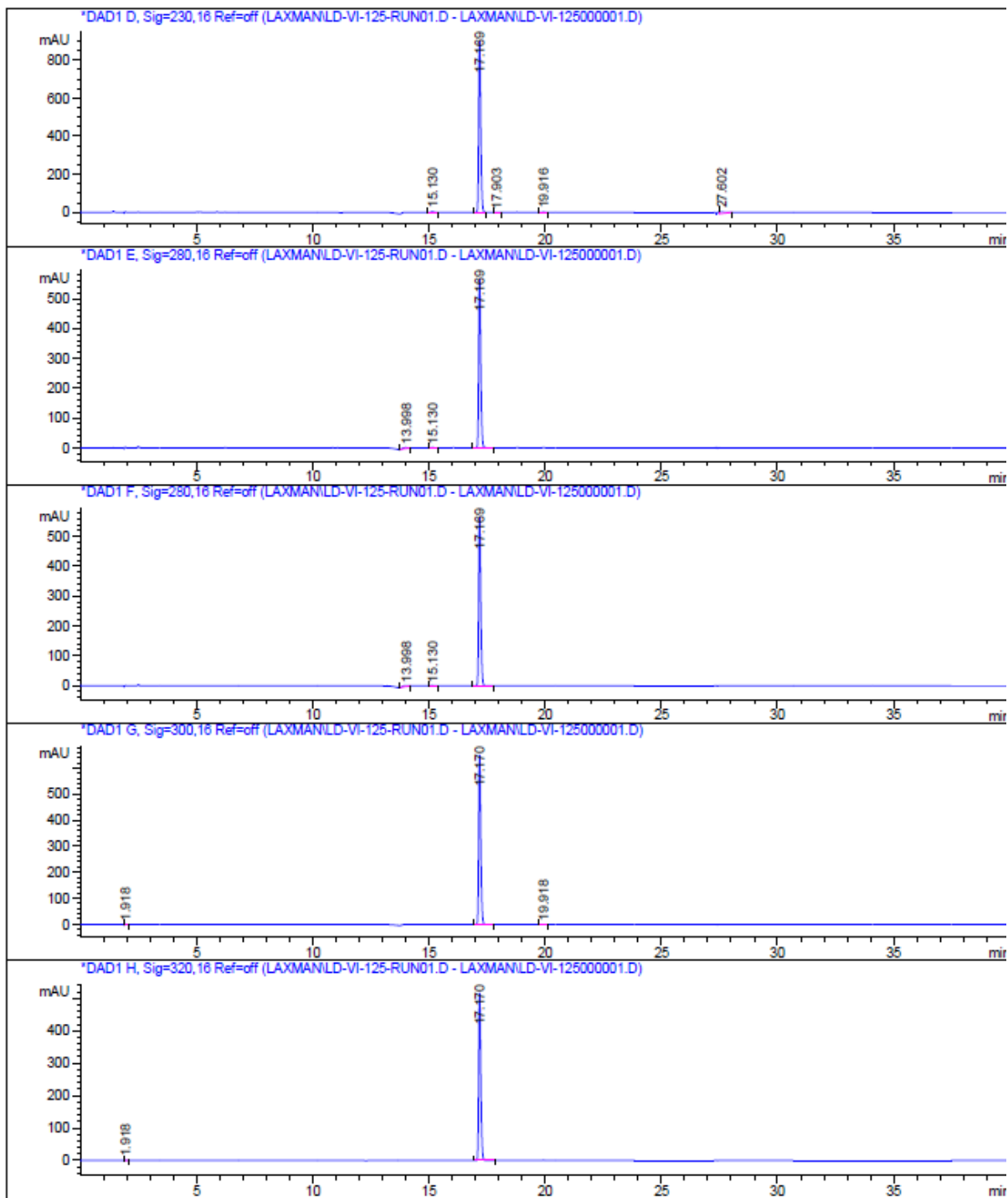
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-125-RUN01.D
Sample Name: LD-VI-125-1A-run1

```
=====================================================================
                        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 Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  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
```

407

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

408

```
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 ***
```
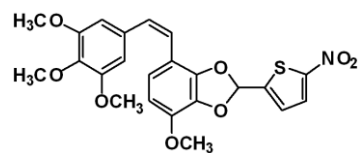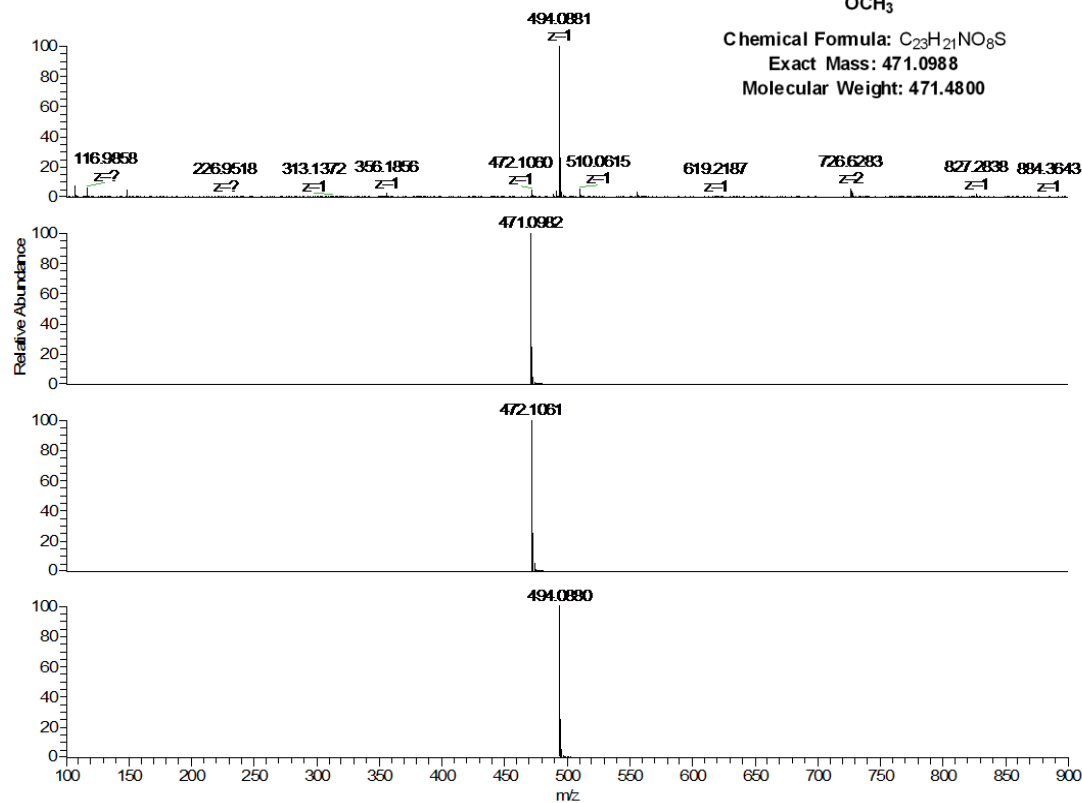
409

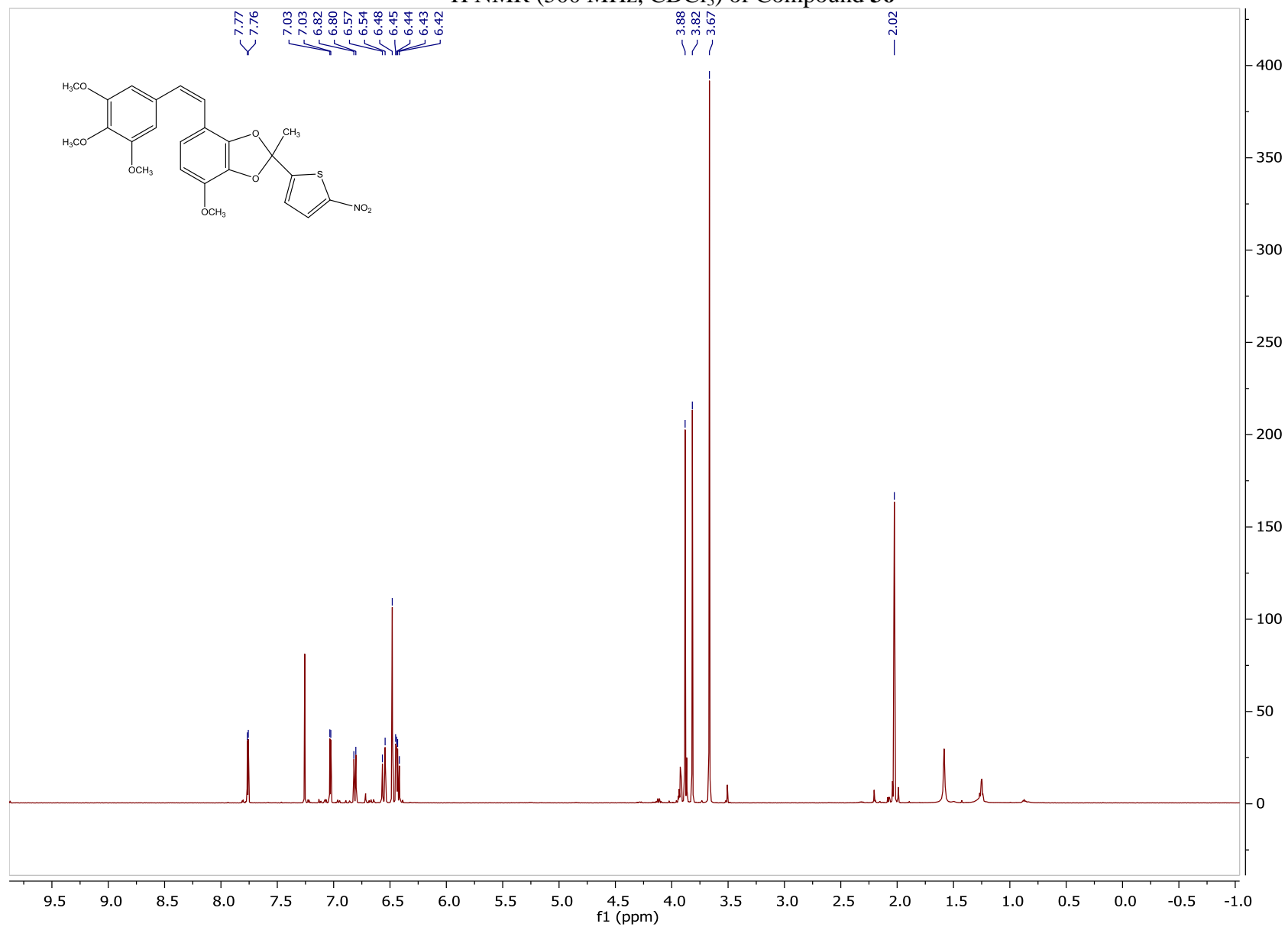# Mass Spectrum for Compound 35

Chemical Formula: $C_{23}H_{21}NO_8S$
Exact Mass: 471.0988
Molecular Weight: 471.4800

NL:
2.44E7
LD-VI-125-1A_Orbi_+
ESI#13 RT:0.11 AV:1
T: FTMS + c ESI Full ms
[100.00-900.00]

494.0881
z=1

116.9858
z=?
226.9518
z=?
313.1372
z=1
356.1856
z=1
472.1080
z=1
510.0615
z=1
619.2187
z=1
726.6283
z=2
827.2838
z=1
884.3643
z=1

NL:
7.23E5
$C_{23}H_{21}NO_8S$:
$C_{23}H_{21}N_1O_8S_1$
pa Chrg 1

471.0982

NL:
7.22E5
$C_{23}H_{21}NO_8S$ +H:
$C_{23}H_{22}N_1O_8S_1$
pa Chrg 1

472.1061

NL:
7.23E5
$C_{23}H_{21}NO_8S$ +Na:
$C_{23}H_{21}N_1O_8S_1Na_1$
pa Chrg 1

494.0880

Relative Abundance

m/z

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **36**

411

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **36**

HPLC trace of Compound **36**

413

```
==================================================================
                      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 Type Width   Area   Height   Area
     #   [min]   [min]   [mAU*s]   [mAU]   %
    ----|-------|----|-------|----------|----------|--------|
     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 Type Width   Area   Height   Area
     #   [min]   [min]   [mAU*s]   [mAU]   %
    ----|-------|----|-------|----------|----------|--------|
     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 Type Width   Area   Height   Area
     #   [min]   [min]   [mAU*s]   [mAU]   %
    ----|-------|----|-------|----------|----------|--------|
     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 Type Width   Area   Height   Area
     #   [min]   [min]   [mAU*s]   [mAU]   %
    ----|-------|----|-------|----------|----------|--------|
     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
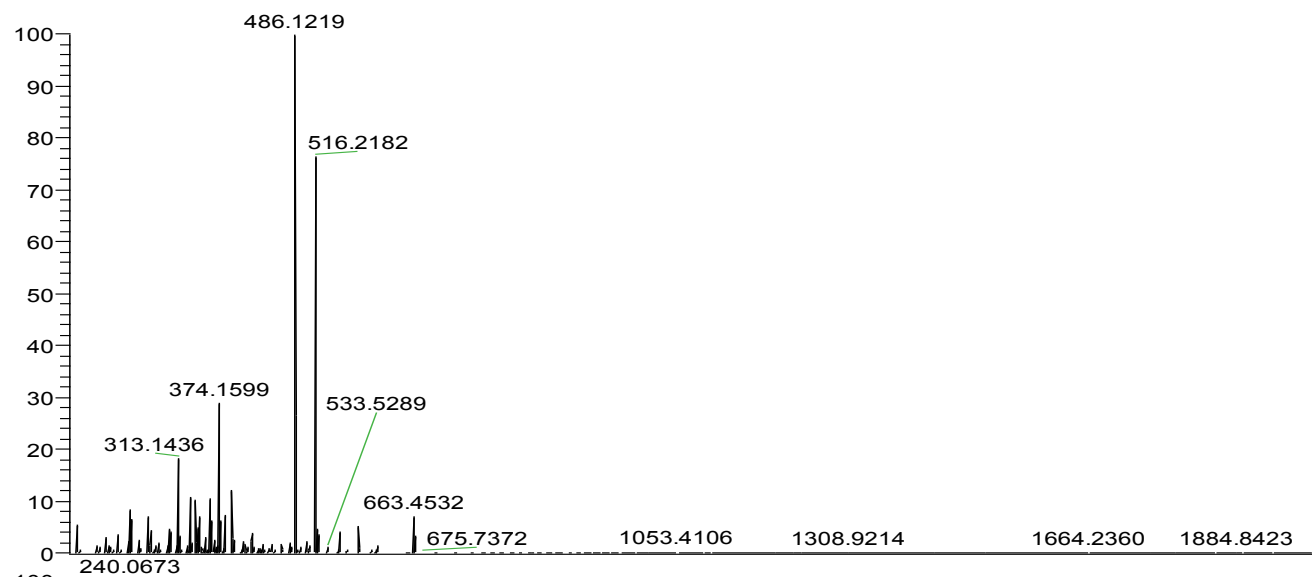

Signal 7: DAD1 G, Sig=300,16 Ref=off
 Signal has been modified after loading from rawdata file!

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

Mass Spectrum of Compound **36**
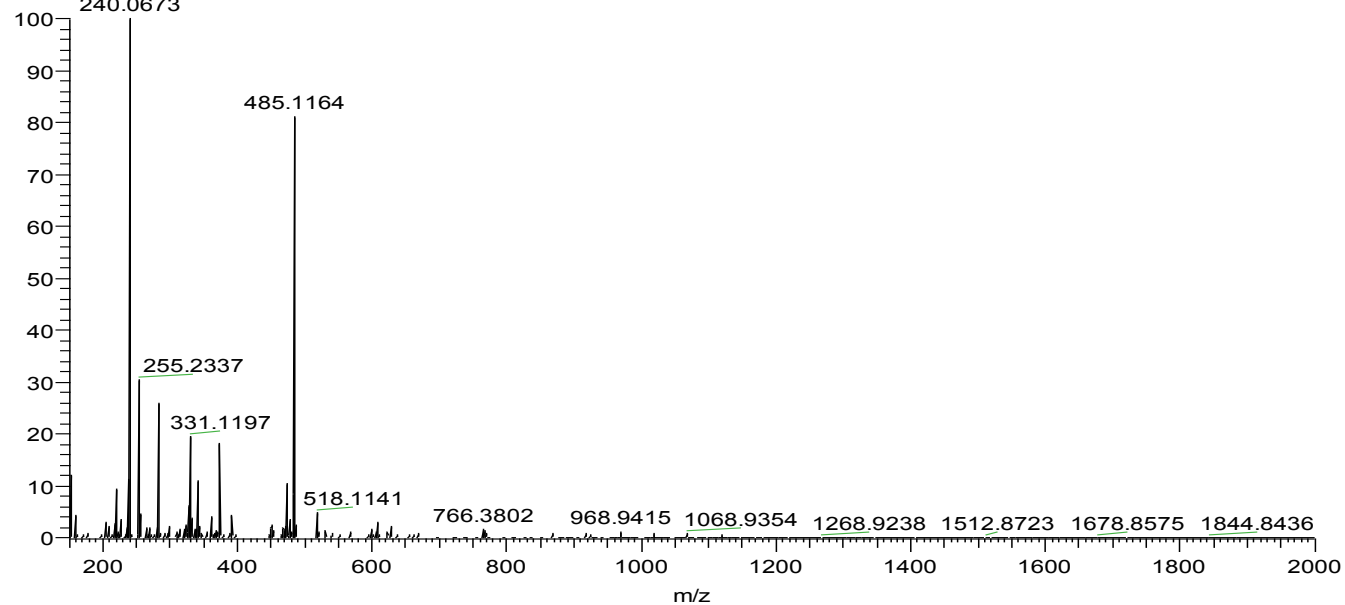
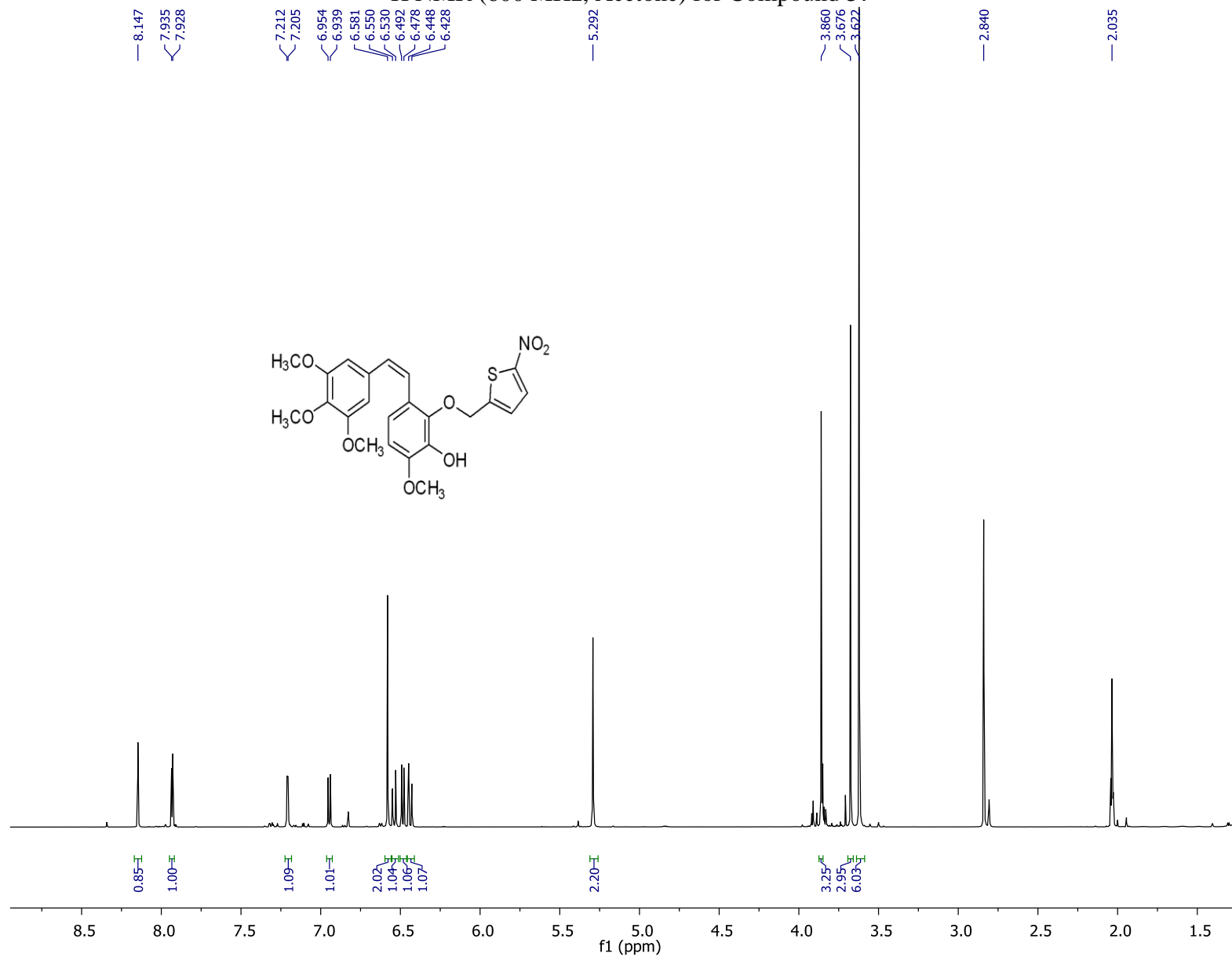^{1}H NMR (600 MHz, Acetone) for Compound **37**

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

153.057
152.270
149.215
148.591
137.440
134.066
132.746
128.977
128.615
126.472
125.078
124.945
117.943
106.195
102.989
68.492
59.639
55.383
55.219

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
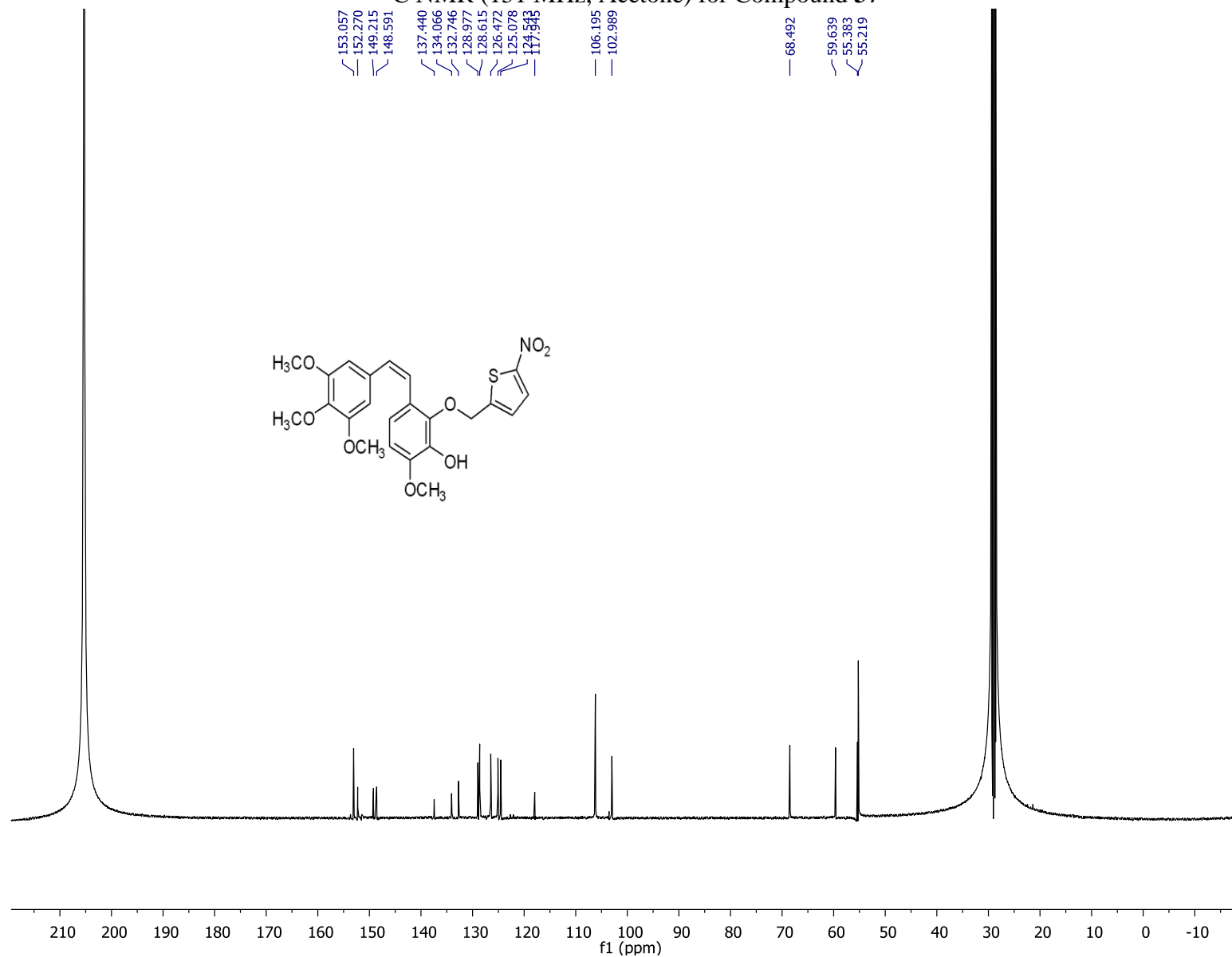
^{13}C DEPT NMR (125 MHz, Acetone) for Compound **37**

128.976
128.615
126.472
125.078
124.542
106.193
102.988
68.492
59.638
55.383
55.219

150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)
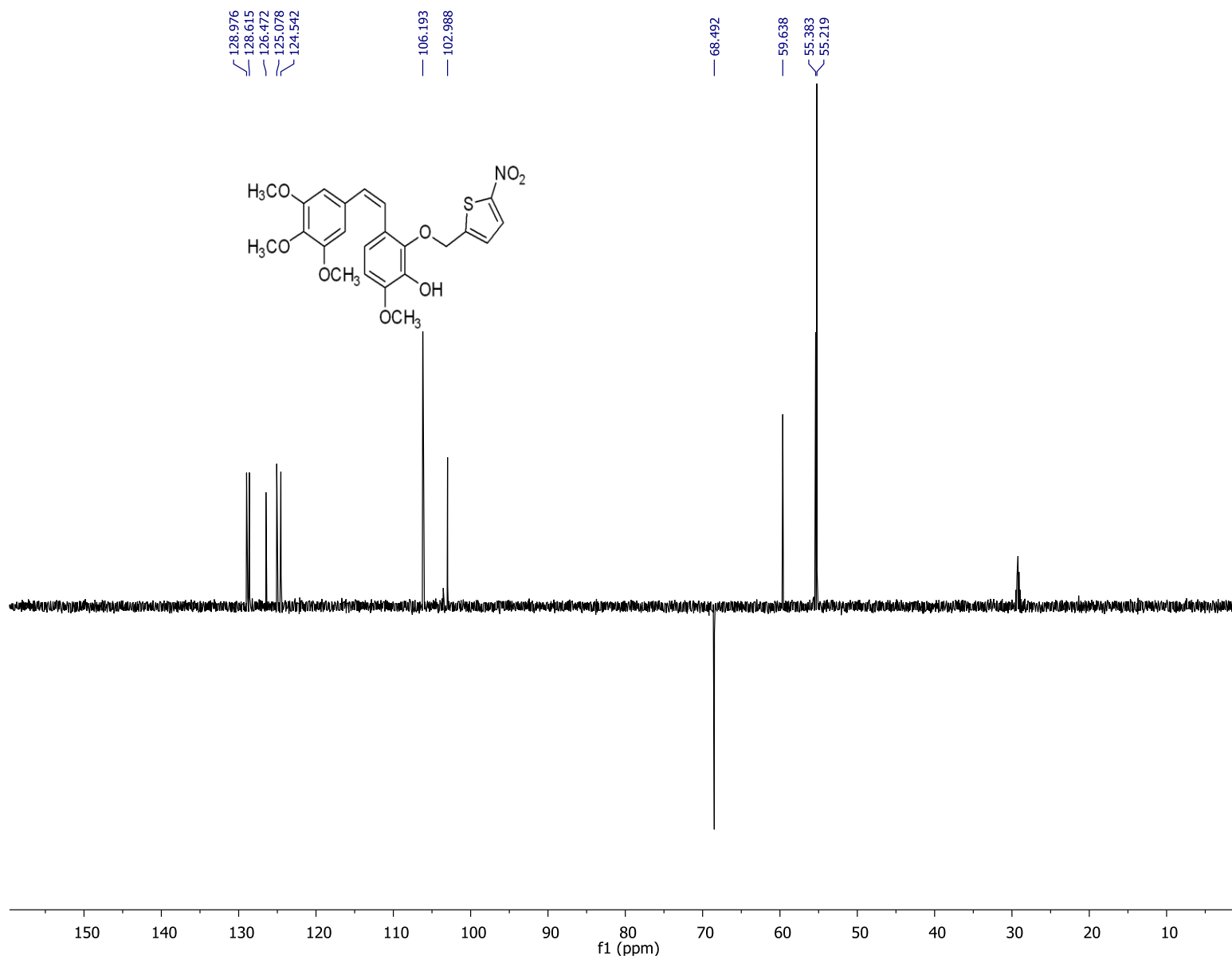
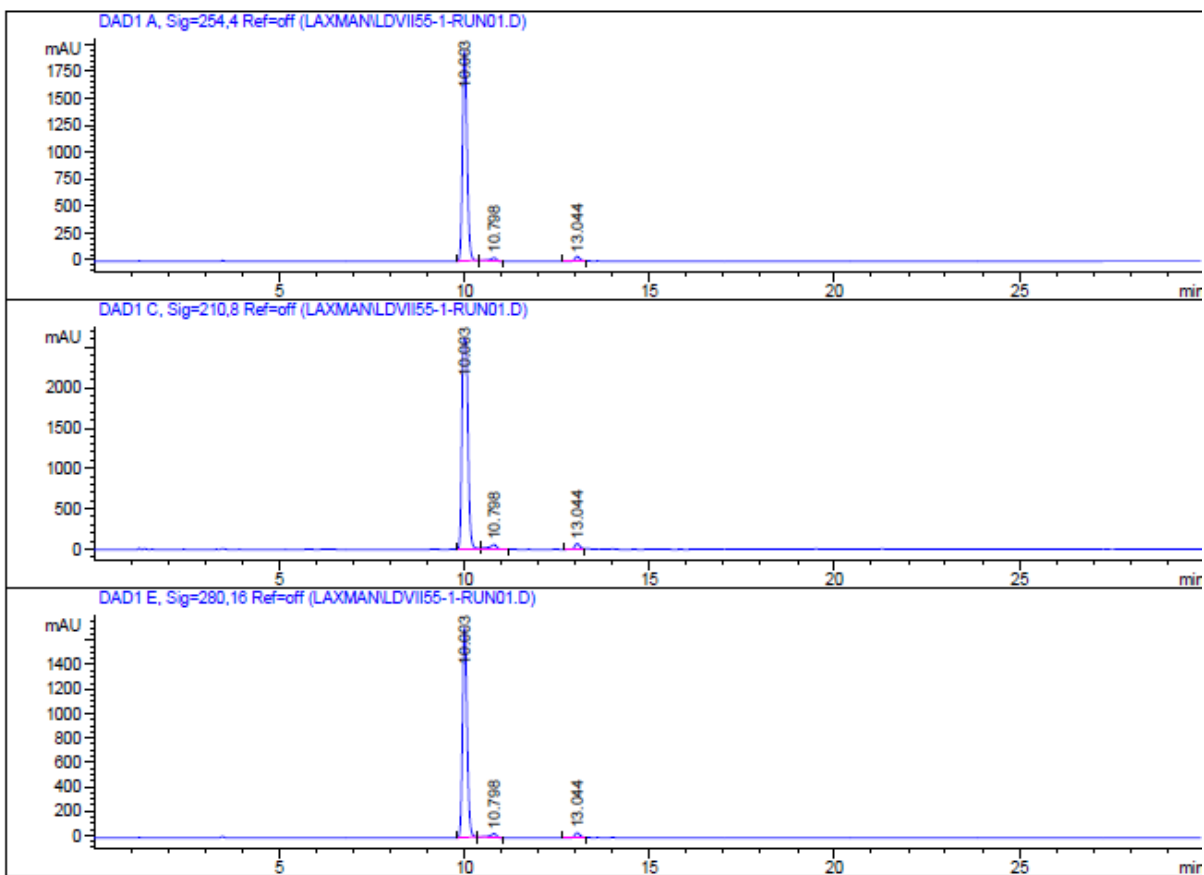# HPLC Traces for Compound **37**

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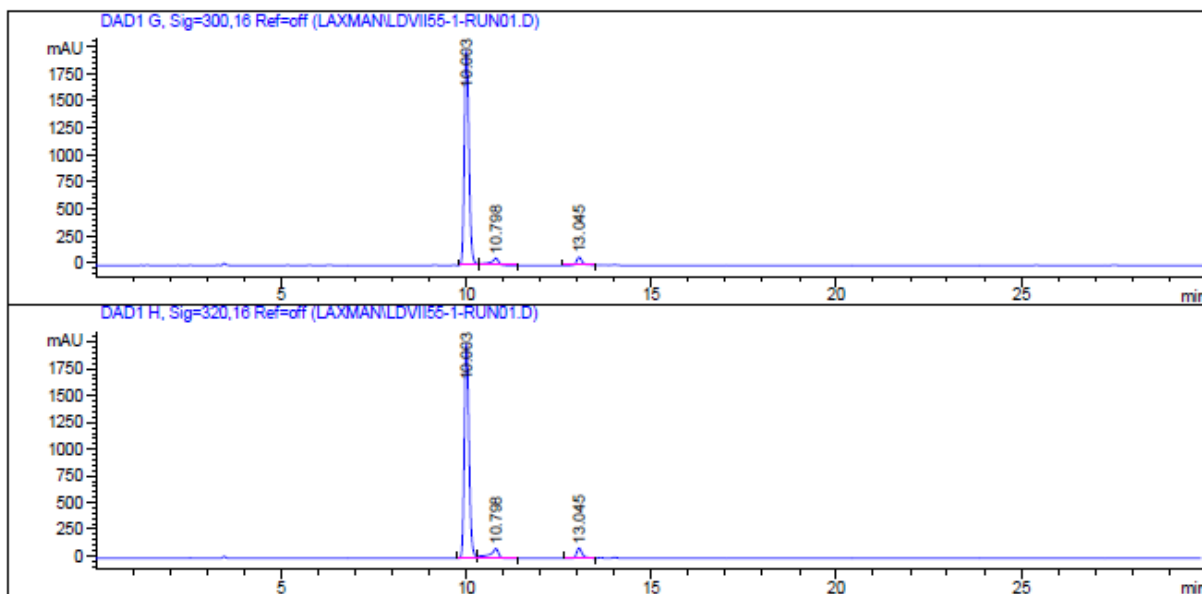
```
==================================================================
                    Area Percent Report
==================================================================


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

Instrument 1 4/9/2015 11:52:45 AM Graham   Page   2 of 3

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

  Peak RetTime Type  Width     Area      Height     Area
   #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
    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 Type  Width     Area      Height     Area
   #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
    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 Type  Width     Area      Height     Area
   #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
    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 Type  Width     Area      Height     Area
   #   [min]        [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
    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 ***
```
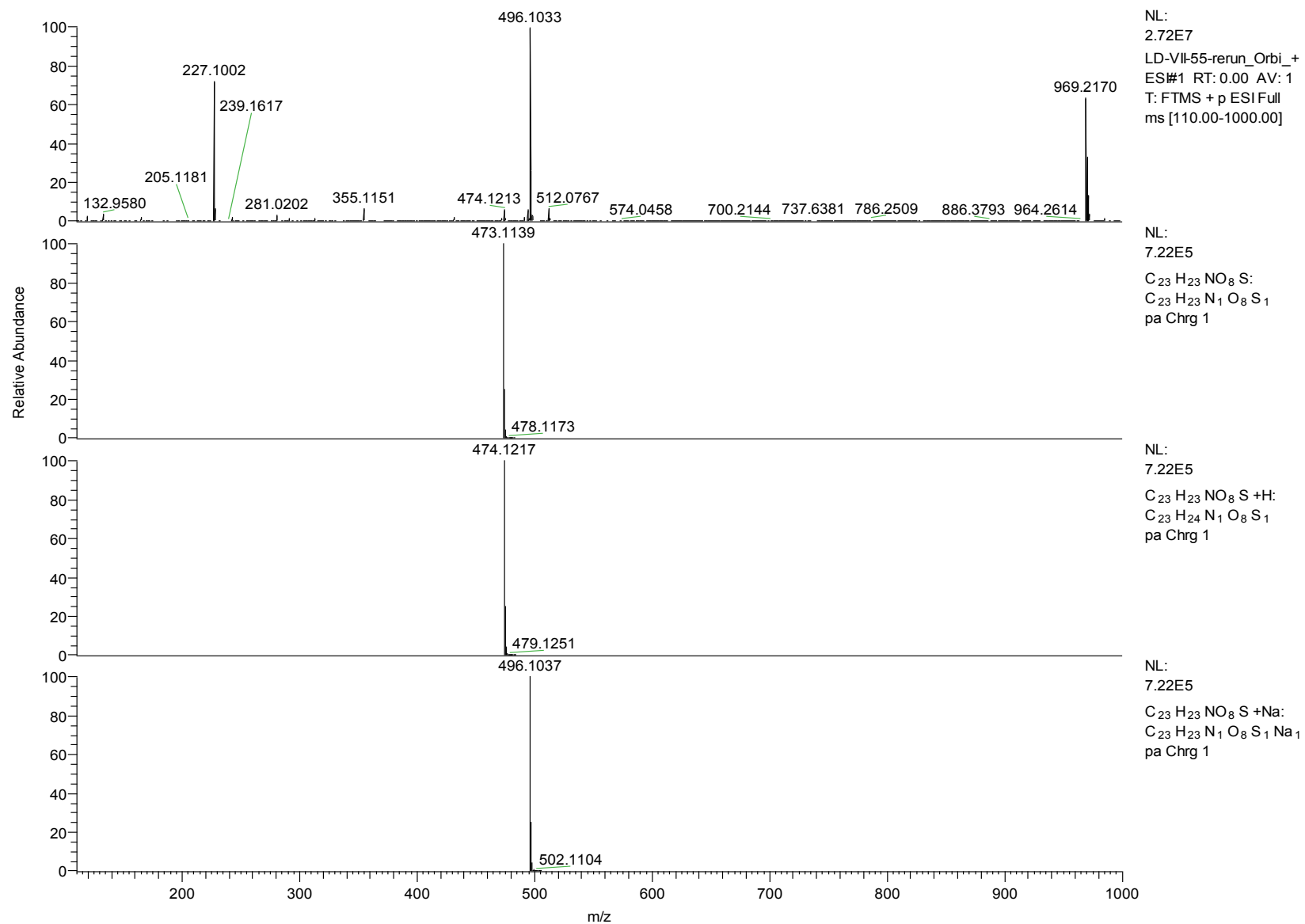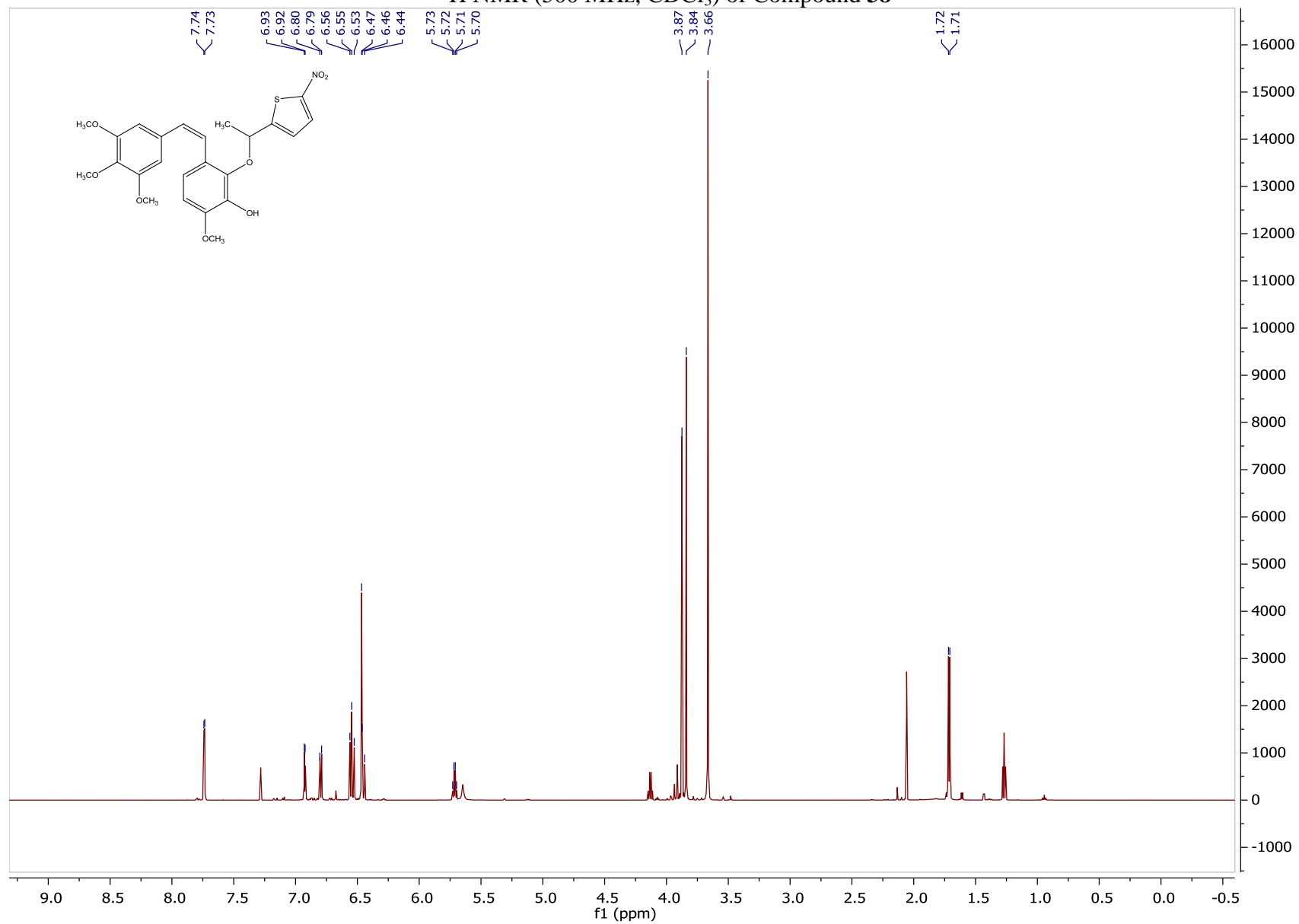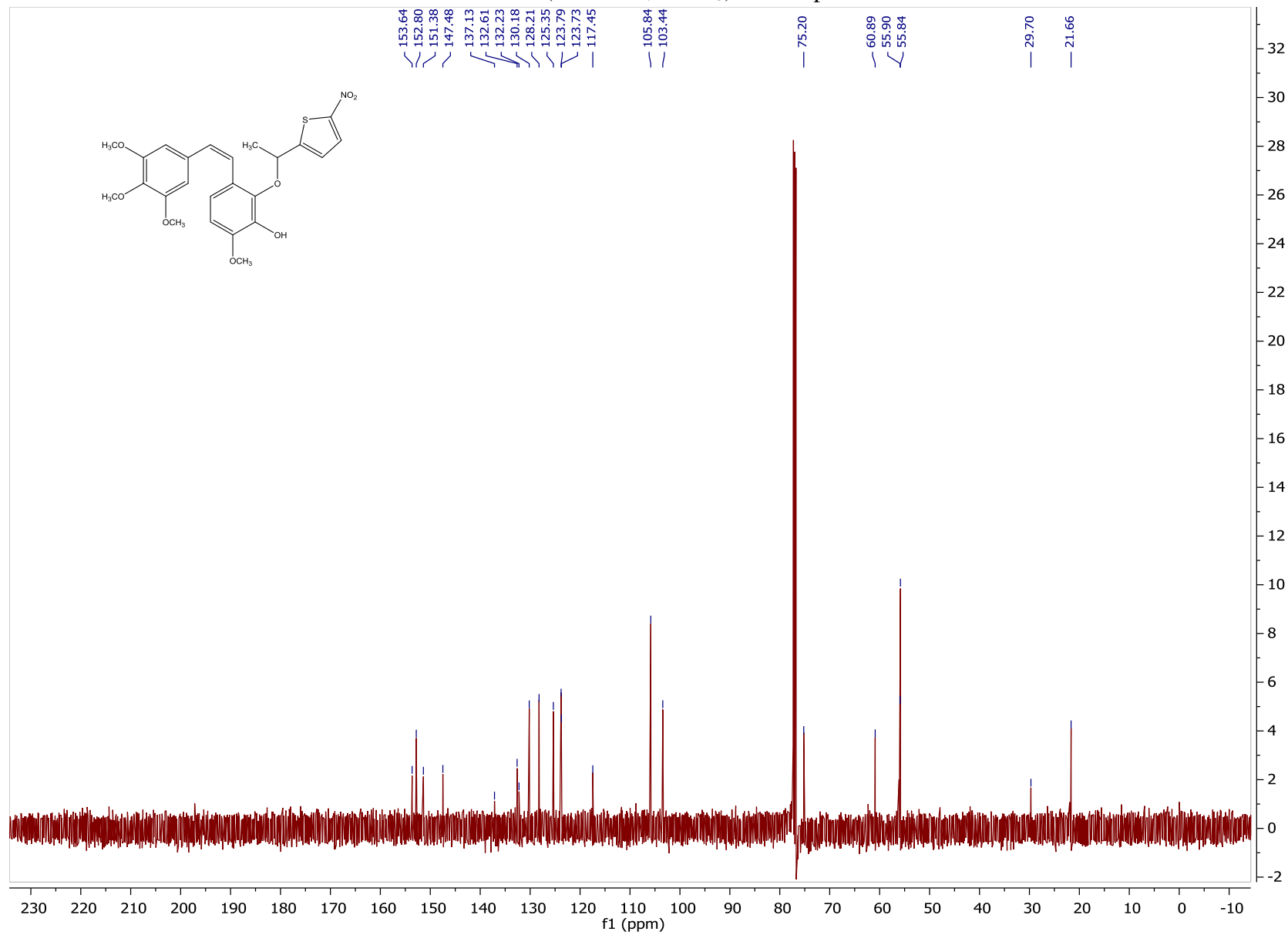
Mass Spectrum of Compound **37**

HPLC trace of Compound **38**

DAD1 F, Sig=320,16 Ref=off (Blake\wash.D)

DAD1 G, Sig=300,16 Ref=off (Blake\wash.D)

DAD1 H, Sig=320,16 Ref=off (Blake\wash.D)

```
=================================================================
                    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 Type  Width     Area        Height     Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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
```

429

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

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 Type  Width     Area       Height    Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
Totals :                  1.53055e4  1447.22120
```

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

```
Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
    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
```

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*** End of Report ***

Mass Spectrum of Compound **38**

Top spectrum (positive mode):

- 282.2789
- 333.1328
- 488.1363
- 516.2174
- 609.1348
- 717.7833
- 997.2480
- 1146.4154
- 1308.9265
- 1622.5002
- 1884.8030
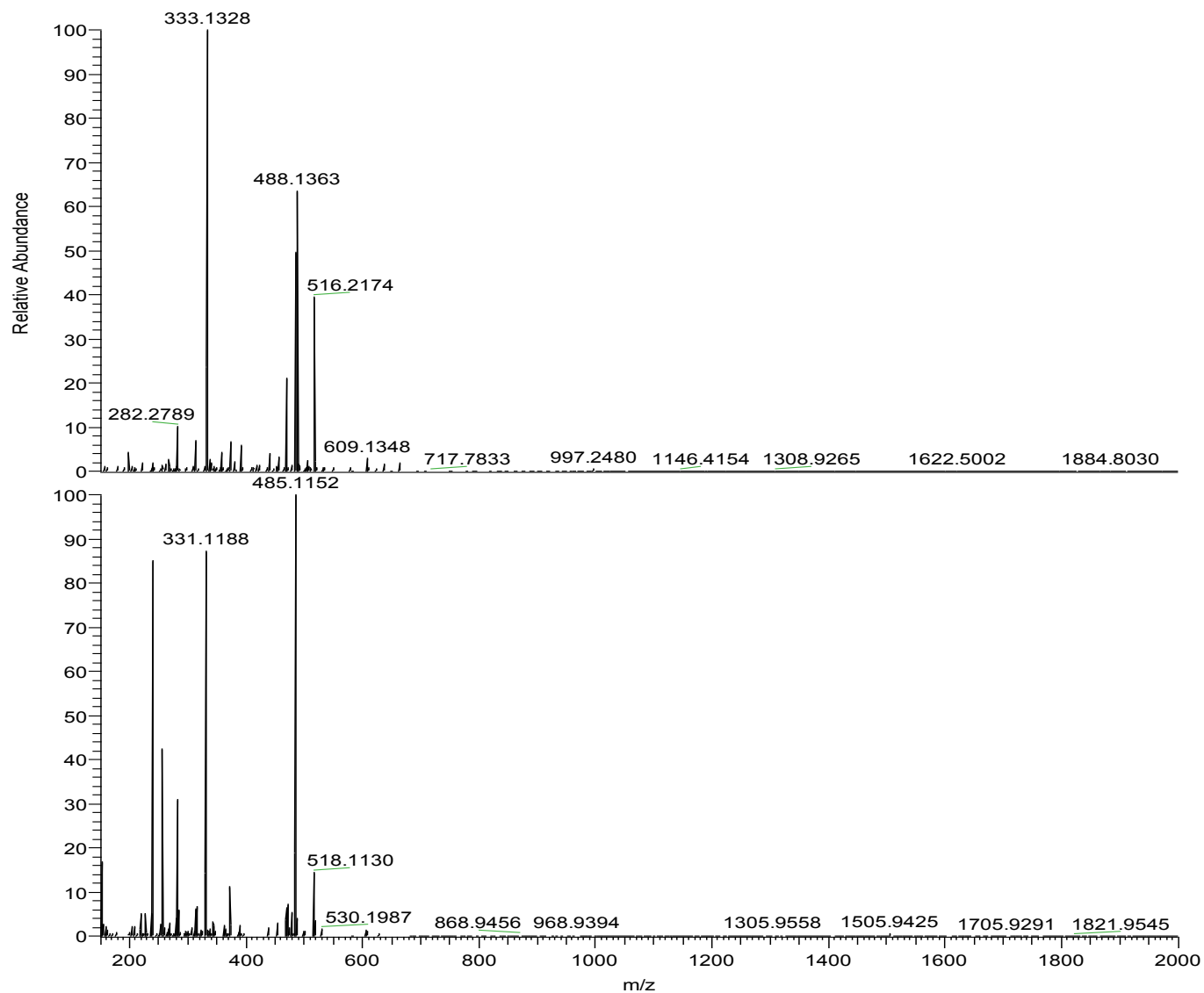
NL: 6.03E6
Mono Major CA1b#1
RT: 0.00 AV: 1 T:
FTMS + p APCI corona
pi Full ms
[150.00-2000.00]

Bottom spectrum (negative mode):

- 331.1188
- 485.1152
- 518.1130
- 530.1987
- 868.9456
- 968.9394
- 1305.9558
- 1505.9425
- 1705.9291
- 1821.9545

NL: 1.49E6
mono major ca1 neg#1
RT: 0.00 AV: 1 T:
FTMS - p APCI corona
pi Full ms
[150.00-2000.00]

¹H NMR (600 MHz, CDCl₃) for Compound **39**

^{13}C NMR (151 MHz, CDCl$_3$) for Compound **39**

152.780
151.818
148.494
147.032
142.636
138.380
137.229
132.380
130.654
128.250
125.539
124.600
124.281
120.497
106.580
105.971
68.689
60.944
56.375
55.845

f1 (ppm)
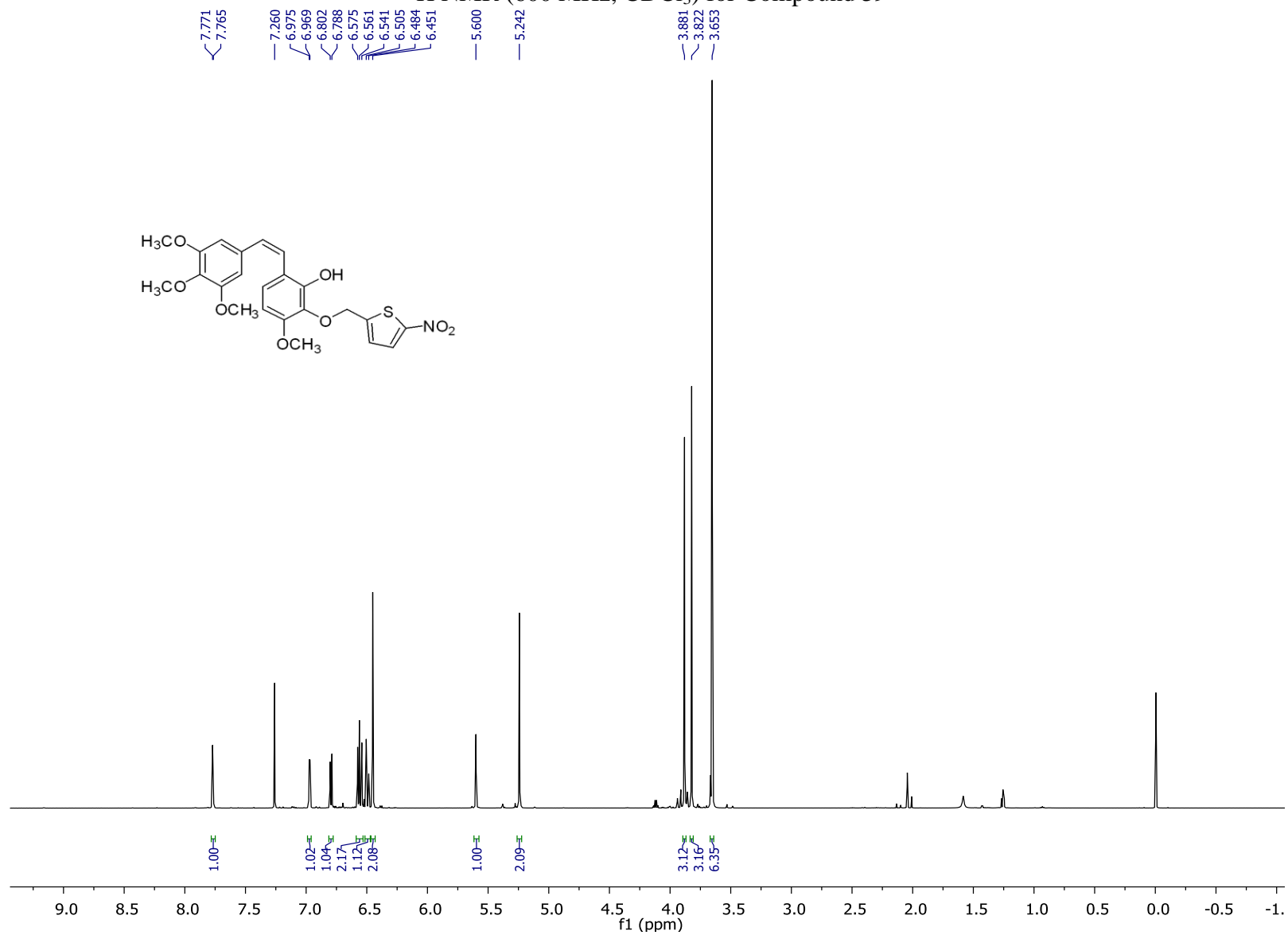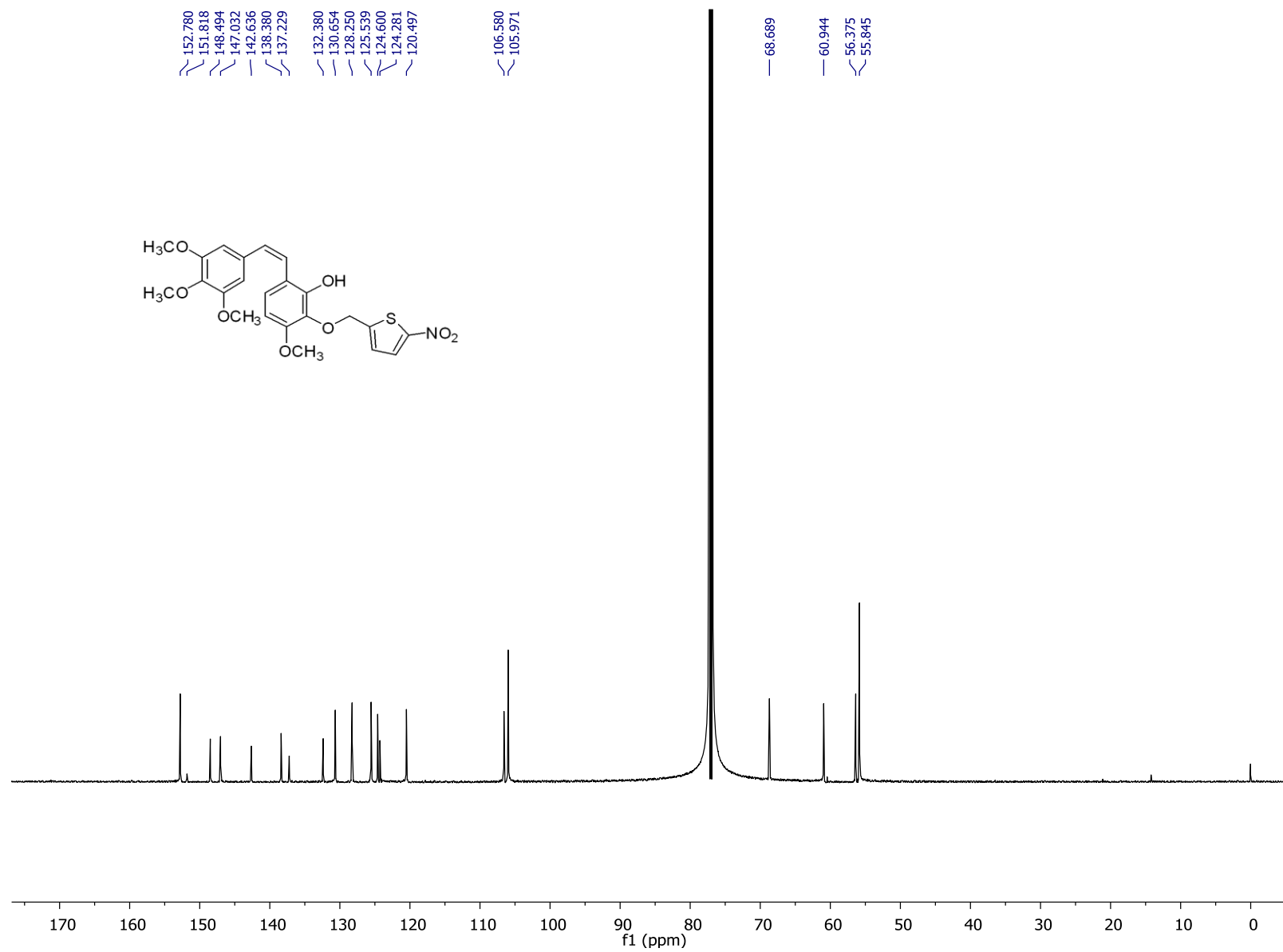
HPLC Traces for Compound **39**

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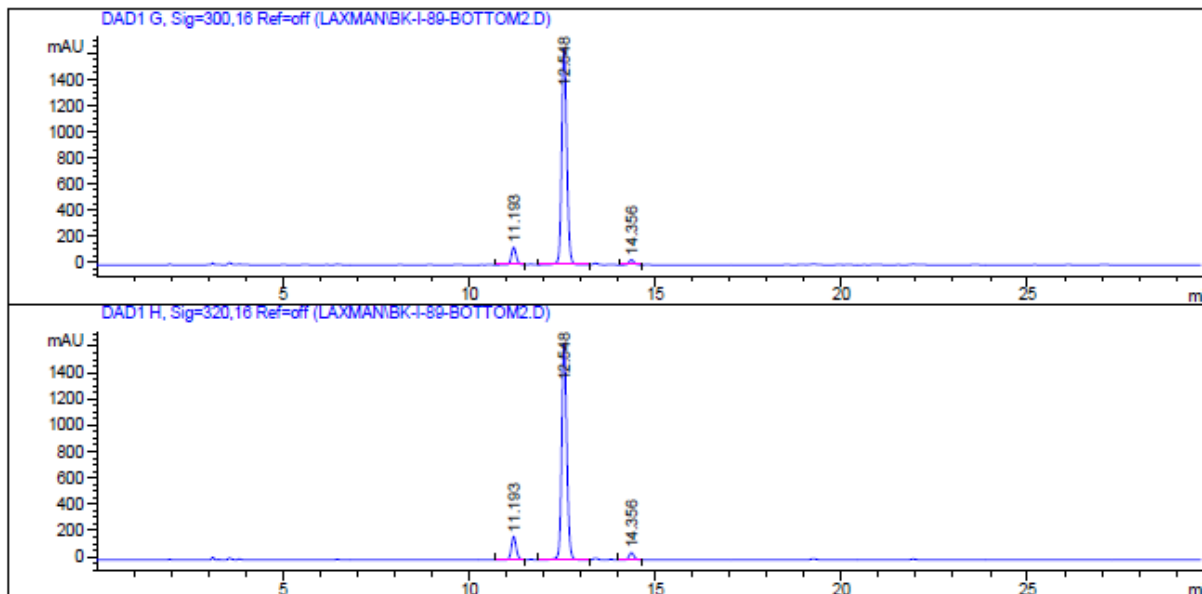
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Acq. Operator   : Laxman
Acq. Instrument : Instrument 1   Location :   -
Injection Date : 7/8/2015 2:42:58 PM
Acq. Method   : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
Last changed   : 7/8/2015 2:37:39 PM by Laxman
Analysis Method : C:\CHEM32\1\DATA\LAXMAN\BK-I-89-BOTTOM2.D\DA.M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

436

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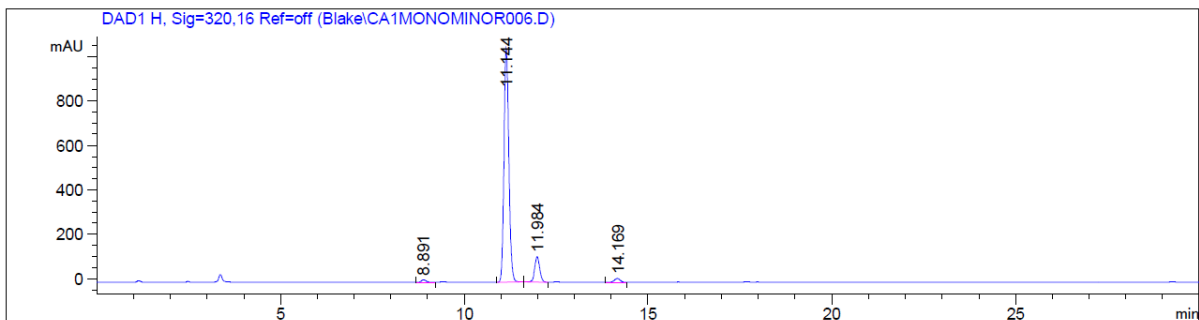
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				3.31032e4	2940.48544	

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 ***

438

Mass Spectrum of Compound **39**

NL:
7.22E5

$C_{23}H_{23}NO_8S$:
$C_{23}H_{23}N_1O_8S_1$
pa Chrg 1

NL:
7.22E5

$C_{23}H_{23}NO_8S$ +H:
$C_{23}H_{24}N_1O_8S_1$
pa Chrg 1

NL:
7.22E5

$C_{23}H_{23}NO_8S$ +Na:
$C_{23}H_{23}N_1O_8S_1Na_1$
pa Chrg 1

439

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

440
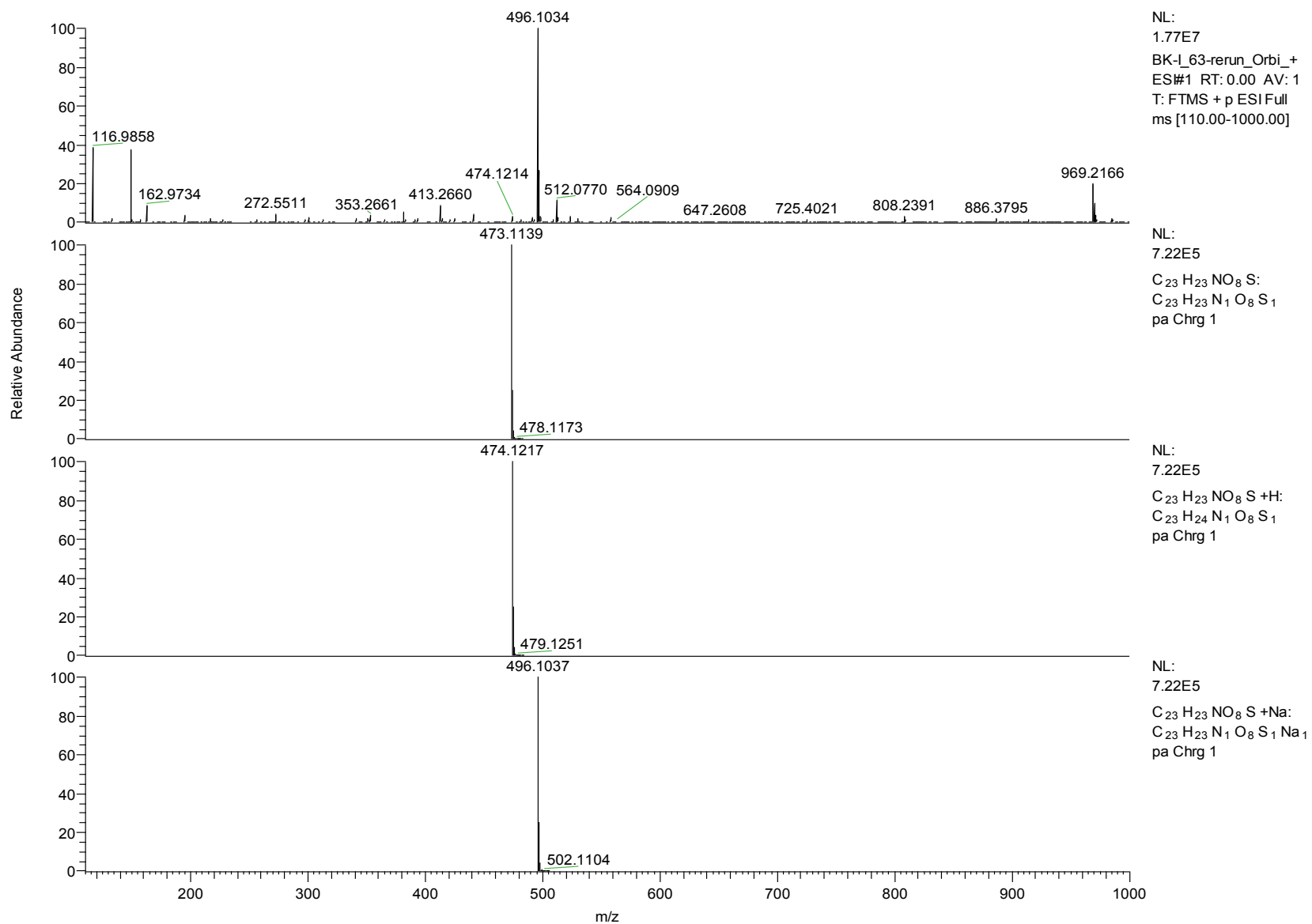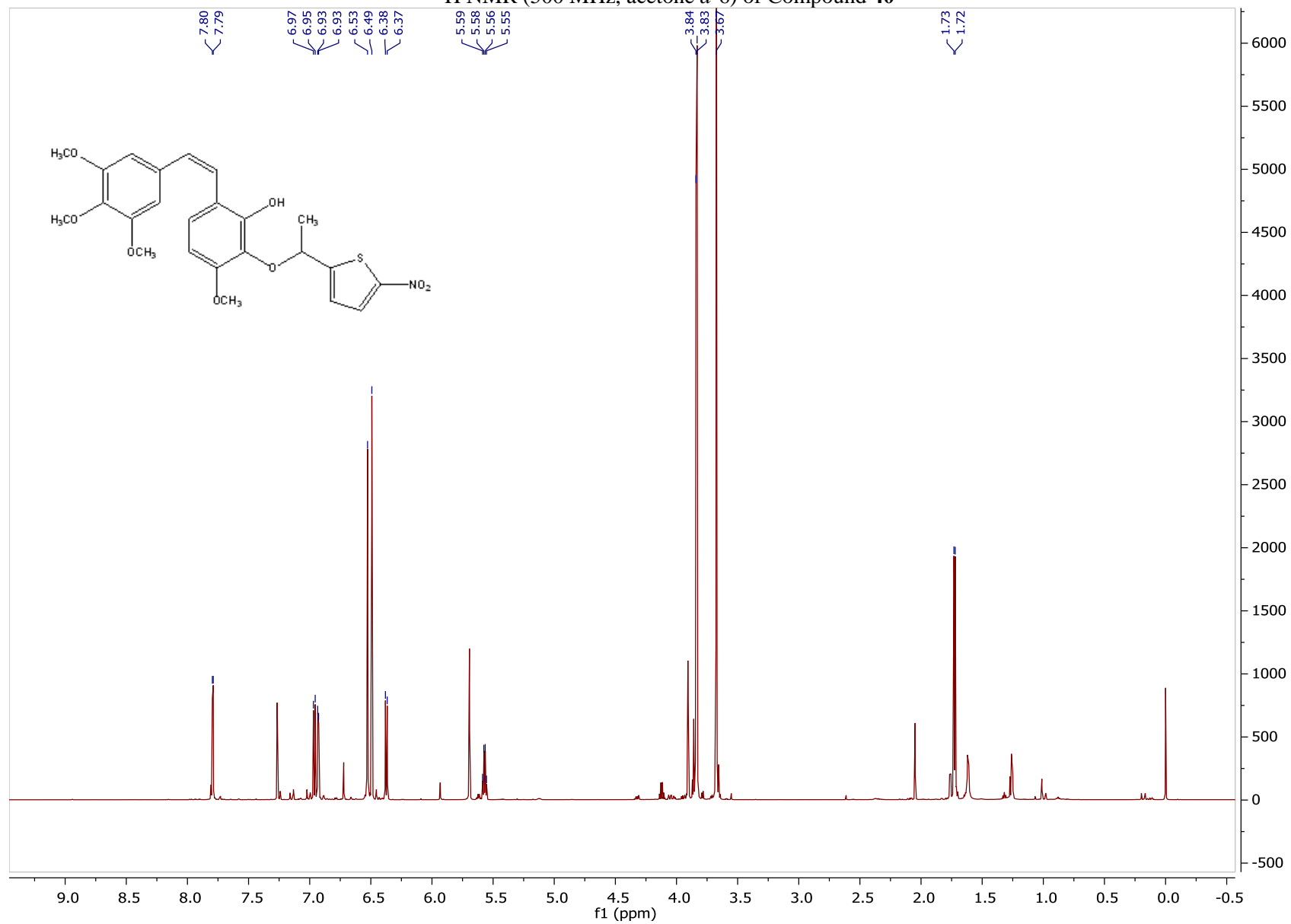
^{13}C NMR (500 MHz, acetone d-6) of Compound **40**
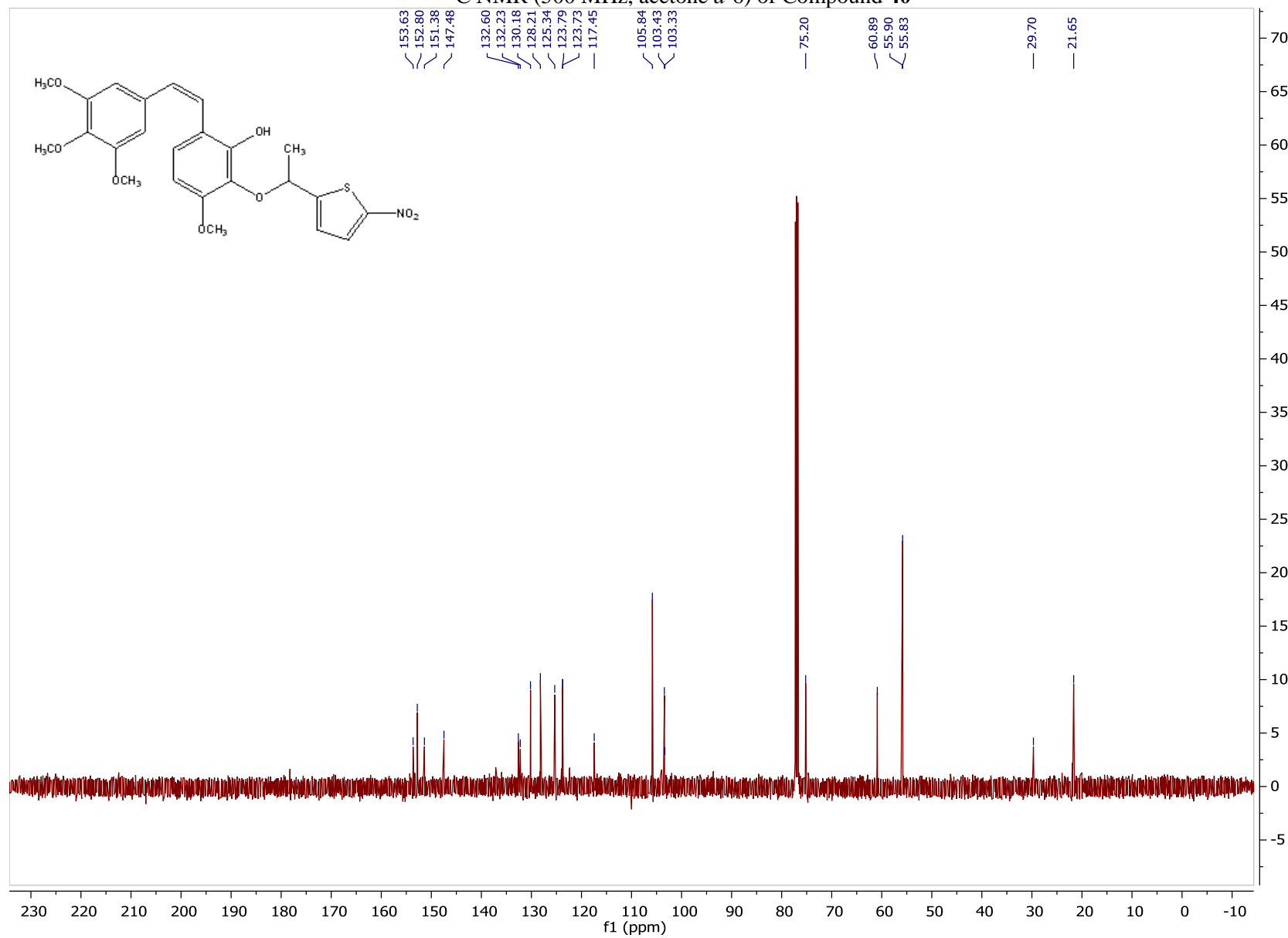
HPLC trace of Compound **40**

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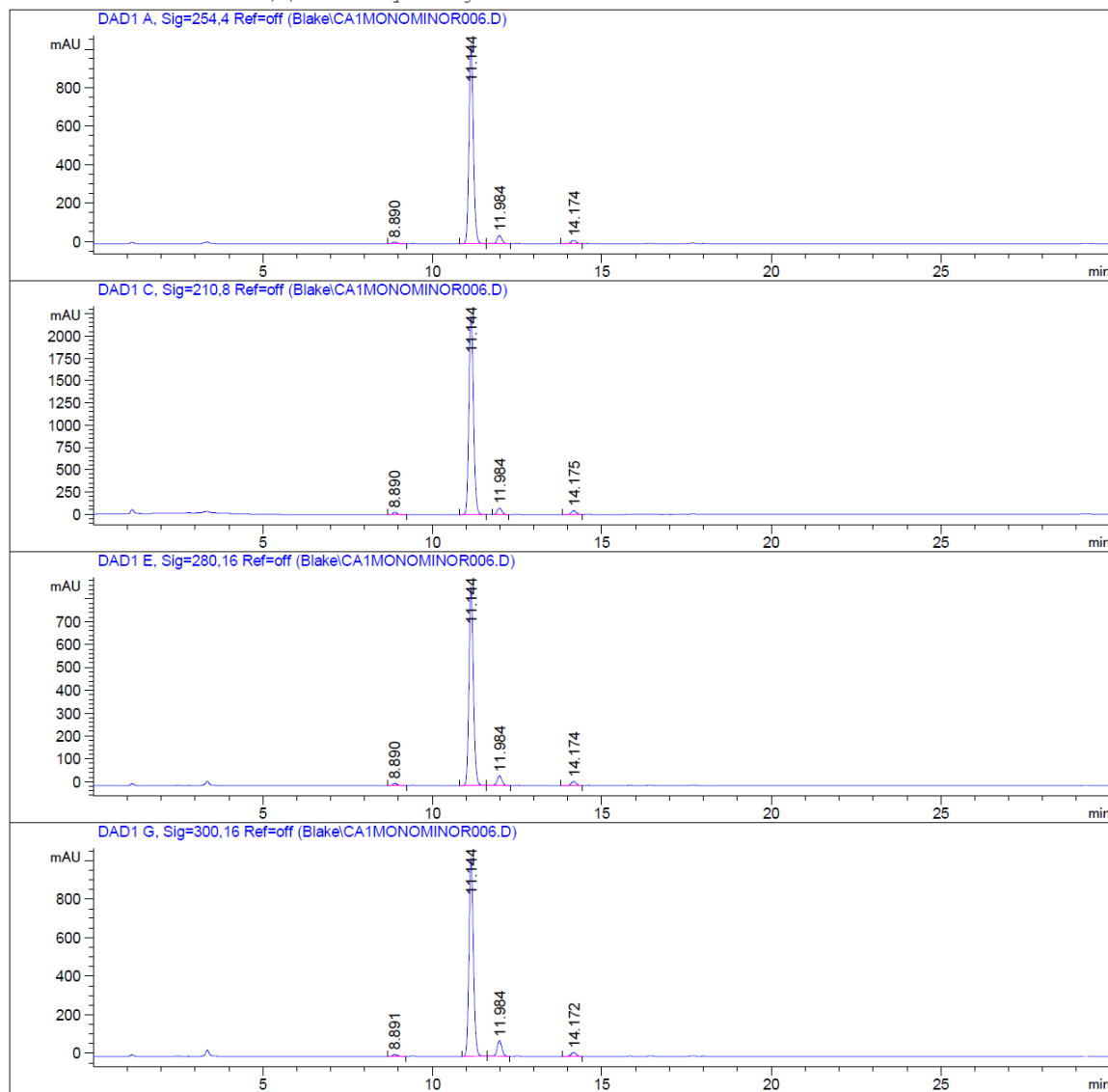
```
===================================================================
                      Area Percent Report
===================================================================

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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
  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 Type  Width     Area       Height     Area
     #   [min]        [min]   [mAU*s]     [mAU]        %
    ----|-------|----|-------|----------|----------|--------|
     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 Type  Width     Area       Height     Area
     #   [min]        [min]   [mAU*s]     [mAU]        %
    ----|-------|----|-------|----------|----------|--------|
     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 Type  Width     Area       Height     Area
     #   [min]        [min]   [mAU*s]     [mAU]        %
    ----|-------|----|-------|----------|----------|--------|
     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

445

(600 MHz, CDCl₃) for Compound **41**

446

^{13}C NMR (151 MHz, CDCl$_3$) for Compound **41**

447

HPLC Traces for Compound **41**

448

```
================================================================
                    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 Type  Width     Area      Height     Area
    #   [min]        [min]   [mAU*s]     [mAU]        %
 ----|-------|----|-------|----------|----------|--------|
    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 Type  Width     Area      Height     Area
    #   [min]        [min]   [mAU*s]     [mAU]        %
 ----|-------|----|-------|----------|----------|--------|
    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 Type  Width     Area      Height     Area
    #   [min]        [min]   [mAU*s]     [mAU]        %
 ----|-------|----|-------|----------|----------|--------|
    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 Type  Width     Area      Height     Area
    #   [min]        [min]   [mAU*s]     [mAU]        %
 ----|-------|----|-------|----------|----------|--------|
    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

===
*** End of Report ***

Mass Spectrum for Compound 41
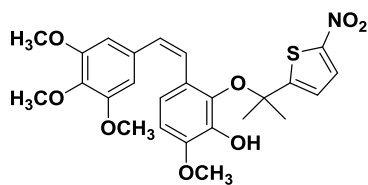
Chemical Formula: $C_{25}H_{27}NO_8S$
Exact Mass: 501.1457
Molecular Weight: 501.5500
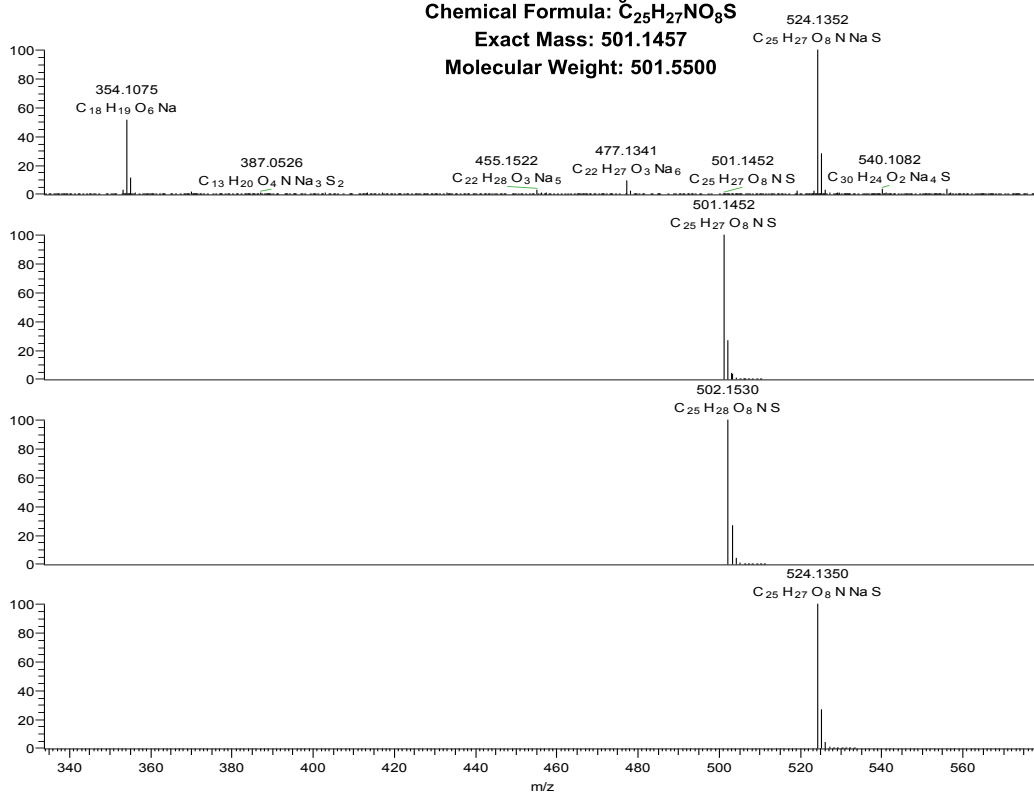
¹H NMR (500 MHz, CDCl₃) of Compound **43**

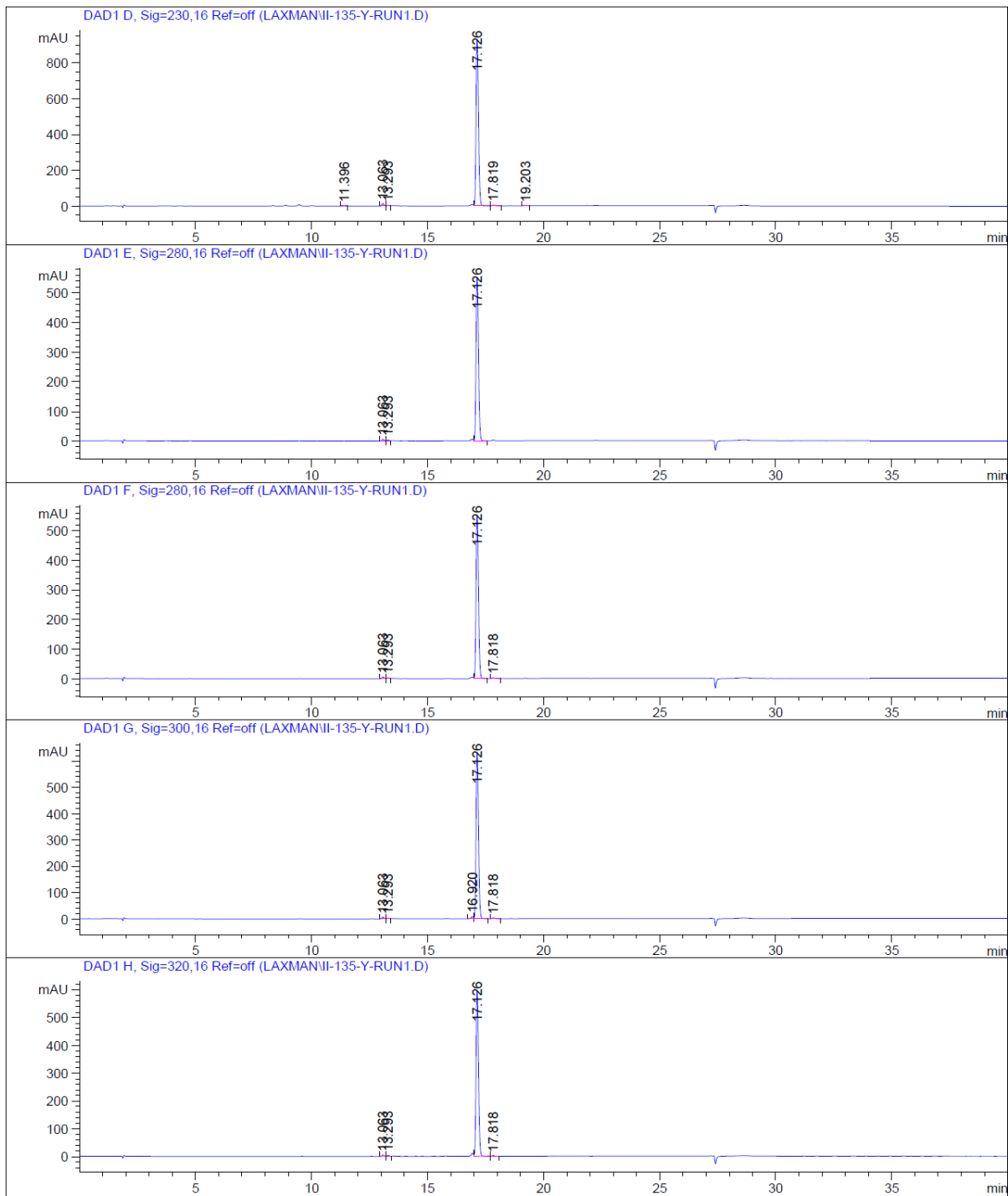^{13}C NMR (500 MHz, CDCl$_3$) of Compound **43**

HPLC trace of Compound **43**

455

456

```
=======================================================================
                          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
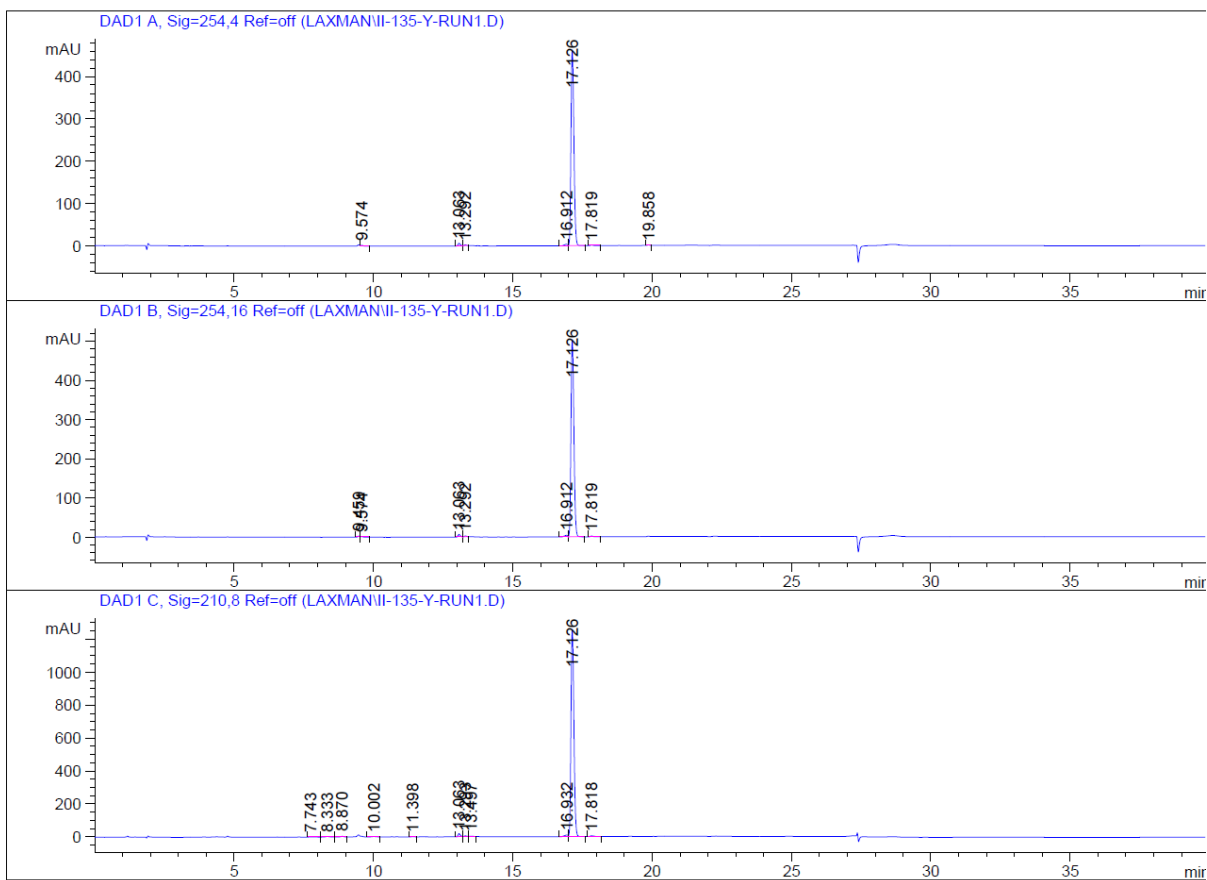
```
Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   9.574 VB    0.0884   12.42534    2.05785   0.3582
   2  13.063 BV    0.0886   36.53085    6.21524   1.0531
   3  13.292 VB    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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
   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 Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
   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
```

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

458

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

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

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

```
Peak RetTime Type  Width     Area      Height    Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   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 Type  Width     Area      Height    Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   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
```

===
*** End of Report ***

Mass Spectrum of Compound **43**

Chemical Formula: $C_{23}H_{23}NO_7S$
Exact Mass: 457.120
Molecular Weight: 457.496

Relative Abundance

480.1088

316.1305

458.1269

338.1125

496.0823

301.1071

285.1122

241.0860

370.2951

441.2975

537.3945

553.3892

604.1819 663.4525 705.6597

792.2444

937.2272

898.4163 953.2014

870.3852

994.5134

200   250   300   350   400   450   500   550   600   650   700   750   800   850   900   950   1000

m/z

460

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **44**

461
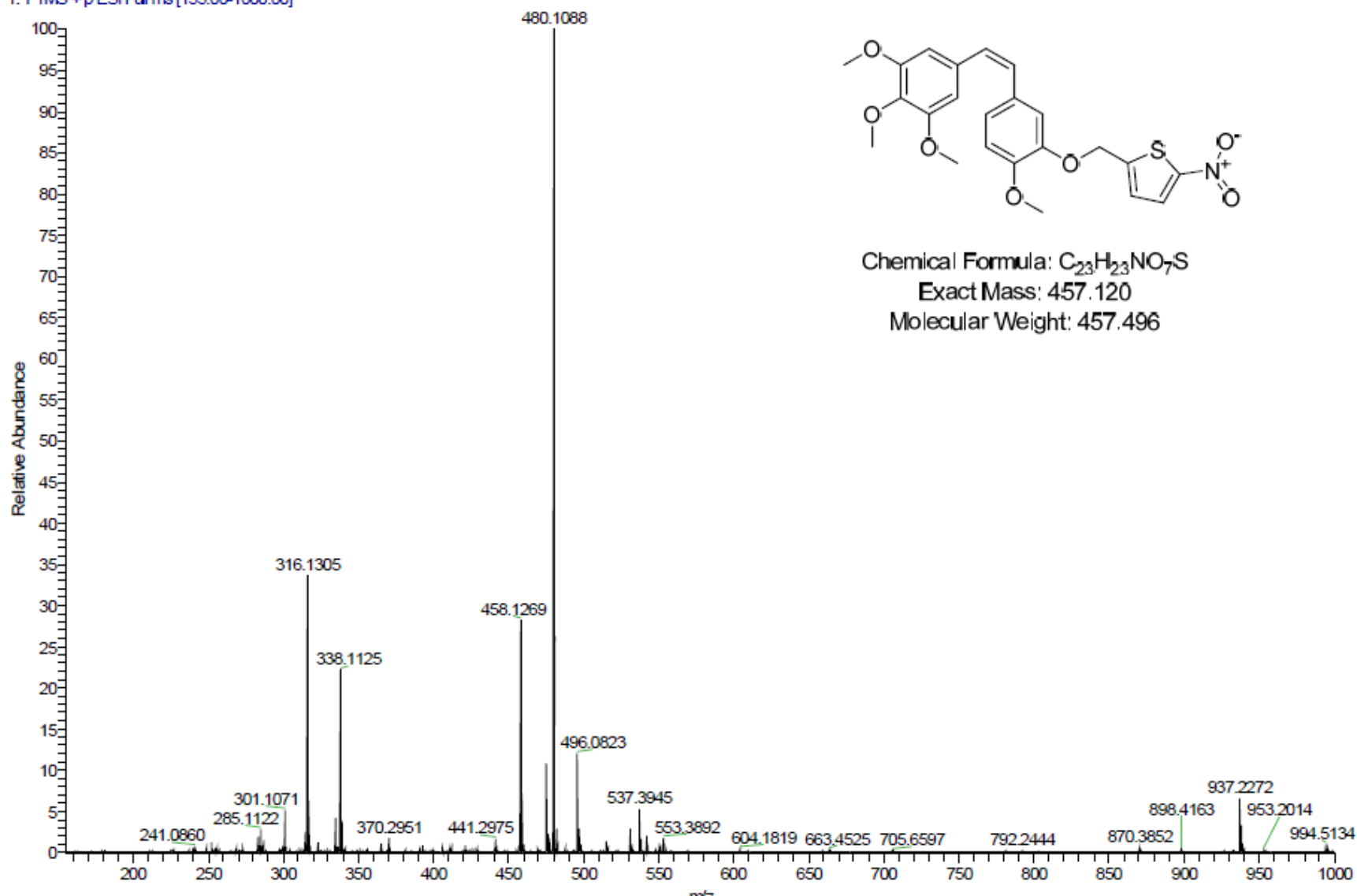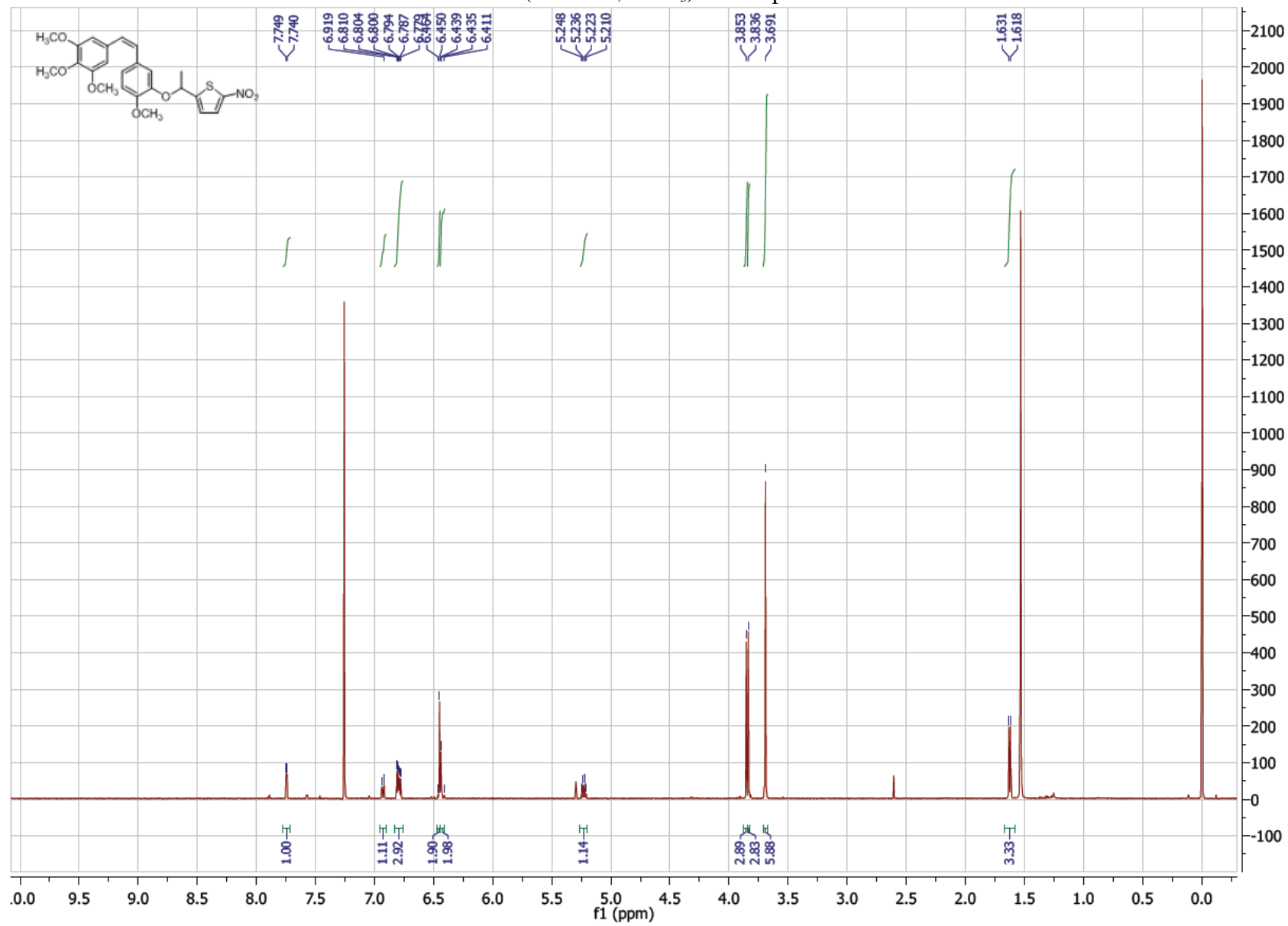
^{13}C NMR (500 MHz, CDCl$_3$) of Compound **44**
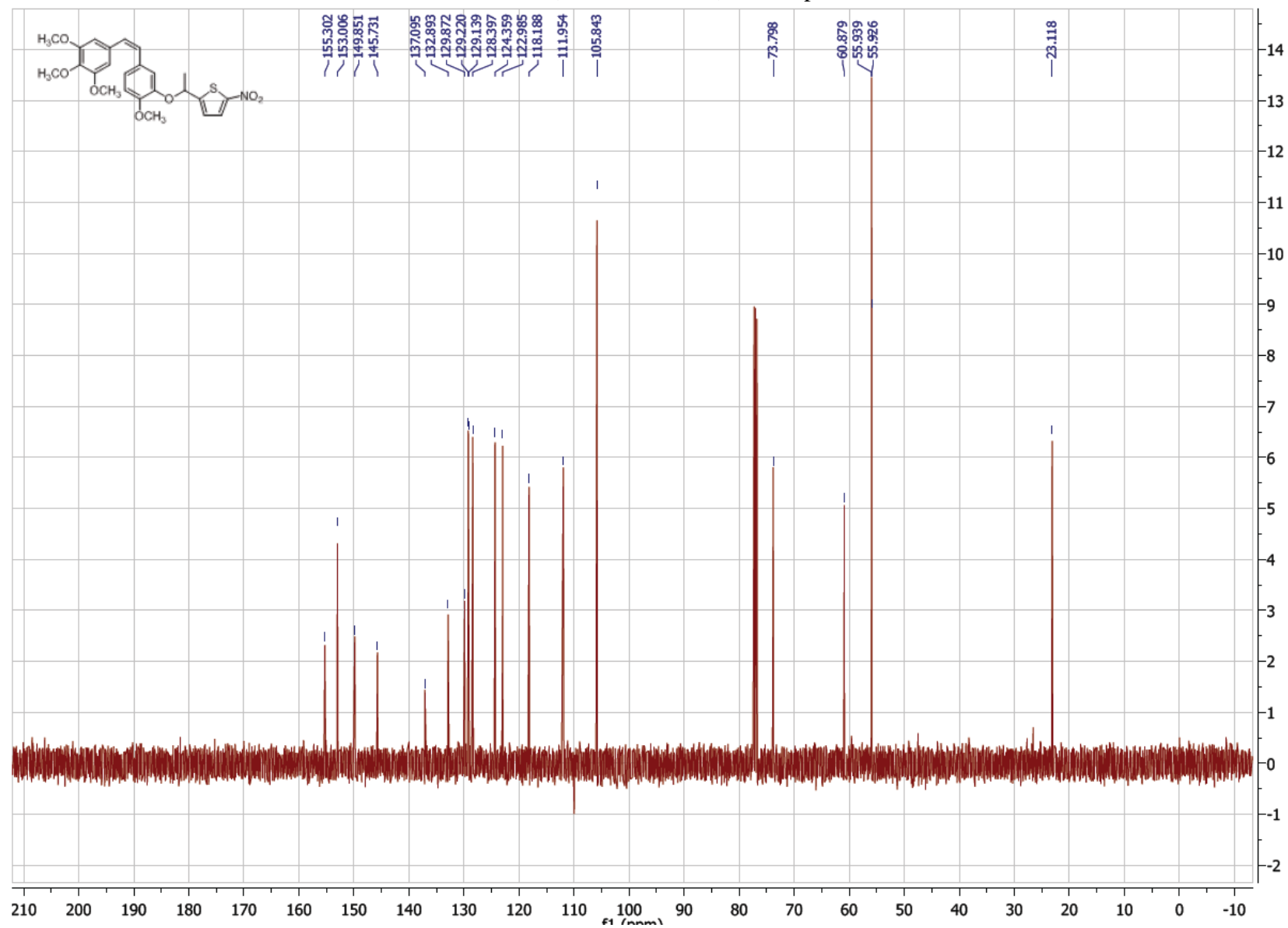
HPLC trace of compound 44

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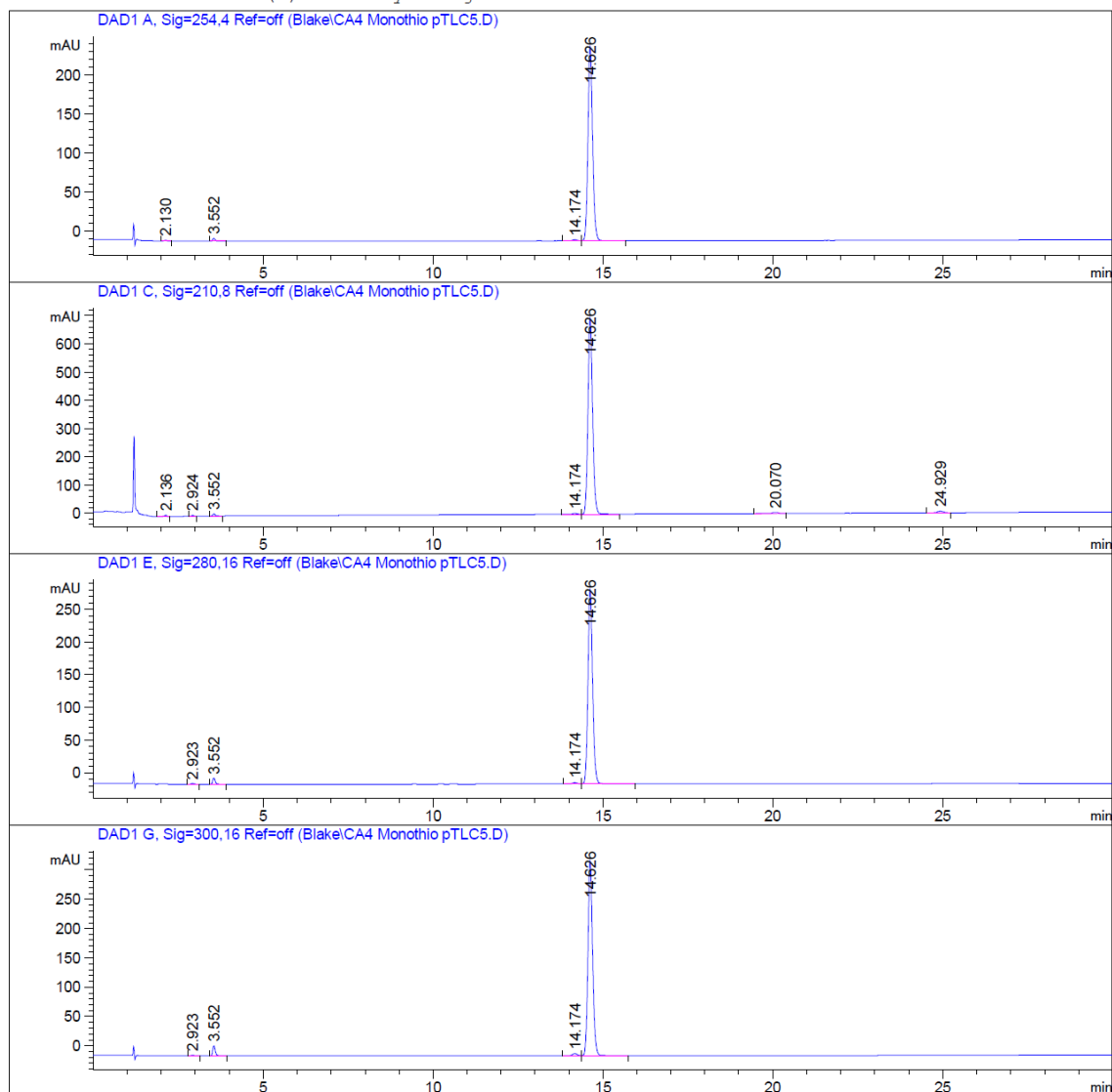

```
=====================================================================
                        Area Percent Report
=====================================================================

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 Type  Width     Area      Height    Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   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 Type  Width     Area      Height    Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   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 Type Width   Area   Height   Area
     #   [min]   [min]   [mAU*s]   [mAU]   %
    ----|-------|----|-------|----------|----------|--------|
       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 Type Width   Area   Height   Area
     #   [min]   [min]   [mAU*s]   [mAU]   %
    ----|-------|----|-------|----------|----------|--------|
       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 Type Width   Area   Height   Area
     #   [min]   [min]   [mAU*s]   [mAU]   %
    ----|-------|----|-------|----------|----------|--------|
       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 ***

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **45**

467

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **45**

161.06
153.21
152.58
143.16
133.12
129.83
129.47
129.41
128.50
125.91
124.17
122.40
112.07
105.93
80.05
77.41
77.16
76.91
61.03
56.08
55.93
28.88
-0.15

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm
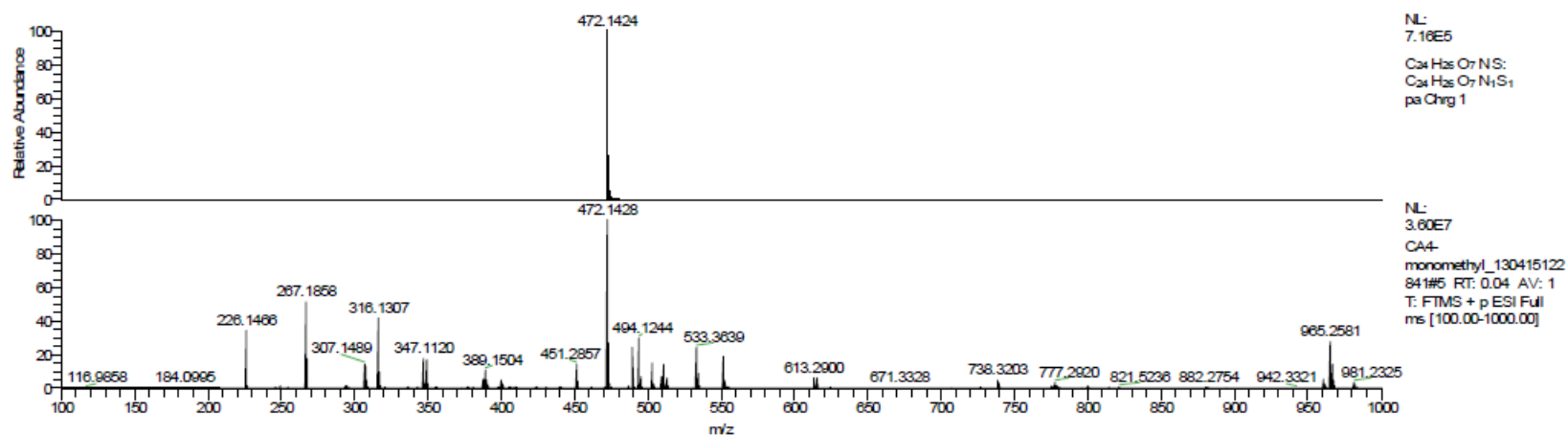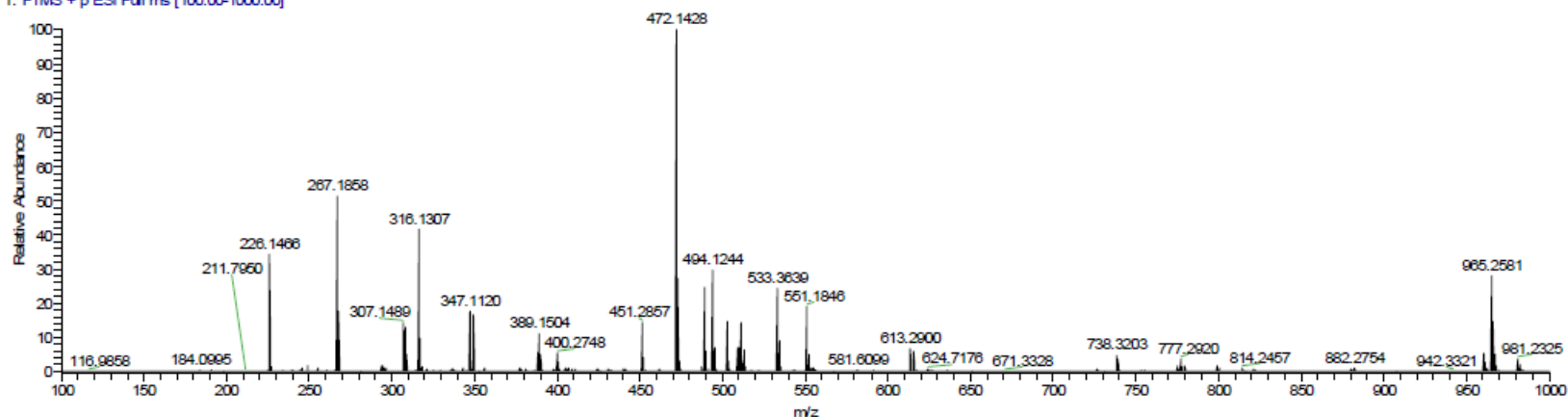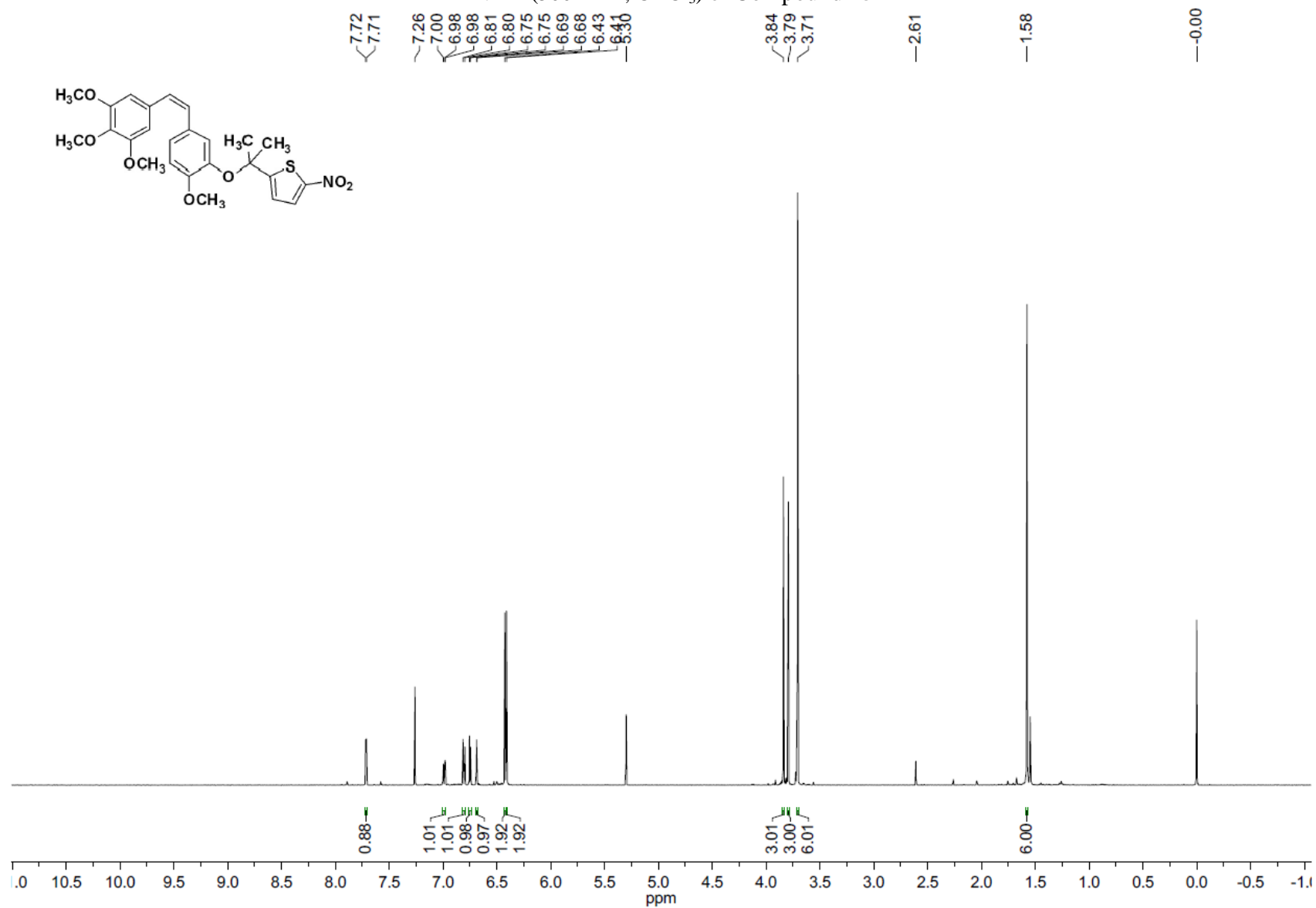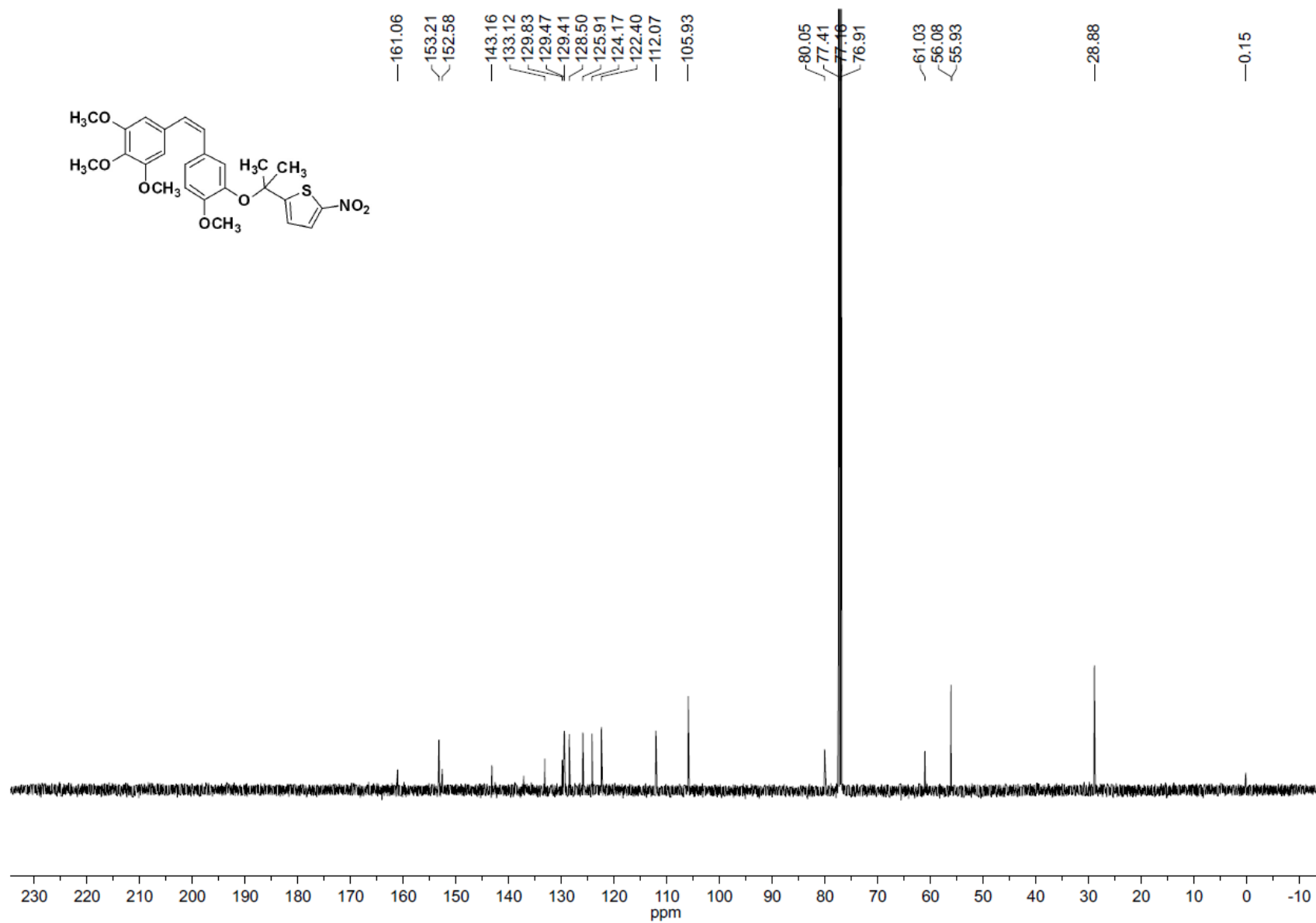
HPLC trace of Compound **45**

Data File C:\CHEM32\1\DATA\MATT MAC\MTM-V-30000028.D
Sample Name: run1

```
    ==================================================================
    Acq. Operator   : Matt Mac
    Acq. Instrument : Instrument 1              Location :    -
    Injection Date  : 10/2/2012 11:02:47 AM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 10/2/2012 10:46:48 AM by Matt Mac
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\DATA\MATT MAC\MTM-V-30000028.D\DA.M (MASTERMETHOD.M)
    Last changed    : 10/2/2012 12:02:27 PM by Matt Mac
    Sample Info     :
```
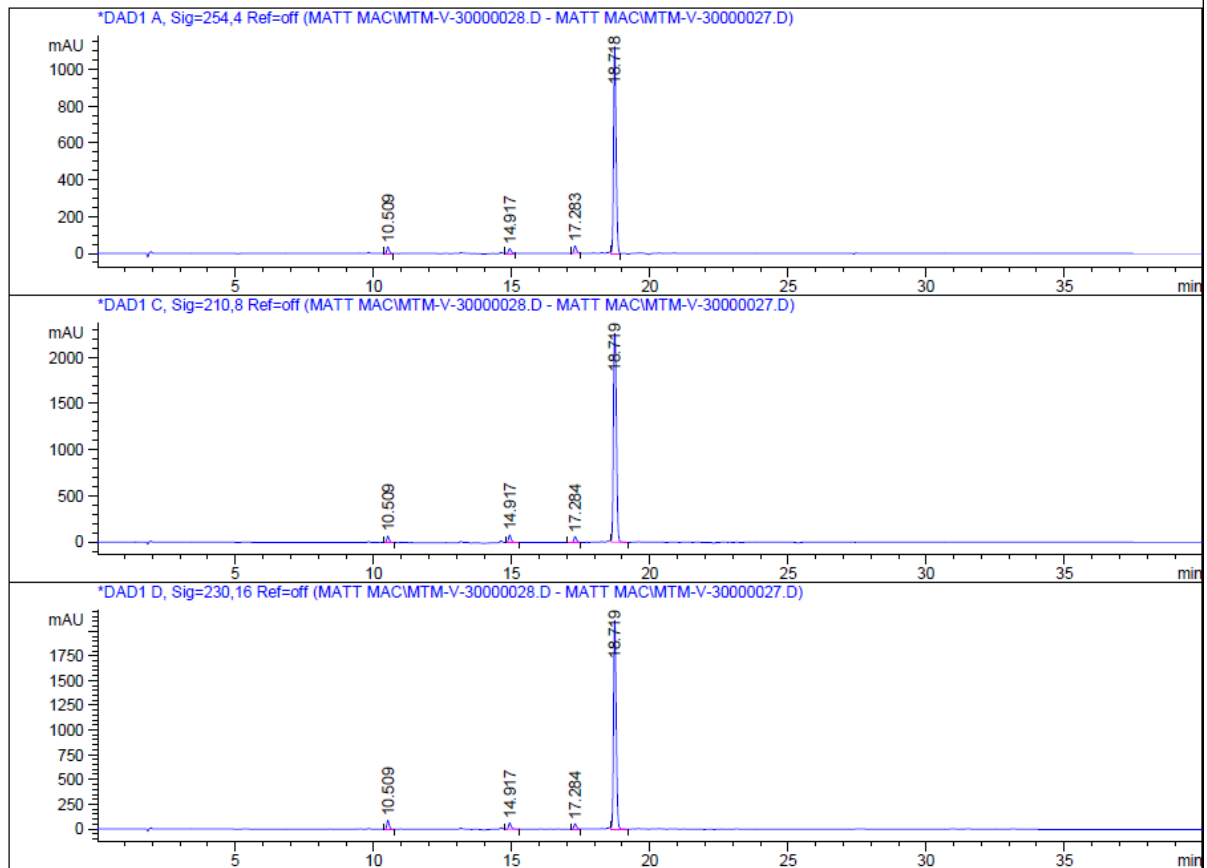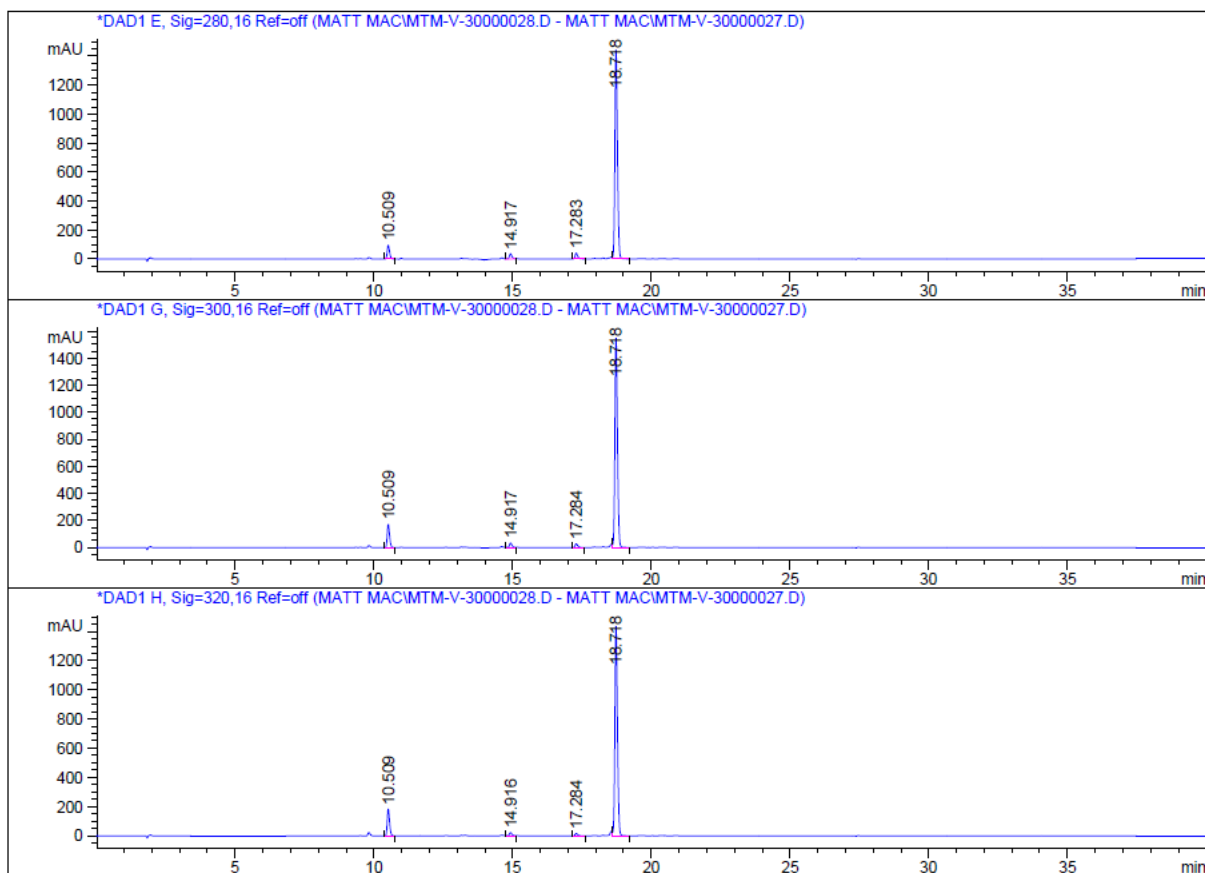
469

```
=================================================================
                     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  10.509 BB    0.0903  206.53261    35.27470    2.6634
   2  14.917 BV    0.0978  166.15672    25.56134    2.1427
   3  17.283 BB    0.0969  259.32324    40.41414    3.3441
   4  18.718 VB    0.0977 7122.55566  1127.88989   91.8498
```

Data File C:\CHEM32\1\DATA\MATT MAC\MTM-V-30000028.D
Sample Name: run1

```
  Peak RetTime Type  Width     Area       Height     Area
   #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
  Totals :                 7754.56824 1229.14007
```

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  10.509 BB   0.0902  406.20374   69.44135   2.1907
   2  14.917 VB   0.1015  576.01318   84.57472   3.1066
   3  17.284 BB   0.0972  398.39267   61.78656   2.1486
   4  18.719 VB   0.1204 1.71613e4  2265.77734  92.5541

  Totals :                 1.85419e4  2481.57997
```

Signal 3: DAD1 D, Sig=230,16 Ref=off
 Signal has been modified after loading from rawdata file!

```
  Peak RetTime Type  Width     Area       Height     Area
   #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  10.509 BB   0.0903  520.65814   88.93446   3.3712
   2  14.917 VV   0.1123  508.84177   65.76926   3.2947
   3  17.284 BB   0.0968  331.46124   51.71173   2.1462
   4  18.719 VB   0.1037 1.40835e4  2114.41040  91.1880

  Totals :                 1.54444e4  2320.82585
```

Signal 4: DAD1 E, Sig=280,16 Ref=off
 Signal has been modified after loading from rawdata file!

```
  Peak RetTime Type  Width     Area       Height     Area
   #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  10.509 BB   0.0903  548.82764   93.68990   5.3169
   2  14.917 VV   0.1077  271.90161   37.01789   2.6341
   3  17.283 BB   0.0981  258.45758   39.62364   2.5039
   4  18.718 VB   0.0984 9243.20996 1448.80347  89.5452

  Totals :                 1.03224e4  1619.13491
```

Signal 5: 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	10.509	BB	0.0903	1010.28589	172.45679	8.9110
2	14.917	BV	0.0966	208.55519	32.61239	1.8395
3	17.284	BB	0.0985	178.25317	27.18596	1.5722
4	18.718	VB	0.0987	9940.42480	1551.60608	87.6772

Totals :   1.13375e4   1783.86122

Signal 6: 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	10.509	BB	0.0903	1076.80786	183.83899	10.2507
2	14.916	BV	0.0991	138.73483	21.00286	1.3207
3	17.284	BB	0.0993	104.35675	15.75329	0.9934
4	18.718	VB	0.0984	9184.83105	1439.67126	87.4352

Totals :   1.05047e4   1660.26641

==
*** End of Report ***

X-Ray Crystallography Data for Compound **45**
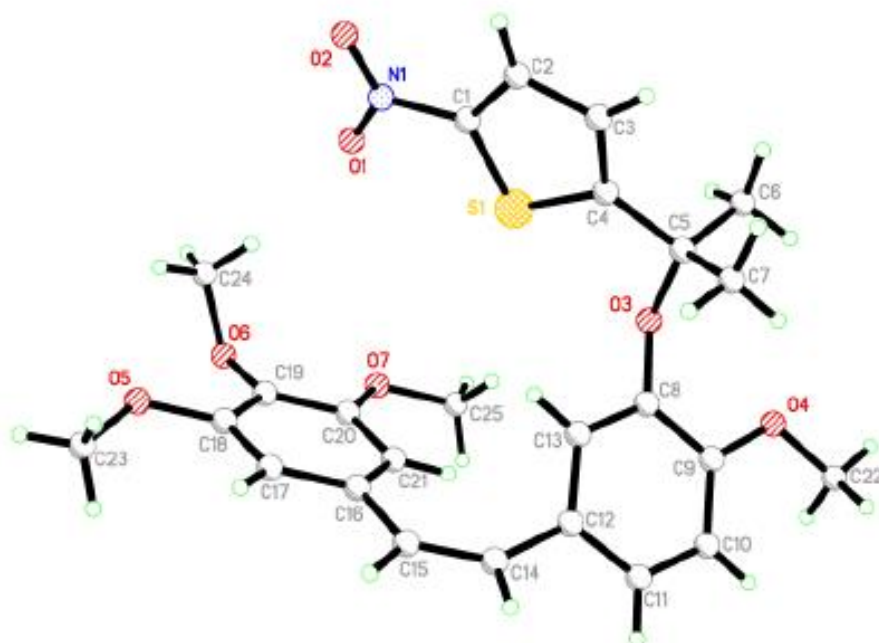
Table 1. Crystal data and structure refinement for kp61.

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 2. 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 3.   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)

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

	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**

MTM-V-30_130210195851 #9   RT: 0.07   AV: 1   NL: 1.29E7
T: FTMS + p ESI Full ms [200.00-2000.00]

NL:
7.08E5

C25 H27 O7 N Na S:
C25 H27 O7 N1 Na1 S1
pa Chrg 1

NL:
1.29E7

MTM-V-
30_130210195851#9
RT: 0.07 AV: 1 T:
FTMS + p ESI Full
ms [200.00-2000.00]
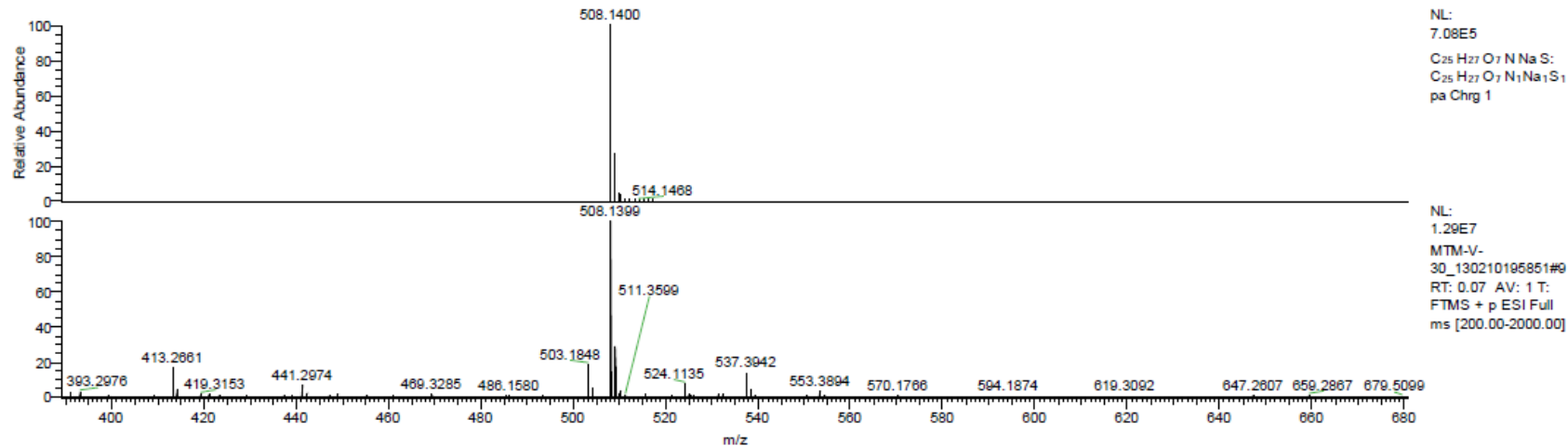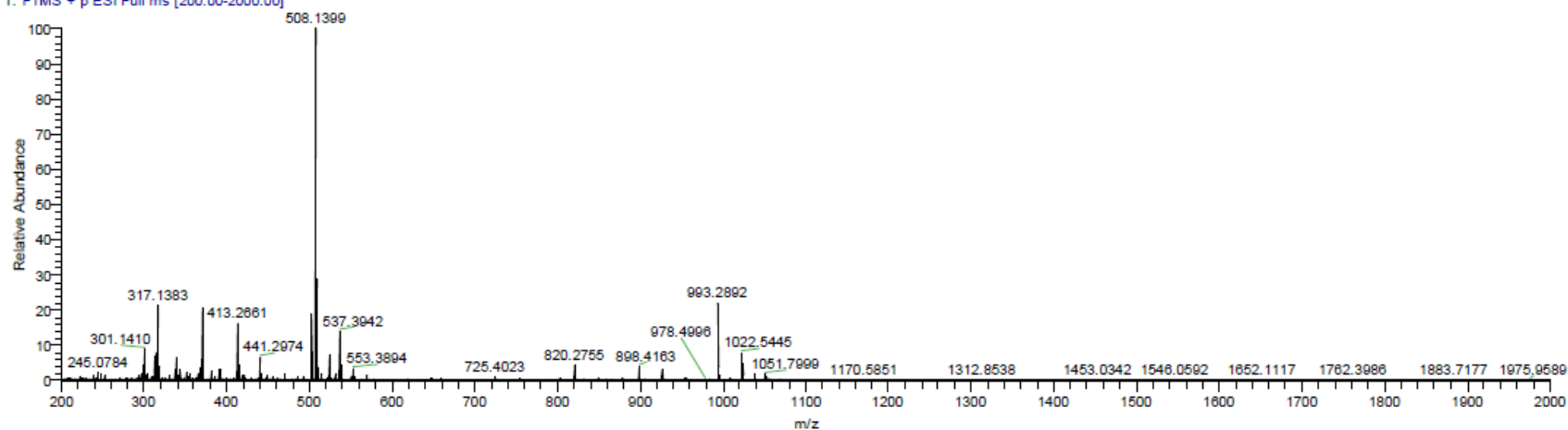
NADPH Cytochrome P450 Oxidoreductase Cleavage Assay

HPLC Conditions:

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

Chromatogram of 100 μM CA-4

CA-4
4.995 min

Chromatogram of 100 μM CA-1

CA-1
3.111 min

Chromatogram of 100 μM KGP370

KGP370
19.942 min

CA4
4.985 min

Absorbance (mau, 300 nm)

Time (min)
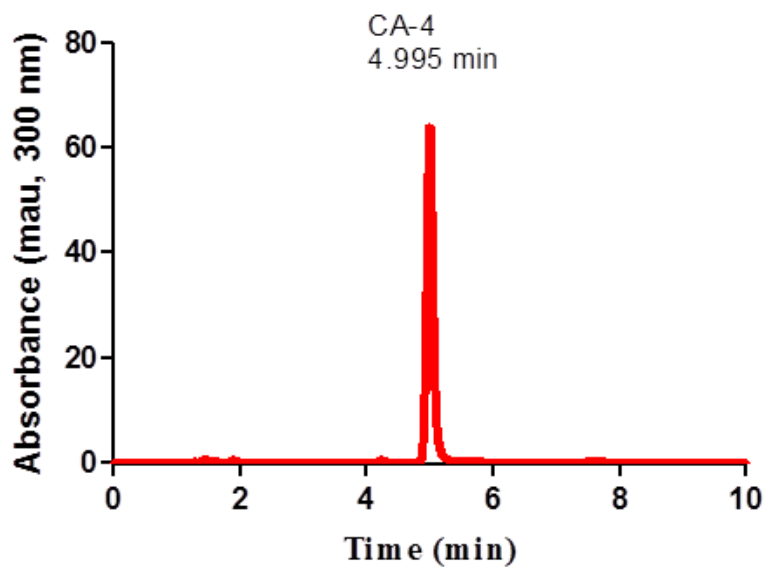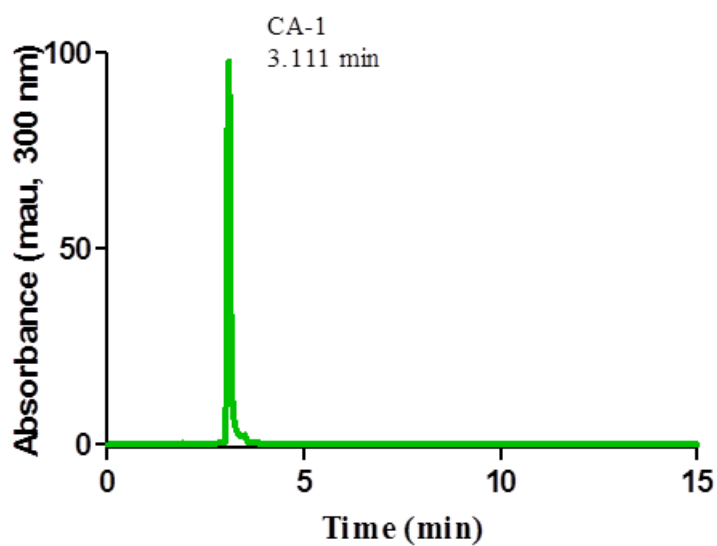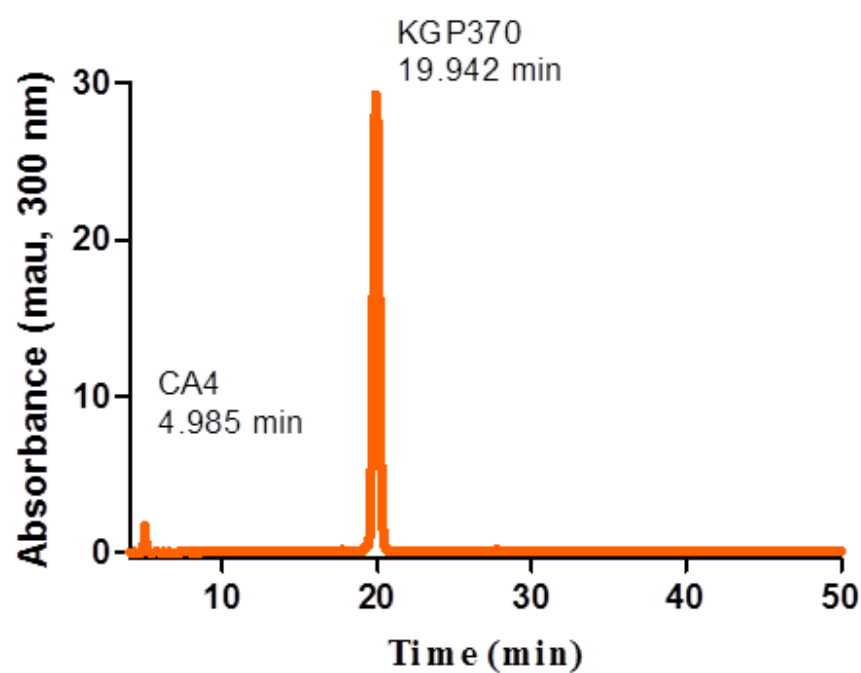
Chromatogram of KGP370 in buffer (+ 0.1% Triton X-100) for 90 min

KGP370
20.024 min

CA4
4.979 min

Absorbance (mau, 300 nm)

Time (min)
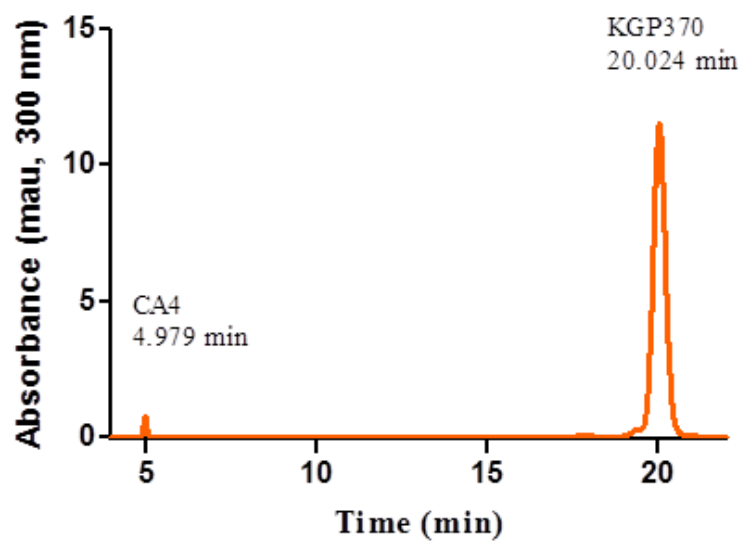
Chromatogram of POR-Treated KGP370 for 90 min

KGP370
20.022 min
A = 279.1

CA4
4.980 min
A = 19

Chromatogram of 100 μM KGP371

KGP371
25.842 min

CA4
4.953 min
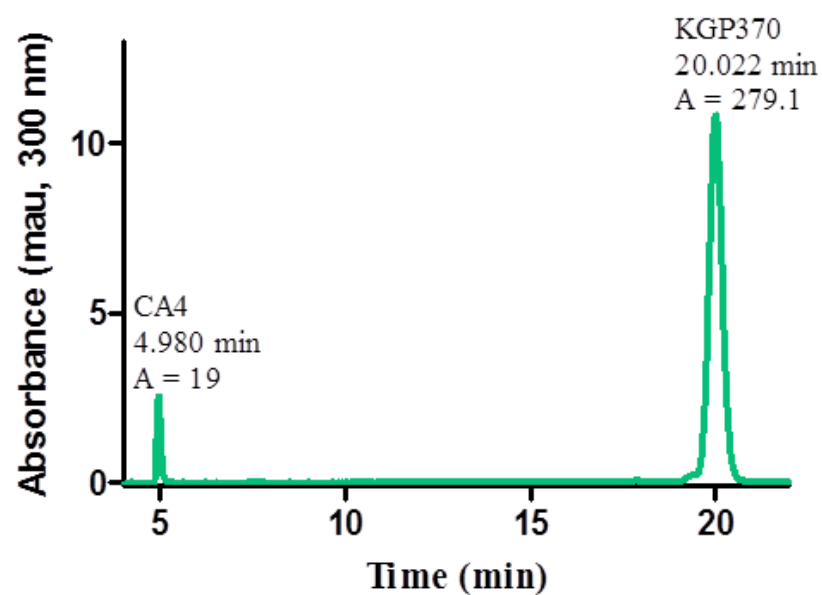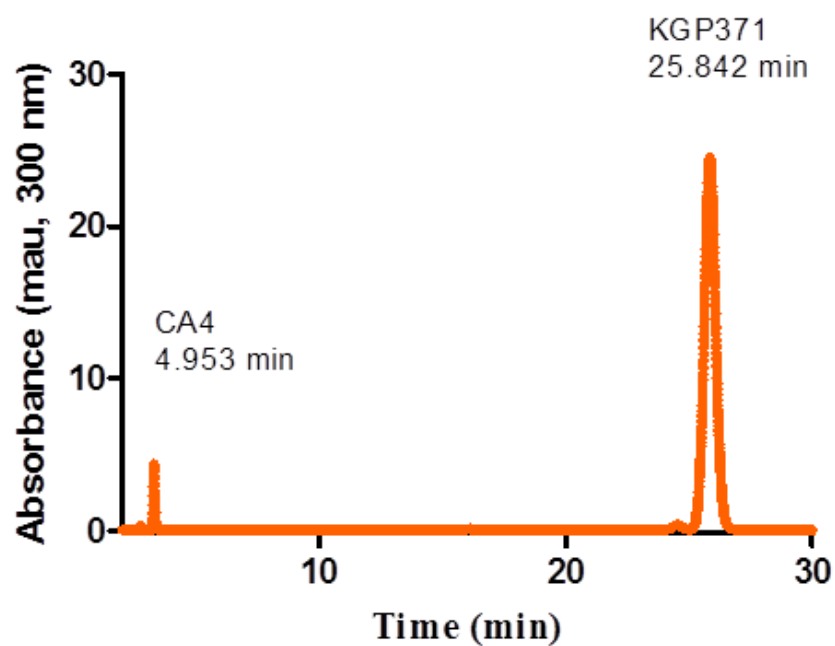
Chromatogram of KGP371 in buffer for 90 min (Control)
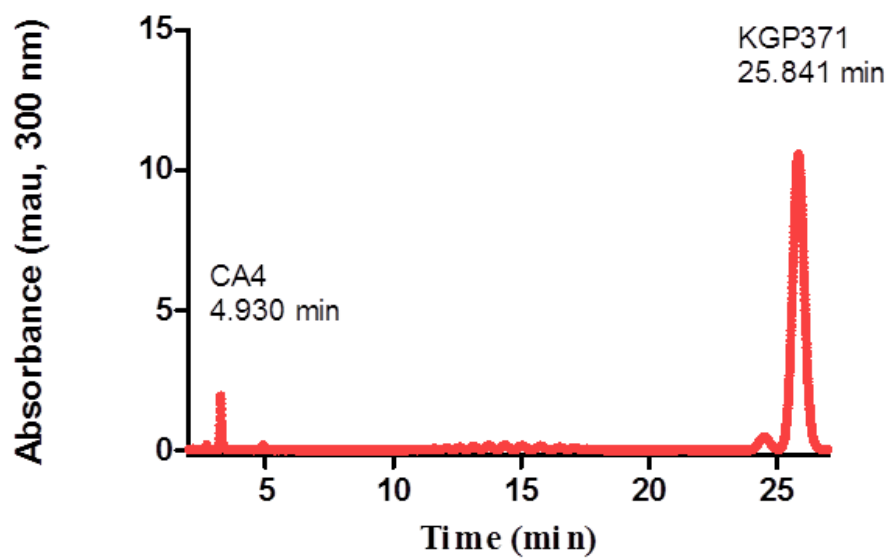
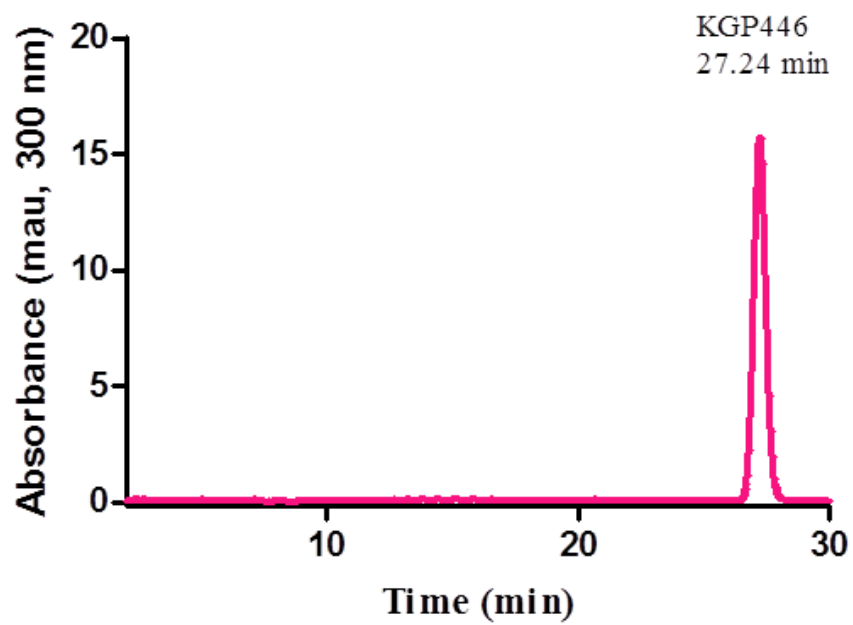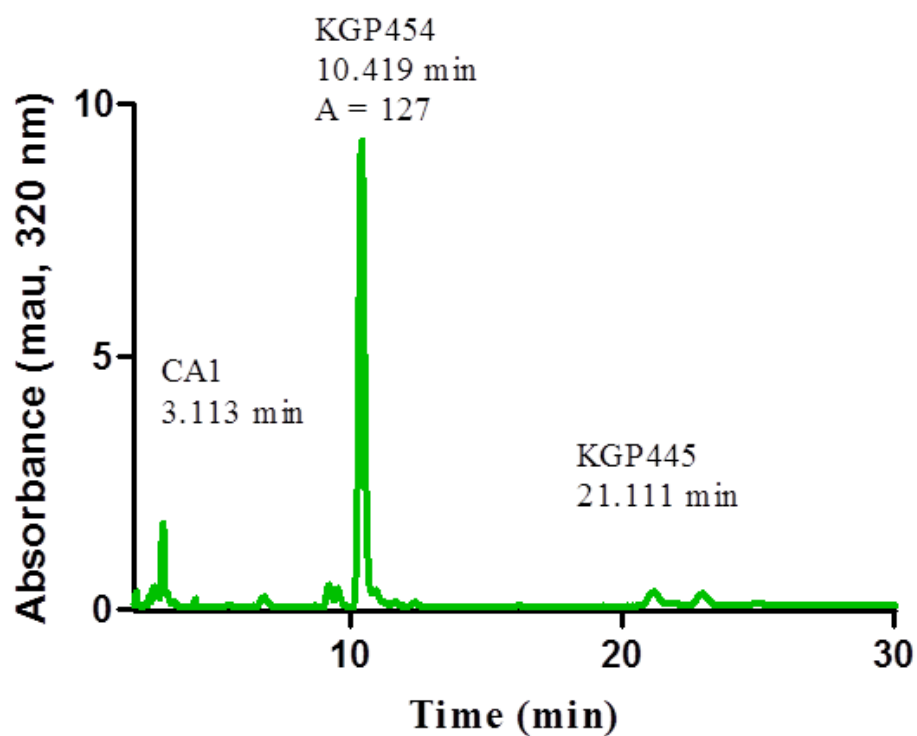## Chromatogram of POR-Treated KGP371 for 90 min
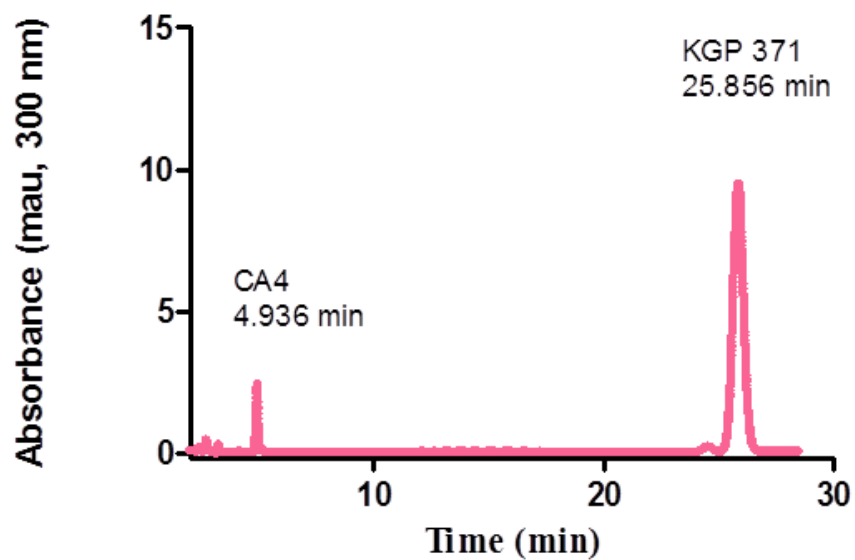
Chromatogram of 100 μM KGP372
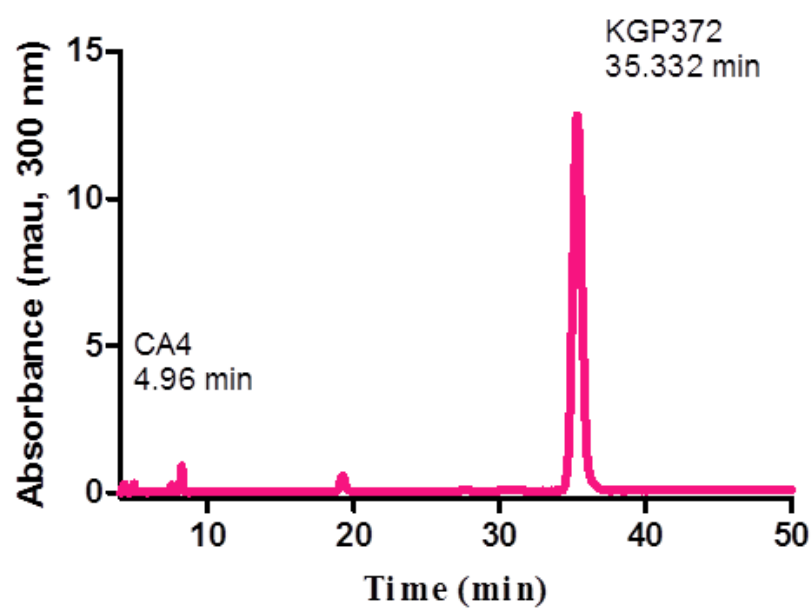
Chromatogram of KGP372 in buffer (+ 0.1% Triton X-100) for 90 min
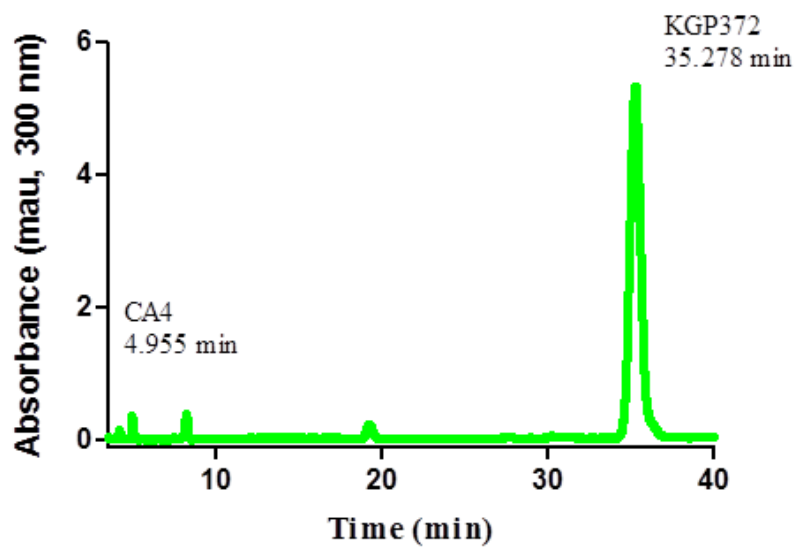
Chromatogram of POR-Treated KGP372 for 90 min

CA4
4.955 min

KGP372
35.214 min
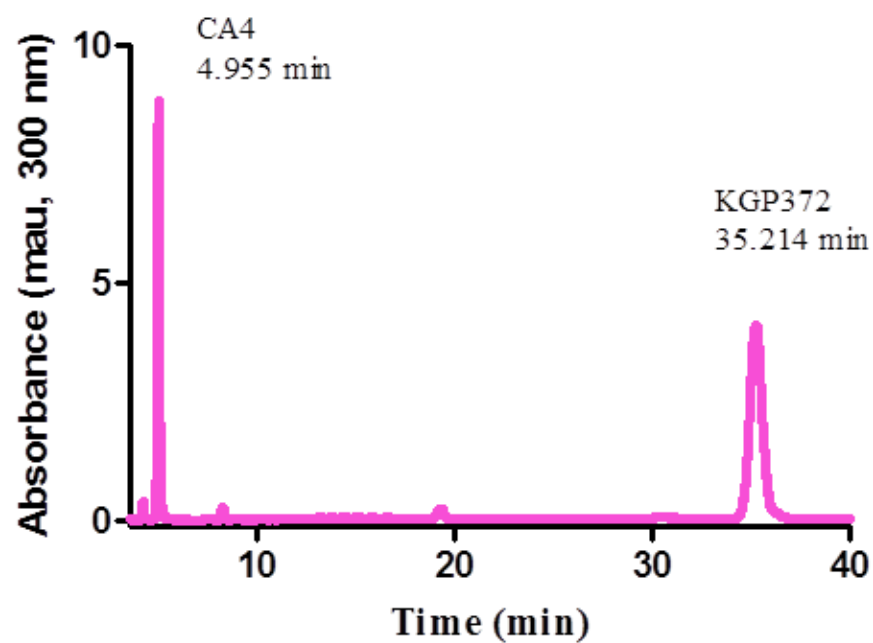
Chromatogram of 100 μM KGP400

KGP400
3.581 min
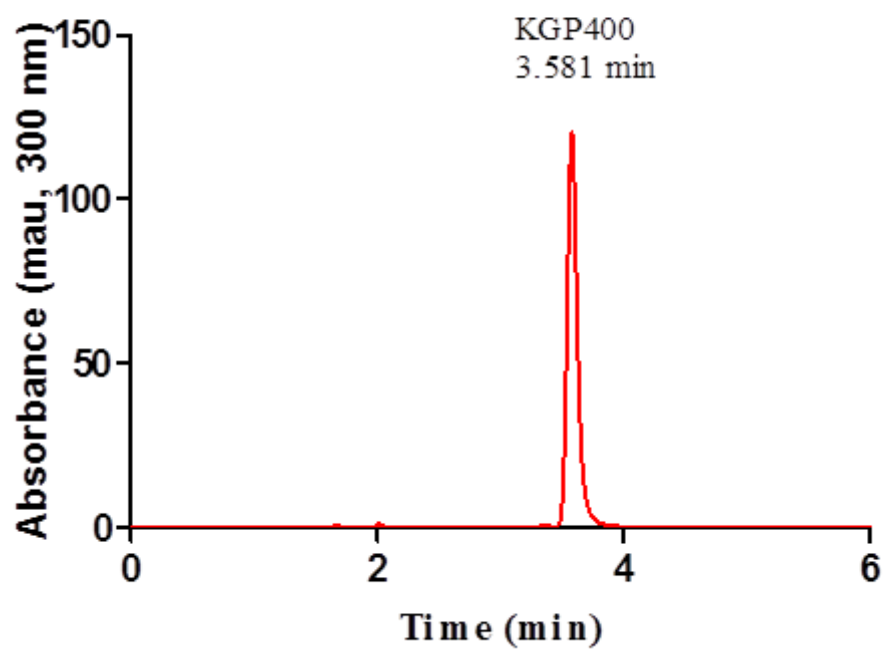
Chromatogram of 100 μM KGP439

Chromatogram of 100 μM KGP440

489
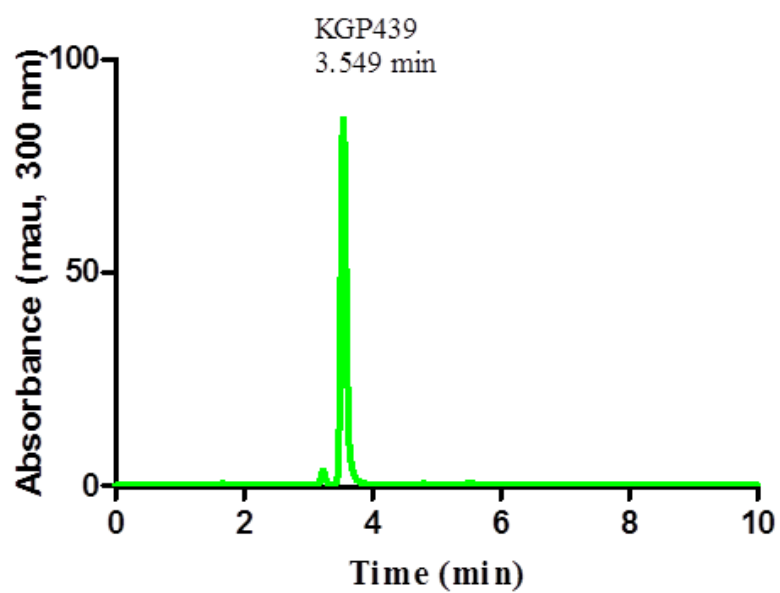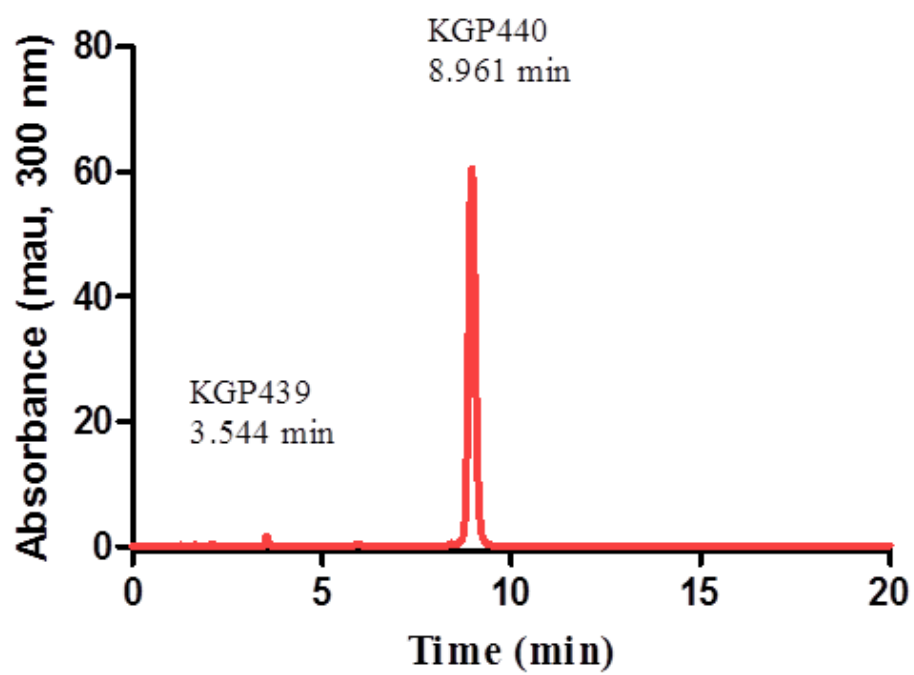
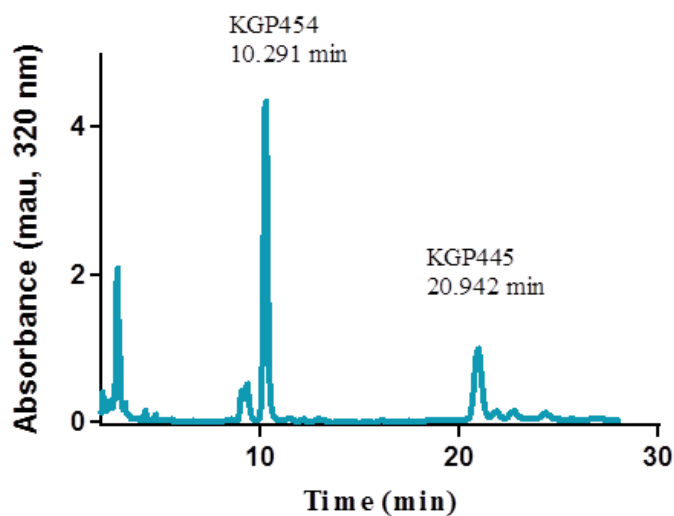**Chromatogram of KGP440 in buffer (approx 50 μM, + 0.1% Triton X-100) for 90 min**
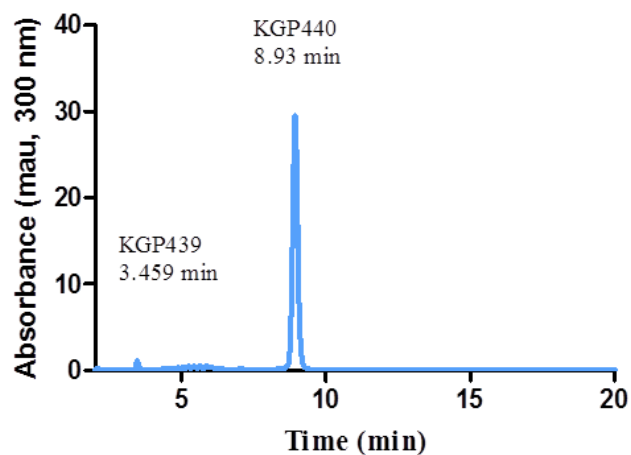
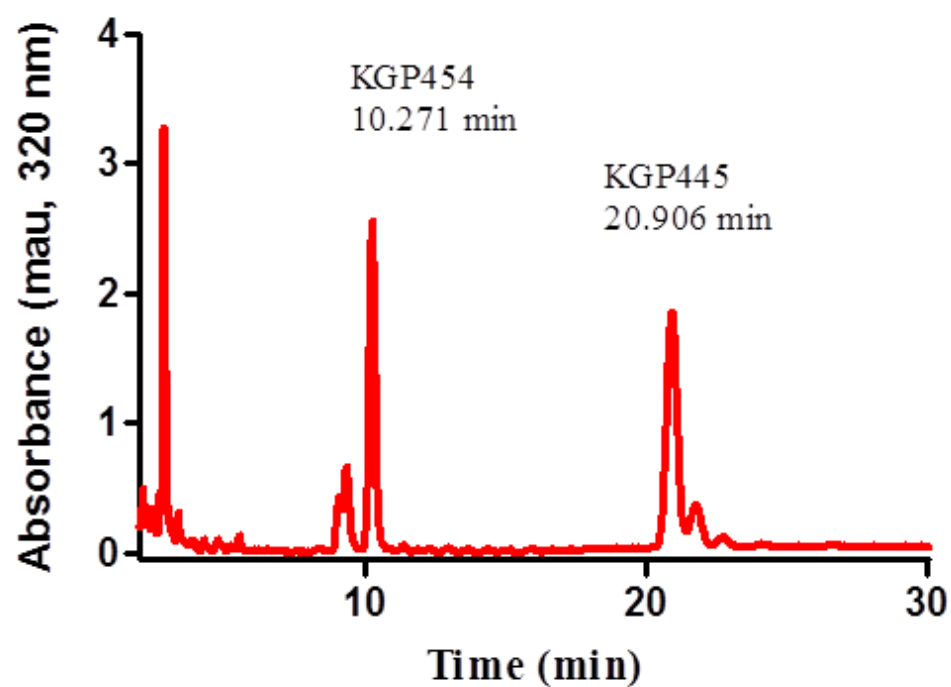**Chromatogram of POR-Treated KGP440 for 90 min**
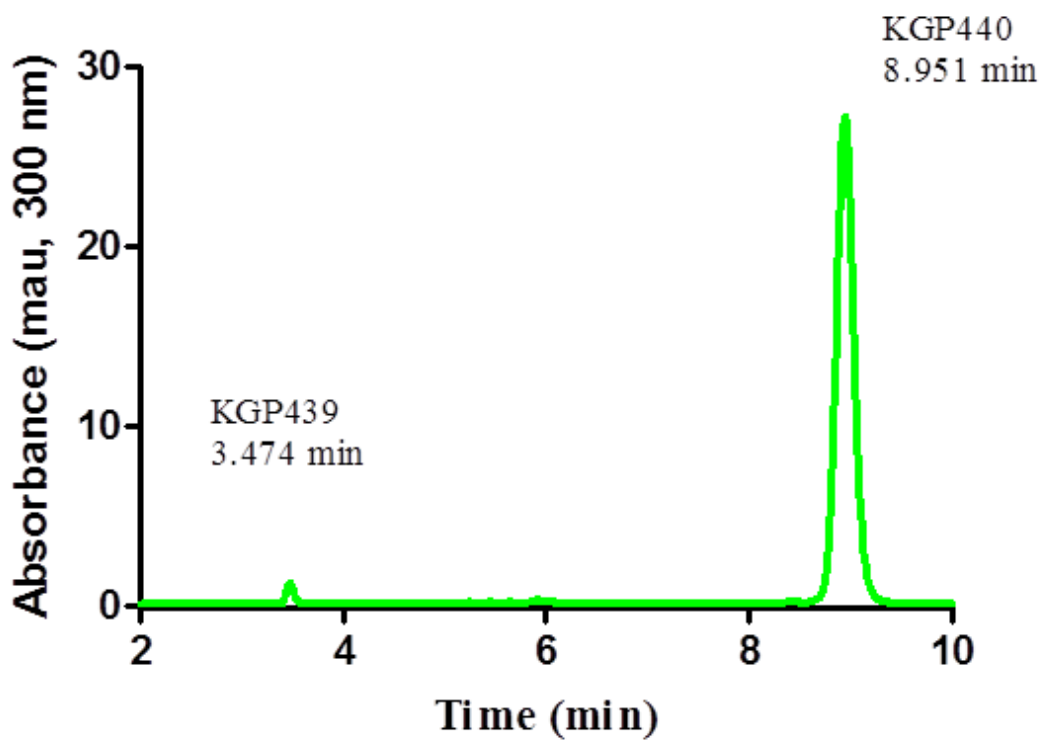
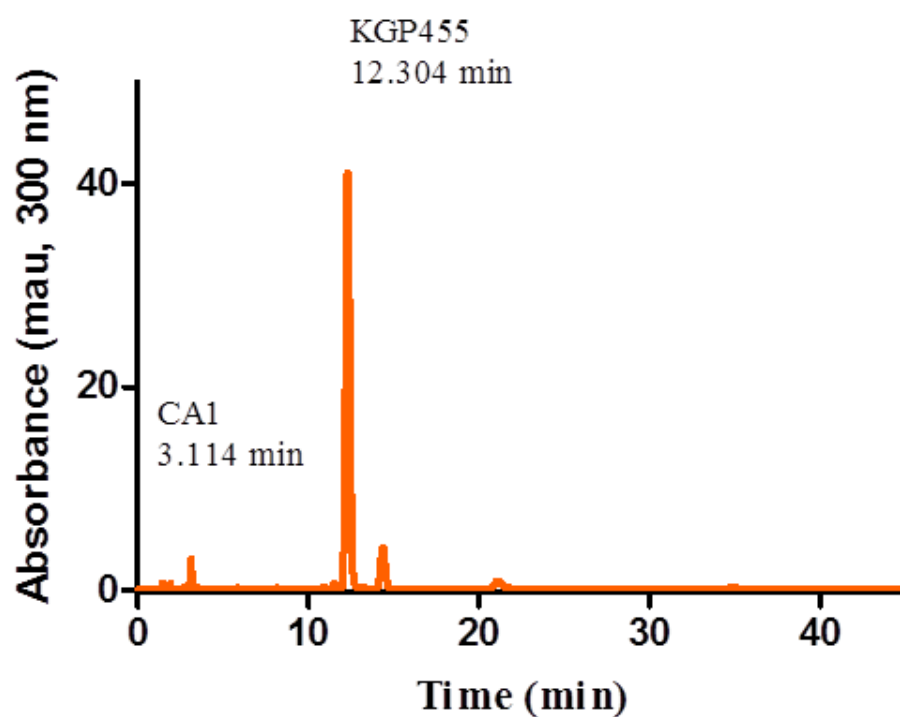**Chromatogram of 100 μM KGP441**
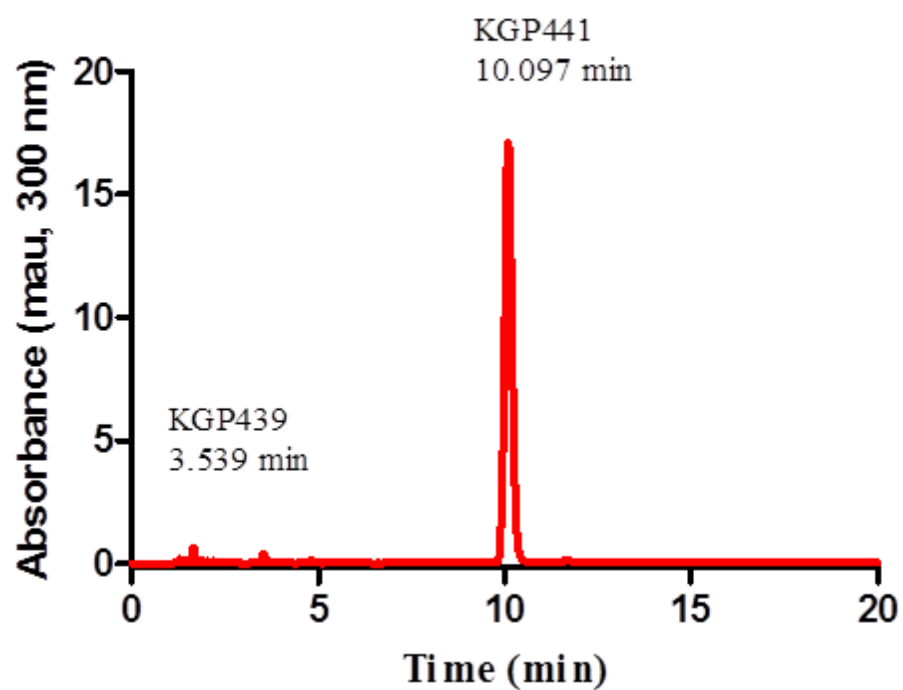
Chromatogram of KGP441 in buffer (approx 50 μM, + 0.1% Triton X-100) for 90 min
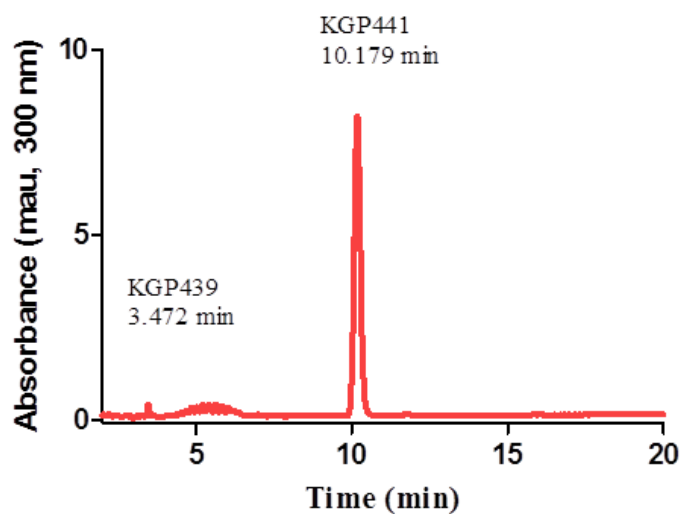
Chromatogram of POR-Treated KGP441 for 90 min
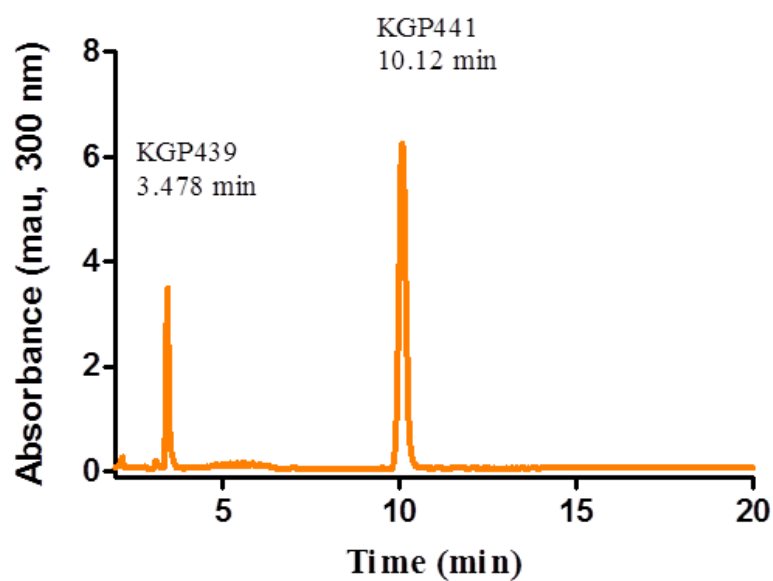
Chromatogram of 100 μM KGP442
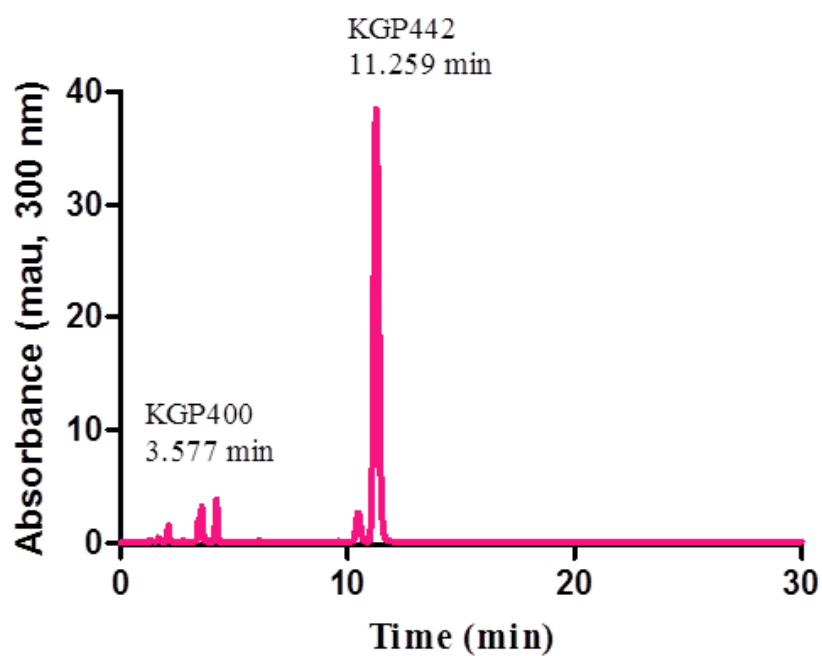
Chromatogram of KGP442 in buffer (+ 0.1% Triton X-100) for 90 min
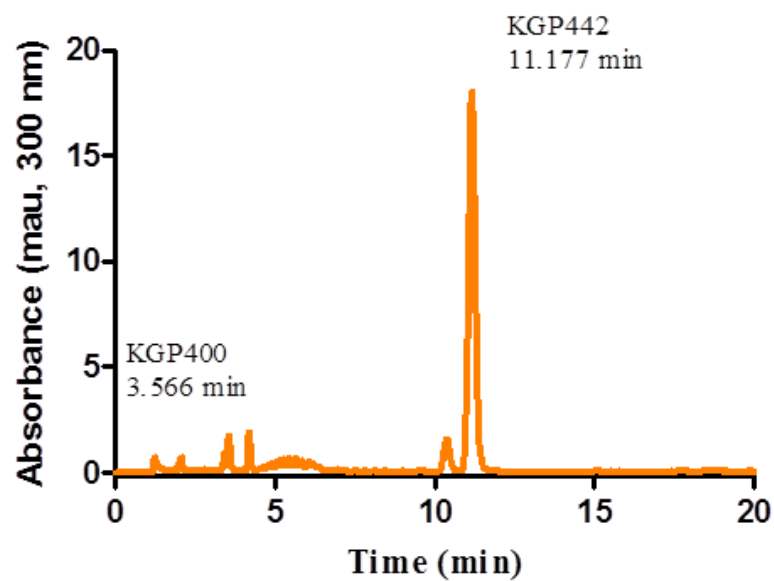
Chromatogram of POR-Treated KGP442 for 90 min
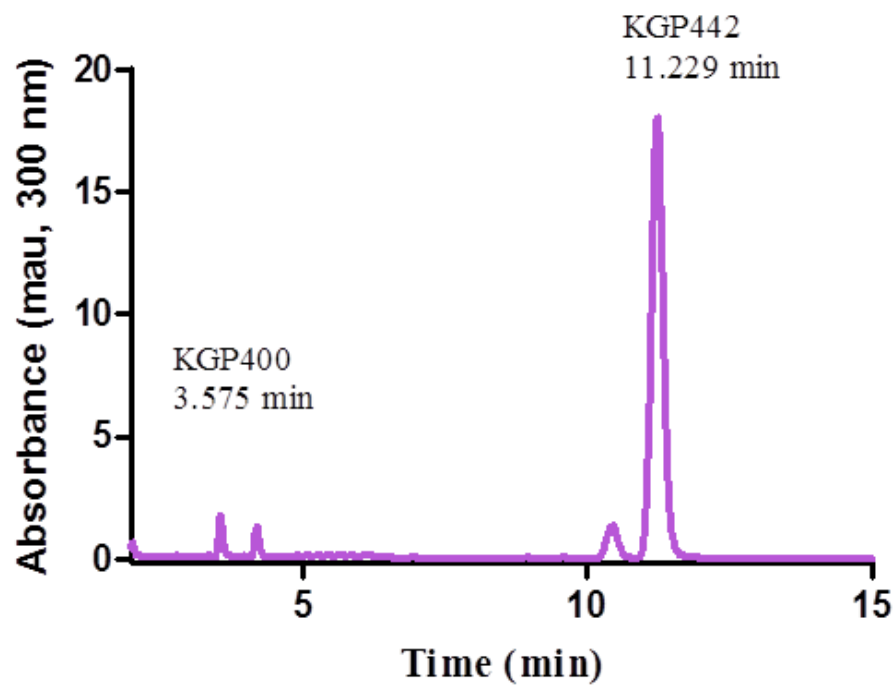
Chromatogram of 100 µM KGP443
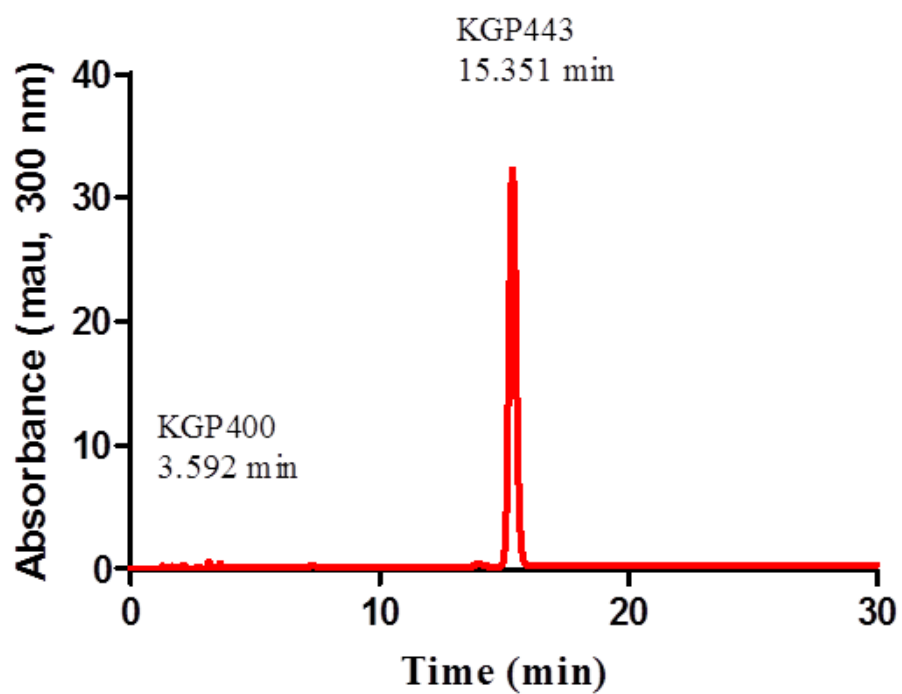
Chromatogram of KGP443 in buffer (+ 0.1% Triton X-100) for 90 min
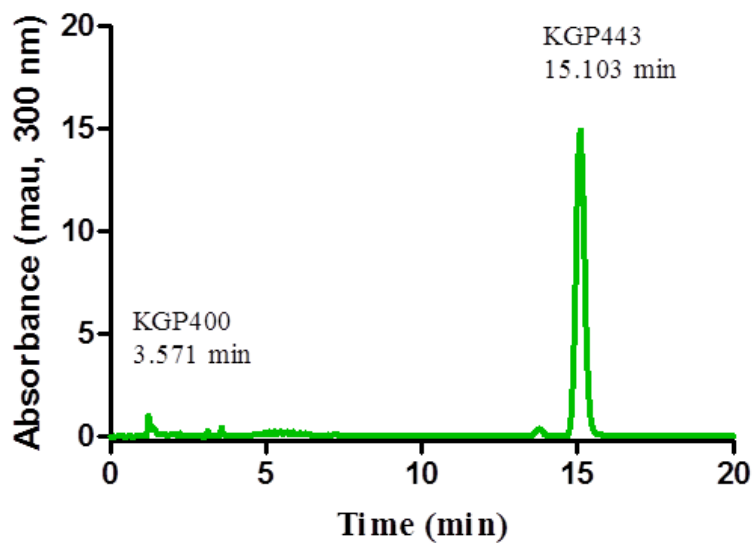
Chromatogram of POR-Treated KGP443 for 90 min
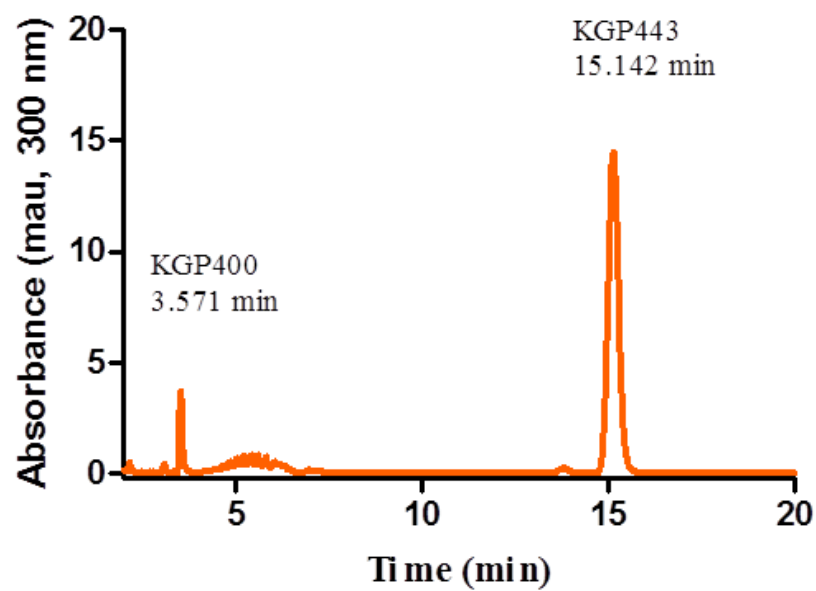
Chromatogram of 100 μM KGP444
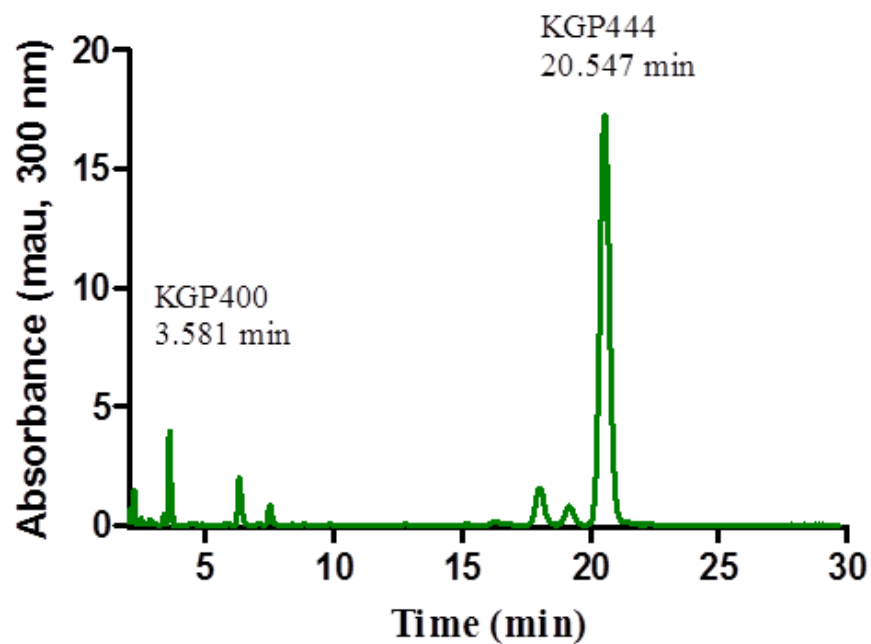
**Chromatogram of KGP444 in buffer (+ 0.1% Triton X-100) for 90 min**
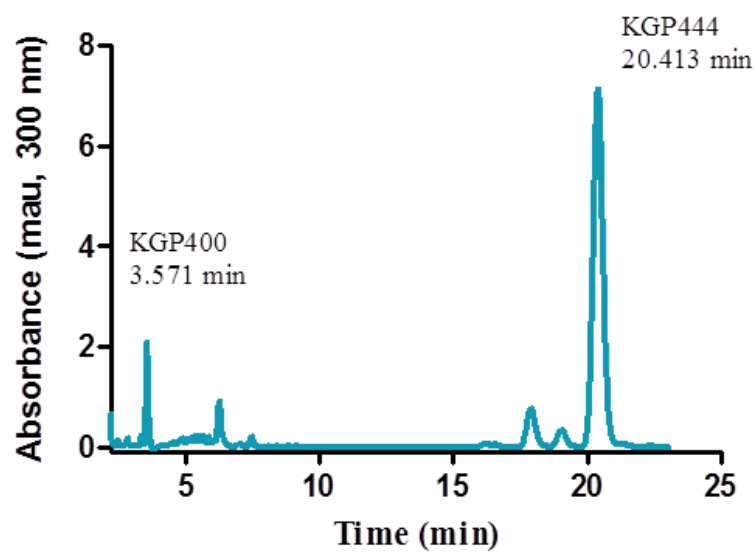
Chromatogram of POR-Treated KGP444 for 90 min

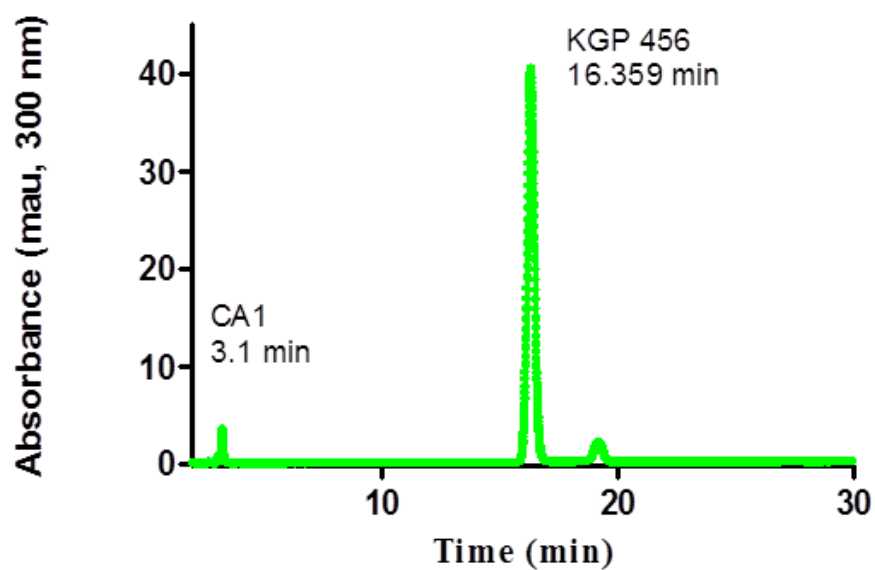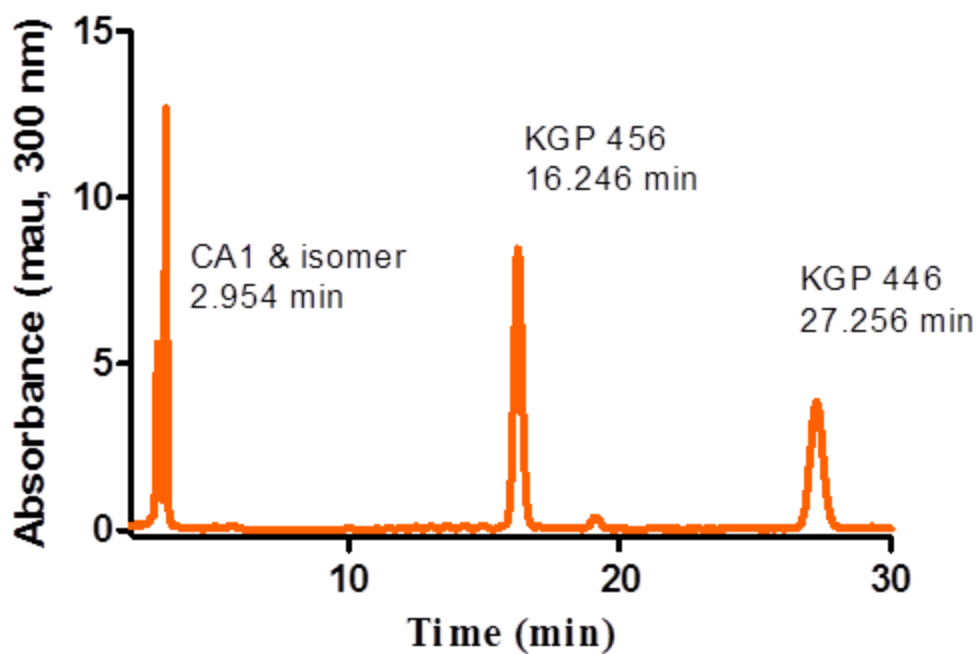# Chromatogram of 100 μM KGP445

KGP445
20.748 min

Absorbance (mau, 300 nm)

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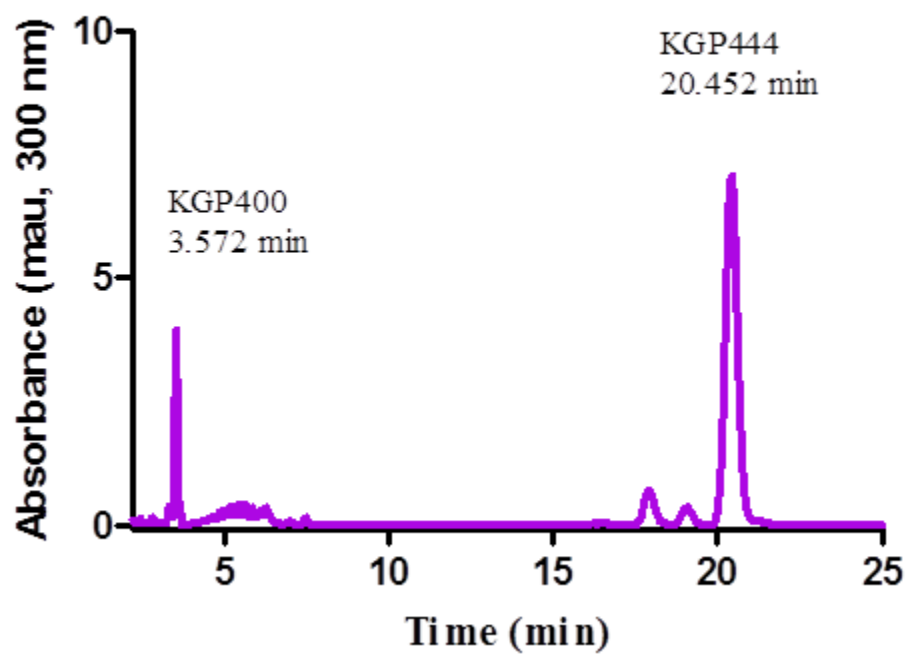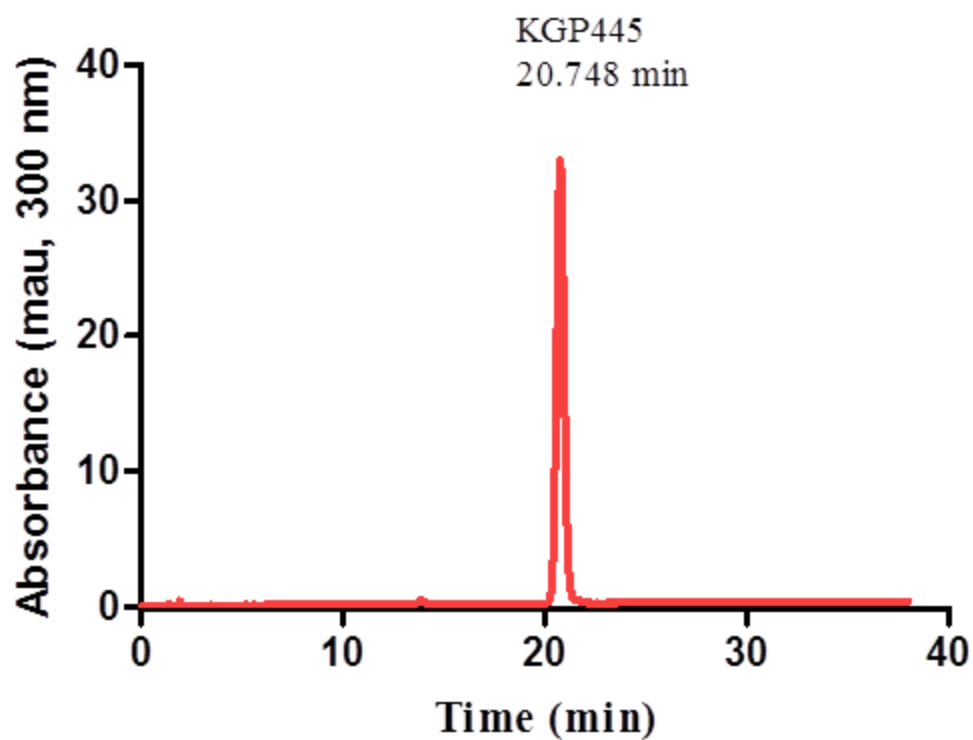
Chromatogram of KGP445 in buffer (+ 0.1% Triton X-100) for 90 min

KGP445
20.641 min

Absorbance (mau, 300 nm)

Time (min)
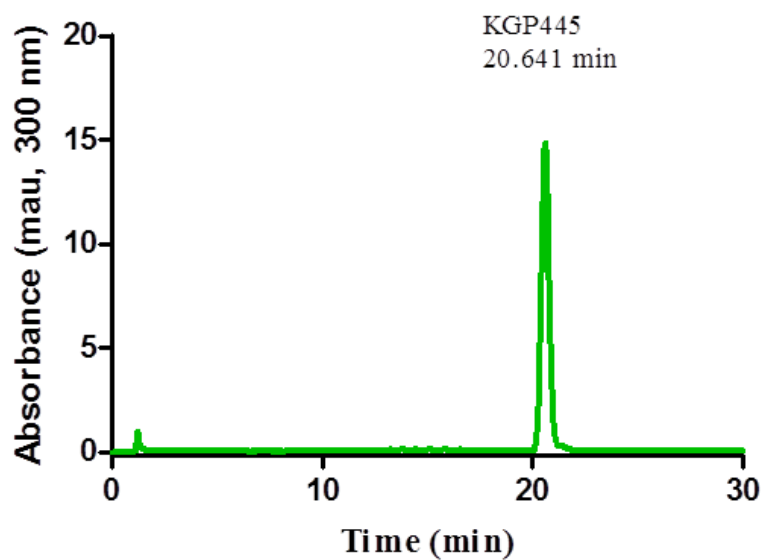
Chromatogram of POR-Treated KGP445 for 90 min

KGP445
20.634 min

Absorbance (mau, 300 nm)

Time (min)

Chromatogram of 100 μM KGP446

KGP446
27.148 min

Absorbance (mau, 300 nm)

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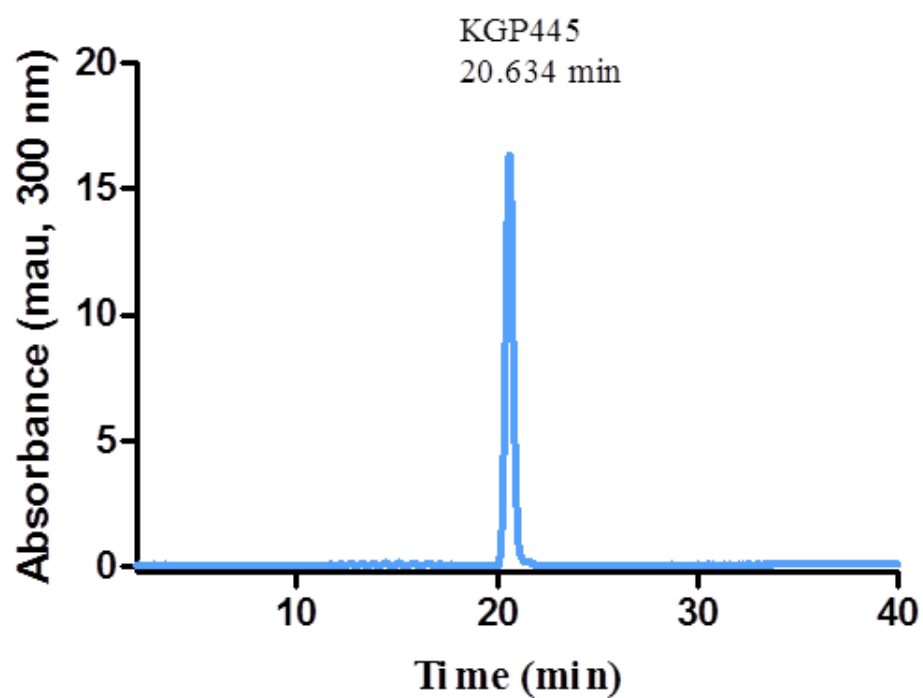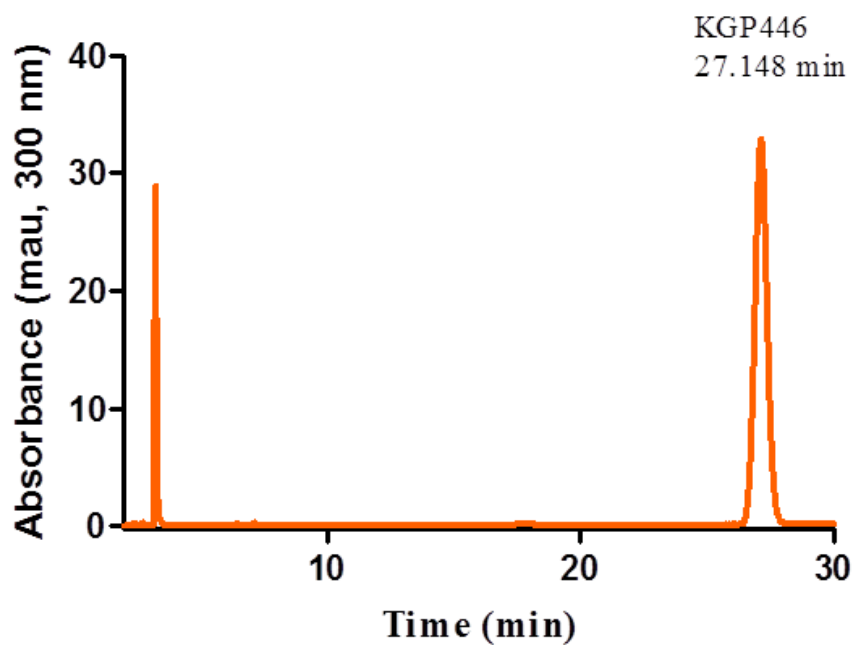
Chromatogram of KGP446 in buffer (+ 0.1% Triton X-100) for 90 min
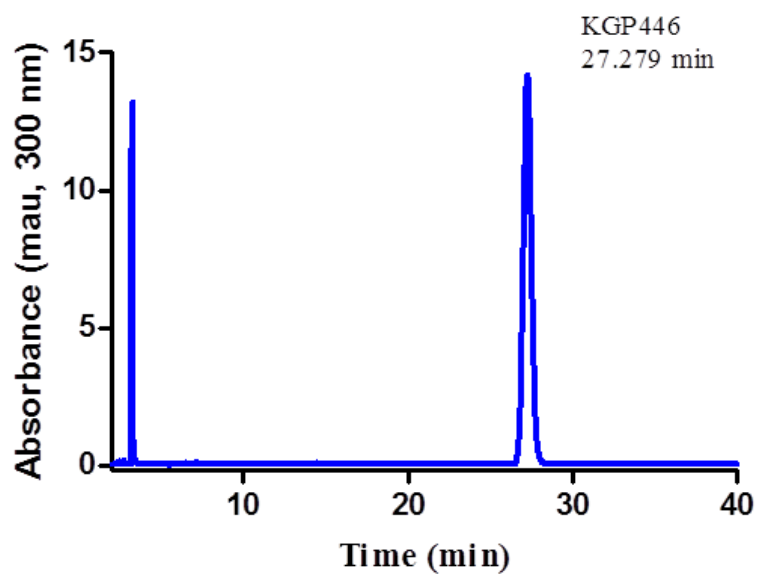
KGP446
27.279 min

Chromatogram of POR-Treated KGP446 for 90 min

KGP446
27.24 min

Chromatogram of 50 μM KGP454

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

Chromatogram of POR-Treated KGP454 for 90 min

KGP454
10.271 min

KGP445
20.906 min

Chromatogram of 100 μM KGP455

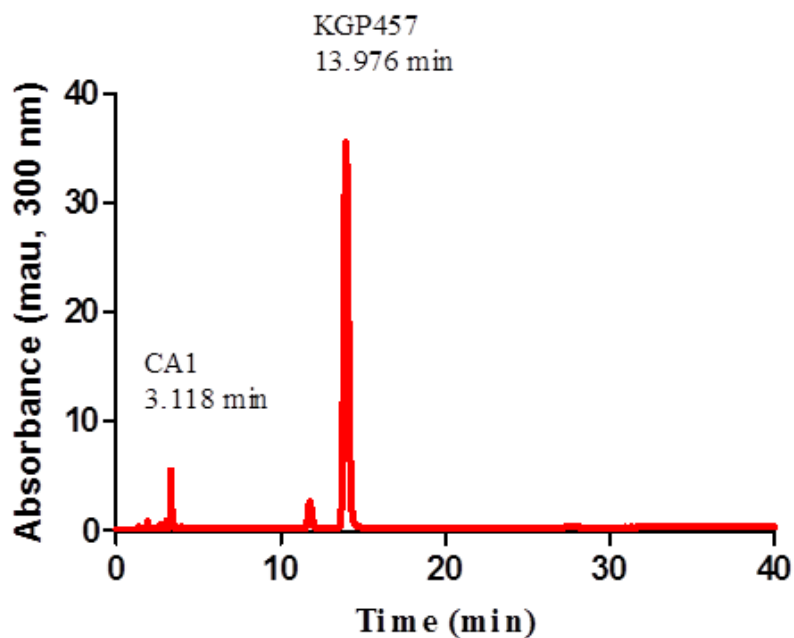# Chromatogram of POR-Treated KGP455 for 90 min

Chromatogram of 100 μM KGP456

KGP 456
16.359 min

CA1
3.1 min
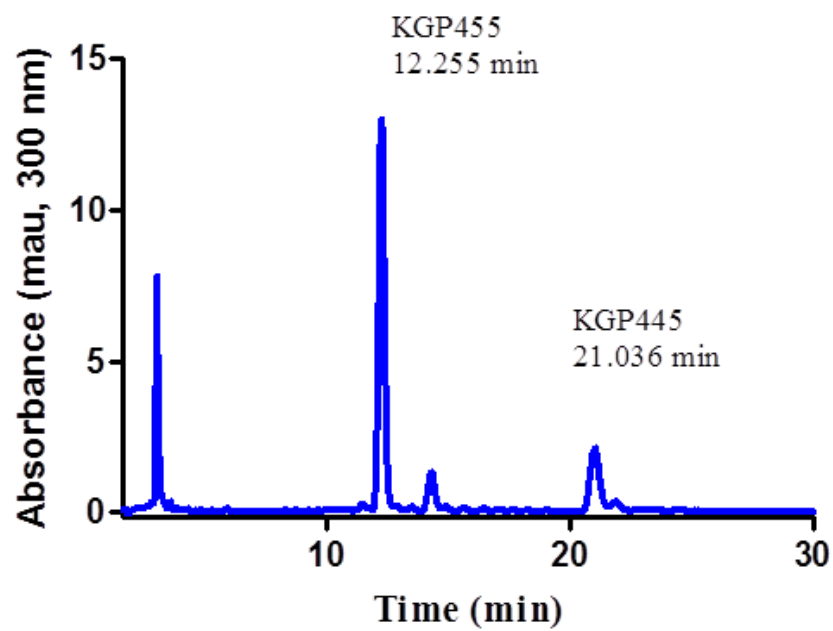
Chromatogram of KGP456 in buffer for 90 min (Control)

CA1 & isomer
2.954 min

KGP 456
16.246 min

KGP 446
27.256 min

Chromatogram of POR-Treated KGP456 for 90 min
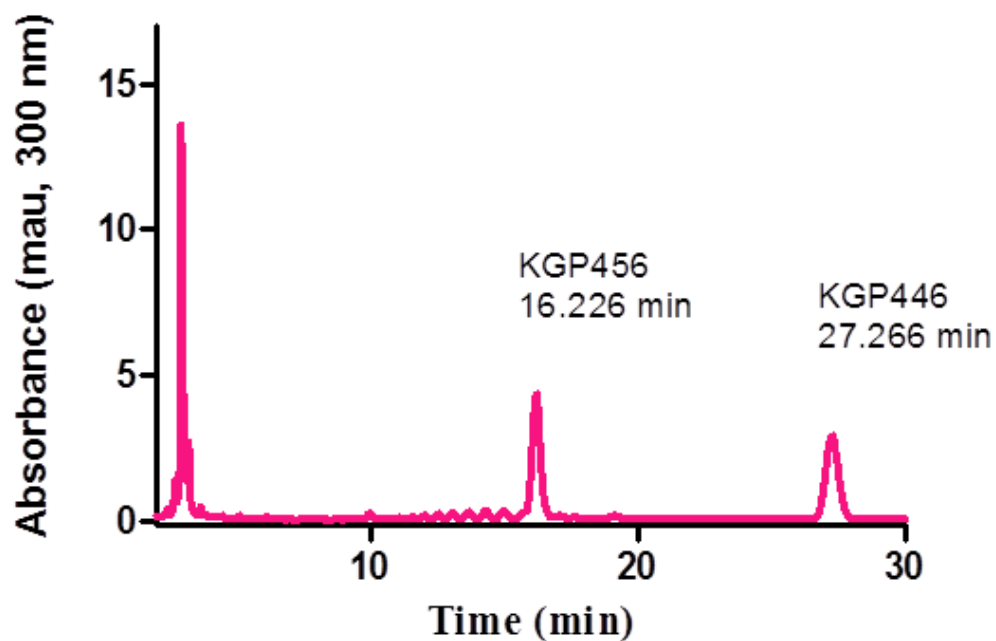
Chromatogram of 100 μM KGP457

Chromatogram of POR-Treated KGP457 for 90 min

KGP457
13.841 min

KGP446
27.463 min

CA1/isomer
2.996 min
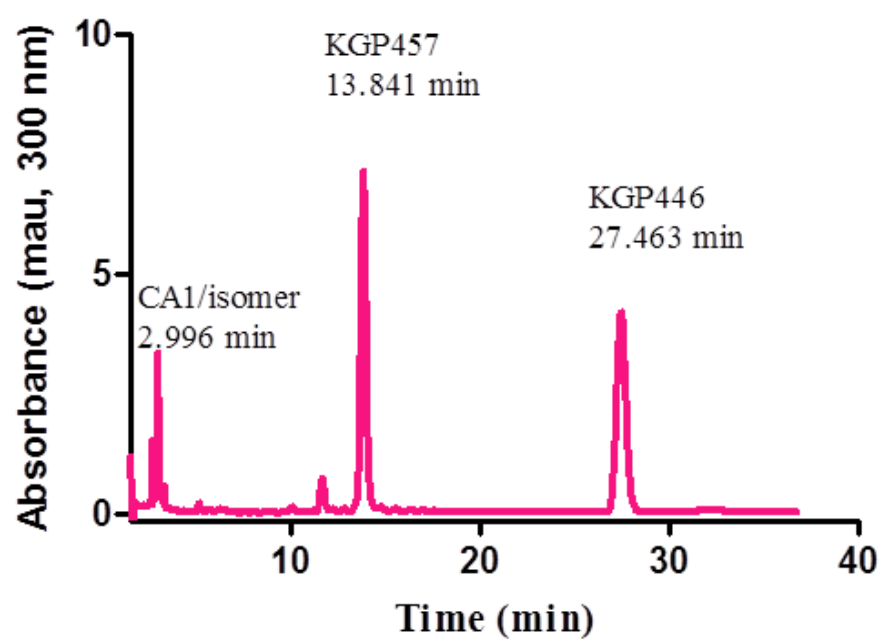
Chromatogram of 50 μM KGP461
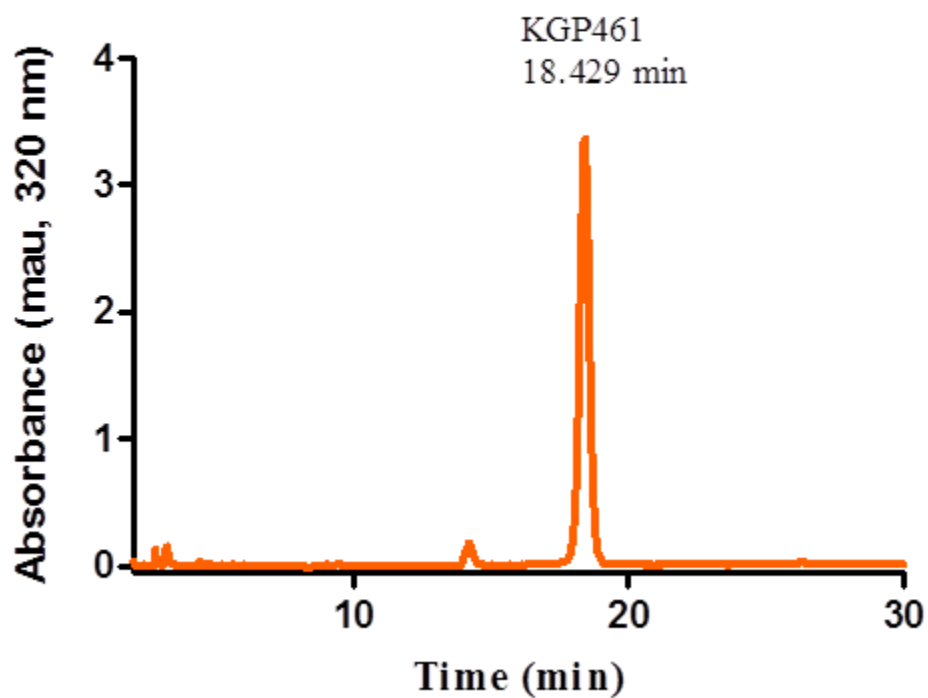
KGP461
18.429 min

Chromatogram of POR-Treated KGP461 for 90 min
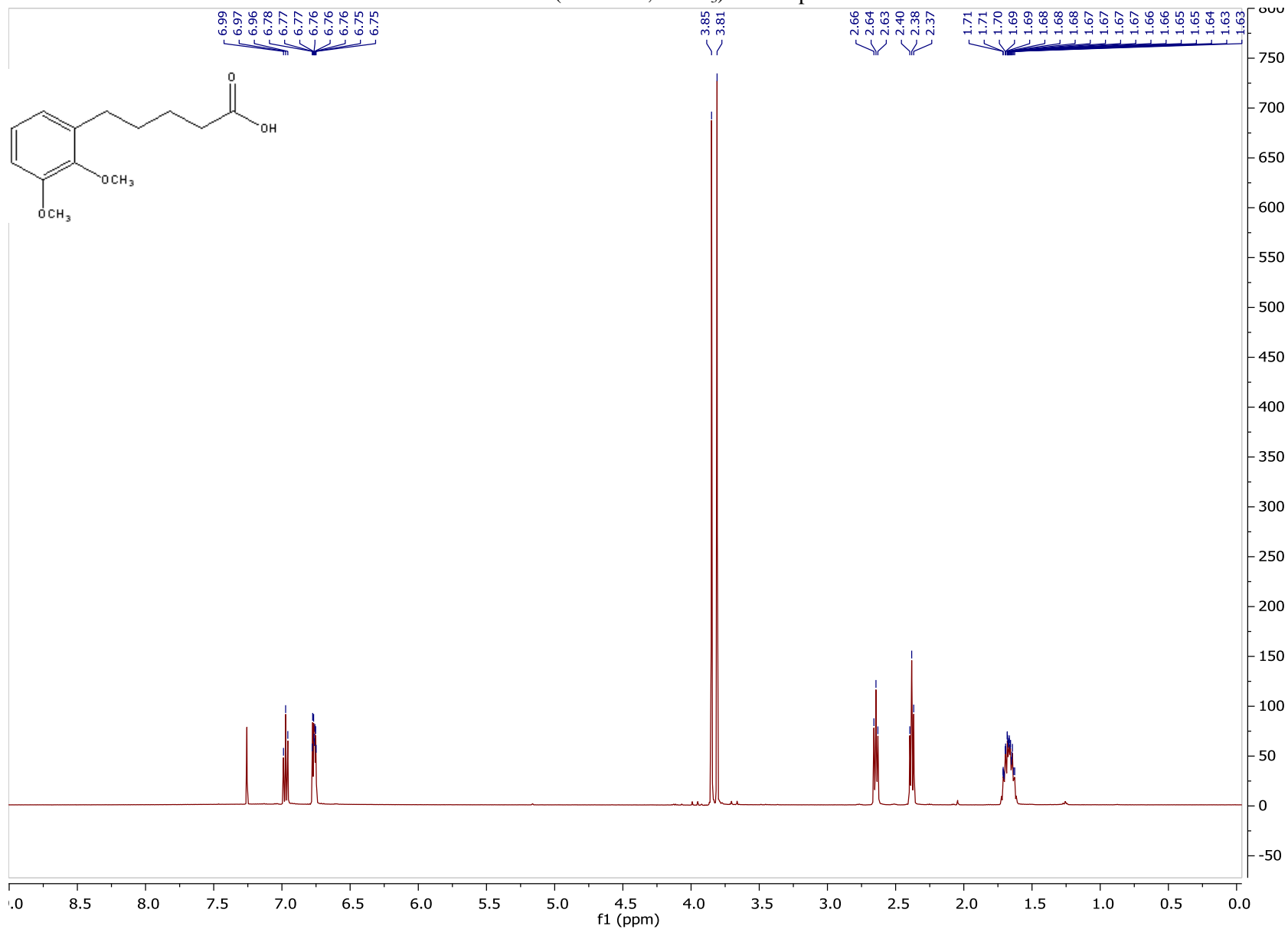

CA1/isomer
2.941 min
A= 40.3
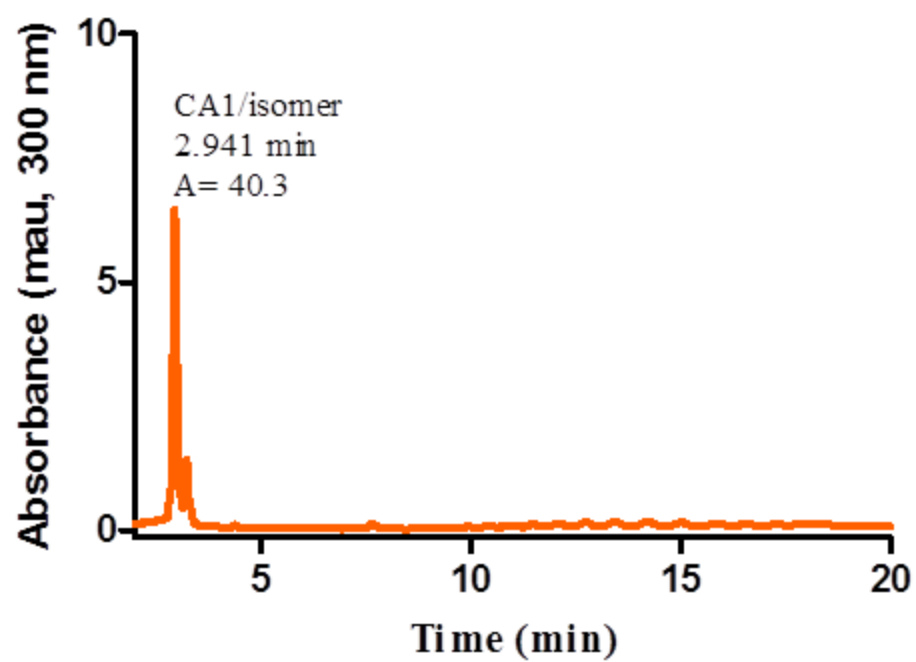
APPENDIX C

Synthesis of KGP18, KGP18 Bioreductively Activatable Prodrug Conjugates, OXi6196
Bioreductively Activatable Prodrug Conjugates, and the Nitroimidazole Trigger
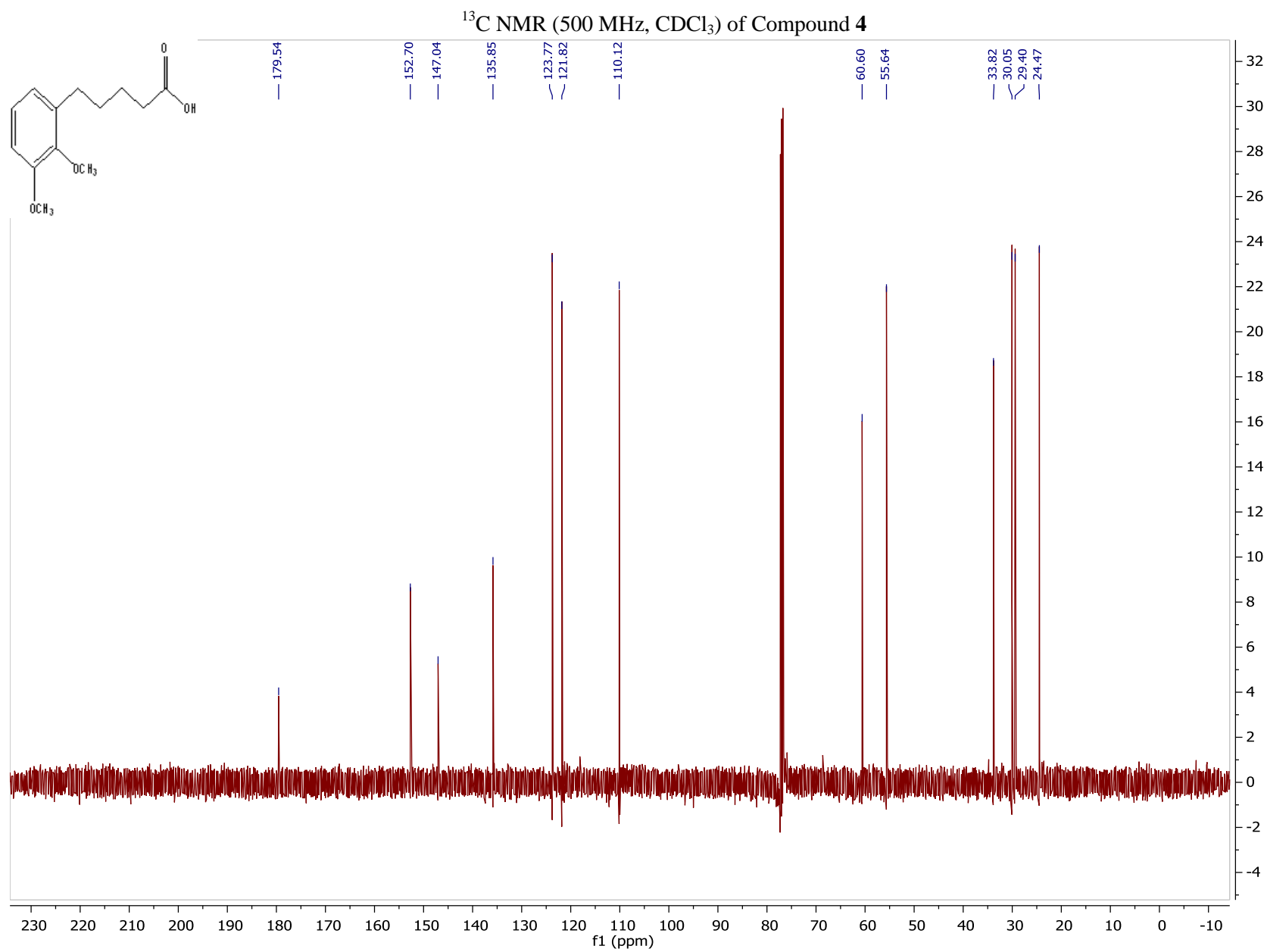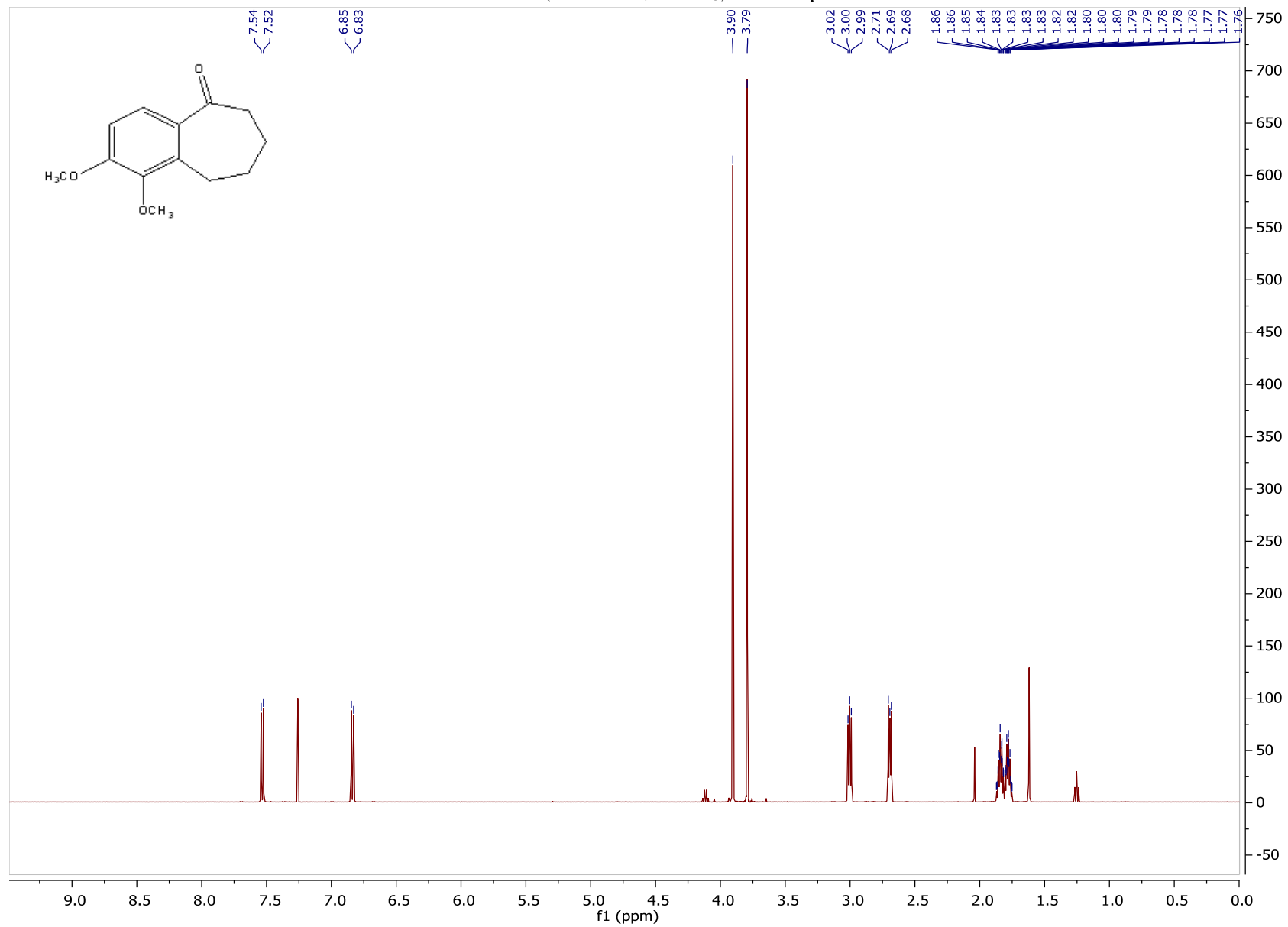
Table of Contents

NMR, HPLC, HRMS Data:

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **4**

509

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **4**

510

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **5**

7.54, 7.52
6.85, 6.83
3.90, 3.79
3.02, 3.00, 2.99, 2.71, 2.69, 2.68
1.86, 1.86, 1.85, 1.84, 1.83, 1.83, 1.83, 1.83, 1.82, 1.80, 1.80, 1.79, 1.79, 1.78, 1.78, 1.77, 1.77, 1.76

f1 (ppm)

511

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **5**

¹H NMR (500 MHz, CDCl₃) of Compound **6**
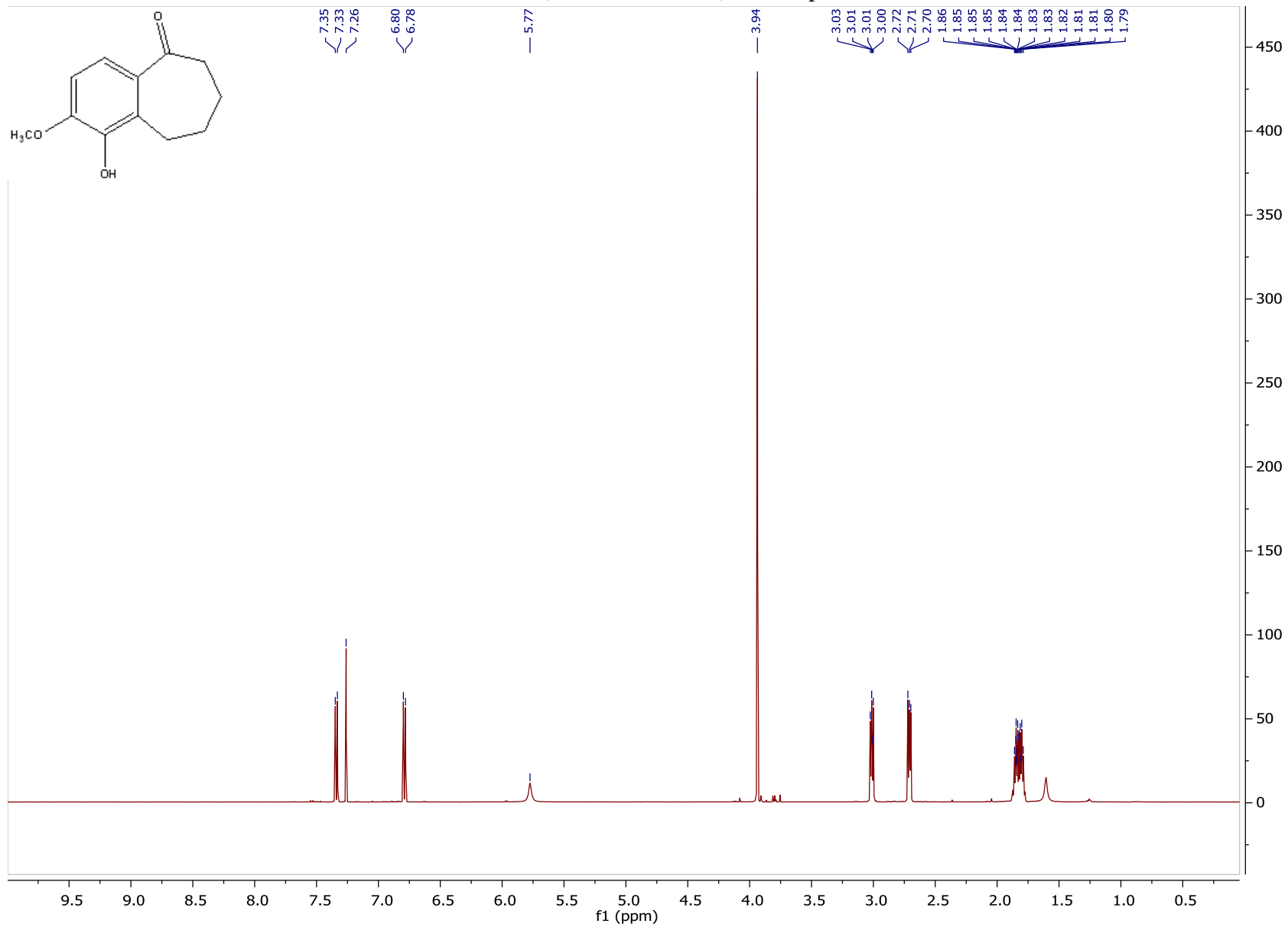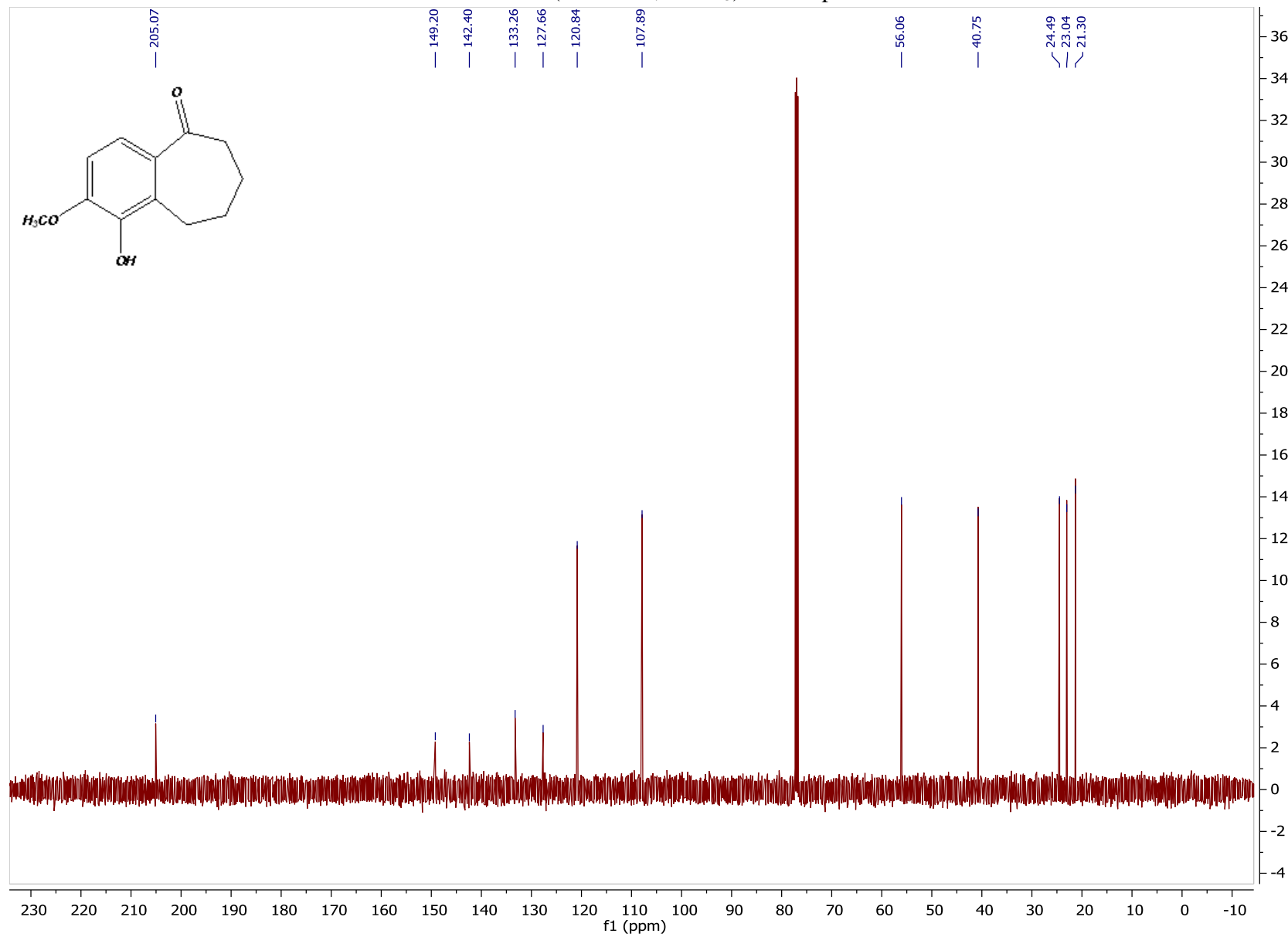
7.35
7.33
7.26
6.80
6.78
5.77
3.94
3.03
3.01
3.01
3.00
2.72
2.71
2.70
1.86
1.85
1.85
1.85
1.84
1.84
1.83
1.83
1.82
1.81
1.81
1.80
1.79

f1 (ppm)

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **6**

205.07

149.20
142.40
133.26
127.66
120.84
107.89

56.06

40.75

24.49
23.04
21.30

514

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **7**

515

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **7**

205.34
153.17
141.71
133.11
133.09
122.30
108.73
54.83
40.69
26.05
25.63
24.65
23.93
21.19
-3.91

516

^{1}H NMR (500 MHz, CDCl$_3$) of Compound 10

6.69 6.68 6.62 6.60 6.48 6.33 6.32 6.30   3.85 3.80 3.79   2.77 2.76 2.75 2.13 2.12 2.10 2.09 2.07 1.97 1.95 1.94 1.92 1.57   1.04   0.23

517
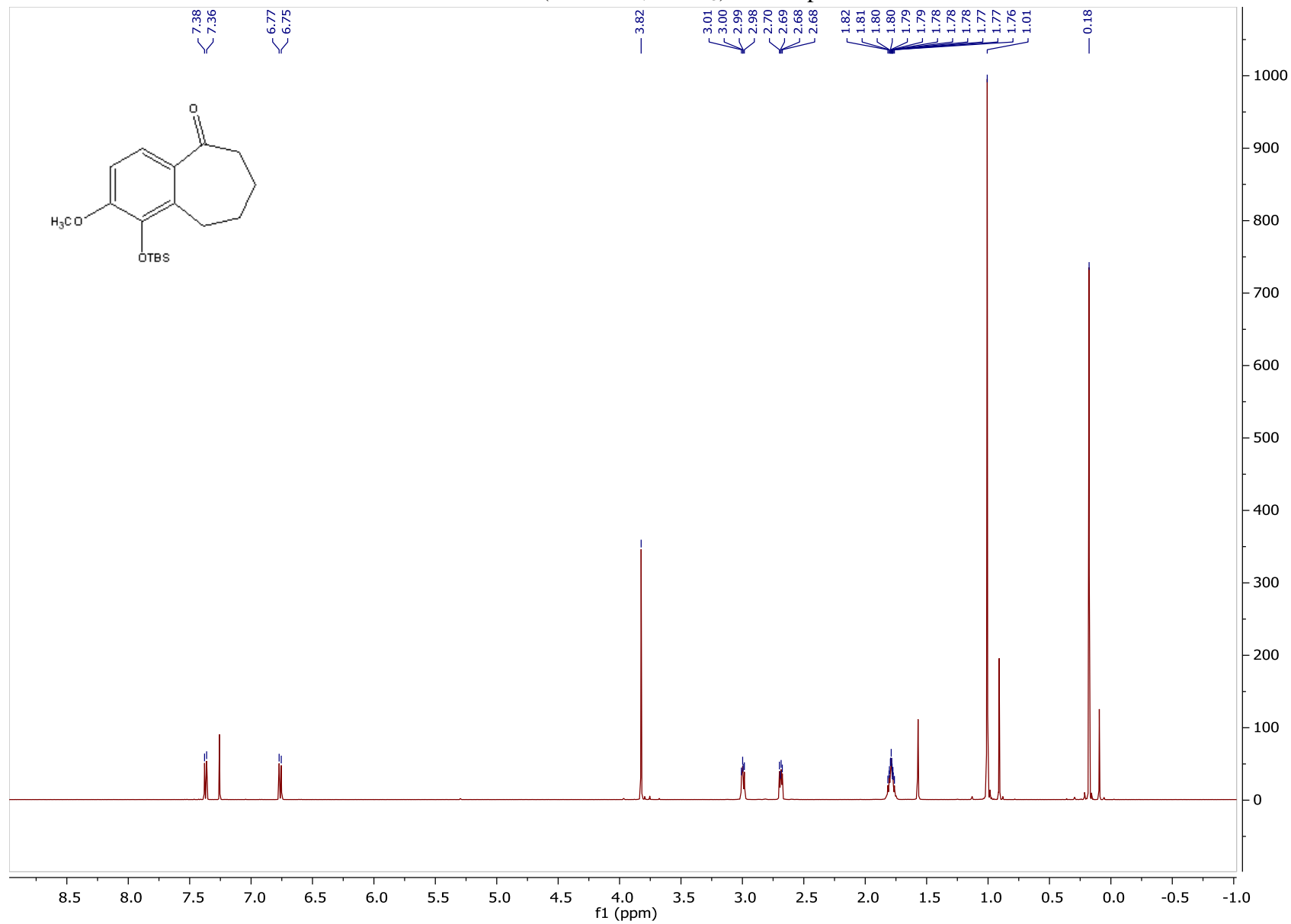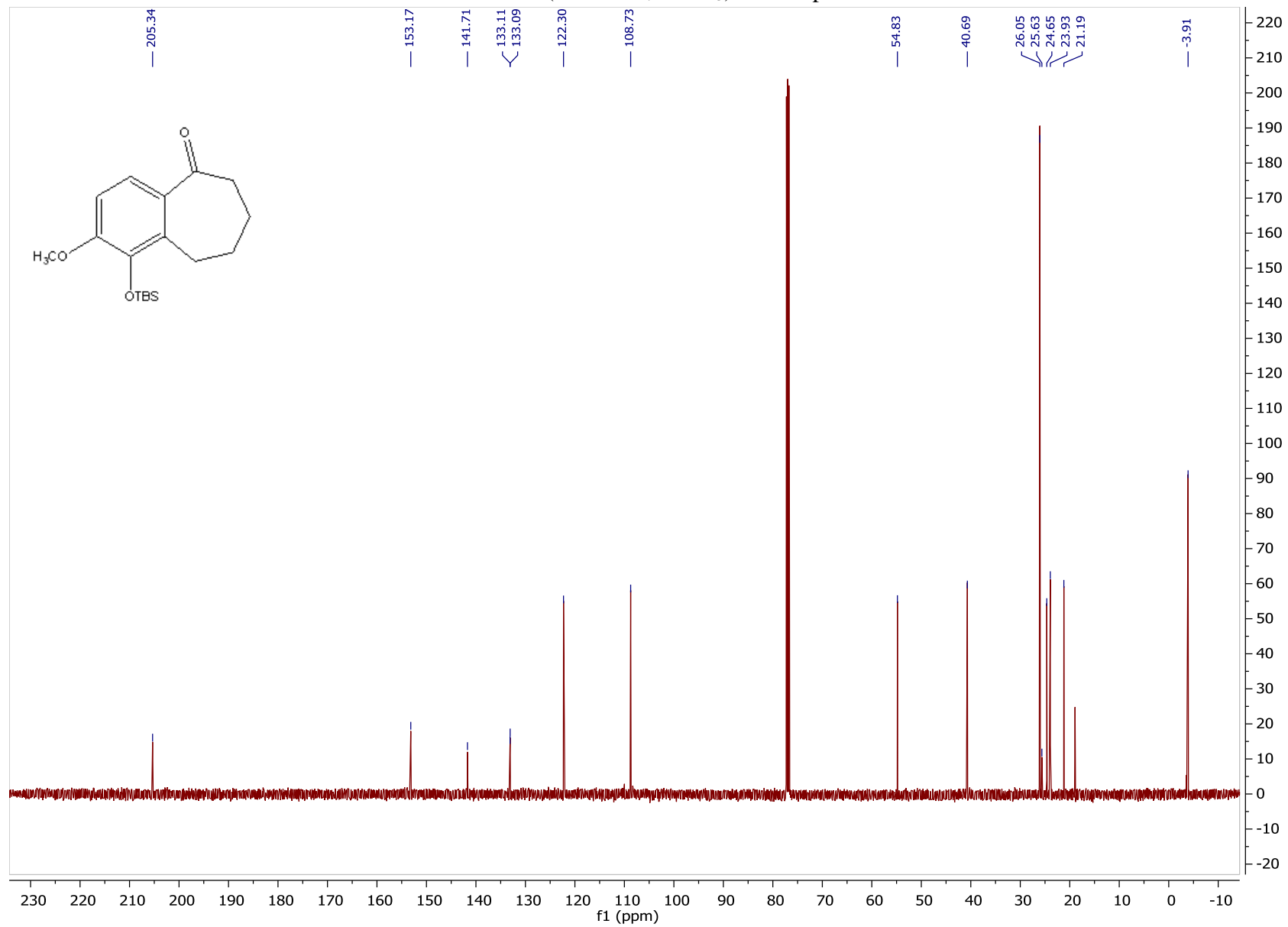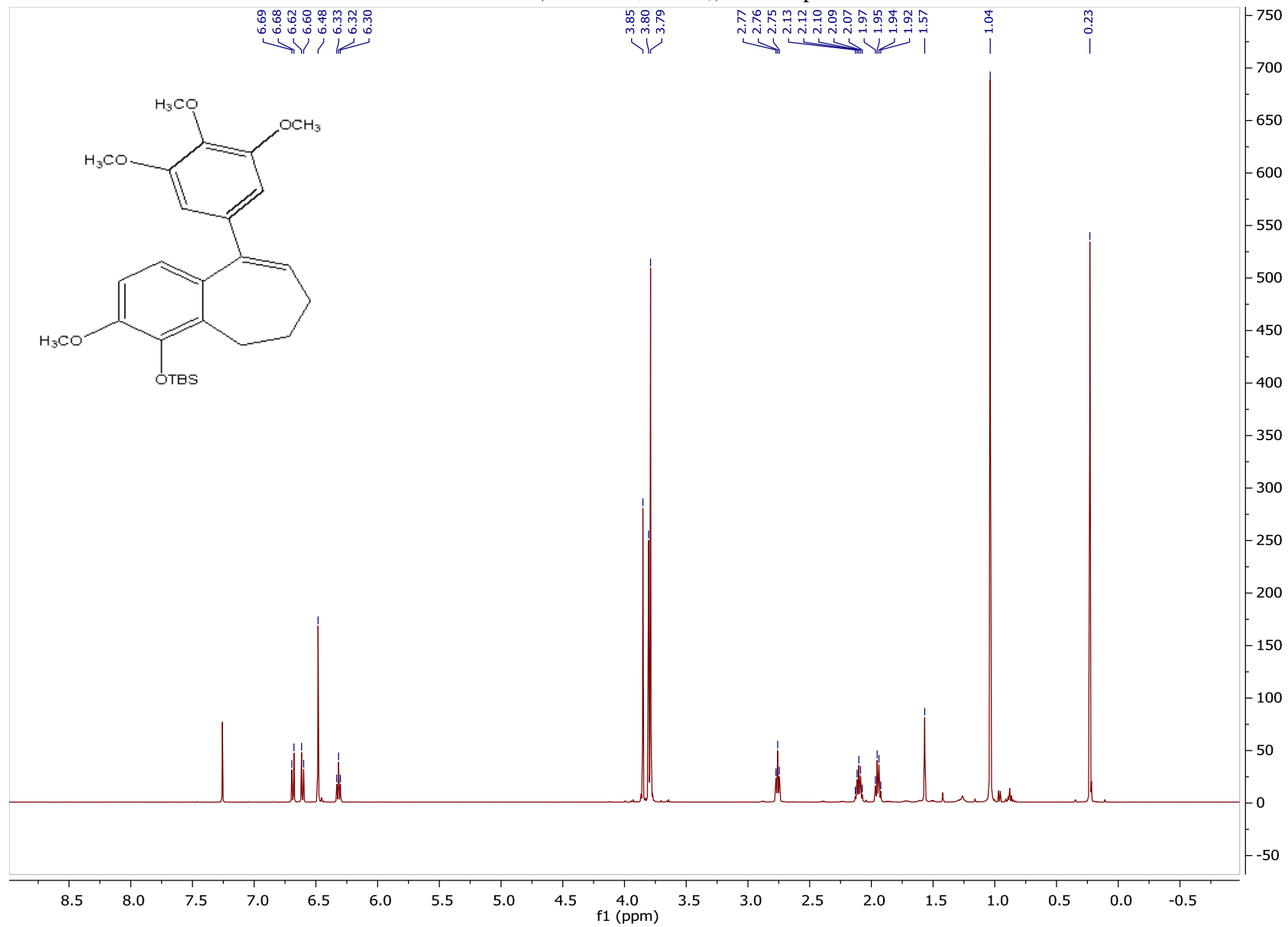
^{13}C NMR (500 MHz, CDCl$_3$) of Compound **10**

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **11**

519

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **11**

520
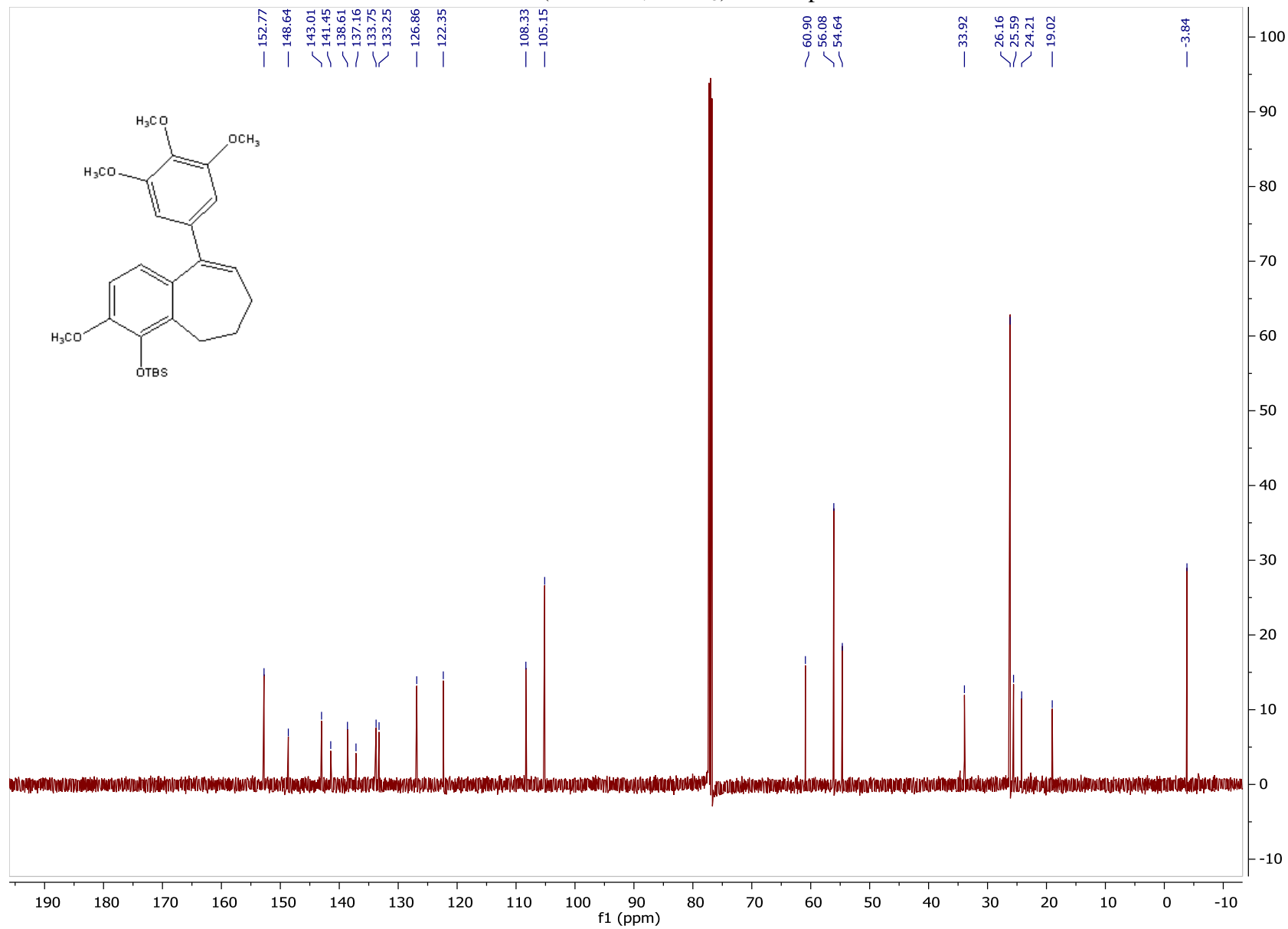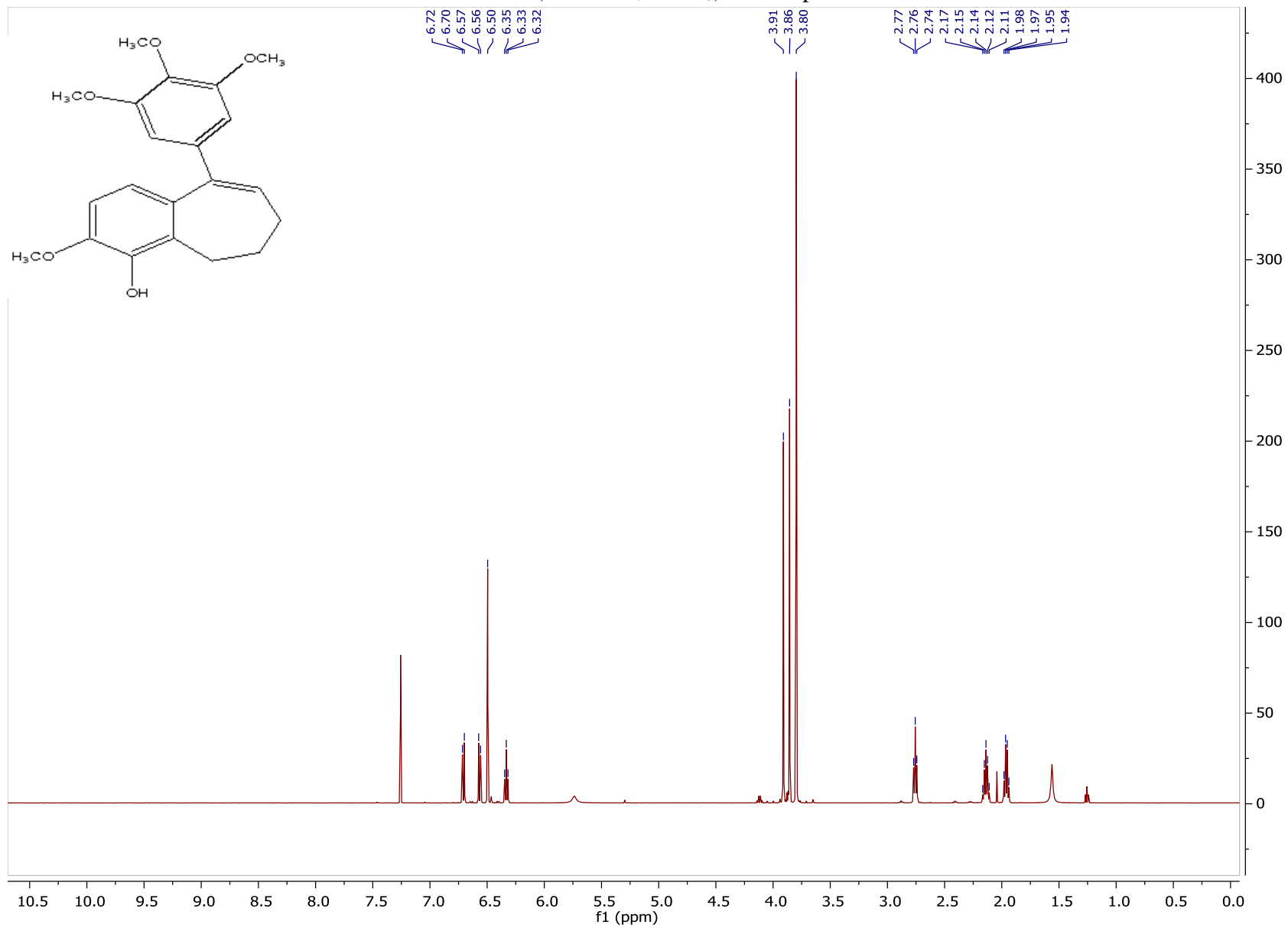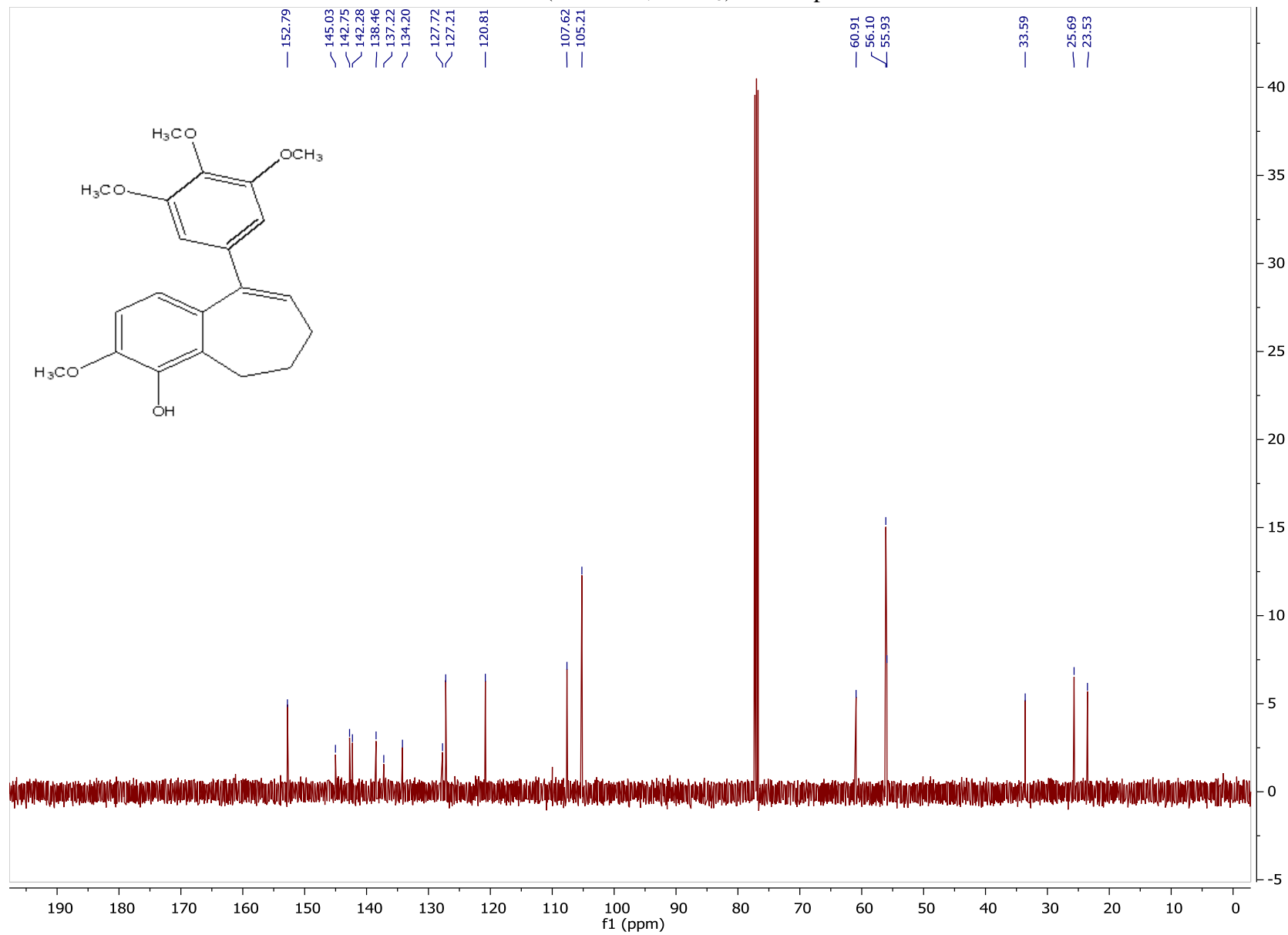
^{1}H NMR (500 MHz, CDCl$_3$) of Compound **13**
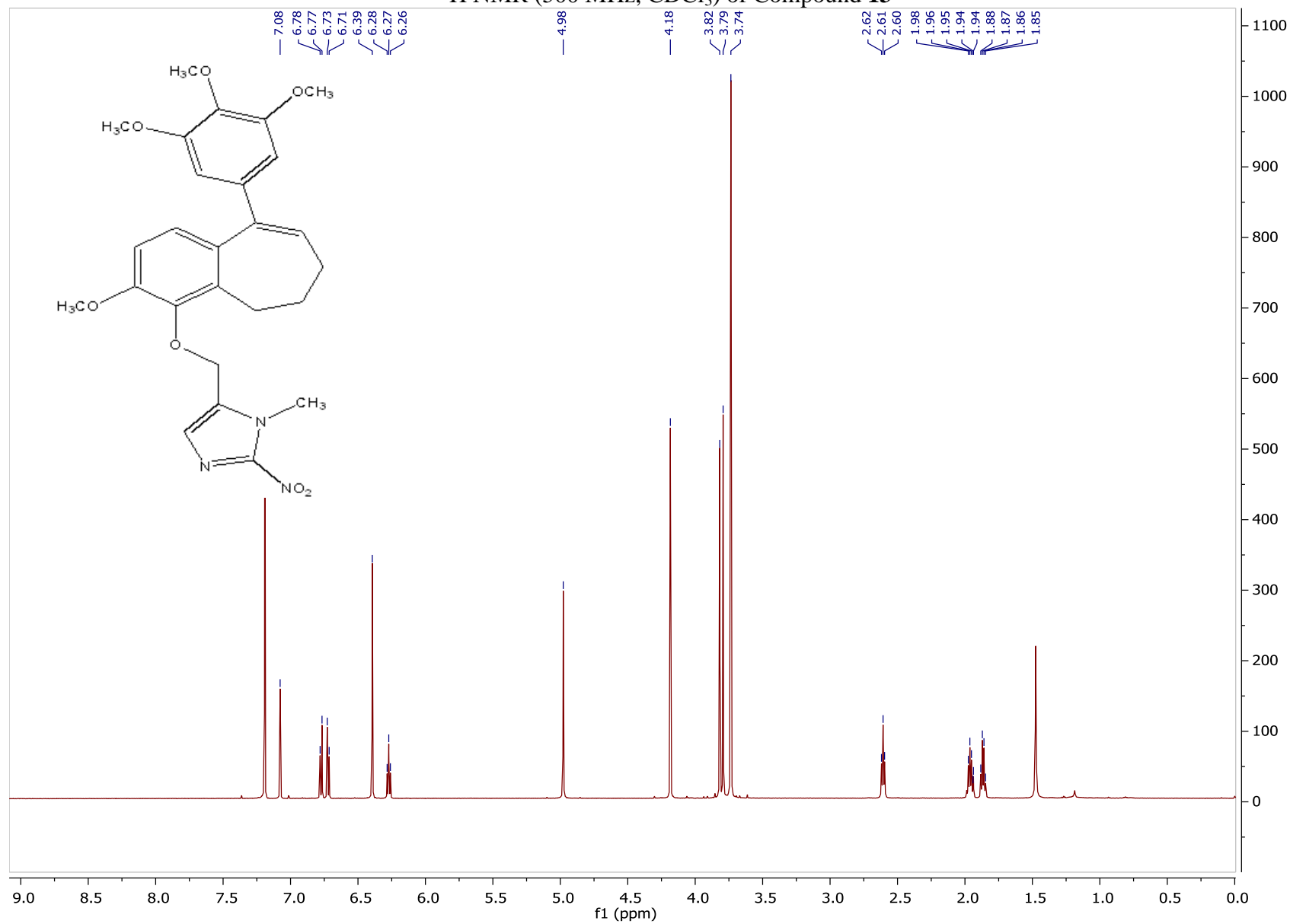
^{13}C NMR (500 MHz, CDCl$_3$) of Compound **13**
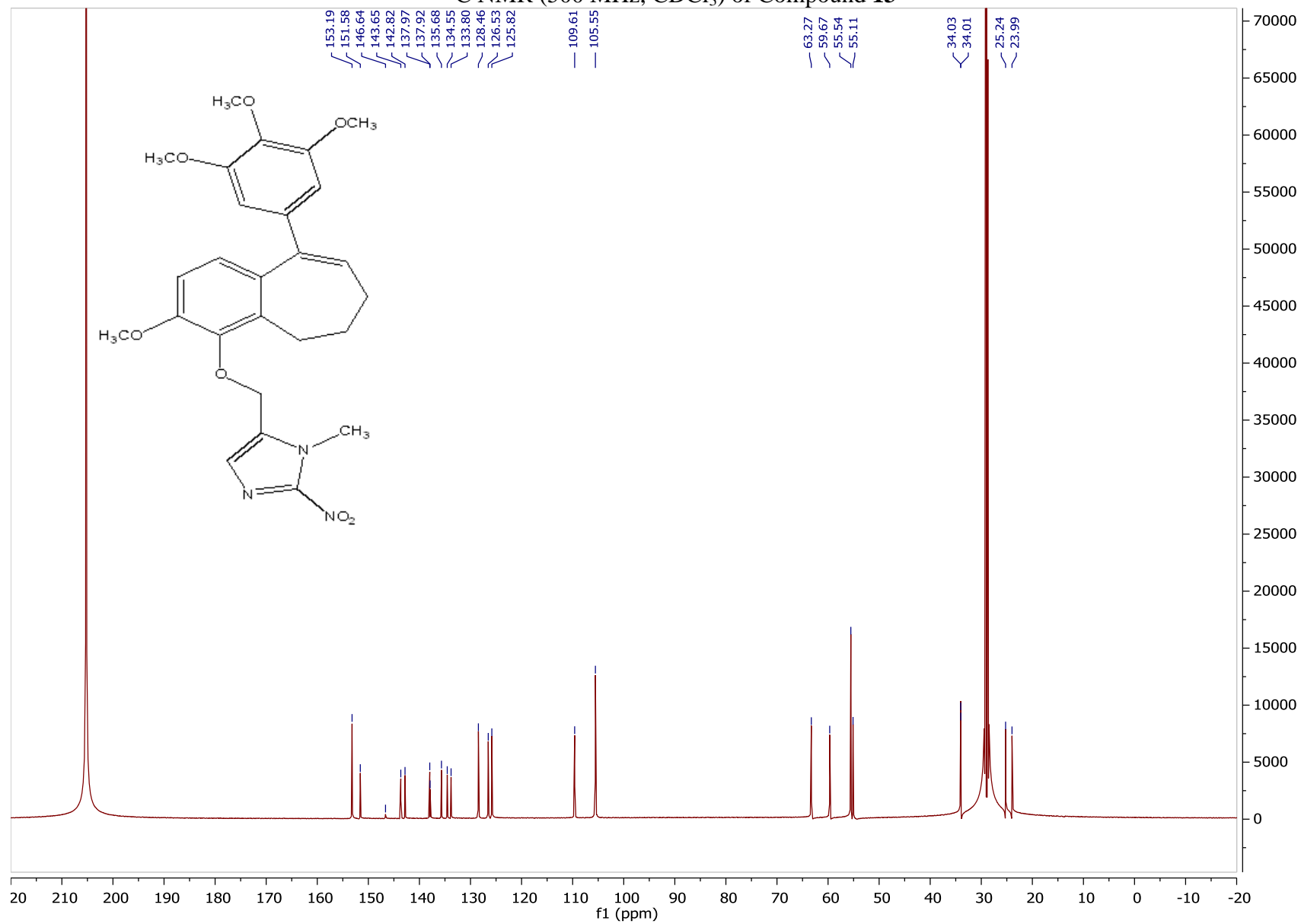
HPLC trace of compound **13**

Data File C:\Chem32\1\Data\ZHE\ZS_IV_39_200021.D
Sample Name: zs_IV_39_2

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Acq. Instrument : Instrument 1                    Location :      -
Injection Date  : 5/25/2016 12:24:32 PM
Acq. Method     : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
Last changed    : 5/25/2016 12:22:35 PM by Zhe
Analysis Method : C:\Chem32\1\Methods\DEF_LC.M
Last changed    : 6/22/2014 3:13:01 PM by SYSTEM
Sample Info     : zs_IV_39_2
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                  normethylnitroimidazole-KGP18
                  GRAD 2 50-90 ACN
                  20160525
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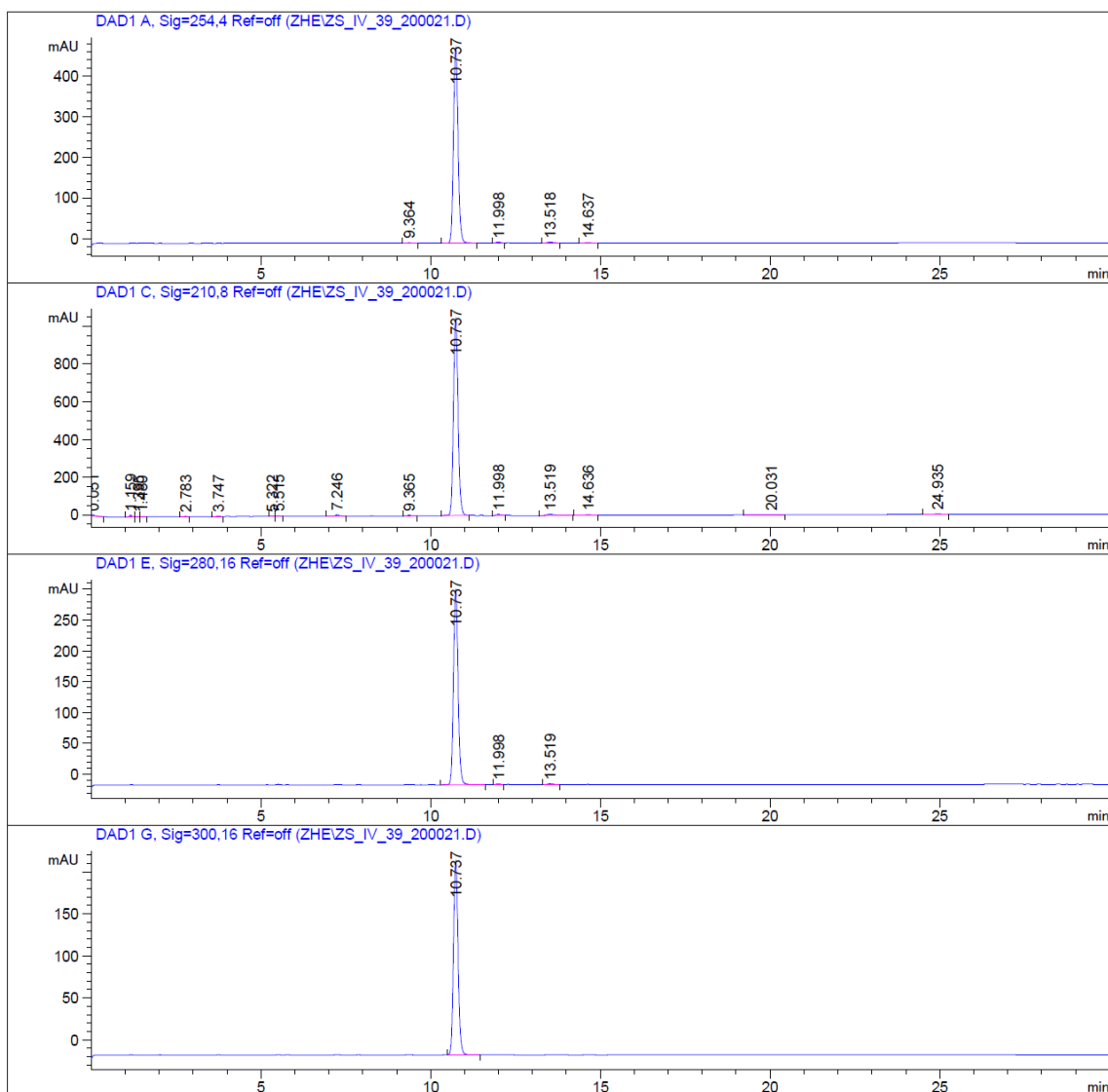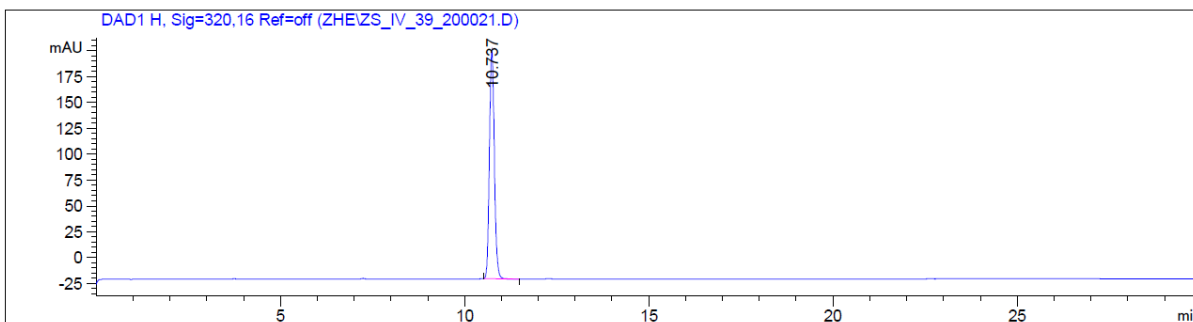
523

Data File C:\Chem32\1\Data\ZHE\ZS_IV_39_200021.D
Sample Name: zs_IV_39_2

DAD1 H, Sig=320,16 Ref=off (ZHE\ZS_IV_39_200021.D)

==
                      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 Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
   1   9.364 BB   0.1481   10.64091   1.10784   0.2329
   2 10.737 BB   0.1424 4483.29248 482.44608 98.1181
   3 11.998 BV   0.1491   26.45956   2.77848   0.5791
   4 13.518 BB   0.1802   33.95763   2.82481   0.7432
   5 14.637 BB   0.1623   14.92925   1.40135   0.3267

Totals :   4569.27982 490.55856

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

Peak RetTime Type Width   Area   Height   Area
  #   [min]   [min]   [mAU*s]   [mAU]   %
----|-------|----|-------|----------|----------|--------|
   1   0.051 BB   0.1066   41.25443   5.68900   0.4048
   2   1.159 BB   0.0634   40.82954   9.26910   0.4006
   3   1.395 BV   0.0595   6.40275   1.64135   0.0628
   4   1.480 VB   0.0833   6.22000   1.01708   0.0610
   5   2.783 BB   0.0793   6.71858   1.32192   0.0659
   6   3.747 BV   0.0903   14.63146   2.42814   0.1436
   7   5.322 VV   0.1091   11.23704   1.53974   0.1103
   8   5.515 VV   0.1104   16.14544   2.18027   0.1584
   9   7.246 BB   0.1306   58.77359   6.68359   0.5767
  10   9.365 BB   0.1478   27.24051   2.89495   0.2673
  11 10.737 BB   0.1425 9707.28418 1044.03503 95.2494
  12 11.998 BB   0.1428   53.50554   5.95838   0.5250
  13 13.519 BV R 0.2067 103.91846   7.27487   1.0197
  14 14.636 BB   0.1658   36.45964   3.38211   0.3577

Data File C:\Chem32\1\Data\ZHE\ZS_IV_39_200021.D
Sample Name: zs_IV_39_2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
15	20.031	BB	0.1974	20.63031	1.56853	0.2024
16	24.935	BB	0.2022	40.18402	3.04033	0.3943

Totals :   1.01914e4   1099.92441

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.737	BB	0.1426	2938.32471	315.77563	99.1208
2	11.998	BB	0.1370	9.60916	1.11037	0.3242
3	13.519	BB	0.1767	16.45451	1.38398	0.5551

Totals :   2964.38837   318.26998

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.737	BB	0.1420	2133.37476	230.35779	100.0000

Totals :   2133.37476   230.35779

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.737	BB	0.1420	2049.19653	221.43524	100.0000

Totals :   2049.19653   221.43524

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                      *** End of Report ***

Mass Spectrum of Compound **13**

526
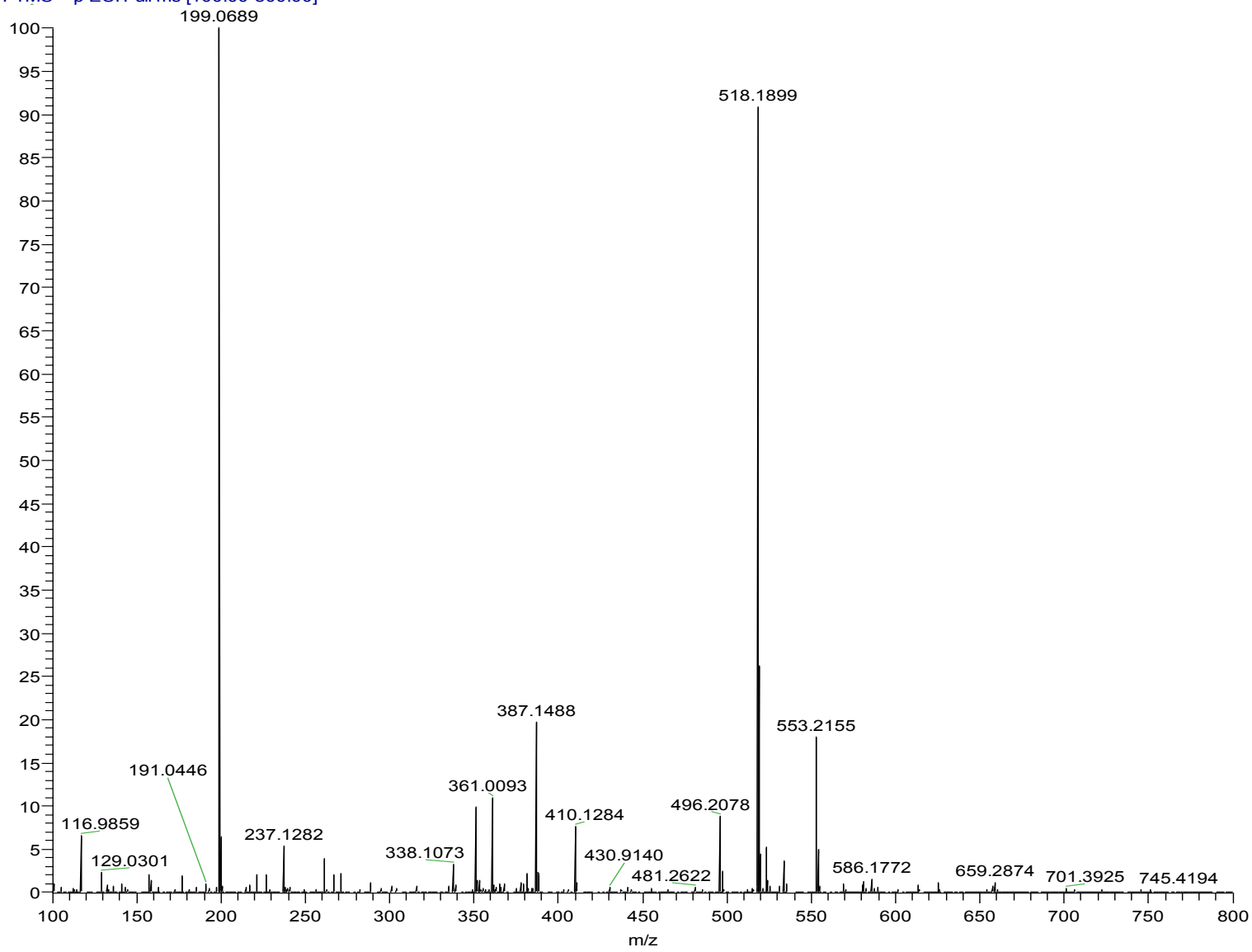
^{1}H NMR (500 MHz, CDCl$_3$) of Compound **15**

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **15**
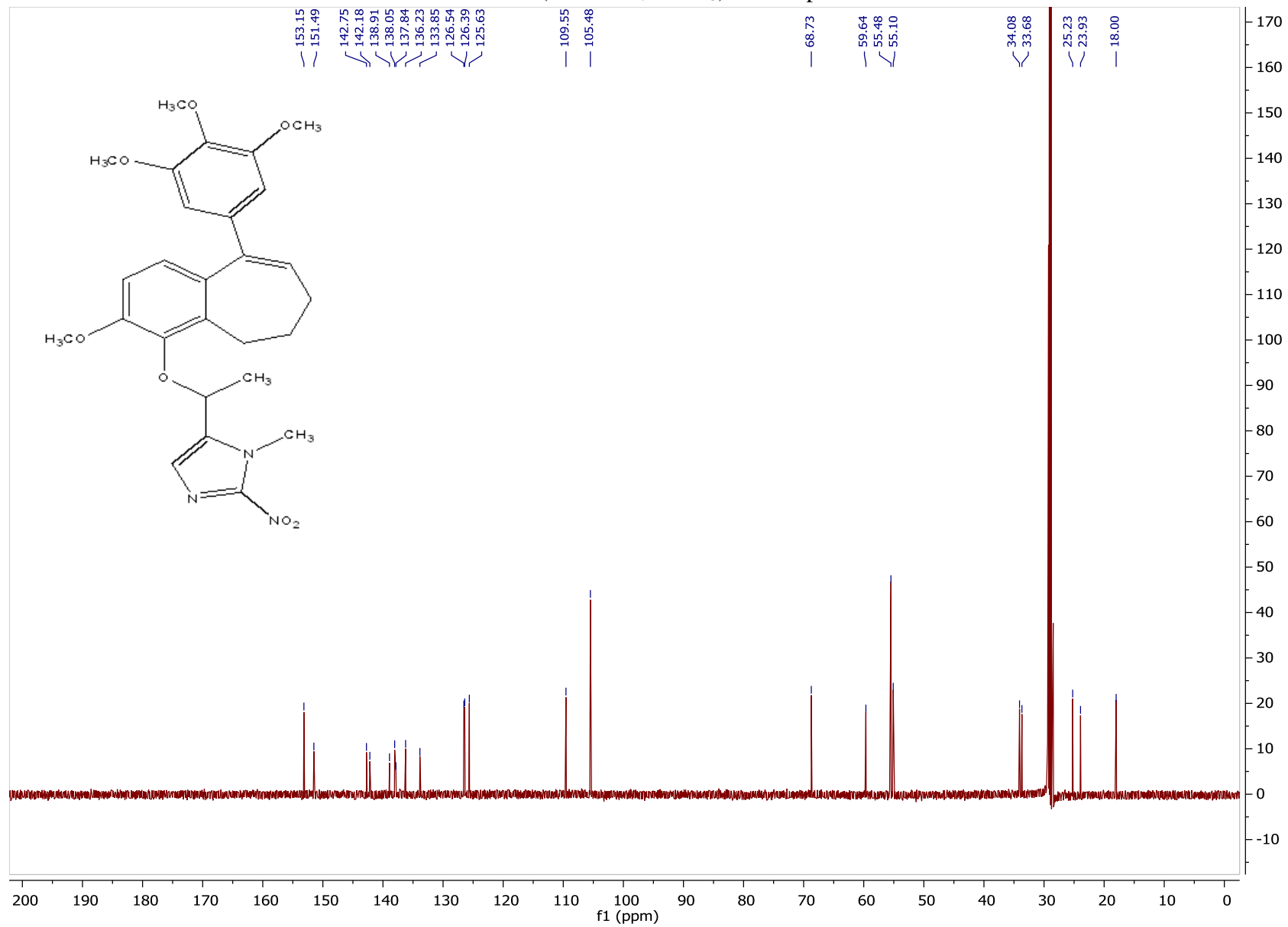
528

HPLC trace of compound 15

```
==================================================================
Acq. Operator   : zhe
Acq. Instrument : Instrument 1                Location :      -
Injection Date  : 5/5/2016 12:03:07 PM
Acq. Method     : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
Last changed    : 5/5/2016 11:51:25 AM by zhe
Analysis Method : C:\Chem32\1\Methods\DEF_LC.M
Last changed    : 6/22/2014 3:13:01 PM by SYSTEM
Sample Info     : zs_IV_38_2
                  KGP18-monomethyl nitroimidazole
                  GRAD2 ACN 50-90
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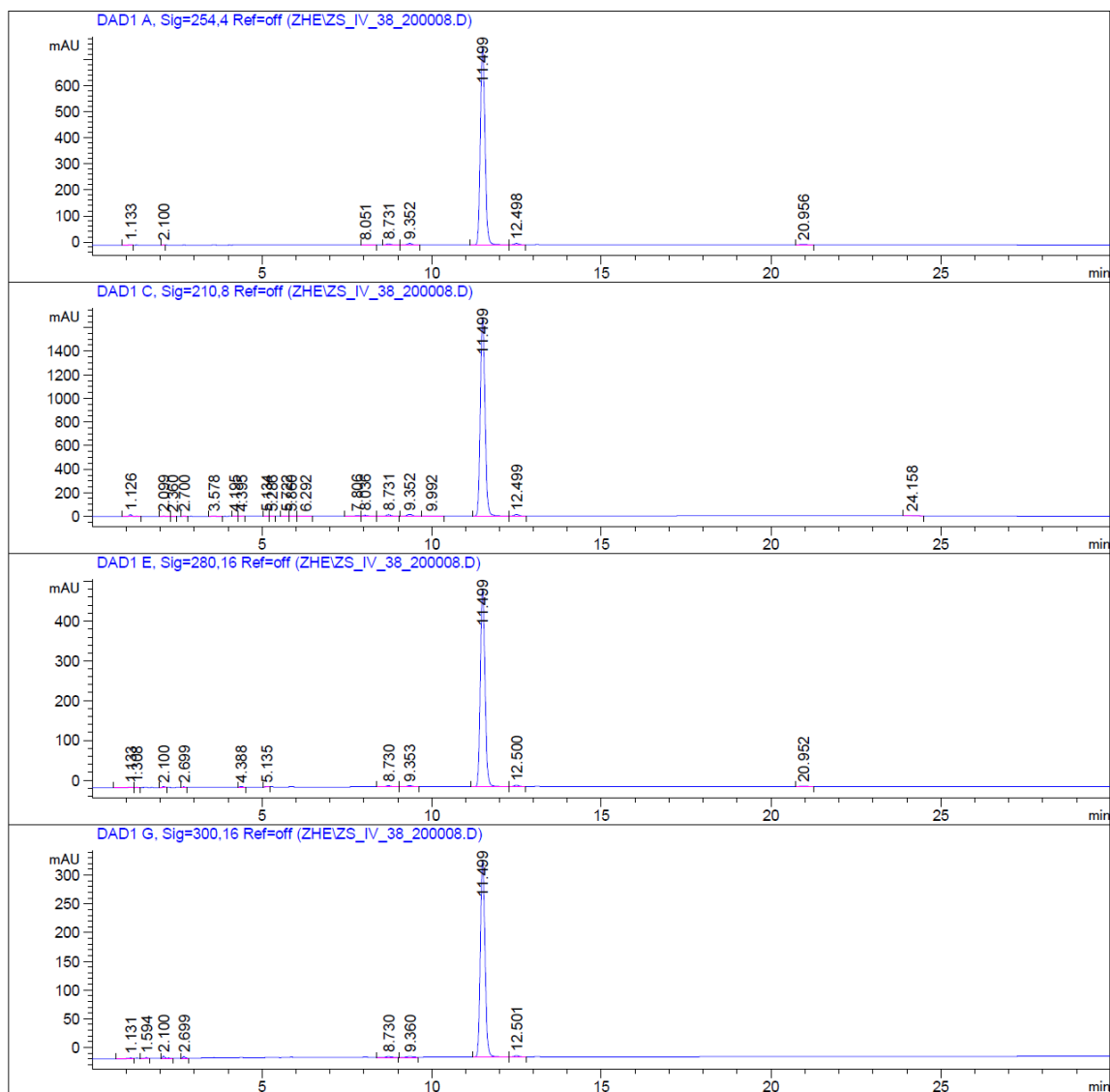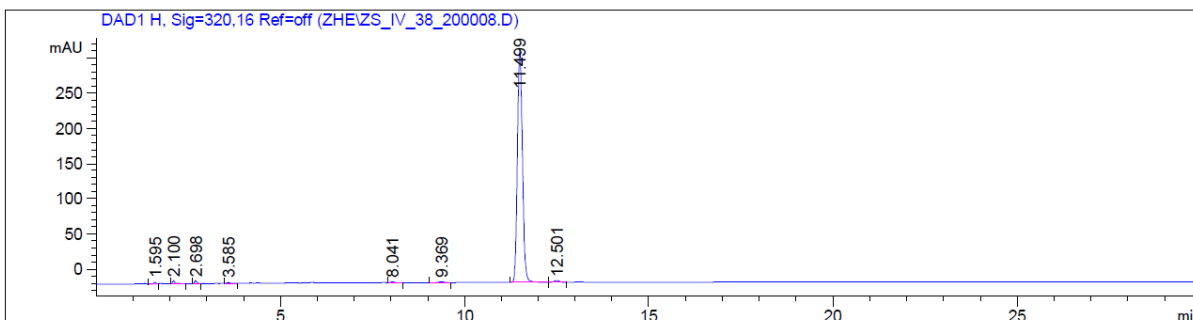
```
===================================================================
                       Area Percent Report
===================================================================

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 Type  Width     Area        Height     Area
  #   [min]        [min]   [mAU*s]       [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.133 BB   0.0924    9.27913    1.45485   0.1268
   2   2.100 BV   0.0580    6.46301    1.71326   0.0883
   3   8.051 BB   0.1354   10.94999    1.23606   0.1496
   4   8.731 BB   0.1383   34.64928    3.87737   0.4734
   5   9.352 BB   0.1515   49.93105    5.04203   0.6822
   6  11.499 BB   0.1455 7137.88574  760.50507  97.5271
   7  12.498 BB   0.1480   44.34357    4.62010   0.6059
   8  20.956 BB   0.2606   25.37336    1.60384   0.3467

Totals :                          7318.87514  780.05257




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

Peak RetTime Type  Width     Area        Height     Area
  #   [min]        [min]   [mAU*s]       [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.126 BV R 0.0926   92.78095   14.90394   0.5555
   2   2.099 BV   0.0776   15.72043    2.88344   0.0941
   3   2.360 VB   0.0651    6.67961    1.58807   0.0400
   4   2.700 BB   0.0674   16.12425    3.66159   0.0965
   5   3.578 BB   0.1048   17.86118    2.51697   0.1069
   6   4.195 VV   0.0911   17.36708    2.84933   0.1040
   7   4.395 VV   0.1013   13.71676    2.07060   0.0821
   8   5.134 BV   0.0937   18.59568    3.02740   0.1113
   9   5.286 VB   0.0980   10.67476    1.68193   0.0639
  10   5.722 BV   0.1337   21.27279    2.18244   0.1274
  11   5.860 VB   0.1106   18.20321    2.45211   0.1090
```

330

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
12	6.292	BB	0.1253	12.25700	1.46969	0.0734
13	7.806	BV	0.1349	45.46371	4.96068	0.2722
14	8.036	VB	0.1438	62.68792	6.53912	0.3753
15	8.731	BB	0.1395	95.28065	10.33574	0.5705
16	9.352	BV R	0.1601	143.08829	13.67217	0.8567
17	9.992	VB E	0.2375	25.20261	1.45927	0.1509
18	11.499	BV	0.1467	1.59298e4	1679.45557	95.3770
19	12.499	VB	0.1524	120.06548	12.03571	0.7189
20	24.158	BB	0.1875	19.08722	1.55162	0.1143

Totals :   1.67019e4   1771.29737

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.133	BV	0.1311	14.51148	1.55142	0.3013
2	1.308	VB	0.0633	5.32972	1.26033	0.1107
3	2.100	BB	0.0581	8.32161	2.20213	0.1728
4	2.699	BB	0.0667	5.14825	1.18580	0.1069
5	4.388	BB	0.0913	6.79379	1.17842	0.1411
6	5.135	BB	0.0890	6.98379	1.25374	0.1450
7	8.730	BB	0.1429	25.02971	2.68118	0.5198
8	9.353	BB	0.1535	30.43494	3.07315	0.6320
9	11.499	BB	0.1455	4662.06055	496.67212	96.8104
10	12.500	BB	0.1472	33.89988	3.55608	0.7040
11	20.952	BB	0.2655	17.14649	1.06684	0.3561

Totals :   4815.66021   515.68121

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.131	BV	0.1201	8.26240	1.00280	0.2495
2	1.594	BV	0.0739	8.13209	1.53150	0.2456
3	2.100	BV R	0.0677	18.48183	4.01500	0.5582
4	2.699	BB	0.0678	12.69180	2.85899	0.3833
5	8.730	BB	0.1446	12.71276	1.31774	0.3839
6	9.360	BB	0.1499	15.29412	1.56715	0.4619
7	11.499	BB	0.1455	3213.04224	342.37991	97.0399
8	12.501	BB	0.1462	22.43438	2.37466	0.6776

Totals :   3311.05162   357.04775

531

Data File C:\Chem32\1\Data\ZHE\ZS_IV_38_200008.D
Sample Name: zs_IV_38_2


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

    Peak RetTime Type Width   Area   Height   Area
     #   [min]   [min]   [mAU*s]   [mAU]   %
    ----|-------|----|-------|----------|----------|--------|
     1   1.595 BV   0.0682   12.10355   2.60457   0.3776
     2   2.100 BV R 0.0740   21.38257   4.15635   0.6671
     3   2.698 BB   0.0675   19.07925   4.31986   0.5952
     4   3.585 BB   0.0842   7.96521   1.40347   0.2485
     5   8.041 VB   0.1351   10.68806   1.20914   0.3335
     6   9.369 BB   0.1389   13.34004   1.45553   0.4162
     7   11.499 BB   0.1454 3099.98291 330.50562 96.7151
     8   12.501 BB   0.1465   20.73283   2.18975   0.6468
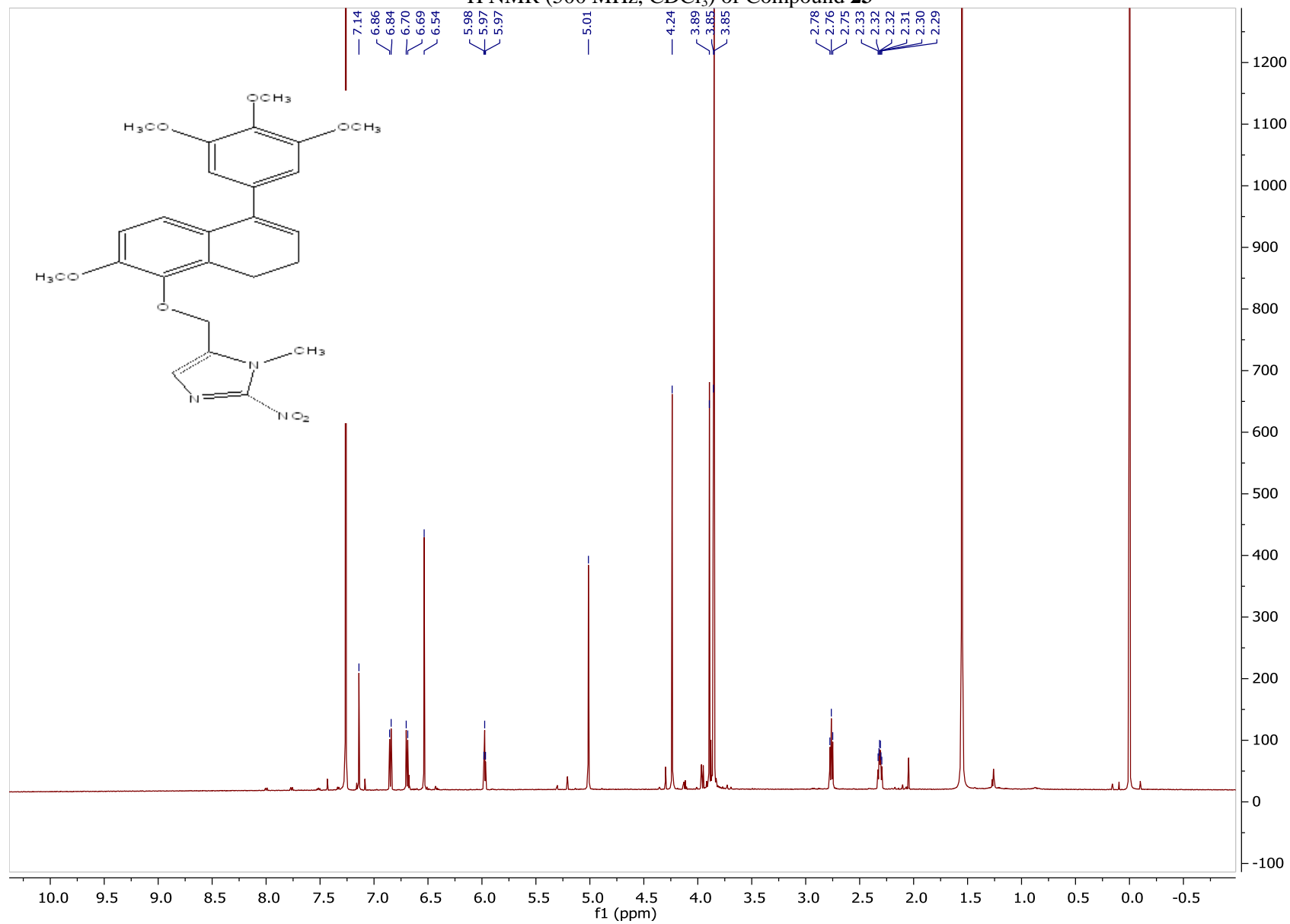
    Totals :   3205.27442 347.84428

    ==
                      *** End of Report ***

Mass Spectrum of Compound **15**

533

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **23**

534
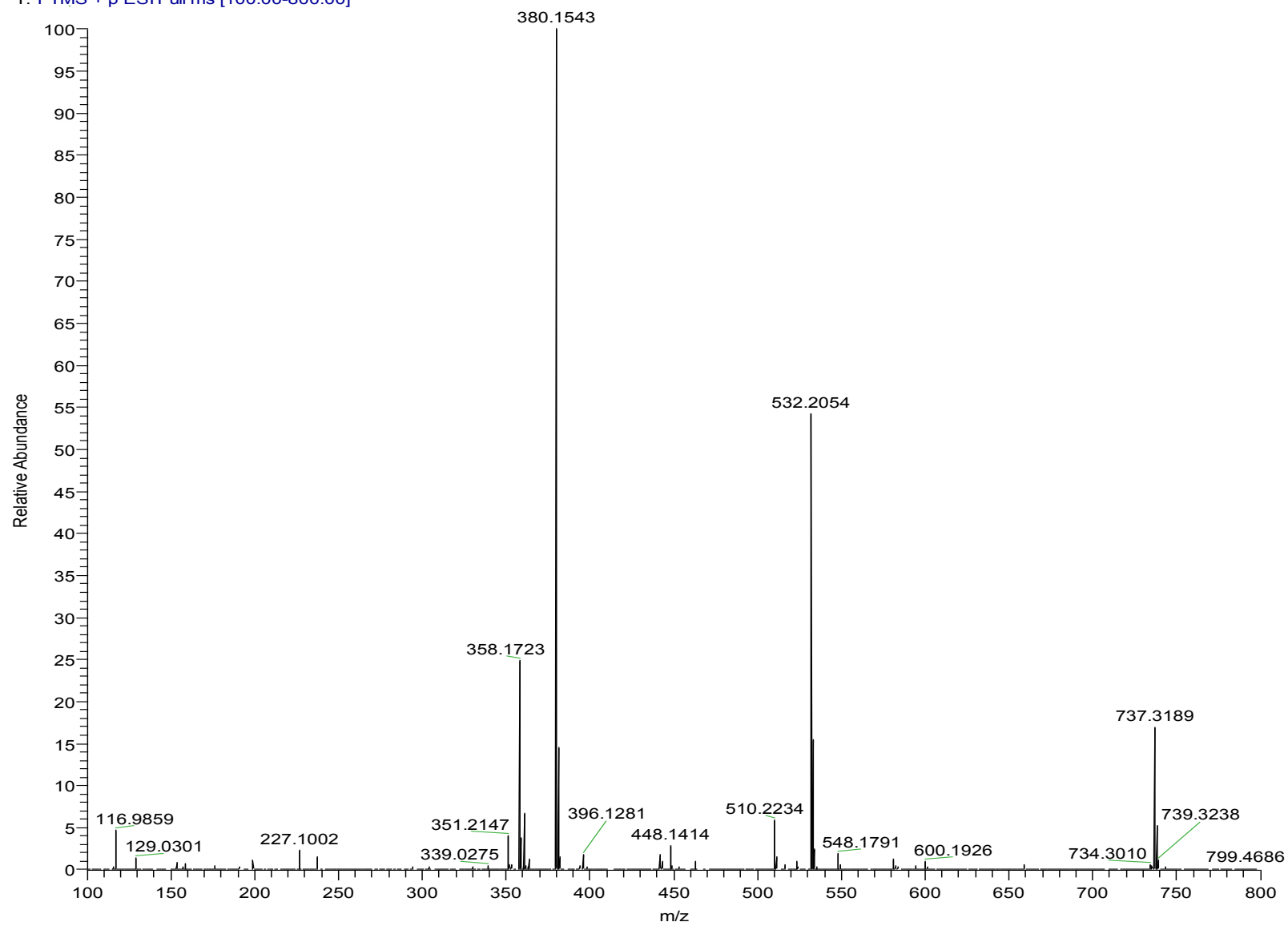
^{13}C NMR (500 MHz, CDCl$_3$) of Compound **23**

HPLC trace of Compound **23**

```
================================================================
Acq. Operator   : zhe
Acq. Instrument : Instrument 1              Location :     -
Injection Date  : 6/13/2016 4:53:29 PM
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\DEF_LC.M
Last changed    : 6/22/2014 3:13:01 PM by SYSTEM
Sample Info     : zs_III_106
                  GRAD 2 50-90 ACN
                  checking for OXi6196
                  20160613
```

536

```
===================================================================
                        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 Type  Width     Area        Height      Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.146 BV   0.1225   14.50789    1.65133   0.9533
   2   6.010 BV   0.1416   10.73883    1.12269   0.7056
   3   7.345 BB   0.1304 1463.31226  170.11348  96.1542
   4   9.683 BB   0.1668   18.27984    1.55920   1.2012
   5  14.543 BB   0.1627   15.00034    1.40349   0.9857

Totals :                         1521.83916  175.85019



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

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]   [mAU*s]       [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.129 BB   0.1194  114.46228   13.99465   2.4845
   2   1.470 BB   0.1120    9.27811    1.08261   0.2014
   3   3.017 BV   0.1166   19.83439    2.44635   0.4305
   4   3.214 VV   0.1009   19.90868    2.87203   0.4321
   5   3.750 VB   0.1196    9.08917    1.15818   0.1973
   6   4.958 BB   0.1201   24.10078    2.98917   0.5231
   7   5.993 BV   0.1532   29.46825    2.88322   0.6396
   8   6.288 VB   0.1122   17.46011    2.41898   0.3790
   9   7.345 BB   0.1320 4174.38184  477.42743  90.6098
  10   7.908 BB   0.1058    8.32523    1.24921   0.1807
  11   8.607 BV   0.1375   42.01498    4.64822   0.9120
  12   9.055 VB   0.1989   19.14094    1.32226   0.4155
  13   9.681 BB   0.2065   65.06004    4.25249   1.4122
  14  14.542 BB   0.1595   36.76067    3.53028   0.7979
```

Data File C:\Chem32\1\Data\ZHE\ZS_III_10600047.D
Sample Name: zs_III_106

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
15	23.035	BB	0.2213	17.70357	1.19172	0.3843

Totals :   4606.98903   523.46678

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.151	BV	0.1316	11.28998	1.12047	0.9390
2	7.345	BB	0.1298	1163.81067	136.00249	96.7981
3	8.607	BB	0.1309	13.15300	1.55119	1.0940
4	14.543	BB	0.1610	14.05339	1.33285	1.1689

Totals :   1202.30703   140.00700

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.345	BB	0.1330	899.99927	101.95317	98.8688
2	8.606	BB	0.1332	10.29763	1.21165	1.1312

Totals :   910.29690   103.16482

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.345	BB	0.1334	852.90222	96.21187	100.0000

Totals :   852.90222   96.21187

==
                    *** End of Report ***

Mass Spectrum of Compound **23**
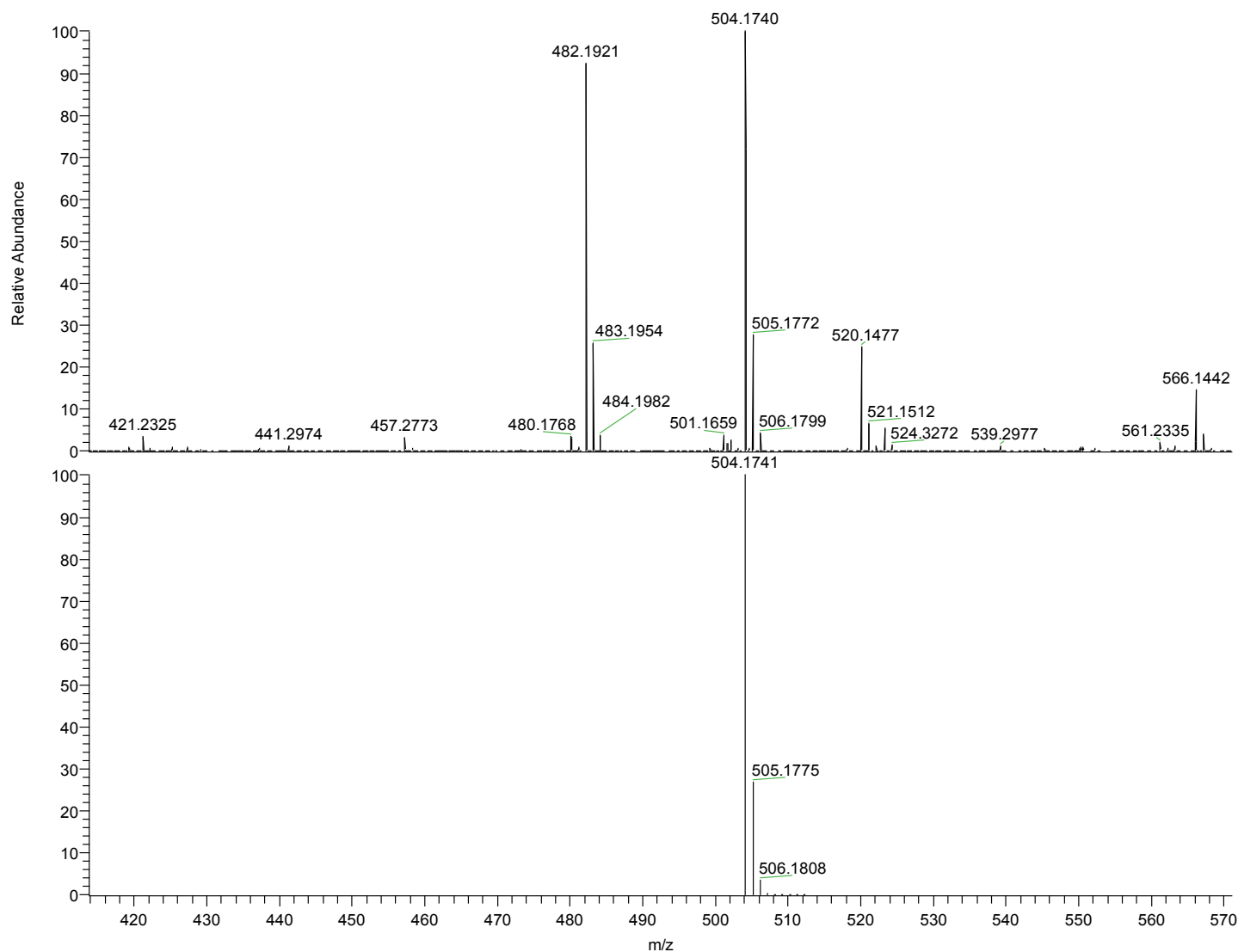
NL:
1.07E7
zs_III_107_+ESI#2-12
RT: 0.01-0.09 AV:
11 T: FTMS + p ESI
Full ms
[200.00-1000.00]

NL:
7.41E5

$C_{25} H_{27} N_3 NaO_7$:
$C_{25} H_{27} N_3 Na_1 O_7$
pa Chrg 1

Top spectrum peaks: 421.2325, 441.2974, 457.2773, 480.1768, 482.1921, 483.1954, 484.1982, 501.1659, 504.1740, 505.1772, 506.1799, 520.1477, 521.1512, 524.3272, 539.2977, 561.2335, 566.1442

Bottom spectrum peaks: 504.1741, 505.1775, 506.1808

m/z

539

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **24**

Peak labels: 153.25, 152.04, 141.62, 139.58, 138.77, 137.61, 136.33, 131.57, 128.73, 126.34, 124.97, 122.04, 109.15, 106.03, 68.85, 59.66, 55.50, 55.06, 34.09, 22.62, 21.65, 17.63
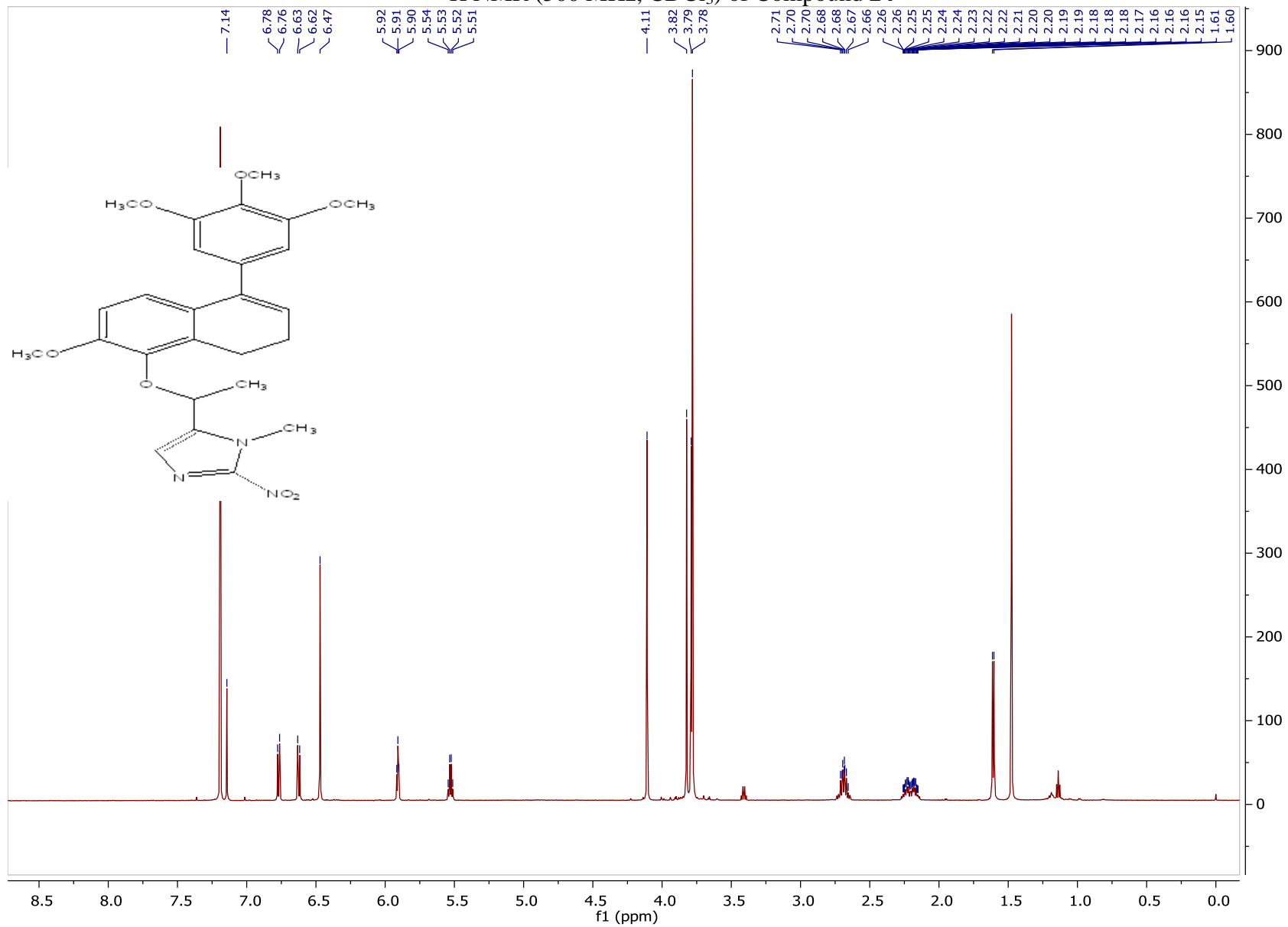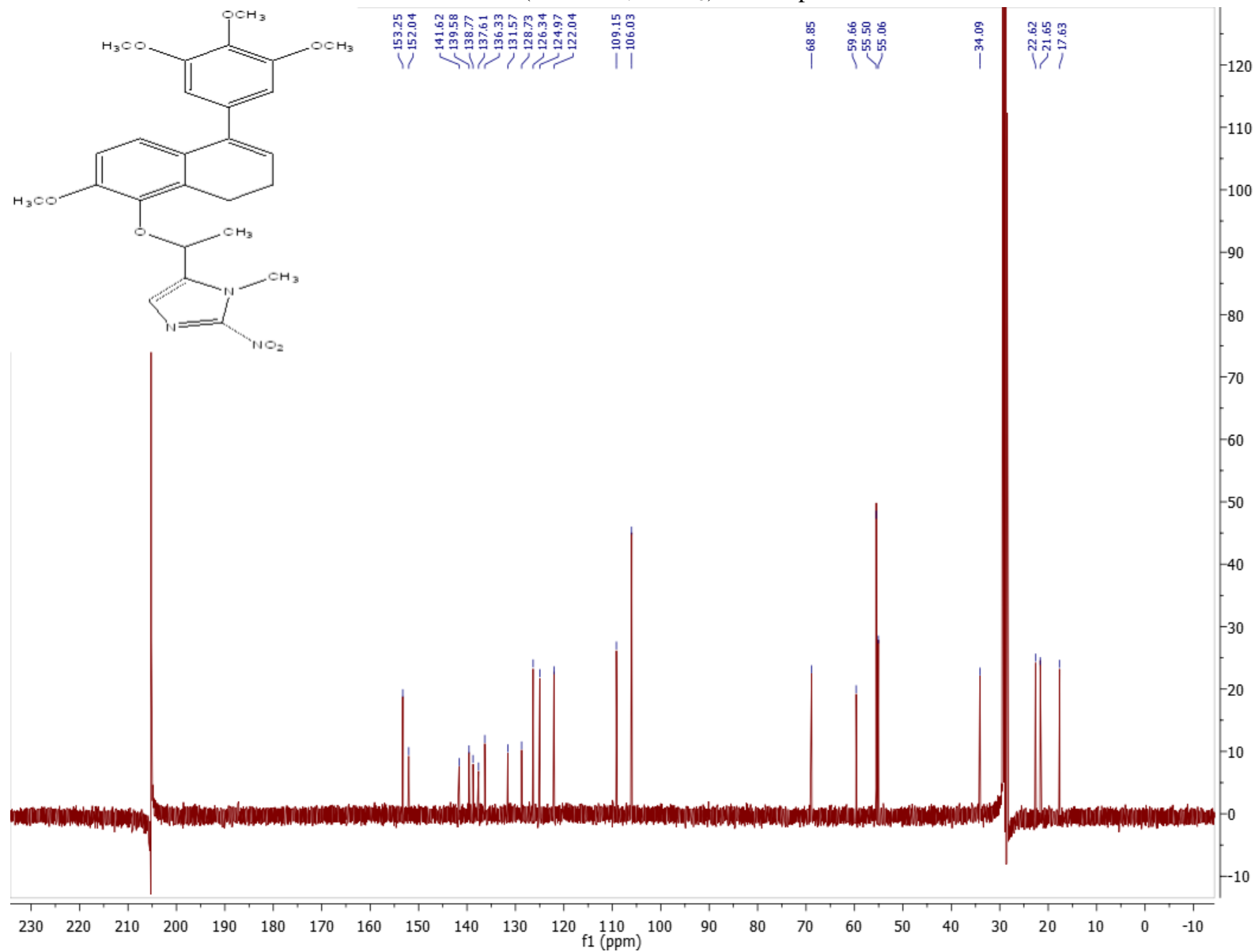
HPLC trace of Compound **24**

Data File C:\Chem32\1\Data\ZHE\ZS_IV_40000050.D
Sample Name: zs_IV_40

```
=================================================================
Acq. Operator   : zhe
Acq. Instrument : Instrument 1              Location :     -
Injection Date  : 6/14/2016 1:13:14 PM
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\DEF_LC.M
Last changed    : 6/22/2014 3:13:01 PM by SYSTEM
Sample Info     : zs_IV_40
                  GRAD 2 50-90 ACN
                  checking for OXi6196
                  20160613
```

Additional Info : Peak(s) manually integrated
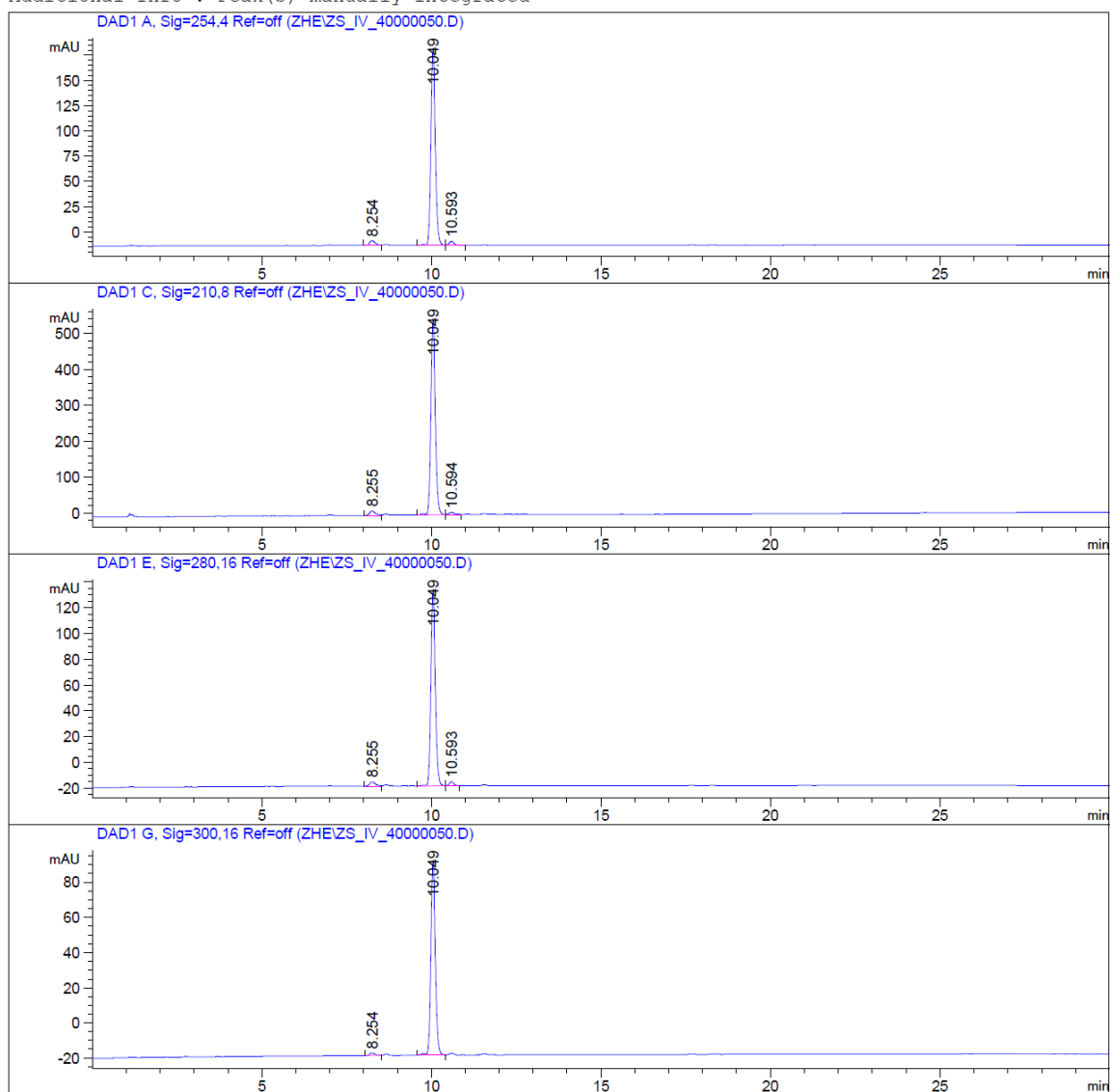
542

Data File C:\Chem32\1\Data\ZHE\ZS_IV_40000050.D
Sample Name: zs_IV_40

DAD1 H, Sig=320,16 Ref=off (ZHE\ZS_IV_40000050.D)
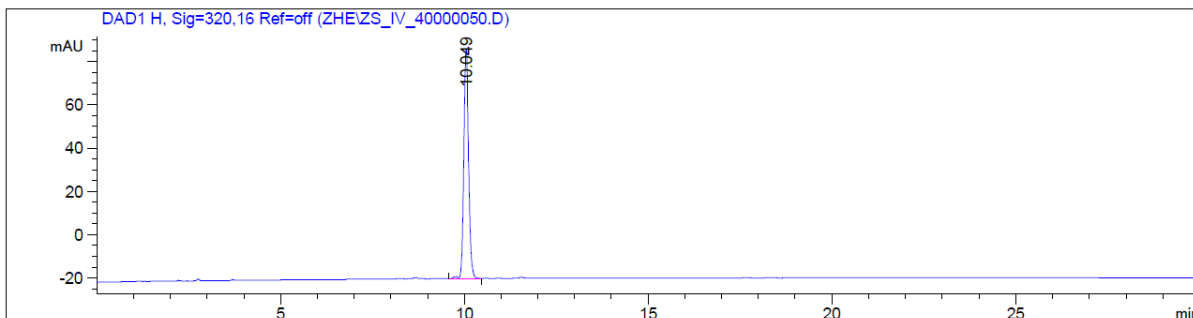
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================================================================
                      Area Percent Report
================================================================

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 Type  Width     Area       Height    Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   8.254 BV    0.1762   56.55766    4.84522   3.0398
  2  10.049 VV R  0.1395 1766.42224  195.27231  94.9382
  3  10.593 VB    0.1461   37.62222    3.91615   2.0220

Totals :                 1860.60212  204.03367




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

Peak RetTime Type  Width     Area       Height    Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   8.255 BV    0.1761  145.61433   12.47793   2.8203
  2  10.049 VV R  0.1392 4952.49658  545.70105  95.9208
  3  10.594 VB    0.1448   64.99823    6.84419   1.2589

Totals :                 5163.10915  565.02318




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

Peak RetTime Type  Width     Area       Height    Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   8.255 BV    0.1770   38.75599    3.30042   2.6811
  2  10.049 VV R  0.1393 1377.34216  152.55774  95.2831
  3  10.593 VB    0.1411   29.42853    3.20475   2.0358
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				1445.52668	159.06291	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.254	BB	0.1619	13.44318	1.26573	1.3209
2	10.049	VB R	0.1397	1004.31458	110.81192	98.6791
Totals :				1017.75776	112.07765	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.049	VB R	0.1390	967.01086	106.53122	100.0000
Totals :				967.01086	106.53122	

===
*** End of Report ***

Mass Spectrum of Compound **24**

NL:
5.19E7
RT_5_110_Orbi+
ESI#1-43 RT:
0.00-0.37 AV: 43 T:
FTMS + p ESI Full ms
[200.00-1000.00]

NL:
7.33E5

$C_{26}H_{30}N_3O_7$:
$C_{26}H_{30}N_3O_7$
pa Chrg 1

Top spectrum peaks: 279.0932, 342.1463, 358.1721, 380.1540, 396.1278, 458.0967, 496.2077, 518.1894, 535.1669, 567.2305, 596.1321, 632.7317, 715.3366, 737.3183, 739.3242, 837.3773, 853.3720, 875.3539, 902.4138, 975.4125, 991.4074

Bottom spectrum peaks: 496.2078, 501.2221

545
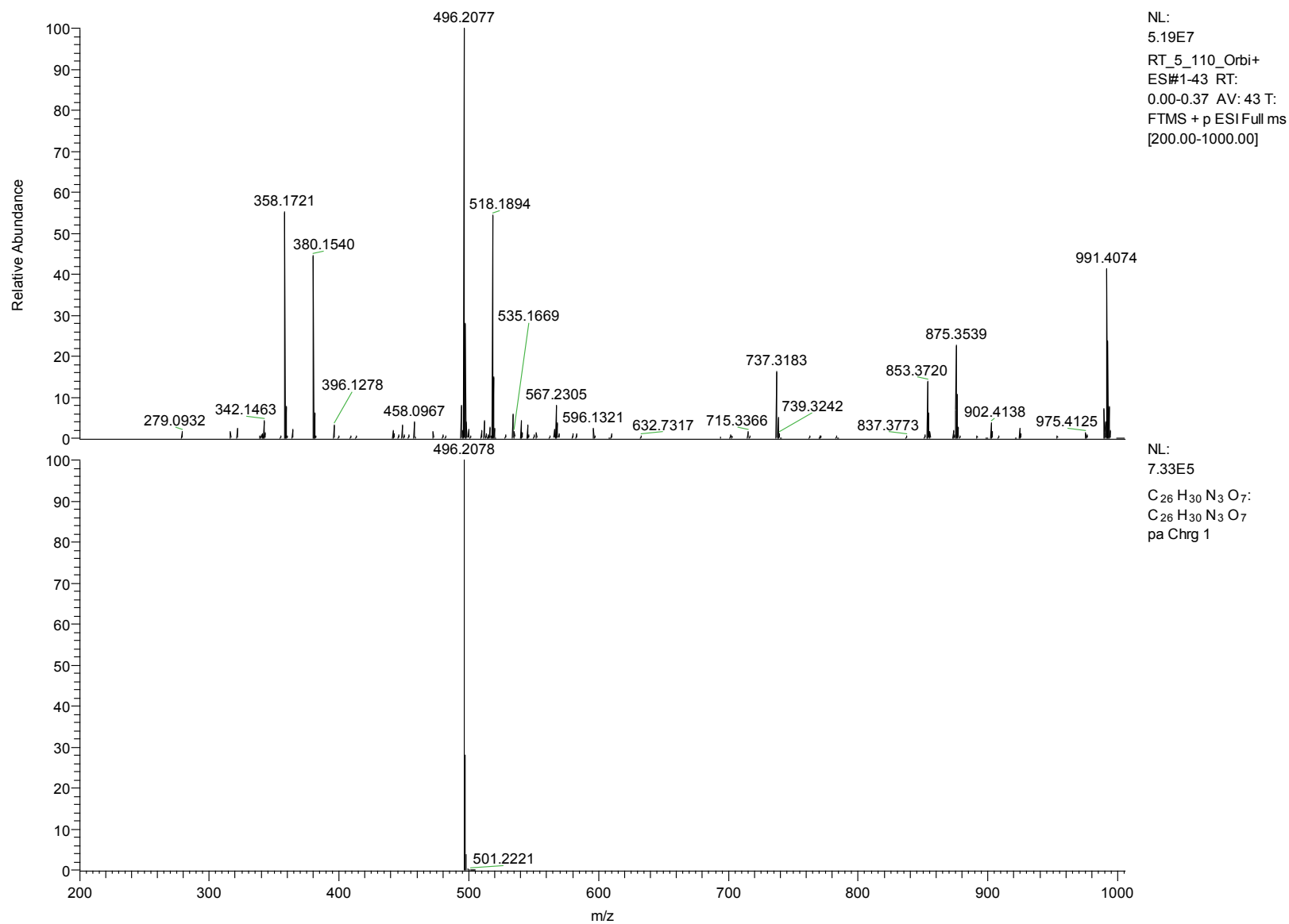
^{1}H NMR (500 MHz, Acetone *d*-6) of Compound **26**
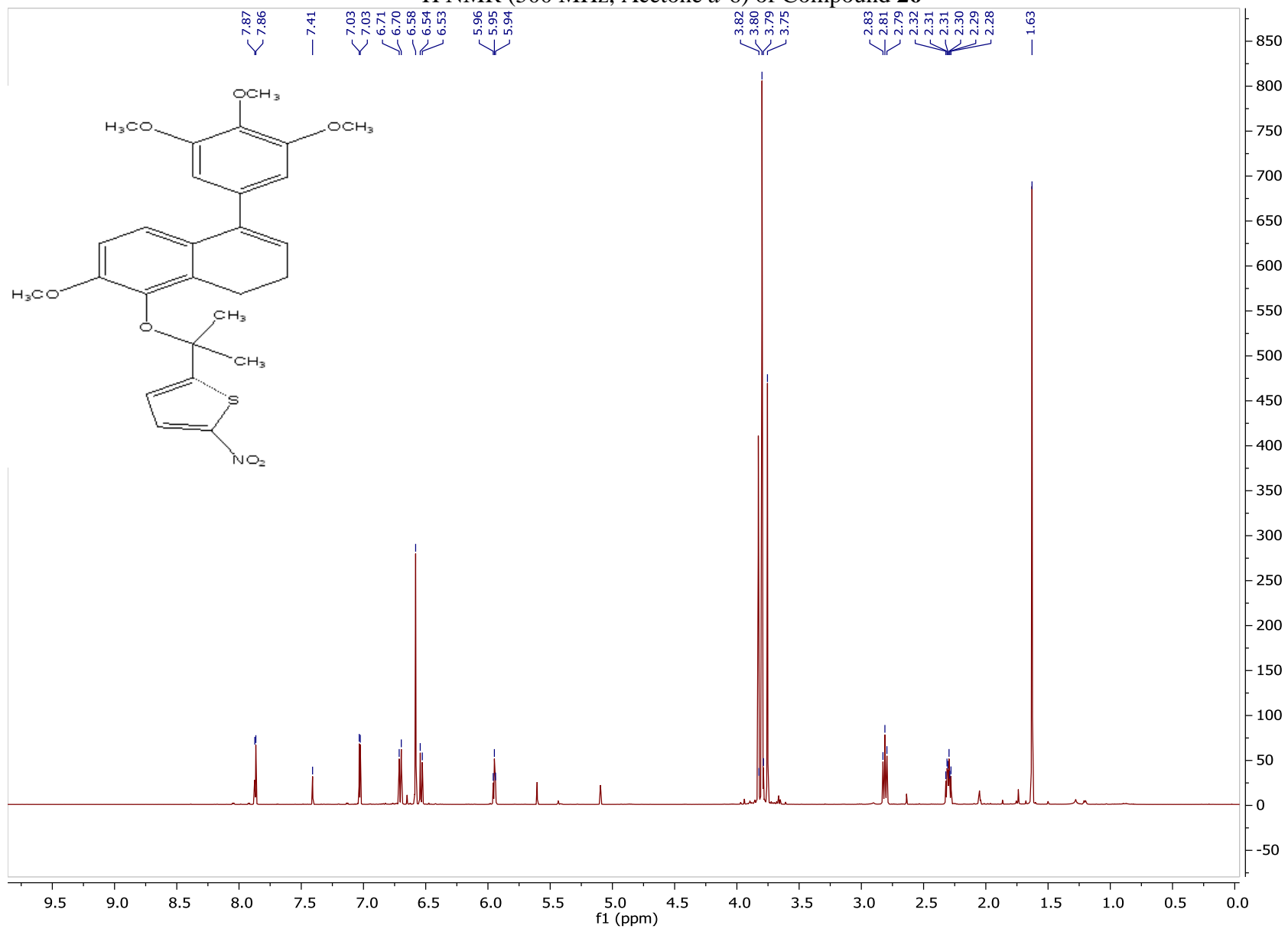
^{13}C NMR (500 MHz, Acetone d-6) of Compound **26**

HPLC trace of compound 26

DAD1 H, Sig=320,16 Ref=off (Blake\BW7 Oxi6196 Gem RC.D)

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

Sorted By             :        Signal
Multiplier            :        1.0000
Dilution              :        1.0000
Use Multiplier & Dilution Factor with ISTDs
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Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.150	BB	0.0810	21.74414	4.02976	0.1085
2	2.526	BV	0.0819	19.27966	3.63409	0.0962
3	2.918	VB	0.0798	21.06863	3.97996	0.1051
4	4.310	BB	0.1394	21.16036	2.10329	0.1056
5	5.728	BB	0.1465	43.51273	4.21120	0.2171
6	6.618	BB	0.1185	96.37733	12.70104	0.4808
7	7.046	BB	0.1352	1.94107e4	2237.72778	96.8423
8	9.382	BB	0.1269	12.60544	1.58271	0.0629
9	10.761	BB	0.1691	31.43010	2.75466	0.1568
10	11.961	BB	0.1444	18.62211	2.04165	0.0929
11	12.667	BB	0.1665	156.38867	14.42549	0.7802
12	19.562	BB	0.1651	190.73715	17.78152	0.9516

```
Totals :                      2.00436e4   2306.97316
```

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.544	BB	0.0636	12.17599	2.98181	0.0321
2	1.731	BV	0.0634	74.16545	18.27028	0.1954
3	1.883	VB	0.0662	147.86836	34.35511	0.3895
4	2.146	BV	0.0880	469.05283	76.04569	1.2356
5	2.346	VB	0.0708	12.12536	2.67804	0.0319
6	2.530	BB	0.0784	109.76604	21.94122	0.2892
7	2.749	BV	0.0872	49.36267	8.56939	0.1300

Data File C:\Chem32\1\Data\Blake\BW7 Oxi6196 Gem RC.D
Sample Name: BW7 Oxi6196 Gem RC

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
8	2.918	VB	0.0797	37.39708	7.07094	0.0985
9	3.344	VB	0.1003	50.03474	7.26759	0.1318
10	3.745	BB	0.1276	11.39088	1.51767	0.0300
11	4.309	BV	0.1319	54.41442	5.99596	0.1433
12	4.659	VV	0.1250	12.91497	1.55343	0.0340
13	4.804	VB	0.1211	12.79491	1.57006	0.0337
14	5.442	BV	0.1321	29.21450	3.33666	0.0770
15	5.728	VB	0.1613	83.36116	7.18853	0.2196
16	6.618	BB	0.1176	301.44446	40.14783	0.7941
17	7.047	BV	0.2078	3.49715e4	2688.12720	92.1243
18	7.902	VV	0.2100	123.94389	8.12347	0.3265
19	8.496	VB	0.1661	59.22626	5.15211	0.1560
20	9.381	BV	0.1367	42.00155	4.76997	0.1106
21	9.626	VV	0.1518	15.92287	1.63308	0.0419
22	9.986	VB	0.1470	13.82489	1.47941	0.0364
23	10.761	BV	0.1798	95.38769	7.63518	0.2513
24	11.962	BB	0.1793	119.56042	9.87117	0.3150
25	12.414	BV	0.1328	32.26881	3.80966	0.0850
26	12.667	VB	0.1623	431.83203	41.20753	1.1376
27	13.498	BB	0.2967	52.24476	2.39301	0.1376
28	19.121	BB	0.1545	17.16413	1.74938	0.0452
29	19.562	BB	0.1660	518.84137	48.05563	1.3668

Totals :   3.79612e4 3064.49704

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.544	VB	0.2223	27.49159	1.55504	0.1911
2	1.883	BB	0.0647	6.14138	1.47195	0.0427
3	2.147	BV	0.0939	28.73404	4.30289	0.1998
4	2.524	VB	0.0820	13.30602	2.50370	0.0925
5	2.752	BV	0.0903	13.37504	2.21877	0.0930
6	2.919	VB	0.0811	38.50901	7.12371	0.2677
7	4.308	BB	0.1187	19.31515	2.43219	0.1343
8	5.903	BB	0.2518	26.66931	1.48306	0.1854
9	6.619	BV	0.1197	33.90186	4.41026	0.2357
10	7.046	VV	0.1336	1.38179e4	1618.53064	96.0746
11	7.904	VV	0.2148	34.61695	2.20964	0.2407
12	8.497	VB	0.1676	16.42806	1.41371	0.1142
13	9.382	BB	0.1276	8.51581	1.06148	0.0592
14	10.761	BB	0.1588	20.12291	1.91218	0.1399
15	11.962	BB	0.1412	10.03933	1.11330	0.0698
16	12.667	BB	0.1666	108.24971	9.97465	0.7527
17	19.562	BB	0.1648	159.14778	14.88236	1.1065

Totals :   1.43825e4 1678.59953

Data File C:\Chem32\1\Data\Blake\BW7 Oxi6196 Gem RC.D
Sample Name: BW7 Oxi6196 Gem RC

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.544	BB	0.0961	7.62694	1.11139	0.1032
2	2.522	BB	0.0811	6.80067	1.29880	0.0921
3	2.753	BV	0.0872	15.37116	2.66771	0.2081
4	2.919	VB	0.0799	57.42009	10.82305	0.7773
5	6.618	BB	0.1181	37.88076	5.01658	0.5128
6	7.046	BB	0.1286	7099.83594	839.95410	96.1135
7	11.963	BB	0.1681	18.23425	1.63518	0.2468
8	12.667	BB	0.1566	35.85839	3.58817	0.4854
9	19.562	BB	0.1642	107.89776	10.13904	1.4607

Totals :   7386.92596   876.23401

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.753	BV	0.0860	11.72718	2.07270	0.7518
2	2.919	VB	0.0793	89.52174	17.04667	5.7388
3	6.618	BB	0.1185	33.24532	4.38226	2.1312
4	7.046	BB	0.1309	1310.35754	154.51758	84.0011
5	11.964	BB	0.1634	11.01170	1.02408	0.7059
6	19.562	BB	0.1639	104.06503	9.79913	6.6711

Totals :   1559.92852   188.84243

===
*** End of Report ***

Mass Spectrum of Compound **26**

Top spectrum (FTMS + p APCI):
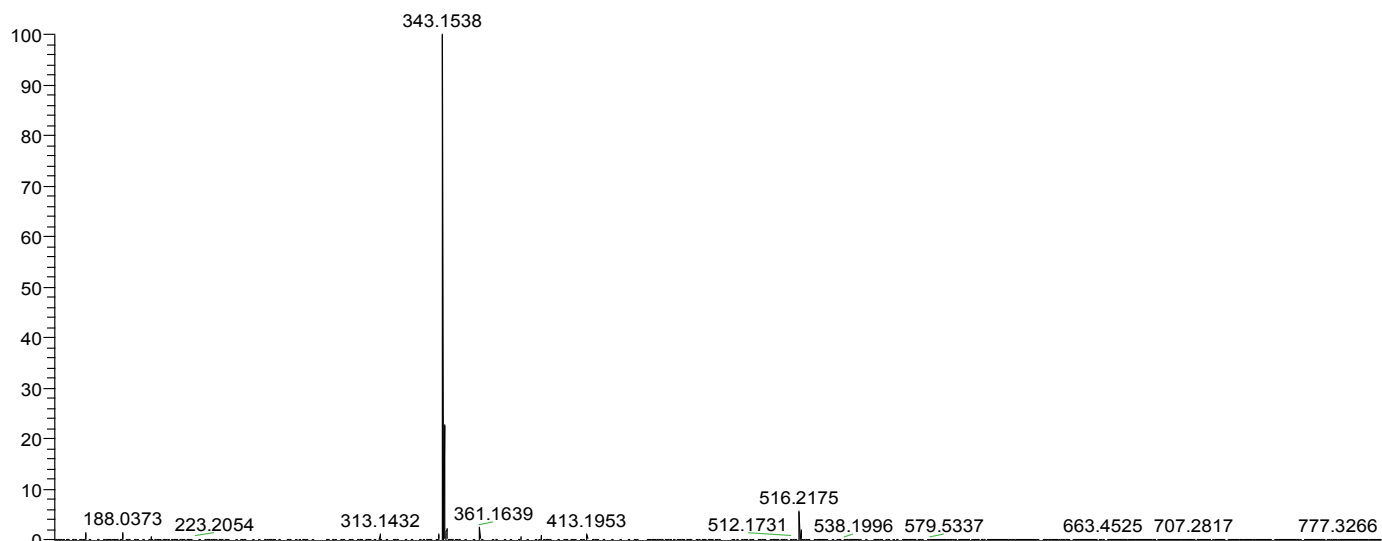
NL: 3.62E7
Oxi6196 Gemb#14
RT: 0.15 AV: 1 T:
FTMS + p APCI
corona pi Full ms
[150.00-2000.00]

Peaks: 188.0373, 223.2054, 313.1432, 343.1538, 361.1639, 413.1953, 512.1731, 516.2175, 538.1996, 579.5337, 663.4525, 707.2817, 777.3266

Bottom spectrum (FTMS - p APCI):
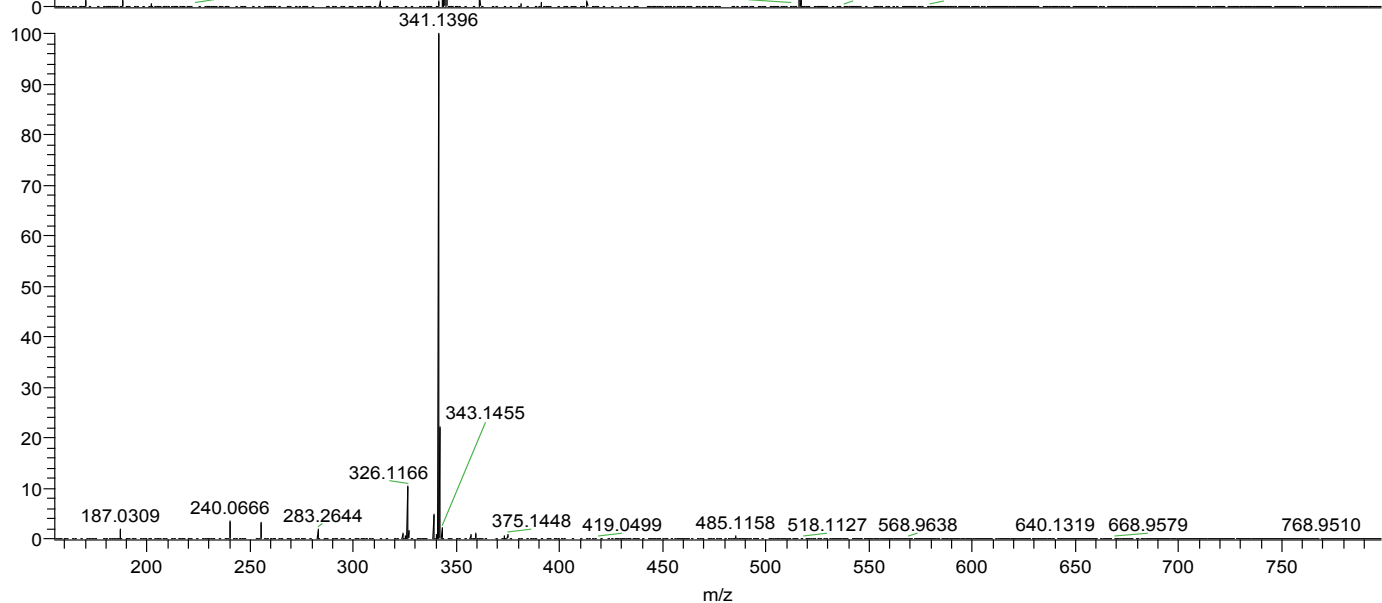
NL: 1.31E7
oxi6196 gem neg#15
RT: 0.15 AV: 1 T:
FTMS - p APCI corona
pi Full ms
[150.00-2000.00]

Peaks: 187.0309, 240.0666, 283.2644, 326.1166, 341.1396, 343.1455, 375.1448, 419.0499, 485.1158, 518.1127, 568.9638, 640.1319, 668.9579, 768.9510

552

^{1}H NMR (500 MHz, CDCl$_3$) of Compound **32**

7.76

4.29

2.57

553

^{13}C NMR (500 MHz, CDCl$_3$) of Compound **32**

554
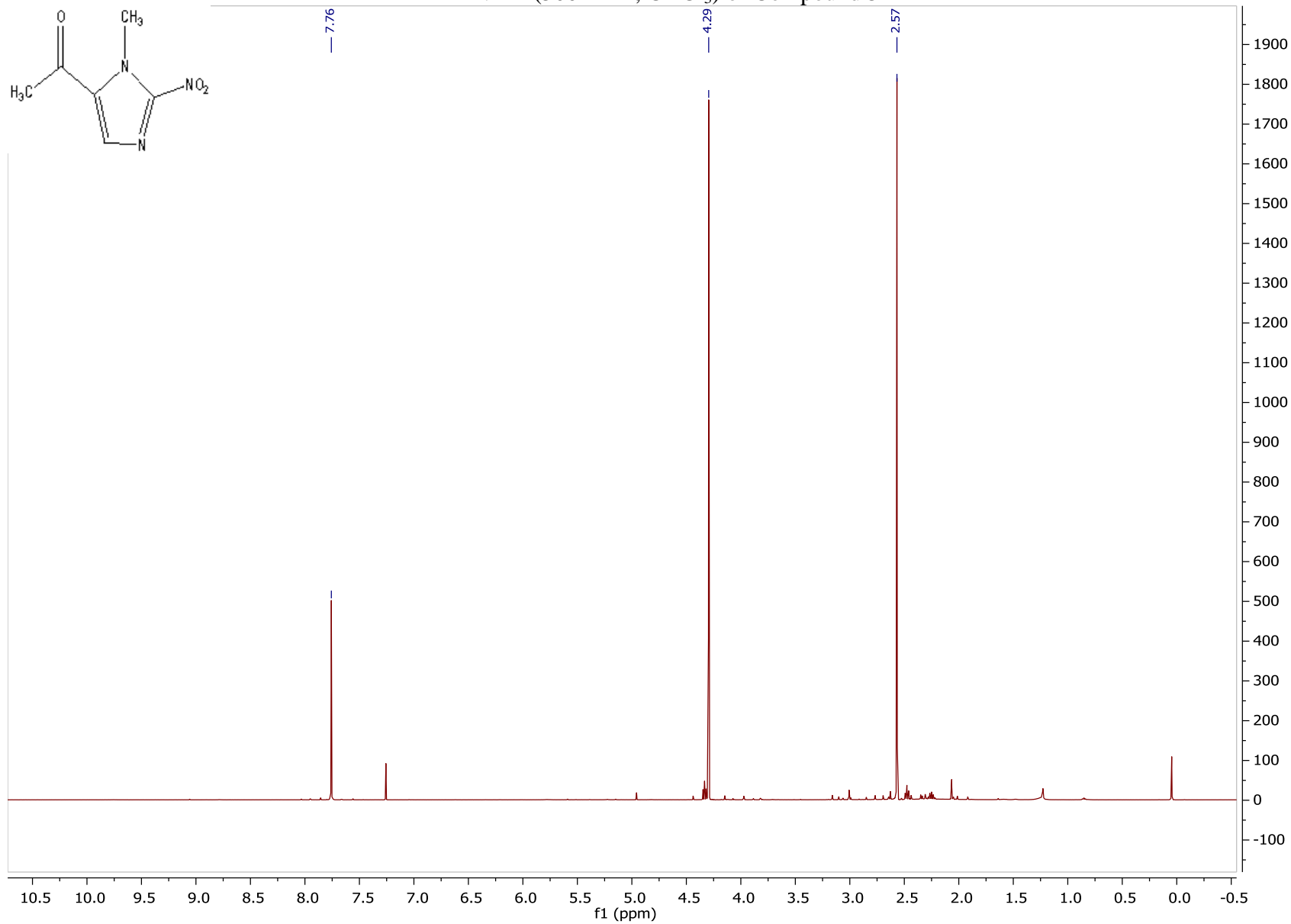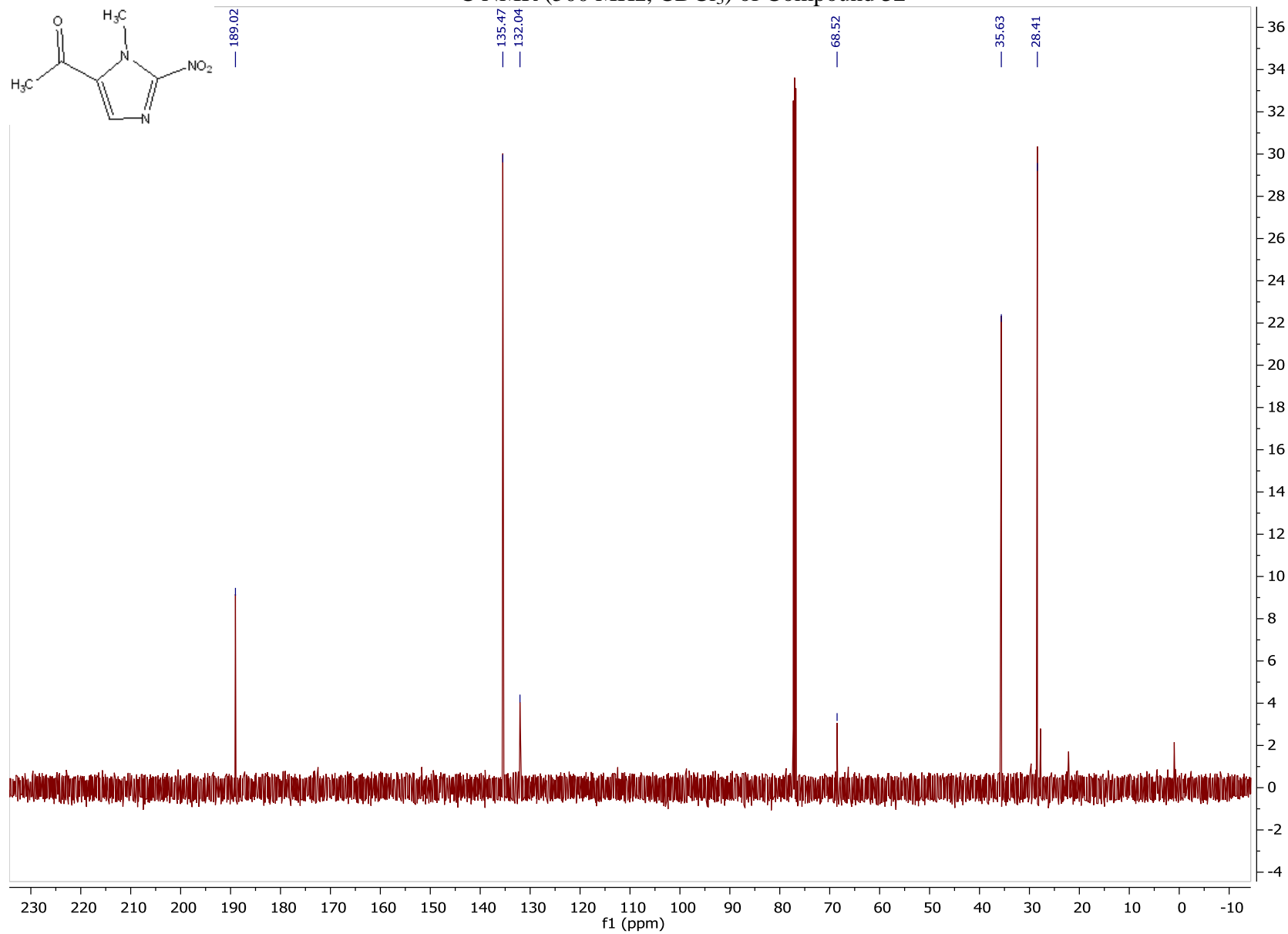
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